Supplemental Materials
Molecular Biology of the Cell

Holenstein et al.
Supplementary Materials and Methods:

Substrate preparation

All experiments involving adherent cells were done using silicone substrates prepared identically. Briefly, PDMS (Sylgard 184, Dow Corning) was mixed in a 60:1 base:crosslinker ratio, which results in a bulk Young’s modulus of 15 kPa (Kenry et al., 2015), degassed and spin coated on top of silicone sheets (SMI Inc.) for the tensile analysis or 25 mm diameter #1 coverslips (Menzel Gläser, Germany) for immunofluorescence and TFM experiments. PDMS was allowed to cure overnight at 45°C and subsequently incubated for 45 minutes in a 10% solution of (3-Aminopropyl)triethoxysilane (APTES, Sigma, 440140) in ethanol, washed and further incubated one hour in 3% glutaraldehyde in PBS. Next, substrates were incubated in a suspension of 0.5 µm red-fluorescent carboxylated beads (1:100; Thermo, F8812) in PBS. Substrates were then washed and incubated in a sulfo-SANPAH (ProteoChem, C1111) solution (0.2 mg/ml) in HEPES buffer (Thermo, 15639-056), illuminated with UV light (Stratalinker UV Cross linker 2400) and immersed in a 50 µl/ml collagen solution (Corning, 354236). After 3 washing steps with PBS and 3 with complete medium, cells were seeded at low density and allowed to attach overnight.

Traction Force Microscopy

Substrates were mounted on a metal holder (Ske Research equipment, Italy) and placed on an inverted spinning disc confocal microscope (iMic, FEI Photonics, Germany) equipped with a 40X (N.A. 0.95) objective. Image stacks of the cell bodies and surface beads were acquired before and after cell detachment of the cells achieved by addition of approx. 0.5 ml of 10% Sodium dodecyl sulfate to the medium (Oakes et al., 2014). After image acquisition, stacks were post-processed in ImageJ (Schneider et al., 2012) in order to obtain single images of the beads in focus (Tseng et al., 2011) per stack. Methods for traction force reconstruction were employed as previously described (Holenstein et al., 2017). Briefly, image pairs were imported into MATLAB (MathWorks, Natick, MA) and aligned to correct for drift (dftregistration.m) and cell traction induced deformation of the substrates was measured using an iterative optical flow tracker called Lukas-Kanade Tracker (KLT). Bead displacements were interpolated on a regular grid with an 8 pixel spacing (1.2 µm) and filtered using a...
two-dimensional Wiener filter. From this displacement field, the traction stresses were calculated using regularized Fourier Transform Traction Cytometry (FTTC) (Butler et al., 2002) with zero-order regularization (Plotnikov et al., 2014), using the same regularization parameter for all experiments. The PDMS substrate was assumed to be incompressible and hence the Poisson’s ratio was set to 0.5.

**Micropillar-based TFM**

Micropillar arrays were used following the manufacturer’s instructions (MicroDuits, Switzerland). Briefly, 25,000 cells were seeded on top of each prepared micropillar array and allowed to adhere for 6 to 10 hours. Cells were then washed twice with pre-warmed PBS, fixed in formalin solution (Sigma, HT501128) for 20 minutes and stained with Coomassie blue R-250 solution (Thermo, 20278) for 30 seconds. Arrays were then washed twice with distilled water and stored at 4°C until imaging was done. Cells were imaged under an inverted bright field microscope (ScanR, Olympus) equipped with a 40X N.A. 0.9 objective and a Hamamatsu ORCA-FLASH 4.0 camera. Finally, the images were analyzed using the open source software Mechprofiler 1.0 (Goedecke et al., 2015).

**Rho-associated proteinkinase (ROCK) activity assay**

Rho-associated protein kinase (ROCK) activity was assessed using a commercially available immunoassay kit (Cell Biolabs) that specifically detects ROCK’s phosphorylation of myosin phosphatase target subunit 1 (Mypt1) at Thr<sup>696</sup>. Briefly, cells were seeded on PDMS substrates, lysed with RIPA buffer, and lysates were stored at -80°C until use. Lysates were thawed on ice, and deposited along with a positive control in a well plates precoated with Mypt1. After incubation, substrates were washed and overlaid with primary antibody specific for phosphorylated Mypt1, washed, and a secondary antibody conjugated with horseradish peroxidase was overlaid. The colorimetric reaction was stopped using stop solution and optical density of each samples was read at 450 nm.

**Western blot**

Semi-confluent cultures of all 4 osteosarcoma cell lines were trypsinized and centrifuged. Cell pellets were resuspended in sample buffer (Bio-Rad, 161747) and run in a 4-15%
polyacrylamide gel (Bio-Rad, 4568086). Proteins were then transferred to a PVDF membrane (Bio-Rad, 1704271) using a Trans-Blot Turbo Tranfer System (Bio-Rad, 1704150). Next, membranes were blocked with 5% non-fat dry milk in TBST, probed with anti-lamin A/C (Santa Cruz Biotechnologies, sc-376248) or anti-lamin B receptor (LBR; Abcam, ab32535) primary antibodies for o/n at 4°C, followed by extensive washing in TBST and incubation with the corresponding secondary antibodies conjugated with horseradish peroxidase (Sigma-Aldrich, anti-mouse, S3070173 and anti-rabbit, SAB370878, respectively). The lamin A/C and LBR bands were detected by a chemiluminescence reaction (34577, Thermo Fisher) and imaged using a ChemiDoc MP documentation system (Bio-Rad). Relative band density was estimated using ImageJ software.

**Flow cytometry**

Osteosarcoma cells were cultured until semi-confluence, trypsinized and fixed in cold 70% ethanol for 30 minutes. Next, cells were stained with propidium iodide solution (containing RNaseA; BD Pharmingen) at room temperature for 15 minutes. Approximately 10,000 cells per sample were recorded using a LSRFortessa (BD Biosciences). Cell cycle analysis was done twice using two independently stored vials of each tested cell line. The percentage of cells in each phase of the cell cycle was determined using FlowJo v.10.

**Data representation and statistical analysis**

If not stated differently, data representation and statistical analysis were done using Prism (v.7, Graphpad, USA) and values of compared groups were tested for significance using a two-tailed Mann-Whitney-U test. Significances are noted as: * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.0001.

**Data and code availability**

The datasets generated in the current study, as well as the source code for their analysis, are available from the corresponding author upon reasonable request.
**Supplementary Figure 1.** Analysis of immunofluorescence images of the 4 cell lines used in the present study in adhering conditions. Confocal images of (A) SaOs-2, (B) LM5, (C) HuO9 and (D) M132 cells stained with phalloidin (red channel) and anti-vinculin (green channel) and NucBlue (blue channel), were used to obtain cell spreading area (bottom left panel), projected area of the nucleus (bottom middle panel) and focal adhesion number (bottom right panel). Scale bar represent 25 μm.

**Supplementary Figure 2.** Analysis of immunofluorescence images of the 4 cell lines used in the present study in free-floating conditions. (A) Confocal stacks of the cell lines used in the present study (left to right: SaOs-2, LM5, HuO9 and M132) stained with NucBlue (red channel), phalloidin (green channel) were used to were reconstructed and segmented to estimate cytoplasmic. Scale bars represent 25 μm.
**Supplementary Figure 3.** Representative Western blot of whole lysates from all 4 cell lines probed against lamin A/C (upper box) and Lamin B receptor (LBR; bottom box). Protein bands were quantitated by densitometry and the ratio between Lamin A/C and LBR calculated (bottom row).

**Supplementary references:**

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Tseng Q, Wang I, Duchemin-Pelletier E, Azioune A, Carpi N, Gao J, Filhol O, Piel M, Théry M, Balland M (2011). A new micropatterning method of soft substrates reveals that different tumorigenic signals can promote or reduce cell contraction levels. Lab Chip 11, 2231–2240.
Supplementary Table 1.- Biomechanical and morphological characterization of osteosarcoma cells. Parameters quantified for both cell pairs are expressed as average ± standard deviation of the mean.

| Parameter                              | SaOs-2            | LM5             | p      | HuO9            | M132            | p      |
|----------------------------------------|-------------------|-----------------|--------|-----------------|-----------------|--------|
| **Circularity**                        | 0.1612 ± 0.056    | 0.1681 ± 0.075  | p=0.9437 | 0.0236 ± 0.016  | 0.0457 ± 0.029  | p=0.0002 |
| **Spreading area [μm²]**               | 2254 ± 955        | 1605 ± 479      | p=0.016 | 1385 ± 453      | 870 ± 304       | p=0.0001 |
| **Nuclear projected area [μm²]**       | 287.9 ± 67.65     | 246.6 ± 64.19   | p=0.035 | 220.9 ± 82.13   | 197.2 ± 43.26   | p=0.5254 |
| **Average FA/cell**                    | 80.4 ± 33.1       | 45.3 ± 12.9     | p=0.012 | 87.8 ± 31.1     | 36.6 ± 19.7     | p<0.0001 |
| **FA/μm²**                             | 0.037 ± 0.0106    | 0.0294 ± 0.008  | p=0.012 | 0.065 ± 0.018   | 0.0417 ± 0.015  | p<0.0001 |
| **Free floating volume [μm³]**         | 2077 ± 747        | 1803 ± 713      | p=0.15  | 2653 ± 947      | 2228 ± 859      | p=0.0380 |
| **Nuclear volume [μm³]**               | 902 ± 485         | 1132 ± 560      | p=0.059 | 1328 ± 493      | 1026 ± 525      | p=0.0084 |
| **Indentation stiffness (PDMS) [kPa]** | 3.9 ± 1.8         | 2.4 ± 1.0       | p<0.0001 | 2.6 ± 1.5       | 5.1 ± 2.2       | p<0.0001 |
| **Indentation stiffness (glass) [kPa]**| 3.5 ± 1.7         | 1.8 ± 0.8       | p<0.0001 | 2.0 ± 0.7       | 4.9 ± 2.0       | p<0.0001 |
| **Free floating stiffness [kPa]**      | 0.95 ± 0.07       | 0.82 ± 0.06     | p=0.0093 | 1.22 ± 0.09     | 1.21 ± 0.06     | p=0.764  |
| **Tensile stiffness [kPa]**            | 14.6 ± 7.44       | 15.8 ± 8.16     | p=0.5025 | 13.14 ± 5.9     | 20.5 ± 13.26    | p<0.0001 |
| **Generated traction/cell [kPa]**      | 0.29 ± 0.44       | 0.15 ± 0.15     | p<0.0001 | 0.24 ± 0.16     | 0.13 ± 0.11     | p<0.0001 |
| **Force generation/cell [nN]**         | 9.18 ± 6.18       | 7.54 ± 3.73     | p=0.0017 | 9.13 ± 5.41     | 7.69 ± 3.4      | p<0.0001 |