Supplementary Materials for

Drosophila H1 Regulates the Genetic Activity of Heterochromatin by Recruitment of Su(var)3-9

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Published 5 April 2013, *Science* **340**, 78 (2013)
DOI: 10.1126/science.1234654

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Materials and Methods

Fly strains, genetics and RNAi

Fly strains for H1 knockdown, rearing conditions and dissection techniques are described (*4*). *UAS:Su(var)3-9–eGFP* and *Su(var)3-9[6]* strains were generous gifts of Gunter Reuter and Sarah Elgin, respectively. Other strains were obtained from the Bloomington Stock Center. For RNAi in cell culture, 500-600 bp fragments of GFP (control) and *Drosophila* H1 coding sequences with T7 promoter overhangs were generated by PCR as described (*26*). Primer sequences are available upon request. dsRNA was generated using Ribomax T7 (Promega) and quantitated by spectrophotometry. $5 \bullet 10^5$ *Drosophila* Kc cells were incubated with 20 μg dsRNA on days 0, 2 and 4 for 1 hr in 1 ml serum-free Schneider medium (Invitrogen), followed by addition of 1 ml Schneider medium containing 20% heat-inactivated FBS. Protein content was examined by semi-quantitative Western blot on days 0-8 (*4*).

Microarray Expression Profiling

RNA expression profiling of the duplicate (Kc) and triplicate (salivary glands) experimental sample groups was performed using Affymetrix Genechip system. Total RNA was extracted using the Trizol reagent (Invitrogen). RNA quality and quantity were determined using Agilent 2100 Bioanalyzer and Nanodrop ND-1000. 100 ng total RNA was used to prepare cRNA by following the Affymetrix 3'IVT Express Kit labeling protocol. Standard array processing procedures including hybridization, fluidics processing and scanning of the Affymetrix Drosophila 2.0 arrays were performed at the AECOM Microarray Facility.

Microarray Data Analysis Methods

The RNA expression profiling data were processed and analyzed using the GeneSpring GX11 software (Agilent Technologies). Briefly, the raw data (.cel) files were normalized at the probe level by Robust Multichip Average algorithm (RMA) (*27*), and the significant differential abundance between

RNAi and corresponding control conditions was performed using T-test statistics (p<0.05, alpha level) and varying fold-change thresholding (ranging from 25-100% of reproducible change, based on the target modulation stringency). The functional annotations of the resulting gene lists were performed using the NIH web-based tool DAVID (*28*).

Immunohistochemistry, northern blot, quantitative real-time RT-PCR and chromatin immunoprecipitation (qChIP)

Indirect immunofluorescence (IF) analyses of polytene chromosomes, real-time RT-PCR and qChIP were carried out as described (*4*). Primer sequences are available upon request. The following additional antibodies were used for IF and ChIP: rabbit polyclonal anti-GFP (1:100, BD Bioscience), mouse monoclonal anti-dimethyl histone H3 (H3K9me2, Abcam), rabbit polyclonal anti-GAL4, ChIP grade (Abcam) and rabbit polyclonal anti-6X His tag antibody, ChIP grade (Abcam). Rabbit polyclonal anti-HA (Y-11, Santa Cruz) and mouse monoclonal anti-HA (F-7, Santa Cruz) were used as antibody isotype controls for ChIPs. For northern blot, total RNA was enriched for small RNA by sequential precipitation with increasing amount of ethanol (mirVana kit, Ambion). ~2 µg RNA was loaded on a 15% TBE-urea gel, electrophoresed and transferred to a positively charged nylon membrane (Schleicher and Schuell). Radioactive (^{32}P) probes were prepared from PCR templates by random-primed labeling. PCR primer sequences are available upon request. The membranes were hybridized with probes overnight at 30°C, washed thrice at RT and exposed to film.

Small RNA library preparation and analyses

Small RNA libraries were generated as described (*29*) with several modifications. In brief, 5 µg total RNA was separated on a polyacrylamide gel, and 18–29 nt small RNAs were isolated for cloning. The RNA was eluted from the gel by electroelution using D-tube Dialyzers (Millipore) (*30*). Libraries were single-end sequenced on an Illumina Genome Analyzer for 36 cycles. After FASTQ to FASTA

conversion, the cloning adapter was trimmed from the 3′-end of each read, and sequences shorter than 15 nt or non-clipped sequences were discarded. The remaining sequences were collapsed into a non-redundant list and mapped to the *D. melanogaster* genome (release Apr. 2006 [BDGP R5/dm3]) to determine the total number of uniquely mapping reads. Up to two mismatches were allowed. Subsequently, all reads were mapped to the *Drosophila* transposon consensus sequences allowing up to three mismatches. All mappings were done using the short read aligner bowtie (*31*). The read count of mapping sequences was normalized to the total number of reads that mapped to the genome and were transformed to reads per million genomic mappers.

Reconstitution of chromatin, HMT assays and *in vitro* qChIP

Oligonucleosomal substrates were reconstituted *in vitro* from supercoiled plasmid DNA (3.2 kb, pGIE-0), native core histones and H1 prepared from *Drosophila* embryos. Core histone to DNA and H1 to nucleosome ratios were titrated in pilot ATP-dependent chromatin assembly reactions (*32*) to achieve ~160 bp nucleosome repeat length (NRL) in H1-free chromatin and ~200 bp NRL in H1-containing chromatin. Oligonucleosomal templates were then reconstituted by gradient salt dialysis based on calculated component ratios in the presence of 0.1 mg/ml BSA. Recombinant Su(var)3-9–His_6 was expressed in *E. coli* BL21(DE3)pLys from pET15b-Su(var)3-9 construct generously provided by Axel Imhof (*33*) and purified on Talon (Clontech) resin. HMT reactions contained 50 nM Su(var)3-9–His_6, 20 nM plasmid DNA (in chromatin-containing reactions only), 800 nM of each individual core histone (in H1– reactions) or 640 nM core histones and ~320 nM H1 (in H1+ reactions), 2 µg/ml BSA and 2.5 µM S-[Methyl-^{3}H]-Adenosyl-L-methionine (^{3}H-SAM, 80.7 Ci/mmole, Perkin Elmer) in 50 µl reaction buffer (50 mM Tris-HCl, pH 8.8, 5 mM $MgCl_2$, 4 mM dithiothreitol). The reaction mixtures were incubated for 60 min at 27°C, loaded on SDS-PAGE and stained with Coomassie or transferred to PVDF for autoradiography. For *in vitro* qChIP, 10% of the reaction volume was diluted to 200 µl with RIPA buffer with 1% formaldehyde, followed by the qChIP protocol described above. In control *in vitro*

ChIP experiments, Su(var)3-9–His_6 was replaced with equimolar amounts of purified recombinant GAL4-VP16 (a generous gift of Jim Kadonaga and Mai Khuoung) (*34*) or MBP-TRR(2199-2410)-His_6 (a generous gift of Thomas Kusch) (*35*).

Protein interactions assays

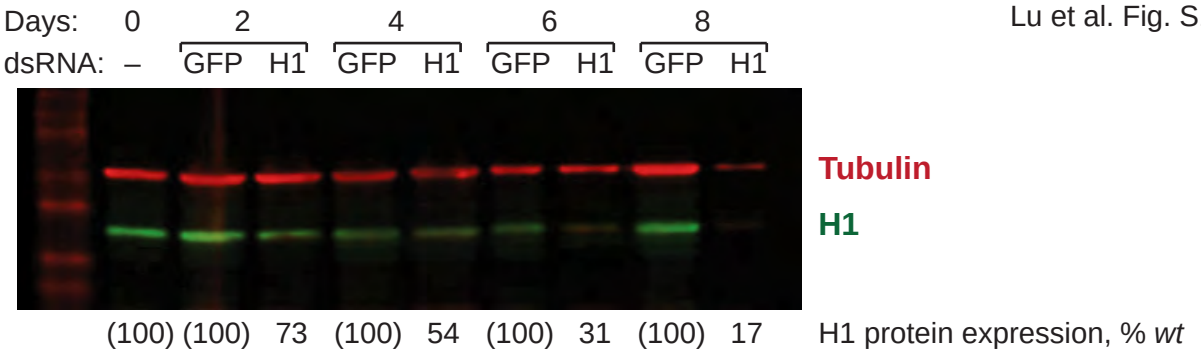
UAS-driven GST fusion constructs for HP1, Su(var)3-9 and PtC (*25*) were prepared by cloning appropriate PCR products into pUAST plasmid; cloning details and primer sequences are available upon request. *Drosophila* S2 cells were co-transfected with a pAc-GAL4 driver construct (a generous gift of Thomas Kornberg) and indicated pUAST-GST constructs using Effectene reagent (Qiagen) and cultured in Schneider medium (Invitrogen) for 48 hr. Typically, $5•10^6$ cells were transfected with 1 μg DNA in 60 mm dishes. They were lysed with a protease-inhibitor cocktail (Invitrogen), and nuclear extracts were prepared according to the instruction of Nuclear Complex Co-IP Kit (Active Motif). To precipitate the complexes, supernatants were incubated for 4 hr with glutathione Sepharose 4B gel (Pharmacia Biotech), the beads were washed five times with RIPA buffer, boiled in SDS-PAGE loading buffer, and precipitated material was analyzed by Western blot (*1*). Alternatively, the lysates were immunoprecipitated with anti-H1 antibody (*4*) as described (*25*). Rabbit anti-H1 (1:50,000) or mouse anti-GST antibodies (1:1,000, Santa Cruz Biotechnology) were used for Western. Full-length Su(var)3-9 was translated *in vitro* from pET15b-Su(var)3-9 in TnT T7 Reticulocyte Lysate System (Promega) in the presence of 20 μCi of EasyTag ^{35}S-L-methionine (>1,000 Ci/mmol, Perkin Elmer). GST fusions of H1, H2A and HP1 were expressed in *E.coli* BL21(DE3)pLys from constructs prepared in pGEX 4T-1 (GE Life Sciences). Primer sequences and cloning details are available upon request. Bacterial lysates were incubated with ^{35}S-labeled Su(var)3-9 or purified Su(var)3-9–His_6 expressed in *E.coli*, and GST fusions were purified on glutathione Sepharose as described above. The purified proteins were analyzed by SDS-PAGE, Coomassie staining and autoradiography or anti-6His Western blot.

Fig. S1. Comparative genome-wide analyses of transcripts in H1-depleted *Drosophila* Kc cells and salivary glands. (**A**) Western blot analysis of H1 knockdown in *Drosophila* Kc cells. H1 protein was depleted by RNAi in cultured cells as described in *Methods*. H1 protein levels were examined by semi-quantitative Western (*1*) at days 0, 2, 4, 6 and 8 of RNAi treatment (green). Tubulin Western (red) was used as loading control. Relative H1 expression levels (%) are shown at the bottom and are normalized to protein loading and control Kc cells (RNAi of unrelated protein, GFP). (**B**) Genome-wide analysis of transcripts in H1-depleted Kc cells. Transcript expression was examined by microarray analyses in H1-depleted (~31% control level) and control (GFP RNAi) Kc cells. The graphs plot signal intensities for transcripts in control (X-axis) versus H1-depleted (Y axis) samples and are the average of two independent experiments. Standard normalization procedures were applied to the raw data. The diagonal lines with a slope of 1 indicate equal expression levels and two threshold activation/repression levels (1.25-fold change). Significantly affected transcripts above or below the threshold (1.25-fold) are indicated by dots. Left panel, signals for protein-coding gene probes. Right panel, signals for probes annotated as TE probes. Numbers in the top left and bottom right corners of each panel represent percentages of transcripts that are up- or down-regulated above the threshold, relative to the total number of probes (18,833 protein coding genes, 79 TEs). (**C**) Genome-wide analysis of transcripts in H1-depleted salivary glands. The numerical data originally presented in **Fig. 1A** was re-analyzed with fold-activation threshold set to 1.25, as in (B) above, for direct comparison.

Fig. S2. *Drosophila* H1 represses transposable elements *in vivo*, and the repression is partially reversed by ectopic overexpression of Su(var)3-9. (**A**) Activation of TEs by H1 depletion in larvae. Transcripts were examined in H1-depleted (~5% wild-type protein level) and control (*Nau* RNAi) animals as in **Fig. 1B**, but RNA samples were prepared from L3 larvae, rather than from salivary glands (SGs). Fold changes were calculated as a ratio of signals for H1-depleted samples to those for control samples. Values were normalized to the expression of RP49 and are representative of three independent experiments. Standard deviations are shown as error bars. *yellow* and *tubulin* (*Tub*) were used as controls. (**B**) Activation of TEs by H1 depletion in larval ovaries. The experiments similar to those in (A) were performed on ovaries dissected from L3 larvae. (**C**) ChIP analyses of dimethyl-H3K9 occupancy at TE regulatory regions in *Su(var)3-9* larvae. The presence of the repressive histone mark ($H3K9Me_2$) in *copia*, *gypsy*, *ZAM*, Eu *Ste*, Het *Ste*, *yellow* and *Tub*, was measured by quantitative ChIP in control (gray bars) and homozygous *Su(var)3-9[6]* (black bars) whole larvae. The ordinate indicates the amounts of specific PCR products in ChIP DNA samples relative to input DNA. All ChIP experiments were performed in triplicate. Error bars, standard deviation. (**D**) ChIP analyses of H1 occupancy at TE regulatory regions in *Su(var)3-9* larvae. H1 presence in in the same TE and control loci as in (**C**) was measured by quantitative ChIP in control (gray bars) and homozygous *Su(var)3-9[6]* (black bars) larvae. (**E**) Distribution of dimethyl-H3K9 and H1 in SG polytene chromosomes of *Su(var)3-9* larvae. SGs from wild-type (WT) and homozygous *Su(var)3-9[6]* larvae were prepared as in (**Fig. 2E**), and polytene spreads were stained with DAPI and antibodies against $H3K9Me_2$ and H1. *Su(var)3-9* mutation strongly reduces the $H3K9Me_2$ mark but does not affect polytene chromosome structure or H1 distribution. (**F**) Re-repression of transposable elements upon ectopic over-expression of *Su(var)3-9* in H1-depleted whole larvae. Total RNA was prepared from control, H1-depleted and H1-depleted, Su(var)3-9-"rescued" (overexpressing UAS:Su(var)3-9–eGFP under the control of Tubulin-GAL4) whole L3 larvae. Real-time RT-PCR assays were performed and analyzed as in (A). Black bars, H1-depleted salivary glands; gray bars, H1-depleted, Su(var)3-9-rescued salivary glands.

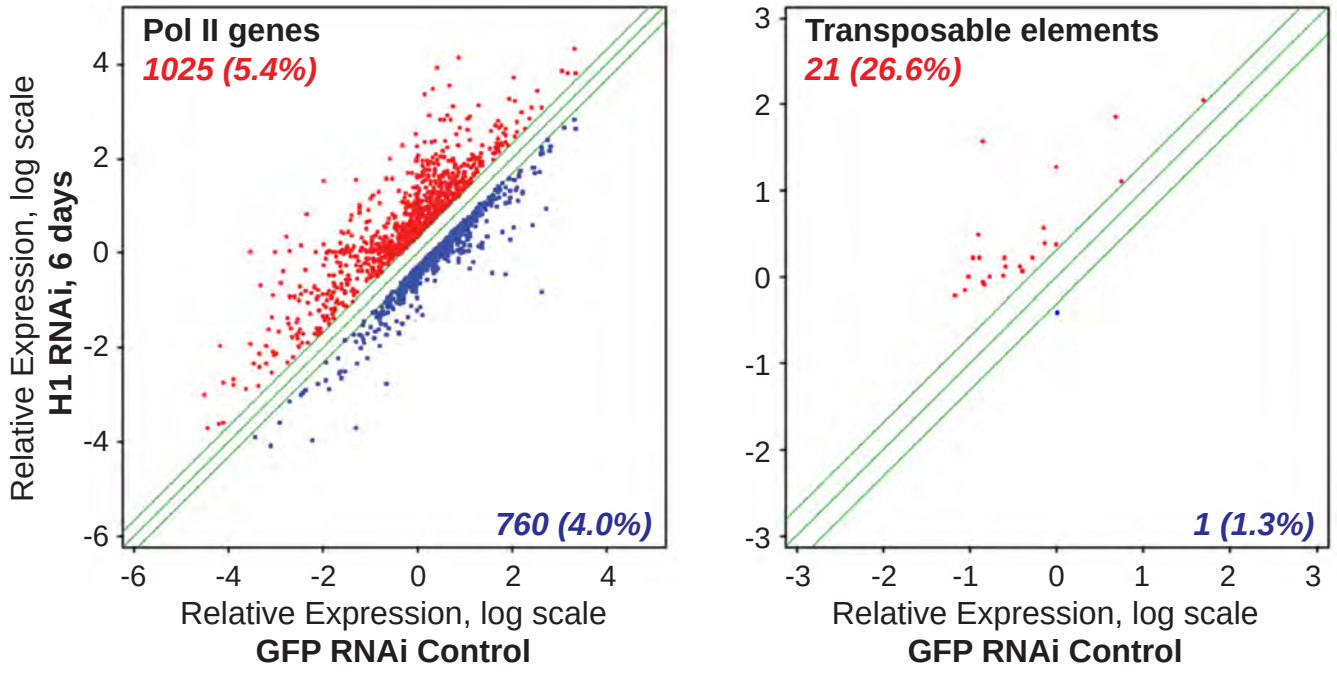
Fig. S3. Su(var)3-9 is tethered to H1-containing chromatin. (**A**) Recombinant Su(var)3-9, GAL4-VP16 and MBP-TRR fragment. 6His-tagged Su(var)3-9 was expressed and purified from bacteria and analyzed by SDS-PAGE and Coomassie staining. Purified recombinant GAL4-VP16 and MBP-TRR(2199-2410)-His_6 were the gifts of Jim Kadonaga, Mai Khuong and Thomas Kusch. Open arrowhead, Su(var)3-9–His_6, GAL4-VP16 or MBP-TRR-His_6 band; molecular mass markers (kDa) are shown on the left. (**B**) Protein composition of *in vitro* reconstituted chromatin. Oligonucleosomes prepared by salt-dialysis from plasmid DNA and core histones with (H1+) or without H1 (H1–) were analyzed by SDS-PAGE and Coomassie staining. Positions of BSA, H1 and core histone bands are indicated on the right; molecular mass markers (kDa) are shown on the left. (**C**) Micrococcal nuclease (MNase) analysis of reconstituted chromatin. Partial digestion with 4 different dilutions of MNase was performed on H1-free (H1–) and H1-containing (H1+) oligonucleosomes. De-proteinated DNA fragments were analyzed by agarose gel electrophoresis and stained with ethidium. Note the increased nucleosome repeat length in H1+ lanes consistent with H1 incorporation. Triangles at the top indicate increasing MNase concentrations; 123 bp ladder was used as a molecular mass marker.

A



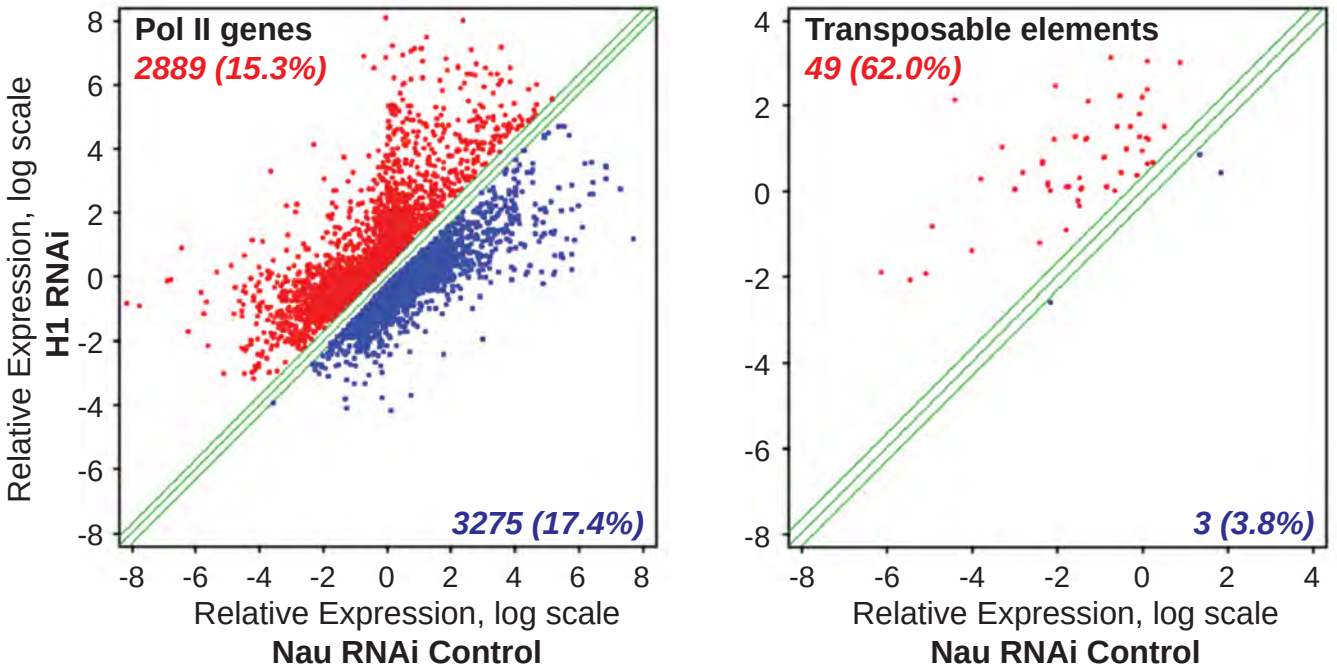
H1 RNAi in Kc cells, Western Blot

B

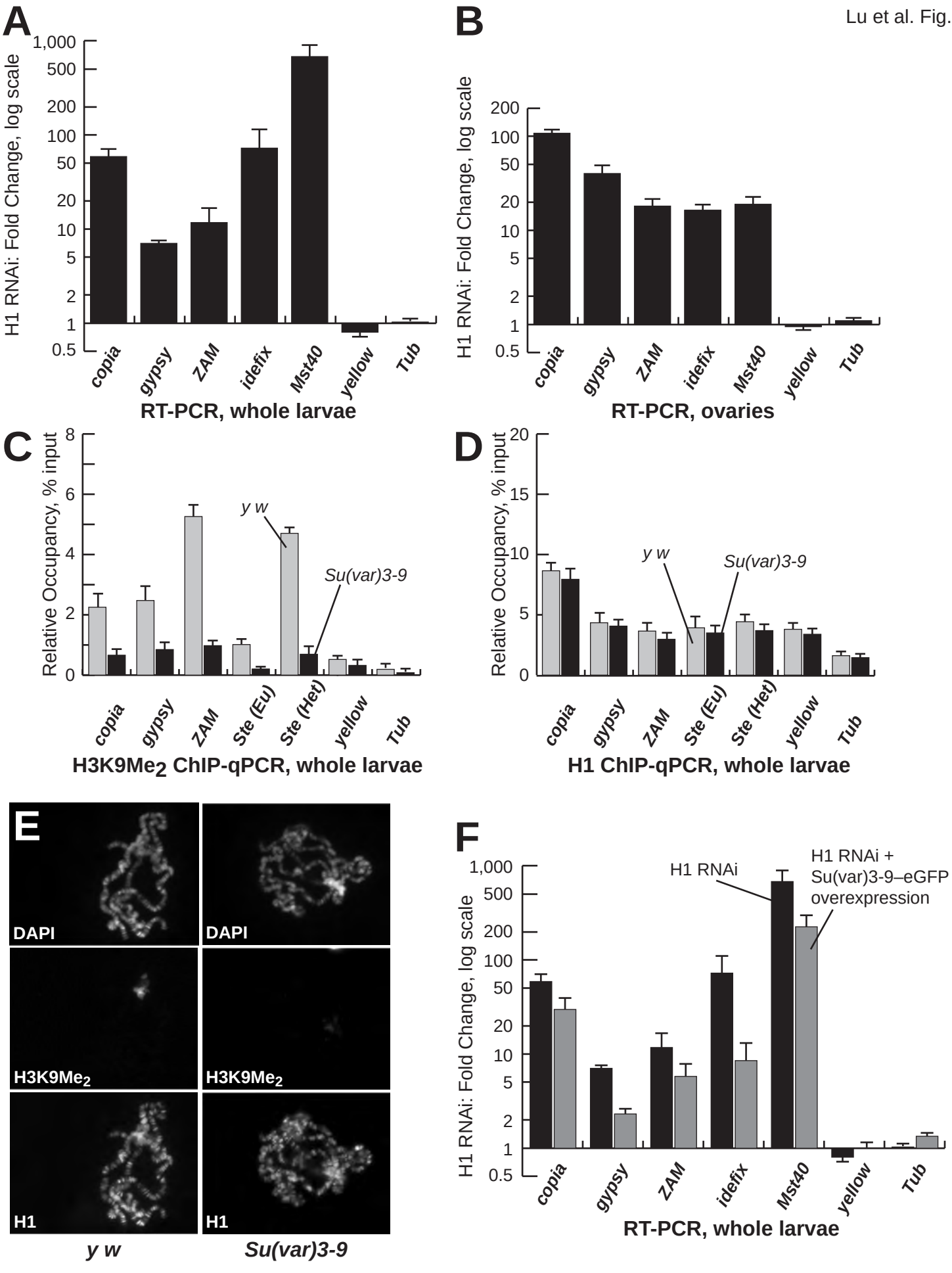


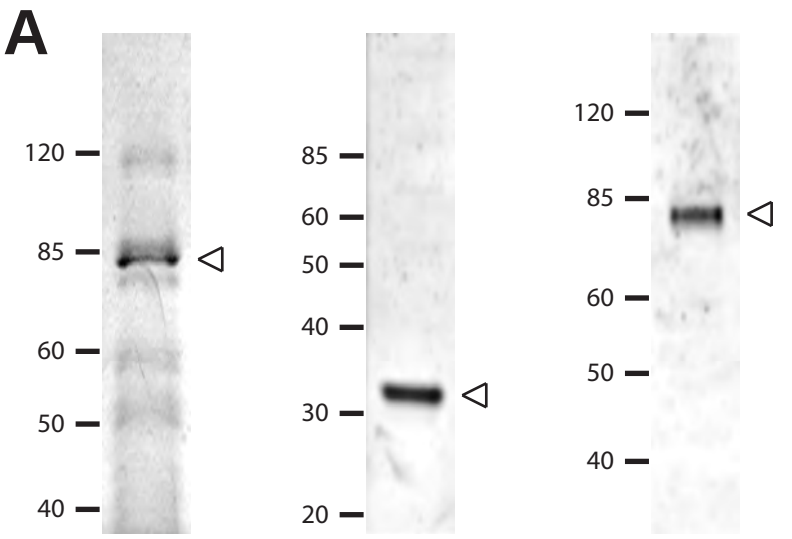
Kc cells, Affymetrix 2 array data

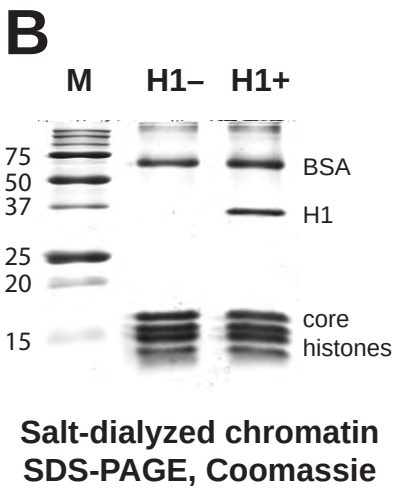
C



L3 salivary glands (SG), Affymetrix 2 array data


A
H1 RNAi: Fold Change, log scale
1,000
500
200
100
50
20
10
5
2
1
0.5
copia
gypsy
ZAM
idefix
Mst40
yellow
Tub
RT-PCR, whole larvae
B
H1 RNAi: Fold Change, log scale
200
100
50
20
10
5
2
1
0.5
copia
gypsy
ZAM
idefix
Mst40
yellow
Tub
RT-PCR, ovaries
C
Relative Occupancy, % input
8
6
4
2
0
y w
Su(var)3-9
copia
gypsy
ZAM
Ste (Eu)
Ste (Het)
yellow
Tub
H3K9Me2 ChIP-qPCR, whole larvae
D
Relative Occupancy, % input
20
15
10
5
0
y w
Su(var)3-9
copia
gypsy
ZAM
Ste (Eu)
Ste (Het)
yellow
Tub
H1 ChIP-qPCR, whole larvae
E
DAPI
DAPI
H3K9Me2
H3K9Me2
H1
H1
y w
Su(var)3-9
F
H1 RNAi: Fold Change, log scale
1,000
500
200
100
50
20
10
5
2
1
0.5
H1 RNAi
H1 RNAi +
Su(var)3-9–eGFP
overexpression
copia
gypsy
ZAM
idefix
Mst40
yellow
Tub
RT-PCR, whole larvae


A
120
85
60
50
40
85
60
50
40
30
20
120
85
60
50
40
Su(var)3-9–His
GAL4-VP16
MBP-TRR–His
SDS-PAGE, Coomassie


B
M
H1–
H1+
75
50
37
25
20
15
BSA
H1
core
histones
Salt-dialyzed chromatin
SDS-PAGE, Coomassie

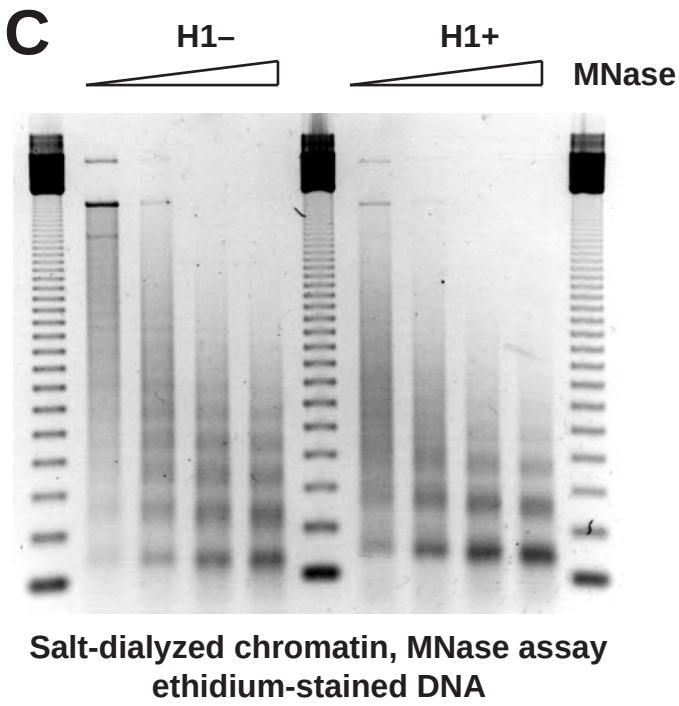

C
H1–
H1+
MNase
Salt-dialyzed chromatin, MNase assay
ethidium-stained DNA

Table S1. Regulation of transposable element activity by H1 knock-down in *Drosophila in vivo* (L3 salivary glands, SG) and Kc cells (Kc).

RNA expression profiling of the duplicate (Kc) and triplicate (SG) experimental sample groups was performed using Affymetrix Genechip system as described in Methods. Both *in vivo* and in cultured cells, H1 depletion activates an overlapping set of transcripts annotated as transposable elements. Samples with >1.25 fold change are highlighted in orange, and samples with >2.0 fold change in expression are highlighted in red. *P*-values of differential abundance between RNAi and corresponding control conditions were calculated by T-statistics, and data with *p*-values >0.05 were excluded from consideration (highlighted in red).

Probe Set ID	Name	Avg SG H1 RNAi vs Nau RNAi	*p*-value	Avg Kc H1 RNAi vs GFP RNAi	*p*-value
1625791_s_at	invader4	89.60	0.00015	2.25	0.01716
1639729_s_at	blastopia	20.40	0.00000	1.53	0.00477
1623960_s_at	GATE	19.81	0.00043	2.24	0.00050
1640448_x_at	ZAM	17.17	0.00204	1.23	0.01175
1630934_at	R2-element	17.04	0.00149	1.42	0.09126
1636749_at	Bari2	14.98	0.00001	1.09	0.57824
1627936_s_at	invader3	9.65	0.00006	2.02	0.01013
1634666_at	ZAM	9.02	0.00388	1.27	0.00557
1635258_s_at	Quasimodo	8.55	0.00113	1.19	0.00350
1641450_s_at	micropia	8.50	0.00387	1.58	0.07927
1631349_s_at	Tabor	7.67	0.00158	1.20	0.05180
1624819_s_at	gypsy	7.65	0.00077	1.45	0.07845
1626392_s_at	17.6	7.40	0.00000	1.43	0.00139
1626966_s_at	Juan	7.18	0.00011	0.89	0.24245
1635696_s_at	McClintock	6.66	0.00018	1.12	0.39400
1634633_s_at	accord	6.58	0.00001	2.13	0.00006
1626205_s_at	G6	6.07	0.00077	0.87	0.09237
1629641_s_at	Ivk	5.64	0.01073	1.63	0.00663
1633959_s_at	Burdock	5.34	0.00017	0.86	0.05810
1635829_s_at	gypsy2	5.12	0.00166	1.70	0.00115
1635017_at	gypsy6	5.10	0.00495	5.22	0.00263
1638469_s_at	opus	4.75	0.00145	1.09	0.14097
1624377_s_at	rover	4.64	0.00219	1.08	0.07366
1630948_s_at	Doc3-element	4.55	0.00000	1.24	0.05100
1640955_s_at	Tirant	4.31	0.00011	0.93	0.35628
1641210_s_at	qbert	3.57	0.00050	2.60	0.00776
1632295_s_at	Doc2-element	3.36	0.00139	1.76	0.00411
1632924_at	Tom1	3.31	0.00608	1.21	0.11064
1635886_s_at	blood	3.28	0.00028	0.93	0.03711
1623158_s_at	Tom1	3.20	0.00256	1.30	0.05554
1633998_s_at	HMS-Beagle	3.03	0.01122	1.71	0.01677
1634187_x_at	Dm88	3.00	0.00853	1.29	0.07544
1640606_x_at	Cr1a	2.95	0.00125	1.61	0.00831
1637622_s_at	Bari1	2.77	0.00032	1.19	0.04960
1632683_s_at	copia	2.57	0.00003	1.09	0.04989
1632902_s_at	G3	2.46	0.00032	1.54	0.08629
1626453_x_at	G4	2.34	0.00050	0.90	0.18014
1640167_s_at	412	2.23	0.00186	1.09	0.00517
1641421_s_at	diver2	2.19	0.05984	2.40	0.01007

1639054_s_at	pogo	2.16	0.00094	1.86	0.03760
1626434_s_at	gypsy5	2.10	0.00064	1.21	0.00916
1623159_at	looper1	2.02	0.00162	1.02	0.89600
1624543_s_at	springer	1.95	0.00494	1.81	0.10221
1624631_x_at	Rt1a	1.92	0.00012	1.14	0.11196
1625050_s_at	1731	1.83	0.00160	1.02	0.67469
1640242_s_at	Transpac	1.83	0.00588	1.91	0.01940
1630420_x_at	baggins	1.75	0.01979	1.13	0.24281
1630262_s_at	transib1	1.66	0.01159	1.36	0.01537
1625195_s_at	mdg1	1.62	0.09036	1.28	0.01754
1630585_s_at	HeT-A	1.45	0.22827	1.38	0.10788
1634508_at	INE1	1.43	0.02611	1.00	0.96696
1624224_at	HeT-A	1.41	0.04022	1.05	0.63617
1628082_at	aurora-element	1.37	0.15253	0.74	0.02667
1627940_at	Stalker3	1.36	0.05480	1.11	0.37582
1637786_s_at	R1-element	1.32	0.07871	1.24	0.00895
1631895_s_at	gypsy3	1.31	0.09286	0.86	0.08256
1637377_x_at	mariner2	1.21	0.77749	1.04	0.21701
1631713_x_at	S-element	1.20	0.06602	1.15	0.22047
1627374_at	transib3	1.20	0.37826	1.12	0.18493
1633970_at	NOF	1.18	0.46404	1.12	0.16582
1635012_x_at	transib3	1.13	0.48394	1.04	0.59613
1627177_x_at	transib4	1.13	0.10484	1.02	0.79763
1638228_s_at	rooA	1.11	0.29276	1.11	0.24498
1634338_x_at	Rt1c	1.10	0.15896	0.81	0.23486
1623349_x_at	Rt1b	1.08	0.28389	1.43	0.01051
1636015_s_at	Stalker2	1.07	0.74723	1.29	0.00551
1626130_s_at	G-element	1.02	0.55548	0.99	0.91311
1626288_at	Circe	1.02	0.34201	1.23	0.12265
1629669_x_at	297	1.01	0.27657	0.77	0.12584
1641296_at	S-element	0.97	0.48514	0.98	0.89069
1623845_s_at	invader5	0.96	0.71349	0.91	0.02330
1623559_s_at	I-element	0.94	0.89535	0.88	0.07895
1625649_s_at	frogger	0.87	0.08939	0.87	0.26529
1631791_at	?	0.82	0.02715	0.92	0.50934
1638428_at	TART-element	0.78	0.01313	1.06	0.77709
1632126_at	Penelope/ORF1	0.77	0.12380	0.90	0.34218
1637055_s_at	gtwin	0.73	0.00444	0.96	0.56529
1629242_x_at	TART-element	0.71	0.00362	1.05	0.48864
1628989_at	P-element	0.40	0.00677	1.06	0.31559

Table S2. H1 knockdown in *Drosophila in vivo* activates the expression of transposable element-related small RNAs.

Small RNA expression in salivary glands and ovaries of control (Nau RNAi) and H1 knockdown (to ~5% control H1 protein level) larvae was analyzed by masive parallel sequencing as described in *Methods.* The read counts are presented as normalized reads per miliion genomic mappers. n.d., not detected in control or experimental samples.

	Saliavry glands, small RNA reads (21-28 nt)			Ovaries, small RNA reads (21-28 nt)		
Name	Nau RNAi	H1 RNAi	**Ratio H1/Nau**	Nau RNAi	H1 RNAi	**Ratio H1/Nau**
invader4	1.756	154.580	**88.043**	100.607	255.346	**2.538**
blastopia	n.d.	n.d.	**n.d.**	n.d.	n.d.	**n.d.**
GATE	5.518	46.992	**8.516**	500.260	1248.957	**2.497**
ZAM	0.502	56.885	**113.399**	354.103	893.264	**2.523**
R2-element	0.251	8.656	**34.513**	30.895	67.196	**2.175**
Bari2	n.d.	n.d.	**n.d.**	n.d.	n.d.	**n.d.**
invader3	0.752	15.458	**20.543**	285.976	653.149	**2.284**
ZAM	0.502	56.885	**113.399**	354.103	893.264	**2.523**
Quasimodo	3.762	35.862	**9.532**	432.925	1052.743	**2.432**
micropia	3.261	108.824	**33.375**	214.284	993.610	**4.637**
Tabor	1.003	4.328	**4.314**	631.366	904.015	**1.432**
gypsy	3.762	167.564	**44.538**	268.548	1404.852	**5.231**
17.6	3.511	19.168	**5.459**	471.742	1532.973	**3.250**
Juan	2.007	46.992	**23.419**	150.514	752.599	**5.000**
McClintock	0.502	2.473	**4.930**	133.878	335.086	**2.503**
accord	1.756	217.030	**123.612**	208.343	751.704	**3.608**
G6	2.007	3.710	**1.849**	428.964	602.975	**1.406**
Ivk	3.261	30.298	**9.292**	312.514	744.536	**2.382**
Burdock	2.007	26.588	**13.250**	115.658	350.317	**3.029**
gypsy2	1.505	6.802	**4.520**	619.087	1739.042	**2.809**
gypsy6	8.779	22.259	**2.536**	1419.978	2174.475	**1.531**
opus	7.775	113.152	**14.553**	472.534	1327.801	**2.810**
rover	2.007	4.328	**2.157**	819.112	1182.656	**1.444**
Doc3-element	1.003	28.443	**28.350**	217.057	650.461	**2.997**
Tirant	0.251	1.237	**4.930**	274.886	34.046	**0.124**
qbert	n.d.	n.d.	**n.d.**	n.d.	n.d.	**n.d.**
Doc2-element	1.003	6.802	**6.779**	225.771	700.634	**3.103**
Tom1	n.d.	n.d.	**n.d.**	0.792	4.480	**5.655**
blood	28.092	310.396	**11.049**	570.764	2118.926	**3.712**
Tom1	n.d.	n.d.	**n.d.**	0.792	4.480	**5.655**
HMS-Beagle	1.756	11.130	**6.339**	313.306	808.149	**2.579**
Dm88	1.756	22.259	**12.678**	233.692	737.368	**3.155**
Cr1a	9.280	161.999	**17.456**	421.835	1262.396	**2.993**
Bari1	0.502	3.092	**6.163**	91.497	305.520	**3.339**
copia	24.580	348.113	**14.162**	329.942	768.727	**2.330**
G3	1.254	12.985	**10.354**	5.149	15.231	**2.958**
G4	0.502	6.801	**13.559**	70.504	206.069	**2.923**
412	10.785	32.771	**3.038**	1230.252	3779.125	**3.072**
diver2	2.508	6.801	**2.712**	200.817	437.224	**2.177**
pogo	35.115	1.855	**0.053**	220.621	387.051	**1.754**
gypsy5	0.251	0.618	**2.465**	274.886	956.876	**3.481**

looper1	0.251	1.855	**7.396**	21.389	43.006	**2.011**
springer	100.077	149.015	**1.489**	323.209	1147.714	**3.551**
Rt1a	1.756	6.183	**3.522**	175.071	371.820	**2.124**
1731	4.013	39.572	**9.861**	223.394	565.346	**2.531**
Transpac	4.013	21.641	**5.393**	51.888	204.277	**3.937**
baggins	3.010	30.298	**10.066**	314.495	784.854	**2.496**
transib1	0.502	16.695	**33.280**	4.753	16.127	**3.393**
mdg1	7.274	59.977	**8.246**	1225.895	3192.276	**2.604**
HeT-A	2.508	6.183	**2.465**	245.179	430.057	**1.754**
INE1	1.003	12.985	**12.942**	n.d.	n.d.	**n.d.**
HeT-A	2.508	6.183	**2.465**	245.179	430.057	**1.754**
aurora-element	1.003	1.855	**1.849**	74.465	198.005	**2.659**
Stalker3	2.007	8.038	**4.006**	n.d.	n.d.	**n.d.**
R1-element	16.554	51.320	**3.100**	n.d.	n.d.	**n.d.**
gypsy3	2.007	3.092	**1.541**	514.915	1333.176	**2.589**
mariner2	0.000	1.855	**INF**	1.584	0.896	**0.565**
S-element	3.261	30.298	**9.292**	44.362	130.809	**2.949**
transib3	n.d.	n.d.	**n.d.**	6.337	39.422	**6.220**
NOF	0.251	0.000	**0.000**	4.357	7.168	**1.645**
transib3	n.d.	n.d.	**n.d.**	6.337	39.422	**6.220**
transib4	0.000	6.183	**INF**	0.396	1.792	**4.524**
rooA	3.010	32.771	**10.888**	242.406	711.386	**2.935**
Rt1c	0.000	1.237	**INF**	23.765	38.526	**1.621**
Rt1b	6.772	39.572	**5.843**	544.226	1014.217	**1.864**
Stalker2	2.007	3.710	**1.849**	558.881	1262.396	**2.259**
G-element	4.264	6.802	**1.595**	371.927	654.045	**1.759**
Circe	3.261	45.137	**13.843**	275.282	682.715	**2.480**
297	10.033	304.213	**30.322**	578.290	2308.868	**3.993**
S-element	3.261	30.298	**9.292**	44.362	130.809	**2.949**
invader5	1.756	10.511	**5.987**	0.792	1.792	**2.262**
I-element	1.254	19.168	**15.284**	267.360	716.761	**2.681**
frogger	0.000	0.618	**INF**	0.792	1.792	**2.262**
?	n.d.	n.d.	**n.d.**	n.d.	n.d.	**n.d.**
TART-element	n.d.	n.d.	**n.d.**	n.d.	n.d.	**n.d.**
Penelope/ORF1	0.251	1.855	**7.396**	n.d.	n.d.	**n.d.**
gtwin	0.752	3.710	**4.930**	1110.237	2350.081	**2.117**
TART-element	n.d.	n.d.	**n.d.**	n.d.	n.d.	**n.d.**
P-element	0.502	14.221	**28.350**	0.000	8.960	**INF**

Table S3. Genetic interactions of *His1* and *Su(var)3-9*.

(Top) Double heterozygous *pINT-H1^{2M}/SM5, Cy; Tub:GAL4/TM3, Sb* transgenic fly lines were established and maintained at 18°C. They were mated with homozygous transgenic *UAS:Su(var)3-9–eGFP* or *yw* (control) flies at indicated temperatures. **(Bottom)** Double heterozygous *pINT-H1^{2M}/SM5, Cy; Tub:GAL4/TM3, Sb* transgenic flies were mated at 26°C to *yw* (control) flies or indicated mutatants. Viability was scored as the number of eclosed *Cy+*, *Sb+* adults relative to the total number of offspring. The expected number of *Cy+*, *Sb+* flies (calculated from the Mendelian distribution) is shown in parentheses. Probability values are calculated by the chi-square two-way test.

pINT-H1^{2M}/SM5; Tub:GAL4/TM3, Sb **cross with**	Temperature		
	23°C	26°C	29°C
UAS:Su(var)3-9–eGFP	19/78 (20)	25/153 (38)	5/231 (58)
yw (control)	40/256 (64)	13/192 (48)	0/147 (37)
p-values	0.08	0.005	0.07

Allele combination	*Df(3R)P47/Dp(3;3)C123.3*	*Su(var)3-9^{1}/TM3, Sb*	*yw* (control)
pINT-H1^{2M}/SM5; Tub:GAL4/TM3, Sb	0/38 (7)	0/61 (10)	13/192 (48)
p-values	0.09	0.04	N/A

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