Supplementary figure legends

Figure S1. Purification of H3.1 histones with reversed-phase chromatography.

Figure S2. Flow cytometry data for HeLa cells released from double thymidine block, which was described in Fig. 1A

Figure S3. A full list of the H3:K27-R40 peptides for Fig. 1. It should be noted that H3K27me2/K36me3 and H3K27me3/K36me2 peptides coelute in reversed-phase chromatography and share identical mass; therefore, we were unable to quantify them individually.

Figure S4. An alternative plot for data in Figure 1. H3K9me2 is established quickly on the newly synthesized histones, which trails the incorporation of H3 histones.

Figure S5. A full list of the H3:K27-R40 peptides for Fig. 2. It should be noted that H3K27me2/K36me3 and H3K27me3/K36me2 peptides coelute in reversed-phase chromatography and share identical mass; therefore, we were unable to quantify them individually.

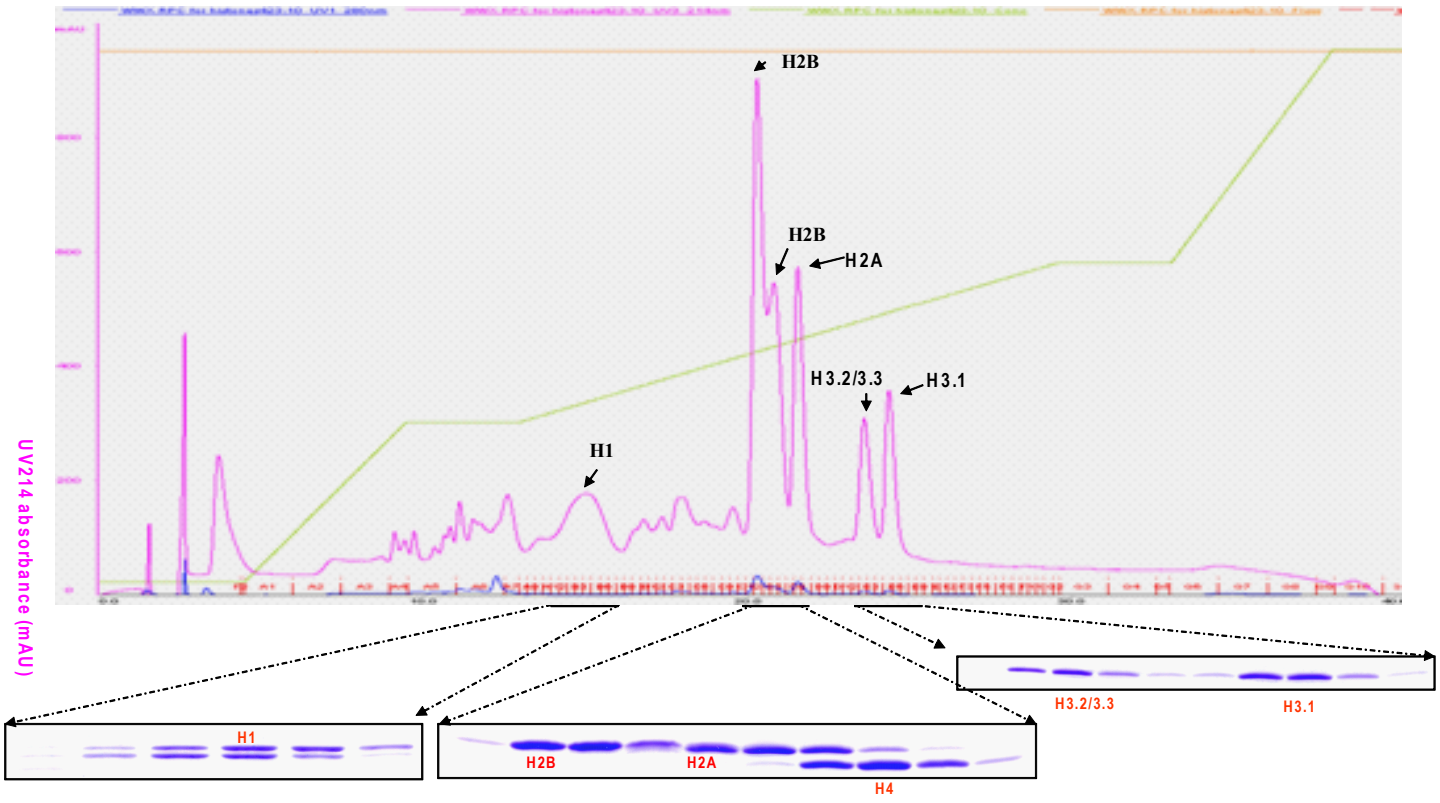
Figure S6. Flow cytometry data for HeLa cells arrested with HU, which was described in Figs. 3 and 4.

Figure S7. A full list of the H3:K27-R40 peptides for Fig. 3. It should be noted that H3K27me2/K36me3 and H3K27me3/K36me2 peptides coelute in reversed-phase chromatography and share identical mass; therefore, we were unable to quantify them individually.

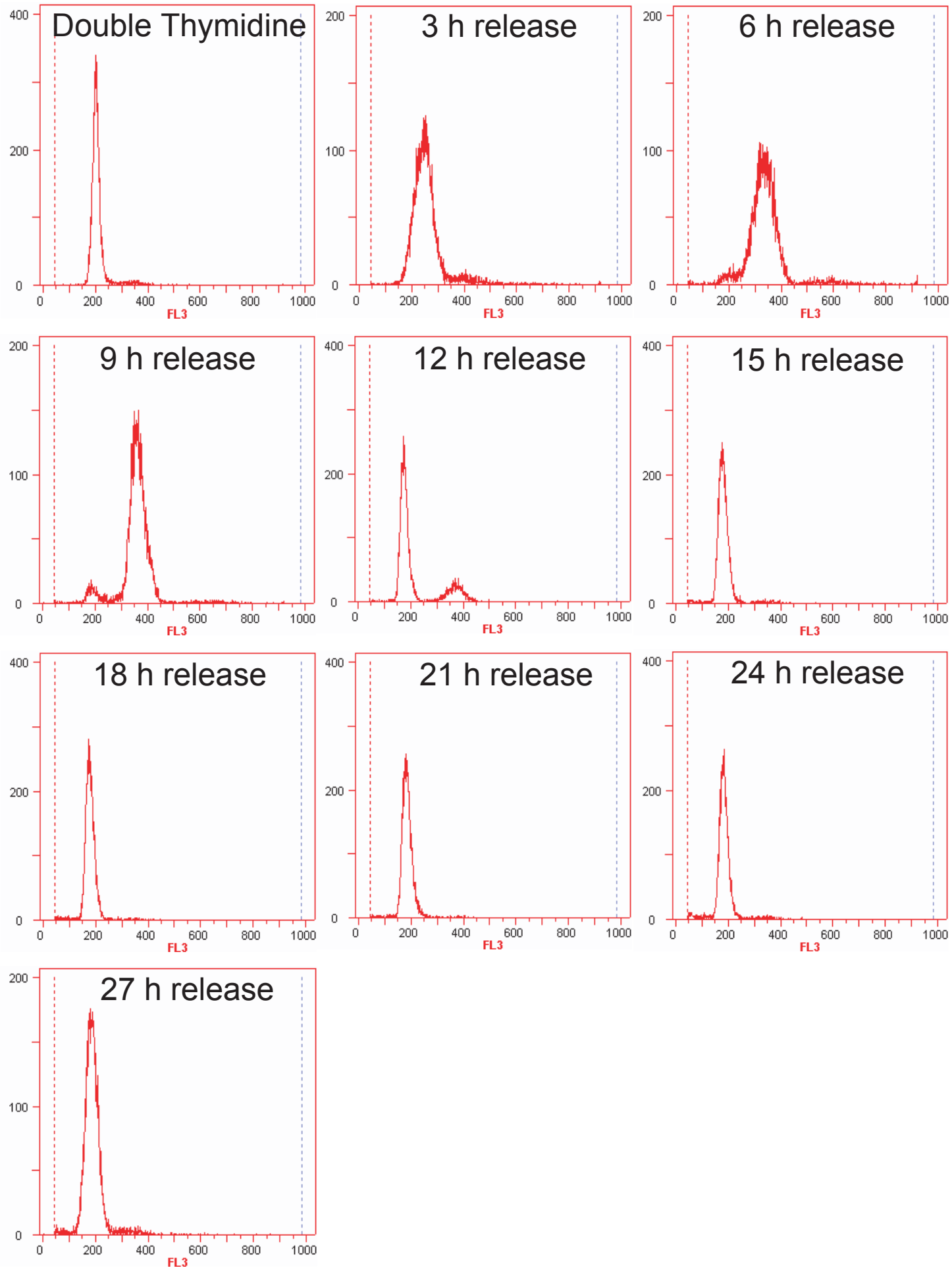
Figure S8. Relative abundance of H3:K9-R17 peptides bearing different modifications. (A) Summary table for three independent experiments. (B) Extracted Ion Chromatograms (XICs) of all H3:K9-R17 peptides.

Figure S9. Mass spectra of the corresponding peptides that are listed in Fig. 4.

Figure S10. Contact inhibition with T24 bladder carcinoma cells and serum starvation with HeLa cells failed to fully arrest the cells as indicated by the incorporation of newly synthesized histone H3.1 (A) Contact inhibition with T24 bladder carcinoma cells for 72 h led to the incorporation of approximately 20% of newly synthesized H3.1 histones. (B) Serum starvation with HeLa cells for 24 h led to the incorporation of approximately 50% of newly synthesized H3.1 histones.



Supplementary Fig. S1



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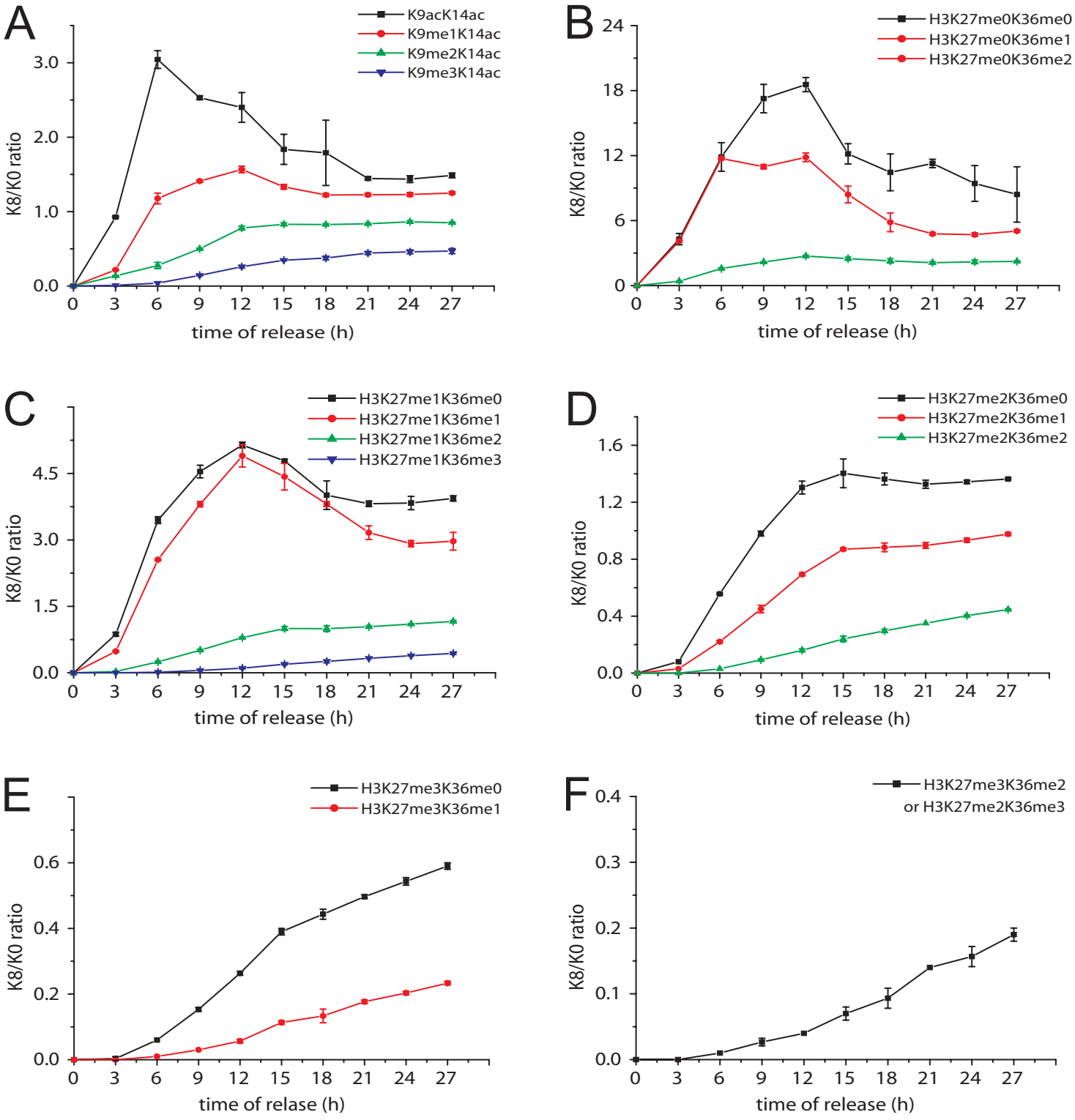

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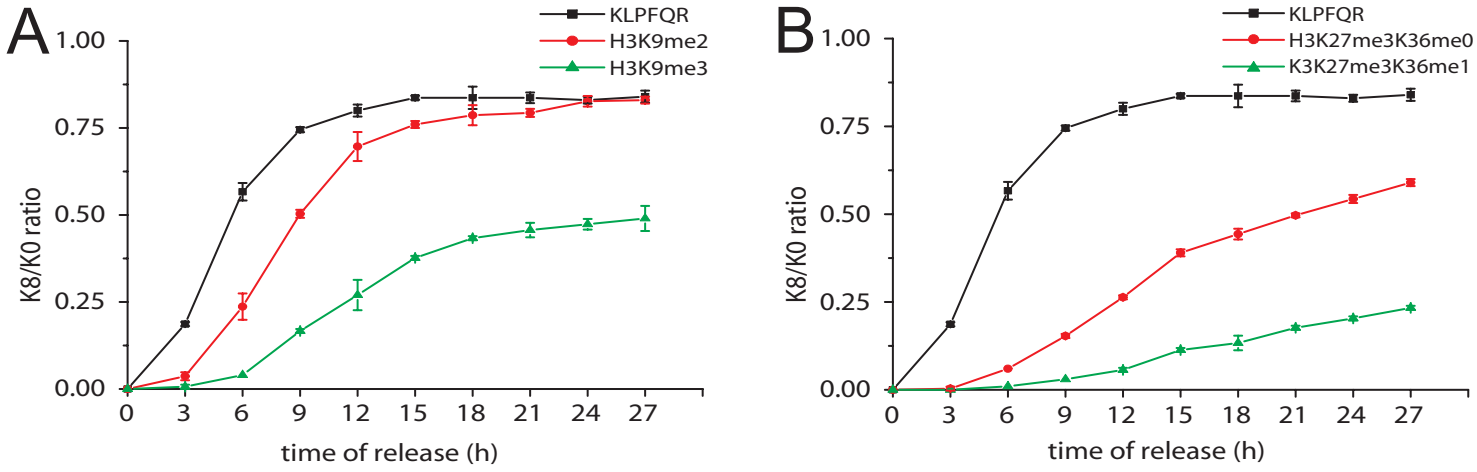

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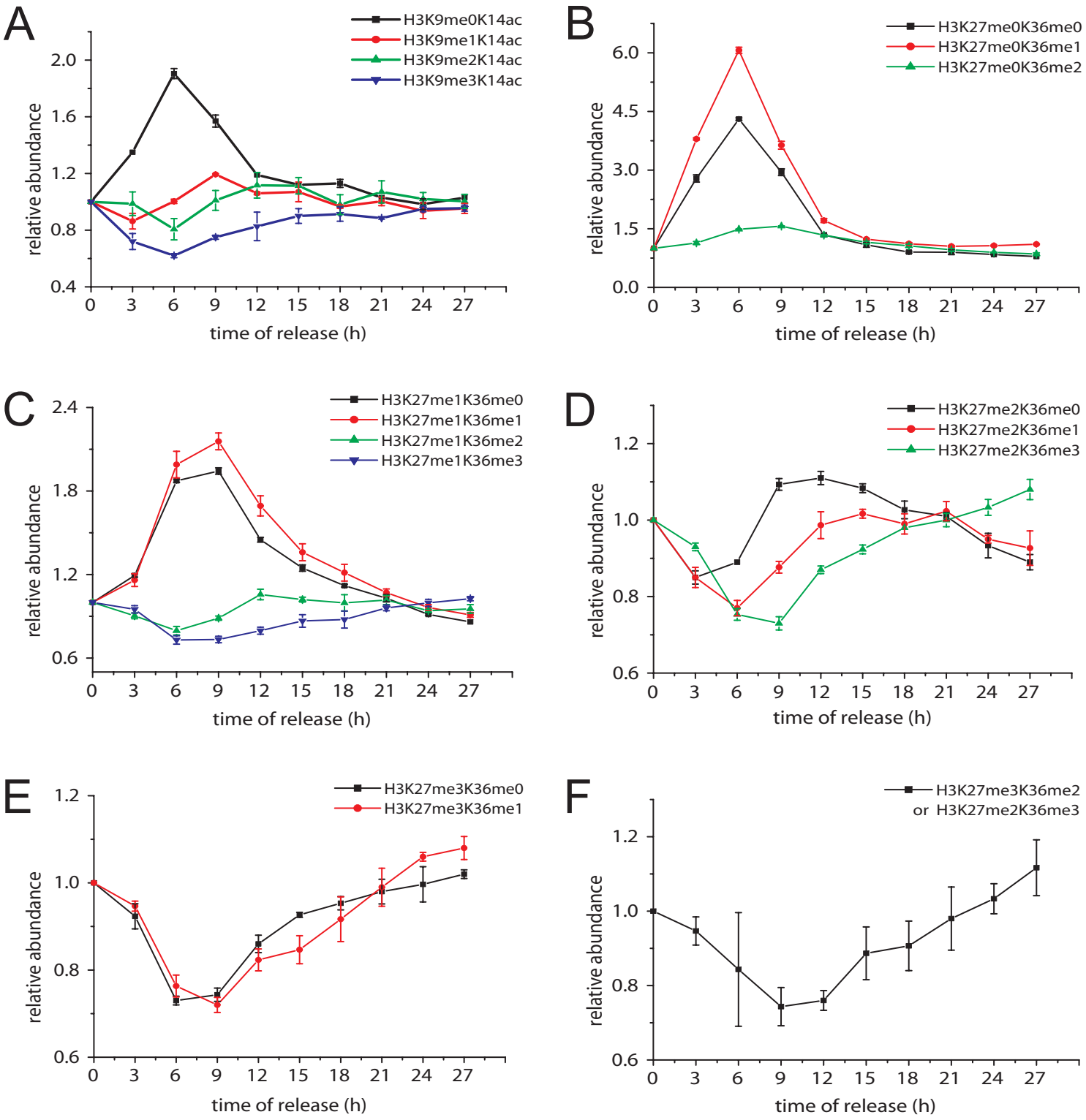


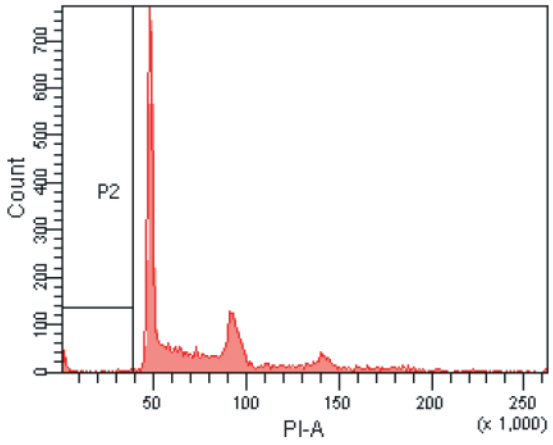
Supplementary Fig. S5

A

Time of HU treatment	The portion of sub-G1 peak
Untreated	1.9%
0 h	1.7%
24 h	1.6%
48 h	4.6%
72 h	4.7%

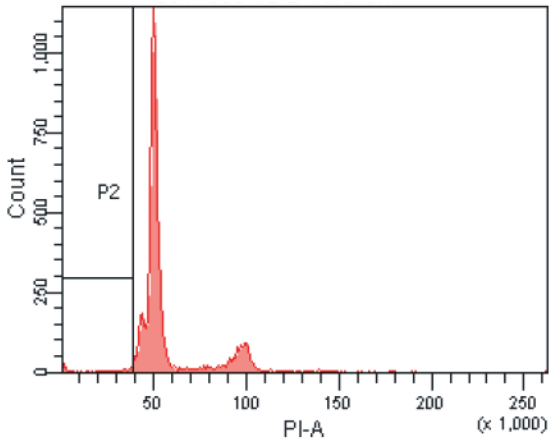
Note: there was already one round of 12h HU treatment before

B



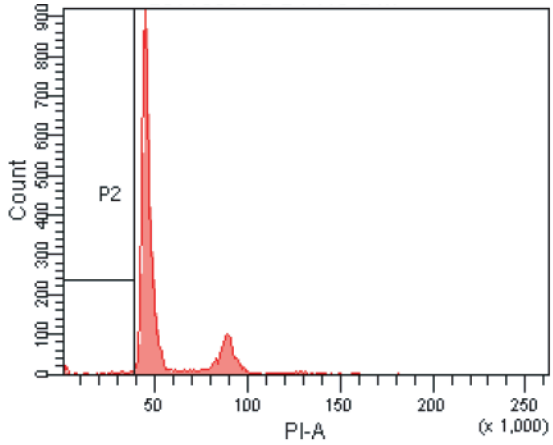
Untreated

C



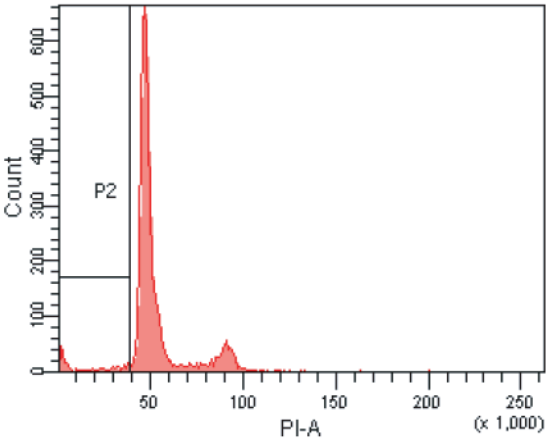
0 h

D



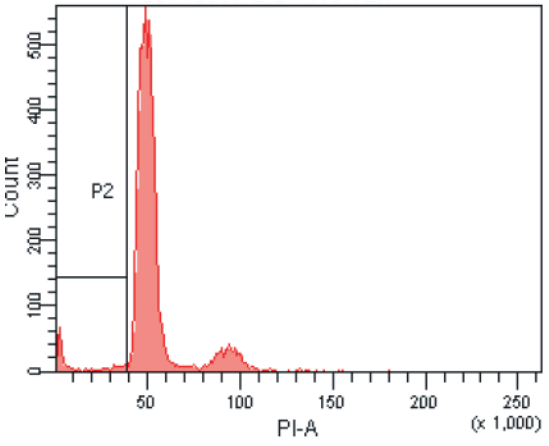
24 h

E



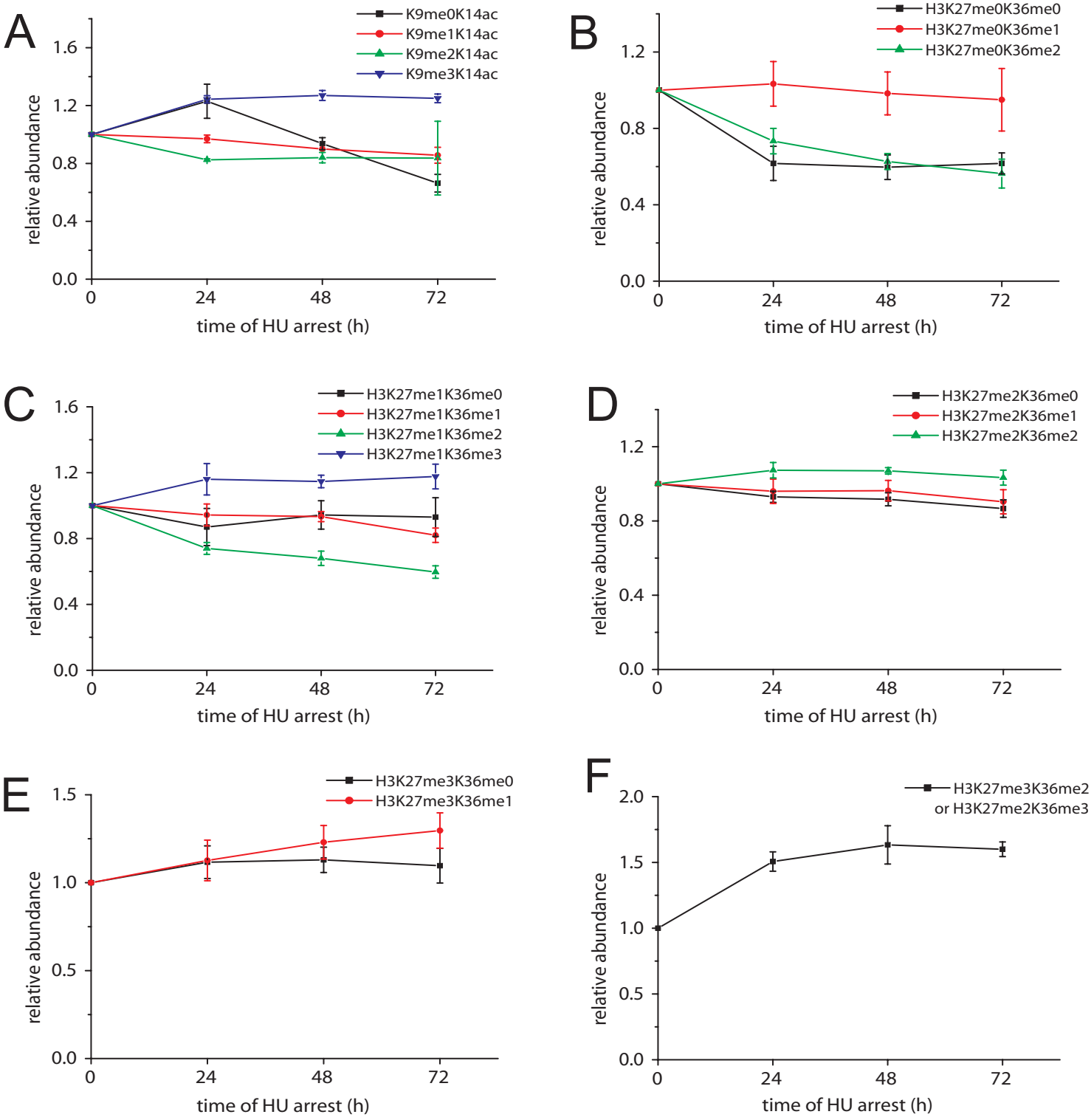
48 h

F



72 h

Supplementary Fig. S6

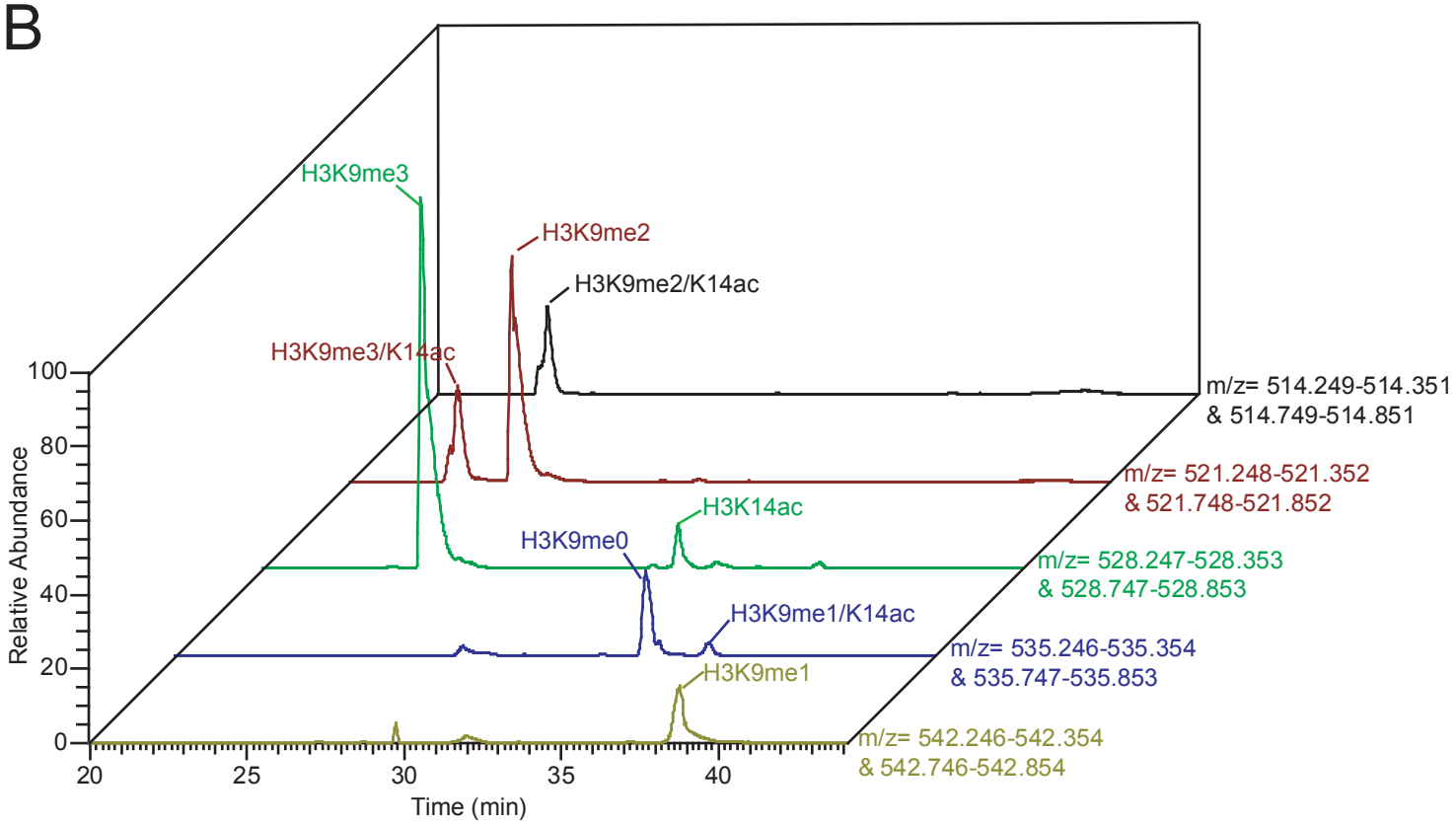


Supplementary Fig. S7

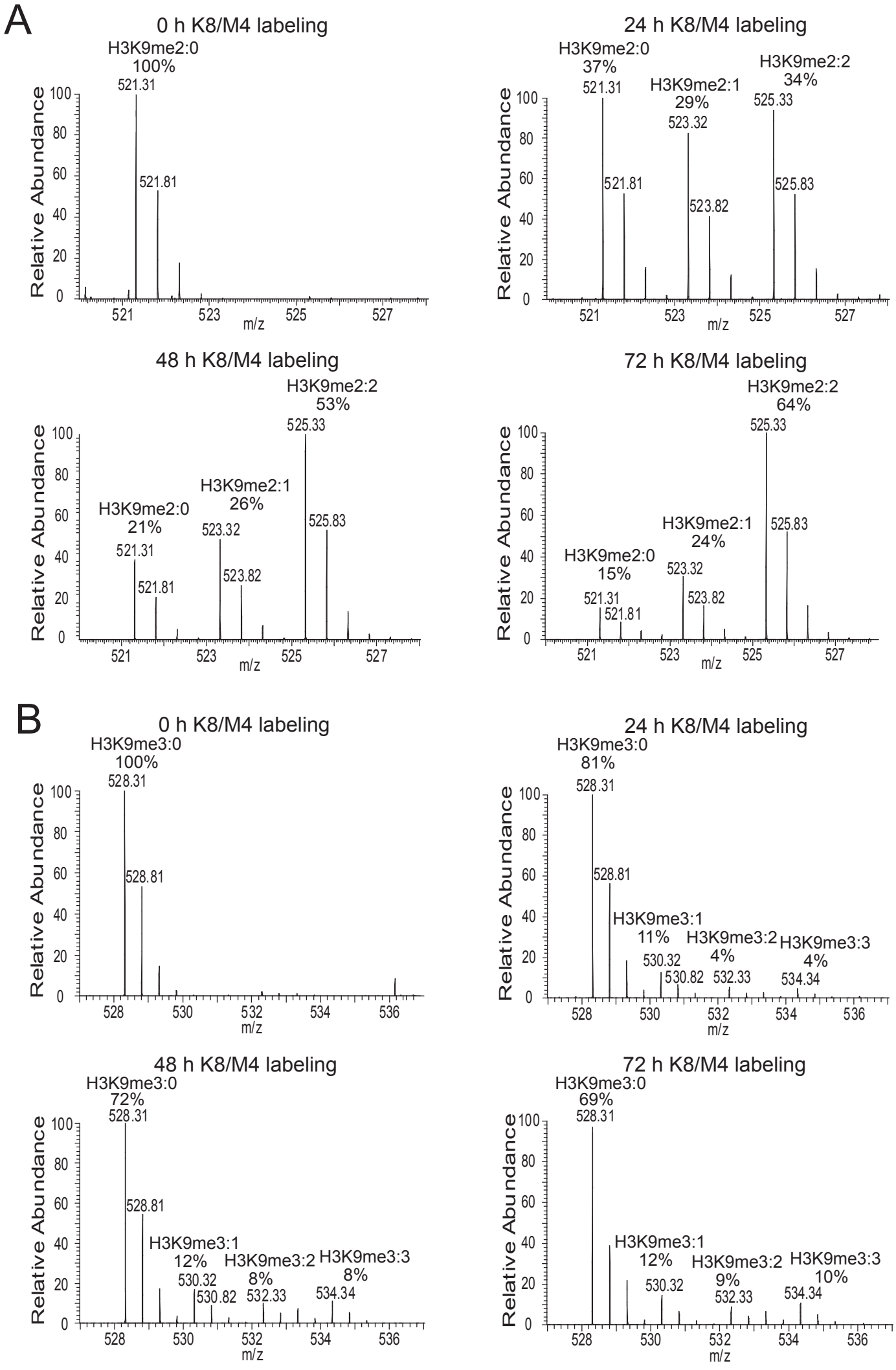
A

Peptide(H3:K9-R17)	Approximate abundance			
	Exp1	Exp2	Exp3	Mean Value
H3K9me0	8.8%	10.8%	9.9%	9.9%±1.0%
H3K9me0/K14ac	2.8%	2.9%	3.4%	3.0%±0.3%
H3K9ac	ND	ND	ND	ND
K3K9me1	6.6%	6.2%	4.3%	5.7%±1.2%
H3K9me1K14ac	1.5%	1.8%	2.0%	1.8%±0.2%
H3K9me2	25.9%	26.3%	27.5%	26.6%±0.8%
H3K9me2K14ac	7.7%	5.0%	5.1%	6.0%±0.5%
H3K9me3	37.5%	40.4%	41.4%	39.8%±2.0%
H3K9me3K14ac	9.2%	6.5%	6.4%	7.3%±1.6%

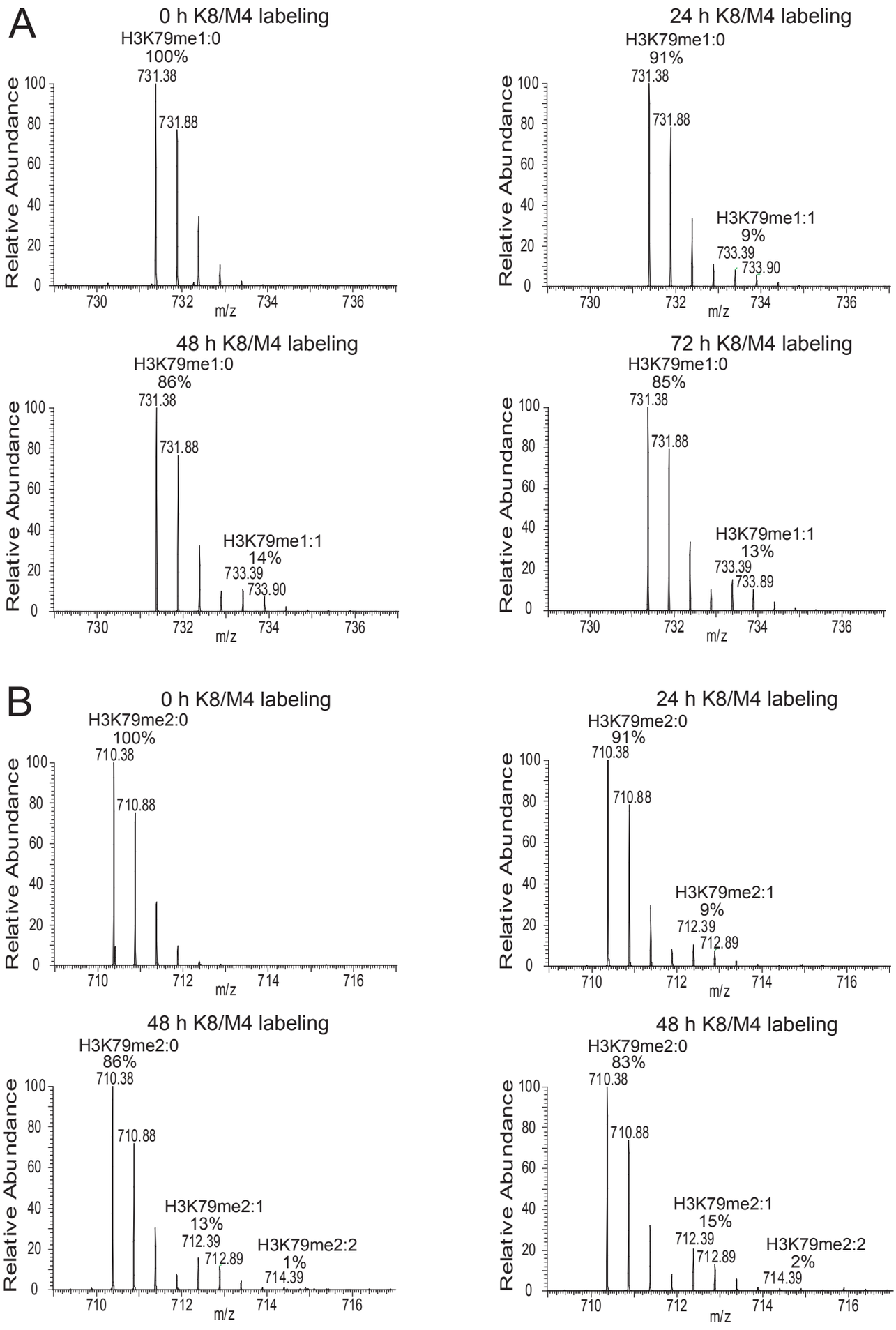
B



Supplementary Fig. S8



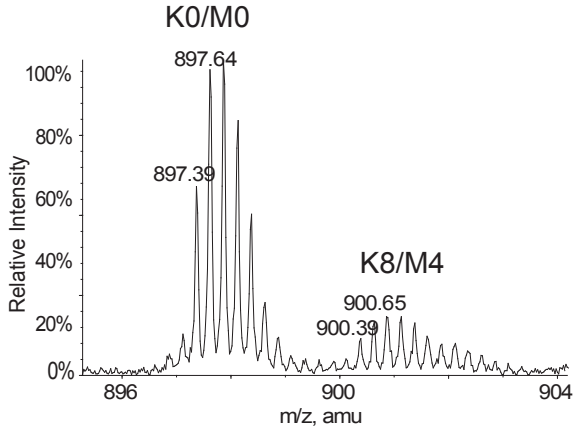
Supplementary Fig. S9 Page1



Supplementary Fig. S9 Page2

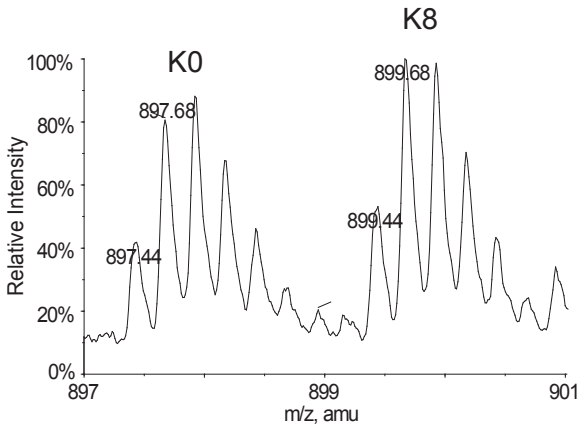
A

Contact Inhibition of T24 cells, 72 hrs
Bulk H3.1 Old: K0/M0 labeled New: K8/M4 labeled
FQSSAVMALQEACAYLVGLFEDTNLCAIHAK



B

Serum Starvation of HeLaS3 cells, 24 hrs
Bulk H3.1 Old: K0 labeled New: K8 labeled
FQSSAVMALQEACAYLVGLFEDTNLCAIHAK



Supplementary Fig. S10

Supplementary methods

Cell culture

For the K8 pulse-labeling experiments shown in Fig. 1, HeLa cells were synchronized in G1/S phase by double thymidine blocking (2mM thymidine for 16 h, release for 10 h, followed by a second thymidine treatment for 12 h). The cells were released into K8-labeling media. Thymidine (2 mM) was added 18 h after cell cycle release to prevent the cells from entering into the next S phase. The cells were harvested every 3 h from G1/S release. The cell culture was performed in the same way to quantify the global levels of histone methylation (shown in Fig. 2), except that the cells were released into K0 media.

To study histone methylation dynamics in long-term arrested cells, HeLa cells were arrested at the G1/S boundary with double thymidine treatment and were further arrested with HU for 12 h. Cells were cultured for 24, 48 or 72 h in HU containing media supplemented with K0/M0 (for relative quantification of overall methylation levels in Fig. 3) or K8/M4 (for analysis of the turnover of methyl groups in Fig. 4).

Acidic extraction of histones

Briefly, cells were lysed with lysis buffer (10 mM Tris-HCl pH 8.0, 1.5 mM KCl, 2 mM $MgCl_2$, 1 mM DTT and 1.5% NP40) and were washed three times with lysis buffer to remove non-chromatin histones. The chromatin was subsequently washed once with wash buffer (10 mM Tris-HCl pH 8.0, 1.5 mM KCl, 2 mM $MgCl_2$ and 1 mM DTT) and was resuspended into 0.2 M HCl and rotated at 4℃ for 2 hrs. The samples were centrifuged at 13,000 rpm at 4℃ for 10 min to pellet the insoluble components. The supernatant was collected as the histone-containing fraction. Histone polypeptides were precipitated with 20% trifluoroacetic acid (TFA) and dissolved with deionized water.

Mass spectrometry analysis

Peptides were separated with a homemade analytical capillary column (50 μm × 12 cm) that was packed with C18 reversed-phase material (5-μm YMC spherical particles). The HPLC gradient was generated with an Agilent 1200 series as follows: 0%-40% B in 70 min,

40%-100% B in 10 min, 100% B for 5 min, and 100%-0% B in 5 min (A = 0.1% formic acid in water; B = 0.1% formic acid, 80% acetonitrile). The eluted peptides were electrosprayed into an LTQ-Orbitrap mass spectrometer (Thermo). Data was acquired in information dependent mode, and a full mass spectrometry scan was collected from m/z 300 - 2000 with a resolution of R equal to 60,000. The top six abundant ions were selected and fragmented in linear ion trap by collision-induced dissociation (CID), and the fragment ions were scanned with an ion trap. Precursor ions were placed into an exclusion list from further selection for 40 sec.

Database search and identification of peptide modifications

The RAW files were converted into DTA files by DTASuperCharge (Mortensen *et al*, 2010). MS/MS spectra were searched against the IPI human database using Mascot server 2.2 (Matrix Science Ltd.). To determine the posttranslational modifications on the histone peptides, the database was searched with the following variable modifications: acetyl on lysine, dimethyl on lysine, trimethyl on lysine, propionyl on lysine and propionyl-methyl on lysine. A maximum missed cleavage sites of two was used; the peptide tolerance was set to 10 ppm to distinguish acetylation and trimethylation; and the fragment tolerance was set to 0.6 Da. Search results were manually verified against the RAW files to define the isobaric ions. MS/MS spectra for all distinct methylation states were verified according to our previous reports (Chen *et al*, 2011; Yuan *et al*, 2011).

Relative quantification

For relative quantification of the K8- or R10-labeling system (Fig. 1), Mascot search results and RAW files were analyzed with the open source software MSquant, and the ratio of heavy/light peptide pairs was calculated based on the extracted ion chromatogram (XIC) (Mortensen *et al*, 2010).

Using R10 histones as the external reference, we calculated the dynamic changes of global levels of specific histone modifications. Briefly, an R0/R10 ratio of a peptide bearing a modification of interest was normalized against an average R0/R10 ratio of unmodified histone backbone peptides; therefore, we obtained its relative abundance. The relative

abundance at each time point was normalized to time zero to create the dynamic curves that are shown in Figs. 3 and 4.

XIC was employed to calculate the relative abundance of peptides bearing various combinations of light and heavy methyl groups. Xcalibur 2.0.7 software (Thermo) was used to extract the XIC of first two isotopic peaks of the doubly charged peptides. The mass tolerance was designated as 0.1 Da, and the mass precision was set to two decimals. XIC peaks from isobaric ions were manually defined according to Mascot search results. For relative quantification, the sum of the XIC peak area from all combinations of a methylated peptide was defined as 100%.

Reference

Chen X, Xiong J, Xu M, Chen S, Zhu B (2011) Symmetrical modification within a nucleosome is not required globally for histone lysine methylation. *EMBO Rep* **12:** 244-251

Mortensen P *et al* (2010) MSQuant, an open source platform for mass spectrometry-based quantitative proteomics. *J Proteome Res* **9:** 393-403

Yuan W, Xu M, Huang C, Liu N, Chen S, Zhu B (2011) H3K36 methylation antagonizes PRC2-mediated H3K27 methylation. *J Biol Chem* **286:** 7983-7989