SUPPORTING INFORMATION

Supplemental Figure 1. Hyperproliferation of PC1 depleted cells is dependent on Intβ1. Growth of Pkd1 knockdown IMCD3 (A) or MDCK (B) cells is suppressed by the inhibition of Intβ1 expression. Constitutive specific targeting of *Pkd1* and *Intβ1* was achieved following transduction of IMCD3 with the using the lentivectors VIRHD/P/siPkd1$_{10071}$ or VIRHD/HY/siIntβ1$_{2363}$ and MDCK with the lentivectors VIRHD/P/siPkd1$_{3211}$ or VIRHD/HY/siIntβ1$_{451}$ followed by selection with and puromycin (2µg/ml) or hygromycin (400µg/ml), respectively[5,6,55]. shLuc from the VIRHD/P/Luc850 lentivector was used as control. Subscript numbers indicate the position of the first nucleotide of the 19-mer sequence in the shRNA on the target mRNA with respect to the starting ATG. Cell proliferation assays were performed using crystal violet dye. Statistical comparison by two-way ANOVA with Bonferroni post-test: *$P<0.0001$.

Supplemental Figure 2. Establishment of immortalized mouse F1 collecting duct cells. The F1 cell line was established following the isolation of renal epithelial cells from kidney papillae of *Pkd1$^{fl/fl}$* mice (B6.129S4-Pkd1^{tm2Ggg}/J, Jackson Laboratory) by immortalization with the lentiviral vector VVPW/mTert expressing the murine telomerase reverse transcriptase (mTert) (kindly provided by Dr. Ronald DePinho), similarly to what described by Steele et al.[36]. After three passages, these cells were subjected to high osmolarity medium (700mOsm/kg) for 24 hours to enrich for collecting duct cells. Multiple clones obtained by limiting dilution. (A) Immunodetection of Aqp2 and Tamm-Horsfall glycoprotein (uromodulin: UMOD) in clones 1-12. Positive expression of Aqp2

and absence of UMOD confirmed the collecting duct origin of the cells. Actin expression was determined as loading control on the stripped membrane. B: bulk culture before cloning; I: IMCD3 cells; T: 293T cells; C: UMOD positive urine control. One representative clone (F1) was chosen for subsequent studies. (B) F1 cells form a monolayer with high transepithelial resistance on transwell membrane (>1000 Ohm/cm^2) characteristics of renal collecting duct epithelia.

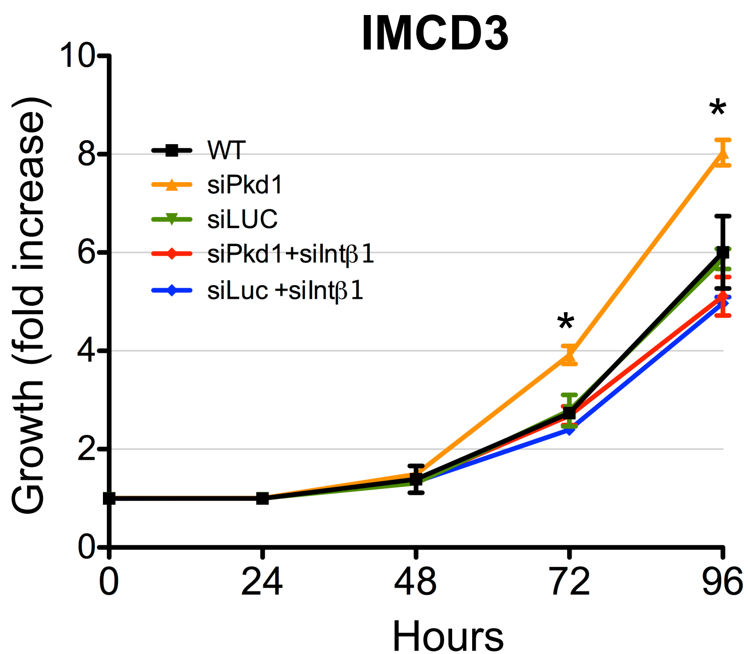
Supplemental Figure 3. Determination of $Pkd1^{fl/fl}$ allele inactivation in kidneys. The genomic structure of the first 5 exons of floxed Pkd1 allele of $Pkd1^{fl/fl}$ (B6.129S4-Pkd1^{tm2Ggg}/J) mice is shown indicating the position of the loxP sites at the 5' of exon 2 and exon 5 and the primers used for the PCR detection of the inactivated alleles. The corresponding transcripts originated before (wild-type) or after (excised) the Cre-mediated excision of the floxed region are shown. Semi-quantitative nested PCR. Total RNA was isolated from kidneys of 3-week old mice with the indicated genotypes and cDNA was prepared using the EcoDry Premix (Clontech) according to manufacturer's instructions. A first round PCR was performed for the amplification of the region spanning exon 1 to exon 5 of Pkd1 using primers E1/F (5'-CGTCAATTGCTCCGGCC-3') and E5/R (5'-TCATGGGCAAAGTAGAAGGG-3'). A second nested PCR was performed on the 1/20th of the product of the first PCR to amplify the region spanning exon 1 and the spliced E1-E5 exons using the E1/F and the E1-5/R primers (E1-5/R: 5'-GACATATTCCTCAC*AGCGCGGT*-3'; underlined sequence is on exon 5, italicized sequence is on exon 1). Amplification of the aquaporin-2 gene transcript (using primers: mAqp2/F2: 5'-GCATTGGCACCCTGGTTCA-3' and mAqp2/R1: 5'-

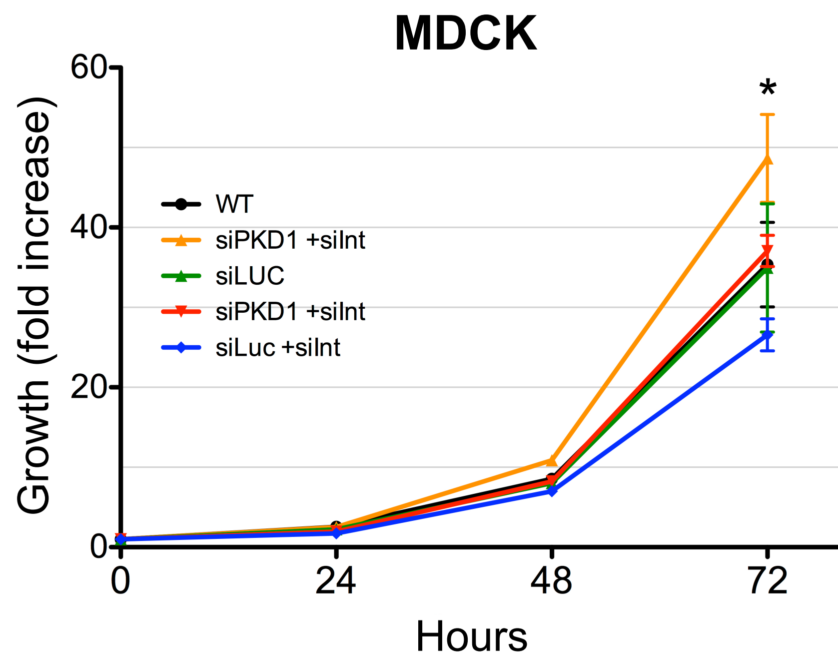
CATTGTTGTGGAGAGCATTGAC-3’ was performed in parallel from the same cDNAs to normalize for the number of collecting duct cells. Separation of the amplified products on 2% TBE agarose gel revealed comparable levels of amplified 85bp and the 221bp bands from the excised Pkd1 and the aquaporin-2 gene, respectively, indicating an equally efficient inactivation of the Pkd1 gene in kidneys from single Pkd1-KO and DKO animals.

Supplemental Figure 4. Representative images of PAS-stained kidneys of Pkd1-KO and DKO at 6 weeks (top panel) and at 7 months (bottom panel), showing much less variability of cystic phenotype in DKO in comparison to the Pkd1-KO kidneys.

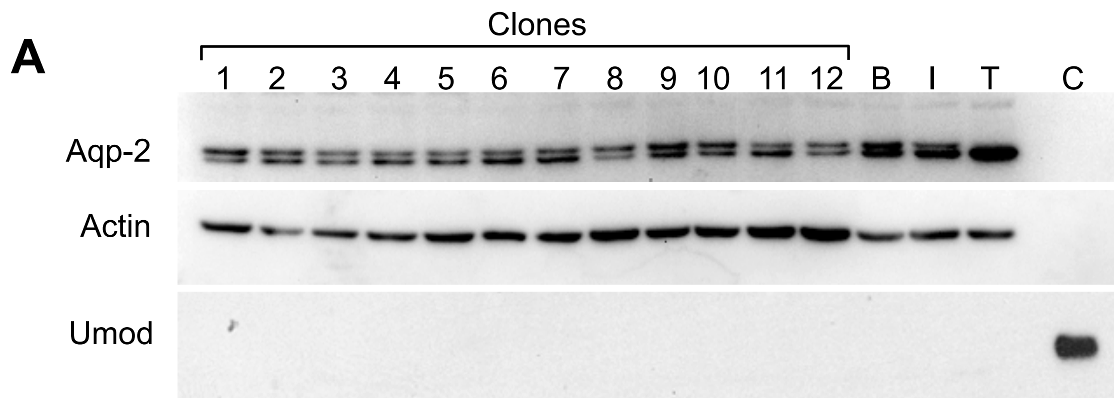
Supplemental Figure 5. Cysts form in the collecting ducts. Co-staining with Aqp-2, DBA, LTA, or Tamm-Horsfall (THP) show co-localization of Aqp-2 and DBA with cystic cells but not LTA or THP. Counts were performed on four to eight chosen fields/slide at a magnification of 200X. Over 6 slides per kidney from each genotype (n=3) at 4 weeks of age were scored. Of a count >60 (Pkd1-KO) and >10 (DKO) cysts all were DBA positive.

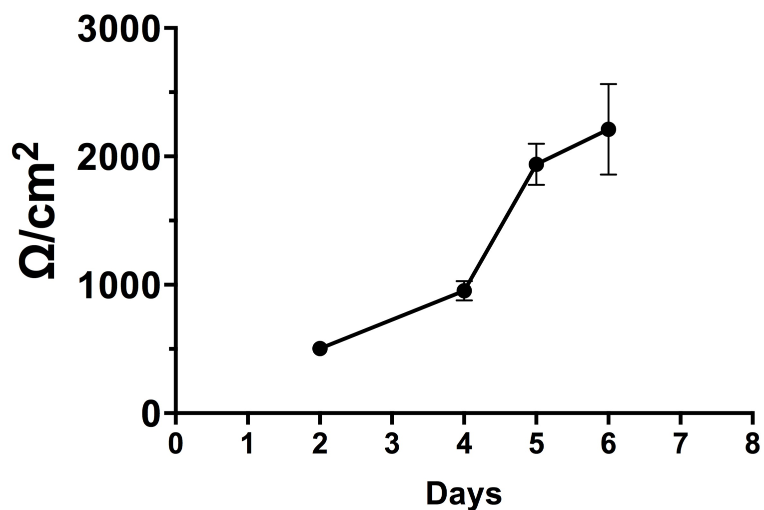
Supplemental Figure 1





Supplemental Figure 2





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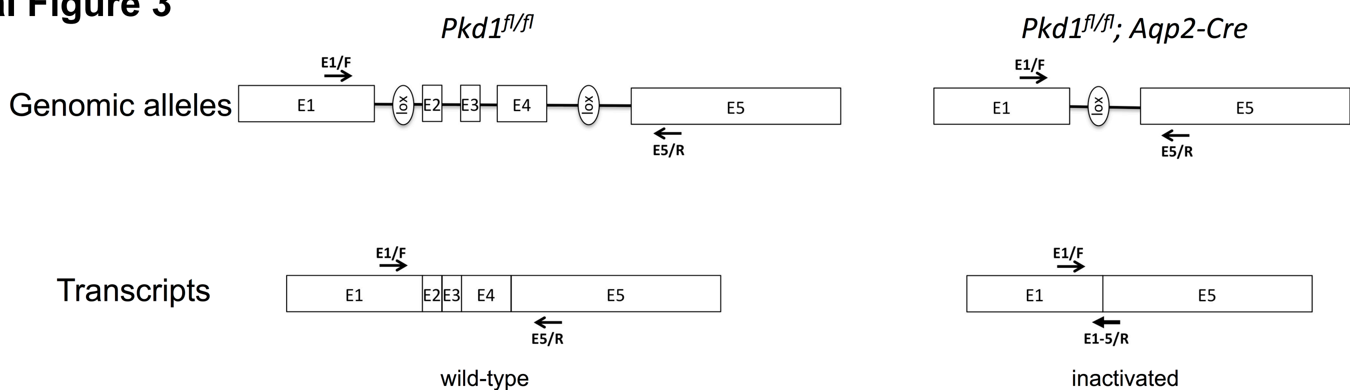

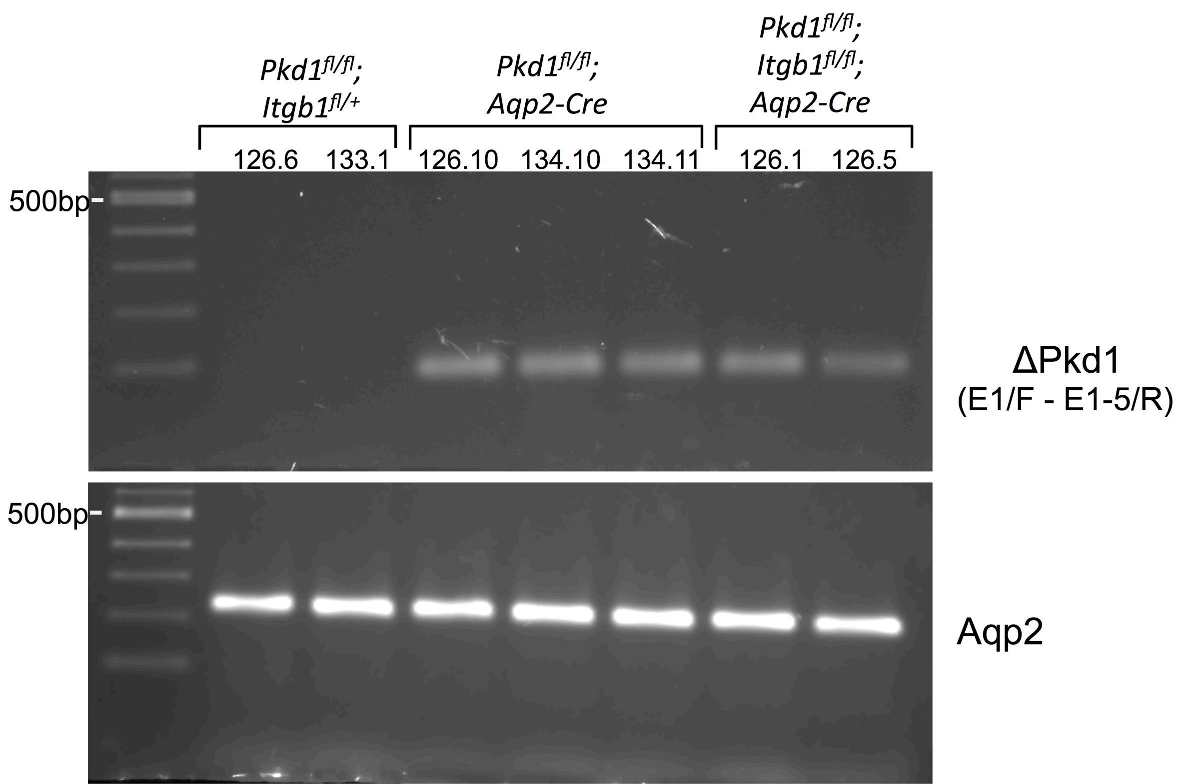

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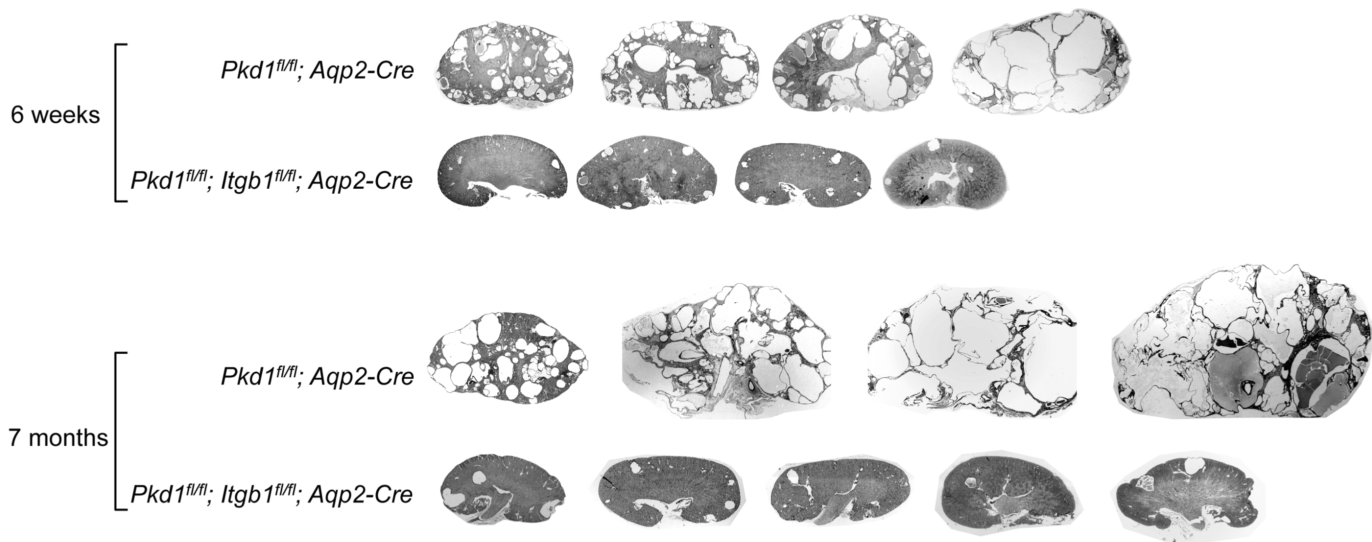

Supplemental Figure 5