Figure EV1. Accumulation of the endogenous and over-expressed PS1 in ERQC of CsA-treated cells.

A Immunocytochemistry performed on CHO cells over-expressing wild-type PS1 and treated with CsA (60 μg/ml), using PS1 NTF and CTF antibodies. PS1 molecules that accumulate in the ringlike structure (arrows) react with both NTF and CTF antibodies. This observation indicates that full-length PS1 molecules form aggregates at an early maturation stage, prior to their self-cleavage, and are deposited in the structure.

B Immunocytochemistry of naïve NIH 3T3 cells treated with CsA (60 μg/ml). Labeling by PS1-CTF antibody showed accumulation of the endogenous PS1 in a ringlike structure (arrow).

C Immunocytochemistry performed on CsA-treated (60 μg/ml) CHO cells over-expressing wild-type PS1 using PS1-NTF and vimentin/ubiquitin antibodies. Vimentin fibers (red channel) do not collapse around the PS1-containing, ringlike structure.

D No ubiquitin signal (red) can be seen in PS1-containing ringlike shape of CsA-treated CHO-PS1 cells.

E Fluorescently tagged H2A, a known marker of the ERQC compartment, was expressed in CHO PS1 cells. Our results show that H2A is evenly distributed in untreated cells.

F Western blot of cyclophilin B and cyclophilin A showing that siRNA toward cyclophilin B or cyclophilin A notably reduces the levels of these proteins in CHO cells within 48 h.
Figure EV2. The substitution of proline 264, 267, or both in the sequence of PS1 leads to its accumulation in the ERQC upon proteasome inhibition.

A  CsA treatment induces the aggregation of P264L/P267S PS1 double mutant (DM). Western blot (WB) of samples prepared from CHO cells stably expressing DM P264L/P267S PS1 and treated with CsA (60 μg/ml), MG132 (10 μM for 5 h), or the vehicle. The cells were homogenized and subjected to high-speed sedimentation assay. Supernatants (S) and pellets (P) were separated and analyzed by WB using the PS1 CTF antibody. CsA and MG132 treatments resulted in the aggregation and sedimentation of DM PS1.

B  The inhibition of cyclophilins by CsA results in the deposition of P264L and P267S PS1 mutants as well as of the DM PS1 in the ERQC (arrows). CHO cells expressing the indicated PS1 mutants were treated with CsA and subjected to immunofluorescence.

C  Immunofluorescence experiment using PS1 NT and CTF antibodies. DM PS1 but not wild-type PS1 molecules accumulate in the ERQC of CHO-PS1 cells following proteasome inhibition by 10 μM MG132.

D, E  Neither D257A (D) nor A246E (E) PS1 mutants accumulate in ERQC of MG132-treated PS1-over-expressing CHO cells as observed by immunostaining using the PS1-NTF antibody.

F  No deposition of YFP-labeled dopamine transporter (DAT) in ERQC could be detected by fluorescence microscopy in CsA-treated CHO cells.
Figure EV3. The substitution of proline 264 or 267 in the sequence of PS1 leads to the attenuation of γ-secretase activity.

A WB analysis using PS1 NTF and PS2 CTF antibodies confirms the absence of these proteins in mouse MEF cells derived from PS1 and PS2 KO animals.

B No detectable γ-secretase activity found by WB analysis of C99-APP myc cleavage in cells lacking PS1 and PS2 (PS1/2 KO cells, lane 3). MEF cells derived from wild-type mouse exhibit normal γ-secretase activity as measured by the cleavage of the C99-APP myc substrate (lane 2).

C WB analysis of C99-APP myc was performed on samples of PS1/2 KO MEF cells. γ-secretase activity was restored in PS1/2 KO cells that were transiently transfected with the wild-type human PS1, partially restored by the transfection of the P264L PS1 mutant, but was not detectable in cells that were transfected with the P267S, DM, or the D257A PS1 mutants.

D Quantification of signal intensities in four independent experiments as in (C) unveils significantly lower restoration of γ-secretase activity in cells expressing the P264L, P267S, D257A, or the DM PS1 constructs compared to cells that express the human wild-type PS1. Error bars represent SEM, P < 0.01 (Student’s t-test).

E WB analysis of C99-APP myc cleavage performed on samples of PS1/2 KO MEF cells treated with CsA. The inhibition of cyclophilins by CsA reduces the rate of γ-secretase activity as measured by the C99-APP myc substrate cleavage assay in PS1/2 KO MEF cells expressing the wild-type PS1.

F A C-terminal β-APP-fluorescence-based activity assay performed on wild-type mouse MEF cells expressing only the endogenous PS1. Calibration experiment using purified membranes containing γ-secretase complex shows concentration-dependent activity.

G A C-terminal β-APP-fluorescence-based activity assay performed on cells that lack either PS1 or PS2. A reduced rate of γ-secretase activity was observed in cells lacking PS1 or PS2 as measured by the cleavage of a fluorescent γ-secretase substrate. Error bars represent SEM.
Figure EV4. Presenilin 2 accumulates in the ERQC of CsA-treated cells.
A, B  Confocal microscopy imaging of cells expressing GFP-tagged presenilin 2 (GFP-PS2) or GFP-PS2 and H2a-RFP. GFP-PS2 accumulates in the ERQC (arrows) of CHO cells stained by DAPI (A) or NIH 3T3 cells (B) following CsA treatment (60 μg/ml, 16 h).
Figure EV5. Cyclophilin B is required for maturation and integrity of PS1 in the mouse brain.

A, B Paraffin-embedded brain sections of wild-type and cyclophilin B KO mice were labeled with CTF PS1 antibody and DAB staining. Lower PS1 signals were observed in the hippocampus (A) and cortex (B) of cyclophilin B KO mice compared to the levels detected in wild-type animals (DG: dentate gyrus; HC: hippocampus).

C Brains of BALB/c mice that were injected with a lentivirus expressing GFP and the wild-type human PS1 or the P264L mutant and sacrificed 5 weeks later. Frozen sections were imaged using PS1 antibody (red) and Hoechst staining. While the wild-type protein appears in a diffuse pattern, P264L PS1 accumulates in well-defined foci (inset, arrows). Scale bar, 25 μm.

D A brain slice of a mouse that was injected with a lentivirus expressing the P264L PS1 mutant (labeled by PS1 antibody and then tagged by rhodamine) and a mask, created by the ImageJ software and used for foci counting and area quantification.