# Supplementary Material for "A Robust Method for the Purification and Characterization of Recombinant Human

# Histone H1 Variants"

Adewola Osunsade<sup>1,2</sup>, Nicholas A. Prescott<sup>1,2</sup>, Jakob M. Hebert<sup>1,2</sup>, Devin M. Ray<sup>1,2,3</sup>, Yazen Jmeian<sup>4</sup>, Ivo C. Lorenz<sup>4</sup>, and Yael David<sup>1,2,5,6</sup>\*

1Chemical Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY

2Tri-Institutional PhD Program in Chemical Biology, New York, NY

3Tri-Institutional MD-PhD Program, New York, NY

4Tri-Institutional Therapeutics Discovery Institute, New York, NY

5Department of Pharmacology, Weill Cornell Medical College, New York, NY

6Department of Physiology, Biophysics and Systems Biology, Weill Cornell Medical College, New York, NY

#### Methods

#### **General Materials and Methods**

Analytical reversed-phase HPLC (RP-HPLC) was performed on a Agilent 1200 series instrument with an Agilent C18 column (5 µm, 4 × 150 mm), employing 0.1 % TFA in water (HPLC solvent A), and 90 % acetonitrile, 0.1 % TFA in water (HPLC solvent B), as the mobile phases. Analytical gradients were 0-70 % HPLC buffer B over 30 min at a flow rate of 0.5 mL/min, unless stated otherwise. Preparative scale purifications were conducted on an Agilent LC system. An Agilent C18 preparative column (15-20 µm, 20 × 250 mm) or a semi-preparative column (12 µm, 10 mm × 250 mm) was employed at a flow rate of 20 mL/min or 4 mL/min, respectively. HPLC Electrospray ionization MS (HPLC-ESI-MS) analysis was performed on an Agilent 6120 Quadrupole LC/MS spectrometer (Agilent Technologies). UV spectrometry was performed on NanoDrop 2000c (Thermo Scientific). Biochemicals and media were purchased from Fisher Scientific or Sigma-Aldrich Corporation unless otherwise stated. T4 DNA ligase, DNA polymerase and restriction enzymes were obtained from New England BioLabs. Primer synthesis and DNA sequencing were performed by Integrated DNA Technologies and Genewiz, respectively, PCR amplifications were performed on a Bio-Rad T100<sup>™</sup> Thermal Cycler. Centrifugal filtration units were purchased from Sartorius, and MINI dialysis units purchased from Pierce. Size exclusion chromatography was performed on an AKTA FPLC system from GE Healthcare equipped with a P-920 pump and UPC-900 monitor. Sephacryl S-200 columns were obtained from GE Healthcare. All the western blots were performed using the primary antibodies and fluorophore-labeled secondary antibodies annotated in Table S1 following the protocol recommended by the manufacture. Blots were imaged on Odyssey CLx Imaging System (Li-Cor).

#### Linker Histone Purification:

All linker histones were purified as follows: The His-SUMO-H1-GyrA-His construct (Table S2) was cloned into a pET21 vector backbone and transformed into Rosetta DE3 E. coli cells. Six liters of culture were grown at 37 °C under ampicillin and chloramphenicol selections until OD600 reached 0.6, followed by the induction of protein expression with 0.5 mM IPTG overnight at 16 °C. Bacteria were harvested by centrifugation at 5000 xg for 20 minutes at 4 °C. Bacterial pellets were resuspended in 15 mL of chilled H1 Lysis Buffer (20 mM Tris pH 7.5, 200 mM NaCl, 1 mM PMSF) and lysed by sonication (amplitude: 25, 1 min 30 s total time, 15 s on and 10 s off). Lysate was then clarified by centrifugation at 17,000 xg for 20 minutes at 4 °C. The lysate was poured onto 3 mL of Ni-NTA beads equilibrated with H1 Lysis Buffer, and incubated with agitation at 4 °C for one hour. The flow-through was discarded, and the beads were then washed with 10 mL of H1 Wash Buffer (50 mM Imidazole, 20 mM Tris pH 7.5, 200 mM NaCl, 1 mM PMSF). The protein was eluted with 5 mL of pre-chilled H1 Elution Buffer (500 mM Imidazole, 20 mM Tris pH 7.5, 200 mM NaCl. 1 mM PMSF). The elution was injected onto a size-exclusion column (GE Life sciences preparative scale S200 10/300). Fractions containing full-length His-SUMO-H1-GyrA-His were pooled, and DTT was added to a final concentration of 1 mM. The sample was treated with the enzyme Ulp-1 (1:100 v/v of 4 mg/mL enzyme) with agitation for one hour at room temperature. followed by a six-hour treatment at room temperature with 500 mM β-ME. Solid urea was added to a final concentration of 8 M, and the  $\beta$ -ME was dialyzed out. The sample was then poured onto 5 mL of Ni-NTA beads equilibrated with H1 Denaturing Buffer (8 M Urea, 20 mM Tris pH 7.5, 200 mM NaCl), and mixed at 4 °C for one hour. The flowthrough was collected and the pH was adjusted to 9. The sample was manually loaded onto an equilibrated HiTrap HP Cation Exchange column, and further purified with a gradient of H1 Purification Buffer A (8 M Urea, 20 mM Tris pH 9, 200 mM NaCl) and H1 Purification Buffer B (8 M Urea, 20 mM Tris pH 9, 1.5 M NaCl). Fractions containing full-length H1 were pooled and refolded in a stepwise manner (8 M  $\rightarrow$  4 M  $\rightarrow$  2 M  $\rightarrow$ 0 M Urea). The final buffer conditions are 20 mM Tris pH 7.5, 200 mM NaCl. H1 concentration

was calculated by using the absorbance at 214 nm and extinction coefficient ( $\mathcal{E}_{214}$ , determined based on each variant's amino acid composition)<sup>1</sup>.

#### **Recombinant histone purification**

Unmodified human histones were purified from E. coli as described before<sup>2</sup>. Briefly, BL-21 cells transformed with the expression plasmid for each of the canonical histones were grown at 37 °C under ampicillin selection in LB to OD<sub>600</sub> = 0.6 and then induced with 0.5 mM final IPTG at 37 °C for 3 hr. Cells were harvested by centrifugation at 4500 xg for 15 minutes at 4 °C. Bacterial pellets were resuspended in 40 mL of lysis buffer (50 mM HEPES pH 7, 300 mM NaCl) and lysed by sonication and passage through a French press. Lysates were cleared by a 20 minute spin at 17,000 xg at 4 °C. The supernatants were discarded and inclusion body pellets were washed once with lysis buffer containing 1 % Triton X-100 and then once with lysis buffer without detergent. The proteins were extracted from the inclusion bodies with extraction buffer (20 mM Tris pH 7.5, 6 M guanidine hydrochloride, 1 mM EDTA, 100 mM NaCl) under agitation for one hour at room temperature. After a 20,000 xg 30 minute centrifugation the supernatant was dialyzed against unfolding buffer A (7 M Urea, 10 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl, 1 mM DTT) overnight at 4 °C. The dialyzed supernatant was then loaded on a pre-washed Hi-Trap SP HP 5 ml column three times after which the unbound was washed with unfolding buffer A. The histones were eluted on a 45 minute gradient between unfolding buffer A and unfolding buffer B (7 M Urea, 10 mM Tris pH 7.5, 1 mM EDTA, 1 M NaCl, 1 mM DTT). The histone-containing fractions were pooled and further purified on a preparative C-18 RP-HPLC and a gradient of 40 -70 % HPLC solvent B. The purified histones were analyzed by RP-HPLC and their masses were verified by ESI-MS.

#### Histone octamer formation

Assembly of histones into a protein octamer was performed as described before<sup>2</sup>. Each purified lyophilized histone was dissolved in unfolding buffer (20 mM Tris pH 7.5, 6 M guanidine hydrochloride, 1 mM DTT). The histone concentration was evaluated by A280 measurements and histones were combined in an equimolar ratio with 5 % excess of H2A and H2B. The total histone concentration was adjusted to 1 mg/ml and the pooled histones were dialyzed against refolding buffer (10 mM Tris pH 7.5, 2 M NaCl, 1 mM EDTA, 1 mM DTT) with three exchanges, one of which was overnight. The mixture was recovered and cleared by a 10-minute centrifugation at 17,000 xg, 4 °C. The supernatant was concentrated on a 10,000 Da concentrator to under 500µl and injected onto a S200 10/300 size exclusion column on an AKTA FPLC. The octamer containing fractions were pooled and concentrated on a 10,000 Da MWCO centrifugal filter unit. 50 % glycerol (v/v) was added, and the final octamer concentration was measured by A280.

#### Linker DNA preparation

A 30 bp linker DNA fragment was added to the 3' end of the nucleosome positioning sequence ('601')<sup>3</sup> by PCR amplification. A 40X PCR reaction was prepared with Phusion polymerase following the manufacturer's protocol in the presence of a 5'-CTGGAGAATCCCGGTGCCGAGG primer and a 3'-

GGCGGCCGCGTAGTACTGGATCTTACATGCACAGGATGTATATATCTGACACG primer. The 40 reactions were pooled and a sample was analyzed on an agarose gel stained with EtBr. The pooled reactions were purified on 15 Qiagen PCR purification columns, following the manufacturer's protocol. Each column was eluted with 100 µL of water, after which the eluents were pooled and lyophilized. The DNA was resuspended in water and quantified by A260.

#### Nucleosome assembly

Nucleosome assembly was performed as described before with minor changes<sup>4</sup>. The reactions were performed at 2  $\mu$ M concentration and 10  $\mu$ L scale in 10 mM Tris pH 7.5, 1 mM EDTA, 1 mM DTT, 2 M NaCl. Reactions were put at 37 °C for 15 minutes after which they were transferred to 30 °C. At subsequent 15 minute intervals, dilution buffer (10 mM Tris pH 7.5, 1 mM EDTA, 1 mM DTT, 10 mM NaCl) was added in the following volumes ( $\mu$ L): 6.7, 5, 3.6, 4.7, 6.7, 10, 30, 20, 100. The DNA:octamer ratio was tested experimentally and determined for each octamer preparation. Nucleosomes were analyzed on a 5 % acrylamide TBE native gel to determine their quality. Nucleosomes of suitable quality were pooled and quantified by A260.

#### Nucleosome-array reconstitution

12-mer Nucleosome arrays were prepared as described previously with minor alterations<sup>5</sup>. Briefly, 12-mer DNA (containing 12 repeats of the 601 sequences with 30 bp of linker DNA between them), octamers, buffer DNA (mouse mammary tumor virus, or MMTV), and linker histones were mixed under high salt conditions (2 M TEN buffer: 10 mM Tris pH 7.6, 0.1 mM EDTA, 2 M NaCl, 1 mM DTT). Preparations were done with 0.25-0.5  $\mu$ M 601 sites in 50-100  $\mu$ L volumes with experimentally determined DNA:octamer ratios. To ensure full saturation with linker histone, H1 was used in 1.5 molar excess relative to 601 sites. The samples were dialyzed at 4 °C in 3.5 kDa MWCO Slide-A-Lyzer MINI units (Fischer Scientific) into 1.4 M TEN buffer (10 mM Tris pH 7.6, 0.1 mM EDTA, 1.4 M NaCl, 1 mM DTT) for one hour. Using a peristaltic pump at a flow rate of 2.5 mL/min, the arrays were diluted into 0.5 M TEN buffer (10 mM Tris pH 7.6, 0.1 mM EDTA, 0.5 M NaCl, 1 mM DTT) over the course of seven hours. Dialysis into 0.1 M TEN buffer (10 mM Tris pH 7.6, 0.1 mM EDTA, 10 mM NaCl, 1 mM DTT) was performed overnight. Assembled arrays were purified from unassembled materials as described previously<sup>5</sup>. The concentration of arrays was determined by A260.

#### **Magnesium Precipitation**

The compaction state of arrays was tested using magnesium precipitation as previously reported<sup>5</sup>. Briefly, purified arrays were treated with  $MgCl_2$  in 0.25 mM increments starting at 0.5 mM and left on ice for 10 minutes. They were then centrifuged at 17,000 xg for 10 minutes at 4 °C. Following this, the supernatant was carefully removed and the A260 was measured. Three technical replicates were performed for each  $Mg^{2+}$  concentration.

#### **MNase Digestion**

Two pmol of 12-mer arrays were mixed with 10 X MNase digestion buffer (500 mM Tris pH 7.9, 50 mM CaCl<sub>2</sub>) and 1  $\mu$ L MNase (2,000 units/ $\mu$ L) in a total volume of 10  $\mu$ L. The reaction was performed on ice for either 10, 60, or 300 seconds, then quenched with MNase quench buffer (0.4 M NaCl, 0.2 % w/v SDS, 20 mM EGTA). The DNA was purified using a PCR purification kit (QIAGEN), and loaded onto a 5 % native PAGE TBE gel in 0.5 X TBE. Gels were run at 130 V for 30 minutes and then stained with ethidium bromide before imaging on an Amersham Al600 imager. The amount of full-length 12-mer DNA remaining at each time point was calculated by measuring the intensity of the top band of the gel by densitometry using Li-Cor Image Studio software.

#### **Biolayer Interferometry**

Biolayer interferometry on an Octet Red96e system (PALL/ForteBio) was used to characterize the binding kinetics between the linker histone variants and mononucleosomes. NCPs with biotinylated linker DNA were immobilized on the sensor surface of streptavidin-coated biosensors (PALL/ForteBio). Sensors were pre-blocked in PBS (pH 7.4). NCPs were diluted to a

concentration of 1 µg/mL with manufacturer-supplied Kinetics Buffer (PBS, pH 7.4 + 0.02 % Tween20, 0.1 % BSA, 0.05 % NaN<sub>3</sub>). A five-step 2-fold dilution series of each linker histone was prepared in manufacturer-supplied Kinetics Buffer, starting at 10 nM. Typical binding kinetics assays were performed at 23 °C with a 180 s association phase and a 1200 s disassociation phase. All experimental samples were referenced against streptavidin sensors in buffer without linker histone added. Data analysis was performed using Octet data analysis software version 11.0 (Pall/ForteBio) using curve fitting to a 1:1 model for estimation of kinetic parameters. Kinetic data reported are derived from global fitting of replicates at five protein concentrations, with standard error values calculated from the analysis software. Goodness of fit was analyzed by examining residual plots for the fitted curves, as well as the R<sup>2</sup> and  $\chi^2$  values of each fit.

#### Circular Dichroism (CD)

Purified H1 proteins were analyzed by Circular Dichroism. Briefly, wavelength scans (190-280 nm; bandwidth, 1 nm; time constant, 100 ms) were performed at ambient temperature on purified protein samples (of varying concentrations) using a high-precision quartz cell (Hellma Analytics, 106-0.20-40; path length, 0.2 mm) and either an AVIV 420 CD instrument or a Chirascan V100 instrument. Three scans were collected for each sample, sampling per nm with an averaging time of 3 s and with no wait time between scans. Scans were averaged for each sample, and the value for each wavelength was subtracted from a value for the protein buffer. Data were analyzed by calculating mean residual ellipticity (MRE), scaling slightly to overlay if needed, and plotted in GraphPad Prism.

#### Supplementary References:

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(3) Lowary, P. T., and Widom, J. (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning11Edited by T. Richmond. *J. Mol. Biol.* 276, 19–42.

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## Supplementary Figures

Unconserved	01	2	3	45	67	789	10	Conserved

	10		)		
H1 0					APAAKPKRAK
H1 1		<mark>se</mark>	TVPPAPAASA	APE-KPLAGK	KAKKPAKAAA
H1_2		<mark>se</mark>	TAPAAPAAAP	PAE-KAPVKK	KAAKKAG
H1_3		<mark>se</mark>	TAPLAP <mark>T</mark> IPA	PAE-KTPVKK	KAKKAGAT
H1_4		<mark>se</mark>	TAPAAP <mark>A</mark> APA	PAE-KTPVKK	KARKSAG-
н1_5		<mark>S E</mark>	TAPAET <mark>A</mark> TPA	PVE-KSPAK <mark>K</mark>	KATKKAAGAG
Hlfnt	- EQALTGEAQ	SRWPRRGG <mark>SG</mark>	AMAEAP GPSG	ESR-GHSATQ	L P A E K T V G G P
Hlt		<mark>se</mark>	TVPAAS <mark>A</mark> SAG	VAA <mark>M</mark> EKLPT <mark>K</mark>	KRGRKPAGLI
Hlx		<mark>SVEL</mark> EE	ALPVTTAEGM	AKK <mark>V</mark> TKAGG <mark>S</mark>	AAL SPSKKRK
Hloo	<mark>APG</mark>	SVTSDISPSS	T S T A G S S R S P	ESEKPGPSHG	GVPPGGPSHS
HILS1	LHASTIWHLR	STPPRRKQWG	HCDPHRILVA	SEVTTE	ITSPTPAP
Consistency	0000000000	0000000055	5454445 <mark>334</mark>	334 <mark>0</mark> 43444 <mark>6</mark>	4545431332
	60	70	80	90	100
H1_0	A S K K S T D H P K	YSD <mark>MIVA</mark> AIQ	AEKNR <mark>A</mark> G <mark>S</mark> SR	QSIQK <mark>Y</mark> IKSH	Y KVGENAD
H1_1	A S K K K P A G <mark>P</mark> S	VSE <mark>LIVQ</mark> AAS	SSKER <mark>g</mark> vsl	AALKK <mark>A</mark> LAAA	G – – YDVEK <mark>NN</mark>
H1_2	GTPRK <mark>AS</mark> G <mark>P</mark> P	VSE <mark>LITKAV</mark> A	A SKER <mark>S</mark> G <mark>V</mark> SL	AALKKA <mark>L</mark> AAA	G – – <mark>YD VE K</mark> NN
н1_3	A G K R K A S G <mark>P P</mark>	VSE <mark>LITKAV</mark> A	A SKER <mark>S</mark> G <mark>V</mark> SL	AALKKA <mark>L</mark> AAA	G – – <mark>YDVEKNN</mark>
H1_4	A A K R K A S G <mark>P P</mark>	VSE <mark>LITKAV</mark> A	A SKER <mark>S</mark> GVSL	AALKKA <mark>L</mark> AAA	G – – <mark>YDVEKNN</mark>
н1_5	AAKRKAT <mark>G</mark> PP	VSE <mark>LITKAV</mark> A	A S K E R <mark>N</mark> G L S L	AALKKA <mark>L</mark> AAG	G – – YDVEK <mark>NN</mark>
Hlfnt	S R G C S S S <mark>V</mark> L R	VSQ <mark>LVLQAIS</mark>	THK <mark>GL</mark> TL	AALKKE <mark>LRNA</mark>	<mark>G</mark> – – <mark>YEVRRKS</mark>
Hlt	S A S R K V P N L S	VSK <mark>LITEALS</mark>	V S Q E R <mark>V G</mark> M S L	VALKK <mark>A</mark> LAAA	G – – YDVEKNN
H1x	N S K K K N Q P <mark>G</mark> K	YSQ <mark>LVVE</mark> TIR	R L G E R N <mark>G S S L</mark>	AKIYT <mark>E</mark> AKKV	PW-FDQQNGR
H100	S L P V G R R H P P	VLR <mark>MVLEAL</mark> Q	AGEQRR <mark>G</mark> TSV	AAIKL <mark>Y</mark> ILHK	Y PT V D V L R F K
HILS1	RAQVCGGQ <mark>PW</mark>	VTV <mark>L</mark> DPLSGH	TGR	EAERHF <mark>ATVS</mark>	I SAVELKYCH
Consistency	55555 <mark>443</mark> 54	775 <mark>9</mark> 755865	<mark>6555628476</mark>	78776 <mark>4</mark> 7555	400 <mark>67666</mark> 45
	11(	) 13	20 13	0 14(	150
H1 0	SOTKISTERI				<b>K</b>
H1 1	SBIKLGIKSL	VSKGTLV0	-TKGTGASGS	FKLNKKASSV	ETKPG
H1 2	SRIKLGLKSL	VSKGTLV0	-TKGTGASGS	FKLNKKAASG	EAKPK
н1 3	SRIKLGLKSL	VSKGTLV0	- TKGTGASGS	FKLNKKAASG	EGKPK
H1 4	SRIKLGLKSL	VSKGTLVQ	- TKGTGASGS	FKLNKKAASG	EAKPK
H1_5	SRIKLGLKSL	VSKGTLVQ	- TKGTGASGS	FKLNKKAASG	EAKPK
H1fnt	GRHEAPRG	QAKATLLR	- <mark>VSGSDAAGY</mark>	FRVWKVPKPR	RKPGRARQEE
Hlt	SRIKLSLKSL	VNKGILVQ	– <mark>TRGTGASGS</mark>	FKLSKKVIPK	STR
H1x	T <mark>YLK</mark> YSIKAL	VQNDTLLQ	– <mark>VKGTGANGS</mark>	F K L N R K K L E G	GGE
H100	YLLK <mark>QALA</mark> TG	MRRGLLARPL	N SKARGATGS	FKL <mark>VPKH</mark> KKK	I Q P R K M A P A T
HILS1	GWRPAGQRVP	SKTATGQRTC	A KPCQKPSTS	KVI LRAVADK	GTCK
Consistency	656 <mark>8</mark> 566755	65677858 <mark>00</mark>	0 <mark>677678788</mark>	878 <mark>4</mark> 764554	4342200000
	160	12	70 18	0 19	200
H1 0			FKKTKKETKK	VATPKKASKP	KKAASKAPTK
H1 1		ASKVATKTKA	TGASKKLKKA	TGASKKSVKT	PKKAKKPAAT
H1 2		VKKAGGTKPK	KPVGAAKKPK	KAAGGATPKK	SAKKTPKKAK
н1 3		AKKAGAAKPR	KPAGAAKKPK	<b>KVAGAATPKK</b>	SIKKTPKKVK
H1 4		AKKAGAA <mark>K</mark> AK	KP A G A A K K P K	<b>KATGAATP</b> KK	SAKKTPKKAK
н1 5		AKKAGAA <mark>K</mark> AK	<b>KPAGATPKK</b> A	<b>KKAAGAKKAV</b>	KKTP <mark>KKA</mark> KKP
Hlfnt	GTRAPWRTPA	APRSSRR <mark>R</mark> RQ	PLRKAARKAR	E <mark>VWRRNAR</mark> AK	AKANARARRT
H1t		<mark>sk</mark> a	KKSVSAKTK <mark>K</mark>	L V L S R D S K S P	KTAK <mark>T</mark> NK <mark>R</mark> AK
H1x		<mark>RR</mark> G	AP <mark>AAATAPA</mark> P	T <mark>A</mark> HKAK <mark>K</mark> AAP	GAAG <mark>S</mark> RR <mark>A</mark> DK
Hloo	APRRAGEAKG	KGPKKPS <mark>E</mark> AK	EDPPNVGKVK	<b>KAAKRPAKV</b> Q	KPPP <mark>K</mark> PG <mark>A</mark> AT
HILS1		YVSLATL <mark>K</mark> KA	VSTTGYDMAR	N <mark>A</mark> YHFKRVLK	GLVD <mark>K</mark> GS <mark>A</mark> G <mark>S</mark>
Consistency	00000000000	2 2 3 2 2 2 2 <mark>6</mark> 4 5	4 4 <mark>5 4 5 5 4 5 4</mark> 6	4 <mark>6 4 3 4 4 5 4 5 4</mark>	4 4 4 4 <mark>5</mark> 4 4 <mark>5</mark> 4 5
		0	20	0 24	)250
H1_0	к <mark>Р</mark>	-KATPVKKAK	KKLAA TPKKA	KKPKTVKAKP	VKASKPKKAK
H1_1	R	KSSKNPKKPK	TVKPKK-VAK	SPAKAKAVKP	-KAAKARVTK
H1_2		FAATVIKKV	KTROPYKAKVA	S DAVAKAAKSAA	- KAVKPKAAK
н1_3 11_4	KDAAAGT	AKVAKSAKKV	KAAK	SPARAKAPKP	- AAAAPKSGK
H1 5	A A CV V	KVAKSPKKA	AAAKPKKAPK	SPAKAKAVKP	-KAAKPKAAK
H1fnt	REARPEAK	EPPCARAKER	AGATAADECE	GOAVKEDTTP	RSGKDKRPS
H1t	KP	-RATTPKTVR	SGRKAKGAKG	KQQQKSPVKA	-RASKSKLTO

H100       EKARKQGGAA KDTRAQSGEA RKVPPKPDKA MRAPSSAGGL SRKAKAKGSR         HILS1       F	H1v	<b>KP</b>	ARGOKPEOR	SHKKGAGAKK	DKGGKAKKTA	- A A G G K K V K K
HILSI       F	H100	RKARKOGGAA	KDTRAOSGEA	REVERKEDEA	MRAPSSAGGL	SPKAKAKCSP
Consistency 5 2 1 0 0 0 0 0 0 0 2 4 4 4 4 3 4 6 4 4 5 3 4 5 4 6 3 4 4 5 4 5 5 4 4 3 5 4 0 7 7 5 7 4 8 3 5 7	HTLS1	F	-TLGKKOASK	SKLKVKRORO	ORWRSGORPE	COHRSLLCSK
	Consistence	521000000	2444434644	5345463445	4545544354	0775748357
	consistency					
H1_0       PVKPKAKSSA       KRAGKKK         H1_1       PKTAKPKKAA       PKKK         H1_2       PKVVKPKKAA       PKKK         H1_3       PKVKKAKAA       KKK         H1_4       PKAAKPKKAA       AKKK         H1       PKAAKPKAA       AKKK         H1_4       PKAAKPKAA       AKKK         H1       PKAAKPKAAK       AKKAAAKKK         H1       HEVTKAKAS       KKAAAKKK         H1       HEVTKAKAS       KKAAAKKK         H1       HEVTKAT       SK         H1       HEVTKAT       SK         H12       QGHKRLIKGV       RVAKESKSK         PTASKVKNGA       ASPTKKVVA       KAKAPKAGG         H12       GGHKRLIKGV       RVAKCHCN			)	0	0	)
H1_1       PKTAKPKKAA       PKKK         H1_2       PKVVKPKKAA       PKKK         H1_3       PKVTKAKKAA       PKKK         H1_4       PKAAKPKKAA       AKKK         H1_4       PKAAKPKAA       AKKK         H1_5       PKAAKPKAA       AKKK         H1       HHEVNVRKAT       SKK         H1       HHEVNVRKAT       SKK         H1x       HAKPSVPKVP       KGRK         H1x       AAKPSVPKVP       KGRK         Consistency 5533646654       4664000000       000000000       000000000         Consistency 5533646654       4664000000       0000000000       0000000000         11_1	н1 0	P V K P K A K S S A	KRAGKKK			
H1_2       PKVVKPKKAA       PKKK         H1_3       PKVTKAKKAA       PKKK         H1_4       PKAAKPKKAA       PKKK         H1_5       PKAAKPKKAA       AKKK         H11       HEVVKAKAK       AKKK         H11       HEVVKAKAK       AKKK         H11       HEVVKAKAK       AKKAAAKKK         H11       HEVVVKAKAK       AKKAAAKKK         H11       HEVVVKAKAK       SKK         H12       GHKXLIKGV       RKPACKON         Consistency       5533646654       4664000000       000000000       000000000       0000000000         Consistency       5533646654       4664000000       0000000000       0000000000       0000000000         11_0	н1 1	PKTAKPKKAA	PKKK			
H1_3       PKVTKARKAA       PKKK         H1_4       PKAAKPKKAA       AKKK         H1_5       PKAAKPKKAA       AKKK         H1_6       PKAAKPKAAK       AKKAAAKKK         H1fnt       SKPREEKQEP       KKAAAKKK         H1fnt       SKPREKQEP       KKAAAKKK         H1k       HEVVVRKAT       SKK         H1x       AAKPSVPKVP       KGRK         Consistency 5533646544664000000000000000000000000000	н1 2	PKVV <mark>KPKKAA</mark>				
H1_4 PKAAKPKKAA AKKK	н1 3	PKVTKAKKAA				
H1_5       PKAAKPKAAK       AKKAAKKK         H1fnt       SKPREEKQEP       KKPAQRTIQ         H1t       HHEVNVRKAT       SKK         H1x       AAKPSVPKVP       KGR         H1x       AAKPSVPKVP       KGRK         H1x       AAKPSVPKVP       KGRK         Consistency 5533646654       4664000000       000000000       000000000         Consistency 5533646654       4664000000       0000000000       0000000000         H1_0	H1 4	PKAAKPKKAA	AKKK			
H1_fnt       SKPREEKQEP       KKPAQRTIQ	н1_5	PKAAKPKAAK	AKKAAAKKK-			
H1t       HHEVNVRAT       SKK	Hlfnt	SKPREEKOEP	KKPAORTIO-			
H1x       AAKPSVPKVP       KGRK         H1co       SSQGDAEAYR       KTKAESKSSK       PTASKVKNGA       ASPTKKKVVA       KAKAPKAGQG         H1LS1       QGHKRLIKGV       RVAKCHCN-	H1t	HHEVNVRKAT	SKK			
Hloo       SS QGDAEAYR       KTKAESKSSK       PTASKVKNGA       ASPTKKKVVA       KAKAPKAGQG         HILS1       QGHKRLIKGV       RRVAKCHCN-            Consistency 55 3 3 6 4 6 6 5 4       4 6 6 4 000000       0000000000       0000000000       0000000000	H1x	AAKPSVPKVP	KGRK			
HILS1       QGHKRLIKGV       RVAKCHCN-	Hloo	SSOGDAEAYR	KTKAESKSSK	PTASKVKNGA	ASPTKKKVVA	KAKAPKAGOG
Consistency 55 3 3 6 4 6 6 5 4 4 6 6 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	HILS1	OGHKRLIKGV	RRVAKCHCN-			
	Consistency	5533646654	4664000000	0000000000	0000000000	0000000000
H1_0			)	0	0	)
H1_1	н1_0					
H1_2	H1_1					
H1_3	н1_2					
H1_4	H1_3					
H1_5	H1_4					
H1fnt <t< td=""><td>H1_5</td><td></td><td></td><td></td><td></td><td></td></t<>	H1_5					
H1t	Hlfnt					
H1x         PNTKAAAPAK         GSGSKVVPAH         LSRKTEAPKG         PRKAGLPIKA         SSSKVSSQRA           H1LS1	H1t					
H100         PNTKANAPAK         GSGSKVVPAH         LSRKTEAPKG         PRKAGLPIKA         SSSKVSSQRA           HILS1	H1x					
HILS1Consistency 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Hloo	PNTKAAAPAK	GSGSKVVPAH	LSRKTEAPKG	PRKAGLPIKA	SSSKVSSQRA
Consistency 000000000 00000000 00000000 0000000 0000	HILS1					
H1_0	Consistency	0000000000	0000000000	00000000000	00000000000	0000000000
H1_0						
H1_0		• •				
	н1_0					
H1_1	H1_1					
H1_2	H1_2					
H1_3	H1_3					
H1_4	H1_4					
H1_5	н1_5					
H1fnt	Hlfnt					
H1t	H1t					
H1x	H1x					
Hloo EA	Hloo	EA				
HILS1	HILS1					
Consistency 0 0	Consistency	7 <mark>0 0</mark>				

<u>Figure S1:</u> Alignment of all H1 variants. PRALINE amino acid sequence alignment of the human H1 variants<sup>6</sup>.



<u>Figure S2:</u> Purification Strategy for Human Linker Histones. All linker histones were purified as described in Materials and Methods. For a representative purification, we used samples of each indicated step in the purification of H1.4. S, SUMO; G, GyrA, green rectangle, 6XHis tag.



WB: anti H1

<u>Figure S3:</u> Western Blot analysis of all H1 variants using a pan-H1 antibody. The pan-H1 antibody derived using H1 from calf thymus does not recognize all H1 variants with equal sensitivity.



**Figure S4: Biolayer interferometry control experiments.** (a) Comparison of signal for sensors loaded with NCPs (cyan) or dipped in buffer instead of loading (grey) before carrying the remainder of the BLI experiment as described in the methods. Non-specific binding of H1.2 to the streptavidin biosensor is negligible. (a) BLI experiment wherein biosensors were loaded with the same biotinylated DNA used to generate NCPs. H1.2 binding to free DNA is orders of magnitude weaker than to NCPs.



<u>Figure S5:</u> Processed Biolayer Interferometry (BLI) data for each of the H1 variants described in Figure 2.





С

d



EtBr

MNase (sec) 0 10 60 300





EtBr

# MNase (sec) 0 10 60 300



**Figure S6:** Representative MNase assay gels. One representative gel of triplicate experiments quantified and presented in Figure 3 showing the MNase digestion of arrays assembled in the presence of H1.1 (a), H1.2 (b), H1.3 (c) H1.4 (d), H1.5 (e), H1x (f) H1.0 (g).

|--|

Host	Epitope	Label	Dilution	Vendor
Rabbit	Anti-H1.0	-	1: 1000	Invitrogen PA530055
Rabbit	Anti-H1	-	1: 1000	Active Motif #39707
Goat	Anti-Rabbit	IRDye 800CW	1: 15000	Li-Cor

# Table S2. Amino Acid Sequence of His-SUMO-H1.4-GyrA-His construct, representative of all other H1 constructs used

Construct	Amino Acid Sequence
Name	
His-Sumo-	MHHHHHHSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLM
H1.4-GyrA-	EAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSETAPA
His	APAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAAL
	KKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKAASGEAK
	PKAKKAGAAKAKKPAGAAKKPKKATGAATPKKSAKKTPKKAKKPAAAAGAKKAK
	SPKKAKAAKPKKAPKSPAKAKAVKPKAAKPKTAKPKAAKPKKAAAKKKCITGDAL
	VALPEGESVRIADIVPGARPNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVYTVR
	TVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCAGFA
	RGKPEFAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDA
	GVQPVYSLRVDTADHAFITNGFVSHATHHHHHHH*

## Table S3. Kinetic values derived from BLI experiments.

H1	К <sub>d</sub> (М)	K <sub>d</sub> Error	<i>k</i> <sub>on</sub> (M⁻¹s⁻¹)	k <sub>on</sub> Error	k <sub>off</sub> (s <sup>-1</sup> )	k <sub>off</sub> Error	X <sup>2</sup>	R <sup>2</sup>
H1.0	1.35E-11	<1.0E-12	2.10E+06	7.30E+03	2.84E-05	5.77E-07	0.8217	0.9983
H1.1	1.31E-11	<1.0E-12	1.57E+06	6.81E+03	2.05E-05	6.64E-07	0.847	0.998
H1.2	1.39E-11	<1.0E-12	2.86E+06	8.93E+03	3.99E-05	5.36E-07	0.7582	0.9981
H1.3	1.44E-11	<1.0E-12	2.17E+06	7.46E+03	3.11E-05	5.56E-07	0.7758	0.9979
H1.4	2.44E-11	<1.0E-12	1.39E+06	6.06E+03	3.40E-05	6.21E-07	0.8217	0.9981
H1.5	1.03E-11	<1.0E-12	1.20E+06	4.80E+03	1.23E-05	3.53E-07	0.509	0.9991
H1x	1.03E-10	1.13E-12	1.52E+06	1.18E+04	1.56E-04	1.22E-06	2.067	0.9925
H1t	3.83E-11	<1.0E-12	1.59E+06	6.52E+03	6.09E-05	6.23E-07	0.9044	0.9977