Supplemental Figure Legends

Supplemental Figure S1. The processing of NotchTM-Gal4p by PS1 F411Y/S438P in the absence of NCT. Yeast cells expressing PS1 wild type or F411Y/S438P mutant, other γ-secretase subunits, and NotchTM-Gal4p were examined for growth after 3 days at 30 ^{o}C on selection (SD-LWHUAde) or nonselection (SD-LWU) media, as indicated. Three independent clones were tested.

Supplemental Figure S2. Reduced stability of F411Y/S438P mutant comparing to wild type PS1 CTF. PS double knockout cells were transfected with PS1pcDNA3.1/Zeo or F411Y/S438PpcDNA3.1/Zeo, and NCT or control siRNAs with Lipofectamine 2000. At 48 hours after transfection, transformants were treated with 100 μM cycloheximide and the cells were incubated at 37^{o}C for 0, 1, 2, 4, 6 hr. Upon cycloheximide chase, PS1 stability was analyzed by immunoblotting using specific antibodies. The asterisks indicate nonspecific bands.

Supplemental Figure S3. Similar subcellular localization of F411Y/S438P and wild type PS1 CTF. PS double knockout cells were transfected with PS1pcDNA3.1/Zeo or F411Y/S438PpcDNA3.1/Zeo, and NCT or control siRNAs with Lipofectamine 2000. At 48 hours after transfection, transformants were homogenized and then fractionated by sucrose gradient sedimentation. 10 fractions were analyzed by immunoblotting using specific antibodies. Wild type or mutant PS1 were recovered in the Golgi and the endoplasmic reticulum (ER)/ the plasma membrane fractions with similar distribution. The asterisks indicate nonspecific bands.

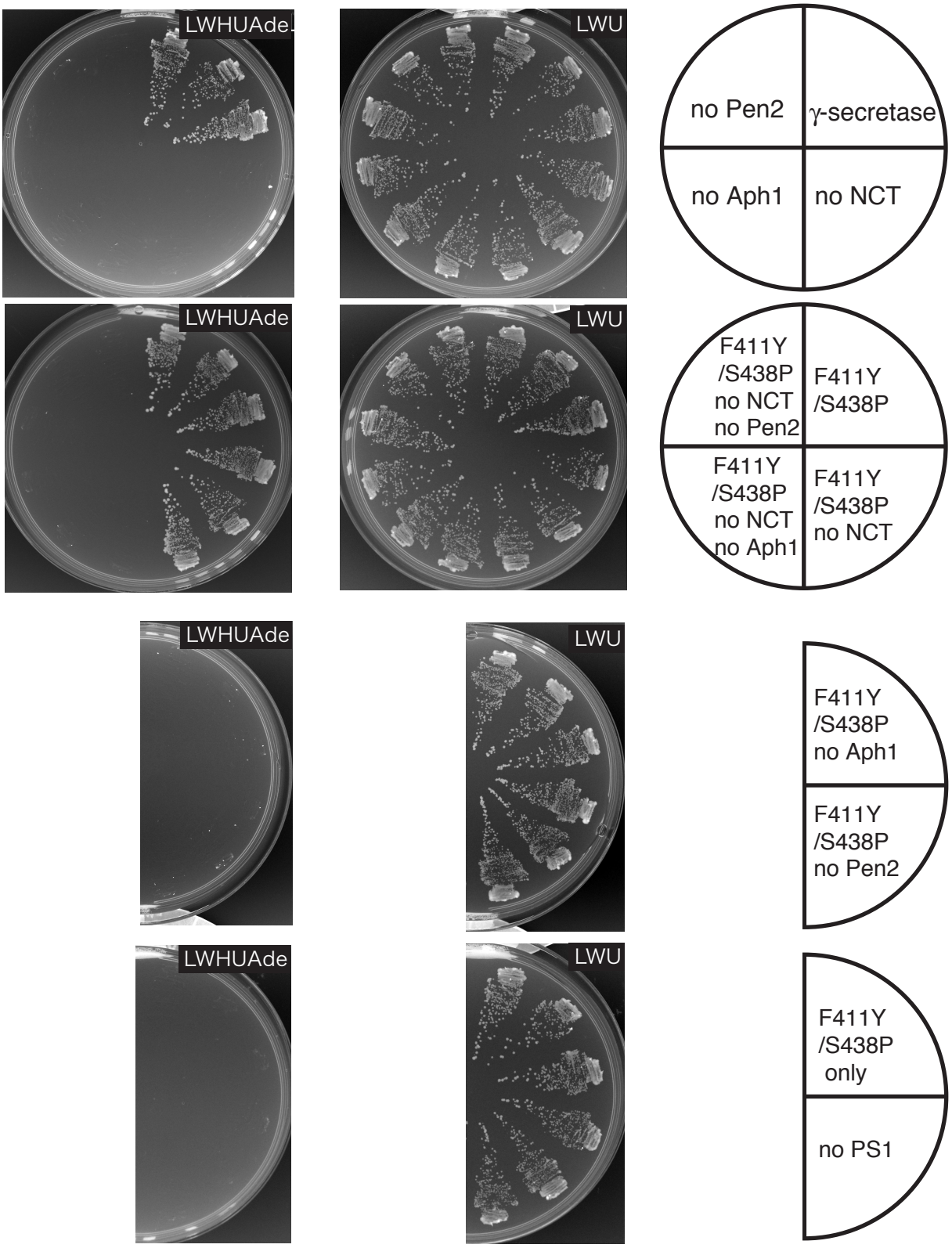
Supplemental Experimental Procedures

Subcellular Fractionation. Fractionation by sucrose gradient was performed as described previously (1), with modifications. Cells were suspended in a final concentration of 1 vol. of cell pellet per 5 vol. of homogenizing solution (0.25 M sucrose, 10mM Tris/Cl (pH 7.4), 1mM magnesium acetate, and a protease inhibitor

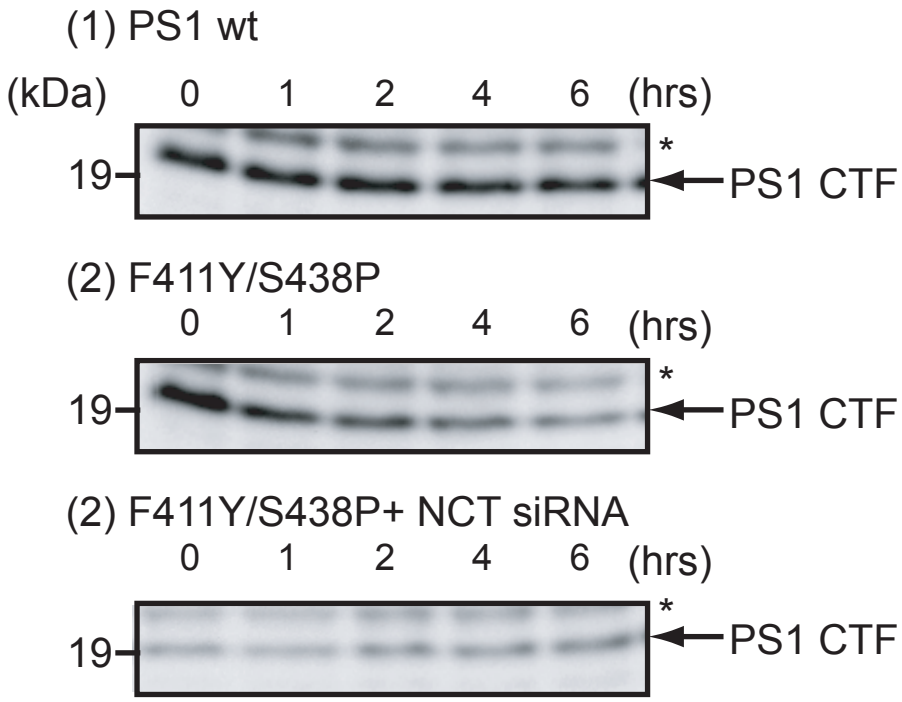
cocktail (Sigma, MO)) and passed through 21 G needles 20 times. Then homogenates were centrifuged at 800 g for 5 min, and the resulting supernatant (0.2 ml) was loaded on 0.2 ml of 2 M sucrose, 0.8 ml of 1.3 M sucrose, 0.6 ml of 1.16 M sucrose, 0.3 ml of 0.8 M sucrose. All solution contained 10mM Tris/Cl (pH 7.4) and 1mM magnesium acetate. The gradients were centrifuged for 18 hours at 100,000 g in a Beckman TLS55 rotor. Ten 0.2 ml fractions were collected from the top of each gradient. Fractions were analyzed by immunoblotting using specific antibodies. Antibodies used were anti-PS1 (G1L3, gift from Dr. T. Iwatsubo), anti-γ-adaptin (the TGN marker; BD Biosciences, MD), anti-Bip (the ER marker; Stressgen, MI), anti-Arf (the vesicles and cytosol marker; 1D9, Affinity BioReagents, IL), and anti-Na+/K+-ATPase (the plasma membrane marker; Abcam, MA).

(1) Greenfield, J. P., Tsai, J., Gouras, G. K., Hai, B., Thinakaran, G., Checler, F., Sisodia, S., Greengard, P., and Xu, H. (1999) *Proc Natl Acad Sci USA.* **96**, 742-747.

Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3