SUPPLEMENTARY MATERIALS

Disruption of mitochondrial electron transport impairs urinary concentration via AMPK-dependent suppression of aquaporin-2

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Supplementary Table S1

Primary and secondary antibodies used for immunofluorescence and immunoblotting.

Antibody / type	Host	Catalog Nr.	Vendor	Dilution	Time
AMPKα (D5A2)	rabbit	5831	Cell Signaling Technology, Danvers, MA, USA	WB 1:1000 IF 1:250	2 hr RT
p-AMPKα (T172) (40H9)	rabbit	2535	Cell Signaling Technology, Danvers, MA, USA	WB 1:1000 IF 1:250	3 hr RT
AQP2 (total) - HRP conjugated	mouse	sc-515770	Santa Cruz Biotechnology, Dallas, TX, USA	IF 1:100, WB 1:200	o/n, 4^0C
AQP2 (total) - FITC conjugated	mouse	sc-515770	Santa Cruz Biotechnology, Dallas, TX, USA	IF 1:100, WB 1:500	1 hr RT
AQP2 (total) - AF647 conjugated	mouse	sc-515770	Santa Cruz Biotechnology, Dallas, TX, USA	IF 1:100, WB 1:500	1 hr RT
AQP2 (total) - non-conjugated	mouse	sc-515770	Santa Cruz Biotechnology, Dallas, TX, USA	IF 1:100	o/n 4^0C
p-AQP2 (S269)	rabbit	p112-269t	PhosphoSolutions, Aurora, CO, USA	WB 1:1000 IF 1:250	1 hr RT
AQP3 (total)	Rabbit	HPA014924	Millipore Sigma, Burlington, MA	WB 1:1000	o/n 4^0C
ENaCγ	rabbit	SPC-405D	StressMarq Bioscience, Victoria, BC, Canada	IF 1:250, WB 1:1000	1 hr RT
Ezrin	rabbit	3145	Cell Signaling Technology, Danvers, MA, USA	IF 1:500	1 hr RT
NA^+/K^+ ATPase α1/ATP1A1 - AF546 conjugated	mouse	sc-514614	Santa Cruz Biotech, Dallas, TX, USA	WB 1:500	1 hr RT
GFP	rabbit	ab13970	Abcam, Waltham, MA, USA	IF 1:200	o/n, 4^0C
tdTomato / RFP	rabbit	600-401-379	Rockland Immunochemicals, Limerick, PA, USA	IF 1:200	o/n, 4^0C
anti-rabbit IgG - HRP conjugated	Goat	111-035-003	Jackson Immunoresearch, West Grove, PA, USA	WB 1:5000	1 hr RT
anti-rabbit IgG AF 647 conjugated	Donkey	711-605-152	Jackson Immunoresearch, West Grove, PA, USA	WB 1:5000	1 hr RT
anti-rabbit IgG (H+L)-Cy3	Donkey	711-166-152	Jackson Immunoresearch, West Grove, PA, USA	IF 1:200	1 hr RT
anti-mouse IgG (H+L)-Cy5	Donkey	715-175-150	Jackson Immunoresearch, West Grove, PA, USA	IF 1:200	1 hr RT
anti-Chicken IgY (H+L) Alexa Fluor 488	Goat	A-11039	Thermo Fisher Scientific, Waltham, MA, USA	IF 1:500	1 hr RT

Abb.: IF, immunofluorescence; o/n, overnight; RT, room temperature; WB, Western blot.

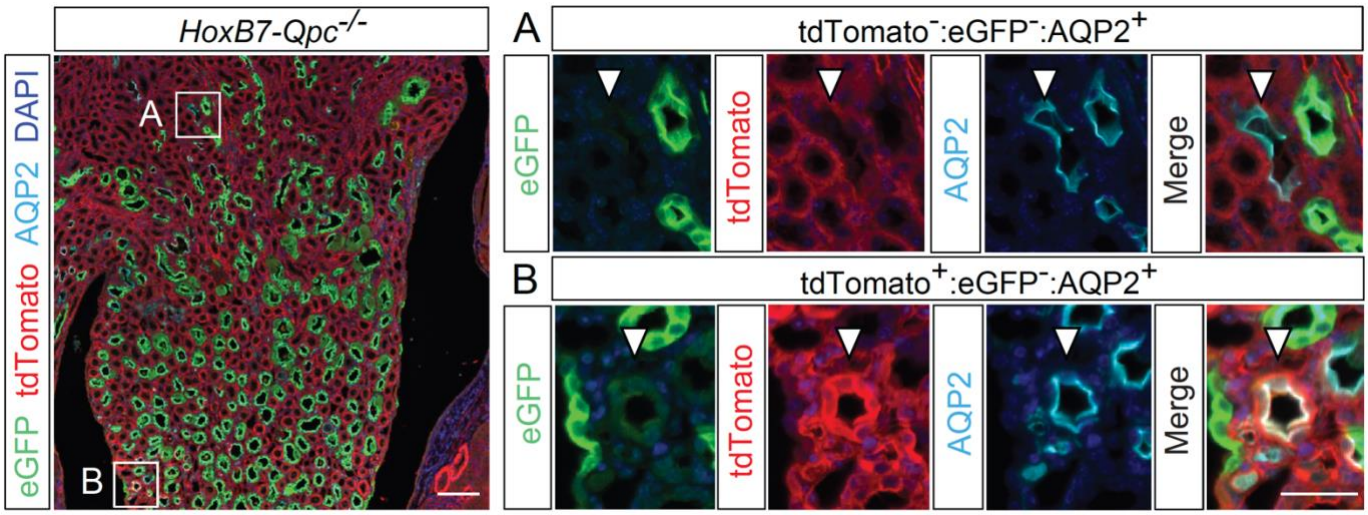
Supplementary Table S2

Serum electrolytes in control and *HoxB7-Qpc*$^{-/-}$ mice.

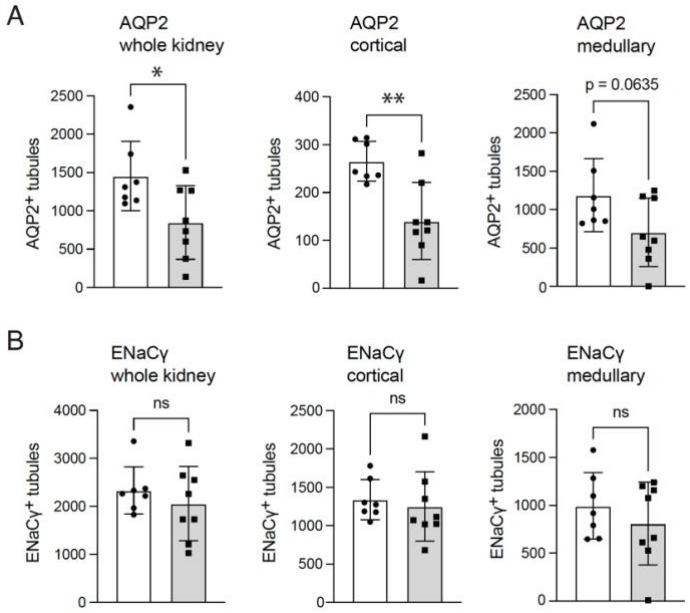
Genotype	nbr	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	iCa^{2+} (mg/dL)	HCO_3^- (mmol/L)	Anion gap
Control	n=3	147.7 ± 0.5	3.2 ± 0.2	107.3 ± 1.2	1.30 ± 0.03	24.3 ± 0.5	20.0 ± 0.8
HoxB7-Qpc$^{-/-}$	n=3	146.7 ± 0.9	3.5 ± 0.5	109.0 ± 1.6	1.30 ± 0.01	22.3 ± 2.1	19.7 ± 0.5

Abb.: nbr, number of mice.

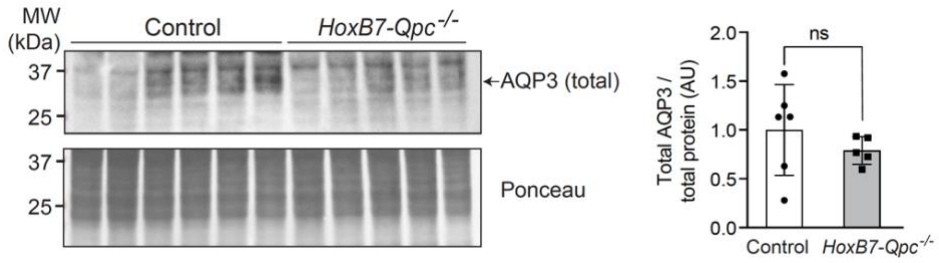
Supplementary Figures



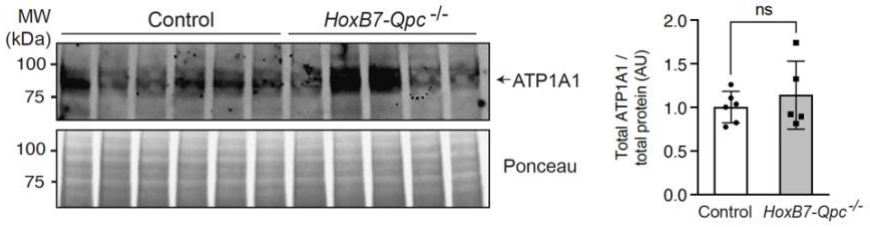
Supplementary Figure S1. Efficient recombination of *Qpc* in *HoxB7-Qpc*$^{-/-}$ kidneys (linked to Figure 1). To investigate whether AQP2(+):eGFP(-) epithelial cells represent CD cells that did not recombine or cells with an inactive Cre-reporter allele, we performed co-staining for tdTomato (tdT) in 6-month-old *HoxB7-Qpc*$^{-/-}$ mice. At baseline (no recombination), the active reporter allele expresses tdT (red fluorescence). Cre-mediated recombination switches the expression from tdT to eGFP. The lack of tdT expression in eGFP(-):AQP2(+) cells (panel A) indicates that the reporter is not active, whereas tdT expression in eGFP(-):AQP2(+) cells (panel B) indicates that Cre-mediated recombination did not occur. Scale bars: 100 µm (low magnification), 50 µm (high magnification). Abb.: AQP2, aquaporin 2; DAPI, 4',6-diamidino-2-phenylindole; eGFP, enhanced green fluorescence protein.



Supplementary Figure S2. AQP2-expressing tubules are reduced in *HoxB7-Qpc*$^{-/-}$ kidneys (linked to Figure 2)**.** (A) Counts of AQP2(+) tubules, and (B) ENaCγ(+) tubules in cortex, medulla, or whole kidney. For each kidney section, all tubules were counted. Counts were then segregated by region; *Cre(-)* littermate control (n=7) and *HoxB7-Qpc*$^{-/-}$ mice (n=8); *Cre(-)* littermate control (n=7) and *HoxB7-Qpc*$^{-/-}$ mice (n=8). Data are represented as average mean values ± SD; two-tailed Student's *t*-test, **P* < 0.05, ***P* < 0.01; ns = not significant.



Supplementary Figure S3. **Total AQP3 protein abundance in *HoxB7-Qpc*$^{-/-}$ mice** (linked to Figure 3). Total aquaporin-3 (AQP3) protein levels in whole kidney lysates from *Cre(-)* littermate control and *HoxB7-Qpc*$^{-/-}$ mice; n = 6 and 5, respectively. Data are represented by mean values ± SD; two-tailed Student's *t*-test, ns = not significant. Abb.: AU, arbitrary units.



Supplementary Figure S4. **Total Na^+/K^+ ATPase protein abundance in *HoxB7-Qpc*$^{-/-}$ mice** (linked to Figure 3). Total Na^+/K^+ ATPase α1 (ATP1A1) protein levels in whole kidney lysates from *Cre(-)* littermate control and *HoxB7-Qpc*$^{-/-}$ mice; n = 6 and 5, respectively. Data are represented by mean values ± SD; two-tailed Student's *t*-test, ns = not significant. Abb.: AU, arbitrary units.