TrkB phosphorylation by Cdk5 is required for activity-dependent structural plasticity and spatial memory

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Supplementary Fig. 1

Supplementary Fig. 1 Schematic representation of the strategy used in generating TrkB<sup>S478A/S478A</sup> knock-in mice. The wild-type (WT) amino acid serine 478 (S478, red) and its immediate adjacent sequence located in exon 12 (blue rectangle) were mutated to an alanine residue (*S478A, red, dominant mutation) and a Pst I restriction site (underlined, silent mutation). All mutated nucleotides are highlighted in blue. The neomycin resistance cassette (PGK-neo) flanked by loxP sites (black arrows) was inserted downstream of exon 12 (EX 12). The presence of the correctly targeted EX 12 in ES cells was confirmed by Southern hybridization using a PstI restriction digest detecting a shift from 4.5Kb in the WT allele to a 2.9 Kb in the S478A allele (data not shown).
Supplementary Fig. 2 Specificity of the phospho-TrkB (S478) antibody. Dissociated hippocampal neurons were co-stained with antibody against phospho-S478-TrkB (red) and PSD-95 (green). Co-localization of phospho-TrkB (S478) immunoreactivity with PSD-95 was indicated by arrows. Specificity of the phospho-TrkB (S478) antibody was indicated by the reduction of red fluorescence signal in the dendrites of hippocampal neurons derived from \(Trkb^{S478A/S478A}\) mutant mice, as compared to control mice.
Supplementary Fig. 3 Normal hippocampus morphology and dendritic arborization in \textit{Trkb}^{S478A/S478A} mutant neurons. (a) Nissl staining showing the hippocampi of adult wild-
type ($Trkb^{+/+}$) and $Trkb^{S478A/S478A}$ mutant mice. (b) Sholl analysis of dissociated hippocampal neurons (14DIV) cultured from control ($Trkb^{WT/WT}$) and $Trkb^{S478A/S478A}$ mutant mice indicated grossly normal dendritic complexity in neurons lacking TrkB S478 phosphorylation (left). The number and length of dendrites were also similar in trkB$^{WT/WT}$ and $Trkb^{S478A/S478A}$ neurons (right; n=15; One-way ANOVA, Tukey multiple comparison test, p>0.05). (c) Golgi staining revealed similar dendritic arborization of CA1 and CA3 pyramidal neurons in the adult hippocampi of wild-type ($Trkb^{+/+}$) and $Trkb^{S478A/S478A}$ mutant mice in vivo (n=20 neurons for wild-type; n=18 neurons for $Trkb^{S478A/S478A}$; Student’s t-test, p>0.05). (d) Both the dendritic protrusion density and the percentage of protrusion with spine heads in CA1 pyramidal neurons of adult hippocampus was similar between the wild-type and $Trkb^{S478A/S478A}$ mutant mice in vivo (n=17 neurons; Student’s t-test, p>0.05).
Supplementary Fig. 4 Cdk5 and its activator p35 are required for BDNF-induced spine morphogenesis. (a) BDNF induced spine formation and maturation in dissociated hippocampal neurons. Incubation of cultured hippocampal neurons (13DIV) expressing GFP with BDNF for 8 or 24 hrs significantly increased the spine density (***p<0.001, one-way ANOVA, Bonferroni’s multiple comparison test). Likewise, the spine area was
increased after BDNF treatment. (b and c) The Cdk5 selective inhibitor Ros, but not DMSO vehicle control, markedly attenuated BDNF-induced increase in spine density and spine area of dissociated hippocampal neurons (**p<0.001, one-way ANOVA, Bonferroni’s multiple comparison test). (d and e) Hippocampal neurons (13DIV) cultured from wild-type (p35+/+) or p35 knockout mice (p35−/−) expressing GFP were treated with BDNF for 24hrs, after which both the spine density and spine area were examined. Treatment with BDNF significantly increased the spine density in wild-type neurons, but not neurons lacking p35 (**p<0.001, one-way ANOVA, Bonferroni’s multiple comparison test). (f) The spine length was not significantly changed after BDNF treatment, and there was no significant difference in spine length between hippocampal neurons (14DIV) cultured from TrkBWT/WT and TrkB S478A/S478A mice (p>0.05, one-way ANOVA, Bonferroni’s multiple comparison test).
**Supplementary Fig. 5** Spine enlargement in response to uncaged MNI-glutamate.

Representative images of dendritic spines of dissociated hippocampal neurons (17DIV) expressing GFP. The selected spines were imaged by time-lapse confocal microscopy every minute before 4 brief trains of 405nm laser in the presence (top) or absence (bottom) of MNI-glutamate (1mM). Increase in spine area was observed after the application of 405nm laser only in the presence of MNI-glutamate. Scale bar=5μm.
**Supplementary Fig. 6**

S478 phosphorylation of TrkB regulates its interaction with TIAM1. (a) Presence of TIAM1 immunoreactivity (red) at the p-S478-TrkB puncta (green) in dissociated hippocampal neurons (22DIV). Scale bars= 10µm. (b) The interaction between TIAM1 and TrkB does not depend on tyrosine phosphorylation of TIAM1, as indicated by the co-immunoprecipitation of TIAM1-Y829F mutant with TrkB when co-expressed in 293T cells. (c) S478 phosphorylation of TrkB regulates the Y829 phosphorylation of TIAM1 when over-expressed in 293T cells. Over-expression of TrkB together with Cdk5/p35 in 293T cells induced tyrosine phosphorylation of TIAM1 at Y829, and the induction was reduced in cells that over-expressed the TrkB S478A mutant construct, as compared to wild-type TrkB. Similar results were observed in three experiments.
Supplementary Fig. 7 Cdk5-mediated phosphorylation of TrkB at Ser-478 is not required for BDNF-induced Cdc42 activity. BDNF (30min) significantly increased the activity of Cdc42 in both Trkb\textsuperscript{WT/WT} and Trkb\textsuperscript{S478A/S478A} cortical neurons. The fold induction of Cdc42 activity (normalized with Cdc42 expression level in the lysate) after BDNF treatment was not significantly different between trkB\textsuperscript{WT/WT} and trkB\textsuperscript{S478A/S478A} neurons (One-way ANOVA, Tukey multiple comparison test, p > 0.05).
Supplementary Fig. 8 Cdk5 and TrkB are required for S6 phosphorylation after activation of NMDA receptor. (a) Induction of S6 ribosomal protein phosphorylation (Ser-235/236) and increased expression of PSD-95 in cultured cortical neurons (15DIV) after bicuculline and glycine treatment (30min). Western blotting with antibody against actin served as the loading control. The induction was mediated by NMDA receptor, since it was abolished in the presence of APV (200μM). (b) The NMDA receptor-induced S6 ribosomal protein phosphorylation and PSD-95 expression required both TrkB and Cdk5. Cortical neurons were pre-treated with the BDNF scavenger TrkB-IgG (1μg/ml) or Cdk5 inhibitor roscovitine (10μM) for 1hr before the addition of bicuculline.
and glycine in the continued presence of the inhibitors. The presence of TrkB-IgG or roscovitine abolished the induction of S6 phosphorylation and PSD-95 expression after activation of NMDA receptor. (c) Phosphorylation of S6 ribosomal protein induced after treatment with bicuculline/glycine for 30min was attenuated in cortical neurons derived from Cdk5 null mice (Cdk5−/−) (3 experiments; *p<0.05, one-way ANOVA, Bonferroni’s multiple comparison test). The increase in PSD-95 expression after stimulation by bicuculline and glycine (30min) was also abolished in Cdk5 knockout neurons (3 experiments; *p<0.05, one-way ANOVA, Bonferroni multiple comparison test). (d) Bicuculline and glycine treatment (30min) significantly increased the expression of PSD-95, but not GluA1 and GluN1, in cultured cortical neurons (3 experiments; ***p<0.001, one-way ANOVA, Bonferroni’s multiple comparison test). (e) Activation of synaptic NMDA receptor increases PSD-95 expression in a mTOR-dependent manner. Cortical neurons were pre-treated with rapamycin (20ng/ml) for 45min before the addition of bicuculline and glycine in the continued presence of the inhibitor. The presence of rapamycin abolished the induction of PSD-95 expression after activation of NMDA receptor (4 experiments; **p<0.01, *p<0.05, One-way ANOVA, Tukey multiple comparison test).
Supplementary Fig. 9 Normal paired pulse facilitation (PPF) in Trkb<sup>S478A/S478A</sup> mutant hippocampal neurons. There was no significant difference in PPF between wild-type (Trkb<sup>++</sup>) and Trkb<sup>S478A/S478A</sup> mutant hippocampal slices (p>0.05 for all time intervals; Two-way ANOVA, Bonferroni multiple comparison test n=23 slices from 7 mice for ++; n=25 slices from 7 mice for trkB<sup>S478A/S478A</sup>).
Supplementary Fig. 10

Normal hippocampus-dependent spatial memory for the control $Trkb^{WT/WT}$ mice in Morris water maze. Wild-type ($Trkb^{+/+}$; n=4) or $Trkb^{WT/WT}$ (n=6) littermates (4-5 months old) were subjected to Morris water maze (2 trials per day for 7 days). The quadrant occupancy in the probe trial (left) and the latency of finding the platform (right) were analyzed. Both wild-type and the control $Trkb^{WT/WT}$ mice spent significantly more time on the target quadrant (Q2) than the other quadrants in the probe trial (one-way ANOVA, Newman-Keuls multiple comparison test), and there was no significant difference in the target quadrant occupancy (p>0.05, Two-way ANOVA, Bonferroni multiple comparison test) between wild-type and the control $Trkb^{WT/WT}$ mice. Chance level (25%) was indicated by the dotted line.
Supplementary Fig. 11 Images of full length blots presented in the main figures.
Supplementary Fig. 11 (continued) Images of full length blots presented in the main figures.