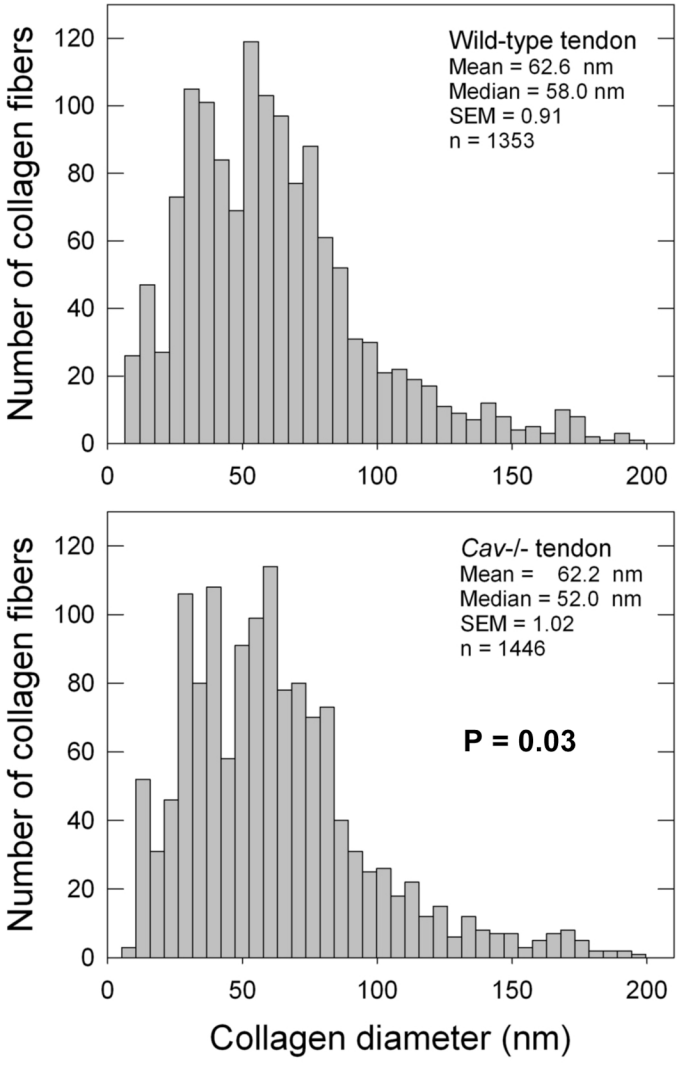
Wild-type tendon
Mean = 62.6 nm
Median = 58.0 nm
SEM = 0.91
n = 1353
Number of collagen fibers
0
20
40
60
80
100
120
0
50
100
150
200
Cav-/- tendon
Mean = 62.2 nm
Median = 52.0 nm
SEM = 1.02
n = 1446
P = 0.03
Number of collagen fibers
0
20
40
60
80
100
120
0
50
100
150
200
Collagen diameter (nm)

Supplemental Figure 1

Algorithm to count and determine the diameters of collagen fibrils using NIH ImageJ software. Transmission electron microscopy images from skin and tendon of wild-type and Cav1-/- mice were median filtered to remove speckle noise, and then thresholded using "Maximum Entropy Thresholding"[1]. Objects were subsequently split using a watershed algorithm[2]. ImageJ's particle analysis was then used to count and measure diameters, area, and area fraction. Resulting data was processed using SigmaPlot, to generate histograms for comparison between large datasets.

The steps of the designed algorithm are the following:

1. **Open the image in NIH ImageJ software.**

Objects (collagen fibers) to be counted need to be in black, and the background in white.

2. **Filtering step**.

Process -> Filter -> Median (2-3 pixels)

3. **Thresholding step**.

Plugins -> Segmentation -> Maximum Entropy Threshold

If the resulting image is not adequate, undo the step, increase the image contrast and repeat step 3.

4. **Separation step**.

Edit -> Invert

Process -> Binary -> Watershed

Edit -> Invert

5. **Measure step**.

Analyze -> Set Scale -> remove scale -> OK

Analyze -> Set Measurements (check the box for Feret's diameter)

Analyze -> Analyze Particles (Under Show select Outlines, and click the boxes for Summarize and Clear results)

Edit->Select All in Results window

Edit->Copy and paste into Sigmaplot or Excel.

1. Sahoo PK, Soltani S, Wong AKC, Chen YC. A survey of thresholding techniques. Comput Vision Graph Image Process 1988; 41:233-60.
2. Beucher S, Lantuéjoul C. Use of watersheds in contour detection. In *International workshop on image processing, real-time edge and motion detection*. France, 1979.