Cell, Volume *126*

Supplemental Data

Presenilin Forms ER Ca^{2+} Leak Channels,

a Function Disrupted by Familial

Alzheimer's Disease-Linked Mutations

Huiping Tu, Omar Nelson, Arseny Bezprozvanny, Zhengnan Wang, Sheu-Fen Lee, Yi-Heng Hao, Lutgarde Serneels, Bart De Strooper, Gang Yu, and Ilya Bezprozvanny

Supplemental Experimental Procedures

Calculations of Thermodynamical Limit for ER Ca^{2+}

The SERCA pump transports 2 Ca^{2+} ions for each ATP molecule hydrolyzed (Pickart and Jencks, 1984), the cytosolic Ca^{2+} concentration is resting cells is 0.1 μM Ca^{2+}, and there is no voltage gradient across the ER membrane. At the steady state the thermodynamic equilibrium for a Ca^{2+} ion moving between cytoplasm and ER:

$$T\Delta S = -\Delta H \quad (1)$$

Where T is the absolute temperature 298K, ΔS is the change in entropy and ΔH is the change in enthalpy of the system as a result of one Ca^{2+} ion movement across ER membrane.

$$\Delta S = R \ln ([Ca^{2+}]_{ER}/[Ca^{2+}]_{cyt}) \quad (2)$$

Where R = 1.987 cal/mol/K is a universal gas constant.

Because hydrolysis of 1 ATP molecule by SERCA leads to transport of 2 Ca^{2+} ions:

ΔH = ½ of ATP hydrolysis energy (3)

In cells the ATP hydrolysis energy is estimated to be –12 kcal/mol (Pickart and Jencks, 1984).

Combining equations 1 – 3 yields:

$$\ln ([Ca^{2+}]_{ER}/[Ca^{2+}]_{cyt}] = -\Delta H/RT = 6{,}000/592 = 10.1 \quad (4)$$

Therefore:

$$[Ca^{2+}]_{ER} = [Ca^{2+}]_{cyt}\, e^{10.1} = 0.1\ \mu M \times 24{,}343 = 2.4\ mM$$

The value of 2.4 mM is an upper limit for the intraluminal ER Ca^{2+} concentration calculated under the assumption that SERCA pump operates with 100% efficiency.

Expression of Presenilins in Sf9 Cells

Wild types and mutant presenilins were expressed in Sf9 cells as previously described for $InsP_3R$ (Tu et al., 2002). Briefly 150 ml of Sf9 cell culture was infected by PS-encoding baculoviruses at multiplicity of infection (MOI) of 5-10. Sf9 cells were collected at 66-72 h post-infection by centrifugation at 4°C for 5 min at 800 rpm (GH 3.8 rotor, Beckman Instruments, Fullerton, CA). The cellular pellet was resuspended in 25 ml of homogenization buffer A (0.25 M sucrose, 5 mM Hepes, pH 7.4) supplemented with protease inhibitors cocktail (1 mM EDTA, 2μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM bezamidine, 2.2 mM 4-(2-aminothyl)-benzenesulfonyl fluoride hydrochloride, 10 μg/ml pepstatin, 0.1 mg/ml phenylmethylsulfonyl fluoride). Cells were disrupted by sonication (Branson Ultrasonic, Danbury, CT) and manually homogenized on ice with glass-Teflon homogenizer. The microsomes were isolated from sf9 cell homogenate by differential centrifugation as previously described (Tu et al., 2002). Briefly 25 ml cells homogenate was centrifuged for 15 min at 4K g_{max} (J 25.50 rotor, Beckman Instruments). The supernatant fluid was filtered through cheese cloth, and the filtrate was centrifuged for 30 min at 90K g_{max} (Ti 50.2 rotor, Beckman Instruments). The pellet from the latter spin was resuspended in 25 ml of high-salt buffer B (0.6 M KCl, 5 mM NaN3, 20 mM Na4P2O7, 1 mM EDTA, 10 mM HEPES, pH 7.2) and manually homogenized on ice using Teflon/glass manual homogenizer and centrifuged for 15 min at 4 K g_{max} (J25.50 rotor, Beckman Instruments). The resulting supernatant fluid was centrifuged for 30 min at 90 K g_{max} (Ti 50.2 rotor, Beckman Instruments). The pellet from the last spin was resuspended in 0.5 ml of the storage buffer (10% sucrose, 10 mM 3-morgholinopropanesulfoni acid, pH 7.0) to typically yield 6 mg/ml of protein (Bradford assay, Bio-rad), aliquoted, quickly frozen in liquid nitrogen, and stored at –80°C.

Planar Lipid Bilayer Experiments

Planar lipid bilayer (BLM) recordings of presenilin-supported currents were performed as previously described for studies of $InsP_3R$ (Tu et al., 2002; Tu et al., 2003; Tu et al., 2005a; Tu et al., 2005b). In these experiments *trans* (intraluminal) side of the BLM contained 50 mM Ba/HEPES (pH 7.35) or 100 mM Cs/HEPES (pH 7.35) or 200 mM Cs/HEPES (pH 7.35) and *cis* (cytosolic) side of the BLM contains 100 mM Tris/HEPES (pH 7.35). Thus, 50 mM Ba^{2+} or 100 mM Cs^+ or 200 mM Cs^+ in the *trans* compartment are main current carriers in these experiments as indicated in the text. The ER microsomes isolated from Sf9 cells infected with PS-encoding baculoviruses were added to the *cis* chamber with the pipet, and fusion of microsomes with the BLM was induced by osmotic pressure resulting from addition of 0.6-1 M KCl to the *cis* chamber. Fusion of ER microsomes to the bilayer leads to incorporation of the channels in such as orientation to lumen of ER (Miller, 1986). Fusion of ER vesicles with the bilayer was registered by the appearance of chloride currents. Once sufficient fusion was achieved ($\geq$100 pA of chloride currents for all experiments), *cis* chamber (cytosolic) was perfused with 20 vol of *cis* recording solution (110 mM Tris dissolved in HEPES, pH 7.35) with stirring. The *cis* chamber was held at virtual ground and the *trans* chamber was voltage clamped (OC-725 bilayer clamp, Warner Instruments) to 0 mV, +10 mV and –10 mV as indicated. The liquid junction potential between *cis* and *trans* recording solutions was compensated before formation of the bilayer. The current across the BLM was amplified (OC-725C), filtered at 5 kHz, digitized (Digidata 1200, Axon Instruments), and stored on computer hard drive and recordable optical discs. For presentation the current traces were digitally filtered at 200 Hz (pClamp 6.0, Axon Instruments)

For off-line computer analysis, stationary noise analysis method (Neher and Stevens, 1977) was used. Using WinEDR V2.4.3 (Dempster, 2001), the currents were filtered at 100 Hz and the mean current (I) and the current variance (δ^2) was determined for the currents measured before addition of ER microsomes (I_{BLM} and $\delta_{BLM}{}^2$) and after fusion of ER microsomes (I_{PS} and

δ_{PS}^2) in the same experiment. The unitary size of PS-mediated currents (i_{PS}) was then estimated for each experiment from the "noise analysis equation" (Neher and Stevens, 1977):

$$i_{PS} = (\delta_{PS}^2 - \delta_{BLM}^2) / (I_{PS} - I_{BLM}) \quad (5)$$

Purification and Reconstitution of His-PS1 and His-PS1-M146V

ER microsomes were prepared from Sf9 cells infected with His-PS1 and His-PS1-M146V baculoviruses as described above. The membrane proteins were extracted from isolated microsomes with 1% CHAPS in the extraction buffer (50 mM phosphate pH 8.0, 150 mM NaCl) at total protein concentration of 1 mg/ml. His-PS1 and His-PS1-M146V proteins were purified by binding to Ni-NTA agarose beads and eluted with 200 mM Imidazole in the same buffer after several washing steps as previously described (Shah et al., 2005). The eluates were dialyzed against 15 mM HEPES, pH 7.5, 150 mM NaCl, 0.5 mM EGTA, 2% CHAPS overnight and then concentrated by Amico Ultra Centrifugal Filter Devices to about 0.7 mg/ml. Purified protein was stored at -80^{o}C in the presence of 10% glycerol before use. In the reconstitution experiments 100 μg of partially purified His-PS1 or His-PS1-M146V protein was added to a mixture of 1 mg sonicated phospholipid mixture (PE:PS:PC=5:1:2) and 2% CHAPS in 0.1 ml NaCl reconstitution buffer (15 mM HEPES, pH 7.5, 150 mM NaCl, 0.8 mM EGTA). After 1 hr incubation on ice, the mixture was dialyzed at 4°C against 2 liters of the reconstitution buffer for 4 days with daily buffer changes. Ergosterol/nystatin protein-free liposomes were prepared by sonicating a mixture of PE:PS:PC:ergosterol lipids (5:2:1:2) in the presence of 100 μg/ml nystatin in the reconstitution buffer. The His-PS1 and His-PS1-M146V proteoliposomes were mixed with equal volume of ergosterol/nystatin protein-free liposomes and mixture was frozen, thawed, and sonicated for 10 s. The cycle was repeated twice and the final proteoliposomes were aliquoted and stored at -80°C with addition of 20% sucrose.

The planar lipid bilayers were formed as described above in the presence of reconstitution buffer (15 mM HEPES, pH 7.5, 150 mM NaCl, 0.8 mM EGTA) in both *cis* and *trans* chambers. The His-PS1 and His-PS1-M146V phospholipid/ergosterol/nystatin proteoliposomes prepared as described above were added with stirring to the *cis* compartment to yield 20-100 μg/ml final protein concentration and the liposomal fusion was induced by addition of 3M NaCl to the *cis* chamber to give a final concentration of 600 mM NaCl on the *cis* side. In agreement with the previous report (Woodbury and Miller, 1990), liposomal fusion was detected as the appearance of transient nystatin channel activity. The liposomal fusion was terminated by perfusion of the *cis* chamber with the buffer composed of 15 mM HEPES, pH 7.5, 600 mM NaCl and 0.8 mM EGTA. The currents across the bilayer were recorded as described above (600 mM NaCl *cis*, 150 mM NaCl *trans*) at +10 mV, 0 mV and –10 mV membrane potentials.

Cytosolic Ca^{2+} Imaging Experiments

The DKO fibroblast cell line and control wild type MEF cell line have been previously described (Herreman et al., 2000). The fibroblast cells culture on poly-D-lysine (Sigma) coated 12 mm round glass coverslips were established by modifying the following published procedures (Herreman et al., 2000). Ca^{2+} imaging experiments with DKO and MEF cells have been performed as previously described for primary cultures of medium spiny neurons (MSN) (Tang et al., 2003). Briefly, the DKO or MEF cells were loaded with 5 μM Fura-2-AM (Molecular Probes) in HCSS buffer (120 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 2 mM $CaCl_2$, 15 mM glucose, and 20 mM HEPES, pH 7.3) for 45 min at 37^{o}C. For Ca^{2+} imaging experiments the coverslips were mounted onto a recording/perfusion chamber (RC-26G, Warner Instrument), positioned on the movable stage of an Olympus IX-70 inverted microscope and washed with HCSS-Ca^{2+} deficient buffer (buffered with EGTA to 50 nM Ca^{2+}). In Ca^{2+} imaging experiments

the cells were intermittently excited by 340 nM and 380 nM UV light (DeltaRam illuminator, PTI) using a Fura-2 dichroic filter cube (Chroma Technologies) and 60× UV-grade oil-immersed objective (Olympus). The emitted light was collected by an IC-300 camera (PTI), and the images were digitalized by ImageMaster Pro software (PTI). Baseline (6 min) measurements were obtained prior to bath application of drugs. The drugs - 300 nM Bradykinin, 1μM ionomycin, or 1 μM Thapsigargin (all – from Sigma) were dissolved in HCSS-Ca^{2+} deficient buffer prior to application to the cells. Images at 340 nm and 380 nm excitation wavelengths were captured every 2 s and shown as 340/380 image rations at time points as indicated. Background fluorescence was determined according to manufacturer's (PTI) recommendations and subtracted.

The absolute values of free cytosolic Ca^{2+} concentrations ($[Ca^{2+}]$) in these experiments were determined from the equation (Grynkiewicz et al., 1985):

$$[Ca^{2+}] = K_d \frac{[R - R_{min}]}{[R_{max} - R]} \frac{}{s_{b,380}} \qquad (6)$$

where K_d = 140 nM is the affinity of Fura-2 for Ca^{2+}, R is the experimentally determined 340/380 ratio, R_{max} is the 340/380 ratio for Fura-2 saturated with Ca^{2+} (determined by application of 20 mM Ca^{2+} and 10 μM ionomycin at the end of the experiment), followed by washout and addition of HCSS buffer containing 10 mM EGTA to determine R_{min} as 340/380 ratio for Ca^{2+}-free Fura-2, and $s_{f,\,380}/s_{b,\,380}$ is the ratio of fluorescence intensity of Ca^{2+}-free and Ca^{2+}-bound form of Fura-2 at 380 nM ($s_{f,380}/s_{b,380}$ = 2 in our experiments).

Microsomal Ca^{2+} Flux Assay

The ER microsomes were prepared from non-infected Sf9 cells and from Sf9 cells infected with PS1 and PS1-M146V baculoviruses as described above. The ER microsomes were prepared from wild type MEFs, DKO MEFs and the DKO MEFs stably transfected with human PS1 rescue construct (HPS1) as previously described for HEK293 cells (Kaznacheyeva et al., 1998). The Sf9 and MEF microsomes were aliquoted and stored at –80°C in the presence of 20% sucrose. The microsomal Ca^{2+} flux assay was performed with constant stirring in a 2 ml quartz cuvette using Felix system (PTI). A freshly thawed ER microsomal fraction was resuspended in the experimental buffer (50 mM HEPES pH 7.2, 110 mM KCl, 10 mM NaCl, 10 mM KH_2PO_4) with addition of 2 mM $MgCl_2$, 1 mM DTT and 2 μM Fura-2 free acid (Molecular Probes). An energy conserving system consisting of creatine kinase (20 units/ml) and phosphocreatine (20 mM) was added. Subsequent addition of ATP to a final concentration of 2 mM resulted in a decrease in the Fura-2 340/380 ratio signal, indicating Ca^{2+} uptake by the microsomes. After Fura-2 340/380 ratio reached steady-state levels (typically 1 hour after addition of ATP), 5 μM Thapsigargin was added to the cuvette to block SERCA pump activity and the passive Ca^{2+} efflux from microsomes was detected as an increase in 340/380 Fura-2 ratio. At the end of each experiment, the 340/380 Fura-2 ratios were calibrated by consecutive additions of 10 μM ionomycin, 2 mM $CaCl_2$ and 20 mM EDTA. The 340/380 Fura-2 ratios were converted to free Ca^{2+} concentration in the cuvette by using the equation from (Grynkiewicz et al., 1985) as described above.

ER Ca^{2+} Measurements

The ER Ca^{2+} measurements in MEF cells were performed according to procedure previously described for BHK fibroblasts (Hofer, 1999; Hofer and Schulz, 1996). Briefly, MEF cells were loaded with 2 μM low affinity Ca^{2+} imaging dye Mag-Fura-2-AM (Molecular Probes) in HCSS buffer (120 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 2 mM $CaCl_2$, 15 mM glucose, and 20 mM

HEPES, pH 7.3) for 45 min at 37^{o}C. The imaging experiments with Mag-Fura-2-loaded MEF cells were performed using PTI Ca^{2+} imaging setup described above. MEFs loaded with Mag-Fura-2 were permeabilized by application of 10 μM Digitonin in the intracellular buffer (125 mM KCl, 25 mM NaCl, 10 mM HEPES, 0.1 mM MgCl2, pH 7.3) containing 170 nM free Ca^{2+} (clamped by 5 mM EGTA) and 3 mM ATP. The Mag-Fura-2 signals were collected as 340/380 ratios for a duration of an experiment. At the end of the experiment the ER membrane was permeabilized with 5 μM ionomycin and cells were washed by ATP-free and Ca^{2+}-free calibration buffer (125 mM KCl, 25 mM NaCl, 10 mM HEPES, pH 7.3) containing 0.8 mM EGTA. The Mag-Fura-2 signals were calibrated in the presence 10 μM ionomycin by using a series of calibration buffers with free Ca^{2+} clamped to defined concentrations (200 μM Ca^{2+}, 400 μM Ca^{2+}, 600 μM Ca^{2+}, 800 μM Ca^{2+}) by 1 mM nitrilotriacetic acid (NTA). The free Ca^{2+} concentration in calibration buffers was calculated by WEBMAXC STANDARD program. Based on these calibration results, the 340/380 Mag-Fura-2 ratios were converted to ER Ca^{2+} concentrations by using an empirical formula:

$$[Ca^{2+}]_{ER} = 141 (R – 0.49) / (1.42 - R) \qquad (7)$$

Where $[Ca^{2+}]_{ER}$ is ER Ca^{2+} concentration in μM and R is the 340/380 ratio reported by Mag-Fura-2 in our experiments following permeabilization of plasma membrane with 10 μM Digitonin.

Supplemental References

Dempster, J. (2001). The laboratory computer: a practical guide for neuroscientists and physiologists, Academic Press).

Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985). A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem *260*, 3440-3450.

Herreman, A., Serneels, L., Annaert, W., Collen, D., Schoonjans, L., and De Strooper, B. (2000). Total inactivation of gamma-secretase activity in presenilin-deficient embryonic stem cells. Nat Cell Biol *2*, 461-462.

Hofer, A. M. (1999). Measurement of free [Ca2+] changes in agonist-sensitive internal stores using compartmentalized fluorescent indicators. Methods Mol Biol *114*, 249-265.

Hofer, A. M., and Schulz, I. (1996). Quantification of intraluminal free [Ca] in the agonist-sensitive internal calcium store using compartmentalized fluorescent indicators: some considerations. Cell Calcium *20*, 235-242.

Kaznacheyeva, E., Lupu, V. D., and Bezprozvanny, I. (1998). Single-channel properties of inositol (1,4,5)-trisphosphate receptor heterologously expressed in HEK-293 cells. J Gen Physiol *111*, 847-856.

Miller, C., ed. (1986). Ion Channel Reconstitution (New York, Plenum Press).

Neher, E., and Stevens, C. F. (1977). Conductance fluctuations and ionic pores in membranes. Annu Rev Biophys Bioeng *6*, 345-381.

Pickart, C. M., and Jencks, W. P. (1984). Energetics of the calcium-transporting ATPase. J Biol Chem *259*, 1629-1643.

Shah, S., Lee, S. F., Tabuchi, K., Hao, Y. H., Yu, C., LaPlant, Q., Ball, H., Dann, C. E., 3rd, Sudhof, T., and Yu, G. (2005). Nicastrin functions as a gamma-secretase-substrate receptor. Cell *122*, 435-447.

Tang, T.-S., Tu, H., Chan, E. Y., Maximov, A., Wang, Z., Wellington, C. L., Hayden, M. R., and Bezprozvanny, I. (2003). Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. Neuron *39*, 227-239.

Tu, H., Miyakawa, T., Wang, Z., Glouchankova, L., Iino, M., and Bezprozvanny, I. (2002). Functional characterization of the type 1 inositol 1,4,5-trisphosphate receptor coupling domain SII(+/-) splice variants and the *opisthotonos* mutant form. Biophys J *82*, 1995-2004.

Tu, H., Nosyreva, E., Miyakawa, T., Wang, Z., Mizushima, A., Iino, M., and Bezprozvanny, I. (2003). Functional and biochemical analysis of the type 1 inositol (1,4,5)-trisphosphate receptor calcium sensor. Biophysical J *85*, 290-299.

Tu, H., Wang, Z., and Bezprozvanny, I. (2005a). Modulation of mammalian inositol 1,4,5-trisphosphate receptor isoforms by calcium: a role of calcium sensor region. Biophys J *88*, 1056-1069.

Tu, H., Wang, Z., Nosyreva, E., De Smedt, H., and Bezprozvanny, I. (2005b). Functional characterization of mammalian inositol 1,4,5-trisphosphate receptor isoforms. Biophys J *88*, 1046-1055.

Woodbury, D. J., and Miller, C. (1990). Nystatin-induced liposome fusion: a versatile approach to ion channel reconstitution into planar bilayers. Biophys J *58*, 833-839.

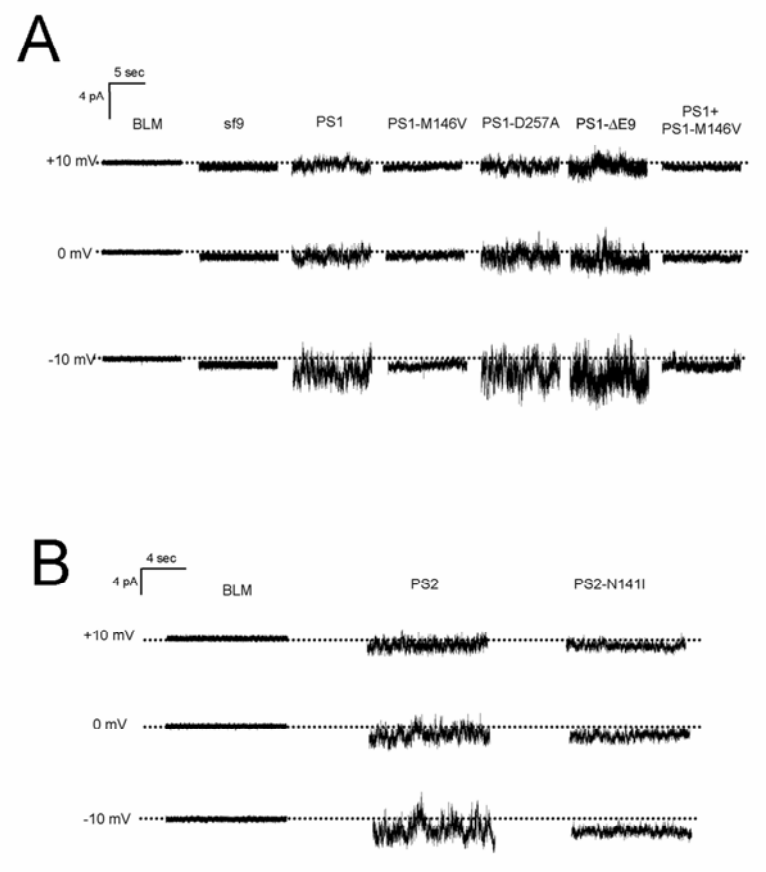

Figure S1. PS1 and PS2 Support Cs^+ Currents in Planar Lipid Bilayers

(A and B) The Cs^+ currents recorded in planar lipid bilayer (BLM) experiments at +10 mV, 0 mV, and –10 mV membrane potentials are shown for empty BLM (BLM), for microsomes from non-infected Sf9 cells (Sf9), and for microsomes from Sf9 cells infected with wild type and mutant PS1 (A) and PS2 (B) baculoviruses as indicated. The dotted lines represent the zero level for the current traces. For each experiment 10 s of continuous current recording is shown. Similar results were obtained in at least three experiments with each construct.

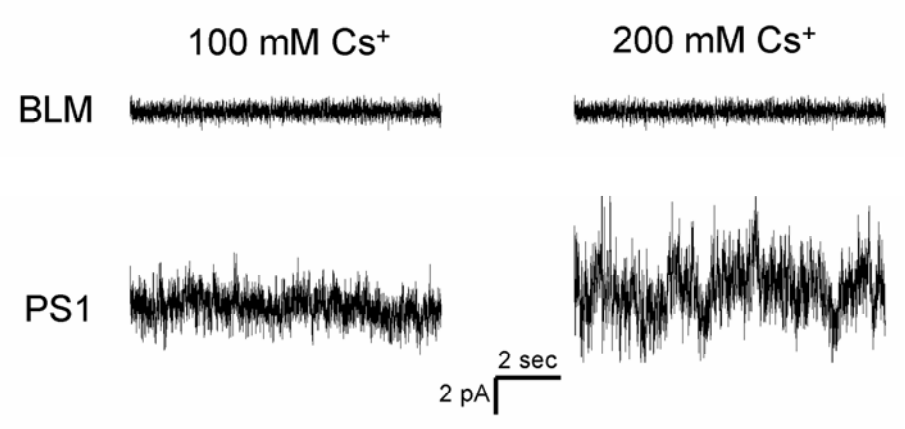

Figure S2. PS1-Mediated Currents with 200 mM Cs^+ as a Current Carrier

The 100 mM Cs^+ and 200 mM Cs^+ currents recorded in planar lipid bilayer experiments at 0 mV membrane potentials are shown for empty BLM (BLM) and following fusion of ER microsomes isolated from Sf9 cells infected with wild type PS1 baculoviruse (PS1). The unitary current for 100 Cs^+ is equal to 0.21 pA and for 200 mM Cs^+ is equal to 0.58 pA. For each experiment 10 s of continuous current recording is shown. Similar results were obtained in at least three independent experiments.

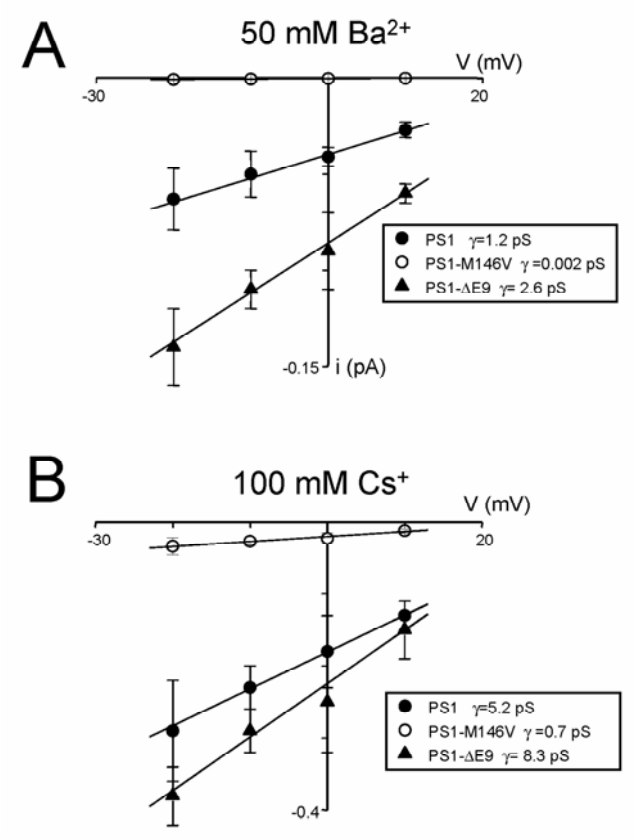

Figure S3. Current-Voltage Relationship for PS1-Mediated Currents

(A) An average estimates of Ba^{2+} unitary current at –20 mV, -10 mV, 0 mV and + 10 mV transmembrane voltages are shown as mean ± SD (n = 3) for experiments with PS1 (solid circles), PS1-M146 (open circles) and PS1-ΔE9 microsomes (solid triangles) . The linear fit to the data (lines) yielded the average microscopic Ba^{2+} conductance (γ_{Ba}) equal to 1.2 pS for PS1, 0.002 pS for PS1-M146V, and 2.6 pS for PS1-ΔE9.

(B) An average estimates of Cs^{+} unitary current at –20 mV, -10 mV, 0 mV and + 10 mV transmembrane voltages are shown as mean ± SD (n = 3) for experiments with PS1 (solid circles), PS1-M146 (open circles) and PS1-ΔE9 microsomes (solid triangles) . The linear fit to the data (lines) yielded the average microscopic Cs^{+} conductance (γ_{Cs}) equal to 5.2 pS for PS1, 0.7 pS for PS1-M146V and 8.3 pS for PS1-ΔE9.

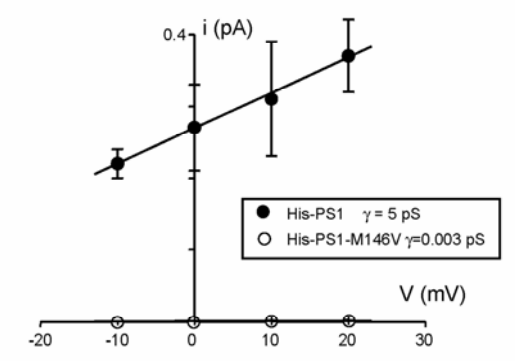

Figure S4. Current-Voltage Relationship for His-PS1-Mediated Currents

An average estimates of Na^+ unitary current at –10 mV, 0 mV, +10 mV and +20 mV transmembrane voltages are shown as mean ± SD (n = 3) for experiments with His-PS1 (solid circles) and His-PS1-M146 (open circles) proteoliposomes. The linear fit to the data (lines) yielded the average microscopic Na^+ conductance (γ_{Na}) equal to 5 pS for His-PS1 liposomes and 0.003 pS for His-PS1-M146V liposomes.

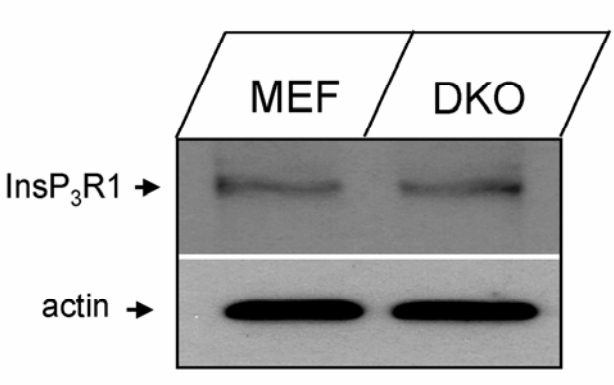

Figure S5. Expression of $InsP_3R1$ in MEF and DKO Cells

Expression of $InsP_3R1$ in MEF and DKO cells. The microsomes prepared from MEF and DKO cells as indicated were analyzed by Western blotting with anti-$InsP_3R1$ rabbit polyclonal antibodies (T443) (upper panel) (Kaznacheyeva et al., 1998) and anti-actin monoclonal antibodies (lower panel).

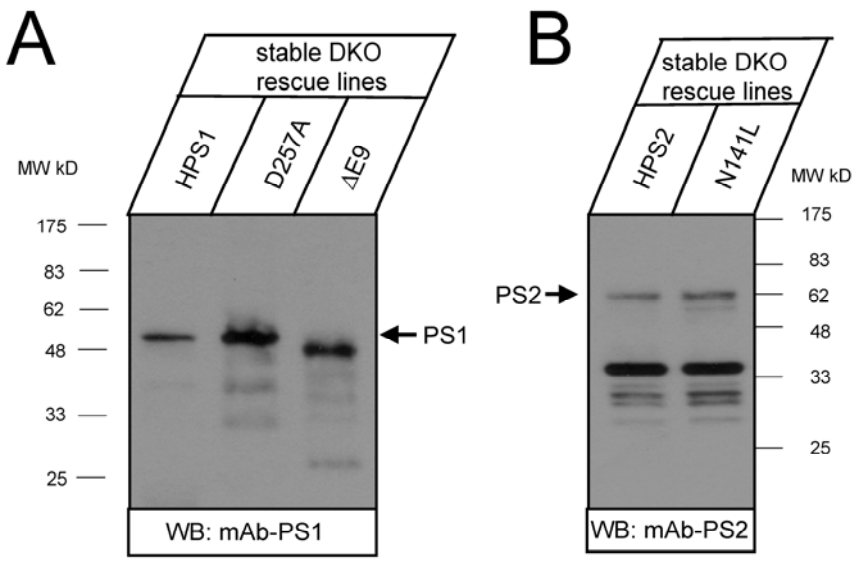

Figure S6. Expression of PS1 and PS2 in Stable DKO Rescue Lines

(A) Western blot analysis of lysates from the DKO cell lines stably transfected with human PS1 rescue construct (HPS1), PS1-D257A construct (D257A) and PS1-ΔE9 construct (ΔE9). Anti-PS1 mouse monoclonal antibody was used for Western blotting.

(B) Western blot analysis of lysates from the DKO cell lines stably transfected with human PS2 rescue construct (HPS2) and PS2-N141I (N141I) construct. Anti-PS2 mouse monoclonal antibody was used for Western blotting.

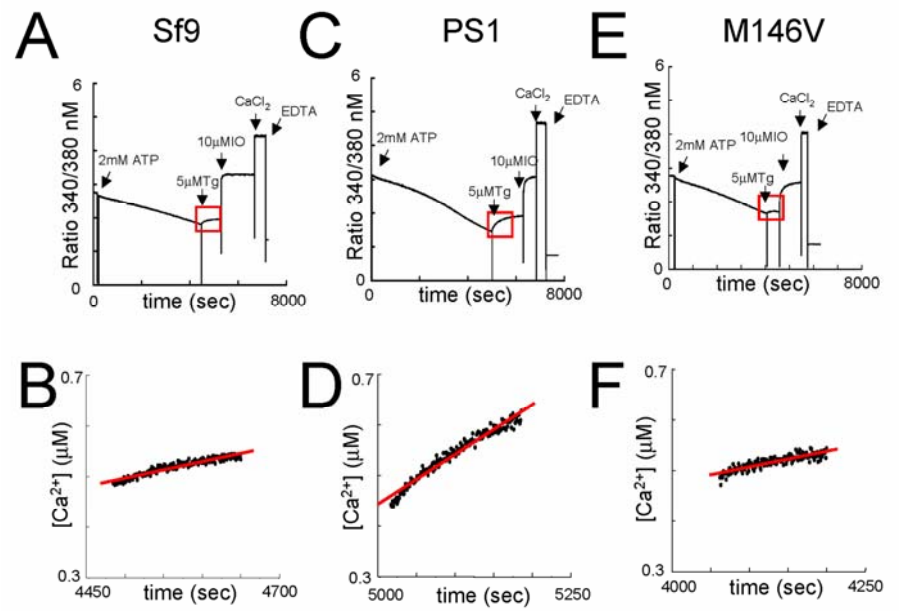

Figure S7. PS1 Facilitates Ca^{2+} Leak in Microsomes from Sf9 Cells

(A) 340/380 Fura-2 ratios are plotted versus time for an experiment with ER microsomes isolated from non-infected Sf9 cells (Sf9) . Ca^{2+} uptake into microsomes is initiated by addition of 2 mM ATP. Times of addition of 5 μM thapsigargin (Tg), 10 μM ionomycin (IO), 2 mM $CaCl_2$ and 20 mM EDTA are also shown.

(B) By using procedure described in Methods Fura-2 340/380 ratios were converted to Ca^{2+} concentrations in the cuvette. The cuvette Ca^{2+} concentration immediately following addition of 5 μM thapsigargin is plotted against time. The slope of the linear fit to these data (red line) provides an estimate for the rate of passive Ca^{2+} leak from the ER microsomes. The rate of Ca^{2+} leak for the experiment shown is equal to 0.38 nM Ca^{2+}/s.

(C and D) Similar experiment as in (A) and (B) was performed with ER microsomes isolated from Sf9 cells infected with PS1 baculovirus. The rate of Ca^{2+} leak for the experiment shown is equal to 0.95 nM Ca^{2+}/s.

(E and F) Similar experiment as in (A) and (B) was performed with ER microsomes isolated from Sf9 cells infected with PS1-M146V baculovirus. The rate of Ca^{2+} leak for the experiment shown is equal to 0.31 nM Ca^{2+}/s.

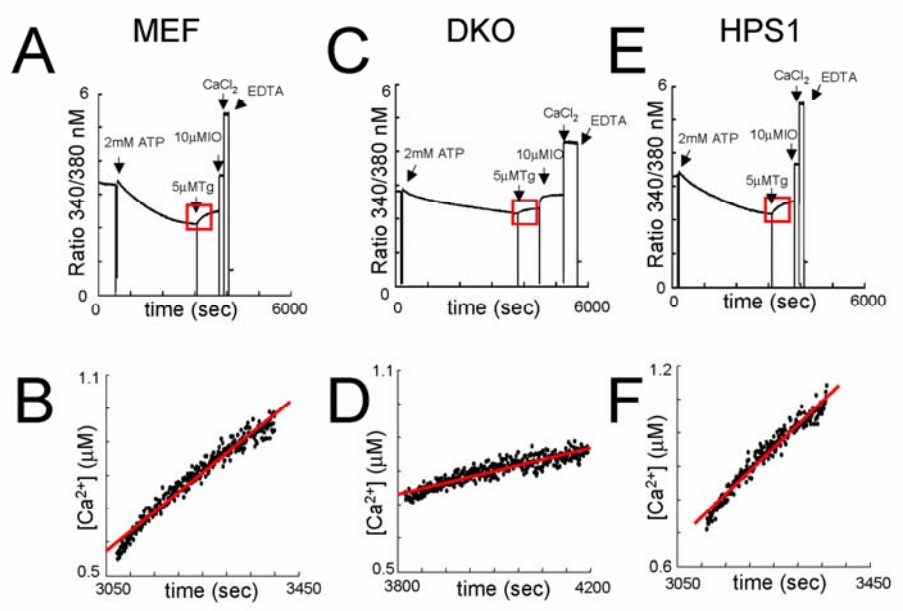

Figure S8. Presenilins Support Passive Ca^{2+} Leak in MEF Microsomes

(A) 340/380 Fura-2 ratios are plotted versus time for an experiment with ER microsomes isolated from wild type MEFs. Ca^{2+} uptake into microsomes is initiated by addition of 2 mM ATP. Times of addition of 5 μM thapsigargin (Tg), 10 μM ionomycin (IO), 2 mM $CaCl_2$ and 20 mM EDTA are also shown.

(B) By using procedure described in Methods Fura-2 340/380 ratios were converted to Ca^{2+} concentrations in the cuvette. The cuvette Ca^{2+} concentration immediately following addition of 5 μM thapsigargin is plotted against time. The slope of the linear fit to these data (red line) provides an estimate for the rate of passive Ca^{2+} leak from the ER microsomes. The rate of Ca^{2+} leak for the experiment shown is equal to 1.17 nM Ca^{2+}/s.

(C and D) Similar experiment as in (A) and (B) was performed with ER microsomes isolated from DKO MEFs. The rate of Ca^{2+} leak for the experiment shown is equal to 0.34 nM Ca^{2+}/s.

(E and F) Similar experiment as in (A) and (B) was performed with ER microsomes isolated from HPS1 DKO stable rescue line. The rate of Ca^{2+} leak for the experiment shown is equal to 1.34 nM Ca^{2+}/s.