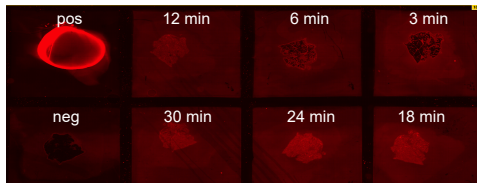
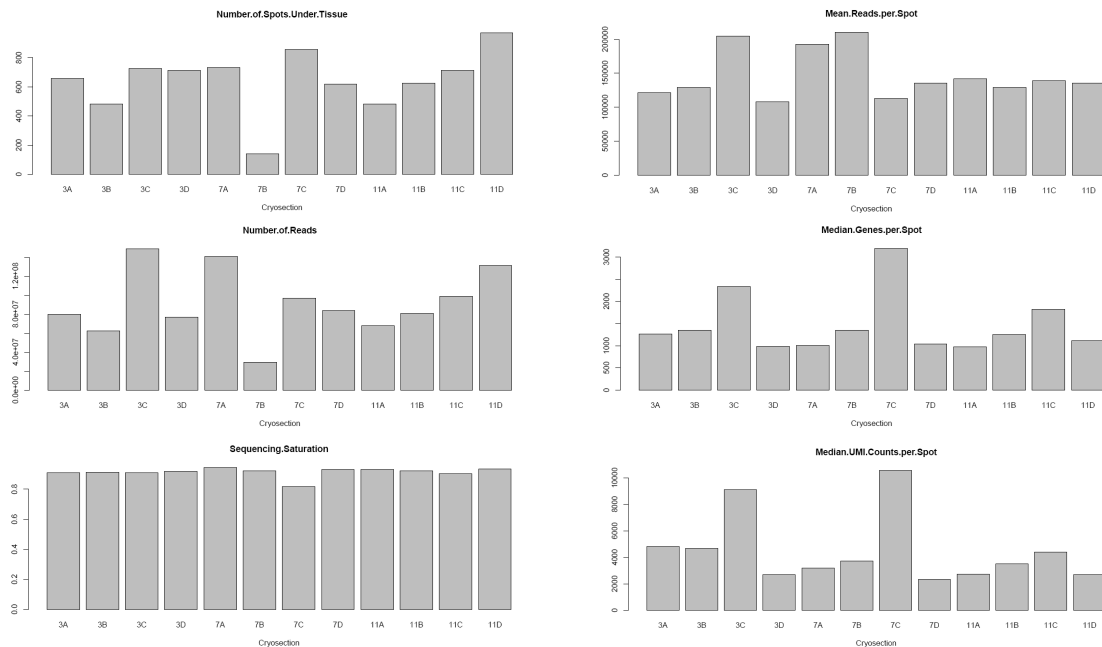
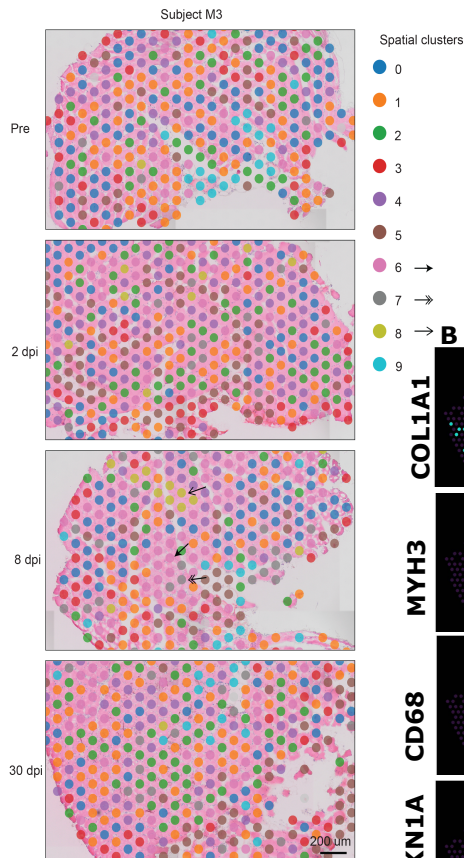


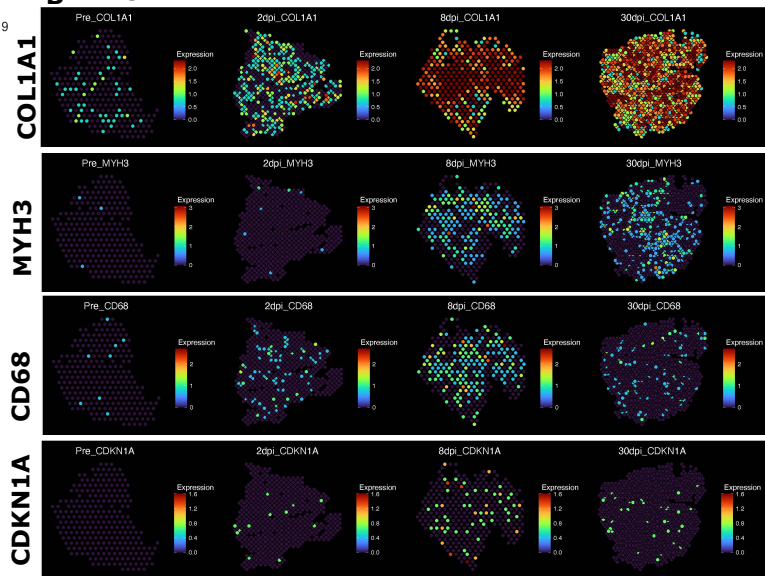
A**B**

Supplementary Figure 1:

A: cDNA staining of skeletal muscle cryosections. Time points represent permeabilization time. B: Quality control of spatial sequencing data. pos and neg = positive and negative control, respectively.

A

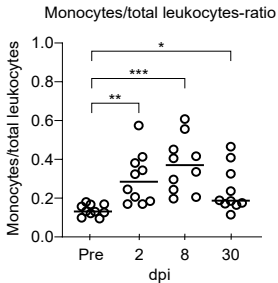
B Pre 2 DPI 8 DPI 30 DPI



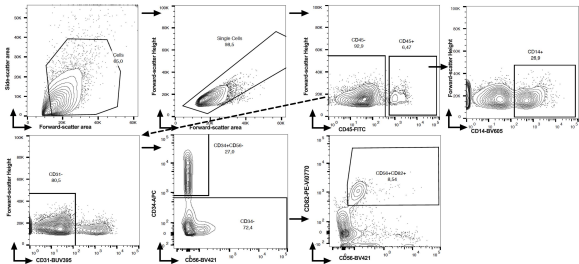
Supplementary Figure 2:

A: Spatial spots divided into nine different clusters based on gene expression illustrating spots containing cluster 6, 7, and 8 are more present after injury. B: Selected genes (Collagen 1A1 (*COL1A1*), embryonic myosin heavy chain (*MYH3*), CD68 (*CD68*) and p21 (*CDKN1A*) and their spatial/temporal expression following muscle injury.

A

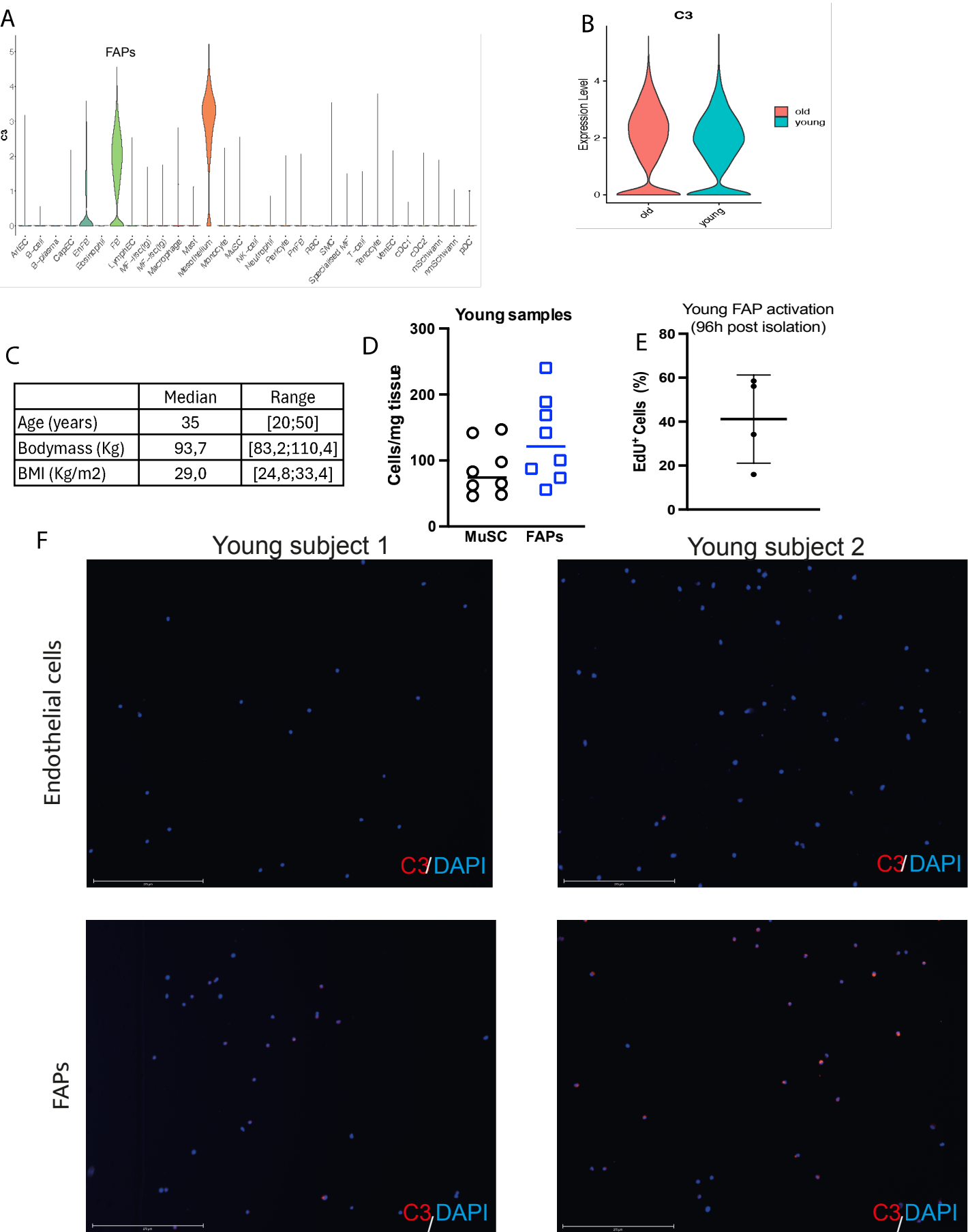


B



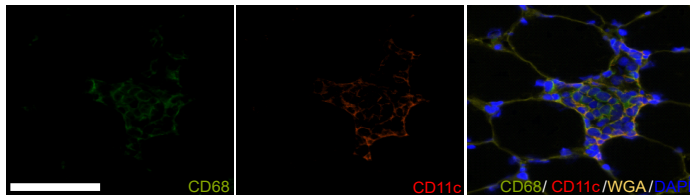
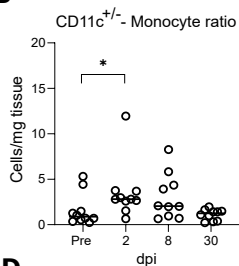
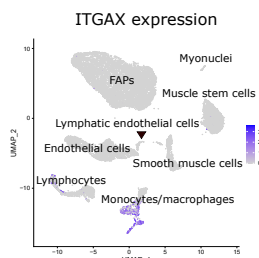
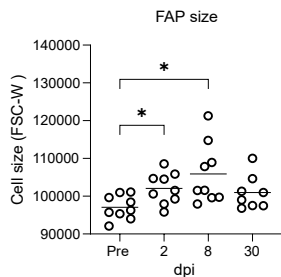
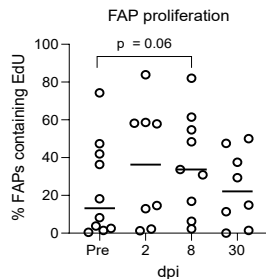
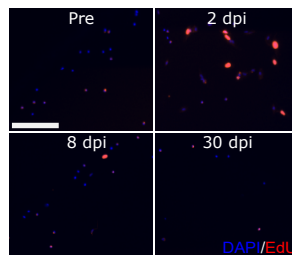
Supplementary Figure 3:

A: Monocytes/total leukocytes-ratio. * = $p < 0.05$. ** = $p < 0.01$. *** = $p < 0.001$. dpi = days post injury (n=10, biological replicates, one-way ANOVA with Dunnett's test to correct for multiple comparisons). B: Flow-plots demonstrating the sorting strategy for Macrophages, Fibro-adipogenic progenitors (FAPs) and Muscle Stem Cells (MuSCs).



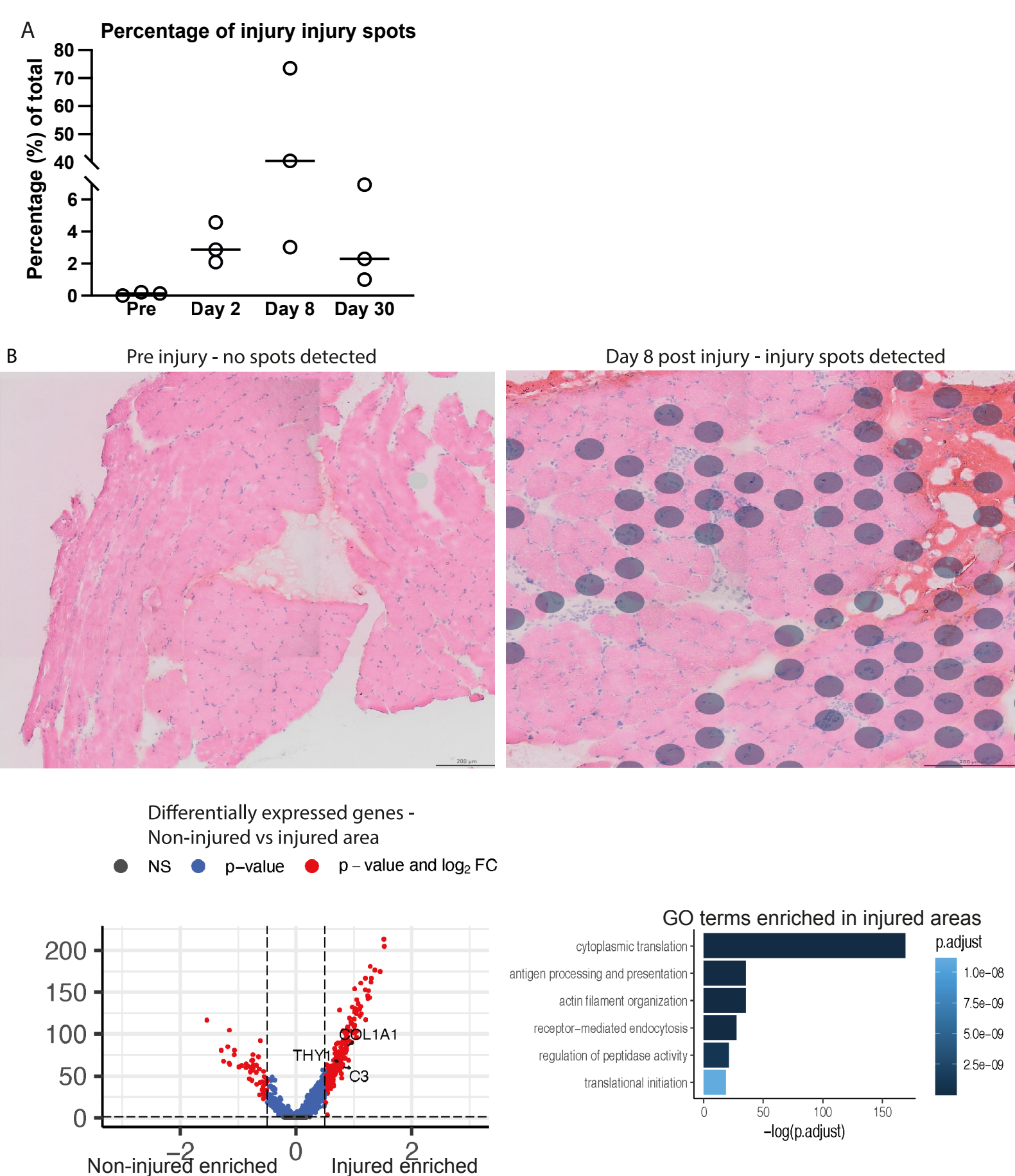
Supplementary Figure 4. FAP content, function and C3 expression in healthy young subjects.

A: Publicly available single nuclei RNA seq data from skeletal muscle shows C3 expression to be specific to FAP or FAP-like cell populations. **B:** Subdivision of the FAP population into young (<40 years) and elderly (>60 years) demonstrates similar levels of C3 expression. **C:** Demographic information on the young cohort (n=8, biological replicates, NCT04998500 trial) from which biopsies were analyzed for FAP content, function and C3 expression. **D:** The content of FAPs in healthy young subjects (n=8, biological replicates, bar indicates mean). **E:** Following tissue digestion and FACS isolation young FAPs engage in the cell cycle (EdU incorporation following 96 hours post isolation, n=6, biological replicates, bar indicates mean). **F:** C3 protein expression is high in freshly isolated young FAPs compared to endothelial cells from the same donor (n=2, biological replicates).

A**B****C****D****E****F**

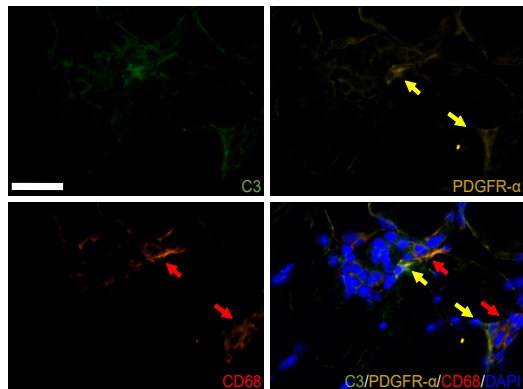
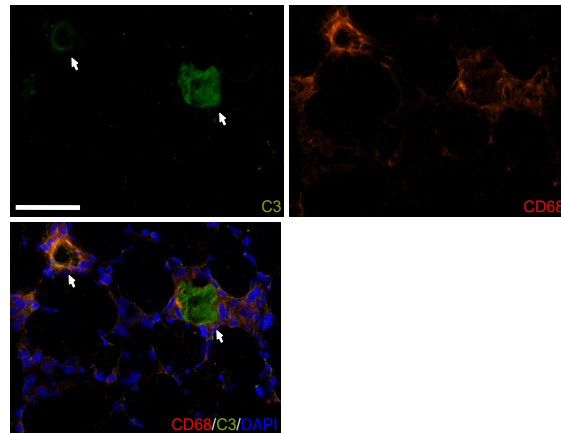
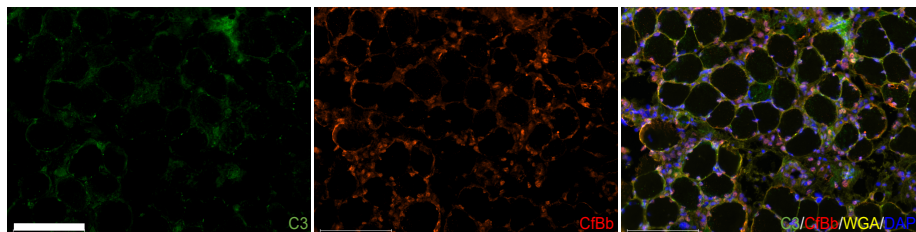
Supplementary Figure 5:

A: Immunostaining of CD68, CD11c, WGA, and DAPI 8 dpi. Scale bar represents 125 μ m. B: CD11c^{+/-}-monocyte ratio (n = 10, biological replicates). C: tSNE-plot of ITGAX expression in single cells. D: FAP size determined by flow cytometry (n = 9, biological replicates, one-way ANOVA and Dunnett's test to correct for multiple comparisons). E +F: EdU-positive FAPs 48h after isolation (n = 9, biological replicates, one-way ANOVA and Dunnett's test to correct for multiple comparisons). Scale bar represents 275 μ m. * = p < 0.05. dpi = days post injury.



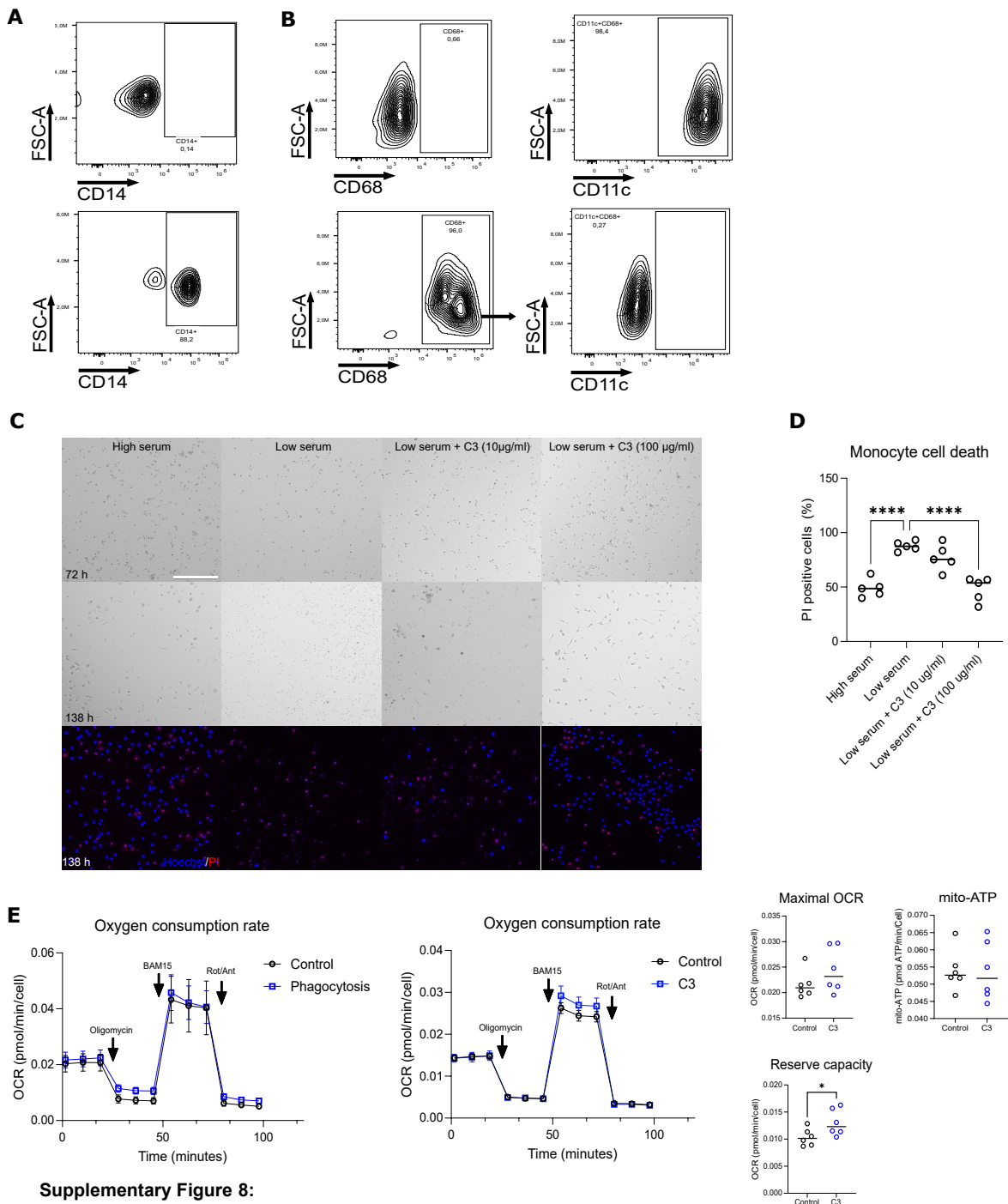
Supplementary Figure 6. Spatial isolation of injury in muscle biopsies

A: Abundance (percentage of total) of spots enriched for genes involved in regeneration at pre, 2, 8 and 30 days post injury (n= 3, , biological replicates, bar indicates mean). B: Example of combining injury identified spots with H&E stainings. No injury spots were identified in pre biopsy, while the injury spots were enriched in areas of regeneration at day 8. C: Volcano plot of differentially expressed genes (DEGs) between injured vs non-injured areas at day 8 post injury (n= 3, , biological replicates). D: Gene ontology (GO) analysis of pathways enriched in injured areas compared to non-injured areas (n= 3, biological replicates).

A**B****C**

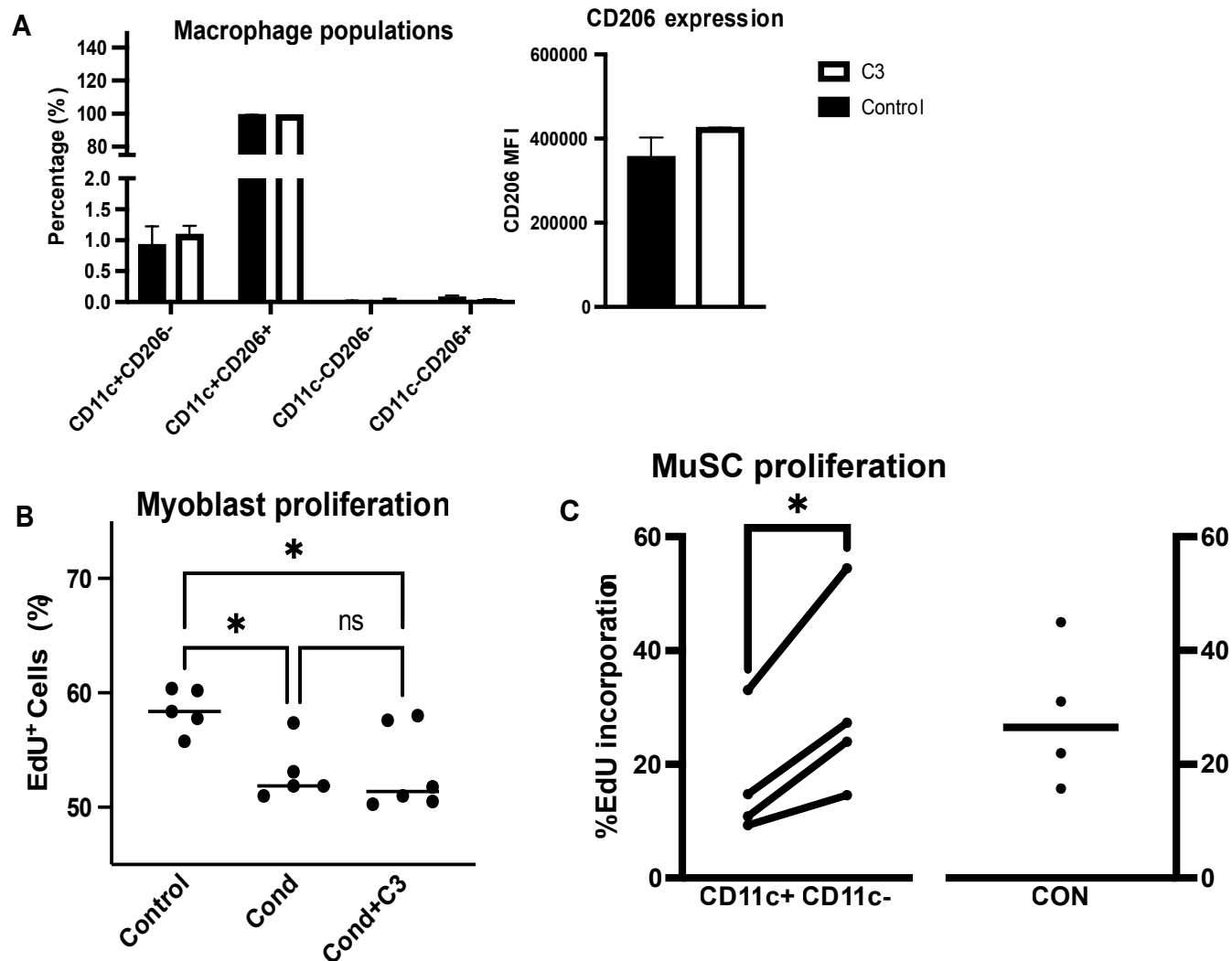
Supplementary Figure 7:

A: Representative immunostaining of C3, PDGFR- α , CD68, and DAPI 8 dpi. Scale bar represents 75 μ m. B: Immunostaining of C3, CD68, and DAPI 8dpi (performed on n=10, biological replicates). Scale bar represents 125 μ m. C: Representative immunostaining of C3, CfBb, WGA, and DAPI 8 dpi (performed on n=10, biological replicates). Scale bar represents 150 μ m.



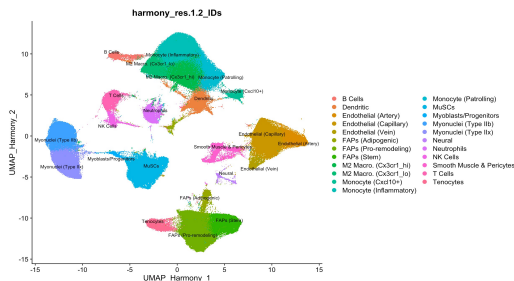
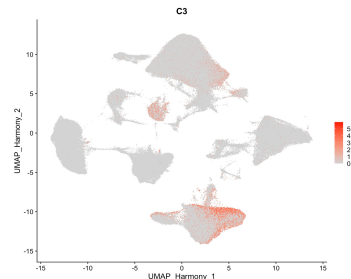
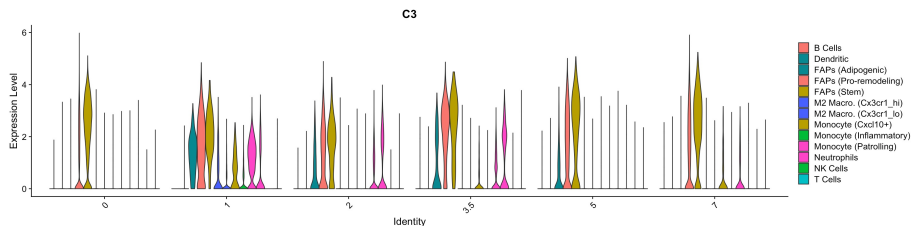
Supplementary Figure 8:

A: Flow plots of directly isolated monocytes from human peripheral blood with unstained control (above) and stained cells (below). B: Flow plots of isolated monocytes from human peripheral blood after 6 days of stimulation with monocyte colony stimulating factor, unstained control (above) and stained cells (below). Flow plots of isolated monocytes from human peripheral blood after 6 days of stimulation with monocyte colony stimulating factor confirming the majority is positive for CD11c, unstained control (above) and stained cells (below). C+D: Cell death of directly isolated monocytes in response to low serum revealing an increased survival with C3 protein and a markedly different morphology. **** = $p < 0.0001$ ($n=5$, biological replicates, one-way ANOVA and Dunnett's test to correct for multiple comparisons). Scale bar represents 275 μm . E: Measurement of oxygen consumption rate (OCR), mitochondrial ATP and reserve capacity in macrophages stimulated with C3 protein alone or undergoing C3 mediated phagocytosis ($n=6$, biological replicates, paired, two-sided t-test).



Supplementary Figure 9. C3 effects on macrophage markers and conditioned media.

A: Primary human macrophages were evaluated for expression of CD11c and CD206 following C3 or control treatment. There was minor differences in the percentages of macrophage subsets, with a minor difference in expression (Median fluorescence intensity, MFI) of CD206 (n=2 (C3) and n=3 (control), biological replicates). B: Myoblasts were exposed to macrophage conditioned media (or control, no cells) with or with prior C3 treatment (n=4, biological replicates one-way ANOVA and Dunnett's test to correct for multiple comparisons). C: CD11c+/- macrophages were isolated from human skeletal muscle to generate conditioned media. Freshly isolated MuSCs were exposed to either control (no cells) or macrophage conditioned media for 48 h EdU incorporation post isolation * denotes $p < 0.05$ (n=4, biological replicates, paired, two-sided test).

A**B****C**

Supplementary Figure 10:

A: UMAP plot of murine single-cell sequencing data from mononuclear cells in skeletal muscle. B: UMAP plot of C3 expression in single cells. C: C3 expression in FAPs and immune cells in murine skeletal muscle injury and regeneration.