Supplemental data

Supplemental Material and Method

Negative Stain Electron Microscopy. Aß oligomer mixtures (see *Materials and Methods*, *Preparation of Aß oligomer mixtures and ELISA quantification*, in the main text.) were spread on formvar-carbon coated nickel slot grids (FCF2010-Ni; Electron Microscopy Sciences, Hatfield, PA), negatively stained with 2% (w/v) phosphotungstic acid, pH 7.0 (Wako Pure Chemical Industries, Osaka, Japan), and viewed in an electron microscope with an ATM digital camera (JEOL JEM-1011; JEOL USA, Inc., Peabody, MA), as described (1).

Supplemental Figure 1

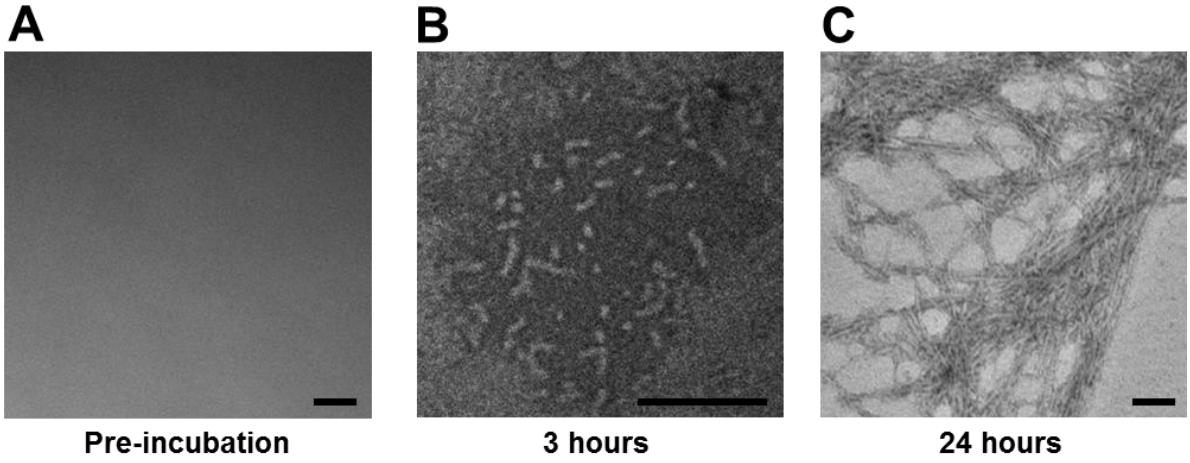

Figure S1. Electron micrograph image of Aß oligomer mixtures.

(A–C) Electron micrograph of negatively stained synthetic human Aß1-42 (0.1 mg/ml) of pre-incubation (A), after incubation at 37 °C for three (B) and 24 (C) hours. (B) After three hours incubation, short and curved protofibrils were observed. (C) Long and straight fibril formation was observed after 24 hours of incubation. Scale bar = 100 nm.

Supplemental Figure 2

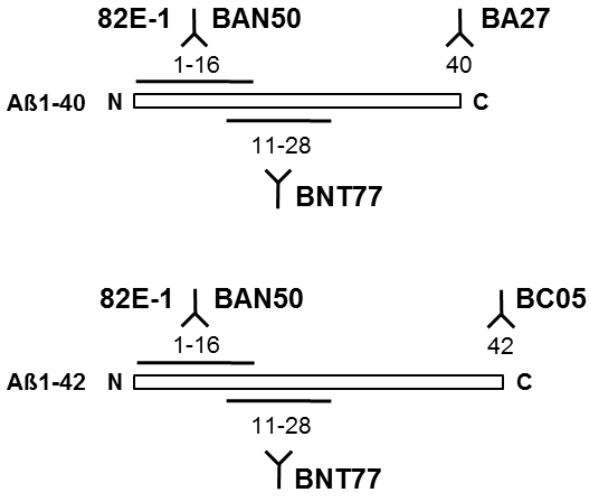

Figure S2. Epitopes recognized by each anti-Aβ antibody.

Epitopes recognized by each anti-Aβ monoclonal antibody (82E1, BAN50, BNT77, and BA27) used in this study. 82E-1 and BAN50 recognize N-terminal (residues 1-16) of human Aβ peptide. BNT77 is a monoclonal antibody against human Aβ11-28, which could also recognize murine Aβ. BA27 and BC05 are end-specific monoclonal antibodies which recognize C-terminus of Aβ40 and Aβ42, respectively.

Supplemental Figure 3

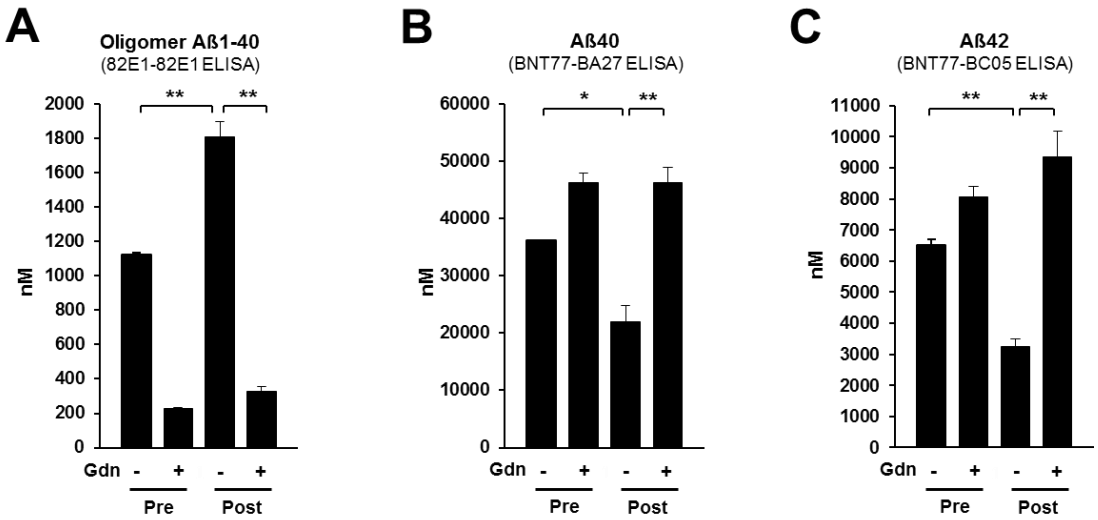

Figure S3. BNT77-BA27 and BNT77-BC05 ELISA preferentially detect monomer (or LMW) Aβ.

(A–C) An Aß oligomer mixture was prepared by incubating synthetic human Aß1-40 (0.2 mg/ml) for 24 hours at 37 °C or Aß1-42 (0.1 mg/ml) for 3 hours at 37 °C. Concentrations of Aß in pre- and post-incubation samples were measured by 82E1-82E1 Aß oligomer-specific ELISA (for oligomer Aβ1-40 (A), BNT77-BA27 ELISA (for Aβ40, (B)) and BNT77-BC05 ELISA (for Aβ42, (C)). Samples were treated with and without guanidine HCl before ELISA measurement. (n = 3–5/ group); *P < 0.05, **P < 0.01. Gdn, guanidine HCl.

Supplemental Figure 4

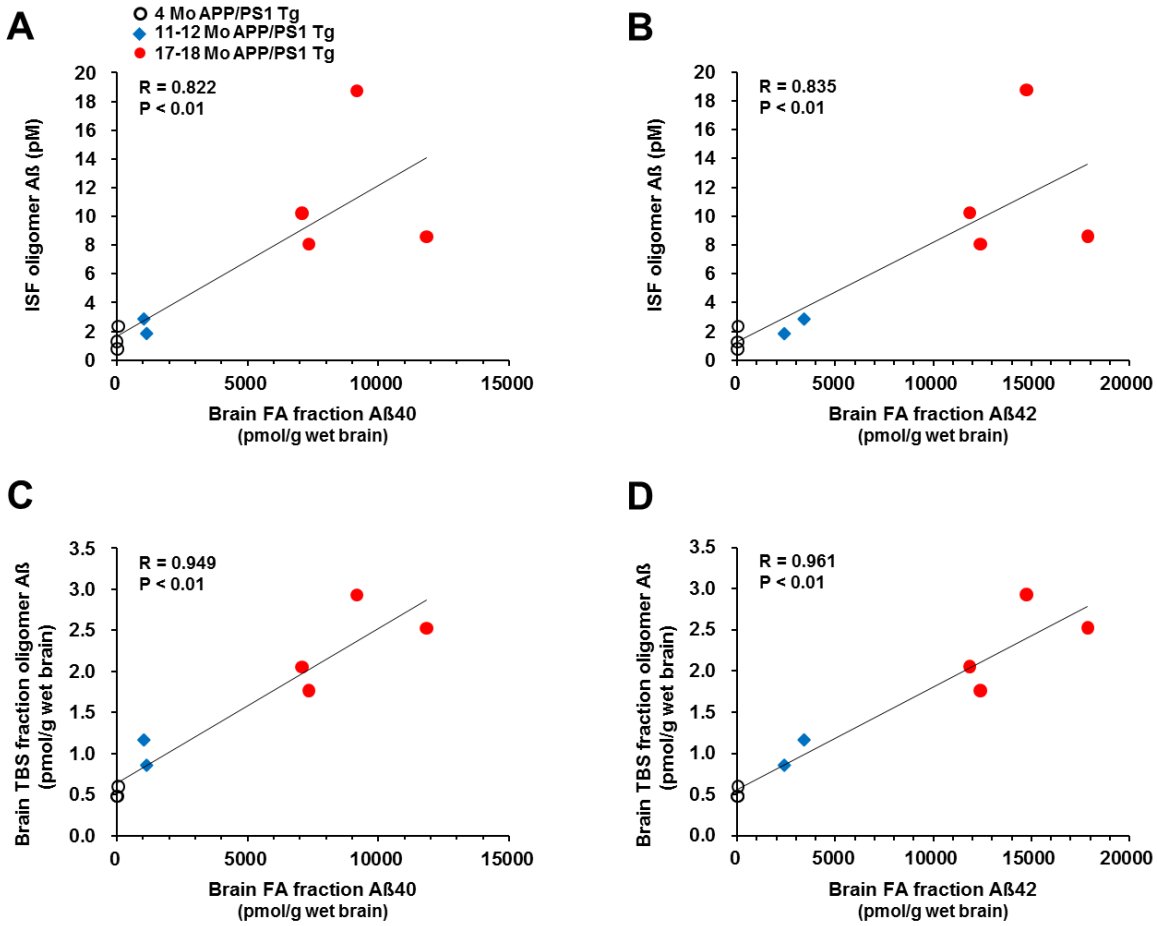

Figure S4. Correlations of brain ISF and TBS fraction Aβ oligomers with FA fraction Aβ levels.

(A, B) The levels of ISF Aβ oligomers (detected by 82E1-82E1 ELISA) positively correlate with the levels of brain FA fraction Aβ40 (A) and Aβ42 (B). (C, D) Brain TBS-soluble Aß oligomer levels (82E1-82E1 ELISA) positively correlated with the levels of brain FA fraction Aβ40 (C) and Aβ42 (D). (n = 9, 4–18 Mo old animals) $P < 0.01$, Spearman rank test. Concentrations of Aß in brain FA fractions were measured by BNT77-BA27 ELISA (for Aβ40, (A, C)) and BNT77-BC05 ELISA (for Aβ42, (B, D)).

Supplemental Figure 5

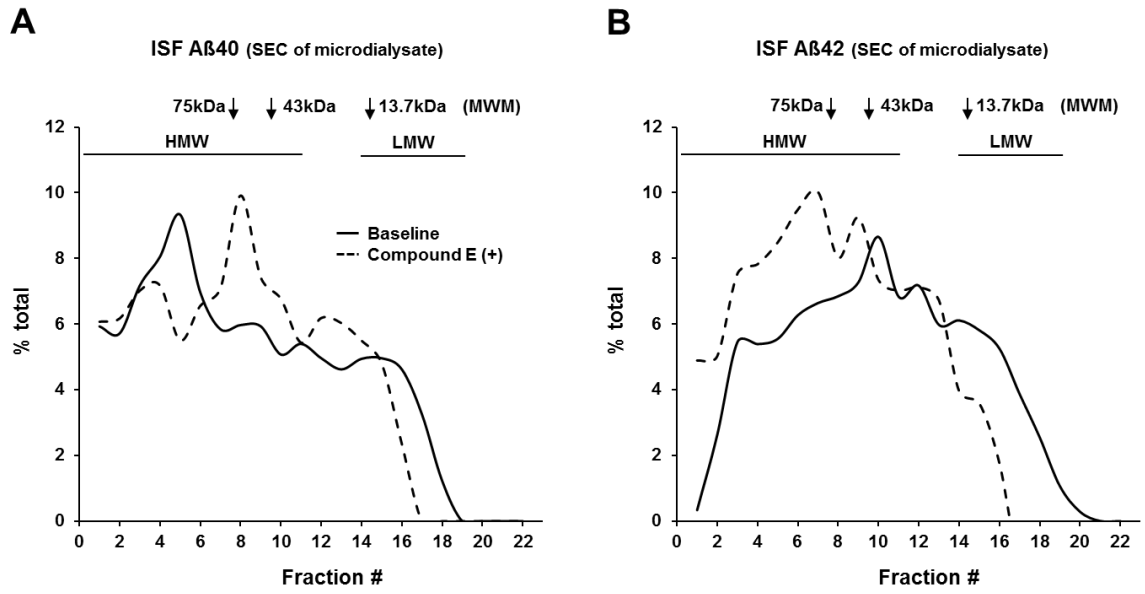

Figure S5. Change in distribution of ISF Aβ between before and after Compound E treatment.

Levels of ISF Aβ in each fraction in Fig. 4, the main text, were normalized to total pM value under each curve [sum of Aβ values (pM) of fractions #1–22] and expressed as "% total" value, which shows differences in molecular weight distribution of ISF Aβ40 (A) and Aβ42 (B) between before (solid line) and after (dashed line) the Compound E treatment (averaged values of three 17–18 month-old APP/PS1 Tg mice were shown). The distribution of ISF Aβ shifted to higher molecular weight fractions after Compound E treatment. HMW, high molecular weight; LMW, low molecular weight; MWM, molecular weight marker.

Reference

Hori, Y., Hashimoto, T., Wakutani, Y., Urakami, K., Nakashima, K., Condron, M. M., Tsubuki, S., Saido T. C., Teplow, D. B., Iwatsubo, T. (2007) The Tottori (D7N) and English (H6R) Familial Alzheimer Disease Mutations Accelerate Aβ Fibril Formation without Increasing Protofibril Formation. *J. Biol. Chem.* **282**, 4916-4923