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Supplemental Information

WNT7A suppresses adipogenesis of skeletal muscle mesenchymal stem cells and fatty infiltration through the alternative Wnt-Rho-YAP/TAZ signaling axis

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Supplemental Figures

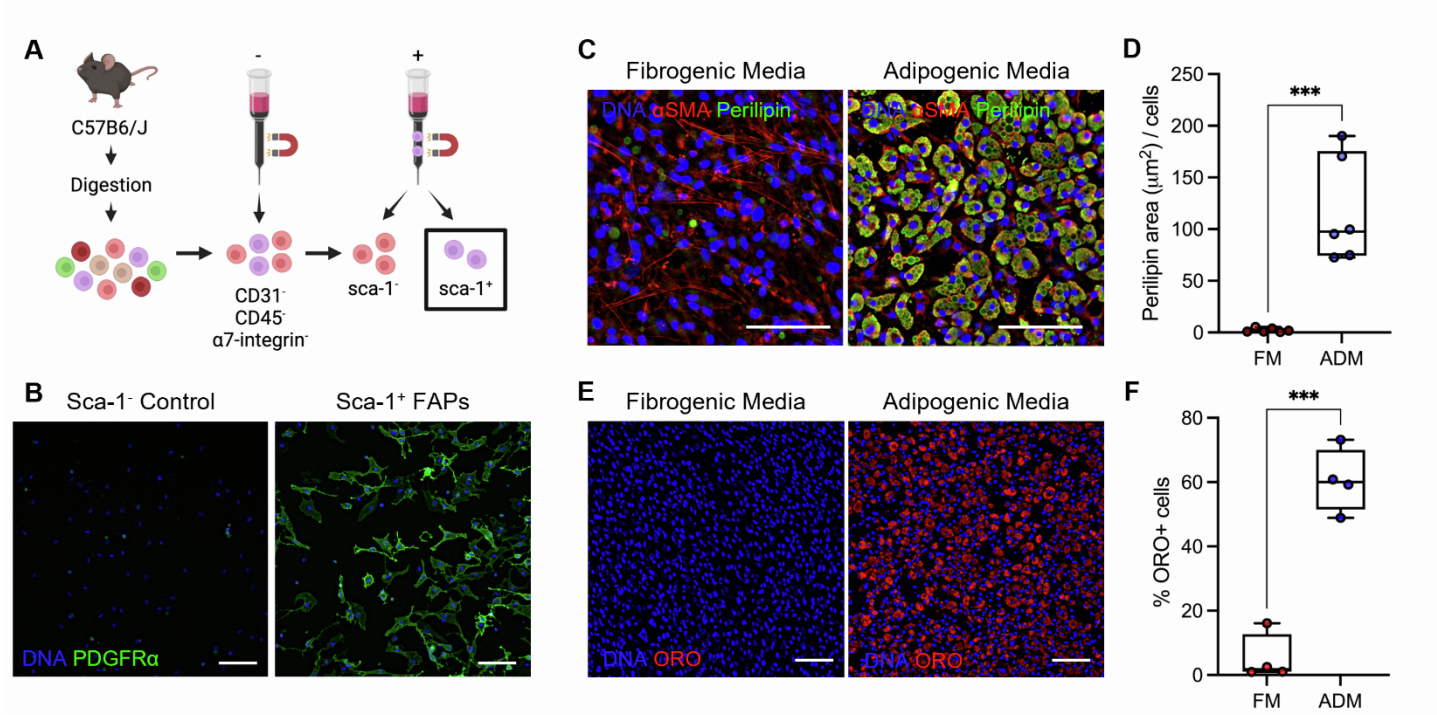

Figure S1. ***(A)*** Schematic of FAPs isolation from C57BL6/J hindlimb muscles via magnetic activated cell sorting. Created with Biorender. ***(B)*** Freshly isolated FAPs express PDGFRα. 24 hours post-seeding. Scale bar: 100 μm. ***(C)*** Representative immunofluorescence images of α-smooth muscle actin (αSMA) and perilipin-labeled FAPs differentiated in fibrogenic and adipogenic differentiation media. Scale bar: 100 μm. ***(D)*** Perilipin area normalized by cell quantity in fibrogenic (FM) and adipogenic (ADM) media. Unpaired t-test. *** $p<0.001$. n=6. ***(E)*** Representative images of Oil Red O (ORO)-labeled FAPs differentiated in fibrogenic and adipogenic differentiation media. ***(F)*** Percent ORO+ cells in fibrogenic (FM) and adipogenic (ADM) media. Unpaired t-test. *** $p<0.001$. n=4.

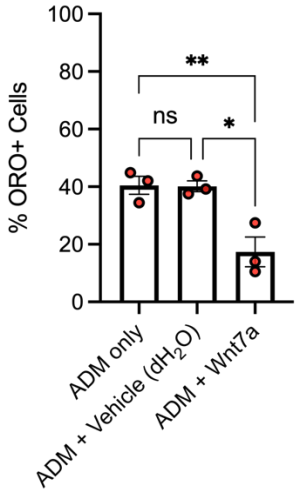

Figure S2. dH_2O vehicle in ADM (0.2% v/v) does not affect percent ORO+ cells compared to the ADM only condition. One-way ANOVA with Tukey's post-hoc analysis. * $p<0.05$; ** $p<0.01$. n=3.

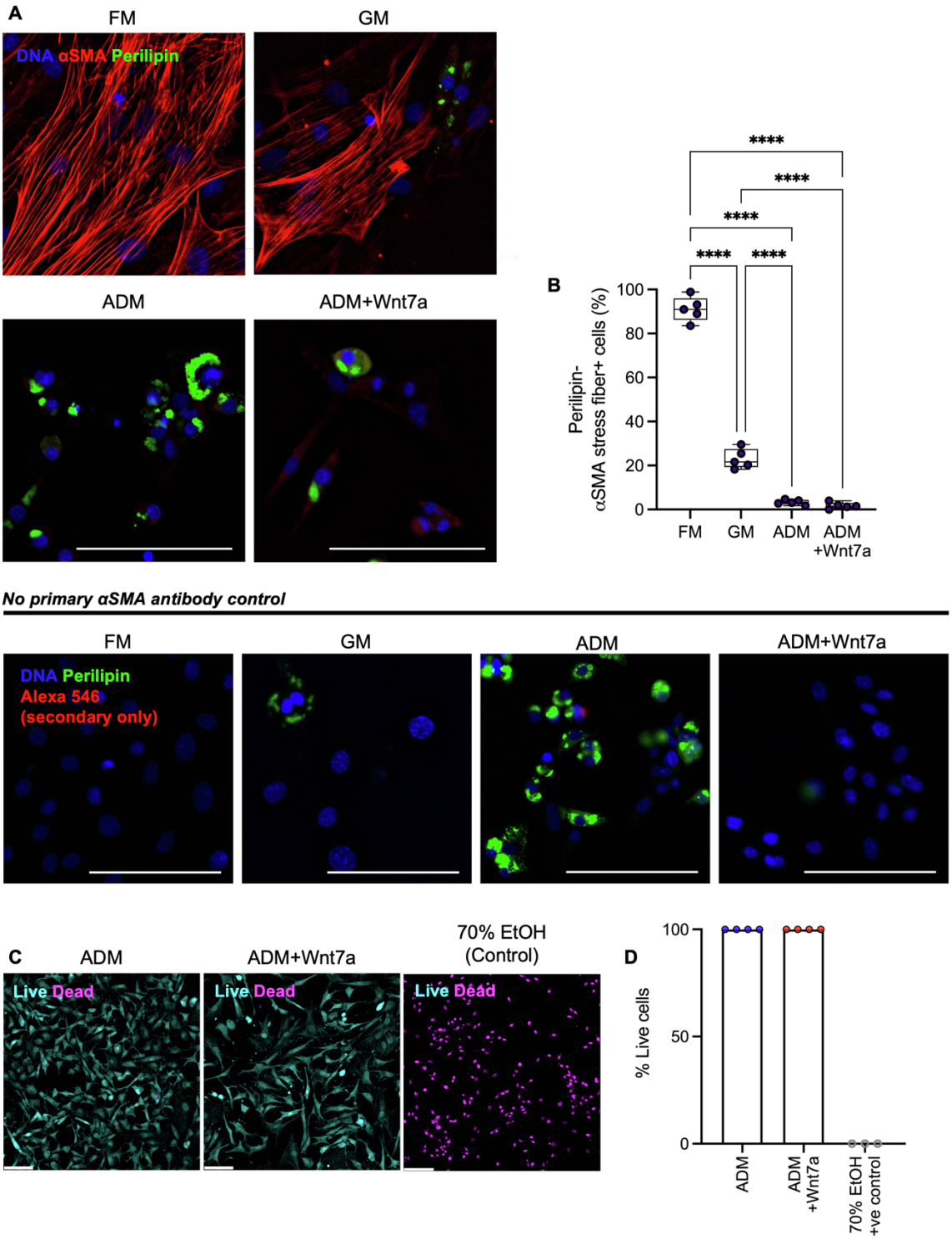

Figure S3. ***(A)*** Representative immunofluorescence images of α-smooth muscle actin (αSMA) and perilipin-labeled FAPs. Scale bar: 100 μm. ***(B)*** Quantification of % αSMA stress fiber positive cells. One-way ANOVA with Tukey's post-hoc analysis. **** p<0.0001. ***(C)*** Representative images of live-dead staining in ADM ± WNT7A conditions after 1-day treatment. A 15-min 70% EtOH treatment was used as a positive control for dead staining. Scale bar: 100 μm. ***(D)*** Quantification of % live cells show minimal cell death with WNT7A (200 ng/ml). n=4.

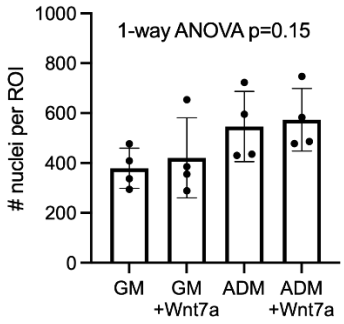

Figure S4. Number of nuclei per image determined using data from Fig. 1F. Freshly isolated FAPS were expanded for 4 days and cultured for additional 3 days in either growth and adipogenic differentiation, with or without Wnt7a.

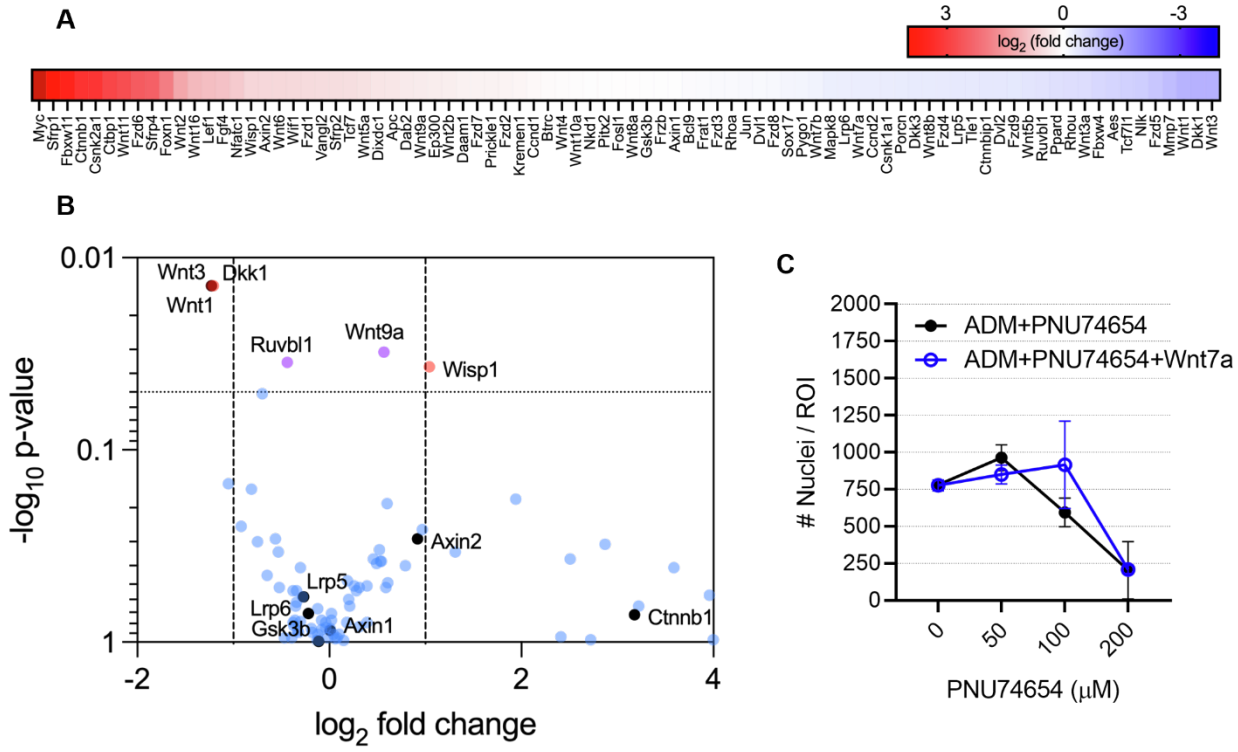

Figure S5. ***(A)*** Gene expression heat map array and ***(B)*** a volcano plot of Wnt-related genes. n=3. ***(C)*** Dose-response assay of PNU74654. Nuclei count begins to decrease around 100 μM after 5 days. n=2 per concentration.

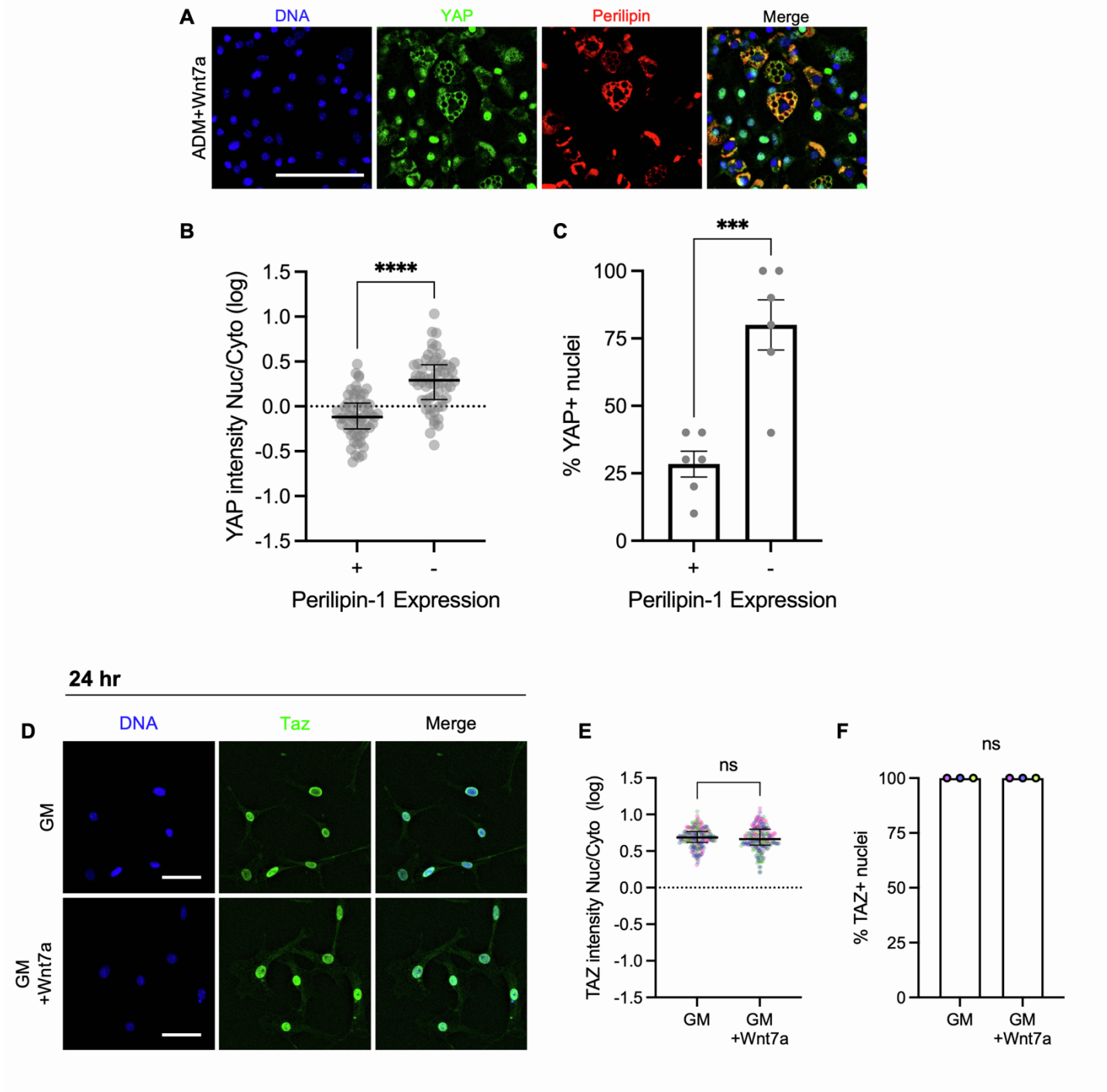

Figure S6. ***(A)*** Representative immunofluorescence images of YAP (detected with Alexa-488) and Perilipin-1 (detected with Alexa-647)-labeled cells after 3-day treatment in ADM + WNT7A (200 ng/ml). Scale bar: 100 μm. ***(B)*** Quantification of YAP nuclear:cytosol intensity ratio. Values were log-transformed. Two-tailed unpaired t-test. Median ± IQR. **** p<0.0001. 120 cells analyzed from 10 replicates. ***(C)*** Percent YAP+ nuclei. Two-tailed unpaired t-test. Mean ± SEM. **** *p*<0.0001. n=10. ***(D)*** Representative immunofluorescence images of TAZ-labeled cells after 24-hour treatment in GM ± WNT7A (200 ng/ml). Scale bar: 50 μm. ***(E)*** Quantification of TAZ nuclear:cytosol intensity ratio at the 24-hour time point. Values were log-transformed. Two-tailed unpaired t-test. Median ± IQR. **** p<0.0001. n=180 cells analyzed from 3 biological replicates. Colors represent biological replicates. ***(F)*** Percent TAZ+ nuclei at the 24-hour time point. Two-tailed unpaired t-test. n=3. Colors represent biological replicates.

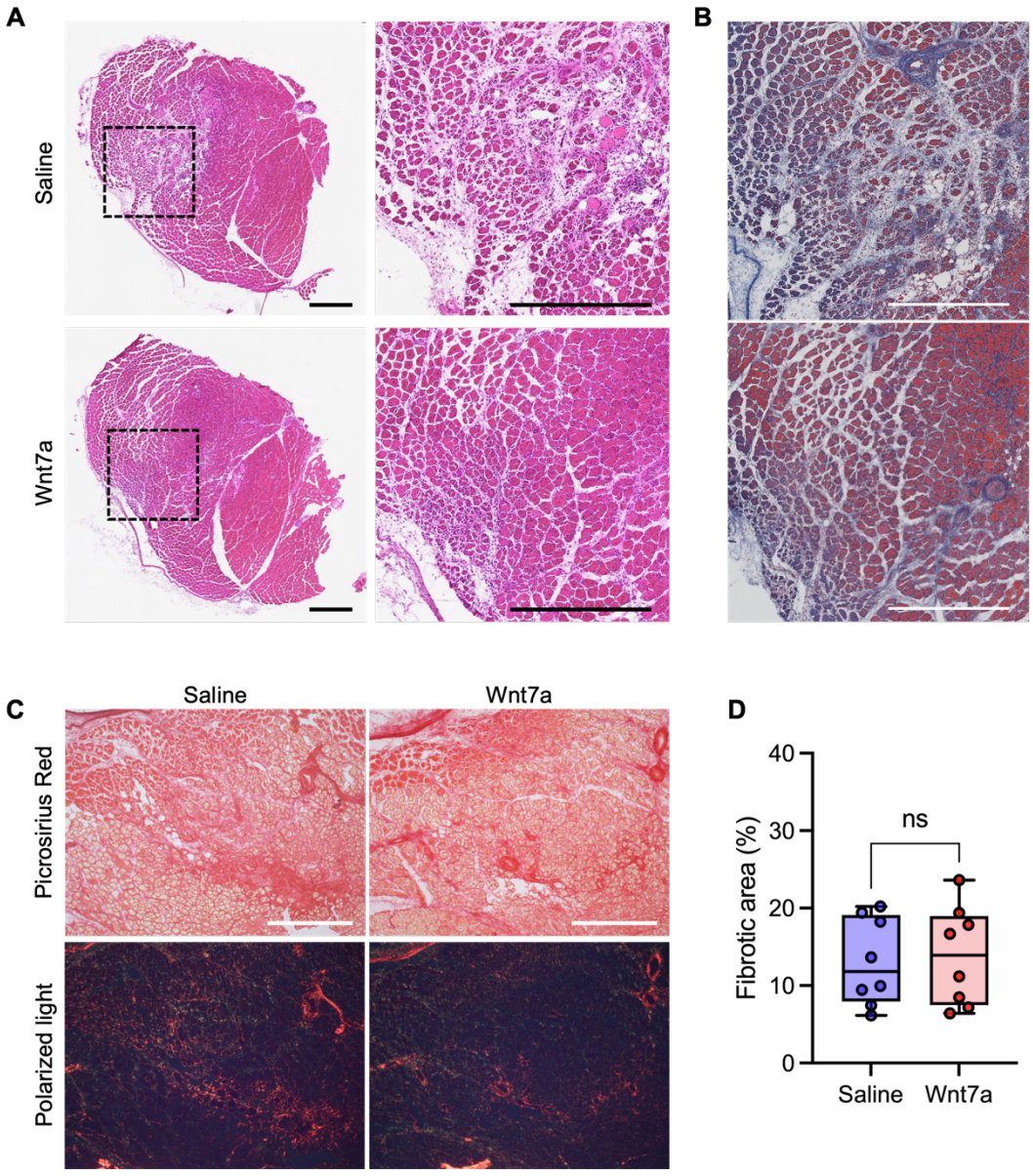

Figure S7. ***(A)*** Representative H&E staining of cross-sectioned TA muscles injected with saline ± WNT7A following glycerol-induced injury. Day 14 post-injury. Scale bar: 500 µm. ***(B)*** Representative trichrome staining of cross-sectioned TA muscles injected with saline (30 µl) ± WNT7A (2 µg/30 µl) following glycerol-induced injury. Day 14 post-injury. Scale bar: 500 µm. ***(C)*** Representative picrosirius red and polarized light images of TA muscles injected with saline ± WNT7A following glycerol-induced injury. Day 14 post-injury. Scale bar: 500 µm. ***(D)*** Quantification of the percent fibrotic area determined from polarized light. Two-tailed paired t-test.

Supplemental Experimental Procedures

Histology and Immunohistochemistry:
Hindlimbs were dissected and fixed for 1 hour at room temperature in 4% paraformaldehyde in PBS. Fixed TA muscles were dissected and frozen in liquid nitrogen-chilled isopentane. 10 μm sections were obtained from the frozen TAs using a cryotome. Tissue sections were incubated using blocking/permeabilization buffer (5.0% goat serum, 2.0% bovine serum albumin, 0.5% Triton X-100 in PBS) for 1 hour at room temperature. The following primary and secondary antibodies were used for tissue immunohistochemistry in this study: anti-perilipin (Abcam; ab3526; 1:200) and goat anti-rabbit Alexa Fluor 488 (ThermoFisher; A11008; 1:500). Hoechst 33342 (ThermoFisher; PI62249; 1:1000) and Alexa Fluor 647 Phalloidin (ThermoFisher; A22287; 1:1000) were used to stain nuclei and F-actin, respectively. Tissue sections were also processed for routine H&E, trichrome, and picrosirius red staining used for polarized light imaging.

Imaging and Image Analysis
Quantifications of perilipin area, % Oil Red O+ cells, % PPARγ+ nuclei, nuclear β-catenin intensity, in vivo perilipin+ area, and in vivo polarized light signal+ fibrotic area were performed using ImageJ in an automated manner. We describe the workflow for each measurement performed below. *Nuclei Count:* (1) Apply auto threshold (Otsu dark method) on *DNA* channel, (2) make binary, (3) apply watershed, (4) apply fill holes, (5) run analyze particle function (size 12-300 μm^2, circularity 0.3-1.0). *Perilipin area:* (1) Apply auto threshold (Otsu dark method) on *perilipin* channel, (2) convert to mask, (3) run analyze particle function (size 10-5000 μm^2). *Nuclear PPARγ count:* (1) Apply auto threshold (Otsu dark method) on *PPARγ* channel, (2) convert to mask, (3) run analyze particle function (size 45-450 μm^2). *Nuclear β-catenin intensity:* (1) make *DNA* channel binary, (2) apply watershed, (4) apply fill holes, (5) run analyze particle function (show overlay, exclude on edges, add to manager, in situ show), (6) select *β-catenin* channel, (7) run measure function from ROI Manager. *In vivo perilipin+ area:* (1) perilipin channel to 8-bit, (2) apply auto threshold (Otsu dark method), (3) set black background, (4) convert to mask, run analyze particle function (size 100-4900 μm^2). *Polarized light signal+ fibrotic area*: (1) convert polarized light image to 8-bit, (2) apply auto threshold (Otsu dark method), (3) run measure function with “area mean limit” on, (4) turn off “area mean limit” and manually outline the muscle section boundary, (5) run measure function to obtain muscle cross-sectional area and determine % fibrotic area. Myofiber areas were manually quantified using ImageJ. Cell shape metrics were manually quantified by outlining individual cells using ImageJ. The measure function was applied to determine cell area, minimum and maximum Feret’s diameter, and Feret’s angle. Note, the maximum Feret’s diameter indicates the longest distance between two points along the cell boundary, and the minimum Feret’s diameter indicates the shortest distance between two points along the cell boundary. Quantification of αSMA stress fiber+ cells was manually determined. YAP/TAZ nuclear-cytoplasmic ratios were manually quantified. Quantification of the percent YAP+ nuclei was determined by counting nuclei exhibiting Log(Nuc/Cyto) > 0.