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Article

C3 Deficiency Leads to Increased Angiogenesis and Elevated Pro-Angiogenic Leukocyte Recruitment in Ischemic Muscle Tissue

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Supplementary Material

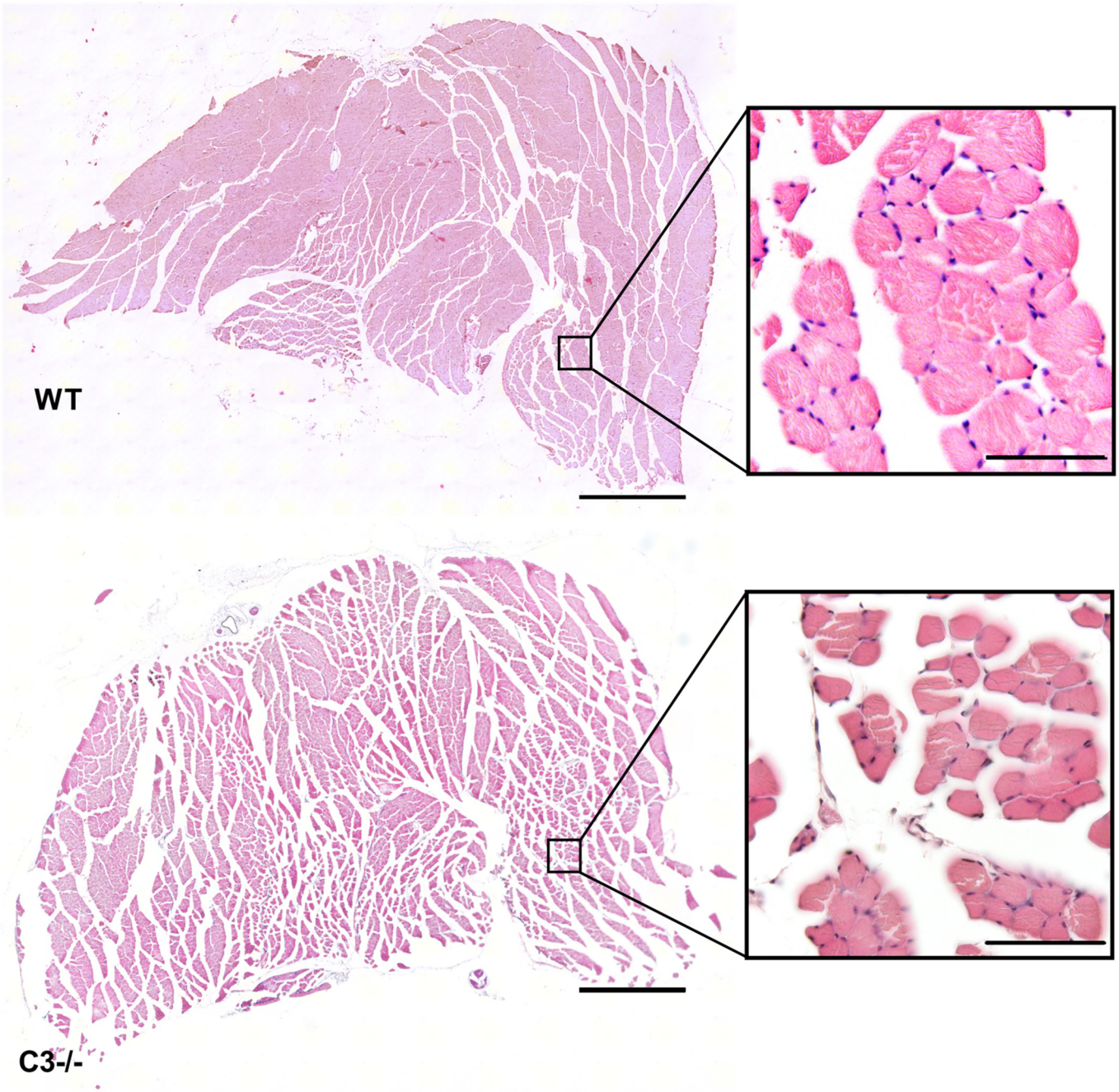

Figure S1. Tissue from sham-operated legs (non-ischemic) show no signs of ischemic damage. Representative pictures of H&E stained gastrocnemius muscles isolated from sham-operated legs of wildtype (WT) mice (upper picture) and C3−/− mice (lower picture) 7 days after FAL. Scale bars: 1000 µm (overview), 50 µm (detail).

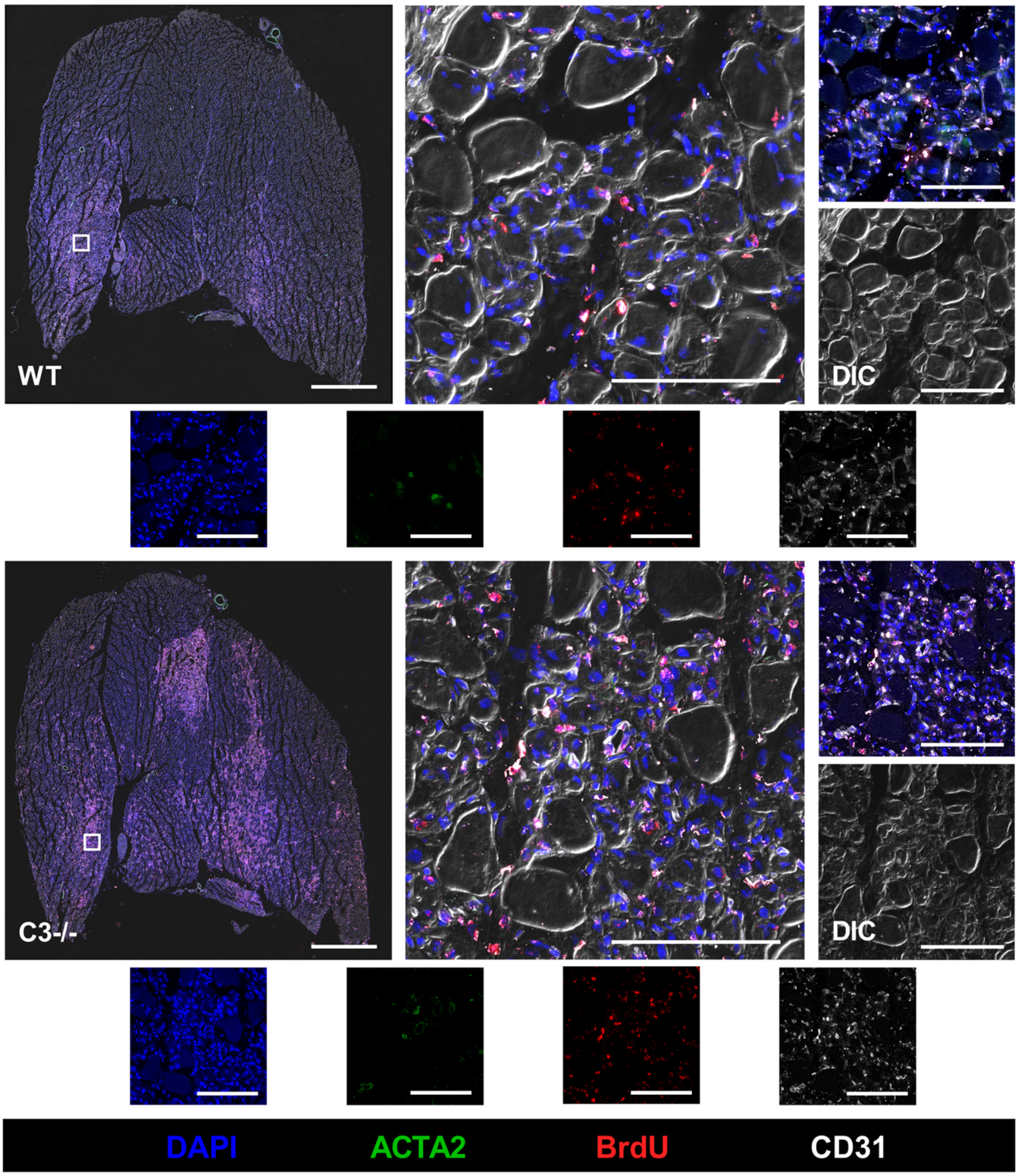

Figure S2. Ischemic gastrocnemius muscles of C3−/− mice show increased angiogenesis. Representative pictures of ischemic gastrocnemius muscles of WT (upper pictures) and C3−/− mice (lower pictures) 7 days after FAL. Immunofluorescence pictures were combined with brightfield images taken with polarized light (differential interference contrast, DIC) to show the muscle fiber structures. ***Left:*** Several pictures were stitched to display the whole sectional area of approx. 20 mm^2. White square indicates detail. ***Middle****:* Detail is displayed as zoomed images of IF+DIC. ***Right:*** Individual IF and DIC pictures are shown, ***below*** IF single channels. Cells were labeled with antibodies targeting endothelial cells ($CD31^+$ (grey)), proliferating cells ($BrdU^+$ (red)), pericytes ($ACTA2^+$ (green)), and stained with DAPI (blue) to label the nuclei. Scale bars: 1000µm (overview), 100µm (detail).

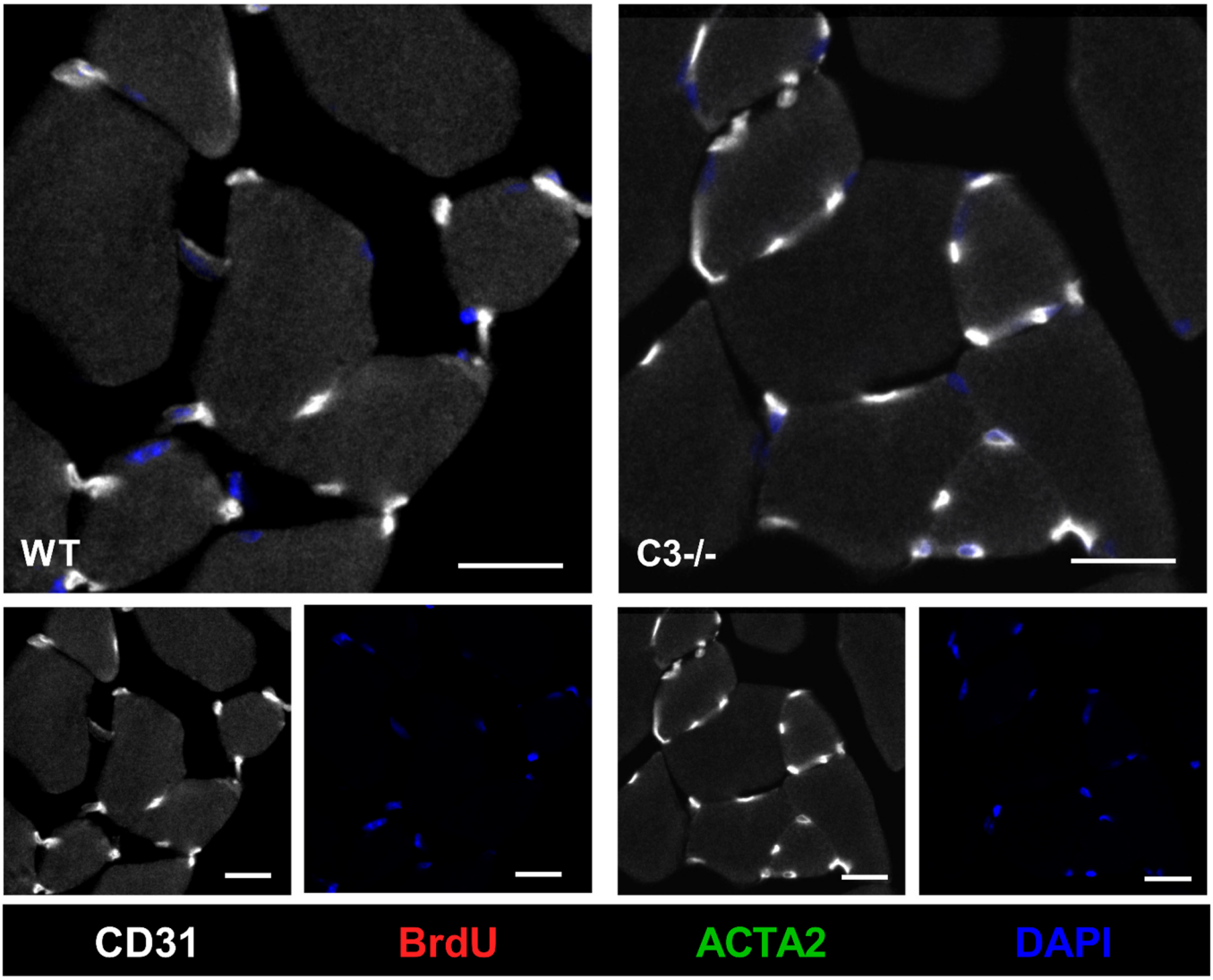

Figure S3. C3−/− and WT mice show a comparable number of endothelial cells per muscle fiber. Immunofluorescence pictures of gastrocnemius muscles isolated from sham-operated (non-ischemic) legs of WT (left) and C3−/− mice (right) 7 days after FAL. No proliferation ($BrdU^+$ cells) is seen. Cells were labeled with antibodies targeting endothelial cells ($CD31^+$ (grey)), proliferating cells ($BrdU^+$ (red)), pericytes ($ACTA2^+$ (green)), and stained with DAPI (blue) to label the nuclei. Only single channels of CD31 and DAPI are displayed as the channels for BrdU and ACTA showed no signal. Scale bars: 20 μm.

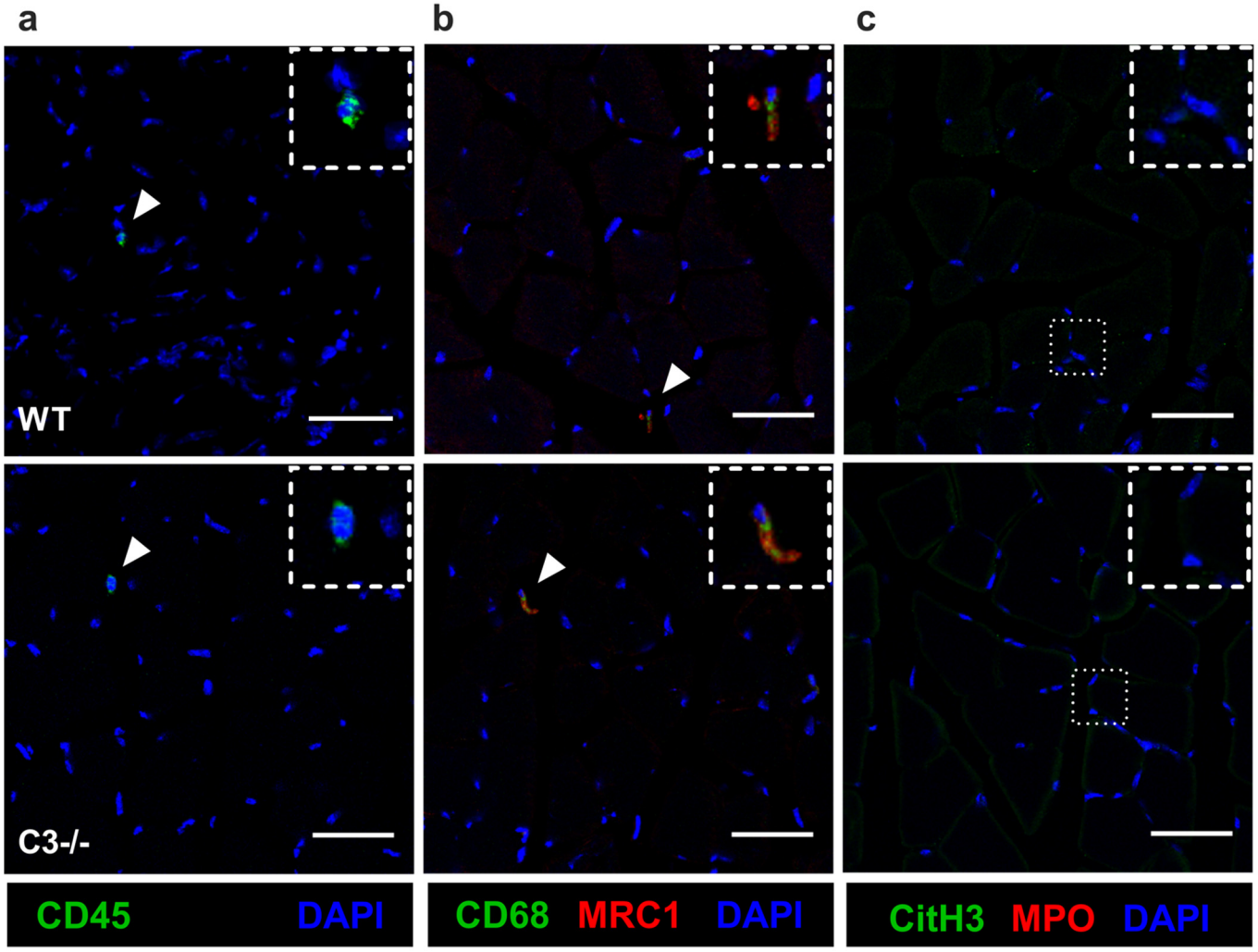

Figure S4. C3−/− and WT show little number of CD45$^+$, CD68$^+$, and MPO$^+$ cells. Representative immunofluorescence pictures of gastrocnemius muscles isolated from sham-operated of WT (upper pictures) and C3−/− mice (lower pictures) 7 days (**a** and **b**) and 24h (**c**) after FAL. (**a**) Cells were labeled with antibodies targeting CD45 (green, pan-leukocyte marker) and with DAPI (blue) to label nuclei. CD45$^+$ cells are indicated by white arrowheads. Scale bars: 50 µm. Detail 30µm × 30µm. (**b**) Cells were labeled with antibodies targeting MRC1 (red, marker for M2-like polarized macrophages), CD68 (green, macrophage marker) and with DAPI (blue) to label the nuclei. CD68$^+$/MRC1$^+$ cells are indicated by white arrowheads. Scale bars: 50 µm. 30µm × 30µm. (**c**) Cells were labeled with antibodies targeting MPO (red, marker for neutrophils), CitH3 (green, citrullinated histone H3) and with DAPI (blue) to label nuclei. No MPO$^+$ cells or signals for CitH3 can be seen in the representative picture and the cropped detail, referring to the very low amount of MPO$^+$ cells in tissue of sham operated legs. Colocalized signals for CitH3 and MPO were not found at all. Scale bars: 50µm. Details 30µm × 30µm.