ELECTRONIC SUPPLEMENTARY MATERIAL (ESI)

Immunofluorescence staining protocol

Abbreviations:
PFA: paraformaldehyde
PBS: phosphate buffered saline (without Ca^{2+}/Mg^{2+})
TX-100: Triton TX-100
DI: deionized water
PVA: polyvinylalcohol

Protocol[S1]:

1. Fix 10 min with 3% PFA in PBS (1 ml/well)
2. Permeabilize cells 5 min with 0.2% TX-100 in PBS (1 ml/well)
3. Wash once with PBS/0.02 TX-100
4. Incubate 60 min with primary antibody in PBS/0.02% TX-100 (250 µl/sample)
5. Wash 3x 5 min with PBS/0.02% TX-100
6. Incubate 60 min with secondary antibody and other dyes in PBS/0.02% TX-100 (250 µl/sample) in darkness
7. Wash 3x 15 min with PBS/0.02% TX-100 in darkness
8. Wash briefly with DI in darkness
9. Mount samples with one drop of PVA on a glass slide, dry PVA overnight, store at 4°C afterwards

All steps performed at room temperature unless otherwise stated.

Antibodies:

primary	dilution	secondary	dilution	color
mouse anti-vinculin (clone hVIN-1)	1:500	Alexa Fluor 488 Goat Anti-Mouse (IgG H+L)	1:250	green

Dyes:

		Alexa Fluor 568 Phalloidin	1:50	red
		4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI)	to 1 µg/ml	blue

Estimation of elastic force

In this study we assume that by applying an external force perpendicular to the middle of the bundle the initial contractile tension does not change during the small deformation regime. Here, we estimate the elastic force that could result from these deformations considering experimental geometrical values and mechanical properties reported in the literature.

Bundles are modelled as cylinders of radius R_c following the linear elastic theory. The elastic force F_e due to stretching can be obtained from the bundle strain ε as

$$F_e = \pi R_c^2 E \varepsilon$$

where E is the elastic modulus. The strain can be written from geometrical considerations

$$\varepsilon = \frac{L - L_0}{L_0}$$

From the literature[S1] E = 0.5 MPa and bundle radius is believed to be in the range 100 - 250 nm. The maximal strain of 4.3% was observed for a bundle with measured tension of 97.8 nN. Estimation of the elastic force for this case gives thus F_e = 2.7 nN (assuming R_c = 200 nm). This value represents 2.8% of the bundle tension. Regarding the average strain of 2.2% for maximal indentations of all the measured bundles and the average tension of 94.8 nN, the average elastic force represents 1.5% of the average tension. The force contribution due to bundle stretching can thus be neglected for the small deformation regime employed in this work.

SUPPLEMENTARY MOVIE CAPTION

Supplementary Movie M1. Simulated displacement field for cantilever designs (width 5, 10 or 20 µm, height 5 µm, length 100 to 1200 µm in 100 µm step). The load (line load 20 nN/µm multiplied by the beam width) applied at the tip of the cantilever was divided by the simulated tip displacement to obtain the spring constant. The simulation was repeated for positive and negative deflections along z, in order to test for the effect of the asymmetry at the anchor. The largest relative difference between positive and negative deflections was 0.54 x10^{-6}**.**

SUPPLEMENTARY REFERENCES

S1. J. Smith-Clerc, and B. Hinz. *Methods in Molecular Biology* (N. J. Clifton), 2010, **611**, 43-57.

S2. S. Degugchi, T. Ohashi, and M. Sato, *MCB Molecular and Cellulare Biomechanics*, 2005, **2**, 125-133.