Supplemental methods

Sample preparation and immunohistochemistry

Mice were transcardially perfused with cold phosphate-buffered saline (PBS, 10 mM, pH 7.4), followed by 4% paraformaldehyde in PBS under ketamine (150 mg/kg) and xylazine (20 mg/kg) anesthesia. Brains were post-fixed in 4% paraformaldehyde for 4 hr at 4 ºC and cryoprotected with 30% sucrose solution in PBS. Coronal sectionsthrough the dorsal hippocampus were prepared using a freezing microtome (40 μm thick).

For immunohistochemistry, sections were washed with PBS and incubated in 0.3% hydrogen peroxide/PBS for 20 min to eliminate endogenous peroxidase activity. After several washes, sections were blocked with 10% normal goat serum in PBS, followed by overnight incubation with mouse anti-STEP antibody (Novus Biologicals, 1:2000), rabbit anti-somatostatin antibody (Peninsula Lab, 1:1000) or rabbit anti-pERK antibody (Cell Signaling, 1:2000). Sections were then processed using the ABC staining method (Vector Labs). Nickel-intensified 3,3´-diaminobenzidine (Vector Labs) was used to visualize the signal.

For immunofluorescent labeling, sections were washed with PBS and blocked with 10% normal goat serum in PBS, followed by overnight incubation with mouse anti-His antibody (Cell Signaling, 1:1000) or rabbit anti-pERK antibody (Cell Signaling, 1:1000). After several washes, sections were incubated with secondary antibodies conjugated with Alexa 488 or Alexa 594 (Molecular Probes, 1:1000) for 2 hrs at room temperature, and then mounted with Gelmount (Biomedia). Fluorescent images were captured using a Zeiss 510 Meta confocal microscope (2 μm-thick optical section).

Estimation of cell number

Cell density in the hilus was measured using the stereological analysis parameters described by Chen et al. (2005) with slight modification. Cells were counted from 6 evenly spaced (rostro-caudal) sections in dorsal hippocampus (stereotaxic coordinate AP: -1.20 to -2.60) and labeled with either STEP or somatostatin primary antibodies followed

by a secondary antibody conjugated with Alexa 488. Sections were cut with a microtome (40 μm thick) and total section thickness was used as height. Only nuclei and somata that did not transect the upper surface of the section were counted. Cells between the upper and lower blades of the dentate gyrus that were not within area CA3 were defined as hilar. To divide the hilar region into the deep hilus and the granule cell layer border area, we assigned regions within 20 μm from the bottom of suprapyramidal or above the infrapyramidal blade in dentate gyrus as border area. Only cells with their somata localized within this area were defined as border cells. Cells > 20 μm from the GCL boarders were defined as deep hilar cells.

Phosphatase assay

In vitro phosphatase assays were performed as previously described (Lombroso et al., 1993). Assays were carried out at 30°C for 30 min in a 100 μl reaction mixture containing 50 mM imidazole buffer (pH 7.0), with varying amounts of STEP fusion proteins and 5 mM of the phosphatase substrate para-nitro phenyl phosphate (pNpp). To determine whether the TAT-STEP [C to S] fusion protein had any effect on wild type STEP phosphatase activity, equal amounts of both fusion proteins were combined and enzymatic activity determined. The reaction was terminated by the addition of 0.9 ml of 0.2 N NaOH and the optical density was measured at 410 nm. Phosphatase activity was expressed as a percentage of wild type STEP activity.

Fluoro-Jade B staining

Sections were initially mounted, dehydrated with 70% ethanol, washed with distilled water and then incubated in 0.06% potassium permanganate solution for 10 min. Next, the sections were incubated in 0.004% Fluoro-Jade B (Chemicon Int.) solution containing 0.1% glacial acetic acid for 20 min at room temperature, and then mounted with DPX (Electron Microscopy Sciences).

Cresyl violet staining

In brief, sections were mounted onto gelatin-coated slides and dried. After dehydrating in a graded alcohol series followed by water, sections were stained with 0.3% Cresyl violet solution. After destaining with 95% ethanol containing 0.1% glacial acetic acid, sections were dehydrated and covered with Permount.

Microinjection and seizure induction

Mice were placed in a stereotaxic frame (David Kopf Instruments) under ketamine (100 mg/kg) and xylazine (15 mg/kg) anesthesia. Guide cannulae (24G) were located in the motor cortex (stereotaxic coordinates: AP: -1.64 mm, ML: +1.00 mm, DV: -0.50mm) or hilus (stereotaxic coordinates: AP: -1.80 mm, ML: +1.20 mm, DV:-2.20 mm). Ten days later, mice were infused (2 min) with FK506 (0.5 μL, 100 μM in 5% DMSO/95% physiological saline), U0126 (0.5 μL, 100 μM in 5% DMSO/95% physiological saline), TAT-STEP (0.5 μL, 100 nM in physiological saline) or TAT-myc (0.5 μL, 100 nM in physiological saline). FK506 was infused 15 min after pilocarpine injection. U0126, TAT-myc and TAT-STEP were infused 1.5 hr before pilocarpine injection.

Estimation of cell number

To quantitate Fluoro-Jade B labeling in the hilus, photomicrographs were captured at 40X magnification using a 16-bit digital camera and cell counts were performed using MetaMorph software (Universal Imaging). The total number of Fluoro-Jade B-positive cells were counted in the ipsilateral hilus (stereotaxic coordinate AP: -1.60 ~ -2.00) from each animal (two sections per animal with sections separated by 200 μm). The number of Fluoro-Jade B positive cells was expressed as the mean ± SEM from 5 mice in each group. In the endopiriform nucleus, Fluoro-Jade B labeling was quantitated (bilaterally) from two sections per animal (200 μm interval:stereotaxic coordinate AP: -1.60 ~ -2.00). The number of Fluoro-Jade B-positive cells was expressed as the mean ± SEM from 6 mice for each group. Cell counts were analyzed statistically using Student's t-test, and significance was accepted for $p<0.05$.

Reference

Lombroso PJ, Naegele JR, Sharma E, Lerner M (1993) A protein tyrosine phosphatase expressed within dopaminoceptive neurons of the basal ganglia and related structures. J Neurosci 13:3064-3074.

Supplemental Data

Files in this Data Supplement:

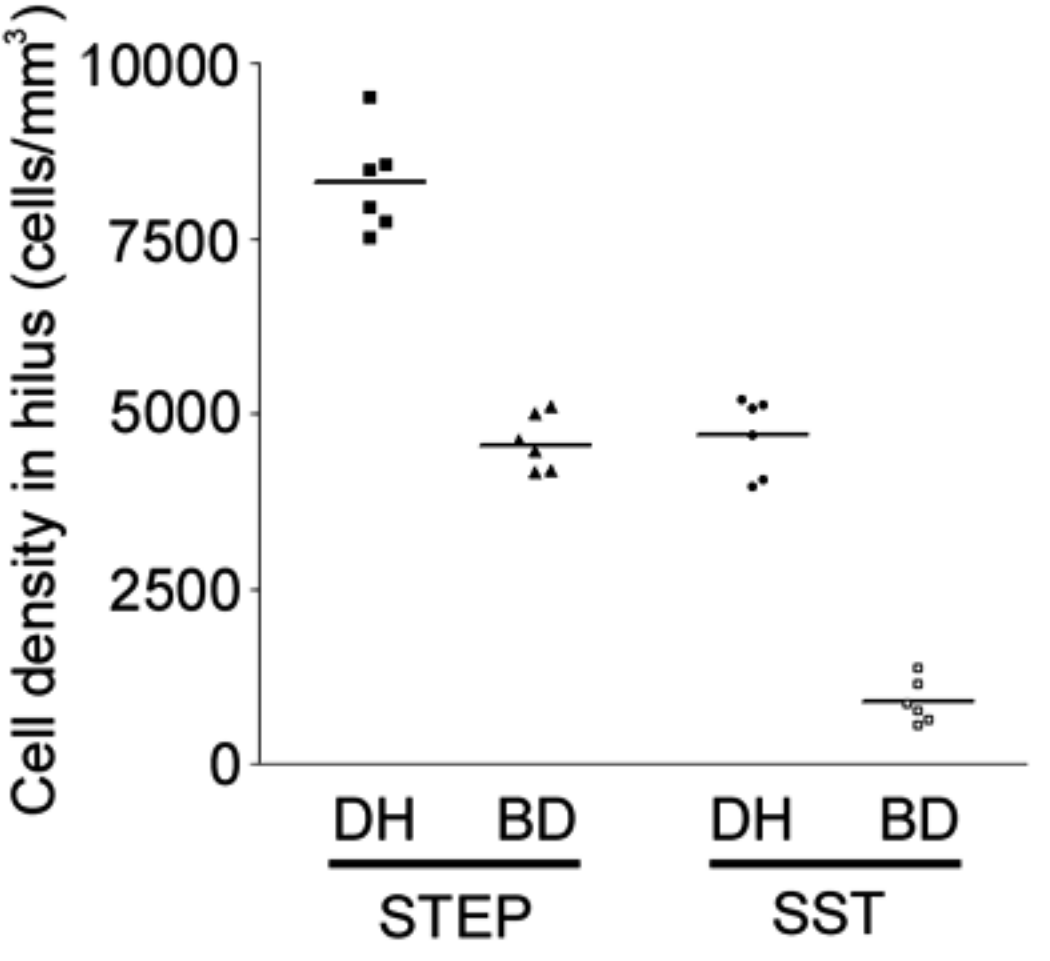
supplemental material - Supplemental Methods

supplemental material - Supplementary Figure 1. Quantitation of STEP- and somatostatin-positive cell density in the hilus. Density of both STEP- and somatostatin (SST)-positive cells was higher in the deep hilus than in the border area. Data were obtained from 6 mice for each group. Symbols represent values for individual mice; Horizontal bars represent mean values. DH: Deep hilus, BD: Border area. Please refer to the Supplementary Methods section for a description of the stereological counting technique.
supplemental material - Supplemental Figure 2. FK506 does not attenuate SE-induced cell death in the endopiriform nucleus. A, Left panel: Cresyl violet-stained section denoting the location of the endopiriform nucleus. Scale bar: 500 μm. Right panel: immunolabeling revealed limited STEP expression in the endopiriform nucleus. Scale bar: 100 μm. B, Mice were injected with pilocarpine and FK506 as described in the main text and killed 6 hr after SE onset. Representative images of Fluoro-Jade B labeling reveal that SE elicited marked cell death in the endopiriform nucleus, and, in contrast to the hilus, FK506 did not increase cell survival. AG: amygdala, EPN: endopiriform nucleus. Scale bar: 100 μm. C, Quantitative analysis of Fluoro-Jade B-(FJB) positive cells in mice injected with pilocarpine and either FK506 or vehicle. Data were obtained from 6 mice in each group and represented by mean ± SEM.
supplemental material - Supplemental Figure 3. TAT-STEP blocks pERK nuclear translocation. A, TAT-STEP (50 nM, 0.5 μL) was infused into the motor cortex, then 1.5 hrs later, mice were injected with pilocarpine and sacrificed 15 min after SE onset. Immunolabeling for His revealed the cytoplasmic localization of TAT-STEP (red). TAT-STEP infusion suppressed pERK (green) translocation into nucleus (arrows). In the contralalateral motor cortex marked pERK nuclear translocation was observed. B, TAT-STEP inhibits ERK activity without effect on the activity of wild type STEP. Phosphatase activity is expressed as a percentage of

wild-type activity. Five µg of wild-type (WT) STEP46 fusion protein, 5 µg of TAT-STEP46 (C-S) and a combination of 5 µg WT STEP46 and TAT-STEP46 (C-S) (WT+CS) were assayed. Data are the mean (± S.D.) of three independent experiments. T test values= TAT-STEP vs WT-STEP; $p < 0.00005$; TAT-STEP vs WT-STEP + TAT-STEP; $p < 0.0005$; WT-STEP vs WT-STEP + TAT-STEP; $p > 0.84$.

supplemental material - Supplemental Figure 4. Microinfusion into the dentate gyrus. A, U0126 (100 µM, 0.5 µL) was unilaterally infused (arrow denotes approximate location of infusion) 1.5 hours before pilocarpine injection, and animals were killed 15 min following SE onset. Immunohistochemical labeling for pERK revealed that U0126 effectively inhibited SE-induced ERK activation in the infused dentate gyrus. In contrast, robust ERK activation was observed in the contralateral hemisphere. Note the robust pERK expression in the ipsilateral mossy fibers. Mossy fibers express high basal levels of pERK; the inability of the MEK1/2 inhibitor U0126 to block mossy fiber pERK expression indicates that ERK was activated prior to U0126 infusion. B, TAT-STEP was infused into the hilus and detected using immunofluorescent labeling for the His tag (red). Note the intracellular localization of TAT-STEP at 2 hrs-post infusion. Tissue was counter labeled with Hoechst 33258 (blue). Arrow denotes approximate location of infusion. GCL: granule cell layer, Hil: hilus.

supplemental material - Supplemental Figure 5. Neuroprotection and the MAPK pathway in the hilus. Mice were unilaterally infused with FK506 (100 µM, 0.5 µL), U0126 (100 µM, 0.5 µL) and/or TAT-STEP (100 nM, 0.5 µL), injected with pilocarpine, and sacrificed 6 hr after SE onset. A, Representative images of Fluoro-Jade B labeling. Boxed regions are magnified below. Compared to the contralateral hilus, cell death was attenuated by FK506. (Below) Representative images reveal that the neuroprotective effects of FK506 were blocked by U0126 and TAT-STEP. In contrast, the control peptide TAT-myc (100 nM, 0.5 µL) did not attenuate FK506-mediated neuroprotection. B, Quantitative analysis of Fluoro-Jade B-(FJB) positive cells in the hilus. Data are presented as the mean ± SEM. ## $p < 0.01$ compared with vehicle-injected mice. ** $p < 0.01$ compared with FK506-injected mice. ** $p<0.01$ compared with TAT-STEP-injected mice.


Cell density in hilus (cells/mm3)
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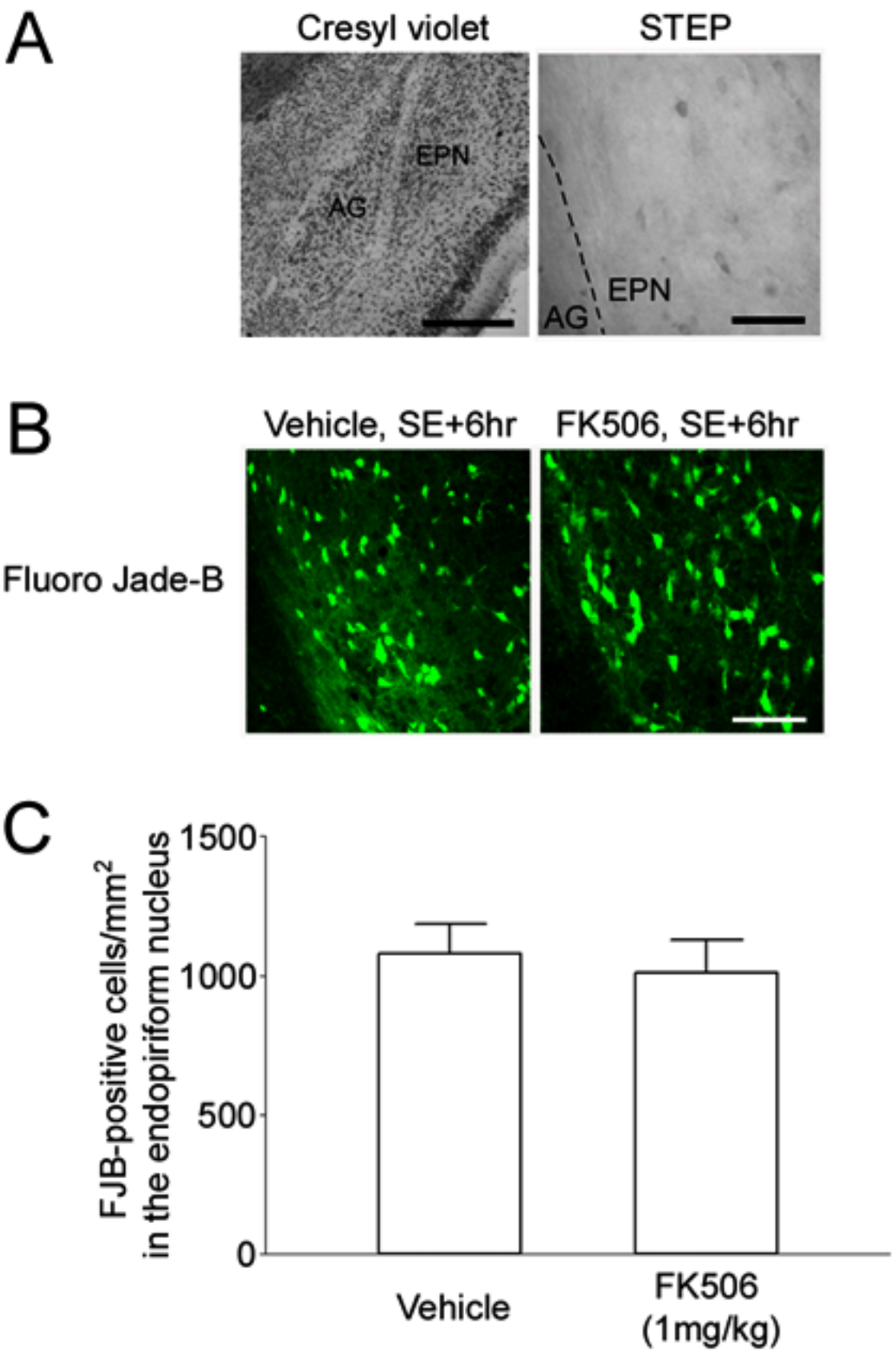
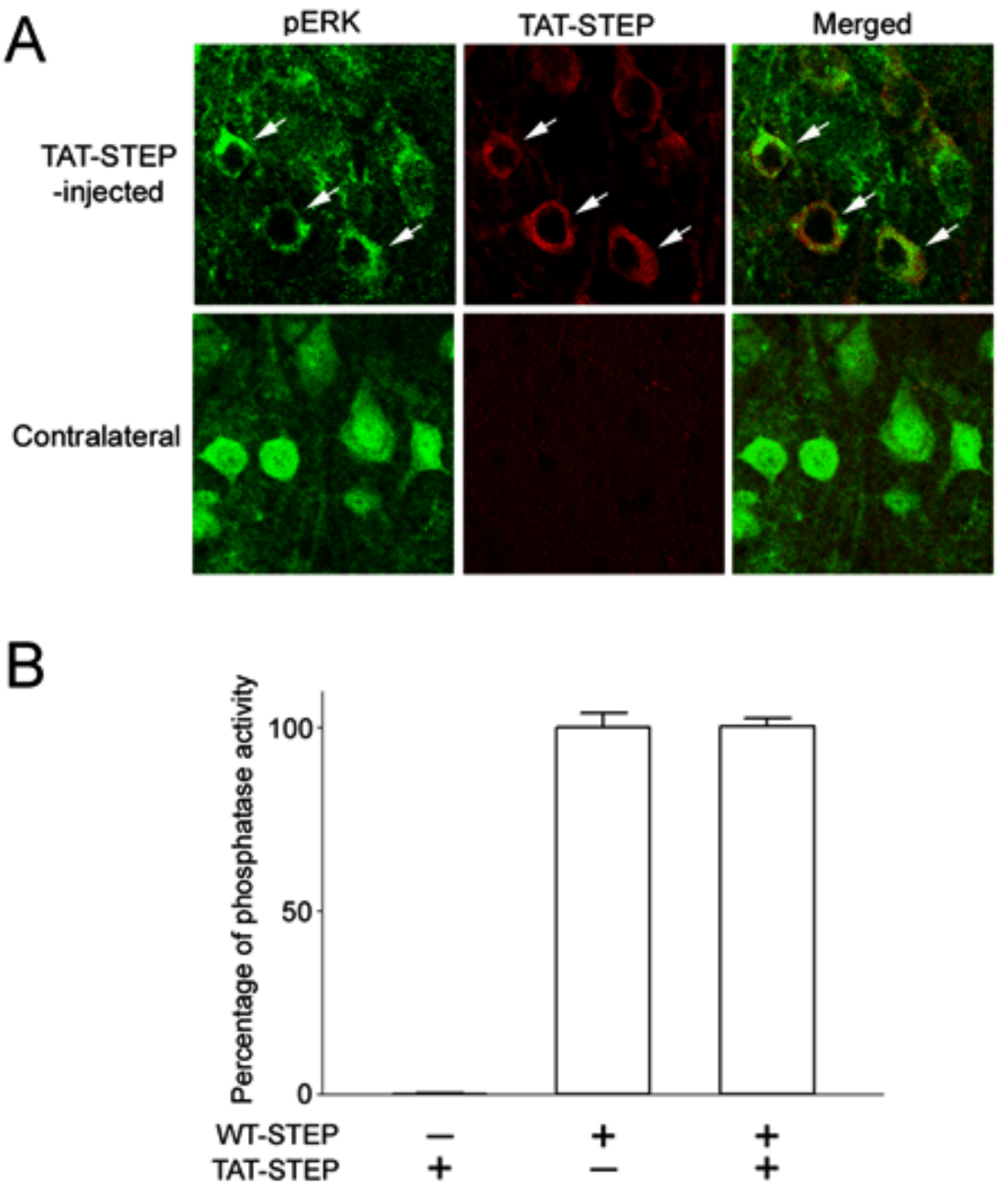
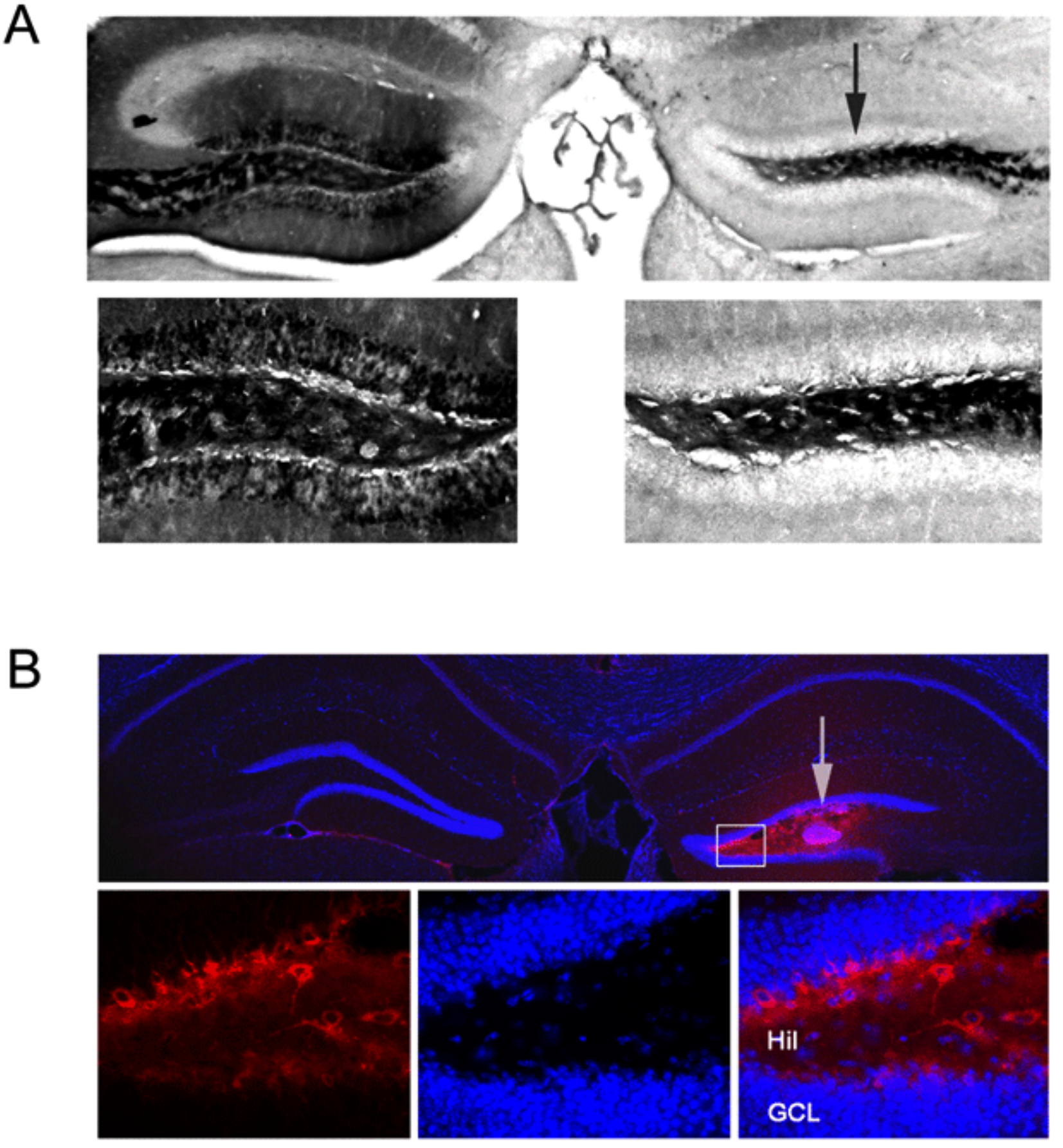
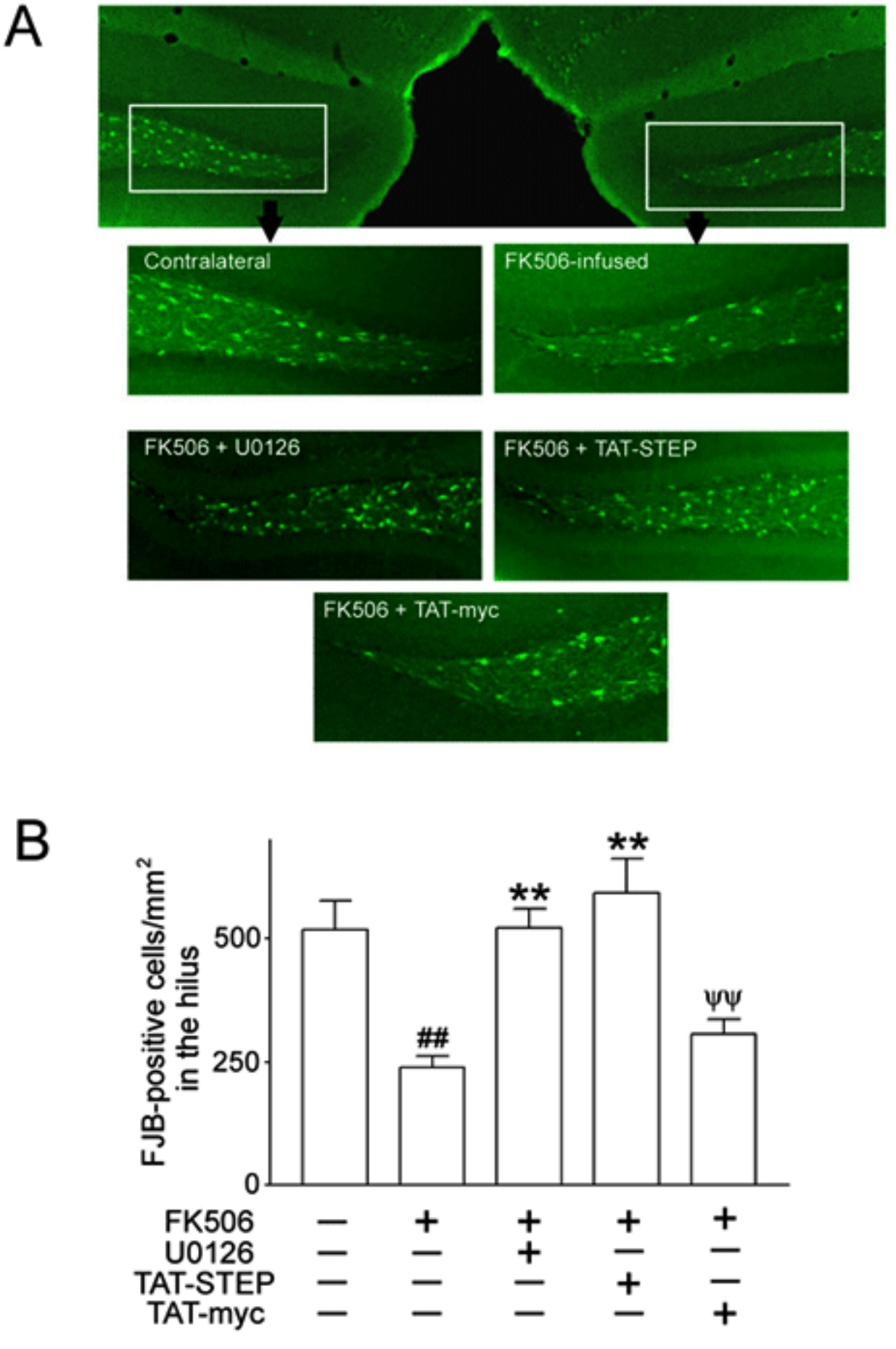
7500
5000
2500
0
DH
BD
DH
BD
STEP
SST


A
Cresyl violet
STEP
EPN
AG
EPN
AG
B
Vehicle, SE+6hr
FK506, SE+6hr
Fluoro Jade-B
C
FJB-positive cells/mm2
in the endopiriform nucleus
1500
1000
500
0
Vehicle
FK506
(1mg/kg)


A
pERK
TAT-STEP
Merged
TAT-STEP
-injected
Contralateral
B
Percentage of phosphatase activity
100
50
0
WT-STEP
TAT-STEP
—
+
+
—
+
+


A
B
Hil
GCL


A
Contralateral
FK506-infused
FK506 + U0126
FK506 + TAT-STEP
FK506 + TAT-myc
B
FJB-positive cells/mm2 in the hilus
500
250
0
##
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ΨΨ
FK506 — + + + +
U0126 — — + — —
TAT-STEP — — — + —
TAT-myc — — — — +