Supporting Information

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SI Materials and Methods

Human Brain Sample Preparation. Frozen human cerebral cortices were provided by C. Lemere (Brigham and Women's Hospital/Harvard Medical School) or M. Frosch (Massachusetts General Hospital/Harvard Medical School) under Institutional Review Board-approved human studies protocols and by M. Farrell (Beaumont Hospital, Dublin, Ireland) in accord with local Ethics Committee guidelines and Ethical Review Committee/Institutional Review Board approval. Samples of temporal or frontal cortex containing white and gray matter were weighed. Freshly prepared, ice cold Tris-buffered saline (TBS) consisting of 20 mM Tris-HCl, 150 mM NaCl, pH 7.4, was added to the frozen cortex at 4:1 (TBS volume:brain wet weight) and homogenized with 25 strokes at a setting of 10 on a mechanical Dounce homogenizer. The homogenate was spun at 175,000 × *g* in a TLA100.2 rotor on a Beckman TL 100. The supernatant (called TBS extract) was aliquoted and stored at −80 °C.

Immunoprecipitation/Western Blot Analysis of Aβ. We used an immunoprecipitation/Western blot protocol described previously (1, 2) to detect Aβ in the TBS extracts or neuronal culture medium. Samples were immunoprecipitated with either polyclonal Aβ antiserum AW7 (1:50) and Protein A Sepharose (PAS; Sigma) or monoclonal Aβ antibodies 3D6 (3 μg/mL, gift of Elan Corporation, plc) or 82E1 (3 μg/mL, IBL) and Protein G agarose (PGA; Roche) plus PAS. After the beads were washed, the immunoprecipitates were eluted with 10 μL 4% lithium dodecyl sulfate (LDS) sample buffer, heated at 65 °C for 5 min, and centrifuged at 18,500 × *g* for 5 min. The supernatant was electrophoresed on a 26-well 4 to 12% bis-Tris gel using Mes running buffer (Invitrogen). Proteins were transferred to 0.2 μm nitrocellulose and Western blotted for Aβ with 1 μg/mL each of 6E10 (Covance) + 2G3 + 21F12 (gifts of Elan Corporation, plc) using the LiCor Odyssey Infrared Imaging System.

Immunoprecipitation-Size Exclusion Chromatography. TBS extracts of AD or control cortex were immunoprecipitated with 3D6 (3 μg/mL), 15 μL PAS, and 15 μL PGA. After the beads were washed, the immunoprecipitates were eluted with 10 μL 4% LDS sample buffer, heated at 65 °C for 5 min and centrifuged at 18,500 × *g* for 5 min. The supernatant was transferred to 500 μL TBS and injected onto a Superdex 75 (10/30 HR) column (Amersham Biosciences) and eluted at a flow rate of 0.8 mL/min into 1-mL size exclusion chromatography (SEC) fractions using 50 mM ammonium acetate, pH 8.5. Next, 750 μL of each fraction were removed and stored at −80 °C. The remaining 250 μL were lyophilized, reconstituted in 15 μL of 2× LDS sample buffer, heated at 65 °C for 5 min, and used for Western blot analysis. Soluble Aβ monomer-rich or dimer-rich SEC fractions from AD-brain TBS extracts (AD-TBS) and the corresponding fractions from control brain extracts (Cont-TBS) were pooled separately and lyophilized before addition to the culture medium of primary cultured hippocampal neurons.

Production and Characterization of Cross-Linked Synthetic Dimers. Aβ40 S26C was synthesized by the Biopolymer Laboratory at the University of California Los Angeles Medical School and the correct sequence and purity confirmed by amino acid analysis, reverse-phase HPLC and mass spectrometry (1). Disulfide-bonded Aβ dimers were generated by atmospheric oxidation of a 20-μM solution of Aβ40 S26C in 20 mM ammonium bicarbonate, pH 8.0, for 4 d at room temperature. To facilitate disassembly of aggregates formed during the oxidation reaction, the peptide solution was lyophilized and subsequently incubated in 5 M GuHCl, 50 mM Tris-HCl, pH 8.0, for 4 h. Disulfide crossed-linked Aβ dimers were separated from unreacted monomer and higher aggregates by SEC. Briefly, two Superdex 75 10/30 HR columns were linked in series and eluted with 50 mM ammonium acetate, pH 8.5, at a flow rate of 0.5 mL/min. Fractions (0.5 mL) were collected, and an aliquot of each was electrophoresed on 16% Tris-tricine polyacrylamide gels and detected by silver staining. Fractions found to contain exclusively dimeric Aβ were pooled and the peptide content determined by comparison with known standards. Samples were stored at −80 °C until use.

Hippocampal Neuronal Cultures. Primary hippocampal cultures were generated from E18 Sprague-Dawley rat embryos. The hippocampus was dissected out in Hank's Balanced Salt Solution buffered with Hepes and dissociated with 0.125% trypsin (Invitrogen) for 15 min at 37 °C, followed by trituration. Dissociated cells were plated at a density of 1.5×10^5 cells/cm^2 in six-well plates precoated with poly-D lysine (100 μg/mL), or 1×10^4 cells/cm^2 on 24-well plates with coverslips coated with poly-D lysine (100 μg/mL). After 4 d culturing in Neurobasal medium with B-27 supplement (Invitrogen) and glutamax, cytosine arabinofuranoside was added to reduce glial proliferation. Half the medium was exchanged every 4 d. The treatment of the hippocampal neurons with Aβ oligomers was begun on various days (7, 14, or 18) after plating, and neurons were fixed for immunostaining and confocal microscopy 1, 2, or 3 d after starting treatment.

DNA Constructs, Lentiviral Generation, and Infection. The pcMV-hTau-EYFP construct was generously provided by George Bloom (University of Virginia). Lentiviral vectors were generated by inserting EGFP or Tau-EYFP cDNAs into pCDH1 (System Biosciences). Lentiviral RNAi constructs were made with the pLenti6/BLOCK-iT-DEST Gateway Vector Kit (Invitrogen). The target sequences were as follows (5′ to 3′): Cont-RNAi: AGGATAGAGTCCAGTCGAA, Tau-RNAi1: GGACAGGAAATGACGAGAA; Tau-RNAi2: AGGATAGAGTCCAGTCGAA. The pCDH1 lentiviral constructs were cotransfected with the helper plasmids δ8.9 and VSV-G; pLenti6 RNAi constructs were cotransfected with ViraPower Packaging Mix (Invitrogen) into 293-FT cells (Invitrogen) using Fugene6 transfection reagent (Roche). Conditioned media were collected 72 h later, centrifuged at 500 × *g* for 5 min to remove suspended cells, and stored at −80 °C. Four hours after plating, hippocampal neurons were infected with lentiviral conditioned medium overnight, then washed with Neurobasal medium with B-27 supplement.

Western Blotting of Cell Lysates. Various cultures in six-well plates were lysed in 0.2 mL lysis buffer [0.1% SDS, 1% Nonidet P-40, 50 mM Hepes, pH 7.4, 2 mM EDTA, 100 mM NaCl, 5 mM Na_3VO_4, 40 μM p-nitrophenyl phosphate, and 1% protease inhibitor mixture set I (Calbiochem)]. The lysates were centrifuged at 13,500 × *g* for 25 min. The supernatants were collected and denatured. The concentration of protein was determined by BCA assay. Twenty micrograms of total protein was loaded in each lane, separated by 4 to 12% SDS/PAGE and blotted onto nitrocellulose membrane. The blot was blocked for 1 h at room temperature, followed by incubation overnight at 4 °C with mouse monoclonal antibodies AT8, AT180, AT270 (Thermo Scientific), 12E8 (gift of E. Mandelkow, Max Planck Unit for Structural Molecular Biology, Hamburg, Germany), Tau1 (Chemicon), GSK3β (Thermo Sci-

entific), GAPDH (Chemicon), or rabbit polyclonal antibodies PHF-1 (Invitrogen), Tau 22690, Tau 39524 (Abcam), Tau K9JA (DAKO), GFP (Molecular Probes), or phospho-GSK3β (Ser-9; Cell Signaling). Membranes were rinsed and incubated for 1 h with fluorescent-conjugated goat anti-rabbit or mouse IgG (1:5,000; Invitrogen). Blots were scanned using the LiCor Odyssey Infrared Imaging System. Intensity of bands was measured by LiCor Odyssey software.

Immunocytochemistry, TUNEL Staining, and Confocal Microscopy. Hippocampal neurons cultured for 18 d were treated under various conditions as specified in the text. After treatment, cells were fixed in cold methanol for 15 min. Neurons were rinsed three times with PBS. After blocking in 5% BSA in PBS, neurons were incubated with rabbit antibody Tau K9JA (DAKO; 1:1,000) and mouse antibodies α-Tubulin (Sigma; 1:1,000), AT8 (Thermo Scientific; 1:200) or 12E8 (1:1,000) at 4 °C overnight. After rinsing with PBS three times, cells were incubated with Alexa-fluor 633 goat anti-rabbit IgG and Alexa-fluor 546 goat anti-mouse IgG (Invitrogen; 1:1,000) at 4 °C overnight. After rinsing three times with PBS, coverslips were placed with mounting medium (Southern Biotech). TUNEL staining was performed using Click-iT TUNEL Alexa Fluor488 kit (Invitrogen). Confocal microscopy was performed on a Zeiss LSM510 microscope. We used a 20×/0.75 objective and scanned the samples in a *z*-stack manner (three stacks, 2-μm interval). Neurons under different conditions were photographically captured in a random manner.

Quantification of Neuritic Degeneration. We used Imaris software to process the *z*-stack images of neurons and measure the total length of Tubulin-positive neurites and the number of tau-positive beads automatically in unbiased fashion. For each condition, at least six microscopic fields from two independent experiments were quantified. The data were expressed as the average density of tau-positive beads along 100-μm lengths of the neurites. Error bars equal SEM. Statistical significance was calculated by Student *t* test.

1. Shankar GM, et al. (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14:837–842.

2. Walsh DM, et al. (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416:535–539.

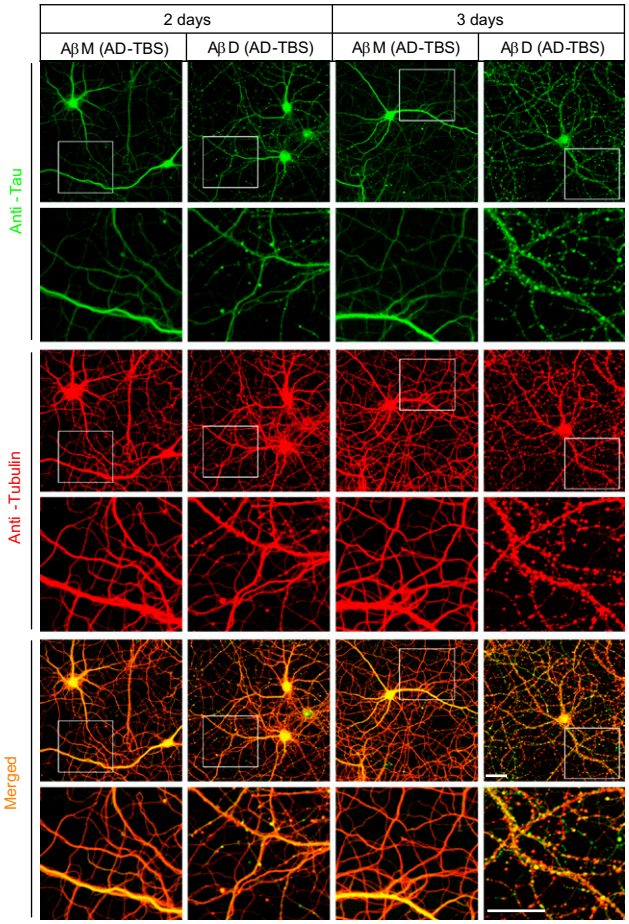

Fig. S1. Higher-power confocal images showing the tau (green) and Tubulin (red) immunoreactivities of the cytoskeleton of hippocampal neurons [days in vitro (DIV) 20 or 21] after 2- or 3-d treatment with Aβ monomers (M) or dimers (D) isolated by SEC from AD-TBS extracts. Regions in the white boxes are shown immediately below at higher magnification. (Scale bar, 50 μm.)

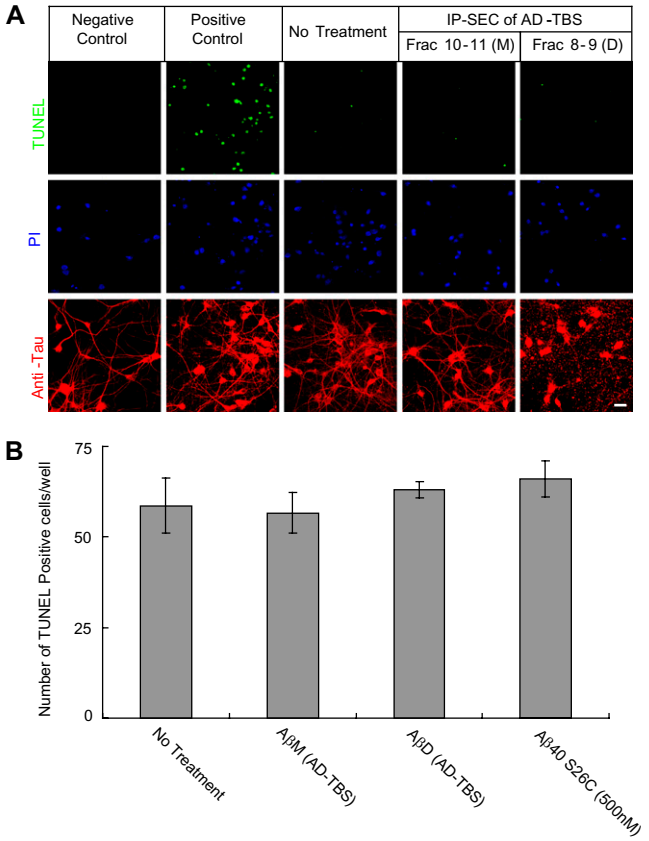

Fig. S2. Soluble Aβ oligomers did not induce significant neuronal apoptosis after 3-d treatment. (*A*) Confocal images showing TUNEL (green), propidium iodide (PI) (blue), and the tau-reactive microtubule cytoskeleton (red) of primary hippocampal neurons (DIV21) after 3-d treatment with SEC fraction 10–11 (M, monomers) or fraction 8–9 (D, dimers) from AD-TBS. Neurons without dUTP incorporation served as negative control, and neurons treated with DNase I after fixation served as positive control. (Scale bar, 50 μm.) (*B*) Histograms of the average numbers of TUNEL-positive cells in each well (~4×10^4 neurons) after the indicated treatments. Means from three independent experiments; error bars, SEM.

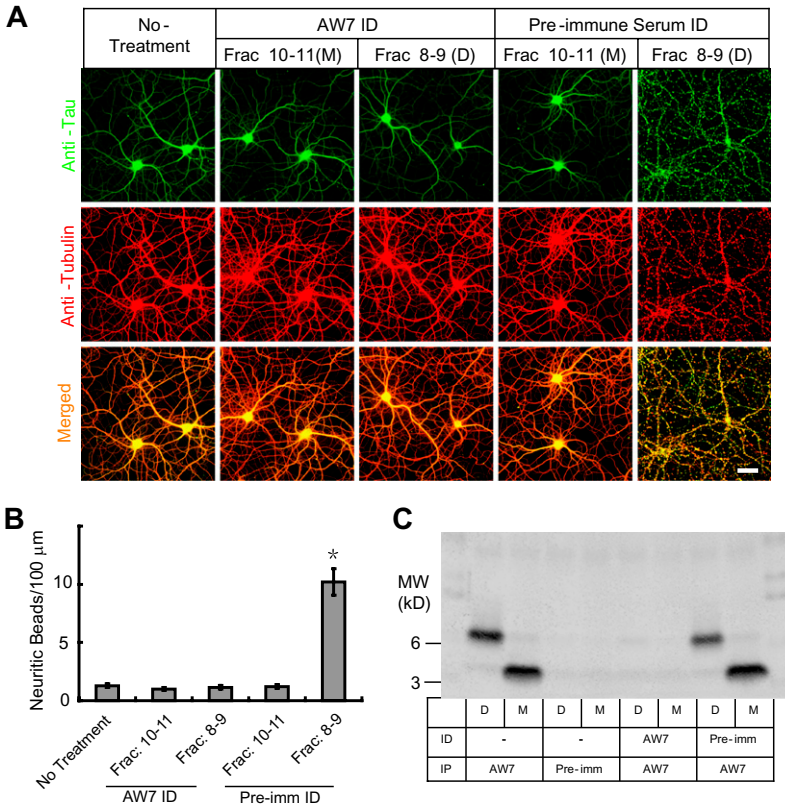

Fig. S3. Immunodepletion (ID) of Aβ dimers prevents neuritic disruption of hippocampal neurons. (*A*) Confocal images showing the tau-reactive (green) and microtubule-reactive (red) cytoskeleton of primary hippocampal neurons (DIV21) after 3-d exposure to Aβ monomers (SEC fractions 10–11, M) or Aβ dimers (SEC fractions 8–9, D) that had been immunodepleted with either AW7 or Preimm. (Scale bar, 50 μm.) (*B*) Histograms represent the average number of tau-positive beads along 100-μm lengths of Tubulin-positive neurites under different conditions. Asterisk indicates data significantly different from those of neurons without treatment ($P < 0.01$ by Student *t* test). Error bars, SEM. (*C*) SEC fractions enriched in Aβ dimers (D) or monomers (M) were immunodepleted (ID) with Aβ antiserum AW7 or its preimmune serum (Preimm) or not immunodepleted (–). After the immunodepletion, the respective supernates were immunoprecipitated with AW7 to visualize any remaining Aβ species.

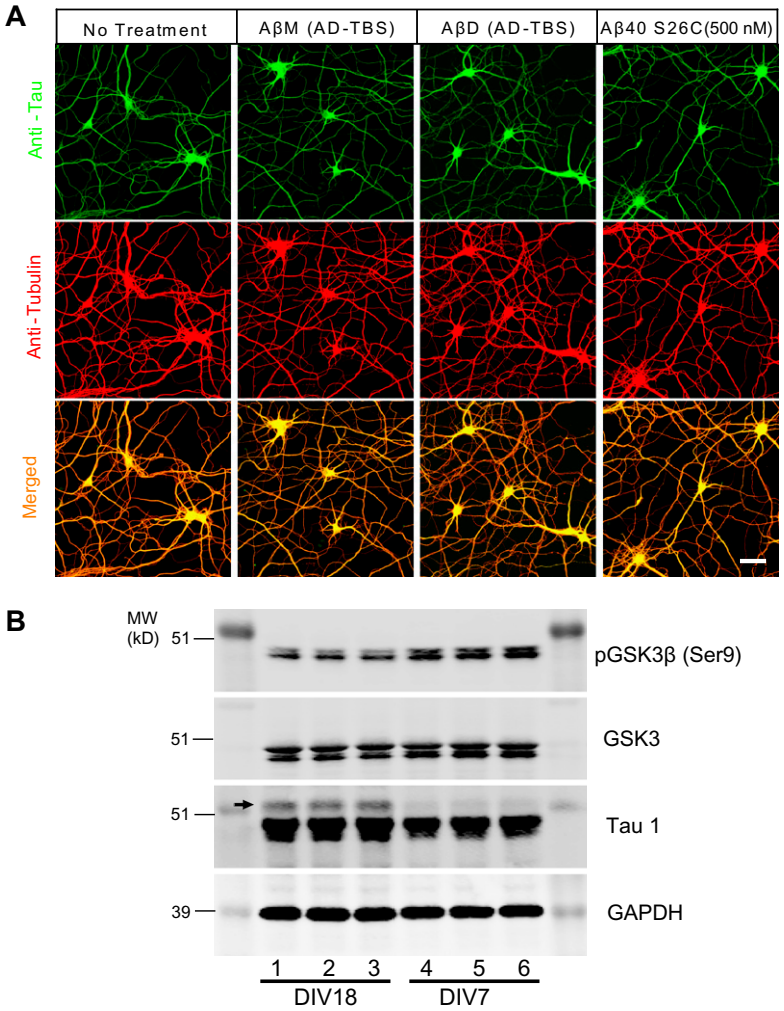

Fig. S4. (*A*) Confocal images showing the tau (green) and tubulin (red) immunoreactivities of the cytoskeleton of hippocampal neurons (DIV10) after 3-d treatment with Aβ monomers (SEC fractions 10–11) or Aβ dimers (SEC fractions 8–9) isolated from AD-TBS, or else with Aβ40 S26C (500 nM). (Scale bar, 50 μm.) (*B*) Representative Western blots showing the phosphorylation of GSK3β (at ser 9), expression of total GSK3, and tau in primary cultures of hippocampal neurons cultured for 18 d (DIV18. lanes 1–3) or 7 d (DIV7, lanes 4–6). Arrow indicates a mature form of tau (~55 kDa). Blotting of GAPDH served as a control.

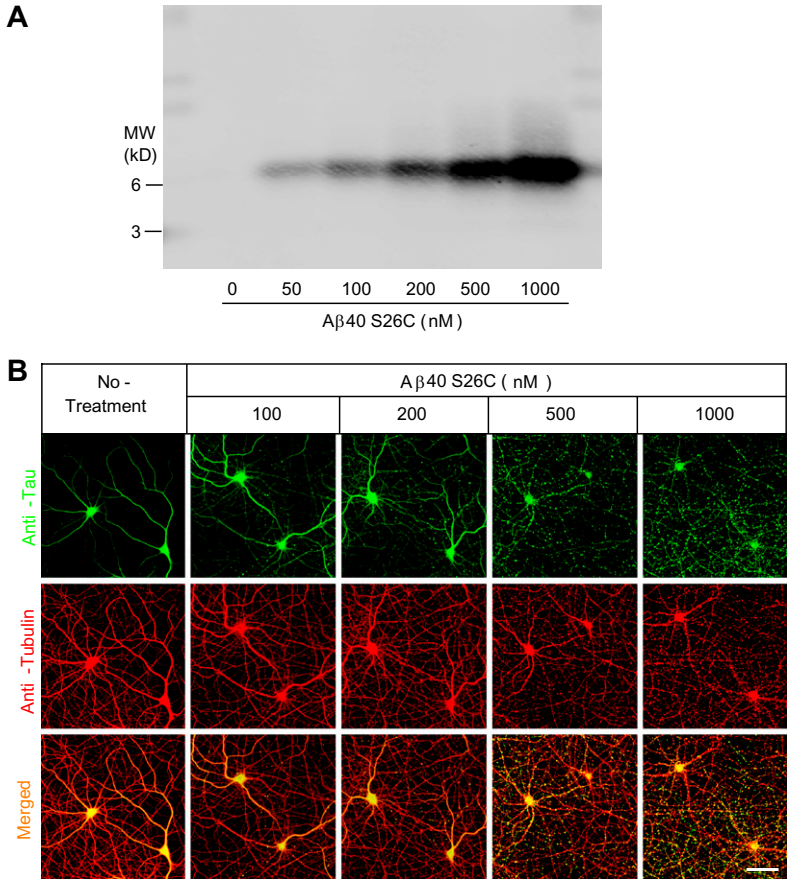

Fig. S5. Disruption of the neuronal cytoskeleton by pure, synthetic dimers of Aβ40. (*A*) Western blotting of culture media (20 μL) containing increasing concentrations (nM) of crosslinked dimers of Aβ40 S26C. (*B*) Confocal images showing the tau-reactive (green) and microtubule-reactive (red) cytoskeleton of primary cultured hippocampal neurons (DIV21) after 3-d treatment with increasing concentrations of Aβ40 S26C dimers. (Scale bar, 50 μm.)

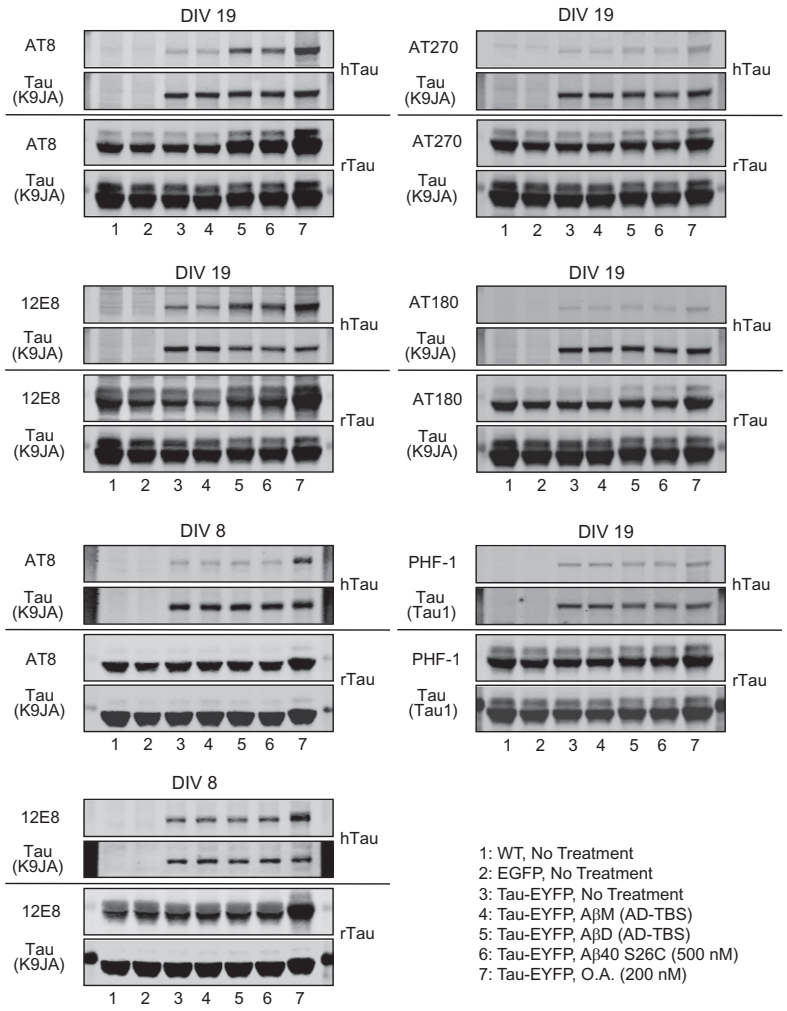

Fig. S6. Representative Western blots showing the phosphorylation of human Tau-EYFP (hTau) and endogenous rat tau (rTau) at different epitopes in primary cultures of hippocampal neurons (DIV19 or DIV8) transduced with or without lentivirus encoding EGFP or hTau-EYFP. Neurons were treated under the indicated conditions (see key) for just 1 d. Western blotting for total tau (K9JA, or Tau1) served as a control. O.A., okadaic acid.

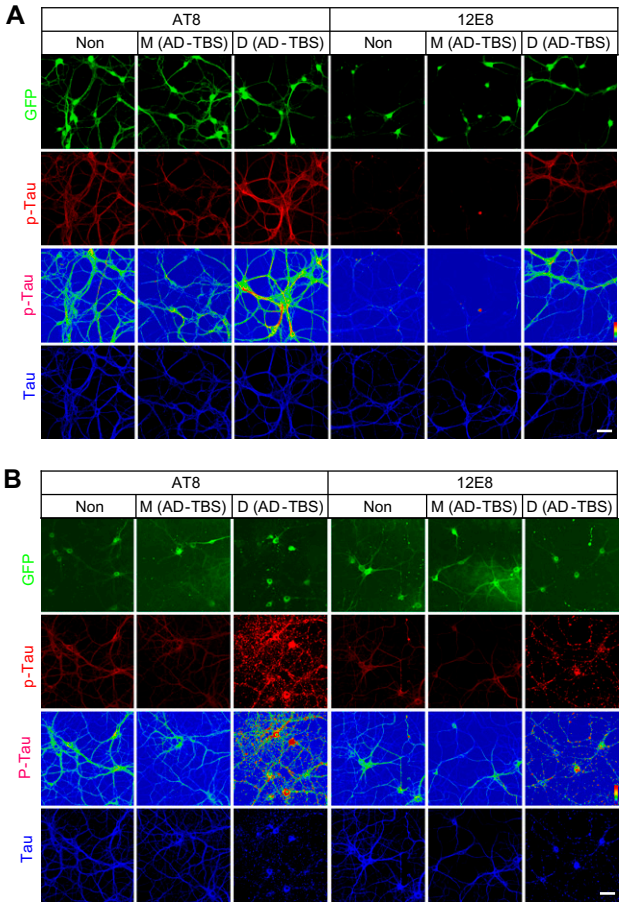

Fig. S7. Confocal images showing GFP fluorescence (green; first row), phospho-tau [red (second row) and pseudocolor image (third row)], and total tau (blue; fourth row) reactivities of the microtubule cytoskeleton in hippocampal neurons transduced with hTau-EYFP (DIV21) after 1-d (*A*) or 2-d (*B*) treatment. Scale bar for the intensity of p-tau staining in the pseudocolored images of the third row is shown at the far right edge of that row. (Scale bars, 50 μm.)

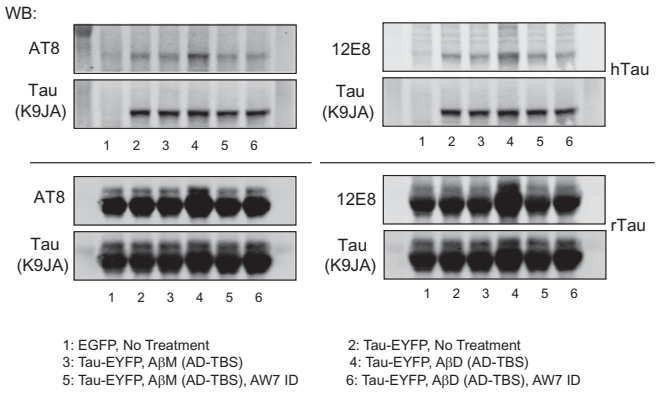

Fig. S8. Representative Western blots showing the phosphorylation of human Tau-EYFP (hTau) and endogenous rat tau (rTau) at the AT8 or 12E8 epitopes in primary hippocampal neurons (DIV18) transduced with lentivirus encoding EGFP or hTau-EYFP. Neurons were treated for 1 d under the conditions indicated in the key. Western blotting of total tau (K9JA) served as a control.