Supplemental Materials

Table of Contents

Supplemental Table 1: Oligonucleotides.

The following oligonucleotides were used to amplify the 5`homology region (HR5), the floxed region (FR), the 3`homology region (HR3), and the Southern Blot probe. The primers used for genotyping are labeled with “geno”.

oligonucleotide	sequence 5' - 3'
HR5_for	GACATTTGTGAGGCTAAAACTCC
HR5_rev	ATCTTCCTAGATGATTCTGGATTACC
FR_for	GTGTTTCCAGCACACTGGCA
FR_rev	ACACCCATAAAAATTTCTCTGTGAC
HR3_rev	CTTCTCAATGACTTCCCTTGC
HR3_for	TGAAAGGTCTTTCATTGATTTAGAATC
probe_for	AGGTGGATCTTTCTTGCCC
probe_rev	AGAAGCCGAAGCTAGCTCTC
geno_WT_for	TCCAATGCAAAGTGTAGGAAGTG
geno_KO_for	TTCTGTTAGTGCCACAACGC
geno_rev	TCATGGCGCTTCCTTTCAACTG

Supplemental Table 2: Antibodies used for Western blotting and immunofluorescence staining.

Ab #	Antibody	Dilution	Vendor	Antibody Registry ID	Application
1	Mouse anti-claudin-2	1:1000	Thermo Fischer Scientific	AB_2533085	Western Blot
2	Rabbit anti-claudin-10	1:1000	Thermo Fischer Scientific	AB_2533386	Western Blot
3	Rabbit anti-aquaporin 2	1:1000	Novus Biologicals	AB_1107363	Western Blot
4	Rabbit anti-NKCC2	1:1000	StressMarq Biosciences	AB_10640877	Western Blot
5	pNCC S71	1:1000	Pineda Anti-bodies Service	NA[5]	Western Blot
6	Rabbit anti-NCC	1:1000	StressMarq Biosciences	AB_10641430	Western Blot
7	Mouse anti-β-actin	1:5000	Sigma-Aldrich	AB_476692	Western Blot
8	Mouse anti-tubulin	1:4000	Sigma-Aldrich	AB_477593	Western Blot
9	Rabbit anti-claudin-2	1:400	Thermo Fischer Scientific	AB_2533911	Immunofluorescence Staining
10	Mouse anti-claudin-10	1:400	Thermo Fischer Scientific	AB_2533510	Immunofluorescence Staining
11	Goat anti-rabbit IgG Alexa 633	1:400	Thermo Fischer Scientific	AB_2535731	Immunofluorescence Staining
12	Goat anti-mouse IgG Alexa 488	1:400	Thermo Fischer Scientific	AB_2534084	Immunofluorescence Staining
13	Mouse anti-claudin-1	1:200	Thermo Fischer Scientific	AB_2533323	Immunofluorescence Staining
14	Mouse anti-claudin-2	1:200	Thermo Fischer Scientific	AB_2533085	Immunofluorescence Staining
15	Rabbit anti-claudin-10	1:200	Thermo Fischer Scientific	AB_2533386	Immunofluorescence Staining
16	Rabbit anti-claudin-12	1:100	NA[1]	NA[1]	Immunofluorescence Staining
17	Rabbit anti-Na-PiIIa	1:500	NA[2]	NA[3]	Immunofluorescence Staining
18	Mouse anti-NHE3	1:500	Millipore	AB_94714	Immunofluorescence Staining
19	Guinea pig anti-NKCC2	1:2000	Pineda Anti-bodies Service	NA[2]	Immunofluorescence Staining
20	Rabbit anti-NCC	1:500	NA[4]	NA[5]	Immunofluorescence Staining
21	Rabbit anti-phospho-S71-NCC	1:20000	Pineda Anti-bodies Service	NA[5]	Immunofluorescence Staining
22	Goat anti-aquaporin 2	1:500	Santa Cruz Biotechnology	AB_10988758	Immunofluorescence Staining
23	Goat anti-rabbit IgGF Cγ2	1:200	Jackson/Dianova	AB_2338747	Immunofluorescence Staining
24	Goat anti-mouse IgGF Cγ3	1:300	Jackson/Dianova	AB_2338694	Immunofluorescence Staining

25	Donkey anti-guinea pig IgGF Cγ3	1:300	Jackson/Dianova	AB_2340467	Immunofluorescence Staining
26	Donkey anti-goat IgGF Cγ3	1:300	Jackson/Dianova	AB_2307341	Immunofluorescence Staining
27	Rabbit anti-occludin	1:100	Thermo Fischer Scientific	AB_2533977	Immunofluorescence Staining
28	Rabbit anti-ZO-1	1:200	Thermo Fischer Scientific	AB_2533456	Immunofluorescence Staining
29	Goat anti-Rabbit IgG Alexa 488	1:400	Thermo Fischer Scientific	AB_2576217	Immunofluorescence Staining
30	Goat anti-mouse IgG Alexa 594	1:400	Thermo Fischer Scientific	AB_2534091	Immunofluorescence Staining

[1]kind gift of Dr. H. Rittner, Würzburg, Germany
[2]Mutig et al., 2007, Am J Physiol Renal Physiol. 293:F1166-F1177
[3]kind gift of Dr. J. Biber, Zurich, Switzerland; see also Bachmann et al., 2004, J Am Soc Nephrol. 15:892-900
[4]kind gift of Dr. D. H. Ellison, Portland, Oregon, USA
[5]see also Mutig et al., 2010, Am J Physiol Renal Physiol. 298:F502-F509

Supplemental Table 3: Transepithelial voltage and resistance of isolated PT segments in NaCl-based control solution.

Transepithelial Voltage (V_{te}, mV)					
		S1	**PCT**	**S2**	**S3**
WT	**MW ± SEM**	-0.02 ± 0.09	-0.32 ± 0.13	-0.24 ± 0.17	-1.18 ± 0.36
	n	11	14	7	10
***Cldn10a* KO**	**MW ± SEM**	0.25 ± 0.16	0.14 ± 0.21	-0.30 ± 0.23	-1.47 ± 0.37
	n	10	11	9	7
	p-value	ns	ns	ns	**0.04**
Transepithelial Resistance (R_{te}, Ω·cm²)					
WT	**MW ± SEM**	6.4 ± 1.5	10.6 ± 2.2	9.3 ± 1.6	9.3 ± 1.20
	n	11	14	8	8
***Cldn10a* KO**	**MW ± SEM**	8.0 ± 1.8	5.9 ± 1.8	5.7 ± 2.0	11.3 ± 2.3
	n	10	11	9	7
	p-value	ns	ns	ns	ns

Supplemental Table 4: Transepithelial voltage and resistance of isolated PT segments in Na-pyruvate-based solution.

Transepithelial Voltage (V_{te}, mV)					
		S1	**PCT**	**S2**	**S3**
WT	**MW ± SEM**	0.30 ± 0.15	-0.03 ± 0.16	-0.87 ± 0.13	-2.74 ± 0.35
	n	16	20	19	16
***Cldn10a* KO**	**MW ± SEM**	0.28 ± 0.13	0.13 ± 0.17	-0.68 ± 0.26	-2.63 ± 0.29
	n	15	11	9	7
	p-value	ns	ns	ns	ns
Transepithelial Resistance (R_{te}, Ω·cm²)					
WT	**MW ± SEM**	5.5 ± 0.6	6.4 ± 1.0	5.6 ± 0.6	7.8 ± 0.7
	n	16	20	19	16
***Cldn10a* KO**	**MW ± SEM**	3.6 ± 0.6	5.9 ± 0.8	5.0 ± 0.7	6.4 ± 0.7
	n	15	11	9	7
	p-value	**0.03**	ns	ns	ns

Supplemental Table 5: Transepithelial voltage, transepithelial resistance, and furosemide inhibitable transport current in cortical thick ascending limb segments.

		Transepithelial Voltage (V_{te}, mV)	Transepithelial Resistance (R_{te}, $\Omega \cdot cm^2$)	$\Delta I'sc_{furo}$ [$\mu A/cm^2$]
WT	**MW ± SEM**	18.0 ± 1.2	11.3 ± 1.0	-1486 ± 129
	n	13	13	13
***Cldn10a* KO**	**MW ± SEM**	16.8 ± 0.9	10.0 ± 0.8	-1728 ± 183
	n	17	17	17
	p-value	ns	ns	ns

Supplemental Table 6A: Reads per kilobase of transcript, per million mapped reads for Claudins 1 to 24 in isolated WT and *Cldn10a* KO PCT and DCT.

	PCT			DCT	
	WT	***Cldn10a* KO**		**WT**	***Cldn10a* KO**
CLDN1	**2.95 ± 0.65**	**2.35 ± 0.55**	CLDN1	0.01 ± 0.00	0.04 ± 0.02
CLDN2	**54.03 ± 5.73**	**56.19 ± 4.16**	CLDN2	0.09 ± 0.04	0.08 ± 0.04
CLDN3	n.d.	n.d.	CLDN3	0.45 ± 0.04	0.34 ± 0.11
CLDN4	n.d.	n.d.	**CLDN4**	**1.45 ± 0.22**	**1.50 ± 0.32**
CLDN5	n.d.	n.d.	CLDN5	n.d.	0.02 ± 0.02
CLDN6	0.34 ± 0.12	0.32 ± 0.19	CLDN6	0.33 ± 0.08	0.66 ± 0.19
CLDN7	n.d.	0.01 ± 0.01	**CLDN7**	**2.19 ± 0.34**	**1.68 ± 0.19**
CLDN8	0.02 ± 0.01	n.d.	**CLDN8**	**29.95 ± 0.84**	**28.91 ± 1.52**
CLDN9	n.d.	n.d.	CLDN9	0.10 ± 0.05	0.17 ± 0.13
CLDN10	**10.11 ± 2.63**	**n.d.**	CLDN10	0.08 ± 0.04	0.07 ± 0.04
CLDN11	n.d.	n.d.	CLDN11	n.d.	n.d.
CLDN12	**7.49 ± 0.50**	**7.94 ± 0.45**	**CLDN12**	**1.72 ± 0.15**	**1.37 ± 0.02**
CLDN13	n.d.	n.d.	CLDN13	n.d.	n.d.
CLDN14	n.d.	n.d.	CLDN14	n.d.	n.d.
CLDN15	0.20 ± 0.06	0.14 ± 0.07	CLDN15	0.32 ± 0.04	0.22 ± 0.04
CLDN16	n.d.	n.d.	**CLDN16**	**1.23 ± 0.38**	**1.60 ± 0.31**
CLDN17	n.d.	n.d.	CLDN17	0.02 ± 0.02	n.d.
CLDN18	0.07 ± 0.01	0.19 ± 0.05	CLDN18	n.d.	n.d.
CLDN19	n.d.	n.d.	CLDN19	0.33 ± 0.13	0.28 ± 0.04
CLDN20	0.07 ± 0.05	0.03 ± 0.03	CLDN20	0.05 ± 0.03	0.04 ± 0.02
CLDN22	n.d.	n.d.	CLDN22	n.d.	n.d.
CLDN23	n.d.	n.d.	CLDN23	n.d.	0.02 ± 0.02
CLDN24	0.02 ± 0.02	n.d.	CLDN24	0.02 ± 0.02	n.d.

means ± SEM; PCT, n = 4 WT and 3 KO mice; DCT, n = 4 WT and 4 KO mice. Values > 1 are highlighted in **bold**.

Supplemental Table 6B: Whole kidney RNA array analysis.

Gene Symbol	Fold-Change *Cldn10a* KO vs. WT	p-value *Cldn10a* KO vs. WT
Cldn1	1.10	0.771
Cldn2	1.31	0.193
Cldn3	1.18	0.560
Cldn4	1.16	0.607
Cldn5	-1.11	0.144
Cldn6	-1.01	0.919
Cldn7	-1.54	0.005
Cldn8	1.45	0.288
Cldn9	-1.18	0.531
Cldn10(a+b)	**-4.39**	**0.089**
Cldn11	-1.07	0.615
Cldn12	1.19	0.379
Cldn13	-1.24	0.272
Cldn14	1.15	0.464
Cldn15	-1.37	0.195
Cldn16	-1.13	0.661
Cldn17	-1.08	0.724
Cldn18	1.25	0.154
Cldn19	1.20	0.345
Cldn20	-1.02	0.947
Cldn22	-1.40	0.032
Cldn23	-1.04	0.858
Cldn24	1.15	0.652
Slc12a1	-1.07	0.403
Slc12a3	-1.01	0.963
Aqp2	-1.35	0.402

RNA was extracted from 3 WT and 3 *Cldn10a* KO whole mouse kidneys using the NucleoSpin RNA/Protein, Mini kit for RNA and protein purification (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. Clariom S Affymetrix mouse array analysis was conducted at the Charité core facility (Charité – Universitätsmedizin Berlin, Germany). No conclusive pattern emerged from these data, as effects on specific nephron segments are diluted in such a whole kidney approach. However, the data suggest that there are no relevant overall changes in any of the claudins, especially not in claudin-16 and -19, which would be decisive to the reabsorption of divalent cations.
Note that this approach is not able to discriminate between *Cldn10a* and *Cldn10b* as it measures total *Cldn10*.
Major transporters found to be upregulated in Western Blots and immunostainings (NKCC2 = Slc12a1, NCC = Slc12a3, Aqp2) were also unaltered in this analysis.

Supplemental Table 7A: Comparison of urine parameters between WT and *Cldn10a* KO mice after treatment with acetazolamide, furosemide, and hydrochlorothiazide, respectively.

Urine												
		Volume (ml/h)	**Osmola-rity (mOsM)**	**Urea (mM)**	**pH**	**Crea-tinine (mM)**	**Na (mM)**	**K (mM)**	**Ca (mM)**	**Mg (mM)**	**Cl (mM)**	**Phos-phate (mM)**
Acetazolamide												
WT	**MW ± SEM**	0.05 ± 0.01	1118.6 ± 44.6	480.1 ± 89.0	7.78 ± 0.08	1.30 ± 0.12	171.8 ± 5.4	208.0 ± 6.9			118.5 ± 8.4	30.4 ± 2.8
	n	8	8	8	8	8	8	8			8	8
***Cldn10a* KO**	**MW ± SEM**	0.04 ± 0.01	1174.1 ± 99.6	531.6 ± 83.5	8.10 ± 0.10	1.13 ± 0.14	175.8 ± 12.1	246.9 ± 13.5			141.3 ± 19.8	26.8 ± 4.7
	n	8	8	8	8	8	8	8			8	8
	p-value	ns	ns	ns	**0.04**	ns	ns	**0.03**			ns	ns
Furosemide												
WT	**MW ± SEM**	0.25 ± 0.03	480.3 ± 24.6	96.1 ± 15.9	6.88 ± 0.27	0.60 ± 0.10	132.3 ± 6.8	47.81 ± 3.97	0.77 ± 0.04		154.5 ± 7.1	
	n	8	8	8	8	8	8	8	8		8	
***Cldn10a* KO**	**MW ± SEM**	0.24 ± 0.04	475.4 ± 57.8	55.6 ± 8.3	8.00 ± 0.30	0.42 ± 0.09	163.5 ± 16.3	38.14 ± 5.05	0.83 ± 0.04		189.4 ± 19.6	
	n	8	8	8	8	8	8	7	8		8	
	p-value	ns	ns	**0.04**	**0.014**	ns	ns	ns	ns		ns	
Hydrochlorothiazide												
WT	**MW ± SEM**	0.13 ± 0.01	688.4 ± 65.3	262.3 ± 48.1		0.95 ± 0.14	172.8 ± 9.4	106.5 ± 13.8		0.57 ± 0.07	172.8 ± 17.5	
	n	8	8	8		8	8	7		4	8	
***Cldn10a* KO**	**MW ± SEM**	0.16 ± 0.03	607.6 ± 41.8	215.1 ± 31.6		0.65 ± 0.09	171.1 ± 4.2	90.8 ± 9.8		0.46 ± 0.08	175.6 ± 6.5	
	n	8	8	8		8	8	8		3	8	
	p-value	ns	ns	ns		ns	ns	ns		ns	ns	

Supplemental Table 7B: Comparison of serum parameters between WT and *Cldn10a* KO mice after treatment with furosemide and hydrochlorothiazide, respectively.

Serum								
		Osmolarity (mOsM)	**Creatinine (µM)**	**Na (mM)**	**K (mM)**	**Ca (mM)**	**Mg (mM)**	**Cl (mM)**
Furosemide								
WT	**MW ± SEM**	307.3 ± 0.9	9.82 ± 1.15	142.6 ± 0.7	4.81 ± 0.27	0.58 ± 0.05		99.4 ± 1.9
	n	7	8	7	8	7		7
***Cldn10a* KO**	**MW ± SEM**	317.5 ± 5.2	12.36 ± 1.63	146.8 ± 2.0	4.31 ± 0.22	0.71 ± 0.07		99.8 ± 1.6
	n	6	7	6	7	6		6
	p-value	**ns**	**ns**	**ns**	**ns**	**ns**		**ns**
Hydrochlorothiazide								
WT	**MW ± SEM**	312.7 ± 2.7	12.5 ± 2.0	143.6 ± 1.0	4.22 ± 0.24	0.83 ± 0.10	0.53 ± 0.06	110.7 ± 2.2
	n	3	7	7	7	7	4	7
***Cldn10a* KO**	**MW ± SEM**	308.0 ± 6.2	12.2 ± 1.2	146.7 ± 2.4	3.96 ± 0.24	0.76 ± 0.09	0.53 ± 0.09	106.8 ± 1.9
	n	2	6	6	6	6	4	6
	p-value	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**

Due to small serum volumes obtained, values are missing for the acetazolamide experiment.

Supplemental Table 7C: Comparison of fractional excretion between WT and *Cldn10a* KO mice. Values after treatment with furosemide and hydrochlorothiazide, respectively.

		Fractional Excretion (%)				
		Na	**K**	**Ca**	**Cl**	**Mg**
Furosemide						
WT	**MW ± SEM**	1.63 ± 0.48	18.91 ± 3.48	2.37 ± 0.65	2.74 ± 0.83	
	n	7	8	7	7	
***Cldn10a* KO**	**MW ± SEM**	5.31 ± 2.00	34.19 ± 4.57	6.13 ± 2.46	9.02 ± 3.38	
	n	7	7	7	7	
	p-value	ns	**0.02**	ns	ns	
Hydrochlorothiazide						
WT	**MW ± SEM**	1.71 ± 0.45	34.74 ± 9.86		2.11 ± 0.52	1.66 ± 0.89
	n	8	7		8	4
***Cldn10a* KO**	**MW ± SEM**	2.82 ± 0.51	43.66 ± 6.76		3.87 ± 0.60	1.59 ± 0.31
	n	8	8		8	3
	p-value	ns	ns		**0.04**	ns

Supplemental Table 7D: Comparison of renal clearance between WT and *Cldn10a* KO mice. Values after treatment with furosemide and hydrochlorothiazide, respectively.

		Clearance (ml/min)					
		Na	**K**	**Ca**	**Cl**	**Mg**	**Creatinine**
Furosemide							
WT	**MW ± SEM**	0.0039 ± 0.0003	0.0423 ± 0.0044	0.0059 ± 0.0008	0.0065 ± 0.0005		0.2923 ± 0.0575
	n	7	8	7	7		8
***Cldn10a* KO**	**MW ± SEM**	0.0041 ± 0.0007	0.0423 ± 0.0093	0.0049 ± 0.0011	0.0071 ± 0.0011		0.1227 ± 0.0334
	n	7	6	7	7		8
	p-value	ns	ns	ns	ns		**0.02**
Hydrochlorothiazide							
WT	**MW ± SEM**	0.0026 ± 0.0002	0.0513 ± 0.0090		0.0033 ± 0.0003	0.0023 ± 0.0004	0.2204 ± 0.0516
	n	8	7		8	4	8
***Cldn10a* KO**	**MW ± SEM**	0.0030 0.0006	0.0512 0.0106		0.0042 0.0007	0.0023 0.0009	0.1247 0.0298
	n	8	8		8	3	8
	p-value	ns	ns		ns	ns	ns

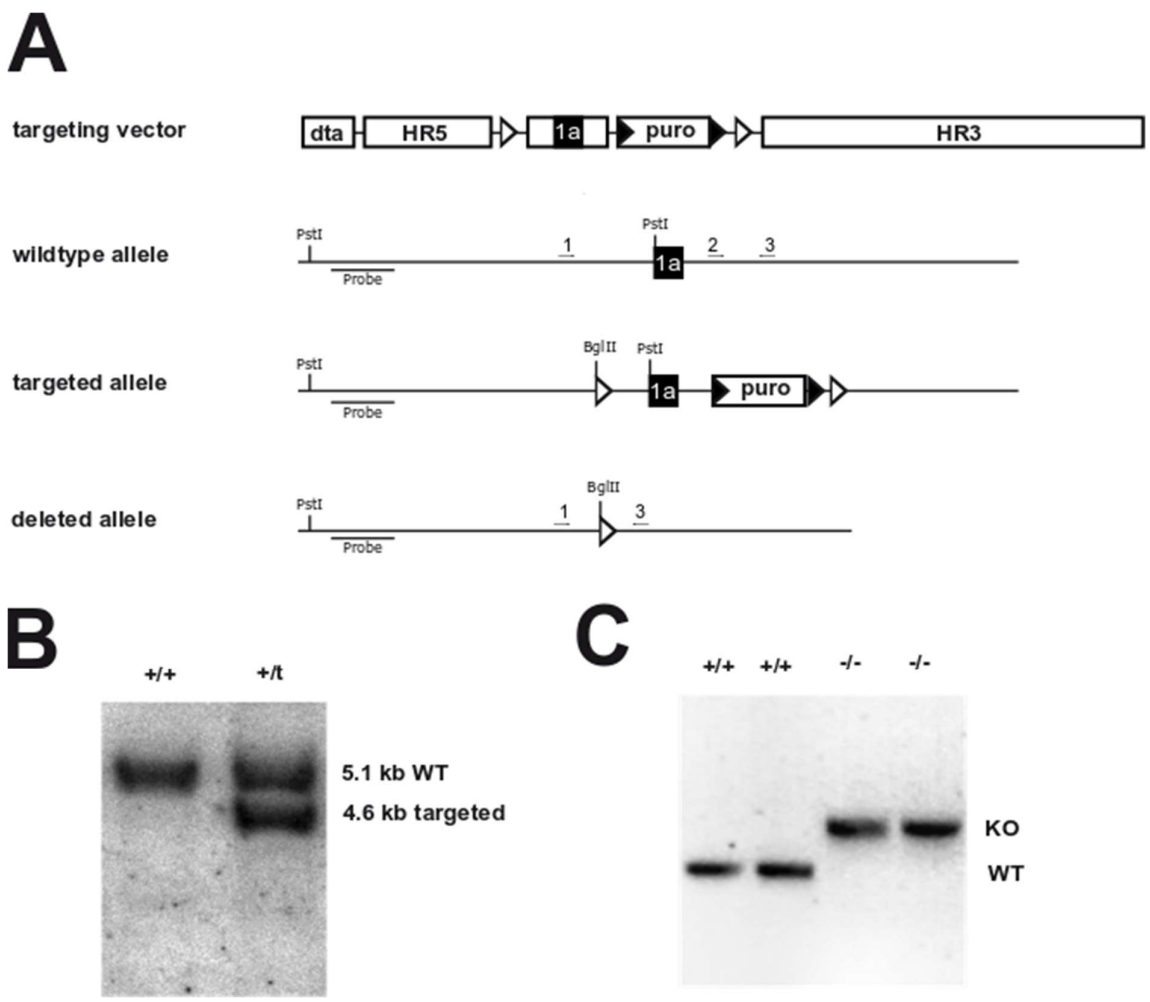
Supplemental Table 8: Enrichment (p-value cutoff 0.01) of GO terms in differentially expressed genes of the PCT samples. The source column refers to the Gene Ontology subdomains: molecular function (MF), cellular component (CC), biological process (BP).

P-value	Term size	Intersection size	Term id	Source	Term name
1.43E-17	1726	52	GO:0044281	GO:BP	small molecule metabolic process
7.95E-16	948	38	GO:0006082	GO:BP	organic acid metabolic process
4.57E-13	898	34	GO:0019752	GO:BP	carboxylic acid metabolic process
7.01E-13	911	34	GO:0043436	GO:BP	oxoacid metabolic process
3.54E-11	425	23	GO:0006631	GO:BP	fatty acid metabolic process
2.04E-10	616	26	GO:0032787	GO:BP	monocarboxylic acid metabolic process
1.06E-09	5913	83	GO:0003824	GO:MF	catalytic activity
2.18E-09	1349	36	GO:0006629	GO:BP	lipid metabolic process
4.29E-09	296	18	GO:0006790	GO:BP	sulfur compound metabolic process
1.10E-08	992	30	GO:0044255	GO:BP	cellular lipid metabolic process
2.54E-06	334	16	GO:0016042	GO:BP	lipid catabolic process
3.62E-06	150	11	GO:0042579	GO:CC	microbody
3.62E-06	150	11	GO:0005777	GO:CC	peroxisome
3.56E-05	368	15	GO:0033218	GO:MF	amide binding
3.97E-05	141	10	GO:0016616	GO:MF	oxidoreductase activity
8.06E-05	152	10	GO:0016614	GO:MF	oxidoreductase activity
9.66E-05	618	19	GO:0044283	GO:BP	small molecule biosynthetic process
0.00011939	109	9	GO:0033865	GO:BP	nucleoside bisphosphate metabolic process
0.00011939	109	9	GO:0033875	GO:BP	ribonucleoside bisphosphate metabolic process
0.00011939	109	9	GO:0034032	GO:BP	purine nucleoside bisphosphate metabolic process
0.00013749	7	4	GO:0019694	GO:BP	alkanesulfonate metabolic process
0.00013749	7	4	GO:0019530	GO:BP	taurine metabolic process
0.00023406	293	13	GO:0016053	GO:BP	organic acid biosynthetic process
0.00026747	831	21	GO:0016491	GO:MF	oxidoreductase activity
0.00029722	59	7	GO:0006749	GO:BP	glutathione metabolic process
0.00038056	818	21	GO:0055114	GO:BP	oxidation-reduction process
0.00051096	169	10	GO:0006575	GO:BP	cellular modified amino acid metabolic process
0.00063433	218	11	GO:0046395	GO:BP	carboxylic acid catabolic process
0.00071955	98	8	GO:0009062	GO:BP	fatty acid catabolic process
0.00090813	226	11	GO:0016054	GO:BP	organic acid catabolic process
0.00096343	181	10	GO:1901605	GO:BP	alpha-amino acid metabolic process
0.00126978	285	12	GO:0046394	GO:BP	carboxylic acid biosynthetic process
0.00143572	258	11	GO:1901681	GO:MF	sulfur compound binding
0.00143855	74	7	GO:0006635	GO:BP	fatty acid beta-oxidation
0.00145554	344	13	GO:0044282	GO:BP	small molecule catabolic process

0.00214532	113	8	GO:0019395	GO:BP	fatty acid oxidation
0.00242128	1978	33	GO:1901575	GO:BP	organic substance catabolic process
0.00261761	116	8	GO:0009410	GO:BP	response to xenobiotic stimulus
0.00309255	5944	68	GO:0043167	GO:MF	ion binding
0.00312254	36	5	GO:0004364	GO:MF	glutathione transferase activity
0.00317573	119	8	GO:0034440	GO:BP	lipid oxidation
0.00360156	121	8	GO:0072329	GO:BP	monocarboxylic acid catabolic process
0.00404628	18	4	GO:0043295	GO:MF	glutathione binding
0.00423085	213	10	GO:0044242	GO:BP	cellular lipid catabolic process
0.00456601	73	6	GO:0031526	GO:CC	brush border membrane
0.00509698	19	4	GO:1900750	GO:MF	oligopeptide binding
0.00513691	15	4	GO:0015936	GO:BP	coenzyme A metabolic process
0.00517996	68	6	GO:0016765	GO:MF	transferase activity
0.00602702	521	15	GO:0010876	GO:BP	lipid localization
0.00634034	11132	105	GO:0005737	GO:CC	cytoplasm
0.00673917	459	14	GO:0006869	GO:BP	lipid transport
0.00773682	134	8	GO:0006690	GO:BP	icosanoid metabolic process
0.00848765	36	5	GO:0009069	GO:BP	serine family amino acid metabolic process

Supplemental Table 9: Separate Excel file of transcriptome data:

pct_wt_vs_ko_diff_expr_genes.xlsx

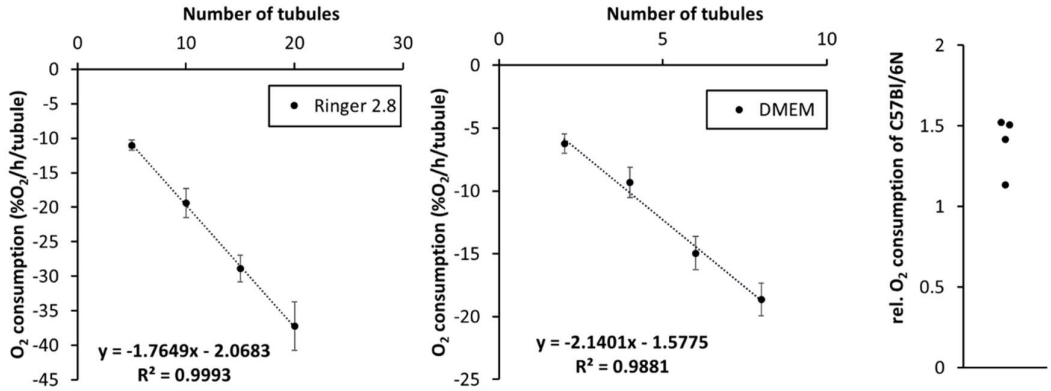


Supplemental Figure 1: Generation of a *Cldn10a* KO mouse.

A Strategy for the targeting of exon 1a of *Cldn10a.* For the generation of a floxed exon 1a of *Cldn10*a targeting construct was generated in which a loxP site was inserted before the floxed region (FR, 1.3 kb) containing exon 1a. The 5´homology region (HR5, 2.0 kb) was flanked by a dta (Diphteria Toxin A) cassette. A puromycin resistance cassette together with the second loxP site was inserted between the FR and the 3´homology region (HR3, 12 kb).

B Southern Blot of the targeted embryonic stem cells clone. The targeted clone showed an additional band of the correct size.

C Genotyping of tissue samples from mice. PCR using the three primers 1,2, and 3 confirmed the absence of the WT allele in *Cldn10a* KO mice.



Supplemental Figure 2: O_2 experiment: calibration curve.

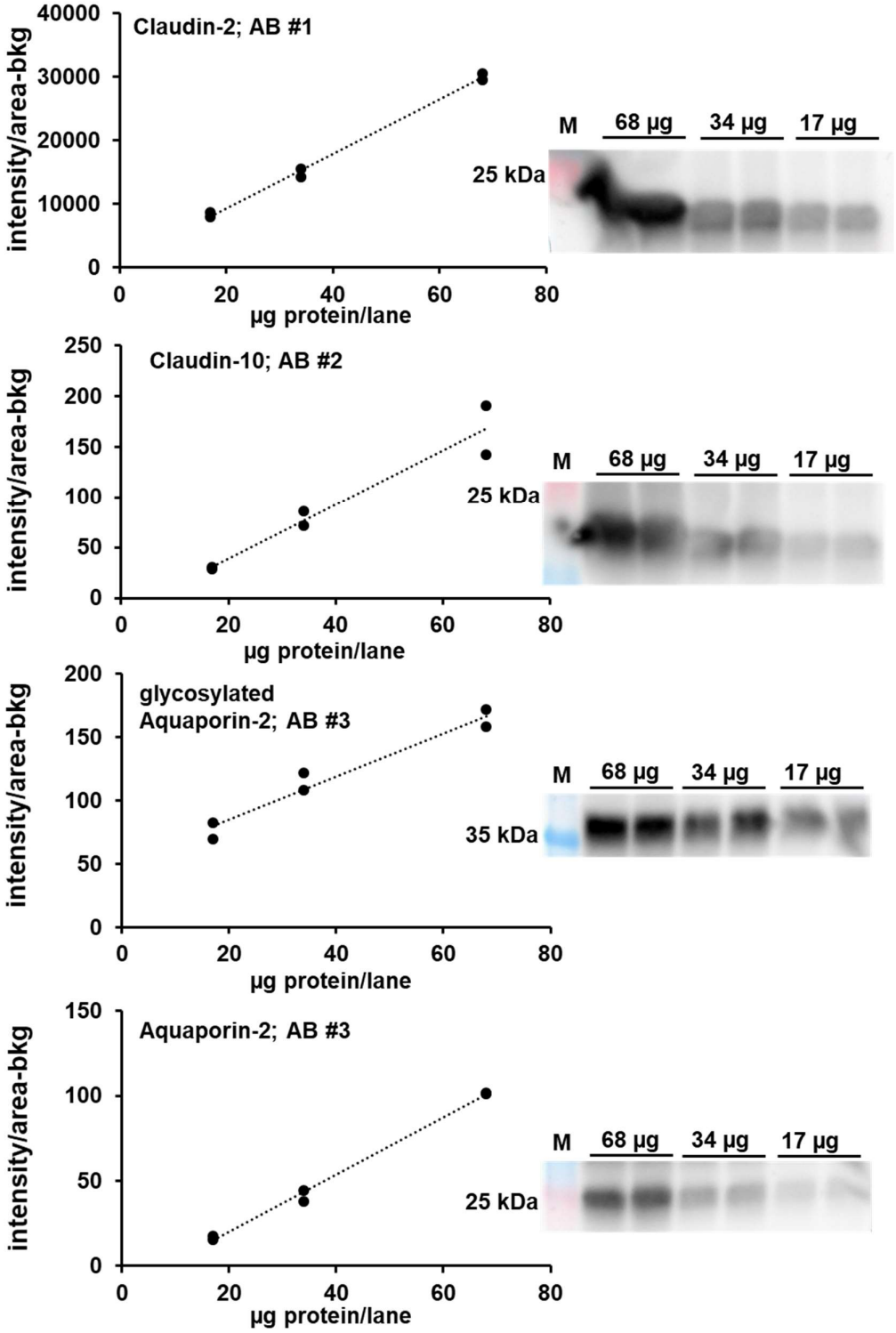
O_2 consumption was calibrated to the number of proximal convoluted tubules (PCT).

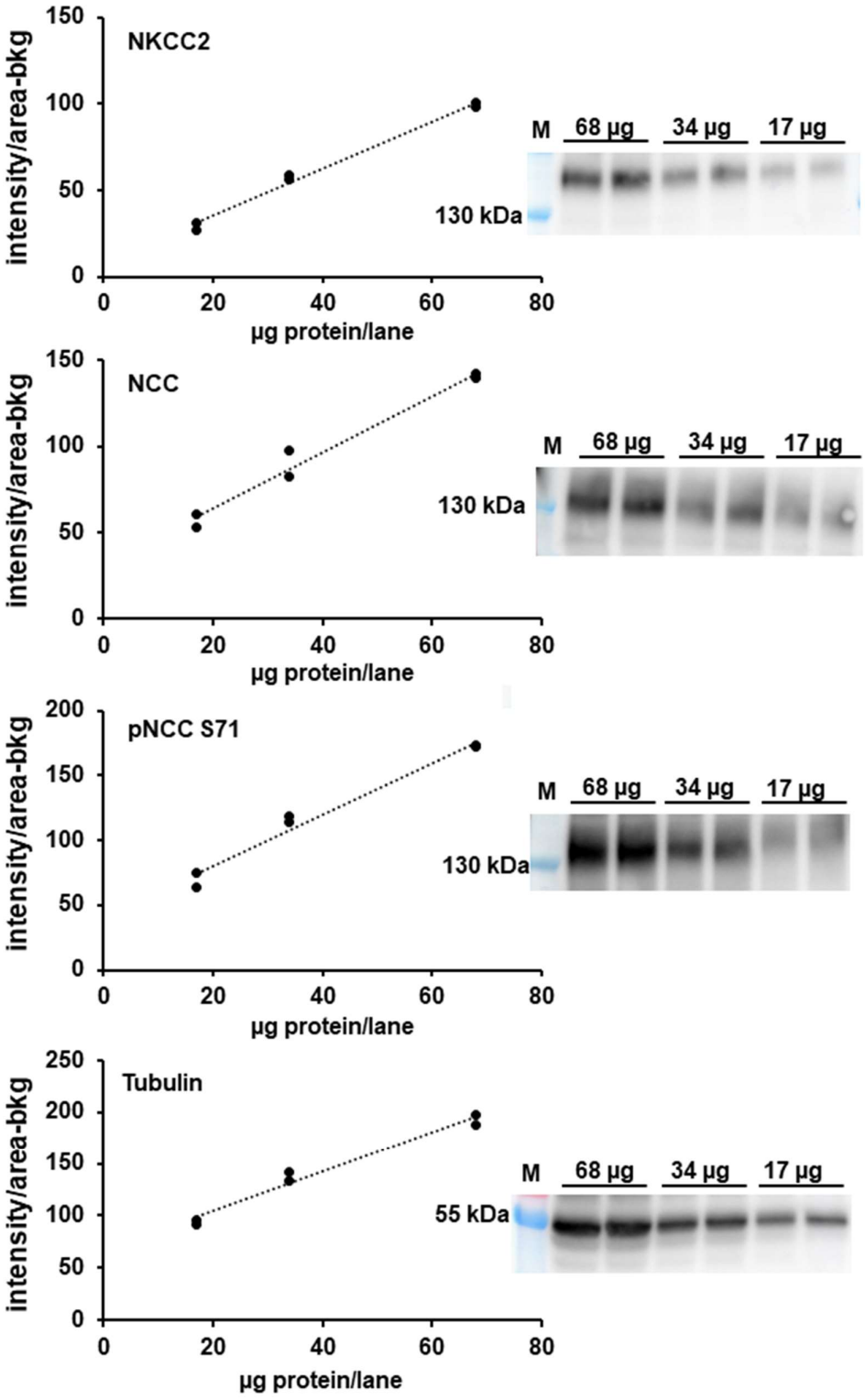
Left panel: minimal solution calibration curve with 5, 10, 15, and 20 PCT.

Middle panel: DMEM calibration curve with 2, 4, 6, and 8 PCT.

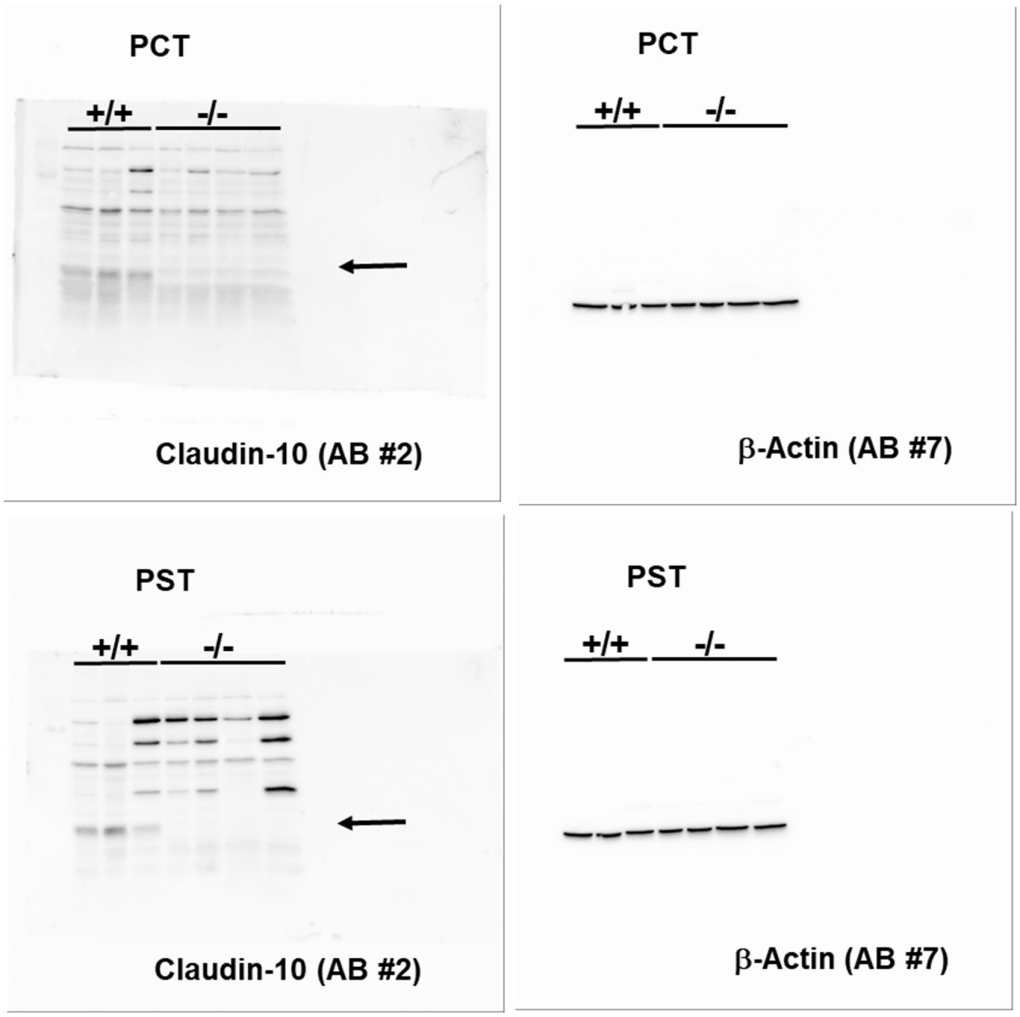
Right panel: calculated relative O_2 consumption as ratio between mean O_2 consumption for all measurements in minimal solution and DMEM, respectively. N = 4 mice.

Supplemental Figure 3: Western blots: linearity.



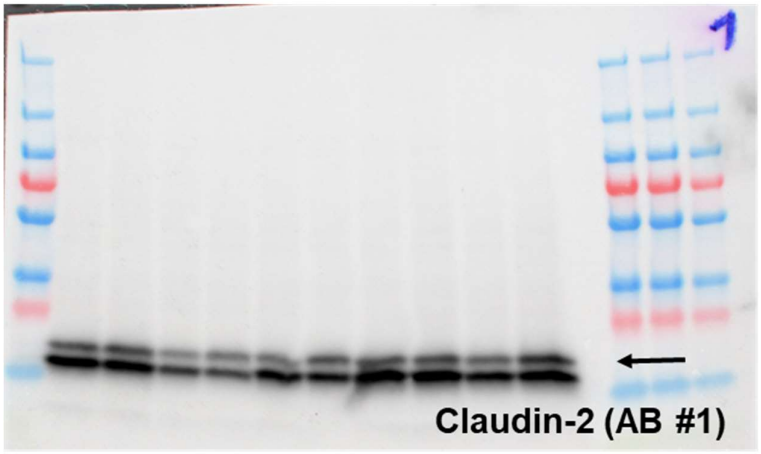

NKCC2
intensity/area-bkg
µg protein/lane
M
68 µg
34 µg
17 µg
130 kDa
NCC
intensity/area-bkg
µg protein/lane
M
68 µg
34 µg
17 µg
130 kDa
pNCC S71
intensity/area-bkg
µg protein/lane
M
68 µg
34 µg
17 µg
130 kDa
Tubulin
intensity/area-bkg
µg protein/lane
M
68 µg
34 µg
17 µg
55 kDa

Supplemental Figure 4: Original Western blots.

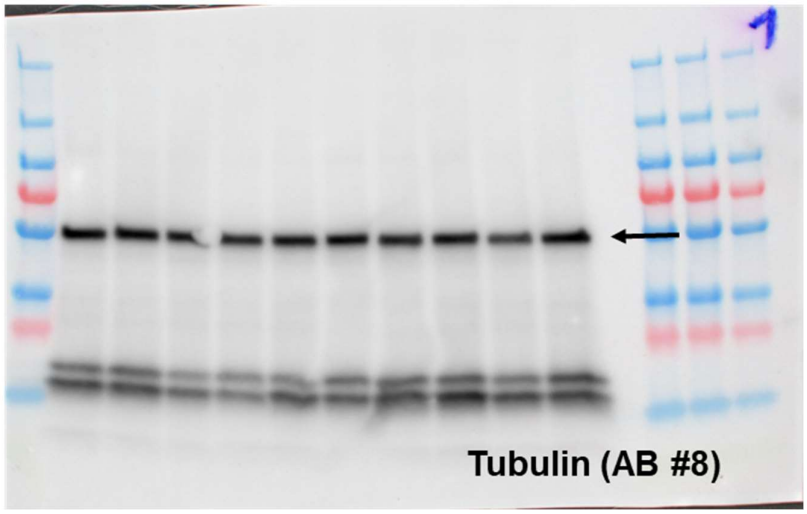


Original blots, Fig. 1

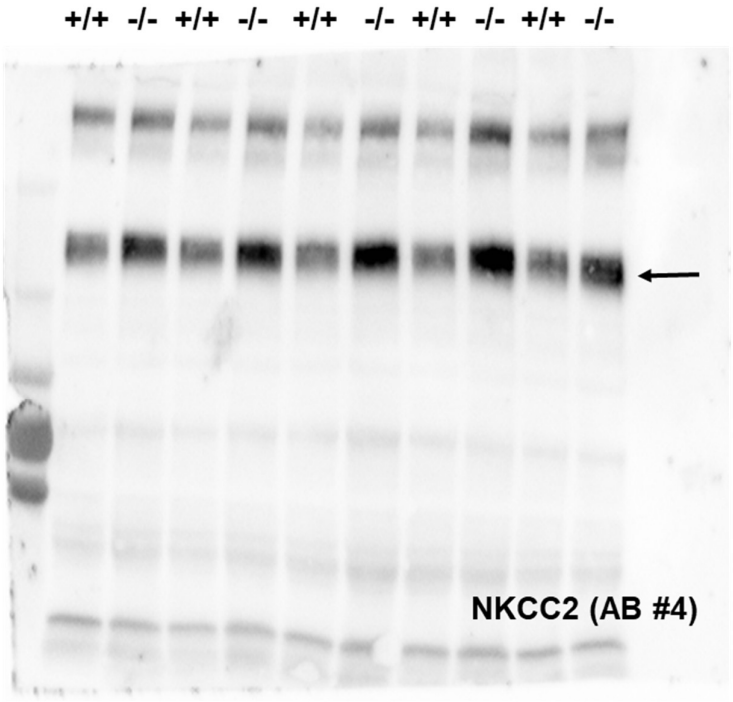
+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-

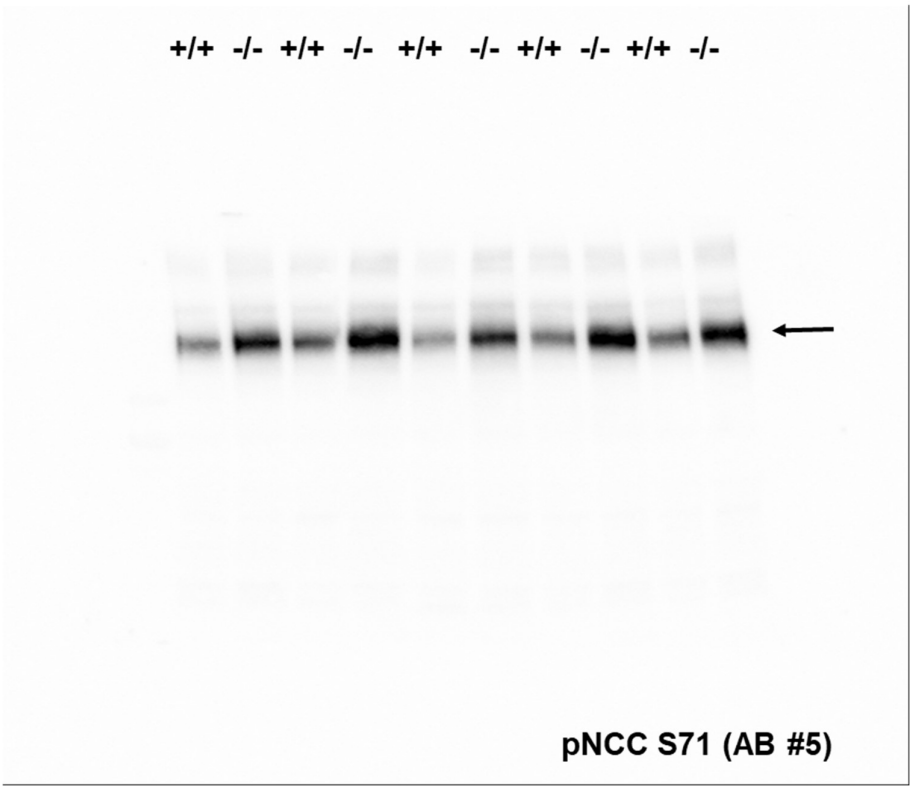


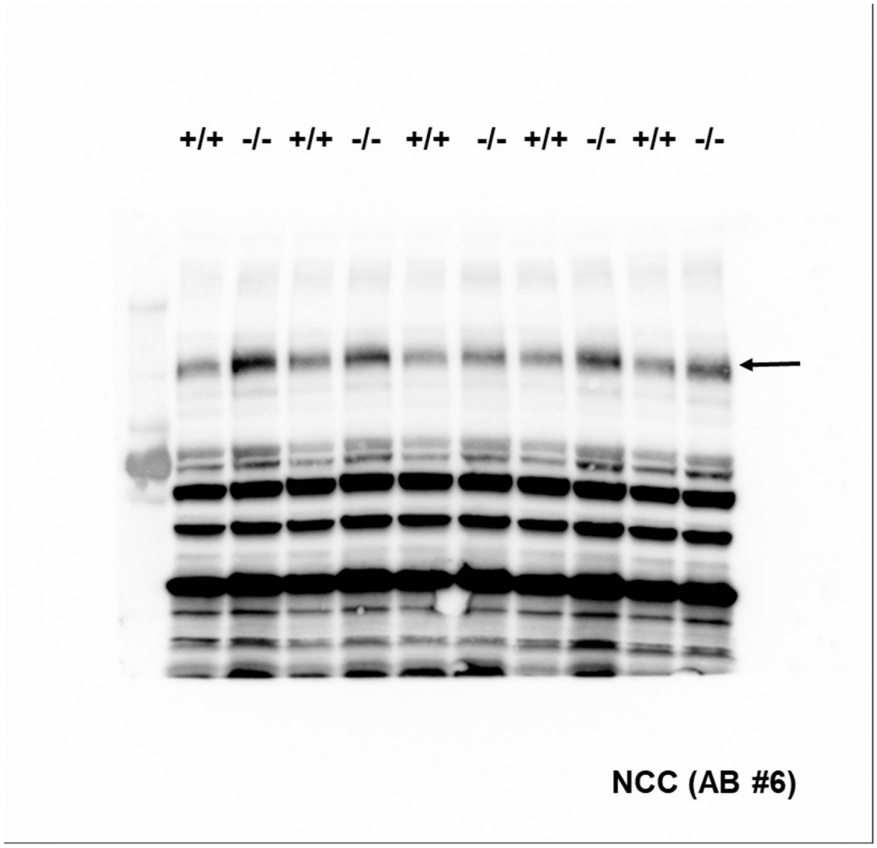
+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-

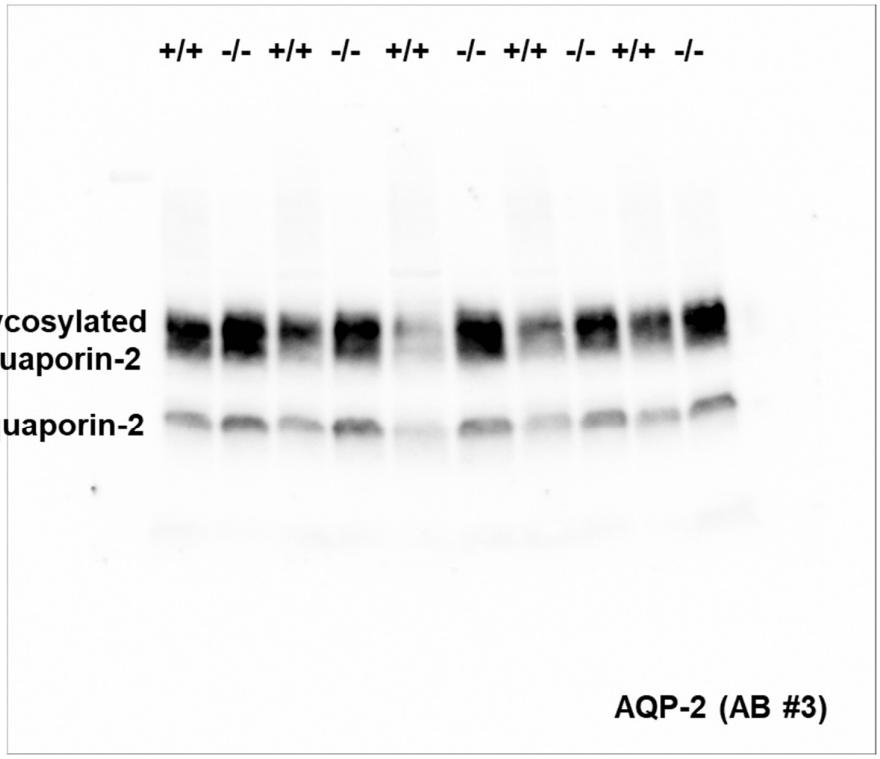


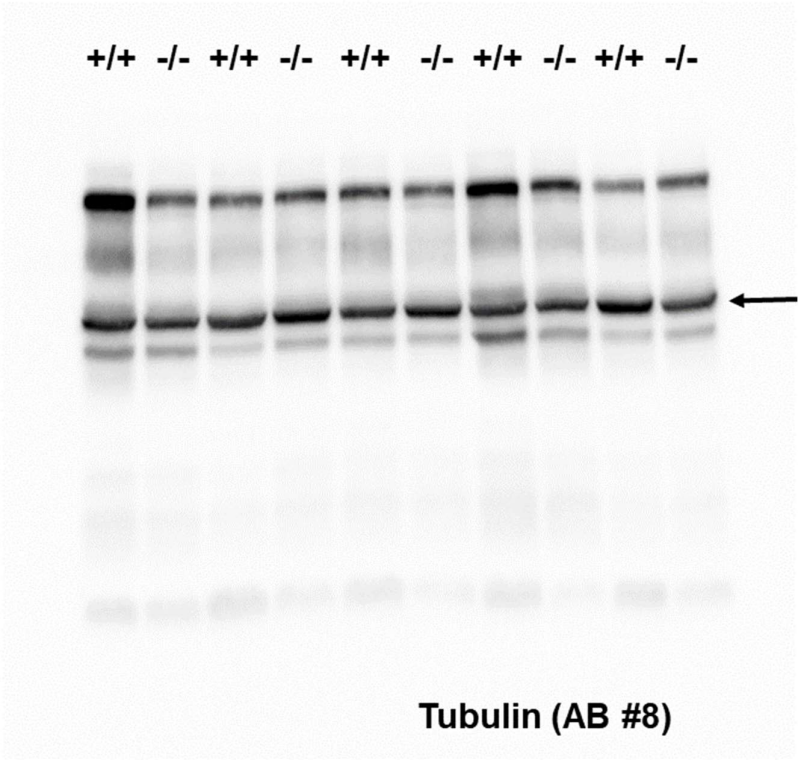
Original blots, Fig. 4


+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
NKCC2 (AB #4)

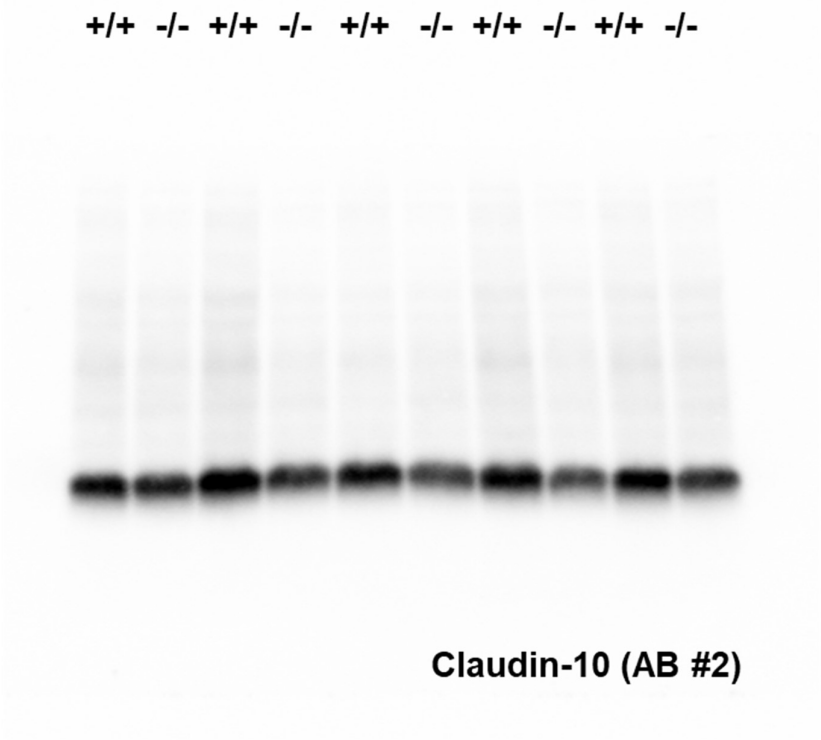

+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
pNCC S71 (AB #5)

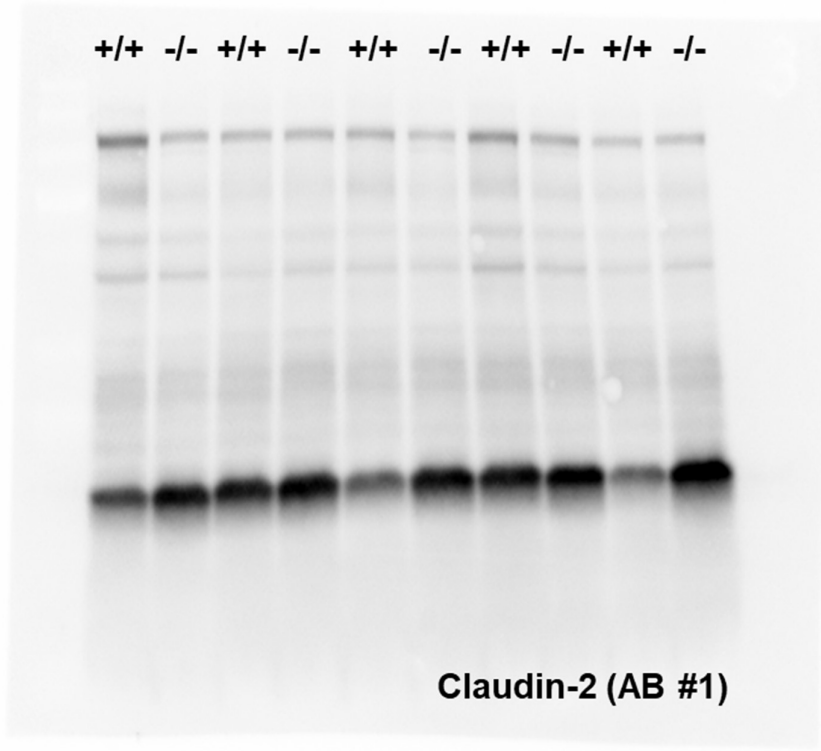

+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
NCC (AB #6)


+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
glycosylated Aquaporin-2
Aquaporin-2
AQP-2 (AB #3)

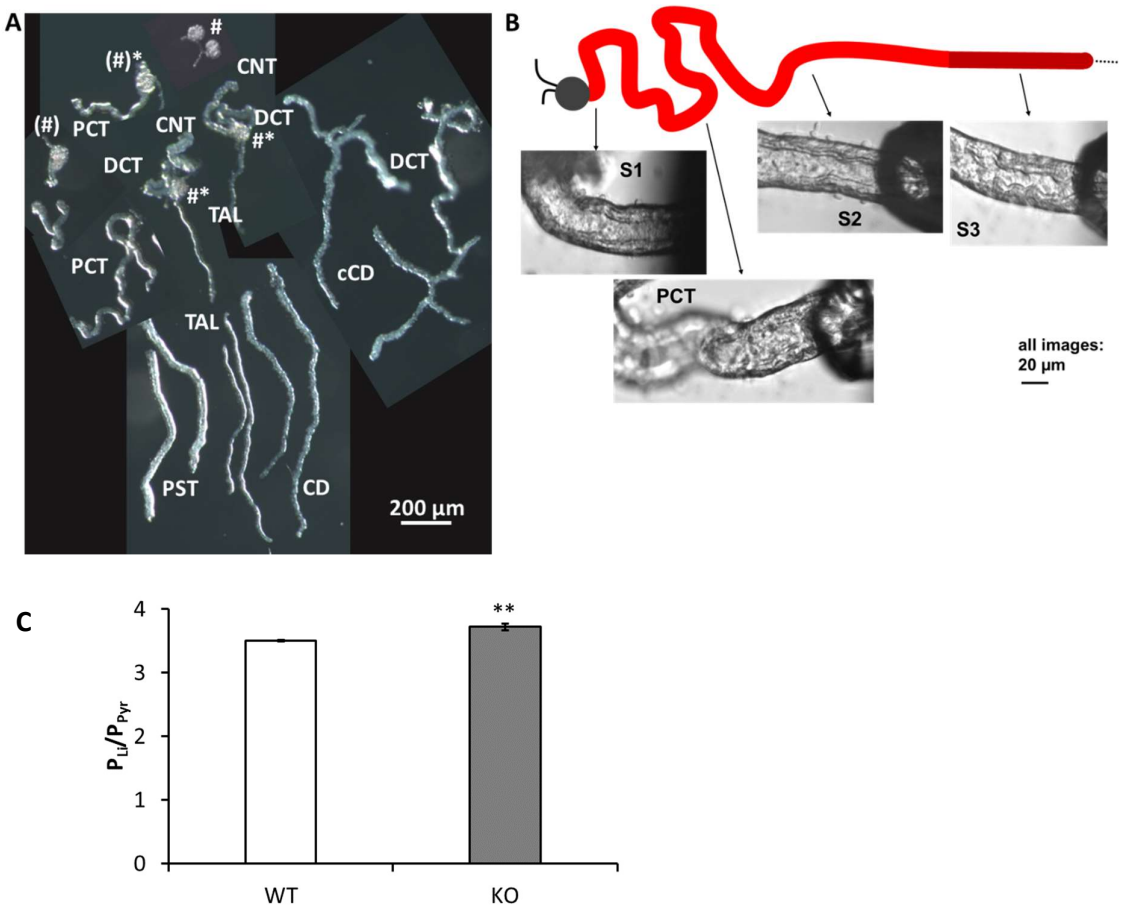


Original blots, Fig. 6





Original blots, Supplemental Figure 9

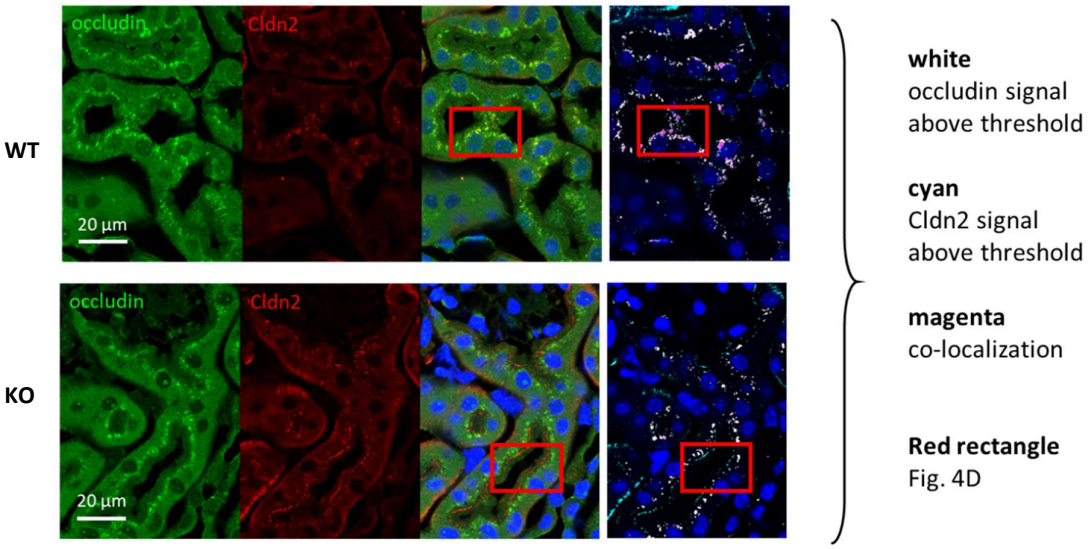


Supplemental Figure 5: Criteria for identification of tubular segments and permeability ratio P_{Li}/P_{Pyr}.

A Enzymatic preparation: glomeruli # – without remains of PT or TAL; vas afferens and efferens normally still attached; glomeruli with beginning S1 segment (#), PCT – long, shiny and smooth, no changes in diameter; PST (=PT S3) – very distinctive: thickest/broadest tubular segment, often milky, uniform cells, often slightly twisted with loose corkscrew like turns; TAL – very distinctive, shiny, thin; DCT – short, attached to TAL (MD, #*) or to CNT/CD, smooth, convoluted and shiny in the beginning; DCT2 and transition to CNT more variable; CNT/CD - branching, cobblestone like appearance (drainage points in cortical part).

B Localization of proximal segments for electrophysiological measurements with schematic proximal tubule (outer stripe of outer medulla in dark red).

C Permeability ratio P_{Li}/P_{Pyr} of isolated S2 segments: we assessed P_{Li}/P_{Pyr} in a subset of perfused PTs. The results showed an increase in paracellular lithium permeability in *Cldn10a* KO PTs, in line with the known Li permeability of *Cldn2*-based paracellular channels (Yu et al. J Gen Physiol 133: 111-127, 2009); **, $p < 0.01$.

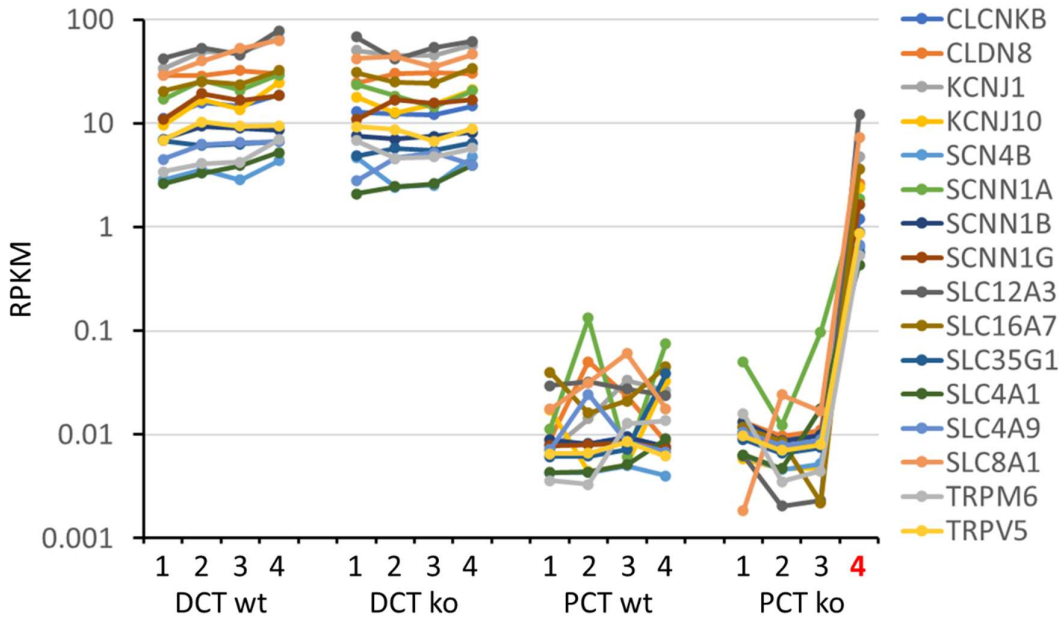


Supplemental Figure 6: Quantification of colocalization between occludin-positive vesicles and claudin-2.

To estimate co-localization between occludin-positive vesicles and claudin-2, images were recorded from regions predominantly containing S1 segments.

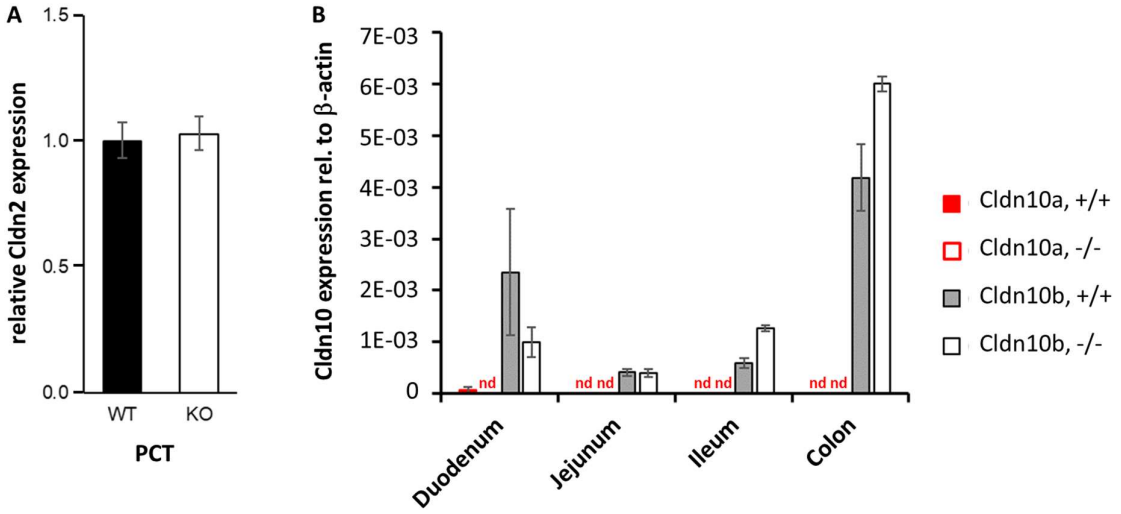
Left panels: Occludin, green, Claudin-2, red; nuclei in merged image, DAPI, blue.

Right panels: Intensity thresholds of 100 (total intensity range of 0 – 255) were chosen for both channels. Pixels with occludin intensities ≥ 100 and claudin-2 intensities <100 are visualized in white; pixels with occludin intensities <100 and claudin-2 intensities ≥ 100 are visualized in cyan; pixels with occludin and claudin-2 intensities ≥ 100 are visualized in magenta; DAPI, blue. Red rectangles are the image sections shown in Fig. 4D.

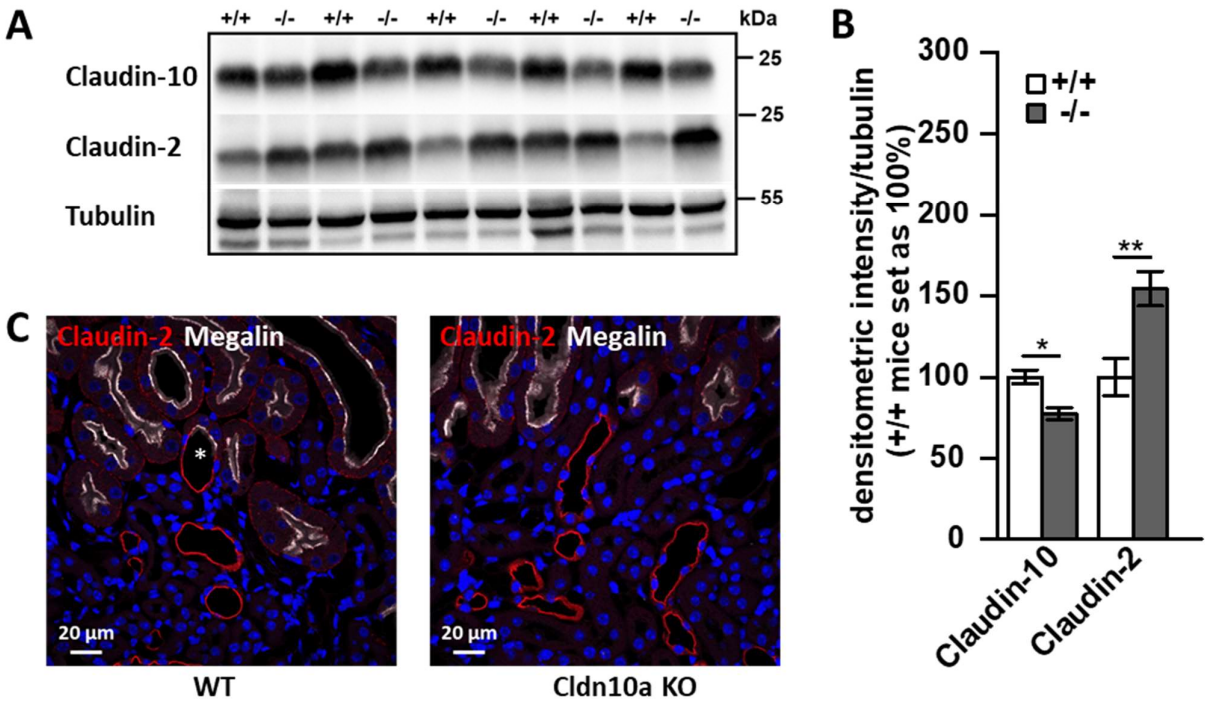


Supplemental Figure 7: Distal nephron segment contamination of *Cldn10a* KO PCT sample #4.

DCT and PCT nephron segments were collected from four WT and four *Cldn10a* KO kidneys and processed for RNA-Seq. RPKM (reads per kilobase of transcript, per million mapped reads) values for 16 genes are shown that are known to be highly expressed in DCT but low in PCT. For all of these genes, sample #4 from *Cldn10a* KO PCTs shows high RPKM values, indicating contamination of the sample with a more distal nephron segment. Consequently, this sample was excluded from further evaluation.



Supplemental Figure 8: Quantitative PCR.
A Relative *Cldn2* expression did not differ between isolated PCT from *Cldn10a* WT and KO animals. **B** With the exception of negligible amounts in the duodenum of WT (+/+) mice, *Cldn10a* was not detected (nd) in the intestine of WT (+/+) and *Cldn10a* KO (-/-) mice. *Cldn10b* was present in all segments and no significant differences were detected between WT and *Cldn10a* KO animals.

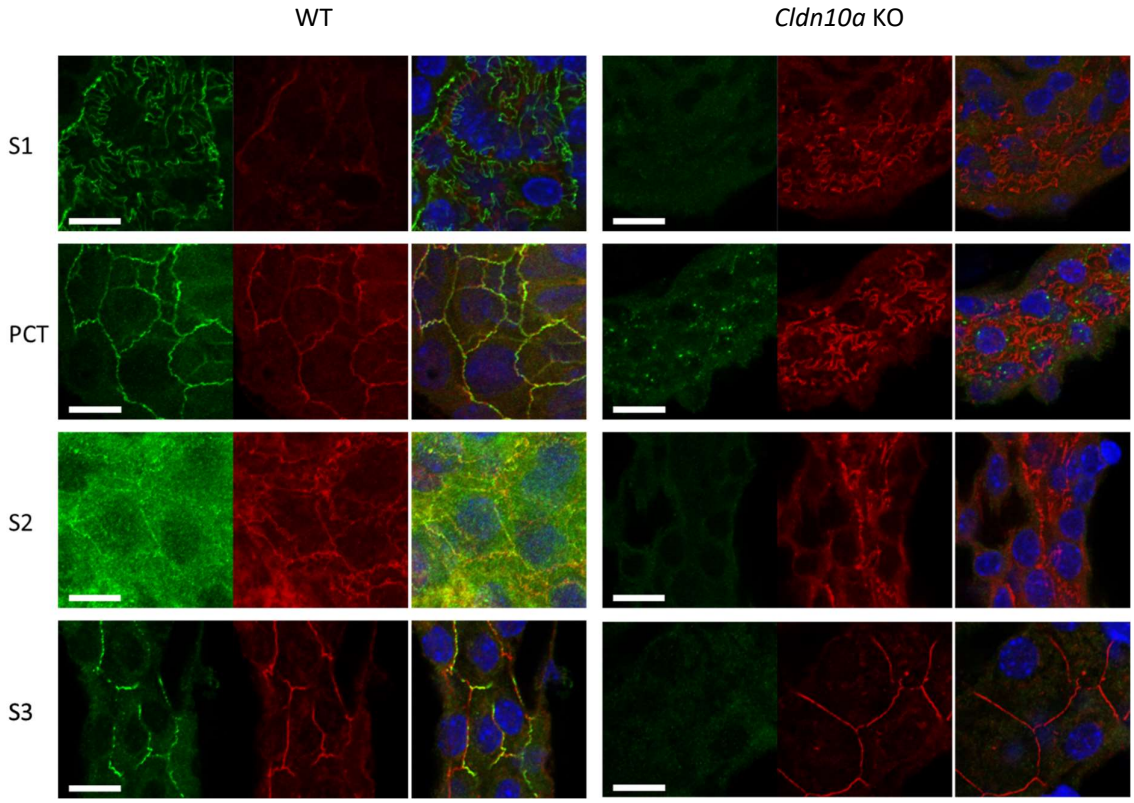


Supplemental Figure 9: Whole kidney claudin-2 and -10 Western blots, PT and non-PT claudin-2 staining.

A Claudin-10 signals in Western Blots of whole kidney protein extracts were reduced in *Cldn10a* KO (-/-) compared to WT (+/+) mice. The residual claudin-10 signal in -/- is due to claudin-10b, as the antibody does not discriminate between the two isoforms. Tubulin, loading control.

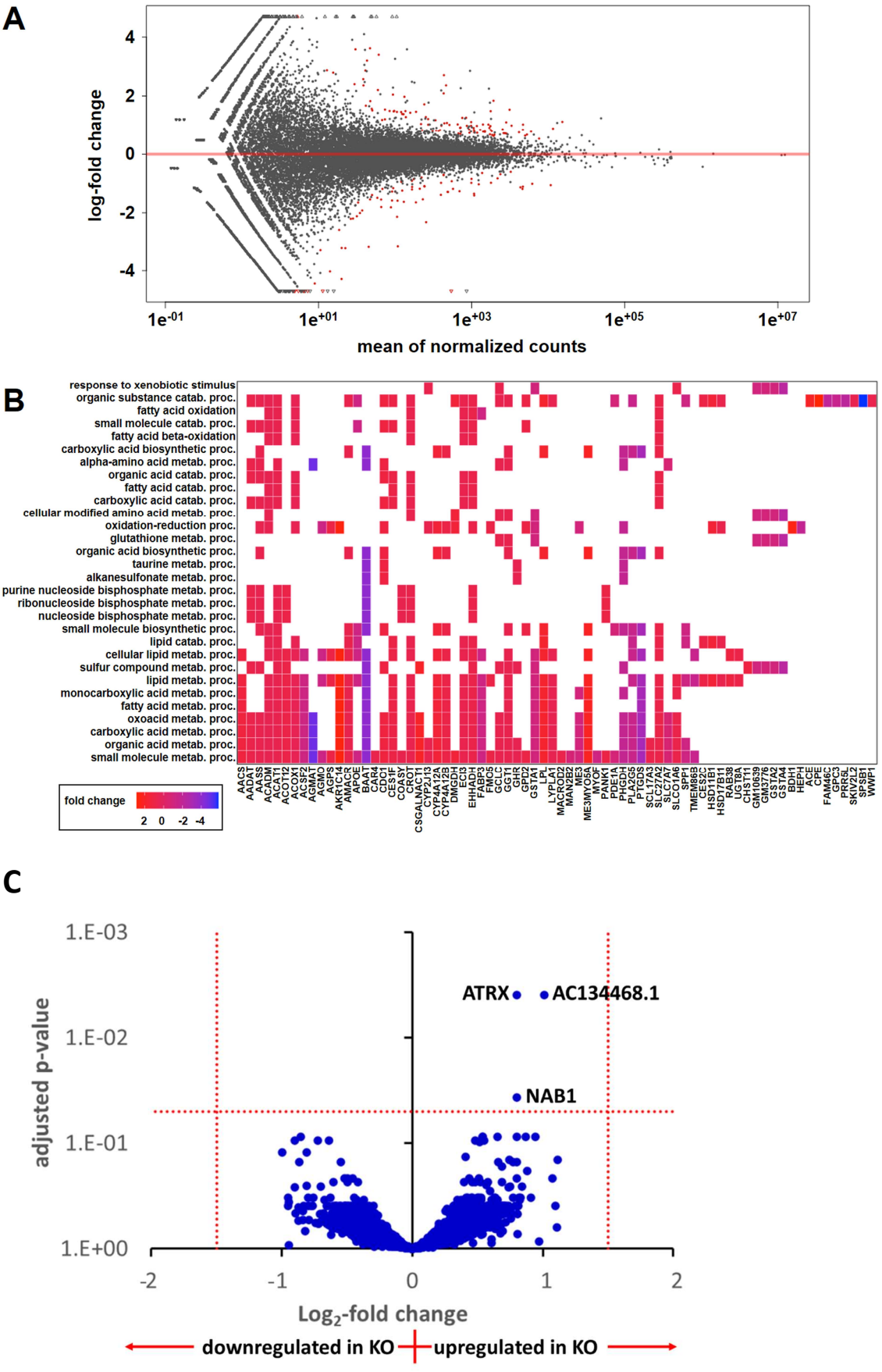
B Densitometric evaluation of the blot shown in **A**.

C Co-staining of claudin-2 (red) and megalin (gray) as PT marker in outer stripe of outer medulla. Megalin-positive tubules mark PT S3 segments. Very strong claudin-2 staining is observed in descending limbs of Henle's loop (megalin negative; * note continuity with S3 segment).



Supplemental Figure 10: Immunohistochemical staining of isolated proximal tubule segments.

Isolated WT and *Cldn10a* KO proximal tubule segments fixed and immuno-stained after dilution potential measurements (green, claudin-10; red, claudin-2; blue, DAPI; bars, 10 µm; merged channels also shown in Fig. 3C).


A
log-fold change
mean of normalized counts
B
response to xenobiotic stimulus
organic substance catab. proc.
fatty acid oxidation
small molecule catab. proc.
fatty acid beta-oxidation
carboxylic acid biosynthetic proc.
alpha-amino acid metab. proc.
organic acid catab. proc.
fatty acid catab. proc.
carboxylic acid catab. proc.
cellular modified amino acid metab. proc.
oxidation-reduction proc.
glutathione metab. proc.
organic acid biosynthetic proc.
taurine metab. proc.
alkanesulfonate metab. proc.
purine nucleoside bisphosphate metab. proc.
ribonucleoside bisphosphate metab. proc.
nucleoside bisphosphate metab. proc.
small molecule biosynthetic proc.
lipid catab. proc.
cellular lipid metab. proc.
sulfur compound metab. proc.
lipid metab. proc.
monocarboxylic acid metab. proc.
fatty acid metab. proc.
oxoacid metab. proc.
carboxylic acid metab. proc.
organic acid metab. proc.
small molecule metab. proc.
fold change
C
adjusted p-value
ATRX
AC134468.1
NAB1
Log2-fold change
downregulated in KO
upregulated in KO

Supplemental Figure 11: RNA-Seq analysis.

A MA-Plot for PCT data. Genes with significantly altered expression levels are marked in red.

B Selection of overrepresented Gene Ontology annotations and their respective differentially expressed genes from the PCT data as heatmap. Fold change is color coded. For a complete list of enriched GO terms see Supplemental Table 8.

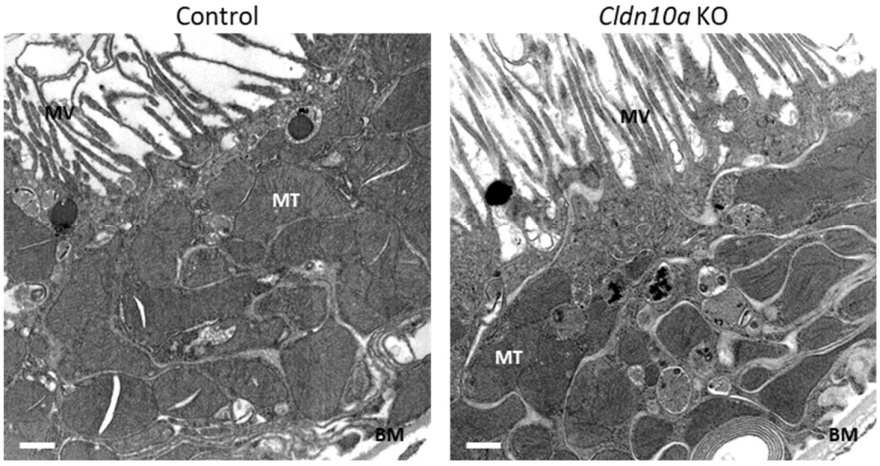
C Plot of $\log_2$-fold change between WT and *Cldn10a* KO DCT against the adjusted p-values. Red lines denote p = 0.05 and $|\log_2\text{-fold change}| = 1.5$. None of $|\log_2\text{-fold change}|$ values exceeded the threshold of 1.5, and only three values exhibited p-values below 0.05:

AC134468.1, unknown function (ENSMUSG00000114147)

ATRX, chromatin remodeler, transcription modulator (ENSMUSG00000031229)

NAB1, Ngfi-A binding protein 1, transcription factor (ENSMUSG00000002881)

None of these genes bears any obvious direct relation to the phenotype of the *Cldn10a* KO mice.



Supplemental Figure 12: Electron microscopy of PT mitochondria.

The morphology of PT mitochondria was not different between WT and *Cldn10a* KO mice in electron micrographs. Scale bars, 0.5 µm. BM, basement membrane; MT, mitochondrion; MV, microvilli.