SUPPLEMENTARY INFORMATION

HSPA9/MORTALIN MEDIATES AXO-PROTECTION BY MODULATING MITOCHONDRIAL DYNAMICS IN NEURONS

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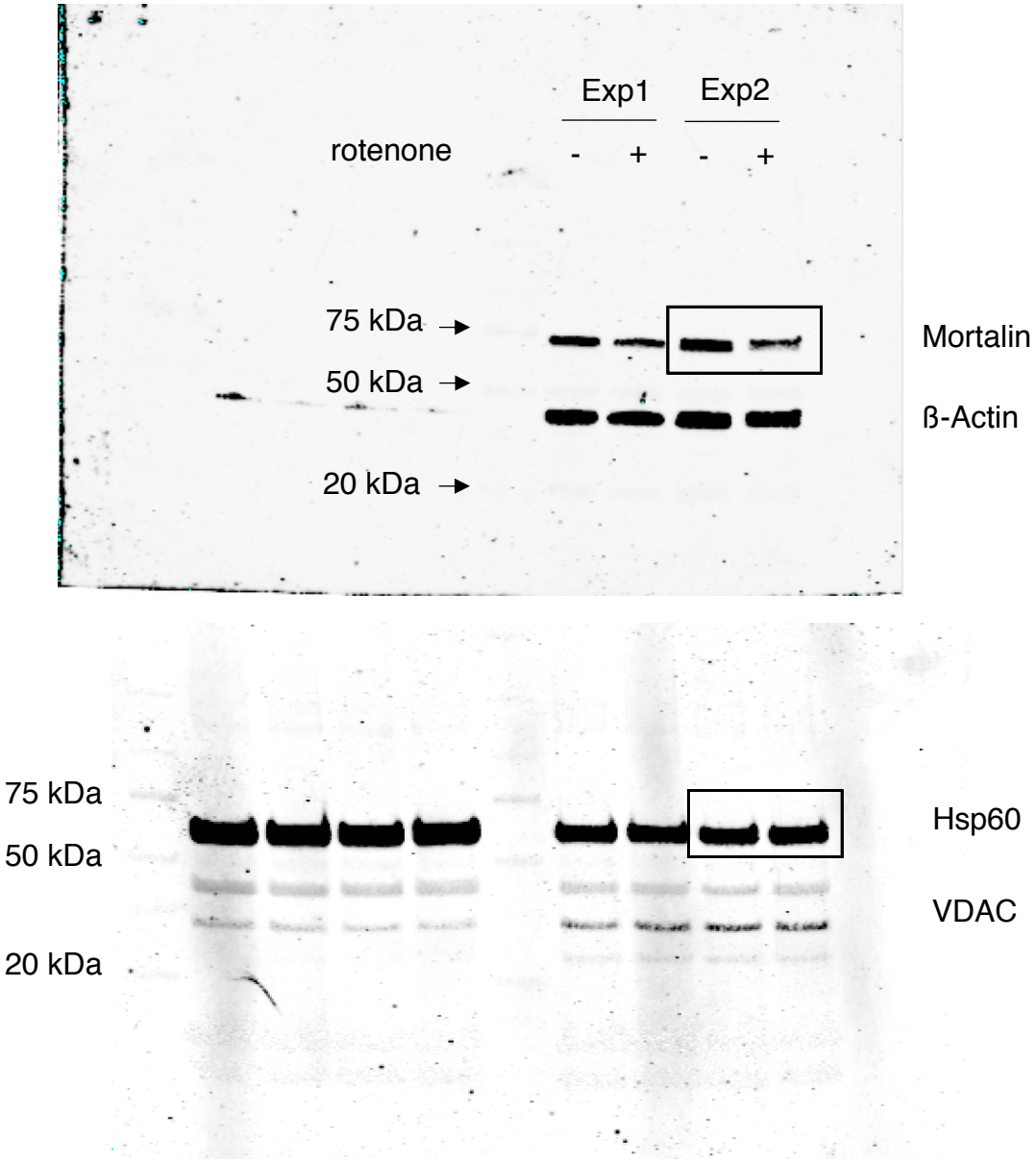
SUPPLEMENTAL METHOD

Measure of mitochondrial membrane potential.

To assess the impact of rotenone and FCCP treatments on mitochondrial respiratory function, neurons were stained with JC-1 dye (2 μM in HBSS; Abcam) following recommendations of the manufacturer. JC-1 mitochondrial aggregation was evaluated through the quantification of fluorescence emission at 530 nm (green) and 590 nm (red), following an excitation at 485 nm using a VarioSkan apparatus (Flash Multimode Reader, Thermo Scientific). The red-to-green ratio was used as an indicator of the mitochondrial membrane potential ($\Delta\Psi m$).

SUPPLEMENTAL FIGURES

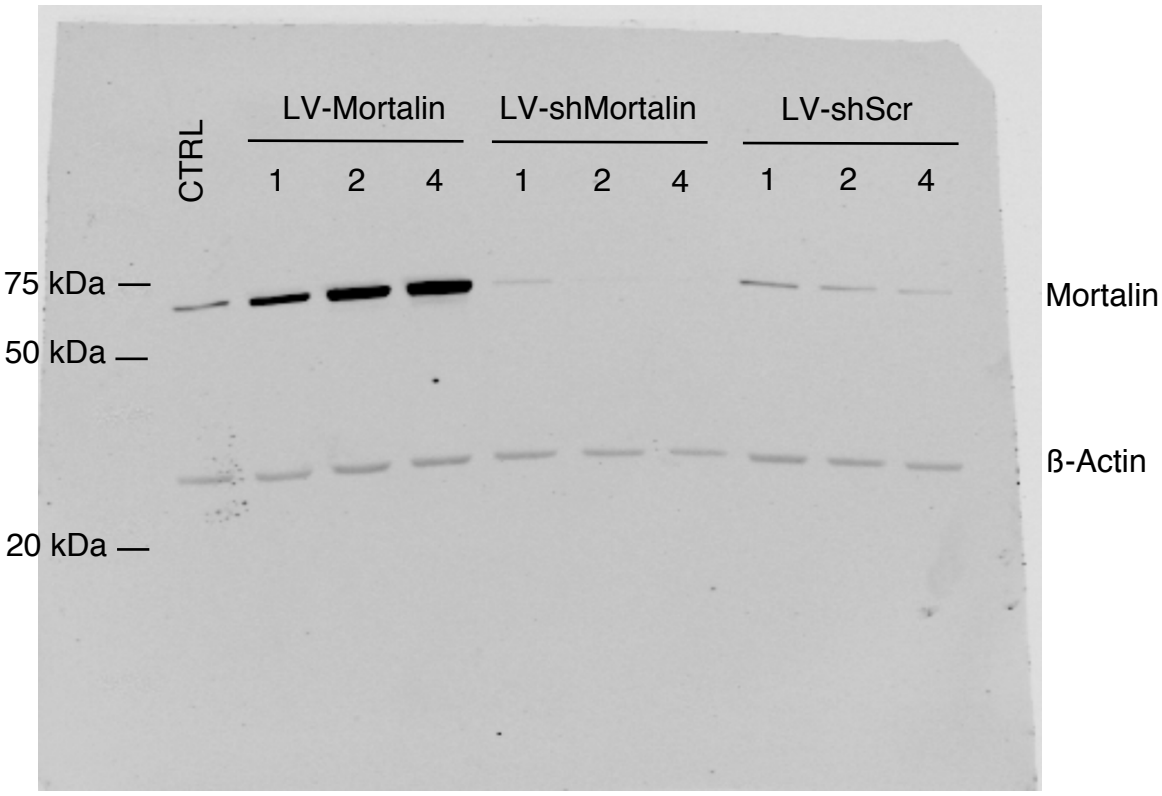
<u>Sup Figure 1</u>: Full western-blots used for Fig 1A



Quantification of Mortalin in mitochondria enriched fractions of cortical neurons with (+) or without (-) rotenone treatment (10nM, 4h).

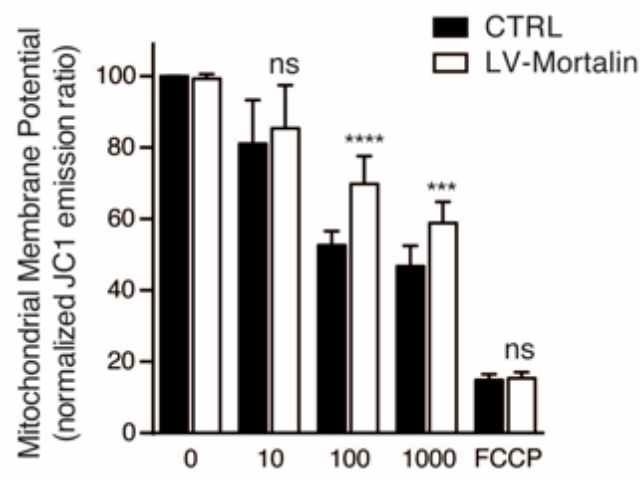
Both ß-Actin and the mitochondrial chaperone Hsp60 were used as loading controls. Staining was performed on 2 different blots because Mortalin and Hsp60 migrate close to each other. The blot stained with Hsp60 antibody was also stained with a VDAC antibody that was not analyzed further in this study. Molecular weight markers are pointed out for each blot. The elements used for Figure 1 are shown in boxes.

<u>Sup Figure 2</u>: Full western-blots used for Fig 1B



Full blot scan of Mortalin quantifications after lentiviral vectors driven over-expression (LV-Mortalin) or down-regulation (LV-shMortalin), using different MOI (1,2,4). A lentiviral vector expressing a scramble sequence of shMortalin (LV-shScr) was used as a control of RNA interference. ß-Actin was used as loading control. Molecular weight markers visible on the membrane are pointed out.

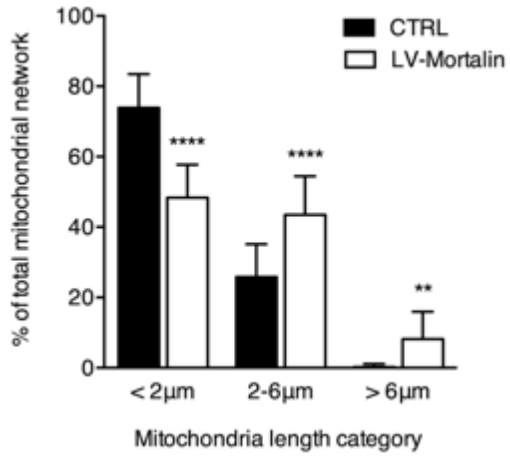
Sup Figure 3



Mortalin overexpression allows to maintain mitochondrial membrane potential in oxidative stress conditions.

Rat embryonic primary cortical neurons were transduced on DIV 3 with lentiviral vectors (LV) to induce overexpression of Mortalin (+100 %, LV-Mortalin). Neurons were then treated or not with rotenone at 10, 100 or 1000 nM for 4 hours, and the mitochondrial membrane potential was assessed by JC-1 staining, followed by measurements of red-to-green fluorescence ratios. These ratios were normalized to control, non-treated neurons. Each experimental case was performed in triplicate and results are presented as means ± SD of 3 independent experiments. *** $p<0.01$, **** $p<0.001$, by Mann-Whitney non parametric t-test.

Sup Figure 4



Mortalin overexpression allows to maintain mitochondrial morphology in oxidative stress conditions.

Rat embryonic primary cortical neurons were transduced (or not) on DIV 3 with lentiviral vectors (LV) to induce over-expression of Mortalin. On DIV 12, neurons were treated with 10 nM rotenone for 4 hours, fixed and both neuronal and mitochondrial networks were visualized by staining with, respectively, Tom20 and βIII-tubulin (βIII-Tub). Pictures were randomly taken and mitochondrial lengths were measured in all neural extensions. For each neuron, the sum of the lengths of all mitochondria was calculated (mitochondrial network), within which the relative proportions of short (<2µm), medium (2-6µm) and long (>6µm) mitochondria were considered. The graph represents means ± SD of 23 (Ctrl) and 28 (LV-Mortalin) neuronal mitochondrial networks from 3 independent neuronal preparations.

** $p<0.1$, **** $p<0.001$, by Mann-Whitney non parametric t-test.