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Supplementary Material

Presenilin-1 Holoprotein is an Interacting Partner of Sarco Endoplasmic Reticulum Calcium-ATPase and Confers Resistance to Endoplasmic Reticulum Stress

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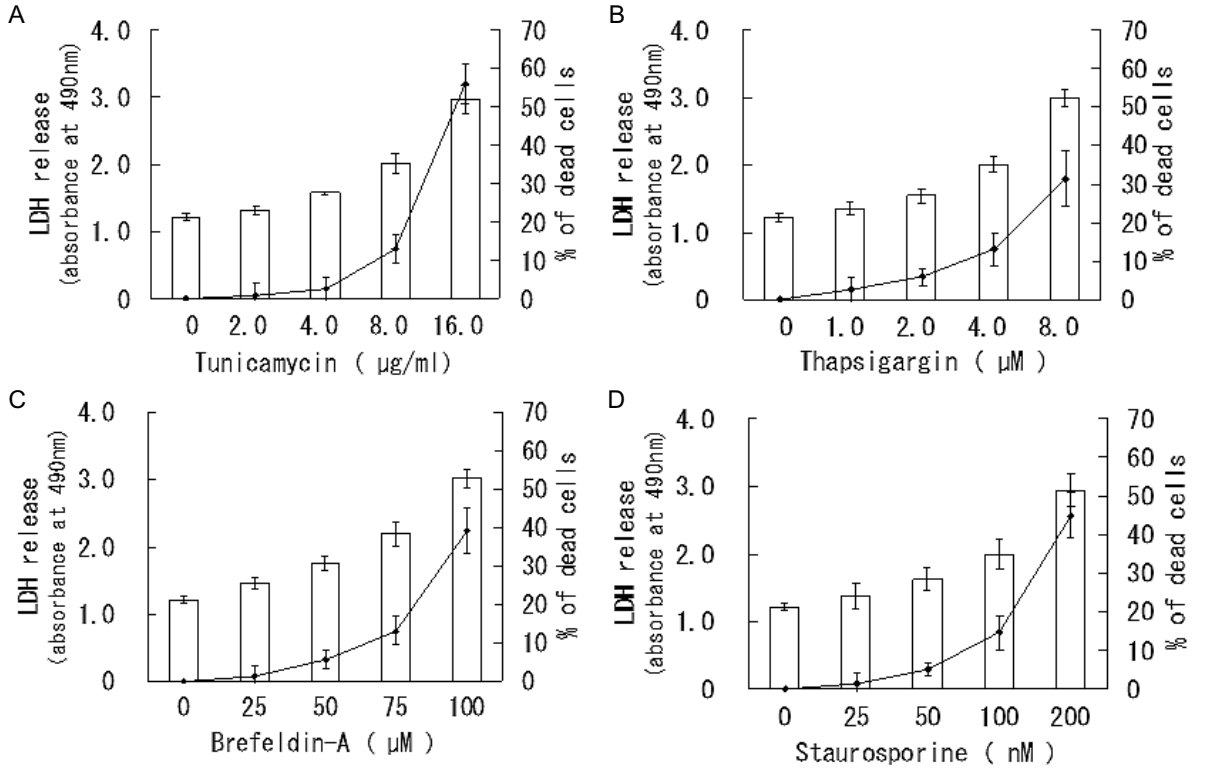
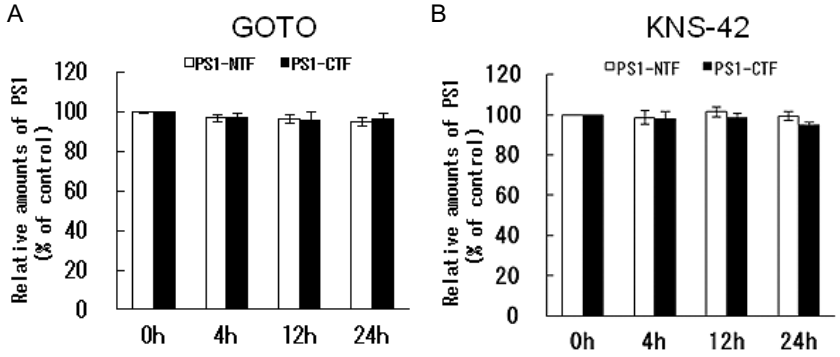
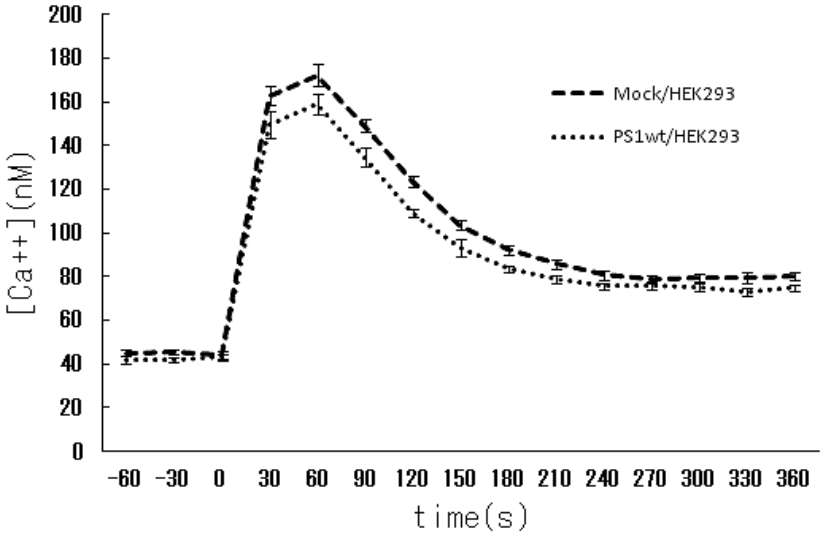


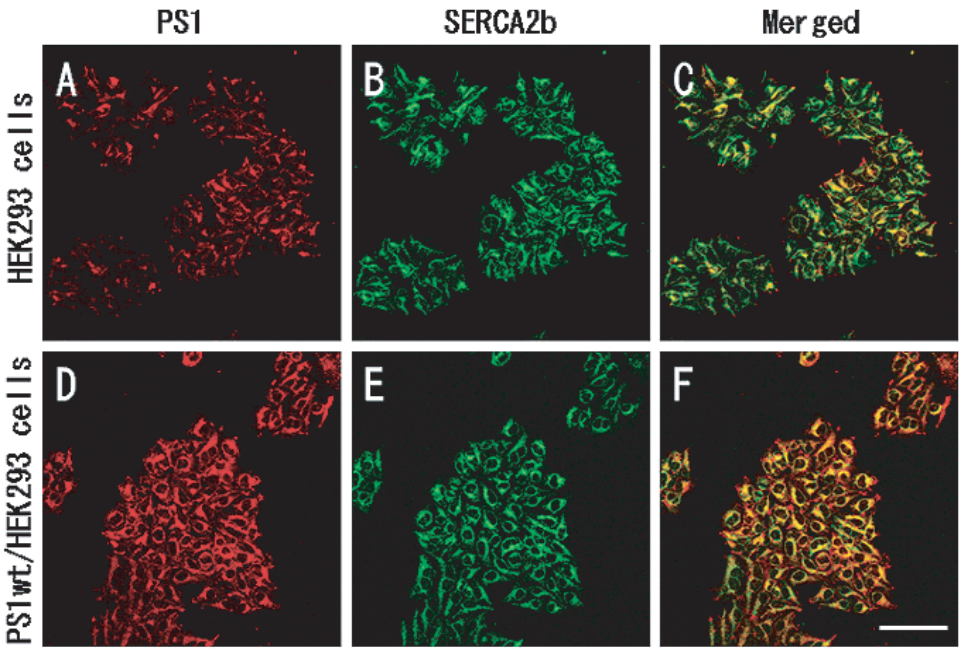
Supplementary Figure 1. LDH release assay and cell viability. HEK293 cells were exposed to various concentrations of the ER stress-inducing chemical compounds tunicamycin (A), thapsigargin (B), brefeldin-A (C), and staurosporine (D). The cells showed increases in both LDH release (bars) and dead cell ratio (lines; % of dead cells) in a dose-dependent manner. GOTO cells and KNS-42 cells were also exposed to the same compounds by the same methods. Bar indicate mean values ± SEM, $n = 3$.



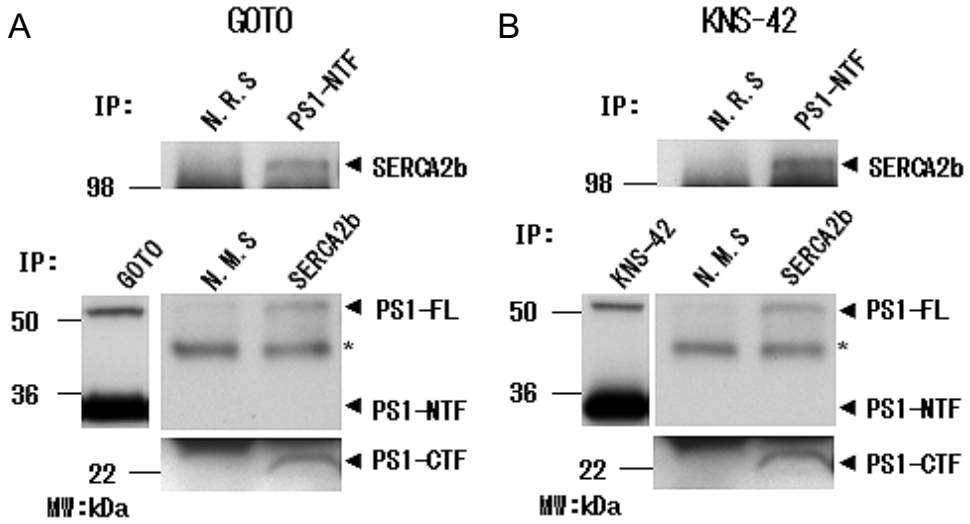
Supplementary Figure 2. Quantification of PSEN1 N- and C-terminal fragments after exposure to tunicamycin. Proteins (total 20 fÌg) were loaded in each lane for Western blotting. The intensity of each band was quantified relative to that observed before the exposure, expressed as 1.0. The relative amounts of PSEN1 CTFs and NTFs in GOTO (A) and KNS-42 (B) cells are indicated as mean values ± SEM. $n = 6$.



Supplementary Figure 3. Cytosolic calcium concentrations in cells overexpressing wild-type PSEN1 holoprotein. Changes in cytosolic calcium levels evoked by thapsigargin in cells stably overexpressing PSEN1 holoprotein or transfected empty vector. ER Ca^{2+} stores were released into the cytosol by application of 1 fÌM thapsigargin with no Ca^{2+} in the bathing solution. Basal cytosolic Ca2+ ($\sim$ 45 nM) levels and peak Ca^{2+} ($\sim$ 160 nM) signals after application of thapsigargin were reduced in cells stably overexpressing PSEN1 holoprotein. $n = 8$



Supplementary Figure 4. Co-localization of PSEN1 and SERCA. HEK293 cells (A, B, C) and HEK293 cells stably overexpressing PSEN1 (D, E, F) were investigated by confocal microscopy after being prepared, fixed, and co-stained with anti-PSEN1 antibody (A, D) or anti-SERCA2b (B, E) as described in the Methods. PSEN1 immunoreactivity shows co-localization with that of SERCA2b (D, F). Co-localization area is indicated in yellow. Bar = 100 μm.



Supplementary Figure 5. Interaction of PSEN1 holoprotein with SERCA. In co-immunoprecipitation experiments in GOTO (A) and KNS-42 (B) cells, PSEN1 holoproteins and CTFs interacted with SERCA channels. In each lane, 5 μl (PSEN1) or 10 μl (SERCA) from 50 μl of immunoprecipitated sample buffer was loaded

Supplementary Table 1
Concentrations of chemical compounds for experiments

Compound	HEK293	GOTO	KNS-42
Tunicamycin	8 μg/ml	6 μg/ml	15 μg/ml
Thapsigargin	4 μM	4 μM	12.5 μM
Brefeldin-A	75 μM	25 μM	100 μM
Staurosporine	100 nM	100 nM	300 nM