Dynamics of chromatin decondensation reveals the structural integrity of a mechanically prestressed nucleus

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Supplementary material

Supplementary Figure 1. No swelling was observed in for cleavage of nucleic acids. At high DNase concentrations (500 mU/microlitre) fragemented bits of chromatin were emitted from the nuclei though there was no expansion in size. Time points are indicated in the figure. Scale-bar = 10 μm.

Supplementary Figure 2. Nuclei extracted with 1% Triton-X-100, and without, both show expansion kinetics on clostripain digestion. (A) The cross-sectional area normalized to the initial are plotted for nuclei extracted with Triton-X-100 (nT1, nT2), and two control nuclei which were extracted similarly but without Triton (nC1, nC2) for digestion by clostripain (2 mU/microlitre). While the initial lag phase seems shorter for the Triton-extracted nuclei, both show entropic expansion. (B) There were a greater number of nuclei with cytoplasmic debri sticking to them in the sample extracted without detergent, and thus we used triton-extracted nuclei in all experiments. The figure shows a sample extracted without triton, which shows a nucleus with cytoplasmic debri and another without. In all cases only clean nuclei like the one on top were used for experiments. Scale-bar = 5 μm.

Supplementary Figure 3. Poly-D-lysine coating does not affect expansion kinetics. (A) The expansion kinetics is shown for three nuclei on a poly-D-lysine coated glass coverslip (nC1, nC2, nC3), and three on an uncoated glass coverslip (nUC1, nUC2, nUC3). No apparent difference in expansion kinetics was observed except the expected heterogeneity between different nuclei.

Supplementary Figure 4. Change in the mean anisotropy value with time for trypsin digestion (△) and control nuclei (○) isolated from H2B-EGFP expressing HeLa cells. The fall in the anisotropy in the trypsin digested nuclei is indicative of the increased rotational mobility of histone (H2B-EGFP) proteins and hence of chromatin decompaction. Anisotropy images of the nuclei were taken on an inverted fluorescence

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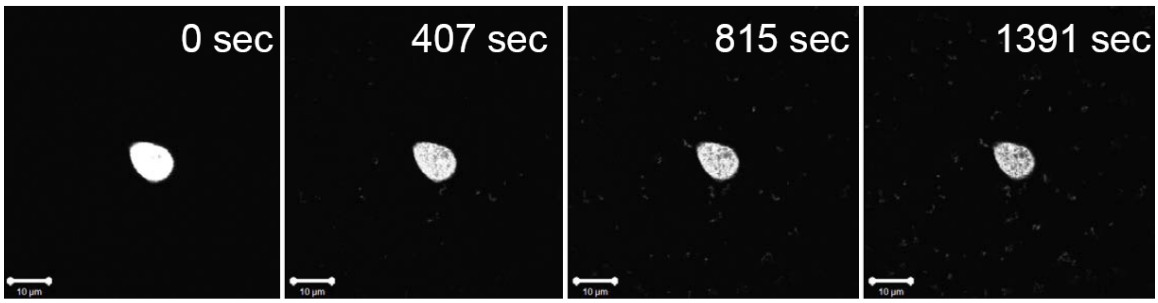
microscope with a 100× 1.4 NA objective and a cooled ICCD (Cascade 650; Roper Scientific, AZ, USA). The light from the mercury arc lamp of the microscope was polarized using a polarizer before exciting the sample. The fluorescence images both in the parallel and the perpendicular polarizations with respect to the excitation polarization were analyzed using V++ (Digital Optics, NZ) and Labview (National Instruments) software. The anisotropy images were plotted by calculating the anisotropy values for each pixel using the parallel and perpendicular polarization images. Anisotropy images of the chromatin swelling were taken using the H2B-EGFP fluorescence. The histograms of the anisotropy for each image were plotted from the anisotropy values at each pixel of the images and the mean anisotropy value was estimated. In brief, the initial anisotropy represents the rotational freedom of histone H2B in the chromatin assembly. Anisotropy is given by $\frac{I_X - I_\perp}{I_X + 2I_\perp}$ where I_X is the parallel component of the emission intensity with respect to the excitation polarization direction and $I_\perp$ is the perpendicular component. Decondensation of chromatin should lead to variation in the rotational freedom of the histone proteins and other chromatin associated proteins. A decrease in anisotropy implies an increase in the perpendicular component of the fluorescence emission and hence an increased rotational freedom of the protein. Hence, anisotropy is a measure of the chromatin decondensation.

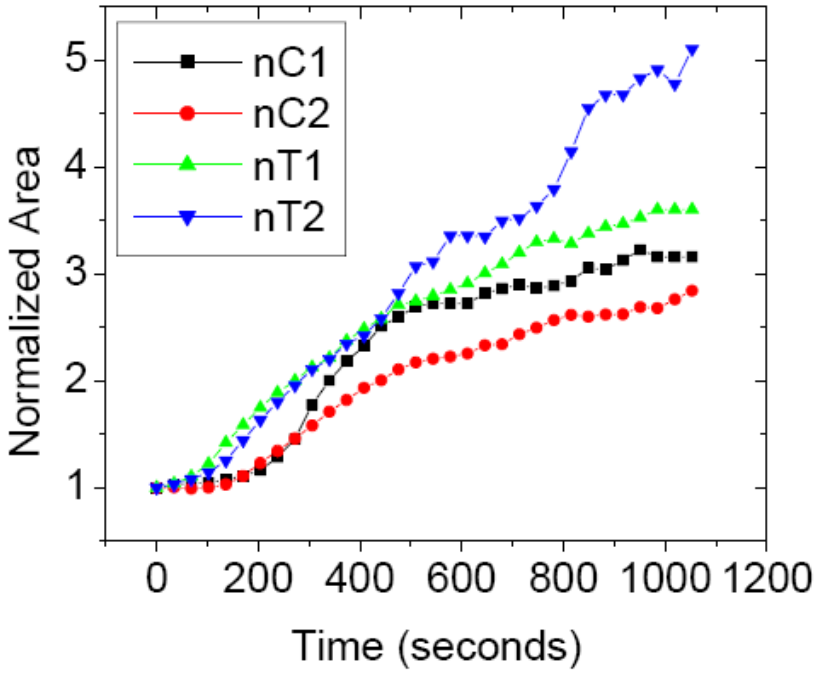
Supplementary Figure 5. Reversible swelling of isolated nuclei under low divalent salt concentrations. Isolated H2B-EGFP HeLa nuclei in PBS buffer were washed four times in deionized water, to show large swelling (quantified in reference 11 of the main-text, though EDTA was used there to chelate divalent ions). The swelling was reversed on adding back PBS, to regain exactly the same configuration as before. No loss of histones was apparent. Scale-bar = 5 μm.

Supplementary Figure 6. Swelling under high salt conditions. (A) Isolated H2B-EGFP HeLa nuclei were treated with 1 M $MgCl_2$. All H2B-EGFP fluorescence was immediately lost, thus DIC images are presented in all cases. The nuclei swelled, and the swelling was not reversed on replacing the buffer with PBS. There was further swelling on four washes with deionized water, which was reversible. Clostripain digestion (2 mU/microlitre) of the same nuclei caused a massive swelling and subsequent rupture. Scale-bar = 10 μm. (B) H3-acetylation is a common mark present in several active regions of chromatin, and cells usually show a robust staining for this modified histone. Antibody staining of similar control and salt-treated, and subsequently fixed nuclei with an antibody against acetylated H3 (Upstate), showed a presence of histone H3 even in the salt-treated nuclei. Thus all core histones are not lost – which might explain the partial swelling of salt-treated nuclei. All image acquisition parameters were kept the same between the top and the bottom panels. (C) Antibody staining of similar control and salt-treated, and fixed nuclei with an antibody against Lamin B1 (Abcam) shows the presence of the lamin scaffold in both conditions. All image acquisition parameters were kept the same between the top and the bottom panels. A rhodamine-labeled secondary antibody was used, and the DNA stained with Hoechst 33342 (Sigma) in both (B) and (C). Scale-bar = 5 μm in both (B) and (C).

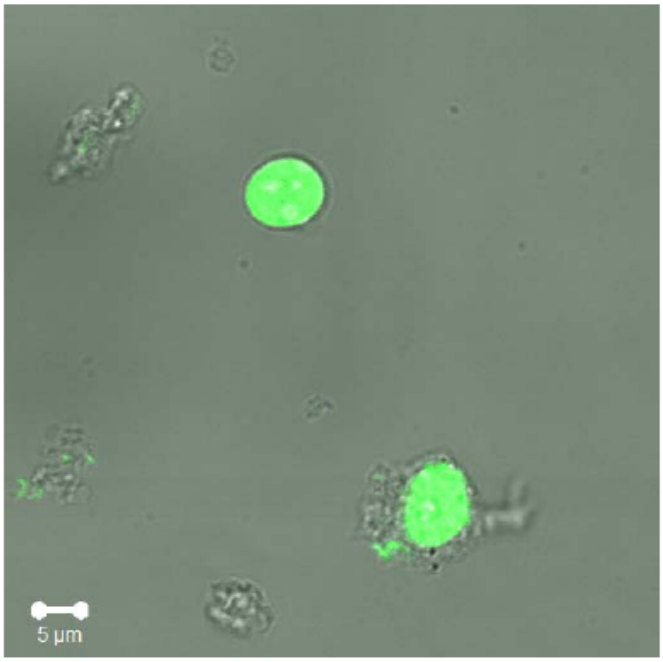


Supplementary Fig. 1: Mazumder, Roopa et al

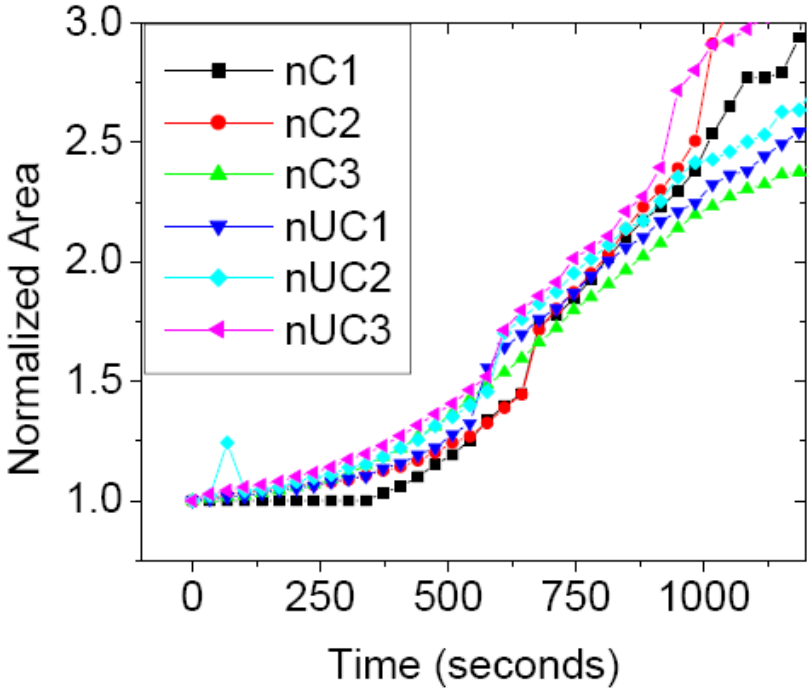
A



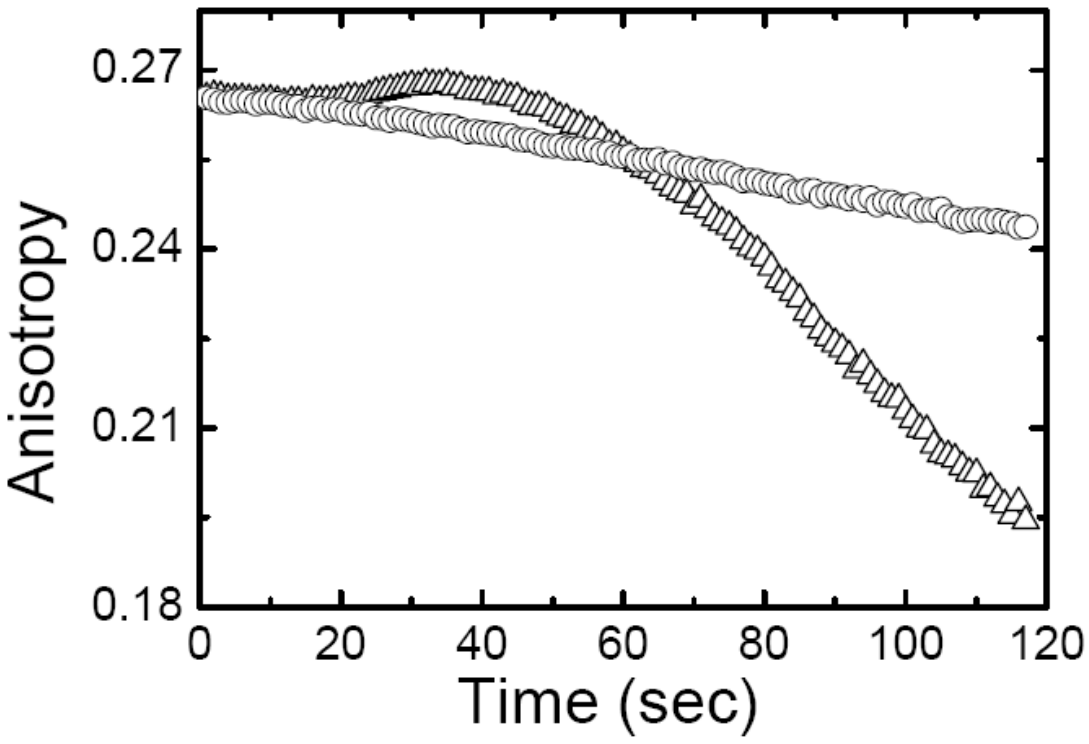
B



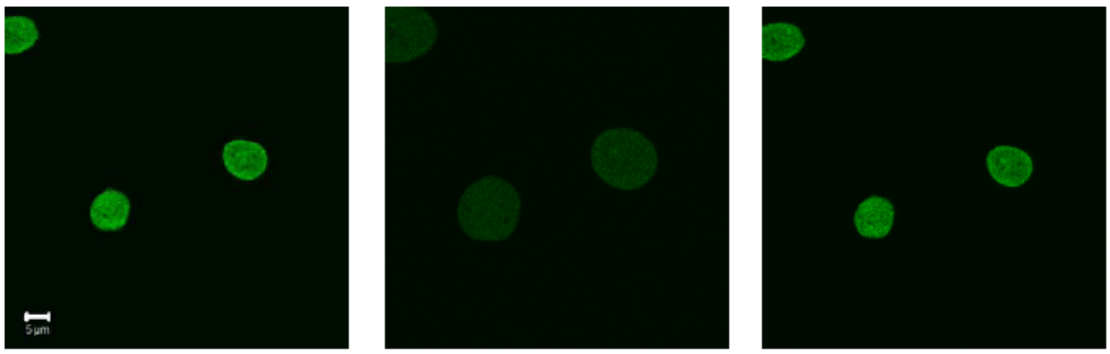
Supplementary Fig. 2: Mazumder, Roopa et al



Supplementary Fig. 3: Mazumder, Roopa et al



Supplementary Fig. 4: Mazumder, Roopa et al

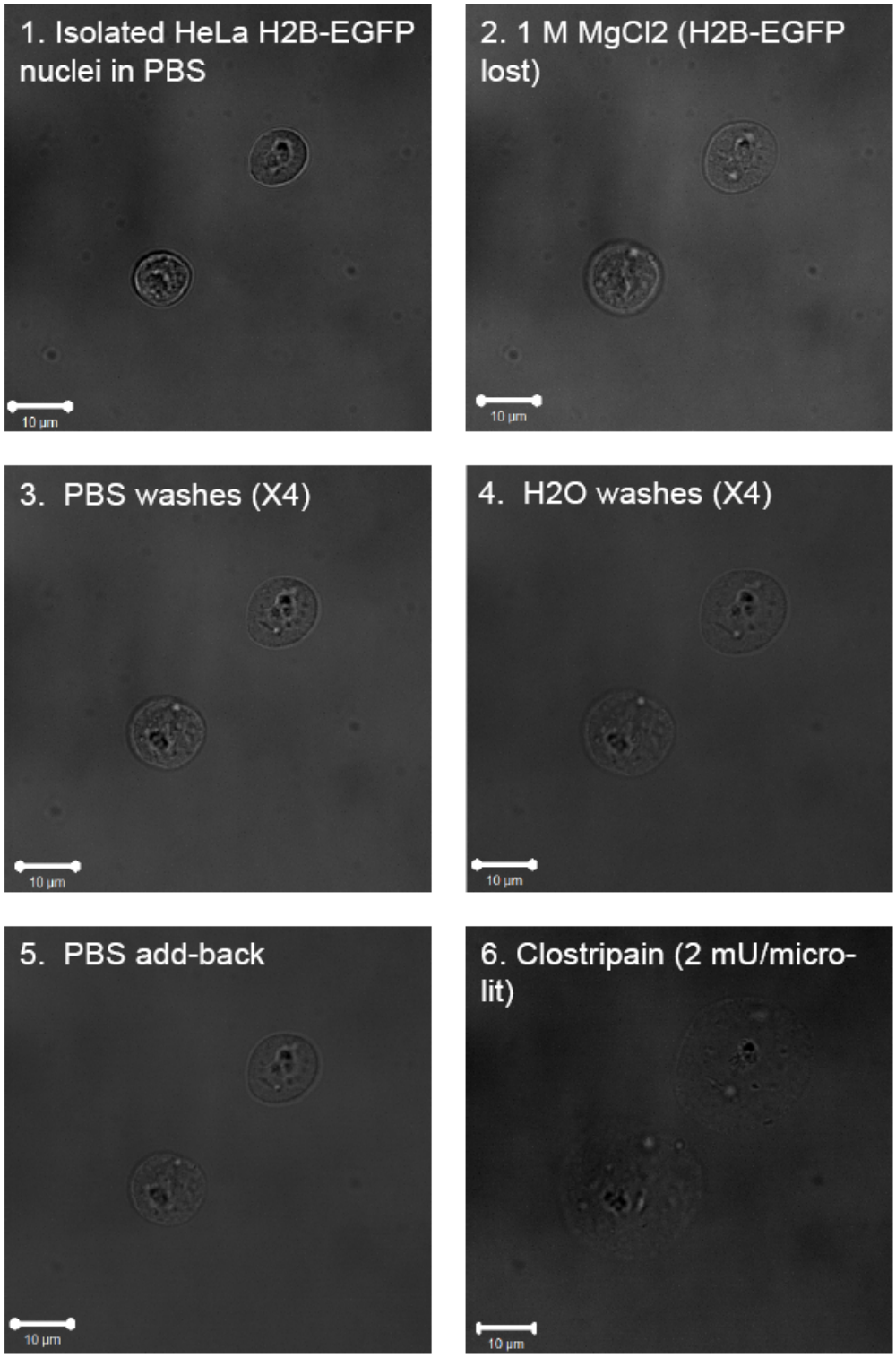


1. Isolated HeLa H2B-EGFP nuclei in PBS

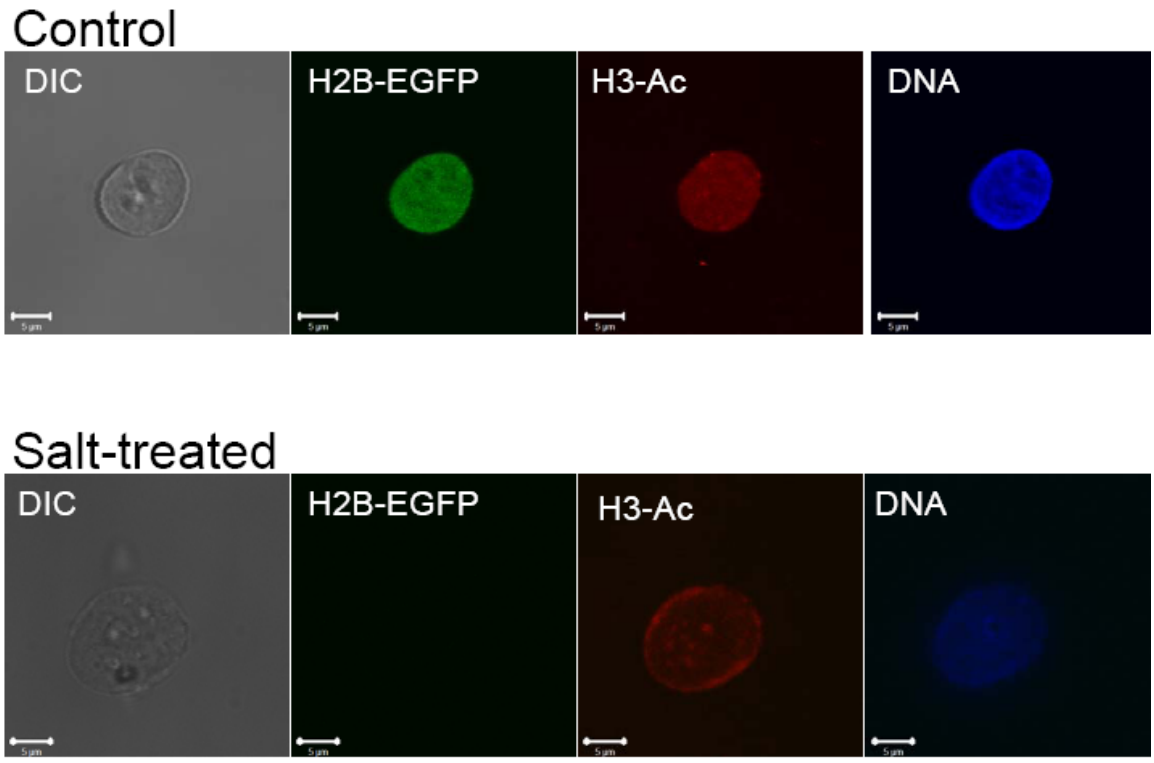
2. H2O washes (X4)

3. PBS add-back

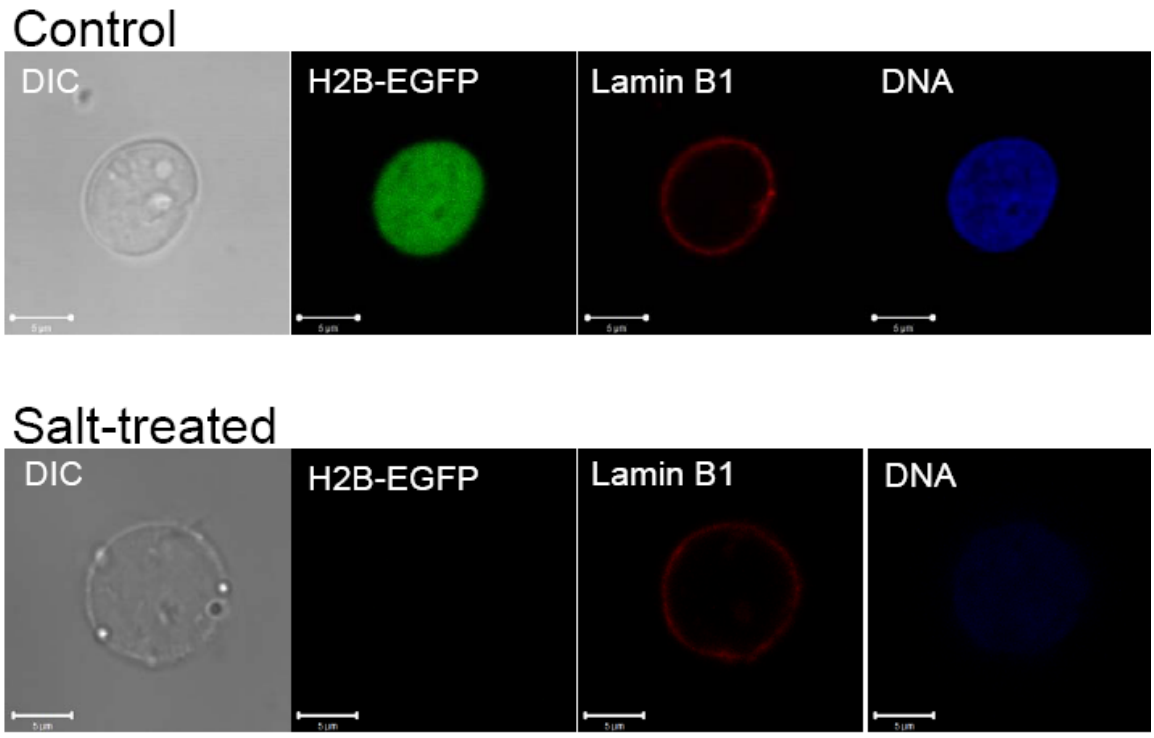
Supplementary Fig. 5: Mazumder, Roopa et al



Supplementary Fig. 6A: Mazumder, Roopa et al



Supplementary Fig. 6B: Mazumder, Roopa et al



Supplementary Fig. 6C: Mazumder, Roopa et al