**Supplemental Data**

**Supplemental Figure Legends**

**Supplemental Figure 1. Double conditional knockout of *Smyd2* and *Pkd1* suppressed cell proliferation but induced apoptosis of the cyst-lining epithelial cells.** (**A**) and (**B**) The histological H&E staining for the corresponding area of each section in Figure 2G and 2H was showed in the right panels. Scale bars, 100 μm.

**Supplemental Figure 2. Knockdown or inhibition of Smyd2 suppressed cell growth *in vitro*. (A** and **B)** Knockdown of Smyd2 with siRNA inhibited cell proliferation of *Pkd1* null MEK cells (**A**) and PN24 cells (**B**) as examined by MTT assay. (**C**) FACS analysis in three independent experiments indicated that inhibition of Smyd2 with its inhibitor AZ505 induced cell-cycle arrest at the G0/G1 phase and decreased S phase entry in *Pkd1* null MEK cells and Smyd2 knockdown *Pkd1* null MEK cells compared to those in the control cells. *p* value indicates the significance of differences in the percentage of cells with AZ505 and/or siRNA versus without AZ505 or siRNA treatment at a given cell-cycle phase. Error bars indicate SEM.

**Supplemental Figure 3. Treatment with AZ505 decreased cyst lining epithelial cell proliferation but induced apoptosis in kidneys from *Pkd1*$^{nl/nl}$ mice and *Pkd1*$^{flox/flox}$:*Tam-Cre* mice.** (**A and C**) AZ505 treatment reduced cyst lining epithelial cell proliferation as shown with Ki67 staining in *Pkd1*$^{nl/nl}$ mice (**A**) and in *Pkd1*$^{flox/flox}$:*Tam-Cre* mice (**C**). The histological H&E staining for the corresponding area of each section in Figure 3E and 4F was showed in the bottom panels. Scale bars, 100 μm. (**B and D**) AZ505 treatment induced cyst lining epithelial cell death in kidneys from *Pkd1*$^{nl/nl}$ mice (**B**) and *Pkd1*$^{flox/flox}$:*Tam-Cre* mice (**D**) as detected by TUNEL assay. The histological H&E staining for the

corresponding area of each section in Figure 3F and 4G was showed in the bottom panels. Scale bars, 100 μm.

**Supplemental Figure 4. Treatment with AZ505 has no effect on the body weights of *Pkd1*$^{nl/nl}$ and *Pkd1*$^{flox/flox}$:*Tam-Cre* mice.** (**A**) Statistic analysis indicated there was no significant difference in body weights between AZ505 treated and DMSO treated *Pkd1*$^{nl/nl}$ mice. Mean body weight is 8.25±1.79 g versus 8.344±1.78 g (n=12 mice per group). (**B**) Statistic analysis indicated that there was no significant difference in body weights between between AZ505 treated and DMSO treated *Pkd1*$^{flox/flox}$:*Tam-Cre* mice. Mean body weight is 22.88±3.14g versus 24.28±3.11g (n=14 mice per group).

**Supplemental Figure 5. Smyd2 regulates the phosphorylation and activation of STAT3 and NF-κB in renal epithelial cells.** (**A**) Overexpression of GFP-tagged Smyd2 increased the expression of the phosphorylation of STAT3 and p65 in mouse IMCD3 cells. (**B** and **C**) Knockdown of Smyd2 with siRNA (**B**) or inhibition of Smyd2 with AZ505 (**C**) decreased the phosphorylation of STAT3 and p65, but did not affect their expression in *Pkd1* null MEK cells. The blots of Smyd2 and actin in **B** and **C** are the same as those in Figure 7A and 7B, respectively.

**Supplemental Figure 6. The expression of STAT3 and NF-κB target genes were analyzed by qRT-PCR in renal epithelial cells.** (**A**) The expression of CyclinD1, c-Myc, and TNFα was increased in *Pkd1* null cells compared to *Pkd1* WT cells. Relative expression level of these genes was normalized to actin. (**B** and **C**) The mRNA expression of STAT3 and NF-κB target genes was decreased in *Pkd1* null cells treated with Smyd2 siRNA for 24 hours (**B**) and AZ505 for 2 hours (**C**) compared to controls. Relative expression levels of these genes were normalized to actin. (**D**) The mRNA expression of STAT3 and NF-κB target genes, CyclinD1, c-Myc, and TNFα, were decreased in PN7 kidneys from

*Pkd1*$^{flox/flox}$:*Smyd2*$^{flox/flox}$:*Ksp-Cre* neonates compared to kidneys from aged matched *Pkd1*$^{flox/flox}$:*Smyd2*$^{+/+}$:*Ksp-Cre* (Smyd2$^{+/+}$) neonates. Error bar represents SEM.

**Supplemental Figure 7. Histone methylation was changed in cystic renal epithelial cells**. (**A** and **B**) Treatment with AZ505 decreased the mono-, di-, or tri-methylation of histone H3 at lysine K4 and K36 in the nuclear fraction of *Pkd1* null MEK cells (**A**) and *Pkd1* homozygous PN24 cells (**B**), but did not affect the methylation of H3 at K9 and K27 in these cells. (**C**) Knockdown of Smyd2 decreased the mono-, di-, or tri-methylation of H3K4 and H3K36 in the nuclear fraction of *Pkd1* null MEK cells. Treatment with AZ505 only slightly further decreased the mono-, di-, or tri-methylation of H3K4 and H3K36 in the nuclear fraction of Smyd2 knockdown cells.

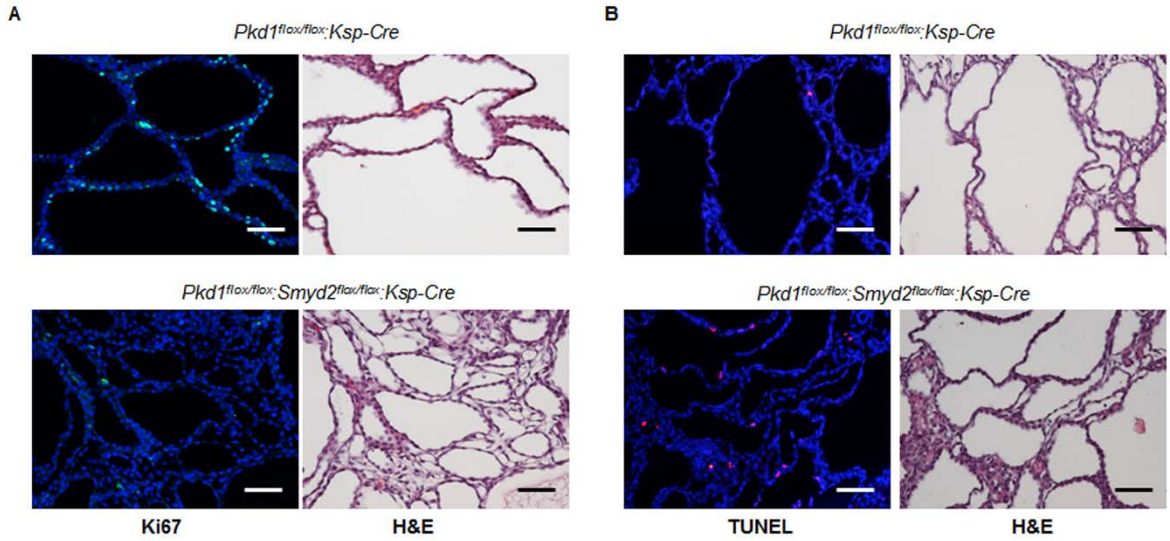
**Supplemental Figure 8. A schematic diagram depicting the Smyd2 ChIP-seq data.** We identified 91 potential Smyd2 target genes in *Pkd1* wild type MEK cells and 116 potential Smyd2 target genes in *Pkd1* null MEK cells. There were 14 genes identified in both *Pkd1* wild type and null MEK cells. Thus, the number of potential Smyd2 target genes in *Pkd1* null MEK cells are 116 - 14 = 102 genes.

**Supplemental Figure 9. The novel Smyd2 target Ptpn13 was downregulated by Smyd2 in *Pkd1* mutant renal epithelial cells.** (**A and B**) Smyd2 bound to the promoter of Ptpn13 as examined by ChIP assay (A) and ChIP-qPCR assay (B), which was performed with anti-Smyd2 antibody or normal goat IgG in *Pkd1* null MEK cells. (**C and D**) qRT-PCR (C) and western blot (D) analysis of the relative mRNA and protein level of Ptpn13 in *Pkd1* heterozygous PH2 (PH2) cells compared to that in *Pkd1* homozygous PN24 (PN24) cells. (**E and F**) qRT-PCR (E) and western blot (F) analysis of the relative mRNA and protein level of Ptpn13 in PN24 cells with or without knockdown of Smyd2 with siRNA.

**Supplemental Figure 10. Smyd2 regulates p53 dependent cystic renal epithelial cell apoptosis.** (**A** and **B**) Knockdown of Smyd2 with siRNA induced apoptosis in *Pkd1* null MEK cells, but not that in *Pkd1* WT MEK cells, as detected by TUNEL assay. Scale bars, 100 μm. (**C** and **D**) Knockdown of Smyd2

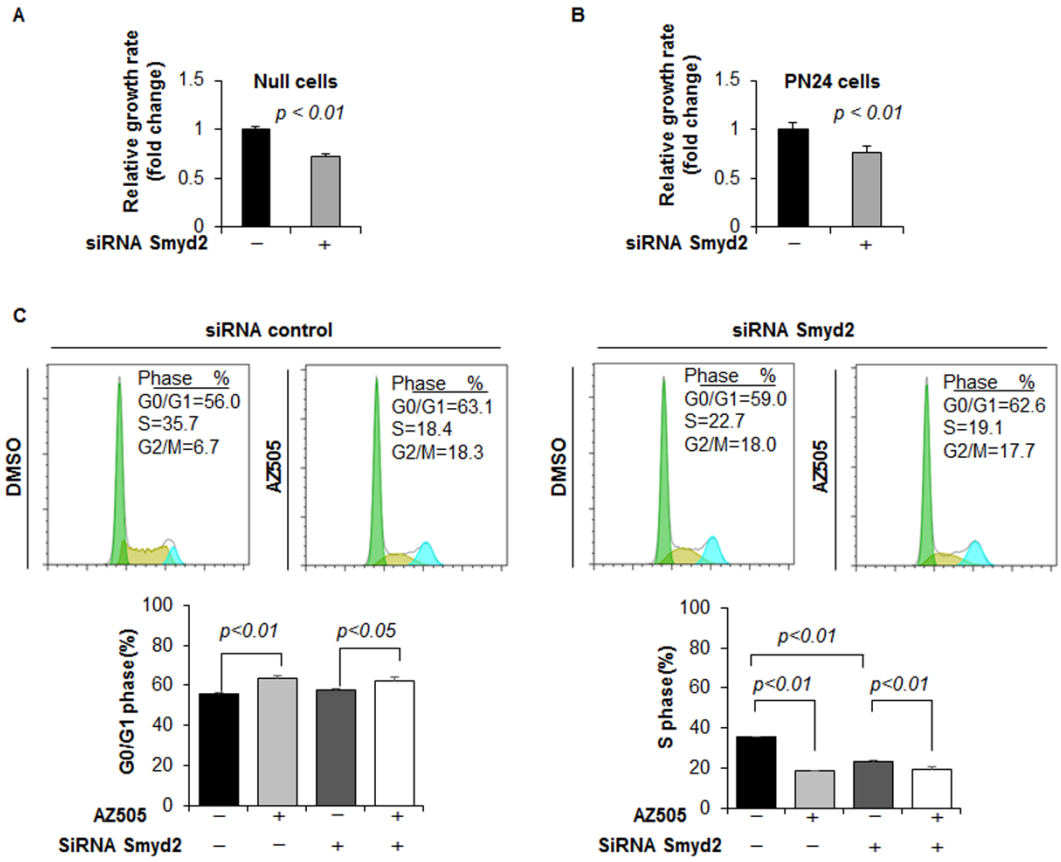
induced apoptosis in *Pkd1* null MEK cells, while knockdown of p53 reduced knockdown of Smyd2 induced apoptosis in these cells. Scale bars, 100 μm. (**E**) Smyd2 interacted with p53 in *Pkd1* WT MEK and *Pkd1* null MEK cells as examined by co-immunoprecipitation assay with anti-Smyd2 and anti-p53 antibody. The methylation of p53 was examined by immunoprecipitation with anti-p53 antibody and then blotted with anti-p53 and anti–pan methyl-lysine antibody in WT MEK and *Pkd1* null MEK cells. IgG was used as a negative control. (**F**) Western blot analysis of p53 and methyl-p53 in *Pkd1* null MEK cells with or without knockdown of Smyd2 with siRNA. (**G**) qRT-PCR analysis of relative p53 mRNA expression in *Pkd1* null MEK cells with or without knockdown of Smyd2 with siRNA. (**H**) Smyd2 bound to the promoter of p53. ChIP–qPCR assay was performed with anti-Smyd2 antibody or normal goat IgG in *Pkd1* null MEK cells. (**I** and **J**) NFκB was involved in Smyd2 mediated cell death in renal epithelial cells. Treatment with Smyd2 inhibitor AZ505 alone or NFκB inhibitor BAY-11-7085 alone induced renal epithelial cell death in *Pkd1* null MEK cells, whereas treatment with both AZ505 and BAY-11-7085 induced more cell death in *Pkd1* null MEK cells compared to those in AZ505 and BAY-11-7085 alone treated cells, as detected by TUNEL assay. Scale bars, 100 μm.

Supplemental Figure 1



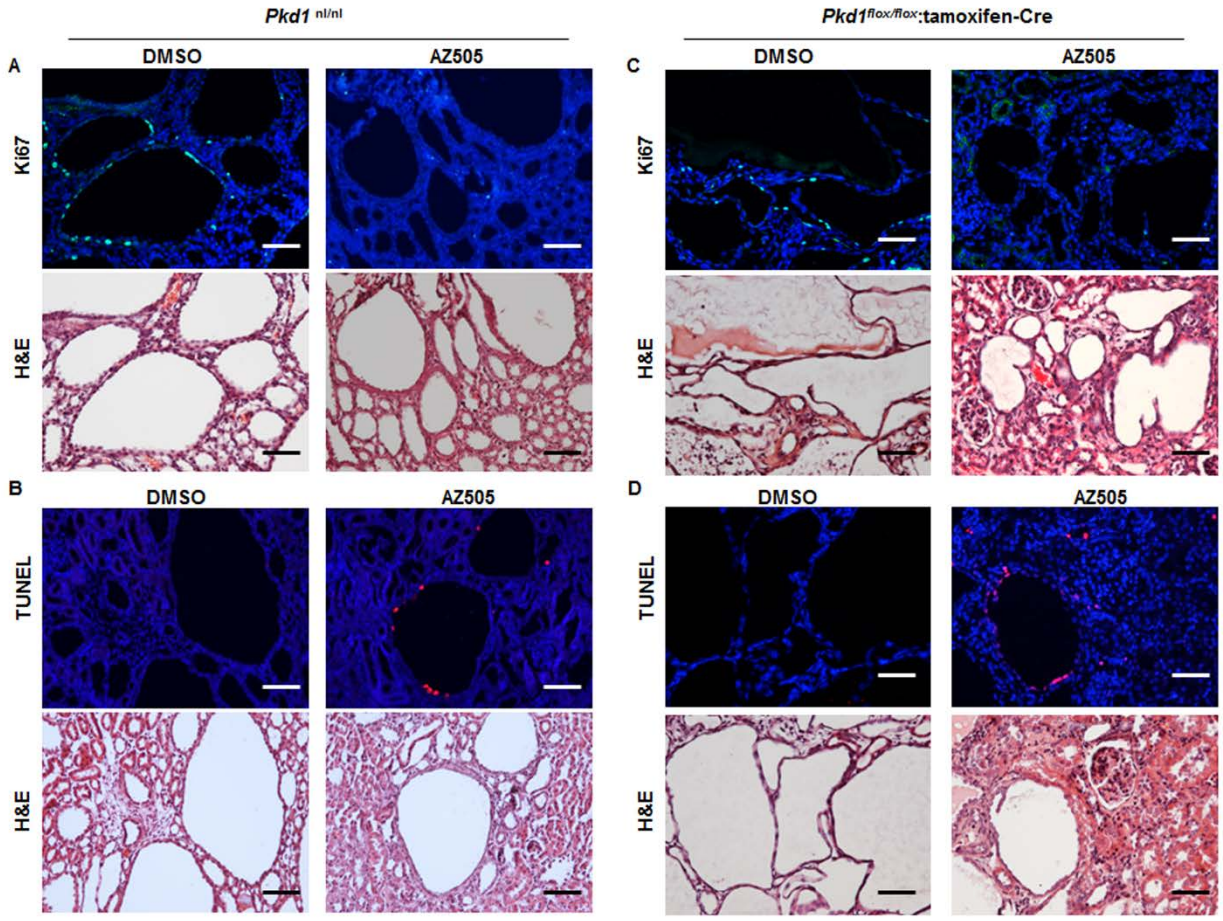

**Supplemental Figure 1. Double conditional knockout of Smyd2 and Pkd1 suppressed cell proliferation but induced apoptosis of the cyst-lining epithelial cells.** (A) and (B) The histological H&E staining for the corresponding area of each section in Figure 2G and 2H was showed in the right panels. Scale bars, 100 μm.

Supplemental Figure 2



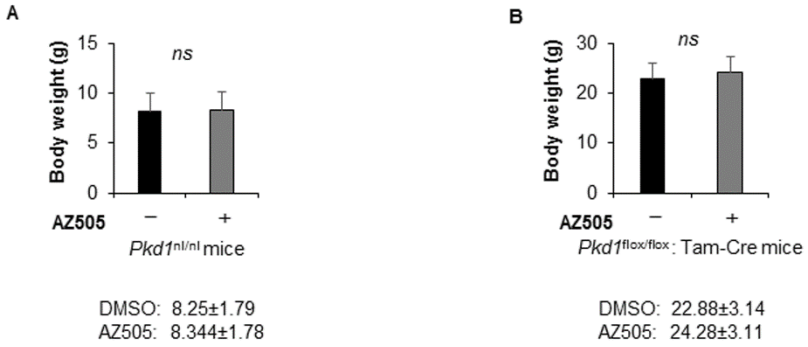

**Supplemental Figure 2. Knockdown or inhibition of Smyd2 suppressed cell growth in vitro.** (A and B) Knockdown of Smyd2 with siRNA inhibited cell proliferation of *Pkd1* null MEK cells (A) and PN24 cells (B) as examined by MTT assay. (C) FACS analysis in three independent experiments indicated that inhibition of Smyd2 with its inhibitor AZ505 induced cell-cycle arrest at the G0/G1 phase and decreased S phase entry in *Pkd1* null MEK cells and Smyd2 knockdown *Pkd1* null MEK cells compared to those in the control cells. p value indicates the significance of differences in the percentage of cells with AZ505 and/or siRNA versus without AZ505 or siRNA treatment at a given cell-cycle phase. Error bars indicate SEM.

Supplemental Figure 3



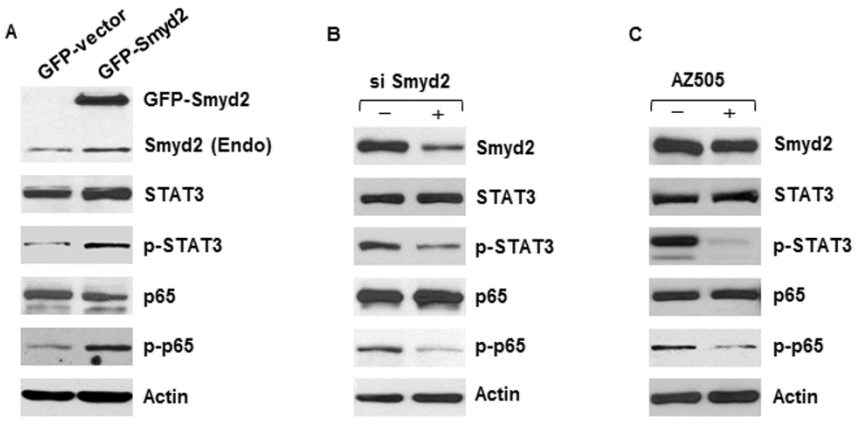

**Supplemental Figure 3. Treatment with AZ505 decreased cyst lining epithelial cell proliferation but induced apoptosis in kidneys from *Pkd1*$^{nl/nl}$ mice and *Pkd1*$^{flox/flox}$:*Tam-Cre* mice.** (**A and C**) AZ505 treatment reduced cyst lining epithelial cell proliferation as shown with Ki67 staining in *Pkd1*$^{nl/nl}$ mice (**A**) and in *Pkd1*$^{flox/flox}$:*Tam-Cre* mice (**C**). The histological H&E staining for the corresponding area of each section in Figure 3E and 4F was showed in the bottom panels. Scale bars, 100 μm. (**B and D**) AZ505 treatment induced cyst lining epithelial cell death in kidneys from *Pkd1*$^{nl/nl}$ mice (**B**) and *Pkd1*$^{flox/flox}$:*Tam-Cre* mice (**D**) as detected by TUNEL assay. The histological H&E staining for the corresponding area of each section in Figure 3F and 4G was showed in the bottom panels. Scale bars, 100 μm.

Supplemental Figure 4



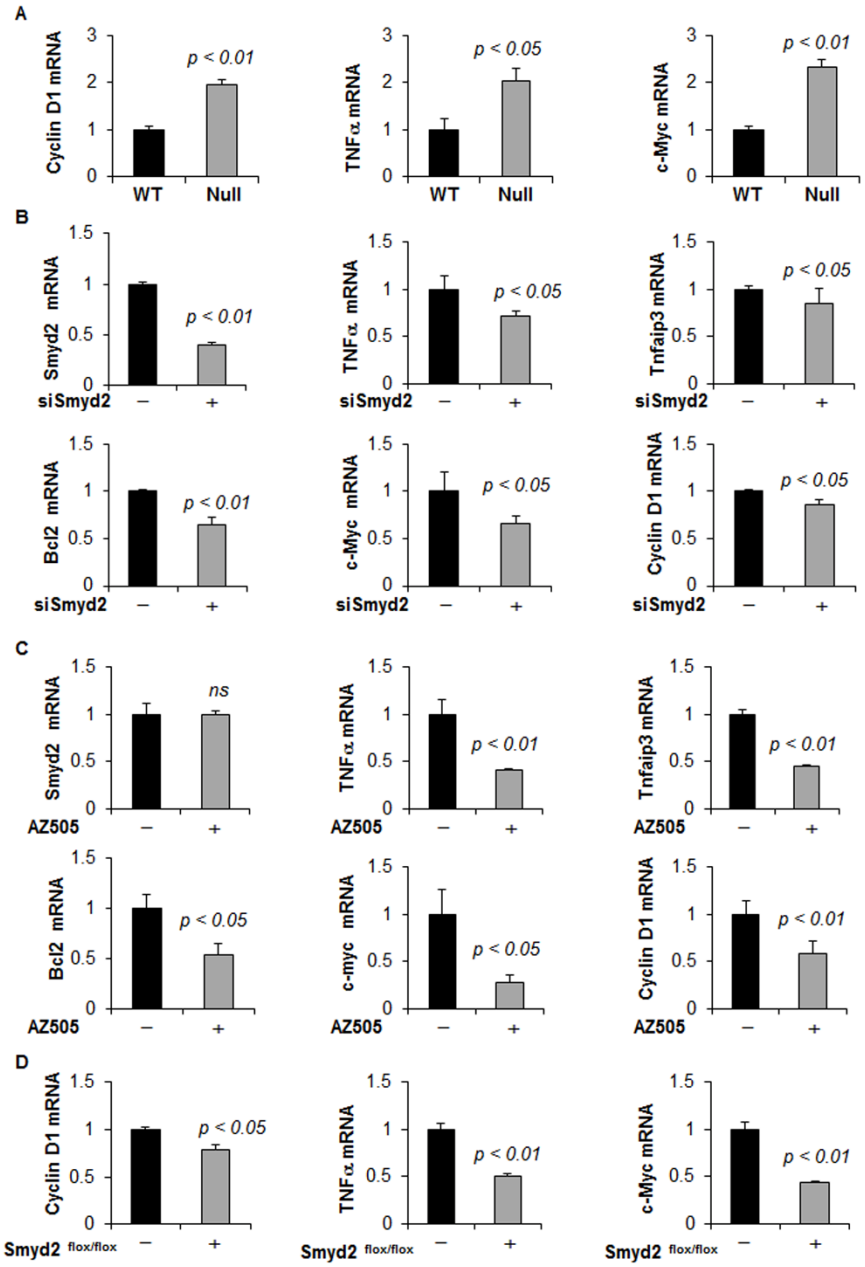

**Supplemental Figure 4. Treatment with AZ505 has no effect on the body weights of *Pkd1*$^{nl/nl}$ and *Pkd1*$^{flox/flox}$:*Tam-Cre* mice.** (**A**) Statistic analysis indicated there was no significant difference in body weights between AZ505 treated and DMSO treated *Pkd1*$^{nl/nl}$ mice. Mean body weight is 8.25±1.79 g versus 8.344±1.78 g (n=12 mice per group). (**B**) Statistic analysis indicated that there was no significant difference in body weights between between AZ505 treated and DMSO treated *Pkd1*$^{flox/flox}$:*Tam-Cre* mice. Mean body weight is 22.88±3.14g versus 24.28±3.11g (n=14 mice per group).

Supplemental Figure 5




**Supplemental Figure 5. Smyd2 regulates the phosphorylation and activation of STAT3 and NF-κB in renal epithelial cells.** (**A**) Overexpression of GFP-tagged Smyd2 increased the expression of the phosphorylation of STAT3 and p65 in mouse IMCD3 cells. (**B** and **C**) Knockdown of Smyd2 with siRNA (**B**) or inhibition of Smyd2 with AZ505 (**C**) decreased the phosphorylation of STAT3 and p65, but did not affect their expression in *Pkd1* null MEK cells. The blots of Smyd2 and actin in **B** and **C** are the same as those in Figure 7A and 7B, respectively.

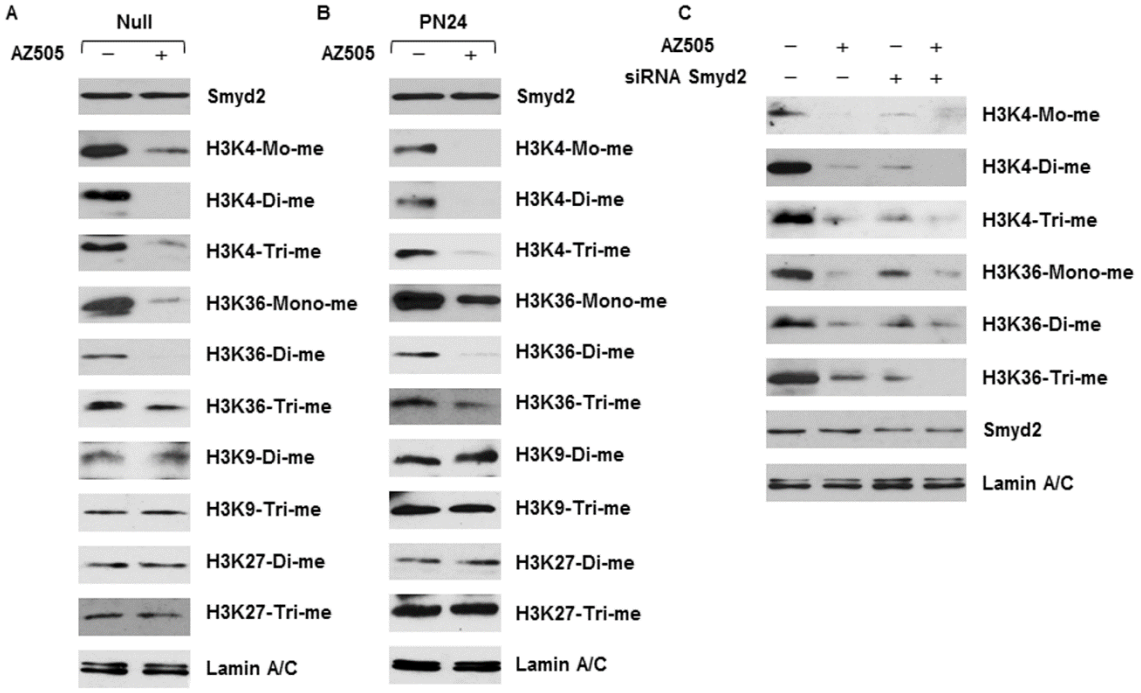
Supplemental Figure 6




**Supplemental Figure 6. The expression of STAT3 and NF-κB target genes were analyzed by qRT-PCR in renal epithelial cells.** (**A**) The expression of CyclinD1, c-Myc, and TNFα was increased in *Pkd1* null cells compared to *Pkd1* WT cells. Relative expression level of these genes was normalized to actin. (**B** and **C**) The mRNA expression of STAT3 and NF-κB target genes was decreased in *Pkd1* null cells treated with Smyd2 siRNA for 24 hours (**B**) and AZ505 for 2 hours (**C**) compared to controls. Relative expression levels of these genes were normalized to actin. (**D**) The mRNA expression of STAT3 and NF-κB target genes, CyclinD1, c-Myc, and TNFα, were decreased in PN7 kidneys from

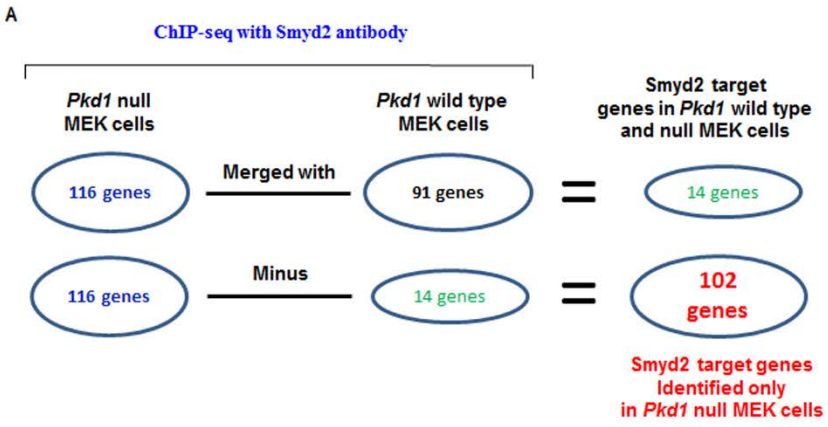
*Pkd1*$^{flox/flox}$:*Smyd2*$^{flox/flox}$:*Ksp-Cre* neonates compared to kidneys from aged matched *Pkd1*$^{flox/flox}$:*Smyd2*$^{+/+}$:*Ksp-Cre* (Smyd2$^{+/+}$) neonates. Error bar represents SEM.

Supplemental Figure 7



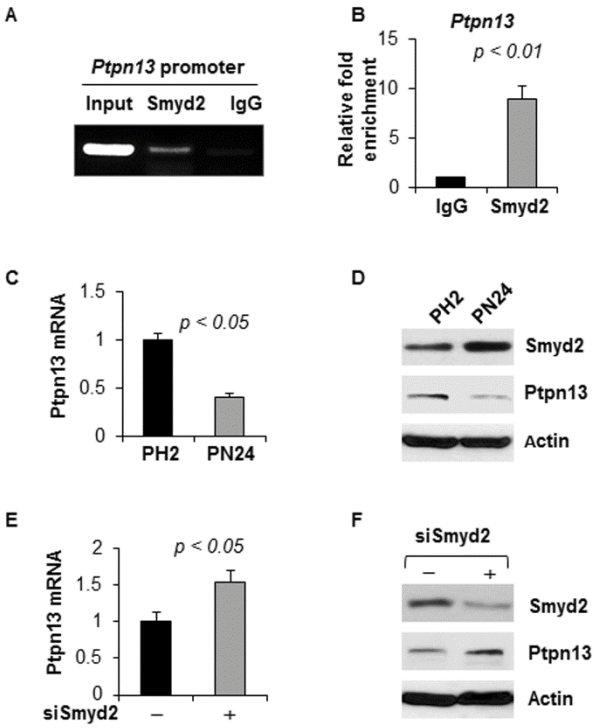

**Supplemental Figure 7. Histone methylation was changed in cystic renal epithelial cells**. (**A** and **B**) Treatment with AZ505 decreased the mono-, di-, or tri-methylation of histone H3 at lysine K4 and K36 in the nuclear fraction of *Pkd1* null MEK cells (**A**) and *Pkd1* homozygous PN24 cells (**B**), but did not affect the methylation of H3 at K9 and K27 in these cells. (**C**) Knockdown of Smyd2 decreased the mono-, di-, or tri-methylation of H3K4 and H3K36 in the nuclear fraction of *Pkd1* null MEK cells. Treatment with AZ505 only slightly further decreased the mono-, di-, or tri-methylation of H3K4 and H3K36 in the nuclear fraction of Smyd2 knockdown cells.

Supplemental Figure 8



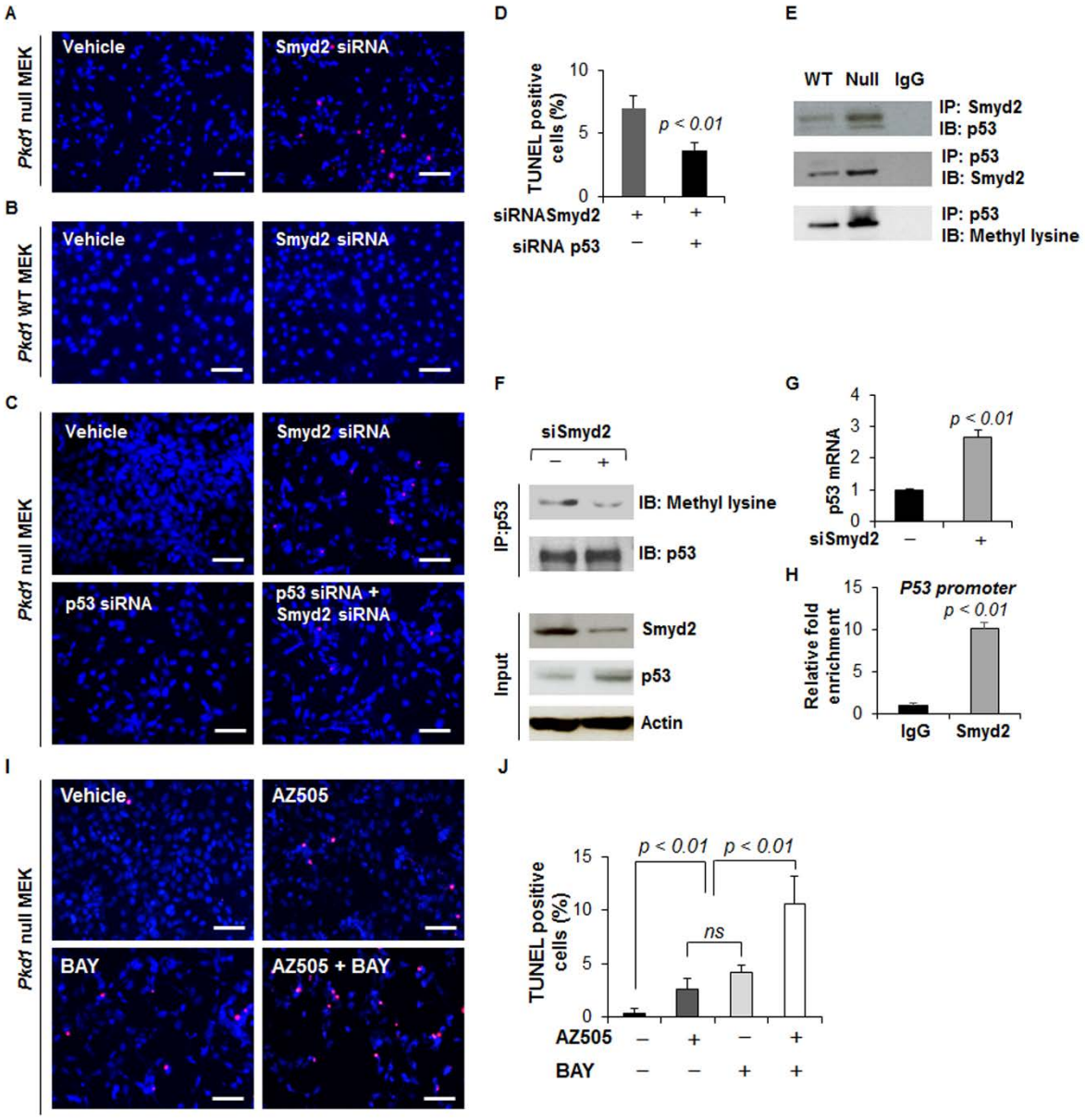

**Supplemental Figure 8. A schematic diagram depicting the Smyd2 ChIP-seq data.** We identified 91 potential Smyd2 target genes in *Pkd1* wild type MEK cells and 116 potential Smyd2 target genes in *Pkd1* null MEK cells. There were 14 genes identified in both *Pkd1* wild type and null MEK cells. Thus, the number of potential Smyd2 target genes in *Pkd1* null MEK cells are 116 - 14 = 102 genes.

Supplemental Figure 9




**Supplemental Figure 9. The novel Smyd2 target Ptpn13 was downregulated by Smyd2 in *Pkd1* mutant renal epithelial cells.** (**A and B**) Smyd2 bound to the promoter of Ptpn13 as examined by ChIP assay (A) and ChIP-qPCR assay (B), which was performed with anti-Smyd2 antibody or normal goat IgG in *Pkd1* null MEK cells. (**C and D**) qRT-PCR (C) and western blot (D) analysis of the relative mRNA and protein level of Ptpn13 in *Pkd1* heterozygous PH2 (PH2) cells compared to that in *Pkd1* homozygous PN24 (PN24) cells. (**E and F**) qRT-PCR (E) and western blot (F) analysis of the relative mRNA and protein level of Ptpn13 in PN24 cells with or without knockdown of Smyd2 with siRNA.

Supplemental Figure 10




**Supplemental Figure 10. Smyd2 regulates p53 dependent cystic renal epithelial cell apoptosis.** (**A** and **B**) Knockdown of Smyd2 with siRNA induced apoptosis in *Pkd1* null MEK cells, but not that in *Pkd1* WT MEK cells, as detected by TUNEL assay. Scale bars, 100 μm. (**C** and **D**) Knockdown of Smyd2 induced apoptosis in *Pkd1* null MEK cells, while knockdown of p53 reduced knockdown of Smyd2 induced apoptosis in these cells. Scale bars, 100 μm. (**E**) Smyd2 interacted with p53 in *Pkd1* WT MEK and *Pkd1* null MEK cells as examined by co-immunoprecipitation assay with anti-Smyd2 and anti-p53 antibody. The methylation of p53 was examined by immunoprecipitation with anti-p53 antibody and then

blotted with anti-p53 and anti–pan methyl-lysine antibody in WT MEK and *Pkd1* null MEK cells. IgG was used as a negative control. (**F**) Western blot analysis of p53 and methyl-p53 in *Pkd1* null MEK cells with or without knockdown of Smyd2 with siRNA. (**G**) qRT-PCR analysis of relative p53 mRNA expression in *Pkd1* null MEK cells with or without knockdown of Smyd2 with siRNA. (**H**) Smyd2 bound to the promoter of p53. ChIP–qPCR assay was performed with anti-Smyd2 antibody or normal goat IgG in *Pkd1* null MEK cells. (**I** and **J**) NFκB was involved in Smyd2 mediated cell death in renal epithelial cells. Treatment with Smyd2 inhibitor AZ505 alone or NFκB inhibitor BAY-11-7085 alone induced renal epithelial cell death in *Pkd1* null MEK cells, whereas treatment with both AZ505 and BAY-11-7085 induced more cell death in *Pkd1* null MEK cells compared to those in AZ505 and BAY-11-7085 alone treated cells, as detected by TUNEL assay. Scale bars, 100 μm.

**Supplemental Table 1. The primers used for quantitative real time PCR and ChIP-PCR.**

| Gene name | Forward (5'- 3') | Reverse (5'- 3') |
|---|---|---|
| ***Smyd2*** | TTCTTTACCTGTGAGTGCCG | GTGCGTATCTGACCATGTCTC |
| ***Tnfaip3*** | ACAGGACTTTGCTACGACAC | CTGAGGATGTTGCTGAGGAC |
| ***Bcl2*** | GGACTTGAAGTGCCATTGGTA | GTTATCATACCCTGTTCTCCCG |
| ***Cyclin D1*** | GCCCTCCGTATCTTACTTCAAG | GCGGTCCAGGTAGTTCATG |
| ***c-Myc*** | GCTGTTTGAAGGCTGGATTTC | GATGAAATAGGGCTGTACGGAG |
| ***c-Fos*** | CCTTTGTCTTCACCTACCCTG | CTTGCCTTCTCTGACTGCTC |
| ***Tnfa*** | CTTCTGTCTACTGAACTTCGGG | CAGGCTTGTCACTCGAATTTTG |
| ***Bicc1*** | ATGCTCTCAACACTTCGGTC | CAGTAGAGGGAAGCATAAGGTG |
| ***Zfp423*** | ATCGGTGAAAGTTGAAGAGGG | ACTTGTCACGCTGTTCCTG |
| ***Foxp3*** | AAGTACCACAATATGCGACCC | TCTGAAGTAGGCGAACATGC |
| ***Bax*** | TTGGAGATGAACTGGACAGC | CAGTTGAAGTTGCCATCAGC |
| ***Pkhd1*** | AGAAACACGGGATGGCTATG | ACAGGCAAGACAACGAAGG |
| ***Ahi1*** | CTTTTCTGGGAGTGAGGATGG | AAGGCAACCATATTCTCCAGG |
| ***Pkd1*** | CCCCGAATGTGGTTTCTATGG | GCCGTCCGATGTATGACTGC |
| ***Actin*** | AAGAGCTATGAGCTGCCTGA | TACGGATGTCAACGTCACAC |
| ***P53 promoter*** | AACACGGTGGTGCGATACCAAG | CCAACACGGGCCCCTAAGTTC |
| ***P53 promoter-NC*** | CACAGGCCTTTAATCCCAGA | CGGCTTCTTCTCAGTCATCC |
| ***Smyd2 promoter*** | AACCCTCTGCACACCAAACTTC | GGAACGCCAAGGAGAAAGC |