

Supplementary Figure Legends

Supplementary Figure 1

(a) Demonstration of erbB4 immunostaining specificity. Immunocytochemistry in the absence of erbB4 primary antibody, but with the applicable secondary antibody, eliminates staining in the dendrites of multipolar interneurons. Scale bar=35uM

(b) Additional traces of DIV28 cortical multipolar interneuron dendritic trees. Axons were omitted for clarity. Neurons expressed either GFP or GFP-tagged kalirin RNAi for 3-days. 1-day posttransfection, neurons were treated with either NRG1 β (5nM) or with vehicle for 2 days. Scale bar=100um.

Supplementary Figure 2

(a) Interneurons expressing GFP-tagged kalirin RNAi show erbB4 immunoreactivity. Scale bar=35uM.

(b) Representative traces of pyramidal neurons transfected with GFP for 3-days and treated one day post-transfection with either vehicle or with NRG1 β (5nM; 2-days). Axons were omitted for clarity. Scale bar=50uM.

(c) Quantification showing that NRG1 β fails to alter the total dendritic length of pyramidal neurons relative to vehicle-treated pyramidal neurons. Data are the mean \pm SEM.

(d) ErbB4 overexpression in hEK293 cells increases active fyn/src levels.

(e) Treatment with the fyn/src activity inhibitor PP2 (10uM; 3-days) reduces active fyn/src levels in neurons. Active erk 1 and 2 are shown as a control.

Supplementary Figure 3

(a) Endogenous fyn is present in the dendrites of multipolar interneurons. Scale bar=35uM.

(b) Overexpressed fyn is targeted to the dendrites of multipolar interneurons. Scale bar=35uM.

(c) Additional traces of DIV28 cultured cortical multipolar interneuron dendritic trees. Axons were omitted for clarity. Neurons were transfected with either GFP, GFP-tagged

kalirin RNAi, fyn, or GFP-tagged kalirin RNAi in combination with fyn. Scale bar=100um.

Supplementary Figure 4

(a) Depiction of the amino acid sequence of the C-terminus of kalirin-7. The C-terminus contains a single tyrosine residue located at the 1662 site (shown in blue), and this residue lies within the PDZ-binding motif of kalirin-7 necessary for its interaction with PSD-95.

(b) Of the phosphorylatable residues in the kalirin-7 PDZ-binding motif, only the Y1662 residue exceeds chance levels of being a kinase substrate. Phosphorylation potential of individual residues was determined by using the Net Phos 2.0 program (Technical University of Denmark). Program details were published in.¹

(c) hEK293 cells overexpressing PSD-95 in combination with WT kalirin-7 or Y1662A mutant kalirin-7 in the absence or presence of fyn. Fyn stimulated WT kalirin-7 but not the Y1662A mutants' ability to increase active Rac1 levels.

(d) hEK293 cells with or without erbB4 overexpression. Both full-length and cleaved erbB4 were detected in cells overexpressing erbB4, while both bands were absent in cells lacking erbB4. The vast majority of erbB4 signal is the full-length receptor.

(e) Additional traces of DIV28 cultured cortical multipolar interneuron dendritic trees. Axons were omitted for clarity. Neurons expressed either GFP alone or in combination with Y1662A kalirin-7 for 3-days. 1-day posttransfection, neurons were treated with either NRG1 β (5nM) or with vehicle for 2 days. Scale bar=100um.

Supplementary materials and methods

Antibodies and Reagents

The following antibodies were used: monoclonal PSD-95 (Neuromab), monoclonal GFP (Chemicon), polyclonal GFP (Richard Huganir, Johns Hopkins University), monoclonal Fyn (BD Transduction Laboratories), monoclonal Rac1 (Millipore), polyclonal anti-phospho fyn/src416 (Cell Signaling Technology), monoclonal phospho-tyrosine (Southern Biotech.). Monoclonal erbB4 antibody against the c-terminus of erbB4 (C-7 clone from Santa Cruz Biotechnology) was used for assessing the co-immunoprecipitation interaction of kalirin-7 with erbB4 in neurons. For assessment of the co-localization between erbB4 and kalirin-7 in interneurons, the HFR antibody clone against erbB4 was used (Santa Cruz Biotechnology). Antibodies against kalirin and kalirin-7 were described previously.²⁻³ Recombinant neuregulin-1 beta 1 (NRG1 β) was purchased from R&D systems and was reconstituted in sterile PBS with 0.4% bovine serum albumin (BSA); sterile PBS containing 0.4% BSA was used for vehicle treatments. PP2 was purchased from Fisher Scientific.

cDNA plasmids

The plasmids encoding myc-kalirin-7, myc-kalirin-5 and myc-delta c-terminus kalirin-7 were described previously.⁴ The Y1662A kalirin-7 mutant was generated by site-directed mutagenesis using the QuickChange II XL mutagenesis kit (Stratagene). The erbB4 plasmid was a gift from Dr. Gabriel Corfas (Harvard University). The fyn, src, and abl constructs were a gift from Dr. Richard Huganir (Johns Hopkins University).

Rac1 activation assay

Active Rac1 levels were assessed using a Rac1 Assay kit from Millipore following the manufacturers' protocol with normalization to total Rac1 as described previously.⁵⁻⁶

Neuronal cultures

Medium and high density cortical neuron cultures were prepared from Sprague-Dawley rat E18 embryos as described previously.⁷ Neurons were plated onto coverslips or 60 mm dishes, coated with poly-D-lysine (0.2 mg/ml, Sigma), in plating media (feeding media plus 5% fetal calf serum). After 1 hr, the media was changed to feeding media (Neurobasal media supplemented with B27 (Invitrogen) and 0.5 mM glutamine). 200 μ M D,Lamino-phosphonovalerate (D,L-APV, Ascent Scientific) was added to the media 4 days later. Dissociated postnatal DIV27-30 cortical cultures were assessed.

Plasmid Transfections

Cortical neurons were transfected with the appropriate cDNAs for 4 hours in Neurobasal media in the presence of Lipofectamine 2000 according to the manufacturer's protocol. Following transfection, neurons were supplanted in Neurobasal media. Neurons were fixed for 10-minutes in 3.7% formaldehyde followed by 10-minutes of methanol pre-chilled to -20°C. Cells were then washed in phosphate buffer saline (PBS), blocked for 1-hour with PBS containing 2% normal goat serum (NGS) and 0.1% Triton. Primary and secondary antibodies were added to cells in PBS containing 2% NGS.

hEK293 cells were transfected overnight with appropriate cDNAs in DMEM media without antibiotic using Lipofectamine 2000. The next morning, DMEM containing 10% fetal bovine serum (FBS) and pen/strep antibiotic was fed to cells. hEK293 cells were transfected when they reached ~40% confluency and were ~95% confluent at the time of harvesting.

Supplementary References

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