

Supplementary Information for

GSAP modulates γ -secretase specificity by inducing conformational change in PS1

Eitan Wong^{1,2}, George P. Liao^{1,3}, Jerry C. Chang², Peng Xu², Yue-Ming Li^{1,3*} and Paul Greengard^{2*}

¹ Chemical Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065

² Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10065

³ Program of Pharmacology, Weill Graduate School of Medical Sciences of Cornell University, New York, NY 10021

This PDF file includes:

Figs. S1-S3

Figure S1

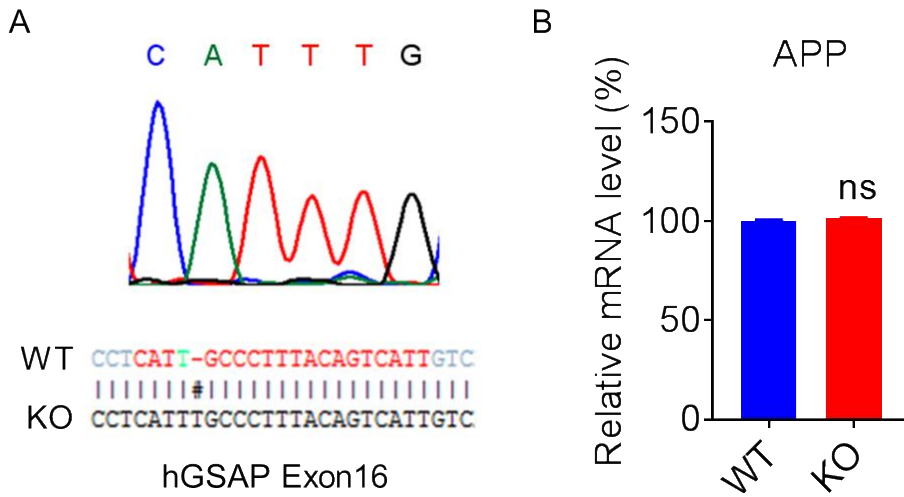


Figure S1. **HEK-APP GSAP KO conformation and APP expression.** CRISPR gRNA targeting exon 16 (A), sequence chromatogram of HEK-APP GSAP KO in the targeted region (upper panel) and sequence alignment of HEK-APP WT versus KO in exon16 (lower panel, CRISPR gDNA in red and last PAM nucleotide in green). APP mRNA levels in HEK-APP WT versus HEK-APP GSAP KO cells (B).

Figure S2

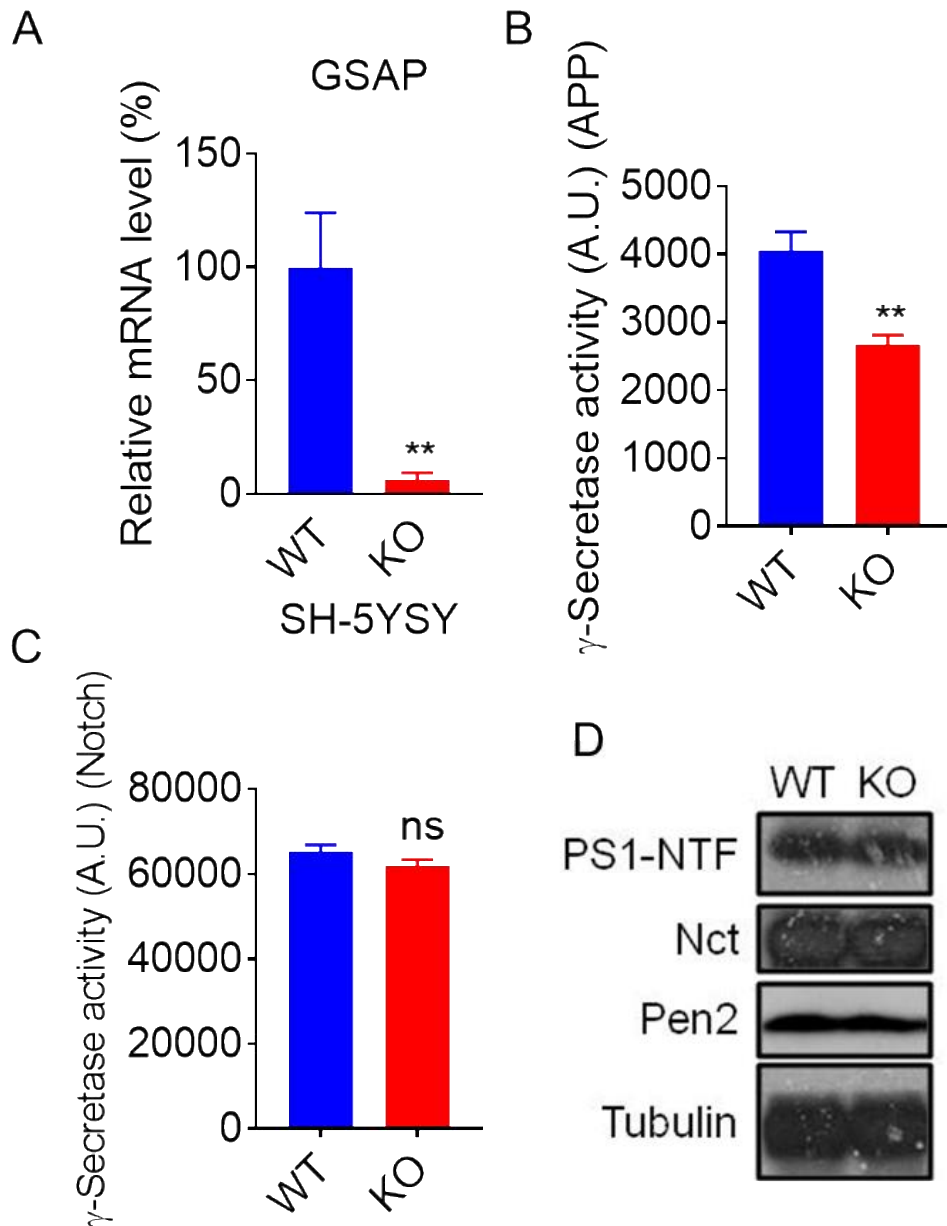


Figure S2. **SH-5YSY GSAP KO reduces γ -secretase activity for APP.** GSAP mRNA levels in SH-5YSY cells versus CRISPR GSAP KO cells (A). γ -Secretase activity levels toward recombinant APP (B) are decreased in GSAP KO SH-5YSY compared to GSAP WT counterpart but remained unchanged for recombinant Notch (C). All data represent means \pm SEM; n=6. (D) Western blot analysis of γ -secretase subunits PS1-NTF, Nct, and Pen2 in SH5-YSY WT and KO (p-value **< 0.01).

Figure S3

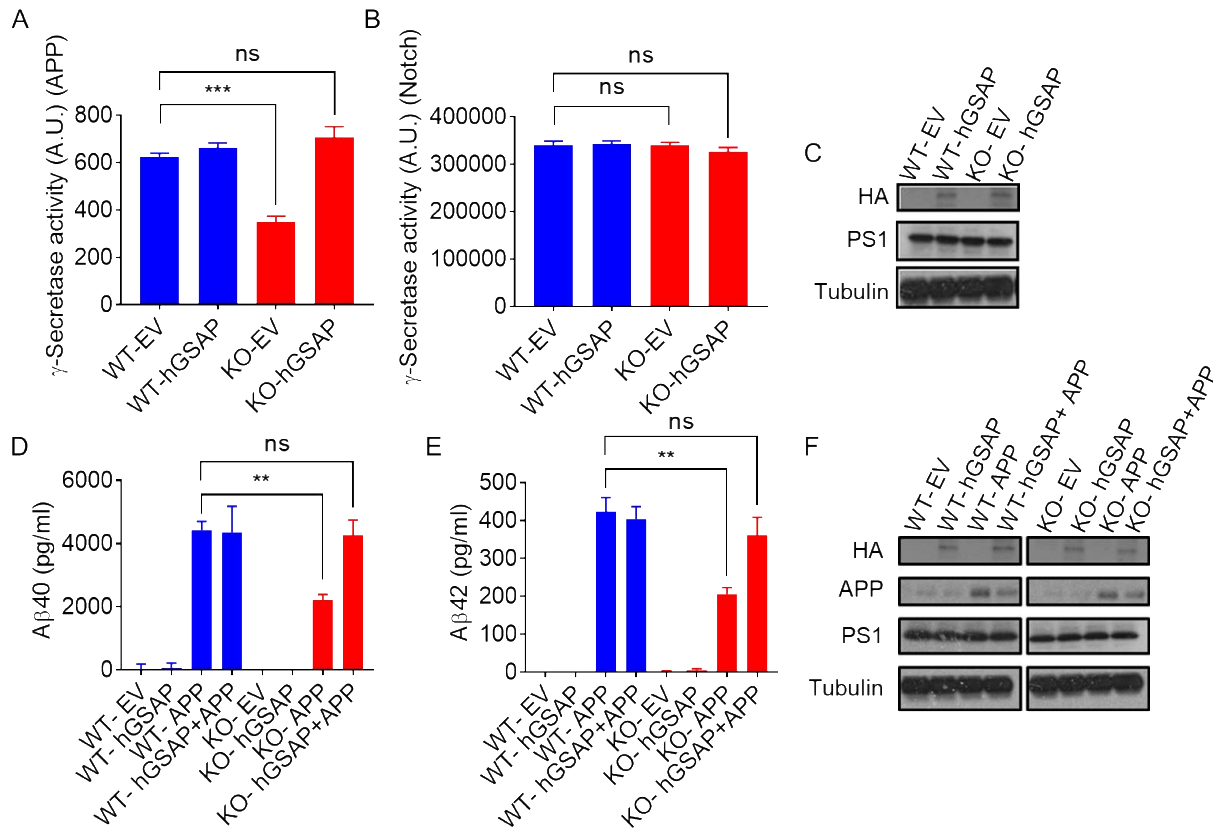


Figure S3. Overexpression of hGSAP in SH-5YSY GSAP KO cells rescues γ -secretase activity and A β secretion. Membrane fractions prepared 48 hours post transfection of EV and hGSAP in SH-5YSY GSAP WT or KO were assayed for γ -secretase activity using recombinant APP (A) or Notch (B). Western blot analysis of HA (hGSAP) and PS1-NTF in SH-5YSY WT and KO confirming GSAP expression which had no effect on PS1 (C). A β analysis was done on SH-5YSY WT or KO transfected with Empty vector (EV) or full-length human GSAP (hGSAP). To detect A β levels the cells were transfected either with APP or hGSAP+APP. A β secretion was measured 48 hours post-transfection using MSD A β detection kit; A β 40 (D) and A β 42 (E). All data represent means \pm SEM; n=3. (F) Western blot analysis of HA (hGSAP), APP and PS1-NTF in SH-5YSY WT and KO transfected with: EV, hGSAP, APP or hGSAP+APP confirming the expression of hGSAP or APP in the indicated cells (p-value ** < 0.01; ***<0.001).