Brief Communication – Supplementary Information

Vascular endothelial growth factor C therapy for polycystic kidney diseases

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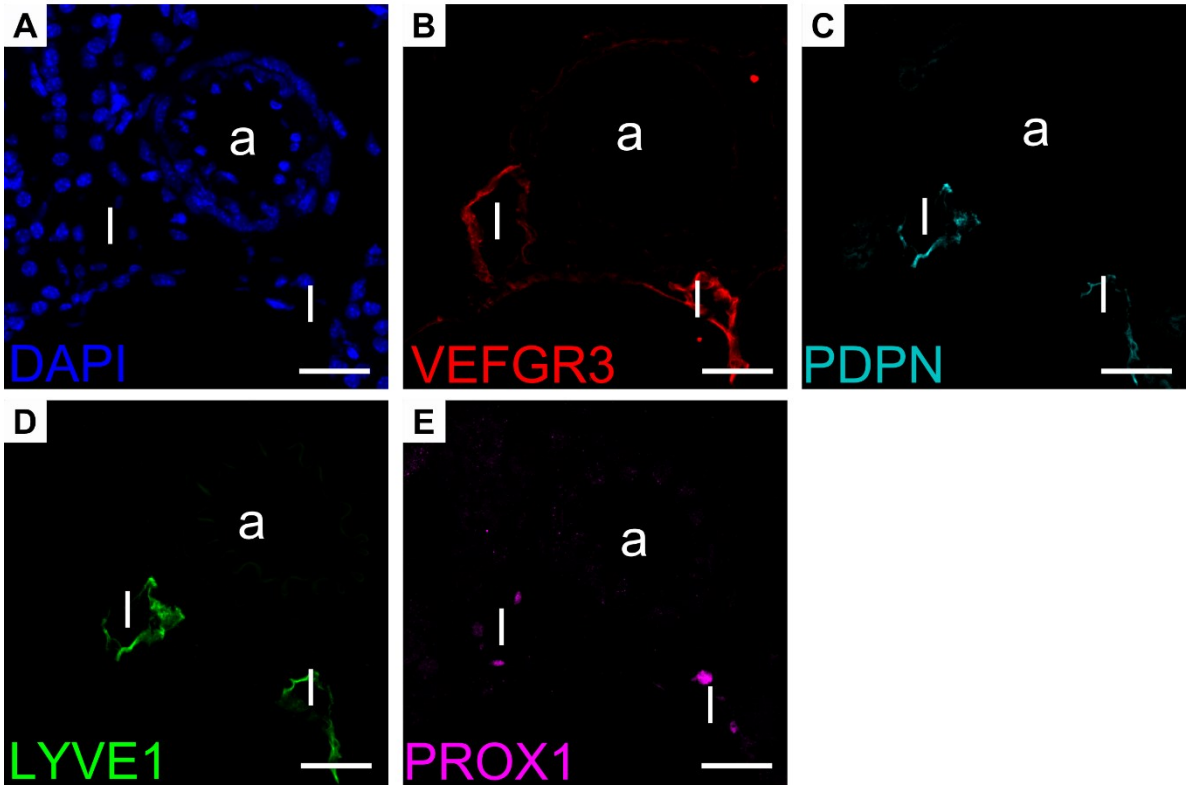
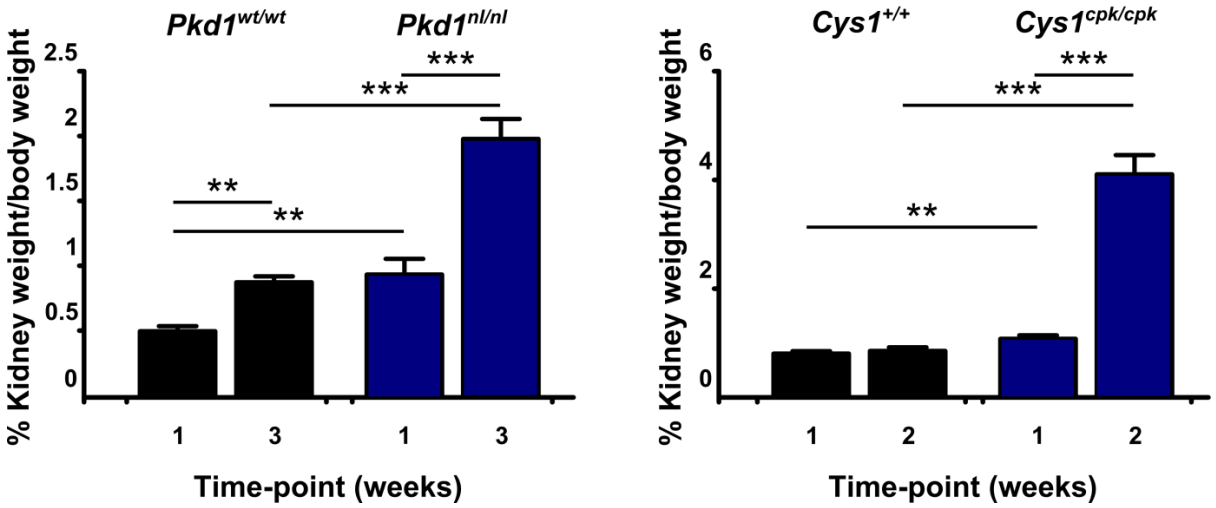
Supplementary figure 1



Supplementary Figure 1. Expression of lymphatic markers in *Pkd1*$^{wt/wt}$mice

VEGFR3 (B), PDPN (C), LYVE1 (D) and PROX1 (E) co-localised to the larger lymphatics (l) in the kidneys of three week old *Pkd1*$^{wt/wt}$ mice. None of the markers were detected in the arteries (a) of the kidney. Bar is 50 μm in all panels.

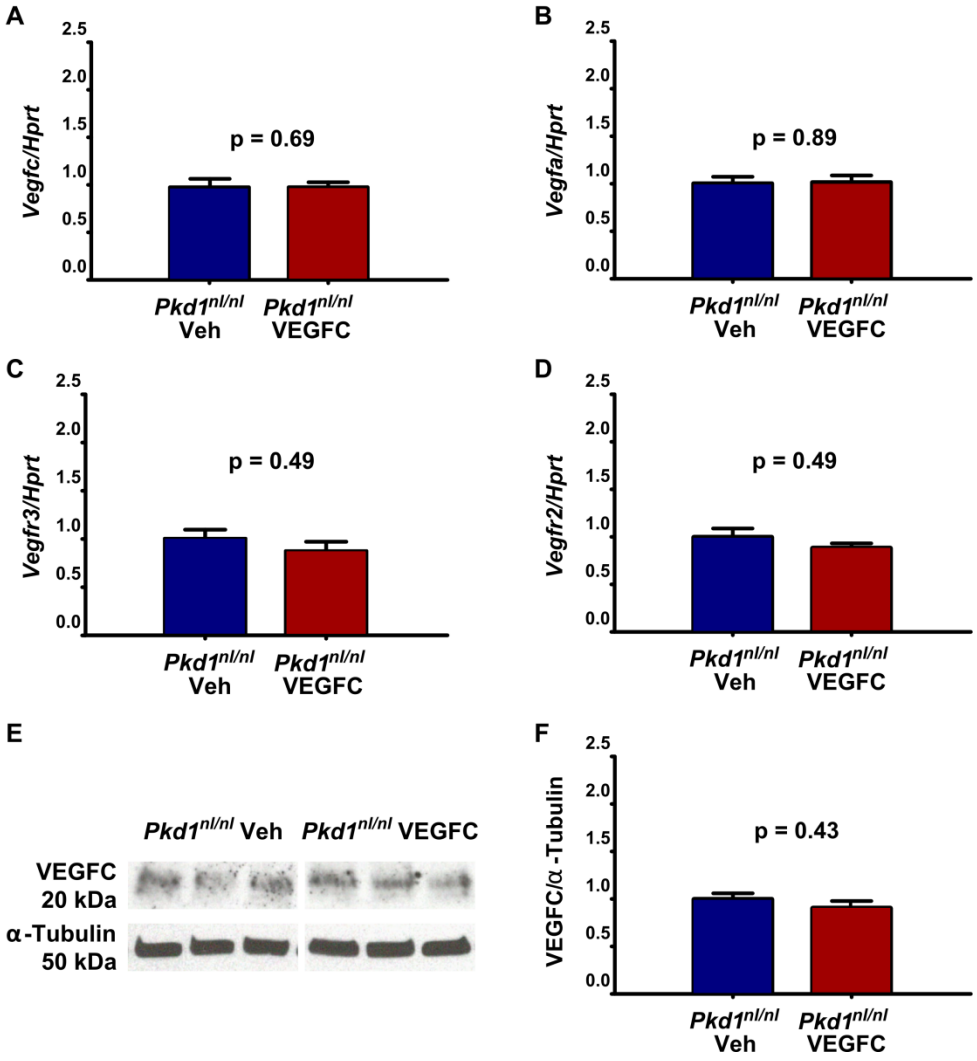
Supplementary figure 2



Supplementary Figure 2. Percentage of kidney weight/body weight in *Pkd1*$^{nl/nl}$ and *Cys1*$^{cpk/cpk}$ mice

The kidneys of *Pkd1*$^{nl/nl}$ mice grew rapidly during 1 to 3 weeks of age, the period when VEGFC treatment was provided, with only a modest growth in *Pkd1*$^{wt/wt}$ kidney (n=4-9 in each group and time-point). Rapid kidney growth occurred during weeks 1 to 2 of age in *Cys1*$^{cpk/cpk}$ when VEGFC was administered, with no change in *Cys1*$^{+/+}$ mice during this period (n=13-17 in each group and time-point). All data is presented as mean ± SEM. ** = p <0.01, *** = p <0.001 between groups.

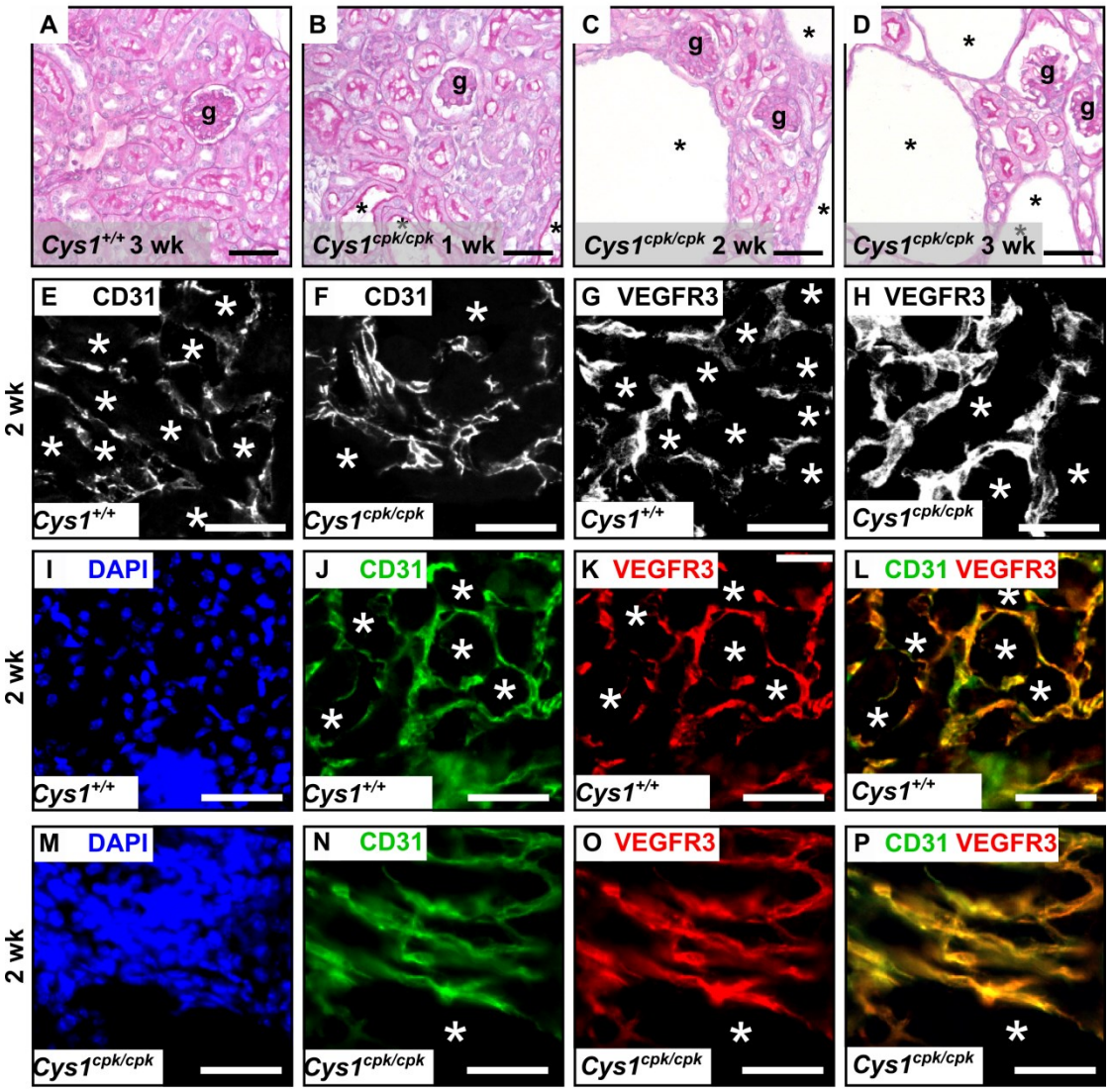
Supplementary figure 3



Supplementary Figure 3. Endogenous Vegfa, Vegfc, Vegfr2 and Vegfr3 levels were not altered in the kidneys of Pkd1$^{nl/nl}$ mice following VEGFC treatment

qRT-PCR comparing mRNA levels of *Vegfa* (A), *Vegfc* (B), *Vegfr2* (C) *and Vegfr3* (D) in *Pkd1*$^{nl/nl}$ mouse kidneys following either vehicle (PBS) or VEGFC treatment (n=4 in each group). (E) Western blotting for VEGFC in *Pkd1*$^{nl/nl}$ mouse kidneys following either vehicle (PBS) or VEGFC treatment (n=3 in each group). Densitometry was performed (F) using α-tubulin as a house-keeping protein. All data is presented as mean ± SEM and presented relative to levels in *Pkd1*$^{nl/nl}$ kidneys administered PBS where average expression was given an arbitrary value of 1.

Supplementary figure 4



Supplementary Figure 4. Disorganisation of the renal microvasculature in *Cys1*$^{cpk/cpk}$ mice.

(A-D) Histology of kidneys obtained from *Cys1*$^{+/+}$ and *Cys1*$^{cpk/cpk}$ mice. Representative images of immunohistochemical staining for CD31 in the kidney of a two week old *Cys1*$^{+/+}$ (E) and *Cys1*$^{cpk/cpk}$ (F) mouse showing the positive vessels surrounding the tubules (*). Staining for VEGFR3 in two week old *Cys1*$^{+/+}$ (G) and *Cys1*$^{cpk/cpk}$ (H) mouse kidneys. Note that the CD31 and VEGFR3 frames shown for *Cys1*$^{+/+}$ and *Cys1*$^{cpk/cpk}$ mice are not of the same section. (I-P) Double-labelling for CD31 and VEGFR3 in the same sections of *Cys1*$^{+/+}$ and *Cys1*$^{cpk/cpk}$ mice demonstrated co-localisation of both markers on vessels surrounding the kidney tubules. Bar is 50 μm in each panel, g = glomerulus.

Supplementary Table 1: Quantification of vascular parameters in the kidneys of *Pkd1*$^{wt/wt}$ and *Pkd1*$^{nl/nl}$ mice administered PBS or VEGFC.

	Pkd1$^{wt/wt}$	*Pkd1*$^{nl/nl}$ PBS	*Pkd1*$^{nl/nl}$ VEGFC
% area positive for CD31	32.0 ± 1.5	44.2 ± 1.8[a]	33.5 ± 2.4[b]
CD31$^+$ Ki67$^+$ cells/cm^2 of DAPI area	48.7± 6.0	13.9 ± 1.5[a]	38.4 ± 4.9[b]
Average size of LYVE1$^+$ Prox1$^+$ (μm^2)	6.8 ± 0.4	7.0 ± 0.9	9.1 ± 0.6[b]
% area positive for VEGFR3	19.8 ± 2.1	38.4 ± 1.3[a]	24.4 ± 2.0[b]
VEGFR3$^+$ Ki67$^+$ cells/cm^2 of DAPI area	44.9 ± 3.7	17.0 ± 1.6[a]	54.1 ± 13.4[b]

Data is presented as mean ± SEM. n=3-6 three week old mice per group. a = $p < 0.05$ comparing *Pkd1*$^{wt/wt}$ with *Pkd1*$^{nl/nl}$ mice administered PBS, b = $p < 0.05$ comparing *Pkd1*$^{nl/nl}$ mice administered PBS or VEGFC.

Supplementary Table 2: Quantification of vascular parameters in the kidneys of *Cys1*$^{+/+}$ and *Cys1*$^{cpk/cpk}$ mice administered PBS or VEGFC.

	Cys1$^{+/+}$	*Cys1*$^{cpk/cpk}$ PBS	*Cys1*$^{cpk/cpk}$ VEGFC
% area positive for CD31	40.8 ± 1.8	44.6 ± 0.3	41.3 ± 1.2
CD31^{+} Ki67^{+} cells/cm^{2} of DAPI area	66.4 ± 6.4	20.3 ± 4.8[a]	40.9 ± 5.2[b]
Average area of LYVE1^{+} Prox1^{+} vessels (μm^{2})	2.9 ± 0.3	4.0 ± 0.5	5.0 ± 0.2
% area positive for VEGFR3	35.3 ± 2.2	43.1 ± 0.4[a]	38.7 ± 1.6[b]
VEGFR3^{+} Ki67^{+} cells/cm^{2} of DAPI area	54.8 ± 5.6	22.7 ± 3.0[a]	38.8 ± 2.5[b]

Data is presented as mean ± SEM. n=3-5 two week old mice per group. a = $p < 0.05$ comparing *Cys1*$^{+/+}$ with *Cys1*$^{cpk/cpk}$ mice administered PBS, b = $p < 0.05$ comparing *Cys1*$^{cpk/cpk}$ mice administered PBS or VEGFC.