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Supplementary infor

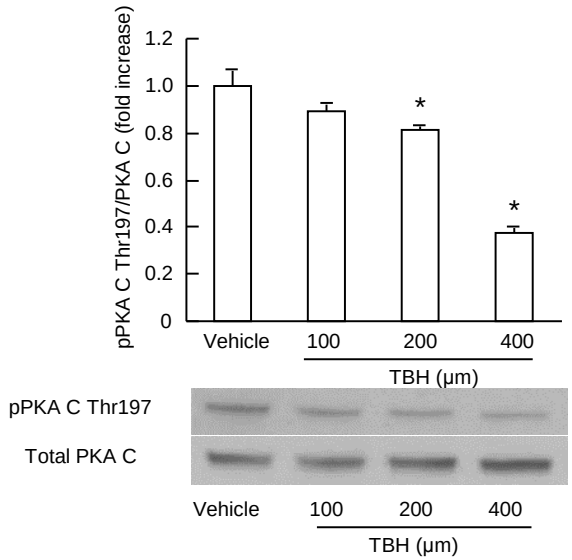

Figure S1: Effects of CypD deficiency on Aβ-induced loss of surface GluR1 cluster.

Surface GluR1 clustering was assessed using nonpermeabilized staining conditions and an antibody recognizing an extracellular epitope of GluR1 (Abcam). Then neurons were fixed in 4% paraformaldehyde for 20 minutes and blocked in 10% goat serum and 0.2% Triton for 30 minutes. Neuronal dendrites were visualized by mouse anti-MAP2 IgG (Boehringer Mannheim) followed by goat anti-mouse IgG conjugated with FITC (Sigma–Aldrich Corp.). GluR1 staining was followed by goat anti-rabbit IgG conjugated with TRICT (Sigma–Aldrich Corp.). Images were taken under a Leica confocal. Post Synaptic density was quantified as the numbers of surface GluR1 positive clusters per micron of dendrite length. After Aβ treatment, GluR1 cluster in nonTg neurons was decreased by 62% in comparison to those in vehicle-treated controls (Fig. S1), while CypD deficiency largely reversed the Aβ-induced decrease in synaptic density (Fig. S1).

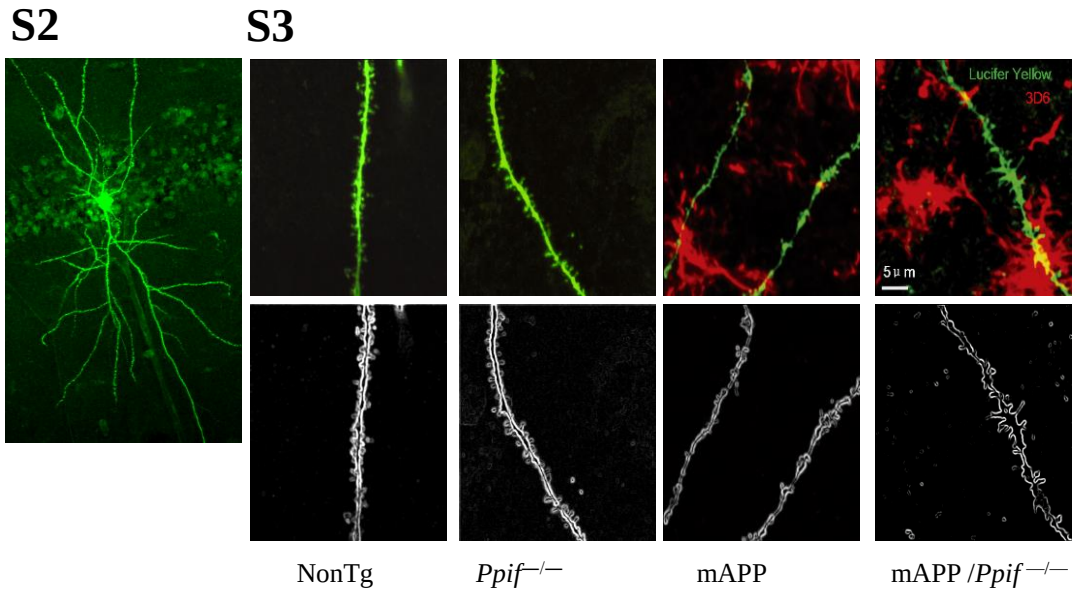

Figure S2. A representative image of a Lucifer yellow stained hippocampal CA1 neuron. Single neuron injection was performed on light fixed brain slices (-2 mm to bregma) as previously described with modification (1). CA1 neurons were filled with 0.1% Lucifer Yellow CH. After injection, brain slices were subjected to anti-Lucifer yellow IgG staining (Sigma) followed by goat anti-rabbit IgG Alexa 488 (Invitrogen). Images were collected under Biorad confocal microscopy.

Figure S3. Enlargements of representative images show CA1 basal dendritic spines and Aβ immunoreactive plaques. Lucifer Yellow stained CA1 neurons were double stained with 3D6 followed by goat anti-mouse IgG Alexa 594 (Invitrogen) to visualize the amyloid beta plaques. The double staining helps to determine the physical proximity of dendritic spines to amyloid beta plaques. Scale bar = 5μm.

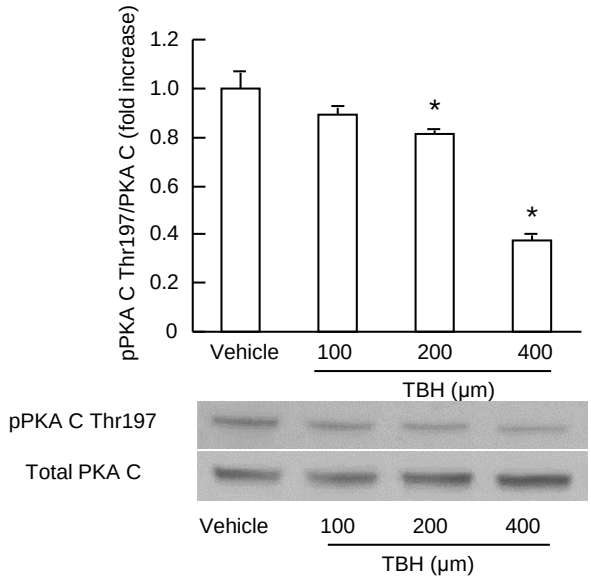

Figure S4. TBH-induced PKAc dephosphorylation in cultured nonTg neurons. We investigated the direct effect of oxidative stress on phosphorylated PKA C (p-PKA C) in neurons. NonTg hippocampal neurons were exposed to tert-butyl hydroperoxide (TBH) at concentrations of 0, 100, 200 and 400 μM for 1 hour, and then subjected to the immunoblotting for PKA C Thr197 phosphorylation. We selected TBH for this study as it is a strong inducer of mitochondrial ROS production, and triggers CypD translocation to mitochondrial inner membrane leading to mPTP formation (2, 3). Indeed, in the presence of TBH, PKA C underwent a dose-dependent decrease in its Thr197 phosphorylation, while there was no change in the levels of total PKA C expression. Levels of p-PKA C relative to the total PKA C demonstrated a substantial decrease ($p<0.05$). These results provide evidence that oxidative stress is a causative factor of PKA C dephosphorylation in neurons.

References:

1. Pace CJ, Tieman DG, & Tieman SB (2002) Intracellular injection in fixed slices: obtaining complete dendritic arbors of large cells. (Translated from eng) *J Neurosci Methods* 119(1):23-30 (in eng).

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