

Supplementary Material

Inhibition of the tyrosine phosphatase STEP₆₁ restores BDNF expression and reverses motor and cognitive deficits in phencyclidine treated mice

Jian Xu, Pradeep Kurup, Tyler D. Baguley, Ethan Foscue, Jonathan A. Ellman, Angus C. Nairn,

Paul J. Lombroso

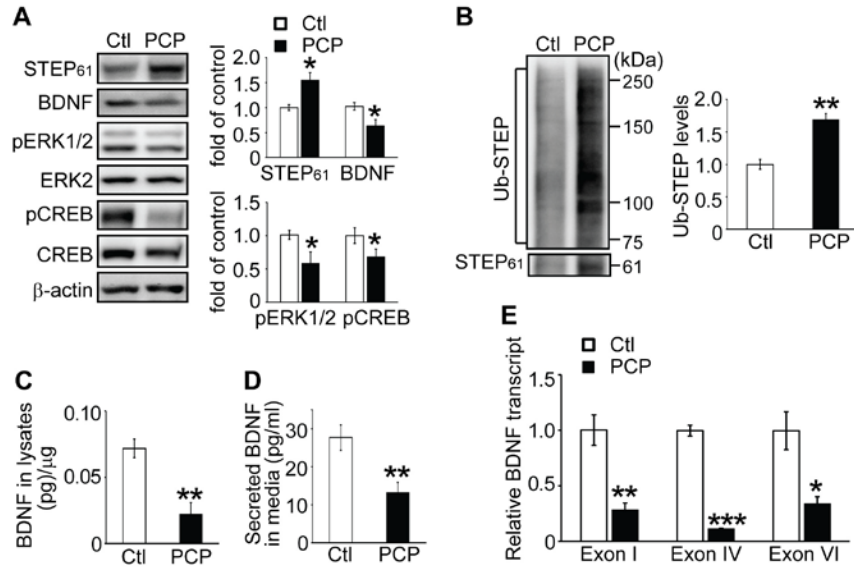


Figure S1 Elevated STEP₆₁ correlates with decreased BDNF protein and BDNF mRNA

expression. **A** Cortical neurons were treated with PCP (10 μM) for 24 h. STEP₆₁ and its substrates were probed with phospho-specific or pan-antibodies, and phospho-levels were normalized to total protein levels and then to β-actin as loading control. **B** PCP treatment leads to increased ubiquitination of STEP₆₁ in culture. BDNF levels in lysates (**C**) and culture media (**D**) were assayed using ELISA. **E** mRNA levels of three BDNF transcripts (Exon I, IV and VI) were measured using quantitative real-time PCR. Target expression levels were normalized to GAPDH as internal control. All data were expressed as mean ± SEM and statistical significance was determined using Student's *t*-test (**p* < 0.05, ***p* < 0.01; *n* = 6 independent batches of cultures for **A**, **B** and **E**; *n* = 8 independent batches of cultures for **C** and **D**)

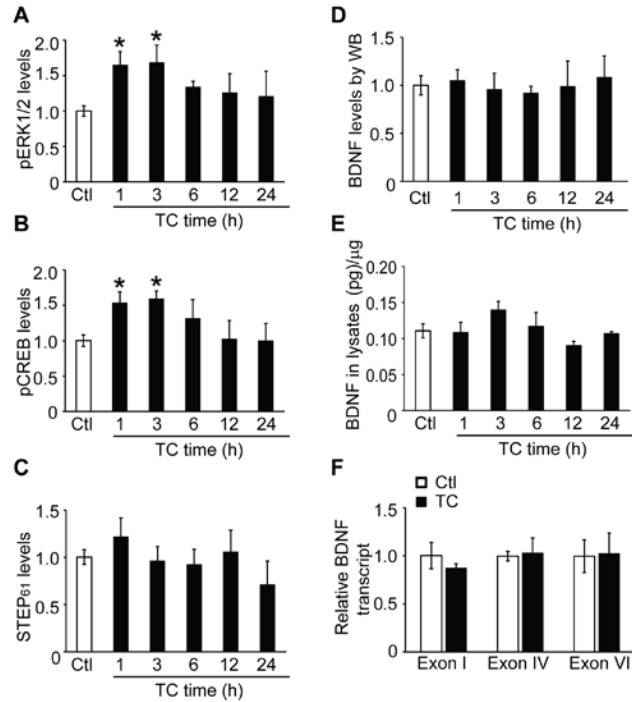


Figure S2 TC-2153 alone did not alter BDNF protein or mRNA levels. Cortical neurons were treated with TC-2153 (1 μ M) for 1-24 h. After treatments, neurons were lysed in 1 \times RIPA buffer for western blotting. TC-2153 induced a transient increase in pERK1/2 (**A**) and pCREB (**B**) levels, without changing total level of STEP₆₁ (**C**) or BDNF (**D**) protein levels in lysates. **E** TC-2153 treatment did not alter BDNF levels as measured by ELISA. **F** Separate cultures were treated with TC-2153 (1 μ M) for 24 h, lysed using the RNeasy kit for RNA extraction and processed for quantitative real-time PCR (qRT-PCR) mRNA levels of 3 BDNF transcripts (Exon I, IV and VI) were measured using qRT-PCR. Target expression levels were normalized to GAPDH as internal control. All data were expressed as mean \pm SEM and statistical significance determined by one-way ANOVA with *post hoc* Bonferroni's test (for **A-E**) or Student's t test (for **F**) (* $p < 0.05$; $n = 6$ independent batches of cultures for all groups)

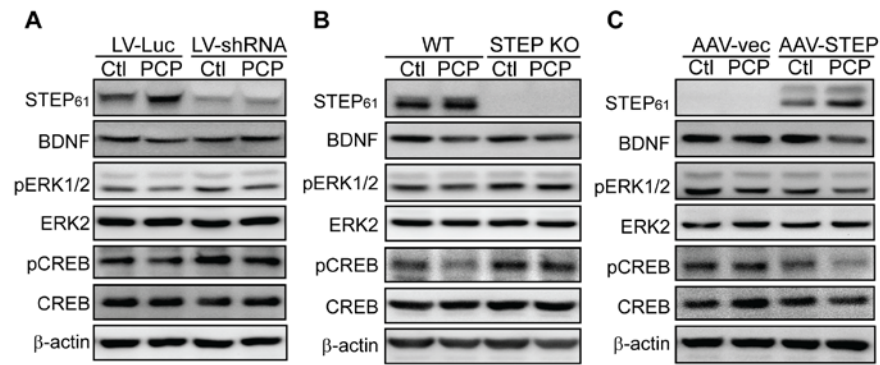


Figure S3 Representative western blots for histograms shown in Figure 3. **A** STEP₆₁ knockdown attenuates PCP-induced decreases in BDNF. Cortical neurons were infected with lentivirus containing luciferase vector (LV-Luc) or STEP shRNA (LV-shRNA) for 7 days, followed by control or PCP (10 μM) for 24 h. **B** Cultured cortical neurons from WT or STEP KO mice were treated with control or PCP (10 μM) for 24 h. **C** Cortical neurons from STEP KO mice were infected with AAV1/2 control vector (AAV-vector) or STEP₆₁ (AAV-STEP₆₁) for 7 days, followed by control or PCP treatment. Neurons were lysed after treatment and subjected to western blotting

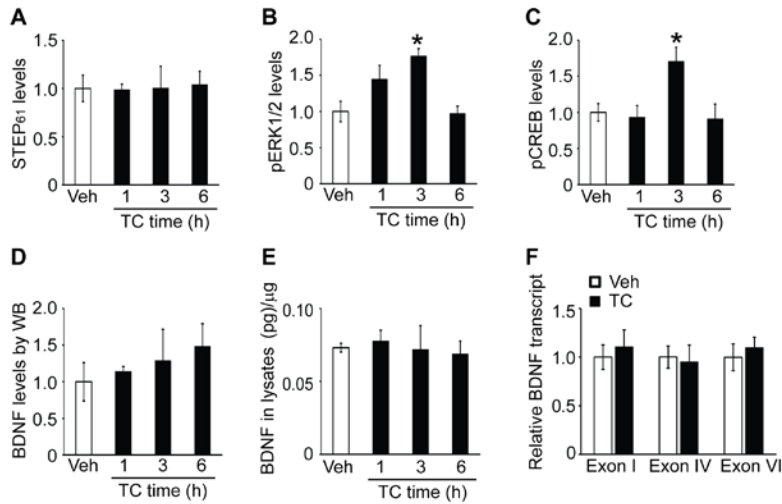


Figure S4 TC-2153 did not alter BDNF protein or mRNA levels *in vivo*. Male C57BL/6 mice were administrated with TC-2153 (10 mg/kg, i.p.) and sacrificed 1-6 h later. Frontal cortex was processed for biochemical analysis. **A** TC-2153 administration did not alter total STEP₆₁ levels. **B, C** TC-2153 administration resulted in a transient increase of pERK1/2 (**B**) and pCREB (**C**) levels. **D, E** TC-2153 administration did not change BDNF expression. BDNF protein levels were measured by western blotting (**D**) and ELISA (**E**). **F** mRNA levels of 3 BDNF transcripts (Exon I, IV and VI) were measured 6 h after TC-2153 injection using quantitative real-time PCR. Data were expressed as mean \pm SEM and statistical significance determined by one-way ANOVA with *post hoc* Bonferroni's test (for **A-E**) or Student's *t*-test (for **F**) (* $p < 0.05$; $n = 4$ C57BL/6 mice for all groups)

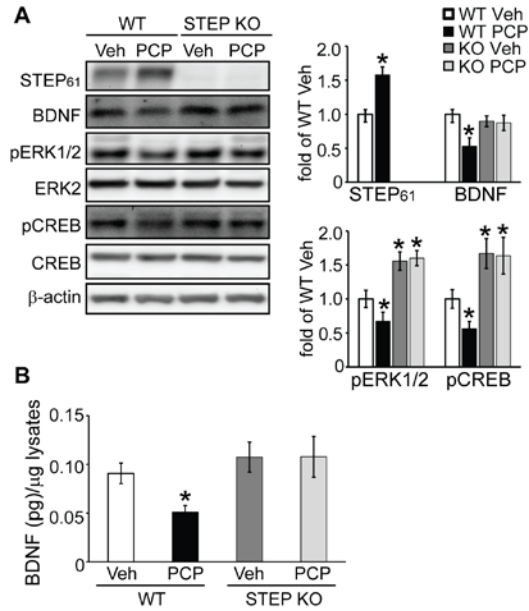


Figure S5 No detectable reduction in BDNF expression in STEP KO mice after acute administration of PCP. **A** Male WT and STEP KO mice (4-6 months old) were treated with PCP (7.5 mg/kg, i.p.) for 1 h. Tissues from frontal cortices were processed by western blotting. Proteins were probed with phospho-specific- or pan-antibodies, and phospho-levels were normalized to total protein levels, and then to β -actin as loading control. **B** BDNF levels were also measured by ELISA. Data were expressed as mean \pm SEM and statistical significance determined using one-way ANOVA with *post hoc* Bonferroni's test ($*p < 0.05$, $n = 6$ mice per group)

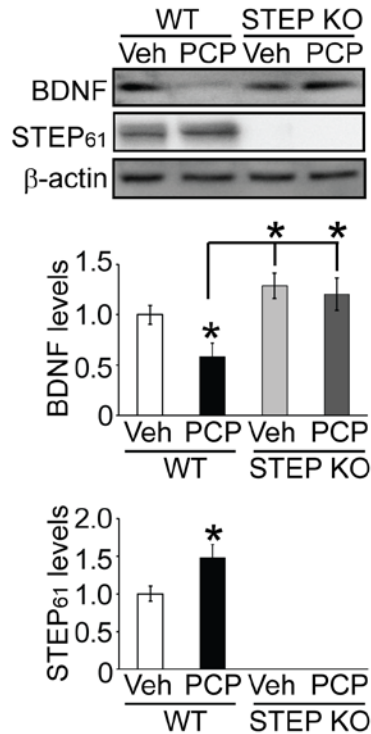


Figure S6 Genetic reduction of STEP prevents PCP-induced reduction of BDNF during NOR consolidation. Male WT and STEP KO mice (3-6 months old) were administered vehicle (Veh) or PCP (5 mg/kg, i.p., twice daily for 5 days, followed by 1 week break). Mice were trained in the NOR task with two identical objects. Nine hours post-training, hippocampi were collected for western blotting. Data were expressed as mean \pm SEM. Two-way ANOVA with *post hoc* Bonferroni's test (for BDNF) and Student's *t*-test (for STEP₆₁) were performed to determine statistical significance (* $p < 0.05$, $n = 6$ mice per group)

Supplementary Table 1. Antibodies used in this study.

Antibody	Immunogen	Host	Dilution	Source
anti-STEP	Residues around Ile ⁴⁴⁰ of human STEP ₆₁	rabbit	1:1000	Cell Signaling Technologies, Danvers, MA
anti-STEP (23E5)	N-terminal of rat STEP ₄₆	Mouse	1:1000	Santa Cruz Biotechnology, Santa Cruz, CA
anti-BDNF	An internal region of human BDNF	rabbit	1:500	Santa Cruz Biotechnology
anti-pERK1/2	Synthetic phosphopeptide around Tyr ²⁰⁴ of human ERK	mouse	1:1000	Santa Cruz Biotechnology
anti-ERK2	C-terminus of rat ERK2	rabbit	1:5000	Santa Cruz Biotechnology
anti-pCREB	Synthetic phosphopeptide around Ser ¹³³ of human CREB	rabbit	1:1000	Cell Signaling Technologies
anti-CREB	N-terminus of human CREB	rabbit	1:1000	Cell Signaling Technologies
anti-phopsho-GluN2B	Synthetic phosphopeptide around Tyr ¹⁴⁷² of rat GluN2B	rabbit	1:1000	Millipore, Billerica, MA
anti-GluN2B	C-terminus (aa 1463-1482) of mouse GluN2B	rabbit	1:1000	Millipore
anti-phospho-Pyk2	Synthetic phosphopeptide around Tyr ⁴⁰² of human Pyk2	rabbit	1:1000	Cell Signaling Technologies
anti-Pyk2	C-terminus of human Pyk2	mouse	1:1000	Cell Signaling Technologies
anti-ubiquitin	Ubiquitin purified from bovine red blood cells	rabbit	1:5000	Thermo Scientific, Fremont, CA
anti-β-actin	gizzard Actin of avian origin	mouse	1:5000	Santa Cruz Biotechnology
anti-rabbit IgG	rabbit IgG (H+L), Peroxidase Conjugated	goat	1:5000	Thermo Scientific
anti-mouse IgG	mouse IgG (H+L), Peroxidase Conjugated	goat	1:5000	Thermo Scientific