SUPPLEMENTAL FIGURES

Figure S1: Generation of mice lacking KCTD1 and of conditional KCTD1 KO mice. Related to Figures 1, 2, 4 and STAR Methods section.

A. Allele map of the Kctd1tm1a$^{(EUCOMM)Wtsi}$ allele, which is a KO first allele: lacZ reporter-tagged insertion with conditional potential.

B. The Kctd1tm1a$^{(EUCOMM)Wtsi}$ allele inserts between exon 2 and 3 of the KCTD1 gene, disrupting the critical BTB domain and leading to non-sense-mediated decay following removal of the floxed region (loxP sites flank exon 3) after crossing these mice with β-actinCre^{+} mice (tm1b allele, homozygous mice hereafter referred to as KCTD1$^{-/-}$ mice). In these mice cells that normally express KCTD1 can be identified by labeling for β-galactosidase (lacZ). Crossing the mice generated from the Kctd1tm1a$^{(EUCOMM)Wtsi}$ ES cell clones with a FLP-deleter mouse strain results in the removal of the lacZ and *neoR* cassette that are flanked by FRT sites, and thereby allowing for normal KCTD1 expression, but maintaining loxP sites flanking the critical exon 3 of KCTD1 (tm1c allele, KCTD1$^{fl/fl}$ mice). These mice were further crossed with β-actinCre^{+} mice to generate KCTD1 KO mice without the lacZ cassette (tm1d). In addition, KCTD1$^{fl/fl}$ mice were crossed with several Cre strains (Six2Cre^{+}, PvalbCre^{+}, Aqp2Cre^{+}, β-actinCreERT2^{+}) to generate conditional KCTD1 KO mice (tm1d).

C. Genotyping of KCTD1$^{-/-}$ and conditional KCTD1 KO mice. Genotyping for exon 3 of KCTD1 reveals absence in KCTD1$^{-/-}$ (KO) mice. KO mice are positive for lacZ (*). Confirmation of floxed alleles in KCTD1$^{fl/fl}$ mice.

D. Lack of KCTD1 expression in kidneys of KCTD1$^{-/-}$ mice. Lack of KCTD1 transcripts in cDNA from kidney lysates of KCTD1$^{-/-}$ mice (36b4 expression shown as housekeeping gene control). PCR primer spanning exon 3 were used that is lacking in KCTD1$^{-/-}$ mice.

E. Cre activity and specificity for all Cre strains were confirmed by crossing Cre strains with *B6.Cg-Gt(ROSA)26Sortm3$^{(CAG-EYFP)Hze/J}$* (Ai3) reporter mice. Representative images are shown from a P0 Six2Cre^{+}Ai3$^{+/WT}$ mouse kidney showing Cre activity (red staining with anti-GFP antibody, white arrow) in Six2^{+} NPCs in the nephrogenic zone but sparing the Aqp2^{+} CDs that are not derived from Six2^{+} NPCs (bottom image: white; green arrow). Scale bars: top image 50 μm; bottom image 20 μm.

F. Top: Cre activity (red staining with anti-GFP antibody, arrow) in a kidney of an adult Aqp2Cre^{+}Ai3$^{+/WT}$ mouse shows co-localization (arrow) with Aqp2 immunolabeling (white). Scale bar: 20 μm. Bottom: Cre activity (red staining with anti-GFP antibody, arrow) in a kidney of an adult PvalbCre^{+}Ai3$^{+/WT}$ mouse. Scale bar: 50 μm.

Figure S2: Efficiency of inactivation of KCTD1 or AP-2β in mutant mice generated. Related to Figure 4 and STAR Methods section.

A. Left: RNA-Seq data of whole kidney lysates show that TAM-induced Cre-mediated recombination effectively eliminates exon 6 of the TFAP2B gene in β-actinCreERT2^{+}TFAP2B$^{fl/fl}$ mice treated with TAM at 6 weeks of age and assessed at 4 months of age. Exon 6 is required for DNA binding and thus for AP-2β transcription factor activity. Right: Sashimi plot for TFAP2B of RNA-Seq data reveal transcripts lacking exon 6 of TFAP2B.

B. Top: In Six2Cre^{+}KCTD1$^{fl/fl}$ mice loxP sites flank the critical exon 3 of KCTD1 and their kidneys show diminished transcripts containing exon 3. Bottom: Sashimi plots for KCTD1 of RNA-Seq data reveal transcripts lacking exon 3 of KCTD1.

C. Semiquantitative RT-PCR for KCTD1 using one primer within exon 3 of the KCTD1 gene demonstrates that kidneys of KCTD1$^{-/-}$ mice have complete absence of functional KCTD1 expression (lack of exon 3-containing KCTD1 transcripts). Kidneys of Six2Cre^{+}KCTD$^{fl/fl}$ mice show a severely diminished expression of exon 3-containing KCTD1 transcripts (the remaining KCTD1 transcripts are derived from the CDs that are not targeted by the Six2Cre strain). Efficient induced inactivation of KCTD1 (Cre-mediated excision of exon 3 of KCTD1) in kidneys of β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice after administration of TAM at either P9 or at 6 weeks of age.

Graphs represent data as mean ± SEM. Semiquantitative RT-PCRs performed in triplicate. P-values are shown (two-tailed, unpaired *t*-test).

Figure S3: Inactivation of AP-2β or KCTD1 in CTs/CDs does not result in significant kidney abnormalities.

Related to Figures 1, 2 and 4.

A. Aqp2Cre^{+}TFAP2B$^{fl/fl}$ mice show no morphological kidney abnormalities. H&E images of kidney of 1-month-old Aqp2Cre^{+}TFAP2B$^{fl/fl}$ mouse compared to its control littermate are shown. Scale bars, 50 μm.

B. EGF KO mice lack EGF immunolabeling in their kidneys, which is observed in their littermate controls (EGF in red). 4-months old mice. Normal kidney histology is observed with normal NKCC2^{+} TALs (NKCC2 in green). Scale bars, 50 μm.

C. Daily administration of EGF to Six2Cre^{+}KCTD1$^{fl/fl}$ mice between P3 and P9 (1μg/gm BW EGF sc daily; [recombinant mouse EGF, Peprotech]) does not rescue the histological renal abnormalities or azotemia (high BUN) when assessed at 6-weeks of age. Scale bars, 50 μm.

D. Inactivation of KCTD1 in CTs/CDs (in Aqp2Cre^{+}KCTD1$^{fl/fl}$ mice) does not affect kidney weight or function. Normal kidney histology in a 7-months-old Aqp2Cre^{+}KCTD1$^{fl/fl}$ mouse. Scale bar, 100 µm.

All graphs show mean ± SEM.

Figure S4: Inactivation of KCTD1 in the nephron proximal to the CDs results in downregulation of mainly TAL/DCT markers when assessed at P8.

Related to Figure 4.

Heatmaps showing DEGs and their nephron segment-specific expression pattern (based on single-cell RNA-Seq data obtained from adult mouse kidneys (Park et al., 2018)) in whole kidney lysates from P8 Six2Cre^{+}KCTD1$^{fl/fl}$ mice versus control littermates (n=4/group). Scale and color coding represent Z-scores. Lack of KCTD1 results at P8 in downregulation of mainly TAL/DCT-specific genes (e.g. EGF, Pvalb and Slc12a3; arrows).

DEGs were defined as having a greater >1.5-fold change and a FDR <0.05. DEGs are shown for which a nephron segment-specific expression pattern was found by single-cell RNA-Seq (Park et al., 2018). A complete list of DEGs and RNA-Seq data is provided in the Supplemental Material. Endo: endothelial, vascular and descending loop of Henle; Podo: podocytes; PT: proximal tubules; LOH: loop of Henle (including TAL); DCT: distal convoluted tubule; CD_PC: collecting duct, principle cells; CD_IC: intercalated cells; CD_Trans: collecting duct, transient cells; Novel1: novel cell type 1; Fib: fibroblast; Macro: macrophage; Neutro: neutrophil; B_lymph: B lymphocyte; T_lymph: T lymphocyte; NK: natural killer cell; Novel 2: novel cell type 2.

Figure S5: Induced inactivation of AP-2β in the adult kidney results in downregulation of mainly TAL/DCT markers.

Related to Figure 4.

Heatmaps showing DEGs (top 157 are shown; left) and their nephron segment-specific expression pattern (based on single-cell RNA-Seq data obtained from adult mouse kidneys (Park et al., 2018)) in whole kidney lysates from 4-months-old β-actinCreERT2^{+}TFAP2B$^{fl/fl}$ mice versus control littermates treated with TAM at 6 weeks of age (n=4/group). Scale and color coding represent Z-scores. Inactivation of AP-2β in the adult results in downregulation of mainly TAL/DCT genes (e.g. SFRP1, EGF, Pvalb; arrows), whereas compensatory upregulation of other genes was observed mainly in PTs and CDs.

DEGs were defined as having a greater >1.5-fold change and a FDR <0.05. DEGs are shown for which a nephron segment-specific expression pattern was found by single-cell RNA-Seq (Park et al., 2018). A complete list of DEGs and RNA-Seq data is provided in the Supplemental Material. Endo: endothelial, vascular and descending loop of Henle; Podo: podocytes; PT: proximal tubules; LOH: loop of Henle (including TAL); DCT: distal convoluted tubule; CD_PC: collecting duct, principle cells; CD_IC: intercalated cells; CD_Trans: collecting duct, transient cells; Novel1: novel cell type 1; Fib: fibroblast; Macro: macrophage; Neutro: neutrophil; B_lymph: B lymphocyte; T_lymph: T lymphocyte; NK: natural killer cell; Novel 2: novel cell type 2.

Figure S6: Gene expression analysis in kidneys of KCTD1$^{-/-}$ mice, Six2Cre^{+}KCTD1$^{fl/fl}$ mice and control littermates, as well as in mice with inducible inactivation of AP-2β in the adult. Related to Figures 3, 4 and 5.

A. Semiquantitative RT-PCR expression analysis of genes in 2-months-old KCTD1$^{-/-}$ and control littermates. Their nephron segment-specific expression location is shown, based on RNA-Seq data of specific nephron segments (Park et al., 2018; Ransick et al., 2019). Average values, p-values and fold-changes (KO versus WT) are shown. These data show a strong downregulation of key TAL/DCT genes, such as NCC, NKCC2, parvalbumin, and EGF. Among the most significantly upregulated genes are pendrin, CDKN1A and the macrophage markers CD68 and F4/80. Color scale indicates genes that are altered >5- or >3-fold. No difference in endothelial cell marker expression, CD31, was observed in these kidneys. Semiquantitative RT-PCR experiments were performed in triplicate with n>7 mice/group. Significantly differentially expressed genes are indicated in bold.

B. Table shows gene expression assessed (including their average values, p-values and fold-changes) in kidneys of P0, P3, P8, 2-months-old and 8-months-old Six2Cre^{+}KCTD1$^{fl/fl}$ mice and control littermates, as well as their nephron specific location based on RNA-Seq data of specific nephron segments (Park et al., 2018; Ransick et al., 2019). Among the most significantly downregulated genes in kidneys of 2-months-old Six2Cre^{+}KCTD1$^{fl/fl}$ mice are NKCC2, Pvalb, EGF, and NCC. Among the most significantly upregulated genes in kidneys of adult Six2Cre^{+}KCTD1$^{fl/fl}$ mice is pendrin. Notably, only few genes showed a moderately reduced expression at P3, while the expression of most genes was normal at P3 and only became reduced at P8 or thereafter. No significant gene expression changes with >3-fold alterations were observed at P3, while these changes were observed in the adult kidneys of Six2Cre^{+}KCTD1$^{fl/fl}$ mice. Values of significant differentially expressed genes are indicated in bold. Color scale indicates genes that

are altered >5- or >3-fold. Semiquantitative RT-PCR experiments were performed in triplicate with n>7 mice/group.

C. Semiquantitative RT-PCR of kidneys from mice with inducible inactivation of AP-2β in the adult, which were also used for RNA-Seq (4-months-old β-actinCreERT2^{+}TFAP2B$^{fl/fl}$ mice versus control littermates treated with TAM at 6 weeks of age). Semiquantitative RT-PCR experiments were performed in triplicate with n>7 mice/group.

D. Increased expression of CDKN1A, observed with premature senescence, is seen in kidneys of mice lacking KCTD1 already at 2-months of age (but not in P8 kidneys). Induced inactivation of KCTD1 at P9 in β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice leads to a strong increase in CDKN1A expression in kidneys of these mice as well (assessed at 2-months of age).

Graphs represent data as mean ± SEM. Semiquantitative RT-PCRs performed in triplicate. P-values are shown (two-tailed, unpaired *t*-test).

E. Top: Inactivation of KCTD1 at P0 results in DCT defects as seen in KCTD1$^{-/-}$ or Six2Cre^{+}KCTD1$^{fl/fl}$ mice with cystic dilatation of DCTs that show complete loss of EGF protein (green arrow), whereas strong EGF immunolabeling is observed in DCTs in TAM-treated control littermates (white arrow). Co-immunolabeling for NCC and EGF in 10-months-old kidneys of β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice that were treated with TAM at P0 are shown.

Bottom: Induced inactivation of AP-2β in adult mice (β-actinCreERT2^{+}TFAP2B$^{fl/fl}$ mice treated with TAM at 6 weeks of age and assessed at 4 months of age) results in dilated abnormal DCTs with reduced expression of NCC and complete loss of EGF (yellow arrows). PTs appear normal (red arrow). Scale bars, 50 μm.

Figure S7: Aged mice lacking KCTD1 develop chronic anemia and systolic hypertension concomitantly with deterioration of kidney function as a consequence of renal fibrosis. Related to Figures 6 and 7.

A. Lack of KCTD1 in the kidney results in extensive polycystic kidney disease with multiple cortical cysts, severe renal fibrosis (Trichrome staining, blue), and tubulointerstitial nephritis. Left image shows a kidney of a 7-months-old Six2Cre^{+}KCTD1$^{fl/fl}$ mouse. Scale bar, 1 mm.

Middle image (Trichrome staining): renal cyst with thin cystic epithelium (black arrow), surrounding fibrosis (blue color [green arrow]), and tubulointerstitial nephritis with an inflammatory infiltrate (right image, H&E) in a 7-months-old Six2Cre^{+}KCTD1$^{fl/fl}$ mouse. Scale bars, 100 μm.

B.-C. Only aged but not young adult Six2Cre^{+}KCTD1$^{fl/fl}$ mice and KCTD1$^{-/-}$ mice develop chronic anemia with reduced hemoglobin (HGB), hematocrit (HCT) and erythrocytes (RBC). No anemia

is detected in aged Six2Cre^{+}KCTD1$^{fl/fl}$ mice that are heterozygous for β-catenin (Six2Cre^{+}KCTD1$^{fl/fl}$β-catenin$^{WT/fl}$ mice). No anemia is observed in aged Aqp2Cre^{+}KCTD1$^{fl/fl}$ mice.

D. Aged mice lacking KCTD1 develop systolic hypertension concomitantly with deterioration of kidney function as a consequence of renal fibrosis. Hemodynamic pressure-volume (PV) loop experiments show that young male KCTD1$^{-/-}$ mice have normal heart function (in the setting of no anemia and no kidney fibrosis). Aged 10-months-old KCTD1$^{-/-}$ mice (with chronic anemia and kidney fibrosis) have systolic hypertension (increased AP max), increased ejection fraction and decreased left ventricular volume (n=5 male mice/group). Induced inactivation of KCTD1 at 6-weeks of age does not induce systolic hypertension or cardiac dysfunction 7 months after KCTD1 inactivation (n=5 male 8.5-months-old mice/group, treated with TAM at 6-weeks of age).

CO: cardiac output; SW: stroke work; SV: stroke volume; EF: ejection fraction; AP: aortic pressure; HR: heart rate; LV V: left ventricular volume; s: systole; d: diastole; dP/dTmax: maximum rate of pressure change in the left ventricle; dP/dTmin: minimum rate of pressure change in the left ventricle; LVP: left ventricular pressure.

E. Western blot of kidney lysates from 5-months-old SFRP1$^{-/-}$ and control mice shows no increase in renal active β-catenin levels in SFRP1$^{-/-}$ mice and normal protein levels of differentiation markers of TALs (NKCC2) and DCTs (Pvalb and NCC). Histology of the contralateral kidneys of these mice shows normal histology and no renal fibrosis. Scale bars, 50 μm.

Supplemental Files:
Related to Figure 4.

RNA-Seq data

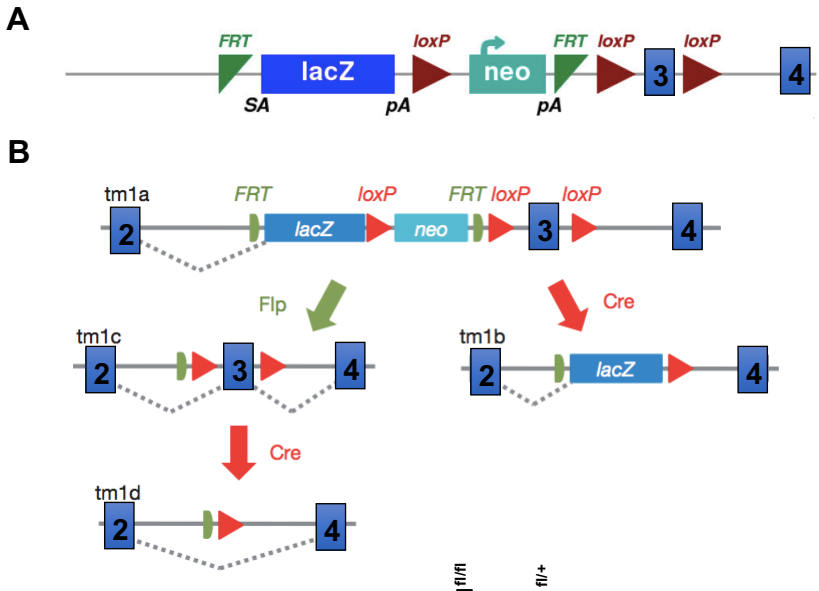
Supplemental Table S5: WT1_vs_KO1_DEG_genes_table.xls

DEGs in whole kidney lysates from 4-months-old β-actinCreERT2^{+}TFAP2B$^{fl/fl}$ mice versus control littermates treated with TAM at 6 weeks of age (n=4/group).

Supplemental Table S6: WT3_vs_KO3_DEG_genes_table.xls

DEGs in whole kidney lysates from P8 Six2Cre^{+}KCTD1$^{fl/fl}$ mice versus control littermates (n=4/group).

RNA-Seq data has been deposited to the GEO database: GSE126326 and GSE130864.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FRT
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FRT
loxP
loxP
lacZ
neo
2
3
4
Flp
Cre
tm1c
tm1b
Cre
tm1d
C
WT
KO
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D
WT
KO
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36B4 cDNA
E
Cre/DAPI
Six2Cre+/WT
Cre/AQP2 (white)
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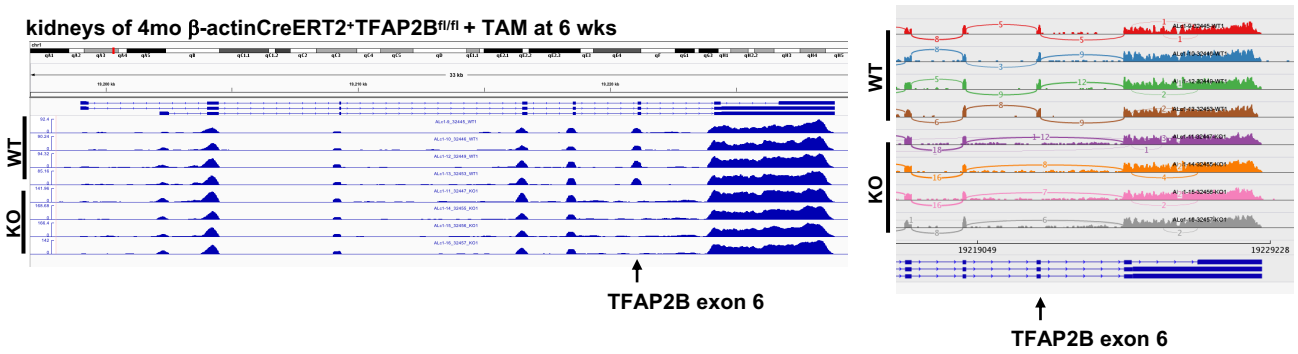
Aqp2Cre+/WT
Cre/phalloidin (white)/DAPI
PvalbCre+/WT

Figure S1

A



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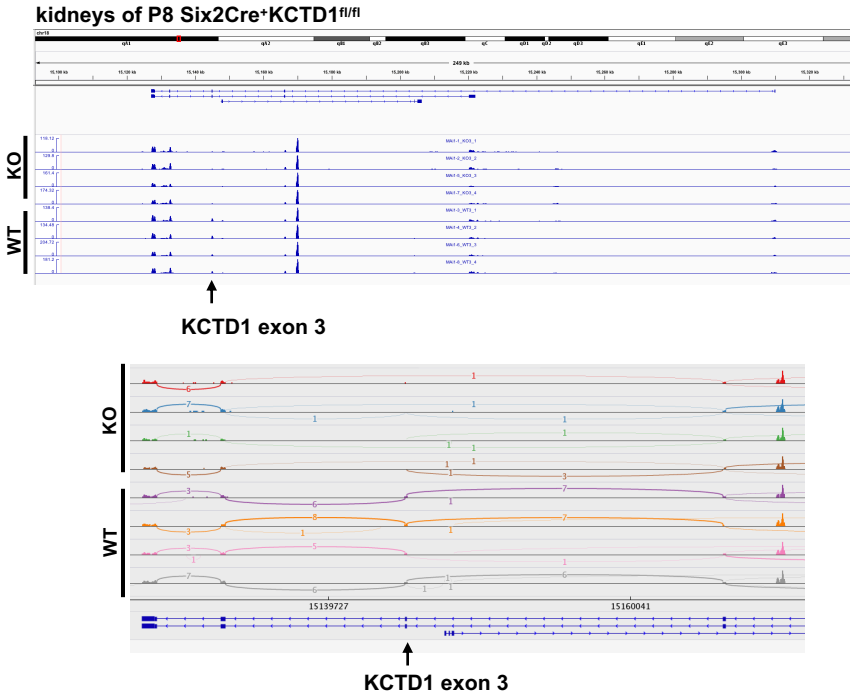

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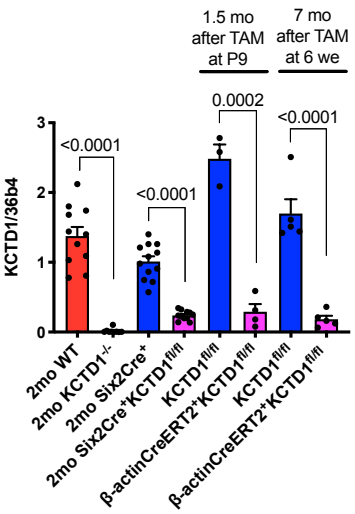
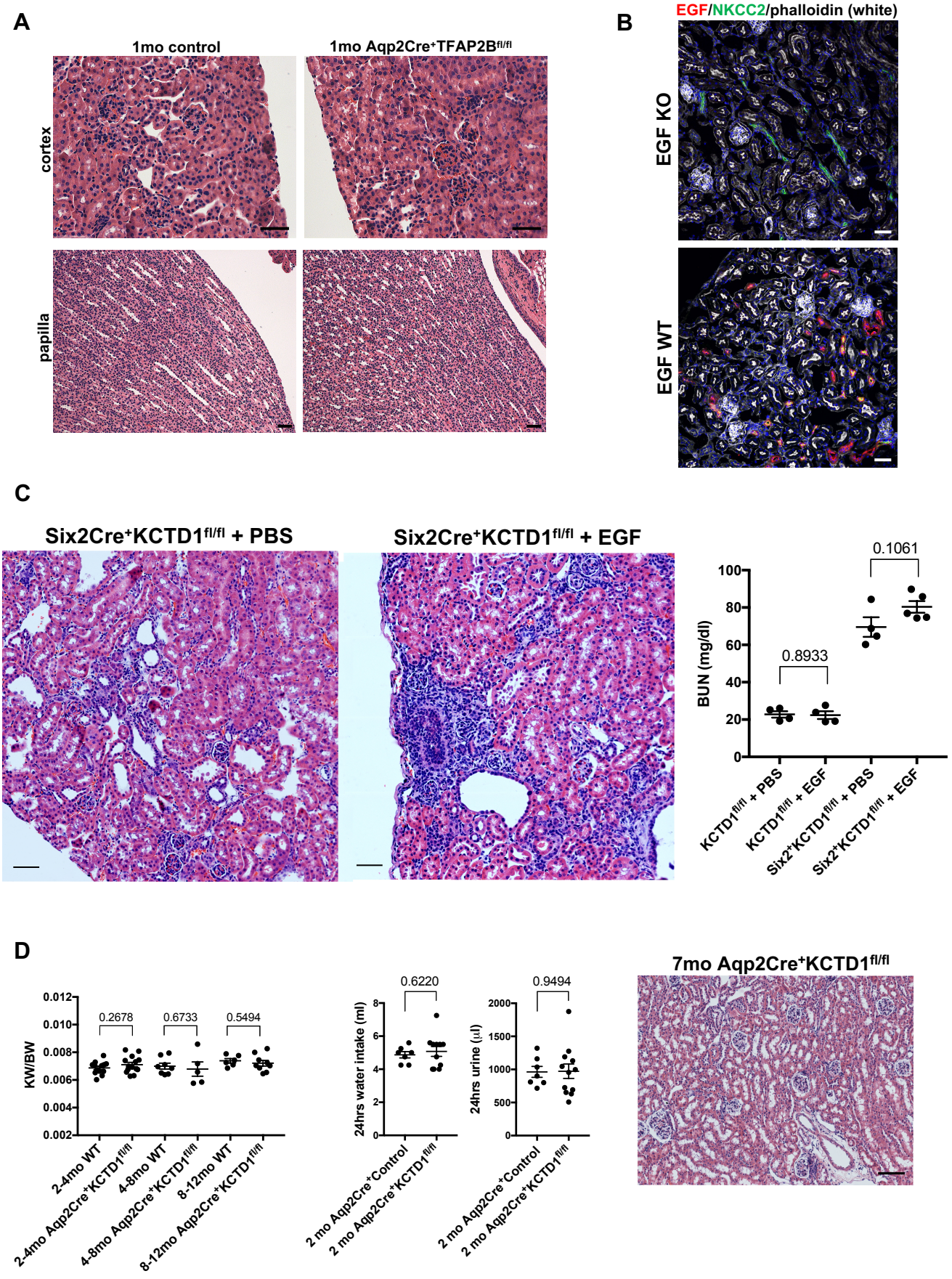


Figure S2


A
1mo control
1mo Aqp2Cre+TFAP2Bfl/fl
cortex
papilla
B
EGF/NKCC2/phalloidin (white)
EGF KO
EGF WT
C
Six2Cre+KCTD1fl/fl + PBS
Six2Cre+KCTD1fl/fl + EGF
BUN (mg/dl)
0.8933
0.1061
KCTD1fl/fl + PBS
KCTD1fl/fl + EGF
Six2+KCTD1fl/fl + PBS
Six2+KCTD1fl/fl + EGF
D
KW/BW
0.2678
0.6733
0.5494
2-4mo WT
2-4mo Aqp2Cre+KCTD1fl/fl
4-8mo WT
4-8mo Aqp2Cre+KCTD1fl/fl
8-12mo WT
8-12mo Aqp2Cre+KCTD1fl/fl
24hrs water intake (ml)
0.6220
2 mo Aqp2Cre+Control
2 mo Aqp2Cre+KCTD1fl/fl
24hrs urine (µl)
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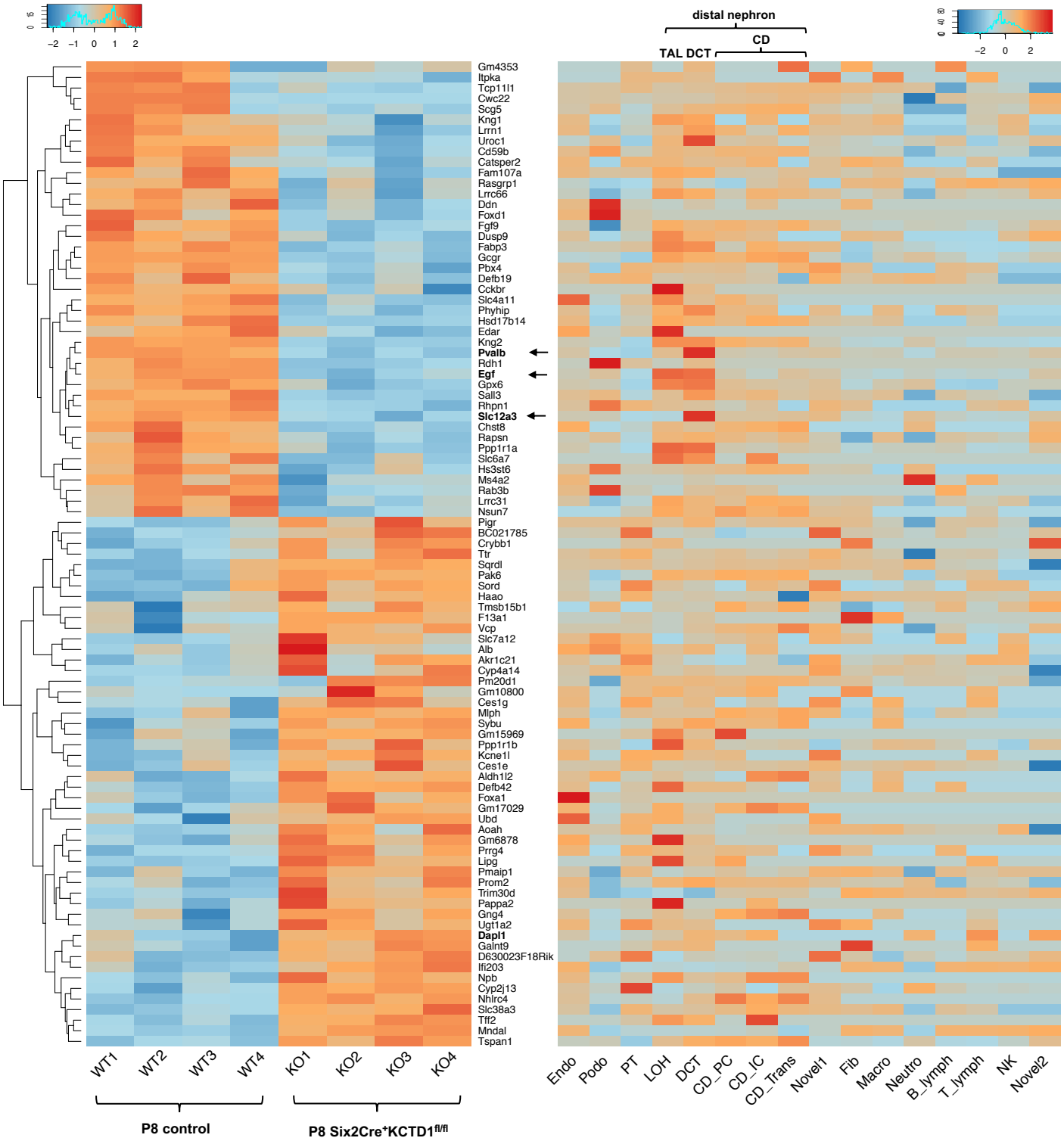
2 mo Aqp2Cre+KCTD1fl/fl
7mo Aqp2Cre+KCTD1fl/fl

Figure S3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Sybu
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Tff2
Mndal
Tspan1
WT1
WT2
WT3
WT4
KO1
KO2
KO3
KO4
P8 control
P8 Six2Cre+KCTD1fl/fl
Endo
Podo
PT
LOH
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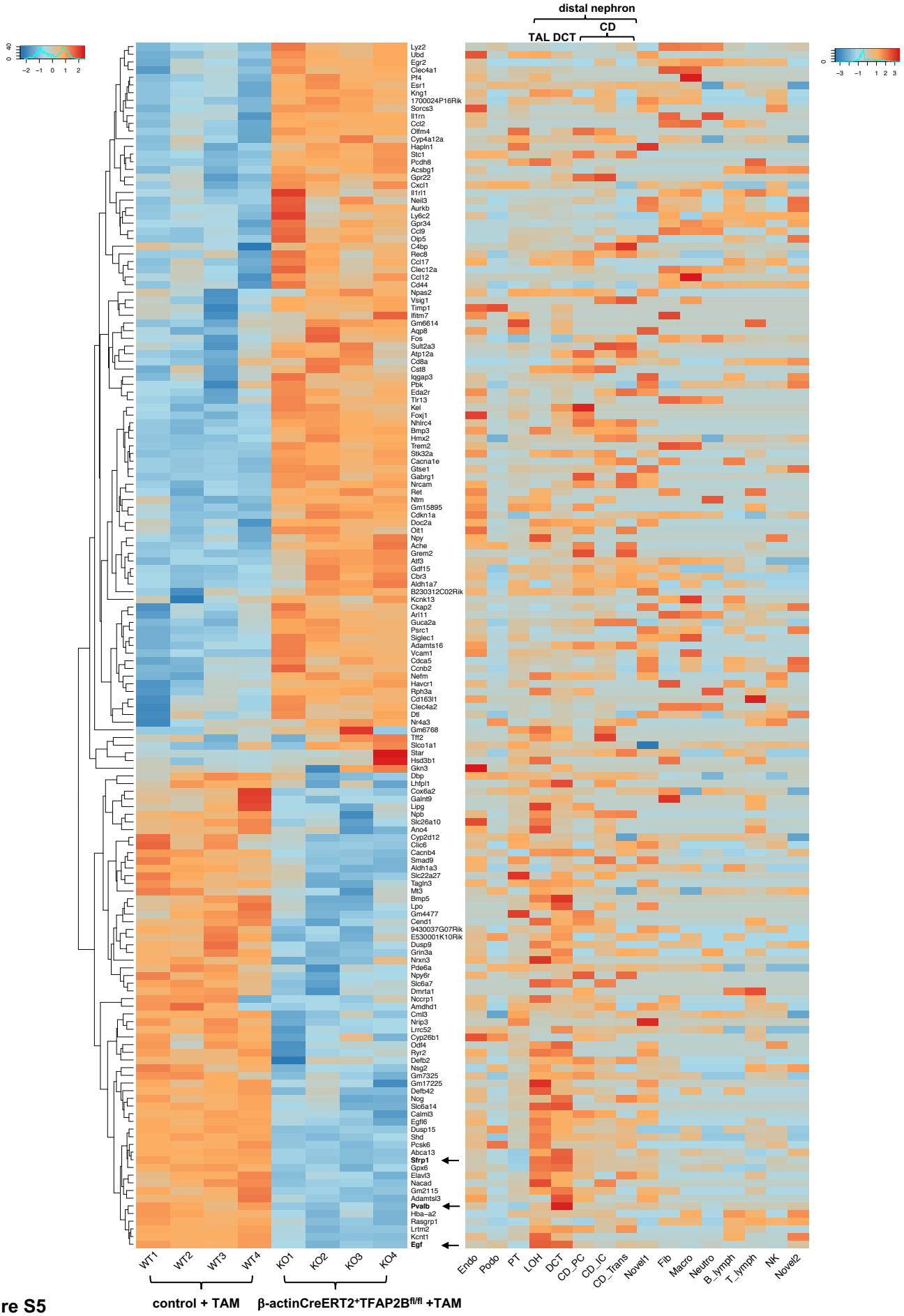
Figure S4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Guca2a
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Siglec1
Adamts16
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Ccnb2
Nefm
Havcr1
Rph3a
Cd163l1
Clec4a2
Dtl
Nr4a3
Gm6768
Tff2
Slco1a1
Star
Hsd3b1
Gkn3
Dbp
Lhfpl1
Cox6a2
Galnt9
Lipg
Npb
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Ano4
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Kcnt1
Egf
WT1
WT2
WT3
WT4
KO1
KO2
KO3
KO4
Endo
Podo
PT
LOH
DCT
CD_PC
CD_IC
CD_Trans
Novel1
Fib
Macro
Neutro
B_lymph
T_lymph
NK
Novel2
control + TAM
β-actinCreERT2+TFAP2Bfl/fl +TAM

Figure S5

A

gene	nephron location	2mo WT	2mo KCTD1$^{-/-}$	P-value	ratio KO/WT
Pvalb	DCT1	1.603	0.006	4.158E-09	0.00
Egf	TAL/DCT	1.500	0.012	3.653E-12	0.01
NCC (Slc12a3)	DCT	0.313	0.042	1.072E-09	0.13
NKCC2 (Slc12a1)	TAL	1.140	0.152	1.23E-09	0.13
Pendrin (Slc26a4)	CT/CD	0.915	6.332	3.138E-05	6.92
Cdkn1a		1.772	10.007	0.0110815	5.65
Cd68	macrophages	0.582	3.181	1.911E-05	5.46
F4/80	macrophages	0.441	1.458	0.0001548	3.31
Scnn1b	CT/CD	0.475	1.552	1.438E-10	3.27
Scnn1g	CT/CD	0.667	1.673	5.911E-07	2.51
EnAc (Scnn1a)	CT/CD>TAL, DCT	1.516	2.890	0.027921	1.91
Cd31	endothelial cells	1.611	1.780	0.643596	1.10
>3-fold	<3-fold	<5-fold	>5-fold		

B

gene	nephron segment	P0 KCTD1$^{fl/fl}$	P0 Six2Cre$^+$KCTD1$^{fl/fl}$	ttest	ratio KO/WT	P3 KCTD1$^{fl/fl}$	P3 Six2Cre$^+$KCTD1$^{fl/fl}$	ttest	ratio KO/WT
NCC (Slc12a3)	DCT	0.043	0.021	0.000139336	0.480	0.146	0.080	0.00028456	0.551
Pvalb	DCT1	0.055	0.027	8.90475E-05	0.496	0.043	0.044	0.974793823	1.012
NKCC2 (Slc12a1)	TAL	0.182	0.173	0.514889606	0.947	0.333	0.290	0.138160845	0.872
EGF	TAL/DCT	0.001	0.000	0.120555892	0.350	0.005	0.004	0.454958508	0.832
Pendrin	CT/CD	0.002	0.002	0.998278425	1.001	0.029	0.018	0.014493508	0.601
Cd68		0.552	0.471	0.210579757	0.854	0.479	0.639	0.292416194	1.334
Cd31		0.516	0.507	0.893371262	0.984	1.151	1.025	0.143070638	0.890
Cdkn1a		4.423	4.283	0.870229969	0.968	3.772	3.267	0.005885496	0.866
Scnn1b	CT/CD>DCT								
Scnn1g	CT/CD					ND	ND		
ENaC (Scnn1a)	TAL/DCT/CT/CD	0.070	0.074	0.769694649	1.063	0.189	0.144	0.002731436	0.763
>3-fold	<3-fold	<5-fold	>5-fold						

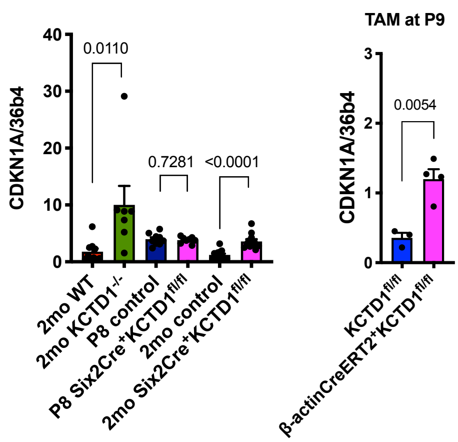
gene	P8 KCTD1$^{fl/fl}$	P8 Six2Cre$^+$KCTD1$^{fl/fl}$	ttest	ratio KO/WT	2mo KCTD1$^{fl/fl}$	2mo Six2Cre$^+$KCTD1$^{fl/fl}$	ttest	ratio KO/WT
NCC (Slc12a3)	0.648	0.367	1.11201E-05	0.566	3.555	0.807	4.4616E-05	0.227
Pvalb	0.546	0.121	2.52629E-07	0.222	11.440	0.208	0.00529527	0.018
NKCC2 (Slc12a1)	0.575	0.457	0.001016765	0.795	3.857	0.723	3.6104E-05	0.188
EGF	0.062	0.013	1.04929E-08	0.216	1.455	0.099	2.5196E-12	0.068
Pendrin	0.026	0.032	0.117223079	1.241	0.813	3.465	4.629E-05	4.262
Cd68	0.876	1.125	0.063394231	1.284	1.830	3.076	0.02154393	1.681
Cd31	1.720	2.047	0.152114792	1.190	1.369	1.249	0.42283777	0.912
Cdkn1a	3.970	3.825	0.726754299	0.963	1.228	3.596	3.9889E-05	2.928
Scnn1b	0.723	0.826	0.459065551	1.143	0.693	2.626	1.2905E-14	3.787
Scnn1g	0.570	0.163	0.039803149	0.286	0.985	1.952	8.3231E-05	1.981
ENaC (Scnn1a)	0.289	0.128	0.002993319	0.443	3.586	5.841	0.13068276	1.629

gene	8mo KCTD1$^{fl/fl}$	8mo Six2Cre$^+$KCTD1$^{fl/fl}$	ttest	ratio KO/WT
NCC (Slc12a3)	1.560	0.085	5.6423E-05	0.055
Pvalb	1.825	0.068	0.00459389	0.037
NKCC2 (Slc12a1)	1.223	0.127	9.2617E-07	0.104
EGF	0.879	0.085	0.00028229	0.097

C

gene	WT + TAM	β-actinCreERT2$^+$ TFAP2B$^{fl/fl}$ + TAM	P-value	ratio KO/WT
Pvalb	4.700	0.061	0.0003	0.01
Sfrp1	1.660	0.233	<0.0001	0.14
Egf	2.010	0.546	0.0003	0.27
NCC (Slc12a3)	1.760	0.695	0.0008	0.39
Cdkn1a	1.410	3.760	0.0043	2.67
pendrin	0.546	1.560	<0.0001	2.86
>3-fold	<3-fold	<5-fold	>5-fold	

D



E

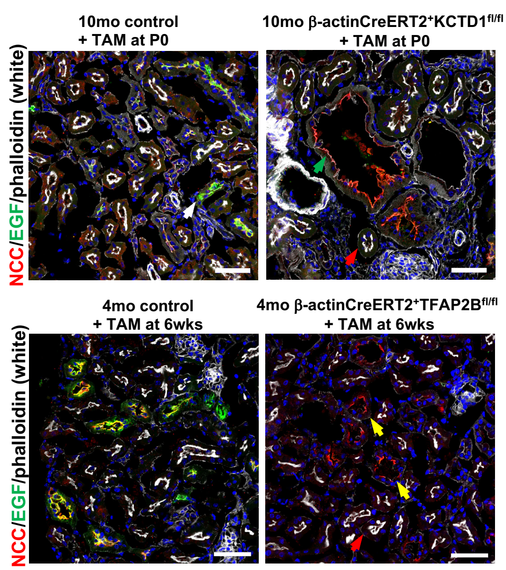
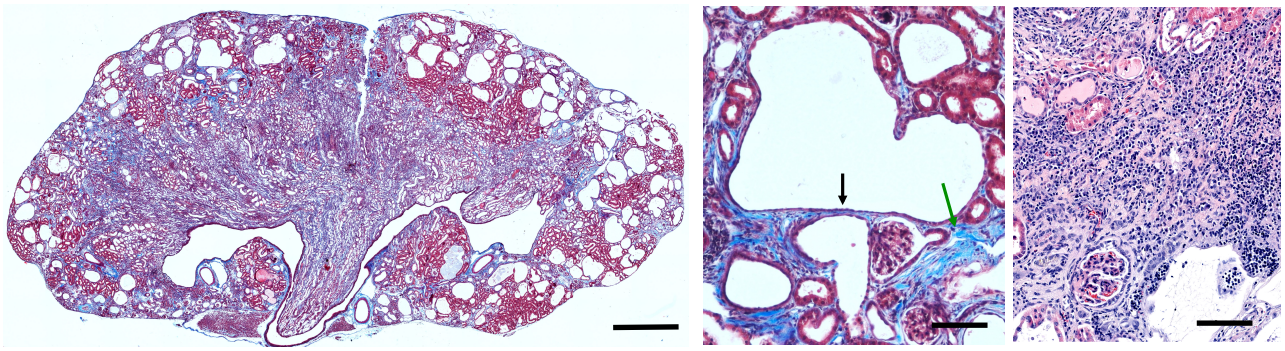


Figure S6

A



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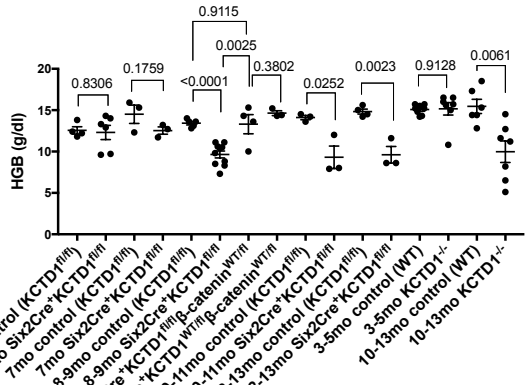

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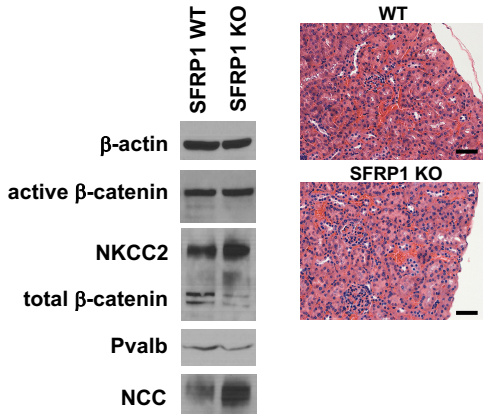

C

		WBC	Lym	mono	gran	lym%	mono%	Gran%	HCT	MCV	RDW%	HGB	MCHC	MCH	RBC	PLT	MPV
control (KCTD1$^{fl/fl}$)	2mo (n=4)	2.23	1.63	0.18	0.43	72.58	6.33	21.10	36.73	47.50	16.23	12.55	34.15	16.25	7.74	166.25	6.68
Six2Cre^{+}KCTD1$^{fl/fl}$	2mo (n=6)	1.77	1.30	0.12	0.35	74.85	5.23	19.92	35.12	43.20	16.87	12.30	35.07	16.28	7.57	101.00	6.30
ttest		*0.563352*	*0.524824*	*0.4329194*	*0.7738652*	*0.5985899*	*0.2897256*	*0.7923917*	*0.6449934*	*0.3285644*	*0.0007235*	*0.830630273*	*0.2572861*	*0.9380631*	*0.8139943*	*0.3494862*	*0.0607471*
control (KCTD1$^{fl/fl}$)	7mo (n=3)	5.83	4.20	0.40	1.23	74.37	5.40	20.23	42.27	45.70	17.63	14.50	34.23	15.67	9.23	771.33	7.23
Six2Cre^{+}KCTD1$^{fl/fl}$	7mo (n=3)	10.40	7.80	0.60	2.00	75.00	5.53	19.47	36.67	44.23	18.27	12.53	34.33	15.20	8.33	754.00	7.33
ttest		*0.1828081*	*0.1328802*	*0.4353309*	*0.3652066*	*0.8615962*	*0.8985625*	*0.7939594*	*0.1339784*	*0.4568676*	*0.5530234*	*0.175922156*	*0.9192688*	*0.6528044*	*0.2661975*	*0.8407025*	*0.8581949*
control (KCTD1$^{fl/fl}$)	8-9mo (n=5)	7.76	5.88	0.54	1.34	76.68	5.94	17.38	41.76	48.54	18.00	13.42	32.24	15.62	8.60	624.80	7.40
Six2Cre^{+}KCTD1$^{fl/fl}$	8-9mo (n=11)	6.20	4.32	0.50	1.38	68.23	7.11	22.77	29.44	43.13	19.77	9.64	34.25	14.74	6.84	549.73	6.63
ttest		*0.4515727*	*0.2889018*	*0.838769*	*0.9254347*	*0.0177372*	*0.1235922*	*0.2355074*	*0.0002133*	*0.0017305*	*0.0640076*	*3.83922E-05*	*0.0150974*	*0.0602317*	*0.0089181*	*0.5306272*	*0.0257896*
control (KCTD1$^{fl/fl}$)	10-11mo (n=3)	9.00	7.33	0.53	1.13	82.07	5.00	12.93	42.07	46.40	17.00	14.10	33.50	15.53	9.06	940.67	6.17
Six2Cre^{+}KCTD1$^{fl/fl}$	10-11mo (n=3)	10.50	7.33	0.67	2.50	69.33	6.53	24.13	26.60	42.33	19.93	9.30	35.23	14.83	6.23	774.00	6.00
ttest		*0.454940*	*1.000000*	*0.301970*	*0.062242*	*0.027126*	*0.166544*	*0.003864*	*0.031596*	*0.150225*	*0.006021*	*0.025159*	*0.108549*	*0.108549*	*0.029384*	*0.004334*	*0.439512*
control (KCTD1$^{fl/fl}$)	12-13mo (n=4)	5.40	3.68	0.45	1.28	66.90	7.45	25.65	43.40	47.05	17.55	14.83	34.23	16.08	9.25	541.25	7.50
Six2Cre^{+}KCTD1$^{fl/fl}$	12-13mo (n=3)	7.10	5.37	0.43	1.30	74.90	5.43	19.67	27.20	41.63	18.80	9.60	35.47	14.77	6.52	412.67	6.20
ttest		*0.305698*	*0.2272412*	*0.8670311*	*0.9171303*	*0.1096981*	*0.0692704*	*0.1519394*	*0.0015375*	*0.0965897*	*0.1089736*	*0.002266533*	*0.0405644*	*0.2612907*	*0.0055728*	*0.4476686*	*0.0080513*
Six2Cre^{+}KCTD1$^{fl/fl}$β-catenin$^{wt/fl}$	8-9mo (n=4)	11.08	8.40	0.68	2.00	76.13	5.40	18.48	39.65	43.75	18.10	13.30	33.63	14.68	9.07	660.25	6.23
Six2Cre^{+}KCTD1$^{wt/fl}$β-catenin$^{wt/fl}$	8-9mo (n=3)	8.40	6.07	0.57	1.77	71.73	6.37	21.90	43.77	46.90	17.20	14.63	33.47	15.70	9.33	636.00	5.83
control (KCTD1$^{fl/fl}$)	8-9mo (n=5)	7.76	5.88	0.54	1.34	76.68	5.94	17.38	41.76	48.54	18.00	13.42	32.24	15.62	8.60	624.80	7.40
Six2Cre^{+}KCTD1$^{fl/fl}$	8-9mo (n=11)	6.20	4.32	0.50	1.38	68.23	7.11	22.77	29.44	43.13	19.77	9.64	34.25	14.74	6.84	549.73	6.63
ttest KCTD1$^{fl/fl}$ vs Six2Cre^{+}KCTD1$^{fl/fl}$		*0.4515727*	*0.2889018*	*0.838769*	*0.9254347*	*0.0177372*	*0.1235922*	*0.2355074*	*0.0002133*	*0.0017305*	*0.0640076*	*3.83922E-05*	*0.0150974*	*0.0602317*	*0.0089181*	*0.5306272*	*0.0257896*
ttest Six2Cre^{+}KCTD1$^{fl/fl}$ vs Six2Cre^{+}KCTD1$^{fl/fl}$β-catenin$^{wt/fl}$		*0.0706106*	*0.0383793*	*0.4657009*	*0.2478912*	*0.0429398*	*0.0587338*	*0.3926732*	*0.0086816*	*0.6485322*	*0.1077799*	*0.002523786*	*0.4729944*	*0.8887268*	*0.0135506*	*0.4189154*	*0.2485945*
control (WT)	3-5mo (n=8)	8.25	6.25	0.46	1.54	77.04	4.65	18.31	47.20	47.61	17.18	15.08	31.91	15.18	9.91	545.13	5.94
KCTD1$^{-/-}$	3-5mo (n=7)	7.67	5.87	0.43	1.37	75.37	5.44	19.19	46.63	45.84	17.64	15.16	32.54	14.91	10.14	498.00	5.86
ttest		*0.6403772*	*0.693249*	*0.6462194*	*0.6502136*	*0.6435815*	*0.1762757*	*0.7788642*	*0.814371*	*0.0097518*	*0.0227224*	*0.912776665*	*0.0278235*	*0.0841963*	*0.6087445*	*0.5064643*	*0.4580928*
control (WT)	10-13mo (n=6)	2.98	1.90	0.18	0.90	63.17	4.43	32.40	48.55	45.67	17.12	15.45	31.83	14.55	10.64	587.17	5.58
KCTD1$^{-/-}$	10-13mo (n=7)	3.77	2.53	0.27	0.97	60.56	7.44	32.00	30.06	42.39	19.19	9.97	33.47	14.13	7.06	388.83	5.85
ttest		*0.6641325*	*0.6452355*	*0.4743504*	*0.8787271*	*0.6905231*	*0.0893166*	*0.9444935*	*0.0038704*	*0.0022299*	*0.0064497*	*0.006072144*	*0.0420353*	*0.0564362*	*0.0102034*	*0.1371694*	*0.0530091*
control (WT)	10-12mo (n=4)	4.55	2.98	0.35	1.23	64.48	6.23	29.30	42.95	43.90	18.15	14.83	34.58	15.18	9.78	656.50	6.00
Aqp2Cre^{+}KCTD1$^{fl/fl}$	10-12mo (n=8)	5.36	3.56	0.43	1.38	65.41	6.93	27.66	44.84	44.85	17.89	15.03	33.55	15.04	9.99	856.63	6.21
ttest		*0.2668243*	*0.5436389*	*0.2135568*	*0.7741534*	*0.9391732*	*0.5043487*	*0.891621*	*0.1550915*	*0.159176*	*0.5289276*	*0.693992784*	*0.1885215*	*0.5853314*	*0.3606232*	*0.2871371*	*0.507488*

D

		MAX	MIN-	EDM-	MAX-	MIN-	SV	CO	EF	SW	DP/DT(mmHg/ Sec)		AP(mmHg)	
	HR	LVP	LVP	LVP	LV_V	LV_V	(ul)	ml/min	(%)		MAX	MIN	MAX	MIN
WT 4 mo	460.8	107	1.2	7.2	41.5	24.4	14.3	6.6	34.7	1542.5	9871	-7966	105.4	71.2
KCTD1$^{-/-}$ 4 mo	445.4	102.6	1.4	7.4	42.1	23.3	15.2	7	36.7	1650.3	10197	-7644	102.8	68.6
ttest	*0.9667*	*0.3951*	*0.9803*	*0.3739*	*0.2118*	*0.0445*	*0.8224*	*0.7206*	*0.5751*	*0.8373*	*0.0846*	*0.1409*	*0.9293*	*0.9988*
WT 10 mo	425.1	107.9	1.1	6.6	49	31.1	14.6	6.2	30.6	1556	7653	-6879	106.4	69.6
KCTD1$^{-/-}$ 10 mo	443.7	114.9	1.9	7.8	40.9	23.2	15.7	7	38.8	1616.7	10750	-8176	115.7	73.2
ttest	*0.5174*	*0.0684*	*0.3025*	*0.3959*	*0.0058*	*0.0015*	*0.2656*	*0.1227*	*0.001*	*0.5434*	*0.0011*	*0.0588*	*0.0259*	*0.1467*
KCTD1$^{fl/fl}$ + TAM	477.99	107.64	-0.33	4.63	45.79	27.25	15.11	7.46	33.04	1692.20	9592.40	-8264.20	105.50	70.90
β-actinCreERT2^{+}KCTD1$^{fl/fl}$ + TAM	529.96	101.40	-0.74	4.28	45.40	27.83	12.51	6.58	27.95	1605.74	11108.67	-6071.17	100.73	67.87
ttest	*0.2130*	*0.0519*	*0.3585*	*0.7991*	*0.9208*	*0.8228*	*0.3394*	*0.5732*	*0.2473*	*0.7530*	*0.2680*	*0.5515*	*0.1013*	*0.1504*

Figure S7

		Na	K	Cl	Na/K ratio	BUN	Creatinine	Crea/BW
control (KCTD1$^{fl/fl}$)	2-3mo (n=8)	151.63	8.43	117.25	18.63	33.06	0.21	0.0081
Six2Cre^{+}KCTD1$^{fl/fl}$	2-3mo (n=12)	148.58	7.10	107.67	21.83	72.90	0.31	0.0127
ttest		*0.0453221*	*0.0877599*	*0.000111076*	*0.1156969*	*2.08087E-07*	*0.010707*	*0.000681*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
control (KCTD1$^{fl/fl}$)	4mo (n=5)	152.00	8.98	116.20	17.20	22.00	0.22	0.0070
Six2Cre^{+}KCTD1$^{fl/fl}$	4mo (n=5)	151.80	8.10	110.00	19.00	90.06	0.60	0.0206
ttest		*0.9409215*	*0.2934006*	*0.052640875*	*0.3166413*	*7.13506E-05*	*0.0074112*	*0.0068015*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
control (KCTD1$^{fl/fl}$)	8-9mo (n=6)	152.00	8.92	116.00	17.33	36.70	0.28	0.0071
Six2Cre^{+}KCTD1$^{fl/fl}$	8-9mo (n=6)	150.17	7.40	105.17	20.67	153.20	0.93	0.0280
ttest		*0.3824761*	*0.0176106*	*0.000302294*	*0.0357328*	*6.37759E-06*	*0.0001171*	*9.955E-05*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
control (WT)	3-4mo (n=5)	148.00	6.00	115.75	26.25	28.53	0.28	0.0138
KCTD1$^{-/-}$	3-4mo (n=5)	145.20	4.54	104.00	33.20	94.83	0.60	0.0406
ttest		*0.1996187*	*0.1563509*	*0.003296552*	*0.1678807*	*0.007628559*	*0.0332544*	*0.0088653*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
control (WT)	10-12mo (n=7)	150.43	6.09	121.86	25.00	34.10	0.20	0.0083
KCTD1$^{-/-}$	10-12mo (n=5)	152.60	6.04	108.60	25.80	249.78	0.90	0.0534
ttest (WT vs KCTD1$^{-/-}$)		*0.246881*	*0.9345713*	*0.001254959*	*0.7355975*	*1.85353E-08*	*2.278E-05*	*1.95E-05*
KCTD1$^{-/+}$	10-12mo (n=5)	158.20	8.42	116.60	18.80	23.58	0.22	*0.0081835*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
control (KCTD1$^{fl/fl}$)	2mo (n=4)	152.25	8.65	116.00	18.00	25.80	0.33	0.0138
Aqp2Cre^{+}KCTD1$^{fl/fl}$	2mo (n=5)	148.20	8.42	111.00	17.80	27.58	0.28	0.0157
ttest		*0.108707*	*0.8105493*	*0.104689121*	*0.919578*	*0.699826575*	*0.5837275*	*0.6125085*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
control (KCTD1$^{fl/fl}$)	10mo (n=3)	152.00	7.87	112.33	19.33	20.83	0.20	0.0069
Aqp2Cre^{+}KCTD1$^{fl/fl}$	10mo (n=6)	153.50	7.55	112.33	20.50	26.48	0.27	0.0100
ttest		*0.3358177*	*0.5444907*	*1*	*0.4216929*	*0.125841219*	*0.214125*	*0.1244395*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
KCTD1$^{fl/fl}$ + TAM	2mo after TAM at P9 (n=3)	152.67	7.23	109.67	21.00	30.73	0.40	0.0189
β-actinCreERT2^{+}KCTD1$^{fl/fl}$ + TAM	2mo after TAM at P9 (n=4)	148.25	7.73	104.00	19.50	68.38	0.55	0.0292
ttest		*0.0051133*	*0.4544826*	*0.012124815*	*0.4018288*	*0.000790034*	*0.1578388*	*0.0318029*
		Na	**K**	**Cl**	**Na/K ratio**	**BUN**	**Creatinine**	**Crea/BW**
KCTD1$^{fl/fl}$ + TAM	7mo after TAM at 6we (n=4)	147.75	7.03	117.75	20.75	28.30	0.20	0.0079
β-actinCreERT2^{+}KCTD1$^{fl/fl}$ + TAM	7mo after TAM at 6we (n=4)	146.50	6.60	115.00	22.25	53.20	0.23	0.0089
ttest		*0.4713606*	*0.2322037*	*0.115077877*	*0.2838799*	*3.13894E-05*	*0.3559177*	*0.4846004*

		Na	K	Cl	Na/K ratio	BUN	Creatinine	Crea/BW
TFAP2B$^{fl/fl}$ + TAM	3.5mo after TAM at 6we (n=5)	150.17	8.82	112.50	17.67	29.32	0.22	0.0083
β-actinCreERT2^{+}TFAP2B$^{fl/fl}$ + TAM	3.5mo after TAM at 6we (n=6)	150.00	7.96	108.40	19.20	66.76	0.44	0.0181
ttest		*0.8720572*	*0.3828292*	*0.015640714*	*0.4537482*	*1.57034E-05*	*0.0742412*	*0.0254875*

		Na	K	Cl	Na/K ratio	BUN	Creatinine	Mg	Crea/BW
control (KCTD1$^{fl/fl}$)	8-9mo (n=6)	152.00	8.92	116.00	17.33	36.70	0.28	2.90	0.0071
Six2Cre^{+}KCTD1$^{fl/fl}$	8-9mo (n=9)	150.00	7.03	104.00	21.29	167.08	0.99	4.36	0.0306
Six2Cre^{+}KCTD1$^{fl/fl}$β-catenin$^{WT/fl}$	8-9mo (n=9)	150.22	7.31	102.56	21.00	121.49	0.64	3.41	*0.0201*
ttest Six2Cre^{+}KCTD1$^{fl/fl}$ vs Six2Cre^{+}KCTD1$^{fl/fl}$β-catenin$^{WT/fl}$		*0.7710039*	*0.893792*	*0.603319666*	*0.8724329*	*0.003699116*	*0.0015857*	*0.0302764*	*0.0349037*
ttest control (KCTD1$^{fl/fl}$) vs Six2Cre^{+}KCTD1$^{fl/fl}$β-catenin$^{WT/fl}$		*0.3780103*	*0.0158484*	*2.06093E-06*	*0.0375476*	*7.20917E-06*	*0.0006754*	*0.2472441*	*0.0020547*
ttest control (KCTD1$^{fl/fl}$) vs Six2Cre^{+}KCTD1$^{fl/fl}$		*0.3824761*	*0.0176106*	*0.000302294*	*0.0357328*	*6.37759E-06*	*0.0001171*	*0.0078625*	*0.0001137*

Table S1: Serum chemistries in experimental mouse groups of different ages.
Related to Figures 2, 3, 5, 6 and 7.

Six2Cre^{+}KCTD1$^{fl/fl}$ mice, KCTD1$^{-/-}$ mice and mice with induced inactivation of KCTD1 at P9 (β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice + TAM) show already in young mice hypochloremia and elevated BUN and serum creatinine/BW levels. With progressing age BUN and serum creatinine levels increase further. Aqp2Cre^{+}KCTD1$^{fl/fl}$ mice (lacking KCTD1 in the CTs/CDs) show no abnormalities, consistent with normal kidney histology and function. Induced inactivation of KCTD1 at P9 (β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice + TAM) and evaluation at 2-months of age phenocopies findings in age-matched Six2Cre^{+}KCTD1$^{fl/fl}$ mice. Induced inactivation of KCTD1 in 6-week-old mice (β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice + TAM) and evaluation 7 months later shows a moderate increase in BUN levels. Inactivation of AP-2β in adult mice results in hypochloremia and increased BUN levels.

BUN in mg/dl, chloride in mEq/l, creatinine in mg/dl, potassium in mEq/l, sodium in mEq/l.

Supplemental Item: Table S1

Supplemental Item: Table S2

		Na	K	Cl	Creatinine	Osmolality	Urea	Na/Crea	K/Crea	Cl/Crea	Urea/Crea	FeNa	FeK	FeCl	Crea Cl	24hr Urine	Urea mass$_U$ (gm)	Cl mass$_U$	Na mass$_U$	K mass$_U$
control (KCTD1$^{fl/fl}$)	4mo (n=5)	185.60	424.50	232.20	76.00	3364.00	5471.40	2.43	5.62	3.07	72.13	0.35	13.80	0.59	296.33	1220.00	65.58	9.91	5.15	20.13
Six2Cre^{+}KCTD1$^{fl/fl}$	4mo (n=5)	32.83	73.33	36.17	12.47	626.83	894.17	2.74	5.97	2.94	72.47	0.99	43.49	1.58	148.79	8154.40	78.19	11.21	6.45	24.64
ttest		*3.04E-06*	*9.69E-06*	*1.99E-06*	*6.25E-07*	*5.92E-06*	*8.06E-06*	*0.238585403*	*0.530352688*	*0.553477168*	*0.950730501*	*0.003426104*	*0.010169424*	*0.010664775*	*0.015143669*	*1.28E-05*	*0.173459947*	*0.34562822*	*0.134506766*	*0.246984115*

		Na	K	Cl	Creatinine	Osmolality	Urea	Na/Crea	K/Crea	Cl/Crea	Urea/Crea	FeNa	FeK	FeCl	Crea Cl	24hr Urine	Urea mass$_U$	Cl mass$_U$	Na mass$_U$	K mass$_U$
control (KCTD1$^{fl/fl}$)	8-9mo (n=6)	104.17	243.58	138.00	51.57	1942.67	3112.83	2.01	4.80	2.65	60.88	0.35	15.45	0.60	310.48	2362.00	68.62	10.75	5.16	20.90
Six2Cre^{+}KCTD1$^{fl/fl}$	8-9mo (n=6)	33.33	61.05	30.50	10.92	514.33	755.00	3.10	5.65	2.82	69.79	1.96	71.76	2.51	99.08	11498.33	85.31	12.19	8.64	26.89
ttest		*0.001038799*	*0.000131485*	*0.000335459*	*4.60E-05*	*6.69E-05*	*5.96E-05*	*0.008465796*	*0.077824284*	*0.605632088*	*0.076180487*	*0.000255486*	*3.64E-05*	*0.000103568*	*0.007311058*	*1.82E-06*	*0.141050315*	*0.514656573*	*0.00293042*	*0.07117485*

		Na	K	Cl	Creatinine	Osmolality	Urea	Na/Crea	K/Crea	Cl/Crea	Urea/Crea	FeNa	FeK	FeCl	Crea Cl	24hr Urine	Urea mass$_U$	Cl mass$_U$	Na mass$_U$	K mass$_U$
control (WT)	3-4mo (n=5)	94.50	398.03	179.75	59.25	2447.38	3388.25	1.60	6.72	3.06	59.11	0.34	38.01	0.85	120.85	574.20	23.62	4.46	1.51	10.94
KCTD1$^{-/-}$	3-4mo (n=5)	40.20	92.66	46.60	14.40	652.50	1068.20	2.80	6.46	3.24	74.83	1.08	92.47	1.79	85.94	4850.20	51.68	8.05	4.47	17.57
ttest		*3.43E-05*	*7.28E-06*	*2.07E-06*	*5.49E-06*	*4.57E-06*	*0.000212616*	*0.00300881*	*0.489307334*	*0.608234657*	*0.160598927*	*0.033975905*	*0.050486412*	*0.116638153*	*0.321989207*	*0.000199369*	*0.016172244*	*0.081344462*	*0.006977808*	*0.15614739*

		Na	K	Cl	Creatinine	Osmolality	Urea	Na/Crea	K/Crea	Cl/Crea	Urea/Crea	FeNa	FeK	FeCl	Crea Cl	24hr Urine	Urea mass$_U$	Cl mass$_U$	Na mass$_U$	K mass$_U$
control (WT)	10-12mo (n=7)	108.43	340.86	167.71	66.53	2475.00	4148.86	1.70	5.09	2.53	62.17	0.23	17.06	0.42	189.90	836.00	34.21	4.86	2.10	11.02
KCTD1$^{-/-}$	10-12mo (n=5)	55.20	65.06	43.20	9.20	516.80	828.60	6.11	7.19	4.77	92.96	3.75	112.24	4.14	20.98	2957.60	23.62	3.86	3.22	7.23
ttest		*0.000935834*	*2.33E-05*	*0.000221531*	*1.90E-06*	*7.54E-06*	*8.05E-06*	*0.002313666*	*0.00024728*	*0.028713064*	*0.000557571*	*0.005628449*	*0.000393022*	*0.006807669*	*0.000200442*	*0.000423452*	*0.13153474*	*0.265673384*	*0.024438569*	*0.120055992*

		Na	K	Cl	Creatinine	Osmolality	Urea	Na/Crea	K/Crea	Cl/Crea	Urea/Crea	FeNa	FeK	FeCl	Crea Cl	24hr Urine	Urea mass$_U$	Cl mass$_U$	Na mass$_U$	K mass$_U$
control (KCTD1$^{fl/fl}$)	2mo (n=4)	98.00	378.63	202.25	55.75	2548.75	4285.00	1.81	6.82	3.65	76.64	0.41	26.53	1.00	220.38	1483.75	63.19	9.08	3.35	18.78
Aqp2Cre^{+}KCTD1$^{fl/fl}$	2mo (n=5)	110.60	398.60	202.40	52.72	2708.00	4406.40	2.10	7.58	3.85	83.38	0.39	25.35	0.96	126.37	948.60	39.87	6.62	2.32	14.39
ttest		*0.481179804*	*0.616644681*	*0.994166645*	*0.53089921*	*0.645504252*	*0.851809942*	*0.408651431*	*0.218278945*	*0.560341603*	*0.434375198*	*0.858160847*	*0.86191251*	*0.851247218*	*0.235679584*	*0.074560585*	*0.069426642*	*0.040876443*	*0.093545975*	*0.050918293*

		Na	K	Cl	Creatinine	Osmolality	Urea	Na/Crea	K/Crea	Cl/Crea	Urea/Crea	FeNa	FeK	FeCl	Crea Cl	24hr Urine	Urea mass$_U$	Cl mass$_U$	Na mass$_U$	K mass$_U$
KCTD1$^{fl/fl}$ + TAM	7mo after TAM at P9 (n=4)	124.67	329.67	161.00	63.87	2330.67	3642.67	2.00	5.25	2.59	57.70	0.27	13.20	0.47	178.55	823.33	30.56	4.00	4.63	7.32
β-actinCreERT2^{+}KCTD1$^{fl/fl}$ + TAM	7mo after TAM at P9 (n=6)	39.20	121.98	57.00	16.88	865.40	1402.00	2.34	7.33	3.40	84.22	0.35	22.70	0.72	220.11	4276.00	60.98	7.04	7.30	13.65
ttest		*0.000178718*	*0.0051591*	*0.008092131*	*4.41899E-05*	*0.00058831*	*0.000252191*	*0.309174979*	*0.126510113*	*0.218935901*	*0.024541161*	*0.300914799*	*0.076021932*	*0.232462084*	*0.36908893*	*0.008105043*	*0.114704719*	*0.233823202*	*0.232915451*	*0.175594562*

		Na	K	Cl	Creatinine	Osmolality	Urea	Na/Crea	K/Crea	Cl/Crea	Urea/Crea	FeNa	FeK	FeCl	Crea Cl	24hr Urine	Urea mass$_U$	Cl mass$_U$	Na mass$_U$	K mass$_U$
KCTD1$^{fl/fl}$ + TAM	7mo after TAM at 1.5mo (n=4)	137.75	444.50	226.00	62.15	3181.25	5481.00	2.29	7.24	3.73	88.39	0.31	20.62	0.63	173.83	864.50	44.11	6.87	2.73	14.11
β-actinCreERT2^{+}KCTD1$^{fl/fl}$ + TAM	7mo after TAM at 1.5mo (n=4)	77.50	258.25	120.50	38.73	1872.50	3305.50	1.97	6.61	3.05	84.79	0.31	22.78	0.62	213.53	1775.00	58.51	7.61	3.13	17.76
ttest		*0.004960628*	*0.013748402*	*0.010724068*	*0.024033398*	*0.015533125*	*0.028009963*	*0.320999182*	*0.405382331*	*0.309543547*	*0.644921447*	*0.985638515*	*0.587660146*	*0.950017675*	*0.33032303*	*0.015224138*	*0.27184871*	*0.764229011*	*0.634840888*	*0.354153906*

		Na	K	Cl	Crea	Osmo	urea	Na/Crea	K/Crea	Cl/Crea	urea/crea	FeNa	FeK	FeCl	Crea Cl	24hrs urine	ureamass	Clmass	Namass	Kmass
control WT + TAM	2mo after TAM at 1.5mo (n=2)	121.50	431.00	217.00	52.65	2601.50	4104.50	2.31	8.19	4.13	77.97	0.38	23.52	0.91	173.18	852.50	46.34	6.90	5.97	12.45
β-actinCreERT2^{+}TFAP2B$^{fl/fl}$	2mo after TAM at 1.5mo (n=4)	41.25	105.20	53.00	12.75	708.75	1067.50	3.22	8.23	4.13	83.84	0.70	36.00	1.25	133.25	5046.00	48.02	6.59	8.01	12.09
t-test		*9.06426E-05*	*2.90171E-05*	*4.71715E-05*	*3.01455E-05*	*4.11823E-06*	*2.64246E-05*	*0.003227865*	*0.931436032*	*0.997085395*	*0.121452829*	*0.141649748*	*0.240742133*	*0.418836345*	*0.417712087*	*3.63E-05*	*0.702492784*	*0.228500757*	*0.01209701*	*0.808364123*

Table S2: Urine chemistries and analyses in experimental mouse groups.
Related to Figures 2, 3, 5, 6 and 7.

Six2Cre^{+}KCTD1$^{fl/fl}$ mice and KCTD1$^{-/-}$ mice with severe polyuria have markedly hypoosmolar urine and reduced urinary electrolyte and urea concentrations (diluted urine due to lack of ability to concentrate the urine). Urine electrolytes and urea normalized to urinary creatinine are shown as well. Fractional excretions of electrolytes are indicated showing that urinary electrolyte loss increases with age progression and deterioration of kidney function in Six2Cre^{+}KCTD1$^{fl/fl}$ mice and KCTD1$^{-/-}$ mice.

Creatinine clearance strongly decreases with progressive deterioration of kidney function in aged Six2Cre^{+}KCTD1$^{fl/fl}$ mice and KCTD1$^{-/-}$ mice. Mass of electrolytes and urea in 24-hour urine collection is shown (in µg, urine concentration x 24-hour urine volume). Unremarkable urine in Aqp2Cre^{+}KCTD1$^{fl/fl}$ mice.

Induced inactivation of KCTD1 in 6-week-old mice (β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice + TAM) and evaluation 7 months later shows hypoosmolar urine with mild polyuria, but normal creatinine clearance. Induced inactivation of AP-2β in 6-week-old mice (β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice + TAM) results in hypoosmolar urine with polyuria.

Sodium, potassium, chloride in mEq/l. Creatinine and urea nitrogen in mg/dl. Osmolality in mOsm/kgH_2O.

Cre strains:	
Six2Cre^{+} strain	targets NPCs: entire nephron except CDs
PvalbCre^{+} strain	targets proximal DCTs (DCT1s, also called early DCTs)
Aqp2Cre^{+} strain	targets principle cells of CTs/CDs
β-actinCreERT2^{+} mice +TAM (CAGGCreERT2^{+} mice)	TAM induces inactivation in all tissues
Reporter mice:	
KCTD1$^{lacZ/WT}$ mice (KCTD1$^{-/+}$ mice)	KCTD1 reporter mice (contain lacZ cassette in endogenous KCTD1 locus)
TCF/Lef:H2B-GFP: 1. KCTD1$^{WT/-}$TCF:LEF-GFP reporter mice 2. KCTD1$^{-/-}$TCF:LEF-GFP reporter mice	reporter mice for β-catenin signaling activity: 1. β-catenin reporter in KCTD1 heterozygotes 2. β-catenin reporter in KCTD1 KO mice
B6.Cg-Gt(ROSA)26Sor$^{tm3(CAG-EYFP)Hze}$/J (Ai3) reporter mice	EYFP identifies cells with Cre activity
Null mice:	
KCTD1$^{-/-}$ mice	KCTD1 deficiency in all cells
EGF$^{-/-}$ mice	EGF deficiency in all cells
SFRP1$^{-/-}$ mice	SFRP1 deficiency in all cells
Cell type-specific mutant mice:	
β-actinCreERT2^{+}KCTD1$^{fl/fl}$ mice + TAM	TAM-inducible KCTD1 KO mice in all cells
β-actinCreERT2^{+}TFAP2B$^{fl/fl}$ mice + TAM	TAM-inducible TFAP2B KO mice in all cells
β-actinCreERT2^{+}KCTD1$^{fl/fl}$TFAP2B$^{fl/fl}$ mice + TAM	TAM-inducible KO of KCTD1 and TFAP2B in all cells
Six2Cre^{+}KCTD1$^{fl/fl}$ mice	KCTD1 deficiency in entire nephron except CDs
Six2Cre^{+}KCTD1$^{fl/fl}$β-catenin$^{fl/WT}$ mice	KCTD1 deficiency and heterozygosity for β-catenin in entire nephron except CDs
PvalbCre^{+}KCTD1$^{fl/fl}$ mice	KCTD1 deficiency in DCT1s
Aqp2Cre^{+}KCTD1$^{fl/fl}$ mice	KCTD1 deficiency in CTs/CDs
Six2Cre^{+}TFAP2B$^{fl/fl}$ mice	TFAP2B deficiency in entire nephron except CDs
PvalbCre^{+}TFAP2B$^{fl/fl}$ mice	TFAP2B deficiency in DCT1s
Aqp2Cre^{+}TFAP2B$^{fl/fl}$ mice	TFAP2B deficiency in CTs/CDs

Table S3: Description of mutant mice used in this study.
Related to STAR methods section.

Supplemental Item:
Table S3

Primer Name	Primer Sequence (5' to 3') UP	Primer Sequence (5' to 3') DW
mouse primers		
36b4	TCACTGTGCCAGCTCAGAAC	AA TTTCAA TGGTGCCTCTGG
KCTD1	CAAATACCCCGAATCCAGAATCG	ACATCTGCCCGTCTCTGTCA
Cd31	CCAGGGAGCACACCGAGAG	TGTCACCTTGGGCTTGGATACG
NKCC2 (Slc12a1)	ATGCCTCGTATGCCAAATCT	CCCACATGTTGTAAATCCCATA
Pvalb	TTCCAGATGGTGGGCCTGAAG	AGACAAGTCTCTGGCATCTGAG
Egf	TTCTCACAAGGAAAGAGCATCTC	GTCCTGTCCCGTTAAGGAAAAC
NCC (Slc12a3)	CAGTGCCTGGTGCTTACAGGGC	CATCATGCAGGACACCAATG
Pendrin	GACTGTAAAGACCCTCTTGATCTGA	GGAAGCAAGTCTACGCATGG
Scnn1g	CTTCTTCACTGGTCGGAAGC	CTGAAGGTGTAGGTGGCACA
Scnn1a	CGGAGTTGCTAAACTCAACATC	TGGAGACCAGTACCGGCT
Scnn1b	CTGCAGTCATCGGAACTTCA	CCGATGTCCAGGATCAACTT
Cd68	AGCTGCCTGACAAGGGACACT	AGAGGACCAGGCCAATGAT
Cdkn1a	AAGTGTGCCGTTGTCTCTTCG	AGTCAAAGTTCCACCGTTCTCG
Pai1	GACACCCTCAGCATGTTCATC	AGGGTTGCACTAAACATGTCAG
Col1a1	GTGCTCCTGGTATTGCTGGT	AAGGACCATCCCACTGTCTG
Tgfb1	AGGACCTGGGTTGGAAGTGGAT	AAGCGCCCGGGTTGTGTT
F4/80	GCCTATTATCTATACCCTCCAGCACATC	TCCATCTCCCATCCTCCACATCAG
human primers		
36B4	GCAATGTTGCCAGTGTCTGT	GCCTTGACCTTTTCAGCAAG
KCTD1	AATGCGCCTGTCCACATTGAT	GATTCAGGGTATTTGGTGAGGG

Table S4 : Primers used for semiquantitative RT-PCRs.
Related to STAR methods section.