Supporting Information

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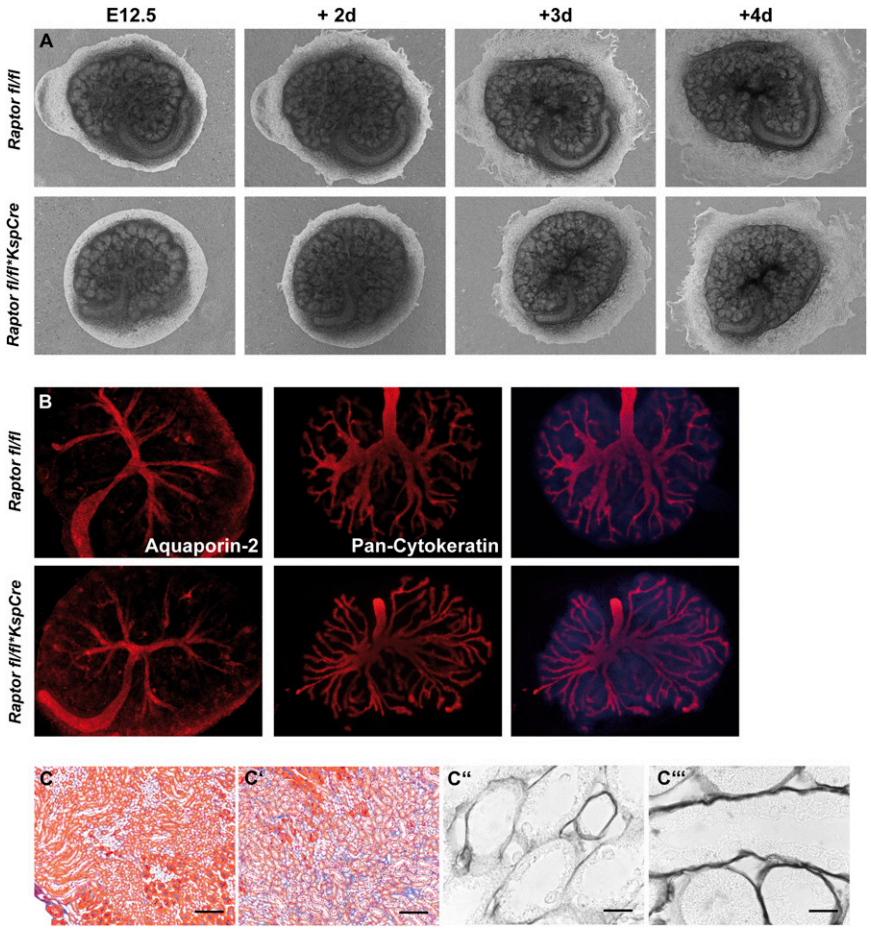

Fig. S1. Use of kidney-cell culture to assess developmental abnormalities in tubulogenesis. (*A* and *B*) Kidney explants from embryonic day (E) 12.5 embryos from *Raptor fl/fl* KspCre* and *Raptor fl/fl* animals were assessed for branching and elongation defects up to E17.5. As evident from the differential interference contrast (*A*) and immunofluorescence (*B*) images, neither tubular elongation nor tubular branching differed in the two genotypes. (*C* and *C′*) Acid fuchsin-orange G (SFOG) staining shows increased fibrous tissue content in *Raptor fl/fl* KspCre* mice compared with wild-type animals. (*C′′* and *C′′′*) Similarly, silver staining shows that basement membranes are thickened around thick ascending limb of Henle (TAL) tissue from knockout mice as compared with TAL tissue from wild-type mice. (Scale bars: *C* and *C′*, 200 μm; *C′′* and *C′′′*, 20 μm.)

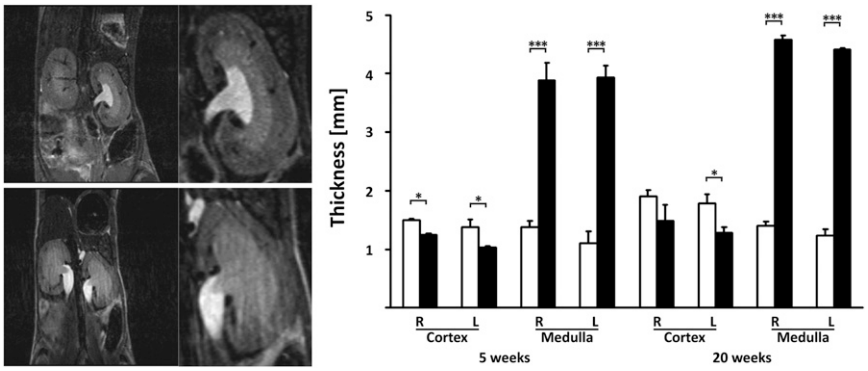

Fig. S2. MRI shows reduced cortical and increased medullary thickness in *Raptor fl/fl*KspCre* mice. T2-weighted MRI sequences show a dramatically increased medullary thickness in kidneys from knockout mice (black bars). This increased thickness seems to be a consequence of compensatory growth mechanisms, which already could be detected in the transcriptomic analysis of kidneys from wild-type versus knockout mice on postnatal day 14 (*$P < 0.05$; ***$P < 0.001$). L, left kidney; R, right kidney.

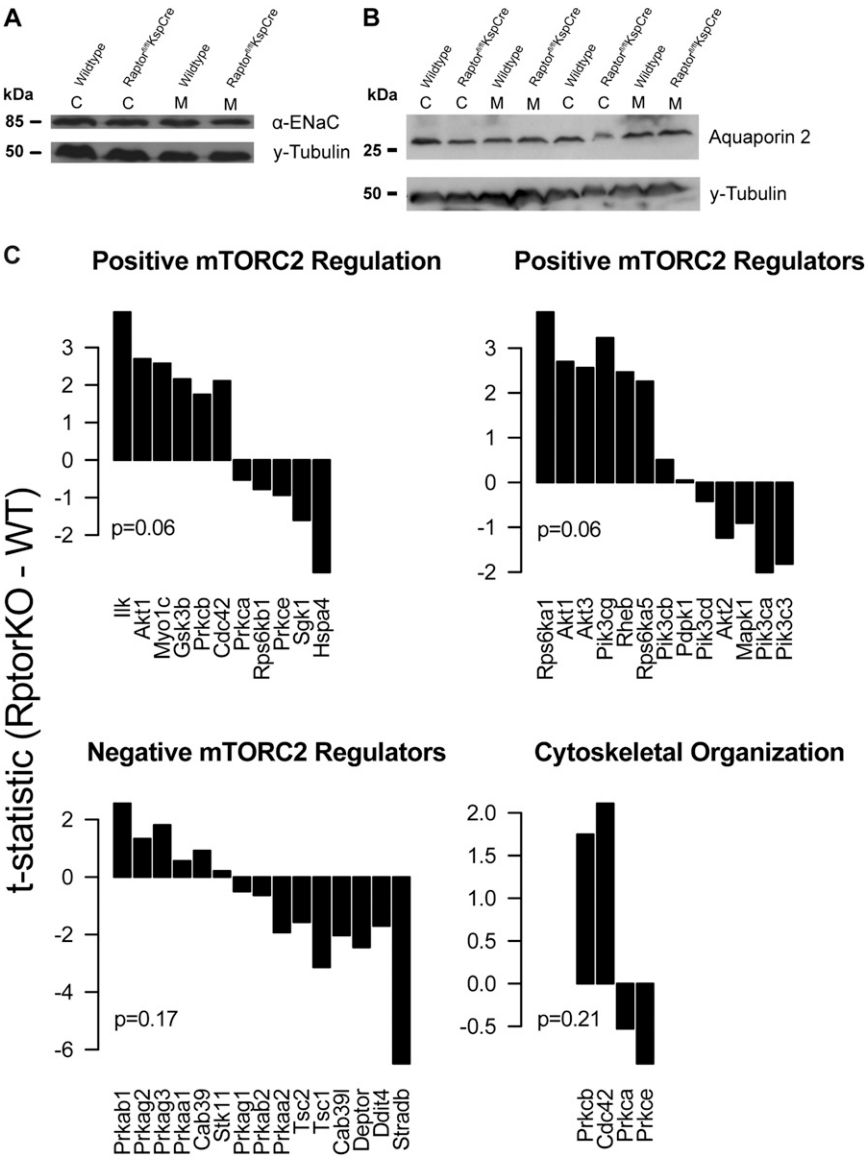

Fig. S3. Positive and negative regulation of mammalian target of rapamycin complex 2 (mTORC2)-related genes. (*A* and *B*) In contrast to TAL and distal convoluted tubule (DCT) transport proteins, we did not detect a difference in α-ENaC (*A*) or aquaporin 2 (*B*). (*C*) Bar plots depict the differential regulation in kidneys in *Raptor*-knockout and wild-type mice. Genes related to positive mTORC2 regulation and positive and negative mTORC2 regulators are preferentially up-and down-regulated, respectively, under *Raptor*-knockout conditions. The *P* values denote the significance of regulation per gene set.

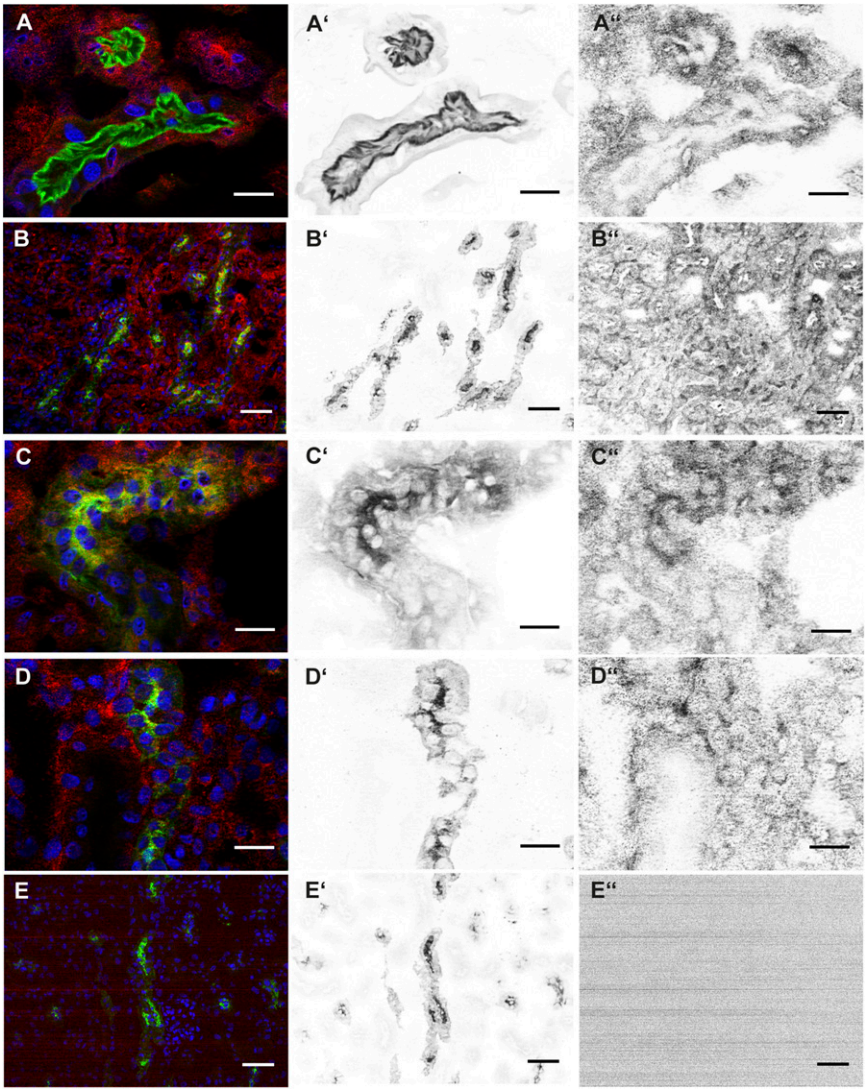

Fig. S4. Expression of total S6 protein (S6P) in mouse renal tubules. (*A*–*D*′′) Total S6P (red) is ubiquitously expressed along the renal tubule, as shown with the segment-specific markers (all in green): (*A*–*A*′′) Lotus tetragonobulus (LTG), proximal tubule; (*B*–*B*′′) Tamm–Horsfall protein (THP), TAL; (*C*–*C*′′) Calbindin-D28K, distal tubule; and (*D*–*D*′′) aquaporin 2, collecting duct. (*E*–*E*′′) Control staining omitting the total S6P primary antibody. Although the exposure time was extended to 20 s, no specific signal of the secondary donkey anti-rabbit 555 antibody can be detected. (Scale bars: *A*–*A*′′, *C*–*C*′′, and *D*–*D*′′, 20 μm; *B*–*B*′′ and *E*–*E*′′, 50 μm.)

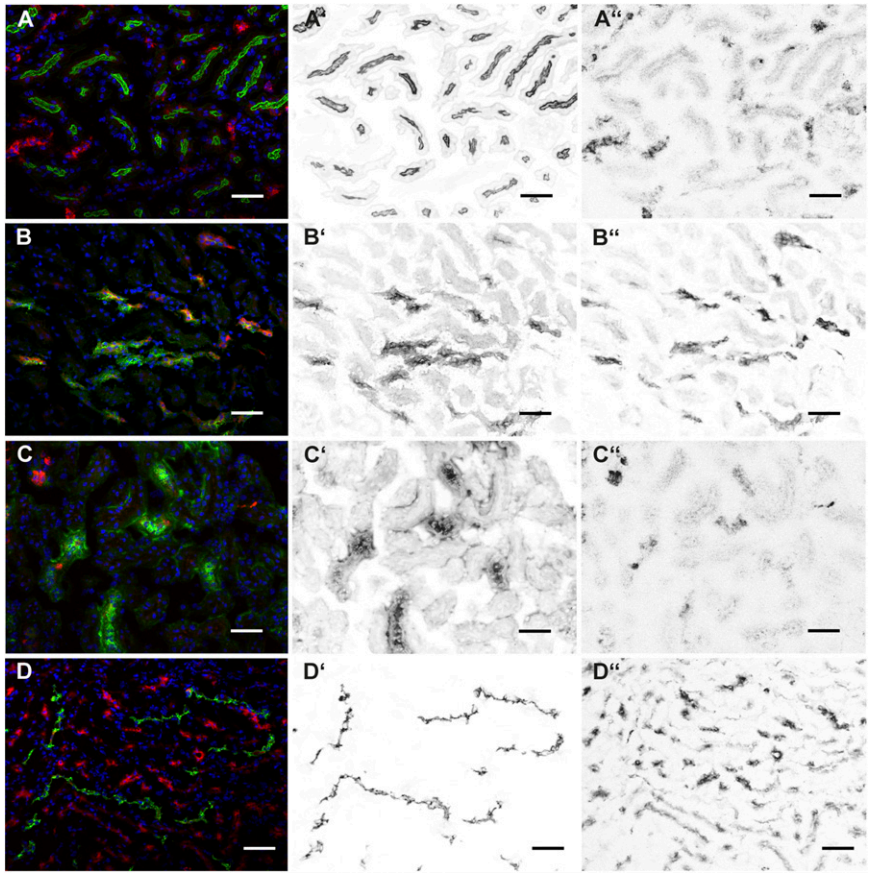

Fig. S5. Expression of phosphorylated S6P (P-S6P) in mouse renal tubules. P-S6P (red) is expressed only very slightly in proximal tubules (*A*–*A*′′) but is strongly expressed in the TAL (*B*–*B*′′). Again, there is a very weak expression in DCT/connecting tubule (*C*–*C*′′) and virtually no expression in collecting ducts (*D*–*D*′′). This expression is consistent with the major TAL phenotype of *Raptor fl/fl*KspCre* mice. Segment-specific markers (all in green) are (*A*–*A*′′) LTG, proximal tubule; (*B*–*B*′′) THP, TAL; (*C*–*C*′′) Calbindin-D28K, distal tubule; and (*D*–*D*′′) Aquaporin 2, collecting duct. (Scale bars: 50 μm.)

Table S1. Physiological parameters of *Raptor fl/fl*KspCre* animals under basal conditions and after diuretic treatment

	Control	Thirst challenge	Vehicle	Furosemide	Thiazide	Triamterene
WT Δbw, g (9 or 10)	N.a.	−1.1 ± 0.2	−0.6 ± 0.0	−2.0 ± 0.1	−0.7 ± 0.1	−1.1 ± 0.1
KO Δbw, g (8)	N.a.	−3.7 ± 0.4***	−1.1 ± 0.2*	−2.2 ± 0.3	−1.42 ± 0.1***	−1.7 ± 0.3
WT food, g (10)	4.1 ± 0.2					
KO food, g (9)	3.8 ± 0.1					
WT drink, mL (10)	3.9 ± 0.1					
KO drink, mL (9)	14.3 ± 1.1***					
WT urine flow, μL/h (10)	64.2 ± 7.8	3.8 ± 1.9	56.8 ± 7.5	369.8 ± 43.0	73.7 ± 11.8	48.0 ± 9.0
KO urine flow, μL/h (9)	832.6 ± 96.3***	176.9 ± 21.9***	115.5 ± 1 9.6**	415.3 ± 56.5	212.5 ± 23.1***	144.8 ± 30.7**
WT urea, mM (10)	9.2 ± 1.2					
KO urea, mM (8)	14.1 ± 1.2**					
WT serum Na, mM (10)	153.4 ± 1.4					
KO serum Na, mM (8)	154.0 ± 0.8					
WT serum K, mM (10)	4.2 ± 0.1					
KO serum K, mM (8)	4.1 ± 0.1					
WT serum Ca, mM (10)	2.3 ± 0.0					
KO serum Ca, mM (8)	2.3 ± 0.0					
WT serum Mg, mM (10)	0.6 ± 0.0					
KO serum Mg, mM (8)	0.7 ± 0.0					
WT urine ADH, pmol/24 h (9)	0.7 ± 0.1					
KO urine ADH, pmol/24 h (9)	0.9 ± 0.2					
WT plasma osmolality, mOsm/kg (9)	306.4 ± 2.2					
KO plasma osmolality, mOsm/kg (7)	316.3 ± 3.3*					
WT urine osmolality, mOsm/kg (10)	2,785.5 ± 252.2	Per 6 h in metabolic cage	616.4 ± 77.6	51.0 ± 2.2	704.2 ± 111.9	419.0 ± 70.3
KO urine osmolality, mOsm/kg (9)	540.7 ± 46.7***		224.4 ± 39.3	275.5 ± 54.9	255.2 ± 33.9	287.1 ± 48.8
WT urine Na, μmol/24 h (10)	370.6 ± 15.7	Per 6 h in metabolic cage	7.7 ± 1.6	192.5 ± 20.8	28.1 ± 7.28	12.3 ± 2.9
KO urine Na, μmol/24 h (9)	326.0 ± 19.4		6.5 ± 1.9	99.8 ± 18.7***	44.6 ± 8.2	16.1 ± 4.3
WT urine K, μmol/24 h (10)	594.0 ± 26.2	Per 6 h in metabolic cage	20.1 ± 4.4	89.6 ± 12.8	25.7 ± 6.9	10.0 ± 2.0
KO urine, μmol/24 h K (9)	521.3 ± 26.7		13.8 ± 3.6	34.4 ± 5.6***	39.9 ± 6.6	19.2 ± 6.1
WT urine Ca, μmol/24 h (10)	2.0 ± 0.1	Per 6 h in metabolic cage	0.5 ± 0.1	1.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
KO urine Ca, μmol/24 h (8)	6.0 ± 1.1***		1.1 ± 0.2**	1.5 ± 0.1	1.8 ± 0.3**	1.9 ± 0.4**
WT urine Mg, μmol/24 h (10)	11.0 ± 0.7					
KO urine Mg, μmol/24 h (8)	16.1 ± 1.1***					

Number of mice is stated in parentheses. Asterisks indicate statistical significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ knockout vs. wild type). bw, body weight; N.a., not applicable.

Table S2. Physiological parameters of *Raptor fl/fl*Rictor fl/fl*KspCre* animals

	Control
WT bw, g (24)	24.1 ± 0.8.
KO bw, g (12)	20.69 ± 1.4*
WT deaths until week 12	0/24
KO deaths until week 12	2/14
WT urine flow, $\mu L \cdot h^{-1} \cdot g^{-1}$ bw (24)	8.5 ± 0.7
KO urine flow $\mu L \cdot h^{-1} \cdot g^{-1}$ bw (12)	19.1 ± 3.1**
WT urea, mM (23)	9.4 ± 0.6
KO urea, mM (12)	18.3 ± 1.5***
WT serum Na, mM (23)	147.8 ± 1.4
KO serum Na, mM (12)	147.1 ± 0.8
WT serum K, mM (23)	5.4 ± 0.1
KO serum K, mM (12)	5.0 ± 0.2
WT serum Ca, mM (23)	2.2 ± 0.1
KO serum Ca, mM (12)	2.2 ± 0.0
WT FE Na, % (23)	0.62 ± 0.2
KO FE Na, % (12)	0.93 ± 0.1
WT FE K, % (23)	16.8 ± 2.2
KO FE K, % (12)	24.9 ± 4.1
WT FE Ca, % (23)	3.8 ± 0.7
KO FE Ca, % (12)	9.3 ± 2.3*
WT FE urea, % (23)	0.5 ± 0.1
KO FE urea, % (12)	0.3 ± 0.1*

Number of mice is stated in parentheses. Asterisks indicate statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ knockout vs. wild type). bw, body weight; FE, fractional excretion.