Supplementary Figures

Figure 1

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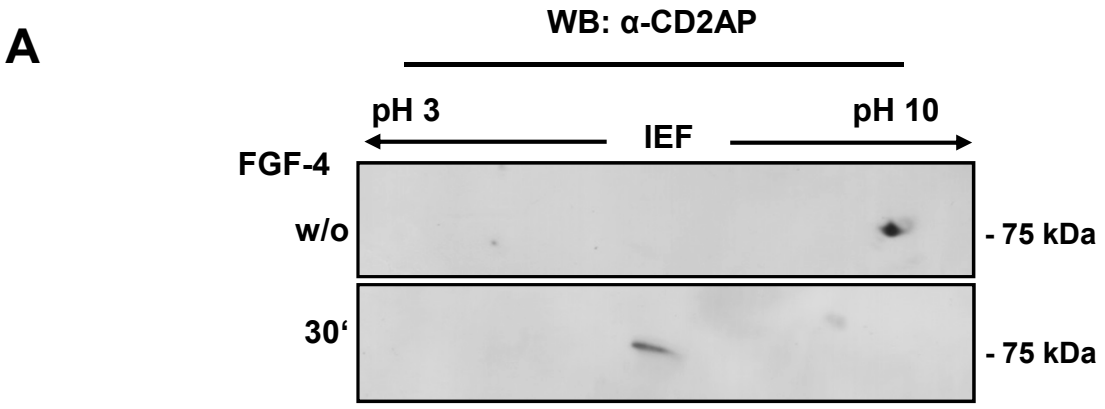

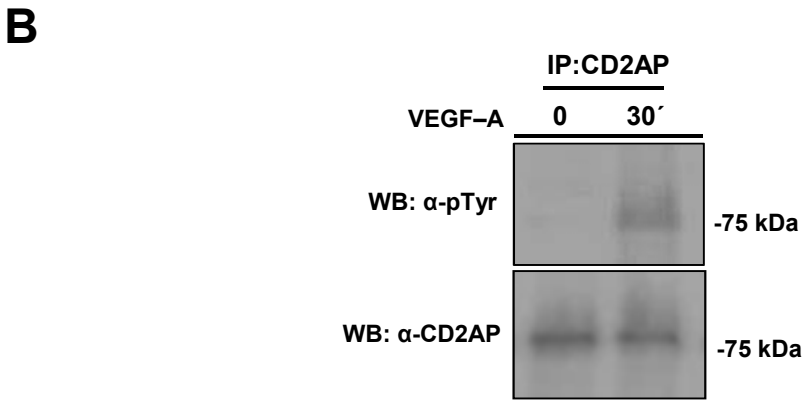

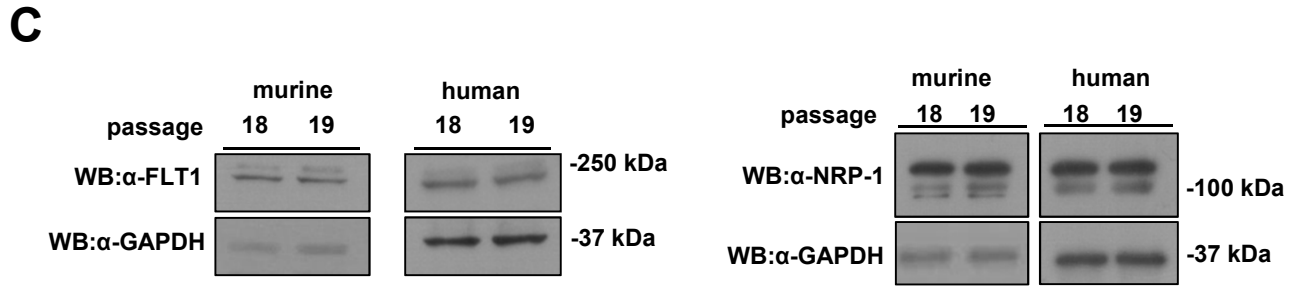

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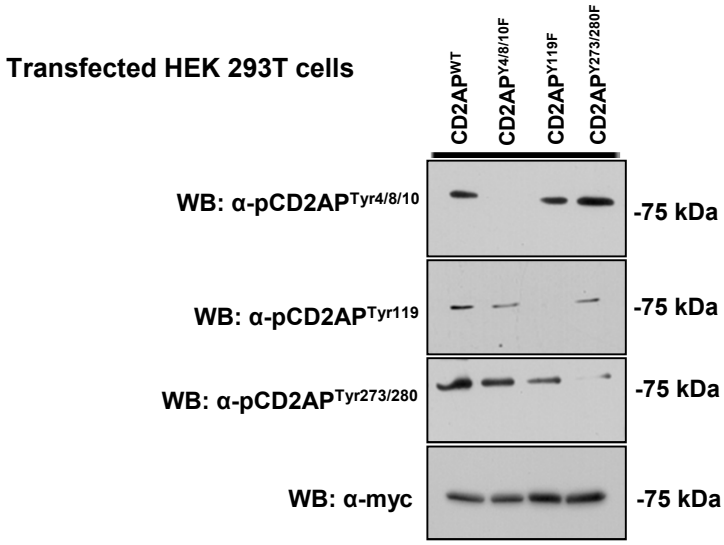
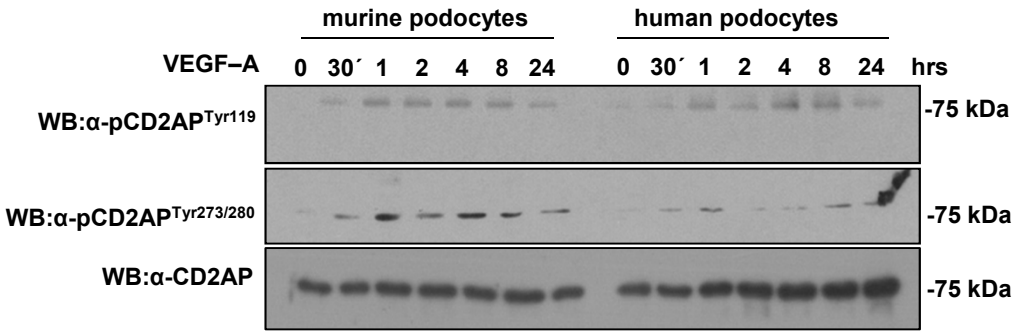
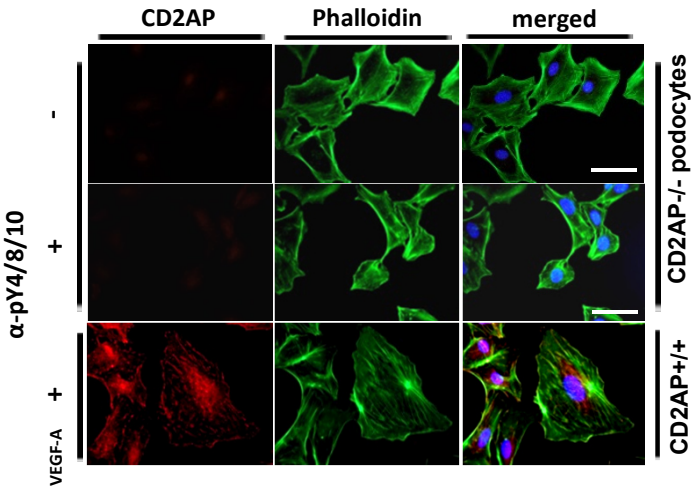

Transfected HEK 293T cells
CD2AP^WT
CD2AP^Y4/8/10F
CD2AP^Y119F
CD2AP^Y273/280F
WB: α-pCD2AP^Tyr4/8/10
WB: α-pCD2AP^Tyr119
WB: α-pCD2AP^Tyr273/280
WB: α-myc
-75 kDa
-75 kDa
-75 kDa
-75 kDa

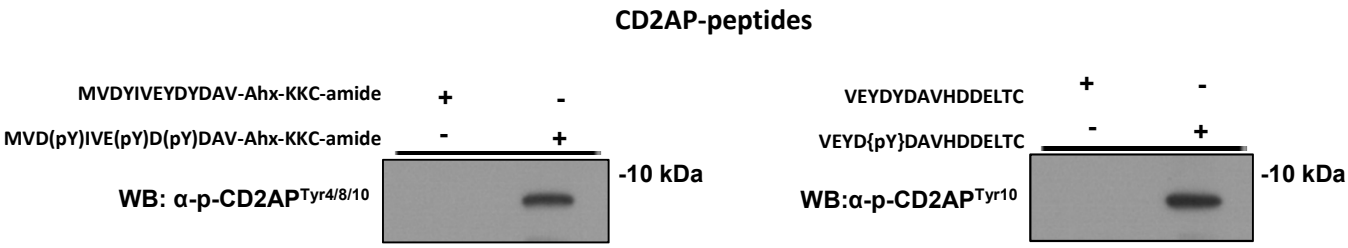
E


murine podocytes
human podocytes
VEGF–A
0 30´ 1 2 4 8 24
0 30´ 1 2 4 8 24 hrs
WB:α-pCD2AP^Tyr119
WB:α-pCD2AP^Tyr273/280
WB:α-CD2AP
-75 kDa
-75 kDa
-75 kDa

F


CD2AP
Phalloidin
merged
α-pY4/8/10
-
+
VEGF-A
+
CD2AP-/- podocytes
CD2AP+/+

G

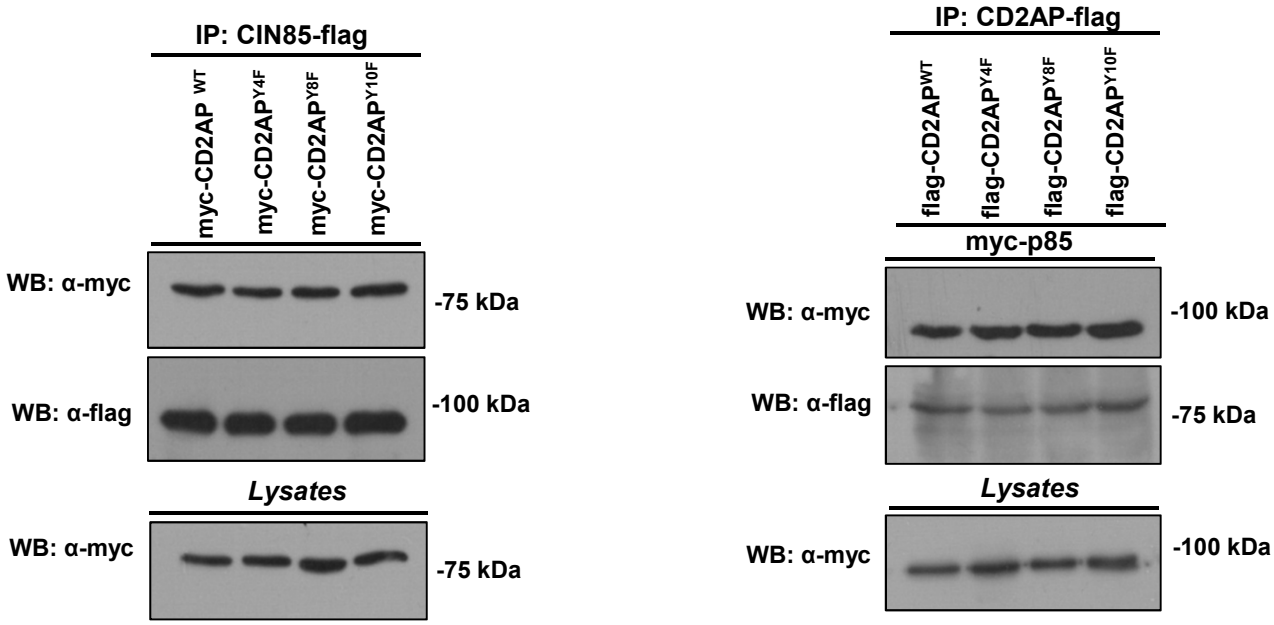

CD2AP-peptides
MVDYIVEYDYDAV-Ahx-KKC-amide
MVD(pY)IVE(pY)D(pY)DAV-Ahx-KKC-amide
WB: α-p-CD2AP Tyr4/8/10
-10 kDa
VEYDYDAVHDDELTC
VEYD{pY}DAVHDDELTC
WB:α-p-CD2AP Tyr10
-10 kDa

H


λ-phosphatase
pervanadate
WB: α-p-CD2AP Tyr4/8/10
-75 kDa
WB:α-CD2AP
-75 kDa
λ-phosphatase
pervanadate
WB:α-p-CD2AP Tyr10
-75 kDa
WB:α-CD2AP
-75 kDa

Figure 2

A


IP: CIN85-flag
myc-CD2AP WT
myc-CD2AP Y4F
myc-CD2AP Y8F
myc-CD2AP Y10F
WB: α-myc
-75 kDa
WB: α-flag
-100 kDa
Lysates
WB: α-myc
-75 kDa
IP: CD2AP-flag
flag-CD2AP WT
flag-CD2AP Y4F
flag-CD2AP Y8F
flag-CD2AP Y10F
myc-p85
WB: α-myc
-100 kDa
WB: α-flag
-75 kDa
Lysates
WB: α-myc
-100 kDa

Supplementary Figure legend

Supplementary Figure 1

CD2AP is tyrosine phosphorylated in response to VEGF-A stimulation. (A) 2D isoelectrical electrophoresis using an anti-CD2AP antibody shows a shift of CD2AP after treatment with FGF-4 after 30 minutes. *Expression of the VEGFR1 and Neuropilin-1 in cultured murine and human podocytes.* (B) Differentiated murine podocytes were treated or untreated with 20 ng/ml VEGF-A for 30 minutes. Lysates were immunoprecipitated with a CD2AP-antibody and western blot was performed with a phosphotyrosine-antibody. CD2AP was used as loading control. (C) Lysates of differentiated murine and human podocytes from two different passages were analyzed by western blot using an FLT-1 and Neuropilin-1 antibody. GAPDH was used as loading control. Specificity of generated phosphor-CD2AP antibodies. (D) Myc-tagged CD2AP DNA was point mutated on indicated tyrosine sites. HEK 293T cells were transfected with CD2AP and CD2AP tyrosine-mutants. Western blot was performed with the appropriate p-CD2AP antibody. Myc was used as loading control. (E) Differentiated murine and human podocytes were treated with 20 ng/ml VEGF-A for up to 24 hours. Western blot analysis of lysates was performed using a p-CD2AP^{tyr119} or p-CD2AP$^{tyr273/280}$ antibody. CD2AP was used as loading control. (F) Differentiated murine CD2AP+/+ and CD2AP-/- podocytes untreated or treated with VEGF-A were stained with the p-CD2AP$^{tyr4/8/10}$ antibody (red) and co-stained with Phalloidin (green) and DAPI (blue). Scale bars 30 µm. (G) Non- and phosphorylated peptides of CD2AP were analyzed by western blot. Appropriate p-CD2AP antibody shows only a signal with the phosphorylated peptides. (H) Differentiated murine podocytes were untreated or treated with pervanadate (10 mM) for 30 minutes. Treatment of the lysates with λ-phosphatase leads to dephosphorylation of CD2AP.

Supplementary Figure 2

Absence of tyrosine 10 does not effect binding of proteins to the proline rich or coild-coil region of CD2AP (A) Myc and flag-tagged CD2AP DNA was point mutated on indicated tyrosine sites. HEK 293T cells transfected with CD2AP- and CIN85- or p85-DNA were lysed and immunoprecipitated with a flag-antibody. Western blot was analyzed with a myc- and flag-antibody.