

## Supplementary Information

### Rehabilitative Exercise and Spatially Patterned Nanofibrillar Scaffolds Enhance Vascularization and Innervation Following Volumetric Muscle Loss

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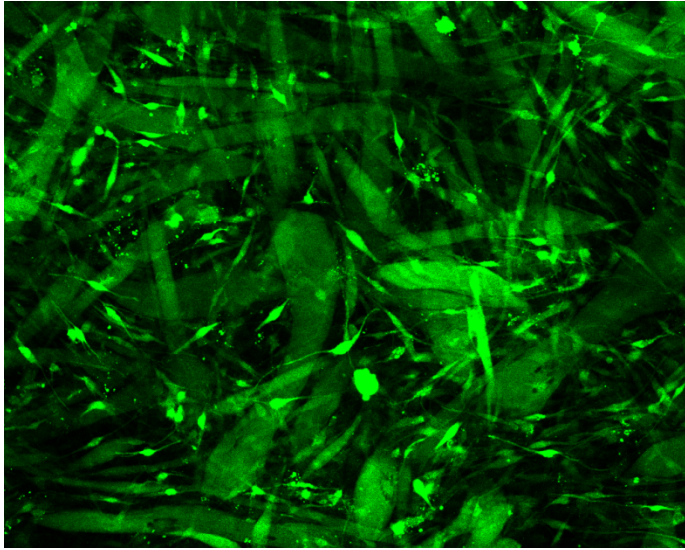
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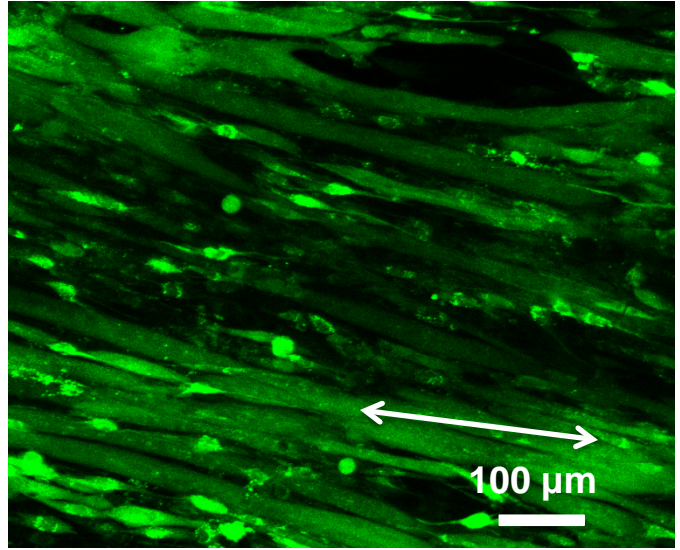
Dr. Ngan F. Huang, PhD

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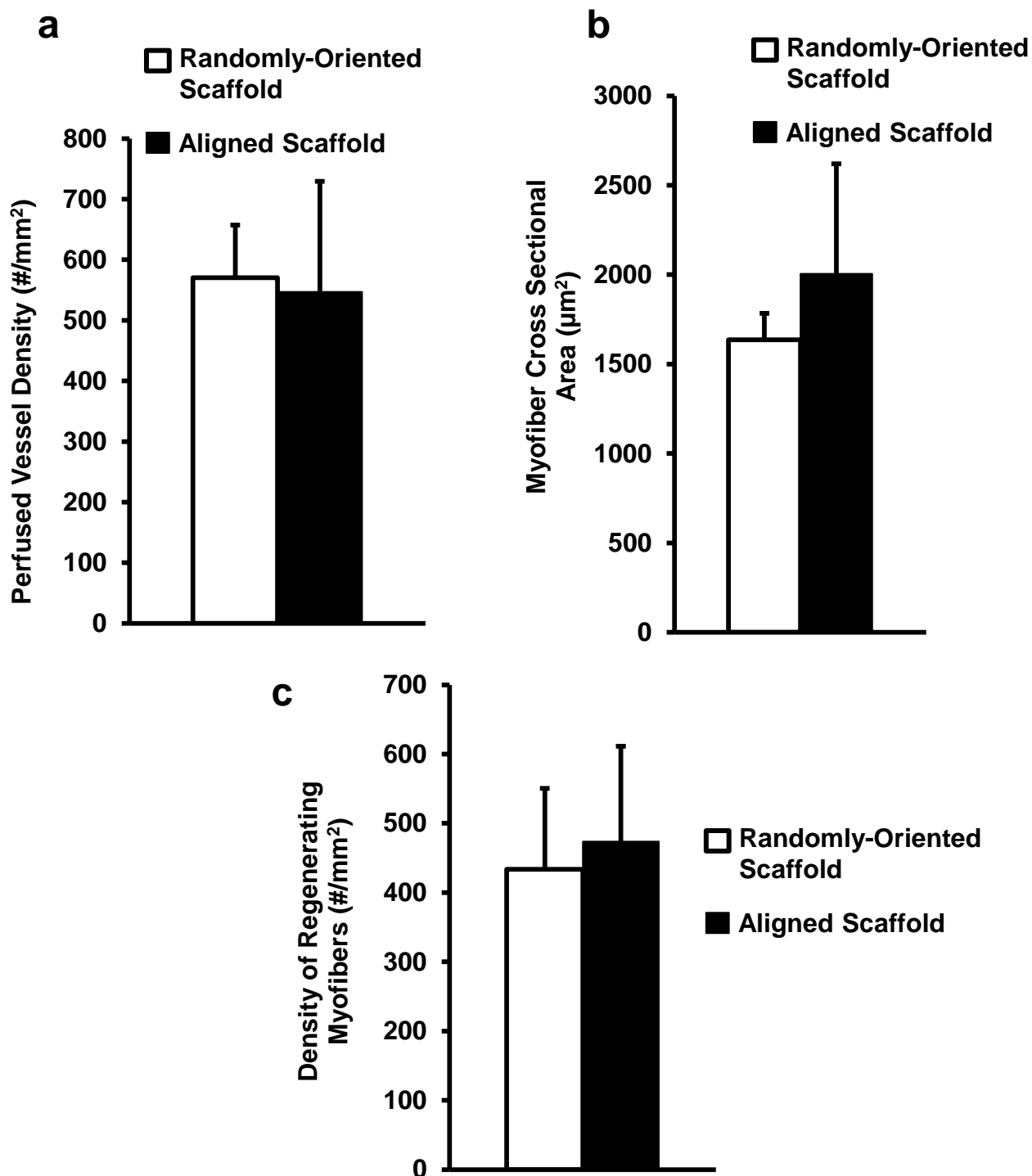
Randomly-Oriented Scaffolds



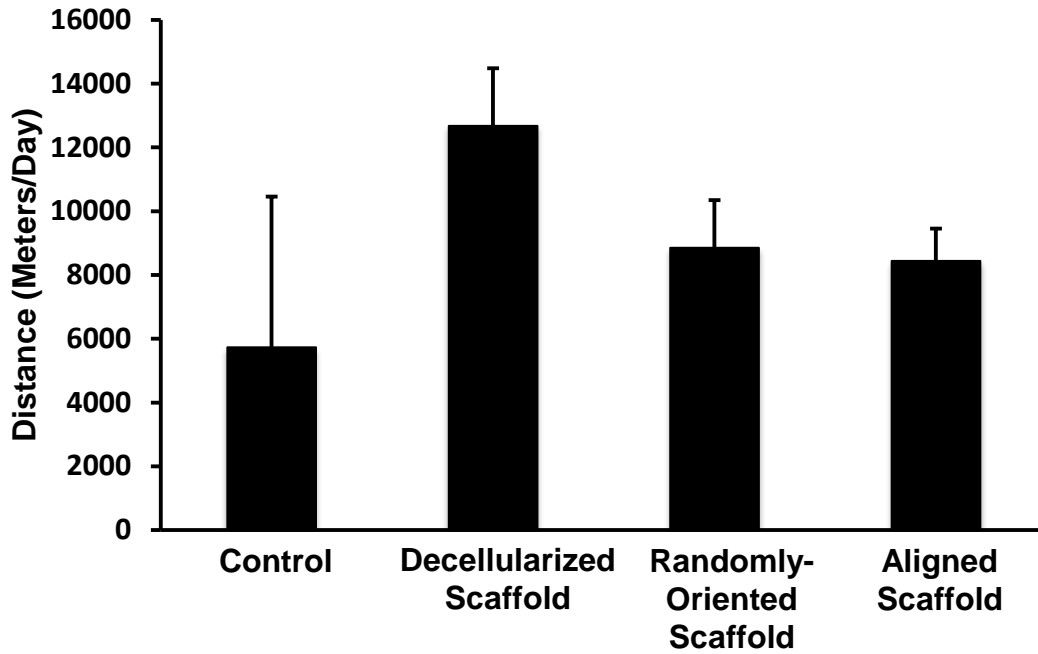
Aligned Nanofibrillar Scaffolds



**Supp Fig 1. Green fluorescence protein-expressing mouse myotubes formed on randomly oriented or aligned scaffolds after 5 days of differentiation.** Double arrows depict orientation of aligned nanofibrils.



**Supp. Fig. 2. Muscle regeneration and revascularization after implantation of aligned or randomly oriented scaffold aggregates in a mouse model of VML. A-C.** Randomly oriented or aligned scaffolds were implanted into the void space of the ablated tibialis anterior muscle. After 3 weeks of implantation in the absence of exercise, immunofluorescence analysis was performed on the tibialis anterior muscle for antibodies against CD31, myosin heavy chain (MHC), and laminin. Within a 500μm distance from the edge of the scaffold, quantification of perfused (CD31<sup>+</sup>/isolectin<sup>+</sup>) vessel density (A), MHC<sup>+</sup> myofiber cross sectional area (B), and *de novo* myogenesis (# MHC<sup>+</sup> myofibers /mm<sup>2</sup>) (C) were performed. Shown are mean ± SD (randomly oriented scaffold (n=4), aligned scaffold (n=6)). Error bars denote standard deviation.



**Suppl. Fig. 3. Caged Wheel running distance (meters).** 7 days after initial transplants, mice were introduced to exercise wheels and running distance was tracked per day. Graph shows the averaged running distance per day for each treatment group (n=3 each group). Error bars denote standard deviation.

## No Exercise

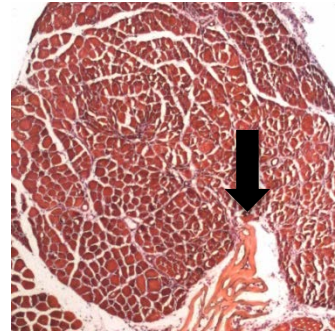
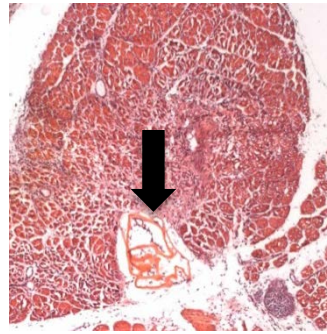
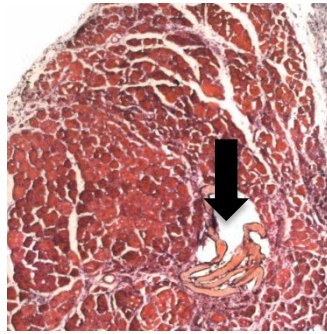
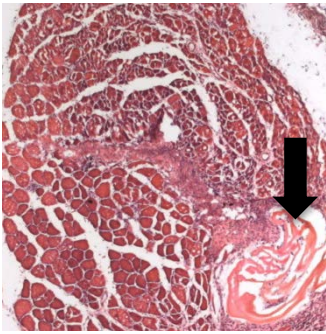
## With Exercise

Randomly-  
Oriented Scaffold

Aligned  
Scaffold

Randomly-  
Oriented Scaffold

Aligned  
Scaffold

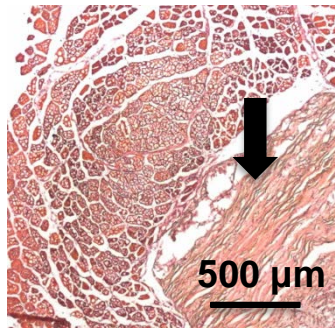
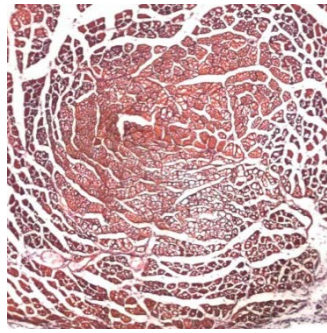
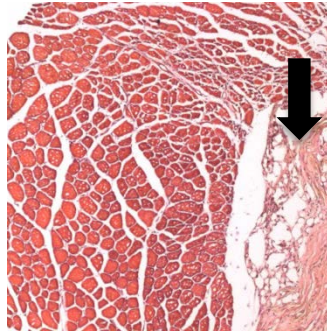
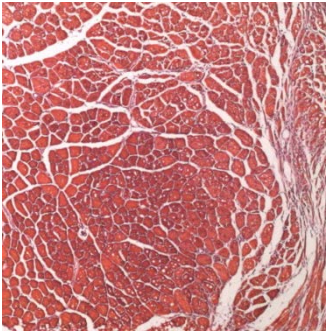


Control

Decellularized  
Scaffold

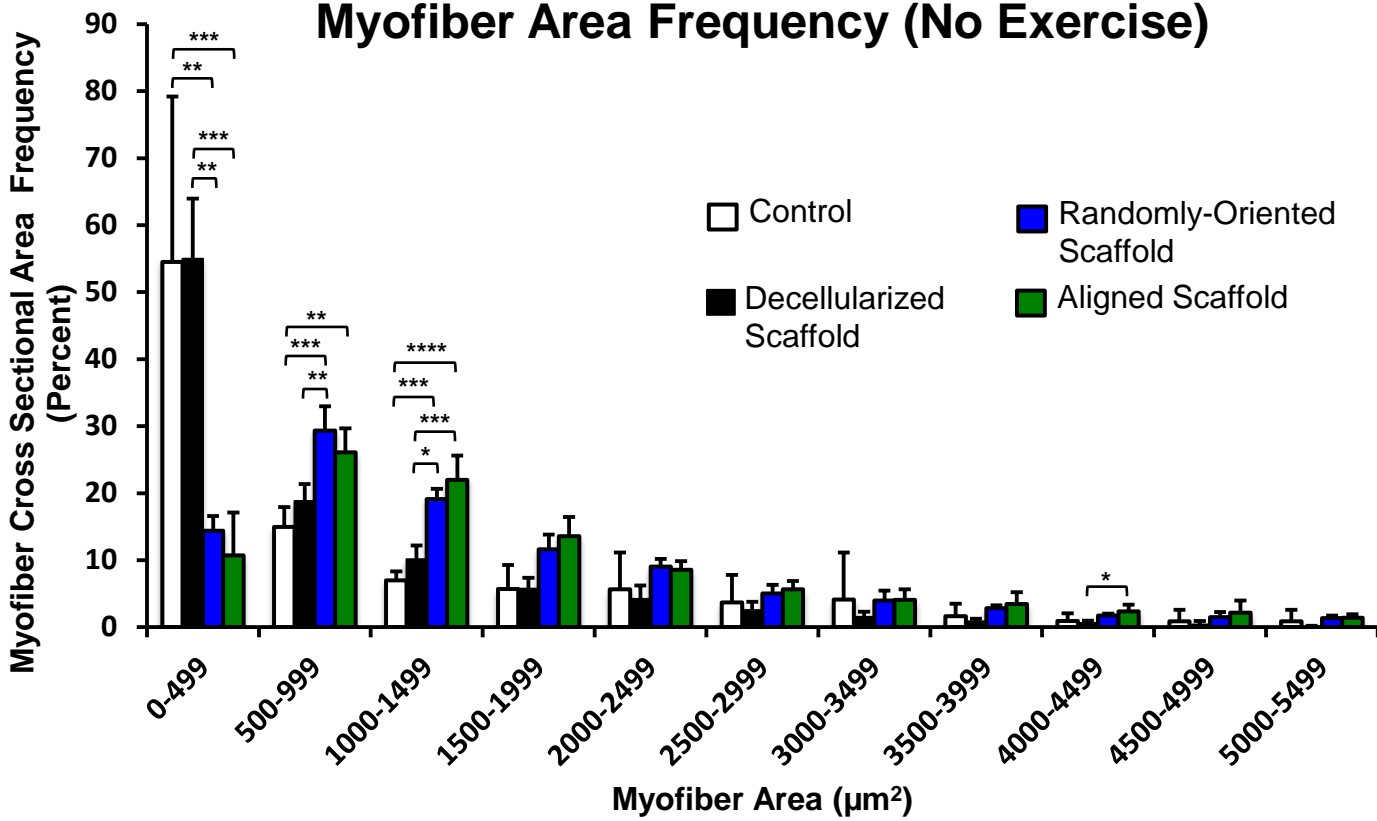
Control

Decellularized  
Scaffold

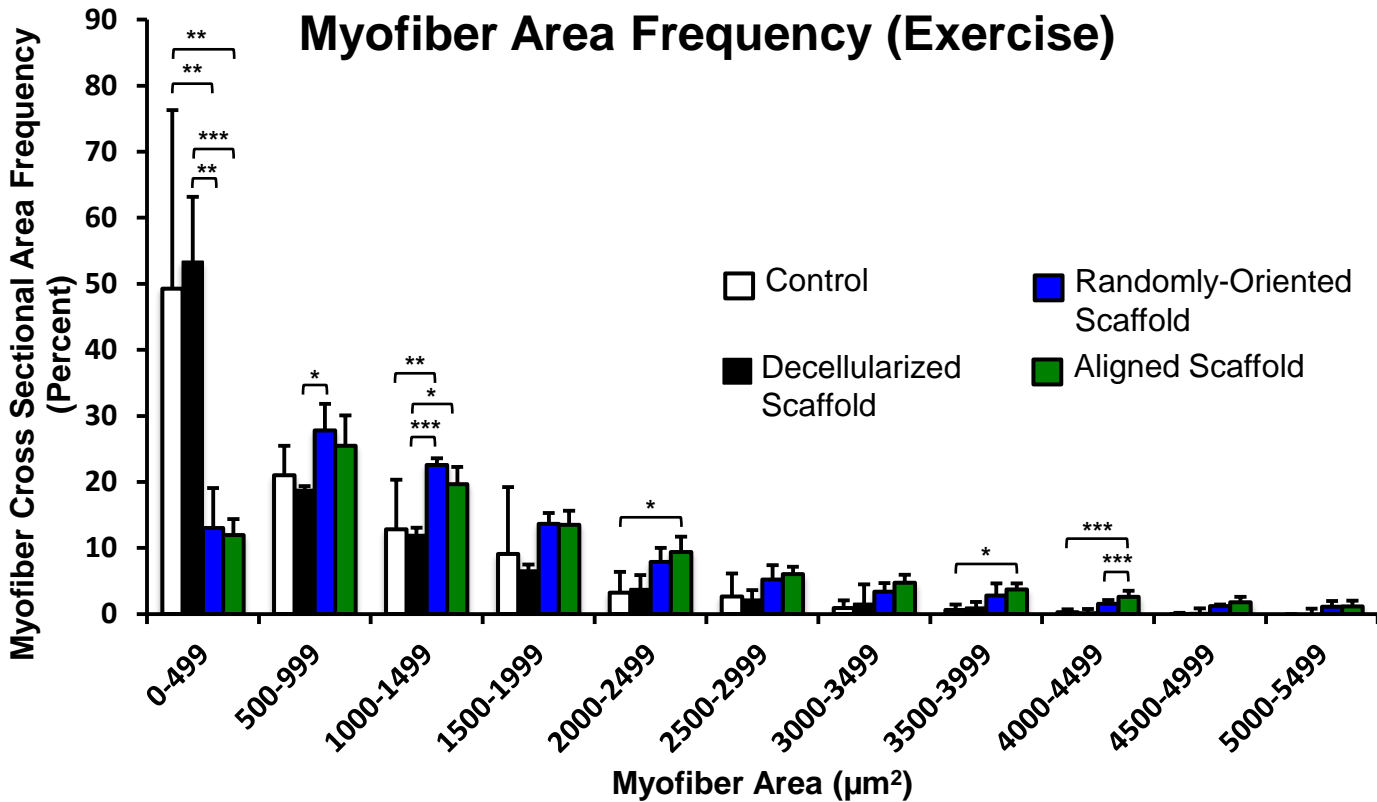


**Suppl. Fig. 4. Histological assessment of tissue cross sections by hematoxylin and eosin stains.** At 3 weeks after implantation into the ablated tibialis anterior muscle, the scaffold-containing muscle was excised. H&E staining was used to visualize the remnants of the scaffold (denoted by arrow). Scale bar: 500  $\mu\text{m}$ .

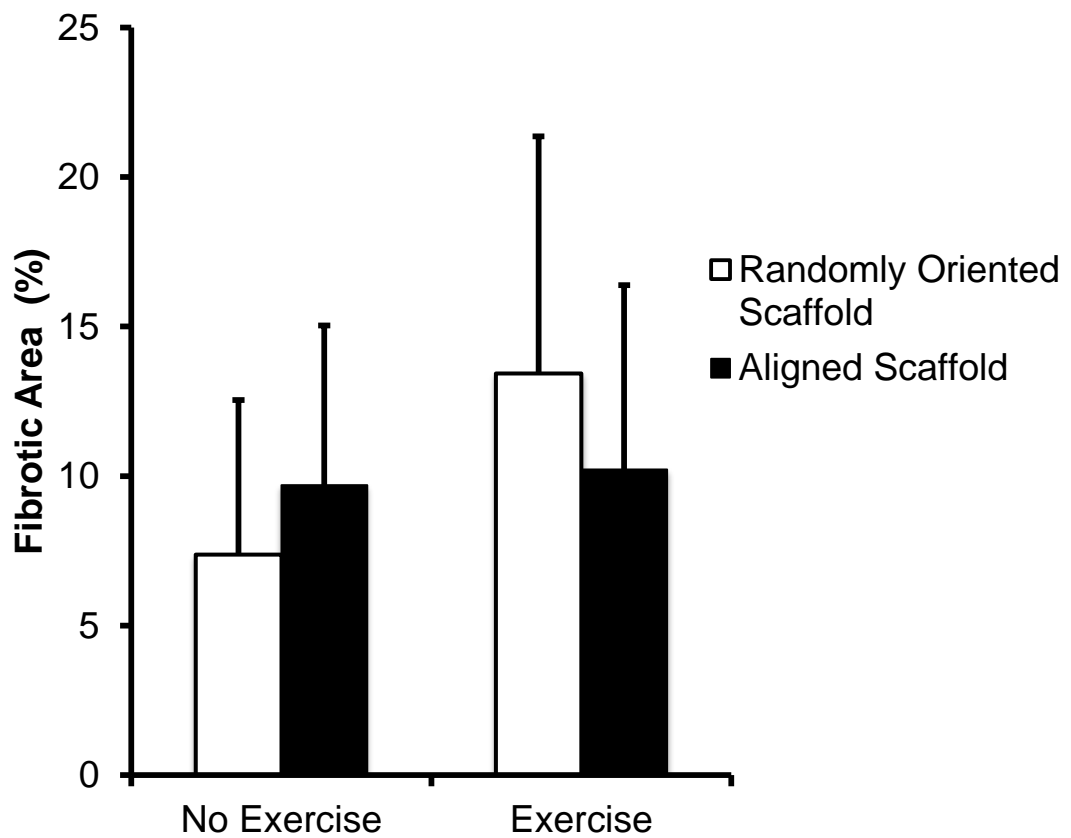
## Myofiber Area Frequency (No Exercise)



## Myofiber Area Frequency (Exercise)



**Suppl. Fig. 5. Histological assessment of regenerating myofiber cross-sectional area.** Frequency distribution of mean myofiber area sizes in the presence or absence of exercise. Shown are mean  $\pm$  SD (aligned scaffold with or without exercise (n=6), randomly oriented scaffold with exercise (n=5), and all other groups (n=4)). Statistically significant comparisons: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001. Error bars denote standard deviation.



**Suppl Fig. 6. Quantification of fibrosis in the injured muscle by the percent area of collagen from Trichrome-stained slides (n=4). Error bars denote standard deviation.**