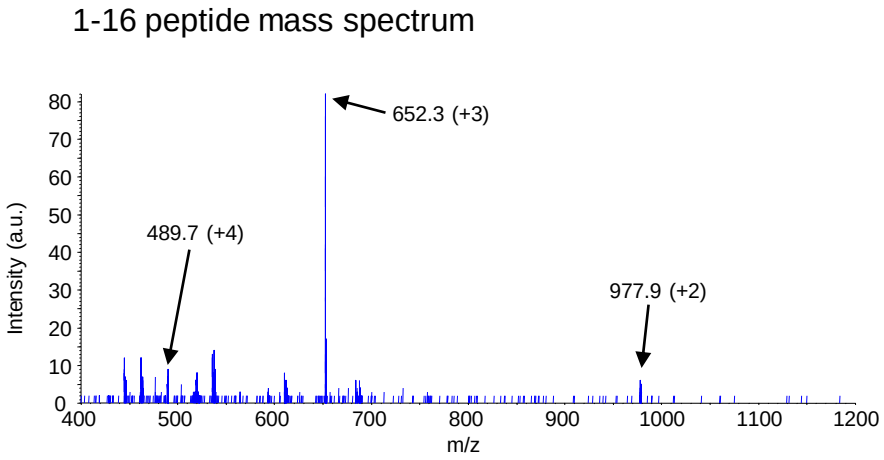
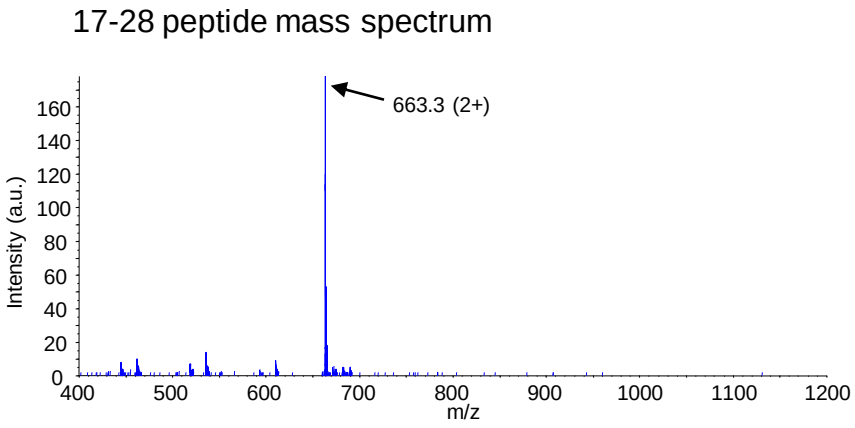
Figure S1 Mass spectrometric analysis of the purified ~8.5/9 kDa band indicates the presence of a non-covalent Aβ1-40 dimer. MALDI-TOF mass spectra for $A\beta_{1\text{-}40}$ peptide fragments resulting from lysC digest of purified 7PA2-derived Aβ from Figure 2C. Masses for individual LC peaks corresponding to peptide fragments 1-16, 17-28 and 29-40. A.U. denotes arbitrary units, and quaternary, tertiary, doubly or singly protonated fragments are indicated by (+4), (+3), (+2) and (+1), respectively.

Figure S1

(A)



(B)



(C)

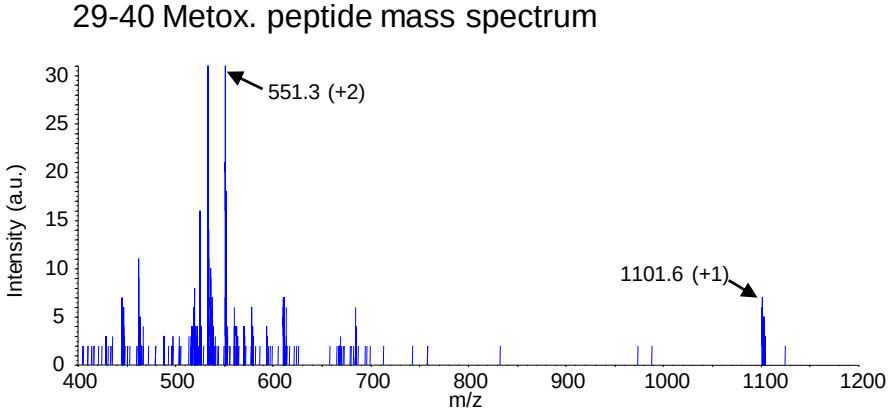


Figure S2 7PA2 CM contains species with the Aβ epitope, but without a free Asp1 Nterminus. SEC fractions (#3-13) of CM from 7PA2 cells and from untransfected CHO- cells were each Western blotted with either 6E10 or 3D6. 6E10 revealed the presence of ~4.5 kDa Aβ monomer and ~5-14 kDa bands, whereas 3D6 detected only the ~4.5 kDa monomer. M, D, and T denote the position where putative Aβ monomer, dimer and trimer migrate, and the migration of molecular weight standards (in kDa) are indicated on the left.

Figure S2

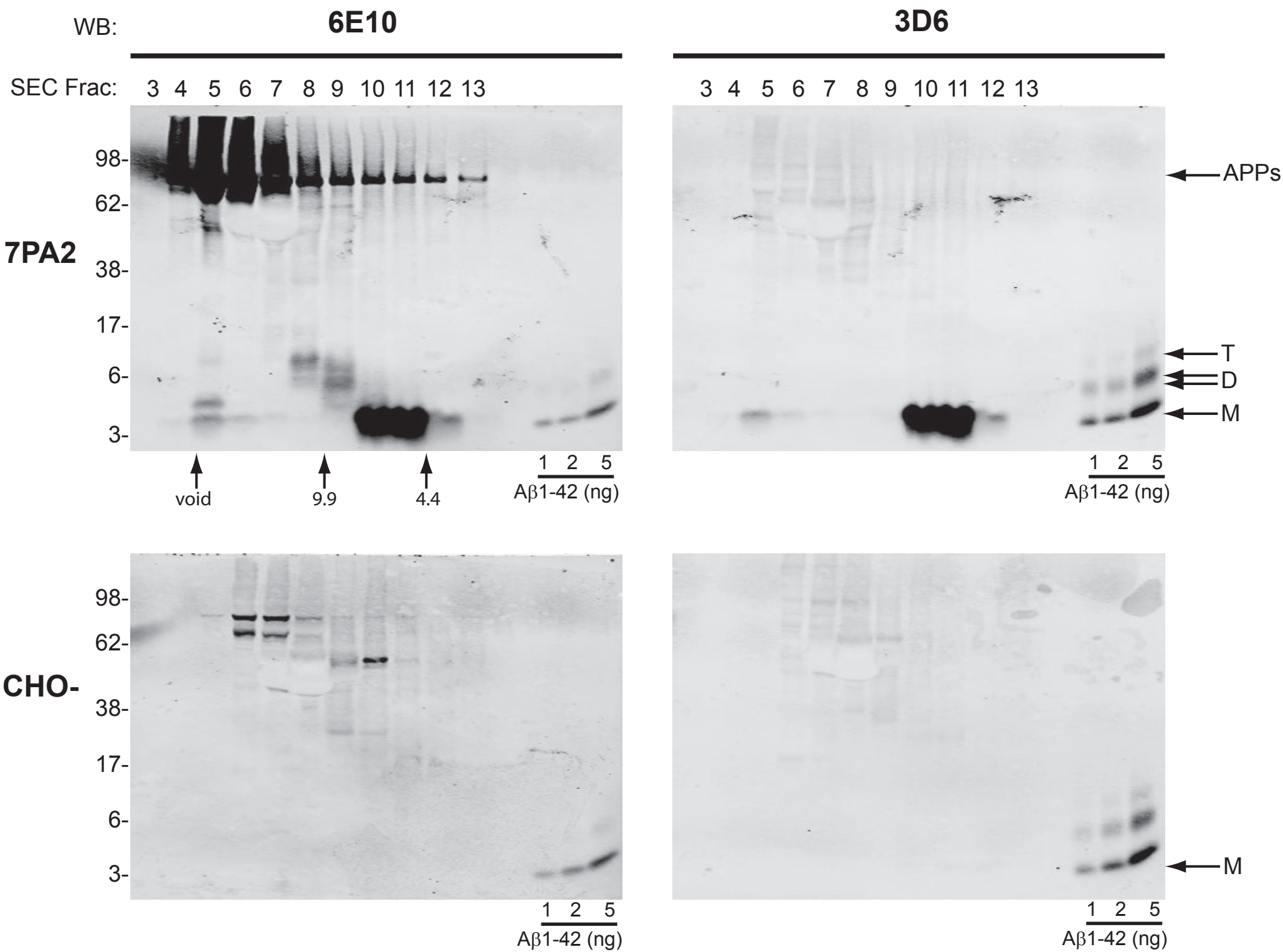

WB:
6E10
3D6
SEC Frac: 3 4 5 6 7 8 9 10 11 12 13
7PA2
98-
62-
38-
17-
6-
3-
APPs
T
D
M
void
9.9
4.4
1 2 5
Aβ1-42 (ng)
CHO-

Fig. S3 Antibody 1G6 detects 6E10-reactive species that migrate between ~8-14 kDa, whereas the Asp1 specific antibody, 82E1 detects only ~4 kDa Aβ monomer. Ten ml of 7PA2 CM was divided into 2 ml aliquots and IP'd with one of the antibodies indicated. Immunoprecipitates were washed, electrophoresed on a NuPAGE gel, transferred to nitrocellulose and Western blotted with 6E10. The Asp1-specific antibody 82E1 precipitates only monomer, whereas the Pre-β and 1G6 antibodies do note precipitate Aβ monomer, but pull down 6E10-reactive species that migrate between ~7-14 kDa. M, D, and T denote the position where putative Aβ monomer, dimer and trimer migrate, and the migration of molecular weight standards (in kDa) are indicated on the left.

Figure S3

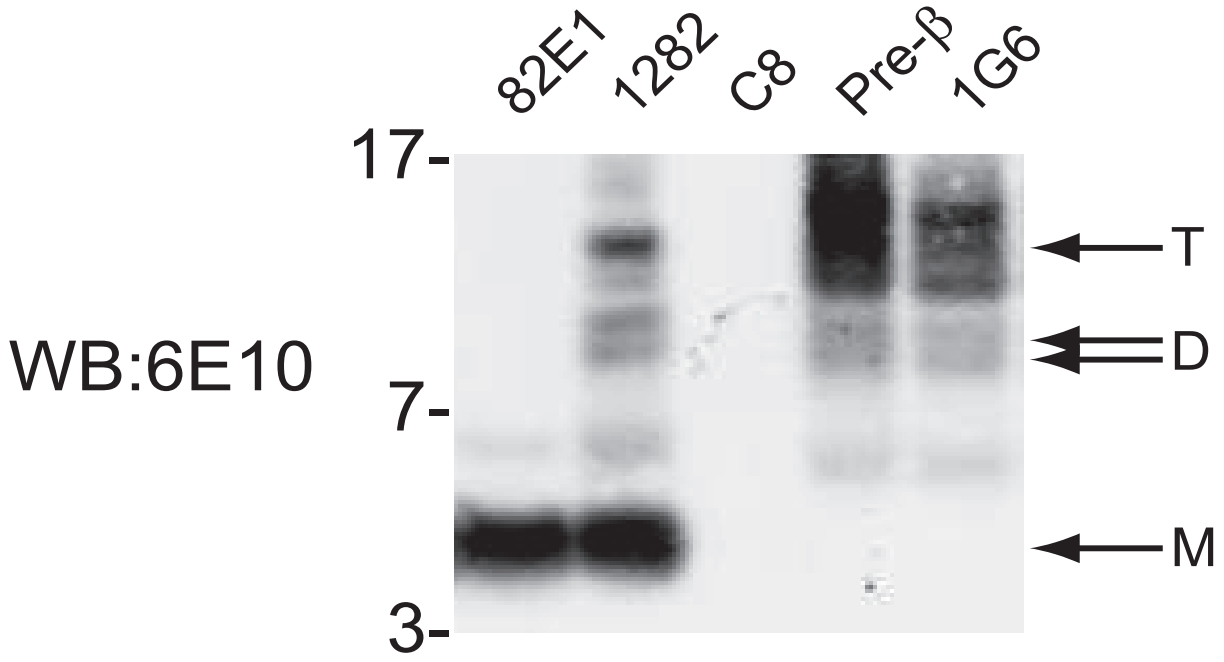
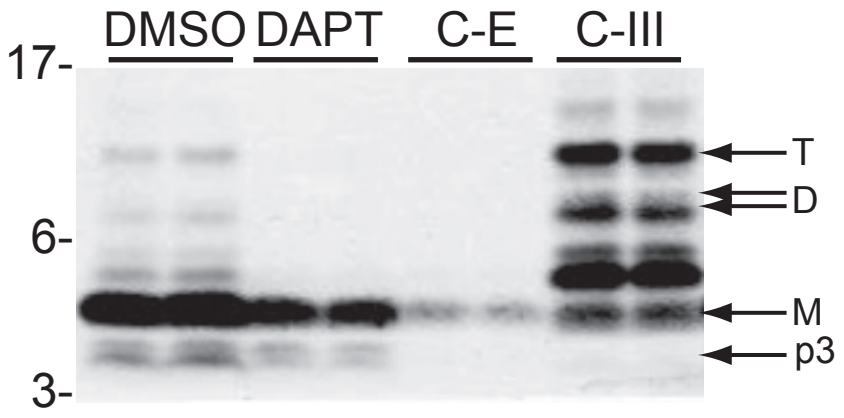


Figure S4 The β-secretase inhibitor, Compound III reduces production of Aβ monomer but increases production of ~5-16 kDa Aβ immunoreactive species. **(A)** and **(B)** Duplicate dishes of 7PA2 cells were conditioned for ~15 h in the presence or absence of the γ-secretase inhibitors, DAPT (10 μM) or Compound E (1 nM); or the β-secretase inhibitor, Compound III (3 μM). CM was collected from cells and used for IP with **(A)** R1282 and and **(B)** pre-β. R1282 IP's were Western blotted with a combination of anti-Aβ monoclonals 6E10, 2G3 and 21F12, and the pre-β IP's were Western blotted with 6E10 alone. Treatment of 7PA2 cells with γ-secretase inhibitors blocks the secretion of most species detectable by both R1282 IP and pre-β IP. In contrast treatment with Compound III (C-III) inhibited production of Aβ monomer but significantly increased the quantity of 6E10 immunoreactive species migrating between ~5-16 kDa. M, D, and T denote the position where putative Aβ monomer, dimer and trimer migrate, and the migration of molecular weight standards (in kDa) are indicated on the left.

Figure S4

(A)

IP: R1282
WB: 6E10/2G3/21F12



(B)

IP: pre-β
WB: 6E10