Supplemental materials

Title: Regulation of cellular dynamics and chromosomal binding site preference of linker histones

Running title: Regulation of histone H1 variants

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Supplemental Figures

Supplemental Fig S1

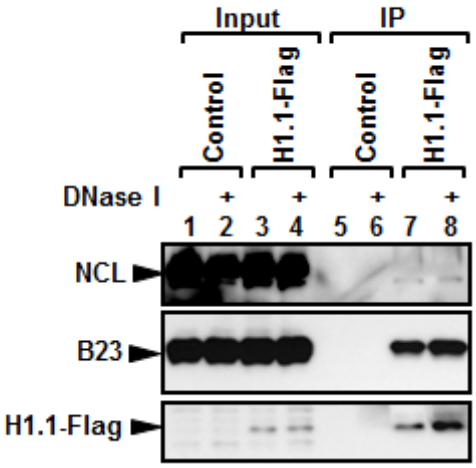

Fig. S1. H1.1-Flag associates with B23 and NCL in the extracts treated with or without DNase I. The nuclear extracts were prepared from control HeLa cells or HeLa cells expressing H1.1-Flag and treated without or with RNase-free DNase I. The extracts were subjected to immunoprecipitation with anti-Flag antibody and the precipitated proteins were analyzed by western blotting.

Supplemental Fig S2

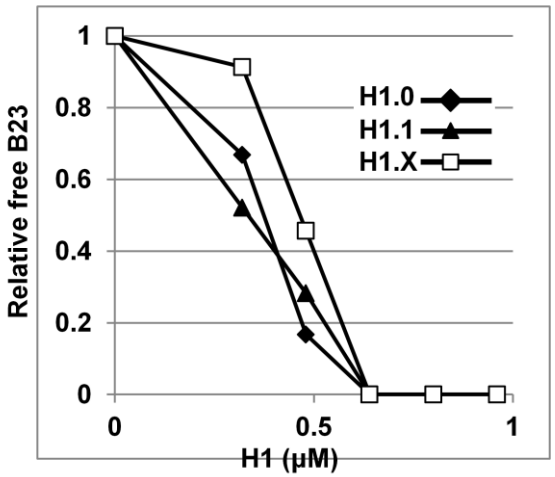

Fig. S2 Quantitative analysis of linker histone binding of B23. Increasing amounts of linker histones were mixed with constant amount of B23, and the complex were separated by 6% native PAGE. Relative free B23 amount shown in Fig, 3E top panel was estimate by Image J software and plotted as a function of the amounts of linker histones.

Supplemental Fig S3

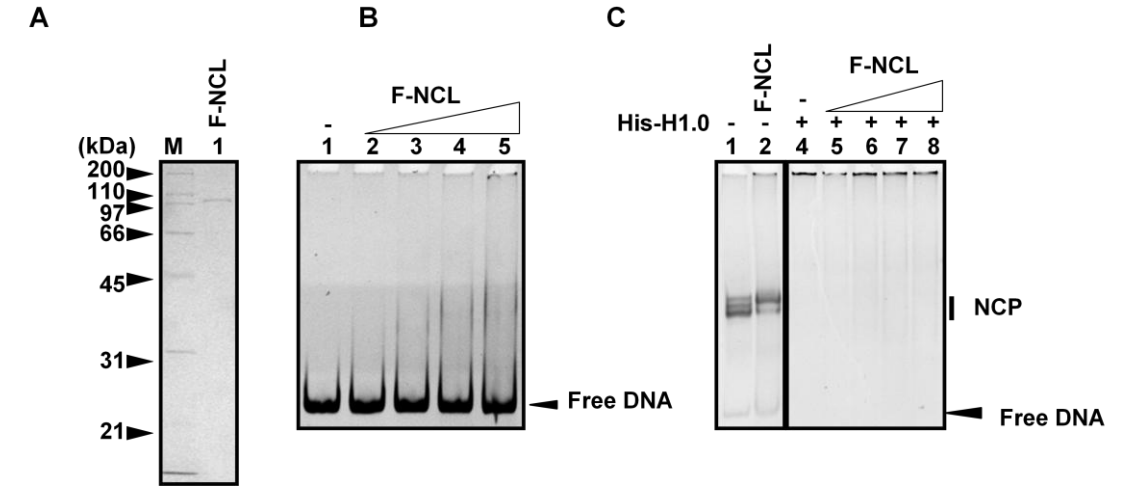

Fig. S3. Linker histone chaperone activity of NCL. A. Recombinant Flag-NCL was expressed in 293T cells and purified with anti-Flag affinity gel. The Flag-NCL protein (200 ng) was separated on 10% SDS-PAGE and visualized with CBB staining. B. DNA binding activity of NCL. Free DNA (0.06 µM) was mixed with increasing amounts of Flag-NCL (0.015, 0.03, 0.06, and 0.12 µM), the complex was separated by native PAGE, and DNA was visualized with GelRed staining. C. Linker histone chaperone activity of NCL. NCPs assembled on 196 bp DNA was incubated without or with NCL (lanes 1 and 2, respectively.) NCPs (0.01 µM) mixed with His-H1.0 (0.06 µM) were incubated without or with increasing amount of Flag-NCL (0.045, 0.09, 0.135, and 0.18 µM), and the complexes were separated by native PAGE.

Supplemental Fig S4

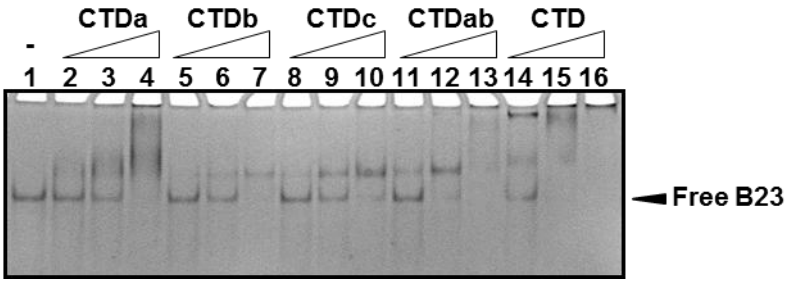

Fig. S4. B23 binding of the CTD peptides. His-B23 (1.6 μM) was incubated without (lane 1) or with increasing amounts of GST-H1.0-CTD peptides (0.6, 1.2, and 1.8 μM), separated by 6% native PAGE, and visualized with CBB staining. Position of free B23 is shown by arrowhead.

Supplemental Fig. S5

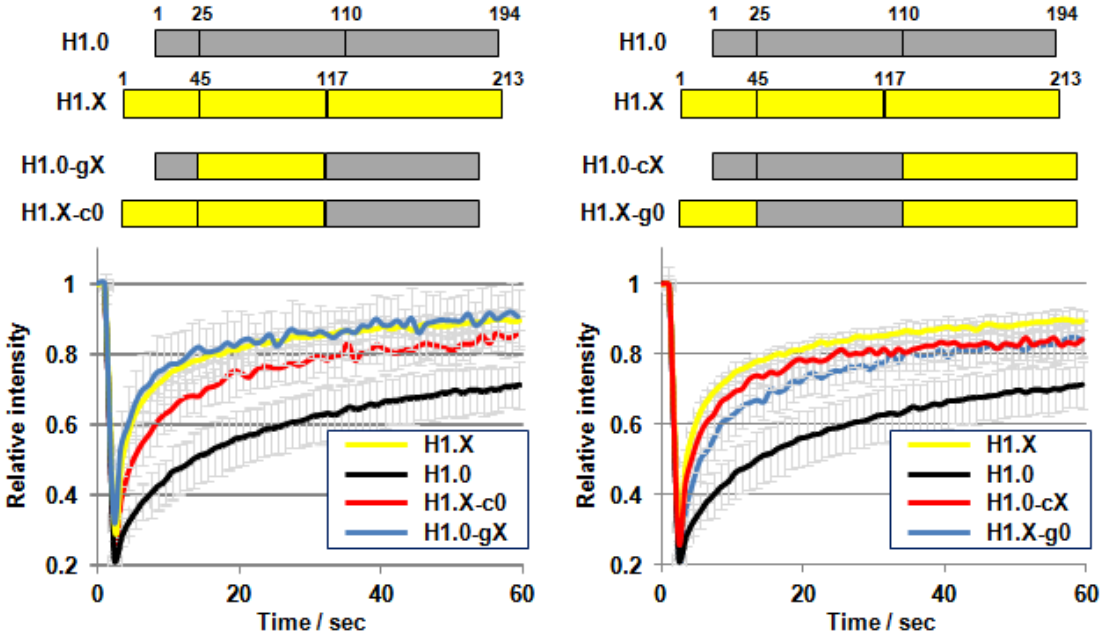

Fig. S5. FRAP assay of the H1.0 and H1.X chimeric proteins. FRAP assays were performed as described in Fig. 5 and the results for H1.0-gX and H1.X-c0 (left panel) and those for H1.0-cX and H1.X-g0 (right panel) are shown in the same graphs to show the effect of the NTD on the mobility of linker histones. H1.0-gX and H1.X-c0 have the GD and CTD of H1.X and H1.0, respectively, whereas H1.0-cX and H1.X-g0 have the GD and CTD of H1.0 and H1.X, respectively. The protein structures are represented schematically at the top of the graphs. The data sets shown are exactly same shown in Fig. 5.

Supplementary Fig. S6

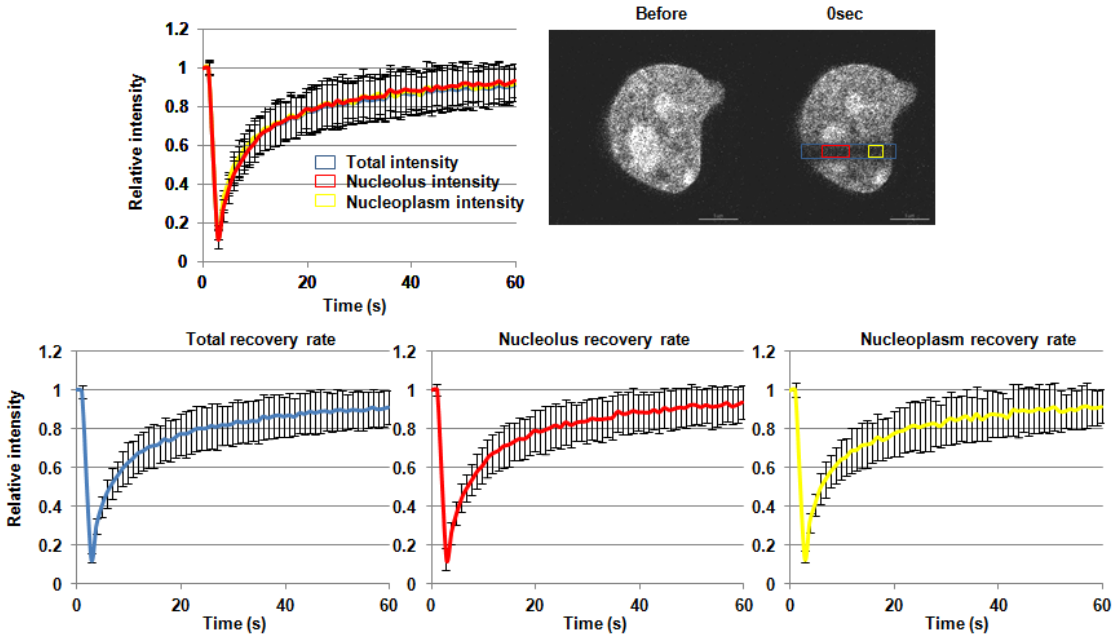

Fig. S6 FRAP assay of H1.X. EGFP-H1.X was transiently expressed in HeLa cells and FRAP assays were performed. The region indicated by a blue box including the nucleolus were bleached with a 488 nm laser line and EGFP intensity in total bleached area (blue), nucleolar region (red), and the nucleoplasmic region (yellow) was quantitatively analyzed. The graph indicated at the top left shows three recovery curves in one graph.

, Supplemental Tables

Primer sequences for plasmid vector construction

Primer	Sequence
H1.0-1	agctggatccgccgccaccatgaccgagaattccacgtc
H1.0-2	agcggatcctcacttcttcttgccggccc
H1.1-1	agctggatccgccgccaccatgtctgaaacagtgcctcc
H1.1-2	agcggatccttacttttttcttgggtgccg
H1.X-1	agctggatccgccgccacccatatgtccgtggagctcgagga
H1.X-2	agcggatcctcacttgcggcccttgggca
H1.0-3	aaaggatccttcaagaagaccaag
H1.0-4	agct gaattc tcaggttggggctttggaggca
H1.0-5	Agct ggatcc aagaaacccaaagccacccc
H1.0-6	agct gaattc tcatttgacagtcttgggttttt
H1.0-7	agct ggatcc gccaagccggtcaaggcatc
H1.X-3	aaaggatccctggcgggcggcggg
H1.0-cX-1	tcttgcggttcttcttgaaggccactga
H1.0-cX-2	cttcaagaagaaccgcaagaagctggag
H1.X-c0-1	ccttcttggtgagcttgaaggaaccgtt
H1.X-c0-2	cttcaagctcaccaagaaggaaatcaag
H1.0-nX-1	aagaagaagaaccaccccaagtattcagac
H1.0-nX-2	atacttggggtggttcttcttcttgctatt
H1.X-n0-1	aagtccacagaccagccgggcaagtacagc
H1.X-n0-2	cttgcccggctggtctgtggacttcttgga
H1.0-gX-1	aagtccacagaccagccgggcaagtacagc
H1.0-gX-2	cttgcccggctggtctgtggacttcttgga
H1.X-g0-1	aagaagaagaaccaccccaagtattcagac
H1.X-g0-2	atacttggggtggttcttcttcttgctatt
H1.X-E73K-F	tacaccaaggccaagaaggttccgt
H1.X-E73K-R	cttcttggccttggtgtagatcttggc
H1.0-K52E-F	tccattcaggagtatatcaagagc
H1.0-K52E-R	cttgatatactcctgaatggactggcg

Table S2. Primer sequences for ChIP assay

Primer	Sequence
5S-F	tccggtaccgtctacggccataccaccctga
5S-R	ggggtaccttcgaaagcctacagcacccggta
RNU2-F	tccaagcttatcgcttctcggccttttgg
RNU2-R	tttggatccgggtgcaccgttcctggagg
CES1-F	ggattaggcaattggcagcg
CES1-R	agtggccaggataaaggcac