**Collagen content calculation**

The presence of collagen was labeled in the biopsies with green colour and was used to identify the contours of muscle fibres (darker regions). Aiming to avoid possible artefacts from the samples, we decided to take regions of interest (ROIs) with circular shape from each image. The ROIs were selected in regions where the tissue was not altered or broken.

To calculate the collagen content in the images we took a maximum of 3 circular ROIs per image. These ROIs had 700 pixels of diameter, and they did not overlap between them. An adaptive threshold was used to differentiate collagen and muscle fibers, binarizing each image depending of theirs levels of intensity.

**Muscle fibres geometric features extraction and nuclei counting**

To calculate geometric characteristics, and counting the number of nuclei from the muscle fibres, we used a unique circular ROI with 2200 pixels of diameter. For these procedures, we selected 10 circular ROIs from each type of muscle in each genotype of mouse.

-Extraction of geometric characteristics.

In order to get quantifiable features of the muscle fibres with accuracy, was necessary a perfect segmentation of the muscle fibres outlines (from the biopsies marked with green colour). It was as follows: first, we run a segmentation script over the selected ROIs using an adaptive threshold in a similar way that the program used for calculating collagen content. Second, we executed a set of morphological operations to split joined fibres and to delete the noise and little artefacts. Finally, a manual correction step (using Adobe Photoshop CS6) was necessary to successfully complete the identification of the fibres outlines.

This final image was used to extract the 8 geometric characteristics from all the full cells (no portions of cells) into the ROI of each image. We extracted these eight geometrical features from the muscle fibres: mean area, standard deviation area, mean minor axis, mean major axis, mean relation between axis, standard deviation relation between axis, mean convex hull and standard deviation convex hull.

The segmentation technique and the geometric features extraction was based in previous papers (Sáez et al., 2013; Sánchez-Gutiérrez et al., 2017).

-Nuclei counting.

We segmented the dapi staining in the biopsy (contained in the ROI) that was labelled in blue colour: (1) We automatically adjusted the contrast of the image highlighting the nuclei presence. Next, the image was converted into a logical matrix, treating any captured information for dapi as 1’s and the rest as background (0’s). (2) We deleted the noise of the image: objects with a really small area. (3) We calculated the range of intensity of the objects representing the nuclei. We stablished two different thresholds for getting the peaks of intensity representing the individual nuclei: first, for small objects the intensity threshold was low, avoiding the disappearance of the real nuclei. For the second one, a major threshold was applied to the bigger objects than the average size objects (several nuclei were joined), for capturing their individual presence. Finally, we counted the number of nuclei captured into the ROI.

All the cited protocols have been developed using Matlab R2014b.

Sáez, A., Rivas, E., Montero-Sánchez, A., Paradas, C., Acha, B., Pascual, A., Serrano, C., and Escudero, L.M. (2013). Quantifiable diagnosis of muscular dystrophies and neurogenic atrophies through network analysis. BMC Med. *11*.

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