Supplemental Data Information - Ronzaud *et al.*

Supplemental methods

Genotyping and recombination PCR. Genotyping and recombination PCR were performed on DNA extracted from tail, kidney and liver as described in (1, 2).

Immunoblotting. NEDD4-2 was detected using antibodies against the amino acids 110-226 (3) diluted 1/1000, or 300-376 (Abcam) diluted 1/500, or the residues surrounding the amino acid 151 (Cell Signaling) (4) diluted 1/1000. NEDD4-1 and β-actin were detected using an anti-NEDD4-1 (5) diluted 1:500 and an anti-actin (Sigma) diluted 1:1000, respectively.

Supplemental references

1. Shi, P.P., Cao, X.R., Sweezer, E.M., Kinney, T.S., Williams, N.R., Husted, R.F., Nair, R., Weiss, R.M., Williamson, R.A., Sigmund, C.D., et al. 2008. Salt-sensitive hypertension and cardiac hypertrophy in mice deficient in the ubiquitin ligase Nedd4-2. *Am J Physiol Renal Physiol* 295:F462-470.
2. Traykova-Brauch, M., Schonig, K., Greiner, O., Miloud, T., Jauch, A., Bode, M., Felsher, D.W., Glick, A.B., Kwiatkowski, D.J., Bujard, H., et al. 2008. An efficient and versatile system for acute and chronic modulation of renal tubular function in transgenic mice. *Nat Med* 14:979-984.
3. Boase, N.A., Rychkov, G.Y., Townley, S.L., Dinudom, A., Candi, E., Voss, A.K., Tsoutsman, T., Semsarian, C., Melino, G., Koentgen, F., et al. 2011. Respiratory distress and perinatal lethality in Nedd4-2-deficient mice. *Nat Commun* 2:287.
4. Kimura, T., Kawabe, H., Jiang, C., Zhang, W., Xiang, Y.Y., Lu, C., Salter, M.W., Brose, N., Lu, W.Y., and Rotin, D. 2011. Deletion of the ubiquitin ligase Nedd4L in lung epithelia causes cystic fibrosis-like disease. *Proc Natl Acad Sci U S A* 108:3216-3221.
5. Staub, O., Dho, S., Henry, P.C., Correa, J., Ishikawa, T., McGlade, J., and Rotin, D. 1996. WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na^+ channel deleted in Liddle's syndrome. *EMBO J.* 15:2371-2380.

Supplemental figures and legends

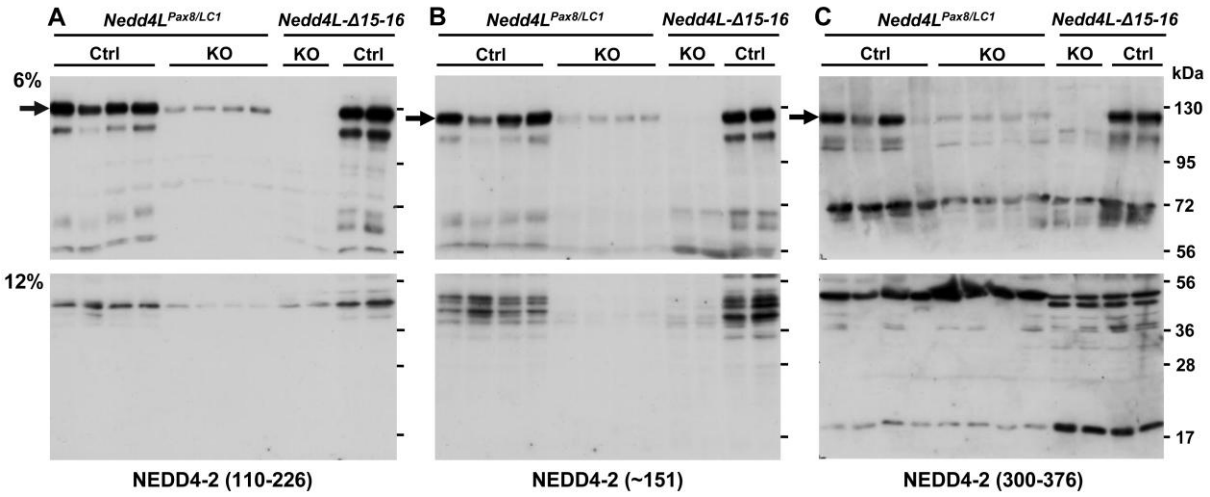

Figure S1

Detailed analysis of NEDD4-2 protein loss in *Nedd4L*$^{Pax8/LC1}$ KO mice using different NEDD4-2 antibodies. Western blots for NEDD4-2 were performed on whole kidney extracts from control and *Nedd4L*$^{Pax8/LC1}$ KO mice using different antibodies against either the amino acids 110-226 **(A)**, or the residues surrounding the amino acid 151 **(B)**, or the amino acids 300-376 **(C)**. Samples were loaded on 6% and 12% polyacrylamide gels to detect high as well as low molecular weight products. Kidney extracts from P19-old total *Nedd4L-Δ15-16* KO mice were used as negative controls. Arrows indicate the specific 130-kDa NEDD4-2 band that was recognized by all antibodies and that was lost in the total KO but remained in the *Nedd4L*$^{Pax8/LC1}$ KO mice at low levels. Ctrl: control mice.

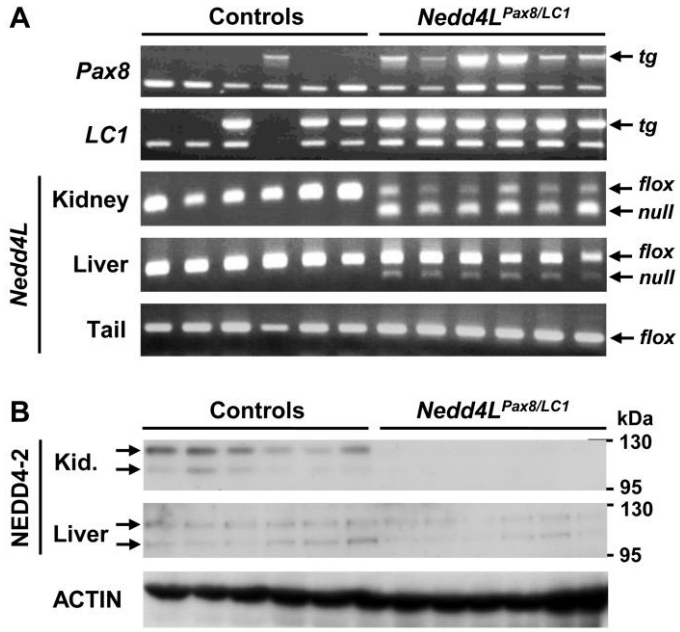

Figure S2

***Nedd4L*$^{Pax8/LC1}$ KO mice show recombination of the *Nedd4L* floxed allele in liver. (A)** PCRs on DNA extracted from kidney, liver and tail show recombination (null band) in kidney as well as in liver of doxycycline-treated *Nedd4L*$^{Pax8/LC1}$ KO mice. **(B)** However, a significant amount of NEDD4-2 protein was still detected in liver of KO mice. Tg: *Pax8-rTA* or *LC1* transgenic band; flox: *Nedd4L* floxed allele band; null: recombined *Nedd4L* allele band.

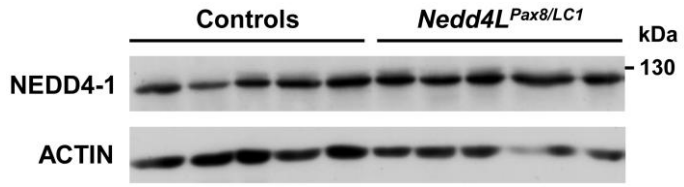

Figure S3

***Nedd4L*$^{Pax8/LC1}$ KO mice show normal NEDD4-1 abundance in the kidney.** Western blot analysis on kidney from control and *Nedd4L*$^{Pax8/LC1}$ KO mice shows unchanged NEDD4-1 protein expression.

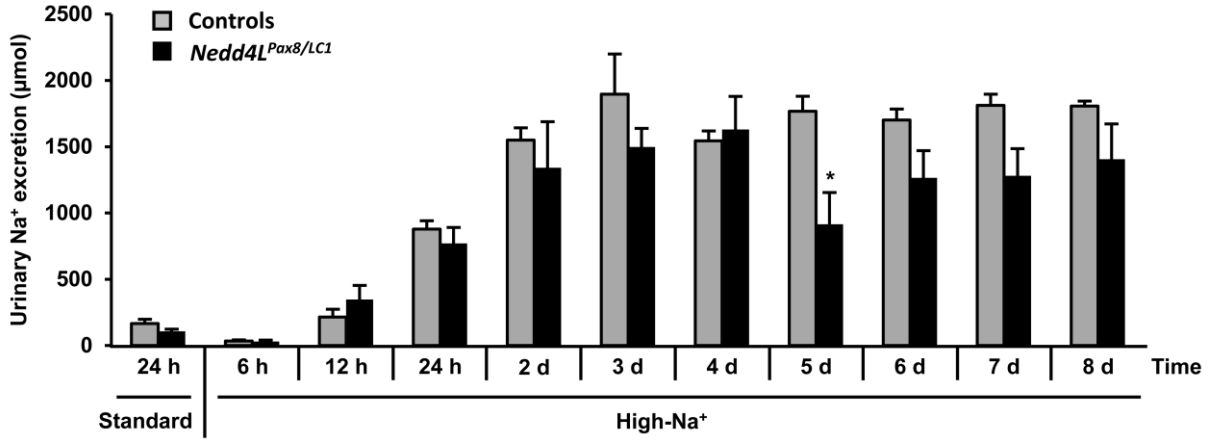

Figure S4

***Nedd4L*$^{Pax8/LC1}$ KO mice do not display any sign of Na^+ retention after switch to high-Na^+ diet.** After equilibration on a standard diet, control (n = 4) and *Nedd4L*$^{Pax8/LC1}$ KO (n = 4) mice were fed a high-Na^+ diet and Na^+ excretion was measured after 6 h, 12 h, 24 h and then daily. Despite a tendency to retain Na^+, KO mice did not show any significant difference in Na^+ excretion compared to control mice, except at day 5. *P<0.05, KO versus controls.

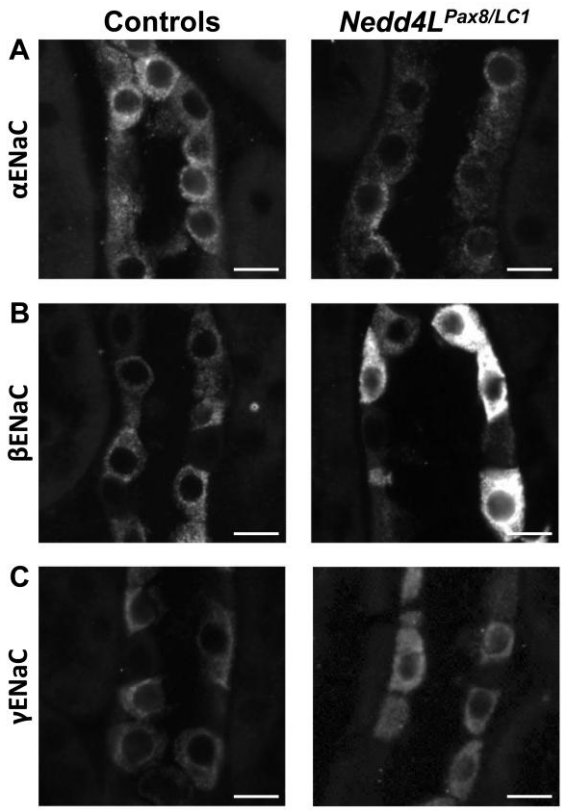

Figure S5

Immunofluorescence for α-, β- and γENaC protein expression. High magnification of immunostaining for α- **(A)**, β- **(B)** and γENaC **(C)** on kidney cryosections of control and *Nedd4L*$^{Pax8/LC1}$ KO mice under high-Na^+ diet. Cytoplasmic β- and γENaC abundance is increased in CD of KO, whereas αENaC is not. Scale bars ~10 µm.

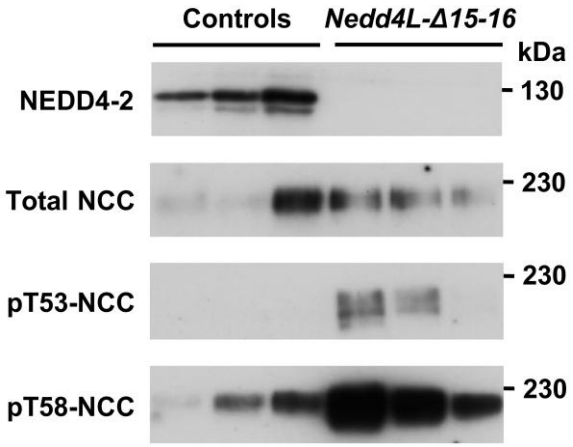

Figure S6

NCC abundance and phosphorylation is increased in the total *Nedd4L-Δ15-16* KO mice. Western blot analysis on kidney from control and total *Nedd4L-Δ15-16* KO mice from Kumar shows increased NCC protein expression and phosphorylation.

Supplemental table

Gene	Assay ID or primer/probe sequences
Aqp2	Mm00437575_m1
Scnn1a	Mm00803386_m1
Scnn1b	Forward: GGGTGCTGGTGGACAAGC Reverse: ATGTGGTCTTGGAAACAGGAATG Probe: CAGTCCCTGCACCATGAACGGCT
Scnn1g	Forward: AACCTTACAGCCAGTGCACAGA Reverse: TTGGAAGCATGAGTAAAGGCAG Probe: AGCGATGTGCCCGTCACAAACATCT
Gapdh	Mm99999915_g1
Slc12a3	Mm00490213_m1
Nedd4L	Mm01258749_m1
Kcnj1	Forward: ACGGATTCAGGTTTGTGACAG Reverse: GATCACTCCAAGAATAGACTGGAAGA Probe: CAGTGTGCCACTGCCATTTTTCTGCTT

Table S1. TaqMan Gene Expression Assay ID numbers (Applied Biosystems) or sequences of primers and probes for real-time quantitative PCR.