PNAS
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Supplementary Information for

H1 linker histones silence repetitive elements by promoting both histone H3K9 methylation and chromatin compaction

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This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.1920725117

SI Materials and Methods

Cell Lines and Culture Conditions

Mouse embryonic stem cells (ESCs) were grown in a humidified incubator at 37°C with 5% CO_2 and maintained on 0.1% gelatin coated plates in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 15% stem cell-qualified fetal bovine serum (Gemini), 0.1 mM non-essential amino acids (ThermoFisher), 50 U/ml penicillin-streptomycin (ThermoFisher), 1 mM sodium pyruvate (Corning), 0.1 mM beta-mercaptoethanol (Sigma) and 1000 U/ml LIF (ESGRO, Millipore). *Suv39-dn* ES cells were a kind gift of Thomas Jenuwein (1). Generation and characterization H1 triple knockout (TKO) ES cells were described previously (2). H1-low ESCs were generated from TKO ES cells by successive rounds of CRISPR/Cas9 genome editing. Short guide RNA (sgRNA) sequences targeting the 5' and 3' ends of *Hist1h1a*, *Hist1h1b* or *H1f0* genes were cloned into pU6-sgRNA-EF1α-puro-T2A-BFP (a gift from Jonathan Weissman, Addgene plasmid #60955; http://n2t.net/addgene:60955; RRID:Addgene_60955). $4x10^6$ cells were co-transfected with pCas9-GFP (a gift from Kiran Musunuru, Addgene plasmid #44719; http://n2t.net/addgene:44719; RRID:Addgene_44719) and the sgRNA plasmids using Lipofectamine3000 (ThermoFisher). Positive transfectants were sorted by flow cytometry and clones were selected and expanded after 7 days. Genomic DNA was purified from clonal populations using the Quick DNA Miniprep Kit (Zymo) and screened by PCR. Positive clones were further characterized by high performance liquid chromatography (described below). H1d rescue lines were generated using CRISPR-Cas9-mediated homology directed repair. Sequences encoding *Hist1h1d*(1-666) or *Hist1h1d*(1-420) with an 5' 3xFlag tag were cloned into a homology directed repair donor vector (H1d_pDonor_HyTK) plasmid containing 3.8 and 2-kb of the 3' and 5' regulatory sequences of the endogenous *Hist1h1d* gene, respectively and a hygromycin resistance cassette. ES cells were transfected as above but with the addition of the HDR plasmid and selected with 200 µg/ml hygromycin (Gibco) following sorting for positive transfectants. Positive clones were additionally screened by western blot for the 3xFlag tag. CRISPR sgRNA sequences are provided in Table S1. Screening primer sequences are available on request.

ChIP-seq analyses

ChIP-seq data was accessed from the GEO database. Reads were aligned to the mouse genome (mm9) using STAR (3) and analyzed using the iterative segmentation of ordered reads (ISOR) algorithm to identify significantly enriched and depleted regions (4). A Fisher's exact test was used to determine the significance of the overlap between each of the ChIP-seq datasets. A p-value<0.01 and a signal>0.5 were used for the overlap analysis. See Chen et al., 2014 for additional details regarding the ISOR algorithm. Enrichment of H3K9me3, Suv39h1, SETDB1 and G9a within H1-enriched ISOR domains was analyzed using the annotatePeaks function of HOMER (5), and matched random genomic regions were generated using BEDtools (6). H3K9me3 domains were

called using the findPeaks function of HOMER, and Suv39-dependent peaks were determined using Bedtools (see **Figure S1**). Overlap of repetitive sequences and H1-enriched or matched control ISOR regions were determined using the genomeOntology function of HOMER. Plots were generated using GraphPad Prism 8.

RNA purification and RT-qPCR

Total RNA was purified using TriReagent (Zymo) and quantified with a NanoDrop 1000 Spectrophotometer (ThermoScientific). 1 µg of total RNA was digested with 2 units TURBO DNaseI (Thermo) for 1 hour at 37°C, and was purified using the RNA Clean and Concentrator-5 kit (Zymo Research). cDNA was prepared from 0.5 µg DNaseI-digested RNA using the GoScript Reverse Transcription Mix (Promega) with random primers. Real-time qPCR amplifications were carried out using were prepared in 384-well optical plates (Axygen) using Power Sybr Green PCR Master Mix (ThermoFisher). Primer sequences are in **Table S2**.

Chromatin Immunoprecipitation (ChIP)

ESCs were harvested and washed once with PBS. $1x10^7$ cells were fixed with 1% formaldehyde (Pierce) for 8 minutes at room temperature and quenched with 125 mM glycine for 5 minutes, also at room temperature. Crosslinked cells were washed twice with ice-cold PBS, snap frozen using liquid nitrogen and stored at -80°C. For preparation of sheared chromatin, $1x10^7$ cells were thawed on ice, lysed in ChIP lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1% SDS) and sonicated using a Biorupter (Diagenode) for 3 cycles (5 minutes per cycle, 30 seconds on, 30 seconds off) on the high power setting at 4°C. After sonication, lysates were centrifuged at 20,000 x g to pellet debris and transferred to new tubes. A portion of sheared chromatin was digested with 10 µg RNaseA (ThermoFisher) at 37°C for 30 minutes and treated with 0.6 mAU proteinase K (Millipore) for 2 hours at 55°C. Following overnight incubation at 65°C to reverse crosslinks, DNA was purified using the ChIP DNA Clean and Concentrator-5 (Zymo Research). Purified DNA was analyzed by agarose gel electrophoresis to ensure equal shearing to an average size of 200-500 bp. For ChIP, sheared chromatin equivalent to $1x10^6$ cells was diluted ten-fold in ChIP dilution buffer (20 mM Tris-HCl pH 8.0, 0.5% Triton X-100, 2 mM EDTA, 150 mM NaCl) and pre-cleared with 10 µL Protein A Dynabeads (ThermoFisher) for 1 hour at 4°C with rotation. Chromatin was then immunoprecipitated with the antibody of interest (anti-H3K9me3, Abcam catalog#: ab8898, 5 µg; anti-H3K27me3, Diagenode catalog#: C15410195, 2 µg; anti-Flag, Sigma catalog#: F1804, 2 µg), or anti-rabbit IgG control (Millipore CS200581, 5 µg) overnight at 4°C with rotation. Antibody-chromatin complexes were then captured with 20 µL protein A Dynabeads for 2 hours at 4°C and washed twice in low-salt ChIP buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 140 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate), then in high salt ChIP buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 500 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate), then in LiCl buffer (10

mM Tris-HCl pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 0.5% sodium deoxycholate) and then in TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Beads were then resuspended in ChIP elution buffer (10 mM Tris-HCl pH 8.0, 5 mM EDTA, 300 mM NaCl, 0.5% SDS) and immunoprecipitated DNA was purified as above. Amounts of each DNA fragment in input and precipitated material were determined using quantitative PCR with primers for the indicated targets. Primer sequences are listed in **Table S3**.

Recombinant Protein Purification

The coding sequences of mouse H1 subtypes were cloned into pET-15b (Novagen) using the BamHI and NdeI restriction sites. Cloning primers are available on request. Constructs were transformed into Rosetta2 cells (EMD Millipore) and protein expression was induced by addition of IPTG (1 mM) at 16°C overnight. Cells were harvested by centrifugation, washed once in ice-cold PBS and processed fresh or frozen in liquid nitrogen and stored at -80°C. Cell pellets were resuspended in lysis buffer (50 mM Tris-HCl pH 8.0, 1 M NaCl, 10% glycerol, 0.5 mM TCEP, 1x EDTA-free protease inhibitors (Sigma), 10 U/ml Universal Nuclease (Pierce), 0.25 mg/ml lysozyme) and incubated at 4°C for 30 minutes. Cells were sonicated using a 150E Sonic Dismembrator (Fisher Scientific) at 50% amplitude, with 2 second pulses for 15 minutes on ice with 10 seconds between each pulse. Lysate was clarified by centrifugation at 30,000 x g twice and then incubated with HisPur Cobalt Resin (Thermo Fisher) overnight. The resin was washed with lysis buffer containing 20 mM imidazole and then bound proteins were eluted in lysis buffer containing 300 mM imidazole. Peak fractions were pooled and further purified by FPLC on 15S Source Sepharose (GE Healthcare) using an Akta chromatography system (Amersham). Purified protein was dialyzed into storage buffer (10 mM Tris-HCl pH 8.0, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol), concentrated, snap frozen in liquid nitrogen and stored at -80°C until further use. GST-tagged linker histone proteins or truncated polypeptides were purified in a single step using Glutathione Sepharose 4B (GE Healthcare) using similar procedures.

Full length 6his-MBP-tagged Suv39h1/2 constructs were a kind gift of Thomas Jenuwein. All other Suv39h1 constructs were cloned into a pET-6his-MBP LIC vector (a kind gift of Scott Gradia, Addgene plasmid #29656) using HiFi DNA Assembly Master Mix (New England Biolabs). Cloning primers are available on request. Constructs were transformed into Rosetta2 cells (EMD Millipore) and protein expression induced by the addition of ITPG (0.1 mM) at 16°C overnight. Cell pellets were resuspended in lysis buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 10% glycerol, 0.2% NP-40 (Calbiochem, Millipore), 1x EDTA-free protease inhibitors (Sigma), 0.5 mM TCEP, 10U Universal Nuclease (Pierce), 0.25 mg/ml lysozyme (Millipore)) and processed as above. Clarified lysate was incubated with amylose resin (New England Biolabs), washed with wash buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 10% glycerol, 0.2% NP-40, 5 mM β-mercaptoethanol) and eluted in wash buffer containing 20 mM maltose. If necessary, further purification was carried out

by FPLC on a 15Q Source Sepharose (GE Healthcare). Peak fractions were pooled, dialyzed into storage buffer (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol), concentrated and snap frozen in liquid nitrogen for storage at -80°C.

All protein concentrations were determined by Coomassie stained SDS-PAGE using BSA standards (Pierce) and quantification with Image Studio (LI-COR Biosciences).

GST Pulldown Assay

Purified recombinant GST or GST-H1 fusion proteins (16 pmol) were bound to glutathione Sepharose for 2 hours at 4°C in binding buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 2 mM DTT, 0.5% NP-40, 10% glycerol), washed once with binding buffer and incubated with 20 pmol of the indicated protein overnight at 4°C. Beads were washed three times with wash buffer (binding buffer + 300 mM NaCl) and boiled in SDS sample buffer to release bound proteins. Bound proteins were separated by SDS-PAGE and analyzed by immunoblotting with anti-6his (1:3,000; Sigma catalog#: H1029) or anti-Flag (1:1,000; Sigma catalog#: F1804) antibodies.

Western blotting

Samples were separated by SDS-PAGE and transferred (wet) to 0.2 µm nitrocellulose membrane. The membrane was blocked with Odyssey blocking buffer (Licor Biosciences) for 1 hour at room temperature and incubated with primary antibody, also for 1 hour at room temperature. Membranes were washed three times with TBS-T and incubated with IRDye 800 or 700CW secondary antibodies (1:10,000; LI-COR Biosciences) for 30 minutes at room temperature. After two additional washes with TBS-T, membranes were visualized using a LI-COR Odyssey Fc (LI-COR Biosciences).

Nucleosome Reconstitution

Mouse recombinant histone octamers were either refolded as previously described (Dyer et al 2004) or purchased (Histone Source, Colorado State University). Template DNA bearing two repeats of the '601' strong nucleosome positioning sequence (a kind gift Tim Richmond) were excised from the parent plasmid by restriction digest and purified by FPLC using Source 15Q Sepharose (GE Healthcare) using an AKTA chromatography system (GE Healthcare). Peak fractions were pooled, ethanol precipitated, resuspended in water and stored at -20°C.

Optimal histone to DNA stoichiometry was determined empirically. Histone octamer and DNA template (1-5 µM each in a final volume of 50-75 µL) were combined in a high salt buffer (2 M NaCl, 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA) on ice. The mixtures were transferred to 3.5K MWCO dialysis cups (Pierce) and dialyzed against 200 mL of NRB1 (1.4M NaCl, 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA) for 1 hour at 4°C. 350 mL low salt buffer (10 mM NaCl, 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA) was added using a peristaltic pump at a rate of ~0.5-1 ml/min for 6-12 hours.

At this step, reconstitutions were removed and heat shifted for 1 hour at 37°C. Finally, reconstitution mixtures were dialyzed into low salt buffer overnight. Reconstitution mixtures were further purified by 5-20% sucrose gradient using an SW-41 rotor (Beckman Coulter, Inc.) for 16 hours at 36,000 rpm at 4°C. Fractions were analyzed by 0.7% agarose gel electrophoresis (0.5x TBE, 120V for 90 minutes at 4°C). Peak fractions, free of unbound template DNA or non-nucleosomal species, were pooled and concentrated. Purified nucleosomes were stored at 4°C until use.

Purified, recombinant H1's were bound to dinucleosomes for 30 minutes on ice in binding buffer (10 mM Tris-HCl pH 8.5, 50 mM NaCl). H1 binding was assessed by non-denaturing agarose gel electrophoresis as above. Optimal H1:nucleosome ratios were determined empirically (see **Figure S3F**).

In vitro Histone Methyltransferase Assays

The indicated enzymes (SETDB1, 50 nM; Suv39h1, 100 nM; G9a, 20 nM; MLL1, 500 nM) were incubated with dinucleosome reconstituted with or without H1 (400 nM nucleosome in 15 µl final reaction volume) in HMT buffer (50 mM Tris-HCl pH 8.5, 10 mM NaCl, 10 mM DTT, 100 µM S-adenosylmethionine) for 1 hour at 30°C. The reaction was terminated by the addition of 4x Laemmli buffer and incubation at 95°C for 5 minutes. Reactions were separated by 15% SDS-PAGE and analyzed by immunoblot for the indicated histone modification. Suv39h1 was purified from bacteria (above); SETDB1 and G9a were purchased from Reaction Biology. MLL1 complex was a generous gift of Yali Dou.

Micrococcal Nuclease Digestion and Chromatin Compaction Analysis

$1x10^6$ cells were resuspended in EZ Nuclei Lysis Buffer (Sigma) and digested with micrococcal nuclease (New England Biolabs) for 1 minute at 37°C. The reaction was terminated by the addition of EDTA. The soluble fraction was isolated by centrifugation and DNA purified using the ChIP DNA Clean and Concentrator-5 kit (Zymo Research) following RNaseA and proteinase K digestion, as above. DNA fragments were analyzed by 1.2% non-denaturing agarose gel electrophoresis. Nucleosome repeat length was determined using the Fragment Analyzer System (Agilent). Chromatin compaction was analyzed using ImageJ (NIH) by comparing the fraction of signal arising from smaller (≤4 nucleosomes) and larger (>4 nucleosomes) DNA fragments normalized to the total signal in each lane.

Analysis of Major Satellite Foci

WT, Suv39-dn and H1-low mESCs were transfected with pTALYM3B15 (a gift from Maria-Elena Torres-Padilla (Addgene plasmid #47878 ; http://n2t.net/addgene:47878 ; RRID:Addgene_47878) and sorted by FACS after 24 hours. Cells were attached to coverslips coated with Laminin-511 (Thermo Scientific), fixed in 3.2% PFA, and washed three times with PBS prior to mounting in

Prolong-Diamond + DAPI. Images were acquired using a oil immersion 100× objective on an epifluorescence Olympus BX83 microscope, with an X-Cite 120 PC lamp (EXFO) and an ORCA-R2 digital charge-coupled device (CCD) camera (Hamamatsu) using FITC-5050A-Zero and DAPI (DAPI-5060C-Zero) filters from Semrock. Exposure times were 300ms and 10ms, respectively. Z stacks spanning the entire volume of the cells were acquired by imaging every 300 nm along the z-axis. Operation of the microscope was achieved using MetaMorph software (Molecular Devices, Inc.).

Image analysis proceeded as follows: first, samples were deconvolved in Matlab R2019a with FISHQUANT (7) using the filtering function of the main interface with a Laplacian of Gaussian filter (size 5, sigma 1). Filtered images were then imported into ImageJ and ROI defined manually by hand. >150 ROI per genotype were then analyzed by measuring the integrated intensities within each ROI. The results were then exported for graph generation in GraphPad Prism.

Isolation and analysis of histones

Histones were isolated exactly as previously described (8). Briefly, ES cells were resuspended in sucrose buffer (0.3 M sucrose, 15 mM NaCl, 20 mM HEPES pH 7.9, 2 mM EDTA, 0.5% NP-40) and lysed by several passes through a B pestle Dounce homogenizer (Wheaton). Nuclei were isolated by centrifugation and lysed in high salt buffer (350 mM KCl, 10 mM Tris-HCl pH 7.2, 0.5 mM $MgCl_2$). Following centrifugation, histones were extracted from the chromatin pellet using 0.2N sulfuric acid overnight at 4°C, precipitated with ethanol and resuspended in water. Histones were quantified by Bradford assay and analyzed by 15% SDS-PAGE followed by Coomassie staining or HPLC. For HPLC, samples were applied to a Vydac 218TP C18 HPLC column using the Waters 2695 Separations Module. The effluent was monitored and peaks recorded using the using the Waters 996 Photodiode Array Detector at 214nm. H1 peak areas were determined using the Waters Empower Pro software (Ver. 2) and normalized to H2B peaks.

Quantification and Statistical Analysis

All statistical analyses were performed using GraphPad Prism 8 and the unpaired, two-tailed Student's t test was applied unless otherwise noted. A p-value≤0.05 was considered statistically significant. P-values are notated as follows: ns = >0.05, * = 0.01 to ≤0.05, ** = 0.001 to <0.01, *** = 0.0001 to <0.001, **** = <0.0001.

Data Availability

All unique reagents generated in this study are available by request. Please contact the corresponding author, Arthur Skoultchi (arthur.skoultchi@einsteinmed.org).

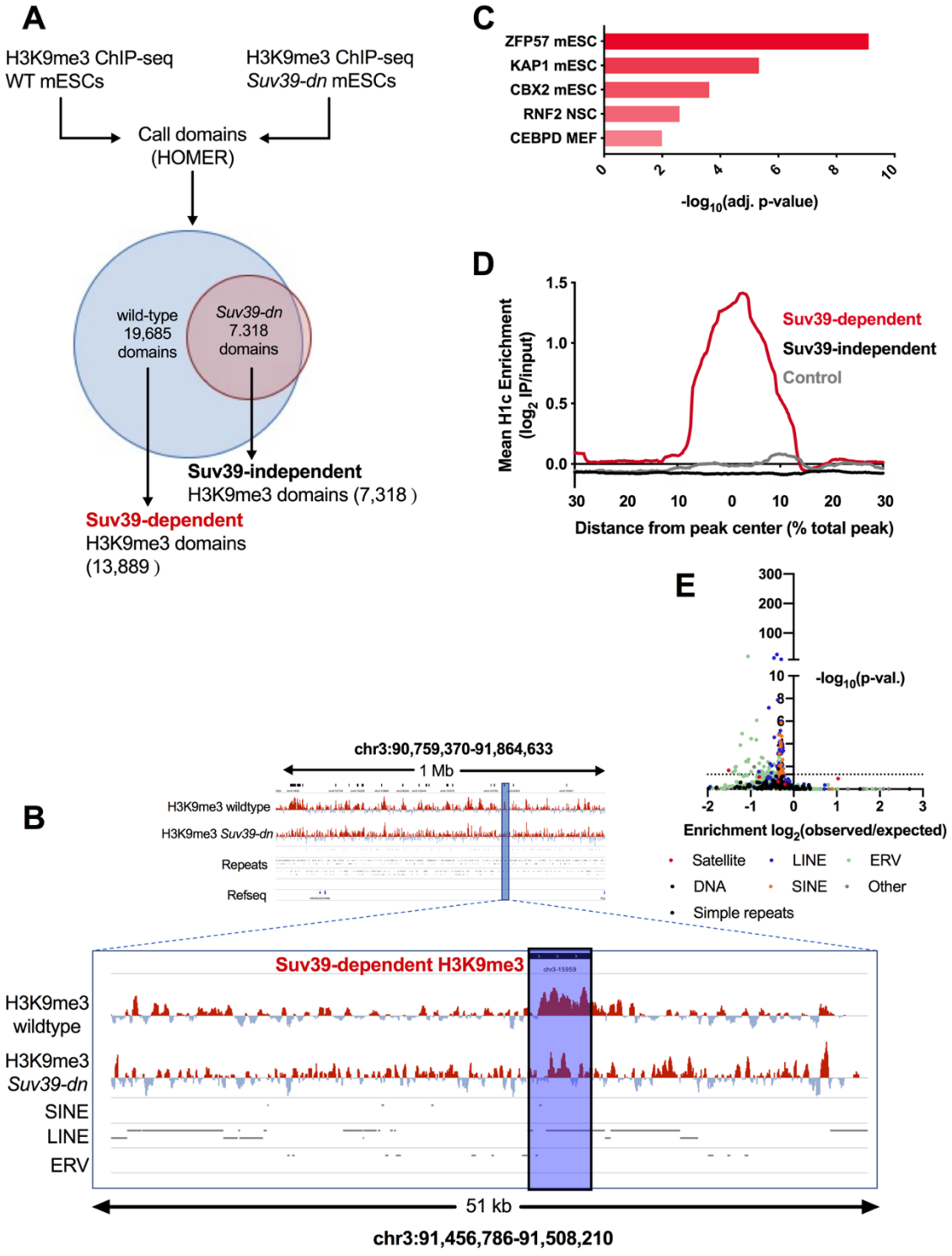

A
H3K9me3 ChIP-seq
WT mESCs
H3K9me3 ChIP-seq
Suv39-dn mESCs
Call domains
(HOMER)
wild-type
19,685
domains
Suv39-dn
7.318
domains
Suv39-independent
H3K9me3 domains (7,318)
Suv39-dependent
H3K9me3 domains
(13,889)
C
ZFP57 mESC
KAP1 mESC
CBX2 mESC
RNF2 NSC
CEBPD MEF
0
2
4
6
8
10
-log10(adj. p-value)
D
Mean H1c Enrichment
(log2 IP/input)
1.5
1.0
0.5
0.0
Suv39-dependent
Suv39-independent
Control
30
20
10
0
10
20
30
Distance from peak center (% total peak)
E
300
200
100
10
8
6
-log10(p-val.)
-2
-1
0
1
2
3
Enrichment log2(observed/expected)
Satellite
LINE
ERV
DNA
SINE
Other
Simple repeats
B
chr3:90,759,370-91,864,633
1 Mb
H3K9me3 wildtype
H3K9me3 Suv39-dn
Repeats
Refseq
Suv39-dependent H3K9me3
H3K9me3
wildtype
H3K9me3
Suv39-dn
SINE
LINE
ERV
51 kb
chr3:91,456,786-91,508,210

Fig. S1. Computational identification of Suv39-dependent H3K9me3 domains and analysis of H1 enrichment. **(A)** Analysis pipeline for identification of Suv39-dependent H3K9me3 domains. H3K9me3 ChIP-seq from wildtype and *Suv39-dn* ES cells was aligned to to the mouse genome and enriched domains called using HOMER. Domains which were present in wildtype but lost in *Suv39-dn* cells were determined using Bedtools. **(B)** Genome browser view of Suv39-dependent H3K9me3. 1 MB region showing H3K9me3 ChIP-seq signal from wildtype and *Suv39-dn* cells. Signal is displayed as $\log_2$(IP/input). Annotated repeats and RefSeq genes are also shown. *Below.* As above but annotated LINEs, SINEs and ERVs are shown. Suv39-dependent H3K9me3 region (an annotated LINE) is highlighted in blue. **(C)** Protein factors significantly enriched in H1 ISOR regions. **(D)** H1c enrichment at Suv39-dependent, Suv39-independent and control genomic regions as in Figure 1D. Enrichment is displayed as $\log_2$(IP/input). **(E)** Overlap of matched control genomic regions and repetitive elements as in Figure 1E.

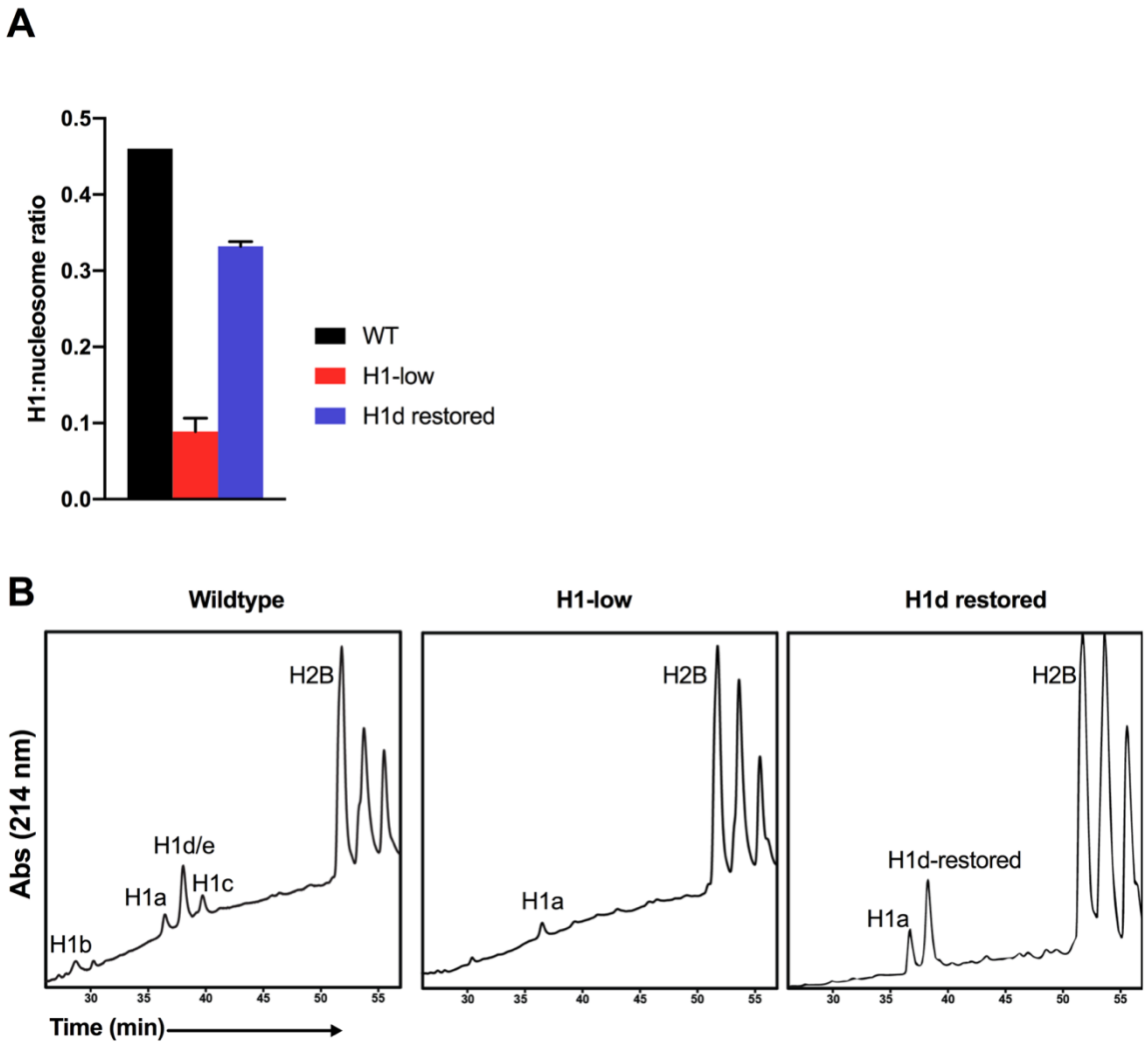
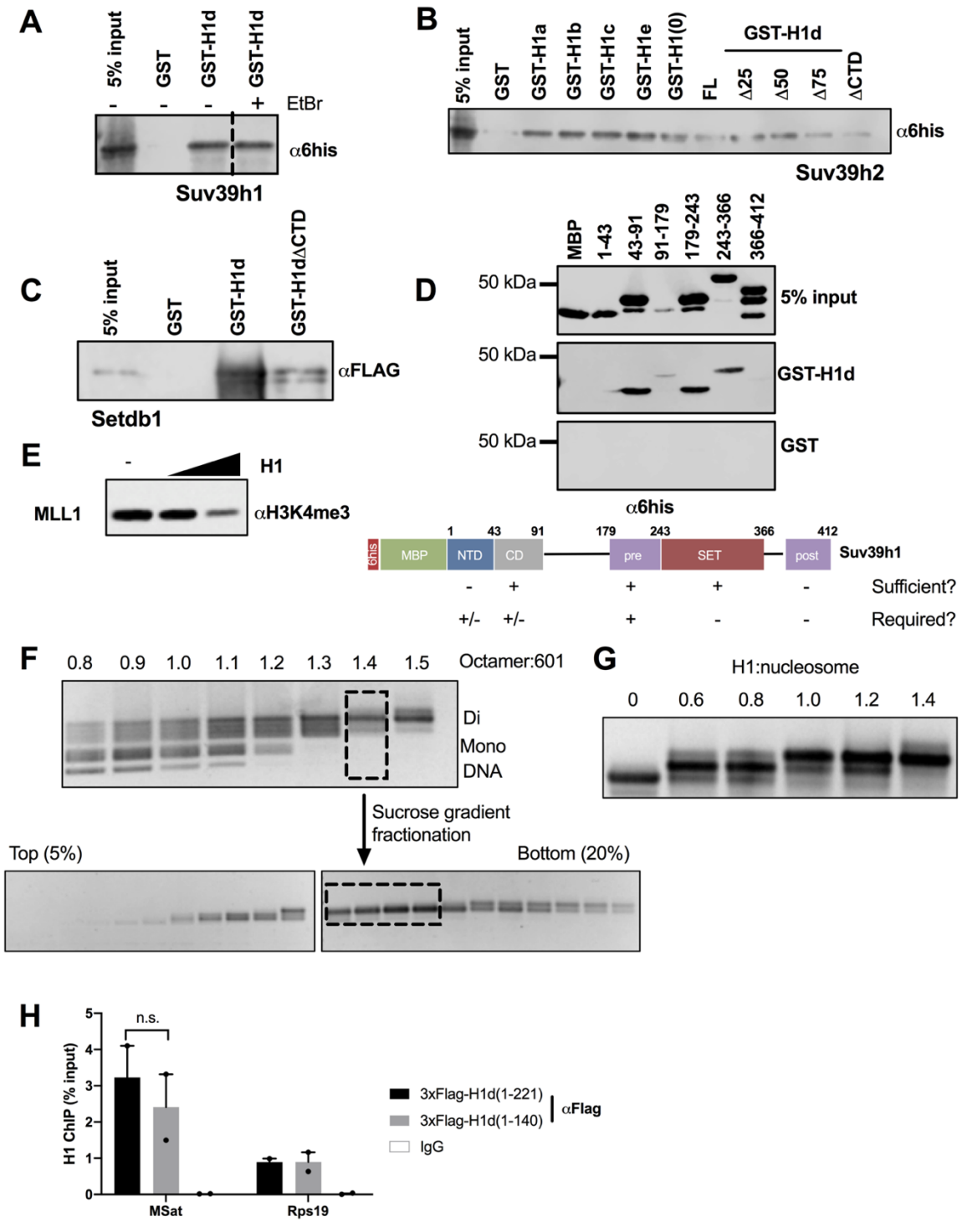


Fig. S2. Characterization of H1 levels in wildtype, H1-low and H1 rescue ES cells. (A) H1:nucleosome ratios in WT, H1-low and H1d restored mESCs. Acid extracted histones were analyzed by quantitative HPLC and the H1:nucleosome ratio determined dividing the area(s) under the H1 A_{214} peak(s) by one-half of the area under the A_{214} H2B peak. Absorbance values of the H1 and H2B peaks were normalized to peptide bond number. **(B)** Representative reverse phase HPLC analyses of approximately 10 µg histone extracts from wildtype, H1-low and H1d-restored mESCs. Abscissae, elution time (minutes); ordinate, absorbance at 214 nm. Identities of the individual peaks are indicated.


A
- 5% input
- GST
- GST-H1d
+ GST-H1d
EtBr
α6his
Suv39h1
B
5% input
GST
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GST-H1b
GST-H1c
GST-H1e
GST-H1(0)
FL
GST-H1d
Δ25
Δ50
Δ75
ΔCTD
α6his
Suv39h2
C
5% input
GST
GST-H1d
GST-H1dΔCTD
αFLAG
Setdb1
D
MBP
1-43
43-91
91-179
179-243
243-366
366-412
50 kDa
5% input
50 kDa
GST-H1d
50 kDa
GST
α6his
6his
MBP
NTD
CD
pre
SET
post
Suv39h1
1
43
91
179
243
366
412
Sufficient?
Required?
E
-
H1
MLL1
αH3K4me3
F
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
Octamer:601
Di
Mono
DNA
Sucrose gradient fractionation
Top (5%)
Bottom (20%)
G
H1:nucleosome
0
0.6
0.8
1.0
1.2
1.4
H
n.s.
H1 ChIP (% input)
3xFlag-H1d(1-221)
3xFlag-H1d(1-140)
IgG
αFlag
MSat
Rps19

Fig. S3. **Interaction studies between H1 and H3K9 methyltransferases and preparation of chromatin *in vitro*. (A)** GST or GST-H1 fusion proteins were expressed and purified from bacteria and incubated with recombinant hexahistidine (6his)-tagged Suv39h1. After washing, bound proteins and 5% input control were separated by SDS-PAGE and analyzed by immunoblotting using a 6his-specific antibody. Where indicated, ethidium bromide was included in binding and wash buffers at 10 µg/ml. Dashed line indicates where the image was cropped to remove the intervening lane. **(B)** Recombinant Suv39h2 was incubated with the indicated purified GST or GST-H1 fusion proteins or polypeptides and analyzed as in (A). 5% input control is shown. **(C)** Flag-tagged, recombinant SETDB1 was incubated with GST-H1d(full-length), GST-H1dΔCTD or GST alone and bound proteins analyzed by immunoblot using an antibody specific for the Flag-tag peptide. **(D)** Individual Suv39h1 domains were expressed and purified from bacteria and incubated with GST or GST-H1d. Bound proteins were analyzed by immunoblot with the indicated antibody (above). Suv39h1 domains structure and summary of binding data (below). **(E)** *In vitro* histone methyltransferase (HMT) assays of MLL1 (500 nM) with reconstituted chromatin in the presence or absence of H1. Experiment performed as in 3E with increasing amounts of H1. Enzymatic activity was detected by immunoblotting for the indicated histone modification. **(F)** In vitro reconstitution of chromatin. *Top*. A DNA fragment bearing two repeats of the synthetic '601' nucleosome positioning sequence was mixed with increasing amounts of recombinant histone octamers and subjected to salt dialysis. The mixtures were analyzed by 0.7% agarose gel electrophoresis followed by staining with ethidium bromide. Numbers above the panel represent molar ratio of octamers to nucleosome positioning sequence. The positions of free DNA, mononucleosomes, and dinucleosomes are indicated on the right. *Bottom*. Reaction mixtures with a histone octamer to DNA ratio of 1.4 were pooled, concentrated and centrifuged on a 5-20% linear sucrose gradient for 18 hours at 36,000 rpm. Individual fractions were analyzed by agarose gel electrophoresis. Properly assembled fractions (indicated by the dashed box) were pooled and concentrated. **(G)** Optimization of H1-dinucleosome loading. Increasing amounts of H1 were incubated with purified dinucleosome and analyzed by agarose gel electrophoresis. A representative gel is shown. **(H)** Exogenous H1d-FL (3xFlag-H1d(1-221) or H1d-Δ75 (3xFlag-H1d(1-140) occupancy at major satellites. Crosslinked chromatin from H1-low cells in which either full-length H1d (H1d-FL) or H1d lacking 75 percent of the CTD (H1d-Δ75) were re-introduced by stable transfection was immunoprecipitated with an anti-Flag or control IgG antibody. Recovered DNA was quantified by PCR using primers for major satellite sequences or the *Rps19* promoter (negative control). Error bars indicate SEM of two independently derived clones. ns, not statistically significant.

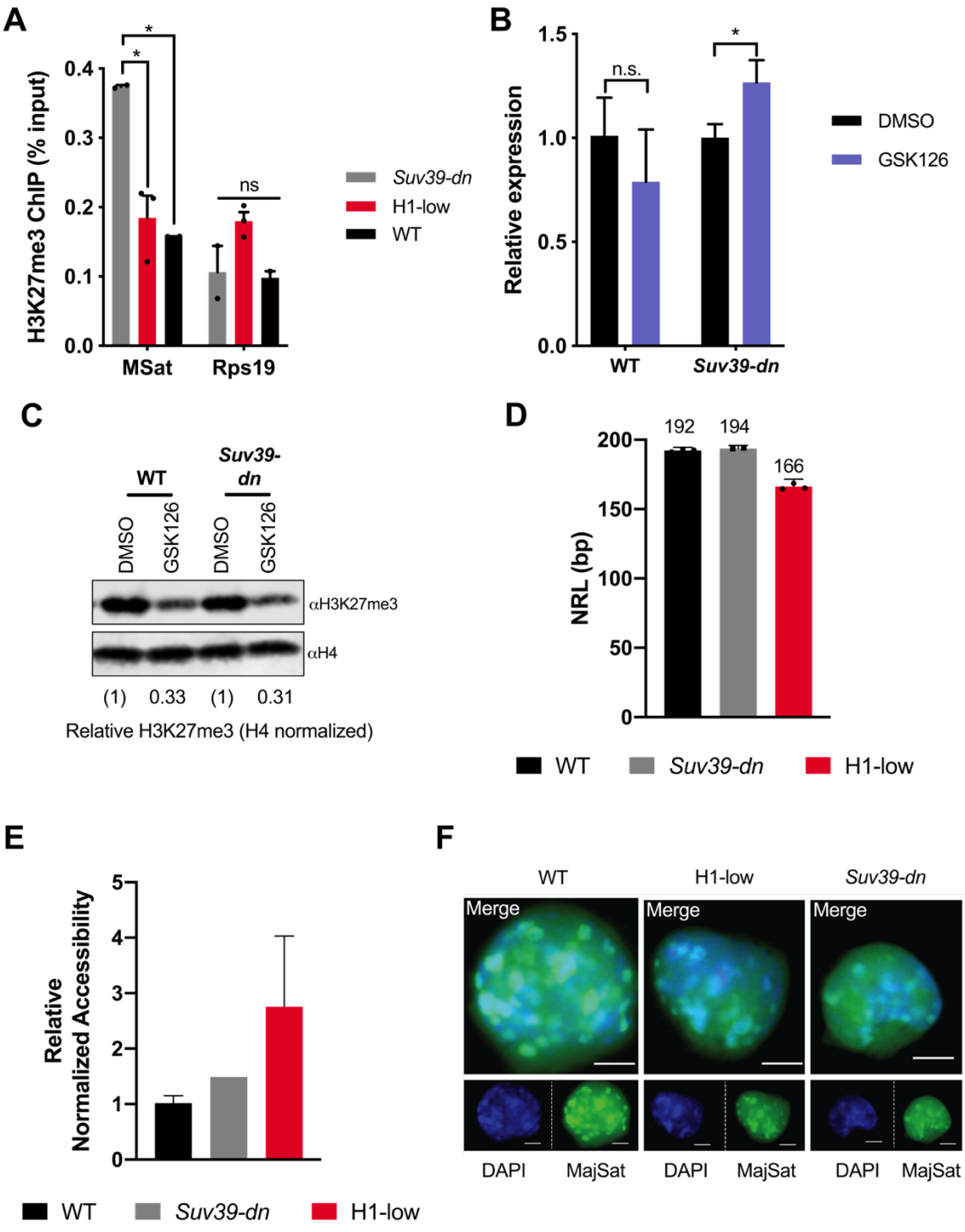
A
H3K27me3 ChIP (% input)
0.4
0.3
0.2
0.1
0.0
*
*
ns
MSat
Rps19
Suv39-dn
H1-low
WT
B
Relative expression
1.5
1.0
0.5
0.0
n.s.
*
WT
Suv39-dn
DMSO
GSK126
C
WT
Suv39-dn
DMSO
GSK126
DMSO
GSK126
αH3K27me3
αH4
(1) 0.33 (1) 0.31
Relative H3K27me3 (H4 normalized)
D
NRL (bp)
200
150
100
50
0
192
194
166
WT
Suv39-dn
H1-low
E
Relative Normalized Accessibility
5
4
3
2
1
0
WT
Suv39-dn
H1-low
F
WT
H1-low
Suv39-dn
Merge
Merge
Merge
DAPI
MajSat
DAPI
MajSat
DAPI
MajSat

Fig. S4. H1-mediated chromatin compaction is the dominant mechanism of major satellite repression. **(A)** H3K27me3 levels at major satellites in wildtype, H1-low and Suv39-dn cells. Cross-linked chromatin from wildtype, H1-low and Suv39-dn ES cells was immunoprecipitated with an H3K27me3 antibody and recovered DNA quantified by PCR using primers for the indicated DNA sequences. Data is shown as a percent of input DNA fragment. Error bars represent SEM of three independent clones (WT, H1-low) or two independent cultures (Suv39-dn). **(B)** Transcript levels of major satellites were measured in wildtype and Suv39-dn ES cells treated with DMSO or GSK126 (5 μM) for 7 days. Values were normalized to *Gapdh* mRNA using the $\Delta\Delta C_t$ method. Error bars represent standard deviation for 3 independent cultures. **(C)** H3K27me3 levels in DMSO and GSK126-treated wildtype and Suv39-dn ES cells. Histone acid extracts were separated by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies. The intensity of each H3K27me3 band was quantified and normalized to the intensity of the corresponding H4 band and the values are shown at the bottom of the panel relative to value of the respective DMSO control sample. **(D)** Quantification of nucleosome repeat length (NRL) in wildtype, Suv39-dn and H1-low ES cells. DNA from digests shown in **Figure 4B** were subjected to Bioanalyzer analysis and the peak corresponding to tri-nucleosome sized DNA was used to estimate NRL. N=2-3 independent clones per genotype. Mean NRL is shown above. **(E)** Major satellite accessibility in wildtype, Suv39-dn and H1-low ES cells. Soluble MNase fragments from the digests shown in **Figure 4B** were isolated by centrifugation, DNA was purified and major satellite DNA quantified by qPCR and normalized to the *Rps19* promoter. Error bars represent SEM from 2-3 independent clones. **(F)** Cells transfected with mClover-MajSat-TAL were sorted, applied to coverslips and fixed. Representative images of fluorescent channels are shown. Scale bar = 5 μm.

Table S1: sgRNA sequences used to generate H1-low and H1-rescue ES lines

Primer name	Sequence (5' – 3')
sgRNA-*Hist1h1b*_5'	TAAACCAGGAGCCTGAGCAA
sgRNA-*Hist1h1b*_3'	ACTCCGTTACGATGGCTGTT
sgRNA-*H1f0*_5'	TCGGAAGGCTCCTTGAACAG
sgRNA-*H1f0*_3'	GATCCCTCATACAGACATTG
sgRNA-*Hist1h1a*_5'	TCTATAAATACTCCGTGCAG
sgRNA-*Hist1h1a*_3'	GCATTTGGGTACAAGCCTGG
sgRNA-*Hist1h1d*_HDR	AGTTAAAAGGTGTTACAGAG

Table S2: RT-qPCR primers used in this study

Primer name	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
Gapdh	AACTTTGGCATTGTGGAAGG	CACATTGGGGGTAGGAACAC
Major satellites	TGGAATATGGCGAGAAAACTG	AGGTCCTTCAGTGGGCATTT
L1 promoter	ACTGCGGTACATAGGGAAGC	TGTGATCCACTCACCAGAGG
L1 ORF1	CACTCCCACCCCACCTAGT	TAACTCTTTAGCAGTGCTCTCCTGT
L1 ORF2	ACCTGGACGAAATGGACAAA	CATCTGGTCCTGGGCTTTT
IAP	AAGCAGCAATCACCCACTTTGG	ATGTTTTGGGGAGGACTGTG
ETn_MusD	GTGCTAACCCAACGCTGGTTC	CTCTGGCCTGAAACAACTCCTG
Nanog	AAGCAGAAGATGCGGACTGT	ATCTGCTGGAGGCTGAGGTA

Table S3: ChIP primers used in this study

Primer name	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
Major satellites	GACGACTTGAAAAATGACGAAATC	CATATTCCAGGTCCTTCAGTGTGC
L1 promoter	ACTGCGGTACATAGGGAAGC	TGTGATCCACTCACCAGAGG
L1 ORF1	CACTCCCACCCCACCTAGT	TAACTCTTTAGCAGTGCTCTCCTGT
L1 ORF2	ACCTGGACGAAATGGACAAA	CATCTGGTCCTGGGCTTTT
IAP	GCTCCTGAAGATGTAAGCAATAAAG	CTTCCTTGCGCCAGTCCCGAG
ETn_MusD	GATTGGTGGAAGTTTAGCTAGCAT	TAGCATTCTCATAAGCCAATTGCAT
Rps19	GCCACATGTCATAGTGCCTTTTCCC	CGGGAACAAGGAGGCGGAAA

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