Molecular Neurobiology

Trimetazidine ameliorates mitochondrial dysfunction in Amyotrophic Lateral Sclerosis SOD1^{G93A} cell models via autophagy activation.

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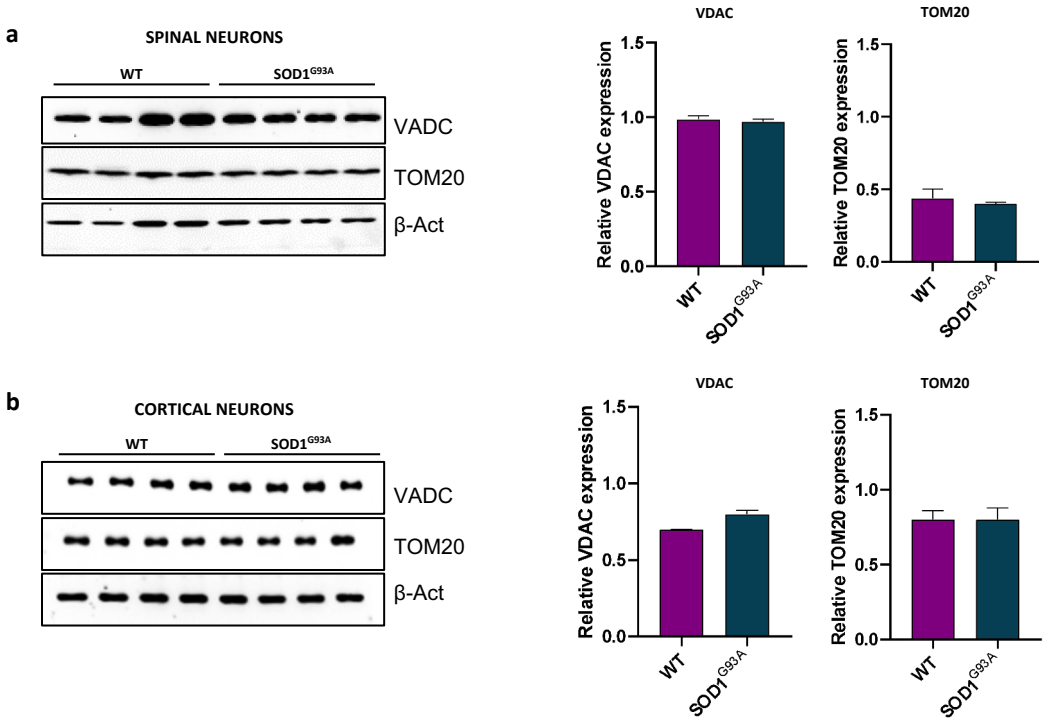

Fig. S1 (a) Representative WB (right) and quantification (left) of VDAC and TOM20 expression in primary spinal neurons wild-type (WT) and SOD1^{G93A} mice. Proteins expression was normalized using β-Actina and represented by arbitrary units. **(b)** Representative WB and quantification as in (a) in primary cortical neurons wild-type (WT) and SOD1^{G93A} mice. Data are presented as mean ± SEM. Statistical analysis was conducted using Student's t test.

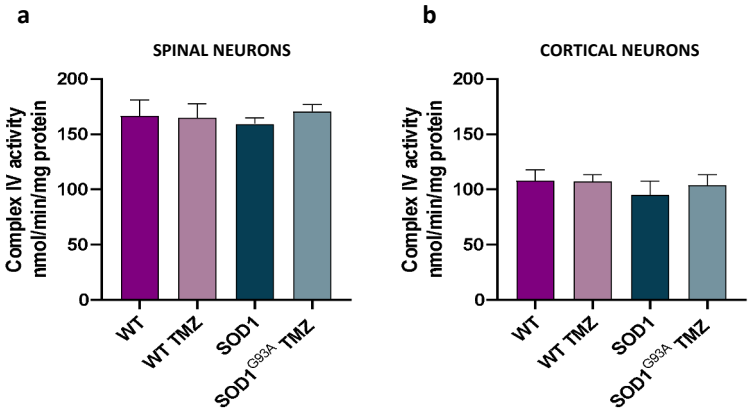

Fig. S2 Representative histograms showing the activity of electron transport chain complex IV in total extract from primary spinal cord **(a)** and cortical **(b)** cell cultures obtained from WT and SOD1^{G93A} mice untreated or treated overnight (ON) with 10μM of Trimetazidine (TMZ). Values are expressed as nmol/min/mg protein, normalised by citrate synthase activity and reported as mean ± S.D. from three independent experiments with each sample in quadruplicate.

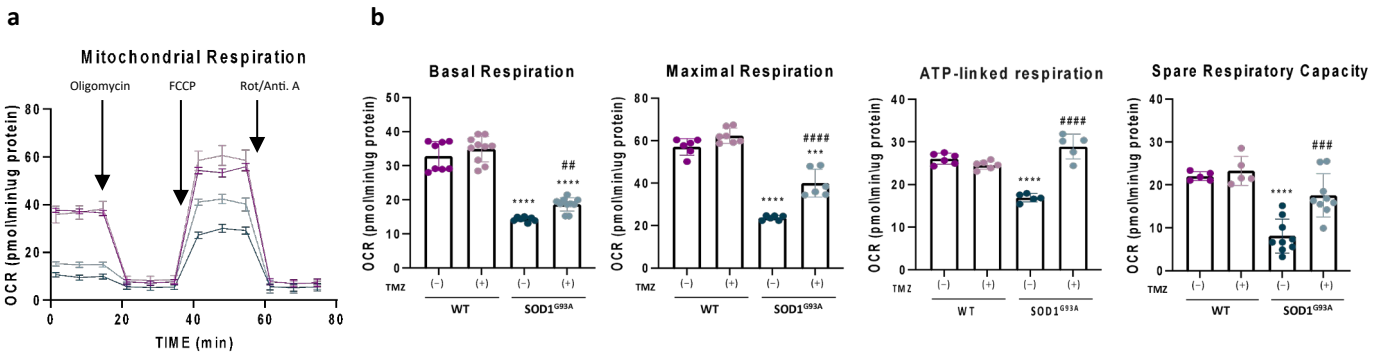

Fig. S3 Trimetazidine treatment restores mitochondrial performances of NSC-34 SOD1^{G93A} cell lines.
(a) Representative profile of measurements of the rate of oxygen consumption ratio (OCR) in NSC-34 cell lines constitutively expressing SOD1 wild type (WT) or mutated (SOD1^{G93A}), untreated or treated with 10 µM TMZ as indicated. **(b)** The histograms show individual parameters for basal respiration, maximal respiration, ATP-linked respiration and spare respiratory capacity, as indicated. Data are presented as means ± SEM, ***p < 0.001, ****p< 0.0001 compared with wild type; ##p < 0.01, ###p < 0.001, ####p < 0.001 compared with untreated SOD1^{G93A}, n = ≥ 3 per group. p values were obtained using parametric one-way ANOVA with Bonferroni post hoc test.

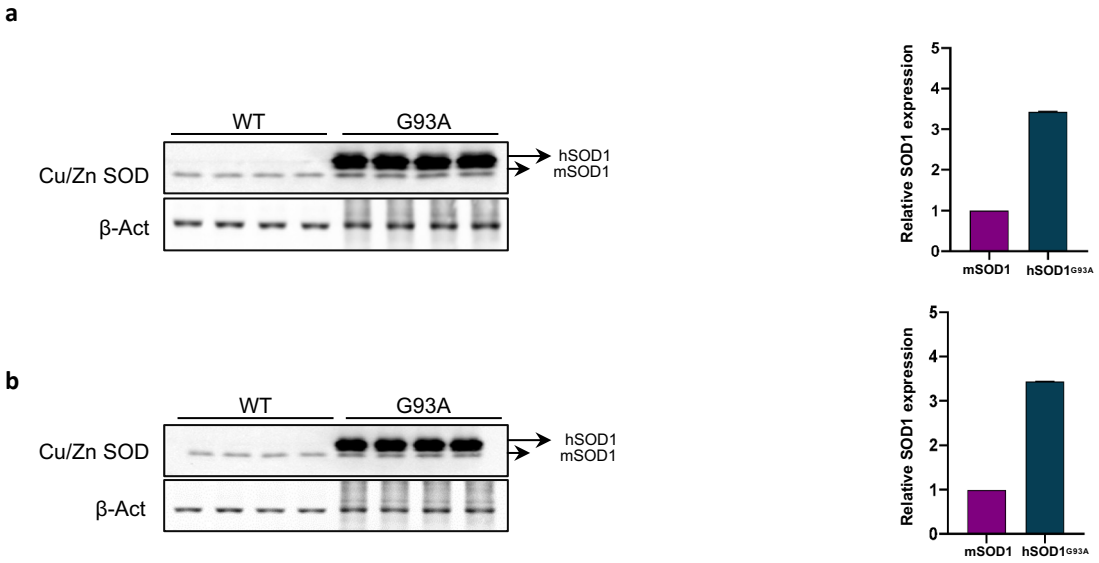

Fig. S4 Representative WB of murine SOD1 (mSOD1) and human SOD1 (hSOD1) expression in primary spinal **(a)** and cortica **(b)** neurons (left) in wild-type (WT) and SOD1^{G93A} (G93A) mice and relative quantification (right) represented by arbitrary units. Data are presented as mean ± SEM. ****p < 0.0001 compared with mSOD1. P values were obtained using Student's t test.

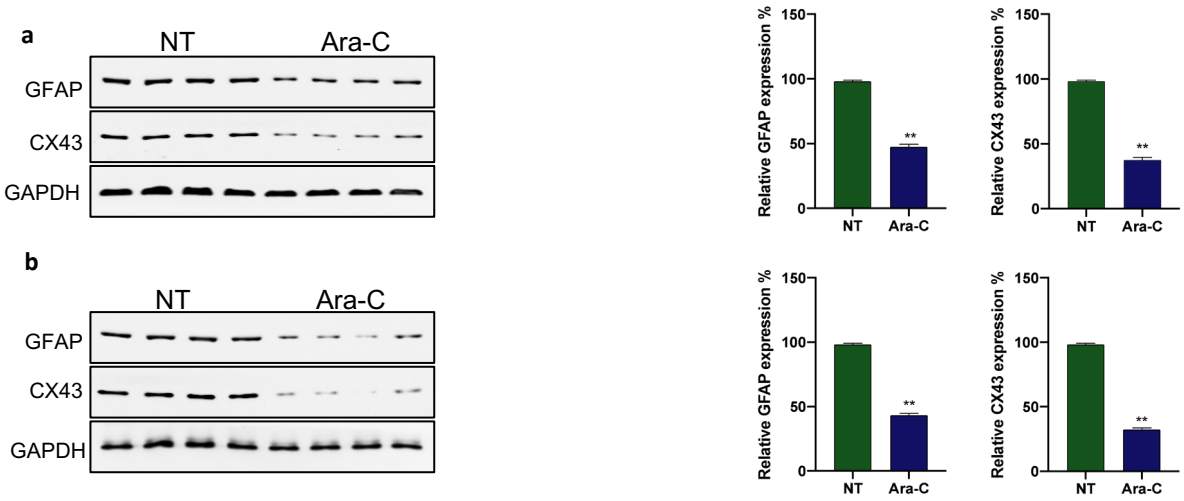

Fig. S5 (a) Representative WB (right) and quantification (left) of GFAP and CX43 in primary spinal neurons untreated (NT) and treated with the astroglial proliferation inhibitor (Ara-C). Proteins expression was normalized using GAPDH. **(b)** Representative WB and quantification as in (a) in primary cortical neurons untreated (NT). Data are presented as mean ± SEM, **p < 0.01 compared with NT. P values were obtained using Student's t test.