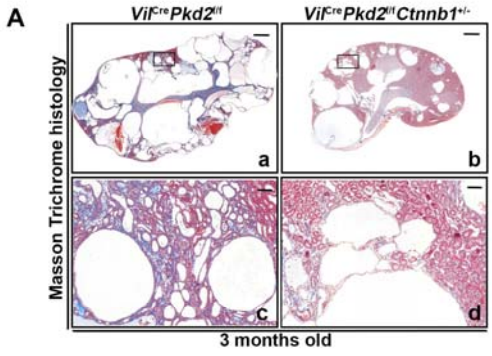
A
Vil^Cre Pkd2^f/f
Vil^Cre Pkd2^f/f Ctnnb1^+/-
Masson Trichrome histology
a
b
c
d
3 months old

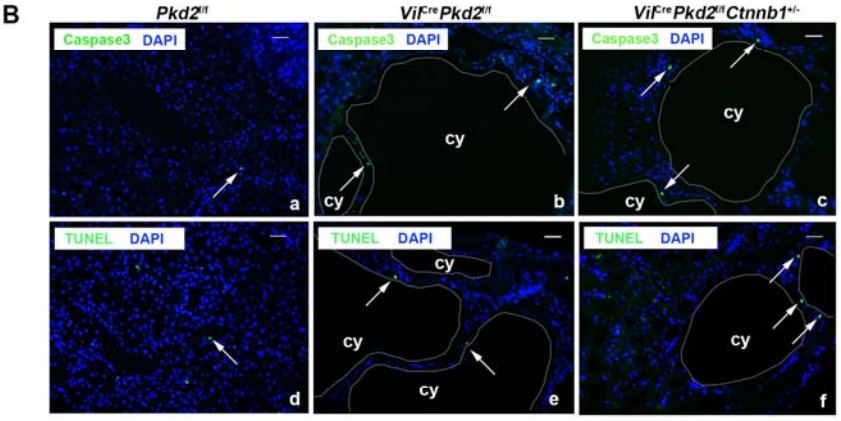
B
Pkd2^f/f
Vil^Cre Pkd2^f/f
Vil^Cre Pkd2^f/f Ctnnb1^+/-
Caspase3 DAPI
TUNEL DAPI
cy
a
b
c
d
e
f

Fig. S1

Fig S1. Effects of allelic reduction of *Ctnnb1*.

(A) Masson trichrome staining images of renal sections from *Vil*^{Cre}*Pkd2*$^{f/f}$ and *Vil*^{Cre}*Pkd2*$^{f/f}$*Ctnnb1*$^{+/-}$ mice were showed. All samples were collected at 3 months of age. Scale bars: 800 μm in a-b, 100 μm in c-d.

(B) Representative images of renal sections stained with an antibody to cleaved caspase-3 (a-c) and by TUNEL assay (d-f). cy: cyst. Scale bars: 60 μm. Data are from 3 animals/group.

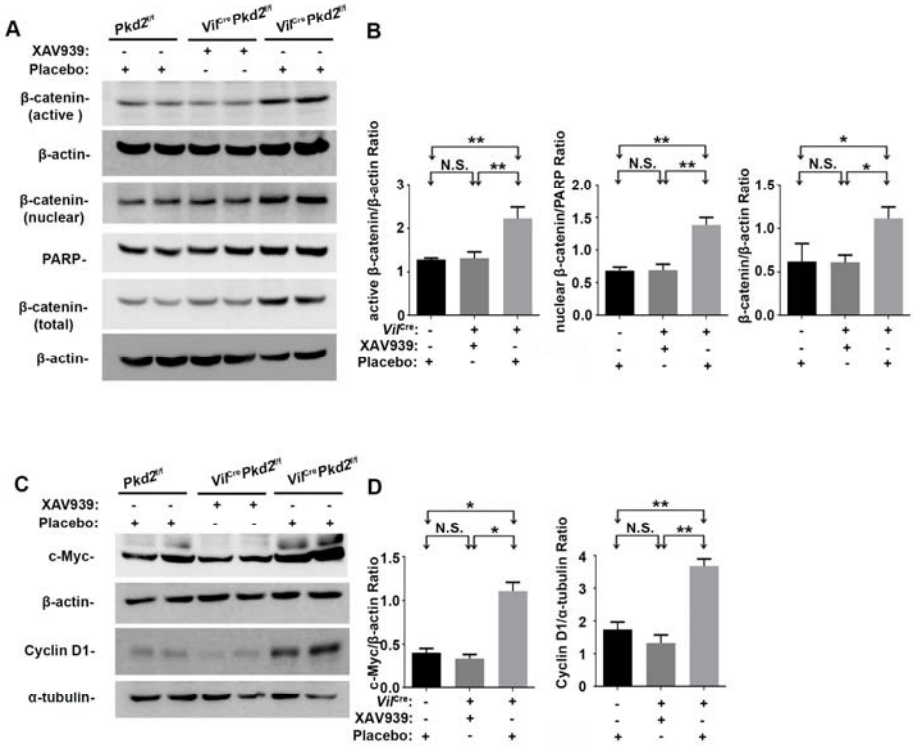
A
PkdZ^f/f
Vil^Cre PkdZ^f/f
Vil^Cre PkdZ^f/f
XAV939:
Placebo:
β-catenin- (active)
β-actin-
β-catenin- (nuclear)
PARP-
β-catenin- (total)
β-actin-
B
active β-catenin/β-actin Ratio
nuclear β-catenin/PARP Ratio
β-catenin/β-actin Ratio
N.S.
**
*
Vil^Cre:
XAV939:
Placebo:
C
c-Myc-
β-actin-
Cyclin D1-
α-tubulin-
D
c-Myc/β-actin Ratio
Cyclin D1/α-tubulin Ratio

Fig. S2

Fig. S2. XAV939 rescues the elevated expression of β-catenin and its target genes induced by PC2 deficiency.

(A-B) Representative western blots for the active, nuclear, and total β-catenin from the renal lysates of *Pkd2*$^{f/f}$, XAV939-treated and DSMO (placebo)-treated *Vil*^{Cre}*Pkd2*$^{f/f}$ mice are shown, along with (B) Normalized quantitative analysis of the densitometry values of the tested tissues.

(C-D) Representative western blots of the same lysates also showed that XAV939 treatment suppressed the β-catenin-mediated transcription (c-Myc and Cyclin D1) activated by PC2 deficiency. Samples were collected at 2 months of age. Data in B and D are presented as mean±SEM (*P<0.05 and **P<0.01, Student's *t* test). Data are from 3 animals/group.

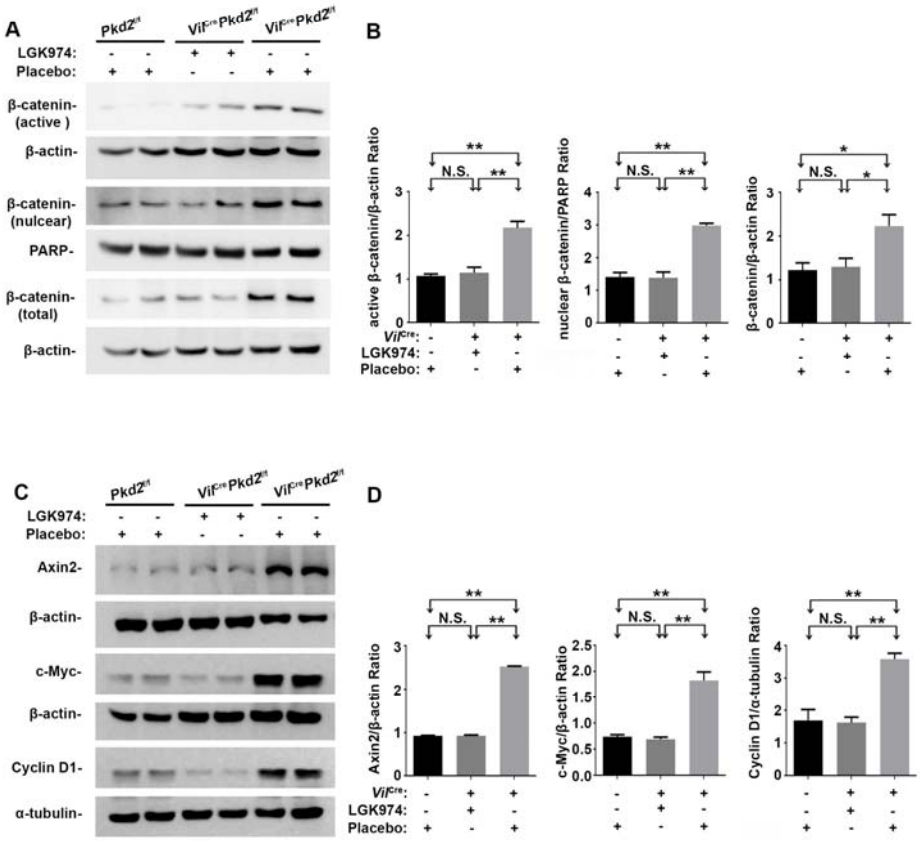
A
Pkdz^f/f
Vil^Cre Pkdz^f/f
Vil^Cre Pkdz^f/f
LGK974:
Placebo:
β-catenin- (active)
β-actin-
β-catenin- (nulcear)
PARP-
β-catenin- (total)
β-actin-
B
active β-catenin/β-actin Ratio
nuclear β-catenin/PARP Ratio
β-catenin/β-actin Ratio
N.S.
**
*
Vil^Cre:
LGK974:
Placebo:
C
Axin2-
β-actin-
c-Myc-
β-actin-
Cyclin D1-
α-tubulin-
D
Axin2/β-actin Ratio
c-Myc/β-actin Ratio
Cyclin D1/α-tubulin Ratio

Fig. S3

Fig. S3. LGK974 rescues the elevated expression of β-catenin and its target genes induced by PC2 deficiency.

(A-B) Representative western blots for the active, nuclear, and total β-catenin from the renal lysates of *Pkd2*$^{f/f}$, LGK974-treated and DSMO (placebo)-treated *Vil*^{Cre}*Pkd2*$^{f/f}$ mice are shown, along with (B) Normalized quantitative analysis of the densitometry values of the tested tissues.

(C-D) Representative western blots of the same lysates also showed that LGK974 treatment suppressed the β-catenin-mediated transcription (including Axin2, c-Myc and Cyclin D1) activated by PC2 deficiency. Samples were collected at 3 months of age. Data in B and D are presented as mean±SEM (*P<0.05 and **P<0.01, Student's *t* test). Data are from 3 animals/group.

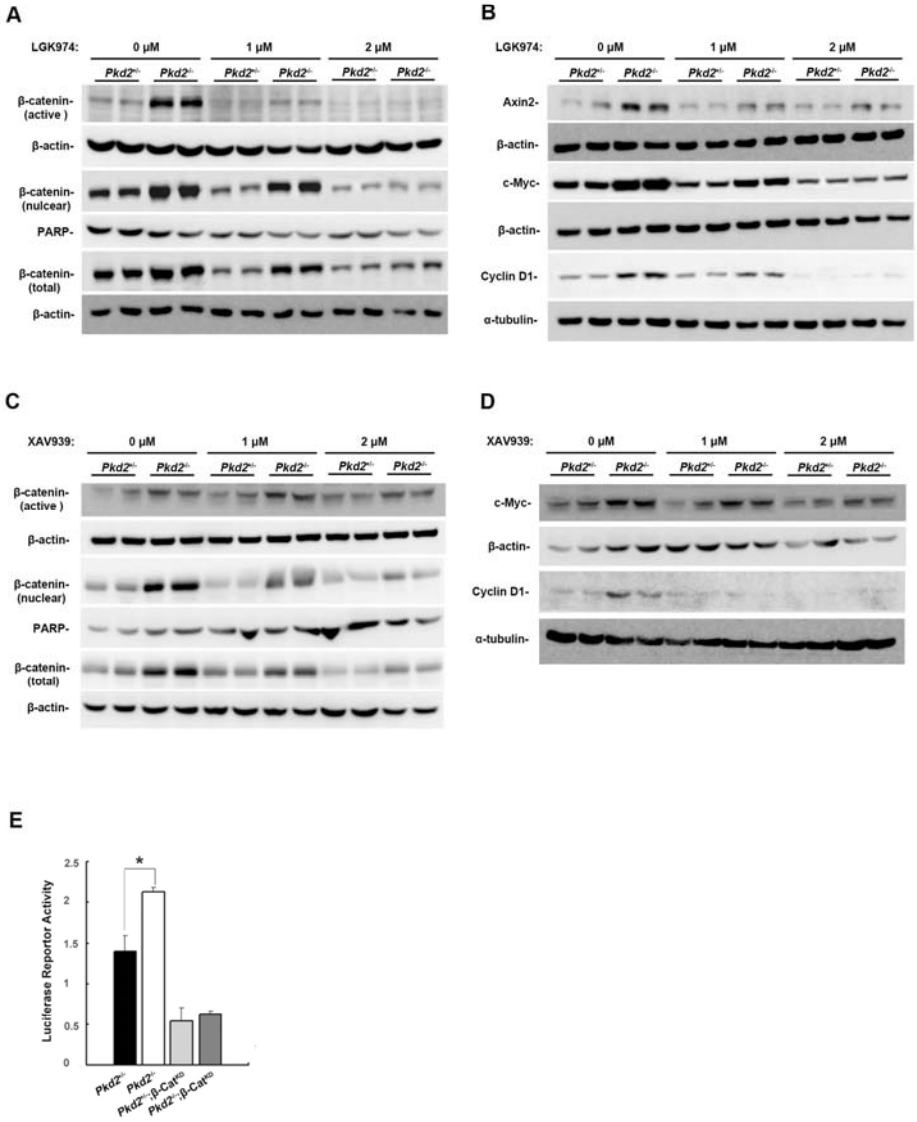
A
LGK974:
0 μM
1 μM
2 μM
Pkd2+/-
Pkd2-/-
β-catenin- (active)
β-actin-
β-catenin- (nuclear)
PARP-
β-catenin- (total)
β-actin-
B
LGK974:
0 μM
1 μM
2 μM
Pkd2+/-
Pkd2-/-
Axin2-
β-actin-
c-Myc-
β-actin-
Cyclin D1-
α-tubulin-
C
XAV939:
0 μM
1 μM
2 μM
Pkd2+/-
Pkd2-/-
β-catenin- (active)
β-actin-
β-catenin- (nuclear)
PARP-
β-catenin- (total)
β-actin-
D
XAV939:
0 μM
1 μM
2 μM
Pkd2+/-
Pkd2-/-
c-Myc-
β-actin-
Cyclin D1-
α-tubulin-
E
Luciferase Reportor Activity
2.5
2
1.5
1
0.5
0
*
Pkd2+/-
Pkd2-/-
Pkd2-/-β-Cat+/-
Pkd2-/-β-Cat+/-

Fig. S4

Fig. S4. LGK974 and XAV939 treatment reduced the active, nuclear, and total β-catenin levels in cultured renal epithelial cells.

(A-B) *Pkd2*$^{+/-}$ and *Pkd2*$^{-/-}$ cells were incubated with 0, 1, or 2 μM LGK974 for 24 hours or (C-D) with 0, 1, or 2 μM XAV939 for 16 hours, and were analyzed by western blotting.

(E) Wnt reporter-gene activity was elevated in *Pkd2*$^{-/-}$ cells. Cells were transfected with the TOP-FLASH Wnt reporter gene Basal in the presence or absence of β-catenin siRNA, and reporter-gene activity was determined 24 hours after transfection. Data are presented as mean±SEM (*P<0.05; n=3, Student's *t* test).

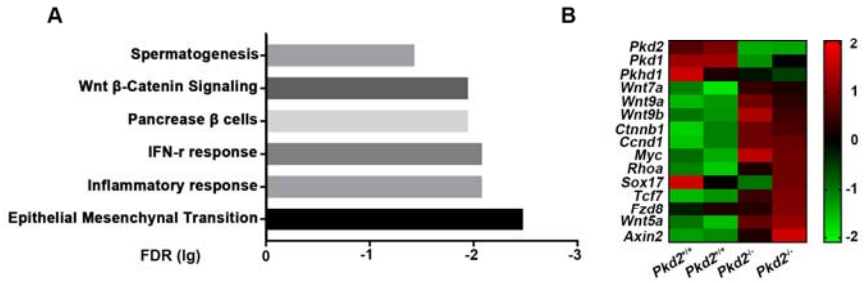
A
Spermatogenesis
Wnt β-Catenin Signaling
Pancrease β cells
IFN-r response
Inflammatory response
Epithelial Mesenchynal Transition
FDR (lg)
0
-1
-2
-3
B
Pkd2
Pkd1
Pkhd1
Wnt7a
Wnt9a
Wnt9b
Ctnnb1
Ccnd1
Myc
Rhoa
Sox17
Tcf7
Fzd8
Wnt5a
Axin2
Pkd2+/+
Pkd2+/+
Pkd2-/-
Pkd2-/-
2
1
0
-1
-2

Fig. S5

Fig. S5. Gene expression analysis of WT and *Pkd2*-null collecting duct cells.

(A) Pathway analysis showed that the gene expression was significantly altered between WT and *Pkd2*-null cells; pathways with significant enrichment scores [log10 (FDR)] are shown.

(B) Comparison of the expression of Wnt pathway–associated genes in *Pkd2*-null and WT cells.

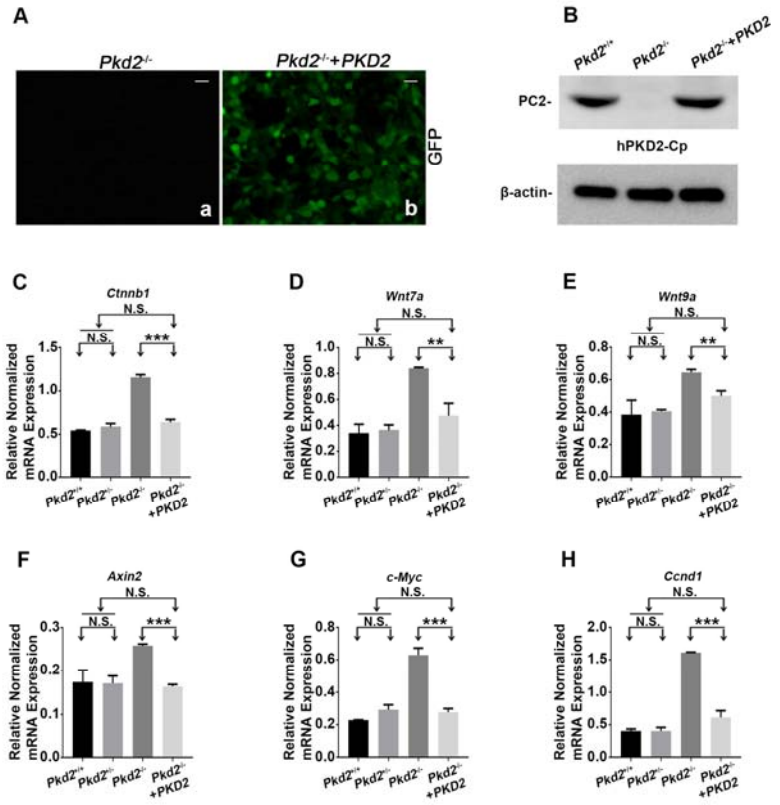
A
Pkd2-/-
Pkd2-/-+PKD2
GFP
a
b
B
Pkd2+/+
Pkd2-/-
Pkd2-/-+PKD2
PC2-
hPKD2-Cp
β-actin-
C
Ctnnb1
D
Wnt7a
E
Wnt9a
F
Axin2
G
c-Myc
H
Ccnd1
Relative Normalized mRNA Expression
N.S.
**
***
Pkd2+/+
Pkd2-/-
Pkd2-/-
Pkd2-/- +PKD2

Fig. S6

Fig. S6. Restoring PC2 in *Pkd2*$^{-/-}$ cells normalizes the dysregulated expression of Wnt signaling genes.

(A-B) Validation of PC2 re-expression: the efficiency of re-expression was demonstrated by (A) GFP presence and (B) PC2 western blots.

(C-H) *PKD2* re-expression reduced the mRNA levels of *Ctnnb1*, *Wnt7a*, *Wnt9a, Axin2*, *c-Myc,* and *Ccnd1,* which were elevated in the *Pkd2*$^{-/-}$ cells. Data are presented as mean±SEM (**P<0.01, and ***P<0.001, N.S.= No Significant; n=3, Student's *t* test).

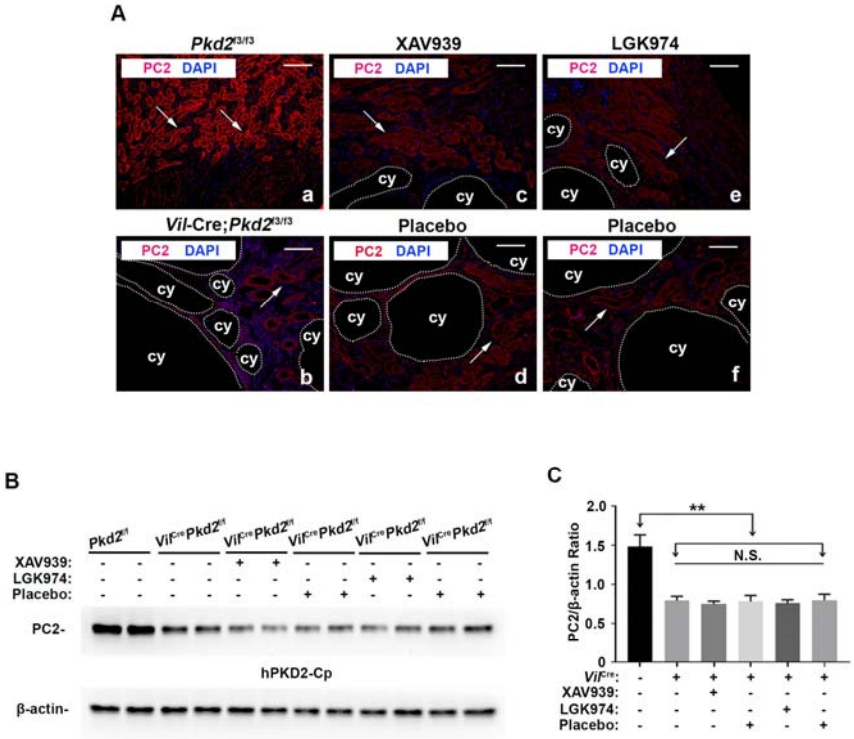
A
Pkd2f3/f3
XAV939
LGK974
PC2 DAPI
cy
a
c
e
Vil-Cre;Pkd2f3/f3
Placebo
Placebo
b
d
f
B
PkdZf/f
ViCre PkdZf/f
XAV939:
LGK974:
Placebo:
PC2-
hPKD2-Cp
β-actin-
C
PC2/β-actin Ratio
2.0
1.5
1.0
0.5
0
**
N.S.
ViCre:
XAV939:
LGK974:
Placebo:

Fig. S7

Figure S7. PC2 expression in the kidneys of *Pkd2*$^{f/f}$ and *Vil*^{Cre}*Pkd2*$^{f/f}$ with and without XAV939- and LGK974-treated.

(A) Using the anti-PC2 (hPKD2-Cp) polyclonal antibodies, immunofluorescence staining (arrows) showed significantly decreased PC2 expression (red) in the *Vil*^{Cre}*Pkd2*$^{f/f}$ kidneys compared to *Pkd2*$^{f/f}$ control (a *vs* b). By the same staining, there was no PC2 expression difference among kidneys with or without LGK974 and XAV939 treatment (c *vs* d and e *vs* f). DAPI dye (blue) was used to stain nuclei. cy: cyst. Bars: 50 μm in A.

(B) Duplicated lysates from the control *Pkd2*$^{f/f}$ kidney and the *Vil*^{Cre}*Pkd2*$^{f/f}$ kidneys with or without XAV939 and LGK974 treatments were used to perform western blot analysis with the same anti-PC2 antibody. Similar results to (A) were observed.

(C) Normalized quantitative analysis of the densitometry values of the tested tissues. Data are presented as mean±SEM (*P<0.01, N.S.= No Significant; Student's *t* test). Data are from 3 animals/group.

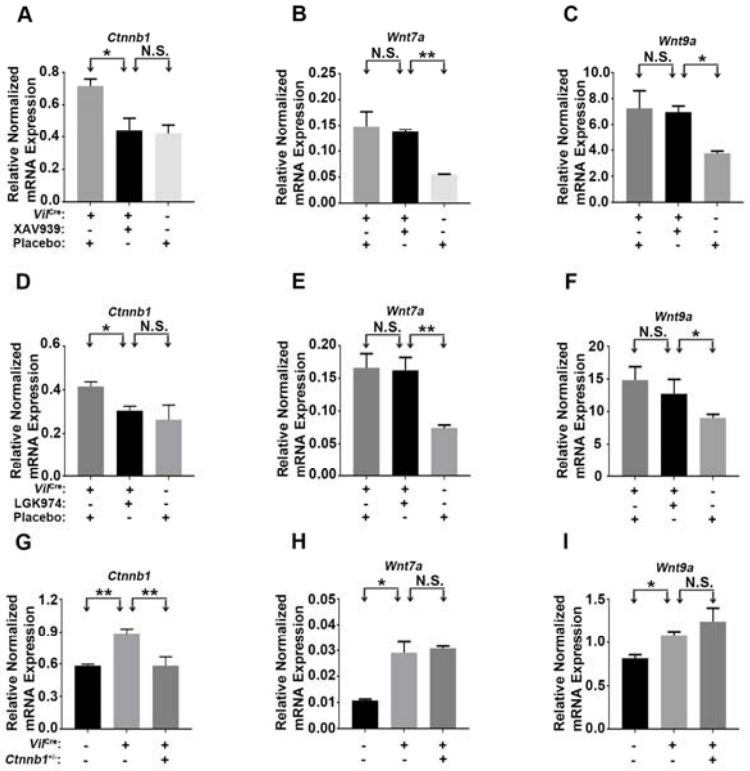
A
Ctnnb1
*
N.S.
Relative Normalized mRNA Expression
0.8
0.6
0.4
0.2
0.0
Vil^Cre:
XAV939:
Placebo:
B
Wnt7a
N.S.
**
0.25
0.20
0.15
0.10
0.05
0.00
C
Wnt9a
N.S.
*
10.0
8.0
6.0
4.0
2.0
0.0
D
Ctnnb1
*
N.S.
0.6
0.4
0.2
0.0
Vil^Cre:
LGK974:
Placebo:
E
Wnt7a
N.S.
**
0.20
0.15
0.10
0.05
0.00
F
Wnt9a
N.S.
*
20
15
10
5
0
G
Ctnnb1
**
**
1.2
0.9
0.6
0.3
0.0
Vil^Cre:
Ctnnb1^+/-:
H
Wnt7a
*
N.S.
0.05
0.04
0.03
0.02
0.01
0.00
I
Wnt9a
*
N.S.
1.5
1.0
0.5
0.0

Fig. S8

Fig. S8. Loss of one *Ctnnb1* allele, or XAV939 or LGK974 treatment, reduces the PC2-loss-associated elevation of renal expression of *Ctnnb1* but not of *Wnt7a* or *Wnt9a*.

Compound treatments and sample collections were conducted as described in Fig. 1 and Fig. S2-S3. Gene expression was analyzed by quantitative RT-PCR. Data are presented as mean±SEM (*P<0.05, **P<0.01, N.S.= No Significant, n=3, Student's *t* test).

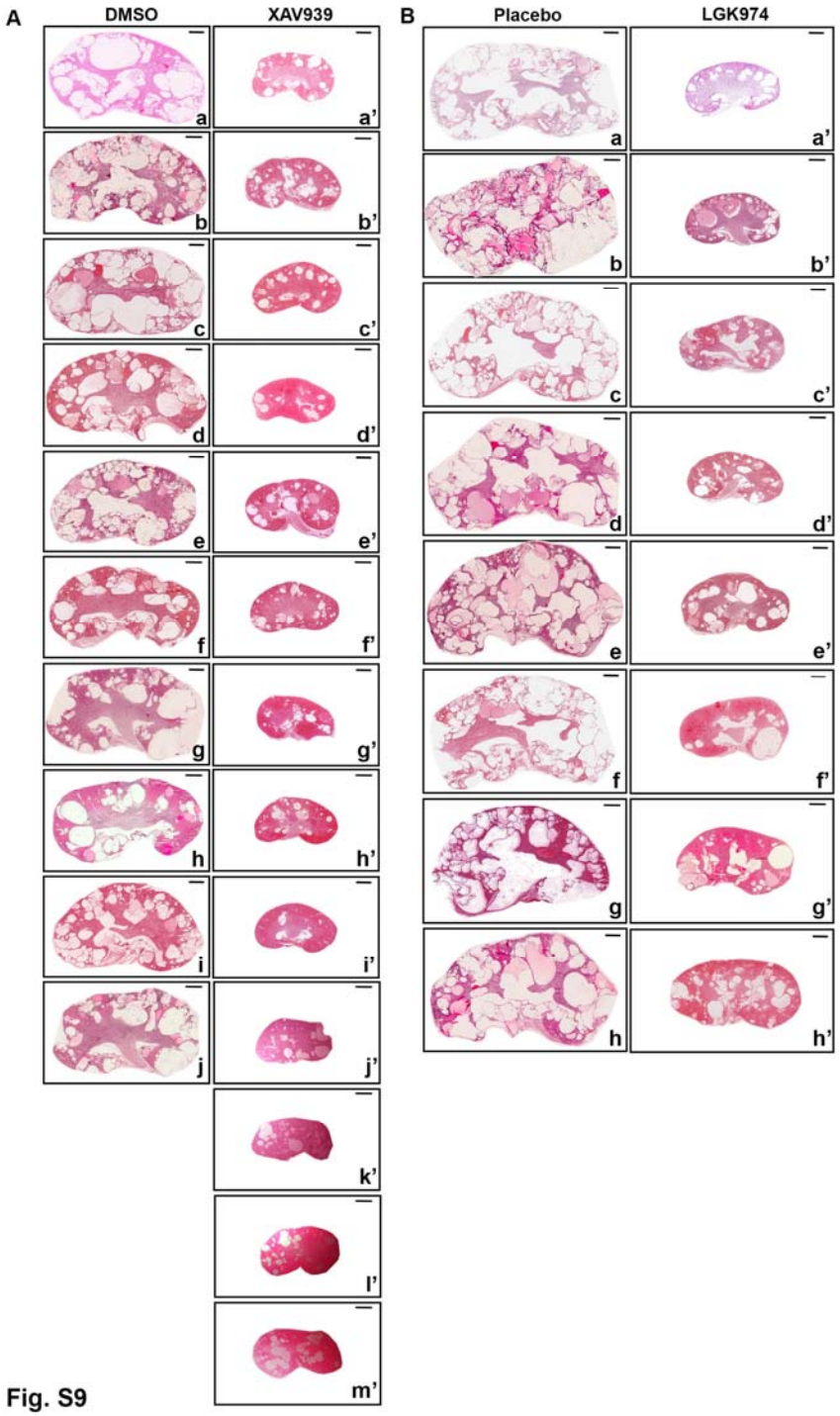
A
DMSO
XAV939
a
a'
b
b'
c
c'
d
d'
e
e'
f
f'
g
g'
h
h'
i
i'
j
j'
k'
l'
m'
B
Placebo
LGK974
a
a'
b
b'
c
c'
d
d'
e
e'
f
f'
g
g'
h
h'

Fig. S9

Fig. S9. Histology of all the kidneys treated with XAV939 or LGK974 and their placebos.

(A) Histology of the kidneys from *Vil*^{Cre}*Pkd2*$^{f/f}$ mice treated with XAV939 (a-j) or placebo (DMSO) (a'-m') was showed. Histology of a and a' have been showed in Fig. 3Bc-d. All samples were collected at 2 months of age.

(B) Histology of the kidneys from *Vil*^{Cre}*Pkd2*$^{f/f}$ mice treated with LGK974 (a-h) or placebo (a'-h') was showed. Histology of a and a' have been showed in Fig. 4Bc-d. All samples were collected at 3 months of age. Scale bars: 600 μm.