Supporting Information

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SI Methods

Animals Used for Proteomic Analysis. Four strains of mice were used in this analysis: single-transgenic pR5 (1, 2), double-transgenic APP/PS2 (3), a crossbreeding of the 2 strains (^{triple}AD) (4), and nontransgenic wild-type littermate controls. From each strain, 6 female mice were killed at the age of 10 months, and forebrains dissected. From each forebrain we obtained a "crude synaptosomal" (vesicular) fraction, followed by lysis and determination of protein content using the DC Protein Assay (BioRad 500-0116). For iTRAQ processing and labeling (see below), 33 μg were obtained from 3 samples and mixed before analysis to reduce the impact of individual outliers. A mix from animals 1–3 (group 1) of each strain was used in the first run and of animals 4–6 (group 2) in the second. Three animals each from groups 1 and 2 were randomly chosen for the third run (Table S2).

Animals Used for Analysis of Mitochondrial Function. Four strains of mice were used: pR5, APP/PS2, ^{triple}AD, and nontransgenic wild-type littermate controls. From each strain, 7–12 female mice were killed at the age of 2, 4, 8, 12, and 16 months to identify the age when functional changes start, and forebrains were then dissected.

Crude Vesicular Extraction and Lysis. To prepare a crude vesicular extract from freshly killed mice, forebrains were separated from brainstem and cerebellum. Forebrains were transferred into 5 mL prechilled preparation buffer (0.32 M sucrose, 1 mM $NaHCO_3$, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$) containing protease inhibitors (Complete, EDTA-free 11873580001; Roche). They were slowly and gently homogenized in a douncer, with 12 up-and-down strokes at 700 rpm. The lysate was then centrifuged at 1,400 $\times$ *g* for 10 min at 4 °C. The supernatant (S1) was saved and the pellet resuspended in 2 mL preparation buffer containing protease inhibitors. The suspension was homogenized further with 3 slow and gentle up-and-down strokes at 700 rpm and then centrifuged at 720 $\times$ *g* for 10 min at 4 °C. The pellet was discarded and the supernatant (S2) combined with supernatant S1. The lysate was centrifuged again at 720 $\times$ *g* for 10 min at 4 °C, and the pellet was discarded. Crude vesicles including synaptosomes were then pelleted at 13,800 $\times$ *g* for 10 min at 4 °C.

The crude synaptosomal preparation was resuspended in 500 μL RIPA buffer [50 mM Tris-HCl (pH 7.5), 1% (vol/vol) Triton X-100, 0.1% (vol/vol) SDS, 15 mM sodium deoxycholate, 375 mM NaCl, 10 mM EGTA] containing EDTA-free Complete protease inhibitors (Roche). Samples were homogenized by passing through a Terumo syringe needle (22 Gauge $\times$ 1 1/2″) and subsequently incubated on ice for 1 h. Lysates were then further passed through a Terumo Insulin syringe (29 Gauge $\times$ 1/2″) and centrifuged at 22,000 $\times$ *g* for 10 min at 4 °C. The pellet was discarded and the supernatant used for further analysis.

Protein Digestion and iTRAQ Labeling. Preparation and labeling was conducted according to the iTRAQ manual (Applied Biosystems). In short, 100 μg of protein was acetone precipitated and resuspended in 20 μL of dissolution buffer (0.5 M triethylammonium bicarbonate) containing 0.1% (vol/vol) SDS. The sample was then reduced by adding TCEP [Tris(2-carboxyethyl)phosphine] to a concentration of 50 mM and incubated at 60 °C for 1 h. Subsequently, the sample was treated with 200 mM MMTS (methyl methanethiosulfonate) for alkylation/cysteine blocking for 10 min at room temperature. The protein sample was then treated with 4 μg trypsin (Trypsin Gold; Promega) per 100 μg protein at 37 °C for 16 to 24 h. Labeling of the samples with iTRAQ labels was done at room temperature for 1 h. Table S2 shows the iTRAQ labels and the corresponding samples. In each iTRAQ run, the different samples were mixed after labeling and dried in SpeedyVac. The dried samples were then stored at −20 °C before 2D HPLC peptide separation and data acquisition.

First-Dimensional HPLC for Peptide Fractionation. To investigate the effect of the first-dimensional HPLC peptide separation, we used both reverse-phase (RP) and strong cation exchange (SCX) to fractionate peptides. RP HPLC was used for Run1; SCX HPLC was used for Run2; RP HPLC and SCX HPLC were both used in parallel for Run3, and this run identified most proteins. The collected fractions from the first-dimensional HPLC were dried in SpeedyVac and then stored at −20 °C before nanoLC-ESI MS/MS data acquisition.

SCX HPLC. An Agilent 1100 quaternary HPLC pump with a PolyLC PolySulfoethyl A (200 mm $\times$ 2.1 mm $\times$ 5 μm, 200A) column was used for strong cation exchange chromatography sample clean up and fractionation. Buffer A was 5 mM phosphate and 25% acetonitrile (pH 2.7), and buffer B was 5 mM phosphate, 350 mM KCL, and 25% acetonitrile (pH 2.7). The dried iTRAQ-labeled sample was resuspended with loading buffer (buffer A) and loaded onto the SCX column. The flow-through was discarded as this contained interfering chemicals for the second-dimensional nanoLC and mass spectrometry. To elute peptides, buffer B gradient was increased from 10% to 45% in 70 min at a flow rate of 300 μL/min. Twenty-five fractions were collected beginning from the start of the gradient.

RP HPLC. The sample was first cleaned up using Strong Anion Exchange resin (BioRad Macroprep High Q Support) to remove neutral or negatively charged chemicals that would interfere with reverse-phase HPLC separation and mass spectrometry data acquisition. The reverse-phase HPLC fractionation used column Phenomenex Jupiter 5u C4 300A 150 $\times$ 2.00 mm. Buffer A was 0.1% TFA in MilliQ water, buffer B was 0.085% TFA in acetonitrile. After 10 min sample loading and desalting, buffer B gradient was increased from 5% to 40% in 25 min and then increased to 90% in 5 min to elute peptides. The fractions were collected at 1-min intervals.

NanoLC-ESI MS/MS Mass Spectrometry. The Agilent 1100 nanoLC system (Agilent) and the QStar XL MS/MS system (Applied Biosystems) were used for nanoLC-electrospray MS/MS. The peptide fractions from first-dimensional HPLC were resuspended with loading/desalting solution (0.1% trifluoroacetic acid, 2% acetonitrile, 97.9% water). The resuspended sample was loaded onto a peptide Captrap column (Michrom Bioresources) and desalted with the desalting solution at 10 μL per minute for 13 min. After desalting, the trap was switched online with a 150 μm $\times$ 10 cm C18 3 μm 300A ProteCol column (SGE). Buffer solution A was 99.9% water/0.1% formic acid, and buffer solution B was 90% acetonitrile/9.9% water/0.1% formic acid. The buffer B concentration was increased from 5% to 90% in 120 min in 3 linear gradient steps to elute peptides. The RP nanoLC eluent was subjected to positive ion nanoflow ESI analysis in an information-dependant acquisition mode (IDA). In the IDA mode, a TOF-MS survey scan was acquired (*m*/*z* 380-1600, 0.5 s), with the 3 most intense multiply charged ions (counts >50) in the

survey scan sequentially subjected to MS/MS analysis. MS/MS spectra were accumulated for 2 s in the mass range m/z 100–1,600 with a modified Enhance All Q2 transition setting favoring low mass ions so that the reporting iTRAQ tag ion (114, 115, 116, and 117 Da) intensities were enhanced for quantification. After peptide elution, the nanoLC column was cleaned with 100% buffer B for 15 min and then equilibrated with buffer A for 30 min before the next sample injection.

Database Processing and Statistics for Quantitative Proteomic Analysis. The experimental nanoLC-ESI MS/MS data were submitted to ProteinPilot (Applied Biosystems, version 1.0) for data processing. The Paragon method was used in thorough ID search effort with Biological modifications selected in ID Focus. The software correction factors provided in the iTRAQ kit were entered in the iTRAQ Isotope Correction Factors table. The detected protein threshold (unused ProtScore) was set as larger than 1.3 (better than 95% confidence). The database used was uniprot sprot20051220 with *Mus musculus* specified as the searched species.

After ProteinPilot data processing, the protein summary listing the identified proteins and their iTRAQ ratios was exported as a tab delimited text file. iTRAQ runs were performed 3 times. In each run, proteins satisfying the criteria (P value <0.01 and iTRAQ ratio <0.82 or >1.20) were highlighted. Then all proteins were aligned and iTRAQ ratios among the 3 runs were compared. The listed proteins (Table 1 and Table S1) have the same ratio changing trend in all runs and satisfy the above criteria in minimum 2 runs. Finally, to compare the runs, all identified proteins from all runs were aligned using an excel macro. To highlight differentially expressed proteins, iTRAQ ratios <0.82 or larger than 1.2 and a P value <0.01 were used as threshold.

BN-PAGE and Tricine-SDS-PAGE. Sample preparation, Blue Native (BN) electrophoresis, 2D tricine-SDS-PAGE were performed as described in ref. 5. Per lane, the n-dodecyl-ß-D-maltoside (7 g/g mitochondrial protein) solubilized fraction of ≈10 mg protein from isolated mouse brain mitochondria was separated in the first dimension (acrylamide gradient 4–3%, sample gel 3.5%). The native lanes were cut and used for 2D SDS/PAGE (16% acrylamide) to separate the subunits of the respiratory chain complexes. Finally the gels were visualized by silver staining as described in ref. 6.

Brain Tissue Preparation for Mitochondrial Analysis. Cellular preparations were obtained to determine the mitochondrial membrane potential (MMP) and mitochondria, to determine respiration rates as described in refs. 7 and 8. For that, mice were killed by decapitation and brains quickly dissected on ice. The cerebellum and one cortical hemisphere (the other hemisphere was directly used for preparation of isolated mitochondria for mitochondrial respiration and complex activities) were separately minced into 1 mL of medium I [138 mM NaCl, 5.4 mM KCl, 0.17 mM Na_2HPO_4, 0.22 mM K_2PO_4, 5.5 mM glucose, 58.4 mM sucrose (pH 7.35)] with a scalpel and further dissociated by trituration through a nylon mesh (pore diameter 1 mm) with a pasteur pipette. The resulting suspension, which contained both neuronal (≈72%) and glial cells (≈26%), was filtered by gravity through a fresh nylon mesh with a pore diameter of 102 μm, and the dissociated cell aggregates were washed twice with medium II [(110 mM NaCl, 5.3 mM KCl, 1.8 mM $CaCl_2$*H_2O, 1 mM $MgCl_2$*6 H_2O, 25 mM glucose, 70 mM sucrose, 20 mM Hepes (pH 7.4)] by centrifugation (400 × g for 3 min at 4 °C). We used 100 μL of the suspension for protein determination. After centrifugation, cells were resuspended in 3 mL DMEM, and then aliquots of 100 μL were distributed per well in a 48-well plate for measurement of the mitochondrial membrane potential. The preparations of cerebellar and cortical cells from transgenic mice and WT littermate controls (crossover design) were made within 2 h under the same conditions and in parallel, and maintained at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Viability was found to be >90% using the MTT assay and Trypan Blue exclusion test. Data are expressed as fluorescence units per mg/mL protein.

Determination of the Mitochondrial Membrane Potential MMP. The membrane potential of the inner mitochondrial membrane was measured using the dye tetramethylrhodamine ethyl ester (TMRE; Molecular Probes) and the dye Rhodamine 123 (R123; Molecular Probes) added to the cell culture medium at a final concentration of 10 μM (TMRE) or 0.4 μM (R123) for 15 min. Cells were washed twice with HBSS (Sigma), and fluorescence was determined with a Victor2 multiplate reader (Perkin–Elmer) at 535 nm/590 nm (TMRE, Ex/Em) or at 490 nm/538 nm (R123, Ex/Em) (7).

ATP Levels. The ATP content of dissociated cells was determined with a bioluminescence assay (ViaLighTM HT; Cambrex Bio Science) according to the instruction of the manufacturer. The enzyme luciferase, which catalyzes the formation of light from ATP and luciferin was used. Dissociated brain cells were lysed before the addition of the reagent. The emitted light is linearly related to the ATP concentration and is measured using a luminometer (7).

ROS Levels. The levels of ROS were measured using the fluorescent probe H2DCF-DA, and the levels of superoxide anion radical were measured using DHE (9). The brain cells were loaded for 15 min with 10 μM H2DCF-DA or for 60 min with 10 μM DHE. After washing twice with HBSS, the formation of the fluorescent product dichlorofluorescein was detected using the Victor2 multiplate reader (PerkinElmer Life Sciences) at 485 nm (excitation)/535 nm (emission). DHE, which is oxidized to the fluorescent ethidium cation by $O_2^{\bullet -}$ was detected using the Victor2 multiplate reader at 490 nm (excitation)/590 nm (emission).

Preparation of Isolated Mitochondria. Mitochondria were isolated from mouse brains as described in ref. 7. Briefly, mice were killed by decapitation, and one brain hemisphere was rapidly dissected on ice and washed in an ice-cold buffer (210 mM mannitol, 70 mM sucrose, 10 mM Hepes, 1 mM EDTA, 0.45% BSA, 0.5 mM DTT, and Complete Protease Inhibitor mixture tablets (Roche Diagnostics). After removing the cerebellum, the tissue sample was homogenized in 2 mL of buffer with a glass homogenizer (10–15 strokes, 400 rpm), and the resulting homogenate was centrifuged at 1,400 × g for 7 min at 4 °C to remove nuclei and tissue particles. The low-speed centrifugation step was repeated once with the supernatant. Then, the supernatant fraction was centrifuged at 10,000 × g for 5 min at 4 °C to pellet mitochondria. The resulting pellet was resuspended in 1 mL of ice-cold buffer and centrifuged again at 1,400 × g for 3 min at 4 °C. Finally, the mitochondria-enriched supernatant was centrifuged at 10,000 × g for 5 min at 4 °C to obtain a mitochondrial fraction. This fraction was resuspended in 100 μL of ice-cold buffer and stored at 4 °C until use, followed by determination of protein content (7).

Mitochondrial Respiration. Mitochondrial oxygen consumption was measured at 37 °C using an Oroboros Oxygraph-2k system. Isolated mitochondria (0.5 mg) were added to 2 mL of a mitochondrial respiration medium containing 65 mM sucrose, 10 mM potassium phosphate, 10 mM Tris-HCl, 10 mM $MgSO_4$, and 2 mM EDTA (pH 7.0) (7). To measure the state 4 of the complex I, 10 mM glutamate and 2 mM malate were added. Then, 2 mM

ADP was added to measure state 3 respiration. After determining coupled respiration, 0.05 μM FCCP [carbonylcyanide p-(trifluoromethoxy) phenylhydrazone] was added and respiration was measured in the absence of a proton gradient. To check the integrity of the mitochondrial membrane, 10 μM cytochrome *c* was added. To inhibit complex I and III activities, 0.5 μM rotenone and 2.5 μM antimycin A, respectively, were added. To avoid oxygen limitation, an intermittent reoxygenation was achieved by partial opening of the oxygraph chamber and oxygen transfer from the gas phase until obtaining a maximum oxygen concentration (10). This standard procedure was used for all experiments after the inhibition of complex I by rotenone. Then, 2 mM ascorbate and 0.5 mM TMPD were added and respiration was measured. Finally, 10 mM sodium azide was added to inhibit complex IV activity (11). Mitochondria from transgenic and control mice were measured in parallel pairs using the same conditions (crossover design).

Activity of Complex I. We solubilized 100 μg of isolated mitochondria in n-dodecyl β-D-maltoside (0.1 mg). NADH:hexaammineruthenium(III)-chloride (HAR) activity was measured at 30 °C in a buffer containing 2 mM Na^+/Mops, 50 mM NaCl, and 2 mM KCN (pH 7.2) using 2 mM HAR and 200 μM NADH as substrates to estimate the complex I content. To determine NADH-ubiquinone oxidoreductase activity, 100 μM *n*-decylubiquinone (DBQ) and 100 μM NADH were used as substrates and 5 μM rotenone as inhibitor, as described in ref. 7. Oxidation rates of NADH were recorded with a Shimadzu MultiSpec-1501 diode array spectrophotometer ($\varepsilon_{340-400\ nm}$ = 6.1 $mM^{-1}{\cdot}cm^{-1}$). Complex I activity was normalized to the complex I content of the mitochondrial preparation and is given as DBQ/HAR ratio.

Activity of Complex IV. Cytochrome *c* oxidase activity was determined in intact isolated mitochondria (50 μg) using the Cytochrome *c* Oxidase Assay kit. The colorimetric assay is based on the observation that a decrease in absorbance at 550 nm of ferrocytochrome *c* is caused by its oxidation to ferricytochrome *c* by cytochrome *c* oxidase. The Cytochrome *c* Oxidase Assay was performed as described in ref. 7. Activity was normalized to the corresponding citrate synthase.

Activity of Citrate Synthase. The reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by citrate synthase at 412 nm (extinction coefficient of 13.6 $mM^{-1}{\cdot}cm^{-1}$) was followed in a coupled reaction with CoA and oxaloacetate as described in ref. 12. Briefly, a reaction mixture of 0.2 M Tris-HCl (pH 8.0) 0.1 mM acetyl-CoA, 0.1 mM DTNB, n-dodecyl-β-D-maltoside (20%) and 10 μg of mitochondrial protein was incubated at 30 °C for 5 min. The reaction was initiated by the addition of 0.5 mM oxaloacetate, and the absorbance change was monitored for 5 min with a Shimadzu MultiSpec-1501 diode array spectrophotometer (11).

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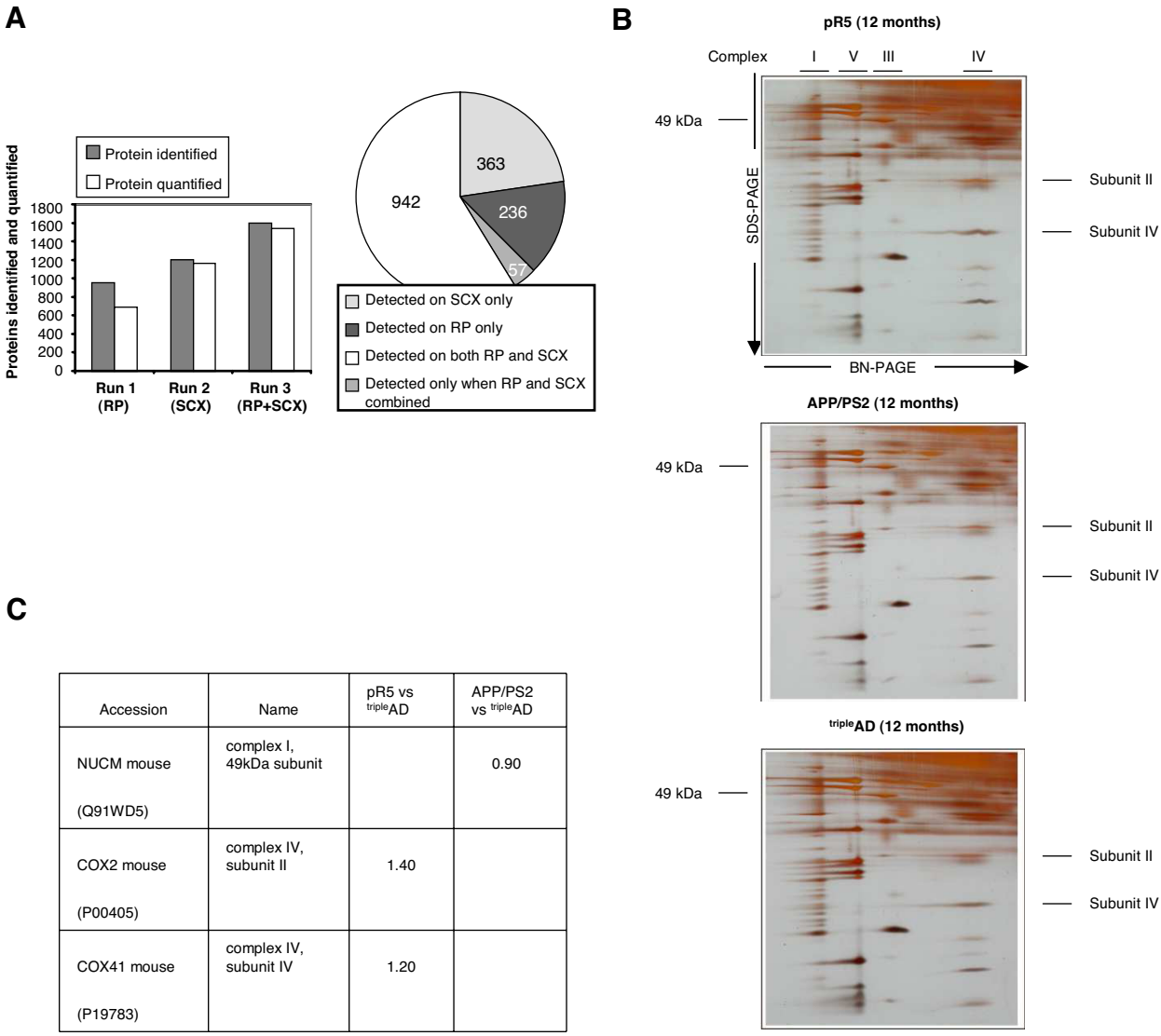

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Accession	Name	pR5 vs ^{triple}AD	APP/PS2 vs ^{triple}AD
NUCM mouse (Q91WD5)	complex I, 49kDa subunit		0.90
COX2 mouse (P00405)	complex IV, subunit II	1.40	
COX41 mouse (P19783)	complex IV, subunit IV	1.20	

Fig. S1. Quantitative proteomics analysis and separation of dodecylmaltoside-solubilized mitochondrial complexes from mouse brain. (*A*) Total of proteins identified and quantified in each run, and proteins identified in Run3 (Table S1) by combined strong cation exchange (SCX) HPLC and reverse-phase (RP) HPLC. (*B*) Solubilized brain mitochondrial complexes (I–III and V) from 12-month-old mice of different genetic background (pR5; APP/PS; ^{triple}AD) were separated on a linear 4%–13% acrylamide gradient gel by BN-PAGE (not shown) and subsequently the individual subunits of the native complexes were separated by tricine-SDS/PAGE using a 16% T, 3% C gel type as detailed in *Materials and Methods*. Proteins were visualized by silver staining. The position of the 49-kDA subunit of complex I and the positions of the complex IV subunits II and IV are indicated. (*C*) The protein expression levels of the 3 subunits were compared between the ^{triple}AD mice, the pR5, and the APP/PS2 mice, respectively. In agreement with the iTRAQ results, the subunit 49 kDa of the complex I (NUCM) was up-regulated in the ^{triple}AD mice compared with the APP/PS2 mice. The subunits II and IV of the complex IV were down-regulated in the ^{triple}AD mice compared with the pR5 mice.

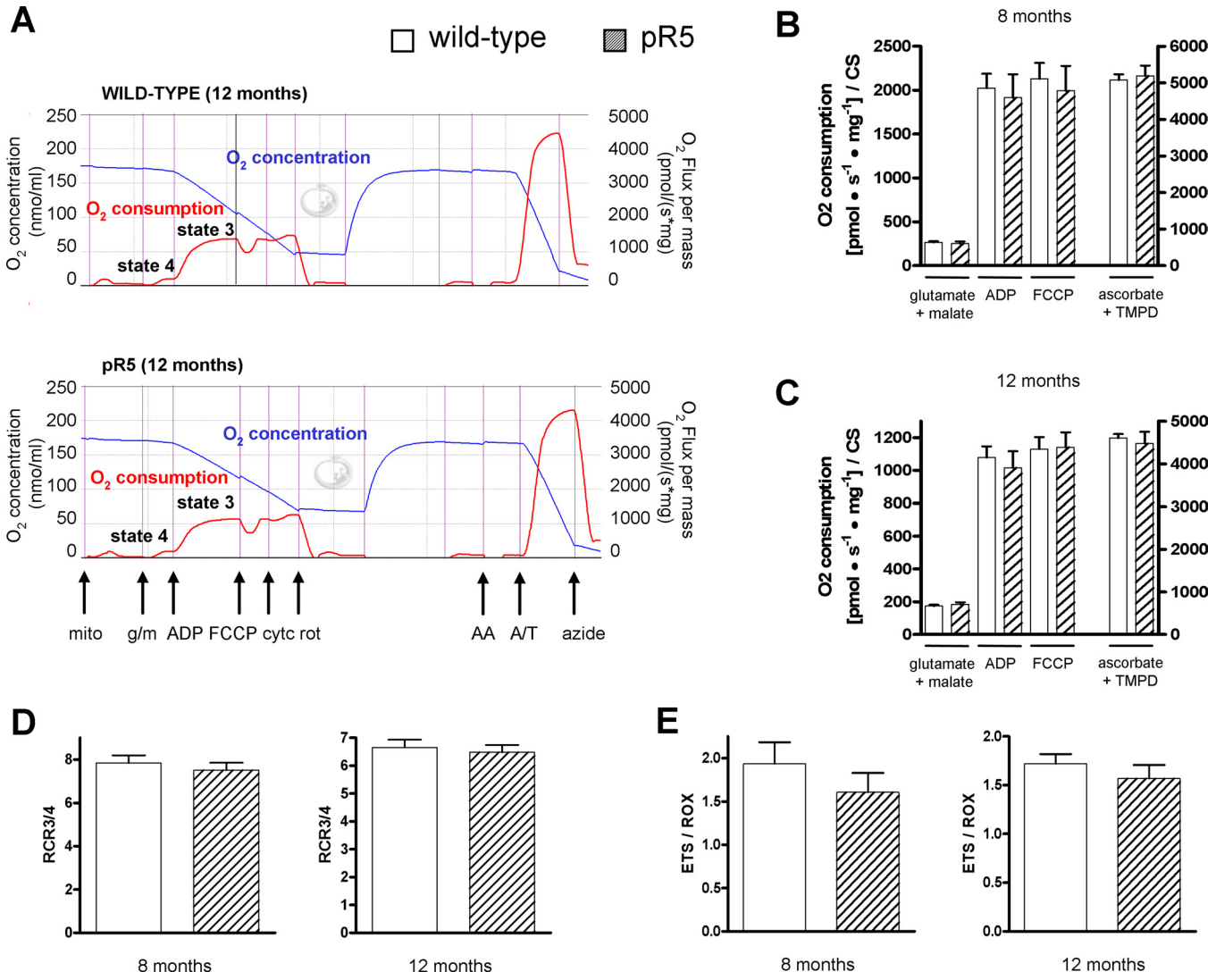

Fig. S2. No reduction of oxygen consumption in pR5 tau mitochondria at 8 and 12 months of age. Measurement of oxygen (O_2) flux and consumption in freshly isolated mitochondria from wild-type and age-matched pR5 cortices. After detecting endogenous respiration (mito), glutamate+malate (g/m) were added to induce state 4 respiration. ADP stimulated state 3 respiration. After determining coupled respiration, FCCP was added and the maximal respiratory capacity measured in the absence of a proton gradient. Cytochrome c (cyt c) demonstrated mitochondrial membrane integrity. To inhibit activities of complexes I–III, rotenone (rot) and antimycine A (AA) were added. Complex IV activity was stimulated by ascorbate/TMPD (A/T) before terminating mitochondrial respiration by adding sodium azide (azide). O_2 consumption was normalized to the corresponding citrate synthase (CS) activity. (*A*) Representative diagrams of O_2 flux and consumption in mitochondria from 12-month-old wild-type and pR5 mice in response to titrated substrates and inhibitors of mitochondrial complexes. No effect of the transgene on respiratory rates of mitochondria from the wild-type and pR5 mice (*B*) at the age of 8 and (*C*) 12 months. (*D*) RCR3/4 (state3/state4 ratio) representing the mitochondrial coupling state and (*E*) ETS/ROX ratio, which yields an index of the maximum oxygen consumption capacity of the electron transport system (ETS) relative to the magnitude of residual oxygen consumption (ROX), did not differ between the wild-type and pR5 mice at the age of 8 and 12 months (n = 7–12 animals/group).

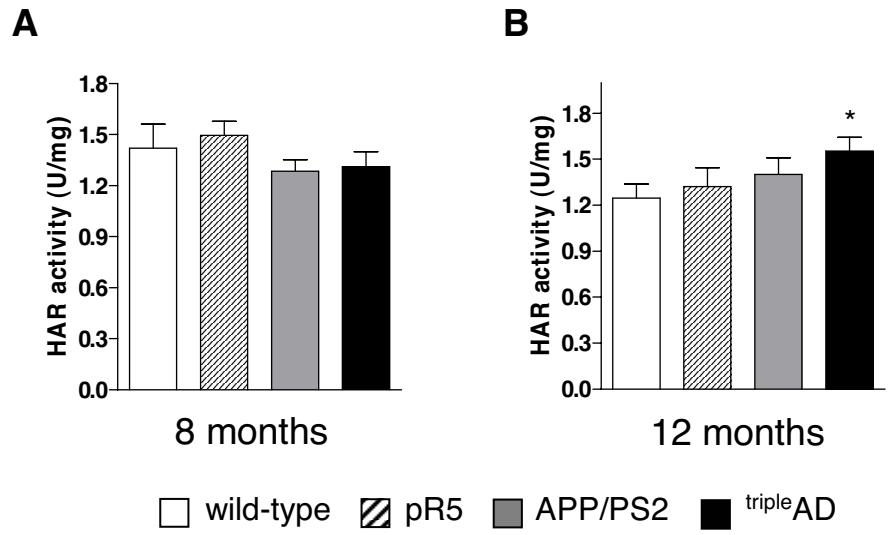

Fig. S3. Increased complex I content in ^{triple}AD cortices. Complex I content (HAR activity) was significantly increased in mitochondria from ^{triple}AD mice at 12 months of age (*B*), but not yet at 8 months of age (*A*) (*, $P < 0.05$ vs. wild-type, Student *t* test). Values represent the means ± SE from $n = 7–12$ animals/group.

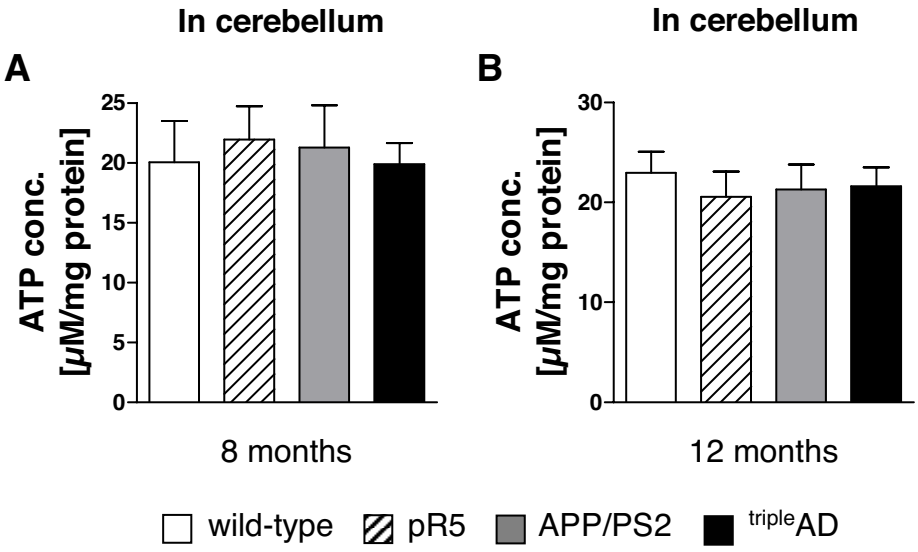

Fig. S4. No drop of ATP synthesis in cerebellar cells. The decrease of ATP levels is brain region-specific because no difference in ATP levels was observed between cerebellar cells from the different mouse models at the age of 8 (*A*) and 12 months (*B*). Values represent the means ± SE from $n = 7–12$ animals/group.

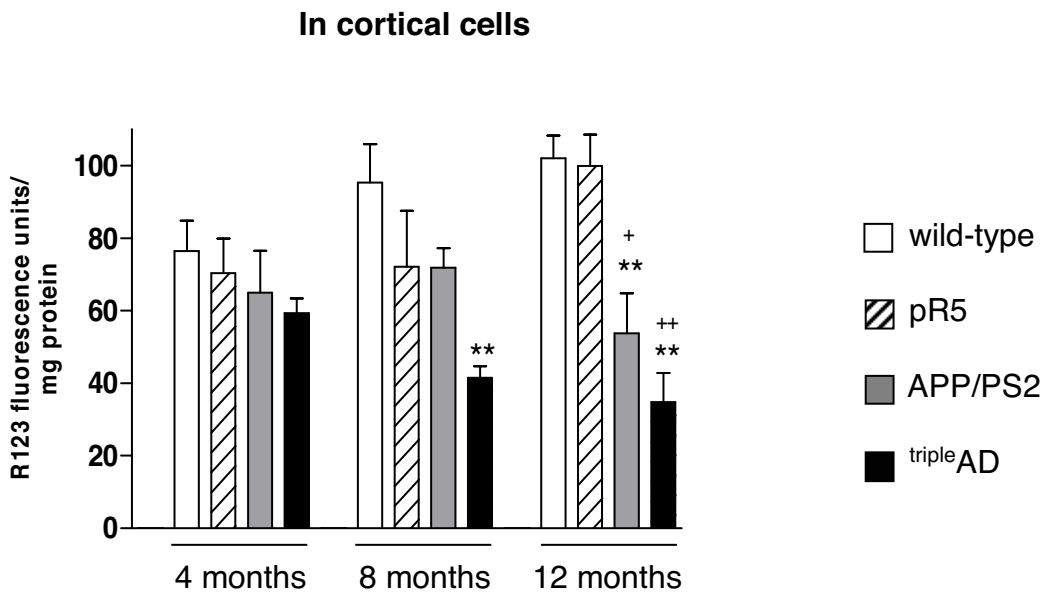

Fig. S5. Reduced MMP in cortical brain cells from ^{triple}AD mice. Basal MMP (R123 fluorescence units/mg protein) was reduced in cortical cells from 8-month-old ^{triple}AD mice. At the age of 12 months, MMP was also reduced in cells from APP/PS2 mice. One-way ANOVA post hoc Tukey's; **, $P < 0.01$; vs. wild-type, $^{+}$, $P < 0.05$, $^{++}$, $P < 0.01$ vs. pR5. Values represent the means ± SE from $n = 7–12$ animals/group.

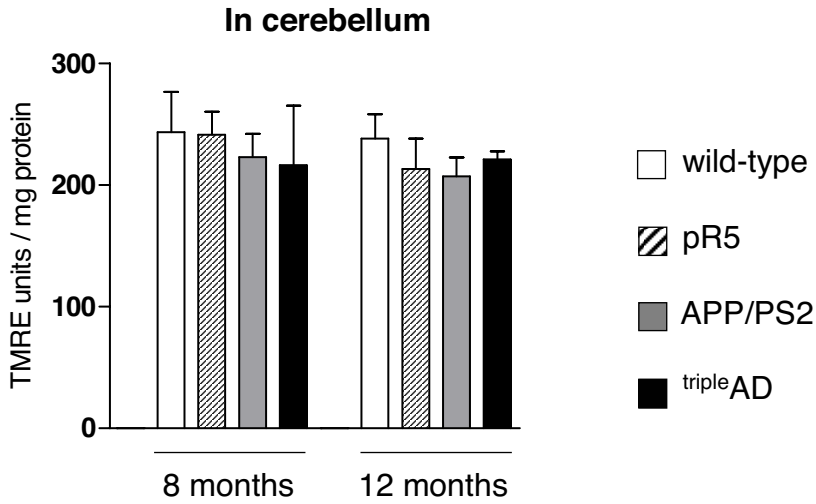

Fig. S6. Unchanged MMP in cerebellar cells from ^{triple}AD mice. The decrease of the basal MMP (TMRE fluorescence units/mg protein) is brain region-specific, because no difference was observed for cells derived from the cerebellum at 8 and 12 months of age. Values represent the means ± SE from n = 7–12 animals/group.

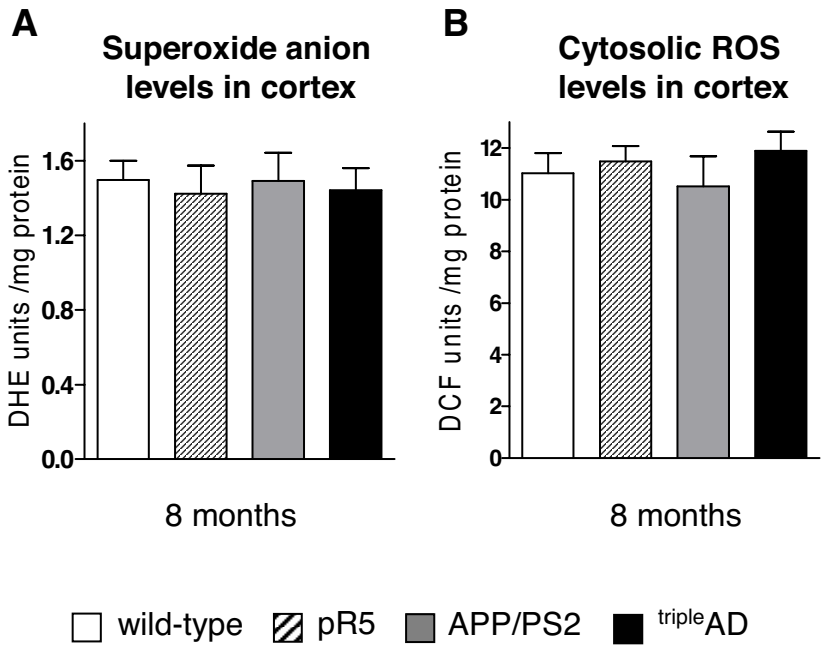

Fig. S7. Unchanged ROS levels in cortical cells from ^{triple}AD mice at the age of 8 months. (*A*) Superoxide anions levels determined by DHE oxidation and (*B*) cytosolic ROS levels measured after incubation with DCF did not increase in cortical cells from ^{triple}AD mice at 8 months of age. Values represent the means ± SE from $n = 7–12$ animals/group.

Table S1. iTRAQ ratios (wild-type vs. ^{triple}AD, wild-type vs. pR5, wild-type vs. APP/PS2, pR5 vs. ^{triple}AD, and APP/PS2 vs. ^{triple}AD) showing differentially expressed proteins observed by iTRAQ experiment

Accession no.	Name	Wild-type vs. ^{triple}AD	Wild-type vs. pR5	Wild-type vs. APP-PS2	pR5 vs. ^{triple}AD	APP/PS2 vs. ^{triple}AD
ANXA5_MOUSE (P48036)	Annexin A5 (Annexin V) (Lipocortin V)	1.28		1.95		0.63
ANXA6_MOUSE (P14824)	Annexin A6 (Annexin VI) (Lipocortin VI)	1.46	0.66	1.39	2.2	
ARF3_MOUSE (P61205)	ADP-ribosylation factor 3			1.56		0.65
BASP_MOUSE (Q91XV3)	Brain acid soluble protein 1 (BASP1 protein)		0.71		1.51	
CALM_MOUSE (P62204)	Calmodulin (CaM)		1.3	1.5	0.78	0.65
COX2_MOUSE (P00405)	**Cytochrome c oxidase subunit 2 (EC 1.9.3.1)**	**1.22**		**1.22**	**1.42**	
COX41_MOUSE (P19783)	**Cytochrome c oxidase subunit IV isoform 1, mitochondrial precursor (EC 1.9.3.1)**	**1.36**		**1.5**	**1.47**	
COX5A_MOUSE (P12787)	**Cytochrome c oxidase polypeptide Va, mitochondrial precursor (EC 1.9.3.1)**	**1.21**	**0.8**	**1.2**	**1.43**	
COX5B_MOUSE (P19536)	**Cytochrome c oxidase polypeptide Vb, mitochondrial precursor (EC 1.9.3.1)**				**1.33**	
CX7A2_MOUSE (P48771)	**Cytochrome c oxidase polypeptide VIIa-liver/heart, mitochondrial precursor (EC 1.9.3.1)**	**1.41**		**1.5**	**1.69**	
HBA_MOUSE (P01942)	Hemoglobin alpha subunit	0.73		0.73	0.69	
HBB1_MOUSE (P02088)	Hemoglobin beta-1 subunit chain)	0.69				0.6
MBP_MOUSE (P04370)	Myelin basic protein (MBP) (Myelin A1 protein)	1.32				1.22
NDKA_MOUSE (P15532)	Nucleoside diphosphate kinase A (EC 2.7.4.6)					0.56
NIDM_MOUSE (Q9DCS9)	**NADH-ubiquinone oxidoreductase PDSW subunit (EC 1.6.5.3)**			**1.5**	**1.39**	**0.66**
NUCM_MOUSE (Q91WD5)	**NADH-ubiquinone oxidoreductase 49 kDa subunit, mitochondrial precursor (EC 1.6.5.3)**			**1.5**		**0.8**
NUIM_MOUSE (Q8K3J1)	**NADH-ubiquinone oxidoreductase 23 kDa subunit, mitochondrial precursor (EC 1.6.5.3)**	**1.22**		**1.27**	**1.39**	
PHB_MOUSE (P67778)	Prohibitin (B-cell receptor associated protein 32) (BAP 32)					0.77
PPIA_MOUSE (P17742)	Peptidyl-prolyl cis-trans isomerase A (EC 5.2.1.8)					1.4
S12A2_MOUSE (P55012)	Solute carrier family 12 member 2 symporter				1.49	
SYN2_MOUSE (Q64332)	Synapsin-2 (Synapsin II)		0.75		1.69	
TAU_MOUSE (P10637)	Microtubule-associated protein tau (neurofibrillary tangle protein)	0.64	0.66			0.6
THY1_MOUSE (P01831)	Thy-1 membrane glycoprotein precursor	1.4	0.7	1.59	1.94	
VA0D_MOUSE (P51863)	Vacuolar ATP synthase subunit d	1.35	0.78	1.76	1.71	0.77

Wild-type and ^{triple}AD mouse samples are compared with other types. Only iTRAQ ratios satisfying the criterion (P value < 0.01 and $0.82 <$ iTRAQ ratio < 1.2) with the same ratio-changing trend in a minimum of 2 of the 3 independent iTRAQ runs and not having ratio-changing trends in any other runs are listed in this table. Deregulated subunits of complexes I and IV are bold.

Table S2. iTRAQ experiment runs and the mouse sample labeling

iTRAQ runs	2DLC	114	115	116	117
Run1	RP-RP	A(1,2,3)	B(1,2,3)	C(1,2,3)	D(1,2,3)
Run2	SCX-RP	A(4,5,6)	B(4,5,6)	C(4,5,6)	D(4,5,6)
Run3	RP-RP	A(1,2,5)	B(2,3,4)	C(3,5,6)	D(2,5,6)

A, wild-type; B, pR5; C, APP/PS2; D, ^{triple}AD.