Supplementary Information

Supplementary Figure Legends

Fig. S1 NRIP expression in the spinal cord and skeletal muscles at 4 and 6 weeks of age in WT and SOD1 G93A mice. (**A**) NRIP expression in the spinal cord at 4 weeks of age in WT and SOD1 G93A mice. Total proteins from L3-L5 spinal cord were subjected to Western blot (WB) analysis for NRIP expression, with GAPDH serving as the loading control. The right panel shows the quantification of NRIP expression conducted through densitometry analysis. (**B**) NRIP expression in the gastrocnemius (GAS) muscles at 4 weeks of age in WT and SOD1 G93A mice. The right panel illustrates the quantification. (**C**) NRIP expression in the tibialis anterior (TA) muscles at 4 weeks of age in WT and SOD1 G93A mice. The right panel displays the quantification. (**D**) NRIP expression in the spinal cord at 6 weeks of age in WT and SOD1 G93A mice. The right panel represents the quantification of NRIP expression. (**E**) NRIP expression in the gastrocnemius (GAS) muscles at 6 weeks of age in WT and SOD1 G93A mice. The right panel illustrates the quantification. (**F**) NRIP expression in the tibialis anterior (TA) muscles at 6 weeks of age in WT and SOD1 G93A mice. The right panel shows the quantification. Each group consisted of N = 3 mice for both WT and SOD1 G93A. Data are presented as mean ± SEM. Statistical analysis was conducted using the Student t-test. $*P < 0.05$ and $**P < 0.01$.

Fig. S2 Immunofluorescence analysis of AAV-GFP expression in the spinal cord of WT mice via intramuscular injection. The analysis was conducted on both control WT mice (without AAV-GFP treatment) and WT mice administered with AAV-GFP through intramuscular injection. The spinal cord was dissected into cervical, thoracic, and lumbar segments. Each segment was co-stained with anti-GFP (green, indicating GFP expression) and anti-NeuN (red, a neuron marker) antibodies. DAPI was used as the nuclear stain. Scale bars: 100 μm.

Fig. S3 Transgene copy number determination in SOD1 G93A mice treated with AAV-NRIP and AAV-GFP. The transgene copy number in SOD1 G93A mice treated with AAV-NRIP and AAV-GFP was determined by real-time PCR. The ΔCt (cycle threshold) of transgene copy number was assessed by comparing a reference gene (mouse *apob*) with the transgene (human *SOD1)*. The AAV-GFP group comprised 3 mice, and the AAV-NRIP group comprised 4 mice. Data are presented as mean ± SEM. Statistical analysis was performed using the Student t-test. ns, no significance.

Fig. S4 Total distance moved and rearing frequency of SOD1 G93A mice with AAV-GFP or AAV-NRIP treatment in males and females. (**A**) The total distance moved by SOD1 G93A mice with AAV-GFP or AAV-NRIP treatment was recorded from 100 to 147 days using EthoVision Video Tracking Software (Noldus) during a 10-minute test. The total distance traveled in the box was measured. In the male group, data are presented as mean ± SEM. **P* =0.045 at the age of 126 days; ns, no significance; one-way ANOVA with Tukey's post hoc test. In the female group, data are presented as mean ± SEM. **P* =0.0496 at the age of 120 days; ns, no significance; one-way ANOVA with Tukey's post hoc test. (**B**) Rearing frequency, indicating the frequency of mice standing on their hindlimbs in the box, was recorded from 100 to 147 days. In the male group, data are presented as mean ± SEM. **P* =0.0166 at the age of 126 days; ns, no significance; one-way ANOVA with Tukey's post hoc test. In the female group, data are presented as mean ± SEM. **P* =0.0381 at the age of 120 days; **P* =0.0429 at the age of 133 days; ns, no significance; one-way ANOVA with Tukey's post hoc test.

Fig. S5 Therapeutic effects of AAV-NRIP on rotarod performance and grip force via intramuscular injection in SOD1 G93A mice. (**A**) Evaluation of AAV-NRIP efficacy in rotarod performance of SOD1 G93A mice at the age of 120 days. Mice were placed on a rotating rod set at 20 rpm, and the time until falling was recorded. N=6 for each group. (**B**) Analysis of grip force. Forelimb strength was measured five times for each mouse at the age of 120 days. N=6 for each group. Data are presented as mean ± SEM. Statistical analysis was conducted using the Student t-test. ns, no significance. (**C**) Kaplan-Meier survival curves comparing AAV-NRIP-treated and AAV-GFP-treated SOD1 G93A mice. Statistical differences were analyzed using the Logrank test. ns, no significance.

Fig. S6 AAV-NRIP gene therapy improves ChAT and NeuN co-positive MN survival in SOD1 G93A mice. (**A**) Quantification of total $ChAT^+/NeuN^+$ MN numbers per spinal anterior horn in WT mice (N=4) and SOD1 G93A mice treated with AAV-GFP (N=4) and AAV-NRIP (N=4). Data are presented as mean ± SEM. ***P* =0.0026 (WT vs. SOD1 G93A+AAV-GFP); ***P* =0.0036 (SOD1 G93A+AAV-GFP vs. SOD1 G93A+AAV-NRIP); one-way ANOVA with Tukey's post hoc test. (**B**) Quantification of $ChAT^+/NeuN^+$ MN numbers with a cell size smaller than 500 μm^2 per spinal anterior horn in WT mice (N=4) and SOD1 G93A mice treated with AAV-GFP (N=4) and AAV-NRIP (N=4). Data are presented as mean ± SEM. Statistical analysis was conducted using the one-way ANOVA with Tukey's post hoc test. ns, no significance.

Figure S1

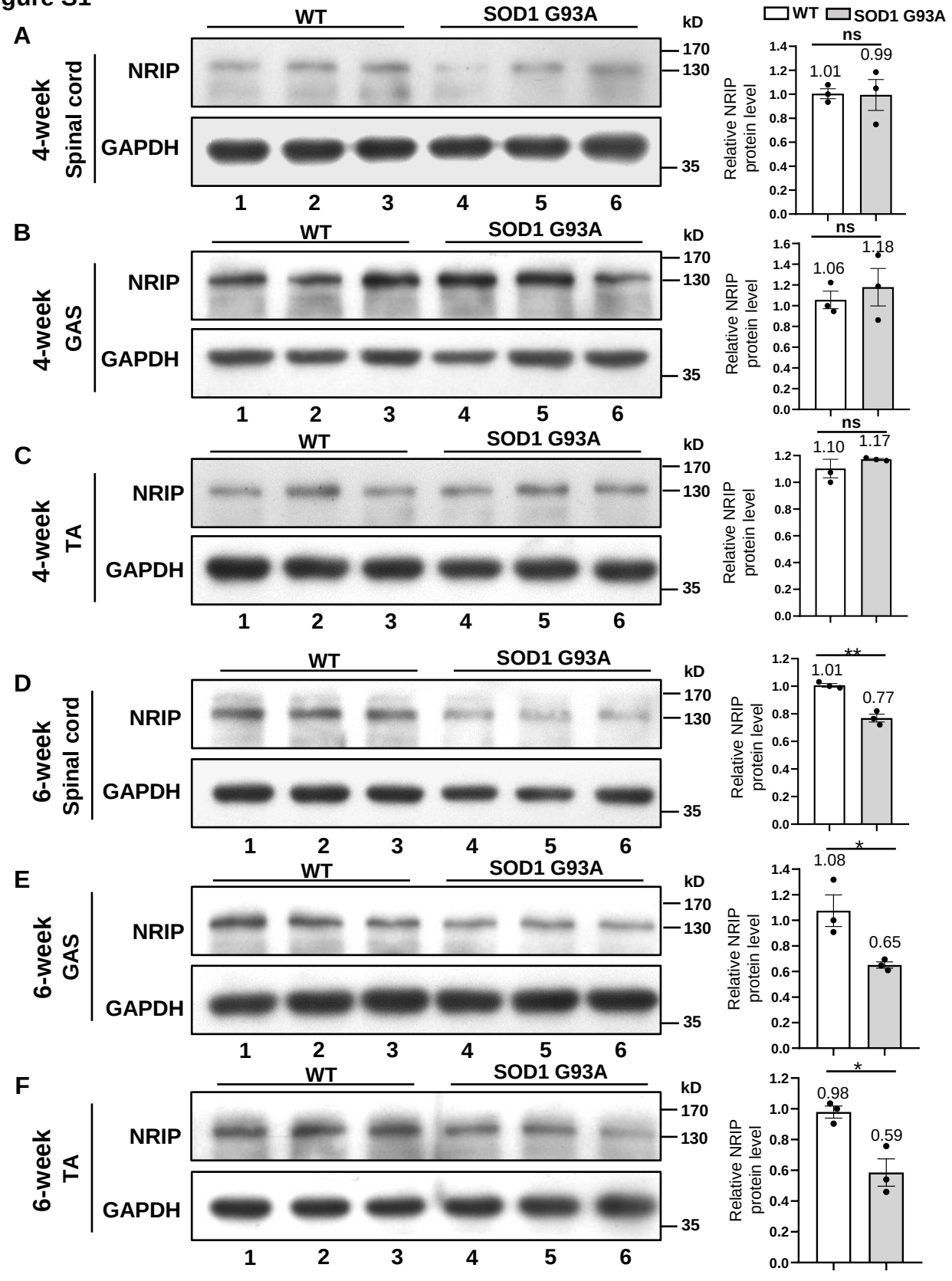

A
4-week Spinal cord
WT
SOD1 G93A
kD
NRIP
GAPDH
170
130
35
1 2 3 4 5 6
WT
SOD1 G93A
ns
1.01
0.99
Relative NRIP protein level
B
4-week GAS
1.06
1.18
C
4-week TA
1.10
1.17
D
6-week Spinal cord
**
1.01
0.77
E
6-week GAS
*
1.08
0.65
F
6-week TA
0.98
0.59

Figure S2

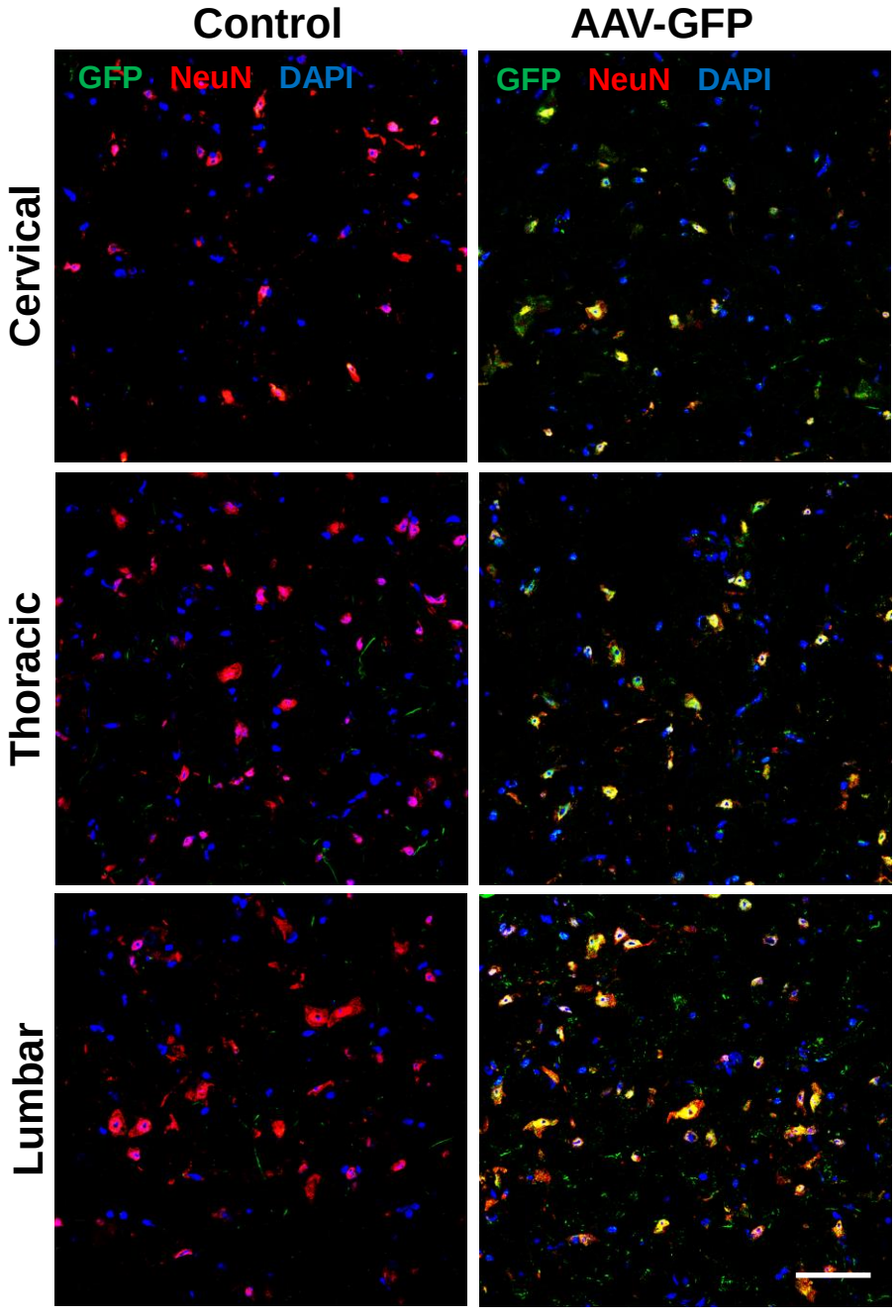

Control
AAV-GFP
GFP NeuN DAPI
GFP NeuN DAPI
Cervical
Thoracic
Lumbar

Figure S3

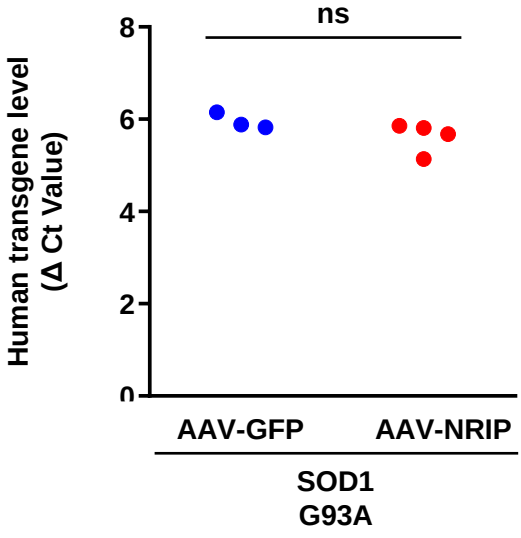

ns
8
6
4
2
0
Human transgene level
(Δ Ct Value)
AAV-GFP
AAV-NRIP
SOD1
G93A

Figure S4

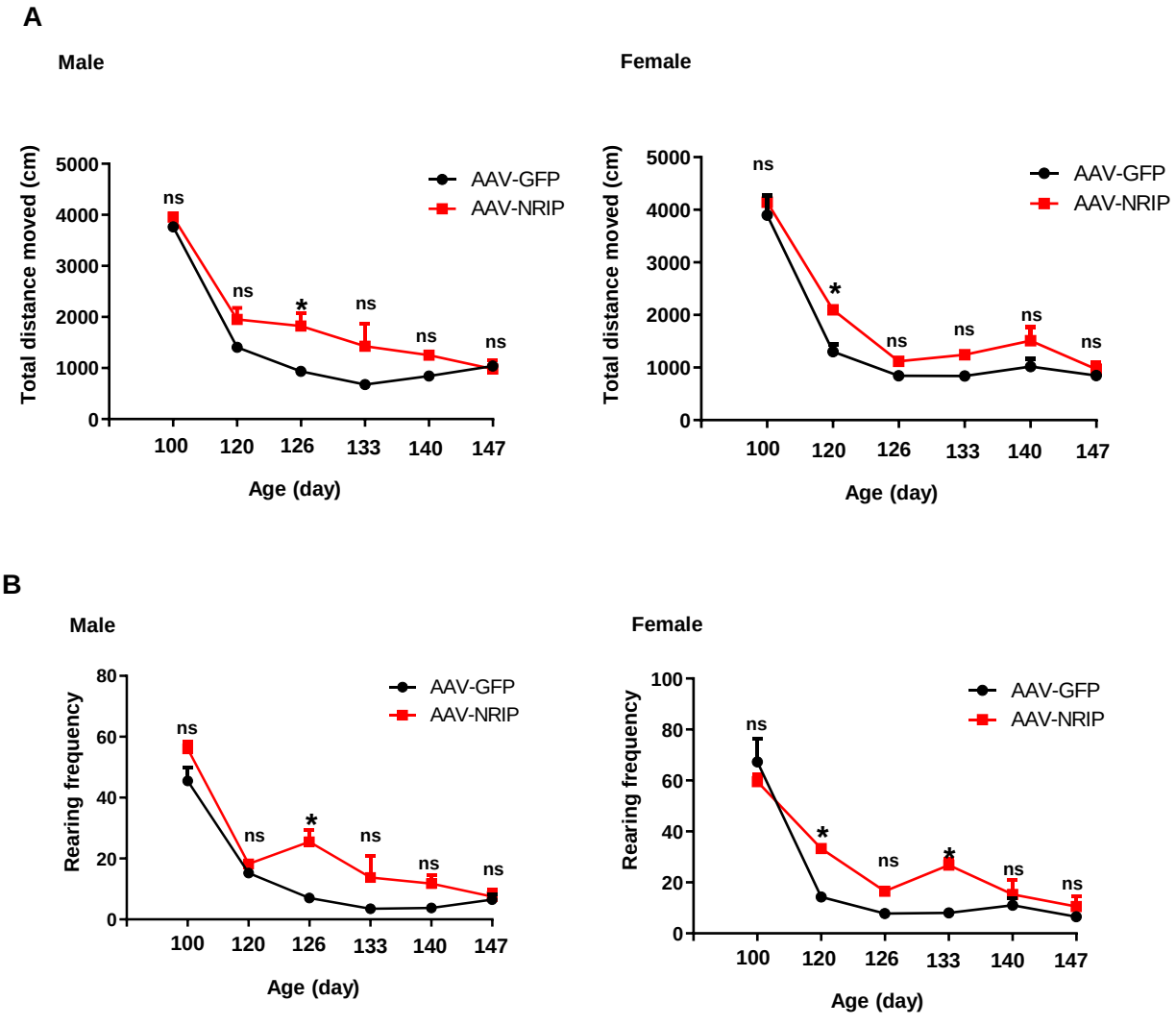

A
Male
Female
Total distance moved (cm)
5000
4000
3000
2000
1000
0
AAV-GFP
AAV-NRIP
ns
*
100
120
126
133
140
147
Age (day)
B
Male
Female
Rearing frequency
80
60
40
20
0
100

Figure S5

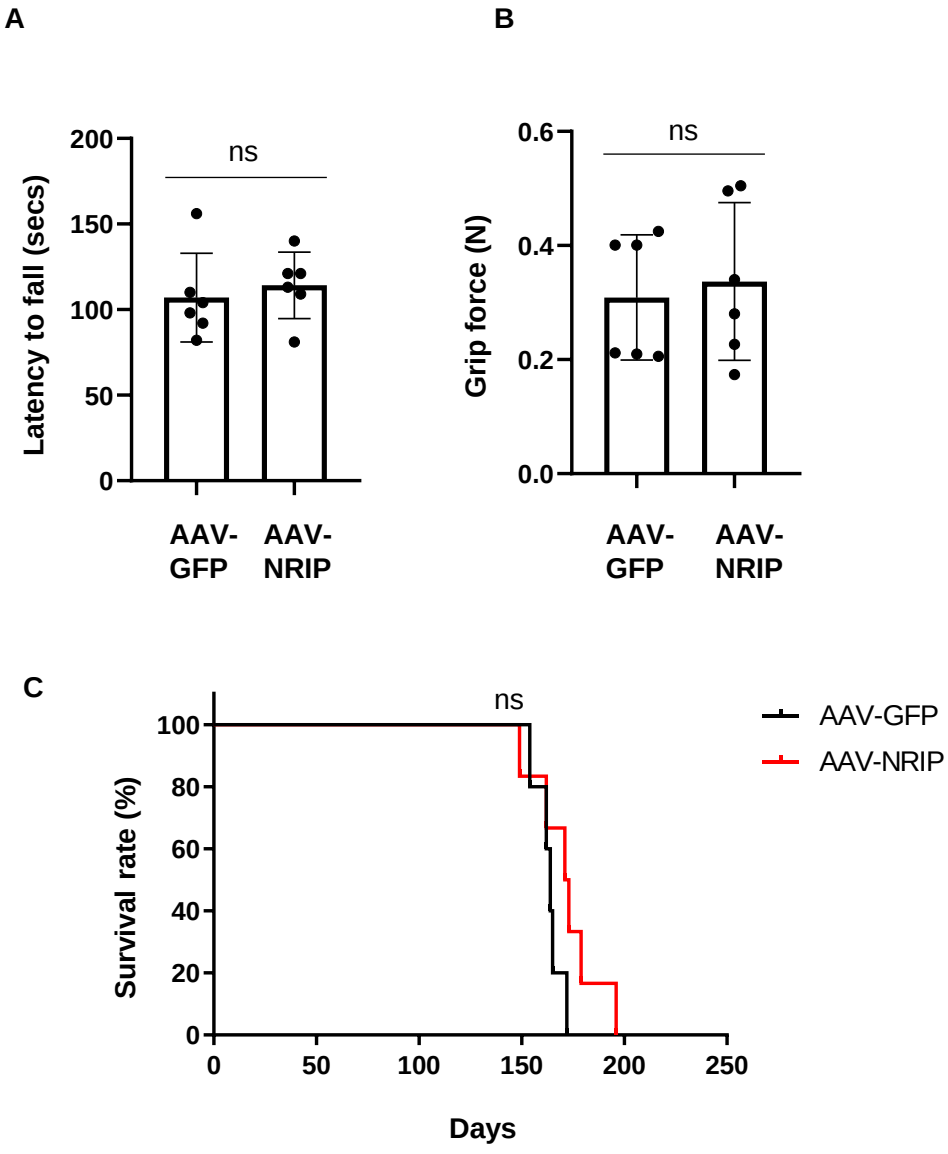
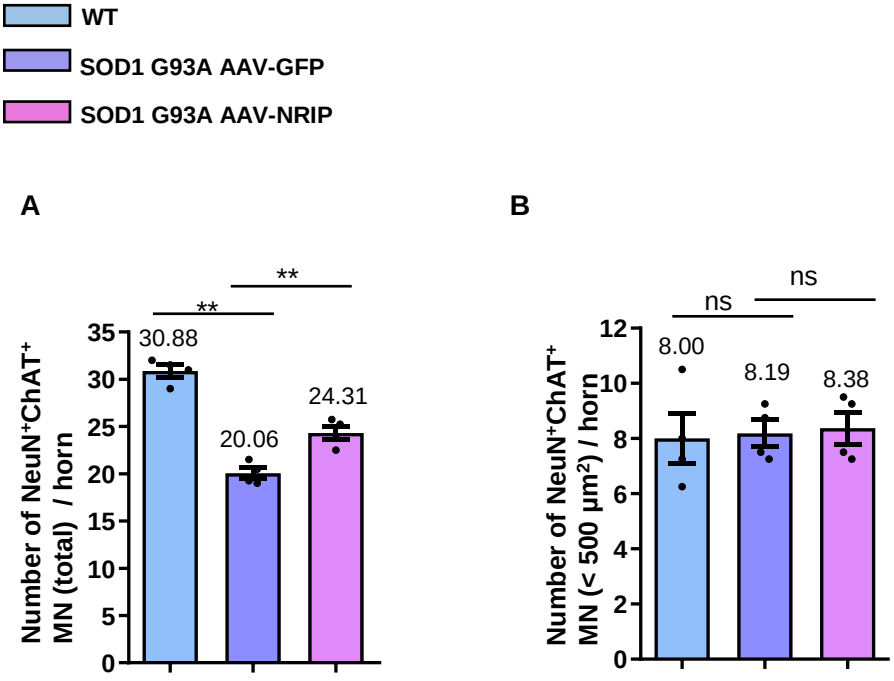

A
B
C
Latency to fall (secs)
200
150
100
50
0
ns
AAV-GFP
AAV-NRIP
Grip force (N)
0.6
0.4
0.2
0.0
ns
AAV-GFP
AAV-NRIP
Survival rate (%)
100
80
60
40
20
0
ns
0
50
100
150
200
250
Days
AAV-GFP
AAV-NRIP

Figure S6


WT
SOD1 G93A AAV-GFP
SOD1 G93A AAV-NRIP
A
B
**
**
30.88
20.06
24.31
Number of NeuN+ChAT+ MN (total) / horn
35
30
25
20
15
10
5
0
ns
ns
8.00
8.19
8.38
Number of NeuN+ChAT+ MN (< 500 µm2) / horn
12
10
8
6
4
2
0

Supplementary Information

Grayscale images of Fig.1D, Fig. 2C, Fig. 2D, Fig. 4A, Fig. 4D, Fig. 5A,Fig. 6A and Fig. S2

Figure 1D
Grayscale

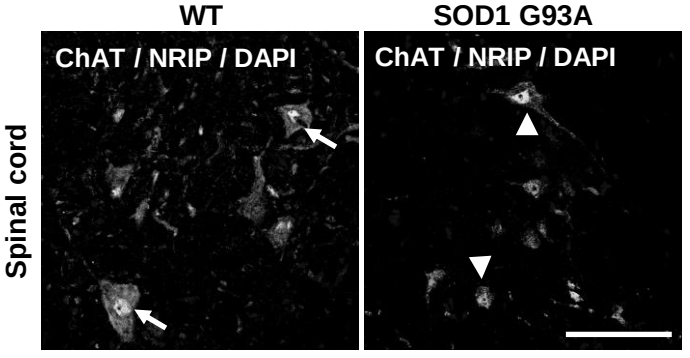


Figure 2C
Grayscale

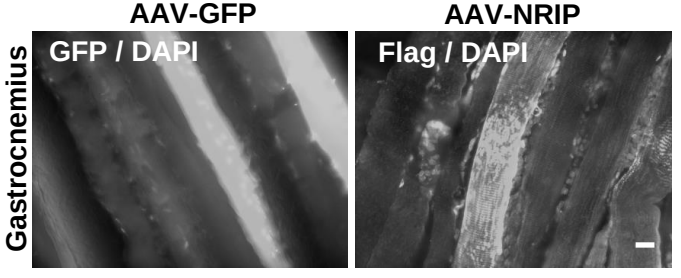


Figure 2D
Grayscale

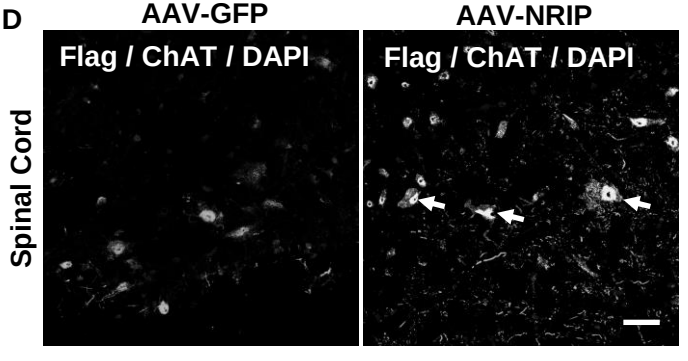


Figure 4A
Grayscale

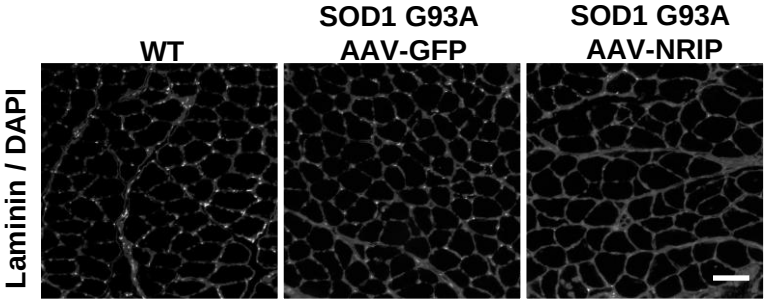


Figure 4D
Grayscale

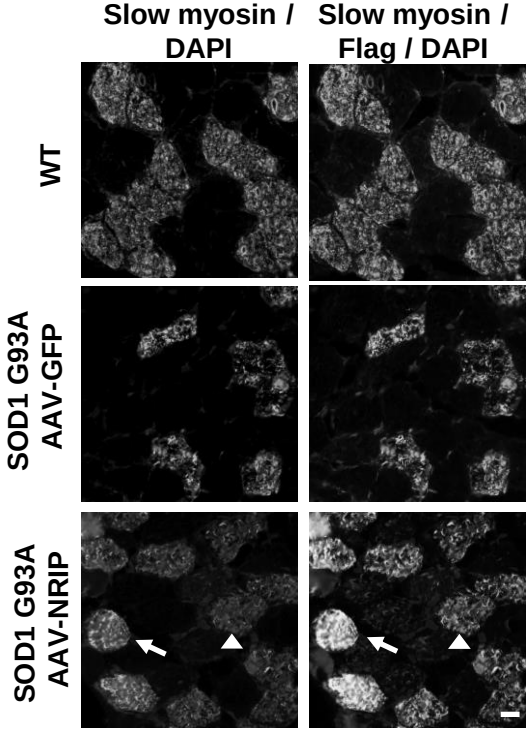


Figure 5A
Grayscale

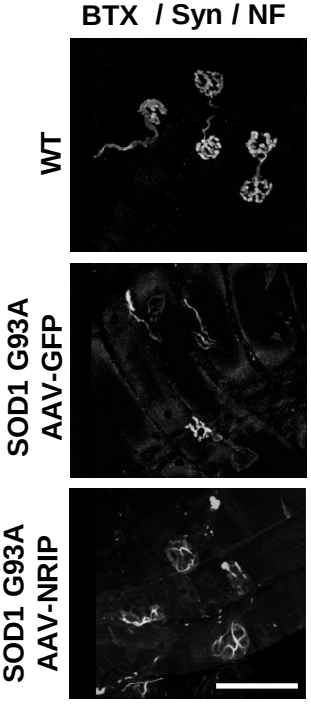


Figure 6A
Grayscale

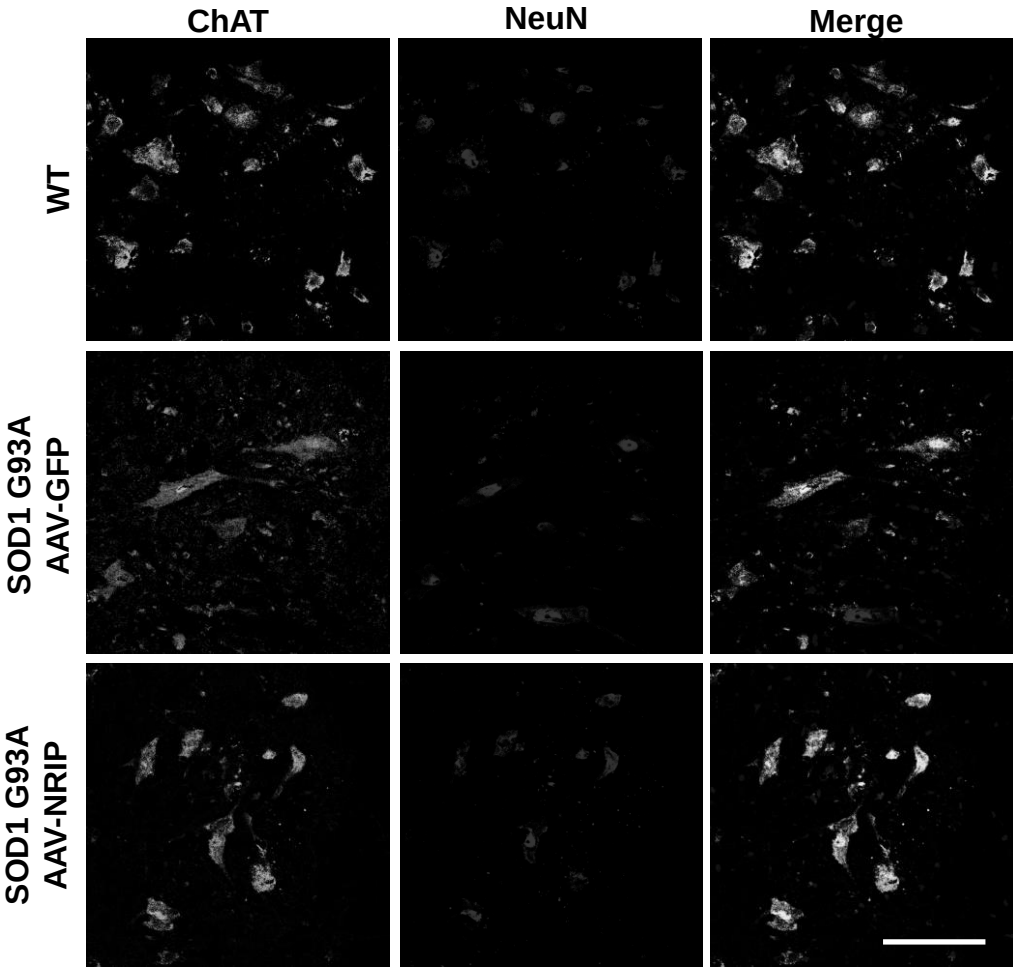


Figure S2
Grayscale