Supplemental Methods

Hybridoma fusions. $(A\beta 1\text{-}40S26C)_2$ immunized animals were sacrificed using CO_2 asphyxiation to avoid introducing anesthetic into the animals bloodstream. The spleen from each animal was aseptically removed and placed in a 100 mm tissue culture dish containing HyClone DMEM/F12 medium with 2.5 mM L-glutamine but without HEPES or serum (medium A, Thermo Fisher Scientific, Logan, UT) pre-warmed to 37 ^{o}C. Lymphocytes were isolated from splenic tissue using an angled and regular forceps. Lymphocytes were separated from red blood cells by lyzing the plasma cells in 20 mM Tris HCl containing 0.14M ammonium chloride, pH 7.2. The purified cells were mixed with a 1.5-fold excess of Sp2/0 cells (American Type Culture Collection, Rockville, MD) in medium A and cells fused by drop-by-drop addition of 50% Hybri-Max™ polyethylene glycol (Kohler and Milstein, 1975; Yokoyama et al., 2006). The resulting hybridomas were diluted to ~2.5 x 10^6 viable cells/ml into medium A containing L-glutamine, HEPES, hybridoma fusion and cloning supplement (Roche Applied Science, Mannheim, Germany), and 5% fetal bovine serum. The cell suspension was plated (~500 µl per well) into eighteen, 24-well tissue culture plates (CELLSTAR®, Greiner Bio-One, Monroe, NC). After 1 day, two drops of a solution of 5 mM hypoxanthine, 0.02 mM aminopterin, and 0.8 mM thymidine (Hybri-Max™ HAT) was added to each well, the cells feed intermittently, and on day 10 hybridoma supernatants were analyzed for anti-$(A\beta 1\text{-}40S26C)_2$ antibodies using microtiter plate-immobilized $(A\beta 1\text{-}40S26C)_2$ in our europium time-resolved fluorescence assay described in the Materials and Methods section. Briefly, hybridoma supernatants were diluted 1:1 with assay buffer and a positive assay signal was defined as twice the mean for all supernatants examined.

Cultured cells and lysates. Chinese hamster ovary (CHO) cells and CHO cells overexpressing APP (1B1), were cultured in 10 cm^2 dishes until nearly confluent in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS, Sigma, St. Louis, MO) and 0.4 mg/mL hygromycin (Invitrogen Corporation, Carlsbad, CA). Whole cell lysates were generated, as described (Walsh et al., 2003)

Immunoprecipitation/Western blot. The ability of murine mAb 3C6 to bind to full-length APP and C-terminal fragments (CTFs) was assessed essentially as done for 3C6 binding to Aβ (see Materials and Methods). Briefly, 450 µl of CHO or 1B1 cell lysate were incubated with 160 µg/ml 3C6 and 35 µg/ml anti-IgM (µ-chain specific; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Murine anti-Aβ N-terminal mAb, 6E10, binding was determined in the same manner using 5 µg/ml 6E10. Immunoprecipitation (IP) of antibody complexes was performed using Protein G sepharose beads (Sigma) and Western blot detection of Aβ run on 10-20% polyacrylamide tris-tricine gels and transferred onto 0.2 µm nitrocellulose (Optitran, Schleicher and Schull, Germany) and Aβ detected by Western blot using 1 µg/ml mAb 6E10 and enhanced chemiluminescence (Pierce; Rockford, IL) (Mc Donald et al., 2010).

Immunodepletion. AD brain TBS extract was depleted of Aβ peptide by 3 sequential rounds of IP using AW7, as described in the Materials and Methods section of the manuscript.

***In vivo* electrophysiology**. *In vivo* studies on urethane anaesthetized male Wistar rats and statistical comparisions were performed as described in the Materials and Methods section of the manuscript.

SI Figure Legends

Supplemental Figure 1 **SDS-PAGE analysis of purified mAbs 3C6 and 4B5.**
Water dialysis and thiophilic affinity chromatography (HiTrap IgM, GE Healthcare) produced highly pure antibody preparations. ~5 μg of total protein were electrophoresed on 12% polyacrylamide tris-glycine gels and proteins visualized by silver staining.

Supplemental Figure 2 **Immunization with (Aβ1-40S26C)$_2$ results in production of a high level of circulating anti-Aβ antibodies.**
Representative antibody titration curves against plate-immobilized Aβ aggregates for two tPA knockout mice immunized with (Aβ1-40S26C)$_2$ (●, □) that had sera titers of ~30,000. Serum from an unimmunized mouse (◊) did not contain antibodies capable of detecting the immobilized peptide.

Supplemental Figure 3 **3C6, but not a control IgM, avidly binds (Aβ1-40S26C)$_2$.**
The antibody binding curves show biotinylated 3C6 (▲) and a control biotinylated IgMκ□ (●), M1520 (Sigma), binding to plate-immobilized (Aβ1-40S26C)$_2$. Binding of biotinylated IgMκ M1520 against plate-immobilized Aβ1-40S26C monomers (○) was also assessed. Studies were carried out at 37^{o}C in PBSA.

Supplemental Figure 4 **The anti-Aβ mAb 3C6 does not bind to APP.** IP/Western blot analysis confirmed that an anti-Aβ N-terminal mAb, 6E10, but not 3C6, recognized full length APP (**A**)

and the C-terminal fragment C99 (**B**) that were present in lysates of the APP over-expressing 1B1 cell line. Western blots were developed using mAb 6E10 and ECL.

Supplemental Figure 5 **The plasticity impairing activity of AD brain extract is mediated by Aβ.** Intracerebroventricular (i.c.v.) injection (*) of AD TBS brain extract inhibited high frequency stimulation (HFS, arrow) induced LTP (100 ± 2 % baseline, n = 5, $p < 0.05$ compared with 135 ± 3 % vehicle injected controls, n = 10), but immunodepleted extract had no effect (131±6% baseline, n=6, $p > 0.05$ compared with vehicle injected controls).

Supplemental Figure 6 **Peptide epitope mapping of anti-Aβ dimer mAbs 3C6 and 4B5.** Antibody binding curves for mAbs 3C6 (A) and 4B5 (B) against plate-immobilized Aβ peptide fragments, full-length WT Aβ1-40, and $(Aβ1\text{-}40S26C)_2$. The symbols represent antibody binding to the different Aβ peptides, as described in Panel C. The binding studies were carried out at 37^{o}C in 1% BSA and PBS containing 0.05% Tween 20, pH 7.4. (C) Schematic of MAbs 3C6 and 4B5 reactivity's against the different Aβ peptides. Stronger antibody binding against a particular peptide is represented by additional '+' symbols.

References

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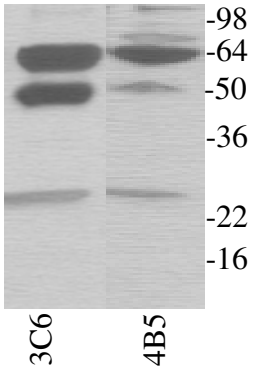
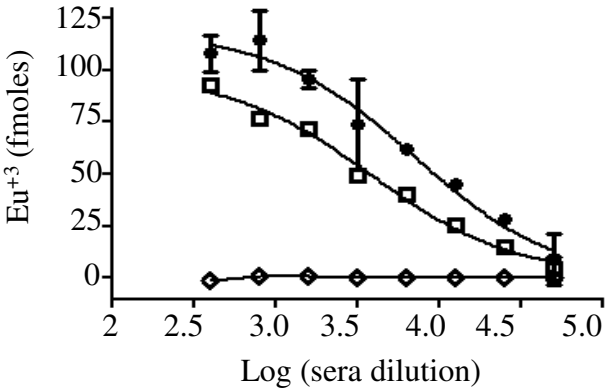
Supplemental Figure 1 O'Nuallain et al.



Supplemental Figure 2 O'Nuallain et al.



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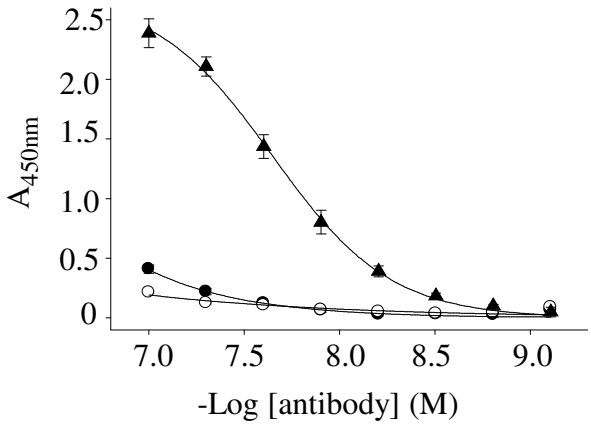

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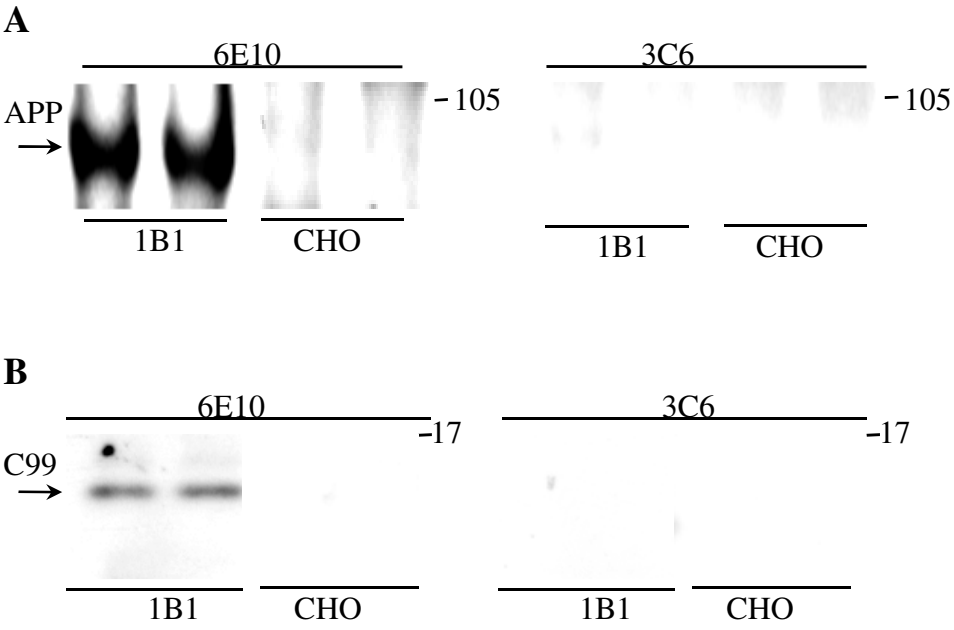
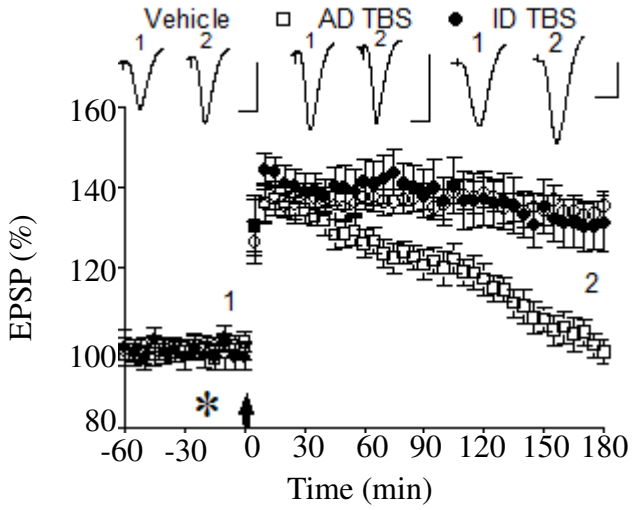
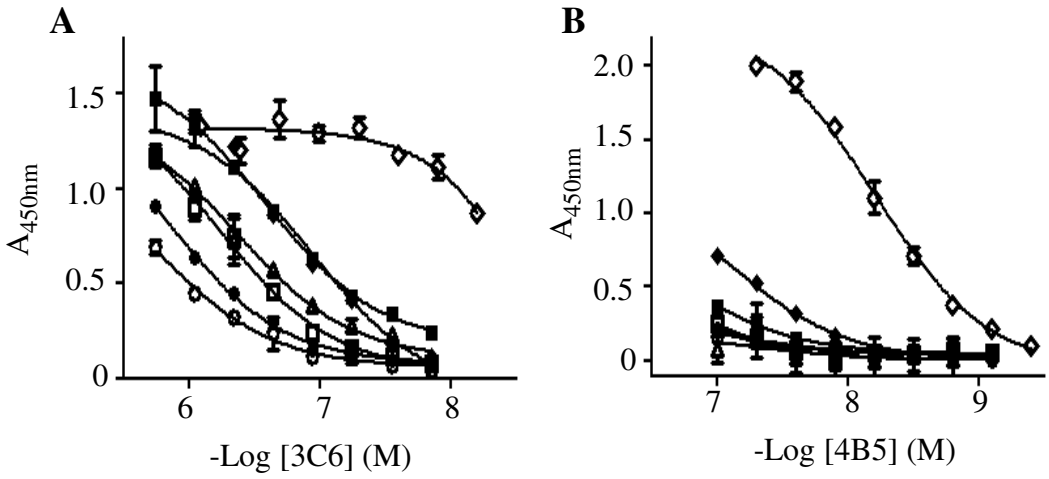


Supplemental Figure 5 O'Nuallain et al.



Supplemental Figure 6 O'Nuallain et al.



C

Aβ peptide	1	10	20	30	40	3C6 Binding	4B5 Binding
	DAEFRHDSGY	EVHHQKLVFF	AEDVGSNKGA	IIGLMVGGVV			
○ 1-7						+	+
● 1-15						+	+
□ 11-25						+	+
■ 19-35						+++	++
Δ 17-40						++	+
♦ 1-40						+++	+++
◊ $(1-40)_2$						++++++	++++++