Supplemental Information

Content of Supplemental Information

1. Supplemental Figures and Figure Legends (5 figures)

2. Supplemental Table (1 table)

Supplemental Figures

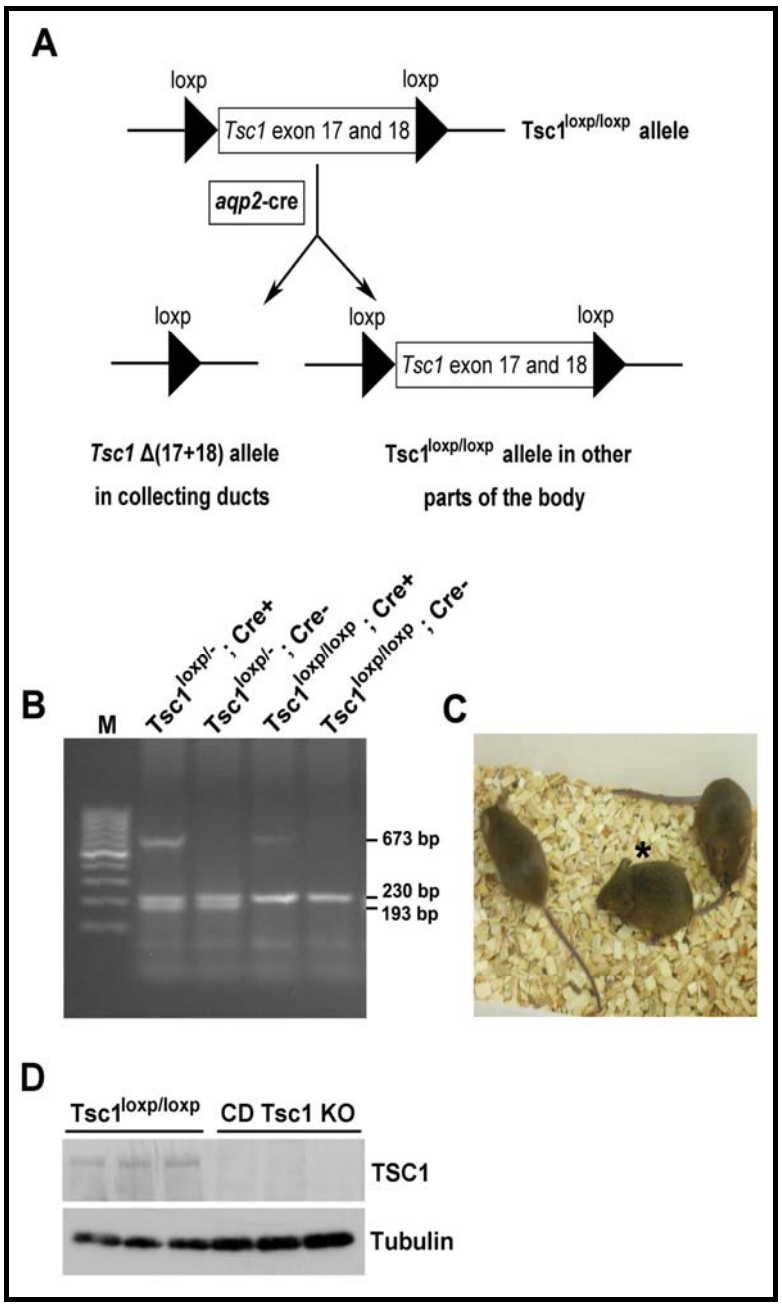

Figure S1. Generation of mice with collecting duct-specific deletion of Tsc1. (A) Schematic of deletion of Tsc1 exon 17 and 18 by Cre-mediated recombination. (B) Genotyping the offspring after mating transgenic cre and loxp mice by PCR. (C) Representative picture of control and KO mice from the same litter at 8 weeks. CDTsc1KO mice (asterisk) were born at normal Mendelian ratios and grew normally. (D) Confirmation of the deletion of Tsc1 by western blot. Protein lysate was from 4-week-old control and KO mice by microdissection.

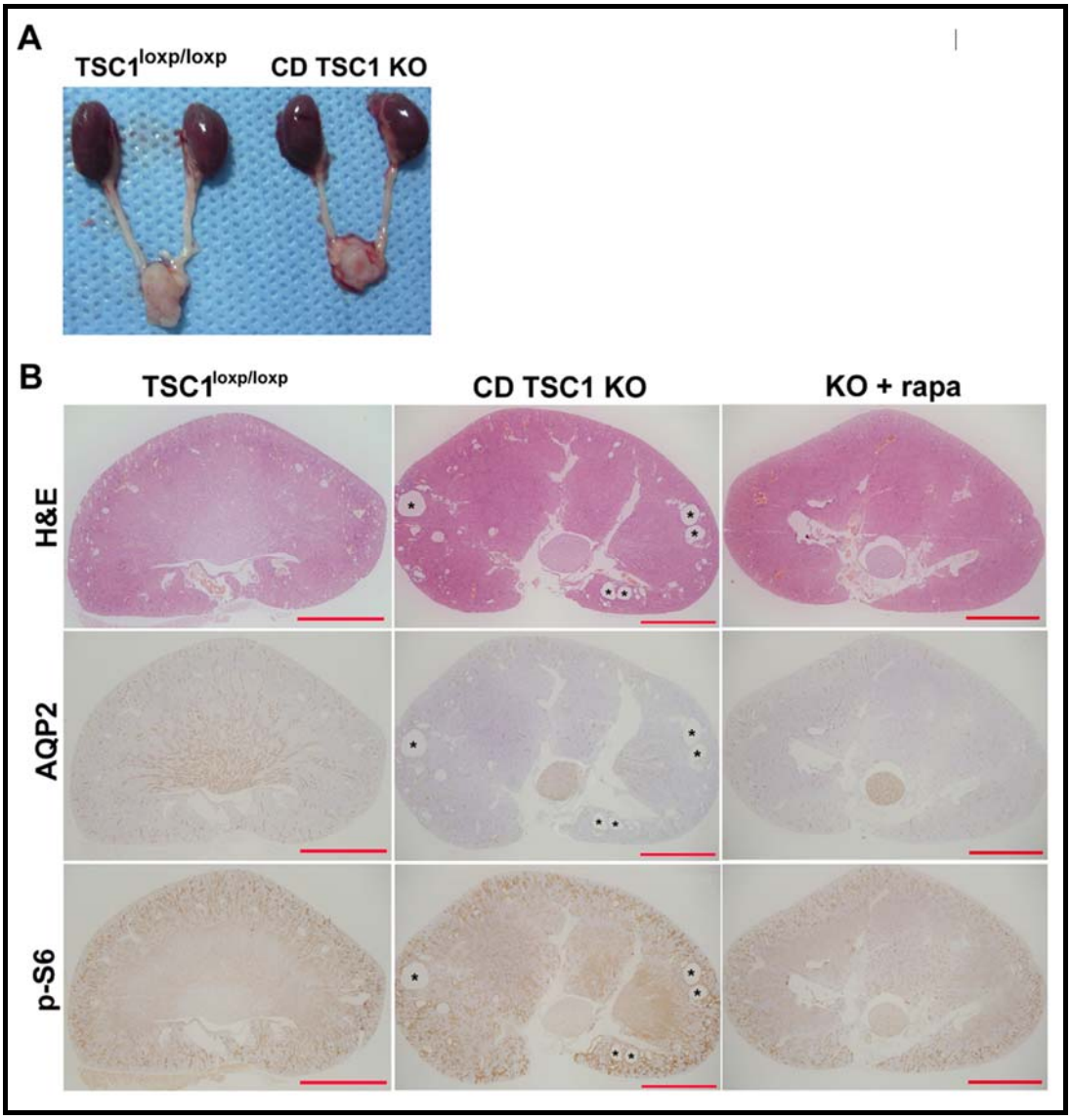

Figure S2. Overview of the global appearance and coronal sections of kidneys of each group. (A) No obvious difference in global appearance of kidneys between control and KO mice. (B) Overview of the coronal sections of kidneys. Note that the enlarged cavities (asterisk) were evident in KO mice under low magnification (×16), and invisible after rapamycin treatment. Scale bar = 2 mm

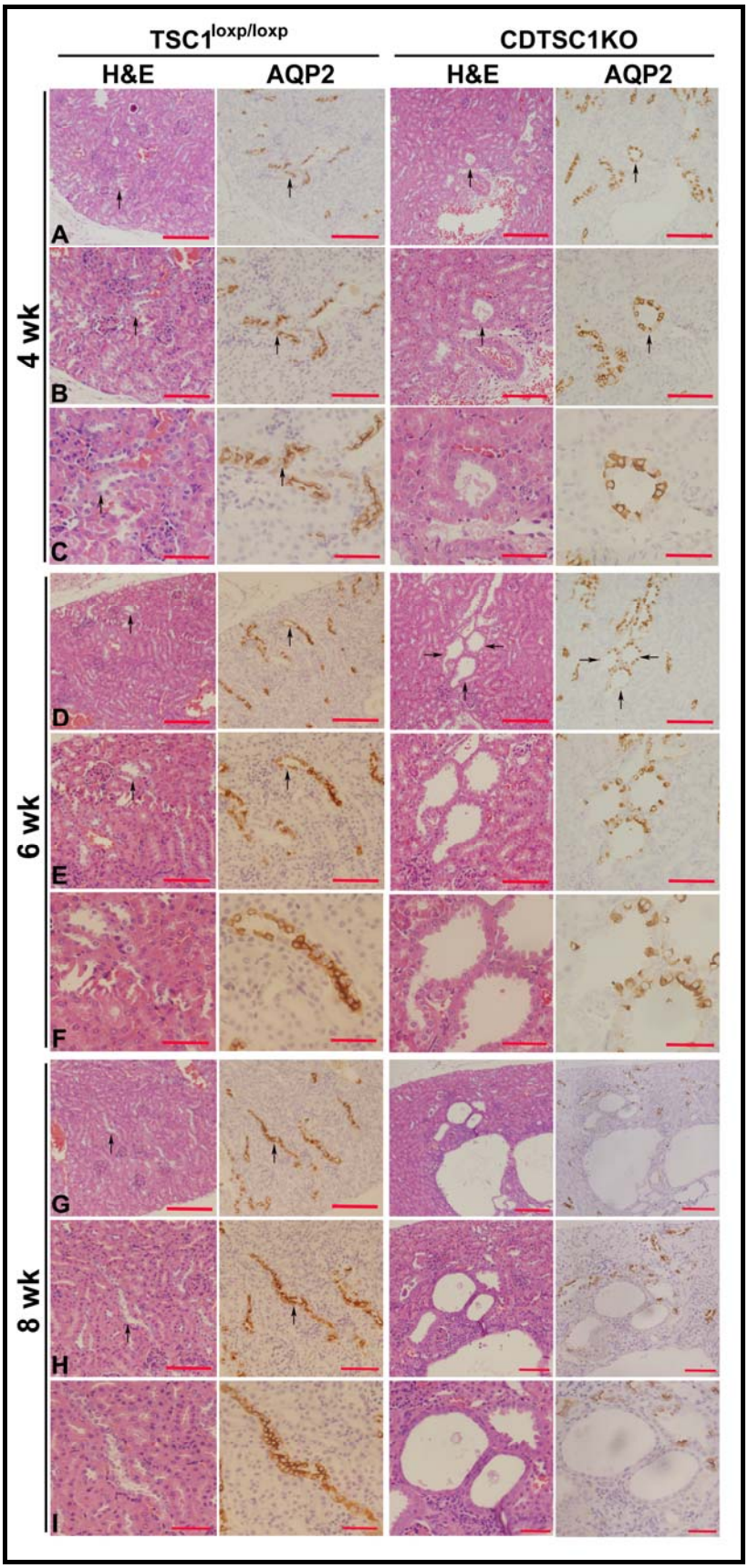

Figure S3. Representative images showed time-dependent CCD expansion in CDTsc1KO mice. Two sequential sections from kidneys of 4-, 6- and 8-week-old

control and KO mice were used respectively for H&E staining and IHC-staining of AQP2. The representative CCD visible on both H&E and IHC stained sections were photographed under serial magnifications. Scale bars = 200 μm for panels A, D and G, 100 μm for panels B, E and H, 50 μm for panels C, F and I.

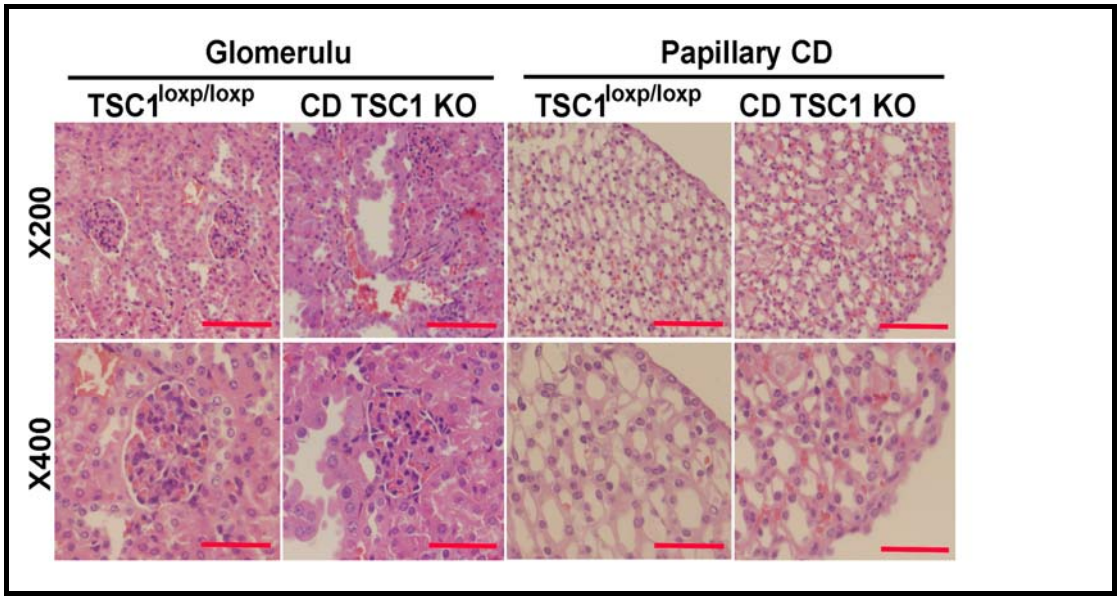

Figure S4. Representative HE images of glomerulu and papillary collecting ducts from control and KO mice. No obvious morphological or structural difference was observed in both glomerulum and papillary collecting duct between control and KO mice. Scale bar = 100 μm for upper panels, 50 μm for lower panels.

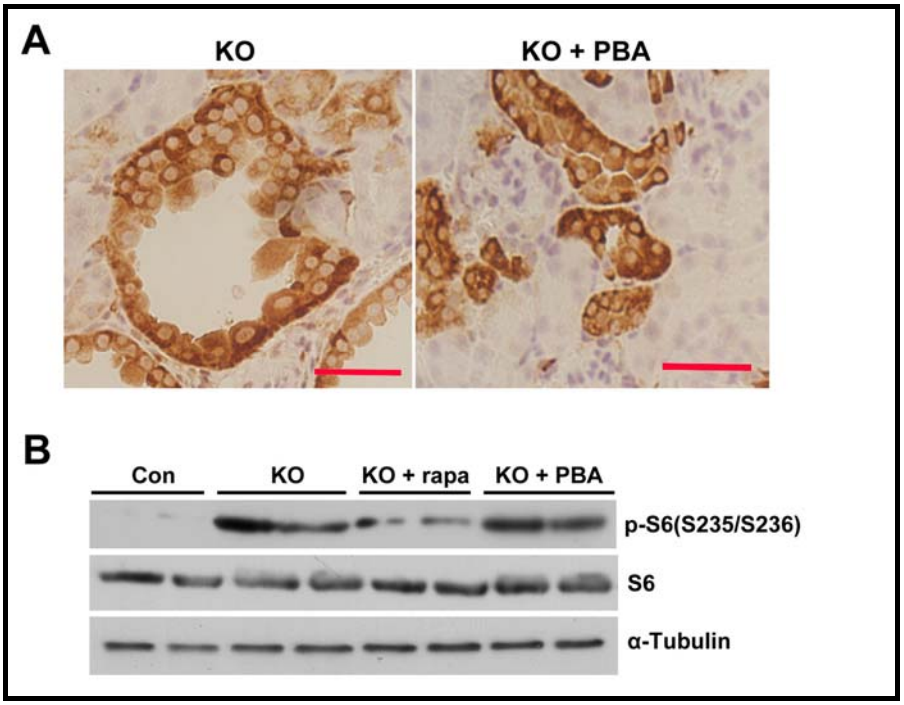

Figure S5. PBA ameliorated the CCD cell expansion and detachment in KO mice, but had little effect on enhanced S6 phosphorylation. (A) Immunohistochemistry of phospho-S6 in KO mice and KO mice with 4 weeks PBA treatment. Scale bar = 50 μm. (B) Immunobloting of phospho-S6 in KO mice and KO mice with PBA. Protein lysate was from microdissection.

Supplemental Table

Table S1. Physical and biochemical parameters of control, CDTsc1KO mice and KO mice with rapamycin or PBA.

	Control	CDTsc1KO	Con + Rapa	KO + Rapa	Con + PBA	KO + PBA
Physical						
Body weight, g	20.8 ± 3.1	20.1 ± 2.1	18.1 ± 1.6	18.0 ± 1.3	17.5 ± 1.8	17.4 ± 1.7
Kidney weight, g	0.10 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Adrenal gland, mg	5.3 ± 1.9	5.2 ± 2.1	5.2 ± 0.5	5.2 ± 0.3	5.1 ± 0.4	5.1 ± 0.4
blood						
Na^+, mmol/l	151.4 ± 3.2	148.2 ± 5.0	151.2 ± 3.1	150.6 ± 1.7	149.7 ± 2.3	146.0 ± 2.7
Cl^-, mmol/l	108.6 ± 3.6	108.5 ± 3.4	110.6 ± 3.7	109.1 ± 4.8	108.0 ± 2.5	107.7 ± 1.0
P, mmol/l	2.6 ± 0.7	3.0 ± 1.5	2.8 ± 0.4	2.9 ± 0.7	2.7 ± 0.3	2.9 ± 0.2
Creatinine, μmol/l	15.6 ± 2.4	16.5 ± 3.7	14.2 ± 1.5	14.6 ± 1.8	13.8 ± 2.2	13.6 ± 2.1
BUN, μmol/l	15.0 ± 1.7	10.7 ± 1.8*	13.7 ± 3.7	9.7 ± 1.7*	11.8 ± 0.6	9.22 ± 1.3
pH (veinous)	7.34 ± 0.09	7.19 ± 0.05*	7.31 ± 0.05	7.27 ± 0.09	7.24 ± 0.05	7.24 ± 0.07
CO_2, mmol/l	15.9 ± 1.1	18.9 ± 1.3*	16.1 ± 1.7	16.6 ± 0.5	15.6 ± 2.0	17.1 ± 2.3
Urine						
Water intake (ml/d)	3.85 ± 0.54	4.71 ± 0.72	—	—	—	—
Urine volume (ml/d)	1.54 ± 0.26	2.03 ± 0.43	—	—	—	—
pH	7.32 ± 0.09	5.28 ± 0.17*	7.30 ± 0.05	7.20 ± 0.09#	7.20 ± 0.12	7.17 ± 0.18#

Note: All mice used were 8-week-old. For KO + Rapa or KO + PBA group, KO mice were administered with rapamycin (1 mg/kg/d) or PBA (500 mg/kg/d) for 4 weeks respectively. Data were expressed as mean ± SD. BUN, blood urea nitrogen; —, not tested; *, $P < 0.05$, *vs* respective control; #, $P < 0.05$, *vs* CDTsc1KO. n = 7-12 per data point.