Presenilin 1 and Presenilin 2 Target γ-Secretase Complexes to Distinct Cellular Compartments

Xavier Meckler and Frédéric Checler

SUPPLEMENTAL DATA

MOVIE LEGENDS

MOVIE 1: **Intracellular trafficking of PS2-containing γ-secretase complexes presenting distinct patterns of distribution.** COS-7 cells were transfected with 4γ-PS2/APH1aS and transiently induced with doxycycline for 2 hours one day before imaging. The cell on the left presents one large and bright structure nearby the nucleus from which many vesicles come in and out, and numerous smaller and highly mobile vesicles/tubules scattered throughout the cell; whereas the cell on the right presents several large vesicular structures around the nucleus and many other smaller vesicles scattered throughout the cell, and a less active vesicular trafficking between these structures. Images were acquired by time-lapse confocal microscopy using a spinning-disk confocal microscope (UltraVIEW Live Imaging System, Perkin Elmer). Frames were taken every second for 2 minutes.

MOVIE 2: **Intracellular trafficking of PS2-containing γ-secretase complexes localized in long tubules.** COS-7 cell transfected with 4γ-PS2/APH1b and transiently induced with doxycycline for 2 hours one day before imaging. Images were acquired by time-lapse confocal microscopy using a spinning-disk confocal microscope (UltraVIEW Live Imaging System, Perkin Elmer). Frames were taken every second for 2 minutes.

SUPPLEMENTAL FIGURES LEGENDS

FIGURE S1. **mΔENotch processing is not altered by the expression of 4γ-PS/APH1 constructs.** HEK293 cells stably expressing myc-tagged mΔENotch were co-transfected with empty vector, or with the indicated 4γ-PS/APH1 constructs, and the transactivator-protein expression plasmid; 7 h after transfection, transgenes expression was induced or not with doxycycline (Dox.) for 16 h; 8 h after induction, cells were placed for 16 h in culture medium containing or not the γ-secretase inhibitor DAPT before cell lysis. Cell lysates were analyzed by Western blot using an anti-myc antibody (9E10) to detect mΔENotch and the γ-secretase cleaved fragment Notch Intracellular Domain (NICD), or specific antibodies for each γ-secretase subunit, or an anti-GFP antibody to detect NCT-EGFP and an anti-2A peptide antibody to detect APH1-T2A. Molecular weights in kDa are indicated on the left of each blot. Note that the relative intensities ratio between mΔENotch band and NICD band was not altered by the expression of specific set of γ-secretase subunits.

FIGURE S2. **PS2-containing γ-secretase complexes are partially detected in early endosomes.** COS-7 cells were co-transfected with the indicated 4γ-PS2/APH1 constructs and the transactivator-protein expression plasmid; transgenes expression was induced with doxycycline for 2 h the next day, cells were fixed one day after induction, and processed for immunocytochemistry using an antibody against the early endosome marker EEA1. Fluorescent signals were acquired using laser confocal microscopy. Insets correspond to magnifications of the boxed areas. Scale bars represent 5 μm.

Figure S1, Meckler and Checler

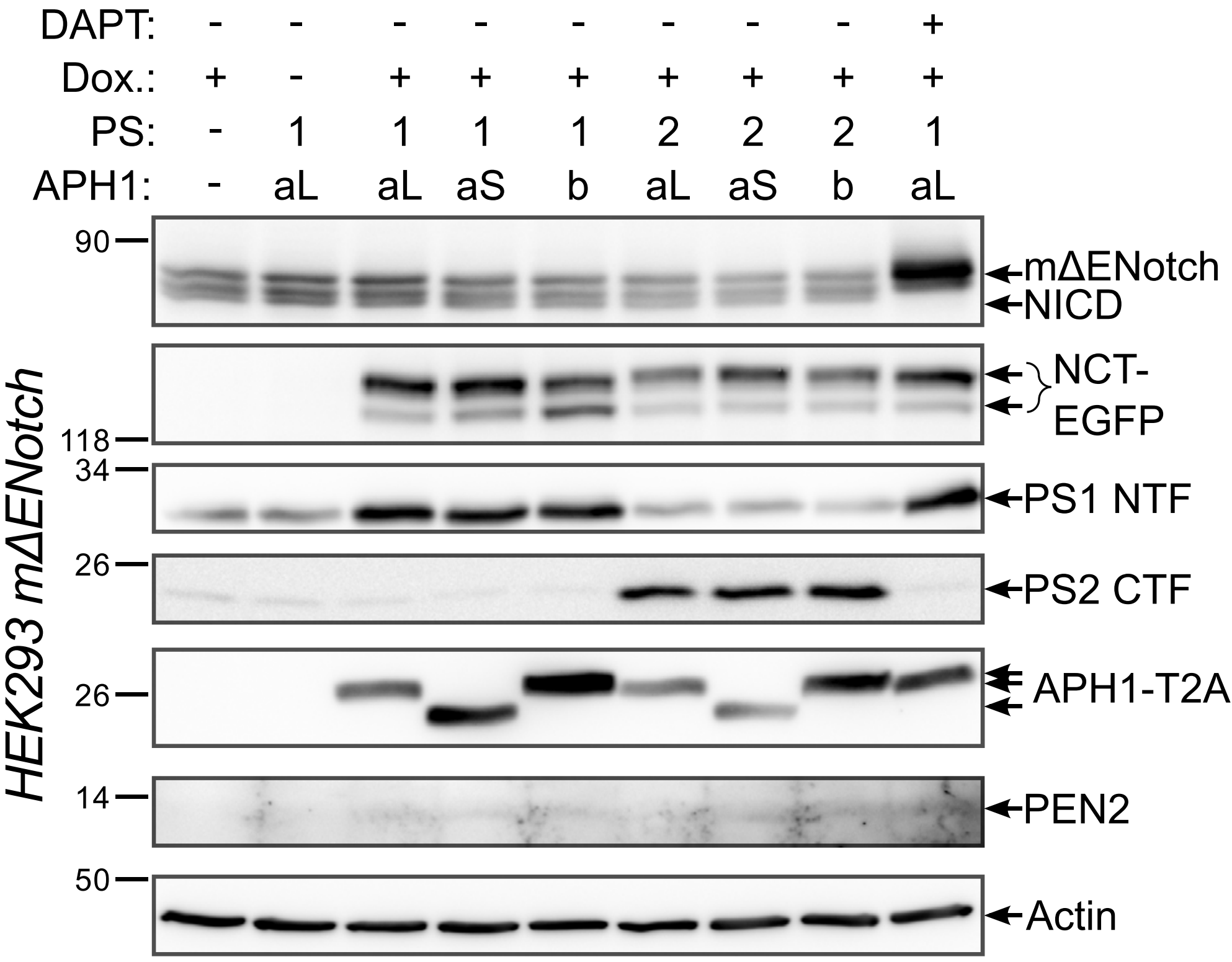

Figure S2, Meckler and Checler