Supplementary Information

Size-Tunable Nanoneedle Arrays for Influencing Stem Cell Morphology, Gene Expression, and Nuclear Membrane Curvature

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**SUPPLEMENTARY FIGURES**

**Figure S1.** (a) Stability of the nanoneedles used for experiments. The structures were immersed in the cell culture medium with 10 % v/v serum and 1% v/v antibiotics, and then kept in the cell culture incubator (37 °C, 5% CO₂ with 100% RH). After that SEM images were taken to check the structure. Scale bar: 2 μm. (b) Cell culture on the nanoneedles up to 19 days. hMSCs were cultured for 10, 14, and 19 days, respectively, and then fixed and immunostained. Green: actin, blue: DAPI. Scale bar: 200 μm.
Figure S2. Proliferation of hMSCs on the structures, as evidenced by (a) percentage of Ki-67 positive nuclei (mean ±SD., N = 4 for i and ii, and N = 3 for v) and (b) qRT-PCR result with MKI67 primers (box plot, maximum/minimum, N = 3).

If Welch’s ANOVA detected a significant effect on gene expression a Games-Howell post hoc test was conducted. Asterisks (*) indicate significantly different (p < 0.05) groups. For Ki-67 staining experiments, hMSCs were cultured for 72 h. For qRT-PCR, hMSCs were cultured for 24 h. i: Flat substrate, ii: nanopillars, v: sharp nanoneedles.

(c-e) LIVE/DEAD® assay performed after 35 days of cell culture: hMSCs on (c) flat substrates, (d) nanopillars, and (e) nanoneedles. Green: Calcein AM, Red: Ethidium homodimer-1 (EthD-1), Scale bars = 500 µm.
Figure S3. High-content-screening results of hMSCs cultured on flat substrate (i), nanopillars- (ii = 718.4 ± 31.7 nm), blunt- (iii = 316 ± 20 nm), less-blunt- (iv = 172 ± 6.2 nm), and sharp- (v = 47 ± 7.0 nm) nanoneedles. (a-b) Cell area and cell eccentricity as a function of substrate. (c-d) Nucleus area and nucleus solidity as a function of substrate. (biolin box plot, maximum/minimum, N > 2500).
Figure S4. Representative immunostaining images of nuclear membrane (Lamin A and Lamin B) staining to confirm the distortion of cell nuclear. hMSCs were cultured for 72 h on (a) nanopillars and (b) sharp nanoneedles, respectively. Scale bar: 10 µm.
Figure S5. Cell orientation a function of experimental condition.

The binary object representing each (cell + protrusions) is fitted with an equivalent ellipse. The angle between the major axis of this ellipse and the positive direction of the x-axis is calculated and assigned as the orientation for that cell. Each facet in the above plot shows the kernel density estimate, i.e. a smoothed representation of the distribution of data. Flat substrates show no preferential cell orientation, hence the flat distribution. Nanostructured substrates show varying degrees of preferential alignment at 0° and ±90°, as per CellProfiler’s standard angle definition. This means that by definition cells aligned to the x-axis are mapped to the same value (0°), hence why the peak looks roughly twice the height of those at ±90°. During imaging for the image-based cell profiling there was a slight uncertainty in the registration of the nanoneedle array to the microscope camera/image frame, hence a slight broadening in peaks in some cases (e.g. the difference in height between the substrates iv and v may not be significant).
Figure S6. The value of the alignment distribution at $0^\circ$ and $90^\circ$ with different tip sharpness. Values are shown as mean and standard deviation at four different time points (6, 12, 24, and 72 h). Mean ± SD, N = 3.
Figure S7. Validation of siRNA-mediated gene silencing: (a-b) Relative gene expressions after 72 h of siRNA transfection. (a) RHOA and (b) RAC1. Gene expression data has been normalized with the control. Mean ± SD, N = 3, ****: p < 0.001 from Student’s paired t-test. (c) Representative immunostained images of Lipofectamine-treated hMSCs cultured on nanoneedles as a control experiment. Scale bars: 25 µm.

Figure S8. Effects of Rac1 and actomyosin contractility inhibitors: Representative immunostained images and alignment quantification after inhibitor treatment to the hMSCs on nanoneedle substrates. (a) Treatment with 10 µM of DMSO only was used as a control. (b) NSC23766 and (c) blebbistatin were treated to inhibit Rac1 and MLC2, respectively (scale bar: 25 µm).
Figure S9. (a-c) Relative expression of ITGAV, ITGA5 and ITGB1 after 24 h culture of hMSCs on each substrate. i: Flat substrate, ii: Nanopillars, v: Sharp nanoneedles. Data were normalized to the gene expression on flat substrate after 6 h culture of hMSCs. Mean ± SD., *: p < 0.05 from Tukey’s honestly significant difference (HSD) post hoc test.

(d-f) Representative confocal immunofluorescence images of integrin β1-stained hMSCs on different structures after 72 h culture. (c) Flat, (d) Nanopillars, and (e) Nanoneedles (scale bar: 25 μm). (g) Quantified fluorescence intensity of focal adhesions. Box plot, minimum/maximum, N = 4-6.
Figure S10. (a) Representative YAP staining results from the hMSCs on different structures with a time point of 6 and 24 h (scale bar: 50 μm), and (b) quantification of YAP intensity ratio, defined as YAP intensity in the nuclei region divided by the intensity in cytoplasmic region. Box plot, minimum/maximum, N > 2000. i: Flat substrate, ii: Nanopillars, iii-iv: Blunt nanoneedles, v: Sharp nanoneedles.

(c-d) Relative gene expression of YAP-target genes, and visualization of YAP in the hMSCs. Box plot, minimum/maximum, N = 3. If Welch’s ANOVA detected a significant effect on gene expression a Games-Howell post hoc test was conducted. Asterisks (*) indicate significantly different (p < 0.05) groups.
Figure S11. Adipogenic and osteogenic differentiation of hMSCs on different substrates:

(a) Oil Red O staining used to visualize lipid vacuoles after 21 days of adipogenic differentiation. Representative fluorescent images of stained lipid (red) in hMSCs cultured on flat, nanopillar and nanoneedle substrates, and quantification of extracted stain using absorbance measurements (mean ± SD, N = 2). This shows that all substrates were able to support chemically stimulated adipogenesis, and there was no evidence of material-driven differentiation under basal conditions. Scale bars = 400 µm.

(b) Alizarin Red S staining used to visualize calcium deposits after 21 days of osteogenic differentiation. Representative digital camera images of stained calcium (red) deposited by hMSCs cultured on flat, nanopillar and nanoneedle substrates, and quantification of extracted stain using absorbance measurements (mean ± SD, N = 2). This shows no evidence of material-driven differentiation under basal conditions, while flat and nanopillar substrates were able to support osteogenesis. Nanoneedles, meanwhile, showed greatly reduced calcium staining, suggesting that this material substrate impairs differentiation down this lineage. Scale bars = 4 mm.
EXPERIMENTAL METHODS

Stiffness of nanopillars and nanoneedles. The theoretical stiffness and deformation profile of nanopillars and nanoneedles were solved using Euler-Bernoulli beam theory. The governing equation relates the applied moment \( M \), the elastic modulus \( E \) of the material, and the second moment of the area \( I \), to the deflection \( v \) of the beam:

\[
M = E \cdot I \cdot \frac{d^2v}{dx^2}
\] (1)

Each of the parameters \( M, E, \) and \( I \) can vary as a function of position \( (x, y, z) \) along the beam. Since the system was modeled for a concentrated load at the apex of the structure, a position-dependent moment is developed, \( i.e. M(x) \). The moment is evaluated as: \( M(x) = F \cdot x \), where \( F \) is the concentrated load and \( x \) is the distance from the applied load. The elastic modulus \( E \) of silicon was assumed to remain constant throughout the etching process; thus, \( E \) is taken to be 129.5 GPa, which is the provided value from the manufacturer. Finally, the second moment of the area, which is a geometric factor, changes along the axis of symmetry, \( i.e. I(x) \). For a solid conical structure, \( I(x) \) is a function of the tip diameter \( A \), base diameter \( B \), and length \( L \):

\[
I(x) = \left( \frac{\pi}{64} \right) \left( A + \left( \frac{B-A}{L} \right) x \right)^4
\] (2)

Implementing these conditions, the governing equation for deflection of a solid conical structure becomes:

\[
M(x) = E \cdot I(x) \cdot \frac{d^2v}{dx^2}
\] (3)

The following boundary conditions were applied:

\[
\frac{dv}{dx} = 0; \ x = L
\] (4)

\[
v = 0; \ x = L
\] (5)

Eq. 4 states that at \( x = L \) the slope of deflection is equal to 0. Furthermore, Eq. 5 states that the deflection at \( x = L \) is equal to 0, \( i.e. \) no deformation at the base of the structure. Following integration and implementation of the boundary conditions, the solution for the deflection of a solid conical beam is:

\[
v = \frac{32FL^2 (2AL^3-6AL^2x+6AB^2Lx^2(2AL-AX+Bx)^2-2Ax^3+3BL^2x-6BLx^2+3Bx^3)}{B^3E(AL-AX+Bx)^2\pi}
\] (6)

The solution was validated using 3 different methods. The first step was to set \( A = B \), \( i.e. \) a cylindrical beam, and to compare the beam deflection of Eq. 6 to the well-known solution\cite{1}:

\[
v = \frac{Fx^2}{6EI} (3L - x)
\] (7)

Figure S12 demonstrates perfect agreement between the solutions.
Figure S12. The solution for the deflection of a conical beam (Eq. 6 – solid lines) is compared to the known solution for the deflection of a cylinder (dashed lines). In the model L = 1 mm, F = 0.01 N, and E = 10 GPa. Additionally, A = B which was set to 0.1 mm (orange) or 0.2 mm (blue). The cylinder is shown in the background for reference but is not drawn to scale.

The second validation step was to compare this generalized solution for deflection at any point along the length of a conical beam to the solution of McCutcheon\textsuperscript{[2]}, which only gives the apical deflection of a solid cone.

\[ v_A = \frac{FL^3}{3EI_A} \left( \frac{A}{B} \right)^3 \]  

(8)

The subscript (A) denotes properties of the tip, i.e. tip deflection \( (v_A) \) and apical second moment of the area \( (I_A) \). Figure S13 again demonstrates perfect agreement with the known solution.

Figure S13. The analytical solution for the deflection of a conical beam (solid line) is compared to the point solution of McCutcheon (star markers). In the model L = 1 mm, B = 0.2 mm, F = 0.01 N, and E = 10 GPa. Additionally, A was set to 0.2 (blue), 0.1 (orange), and 0.05 mm (black).
The final validation step was to demonstrate that the solution is accurate for any point along the length of the beam and thus gives a deflection profile of the entire beam. To achieve this the beam was modeled, meshed, and solved using FEBio\textsuperscript{[3]}, an open-source finite element solver. Each beam was meshed with 100 elements in the longitudinal axis (x) and 64 radial elements. The material was modeled as an isotropic linear elastic solid with a Poisson’s ratio of 0.3, and physical dimensions: $L = 1$ mm, $A = 0.1$ mm, $B = 0.2$ mm.

A traction force ($F = 0.01$ N) was imposed at the apex of each cone with the base remaining fixed (zero displacement and rotation). Deformation profiles for each element along the longitudinal axis are plotted in Figure S14 and demonstrate good agreement. It should be noted that discrepancies do exist between the two solutions due to the modeling assumptions, e.g. Euler’s solution assumes infinitesimal strains and small rotations. Therefore Eq. 6 and any solutions based on Euler’s beam theory are only valid for small deflections. While it is not proven here, we assume that the hMSCs produce small traction forces and thus small beam deflections allowing us to use Eq. 6.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{beam_deflection.png}
\caption{Comparison of beam deflection between FEBio (red) and the analytical solution Eq. 6 (black). In the model $L = 1$ mm, $A = 0.1$ mm, $B = 0.2$ mm, $F = 0.01$ N, and $E = 10$ GPa. Cylinders with diameters equal to A or B set the bounding limits and are shown in grey.}
\end{figure}
High-content screening of cell images and modeling of cell features. The following describes both the image analysis, subsequent data handling, and then modeling work. This was carried out broadly in two sections:

1. Automated image analysis using a custom image-analysis pipeline in CellProfiler (CellProfiler 3.0.0, Broad Institute).[4]
2. Data analysis and modeling using custom scripts written in R (R 3.5.2, R Core Team: http://www.R-project.org/).

Image-based cell profiling has been conducted as stated below:

Pre-processing of images:
Each microscope image was pre-processed using ImageJ, to split each combined field-of-view into separate image files for each channel, using a macro written in ImageJ.[5]

Pipeline construction:
Immunofluorescent microscopy images were imported into CellProfiler. Initially, a small test set of images were manually pre-selected, representative of each of the experimental conditions to manually tune the pipeline parameters. In the following, parameters were tuned interactively using CellProfiler’s graphical user interface.

Summary of pipeline (bold text refers to binary objects generated by the pipeline):
- File import:
  - Metadata is assigned to image files, channels are mapped to their respective fields-of-view.
  - Pixel intensities are rescaled to the range 0 and 1 for calculation purposes (default approach for CellProfiler).
- Primary segmentation of nucleus
  - Cell nuclei were identified from the image of DAPI fluorescence. The thresholding method was a two-class adaptive Otsu threshold. An adaptive thresholding approach, using a window size of 50 pixels, was qualitatively observed to be more robust across a wide variety of substrate types.
  - Objects smaller than 4.1 µm and larger than 20.3 µm were considered to be a segmentation error and discarded.
- Secondary segmentation of cell body
  - The cell body for each cell was identified by using the nucleus as a kernel to perform a secondary segmentation on the tubulin α channel image.
A global minimum cross-entropy thresholding approach, combined with a propagation algorithm to help distinguish between clumped objects, was found to give the best results.

The threshold of this step was tuned so that the resulting binary object captured the majority of the cell body region, but not necessarily all protrusions.

Secondary segmentation of **cell body + cell protrusions**
- Using the **cell body** as a kernel, another secondary segmentation was performed on a maximum projection of the actin, tubulin α, and YAP channels. Prior to the projection, all channels were normalized to their maximum/minimum values to maximize the contribution of each to the projection. This projection was only used for this segmentation, not for any subsequent intensity measurements.
- Segmentation was performed using an adaptive Otsu algorithm, two-class, with a window-size of 10 pixels.
- This object incorporates both the cell body and total protrusion area.

Tertiary segmentation of **cytoplasm**
- The cytoplasmic region of each cell was defined by subtracting the **nucleus** from the **cell body**.

Tertiary segmentation of **cell protrusions**
- **Cell protrusions** were defined by subtracting the **cell body + cell protrusions** from the **cell body**.

Calculation of the **Voronoi cell area**
- A Voronoi tessellation was performed on each cell using the **nucleus** as the kernel, to produce a binary object that represents a measure of the free area around each nucleus.

Background intensity determination
- Per-image, per-channel background intensities were determined by applying a global, minimum cross-entropy threshold, to identify foreground regions of each image.
- Summary statistics for the background pixel intensities were calculated.
- In addition, the required threshold level was also recorded.

Filtering of edge objects
- Any cell where any part of the **nucleus, cell body, cytoplasm, cell body + cell protrusions, cell protrusions**, or **Voronoi cell area** touched the edge of the image frame were removed from the analysis, to avoid the risk of artifacts arising from artificial cell shapes.

Per-image intensity measurements
Summary statistics for intensity values in each field-of-view and each channel were recorded.

- Per-object intensity measurements
  - Summary statistics for intensity values of each of the binary objects defined above, for each channel, were recorded.

- Per-object morphology measurements
  - Morphological descriptors (geometric and shape parameters such as area, form factor, etc.) were measured for each of the binary objects defined above.

- Quality-control images
  - Composite images showing the outline of the final binary objects, on top of the original microscopy images were generated for quality-control purposes, and saved as compressed JPEG files to reduce the amount of disk space required.

- Data export
  - All of the data generated by the pipeline, including metadata, per-image and per-object based measurements was exported as a series of comma-separated values (csv) files.

Additional measurements made by the pipeline, but not included in analysis:

- Perinuclear area determination:
  - Commonly, the perinuclear area, as defined by a fixed-width ring surrounding the nucleus, is used to normalize for cell thickness variations.[6]
  - For highly rounded or elongated cells on nanoneedles, in particular where the cell nucleus is frequently collocated with the cell membrane in highly elongated cells, it was found particularly challenging to consistently define a peri-nuclear without introducing segmentation artifacts, hence this approach was not used here.

- Per-cell skeletonization
  - A morphological skeleton of each cell was generated by performing a binary skeletonization operation on the cell body + protrusion area, using a Poisson equation based erosion function.
  - Not included as the summary statistics measured failed to capture some of the nuances associated with cells on nanoneedles.

- Per-object neighbor measurements
  - The proportion and number of neighboring cells was calculated for each cell body and each cell body + cell protrusion.
It was found that the relatively low neighborhood contacts on nanoneedle substrates strongly skewed the distributions of these data towards zero, making appropriate transformation for statistical analysis via linear discriminant analysis impossible. Local cell density (derived from the Voronoi cell area) was found to be a better measure for the purposes of analysis.

- Per-object texture measurements
  - Haralick texture features for cell objects, for the actin, tubulin $\alpha$, YAP channels, were measured, using a scale factor of 3.
  - Initial tests identified that while many features, such as intensity texture parameters, provided good class separation during linear discriminant analysis, these features were also convoluted with the appearance of the nanoneedle array in the underlying image data. Hence it was unclear if the signal being measured was from the cell texture, from the pattern of nanoneedles, or most likely, a mixture of both.

Batch analysis:

Due the number of images involved, pipeline processing was sub-divided into smaller batches using a custom script written in Microsoft Powershell. Each batch was called as single command-line call to a new CellProfiler instance, which was automatically assigned asynchronously to individual logical processor cores of a server (Microsoft Windows Server 2012 R2, Intel Xeon CPU E5-2630 @ 2.6 GHz with 12 logical cores, 96 GB RAM). Analysis took approximately 19.5 hours, after which the individual data files were concatenated using another PowerShell script. This resulted in a single csv file for each binary object, plus additional folders and files containing image and pipeline metadata.

General note on data plots derived from measurements from image-based cell profiling:

Data shown in the manuscript was exported from R as csv files and plotted using OriginPro. Other plots were generated using the package ggplot2[7] and ggcorrplot (https://CRAN.R-project.org/package=ggcorrplot) in R. The majority of plots are categorical, showing the distribution of data points for different experimental conditions. In these plots, a symmetric kernel density estimate is used to illustrate the shape of each distribution. A box plot is overlaid on top of the density estimate, to provide information about summary statistics. The upper and lower limits of the box indicate the 25th and 75th percentiles, the line indicates the median.

Data analysis was conducted following the procedures below:

Data analysis pipeline:
A custom script was written in R, using an integrated development environment (R Studio 1.1.463, R Studio Inc. http://www.rstudio.com/) as an integrated development environment. The following R packages were used (list here does not include dependencies automatically loaded by their respective packages):

- MASS (7.3-51.1)[8]
- tidyverse (1.2.1): https://CRAN.R-project.org/package=tidyverse, a collection of packages comprising:
  - ggplot2 (3.1.0),[7]
  - dplyr (0.8.0.1): https://CRAN.R-project.org/package=dplyr
  - tidyr (0.8.2): https://CRAN.R-project.org/package=tidy
  - readr (1.3.1): https://CRAN.R-project.org/package=readr
  - purrr (0.3.0): https://CRAN.R-project.org/package=purrr
  - tibble (2.0.1): https://CRAN.R-project.org/package=tibble
  - stringr (1.4.0): https://CRAN.R-project.org/package=stringr
  - forcats (0.4.0): https://CRAN.R-project.org/package=forcats
- FSA (0.8.22): https://github.com/droglenc/FSA
- gtable (0.2.0): https://CRAN.R-project.org/package=gtable
- e1071 (1.7-0.1): https://CRAN.R-project.org/package=e1071
- caret (6.0-81),[9]
- ggcorrplot (0.1.2): https://CRAN.R-project.org/package=ggcorrplot
- here (0.1): https://CRAN.R-project.org/package=here
- scales (1.0.0): https://CRAN.R-project.org/package=scales

Data cleaning:
After loading the data into R, multiple checks and filters were applied to ensure data integrity and remove image analysis artifacts:

- Pipeline errors
  - CellProfiler metadata was checked to confirm no errors had occurred in any measurement modules, and that all images were scaled from the same 16-bit image depth (to ensure appropriate handling of intensity values). No errors were reported in the dataset presented here.
- Over-segmentation
  - Given the large number of measurements, automated methods for identifying image analysis artifacts are required. Here, over-segmented cells were identified by visually
inspecting the distribution of measured areas, raw data files, and corresponding quality control images to determine a reasonable threshold for outliers caused by segmentation errors.

- A threshold of 75,000 square pixels as the cutoff for cell body + cell protrusions area (~12,400 µm²). Note: this threshold was set to remove image analysis outliers only, not biological outliers.

- Under-segmentation
  - Similarly under-segmentation was identified by removing any cells where the cell body area was less than 1.05 times larger than the corresponding nucleus area.
  - Cells where the area of the cell body + cell protrusions is equal to zero were deemed segmentation errors (by definition, cell body + cell protrusions area ≥ cell body area), and removed.
  - Any cell where the median intensity of either the nucleus or cytoplasm, on any of the actin, tubulin α, or YAP channels, was less than double the channel background intensity were removed due to the poor signal-to-noise ratio.
  - Finally, all object datasets were cross-referenced to remove any cell that did not have a complete set of measurements across all features and objects, to ensure one-to-one mapping between datasets in the analysis.

- Image and cell numbers:
  - 5,372 microscopy images were analyzed by the image pipeline
  - 111,832 cells were identified by the image pipeline
  - 107,257 cells remained for analysis, after data cleaning.

**Feature aggregation:**

Several features were aggregated to produce new variables to aid with the analysis:

- YAP ratio
  - This is the ratio of YAP intensity in the nucleus and cytoplasm calculated on a per-cell basis.
  - It represents the relative location of YAP within the cell. It is expressed as a logarithm for ease of displaying and comparing ratios of less than one.
  - Median intensity values were chosen to mitigate the impact of outliers.

  \[
  \text{YAP ratio} = \log_{10} \left( \frac{\text{Nucleus median YAP intensity}}{\text{Cytoplasm median YAP intensity}} \right)
  \]

- Actin/tubulin α ratio
This is the ratio of actin intensity in the cytoplasm divided by the intensity of tubulin α, calculate on a per-cell basis.

It represents the relative ratio of actin and tubulin α, comparable to other cells in the analysis.

\[
\text{Actin/tubulin } \alpha \text{ ratio } = \log_{10} \left( \frac{\text{Cytoplasm median actin intensity}}{\text{Cytoplasm median tubulin } \alpha \text{ intensity}} \right)
\]

- **Local cell density**
  - This is the inverse of the Voronoi cell area.
  - It represents a measure of the free space around the nucleus, and is hence a measure of cell density.
  - The measurement is calibrated to units of cells per µm².

\[
\text{Local cell density } = \frac{1}{\text{Voronoi cell area}}
\]

- **Cell protrusion ratio**
  - This is the ratio of the cell body area to the cell body + cell protrusions area.
  - By definition, a value of 1 indicates no protrusions, and values < 1 indicate an increasing proportion of protrusions.

\[
\text{Cell protrusion ratio } = \frac{\text{Cell body area}}{\text{Cell body + cell protrusions area}}
\]

- **Corrected total cell fluorescence (CTCF)**
  - This is a method for correcting the integrated intensity (the sum of all pixel intensities within a given object).
  - It estimates the total sum of pixel intensities from the background signal, from a combination of the object area and an estimate of the mean background intensity (derived from measurements in the image analysis pipeline, see above for details).
  - For low cell densities, where the majority of any given field-of-view is background, the mean pixel intensity of this region is used.
  - For high cell densities, where the majority of the field-of-view is occupied by cells, the mean background intensity tends to zero. In these cases, the value of the global threshold used to split the foreground and background is used as an estimate of the background signal level.
  - Background correction is performed on a per-image basis.

\[
\text{CTCF } = \text{ Integrated intensity } - (\text{Background intensity estimate } \times \text{ Object area})
\]
**Feature selection:**
The measurement modules included in CellProfiler produce a very large number of data fields per cell (> 1,500 for the pipeline used here). Not all of these are useful, and some features are highly correlated, which can cause significant problems in the proper modeling of the data. Some features are discrete, or cannot be appropriately transformed for the modeling undertaken here (for example the Euler number of the binary objects). These features were excluded from the analysis. Hence, the number of features was reduced by selecting those which form a complementary set of data about each cell. Table S1 details the features selected.

**Data transformation and normalization:**
Many of the measured features, in particular the shape parameters, have highly skewed distributions. Such distributions violate the assumption of normally distributed data for many statistical techniques.

Measurements were transformed to become more normal-like by applying a generalized logarithm function, as described by Laufer et al.[10, 11]:

\[
\text{Generalized logarithm} = \log_{10} \left( \frac{x + \sqrt{x^2 + c^2}}{2} \right)
\]

where \(x\) is the data point to be transformed and \(c\) is a scaling factor. The scaling factor is the third percentile of the empirical distribution function of the measured variable. At \(c = 0\) this equation is the same as a normal logarithm, but for values of \(c > 0\) this function transforms the output for values of \(x < 0\), avoiding infinity errors in the subsequent logarithm.

In addition, a robust Z-score was used to normalize batch-to-batch variations in the three technical replicates included in the image-based cell profiling. Using the flat substrate of each replicate as the control, a robust Z-score for each feature was calculated as:

\[
\text{Robust Z-score} = \frac{(\text{Value of data point} - \text{Median of the same variable on a flat control})}{(\text{Median absolute deviation of the same variable on the flat control})}
\]

The robust Z-score uses the median and median absolute deviation, rather than the mean and standard deviation typically used in a Z-score, to minimize the effect of outliers.
Identification and removal of highly-collinear features:
Before the data can be modeled, highly correlated features should be removed. Manual feature selection (described above) helps to reduce the risk of multicollinearity between features during modeling. However, this alone may not identify all strongly correlated features. Failing to remove these features can result in highly unstable models, making their interpretation meaningless. Multiple techniques exist for tackling this problem, here we use an approach discussed by Caicedo et al.[10] The Spearman’s rank correlation coefficient was calculated for all pairs of features, an example is shown in Figure S13 for reference. High absolute values of the correlation coefficient indicate highly correlated features. Using a correlation coefficient threshold of 0.75, each pair of features with a correlation greater than this were compared. For a given feature in the pair, the mean absolute correlation of this feature with all other features was calculated. Whichever feature in the pair had the greatest mean absolute correlation was removed, and the process repeated until no features had an absolute threshold greater than 0.75. Figure S13 also shows an example of a reduced correlation matrix, calculated for a five-class model (discussed below).

Figure S13. Correlation matrix for features before (left) and after (right) automated removal of highly correlated features, showing intense colors (red or blue) where features are highly correlated. Note: the diagonal stripe represents each feature correlated with itself, hence is 1 by definition. These matrices are for the five-class model and are shown for illustrative purposes only, see Table S1 for the full list of input features, and Supplementary Table 2 for list of which features were kept in each model.

Linear discriminant analysis model construction and validation:
We are most interested in the ultimate behavior of cells on the substrate, so the modeling here considered only cells from the 72-hour timepoint. The following analysis uses the cleaned, transformed and normalized data, as described above.
Linear discriminant analysis (LDA) was used here, as we have a number of non-metric classes (different sharpness nanoneedles and a flat control) with a large number of metric variable measurements, plus the resulting model can be interpreted to infer information about the underlying features.\[12\]

LDA acts to maximize the separation between data points belonging to different classes (in this case different substrates). It does this by creating one or more discriminant functions, that can be used to score each cell. Each function is a linear combination of variables, where each variable here represents one of the features that was included in the model (e.g. cell area, nucleus solidity, etc.). Each variable is weighted by a coefficient, which represents the degree to which that variable contributes to the discriminant function. For a given cell, the discriminant function scores represent the class the cell either belongs too (during supervised training) or has been assigned to (during model validation).

To build each model, the relevant dataset was randomly split into two halves. Fifty percent of the cells were designated as a training dataset. The remainder were designated as the test (also known as holdout) dataset. An LDA based classifier was trained on the training dataset, using the MASS R package.\[9\] The trained classifier was then used to predict the class of each cell in the test dataset. This approach allows the specificity and sensitivity of the model to be directly assessed, by measuring how many cells were correctly and incorrectly classified, as we ultimately know which substrate they belonged to. As a further validation, each model was trained again on a dataset with randomized class labels, which resulted in a model with no significant separation of the test dataset. The stability of the model was assessed by running the script three times, using different seed values for the random number generator in R. The random number generator is used to sample data values to create the training and test datasets, so this is a check to make sure that our interpretation of the results is valid for more than just a single training/test dataset. The overall accuracy of the five-, three- and two-class models, remained within 2 % agreement for all three iterations, i.e. building the model from a sample of the data doesn’t overly affect the final model accuracy. Figure 2c in the manuscript illustrates how this translates into the model interpretation by showing the mean composite potency index (from the three iterations), plus the minimum and maximum values achieved by resampling.

Three models were constructed, including a differing number of classes:

- Five-class model - incorporating cells from five substrate types (sharp nanoneedles, 47 ± 7 nm; blunt nanoneedles, 172 ± 6 nm; blunt nanoneedles, 316 ± 20 nm; nanopillars, 718 ± 32 nm; flat substrate).
- Three-class model - incorporating cells from three substrate types (sharp nanoneedles, nanopillars, flat substrate).
- Two-class model - incorporating cells from two substrate types (sharp nanoneedles, flat substrate).

**Figure S14** illustrates the five-class model, and the separation achieved by the first discriminant function (there are four in total) on the test dataset. For clarity, the distribution of the scores of each cell is plotted, rather than individual data points. The plot illustrates how the first discriminant function does a reasonable job in particular separating the sharp nanoneedles, nanopillars and flat substrates.

In each case, the optimum number of discriminant functions for each model was one minus the number of classes, *i.e.* four, two and one respectively, as checked using the caret R package.[9]

**Figure S15** shows the confusion matrices for each of the three models. These matrices represent how many cells were correctly identified, or if misclassified, into which class they were incorrectly assigned. The three models together illustrate how it is relatively easy to identify cells on flat, nanopillar and sharp nanoneedles (the three extreme cases), classification becomes considerably harder for sharp, and blunt types of nanoneedles. This is consistent with flat, nanopillar and sharp nanoneedles being the most distinctly different substrates. Further details about the specificity and sensitivity (figures of merit derived from the confusion matrix) for each model and each class is shown **Tables S3 to S5**.
Figure S15. Confusion matrices (heatmaps) for the five-, three- and two-class models respectively. The number and color fill of each square indicates the number of cells that were classified to a given class.

Interpretation of the LDA model:
In order to simplify the interpretation of the discriminant functions, discriminant loadings were calculated. For each model, the scores assigned to each cell in the test dataset were correlated with the original measurements of each feature to produce the discriminant loading. These loadings represent the strength of the correlation between the discriminant function and the underlying data.

The five- and three-class models are described by more than one discriminant function. To further aid the interpretation of these models, each discriminant loading was weighted by the relative contribution of that discriminant function. For example, in the three-class model, the first discriminant function represents accounts for roughly 90% of the between-class variance, hence the discriminant loadings attributed to this function contribute more to the overall class separation.

Here we use a weighted approach reported by Hair et al. referred to as the potency value:\cite{12}

\[
\text{Potency value of variable } i \text{ on function } j = (\text{Discriminant loading}_{ij})^2 \times \frac{\text{Eigenvalue of discriminant function } j}{\text{Sum of all eigenvalues across all significant functions}}
\]

which can be combined to form a composite potency index for each variable:

\[
\text{Composite potency index of variable } i = \frac{\text{Sum of potency values of variable } i \text{ across all significant discriminant functions}}{}
\]

The composite potency index is a relative measure of the importance of a given variable to the overall discriminant functions.
**SUPPLEMENTARY TABLE**

**Table S1.** Description of main image-based cell profiling features included as initial inputs to the LDA model

| Binary object and variable category | Feature          | Description                                                                                                                                 |
|-------------------------------------|------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| **Cell body morphology**            | Cell area        | Cell body area                                                                                                                                |
|                                     | Cell compactness | The mean squared distance between pixels within the cell body and its geometric center, divided by the cell body area. A circle has a compactness of one, irregular shapes have values > 1. |
|                                     | Cell eccentricity| Eccentricity of the equivalent ellipse fitted to the cell body. A circle has a eccentricity of 0, a line segment has an eccentricity of 1. |
|                                     | Cell extent      | Considering a bounding box around the object (a rectangular box the contains the entire cell body), extent is the cell body area divided by the bound box area. A rectangular cell would have an extent of 1, irregularly shaped cells have values less than 1. |
|                                     | Cell form factor | Calculated as: $4\pi \times \frac{\text{Cell body area}}{\text{Cell body perimeter}^2}$ The form factor equals 1 for a circle, and less for irregularly shaped cells. |
|                                     | Cell mean radius | Calculated as the mean of all the distances between every pixel inside the cell body and the nearest pixel outside of the cell body.          |
|                                     | Cell perimeter   | Perimeter of the cell body.                                                                                                                   |
|                                     | Cell major axis length | An equivalent ellipse, is fitted to the cell body. The properties of this ellipse are used to determine a number of parameters, including major and minor axis length, and the orientation of the cell with respect to the image frame. |
|                                     | Cell minor axis length | These values provide information about the size, elongation, and relative orientation of cells. |
|                                     | Cell orientation | Note: measures of orientation are not included in modeling due to uncertainty in absolute orientation, but the description is included here for completeness. |
|                                     | Cell solidity     | The convex hull of the cell body is calculated. Here, the convex hull can be visualized by imaging a rubber band stretched around the cell, defining a region that fully encloses the cell body. The solidity is then the ratio: $\frac{\text{Cell body area}}{\text{Cell body convex hull}}$ A solidity of 1 represents a cell where the edge does not fold back in on itself. Irregularly shaped cells have solidities of less than 1. |
| **Nucleus morphology**              | Nuclear area     | As above, but for the nucleus.                                                                                                               |
|                                     | Nuclear compactness |                                                                                                                                            |
|                                     | Nuclear eccentricity |                                                                                                                                           |
|                                     | Nuclear extent   |                                                                                                                                             |

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### Table S2. Output/diagnostic parameters from the five-class and three-class LDA models.

| Model name | Accuracy | 95% confidence intervals | No information rate | P-value (accuracy > no information rate) | Kappa | McNemar’s Test p-value |
|------------|----------|--------------------------|---------------------|------------------------------------------|-------|------------------------|
| Five-class | 0.5527   | (0.5436, 0.5618)         | 0.2705              | < 2.2 x 10^-16                           | 0.431 | < 2.2 x 10^-16         |
| Three-class | 0.7729  | (0.7626, 0.7829)         | 0.4655              | < 2.2 x 10^-16                           | 0.6452 | 2.827 x 10^-10        |
| Two-class  | 0.945    | (0.9384, 0.9512)         | 0.6231              | < 2 x 10^-16                            | 0.8827 | 0.251                 |

### Model name Features included in the model after removal of highly correlated features
(see table above for details of each feature)

| Model name | Features included in the model after removal of highly correlated features |
|------------|---------------------------------------------------------------------------|
| Five-class | Cell eccentricity, cell major axis length, cell mean radius, cell minor axis length, cell solidity, local cell density, cell protrusion ratio, cell CTCF actin, cell CTCF tubulin α, cell mass displacement actin, cell mass displacement tubulin α, cell mass displacement YAP, cytoplasm ratio of actin to tubulin α, nuclear extent, nuclear form factor, nuclear minor axis length, nuclear solidity, nuclear/cytoplasm YAP ratio |
| Three-class | Cell eccentricity, cell major axis length, cell mean radius, cell minor axis length, cell solidity, cell protrusion ratio, cell CTCF actin, cell mass displacement actin, cell mass displacement tubulin α, cell mass displacement YAP, cytoplasm ratio of actin to tubulin α, nuclear extent, nuclear form factor, nuclear minor axis length, nuclear solidity, nuclear/cytoplasm YAP ratio |
| Two-class  | Cell eccentricity, cell extent, cell form factor, cell major axis length, cell mean radius, cell minor axis length, cell local cell density, cell protrusion ratio, cell CTCF actin, cell mass displacement actin, cell mass displacement tubulin α, cell mass displacement YAP, cytoplasm ratio of actin to tubulin α, nuclear extent, nuclear form factor, nuclear minor axis length, nuclear solidity, nuclear/cytoplasm YAP ratio |
Table S3. Sensitivity and specificity of five-class model

|                | i: Flat | ii: Nanopillars (718 ± 32 nm) | iii. Blunt nanoneedles (316 ± 20 nm) | iv. Blunt nanoneedles (172 ± 6 nm) | v. Sharp nanoneedles (47 ± 7 nm) |
|----------------|---------|-------------------------------|-------------------------------------|-----------------------------------|----------------------------------|
| Sensitivity    | 0.8361  | 0.44287                       | 0.41034                             | 0.5374                            | 0.5494                           |
| Specificity    | 0.9283  | 0.93212                       | 0.90633                             | 0.8920                            | 0.7664                           |
| Positive Predicative Value | 0.6951 | 0.52937                       | 0.49047                             | 0.6094                            | 0.4659                           |
| Negative Predicative Value | 0.9666 | 0.90657                       | 0.87492                             | 0.8602                            | 0.8210                           |
| Prevalence     | 0.1636  | 0.14706                       | 0.18015                             | 0.2387                            | 0.2705                           |
| Detection Rate | 0.1368  | 0.06513                       | 0.07392                             | 0.1283                            | 0.1486                           |
| Detection Prevalence | 0.1968 | 0.12303                       | 0.15072                             | 0.2104                            | 0.3190                           |
| Balanced Accuracy | 0.8822 | 0.68749                       | 0.65834                             | 0.7147                            | 0.6579                           |

Table S4. Sensitivity and specificity of three-class model

|                | i: Flat | ii: Nanopillars (718 ± 32 nm) | v: Sharp nanoneedles (47 ± 7 nm) |
|----------------|---------|-------------------------------|---------------------------------|
| Sensitivity    | 0.8398  | 0.5536                        | 0.8516                          |
| Specificity    | 0.8970  | 0.8821                        | 0.8784                          |
| Positive Predicative Value | 0.7616 | 0.6139                        | 0.8591                          |
| Negative Predicative Value | 0.9346 | 0.8536                        | 0.8718                          |
| Prevalence     | 0.2815  | 0.2530                        | 0.4655                          |
| Detection Rate | 0.2364  | 0.1401                        | 0.3964                          |
| Detection Prevalence | 0.3104 | 0.2282                        | 0.4614                          |
| Balanced Accuracy | 0.8684 | 0.7178                        | 0.8650                          |

Table S5. Sensitivity and specificity of two-class model

|                |          |
|----------------|----------|
| Sensitivity    | 0.9218   |
| Specificity    | 0.9591   |
| Positive Predicative Value | 0.9317 |          |
| Negative Predicative Value | 0.9530 |          |
| Prevalence     | 0.3769   |
| Detection Rate | 0.3474   |
| Detection Prevalence | 0.3728 |          |
| Balanced Accuracy | 0.9404 |          |
Table S6. Relative gene expression of various genes with different timepoint

A. Cytoskeleton-related & focal adhesion-related genes:

|       | ACTB | TUBA1A | DSTN | MACF |
|-------|------|--------|------|------|
|       | Avg  | STD    | Avg  | STD  |
| 6 h   |      |        |      |      |
| Flat  | 1.000 | 0.123  | 1.000 | 0.103 |
| Nanopillars (718 ± 32 nm) | 1.374 | 0.115  | 1.182 | 0.421 |
| Blunt nanoneedles (316 ± 20 nm) | 0.779 | 0.167  | 0.915 | 0.082 |
| Blunt nanoneedles (172 ± 6 nm) | 0.445 | 0.165  | 0.980 | 0.323 |
| Sharp nanoneedles (47 ± 7 nm) | 0.779 | 0.400  | 0.883 | 0.107 |

|       |      |        |      |      |
| 24 h  |      |        |      |      |
| Flat  | 1.084 | 0.118  | 2.294 | 0.474 |
| Nanopillars (718 ± 32 nm) | 0.442 | 0.106  | 1.076 | 0.133 |
| Blunt nanoneedles (316 ± 20 nm) | 0.359 | 0.172  | 1.033 | 0.284 |
| Blunt nanoneedles (172 ± 6 nm) | 0.211 | 0.030  | 0.968 | 0.097 |
| Sharp nanoneedles (47 ± 7 nm) | 0.108 | 0.031  | 0.529 | 0.147 |

B. Integrin clusters

|       | ITGAV | ITGA2 | ITGA5 |
|-------|-------|-------|-------|
|       | Avg   | STD   | Avg   | STD   | Avg   | STD   |
| 6 h   |       |       |       |       |       |       |
| Flat  | 1.000 | 0.300 | 1.000 | 0.296 | 1.000 | 0.210 |
| Nanopillars (718 ± 32 nm) | 0.804 | 0.193 | 1.889 | 0.788 | 1.202 | 0.369 |
| Blunt nanoneedles (316 ± 20 nm) | 0.684 | 0.174 | 3.553 | 2.361 | 1.054 | 0.553 |
| Blunt nanoneedles (172 ± 6 nm) | 0.495 | 0.157 | 0.512 | 0.088 | 0.444 | 0.053 |
| Sharp nanoneedles (47 ± 7 nm) | 0.777 | 0.087 | 1.098 | 0.313 | 1.057 | 0.230 |

| 24 h  |       |       |       |       |       |       |
| Flat  | 1.008 | 0.166 | 1.278 | 0.147 | 0.541 | 0.028 |
| Nanopillars (718 ± 32 nm) | 1.052 | 0.245 | 3.965 | 0.960 | 0.629 | 0.136 |
| Blunt nanoneedles (316 ± 20 nm) | 0.849 | 0.103 | 4.256 | 2.015 | 0.564 | 0.085 |
| Blunt nanoneedles (172 ± 6 nm) | 0.988 | 0.190 | 4.009 | 0.046 | 0.627 | 0.089 |
| Sharp nanoneedles (47 ± 7 nm) | 0.602 | 0.085 | 1.295 | 0.177 | 0.258 | 0.053 |
## C. YAP target genes & Lamin genes:

| ANKRD1 | CTGF | LMNA | LMNB |
|--------|------|------|------|
| Avg    | STD  | Avg  | STD  | Avg  | STD  | Avg  | STD  |
| Flat   | 1.000| 0.206| 1.000| 0.211| 1.000| 0.065| 1.000| 0.118|
| Nanopillars (718 ± 32 nm) | 1.531| 0.120| 0.916| 0.057| 1.197| 0.125| 1.290| 0.107|
| Blunt nanoneedles (316 ± 20 nm) | 0.860| 0.073| 0.710| 0.086| 1.794| 0.358| 1.456| 0.220|
| Blunt nanoneedles (172 ± 6 nm) | 0.747| 0.157| 0.783| 0.103| 1.227| 0.212| 1.716| 0.703|
| Sharp nanoneedles (47 ± 7 nm) | 1.226| 0.147| 1.335| 0.159| 1.675| 0.577| 1.251| 0.317|
| Flat   | 131.099| 14.063| 11.574| 4.717| 0.488| 0.357| 2.868| 2.239|
| Nanopillars (718 ± 32 nm) | 25.840| 3.000| 5.028| 1.083| 1.069| 0.141| 0.877| 0.236|
| Blunt nanoneedles (316 ± 20 nm) | 23.505| 7.051| 2.572| 0.299| 0.749| 0.287| 1.265| 0.527|
| Blunt nanoneedles (172 ± 6 nm) | 17.394| 2.112| 2.194| 0.130| 1.097| 0.279| 0.599| 0.115|
| Sharp nanoneedles (47 ± 7 nm) | 13.114| 5.497| 1.458| 0.237| 2.821| 0.082| 0.624| 0.019|

## D. Nuclear envelope genes:

| DNM2 | SYNE2 | SYNE3 | SUN2 |
|------|-------|-------|------|
| Avg  | STD   | Avg   | STD  | Avg  | STD  | Avg  | STD  |
| Flat | 1.000 | 0.153 | N.A. (did not expressed for all samples) | 1.000 | 0.062 | 1.000 | 0.217|
| Nanopillars (718 ± 32 nm) | 1.085 | 0.082 | 1.274 | 0.167 | 0.744 | 0.378|
| Blunt nanoneedles (316 ± 20 nm) | 0.552 | 0.049 | 0.929 | 0.099 | 0.734 | 0.220|
| Blunt nanoneedles (172 ± 6 nm) | 0.470 | 0.083 | 0.762 | 0.086 | 0.894 | 0.147|
| Sharp nanoneedles (47 ± 7 nm) | 0.533 | 0.086 | 0.861 | 0.094 | 0.608 | 0.123|
| Flat | 1.553 | 0.228 | 1.918 | 0.307 | 0.372 | 0.120|
| Nanopillars (718 ± 32 nm) | 0.126 | 0.025 | 1.042 | 0.167 | 2.437 | 1.133|
| Blunt nanoneedles (316 ± 20 nm) | 0.536 | 0.307 | 1.270 | 0.145 | 1.177 | 0.007|
| Blunt nanoneedles (172 ± 6 nm) | 0.700 | 0.059 | 3.100 | 0.139 | 2.113 | 0.280|
| Sharp nanoneedles (47 ± 7 nm) | 0.276 | 0.092 | 1.387 | 0.251 | 1.032 | 0.000|
E. RhoGTPase related genes:

|       | RHOA Avg | ROCK Avg | RAC1 Avg | CDC42 Avg |
|-------|----------|----------|----------|-----------|
| Flat  | 1.000    | 1.000    | 1.000    | 1.000     |
| 6 h   | 1.435    | 0.907    | 0.973    | 1.765     |
| Nanopillars (718 ± 32 nm) | 0.170 | 0.150    | 0.096    | 0.296     |
| Blunt nanoneedles (316 ± 20 nm) | 0.145    | 0.128    | 1.010    | 3.167     |
| Sharp nanoneedles (47 ± 7 nm) | 0.108    | 0.769    | 0.146    | 1.164     |
| 24 h  | 1.449    | 0.394    | 1.296    | 1.572     |
| Flat  | 1.000    | 1.000    | 1.000    | 1.000     |
| Nanopillars (718 ± 32 nm) | 0.400    | 1.000    | 0.153    | 3.073     |
| Blunt nanoneedles (316 ± 20 nm) | 0.291    | 1.882    | 0.147    | 0.170     |
| Sharp nanoneedles (47 ± 7 nm) | 0.082    | 1.147    | 0.088    | 2.014     |
| 6 h   | 0.941    | 0.951    | 1.088    | 1.872     |
| Flat  | 1.449    | 0.394    | 1.296    | 1.572     |
| Nanopillars (718 ± 32 nm) | 0.400    | 1.000    | 0.062    | 1.164     |
| Blunt nanoneedles (316 ± 20 nm) | 0.181    | 1.702    | 0.141    | 0.170     |
| Sharp nanoneedles (47 ± 7 nm) | 0.082    | 1.009    | 0.164    | 2.014     |
| 24 h  | 0.466    | 1.169    | 0.136    | 1.140     |
| Flat  | 1.449    | 0.394    | 1.296    | 1.572     |
| Nanopillars (718 ± 32 nm) | 0.400    | 1.000    | 0.217    | 0.378     |
| Blunt nanoneedles (316 ± 20 nm) | 0.181    | 1.702    | 0.141    | 0.170     |
| Sharp nanoneedles (47 ± 7 nm) | 0.082    | 1.009    | 0.164    | 2.014     |

F. Proliferation and endocytosis related genes:

|       | MKI67 Avg | PCNA Avg | CAV1 Avg |
|-------|-----------|----------|----------|
| Flat  | 1.000     | 1.000    | 1.000    |
| 6 h   | 1.085     | 1.274    | 0.744    |
| Nanopillars (718 ± 32 nm) | 0.082    | 0.167    | 0.378    |
| Blunt nanoneedles (316 ± 20 nm) | 0.400    | 0.099    | 0.220    |
| Sharp nanoneedles (47 ± 7 nm) | 0.174    | 0.086    | 0.123    |
| 24 h  | 1.553     | 1.918    | 0.307    |
| Flat  | 1.000     | 1.000    | 1.000    |
| Nanopillars (718 ± 32 nm) | 0.082    | 0.167    | 0.217    |
| Blunt nanoneedles (316 ± 20 nm) | 0.400    | 0.099    | 0.378    |
| Sharp nanoneedles (47 ± 7 nm) | 0.174    | 0.086    | 0.123    |
| 6 h   | 0.552     | 0.929    | 0.734    |
| Blunt nanoneedles (316 ± 20 nm) | 0.049    | 0.099    | 0.220    |
| Sharp nanoneedles (47 ± 7 nm) | 0.762    | 0.086    | 0.147    |
| 24 h  | 0.533     | 0.861    | 0.608    |
| Flat  | 1.553     | 1.918    | 0.307    |
| Nanopillars (718 ± 32 nm) | 0.082    | 0.167    | 0.378    |
| Blunt nanoneedles (316 ± 20 nm) | 0.400    | 0.099    | 0.220    |
| Sharp nanoneedles (47 ± 7 nm) | 0.174    | 0.086    | 0.123    |

Table S7. Secondary antibodies

| Target  | Host   | Supplier          | Product # | Dilution |
|---------|--------|-------------------|-----------|----------|
| YAP     | Mouse  | Santa Cruz Biotech | sc-101199 | 1:500    |
| Vinculin| Mouse  | Sigma Aldrich     | V9131     | 1:200    |
| pPAX    | Rabbit | Abcam             | ab4833    | 1:1000   |
| α Tubulin| Rabbit | Abcam             | ab52866   | 1:1000   |
| Ki-67   | Rabbit | Abcam             | ab15580   | 1:700    |
| Integrin β1 | Mouse | Abcam             | ab24693   | 1:500    |
| Gene name | Accession number | Forward / Reverse primer 5'-3' | Amplicon (bp) |
|-----------|------------------|--------------------------------|--------------|
| HPRT1     | NM_000194.2      | TGACACTGGCAAAACAAATGCA GTTCTCTTTTCAACCGACAGCT | 94           |
| PPIA      | NM_021130        | CTTCACACGGCCATAATGGC GTTACATCTTCTGCTGTCTTGG | 273          |
| RPL13a    | NM_012423        | AAGTACCAAGGCTGACAG CCGTTCATCCGATGCTCATTAG | 100          |
| ACTB      | NM_001101        | ATCATGTGGAGACCTCTCAACATCTGGTAGATGCTCGAAT | 318          |
| ANKRD1    | NM_014391.2      | AGTAGAGGAACTGGTCACTTGGGGCTAGAAGTGTCTTCAT | 138          |
| CAV1      | NM_001753.4      | GCAGAACAACACCTTTGGCGGGTGTTAGAGATGTCCCTCCGA | 209          |
| CDC42     | NM_001791.3      | GGTGGAGAAGCTGAGGTCAT CATCGTCCTAGCCTGTCCTAATCG | 99           |
| CLTA2     | NM_007096.3      | AAAAGCAAACACAGGGGTGCGGCTGTCTAGCCTGTCCTAATC | 107          |
| CTGF      | NM_001901.2      | AGGAGTGGGTGTGTGACAG CCAGGGTGTGACAGCTTGCTGCC | 117          |
| DNM2      | NM_001005360.2   | CCAATCACGGGGAGATGCTAACGCGTTCCTCCGATCAGACAC | 200          |
| DSTN      | NM_006870.3      | CGAAGATGGCCCTAGGAGATG TCTGTTGTCAGCATTATCAG | 85           |
| ITGA2     | NM_002203.3      | TTAGCGCTACAGTCAAGGCAT CCGTTCCTAGGGAGACATCACT | 179          |
| ITGA5     | NM_002205.2      | AGACTTTTCTGCTGCTGCC CGTGTCCTGCGGTGGTAGACAT | 174          |
| ITGAV     (CD51) | NM_002210.4  | TCCGATTCTCAAAGCTGGGAGCG AAGGGCCACTGAAAGATGGGAC | 137          |
| ITGB1     | NM_002211.3      | GCCGCGAAAAGATGAATATTTACAAC ATCTGGAGGGCAACCCCTTCT | 245          |
| ITGB3     | NM_000212.2      | ACCAGTAAACCTGGGATGCG TCCGACACACTCTCCTGCTT | 208          |
| LMNA      | NM_170707.3      | CTCGACTGCCCAGGGAAATG TCTGGACCTGAGCATTGACGAG | 275          |
| LMNB      | NM_005573.3      | GACTGCCAACCACCCGTG TAGCATAGGTGAGGAGACG | 362          |
| MACF1     | XM_024446116     | AGACAAGTGTCAGTGCAA GTTCTACCTCTGTCTGCCG | 218          |
| MAP4      | XM_017006409.2   | GCCAATTGTTGTGACATGGAGT GTTGCAGGGAGACATACCGTAA | 294          |
| MAP6      | XM_006718556.4   | GCTTTTTCAGCTAGCATCAGGG CGTTTTAGCTCAGCTGTTTC | 83           |
| MKI67     | NM_002417.5      | TGACTTCTTCTCATTCTGAAGAC TCAGGTGTATTGTAGAGGCC | 109          |
| NANO      | NM_024865.2      | AATACCCTCGCCCTGACCAGATGC TGCGTCACACATGGTTGGC | 148          |
| PCNA      | NM_182649.2      | AAGAGAGTGAGTGGCTTGGT TGTGATAAAGAGGAGGAAGG | 292          |
| PTK2      | NM_153831.3      | CAGTGCTCTTCTGGAGCTTTTG GTCGATAAAGAGGAGGAAGG | 107          |
| PXN       | XM_024449110.1   | TGAGACGCCCTACCTGTGGAG AAGAAGTTTCCAGGTGGTGC | 125          |
| RAC1      | NM_006908.4      | AAACCGGTGAATCTGGGCTT AGGAAACACATCTGGTTGCGGA | 91           |
| RHOA      | NM_001664.3      | TGAGACGCCCTCAGATGTTAGAAGAAA TCTGAGTTTCCACCGGTCTC | 190          |
| Gene   | Accession    | Sequence                          | Length |
|--------|--------------|-----------------------------------|--------|
| ROCK   | NM_005406.2  | GGTGCTGGTAAGAGGGCATT AGCATCCAATCCATCCAGCAA | 145    |
| SUN2   | NM_001199579.1 | GCAGCAGATTCTCTTCAGGG AGGTGTGTGCATCAGAGGAC | 246    |
| SYNE2  | NM_182914.2  | CTCCCCGAGCAAAGTTCAGA AGCATGGGGTAAAAGGACCG | 238    |
| SYNE3  | NM_152592.3  | TCGTTGTACCCACCAGCAAAAG GTAGAAGATTTGCCTTCG | 399    |
| TUBA1A | NM_006009.3  | GAAGCAGCAACCATGCGTGA TGCCAGTGCAGACTTCATCA | 265    |
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