Supplementary Material and Methods

siRNA sequences :

The oligonucleotides corresponding to the sense-strand sequences of siRNA were: Human specific hnRNP U-siRNA: r(AAAGACCACGAGAAGAUCAUG)dTdT ; shared human/mouse hnRNP U-siRNA r(GAGGUGGAAUGCCCAACAG) dTdT ; Mouse-specific hnRNP U-siRNA r(CAGUGUCUUGGCAAGUUU) dTdT and control, irrelevant siRNA (Qiagen).

Primers sequences :

The qRT–PCR primer sequences were as follows: hnRNP U 5'-AACAGAGGTGGTGGCCATAG-3' and 5'-GTAACTACCACGGCCAGGAA-3' and 5'-GGAAGTACGGCCTGAGAGGT-3'; HP1α 5'-CTAGACAGGCGCGTGGTTAAG- and 5'-GCTCAGGGCAATCCAAGTTTTT-3' : and GAPDH : 5'-GGACCTGACCTGCCGTCTAGAA-3' and 5'-GGTGTCGCTGTTGAAGTCAGAG-3'

Antibodies :

Western-blot : Mouse anti-hnRNP U (sc-32315; Santa Cruz Biotechnology) ; Mouse anti-HP1α (2HP-2G9-AS ; Euromedex) ; rat anti-HA (cat# 1867423, Roche) ; anti-Suv39h1 (07-550, Upstate) ; β tubulin (T5202, Sigma) ; goat anti-DNMT3A (sc-10231, Tebu-Bio) ; rabbit anti-HP1α (C7F11, Cell Signaling).

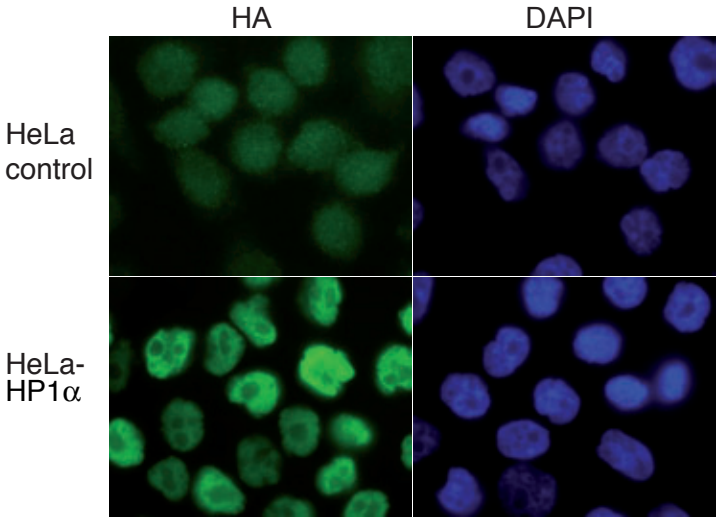
HP1α peptides S	HP1α peptides C	hnRNP U peptides S	hnRNP U C peptides C
DTDEADLVLAK	REQSNDIAR	FIEIAAR	DIDIHEVR
SNFSNSADDIK	DTDEADLVLAK	DIDIHEVR	LSASSLTMESFAFLWAGG
KSNFSNSADDIK	SNFSNSADDIK	YNILGTNTIMDK	
WKDTDEADLVLAK	KSNFSNSADDIK	LLEQYKEESKK	NFILDQTNVSAAAQR
LTWHAYPEDAENK	WKDTDEADLVLAK	NFILDQTNVSAAAQR	YNILGTNTIMDK
GFSEEHNTWEPEK	LTWHAYPEDAENK	SSGPTSLFAVTVAPPGAR	
LTWHAYPEDAENKEK	IIGATDSCGDLMFLMK	EKPYFPIPEEYTFIQNVPLED R	
TADSSSSEDEEEYVVEK	LTWHAYPEDAENKEK		
RTADSSSSEDEEEYVVEK	TADSSSSEDEEEYVVEK		
LTWHAYPEDAENKEKETA K	RTADSSSSEDEEEYVVEK		
	LTWHAYPEDAENKEKETAK		
	LTWHAYPEDAENKEKETAK S		

Table S1 : Mass spectrometry identified peptides for HP1 and its interacting partner hnRNP U in nuclear soluble (S) and chromatinian fractions (C).

	scr/Gal4DBD	si U1/Gal4DBD	scr/Gal4HP1
Luciferase/Renilla	0,0063	0,0061	0,0009
	scr/Gal4DBD	si U2/Gal4DBD	scr/Gal4HP1
Luciferase/Renilla	0,03	0,029	0,008

Table S2: *Luciferase*/*Renilla* ratios of two representative experiments are shown.

A



B

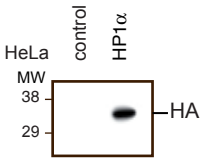


Figure S1, Ameyar-Zazoua et al.

Figure S1: Flag-HA tagged-HP1α-localizes in the nucleus. (A) Control HeLa cells (upper panel) or HeLa cells expressing Flag-HA-HP1α (lower panel) were subjected to immunofluorescence using anti-HA antibody. Cells were counter-stained with DAPI. (B) HeLa cells stably expressing human HP1α or control HeLa cells were labelled with anti-HA antibody and analyzed by western blotting

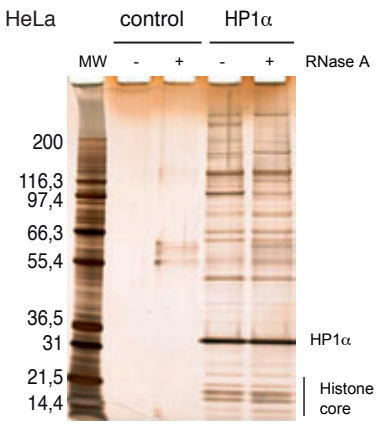


Figure S2, Ameyar-Zazoua et al.

Figure S2: Effects of RNase A treatment on nuclear soluble HP1α-complex. Flag immunoprecipitates from HeLa cells expressing HP1α-tagged protein or from control cells were treated, or not, with RNase A prior to incubation with HA-agarose beads. Eluates were run on 4-12% SDS PAGE and silver stained. Molecular masses of protein markers are indicated on the left.

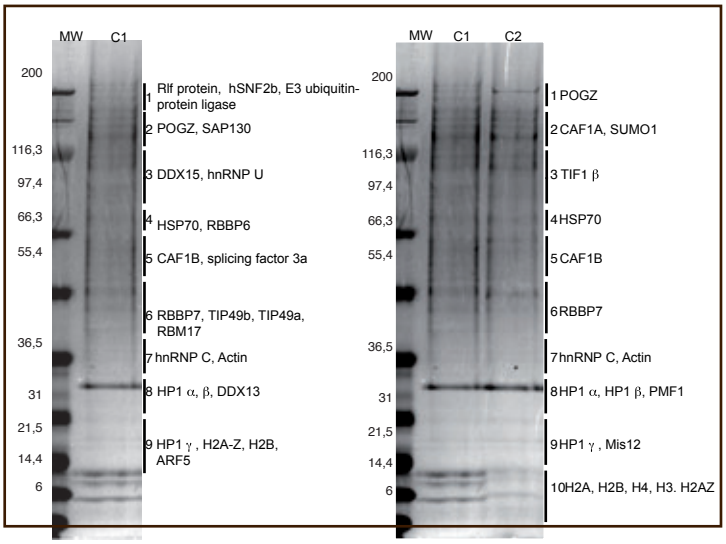
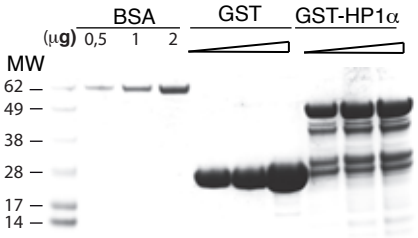


Figure S3, Ameyar-Zazoua et al.

Figure S3: hnRNP U co-fractionate with RNA binding proteins in the insoluble HP1 α-complex. The insoluble HP1α complex was fractionated on glycerol gradients and fractions presenting similar profiles (as visualized on silver stain) were pooled and analyzed by MS. hnRNP U was found specifically in the fraction C1 (compare with C2). C1 corresponds to fractions ranging between 33 to 37% glycerol. C2 corresponds to fractions ranging between 27 to 31% glycerol.

A



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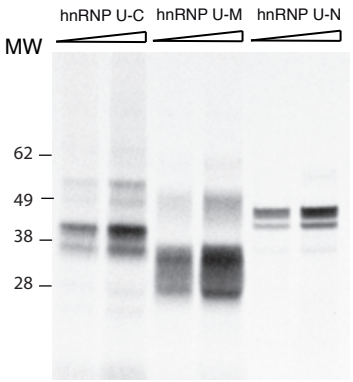

Figure S4, Ameyar-Zazoua et al.

Figure S4: Control of protein amounts in the GST pull-down experiments shown on Figures 2C. (A) Serial dilutions of recombinant proteins, GST and GST-HP1α were revealed by coomassie staining. (B) Serial dilutions of recombinant hnNRP U proteins were revealed by autoradiography.