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Supplemental information

PSD-95 protects synapses from β-amyloid

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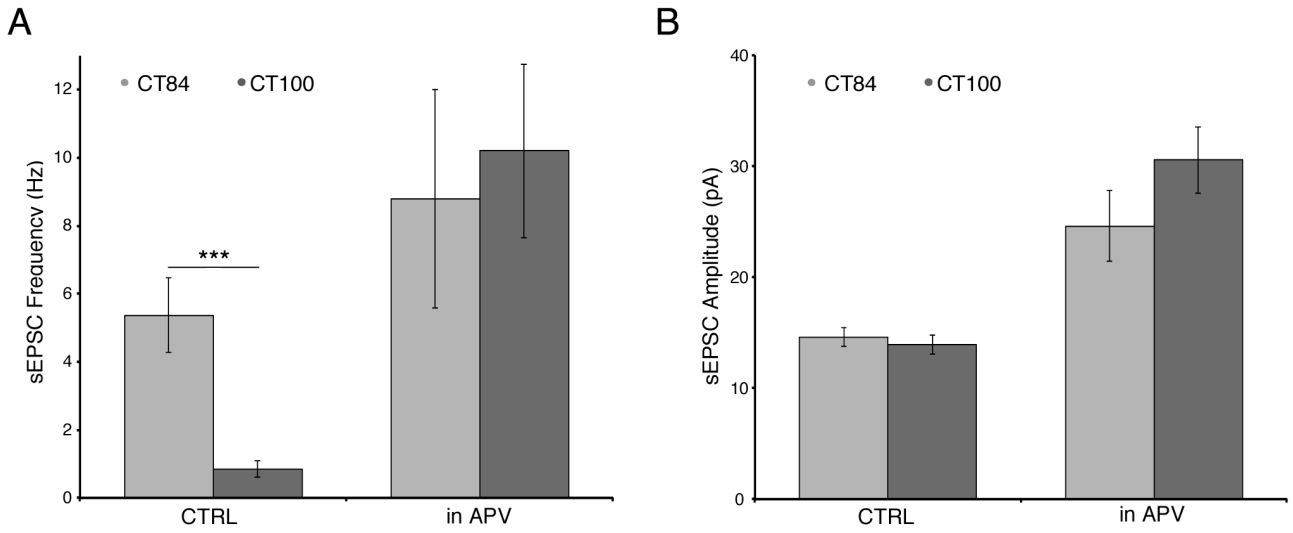

Figure S1 (Related to Figure 2): CT100 depresses synaptic transmission in primary hippocampal neurons, which is blocked by glutamate-competitive NMDAR antagonist.

(A) Frequency of spontaneous EPCSs (sEPSC) recorded in 8-12DIV hippocampal neurons. CT100 significantly decreased sEPSC frequency in control neurons (CT84: N=15; CT100: N=19 ***, $p<0.001$), but had no effect in the presence of APV (CT84: N=11; CT100: N=9; 100μM).

(B) Amplitude of sEPSC recorded in (A). CT100 does not affect sEPSC amplitude in control neurons and neurons incubated in APV.

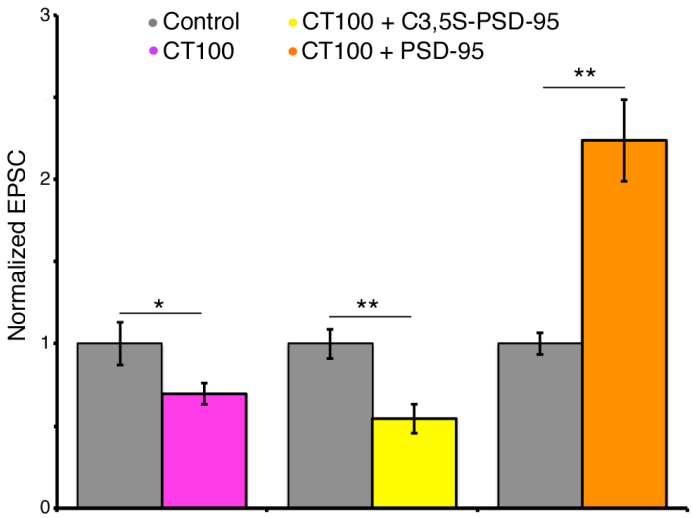

Figure S2 (Related to Figure 1 and 2): PSD-95 palmitoylation is required for blocking the effects of CT100.

Dual-patch recordings in WT rat organotypic hippocampal slices indicated synaptic depression in the presence of CT100 and CT100 + C3,5S-PSD-95 in contrast with neurons expressing CT100 and WT PSD-95 (data from Figure 1A) where synaptic potentiation was observed. N≥11; *, $p<0.05$; **, $p<0.01$, values compared to uninfected control neurons.

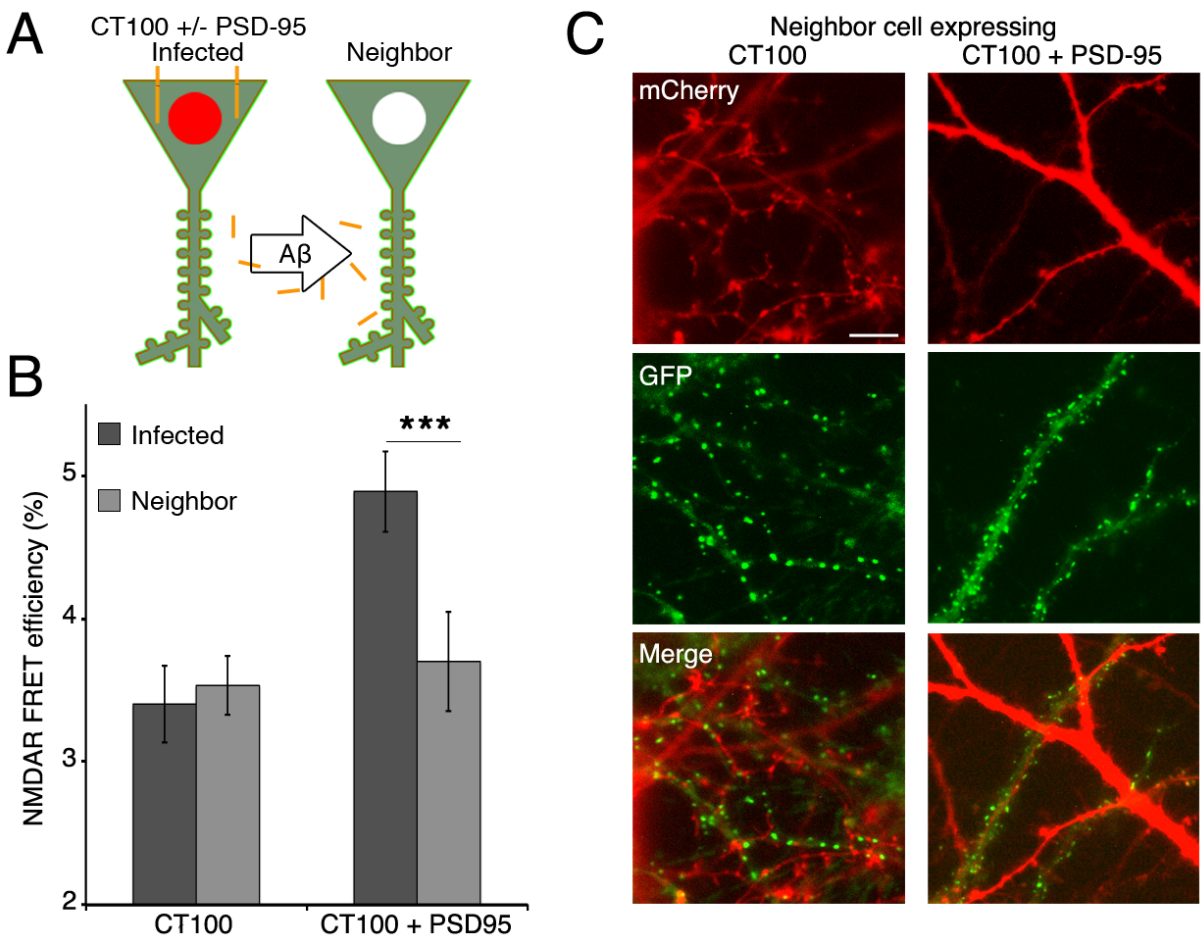

Figure S3 (Related to Figure 2): CT100 expressing neurons drive movement of the NMDAR-CTD in neighboring neurons

(A) Experimental configuration (see main text for details).

(B) Bar graph of spine FRET efficiency of GluN1-GFP/GluN1-mCherry expressing neurons infected with CT100 or CT100 + PSD-95, or located nearby infected cells (Neighbor); N > 20 neurons; > 400 spines (for each condition); *** $p < 0.0001$, unpaired t-test.

(C) Example images of dendrites of uninfected neurons with 'Neighbor' cells expressing CT100 (left) or CT100 + PSD-95 (right). Scale bar is 5µm.

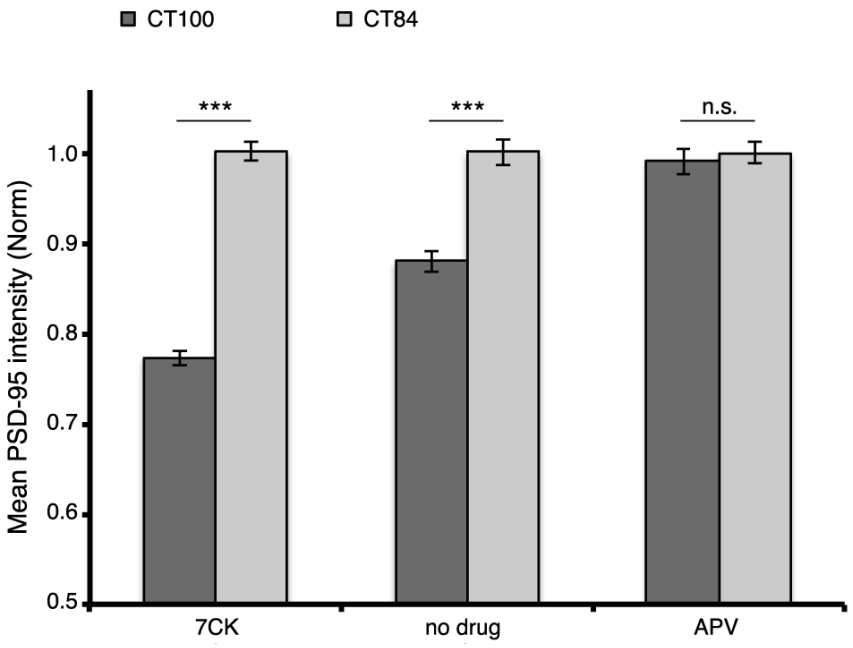

Figure S4 (Related to Figure 4): CT100 reduction of PSD-95 in spines requires ligand binding to the glutamate site of the NMDAR but not NMDAR ion-flux.

Bar graph of PSD-95 average intensity in spines expressing GFP and CT100 or CT84, (normalized to PSD-95 intensity values in all CT84-expressing spines). Spines contours were manually traced with the GFP channel (See 'Immunohistochemistry' and 'Quantification' sections in METHODS for more information). The 7CK dataset is the same as in Figure 4B and 4C. The 'no drug' and 'APV' datasets were acquired from neuronal cultures processed and imaged at the same time. The partial block of AMPAR transmission by 7CK appears not to prevent metabotropic NMDAR effects permitted by 7CK. N>2000 spines per condition; *** $p<0.0001$, unpaired T-test, n.s. = non significant.

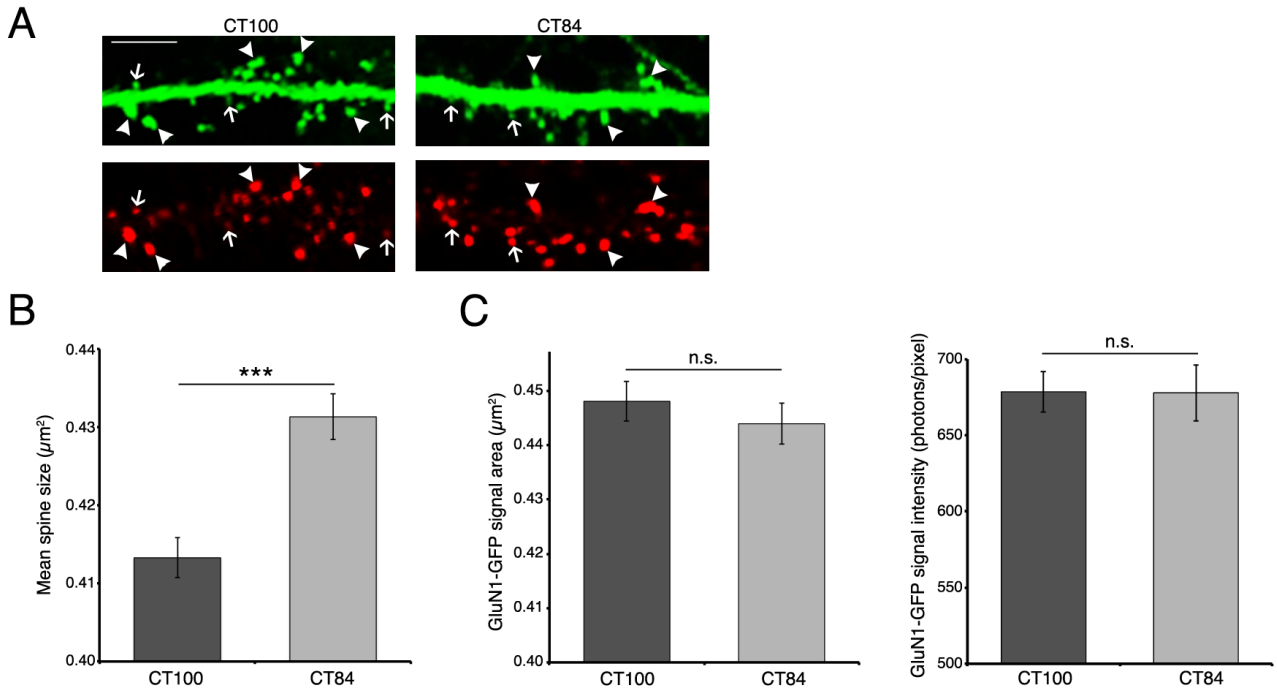

Figure S5 (Related to Figure 4): CT100 reduces spine size but not GluN1-GFP density

(A) Unmasked images of PSD-95 immunostaining in neurons expressing GFP and CT100 (left) or CT84 (right). Scale bar is 5μm.

(B) Average spine size as measured with cytoplasmic GFP for neurons expressing CT100 or CT84, same dataset as in Figure 4B and 4C. N>3000 spines per condition; *** $p<0.0001$, unpaired T-test.

(C) (left) Average GluN1-GFP signal area in spines expressing CT100 or CT84, (right) Average GluN1-GFP signal intensity in spines expressing CT100 or CT84; same dataset as in Figures 2C and 4D. N>2500 spines per condition; n.s. = non significant, unpaired T-test.

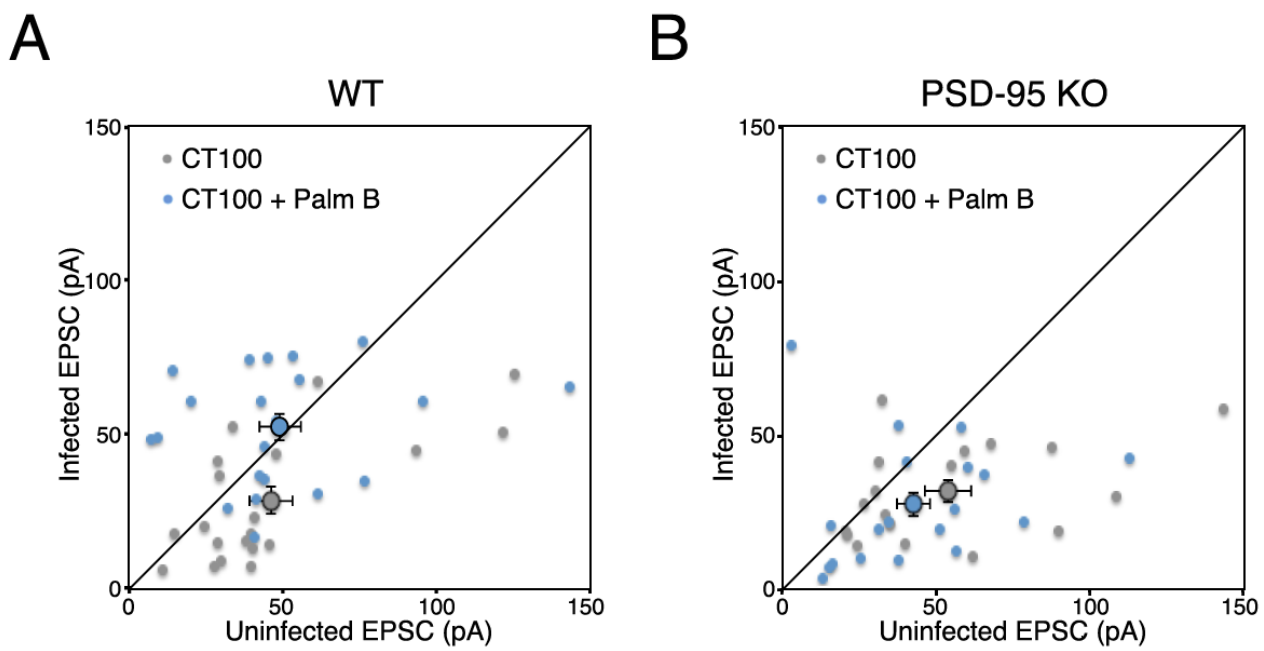

Figure S6 (Related to Figure 5): Palm B rescue CT100 induced synaptic depression in WT mice but has no effect in PSD-95 KO mice.

(A) Dot-plot of excitatory post-synaptic current (EPSC) dual-patch recordings in organotypic slices made from WT mice. Infected neurons are expressing CT100, Palm B treated slices were incubated with 1μM Palm B for 3 hours; group average indicated by larger black outlined symbols. Same dataset as in Figure 5A. $N \geq 20$ for each group

(B) Dot-plot of excitatory post-synaptic current (EPSC) dual-patch recordings in organotypic slices made from PSD-95 KO mice. Infected neurons are expressing CT100, Palm B treated slices were incubated with 1μM Palm B for 3 hours; group average indicated by larger black outlined symbols. Same dataset as in Figure 5B. N=19 for each group.

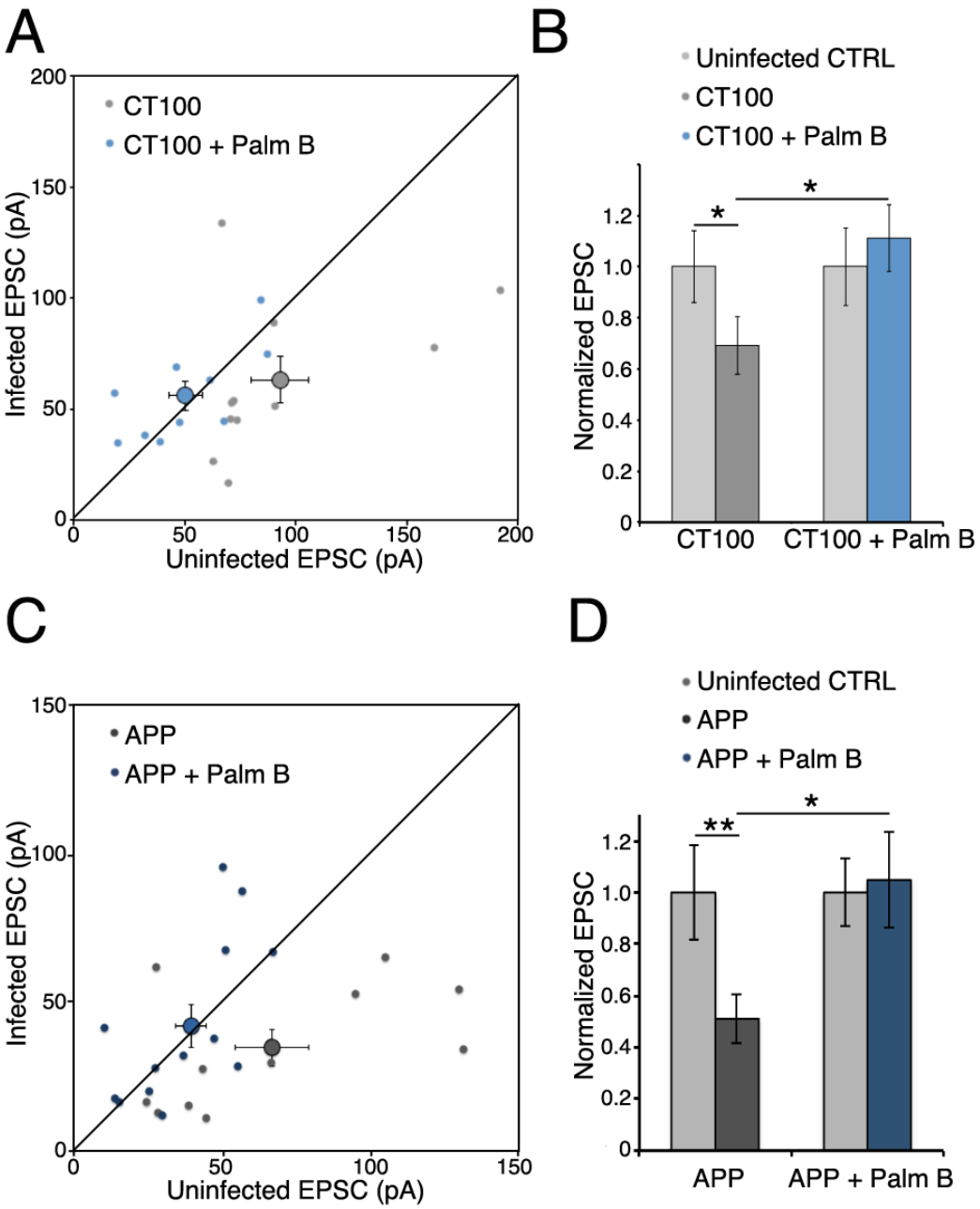

Figure S7 (Related to Figure 5): Palm B rescue CT100 and APP-induced synaptic depression in rat hippocampal slices

(A) Dot-plot of excitatory post-synaptic current (EPSC) dual-patch recordings in organotypic slices. Infected neurons are expressing CT100, Palm B treated slices were incubated with 1μM Palm B for 3 hours; group average indicated by larger black outlined symbols.

(B) Bar-graph of dual-patch recordings for indicated groups; responses normalized to uninfected controls (light gray). * $p < 0.05$, paired or unpaired t-test; $N \geq 10$ for each group.

(C) Dot-plot of excitatory post-synaptic current (EPSC) dual-patch recordings in organotypic slices. Infected neurons are expressing full-length human APP, Palm B treated slices were incubated with 1μM Palm B for 3 hours; group average indicated by larger black outlined symbols.

(D) Bar-graph of dual-patch recordings for indicated groups; responses normalized to uninfected controls (light gray). ** $p < 0.01$ paired t-test, * $p < 0.05$ unpaired t-test; $N \geq 11$ for each group.

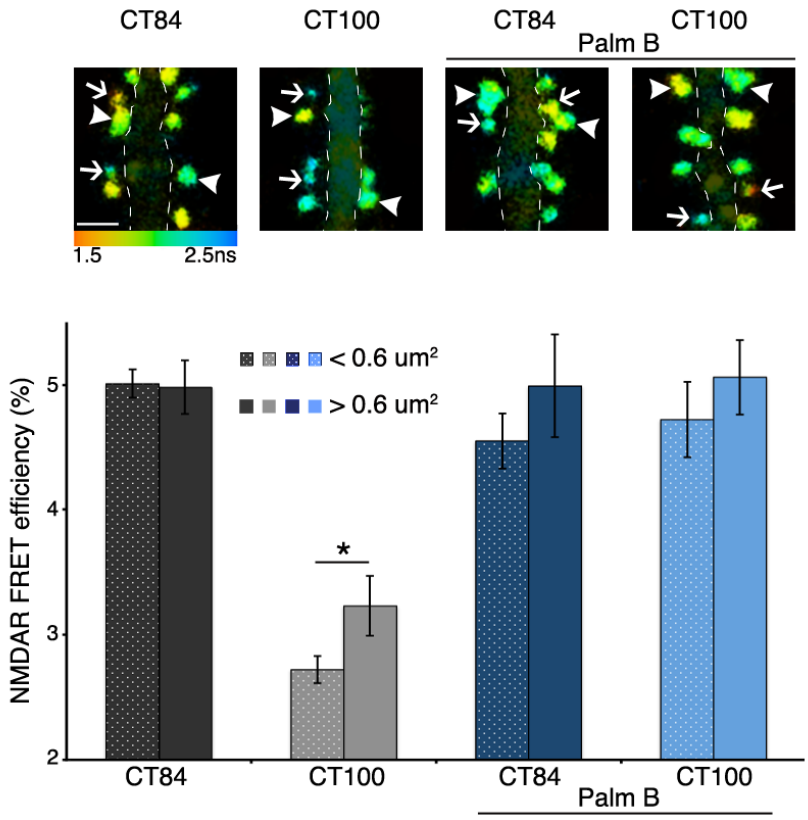

Figure S8 (Related to Figure 5): Palm B rescue Aβ-driven movement of the NMDAR-CTD in small and big dendritic spines

(Top) Representative FLIM images of neurons expressing the NMDAR FRET probes and the indicated constructs. Small spines are shown with arrows and big spines with arrowheads. Scale bar is 2μm, dendrites masked for clarity.

(Bottom) FRET efficiency of small (dotted pattern) and big spines expressing CT84 or CT100. 1μm Palmostatin B (Palm B) for 3 hours rescued NMDAR FRET efficiency of CT100 expressing neurons. N > 600 spines per condition; * $p < 0.05$, unpaired t-test.