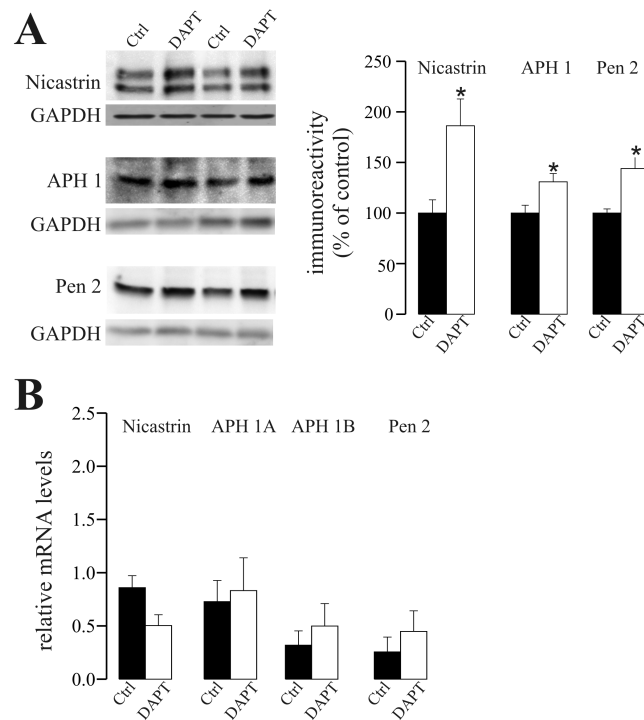


Supplementary Figures

Inhibition of γ -secretase leads to an increase in presenilin-1

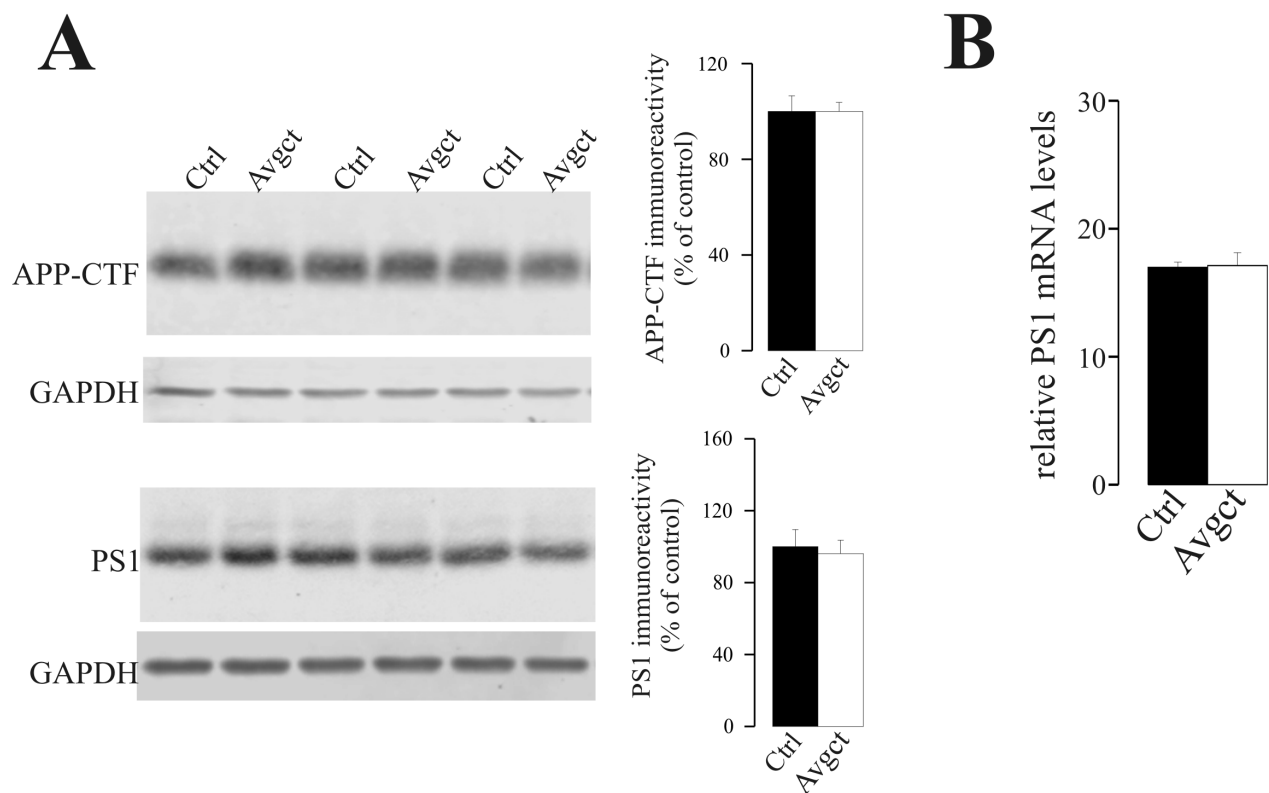
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Supplemental Figure 1. GSI DAPT augments γ -secretase subunits in SH-SY5Y cells. (A) SH-SY5Y cells were treated for 18 h (acutely) with DAPT (5 μ M) or the vehicle alone (control; Ctrl), as indicated in Fig. 1. Cell extracts were probed for subunits of the γ -secretase complexes: nicastrin, PEN2, and APH1 (with an antibody that recognizes both the APH1A and APH1B forms). Equivalent amounts of protein were loaded in each lane and GAPDH was used as a loading control. Representative blots and densitometric quantification of the immunoreactivity are shown. For nicastrin the upper band is the mature form [Kimberly *et al* 2003]^{\$} and the band quantified. (B) Relative expression of nicastrin, PEN2 and APH1 (homologs APH1A and APH1B) mRNA was analyzed by *q*RT-PCR. Transcript levels were calculated by the comparative $2^{-\Delta C_t}$ method with respect to GAPDH cDNA. The data represent the means $\pm$ SEM of at least n=10 independent determinations (obtained from two independent set of experiments): * $p < 0.05$.

^{\$} Kimberly WT, LaVoie MJ, Ostaszewski BL, Ye W, Wolfe MS, Selkoe DJ (2003) Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. *Proc Natl Acad Sci USA* 100:6382-6387.



Supplemental Figure 2. Effect of avagacestat on APP-CTF and PS1 levels in the cortex of rats treated for 4 days. Rats were treated daily with the GSI avagacestat (40 mg/kg, Avgct) or the vehicle alone (control; Ctrl) for 4 days (n= 10 per group), and they were sacrificed 4-5 hours after the last dose. **(A)** The APP-CTF and PS1 were evaluated in Western blots of extracts from brain hemi-cortices. Representative blots probed for APP-CTF and PS1, and the densitometric quantifications, are shown. GAPDH was used as a loading control. **(B)** Relative PS1 mRNA was analyzed by *q*RT-PCR in rat hemi-cortices. The data are presented relative to the control rats, expressed as the means $\pm$ SEM: * $p < 0.05$.