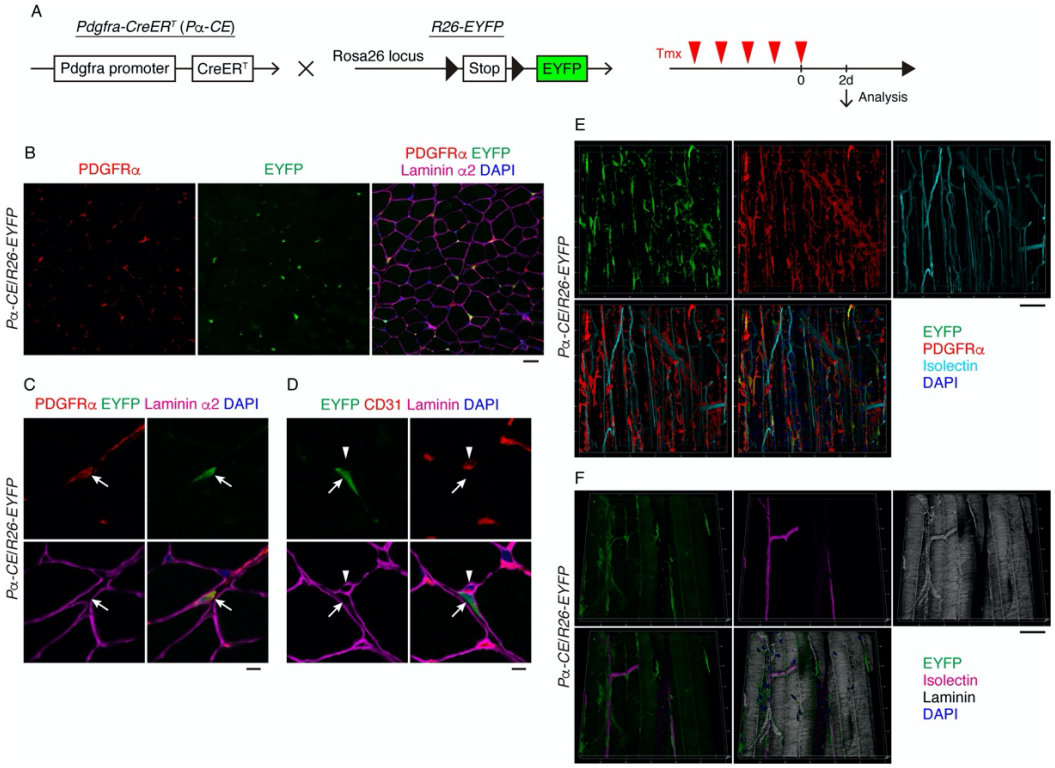
Supplemental Data

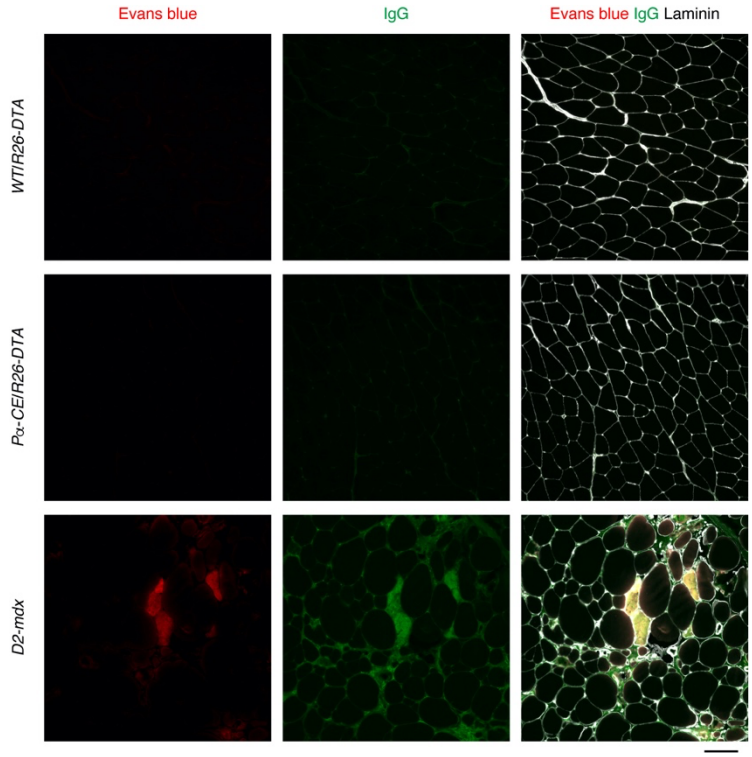


Supplemental Figure 1. Specific recombination in mesenchymal progenitors induced in the *Pdgfra-CreER* mice

(A) Generation of *Pα-CE*/*R26-EYFP* mice.

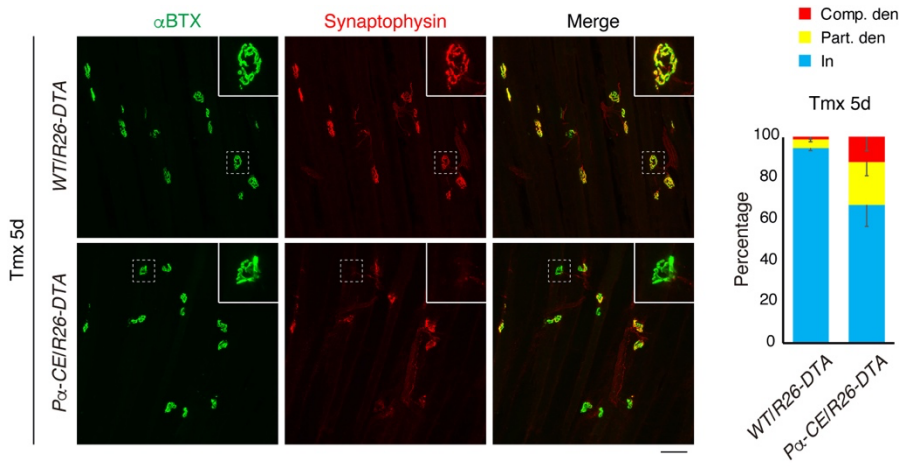
(B–D) Immunofluorescence staining of *Pα-CE*/*R26-EYFP* muscle for PDGFRα, EYFP, and laminin α2 (B, C) and EYFP, CD31, and laminin (D). Note that specific recombination was induced in PDGFRα$^+$ cells, which were localized outside the basal lamina. Arrows indicate mesenchymal progenitors located in interstitial space, and arrowheads indicate capillaries surrounded by a basal lamina.

(E and F) Whole-mount immunofluorescence staining of *Pα-CE*/*R26-EYFP* EDL muscle for EYFP and PDGFRα (E), and EYFP and Laminin (F). Fluorescently-labeled isolectin was used to visualize vasculature. Scale bars, 20 μm (B), 5 μm (C, D), and 50 μm (E, F).



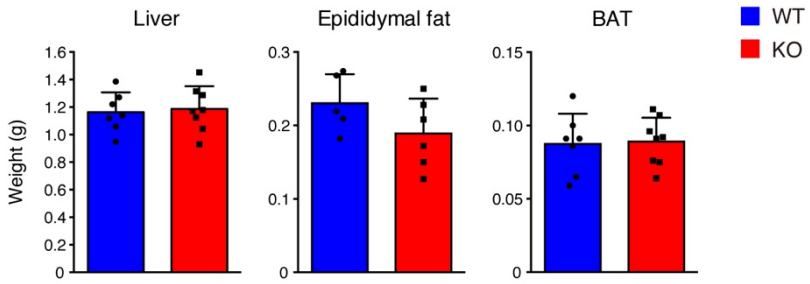
Supplemental Figure 2. Mesenchymal progenitor depletion does not damage myofibers

Fluorescence images of Evans blue dye, IgG, and laminin. *D2-mdx* mice were used as a positive control for the assessment of myofiber damage. Scale bar, 100 μm.



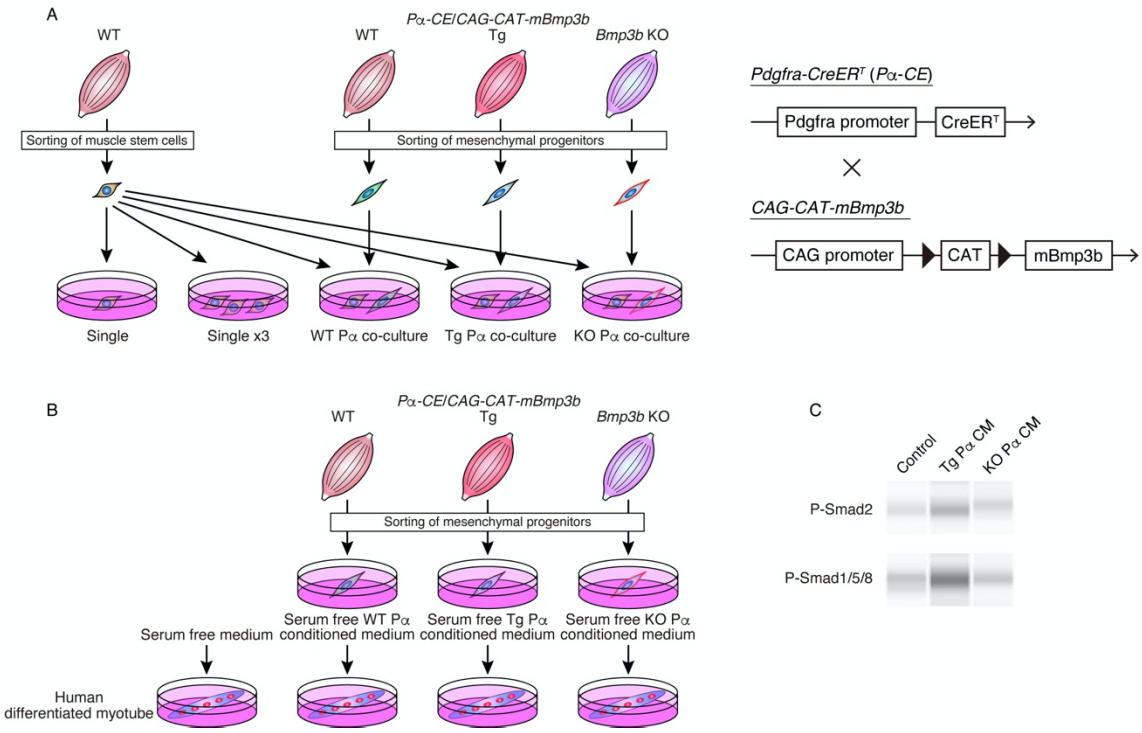
Supplemental Figure 3. NMJ status after mesenchymal progenitor depletion

Fluorescence images of acetylcholine receptor (αBTX) and synaptophysin. Insets show magnified images of boxed regions. Ratios of completely denervated (Comp. den), partially denervated (Part. den), and innervated (In) NMJ were calculated at 5 days after Tmx treatment. n = 4 for *WT/R26-DTA*, n = 6 for *Pα-CE/R26-DTA*.



Supplemental Figure 4. Liver weight and adipose tissue weight of *Bmp3b* KO mice are comparable with those of WT mice

Liver, epididymal fat, and scapular brown adipose tissue (BAT) were dissected from *Bmp3b* KO or WT mice. The weight of each tissue was measured. Liver and BAT: $n = 7$ for WT, $n = 8$ for KO. Epididymal fat: $n = 5$ for WT, $n = 6$ for KO mice. Data represent the means ± SD; two-sided unpaired t-test.

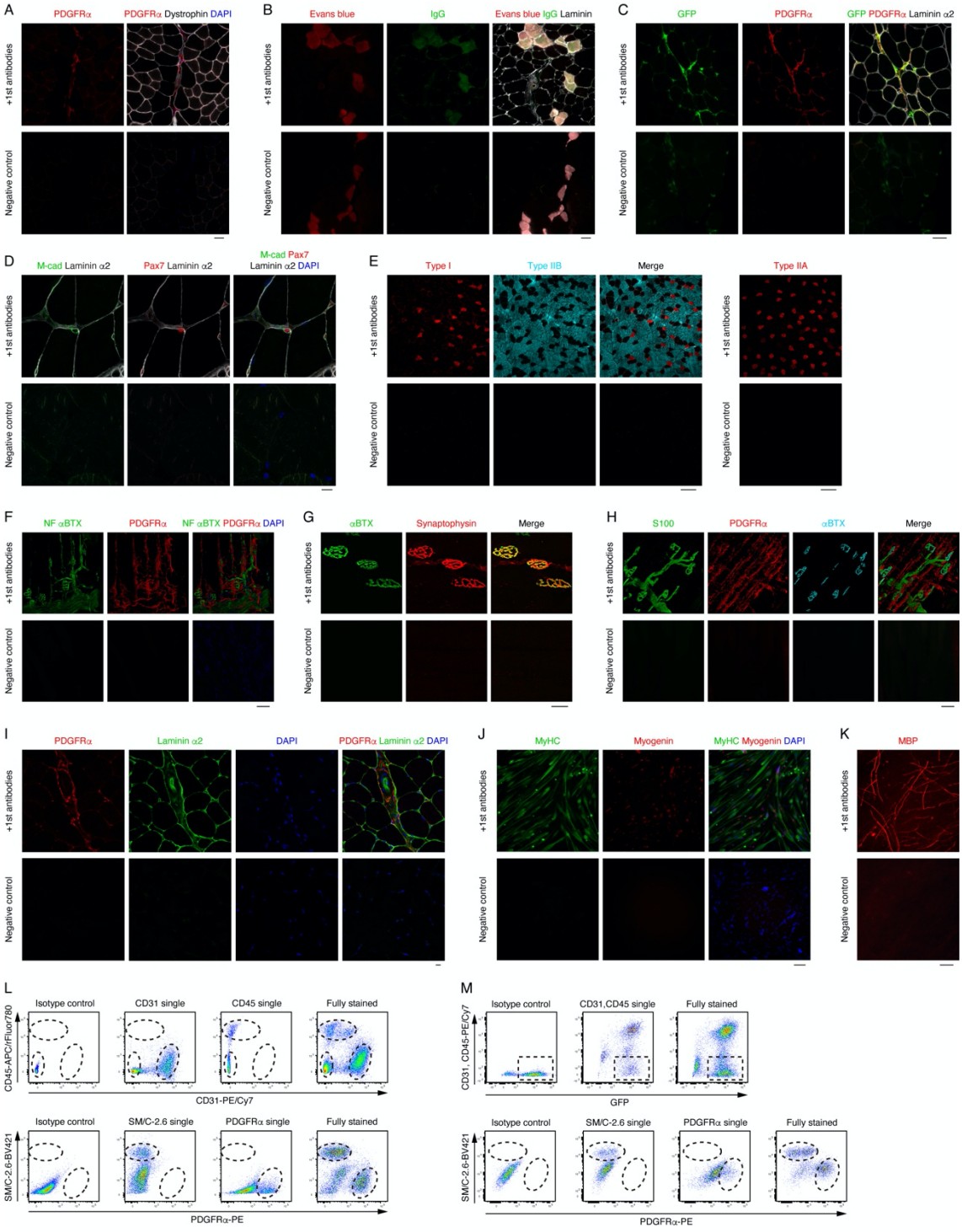


Supplemental Figure 5. Culture experiments to assess the effect of *Bmp3b* derived from mesenchymal progenitors on muscle cells

(A) Scheme of the co-culture experiments and generation of *Pα*/*Bmp3b* Tg mice.

(B) Scheme of the culture experiments using the conditioned medium.

(C) Phosphorylation of Smad2 and Smad1/5/8 after treatment with the conditioned medium was analyzed by capillary-based immunoassay.



Supplemental Figure 6. Negative immunostaining controls

(A) Immunostaining for PDGFRα and dystrophin (upper panels), and negative control staining (lower panels). (Related to Figure 1B). (B) Immunostaining for mouse IgG and

laminin (upper panels), and negative control staining (lower panels). Evans blue fluorescence was detected in both positive and negative staining sections. (Related to Supplemental Figure 2). (C) Immunostaining for GFP, PDGFRα, and laminin α2 (upper panels), and negative control staining (lower panels). (Related to Figure 2H). (D) Immunostaining for M-cadherin (M-cad), Pax7, and laminin α2 (upper panels), and negative control staining (lower panels). (Related to Figure 3A). (E) Immunostaining for type I, IIB, and IIA myosin heavy chain (upper panels) and negative control staining (lower panels). (Related to Figure 3B and 6E). (F) Immunostaining for neurofilament (NF), acetylcholine receptor (αBTX), and PDGFRα (upper panels), and negative control staining (lower panels). (Related to Figure 4A). (G) Immunostaining for acetylcholine receptor (αBTX), synaptophysin (upper panels), and negative control staining (lower panels). (Related to Figure 4B and 6G). (H) Immunostaining for S100, PDGFRα, and acetylcholine receptor (αBTX) (upper panels), and negative control staining (lower panels). (Related to Figure 4C and 6H). (I) Immunostaining for PDGFRα and laminin α2 (upper panels) and negative control staining (lower panels). (Related to Figure 5A). (J) Immunostaining for myosin heavy chain (MyHC) and myogenin (upper panels), and negative control staining (lower panels). (Related to Figure 7A-C). (K) Immunostaining for myelin basic protein (MBP) (upper panels) and negative control staining (lower panels). (Related to Figure 8B). (L and M) Isotype and single staining controls for FACS experiments. (Related to Figure 1D and 5B [L], and 2G [M]). Scale bars, 30 μm (A-C, G), 10 μm (D, I), 100 μm (E), 50 μm (F, H), 75 μm (J), and 25 μm (K).

Supplemental Table 1. List of genes that were specifically expressed in mesenchymal progenitors and downregulated in mesenchymal progenitor-depleted muscle and aged mesenchymal progenitors

				Young			Aged			
Symbol	RefSeq	WT/ R26-DTA	Pα-CE/ R26-DTA	Pα	SC	CD31, CD45	Pα	SC	CD31, CD45	Gene Ontology (Molecular function)
Kera	NM_008438	329	91	1805	71	25	196	69	10	-
Wfdc1	NM_023395	983	390	456	16	97	150	26	201	peptidase inhibitor activity; serine-type endopeptidase inhibitor activity
Il11ra1	NM_010549	9	3	213	10	2	80	9	2	cytokine binding; cytokine receptor activity; interleukin-11 binding; interleukin-11 receptor activity
Gdf10	NM_145741	54	14	805	2	5	306	11	3	cytokine activity; growth factor activity
Nmb	NM_026523	211	29	847	17	44	363	16	38	neuromedin B receptor binding; neuropeptide hormone activity
Glt8d2	XM_483930	21	6	267	7	2	123	20	4	transferase activity; transferase activity, transferring glycosyl groups
Col1a2	NM_007743	2286	851	18341	554	228	9027	1591	116	SMAD binding; extracellular matrix structural constituent; extracellular matrix structural constituent conferring tensile strength; identical protein binding; metal ion binding; platelet-derived growth factor binding; protease binding; protein binding; protein binding, bridging
1500015O10Rik	NM_024283	56	20	663	44	6	328	170	5	G1 to G0 transition; anaphase-promoting complex-dependent catabolic process; cellular senescence; central nervous system development; negative regulation of cell proliferation; neuropeptide signaling pathway; positive regulation of corticosterone secretion; positive regulation of corticotropin secretion; positive regulation of corticotropin-releasing hormone secretion; regulation of cell proliferation; response to wounding; vasopressin secretion

Pα-CE: Pdgfra-CreER^{T}, Pα: PDGFRα^{+} cells, SC: Satellite cells, CD31, CD45: CD31^{+} or CD45^{+} cells
Normalized gene expression values of each sample are presented.

Supplemental Table 2

Primary antibodies

Antibody	Clone	Conjugate(s) Used	Dilution	Source, catalog number
rat anti-CD31	390	PE/Cy7	1:200	BioLegend
rat anti-CD45	30-F11	PE/Cy7	1:200	BioLegend
rat anti-CD45	30-F11	APC-eFluor 780	1:200	Thermo Fisher Scientific
rat anti-Satellite cells	SM/C-2.6	biotin	1:200	A gift from S. Fukada[1]
goat anti-PDGFRα	polyclonal	unconjugated	2.5 μg/ml	R&D, AF1062
goat anti-PDGFRα	polyclonal	PE	15 μl/test	R&D, FAB1062P
rabbit anti-Laminin	polyclonal	unconjugated	1:400	Sigma, L9393
rat anti-Laminin α2	4H8-2	unconjugated	1:400	Santa Cruz Biotechnology
rabbit anti-M-cadherin	polyclonal	unconjugated	1:800	A gift from S. Takeda[2]
mouse anti-Pax7	PAX7	unconjugated	1:2	DSHB
rabbit anti-GFP	polyclonal	unconjugated	1:800	MBL, 598
goat anti-GFP	polyclonal	unconjugated	1:200	OriGene, AB0020
rabbit anti-Dystrophin	polyclonal	unconjugated	1:800	Abcam, ab15277
mouse anti-Type I MyHC	NOQ7.5.4D	unconjugated	1:200	Sigma
mouse anti-Type IIA MyHC	SC-71	unconjugated	1:200	Developmental Studies Hybridoma Bank
mouse anti-Type IIB MyHC	BF-F3	unconjugated	1:10	Developmental Studies Hybridoma Bank
mouse anti-Neurofilament	SMI312	unconjugated	1:1000	BioLegend
rabbit anti-Synaptophysin	polyclonal	unconjugated	1:200	Abcam, ab14692
rabbit anti-S100	polyclonal	unconjugated	1:2	Dako, IS504
mouse anti-MyHC	MF-20	unconjugated	1:2	Developmental Studies Hybridoma Bank
mouse anti-Myelin basic protein	SMI99	unconjugated	1:2000	BioLegend

α-Bungarotoxin		Alexa 488, Alexa647	1:1000	Thermo Fisher Scientific, B13422, B35450
rabbit anti-phospho-Smad2	138D4	unconjugated	1:1000	Cell Signaling
rabbit anti-phospho-Smad1/ Smad5/ Smad9	D5B10	unconjugated	1:1000	Cell Signaling
rabbit anti- phospho-Akt	D9E	unconjugated	1:2000	Cell Signaling
Isolectin GS-IB_4		Alexa 647	1:100	Thermo Fisher Scientific, I32450

Secondary reagents used

Secondary Reagent	Conjugate(s) Used	Dilution	Source
streptavidin	BV421	1:200	BioLegend
donkey polyclonal anti-goat IgG	Alexa 568	1:1000	Molecular Probes
donkey polyclonal anti-mouse IgG	Alexa 488, Alexa 568	1:1000	Molecular Probes
donkey polyclonal anti-rabbit IgG	Alexa 488, Alexa 568, Alexa 647	1:1000	Molecular Probes
chicken polyclonal anti-rat IgG	Alexa 647	1:1000	Molecular Probes
donkey polyclonal anti-mouse IgG1	Alexa 555	1:1000	Molecular Probes
donkey polyclonal anti-mouse IgM	Alexa 647	1:1000	Molecular Probes
goat polyclonal anti-rabbit IgG	HRP	1:3000	Cell Signaling

Supplemental Table 3

Primer sequences

Gene	Forward	Reverse	Size
mouse Fbxo32	5' GCAGAGAGTCGGCAAGTCTG 3'	5' GTAAGCACACAGGCAGGTCG 3'	155
mouse Jun	5' GTCTCAGGAGCGGATCAAGG 3'	5' TGCGCTTTCAAGGTTTTCAC 3'	126
mouse Sox2	5' GACCAGCTCGCAGACCTACA 3'	5' CCTCGGACTTGACCACAGAG 3'	113
mouse Id2	5' AGTCCGGTGAGGTCCGTTAG 3'	5' ACCAGTTCCTTGAGCTTGGAG 3'	137
mouse Mbp	5' AGACCCTCACAGCGATCCAA 3'	5' CCCGATGGAGTCAAGGATG 3'	108
mouse Gjb1	5' CGGCTGGTCAAGTGTGAAG 3'	5' GGCTGCGAGCATAAAGACAG 3'	102
mouse Cmas	5' CAAAGGCATCCCACTGAAGA 3'	5' CCCACACACTCTGGAAGACC 3'	104
mouse Bmp3b	5' CTTTGACGCCTACTACTGTGCTG 3'	5' AAGGGAGTTCATCTTGTCTGGAA 3'	157
mouse Hsbp1	5' CAAGACCATGCAGGACATCAC 3'	5' AGGTCAGCGATATTCTTCTCCA 3'	147
human BMP3B	5' CGGCTGGAATGAATGGATAA 3'	5' TGACAATGCTCTGGATGGTG 3'	125
human NDUFA13	5' CGTCAAAGGTGAAGCAGGAC 3'	5' CTCCAGTGCCCGTAGATCAG 3'	139
rat Jun	5' TGGGCACATCACCACTACAC 3'	5' GCGTATTCTGGCTATGCAGTTC 3'	125
rat Mbp	5' AGAGACCCTCACAGCGACAC 3'	5' CCCGATGGAGTCAAGGATG 3'	116
rat Egr2	5' TCTGCGCCTAGAAACCAGAC 3'	5' CAGAGGTGACGCTGGAAGAG 3'	169
rat GAPDH	5' ATGGCCTTCCGTGTTCCTAC 3'	5' CCTGCTTCACCACCTTCTTG 3'	103

References for Supplemental Data

1. Fukada S, Higuchi S, Segawa M, Koda K, Yamamoto Y, Tsujikawa K, et al. Purification and cell-surface marker characterization of quiescent satellite cells from murine skeletal muscle by a novel monoclonal antibody. *Exp Cell Res.* 2004;296(2):245-55.
2. Ojima K, Uezumi A, Miyoshi H, Masuda S, Morita Y, Fukase A, et al. Mac-1(low) early myeloid cells in the bone marrow-derived SP fraction migrate into injured skeletal muscle and participate in muscle regeneration. *Biochem Biophys Res Commun.* 2004;321(4):1050-61.