PNAS

Supporting Information for

Mechano-epigenetic regulation of extracellular matrix homeostasis via Yap and Taz

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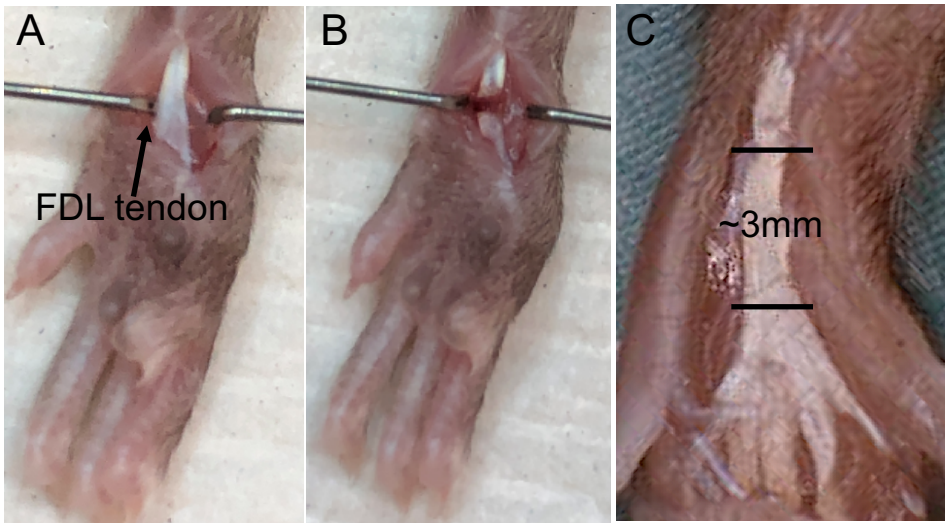
This PDF file includes:

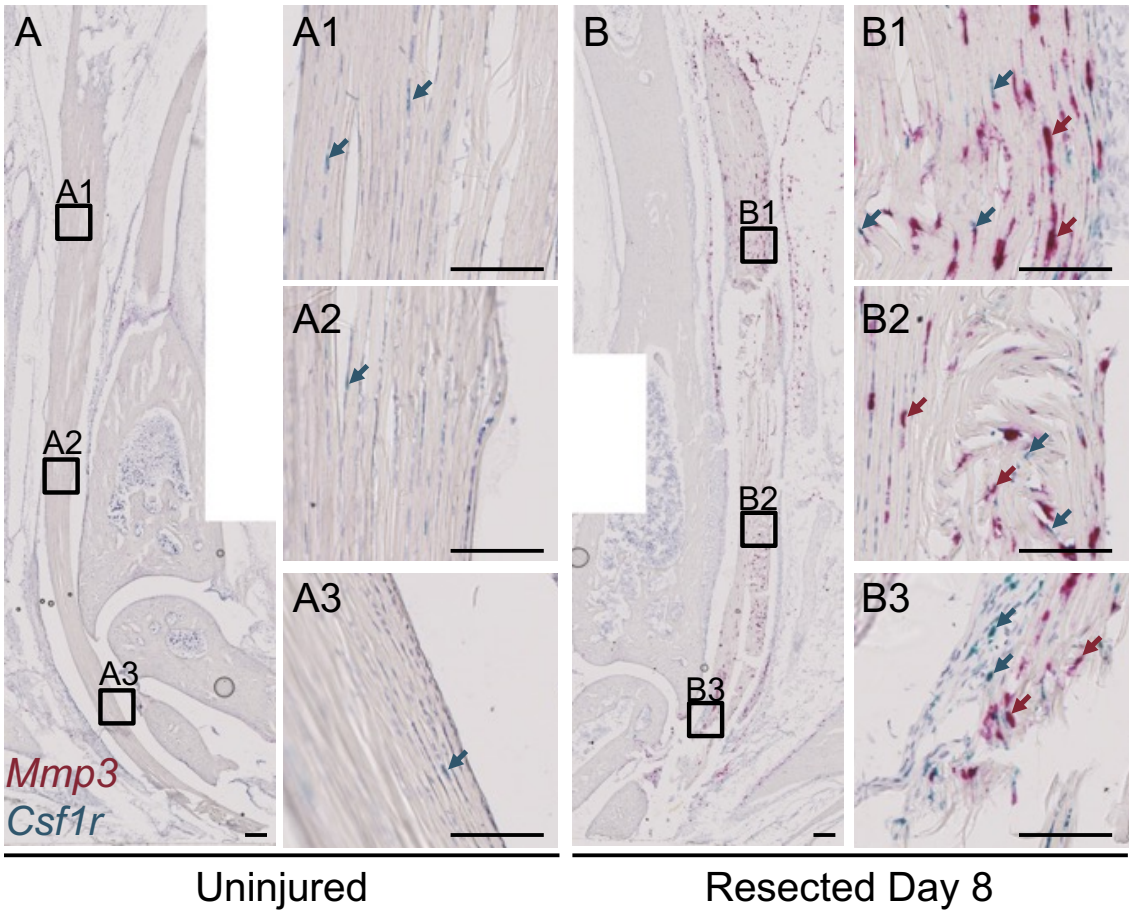
Figures S1 to S5
Tables S1
Legends for Datasets S1 to S2

Other supporting materials for this manuscript include the following:

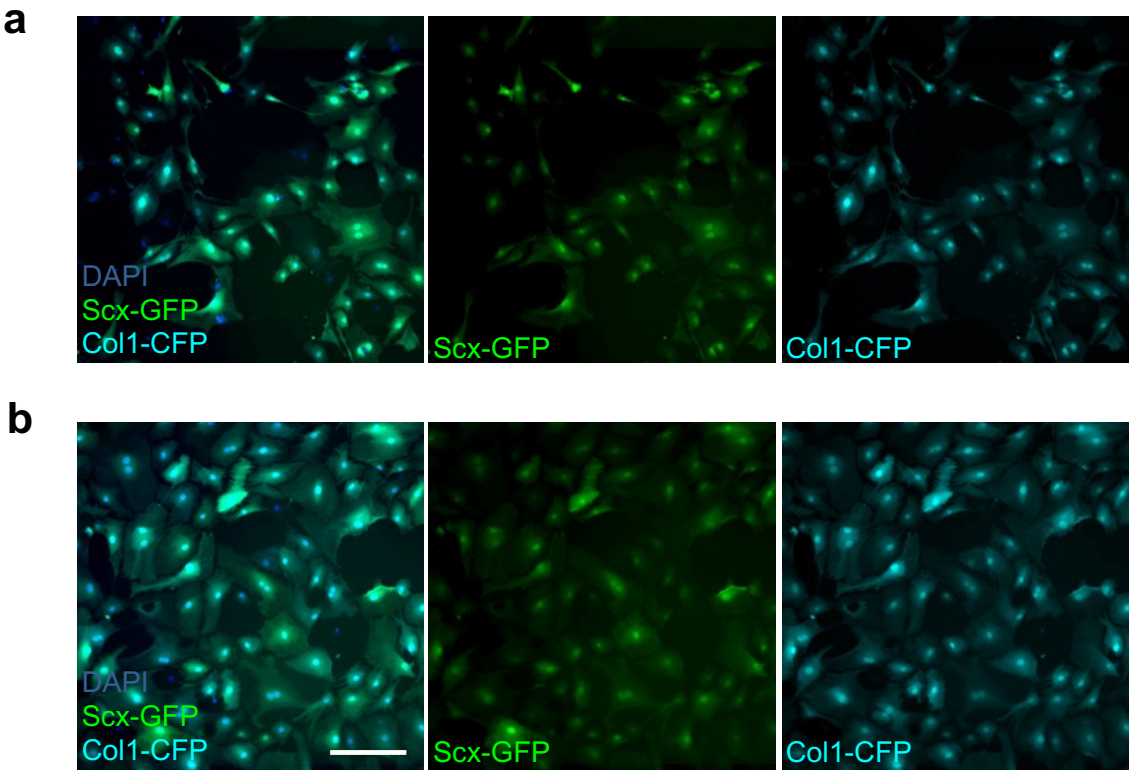
Datasets S1 to S2



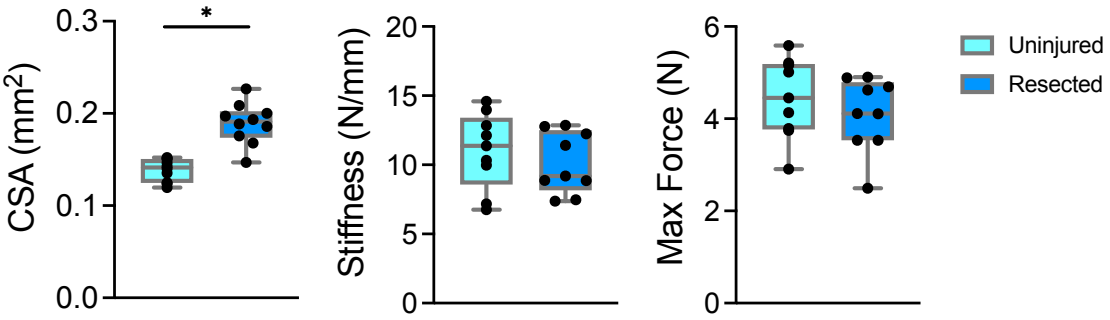
Supplemental Figure 1: FDL tendon resection surgery.
While the animal was lying in a prone position with the ankle in plantar flexion, the distal region of the FDL tendon was accessed via an incision through the palmar region of the paw (A). The tendon was gripped and cut using scissors, resulting in ~3mm of tendon being removed just proximal to the bifurcation point (B). Panel C is from another animal and had the skin reflected further to demonstrate the ~3 mm region of the FDL that removed. For clarity, these images were taken on a cadaveric specimen. However, in live animals, the proximal tendon region significantly retracted following resection.



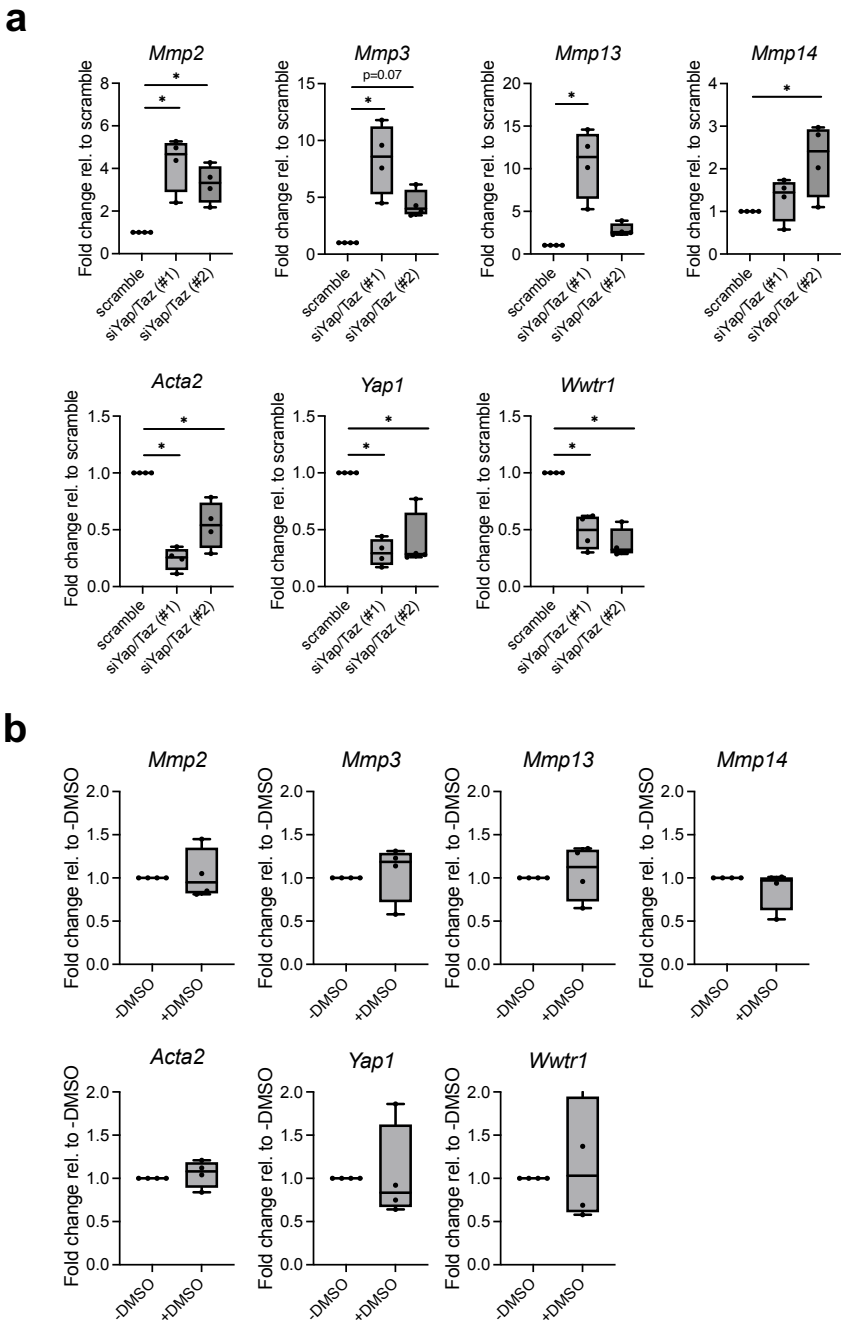
Supplemental Figure 2: Myeloid cells are not the primary source of Mmp3 expression in the FDL tendon.
Representative images of RNAscope duplex in situ hybridization staining for Mmp3 (red) and Csf1r (teal) in contralateral uninjured (A) and resected FDL tendons(B) on day 8 following resection. The vast majority of Mmp3 expression is by fibroblastic cells that do not express Csf1r, which is a myeloid cell marker.



Supplemental Figure 3: Cells isolated from tail tendon digests predominantly express tendon fibroblast reporters.
Representative images from two separate tail tendon cell cultures at passage 0 from the Scx-GFP;Col1a1-CFP dual reporter mouse line. Both Scx-GFP and Col1a1-CFP are abundantly expressed by tendon fibroblasts and nearly all the cells express both reporters. These cultures were created using the same isolation protocol as the CD1 and YAP-CA mouse lines reported in this paper. Scale bar is 100 um.



Supplemental Figure 4: Structural properties of FDL tendons following resection. Viscoelastic and uniaxial failure tests were conducted on FDL tendons at day 8 post-resection. The cross-sectional area was higher in the resected tendons compared to contralateral controls while the stiffness and maximum force were not different from one another (n=9). *P<0.05 evaluated by paired t-test. Error bars represent standard deviation.



Supplemental Figure 5: Validation of siRNA targeting Yap/Taz and effect of DMSO on target gene expression

(a) qRT-PCR analysis of mouse tendon fibroblasts following administration of siRNA targeting Yap and Taz for 3 days. Each group indicates a different single siRNA oligo targeting both Yap and Taz (n=4). *p<0.05 evaluated by unpaired t-test. Error bars represent standard deviation. (b) qRT-PCR analysis of mouse tendon fibroblasts following administration of DMSO for 24 hours. (n=4). No comparisons reached statistical significance, evaluated by unpaired t-test. Error bars represent standard deviation.

Table S1: Primer sequences used for qRT-PCR.

Gene Name	Direction	Sequence
Gapdh	Forward	GTGGAGTCATACTGGAACATGTAG
	Reverse	AATGGTGAAGGTCGGTGTG
Col1a1	Forward	CCTCAGGGTATTGCTGGACAAC
	Reverse	CAGAAGGACCTTGTTTGCCAGG
Acta2	Forward	GTCCCAGACATCAGGGAGTAA
	Reverse	TCGGATACTTCAGCGTCAGGA
Cyr61	Forward	CTGCGCTAAACAACTCAACGA
	Reverse	GCAGATCCCTTTCAGAGCGG
Mmp13	Forward	CTTCTTCTTGTTGAGCTGGACTC
	Reverse	CTGTGGAGGTCACTGTAGACT
Mmp14	Forward	CAGTATGGCTACCTACCTCCAG
	Reverse	GCCTTGCCTGTCACTTGTAAA
Mmp3	Forward	ACATGGAGACTTTGTCCCTTTTG
	Reverse	TTGGCTGAGTGGTAGAGTCCC

Dataset S1: Gene ontology analysis from genes downregulated from blebbistatin treatment.

Dataset S2: Gene ontology analysis from genes upregulated from blebbistatin treatment.