Supplementary material

Supplementary material and methods

Biochemical experiments

Crude brain mitochondria of *Afg3l2* constitutive knockout and wild type mice were isolated as described (Wieckowski et al, 2009). Neurons were lysed in RIPA buffer (150 mM NaCl, 50 mM Tris–HCl pH 7.4, 5 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail (Roche) on ice. 20 µg of mitochondrial proteins or 10 µg of total proteins were used for western blot. Anti-phospho-GSK-3α/β (Ser21/9), anti- GSK3 β, anti-p35/25 antibodies, anti-phospho-MARK Family (Activation Loop) were obtained from Cell Signaling; anti-CDK5 from Abcam; anti-MnSOD (SOD2), anti-GAPDH, anti-Tau1 and anti-Actin from Millipore; anti-complex II 70 kDa from Invitrogen; AT8 antibody from Pierce; anti-acetylated tubulin, anti-tyrosinated tubulin, anti-β-tubulin from Sigma-Aldrich; anti-calnexin from Enzo life science. Anti-AFG3L2 antibodies were previously described (Koppen et al, 2007). Detection of carbonylated proteins was performed using the Oxyblot kit (Millipore), according to the manufacturer's instructions.

Afg3l2, *Opa1*, *Mapt*, and RNA interference

To downregulate *Afg3l2,* we used Stealth siRNAs synthesized by Invitrogen with the following sequences:

siRNA B: 5'-GCGUUCUCUGCUGAGGGAUGUAAUU-3'

siRNA C: 5'-GGUUGAUGGGCAAUACGUCUGGUUU-3'

To downregulate *Opa1,* we used Stealth siRNAs synthesized by Invitrogen with the following sequences:

siRNA A: 5'-CAAGAGCAGUGUGUUCACAACGAAA-3'

siRNA B: 5'- CAGUGUUCUGUAUUCAGGAACGCUU-3'

To downregulate *Mapt,* we used Stealth siRNAs synthesized by Invitrogen with the following sequence:

siRNA 36: 5'-CAGUCGAAGAUUGGCUCCUUGGAUA-3'

siRNA 37: 5'-CAGGAGGUGGCAAGGUGCAGAUAAU-3'

siRNA 38: 5'-CAGGAGGUGGCCAGGUGGAAGUAAA-3'

A construct containing the full length sequence of rat *Mapt* cDNA in the vector pExpress-1 was used for overexpression in MEFs (kind gift of Dr. Walter Becker).

Quantification of mitochondrial membrane potential with TMRM

Primary cortical neuronal cultures were stained with 10 nM TMRM (Sigma) for 3 hours. As a positive control, 20 µm CCCP (sigma) was added for two hours after adding TMRM. Images were obtained at a excitation wavelength of 561 nm. Average pixel intensities of TMRM were quantified in the soma of neurons by using Volocity 6.1 (Perkin-Elmer).

Transfection of parkin-mCherry

parkin-mCherry (400 ng) and mito-GFP (200 ng) constructs were co-transfected together with 100 nM of the respective siRNA by electroporation. Primary cortical neuronal cultures treated with 100 µM antimycin A for 40 min

acted as a positive control. Images of the axons were collected at excitation wavelength of 488 nm (mito-GFP) and 561 nm (parkin-mCherry).

CellROX experiments

To measure cellular ROS, primary cortical neurons were stained with 2.5 μM CellROX green (Life Technologies) for 30 min. The neurons were washed twice with pre-conditioned neuronal medium and imaged in the same. As a positive control for ROS production, 100 μm menadione (Sigma) was added for 45 min prior to addition of CellROX green reagent. NAC (1 mM) or vitamin E (200 μM) were added with menadione to the neuronal medium. Average pixel intensities of CellROX green were quantified in the soma of neurons by using Volocity 6.1 (Perkin-Elmer).

Supplementary References

Koppen M, Metodiev MD, Casari G, Rugarli EI, Langer T (2007) Variable and tissue-specific subunit composition of mitochondrial m-AAA protease complexes linked to hereditary spastic paraplegia. *Mol Cell Biol* **27:** 758-767

Wieckowski MR, Giorgi C, Lebiedzinska M, Duszynski J, Pinton P (2009) Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells. *Nat Protoc* **4:** 1582-1590