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Supplementary Data

Grape Seed Polyphenolic Extract Specifically Decreases Aβ*56 in the Brains of Tg2576 Mice

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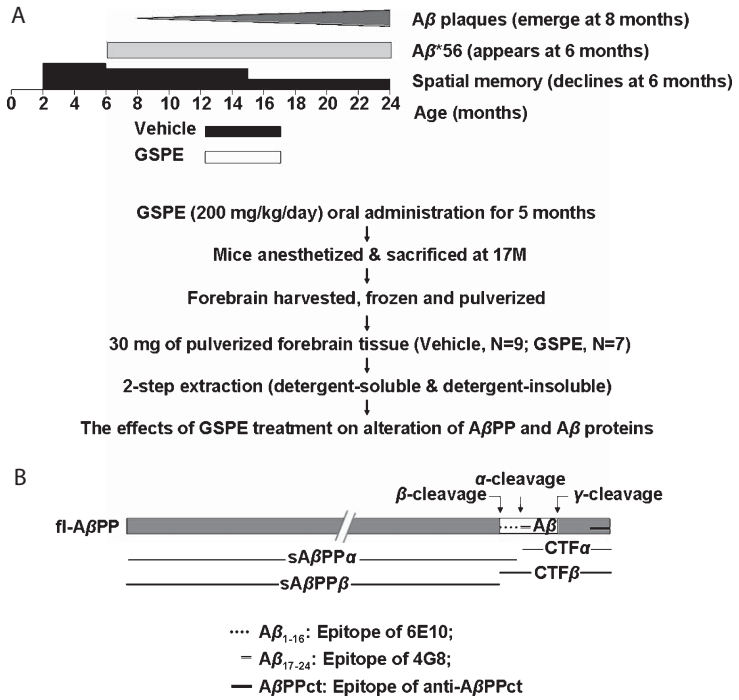
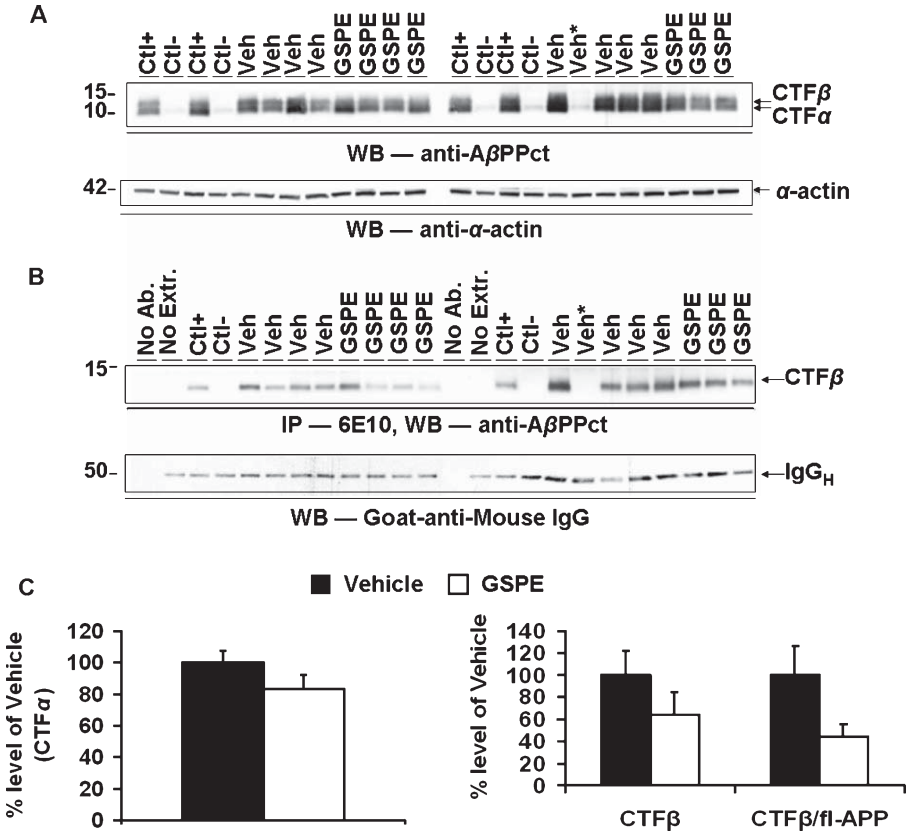
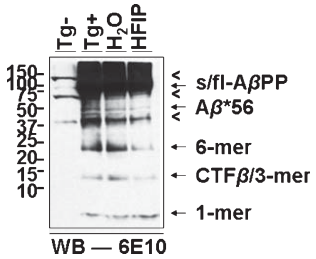


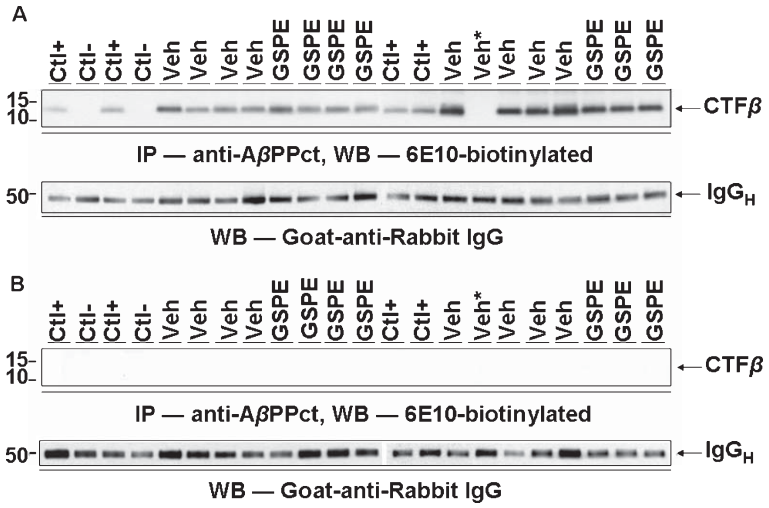
Supplementary Figure 1. Experimental Design. A) Timeline (upper) showing the development of neuropathology and memory deficits in Tg2576 mice and flowchart (lower) showing the aging and treatment of Tg2576 mice and subsequent brain tissue collection, protein extraction and biochemical analyses. B) Schematic diagram illustrating the epitopes recognized by the antibodies used in this study (fl -AβPP: full-length AβPP; α-, β- and γ-cleavage: cleavage sites of α-, β- and γ-secretases, respectively).



Supplementary Figure 2. Processing of transgenic AβPP. A) α-secretase processing. AβPP C-terminal fragment CTFα was detected (upper panel) by rabbit polyclonal antibody anti-AβPPct in western blots of detergent-soluble fractions; α-actin was used as a loading control (lower panel). B) β-secretase processing. Detergent-soluble extracts were immunoprecipitated using 6E10, and immunoblots were probed with anti-AβPPct. Upper panel reveals AβPP C-terminal fragment CTFβ, and lower panel reveals the heavy chain of the capture antibody (mouse IgG_H). C) Quantification. The levels of CTFα were first normalized to α-actin. CTFα, CTFβ and the ratio of CTFβ/fl-AβPP were not significantly different between mice treated with GSPE ($n = 7$) and those treated with vehicle ($n = 8$). Veh*, sample from mis-genotyped mouse, not included in quantitative analyses; Tg2576 (Ctl+) and transgene-negative (Ctl-) mice bred and maintained at the University of Minnesota that served as additional controls (see *Materials and Methods*); $Aβ_{40/42}$: synthetic $Aβ_{1-40}$ and $Aβ_{1-42}$ as positive controls for Aβ monomers and low-*n* Aβ oligomers; No Ab.: no 6E10 was included in immunoreactions; No Extr.: no protein extracts were included in immunoreactions.



Supplementary Figure 3. Confirmation of the identity of Aβ oligomers. Two equally-prepared samples were treated with either water or 100% hexafluoroisopropanol (HFIP). HFIP treatment led to dissociation of the bands tentatively identified as Aβ*56 and Aβ hexamers (6-mer) and to an increase in monomeric Aβ peptide (1-mer), as would be expected if these bands represented Aβ oligomers. Untreated samples (Tg+ and Tg-) served as additional controls. Arrowheads show non-specific bands.



Supplementary Figure 4. Immunodepletion of CTFβ. In order to determine levels of Aβ trimers, it is necessary to deplete samples of CTFβ, which has a similar electrophoretic mobility. Two rounds of immunodepletion using anti-AβPPct were required to completely remove CTFβ. A) First round of immunodepletion. Samples were immunodepleted using anti-AβPPct, as described in *Materials and Methods*, and then immunoprecipitated using anti-AβPPct to capture any remaining AβPP CTFs. After one round of immunodepletion, CTFβ is still detectable in blots probed with biotinylated 6E10 (upper panel). Lower panel shows the heavy chain of the capture antibody (IgG_H). B) Second round of immunodepletion. After a second round of immunodepletion and immunoprecipitation, CTFβ is no longer detectable. Blots of IgG_H show that equal amounts of capture antibody were used to react with each sample.