Supporting Information

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Quantification of the PFN2a Knockdown

The knockdown of PFN2a was quantified using a Zeiss 510 META confocal microscope with a 40× 1.3-N.A. oil immersion objective and zoom of 2. Mean pixel intensity of the neuronal soma was measured for shPFN2a-transfected cells (18 neurons of three independent experiments) and untransfected neighbor neurons (18 neurons of three independent experiments) stained with anti-PFN2a. The average pixel intensity of untransfected cells was set to 100%.

Pull-Down Assay

Expression and purification of both profilin isoforms from *Escherichia coli* extracts using poly-L-proline affinity chromatography were determined as described for human PFN1 and mouse PFN2a (1, 2). Brains from adult mice were excised, frozen in liquid nitrogen, and homogenized in ice-cold solubilization buffer (50 mM Hepes, 150 mM NaCl, 2 mM EGTA, 0.5% Triton X-100, 1 mM PMSF). Particulate matter was removed via centrifugation for 30 min at 20,000 × *g* (4 °C). For pull-down experiments, the mouse mDia1 fragments mDia1-448 and mDia456-761 (3) were expressed as recombinant His-tagged proteins and purified on Ni-NTA agarose (Qiagen). The bead-bound mDia1 fragments (100 μL) were incubated overnight with 10 nmol of purified profilin 1 or 2a and 0.5 mL of brain extract (protein concentration of 10 mg/mL), respectively. Beads were sedimented after washing and processed for SDS/PAGE and immunoblotting. The bound profilins were detected by polyclonal anti-PFN1 (P7624; Sigma) and anti-PFN2a (AS361) (4) antibodies.

Immunoprecipitation from Mouse Brain

Brains from adult mice were excised, frozen in liquid nitrogen, and homogenized in ice-cold solubilization buffer (50 mM Hepes, 150 mM NaCl, 2 mM EGTA, 0.5% Triton X-100, 1 mM PMSF). Particulate matter was removed via centrifugation for 30 min at 20,000 × *g* (4 °C). Protein-protein interactions in the brain extract were stabilized before immunoprecipitation by treatment with the chemical cross-linking agent dithiobis (succinimidyl propionate) (DSP; Pierce). For cross-linking, DSP was added to a final concentration of 0.5 mg/mL (freshly prepared in dimethylsulfoxide at 20 mg/mL). After 30 min on ice, the reaction was stopped by the addition of Tris pH 8 (final concentration of 25 mM). Brain extract (20 mg of total protein) was then incubated with 120 μL of Protein A/G-agarose (Santa Cruz) plus 70 μg of polyclonal anti-mDia2 antibody (sc-10889; Santa Cruz) or a polyclonal anti-mouse IgG antibody (F9006; Sigma) as a control for 4 h with end-over-end rotation at 4 °C. After six washes with 2.0 mL of ice-cold solubilization buffer, the immunoprecipitated proteins were eluted from the beads via incubation with SDS/PAGE sample buffer and visualized via Western blotting using polyclonal anti-PFN1 (P7624), anti-PFN2a antibody (AS361), and anti-mDia2 (sc-10889) antibodies.

Quantification of Profilin Isoforms from Mouse Brain

PFN1 and PFN2a protein levels in brain extracts of adult mice (C57/Bl6 and $p75^{NTR}$ KO) were detected by immunoblotting. Twenty micrograms of total protein was subjected to SDS/PAGE and blotted onto nitrocellulose. Immunoreactivity was obtained with anti-PFN1 (P7624), anti-PFN2a (AS361), and tubulin (DM1A; Sigma). Densitometric quantification of the proteins was performed using ImageJ 1.42 (National Institutes of Health), and values were normalized to tubulin.

1. Lambrechts A, et al. (1997) The mammalian profilin isoforms display complementary affinities for PIP2 and proline-rich sequences. *EMBO J* 16:484–494.
2. Wittenmayer N, et al. (2004) Tumor suppressor activity of profilin requires a functional actin binding site. *Mol Biol Cell* 15:1600–1608.
3. Krebs A, Rothkegel M, Klar M, Jockusch BM (2001) Characterization of functional domains of mDia1, a link between the small GTPase Rho and the actin cytoskeleton. *J Cell Sci* 114:3663–3672.
4. Murk K, Buchmeier S, Jockusch BM, Rothkegel M (2009) In birds, profilin-2a is ubiquitously expressed and contributes to actin-based motility. *J Cell Sci* 122:957–964.

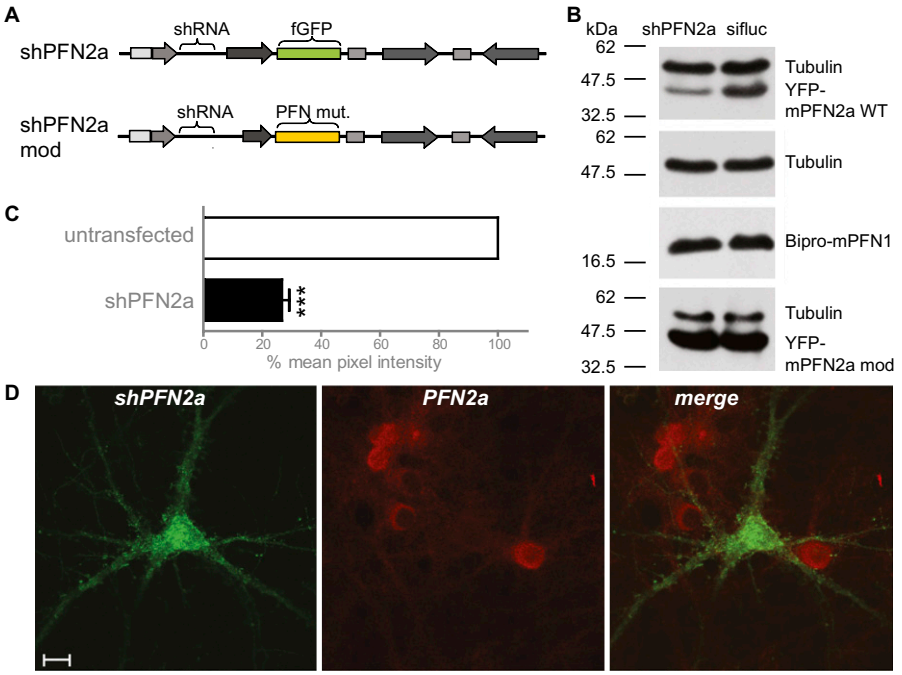

Fig. S1. Knockdown of the brain-specific form of PFN2a via RNAi. (*A*) Polycistronic vector constructs targeting PFN2 amRNA and expressing fGFP or a modified form of PFN2a resistant to shRNA binding (YFP-PFN-mut) both under a truncated CMV promoter. (*B*) Specific knockdown of exogenous PFN2a expressed in HeLa cells. BiPro-tagged PFN1 levels are unaffected by the knockdown, control plasmid sifluc does not alter PFN2a expression levels, and modified PFN2a is resistant to the knockdown. (*C*) Mean pixel intensity (neuronal soma) of primary hippocampal neurons (14 DIV) transfected with shPFN2a (n = 18) or untransfected neighboring cells (n = 18) stained with anti-PFN2a. The pixel intensity of control cells was set to 100%, and PFN2a protein levels are reduced to 26.7 ± 2% (18 neurons of three independent experiments). ***$P < 0.001$. (Scale bar: 10 μm.) (*D*) Fluorescence images of primary hippocampal neurons (14 DIV) transfected with shPFN2a (green) and labeled with anti-PFN2a (red).

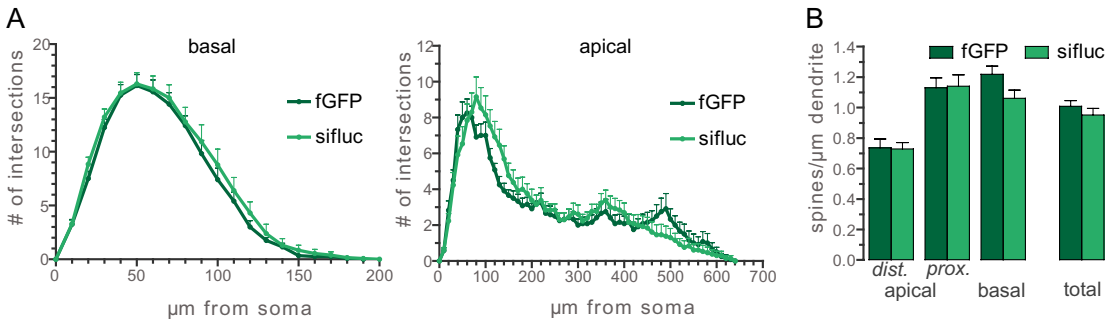

Fig. S2. Similar dendritic morphology and spine density of cells transfected with control plasmids. Sholl analysis (*A*) and spine density (*B*) of CA1 pyramidal neurons (organotypic cultures) used as control cells expressing fGFP (n = 12) or sifluc (n = 13). Because no morphological alterations between the two control groups were observed, they were combined for further analysis. dist., distal; prox., proximal.

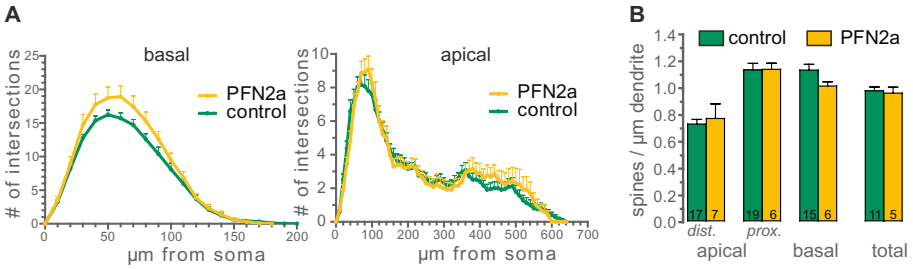

Fig. S3. Overexpression of PFN2a leaves dendrite morphology unaffected. (*A*) Sholl analysis (basal and apical dendrites) of control cells and neurons transfected with PFN2a, with an expression time of 48 h (n = 13). (*B*) Spine density of PFN2a-overexpressing cells. dist., distal; prox., proximal.

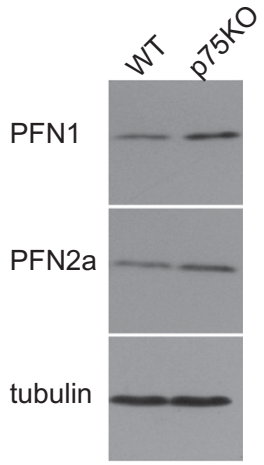

Fig. S4. Expression of both profilin isoforms is changed in the brain of $p75^{NTR}$ KO mice. Whole-brain lysates from WT and $p75^{NTR}$ KO mice were analyzed for both profilin isoforms using immunoblotting. PFN1 and PFN2a levels were found to be increased in $p75^{NTR}$ KO mice by factors of 1.2-fold and 1.14-fold, respectively, compared with WT mice (averaged values of two independent experiments).

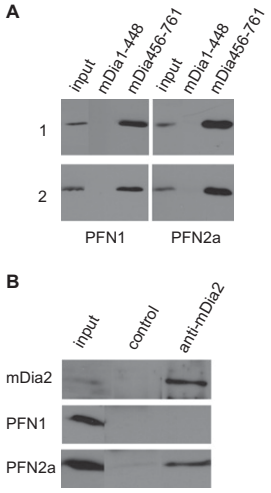

Fig. S5. Interaction of profilin isoforms with formins. (*A*) Both profilin isoforms interact with mDia1. Ni-NTA beads coated with the recombinant mDia1 fragments comprising the FH1 (mDia456–761) or RBD-FH3 (mDia1–448) domain were used in pull-down experiments. Precipitates were subjected to SDS/PAGE and immunoblotting with polyclonal antibodies against PFN1 and PFN2a (1). Recombinant PFN1 and PFN2a (input: 50 ng each) both react specifically with the polyproline stretch-containing FH1 (2). The same specificity for this mDia1 domain was seen with genuine PFN1 and PFN2a present in brain lysates (input: 50 μg of total protein). (*B*) PFN2a reacts preferentially with mDia2 in brain lysates. Immunoprecipitation was performed with a polyclonal antibody against mDia2 or an anti-mouse IgG (control) and protein A/G agarose using total protein from brain lysates pretreated with the cross-linker DSP to stabilize protein complexes in vivo. Precipitates were subjected to SDS/PAGE and immunoblotting with antibodies against mDia2, PFN1, and PFN2a. (*Left*) Input shows the presence of mDia2 and both profilin isoforms in the lysate. (*Right*) Immunoprecipitates contain only PFN2a and mDia2, and PFN1 is not detectable.

Table S1. Spine numbers of organotypic cultures

Experiment	Distal apical	Proximal apical	Basal	Total
Control (Fig. 2)	0.73 ± 0.03	1.14 ± 0.05	1.14 ± 0.04	1.02 ± 0.02
shPFN2a (Fig. 2)	0.72 ± 0.06	0.88 ± 0.05 [0.004]	0.93 ± 0.10 [0.04]	0.82 ± 0.07 [0.006]
shPFN2a and PFN1 (Fig. 3)	0.71 ± 0.05	1.09 ± 0.06	1.13 ± 0.06	0.97 ± 0.04
shPFN2a-mod (Fig. 3)	0.63 ± 0.05	1.10 ± 0.06	1.18 ± 0.04	1.00 ± 0.04
PFN2a-overexpressed (Fig. S3)	0.73 ± 0.10	1.14 ± 0.04	1.01 ± 0.07	0.96 ± 0.03

Spine numbers are shown as spines per micrometer of dendrite. *P* values in brackets are always compared with control experiments.

Table S2. Number of dendritic ends and spines of primary hippocampal neurons

Experiment	Ends	Spines
fcherry (Fig. 4)	56.6 ± 3.4	0.66 ± 0.03
p75 (Fig. 4)	38.4 ± 2.5 [0.0003]	0.57 ± 0.02 [0.03]
PFN2a (Fig. 4)	64.7 ± 5.6	0.50 ± 0.02 [0.001]
PFN2a and p75 (Fig. 4)	57.6 ± 6.9	0.57 ± 0.02 [0.04]
PFN1 (Fig. 4)	39.0 ± 2.3 [0.0003]	0.66 ± 0.05
PFN1 and p75 (Fig. 4)	43.1 ± 2.6 [0.006]	0.62 ± 0.04

Spine numbers are shown as spines per micrometer of dendrite. *P* values in brackets are always compared with control experiments.