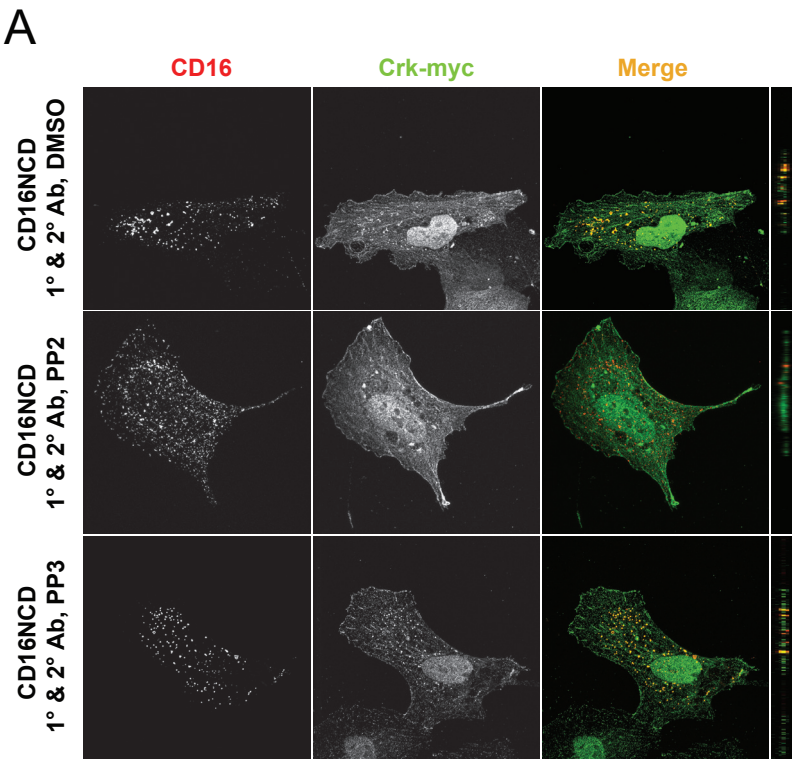
Supplements



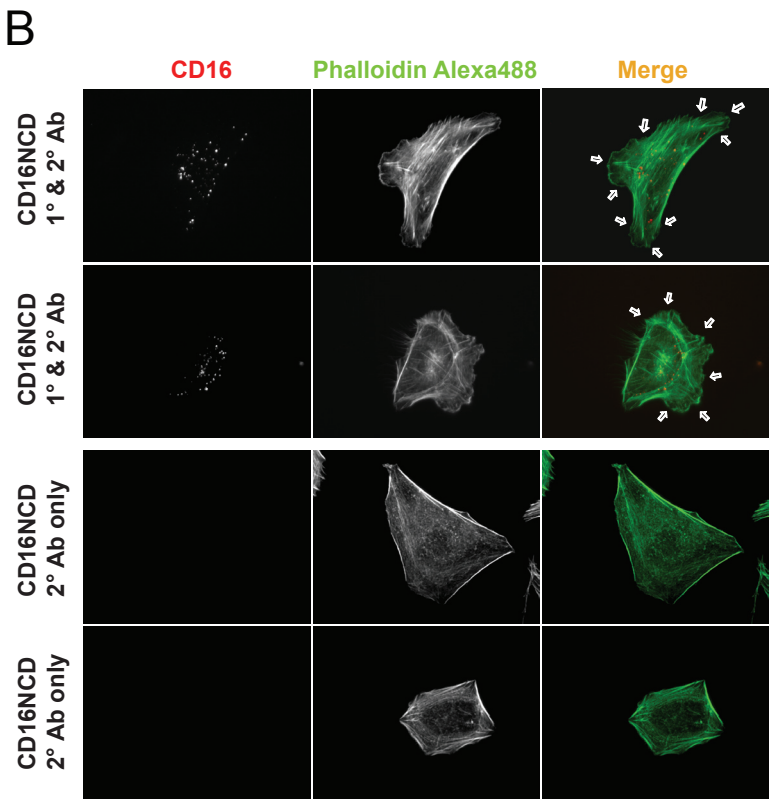


Figure S1.

(A) Src kinase activity is necessary for recruitment of Crk to Nephrin cytoplasmic domain. Human podocytes expressing CD16/7-NephrinCD (CD16NCD) were treated with solvent control (DMSO), Src kinase inhibitor PP2 or inactive control compound (PP3) before clustering with anti-CD16 antibody (1°) and secondary anti-mouse IgG antibody (Texas Red). Crk-myc was stained with anti-myc antibody and Alexa Fluor 488-labeled secondary IgG antibody. Co-localization was analyzed by confocal microscopy. YZ planes were reconstructed on the far right. Magnification: 630 X. (B) Clustered, activated CD16/7-NephrinCD induces lamellipodia formation in cultured human podocytes. CD16/7-NephrinCD was expressed in human podocytes and was activated as descibed above. Lamellipodia were visualized by staining actin with fluorophore-coupled phalloidin (green). Arrows indicate lamellipodia. Magnification: 630 X.

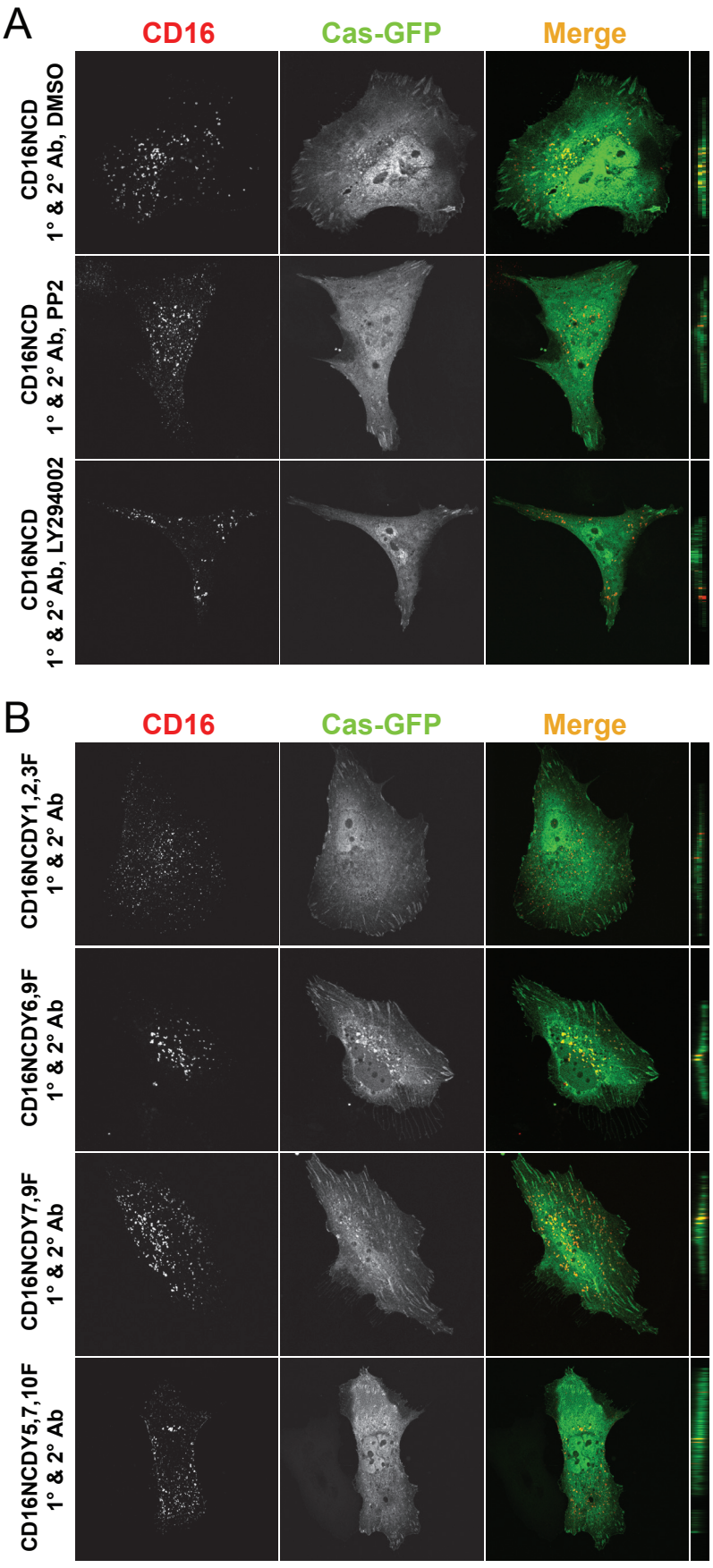


Figure S2.

Cas is recruited to Nephrin in a Src and pI-3 kinase dependent manner. (A) Human podocytes expressing CD16/7-NephrinCD and Cas-GFP were treated with solvent control (DMSO), Src kinase inhibitor PP2 or pI-3 kinase inhibitor LY294002 and activated as described above. (B) Podocytes expressing CD16/7-NephrinCD or indicated mutants (red) and Cas-GFP (green) were activated and co-localization was evaluating by confocal microscopy. YZ planes are reconstructed on the far right. Magnification: 630 X.

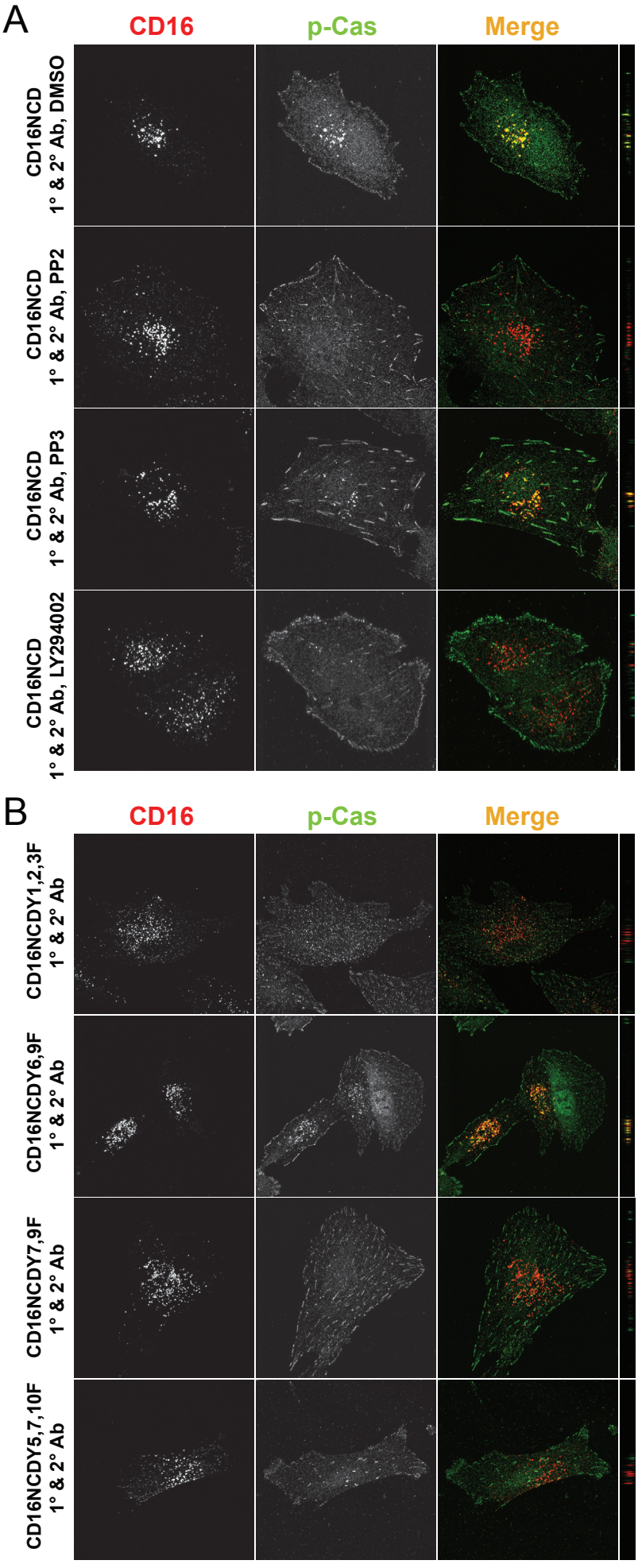


Figure S3.

Phosphorylation of Cas in Nephrin clusters depends on pI-3 kinase and Nephrin tyr residues Y5, 7, 10 (A) Human podocytes expressing CD16/7-NephrinCD were treated with DMSO (solvent control), Src kinase inhibitor PP2, inactive control compound PP3 or pI-3 kinase inhibitor LY294002 prior to clustering and p-Cas was stained with anti-p-Cas and secondary IgG antibody (Alexa Fluor 488). (B) Podocytes expressing CD16/7-NephrinCD mutants as indicated were stained and activated as in (A) and analyzed by confocal microscopy. YZ plane reconstructions are shown on the far right. Magnification: 630 X.

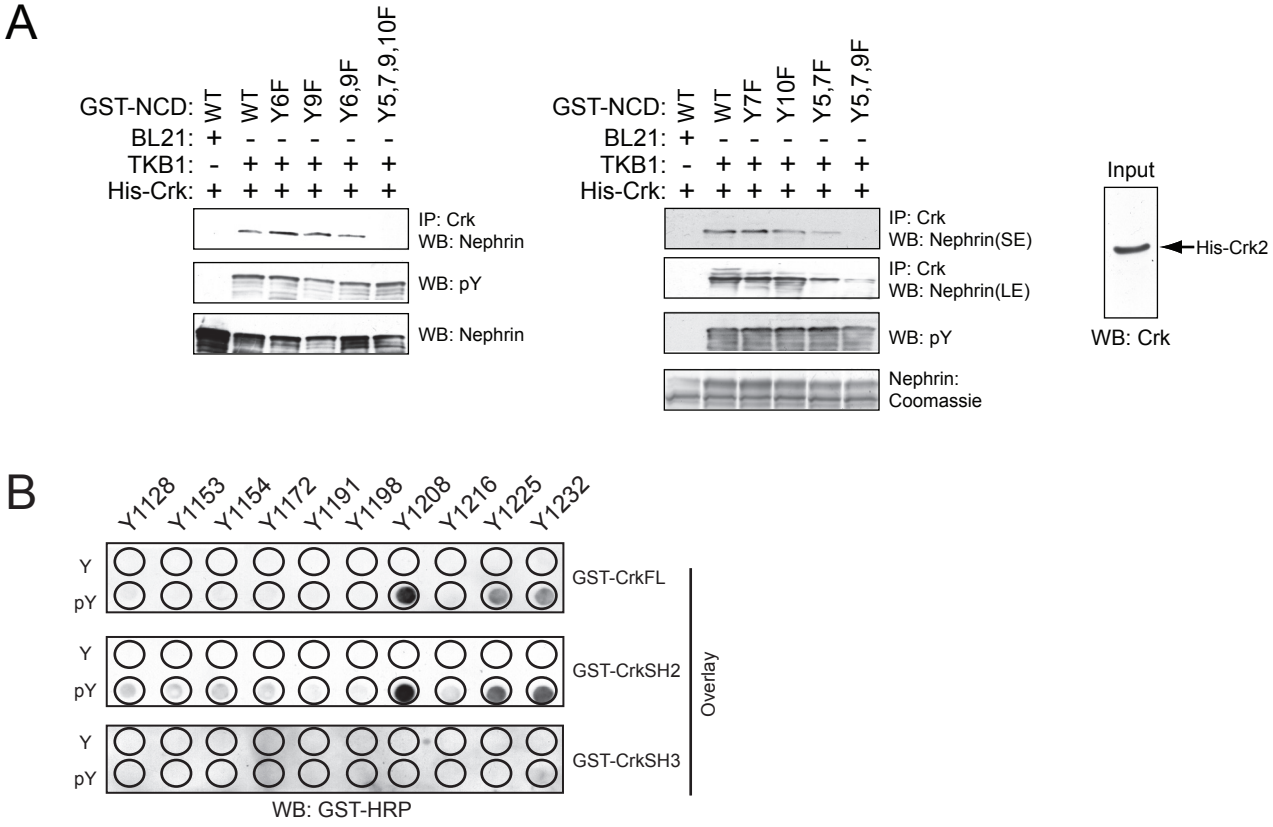


Figure S4.

Nephrin and Crk exhibit interaction affinity in vitro. (A) Purified recombinant GST-NephrinCD (GST-NCD) or indicated tyrosine residue mutants were expressed in BL21 or TKB1 *E.coli*. As shown, expression of these recombinant proteins in TKB1 cells results in Nephrin tyrosine phosphorylation. Indicated proteins were incubated with purified recombinant His-Crk2 and pulled down using glutathione agarose. (B) GST-Crk overlay. Nephrin oligopeptides were synthesized with and without phosphorylated tyrosine residues as indicated and arrayed on a nylon membrane. These membranes were incubated with purified recombinant full-length GST-Crk2, or fragments containing only the Crk2 SH2 or SH3 domain. The overlay was assayed with anti-GST antibody conjugated with horseradish peroxidase (HRP).

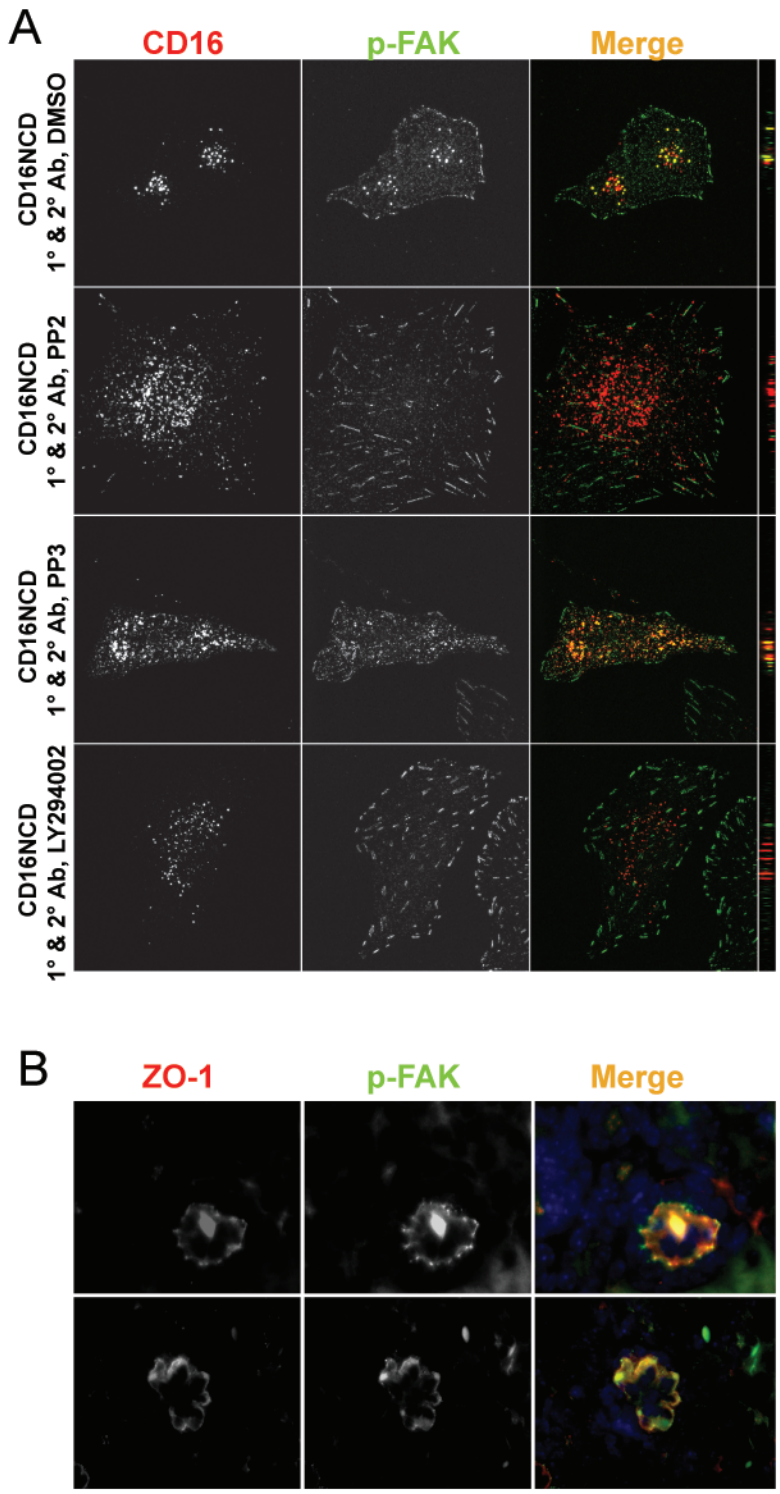


Figure S5.

Phosphorylated focal adhesion kinase (FAK) is present in activated Nephrin clusters in the CD16/7-NephrinCD model and at the podocyte precursor intercellular junction in newborn mouse. (A) Human podocytes expressing CD16/7-NephrinCD were treated with solvent control (DMSO), Src kinase inhibitor PP2, inactive control compound or pI-3 kinase inhibitor prior to clustering with anti-CD16 antibody (1°) and Texas Red conjugated secondary IgG antibody. Endogenous p-FAK was stained with phospho-FAK antibody and detected with Alexa Fluor 488 conjugated antibody (green). Co-localization was evaluated by confocal microscopy. (B) Indirect immunofluorescence: Paraffin-embedded mouse newborn kidney sections (4 µm) were stained with p-FAK or ZO-1 antibody showing that p-FAK is targeted to the podocyte precursor intercellular junction starting at the late capillary loop stage. Magnification: 630 X.

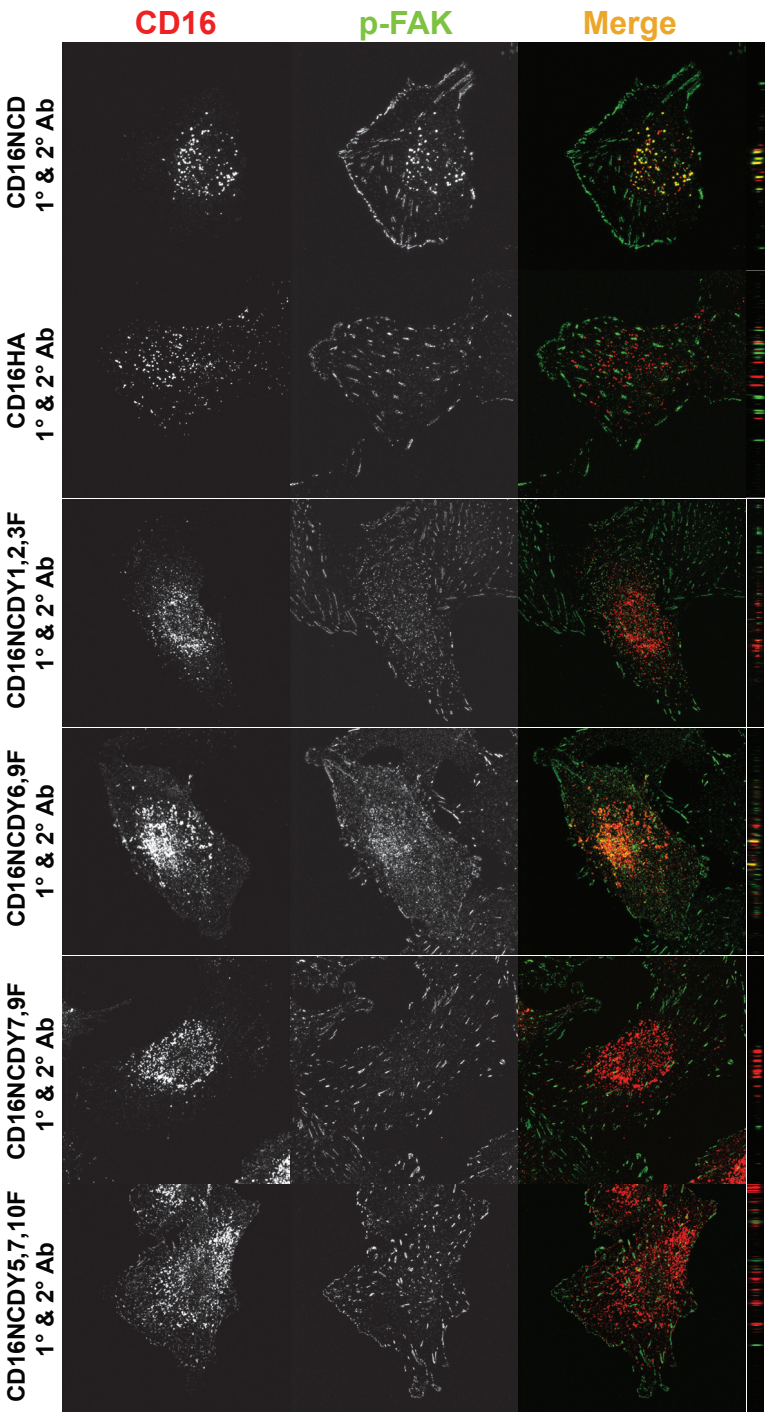


Figure S6.

Phosphorylation of FAK in Nephrin clusters requires pI-3 kinase and Nephrin tyr residues Y5, 7, 10. Podocytes expressing CD16/7-NephrinCD or mutants as indicated were activated as previously described (red) and p-FAK was detected by indirect immunofluorescence (green). Co-localization of p-FAK and Nephrin was analyzed by confocal microscopy. Magnification: 630 X.

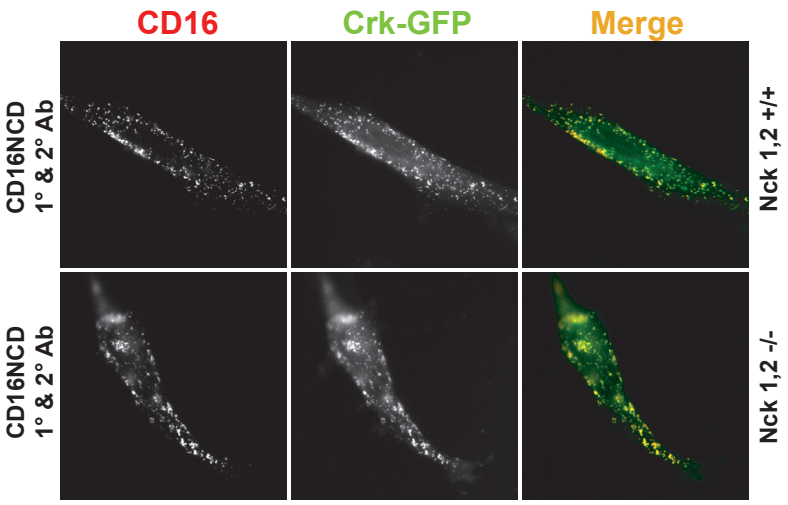


Figure S7.

Nck1/2 is not necessary for Crk recruitment to CD16/7-NephrinCD. Nck wild type (Nck1/2$^{+/+}$) or Nck1 and Nck2 double null MEF (Nck1/2$^{-/-}$) were transfected with plasmid encoding CD16/7-NephrinCD and Crk-GFP and cells were activated as described above. Note that Nephrin and Crk co-localize in Nck1/2 double null MEF (merged images on the right). Magnification: 630 X.

Supplementary Table 1

Human Crk shRNA sequences.

	Sequence
Crk shRNA1	**CCGGCCTCTTTGACTTTAATGGGAACTCGAGTTCCCATT AAAGTCAAAGAGGTTTTT**
Crk shRNA2	**CCGGCATCTTG AGAATCCGGGACAACTCGAGTTGTCCCGGATTCTCAAGATGTTTTT**
Crk shRNA3	**CCGGGCTTTACTGGAATTCTACAAACTCGAGTTTGTAGAATTCC AGTAAAGCTTTTT**
Crk shRNA4	**CCGGCGCCTCAGTATCGGCT CTGATCTCGAGATCAGAGCCGATACTGAGGCGTTTTT**
Crk shRNA5	**CCGGGCGAGCCCTCTTTGACTTTAACTCGAGTTAAAGTCAAAGAG GGCTCGCTTTTT**

Supplementary Table 2

Supplementary Table 2 displays sequences of arrayed oligopeptides used in the overlay experiment shown in Supplementary Figure 4B.

Y#	Tyrosine#	Sequence
Y1	Y1128	DRIRNE**Y**EESQWT
pY1	pY1128	DRIRNE**pY**EESQWT
Y2	Y1153	AEVDPH**YY**SMRDFS
pY2	pY1153	AEVDPH**pY**YSMRDFS
pY3	pY1154	AEVDPHY**pY**SMRDFS
Y2,3F	Y1153,1154F	AEVDPH**FF**SMRDFS
Y4	Y1172	TLEEVS**Y**RQAFTG
pY4	pY1172	TLEEVS**pY**RQAFTG
Y5	Y1191	AFPGHL**Y**DEVERV
pY5	pY1191	AFPGHL**pY**DEVERV
Y6	Y1198	DEVERV**Y**GPPGVW
pY6	pY1198	DEVERV**pY**GPPGVW
Y7	Y1208	PGVWGPL**Y**DEVQMDP
pY7	pY1208	PGVWGPL**pY**DEVQMDP
Y8	Y1216	EVQMDP**Y**DLRWPE
pY8	pY1216	EVQMDP**pY**DLRWPE
Y9	Y1225	RWPEVK**Y**EDPRGI
pY9	pY1225	RWPEVK**pY**EDPRGI
Y10	Y1232	EDPRGI**Y**DQVAAD
pY10	pY1232	EDPRG**pY**DQVAAD