SUPPLEMENTARY METHODS

Behavioural analyses

Starting from the age of 84 days, mice were tested once a week for motor behavioural deficits by the grip test. Animals were posed on a horizontal grip; the grip was then inverted and the time (sec) at which the animal fell from the grip was recorded for a maximum of 90 sec. Animals performed three trials and the time of the best trial was considered for the statistical analysis. Body weight of each animal was recorded before the test. Time of survival was detected as previously described (Peviani, 2007).

For the statistical analyses, the result of each group of animals at each test session was expressed as mean ± standard error (S.E.). Missing data, due to death of animals, were taken into account in the statistic analysis as follows: when an animal died, its performance was considered equal to the last recorded value for all subsequent test sessions, until the death of the last animal of the group. The patterns in the progression of motor deficits and body weight were evaluated by a repeated measure two-way ANOVA. The survival of SOD1G93A and GFP1/SOD1G93A mice was analyzed by the log-rank test.

Table 1

Gene	Accession n°	TaqMan Gene expression assay
β-actin (Actb)	NM_007393	4352341E
GFAP	NM_010277	Mm00546086_m1
CD68	NM_009853	Mm00839636_g1
TNF α	NM_013693	Mm00546086_m1

Table 2

Gene Accession	n°	Forward (5'_ 3')	Reverse (5'- 3')
GFP	M62654	CAT GCC CGAAGG CTA CGT	GCT TGT GCC CCA GGA TGT
β-actin (Actb)	NM_007393	GCCCTGAGGCTCTTTTCCAG	TGCCACAGGATTCCATACCC
β 1 subunit of 20S (Psmb1)	NM_011185	TAATTGGCTGCAGTGGTTTCC	AAGCGCCGTGAGTACAGGAT

β 2 subunit of 20S (Psmb2)	NM_011970	GATGAAGGACGATCATGACAAGAT	TGGGAGACAATTCATATCCATTCC
β 5 subunit of 20S (Psmb5)	NM_011186	CGCAGCAGCCTCCAAACT	GAAGGCGGTCCCAGAGATC
LMP2 subunit of 20S (Psmb9)	NM_013585	TAGCTGACATGGCCGCCTA	TGGTCCCAGCCAGCTACTATG
LMP7 subunit of 20S (Psmb8)	NM_010724	AAGGATGAACAAAGTGATCGAGATT	TGCTGCAGACACGGAGATG
LMP10 subunit of 20S (Pmsb10)	NM_013640	GGAACCCACAGGAGGCTTCT	GTCCGCTCCCAGGATGACT
α5 subunit of 20S (Psma5)	NM_011967	AGTCCTCGCTCATCATCCTCA	ACGGCTCCTTCTTAAATGTCCTT
S1 subunit of 19S (Psmd1)	NM_027357	TGTTGGATAATCCAGCACGAGT	GGTTCCACGAGTTCTTCAACGT
α Subunit of 11S (Psme1)	NM_011189	GGCTTCCACACGCAGATCTC	TCTCCATGACCATCAGACGG
POMP	NM_025624	GTGTGCTCAGCATAGTTGCCTG	AGCTTTGCTATAACCTCAGAACCAC
CD8 a chain (Cd8a)	NM_001081110	CTATTCAAACCACTGCCGCAG	CCGAGGTTCGACCTGACATTAC

LEGENDS TO SUPPLEMENTARY FIGURES

Fig. S1 Representative microphotographs of GFP immunostaining in the ventral (a, c, e) and dorsal horns (b, d, f) of the lumbar spinal cord of NTg (a, b), GFP1 (c, d) and GFP2 (e, f) mice. Scale bar 100 μm. A low background staining appears in NTg and GFP2 sections, while higher immunoreactivity occurs in GFP1 mice.

Fig. S2 Behavioural analysis of body weight (A) and grip test (B) of SOD1G93A (n=13) compared to GFP1/SOD1G93A mice (n=13) shows no changes in the progression of disease. Each point represents the mean ± S.E. Statistical analysis was done by two-way ANOVA for repeated measures. Panel C compares the survival curves for SOD1G93A and GFP1/SOD1G93A animals. Log-rank analysis of probabilities showed no significant difference.

SUPPLEMENTARY FIGURES

Fig S1

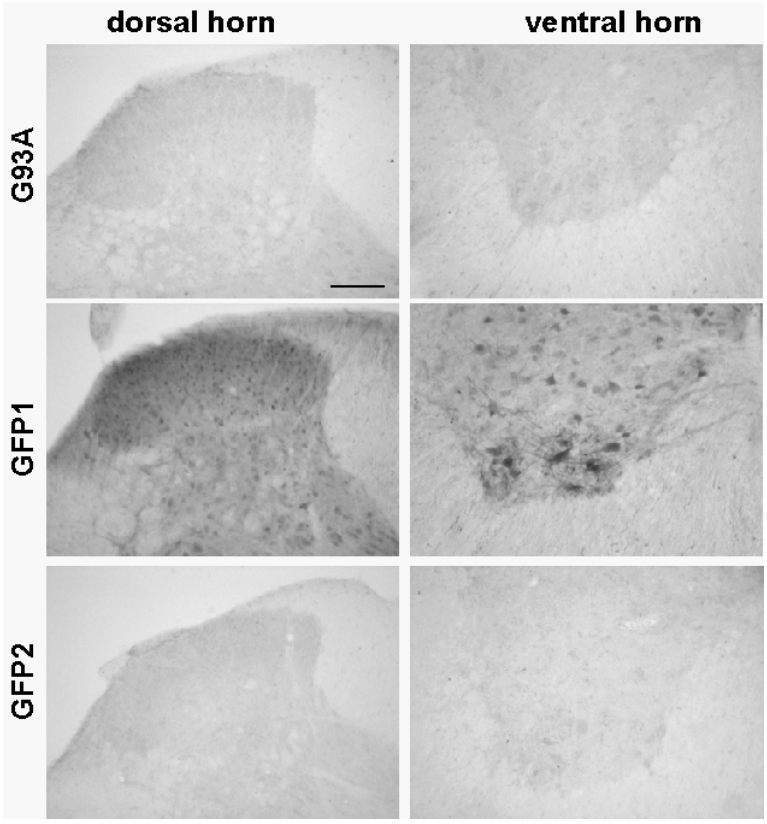
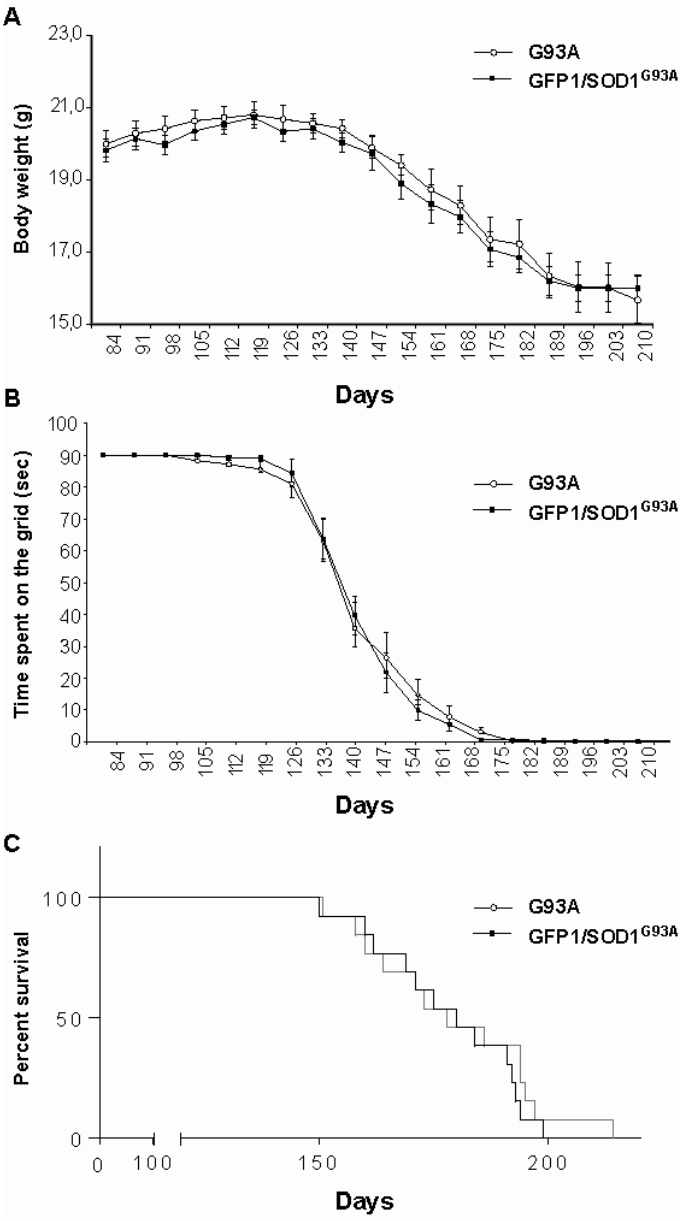


Fig S2


A
Body weight (g)
23,0
21,0
19,0
17,0
15,0
G93A
GFP1/SOD1G93A
84 91 98 105 112 119 126 133 140 147 154 161 168 175 182 189 196 203 210
Days
B
Time spent on the grid (sec)
100 90 80 70 60 50 40 30 20 10 0
G93A
GFP1/SOD1G93A
84 91 98 105 112 119 126 133 140 147 154 161 168 175 182 189 196 203 210
Days
C
Percent survival
100
50
0
G93A
GFP1/SOD1G93A
0 100 150 200
Days