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Supporting Information for

Fibroblast growth factor 8b (FGF-8b) enhances myogenesis and inhibits adipogenesis in rotator cuff muscle cell populations in vitro

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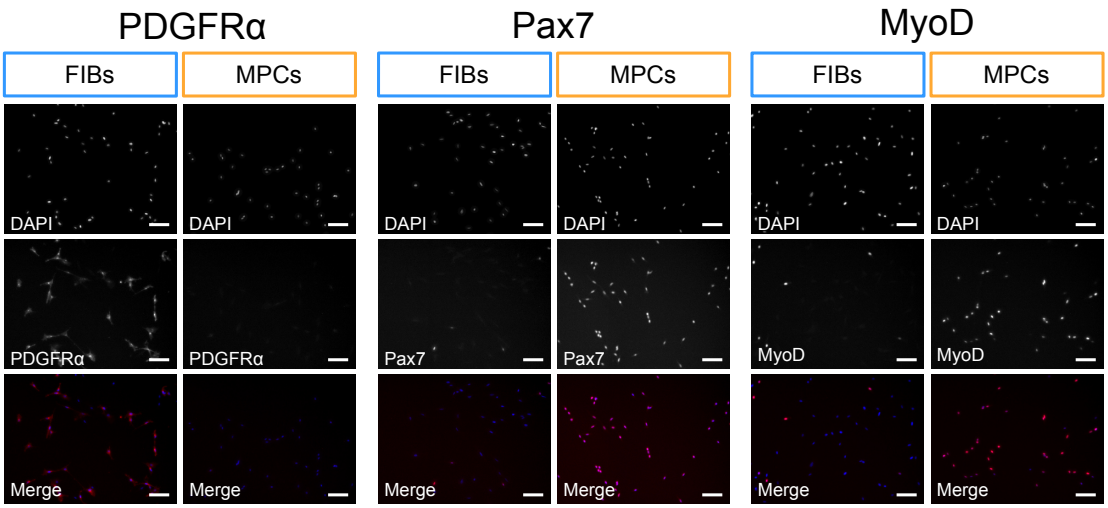

Fig. S1. Representative fluorescent images of FIBs and MPCs stained with PDGFRα, Pax7, and MyoD. The quantification of cell numbers is shown in Fig 1K.

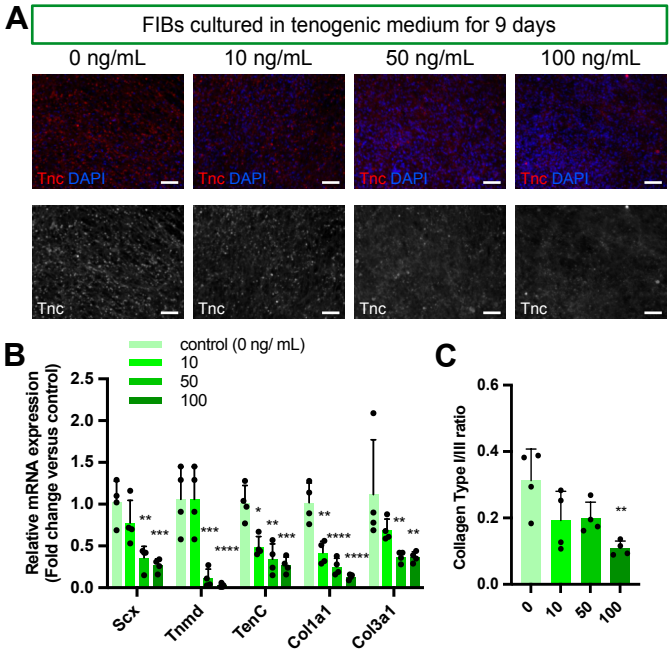

Fig. S2. FGF-8b suppresses tenogenesis of FIBs. (A) Representative fluorescent images stained with Tenascin C (Tnc) after 9 days of culture at the different concentrations of FGF-8b. (B) mRNA expression of tenogenic marker genes (Scx, Tnmd, and Tnc) and tendon extracellular matrix proteins (Col1a1 and Col3a1). Data are expressed as fold change relative to the tenogenic control condition (0 mg/mL) (n = 4). (C) The ratio of Col1a1/Col3a1 mRNA expression level at the different concentrations of FGF-8b (n = 4). Scale bar: 100 μm.

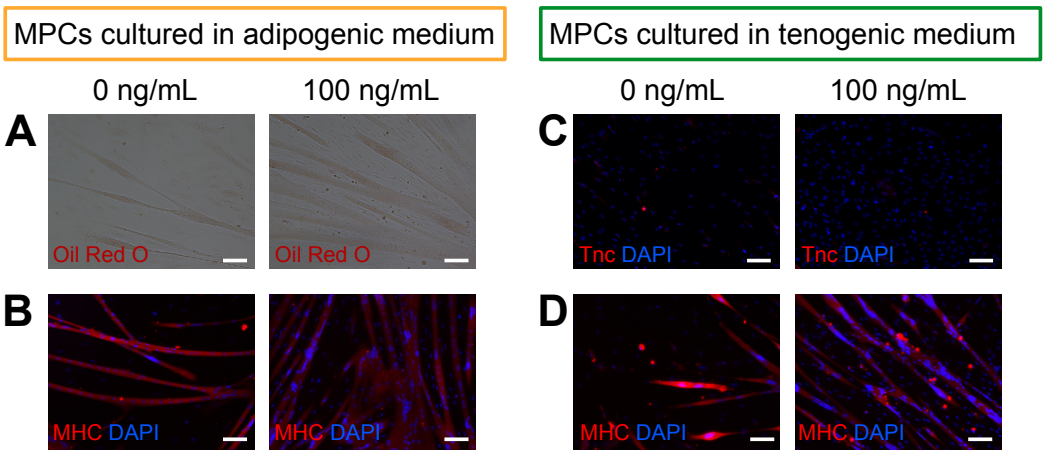

Fig. S3. MPCs did not undergo adipogenic or tenogenic differentiation. (A) Representative images of the stained lipid droplets after 9 days of culture with or without FGF-8b under adipogenic induction. (B) Representative fluorescent images stained with MHC after 9 days of culture with or without FGF-8b under adipogenic induction. (C) Representative fluorescent images stained with TenC after 9 days of culture with or without FGF-8b under tenogenic induction. (D) Representative fluorescent images stained with MHC after 9 days of culture with or without FGF-8b under tenogenic induction.

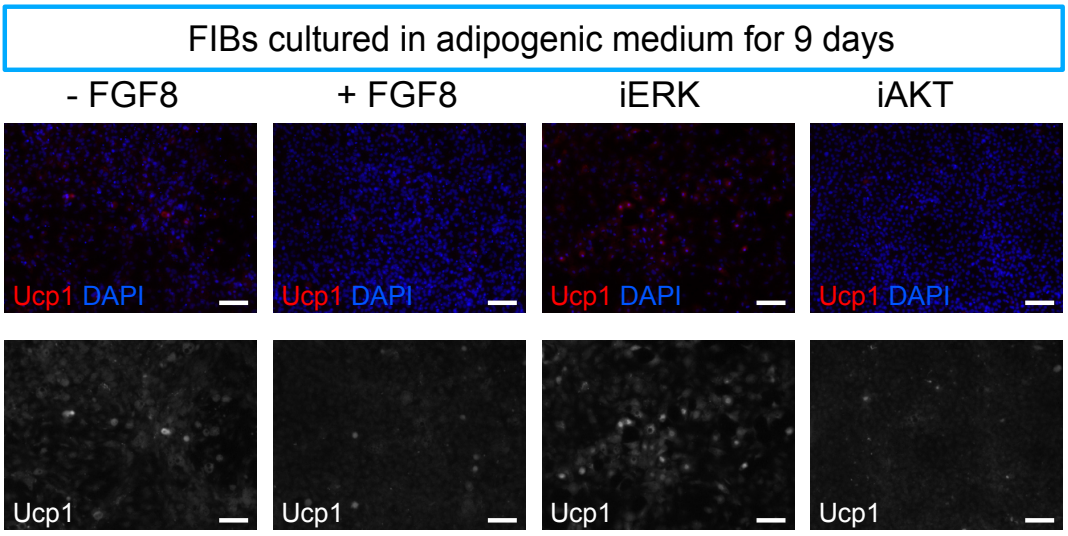

Fig. S4. Representative fluorescent images stained with Ucp1 after 9 days of culture.

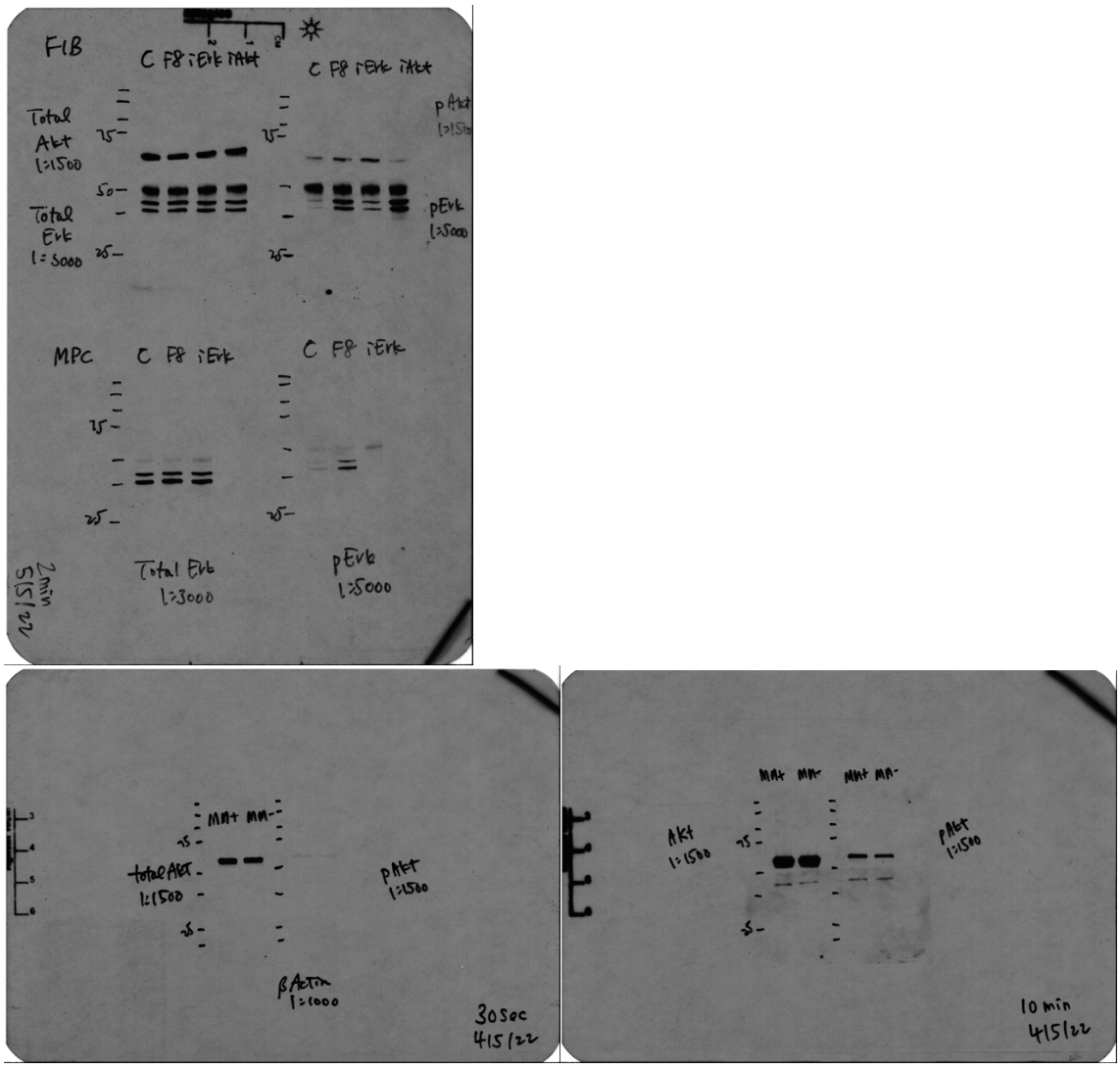

Fig. S5. Full blot images shown in this study.

Table S1. TaqMan Assay ID used for quantitative real-time PCR.

Gene Symbol	TaqMan Assay ID	Entrez Gene ID	GenBank ID	Gene Name
Pparg	Rn00440945_m1	25664	NM_013124.3	peroxisome proliferator-activated receptor gamma
Fabp4	Rn00670361_m1	79451	NM_053365.1	fatty acid binding protein 4
Adipoq	Rn00595250_m1	246253	NM_144744.3	adiponectin
Lep	Rn00565158_m1	25608	NM_013076.3	leptin
Pdgfra	Rn01417537_m1	25267	NM_012802.1	platelet derived growth factor receptor alpha
Ucp1	Rn00562126_m1	24860	NM_012682.2	uncoupling protein 1
Scx	Rn01504576_m1	680712	NM_001130508.1	scleraxis bHLH transcription factor
Tnmd	Rn00574164_m1	64104	NM_022290.1	tenomodulin
Tnc	Rn01454948_m1	116640	NM_053861.1	tenascin C
Col1a1	Rn01463848_m1	29393	NM_053304.1	collagen type I alpha 1 chain
Col3a1	Rn01437681_m1	84032	NM_032085.1	collagen type III alpha 1 chain
Pax7	Rn01518732_m1	500574	NM_001191984.1	paired box 7
Myod1	Rn00598571_m1	337868	NM_176079.1	myogenic differentiation 1
Myog	Rn00567418_m1	29148	NM_017115.2	myogenin
Myf5	Rn01502778_m1	299766	NM_001106783.1	myogenic factor 5
Des	Rn00574732_m1	64362	NM_022531.1	desmin
Tnnt1	Rn00592835_m1	171409	NM_134388.2	troponin T1, slow skeletal type
Fgfr1	Rn00577234_m1	79114	NM_024146.1	fibroblast growth factor receptor 1
Fgfr2	Rn01269940_m1	25022	NM_012712.1	fibroblast growth factor receptor 2
Fgfr3	Rn00584799_m1	84489	NM_053429.1	fibroblast growth factor receptor 3
Fgfr4	Rn01441815_m1	25114	NM_001109904.1	fibroblast growth factor receptor 4
Gapdh	Rn01775763_g1	24383	NM_017008.4	glyceraldehyde-3-phosphate dehydrogenase
Fgf8	Rn00590996_m1	29349	NM_133286.1	fibroblast growth factor 8
Ucp1	Rn00562126_m1	24860	NM_012682.2	uncoupling protein 1 (mitochondrial, proton carrier)

Table S2. List of antibodies.

Antibodies	Product code	Manufacturer	Nature of antibodies	Dilution
Erk1/2	4695 [137F5]	Cell Signaling Technology	Monoclonal (rabbit)	1:3000
Phospho-Erk1/2	4370 [D13.14.4E]	Cell Signaling Technology	Monoclonal (rabbit)	1:5000
Akt	9272	Cell Signaling Technology	Polyclonal (rabbit)	1:1500
Phospho-Akt	9271	Cell Signaling Technology	Polyclonal (rabbit)	1:1500
Anti-rabbit (HRP)	972-4446	Bio-rad	Polyclonal (goat)	1:3000
Vimentin	ab92547 [EPR3776]	Abcam	Monoclonal (rabbit)	1:250 (2 µg / mL)
αSMA	MA5-11547 [1A4]	Invitrogen	Monoclonal (mouse)	1:100 (0.4 µg / mL)
PDGFRα	ab203491 [EPR22059-270]	Abcam	Monoclonal (rabbit)	1:500 (1.214 µg / mL)
Pax7	PAX7	DSHB	Monoclonal (mouse)	2 µg / mL
MyoD	LS-C392310 [SPM427]	LSBio	Monoclonal (mouse)	1:200 (1 µg / mL)
Desmin	14-9747-82 [DE-U-10]	Invitrogen	Monoclonal (mouse)	1:100 (5 µg / mL)
TnC	NB110-68136 [4C8MS]	Novus Biologicals	Monoclonal (mouse)	1:200 (5 µg / mL)
MHC	MF20	DSHB	Monoclonal (mouse)	2 µg / mL
Ucp1	bs-1925R	Bioss Antibodies	Polyclonal (rabbit)	1:200 (5 µg / mL)
Anti-Rabbit IgG Alexa Fluor® 594	ab150080	Abcam	Polyclonal (goat)	1:1000
Anti-Mouse IgG Alexa Fluor® 594	ab150116	Abcam	Polyclonal (goat)	1:1000