SUPPLEMENTAL MATERIAL

Supplemental methods:

Immunofluorescence microscopy: Immunofluorescence microscopy for sheep IgG, mouse IgG, and mouse C3 was performed as described previously [1,2]. Briefly, 5 μm cryostat kidney sections were stained with fluorescein- or rhodamine-conjugated IgG fractions of monospecific antisera. The following antibodies were used: fluorescein isothiocyanate (FITC)-conjugated rabbit anti-sheep IgG, FITC-conjugated goat anti-mouse C3 (both MP Biomedical; Santa Ana, CA), Cy3-conjugated goat anti-mouse IgG (Cell Signaling Technology; Danvers, MA). The immunofluorescence signals from whole glomeruli were evaluated using AxioObserver-100 (Zeiss) fluorescence microscope using AxioVision 4.8 software. Quantification of glomerular immunofluorescence intensity was carried out using ImageJ software (Arch Version, ImageJ-win32). All images were processed by using Adobe Photoshop CS5 and assembled in Adobe Illustrator CS5.

Transmission electron microscopy: Kidneys from 3-month-old control Vangl2$^{Flox/Flox}$;Cre^{-} and mutant Vangl2$^{Flox/\Delta Ex4}$;Pod-Cre were collected, cortices were sliced into small 2 mm cubes and placed in a standard EM buffer. The further processing and imaging was serviced to the Department of Pathology & Laboratory Medicine at Mount Sinai Hospital in Toronto, Canada. Images were analyzed using ImageJ64 (version 1.46) Software. Approximately 15 images per mouse were analyzed. The GBM length along the segments was measured using the "freehand line" and "measure" functions. Number of foot processes was counted using the "multi-point selections" tool. Average FP width was calculated by dividing FP number by the length along the GBM. The student's two-tailed t-test was used to calculate significance between groups.

Supplemental Figure Legends:

Supplemental Figure 1. ***Analysis of glomerular development in the Looptail mouse by light microscopy.*** **A.** Hematoxylin & Eosin staining of paraffin sections of E17.5 wildtype and homozygous *Looptail* embryos; scale bar is 20 μm. **B**. High magnification of H&E stained immature (*left* panel) and mature (*right* panel) glomeruli. Notice the characteristic cuboidal shape of immature podocytes at the periphery of developing glomeruli. **C.** Percentage of histological immature and mature glomeruli in wildtype vs. *Lp-/-* embryos. Total >200 glomeruli in 3 embryos per each genotype were scored. Means and standard errors of the means are presented, chi-squared test was used, p=1.005E-7. **D.** Quantification of glomerular tuft area in wildtype and *Lp-/-* glomeruli. Glomeruli were subdivided into small (<15 x 10^3 μm^2) medium size (15-25 x 10^3 μm^2) and large (>25 x 10^3 μm2) glomeruli. Total >140 glomeruli in 3 embryos per each genotype were scored. Means and standard errors of the means are presented, chi-squared test was used. Statistically significant differences were detected for small and medium size, but not large size glomeruli.

Supplemental Figure 2. ***Glomerular deposition of antibodies and complement in anti-GBM nephritis.*** **A.** Cryosections from Vangl2$^{Flox/Flox}$;Cre^{-} and Vangl2$^{Flox/\Delta Ex4}$;Pod-Cre kidneys prepared from kidneys 4 weeks after injections with sheep anti-GBM antibody were stained with antibodies to sheep IgG (green), mouse C3 (green) and mouse IgG (red); scale bar is 5 μm. **B.** Quantification of immunofluorescence intensity of antibody staining in control Vangl2$^{Flox/Flox}$;Cre^{-} vs. Vangl2$^{Flox/\Delta Ex4}$; Pod-Cre mutant kidneys; n=3 kidneys per genotype were examined; means and standard errors of the means are shown, two-tailed unequal variance t-test was used. No statistically significant differences were present, indicating that deposition of antibodies and complement was comparable in mice of both

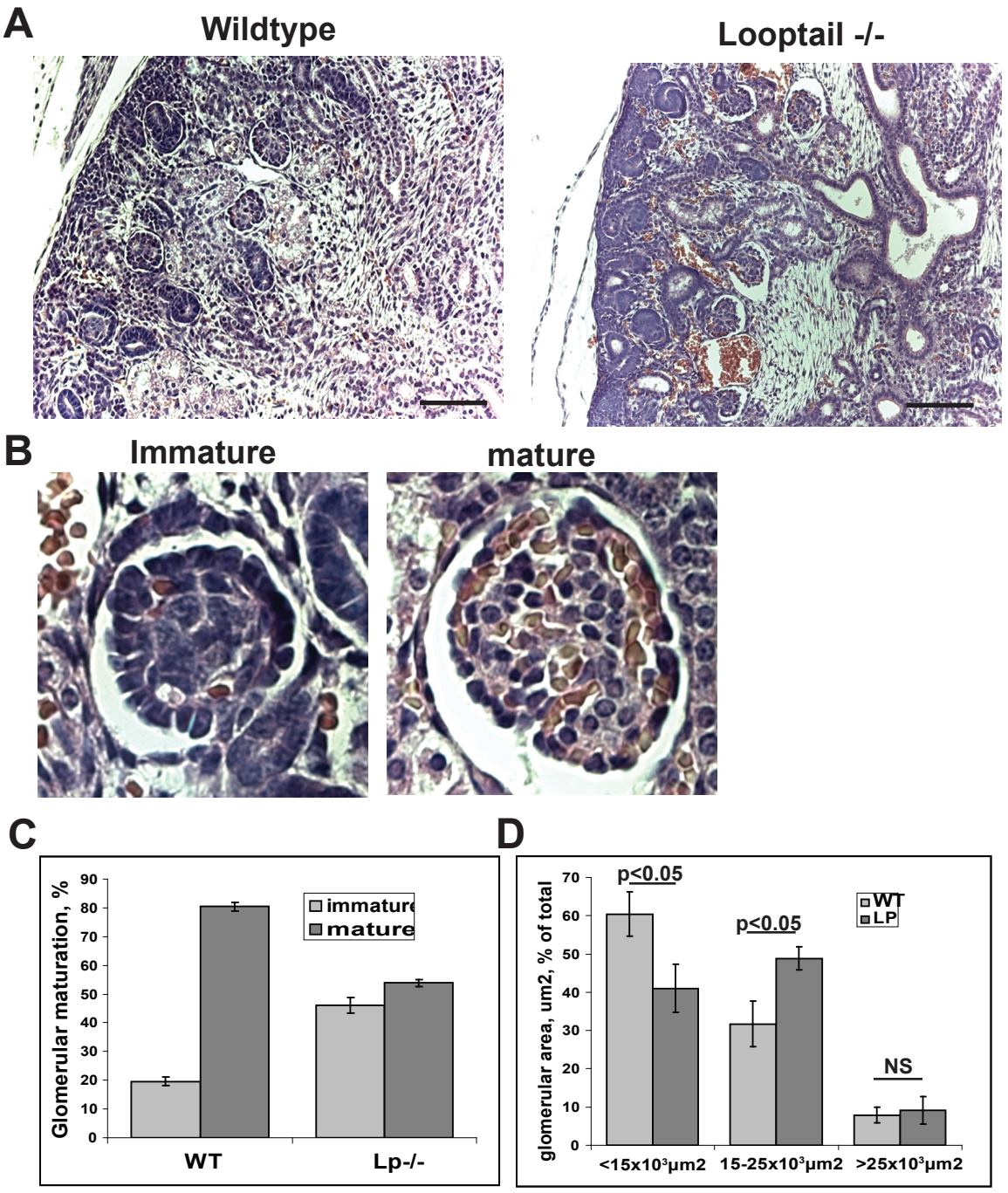
genotypes. The C3 staining of Bowman's capsule (surrounding the glomerulus) is observed in normal mouse kidneys, and is not due to the administration of anti-GBM antibody.

Supplemental Figure 3. ***Vangl2 is dynamically expressed in the developing glomerulus.*** Co-immunofluorescent staining of E17.5 C57/Bl6 wildtype kidney sections with anti-Vangl2 antibody (red or white) and antibodies against podocalyxin and nephrin (green) (presented in Figure 1). Scale bars are 5 μm. The Vangl2 red channel from the color images was converted into a grayscale to improve visualization of Vangl2 expression.

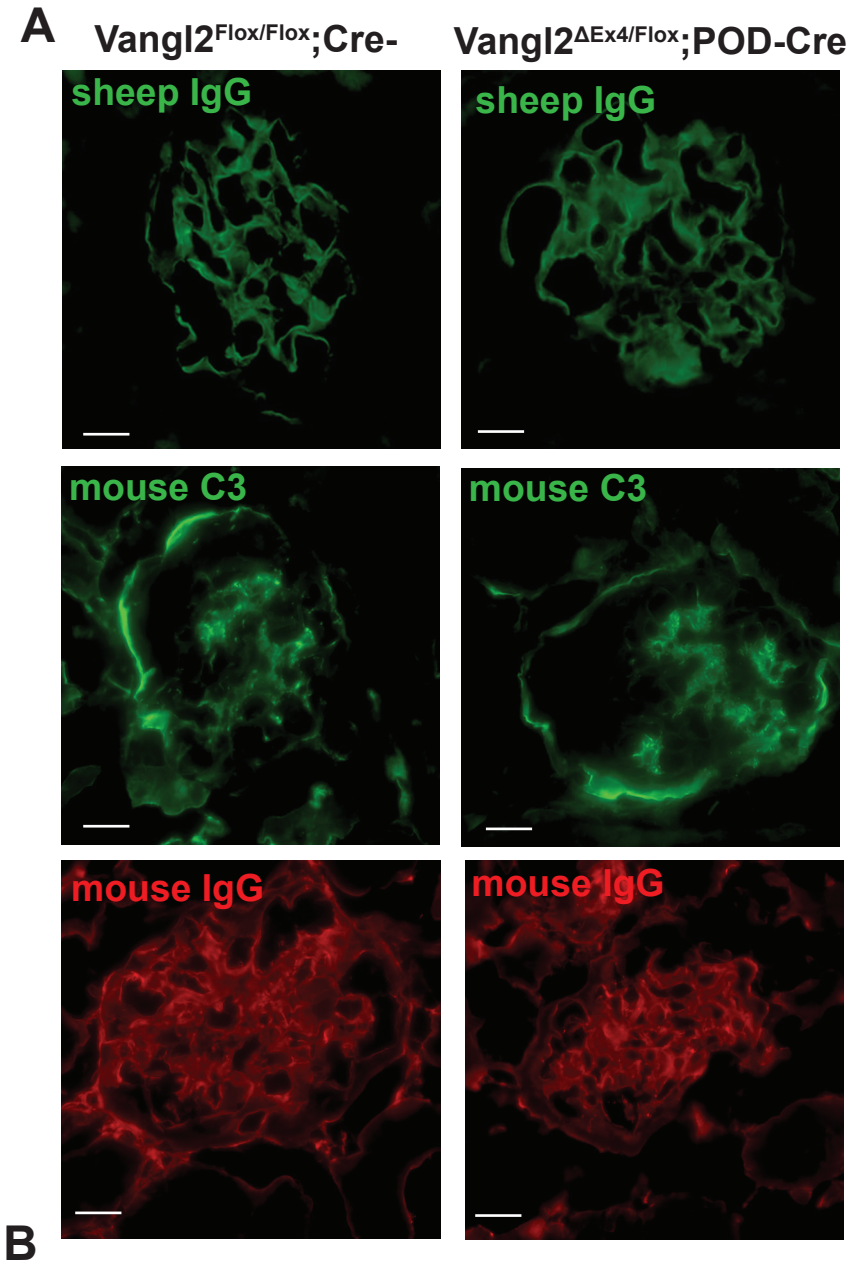
Supplemental Figure 4. ***Podocyte-specific excision of Vangl2 gene does not affect tubular morphology in neonatal mice or podocyte foot process morphology in adult mice under normal physiological conditions.*** **A.** Hematoxilin & Eosin staining of P14 control $Vangl2^{Flox/Flox}$;Cre^{-} and mutant $Vangl2^{Flox/\Delta Ex4}$;Pod-Cre paraffin-embedded kidney sections, scale bar 30 μm. **B.** Transmission electron microscopy of renal glomeruli of control and mutant 3 month-old animals. We could not see any important differences between the two genotypes; scale bar 500nm. **C.** Statistical analysis of average foot process width. 15 images per genotype were analyzed. Mean and standard errors of the mean are shown, unequal variance t-test was performed.

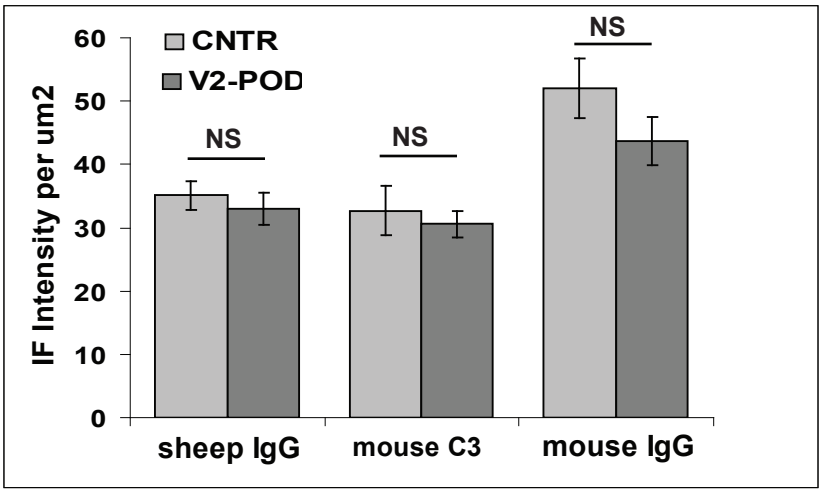
References

1. Cybulsky AV, *et al.* (2002) Complement C5b-9 membrane attack complex increases expression of endoplasmic reticulum stress proteins in glomerular epithelial cells. (Translated from eng) *J Biol Chem* 277(44):41342-41351 (in eng).
2. Salant DJ & Cybulsky AV (1988) Experimental glomerulonephritis. (Translated from eng) *Methods Enzymol* 162:421-461 (in eng).

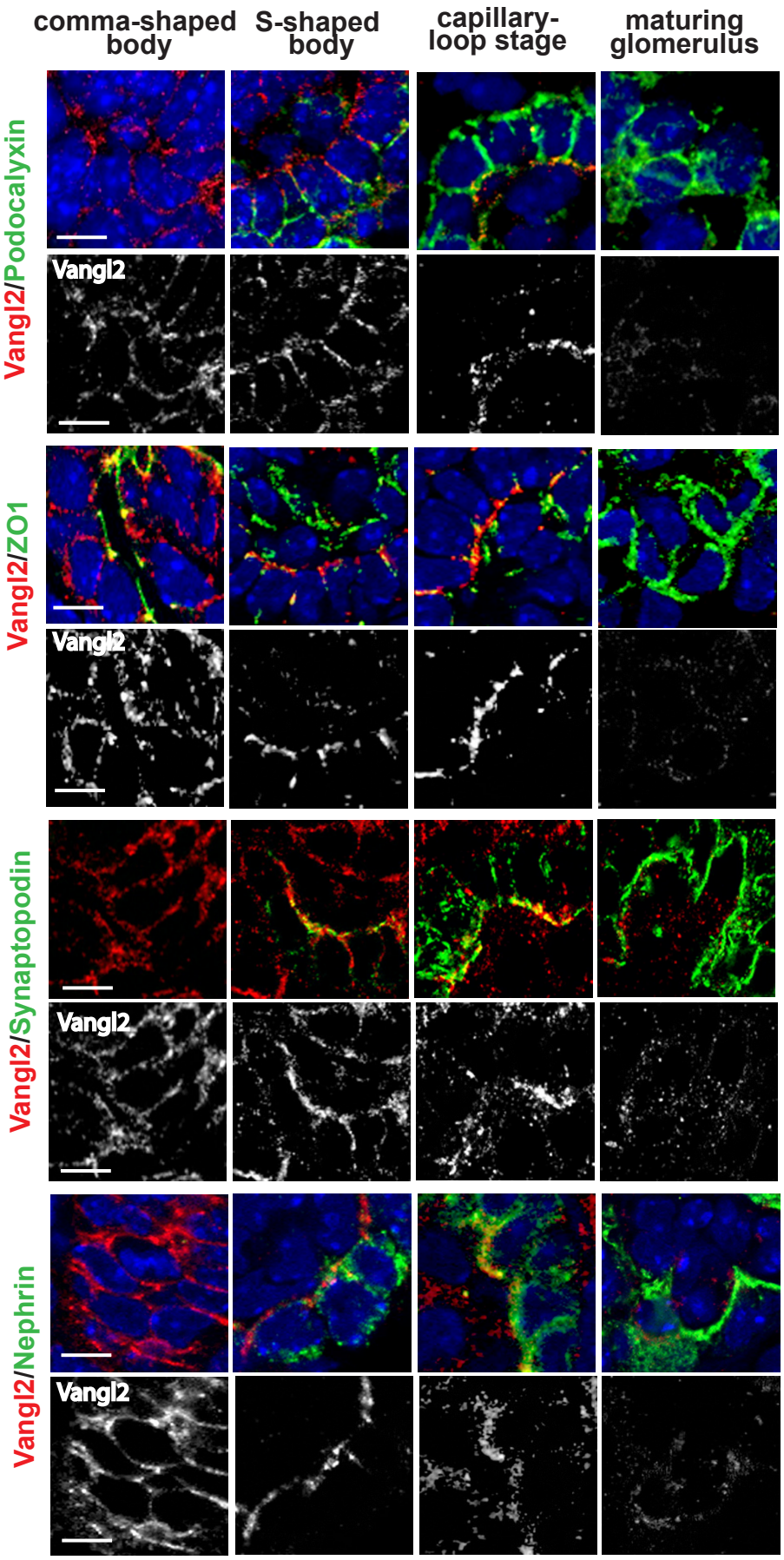


Supplemental Figure1





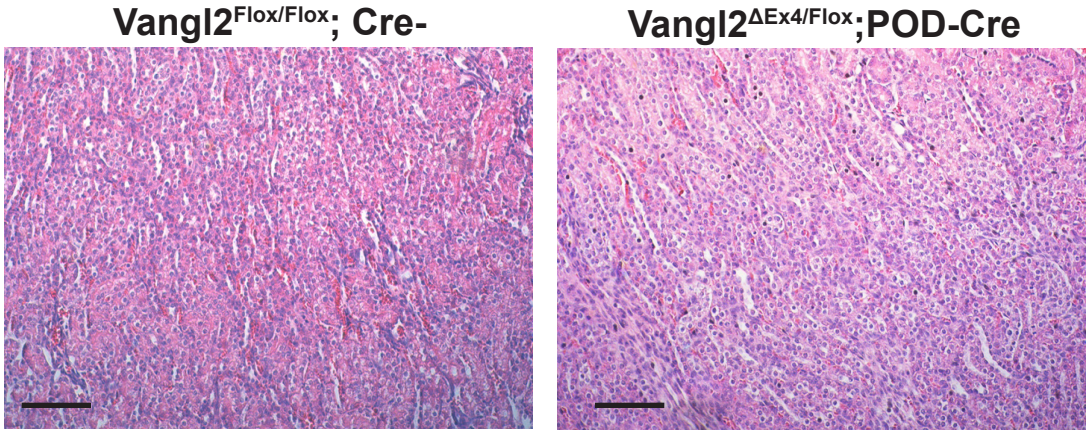
Supplemental Figure 2



Supplemental Figure 3

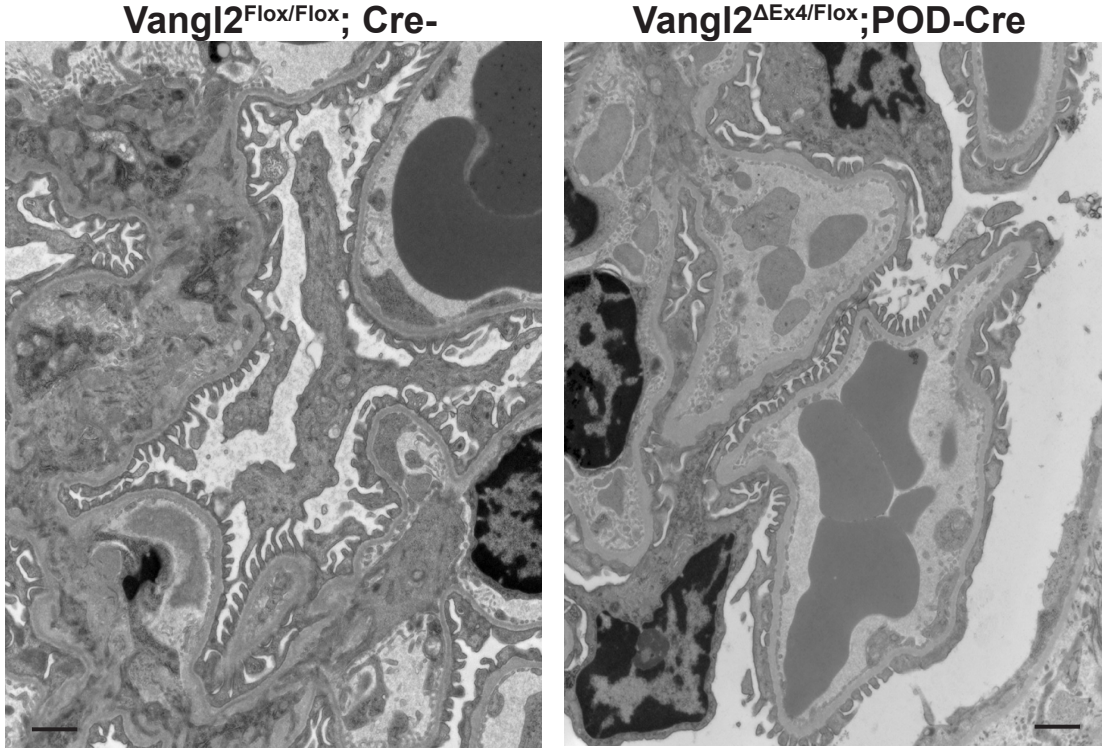
A

P14 kidney tissues stained with H&E

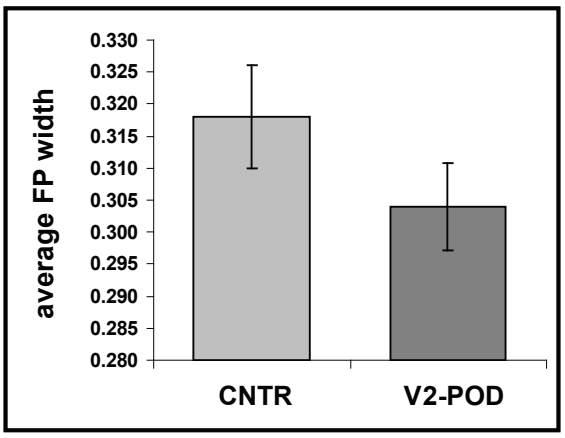


B

TEM of glomeruli in 3-month-old animals



C



Supplemental Figure 4