Supporting Information

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SI Materials and Methods

***In Vitro* Synthesis of the $F_1\beta$ Precursor Protein.** A pTZ18U plasmid containing cDNA of the $pF_1\beta$ subunit of the ATP synthase from *N. plumbaginifolia* was used for the *in vitro* expression of the precursor protein. The protein was produced in a coupled transcription-linked translation reticulocyte lysate TNT system (Promega) in the presence of [^{35}S]-labeled methionine (GE Healthcare AB) in accordance with the Promega protocol (1).

Immunoblotting. After import assay, the mitochondrial pellet was dissolved in Laemmli sample buffer (2) and boiled for 5 min. Samples were loaded on 10 to 20% TricineGlycine SDS-PAGE gels (BioRad) and then transferred to nitrocellulose membrane. The membrane was then boiled in PBS for 5 min and blocked in 5% nonfat milk and incubated with primary antibody overnight (1:500 of αAβ40, αAβ42, or 4G8, Table S1). The membranes were subsequently incubated with secondary antibody (HRP-linked antibody, Amersham Biosciences, GE Healthcare AB) and developed using the Pierce Super Signal West Pico chemiluminescence substrate kit (Pierce, Thermo Fisher Scientific).

Quantification of Gold Particles After Import Assay by Immunoelectron Microscopy. Images were taken randomly at a primary magnification of 21,500 × and individual mitochondria were taken at a primary magnification of 60,000 × and printed. To evaluate the distribution of labeling within the fraction, the gold particles over mitochondria and the gold particles outside mitochondria were counted and the corresponding area was estimated by point counting on printed images and expressed as gold particles/μm^2 (3). On higher magnification the gold particles at cristae membrane, in mitochondria matrix or at the periphery (outer membrane) in individual mitochondria, were counted and the distribution was calculated.

Immunohistochemical Staining. Seven μm thick sections were deparaffinised and manually immunostained with antibody directed to Aβ. The labeled streptavidin-biotin method (Histostain-Plus Kit, Zymed) was used with Romulin 3-amino-9-ethylcarbazole (Romulin AEC) chromogen (Biocare Medical). The sections were counterstained with Harris' hematoxylin (Merck), dehydrated, and mounted in DePex (BDH Laboratory Supplies). Omission of primary antibodies revealed no detectable staining. The stained sections were assessed under light microscopy at 100 to 200× magnifications and search for fleecy, diffuse, and dense plaques, as well as for amyloid angiopathy.

1. Boutry M, Chua NH (1985) A nuclear gene encoding the beta subunit of the mitochondrial ATP synthase in *Nicotiana plumbaginifolia*. *Embo J* 4:2159–2165.
2. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.
3. Weibel, E (1979) *Practical Methods for Biological Morphometry* (Academic, London), Vol. 1.

Table S1. Clinical data of patients from whom brain biopsies were collected during neurosurgery of normal pressure hydrocephalus

Case	PAD	Aβ in mitochondria	Age at biopsy	Gender	Leading symptoms	NPH	Cognition	MMSE	Duration of cognitive symptoms	Other disease
1	Parenchymal amyloid aggregation	+	73	Male	Cognitive impairment, gate difficulty	+	Mild decline	25	2 years	DM, hypertension, cardiovascular disease
2	Parenchymal amyloid aggregation	+	75	Male	Cognitive impairment, gate difficulty	−	Severe dementia	<10, not reliable evaluation due to poor co-operation (bedridden in old people's home)	Clear decline in 1 year	Parkinson symptoms several years, epilepsy
3	Intracellular hyperphos-phorylated tau	−	80	Male	Gate difficulty	+	Mild dementia	14	2–3 years	Mainly motor symptoms (suspected PSP) DM, cardiovascular disease
4	Parenchymal amyloid aggregation	+	76	Female	Gate difficulty	+	Mild decline	27	0	Post polio
5	Parenchymal amyloid aggregation	+	58	Female	Gate difficulty, cognitive impairment	+	Normal/ only mild decline	>27	6 months	−
6	No pathology	−	53	Male	Cognitive impairment	+*	Mild decline	27	1 year	Glaucoma
7	Parenchymal amyloid aggregation	+	71	Male	Cognitive impairment, gate difficulty	−	Moderate to severe dementia	14	2–3 years	TIA, cardiovascular disease

*Obstructive hydrocephalus (chronic); DM, diabetes mellitus; MMSE, mini-menatal state examination; NPH, normal pressure hydrocephalus; PSP, progressive supranuclear palsy; TIA, transient ischemic attack.

Table S2. List of antibodies used in this study

Antibody	Raised towards	Name	Company/gift giver	Purpose
α-Aβ	Amino acids 17–24	4G8	Signet	Detection of Aβ
α-Aβ40	Aβ_{40} C-terminus	44–348	Biosource	Detection of Aβ40
α-Aβ42	Aβ_{42} C-terminus	44–344	Biosource	Detection of Aβ42
$JN_{1\text{-}42}$	Aβ_{42} C-terminus		Dr. Jan Näslund	Detection of Aβ42 by iEM
α-Tom20	Amino acids 1–145	FL-145 SC-11415	Santa Cruz	Inhibition of Tom 20
α-Tom40	Amino acids 62–361	H300, SC11414	Santa Cruz	Inhibition of Tom 40
α-Tom70	C-terminus	C18, SC26495	Santa Cruz	Inhibition of Tom 70
α-VDAC	Amino acids 185–197	Anti-Porin (Ab-5) PC-548T	Calbiochem	Inhibition of VDAC
α-Grp75	C-terminus		Prof. Elzbieta Glaser	Purity of fractionated mitochondria
α-NDUFS4	Full-length	MS104	MitoSciences	Purity of fractionated mitochondria