Supplementary Figure 1

Basal activity of Ptpn11<sup>N308D/+</sup> and Ptpn11<sup>D61G/+</sup> mutants.

a. Ptpn11<sup>N308D/+</sup> mutants and WT controls show comparable latencies to the target platform in the visible-version of water maze training. Ptpn11<sup>N308D/+</sup> mice and WT showed comparable performance in the visible platform version of the water maze. \( F_{1, 18} = 0.003, P = 0.954. \)

b-c) In an open field analysis (20 min duration), Ptpn11<sup>N308D/+</sup> mutant mice (n=16) and WT (n=22) controls showed comparable speed and travel distance (speed, t-test, \( P = 0.194; \) distance, t-test, \( P = 0.225 \)).

d. Ptpn11<sup>D61G/+</sup> mutants showed significantly longer latency to the platform during training compared to WT controls in the visible version of water maze. Repeated measures ANOVA with genotype as between-subjects factor, \( F_{1, 23} = 32.99, P < 0.0001. \)

f. In an open field analysis (20 min duration), Ptpn11<sup>D61G/+</sup> mutant mice (n=10) showed significantly slower speed and less travel distance than WT controls (n=15). t-test, *** \( P < 0.0001. \)
Supplementary Figure 2

Probe trials after extended training.

(a-b) *Ptpn11*^<del>N308D</del>^ and WT controls show comparable performance in probe trials after extended training. Quadrant occupancy (a) and proximity analysis (b) for the probe trial conducted after 5 days of training shows that there is no significant difference between *Ptpn11*^<del>N308D</del>^ mutants and WT controls.

(c-d) *Ptpn11*^<del>D61G</del>^ show spatial memory deficits even with additional training. c. Quadrant occupancy for the probe trial conducted after 7-days of training reveals that *Ptpn11*^<del>D61G</del>^ mice (n=10) show no preference for the target quadrant, unlike their WT littermates (n=15) (F<sub>3,36</sub> = 1.824, P = 0.160 and F<sub>3,56</sub> = 36.04, ***P < 0.0001 for *Ptpn11*^<del>D61G</del>^ and WT, respectively; one-way ANOVA). In addition, *Ptpn11*^<del>D61G</del>^ mice also spent significantly less time in the target quadrant than WT mice (*Ptpn11*^<del>D61G</del>^, 33.50 ± 6.27 %; WT, 46.79 ± 3.17, "P < 0.05; t-test). Pool quadrants; target (T), adjacent right (AR), opposite (O), and adjacent left (AL) quadrant. d. *Ptpn11*^<del>D61G</del>^ showed significantly longer proximity to the target platform than WT mice in the probe trial given after 7 days training (*Ptpn11*^<del>D61G</del>^, 48.34 ± 4.11 cm; WT, 38.77 ± 2.01 cm, "P < 0.05; t-test).
Supplementary Figure 3

*Ptpn11* N308D/+ mutants show deficits in contextual fear conditioning.

Mice were trained with two shocks (0.5 mA, 2 s, 1 h interval) for two days and contextual fear memory was assessed for 3 min in the training chamber on the 3rd day. Freezing (%): WT, 58.91 ± 2.50, n=20; *Ptpn11* N308D/+, 43.20 ± 6.82, n=15; * P < 0.05; t-test.
Basal synaptic transmission and paired-pulse facilitation in NS mice.

a. Basal synaptic transmission was not altered in *Ptpn11*<sup>D61G/+</sup> mice (wild type, *n*=9 slices from 7 mice; *Ptpn11*<sup>D61G/+</sup>, *n*=9 slices from 6 mice; Repeated-measures ANOVA, *F*<sub>1, 16</sub> = 0.502, *P* = 0.489). Plot shows the fEPSP slope as a function of stimulation intensity.
b. Presynaptic fiber volley sizes were not different between WT and *Ptpn11*<sup>D61G/+</sup> mice (Repeated-measures ANOVA, *F*<sub>1, 16</sub> = 0.104, *P* = 0.751). Plot shows the fiber volley size as a function of stimulation intensity.
c. Paired-pulse facilitation was not changed in *Ptpn11*<sup>D61G/+</sup> mice (Repeated-measures ANOVA, *F*<sub>1, 15</sub> = 0.183, *P* = 0.674).
d. Basal synaptic transmission was normal in *Ptpn11*<sup>N308D/+</sup> mice (wild type, *n*=13 slices from 7 mice; *Ptpn11*<sup>N308D/+</sup>, *n*=11 slices from 6 mice; Repeated-measures ANOVA, *F*<sub>1, 22</sub> = 0.194, *P* = 0.664).
e. Presynaptic fiber volley sizes were not different between WT and *Ptpn11*<sup>N308D/+</sup> mice (Repeated-measures ANOVA, *F*<sub>1, 22</sub> = 0.067, *P* = 0.798). Plot shows the fiber volley size as a function of stimulation intensity.
f. Paired-pulse facilitation was normal in *Ptpn11*<sup>N308D/+</sup> mice for different inter-stimulus intervals (wild type, *n*=8 slices from 6 mice; *Ptpn11*<sup>N308D/+</sup>, *n*=9 slices from 5 mice; Repeated-measures ANOVA, *F*<sub>1, 15</sub> = 0.0146, *P* = 0.905).
Supplementary Figure 5

*Ptpn11*<sup>N308D/+</sup> mutants show LTP deficits with a 2-burst TBS induction protocol.

LTP induced by a 2 TBS protocol was significantly reduced in the hippocampal slices from *Ptpn11*<sup>N308D/+</sup> mice compared with their WT littermates (WT, n=10 slices from 7 mice; *Ptpn11*<sup>N308D/+</sup>, n=11 slices from 6 mice; Repeated-measures ANOVA: *F*<sub>1, 19</sub> = 7.448, *P* < 0.05; last 10 min of recording, WT, 131.3 ± 3.36 %, *Ptpn11*<sup>N308D/+</sup>, 117.0 ± 2.02 %, t-test, *P* < 0.01). fEPSP slopes normalized to the average baseline response before LTP induction (at time 0) are plotted in 2-min blocks. Sample traces show responses during baseline (gray) and the last 10 min (black) of the recording (average of ten traces). Scale: vertical bar, 0.5 mV; horizontal bar, 4 ms. Error bars represent s.e.m.
**Supplementary Figure 6**

**Viral overexpression of AAV-PTPN11<sup>D61G</sup>**.

a. Western blot analyses confirmed the overexpression of SHP2 (255.6 ± 27.69 % in PTPN11<sup>D61G</sup>-expressing hippocampus compared to GFP-expressing hippocampus, n = 5 per group, P < 0.001). b. PTPN11<sup>D61G</sup>-expressing slice was stained with SHP2 antibody together with Gad67 antibody as an inhibitory neuronal marker. Most of the SHP2 staining (red) did not overlap with Gad67 (green).
**Supplementary Figure 7**

**Effect of *PTPN11*°61G overexpression and SL327 treatment on behavior and basal synaptic transmission.**

(a-b) Effects on the acquisition of water maze or swimming speed. a. For the latency to the platform during training, repeated-measures ANOVA revealed no difference among the groups ($F_{3, 34} = 0.618, P = 0.608$). b. Neither mutant *PTPN11* overexpression nor SL327 treatment affect swimming speed in the probe trial (effect of virus, $F_{1, 37} = 0.054, P = 0.818$; effect of treatment, $F_{1, 37} = 0.240, P = 0.627$).

(c-d) Basal synaptic transmission and paired-pulse facilitation in *PTPN11*°61G overexpressing slices. c. Overexpression of *PTPN11*°61G or SL327 treatment did not affect the basal synaptic transmission in CA3-CA1 synapses. Repeated-measures ANOVA, $F_{3, 36} = 0.175, P = 0.912$. d. Paired-pulse facilitation was not affected by either *PTPN11*°61G overexpression or SL327 treatment. Repeated-measures ANOVA, $F_{3, 35} = 0.356, P = 0.785$. 

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WT PTPN11 overexpression does not affect either basal p-Erk level or learning and memory in water maze tests.

a. Western blot analysis confirmed the overexpression of SHP2 (711.4 ± 42.2 % in wild type PTPN11- transfected hippocampus compared to GFP-transfected hippocampus, n = 5 per group, P < 0.001)

b. Wild-type AAV-PTPN11 overexpression does not affect basal p-Erk level in the hippocampus. (Normalized p-Erk: PTPN11, 98.44 ± 11.48 %, n=5; WT, 100.00 ± 7.53 %, n=4)

c. For the latency to the platform during training in the hidden-platform version of Morris water maze, repeated-measures ANOVA revealed no difference between GFP (n=9) and PTPN11-transfected (n=12) mice (F₁,₁₉ = 1.518, P = 0.233).

d-e) Wild-type PTPN11- and GFP- transfected controls show comparable memory in the probe trial. Quadrant occupancy (d) and proximity analysis (e) shows that there is no significant difference between PTPN11- and GFP- transfected controls.
Supplementary Figure 9

Effects of SL327 treatment on p-Erk levels in the hippocampus.

a. Hippocampi were isolated 1 h after SL327 (0, 3, 10, 30, 40, and 50 mg/kg) injection (n=2 – 6 per dose). p-Erk levels were normalized to the controls (vehicle injected) and fitted using a variable slope model in Graphpad Prism. b. SL327 treatment reverses increased Erk activation in PTPN11<sup>D61G</sup>-transfected hippocampi. **Left**, Representative immunoblot showing p-Erk (upper) and total Erk (lower) in PTPN11<sup>D61G</sup>-transfected and GFP-transfected hippocampi. **Right**, Bar graph displaying normalized p-Erk levels (mean ± s.e.m.). n=7-8 per group. t-test, *P <0.05. c. p-Erk is not significantly increased in the hippocampus of Ptpn11<sup>N308D</sup> mice compared to WT. p-Erk level normalized to WT, 86.08 ± 10.36 %. n=5 slices from 5 mice per group. t-test, P = 0.330.
SL327 treatment reversed memory deficits in Ptpn11D61G/+ mice in Morris water maze.

Quadrant occupancy analysis for the probe trial after the 5th day of training reveals that Ptpn11D61G/+veh mice showed no specific preference for the target quadrant (Dunnett’s Multiple Comparison Test after one-way ANOVA, P > 0.05 for T vs AL, T vs O). Ptpn11D61G/+SL327 groups selectively searched in the target quadrant (one-way ANOVA, F₃,₃₁ = 15.03, *** P < 0.001; T vs. all other quadrants, Dunnett’s Multiple Comparison Test, *** P < 0.001).
Supplementary Figure 11

Full-length pictures of the blots presented in the main figures.
**Supplementary Table 1: Progeny from NS mutant breeding**

<table>
<thead>
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<th>WT</th>
<th>Heterozygote</th>
<th>Litter size (Average ± SEM)</th>
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<td>4.3 ± 0.2</td>
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<tr>
<td>$Ptpn11^{N308D/+}$ x WT</td>
<td>501</td>
<td>181</td>
<td>5.7 ± 0.2</td>
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