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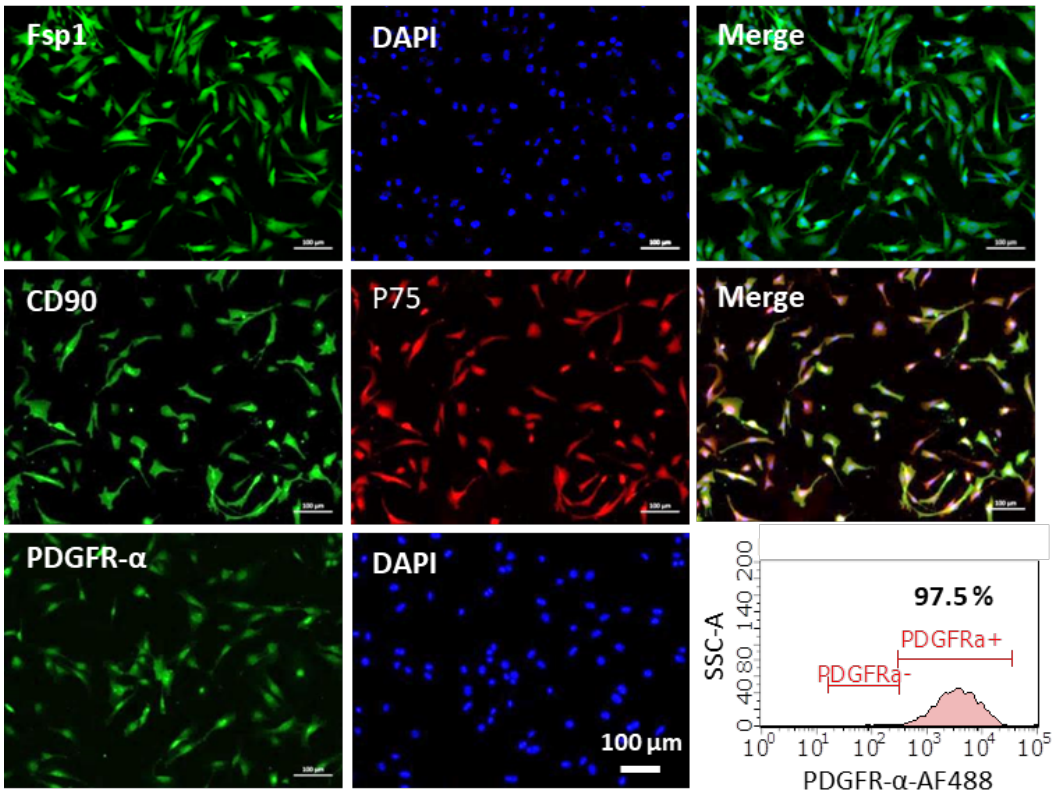


Fig. S1 | Characterization of primary dermal fibroblast-like cells. Immunofluorescence analysis of fibroblast marker (Fsp1, CD90, P75 and PDGFR-α) expression, and flow-cytometry analysis of PDGFR-α after overnight seeding of dermal cells in Fb medium. All the experiments were performed in triplicate.

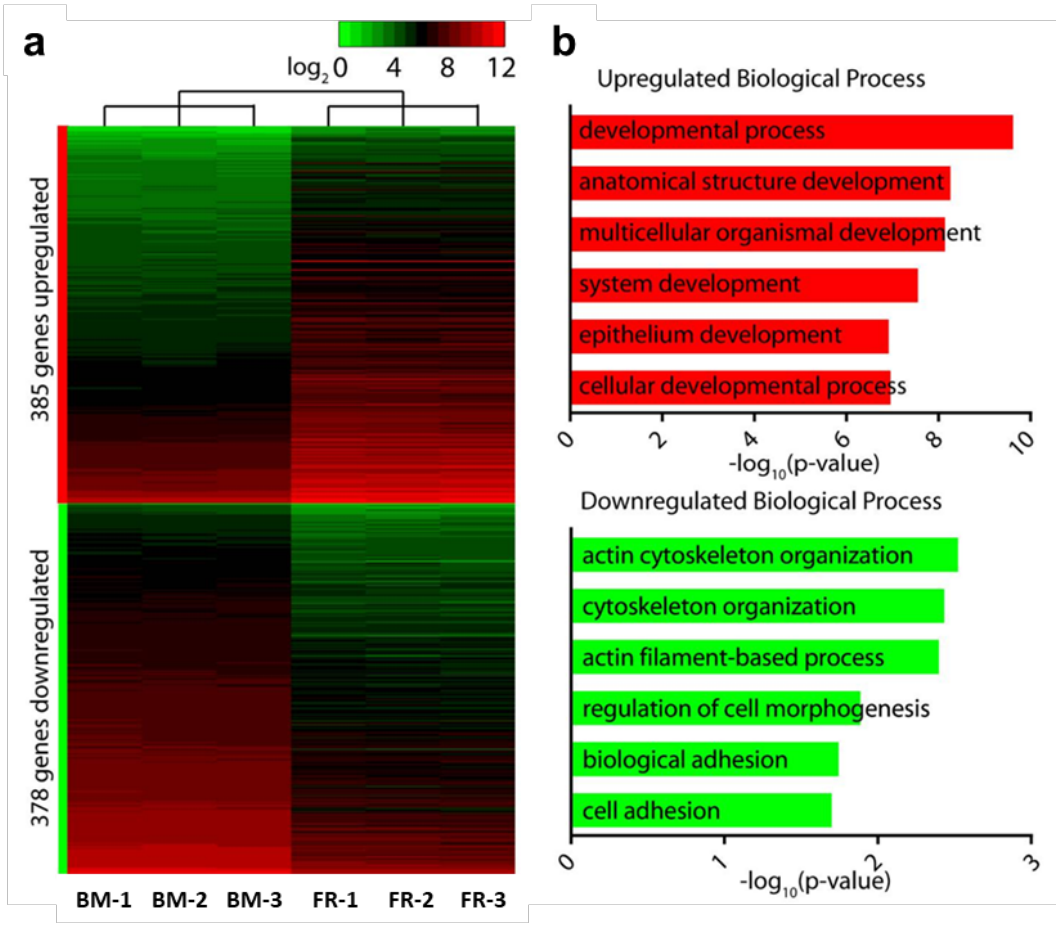


Fig. S2 | DNA microarray analysis of dermal cells treated with FR medium for 2 days. a, Heat map of upregulated and downregulated genes (> two-fold, FDR-adjusted $p < 0.05$, $n = 3$). **b**, Enriched gene ontology terms for upregulated and downregulated biological processes.

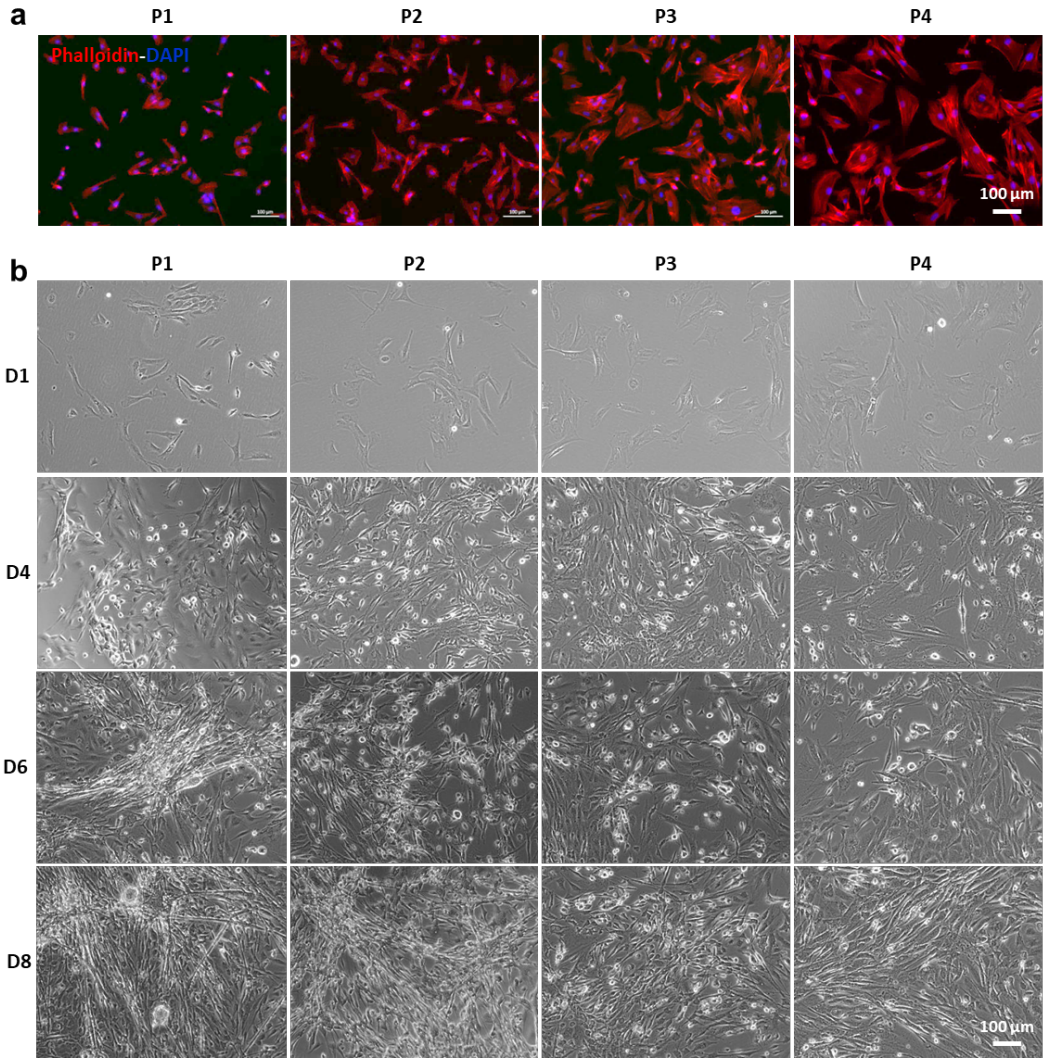


Fig. S3 | Chemical-induced myotube formation from dermal cells at different passages. a, The morphology of dermal cells at various passages stained with phalloidin and DAPI one day after plating. **b**, Bright-field images show myotube formation in FR medium-treated dermal cells at different passages. All the experiments were performed in triplicate.

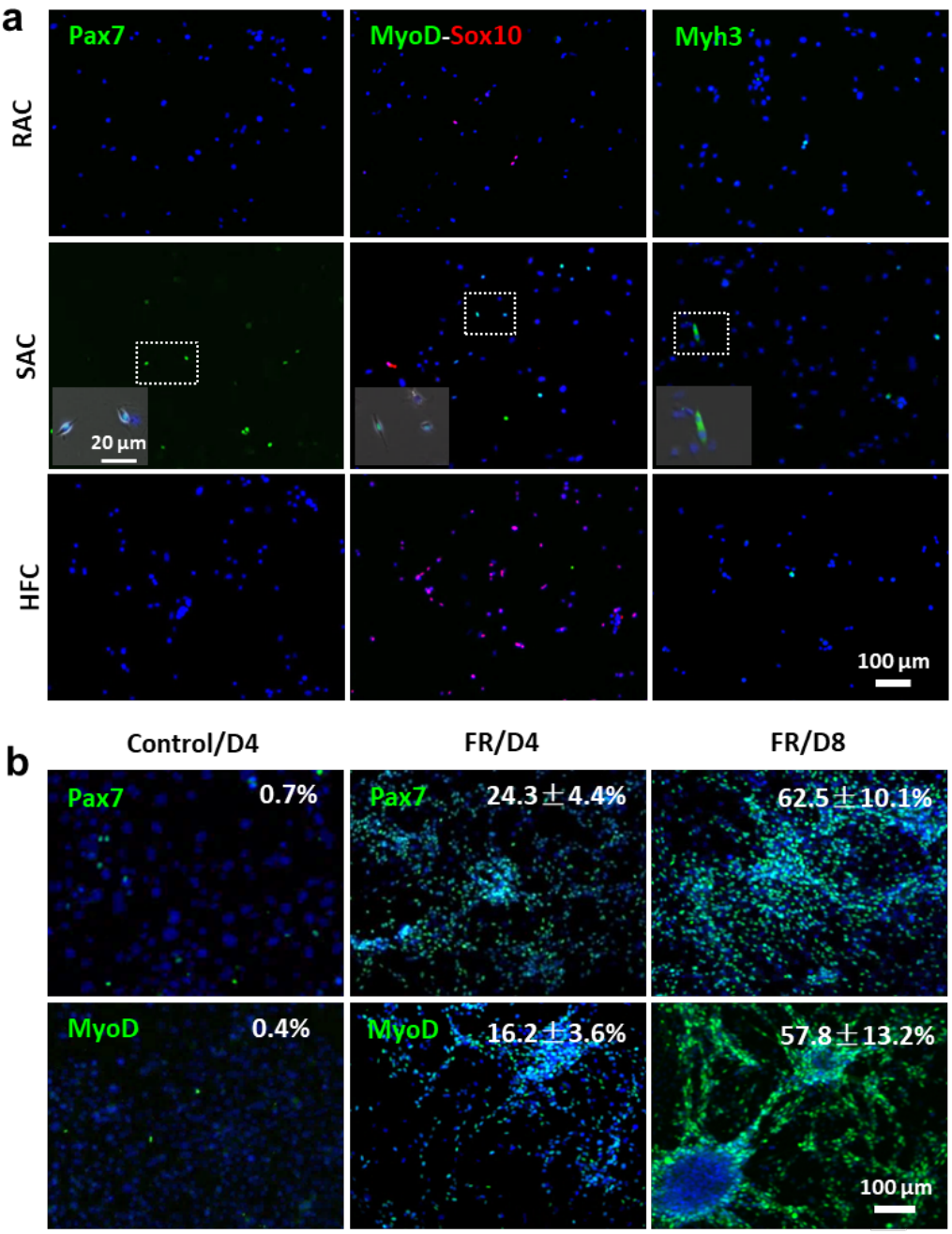


Fig. S4 | Slow-adhering cell population contributes to the expanded CiMCs. a, Immunofluorescence analysis of skeletal muscle markers (Pax7, MyoD, Myh3) and skin NCSC marker Sox10 in rapidly adhering cells (RACs), slow-adhering cells (SACs) and hair follicle cells (HFCs) after overnight seeding in basic medium. Insets are magnified regions within the white rectangle. **b**, Immunofluorescence staining for Pax7 and MyoD in CiMCs induced from SACs. Numbers denote the percentage of positive cells. All the experiments were performed in triplicate.

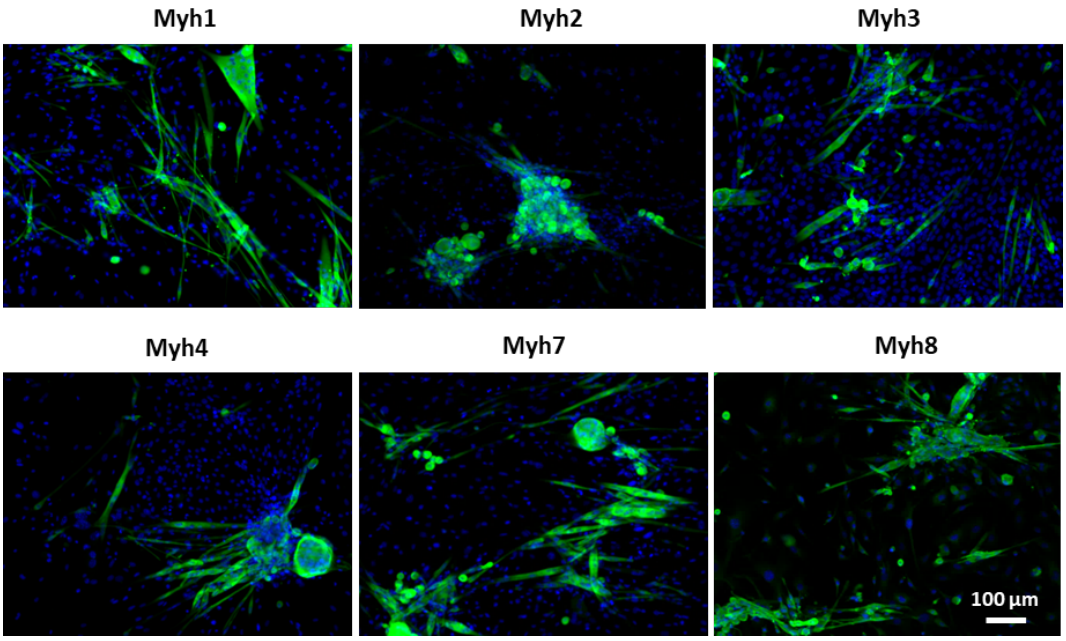


Fig. S5 | Characterization of skeletal muscle MHC expression in CiMCs. Cells were treated with the chemical cocktail for 6 days, and stained for myosin heavy chain markers following fixation and permeabilization. All the experiments were performed in triplicate.

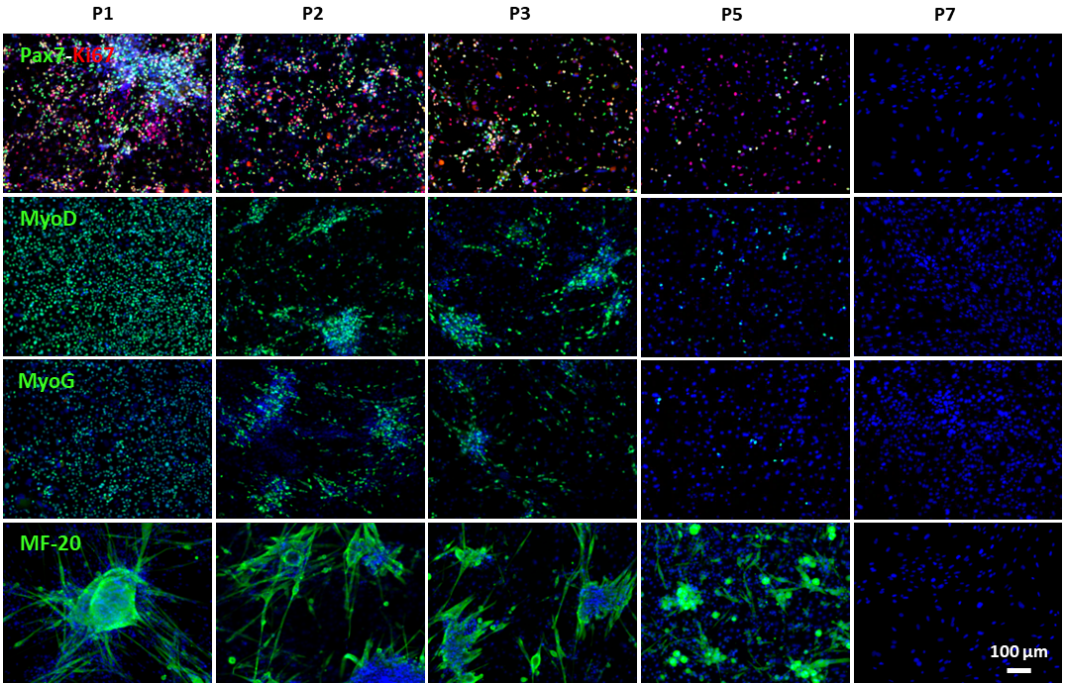


Fig. S6 | Myogenic expansion and differentiation potential of CiMCs at different passages. CiMCs were passaged every 3 days in FR medium, and the myogenic ability of CiMCs at different passages was evaluated after 8 days of culture in FR medium. All the experiments were performed in triplicate.

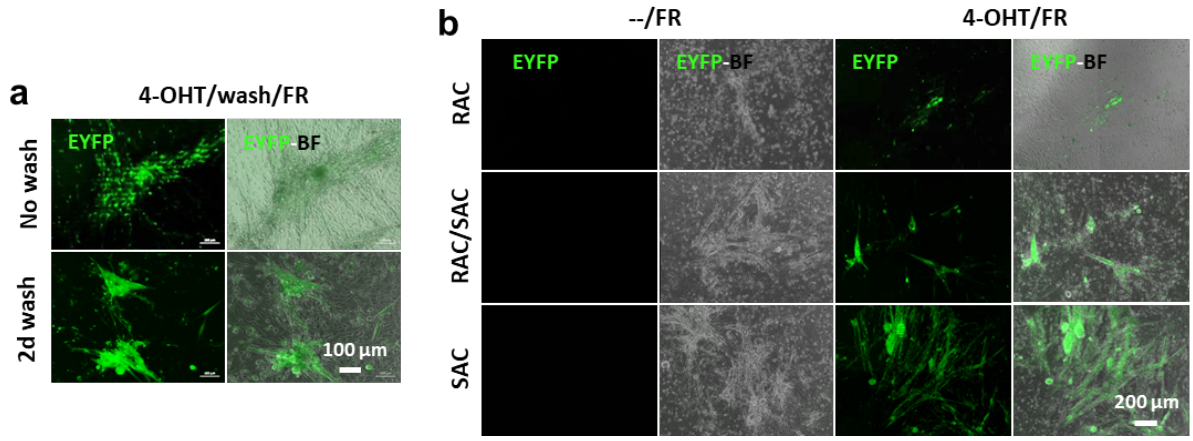
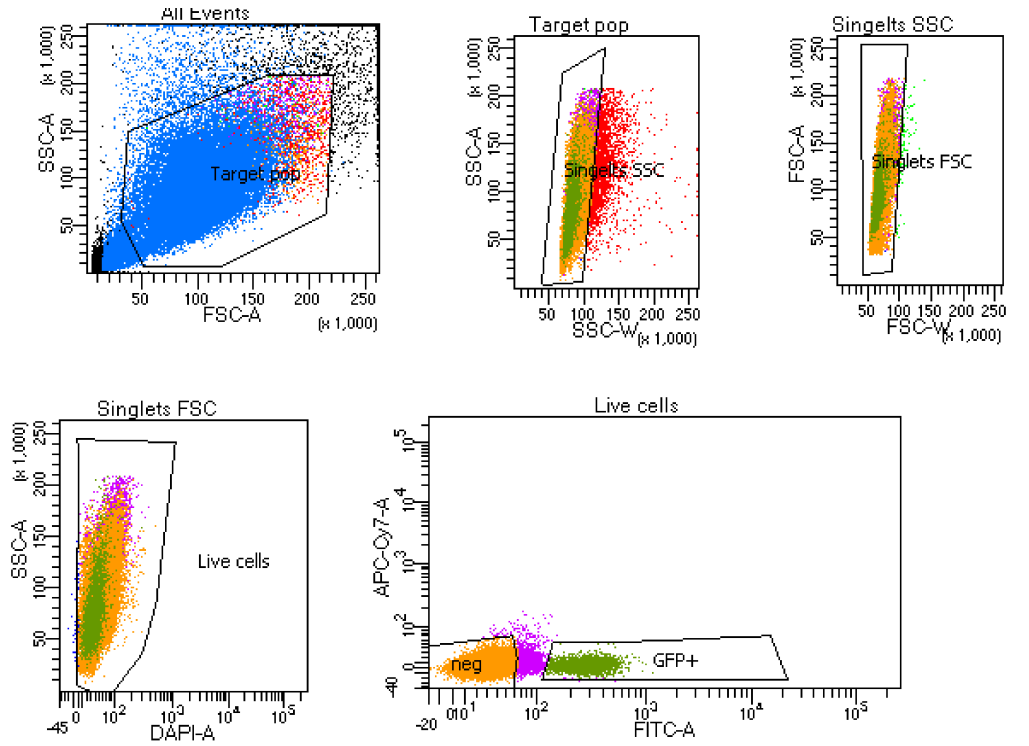


Fig. S7 | Chemical cocktail induced myogenesis from transgenic Pax7-CreER:Rosa26-EYFP dermal cells. a, Dermal cells from Pax7-CreER:Rosa26-EYFP mice were seeded in basic Fb medium containing 4-OTH for 1 day. The medium was replaced with fresh Fb medium for 2 days, and then changed to FR medium for an additional 8 days. **b**, RACs, RACs/SACs, and SACs of Pax7-CreER:Rosa26-EYFP dermal cells were treated with and without 4-OHT and cultured in FR medium for 8 days. All the experiments were performed in triplicate.



Tube: Pax 7 GFP

Population	#Events	%Parent	%Total
All Events	62,440	####	100.0
Target pop	50,000	80.1	80.1
Singelts SSC	47,560	95.1	76.2
Singlets FSC	47,475	99.8	76.0
Live cells	47,368	99.8	75.9
neg	42,510	89.7	68.1
GFP+	2,656	5.6	4.3
P8	3,021	6.4	4.8
P9	0	0.0	0.0
P10	5	0.0	0.0
P11	5,418	11.4	8.7
Q1	0	0.0	0.0
Q2	1	0.0	0.0
Q3	47,150	94.3	75.5
Q4	2,849	5.7	4.6
P7	51,520	82.5	82.5

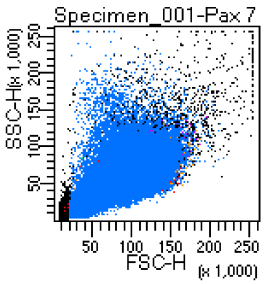


Fig. S8 | Gating strategy for FACS of EYFP$^-$ and EYFP$^+$ cells. The sorted cells were day 4 Pax7 lineage-tracing CiMCs, by BD FACSDiva Software Version 8.0.2.

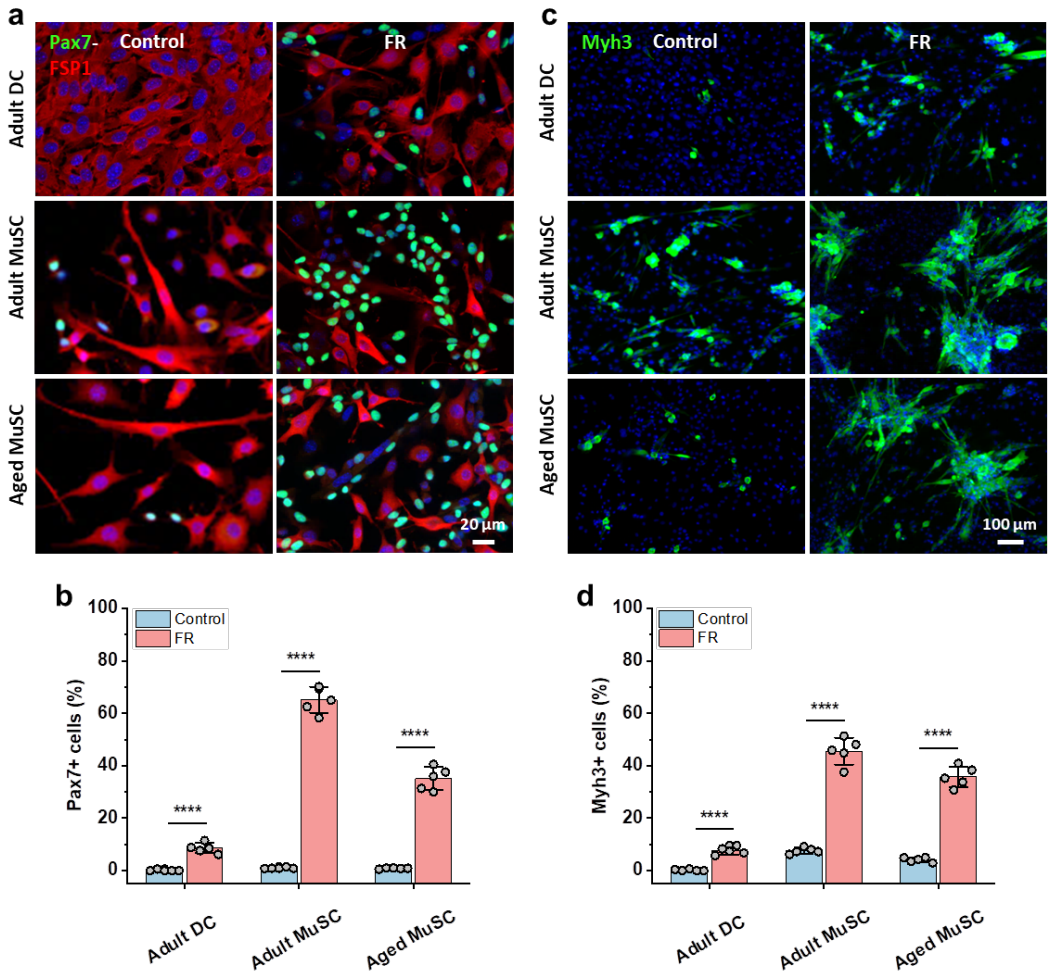


Fig. S9 | Chemical-induced myogenic expansion in adult dermal cells (DC) and MuSCs. a, Immunofluorescence staining for Pax7 and FSP1 in adult DCs, and adult and aged MuSCs at D8 in control Fb media (left panels) and FR media (right panels). **b**, Percentage of Pax7$^+$ cells in (**a**) ($n = 5$ samples per group). **c**, Immunofluorescence staining for Myh3 in adult dermal cells and adult and aged MuSCs at D8 in control Fb media (left panels) and FR media (right panels). **d**, Percentage of Myh3$^+$ cells in (**c**) ($n = 5$ samples per group). Data are presented as mean ± SD. Two-tailed Student's t-test. ****$P < 0.0001$.

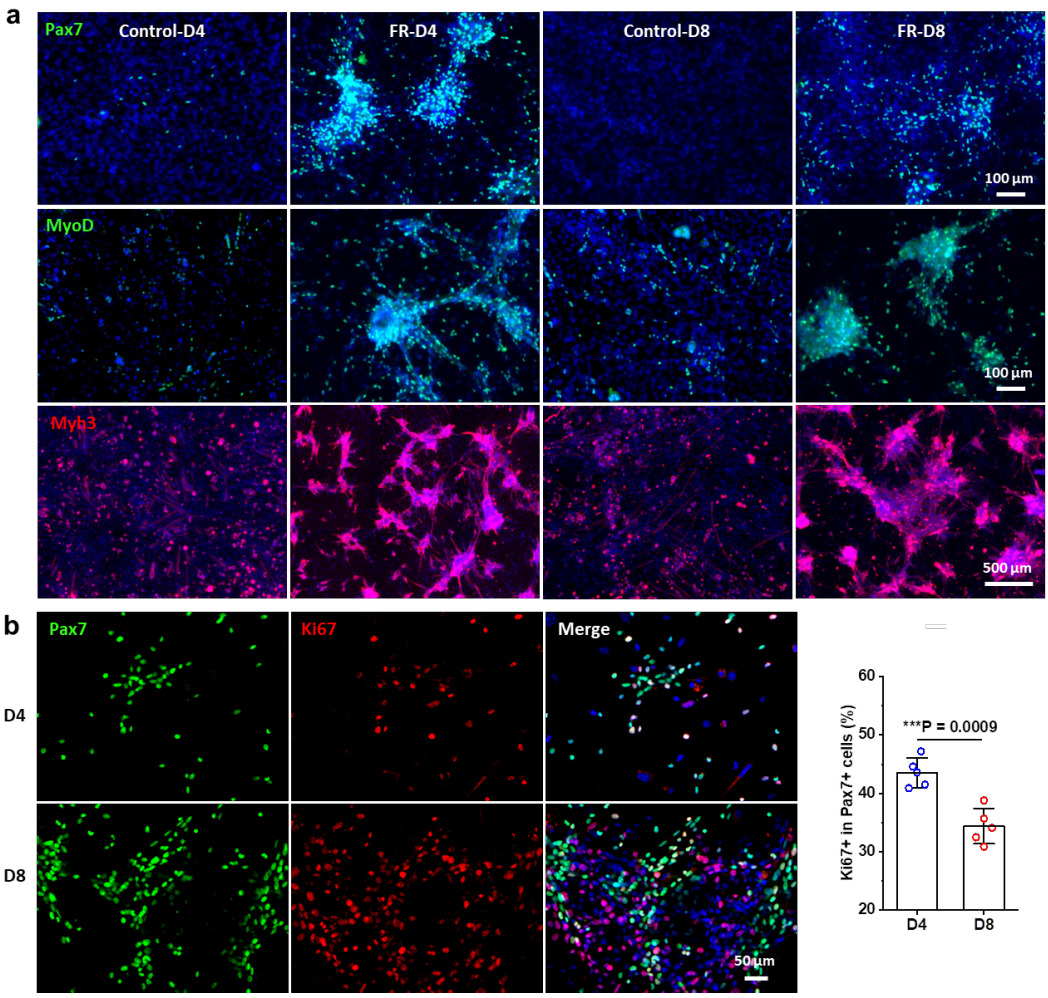


Fig. S10 | Chemical-induced myogenic expansion of adult MuSCs. a, Immunofluorescence staining for Pax7, MyoD and Myh3 in mouse adult MuSCs treated with control Fb media or FR media for 4 days and 8 days ($n = 3$ samples per group). **b**, Immunofluorescence staining for Pax7-Ki67 in CiMCs expanded from adult mouse MuSCs and the percentage of proliferating $Pax7^+$ cells based on Ki67 expression ($n = 5$ samples per group). Data are presented as mean ± SD. Two-tailed Student's t-test. ***$P < 0.001$.

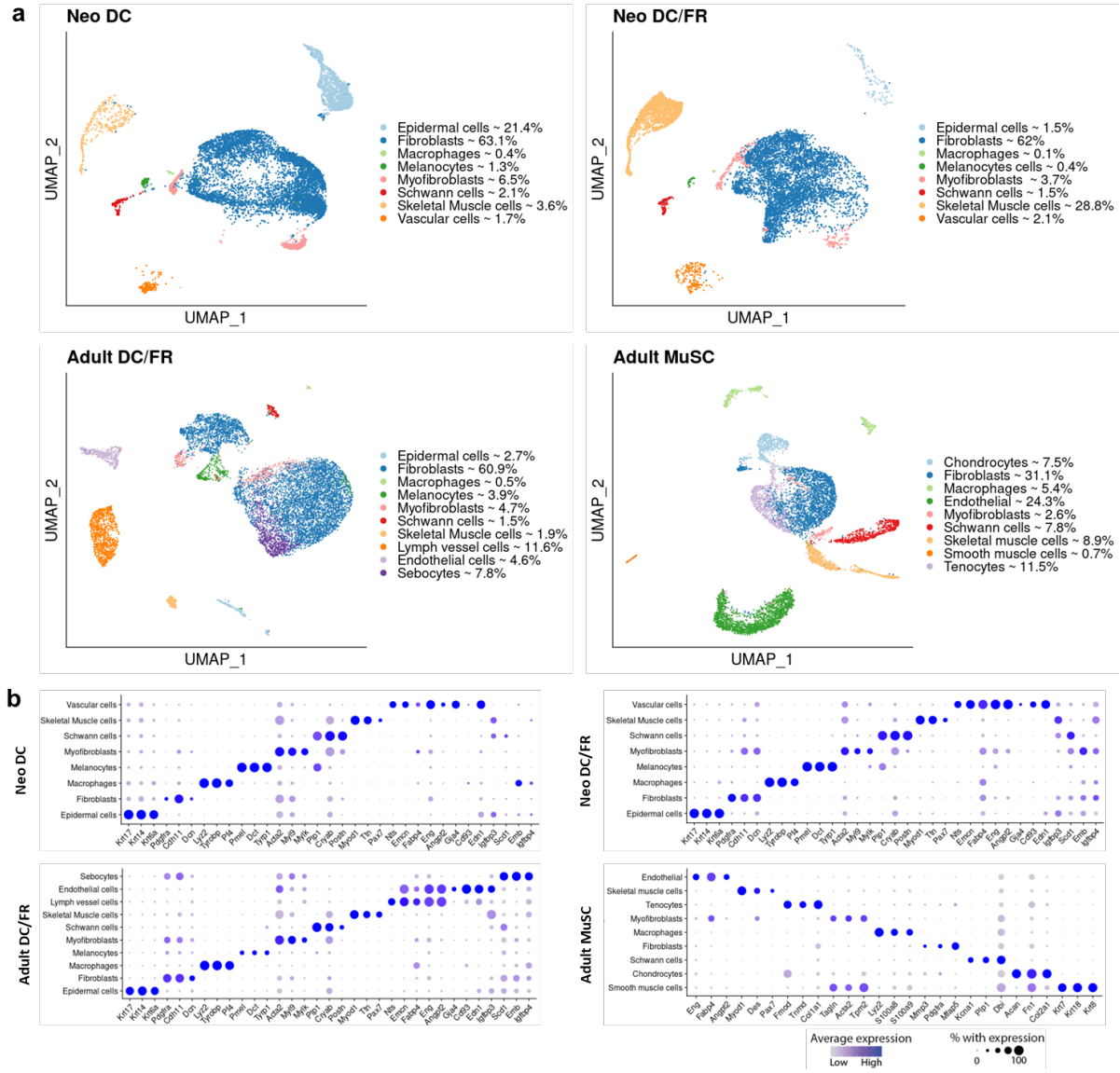
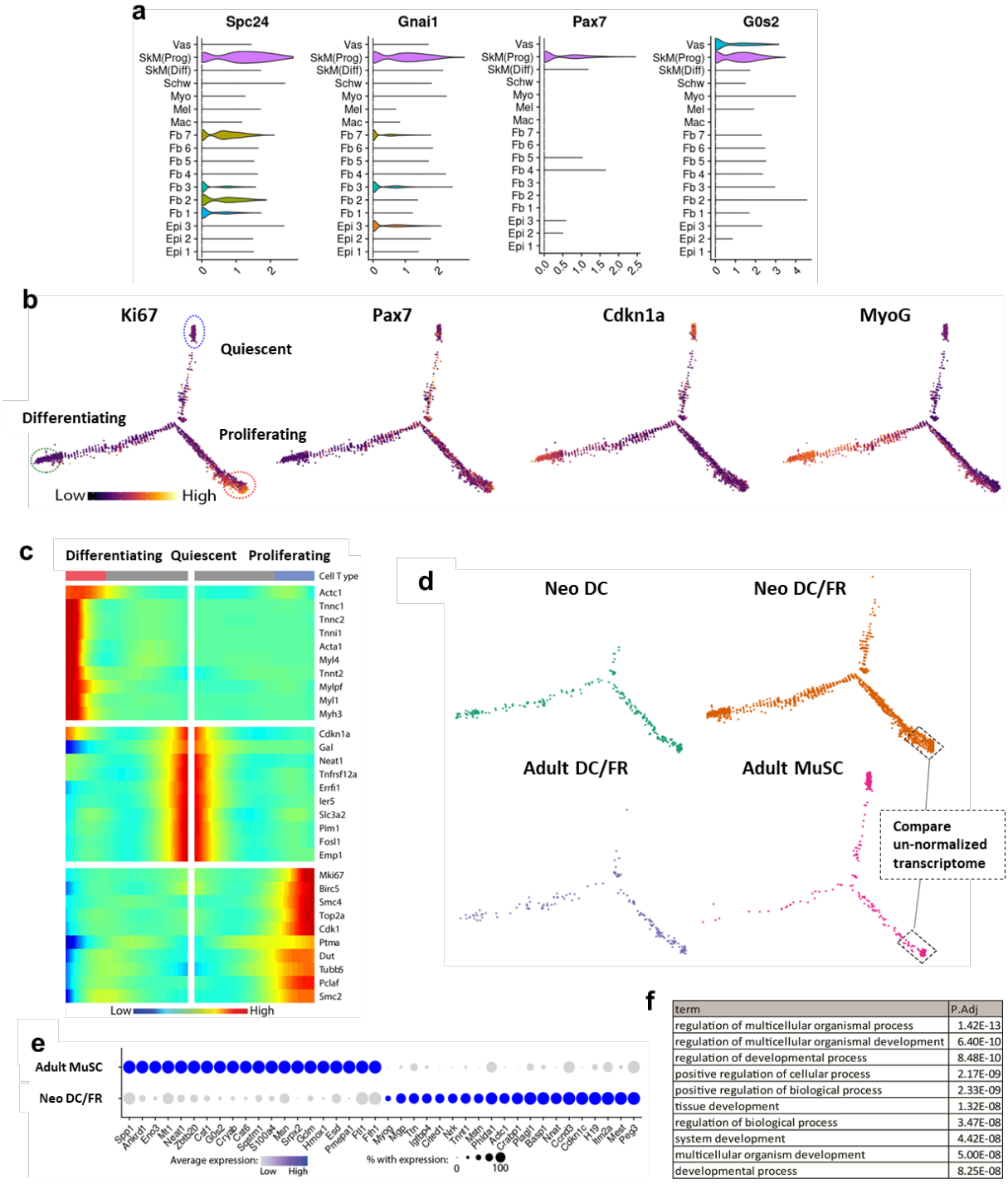


Fig. S11 | scRNA-seq analysis of chemical-treated dermal cells and endogenous MuSCs. a, UMAP plot showing the integration of the neo DC, neo DC/FR, adult DC/FR and adult MuSC. Numbers indicate cell percentage in total cells. **b**, Marker genes used for identifying the different cell clusters.



f

term	P.Adj
regulation of multicellular organismal process	1.42E-13
regulation of multicellular organismal development	6.40E-10
regulation of developmental process	8.48E-10
positive regulation of cellular process	2.17E-09
positive regulation of biological process	2.33E-09
tissue development	1.32E-08
regulation of biological process	3.47E-08
system development	4.42E-08
multicellular organism development	5.00E-08
developmental process	8.25E-08

Fig. S12 | scRNA-seq analysis reveals heterogeneous cell identities. a, Identification of other marker genes for Pax7$^+$ cell clusters in neonatal dermal cells. Neonatal dermal cells were clustered with higher resolution, and differential gene expression test was performed between the Pax7$^+$ cell clusters with other cell clusters, and identified another 3 genes that were highly expressed in Pax7$^+$ cell clusters. Vas: Vascular cells, Skm(Prog): Skeletal muscle progenitor cells, Skm(Diff): Skeletal muscle differentiating cells, Schw: Schwann cells, Myo: Myofibroblasts, Mel: Melanocytes, Mac: Macrophages, Fb1-7: 7 sub-clusters of fibroblasts, Epi 1-3: 3 sub-clusters of epidermal cells. **b**, Pseudotime analysis of myogenic cells from all samples. The color gradient indicates the expression level of the respective genes along pseudotime trajectory. **c**, Heatmap showing upregulated genes in the differentiating, quiescent and proliferating myogenic cells. **d**, Pseudotime trajectory broken down into the respective samples. Cells from early pseudotime were identified and their un-normalized gene expression data were tested for differential expression testing. **e**, Top 20 up and down-regulated gene identified from in Neo DC/FR and endogenous adult MuSC**. f**, Gene ontology biological process terms enriched for the differentially expressed genes of Neo DC/FR and endogenous adult MuSC.

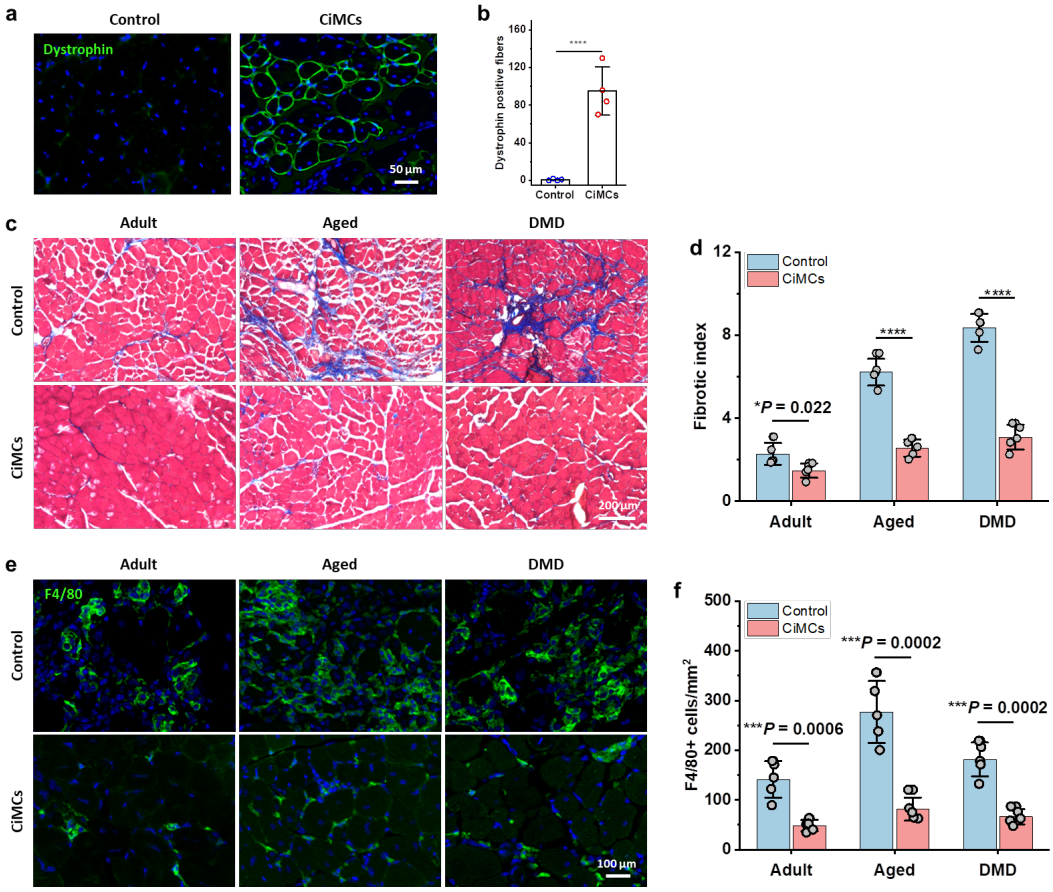


Fig. S13 | *In vivo* analysis of CiMCs transplantation in CTX-injured adult, aged and mdx mice. a, Dystrophin expression in dermal cells (negative control) and CiMCs implanted in mdx TA muscles for 4 weeks ($n = 4$ mice per group). **b**, The amount of dystrophin$^+$ fibers in (**a**) ($n = 4$ mice per group). **c**, Representative images of Masson Trichrome stained sections in adult, aged and mdx TA muscles treated with control cells or CiMCs for 4 weeks. **d**, Quantification of the fibrotic index. The fibrotic index was derived as the area of fibrosis divided by the total area of muscle, normalized to untreated normal muscle ($n = 5$ mice per group). **e**, Macrophage staining with F4/80 in adult, aged and mdx TA muscles at 4 weeks after CiMC transplantation. **f**, Quantification of F4/80 expressing cells in adult, aged and mdx TA muscles at 4 weeks after CiMC transplantation ($n = 5$ mice per group). Data are presented as mean ± SD. Two-tailed Student's t-test. *$P < 0.05$, ***$P < 0.001$ and ****$P < 0.0001$.

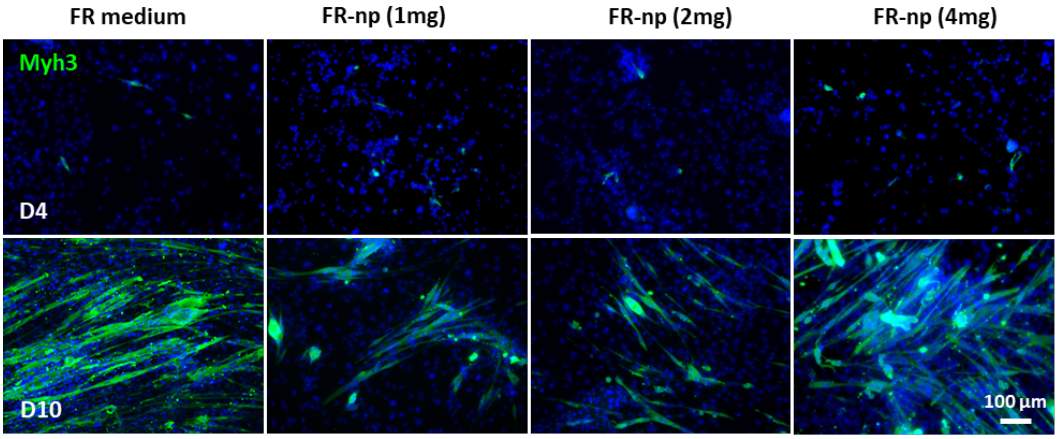


Fig. S14 | Drug-loaded nanoparticles induce *in vitro* myogenesis. Immunofluorescence staining for Myh3 in SACs treated with different doses of FR-np. All the experiments were performed in triplicate.

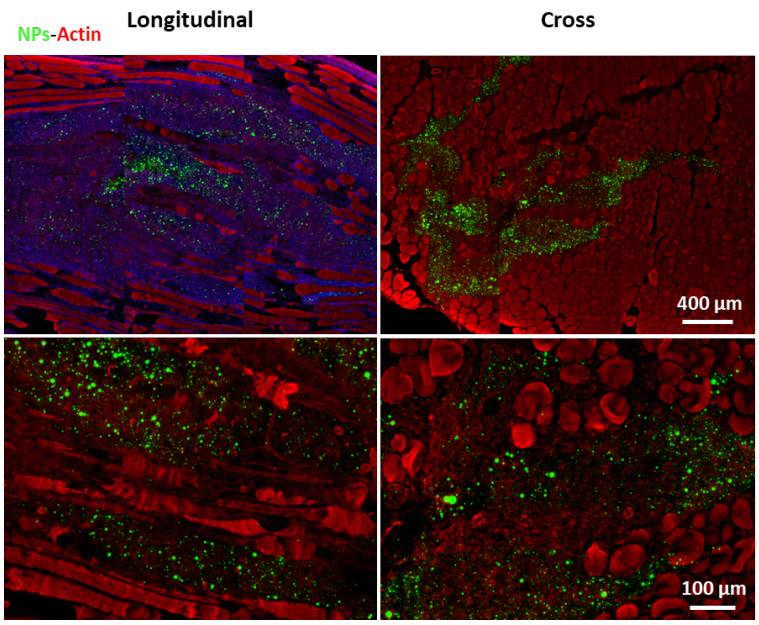


Fig. S15 | Nanoparticle distribution in TA muscles after injection. Immunofluorescent images show the distribution of green fluorescence-labeled nanoparticles (NPs) in longitudinal and cross-sections of TA muscles 2 days after injection. All the experiments were performed in triplicate.

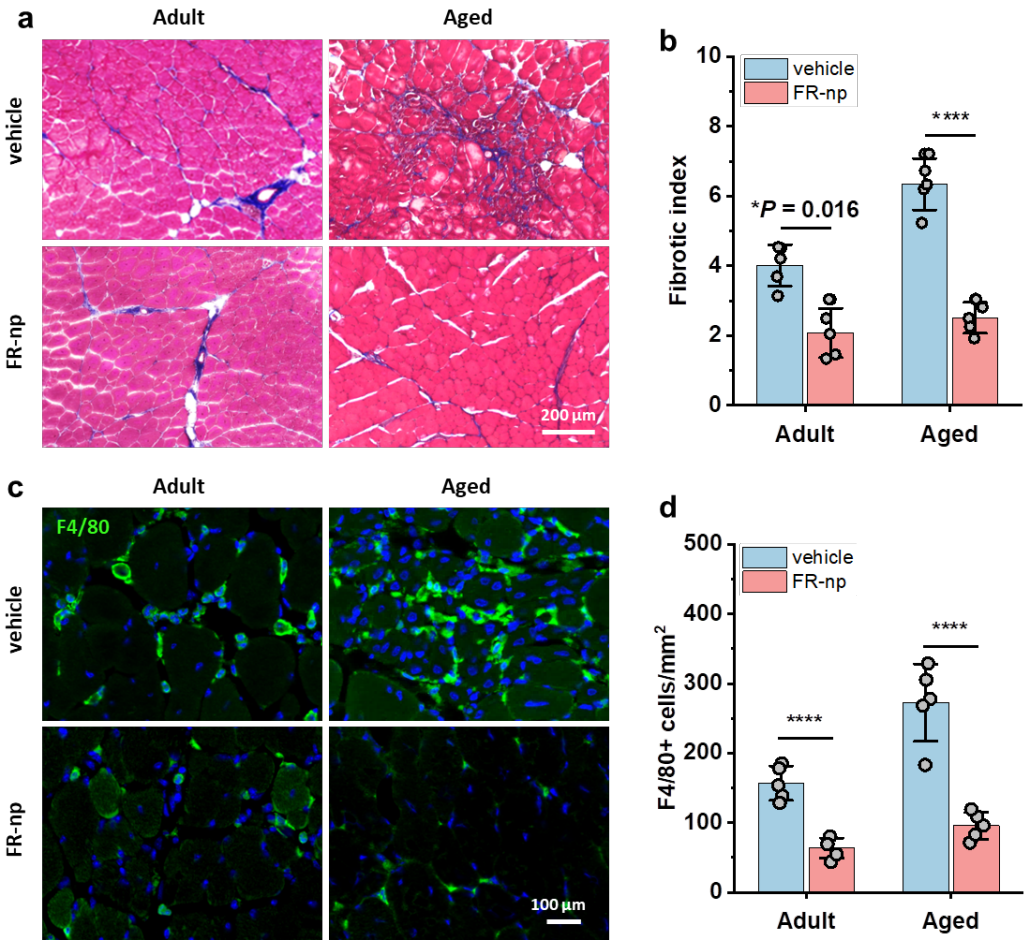


Fig. S16 | FR-np promotes muscle regeneration by suppressing fibrosis and inflammation. a, Representative images of Masson Trichrome stained sections in adult and aged TA muscles treated with vehicle or FR-np for 4 weeks. Vehicle refers to np without drugs and served as a control. **b**, Quantification of the fibrotic index for conditions indicated in (**a**). The fibrotic index is calculated as the area of fibrosis divided by the total area of muscle, normalized to untreated normal muscle ($n = 5$ mice per group). **c**, Macrophage staining with F4/80 in adult and aged TA muscles treated with or without FR-np for 4 weeks. **d**, Quantification of F4/80 expressing cells in adult and aged TA muscles treated with or without FR-np for 4 weeks ($n = 5$ mice per group). Data are presented as mean ± SD. Two-tailed Student's t-test. *$P < 0.05$ and ****$P < 0.0001$.

Supplementary Table S1 | Primer sequences for RT-qPCR analysis.

Gene	Forward	Reverse
Pax7	CGTAAGCAGGCAGGAGCTAA	ACTGTGCTGCCTCCATCTTG
Mrf5	AAGGCTCCTGTATCCCCTCAC	TGACCTTCTTCAGGCGTCTAC
MyoD	CTGCTCTGATGGCATGATGGA	CACTGTAGTAGGCGGTGTCG
Mymk	TTCCTCCCGACAGTGAGCAT	GCACAGCACAGACAAACCAG
MyoG	GTGCCCAGTGAATGCAACTC	CGAGCAAATGATCTCCTGGGT
Myh3	CTCTGTCACAGTCAGAGGTGT	TTCCGACTTGCGGAGGAAAG
Nanog	TCTTCCTGGTCCCCACAGTTT	GCAAGAATAGTTCTCGGGATGAA
Oct4	GGCTTCAGACTTCGCCTCC	AACCTGAGGTCCACAGTATGC
Aggrecan	CCTGCTACTTCATCGACCCC	AGATGCTGTTGACTCGAACCT
Pparg	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
Runx2	AACGATCTGAGATTTGTGGGC	CCTGCGTGGGATTTCTTGGTT
Hand2	CACCAGCTACATCGCCTACC	TCTCATTCAGCTCTTTCTTCCTCT
B2M	CTCGGTGACCCTGGTCTTTC	TTGAGGGGTTTTCTGGATAGCA

Supplementary video S1. Spontaneous beating of CiMC myotube derived from dermal cells treated with FR medium for 4 days.

Supplementary video S2. Spontaneous beating of CiMC myotube derived from dermal cells treated with FR medium for 8 days.

Supplementary video S3. Spontaneous beating of CiMC myotube derived from dermal cells treated with FR medium for 14 days.

Supplementary video S4. Spontaneous beating of CiMC myotube derived from dermal cells treated with FR medium for 16 days.