Supplemental Data.

Methods

RNA isolation and real time RT-PCR analysis. Total RNA from the brains of PPT1-KO and control mice was isolated using TriZol reagent (Invitrogen) and further purified by QIAGEN RNeasy Mini Kit and treated with DNAse (DNAse I, 30 U/μg total RNA) (QIAGEN) then reverse transcribed using SuperScript III First-Strand Synthesis System (Invitrogen). Expression of mRNA was quantitated by using SYBR Green PCR Master Mix, performed with ABI Prism 7000 Sequence Detection System (Applied Biosystems) with cDNA equivalent to 10 ng of total RNA for VAMP-2, Syntaxin-1, SNAP-25, Syt-I, and GAD-65. The primers used for these genes are presented in ***Supplemental Table 1***. The data were analyzed using ABI Prism Software version 1.01 (Applied Biosystems). The final data were normalized to β-actin and presented as fold change in PPT1-KO mice compared with those of WT littermates. Each experiment was repeated at least three times and the results are expressed as the mean $\pm$ were done in triplicate for the PPT1-KO and WT mice. Data are analyzed by student's t test and a p value of <0.05 is considered significant.

Mouse astroglial cell culture. Mouse astroglial cells were isolated from the brains of 15-day-old fetuses of PPT1-KO and WT littermates. The cells were cultured in Minimum Essential Medium (MEM, invitrogen) containing 10% FBS at 37^{o}C under an atmosphere of 5% CO_2 and 95% air.

^{3}H-Palmitate labeling of proteins and immunoprecipitation. Cells were metabolically labeled prior to immunoprecipitation with [^{3}H] palmitic acid (200 μCi/ml palmitate, Amersham Pharmacia Biotech) for 3 hr. Labeled cells were washed two times with ice-cold PBS and treated with MemPER Eukaryotic Membrane Protein Extraction Kit (Pierce) according to the supplier's protocol. For immunoprecipitation, the samples were then incubated overnight with primary antibodies at 4°C. After addition of protein A Sepharose beads (Amersham Pharmacia Biotech), samples were incubated for 1 hr at 4°C. Immunoprecipitates were washed three times with buffer containing 50 mM Tris-HCl, pH 7.2; 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, and 0.3% Triton X-100, boiled in SDS-PAGE sample buffer (80 mM Tris, pH 6.8, 10% glycerol, 5% SDS, 150 mM dithiothreitol, 0.005% bromophenol blue) for 5 min. For autoradiography, protein samples were separated by SDS-PAGE and dried using vacuum dryer. And gel was exposed to BAS-IIIs Imaging plate (Fuji) for 2 weeks, and the image was processed using the BAS-1500 Reader (Fuji) with the Image Reader V1.4E and Image Gauge V3.0 programs (Fuji).

Western blot analysis. The brains were homogenized in protein extracting buffer (50 mM Tris-HCI, 150mM NaCl, 0.25% SDS, 1mM EDTA and 1%NP-40) containing protease-inhibitor cocktails (Sigma-Aldrich). For the total lysate preparation, mouse neurospheres were treated in Phosphosafe extraction reagent (EMD biosciences). Soluble and membrane proteins were prepared by Mem-PER Eukaryotic Membrane Protein Extraction Kit (Pierce). Twenty micrograms of the total proteins from each sample were resolved by electrophoresis using 4-15% SDS-polyacrylamide gels (Bio-Rad) under

denaturing and reducing conditions. Proteins were then electrotransferred to nitrocellulose membrane (Bio-Rad). The membranes were blocked with 5% non-fat dry milk (Bio-Rad) and then subjected to immunoblot analysis using the methods as described before. The primary antibodies used in the present study are: anti-synaptotagmin, anti-GAD65 (Santa Cruz biotechnology), anti-PSD-95, anti-sodium potassium ATPase, anti-VAMP2, anti-SNAP-25, anti-syntaxin-1 (Abcam) and anti-β-actin (USBiological). The second antibodies used in the study are: goat anti-rabbit IgG, rabbit anti-mouse IgG (Santa Cruz Biotechnology). Chemiluminescent detection was performed by using Supersignal west pico luminol/enhancer solution (Pierce) according to the manufacturer's instructions. Densitometric analysis was performed by Quantity One software (Bio-rad). Error bars indicate SD (n=3).

Co-immunoprecipitation. Cell extracts were prepared using buffer containing 0.5% Triton X-100. The cell debris was centrifuged at 15,000×g for 30 min at 4^{o}C, and the supernatant was used for immunoprecipitation. Protein G–agarose beads (Sigma) with attached anti-Syntaxin-1 antibody (Santa Cruz biotechnology) were incubated with the cell detergent extracts for 6h at 4^{o}C, and then extensively washed. Protein bands on western immunoblots were detected using SNAP-25 antibodies (Abcam) and the Supersignal west pico luminol/enhancer solution (Pierce) according to the manufacturer's instructions.

Supplemental Figure Legends

Supplemental Figure 1: PPT1-deficiency causes reduced levels of synaptic vesicle-associated proteins in the brain. Western blot analyses of lysates of postmortem brain tissues from a normal control (*left lanes*) and from an INCL patient (*right lanes*) for Syt I (**panel A**) and GAD-65 (**panel B**). **1**, normal hippocampus, **2**, normal cortex, **3**, INCL patient hippocampus, 4, INCL patient cortex. Western blot analyses of brain lysates from WT (*left lanes*) and those of their PPT1-KO littermates (*right lanes*) for Syt I (**panel C**) and GAD-65 (**panel D**). 1M, 1 month; 3M, 3 months; 6M, 6 months.

Supplemental Figure 2: The mRNA levels of various SV-proteins in the cerebral cortex of PPT1-KO mice and their WT littermates. The mRNA levels were quantitated by real time PCR (see *Supplemental Table 1* for specific primers used). The mRNA levels of the following markers were quantitated: VAMP2 (***panel A***), Syntaxin-1 (***panel B***), SNAP-25 (***panel C***), Syt-I (***panel D***) and GAD-65 (***panel E***). Results are expressed as the mean (n=3) $\pm$ SD. Note no significant differences in the mRNA levels between WT and PPT1-KO mice littermates.

Supplemental Figure 3: Cultured neurons differentiated from PPT1-KO neurospheres show decreased levels of SV-associated proteins. **(A)** Western blot analysis of Syt-I protein in the WT (*left lane*) and PPT1-KO (*right lane*) neurospheres. **(B)** Western blot analysis of GAD-65 in the WT *(left lane*) and PPT1-KO (*right lane*) neurons.

Supplemental Figure 4: (**A**) Western blot analysis of purified synaptosomal/SV membrane fractions. Syt-I and GAD-65 were detected by Western blot analysis. Na/K-ATPase was used as a protein-loading standard. Proteins in the WT (*left lane*) and PPT1-KO (*right lane*). Densitometric analysis of Syt-I and GAD-65 show on the right side of images. (**B)** The levels of readily-releasable SV-pools in WT (*upper panels*) and PPT1-KO (*lower panels*) using primary cultures of cortical neurons from PPT1-KO and WT mice. Active (readily releasable) vesicles were detected by loading of the styril dye FM1-43FX (*green*) in the WT (*upper panels*) and PPT1-KO (*lower panels*) neurons. Nuclei were stained by DAPI; scale bar, 20μm.

Supplemental Figure 5: (A) Co-immuno-precipitation with Syntaxin-1 and SNAP-25 proteins. Cell lysate subjected to IP using anti-Syntaxin-1 antibody and were analyzed by SDS-PAGE/ immunoblotting (IB) using anti-SNAP-25 antibody. **(B)** Western blot analysis of post synaptic density protein-95 (PSD95) in the WT (*left lane*) and PPT1-KO (*right lane*) neurons. Note the virtually identical protein levels in the soluble and membrane fractions. β-actin: soluble fraction loading control, Na/K-ATPase: membrane fraction loading control.

Supplemental Reference:

Bjohrs, M., Rickman, C., Binz, T. and Davletov, B. (2004) A molecular basis underlying differences in the toxicity of botulinum serotypes A and E. EMBO Reports 5: 1090-1095.

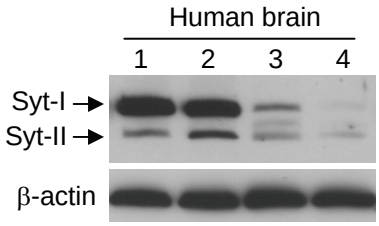
Supplemental Table 1: PCR Primers used for quantitative RT-PCR

VAMP2-F	5'-GACAAAACAGAATCCCCCTAATTC-3'
VAMP2-R	5'-AGTCGAACCTCTAGCAAGGATGA-3'
Syntaxin-1-F	5'ATGCTGGTGGAGAGCCAGG-3'
Syntaxin-1-R	5'-GGTACTTGACGGCCTTCTTGG-3'
SNAP-25-F	5'-AGGCGAACAACTGGA ACGC-3'
SNAP-25-R	5'-GCCCGCAGAATTTTCCTAGG-3'
Syt-I-F	5'-ATAGTTGCGGTCCTTCTAGTCGT-3'
Syt-I-R	5'GAACAAACATTTCTTACAGACACAGAA-3'
GAD-65-F	5'-CACTCTGGAAGACAATGAAGAGAGA-3'
GAD-65-R	5'-CATTCTGGCTTTAATCACTGGC-3'

F, forward; R, Reverse.

Supplemental Figure 1

A



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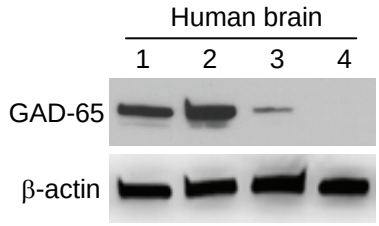

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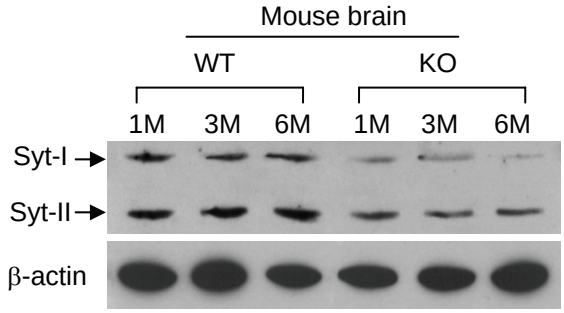

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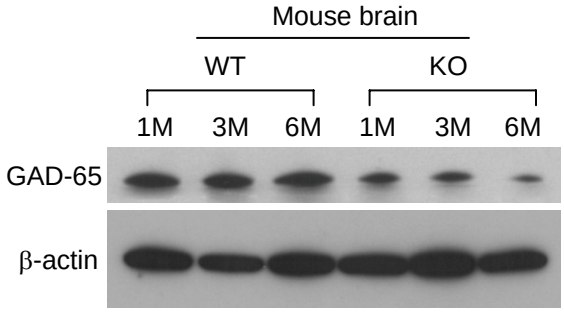

Supplemental Figure 2

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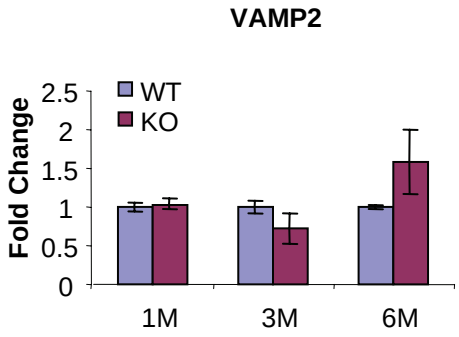

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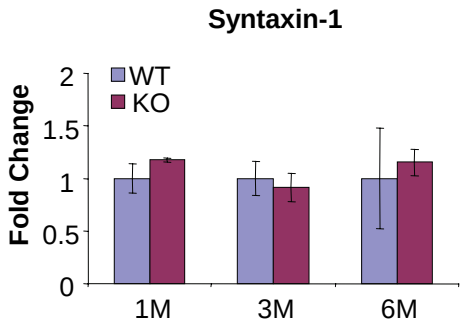

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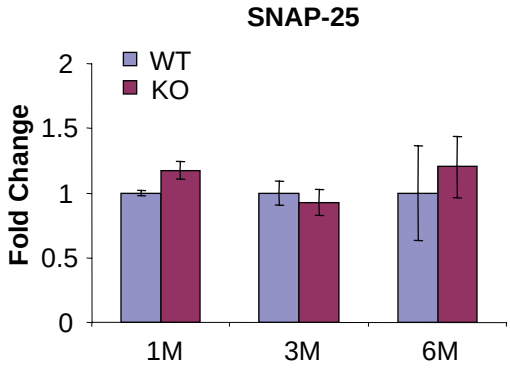

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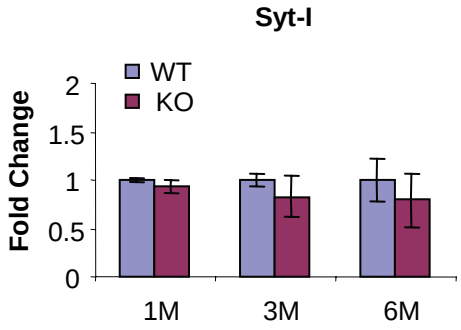

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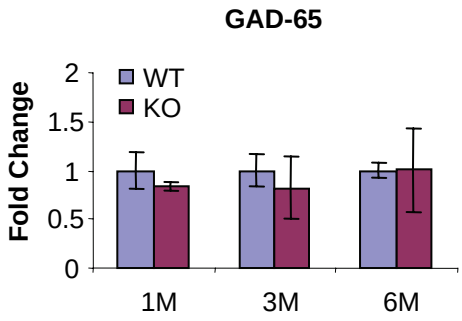

Supplemental Figure 3

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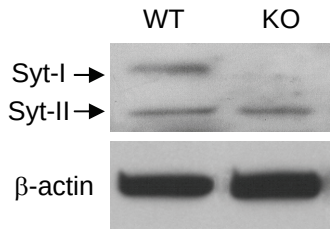

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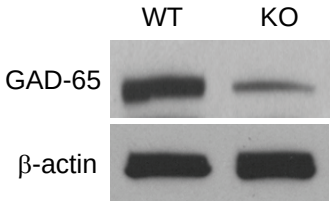

Supplemental Figure 4

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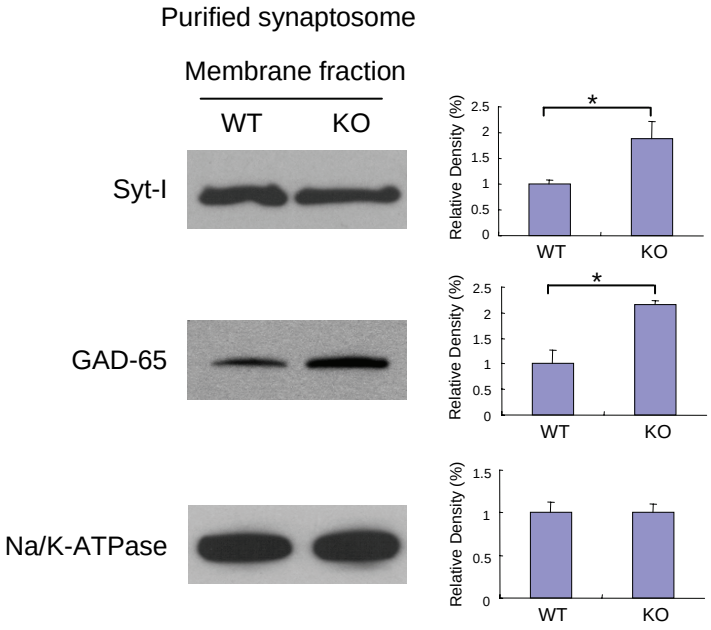

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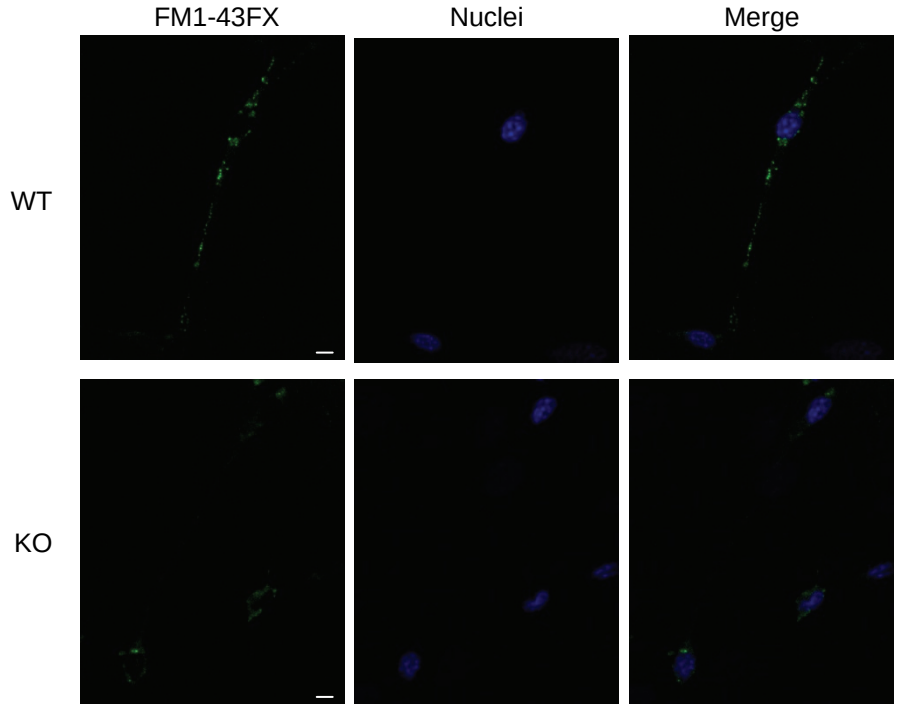

Supplemental Figure 5

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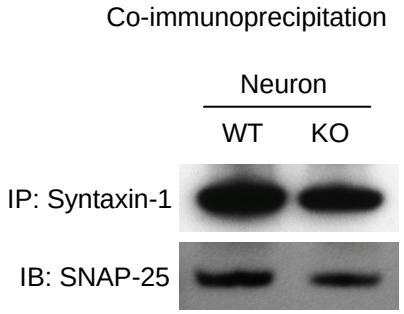

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