Supplementary experimental procedures

Neurobehavioral examination of subjects

The cases included represent subjects who have come to autopsy between 1985 and 2006 with either a clinical diagnosis of probable or possible AD, or normal controls. Mean scores are reported for three commonly used measures of global cognitive status: the Blessed Information-Memory-Concentration (BIMC), the MMSE and the Clinical Dementia Rating (CDR) score. Most of the patients had received the comprehensive neuropsychological battery administered through the University of California, San Diego Alzheimer's disease Research Center (UCSD ADRC) as part of their structured annual evaluation [1]. The MCI cases displayed symptoms of memory or other cognitive decline, and detailed psychometric testing demonstrated mild deficits in one or more areas of cognition, but the cognitive and functional deficits were not severe enough to meet a clinical definition of dementia [2]. Only subjects that received cognitive testing within 12 months of death were included in the analyses.

Neuropathological examination

For each case, paraffin sections from 10% buffered formalin-fixed neocortical, limbic and subcortical material were stained with haematoxylin and eosin (H&E), and thioflavine-S; then the sections were used for routine neuropathological analysis. Analyses included assessment of plaque and tangle density in the neocortex and hippocampus [3, 4] and Braak Stage [5]. All cases met the Consortium to Establish a Registry for AD (CERAD) and National Institute of Aging (NIA) criteria for diagnosis, and displayed neuritic plaques and tangle formation in the neocortex and limbic system [6, 7].

Immunoblot analysis

Samples (20µg) were loaded onto a 4-12% Bis-Tris gel (Invitrogen, Carlsbad, CA) and run with NuPAGE MES SDS running buffer (Invitrogen) at 200V for 45 minutes on ice. Gels were transferred to a 0.2µm nitrocellulose membrane, in transfer buffer (1L Tris Glycine buffer containing 20% methanol) at 400mA for 1.5 hours. Membranes were boiled in phosphate-buffered saline for 5 minutes, blocked with 3%

bovine serum albumin in Tris-buffered saline and 0.05% Tween 20 (TBS/T) for one hour, and incubated overnight at 4°C with either the specific Aβ antibodies or synaptic protein antibodies. Membranes were washed in Tris-buffered saline and 0.05% Tween 20 (TBS/T) for one hour, and incubated in secondary antibody for one hour at room temperature. Membranes were washed for 30 minutes in TBS/T and washed for five minutes in Tris-buffered saline (TBS). Detection was carried out with Western Lightning Chemiluminescence Reagent Plus (PerkinElmer, Waltham, MA) and visualized by enhanced chemiluminescence. Membranes were analyzed with a Versadoc XL imaging apparatus (BioRad, Hercules, CA). Analysis of actin levels was used as a loading control.

Primary neuronal cultures

Hippocampal neuron cultures were prepared from P1 mouse hippocampi. Briefly, for immunostaining experiments, mouse hippocampi were dissected in HBSS dissecting media containing 4 mM $NaHCO_3$ (7.5%) and 10mM HEPES buffer. Neurons were then dissociated by enzymatic treatment with 0.25% trypsin in dissecting media for 15 min at 37ºC, and subsequent mechanical disruption. Neurons were plated at medium density (45,000 cells/cm^2) on poly-L-lysine coated coverslips (12mm in diameter) in MEM plating media containing 1mM Sodium Pyruvate, 0.6% glucose, 10% horse serum, 2mM glutamine. Cultures were placed in an incubator at 37°C under a humidified atmosphere of 5% CO_2 in air for 2 hours. The plating culture medium was then replaced and cultures were maintained in B27 supplemented Neurobasal media (Invitrogen) until 12 days *in vitro* (DIV).

Multidimensional Protein Identification Technology (MudPIT) and LTQ

The protein digest was pressure-loaded onto a 250--µm i.d capillary packed with 2.5cm of 10-µm Jupiter C18 resin (Phenomenex, Torrance, CA, USA) followed by an additional 2.5cm of 5-µm Partisphere strong cation exchanger (Whatman, Clifton, NJ). The column was washed with buffer containing 95% water, 5% acetonitrile, and 0.1% formic acid. After washing, a 100--µm i.d capillary with a 5-µm pulled tip packed with 15 cm 4-µm Jupiter C18 resin (Phenomenex, Torrance, CA, USA) was attached to the

filter union and the entire split-column (desalting column–filter union–analytical column) was placed inline with an Agilent 1100 quaternary HPLC (Palo Alto, CA) and analyzed using a modified 5-step separation described previously [8]. The buffer solutions used were 5% acetonitrile/0.1% formic acid (buffer A), 80% acetonitrile/0.1% formic acid (buffer B), and 500 mM ammonium acetate/5% acetonitrile/0.1% formic acid (buffer C). Step 1 consisted of a 75 min gradient from 0-100% buffer B. Steps 2-5 had a similar profile except 3 min of 100% buffer A, 5 min of X% buffer C, a 10 min gradient from 0-15% buffer B, and a 102 min gradient from 15-45% buffer B. The 5 min buffer C percentages (X) were 10, 40, 60, 100% respectively for the 5-step analysis. As peptides eluted from the microcapillary column, they were electrosprayed directly into an LTQ mass spectrometer (ThermoFinnigan, Palo Alto, CA) with the application of a distal 2.4 kV spray voltage. A cycle of one full-scan mass spectrum (400-1400 m/z) followed by 3 data-dependent MS/MS spectra at a 35% normalized collision energy was repeated continuously throughout each step of the multidimensional separation. Application of mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcaliber datasystem.

As peptides eluted from the microcapillary column, they were electrosprayed directly into an LTQ 2-dimensional ion trap mass spectrometer (ThermoFinnigan, Palo Alto, CA) with the application of a distal 2.4 kV spray voltage. A cycle of one full-scan mass spectrum (400-1400 m/z) followed by 8 data-dependent MS/MS spectra at a 35% normalized collision energy was repeated continuously throughout each step of the multidimensional separation. Application of mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcalibur datasystem.

Analysis of Tandem Mass Spectra

MS/MS spectra were analyzed using the following software analysis protocol. Poor quality spectra were removed from the dataset using an automated spectral quality assessment algorithm [9]. MS/MS spectra remaining after filtering were searched with the ProLuCID algorithm against the immunoprecipitated Aβ transgene and for Aβ interacting proteins concatenated to a decoy databases in which the sequence for

each entry in the original database was reversed [10]. All searches were parallelized and performed on a Beowulf computer cluster consisting of 100 1.2 GHz Athlon CPUs [11]. Only peptides with at least 1 tryptic termini were considered. Searches were performed with Cystein carbamidomethylation as a fixed modification.

ProLuCID [12] results were assembled and filtered using the DTASelect (version 2.0) program [13]. DTASelect 2.0 uses a linear discriminant analysis to dynamically set XCorr and DeltaCN thresholds for the entire dataset to achieve a user-specified false positive rate (2.5% in this analysis). False positive rates are estimated by the program from the number and quality of spectral matches to the decoy database.

Supplementary references

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Supplementary figure legends

Fig. S1. Linear regression analysis between levels of Ab dimers, synaptic proteins and dementia scores. *A & B.* Increased Aβ dimer levels correlated with increased Blessed score (A) and Braak stage (B). *C & D.* Higher levels of Aβ dimer correlated with reduced levels of VAMP2 (C) and PSD95 (D). *E & F.* Higher Blessed score (E) and Braak stage (F) correlated with reduced levels of PSD95.

Fig. S2. Comparative immunoblot analysis for APP/Aβ in the cortex of APP tg mice. Samples were fractioned into membrane and cytosolic fractions and probed with anti-Aβ antibodies (82E1 and 6E10). *A & B.* In samples homogenized using Buffer A, compared to nontg controls, in APP tg samples multiple bands representing Aβ monomers and multimers were identified at molecular weights ranging from 4 to 28 kDa band in the membrane fraction. *C & D.* In samples homogenized using Buffer B, compared to nontg mice, in APP tg samples the majority of the Aβ was identified as a 4 kDa band in the membrane fraction.

Fig. S3. Double immunolabeling analysis for MAP2 and PSD95 in primary neuronal cultures treated with conditioned media containing Aβ oligomers. Hippocampal neuronal cells from P1 mice were treated for 6 or 24 hrs with conditioned media from APP-expressing CHO cells (80 pM, a sublethal dose). Fixed coverslips were immunolabeled with antibodies against MAP2 (green channel) and PSD95 (red channel) and analyzed with a laser scanning confocal microscope. All images are from cells treated for 24 hrs; the graph represents data from both 6 and 24 hr timepoints. *A & B.* Confocal images showing neurons after 24 hrs of treatment with vehicle (A) or Aβ (B). Compared to vehicle-treated cells, Aβ treatment resulted in a reduction in PSD95-positive punctae along the dendrites. *C.* Analysis of levels of PSD95 and MAP2-immunoreactive structures after 6 and 24 hrs of treatment with vehicle or Aβ. *D & E.* Confocal images at higher power showing the detail of MAP2-labeled dendritic branches and PSD95-immunoreactive punctae (arrows) along the dendrites. *F.* Cell death in the 24 hrs following treatment with vehicle or Aβ was assessed using the lactate dehydrogenase (LDH) assay.

Scale bar in panel *B* equals 20 μm for panels *A & B*; scale bar in panel *E* equals 10 μm for panels *D & E*. *P<0.05 compared to vehicle-treated controls by unpaired, two-tailed Student's t-test.

Supplementary figures

Fig. S1.

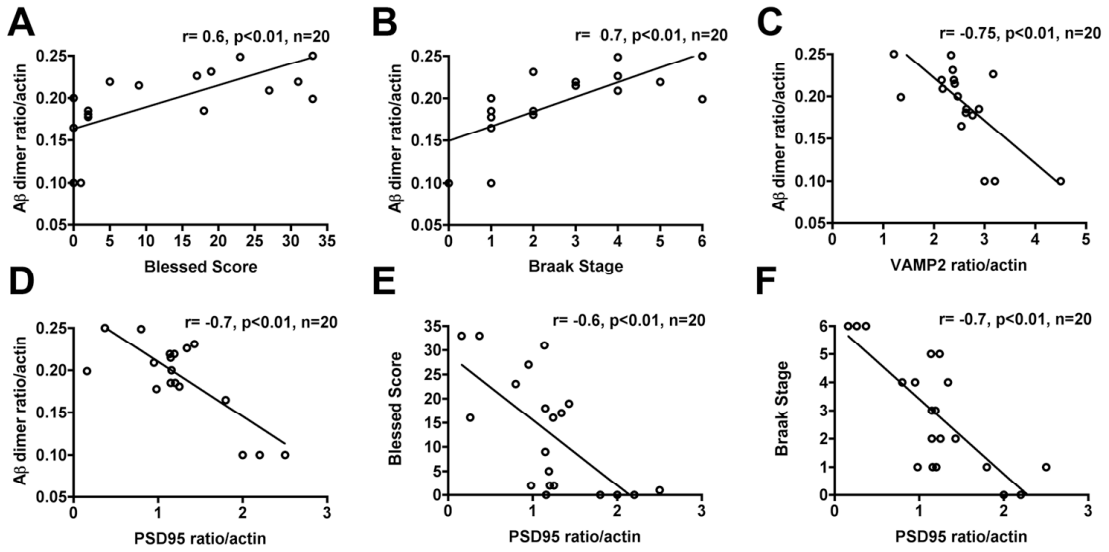


Fig. S2.

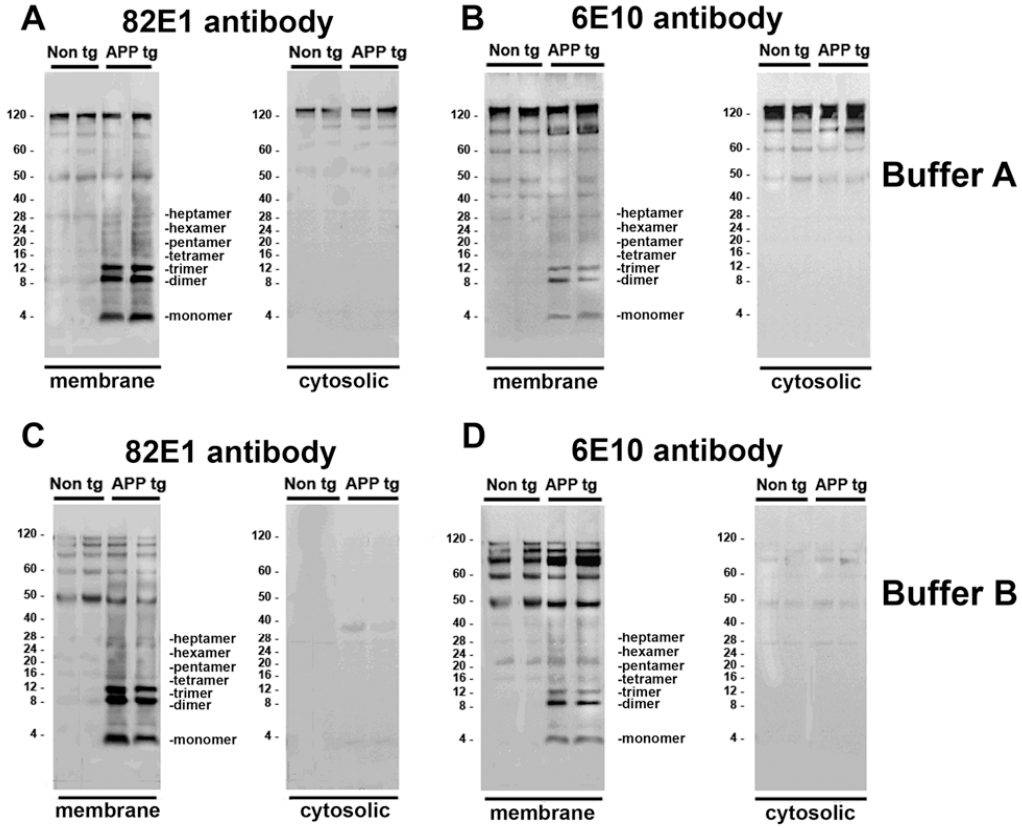
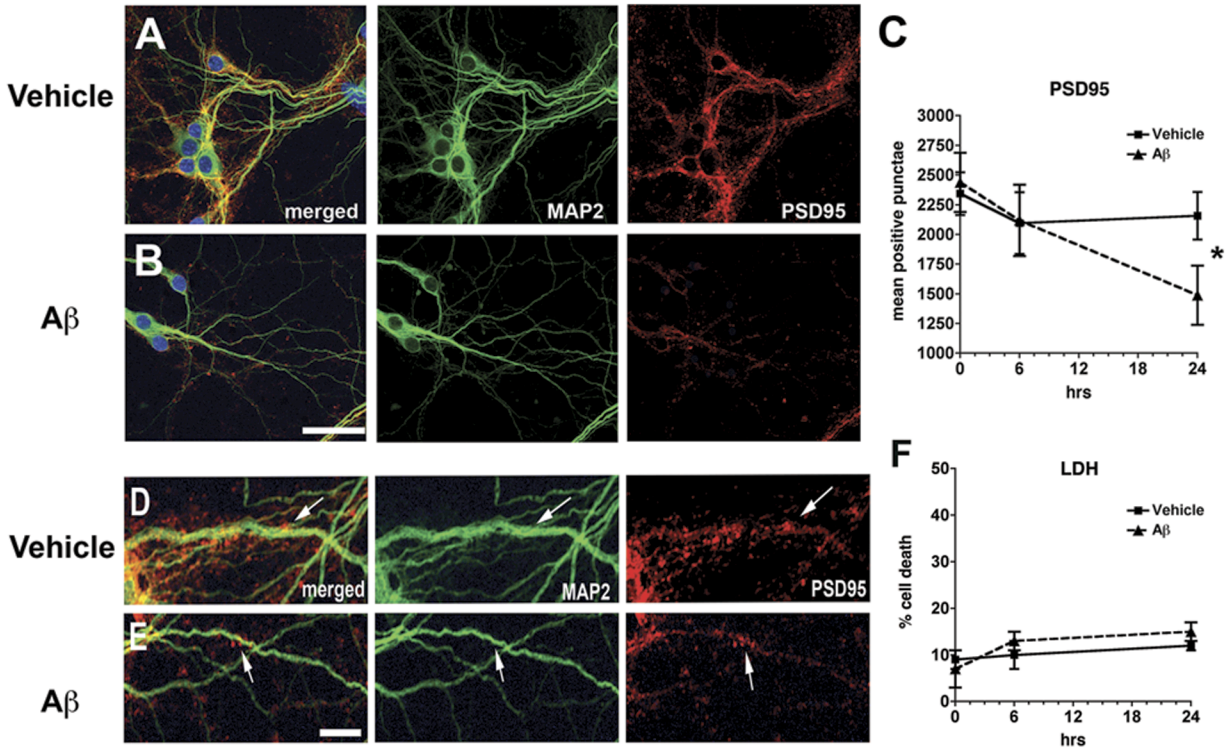


Fig. S3.


A
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MAP2
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MAP2
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