

# **SUPPLEMENTAL MATERIAL**

**Table S1. Major Resources.**

<b>Antibody/reagent</b>	<b>Vendor or Source</b>	<b>Catalog #</b>	<b>Working concentration</b>	<b>Lot #</b>
CD11b	Cell Sciences	MON1019	1 µg/mL	491019X
CD163	Hycult Biotech	HM2157	5 µg/mL	24926M0818-A
CD206	R&D Systems	AF2534	1 µg/mL	VKG0112021R
CD16B/FCGR3B	LifeSpan BioSciences	C409441-50	1:50	163838
4',6-diamidino-2-phenylindole (DAPI)	Thermo Fisher Scientific	D1306	0.5 µg/mL	
Pax7	Developmental Studies Hybridoma Bank	PAX7-c	3 µg/mL	
Laminin	Millipore Sigma	L9393	6.5 µg/mL	103M4779
Wheat-germ agglutinin	Thermo Fisher Scientific	W21405	20 µg/mL	
Lectin	Sigma-Aldrich	L4889	20 µg/mL	063M4010V
Biotinylated goat anti-mouse IgG1	Jackson ImmunoResearch	115-065-205	1.2 µg/mL	131562
Biotinylated rabbit anti-goat IgG	Vector Laboratories	BA-5000	3 µg/mL	
Streptavidin-horse radish peroxidase	Thermo Fisher Scientific	S911	5 µg/mL	1880067
Streptavidin AlexaFluor594	Thermo Fisher Scientific	S32356	10 µg/mL	1902487
Superboost TSA AlexaFluor488	Thermo Fisher Scientific	B40953	Per manufacturer's instruction	1910748
Anti-Rabbit AlexaFluor568	Thermo Fisher Scientific	A-11036	10 µg/mL	2045347
Anti-Rabbit AlexaFluor488	Thermo Fisher Scientific	A-11008	10 µg/mL	2110498
Ulex europaeus (UAE1), biotinylated	Vector Laboratories	B-1065	20 µg/mL	ZF-0904
Griffonia simplicifolia (GSL1, BSL1), biotinylated	Vector Laboratories	B-1105	20 µg/mL	ZF-0717
pan-Collagen	Thermo Fisher Scientific	PA1-85324	20 µg/mL	UG2813859

**Table S2. Relationship between muscle features and ABI in PAD patients.**

	ABI	
	r (95% CI)	p-value
Total macrophages (per fiber)	-0.071 (-0.460, 0.344)	0.745
CD11b+CD206- (per fiber)	-0.159 (-0.526, 0.264)	0.463
CD11b+CD206+ (per fiber)	-0.037 (-0.433, 0.373)	0.866
CD11b+CD206+ (% of total macrophages)	0.031 (-0.378, 0.428)	0.888
Satellite cells (per 100 fibers)	-0.132 (-0.506, 0.289)	0.543
Mean fiber size ( $\mu\text{m}$ )	-0.118 (-0.496, 0.302)	0.586
ECM (% Total Area) †	0.175 (-0.259, 0.545)	0.429
SR (% Total Area) †	0.049 (-0.372, 0.451)	0.827
Capillary density (per fiber) †‡	-0.169 (-0.540, 0.265)	0.446

ABI measures are only from the leg with the lower ABI, which is the leg used to obtain muscle biopsies. P-value adjusted for race, \*statistically significant  $p < 0.05$ . r = Pearson's partial correlation coefficient; n=25 PAD unless noted.

† Based on n=24 PAD

‡ Mean values previously reported.

Note: Confidence intervals for the correlation coefficients are from Fisher's z transformation.

**Table S2. Correlations among muscle features identified on gastrocnemius cross-sections from PAD patients.**

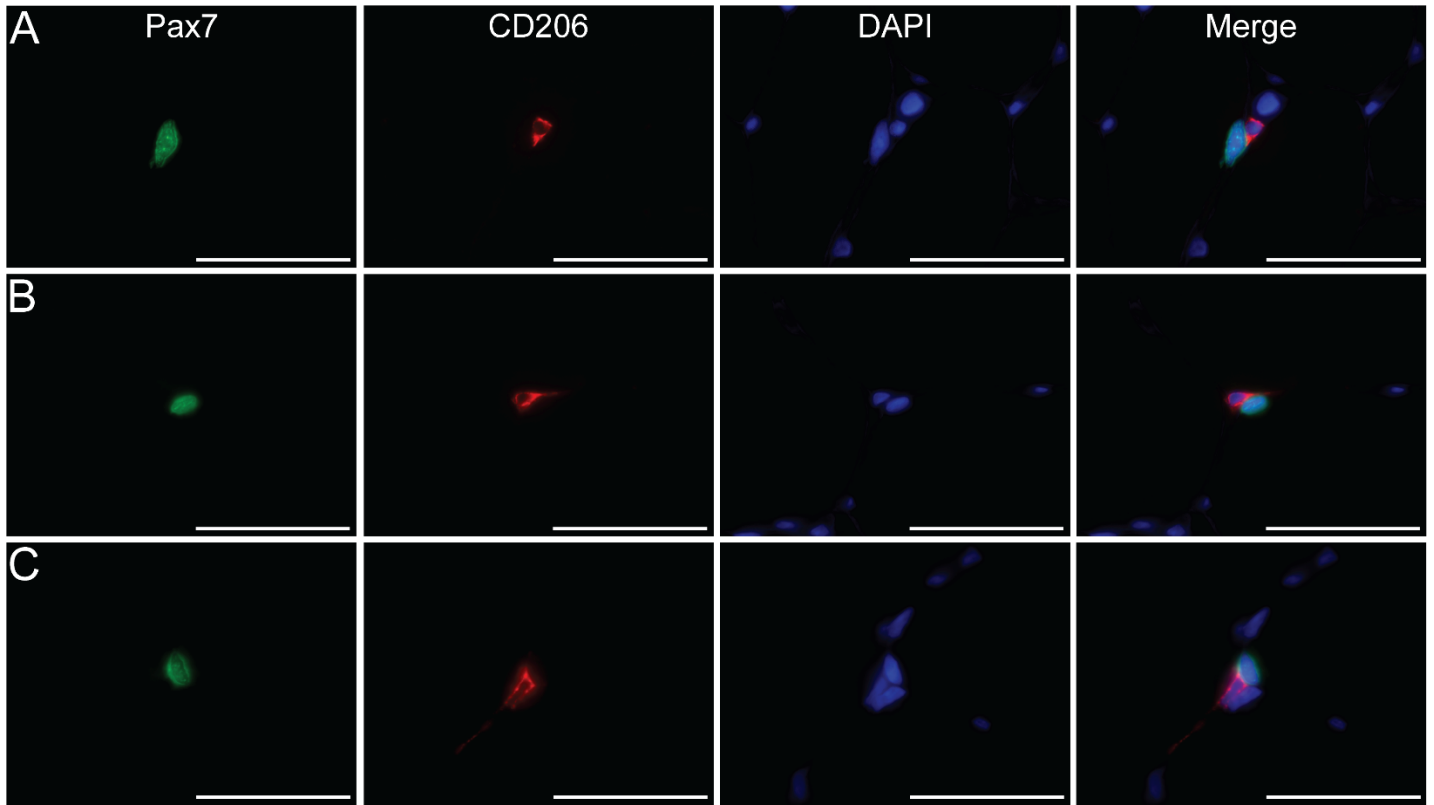
	Total macrophages (per fiber)		CD206- (per fiber)		CD206+ (per fiber)		CD206+ (% of Total)		Satellite Cells (per 100 fibers)		Mean fiber size (µm)		Capillary density (lectin+/fiber) †	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
	95% CI		95% CI		95% CI	95% CI		95% CI		95% CI	95% CI		95% CI	
Total macrophages (per fiber)			<b>0.841</b> ( <b>0.652,</b> <b>0.927</b> )	<b>&lt;0.001*</b>	<b>0.984</b> ( <b>0.961,</b> <b>0.993</b> )	<b>&lt;0.001*</b>	0.213 (-0.213, 0.564)	0.322	<b>0.405</b> ( <b>0.011,</b> <b>0.690</b> )	<b>0.049*</b>	0.292 (-0.133, 0.618)	0.169	0.010 (-0.404, 0.420)	0.965
CD206- (per fiber)	<b>0.841</b> ( <b>0.652,</b> <b>0.927</b> )	<b>&lt;0.001*</b>			<b>0.730</b> ( <b>0.451,</b> <b>0.872</b> )	<b>&lt;0.001*</b>	-0.281 (-0.611, 0.144)	0.186	0.152 (-0.271, 0.521)	0.484	0.121 (-0.299, 0.498)	0.578	-0.044 (-0.447, 0.376)	0.844
CD206+ (per fiber)	<b>0.984</b> ( <b>0.961,</b> <b>0.993</b> )	<b>&lt;0.001*</b>	<b>0.730</b> ( <b>0.451,</b> <b>0.872</b> )	<b>&lt;0.001*</b>			0.362 (-0.056, 0.664)	0.082	<b>0.461</b> ( <b>0.060,</b> <b>0.724</b> )	<b>0.022*</b>	0.328 (-0.094, 0.642)	0.118	0.027 (-0.390, 0.434)	0.905
CD206+ (% of Total)	0.213 (-0.213, 0.564)	0.322	-0.281 (-0.611, 0.144)	0.186	0.362 (-0.056, 0.664)	0.082			<b>0.416</b> ( <b>0.006,</b> <b>0.697</b> )	<b>0.042*</b>	0.397 (-0.016, 0.685)	0.054	0.179 (-0.255, 0.548)	0.417
Satellite cells (per 100 fibers)	<b>0.405</b> ( <b>0.011,</b> <b>0.690</b> )	<b>0.049*</b>	0.152 (-0.271, 0.521)	0.484	<b>0.461</b> ( <b>0.060,</b> <b>0.724</b> )	<b>0.022*</b>	<b>0.416</b> ( <b>0.006,</b> <b>0.697</b> )	<b>0.042*</b>			<b>0.557</b> ( <b>0.186,</b> <b>0.779</b> )	<b>0.004*</b>	-0.065 (-0.464, 0.358)	0.770
Mean fiber size (µm)	0.292 (-0.133, 0.618)	0.169	0.121 (-0.299, 0.498)	0.578	0.328 (-0.094, 0.642)	0.118	0.397 (-0.016, 0.685)	0.054	<b>0.557</b> ( <b>0.186,</b> <b>0.779</b> )	<b>0.004*</b>			0.138 (-0.294, 0.518)	0.535
Capillary density (lectin+/fiber) †	0.010 (-0.404, 0.420)	0.965	-0.044 (-0.447, 0.376)	0.844	0.027 (-0.390, 0.434)	0.905	0.179 (-0.255, 0.548)	0.417	-0.065 (-0.464, 0.358)	0.770	0.138 (-0.294, 0.518)	0.535		

Mean fiber size was determined by minimum feret diameter. P-value adjusted for race, \*statistically significant p<0.05. r = Pearson's partial correlation coefficient; n=25 PAD unless noted.

† Based on n=24 PAD

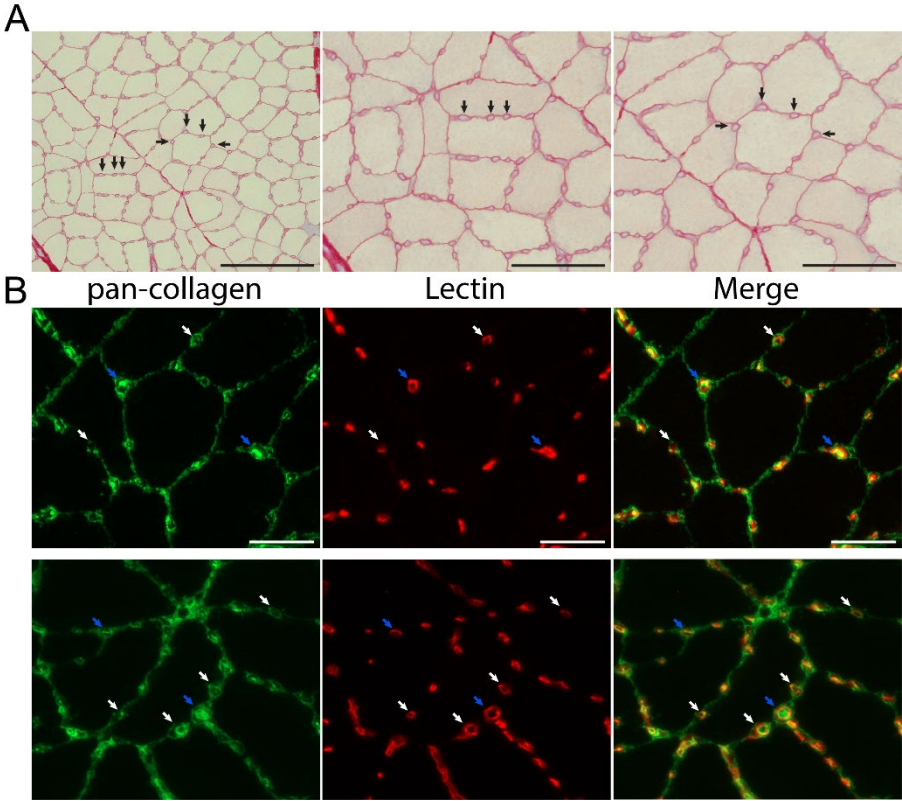
Note: Confidence intervals for the correlation coefficients are based on Fisher's z transformation.

Figure S1. Macrophages in close proximity to satellite cells.



Representative images showing gastrocnemius muscle sections from three different peripheral artery disease (PAD) patients. Satellite cell (paired box 7 (Pax7)+, green); cluster of differentiation 206 (CD206)+ macrophage (red); 4',6-diamidino-2-phenylindole (DAPI)+ cell nuclei (blue). Scale bar = 50 μm.

Figure S2. Collagen in and around capillaries in gastrocnemius from a peripheral artery disease (PAD) patient.



A) Sirius Red histochemistry showing collagen deposition around capillaries (black arrows) of a highly capillarized PAD patient. Left: 200x magnification, scale bar = 200  $\mu$ m. Right two panels: 400x magnification, scale bar = 100  $\mu$ m. B) Collagen immunohistochemistry showing collagen (green) surrounding red, lectin+ capillaries (white arrows). Blue arrows point to cross-sectional capillaries that appear to be filled with collagen. Scale bar = 50  $\mu$ m.