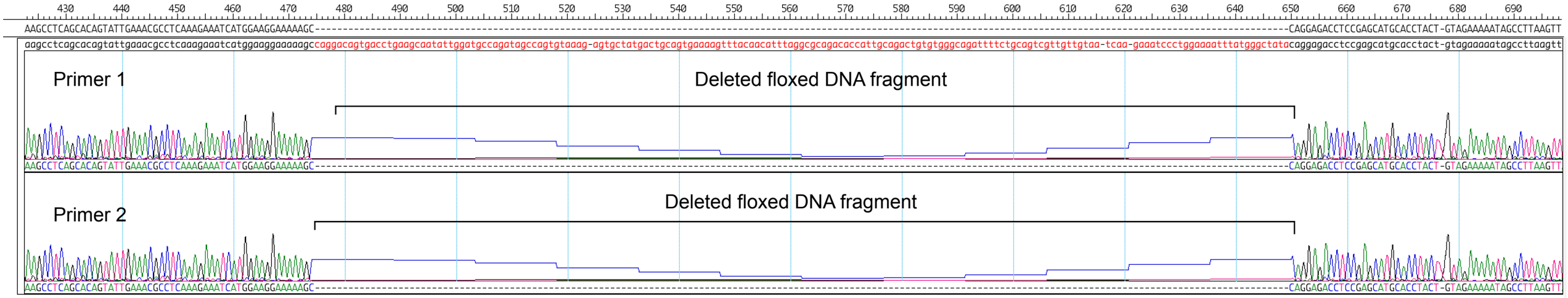
Online Supplement

Deletion of the Vps34 Downstream Effector PIKfyve Establishes Distinct Requirements for PIKfyve in Podocytes versus Proximal Tubular Cells

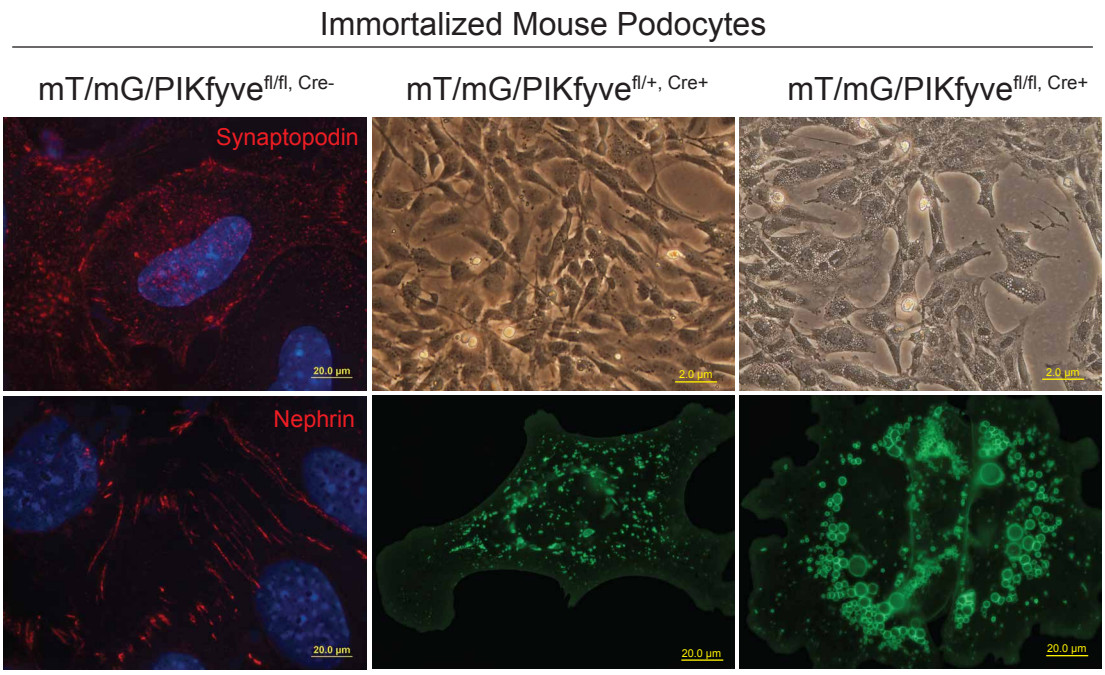
Madhusudan Venkatareddy[1], Rakesh Verma[1], Anne Kalinowski[1], Sanjeevkumar Patel[1], Assia Shisheva[2] and Puneet Garg[1]

[1]Division of Nephrology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, [2]Department of Physiology, Wayne State University School of Medicine, Detroit, MI

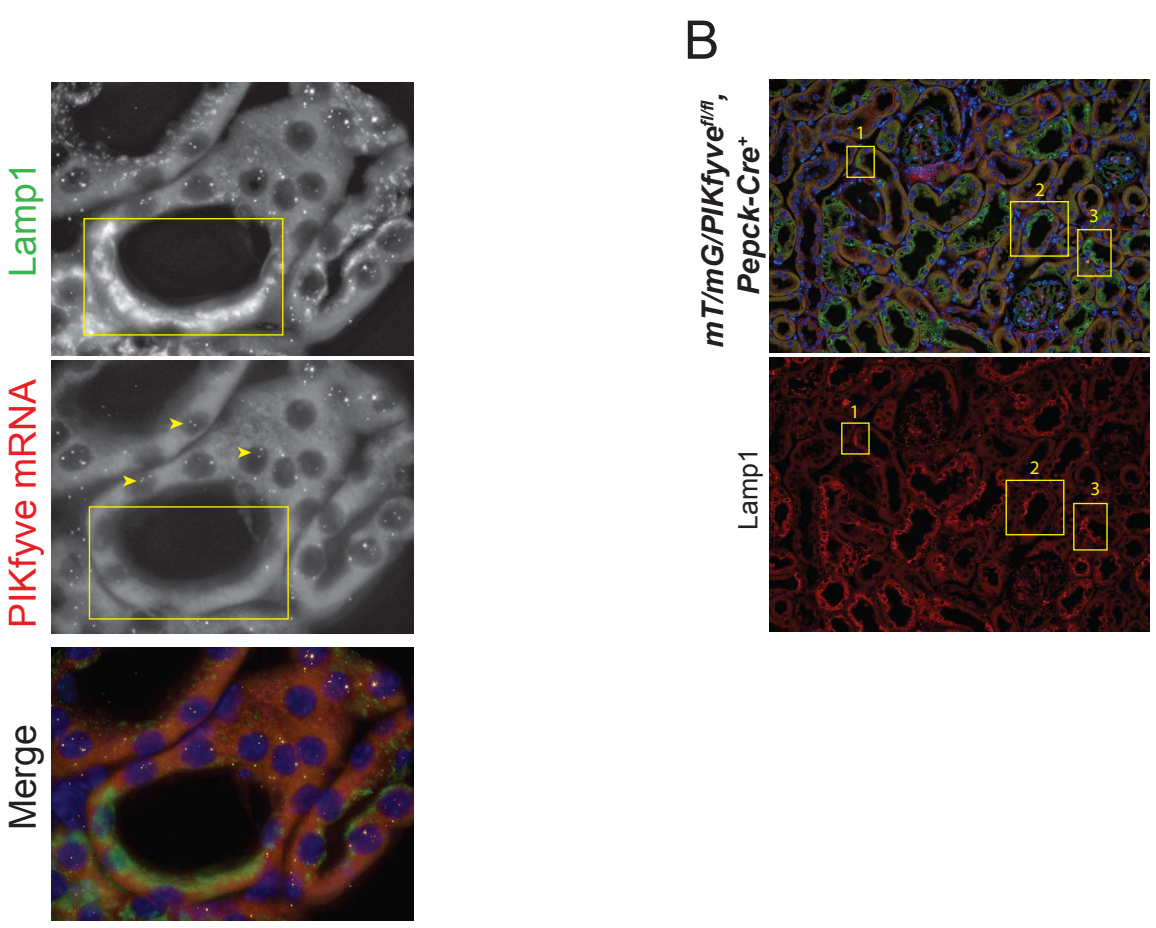
Running Title: *PIKfyve knockout in glomeruli versus proximal tubules*



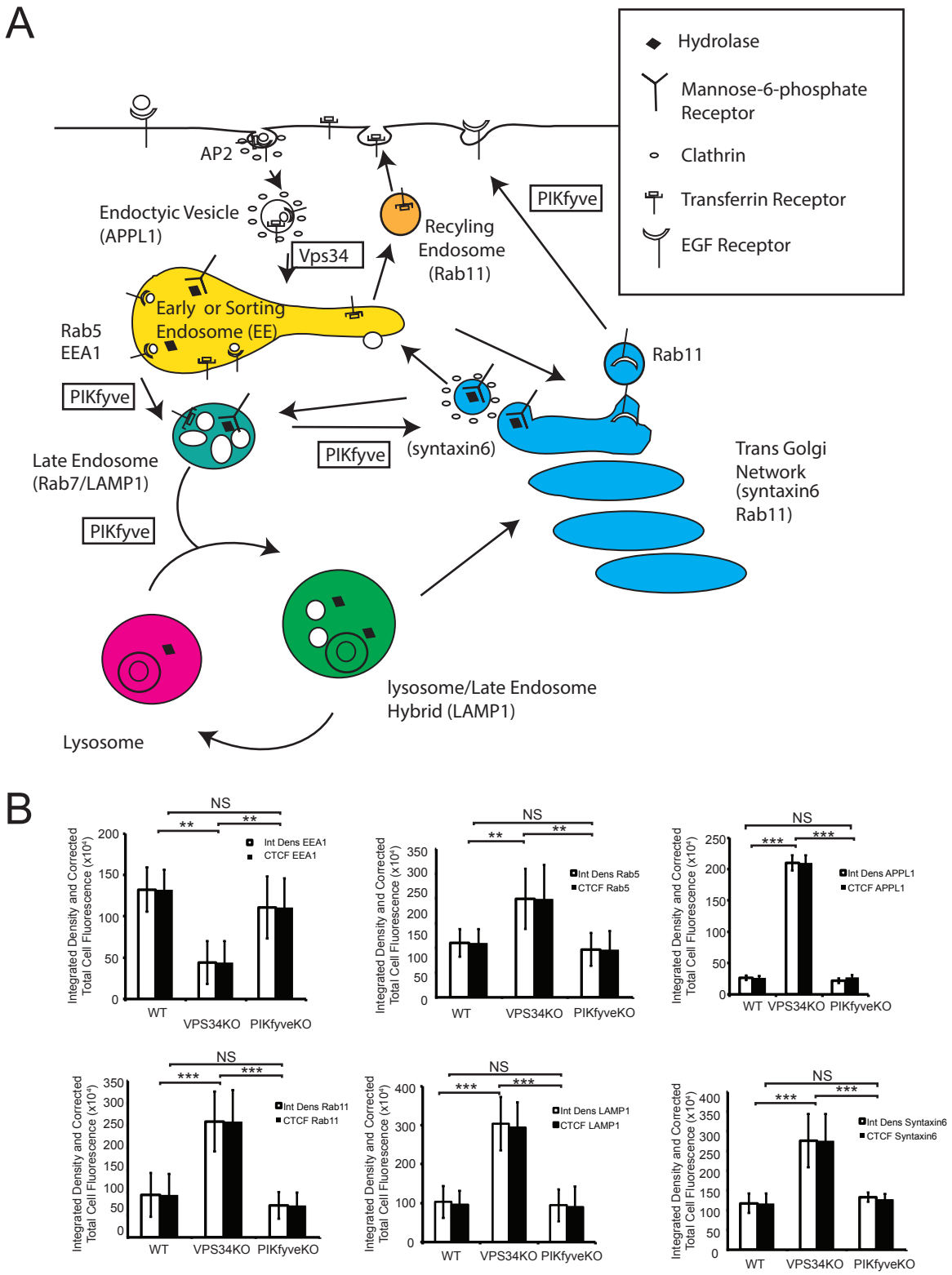
Supplementary figure 1. Chromatogram showing deletion of the floxed segment of PIKfyve.



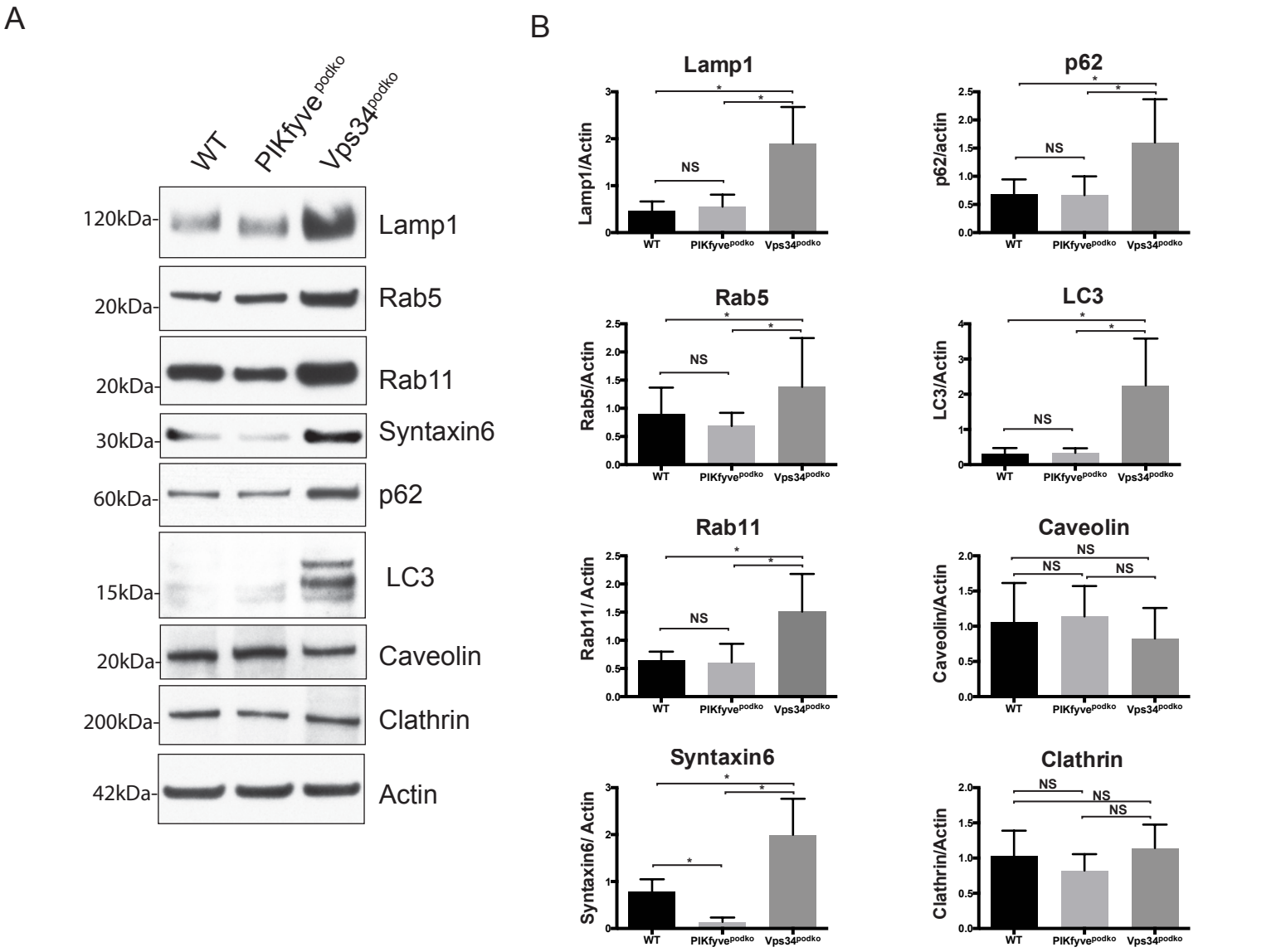
Supplementary figure 2. Immortalized mouse podocyte cell line generated from a mTmG/PIKfyvefl/fl mouse using thermosensitive SV-40 T antigen. Cells were grown at 37oC for 14 days prior to infecting with adenovirus particles carrying Cre. The cell line was characterized by staining with podocyte-specific proteins synaptopodin and nephrin. Following introduction of Cre, the PIKfyvefl/fl homozygous cell line develops large vacuoles in contrast to the control cell line heterozygous for the PIKfyve floxed allele. Scale bar, 20 µm.



Supplementary figure 3. (A) *In-situ* hybridization using RNAscope probes against exon 6 of PIKfyve. Each individual dot indicates (arrowheads) a mRNA strand. Lamp1 staining is used to identify cells with PIKfyve deletion (box). (B) Lamp1 is a marker for PIKfyve deleted cells. Merged images (green, red and blue) of paraffin embedded sections showing expression of GFP following recombination. Red signal is seen due to auto-fluorescence from paraffin. The same section was stained for Lamp1 (red) following de-paraffinization and epitope retrieval. Green signal is lost during this process. Compare the sections in squares, which show overlap of GFP expression and Lamp1 staining.

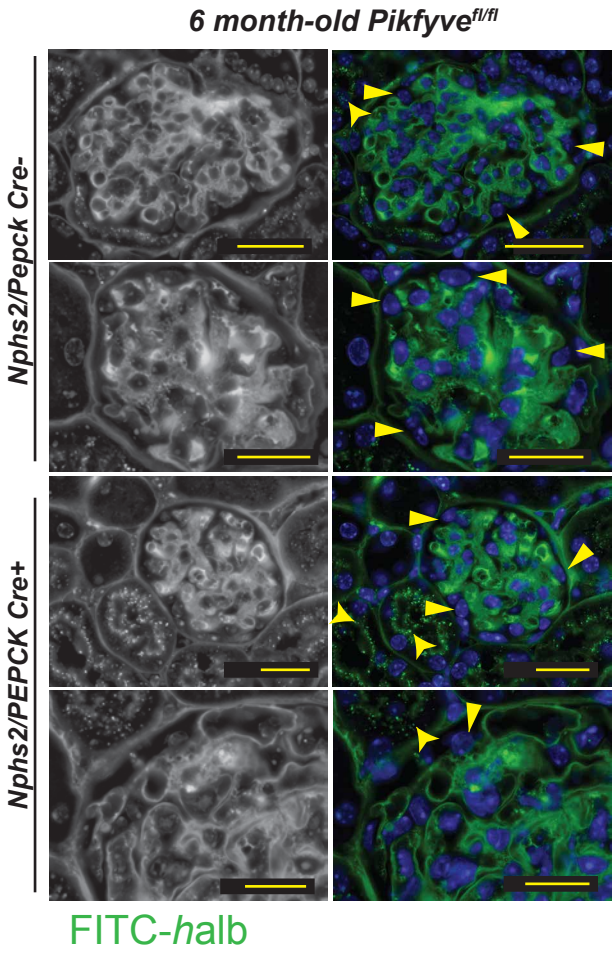


Supplementary figure 4. (A) A simplified schematic of endocytic trafficking and compartment markers. APPL1 is a marker for pre-sorting endosome (early endosome) stage vesicles that are formed from macropinocytosis and clathrin-dependent endocytosis. EEA1 (early endosome antigen1) and Rab5 mark the sorting or early endosomes. Syntaxin6 labels the endosomes originating from the Golgi, which traffic to the sorting or late endosomes, whereas Rab11 marks the TGN membranes and recycling endosomes that move to the cell surface. Lamp1 is a marker of late endosomes and lysosomes. (B) Quantification of fluorescence of the endocytic compartment markers in Vps34- and PIKfyve-deleted mouse podocytes. Using ImageJ software, quantification of fluorescence shows accumulation of APPL1, LAMP1, LC3, Rab5, Rab11 and Syntaxin 6 in the Vps34-deleted podocytes. EEA1, a marker of early endosomes, was decreased in the Vps34-deleted podocytes. Fluorescence of these proteins in podocytes of PIKfyve deleted mice and wild type were similar. Results are expressed as integrated density and corrected total cell fluorescence. NS (P value not significant), **P<0.01, ***P<0.001, scale bars, 20 μm: 5μm in enlarged sections. Error bars, S.E.



Supplementary figure 5. (A) Western blot of lysates from isolated podocytes following Cre-mediated deletion of PIKfyve and Vps34. Increased levels in Lamp1, Rab5, Rab11, Syntaxin6, p62 and LC3 are seen following Vps34 deletion. Lysates from PIKfyve deleted and wild-type cells have similar levels of the above proteins. Caveolin and clathrin levels are unchanged in both PIKfyve and Vps34 deleted podocytes. (B) Quantification of blots using densitometry from 4 separate experiments using ImageJ software. *P<0.001, NS not significant.

A



Supplementary figure 6. (A) Perfusion of 6-month-old mouse kidneys of PIKfyvefl/fl, Nphs2-Cre+/PEPCK-Cre+ double knockout mice and their littermate controls shows complete absence of FITC-*h*alb in podocytes (narrow arrowheads). There is evidence of FITC-*h*alb accumulation in PIKfyve-deleted proximal tubules (wide arrowheads).
(B) Quantification of FITC-*h*alb in podocytes and tubules of Vps34-and PIKfyve deleted mouse kidneys. Using Image J software, quantification of green fluorescence in 3-week-old and 6-week-old vps34-deleted mouse podocytes shows increase accumulation of FITC-*h*alb in the 6-week-old vps34 deleted mouse podocytes compared to wild type and 3-week-old mice. There is a marked increase in FITC-*h*alb accumulation in the Vps34podko mouse tubular cells (tubular cells are not deleted of vps34) as a result of tubular uptake in heavily proteinuric mice. Uptake of FITC-*h*alb in PIKfyve-deleted podocytes was similar to control but higher in tubular cells that are also deleted of PIKfyve. NS (P value not significant), **P<0.01 and ***P<0.001. Error bars, S.E.

B