Supplementary Information

The Sympathetic Nervous System Regulates Skeletal Muscle Motor Innervation and Acetylcholine Receptor Stability

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Table S1

Antibodies used for immunoblot

Antibody	Dilution	Source
β2-AR	1:750	Abcam (#13989)
AChR	1:1,000	Biolegend (#838301)
Akt-Phospho	1:1,000	Cell Signaling (#3787)
Akt-Total	1:1,000	Cell Signaling (#9271)
FoXO1-Phospho	1:1,000	Cell Signaling (#9461)
FoXO1-Total	1:1,000	Cell Signaling (#2860)
$G_{\alpha i1, 2, 3}$	1:200	Santa Cruz (#136478)
$G_{\alpha i 2}$	1:200	Santa Cruz (#13534)
GAPDH	1:40,000	Genetex (#GTX627408)
HDAC4	1:2,000	Cell Signaling (#7628)
IκB-Phospho	1:200	Santa Cruz (#8404)
IκB-Total	1:200	Santa Cruz (#371)
MuRF1	1:1,000	R&D Systems (#AF5366)
Myogenin	1:150	Developmental Studies Hybridoma Bank (DSHB)
NFκB-Phospho	1:200	Santa Cruz (#136548)
NFκB-Total	1:200	Santa Cruz (#8008)
PKA (RIα)	1:1,000	BD Biosciences (#610165)
PKA (RIIα)	1:1,000	BD Biosciences (#612242)
Rapsyn	1:500	Sigma (#R2029)
Tubulin	1:1,000	Sigma (#T6199)
Neurofilament-H	1:1,000	Biolegend (SMI 32)
Phosphorylated neurofilament-H	1:1,000	Biolegend (SMI 31P)
Neurofilament-M	1:1,000	Sigma (#N 5264)
Neurofilament-L	1:1,000	Sigma (#N 5139)
PP2A (clone 1D6)	1: 1000	Sigma (#05-421)
PP1a (clone 5E9)	1:1000	Fisher (#PIMA517155)

Table S2

qPCR Primers

GENES	**Primers***
SKELETAL MUSCLE	
Akt1	Mm01331626_m1
Myod1	Mm00440387_m1
Crebbp	Mm01342452_m1
Foxp1	Mm00474848_m1
Per1	Mm00501813_m1
Vti1b	Mm00444004_m1
Ube2g2	Mm00502312_m1
Neurl1a	Mm00480473_g1
Vamp3	Mm01268442_g1
Chrna1	Mm00431629_m1
Chrng	Mm00437419_m1
Myog	Mm00446194_m1
Stxbp1	Mm00436837_m1
Musk	Mm01346929_m1
FoxO1	Mm00490671_m1
FoxO3	Mm01185722_m1
MuRF1	Mm01185221_m1
Hdac4	Mm01299557_m1
Gadd45a	Mm00432802_m1
Fbxo32 (atrogin1)	Mm01207878_m1
SYMPATHETIC GANGLIA	
Ngfr	Mm00446296_m1
Ap1b1	Mm01187764_m1
Mpz	Mm00485141_g1
Mbp	Mm01266402_m1
Egr2	Mm00456650_m1
Ncam1	Mm01149710_m1
Ngf	Mm00443039_m1
Bdnf	Mm04230607_s1
Gdnf	Mm00599849_m1
Gapdh	Mm99999915_g1

* ThermoFisher Scientific.

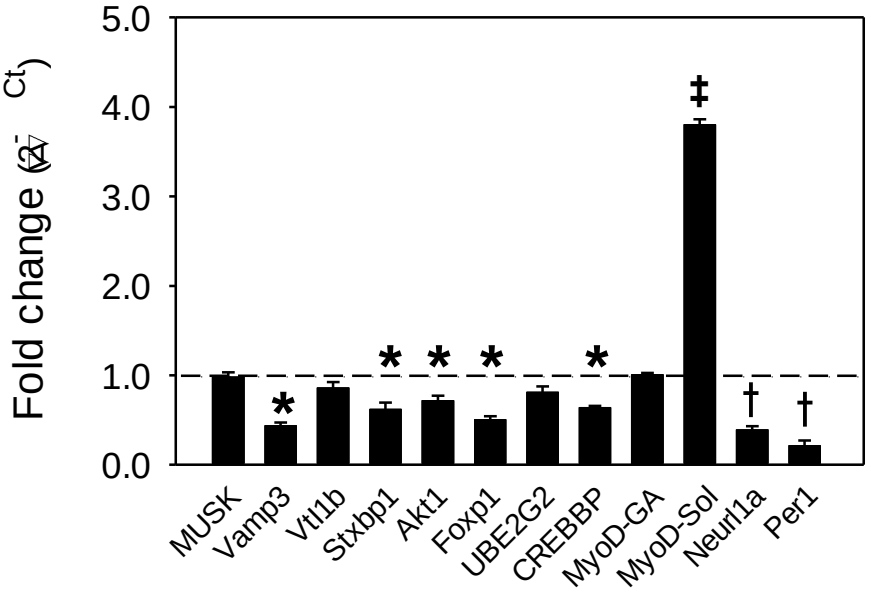

Figure S1. Fold-change ($2^{\Delta\Delta Ct}$) of mRNA expression in sympathectomized compared to sham mice. *MyoD-Sol significantly increased, but Vamp3, Stxbp1*, *Akt1*, *Foxp1*, *CREBBP*, *Neurl1a*, and *Per1* gene expression significantly decreased 7 days after sympathectomy. The difference between sympathectomized and sham mice GA muscle was not significant for all the other genes. N = 4 GA or Soleus muscles from 4 mice per group. * $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$

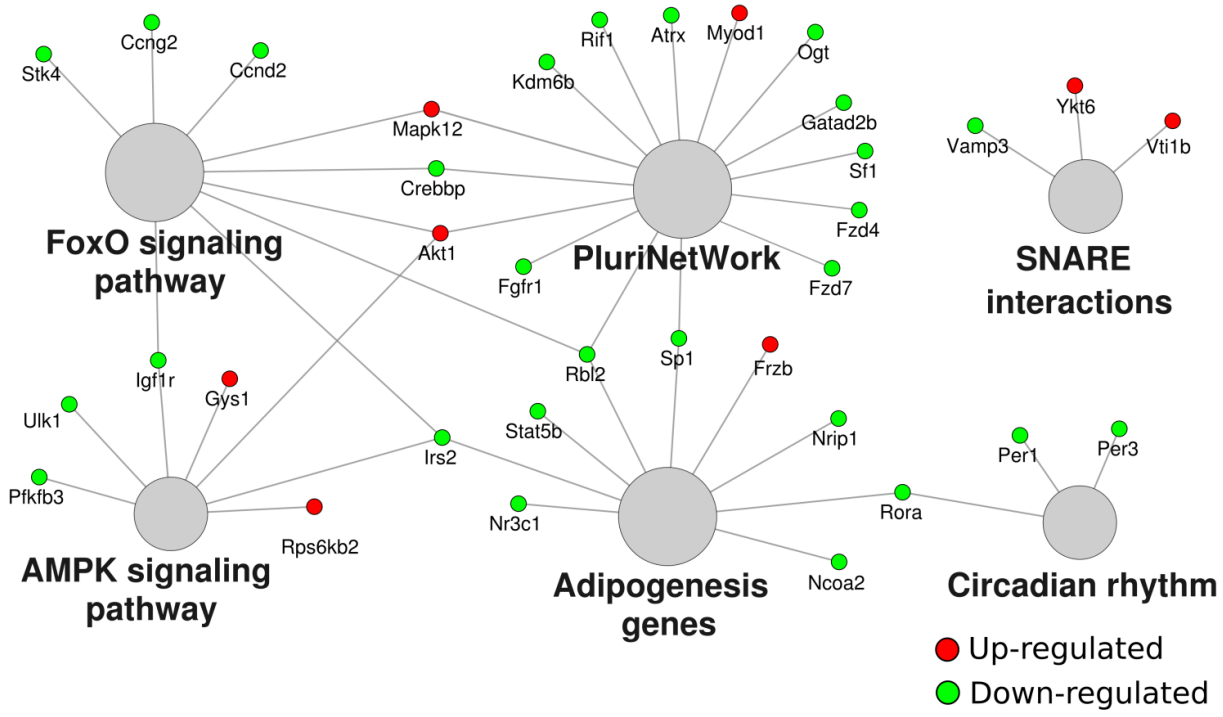

Figure S2. CluePedia networks of the main functionally enriched pathways and differentially expressed genes in sympathetic denervated and sham mice.

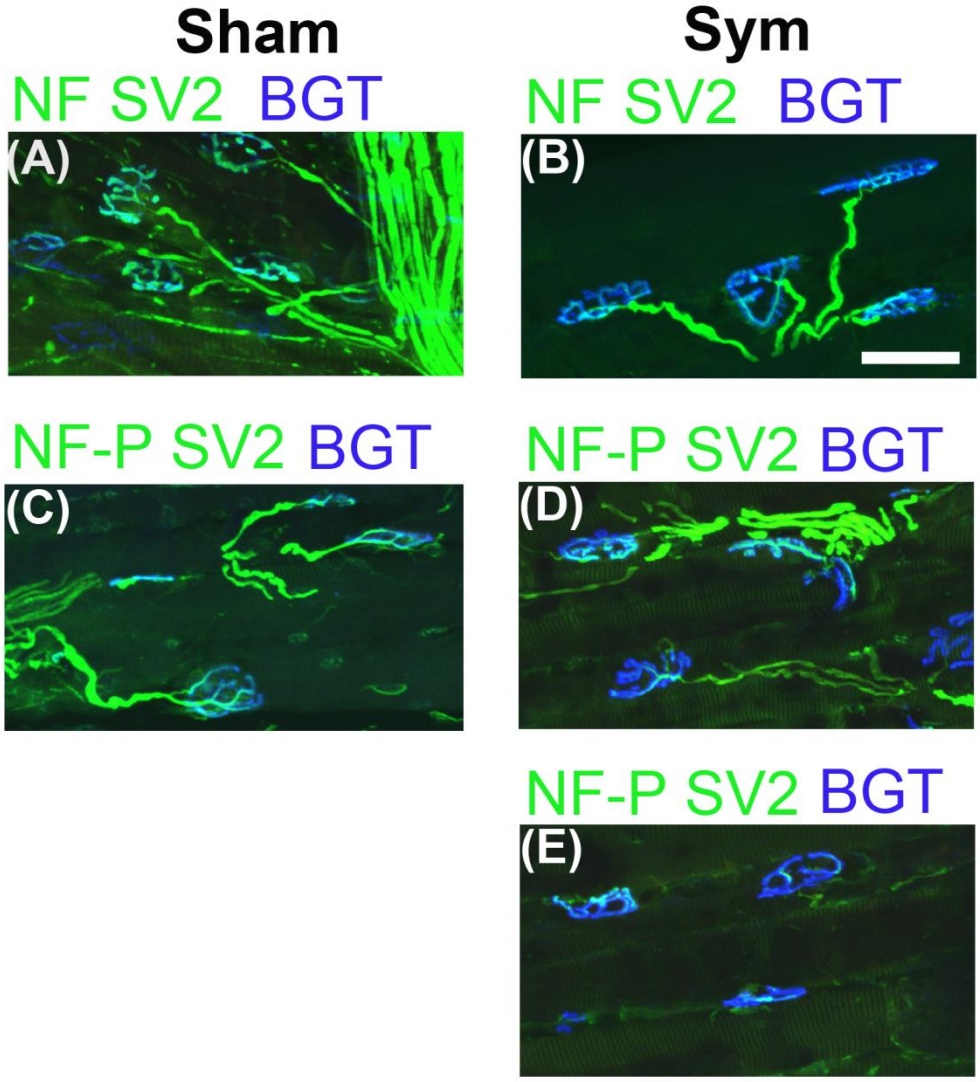

Figure S3. Compromised NF phosphorylation in the Extensor Digitorum Longus (EDL) muscle after lumbar sympathectomy. Z-stack confocal microscopy images of EDL muscles from sham-operated (**A, C**) and 7 day lumbar sympathectomized (**B, D, E**) mice. Panels **A** and **B** show NMJ staining with nonphosphorylated NF (SMI 311 Ab) plus SV2 Ab (green, AF488) and BGT-680 (blue, cy5.5); **C, D** and **E** show staining of phosphorylated NF (SMI 312 Ab)) plus SV2 Ab (green, AF488) and BGT-680 (blue, cy5.5). Light blue indicate NF-SV2 and BGT co-registration. Immunoreactivity to NF-P and SV2 antibodies is positive in the muscle from a sham mouse (**c**) but variable 7 days after sympathectomy (**D, E**). Images are representative of results for 105 NMJs in 3 EDL muscles from 3 sham mice and 69 NMJs in 3 muscles from 3 sympathectomized mice. Calibration bar = 50µm.

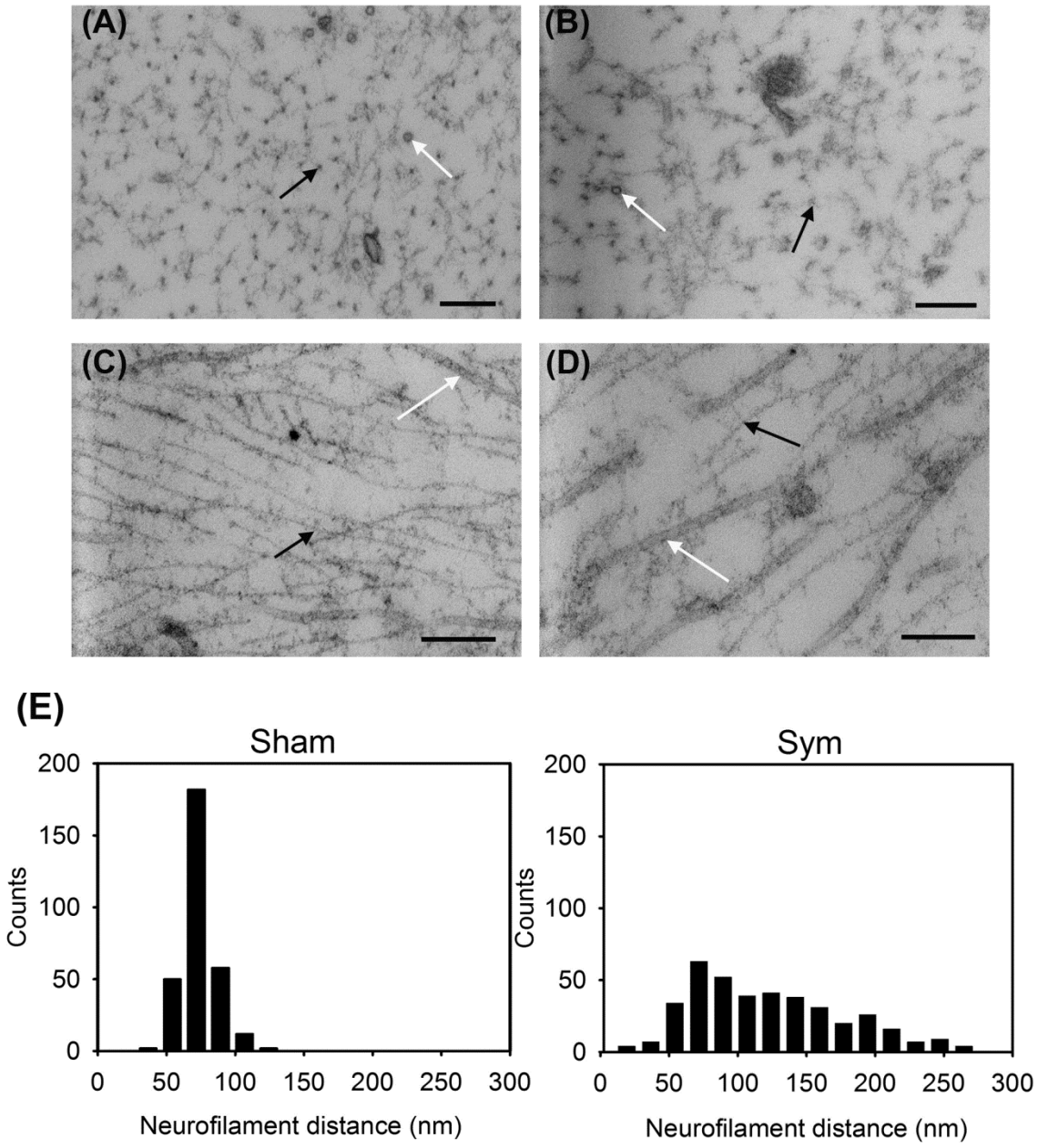

Figure S4. Transmission electron micrographs of neurofilament (black arrows) and microtube (white arrows) disorganization in the tibioperoneal nerve from sympathectomized mice. Representative tibioperoneal nerve cross-sections (**A, B**) and longitudinal (**C, D**) sections from sham (**A, C**) and sympathectomized (**B, D**) mice. Bar = 100 nm. Quantification of the distance between neurofilaments in 5-7 axons in nerves from sham (n = 3) and sympathectomized (3) mice. (**E**).

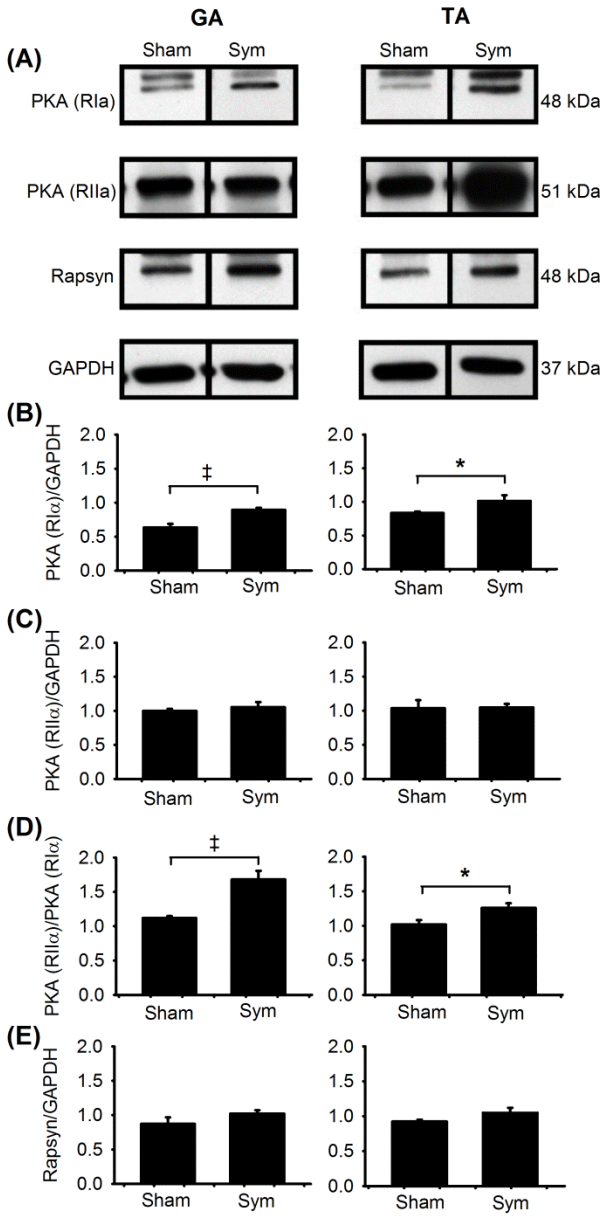

Figure S5. SNS ablation increases PKA (RIa) but decreases the PKA(RIIα)/ PKA(RIα) ratio, with no changes in rapsyn. A. Illustrations of PKA(RIα), PKA(RIIα), and rapsyn in GA and TA muscles. Quantification of PKA (RIa) **(B)**, PKA(RIIα) **(C)**, the PKA(RIIα)/ PKA(RIα) ratio **(D)**, and rapsyn **(E)** in TA and GA muscles. N = 4 GA or TA muscles from 4 mice per group. * $P < 0.05$ and ‡ $P < 0.001$

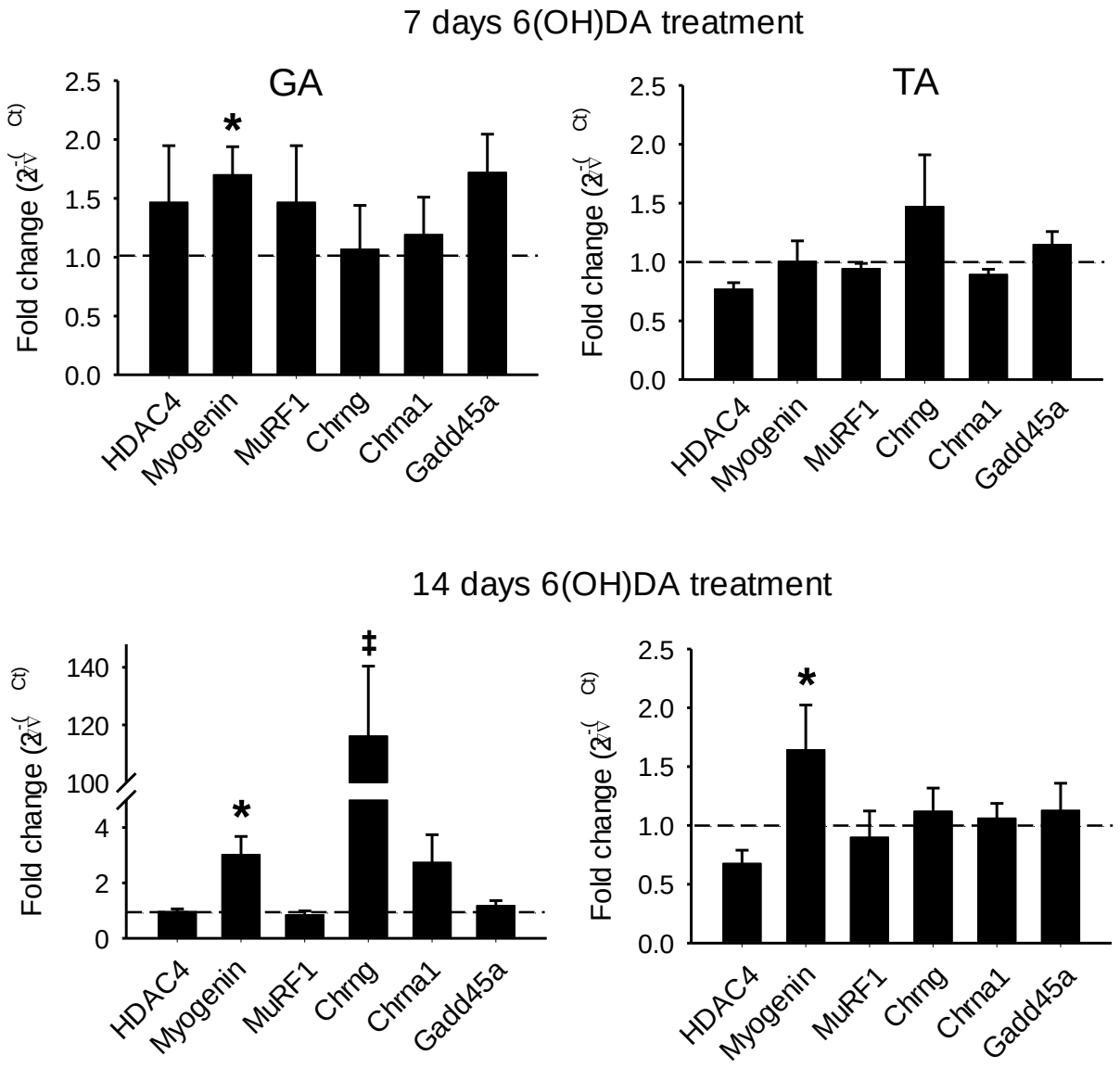

Figure S6. Increased expression of genes associated with skeletal muscle denervation in chemically sympathectomized mice. Gene expression in GA and TA muscles from 8-week old C57BL6 mice injected with 6(OH)DA (n=4 mice) or vehicle (n = 4) on alternate days for 7 or 14 days. All samples were run in triplicate. * $P < 0.05$ and ‡ $P < 0.001$

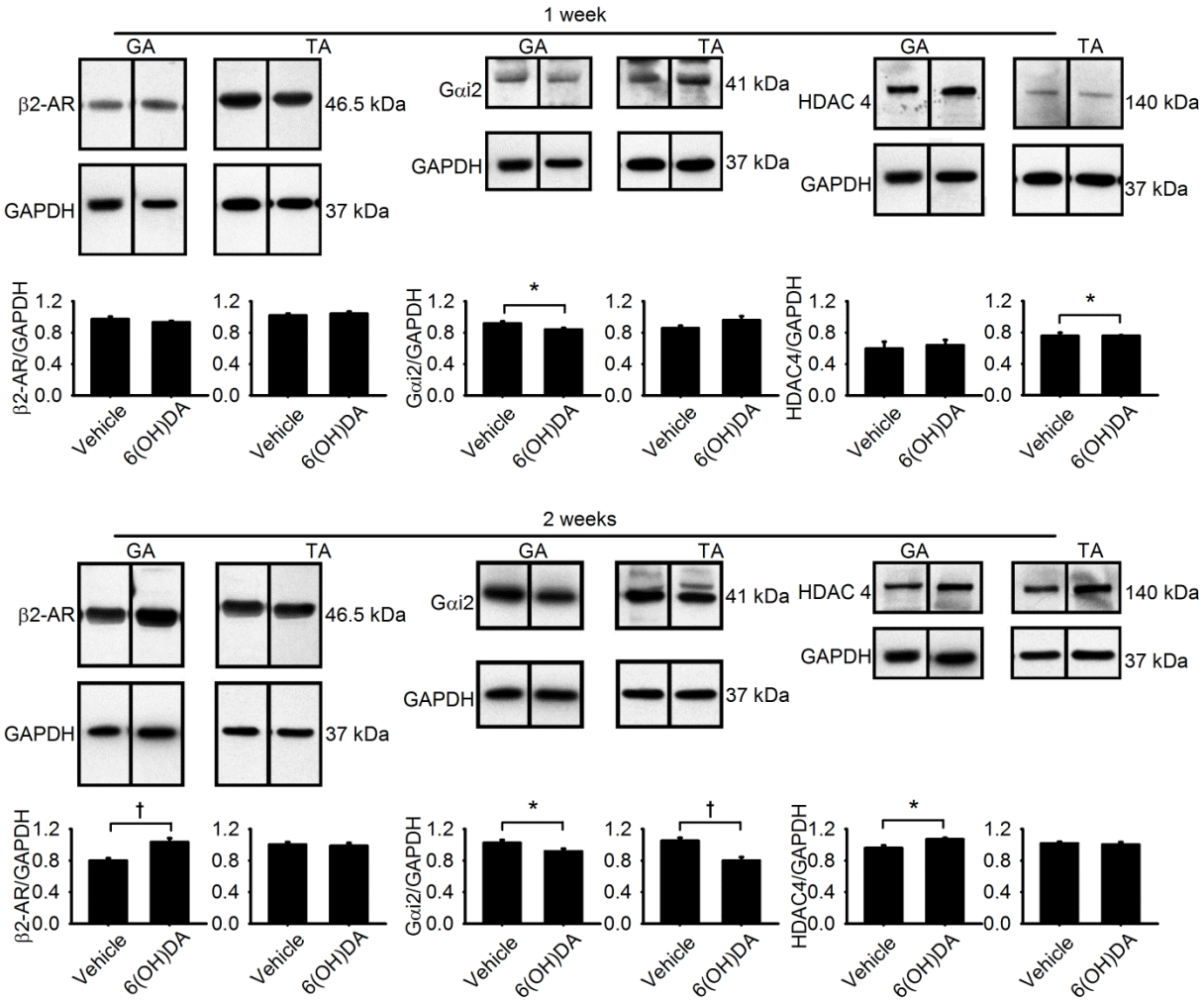

Figure S7. β2-AR, $G\alpha_{i2}$ and Hdac4 protein levels in chemically sympathectomized mice.

Protein levels in GA and TA muscles from 8-week old C57BL6 mice injected with 6(OH)DA (n=4 mice) or vehicle (n = 4) on alternate days for 7 or 14 days. * $P < 0.05$ and † $P < 0.01$

Video 1:

Spatial relationship between sympathetic axons and α-BGT postterminals

The video ***Lumbricalis iDISCO 40XTH BGT 031516.avi*** shows the relationship between sympathetic axons (green) and α-BGT+ (red) postterminals in a lumbricalis muscle. The Z-stack, consisting of 33 images of 2.32 µm/optical slice (image size: 211 x 211 µm, resolution: 0.132 µm/pixel, 12 bits/pixel) were recorded at a sampling speed of 2µs/pixel with an Olympus FV1200/IX83 spectral laser scanning confocal microscope using a UPLFLN40X0 objective, NA 1.3.

RNA Microarrays for sympathectomized and sham mice:

RNA Microarrays in GA muscle from both mice groups have been uploaded as Excel spreadsheets (***Microarray_Sym vs Sham***). The worksheet *Sym_vs_Sham* lists the 182 most significantly modified genes (**see Figures 2 and S2**).

Comparison of RNA microarrays for sympathectomized and sciatic denervated [44] mice.

The Excel file ***twoDataSets_comparison_GSE1893 and WF*** provides a detailed analysis of up- and down-regulated genes in both datasets. Using DAVID functional assessment, these genes clustered into 60 gene ontology (GO) term enrichment and KEGG pathways.