Supplementary Figure 1

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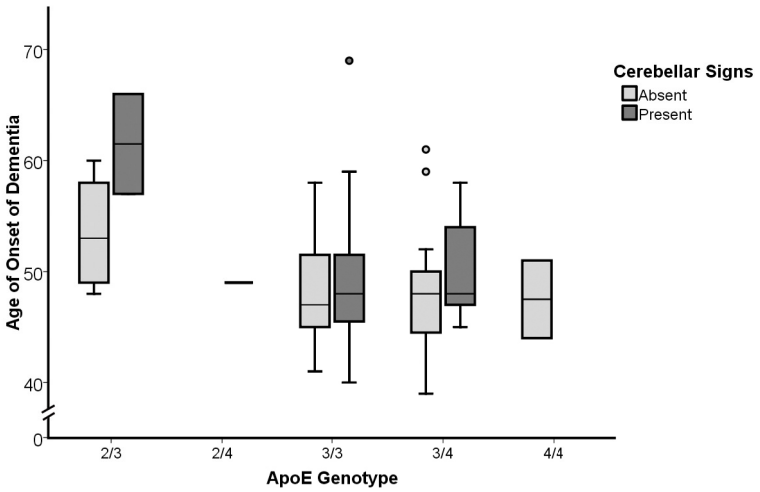

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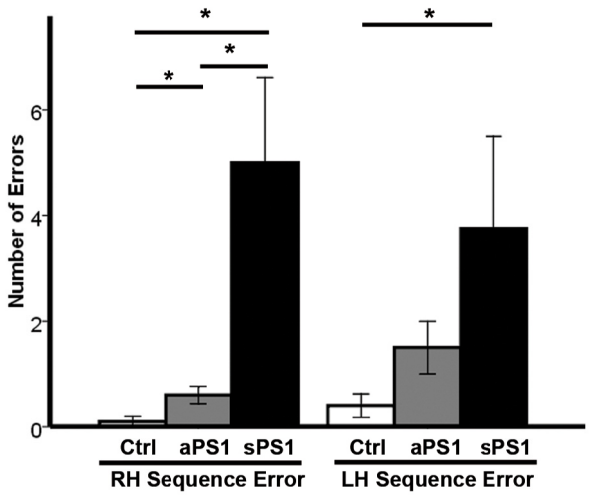

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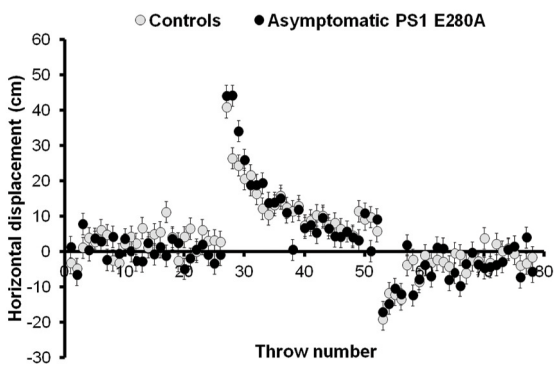
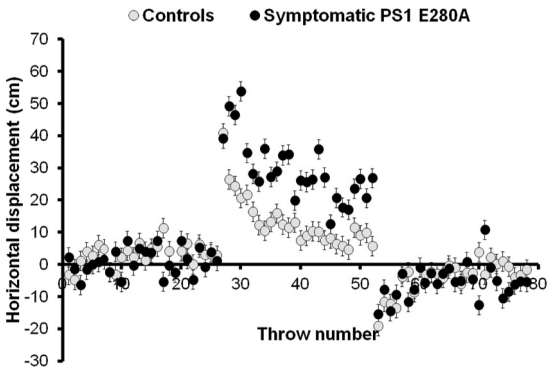
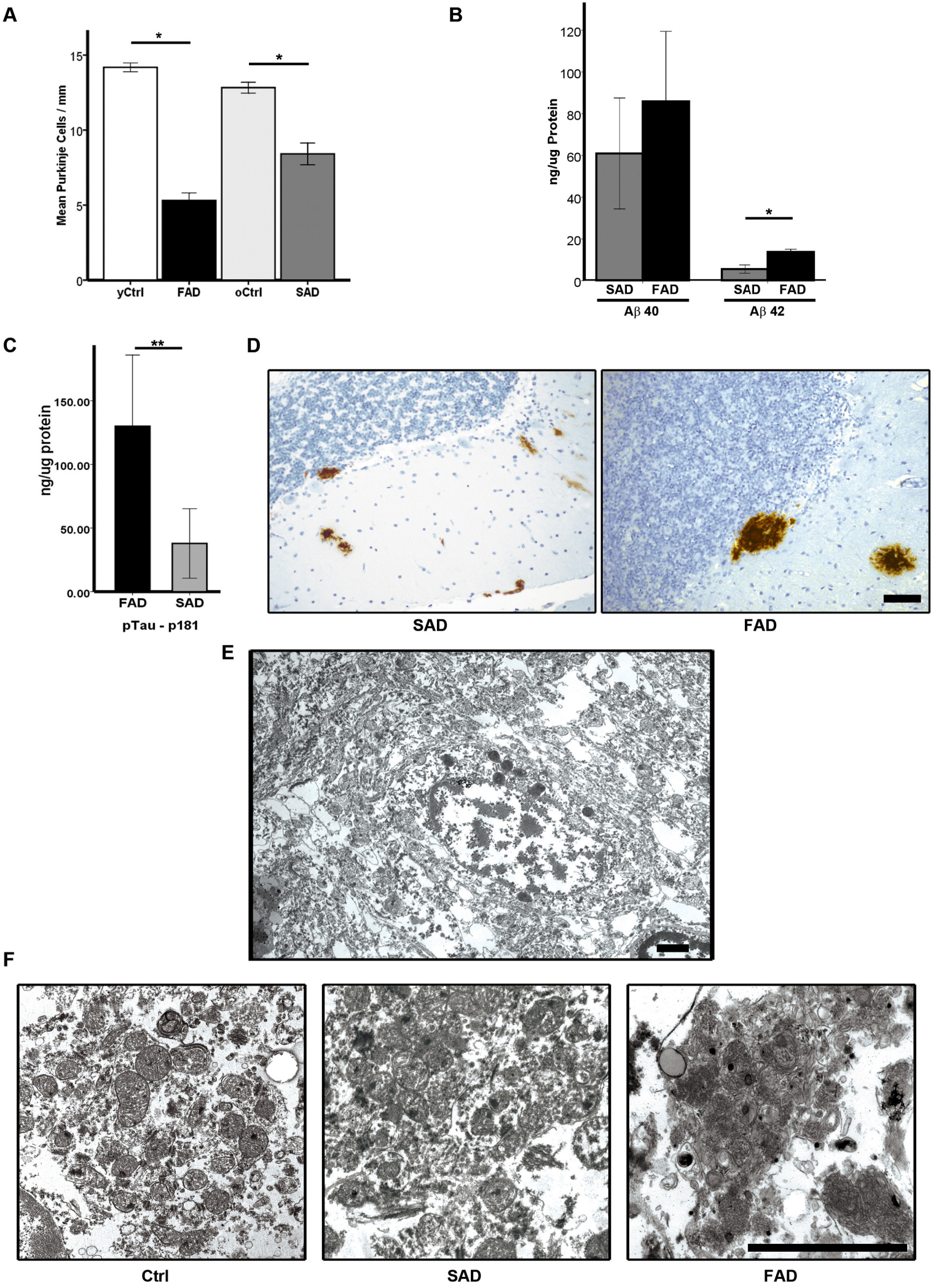
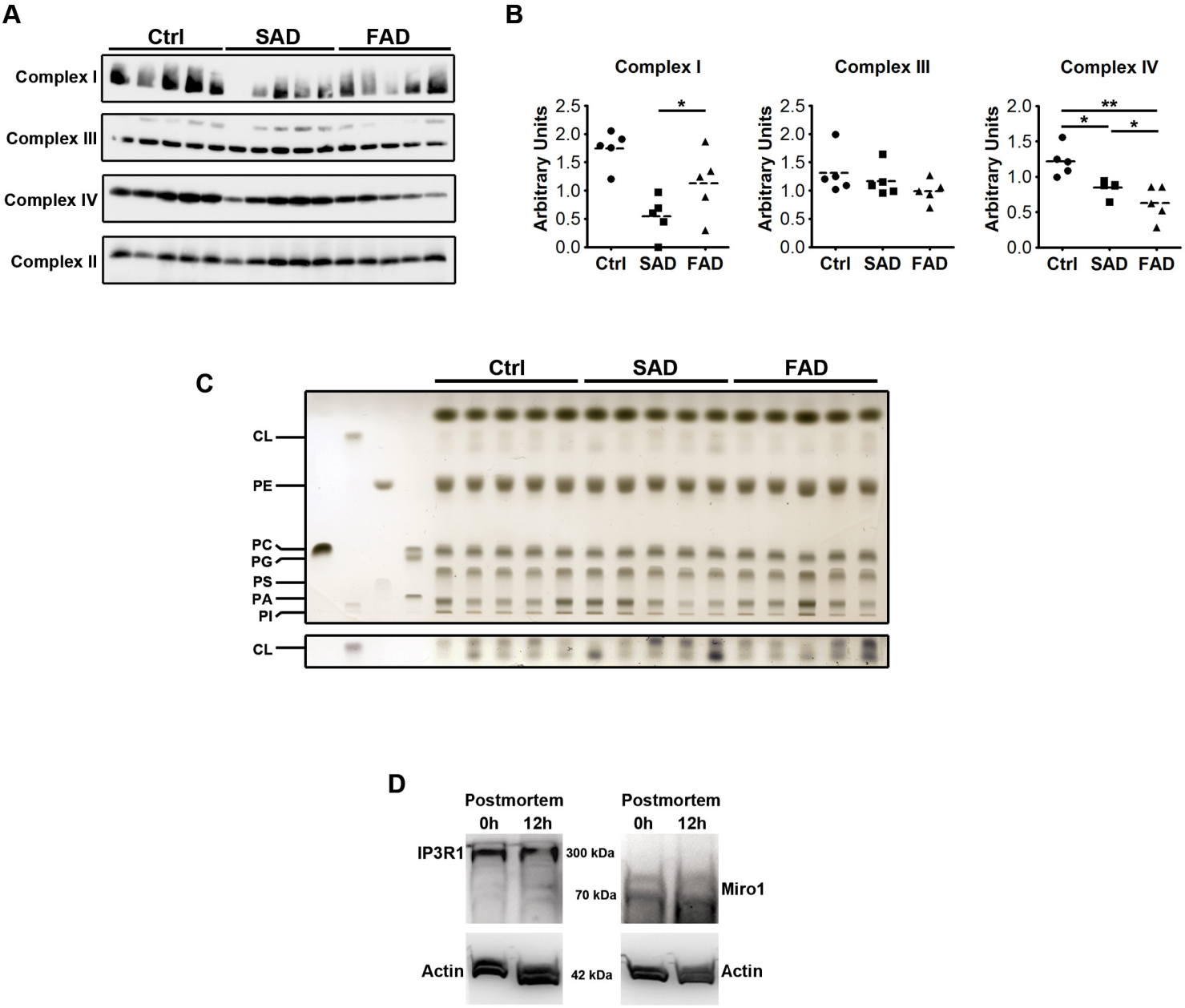




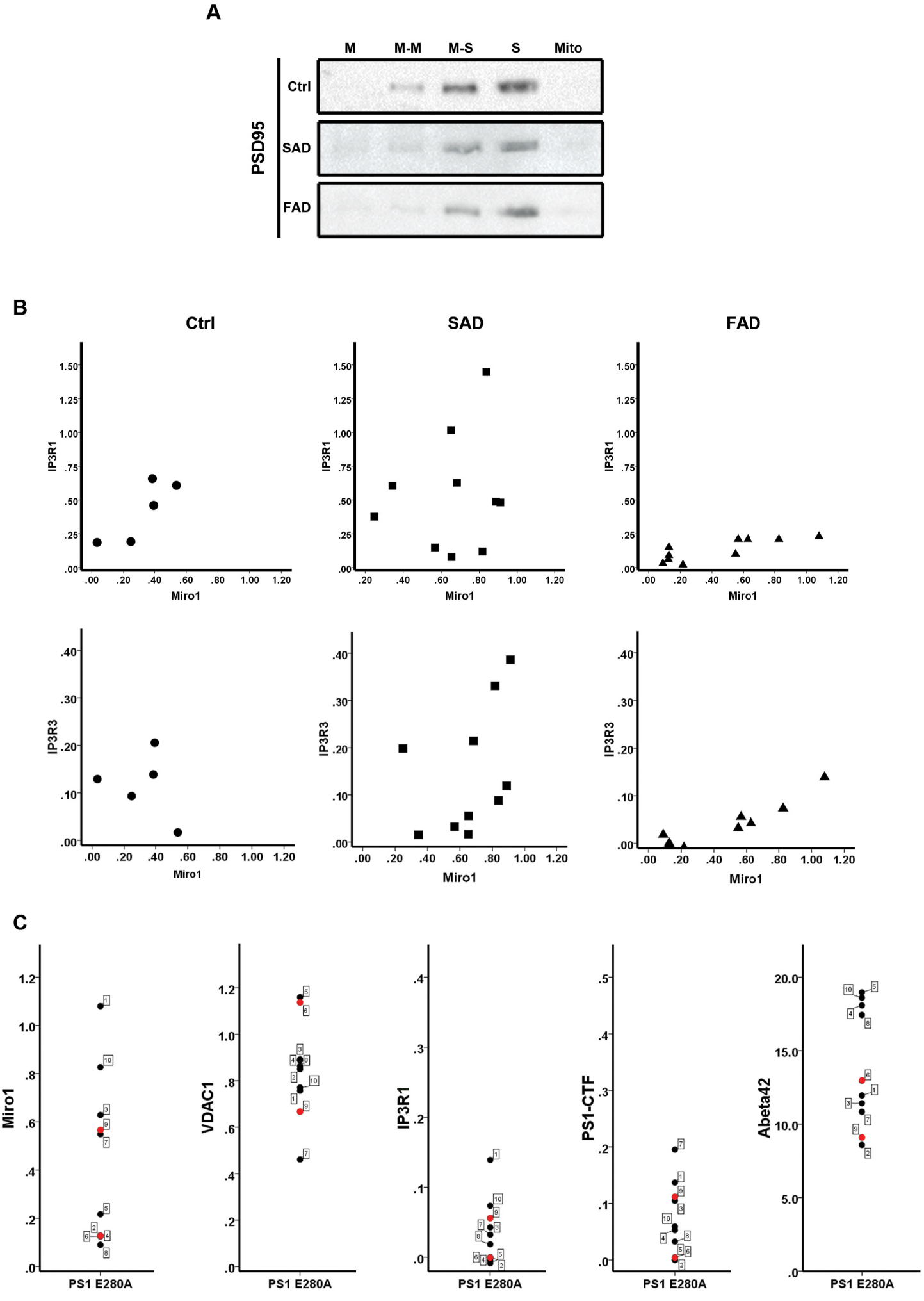
Supplementary Figure 2



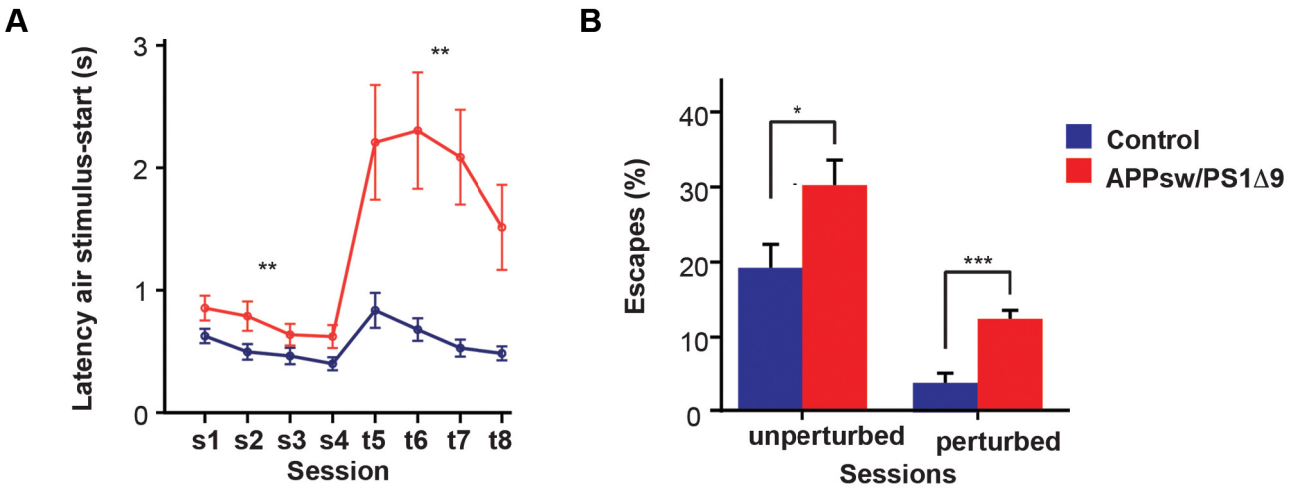
Supplementary Figure 3



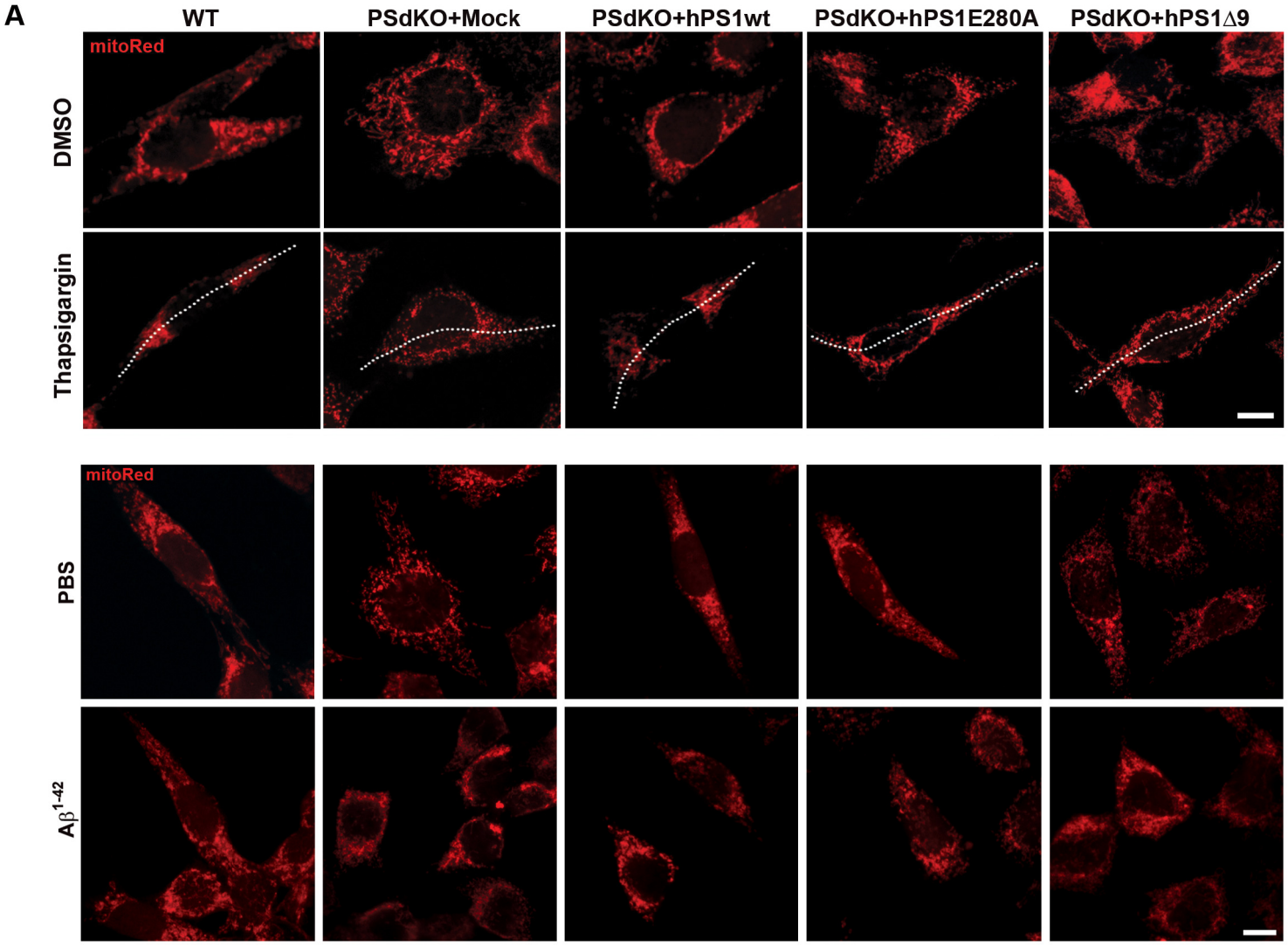
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figures legends

Supplementary Figure 1: Cerebellar dysfunction in E280A carriers.

(A) Box plots represent age of onset of dementia in E280A-FAD patients according to presence or absence of cerebellar signs and ApoE genotype status. (ApoE2/3 without n=6 and with cerebellar signs n=2, ApoE2/4 with cerebellar signs n=1, ApoE3/3 without n=47 and with cerebellar signs n=48, ApoE3/4 without n=16 and with cerebellar signs n=13, ApoE4/4 without n=2). Whiskers represent distribution, horizontal lines mean values and circles outliers.

(C) Number of errors during 9-HPT completion with right and left hands (RH, LH). Statistics: two tailed t-test, *=$p<0.05$, data are means with +/- s.e.m.

(D) Horizontal throws displacement in PAT. Each dot represents mean values with +/- s.e.m.

Supplementary Figure 2: Morphological assessment of cerebellar pathology in E280A-FAD.

(A) Quantification of PCs using Calbindin as a marker in cerebellar vermis of age matched controls for each AD group (yCtrl=young Ctrl, n=5, mean age of death=57 years old, SD=4.80; oCtrl=old Ctrl, n=5, mean age of death=69.4 years old, SD=4.23; and FAD n=10, SAD n=11). Statistics: U Mann-Whitney test, *=$p<0.05$, **=$p<0.01$, data are means +/- s.e.m.

(B) ELISA measurement of $A\beta_{1-40}$ (Aβ40) and Ab_{1-42} (Aβ1-42) levels in the cerebellum of E280A-FAD (n=10) and SAD (n=11) patients. Statistics: U Mann-Whitney test, *=$p<0.05$, data are means +/- s.e.m.

(C) ELISA measurement of pTau-p181 measured levels in the cerebellum of E280A-FAD (n=10) and SAD (n=11) patients. Statistics: U Mann-Whitney test, **=$p<0.01$, data are means +/- s.e.m.

(D) Immunohistochemistry for Aβ plaques with 6E10 antibody in the cerebella of E280A-FAD and SAD patients. Representative pictures. Scale bar=50μm.

(E) Example of ultrastructural analysis images of E280A-FAD cases used for the study. Note the absence of plaques in the vicinity of a PC cell showing abnormal mitochondria. Scale bar =5 μm.

(F) Ultrastructural analysis of PCs of age matched Ctrl, SAD and FAD (all groups n=3, representative pictures) showing clustering of mitochondria. E280A-FAD shows significantly more abnormal mitochondria with electron dense material. Scale bar =5 μm.

Supplementary Figure 3: Biochemical analysis of mitochondrial function in the cerebella of E280A-FAD patients.

(A) Blue native gels for mitochondrial respiratory Complexes I to IV in Ctrl (n=5), SAD (n=5), and FAD (n=5). Right panel shows densitometric quantification of Complexes I to IV normalized to Complex II levels.

(B) Thin layer chromatographic analysis of mitochondrial phosholipids in Ctrl (n=5), SAD (n=5), and FAD (n=5). Cardiolipin (CL), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidic acid (PA) and phosphatidylinsitol (PI).

(C) Western blots of IP3R1 and Miro1 from human temporal cortex biopsies homogenized fresh and after 12 hours.

Supplementary Figure 4: Synaptosome enriched fractions from cerebella and correlation analysis of affected proteins in E280A-FAD cases

(A) Subcellular fractionation on Percoll density gradient and representative immunoblots with PSD95 antibody for synaptosomal-enriched fractions (M=membranes, M-M=membranes and myelin, M-S=membranes and synaptosomes, S= synaptosomes, Mito= mitochondria) from cerebella of Ctrl (n=4), SAD (n=5), and FAD (n=5).

(B) Correlation scatter plot for IP3R1 and IP3R3 and Miro1 levels in Ctrl (n=5), SAD (n=10), and FAD (n=10) patients.

(C) Scatter plots for distribution of densitometry values of Miro1, VDAC1, IP3R1, PS1-CTF and Aβ42 levels for PS1-E280A patients (n=10). Patients under treatment with phenytoin or carbamazepine are depicted in red.

Supplementary Figure 5: Cognitive deficits in APPsw/PS1Δ9 tested with the Erasmus Ladder during unperturbed and perturbed sessions

(A) Time spend inside the box (after the air stimulus is on) before animals start to run on the ladder (unperturbed sessions and perturbed sessions, repeated measures of pre and post-steptimes measurements for control group (n=12) and APPsw/PS1Δ9 mice (22-24 weeks of age, n=8).

(B) Frequency of escapes during unperturbed and perturbed sessions. Statistics: One-way ANOVA test, *=p<0.05, **=p<0.01, ***=p<0.001, data are means +/- s.e.m.

Supplementary Figure 6: Mitochondrial distribution in PS1/PS2 deficient cells after Thapsigarging or Aβ1-42 treatment

Representative confocal fluorescent micrographs of WT MEFs and PSdKO MEFs transfected with human wt PS1 (PSdKO+hPS1wt) or PS1 mutants (PSdKO+hPS1E280A, PSdKO+hPS1Δ9) labeled with Mitotracker Red. Basal conditions (vehicle=DMSO or PBS) and Thapsigarging or Aβ1-42 30' treatment. Scale bar =20 μm.

Supplementary Table 1

Frequency of cerebellar symptoms in AD patients

	E280-FAD n=135		SAD n=85		
Variable	**Count**	**%**	**Count**	**%**	**p**
Cerebellar Symptoms	64	47.4	3	3.5	0.000
Incoordination	14	10.4	---	---	0.000
Dysdiadochokynesia	42	31.1	1	1.2	0.000
Intention Tremor	24	29.7	2	2.4	0.001
Ataxia	2	1.5	---	---	0.000

Supplementary Table 2

Minimental Score of patients tested for cerebellar function

	Controls n=10		aPS1 n=10		sPS1 n=9	
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Minimental	29.80	0.42	29.40	1.08	23.78	3.42

Supplementary Table 3 Demographics and sample distribution for tissue analysys

Group	Case	Source	Gender	AO	AoD	DD	Braak stage	CERAD score	ApoE Haplotype	PI	CB-Aβ plaques	PCs Qu	Abeta /pTau ELISA	EM analysis	MF analysis	WTH analysis	SP analysis	IF
FAD	1	GNA	F	47	54	7	VI	C	3/3	5.5	+	+	+	+	+	+	+	+
FAD	2	GNA	M	44	52	8	VI	C	3/3	4.8	+	+	+	---	---	+	---	---
FAD	3	GNA	M	47	56	9	VI	C	3/3	3.3	+	+	+	---	---	+	---	---
FAD	4	GNA	F	39	59	20	VI	C	3/4	3.7	+	+	+	+	+	+	+	+
FAD	5	GNA	F	46	67	21	VI	C	3/4	4	+	+	+	+	+	+	+	+
FAD	6	GNA	F	49	62	13	VI	C	3/3	4	+	+	+	---	---	+	---	---
FAD	7	GNA	F	48	64	16	VI	C	3/3	3	+	+	+	---	+	+	+	---
FAD	8	GNA	F	43	48	5	VI	C	3/3	4	+	+	+	---	---	+	---	---
FAD	9	GNA	F	37	47	10	VI	C	3/3	2.3	+	+	+	---	+	+	+	---
FAD	10	GNA	F	50	60	10	VI	C	3/3	2.8	+	+	+	---	---	+	---	---
% or Mean (SD)			F=80%	45 (4.3)	56.9 (6.7)	11.9 (5.5)			E4=20%	3.7 (0.9)								
SAD	11	GNA	M	NA	67	NA	V	C	3/3	9.3	---	+	+	---	---	+	---	---
SAD	12	GNA	M	80	86	6	IV	C	NA	18.3	+	+	+	---	+	+	+	---
SAD	13	GNA	F	55	70	15	IV	B	3/4	11.8	+	+	+	---	---	+	---	---
SAD	14	GNA	F	79	87	8	V	C	3/4	2.8	+	+	+	---	---	+	---	---
SAD	15	GNA	F	82	91	9	VI	C	3/3	4.5	+	+	+	+	+	+	+	+
SAD	16	GNA	F	58	79	21	II	C	NA	10.3	+	+	+	---	---	+	---	---
SAD	17	GNA	F	65	74	9	IV	C	3/3	2.5	+	+	+	+	+	+	+	+
SAD	18	GNA	F	65	76	11	V	C	4/4	4	+	+	+	+	+	+	+	+
SAD	19	GNA	F	69	76	7	VI	C	3/4	8	+	+	+	---	+	---	+	---
SAD	20	GNA	M	NA	83	NA	VI	C	3/2	4.5	+	+	+	---	---	+	+	---
SAD	21	GNA	F	NA	61	NA	VI	C	3/3	7.7	+	+	+	---	---	+	---	---
% or Mean (SD)			F=72.7%	69.1	77.3 (9.1)	10.8 (5.0)			E4=22.5%	7.6 (4.7)								
Ctrl	22	GNA	F	---	61	---	---	---	NA	4	---	+	---	+	+	+	+	+
Ctrl	23	GNA	M	---	60	---	---	---	NA	4	---	+	---	+	+	+	+	+
Ctrl	24	GNA	F	---	60	---	---	---	NA	4	---	+	---	+	+	+	+	+
Ctrl	25	NBB	F	---	54	---	---	---	NA	8	---	+	---	---	+	+	---	---
Ctrl	26	NBB	F	---	50	---	---	---	NA	4.1	---	+	---	---	+	+	+	---
Ctrl	27	UKE	F	---	67	---	---	---	NA	24	---	+	---	---	---	---	---	---
Ctrl	28	UKE	M	---	75	---	---	---	NA	24	---	+	---	---	---	---	---	---
Ctrl	29	UKE	F	---	65	---	---	---	NA	24	---	+	---	---	---	---	---	---
Ctrl	30	UKE	M	---	63	---	---	---	NA	24	---	+	---	---	---	---	---	---
Ctrl	31	UKE	F	---	77	---	---	---	NA	24	---	+	---	---	---	---	---	---
% or Mean (SD)			F=70%		63.2 (8.4)					4.8 (1.8)*								

AO=Age of onset, AoD=Age of death, DD=Disease duration, PI=Postmortem index, CB=Cerebellum, Qu=Quantification, MF=Mitochondrial fractions, WTH=Whole tissue homogenate, SP=Synaptosomal preparation, GNA = Neuroscience Group of Antioquia, NA = Not available, NBB = Netherlands Brain bank, UKE = Universitätsklinikum Hamburg-Eppendorf, IF=Immunofluorescence, F=Female, M=Male, SD=Standard deviation, *=Valid for cases 22 to 26.

Supplementary Table 4
Treatment and cause of death for AD patients

Drug Code	Description
1	Antipsychotic
2	Sedative/hypnotic
3	Ca2+ Blocker
4	Antihistaminic
5	Antiepileptic
6	Parasympathomimetic
7	Anxiolytic
8	Vitamin B12
9	Steroids
10	Antidepressant
11	Benzodiazepine
12	Antiparkinsonian
13	Barbiturate
14	Antihypertensive
15	Diuretic
16	Cardiac glycoside
17	NMDA blocker
18	Opioids
19	NSAID
20	Corticosteroids

Type	Patient code	Treatment	Antiepileptic	Cause of Death
FAD	87	5	Valproate	Septic Shock
FAD	91	13	--	Septic Shock
FAD	114	1, 5, 11	Valproate	Septic Shock
FAD	120	5, 17	Valproate	Septic Shock
FAD	126	1,5	Valproate	Multiple Organ Failure
FAD	127	1,5,6,10,	Valproate	Septic Shock
FAD	130	1,5,11	Phenytoin, Clonazepam	Septic Shock
FAD	131	1,5,6,10,11	Carbamazepine	Multiple Organ Failure
FAD	134	1,5,6,10,	Phenytoin	Septic Shock
FAD	136	1	--	Septic Shock
SAD	72	1,2,3,4,5	Phenytoin	Septic Shock
SAD	75	1,6,7,8,9,10,11	--	Multiple Organ Failure
SAD	78	1,12,	--	Septic Shock
SAD	95	14,15,16	--	Myocardial infarction
SAD	99	10	--	Pulmonary Tromboembolism
SAD	106	1, 11, 12,	--	Multiple Organ Failure
SAD	112	6	--	Septic Shock
SAD	117	6,10	--	Septic Shock
SAD	122	NA	--	NA
SAD	133	6	--	Septic Shock
SAD	145	6,10,17	--	Multiple Organ Failure
Ctrl	34	NA	--	Trauma
Ctrl	37	NA	--	Trauma
Ctrl	38	NA	--	Trauma
Ctrl	14	11, 18,19,20	--	Renal Failure
Ctrl	73	11,18,19,20	--	Multiple Organ Failure

Supplementary Table 5
Summary of changes observed in SH-SY5Y cells expressing PS1

Variable	Conditions	PS1 wt	PS1 E280A	PS1 Δ9
Ca^{2+} stored in the ER	Basal	-	Increased?*	Decreased (28)
	Ca^{2+} overload	Release	> release	< release
ER/Mito tethering	Basal	High	low	low
	Ca^{2+} overload	Low	High	low
Mitochondrial transport	Basal	-	Increased	-
IP3R1 levels	Basal	-	Increased	-
	Chronic Ca^{2+} overload	-	Decreased	-
Miro1 levels	Basal	-	Increased	-
	Chronic Ca^{2+} overload	-	Decreased	Increased

** This variable has not been measured for PS1 E280A in cell models. However, most of evaluated PS1 mutations produce a loss of Ca^{2+} ER leak function (28).*

Supplementary Table 6 Antibodies used in the study

	kDa	Type	Host	Isotype	Specificity	Immunogen	Brand	Serial	Application (concentration)
6E10	4-95	Monoclonal	Mouse	IgG1	Human	aa 1-16 Abeta	Covance	SIG-39320	IH(1:100)
CACNA1A	250-280	Polyclonal	Rabbit	IgG	Human	Cytoplasmic domain	Abcam	ab81011	WB(1:500)
Calbindin	28	Monoclonal	Mouse	IgG1	Human	Full length	Novocastra	NCL-calbindin	WB(1:500), IH(1:100)
Fis1	15-21	Monoclonal	Mouse	IgG2b	Human	Full length	Sigma-Aldrich	WH0051024M1	WB(1:500)
Pink1	50-60	Monoclonal	Mouse	IgG1	Human	N-terminus	Cell Signaling	#5660	WB(1:500)
IP3R1	300	Polyclonal	Rabbit	IgG	Hu, Ms, Rat	Cytoplasmic domain	Millipore	ABS55	WB(1:500)
IP3R3	300	Polyclonal	Rabbit	IgG	Hu, Ms, Rat	C-terminal	Millipore	AB9076	WB(1:500)
MFN2	86	Polyclonal	Rabbit	IgG	Human	372-496	Sigma-Aldrich	HPA030554	WB(1:500)
Opa1	80-100	Monoclonal	Mouse	IgG1	Human	708-830	BD biosciences	612607	WB(1:1000), IF(1:50)
Presenilin 1	22	Polyclonal	Rabbit	IgG	Hu, Ms, Monkey	C-terminal	Cell Signaling	#36225	WB(1:500)
Presenilin 1	55	Polyclonal	Rabbit	IgG	Hu, Ms, Monkey	aa 298-367	Abcam	ab12272	IF(1:50)
PSD95	95	Monoclonal	Rabbit	IgG	Hu, Ms, Rat	N-terminal	Millipore	04-1066	WB(1:500), IF(1:50)
Miro1	75/80	Polyclonal	Rabbit	IgG	Human	90aa	Atlas Antibodies	HPA010687	WB(1:500), IF(1:50)
VDAC1	34	Polyclonal	Rabbit	IgG	Human	ser 104 peptide	Calbiochem	ap1059	WB(1:1000)
VDAC1 (porin 31HL)	34	Monoclonal	Mouse	IgG2a	Human	Full length	Calbiochem	529532	IF(1:50)
pDRP1	78	Monoclonal	Rabbit	IgG	Hu, Ms, Rat	DRP1 Phosphorylated Serine 637	Cell Signaling	#6319	WB(1:500)
Kif5C	108	Polyclonal	Rabbit	IgG	Human	PrEST sequence	Atlas Antibodies	HPA035210	WB(1:500)
Syntaphilin	53/70	Monoclonal	Mouse	IgG1	Hu, Ms, Rat	N-terminal	ECM Biosciences	SM3711	WB(1:500)
Calretinin	29-30	Polyclonal	Rabbit	IgG	Hu, Ms, Rat	N-terminal	Swant	CR7699/3H	WB(1:500)
SERCA2	100	Monoclonal	Mouse	IgG2a	Human	501-621	Sigma-Aldrich	WH0000489M1	WB(1:500)
PDI	58	Polyclonal	Rabbit	IgG	Hu, Ms, Rat	Full length	StressGen	SPA-890	IF(1:50)
KDEL	78-94	Monoclonal	Mouse	IgG1	Hu, Ms, Rat, Monkey, Dro, Yeast	KDEL sequence	Millipore	420400	IF(1:50)
Complex I, subunit (NDUFA9)	42.5	Monoclonal	Mouse	IgG1	Hu, Ms, Rat, Bovine	Bovine Heart Complex I	Invitrogen	459100	WB (1:1000)
Complex II, subunit (SDHA)	72.7	Monoclonal	Mouse	IgG1	Hu, Ms, Rat, Bovine	Bovine Heart Complex II, with traces of Complex III	Invitrogen	459200	WB (1:1000)
Complex III, core 2 (UQCR2)	48.5	Monoclonal	Mouse	IgG	Hu, Ms, Rat, Bovine	Bovine Heart Comp. III	Invitrogen	A11143	WB (1:1000)
Complex IV, subunit II (COX2)	25.6	Monoclonal	Mouse	IgG2a	Hu	Human Complex IV subunit II, from SDS-PAGE	Invitrogen	A6404	WB (1:1000)

Cerebellar dysfunction in PS1 E280A – Sepulveda-Falla et al. 2013

Complete Methods

Human subjects and samples

All living participants of the study signed informed consent for clinical examination, clinical data handling and functional tests. Post mortem tissue was obtained after donor consent according to pertaining laws in Colombia, Netherlands and Germany, and approved by local ethical boards, if appropriate, by the University of Antioquia, Medellin, Colombia and NBB, Amsterdam, Netherlands. Formalin fixed and frozen cerebellar tissue from AD patients and controls, was handled as previously described (1) and used for experiments according to availability and sample status (Supplementary Table 3).

Genotypification

Colombian PS1 EOFAD patients were genotyped for E280A using the following primers: 5'-AACAGCTCAGGAGAGGAATG-3' and 5'-GATGAGACAAGTNCCNTGAA-3´. Also they were genotyped for ApoE alleles with 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' and 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'.

Clinical Data Collection

Retrospective analysis of clinical records from 220 AD patients (Supplementary Table 1) was performed including all registered medical and neurological evaluations. Cerebellar signs were identified and assigned as present when two or more evaluations confirmed them. Ambiguous evaluations indicating other motor disturbances were excluded.

Prism adaptation test

Motor performance was tested in 29 subjects (Supplementary Table 2) threw clay balls (weight: 10 g) at a 12 cm by 12 cm cross drawn on a large sheet of parcel paper centered at shoulder level and placed 2m away in front of them. Subjects were instructed to make each toss overhand during the whole experiment, to use the right hand, and to throw the balls to the location where they saw the target. They were seated with the head restrained in a device in which the prism is placed and they had an

unobstructed view of the target during the entire session. No directions about trunk, shoulder or head/neck posture were given. However, they were not allowed to look down at their hand as they collected the next ball from a tray located next to them. Subjects were asked to throw the ball at their own pace, so they were free to take rests if they felt tired. If such event occurred, they were asked to remain still as possible with their eyes closed. The experiment had three conditions. Under each condition the subjects threw 26 balls. During the baseline condition (PRE) subjects did not wear prisms. After finishing the baseline condition subjects were tested in the PRISM condition, where they wore 20 diopter optic prism that produce a light refraction to the right. Once that condition was finished, subjects had the prisms removed and started the POS condition where they continued throwing balls. The position at which the balls made an impact on or around the target was marked immediately after each throw with a marker pen by an experimenter standing outside the visual field of the subject. The location of the balls impacts were plotted sequentially by trial number (abscissa) versus horizontal displacement (in centimeters) from a vertical line passing through the target centre (ordinate). Impacts to the left of the target were plotted as negative values and impacts to the right were plotted as positive values. The three experimental conditions were carried out consecutively after the donning or doffing of the prisms was completed.

Three additional measures were calculated from the collected data of both experiments. First, a motor performance coefficient (PQ), or called variable error (2;3) was calculated from the baseline phase. To obtain the PQ, the horizontal errors (distance from each impact location to a vertical line passing through the target) of the PRE trials were measured. The PQ is the standard deviation obtained from these errors (2). Second, an adaptation magnitude was obtained by subtracting the horizontal distance to the target on the final throw from that on the initial throw while wearing the prisms (PRISM condition). Third, an aftereffect measure was defined as the ball's impact horizontal distance to the target on the first throw after removing the prisms (2).

Nine-hole pegboard test

A modified version of the 9-hole pegboard test was applied (4). Using the Rolyan 9-Hole Peg Test Kit (United Kingdom), 29 subjects (Supplementary Table 2) were seated at a table with the board that includes a small, shallow container holding the nine pegs and the nine empty holes. On a start command, the patients must pick up the nine pegs, one to one, as quickly as possible, and must put them in the nine holes, following a predetermined order of holes. Once all pegs are placed in the holes, the patients must remove them again as quickly as possible, one to one, following the same order of picking up and placing them again into the shallow container. Both the dominant and non-dominant hands were tested. If the patient drops a peg out of the board or violates the order of placement or removal of the pegs, the examiner stops the timer and counts that as an performance error. Then the patient starts the test once again from the beginning. Timing begins when the first peg is picked up and ends when the last peg is placed into the shallow container, which is analyzed as the administration time.

Neuropathological Methods

Histological methods

Immunohistochemistry was performed as previously described (1) in 30 human cerebellar vermix samples (Supplementary Table 3). Staining was performed on formalin-fixed, paraffin embedded tissue. Sections were cut (3-5 µm), deparaffinized, pre-treatment for antigen retrieval and probed with mouse monoclonal Calbindin antibody and anti-Aβ antibody (see antibody data in Supplementary Table 6) Primary antibodies were visualized using a standard diaminobenzidine streptavidin-biotin horseradish peroxidase method (Sigma Aldrich, Hamburg, Germany). PCs were quantified in 3 different optical fields of 3 consecutive slides. Total number of PCs was divided by total measured length of the PC layer. Immunohistochemistry in mice tissue followed similar protocols using Calbindin and 6E10 primary antibodies (Supplementary Table 4).

Cerebellar tissue immunofluorescence

For double-labeling immunofluorescence floating thick sections (100 µm) from formalin fixed cerebellar vermix from 9 individuals were prepared (SupplementaryTable 3). Sections were bleached with a UV light for 1 hour. Then, sections were blocked in NH_4Cl 15 minutes followed by overnight blocking in FBS 10% in PBST. Posteriorly, sections were incubated at 4ºC overnight with anti-VDAC1 and anti-PDI monoclonal antibodies as primary antibodies (SupplementaryTable 4). After washing in PBS, the sections were incubated in the dark for 45 minutes at room temperature with the cocktail of secondary antibodies diluted in the same vehicle solution as the primary antibodies. Negative controls included omission of primary antibodies. After washing in PBS, the sections were stained with a saturated solution of Sudan black B (Armin Baack, Schwerin, Germany) for 30 minutes to block the autofluorescence of lipofuscin granules present in nerve cell bodies, rinsed in PBS, and washed in distilled water. Finally, sections were mounted in Fluoromount G (Southern Biotech, Birmingham, AL, USA), sealed, and dried overnight. Sections were visualized in a Leica Confocal System TCS-SP2 (Wetzlar, Germany).

ELISA

Sandwich enzyme-linked immunosorbent assay (ELISA) for Aβ40, Aβ42 was performed as recommended by the manufacturer (Invitrogen, Carlsbad, CA, USA). Briefly, 100 mg of snap-frozen tissue from frontal cortex and cerebellum were homogenized in 800 µL of 5 M guanidine HCl/50 mM Tris HCl, for Aβ solubilization. Homogenate was mixed for 4 hrs at room temperature, diluted in phosphate-buffered saline/5% bovine serum albumin/0.03% Tween 20 and centrifuged at 16 000 x g for 20 minutes at 4°C. Supernatant was collected and probed with the ELISA kit for each antigen. Samples were measured at 450 nm in a Bio-tek µQuant spectrophotometer (Winooski, VT, USA) and expressed as ng/mg of total protein.

Ultrastructural analysis

Ultrastructural analysis was performed in glutaraldehyde fixed cerebellar tissue from 9 patients and mice as previously described (12). Cerebellar tissues were fixed in

glutaraldehyde and chrome-osmium, dehydrated in ethanol and embedded in Epon 812 (Serva Electrophoresis GmbH, Heidelberg, Germany). After polymerization, 1-µm-thick sections were cut, stained with toluidine blue and checked for presence of amyloid plaques. Relevant specimens were further processed for electron microscopy by cutting 60- to 80-nm-thick sections which were contrasted with uranyl acetate and lead solution. Sections were viewed in a LEO EM 912AB electron microscope (Zeiss, Oberkochen, Germany). Criteria for defining autolytic post mortem changes are: (i) rounded and swollen mitochondria, (ii) loss of matrix, (iii) disrupted cristae, (iv) amorphous densities inside mitochondria; for abnormal mitochondria resembling mitophagy are: (i) shrunken mitochondria, (ii) electron dense amorphous content, (iii) compacted cristae).The PCs that were evaluated for aberrant mitochondria did not contain senile plaques or were present in the microscopy field at used magnifications.

Biochemical Methods

Analysis of mitochondrial preparation from human cerebellar tissue

For mitochondrial isolation cerebellar tissue from patients (SupplementaryTable 3) were cut into small pieces and homogenized with 10 strokes at 1000 rpm in a motor-driven homogenizer (B. Braun Biotech International). The crude homogenate was centrifuged at 1000 x g for 10 min, mitochondria were pelleted by centrifugation of the supernatant at 8000 x g for 10 min and resuspended in freezing buffer. Protein concentration was determined using the Bradford protein assay. For mitochondrial complexes determination, Blue-Native PAGE analysis was apllied to crude mitochondria (100 µg) solubilized at a concentration of 2.5 mg/ml in solubilization buffer supplemented with detergent (0.8% DDM). Mitochondrial extracts were loaded onto polyacrylamide-gradient gels (3-13%). Proteins were transferred onto a PVDF membrane. Alternatively, Thin layer chromatographic analysis of mitochondrial phospholipids was performed after lipid extraction from crude mitochondria and determination of the phosphate concentration of extracted lipids was performed as described earlier (5). Briefly, 15 nmol mitochondrial phospholipids were spotted on silica plates. The plates were developed in chloroform/methanol/25% (v/v) ammonia solution (50:50:3). Phospholipid spots were

visualized by immersion in $CuSO_4$ in 8.5% o-phosphoric acid and subsequent charring at 180°C.

Whole cerebellar tissue western blot analysis

Proteins were isolated from human cerebellum (vermis) from AD cases and controls (Supplementary Table 3). For this, 500 mg of tissue were cut in small pieces, poured into a glass Dounce tissue grinder type B and homogenized in 4 mL of homogenizing buffer with ten even strokes. The homogenate was centrifugued at 1000 x g for 10 min at 4ºC. The supernatant was diluted with lysis buffer. Protein electrophoresis and immunoblot was performed as previously described (65). Briefly, once proteins were quantified, gel electrophoresis sodium dodecyl sulfate polyacrylamide (SDS-PAGE) was carried out using a miniprotean system (BioRad, Hercules, CA, USA) with a molecular weight marker of standard range (Fermentas, Germany). Proteins (50 µg) were loaded into each well with loading buffer (0.375 M Tris pH 6.8, 50% glycerol, 10% SDS, 0.5 M DTT and 0.002% bromophenol blue) and heated to 95ºC for 3 minutes before loading on the gel. After electrophoresis, proteins were transferred to nitrocellulose membranes (BioRad) using an electrophoretic transfer system (Mini Trans-blot Electrophoretic Transfer Cell, BioRad) at 350 mA for 2 hrs The membranes were incubated for 1 h in 5% non-fat milk dissolved in TTBS (20 mM Tris pH 7.5, 500 mM NaCl, 0.02% Tween-20). Then, the membranes were incubated overnight at 4°C with primary antibody (Supplementary Table 4). The membranes were washed with TTBS and incubated with secondary antibody (anti-IgG mouse or anti-rabbit IgG 1:2500, Invitrogen, Germany) coupled to horseradish peroxidase for 1 hr at room temperature. Immunoreactive signal was developed with the ECL Western Blotting chemiluminescence system (SuperSignal West Pico Chemiluminiscent Substrate, Pierce, Rockford, IL USA) and detected with a ChemiDoc system (BioRad). Key proteins were tested in temporal cortex biopsies homogenized fresh and after 12 hours in order to assess the stability of proteins in the range of the postmortem time for samples used in the study. (Supplementary figure 3C). The images were analyzed using the quantification software ImageJ (version 1.45, NHI). All proteins were studied at least twice; the results of each sample were

normalized with respect to the values of actin and compared between groups. To minimize interassay variation, the samples from all experimental groups were processed in parallel.

Synaptosomal and mitochondrial fractioning

The preparation of synaptosomes from human cerebellar tissue was done following the protocol described by Dunkley et al. (7) with some modifications. In brief, 1 g of human cerebellum (vermis) was washed with ice-cold homogenizing buffer, cut in small pieces and homogenized in 9 mL of homogenizing buffer using a tissue grinder. The homogenate was centrifuged at 1.000 x g for 10 min at 4ºC. The supernatant (S1) was collected and diluted to 5,4 mL with homogenizing buffer. The S1 fraction was pipetted over a percoll gradient (F1: 5%, F2: 10%, F3: 15%, F4: 28%) and centrifuged at 31.000 x g for 5 min at 4ºC in a 50 Ti rotor (Beckman Coulter, Krefeld, Germany). All fractions were collected and diluted with lysis buffer (150 mM NaCl, 20 mM Tris pH 7.4, 1 mM EDTA, 10% Glycerol, 1% NP40) containing phosphatase and protease inhibitors (Roche, Mannheim, Germany). The protein content was determined using the bicinchoninic acid method (BCA Protein Assay Kit, Pierce, Rockford, IL, USA). Fractions F1 to F5 were identified as M=Membranes, M-M=Membranes and myelin, M-S=Membranes and synaptosomes, S=Synaptosomes and, Mito=Mitochondria. Western blot was performed loading 60 µg of protein per fraction, using VDAC1, MIRO1, Syntaphilin and PSD95 primary antibodies. Densitometric analysis as performed on the fractions assigning a value of 100% to the total sum of signal from all fractions and percentages accordingly to each fraction.

Cellular Assays

Plasmids and Cloning

Human presenilin-1 cDNA was purchased from GeneCopeia (GenBank: L76517.1). Presenilin-1 cDNA was extracted from the shuttle vector with Bsp119I, blunted with Klenow polymerase and the product digested with XhoI. The cDNA was subcloned into the pcDNA3.1+/Zeo vector with/without GFP using the XhoI and EcoRV restriction sites.

GFP containing vector was developed in Doug Golenbock's lab and it is porvided as plasmid 13031 by Addgene (http://www.addgene.org/13031/). After cloning, the point mutation E280A was introduced by site-directed mutagenesis (QuikChange Lightning Site-Directed Mutagenesis Kit, Stratagene, La Jolla, CA, USA) of wild type human presenilin-1 cDNA employing the primers 5'…CAGCTCAGGAGAGAAATGCAACGCTTTTTCCAGCTCTC…3' and its exact inverse. The presenilin-1 variant lacking amino acids 290-319 (PS1Δ9) was generated by PCR. Two fragments were amplified, the first flanking from 200 nt before the ORF up to the beginning of the exon 9 (Primers F1 5'…AACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCG…3' and 5'…CTTGTGACTCCCTTTCTGTGCAGGAGTAAATGAGAGCTGGA…3') and the second from the start of the exon 10 up to 200 nt after the end of the ORF (Primers F2 5'…TCCAGCTCTCATTTACTCCTGCACAGAAAGGGAGTCACAAG…3' and 5'…CAGAATAGAAATGACACCTACTCAGACAATGCGATGC…3'). The fragments were purified using a PCR purification kit (Fermentas, St. Leon-Rot, Germany) and then a new PCR was performed using the F1 forward primer and the F2 reverse primer to homologize the halves. The product was amplified using the CloneJet system (Fermentas) following the recommendation of the manufacturer. The PS1Δ9 cDNA was subcloned into the pcDNA3.1+/Zeo vector using the XhoI and EcoRI restriction sites.

Stress assays in MEF cell culture

As a pilot study we evaluated mitochondrial redistribution under stress conditions in Mouse Embryonic Fibroblasts (MEFs) from wild type and presenilin-1/2 deficient mice were grown in supplemented DMEM and MEF PS1/2$^{-/-}$ were transfected with pcDNA3.1+/Zeo empty vector, pcDNA3.1+/Zeo-PS1^{wt}, pcDNA3.1+/Zeo-PS1^{E280A}, pcDNA3.1+/Zeo-PS1$^{\Delta 9}$ using Lipofectamine 2000 (Invitrogen) for 24 hrs Cultured cells were treated with 1 ng/mL Thapsigargin (Sigma, Saint Louis, MO, USA) for 30 min or 1 ng/mL synthetic Aβ42 (Bachem AG, Bubendorf, Switzerland) for 4 h. DMSO or PBS were used as vehicle for Thapsigargin and Aβ42 respectively. After treatment, cells were incubated with 250 nM MitoTracker Red CMXRos (Invitrogen) for 30 min, washed, fixed and permeabilized. Mitochondri distribution varied visibly under Ca2+ stress in

PS1wt and less in PS1 mutants while MEF PS1/2$^{-/-}$ showed no mitochondrial redistribution. Aβ42 produced invariable redistribution of mitochondria to the nucleus without differences among cell lines (Supplementary Fig 6).

Ca^{2+} overload assays in SH-SY5Y cell culture

SH-SY5Y human neuroblastoma cells were grown in supplemented DMEM-F12 and were transiently transfected with pcDNA3.1+/Zeo-PS1^{wt}, pcDNA3.1+/Zeo-PS1^{E280A}, pcDNA3.1+/Zeo-PS1$^{\Delta 9}$ using Lipofectamine 2000 (Invitrogen) for 24 hrs. Cultured cells were treated with 7 nM Calcimycin (Sigma, Saint Louis, MO, USA) for 30 min or 16 h. DMSO was used as vehicle. After treatment, cells were incubated with 250 nM MitoTracker Red CMXRos (Invitrogen) for 30 min, washed, fixed and permeabilized. For chronic Ca^{2+} overload assay, cells were transfected for 24 hrs. and then treated with Calcimycin 7nM or DMSO during 16 hrs. Cells were collected and incubated 30 min. in lysis buffer containing proteases and phosphatases inhibitors. Lysates were centrifuged at 13000 rpm for 15 min, and supernatant collected for experiments. Total protein concentration was evaluated with BCA assay and samples were prepared for western blotting as described for tissue samples.

Immunofluorescence in transfected SH-SY5Y

After 30 min treatment with Calcimycin SH-SY5Y cells were incubated 30 min with MitoTracker red CMXRos and washed with PBS 3 times, fixed with cooled 95% ethanol and 5% glacial acetic acid for 10 min. Fixed cells were washed 3 times and permeabilized with PBS and 0.2% Triton X-100 for 10 min. The cells were washed 3 times with PBS and incubated overnight with primary antibody (KDEL mouse monoclonal antibody 1:100, Invitrogen) diluted in incubation buffer (PBS, 0.3% Triton X-100, 1% BSA) at 4ºC in a wet chamber. Then the cells were washed 3 times with PBS and incubated with Alexa Fluor 350 secondary antibody (1:2500, Invitrogen) diluted in incubation buffer for 1 h. After that the cells were washed 4 times with PBS and mounted in slides using Fluoromount-G (Southern Biotech, Birmingham, AL, USA). Cells were examined under a confocal microscope (Leica TCS SP2, Wetzlar, Germany). Confocal images were analyzed individually to confirm the transfection and

to observe the mitochondrial distribution. Cells incubated only with secondary antibodies were used as negative control.

Evaluation of mitochondrial transport in SH-SY5Y

SH-SY5Y cells were plated and transfected in 3 cm glass bottom cell cultures dishes and incubated 30 min with MitoTracker red CMXRos. Cells were live recorded during 4 min using and spinning disc microscope (Perkin Elmer Ultraview Vox, Waltham, MA, USA). Recordings were analyzed and mitochondrial movement evaluated in single tracks for selected cells once successful transfection was assessed with GFP positive fluorescence. At least 10 recordings obtained in three independent experiments were studied and kymographs were prepared from each track using ImageJ software and Kymograph plugin. Finally, number of movable mitochondria for each group was calculated as percentage of total visible mitochondria.

Mice model experiments

Animals

The experiments were carried out in 20-36 weeks old female APPswe/PS1dE9 (APPsw/PS1Δ9) transgenic mice and B6 controls (Jackson Laboratory, Bar Harbor, Maine, USA). The generation of the APP/PS1 mouse line was described previously (8). They were maintained under standard animal housing conditions in a 12-h dark-light cycle with free access to food and water. Animal care was in accordance with ethical guidelines (European Communities Council Directive 86/609/EEC) and approved by the Local Ethical Committee (ER).

Behavioral and motor studies in APPsw/PS1Δ9 mice

APPsw/PS1Δ9 mice were tested with the Erasmus ladder, a fully automated test for detecting motor performance, associative motor learning deficits and cognitive phenotypes in mouse models and learning deficits. It is used to analyze cerebellar

function in multiple mouse mutants, including ataxic Lurcher mice, and also Cx36–/– mice, which show cerebellar motor learning deficits in the absence of ataxia (9;10). The Erasmus ladder consists of a horizontal ladder in between two shelter boxes, which are equipped with two pressurized air outlets (Pneumax, 171E2B.T.A.0009, Gosport, UK) and a bright white LED spotlight in the roof (both light and air are used of departure). The air outlets are also used to control the speed of the mouse and to prevent unauthorized departures (escape). The ladder has 2 × 37 rungs for the left and right side. All rungs are equipped with pressure sensors (produced at Erasmus MC), which are continuously monitored and which can be used to register and analyze the walking pattern of the mouse instantaneously. Moreover, based upon the prediction of the walking pattern, the rungs can be moved up or down by a high-speed pneumatic slide (Pneumax, 2141.52.00.36.91) with a maximum of 13 mm at any moment in time. The computer system (National Instruments, Texas, USA) that runs the real-time system to record sensor data, adjusts air pressure, predicts future touches, calculates interventions, repositions slides and stores data, operates in a fixed cycle of 2 milliseconds. Details of the device and its operations have been published (10). During the first 4 days (unperturbed sessions), mice were trained with the even-numbered rungs on the left side and the odd-numbered rungs on the right side in a descended position so as to create an alternated stepping pattern with 30 mm gaps. Mice were trained to walk the ladder for 72 runs per day. We calculated the number of missteps that are sensed by the descended rungs and steptime which is defined as the time needed to place one of the front paws from one rung to the other. Associative motor learning trials (perturbed sessions) started on day 5 using a 15 kHz tone (Voltcraft, Barking, UK), which gradually increases over 20 milliseconds to 100 dB and which lasts up to 300 milliseconds as the conditioned stimulus (CS), whereas a rising rung, which ascends 12 mm, was used as the unconditioned stimulus (US). The inter-stimulus interval was fixed on 285 milliseconds. To keep this time period constant we observed the real-time speed of the mouse and calculated which rung would rise. Mice typically learn that increasing walking speed avoids being hit by the rung; consequently mice will decrease their steptimes over time. Besides motor coordination deficits estimated in terms of motor performance and associative motor learning parameters, the Erasmus

Ladder is capable of detecting cognitive phenotypes. Cognition is measured determining the capability of mice to recognize the cues of departure (light or air) and to modify their reaction to the given cues with the purpose of reduce their exposition to a stressful situation (avoidance discrimination) on the ladder, which is created by the presence of the US and the CS stimuli during the perturbed sessions.

In vivo PC electrophysiology in APPsw/PS1Δ9 mice

APPsw/PS1Δ9 mice (21.9 ± 0.3 weeks old) were surgically prepared for in vivo electrophysiological recordings. In short, mice were anesthetized and an immobilizing construct holding a small magnet (4x4x1 mm) was placed on the frontal and parietal bones using Optibond (Kerr, Salerno, Italy) and Charisma (Heraeus Kulzer, Hesse, Germany) (11). A craniotomy was made in the interparietal bone and a recording chamber was placed around it, allowing in vivo electrophysiological recordings (12). After at least 24 hrs of recovery, mice were head-fixed, their body restrained in a custom-made tube, and the extracellular Purkinje cell activity was recorded using borosilicate glass single barrel (OD 2.0 mm, ID 1.16 mm), or double barrel pipettes (Septum Theta, OD 1.5mm, ID 1.02mm; both WPI, Sarasota, FL, USA), filled with 2 M NaCl for recordings, and the 2nd barrel with 0.1-0.2 % Alcian Blue solution Electrodes were advanced into cerebellar lobules I to V by a hydraulic micro-drive (Narishige, Tokyo, Japan). Purkinje cells were identified by the firing of complex spikes and were confirmed to be from a single unit by the presence of a pause after each complex spike (not different in APP/SP1 mutant vs. control mice, data not shown). Minimum recording duration was 120 s and several dye injections, followed by histochemical analysis, confirmed that the recordings were from lobules I to V. Raw recorded signals were amplified, filtered (30 Hz to 10 kHz with notch filter, CyberAmp, Axon, USA), digitized (Power1401, CED, UK) and stored for off-line analysis (sampled at 25 kHz, Spike2, CED). Analysis of in vivo extracellular Purkinje cell recordings was performed using the Spiketrain Toolbox (Neurasmus B.V., Rotterdam, www.neurasmus.com) for Matlab (Mathworks, Natick, MA). For each cell the mean firing rate, mean and median CV2 were determined for simple and complex spikes. CV2 is a measures for the regularity of

the firing from spike to spike, calculated as the mean or median of 2·|(ISI)n+1 - ISIn|/ (ISIn+1 + ISIn), with ISI being the inter-spike interval.

Statistical Analysis

Data was tabulated in Microsoft Excel datasheets and analyzed using SPSS 17 statistical software (SPSS Inc., Chicago IL, USA) or GraphPad Prism 5 (GraphPad Software, La Jolla CA, USA). Data was analyzed for determining normal distribution with Kolmgorov-Smirnov and Shapiro-Wilk tests. Mean comparison of parametric variables were performed with Student's T-Test and One-way ANOVA. Non-parametric variables were studied with U-Mann-Whitney and Kruskall Wallis test. Intensity colocalization analysis was performed using Pearson's coefficient and its significance assigned according to number of samples analyzed per group. Binomial variables comparison was performed using χ^2 test. Correlation analysis for continuous variables was performed using Spearman's Rank Coefficient. Statistical significance of all analysis was determined at $p<0{\cdot}05$.

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