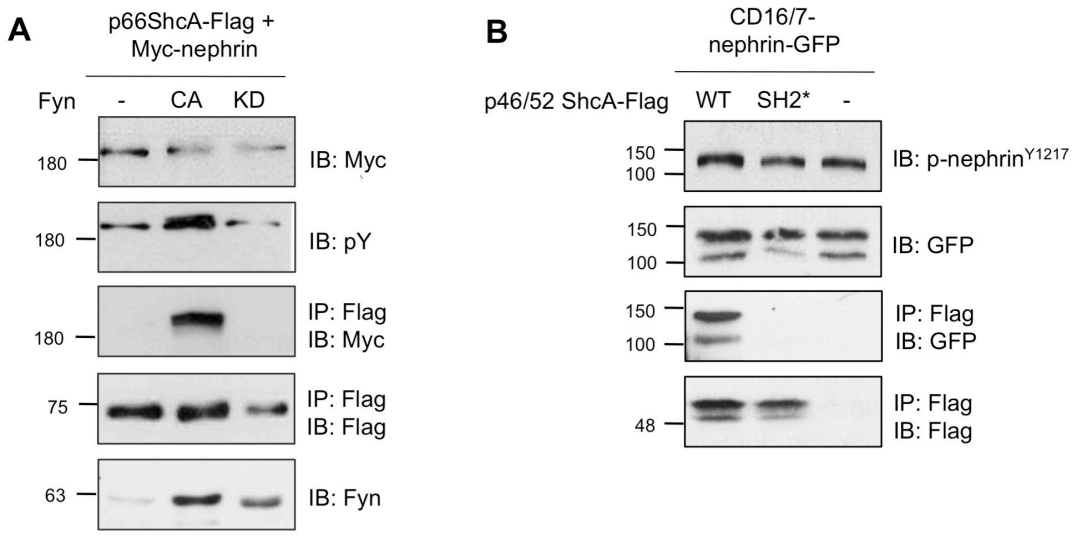
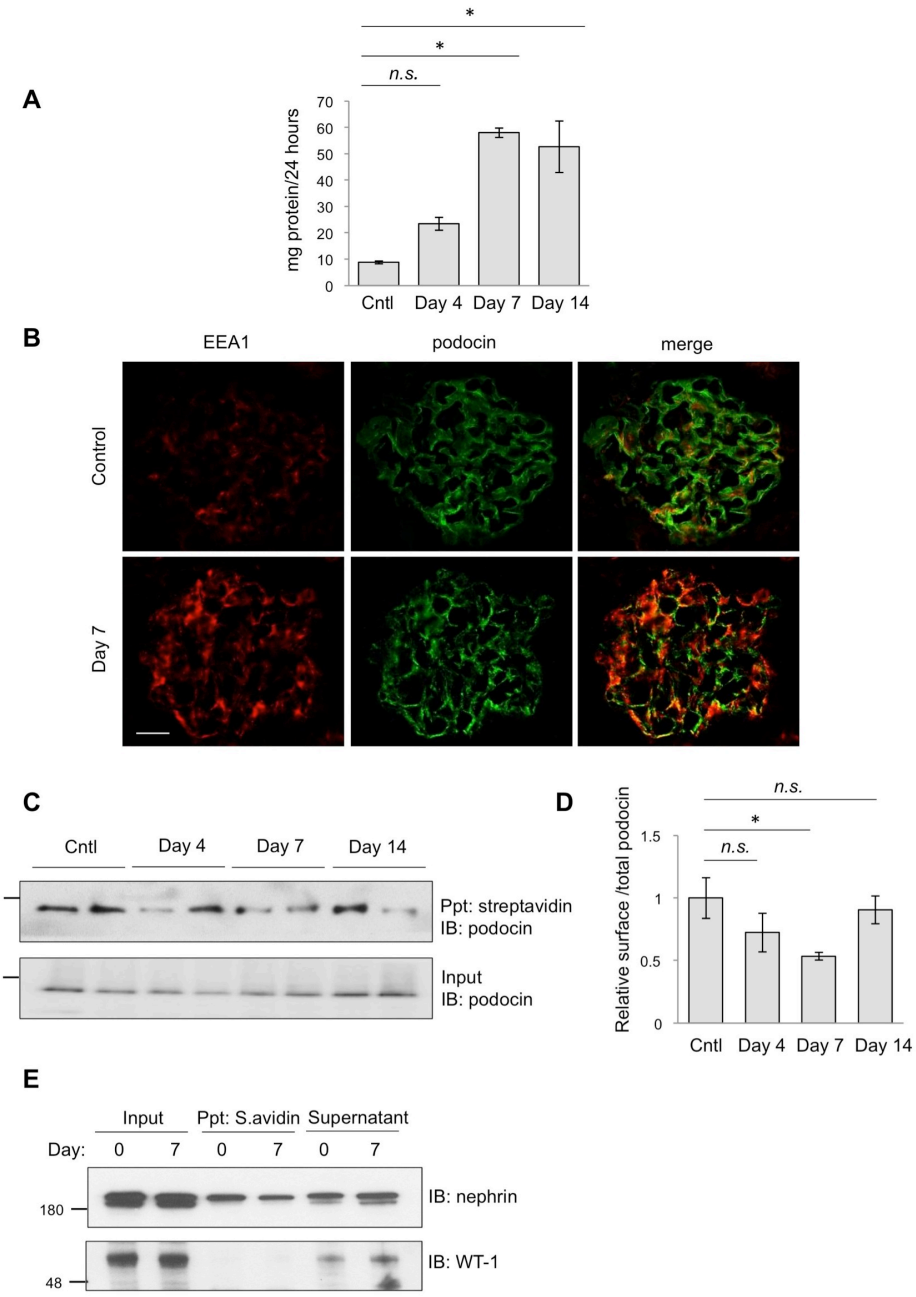
Supplemental information for Martin et al. 2017

"ShcA adaptor protein promotes nephrin endocytosis and is upregulated in proteinuric nephropathies."



Supplemental Figure 1. p66 ShcA also binds tyrosine phosphorylated nephrin and p46/52 ShcA recruitment to phosphorylated nephrin is disrupted via mutation to its SH2 domain. (A) Lysates from HEK293T cells transiently coexpressing p66 ShcA-Flag, Myc-nephrin and constitutively active (CA) or kinase dead (KD)-Fyn were immunoprecipitated (IP) with Flag and immunoblotted (IB) as indicated. p66 ShcA coimmunoprecipitated with phosphorylated nephrin. (B) Lysates from HEK293T cells transiently coexpressing WT p46/52 ShcA-Flag or an SH2* variant that cannot bind nephrin and CD16/7-neprhin-GFP. Stimulation with CD16 resulted in co-immunoprecipitation of nephrin and WT ShcA, but not the SH2* mutant.



Supplemental Figure 2. EEA1 and podocin colocalization is enhanced at the peak of PAN nephrosis. (A) Urinalysis of control (Cntl) and PAN-injected (Day 4, 7, 14) rats showing the characteristic induction of proteinuria. (B) Dual immunofluorescence staining for EEA1 (red) and podocin (green) on kidney sections of control and PAN-injected rats at Day 7 post-injection. Merged images show increased colocalization of EEA1 and podocin at Day 7 compared to control. Scale bar, 20 μm. (C) Biotin-labelled and input samples from Figure 5B were immunoblotted (IB) with podocin. (D) Densitometric quantitation showing a transient decrease in surface podocin levels during the injury timecourse. (n=3). *P<0.05 by two-tailed Student's t-test. (E) Samples from control and Day 7 of the PAN timecourse were immunoblotted (IB) with nephrin (as in Figure 5B) or WT-1, to verify that a podocyte-specific cytosolic marker is not labeled with biotin.

Supplemental Table 1: Dompep prediction of SH2 domain-containing binding partners for nephrin phosphotyrosine residues (human nephrin numbering system).

Position	SH2 domain	Value
977	FYN	0.48
	SHCA	-0.41
1114	FYN	0.64
	NCK	0.04
	SHCA	-0.58
1139	CRK	-0.62
	SHCA	-0.72
	CRK	-0.29
	SHCA	-0.72
1176	CRK	-0.29
	CRKL	-0.57
	FYN	0.25
	NCK	2.02
	SHCA	-0.21
1193	CRK	-0.37
	FYN	0.02
	NCK	2.07
	SHCA	-0.02
1210	CRK	0.23
	CRKL	0.79
1217	CRK	-0.23
	NCK	-0.08
	SHCA	-0.6

Supplemental Table 2: Patient information at the time of biopsy.

Sample	Age/sex	Urine PCR (g/mmol) except FSGS3	Serum creatinine (μmol/L)	Notes
Control 1	59 M	Negative	110	
Control 2	-	Negative	-	Deceased kidney donor
Control 3	31 M	Negative	85	
FSGS1	31 F	1.86	84	
FSGS2	24 M	2.99	85	
FSGS3	12 M	>3 g/L	65	
FSGS4	30 F	0.32	95	
MCD1	56 M	1.33	97	
MCD2	54 M	1.07	176	Acute tubular necrosis
MCD3	31 M	0.97	90	