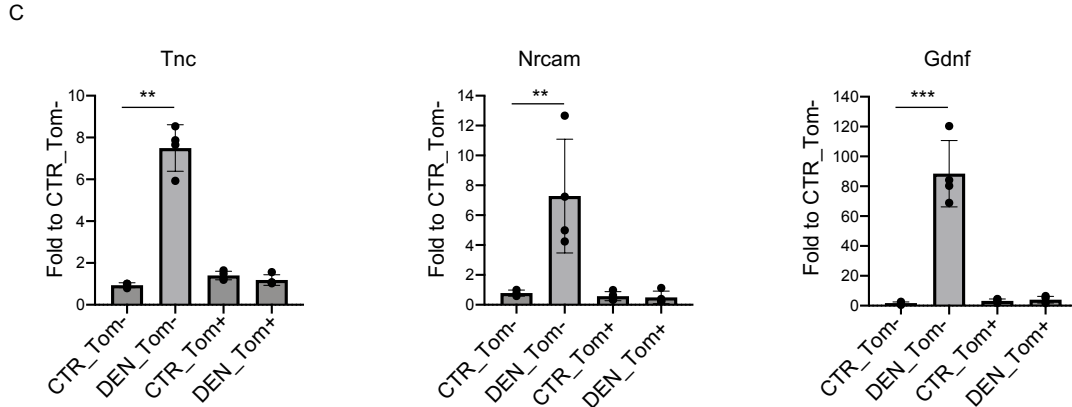
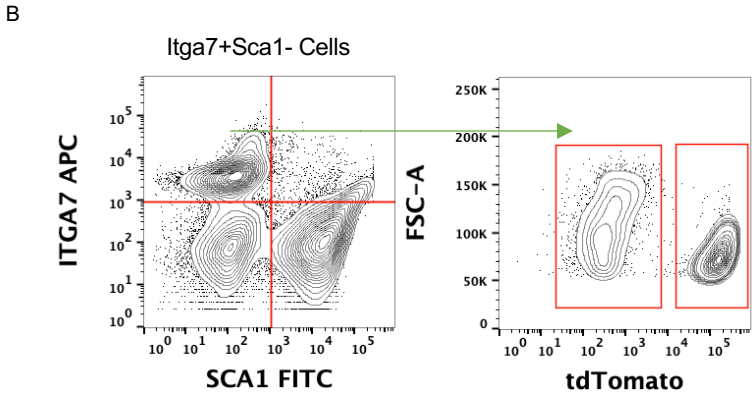
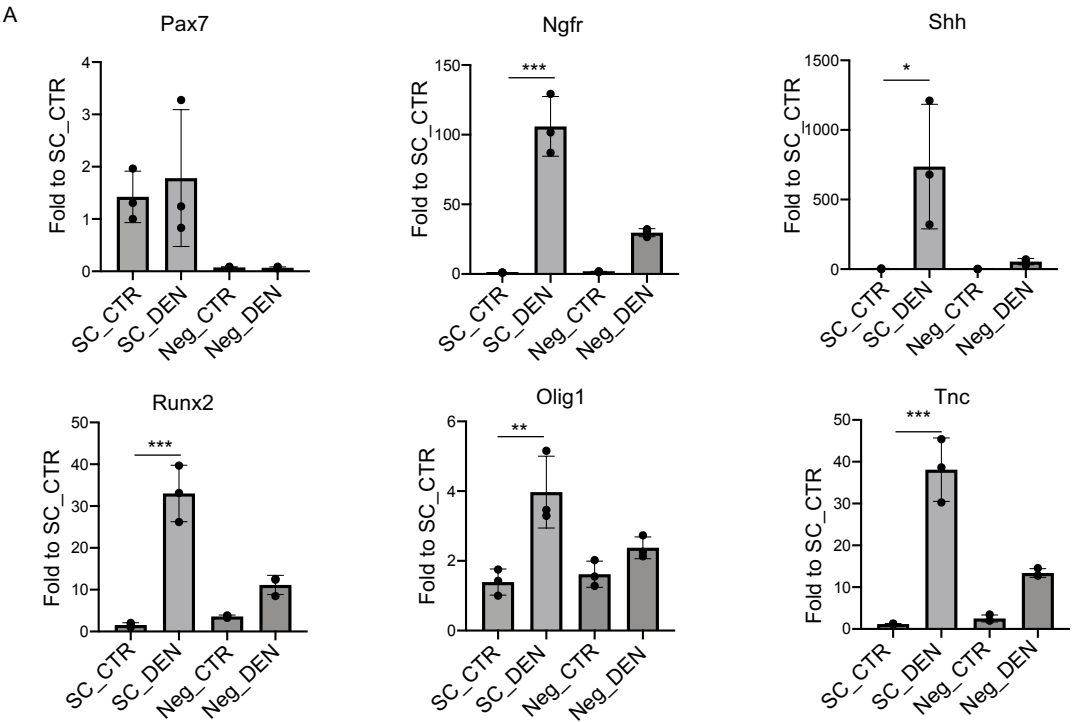


SUPPLEMENTAL DATA

Activation of skeletal muscle-resident glial cells upon nerve injury

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Sacco A.⁷, Puri PL.⁷ and Madaro L.^{2-8*}

Supplementary Figure 1

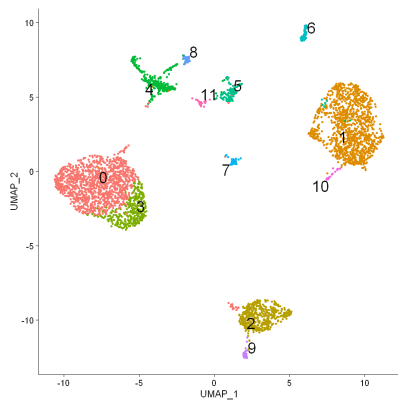


Supplementary Figure 1.

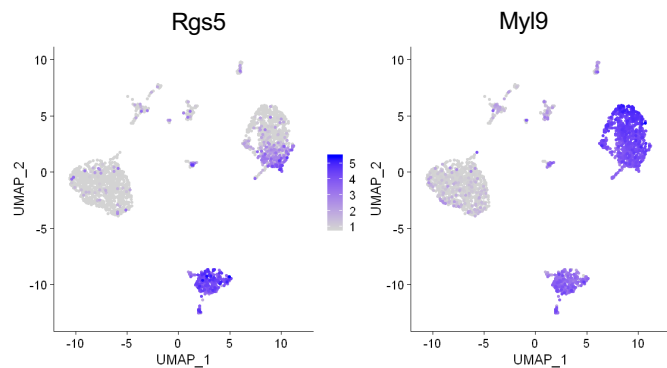
A) qPCR analysis for the expression of Pax7, Ngfr, Shh, Runx2, Olig1 and Tnc in freshly isolated cells from control and denervated muscle isolated with *Satellite Cells isolation kit – Miltenyi Biotec* (SC) or the negative fraction (Neg). Gapdh was used as housekeeping gene (n=3, Values represent mean $\pm$ s.d. *P < 0.05, **P < 0.01 and ***P < 0.001; by One Way Anova Tukey's Multiple Comparisons test). **B)** FACS plot profile of Itga7⁺ cells freshly isolated from tamoxifen treated PAX7.Cre_tdTomato mice. **C)** qPCR analysis for the expression of Tnc, Nrcam and Gdnf in freshly isolated Tomato⁺ and Tomato⁻ cells derived from control and 3 days denervated muscle of tamoxifen treated PAX7.Cre_tdTomato mice. Gapdh was used as housekeeping gene (n=4, Values represent mean $\pm$ s.d. **P < 0.01 and ***P < 0.001; by One Way Anova Tukey's Multiple Comparisons test).

Supplementary Figure 2

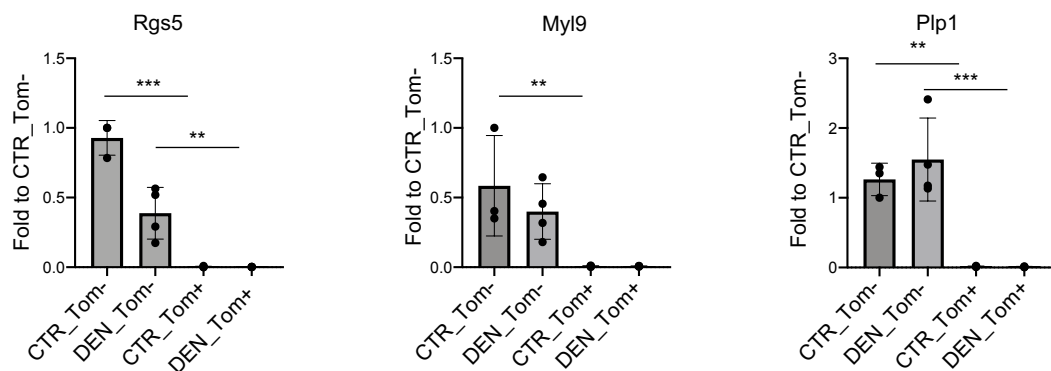
A



B



C

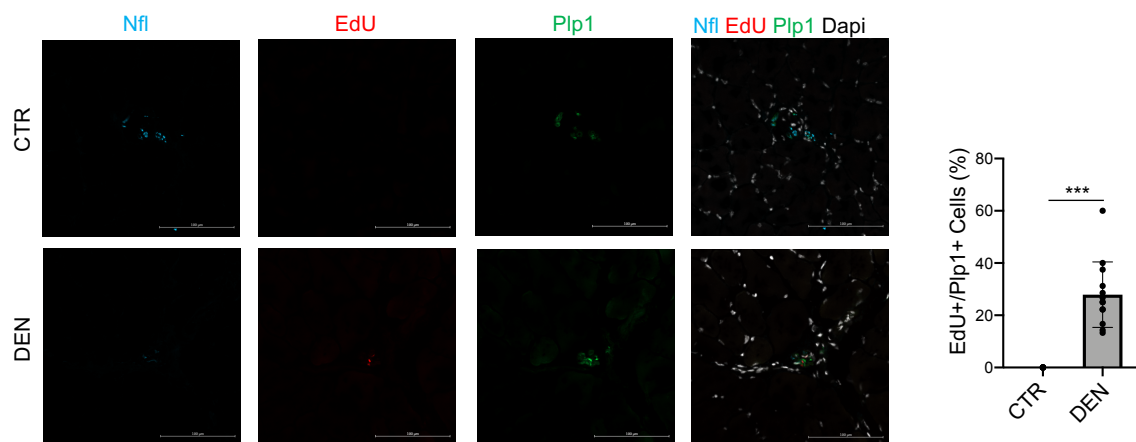


Supplementary Figure 2.

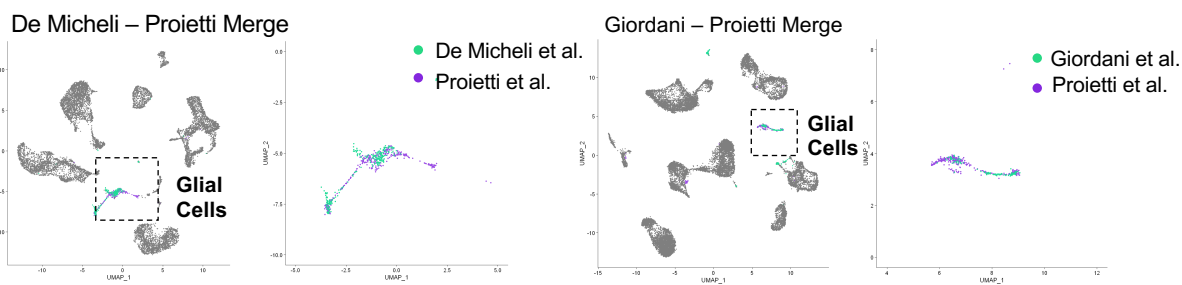
A) Uniform Manifold Approximation and Projection (UMAP)-derived clusters of single cells (Single Cells RNA-seq) of Itga7+Sca1-Ln⁻ isolated cells from control muscle. **B)** Distribution of Rgs5 and Myl9 transcripts in Uniform Manifold Approximation and Projection (UMAP)-derived clusters of single cells (Single Cells RNA-seq) of Itga7+Sca1-Ln⁻ isolated cells from control muscle. **C)** qPCR analysis for the expression of Rgs5, Myl9 and Plp1 in freshly isolated Tomato⁺ and Tomato⁻ cells derived from control and 3 days denervated muscle of tamoxifen treated PAX7.Cre_tdTomato mice. Gapdh was used as housekeeping gene (n=4, Values represent mean $\pm$ s.d. **P < 0.01 and ***P < 0.001; by One Way Anova Tukey's Multiple Comparisons test).

Supplementary Figure 3

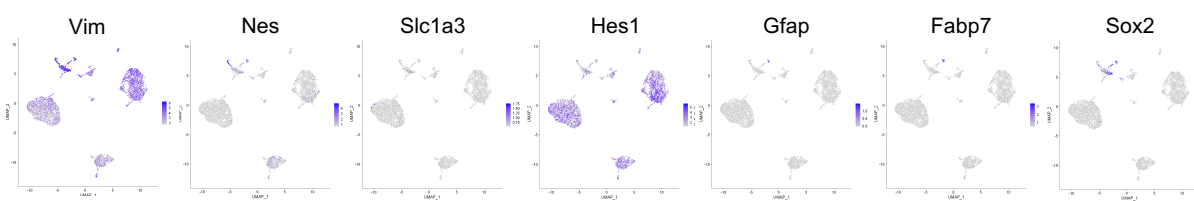
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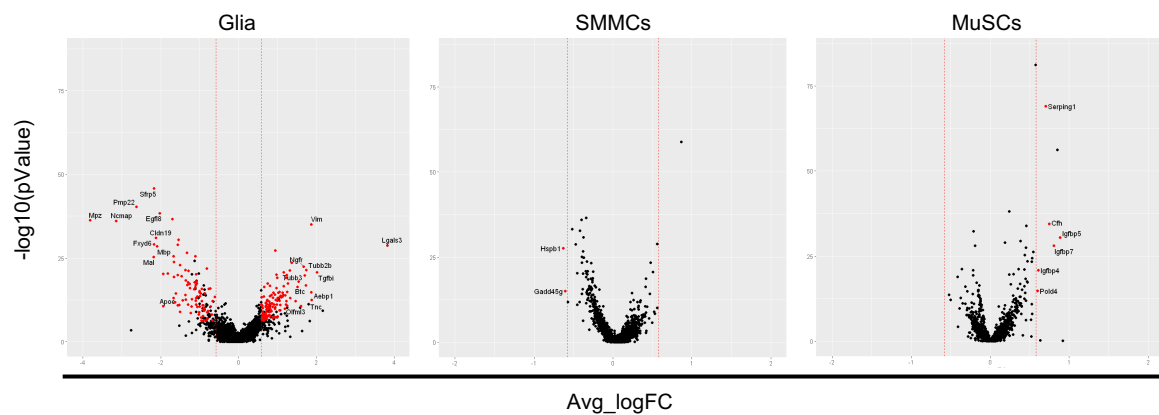
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C



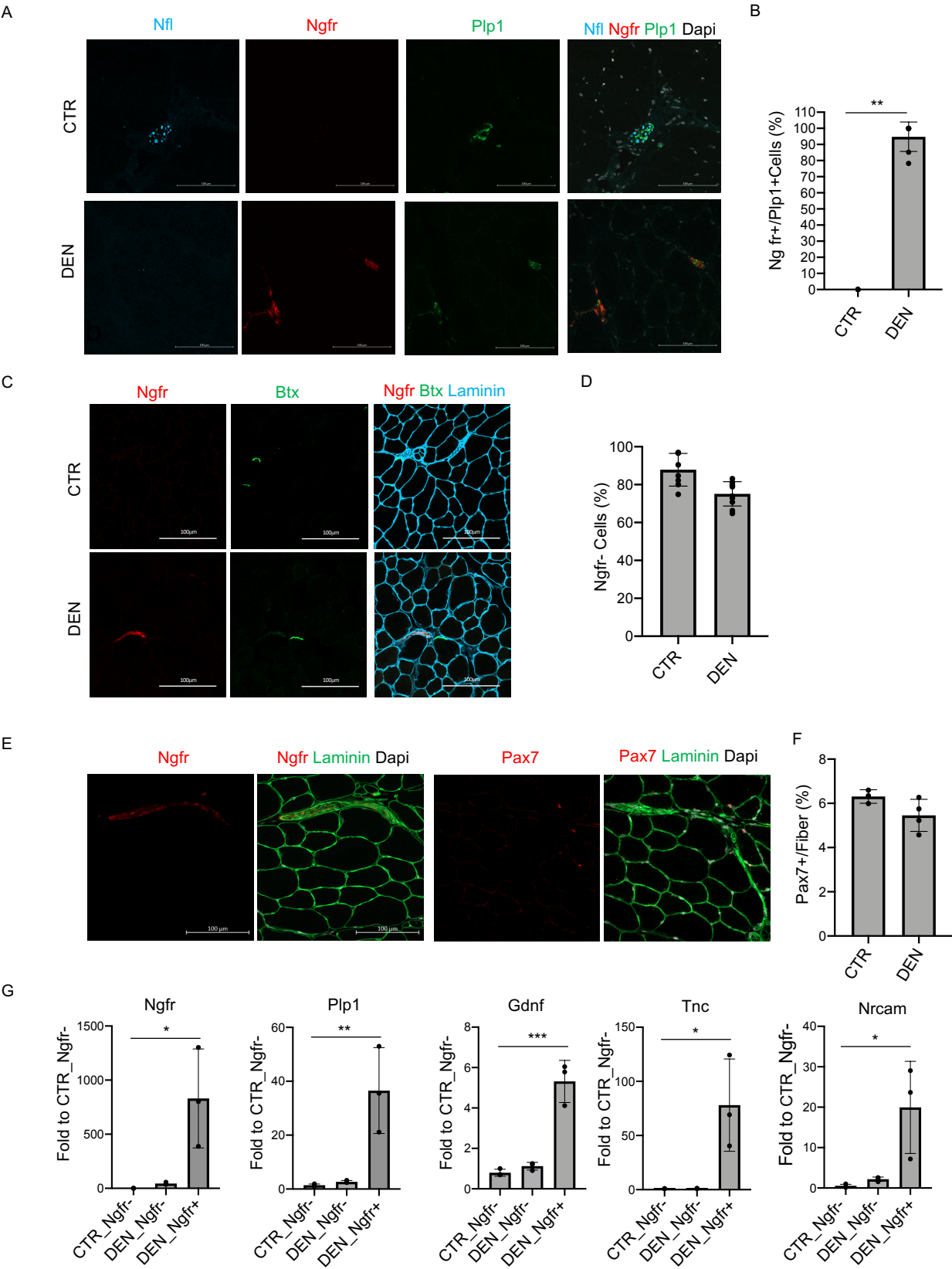
D



Supplementary Figure 3.

A) Representative immunofluorescence analysis of TA muscle cryosection derived from control and denervated muscles, stained for Neurofilament-L (Nfl, Cyan), EdU (red) and Plp1 (green). Nuclei were counterstained with dapi. Scale bar = 100 μ m. (right) Graph representing the percentage of Plp1+EdU+ cells in cryosection of control and denervated muscle. (n=9 slide of 3 different animals. ***P < 0.001; by student t-test) **B)** Uniform Manifold Approximation and Projection (UMAP)-derived clusters of single cells. Single Cells RNA-seq overlap of Plp1 cluster with previously published dataset from uninjured muscle (De Micheli et al. – Day 0 and Giordani et al. **C)** Distribution of Vim, Nes, Slc1a3, Hes1, Gfap, Fabp7 and Sox2 transcripts in Uniform Manifold Approximation and Projection (UMAP)-derived clusters of single cells (Single Cells RNA-seq) of Itga7+Sca1-Ln- isolate cells from control muscle. **D)** Volcano plots for differentially expressed transcripts represents the logarithm of average fold change (Avg_logFC) on x-axis and log10 of the q-value of each transcripts on y-axis; The significative changes induced by denervation was shown for Glia, SMMCs and MuSCs.

Supplementary Figure 4

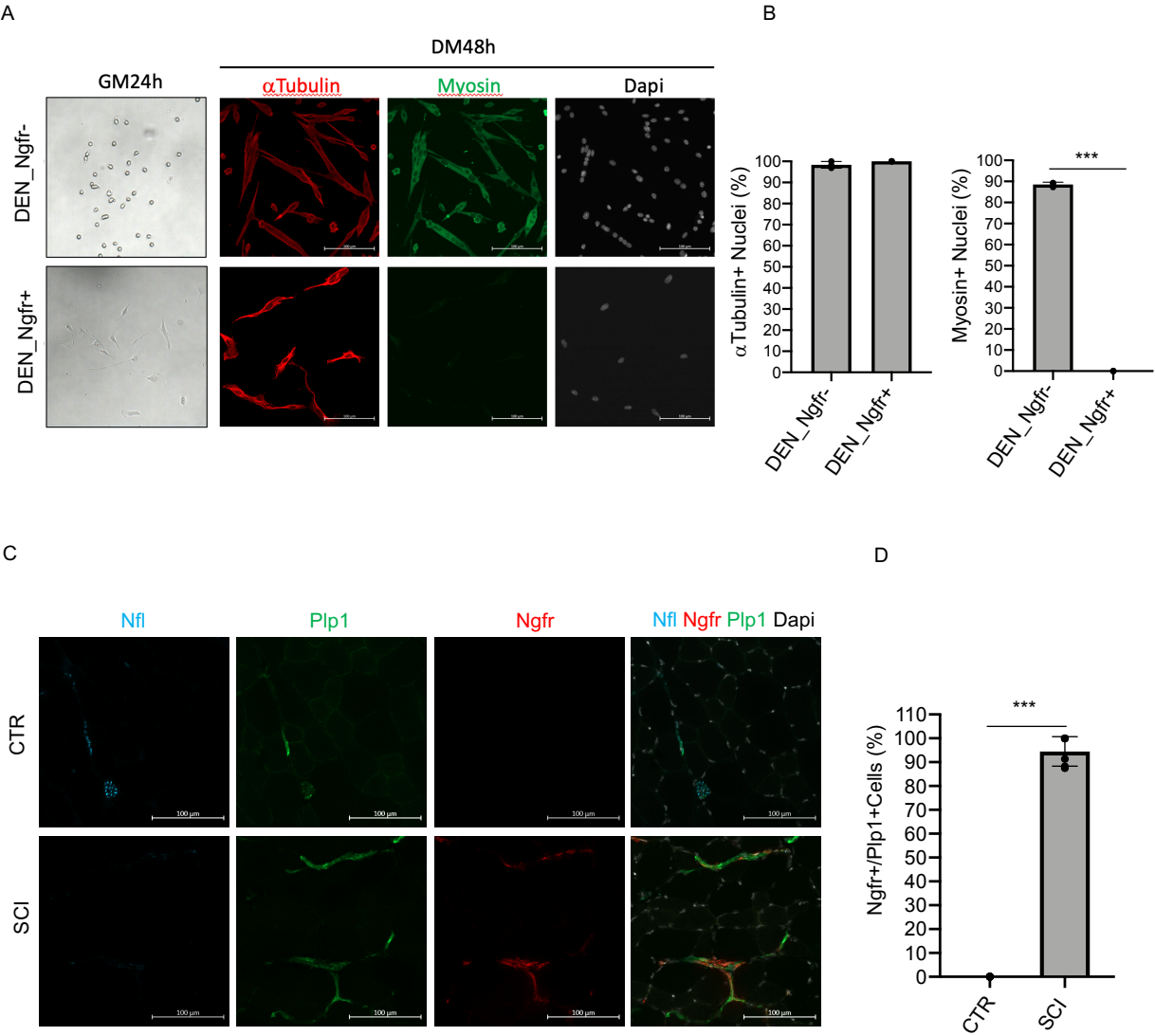


Supplementary Figure 4.

A) Representative immunofluorescence analysis of TA muscle cryosection derived from control (CTR) and denervated (DEN) muscle, stained for Neurofilament-L (Nfl, Cyan), Ngfr (red), Plp1 (green). Nuclei were counterstained with dapi. Scale bar = 100 μ m. **B)** Quantification of Ngfr+/Plp1+ cells in control (CTR) and denervated (DEN) muscle (n=7, Values represent mean $\pm$ s.d. **P < 0.01; by Mann-Whitney test)

C) Representative immunofluorescence analysis of TA muscle cryosection derived from control (CTR) and denervated (DEN) muscle, stained for Ngfr (Red), Bungarotoxin (Green) and Caveolin-3 (Cyan). Nuclei were counterstained with dapi. Scale bar = 100 μ m. **D)** Quantification of Itga7+Ngfr- cells was shown in the graphs (n=8 CTR, n=10 DEN, Values represent mean $\pm$ s.d.). **E)** Representative immunofluorescence analysis of TA muscle cryosection derived from denervated muscle, stained for Ngfr (red, left), Pax7 (red, right), Laminin (green). Nuclei were counterstained with dapi. Scale bar = 100 μ m. **F)** Quantification of Pax7+ cells in control and denervated muscles (n=3, Values represent mean $\pm$ s.d.). **G)** qPCR analysis for the expression of Ngfr, Plp1, Gdnf, Tnc, Nrcam in freshly isolated Ngfr+ or Ngfr- cells derived from control and 3 days denervated muscle. Gapdh was used as housekeeping gene (n=3, Values represent mean $\pm$ s.d. *P < 0.05, **P < 0.01 and ***P < 0.001; by One Way Anova Tukey's Multiple Comparisons test).

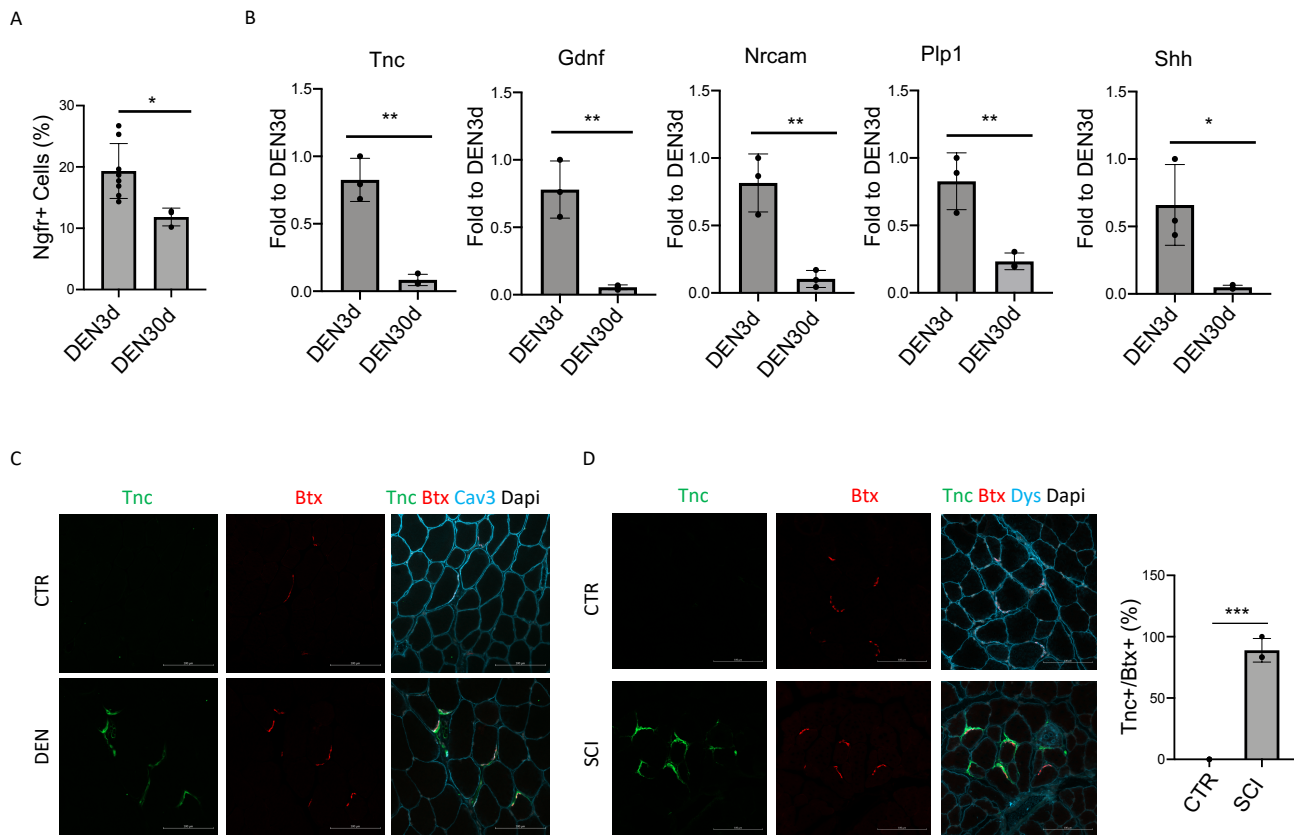
Supplementary Figure 5



Supplementary Figure 5.

A) *In-vitro* culture of both Ngfr- and Ngfr+ cells from denervated muscle. Phase contrast images of proliferating cells was shown (left panel). Representative immunofluorescence analysis for α Tubulin (red) and Myosin (green) was performed 48h after myogenic differentiation. Nuclei were counterstained with dapi. Scale bar = 100 μ m. **B)** Quantification of α Tubulin+ (left) and Myosin+ (right) nuclei (n=3, Values represent mean $\pm$ s.d. ***P < 0.001; by student t-test). **C)** Representative immunofluorescence analysis of control and spinal cord injury (SCI) mice muscle cryosections, at 7-days post injury, stained for Neurofilament-L (Nfl, Cyan), Ngfr (red) and Plp1 (green). Nuclei were counterstained with dapi. Scale bar = 100 μ m. **D)** Quantification of Ngfr+/Plp1+ cells in control (CTR) and spinal cord injury (SCI) mice muscle (n=6, Values represent mean $\pm$ s.d. ***P < 0.001; by Mann-Whitney test).

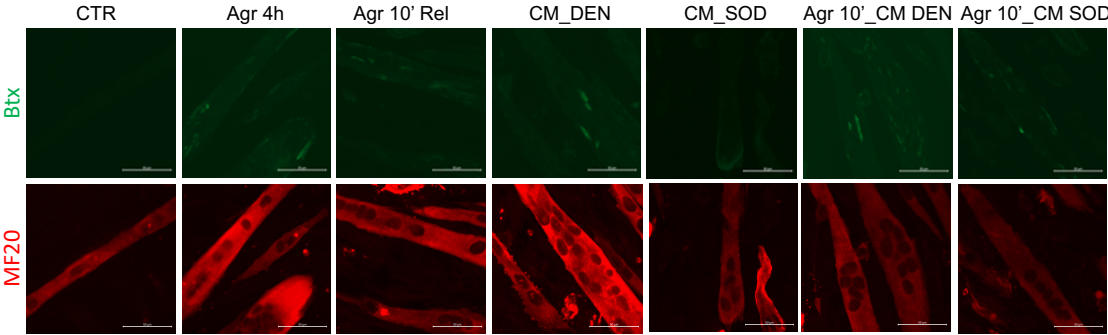
Supplementary Figure 6



Supplementary Figure 6.

A) Ngfr+ cell quantification, shown in the graphs as a percentage of Itga7+Sca1-Ln- population, in denervated muscle at 3- and 30-days post nerve lesion ($n \geq 3$, Values represent mean $\pm$ s.d. * $P < 0.05$; by student t-test). **B)** qPCR analysis for the expression of Tnc, Gdnf, Nrcam, Plp1 and Shh in freshly isolated Ngfr+ cells derived from denervated muscle at 3- and 30-days post nerve lesion ($n=3$, Values represent mean $\pm$ s.d. * $P < 0.05$ and ** $P < 0.01$; by student t-test). **C)** Representative immunofluorescence analysis of TA muscle cryosection derived from control and denervated muscle, stained for Tnc (green), Bungarotoxin (Btx, red) and Caveolin-3 (Cav3, Cyan). Nuclei were counterstained with dapi. Scale bar = 100 μ m. **D)** Representative immunofluorescence analysis of control and spinal cord injury (SCI) mice muscle cryosection, at 7-days post injury, stained for Tnc (green), Bungarotoxin (Btx, red) and Dystrophin (Dys, Cyan). Nuclei were counterstained with dapi. Scale bar = 100 μ m. Quantification of the co-localization Bungarotoxin (Btx) and Tnc data on the right ($n=3$, Values represent mean $\pm$ s.d. *** $P < 0.001$; by student t-test).

Supplementary Figure 7



Supplementary Figure 7.

a) Representative immunofluorescence analysis of C2C12 myotubes stained for Bungarotoxin (Btx, green) and Myosin (MF20, red). Scale bar = 50 μ m.