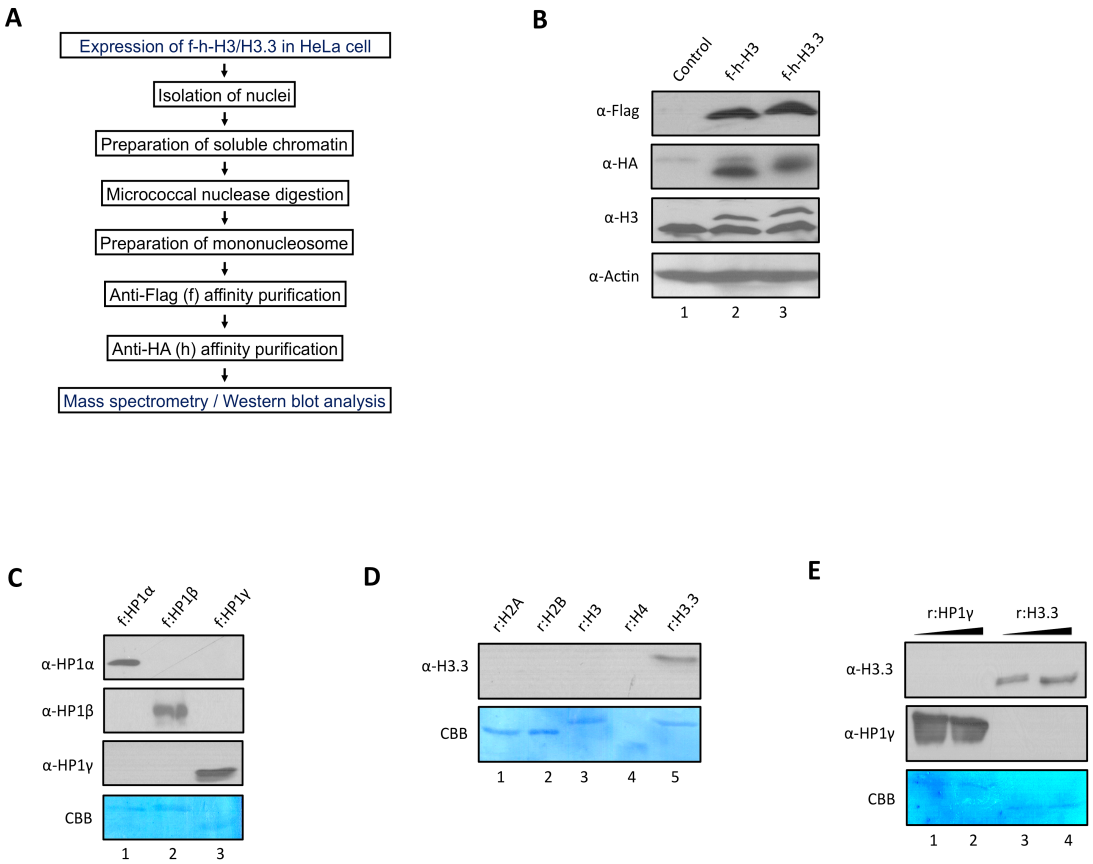

Figure S1. Preparation of H3/H3.3 nucleosomes. (A) HeLa cells were transfected with Flag-HA-H3 (f-h-H3) or Flag-HA-H3.3 (f-h-H3.3) constructs for 48 h. After shearing the nuclei to solubilize chromatin, mononucleosomes were prepared by micrococcal nuclease digestion of the soluble chromatin fractions. Ectopic H3/H3.3-containing mononucleosomes and their interacting proteins were sequentially immunoprecipitated with anti-Flag and anti-HA antibodies. (B) Expression levels of ectopic H3 and H3.3 were assessed by Western blots of cell lysates using indicated antibodies and β-Actin was used as a loading control (lane 1-3) and (C) Recombinant HP1 isoforms purified from bacteria were loaded on 15% SDS-PAGE followed by Western blot with antibodies recognizing each HP1 isoform. (D) Recombinant histones, H2A, H2B, H3, H4, and H3.3 purified from bacteria were loaded on 15% SDS-PAGE and followed by Western blot using H3.3 antibody to show the specificity of the antibody. (E) HP1γ and H3.3 purified from bacteria were loaded on 15% SDS-PAGE followed by Western blot using HP1γ or H3.3 specific antibodies to show that there is no cross-reactivity between the antibodies.

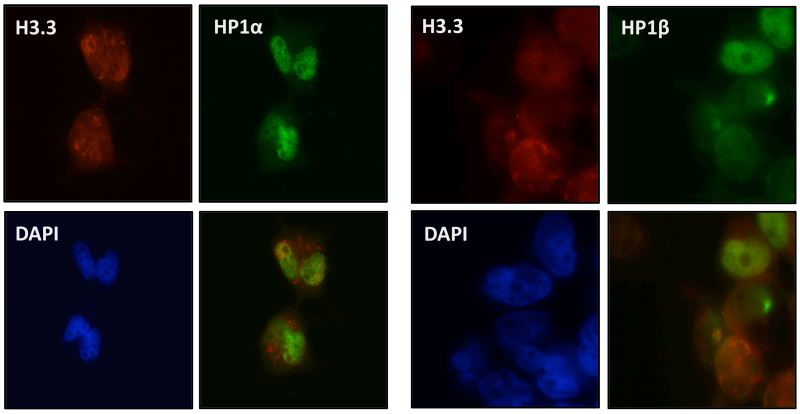

Figure S2. Immunostaining of HP1 isoforms. Cells were immunostained with HP1α, HP1β antibodies (green channel), and H3.3 antibody (red channel). The nuclei were also stained with DAPI (blue).

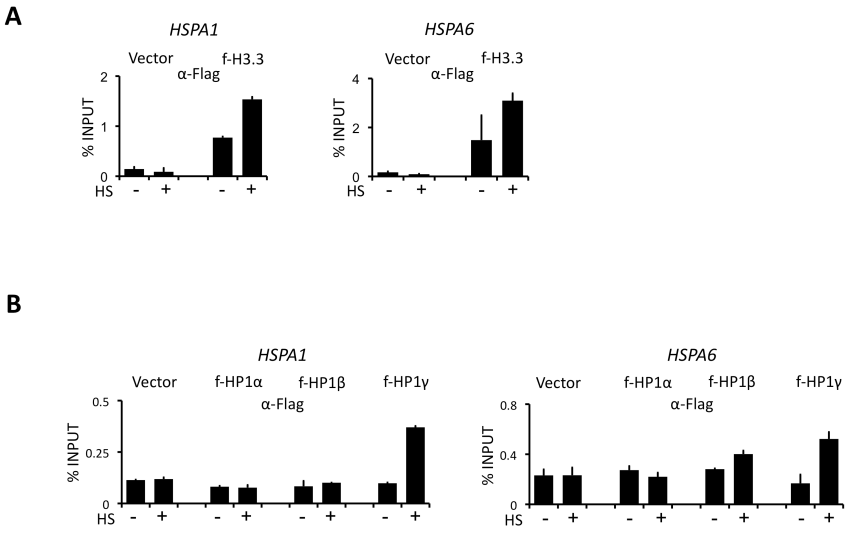

Figure S3. Co-localization of ectopic H3.3 and HP1γ at *HSP70* promoters. (A) Cells were transfected with Flag-H3.3 (f-H3.3) for 48h and treated with heat shock at 42°C for 30 min and then, analyzed by ChIP analysis on *HSPA1* and *HSPA6* promoters as described in Figure 1D. (B) FLAG-tagged versions of HP1 isoforms were expressed in cells and Chip assays were performed as in Figure 1D.

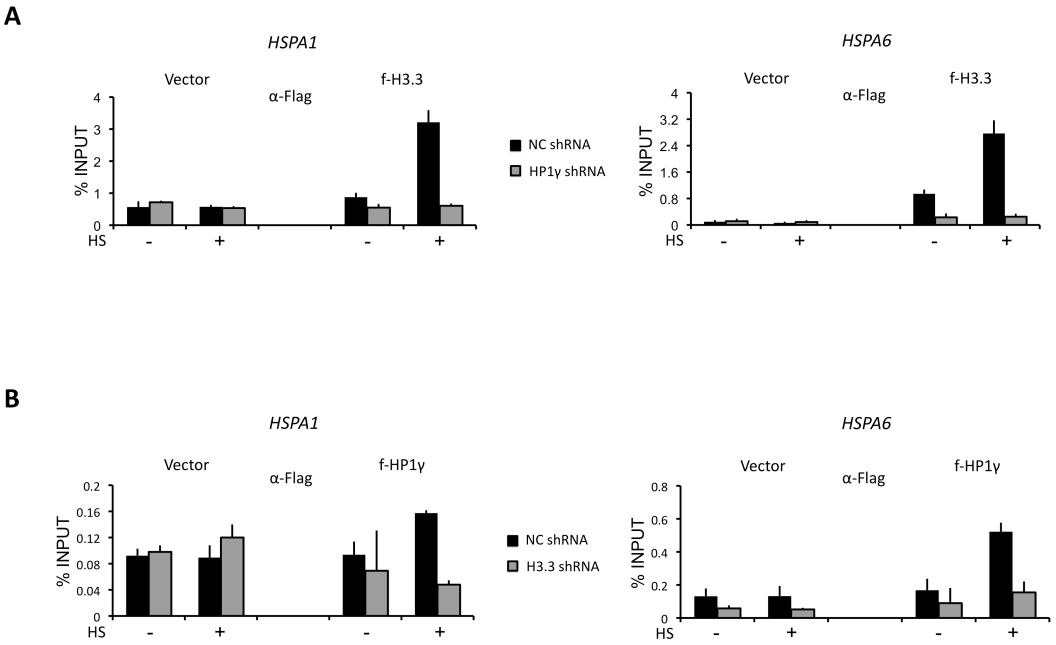

Figure S4. Interdependent promoter occupancy of ectopic H3.3 and HP1γ. H3.3-depleted and HP1γ-depleted cells were transfected with ectopic H3.3 and HP1γ, then ChIP assays were performed using Flag antibody after heat-shock as in Figure 3.

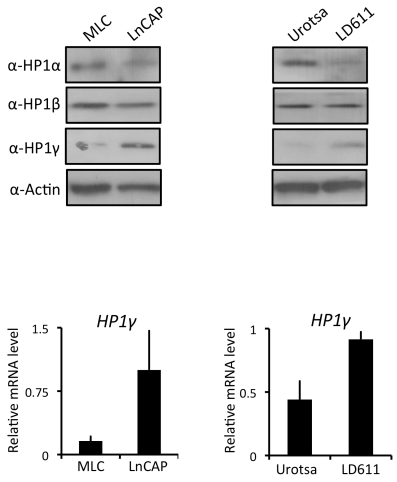

Figure S5. Differential expression patterns of the HP1 proteins in cancer cells. Levels of three HP1 isotypes in untransformed bladder (Urotsa) and prostate (MLC) cells were compared with those in bladder cancer (LD611) and prostate cancer (LNCaP) cells by Western blotting and qRT-PCR as in Figure 6A.

Table S1. Primer sequences for RT-PCR

Primer	Strand	Primer sequence (5′ → 3′)
HSPA6	Forward	CCAAATGCAAGACAAGTGTCG
	Reverse	TTCTAGCTTTGGAGGGAAAG
H3.3A	Forward	GCAAGAGTGCGCCCTCTACTG
	Reverse	GGCCTCACTTGCCTCCTGCAAA
H3.3B	Forward	GTGGCGCTTCGAGAGATTC
	Reverse	GCGAGCCAACTGGATGTCTT
HP1γ	Forward	GTGTAGTGAATGGGAAAGTGG
	Reverse	GGTTCCCAAGTATTGTCAGC
ACTIN	Forward	GTGGGGCGCCCCAGGCACCA
	Reverse	CTCCTTAATGTCACGCACGATT
GAPDH	Forward	GGCCTCCAAGGAGTAAGACC
	Reverse	AGGGGAGATTCAGTGTGGTG

Table S2. Primer sequences for ChIP analysis

Primer	Strand	Primer sequence (5′ → 3′)
HSPA6-P	Forward	GGCCATTCACTAAGGAACCA
	Reverse	AGCAGCAACTTTAGAGGCAAG
HSPA6-C	Forward	GGCAAGGAGCTGAACAAGAG
	Reverse	GAAAGTCTGGGTCTGCTTGG

Table S3. shRNA sequence

Gene	Target sequence (5′ → 3′)
H3.3	TGAAGATACCAATCTGTGTTC