Supplementary Figures legends

Supplementary figure 1: Nuclear stiffening in response to force applied to nesprin-1.

Typical displacement plot for control sh (a – left panel), emerin sh2(b) and laminA/C sh1 (c). Displacement curve transformed into compliance with Jeffrey's model fit (a – right panel) (Jeffrey's model description in supplementary figure 2a).

Supplementary figure 2: Viscoelastic behavior of the nucleus.

To examine the stiffness of the nucleus in response to an applied force, the time-dependent compliance of the nucleus was calculated from the time-dependent displacement using: $J(t) = 6\pi a\, x(t)/F(t)$, where a is the bead radius.

a, The viscoelastic response of the nucleus was characterized by fitting the compliance during force application to a Jeffrey's model, a mechanical circuit model used to describe viscoelastic material. The Jeffrey's model is formed by an elastic spring and viscous dashpot in parallel with a dashpot in series. **b**, Example bead displacement curve. **c**, Example bead displacement curve transformed into compliance with overlay of Jeffrey's model fit.

Supplementary figure 3: Characterization of the nuclear stiffening in response to force.

a, Nuclei isolated from Hela cells were incubated with anti nesprin-1-coated magnetic beads and stimulated with a permanent magnet for different amounts of time. Active RhoA (RhoA–GTP) was isolated with GST–RBD (Rho-binding domain) and analyzed by western blotting. All results are representative of at least three independent experiments.

b, Stable cell lines depleted for Lamin A/C, LAP2α, emerin, SUN1 or SUN2 were generated using shRNA. Efficiency of the knockdown was assessed by western blot. All results are representative of at least three independent experiments.

c, Change in bead displacement between the first and the 6th pulse of force applied to beads coated with anti nesprin-1 antibody bound to nuclei isolated from cells transfected with control siRNA (n=12 beads) for MAN1 siRNA (n=15 beads) and LBR siRNA (n=17 beads). Displacements were calculated relative to the first pulse of force (error bars represent s.e.m., *P<0.05, data were collected from 3 independent experiments and analyzed by two-tailed unpaired t-test).

d, Efficiency of siRNA knockdowns for MAN1 and LBR were assessed by western blot. All results are representative of at least three independent experiments.

Supplementary figure 4: SFK(s) mediate the nuclear stiffening to force.

a, Nuclei isolated from Hela cells were incubated with anti nesprin-1-coated magnetic beads and stimulated with a permanent magnet for different amounts of time. Emerin was immunoprecipitated and its tyrosine phosphorylation was analyzed by western blot. All results are representative of at least three independent experiments.

b, Nuclei isolated from Hela cells were incubated with anti nesprin-1-coated magnetic beads and pretreated 30 min with Gleevec (10 µM), SU66056 (2.5 µM) or FAK inhibitor (5 µM). After stimulation with a permanent magnet for 3 min, tyrosine phosphorylation of nuclear proteins was analyzed by western blot. All results are representative of at least three independent experiments.

c, Nuclei isolated from Hela cells were incubated with anti nesprin-1-coated magnetic beads and stimulated with a permanent magnet for 3 min. Src expression, Src phosphorylation on Y416, FAK expression and FAK phosphorylation on Y397 were assessed by western blot. All results are representative of at least three independent experiments.

d, Change in bead displacement between the first and 6th pulse of force applied to beads coated with anti nesprin-1 antibody bound to nuclei incubated with no ATP (n=14 beads) or with SU6656 (n=22 beads) for 30 min (untreated control n=13 beads). Displacements were calculated relative to the first pulse of force applied to untreated nuclei (Error bars represent s.e.m., *P<0.05, data were collected from 3 independent experiments and analyzed by two-tailed unpaired t-test).

Supplementary Figure 5: Tension induces emerin phosphorylation.

a, Emerin tyrosine phosphorylation was analyzed after immunoprecipitation in MRC5 cells during adhesion to fibronectin or treated with blebbistatin. ("total" refers to the emerin level in nuclear lysates). All results are representative of at least three independent experiments.

b, MRC5 cells were incubated with fibronectin-coated magnetic beads for 30 min. A permanent magnet was used to generate tensional force for different amounts of time. After cell lysis, emerin tyrosine phosphorylation was analyzed. All results are representative of at least three independent experiments.

c, Invasion of emerin knockdown Hela cells and emerin knockdown Hela cells re-expressing WT or 74-95FF emerin mutant was evaluated by Transwell migration assays. Cells were plated in the upper chamber of the filters and after 8 hours cells that had migrated to the underside of the filters were fixed. Relative cell migration was determined by the number of cells that had migrated to the underside of the filter normalized to the total number of cells. A number of n=24 fields were observed per condition. The value from control shRNA Hela cells was arbitrarily set at 100% (Error bars represent s.e.m., # $P<0.05$ compared to control sh, *$P<0.05$ compared to WT, data were collected from 4 independent experiments and analyzed by one way ANOVA).

d, Emerin knockdown MRC5 cells re-expressing WT or 74-95FF emerin mutant were grown on fibronectin-coated coverslips for 6 hours, fixed, permeabilized and stained for YAP/TAZ and myc tagged emerin. To quantify YAP or TAZ nuclear localization (panel d) we calculated the percentage of cells with a predominant nuclear staining (delimited by DAPI staining) among the total cell number. n=36 myc positive cells expressing WT emerin and n=34 myc positive cells expressing 74-95FF were analyzed (Error bars represent s.e.m., *$P<0.05$, data were collected from 3 independent experiments and analyzed by two-tailed unpaired t-test). Bar scale=25 µm.

e, Emerin knockdown MRC5 cells re-expressing WT or 74-95FF emerin mutant were incubated with fibronectin-coated magnetic beads for 20 min. A permanent magnet was used to generate tensional force for 90 min. IEX1 and GAPDH mRNA levels were analyzed using real-time qPCR (error bars represent s.e.m., #$P<0.05$ compared to WT control, data were collected from n=3 independent experiments and analyzed by two-tailed unpaired t-test).

Supplementary Figure 6

Uncropped scans from figure 2d, 3a and 3b.

Supplementary Figure 7

Uncropped scans from figure 3d and 3e.

Supplementary Table 1

Number of beads that showed displacements of less than 10 nm and were excluded from the analysis (see "methods" section).