



Figure 5—figure supplement 2

Endocytic function and distribution patterns of CIP4 protein in 0.1 kPa neuronal cultures

A. CIP4 knockdown suppresses endocytic activity of neurons grown on 0.1 kPa gels. (A1) Western blot showing efficiency of shRNA-mediated CIP4 knockdown. HEK293T cells were transfected with plasmids encoding scrambled shRNA (“scr”) or one of four CIP4 shRNAs (sequence A, B, C, or D) targeting to different regions of the CIP4 sequence. (A2) Representative time-lapse images of FM4-64 uptake in 5 hr neurons grown on 0.1 kPa gels. Time-lapse images (20 frames; 2-minute intervals) of neurons isolated from E17.5 rat cortices which were transfected in utero at E16 with constructs encoding scrambled control or CIP4 (“CIP4 shRNA A-D”) shRNA, and/or CIP4-GFP, cultured on 0.1 kPa for 5 hr, followed by endocytosis assay. Dashed circles surround the region of interest (ROI) for quantitative FM4-64 measurements. Asterisk marks non-transduced neighboring cells. Graph at right summarizes the accumulation curves of FM4-64 signal (\pm SEM, $n > 3$ independent experiments, 5-10 cells per group, normalized to $t = 0$ value; compared to that of scrambled control experiment “*”, $p < 0.05$; multiple t tests for each time point).

B. Images of neurons transfected with plasmids encoding CIP4-GFP, stained with phalloidin and DAPI. Right-most panels show the region of interest (ROI, dashed box) of neurite tips at a higher magnification. Note that CIP4-GFP was distributed along the enlarged lamella edge when neurons were cultured on 20 kPa gels.

C. Fluorescence images (**C1**) of P0 rat cortices transfected in utero at E16 with IRES constructs harboring GFP control or CIP4-GFP. The bottom panels show 4x magnifications of boxed regions corresponding to the P0 cortex in the top panels. Bar, 100 μ m. (**C2**) Histograms showing the percentages (\pm SEM, $n > 3$ cortices each; “n.s.”, not significant, multiple t test) of neurons residing in the cortical plate (“CP”), intermediate zone (“IZ”), or subventricular zone (“SVZ”) regions. (**C3**) Histograms showing the percentage (\pm SEM, $n > 150$ cells per cortex, > 3 cortices each; “n.s.”, not significant, multiple t test) of transfected cortical neurons exhibiting unipolar/bipolar polarized processes (“polarized”), multiple short neurites without a long tailing process (“unpolarized”), or no process (“no neurite”) in cortices.