Supporting Information

Comparative Analysis of the Extracellular Matrix Proteome Across the Myotendinous Junction

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Figure S1

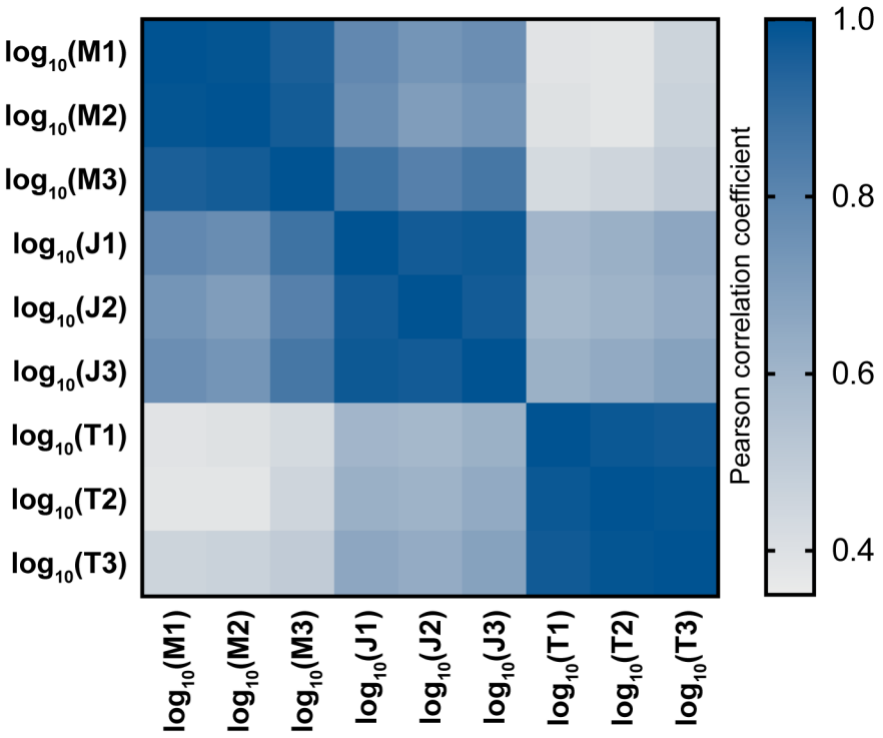

Figure S1. Pearson correlation plot based on raw intensity of ECM proteins found in GuHCl homogenate. The correlation between M and T tissues was lower than M vs J and J vs T, where the ECM in J more highly correlated with M than T. Normalized raw intensities were $\log_{10}$ transformed and Pearson correlation coefficients were calculated between samples.

Figure S2

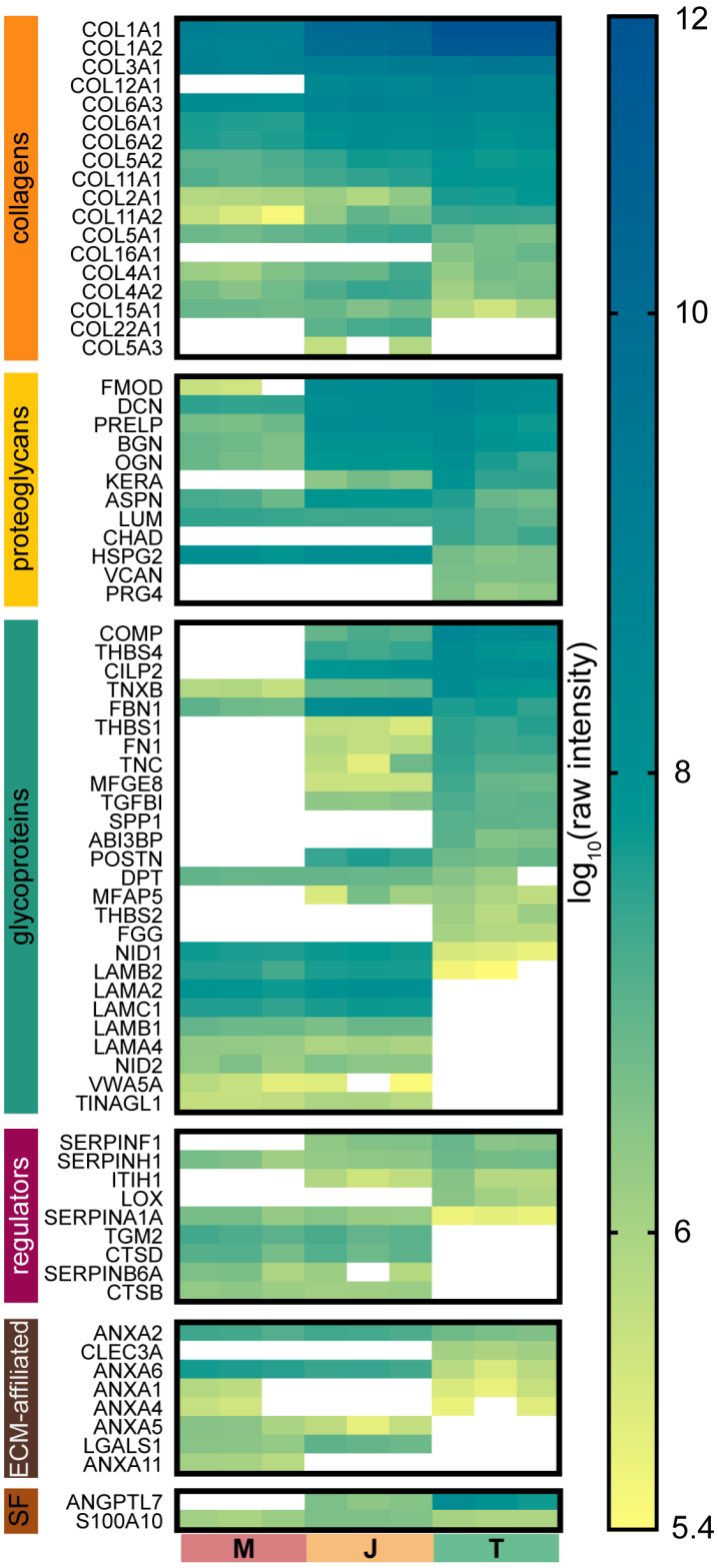
collagens
COL1A1
COL1A2
COL3A1
COL12A1
COL6A3
COL6A1
COL6A2
COL5A2
COL11A1
COL2A1
COL11A2
COL5A1
COL16A1
COL4A1
COL4A2
COL15A1
COL22A1
COL5A3
proteoglycans
FMOD
DCN
PRELP
BGN
OGN
KERA
ASPN
LUM
CHAD
HSPG2
VCAN
PRG4
glycoproteins
COMP
THBS4
CILP2
TNXB
FBN1
THBS1
FN1
TNC
MFGE8
TGFBI
SPP1
ABI3BP
POSTN
DPT
MFAP5
THBS2
FGG
NID1
LAMB2
LAMA2
LAMC1
LAMB1
LAMA4
NID2
VWA5A
TINAGL1
regulators
SERPINF1
SERPINH1
ITIH1
LOX
SERPINA1A
TGM2
CTSD
SERPINB6A
CTSB
ECM-affiliated
ANXA2
CLEC3A
ANXA6
ANXA1
ANXA4
ANXA5
LGALS1
ANXA11
SF
ANGPTL7
S100A10
M
J
T
log10(raw intensity)
12
10
8
6
5.4

Figure S2. Heat map by ECM category of matrisome proteins identified in GuHCl homogenate from M, J and T tissue. Raw intensities were $\log_{10}$ transformed and individual boxes represent each biological replicate for *n* = 3 M, T and J samples (see also Table S1). Tissue was harvested from 5-month-old murine soleus muscle-tendon units. Rows were manually by ECM category.

Figure S3

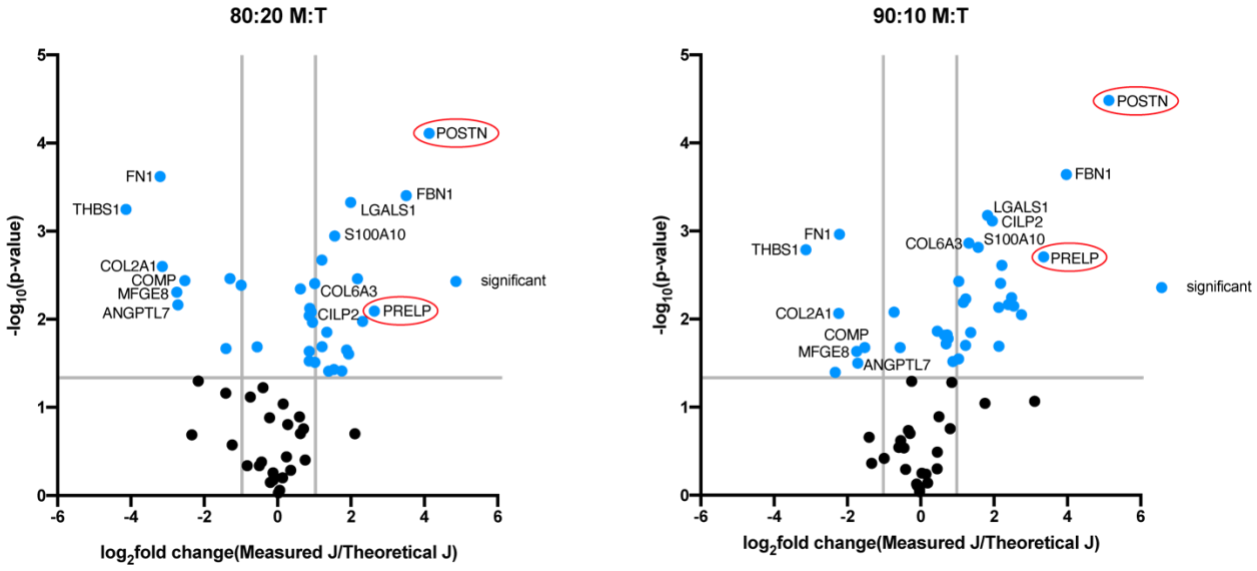

Figure S3. Identification of ECM enriched in J. The measured raw intensities of ECM in GuHCl-extracted J samples were compared with the theoretical raw intensity if the J was made up of an average of M and T tissue. We estimated ~ 88% M in J based on the distribution of the matrisome categories; therefore, two ratios were considered in the calculation of the theoretical raw intensities for each ECM: 80:20 and 90:10 M:J. ECM identified in the upper right portion of the volcano plots indicate ECM that are enriched in J tissues. A subset of proteins that were significantly different are labeled to show how distribution changed depending on the ratio of M:J. IHC of circled ECM can be found in Figure 4C, D. Significance was based on $p < 0.05$ and |fold change| > 2 (grey lines; $n = 3$ biological replicates).

Figure S4

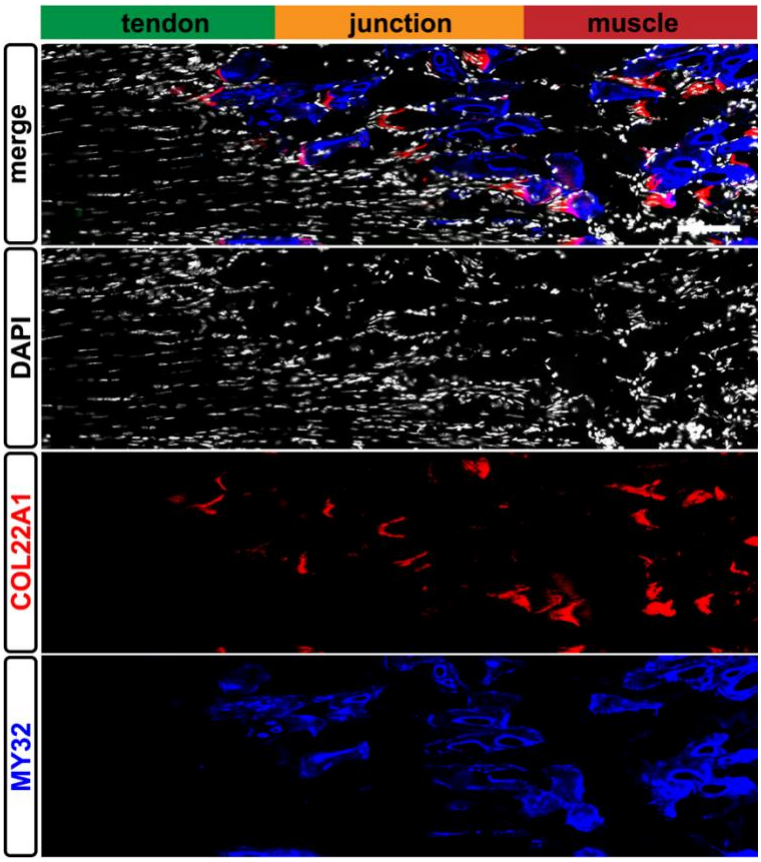

Figure S4. Immunolocalization of COL22A1 and a muscle-specific marker. Type XXII collagen (COL22A1, red) was found only at the interface between myofibers (MY32, blue) and T. The regions were distinguished by the differential distribution of nuclei (DAPI, white). T: aligned parallel arrays of nuclei; M: heterogeneous distribution of nuclei, localized to the periphery or in between myofibers (MY32). Images representative of n = 3 biological replicates; bar = 100 μm.

Table S2

		M vs T	M vs J	J vs T	2-way ANOVA
GuHCl Homogenate	*Cytosolic*	****	ns	****	tissue: ns
	Nuclear	****	ns	***	
	Membrane	****	ns	****	compartment: ****
	Cytoskeletal	****	****	****	interaction: ****
	Matrisome	****	****	****	
CS Fraction	*Cytosolic*	****	ns	****	tissue: ns
	Nuclear	****	ns	****	
	Membrane	****	ns	****	compartment: ****
	Cytoskeletal	****	****	****	interaction: ****
	Matrisome	****	****	****	
Insoluble Fraction	*Cytosolic*	ns	ns	ns	tissue: ns
	Nuclear	ns	ns	ns	
	Membrane	ns	ns	ns	compartment: ****
	Cytoskeletal	****	****	****	interaction: ****
	Matrisome	****	****	****	

Table S2. Statistical analysis of the distribution of cellular compartment as a function of tissue type. Two-way ANOVA was performed on $n = 3$ biological replicates of data represented in Figure 2A and Tukey's multiple comparisons was used for post hoc analysis. *** $p < 0.001$; **** $p < 0.0001$; ns = not significant ($p > 0.05$).

Table S5

		M vs T	M vs J	J vs T	2-way ANOVA
GuHCl Homogenate	*Secreted factors*	****	****	ns	tissue: ns compartment: **** interaction: ****
	ECM-affiliated proteins	ns	ns	ns	
	ECM regulators	ns	ns	ns	
	Glycoproteins	**	ns	ns	
	Proteoglycans	*	ns	ns	
	Collagens	****	****	*	
CS Fraction	*Secreted factors*	ns	ns	ns	tissue: ns compartment: **** interaction: ****
	ECM-affiliated proteins	ns	ns	ns	
	ECM regulators	ns	ns	ns	
	Glycoproteins	****	****	ns	
	Proteoglycans	****	****	*	
	Collagens	****	****	**	
Insoluble Fraction	*Secreted factors*	ns	ns	ns	tissue: ns compartment: **** interaction: ****
	ECM-affiliated proteins	ns	ns	ns	
	ECM regulators	ns	ns	ns	
	Glycoproteins	****	**	ns	
	Proteoglycans	ns	ns	ns	
	Collagens	****	***	ns	

Table S5. Statistical analysis of the distribution of different ECM categories as a function of tissue type. Two-way ANOVA was performed on $n = 3$ biological replicates of data represented in Figure 2B and Tukey's multiple comparisons was used for post hoc analysis. * $p<0.05$; ** $p<0.01$*** $p<0.001$; **** $p<0.0001$; ns = not significant ($p>0.05$).

Table S7: Experimental details on reagents used for IHC

Antigen	Host	Company	Product number	Lot number	Initial concentration	Dilution	RRID
COL5A3	rabbit polyclonal	Invitrogen	PA5-77257	UJ2851627	1 mg/mL	1:100	AB_2720984
COL5A1/A2/A3	goat polyclonal	Southern Biotech	1350-01	A1517WE68Z	0.4 mg/mL	1:100	AB_2794740
COL22A1	rabbit polyclonal	Abcam	ab121846	GR223910-16 GR223910-17	0.3 mg/mL	1:250	AB_11128704
LAMA2	rat monoclonal IgG1 (clone 4H8-2)	Santa Cruz Biotechnology	sc-59854	A1312	0.2 mg/mL	1:100	AB_784266
NID2	mouse monoclonal IgG1 kappa (clone F-2)	Santa Cruz Biotechnology	sc-377424	E1618	0.2 mg/mL	1:50	AB_2819357
anti-skeletal muscle myosin heavy chain (MY32)	mouse IgG1 monoclonal; (clone MYSN02)	Thermo Fisher Scientific	MA5-11748	1634061A	0.126 mg/ml	1:100	AB_10979315
PRELP	sheep polyclonal	Invitrogen	PA547841	VF18802A	0.2 mg/mL	1:300	AB_2608591
POSTN	rabbit polyclonal	Abcam	AB14041	GR3303878-4	0.5 mg/ml	1:300	AB_2299859
TNC	rat monoclonal IgG2A (clone 578)	R&D Systems	MAB2138	KLC0718071 KLC0719091	0.5 mg/mL	1:100 - 1:200	AB_2203818
TNC	rabbit polyclonal	Sigma-Aldrich	AB19013	3257092	0.5 mg/mL	1:200	AB_2256033
Donkey-anti-rabbit IgG1 488		Invitrogen	A21206	2072687	2 mg/ml	1:500	AB_2535792
Goat-anti-rabbit IgG1 488		Invitrogen	A-11034	1971418	2 mg/ml	1:500	AB_2576217
Donkey-anti-rat 488		Invitrogen	A-21208	1979698 2180272	2 mg/mL	1:500	AB_141709
Donkey-anti-rat IgG1 550		Dylight	SA5-10027	Te2575666	2 mg/mL	1:250	AB_2556607
Donkey-anti-rabbit 546		Invitrogen	A-10040	1833519	2 mg/mL	1:500	AB_2534016
Goat-anti-mouse IgG1 546		Invitrogen	A-21123	2132524	2 mg/mL	1:500	AB_2535768
Donkey-anti-goat IgG1 647		Invitrogen	A-21447	2045332	2 mg/mL	1:500	AB_2535853
Chicken-anti-rat IgG 647		Invitrogen	A-21472	1889323	2 mg/ml	1:500	AB_2535875
Donkey-anti-sheep IgG 647		Invitrogen	A-21448	20405339	2 mg/ml	1:500	AB_2535865
Goat-anti-mouse IgG1 633		Invitrogen	A-21126	1840916 2128996	2 mg/mL	1:500	AB_2535768
DAPI		Biotium	40011	n/a	1 mg/ml	1:500	
Mouse on Mouse (M.O.M.) Ig blocking buffer		Vector Laboratories	30035	ZE1226, ZE0723, ZF3620, ZF0620, ZF0920,	90 µl: 2.5 ml 1× PBS		
M.O.M. protein diluent		Vector Laboratories	30045	ZF0620, ZF0417	600 µl: 7.5 ml 1× PBS		