## Osmotic Challenge Drives Rapid and Reversible Chromatin Condensation in Chondrocytes

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Supporting Material



FIGURE S1 Monolayer cultured chondrocyte nuclei with hyper- (A1) and normally- (A2) condensed chromatin as the source images, both with a region of interest drawn across the nuclei (*yellow*). Image after Sobel algorithm (*B*), Sobel image post-thresholding (*C*), thresholded image post-thinning morphological operation (*D*), region of interest (*yellow*) showing the inner part of the nucleus (*E*). From this quantification method, the nucleus with hyper-condesed chromatin has an chromatin condensation parameter of 4.86%, whereas the nucleus without hyper-condensed chromatin has an chromatin condensation parameter of 0.16%. (*F*) The intensity profile across the nucleus with hyper- (A1, \_\_\_\_\_) and normally-(A2, \_\_\_\_\_) condensed chromatin, *arrows* showing the intensity dips (Bar: 5 µm).



FIGURE S2 Images of monolayer cultured chondrocytes expressing H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed by hypoosmotic challenge (100 mOsm/kg), which was then brought back to the isoosmotic conditions. Images were thresholded using the Iterative Self Organizing Data algorithm (Bar:  $5 \mu$ m).



FIGURE S3 Images of monolayer cultured chondrocytes expressing H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed by isoosmotic challenge (300 mOsm/kg), which was then brought back to the isoosmotic conditions. Images were thresholded using the Iterative Self Organizing Data algorithm (Bar: 5  $\mu$ m).



FIGURE S4 Images of monolayer cultured chondrocytes expressing H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed by hyperosmotic challenge (400 mOsm/kg), which was then brought back to the isoosmotic conditions. Images were thresholded using the Iterative Self Organizing Data algorithm (Bar:  $5 \mu$ m).



FIGURE S5 Images of monolayer cultured chondrocytes expressing H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed by hyperosmotic challenge (500 mOsm/kg), which was then brought back to the isoosmotic conditions. Images were thresholded using the Iterative Self Organizing Data algorithm (Bar: 5  $\mu$ m).



FIGURE S6 Images of monolayer cultured chondrocytes expressing H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed by hyperosmotic challenge (700 mOsm/kg), which was then brought back to the isoosmotic conditions. Images were thresholded using the Iterative Self Organizing Data algorithm (Bar: 5  $\mu$ m).



FIGURE S7 Average projection images of monolayer cultured chondrocytes expressing actin-RFP and H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed with hypoosmotic challenge (100 mOsm/kg) (Bar:  $10 \mu m$ ).



FIGURE S8 Average projection images of monolayer cultured chondrocytes expressing actin-RFP and H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed with isoosmotic challenge (300 mOsm/kg) (Bar: 10 µm).



FIGURE S9 Average projection images of monolayer cultured chondrocytes expressing actin-RFP and H2B-GFP fusion-protein in an isoosmotic environment (300 mOsm/kg), followed with hyperosmotic challenge (500 mOsm/kg) (Bar: 10 µm).