Legends of supplementary figures:

Figure S1: Urinary Na^+ excretion and urine osmolality of control and mutant mice

(A) Urinary Na^+ excretion under normal (ND), high K (HKD) and low K (LKD) diets showing no significant difference between both groups (n: 8 control and 7 KO mice). (B) Urine osmolality after 2 weeks of LKD. No significant difference between control and mutant animals was observed (n: 6 mice in each group).

Figure S2: Dietary potassium controls NEDD4-2 phosphorylation and total expression

(A) WB analysis of NEDD4-2 phosphorylation and total expression in mice fed either with high K^+ diet (HKD) for 2 days or with low K^+ diet (LKD) for 2 weeks. (B) Protein quantification from (A) showing significant decrease of NEDD4-2 phosphorylation at S222 and S328 under LKD with no change in total NEDD4-2 expression (n: 6 mice in each group; **: p value < 0.01). (C, D) Co-immunostaining of total NEDD4-2 (green) and AQP2 (red) in WT animals fed with HKD and LKD (C) and quantification of NEDD4-2 fluorescence intensity in the CNT/CCD segments (D). NEDD4-2 abundance was significantly increased in CNT/CCD cells in mice fed with LKD (n: 2 mice in each group; *: p value < 0.05).

Figure S3: ENaC expression and activity in *Nedd4L*$^{Pax8/LC1}$ mice after 2 weeks of LKD.

(A) Q-RT-PCR analysis (TaqMan) showing normal levels of αENaC (Scnn1a), βENaC (Scnn1b) and γENaC (Scnn1g) mRNA in *Nedd4L*$^{Pax8/LC1}$ mice compared to control after 2 weeks of LKD (n: 6 control and 5 KO animals). (B) Immunostaining of βENaC in control and *Nedd4L*-KO mice showing a similar pattern in both genotypes. (C) Benzamil treatment results

in a comparable increase of urine volume excreted by control and KO mice (n: 7 animals in each group).

Figure S4: AQP2 expression and CNT/CD cell size in control versus mutant mice.

(A) WB analyses showing AQP2 protein in control and mutant mice. Fg: Fully glycosylated, Ng: Non glycosylated (B) Quantification of A, showing no significant difference between both groups (n: 5 animals per group). (C) Histogram illustrating mean cell size in the CNT/CD of control and *Nedd4L*$^{Pax8/LC1}$ mice (n= 150 control and 200 mutant cells), showing no significant difference between groups.

Figure S1

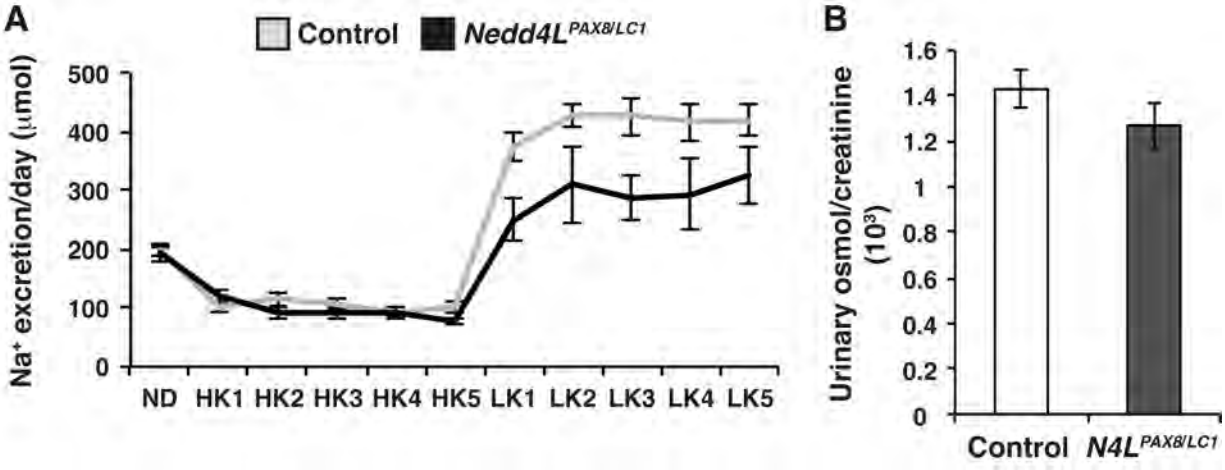

A
Control
Nedd4L PAX8/LC1
Na+ excretion/day (µmol)
500
400
300
200
100
0
ND HK1 HK2 HK3 HK4 HK5 LK1 LK2 LK3 LK4 LK5
B
Urinary osmol/creatinine (10³)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
N4L PAX8/LC1

Figure S2

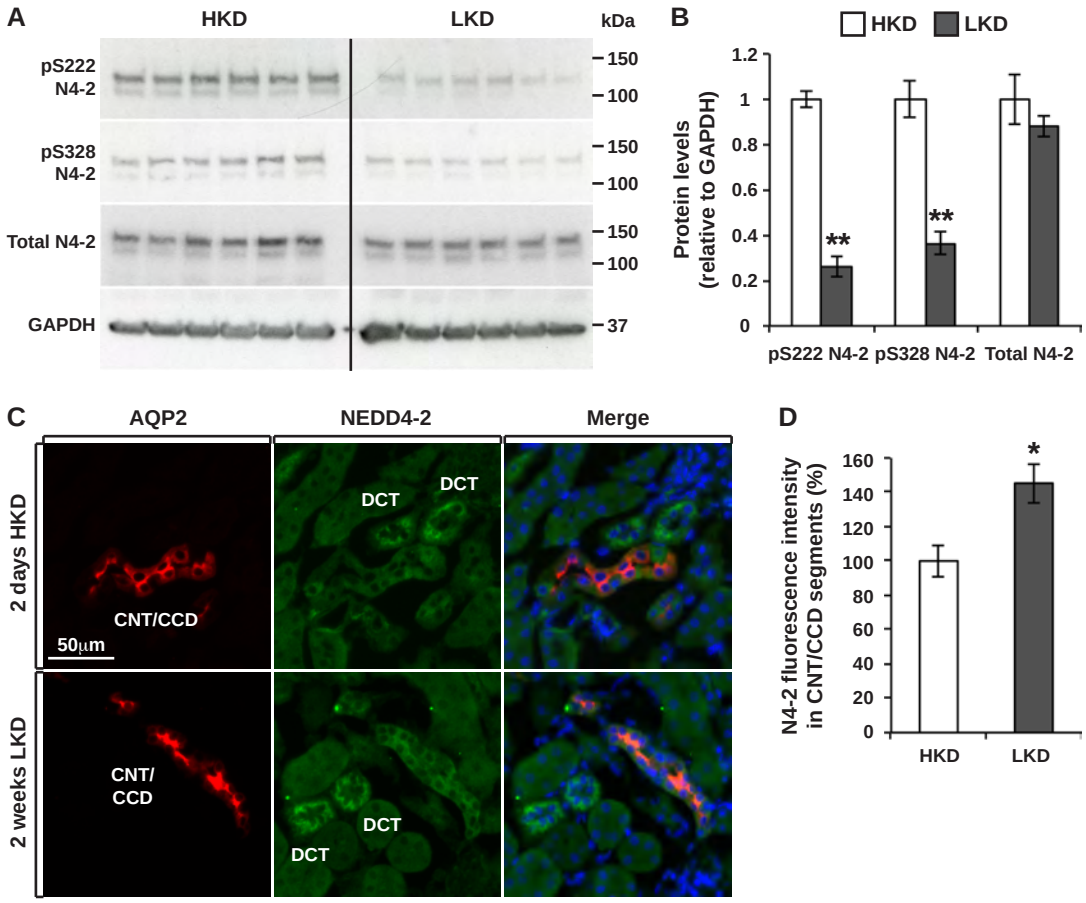

A
HKD
LKD
kDa
pS222 N4-2
pS328 N4-2
Total N4-2
GAPDH
150
100
150
100
150
100
37
B
HKD
LKD
Protein levels (relative to GAPDH)
1.2
1
0.8
0.6
0.4
0.2
0
**
**
pS222 N4-2
pS328 N4-2
Total N4-2
C
AQP2
NEDD4-2
Merge
2 days HKD
2 weeks LKD
CNT/CCD
50μm
CNT/ CCD
DCT
DCT
DCT
DCT
D
N4-2 fluorescence intensity in CNT/CCD segments (%)
160
140
120
100
80
60
40
20
0
*
HKD
LKD

Figure S3

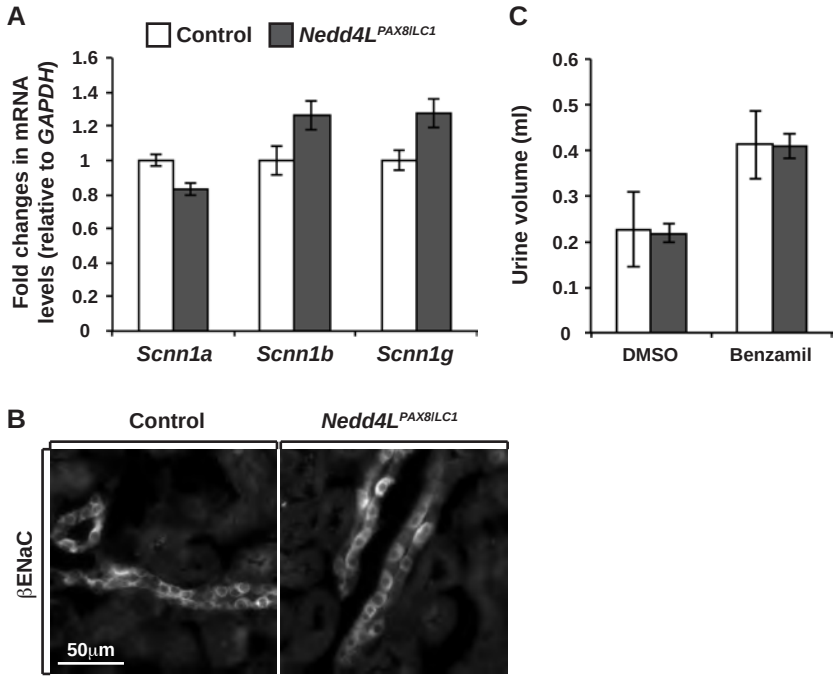
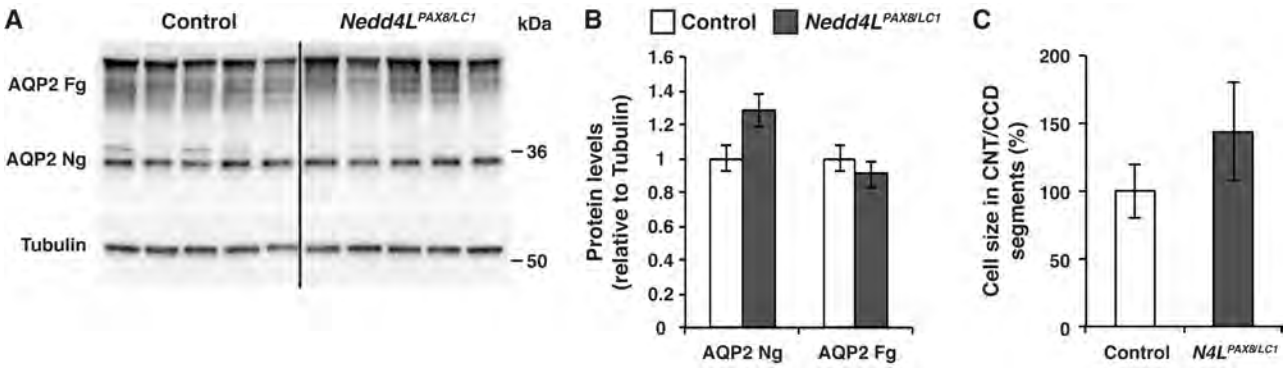

A
Control
Nedd4L PAX8/LC1
Fold changes in mRNA levels (relative to GAPDH)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Scnn1a
Scnn1b
Scnn1g
C
Urine volume (ml)
0.6
0.5
0.4
0.3
0.2
0.1
0
DMSO
Benzamil
B
Control
Nedd4L PAX8/LC1
βENaC
50μm

Figure S4


A
Control
Nedd4L PAX8/LC1
kDa
AQP2 Fg
AQP2 Ng
Tubulin
36
50
B
Control
Nedd4L PAX8/LC1
Protein levels (relative to Tubulin)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AQP2 Ng
AQP2 Fg
C
Cell size in CNT/CCD segments (%)
200
150
100
50
0
Control
N4L PAX8/LC1

	Normal diet (0.3 %)		Low K+ diet (<0.03%)	
	WT	KO	WT	KO
Body weight (g)	24.10 +/- 0.21	22.62 +/- 0.66	21.0 +/-0.6	19.8 +/-0.5
Food intake (g/g BW.24h)	0.16 +/- 0.01	0.17 +/-0.01	0.13 +/- 0.002	0.11 +/-0.01
Water intake (ml/g BW.24h)	0.20 +/- 0.01	0.20 +/- 0.01	0.55 +/-0.04	0.63 +/- 0.08
Urine volume (ml.24h)	1.57 +/- 0.14	1.94 +/- 0.21	5.81 +/- 0.73	5.24 +/-0.87

Table S1: Body weight, food and water intake and urine volume are not changed between control and mutant mice after 2 weeks of LKD

Primary antibodies	Host	Dilution for WB	Dilution for IF	References
Total AQP2	Goat		1/1000	Santa Cruz Biotechnology (C-17):sc-9882
Total AQP2	Rabbit	1/1000		Wagner CA., AJP-renal Physiol 2008
Total NCC	Rabbit	1/500	1/500	Sorensen MV., Kidney international 2013
Total NCC	Guinea-Pig		1/500	Kindly provided by Johannes Loffing
pT53 NCC	Rabbit	1/500	1/500	Sorensen MV., Kidney international 2013
pT58 NCC	Rabbit	1/500		Sorensen MV., Kidney international 2013
pT91 NCC	Sheep	1/700		Richardson C., J Cell Science 2008
3pNCC S(45-55-60)	Sheep	1/700		Kindly provided by Dario Alessi
pT233 SPAK	Sheep		1/100	Richardson C., J Cell Science 2008
pS373 SPAK	Rabbit	1/1000		Millipore (072273)
Total SPAK	Rabbit		1/100	Millipore (072271)
WNK1 (Ex12)	Rabbit	1/100	1/50	RoyA., JCI 2015
WNK4	Mouse		1/50	abcam (52847)
WNK4	Rabbit	1/1000		Novus Biologicals (NB600-284)
αENaC	Rabbit	1/500	1/500	Sorensen MV., Kidney international 2013
βENaC	Rabbit	1/500	1/500	Wagner CA., AJP-renal Physiol 2008
γENaC	Rabbit	1/500	1/500	Wagner CA., AJP-renal Physiol 2008
pS222 NEDD4-2	Sheep	1/600		Faresse N, AJP-renal Physiol 2012
pS328 NEDD4-2	Rabbit	1/500		Flores S., JASN 2005
Total NEDD4-2	Rabbit	1/1000	1/50	Kamynina E., FASEB J 2001
ROMK	Guinea-Pig	1/200	1/50	Al-Qusairi L., AJP-renal physiol 2016
Maxi K α	Rabbit	1/500	1/100	Alomone labs (APC-151)
Maxi K β1	Rabbit	1/1000		abcam (3587)
Maxi K β4	Rabbit	1/1000		Alomone labs (APC-061)
GAPDH	Mouse	1/5000		Millipore (MAB374)
Actin	Mouse	1/1000		Sigma-Aldrich (A5316)
Tubulin	Mouse	1/1000		Sigma-Aldrich (T5201)

Secondary antibodies	Host	Dilution for WB	Dilution for IF	References
Goat, AlexaFluor® 555	Donkey		1/500	Invitrogen (A-21432)
Guinea-Pig, AlexaFluor® 546	Goat		1/500	Invitrogen (A-11074)
Mouse, AlexaFluor® 555	Goat		1/500	Invitrogen (A-21422)
Rabbit, AlexaFluor® 488	Goat		1/500	Invitrogen (A-11034)
Rabbit, AlexaFluor® 488	Donkey		1/500	Invitrogen (A-21206)
Sheep, AlexaFluor® 546	Donkey		1/500	Invitrogen (A-21098)
Guinea-Pig, HRP	Donkey	1/10000		Jackson ImmunoReasearch (706-035-148)
Mouse, HRP	Sheep	1/10000		GE Healthcare UK Limited (NA931V)
Rabbit, HRP	Donkey	1/10000		GE Healthcare UK Limited (NA934V)
Sheep, HRP	Rabbit	1/10000		Milipore (12-342)

Table S2: List of the antibodies used in this study

References for Table S2:

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8. Al-Qusairi, L, Basquin, D, Roy, A, Stifanelli, M, Rajaram, RD, Debonneville, A, Nita, I, Maillard, M, Loffing, J, Subramanya, AR, Staub, O: Renal tubular SGK1 deficiency causes impaired K+ excretion via loss of regulation of NEDD4-2/WNK1 and ENaC. *Am J Physiol Renal Physiol,* 311**:** F330-342, 2016.