Mouse kidney tubular ablation of *Ocrl*/*Inpp5b* phenocopies Lowe syndrome tubulopathy

Supplemental experimental procedure

Antibodies and plasmids. Rabbit anti-OCRL (Sigma-Aldrich); Mouse anti-β-actin (Sangene Biotech); Rabbit anti-retinol binding protein (DAKO); Rabbit anti-vitamin D binding protein (Novus Biological Inc); FITC-conjugated Lotus tetragonolobus agglutinin (Vectors Laboratories); Rabbit anti-bovine serum albumin (BSA) (EMD Millipore Corporation); Rabbit anti-parathyroid hormone (PTH) receptor 1 (Abcam); Rabbit anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cell signaling technology); Alexa Fluor 488 goat anti-mouse IgG antibody (Invitrogen) ; and Alexa Fluor 594 goat anti-rabbit IgG antibody (Invitrogen) were purchased commercially. Rabbit anti-type 2a sodium phosphate cotransporter was a kind gift Dr. H. Murer (University of Zurich, Zurich, Switzerland). Rabbit anti-megalin was a kind gift Dr. D. Biemesderfer (Yale University, Connecticut USA). Mouse anti-NHE3 was a kind gift Dr. Peter S. Aronson (Yale University, Connecticut USA).

Biochemical analysis of plasma and urine. Urine samples were collected and analyzed at 0, 1, 2, 3, 4 months after the two weeks of doxycycline induction. The following parameters were measured in duplicate by ELISA kits: urine calcium (Cayman Chemical), urine phosphate (Abcam), urine albumin (Bethyl Laboratories Inc), plasma and urine creatinine, and PTH, (Bioassay Systems, Hayward, CA and My BioSource Inc, respectively), and plasma C-terminal fibroblast growth factor 23 (cFGF23) (Immunotopics Inc). Plasma bicarbonate and pH were measured by using VetScan i-STAT 1 analyzer (ABAXIS).

Quantitative PCR analysis. Total RNA was extracted from the kidney cortex using Trizol (Thermo Fisher scientific). Its concentration was measured by spectrophotometry (Nanodrop

Technologies, Montchanin, DE). One microgram of total RNA was used for reverse-transcription by a high-capacity cDNA Synthesis Kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Quantitative PCR amplifications were performed using power SYBR green mix (Applied Biosystems) with a 7300 AB real-time PCR machine (Applied Biosystems).

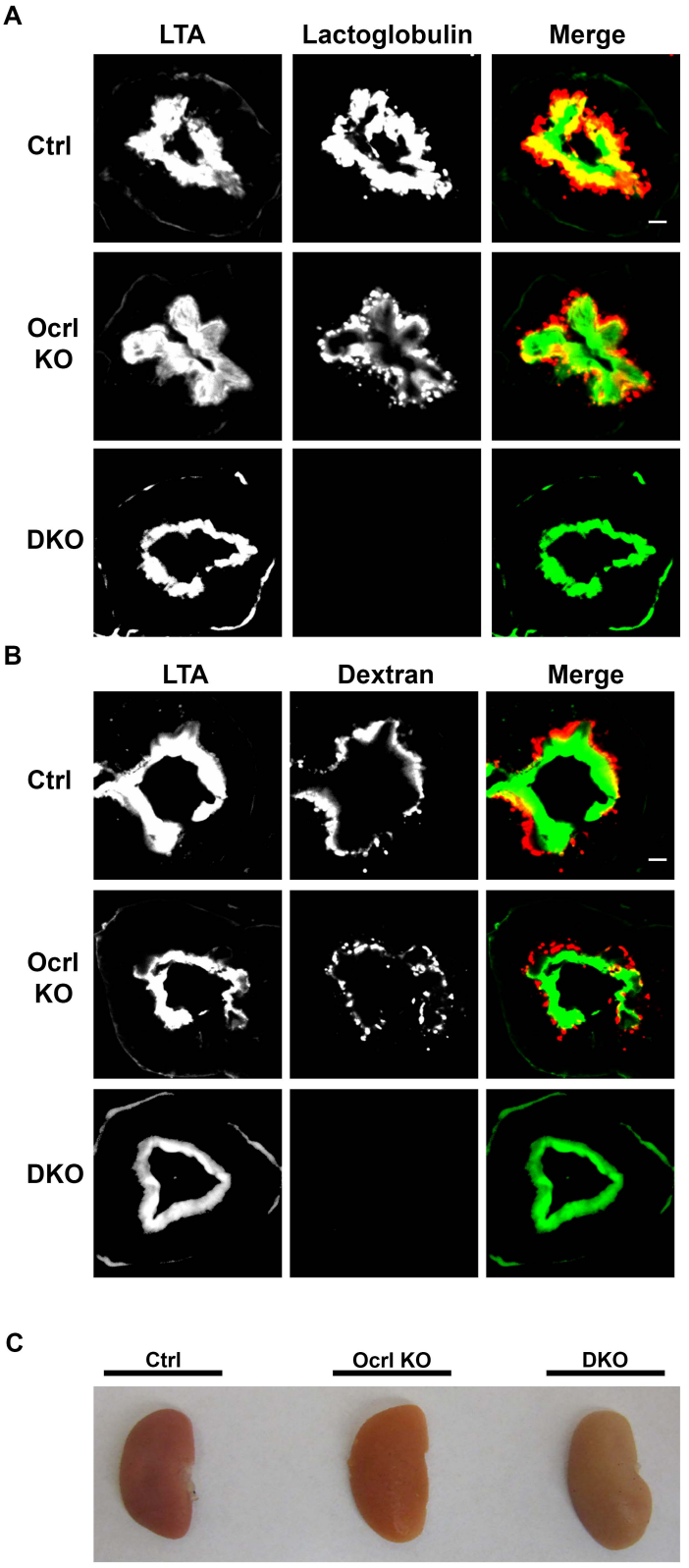
Immunoblotting. Freshly isolated kidney cortex were lysed in lysis buffer containing 50 mM Tris-HCl (pH 7.6), 500 mM NaCl, 0.1 % SDS, 0.5 % deoxycholate, 1 % Triton X-100, 0.5 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM NaF, 1 mM PMSF and protease inhibitor cocktail (Roche Diagnostics). Protein concentrations were quantified using the Bio-Rad protein assay. Equal amount of kidney cortex proteins, renal brush border proteins, or urine proteins normalized to urine creatinine were separated by SDS-PAGE and transferred on PVDF membranes (EMD Millipore). Membranes were incubated with 5% nonfat milk in Tris-buffered saline and Tween 20 (TBST), incubated with the appropriate primary antibody at 4°C overnight, washed with TBST, and incubated with the appropriate HRP-labeled anti-IgG secondary antibody (Bio-Rad). HRP signal was developed using enhanced chemiluminescence reagents (Bio-Rad) and detected with Odyssey (LI-COR bioscience). For quantification, densitometry was performed using Image J software (NIH).

Histology and immunofluorescence. Mice were anesthetized by intraperitoneal injection of ketamine and xylazine followed by perfusion fixation with 4% paraformaldehyde with or without glutaldehyde through the left ventricle. Histology [H & E, periodic acid-Schiff (PAS), and Masson's trichrome staining] was performed by the Yale Pathology Core Tissue Services. To evaluate interstitial fibrosis, Masson stained kidney sections were assessed as previously described [1]. For immunofluorescence, kidney cryosections (4 μm) were exposed to 0.01%

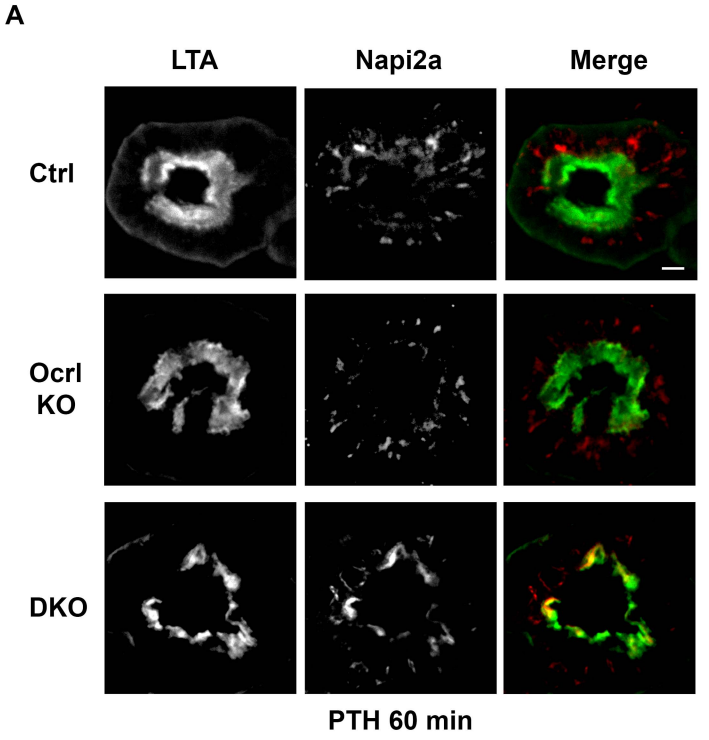
sodium borohydride (Sigma) for 30 minutes, permeablized with 0.2% Triton X-100 in TBS for 30 minutes and incubated with 3% BSA in TBS for 1 hour. Immunostaining was performed with primary antibodies overnight at 4°C, followed by Alexa Fluor 488– and/or Alexa Fluor 594–conjugated secondary antibodies and/or FITC conjugated LTA, washed, and mounted with Fluoro Gel with Tris buffer (Electron Microscopy Science). Images were acquired by confocal microscopy. For quantification, immunofluorescence intensity in regions of interest was measured by Image J software (NIH).

References

1. Hassan, H, Tian, X, Inoue, K, Chai, N, Liu, C, Soda, K, Moeckel, G, Tufro, A, Lee, AH, Somlo, S, Fedeles, S, Ishibe, S: Essential Role of X-Box Binding Protein-1 during Endoplasmic Reticulum Stress in Podocytes. *J Am Soc Nephrol*, 2015.



Supplemental Figure. 1



Supplemental Figure. 2

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Supplemental figure legend

Supplemental Figure 1. Endocytic defect in proximal tubules at 2 weeks following doxycycline induction. (A) Representative images showing uptake of Alexa 546-labeled β-lactoglobulin (red) of control and *Ocrl* KO kidney proximal tubule by immunofluorescence at 15 minutes, but was not observed in the cDKO at identical exposure time. FITC-LTA (green). Scale bars: 10 μm. (B) Representative images showing uptake of Alexa 546–labeled Dextran (red) in the control and *Ocrl* KO kidney proximal tubule by immunofluorescence at 10 minutes, but was not observed in the cDKO at identical exposure time. FITC-LTA (green). Scale bars: 10 μm. (C) Whole kidneys of control, *Ocrl* KO, and cDKO mice in (A) revealing reduced uptake of Alexa 546-labeled β-lactoglobulin in the cDKO mice

Supplemental Figure 2. Uptake of NaPi2a after PTH treatment is markedly delayed in the cDKO mice. (A) Representative immunofluorescence images of kidney proximal tubules stained with NaPi2a (red) 60 minutes after PTH injection in control, *Ocrl* KO, and cDKO mice, FITC-LTA (green) Scale bars: 10 μm. (B) Expression of PTH receptor 1 (PTHR1) in renal brush border membrane detected by immunoblotting. (C) Plasma C-terminal FGF23 (cFGF23) levels in control, *Ocrl* KO, and cDKO mice. *$p<0.05$ N=4.