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Supplementary Materials for

Familial Alzheimer's disease–associated presenilin 1 mutants promote γ-secretase cleavage of STIM1 to impair store-operated Ca^{2+} entry

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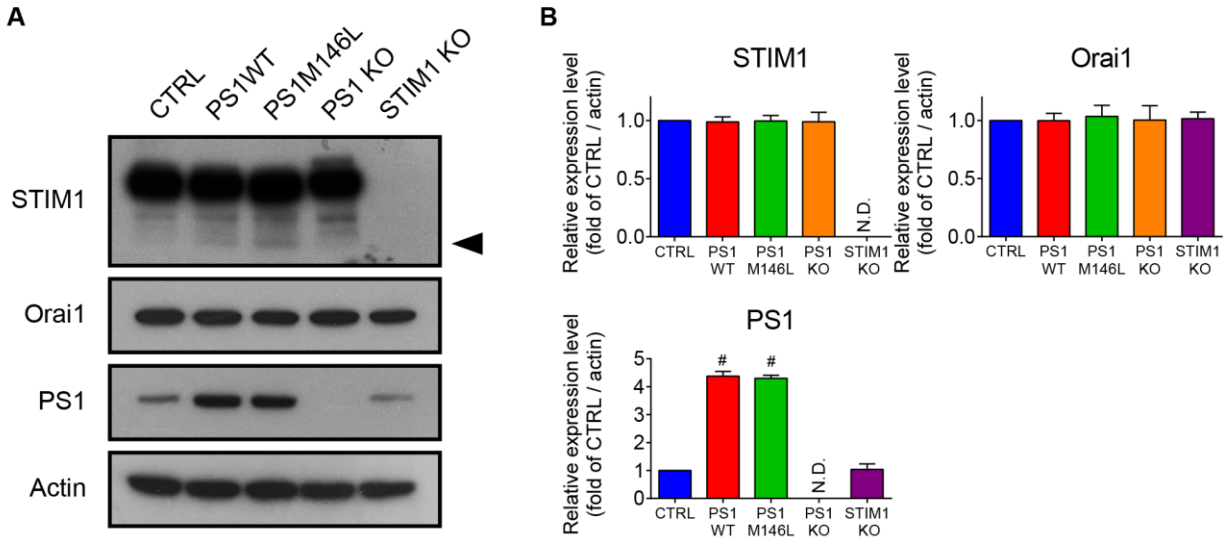

Fig. S1. Mutant PS1 or PS1 knockout does not grossly affect STIM1 or ORAI1 abundance in SH-SY5Y cells. (A) Representative Western blots showing the abundance of STIM1 and ORAI1 in stable SH-SY5Y cells expressing empty vector (CTRL), PS1WT or PS1M146L. CRISPR-mediated PS1 knockout (PS1KO) or STIM1 knockout (STIM1KO) SH-SY5Y cells were included to verify that endogenous PS1 or STIM1 had been knocked out. Arrow indicates a STIM1 immunoreactive band with lower molecular weight (~ 60 kDa). (B) Densitometric analyses of protein abundance of STIM1, ORAI1 and PS1 in SH-SY5Y cell lines normalized to actin. Data summarized as mean ± SEM from 3 separate experiments. Data were analyzed by one-way ANOVA with Bonferroni's test compared to CTRL. N.D: protein band was not detected in the immunoblot.

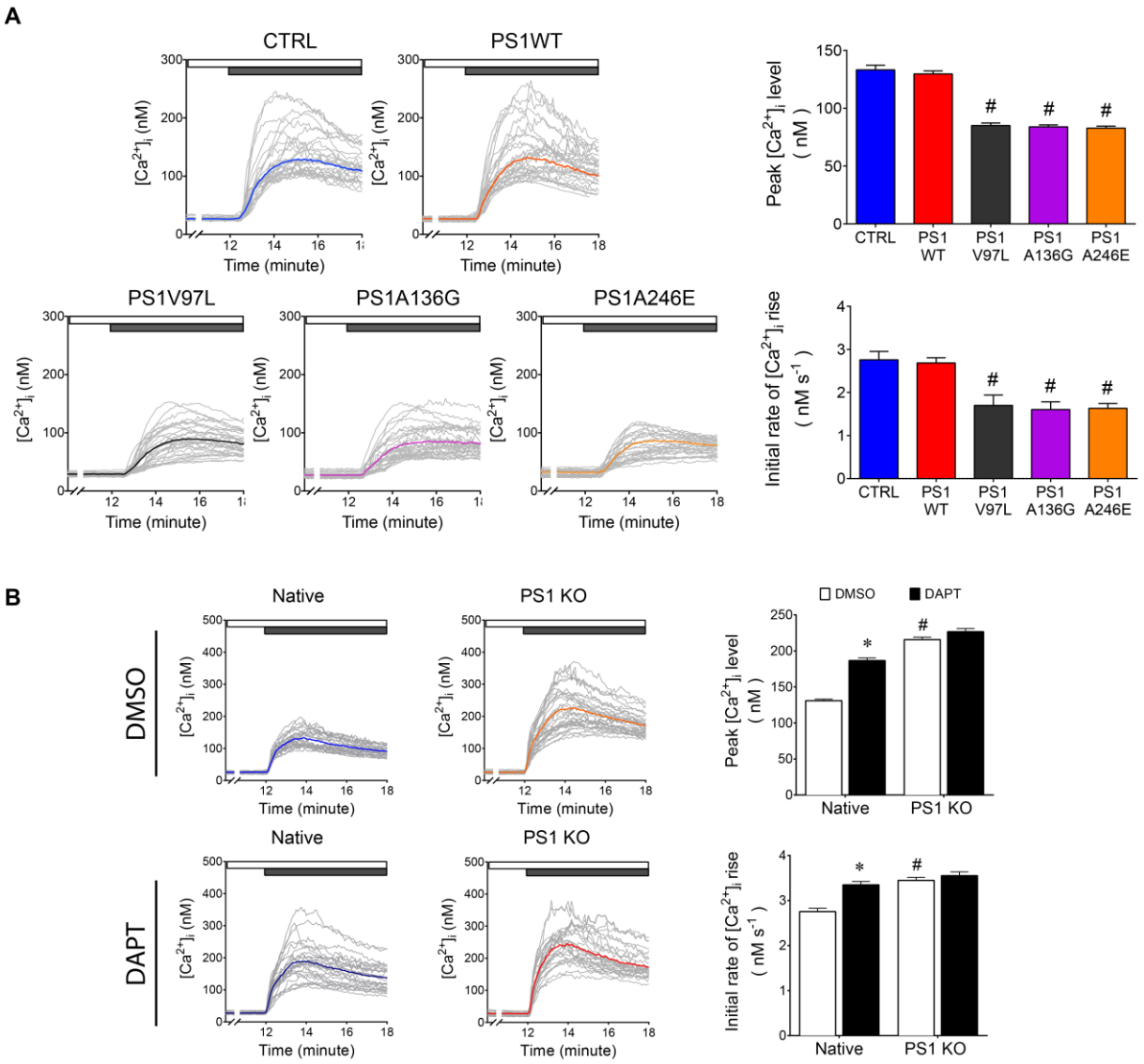

Fig. S2. Impaired CCE in mutant FAD-PS1–expressing cells or PS1 knockout cells. (A) Cytoplasmic Ca^{2+} concentrations ($[Ca^{2+}]_i$) in SH-SY5Y cells stably expressing empty vector (CTRL), wild-type (PS1WT), mutant PS1-V97L, PS1-A136G or PS1-A246E. CCE was triggered by depleting ER Ca^{2+} stores using 50 μM carbachol in Ca^{2+}-free HBSS (white bar), followed by re-addition of 2 mM Ca^{2+} to the extracellular solution (dark grey bar). The grey lines depicted individual cellular $[Ca^{2+}]_i$ while the color lines depicted the mean $[Ca^{2+}]_i$ of the particular cell line. Bar charts summarize statistical analyses of peak $[Ca^{2+}]_i$ or initial rate of Ca^{2+} entry during CCE. Experiments were repeated 5 times for each cell line, with 30 cells analyzed in each experiment. Bars: mean ± SEM. #, $P < 0.01$, one-way ANOVA with Tukey's tests compared to CTRL and PS1WT groups (B) Analogous CCE experiments performed in native SH-SY5Y or PS1KO SH-SY5Y cells with or without DAPT treatment. Bar charts summarized statistical analyses of peak $[Ca^{2+}]_i$ or initial rate of Ca^{2+} entry in cells during CCE with or without DAPT treatment. Data summarized as mean ± SEM from 3 separate experiments with 30 cells analyzed in each experiment. See also table S1. *: $P < 0.01$, Student's *t*-test compared with DMSO treatment. #: $P < 0.01$, Student's *t*-test compared to DMSO treatment of the native SH-SY5Y cells.

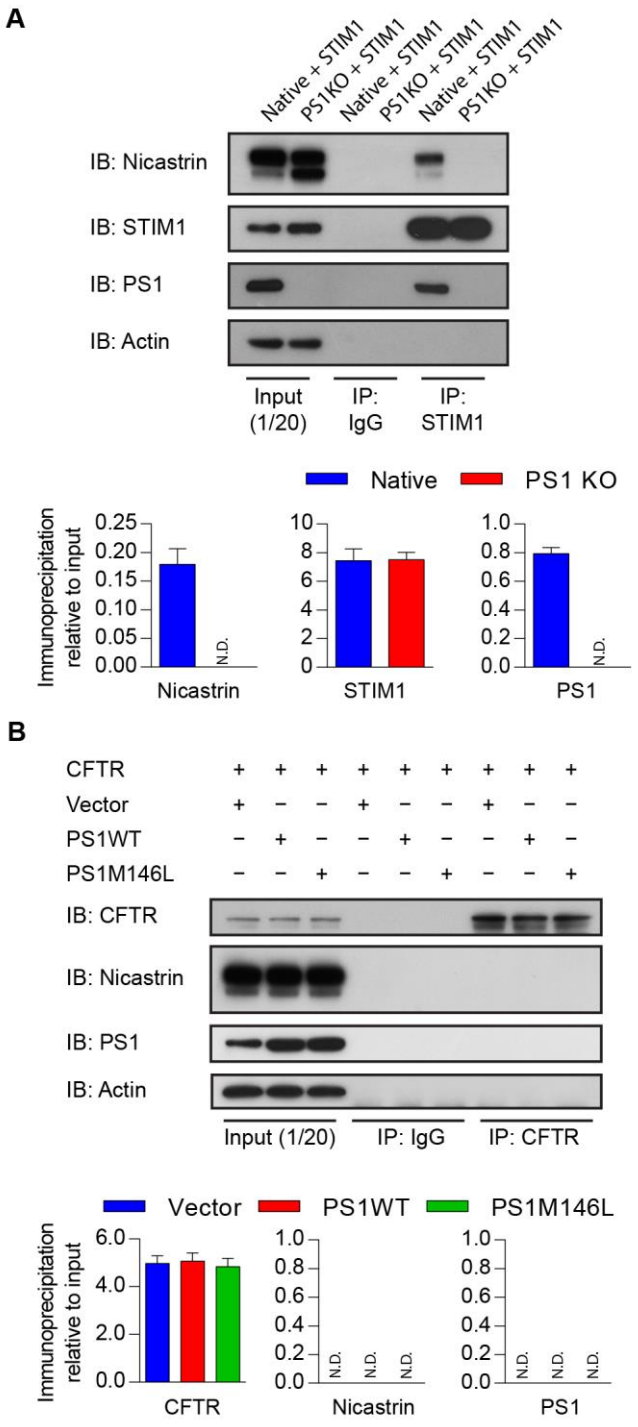

Fig. S3. PS1 interacts with STIM1 but not with CFTR. (A) Native or PS1KO SH-SY5Y cells transfected with STIM1. Input (1/20) shown in the first two lanes, IgG immunoprecipitates shown in second two lanes and STIM1 immunoprecipitates shown in the last two lanes. (B) PS1 and CFTR in SH-SY5Y cells coexpressing CFTR with empty vector, PS1WT, or PS1M146L. Input (1/20) shown in first three lanes, IgG immunoprecipitates (IP: IgG) shown in the middle three lanes and CFTR immunoprecipitates (IP: CFTR) shown in last three lanes. Experiments were repeated 3 times in both A & B and band intensities of coimmunoprecipitated proteins were normalized with their respective input intensities. N.D. indicates that an immunoreactive band was not detected.

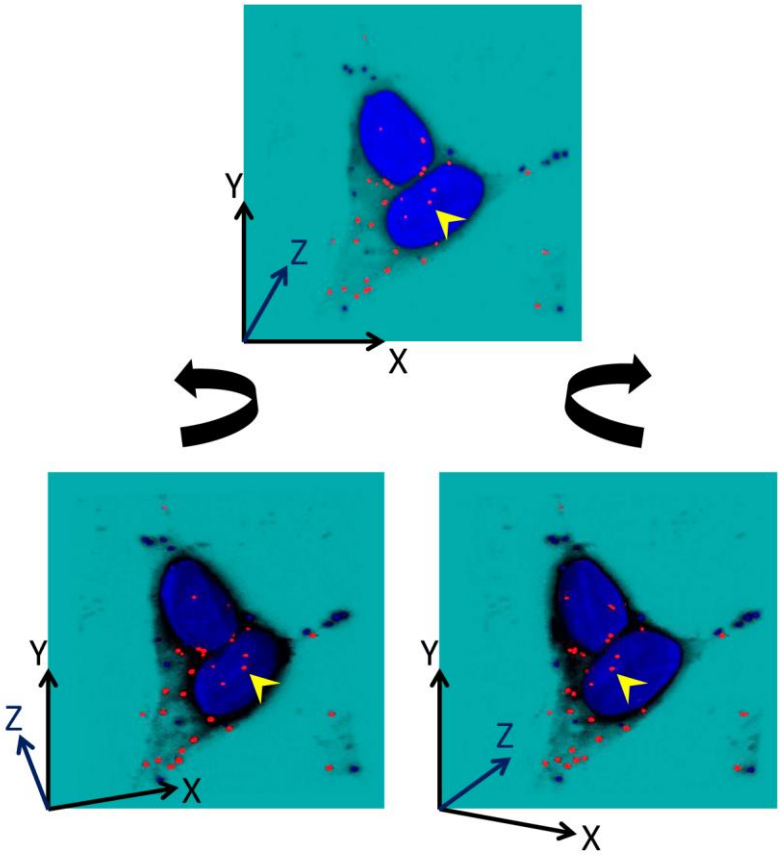

Fig. S4. In situ PLA demonstrates that endogenous PS1 and STIM1 interact. 3D reconstructed confocal images of native SH-SY5Y cells showing positive PLA fluorescent dots (red). Rotation of reconstructed images on Y-axis showing PLA signals displaced along with the rotation of nucleus. One of these PLA signals was indicated by yellow arrow as an example. The signals were localized around the nucleus, resembling a typical ER localization.

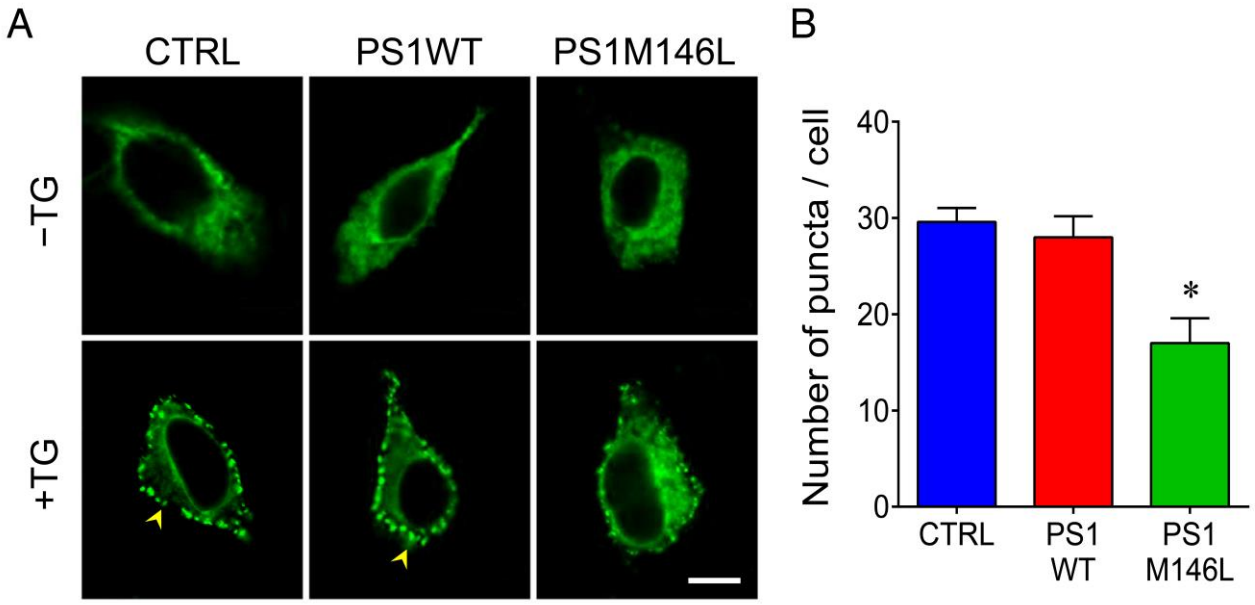

Fig. S5. FAD-linked mutant PS1 impairs STIM1 puncta formation. (A) Representative confocal images depicted localization of YFP labeled STIM1 in stable SH-SY5Y cells expressing empty vector (CTRL), PS1WT or PS1M146L before (-TG) or after (+TG) ER Ca^{2+} store depleted by thapsigargin. Store depletion caused puncta formation near plasma membrane (arrows). Images taken at 60X magnification. Scale bar: 10 μm. (B) Summary of the number of puncta in cells after store depletion. Data summarized as mean ± SEM, n = 5 cells in each group. *: $P < 0.01$ by one-way ANOVA with Tukey's tests compared to CTRL and PS1WT groups.

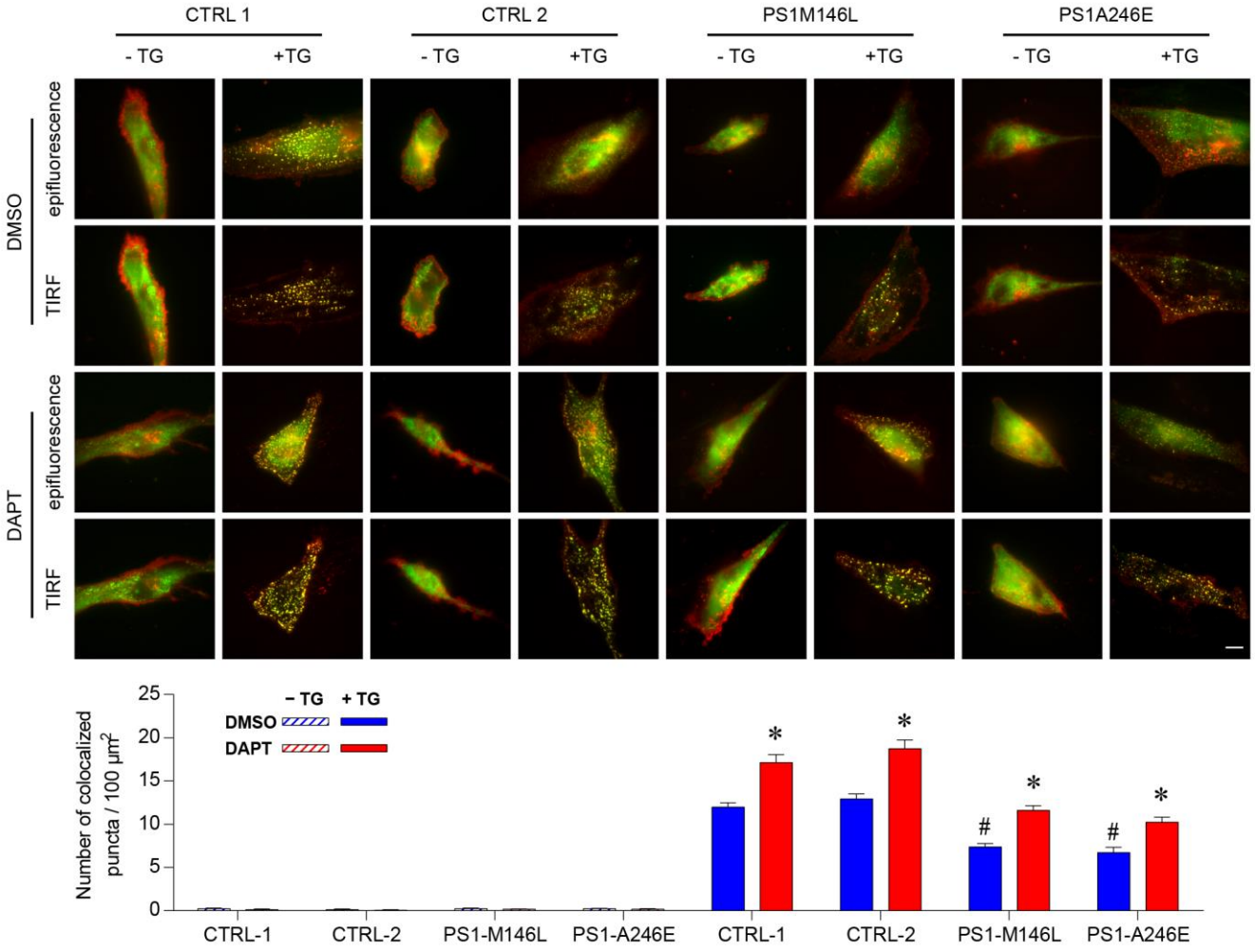

Fig. S6. Mutant PS1 attenuates the STIM1-ORAI1 interaction in fibroblasts from FAD patients. (A) Representative epifluorescence and total internal reflection fluorescent (TIRF) images depicting interaction of STIM1-GFP and ORAI1-mCherry in human skin fibroblast from FAD patients with PS1M146L or PS1A246E mutation or two control fibroblasts without PS1 mutation. ER Ca^{2+} in cells was depleted by addition of 3 µM thapsigargin (TG) in Ca^{2+}-free buffer. Analogous experiments were performed in cells pretreated with 1 µM DAPT for 48 hr. (B) Quantification of STIM1-ORAI1 colocalized puncta in TIRF microscopy in fibroblasts normalized with their respective TIRF footprints before or after ER Ca^{2+} depletion by TG. Bars: mean ± SEM from 3 separate experiments with more than 10 cells analyzed. See also table S3. *: $P < 0.01$, Student's *t*-test compared to respective DMSO treatment. #: $P < 0.01$ by one-way ANOVA with Tukey's tests compared to CTRL1 and CTRL2.

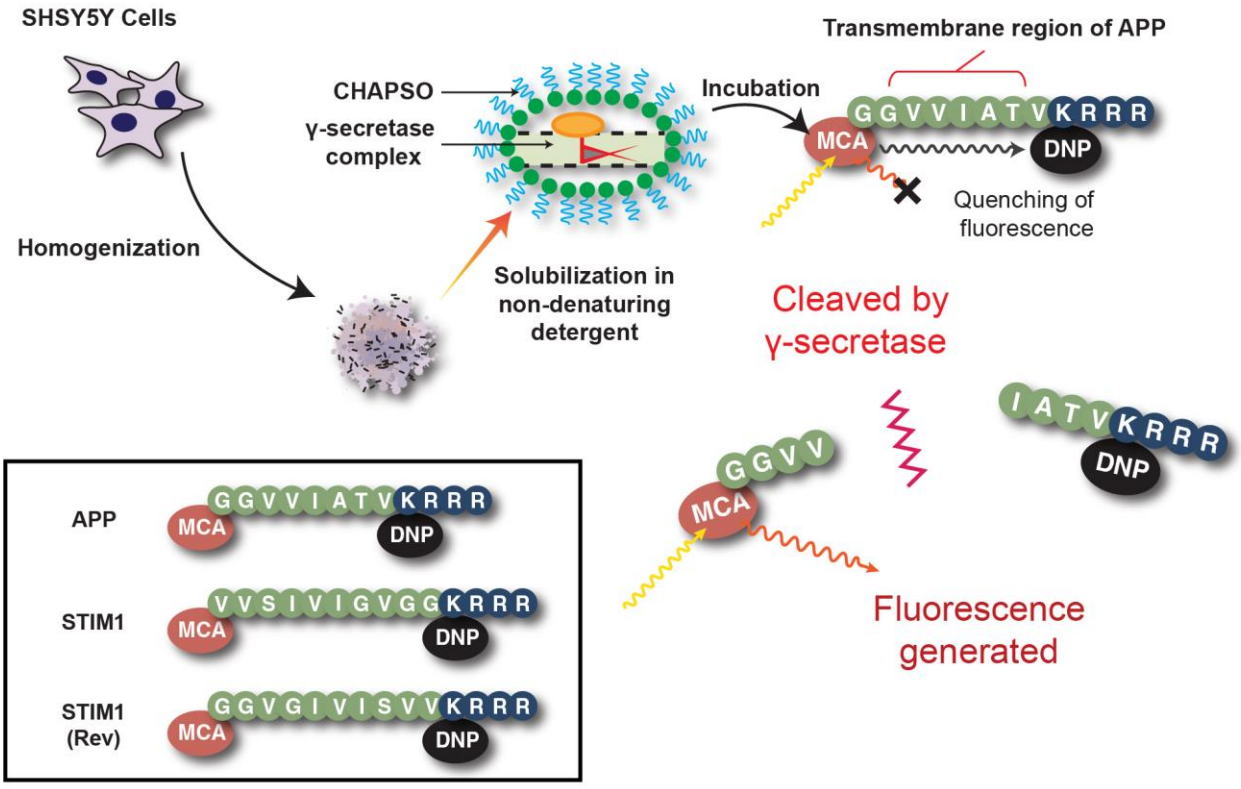

Fig. S7. In vitro fluorogenic γ-secretase cleavage assay. Cartoon illustrating in vitro fluorogenic assay employed to investigate γ-secretase-mediated cleavage of STIM1. Stable SH-SY5Y lines were homogenized and the cell γ-secretase complex was solubilized and extracted by CHAPSO detergent. Peptides with APP, STIM1 or reversed STIM1 [STIM1 (Rev)] transmembrane sequence were synthesized as shown. Synthetic peptides with transmembrane sequence of Itgβ1 and NPR-A served as controls. All the synthetic peptides were tagged with MCA, a fluorophore, and DNP, a fluorescence quencher. For uncleaved peptides, DNP quenches fluorescence emission from MCA, whereas γ-secretase cleavage enhances fluorescence by relieving quenching.

Table S1. The effects of PS1 on CCE.

Corresponding Fig.	Cell and construct	No. of cells analyzed	CCE		Treatment	
			Peak $[Ca^{2+}]_i$	Initial rate of Ca^{2+} entry	DMSO	DAPT
Fig. 1	SH-CTRL	90	132.9 ± 2.37	2.81 ± 0.06	✓	-
			175.66 ± 4.1*	3.2 ± 0.07*	-	✓
	SH-PS1WT	90	130.29 ± 2.43	2.75 ± 0.05	✓	-
			176.82 ± 4.62*	3.11 ± 0.06*	-	✓
	SH-PS1M146L	90	87.18 ± 2.06#	1.71 ± 0.04#	✓	-
			165.84 ± 3.5*	2.93 ± 0.04*	-	✓
Fig. 1	HF-CTRL1	90	173.47 ± 5.17	3.43 ± 0.11	✓	-
			213.51 ± 6.1*	5.16 ± 0.14*	-	✓
	HF-CTRL2	90	163.42 ± 5.17	3.35 ± 0.13	✓	-
			217.58 ± 6.62*	4.96 ± 0.13*	-	✓
	HF-PS1M146L	90	87.51 ± 4.21#	2.17 ± 0.11#	✓	-
			195.07 ± 5.16*	3.56 ± 0.12*	-	✓
	HF-PS1A246E	90	93.16 ± 4.15#	2.46 ± 0.11#	✓	-
			186.86 ± 5.42*	3.24 ± 0.12*	-	✓
fig. S2A	SH-CTRL	150	133.27 ± 4.01	2.76 ± 0.2	-	-
	SH-PS1WT	150	129.69 ± 2.53	2.69 ± 0.12	-	-
	SH-PS1V97L	150	85.11 ± 2.1#	1.7 ± 0.24#	-	-
	SH-PS1A136G	150	83.95 ± 1.71#	1.6 ± 0.18#	-	-
	SH-PS1A246E	150	82.84 ± 1.68#	1.63 ± 0.11#	-	-
fig. S2B	Native SH-SY5Y	90	130.88 ± 2.16	2.75 ± 0.08	✓	-
			186.51 ± 3.65*	3.35 ± 0.08*	-	✓
	PS1KO SH-SY5Y	90	215.69 ± 3.42#	3.45 ± 0.07#	✓	-
			226.58 ± 4.44	3.55 ± 0.09	-	✓

The summary of the results of single-cell Ca^{2+} imaging of CCE in cells expressing different forms PS1. SH: SH-SY5Y; HF: human skin fibroblast.

For data of Fig. 1: * indicates $P < 0.01$ by Student's *t*-test compared to DMSO treatment within the same cell type (DAPT compared to DMSO). # indicates $P < 0.01$ by one-way ANOVA with Tukey's post hoc tests compared to CTRL and PS1WT in SH-SY5Y or CTRL1 and CTRL2 in human fibroblasts.

For data of fig. S2A: # indicates $P < 0.01$ by one-way ANOVA with Tukey's post hoc tests compared to CTRL and PS1WT.

For data of fig. S2B: * indicates $P < 0.01$ by Student's *t*-test compared to DMSO treatment within the same cell type (DAPT compared to DMSO). # indicates $P < 0.01$ by Student's *t*-test compared to DMSO treatment of native SH-SY5Y cells.

Table S2. The amount of PLA red fluorescent dots in SH-SY5Y cells.

	Native SH-SY5Y	Native SH-SY5Y	PS1KO SH-SY5Y	STIM1KO SH-SY5Y
Rabbit antibody	PS1	PS1	PS1	PS1
Mouse antibody	STIM1	Nicastrin	STIM1	STIM1
PLA signals / Cell	31.8 ± 1.58	36.73 ± 1.98	7.13 ± 0.8#	5.6 ± 0.42#

The summary of the results of in situ PLA assay performed in native, PK1KO or STIM1 KO SH-SY5Y cells.
# indicates $P < 0.01$ by one-way ANOVA with Bonferroni's post hoc tests compared to PLA performed in native SH-SY5Y cells using antibodies against PS1 and STIM1.

Table S3. The effects of PS1 on the STIM1-ORAII interaction after ER Ca^{2+} depletion by thapsigargin.

Corresponding figure	Cell and construct	No. of cells analyzed	Colocalized puncta per 100 μm² TIRF footprint	Treatment	
				DMSO	DAPT
Fig. 4A	SH-CTRL	12	10.36 ± 0.48	✓	-
		12	14.13 ± 0.53*	-	✓
	SH-PS1WT	12	9.37 ± 0.49	✓	-
		12	13.42 ± 0.52*	-	✓
	SH-PS1M146L	12	7.54 ± 0.49#	✓	-
		12	10.07 ± 0.47*	-	✓
Fig. 4B	Native SH-SY5Y	12	10.06 ± 0.51	✓	-
		12	13.82 ± 0.49*	-	✓
	PS1KO SH-SY5Y	12	14.04 ± 0.47#	✓	-
		12	14.98 ± 0.47	-	✓
Fig. S6	HF-CTRL1	12	11.97 ± 0.52	✓	-
		12	17.14 ± 0.9*	-	✓
	HF-CTRL2	12	12.93 ± 0.56	✓	-
		12	18.73 ± 1.04*	-	✓
	HF-PS1M146L	12	7.36 ± 0.4#	✓	-
		12	11.59 ± 0.56*	-	✓
	HF-PS1A246E	12	6.71 ± 0.6#	✓	-
		12	10.23 ± 0.57*	-	✓

Summary of the TIRF images showing colocalized STIM1-ORAI1 puncta in cells after ER Ca^{2+} store depleted by thapsigargin. SH: SH-SY5Y cells; HF: human skin fibroblasts.

For data of Fig. 4A: * indicates $P < 0.01$ by Student's *t*-test compared to DMSO treatment within same the cell type (DAPT vs DMSO). # indicates $P < 0.01$ by one-way ANOVA with Tukey's post hoc test compared to CTRL and PS1WT.

For data of Fig. 4B; * indicates $P < 0.01$ by Student's *t*-test compared to DMSO treatment within same the cell type (DAPT vs DMSO). # indicates $P < 0.01$ by Student's *t*-test compared to DMSO treatment of native SH-SY5Y.

For data of fig. S6: * indicates $P < 0.01$ by Student's *t*-test compared to DMSO treatment within the same cell type (DAPT vs DMSO). # indicates $P < 0.01$ by one-way ANOVA with Tukey's post hoc test compared to DMSO treatment groups of CTRL1 and CTRL2.

Table S4. The effects of PS1 on spine stability in isolated rat hippocampal neurons.

Neuron and construct	No. of neurites analyzed	Mature spine density (spines/μm)	Mature spine population (%)	Treatment		
				DMSO	DAPT	STIM1 transfection
CTRL	36	0.23 ± 0.0032	59.57 ± 0.58	✓	-	-
	36	0.27 ± 0.0044*	67.12 ± 0.55*	-	✓	-
	36	0.29 ± 0.0041*	67.19 ± 0.42*	-	-	✓
PS1WT	36	0.22 ± 0.0026	57.75 ± 0.66	✓	-	-
	36	0.28 ± 0.0038*	66.26 ± 0.28*	-	✓	-
	36	0.3 ± 0.0038*	67.62± 0.34*	-	-	✓
PS1M146L	36	0.14 ± 0.0046#	48.80 ± 0.78#	✓	-	-
	36	0.19 ± 0.0048*	56.64 ± 0.32*	-	✓	-
	36	0.22 ± 0.0033*	59 ± 0.43*	-	-	✓

Summary table of the effects of PS1 on spine stability. Neurons were transfected with empty vector (CTRL), PS1WT or PS1M146L. * indicates $P < 0.01$ by one-way ANOVA with Bonferroni's test compared to respective DMSO treatment within the same cell type. # indicates $P < 0.01$ by one-way ANOVA with Tukey's tests compared to DMSO treatments of CTRL and PS1 WT.