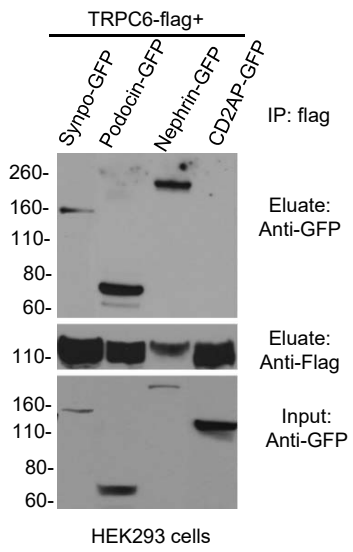
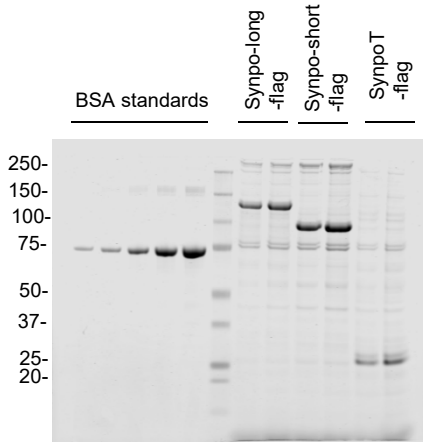
Supplemental Figure 1

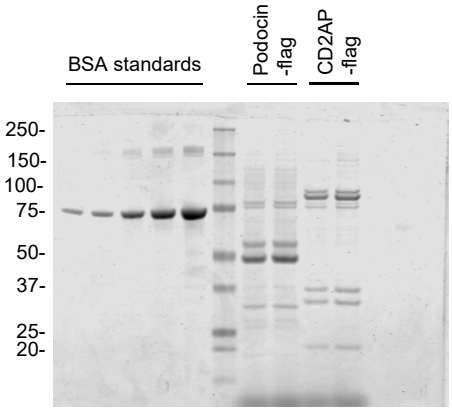
A.



B.

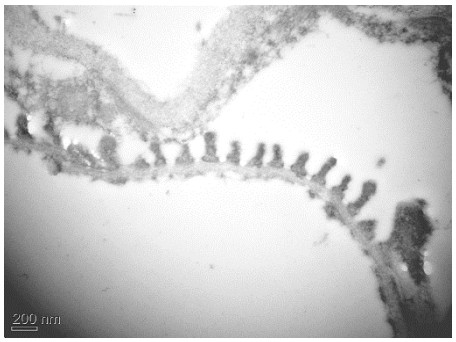


C.

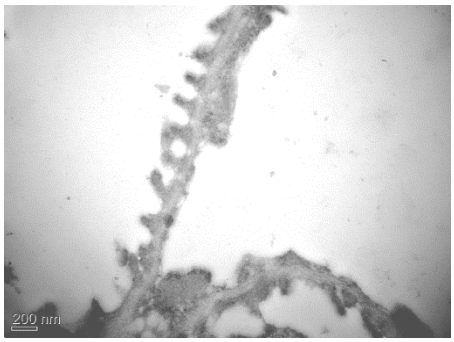


D.

Rabbit IgG + Goat IgG
with secondary antibodies



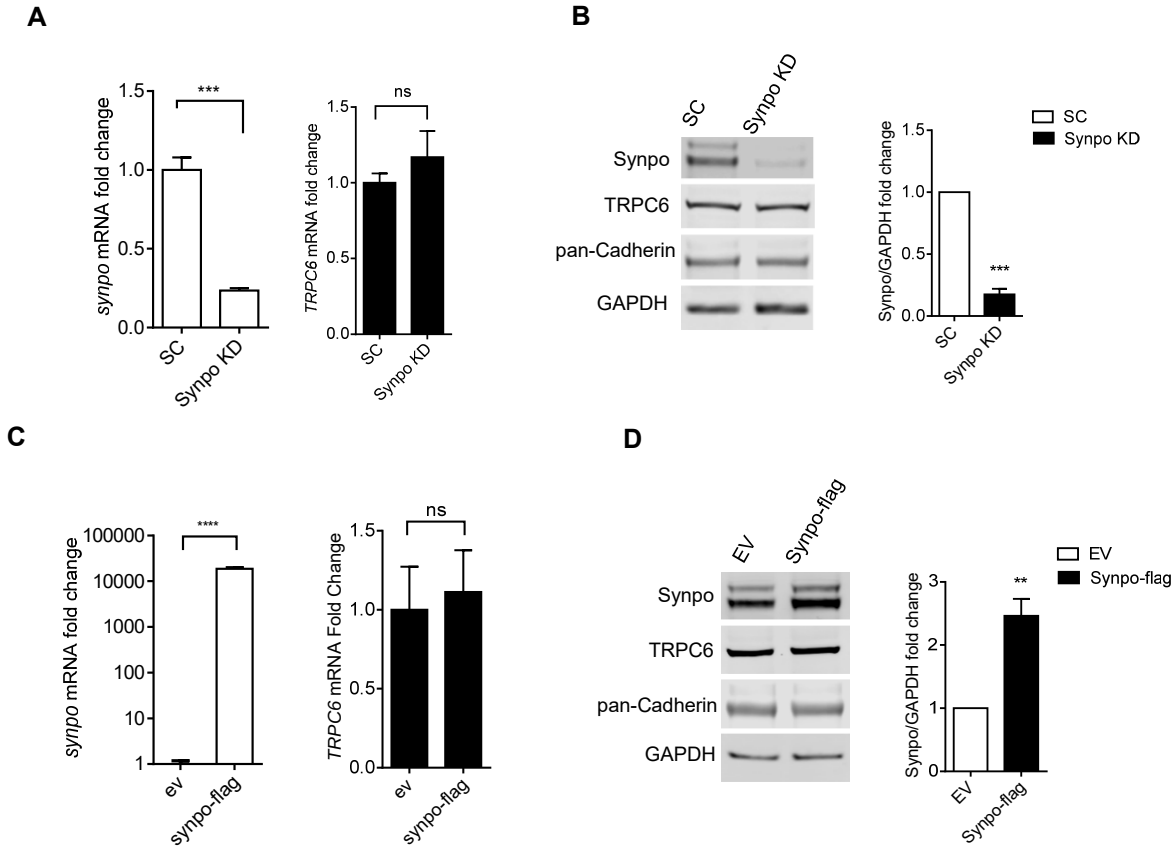
Secondary antibodies only



Control type	Number of gold particles				Total area examined (μm^2)
	In cell structure		Out of cell structure		
	15nm	10nm	15nm	10nm	
Rabbit IgG and goat IgG	1	2	1	0	36
Secondary antibodies only	0	0	1	0	36

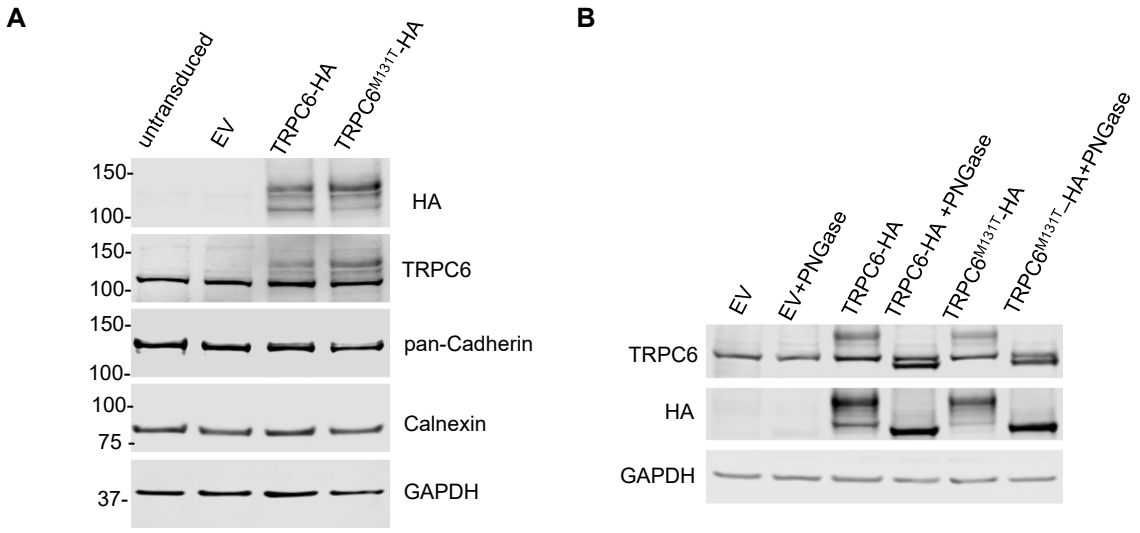
Supplemental figure 1. Additional results are shown for Figure 1. (A). Co-immunoprecipitation in HEK 293 cells confirming interaction of TRPC6 with synaptopodin, podocin and nephrin. CoIP was performed in HEK293 cells co-transfected with TRPC6-flag and GFP tagged synaptopodin, podocin, nephrin and CD2AP. (B) and (C). GelCode Blue Stain showing purified Synpo-long-flag, Synpo-short-flag, SynpoT-flag, Podocin-flag, CD2AP-flag proteins. Gels were scanned by Li-cor Odyssey imaging system. BSA standards were used for calculation of the concentrations of the purified flag-tagged proteins. (D). Negative controls for Immunogold double labeling experiment. For isotype control, TRPC6 primary antibody was replaced with rabbit IgG and synaptopodin primary antibody was replaced with goat IgG. secondary antibody control, samples were incubated with antibody diluent with no primary antibodies then incubated with secondary antibodies. All the other conditions and procedures remained the same.

Supplemental Figure 2



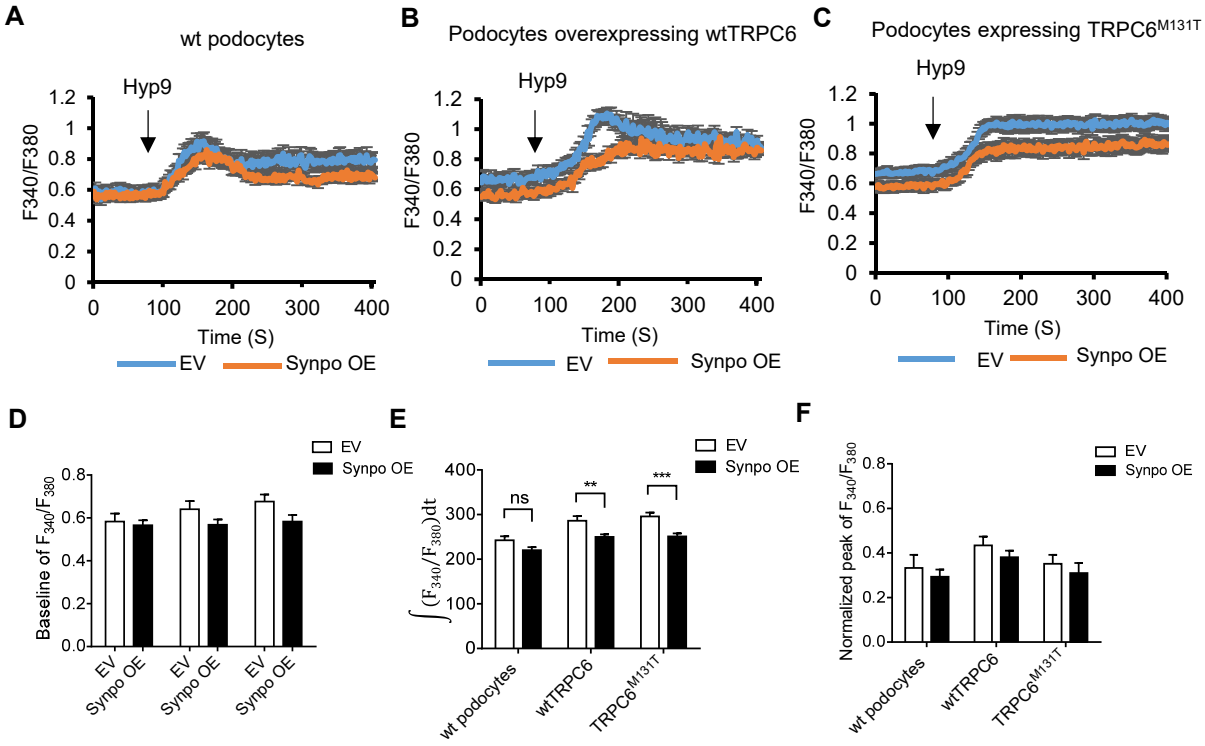
Supplemental figure 2. Real-time PCR and western blot results showing synaptopodin knockdown and overexpression. (A) Significant down-regulation of *synpo* mRNA in *synpo* shRNA transduced podocytes was detected by real-time PCR. TRPC6 transcripts levels show no difference. n=6. (B) Representative western blot showing down-regulation of synaptopodin (Synpo) protein in *synpo* shRNA transduced (Synpo KD) podocytes Quantification of Synpo fold change was based on 3 repeated experiments. TRPC6 and pan-cadherin were also blotted. (C) Significant increase in *synpo* mRNA levels in synpo-flag transduced podocytes was detected by real-time PCR. TRPC6 transcripts levels show no difference. n=6. (D) Representative western blot showing up-regulation of Synpo protein in synpo-flag transduced podocytes Quantification of Synpo fold change was based on 3 repeated experiments. TRPC6 and pan-cadherin were also blotted. Student's t test was used. **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$ graphs represent mean± s.e.m

Supplemental Figure 3



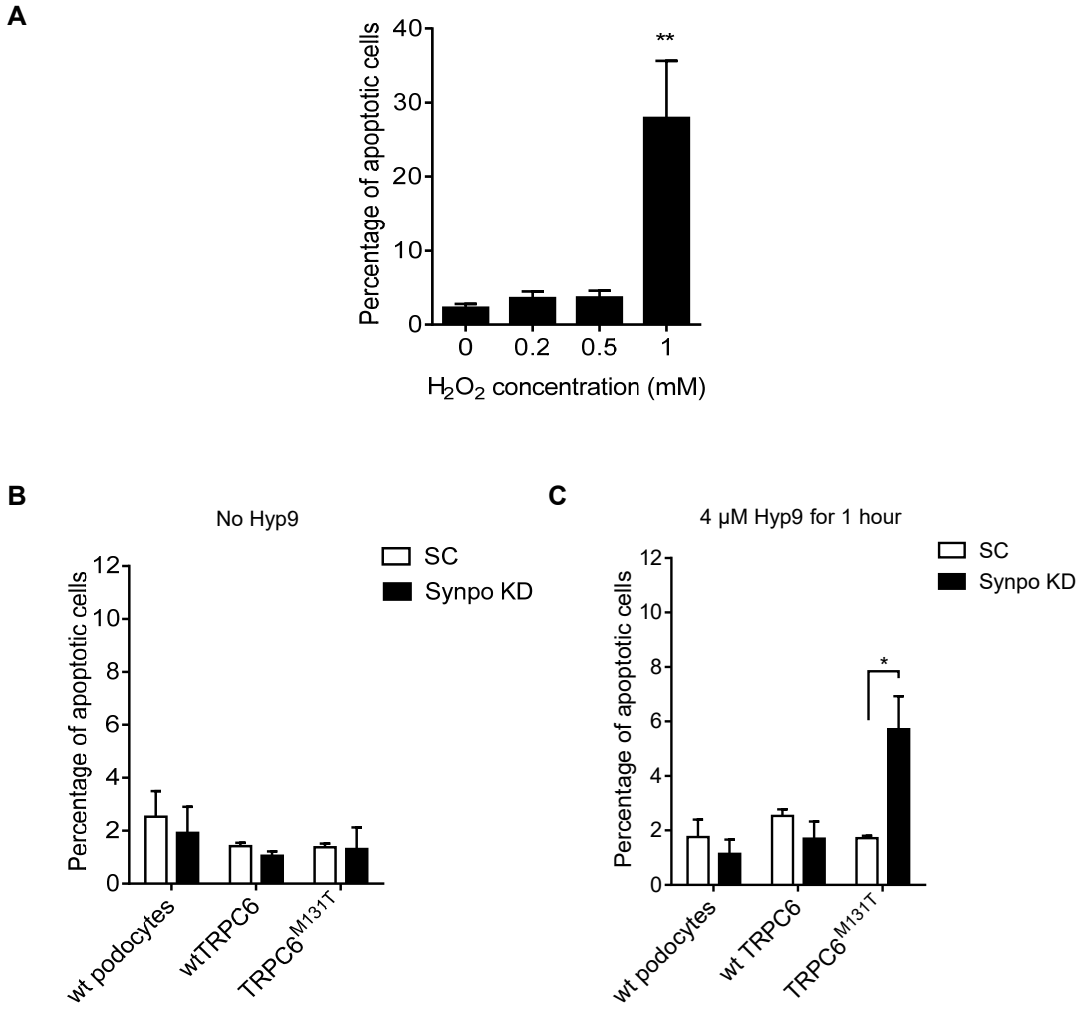
Supplemental figure 3. Overexpression of wild-type TRPC6 and mutant TRPC6^{M131T}. (A) wtTRPC6-HA, TRPC6^{M131T}-HA expression in cultured mouse podocytes by lentiviral infection. Pan-Cadherin expression was not affected by overexpression of wtTRPC6 or TRPC6^{M131T}. (B) The additional TRPC6 (HA) bands above the endogenous TRPC6 band caused by lentiviral expression of wtTRPC6-HA and TRPC6^{M131T} –HA were removed by treatment of glycosydase PNGase F indicating that the higher molecular weight forms are glycosylated TRPC6 protein.

Supplemental Figure 4



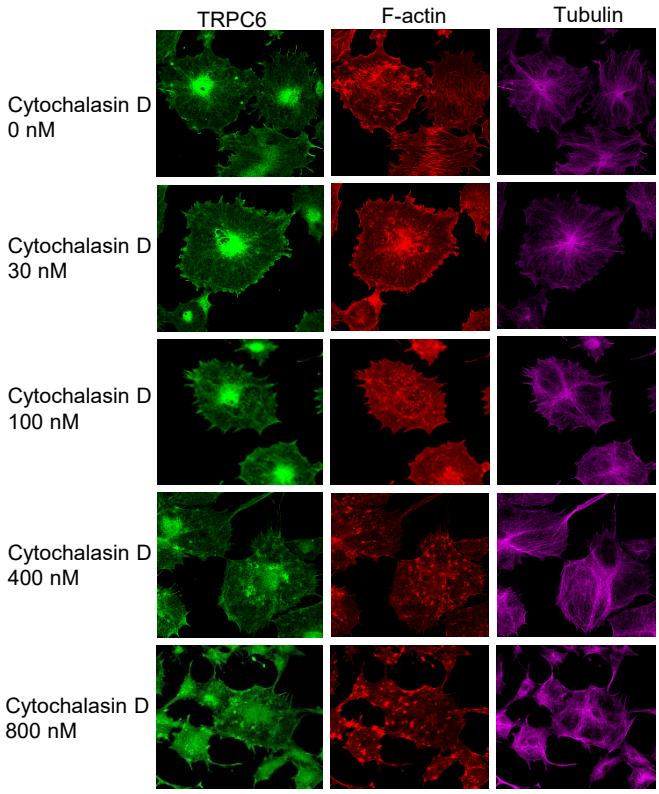
Supplemental figure 4. TRPC6-mediated calcium influx is altered by synaptopodin in podocytes with overexpressed synaptopodin. Calcium influx is demonstrated by Fura-2AM calcium imaging and is represented by 340nm to 380nm ratios in (A)EV and Synpo OE podocytes, (B)EV- wtTRPC6 and SynpoOE-wtTRPC6 podocytes, EV-TRPC6^{M131T} and (C)SynpoOE-TRPC6^{M131T} podocytes. (D) Baselines of 340nm/380nm ratios (average of the ratios before adding Hyp9) showed no significant differences. (E) Quantification of 340nm/380nm ratio changes from 90s (the time point of adding hyp9) to 400s. $\int(F_{340}/F_{380})dt$ integrated 340nm/380nm ratio during 90s and 400s. (F) peak values of 340nm/380nm ratio normalized to the according baselines showed no significant differences. GraphPad's multiple t tests *: $p<0.05$ **: $p<0.01$ ***: $p<0.001$; graphs represent mean ± s.e.m

Supplemental Figure 5



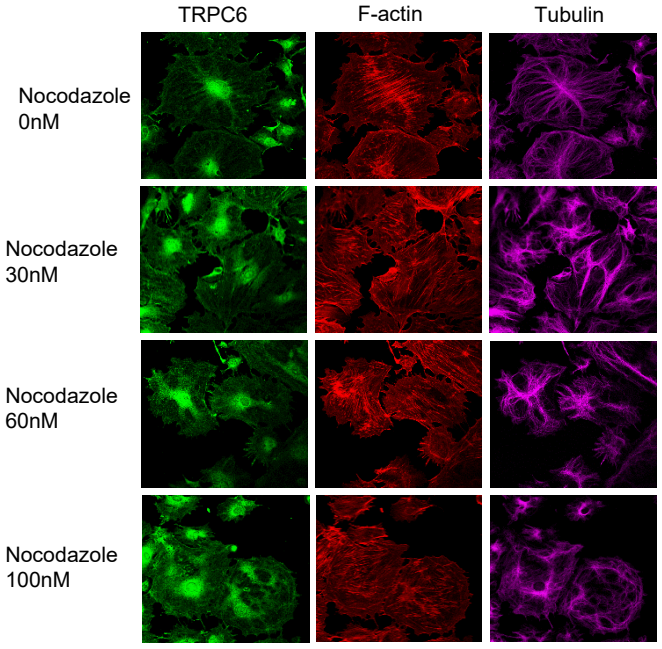
Supplemental figure 5. Additional apoptosis assays for Figure 4. (A) Apoptosis inducer H_2O_2 was used as positive control for the apoptosis assay and was also used to set up the cutoffs of positive Annexin V and PI staining. (B) and (C) Percentages of early apoptotic cells in SC, Synpo KD, SC-wtTRPC6, SynpoKD-wtTRPC6, SC-TRPC6^{M131T} and SynpoKD-TRPC6^{M131T} podocytes without Hyp9 treatment (B), or with 4 μM Hyp9 for 1 hour (C). No significant difference was found in cells without addition of the TRPC6 activator (B). Apoptosis was and was only induced in SynpoKD-TRPC6^{M131T} podocytes with 1 hour of Hyp9 treatment (C).

Supplemental Figure 6



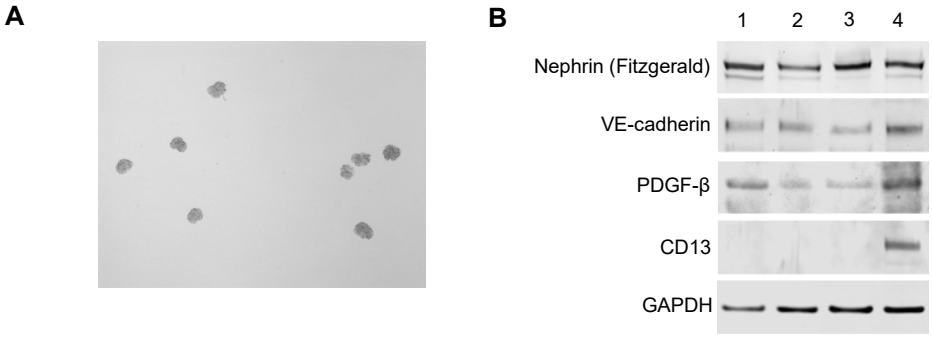
Supplemental figure 6. Representative immunocytochemistry images by confocal microscopy show patterns of TRPC6, F-actin and tubulin in cytochalasin D treated podocytes. TRPC6 and actin filament patterns show a dose-dependent response to cytochalasin D treatments.

Supplemental Figure 7



Supplemental figure 7. Representative immunocytochemistry images by confocal microscopy show patterns of TRPC6, F-actin and tubulin in nocodzole treated podocytes. TRPC6 and microtubule pattern show a dose-dependent response to nocodazole treatments.

Supplemental Figure 8

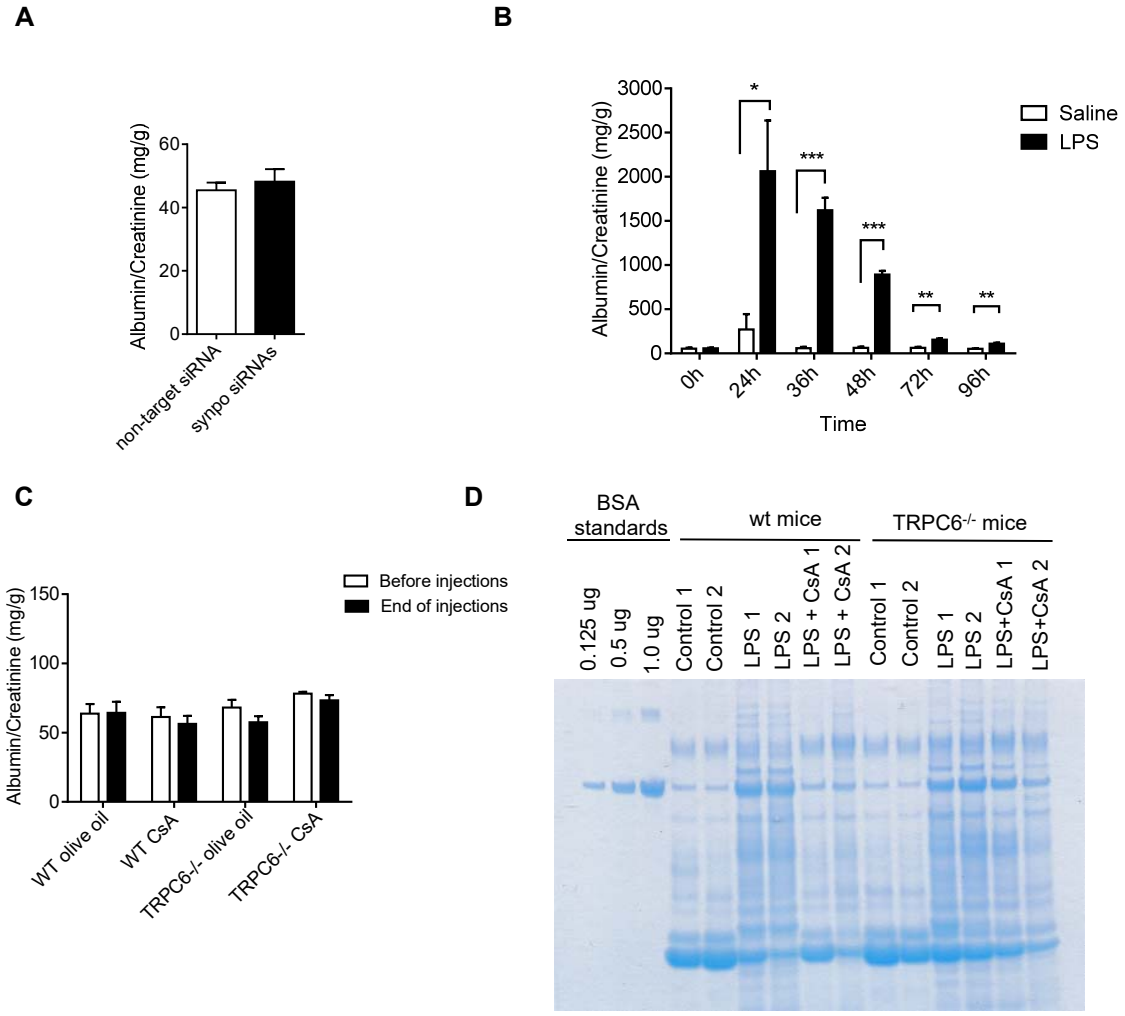


Supplemental figure 8. *In vivo* podocytes are enriched by immunopanning. (A) purified glomeruli after enzyme digestion of the kidneys and purification of the digested tissue with two sizes of cell strainers as described in methods. (B) The enrichment of podocytes from single cell preparation of the isolated glomeruli was done as described in methods. Nephrin (antibody from Fitzgerald, Acton MA was used), VE-cadherin, PDGF-β, CD13 were probed as specific markers for podocytes, endothelial cells, mesangial cells and proximal tubular cells respectively.

1. Single cells digested from glomeruli (obtained by enzyme digestion) before immunopanning
2. Cells collected after immunopanning with podocalyxin antibody
3. Cells collected after immunopanning with nephrin (extracellular) (sigma-aldrich) and podocalyxin antibodies.
4. Single cells digested from glomeruli preparation by sequential sieving.

High purity of podocytes was obtained by immunopanning with both nephrin and podocalyxin antibodies. Nephrin and podocalyxin double immunopanning was then used to enrich podocytes for *in vivo* experiments in this study.

Supplemental Figure 9



Supplemental figure 9. Additional urinary albumin levels are shown by ELISA results or gel staining. (A) Urinary albumin (mg) to creatinine (g) ratios from mice injected with non-target siRNA and *synpo* siRNAs. No significant difference was found. 5 mice in each group. (B) urinary albumin (mg) to creatinine (g) ratios in saline and LPS injected mice at 24h, 36h, 48h, 72h and 96h after injection showing highest average level at 24h with large standard errors in both saline and LPS groups. 36h was selected as the time point for urine analysis in the study for higher consistency (smaller standard errors). 4 mice were used in each group.(C) Urinary Albumin (mg) to creatinine (g) ratios in cyclosporine A treated wild-type and TRPC6$^{-/-}$ mice compared to olive oil controls. No significant differences were found. Student's t test was performed in (A). GraphPad's multiple t tests were performed in (B). Two-way ANOVA was performed in (C) *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ graphs respresent mean± s.e.m. (D) Representative urine samples (1 µl/lane) from the indicated mice were run on a NuPAGE 4-12% Bis-Tris gel and stained with GelCode Blue Stain (Thermo Scientific)