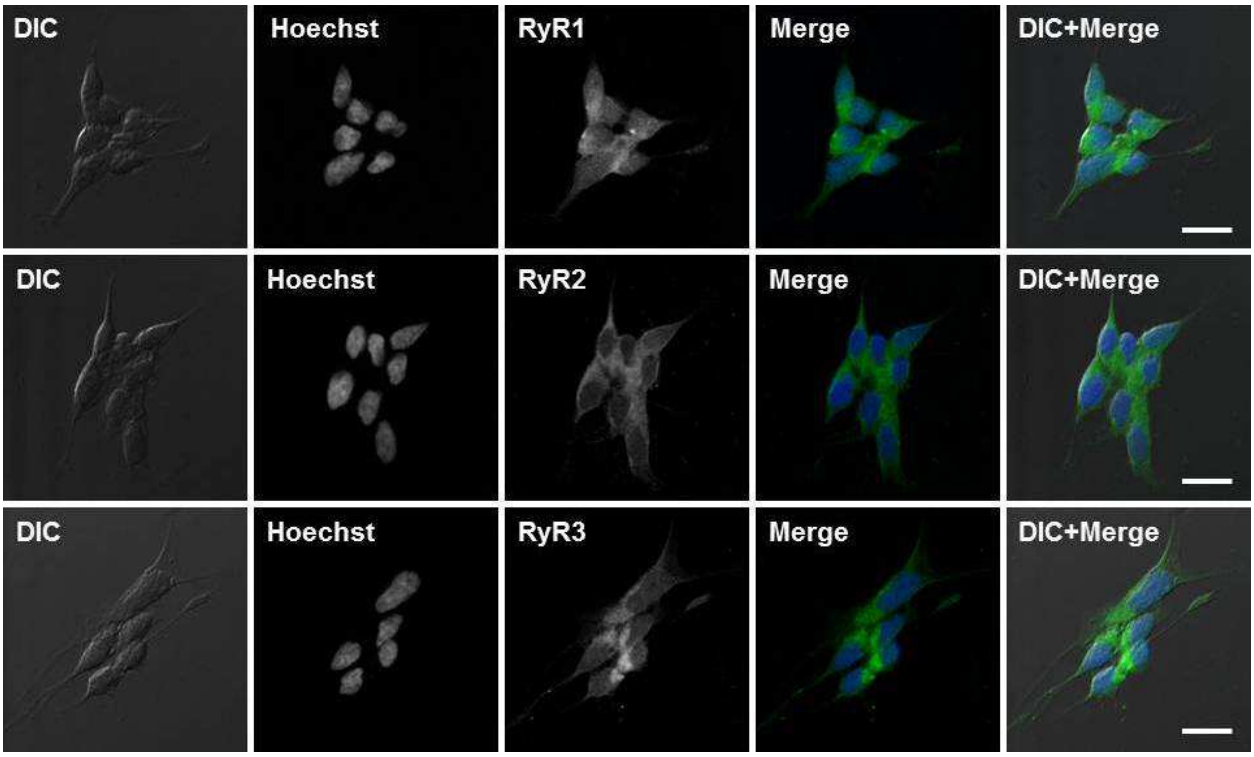
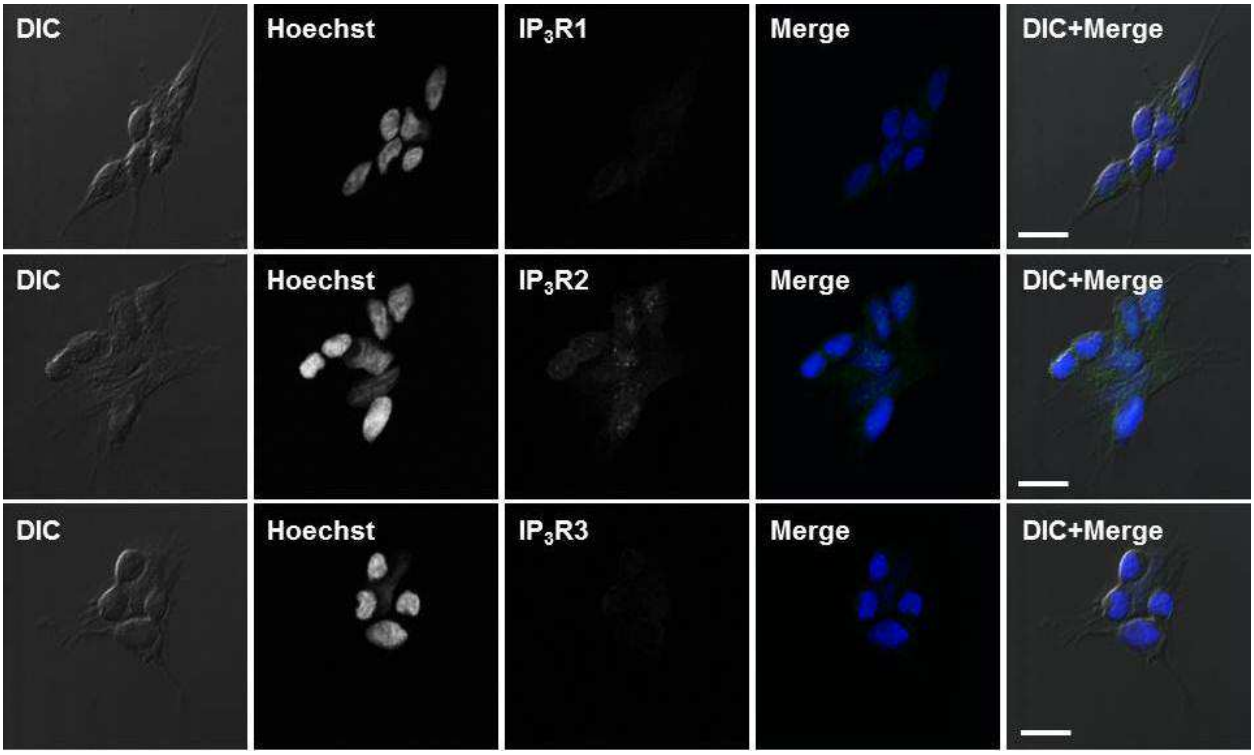
Supplementary Figures

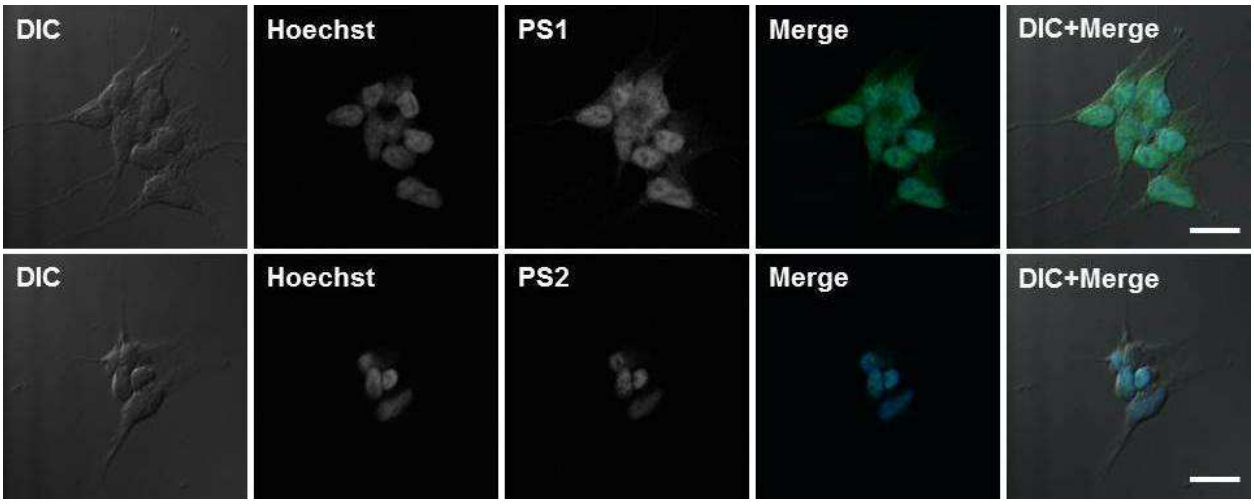


Supplementary Figure 1. Ryanodine receptors are the major intracellular calcium release channels of SH-SY5Y cells. SH-SY5Y cells stained with anti-RyR antibodies (third column). Top row is RyR1 (rabbit anti-RyR1, AB9078, 1:200), middle row is RyR2 (rabbit anti-RyR2, AB9080, 1:200), and bottom row is RyR3 (rabbit anti-RyR3, AB9082, 1:200; green fluorescence in merge columns) immunoreactivity. Cells were counterstained with Hoechst 33258 to identify cell nuclei (second column, blue fluorescence in merge columns). DIC, differential interference contrast. Scale bar is 10 µm.

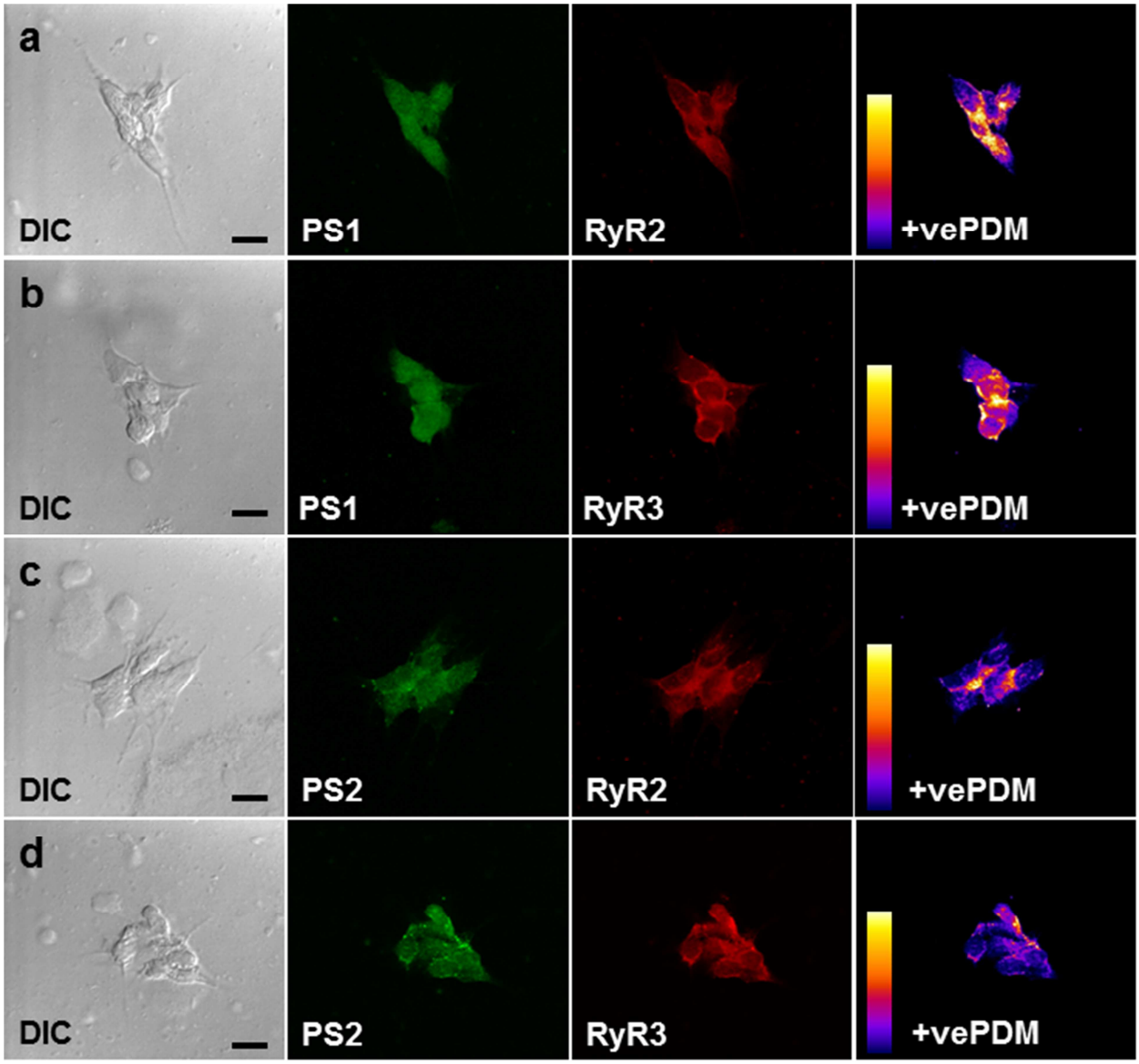


Supplementary Figure 2. SH-SY5Y cells express minor amounts IP_3 receptors.

SH-SY5Y cells were probed with antibodies against IP_3R. Top row is IP_3R1 (rabbit anti-IP_3R1, sc-6093, 1:50), middle row is IP_3R2 (rabbit anti-IP_3R2, sc-7278, 1:50), bottom row is IP_3R3 (rabbit anti-IP_3R3, sc-7277, 1:50) immunoreactivity. Small amounts of IP_3R2 immunoreactivity were detected in the nuclear region but neither IP_3R1 nor IP_3R3 immunoreactivity were detected (green in merge columns). Counter stained with Hoechst 33258 (blue fluorescence in merge columns). Scale bar is 10 µm.

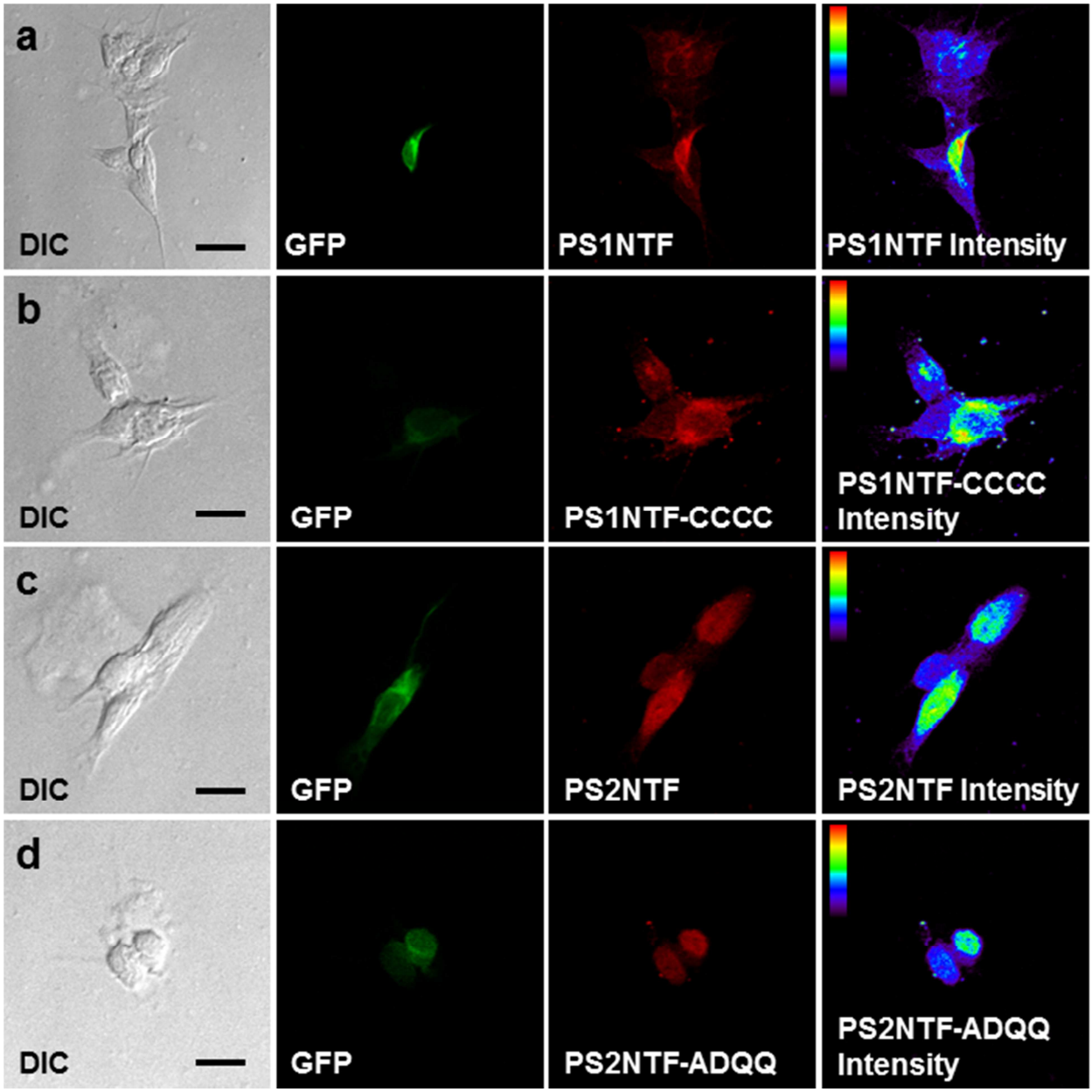


Supplementary Figure 3. Presenilins are endogenously expressed by SH-SY5Y cells. PS1 (top row, rabbit anti-PS1 ab53717, 1:100) and PS2 (bottom row, rabbit anti PS2 P6300-40H, 1:100) immunoreactivity were both detected in SH-SY5Y cells (third column; green fluorescence in merge columns). PS1 was ubiquitous while PS2 immunoreactivity was detected in nuclei. Counter stained with Hoechst 33258 (blue fluorescence in merge columns). Scale bar is 10 µm.



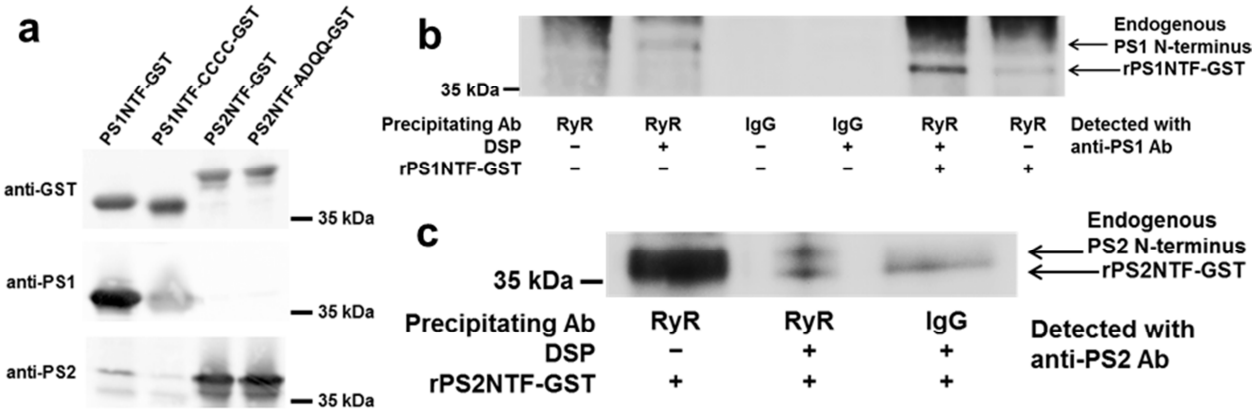
Supplementary Figure 4. Endogenous PS immunoreactivity was colocalized with RyR immunoreactivity in SH-SY5Y cells. SH-SY5Y cells stained with antibodies for PS1 or PS2 and RyR2 or RyR3 for immunocytochemistry colocalization analysis by the method of Manders (1). Manders' coefficient, a measure of overlap of red vs. green (M1) or green vs. red (M2) pixels, was >0.95 in all cases, indicating a high degree of overlap of endogenous PS and RyR proteins. Manders' coefficient of 1 is full colocalization, 0 is random, and -1 is no colocalization. Columns from left to right- DIC

with 10 µm scale bar, PS immunofluorescence, RyR immunofluorescence, and positive product of the differences from the mean (+vePDM). +vePDM is a measure of the variances of red and green immunoreactivity overlap, shown here as a heat map with blue indicating low alignment and yellow high alignment. **A)** PS1 vs. RyR2 (mouse anti-PS1, mab5235, 1:100; rabbit anti-RyR2, AB9080, 1:200). Manders' coefficients- M1= 0.98, M2= 0.998. **B)** PS1 vs. RyR3 (rabbit anti-RyR3, AB9082; 1:200); M1= 0.966, M2= 0.99. **C)** PS2 vs. RyR2 (Mouse anti-PS2, APS26, 1:200); M1= 0.95, M2= 0.991. **D)** PS2 vs. RyR3; M1= 0.988, M2= 0.995.

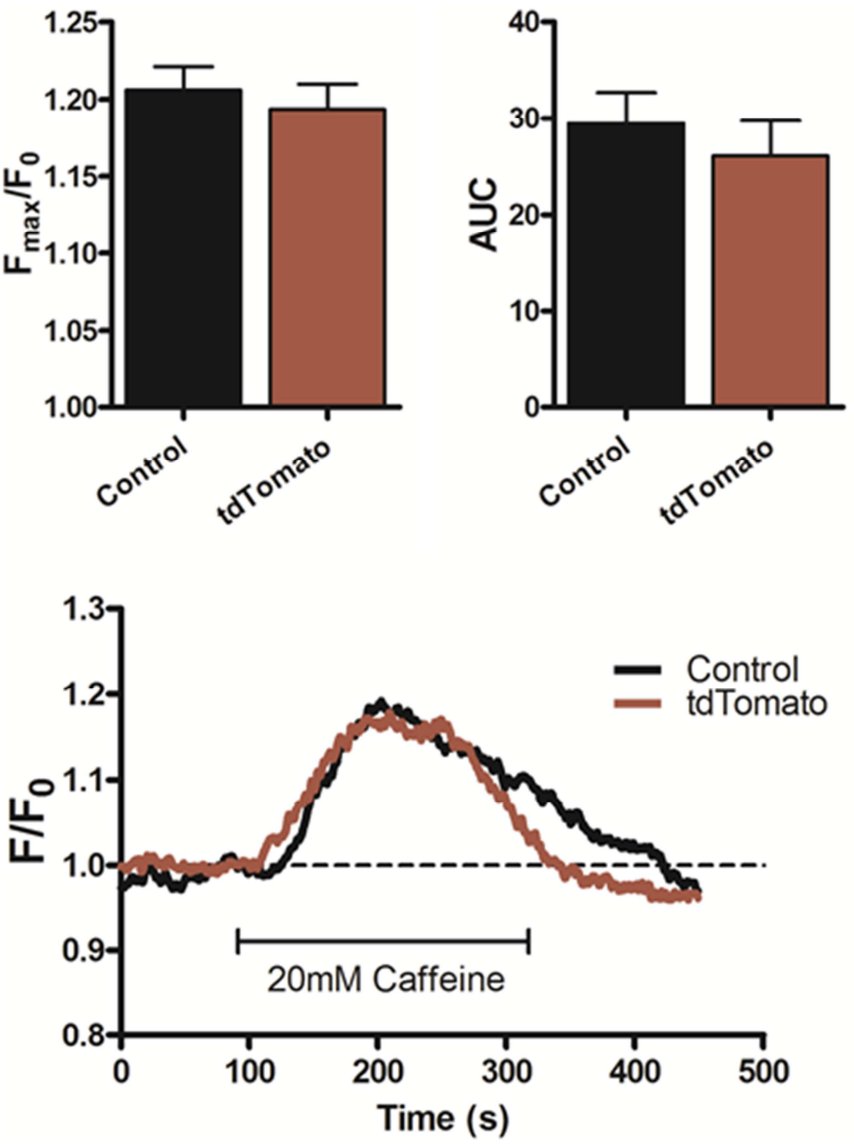


Supplementary Figure 5. SH-SY5Y cells cotransfected with PS-NTF construct and GFP marker. Cells transfected by electroporation with 4µg PS-NTF construct plasmid DNA and 1µg pmaxGFP plasmid DNA (transfection marker) had increased immunofluorescence of the presenilin fragment. Columns from left to right- DIC with 10 µm scale bar, GFP fluorescence, PS-NTF immunofluorescence, pseudo-color heat map to show the increased PS immunofluorescence of the cells expressing the GFP

cotransfection marker (increasing redness indicates increased PS immunofluorescence intensity). **A)** PS1NTF, rabbit anti-PS-1 (sc-7860, 1:50). **B)** PS1NTF-CCCC, stained with rabbit anti-PS-1 (sc-7860, 1:50). **C)** PS2NTF, stained with rabbit anti-PS-2 (sc-7861, 1:50). **D)** PS2NTF-ADQQ, stained with rabbit anti-PS-2 (sc-7861, 1:50).

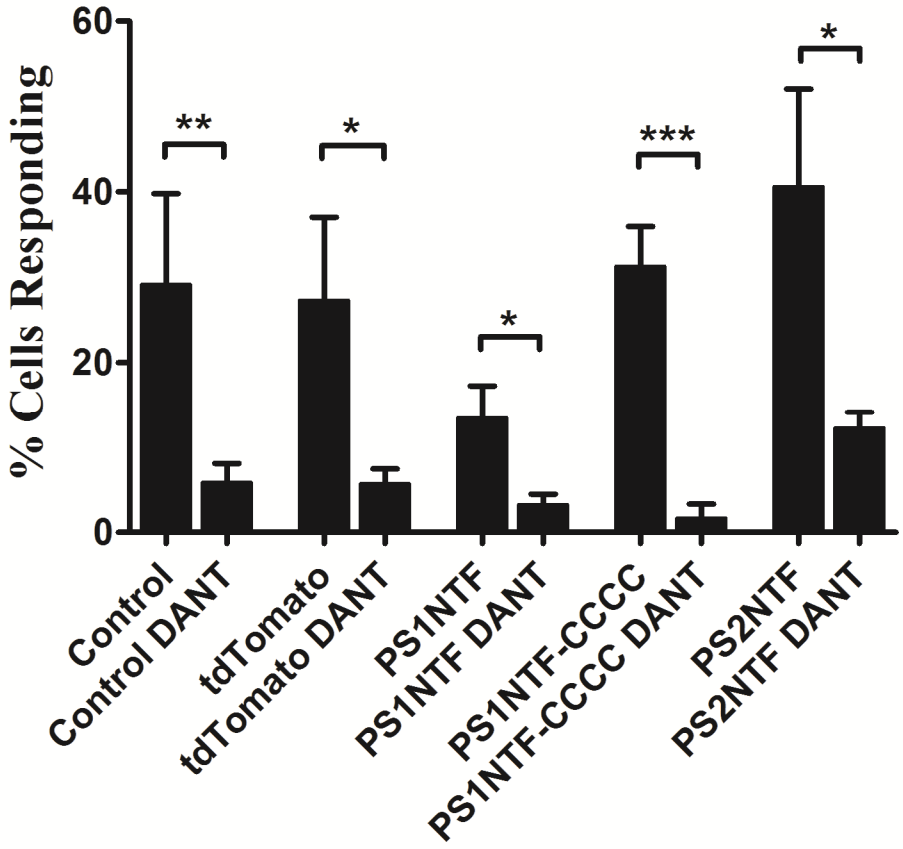


Supplementary Figure 6. PS-NTFs co-immunoprecipitated with RyR. A) GST tagged PS-NTF proteins (PS1NTF-GST, PS1NTF-CCCC-GST, PS2NTF-GST, and PS2NTF-ADQQ-GST; 36 kDa) were expressed in *E. coli* and probed by Western blot (WB) with goat anti-GST (ab6613; 1:8,000; top), rabbit anti-PS1 (sc-7860; 1:200; middle), and rabbit anti-PS2 (sc-7861; 1:200; bottom). Molecular weights were as expected with the PS2NTF fragment running slightly heavier than predicted as reported previously (2, 3). **B)** SH-SY5Y cell homogenates with and without addition of recombinant PS1NTF-GST (rPS1NTF-GST) and/or 1mM DSP, a reversible linker compound that aids formation of cysteine bridges, were co-immunoprecipitated with rabbit anti-RyR2 (AB9080; 1:200). Bound PS1 was detected with rabbit anti-PS1 (sc-7860; 1:200). **C)** Similar samples as in B were incubated with recombinant PS2NTF-GST (rPS2NTF-GST), co-immunoprecipitated with rabbit anti-RyR2, and probed with rabbit anti-PS2 (sc-7861; 1:200). In both panels (B & C), endogenous PS1 & PS2 N-terminal cleavage products (34 kDa) (4, 5) were detected along with the recombinant PS-NTF constructs (36kDa).



Supplementary Data Figure 7. Transfection does not alter the cellular response to pharmacological activation of RyR. A) Comparison of calcium transients in SH-SY5Y cells and tdTomato transfected SH-SY5Y cells showed no change in the maximum amplitude (F_{max}/F_0; n=8 and 10, respectively) of RyR-mediated intracellular Ca^{2+} release in response to a 20 mM caffeine stimulus (*P* = 0.59, two-tailed Student's *t*-test). **B)** The Area Under the Curve (AUC) of calcium transients, a measure of total intracellular calcium release, was not significantly different in SH-SY5Y cells transfected with

tdTomato compared to control SH-SY5Y cells (P = 0.52, two-tailed Student's t-test). Data is shown as mean ± SEM. **C)** Representative traces of untransfected SH-SY5Y cells and SH-SY5Y cells transfected with tdTomato. Fluorescent signals normalized to baseline resting calcium concentrations determined as the average fluorescence during T=70-100 seconds prior to the caffeine stimulated intracellular calcium release initiated at approximately T=120. Time parameters can be found in Supplementary Data Table 3. [* P <0.05; ** P <0.01; *** P <0.001]



Supplementary Figure 8. Dantrolene blocks caffeine induced calcium release in SH-SY5Y cells. SH-SY5Y cells pretreated with 20 μM dantrolene (DANT) were stimulated with 20 mM caffeine. The number of responding cells (20% threshold) was divided by the total number of cells per coverslip and the coverslip percentages were pooled by transfection group. Data is shown as mean ± SEM. Significance determined by one-way ANOVA with Bonferroni's post hoc test. [* P <0.05; ** P <0.01; *** P <0.001]

Supplementary Data Tables

	F_{max}/F_0	SEM	n
Control	1.206	±0.015	8
tdTomato	1.193	±0.016	10
PS1NTF	1.126	±0.007	8
PS1NTF-CCCC	1.162	±0.013	8
PS2NTF	1.198	±0.014	11
PS2NTF-ADQQ	1.111	±0.014	8

	tdTomato	PS1NTF	PS1NTF-CCCC	PS2NTF	PS2NTF-ADQQ
Control	n.s.	**	n.s.	n.s.	***
tdTomato	-	*	n.s.	n.s.	**
PS1NTF	-	-	n.s.	**	n.s.
PS1NTF-CCCC	-	-	-	n.s.	n.s.
PS2NTF	-	-	-	-	***

Supplementary Data Table 1. Amplitude (F_{max}/F_0) of pharmacologically elicited intracellular calcium release in SH-SY5Y cells transfected with PS-NTF constructs. SH-SY5Y cells were treated with 20mM caffeine for 270 seconds to elicit RyR-mediated intracellular calcium release. Maximum amplitude (F_{max}/F_0) was measured per cell, averaged by coverslip (n) (approximately 30-60 cells for each n), and pooled by experimental group (plasmid transfection). F_{max}/F_0 is expressed in units of relative fluorescence using a baseline calculated for each transient (average fluorescence T = 70-100 s). Data shown as mean ± SEM, asterisks indicate

significance by one-way ANOVA with Bonferroni's post-hoc test for multiple comparisons. [n.s. no significance; * P <0.05; ** P <0.01; *** P <0.001]

	AUC	SEM	n
Control	29.44	±3.23	7
tdTomato	26.09	±3.65	9
PS1NTF	15.01	±1.30	8
PS1NTF-CCCC	22.50	±1.95	7
PS2NTF	30.78	±3.36	11
PS2NTF-ADQQ	16.28	±3.08	8

	tdTomato	PS1NTF	PS1NTF-CCCC	PS2NTF	PS2NTF-ADQQ
Control	n.s.	*	n.s.	n.s.	*
tdTomato	-	n.s.	n.s.	n.s.	n.s.
PS1NTF	-	-	n.s.	**	n.s.
PS1NTF-CCCC	-	-	-	n.s.	n.s.
PS2NTF	-	-	-	-	***

Supplementary Data Table 2. AUC, a measure of total intracellular calcium release, of calcium transients elicited by caffeine in PS-NTF transfected cells. SH-SY5Y cells were treated with 20mM caffeine for 270 seconds followed by 140 seconds of buffer wash to return signal to baseline. Area under the curve (AUC) of calcium transients is a correlated, relative measure of the amount of calcium release from the ER into the cytosol in response to caffeine stimulus. AUC was determined only for calcium transients at least 5% above baseline and was measured dynamically per cell (approximately from T= 120 seconds to the return to baseline at T= 400 seconds; Supplementary Data Table 3). AUC values were averaged by coverslip (n) with approximately 30-60 cells for each n, and pooled by experimental group (plasmid transfection). Data is shown as mean ± SEM, asterisks indicate significance by one-way ANOVA (P<0.01) with Bonferroni's post-hoc test for multiple comparisons [n.s. no significance; * P <0.05; ** P <0.01; *** P <0.001].

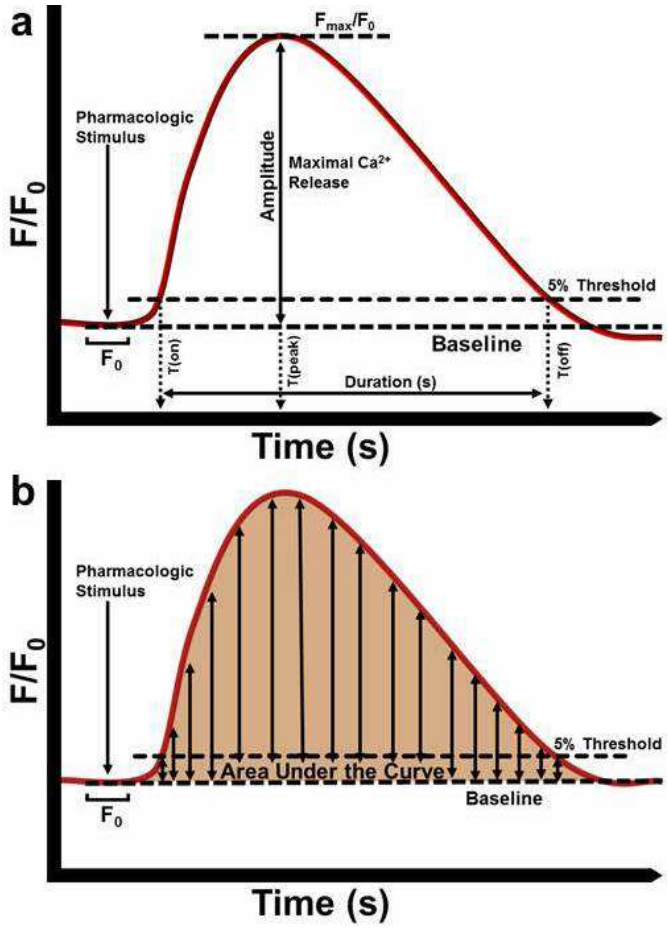
	$Time_{ON}$	SEM	n	$Time_{OFF}$	SEM	n	$Time_{PEAK}$	SEM	n
Control	123.6	±7.53	8	397.7	±17.66	8	247.7	±21.68	8
tdTomato	136.3	±14.03	10	401.3	±19.54	10	274.8	±25.14	10
PS1NTF	123.6	±6.367	9	340.4	±17.92	9	234.5	±20.5	9
PS1NTF-CCCC	122.6	±10.94	8	395.3	±20.98	8	239.5	±21.97	8
PS2NTF	120.5	±6.57	11	396.6	±17.31	11	235.9	±12.46	11
PS2NTF-ADQQ	149.7	±11.61	9	391.3	±28.66	9	264.2	±19.87	9
***P* Value (ANOVA)**	0.28 (n.s.)			0.31 (n.s.)			0.63 (n.s.)		

Supplementary Data Table 3. Time course parameters of pharmacologically elicited intracellular calcium transients in SH-SY5Y cells. Calcium release was stimulated by 20mM caffeine perfused over cells for 270 seconds starting at T=30 seconds, followed by wash with ECS buffer for 140 seconds. $Time_{ON}$ was the time in seconds the caffeine response rose 5% above baseline. $Time_{OFF}$ was the time point where F/F_0 returned to baseline. $Time_{PEAK}$ was the time point in seconds where the calcium response reached F_{max}/F_0 (amplitude). Time values were averaged by coverslip (n) with 30-60 cells for each n and pooled by experimental group (plasmid transfection). Data is shown as mean ± SEM, one-way ANOVA found no significant difference among experimental groups. [n.s., no significance]

	tdTomato	PS1NTF	PS1NTF-CCCC	PS2NTF	PS2NTF-ADQQ
Control	n.s.	***	*	n.s.	***
tdTomato	-	***	n.s.	**	***
PS1NTF	-	-	*	***	n.s.
PS1NTF-CCCC	-	-	-	*	**
PS2NTF	-	-	-	-	***

Supplementary Data Table 4. Chi squared test (χ^2) of statistical significance of binned F_{max}/F_0 values for each experimental group of Figure 4. The F_{max}/F_0 values were sorted into 5% bins and compared by contingency table with Pearson's χ^2 test for significance. [n.s. no significance; * *P* <0.05; ** *P* <0.01; *** *P* <0.001]

Methods Figures



Methods Figure 1. Diagram of an intracellular fluorescent calcium transient identifying key parameters for quantitative assessment of cellular activity. A) F_{max}/F_0, maximum amplitude, maximal Ca^{2+} release; Baseline, average fluorescent signal for T=70-100 (F_0); Threshold, 5% above baseline ($F/F_0 > 1.05$); T(on), time (s) fluorescent signal rises 5% above baseline; T(peak) time (s) at $Fmax/F_0$; T(off), time (s) fluorescent signal returns to baseline. Abscissa, Time (s); ordinate, F/F_0 (relative fluorescence normalized to baseline). **B)** Area under the curve (AUC) is a surrogate marker for total intracellular calcium release. AUC calculation required the peak to rise

above 5% (inclusive threshold), extend for at least 100 seconds, and return to baseline value during the second ECS wash step (T=300-440). AUC values are unit-less but can signify relative amounts of calcium released during caffeine stimulation.

Name	Sequence (5' - 3')	%GC Tm	Therm Tm
PS1NTF Sense	ACATGGATCCGCCACCATGACAGAGATACCTG CACCTTTGTCCTACTTC	58	63.7
PS1NTF Antisense	ACATGAATTCTTATTAGACATGCTTGGCTCCAT ATTTCAATGTCAGC	56.6	66.6
PS2NTF Sense	ACATGGATCCGCCACCATGCTCGCATTCATGG CCTCTGACAGC	57.7	68.5
PS2NTF Antisense	ACATGAATTCTTATTAATGCTTCGCCCCATACTT GAGGGTC	55.9	64
PS1.A14C	CCTTTGTCCTACTTCCAGAATTGCCAGATGTCT GAGGACAGCC	65.2	76.5
PS1.D31C	GCGCCATCCGGAGCCAGAATTGCAGCCAAGAA CGGCAGCAGC	70.2	86.9
PS1.Q56C	CCAATATCTAATGGGCGGCCCTGTAGTAACTC AAGACAGGTGG	65.2	75.6
PS1.Q65C	CTCAAGACAGGTGGTGGAATGCGATGAGGAG GAAGACGAAG	65.4	77.8
PS2.C14A	CTGACAGCGAGGAAGAGGTGGCTGATGAGCG GACGTCCTTG	68.4	81.7
PS2.C31D	GAGCCCCACATCTCGCTCCGACCAGGAAGGCA GGCCAGGCCC	73.1	87.6
PS2.C56Q	CTCAGGAGAGCGAAGAAGACCAGGAAGAGGA CCCGGACCGCTACG	70.4	82.4
PS2.C65Q	GGACCCGGACCGCTACGCACAAAGTGGGGCT CCTGGGCGACC	73.1	87.5
tdTomato Sense	ACATGGATCCGCCACCATGGTGAGCAAGGGC GAGGAGGTC	57.4	65.2
tdTomato Antisense	ACATGAATTCTTATTAGTCCATGCCGTACAGGA ACAGGTGG	57.5	64.2

Methods Table 1. DNA primers used for cloning reactions. All primers were supplied by Integrated DNA Technologies (IDT, Coralville, IA). Melting temperatures (Tm) determined by thermogenic and %GC content methods by Vector NTI (Invitrogen, Carlsbad, CA) and given as °C.

Epitope	Host	Supplier	Cat#	Use/Dilution	Reference
anti-GST	Gt	(Abcam, Cambridge, MA)	AB6613	WB 1:8,000	Maeda Y et al. (2006)
anti-IP3R1	Gt	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-6093	ICC 1:50	Nalaskowski MM *et al*. (2011)
anti-IP3R2	Gt	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-7278	ICC 1:50	Nalaskowski MM *et al*. (2011)
anti-IP3R3	Gt	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-7277	ICC 1:50	Nalaskowski MM *et al*. (2011)
anti-PS1	Rb	(Oncogene, EMD Millipore, Bellerica, MA)	PC267 (303-316)	ICC 1:100	Sherrington R *et al*. (1995)
anti-PS1	Rb	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-7860	WB 1:200; ICC 1:50	Ong CT *et al*. (2008)
anti-PS1	Rb	(Abcam, Cambridge, MA)	Ab53717	ICC 1:100	
anti-PS1-CTF	Ms	(EMD Millipore, Bellerica, MA)	MAB5232	ICC CoLoc 1:100	Runz H *et al*. (2002)
anti-PS2	Rb	(US Biologicals, Swampscott, MA)	P6300-40H	ICC 1:100	Yagi T *et al*. (2008)
anti-PS2	Rb	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-7861	WB 1:200; ICC 1:50	Ong CT *et al*. (2008)
anti-PS2-CTF	Ms	(Pierce, Thermo Fisher Scientific, Rockford, IL)	APS26	ICC CoLoc 1:100	Diehlmann A *et al*. (1999)
anti-RyR1	Rb	(EMD Millipore, Bellerica, MA)	AB9078	ICC 1:200	Medina-Ortiz WE *et al*. (2007)
anti-RyR2	Rb	(EMD Millipore, Bellerica, MA)	AB9080	ICC 1:200	Medina-Ortiz WE *et al*. (2007)
anti-RyR3	Rb	(EMD Millipore, Bellerica, MA)	AB9082	ICC 1:200	Medina-Ortiz WE *et al*. (2007)

Methods Table 2. List of primary antibodies. Gt – Goat, Rb – Rabbit, Ms – Mouse, WB - Western Blot, ICC - Immunocytochemistry, CoLoc – Colocalization.

Epitope	Host	Label	Supplier	Cat#	Use
anti-Goat IgG	Donkey	Alexa Fluor 488	(Life Technologies, Carlsbad, CA)	A-11055	ICC 1:2,000
anti-Goat IgG	Donkey	Alexa Fluor 594	(Life Technologies, Carlsbad, CA)	A-11058	ICC 1:2,000
anti-Goat IgG	Donkey	HRP	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-2020	WB 1:5,000
anti-Mouse IgG	Donkey	Alexa Fluor 488	(Life Technologies, Carlsbad, CA)	A-21202	ICC 1:2,000
anti-Mouse IgG	Donkey	Alexa Fluor 594	(Life Technologies, Carlsbad, CA)	A-21203	ICC 1:2,000
anti-Mouse IgG	Donkey	HRP	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-2314	WB 1:5,000
anti-Rabbit IgG	Donkey	Alexa Fluor 488	(Life Technologies, Carlsbad, CA)	A-21206	ICC 1:2,000
anti-Rabbit IgG	Donkey	Alexa Fluor 594	(Life Technologies, Carlsbad, CA)	A-21207	ICC 1:2,000
anti-Rabbit IgG	Donkey	HRP	(Santa Cruz Biotechnology, Santa Cruz, CA)	sc-2314	WB 1:5,000

Methods Table 3. List of secondary antibodies. Secondary antibodies were applied concomitant with nuclear counterstain by Hoechst 33258 at 0.12 µg/ml. WB - Western Blot, ICC – Immunocytochemistry, HRP – Horse radish peroxidase

1. Manders EMM, Verbeek FJ, Aten JA. Measurement of co-localization of objects in dual-colour confocal images. Journal of Microscopy. 1993;169:375-82.
2. Hayrapetyan V, Rybalchenko V, Rybalchenko N, Koulen P. The N-terminus of presenilin-2 increases single channel activity of brain ryanodine receptors through direct protein-protein interaction. Cell Calcium. 2008;44(5):507-18.
3. Rybalchenko V, Hwang SY, Rybalchenko N, Koulen P. The cytosolic N-terminus of presenilin-1 potentiates mouse ryanodine receptor single channel activity. Int J Biochem Cell Biol. 2008;40(1):84-97.
4. Murayama O, Honda T, Mercken M, Murayama M, Yasutake K, Nihonmatsu N, et al. Different effects of Alzheimer-associated mutations of presenilin 1 on its processing. Neurosci Lett. 1997;229(1):61-4.
5. Thinakaran G, Borchelt DR, Lee MK, Slunt HH, Spitzer L, Kim G, et al. Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. Neuron. 1996;17(1):181-90.