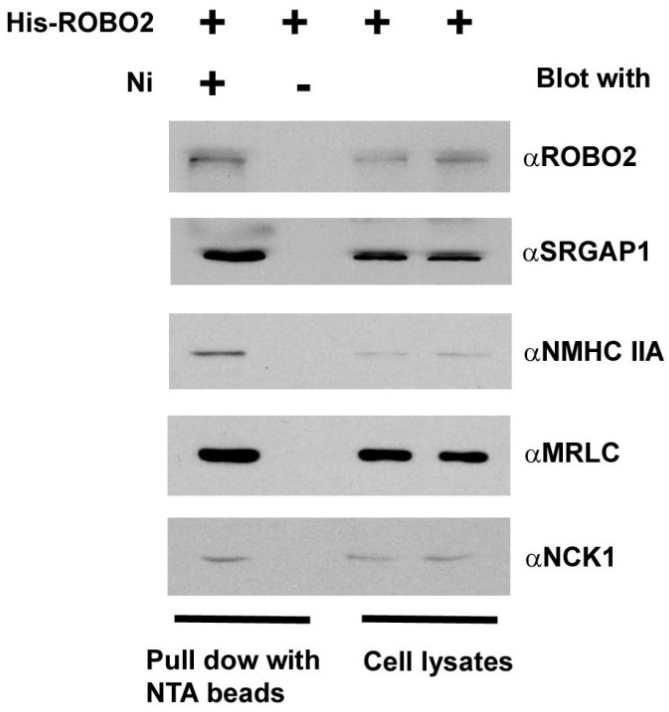
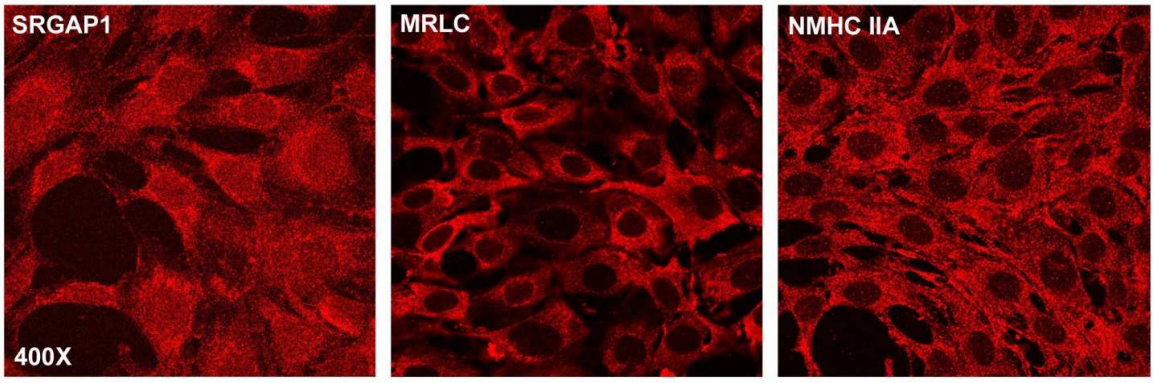
Supplemental material

Supplemental Figure 1



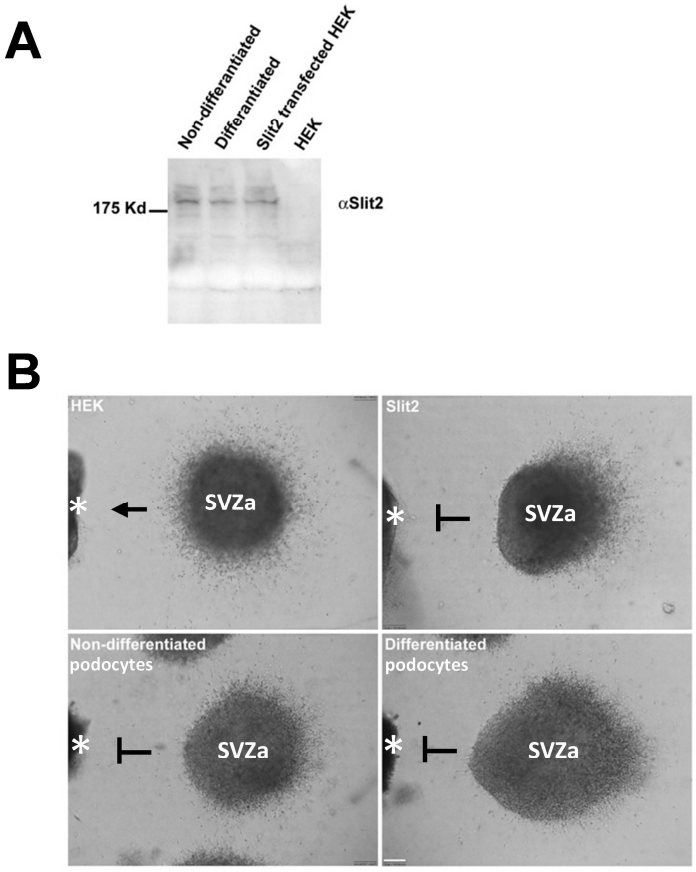
Supplemental Figure 1. ROBO2 co-precipitates with SRGAP1, NMHC IIA, MRLC, and NCK1 in mouse podocyte cell line. His-ROBO2 was stably expressed in mouse podocytes and precipitated with Ni-NTA beads (lanes 1) or control beads (lane 2) in the presence of SLIT2. Full-length ROBO2 co-precipitates with SRGAP1, NMHC IIA, MRLC, and NCK.

Supplemental Figure 2



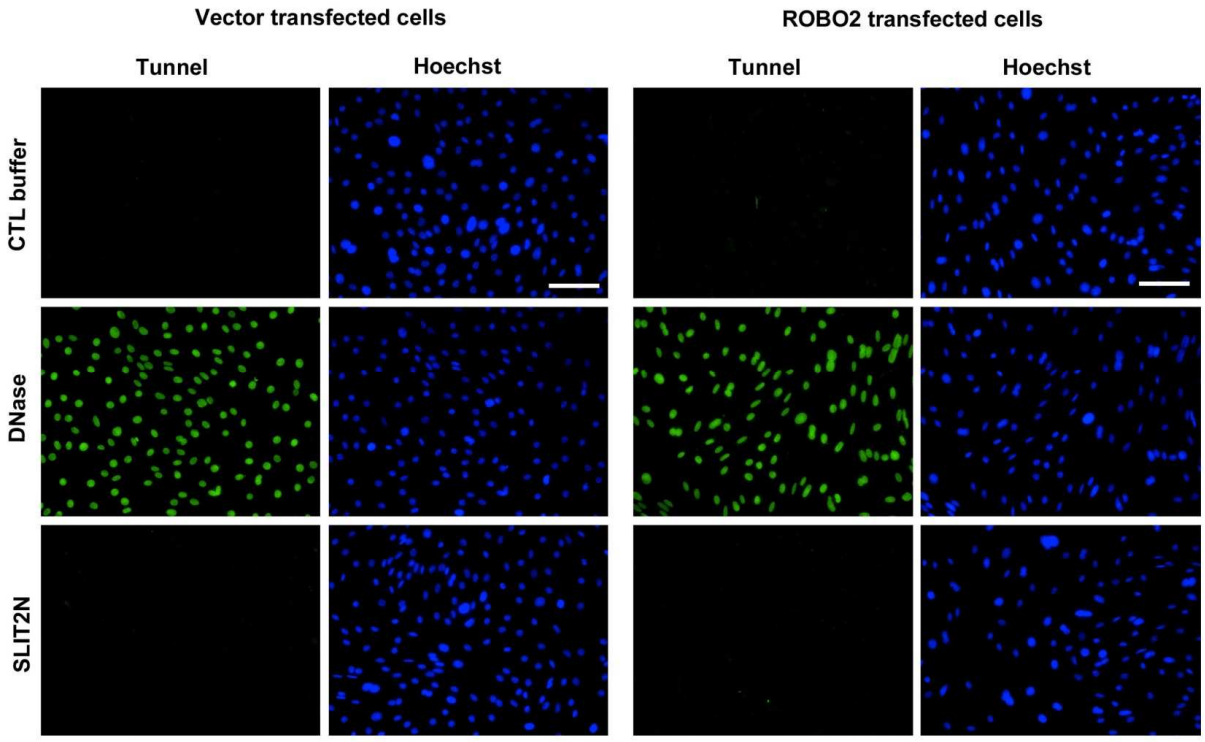
Supplemental Figure 2. SRGAP 1, MRLC, NMHC IIA expression in podocytes cultured at high confluence. Differentiated mouse podocyte cells are cultured to higher confluence to permit cell to cell contacts, and are then stained with antibodies against SRGAP1, MRLC, and NMHC IIA. 400X magnification.

Supplemental Figure 3



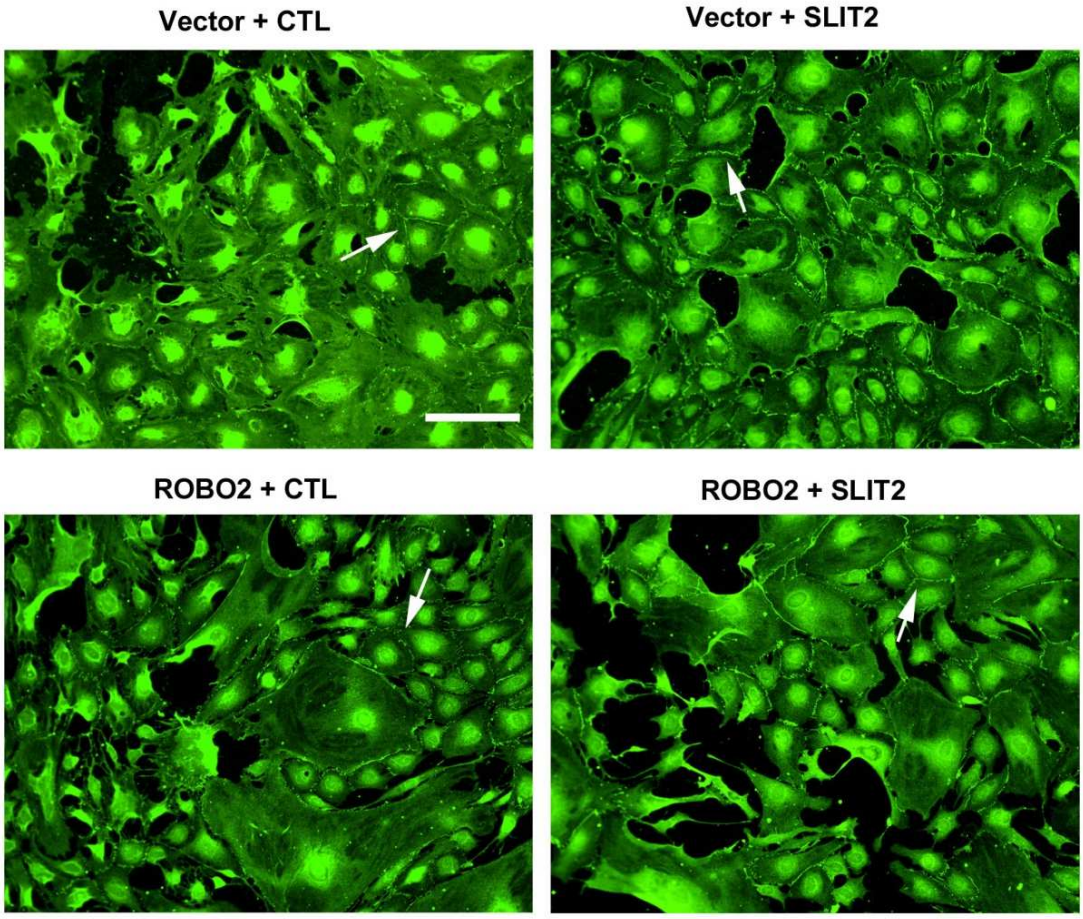
Supplemental Figure 3. Podocyte cells secrete functional SLIT2 that repels SVZa neuron migration. (**A**) Western blot analysis show SLIT2 is expressed in differentiated, non-differentiated cultured podocytes, as well as HEK cells transfected with SLIT2 cDNA. (**B**) Co-culture cell migration assays of explants from rat anterior subventricular zone (SVZa) neurons and cell aggregates (asterisk at left side in each panel) of non-differentiated and differentiated cultured podocytes show that SVZa neurons migrated away from podocyte cell aggregates (lower panels) after 24 hours culture on collagen. HEK cells transfected with SLIT2 was used as a positive control and HEK cells without SLIT2 was used as a negative control for SVZa neuronal migration (upper panel).

Supplemental Figure 4



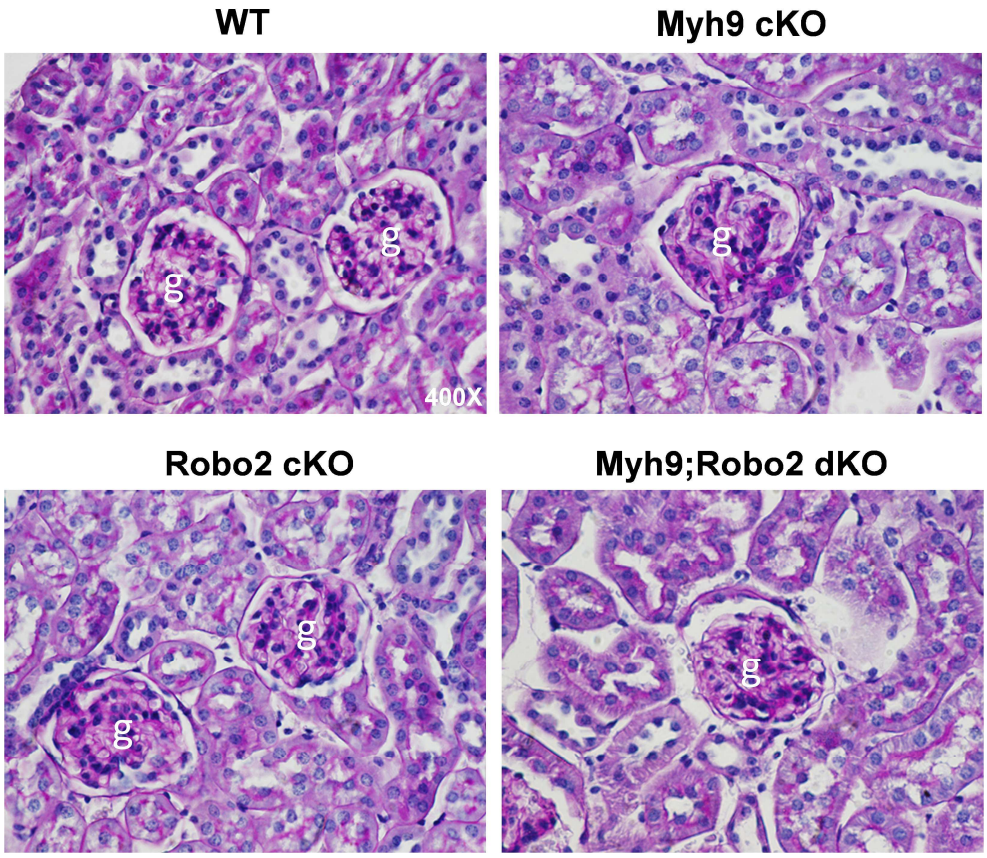
Supplemental Figure 4. Activation of SLIT2-ROBO2 pathway does not cause apoptosis of podocytes. No apoptotic cells were observed in differentiated mouse podocytes with either SLIT2 treatment for 1hr (lower SLIT2N panels) or ROBO2 overexpression in podocytes (right ROBO2 transfected cells columns). Vector transfected cells with control buffer (upper left two panels) were used as negative control. DNase treatment of podocytes (middle row) was used as positive control with numerous positive green TUNEL reaction signals. Scale bar: 121 μm.

Supplemental Figure 5



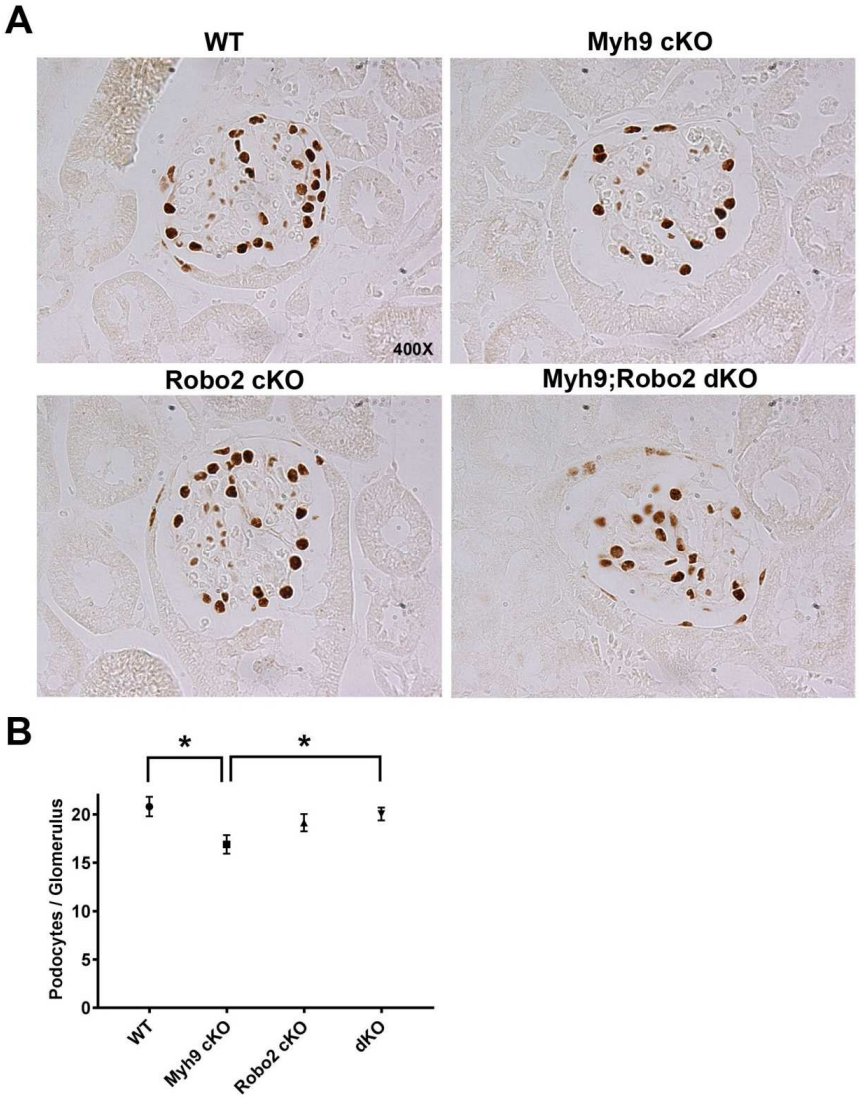
Supplemental Figure 5. There are no changes in ZO1 distribution at the tight junctions among vector controls and ROBO2 over-expression podocytes with or without SLIT2 treatment. Representative images of immunostaining of cell-cell junction protein ZO1 (arrows) in differentiated vector control cells (upper two panels) or ROBO2 overexpressing mouse podocytes (lower two panels) treated with buffer (CTL) or SLIT2-N. Scale bar: 121 μm.

Supplemental Figure 6



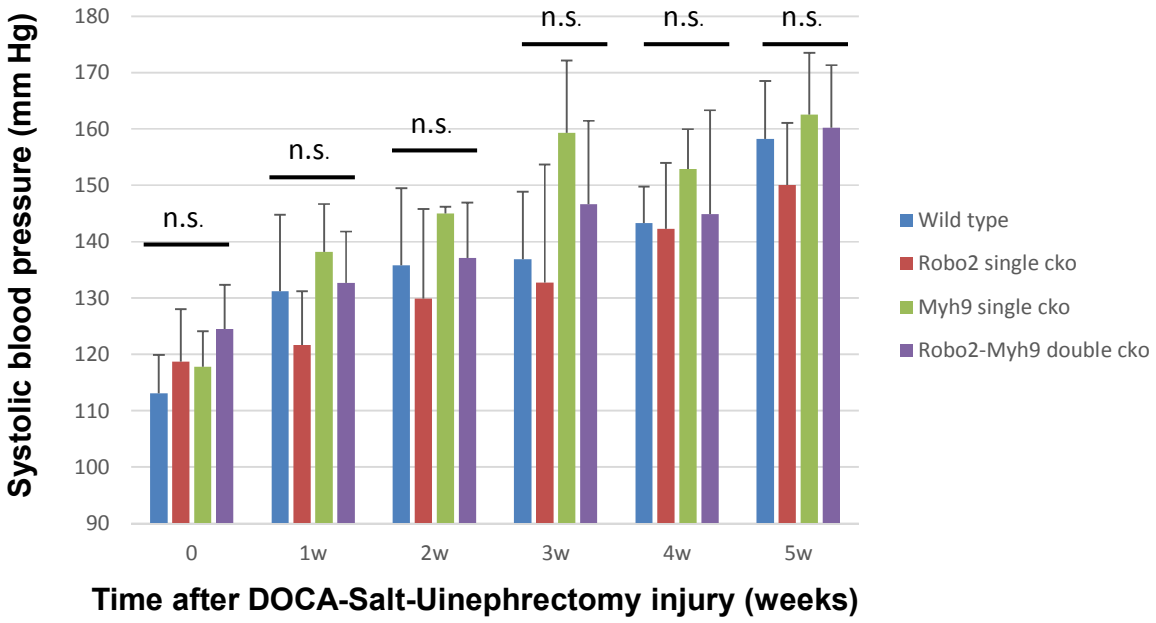
Supplemental Figure 6. Representative images of Periodic acid-Schiff (PAS) staining of mouse kidney tissues before DOCA-salt-uninephrectomy injury. Normal histology are shown in wild-type, Myh9 cKO, Robo2 cKO, and Myh-Robo2 double KO mice. Magnification: 400x; g: glomerulus.

Supplemental Figure 7



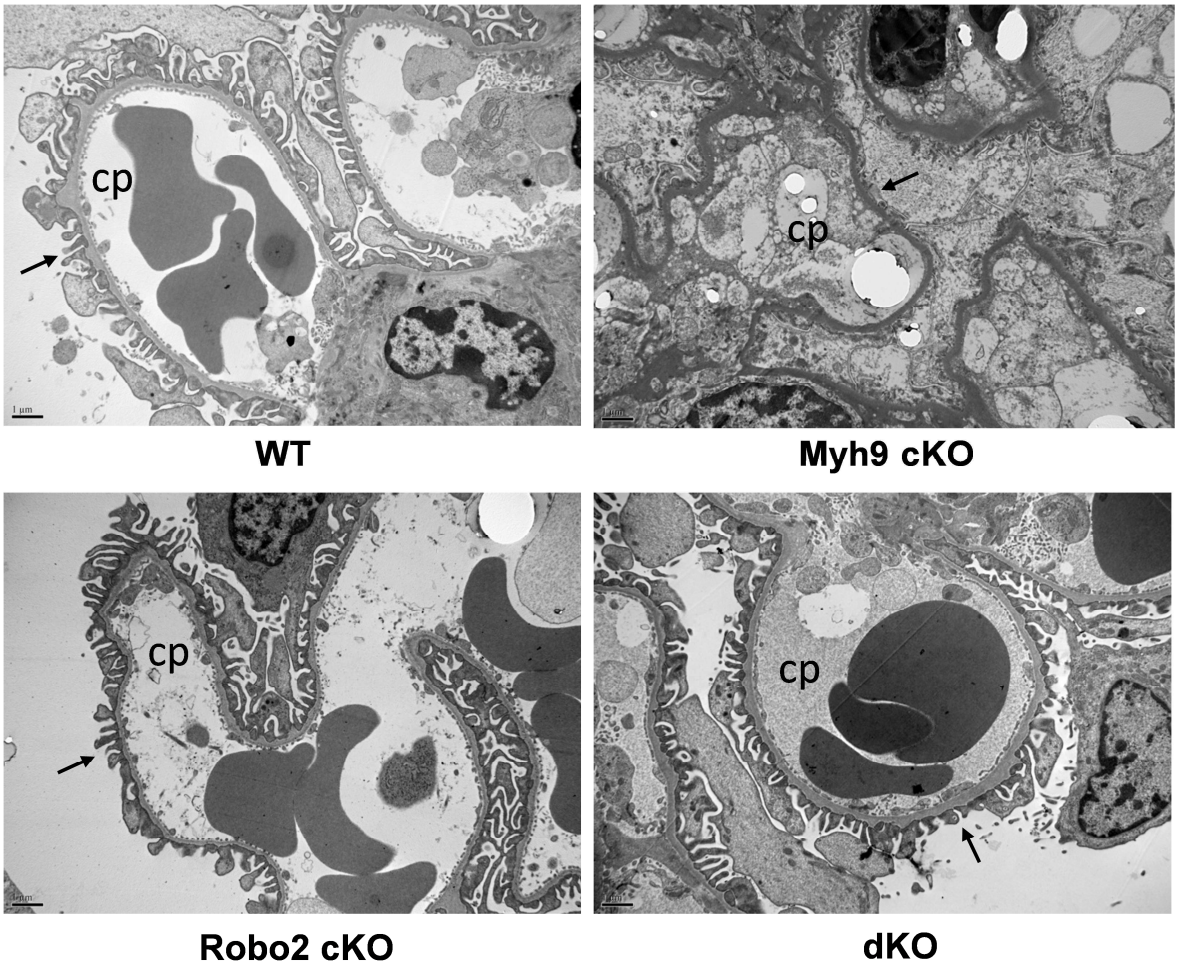
Supplemental Figure 7. *Myh9* knockout mice have fewer podocytes than wild-type mice while *Robo2* knockout partially rescues this podocyte loss. (**A**) Representative images of WT1 staining of the kidney glomeruli from the 10 weeks old *Myh9*$^{flox/flox}$;*Nphs2-Cre*$^{+}$ single knockout mice (Myh9 cKO), *Robo2*$^{flox/flox}$;*Nphs2-Cre*$^{+}$ single knockout mice (Robo2 cKO), *Robo2*$^{flox/flox}$;*Myh9* $^{flox/flox}$;*Nphs2-Cre*$^{+}$ double knockout mice (dKO), and their wild-type littermate controls (WT). (**B**) Quantification of podocytes per glomerulus in the WT1 stained kidney sections. Parietal epithelia cells on the Bowman's capsule were excluded from counting. Data are represented as mean ± SEM; n=4 animals; **p* <0.05, ANOVA.

Supplemental Figure 8



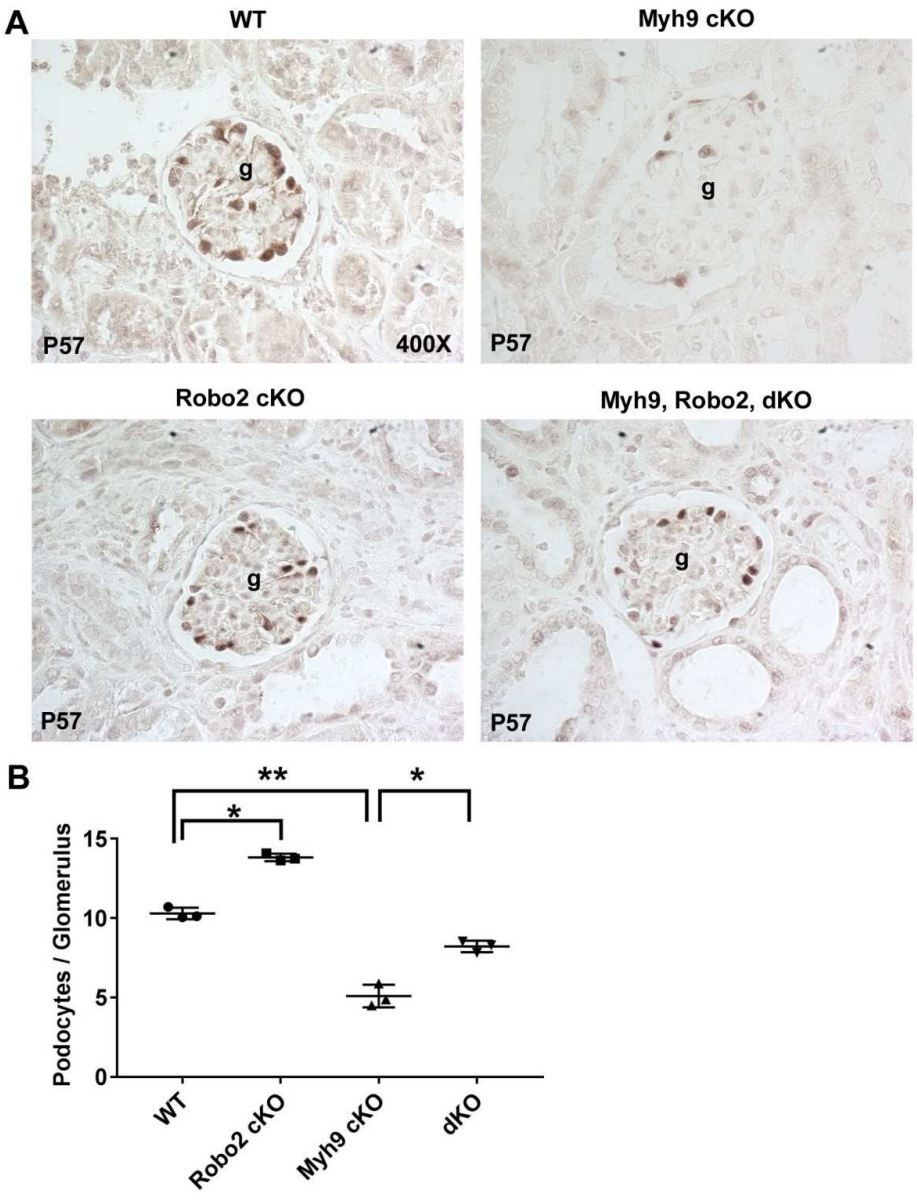
Supplemental Figure 8. All groups of *Robo2* / *Myh9* single and double cKO mice and the wild-type control developed hypertension after DOCA-salt-uninephrectomy injury. Week 0 is the baseline blood pressure before DOCA-salt-uninephrectomy injury. Ten-week old mice were used in the experiment. Mice were implanted with a 60-day, slow-release pellet containing 150 mg of DOCA. All animals received standard rodent diet and were provided with isotonic saline as drinking water (0.9% NaCl). Blood pressure was measured three times a week by non-invasive tail-cuff method for total 5 weeks before mice were sacrificed for analysis. No significant (n.s.) differences of blood pressure were found within groups at each time point (Data are represented as mean ± SEM; n=3 mice in each group; n.s. non-significant, ANOVA).

Supplemental Figure 9



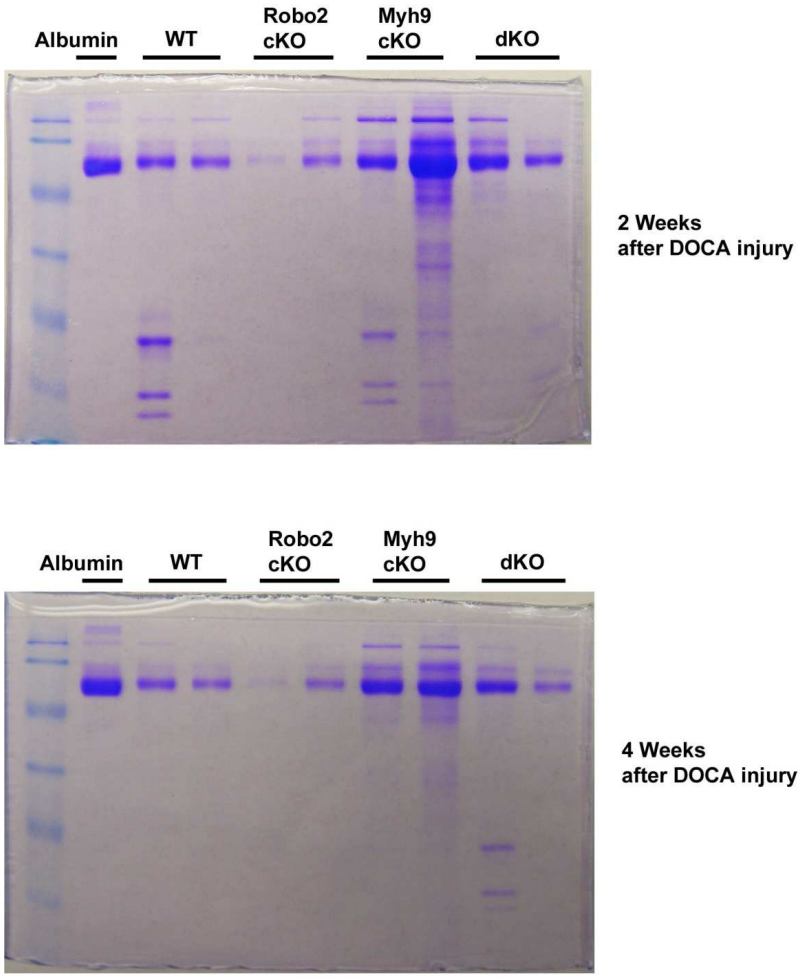
Supplemental Figure 9. Representative electron micrographs of kidney glomeruli from mice with DOCA-salt-uninephrectomy induced podocyte injury. Representative transmission EM images of kidney glomeruli from mice after 5-week DOCA-salt-uninephrectomy hypertension injury in wild-type (WT), Myh9 single cKO (Myh9 cKO), Robo2 single cKO (Robo2 cKO), and Myh9-Robo2 double KO (dKO) mice. Ultrastructure images show severe foot process enfacement in Myh9 single cKO, but relatively preserved foot process (arrow) in Myh9-Robo2 double KO (dKO) kidney in comparison to the foot processes (arrows) in wild-type (WT) and Robo2 single cKO. Scale bar: 1 μm. Abbreviation: cp, capillary loop.

Supplemental Figure 10



Supplemental Figure 10. Podocyte specific P57 staining confirms *Robo2* deficiency protects mice from DOCA-salt-uninephrectomy induced podocyte loss. (**A**) Representative images of P57 positive podocytes in the glomeruli isolated from four groups of mice with Myh9 or Robo2 single cKO, Myh9-Robo2 double cKO (dKO) and wild-type (WT) genotype after 5-week DOCA-salt-uninephrectomy injury. Magnification: 400x. (**B**) Quantification of podocyte number per glomerulus. Data are represented as mean ± SEM; n=3 animals per group; minimum 50 glomeruli are counted in each animal. *p<0.05, **p<0.01, ANOVA.

Supplemental Figure 11



Supplemental Figure 11. Albuminuria from 2 weeks and 4 weeks old mice after DOCA-salt-uninephrectomy injury detected by acrylamide gel and Coomassie blue staining. Albuminuria from mice 2 weeks and 4 weeks after DOCA-salt-uninephrectomy injury detected by acrylamide gel and Coomassie blue staining. WT: wild-type; Robo2 cKO: *Robo2*$^{flox/flox}$;*Nphs2-Cre*$^{+}$ single knockout mice; Myh9 cKO: *Myh9* $^{flox/flox}$;*Nphs2-Cre*$^{+}$ single knockout mice; dKO: *Robo2*$^{flox/flox}$;*Myh9* $^{flox/flox}$;*Nphs2-Cre*$^{+}$ double knockout mice.