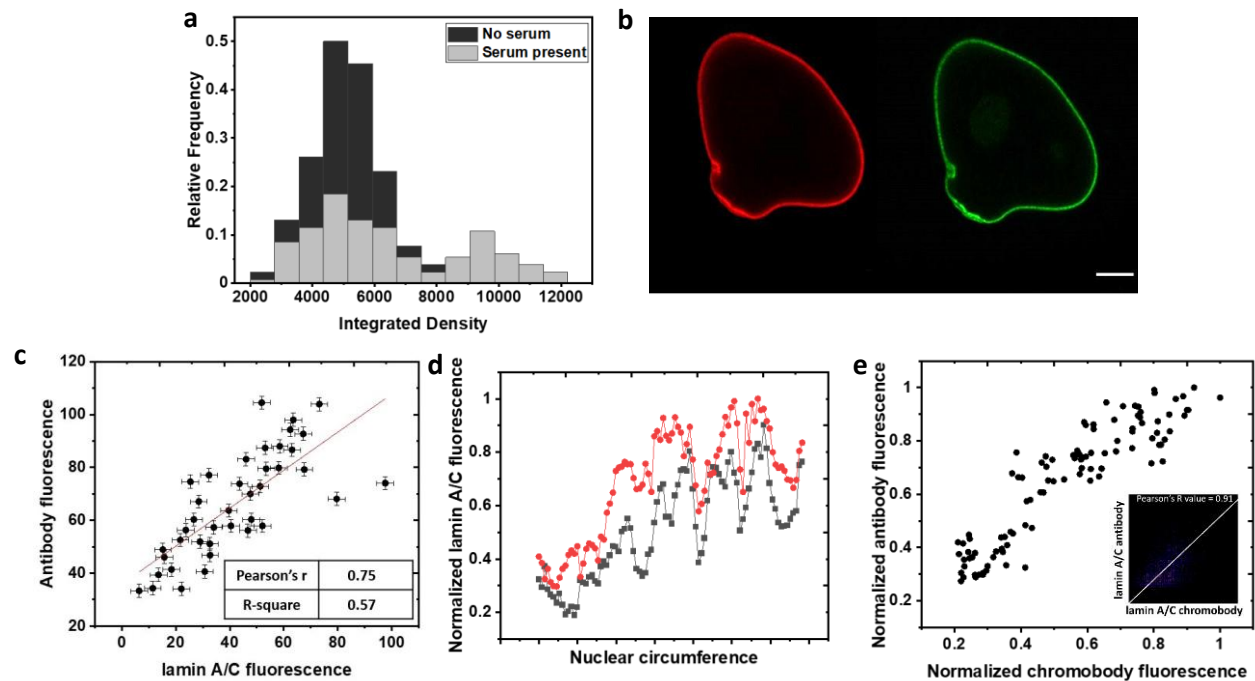
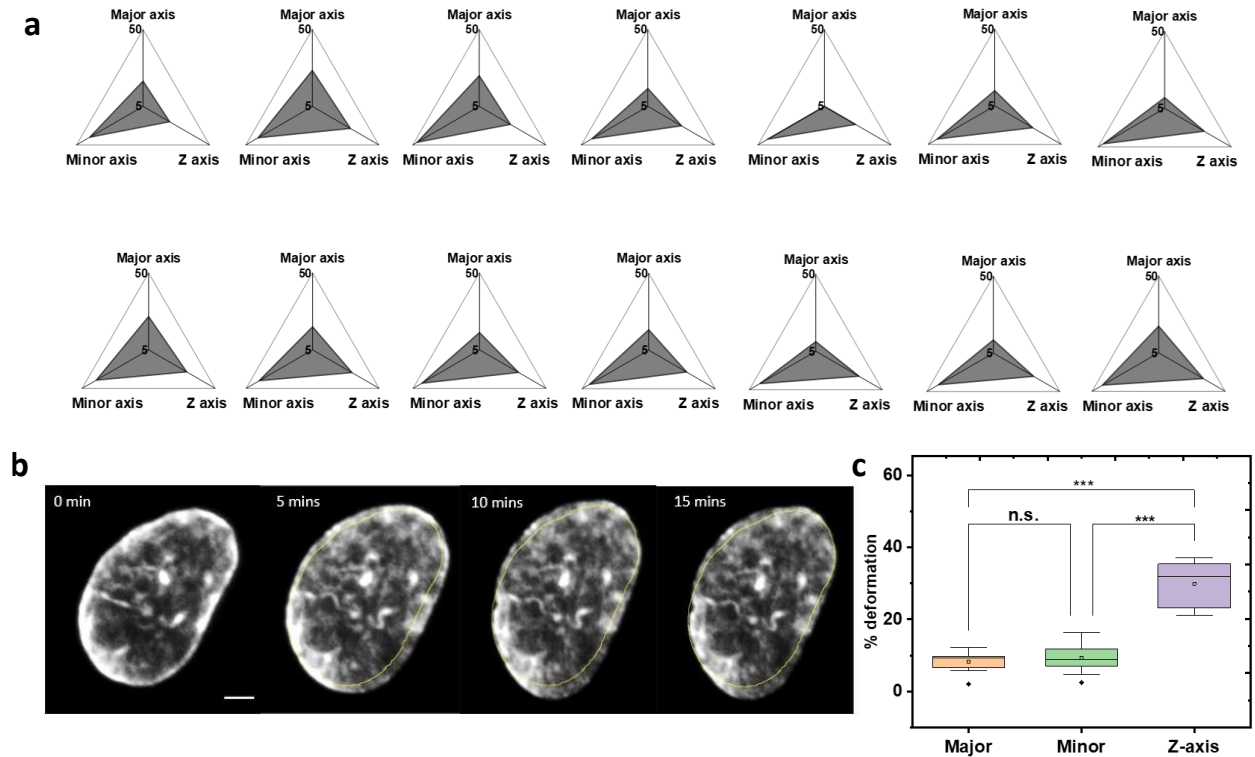


## Supplementary information

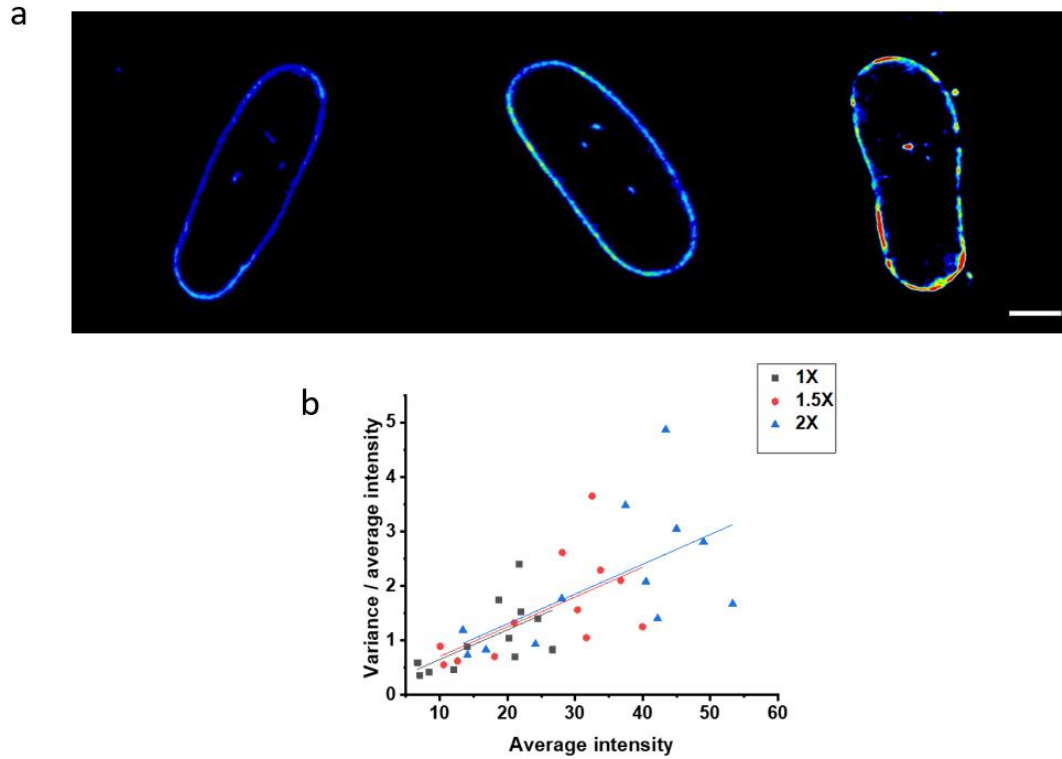


**Figure S1. Spatial correlation between lamin A/C antibody and lamin A/C chromobody in synchronized cells.**

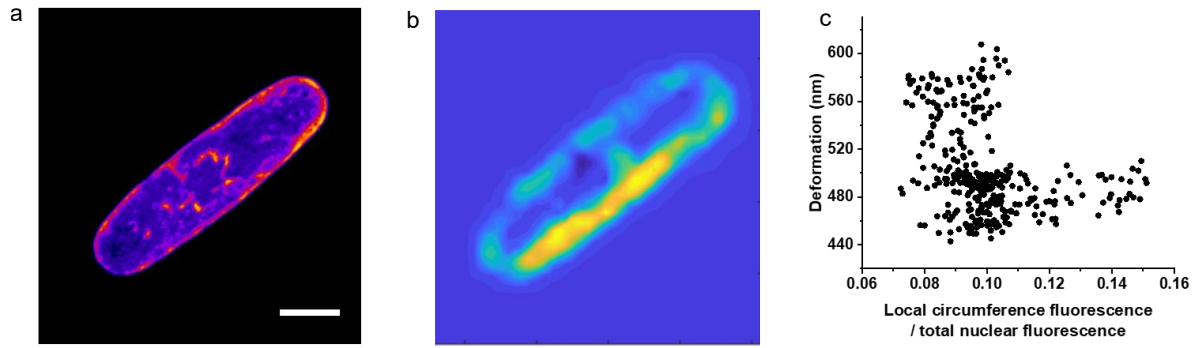
**a**, Narrow peak of fluorescence in serum starved cells indicate that cells are in the same cell cycle phase compared to a broader peak in cells grown in serum containing media to reduce the variation in overall lamin A/C expression due to different phases of cell cycle (n=130; data from three independent experiments). **b**, Nucleus stained with antibody tagged with Atto-647N (left) and GFP-tagged lamin A/C chromobody (right) (scalebar = 5 $\mu$ m). **c**, Relationship between overall antibody and chromobody fluorescence across unique cells (n=40; data from two independent experiments) showing strong correlation in overall antibody and chromobody fluorescence. **d**, lamin A/C antibody (black) and lamin A/C chromobody (red) fluorescence of the same points along the nuclear circumference indicating local lamin A/C fluorescence to be correlated. **e**, Correlation between antibody and chromobody fluorescence of the same points along the nuclear circumference from **S1b** and an example of colocalization analysis of lamin A/C chromobody and lamin A/C antibody has been shown in the inset indicating a strong correlation even between local antibody and chromobody fluorescence.



**Figure S2. Anisotropy in nuclear compression.** **a**, Nuclear deformation of 14 nuclei (data from a single experiment) along major, minor and z-axis showing the largest deformation along the z-axis. **b**, Z-projections of wild type nucleus stained with lamin A/C chromobody at different time intervals with the original zero pressure image overlaid as a visual aid (scalebar = 2µm). **c**, percent nuclear deformation in nuclei that have been removed from cells and isolated, indicating more pronounced deformation in Z axis (n=10 cells, \*\*\*p<=0.001; n.s.= non-significant; data from a single experiment)



**Figure S3. Local nuclear deformation as a function of lamin A/C protein in 3T3 fibroblast cells.** **a**, Increasing lamin A/C heterogeneity with an increase in lamin A/C fluorescence (scale bar = 5 $\mu$ m). **b**, Normalized variance of lamin A/C chromobody fluorescence (n=12; data from a single experiment) showing that spatial heterogeneity of lamin A/C increases with expression levels and the slope does not change after increasing imaging gain by factors of 1.5 and 2, illustrating that imaging parameters do not increase lamin A/C heterogeneity quantification.



**Figure S4. Example local nuclear deformation as a function of lamin A/C.** **a**, lamin A/C distribution along the nuclear circumference in the X-Y plane (scale bar = 5 $\mu$ m). **b**, Nucleus strain map in the X-Y plane. **c**, Nuclear deformation as a function of lamin A/C fluorescence showing a third population with less local deformation in areas of high lamin A/C density