Supporting Information

De Felice *et al.* 10.1073/pnas.0809158106

SI Text

ADDL and Scrambled Aβ_{1-42} Preparation. ADDLs were prepared from Aβ_{1-42} as previously described (1, 2). Briefly, the peptide was dissolved in HFIP to 1 mM and stored as a dried film at −80 °C after solvent evaporation. The film was resuspended in DMSO to a final concentration of 5 mM, thoroughly vortexed and sonicated for 10 min. The solution was then diluted with ice-cold phenol red-free Ham's F12 medium to 100 μM and left at 4 °C overnight. The solution was centrifuged at 14,000 × *g* for 10 min and the supernatant was collected. Solutions of the scrambled peptide were prepared and treated under conditions identical to those used to obtain ADDLs.

Surface Biotinylation-Based Western Blot Assay. Neuronal cultures treated with vehicle or ADDLs were washed with ice-cold PBS and incubated for 15 min at 4 °C in 1.5 mg/mL of the cell-impermeant sulfo-NHS-SS-biotin reagent. Unreacted biotinylation reagent was quenched and removed by washes with ice-cold TBS. Cultures were harvested with 1% Triton X-100, 0.1% SDS in PBS + a protease inhibitor mixture. Lysates were incubated on ice for 30 min with 5-sec vortexing every 5 min and centrifuged at 10,000 × *g* for 5 min at 4 °C. Protein concentration in the supernatant was determined by the BCA assay. Fifty micrograms of protein (from soluble lysates) were mixed with 50 μL of agarose-immobilized streptavidin or neutravidin at 23 °C under agitation for 1 h. Samples were centrifuged at 5,000 × *g* for 2 min and the supernatants containing the nonbiotinylated (nonsurface) proteins were collected. Pellets containing streptavidin- or neutravidin-conjugated beads were washed with lysis buffer and biotinylated (surface) proteins were eluted from the beads with Laemli sample buffer + 50 mM DTT or 2% β-mercaptoethanol for 30 min at 23 °C. Surface, nonsurface, and total proteins were analyzed by Western blot using anti-IRα or anti-Na^+,K^+-ATPase antibodies. In a parallel set of experiments, total biotinylated surface proteins from control or ADDL-treated cultures were determined by dot immunoblots probed with HRP-conjugated streptavidin.

Oxidative Stress. Formation of reactive oxygen species (ROS) was evaluated in live neurons using dihydroethidium (DHE), a fluorescent probe sensitive to superoxide anion formation. Cultures were incubated in neurobasal medium without phenol red for 4 h at 37 °C with vehicle, ADDLs, or insulin + ADDLs and were immediately imaged. DHE fluorescence was analyzed using National Institutes of Health (NIH) Image J software (3) as previously described (4). Sixteen images were analyzed in each experimental condition (carried out in quadruplicate from 2 independent experiments) and were combined to allow quantitative estimates of changes in neuronal ROS levels.

Data Analysis. IR and ADDL binding immunofluorescence intensities were analyzed in 3–6 experiments (see figure legends) using independent neuronal cultures. Twenty to 30 images were acquired in each experimental condition per experiment. Histogram analysis of fluorescence intensities at each pixel across the images was performed using NIH Image J (3) as described (4). Cell bodies were digitally removed from the images so that only ADDL or IR immunostaining on dendritic processes was quantified.

1. Gong Y, *et al.* (2003) Alzheimer's disease-affected brain: presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc Natl Acad Sci USA* 100:10417–10422.
2. Lambert MP, *et al.* (1998) Diffusible, nonfibrillar ligands derived from A beta1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA* 95:6448–6453.
3. Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with Image J. *Biophoton Int* 11:36–42.
4. De Felice FG, *et al.* (2007) A beta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem* 282:11590–11601.

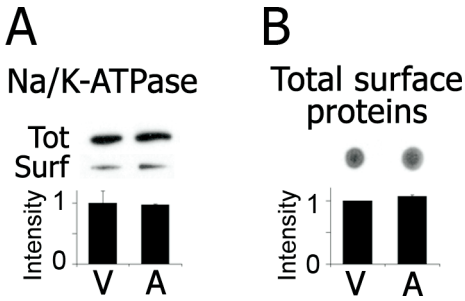

Fig. S1. Levels of surface-exposed membrane proteins in ADDL-treated neurons. (*Left*) Surface abundance of Na^+/K^+-ATPase in hippocampal neurons exposed to vehicle (V) or 100 nM ADDLs (A) for 3 h, assessed by surface biotinylation (see *Methods*) followed by probing with anti-Na/K-ATPase antibody. Total and surface-exposed Na^+/K^+-ATPase levels were determined by densitometry using NIH Image J. (*Right*) Overall abundance of surface-exposed proteins in hippocampal neurons exposed to vehicle (V) or 100 nM ADDLs (A) for 3 h, assessed by surface biotinylation (see *Methods*) followed by probing using HRP-conjugated streptavidin. Intensities were determined by densitometric analysis of dot blots.

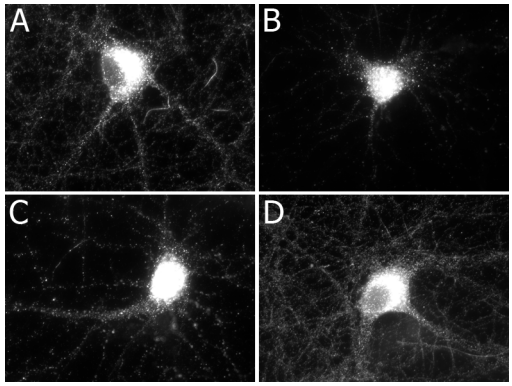

Fig. S2. CK2 and CaMKII mediate ADDL-induced loss of insulin and NMDA receptors. (*A–D*) Representative images of IRα labeling in hippocampal neurons treated for 3 h with vehicle (*A*), 100 nM ADDLs (*B*), 100 nM ADDLs + 5 μM KN93 (*C*), or 100 nM ADDLs + 10 μM DMAT (*D*).

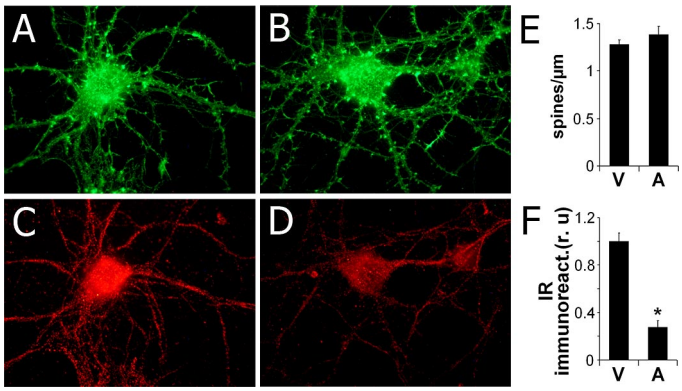

Fig. S3. Lack of overall impact on synapses by short-term exposure to ADDLs. (*A* and *B*) Representative images from hippocampal neurons treated with vehicle (*A*) or 100 nM ADDLs (*B*) for 3 h. Spines were labeled using phalloidin (green) in tandem with the IR antibody (red). (*C* and *D*) IRα labeling in the same neurons as in *A* and *B*, respectively. (*E* and *F*) Quantitation of spine numbers per unit dendrite length (*E*) and integrated IRα immunofluorescence intensities (*F*). Asterisk indicates statistically significant (*, $P < 0.001$) difference relative to vehicle-treated cultures. Note that short-term exposure to ADDLs eliminates dendritic insulin receptors in the absence of spine loss.