

**Biophysical Journal**

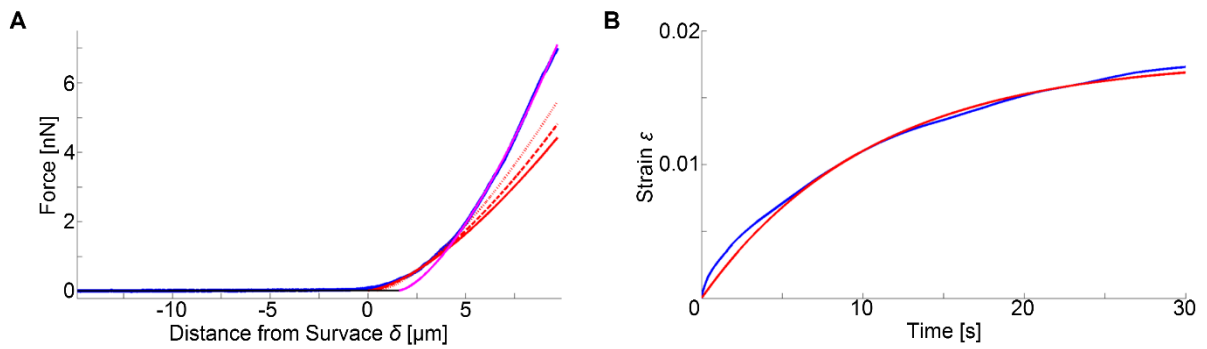
**Supporting Material**

**CNS Cell Distribution and Axon Orientation Determine Local Spinal Cord Mechanical Properties**

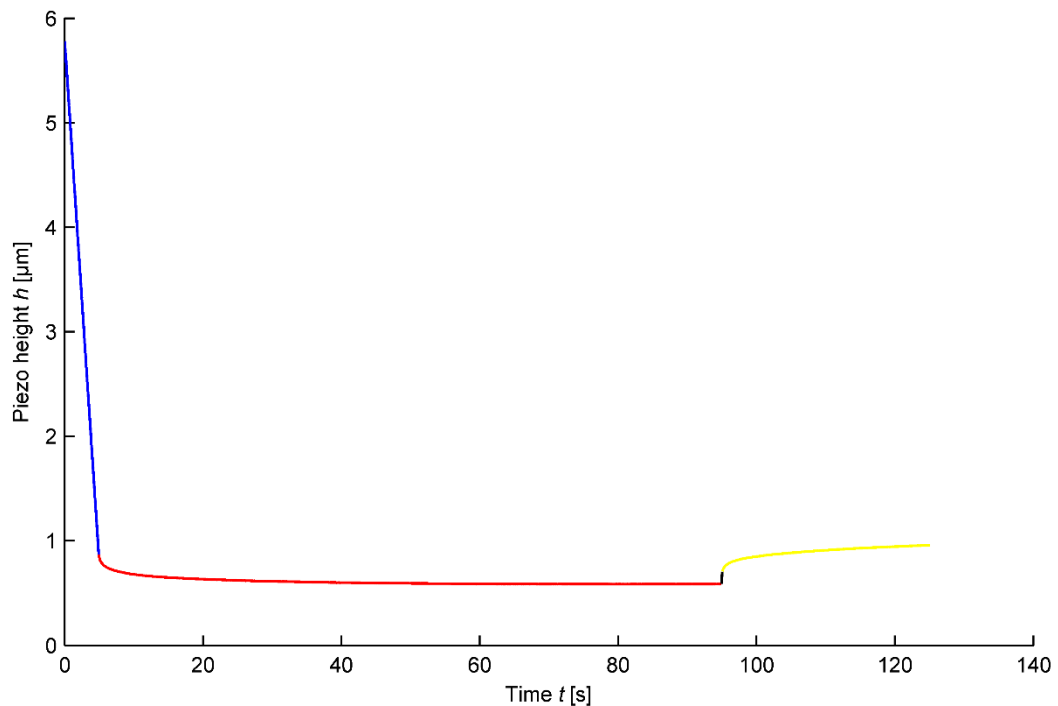
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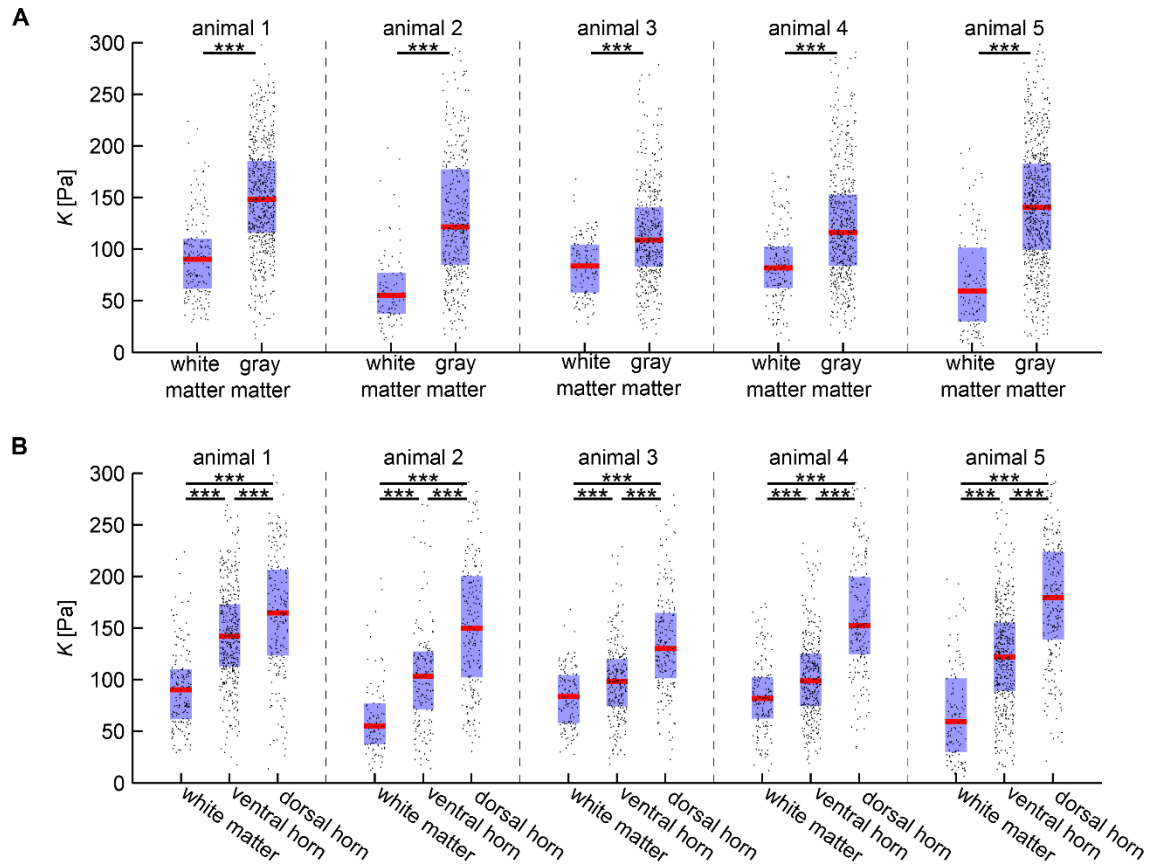
## SI Figures:



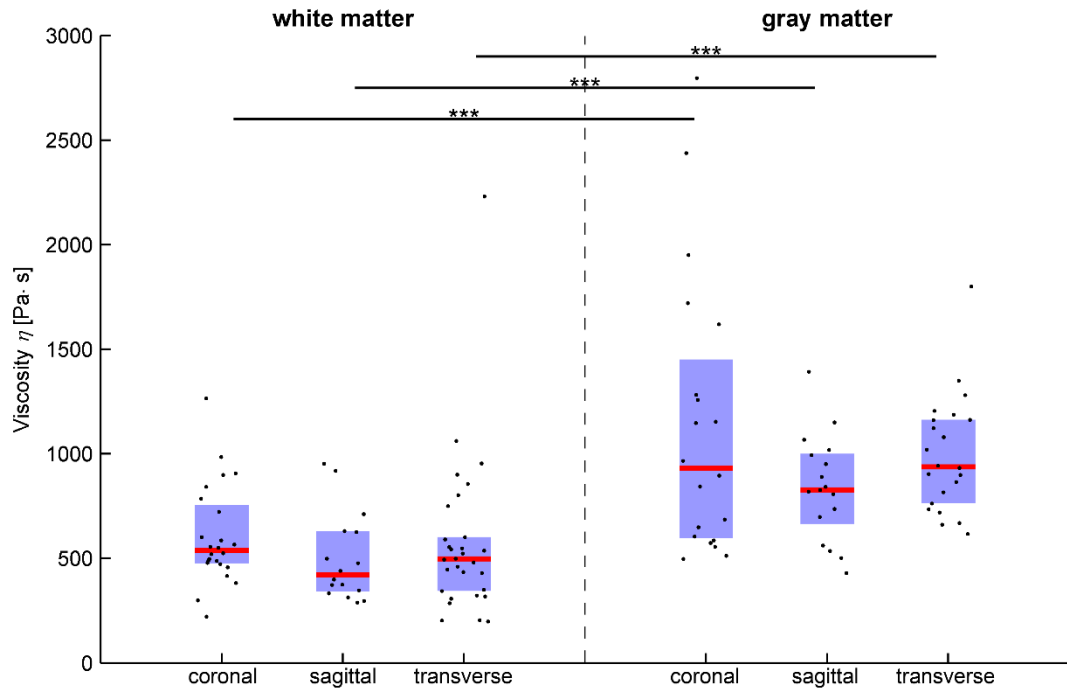
**FIGURE S1** Raw indentation and creep data with fits. (A) Representative force-distance curve of an indentation experiment in *blue*. *Black line* shows baseline fit for each indentation. The 2  $\mu\text{m}$ , 2.5  $\mu\text{m}$ , 3  $\mu\text{m}$  and full indentation Hertz model fits (Eq. 1) in *solid red*, *dashed red*, *dotted red* and *magenta*, respectively. (B) Representative strain-time curve of a creep experiment in *blue*. The Kelvin-Voigt model fit (Eq. 2) in *red*.



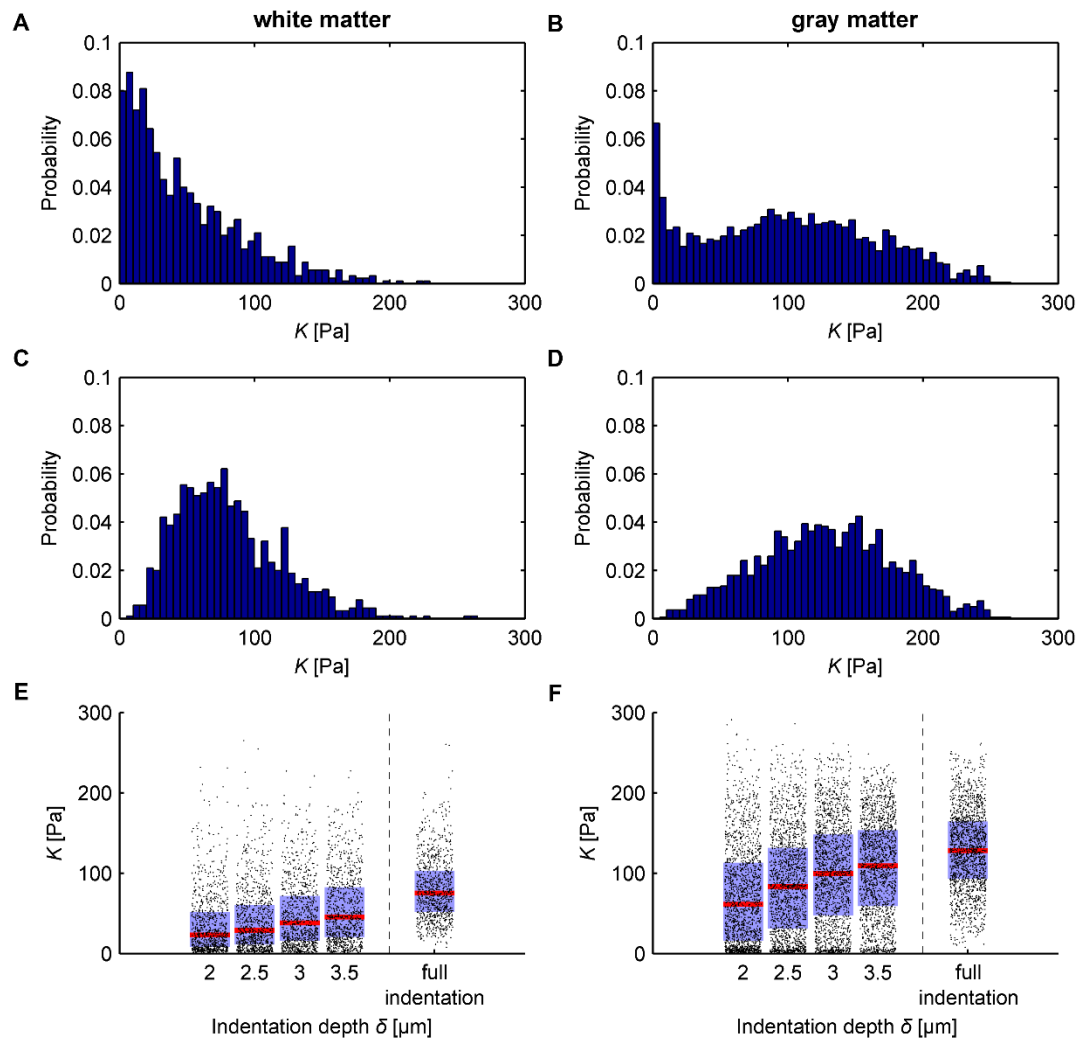
**FIGURE S2** Representative height-time curve of a creep experiment. The four parts of the experiment in *blue*, *red*, *black* and *yellow*, respectively. First part: normal approach till 7 nN. Second part: 90 s constant 7 nN push-force. Third part: fast retraction till -1 nN. Fourth part: 30 s constant -1 nN pull-force (actual creep experiment; see S1 B).



**FIGURE S3** Interindividual differences of  $K$ . Combined box and jittered scatter plots of  $K$  for the sagittal sections of all 5 animals displaying the difference between white and gray matter (A) and dorsal horn, ventral horn and white matter (B). Red line, blue box and black dots represent the median,  $Q_1$ - $Q_3$  percentile, and single data points, respectively. \* ( $p < 0.05$ ); \*\* ( $p < 0.01$ ); \*\*\* ( $p < 0.001$ ).



**FIGURE S4** Viscosity from creep experiments. Combined box and jittered scatter plots of the viscosity  $\eta$  of white and gray matter for coronal (2 sections,  $n_g = 20$ ,  $n_w = 24$ ), sagittal (2 sections,  $n_g = 17$ ,  $n_w = 15$ ) and transverse sections (3 sections,  $n_g = 22$ ,  $n_w = 30$ ). Red line, blue box and black dots represent the median,  $Q_1$ - $Q_3$  percentile, and single data points, respectively. \* ( $p < 0.05$ ); \*\* ( $p < 0.01$ ); \*\*\* ( $p < 0.001$ ).



**FIGURE S5** Strain stiffening of spinal cord white and gray matter. (A-D) Histograms of  $K$  in the coronal plane for white (A, C) and gray matter (B, D) at 3  $\mu\text{m}$  (A, B) and full indentation (C, D). (E, F) Combined box and jittered scatter plots of  $K$  for white and gray matter for 2  $\mu\text{m}$ , 2.5  $\mu\text{m}$ , 3  $\mu\text{m}$ , 3.5  $\mu\text{m}$  and full indentation depth  $\delta$  in the coronal plane. Red line, blue box and black dots represent the median,  $Q_1$ - $Q_3$  percentile, and single data points, respectively. At low indentations a shift of the median  $K$  towards higher values with growing indentation depth.