Supplementary figure 2:
Reduced tyrosine phosphorylation of Tyr-1472 (a Fyn phosphorylation site; Nakazawa et al., 2001) in NMDA-R 2B (NR2B) subunit in Ptpra−/− hippocampus. Adult hippocampi were dissected on ice and immediately homogenized using a polytron in RIPA buffer containing 0.5% SDS (1% NP-40, 1% sodium deoxycholate, 0.5% SDS, 50 mM Tris pH 8.0, 120 mM NaCl, 5 mM EDTA, 1 mM sodium orthovanadate, 1 μM PMSF, 10 μg/ml aprotinin + leupeptin). Lysates were boiled for 3 min and diluted with 4 volumes of RIPA-buffer lacking SDS.
A. Total hippocampal lysates blotted with anti-P-tyr (P-tyr-100, Cell Signaling Technology).
B. Total hippocampal lysates blotted with anti-RPTPα.
C. Equal amounts of protein were immune precipitated with an antibody to NR 2A/2B (Chemicon), and precipitates subjected to immuno-blotting with an antibody (Nakazawa et al., 2001) to Tyr-1472-phosphorylated NR2B (top) or to NR 2A/2B (bottom).
D. Statistical analysis of Tyr-1472-phosphorylated NR2B. The experiment described under C was repeated on 10 pairs of mice. Phospho-Tyr-1472 reactivity was normalized to the amount of precipitated NR 2A/2B by densitometry (WT=1; error bar indicates standard deviation; p<0.05).