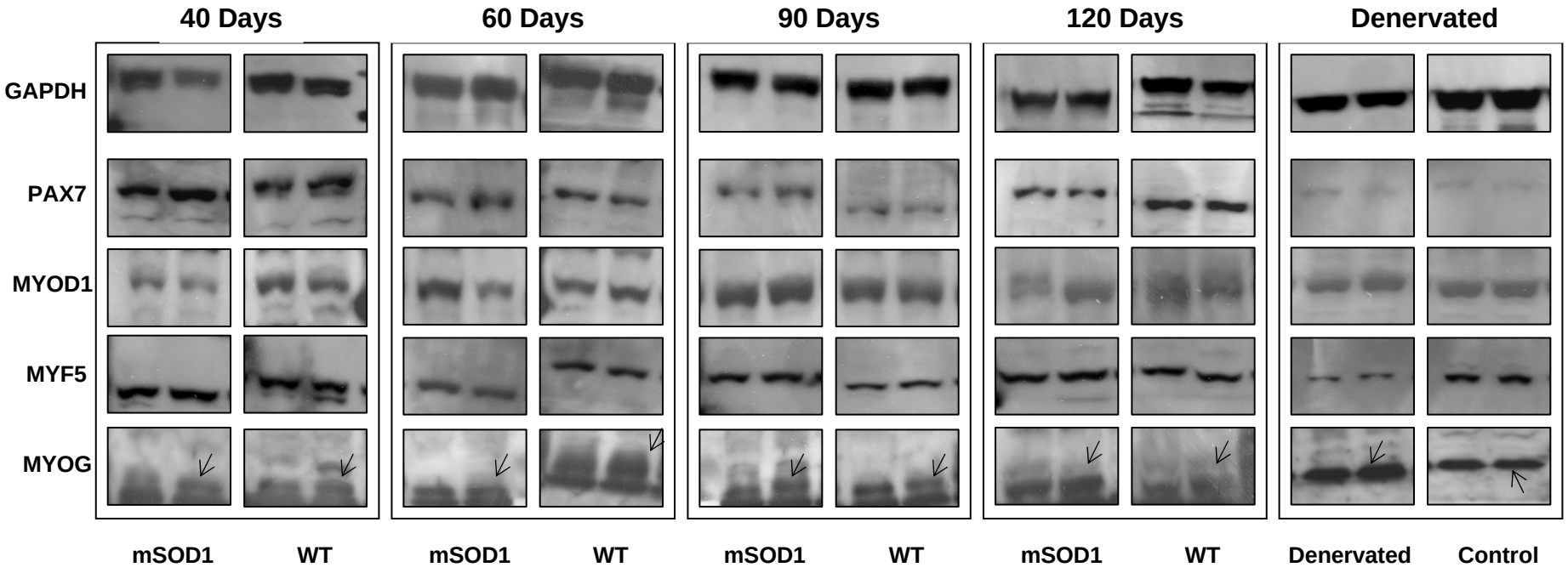
Supplementary Figure 1 (Manzano et al., 2010)



Supplementary Figure 1 Representative western blots from each antibody used. SDS-PAGE Separated protein extracts from two SOD1-G93A (mSOD1) and wild type (WT) non-transgenic littermates are show for each age. The arrow on MYOG blots indicated the 32 kDA band used to quantify MYOG levels (see Supplementary Materials and methods for details).

Supplementary Table 1.

Supplementary Table 1 The list and properties of TaqMan probes used for the quantitative gene expression studies.

Target	**Assay ID[$]**	**Length***	**$R^{2\wedge}$**	**Slope#**	**Ex[&]**
Rn18S	4352930E	187 bp	1.0000	-3.4744	0.940
Gapdh	4352932E	107 bp	0.9777	-3.3763	0.978
Actb	4352933E	115 bp	0.9981	-3.4636	0.944
Pax7	Mm00834079_m1	64 bp	0.9986	-3.1442	1.079
Myod1	Mm00440387_m1	86 bp	0.9848	-3.2183	1.045
Myog	Mm00446194_m1	69 bp	0.9789	-3.1077	1.098
Myf5	Mm00435125_m1	71 bp	0.9927	-3.0299	1.138
Rrad	Mm00451053_m1	71 bp	0. 9938	-3.3963	0.970
Chrna1	Mm00431627_m1	67 bp	0.9951	-3.3974	0.995

[$] TaqMan Gene expression Assays identification number, * Amplicon length in base pairs, ^Coefficient of correlation, # Slope of Ct against 4-log dilution range [&] Amplification efficiency.

Supplementary materials and methods

Time course of the disease progression in mSOD1 mice. Please see the references listed at the end of the supplement. Some molecular markers of the disease in SOD1-G93A mice, including muscle mSOD1 aggregation, are observed as early as 30-40 days of age [1-4]. Loss of MN terminals commence at pre-symptomatic age of 40-60 days, those of fast muscles being first affected [5-9]. This is accompanied with decreased fast muscle contractile force and fiber cross-sectional area [1,10]. Clinical symptoms in SOD1-G93A mice, such as weakness, tremors and muscle wasting start to develop at about 90 days of age at which stage also slow muscle contractile force and ventral root neurons are affected [1,6,7,11,12]. At this stage, elevated levels of denervation marker Chrna1 has been described [13]. Hind-limb paralysis commences at about 120 days of age at which stage both fast and slow muscle fiber diameter is reduced and denervation is severe [1]. The SOD1-G93A become terminally paralysed at 4-5 months of age [11,12].

Calculations of relative gene expression. Relative gene expression was determined using the $2^{-\Delta\Delta CT}$ method where the data are presented as a fold-change in gene expression normalized to the reference genes and relative to the non-transgenic age-matched calibrator. Briefly, the difference between the cycle threshold values (ΔCt) for the gene of interest (goi) and the geometric mean of the reference Ct-values for the same sample ($\Delta Ct = Ct_{goi} - Ct_{geom.\ mean\ of\ references}$) was calculated first for each mutant (n=5 per age) and age-matched wild type sample (n=5 per age). To obtain the difference (ΔΔCt) between the wild type (non-transgenic) calibrator and the mSOD1 samples, the mean of the ΔCt values of the wild type samples were subtracted from the mean ΔCt of the mSOD1 samples ($\Delta\Delta Ct$= Mean ΔCt_{mutant} - Mean ΔCt_{wt}). Because ΔCt is an exponential term, the linear difference in the expression was finally obtained by conversion to $2^{(-\Delta\Delta CT)}$. We designed the experiment to include calibrators and SOD1-G93A animals of one age in a single 96-well plate to exclude inter-run variations within each time point.

Western blot. PAX7 and MYOD displayed expected molecular weight of 60 kDa and 45 kDa, respectively. MYOG and MYF5 showed bands of approximately 32 and 35 kDa, respectively, when their expected sizes were 36 and 32 kDa.

Supplementary References

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