Supporting information

Title

Actin cytoskeleton differentially alters the dynamics of lamin A, HP1α and H2B core histone proteins to remodel chromatin condensation state in living cells

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Materials and Methods

Mathematical equations and calculations

Fluctuation of fluorescence intensity *I* (t) over a time series was measured and the autocorrelation function (adapted from the Confocal Zeiss ZEN software, Carl Zeiss MicroImaging GmbH) was calculated. This function includes both the molecular triplet state component and the molecular translation component, $G(\tau) = 1 + G_t * G_d$, where

$$G_t(\tau) = \left(1 + \frac{T_t * e^{-\tau/\tau_t}}{1 - T_t}\right) \quad \text{--- eq. (1)}$$

T_t: the triplet fraction, τ_t is the triplet relaxation time, and

$$G_d(\tau) = \sum_{i=1}^{3} \frac{\Phi_i}{\left(1 + \left(\frac{\tau}{\tau_{d,i}}\right)^{\alpha_i}\right)^{e_{d1}} * \left(\left(1 + \left(\frac{\tau}{\tau_{d,i}}\right)^{\alpha_i}\frac{1}{S}\right)0.5\right)^{e_{d2}}} \quad \text{---eq(2)}$$

$$\Phi_i = \frac{f_i * \sigma_i^2}{\sum_{i=1}^{3} (f_i * \sigma_i)^2}$$

i: Index of component (1, 2, 3), : Fractional intensity, f_i is the fraction of molecules, and σ_i is the molecular brightness. $\tau_{d,i}$: Diffusional correlation time. $S = \frac{\omega_z}{\omega_r}$: Structural parameter, ω_z is the axial focus radius, and ω_r is the lateral focus radius. e_{d1} and e_{d2} are both dependent on the dimension of the translation component. e_{d1} = 0.5, 1, 1, and e_{d2} = 0, 0, 1 for 1D, 2D, and 3D respectively. α_i: Anomaly parameter. τ_D is related to the diffusion constant D through the equation $D = \omega^2/4\tau_D$. This equation can be used to estimate the diffusion constant of eGFP in cell nucleus by knowing the diffusion constant of the known dye (e.g. atto 488 from ATTO-TEC GmbH), the diffusion time of the dye, and the diffusion time of eGFP. In our experiment, atto 488 dye has $D_{atto488} = 420\ \mu m^2 s^{-1}$, $\tau_{atto488,} = 31 \pm 5\ \mu s$ at 25°C, and that of $\tau_{eGFP} = 370 \pm 15\ \mu s$ at 37 °C, therefore $D_{eGFP} = 35.2\ \mu m^2 s^{-1}$.

Immunofluorescence

Cells were rinsed with 1X PBS, followed by 4% PFA in 1X PBS for 15 minutes. Cells were rinsed again with 1X PBS for three times followed by blocking and permeabilization using 5% Bovine Serum Albumin (BSA) (Sigma-Aldrich), 0.3% Triton™ X-100 in 1X PBS. The primary antibody

(1:100 dilution) was incubated for 45-60 min, followed by secondary antibody (1:100 dilution) incubated for 45-60 mins. To add another primary antibody, the previous steps were repeated. Finally, Hoechst-33342 (Invitrogen™ Molecular Probes®) of 2 µg/ml diluted to 1:1000 was used to stain the nucleus. The primary antibodies used were mouse lamin A/C (abcam®; ab8984), goat HP1α (abcam®; ab77256), Rabbit H3 (trimethyl K9) (abcam®; ab8898).

Figure Legends

Figure S1. Cell geometric constraints demonstrate architectural modulation to nuclear lamin A/C protein. (**A**) Representative images of fibronectin coated 1800 μm^2 rectangular-shaped and 500 μm^2 circular-shaped micropatterns. Scale 20μm. **(B)** Alteration in nuclear lamina projected area due to changes in cellular geometry ($p<0.001$). **(C)** Enhanced heterogeneity in nuclear lamina shape variation across basal-apical confocal nuclear sections ($p<0.05$). **(D)** Enhanced variation in nuclear lamina intensity variation in cells with perinuclear actin perturbed by cellular geometry. n=15-20; $p<0.001$.

Figure S2. Description of the two component 3D diffusion fit for FCS autocorrelation study**. (A-C)** FCS autocorrelation curves for nuclear proteins lamin A, HP1α and H2B, along with their respective curved fittings incorporated with two time components. The black curves represent the autocorrelation curves for the proteins, the red curves represent the two-time component fitting, and the green curves represent the fitting residue. (D-E) Representative confocal images of a NIH3T3 mouse fibroblast cell transfected with the lamin A-mCherry and HP1α-eGFP; and line profile of the proteins across the nucleus. Scale bar = 5 μm.

Figure S3. Modulation in interaction times as measured by FCS for nuclear proteins in serum starved cells. **(A)** Representative apical-basal actin images of cells in serum starved condition. **(B-C)** Distribution profiles of interaction times for nuclear proteins HP1α and H2B respectively for cells in serum starved condition demonstrate alteration in protein dynamics.

Figure S4. Perinuclear actin architecture differentially controls chromatin spatial organization. **(A)** Quantitative assessment of alteration in heterochromatin/euchromatin intensity ratio with changes in cellular geometry ($p<0.001$); n=15-20.

(A)

	Model	Triplet state (μs)	Comp1 (μs)	Fraction1 (%)	Anomaly Factor1	Comp2 (μs)	Anomaly Factor2	Fitting (chi^2, x 10^{-6})
Lamin A	3D 1C		2099	100				6.99
	3D1C+T	245	3588	100				3.06
	3D2C+T	72	1373	71		10789		2.72
	3D1C+A		1717	100	0.74			2.89
	3D1C+T+A	70	2158	100	0.81			2.70
	3D2C+T+A	0.4	68	6	8.82	2151	0.81	2.55

(B)

	Model	Triplet state (μs)	Comp1 (μs)	Fraction1 (%)	Anomaly Factor1	Comp2 (μs)	Anomaly Factor2	Fitting (chi^2, x 10^{-6})
HP1a	3D 1C		5378	100				7.01
	3D1C+T	482	15007	100				3.07
	3D2C+T	31	1404	54		32601		2.50
	3D1C+A		3089	100	0.56			2.71
	3D1C+T+A	27	4341	100	0.64			2.51
	3D2C+T+A	32	850	33	1.31	14449	0.86	2.54

(C)

	Model	Triplet state (μs)	Comp1 (μs)	Fraction1 (%)	Anomaly Factor1	Comp2 (μs)	Anomaly Factor2	Fitting (chi^2, x 10^{-5})
H2B	3D 1C		619	100				12.0
	3D1C+T	286	4167	100				4.77
	3D2C+T	88	395	82		16985		4.20
	3D1C+A		413	100	0.65			5.07
	3D1C+T+A	0.16	410	100	0.65			4.58
	3D2C+T+A	90	478	69	1.3	14647	0.9	4.28

3D1C: 3D diffusion with 1 component
3D1C+T: 3D diffusion with 1 component and a triplet state
3D2C+T: 3D diffusion with 2 components and a triplet state
3D1C+A: 3D diffusion with 1 component and an anomaly factor
3D1C+T+A: 3D diffusion with 1 component, a triplet state and an anomaly factor
3D2C+T+A: 3D diffusion with 2 components, a triplet state and two anomaly factors

Table S1 (A-C) are tables showing different fitting models that were used to fit the FCS data for lamin A, HP1α and H2B, respectively. The analysis shows that 3D diffusion model with two components (fast and slow) and a triplet state fitted all FCS curves well in all cases.

eGFP – tagged protein in the nucleus	First diffusion time in fitted data T1 (µs)	(Effective) Diffusion constant D ($\mu m^2 s^{-1}$)
eGFP	370 ±15	35.2[a], 21.5[b], 23.5[c]
Lamin A	1064 ± 520	12.2[a]
HP1α	1109 ± 384	11.7[a], 7.7[b]
H2B	633 ± 217	20.6[a],
	Second diffusion time in fitted data T2 (ms)	
Lamin A	12.2 ± 5.4	1.0[a], 0.38[d]
HP1α	24.6 ± 9.5	0.53[a], ~ 0.4[e]
H2B	26.1 ± 11.0	0.50[a]

[a]from our FCS experimental data, [b]from[1], , [c]from [2] , [d]from[3], [e]from RICS data in [4] and [5]

Table S2: A summary of the diffusion time and diffusion constant of proteins in the cell nucleus estimated using the two component 3D diffusion fitting model. The summary of the interaction time for each protein is given in the lower half of the table.

	Lamin A (percentage)	HP1α (percentage)	H2B (percentage)
Un-patterned/NoDrugs	54±9	51±8	26±12
Rectangle	55±11	42±8	24±7
Jasplakinolide	54±8	47±7	21±11
Circle	55±10	44±7	28±14
Latrunculin A	51±9	48±6	22±11

Table S3: The percentages of interaction times for lamin A, HP1α, and H2B in different experimental conditions.

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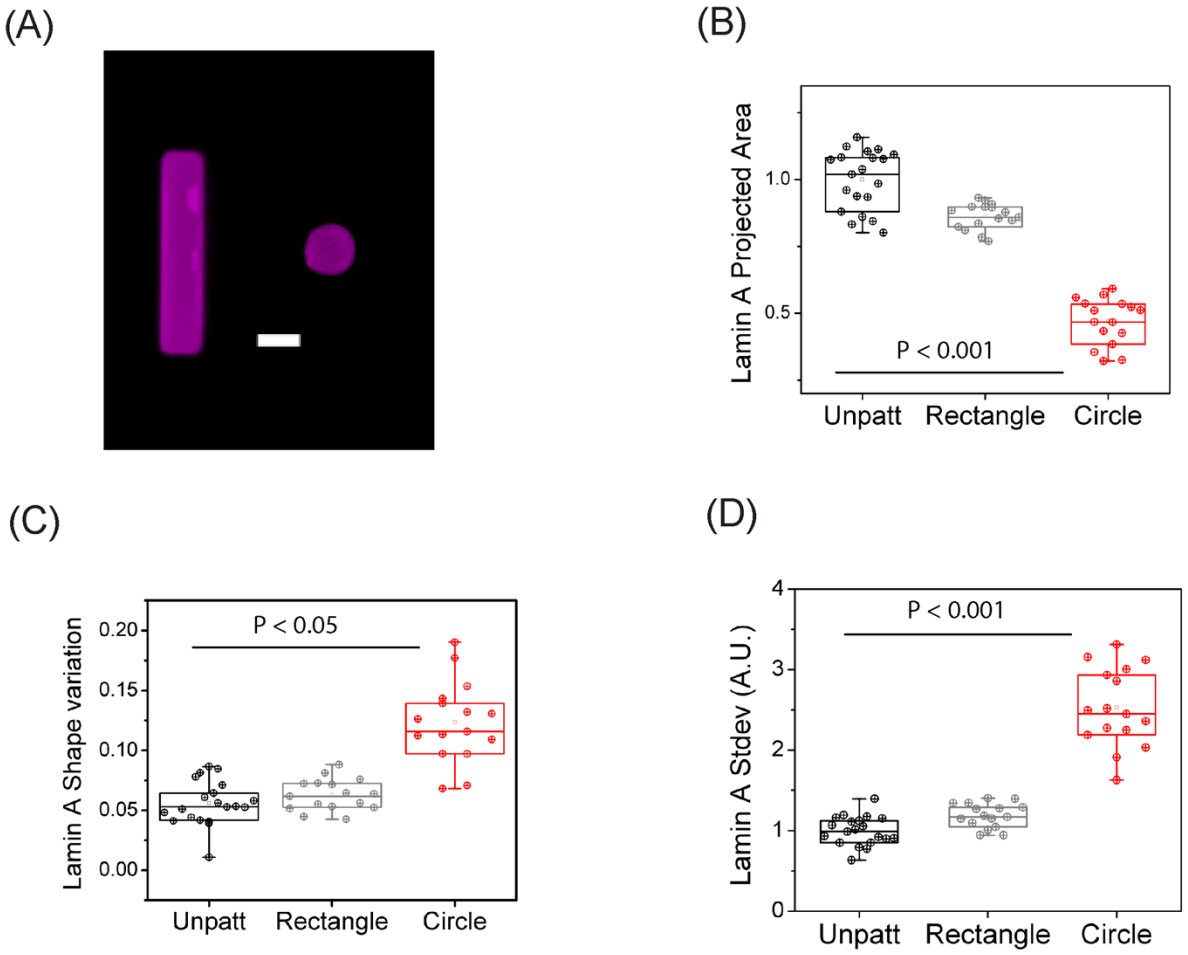
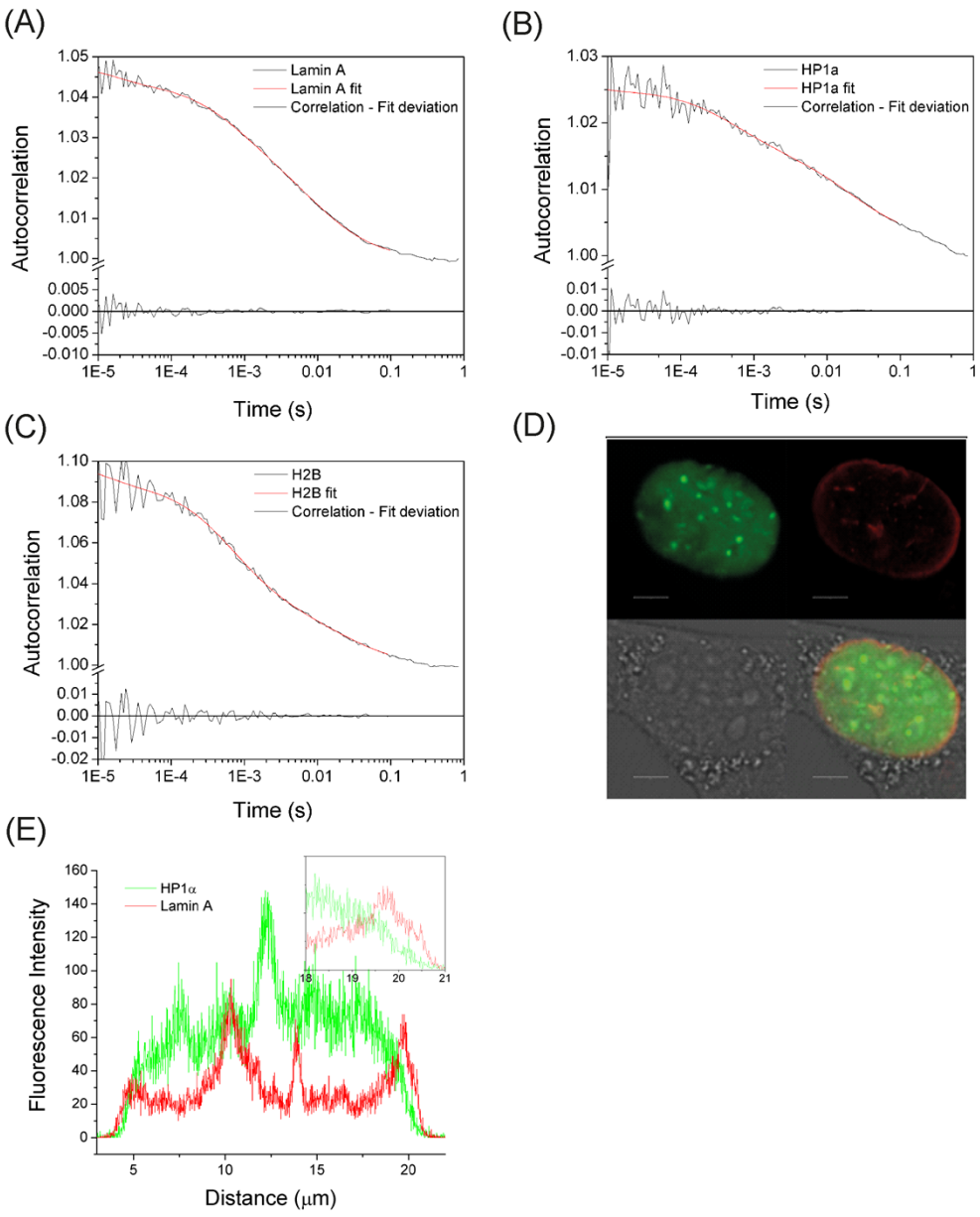


Suppl Figures S1.



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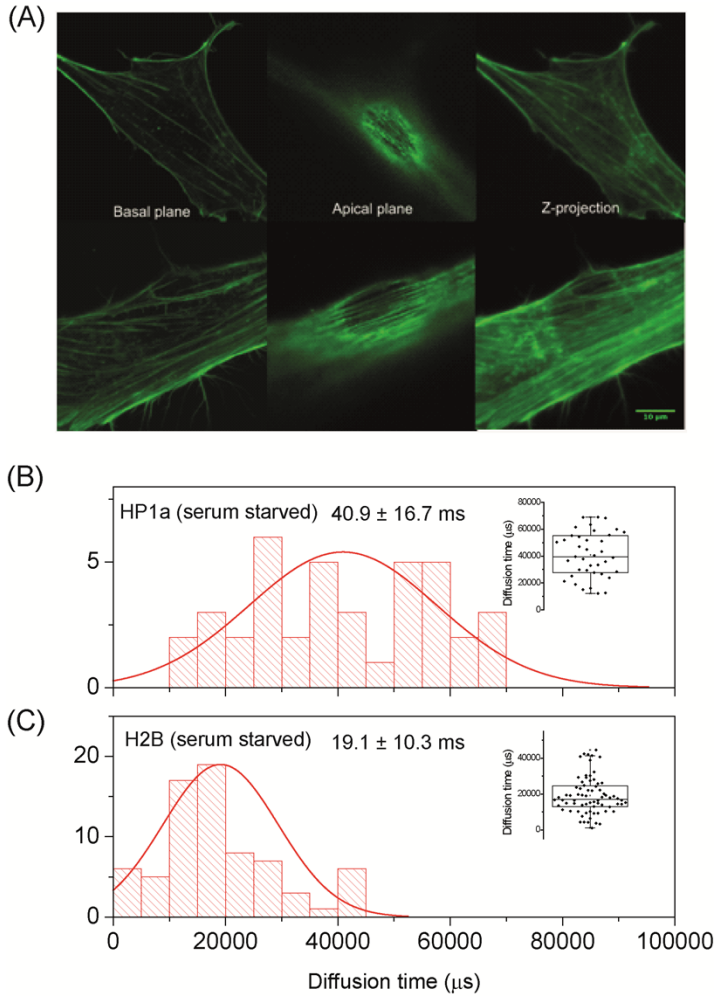

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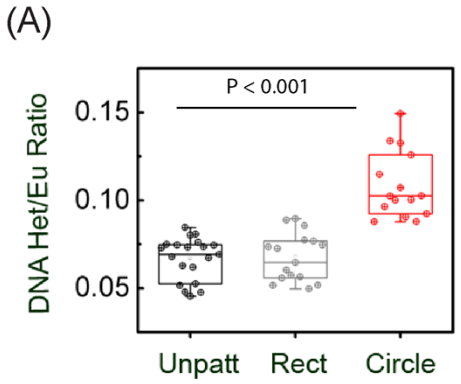

Suppl Figure S4.