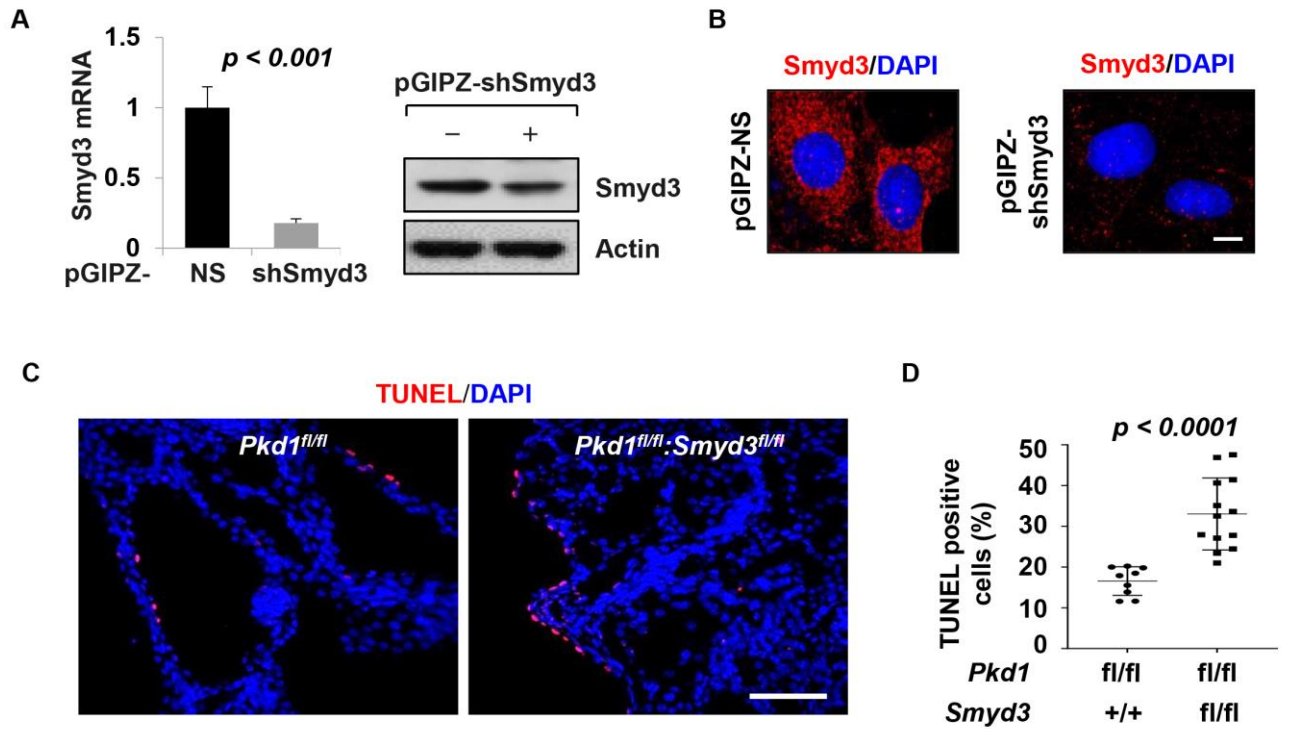
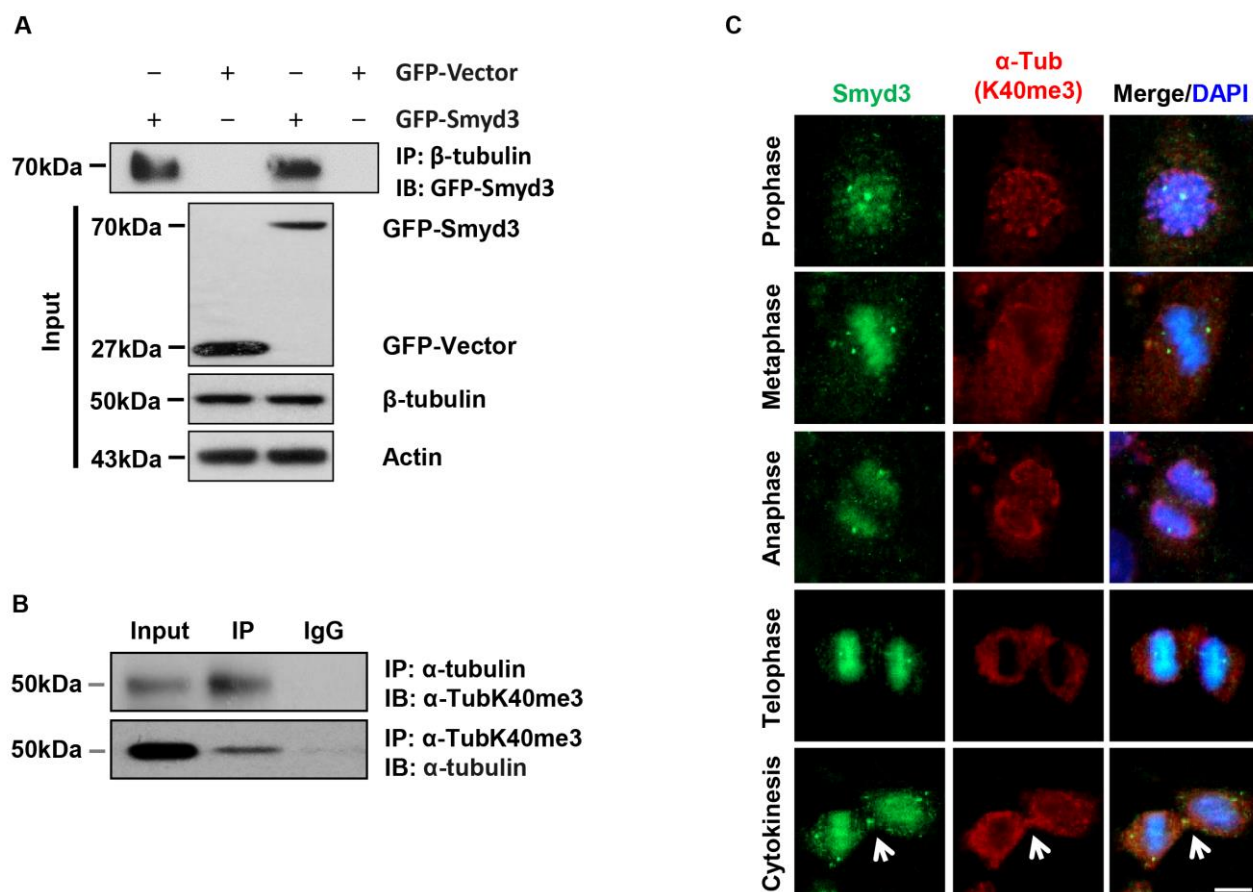


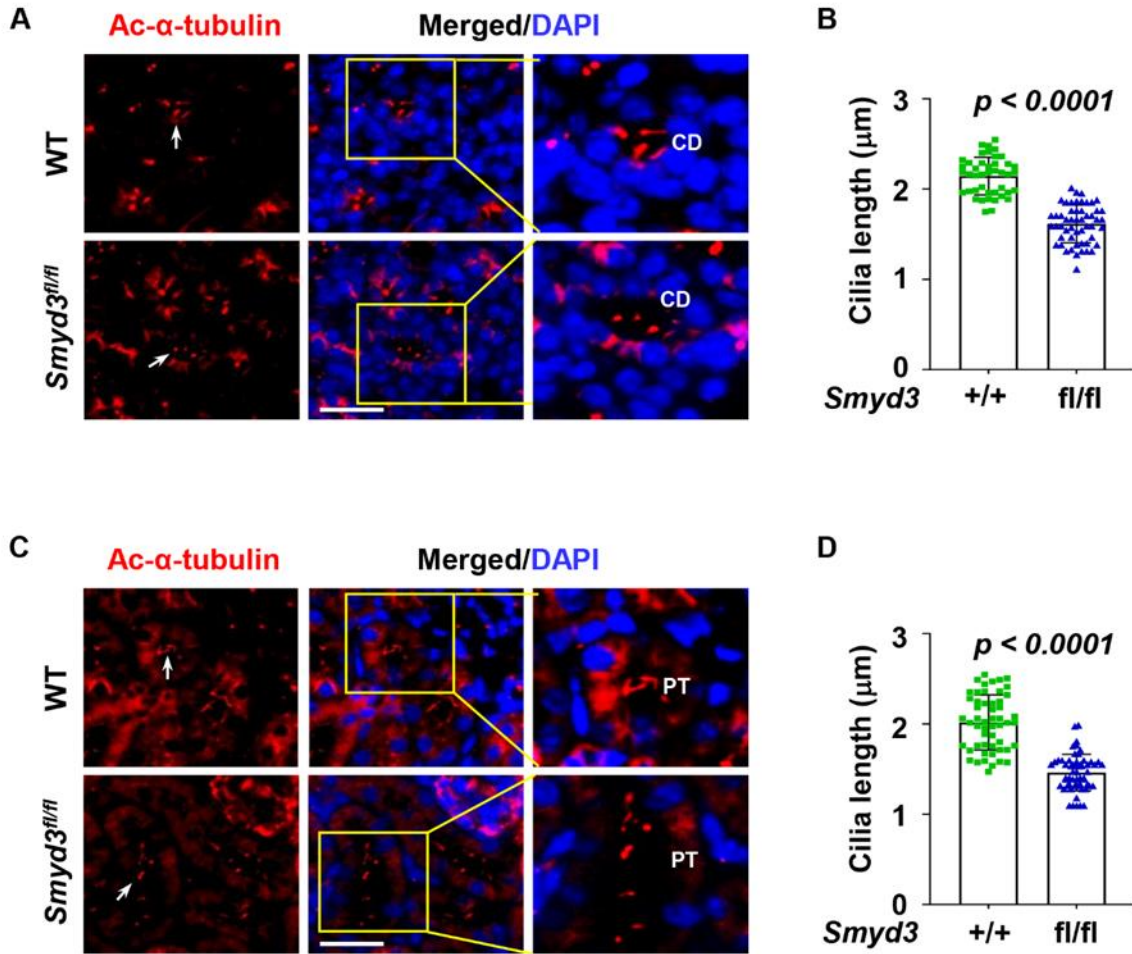
**Supplemental Figure S1.** Single conditional knockout of SMYD3 has no kidney phenotype. (A) Western blot analysis of SMYD3 expression in P7 kidneys from *Pkd1<sup>+/+</sup>;Smyd3<sup>+/+</sup>;Ksp-Cre* wild type (WT) neonates and *Pkd1<sup>+/+</sup>;Smyd3<sup>fl/fl</sup>;Ksp-Cre* SMYD3 single knockout (*Smyd3<sup>fl/fl</sup>*) neonates. (B) Representative kidneys from WT and SMYD3 single knockout neonates. Ruler tick marks are in inches. (C) KW/BW ratios were not significantly different in P7 *Pkd1<sup>+/+</sup>;Smyd3<sup>+/+</sup>;Ksp-Cre* versus *Pkd1<sup>+/+</sup>;Smyd3<sup>fl/fl</sup>;Ksp-Cre* neonates. (D) Representative images for H&E-stained kidney sections from *Pkd1<sup>+/+</sup>;Smyd3<sup>+/+</sup>;Ksp-Cre* and *Pkd1<sup>+/+</sup>;Smyd3<sup>fl/fl</sup>;Ksp-Cre* neonates. Images demonstrate non-cystic kidneys at P7. Scale bar, 1000  $\mu$ m.



**Supplemental Figure S2.** Double conditional knockout of *SMYD3* and *PKD1* induced cyst-lining epithelial cell apoptosis. (**A** and **B**) Mouse IMCD3 cells were transduced with Lentivector-mediated *SMYD3* shRNA, pGIPZ-sh*SMYD3*, and the control vector, pGIPZ-NS, respectively. The knockdown efficiency of *SMYD3* was confirmed by qRT-PCR and western blot (**A**), and by immunostaining (**B**) with *SMYD3* antibody (red) and DAPI (blue). Scale bars, 10  $\mu$ m. (**C** and **D**) Knockout of *SMYD3* induced cyst-lining epithelial cell death in kidneys from P7 *Pkd1<sup>fl/fl</sup>:Smyd3<sup>fl/fl</sup>:Ksp-Cre* neonates. Representative images (**C**), and quantitative analysis (**D**) for the percentage of apoptotic positive cells in kidneys from *Pkd1<sup>fl/fl</sup>:Smyd3<sup>+/+</sup>:Ksp-Cre* neonates, as detected by TUNEL assay. Scale bars: 20  $\mu$ m.



**Supplemental Figure S3.** SMYD3 forms a complex with  $\alpha$ -tubulin and  $\beta$ -tubulin. (A) Co-immunoprecipitation analysis reveals the interaction between GFP-SMYD3 and  $\beta$ -tubulin in HEK293T cells, and endogenous SMYD3 and  $\beta$ -tubulin in PN24 cells. (B) Co-immunoprecipitation analysis reveals the interaction between  $\alpha$ -tubulin and methylated tubulin at K40 in HEK293T cells. (C) SMYD3 co-localizes with methylated  $\alpha$ -tubulin during cytokinesis. Representative images of IMCD3 cells co-stained with methylated  $\alpha$ -tubulin antibody  $\alpha$ -TubK40me3 (red), and Smyd3 (green). Images show co-localization of SMYD3 and  $\alpha$ -TubK40me3 during cytokinesis. Scale bar, 20  $\mu$ m.



**Supplemental Figure S4.** Single conditional knockout of *SMYD3* inhibits ciliogenesis. (**A – D**) *Smyd3* regulates ciliogenesis in kidney tissues. Paraffin-embedded kidney sections were immunostained with the ciliary marker acetylated  $\alpha$ -tubulin (Ac- $\alpha$ -tub) and cilia are indicated by white arrows. Representative images (**A**), and quantitative data (**B**), of cilium length in kidney collecting ducts (CD) in *Pkd1<sup>+/+</sup>:Smyd3<sup>+/+</sup>:Ksp-Cre* (WT, n=45) and *Pkd1<sup>+/+</sup>:Smyd3<sup>fl/fl</sup>:Ksp-Cre* (*Smyd3<sup>fl/fl</sup>*, n=45) kidneys at P7. Representative images (**C**) and quantitative data (**D**), of cilium length in kidney proximal tubules (PT) in *Pkd1<sup>+/+</sup>:Smyd3<sup>+/+</sup>:Ksp-Cre* (WT, n=40) and *Pkd1<sup>+/+</sup>:Smyd3<sup>fl/fl</sup>:Ksp-Cre* (*Smyd3<sup>fl/fl</sup>*, n=50) kidneys at P7. The quantitative data of cilium lengths were measured from 5 to 10 regions for each mouse kidney, (n = 3). Scale bar, 20  $\mu\text{m}$ .