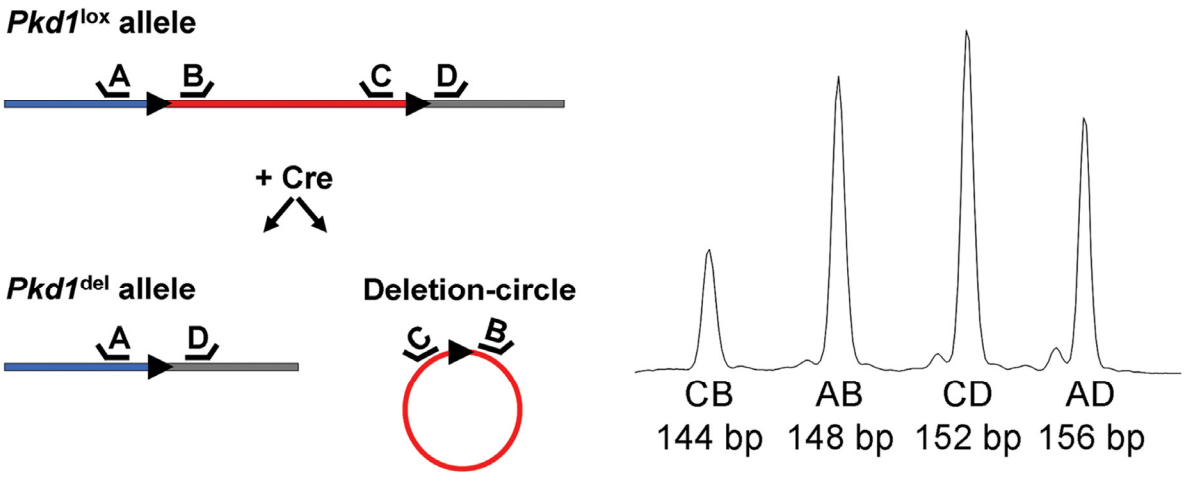
Supplemental Figure 1.

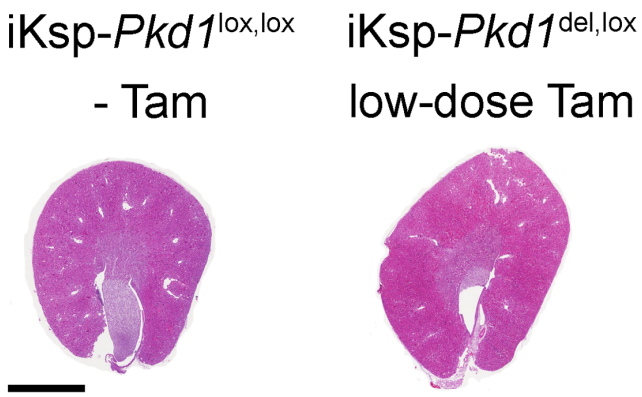


Supplemental Figure 1. Extension-multiplex ligation-dependent probe amplification (eMLPA) strategy used to quantify Cre-mediated recombination efficiency[1]. Probes with universal end-sequences that are used for amplification are hybridized onto the target DNA as indicated. After an extension step of the forward probe using Stoffel-Taq polymerase to fill the gaps between the probes, a ligation step finalizes the templates for PCR. In a PCR reaction using a single primer pair, of which the forward primer is fluorescently labeled, all products are simultaneously amplified. The products are separated and visualized with a capillary sequencer. The relative peak-heights are used to quantify the Cre-mediated recombination efficiency.

REFERENCES

1. Leonhard WN, Roelfsema JH, Lantinga-van Leeuwen IS, Breuning MH, Peters DJ: Quantification of Cre-mediated recombination by a novel strategy reveals a stable extra-chromosomal deletion-circle in mice. *BMC Biotechnol* 8:18, 2008

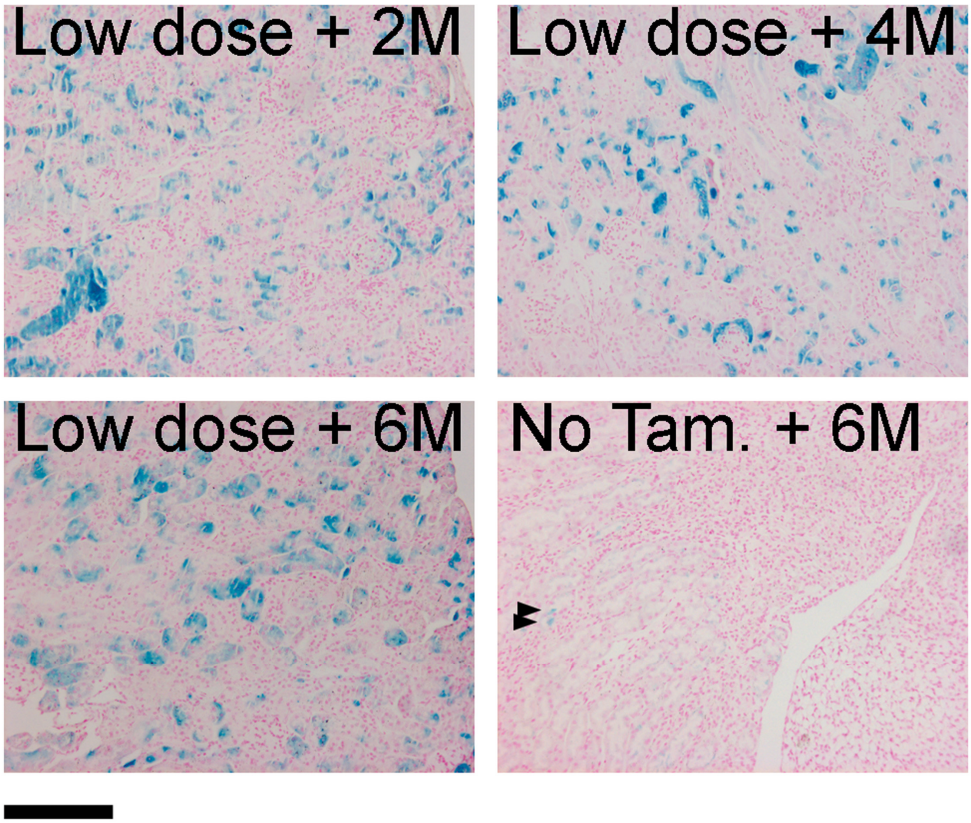
Supplemental Figure 2.



Supplemental Figure 2. Scattered *Pkd1* deletion does not lead to a cystic phenotype in iKsp-*Pkd1*^{del,lox} mice within 6 months.

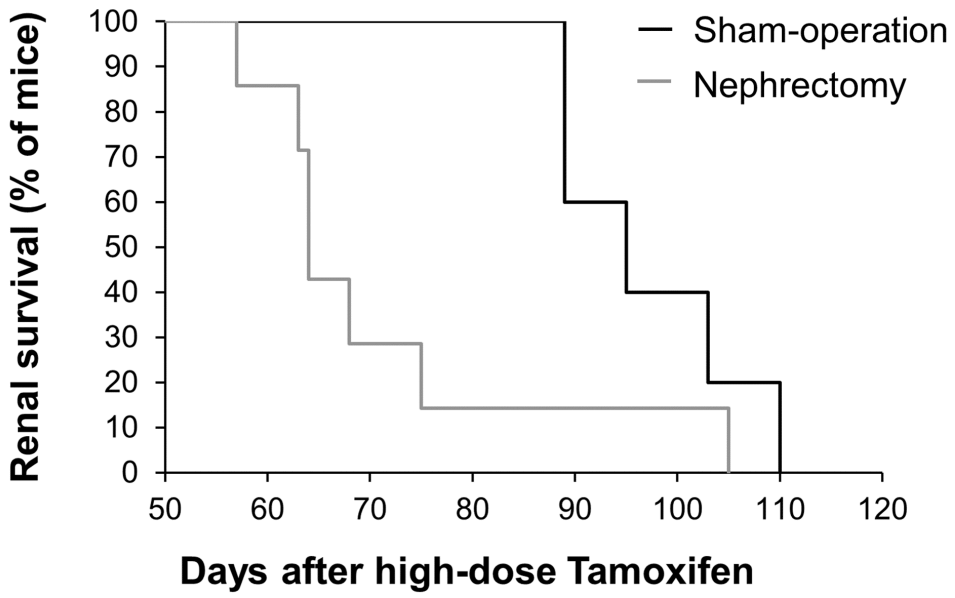
Left; 7-month old iKsp-*Pkd1*^{lox,lox} mouse without tamoxifen (- Tam). Right; 7-month old iKsp-*Pkd1*^{del,lox} mouse treated with low-dose tamoxifen at PN40 (i.e. 0.25 mg; low-dose Tam). No cysts could be observed in these kidney sections. Scale bar, 2 mm.

Supplemental Figure 3.



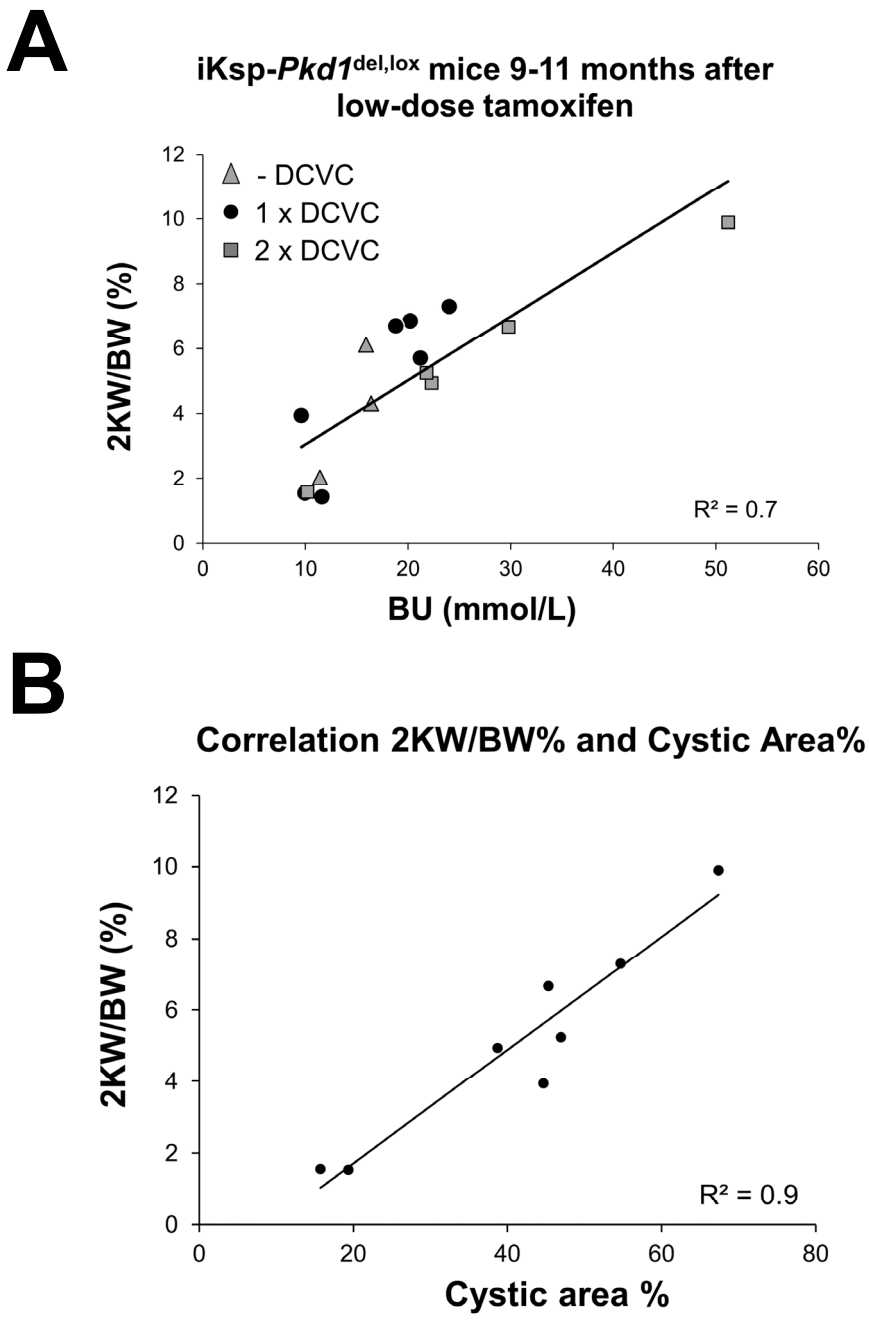
Supplemental figure 3. The pattern of *Pkd1*-deleted cells is stable over time. iKsp-*Pkd1*^{lox,lox} mice were crossbred with a LacZ reporter strain and then treated with low-dose tamoxifen (i.e. 0.25 mg). Kidney sections were stained for LacZ at 2, 4 and 6 months (M) following treatment. Note that LacZ-positive cells were rare in the mice that were not treated with tamoxifen (arrowheads). Scale bar 200 μm

Supplemental Figure 4.



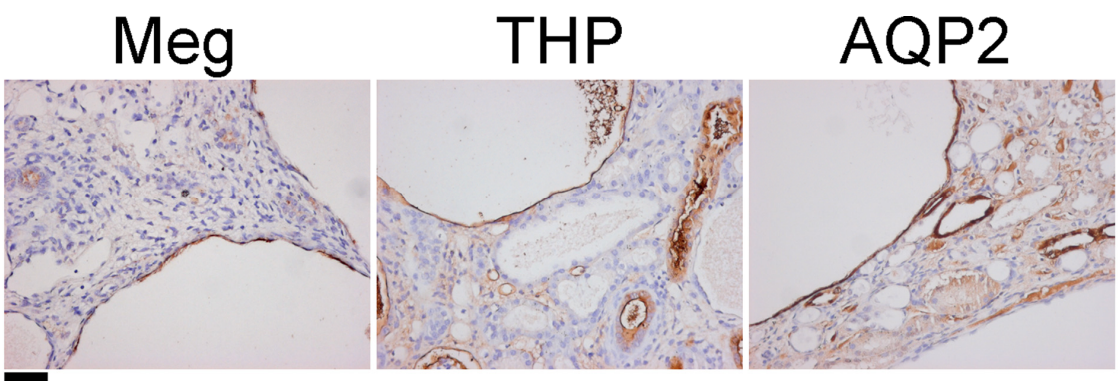
Supplementary Figure 4. Unilateral nephrectomy accelerates the progression of PKD in high-dose (5 mg) tamoxifen treated iKsp-*Pkd1*^{lox,lox} mice. One week following high-dose (5 mg) tamoxifen treatment at PN40, mice underwent unilateral nephrectomy or a sham operation. Mice were sacrificed when the blood urea concentration was higher than 20 mmol/L, indicating the onset of renal failure. Nephrectomized mice (n=7) entered renal failure faster than the sham operated mice (n=5); $p<0.05$, generalized Wilcoxon test.

Supplemental Figure 5.



Supplementary Figure 5. Increased kidney weight correlates with renal function decline and cystic index. **(a)** 2KW/BW% correlates with renal function (expressed as Blood Urea (BU) concentration in mmol/L) of low-dose tamoxifen treated iKsp-*Pkd1*^{del,lox} mice with zero, one or two additional DCVC treatments and sacrificed 9-11 months after *Pkd1*-deletion (also shown in Figure 4D). **(b)** 2KW/BW% also correlated with the cystic index (expressed as % of cyst area)

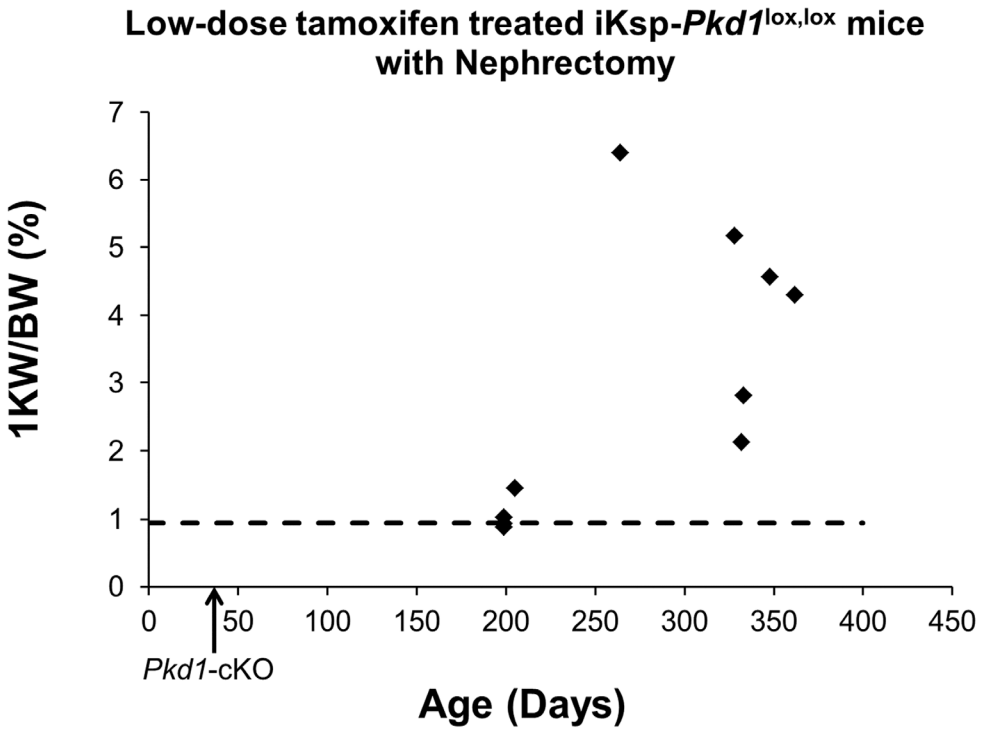
Supplemental Figure 6.



Supplementary Figure 6. Segment specific marker staining on renal sections of mice with scattered *Pkd1*-deletion with severe PKD.

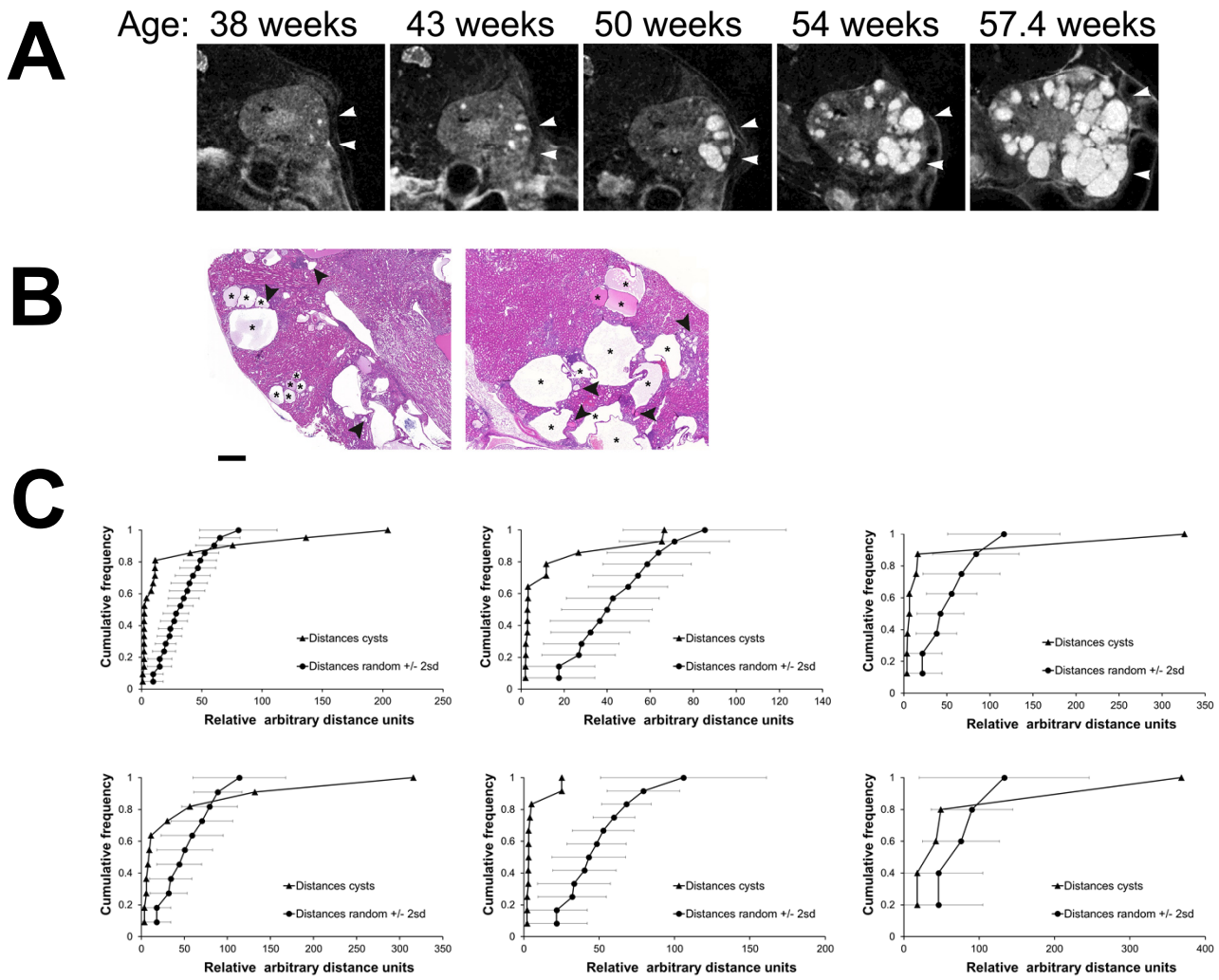
Immunostaining of tubular segment-specific markers reveals cysts that were derived from all segments. The anti-Megalin (Meg) antibody was used as a marker for the proximal tubules, the anti-Tamm Horsfall Protein (THP) antibody was used as a marker for the distal tubules and loops of Henle, and the anti-Aquaporin-2 (AQP2) antibody was used as a marker for the collecting ducts. Note the dilated tubules and smaller cysts near the larger cysts. Scale bar, 50 μm

Supplemental Figure 7.



Supplementary Figure 7. Delayed cyst formation in mice with scattered *Pkd1*-deletion and unilateral nephrectomy. The 1KW/BW% of the iKsp-*Pkd1*^{lox,lox} mice with low-dose tamoxifen at PN40 and unilateral nephrectomy from figure 5A, plotted against age. At an age of 200 days, none of the mice developed severe PKD (n=4) whereas all mice developed severe PKD thereafter (n=6).

Supplemental Figure 8.



Supplementary Figure 8. Clustered cyst formation in mice. **(a)** MRI analysis of one iKsp-*Pkd1*^{lox,lox} mouse treated with low-dose tamoxifen at 6 weeks of age (PN40-42). Transverse T2-weighted scans of the same region of the kidney at 38, 43, 50, 54 and 57.4 weeks of age. Arrowheads point at clusters of cysts that are growing, both in size and number of visible cysts. The mouse was euthanized at an age of 57.4 weeks with a 2KW/BW% of 6.4 (also indicated in figure 4D) and BU of 19.4 mmol/L. **(b)** Images of two different iKsp-*Pkd1*^{del,lox} mice treated with low-dose tamoxifen (at PN40) and DCVC (at PN50) and euthanized ten months after the tamoxifen treatment. Some regions contain many cysts, whereas other regions are not cystic. Note that small cysts are frequently located near larger cysts (examples indicated by arrowheads). Scale bar, 500 μm. (c) Nearest neighbor analysis of kidney sections from 6 mildly affected mice in which distances of nearest neighboring cysts within a kidney section are compared to nearest distances of the average of twenty simulations of a similar number of randomly distributed points within a similar sized area. When the plot of nearest distances of cysts is located left from the random plot, it indicates a clustered distribution of cysts. Error bars of the random plots are 2x the standard deviation (2sd) of 20 random simulations.