STriatal-Enriched protein tyrosine Phosphatase (STEP) Regulates the PTPα/Fyn Signaling Pathway

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Supplemental Information

Materials and Methods

Cholesterol and protein content quantification

Sequential fractionations of DMS were obtained by sucrose gradient fractionation. Total cholesterol level in each fraction was measured by the Amplex Red cholesterol assay kit (Invitrogen) following manufacturer's protocol. Briefly, 50 μl samples were incubated with equal amount of Amplex Red solution (300 μM Amplex Red reagent containing 2 U/mL HRP, 2 U/mL cholesterol oxidase and 0.2 U/mL cholesterol esterase). The reactions were incubated at 37° C for 30 min. Cholesterol content was measured by absorbance at 562 nm using a microplate reader (BioTek, Winooski, VT). Total protein concentration was quantified using BCA protein assay kit (Pierce) following manufacturer's protocol. Cholesterol concentration was normalized to protein content.

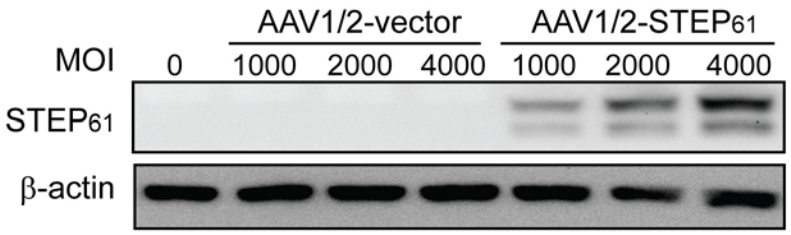

Figure S1. Determination of AAV1/2-$STEP_{61}$ virus titer. STEP KO mouse corticostriatal cultures were infected with AAV1/2 hybrid virus containing $STEP_{61}$ sequence at several multiplicity of infection at DIV 5 for 10 days. $STEP_{61}$ expression was evaluated with anti-STEP antibody. β-actin was probed as a loading control. Representative blots were shown from four independent experiments (n = 4).

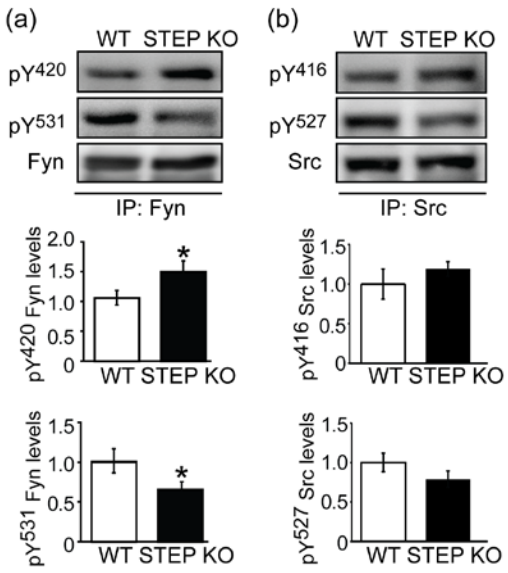

Figure S2. Phosphorylation of Fyn but not Src is altered in STEP KO mouse brain. Fyn (a) or Src (b) was immunoprecipitated from WT and STEP KO striatal homogenates using specific antibodies. Phosphorylation of regulatory sites (pY^{420} and pY^{531} in Fyn; pY^{416} and pY^{527} in Src) was determined using phospho-specific antibodies. Quantification of phosphorylation levels were normalized to immunoprecipitated Fyn or Src. Data were expressed as mean ± SEM (*p < 0.05, Student's t-test; n = 6).

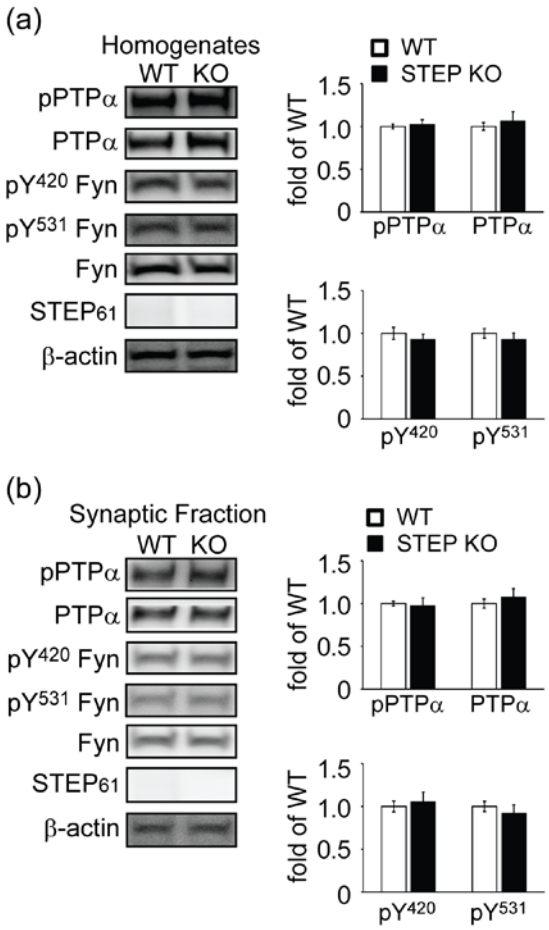

Figure S3. Phosphorylation level of PTPα at Tyr^{789} does not change in STEP KO mouse cerebellum. Total homogenates (a) or crude synaptic membrane fractions (b) from wild-type (WT) and STEP KO (KO) mouse cerebellum were used to determine tyrosine phosphorylation levels of PTPα (pY^{789}), Fyn (pY^{420} and pY^{531}), and total protein levels as indicated in the figure. Quantification of phosphorylation levels were normalized to total protein levels and then to β-actin as a loading control. All data were expressed as mean ± SEM (*$p < 0.05$, **$p < 0.01$, Student's *t*-test; n = 6).

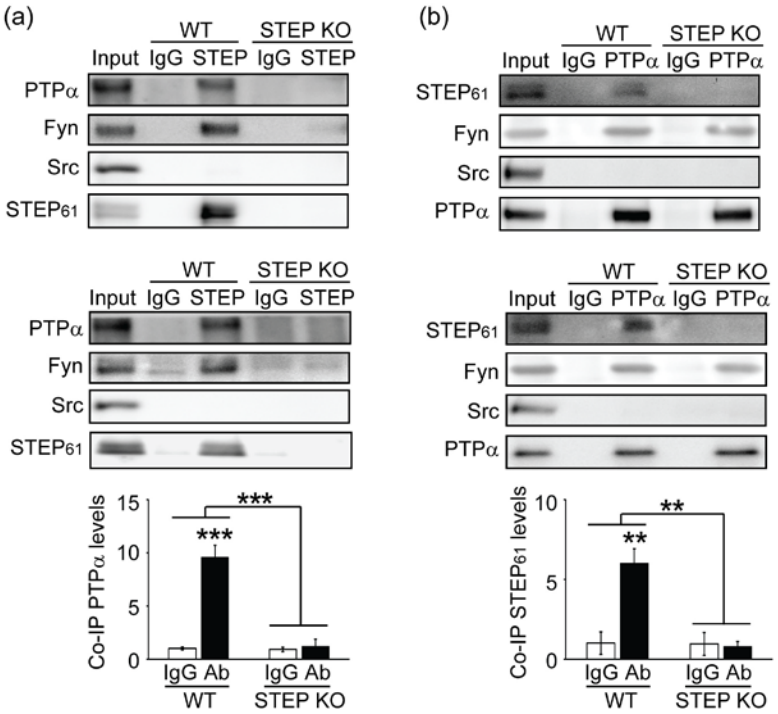

Figure S4. Replicates of reciprocal co-immunoprecipitation of $STEP_{61}$ and PTPα in mouse striatal lysates. WT or STEP KO mouse striatal lysates (300 μg) were incubated with mouse IgG and anti-STEP (23E5) mouse monoclonal antibody (a) or goat IgG and anti-PTPα goat polyclonal antibody (b) overnight at 4 °C. Co-immunoprecipitation of STEP interacting proteins (a) or PTPα interacting proteins (b) was probed with antibodies indicated in the figure. Data were expressed as mean ± SEM (**$p < 0.01$, ***$p < 0.001$, two-way ANOVA with *post hoc* Tukey's test; n = 3).

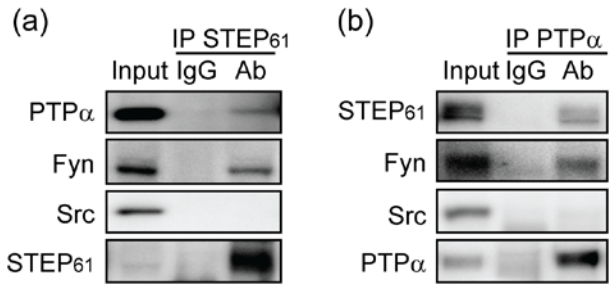

Figure S5. $STEP_{61}$ is associated with PTPα in rat corticostriatal cultures. Rat primary corticostriatal lysates (300 μg) were incubated with mouse IgG and anti-STEP (23E5) mouse monoclonal antibody (a) or goat IgG and anti-PTPα goat polyclonal antibody (b) overnight at 4 °C. Co-immunoprecipitation of STEP interacting proteins (a) or PTPα interacting proteins (b) was probed with antibodies indicated in the figure. Representative blots were shown from three independent experiments (n = 3).

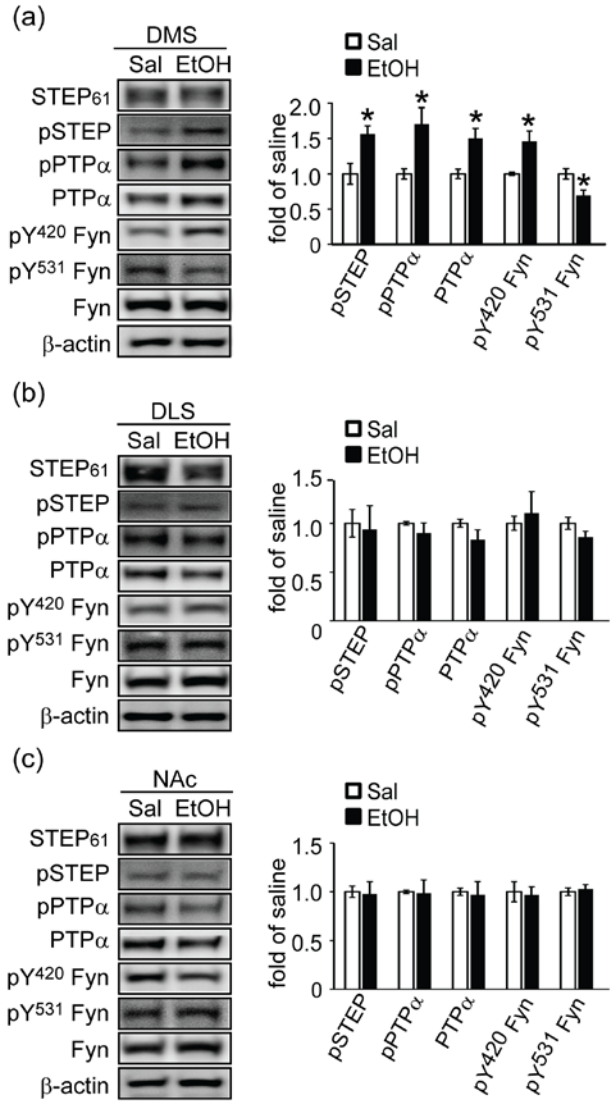

Figure S6. Repeated ethanol administration results in increased phosphorylation of PTPα at Tyr^{789} through phosphorylation and inactivation of $STEP_{61}$. Male C57BL/6 mice (2-3 months) were treated with ethanol (EtOH) subchronically (2g/kg, i.p. daily for 7 days). Synaptosomal fractions (P2) from dorsomedial striatum (a), dorsolateral striatum (b) or nucleus accumbens (c) were used for western blotting with phospho-specific and pan antibodies as shown in the figure. Quantification of phosphorylation levels were normalized to total protein levels and then to β-actin as a loading control. Data were expressed as mean ± SEM (*$p < 0.05$, **$p < 0.01$, Student's *t*-test; n = 5).

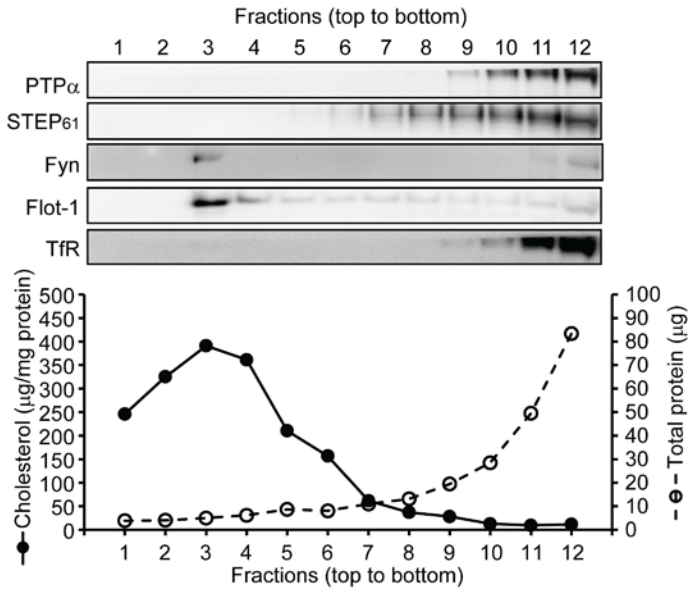

Figure S7. Lipid rafts preparation from mouse DMS. DMS from male C57BL/6 mice (2-3 months) was subjected to sucrose gradient fractionation (details in Methods) to obtain 12 subsequential fractions. Distribution of PTPα, $STEP_{61}$ and Fyn was visualized by specific antibodies. Flotillin-1 (Flot-1) and transferrin receptor (TfR) were used as markers for rafts and non-rafts fractions, respectively. Cholesterol content was measured by the Amplex Red cholesterol assay kit (Invitrogen) and total protein content was quantified using BCA protein assay kit (Pierce) following manufactures' protocols.

Table S1. Antibodies used in this study

Antibody	Immunogen	Host	Dilution	Source
anti-STEP (23E5)	N-terminal of rat $STEP_{46}$	Mouse	1:1000	Santa Cruz Biotechnology, Santa Cruz, CA
anti-pSTEP	Synthetic phosphopeptide around Ser^{221} of mouse $STEP_{61}$	rabbit	1:500	Paul *et al.*, 2003
anti-pPTPα	Synthetic phosphopeptide around Tyr^{789} of human PTPα	rabbit	1:1000	Cell Signaling Technologies, Beverly, MA
anti-PTPα	N-terminal GST-tagged fusion protein corresponding to residues 505-793 of human PTPα	rabbit	1:2000	Millipore, Billerica, MA
anti-phospho-Src family (Tyr^{416})	Synthetic phosphopeptide around Tyr^{416} of human Src	rabbit	1:1000	Cell Signaling Technologies
anti-phospho-Src family (Tyr^{527})	Synthetic phosphopeptide around Tyr^{527} of human Src	rabbit	1:1000	Cell Signaling Technologies
anti-Fyn	N-terminus of human Fyn	rabbit	1:1000	Santa Cruz Biotechnology
anti-Src	N-terminus of human Src	rabbit	1:1000	Santa Cruz Biotechnology
anti-ERK2	C-terminus of rat ERK2	rabbit	1:5000	Santa Cruz Biotechnology
anti-GST	Recombinant glutathione S-transferase from Shistosoma japonicum expressed in *E. coli*	rabbit	1:5000	Santa Cruz Biotechnology
anti-Flot-1	A synthetic peptide corresponding to residues in human Flotillin-1	rabbit	1:1000	Millipore
anti-TfR	N-terminus (aa3-28) of human transferrin receptor	mouse	1:1000	Life Technologies, Gaithersburg, MD
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, Fremont, CA
anti-mouse IgG	mouse IgG (H+L), Peroxidase Conjugated	goat	1:5000	Thermo Scientific