

Sympathetic neuropathology is revealed in muscles affected by

Amyotrophic Lateral Sclerosis

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Sympathetic degeneration in Amyotrophic Lateral Sclerosis

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Figure Legends

Supplementary Figure 1. Histopathology of normal murine muscles processed with Neuron Detection Protocol (NDP).

(**A-B**) Haematoxylin-eosin staining of sections of tibialis anterior, harvested from normal 5-monthold C57BL/6J male mice, either processed with our improved NDP (**A**) or directly frozen in liquid nitrogen (**B**).

Supplementary Figure 2. Detection of sympathetic neuron processes in murine muscles.

Confocal immunofluorescence of tibialis anterior sections, processed with NDP, co-stained with antibodies to tyrosine hydroxylase (TH, red) and neurofilament-H (NF-H, green).

Supplementary Figure 3. Sympathetic innervation topology in murine muscles.

(A) Confocal immunofluorescence of tibialis anterior sections, processed with NDP and co-stained with antibodies to tyrosine hydroxylase (TH, purple) and Smooth Muscle Actin (SMA, green), in combination with Cy3-cnjugated phalloidin. (B) Confocal immunofluorescence of *Tibialis anterior* sections, processed with NDP, co-stained with antibodies to tyrosine hydroxylase (TH, green) and anti-myosin-IIa (red).

NDP, tibialis anterior



Supplementary Figure 1

A





Supplementary Figure 2



Supplementary Figure 3

Primary Antibodies	Supplier	Code	Diluition
Mouse anti sarcomeric α -actinin	Sigma-Aldrich	A7732	1:200
AlexaFluor 647-conjugated BTX	Invitrogen	B35450	1:1000
Mouse anti Myosin-I	Developmental Studies Hybridoma Bank	BA-D5	1:100
Mouse anti Myosin-IIa	Developmental Studies Hybridoma Bank	SC-71	1:100
Mouse anti Myosin-IIb	Developmental Studies Hybridoma Bank	BF-F3	1:100
Rabbit anti Neurofilament-H	MyBioSource	MBS4152815	1:1000
Alexa Fluor 568-conjugated Phalloidin	Invitrogen	A12380	1:400
Rabbit anti-Tyrosine Hydroxylase	Millipore	Ab152	1:400
FITC-conjugated Smooth Muscle Actin	Invitrogen	A12380	1:400

Supplementary Table 1. List of primary antibodies and dyes used in this study. BTX, bungarotoxin.