Table S1. Description of cell lines used in this work

Cell line	Cell type	Source	Phenotype	Age	Sex	^{3}H-Chol	LipidTox	Oil Red O	^{3}H-Ser	Cin/Dura	FL-SA-Ro	EM	Co-loc
ORH	Fibroblast	This work	Control	36	M	2	1		>3	>3	1	1	4
LUI	Fibroblast	This work	Control	31	M				>3	1			
ESP	Fibroblast	This work	Control	31	F	1	1		1	1	1		1
AG07871	Fibroblast	Coriell	Control	49	F	1	1		1		1		
AG08379	Fibroblast	Coriell	Control	60	F	1	2		1	1			
AG08509	Fibroblast	Coriell	Control	73	M	2	4		1				5
AG08517	Fibroblast	Coriell	Control	66	F		1	1	1	1	1		
AG08525	Fibroblast	Coriell	Control	82	F					1			
SW7	Fibroblast	Richard Cowburn	Control	59	M		1						
AG06840	Fibroblast	Coriell	FAD-PS1(A246E)	52	M	1	4		3	1	1	1	
AG06848	Fibroblast	Coriell	FAD-PS1(A246E)	<49	F	1	1	1	2	1	1		
EB	Fibroblast	Tom Bird	FAD-PS1(G209V)	41	F	1	1	1	1	1	1		
GG3	Fibroblast	Gary Gibson	FAD-PS1(M146L)	18	M	1	1		2	1			5
WA	Fibroblast	Tom Bird	FAD-PS1(L418F)	33	M	1	1		1	1	1		
DD	Fibroblast	Tom Bird	FAD-PS2(N141I)	48	M		1		2		1		
SW5	Fibroblast	Richard Cowburn	FAD-APPswe (K670N/M671L)	58	F					1			
SW6	Fibroblast	Richard Cowburn	FAD-APPswe (K670N/M671L)	64	M		1			1			1
A51486	Fibroblast	Tom Bird	SAD	?	?	1	1		1	1			6
AG05770	Fibroblast	Coriell	SAD	<62	M	1	1		1				4
AG06263	Fibroblast	Coriell	SAD	<60	F	1	4		1	2	1	1	
AG06869	Fibroblast	Coriell	SAD	59	F	1	2		1	2	1		
AG07374	Fibroblast	Coriell	SAD	73	M	1	1		1	1			
AG07375	Fibroblast	Coriell	SAD	<62	M	1	1		1	1			1
AG08243	Fibroblast	Coriell	SAD	72	M	1	1		1	1			3
AG08259	Fibroblast	Coriell	SAD	87	M	1	1		1	1			4
AG21158	Fibroblast	Coriell	SAD	69	F	1	2		1	1	1		
Ps-WT	MEF	Bart De Strooper	Wild-type control	NA	NA	>3	>3	1	>3	>3	1	1	>3
Ps1-KO	MEF	Bart De Strooper	Ps1-KO	NA	NA	>3	>3	1	>3	3	1		>3
Ps2-KO	MEF	Bart De Strooper	Ps2-KO	NA	NA	>3	>3	1	>3	3	1		>3
DKO	MEF	Bart De Strooper	Ps1+Ps2 double KO	NA	NA	>3	>3	1	>3	>3	1	1	>3
M3	MEF	This work	PS1-KD control	NA	NA	3	>3	1	>3	3	1		
M2x2	MEF	This work	PS1-KD	NA	NA	3	>3	1	>3	3	1		
M2x2+WT	MEF	This work	Ps1-KD + Human WT-PS1	NA	NA		>3	1	3	1	1		
M2x2+A246E	MEF	This work	Ps1-KD + Human A246E-PS1	NA	NA		>3	1	3	1	1		
510	MEF	David Chan	Wild-type control	NA	NA	1	>3		3				
511	MEF	David Chan	Mfn2-KO	NA	NA	1	>3		3				
516	MEF	David Chan	Mfn2-KO	NA	NA		>3						
518	MEF	David Chan	Wild-type control	NA	NA		>3						

See text for description of the various experiments. Numbers denote the number of times the indicated cell was used in any particular experiment. NA, not applicable.

Fig. S1

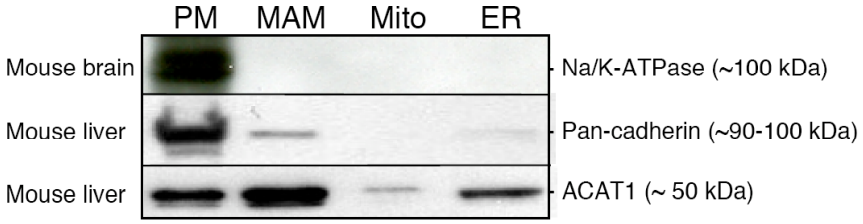

Figure S1. Western blot to detect the indicated proteins in standard subcellular fractionation of mouse tissues to isolate PM, MAM, mitochondria, and ER fractions. Na/K-ATPase and pan-cadherin are enriched in the PM; ACAT is enriched in the MAM. Note that the MAM fraction is essentially devoid of the PM marker.

Fig. S2

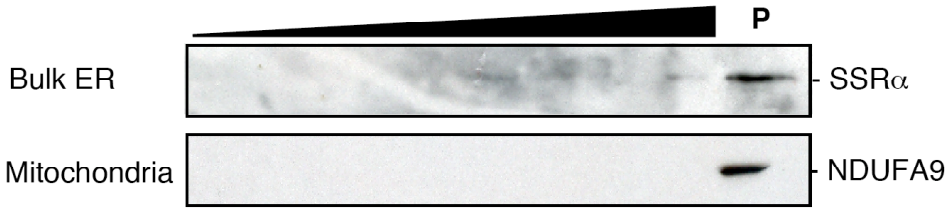

Figure S2. Western blot to detect SSRα (signal sequence receptor α; a marker for bulk ER) and NDUFA9 (a subunit of complex I of the respiratory chain; a marker for mitochondria) in fractions from a 5%-30% sucrose gradient (triangle denotes increasing density from left to right) of purified bulk ER and mitochondria after treatment with 1% Triton X-100 at 4°C for 1 h. Note than neither bulk ER nor mitochondria contain low density DRM's; almost all of both fractions, as determined by the marker proteins, was in the detergent-soluble pellet (P).

Fig. S3

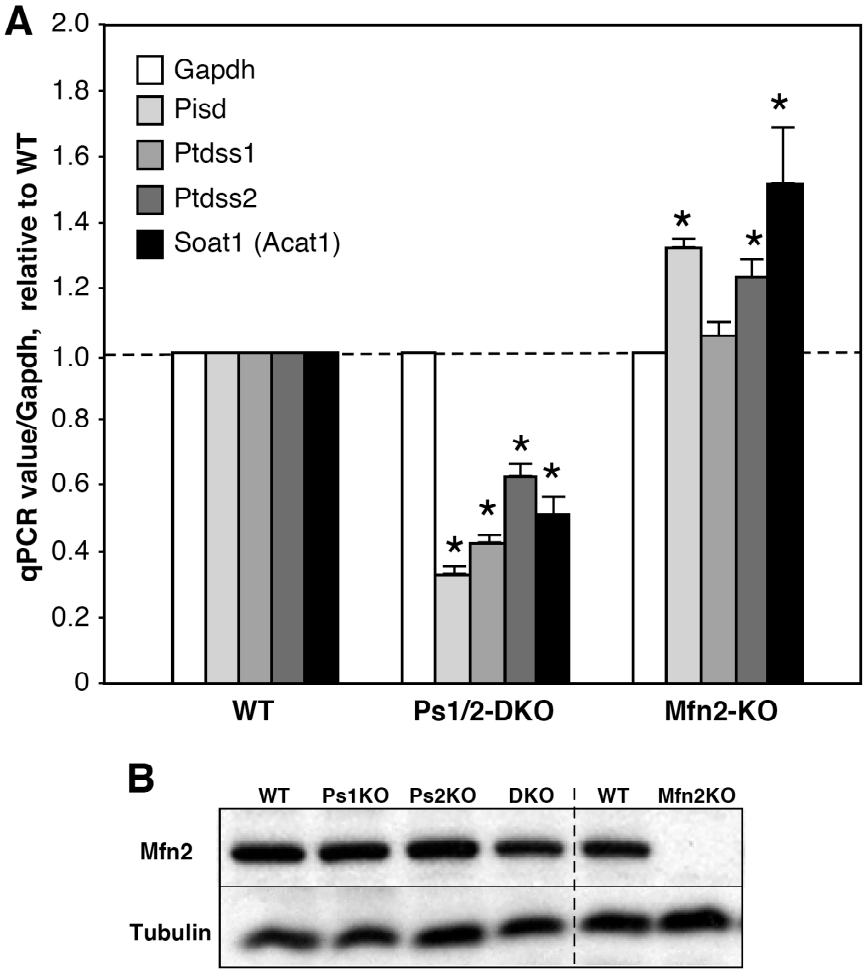

Figure S3. (**A**) Quantitative PCR of phospholipid-related mRNAs in Ps1/2 DKO and Mfn2-KO MEFs. Phosphatidylserine synthase-1 (*Ptdss1*) and -2 (*Ptdss2*) are MAM-localized proteins involved in the interconversion of PtdSer with PtdCho and PtdEtn, respectively; phosphatidylserine decarboxylase (*Pisd*) is a mitochondrial matrix protein that converts PtdSer to PtdEtn; acyl-CoA:cholesterol acyltransferase (Acat1, gene *Soat1* [sterol O-acyltransferase 1]), is a MAM-localized protein that converts free cholesterol to cholesteryl esters. All data were normalized to the qRT-PCR for *Gapdh*. (**B**) Western blot to detect Mfn2 in Ps-KO MEFs. Note that ablation of presenilins has little or no effect on Mfn2 expression.

Fig. S4

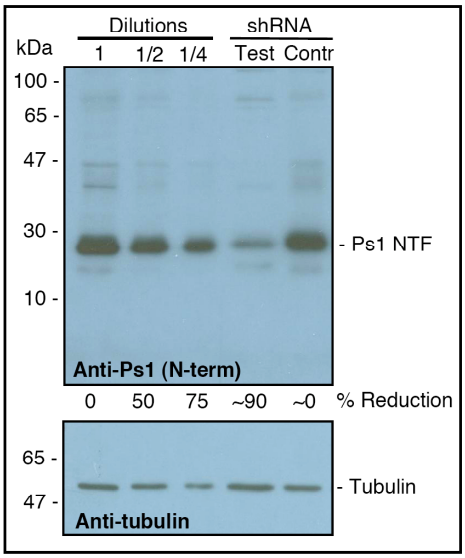

Figure S4. Characterization of Ps1-KD MEFs. Western blot analysis of shRNA clones. Lanes 1-3, dilutions to quantitate Ps1; lane 4, knockdown of Ps1 compared to mismatch control in lane 5. Anti-tubulin loading controls at bottom. The specificity of the shRNA primer was confirmed by transducing a mismatched shRNA. NTF, N-terminal fragment.

Fig. S5

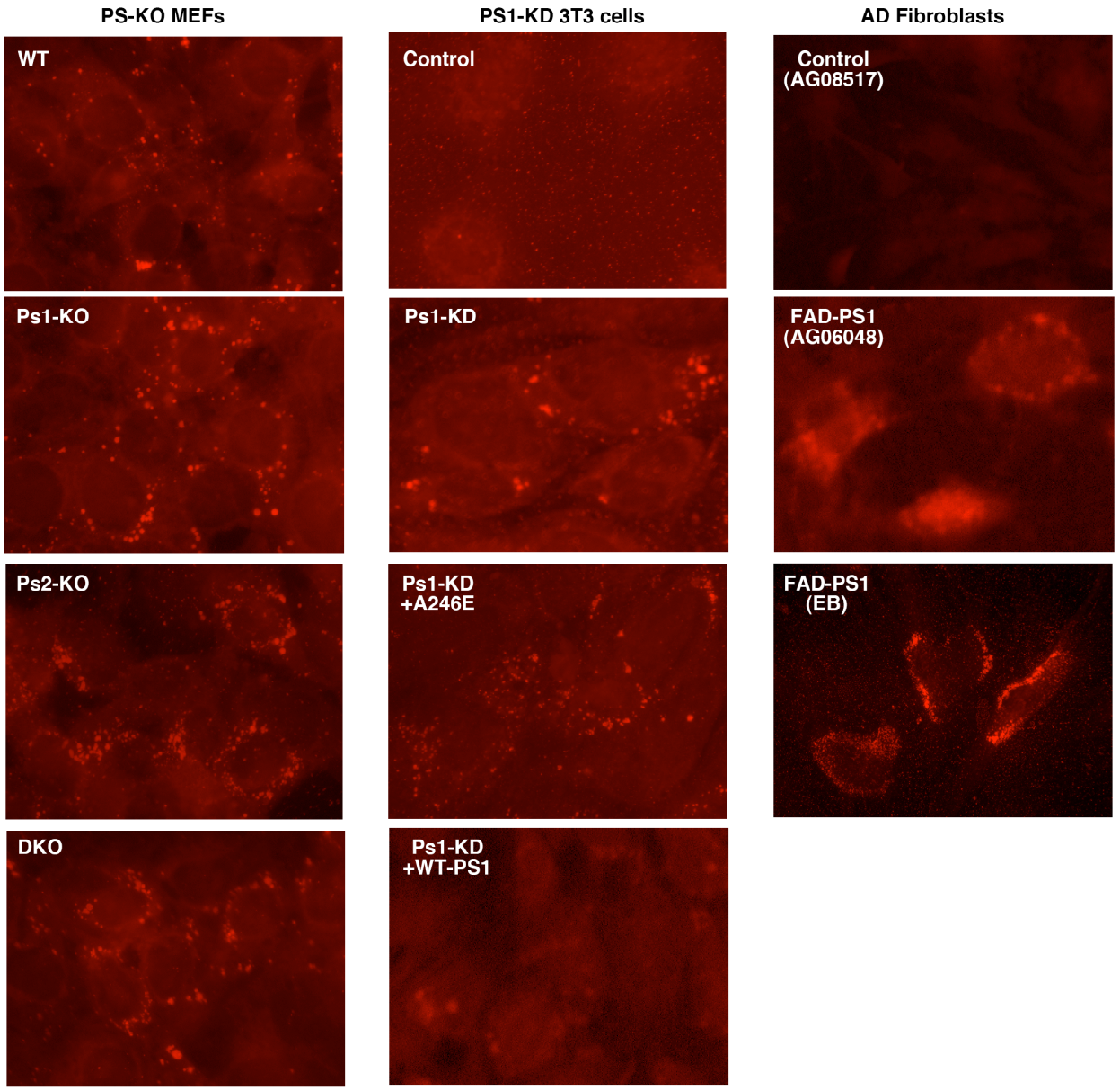

Figure S5. Examples of Oil Red O staining of the indicated cells. Note that the increase in punctate staining in the mouse PS1-KD MEFs was rescued by overexpression of human WT-PS1 but not by A246E mutant PS1.

Fig. S6

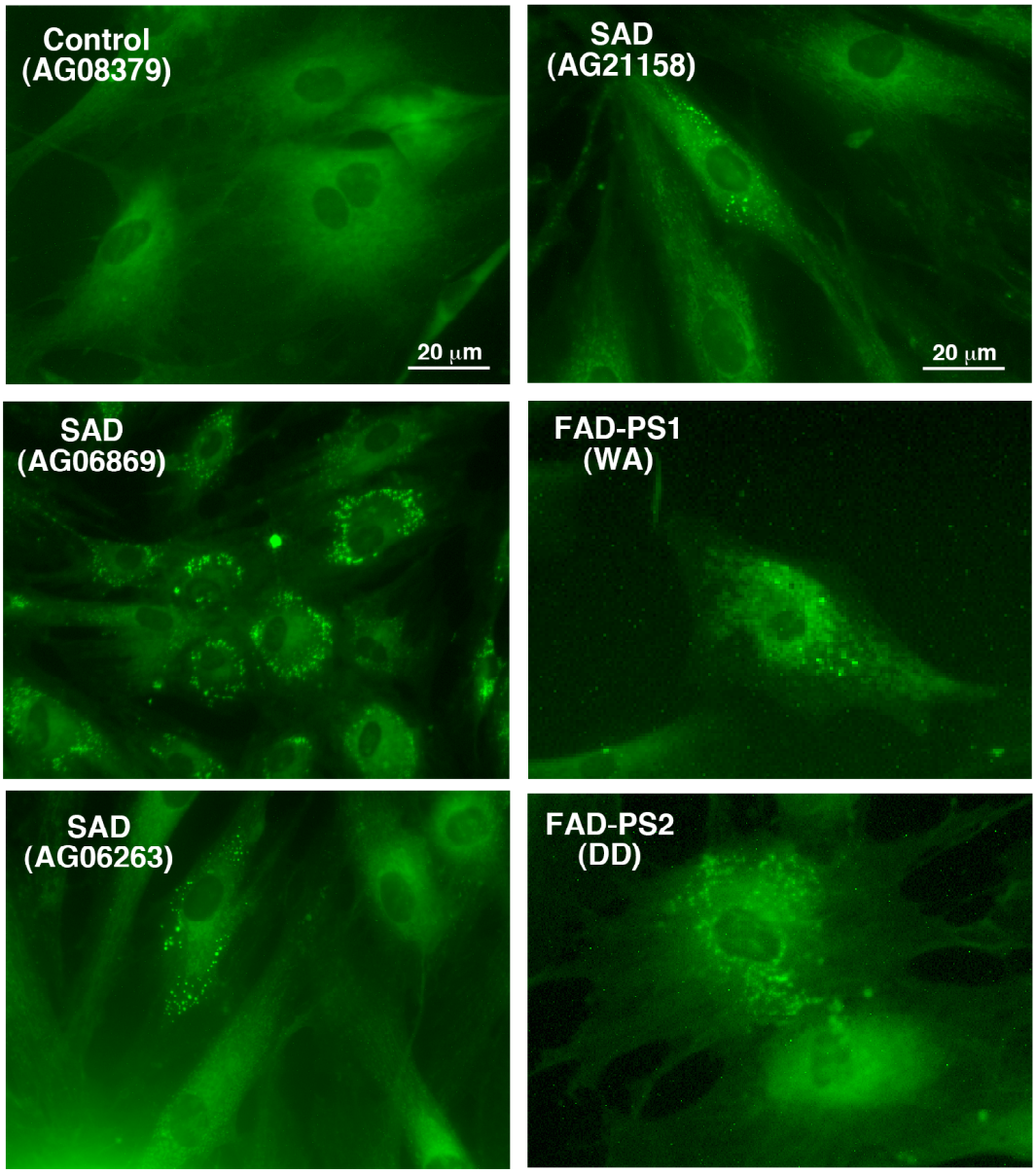

Figure S6. Examples of LipidTox staining of the indicated fibroblasts.

Fig. S7

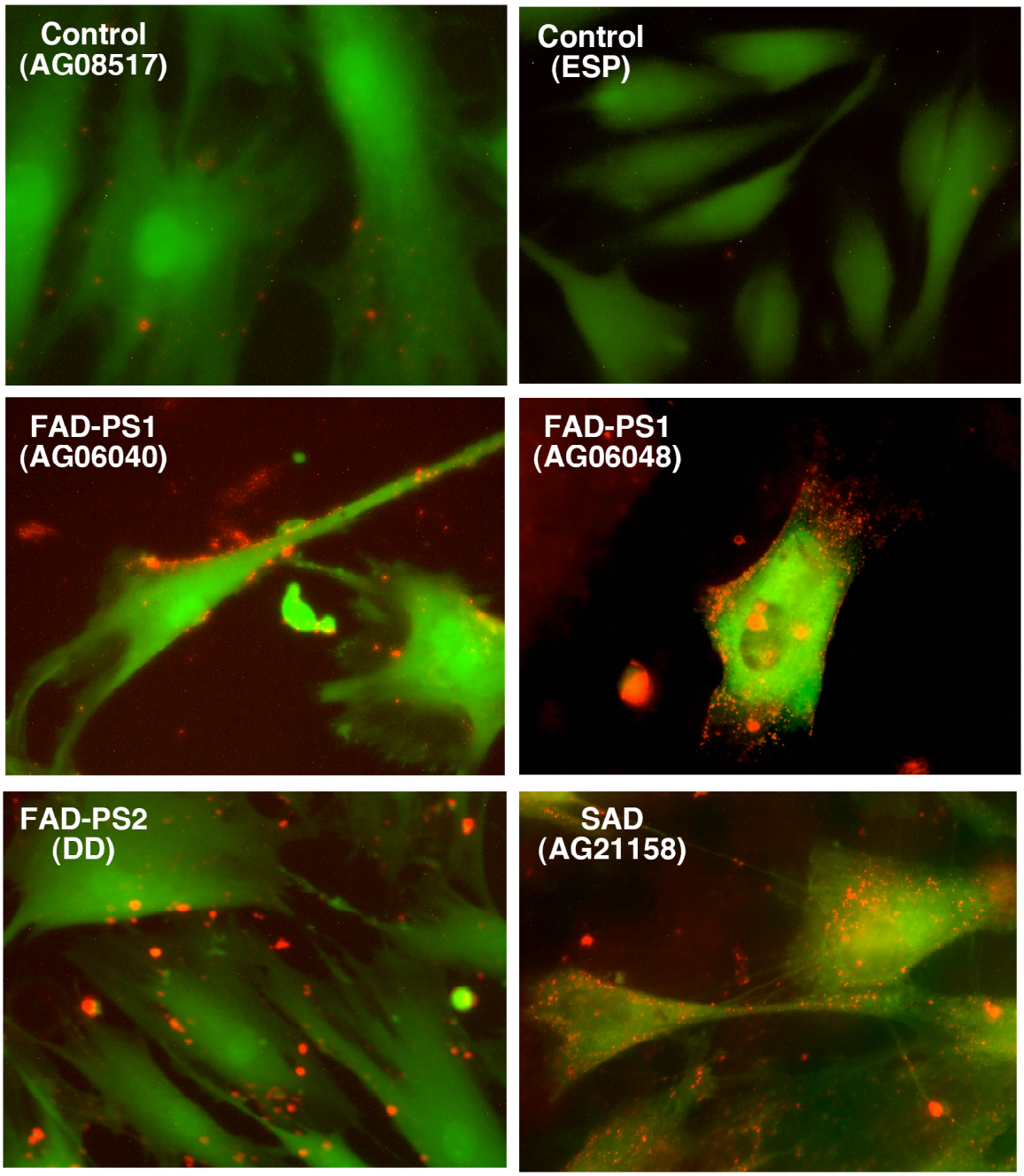

Figure S7. Examples of staining of AD fibroblasts with fluorescent cinnamycin (FL-SA-Ro). To visualize overall cell morphology, cells were counterstained with calcein.

Fig. S8

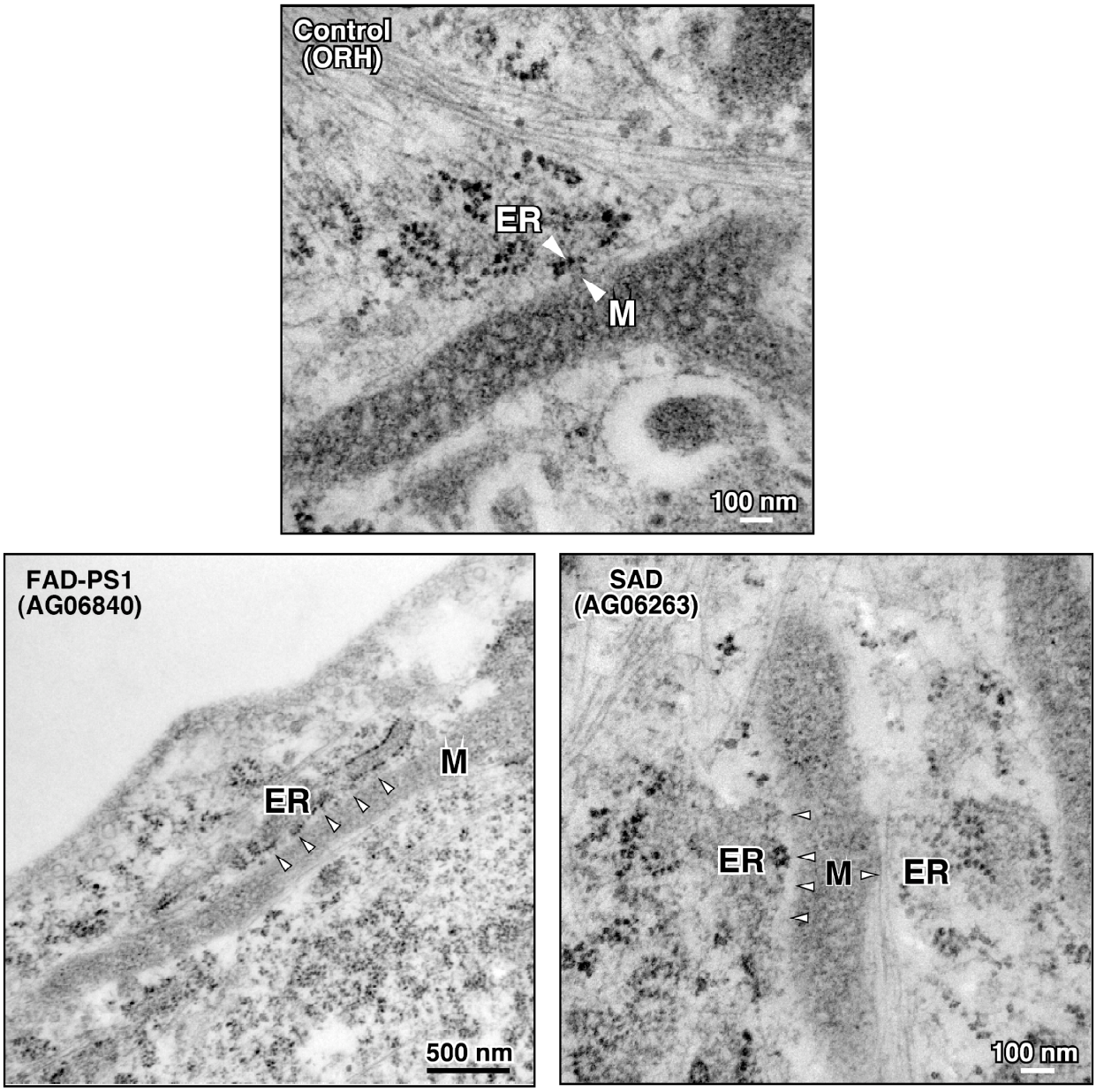

Figure S8. Representative electron micrographs of ER apposed to mitochondria (M) (i.e. MAM) in fibroblasts from a control subject, an FAD patient, and an SAD patient. Note the unusually large area of apposition in the FAD patient, extending for more than 1000 nm (arrowheads).

Fig. S9

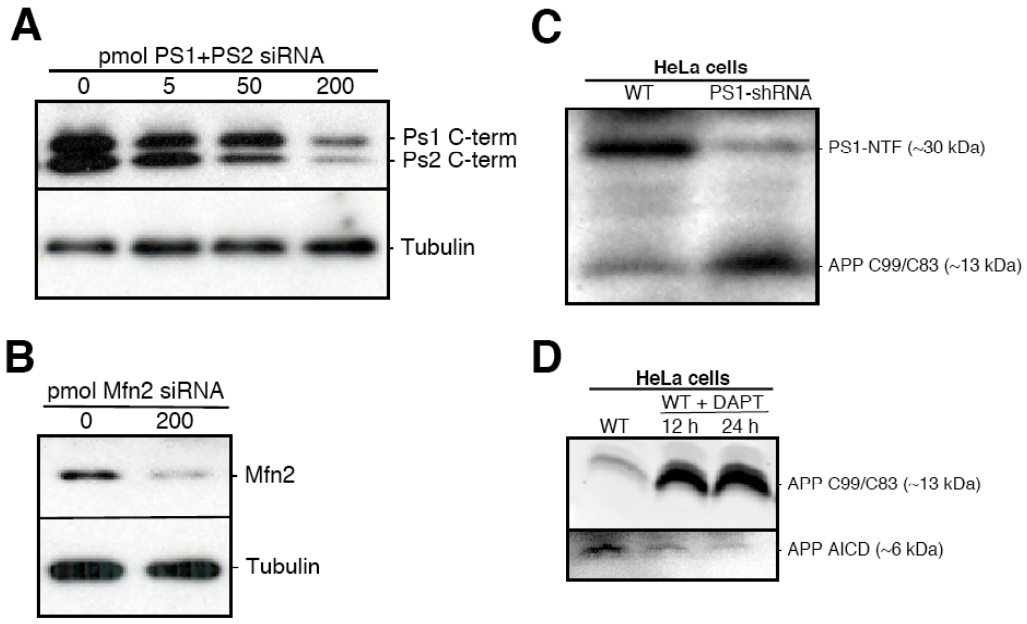

Figure S9. Western blot analysis of shRNA knockdown constructs and of DAPT-treated cells. **(A)** Co-transfection of mouse *Ps1* and *Ps2* shRNAs into WT- and Mfn2-mutant MEFS. Expression using 200 pmol was reduced ~80%. **(B)** Transfection of mouse *Mfn2* shRNA into WT- and Ps-mutant MEFs. Expression was reduced ~80%. **(C)** Transfection of a PS1 shRNA construct into HeLa cells. Expression was reduced ~70%, with a concomitant increase in the unprocessed C-terminus of APP (C99 and C83) (see Fig. 8C). **(D)** Western blot to detect the APP C-terminus and AICD (see Fig. 8C) in HeLa cells. Treatment with DAPT for 12 or 24 h reduced AICD by ~80%, with a concomitant increase in the amount of uncleaved C99 or C83, consistent with inhibition of γ-secretase activity by the drug.

Fig. S10

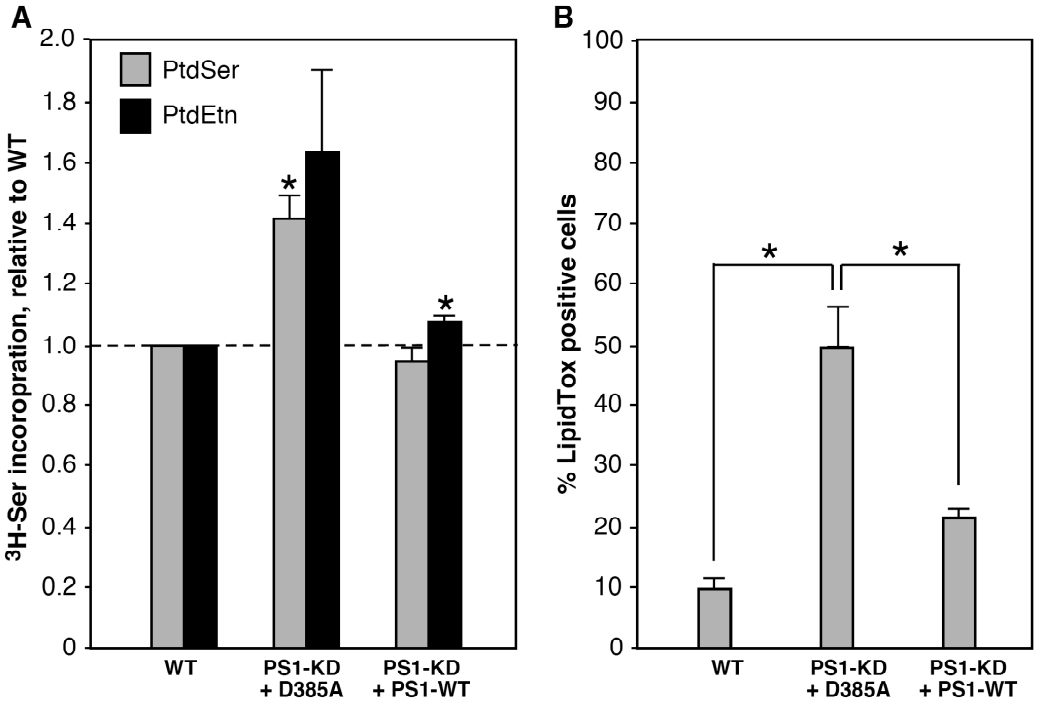

Figure S10. MAM function in HeLa cells in which PS1 had been knocked down (KD) by shRNA, and then subsequently transfected with either WT or "catalytically dead" PS1 (i.e. D385A mutation). **(A)** Phospholipid transport; data are averages (±SE) of ^{3}H-Ser incorporation after 3 and 5 h. **(B)** Lipid droplet formation (average of 5 images (~500 cells analyzed) ±SE.