

Cell Reports Medicine, Volume 2

Supplemental information

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and diseased tendon progenitor cells**

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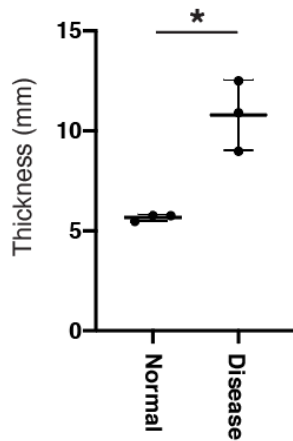
SUPPLEMENTAL INFORMATION

Single-Cell transcriptomic profiling reveals distinct mechanical responses between normal and diseased tendon progenitor cells

Chris Still II^{1*}, Wen-Teh Chang^{2*}, Seth L. Sherman², Kyle R. Sochacki², Jason L. Dragoo³, Lei S. Qi^{4,5,6,7,8}

SUPPLEMENTAL FIGURES

A Tendon Thickness



B

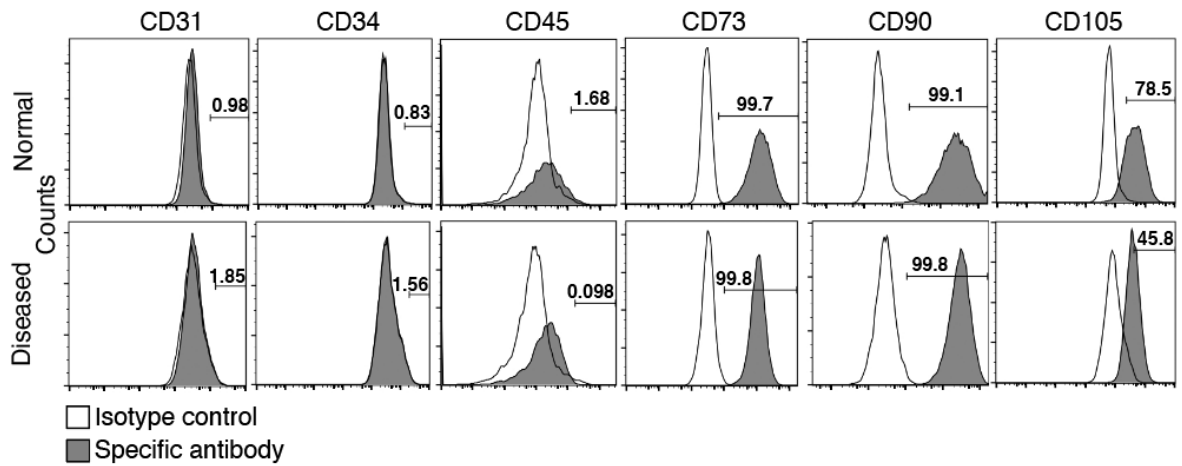


Figure S1: Thickness of tendon tissue from normal and disease cohorts and TPC marker expression. A) Thickness of tissue measured during tendon tissue extraction. Measurements are made in millimeters (3 biological replicates per cohort). * denotes a p value <0.05. B) TPC marker antibody staining panel for patellar tendon derived TPC patient samples (plots are representative of one biological sample per cohort). These tendon samples and TPCs derived therein are the cell source for data in **Figure 1**. Related to **Figure 1**.

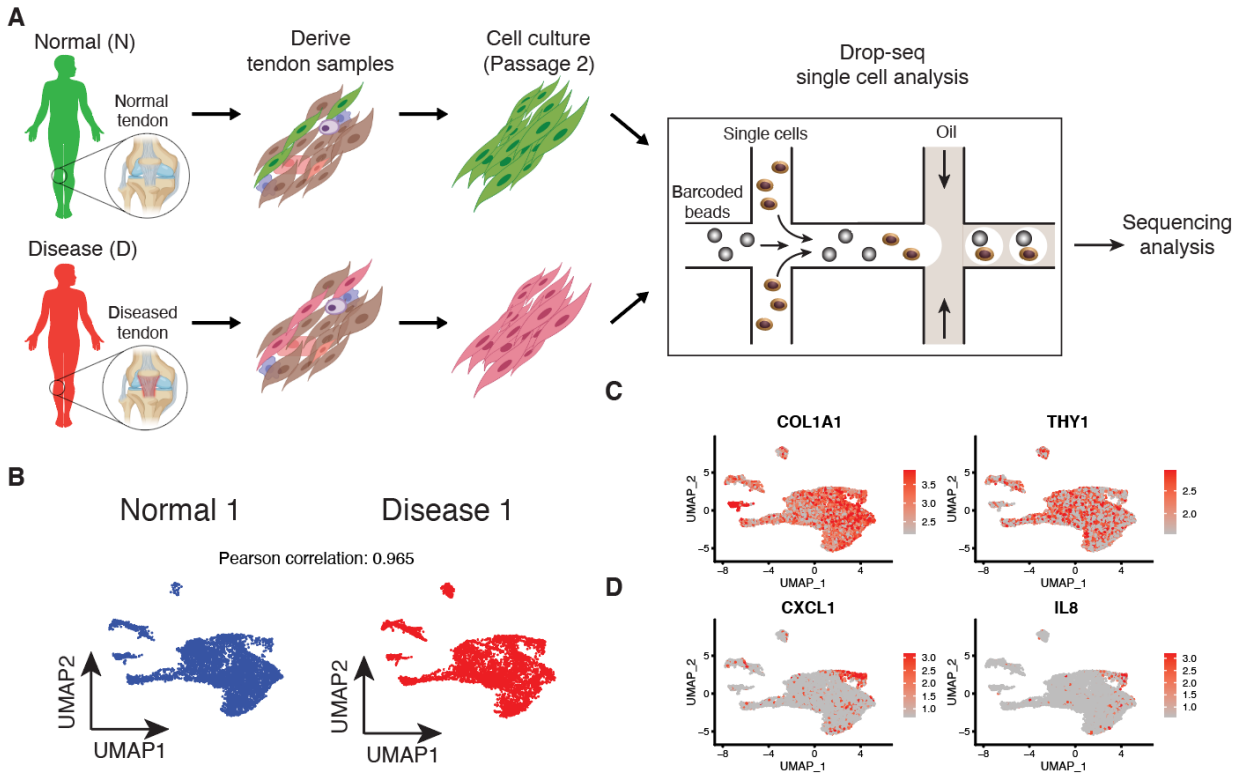


Figure S2: Single cell transcriptomic profiling of the resting TPCs from normal and diseased samples reveal TPC subsets. (A) Overview of scRNA-seq experiment. (B) UMAP plots of cells from the normal cohort and diseased cohort. One biological replicate per cohort (C) UMAP plot of expression of *COL1A1* and *THY1*, general TPC markers. (D) UMAP plot of expression of *CXCL1* and *IL8* in the dataset. (C) and (D) show cells from both the normal and diseased sample. Related to **Figure 1** and **Figure 2**.

TPC correlations

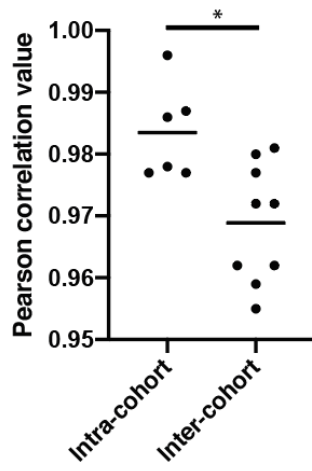


Figure S3: Intra vs inter cohort Pearson correlation comparison. Pearson correlation scores for intra vs inter cohort comparisons were compared. Each dot represents a Pearson correlation. * denotes a p value < 0.05. Related to Figure 1D.

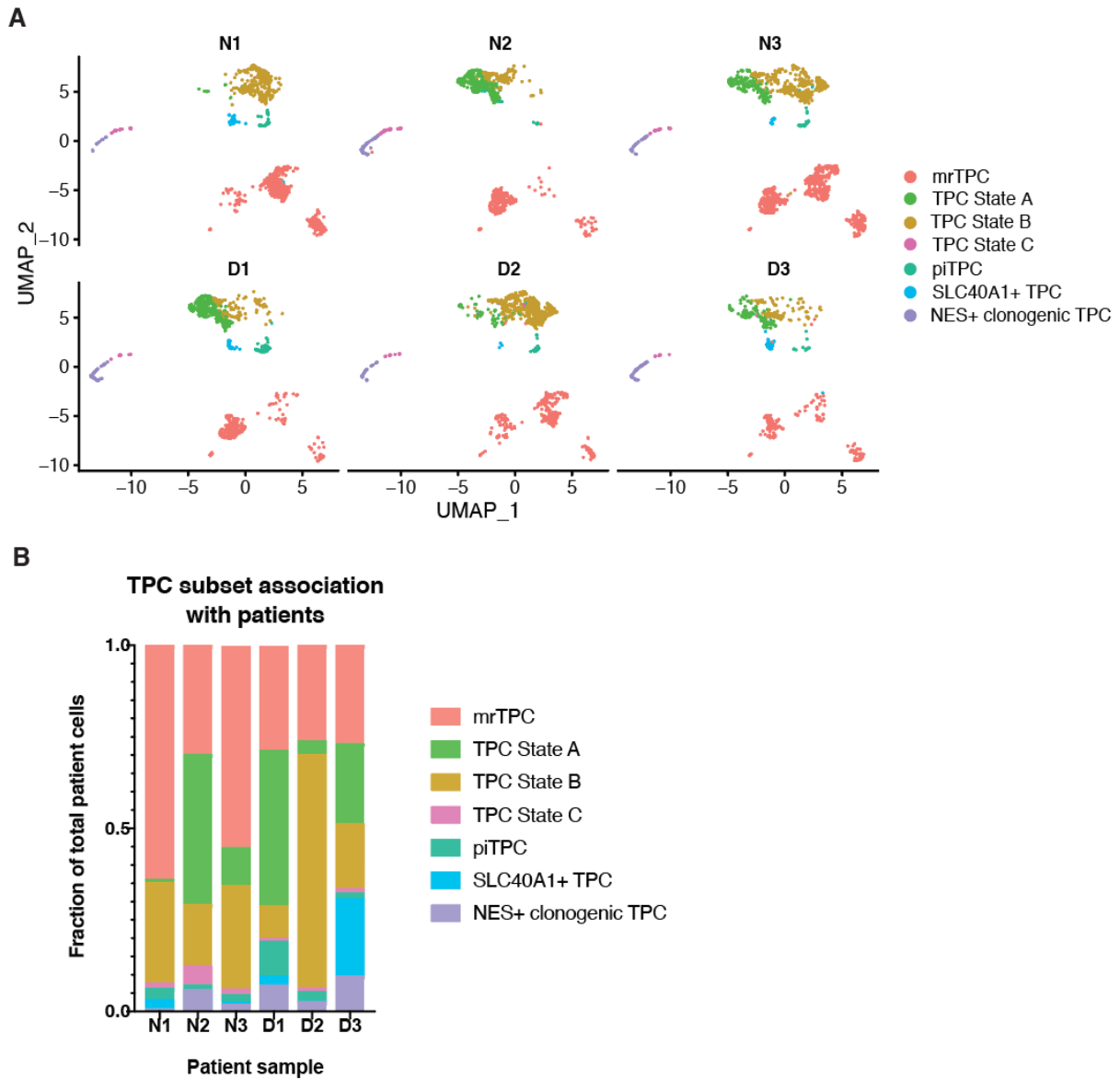


Figure S4: Patient variability in TPC study. A) UMAP plots depicting TPC subsets in each patient. B) Semi-quantitative display of patient TPC levels. One technical replicate per biological sample. Related to **Figure 1B-D**.

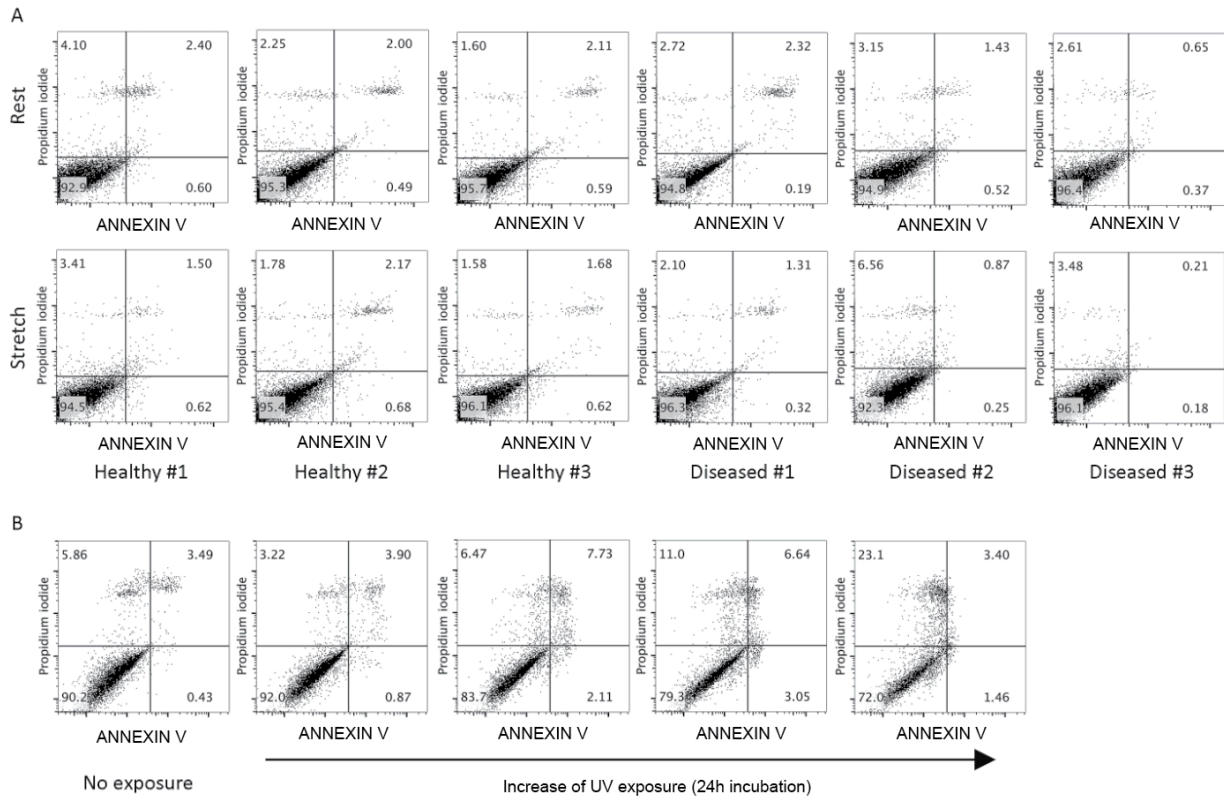


Figure S5: Annexin V and PI staining. Flow cytometric analysis of human TPCs for apoptosis detection in resting or after stretching conditions. A) Human TPCs were under cyclic stretch for 12 h per day/3 days, followed by a 24h resting, or left in resting condition for 4 days, then subjected to apoptosis detection by annexin V/propidium iodide staining. B) Human TPCs were either un-exposed or exposed to increased dosages of UVC ($50 \text{ J/m}^2 - 400 \text{ J/m}^2$), and the same apoptosis detection was applied after a 24 hour incubation. Each panel shows a different biological replicate for the mentioned cohort. Single technical replicate per biological sample. Related to **Figure 2H**.

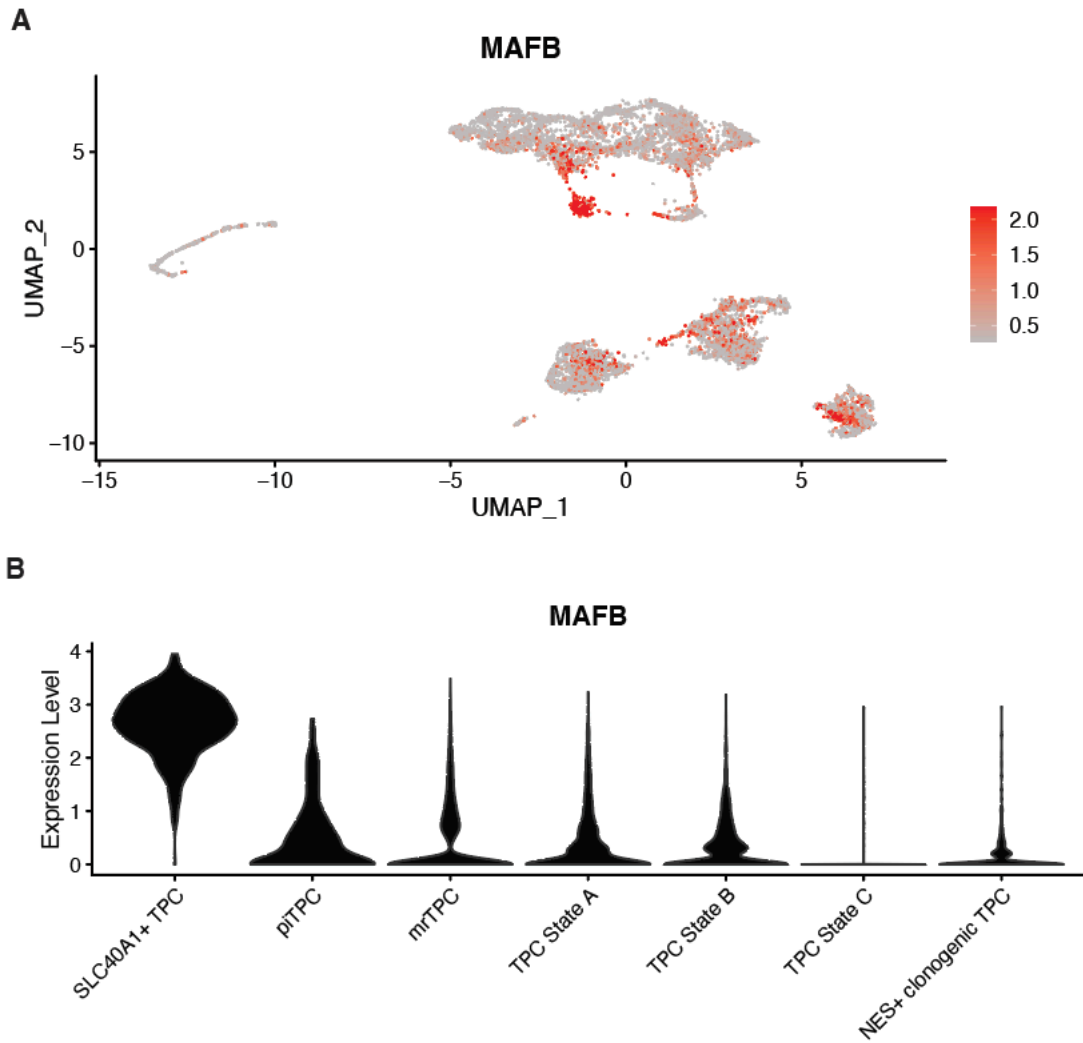


Figure S6: SLC40A1+ TPCs are enriched in MAFB expression. A) Gene expression plot showing expression of *MAFB*. B) Violin plot showing exceptionally high expression of *MAFB* among SLC40A1+ TPCs. Plot and violin plot constitute all 6 biological samples across both cohorts. Related to **Figure 3**.

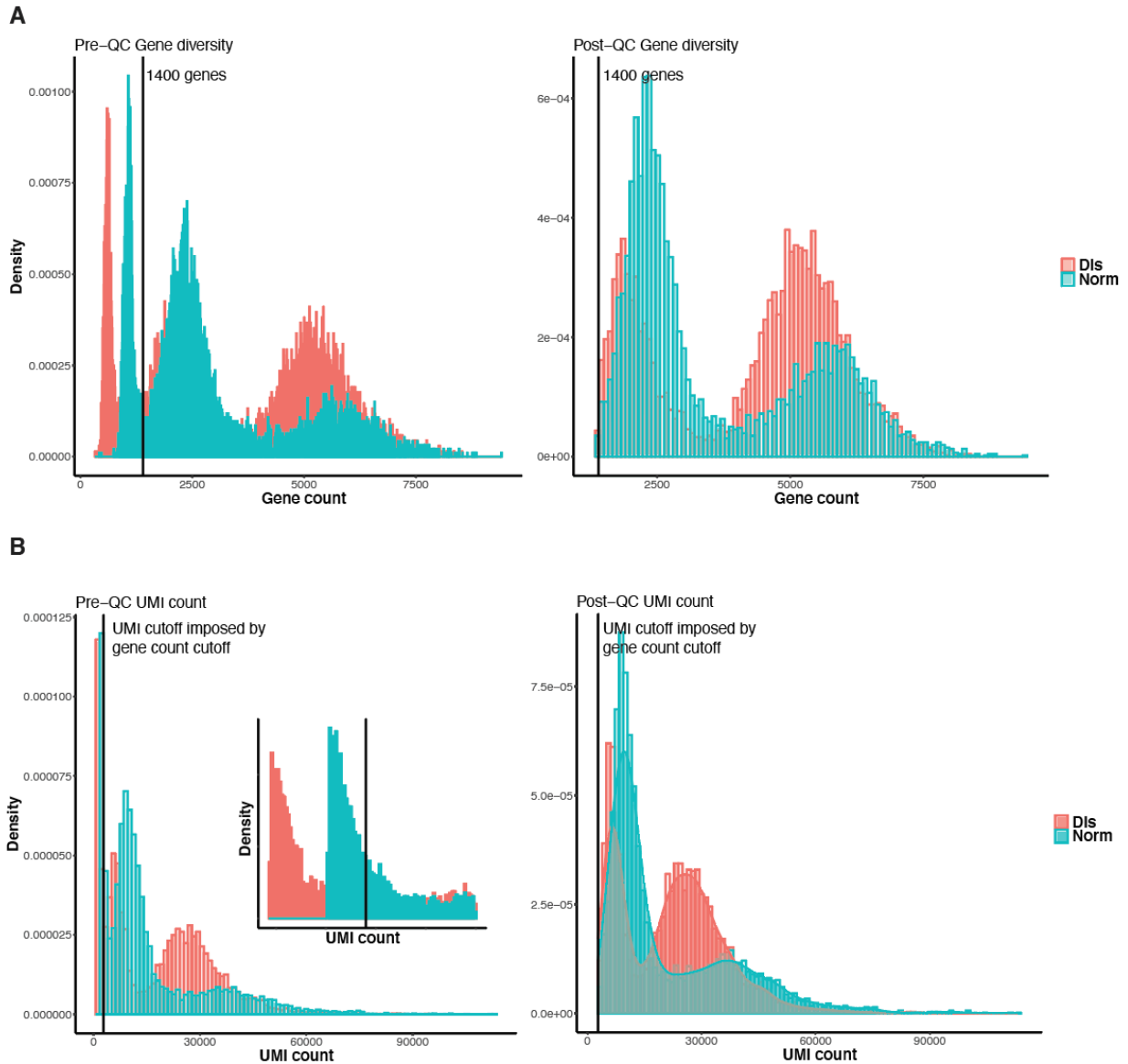


Figure S7: QC metrics for UMI counts and gene diversity cutoff. A) Histograms of gene diversity before and after QC cutoffs are imposed on stretched TPC data. Vertical line represents the 1400 gene cutoff used. B) Histograms of UMI counts before and after QC cutoffs are imposed on stretched TPC data. Vertical line represents the UMI cutoff imposed by the gene diversity cutoff. Related to **Figure 1**.

SUPPLEMENTAL TABLES

Table S1: Sample Information. N and D in the sample column refers to normal and disease respectively. M in the gender column refers to male. Classification grade is based on the Popkin-Golman classification scale for tendon rupture. Related to **Figure 1**.

Sample	Age (years)	Gender	Cohort	Cell viability before scRNAseq	scRNA-seq experiment	Mechanical Stress	Hash antibody Barcode	Traumatic vs. Insidious	Classification Grade	Duration of symptoms	Thickness of tendon tissue (mm)
N1	30	M	Normal	77%/77%	10X Chromium controller 3'/ Drop-seq	Stretched/ Resting	TGATGGCCTATTGGG (Biologend cat#: 394603)				5.75
N2	19	M	Normal	88%	10X Chromium controller 3'	Stretched	GTCAACTCTTTAGCG (Biologend cat#: 394601)				5.47
N3	33	M	Normal	65%	10X Chromium controller 3'	Stretched	AGTAAGTTCAGCGTA (Biologend cat#: 394607)				5.77
D1	33	M	Tendinopathy	85%/96%	10X Chromium controller 3'/ Drop-seq	Stretched/ Resting	TGATGGCCTATTGGG (Biologend cat#: 394603)	Insidious	3	2 years	8.98
D2	26	M	Tendinopathy	81%	10X Chromium controller 3'	Stretched	AGTAAGTTCAGCGTA (Biologend cat#: 394607)	Traumatic	4	1 year	10.9
D3	22	M	Tendinopathy	87%	10X Chromium controller 3'	Stretched	GTCAACTCTTTAGCG (Biologend cat#: 394601)	Insidious	3	2 years	12.5

Table S2: TPC surface marker expression among patient TPCs. Immunophenotyping of human TPC cell surface markers related to MSC, hematopoietic stem cells and endothelial cells. Related to **Figure 1**.

Cohort	Antibody	CD31+	CD34+	CD45+	CD73+	CD90+	CD105+
Healthy	Specific	1.75 ± 1.56	1.47 ± 1.26	2.11 ± 1.09	99.87 ± 0.15	99.47 ± 0.15	78.3 ± 7.60
	Isotype	1.46 ± 1.07	1.48 ± 1.15	0.75 ± 0.58	1.46 ± 0.96	0.46 ± 0.45	1.80 ± 1.66
Diseased	Specific	2.76 ± 1.86	2.29 ± 1.71	0.25 ± 0.16	99.67 ± 0.23	99.83 ± 0.06	60.97 ± 17.16
	Isotype	2.25 ± 1.75	2.09 ± 1.67	0.8 ± 0.17	1.14 ± 0.7	3.64 ± 4.5	2.24 ± 1.93

Table S3: Stretched TPC subpopulation levels for each patient. N and D refer to normal and disease respectively. Numbers are fractions of the total patient population cells. Related to **Figure 1**.

	N1	N2	N3	D1	D2	D3
mrTPC	0.638	0.298	0.551	0.286	0.259	0.268
TPC State B	0.274	0.168	0.282	0.088	0.638	0.177
TPC State A	0.009	0.408	0.104	0.424	0.038	0.219
piTPC	0.030	0.013	0.020	0.095	0.025	0.016
SLC40A1+ TPC	0.026	0.000	0.006	0.025	0.002	0.211
NES+ clonogenic TPC	0.008	0.061	0.021	0.073	0.027	0.098
TPC State C	0.015	0.051	0.015	0.009	0.010	0.011