**Auxetic nuclei in embryonic stem cells exiting pluripotency**

1Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge, CB3 0HE, UK

2Department of Physiology, Development and Neuroscience, Downing Street, University of Cambridge, Cambridge, CB2 3DY

3Wellcome Trust/Medical Research Council Centre Cambridge Stem Cell Research Institute, Tennis Court Road, University of Cambridge, Cambridge, CB2 1QR, UK.

4Wellcome Trust/Medical Research Council Cambridge Stem Cell Research Institute and Madingley Road, University of Cambridge, Cambridge, CB3 0ES, UK

5Department of Engineering, University of Cambridge. Cambridge, CB2 1PZ

†Authors contributed equally to this work.

*Correspondence to. E-mail: kc370@cam.ac.uk
Supplemental Methods

**Cytochalasin D and Trichostatin A treatment.** ESCs were treated with 1 µM of Cytochalasin D for 10 minutes prior to measurement, and 300 nM of Trichostatin A for three hours prior to measurement. The treatment protocols were based on previous work.

**Atomic force microscopy.** Monodisperse polystyrene beads (diameter 37.28 +/- 0.34 µm, microParticles GmbH, Berlin, Germany) were glued to tipless silicon cantilevers (Arrow-TL1, spring constant ~0.02 N/m; NanoWorld, Neuchatel, Switzerland). Cantilevers were mounted on a JPK CellHesion 200 AFM (JPK Instruments AG, Berlin, Germany), which was set up on an inverted optical microscope (Zeiss Axio Observer A1, Zeiss, Germany equipped with a Ph2 Plan-Neofluar objective with 40× magnification and N.A.=0.75). Cantilever spring constants were determined via the thermal noise method included in the AFM software.

Cells were kept at 37 °C for the duration of the experiments. The cantilever was positioned above individual cells using phase contrast. Force distance curves were taken with a maximum indentation force of 30 nN at a tip speed of 20 µm/s and analyzed using the Hertz model at different indentation depths:

\[
F = \frac{4}{3(1 - \nu^2)} \sqrt{E} \delta^{3/2},
\]

where \(F\) is the force, \(E\) the Young’s modulus, \(\nu\) the Poisson's ratio, \(R\) the radius of the probe, and \(\delta\) the indentation. The apparent reduced modulus \(K = \frac{E}{1 - \nu^2}\) was used to characterize the mechanical properties of a sample. For the study of nuclear deformation under compressive load, the illumination was changed to epifluorescence, and an image of the cells before they made contact with the cantilever recorded. The cantilever was then approached to the surface of the cell with
a defined setpoint of $F = 0.1$ nN and moved further down by 2 $\mu$m, ensuring the same axial deformation in all nuclei. At this position, a second picture was taken, and after retraction, a third fluorescence image of relaxed cells was recorded.

**Statistical analysis.** 1-way ANOVA was used for all statistical analysis, including reproducibility analysis, on Matlab (Natick, MA, USA) and OriginPro (OriginLab, Northampton, MA, USA). Prior to statistical analysis, outliers were removed. Reproducibility was verified before pooling data. At all times in the text, the number of experiments is given by $M$ and the total cell numbers is given by $m$. N-way ANOVA was used in Matlab for the passage time analysis, with two groups – time and cell type. *** indicates $P < 1E-5$ at all points in text and figure captions, and ** indicates $P < 0.01$.

**Gene expression analysis.** Relative gene expression analysis was performed by two-step real time reverse transcription PCR$^{5,6}$. Samples of Rex1-GFPd2 mouse ESCs$^7$ cultured on 12 well plates, were harvested in triplicate, by direct lysis using Buffer RLT (Qiagen), homogenized using QIAshredder columns (Qiagen), immediately followed by RNA extraction using the RNeasy Mini Kit (Qiagen) with on-column DNAse I digestion with the RNase-Free DNase Set (Qiagen). RNA was quantitated using a nanodrop spectrophotometer, and 2 $\mu$g of RNA per sample was reverse transcribed to cDNA in a 20$\mu$L reaction, using 200U SuperScript III reverse transcriptase, with 150ng random primers and 40U RNaseOUT, according to the manufacturer’s instructions (Invitrogen). Negative control reactions without reverse transcriptase were processed concurrently, for the first replicates of each timepoint. cDNA and negative control reactions were diluted ten-fold with RNase-DNase-free water (Gibco), and amplification of 1 $\mu$L diluted cDNA per reaction was performed by real-time PCR using Fast SYBR Green Master Mix (Applied Biosystems) in a 5$\mu$L
reaction volume with 1µM of each gene-specific primer pair, on a StepOnePlus Real-Time PCR System thermal cycler (Applied Biosystems) running StepOne Software v2.0 with the following program: 95°C for 20s, then 40 cycles of 95°C for 3s and 60°C for 30s, followed by melting curve analysis. Default settings were used to determine Ct values for each sample and control PCR. Primer sequences are listed in Table S1. PCRs were performed as technical duplicates, and the average Ct for each sample (m=3) was normalized to the average Ct of the L19 and Ywhaz endogenous control genes at each timepoint, to determine relative gene expression using the comparative Ct method. Negative control reactions (without reverse transcriptase) were analysed and compared to experimental sample reactions, to determine background gene expression levels for each primer pair.

**Nuclear Envelope Structure Analysis.** The cells used for the structure analysis were mESC H2B-eGFP between passage 15 and 20 and were maintained in T25 gelatinized flasks in 2i/LIF media. H2B-eGFP was used to demarcate the nuclear boundary. Cells were cultured into a plastic bottomed 35mm Ø ibidi dish (Thistle Scientific IB-81156). Cells were seeded directly into N2B27 for the 24hr T state and the 48hr P state (Fig. S16). Cells were imaged through a high magnification objective (63×, 1.4 N.A.) and an Andor Revolution XD spinning disk confocal with a Yokogawa CSU-X1 head, in an environmental chamber providing 5% CO₂ and a temperature of 37°C for in-vivo experiments. Cells were imaged every 20 seconds for 10 minutes using the 488nm line at 15% power.

Images were imported into MATLAB as single cell videos and converted to binary – thresholding for the nuclear boundary. The nuclear boundary coordinates were extracted in polar form, \( R(\theta) \), from the centre of mass of the nucleus. \( R(\theta) \) was resampled for equal spacing of \( \theta \) and the DC component (i.e. 0th order of the Fourier
transform) removed by subtracting $\bar{R}$ (i.e. the average radial position of the boundary from the centre of mass of the nucleus) before taking the fast (discrete) Fourier transform (FFT). The wavelength, $\lambda$, of the Fourier components were calculated as follows:

$$\lambda_n = A \frac{2\pi \bar{R}}{n} \text{ for } 1 \leq n \leq N$$

where $n$ is the $n^{th}$ Fourier component, $A$ is a scaling factor determined by the microscope and $N$ the number of Fourier components.

The Fourier components for individual cells were averaged over all frames and then averaged again across all cells (~25 in each state) into three bins as a function of $\lambda$. $\lambda > 8 \mu m$ describes nuclear shape, specifically deviations from a circle. $3 < \lambda < 8 \mu m$ compares the amount of wrinkles in the nuclear boundary.

**Hoechst and fluorescein assays.** Cells were incubated in 10ug/mL Hoechst 33342, which labels nucleic acids, for 30min before trypsinization. Cell suspension was collected and resuspended in PBS for the experiments. Images were collected on a Leica SP5 Confocal using a 20×, 0.5 N.A. objective and blue epifluorescence.

For the fluorescein assay E14 wildtype mESCs were passaged and plated at approximately seventy percent confluence into naive, transition and primed conditions. Cells were resuspended in media at $1 \times 10^6$ cells/mL and incubated at 37°C with 100uM fluorescein sodium salt for 1hr. Cell suspension was collected and resuspended in PBS for the experiments. Images were collected on a Leica SP5 Confocal using a 40×, 1.25 N.A. oil objective and green epifluorescence with a 4×4 pixels binning.
**Electron microscopy.** The cell samples were fixed in a 1:1 mix of media and 4% glutaraldehyde solution for 2 h at RT, scraped off and washed with PBS. The cell pellets were post-fixed in osmium tetroxide (2% OsO4 in 0.1M phosphate buffer) overnight at 4 °C. The samples were then dehydrated with ethanol (50%, 70%, 90%, 3× 100% ethanol, 2× 100% propylene oxide, all 20 min), infiltrated with resin (TAAB embedding resin) (1:3, 1:1 & 3:1 resin:propylene oxide), all 2 hours, then 2×100% resin 2 & 8 hours respectively and polymerised in fresh resin at 60 °C for 48 hours. The blocks were sectioned on a Leica Ultracut E set to 80nm (silver/gold sections) and picked up on copper grids. The sections were stained with saturated ethanolic uranyl acetate (30 min) and 1% lead citrate (20 min) and imaged on a Hitachi H-600 electron microscope at 5,000× magnification. Developed films were scanned at 1200dpi. For a quantitative analysis of global chromatin condensation electron microscope images of single N- and T-ESCs and P-cells were 2-D Fourier transformed and the wavelengths of the Fourier components were divided in low frequencies (corresponding to wavelengths, \( \lambda > 100 \text{nm} \)) and high frequencies (corresponding to wavelengths, \( 10 < \lambda < 100 \text{nm} \)). The ratio \( \nu_L / \nu_H \) is a measure of uniformity, the closer to 1 this ratio is, the more evenly spaced the spatial frequencies, the higher the ratio, the more the distribution is clustered.

**Simultaneous measurement of cell and nuclear deformation**

The Syto13 labelling protocol allows simultaneous observation of both the cytoplasm and the nucleus, but we found the intensity of the signal to be between 3-4 times higher in the nucleus than the cytoplasm. In order to distinguish between the cell and nucleus in imaging, we developed a thresholding protocol in Fiji (NIH, Bethesda, MD, USA) that reliably discerned the difference between the two. For the cytoplasm, we use Li’s minimum cross entropy algorithm\(^8\), and for the nucleus we used the
minimum thresholding method⁹. This algorithm was refined through analysis of high resolution images of ESCs stained with Syto13 visualised in epi-fluorescence using 63x, 1.4 N.A. oil immersion microscopy on an inverted Leica SP5. We iteratively applied Li’s algorithm and the minimum thresholding algorithm and applied the edge found by each to both a phase-contrast image and the same cell co-stained with Hoechst 33342 (Invitrogen) (Fig. S4). The thresholding technique’s accuracy was confirmed by using edge detection for the phase contrast and Hoechst images and integrating the area of both these and the edge found by our thresholding algorithm. The difference was never more than 5%. We also applied this iterative image analysis approach to transition cells, which further validated our approach.

**Theoretical characteristics of linear elastic solids**

According to the theory for linear isotropic elastic solids, the nuclear axial stretch due to cytoskeletal deformation in the absence of lateral constraints can be modeled as a uniaxial tension; for a material defined by its Young's modulus $E$ and Poisson ratio $\nu$, an axial stretch deformation of amplitude $S_A$ leads to transverse deformation of amplitude $S_T = -\nu S_A$. The effect of the confining walls on large nuclei is closer to a biaxial compression in the transverse direction. If lateral confinement of the nucleus causes a transverse deformation of amplitude $S_T$, the resulting deformation in the axial direction (ignoring the cytoskeletal contribution) is determined by $S_A = -\frac{2\nu}{1-\nu} S_T$. The slope of the lines on Fig. 3b are calculated for $\nu = 0.4$. 


Fig. S1. Differentiation stages. (a) Rex1/GFPd2 profiles of ESCs at different stages during differentiation, in 2i+LIF medium (black) and after 24 (red), 28 (magenta), 36 (cyan) and 48 hours (blue) in N2B27 medium. The T-ESCs are defined at a time point shortly before Rex1 expression starts changing while ESCs cultured in N2B27 between 24 and 48 hours are a heterogeneous mix of high Rex1 and low Rex1 expressing cells. (b) Cell morphology varies according to culture conditions with (left) a tight colony-forming structure in 2i medium, (middle) a loose colony formation after 24h in N2B27, and (right) cells that start to individuate and form processes after 48h in N2B27. Scale bar: 50 µm.
Fig. S2. Analysis of gene expression in ES cells by real-time quantitative reverse transcription PCR. Expression levels of nuclear lamin genes \textit{Lmna} and \textit{Lmnb1} were compared to known markers of pluripotency \textit{Oct4/Pou5f1}, \textit{Nanog}, and \textit{Rex1/Zfp42}, and the primed state marker \textit{Tcf15} in \textit{Rex1GFPd2} ESCs. Lamin gene expression in ESCs cultured in a naïve pluripotent state in N2B27 media under 2i+LIF conditions was compared to that of cells induced to differentiate by removal of 2i+LIF, harvested 24 or 48 hours later. Error bars indicate standard deviation. Primer pairs used for each gene are labeled on the horizontal axis. Values shown are the mean of three replicates, except for the \textit{LmnA-MEM} and \textit{Rex1} experimental samples, which are the mean from duplicate samples. Relative expression was compared pair-wise between values for each of the three timepoints per primer pair using a two-tailed T-test; significant differences are noted above the bars (*, \(p<0.05\); **, \(p<0.01\); ***, \(p<0.001\)). The –RT negative control values represent the mean from one replicate combined from each of the three timepoints. Where the relative gene expression value for any given experimental sample was not significantly higher than background level (given by the –RT negative control), this is denoted by NS; all other samples are considered to show positive gene expression; \(p<0.05\) (one-tailed T-test). Although T-ESCs have not downregulated \textit{Rex1}, they have downregulated the pluripotency factor \textit{Nanog}, and can be returned to N-ESCs in 2i+LIF medium for an indefinitely self-renewing ESC culture (personal communication with Austin Smith). No consistent changes are observed in the expression of \textit{Lamin A} or \textit{Lamin B1} across the cell groups.
Fig. S3. **Cellular and nuclear cross sectional area.** High resolution analysis of the cellular (a) and nuclear (b) area for naïve, transition and primed cells stained with Syto13 and imaged through a 40×, 0.75 N.A. objective. The bottom and top of the box are the first and third quartiles, the band inside the box is the median. The square is the mean, the star and the asterisk are the minimum and maximum, respectively, the downward and upward triangles represents 1% and 99%, respectively. Cell and nuclear sizes of N- and T-ESCs are very similar while P-cells present slightly larger sizes though the differences are not statistically significant.

Fig. S4. **Simultaneous measurement of cell and nuclear cross-section.** Both the cytoplasm and nucleus can be measured using Syto13. The thresholding algorithm (explained in the text) finds the edges of the cytoplasm and nucleus with high fidelity. (a) ESCs stained with Syto13 under 60× magnification using oil immersion microscopy in epi-fluorescence mode. Scale bar: 10 μm. (b) The threshold for the cell and nucleus found from the images of 4 cells stained with Syto13 (first column) matches with the phase contrast (second column) and the Hoechst image (third column), respectively. Scale bar: 10 μm. (c) The thresholding algorithm is applied to the Syto13 stained cell as imaged by the 40× magnification objective used in the microfluidic assay. Scale bar: 20 μm.
Fig. S5. AFM measurement of normal and auxetic responses. Box plot of the change in nuclear cross-sectional area upon compression by 2 µm with the AFM probe on naïve, naïve treated with Trichostatin A (TSA), naïve treated with Cytochalasin D (CytoD), transition, transition treated with CytoD ESCs and primed and lineage restricted extraembryonic (XEN) cells. The cytoplasm data include measurements on all four classes of cells, namely naïve, transition, primed and lineage restricted cells. The dashed line denotes a cross-sectional area change of zero. Materials below this line are auxetic. M=8, 3, 2, 2, 3, 2 and m=52, 98, 98, 33, 117, 30, 53, 62. The cytoplasm of all investigated cell types, as well as the nuclei of N-ESCs, P- and lineage restricted extraembryonic (XEN) cells, significantly increased in cross-sectional area by ~5-10% upon compression (squares above the dashed line). Remarkably, however, the nuclei of T-, CytoD treated T- and TSA treated N-ESCs became smaller in cross-sectional area upon compression (squares below the dashed line), which suggested that these nuclei have a negative Poisson’s ratio, i.e., they are auxetic.
**Fig. S6. Control and confinement of a single ESC.** (a) A cell is pushed in the channel from the left by applying a constant pressure (arrow). (b) Once in the channel, a counter-pressure from the right is applied to equilibrate the pressure from the left and blocking the cell in the channel. The cell is kept confined for more than one minute. (c) The counter-pressure is decreased to move the cell forward. (d) The counter-pressure is increased to push the cell back in the channel and (e) in the reservoir. Scale bar: 20 µm.

**Fig. S7. Cells can be cultured and analysed subsequent to microfluidic interrogation.** ESCs were removed from the culture plate, and half were interrogated in the microfluidic assay (Microfluidics group) while half were stored in PBS for the same amount of time (Control group). Another group of cells (ES culture group) was
cultured and passaged in 2i/LIF as normal. The three groups were (re)-cultured for three days, then passaged and analysed in flow cytometry and imaged two days after passage. (a) Comparison of the Rex1-GFP signal for the three groups. (b) A 20× phase contrast image of the Microfluidics group indicating their ability to form stem cell colonies after microfluidic interrogation (cf. Fig. 2a). Scale bar: 25 µm. (c) Phase contrast and Rex1-GFP image of a single colony in the control groups. Scale bar: 10 µm. (d) Phase contrast and Rex1-GFP image of two different colonies in the Microfluidics group. Scale bar: 10 µm. (c) and (d) were imaged with 60× oil immersion in laser scanning confocal mode to maximize the rather weak GFP signal.

Fig. S8. Channel translocation time. Time spent by the cells to translocate the channel from the inlet to the outlet reservoir. Each data point and error bar is the mean and standard error of the mean over three measurements of 30 cells each. Counts are normalized by 30 the number of cells measured in each experiment on each class of cells. Dashed lines are guides-for-the-eye. T- and CytoD treated T-ESCs translocated the channels faster than N- and P-ESCs indicating a higher overall deformability in T-ESCs when stretched. Statistics was done with n-way ANOVA, the groups were time and cell group. Time was a significant interaction effect in all cases, P-values in figure indicate differences between cell group.
Fig. S9. Cellular and nuclear volume change in the channel. (a) Relative cellular and (b) nuclear volume change for cells in the channel with respect to the corresponding measurement when the cell is in the inlet. The volumes - both in the inlet and in the channel - have been evaluated by measuring major and minor axes for both the cell and nucleus (see Fig. 2e) from the fluorescent image of the cell after using the two thresholding algorithms described in Fig. S4 and assuming that cells are ellipsoids with two equal minor axis in the two directions perpendicular to the flow in the chip. The bottom and top of the box are the first and third quartiles, the band inside the box is the median. The square is the mean, the star and the asterisk are the minimum and maximum, respectively, the downward and upward triangles represents 1% and 99%, respectively. The dashed line denotes no volume change. There are not significant differences in overall cell deformation in the channels across cell types, though there is an overall loss of volume in all cells as expected because of increased pressure in the channel due to decreased cell curvature. Nuclear deformation in the channel, however, strongly depends on the ESC stage, T-ESCs exhibiting a positive nuclear volume change, thus their nuclei expand when they are stretched in the microchannel.
Fig. S10. Measured strains for different ESC nuclei in the channel. Transverse (top) and axial (bottom) nuclear strain for small and large nuclei (open circles and filled squares, respectively) of naïve, naïve treated with Trichostatin A (TSA), transition, transition treated with Cytochalasin D (CytoD), primed and lineage restricted cells. Each data point and corresponding error bar is the mean and standard deviation of the measurements performed on n single cells in M independent experiments. M=10, 4, 7, 6, 4, 2 and m=320, 188, 202, 189, 120, 177, respectively. The dashed line indicates zero transverse strain. The arrows point from small to large nuclei. $S_T$ is positive for small nuclei of both T- and TSA treated N-ESCs indicating auxeticity. Moreover the nuclear $S_A$ being larger for smaller nuclei than for larger nuclei (downward pointing arrows) for T-, CytoD treated T- and TSA treated N-ESCs is a further evidence of auxeticity. For non-auxetic materials like N-ESCs, P- and lineage restricted cells $S_A$ is larger for larger nuclei than for smaller nuclei (upward pointing arrows).
Fig S11. Nuclear strains for other cell lines. Scatter plots correlating the transverse nuclear strain $S_T$ in the channel to the initial nuclear minor axis for HL60 (orange diamonds), HeLa (black squares) and XEN cells (red circles). HL60 cells are a myeloid precursor cell cultured as in $^{10}$. HeLa cells are cultured as in $^{11}$, and XEN cells are an extraembryonic lineage cultured as in $^{12}$. Each point denotes the measurement for a single cell squeezing through channel under an applied pressure gradient of ~10 mbar. A negative (positive) strain denotes a decrease (increase) of the nuclear minor axis as the cell squeezes through the channel. The horizontal lines denotes a nuclear $S_T$ of zero, while the vertical line is the population median for each data set used to separate the population of each class of cells in small and large nuclei categories.
Insets: corresponding correlation between the mean nuclear axial and transverse strains. Small and large symbols denote nuclear sizes at the left and right of the population median, respectively. The error bars are omitted for clarity, the relative error typically being less than 15%. Only 7%, 10% and 4% of the small nuclei (on the left of the median of the populations) of HL60, HeLa and XEN cells exhibit a significant (>0.05) positive $S_T$. Also $S_A$ is larger for larger nuclei than for smaller nuclei for the three classes of cells. These data underline the fact that the auxeticity we see in the transition nuclei is an unusual property that may be rare in other cells.

Fig. S12. Confocal microscopy. 3D volume reconstructions of the T nuclei - outside (a) and inside (b) of the channel - and the N nuclei (c-d) stained with Syto13 (cytoplasm is thresholded out for clarity). The star in (b) denotes $p < 0.05$. Scale bar: 5 µm. The reported nuclear volumes are the mean and standard error of the mean of the measurements over 10 different cells, upon correcting each 3D volume reconstruction by deconvoluting each image in the z-stack with the objective point spread function. (e) Inverse correlation between initial volume and volume change for nuclei of T- and N-ESCs (red circles and black squares, respectively). Nuclei of T-ESCs significantly expand when stretched in the microfluidic channel as reported in Fig. S9, suggesting auxeticity. On the contrary, the non-auxetic nuclei of N-ESCs do not exhibit a significant volume change. Moreover the nuclear volume change upon entering the channel inversely correlates with the initial nuclear volume for T nuclei: the smaller the nuclei, the larger the volume change. Indeed, for small auxetic nuclei the axial tension due to the cytoskeletal stretch is likely larger than the compression due to the channel walls. Therefore, the volume of small auxetic nuclei expand upon entering the microfluidic channel. On the contrary, for large auxetic nuclei transverse compression would be more balanced with axial tension. Therefore, large auxetic nuclei do not present a significant volume expansion.
**Fig. S13. Hoechst-based assay.** Change in area (a) and in greyscale intensity (b) upon entering the microfluidic channel for naïve (black squares) and transition cells (red circles) incubated in 10µg/mL Hoechst 33342 for 30min before trypsinization. Imaging was through a 0.5 NA, 20× objective to integrate over all fluorophores in the nucleus. (M, m) are (2,92) for N and (2,75) for T. Dashed blue lines indicates no change in area or greyscale intensity. Insets: images of a T-ESC outside (left) and inside the microfluidic channel (right). Scale bars: 5 µm. Hoechst 33342 stains nucleic acids most of which are present in the cell nucleus (cf the third column in Fig. S4b). The Hoechst signal decreases for the small nuclei of T-ESCs stretched in the microfluidic channel most likely due to a dilution of the Hoechst concentration indicating a flow of fluid into the nucleus, further confirming the auxeticity of such nuclei. Indeed, stretched auxetic materials experience a significant volume expansion. On the contrary the Hoechst signal does not decrease for large nuclei of T-ESCs and for both small and large nuclei of N-ESCs, confirming that these do not expand in volume (cf Fig. S12).
Fig. S14. Normal and auxetic materials under stretching. Normal materials decrease their cross section perpendicular to the stretching axis (left) while auxetic materials expand in cross section (right). Blue and red arrows denote the stretching and change in cross section, respectively.
Fig. S15. Illustration of the fluorescein assay. We extracted three quantities from each intensity profile (Fig.3e-h): the axial length of the cell $s$, the standard deviation $\sigma$ of the intensity distribution, and its kurtosis $k$, defined as the fourth moment of the distribution normalised by $\sigma^4$. To do so a line was drawn along the cell from where the intensity was 20 or above (empirical threshold), the size of the line being the axial length of the cell $s$; the standard deviation and kurtosis of the intensity distribution were calculated as:

$$
\sigma = \sqrt{\frac{\sum (x_i - \bar{x})^2 I(x_i)}{\sum I(x_i)}}, \quad k = \frac{\sum (x_i - \bar{x})^4 I(x_i)}{\sum I(x_i)} / \sigma^4
$$

where $I(x_i)$ is the intensity at the $i^{th}$ position and $\bar{x} = \frac{\sum x_i I_i(x_i)}{\sum I_i(x_i)}$.

Qualitatively, the kurtosis characterises the flatness of a distribution; the lower the kurtosis, the flatter it is. For a given cell size, if fluorophores move towards the centre of a cell we expect that $\sigma$ will decrease, and that $k$ will increase, which is depicted in the illustration above. (a) Top: scheme of a cell loaded with fluorescein: the signal is significantly more concentrated in the cytoplasm (light green) than in the nucleus (dark green) due to the fact that there is more available space in the cytoplasm (cf. Fig.
3c-d). Bottom: corresponding intensity profile. Images are integrated along the cell thickness and the measured signal is expected to be peaked in the center where the cell is thicker. (b) An hypothetical cell with nucleus and cytoplasm with the same stiffness undergoes a uniform axial stretch upon entering the microfluidic channel. As a result the ratio between cell sizes outside and inside the channel is equal to the standard deviations, \( \sigma \), of the corresponding intensity profiles, the profile kurtosis, \( k \), inside the channel being less than or equal to the one outside. (c) In an actual cell the nucleus gets less elongated upon entering the channel thus the intensity profile is expected to become flatter, \( \sigma \) and \( k \) increasing and decreasing, respectively. (d) For a cell with an auxetic nucleus fluorophores move into the nucleus towards the center of the cell, thus \( \sigma \) and \( k \) are expected to decrease and increase, respectively. The prediction depicted in the figure is borne out by the data presented in Fig. 3 in the manuscript. Fig. 3i shows the ratio of sigma values inside and outside the channel \( \sigma_{in}/\sigma_{out} \), normalised by the cell axial strain \( s_{in}/s_{out} \), for small cells \( (s_{out} < 15 \text{ microns}) \) and large cells \( (s_{out} \geq 15 \text{ microns}) \). We found, as expected, that small T cells exhibit a significantly smaller value of the sigma ratio, whereas its value for small N cells remains close to 1, if not slightly larger, probably due to the fact that the cell nucleus is stiffer than the cytoplasm. Large cells show no significant contrast in their sigma ratio, consistently with the fact that the auxetic response is prevented by the lateral confinement of the nucleus. This analysis is confirmed by the statistics on the kurtosis ratio \( k_{in}/k_{out} \) reported on Fig. 3j. All cells show a kurtosis ratio lower than one (i.e. distributions are flatter), consistent with the fact that cells are laterally confined in the channel. However, small T cells have a significantly higher kurtosis ratio; this indicates that comparatively to the N cells, fluorophores tend to be located closer to the centre, nearly compensating the flattening effect of the channel. The analysis of the second and fourth moments of these intensity distributions therefore provide evidence that fluorophores are driven towards the centre of the cell while it is stretched, which supports the fact that fluorophores enter the cell nucleus while it swells upon deformation.
Fig. S16. Nuclear envelope wrinkling. (a)-(c) Fluorescence microscopy images of H2B-eGFP in naive, transition and primed nuclei, respectively. Scale bar: 3 μm. (d) Corresponding Fourier component analysis of nuclear boundary structure and shape. The third bin (λ > 8 μm) describes deviation of nuclear shape from circular, the second (3 < λ < 8 μm) quantifies the amount of wrinkling and the first bin (λ < 3 μm), which is not shown, contains high frequency information and is dominated by noise. Each bin is the mean and standard error over measurements for 25 different cells. The inset presents the ratio of the nuclear perimeter divided by the square root of the area of the nuclei in the three classes of cells. From these data, we concluded that the nuclear envelope of the T-nuclei is significantly less wrinkled than the N- and P-nuclei, but the perimeter is conserved between N- and T-nuclei.
Table S1. Primers used for gene expression analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer name</th>
<th>Primer sequence (5’ to 3’)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lmna</td>
<td>LmnA-PS-F</td>
<td>TCTTCTGCCTCCAGTGTACAG</td>
<td>Sehgal et al. (2013)¹³</td>
</tr>
<tr>
<td></td>
<td>LmnA-PS-R</td>
<td>CATGATGCTGCAGTTCTGGGAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LmnA-MEM-F</td>
<td>GCCTTCGACCGGCTCTCATCA</td>
<td>Eckersley-Maslin et al. (2013)¹⁴</td>
</tr>
<tr>
<td></td>
<td>LmnA-MEM-R</td>
<td>TGGCTGAGCGCCAGTTGTACT</td>
<td></td>
</tr>
<tr>
<td>Lmna</td>
<td>LmnA/C-PS-F</td>
<td>CTT CGACCCAGCTCTCATCAAC</td>
<td>Sehgal et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>LmnA/C-PS-R</td>
<td>GCGGCGGCTGCCACCTCACAC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LmnA/C-MEM-F</td>
<td>GGCTGTGGGAGCAACCTTCAG</td>
<td>Eckersley-Maslin et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>LmnA/C-MEM-R</td>
<td>GGCTGCCACTCACACAGGTGG</td>
<td></td>
</tr>
<tr>
<td>Lmnb1</td>
<td>LmnB1-F</td>
<td>AGCGCGCCAAGCTCCAGATC</td>
<td>Eckersley-Maslin et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>LmnB1-R</td>
<td>GTAGCCAGCGCCGCATCTCTT</td>
<td></td>
</tr>
<tr>
<td>Tcf15</td>
<td>Tcf15-F</td>
<td>GTGTAAGGACAGGAGGACAA</td>
<td>Davies et al. (2013)¹⁵</td>
</tr>
<tr>
<td></td>
<td>Tcf15-R</td>
<td>GATGGCTAGATTGGTCCTTG</td>
<td></td>
</tr>
<tr>
<td>Oct4/Pou5f1</td>
<td>Oct4-F</td>
<td>ACCTCAGGTGGACTGGCCCTTA</td>
<td>Azuara et al. (2006)¹⁶</td>
</tr>
<tr>
<td></td>
<td>Oct4-R</td>
<td>GCCTCGAAAGCCACAGTTGATGTT</td>
<td></td>
</tr>
<tr>
<td>Nanog</td>
<td>Nanog-F</td>
<td>GCATCTTCTGTCTCTGGCCTA</td>
<td>Azuara et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Nanog-R</td>
<td>GAACATTTCTTGCTTACAAGGGGTCTGC</td>
<td></td>
</tr>
<tr>
<td>Rex1/Zfp42</td>
<td>Rex1-F</td>
<td>CTCCAGGCCTAGATTTCCTA</td>
<td>Percharde et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Rex1-R</td>
<td>CGTGTCACAGCTTCTAGTCCATT</td>
<td></td>
</tr>
<tr>
<td>Ywhaz</td>
<td>Ywhaz-F</td>
<td>CGTTGTAGGAGCCCTAGGTCAT</td>
<td>Azuara et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Ywhaz-R</td>
<td>TCTGGTTGCGAAGCATTGGG</td>
<td></td>
</tr>
<tr>
<td>L19</td>
<td>L19-F</td>
<td>TGATCGTCTGACGGAGTTG</td>
<td>Percharde et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>L19-R</td>
<td>GGAAAGAGGTCTGGTGGG</td>
<td></td>
</tr>
</tbody>
</table>