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Supplemental Data

A Polymorphism in CALHM1 Influences

Ca^{2+} Homeostasis, Aβ Levels,

and Alzheimer's Disease Risk

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Supplemental Results

Tissue expression profiles of CALHM2 and CALHM3

We used TissueInfo (Skrabanek and Campagne, 2001) to predict the expression of CALHM2, and CALHM3 in human tissues. CALHM2 is expressed most abundantly in the uterus. CALHM2 expression was also detected in: "pancreas, dorsal root ganglion, ganglion, muscle, corpus callosum, leukocyte, kidney, liver, gland, pancreatic islets, prostate, fibroblast, colon, mammary gland, amygdala, lung, thalamus, stem cell, artery, spleen, hippocampus, alveolar macrophage, thymus, eye, gut, skin, optic nerve, adrenal gland, heart, hypothalamus, ovary, cartilage, medulla oblongata, brain, placenta, testis, cervix, oligodendrocyte, subthalamus, bone, breast, adipose, epithelium, head, astrocyte, T cell, central nervous system". CALHM3 is expressed most abundantly in the placenta. CALHM3 expression was also detected in: "lymphocyte, cervix".

Copy number variation (CNV) analysis in the *CALHM1* gene

To analyze *CALHM1* gene dosage, we used quantitative multiplex PCR of short fluorescent fragments (QMPSF), a method based on the simultaneous amplification of multiple short genomic sequences with dye labeled primers under quantitative conditions (Casilli et al., 2002). An assay based on four amplicons located in the promoter, exon 1, exon 2, and 3'-UTR of the *CALHM1* gene revealed a variation in one out of 55 unrelated controls located within the 3'-UTR region. This observation was consistent with an initial report of a potential CNV in *CALHM1* in Yoruba individuals (frequency of 1.6%) (Conrad et al., 2006). Our data do not support the view that a common CNV encompassing the rs2986017 locus disrupt the Hardy-Weinberg equilibrium for this marker. However, we cannot exclude that the deletion of a short-sized segment around rs2986017 might be involved.

Supplemental Experimental Procedures

Bioinformatics analyses

Sequence alignment and phylogeny reconstruction. Orthologs of CALHM1 were obtained from complete genomes available from Ensembl build 36 (Kasprzyk et al., 2004). A multiple sequence alignment of human CALHM1, CALHM2, CALHM3 and CALHM1 orthologs was constructed with T-coffe v 4.45 (Notredame et al., 2000) and manually inspected. Phylogenetic trees constructed with JalView indicated an erroneous mouse ortholog assignment. The most likely

CALHM1 mouse ortholog was found to be RefSeq XP_921421. This sequence was used for constructing the phylogenetic tree shown in Fig. 1. The phylogenetic tree was created with Phylip (Felsenstein, 2005) and the tree rendered as an unrooted tree with Phylodendron (http://iubio.bio.indiana.edu/treeapp/treeprint-form.html).

Materials and antibodies

PNGase F, SNX-482, mibefradil, nifedipine, ω-conotoxin MVIIC, $CoCl_2$, $NiCl_2$, β-mercaptoethanol, and retinoic acid were obtained from Sigma. Endoglycosidase H, xestospongin C, 2-APB, and dantrolene were from Calbiochem. Anti-Myc antibody (clone 9E10) was from Chemicon and anti-V5 antibody from Invitrogen. Anti-actin and anti-N-cadherin antibodies were from BD Transduction Laboratories and anti-GRP78 antibody from Abcam. Anti-$A\beta_{1-17}$ (6E10) and anti-APP (LN27) monoclonal antibodies were from Signet and Zymed, respectively. Anti-PS1/CTF (33B10) monoclonal antibody was provided by Dr. N.K. Robakis, Mount Sinai School of Medicine, New York, NY (Georgakopoulos et al., 1999).

RT-PCR

Total human RNA (Clontech) from several brain regions and 20 tissues (Human Total RNA Master Panel II) was subjected to RT reactions using M-MLV-RT and random hexamer primers (Invitrogen). PCR reactions were performed using GoTaq Flexi DNA polymerase (Promega). Primers for *β-actin* were CTC CTT AAT GTC ACG CAC GAT TTC (sense) and GCC AAC CGC GAG AAG ATG ACC (antisense) (Maxim Biotech) and for *CALHM1*, TGC TTC CTC TGT GCC TTC TG (sense) and CTC CAG GTC ATG GTT CAT GG (antisense).

Real-time quantitative RT-PCR

Relative expression levels of *CALHM1* were assessed by probe-based real-time quantitative RT-PCR (qRT-PCR). Total RNA was extracted from cells using RNeasy Minikit (Qiagen). RNA samples were further treated with DNA-free™ Kit (Ambion). 1 µg RNA was reverse transcribed using M-MLV-RT (Invitrogen) and random hexamers as primers. Amplifications of 100 ng cDNA were performed in triplicates in 25 µl reaction mixtures containing 200 nM primers and 100 nM probe (Universal Probe Library, Roche). Primers and probes were as follows: Forward 5'-TGA CCT TGA TTT ATT TTG CAT ACC-3' and reverse 5'-CGA GCA AGA CGT TCA GTC CT-3' with probe #73 for *HPRT* (house keeping gene); forward 5'-TAC CTC CGC TGC ATC TCC -3' and reverse 5'-ACA GAG CGC ACC ACG AAT -3' with probe #26 for *CALHM1*. The PCR was run on an ABI 7900HT Sequence Detection System (Applied Biosystems). Relative gene expression was determined with the $2^{-\Delta\Delta Ct}$ method.

Cell cultures, transfections, and oocyte injections

HT-22 cells were provided by Dr. D. Schubert (Salk Institute, La Jolla, CA). N2a cells stably transfected with human APP_{695} harboring the Swedish double mutation ($SwAPP_{695}$-N2a) were obtained from Dr. G. Thinakaran (University of Chicago, Chicago, IL). SH-SY5Y cells stably transfected with human APP_{695} (APP_{695}-SH-SY5Y) were obtained from Dr. L. Buée (INSERM U815, Centre Jean-Pierre Aubert, Lille, France) (Vingtdeux et al., 2007). WT and PS-deficient MEFs were provided by Dr. B. De Strooper (K.U. Leuven and VIB, Leuven, Belgium). All cell lines were tested negative for mycoplasma using MycoSensor PCR Assay Kit (Stratagene). HT-22, human embryonic kidney 293 (HEK293), and Chinese hamster ovary (CHO) cell lines were maintained in Dulbecco's Modified Earle's Medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone), 2 mM L-glutamine, and penicillin and streptomycin (Invitrogen). $SwAPP_{695}$-N2a and APP_{695}-SH-SY5Y cells were maintained in 1:1 DMEM / Opti-MEM supplemented with 10%

fetal bovine serum, penicillin and streptomycin, and 0.2 mg/ml G418. PC12 cells were grown in F-12K medium (ATCC) supplemented with 15% horse serum (Invitrogen), 2.5% fetal bovine serum, and penicillin and streptomycin. All cell lines were transiently transfected with WT or mutated *CALHM1* cDNA using Lipofectamine PLUS reagent (Invitrogen) for HEK293 and CHO cells or Lipofectamine 2000 (Invitrogen) for HT-22, $SwAPP_{695}$-N2a, and PC12 cells. For electrophysiology, CHO-K1 cells, chosen because they have little endogenous ionic currents (Gamper et al., 2005), were grown in Kaighn's F-12 medium (ATCC) supplemented with 10% fetal bovine serum (Hyclone) and 1% antibiotic-antimytotic (Invitrogen). One day prior to transfection, cells were plated onto glass cover slips and transfected with a 1:1 (v/v) mixture of Lipofectamine 2000 and cDNAs (1 μg/μl) coding for either WT- or P86L-CALHM1 in pIRES2-EGFP vector (Clontech). Stage VI oocytes isolated from *Xenopus laevis* (NASCO) were defolliculated by collagenase treatment, injected with human CALHM1 cRNA (46 ng; m-MESSAGE mMACHINE, Ambion) or 50 nl water, and maintained in standard oocyte solution (100 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, and 1 mM $MgCl_2$, pH 7.6) supplemented with 50 μg/ml gentamycin at 16°C.

SH-SY5Y cell differentiation and RNA interference

APP_{695}-SH-SY5Y cells were differentiated with retinoic acid (10 μM) in serum-free 1:1 DMEM / Opti-MEM for the indicated times. RNA interference (RNAi) was obtained using the following siRNA sequences targeting human *cyclophilin B* (Accell #D-001920-01, Dharmacon), or human *CALHM1*: CUAAUGUAUUUGUUGAUUU; UGGUCAUGAACAACAACGU; GUGUUGCGCUACAUGUUCU; CCCAUGAGCAGUAUUAGUC (Accell SMARTpool, Dharmacon). Three days after siRNA treatments, cells were challenged with Ca^{2+} add-back conditions and analyzed for Aβ levels, as described previously; control cells were not challenged with Ca^{2+} add-back conditions. Cells were also analyzed for *CALHM1* mRNA levels by qRT-PCR, as described above.

Multimerization analyses and immunoprecipitations (IPs)

Cells were harvested six hours after transfection with the indicated *CALHM1* cDNAs. Cells were solubilized for 2 h at 4°C in HEPES buffer containing 1% Nonidet P-40. Cell extracts were pre-cleared by centrifugation at 10,000 rpm for 5 min. For multimerization analyses, cell extracts were analyzed by western blotting (WB) in the absence (non-reducing conditions) or presence of 5% β-mercaptoethanol (reducing conditions). For IPs, supernatants were incubated with immobilized anti-Myc antibody, as per supplier's instructions (ProFound Mammalian c-Myc Tag IP Kit, Pierce). Total extracts and immunoprecipitated proteins were then analyzed by WB.

Western blotting (WB)

Cells were washed with PBS and solubilized in ice-cold HEPES buffer (25 mM HEPES, pH 7.4, 150 mM NaCl, 1X Complete protease inhibitor cocktail, Roche) containing 1% SDS. Ten micrograms of extracts were analyzed by SDS-PAGE.

Immunofluorescence analysis

CHO cells grown on glass coverslips were transfected as described above. Cells were fixed five hours after transfection with 4% paraformaldehyde in PBS. Cells were permeabilized with 0.1% Triton X-100 and blocked with Pierce Superblock in PBS. Cells were incubated with anti-Myc (1:100) and anti-GRP78 (1:800) primary antibodies, and with Alexa Fluor 488 and 594 anti-IgG secondary antibodies (1:1000, Molecular Probes). Cells were then visualized under a Nikon Eclipse TE2000-S fluorescent microscope.

CALHM1 deglycosylation
CALHM1-transfected HT-22 cell lysates were incubated in the absence or presence of PNGase F or endoglycosidase H for 16 h at 37 °C in NaH_2PO_4 digestion buffers supplemented with SDS and β-mercaptoethanol, as per manufacturers' instructions (Sigma and Calbiochem, respectively). Cell lysates were then analyzed by WB using anti-Myc antibody.

Cell Surface Biotinylation
Biotinylation was performed using Sulfo-NHS-SS-Biotin (EZ-Link® Sulfo-NHS-SS-Biotin, Pierce), as per supplier's recommendations (Cell Surface Protein Isolation Kit, Pierce), with the following modifications. CALHM1-transfected HT-22 cell cultures were biotinylated in one 100-mm dish. Precipitated proteins were eluted from the avidin beads with 150 µl of Laemmli buffer containing 50 mM DTT, and heated for 5 min at 95°C.

Cytoplasmic Ca^{2+} measurements using Fura-2
Undifferentiated PC12 cells were transiently transfected with pIRES vector to express EGFP and CALHM1, EGFP and mutant CALHM1 (P86L), or EGFP only. 24 hours later, the cells were loaded with Fura-2 AM (Molecular Probes; 2 µM) for 1 hour at room temperature. Transfected cells were identified by their green fluorescence. The cells were continuously perfused with Hanks' Balanced Salt Solution (HBSS) (Sigma) containing 1.8 mM $CaCl_2$ and 0.8 mM $MgCl_2$, pH = 7.3. Extracellular Ca^{2+} was removed by perfusing the cells with HBSS supplemented with 2.6 mM $MgCl_2$ and 0.5 mM EGTA. Fura-2 was alternately excited at 340 and 380 nm, and the emitted fluorescence filtered at 510 nm was collected and recorded using a CCD-based imaging system running Ultraview software (Perkin-Elmer). Images were taken every 15 seconds. Dye calibration was achieved by applying experimentally determined constants to the standard equation: $[Ca^{2+}] = Kd\ \beta(R-R\text{min})/(R\text{max}-R)$.

Cell viability and cell cytotoxicity assays
Trypan blue exclusion assay. HT-22 cells were seeded onto six-well culture plates. 6.5 hr post-transfection cells were challenged with Ca^{2+} add-back conditions, as described previously; control cells were not challenged. Cells were then stained with trypan blue and counted.

Lactate dehydrogenase (LDH) release measurements. HT-22 cells were seeded onto 24-well culture plates (6×10^4 cells/well). 6.5 hr post-transfection, cells were challenged with Ca^{2+} add-back conditions; control cells were not challenged. Culture medium was collected and measurements of LDH release were performed, as per manufacturer's instructions (CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega). Absorbance measurements were obtained using a Tecan GENios Pro plate reader at 492 nm.

***CALHM1* sequencing**
CALHM1 ORF was amplified by PCR using the following primer sequences: FX1US 5'-TCT TGG AGG CAG CAG TGA GT-3' and FX1DSa 5'- TTT TGA GAG GTA GGG GGA TAG G-3'(exon1); FX2US 5'-GCT TTG GGA GTC TGA ACA GG -3' and FX2DS 5'-TCC TTT TTC CAC CTG GTT TG-3' (exon2).

SNP analyses
Populations:
France I case-control study. The AD and control subjects were all Caucasian (AD cases n = 710, age at study = 72.1 ± 7.7 years, age at onset = 68.7 ± 8.1 years, 39% male; Controls n = 565, age =

72.1 ± 8.0 years, 39% male). A diagnosis of probable AD was established according to DSM-III-R and NINCDS-ADRDA criteria. Caucasian controls were defined as subjects without DMS-III-R dementia criteria, with integrity of cognitive function and with a MMS score ≥25. Controls were recruited from retirement homes or from electoral rolls (altruistic volunteers). Each individual or next of kin gave informed consent. Control subjects with a family history of dementia were excluded.

France II case-control study. All subjects were Caucasian originating mainly from the West of France. (AD cases n = 645, age = 69.0 ± 9.7 years, age at onset = 64.6 ± 9.6 years, 39% male; Controls n = 483, age = 67.5 ± 10.2 years, 47.5% male). Clinical diagnosis of probable AD was established according to the DSM-III-R and NINCDS-ADRDA criteria. Control subjects (mainly spouses of patients) were required to have a MMS score above 28.

UK case-control study. The UK AD and control subjects were all Caucasian from Greater Birmingham and Manchester (AD cases n = 492, age at study = 74.5 ± 7.9 years, 44 % male; Controls n = 205, age = 71.8 ± 10.5 years, 38% male). Three hundred sixty five individuals presented a clinical diagnosis of probable AD according to the DSM-III-R and NINCDS-ADRDA criteria. Age at onset was not available for these 365 probable AD cases. One hundred twenty seven individuals (age at onset = 63.9 ± 10.4 years) were autopsied and a definitive diagnosis of AD was established. Control subjects were assessed using either DSM-III-R questionnaire or had a MMSE score above 28.

Italy case-control study. All subjects were Caucasian originating mainly from Northern Italy. (AD cases n = 150, age = 76.8 ± 7.9 years, age at onset = 73.8 ± 8.7 years, 36% male; Controls n = 85, age = 70.8 ± 9.0 years, 38% male). Patients were diagnosed according to the DSM-III-R and NINCDS-ADRDA criteria. Control subjects had a MMSE score above 28.

Genotyping. In the France I population, the P86L genotype was determined by genomic DNA amplification of (i) a 114 bp fragment using the forward mismatched primer 5'-GAAGAGTGGAAGCGGCCAC-3' and reverse primer 5'-GACGGCCACCCAGACGACA-3' followed by Bsr I digestion and/or (ii) a 141 bp fragment using the forward mismatched primer 5'-GAAGAGTGGAAGCGGCAGC-3' and reverse primer 5'-GAGGAAGCATTTGCCGTCG- 3' followed by Alu I digestion. The genotyping of 176 individuals was checked by direct sequencing of a 207 bp fragment amplified using the forward primer 5'-CCTGGTGCTCTTTCTGCTTG-3' and reverse primer 5'-CAGAAGGCAGAGGAAGCA-3'. Only two discrepancies were observed between CC and CT genotypes. In the UK, France II, and Italy populations, the P86L genotype was entirely determined by direct sequencing as described above.

Quantitative multiplex PCR of short fluorescent fragments (QMPSF)

Total genomic DNA was prepared from peripheral blood lymphocytes. Short genomic fragments (between 90 and 260 bp) of the *CALHM1* gene were simultaneously amplified within one multiplex PCR using 6-FAM labeled primer pairs (primer sequences are available upon request). PCRs were performed in 25 µl containing 100 ng of genomic DNA, 0.12 to 0.6 µM of each pair of primers and 1.5 units of Thermoprime plus DNA polymerase (Abgen). The PCR consisted of 23 cycles of 20 seconds at 94°C, 30 seconds at 57°C, and 30 seconds at 72°C. DNA fragments generated by QMPSF were separated on an ABI Prism 3100 DNA sequencer (Applied Biosystems) and the resulting fluorescence profiles were analyzed using the Genescan 3.7 Software (Applied Biosystems). For each patient, the QMPSF profile was superimposed on that generated from a reference subject by matching the level of the peaks obtained for the control amplicons corresponding to short exonic fragments of the PCBD2, HMBS, and APP genes, located on

chromosome 5, 11, and 21, respectively. A 50% reduction of a peak area indicates a heterozygous deletion of the corresponding genomic fragment.

Electrophysiology

Xenopus oocyte plasma membrane conductance was recorded 24-72 hours after cRNA injection. Single oocytes were placed in a 1 ml chamber containing LCa96 solution (96 mM NaCl, 1 mM KCl, 0.2 mM $CaCl_2$, 10 mM HEPES, 5.8 mM $MgCl_2$, pH 7.5). In some studies, Na^+ was replaced with NMDG. Conventional two-electrode voltage clamp was performed at room temperature using an amplifier (OC-725C, Warner Instrument Corp.) connected to a PowerMac 8100 via an ITC-16 interface (Instrutech Corp.) and glass electrodes (1B150F-4, WPI Inc.) filled with 3 M KCl (0.5–2.5 MΩ). *Pulse+PulseFit* software (HEKA Elektronik) was used to ramp the applied transmembrane potential (V_m) at 10-s intervals from -80 to 80 mV at 16 mV/s, and acquire data. V_m was clamped at the resting membrane potential between voltage ramps. Transmembrane current (I) and V_m were digitized at 200 Hz and recorded directly to hard disk. To determine the reversal potential V_{rev}, a fifth-order polynomial was fitted to the raw I-V_m data acquired during each voltage ramp, using macros developed in Igor Pro software (WaveMetrics Inc.).

Whole cell recordings of CHO cells were performed with 2-5 MΩ pipettes using an Axopatch 200-B amplifier (Axon Instr. Inc.), connected to a Mac G4 computer via an ITC-16 interface. *Pulse* software was used to deliver voltage protocols and acquire data for off-line analysis. Current-voltage (I-V) relationships were acquired in response to voltage ramps (±100 mV, 2 s duration). Currents were filtered at 1 kHz with a built-in four-pole Bessel filter and acquired at a sampling rate of 2 kHz. The recording chamber was continuously perfused with bath solution (2 ml/min).

The following solutions were used (in mM): For bi-ionic Na^+-Cs^+ experiments [Bath: 120 NaCl, 5 KCl, 1 $CaCl_2$, 5 glucose, 20 sucrose, 5 HEPES, pH 7.4 (adjusted with NaOH); 274 mOsm]; [Pipette: 122 CsCl, 5 NaCl, 2 MgATP, 10 EGTA, 5 glucose, 10 sucrose, 10 HEPES, pH 7.4 (adjusted with CsOH); 288 mOsm]. For zero-current experiments [Bath: 120 NMDG-aspartate, 1 Ca-aspartate, 5 glucose, 25 sucrose, 5 HEPES, pH = 7.4 (adjusted with NMDG); 272 mOsm]; [Pipette: 120 NMDG-aspartate, 10 EGTA 5 glucose, 30 sucrose, 10 HEPES, pH 7.4 (adjusted with NMDG); 298 mOsm]. For bionic Ca^{2+}-Cs^+ experiments [Bath: 140 aspartic acid, 100 NMDG, 20 $Ca(OH)_2$, 5 glucose, 40 sucrose, 5 HEPES, pH = 7.4 (adjusted with NMDG); 294 mOsm]; [Pipette: 120 aspartic acid, 120 CsOH, 10 EGTA, 5 glucose, 30 sucrose, 10 HEPES, pH = 7.4 (adjusted with CsOH); 292 mOsm].

Accession numbers

CALHM3 (previously annotated FAM26A) has Ensembl accession code ENSG00000183128; Uniprot Q86XJ0). CALHM2 (FAM26B; Ensembl ENSG00000138172; Uniprot Q9HA72). Genes with significant sequence similarity to CALHM1 in human include FAM26D (Uniprot Q5JW98), FAM26E (Uniprot Q8N5C1), and FAM26F (Uniprot Q5R3K3). Ensembl accession codes refer to Ensembl release 43.

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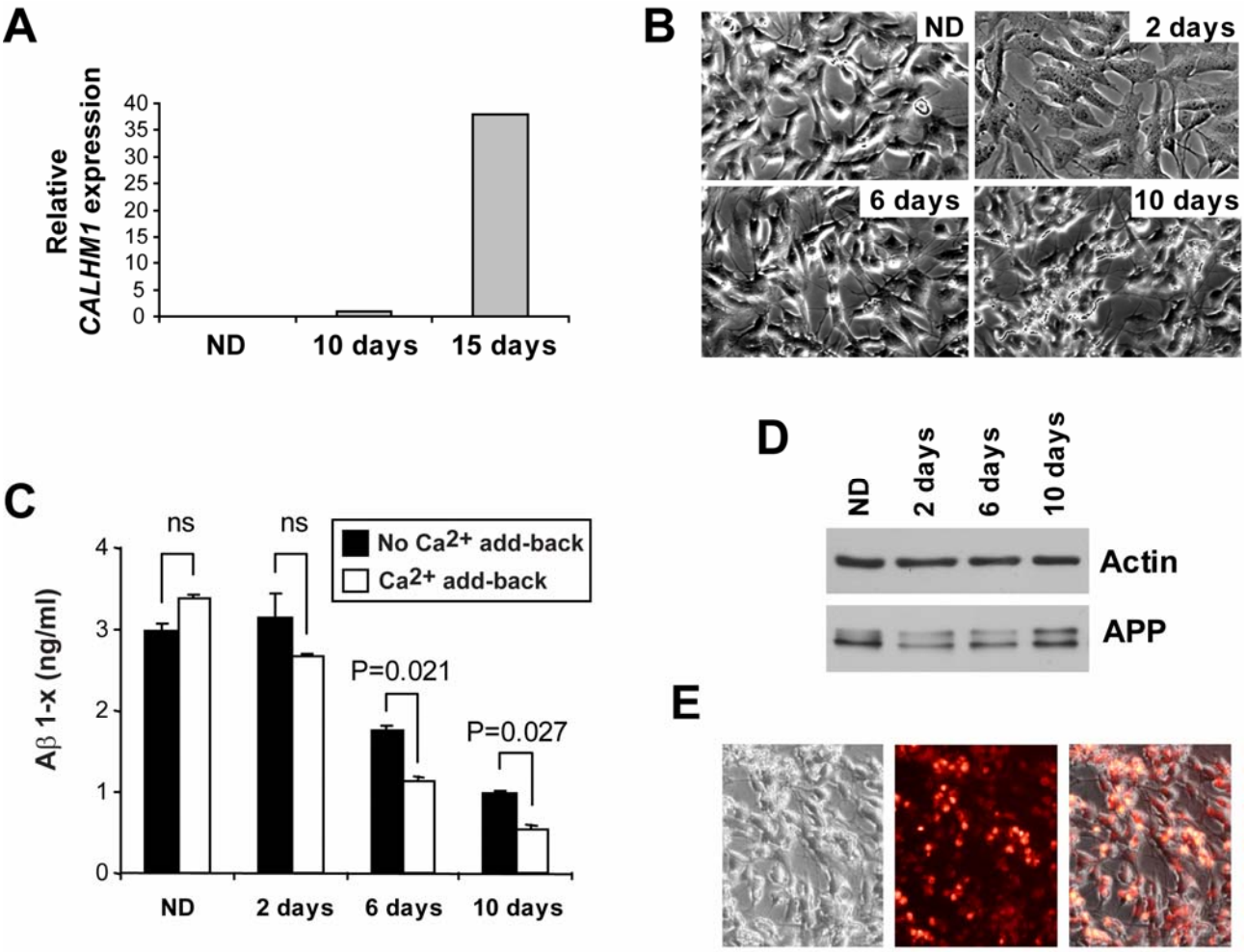

Figure S1. Endogenous CALHM1 controls Aβ levels in retinoic acid-differentiated SH-SY5Y cells

(A) Real-time qRT-PCR analysis of *CALHM1* mRNA in APP_{695}-SH-SY5Y cells differentiated for the indicated times with retinoic acid (10 μM). *CALHM1* expression was normalized to reference gene *HPRT*. ND, non-differentiated. The mRNA level measured in cells differentiated for 10 days was set to 1.

(B) Phase contrast microscopy of APP_{695}-SH-SY5Y cells differentiated for the indicated times with retinoic acid.

(C and D) APP_{695}-SH-SY5Y cells were differentiated for the indicated times with retinoic acid. Medium was changed and cells were incubated for 90 min in the absence or presence of Ca^{2+} add-back conditions, as described in Experimental Procedures. Total secreted Aβ1-x was quantified by ELISA (n = 3; Student's *t* test; ns, non-significant) (C). APP and actin levels were analyzed by WB in the presence of Ca^{2+} add-back conditions (D).

(E) Uptake of Accell control siRNAs in APP_{695}-SH-SY5Y cells differentiated for 15 days with retinoic acid. Cells were treated for 3 days with Accell red fluorescent control siRNAs directed against human *cyclophilin B*. Left panel: Phase contrast microscopy. Middle panel: Fluorescent microscopy. Right panel: Merged images.

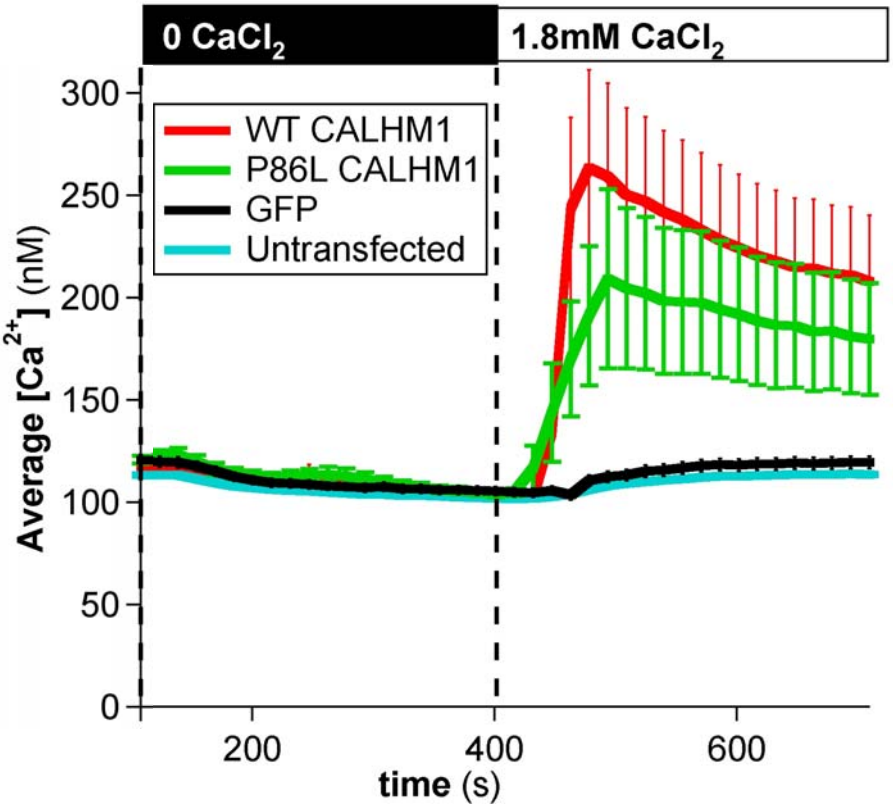

Figure S2. CALHM1 expression increases cytoplasmic Ca^{2+} concentrations. Effect of the CALHM1 P86L polymorphism

Cytoplasmic Ca^{2+} measurements using Fura-2 loading and Ca^{2+} add-back assays in PC12 cells transiently transfected with CALHM1 and EGFP, or EGFP alone. Ca^{2+} in incubation buffer was removed for 5 minutes (0 $CaCl_2$; see Supplemental Experimental Procedures). Cells were then challenged with physiological extracellular Ca^{2+} concentrations (1.8 mM $CaCl_2$). Each trace is the average of $[Ca^{2+}]_i$ measurements ± SEM (n = 64, WT-CALHM1-transfected; n = 51, P86L-CALHM1-transfected; n = 27, EGFP-transfected; and n = 268, untransfected cells).

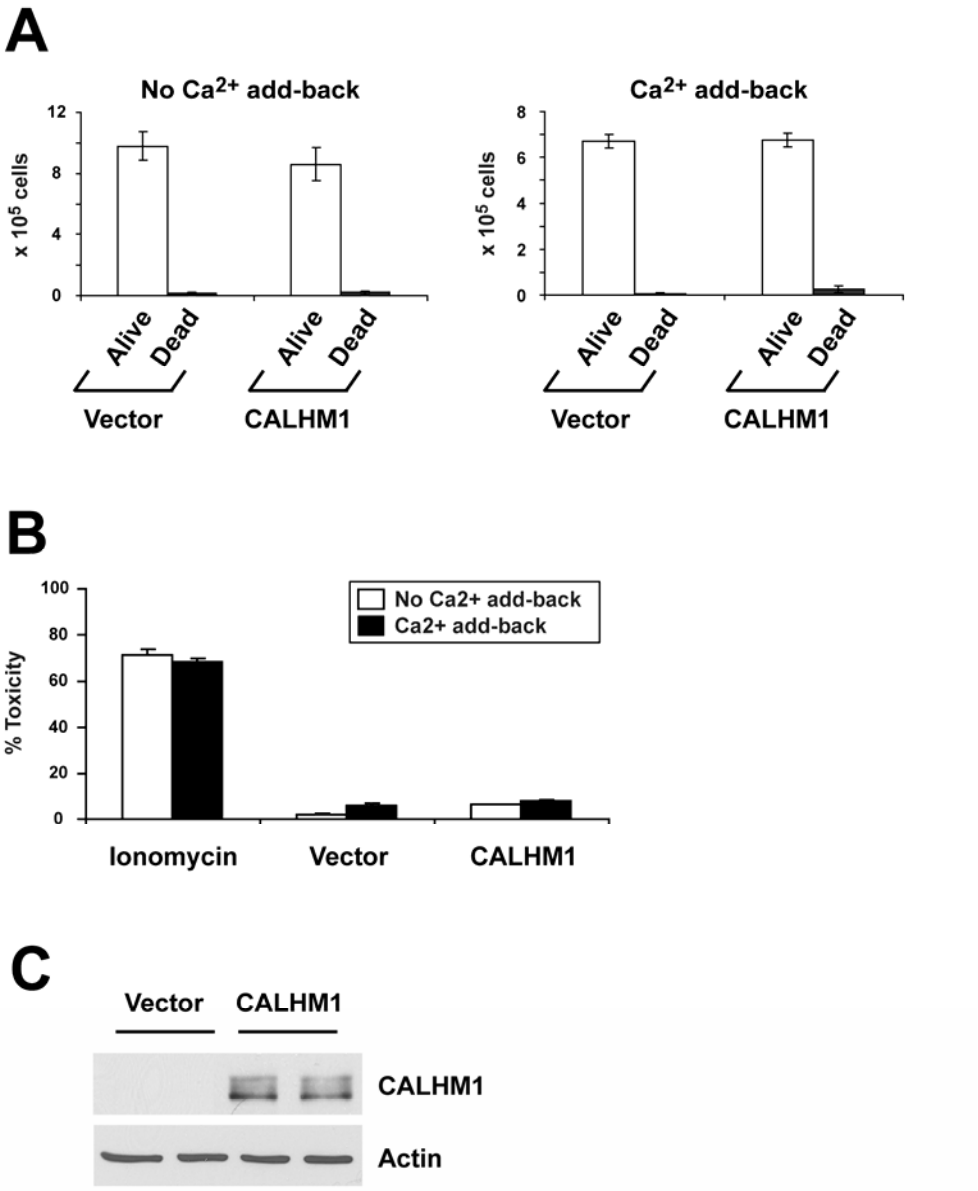

Figure S3. Cell viability and cell cytotoxicity in CALHM1-expressing cells

(A and B) Trypan blue exclusion assay (A) and LDH release measurements (B) in HT-22 cells transiently transfected with Myc-CALHM1 or control vector, in the absence (No Ca^{2+} add-back) or presence (Ca^{2+} add-back) of Ca^{2+} add-back conditions, as described previously. Histograms illustrate the mean values ± S.D. of three (A) and four (B) independent experiments. The Ca^{2+} ionophore ionomycin (10 μM) was used as a positive control for cytotoxicity (B).

(C) WB with anti-Myc (upper panel) and anti-actin (lower panel) antibodies of protein extracts obtained from cells transfected as in (A) and (B).

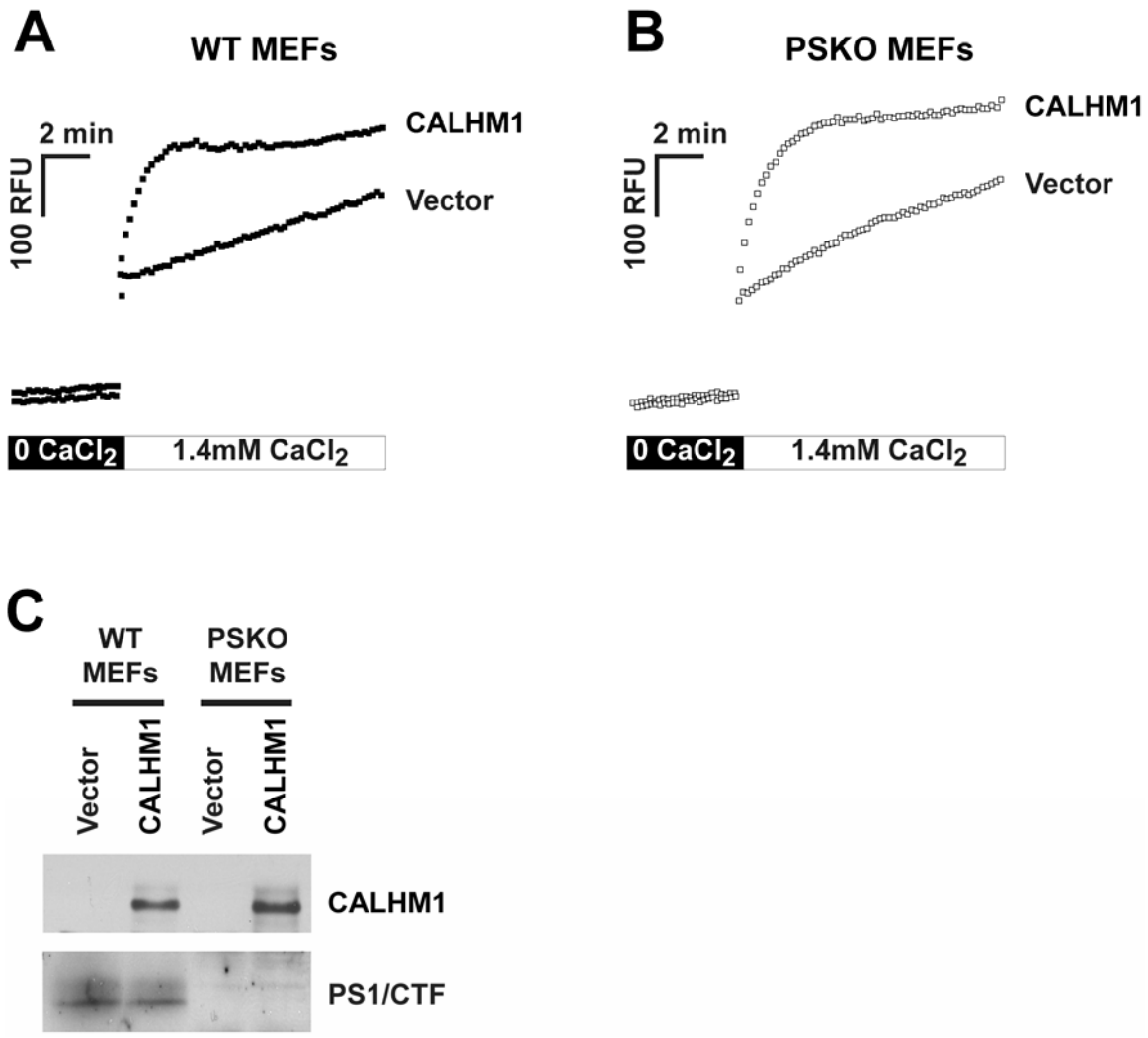

Figure S4. Presenilin deficiency does not affect CALHM1-mediated Ca^{2+} influx
(A and B) Cytoplasmic Ca^{2+} measurements using Fluo-4 loading and Ca^{2+} add-back assays in WT (A) or presenilin-deficient (PSKO; B) mouse embryonic fibroblasts (MEFs) transiently transfected with Myc-CALHM1 or control vector. Cells were first incubated in Ca^{2+}-free buffer (0 $CaCl_2$) and then challenged with physiological extracellular Ca^{2+} concentrations (1.4 mM $CaCl_2$) to monitor the progressive restoration of basal $[Ca^{2+}]_i$. Graphs illustrate representative measurements of 3 independent experiments.
(C) WB of the corresponding cell lysates probed with antibodies directed against Myc (upper panel) or presenilin-1 C-terminal fragment (PS1/CTF; 33B10 antibody; lower panel).