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Supplementary Information for

Cytotoxic CD8$^+$ T lymphocytes expressing ALS-causing SOD1 mutant selectively trigger death of spinal motoneurons

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Figs. S1 to S13

SI reference citations

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Supplementary Information Text

Materials and Methods

Animal experimentation. All animal experiments were approved by the national ethics committee on animal experimentation and were done in compliance with the European community and national directives for the care and use of laboratory animals (authorization #6224-2016072711103977v3). B6.Cg-Tg(SOD1*G93A)1Gur/J (*SOD1*^{G93A}), B6.129S2-Cd8a^{tm1Mak}/J **(***Cd8a*$^{-/-}$) mice were purchased from the Jackson Laboratory. *SOD1*^{G93A}, *Cd8a*$^{-/-}$ and *Hb9::GFP mice* (T.M. Jessell's laboratory, Columbia University, NY, USA) were maintained on a C57BL/6 background under specific-pathogen-free conditions.

For weight curves, mice were weighed once a week starting at 28 days of age and thrice a week from 56 days until end stage. For grid test analysis, mice were tested once a week starting at 56 days of age. Starting with a 40-gram metal grid (followed by 30, 20 and 10 g grids), the time the animal held on the grid before dropping was measured, with a plateau of 30 sec. The experiment was repeated three times with each grid. Muscle strength (arbitrary units) was quantified with the following formula: (40 g x best time) + (30 g x best time) + (20 g x best time) + (10 g x best time)(1, 2). The mortality was defined as the point in time when the mice are unable to right themselves within 15 sec after being placed upon their back. All behavioral studies were done in a blinded manner. All experimental groups were evenly sex-matched to avoid any sex-dependent influences.

CD8$^+$ T cell isolation. For isolation of T cells from lymph nodes, cervical, axillaries, brachial, inguinal and mesenteric lymph nodes were dissected, crushed onto a nylon membrane and passed through a 70-µm cell strainer. Cells were then collected in cold phosphate-buffered saline (PBS). For isolation of infiltrated T cells, spinal cord and brain were removed and crushed on a 70-µm cell strainer. Cells were resuspended in PBS and separated in Percoll density gradient (3). Cells were then processed for immunopurification or cytometry analysis.

For cell culture, CD8$^+$ T cells were isolated by negative selection using magnetic cell isolation technology. The CD8a$^+$ T cells isolation kit (Miltenyi Biotec) was used according to manufacturer's

instruction. Briefly, cells were suspended in PBS containing 0.5% BSA with a mixture of biotin-conjugated antibodies directed against CD4, CD11b, CD11c, CD19, CD45R (B220), CD49b (DX5), CD105, MHC Class II, Ter-119 and TCRγ/δ. Following incubation with anti-biotin microbeads, "untouched" $CD8^+$ T cells were collected as the negative fraction following magnetic separation. $CD8^+$ T cell purity, as determined by CD3/CD8 immunolabelling of sorted cells was 92.1 ± 3.2% (mean ± S.D, n = 3). Viability of $CD8^+$ T cells in neuronal culture medium over time was assessed by Calcein-AM viability assay (R&D systems): 20 min post-seeding, 100 ± 7.3%, after 48 h *in vitro*, 107.2 ± 4.7% and after 72 h *in vitro*, 113.3 ± 2.9%.

Fluorescence-activated cell sorting analysis. Single cell suspensions from blood, spinal cord and brain were prepared as previously described (3, 4), and stained with the following antibodies anti-CD3-BV785 (BioLegend); anti-CD4, anti-CD45-BV650, anti-CD90.2 (Thy1.2)-V500, anti-CD8a-APC, anti-CD49b-PE-CF594, anti-CD62L-PerCP-Cy5.5, anti-CD44-FITC, anti-CD25-BV421 (BD Biosciences); and anti-KLRG1-PE-Cy7 (eBioscience). Viable, single event cells were defined according to forward scatter and side scatter parameters. Cells were analyzed on a FACSCanto II or an LSR Fortessa apparatus using FACSDiva software (BD Biosciences). For the CNS, between $2x10^4$ and $1.2x10^5$ events were analyzed; for peripheral tissues between $2x10^5$ and $2x10^6$ events were analyzed.

For intracellular IFNγ analysis, after incubation with gating antibodies (CD45-FITC, CD11b-PE-Cy7, CD19-APC-Cy7, NKp46-APC, CD4-V500, CD8-PE), cells were fixed and permeabilized with Cytofix/Cytoperm (BD Bioscience) buffer prior incubation for 30 min with PerCP-Cy5.5 rat anti-mouse IFNγ (Clone XMG1.2, BD Bioscience). Intracellular IFNγ staining in $CD8^+$ T cells was analyzed on BD FACS Canto II, using FACSDiva software (BD Biosciences). Flow cytometry data analysis was performed using FlowJo v10.0.8.

For analysis of $CD8^+$ T cell depletion and *CD8a*$^{-/-}$ mutant mice, blood, lymph nodes and CNS samples were analyzed by FACS using antibody against CD45 (clone 30-F11, BD Biosciences), CD3e (clone 145-2C11, BD Biosciences), CD8a (clone 53-6.7, BD Biosciences), CD4 (clone

GK1.5, BD Biosciences), CD11b (clone M1/70, BD Biosciences), and CD19 (clone 1D3, BD Biosciences) to confirm efficacy and selectivity of the depletion. Cells were analyzed using Accuri C6 cytomer (BD Biosciences). In each set of experiments, mixed-sex cohorts of mice per experimental group were used.

***In situ* hybridization.** Sense and antisense digoxigenin (DIG)-labeled RNA probes were generated from a full length CD8a cDNA clone (Biovalley, Nanterre, France) in a 20 µl reaction containing 1 µg of linearized plasmid (linearized with BamH1 and NotI respectively) using the DIG RNA labeling mix (Roche Diagnostics) and T7 and T3 RNA polymerase respectively (Promega) following the manufacturer's instructions. Probes were purified on MicroSpin G50 columns (GE Healthcare). Mice were transcardially perfused with sterile PBS, spinal cords were isolated and post-fixed for 1h at room temperature (RT) by immersion in 4% paraformaldehyde (PFA). Spinal cords were rinsed twice in PBS before incubation overnight in 30% sucrose/PBS at 4°C and frozen in OCT compound. Transverse sections of 14 µm were cut on a cryostat and collected on Superfrost Plus slides (ThermoFisher Scientific). Sections were treated with acetylation solution (295 ml DEPC-treated water, 4ml of triethanolamine, 0,525 µl HCl (37%), 750 µl acetic anhydride) for 10 min at RT. After washing in sterile PBS, sense or antisense DIG-labeled RNA probes were mixed in hybridization buffer (50% formamide, 10% dextran sulfate, 10% yeast tRNA (10 mg/ml) and 2% Denhardt's 50X in DEPC-treated water) and applied to sections. After hybridization at 65°C overnight, sections were washed twice in 50% formamide, 1X SSC, 0.1% tween-20 for 30 min at 65°C and twice in MABT buffer (MAB 1X, 0.1% tween-20 in water) for 30 min at RT. Sections were blocked in MABT with 20% sheep serum and 2% of blocking reagent (Roche diagnostics) for 2 h at RT. After overnight incubation with anti-DIG-antibody conjugated with alkaline phosphatase (Roche diagnostics, diluted 1/2000) at 4°C, slides were washed and incubated with NBT-BCIP (Roche Diagnostics) staining solution with endogenous alkaline phosphatase inhibitor (levamisole 2 mM) according to the manufacturer's instructions. The reaction was stopped by washing in water. Image acquisitions were done using AxioImager D1 microscope (Carl Zeiss).

Primary neuron cultures. Motoneurons from the spinal cords of E12.5 *Hb9::GFP, SOD1^{G93A}* or control C57BL/6 embryos were isolated as we previously described (5, 6), using iodixanol density gradient centrifugation. Motoneurons were plated on poly-ornithine/laminin-treated wells in the presence of neurotrophic factors including glial-derived neurotrophic factor (0.1 ng/ml), brain-derived neurotrophic factor (1 ng/ml) and ciliary neurotrophic factor (10 ng/ml) in supplemented Neurobasal medium (2% (vol/vol) horse serum, 2% (vol/vol) B-27 supplement (ThermoFisher Scientific), 50 µM L-glutamine, 25 µM L-glutamate, 25 µM 2β-mercaptoethanol and 0.5% penicillin/streptomycin). Hippocampal, striatal and cortical neurons were isolated from E17.5 embryos (C57BL/6) as described (5, 7). Neurons were plated on poly-ornithine/laminin-treated wells and cultured in Neurobasal medium complemented with 1 mM sodium pyruvate, 2% B-27 supplement. All these neuronal types were seeded at the density of 1,500 cells/cm^2.

Neuron-CD8$^+$ T cell co-cultures. For co-culture experiments, CD8$^+$ T cells were plated either with motoneurons or in the upper chamber of Nunc transwell insert (0.4 µm pore, ThermoFisher Scientific) just after motoneuron seeding (otherwise indicated), at a ratio of 10 CD8$^+$ T cell for 1 motoneuron. Unless otherwise indicated, co-culture experiments were done with CD8$^+$ T cells isolated from symptomatic mice (130-150 days). When indicated, cells were treated with the following reagents: anti-MHC-I H2-D^b antagonistic antibody (clone 28.14.8, ThermoFisher Scientific), z-AAD-cmk (368050, Merck), recombinant Fas (human)-Fc (human)(ALX-522-002, Enzo Life Sciences), Ac-LEHD-cmk (218728, Merck), anti-IFNγ antibody (clone R4-6A2, ThermoFisher Scientific), mouse recombinant IFNγ (5222SF, Cell Signaling Technology), recombinant LT-βR (human):Fc (human)(ALX-522-041, Enzo Life Sciences). Surviving motoneurons were directly counted by fluorescence or phase contrast microscopy according to morphological criteria as previously described (5, 6). For cortical, hippocampal and striatal neuron-CD8$^+$ T cell co-cultures, lymphocytes were added to neuronal culture at the time of seeding at a ratio of 10:1 (T cell:neuron) and surviving neurons were directly counted under light microscope 72 h later.

Immunolabeling. For immunocytochemistry, motoneurons and motoneuron-T cells co-cultures were plated on poly-ornithine/laminin-treated glass coverslips. Cells were fixed on ice for 20 min in 4% PFA, washed with PBS and incubated for at least 1 h in blocking solution containing 3% BSA, 3% donkey serum, 0.1% Triton X-100 at RT. Cells were incubated overnight at +4°C with the following primary antibodies diluted in the blocking solution: Rat anti-CD8 alpha (clone 53-6.7, R&D systems) and rabbit anti-GFP (TP401, Torrey Pines Biolabs), mouse anti-MHC-I (clone 28-14-8, Antibodies-online) and rabbit anti-β_2m (clone EP2978Y, Abcam). Prior to MHC-I and β_2m immunolabelling, motoneurons were treated with mouse IFNγ (5222SF, Cell Signaling Technology). After PBS washes, cells were incubated with fluorochrome-conjugated secondary antibodies diluted in blocking solution for 1h at RT. Cells were then washed in PBS, and incubated 5 min with PBS containing 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI). Coverslips were mounted onto glass slides using Mowiol medium.

For immunostaining of lumbar spinal cord samples, animals were transcardially perfused with PBS and then with 4% PFA. Spinal cords were dissected and post-fixed by immersion for 45 min at +4°C. Spinal cord were incubated overnight in 30% sucrose solution and imbedded in Tissue-Tek O.C.T. compound (Sakura Finetek). Cryosections of the spinal cord (16 µm) were collected onto Superfrost Plus slides and rinsed with PBS before being incubated for 2 h at RT in blocking solution (3% BSA, 3% Donkey Serum, 0.1% Triton X-100). Sections were incubated with goat anti-ChAT (AB144P, Merck) diluted in blocking solution overnight at 4°C. After washing in PBS, sections were incubated with Alexa Fluor 594-conjugated donkey anti-goat secondary antibody (ThermoFisher Scientific) diluted in blocking solution for 1 h at RT. Slides were mounted in Mowiol medium. For cresyl violet staining, following cryosectioning, sections were rinsed for 5 min in PBS and stained with cresyl violet for 30 min, then rinsed with water before dehydration in ethanol and mounted in Mountex medium (Histolab). Images were acquired using a Zeiss LSM510 laser scanning confocal microscope. Fluorescence analysis was performed using the NIH ImageJ software.

CD8⁺ T cells depletion. The CD8⁺ T cell population was depleted in *SOD1^{G93A}* mice by administration of depleting antibody (clone YTS169.4, BioXCell). As a control, a monoclonal isotype-matched IgG2a (clone LTF-2, directed against keyhole limpet hemocyanin) was injected. Each mouse received intraperitoneal administration of 100 µg of antibody weekly, starting at 100 days, until end stage.

Atomic force microscopy-based single-cell force spectroscopy. For better adhesion during AFM experiments, *Hb9::GFP* motoneurons were plated on a collagen- and laminin-coated dish, at a density of 1000 cells/cm². CD8⁺ T cells were seeded in a separate dish in RPMI 1640 medium (ThermoFisher Scientific). The individual CD8⁺ T cells were attached to the surface activated cantilever using Concanavalin-A-mediated linkage (8). During a force measurement cycle, a CD8⁺ T cell immobilized at the end of the cantilever was brought into contact with a motoneuron. Each cycle consisted of approaching the cell-decorated cantilever to the neuronal layer until the preset deflection was reached and pulling it back to the initial position. All experiments were conducted in serum-free Leibovitz medium (LifeTechnologies) at 33°C. All measurements were carried out with an Asylum Research MFP-3D head (Asylum Research; driving software IgorPro 6.37, Wavemetrics) mounted on an Olympus ix-71 microscope, using soft gold-coated tipless cantilevers (CSC38, MikroMasch, Sofia, Bulgaria) with a nominal spring constant of 30 pN/nm and a resonant frequency of 10 kHz in air that drops to 3 kHz in water. Constant loading speeds of 3 µm/s were used with a total force distance of 8 µm. Before each measurement the spring constant of the cantilever was calibrated by a combination of thermal noise and Sader methods (9, 10). The compression force has been determined at 500 pN, at which force a large contact surface is obtained without significant cell deformation (8).

For experiments with blocking anti-MHC-I antibody, measurements were first collected without any anti-MHC-I antibody, cells were incubated for two hour with 1 µg/ml of antibody and then another series of measurements were performed.

Reverse transcription quantitative polymerase chain reaction. Total RNA was isolated from spinal cord of 90- and 150-day-old *SOD1*^{G93A} and 150-day-old wildtype mice using the RNeasy Mini Kit (Qiagen). The eluted RNA was quantified by spectrophotometry (Nanodrop). Following elimination of genomic DNA, reverse transcription (RT) was performed with the quantitect RT kit (Qiagen). Quantitative PCR was carried out SYBR Green (Qiagen) detection on LightCycler 480II (Roche Diagnostics). After an initial denaturation at 95°C for 15 min, amplification was done via 45 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. After PCR amplification, a melting curve analysis was carried out to verify the specificity of the PCR. Polymerase (RNA) II polypeptide J (PolJ) levels were used to normalize the amounts of cDNA. ΔCt was calculated as the difference between the Ct values, determined with the equation $2^{-\Delta Ct}$. The primer sequences are as follows: PolJ forward: 5'-ACCACACTCTGGGGAACATC-3'; PolJ reverse: 5'-CTCGCTGATGAGGTCTGTGA-3'; β_2m forward: 5'- TCTCACTGACCGGCCTGTAT -3'; β_2m reverse: 5'-GATTTCAATGTGAGGCGGGTG-3'; H2-D^b forward: 5'-CTGAAAACGTGGACGGCGGC-3' and H2-D^b reverse : 5'-GGCCCAGCACCTCAGGGTGA-3'.

TCR repertoire analysis. For analysis of the clonal profile of CD8$^+$ T cells, total lymphocytes were isolated from lymph node of 150-day-old wildtype mice, and from lymph nodes and CNS of 150-day-old *SOD1*^{G93A} mice. CD8$^+$ T cells were purified from total cells as described above. A mean of $2.32x10^6$ CD8$^+$ T cells from lymph nodes and $3.24x10^4$ CD8$^+$ T cells from CNS were resuspended in TRIzol Reagent (ThermoFischer Scientific) and kept a -80°C until analysis. RNA was then quantified by Nanodrop. After reverse transcription using MMLV Reverse transcriptase (ThermoFischer Scientific), qualitative analysis of 18 TRBV families was performed using previously described procedures (11-13). Briefly, the CDR3 region was amplified by PCR in a Veriti Thermal Cycler (ThermoFischer Scientific), in separate reactions using a common reverse Cβ primer and different forward Vβ-specific primers (11). Amplicons generated after two semi-nested PCRs were labeled using a FAM-tagged Cβ primer, and the CDR3-length distribution (CDR3-LD) was determined with an AB3037 DNA sequencer and analyzed using GeneMapper software

(ThermoFischer Scientific). A software was developed to automatically describe individual TRBV profiles and to compare the TRBV usage between separate anatomical compartments. As previously described (11), four typologies of CDR3-LD were observed: monoclonal, oligoclonal, polyclonal with major peak, and polyclonal. A correlation coefficient and a distance score were used to compare TRBV usage between lymph node purified $CD8^+$ T cells and CNS-infiltrating cells (11). Highly similar profiles are characterized by a high correlation coefficient and a low-distance score. The correlation coefficient and the distance score were very effective at distinguishing close or very different distributions, but not those in between.

Statistical analysis. Statistical significance was determined when appropriate by unpaired two-tailed *t* test, one-way analysis of variance (ANOVA) followed by a Tukey-Kramer's *post hoc* tests or two-way repeated-measure ANOVA followed by a Newman-Keuls's *post hoc* test. For the Kaplan-Meier survival analysis, the log-rank test was used. Statistical analyses were done with Graphpad Prism software (GraphPad Software). Significance was accepted at the level of $P < 0.05$.

Figure S1

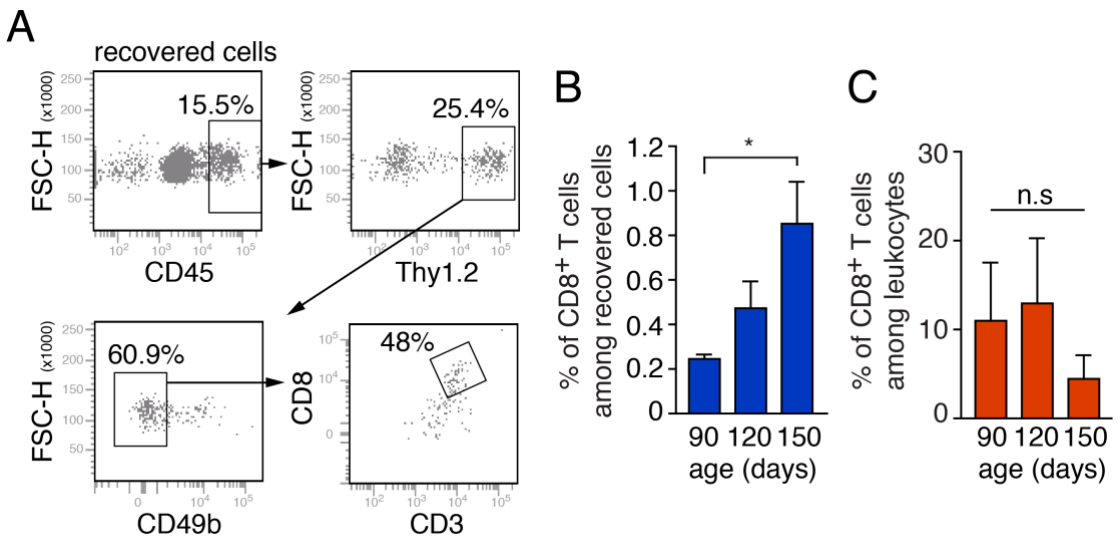

Fig. S1. (*A*) Gating strategy for flow cytometry analysis. Viable, single event cells (recovered cells) from the CNS were first gated for their expression of CD45 as a marker of the hematopoietic lineage. $CD45^+$ cells were analyzed for T cell lineage marker expression using Thy1.2, and were further analyzed for CD49b which is expressed at the surface of NKT cells. $CD49b^-$ cells were then analyzed for CD3 and CD8 expression. FSC-H, forward light scatter height. The relative proportion of cells is indicated in relevant quadrants. (*B*,*C*) Flow cytometry analysis of $CD8^+$ T cells in the CNS and blood of $SOD1^{G93A}$-expressing mice at indicated ages. The percentage of $CD45^+Thy1.2^+CD49b^-CD3^+$-gated $CD8^+$ among recovered cells in the CNS (*B*) and leukocytes (*C*) of ALS mice is represented. (*A*) Representative cytometry profiles of 3 independent experiments, (*B*,*C*) histograms show mean values ± standard error of the mean (SEM), $n = 3$ for each time point, *$P < 0.05$, n.s, non-significant, ANOVA with Tukey-Kramer's *post hoc* test.

Figure S2

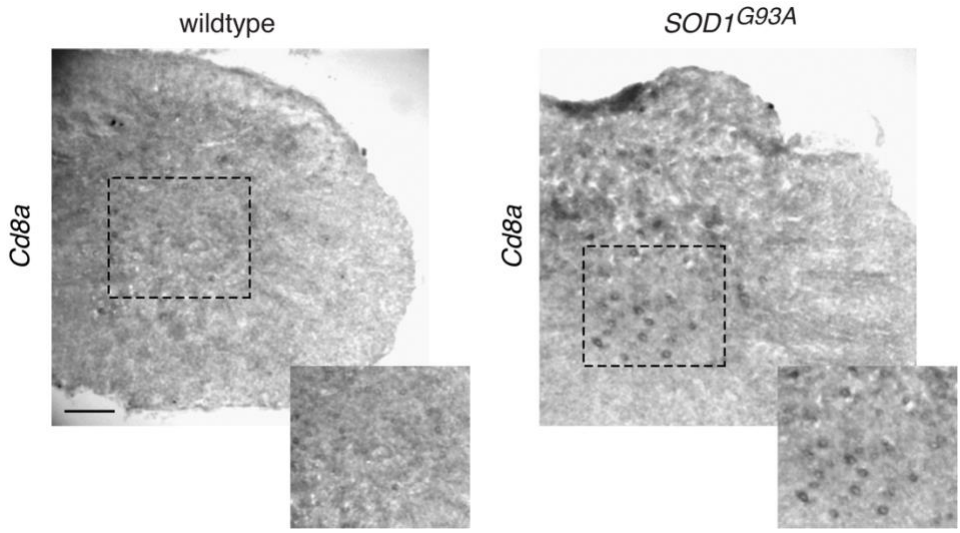

Fig. S2. Detection of infiltrating cytotoxic T cells by *in situ* hybridization with *Cd8a* antisense probe in the spinal cord of 150-day-old *SOD1*^{G93A} mice and age-matched wildtype mice. Lower right-hand corner shows an area (dotted line) at higher magnification. Sense probe did not show any specific signal. Scale bar, 200 µm.

Figure S3

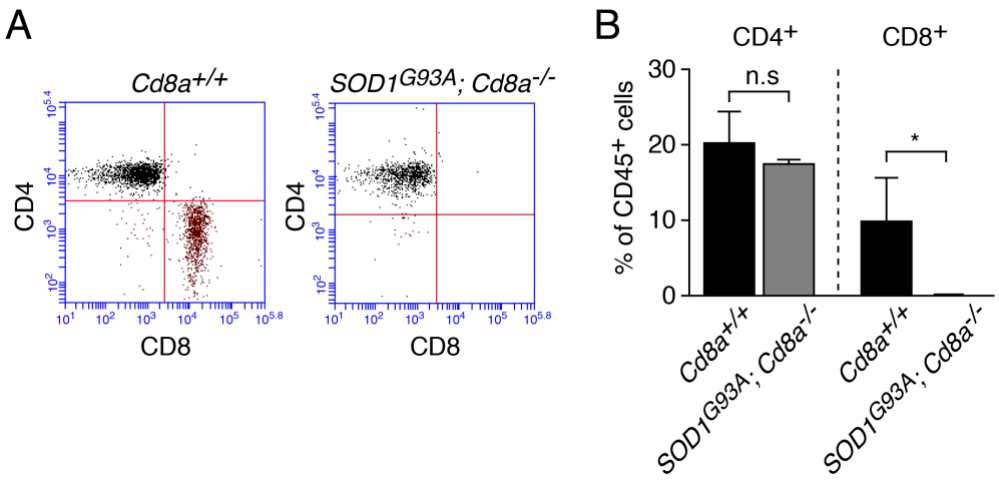

Fig. S3. (*A*) Representative dot plot of $CD8^+$ and $CD4^+$ T cells in the lymph nodes of wildtype (*Cd8a*$^{+/+}$) and *SOD1*^{G93A},*Cd8a*$^{-/-}$ mice showing the depletion of CD8 T cells. (*B*) The percentage of $CD4^+$ and $CD8^+$ T lymphocytes among $CD45^+$ cells was determined in *Cd8a*$^{+/+}$ and double *SOD1*^{G93A},*Cd8a*$^{-/-}$ mutant mice by flow cytometry. Values are means ± SEM, ANOVA with Tukey-Kramer's *post hoc* test.

Figure S4

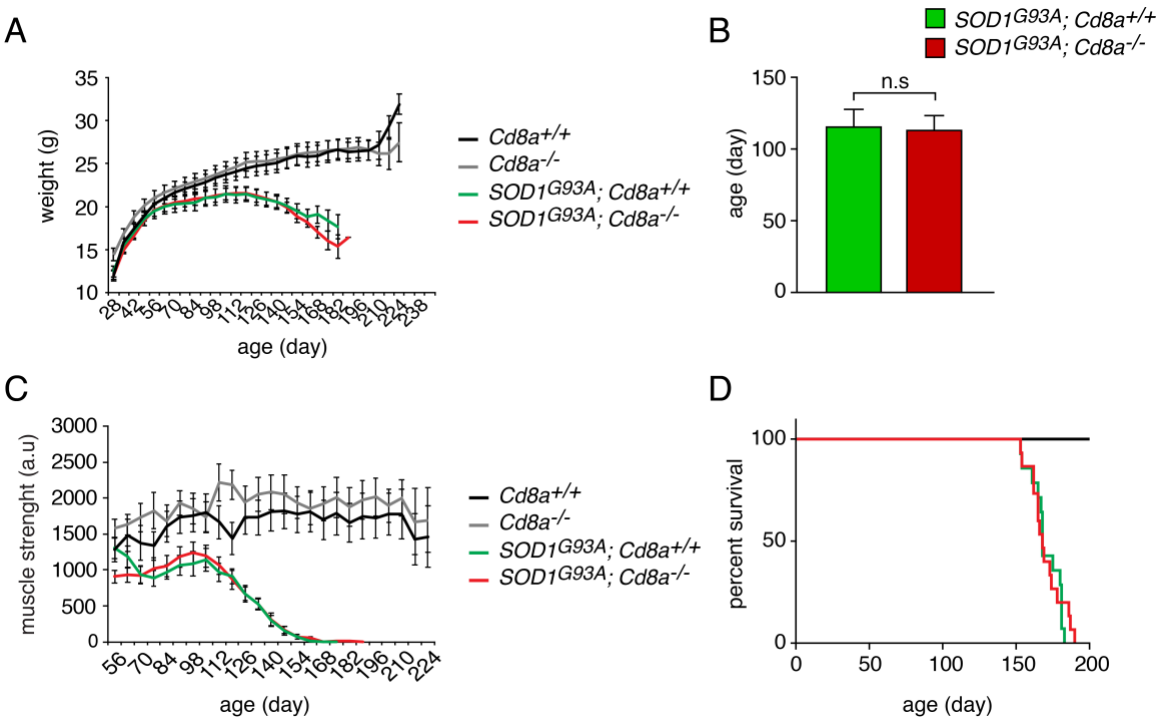

Fig. S4. (*A*) Body weight curves of *Cd8a*$^{+/+}$, *Cd8a*$^{-/-}$, *SOD1*^{G93A}*;Cd8a*$^{+/+}$ *and SOD1*^{G93A}*;Cd8a*$^{-/-}$ mice (*n* = 15). Mice were weighed weekly starting at 28 days of age. Values are means ± SEM. (*B*) The mean age of onset of *SOD1*^{G93A}*;Cd8a*$^{+/+}$ *and SOD1*^{G93A}*;Cd8a*$^{-/-}$ mice was determined by the peak of the weight curve. Values are means ± SD, unpaired two-tailed *t* test. (*C*) Muscular strength of mice with indicated genotype was evaluated weekly starting at 56 days of age by grid test. Values are expressed as the mean ± SEM. (*D*) Kaplan-Meier survival curves for *Cd8a*$^{+/+}$, *Cd8a*$^{-/-}$, *SOD1*^{G93A}*;Cd8a*$^{+/+}$ *and SOD1*^{G93A}*;Cd8a*$^{-/-}$ mice (*n* = 15). Log-rank test, *P* > 0.05. We used mixed-sex cohorts of mice and means survival were : female *SOD1*^{G93A}*;Cd8a*$^{+/+}$, 168.4 ± 3.6, *n* = 7; male *SOD1*^{G93A}*;Cd8a*$^{+/+}$, 172 ± 3.6, *n* = 8; female *SOD1*^{G93A}*;Cd8a*$^{-/-}$, 170.4 ± 4.2 , *n* = 8*;* males *SOD1*^{G93A}*;Cd8a*$^{-/-}$, 169.9 ± 4.3, *n* = 7; (means ± SEM, n.s, ANOVA Tukey-Kramer's *post hoc* test).

Figure S5

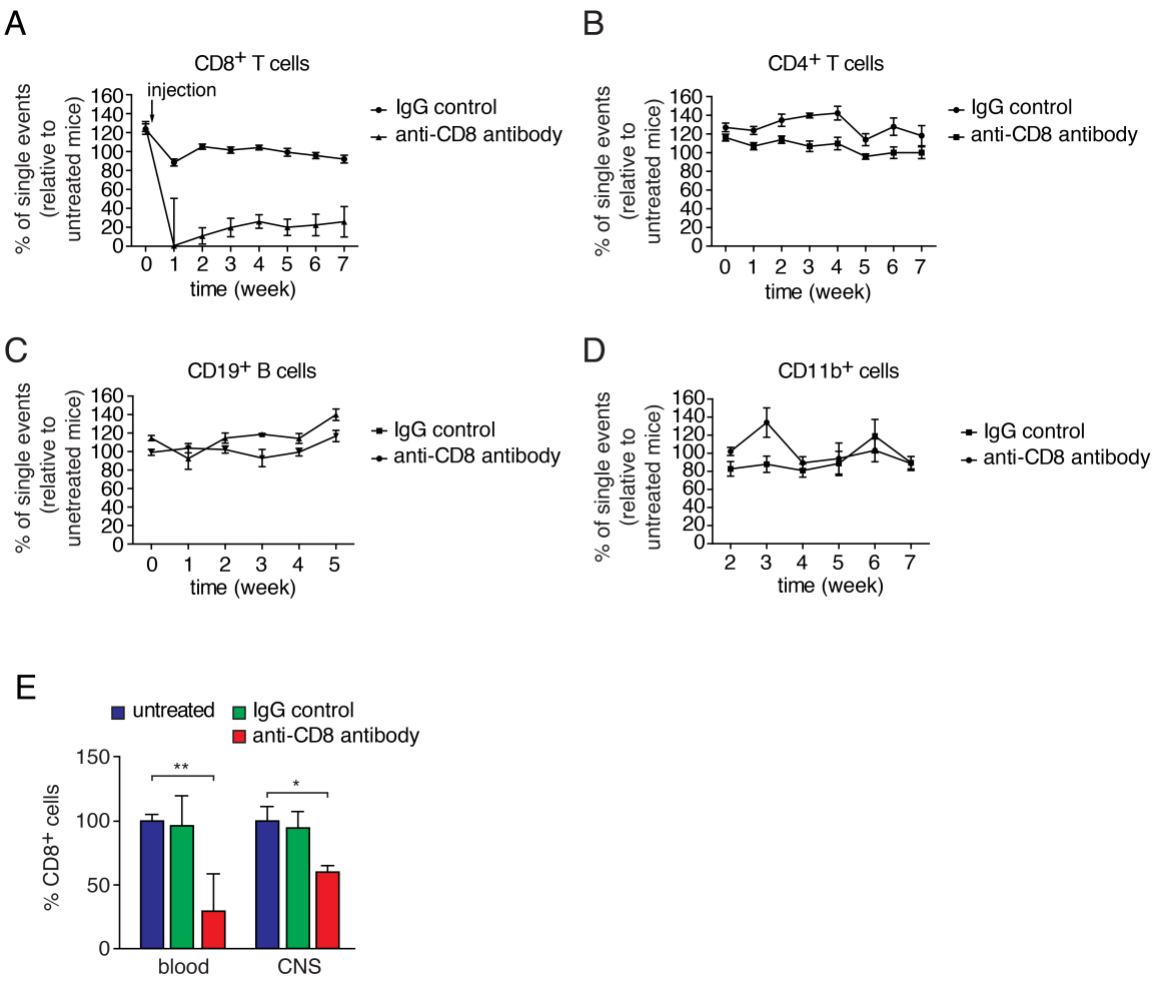

Fig. S5. (*A*) Chronic depletion of circulating CD8$^+$ T cells in *SOD1^{G93A}* mice is based on weekly injection of a depleting anti-CD8 monoclonal antibody (clone YTS169.4, rat IgG2b isotype). IgG2b isotopic control (clone LTF-2) is an IgG2b rat anti-keyhole limpet hemocyanin. Wildtype mice were injected (or not) intraperitoneally with indicated antibody (100 µg) at the age of 100 days. The percentage of circulating (blood) CD8$^+$ T cells was determined by flow cytometry at indicated weeks post-injection and expressed relative to the number of single events determined in the untreated condition (non-injected mice), at each time point. Of note, treatment with 200 µg of antibody did not result in an increased depletion of CD8$^+$ T cells. (*B-D*) Cytometry analysis of circulating CD3$^+$CD4$^+$ T cells (*B*), CD19$^+$ B-cells (*C*) and CD11b$^+$ macrophages (*D*) from untreated, irrelevant IgG control

and anti-CD8 antibody treated animals at indicated time. (*E*) Anti-CD8 depleting antibody and IgG control antibody were administrated to 100-day-old *SOD1*^{G93A} mice for 12 weeks. CD8^{+} T cells were then isolated from the blood and the CNS of mice that received or not antibody and the percentage of T cells was determined (expressed relative to untreated mice)(*n* = 3). Values are means ± SEM, ANOVA with Tukey-Kramer's *post hoc* test, $^{**}P < 0.01$.

Figure S6

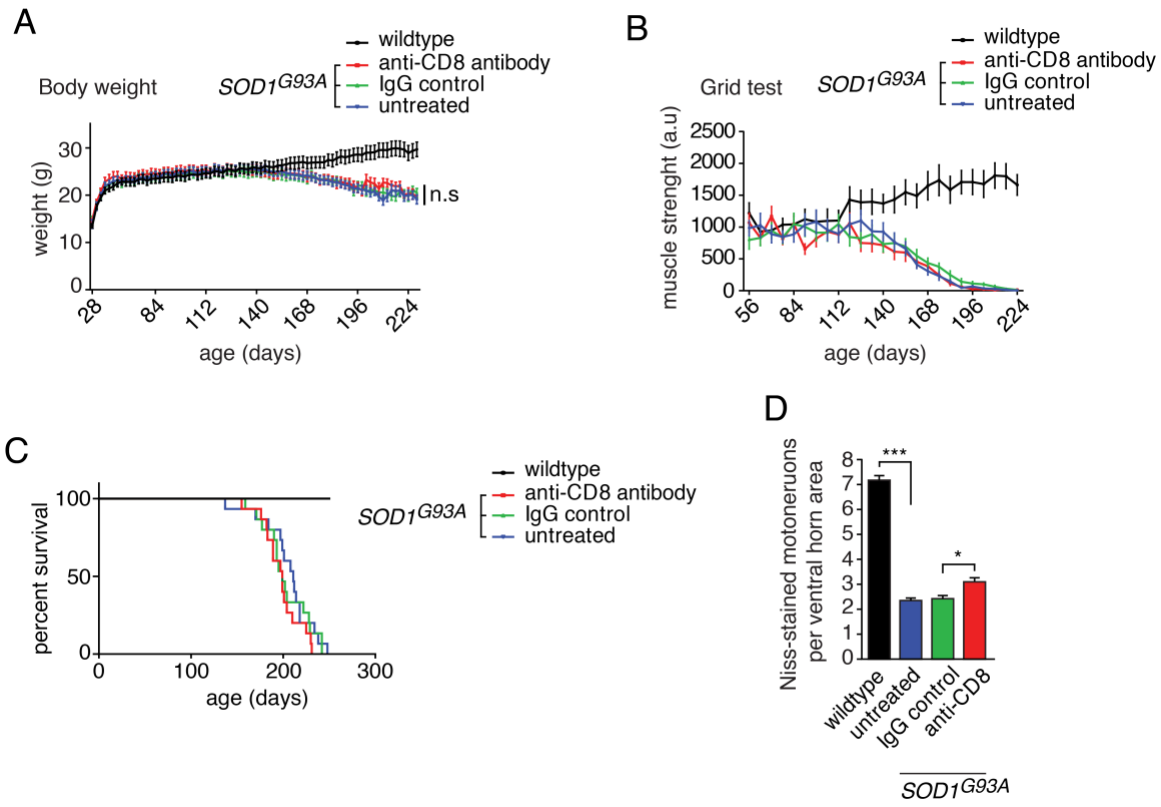

Fig. S6. (*A*) Anti-CD8 antibody and IgG control antibody was administrated to *SOD1*^{G93A} mice weekly by intraperitoneal injection (*n* = 15 for each group), starting at 100 days of age. *SOD1*^{G93A} and wildtype mice were weighed weekly starting at 28 days of age. (*B*) Motor function of wildtype, untreated *SOD1*^{G93A} mice and *SOD1*^{G93A} mice that were administrated weekly with IgG control and anti-CD8 antibody (100 µg each antibody) was determined by grid test at the indicated age. There were no statistical difference between groups of *SOD1*^{G93A} mice (ANOVA with repeated measures Newman-Keuls's *post hoc* test). (C) Kaplan-Meier survival curves of wildtype, untreated, IgG control- and anti-CD8-treated *SOD1*^{G93A} mice ($P > 0.05$ by Log-rank test). (*D*) Spinal cord sections from wildtype, untreated, IgG control- and anti-CD8-treated *SOD1*^{G93A} mice were stained with Cresyl violet (Nissl-staining), and the number of surviving motoneurons with distinct nucleoli was determined. Values are means ± SD, *n* = 4, ANOVA with Tukey-Kramer's *post hoc* test, ***$P <$ 0.001.

Figure S7

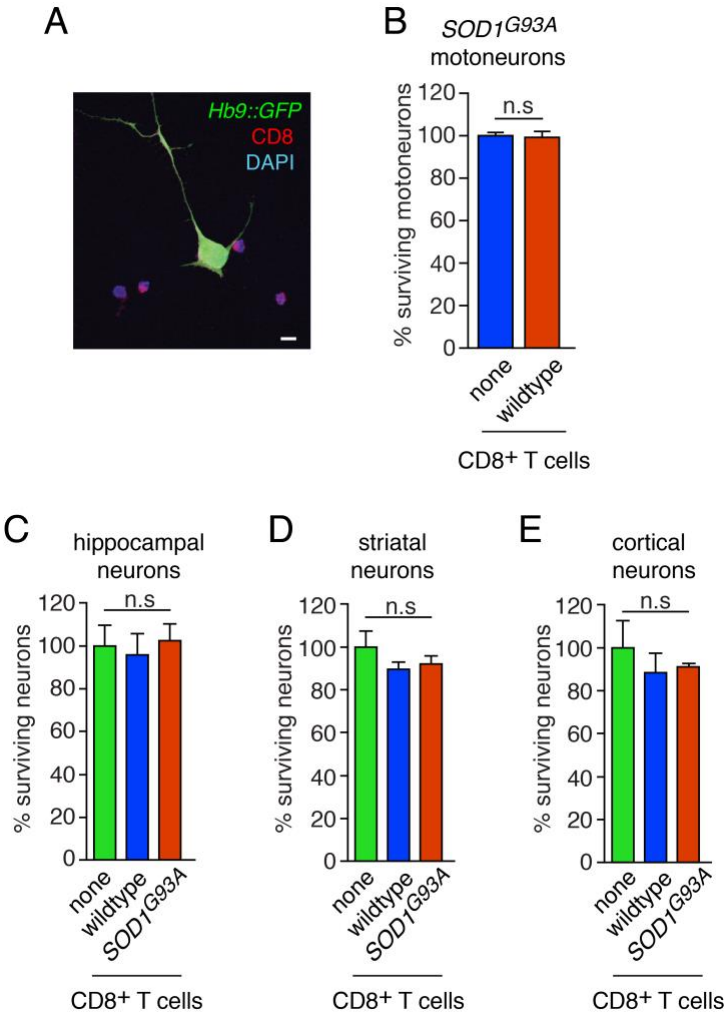

Fig. S7. (*A*) *Hb9::GFP* motoneurons (in green) were co-cultured with CD8$^+$ T cells isolated from ALS mice and immunostained with anti-CD8 antibodies (in red, DAPI in blue). Scale bar, 10 µm. (*B*) SOD1^{G93A}-expressing motoneurons were co-cultured (or not) for 72 h with wildtype CD8$^+$ T lymphocytes and survival was expressed relative to the survival in the absence of T cells. (*C-E*) Hippocampal (*C*), striatal (*D*) and cortical (*E*) neurons were isolated and seeded (or not) with CD8$^+$ T lymphocytes isolated from the lymph nodes of wildtype or *SOD1^{G93A}* mice. The number of surviving neurons, determined 72 h after, is expressed as a percentage of the number of neurons

in the control condition (none). Results shown are the mean values ± SD of at least three independent experiments performed in triplicate. (*B*) unpaired two-tailed *t* test, (*C*-*E*) ANOVA with Tukey-Kramer's *post hoc* test.

Figure S8

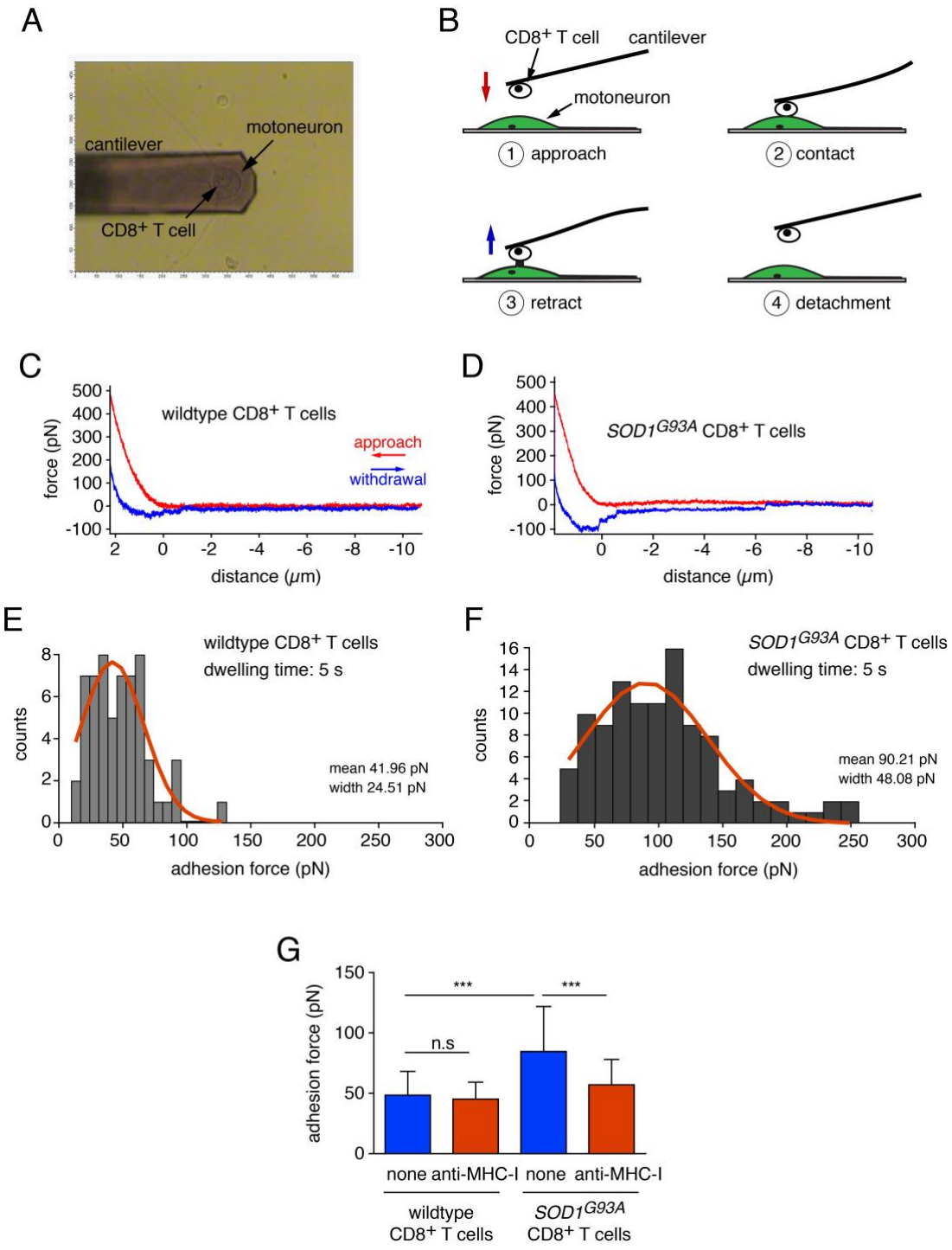

Fig. S8. (*A*) Representative image of a single CD8⁺ T cell attached on a functionalized cantilever and positioned above one motoneuron. (*B*) Schematic representation of the principle of AFM-

SCFS. A single $CD8^+$ T cells is immobilized at the apex of an AFM tipless cantilever coated with concanavalin A. Cantilever is positioned above a motoneuron and approached (1) until the pre-defined contact force is reached (2). After a pre-set dwell time, the cantilever is retracted (3) and T cell detaches from the motoneuron (4). Interaction forces are determined according to deflection of the cantilever. (*C,D*) Representative force-distance curves for wildtype and mutant $CD8^+$ T cell-wildtype motoneuron interaction. Red curve represent the approach, and blue curve represent the cantilever retraction phase. (*E*,*F*) Histogram indicating the number of counts (frequency) for different adhesion forces (pN) between a motoneuron and wildtype (*E*) and $SOD1^{G93A}$-expressing $CD8^+$ T cell (*F*). Here, a dwell time of 5 sec was chosen. (*G*) Mean adhesion force between motoneuron and wildtype or $SOD1^{G93A}$-expressing $CD8^+$ T cell was measured prior to the addition of function-blocking anti-MHC-I antibody (1 µg/ml, each genotype). After 2 h of incubation, measurements of adhesion force were pursued with a dwell time of 5 sec. Values are means of Gaussian fits ± SD, 7 to 14 pairs from 2 to 4 independent cell cultures ANOVA with repeated measures, Newman-Keuls's *post hoc* test.

Figure S9

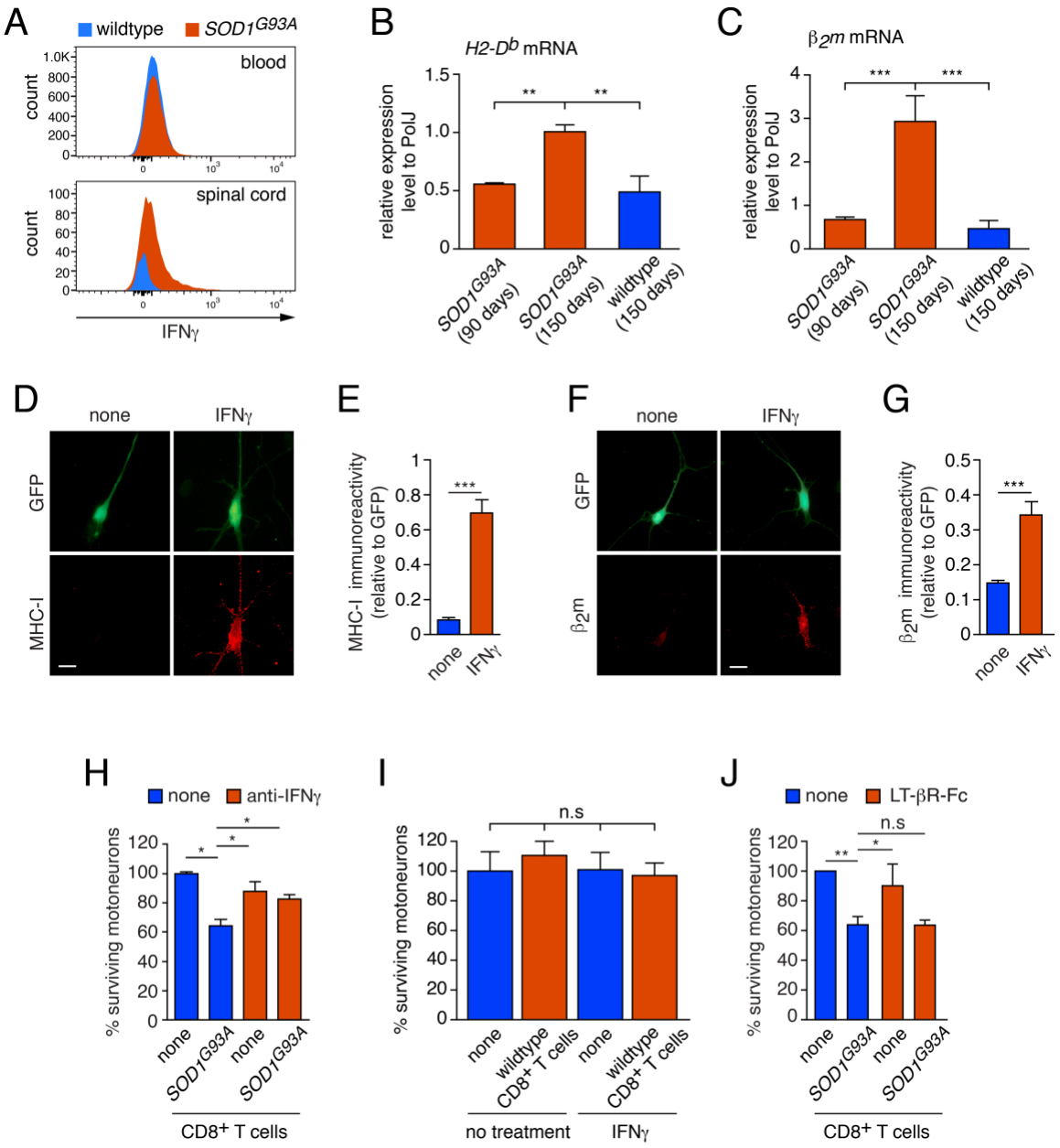

Fig. S9. (*A*) Mean fluorescence intensity of intracellular IFNγ was determined by flow cytometry in CD8⁺ T cells isolated from the blood and spinal cord of wildtype and *SOD1*^{G93A} mice (*n* = 4, total number of cells analyzed: blood wildtype, 21953; blood *SOD1*^{G93A}, 19242; spinal cord wildtype, 624; spinal cord *SOD1*^{G93A}, 2808). (*B*,*C*) Transcripts levels of *H2-D*^{b} (*B*) and *β*$_2$*m* (*C*) in the spinal cord were determined by RT-qPCR. Total RNA was isolated from lumbar spinal cord of 90- and

150-day-old *SOD1*^{G93A} mice and 150-day-old wildtype mice. Results are expressed relative to polymerase (RNA) II (DNA directed) polypeptide J (PolJ) transcript. (*D*-*G*) *Hb9::GFP* motoneurons (in green) were treated or not with 10 ng/ml recombinant IFNγ for 72 h and immunostained with antibodies against MHC-I (*D*) or β_2m (*F*)(in red). MHC-I (*E*) and β_2m (*G*) immunoreactivity was quantified at the somatic level of motoneurons and expressed relative to GFP intensity for each condition. Scale bar, 20 µm. (*H*) Motoneurons were co-cultured for 72 h with mutant SOD1 CD8$^+$ T cells in the presence or not of antagonistic anti-IFNγ antibody (100 ng/ml). (I) Wildtype motoneurons were incubated with mouse recombinant IFNγ (10 ng/ml) for 24 h prior addition (or not) of wildtype CD8$^+$ T cells (for 72 h). (*J*) Motoneurons were co-cultured for 72 h with mutant SOD1 CD8$^+$ T in the presence (or not) of the soluble decoy receptor LT-βR-Fc (100 ng/ml). (*H*-*J*) Survival of motoneurons is expressed as the percentage of the number of motoneurons surviving in the absence of any treatment and T cells. Values are means ± SD, *n* = 3, (*E*,*G*) unpaired two-tailed t test, otherwise ANOVA with Turkey-Kramer's *post hoc* test.

Figure S10

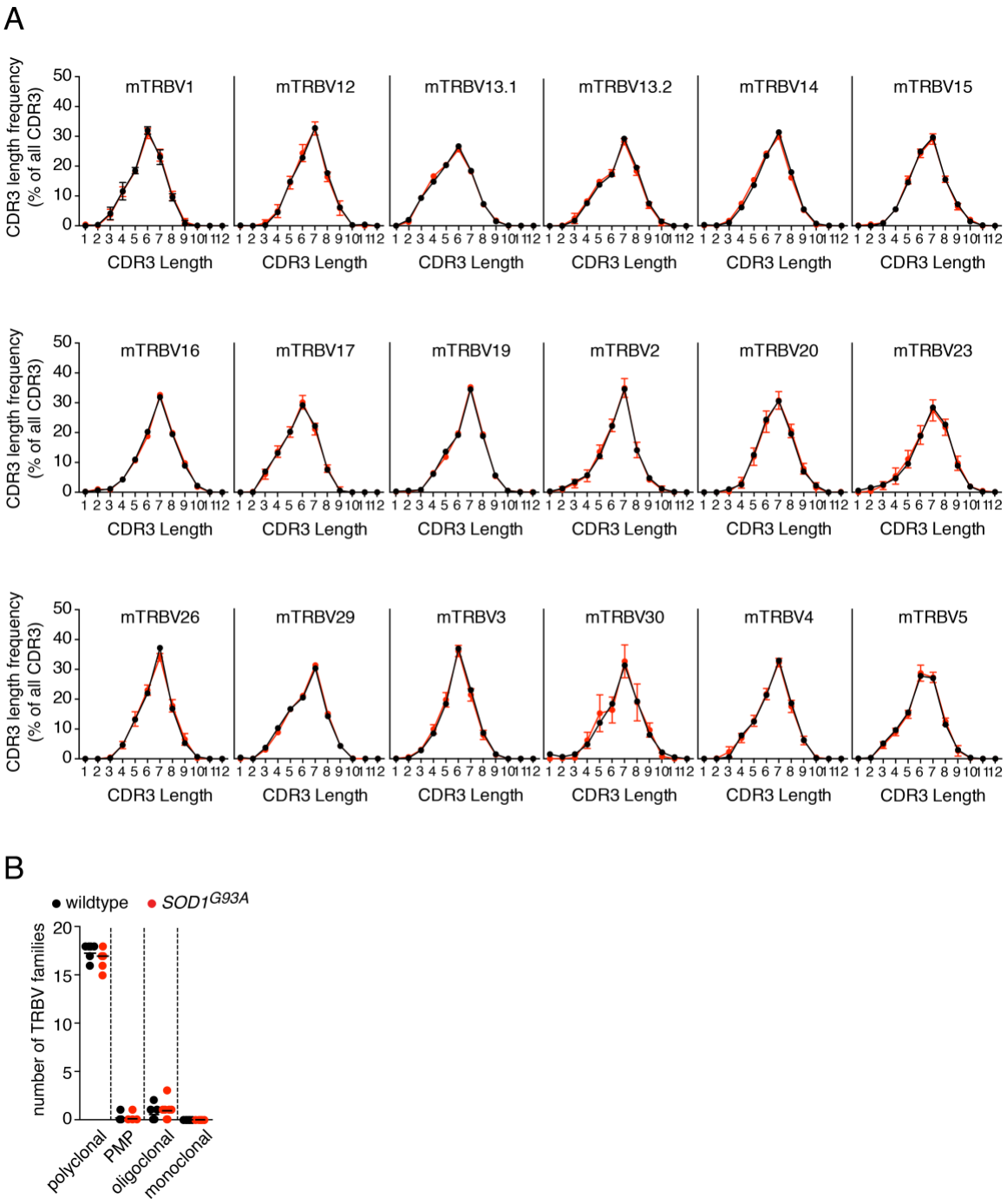

Fig. S10. (*A*) CDR3 length of 18 TRBV (mTRBV) was analyzed in CD8$^+$ T cells isolated from lymph nodes of wildtype (black line, $n = 9$) and *SOD1*^{G93A} mice (red line, $n = 9$). CDR3 length is expressed

as percentage of all CDR3 length measured for each TRBV. CDR3 length is expressed as multiple of nucleotide triplets. (*B*) Comparison of CDR3-LD of 18 TRBV in $CD8^+$ T cells isolated from the lymph nodes of wildtype (black dots)(*n* = 9) and *SOD1*^{G93A} (red dots)(*n* = 9) mice at 150 days of age. CDR3-LD can follow a Gaussian/polyclonal; polyclonal with major peak (PMP), oligoclonal and monoclonal distribution.

Figure S11

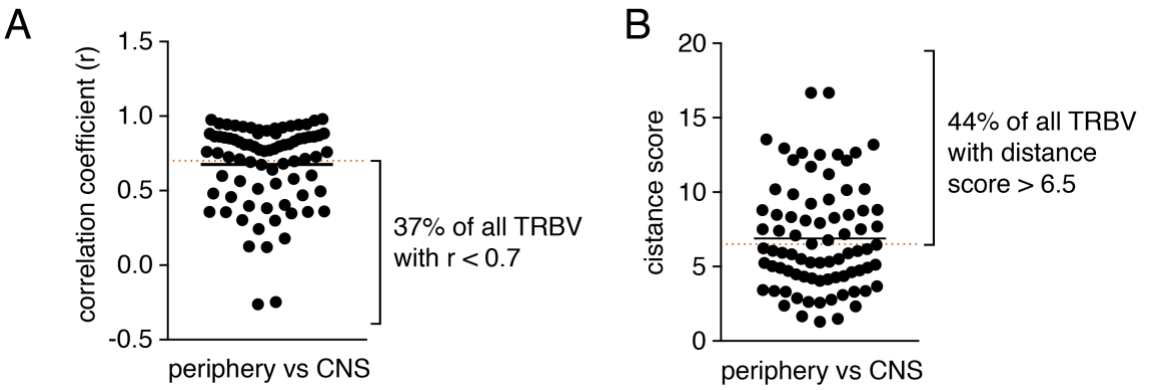

Fig. S11. (*A*) A linear correlation was determined for each TRBV between the periphery (lymph nodes) and the CNS and r value plotted. Low correlation (r < 0.7) indicates difference between the two compartments. (*B*) Distance score determines the distribution of distances between two peaks of CDR3 length. A small distance score indicating a close distribution. The r value of 0.7 and distance score of 6.5 was empirically determined, as described previously (11).

Figure S12

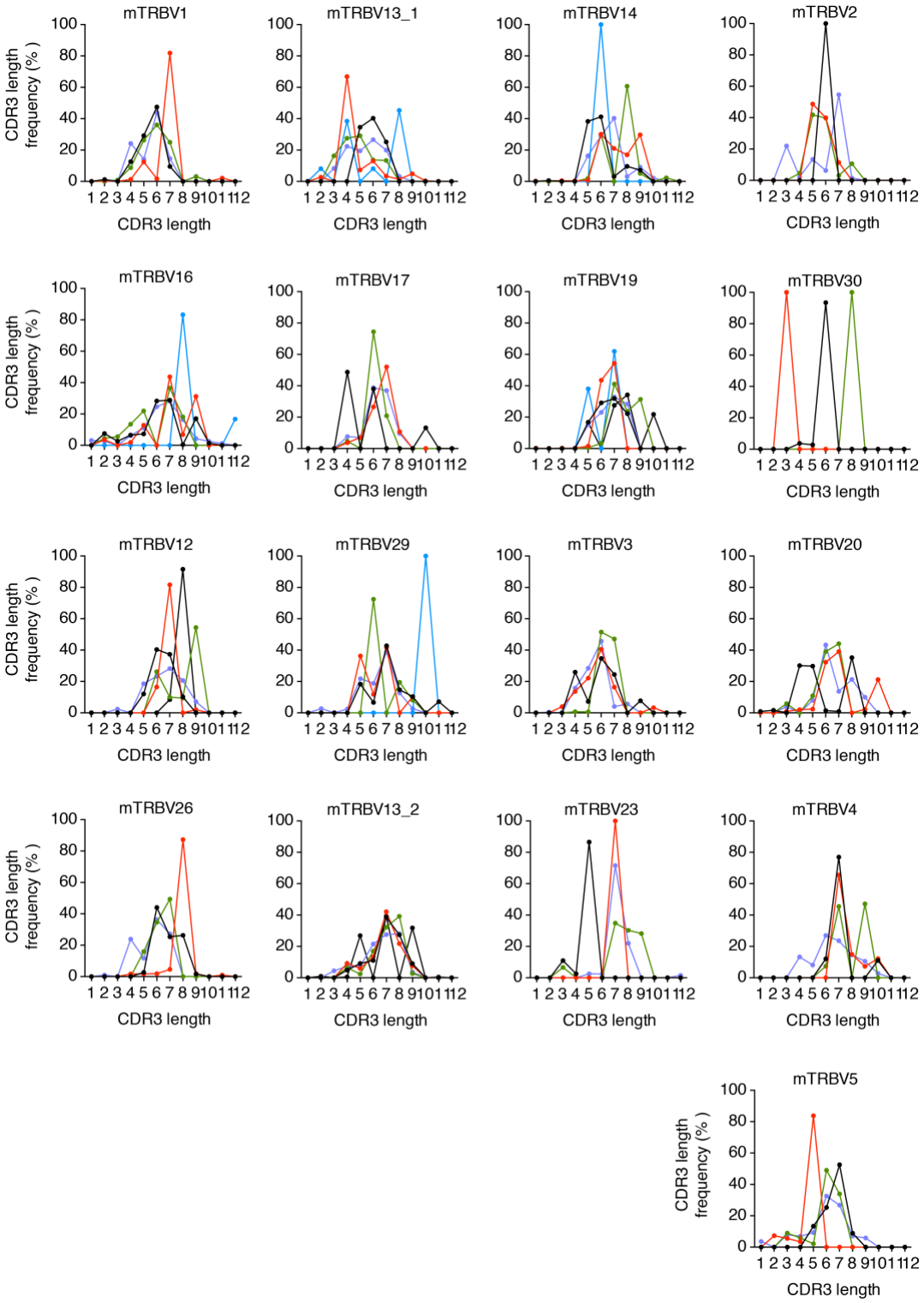
mTRBV1
mTRBV13_1
mTRBV14
mTRBV2
mTRBV16
mTRBV17
mTRBV19
mTRBV30
mTRBV12
mTRBV29
mTRBV3
mTRBV20
mTRBV26
mTRBV13_2
mTRBV23
mTRBV4
mTRBV5
CDR3 length frequency (%)
CDR3 length
0 20 40 60 80 100
1 2 3 4 5 6 7 8 9 10 11 12

Fig. S12. The frequency of CDR3 length was determined for mTRBV1, mTRBV13_1, mTRBV14, mTRBV2, mTRBV16, mTRBV17, mTRBV19, mTRBV30, mTRBV12, mTRBV29, mTRBV3, mTRBV20, mTRBV26, mTRBV13_2, mTRBV23, mTRBV4 and mTRBV5 in $CD8^+$ T that are recruited to the CNS of *SOD1*^{G93A} mice (*n* = 4-6).

Figure S13

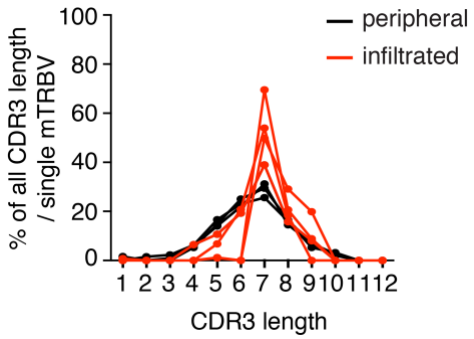

Fig. S13. Distribution of CDR3 length of TRBV15 in lymph nodes (peripheral) and CNS (infiltrated). Each line corresponding to one mouse (*n* = 4).

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