

# ATH-1105, a Small-Molecule Positive Modulator of Hepatocyte Growth Factor (HGF)/MET, Is Neuroprotective in a TDP-43 Mouse Model of Amyotrophic Lateral Sclerosis

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\*These authors contributed equally to this work.

## CONCLUSIONS

Treatment with ATH-1105 in TDP-43<sup>A315T</sup> ALS mice resulted in

- 1 Improvement in motor function, and protection against body weight reduction
- 2 Preservation of nerve function and structure
- 3 Reduction of plasma biomarkers of systemic inflammation and neurodegeneration
- 4 Prolonged survival and delayed time to first mortality

## KEY TAKEAWAY

This study highlights the therapeutic potential of ATH-1105 in a mouse model of ALS and supports further investigation of ATH-1105 in this disease indication



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### Acknowledgments

This study was sponsored by Athira Pharma, Inc. Medical writing support was provided by Ashley Thoma, PharmD, of ApotheCom, and funded by Athira Pharma, Inc.

### Disclosures

Andrée-Anne Berthiaume, Kayla Kleist, Jewel Johnston, Sherif Reda, Hans J. Moebius, and Kevin J. Church are employees and stockholders of Athira Pharma, Inc.

### Disclaimers

ATH-1105 is an investigational therapy that has not received FDA approval and has not been demonstrated safe or effective for any use.

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## INTRODUCTION

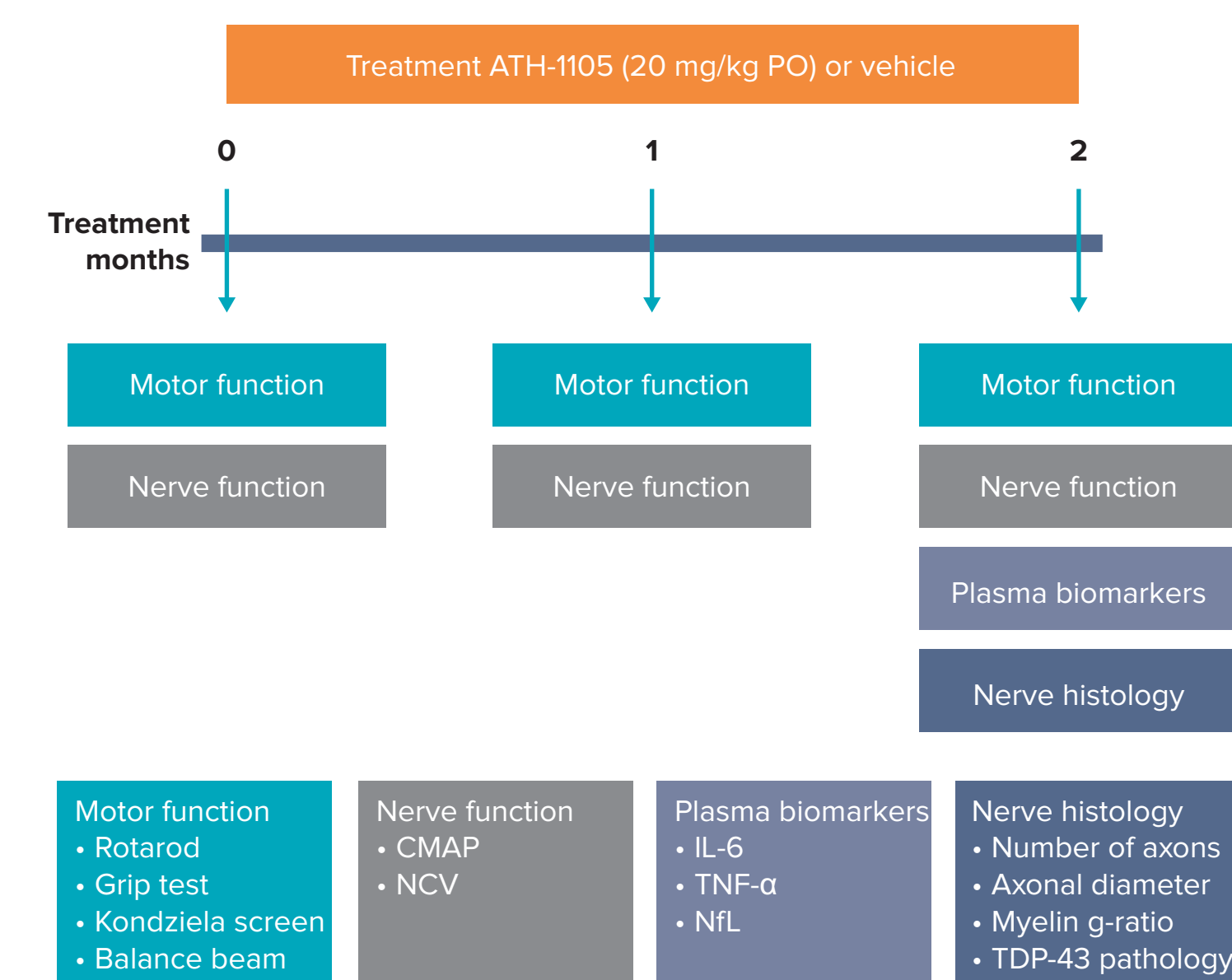
- ALS is characterized by progressive motor neuron degeneration, demyelination, and systemic inflammation<sup>1,2</sup>
  - Up to 97% of people with ALS exhibit TDP-43 proteinopathy<sup>3</sup>
- Beginning at 2 months of age, TDP-43<sup>A315T</sup> mice develop ALS-like deficits in motor and nerve function, motor neuron loss, and systemic inflammation, contributing to early mortality<sup>4</sup>
- Enhancing HGF/MET signaling may counteract the neurodegeneration observed in ALS via neuroprotective mechanisms that counteract neurotoxicity, inflammation, and oxidative stress-induced damage<sup>5-8</sup>
  - See supplemental information for more details (QR code)

## OBJECTIVE

To evaluate the neuroprotective effects of ATH-1105, a small-molecule positive modulator of the HGF/MET system, on function and survival in a TDP-43 mouse model of ALS

## METHODS

Figure 1. Study design

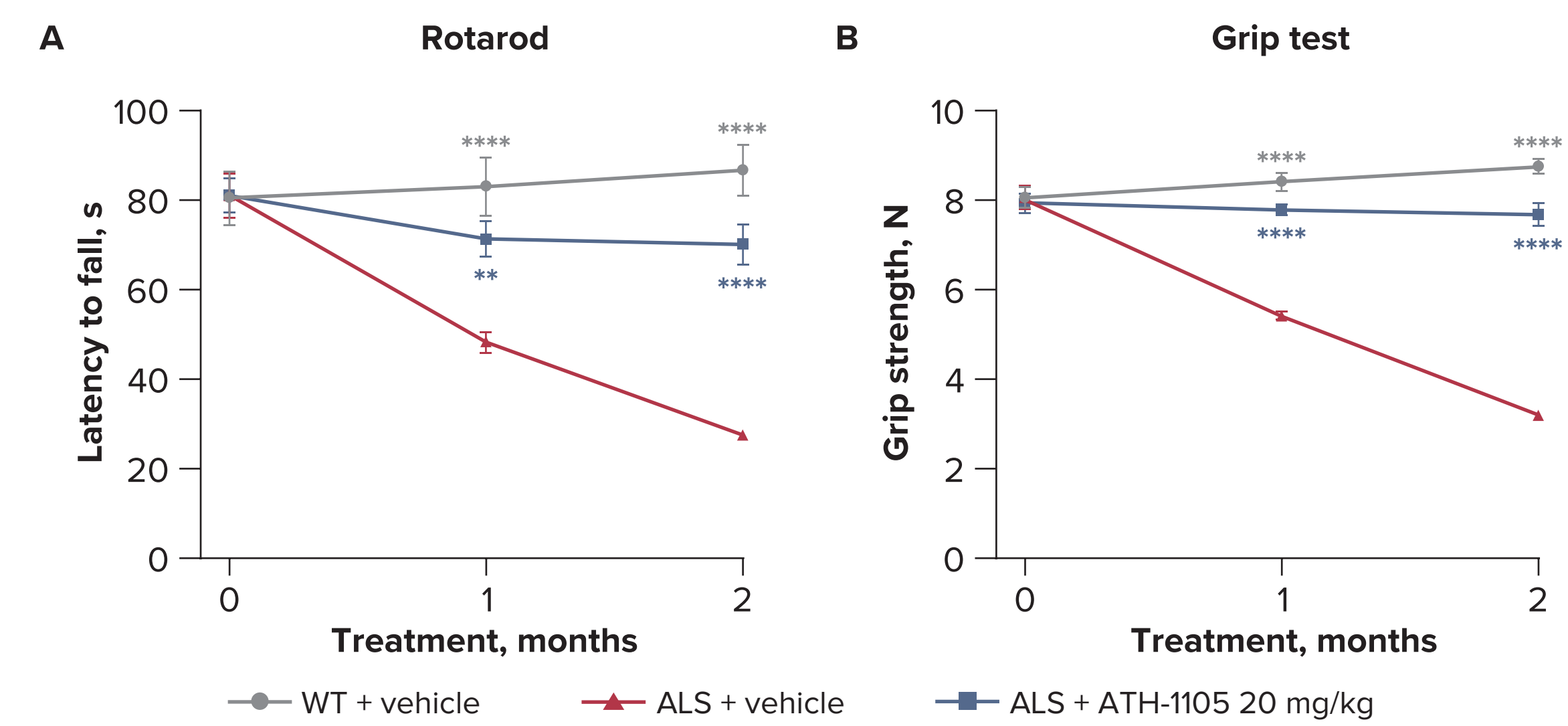


- One-month-old male mice were sorted into 3 groups of 10 animals each and given daily treatment from 1-3 months of age (total of 2 treatment months)
  - WT + vehicle (healthy control): WT mice treated with vehicle (PO)
  - ALS + vehicle (disease control): TDP-43<sup>A315T</sup> mice (JAX #010700) treated with vehicle (PO)
  - ALS + ATH-1105 20 mg/kg: TDP-43<sup>A315T</sup> mice treated with ATH-1105 (PO)
- Behavioral tests, sciatic nerve electrophysiology, histology, and plasma biomarker analyses were carried out as described in the supplemental information (QR code)
- No mortality was observed at or before 3 months of age

- To evaluate effects on survival, a separate cohort of 1-month-old TDP-43<sup>A315T</sup> mice were sorted into groups of 20 animals each and given daily treatment from 1-5 months of age (total of 4 treatment months)
  - ALS + vehicle (PO)
  - ALS + ATH-1105 20 mg/kg (PO)
- The governing ethics committee required humane killing at 5 months of age for this ALS model

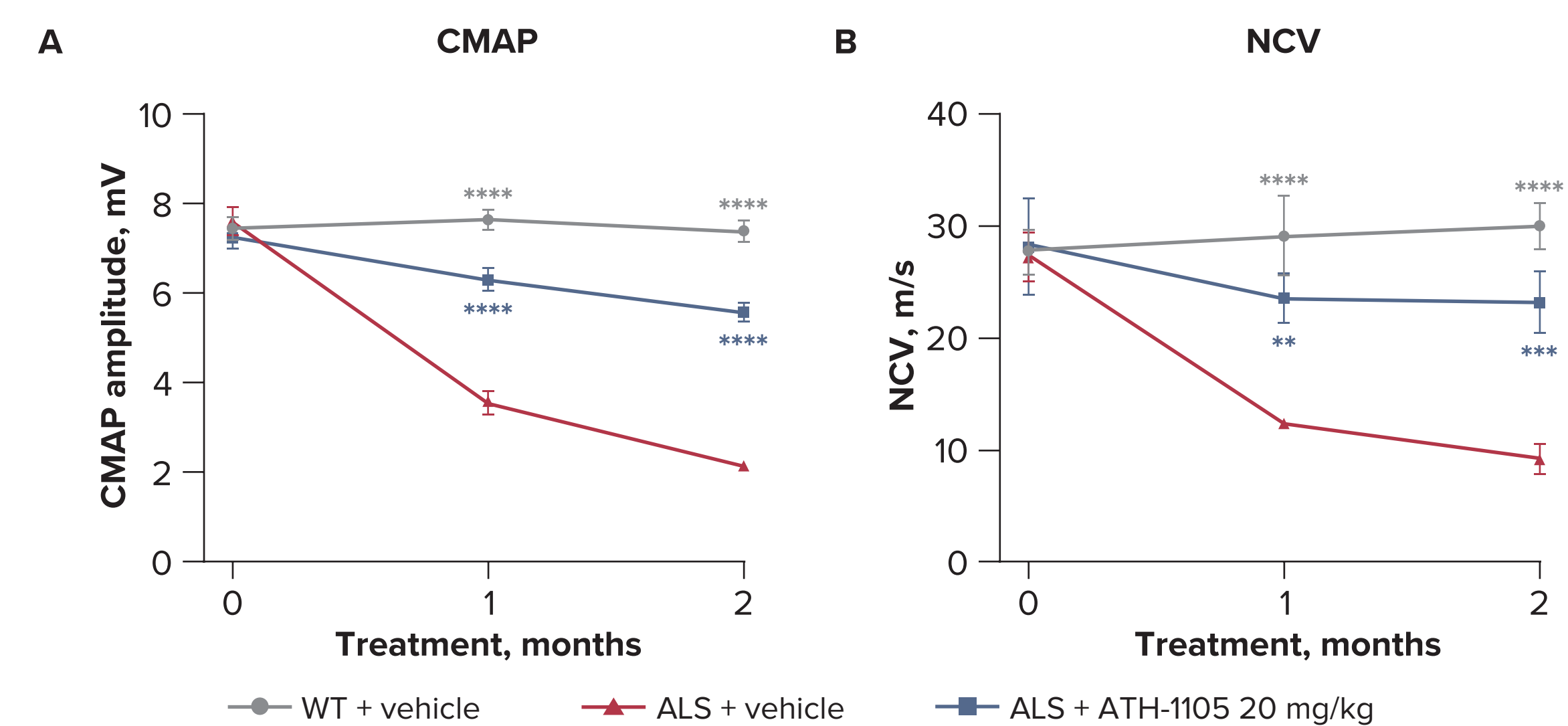
## RESULTS

Figure 2. ATH-1105 significantly improves balance, coordination, and muscle strength



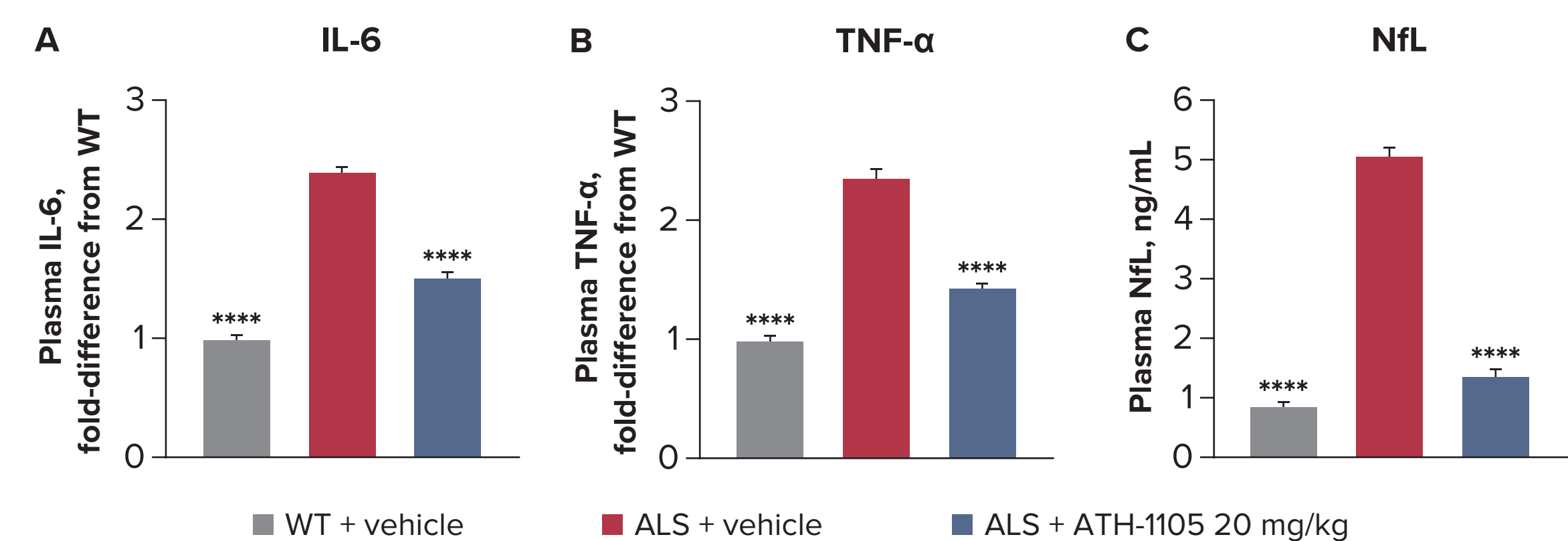
Graphical representation of (A) rotarod latency to fall and (B) grip strength at baseline and after 1 and 2 months of treatment. Significant effects in balance beam cross time and Kondziela screen latency to fall were also observed, as well as significant protection from body weight reduction; these results are presented in the supplemental information (QR code). Data presented as mean ± SEM. Statistical significance was determined by 2-way ANOVA with Dunnett's test versus ALS + vehicle. \*\**p* < 0.01; \*\*\*\**p* < 0.0001.

Figure 3. ATH-1105 significantly improves muscle and nerve function



Graphical representation of (A) CMAP amplitude and (B) NCV at baseline and after 1 and 2 months of treatment. Data presented as mean ± SEM. Statistical significance was determined by 2-way ANOVA with Dunnett's test versus ALS + vehicle. \*\**p* < 0.01; \*\*\**p* < 0.001; \*\*\*\**p* < 0.0001.

Figure 4. ATH-1105 reduces levels of plasma biomarkers of inflammation and neurodegeneration

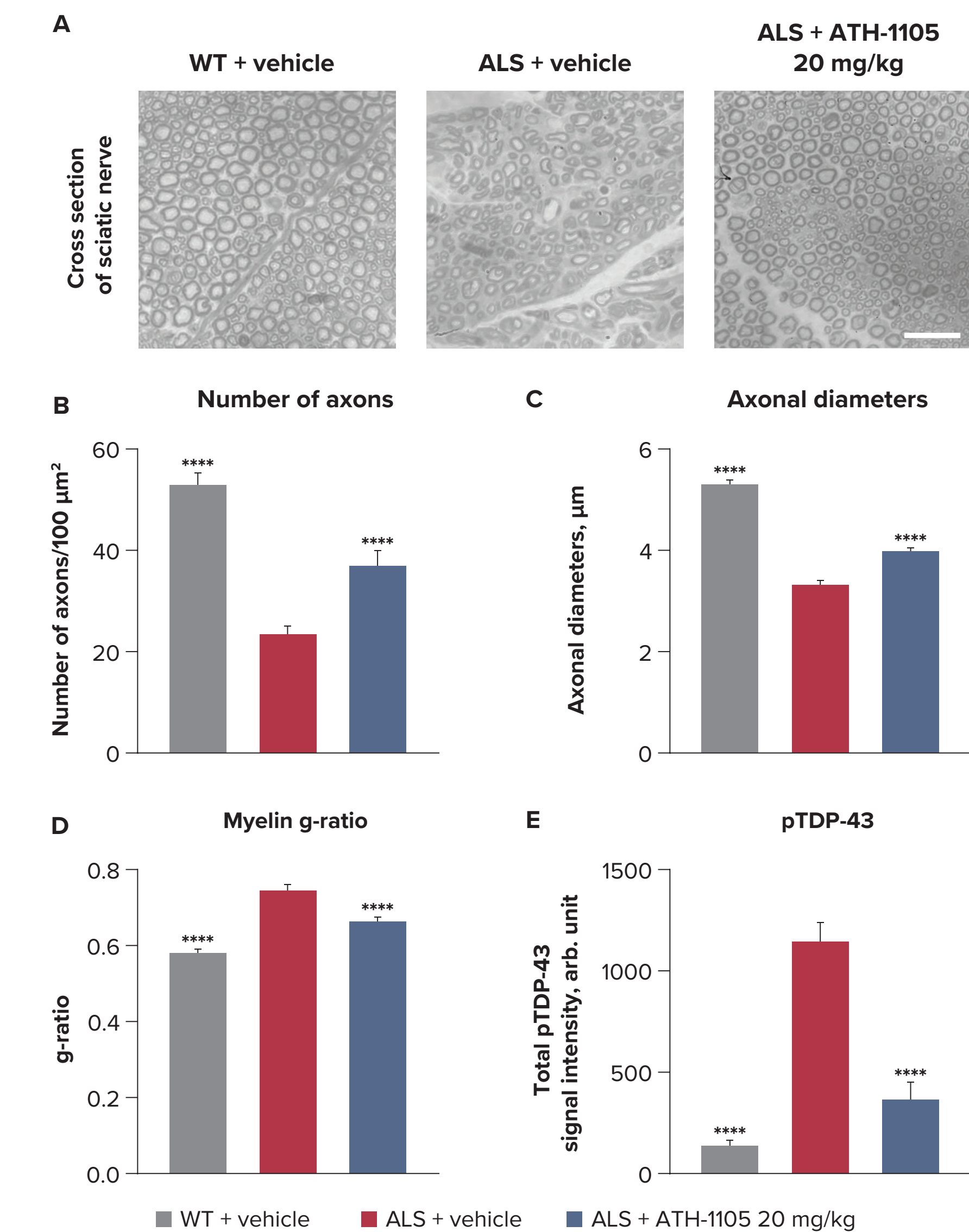


Graphical representation of plasma (A) IL-6 and (B) TNF-α, in fold-difference over the WT + vehicle group at 2 months of treatment. (C) Plasma NFL concentration at 2 months of treatment. Data presented as mean ± SEM. Statistical significance was determined by 1-way ANOVA with Dunnett's test versus ALS + vehicle. \*\*\*\**p* < 0.0001.

**Abbreviations** ALS, amyotrophic lateral sclerosis; ANOVA, analysis of variance; arb, arbitrary; CMAP, compound muscle action potential; HGF, hepatocyte growth factor; IHC, immunohistochemistry; IL-6, interleukin 6; NCV, nerve conduction velocity; NFL, neurofilament light chain; PO, oral gavage; pTDP-43, phosphorylated TDP-43; SEM, standard error of the mean; TDP-43, TAR DNA-binding protein 43; TNF-α, tumor necrosis factor α; WT, wild type.

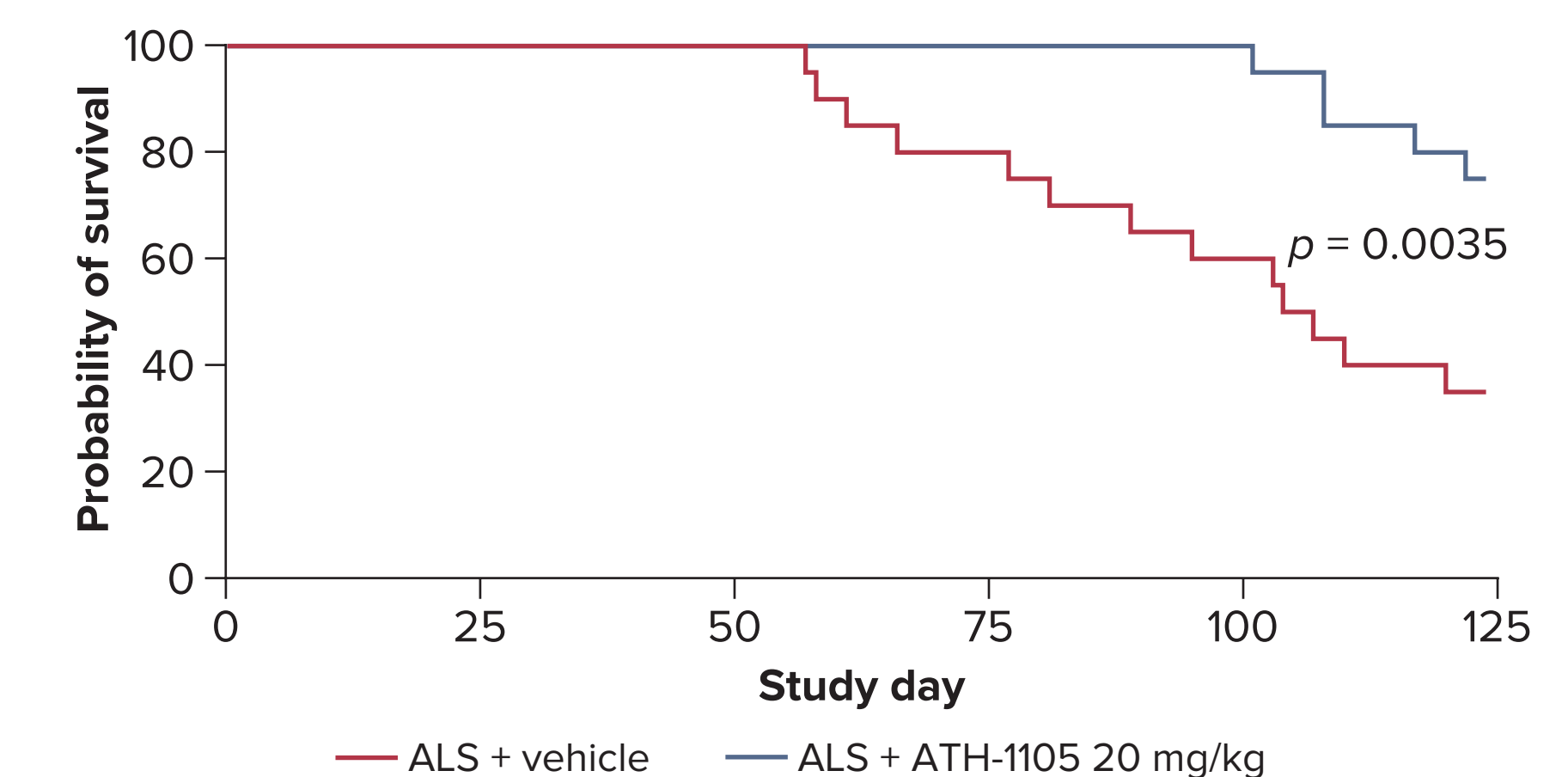
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Figure 5. ATH-1105 protects against axon degeneration and demyelination and reduces pTDP-43 accumulation



(A) Histology images of sciatic nerve cross sections stained with toluidine blue to label myelin. Scale is 10 μm (all panels). Graphical representation of (B) the number of axons, (C) axonal diameters, and (D) myelin g-ratio (defined as the ratio of the inner axonal diameter to the total axonal diameter) after 2 months of treatment. (E) Quantification of pTDP-43 in sciatic nerve sections dual labeled for pTDP-43 (Ser409/410) and the axonal marker Tuj1 by IHC; representative images are shown in the supplemental information (QR code). Data presented as mean ± SEM. Statistical significance was determined by 1-way ANOVA with Dunnett's test versus ALS + vehicle. \*\*\*\**p* < 0.0001.

Figure 6. ATH-1105 prolongs survival and delays time to first mortality



Survival curves up to 4 months of treatment (study day 124; 5 months of age). Time to first mortality with ALS + vehicle was 59 days versus 101 days with ALS + ATH-1105 20 mg/kg. Data presented as Kaplan-Meier survival curves. Statistical significance was determined by the log-rank (Mantel-Cox) test.

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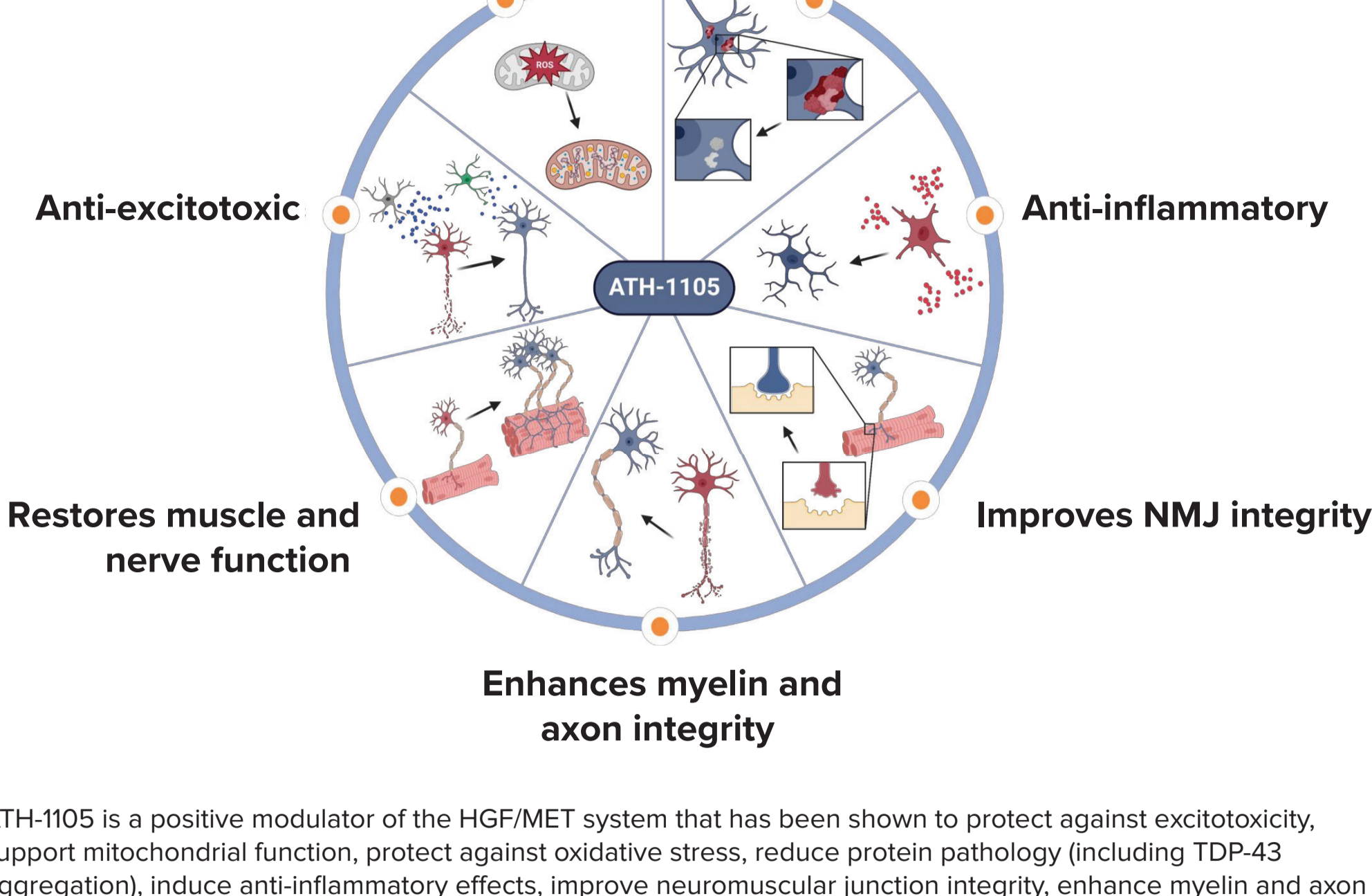
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## SUPPLEMENTAL INFORMATION

### Supplemental Figure S1. Positive modulation of HGF/MET signaling has therapeutic potential in ALS



ATH-1105 is a positive modulator of the HGF/MET system that has been shown to protect against excitotoxicity, support mitochondrial function, protect against oxidative stress, reduce protein pathology (including TDP-43 aggregation), induce anti-inflammatory effects, improve neuromuscular junction integrity, enhance myelin and axon integrity, and ultimately restore muscle and nerve function.

#### Motor function tests

- Rotarod latency: A rotating rod apparatus was used to measure walking performance, coordination, and balance. Latency to fall was measured at successively increased speeds from 4 to 40 rpm, over a 300-second maximum time period
- Grip test: Muscular strength was assessed using standardized grip strength tests for all limbs. All-limb grip strength was measured by placing the animal on a horizontal grid that was connected to a force meter and then pulling the animal's tail until the animal could no longer maintain its grip
- Kondziela inverted screen test: Muscular strength and proprioception was assessed. A vertically positioned grid box allowed mice to grab on to the grid as they climbed down. The latency to fall was quantified
- Balance beam: Animals crossed from one end of a narrow, elevated beam to the other to test balance and coordination. The time necessary to cross the beam was quantified
- For all behavioral tests, an average score from 3 trials was taken for each mouse

#### Sciatic nerve electrophysiology

- CMAP was recorded from the intrinsic foot muscles of anesthetized mice using steel-needle electrodes (MLA1302; AD Instruments)
- Amplitude and latency of CMAP were determined
- The distance between the 2 sites of stimulation was measured alongside the skin surface with the animal's legs fully extended, and NCV was calculated from latency measurements

#### Plasma biomarkers

- Quantification of IL-6, TNF- $\alpha$ , and NFL was performed in duplicate for each animal in 96-well plates by use of ELISA (RAB0308 and RAB0477; Sigma Aldrich and NBP2-80299; Novus Biologicala)

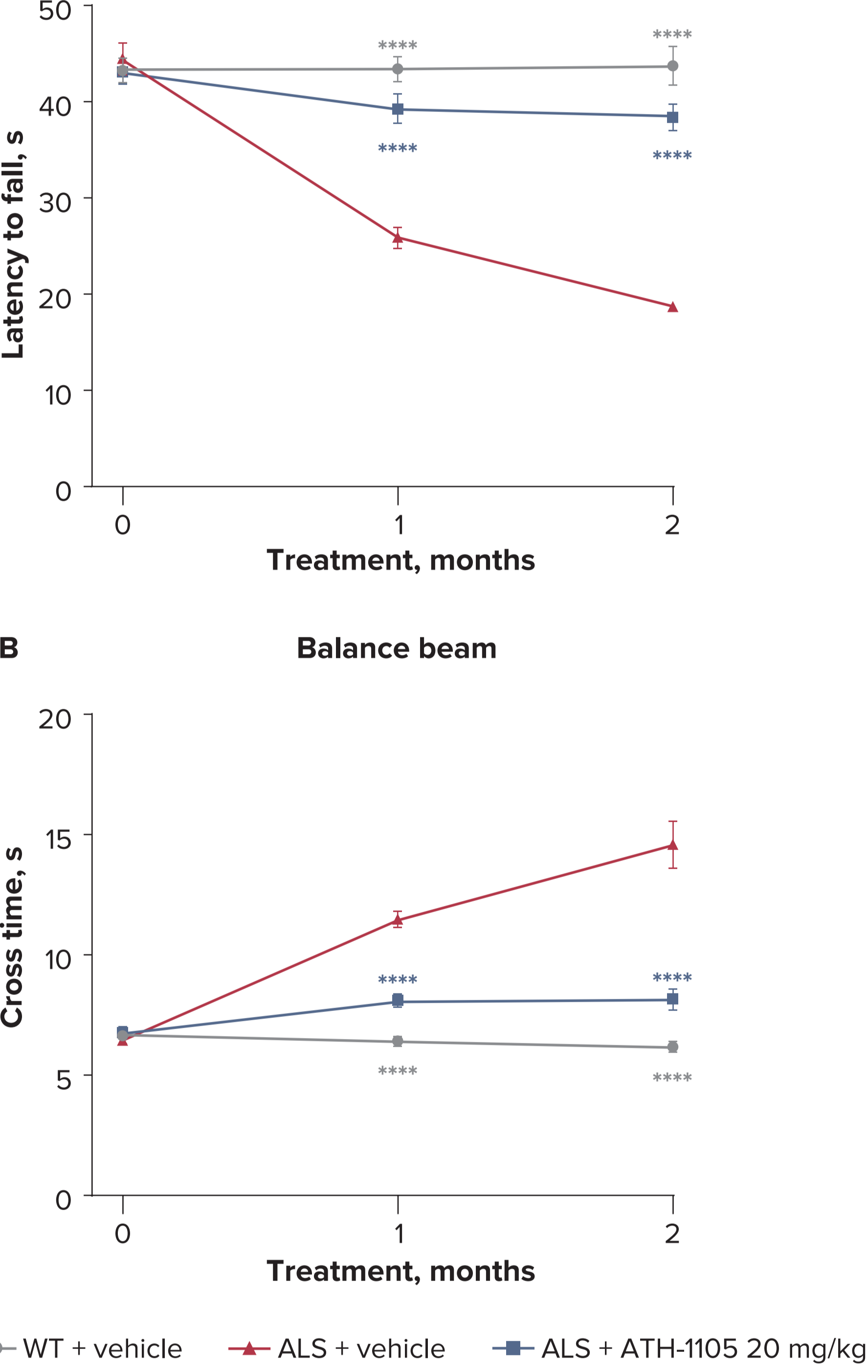
#### Sciatic nerve histology (toluidine blue staining)

- Semi-thin cross sections of fixed sciatic nerves of the left side were cut and stained with toluidine blue, 0.5%, + borax, 1%, + MilliQ water 100 mL
- The axonal diameter, number of myelinated motor axons per 100  $\mu\text{m}^2$ , and the myelin g-ratio were quantified using the ImageJ g-ratio plug-in (<http://gratio.efil.de/>)

#### Supplemental Table S1. Study groups

Group	Genotype	Treatment	Dose	Administration route	Treatment timing	No. of mice (baseline)	No. of nerves for toluidine blue histology	No. of nerves for pTDP-43 IHC
Group 1 healthy control	WT	Vehicle	–	PO	1-3 months old	10	10	7
Group 2 ALS control	TDP-43 <sup>A315T</sup>	Vehicle	–	PO	1-3 months old	10	10	7
Group 3 ALS + ATH-1105	TDP-43 <sup>A315T</sup>	ATH-1105	20 mg/kg	PO	1-3 months old	10	10	7
Group 4 survival ALS control	TDP-43 <sup>A315T</sup>	Vehicle	–	PO	1-5 months old	20	–	–
Group 5 survival ALS + ATH-1105	TDP-43 <sup>A315T</sup>	ATH-1105	20 mg/kg	PO	1-5 months old	20	–	–

### Supplemental Figure S2. ATH-1105 significantly improves balance, coordination, and muscle strength in several behavioral assays

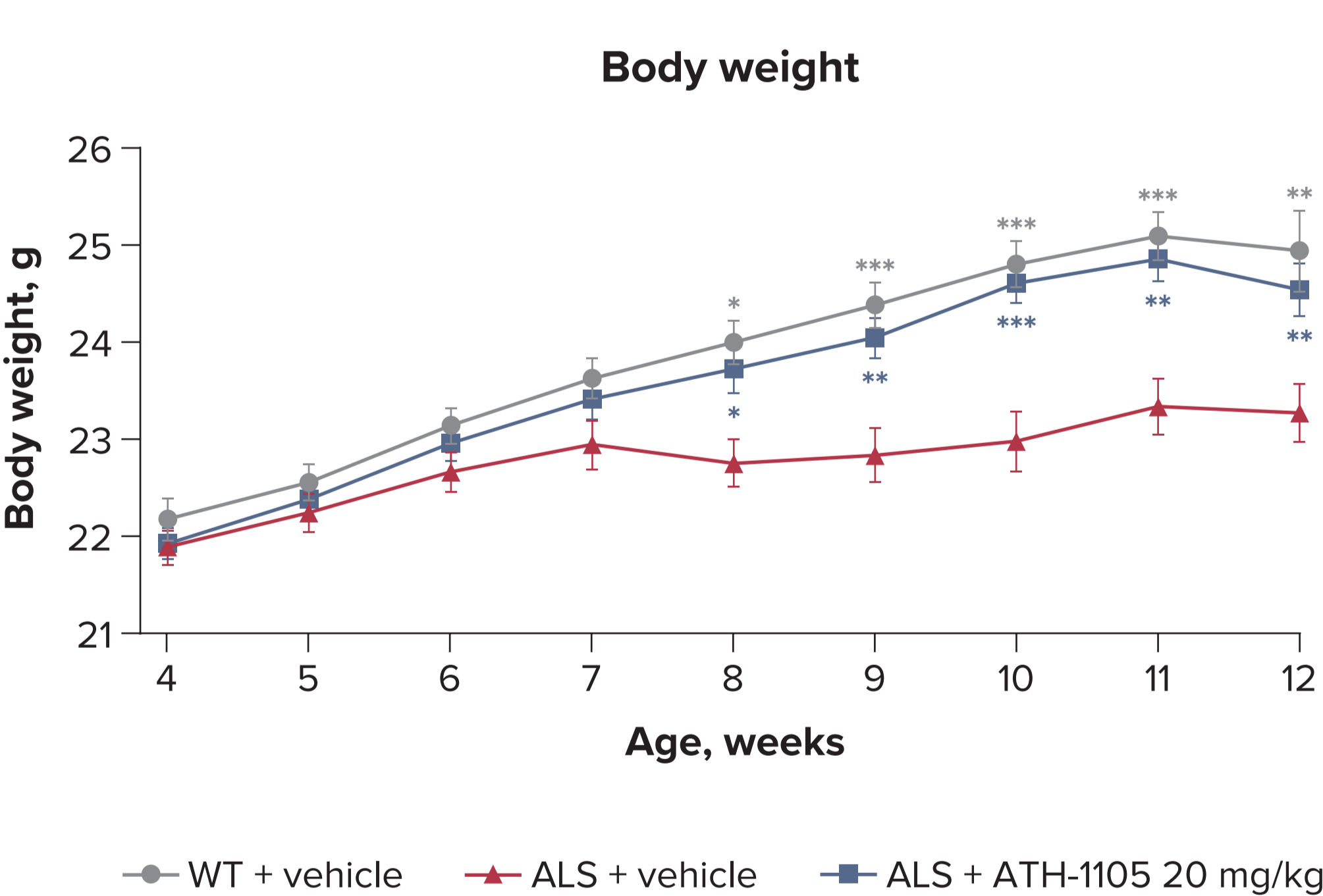


Graphical representation of (A) Kondziela screen latency to fall and (B) balance beam cross time at baseline and after 1 and 2 months of ATH-1105 treatment.

Data presented as mean  $\pm$  SEM. Statistical significance was determined by 2-way ANOVA with Dunnett's test versus ALS + vehicle.

\*\*\*\* $p < 0.0001$ .

### Supplemental Figure S3. ATH-1105 significantly protects against loss of body weight

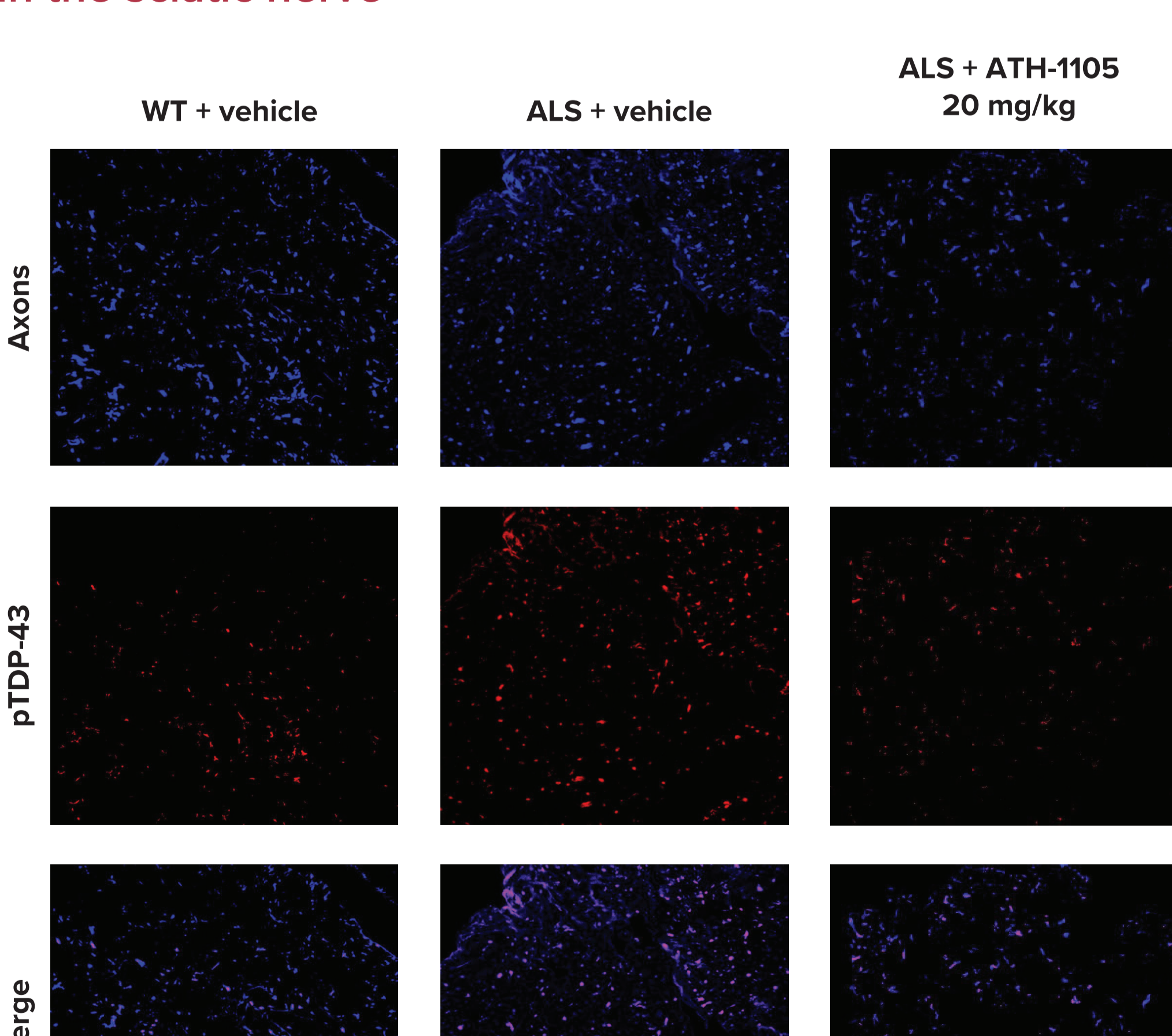


Graphical representation of animal body weight over time.

Data presented as mean  $\pm$  SEM. Statistical significance was determined by 2-way ANOVA with Dunnett's test versus ALS + vehicle.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

### Supplemental Figure S4. ATH-1105 reduces pTDP-43 levels in the sciatic nerve



10- $\mu\text{m}$  thick cross sections of fixed sciatic nerves of the left side ( $n = 7$  mice/group) were cut and labeled with anti-Tuj1, an axonal marker, and anti-pTDP-43 (Ser409/410), a marker of TDP-43 aggregates. Intensity of pTDP-43 labeling was quantified. Representative images are shown. Scale is 50  $\mu\text{m}$  (all panels).

**Abbreviations** ALS, amyotrophic lateral sclerosis; ANOVA, analysis of variance; CMAP, compound muscle action potential; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; IL-6, interleukin 6; NCV, nerve conduction velocity; NFL, neurofilament light chain; NMJ, neuromuscular junction; PO, oral gavage; pTDP-43, phosphorylated TDP-43; SEM, standard error of the mean; TDP-43, TAR DNA-binding protein 43; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; Tuj1, class III beta-tubulin; WT, wild type.

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