Supplemental material

Supplemental methods

From all muscle biopsy samples, 5 μm thick cross-sections were cut at -20 ºC using a cryostat. Muscle samples collected from one participant of each group were mounted together on uncoated glass slides, and air-dried for 3 h at room temperature before being stored at –20 ºC for subsequent analyses. Muscle cross-sections were stained to determine muscle fiber type distribution, i.e. type I and type II muscle fibers, myonuclear and satellite cell content.

First antibodies used were directed against MHC-I (A4.951, Developmental Studies Hybridoma Bank [DSHB], Iowa City, IA; dilution 1:20), laminin (polyclonal rabbit anti-laminin; Sigma, Zwijndrecht, The Netherlands; dilution 1:50), and CD56 (BD biosciences, San Jose, CA; dilution 1:40). CD56 has been extensively used to identify satellite cells (1-4). Appropriate secondary antibodies were applied: goat anti-rabbit IgG AlexaFluor555 (Molecular Probes: dilution 1:50), goat anti-mouse IgG1 Alexa488, Goat anti-mouse biotine (Vector Lab: dilution: 1:133), and streptavidine Alexa 488 (Molecular Probes: dilution 1:200). Nuclei were stained with 4-,6-diamidino-2-phenylindole (DAPI; Molecular Probes; 0.238 μM). The immunohistochemical staining procedures for satellite cell content were adapted from previously published methods (1, 3, 5). Fiber type staining resulted in laminin stained in red, nuclei in blue and MHC-I green. In addition, satellite cell staining resulted in laminin stained in red, nuclei in blue, and CD56 in green (**Figure 1**).

From the biopsy slides, all images were captured using a Nikon E800 fluorescence microscope (Nikon Instruments Europe, Badhoevedorp, the Netherlands) outfitted with a Basler A113 C progressive scan color CCD camera with a Bayer color filter. Image processing and quantitative analyses were done using the Lucia 4.81 software package, as described previously (1, 2, 5). Images were captured at 120x magnification. Laminin was used to determine cell borders, and for all muscle fibers within each image, type I (green), and type II (black) fibers were identified. Within each image the number of muscle fibers and the mean fiber cross-sectional area (CSA) were measured. Fiber circularity was calculated as $(4\pi \cdot CSA)/(perimeter)^2$ to confirm fiber cross-sectional orientation. No differences were observed in fiber circularity between groups or fiber types. For muscle fiber size a mean total of 425±48 (mean ± SE) muscle fibers were analyzed for each muscle biopsy sample collected from healthy young, healthy elderly and hip fracture patients. The frequency distribution was calculated to acquire further insight into the distribution and variability of muscle fiber size. Intervals of 1000 μm^2 were defined and the percentage of muscle fibers in each interval was determined for the type I and type II muscle fibers separately.

Images were captured at a 240x magnification to allow clear satellite cell localization from the satellite cell stained muscle cross-sections. Laminin was used to visualize cell borders. Satellite cells were determined at the periphery of each fiber and stained positive for both DNA (DAPI) and CD56.

Fiber typing was determined by matching type I and type II muscle fibers in the serial muscle fiber type slides (**Figure 1**). The number of satellite cells per muscle fiber was calculated for type I and type II muscle fibers separately. Moreover, the number of myonuclei and central myonuclei per muscle fiber, as well as the mean myonuclear domain (i.e. fiber CSA/#myonuclei per fiber) were assessed for the type I and type II muscle fibers within each image. According to Mackey *et al.* (6), at least 150 fibers are needed to accurately assess muscle fiber satellite cell content. In this study, we evaluated a mean total of 397±28 muscle fibers for muscle fiber type-specific analyses of satellite cell content per muscle sample.

References

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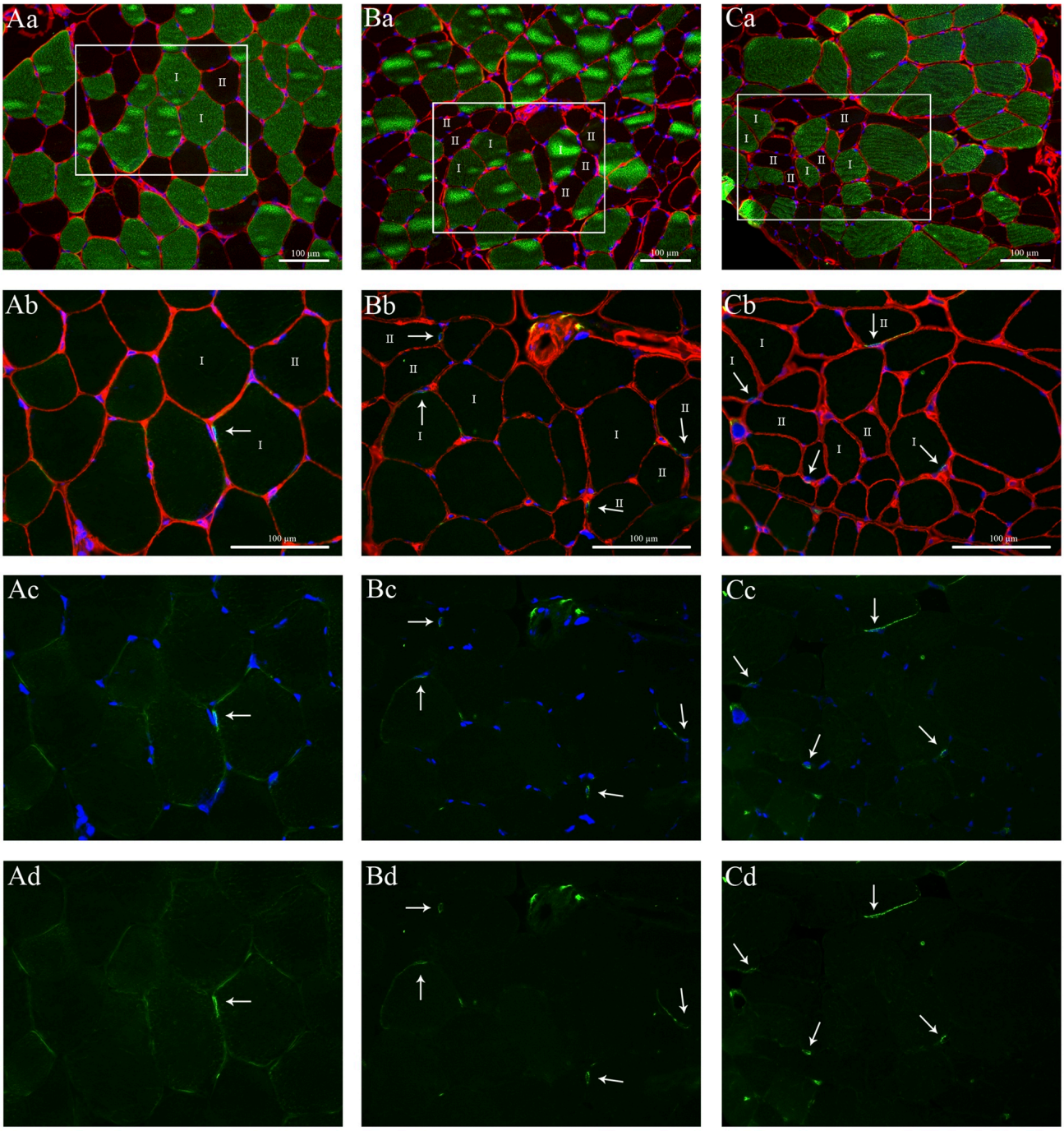
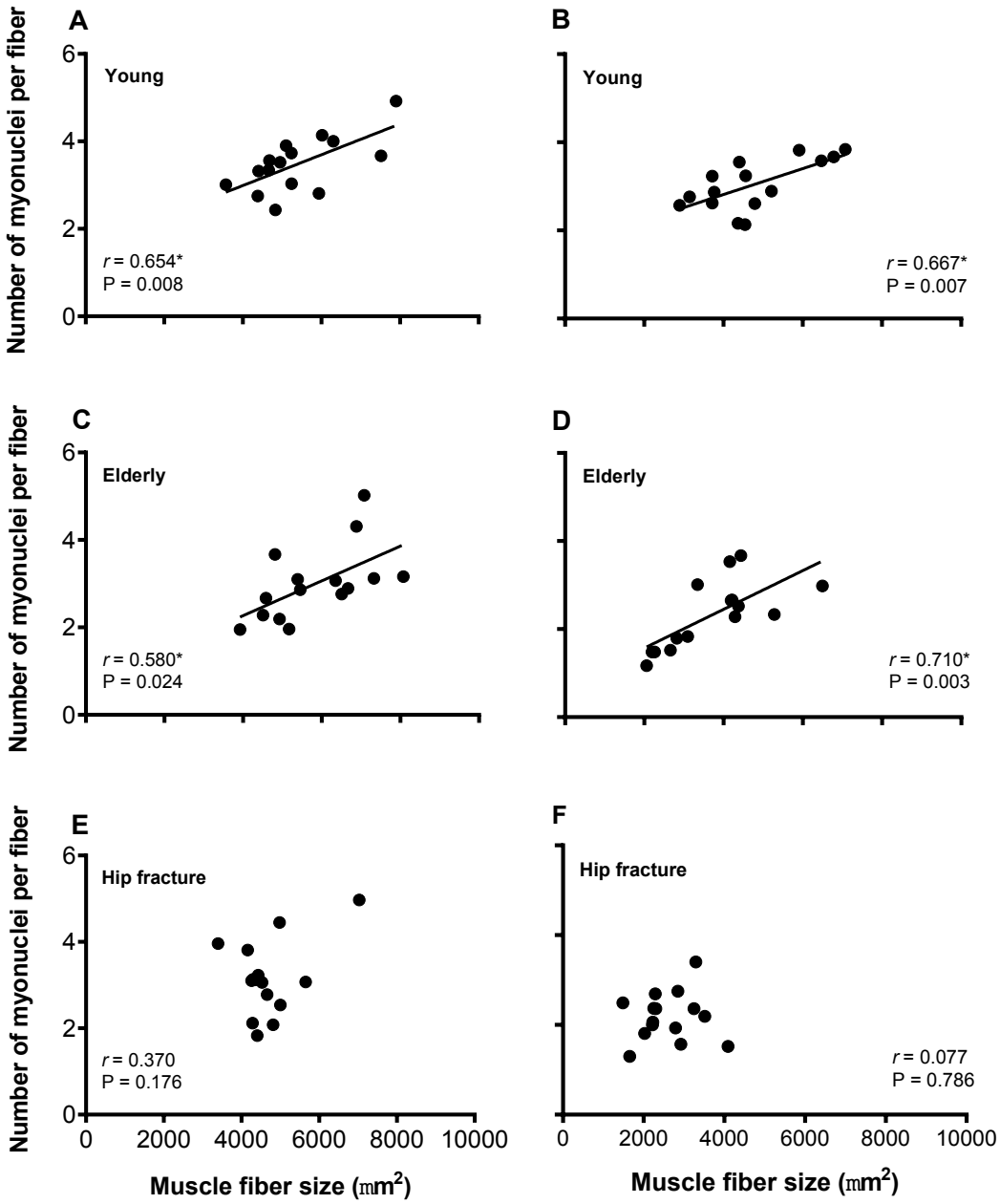
Supplemental Figure 1



Supplemental Figure 1. Fiber type-specific analyses of skeletal muscle satellite cell content in the young (**A**), elderly (**B**), and hip fracture group (**C**). (**Aa-Ba-Ca**) MHC-1(green) +laminin (red) +dapi staining (blue); the marked area represents the same area as presented in frames Ab-Cd. (**Ab-Bb-Cb**) CD56 (green) +dapi (blue) +laminin (red). (**Ac-Bc-Cc**) CD56+dapi staining. (**Ad-Bd-Cd**) CD56 staining. Numbers indicate type I and type II muscle fibers. Arrows point at the satellite cells.

Supplemental Figure 2



Supplemental Figure 2. Scatter plot for the relation between the number of myonuclei per muscle fiber and muscle fiber size for the healthy young (**A** + **B**), healthy elderly (**C** + **D**), and hip fracture group (**E** + **F**) for both type I and type II muscle fibers separately. *r:* Pearson correlation coefficient. * significant correlation (P<0.05).