

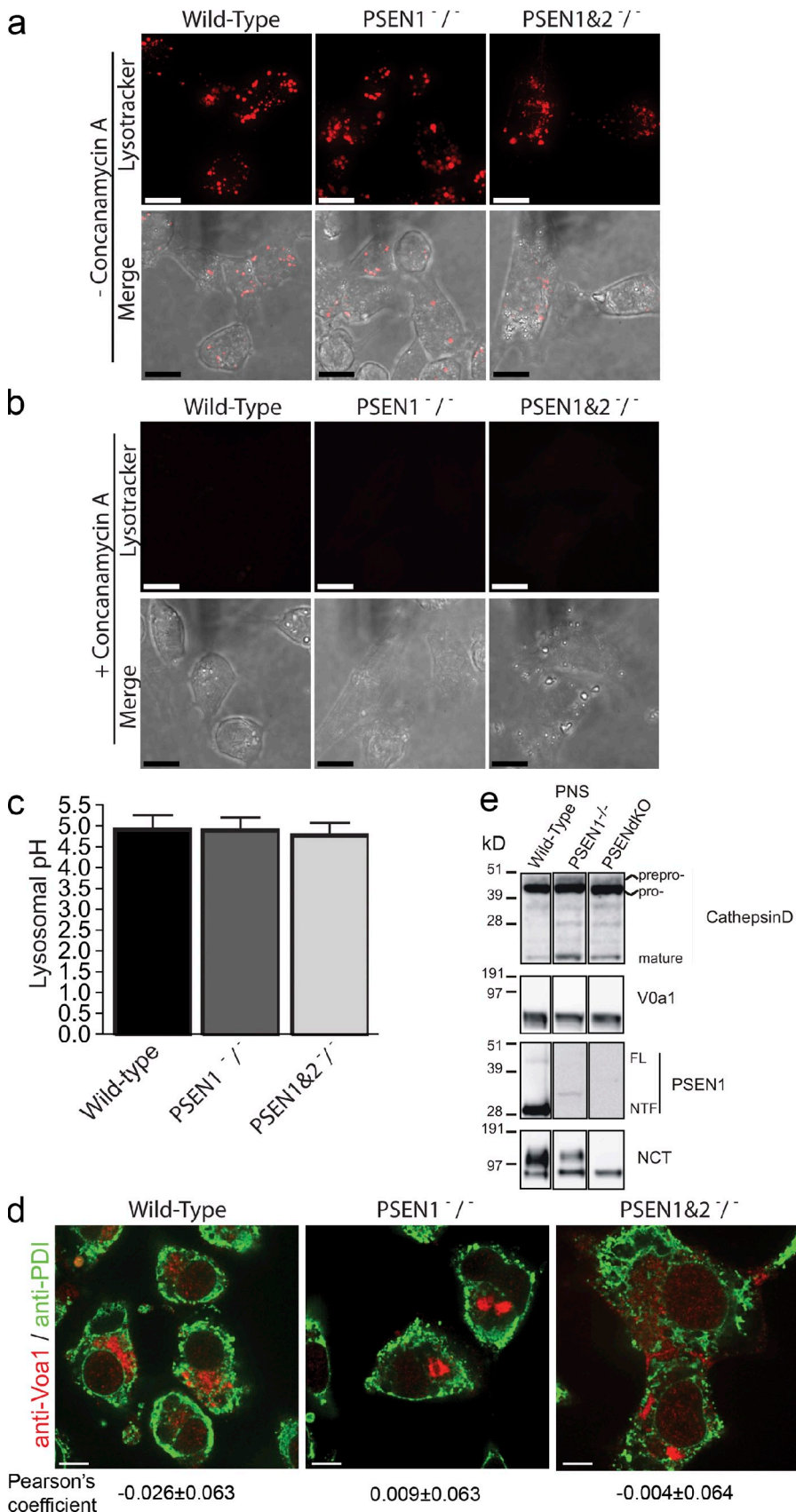
Coen et al., <http://www.jcb.org/cgi/content/full/jcb.201201076/DC1>

Figure S1. Characterization of V-ATPase function in blastocyst-derived extraembryonic endoderm cells that lack PSENs. (a and b) Blastocysts derived from WT, PSEN1^{-/-}, and PSENdKO mice were analyzed by LysoTracker staining with (b) and without (a) concanamycin A treatment. The top panels represent z projections of the LysoTracker fluorescence, whereas the bottom panels depict merged z projections of cells imaged for LysoTracker fluorescence and by brightfield. Bars, 18 μ m. (c) The bar graph represents the measured lysosomal pH for WT, PSEN1^{-/-}, and PSENdKO blastocysts (error bars indicate means \pm SEM; $n = 2-3$, 30-40 cells). (d) The cellular distribution of the V0a1 subunit of the V-ATPase and the resident ER protein, PDI, were analyzed by immunofluorescence. Shown are representative xy confocal images for WT, PSEN1^{-/-}, and PSEN1&2^{-/-} blastocysts with V0a1 antibody staining in red and PDI antibody staining in green. The mean Pearson's coefficient \pm SD for colocalization of V0a1 with PDI is displayed below each image. Bars, 10.1 μ m. (e) Western blot analysis of PNS derived from WT, PSEN1^{-/-}, and PSENdKO blastocysts for endogenous cathepsin D. Immunoreactive bands representing pre- and pro-cathepsin, and in particular mature processed cathepsin D, are reminiscent in all three cell lines, which indicates normal maturation and levels of cathepsin D in WT and PSEN-deficient blastocysts. Expression levels of V0a1 were also not affected by PSEN deficiency. NCT is used as a control to indicate decreased levels of mature NCT in PSEN1^{-/-} and only immature NCT in PSENdKO blastocysts.

a

Cytoplasmic

mouse	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
rat	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
human	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
orangutan	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
cow	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
chicken	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
frog	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
fly	1	MGELFRSEEMTLAQLFLQSEAAAYCCVSELGELGKQVFRDLNPDVNFVORFKVNEVRRCEB	
mouse	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
rat	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
human	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
orangutan	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
cow	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
chicken	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
frog	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
fly	61	MDRKLRFVKEIRKANTIPMDTGENPEVPPFRDMIDLEANFEKIELELKEINTNOEALKR	
mouse	121	NFLELTTELKFIIRKTOQDFDE-----NADDDLLESSSLLEPN	
rat	121	NFLELTTELKFIIRKTOQDFDE-----NADDDLLESSSLLEPN	
human	121	NFLELTTELKFIIRKTOQDFDEAELHQQ-----QADDDLLESSSLLEPN	
orangutan	121	NFLELTTELKFIIRKTOQDFDE-----NADDDLLESSSLLEPN	
cow	121	NFLELTTELKFIIRKTOQDFDE-----NADDDLLESSSLLEPN	
chicken	121	NFLELTTELKFIIRKTOQDFDE-----NADDDLLESSSLLEPN	
frog	121	NFLELTTELKFIIRKTOQDFDE-----NADDDLLESSSLLEPN	
fly	121	NFLELTTELKFIIRKTOQDFDESVPTIVKSSGAYSSSKRYRPOADNNQNDQALIGEE	
mouse	159	EMGRGAE-----LRLGFAVGINRERIPTFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
rat	159	EMGRGAE-----LRLGFAVGINRERIPTFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
human	166	EMGRGAE-----LRLGFAVGINRERIPTFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
orangutan	159	EMGRGAE-----LRLGFAVGINRERIPTFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
cow	159	EMGRGAE-----LRLGFAVGINRERIPTFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
chicken	159	EMGRGAE-----LRLGFAVGINRERIPTFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
frog	159	EMGRGAE-----LRLGFAVGINRERIPTFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
fly	181	GURASQEQNLRLGFAVGINRERIPFERMLNRVCRGNVFLRQAEINLEDPVTDGVV	
mouse	216	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	N-glyc
rat	216	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	
human	223	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	
orangutan	216	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	
cow	216	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	
chicken	216	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	
frog	216	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	
fly	241	HKSVPILFFQDGLKLRVKKICEGFRASLYPCPETPOERKEMASGVNTRIDDLQMLVNG	
mouse	276	EDHRORVLOAAAKNIRVWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
rat	276	EDHRORVLOAAAKNIRVWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
human	283	EDHRORVLOAAAKNIRVWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
orangutan	276	EDHRORVLOAAAKNIRVWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
cow	276	EDHRORVLOAAAKNIRVWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
chicken	276	EDHRORVLOAAAKNIRVWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
frog	276	EDHRORVLOAAAKNIRVWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
fly	301	GDHRFVILVAAKNIINRWFVKRKKKAIYHFLNLCNDIDVQKCLIAEWCPCVDDLSIQF	
mouse	336	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	N-glyc
rat	336	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	
human	343	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	
orangutan	336	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	
cow	336	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	
chicken	336	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	
frog	336	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	
fly	361	ALRRGTEHSGSVPSILNRMOTNORPEPTYNQNKETGCFONIVDAYGIGTYRINPAFYT	
mouse	396	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	TMD I
rat	396	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	TMD II
human	403	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	TMD III
orangutan	396	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	
cow	396	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	
chicken	396	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	
frog	396	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	
fly	421	IIITPPFLFAVMFGDFGHGILMTLFAVMVLRRESRILSQKNENEMFSVFGSRYITILLMGL	

Luminal

mouse	456	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA	TMD III	N-glyc	PGVFGGPE
rat	456	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA			
human	463	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA			
orangutan	456	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA			
cow	456	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA			
chicken	456	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA			
frog	456	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA			
fly	481	FSVYTGILYNDCFKSLNIFGSSWSVREPMFGQVNTBETLLESSVQLNPA			
mouse	516	PFGLDPIWNIA-----INKLIFFLNSFKMMSVILGITHMLFGVSLSLFNHIFPKPLNIYFGFI	TMD IV	TMD V	
rat	516	PFGLDPIWNIA-----INKLIFFLNSFKMMSVILGITHMLFGVSLSLFNHIFPKPLNIYFGFI			
human	522	PFGLDPIWNIA-----INKLIFFLNSFKMMSVILGITHMLFGVSLSLFNHIFPKPLNIYFGFI			
orangutan	516	PFGLDPIWNIA-----INKLIFFLNSFKMMSVILGITHMLFGVSLSLFNHIFPKPLNIYFGFI			
cow	516	PFGLDPIWNIA-----INKLIFFLNSFKMMSVILGITHMLFGVSLSLFNHIFPKPLNIYFGFI			
chicken	516	PFGLDPIWNIA-----INKLIFFLNSFKMMSVILGITHMLFGVSLSLFNHIFPKPLNIYFGFI			
frog	516	PFGLDPIWNIA-----INKLIFFLNSFKMMSVILGITHMLFGVSLSLFNHIFPKPLNIYFGFI			
fly	533	PFGLDPIWVAGANKIIFNFAIIMGISTIFGITHMLFGVSLSLFNHIFPKPLNIYFGFI			
mouse	575	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE	TMD V		ESG
rat	575	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE			ESG
human	581	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE			ESG
orangutan	574	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE			ESG
cow	575	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE			ESG
chicken	575	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE			ESG
frog	574	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE			ESG
fly	593	PEIIFMTSLFGVIVLILFYKWTAYDAHSR-----NAPSLLIHFIMNMFVSYE			ESG
mouse	626	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE	TMD VI		
rat	626	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE			
human	632	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE			
orangutan	625	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE			
cow	626	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE			
chicken	626	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE			
frog	625	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE			
fly	653	NAMLYSGQKIQCFILVVALICVPMMLLKFPLVLRQYLRKHLGTLINFGGIRVNGPTE			
mouse	686	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL	TMD VII	TMD VIII	
rat	686	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL			
human	692	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL			
orangutan	686	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL			
cow	686	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL			
chicken	686	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL			
frog	685	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL			
fly	707	EDAEIIHQDQLSTHSEDAEPEDEVDVFDGDMVHOAHTIIEYCLGICISNTASYLRLWAL			
mouse	746	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR	TMD VIII	TMD IX	
rat	746	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR			
human	746	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR			
orangutan	745	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR			
cow	746	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR			
chicken	746	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR			
frog	739	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR			
fly	760	SLAHAQSEVLWTRVHIGLIVRSLAGGLLEFFFAAFATLVAALLIMEGLSAPLHALR			
mouse	806	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			
rat	806	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			
human	806	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			
orangutan	805	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			
cow	806	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			
chicken	806	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			
frog	799	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			
fly	820	LHWVERONKPYGTFKFLPSEFHIREGKFDPE---			

b

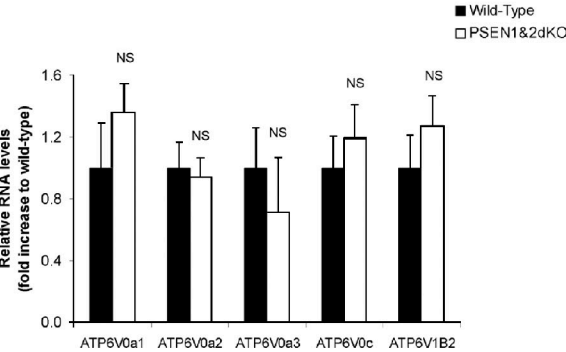
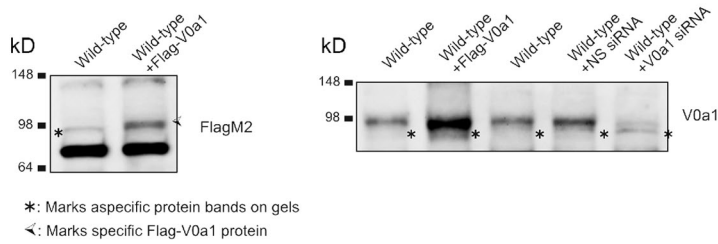


Figure S2. **Sequence alignment and expression levels of V0a1 subunit orthologues.** (a) Sequence alignment of V0a1 subunit orthologues predicts one conserved N-glycosylation site. Four potential N-glycosylation sites were present in the V-ATPase subunit V0a1, of which only one (N₄₈₉WTE) was available in the ER lumen. Multiple sequence alignment of the V0a1 subunit (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) of mouse (NCBI Protein database accession no. NP_058616.1), rat (NP_113792.2), human (NP_001123492.1), orangutan (NP_001126661.1), cow (NP_777179.1), chicken (NP_990055.1), frog (NP_001080294.1), and fly (NP_001163768.1) shows high conservation over the shown species (black). Transmembrane domains (TMD) are indicated with gray bars (Nishi and Forgac, 2000). N-glycosylation motifs were predicted using the NetNGlyc 1.0 software (<http://www.cbs.dtu.dk/services/NetNGlyc/>). According to the current topology model for the V-ATPase, nonfunctional N-glycosylation sites present in the N-terminal cytoplasmic domain are depicted with red bars, whereas the N-glycosylation motif between TMD III and IV, present in the luminal part of the V0a1 subunit, is depicted in green. Note that Asn-Xaa-Ser/Thr motifs are highlighted in red. (b) RNA levels of V0a subunits are not significantly altered between PSEN-deficient and WT MEFs. RNA levels for V0a1, V0a2, and V0a3 were measured by RT-qPCR and quantified according to the comparative $\Delta\Delta C_t$ method in MEF WT versus PSENdKO. V0c and V1B2 were used as a control (error bars indicate mean \pm SEM; $n = 2-5$; NS, one-way ANOVA).

a Novex Tris-Glycine 4-20% gel

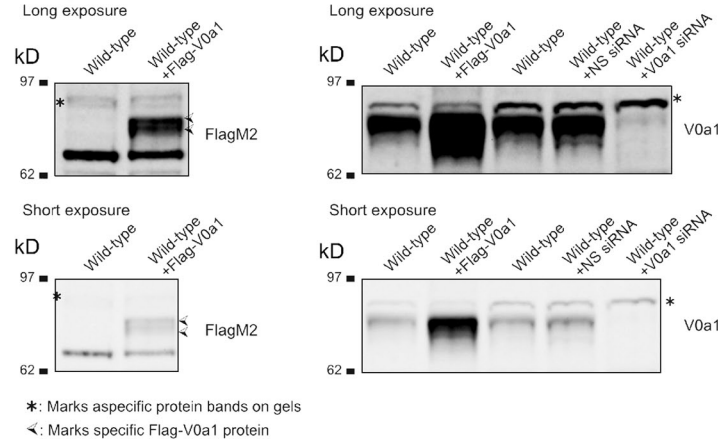
Running conditions: Tris-Glycine SDS running buffer 100min, 125V, 400mA
Blotting: 2h, 25V, 20% MeOH in Tris-Glycine transfer buffer; 15min exposure



b

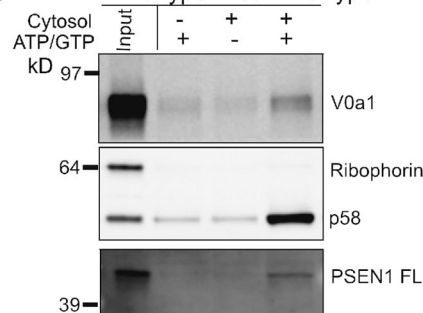
NuPAGE Bis-Tris 4-12% gel

Running conditions: MOPS [3-(N-morpholino) propane sulfonic acid] SDS running buffer 90min, 200V, 400mA
Blotting: 2h, 60V, 125mA, 20% MeOH in NuPAGE transfer buffer



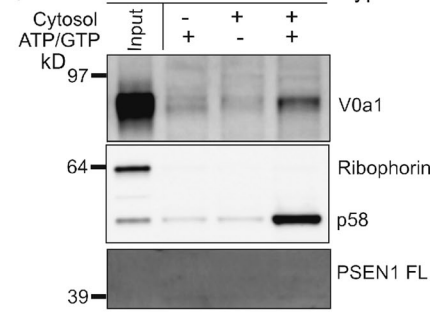
c

Wild type+V0a1wild-type



d

PSENdKO+V0a1wild-type



e

Wild-type+V0a1N489Q

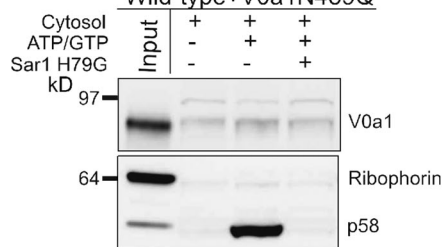


Figure S3. **Characterization of V0a1 subunit in total cell extracts and in vitro ER exit assays.** (a and b) Western blot analysis of V-ATPase subunit V0a1 on different gel types and running buffers. (a) A Novex Tris-Glycine 4–20% gel run in Tris-Glycine SDS running buffer (conditions used as in Lee et al., 2010) and (b) a NuPAGE Bis-Tris 4–12% gel run in MOPS SDS running buffer were used for the Western blotting of total cell lysates from WT MEFs (either non-transfected, overexpressing V0a1, or siRNA down-regulated). Cells were seeded for 24 h (overexpression) or 48 h (down-regulation) before total lysates were prepared. (c–e) The V0a1 subunit is actively transported out of the ER in WT and PSENdKO cells. Western blot analysis of in vitro ER-budding assays performed in permeabilized (c) WT and (d) PSENdKO MEFs, with the input (first lane) and budding reactions (lanes 2–4). No COPII budding vesicles were recovered when cytosol was omitted (lane 2), or in the absence of an ATP/GTP regenerating system (lane 3). Full budding was reconstituted in the presence of cytosol and ATP/GTP (lane 4), as indicated by the release of p58/ERGIC-53 in the vesicle fraction. ER budding is selective, as Ribophorin I, a component of the OST complex and resident protein in the ER, was not sorted into COPII vesicles. Exogenously expressed V0a1 subunit readily exits the ER in both WT (c) and PSENdKO MEF (d). For WT cells, ER exit of full-length PSEN1 is shown as an additional positive control. (e) An in vitro ER-budding assay demonstrating that the exogenously expressed glycosylation-deficient V0a1 N₄₈₉Q mutant also exits the ER. Release in de novo COPII-coated vesicles was inhibited by the addition of Sar1^{H79G}, a dominant-active Sar1 GTPase that selectively blocks ER budding, as also observed for p58/ERGIC-53.

Table S1. **Specific siRNA oligos against mouse ATP6 V0a1**

Oligo	Sequence
V0a1 oligos	5'-UGAUUAACCGGGAGCGGAU-3' 5'-AGACAUCUUAUCCGAAUA-3' 5'-CGAGAUGGGAAGAGGCGCA-3' 5'-GCUAAGAACAUCCGCGUCU-3'
Non-specific siRNA oligo	5'-CGUACGCGAAUACUUCGAdTdT-3'

Table S2. **Primers for qPCR**

Gene isoform	Sense/antisense	Primers	Nucleotides	Accession no.
HPRT-1	Forward	5'-GCTTCCCTGGTTAAGCAGTACA-3'	23	NM_013556.2
	Reverse	5'-GAGAGGTCCTTTTACCAGCAA-3'	22	
GUSB	Forward	5'-TCGCCGACTTCATGACGAA-3'	19	NM_010368.1
	Reverse	5'-GCTGTCTCTGGCGAGTGAAGA-3'	21	
ATP6 V0a1	Forward	5'-GAAATGGCTCCGGAGTCAA-3'	20	NM_001243050.1
	Reverse	5'-TGGTCCTCCGCTGATTGAGA-3'	21	
ATP6 V0a2	Forward	5'-AGCAAGACACACGGGCTCTAC-3'	21	NM_011596.4
	Reverse	5'-ACCGTGAGAGCCACCAACAC-3'	20	
ATP6 V0a3	Forward	5'-GGGAGCTGCTGGGCTAGAAGCAA-3'	23	NM_016921.3
	Reverse	5'-GCCGGACGTCTACCAGAAAGC-3'	21	
ATP6 V0a4	Forward	5'-GTCCAGCCATTGAGGCATTC-3'	22	NM_080467.3
	Reverse	5'-AGCAATACGCAGCCTCCACCTG-3'	22	
ATP6 V0c	Forward	5'-GTCCGCCATGGTCTTCAG-3'	18	NM_009729.3
	Reverse	5'-CAGCTCTGGCCTCATGACT-3'	19	
ATP6 V1B2	Forward	5'-CAATGTCTGCCTTTTTTGAATCTG-3'	25	NM_007509.3
	Reverse	5'-GCCAGGCGAGGAGTGATG-3'	18	

References

- Lee, J.H., W.H. Yu, A. Kumar, S. Lee, P.S. Mohan, C.M. Peterhoff, D.M. Wolfe, M. Martinez-Vicente, A.C. Massey, G. Sovak, et al. 2010. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell*. 141:1146–1158. <http://dx.doi.org/10.1016/j.cell.2010.05.008>
- Nishi, T., and M. Forgac. 2000. Molecular cloning and expression of three isoforms of the 100-kDa a subunit of the mouse vacuolar proton-translocating ATPase. *J. Biol. Chem.* 275:6824–6830. <http://dx.doi.org/10.1074/jbc.275.10.6824>