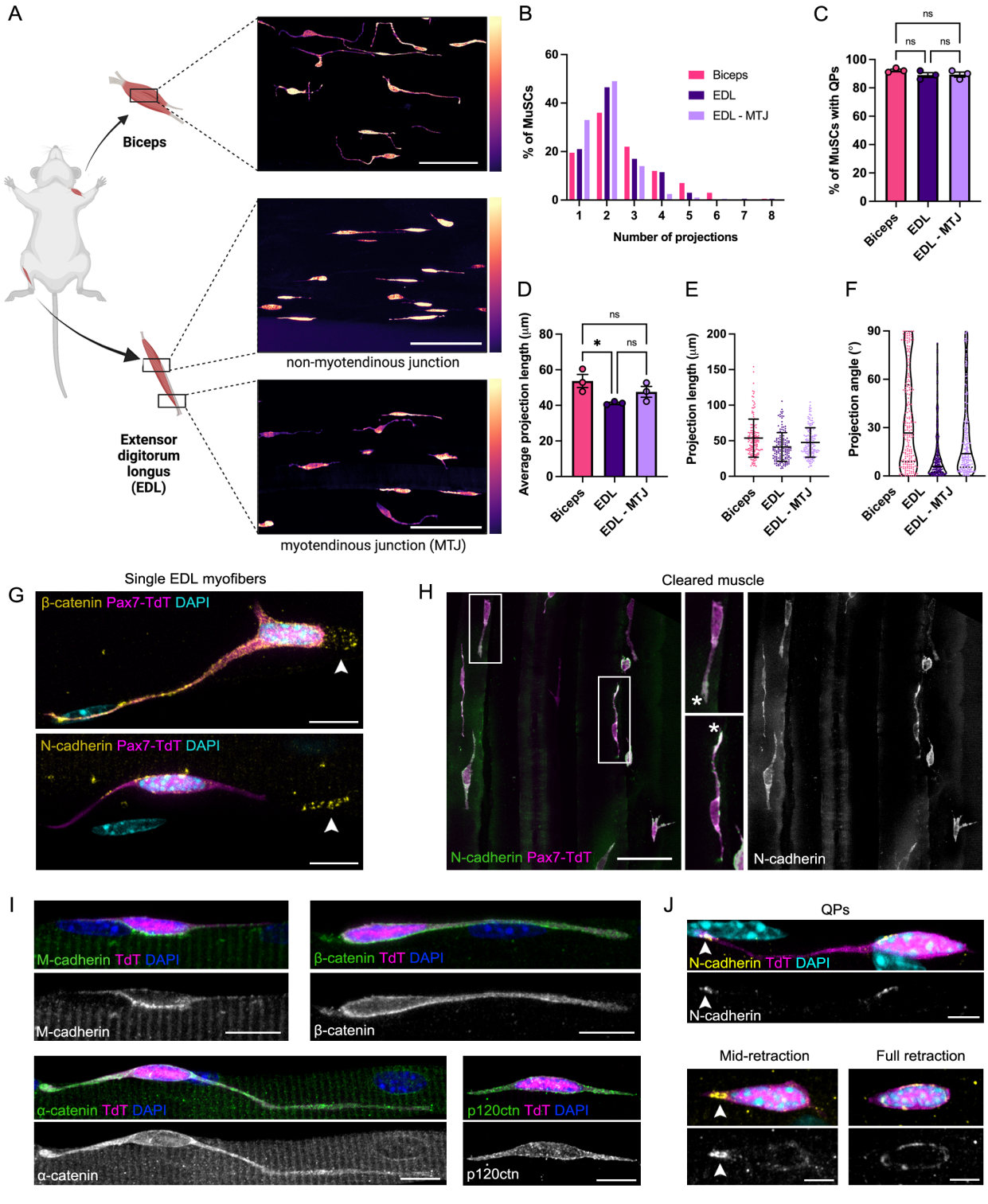
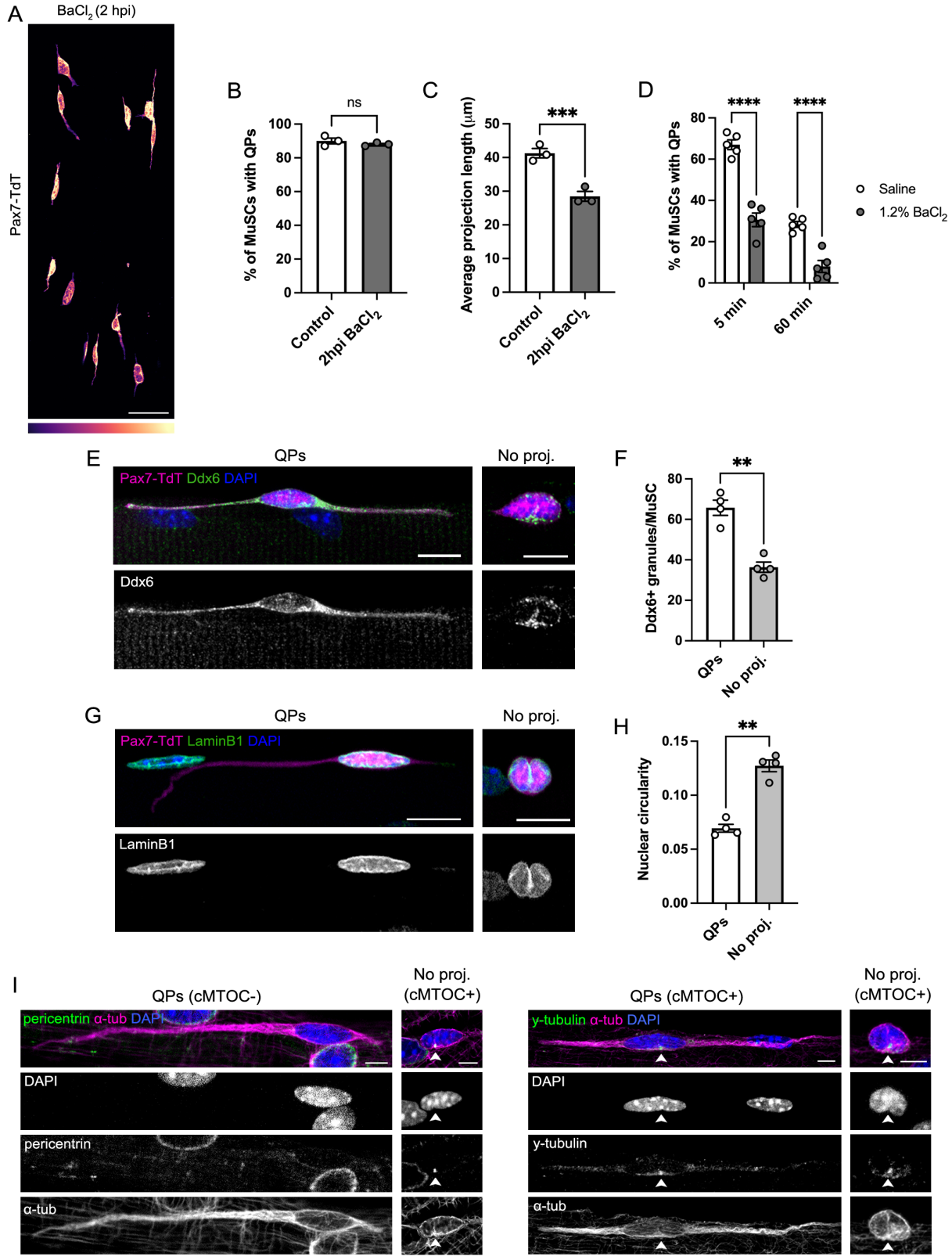


SUPPLEMENTAL FIGURE S1

Supplemental Figure S1. Characterization of quiescent MuSCs; related to Figure 1.

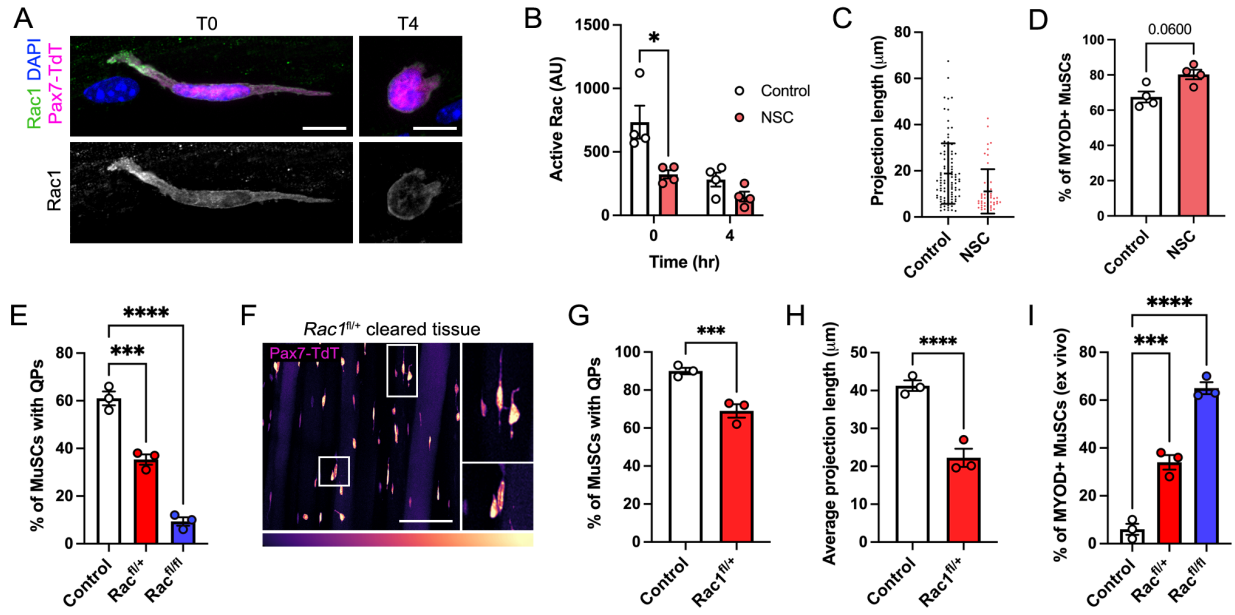
(A) Schematic and images of Pax7-TdT+ MuSCs from (top to bottom) biceps, EDL muscle, and the myotendinous junction area of the EDL. LUT indicates signal intensity. **(B-F)** Quantifications of projection complexity (B), projection frequency (C), QP lengths (D-E), and projection angle (F) across muscle types. **(G)** Images of immunofluorescence on single myofibers showing: β-catenin (top) and N-cadherin (bottom) puncta on myofibers. **(H)** Image of immunofluorescence for N-cadherin in cleared muscle. Asterisks indicate protein enrichment at the ends of quiescent projections. **(I)** Immunofluorescence images of the adherens junction components (clockwise): M-cadherin, β-catenin, p120-catenin, and α-catenin. **(J)** Immunofluorescence images showing N-cadherin redistribution during QP retraction. Arrowheads indicate N-cadherin puncta. Data represent *n*=3 mice and show mean ± s.e.m. Comparisons by one-way ANOVA with Bonferroni's multiple comparisons test (C-D); ns= not significant, *=p<0.05. Scale bars: (A) 75μm, (G,I) 10μm, (H) 50μm, (J) 5μm.



SUPPLEMENTAL FIGURE S2

Supplemental Figure S2. MuSCs with quiescent projections are in a deeper state of quiescence than those without; related to Figure 2.

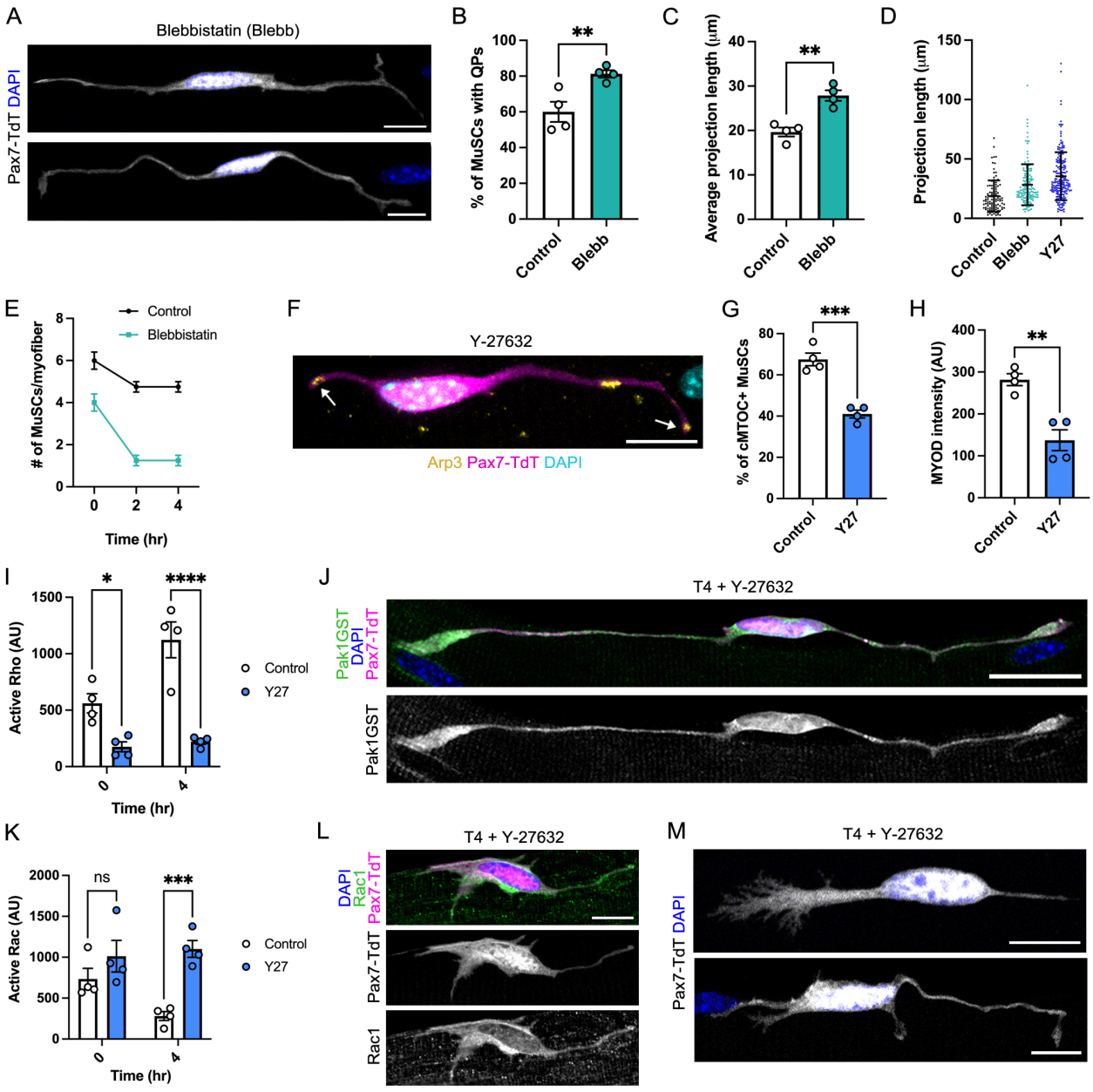
(A) Image of cleared EDL muscle 2 hours post-$BaCl_2$ (2hpi) injury to the neighboring tibialis anterior muscle. LUT indicates signal intensity. **(B-C)** Quantifications of QP frequency (B) and average length (C) in control vs. 2hpi muscle. Control data are shared with Fig. 1E-F and Fig. 6G. **(D)** Quantification of the percentage of MuSCs with QPs on single myofibers treated with saline or 1.2% $BaCl_2$ for 5 or 60 minutes. **(E-F)** Representative images (E) and quantification (F) of Ddx6+ granules in MuSCs ± quiescent projections (QPs). **(G-H)** Representative images (G) and quantification (H) of nuclear circularity in MuSCs ± QPs. Y-axis range is 0-1, in which 0 is a straight line and 1 is a perfect circle. **(I)** Representative images of MuSCs ± a centrosomal microtubule organizing center (cMTOC), labeled by pericentrin (left) or γ-tubulin (right) immunofluorescence in MuSCs ± QPs. Arrowheads indicate cMTOC formation at a site of nuclear indentation. Data represent *n*=3-4 mice and show mean ± s.e.m. Comparisons by one-way ANOVA with Bonferroni's multiple comparisons test (B-C), two-way ANOVA with Šídák's multiple comparisons test (D), or unpaired *t*-tests with Welch's correction (F,H); ns=not significant; **=p<0.01, ***=p<0.001, ****=p<0.0001. Scale bars: (A) 25μm; (E,G) 10μm; (I) 5μm.



SUPPLEMENTAL FIGURE S3

Supplemental Figure S3. Rac1 is downregulated in MuSCs during the quiescence-to-activation transition; related to Figure 3.

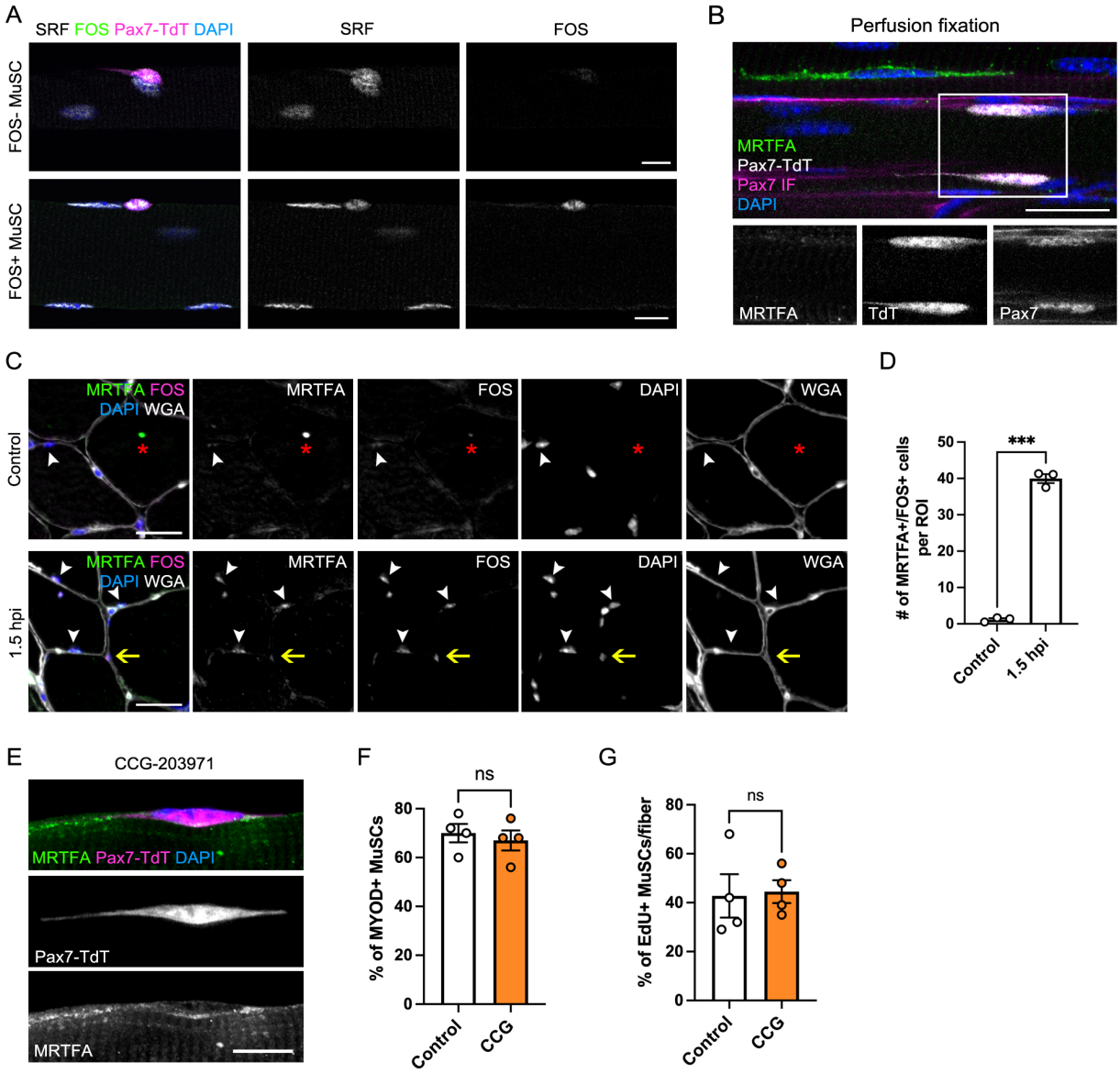
(A) Representative images of Rac1 immunofluorescence at T0 and T4. **(B-D)** Quantifications of active Rac levels (B), QP length (C), and MYOD+ MuSCs (D) in control vs. NSC23766 (NSC)-treated MuSCs. **(E)** Quantification of QP frequency in control vs. *Rac1*$^{fl/+}$ vs. *Rac1*$^{fl/fl}$ MuSCs. **(F)** Representative image of cleared EDL muscle from *Rac1*$^{fl/+}$ mice. LUT indicates signal intensity. **(G-H)** Quantification of control vs. *Rac1*$^{fl/+}$ cleared muscle showing the percentage of MuSCs with QPs (G) and average QP length (H). **(I)** Quantifications of MYOD+ MuSCs on control vs. *Rac1*$^{fl/+}$ vs. *Rac1*$^{fl/fl}$ myofibers. Control data are shared with Fig. 4 and S4. Data represent *n*=3-4 mice and show mean ± s.e.m (B,D-E,G-I) or mean ± s.d. (C). Comparisons by two-way ANOVA with Šídák's multiple comparisons test (B), one-way ANOVA with Bonferroni's multiple comparisons test (D-E,G-I); *= p<0.05, ***=p<0.001, ****=p<0.0001. Scale bars: (A) 10μm; (F) 100μm.



SUPPLEMENTAL FIGURE S4

Supplemental Figure S4. ROCK/MLC inhibition maintains quiescent morphology; related to Figure 4.

(A) Representative images of Blebbistatin-treated MuSCs at T0. **(B-C)** Quantifications of the percentage of MuSCs with quiescent projections (QPs) (B) and average QP length (C) of control vs. Blebbistatin-treated cells at T0. **(D)** Quantifications of QP length between control, Blebbistatin, and Y27-treated MuSCs. **(E)** Time course showing a loss of MuSCs over four hours during Blebbistatin treatment. **(F)** Representative image of Arp3 immunofluorescence in Y27-treated MuSC (arrows indicate Arp3 localization at QP tips). **(G)** Quantification of cMTOC formation in control vs. Y27-treated MuSCs. **(H)** Quantification of MYOD intensity at T8 in control vs. Y27-treated MuSCs. **(I)** Quantification of Rho activity in control and Y27-treated MuSCs at T0 and T4. **(J)** Representative image of active Rac (Pak1GST) in a T4 Y27-treated MuSC. **(K)** Quantification of active Rac levels in control and Y27-treated MuSCs at T0 and T4. **(L-M)** Representative images showing elevated Rac1 protein via immunofluorescence (L) or increased projection complexity and outgrowth (M) in T4 Y27-treated MuSCs. Control data are shared with Fig. 3 and S3. Data represent *n*=3-4 mice and show mean ± s.e.m. (B-C,E,G-H,I,K) or mean ± s.d. (D). Comparisons by one-way ANOVA with Bonferroni's multiple comparisons test (B-C,G), unpaired *t*-test (H), and two-way ANOVA with Šídák's multiple comparisons test (I,K); ns=not significant; *= $p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$. Scale bars: 10μm.



SUPPLEMENTAL FIGURE S5

Supplemental Figure S5. MRTFA translocation initiates FOS induction; related to Figure 5.

(A) Immunofluorescence images showing SRF and FOS protein in T0 MuSCs on single myofibers. **(B)** Immunofluorescence image showing Pax7+ MuSCs with no nuclear MRTFA in perfusion-fixed muscle bundles. The MRTFA-positive cell (green) is an interstitial cell of unknown identity. **(C-D)** Tissue cross-sections (C) and quantifications (D) demonstrating an increase in numbers of MRTFA+/FOS+ cells between control and recently-injured (1.5 hours post-$BaCl_2$ injection) mice. White arrowheads indicate MRTFA+/FOS+ cells under the basal lamina and yellow arrows indicate MRTFA+/FOS+ interstitial cells. Red asterisk indicates nonspecific background signal. ROI, region of interest. **(E)** Immunofluorescence image of a CCG-203971 (CCG)-treated MuSC showing no nuclear MRTFA signal. **(F-G)** Quantifications of MYOD+ MuSCs at T4 (F) or EdU+ MuSCs at T30 (G) in control and CCG-treated conditions. Comparisons by unpaired *t* test with Welch's correction (D,F-G); ns=not significant, ***=$p<0.001$. Scale bars: (A,E) 10μm; (B-C) 25μm.