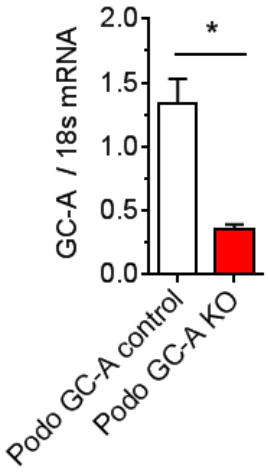
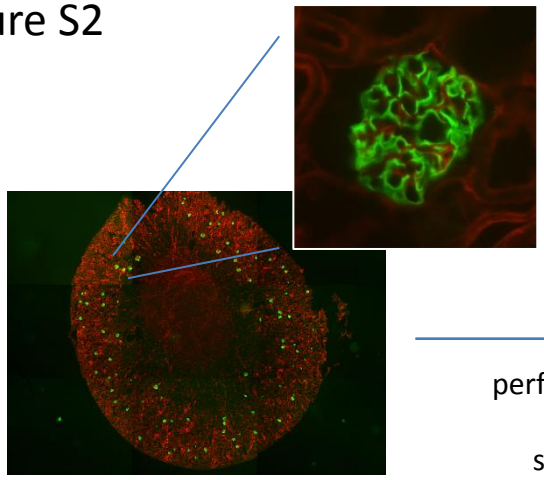
Supplement
Figure S1



GC-A mRNA expression in glomeruli that had been isolated using the magnetic bead method from kidneys of mice with podocyte-specific deletion of GC-A (Podo GC-A KO; NPHS2-Cre x GC-$A^{flox/flox}$) or their control littermates (Podo GC-A control; NPHS2-Cre x GC-$A^{WT/WT}$)(n=4 each genotype).

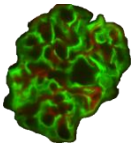
GC-A mRNA expression in isolated Podo GC-A KO glomeruli was reduced to 25% of that of Podo GC-A control.

Supplement
Figure S2



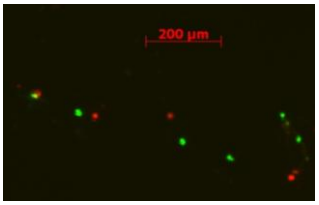
vessels, tubuli, interstitial cells
„**kidney**“

glomeruli



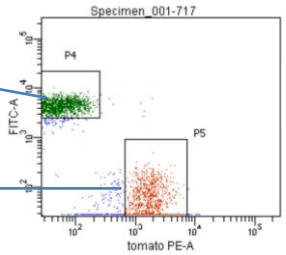
perfusion with magnetic beads
digestion
separation using magnet

digestion



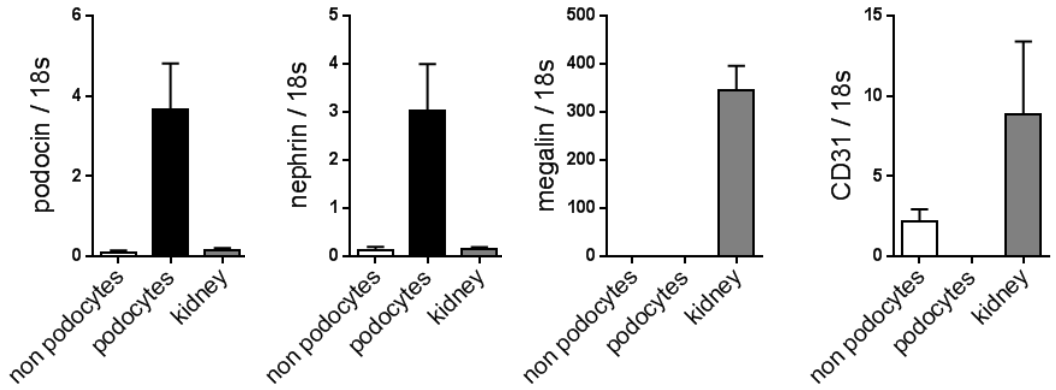
single cell suspension
of glomerular cells

FACS



„podocytes“

„non podocytes“



Supplemental figure 1: Isolation of podocytes by FACS

Upper panel: For separation of podocytes from other glomerular cells by FACS, NPHS2-Cre x GC-A$^{flox/WT}$ were crossed with a double fluorescent Cre- reporter mouse (*Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo/J}$*, mG/mT)[1]. After further crossing steps, Podo GC-A control and Podo GC-A KO mice with mG/mT expression were received. Since podocytes of these mice express GFP they show green fluorescence, while all other cells have red fluorescence. Glomeruli were isolated essentially using the magnetic bead method published previously [2, 3,4]. In brief, mice were anesthetized (12 mg/kg xylazine + 80 mg/kg ketamine) and a perfusion cannula was inserted into the abdominal aorta. After ligation of side branches and the aorta proximal to the right renal artery, 15 ml magnetic bead suspension (37°C, 2× 10^6 beads/ml; Invitrogen) was infused at a pressure of 100 mmHg. After further processing of the kidneys (digestion, cell strainer), the glomeruli were separated from the other kidney tissue by a magnet[2,4]. Glomeruli were further processed to receive a single cell suspension, suitable for fluorescence-activated cell sorting (FACS), as has been described in detail previously[2]. Approximately 250000 podocytes per mouse were harvested.

Lower panel: In order to test for the purity of the preparation, mRNA expression levels of podocin, nephrin, megalin and CD31 were determined in sorted podocytes, “non-podocyte” glomerular cells (red fluorescent cells which had been isolated from glomeruli by FACS) and kidney tissue (remaining tissue after isolation of glomeruli) using real time PCR. While a strong enrichment of podocin and nephrin mRNA in podocytes was detected, no expression of megalin (marker for proximal tubule) and CD31 (marker for endothelial cells) was detectable in the podocyte fraction.

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