Supplemental Materials

Molecular Biology of the Cell

Shimamoto et al.

Supplementary Information for

Nucleosome–nucleosome interactions via histone tails and linker DNA regulate nuclear rigidity

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This file includes:

Figures S1-6
Table S1
Caption for Movie S1

Other Supplementary Materials for this manuscript include the following:

Movie S1

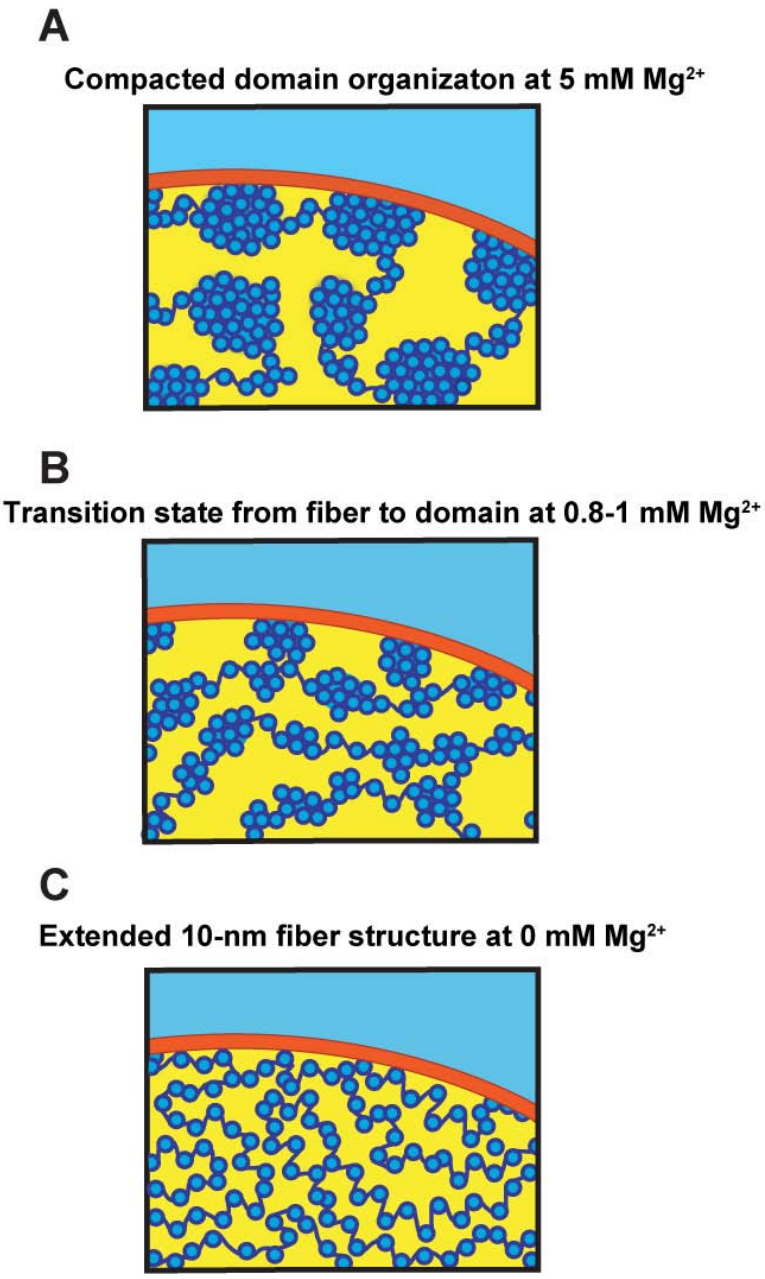

Figure S1. Chromatin organization in the cell nucleus.
Schematics showing possible folding architecture of the chromatin strand in the nucleus at different Mg^{2+} concentrations. Dark blue, DNA; light blue, core histones; red, nuclear envelope. (**A**) At 5 mM Mg^{2+}, chromatin is irregularly folded and assembles into compacted domain structures. (**B**) At 0.8-1 mM Mg^{2+}, chromatin is in a transition state. (**C**) At 0 mM Mg^{2+}, chromatin takes a highly extended form and exhibits an elongated 10-nm fiber structure.

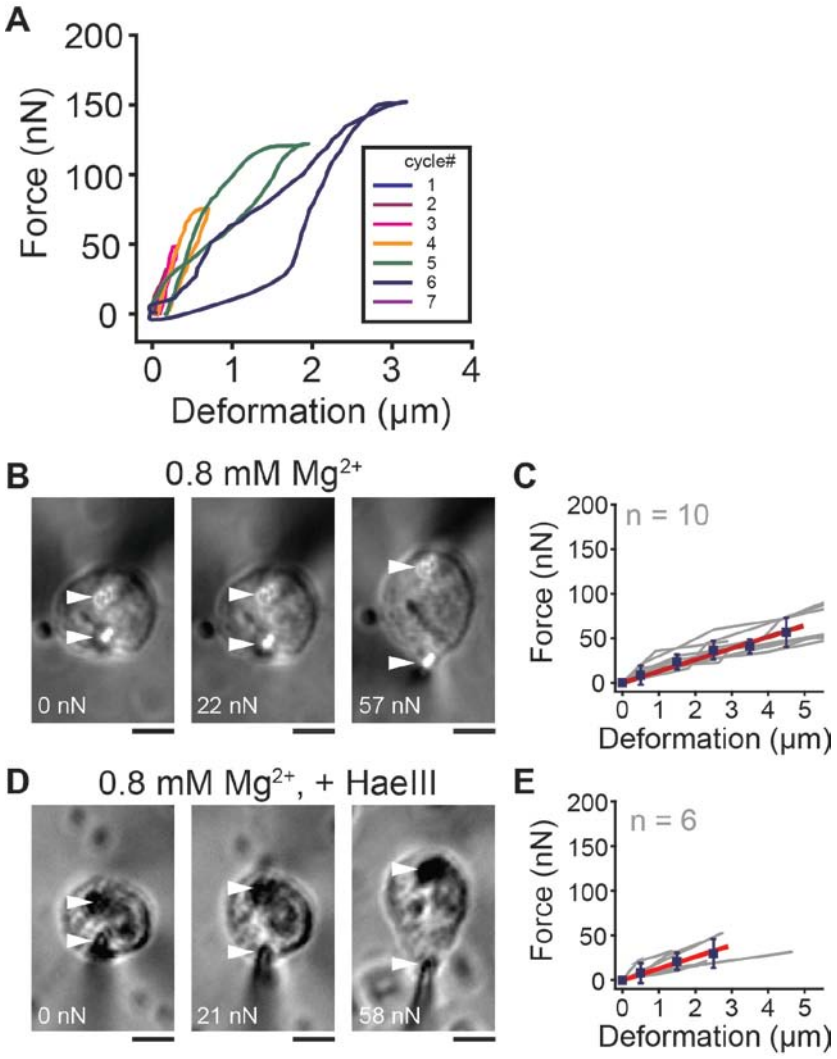

Figure S2. Additional data for nuclear micromechanics.
(**A**) Force−extension curve obtained from a nuclear mechanical response measurement. An isolated HeLa cell nucleus was exposed to 5 mM Mg^{2+} buffer and subjected to 7 cycles of stretch-hold-release micromanipulation with varying force magnitudes, using the microneedle-based setup. Time recordings of force and deformation were re-plotted to generate the trajectories. Colors indicate each cycle of micromanipulation. The openness of the loop in each trajectory represents the degree of viscous response. (**B-E**) Nuclear mechanical response measured at 0.8 mM Mg^{2+}. (**B**) DIC images showing an isolated HeLa cell nucleus, which was exposed to 0.8 mM Mg^{2+} buffer and stretched by the microneedle-based setup at indicated force magnitudes. Arrowheads indicate position of the microneedle tips. (**C**) Force–deformation relationship obtained from n = 10 nuclei at this condition. Data from individual nuclei (grey lines) were pooled for 0.5-μm bins and averaged (blue circles; bars are S.D.). Similar analysis was performed for HaeIII-treated nuclei (n = 6) (**D, E**). Linear regression yielded slopes of 12.8 (R^2 = 0.99) and 12.5 (R^2 = 0.99) for control and HaeIII-treated samples, respectively (red solid lines). Scale bars are 5 μm.

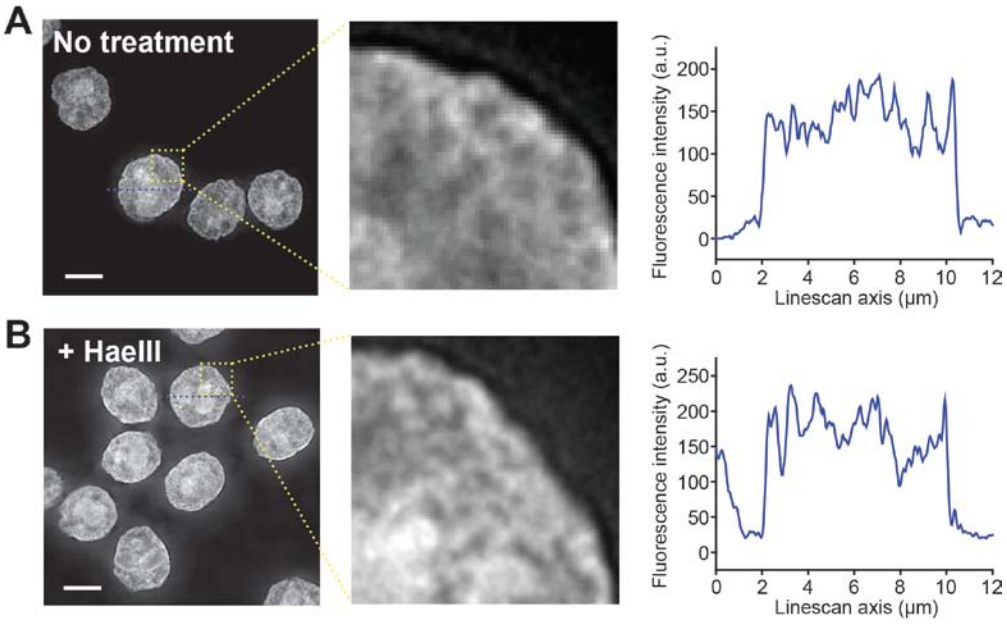

Figure S3. DNA staining patterns in control and HaeIII-treated nuclei.
For fluorescence images of DAPI-stained nuclei from untreated control (**A**) and HaeIII-treated samples (**B**) in Fig. 2C, magnified views in the highlighted areas (dashed yellow squares) and line-scan profiles across the nuclei (blue dotted lines) are presented. Scale bars are 5 μm.

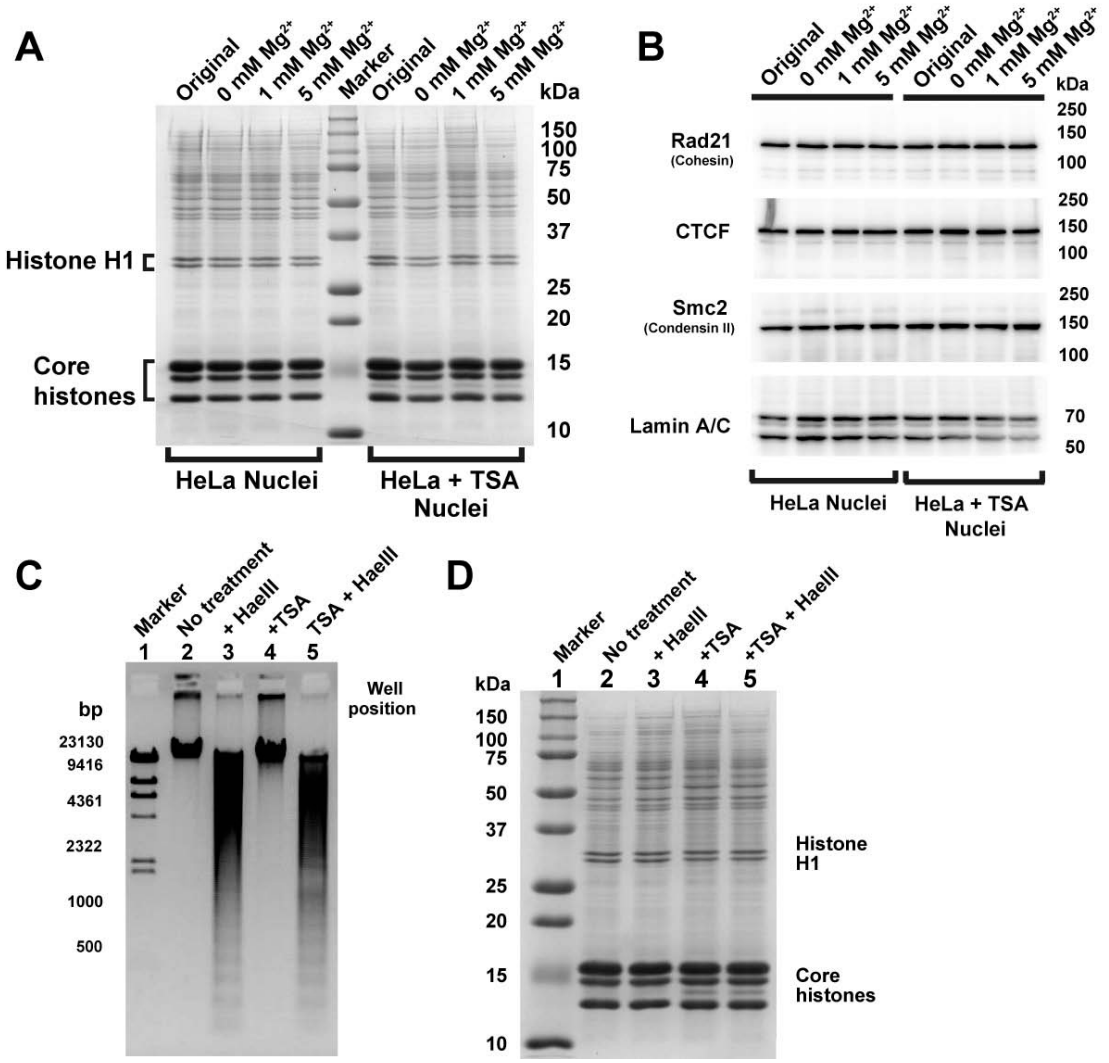

Figure S4. Additional data for biochemical analyses of nuclear components.
(**A**) Maintenance of nuclear components upon TSA treatment. Coomassie brilliant blue (CBB) stain showing the protein composition of untreated control (left four lanes, also provided in Fig. 1P) and TSA treated (right four lanes) nuclei at different concentrations of Mg^{2+}. (**B**) Maintenance of chromatin assembly factors. Western blot analysis showing the maintenance of Rad 21 (cohesin), CTCF, Smc2 (condensin II), and Lamin A/C in untreated control (left four lanes) and TSA-treated (right four lanes) nuclei examined at different concentrations of Mg^{2+}. Data in lanes 1-4 are also provided in Fig. 1Q. Data in lanes 4 and 8 are also provided in Fig. 3C. (**C**) Analysis of nuclear DNA digestion by HaeIII. Agarose gel electrophoresis analysis of DNA fragments purified from untreated control (lanes 2 and 3, also provided in Fig. 2A along with lane 1) and TSA-treated (lanes 4 and 5) nuclei before and after HaeIII digestion. (**D**) Maintenance of nuclear components upon TSA and HaeIII treatment. CBB stain showing the maintenance of major nuclear components in HaeIII-treated (lane 3) and TSA- and HaeIII-treated (lane 5) samples. Also shown are those of untreated control samples (lanes 2 and 4).

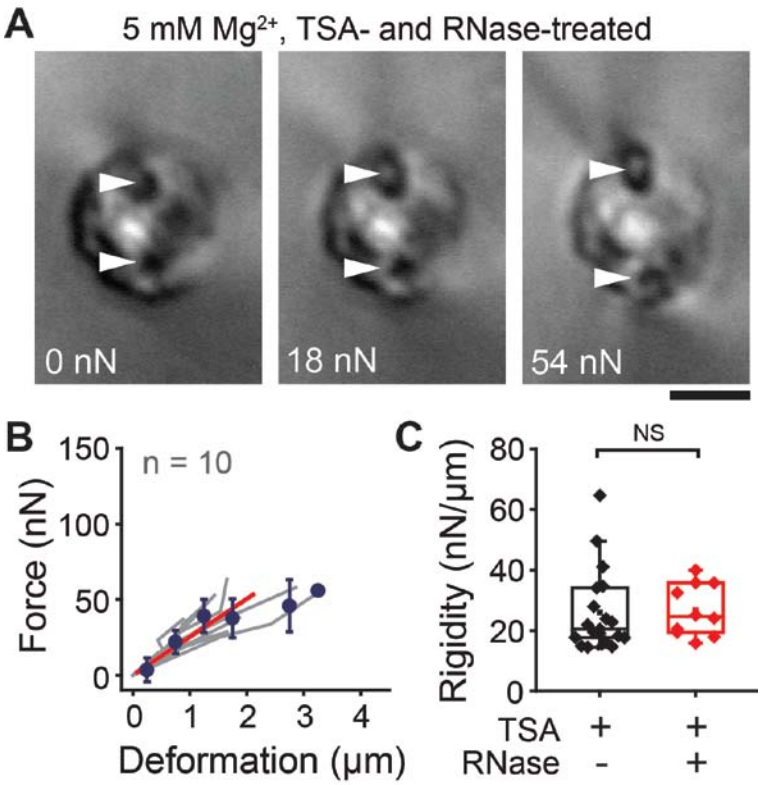

Figure S5. Mechanical response of isolated HeLa nuclei treated with RNase.
(**A**) DIC images showing an isolated HeLa cell nucleus treated with 100 μg/ml RNase A and then stretched by the microneedle-based setup at indicated force magnitudes. In this experiment, nuclei prepared from TSA-treated cells were treated with RNase and exposed to 5 mM Mg^{2+} buffer. Arrowheads indicate position of the microneedle tips. (**B**) Force–deformation plot obtained from n = 10 nuclei. Data from individual nuclei (grey lines) were pooled for 0.5-μm bins and averaged (blue circles; bars are S.D.). Linear regression yielded a slope of 25.2 (R^2 = 0.96) (red solid line). (**C**) Nuclear rigidity determined for control (n = 19, reproduced from Figure 3H) and RNase-treated nuclei (n = 10). Scale bar is 5 μm.

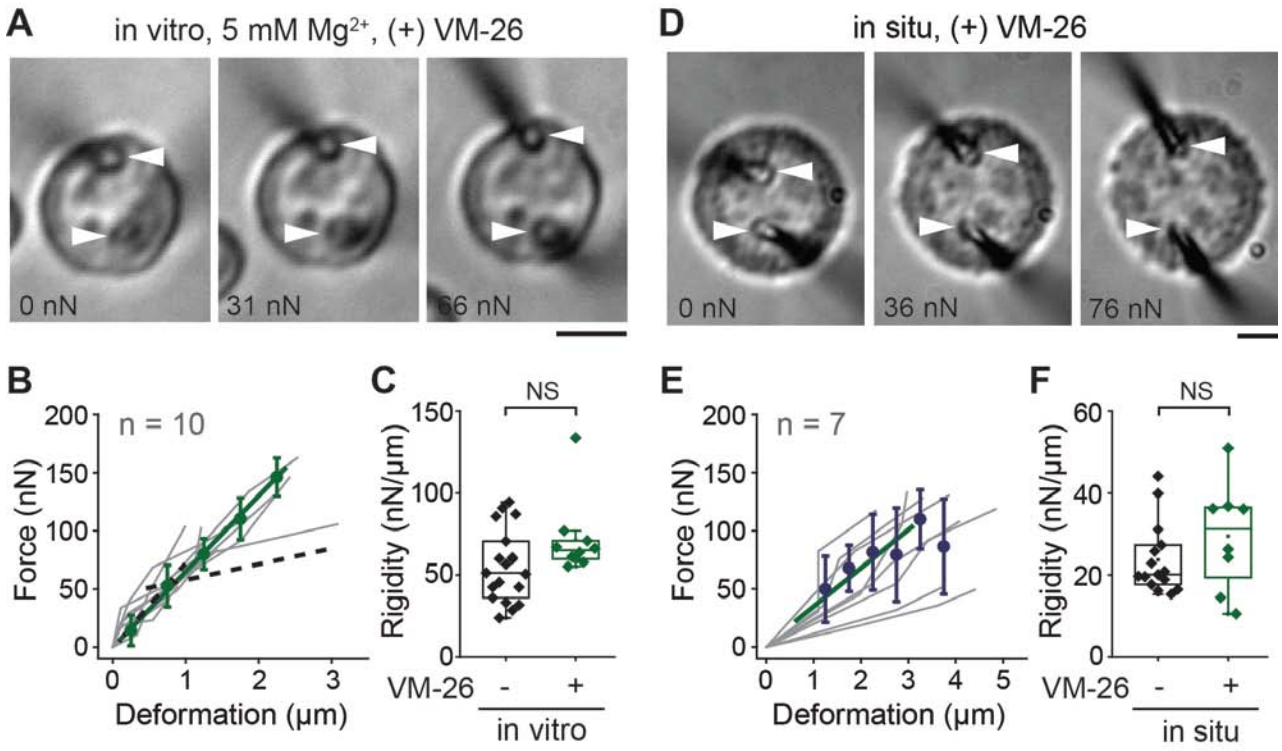

Figure S6. Mechanical response of nuclei upon inhibition of topoisomerase II.
(**A-C**) Mechanical response of isolated nuclei prepared from VM-26-treated HeLa cells. (**A**) DIC images showing an isolated nucleus prepared from a HeLa cell line after 2 h of 20 μM VM-26 treatment and then stretched by the microneedle-based setup at indicated force magnitudes. The nucleus was exposed to 5 mM Mg^{2+} buffer. The deformation arising in the structure was as large as that observed in the non-treated control. Arrowheads indicate position of the microneedle tips. (**B**) Force–deformation plot obtained from n = 10 nuclei. Data from individual nuclei (grey lines) were pooled for 0.5-μm bins and averaged (green circles; bars are S.D.). Linear regression yielded a slope of 64.5 (R^2 = 0.99) (green solid line). Dotted line shows the data analyzed for untreated control nuclei at the identical Mg^{2+} level (reproduced from Figure 1D). VM-26 treatment suppressed viscous softening of nuclei, which were typically observed at large deformations of non-treated nuclei samples. (**C**) Nuclear rigidity determined for untreated control (n = 19, reproduced from Figure 3H) and VM-26 treated nuclei (n = 10) within 1-μm deformation range. NS, not significant ($p > 0.1$) (Student's *t*-test). (**D-F**) *In situ* measurement of nuclear mechanical response upon VM-26 treatment. Experiments were performed in a setting similar to that described in Figure 4. (**D**) DIC images showing a VM-26 treated cell, in which the nucleus was stretched by inserting a pair of microneedles into the cell near the nuclear periphery (white arrowheads). (**E**) Force–deformation plot obtained from n = 8 cells. (**F**) Nuclear rigidity for untreated control (reproduced from Figure 4C) and VM-26 treated cells (n = 6). NS, not significant ($p > 0.1$) (Student's *t*-test). Scale bars are 5 μm.

TSA-treated H3

Histone H3.3 OS=*Homo sapiens* GN=H3F3A PE=1 SV=2 H33_HUMA
Sequence coverage: 57%
Modifications: Global: Carbamidomethyl (C) Optional: Acetyl (K) Oxidation (M)

Range: Start — End	Meas. M/z	Calc. MH+	Dev.(Da)	Sequence
11-19	901.504	901.521	-0.017	STGGKAPRK
11-19	985.599	985.542	0.057	STGGKAPRK 5: Acetyl (K) 9: Acetyl (K)
20 - 28	1070.701	1070.632	0.069	QLATKAARK 5: Acetyl (K) 9: Acetyl (K)
28 - 41	1449.868	1449.828	0.04	KSAPSTGGVKKPHR
29 - 41	1363.825	1363.744	0.081	SAPSTGGVKKPHR 9: Acetyl (K)
29 - 37	845.522	845.436	0.085	SAPSTGGVK 9: Acetyl (K)
42 - 50	1032.659	1032.595	0.064	YRPGTVALR
54 - 64	1406.85	1406.811	0.039	RYQKSTELLIR
55 - 65	1420.862	1420.816	0.047	YQKSTELLIRK 3: Acetyl (K)
58 - 64	831.51	831.493	0.017	STELLIR
66 - 73	1028.665	1028.636	0.028	LPFQRLVR
74 - 84	1335.79	1335.69	0.1	EIAQDFKTDLR
117 - 123	860.516	860.502	0.014	RVTIMPK 5: Oxidation (M)
117 - 123	902.536	902.513	0.024	RVTIMPK 5: Oxidation (M) 7: Acetyl (K)

Search Parameter: Title: Charge=1+ MS Tol.:200.000000 ppm MSMS Tol.:0.000000 Trypsin Mascot 2.5.1 SwissProt SwissProt_2016_11.fasta

TSA-treated H4

Histone H4 OS=*Homo sapiens* GN=HIST1H4A PE=1 SV=2 H4_HUMAN

Protein sequence coverage: 71%

Modifications: Global: Carbamidomethyl (C) Optional: Acetyl (K) Oxidation (M)

Range: Start — End	Meas. M/z	Calc. MH+	Dev.(Da)	Sequence
10-18	927.574	927.537	0.037	GLGKGGAKR 4: Acetyl (K) 8: Acetyl (K)
25 - 36	1325.851	1325.754	0.097	DNIQGITKPAIR
46 - 56	1336.827	1336.722	0.105	RISGLIYEETR
47 - 56	1180.706	1180.621	0.085	ISGLIYEETR
61 - 68	989.632	989.578	0.054	VFLENVIR
69 - 78	1134.598	1134.543	0.055	DAVTYTEHAK
80 - 92	1438.96	1438.797	0.162	KTVTAMDVVYALK
81 - 93	1482.955	1482.798	0.156	TVTAMDVVYALKR 5: Oxidation (M)
93 - 103	1211.774	1211.628	0.146	RQGRTLYGFGG

Search Parameter: Title: Charge=1+ MS Tol.:200.000000 ppm MSMS Tol.:0.000000 Trypsin Mascot 2.5.1 SwissProt SwissProt_2016_11.fasta

Table S1 Mass spectrometry analysis data for histone H3 and H4 upon TSA-treatment of HeLa cells

Movie Caption

Movie S1

Time-lapse movie showing an isolated single HeLa nucleus that was exposed to 5 mM Mg^{2+} buffer and stretched by the microneedle-based setup. The motion of the upper microneedle resulted in stretching of the nucleus. The tip of the lower microneedle responded to this motion, generating a calibrated force, the magnitude of which is proportional to the tip's displacement from equilibrium. In this example, mechanical stretches were applied a total of seven times, with a force magnitude of 13, 23, 33, 45, 65, 77, and 90 nN. The movie was recorded at a sampling rate of 10 frames/s and is presented at 2× speed. Scale bar is 5 μm.