The Heterochromatin Protein 1 positively regulates euchromatic gene expression by RNA binding

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HP1 is a well known conserved protein involved in heterochromatin formation and gene silencing in different species including humans[1-4]. A general model has been proposed for heterochromatin formation and epigenetic gene silencing in different species that implies an essential role for HP1. According to the model, histone methyltransferase enzymes (HMTases) methylate the histone H3 at lysine 9 (H3-MeK9), creating selective binding sites for itself and the chromodomain of HP1[5]. This complex is thought to form a higher order chromatin state that represses gene activity. It has also been found that HP1 plays a role in telomere capping[6]. Surprisingly, recent data have suggested an association of HP1 in gene activity[7-10] but the nature of this interaction is still completely obscure. Here we show, that HP1 is required for positive regulation of more than one hundred euchromatic genes by its association with the corresponding RNA transcripts and by its interaction with the well known proteins DDP1[11], HRB87F[12] and PEP[13], which belong to different classes of heterogeneous nuclear ribonucleoproteins (hnRNPs) involved in RNA processing . We also found that all these hnRNP proteins also bind heterochromatin and are dominant suppressors of position effect variegation.

Our data together, show novel and unexpected functions for HP1 and hnRNPs proteins. All these proteins are in fact involved in both RNA transcript processing and in heterochromatin formation. This suggests that, in general, similar epigenetic mechanisms have a significant role in the metabolism of both RNA and heterochromatin.

HP1 is a chromosomal protein first discovered in *Drosophila melanogaster* through its association with the heterochromatin[1,2]. Molecular studies have shown that HP1 has two prominent structural motifs, the chromo domain[14] and chromoshadow domain[15], important for chromatin binding and protein interactions respectively. In *Drosophila*, HP1 is encoded by the *Su(var)2-5* locus, a dosage-dependent modifier of position effect variegation (PEV)[16]. In addition to being required for heterochromatin formation, HP1 also plays a critical role in telomere capping and the telomere transcriptional repression in *Drosophila*[6,17,18]. A detailed cytological analysis on polytene chromosomes of *Drosophila melanogaster* has shown that HP1 is present at about 190 euchromatic sites, including the developmental and heat shock induced puffs[19] and another study has shown that this protein can be removed from euchromatic sites by *in vivo* RNase treatment[7]. Intriguingly, recent experiments have suggested also a positive role of HP1 in gene expression. It has been shown that HP1 is positively involved in *Hsp70* gene activity[7]. Many euchromatic genes in *Drosophila* are down-regulated in HP1 deficient larvae[8] or cultured cells[9] although it is still unknown how many of these genes could be direct targets of HP1. High-resolution mapping experiments have also shown that HP1 is associated with transcriptionally active chromatin in *Drosophila*[10]. All these data together have opened the possibility that HP1 directly associates with the transcripts of many active genes modulating their

expression. To test this hypothesis, we firstly immunostained the polytene chromosomes of salivary glands with an antibody directed against HP1, and an antibody against the active form of Pol II (Phospho Ser2). As shown in Figure 1a (see also Supplementary Fig. 2a), HP1 and Pol II have an extensive co-localization. These results are confirmed by co-immunoprecipitation of Pol II with HP1 antibody (Figure 1b). We then verified a direct interaction of HP1 with RNA transcripts through *in vivo* and *in vitro* experiments using Hsp70 RNA. The ability of HP1 to interact with the Hsp70 RNA *in vivo* was confirmed by primer extension on the population of RNA immunoprecipitated from SL-2 cells with the C1A9 (anti-HP1) antibody (Figure 1c). By gel shift assay using different HP1 fragments[17], we found that only the HP1 fragments containing the chromodomain are capable of producing a gel shift of RNA (Figure 1d). These results indicate that HP1 associates with active genomic regions by its chromodomain binding to transcripts.

To identify HP1 targets, we performed a RIP-chip assay (RNA-immunoprecipitation on microarrays) in S2 cells for a wide screening (Supplementary information, section A). Using a stringent cutoff (10% top rank), we identified 105 genuine transcript targets (Supplementary Table 3). Although these transcripts were identified in cultured somatic cells, their genes correspond for the most part with HP1 immunosignals along the polytene chromosomes of larval salivary glands (Fig. 2a). We analyzed, by real time RT-PCR, the expression of 17 genes corresponding to the HP1 target transcripts in *Su(var)2-5*^{02}*/Su(var)2-5*^{05} mutant larvae, which express an HP1 with a functionally inactive chromodomain (see Methods). We chose 12 genes that comap with HP1 immunosignals on polytene chromosomes and five others located in regions apparently devoid of HP1 immunosignals. We found a significant reduction in transcripts of the 12

genes that comap with HP1 (Fig. 2b). Three of the genes that do not overlap with any HP1 signal did not show any significant variation between mutant and wild type larvae (Fig. 2c). These genes may be regulated by HP1 in cultured S2 cells but not in larval cells. For the other two genes which do not comap with HP1 on polytene chromosomes, we observed a reduction in transcripts, probably due to a down-regulation of their expression in other larval tissues but not in salivary glands. As a negative control, we tested five genes that do not comap with HP1 in salivary glands and whose transcripts were not HP1 targets in SL-2 cells. The results of RT-PCR analysis in HP1 mutant larvae clearly show no effect on the amount of their transcripts (Fig. 2d), implying that the lack of HP1 induces a specific effect in gene expression and not a general effect in gene expression due to the larval lethality induced by the mutation. Previous observations have shown a spreading of H3K9 methylation in salivary glands of HP1 null mutant larvae[20], suggesting a general effect on gene transcription following the complete loss of HP1. To test this possibility, we analyzed the H3K9 methylation along the polytene chromosomes in *Su(var)2-5*^{02} mutants and found no spreading of H3K9 methylation (Supplementary Fig. 1) thus supporting the view that the *Su(var)2-5*^{02} mutation affects the amount of transcripts of specific genes. This approach permitted us for the first time to systematically identify the direct targets of HP1 in the euchromatin and to determine HP1's positive regulatory role on the corresponding genes.

The direct association of HP1 with RNA transcripts suggests that HP1 could also interact with other RNA-binding proteins. Previous data have shown an interaction between HP1 and DDP1, a multi-KH-domain vigilin that binds single-stranded nucleic acids with high affinity in vitro[11,21]. The KH-domain is a motif identified for the first time in the human heterogeneous nuclear ribonucleoprotein K (hnRNP K)[22]. In

Drosophila, HP1 extensively colocalizes with DDP1 protein on sub-regions of the heterochromatin and in many sites along the euchromatic arms of polytene chromosomes[11] (Fig. 3a and Supplementary Fig. 2b). We asked whether HP1 might also interact with the hnRNP proteins HRB87F and PEP. Both these proteins associate with Hrb57A, another hnRNP protein that, like DDP1, is closely related to the human hnRNP K[23]. HRB87F is the closest *Drosophila* homolog to mammalian A/B type hnRNP[7], which can bind both RNA and single-stranded DNA[24]. PEP (Peptide on Ecdysone Puffs) is a unique zinc finger protein[8] which can bind DNA and, with higher affinity, RNA[25]. We found that HP1 colocalizes on polytene chromosomes and coprecipitates (Fig. 3b-c and Supplementary Fig. 2c-d) with both HRB87F and PEP proteins and DDP1. To analyze possible interdependencies in chromosomal localization among all these proteins we immunolocated each protein on the polytene chromosomes of larvae mutant for genes encoding each of the other proteins and we found that the correct localization of each protein on the euchromatin depends on presence of the others according to a hierarchical order with DDP1 on the top: DDP1>HRB87F>HP1>PEP (Supplementary Fig. 3). We conclude that all these proteins interact for their localization in an ordered manner.

Different classes of hnRNP proteins are involved in different aspects of RNA metabolism. Our data seem to exclude a major role of HP1 in transcript elongation, nuclear export and surveillance mechanism (Supplementary information, section B and Supplementary Fig. 4). We think that the marked effect of HP1 mutations on the amount of RNA transcripts, together with its association with different type of hnRNPs, that apparently play a central role in RNA packaging and stability[25,26], suggest that HP1 is also mainly involved in this function.

The immunofluorescence patterns of all the hnRNP proteins we analyzed show that these proteins not only share common sites in the euchromatin of polytene chromosomes, but with different intensities they are also present on the heterochromatic chromocenter. The heterochromatic convergence of these hnRNPs opens the possibility for their role in heterochromatin formation. A common approach to test the involvement of a gene in heterochromatin formation is to analyze its mutations for their effects on the heterochromatin-induced gene silencing (PEV). The gene for DDP1 has already been shown to be a suppressor of PEV[21]. We tested *Hrb87F* and *Pep* mutations for their effects on the variegation of the *Stubble (Sb)* gene associated with $T(2;3)Sb^{v}$ [27]. In this translocation, the dominant neomorphic *Sb* mutation is relocated adjacent to the pericentromeric heterochromatin of the second chromosome. Flies carrying the translocation have a mosaic phenotype with *Sb* and wild type bristles. The results reported in Table 1, clearly show that mutations at *Hrb87F* and *Pep* are dominant suppressors of PEV: they significantly increase the frequency of *Sb* bristles with respect to the control. We also tested *Hrb87F* and *Pep* mutations for their effects on variegation of the *white (w)* gene associated with the $In(1)w^{m4(28)}$ and *Tp(3;Y)BL2*, a Y chromosome rearrangement carrying the *Hsp70-lacZ* inducible transgene inserted into its centromeric region[29]. The heterochromatic location causes a variegation for the inducible *lac-Z* in salivary glands of larval males[30]. Also in these two cases we found a dominant suppressive effect of all the tested mutations at both loci (Supplementary text section C and Supplementary Fig. 5). These results open a window on new and unsuspected - functional properties of hnRNP proteins in heterochromatin formation and of HP1 in gene expression. It is evident that HP1 is a functionally multifaceted adaptor involved not only in heterochromatin formation, gene silencing and telomere capping , but also in

the regulation of gene expression. What molecular mechanisms are responsible for the functional versatility of HP1? Either HP1 possesses several modes of action, or HP1 always performs the same activity but with different partners in different contexts. In both cases, we think that conformational changes due to post-translational modifications, generating a sort of an epigenetic sub-code, would permit different interactions of HP1 in different contexts. We propose that, regardless of the mechanisms of action, the main function of HP1 is nucleic acid compaction: HP1's interaction with modified histones and specific hnRNP proteins produces a compaction of DNA that is the basis for heterochromatin formation and gene silencing, while HP1 interaction with RNA-packaging hnRNP proteins induces a compaction of RNA with the consequent stabilization that reinforces gene expression.

Methods Summary

Immunofluorescence[2,15], Fluorescence in situ hybridization (FISH) with oligodT-Cy3[33], Histochemical localization of β-galactosidase in PEV analysis[30], Purification of HP1 protein constructs and Electrophoretic mobility shift assay (EMSA)[14,20,34], Preparation of nuclear extracts for RIP-Chip[35], Quantitative Real-Time PCR[14], Co-immunoprecipitation of hnRNPs and Western blot[38].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature

Acknowledgments We thank E. Swanson for her critical reading of the manuscript and suggestions. We thank G. Reuter, F. Azorin, S. Haynes, J. Eissenberg and the Bloomington and Szeged stocks Center for mutant strains. We thank H. Saumweber for HRB87F and PEP antibodies, F. Azorin for DDP1 antibody, S. Elgin for HP1 antibody

and E. Andrulis for Rrp6 antibody. We thank W. Strätling for pBS-hsp70-SalI-SalI plasmid. We thank P. Spierer and G. Reuter for *Su(var)2-5* gene fragments.

Author Contribution L.P. and S.P. designed experiments and S.P. wrote the paper. L.F. performed immunofluorescence and PEV experiments. L.P. performed RIP-chip, RT-PCR and FISH experiments. R.N. and V.D.V. disegned and performed micro arrays data analysis. L.P. and A.F. performed primer extention and band shift.

Author Information Microarray data are available in the ArrayExpress database, http://www.ebi.ac.uk/miamexpress, under accession number E-MEXP-1556 (ChIP-chip records). Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to L.P. (lucia.piacentini@uniroma1.it) or S.P. (sergio.pimpinelli@uniroma1.it).

Methods

***Drosophila* strains.** The *Ore-R, In(1)w^{m4}*, *T(2;3)Sb^{v}* and *T(3;Y)BL2* stocks used here have been kept in our laboratory for many years. The *Su(var)2-5* mutant strains were obtained from G. Reuter. *Su(var)2-5^{05}* is a null mutation and *Su(var)2-5^{04}* encodes a truncated HP1 protein that lacks part of the domain required for its nuclear localization so that the protein results absent in the mutant nuclei[11,31]. *Su(var)2-5^{02}* is a point mutation in the chromodomain[32]. The *ddp1* strain was obtained from F. Azorin. The mutant strain carrying a null *Hrb87F* mutation, called *Df(3R)Hrb87F*, was obtained from S. Haynes. The other *Drosophila* stocks were obtained from Bloomington and

Szeged Stock Centers where are also described. Cultures were maintained at 24°C on standard cornmeal-sucrose-yeast-agar medium.

Immunofluorescence. Salivary glands were dissected in Cohen and Gotchell medium G containing 0.5% Nonidet P-40 and incubated in formaldehyde fixative solution for 25 minutes. For DRB treatments, one gland of the pair was dissected in medium G, the other was incubated in medium G plus 140µM DRB (5,6-Dichloro-l-β-D-ribofuranosylbenzimidazole). For ribonuclease digestion, dissected glands were incubated at room temperature with 50µg/ml DNase-free RNaseA in medium G. The preparations were incubated with primary antibodies: goat anti-HP1 (1:50) (Santa Cruz) or monoclonal mouse anti-HP1 C1A9, monoclonal mouse H5 (1:50) to the Ser-2 phosphorilated CTD of RNA Pol II (Covance), mouse anti-DDP1 (1:50), mouse anti-PEP (1:2), mouse anti-HRB87F (1:10) alone or in various pairwise combinations, overnight at 4°C in a humid chamber. The slides were washed in TBST (10mM Tris-HCl, pH 7.15, 150mM NaCl and 0.05% Tween 20) three times for 5 min and incubated with secondary antibodies (1:100 diluition of FITC-conjugated donkey anti-mouse and 1:400 dilution Cy3-conjugated rabbit anti-goat) (Jackson ImmunoResearch Laboratories) for 1 hour at room temperature in a humid chamber. Finally the slides were washed three times in TBST at 4 °C, stained with 4,6-diamidino-2-phenilindole (DAPI) at 0,01□g /ml, and mounted in antifading medium. Chromosome preparations were analyzed using a computer-controlled Eclipse epifluorescence microscope (model E1000, Nikon) equipped with a CCD camera (Coolsnap). The fluorescent signals, recorded separately as greyscale digital images, were pseudocolored and merged using Adobe Photoshop. For colocalization of HP1 with each protein, salivary glands from 10 larvae were prepared and about 3 good polytene nuclei of each larva were examined.

The colocalization was performed by considering the fluorescent signals whose patterns were stably conserved among the different preparations. To determine the immunopatterns in the different mutants, same CCD camera exposure times were used (0.2 sec for FITC or Cy3 and 0,05 sec for DAPI). When required, the fluorescence of the signals was measured with the Adobe Photoshop program.

RNA immunoprecipitation and Reverse Transcription. For RNA immunopreciptation, the nuclear extract was incubated with 50µg of monoclonal C1A9 anti-HP1 antibody at 4°C overnight under continuous gentle movement. One-hundred microliters of protein G-Sepharose (Sigma) suspension (50% packed Sepharose in Buffer C) was added and the incubation was continued overnight as described. The beads were pelleted by 2 min centrifugation at 240 g at 4°C; the pellet was briefly washed three times with 1mL of IP Wash Solution (150mM NaCl, 50mM Tris pH 7.5, 0.5% NP40) and the Sepharose was transferred for elution into a fresh plastic tube and pelleted again. The supernatant was then completely removed. To elute the immunocomplexes for protein analysis, an aliquot of beads was suspended in 50µL SDS-PAGE sample buffer and incubated for 10 min at 90°C; following centrifugation the supernatant was removed and used in Western blot for testing the presence of HP1 protein. To elute the immunoprecipitated RNAs, the pelleted beads were boiled in 200µL of DEPC water for 5 min, spun, and the supernatant recovered; 1mL of Trizol (Invitrogen) was added to 200 µL of supernatant and mixed, followed by the addition of 200µL of chloroform. This mixture was incubated at 4°C for 5 min and then centrifuged at 12000 g for 15 min; the RNAs in the aqueous phase were precipitated with half volume of isopropanol; after precipitation, the RNAs were resuspended in 10µL of DEPC water. Contaminating DNA was digested with RNase-free DNase I (Sigma). The

RNA purified from the previous step was used as a template to synthesize cDNA using oligo dT, random hexamers and SuperScript reverse transcriptase III (Invitrogen) according to the manufacturer's protocol.

cDNA amplification and labeling. The cDNA was used as template for a two-step random PCR amplification[36]; in Round A, Sequenase is used to extend randomly annealed primers (Primer A) to generate templates for subsequent PCR; during Round B, the specific primer B is used to amplify the templates previously generated and finally round C consists of additional PCR cycles to incorporate the aminoallyl dUTP nucleotide. About 25 ng of each cDNA sample was used for two 8 min extensions with 2.7 mM Round A primer (5'-GTT TCC CAG TCA CGA TCN NNN NNN NN-3', N being a mixture of all four nucleotides with 60% A+T and 40% G+C) at 37°C with 267U/ml Sequenase version 2.0 (usb). DNA was denatured at 94°C for 2 min and cooled to 10°C , and Sequenase 2.0 was added between extensions. The resulting products were used as template for 25 cycles of PCR using 1U/100µl *Taq* polymerase (*Platinum Taq* Invitrogen) and 10mM Round B primer (5'-GTT TCC CAG TCA CGA TC-3'). Finally this DNA was used as template for 25 cycles PCR to incorporate the amino allyl dUTP nucleotides to which the fluorescent dye may be attached[37]. To remove Tris buffer which interferes with the indirect coupling, the aminoallyl-cDNA samples were desalted by filtering through a Microcon 30 and then mixed with the succinimidyl esters of the Cy3 or Cy5 dyes (Amersham Biosciences) in 0.1M sodium bicarbonate buffer (pH 9); the coupling reaction was incubated overnight in the dark at room temperature. Each dye-labeled sample was purified by AutoSeq MicroSpin G-50 columns (Amersham Biosciences) following the manufacturer's directions.

Microarray hybridization and scanning. For hybridization and washing of arrays we followed the Canadian Drosophila Microarray Center protocols (www.flyarrays.com). For scanning we used a ScanArray Lite Microarray Scanner (Packard Bioscience) with laser intensities chosen to maximize signals while avoiding pixel saturation. ScanArray express software was used to quantify hybridization signals; bad spots were flagged automatically by the software and subsequently each slide was inspected manually. Since each gene is represented by two replica spots on the array, data were treated with GEPAS on-line tool (http://gepas.bioinfo.cnio.es) which averaged the two spots. The arrays we scanned are produced by the Canadian Drosophila Microarray Centre located at the University of Toronto (www.flyarrays.com). The 12k1 platform is a primarily cDNA-based glass microarray. The array features 11,018 Berkeley Drosophila Genome Project cDNAs, 297 NIH Testis cDNAs and 432 gene sequences that were amplified from genomic DNA. Approximately 10,500 unique genes are represented by the above, corresponding to roughly 78% of the predicted genes in *Drosophila melanogaster* (FlyBase annotation release 3.2).

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Figure Legends

Figure 1. HP1 can directly bind RNA *in vitro* and *in vivo* and interacts with Pol II. (a) Immunolocalization of HP1 and active Pol II on polytene chromosomes of *D. melanogaster*. Signals produced by the two antibodies show an extensive colocalization. (b) Coimmunoprecipitation of HP1 and Pol II by the C1A9 anti-HP1 antibody. To test the specificity of HP1 with Pol II interction, we probed also with an antibody against α-

actin. (c) Primer extension of RNA immunoprecipitated from SL-2 cells with CIA9 antibody. Two signals are present only in HP1 immunoprecipitates of heat shocked cells (HS). (d) Electromobility shift assay. Left, a diagram of the HP1 fragments used in the gel shift assay. Right, the results of EMSA of radiolabelled HSP70 RNA using the different HP1 fragments. The absence of shift (lane d) using the HP1 fragment lacking the chromo-domain strongly suggests that this part of the protein is responsible for the binding of HP1 to the RNA transcripts.

Figure 2. The genes corresponding to transcript targets of HP1 in S2-L cells show a good overlap with HP1 binding sites on polytene chromosomes and appear down-regulated in HP1 mutants larvae. (a) Localization of HP1 binding sites and the genes corresponding to the HP1 target transcripts along polytene chromosomes of *Drosophila* wild type larvae. Blue bars represent sites where the HP1 targets genes overlap with HP1 immunosignals; orange bars indicate the localization of HP1 target genes that do not overlap with HP1 immunosignals. (b) Quantitative RT-PCR analysis of the expression, in wild type and HP1 mutant larvae, of a sub-set of target genes that overlap with HP1 whose position is indicated in (A) by blue bars marked with asterisks. (c) Quantitative RT-PCR analysis of the expression, in wild type and HP1 mutant larvae, of a sub-set of HP1 target genes that do not overlap with HP1 whose position is indicated in (a) by orange bars marked with asterisks. (d) Quantitative RT-PCR analysis of the expression, in wild type and HP1 mutant larvae, of a sub-set of genes that were not found among the HP1 target genes in S2-L cells and do not co-map with any of the HP1 immunosignals along the polytene chromosomes.

Figure 3. HP1 associates with, and colocalizes on polytene chromosomes with, DDP1, HRB87F and PEP hnRNP proteins. (a - c) A part of the right arm of a wild type polytene second chromosome simulteneously immunostained with the anti-HP1 antibody and an antibody against: (a) DDP1; (b) PEP and (c) HRB87F. There is an extensive colocalization of HP1 with each of the other proteins (arrows). We could not perform simultaneous immunostaining among the DDP1, PEP and HRB87F because the available specific antibodies were made in mouse. However, the colocalization of each protein with HP1 in same regions indicated that all the proteins colocalize in such regions. We made an approximate estimation of the colocalization extent by counting the number of overlapping sites and we found that about 70% of HP1 signals overlap with those of both proteins. (d) Coimmunoprecipitation of HP1 with DDP1, PEP and HRB87F proteins by the C1A9 anti-HP1 antibody.

Table 1. Dominant effects of *Hrb87F* and *PEP* mutations on *Stubble* variegation of *T(2;3)Sb*^{v} [(a)]

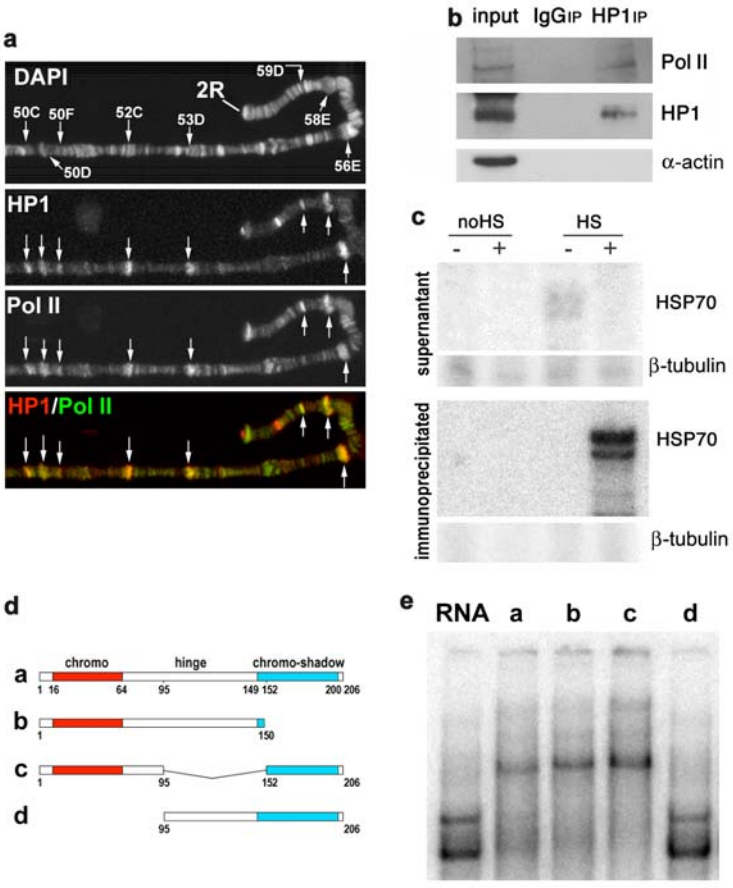
Mutant	No. of flies	total bristles[(b)]	% of *Sb* bristles	*P*[(c)]
+ (control)	102	1428	16.8	
TM3, Ser[(d)]	262	3668	16.4	
Hrb87F^{KG02089}	67	938	37.5	0,0001<*P*<0,005
Df(3R)Hrb87F	99	1386	37.0	0,0001<*P*<0,005
Pep^{KG00294}	52	728	27.6	0,0001<*P*<0,005
Df(3R)Pep^{81k19}	51	714	26.5	0,0001<*P*<0,005

[a] Crosses were *T(2;3)Sb*^{v} males to either *Hrb87F*^{KG02089}*/TM3, Df(Hrb87F)/TM3,* *Pep*^{KG00294}*/TM3, Df(Pep)/TM3* females. The number of *Sb* and *Sb*$^{+}$ bristles in flies carrying the *T(2;3)Sb*^{v} alone with flies who had the translocation and were also heterozygotes for either *Hrb87F*^{KG02089}, *Df(Hrb87F),* *Pep*^{KG00294}, *Df(Pep)* or *TM3* balancer chromosome, were compared.

[b] The bristles examined were the seven pairs of major dorsal bristles: posterior supraalars, anterior postalars, posterior dorsocentrals, anterior and posterior scutellars, and anterior and posterior sternopleurals (Sinclair et al., 1983).

[c] *P* values have been calculated using a χ^2 contingency test.

[d] Total number of bristles counted in flies carrying the *T(2;3)Sb*^{v} and the *TM3* balancer chromosome from all the crosses. Note that the proportion of the *Sb* bristles is similar to that of the control.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59D
2R
50C
50F
52C
53D
58E
50D
56E
HP1
Pol II
HP1/Pol II
b
input
IgG IP
HP1 IP
Pol II
HP1
α-actin
c
noHS
HS
- +
- +
supernatant
HSP70
β-tubulin
immunoprecipitated
HSP70
β-tubulin
d
chromo
hinge
chromo-shadow
a
1 16
64
95
149 152
200 206
b
1
150
c
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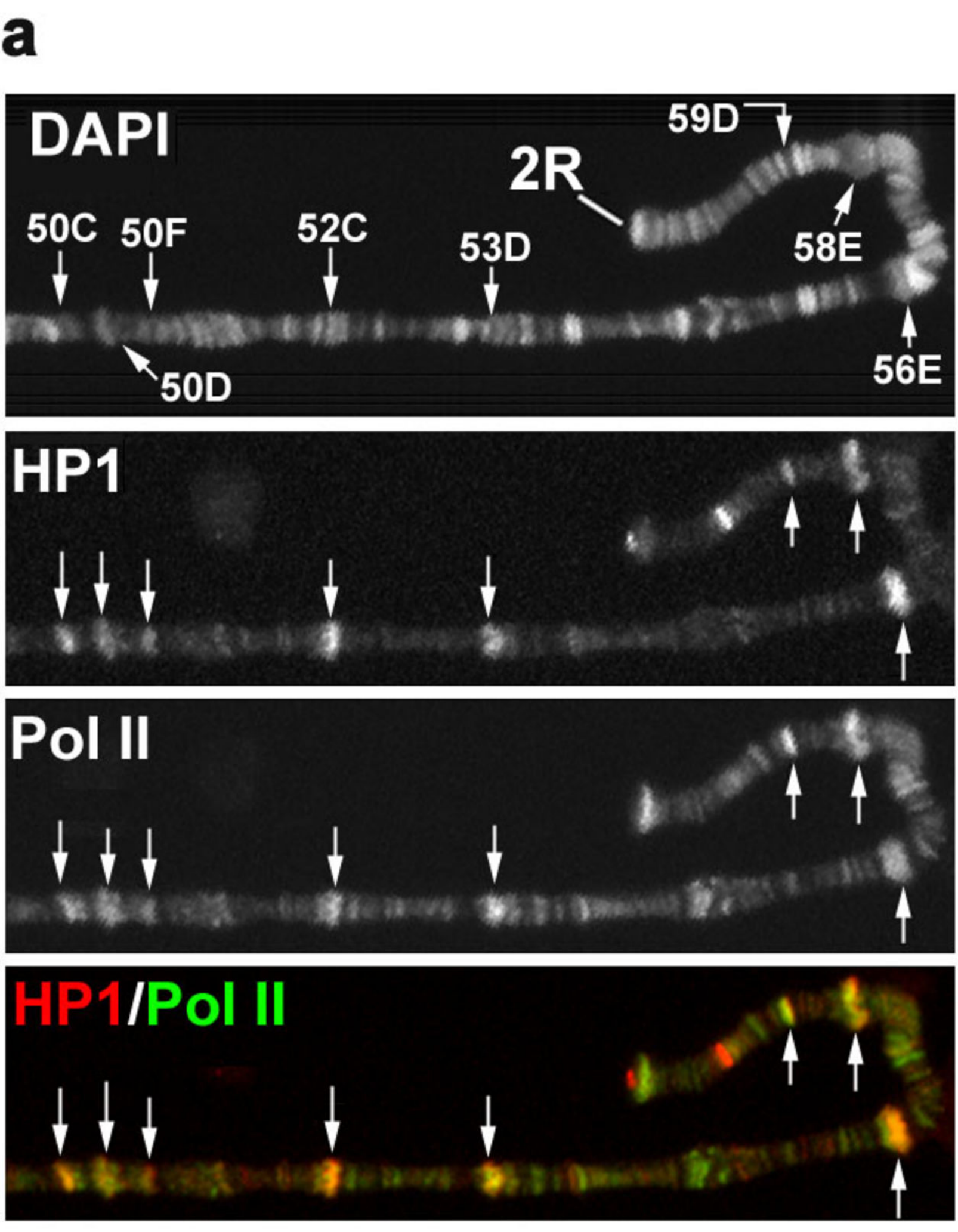
d
95
206
e
RNA
a
b
c
d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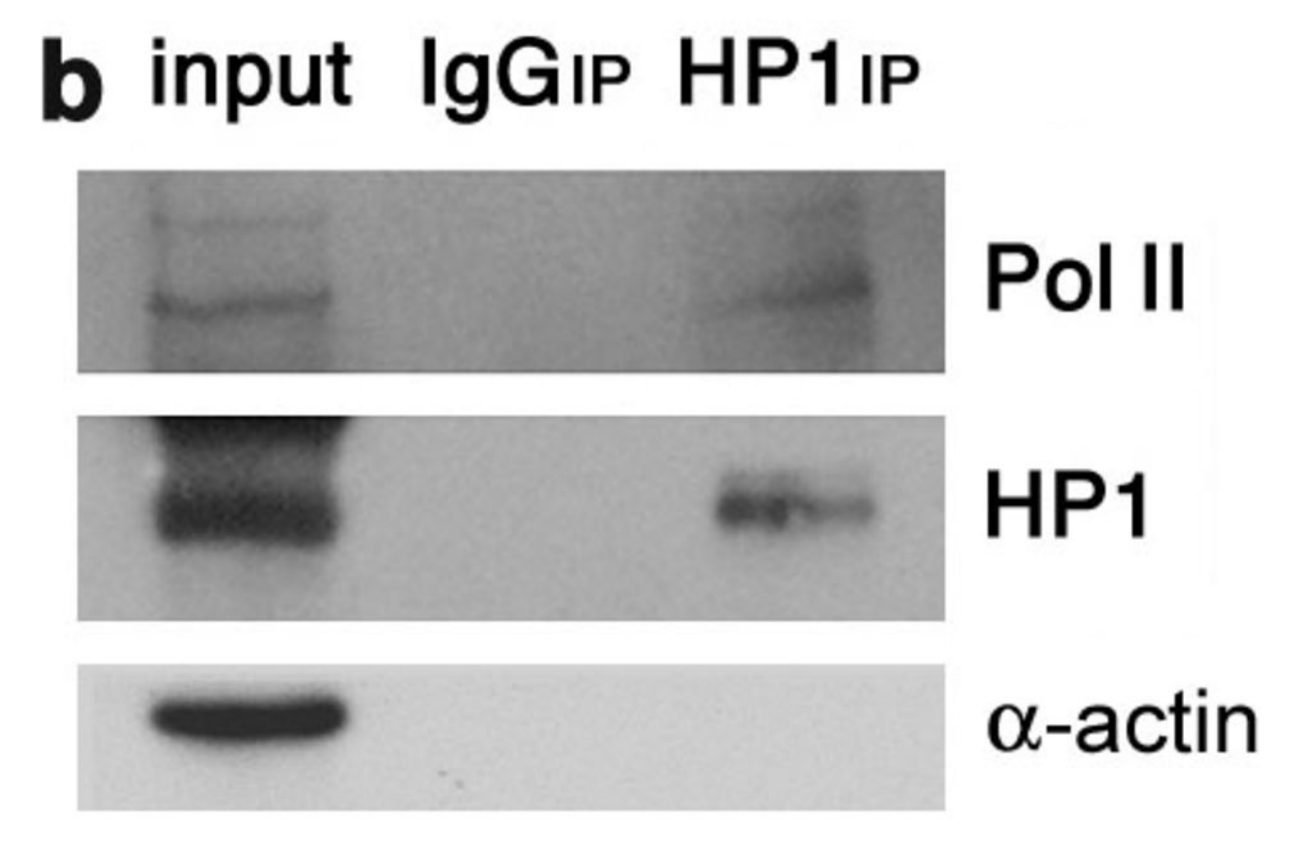
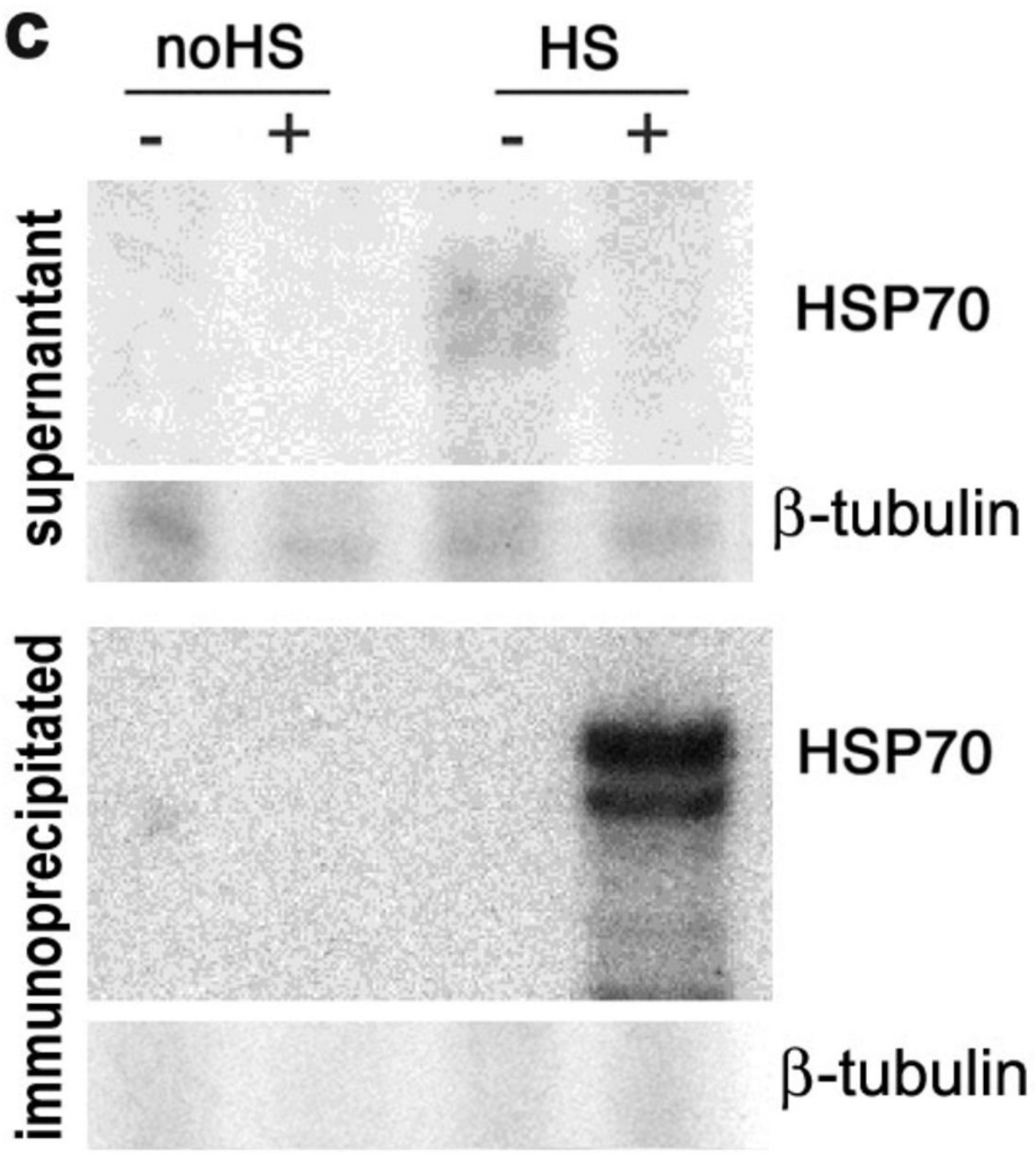
52C
53D
58E
50D
56E
HP1
Pol II
HP1/Pol II


b input IgG IP HP1 IP
Pol II
HP1
α-actin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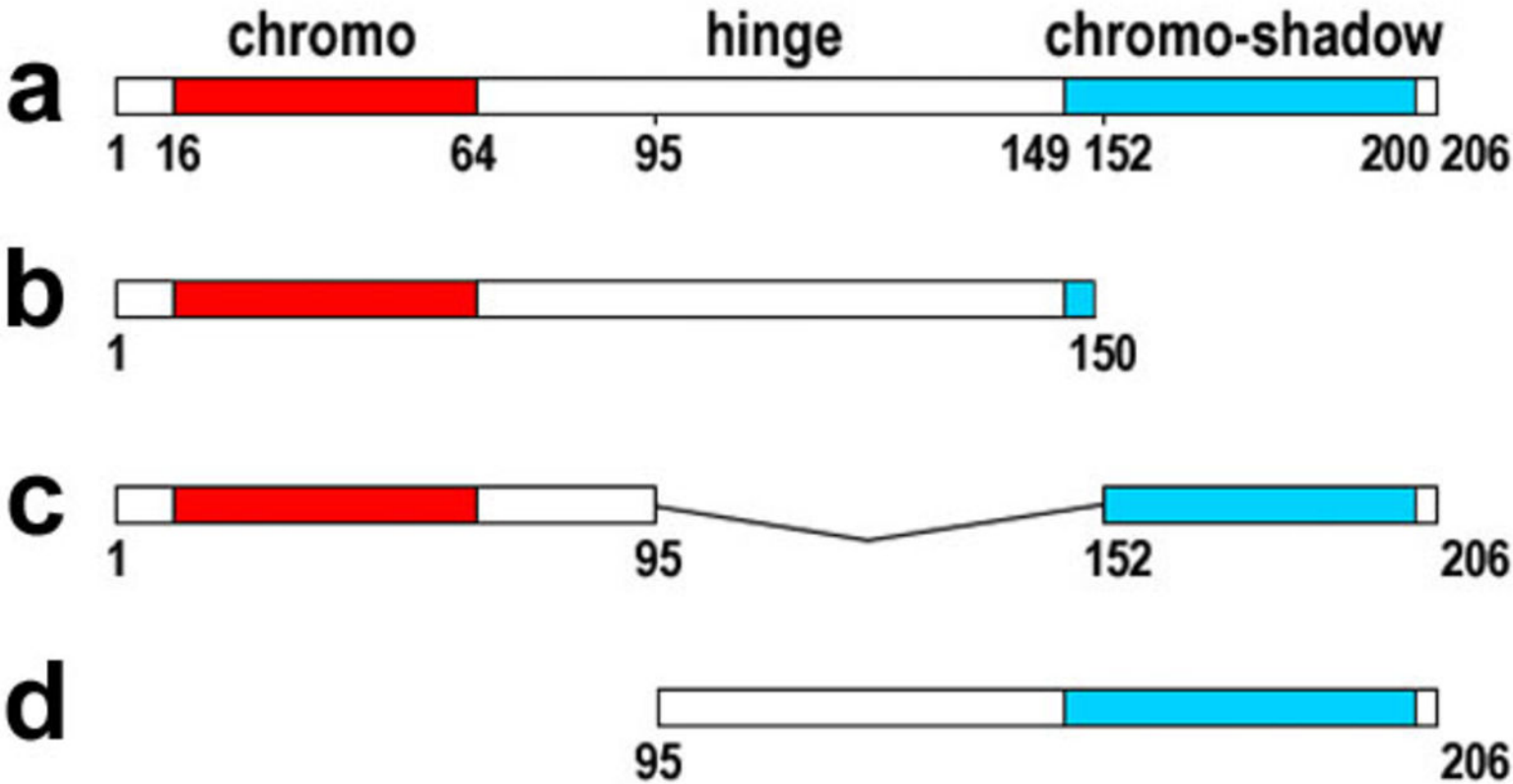
HS
-
+
-
+
supernantant
HSP70
β-tubulin
immunoprecipitated
HSP70
β-tubulin

d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hinge
chromo-shadow
a
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16
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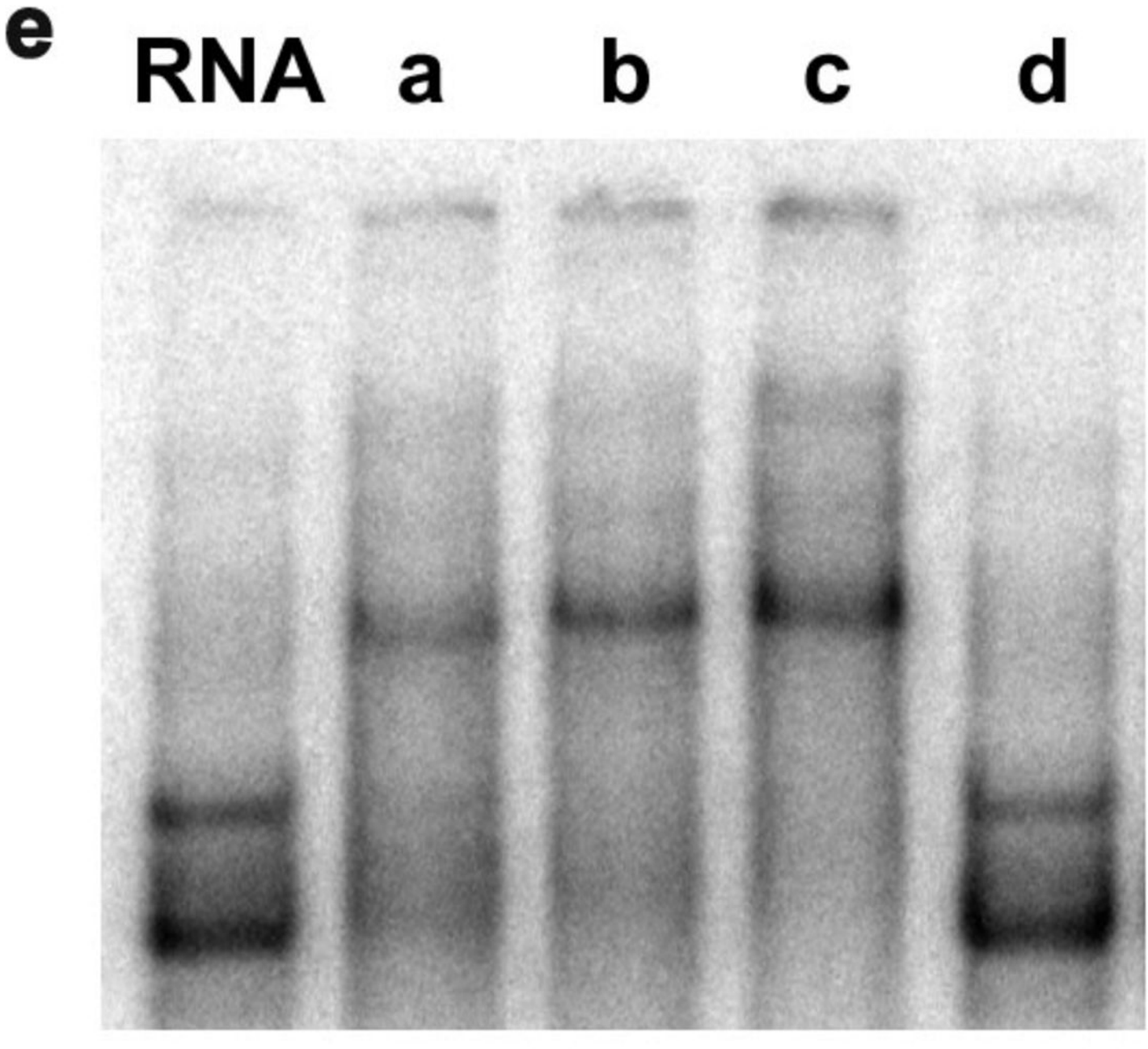
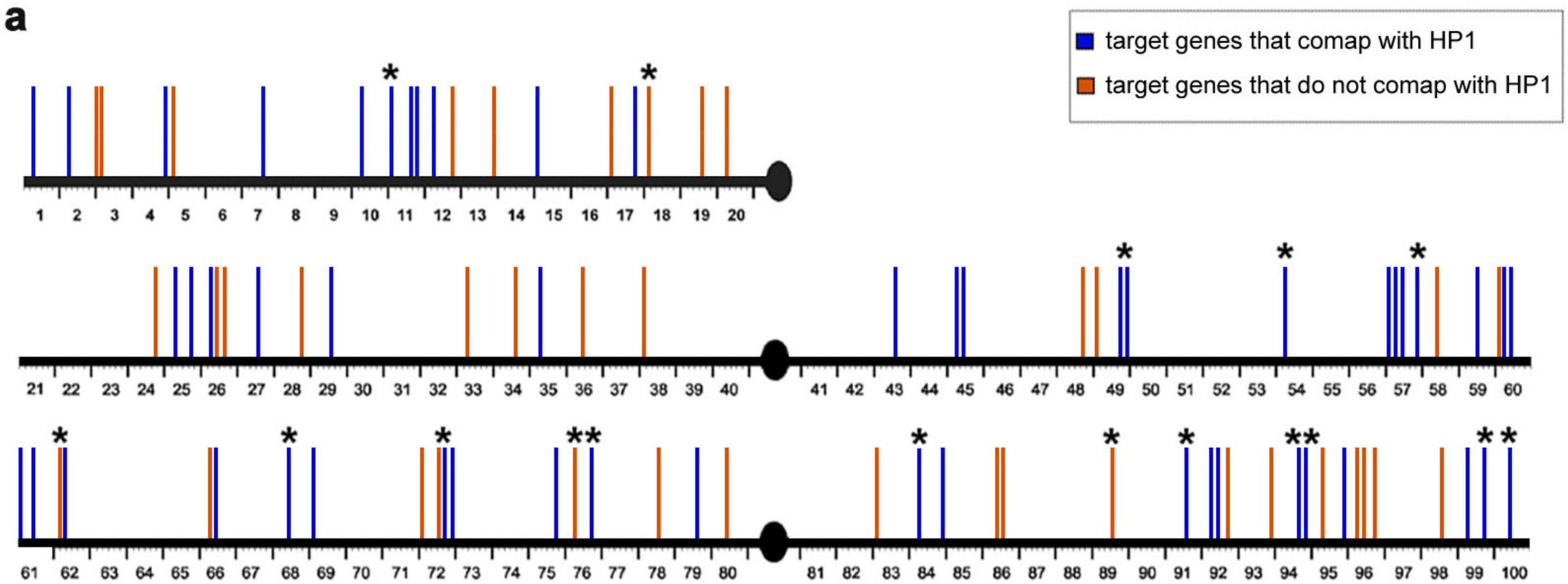
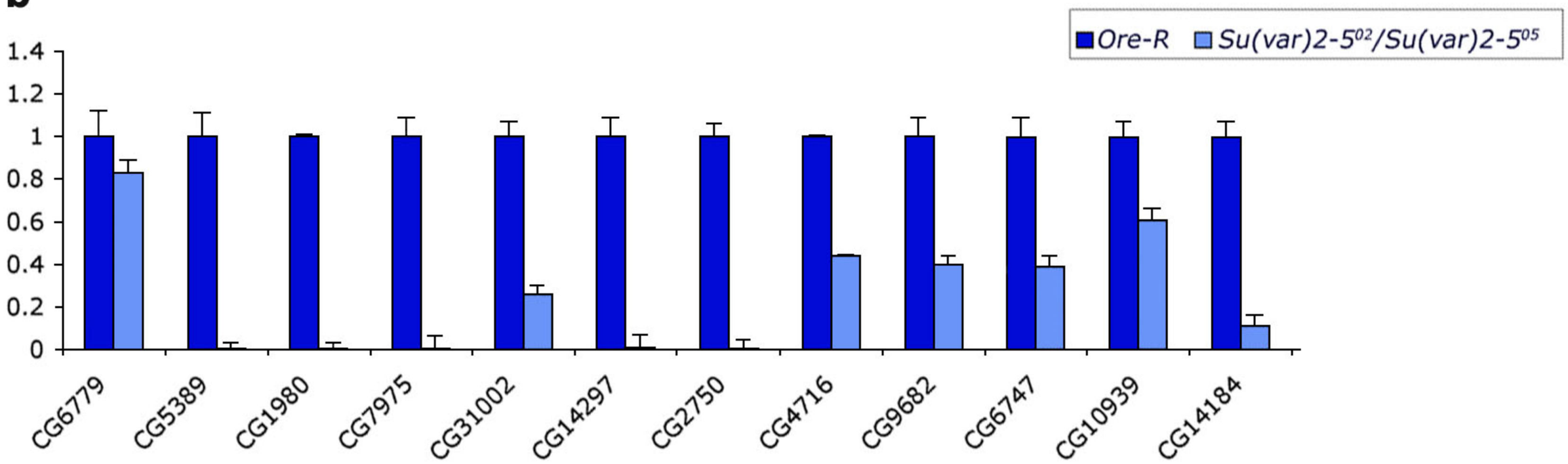
152
200
206
b
1
150
c
1
95
152
206
d
95
206


e
RNA
a
b
c
d

a


target genes that comap with HP1
target genes that do not comap with HP1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

b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1.2
1
0.8
0.6
0.4
0.2
0
CG6779
CG5389
CG1980
CG7975
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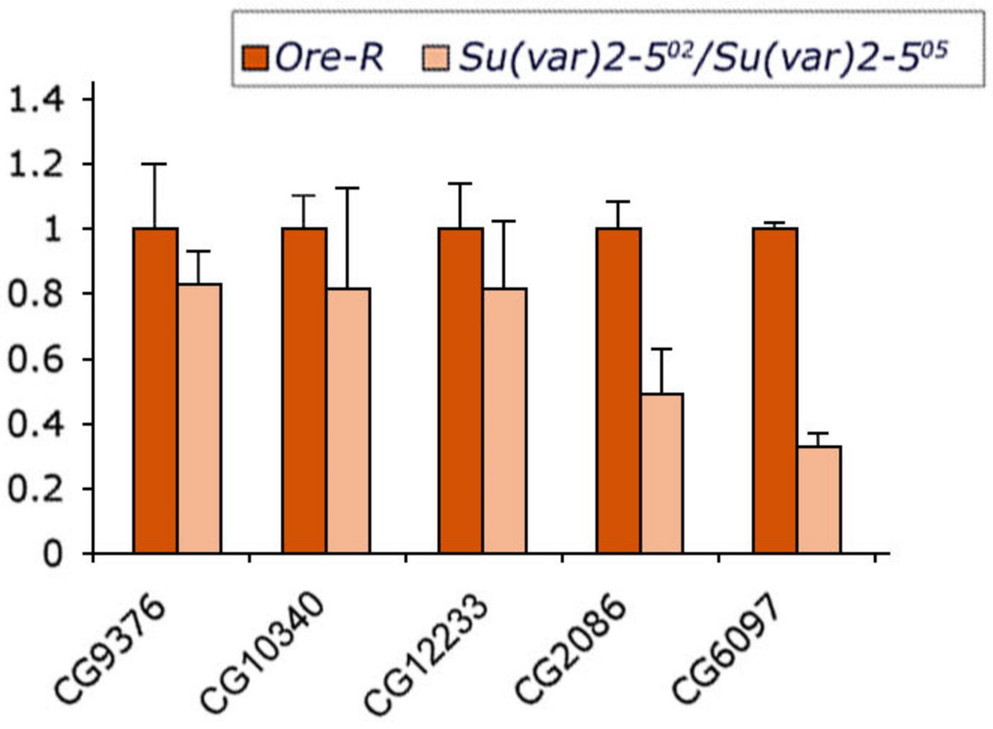
CG10939
CG14184

c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0.8
0.6
0.4
0.2
0
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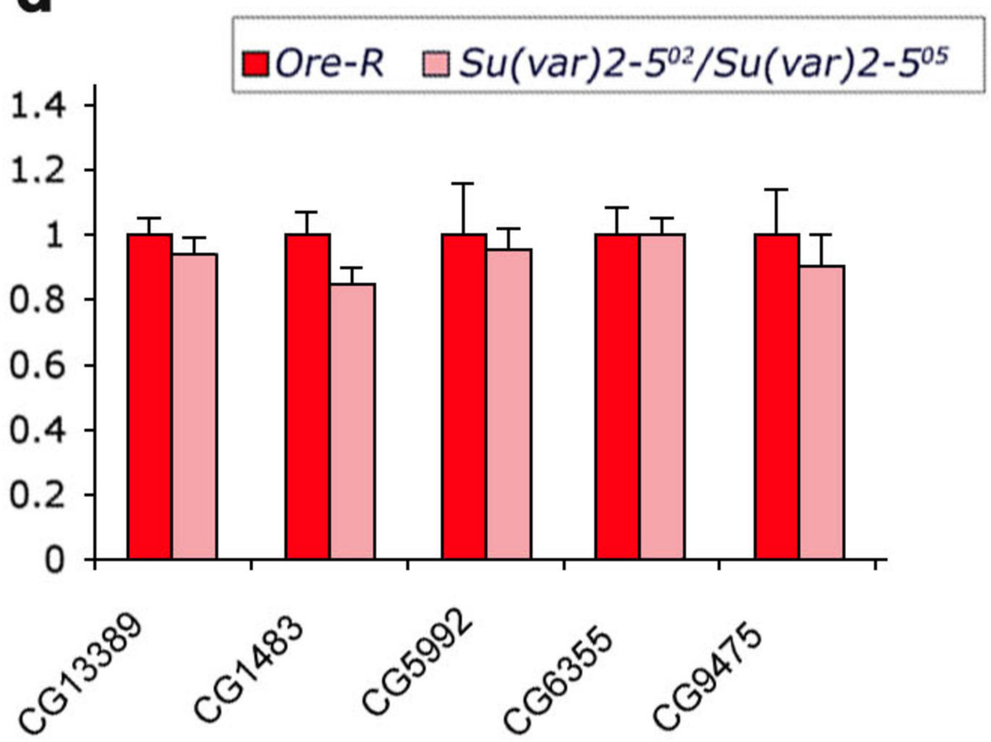
CG10340
CG12233
CG2086
CG6097

d


Ore-R
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0.6
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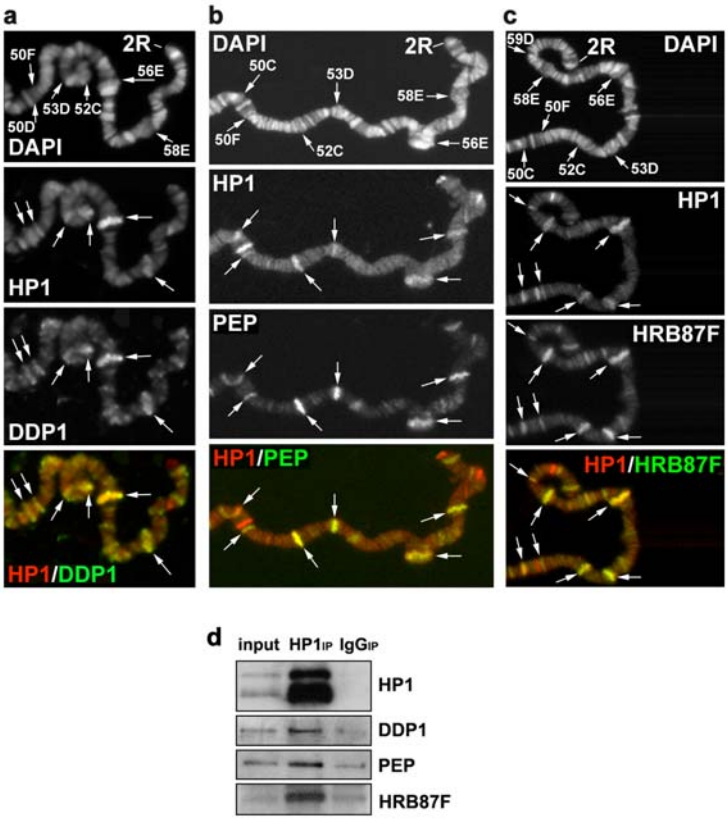
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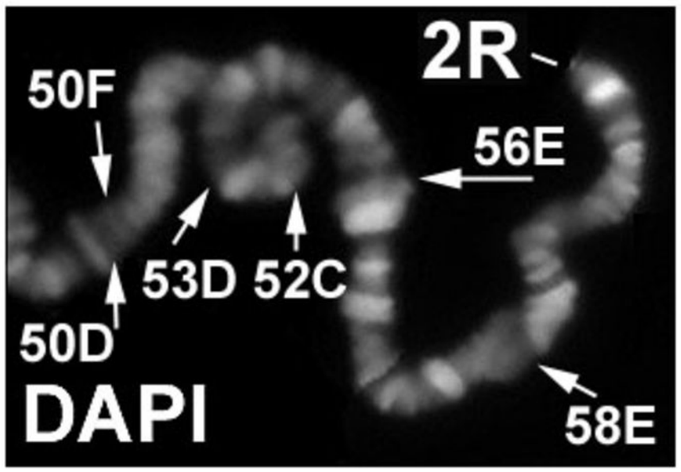
56E
53D
52C
50D
DAPI
58E
HP1
DDP1
HP1/DDP1
b
DAPI
2R
50C
53D
58E
50F
52C
56E
HP1
PEP
HP1/PEP
c
59D
2R
DAPI
58E
50F
56E
50C
52C
53D
HP1
HRB87F
HP1/HRB87F
d
input
HP1IP
IgGIP
HP1
DDP1
PEP
HRB87F

a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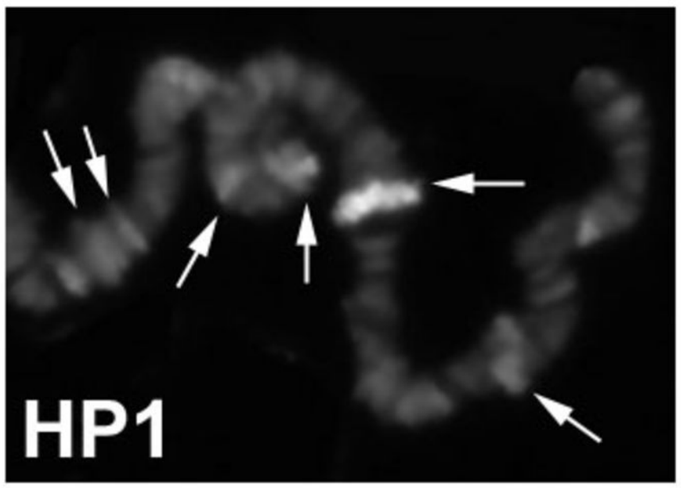
DAPI
58E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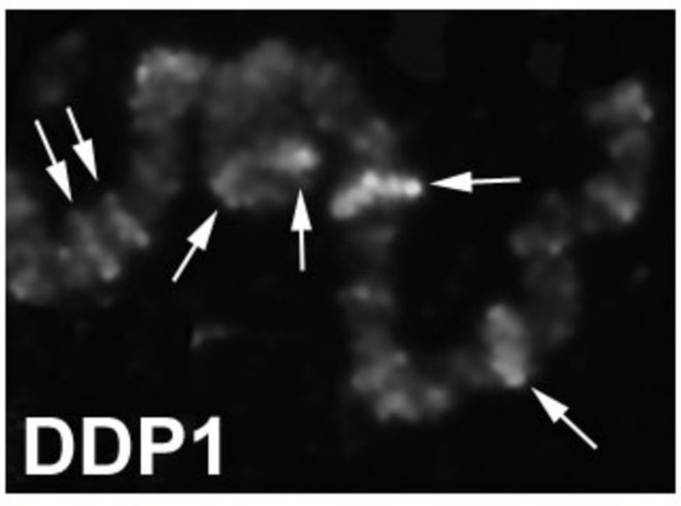
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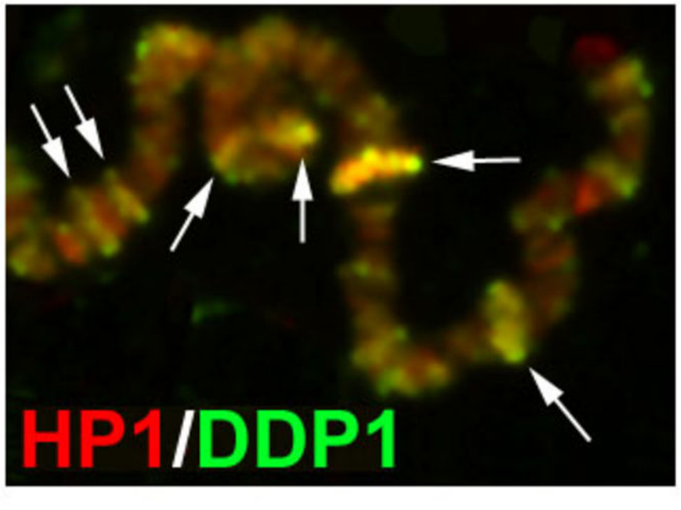
HP1/DDP1

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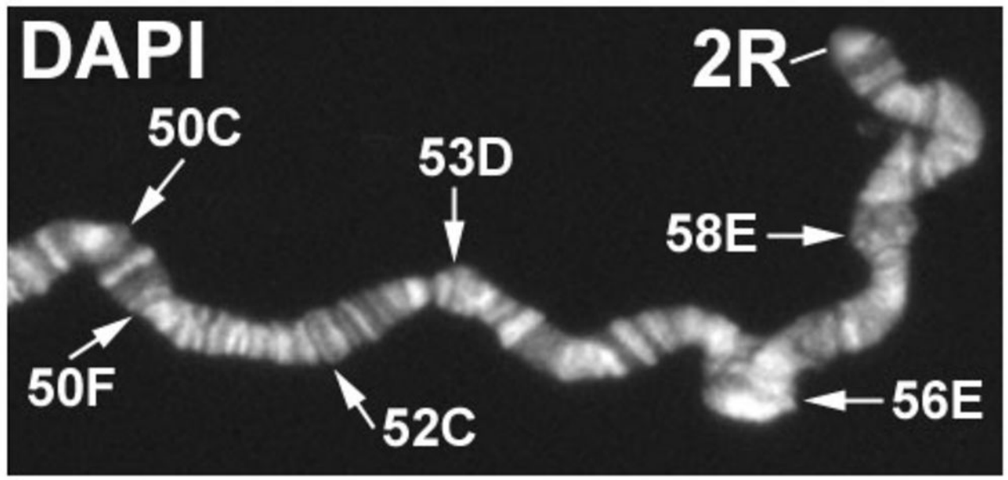
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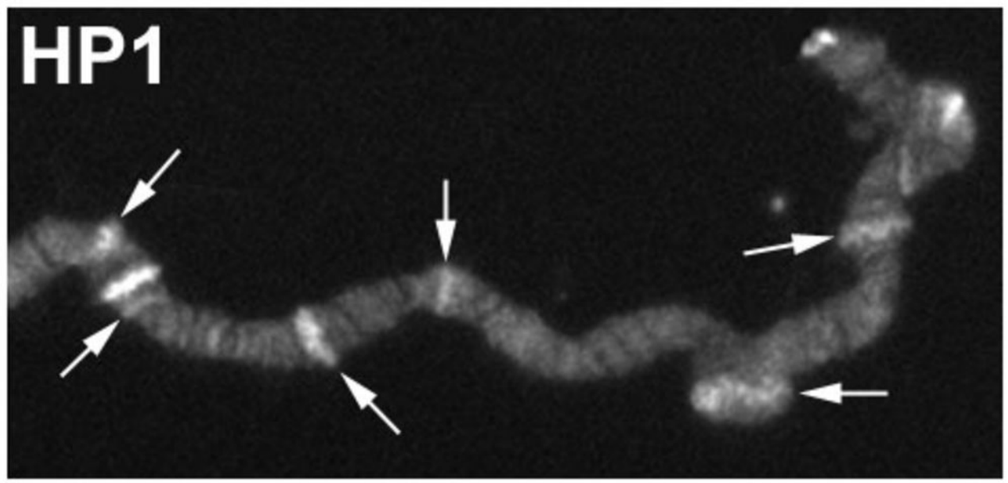
50C
53D
58E
50F
52C
56E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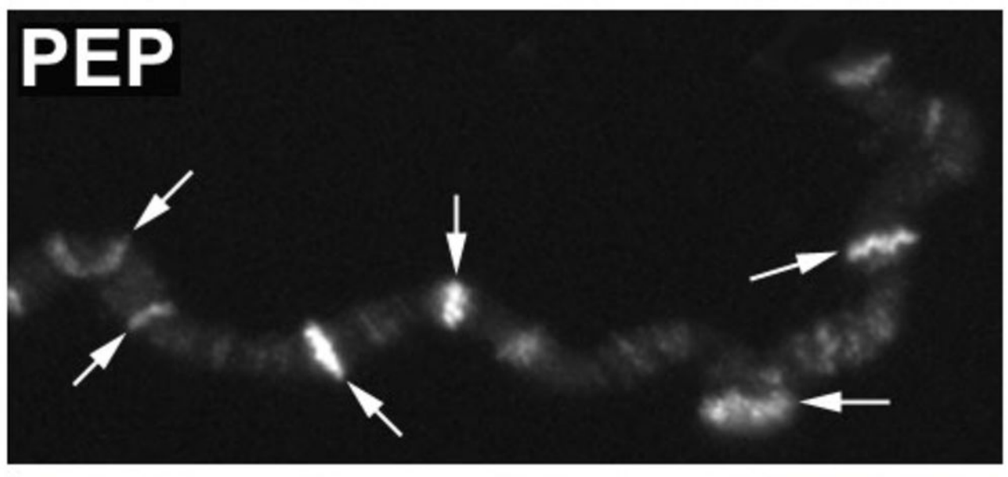
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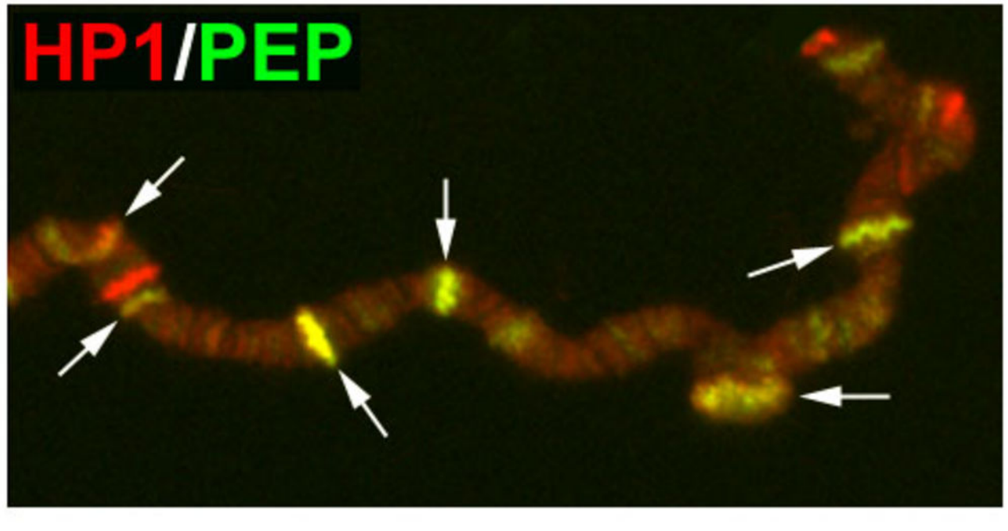
HP1/PEP

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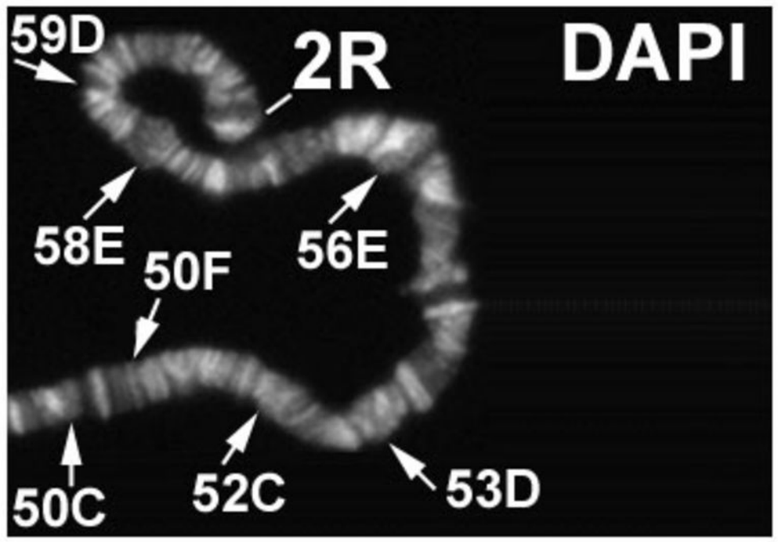
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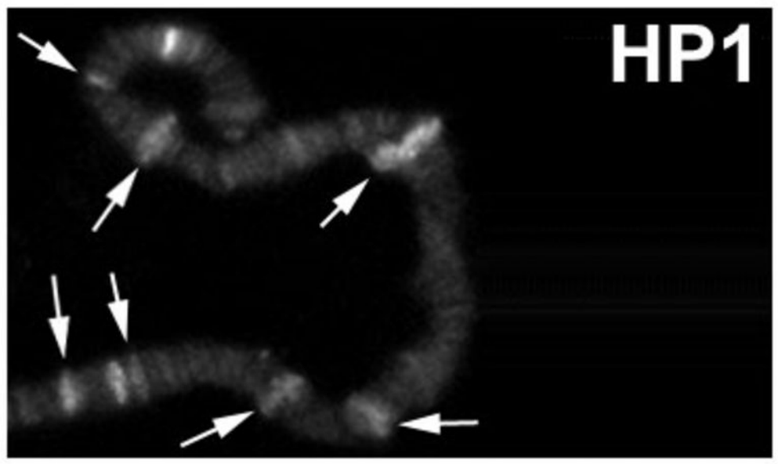
2R
DAPI
58E
50F
56E
50C
52C
53D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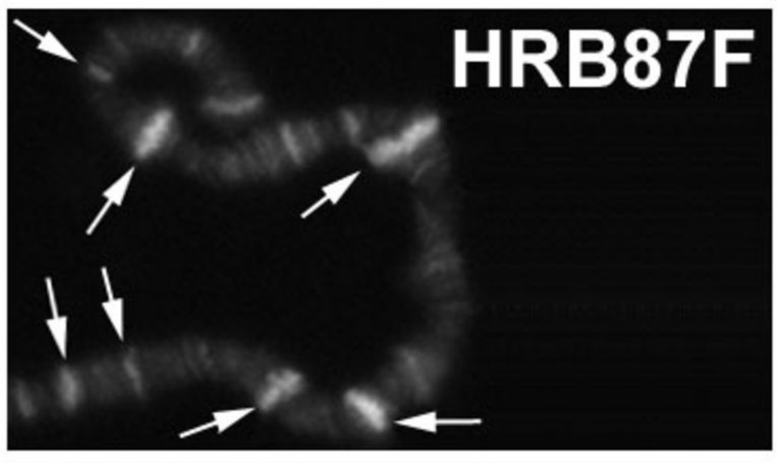
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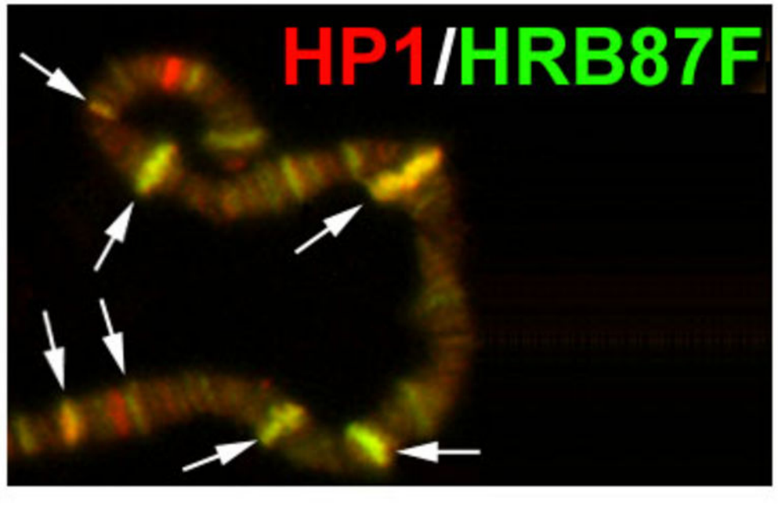
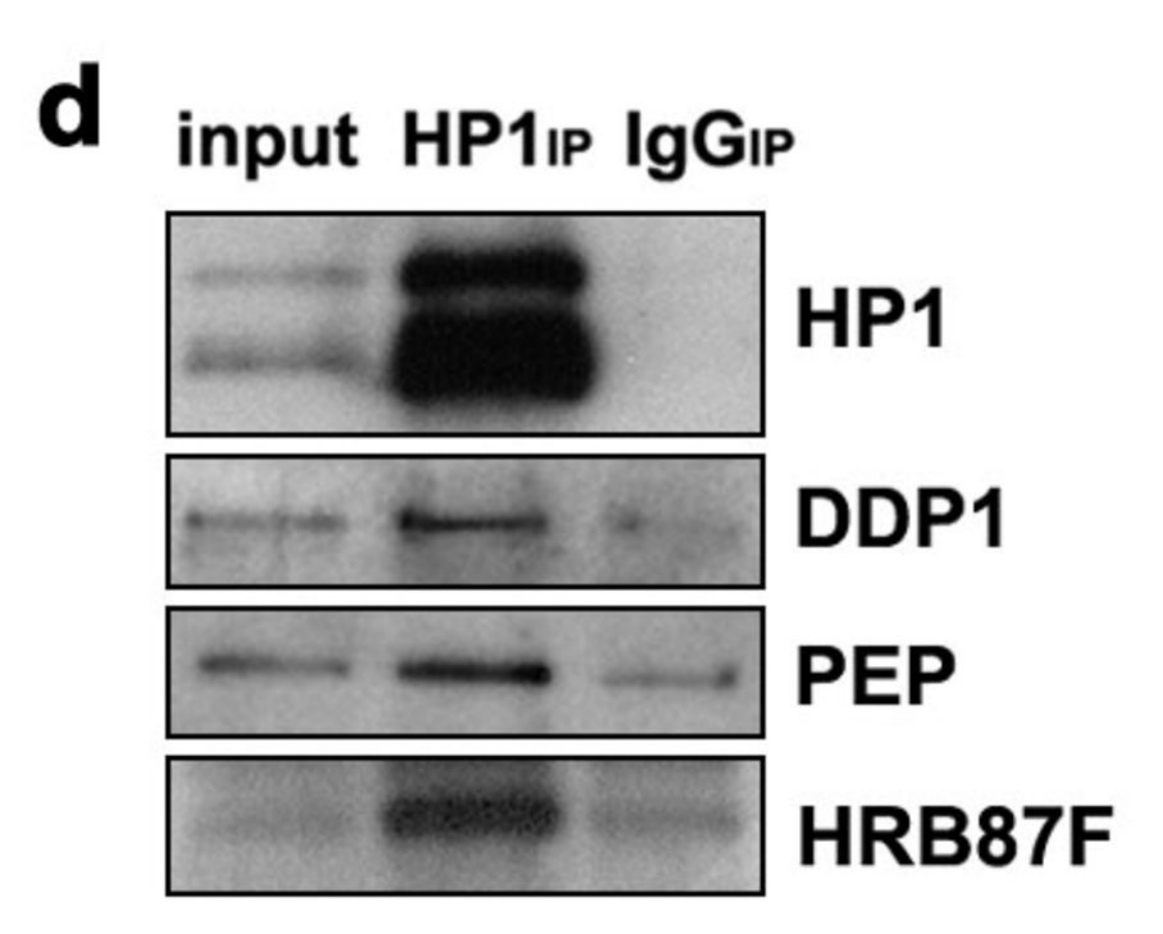

HP1/HRB87F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HP1
DDP1
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