Figures and figure supplements

Inducible depletion of adult skeletal muscle stem cells impairs the regeneration of neuromuscular junctions

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Figure 1. Depletion of Pax7+ SCs in P7DTA skeletal muscles. (A) Scheme demonstrating time of Tmx treatment, Sciatic nerve transection (SNT) surgery, and harvest of tissue. Representative images of TA transverse sections, stained with anti-Pax7 (red), anti-Laminin (white) and DAPI (blue). Red arrowheads indicate Pax7+ cells. (B) Quantification of Pax7+ satellite cell (SC) number from Ctrl and P7DTA TA muscles 6 weeks after sham or SNT surgery. N = 3 mice, 3 sections/mouse, 6 fields/section. Scale bar = 50 μm. *p < 0.05 compared to Ctrl, **p < 0.05 compared to Ctrl sham, ANOVA/Bonferroni multiple comparisons test. DOI: 10.7554/eLife.09221.003
Figure 2. SC depletion exacerbates neuromuscular disruption induced myofiber atrophy. (A) Representative images of TA muscles. Scale bar = 5 mm. (B–D) Quantification of (B) TA (C) EDL and (D) Soleus (SOL) muscle wet weight after SNT as a percentage of contralateral sham. N = 6 for Ctrl and 8 for P7DTA. (E) Representative TA sections stained with anti-Laminin (white) and DAPI (blue). Scale bar = 50 μm. (F) Quantification of TA myofiber size as a percentage of contralateral sham. (G) Histograms of TA myofiber size distributions. N = 4 mice, ≥1000 myofibers/mouse. *p < 0.05, t-tests. DOI: 10.7554/eLife.09221.004
Figure 2—figure supplement 1. Retention of myofibers and modest loss of myonuclei 6 weeks after SNT. (A) Representative images of single isolated myofibers from EDL muscles (fixed prior to isolation from lower limbs), stained with DAPI. (B, C) Quantification of EDL (B) myofiber number and (C) myonuclei number per 500 μm. N = 4 mice, 32 myofibers/mouse, *p < 0.05 compared to Ctrl-sham, **p < 0.05 compared to Ctrl-sham, Ctrl-SNT, and P7DTA-sham ANOVA/Bonferroni multiple comparisons test and t-tests.
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**Figure 3.** SC depletion induces connective tissue accumulation in skeletal muscles after neuromuscular disruption. (A) Representative images of TA sections stained with H&E; scale bar = 100 μm. (B) Representative images of TA sections stained Sirius Red and pseudocolor images generated by VisioPharm software; numbers indicate myofiber connective tissue (MCT) (red) content in each representative image; scale bar = 50 μm. (C) Quantification of MCT content in TA muscles. N = 4 mice. *p < 0.05 compared to Ctrl-sham, P7DTA-sham and Ctrl-SNT, ANOVA/Bonferroni multiple comparisons test. DOI: 10.7554/eLife.09221.006
Figure 4. SC depletion aggravates myofiber type transitions connected to neuromuscular disruption. (A) Representative images of Ctrl and P7DTA inner TA/EDL muscle regions 6 weeks after sham and SNT surgery stained as indicated with anti-MyHC IIX, all MyHCs except IIX, MyHC IIA, MyHC IIB and MyHC I. Also depicted in Merge IIX+/IIX-, MyHC IIB/IIA and MyHC I labeled images are stains for anti-Laminin (white) and DAPI (blue). (B) Quantification of type IIX pure (green only) and hybrid (green and red, labeled with asterisks) myofiber percentages. (C–E) Quantification of (C) Type IIB (D) Type IIA and (E) Type I fiber percentage. N = 4 mice, 3 sections/mouse, 3 fields/section. Scale bar = 50 μm. *p < 0.05 compared to Ctrl-sham and P7DTA-sham, **p < 0.01 compared to Ctrl-sham, P7DTA-sham and Ctrl-SNT, ANOVA/Bonferroni multiple comparisons test.

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Figure 5. SC depletion leads to declines in force generation of skeletal muscles after neuromuscular disruption. (A) Average time to peak tension (TTP) during 150 Hz stimulation in EDL muscles. *p < 0.05 compared to Ctrl and P7DTA sham. ANOVA/Bonferroni multiple comparisons test, N = 4–6. (B) Absolute and (C) Specific force frequency curves for Ctrl and P7DTA EDL muscles 6 weeks after SNT surgery. *p < 0.05 compared to Ctrl SNT at indicated frequency, t-tests, N = 4–6 mice.

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Figure 5—figure supplement 1. Reduced contractile force and slowed force development in EDL muscles following SNT. (A). Representative traces for specific force from in vitro muscle contraction measurements in EDL muscles stimulated at 150Hz for 500ms. (B). Same traces as (A) but normalized to the corresponding peak and expanded for the first 200ms of stimulation to show the delayed force development in muscles following SNT.

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Figure 6. Limited SC proliferation in skeletal muscles upon neuromuscular disruption. (A) Strategy to BrdU label SCs in adult mice 4 weeks after sham or SNT surgery and representative TA sections stained with anti-Pax7 (red), anti-BrdU (green) and anti-Laminin (white). Red arrowheads indicate Pax7+ cell; green arrowhead indicates BrdU+ cell; yellow arrowhead indicates BrdU+/Pax7+ cell. (B) Quantification of BrdU+/Pax7+ percentage. N = 3 mice, 3 sections/mouse, 6 fields of view/section. *p < 0.05, t-tests. DOI: 10.7554/eLife.09221.010
Figure 7. Neuromuscular disruption stimulates SC contribution in the vicinity of NMJs. (A) Scheme demonstrating time of tamoxifen treatment, SNT surgery, and harvest of tissue. Representative images to examine GFP label in myofibers and Pax7+ SCs of P7mTmG skeletal muscles 4 weeks after sham and SNT surgery. (B) Quantification of the percentage of GFP + myofibers from midbelly of EDL muscles 4 weeks after sham or SNT surgery. Scale bar = 50 μm. N = 3 mice, 3 sections/mouse, 6 fields/section. *p < 0.05, t-tests. (C, D) Representative images of single isolated P7mTmG EDL myofibers with no GFP (RFP), GFP at ends (End) or GFP in middle portions where neuromuscular junctions (NMJs) are located (Mid) after (C) sham or (D) SNT surgery. Magnified inset images show SCs (Pax7+) or NMJs (Btx, AChRs). Scale bar for myofibers = 200 μm for inset = 25 μm. (E) Quantification of GFP + fiber percentage and distribution. Note a higher percentage of myofibers after SNT express GFP primarily in the Mid regions, the location of NMJs. N = 4 mice, 25 myofibers examined per mouse. *p < 0.05 for all GFP + groups, **p < 0.05 for Mid GFP, t-tests.

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Figure 7—figure supplement 1. P7mTmG myofibers are not GFP+ when examined immediately after Tmx administration. (A) Scheme demonstrating time of Tmx treatment, SNT surgery, and harvest of tissue. Representative images of adult P7mTmG TA muscle, taken 24 hr after Tmx treatment, sections stained for anti-GFP and anti-Pax7. (B) Images of Ctrl and P7mTmG TA muscle sections, note the lack of GFP+ myofibers. (C) Proportion of central nucleated myofibers (CNF) and GFP+ CNFs after SNT, 3875 myofibers examined.
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Figure 8. Reductions in NMJ reinnervation, post-synaptic morphology, and post-synaptic myonuclei in SC depleted skeletal muscle. (A) Representative confocal IF images and 3-D Amira based reconstructions of Ctrl and P7DTA NMJs 6 weeks after sham or SNT surgery, stained for post-synaptic (AChRs labeled with Btx, green), pre-synaptic markers (SV2, Syt-2, neurofilament, red) and myonuclei (DAPI, blue). Post-synaptic myonuclei are indicated with asterisks. (B) Quantification of NMJ reinnervation: partially denervated (Part) and fully denervated (Full). (C) Quantification of degenerated NMJs based on post-synaptic morphology. (D) Percentage distribution of NMJs based on number of post-synaptic myonuclei. Scale bar = 10 μm. N = 4 mice, Figure 8. continued on next page
20 NMJs/mouse. *p < 0.05 compared to Ctrl and P7DTA sham, **p < 0.05 compared to Ctrl-sham, P7DTA-sham and Ctrl-SNT, ANOVA/Bonferroni multiple comparisons test.

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**Figure 8—figure supplement 1.** Loss of post-synaptic myonuclei is a feature of NMJ degeneration. (A, B) Quantification of Ctrl NMJ post-synaptic myonuclei: (A) Comparison between denervated and reinnervated NMJs; (B) Comparison between pretzel-like and plaque-like (degenerated) NMJs based on their post-synaptic AChR apparatus shape.

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