Figure EV1. Transduction of intravenously injected AAV-D7 is prominent in muscle, but only weakly and faintly detectable in spinal cord and brain, respectively, in ALS mice.

A–C Als mice treated with 1.2 × 10^{12} vg of AAV-D7 at P90 were analyzed at P120. The expression of Dok-7-EGFP protein was monitored by EGFP fluorescence (green) and nuclei were visualized by DAPI (blue). High transduction was observed in the indicated skeletal muscles (A), but weak or faint transduction was detectable in the ventral (faint) and dorsal (weak) horns of the spinal cord (B) and in the cerebellum (faint) (C). Scale bars, 100 μm. EDL, extensor digitorum longus.

Figure EV2. DOK7 gene therapy shows no obvious effects on motor neuron survival and motor axon atrophy in ALS mice.

WT or ALS mice treated or not with 1.2 × 10^{12} vg of AAV-D7 at P90 were analyzed at P120.

A Nissl staining of the L4-L5 spinal segment. Scale bars, 200 μm. Insets show higher magnification of the ventral horn in the boxed regions (scale bars, 100 μm). NT, not treated.

B Quantification of surviving motor neurons (n = 5 mice). Values are means ± SEM. ***P < 0.0001 by one-way ANOVA with Bonferroni’s post hoc test. n.s., not significant.

C Anti-neurofilament-H staining of the L4 ventral roots. Insets show higher magnification of the boxed regions. Scale bars, 50 μm.

D Size distribution of motor axons (n = 5 mice). Values are means ± SEM.
Figure EV2.
Figure EV3. Treatment with AAV-EGFP does not prolong survival in ALS mice.

ALS mice were treated or not with $1.2 \times 10^{12}$ vg of AAV-EGFP at individually defined disease onset.

A Kaplan–Meier survival curves after birth of untreated ALS mice ($n = 15$ mice, $158.4 \pm 1.9$ days, mean ± SEM) and AAV-EGFP-treated ALS mice ($n = 14$ mice, $162.1 \pm 2.5$ days). n.s., not significant by log-rank test. NT, not treated.

B Kaplan–Meier survival curves after onset in untreated ALS mice ($n = 15$ mice, $57.3 \pm 2.4$ days, mean ± SEM) and AAV-EGFP-treated ALS mice ($n = 14$ mice, $59.8 \pm 3.0$ days). n.s., not significant by log-rank test.

C Ages of onset for untreated ($n = 15$ mice) and AAV-EGFP-treated ALS mice ($n = 14$ mice). Mean ages of onset were indicated by horizontal bars. n.s., not significant by Student’s t-test.

D Forelimb grip strength of ALS mice at onset in untreated ($n = 15$ mice) and AAV-EGFP-treated groups ($n = 14$ mice). Values are means ± SEM. n.s., not significant by Student’s t-test.

E The difference in cycle threshold (ΔCt) between the human SOD1-G93A transgene and the reference mouse apob gene. To calculate the human transgene level, the ΔCt value of hSOD1 was subtracted from the ΔCt value of apob (ALS-NT, $n = 15$ mice; ALS-AAV-EGFP, $n = 14$ mice).
**Figure EV4.** Treatment with AAV-D7 at individually defined disease onset shows no obvious effects on motor neuron survival or grip strength in ALS mice.

WT or ALS mice were treated or not with $1.2 \times 10^{12}$ vg of AAV-D7 at individually defined disease onset.

A Nissl staining of the L4-L5 spinal segment at P150. Scale bars, 200 µm. Insets show higher magnification of the ventral horn in the boxed regions (scale bars, 100 µm). NT, not treated.

B Quantification of surviving motor neurons at P150 (WT-NT, $n = 3$ mice; ALS-NT, $n = 4$ mice; ALS-AAV-D7, $n = 4$ mice). Values are means ± SEM. ***$P < 0.0001$ by one-way ANOVA with Bonferroni’s post hoc test. n.s., not significant.

C Forelimb grip strength analysis at the indicated ages for ALS-NT ($n = 5$ mice) and ALS-AAV-D7 ($n = 7$ mice). Values are means ± SEM. n.s., not significant by Student’s $t$-test.