Supplemental Methods

Ultrasound Acquisition with Robotic Scanner

Robotic US was performed using a Vega imager (SonoVol, Inc., Durham, NC, USA). This system robotically manipulates US transducers underneath rodents which are positioned on an acoustically transparent membrane. The transducers can move in two axes, allowing widefield scans to be acquired[1,2]. Mice were anesthetized with inhaled isoflurane (1.5-2%), chemically depilated if necessary, and placed in the supine position on the imaging bay with a thin layer of warm water for acoustic coupling. Widefield 3D B-mode imaging was performed in rapid succession with two on-board transducers: a 128-element linear array (24 MHz), and a dual-element mechanically swept transducer (2 MHz/35 MHz). Elevational frame spacing for these two volumetric acquisitions was 0.1 mm and 0.13 mm, respectively. For contrast enhanced studies, Acoustic Angiography mode was used[3]. A bolus of microbubbles (0.1 mL diluted to 4.16 x 10^9 #/mL in saline), formulated as previously described[2], was infused through the tail vein immediately prior to imaging. Scanning regions of interest (ROIs) were defined across the entire abdomen of the animals using the robotic system's camera display. Following acquisitions, the presence of both kidneys within the ROI was verified by the user. B-mode acquisitions required 24 seconds per transducer, while contrast-enhanced imaging required an additional 200 seconds. An example of ROI placement and data acquisition workflow is provided (Supp. Video 1). Following acquisition of the images, data were analyzed offline within the system's software (SonoEQ™, SonoVol, Inc., Durham, NC, USA).

MRI Acquisition

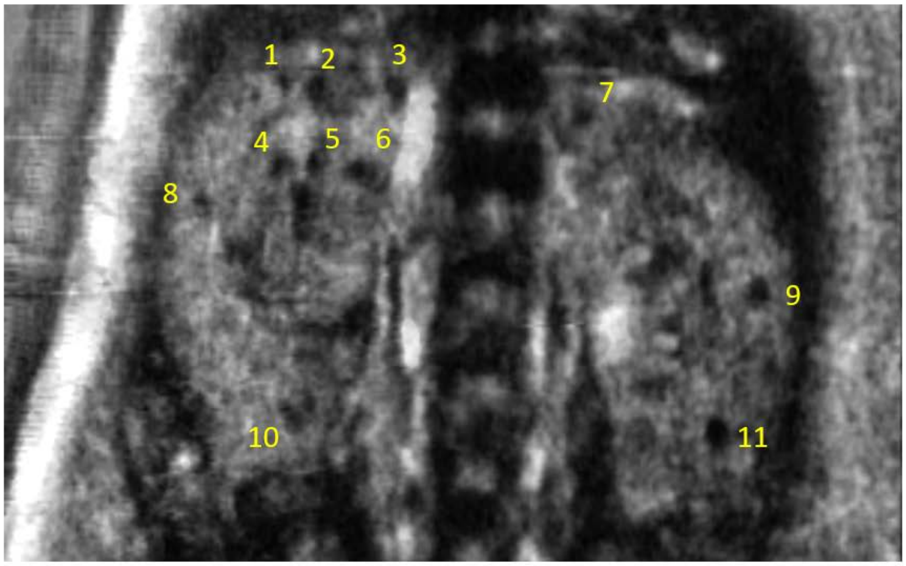
Mice from Cohort 1 were imaged using an Avance DRX 700WB (Bruker BioSpin, Billerica, MA, USA) spectrometer. The scanner has a 16.4 Tesla vertical wide bore magnet and is equipped with a 38-mm-diameter radiofrequency coil. Prior to scanning, the mice were first anesthetized in a chamber using 2.5% isoflurane. The mice were then transferred to the coil's animal holder and anesthesia was maintained throughout the scan at 1.5% isoflurane. A control system (Model 1030; SA Instruments Inc., Stony Brook, New York, USA) was used for respiratory monitoring and gating of the signal generated by a balloon sensor.

Scout images were first obtained in three planes (axial, coronal, and sagittal) to locate the kidneys and prescribe the volumetric scan. Complete coverage of the kidneys was obtained by an axial TurboRARE T2-weighted acquisition reconstructed with 0.1 mm in plane resolution, and 1mm slice thickness (matrix size = 256 x 256 x Z, with Z chosen large enough to cover the full extent of the kidneys). The pulse sequence had the following parameters: TE=25 ms, TR=1500 ms, and FA=180^{o}. After scanning, the animals were removed from the scanner, and placed on a heating pad and allowed to fully recover before returning to their housing cage.

Conventional Ultrasound Acquisition

A transducer attached to a 1-axis linear actuator (Vevo 3100, VisualSonics Inc., Toronto, Canada) was used for acquiring conventional US images on the PKD mouse cohort. The mouse was first anesthetized with isoflurane. Hair on the lower back/flank was shaved and then chemically depilated. The mouse was then placed on the imaging platform with a heating pad and anesthesia maintained through a nosecone (THM150, VisualSonics). Once the mouse was positioned on the platform, acoustic coupling gel (Aquasonic® 100, Parker Laboratories, Inc., Fairfield, NJ) was applied to the mouse's back. Then the transducer (40 MHz, MX550S, VisualSonics), was manually positioned to take one linear step scan over each of the mouse kidneys. Afterward, the gel was wiped off the mouse, and the mouse was returned to its cage to recover. The procedure took approximately 30 min per mouse.

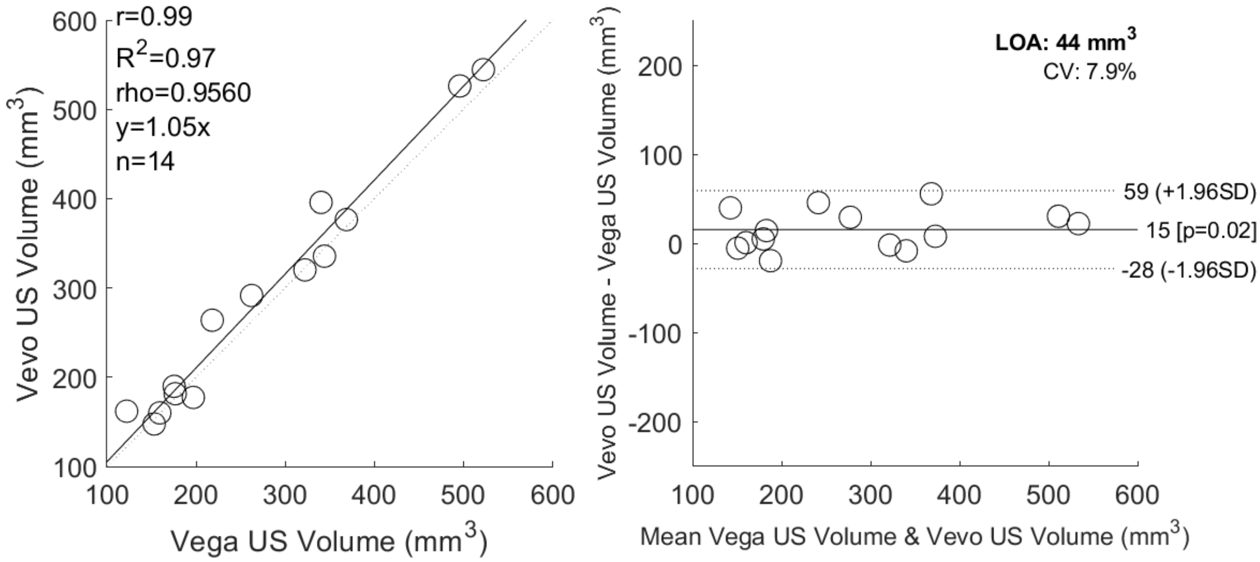
Supplemental Figure S1:



Cyst	Diameter (mm)
1	0.41
2	0.62
3	0.61
4	0.56
5	0.5
6	1.25
7	0.66
8	0.6
9	0.74
10	1.00
11	0.79

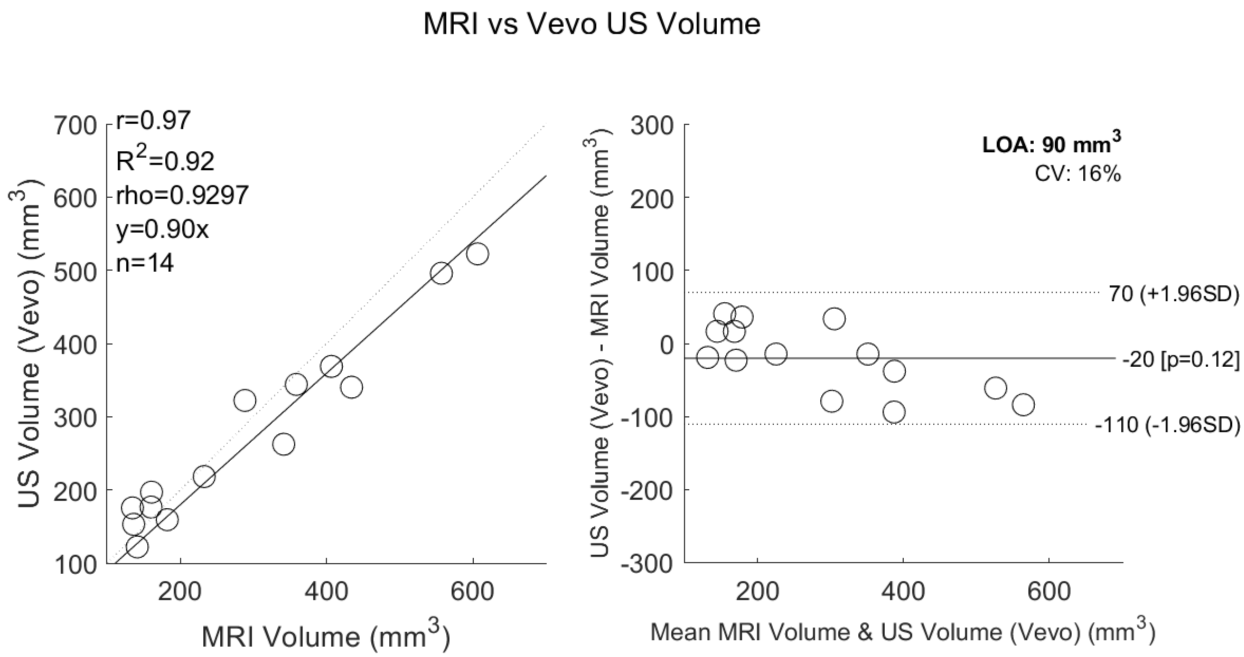
Analysis of cystic diameter measured from a representative coronal plane. 11 cysts were identified and their left-right diameters in the plane were measured using software calipers. Cyst diameter could be utilized in future studies as an additional biomarker for disease progression.

Supplemental Figure S2:



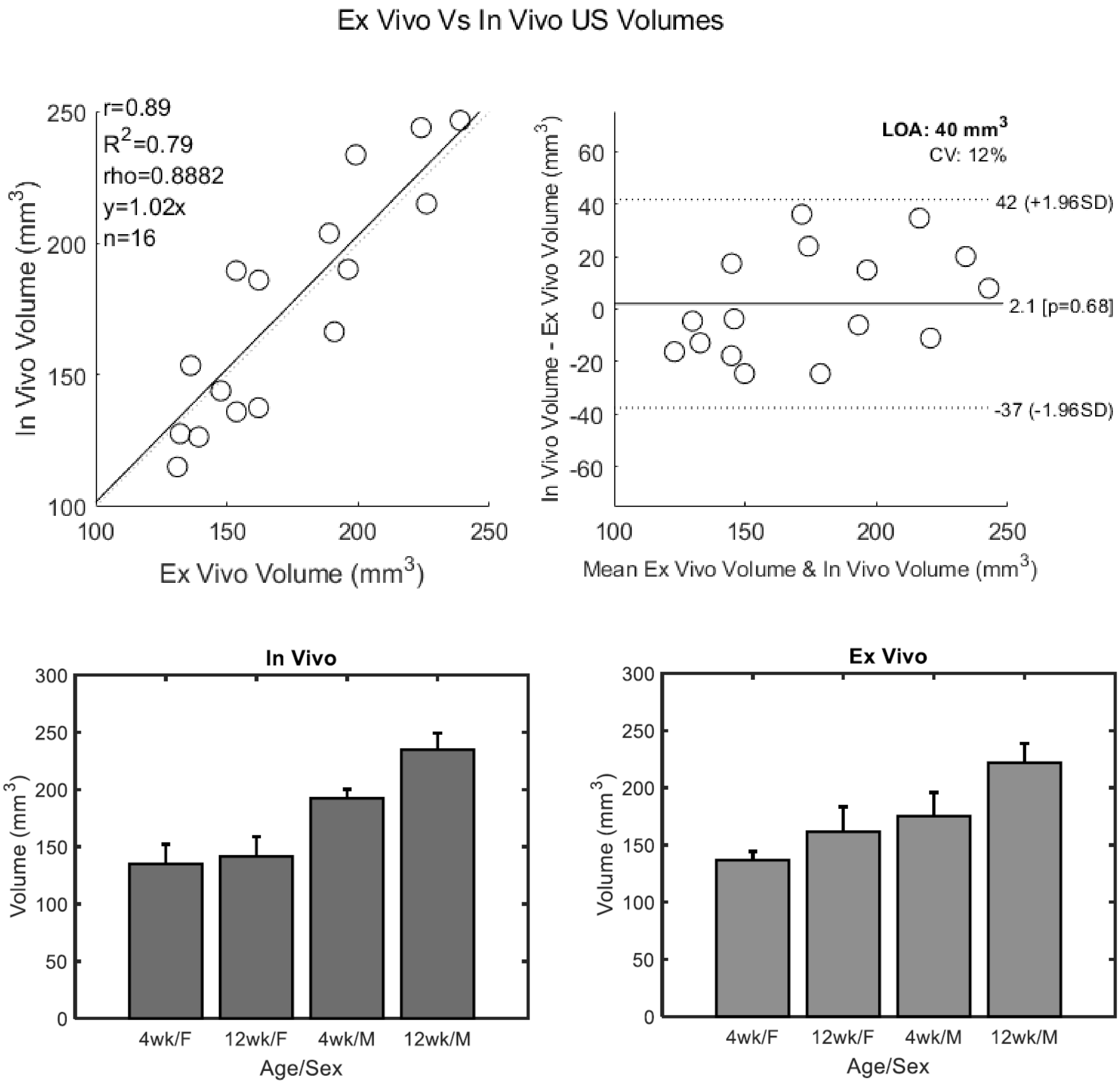
Regression and Bland-Altman plots comparing *in vivo* measurements of kidney volume between robotic ultrasound to conventional ultrasound ("Vega" and "Vevo" systems, respectively). *r*, Pearson correlation coefficient; R^2, coefficient of determination; *rho*, Spearman correlation coefficient; *LOA*, limits of agreement; *CV*, coefficient of variation

Supplemental Figure S3:



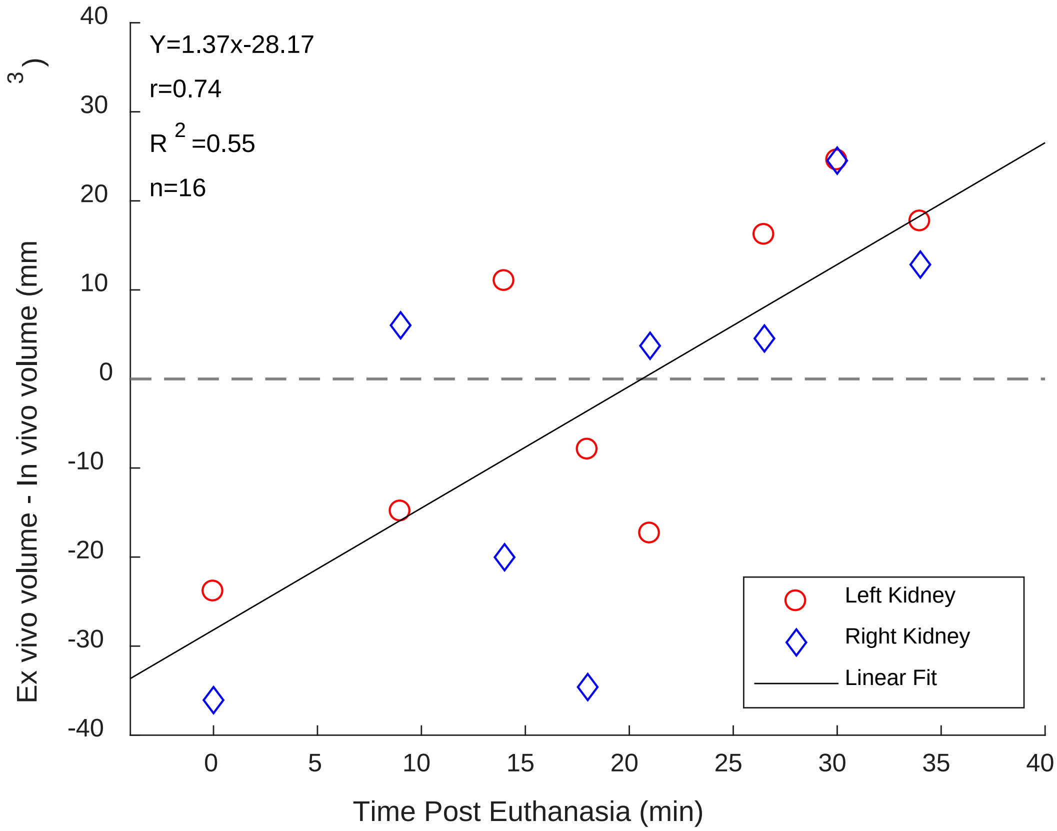
Regression and Bland-Altman plots comparing *in vivo* measurements of kidney volume between conventional ultrasound to MRI. *r*, Pearson correlation coefficient; R^2, coefficient of determination; *rho*, Spearman correlation coefficient; *LOA*, limits of agreement; *CV*, coefficient of variation

Supplemental Figure S4:



Comparing *in vivo* to ex *vivo* kidney volumes measured with robotic US scans. (Top row) Regression and Bland-Altman plots comparing *in vivo* and *ex vivo* kidney volume measurements from the robotic ultrasound scanner. (Bottom row) Bar plots showing ultrasound-derived measurements taken *in vivo* and *ex vivo* of kidney volume for Cohort 2, grouped by age and sex of animal. *r*, Pearson correlation coefficient; R^2, coefficient of determination; *rho*, Spearman correlation coefficient; *LOA*, limits of agreement; *CV*, coefficient of variation; *wk*, weeks; *M*, male; *F*, female.

Supplemental Figure S5:



Scatter plot showing the difference between *in vivo* and *ex vivo* kidney volume as function of time post euthanasia. Both *in vivo* and *ex vivo* measurements were derived from robotic US imaging. *Ex vivo* volume appears to increase if kidneys are excised at a later time following euthanasia. R^2, coefficient of determination; *r*, Pearson's correlation.

Supplementary Table 1: Mice Used for *In Vivo* MRI Validation Studies (Cohort 1)

Mouse ID	60A	60B	570A	570B	601	603	607
Age (weeks)	14	42	14	14	30	26	14
Sex	F	F	M	M	M	F	M
Strain	C57blk6J	C57blk6J	C57blk6J	C57blk6J	C57blk6J	C57blk6J	C57blk6J
Gene Modification	*Pkd1*$^{(RC/RC)}$ *nbce1A*$^{(+/-)}$	*Pkd1*$^{(RC/RC)}$ *nbce1A*$^{(+/+)}$	*Pkd1*$^{(RC/RC)}$ *nbce1A*$^{(+/-)}$	*Pkd1*$^{(RC/+)}$ *nbce1A*$^{(-/-)}$	*Pkd1*$^{(+/+)}$ *nbce1A*$^{(-/-)}$	*Pkd1*$^{(RC/+)}$ *nbce1A*$^{(+/-)}$	*Pkd1*$^{(RC/+)}$ *nbce1A*$^{(+/-)}$

Supplemental References:

1. Czernuszewicz TJ, Papadopoulou V, Rojas JD, Rajamahendiran RM, Perdomo J, Butler J, Harlacher M, O'Connell G, Zukić D, Aylward SR, Dayton PA, Gessner RC: A new preclinical ultrasound platform for widefield 3D imaging of rodents. *Rev. Sci. Instrum.* 89: 075107, 2018

2. Rojas JD, Papadopoulou V, Czernuszewicz TJ, Rajamahendiran RM, Chytil A, Chiang YC, Chong DC, Bautch VL, Rathmell WK, Aylward S, Gessner RC, Dayton PA: Ultrasound Measurement of Vascular Density to Evaluate Response to Anti-Angiogenic Therapy in Renal Cell Carcinoma. *IEEE Trans. Biomed. Eng.* 66: 873–880, 2019

3. Gessner RC, Frederick CB, Foster FS, Dayton PA: Acoustic angiography: A new imaging modality for assessing microvasculature architecture. *Int. J. Biomed. Imaging* 2013: 2013