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Supplementary information

Micromechanics of osteopontin-deficient bone

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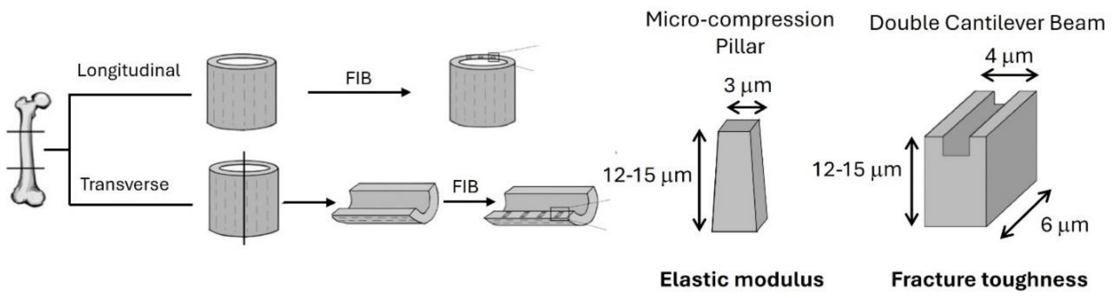

Figure S1. Orientation of longitudinal and transverse pillars for micro-compression and double cantilever beam testing.

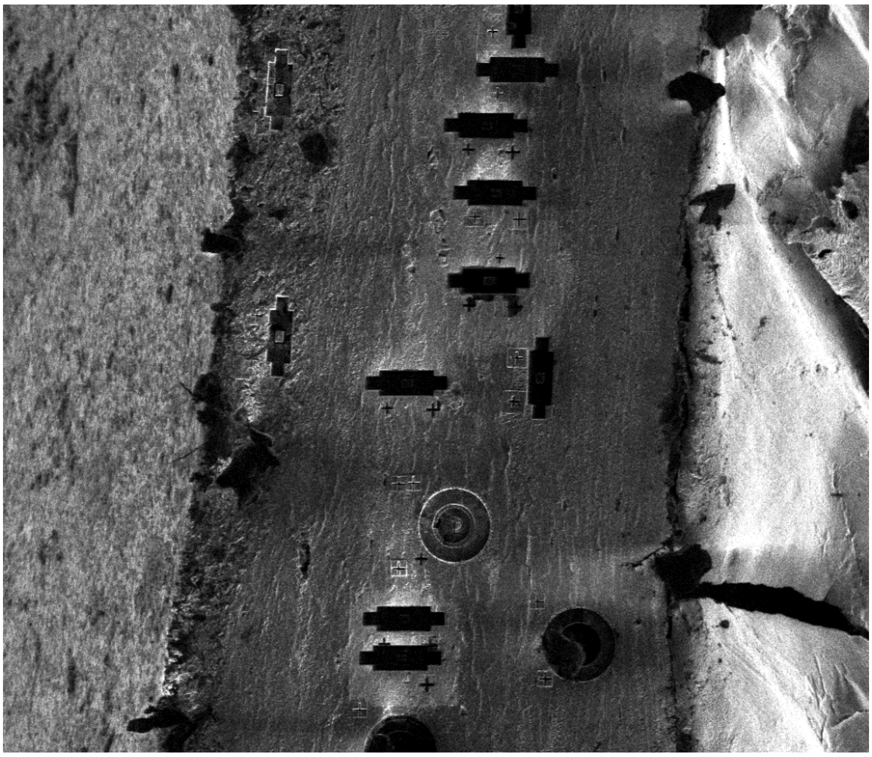

Figure S2. A cross section through a femur showing the region of bone that the DCBs were milled from.

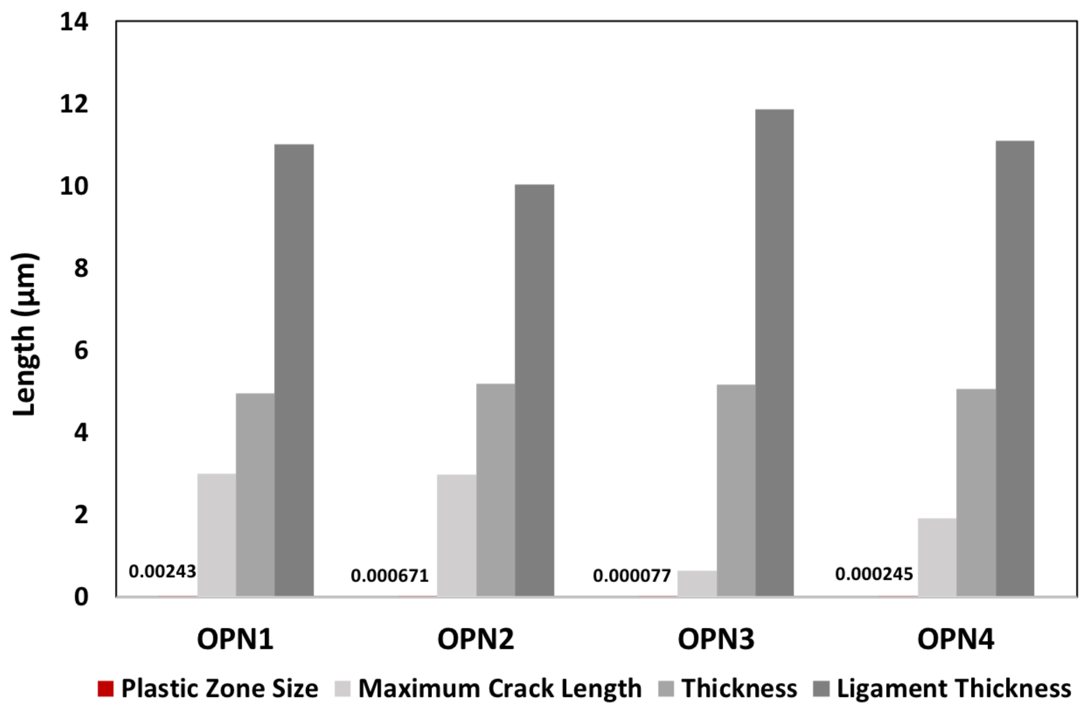

Figure S3. Plastic zone measurements. Maximum crack length of each tested OPN-deficient DCBs in comparison to the estimated size of plastic zone. The size of the plastic deformation zone for mode I deformation needs to be estimated as: $r_{0\sigma} \approx \frac{1}{6\pi}\left(\frac{K_{IC}}{\sigma_0}\right)^2$, where $r_{0\sigma}$ is the size of plastic zone, K_{IC} is the critical stress intensity factor and σ_0 is the ultimate stress measured from the stress-strain curve of micro-compression tests, taken as $\sigma_0 = 0.6\ GPa$ for transverse orientation of $OPN^{-/-}$ tissue.

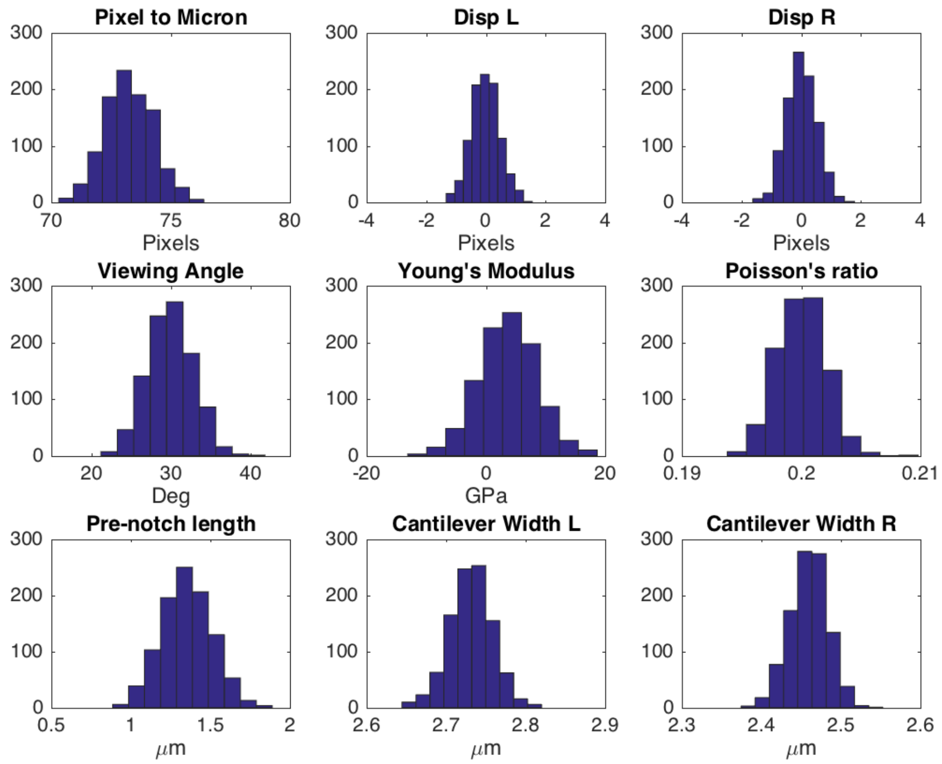

Figure S4. Gaussian distribution of different variables for one of the osteopontin-deficient DCBs. The Monte Carlo error propagation method was applied to generate probability distributions after placing the random inputs used for the Monte Carlo error propagation analysis. Variables with plausible uncertainty were evaluated to acquire a Gaussian distribution with a standard deviation. For each test, the centre of each distribution is the mean measurement, while the standard deviation is the margin of experimental error. The variable terms are defined as follows: Pixel to micron refers to the pixel to micron conversion ration from the scaled test image; Disp L and R refer to the left and right cantilever displacement factor that is used to account for the bending of the cantilever; Viewing angle refers to the correction factor for the recorded SEM images with respect to the axis of the mechanical stage; Young's modulus refers to the uncertainty in the calculated elastic modulus in the micro-compression tests; Poisson's ratio refers to the uncertainty in the reported ratio; Cantilever widths L and R refer to the measured cantilever thicknesses; Pre-notch length refers to the uncertainty associated with a chosen contact point between the wedge tip and the cantilever to obtain the real crack length.

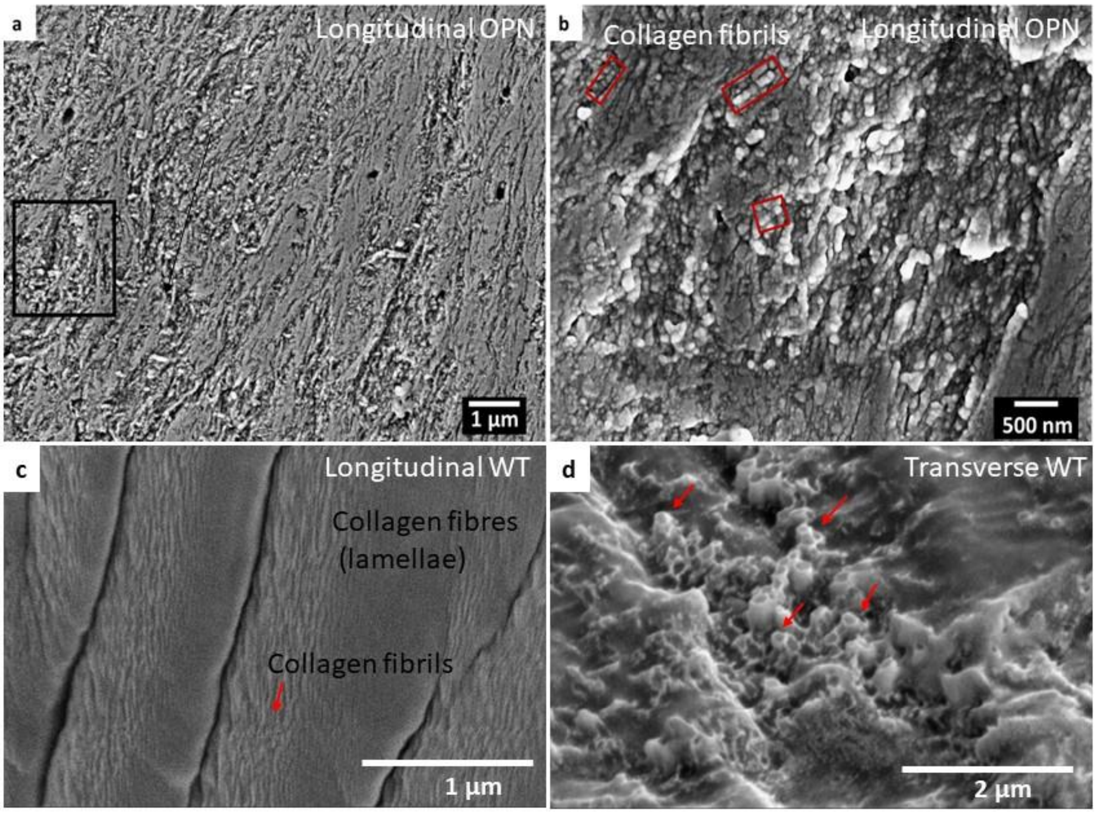

Figure S5. Comparison of Figure 3. **(a)** and **(b)** SEM image of the OPN deficient bone tissue and plasma ion beam FIB-SEM images of wild type (WT) bone tissue taken in the **(c)** longitudinal and **(d)** transverse directions showing highly organised collagen fibres and fibrils in the longitudinal direction. Image **(d)** shows the collagen fibrils in the transverse direction (marked by arrows). Images **(c)** and **(d)** are acquired using a plasma focussed ion beam SEM.

Supplementary Methods

The cross-section of the sample was analysed using a dual-beam plasma Focused Ion Beam (pFIB) system (Helios pFIB, ThermoFisher) equipped with an Xe^+ ion beam column, an electron beam column, and an energy dispersive spectrometer (EDS). Before loading the sample into the pFIB, it was sputter-coated with a 30 nm layer of Au. The region of interest (ROI) was then coated with a 1 µm layer of Pt as a protective coating using the in situ chemical vapor deposition system (FIB-CVD) with an ion beam energy of 8 kV and a current of 28 nA at a 52-degree stage tilt. A higher ion beam energy of 30 kV and 59 nA was used for rough trenching 15 µm away from the ROI. Subsequently, lower ion beam energies of 16 kV and 10 kV with currents ranging from 74 pA to 15 nA were employed for progressive milling toward the ROI. The final ion beam milling was performed at 10 kV, followed by electron beam imaging at 2–5 kV and EDS analysis at 5 kV.