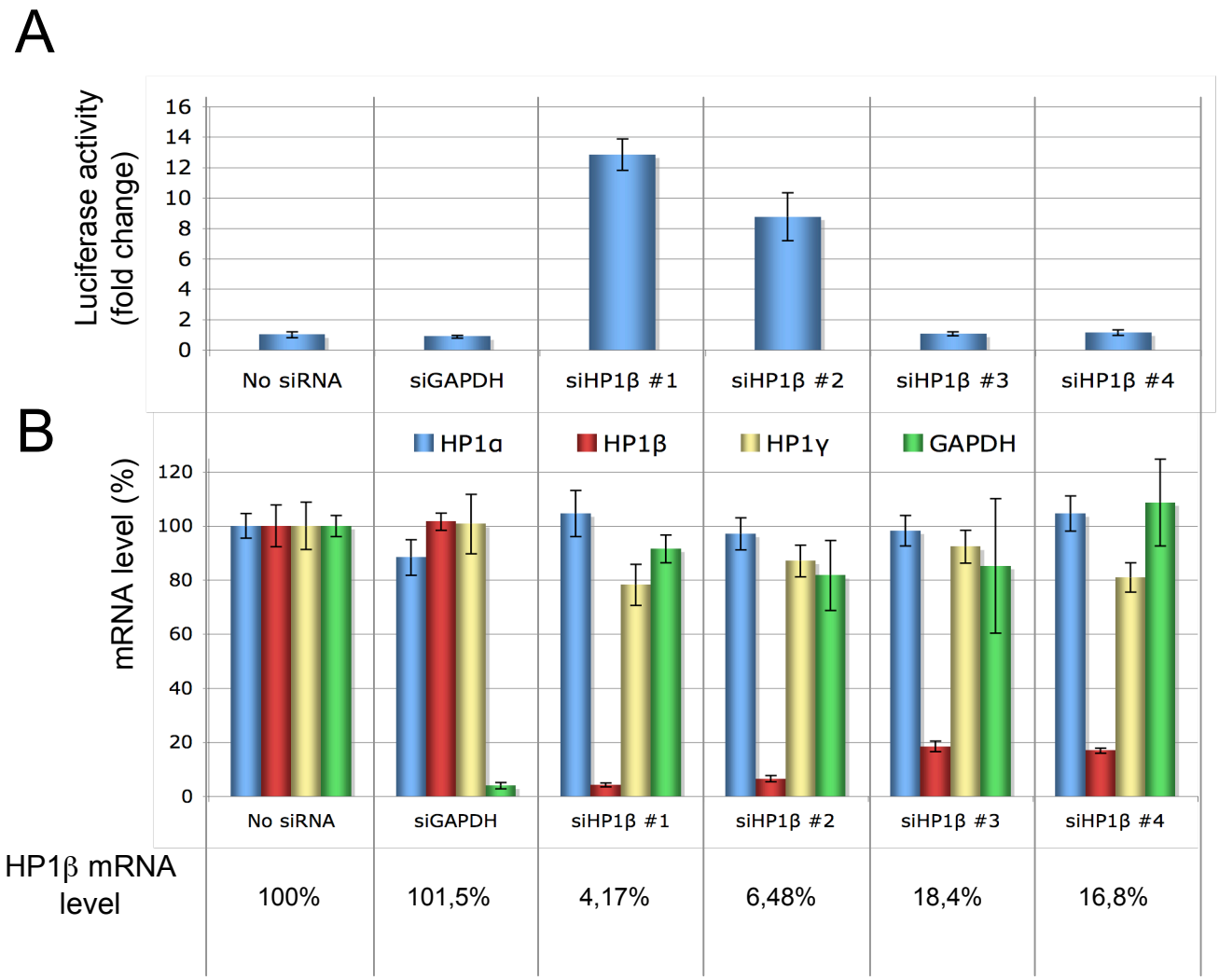

Figure S1: **Efficient HP1β knockdown induces activation of the HIV1 LTR.** Four different HP1β siRNAs (#1 : this study, #2 and #3 : from Dharmacon, #4 : Du Chéné et al 2007) or a GAPDH siRNA were transfected in HeLa LTR-Luc cells. After 48 hours, luciferase activity (**A**) and mRNA levels (**B**) were quantified for each HP1 isoform. Levels were considered to be 100% in cells transfected with lipofectant only. Measures were normalized to cyclophilin B mRNA levels.

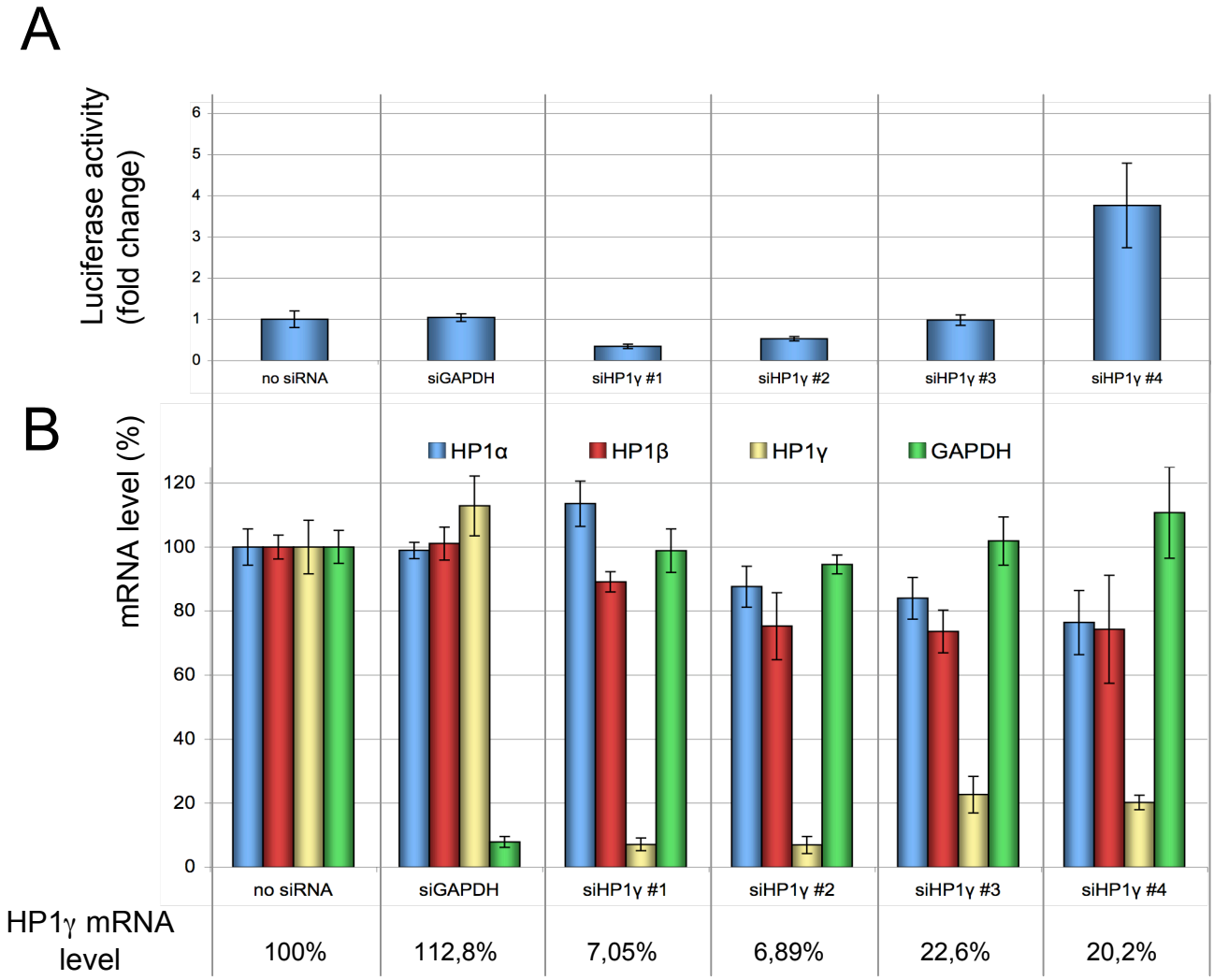

Figure S2: **Efficient HP1γ knockdown induces repression of the HIV1 LTR.** Four different HP1γ siRNAs (#1 : this study, #2 and #3 : from Dharmacon, #4 : Du Chéné et al 2007) or a GAPDH siRNA were transfected in HeLa LTR-Luc cells. After 48 hours, luciferase activity (**A**) and mRNA levels (**B**) were quantified for each HP1 isoform. Levels were considered to be 100% in cells transfected with lipofectant only. Measures were normalized to cyclophilin B mRNA levels.

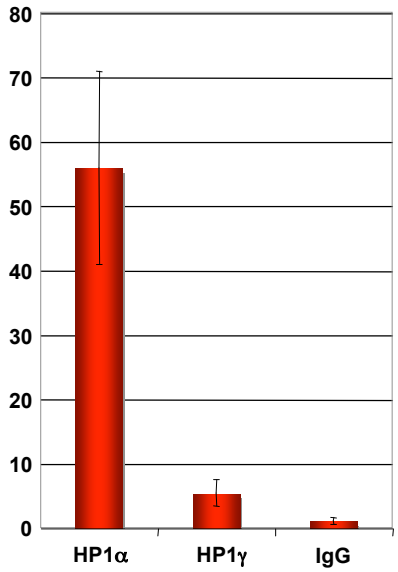

Figure S3 : Positive control for ChIP assays with the anti-HP1α antibody. Extracts from HeLa cells were used in Chromatin Immunoprecipitation assays with anti-HP1α or anti-HP1γ antibodies, or with IgG as a control. Enrichment in satellite regions was quantified by qPCR using the following primers :
hsSat2F: ATCGAATGGAAATGAAAGGAGTCA
hsSat2R: GACCATTGGATGATTGCAGTCA

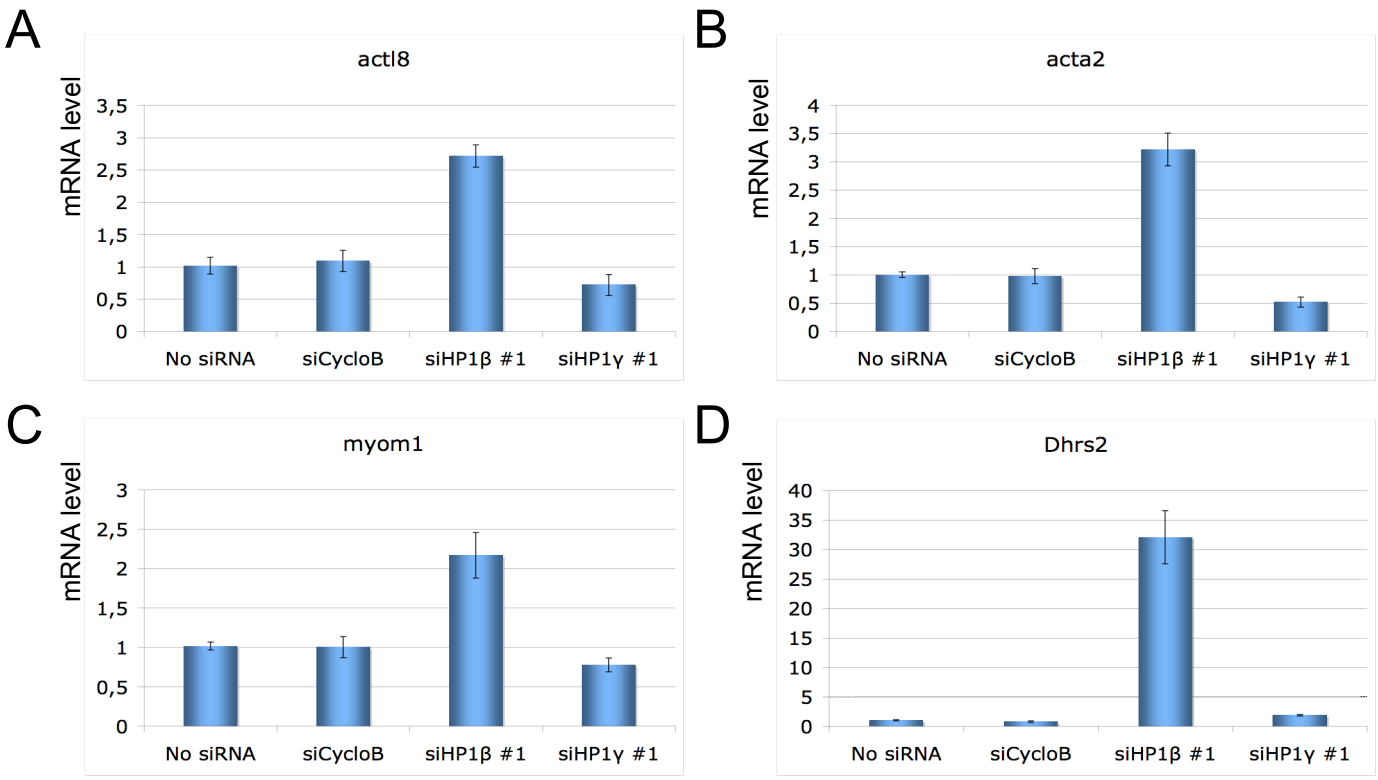

Figure S4 : Cellular genes candidate for a regulation by the HP1β-HP1γ switch. HeLa LTR-luc cells were transfected with the indicated siRNAs (10 nM) and levels of actl8 (**A**), acta2 (**B**), myom1 (**C**), Dhrs2 (**D**) were quantified by qRT-PCR. Transcript levels were normalized to GAPDH mRNA levels.

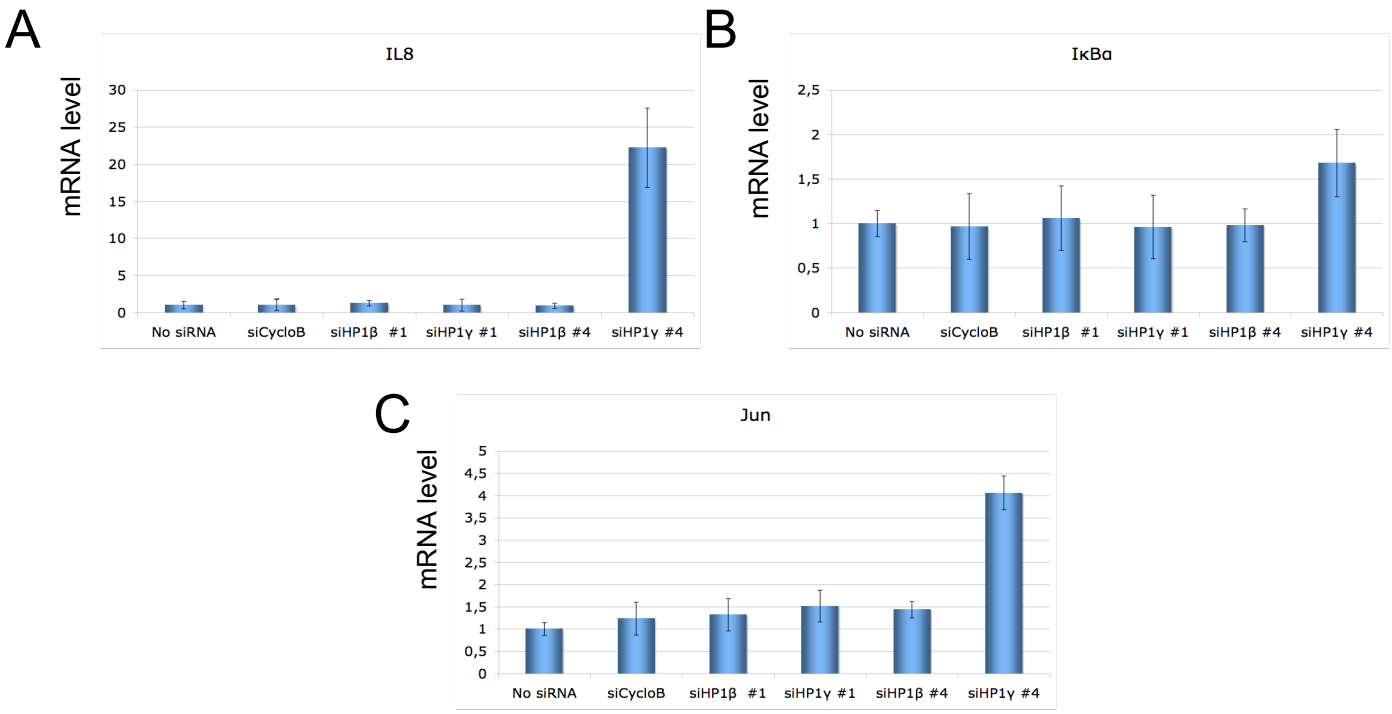

Figure S5 : the anti-HP1γ siRNA #4 activates several genes involved in stress response. HeLa LTR-Luc cells were transfected with siRNAs (10 nM) from this study (siHP1ß #1 and siHP1γ #1) or from the study of Chéné *et al.* 2007 (siHP1ß #4 and siHP1γ #4). Levels of IL8 (**A**), IκBα (**B**), and c-Jun (**C**) mRNA were then quantified by qRT-PCR. Transcript levels were normalized to GAPDH mRNA levels. We note that non-specific activation of IL-8 by siRNAs has been reported earlier (Pauls *et al.* 2006) and that activation of IL-8 is known to stimulate replication of HIV-1 (Lane *et al.* 2001).

References:

Chéné I, Basyuk E, Lin YL, Triboulet R, Knezevich A, Chable-Bessia C, Mettling C, Baillat V, Reynes J, Corbeau P, Bertrand E, Marcello A, Emiliani S, Kiernan R, Benkirane M (2007) Suv39H1 and HP1gamma are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-integration latency. *Embo J* **26:** 424-435

Lane BR, Lore K, Bock PJ, Andersson J, Coffey MJ, Strieter RM, Markovitz DM (2001) Interleukin-8 stimulates human immunodeficiency virus type 1 replication and is a potential new target for antiretroviral therapy. *J Virol* **75:** 8195-8202

Pauls E, Senserrich J, Bofill M, Clotet B, Este JA (2007) Induction of interleukins IL-6 and IL-8 by siRNA. *Clinical and experimental immunology* **147:** 189-196

SUPPLEMENTAL METHODS :

siRNA targeting the following sequences were used :

HP1α

#1 CACAAATTGTGATAGCATT

HP1β

#1 AGCTCATGTTCCTGATGAA

#2 GCCCACAGGTTGTCATATC

#3 CAGAAGAGAACCTGGATTG

#4 GACTCCAGTGGAGAGCTCATG

HP1γ

#1 ATCTGACAGTGAATCTGAT

#2 AGTACTAGATCGACGTGTA

#3 TCAGAAAGCTGGCAAAGAA

#4 GAGGCAGAGCCTGAAGAAT

GAPDH and cyclophilin B controls siRNAs were purchased from Dharmacon. siRNAs were delivered into HeLa LTR-luc cells using dharmafect I (Dharmacon). Type #1 siRNA were used in figure 1.

Primers for qPCR

HP1α

S: 5'-AACAGTGCCGATGACATCAAA-3'

AS: 5'-GCCCCAATGATCTTTTCTGGT-3')

HP1β

S: 5'-GCCGGAGCGGATTATTGGAG-3'

AS:5'-GTGGGCACTTGACATTGGC-3')

HP1γ

S: 5'- TGCCAGAGGTCTTGATCCTGA-3'

AS: 5'-TCTTTCGCCAGCACCAAGTCT-3')

GAPDH

S: 5'-GGACCTGACCTGCCGTCTAGAA-3'

AS: 5’-GGTGTCGCTGTTGAAGTCAGAG-3’

actl8

S: 5'-GTGCAGTACCTCTGGTCATTTG-3’

AS: 5’-GAGGACCGATGGGACATGC-3

acta2

S: 5’-GTGTTGCCCCTGAAGAGCAT-3’

AS: 5’-GCTGGGACATTGAAAGTCTCA-3’

myom1

S: 5’-AACCGAGACATATCATGCCAAG-3’

AS: 5’-CCTGCTATGGAGCAATGCAATTT-3’

dhrs2

S: 5’-GTAGGGAGCACTCTGGGGAC-3’

AS: 5’-CTCCATGTAGGGCAGCAACT-3’

Alternative Chromatin immunoprecipitation (ChIP) protocole and HIV1 primers

When using anti-HP1 and anti-RNAPII antibodies, a modified protocol “IF-Chip” protocol was used. Briefly, J-lat A1 cells were fixed in PBS containing 1% formaldehyde (Sigma) during 10 minutes at room temperature. Crosslink was stopped using PBS containing 125 mM glycine. Following two washes with ice cold PBS, cells were extracted with PBS containing 0.3 % Triton X100 and protease inhibitors (PI - Roche) for 15 min on ice. For one immunoprecipitation, $1x10^7$ cells were resuspended in PBS 10% fetal calf serum containing PI and one of the following antibodies (1/500 dilution) during 16h at 4°C on a rotating wheel: anti-HP1α (1H5), anti-HP1β (1A9), and anti-HP1γ (1G6) from Euromedex, anti-RNA polymerase II (N20 - Santa Cruz.). Cells were washed twice in cold PBS 10 minutes at 4°C on a rotating wheel. Antibody-antigen complex was cross-linked by incubating cell in PBS containing 1% formaldehyde during 5 minutes at 4°C, followed by a 125 mM glycine block and two washes with ice cold PBS. For one immunoprecipitation, $1x10^7$ cells were centrifugated, and the resulting pellet was resuspended in 300 μL of IP buffer (0.1% SDS, 1% Triton, 0.1% Na-deoxycholate, 10 mM TRIS pH 8, 150 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1x PI), and sonicated using a Bioruptor (Diagenode) to a final average size of 500 bp. Lysate was diluted with 3 volume of IP buffer containing no detergents (input) and was incubated with protein A/G beads (Santa Cruz) saturated with

sonicated Herring Sperm DNA during 1 hour at 4°C. Beads were washed extensively and bound material was eluted with 10 mM TRIS pH8, 1 mM EDTA, 1% SDS at RT during 15 min. Eluted complexes were treated with proteinase K during 30 minutes at 55°C then cross-linking was reversed overnight at 65°C. Nucleic acids were isolated by the phenol/chloroform method, ethanol precipitated in presence of glycoblue (Ambion) and resuspended in DNAse free water. Aliquots were used for Quantitative real time PCR. For each amplification, immunoprecitated DNA quantity was reported in percentage of DNA present in the corresponding input.
The qPCR primers on the HIV1 LTR were:
-1.5kb TGCAATTGGAGTCACCATCTGCCT-ACATTCCTGGAGAGTTGGCAGAGA,
-0.5kb GGCTAATTCACTCCCAACGAAG-GGCCCTGGTGTGTAGTTC,
TS CAGCTGCTTTTTGCCTGTACTG-TCCACACTGACTAAAAGGGTCTGA,
+0.5kb CAGTAGCAACCCTCTATTGTGTGC-CTTGCTGTGCGGTGGTCTTACTTT,
+1.5kb ATGGAGCCAGTAGATCCTA-TGCTTTGATAGAGAAACTTGATG,
+2.5kb CCTACGGCGTGCAGTGCTT-GGCATGGCGGACTTGAAGA.

GST pulldown assays. 3 μg of bacterially expressed GST fusion proteins immobilized on glutathione-agarose beads (Sigma) in GST-binding buffer (20 mM Tris-HCl, pH 7.9, 150 mM NaCl, 0.2 mM EDTA, 0.1% Nonidet P-40, 1 mM DTT, protease inhibitor cocktail (Roche), and 0.3 mg/mL BSA) were incubated at 4°C for 2 h with 500 ng of bacterially expressed, purified Hisx6-HA-tagged HP1β. After three washes in GST-binding buffer adjusted to 0.3% Nonidet P-40, samples were resolved by SDS-PAGE, transferred onto nitrocellulose membrane, and stained with Ponceau S. HP1β retained on beads was visualized by western blot with an anti-HA antibody (12CA5), followed by an HRP-linked secondary antibody and ECL detection (Pierce).

Immunoprecipitation and immunoblotting. HeLa S3 cells, or HeLa S3 stably expressing Flag-HA-6xHis-tagged HP1β or HP1γ were grown as spinners in DMEM supplemented with 5% FCS. Nuclear extract were obtained according to the method of Dignam et al. (1983). For immunoprecipitation assays, 1 mg of nuclear extract was diluted to 150 mM salts and incubated at 4°C for 2 h with 20 μL of equilibrated Flag M2 agarose bead slurry (Sigma). After four washes in wash buffer (20 mM Tris-HCl, pH 7.9, 150 mM NaCl, 0.2 mM EDTA, 0.3% Nonidet P-40, 1 mM DTT, protease inhibitor cocktail (Roche), 20 mM b-glycerophosphate, 0.5 mM sodium

orthovanadate), samples were resolved by SDS-PAGE, and transfered onto nitrocellulose membrane. Proteins retained on beads were then visualized by western blot with indicated primary antibodies followed by an HRP-linked secondary antibody and ECL detection (Pierce). Antibodies used were 8WG16, H5, H14 (Covance), for the detection of non-phosphorylated RNAPII, phospho-Ser2-CTD, and phospho-Ser5-CTD, respectively. Flag-tagged HP1s were detected with polyclonal anti-Flag antibody (Sigma).

Reference:

Dignam JD, Lebovitz RM, Roeder RG (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res*. **11**:1475-89