



## **Figure 4– figure supplement 1**

### **Paxillin associates with the F-BAR domain of CIP4**

**A.** Structures of the vinculin tail (PDB ID: 1QKR; top panel) and the CIP4 F-BAR domain (PDB ID: 2EFK; bottom panel) constructed by JMOL, version 14.4.4. Yellow marks indicate the predicted paxillin-binding subdomain (PBS) for vinculin (951K to 970Q) or CIP4 (35R to 55P). Secondary structures were predicted by the DSSP or STRIDE databases.

**B.** Mapping of paxillin domains interacting with CIP4 or vinculin. GST pull-down and immunoblotting of vinculin and myc-CIP4 in lysates of myc-CIP4-expressing HEK293T cells. Histograms reflect quantification of levels of proteins pulled-down by GST fusions of full-length (“FL”) or LD motif-deleted forms of paxillin, all from experiments similar to those shown in top panels ( $\pm$ SEM,  $n \geq 3$  independent experiments; “\*\*\*”,  $p < 0.01$ ; “\*\*\*\*”,  $p < 0.001$ ; multiple  $t$  test). Schematic of GST fusion proteins and table summarizing relative CIP4 or vinculin (“Vin”) binding by paxillin deletion mutants or full-length protein shown in the top panel. Binding strength relative to full-length paxillin indicated as: “++++” > 75% > “+++” > 50% > “++” > 25% > “+” > 5% > “+/-”. Note that although CIP4 primarily associates with LIM domains, deletion of the paxillin LD1-3 domain reduced its affinity for CIP4.

**C.** Mapping of CIP4 domains interacting with paxillin. GST fusion proteins of full-length CIP4 (“FL”) or its variants with F-BAR domain, HR1 and/or SH3 domain truncations were subjected to GST pull-down assays in HEK293T cell lysates, followed by immunoblotting for paxillin and actin. Histogram reflects quantitative measurement of relative protein levels ( $\pm$  SEM,  $n \geq 3$  independent experiments; compared to that of FL experiment; “\*\*\*”,  $P < 0.01$ ;  $t$ -test) pulled down by GST-CIP4, as indicated.

**D.** Schematic of GST fusion proteins used in **B**. Table summarizes relative paxillin binding by CIP4 deletion mutants or full-length protein. Binding strength relative to that of full-length CIP4 is represented as: “++++” > 75% > “+++” > 50% > “++” > 25% > “+”.

**E.** *In vivo* protein interactions in HEK293T cells co-transfected with plasmids encoding paxillin-GFP and myc-tagged CIP4 deletion mutants or full-length protein, as indicated. Cell lysates were immunoprecipitated by myc antibody and blotted with paxillin or myc antibodies. Histograms show relative protein levels as determined by immunoblotting for paxillin co-immunoprecipitated by myc antibody. Data represents mean ( $\pm$ SEM from more than three independent experiments; compared to that of F-BAR experiment; “\*”,  $P < 0.05$ ;  $t$ -test).

**F.** *In vitro* protein interaction and competitive binding assays in HEK293T cells transfected with various amounts (1, 6, and 12  $\mu$ g) of plasmids encoding myc-tagged CIP4 protein (myc-CIP4) and/or control vectors, as indicated. Cell lysates were subjected to a GST pull-down assay with GST-PXN<sup>FL</sup>, GST-PXN <sup>$\Delta$ LD1</sup>, GST-PXN <sup>$\Delta$ LIM3-4</sup> or GST alone, and immunoblotted with vinculin and myc antibodies. Line chart depicts averaged protein levels as determined by immunoblotting of CIP4 or vinculin pulled-down by GST-PXN variant ( $\pm$ SEM, n=3-4; normalized to band intensity of corresponding GST-paxillin variant).