Supplementary Materials

Figure S1 - Mouse positioned during the cryotherapy session

Figure S2 - User's Guide: Using confocal microscopy for analysis of neuromuscular junction (NMJ)

This user guide comprises a workflow designed for use in conjunction with Figure 3 and Table 2 of the manuscript. The following descriptions guide the user through the analysis steps of each NMJ using *Image J software*. The instructions in italics refer to the functions selectable from the *Image J software* menus. See the manuscript for further explanations and definitions of individual terms. Contact Castro, P.A.T.S. by email: paula.soupat@gmail.com.

1.Determining the working scale

Open your image by *File - Open*, then on the bar scale of your figure make an overlap of the size using the / *(straight line)* icon. Click *Analyze - set scale* and enter the bar scale value in the *Know distance* item and set the *unit of length in µm* item. To analyze all the figures in the study, click *Global* and standardize the scale of the complete experiment. Click on *Analyze - set measurements* and define which statistical measures you want to use to analyze your study of the figure (in our study, *area* parameters were used for Figure 3).

2.Selecting your image

After viewing the RGB image, via *Image - Color - Split channel*, select the channel (*red, green or blue)* you want to work on.

3.Treshold Targeting

Particle analysis requires the image to be binary. For this image transformation, *Image - Adjust - Threshold*. Pixels within the threshold range are displayed in red. The user changes to *Dark background - Apply* and then the image threshold is converted to a binary image.

4. Defining the background area

To define the *area*, draw a rectangle in the *background area* and press *Ctrl+C*, then go to *File - New - Internal Clipboard*. A *Clipboard* window will open. In the *Clipboard* window, press *Ctrl+M* and save *the result of the background area* (it will be used as a normalizer).

5. Definition of total sample area

Analysis of the *sample area* requires the parameters of the *Analyze - Set scale* in *area*. To define the area, draw a rectangle in the desired *sample area* and press *Ctrl+C*, then go to *File*

- *New - Internal Clipboard*. A Clipboard window will open. In the *Clipboard window*, press *Ctrl+M* and save the result of the *sample area*.

6.Definition of fragmented area

With the same rectangle established for the *sample area*, the *fragmented area* is defined using the *Wand (tracing) tool*. In the *Clipboard window*, press *Ctrl+M* and save the result of the *fragmented area*.

87 **Figure S3 - Oligonucleotide primers used for real-time PCR amplification of reverse**

88 **transcribed RNA.**

Housekeeping	GAPDH foward	CCTTTTCAAGCGTCTTCCTG
	GAPDH reverse	CTCCTGGCTTCTGTCTTTGG
	HPRT foward	GTGCAACCATTGCCCTAAGT
	HPRT reverse	CAGCCAGCATCTCAGGTGTA
	BETA ACTINA foward	GACGGCCAGGTCATCACTAT
	BETA ACTINA reverse	AAGGAAGGCTGGAAAAGAGC
	B2M foward	ATGGGAAGCCGAACATACTG
	B2M reverse	CAGTCTCAGTGGGGGTGAAT
Nicotinic Acetylcholine Receptors (nAChR)	γ-nAChR foward	CCACCAGAAGGTGGTGTTCT
	γ-nAChR reverse	TGAGGAGATGAGCACACAGG
	α1-nAChR foward	TCCCAGCATCTAGCACACAG
	α1-nAChR reverse	GAAAGGCACCACCAAACAGT
	ε-nAChR foward	CCAGGAAACCCTCTTCTTCC
	ε-nAChR reverse	ATGACTGTGGAAGGGTCAGG
Neuromuscular Signaling Pathway	AGRIN foward	GCACACCTTCGAGAGAGACC
	AGRIN reverse	CATGTGGAGTTGTGGGAGTG
	MUSK foward	AGCCGATGTGTCTGCTCTTT
	MUSK reverse	AGTGTGCGGGGACATACTTC
	RAPSYN foward	ACGGACAGGTCCACAAACTC
	RAPSYN reverse	AGCAGGTCCAGCCTCTACAA
Atrophy Pathway	MURF-1 foward	CTCCCCTTTGTGGTGTGTCT
	MURF-1 reverse	GAGGCAGGAGGCACACTTAG
	ATROGIN-1 foward	CTCACGGAACACTTTGCTGA
	ATROGIN-1 reverse	CACCTTCACCTGACACATGG
	α-DISTROBREVIN foward	CCTATCTGCACCAGCCTAGC
	α-DISTROBREVIN reverse	AGCGGAGTTAGGCACTGAAA
	UTROPHIN foward	AGCACAGGGAAGCTGGAGT
	UTROPHIN reverse	GGAAGTTGAAGCAGGTGAGC

89

90

91