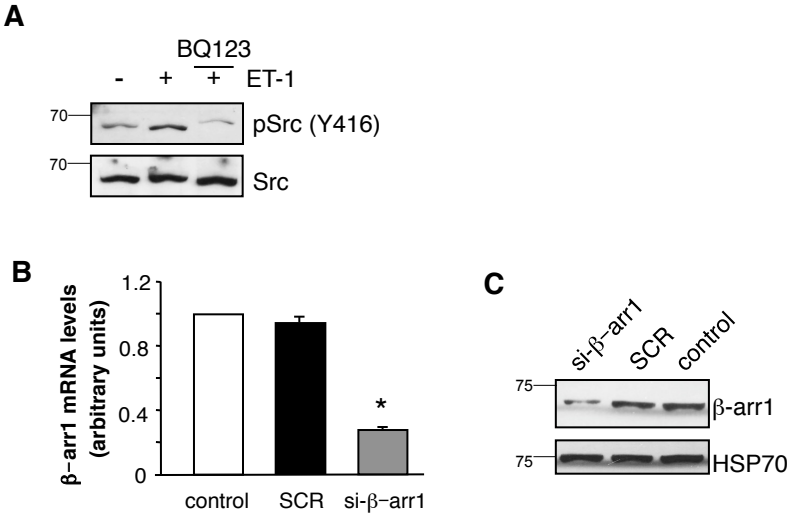

Figure S1. (A) Representative IB analysis of cell extracts from podocytes exposed to control medium, ET-1 (100 nM, 15 minutes) alone or in the presence of BQ123 (1 μM). The phosphorylation status of Src was verified by using anti anti-phospho-Src (Y416) and anti-Src antibodies. (B,C) β-arrestin-1 mRNA and protein expression evaluated by real time-PCR (B) and IB (C) in untransfected (control), scrambled (SCR) and β-arrestin-1 (si-β-arr1) siRNA transfected podocytes. *P<0.001 versus control and SCR. Anti-HSP70 antibody was used to confirm equal protein loading. Molecular mass is indicated in kilodaltons.

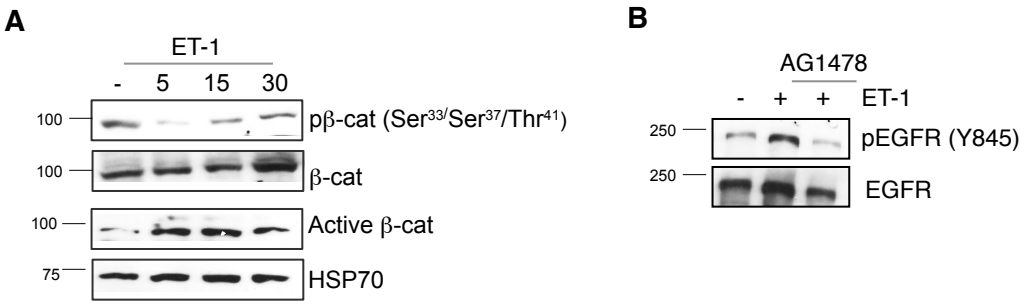

Figure S2. (A) Cell lysates from podocytes incubated with control medium or ET-1 (100 nM) for 5, 15 or 30 minutes were immunoblotted with anti-phospho-β-catenin (Ser33/37/Thr41) and anti-β-catenin antibodies. (B) The effect of the EGFR inhibitor AG1478 was analyzed by IB in podocytes exposed for 15 minutes to control medium, ET-1 (100 nM) alone or in the presence of AG1478 (1 μM). The phosphorylation status of EGFR was assessed by using anti-phospho-EGFR (Y845) and anti-EGFR antibodies. Molecular mass is indicated in kilodaltons.

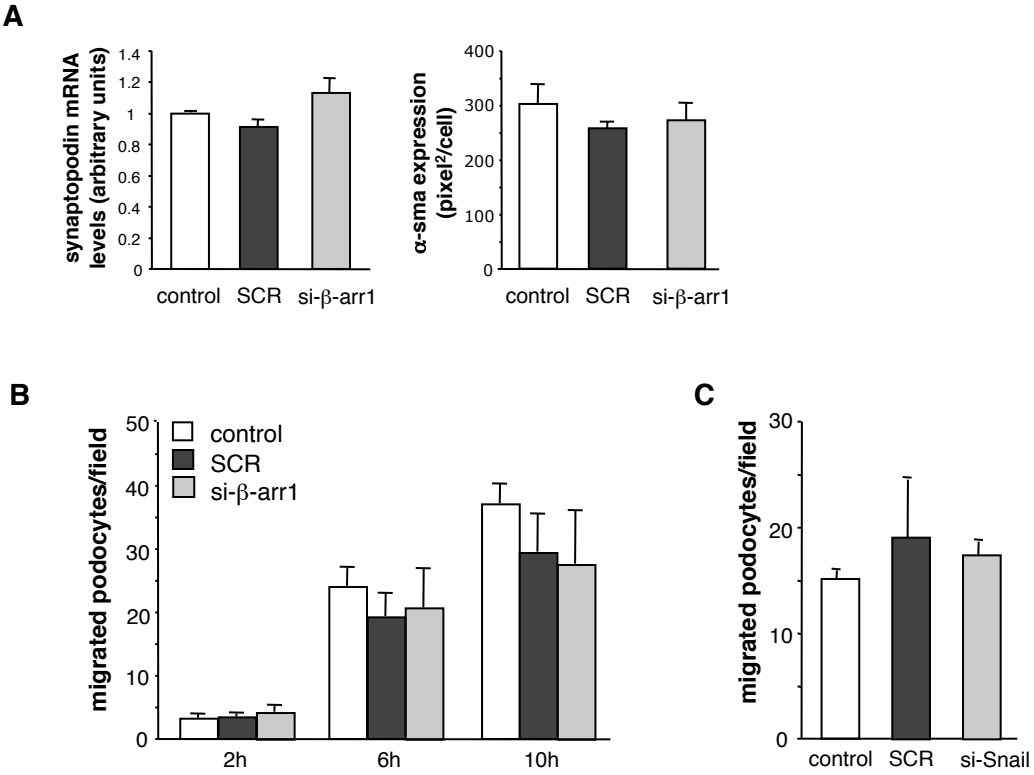

Figure S3. (A) Real time RT-PCR of synaptopodin mRNA expression (left) and quantification of α-sma immunofluorescence staining (right) in untransfected (control), scrambled (SCR) and β-arrestin-1 (si-β-arr1) siRNA transfected podocytes exposed to control medium for 6 hours. Data are mean ± SE (n=3 experiments). (B) Wound healing assay of untransfected (control), scrambled (SCR) and β-arrestin-1 (si-β-arr1) siRNA transfected podocytes exposed to control medium. Mean values ± SE of podocytes migrated into the wound track after 2, 6 and 10 hours (n=4 experiments). (C) Wound healing assay of untransfected (control), scrambled (SCR) and Snail (si-Snail) siRNA transfected podocytes exposed to control medium for 6 hours. Results are mean ± SE (n=6 for control; n=8 for SCR; n=10 for si-Snail).

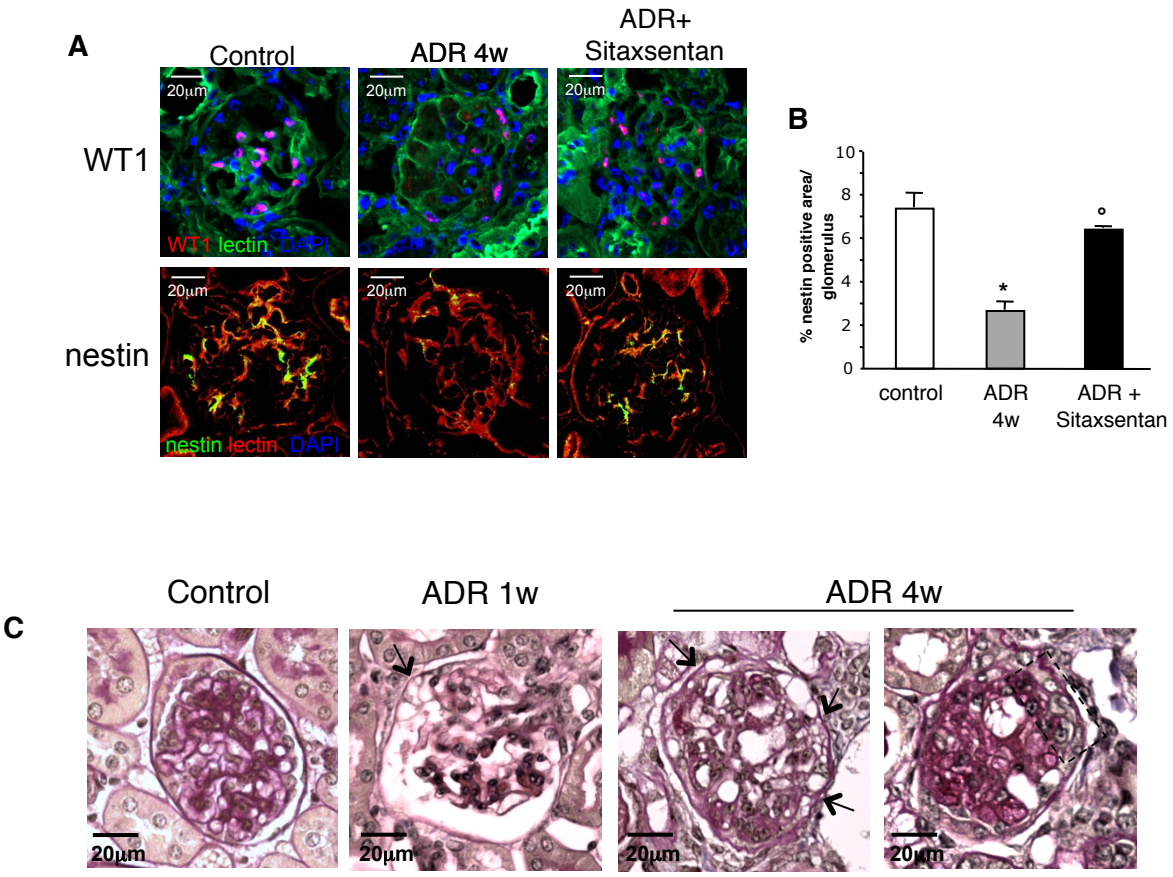

Figure S4. (A) Representative images of WT1 (red) and FITC-WGA-lectin (green) (top) or nestin (green) and rhodamine-WGA-lectin (bottom) in control and ADR-treated mice at 4 weeks receiving or not sitaxsentan. Nuclei were stained with DAPI (blue). (B) Nestin positive area evaluated as percentage of the total glomerular area (mean value ± SE) of at least 15-20 glomeruli/animal (n=3 animals/group). *P<0.001 vs control,−P<0.01 vs ADR 4w. (C) Renal histology of control and ADR-treated mice at 1 and 4 weeks. Arrows indicate synechiae and the box defines a pseudo-crescent. Scale bars are indicated.

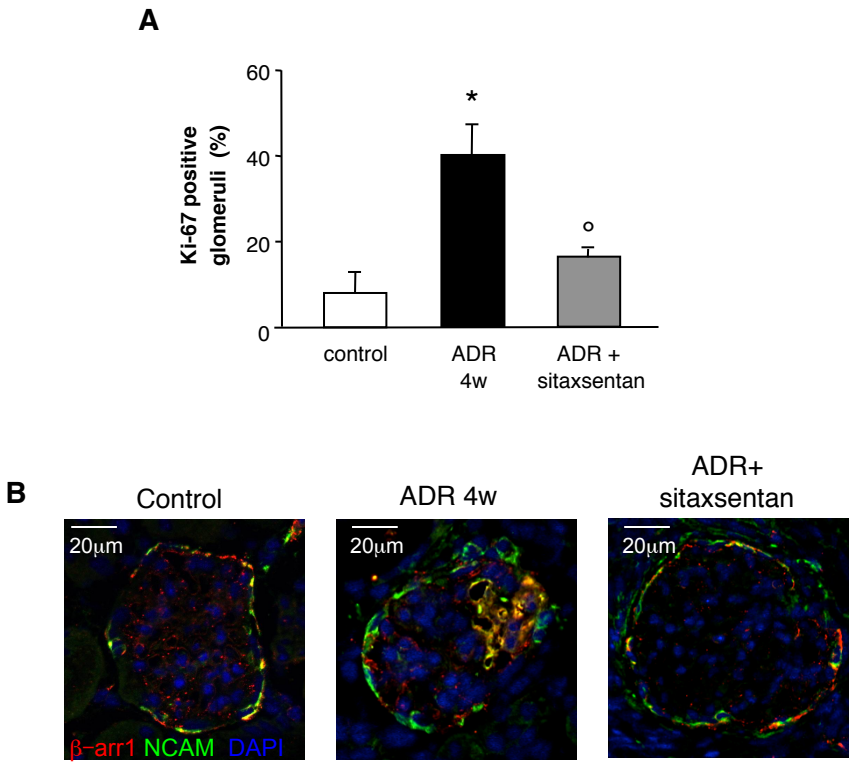

Figure S5. (A) ADR-induced nephrosis is characterized by glomerular cell proliferation. Percentage of glomeruli positive for the proliferation marker Ki-67 in control and ADR-treated animals at 4 weeks receiving or not sitaxsentan. Data are expressed as mean value ± SE of at least 25 glomeruli/animal (n=3 animals/group). * $P<0.01$ vs control, −$P<0.05$ vs ADR 4w. (B) Representative images of β-arrestin-1 (red) and NCAM (green) co-staining in glomeruli from control and nephritic mice treated or not with sitaxsentan. Nuclei were stained with DAPI (blue). Scale bars are indicated.