SUPPLEMENTAL MATERIALS

Tolvaptan plus Pasireotide Shows Enhanced Efficacy in a PKD1 Model

Katharina Hopp*, Cynthia J Hommerding*, Xiaofang Wang*, Hong Ye*, Peter C Harris*[+] and Vicente E Torres*

*Division of Nephrology and Hypertension, [+]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota

Corresponding Author:

Vicente E. Torres

Division of Nephrology and Hypertension

Mayo Clinic

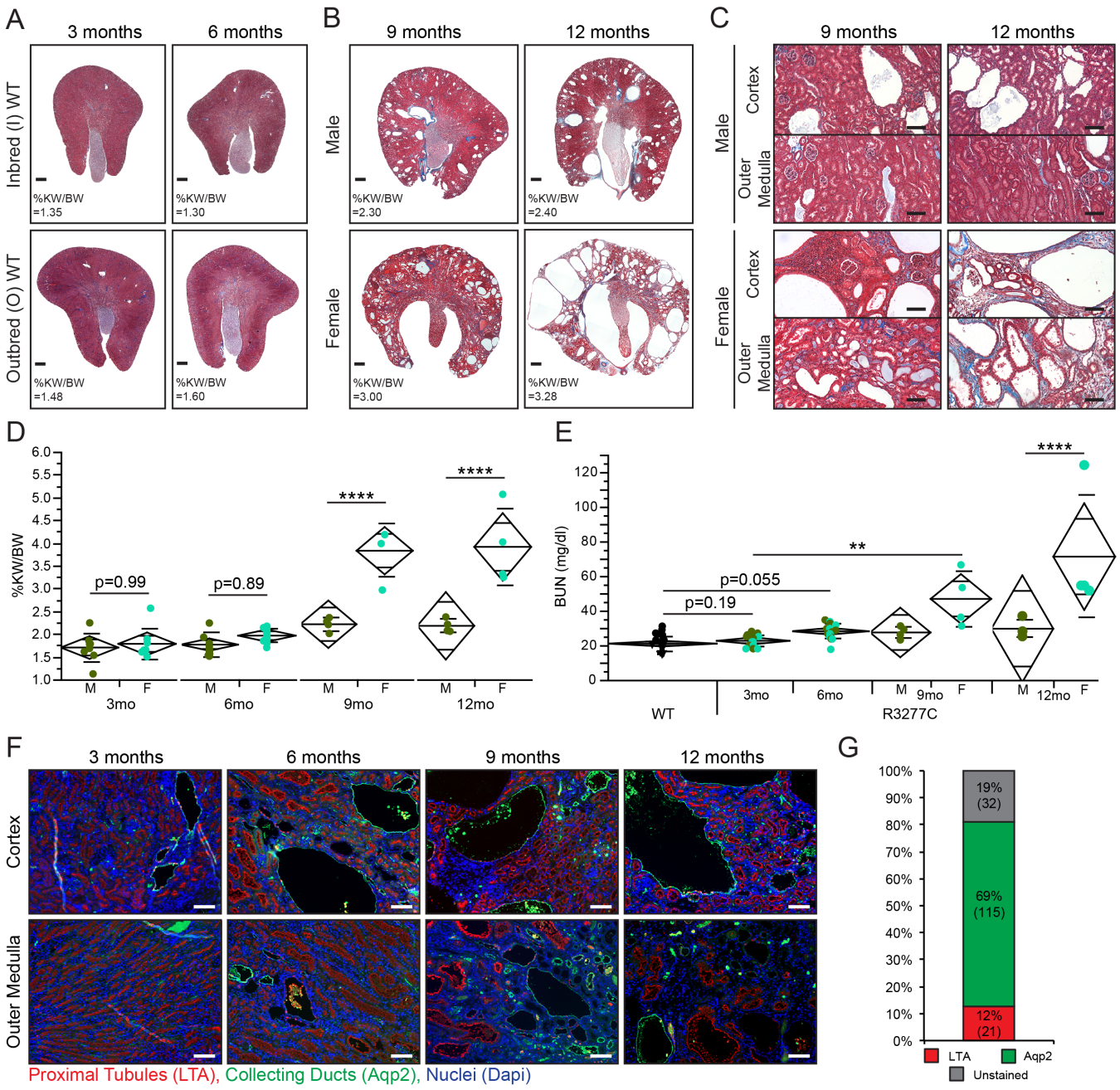
200 First Street SW

Rochester, MN 55905

Phone: 507-284-5009

Fax: 507-266-9315

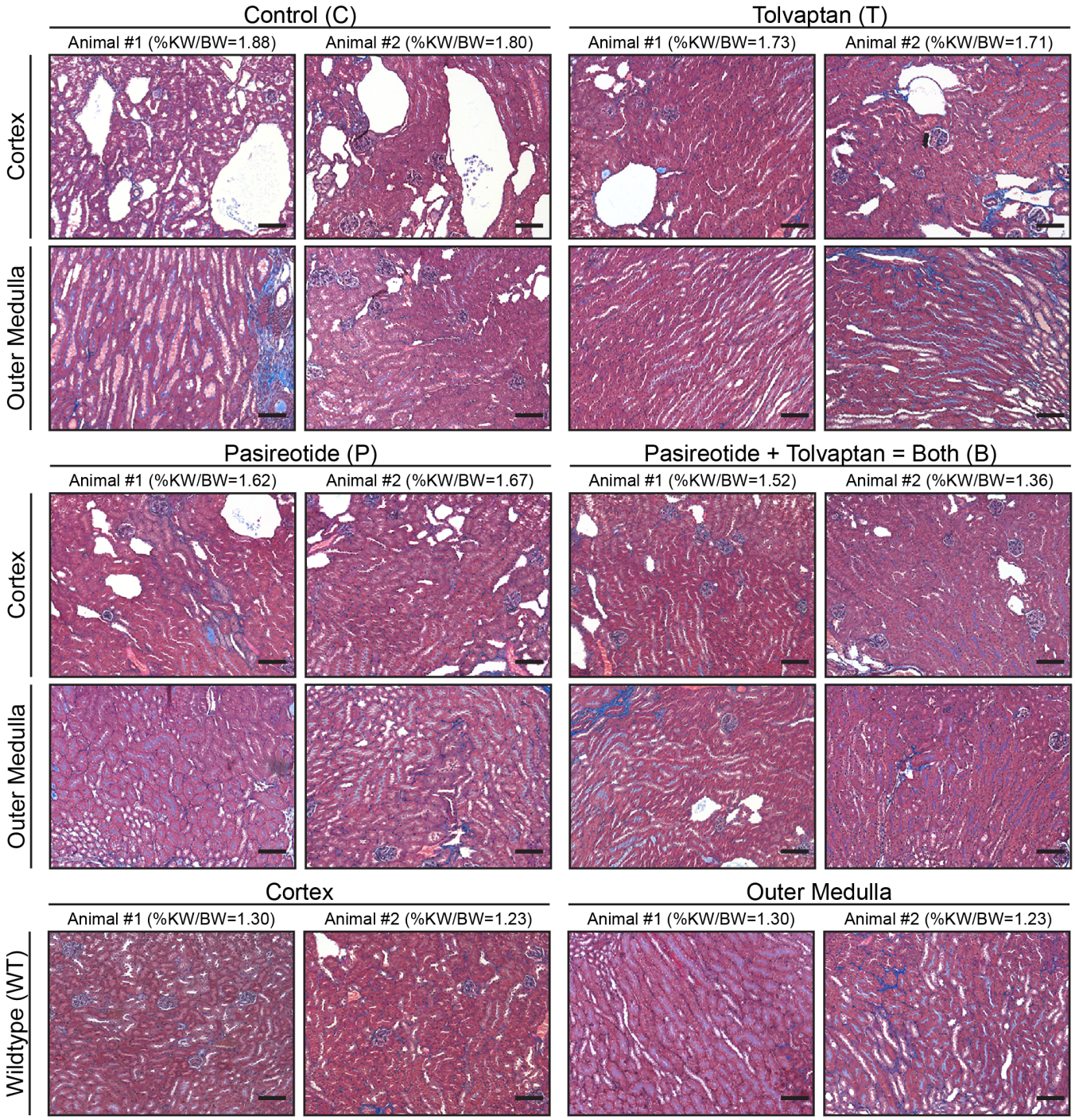
email: torres.vicente@mayo.edu



Supplemental Figure 1 | Analysis of the inbred *Pkd1*$^{RC/RC}$ phenotype up to one year

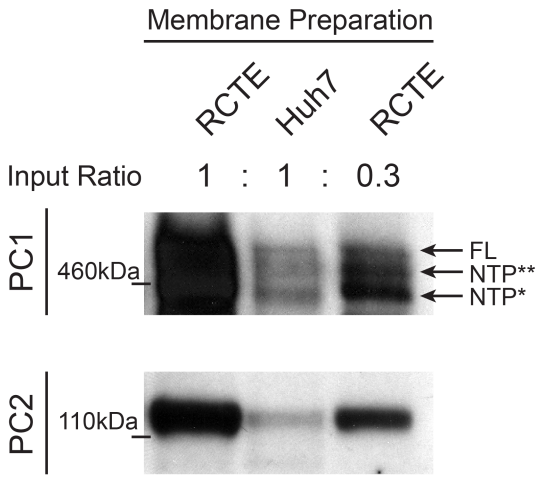
(A) Masson Trichrome stained cross-sections of WT inbred (I) and outbred (O) animals highlight the larger kidney size of outbred animals compared to inbred mice. This background effect may in part drive the larger %KW/body wt of *Pkd1*$^{RC/RC}$ outbred animals compared to inbred mice. Scale bar: 500µm. (B) Masson Trichrome stained cross-sections of 9m and 12m old inbred *Pkd1*$^{RC/RC}$ mice show continuous cyst progression with age. A marked difference of disease severity was noted between these older inbred females and males. Scale bar: 500µm (C) Close up of (B) reinforcing the gender difference and showing that cyst development and growth, rather than just dilated tubules, is the major disease manifestation in this model. Scale bar: 100µm. (D/E) %KW/body wt and BUN depicted as mean diamonds and SD. (D) %KW/body wt of inbred *Pkd1*$^{RC/RC}$ separated by males (M, dark green) and females (F, light green) illustrates the significance of the gender difference at 9m and 12m. This is likely a background effect as it was not noted in the outbred strain[1] (E) BUN analysis, which becomes significant at 9m in females, highlights a decline in renal function with advanced disease.

(F) Immunofluorescence staining of inbred *Pkd1*$^{RC/RC}$ animals shows that the majority of cysts are of collecting duct (Aqp2) origin. A few dilated tubules/cysts stain positive for LTA (proximal tubule) especially at 9m and 12m of age. Scale bar: 100µm. (G) Quantification of the number of cysts per cross-section (avrg. in parentheses) positive for LTA, Aqp2 or neither of 6m old mice (n=6). Collecting duct cysts account for the majority (69%) of cysts and are significantly more frequent then unstained or LTA positive cysts ($p=9.85x10^{-6}$). Only 12% of LTA positive cysts (avrg. 3/21) have a diameter greater 100µm, highlighting that the majority are dilated tubules. Scale bar: 100µm. p-values: **<0.01, ****<0.0001. %KW/BW, %KW/body wt.



Supplemental Figure 2 | High magnification histology images of tolvaptan/pasireotide preclinical trial mice.

Cortical and outer medullary images were taken of Masson Trichrome stained kidney cross-sections from two animals per treatment group with the average %KW/body wt. Untreated control group images show significant cyst burden in the cortex with some dilated tubules in the outer medulla (Animal #1). Cyst burden and size decreased and tubular dilation were less common in animals treated with either drug. The tubular structure and parenchyma of animals treated with both drugs was comparable to WT in many aspects. Scale bar: 100μm. %KW/BW, %KW/body wt.



Supplemental Figure 3 | Hepatocytes express PC1 and PC2.
Membrane preparations of the hepatocellular carcinoma cell-line Huh7, an accepted hepatocyte culture model, show that both PC1 (7e12) N-terminal glycoforms (NTP), and the full length (FL) protein are expressed, as characterized previously[1]. This has been shown at the mRNA level[2]. The cells also express PC2 (Yce2). Both proteins are present at a much lower level when compared to a renal cortical tubular epithelial cell-line (RCTE).

Supplemental Table 1 | Urine volume and osmolality analysis

	12h Urine Volume[1] (ml)	Urine Osmolality (mOsm/kg)
Control	0.84±0.51	1124.85±147.25
Tolvaptan	1.38±0.47[A]	902.64±61.49[B]
Pasireotide	0.63±0.39[A]	1328.88±417.15[B]
Both	0.87±0.28	1082.50±176.27

[1] Urine was collected overnight for 12h in metabolic cages housing 2-4 animals, provided measurements are per animal

[A] Tukey-Kramer HSD: T vs. P p=0.0197, all other comparisons were not significant

[B] Tukey-Kramer HSD: T vs. P p=0.0185, all other comparisons were not significant

References

1. Hopp, K, Ward, CJ, Hommerding, CJ, Nasr, SH, Tuan, HF, Gainullin, VG, Rossetti, S, Torres, VE, Harris, PC: Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *J Clin Invest,* 122**:** 4257-4273, 2012.
2. The European Polycystic Kidney Disease Consortium: The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell,* 77**:** 881-894, 1994.