Supplementary Figure legends

Figure S1. (A, B) Expression level of the Flag-tagged K to R mutants do not exceed 10% of the corresponding endogenous histones. Histone samples were loaded at three distinct amounts, which are labeled as 1X, 3X and 9X; the upper panels were detected with anti-Flag to indicate the expression of the exogenous histones; the lower panels were detected with anti H3 or anti-H4 histones to compare the levels of exogenous histones with the endogenous histones. (C) Coomassie blue staining of the Flag-H3K4R mono-nucleosomes show comparable amounts of Flag-H3K4R and the endogenous H3. (D) Western analysis show close to 1:1 ratio of the Flag-H3 histones and the endogenous H3. (E, F) Antibodies against H4 react poorer on Flag-H4K20R than Flag-H4 and H4 histones.

Figure S2. The Flag-tagged histones are incorporated into representative regions of the chromatin. (A) Schematic location of the tested regions. (B) Primer sequence and location on the genome. Amplicon used for normalization is labeled in red. Testing amplicons in coding genes are labeled in blue. Test amplicons in intergenic regions are labeled in black. (C) Quantitative PCR results of above mentioned amplicons with HeLa mono-nucleosomes and affinity purified Flag-tagged K to R mutants containing mono-nucleosomes as templates.

Figure S3. Explanatory illustration for quantitative mass spectrometry.

Figure S4. The K8 labeling efficiency of HeLa cells is above 90%. Two H3 backbone peptides from HeLa cells labeled with K8 displayed 93% and 90% labeling efficiency.

Figure S5. Symmetric methylation is not required for H3K4. (A) Summary table of the quantified peptides of methylated H3K4; (B) Mass spectra for the quantified peptides including the backbone peptide and H3K79me1/2/3 peptides. (C) Western analysis quantifying relative abundance of H3K4me2/3. amu is the abbreviation of atomic mass unit.

Figure S6. Symmetric methylation is not required for H3K36. (A) Summary table of the quantified peptides of methylated H3K36; (B) Mass spectra for the quantified peptides including the backbone peptide and H4K20me1/2 peptides. (C) Western analysis quantifying relative abundance of H3K36me3. amu is the abbreviation of atomic mass unit.

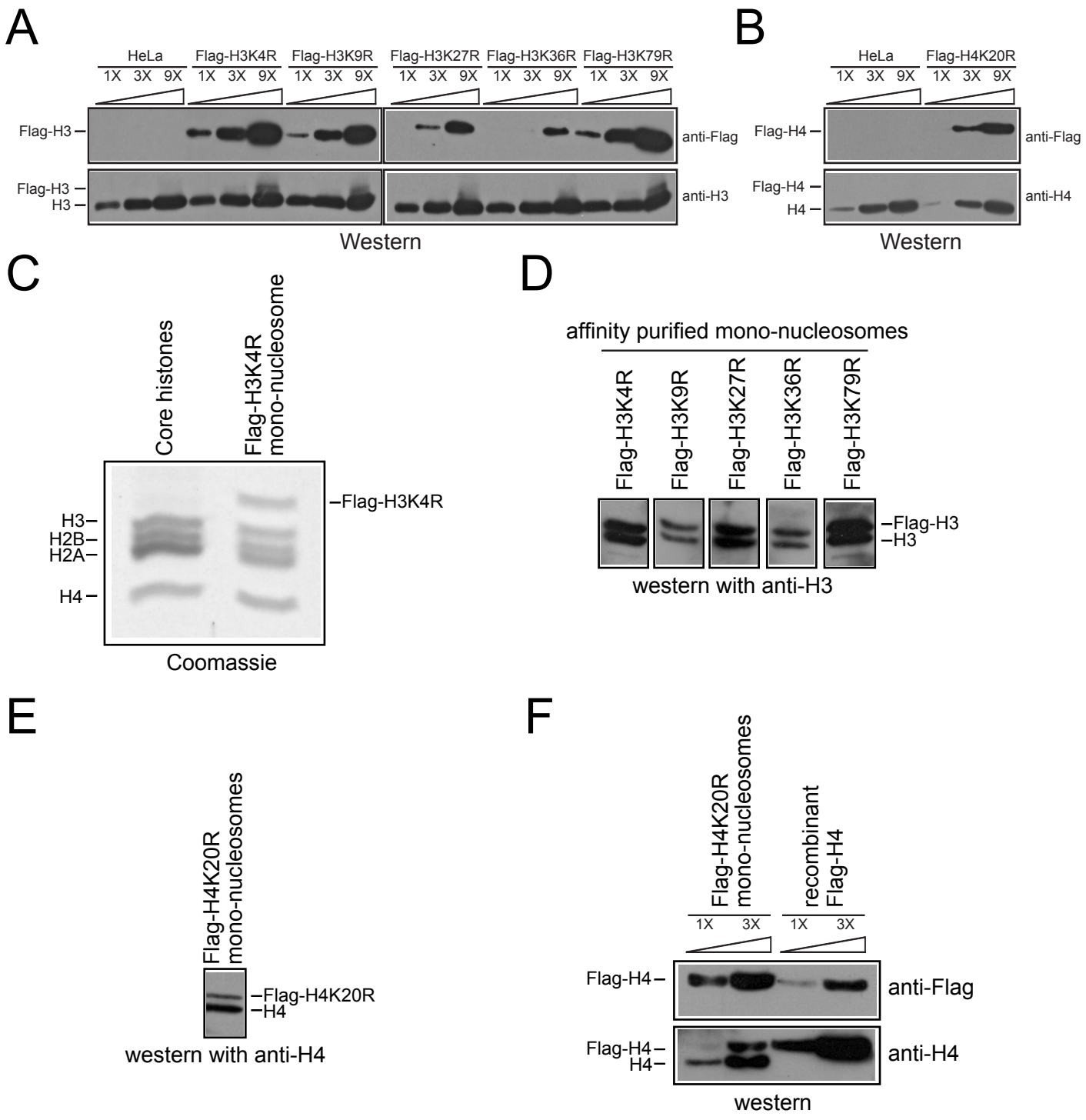
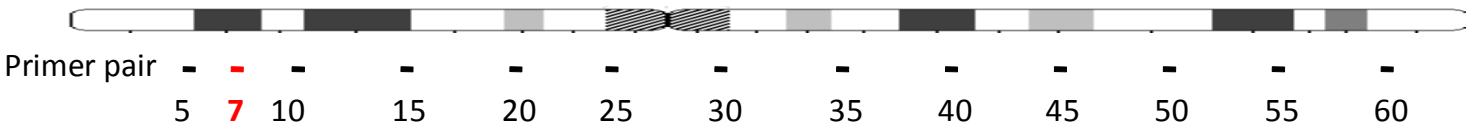

A
HeLa
Flag-H3K4R
Flag-H3K9R
Flag-H3K27R
Flag-H3K36R
Flag-H3K79R
1X 3X 9X
Flag-H3
anti-Flag
H3
anti-H3
Western
B
HeLa
Flag-H4K20R
Flag-H4
H4
Western
C
Core histones
Flag-H3K4R mono-nucleosome
H3
H2B
H2A
H4
Flag-H3K4R
Coomassie
D
affinity purified mono-nucleosomes
Flag-H3K4R
Flag-H3K9R
Flag-H3K27R
Flag-H3K36R
Flag-H3K79R
Flag-H3
H3
western with anti-H3
E
Flag-H4K20R mono-nucleosomes
Flag-H4K20R
H4
western with anti-H4
F
Flag-H4K20R mono-nucleosomes
recombinant Flag-H4
1X 3X
Flag-H4
anti-Flag
H4
anti-H4
western

Figure S1

A



B

position	primer sequence	locus information
7Mbp	CTTGCAGCTAGGCAATACAC	291235 bp at 5' side: bone morphogenetic protein 2 preproprotein preproprotein
	GTGACCTCTGCAACACACAG	813197 bp at 3' side: hydroxyacid oxidase 1
5Mbp	TCTCCTTCCCTTTGTGATGC	122675 bp at 5' side: solute carrier family 23 (nucleobase transporters), member 2
	TTGCCCAGTCTACAAAGAGC	45478 bp at 3' side: hypothetical protein LOC29058 isoform 1
10Mbp	CACAGGCCAGTCATTTGATC	55991 bp at 5' side: ankyrin repeat domain protein 5
	GGCAGAGATTTTGCCAAGAG	163775 bp at 3' side: synaptosomal-associated protein 25 isoform SNAP25A
15Mbp	GCCTGCTCTCCACCTATTTC	MACRO domain containing 2 isoform 1
	ACCCACTTGAGACCAGAGTG	
20Mbp	GTGAAGGCAGGGCTTAAGTC	hypothetical protein LOC26074
	TGCTTTGCCTGAGATGACAG	
25Mbp	TCTTTGATGCGACCCAACTG	430 bp at 5' side: visual system homeobox 1 isoform b
	CATATATGGGGTAGCCATCG	113263 bp at 3' side: ectonucleoside triphosphate diphosphohydrolase 6 isoform 2
30Mbp	TTCTAAAAGCCCTGGGGCTC	1098 bp at 3' side: hypothetical protein XP_002344254
	CGGGTTTGCTCCTGTTTCTC	
35Mbp	GAACAAGCTAACATCGCCTG	erythrocyte membrane protein band 4.1-like 1 isoform a
	CTTATGTCCCATGCCTGATG	erythrocyte membrane protein band 4.1-like 1 isoform b
40Mbp	GGGAGGCAGGAAGCAATTAC	1250083 bp at 5' side: DEAH (Asp-Glu-Ala-His) box polypeptide 35
	TGTCTAGCAGGCAGGGAACT	399182 bp at 3' side: transcription factor MAFB
45Mbp	GGAATACTCTTGCTCGCATG	43554 bp at 5' side: CD40 antigen isoform 1 precursor
	ACTTCAACTGGGTCTCGAAG	1849 bp at 3' side: cadherin 22 precursor
50Mbp	TATCGGCTCTTGTCATGGAG	180007 bp at 5' side: potassium voltage-gated channel, subfamily G, member 1
	ATTTGCTCCTGTGTGCAGAC	200976 bp at 3' side: nuclear factor of activated T-cells, cytoplasmic, calcine...
55Mbp	TGTGGTCCTCAGACAGCATC	221416 bp at 5' side: cerebellin 4 precursor
	GAACGGGGTCAGTGCTATTC	23193 bp at 3' side: melanocortin 3 receptor
60Mbp	GTATCCGGGTCAGATTCCTG	1138997 bp at 5' side: similar to hCG2017976
	ATTTCCTAGGGCCAGTTCTC	24846 bp at 3' side: cadherin 4, type 1 preproprotein preproprotein

C

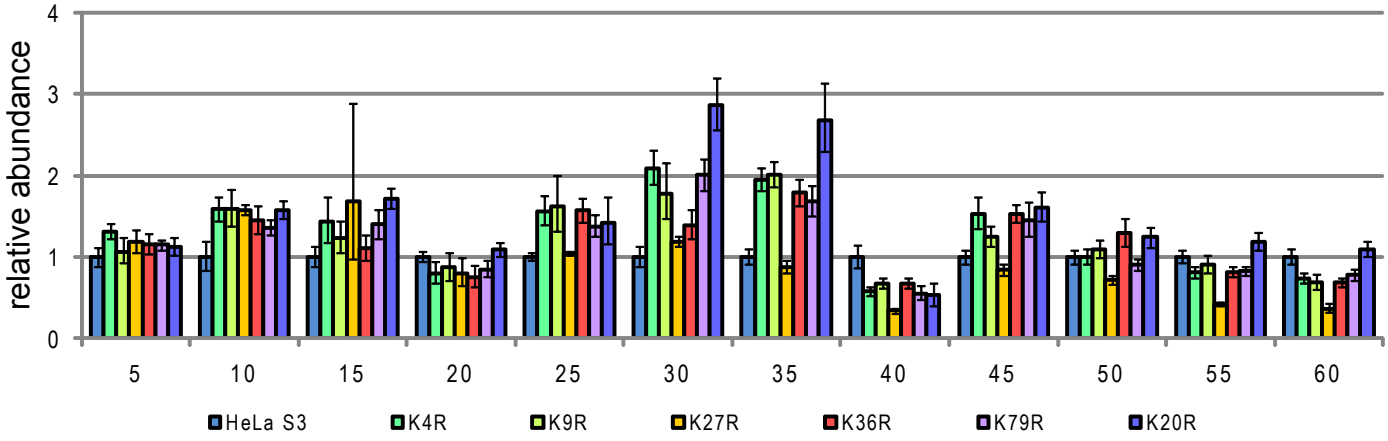
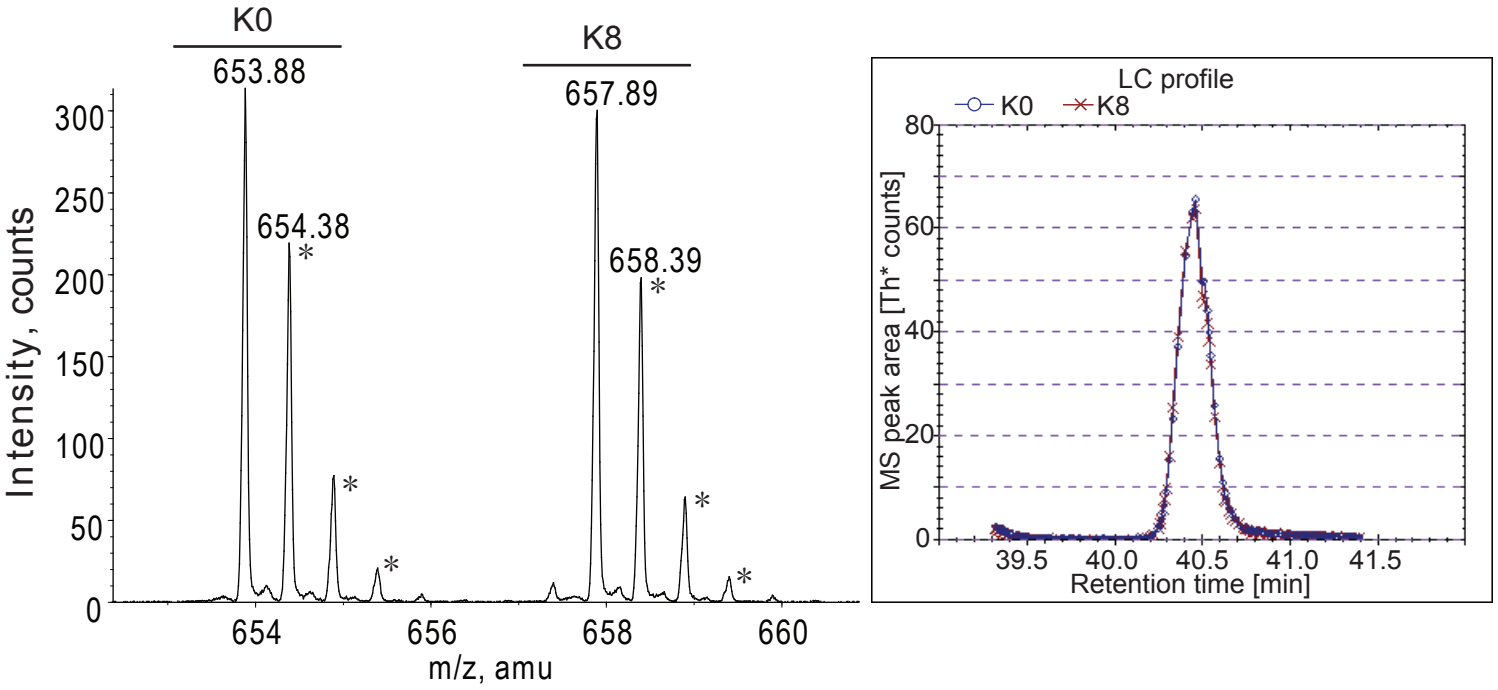


Figure S2

YQK(pro)STELLIR, $2H^+$, K8/K0=0.98



Explanatory illustration for comparative quantification of SILAC based mass spectrometry

A K0 peptide and its corresponding K8 peptide with identical amino acid sequence were co-eluted from the reverse-phase HPLC column, got ionized on the mass spectrometer and displayed in the MS spectrum as a peptide pair with defined mass difference of 8 Da (left panel). In the LC-MS/MS run, the mass spectrometer cycled between a MS scan and three MS2 scans in every 2 sec, thus a number of MS spectra were obtained for a given peptide across its LC profile (LC peak width ranges between 60 to 120 sec in general). Peak areas of the K0/K8 peptide pair in each MS spectrum were plotted against the elution time to generate the extracted ion chromatograms (XICs) (right panel). The individual peak areas of the K0 and K8 pair from all available MS spectra were summed to calculate the relative abundance ratio.

*Note: ^{13}C is a stable isotope that exists at certain percentage in nature. Peptides containing one ^{13}C atom have 1 Da mass shift; peptides containing two ^{13}C atoms have 2 Da mass shift. In this sample case, the peptides carry two protons, thus the native isotopic peaks (∗) have 0.5 Da shift for their m/z values.

Fig. S3

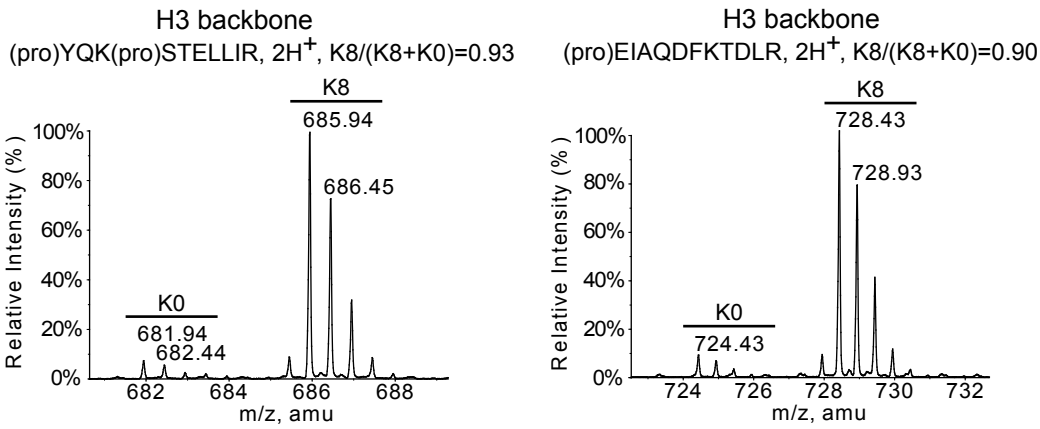

H3 backbone
(pro)YQK(pro)STELLIR, 2H+, K8/(K8+K0)=0.93
K8
685.94
686.45
K0
681.94
682.44
Relative Intensity (%)
100%
80%
60%
40%
20%
0%
682
684
686
688
m/z, amu
H3 backbone
(pro)EIAQDFKTDLR, 2H+, K8/(K8+K0)=0.90
K8
728.43
728.93
K0
724.43
Relative Intensity (%)
100%
80%
60%
40%
20%
0%
724
726
728
730
732
m/z, amu

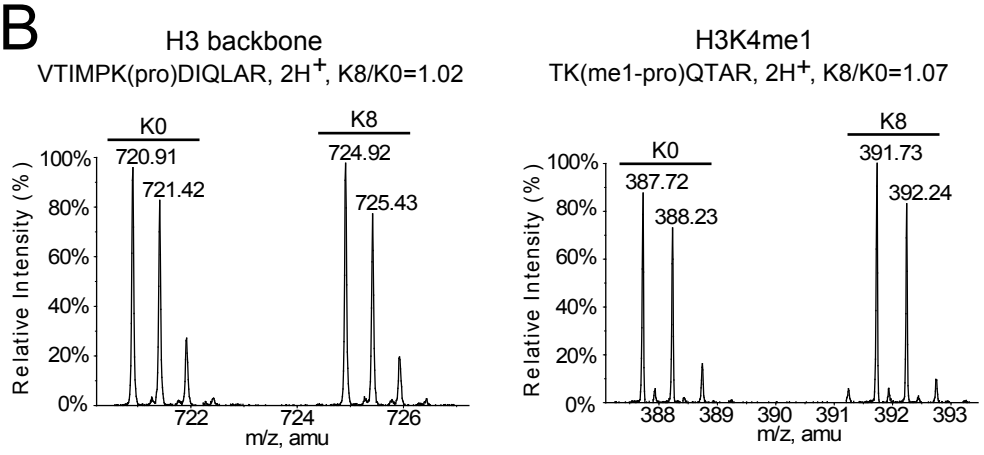
Figure S4

A

	K8 labeled HeLa H3 / K0 labeled partner H3 in Flag-H3K4R mono-nucleosomes	
	raw ratio	normalized ratio
H3 backbone		
VTIMPK(pro)DIQLAR	1.02	
H3K4me1		
TK(me1-pro)QTAR	1.07	1.05

pro: propionylation

B



C

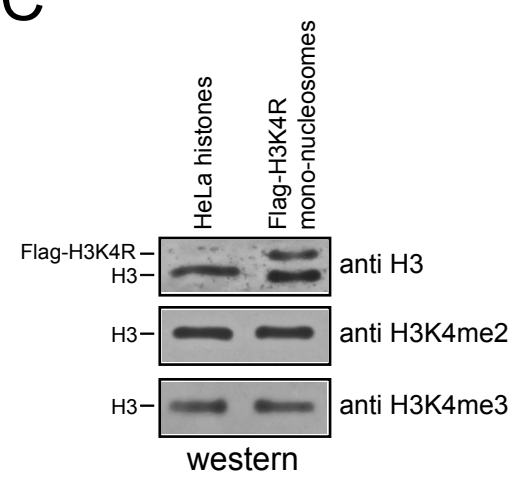


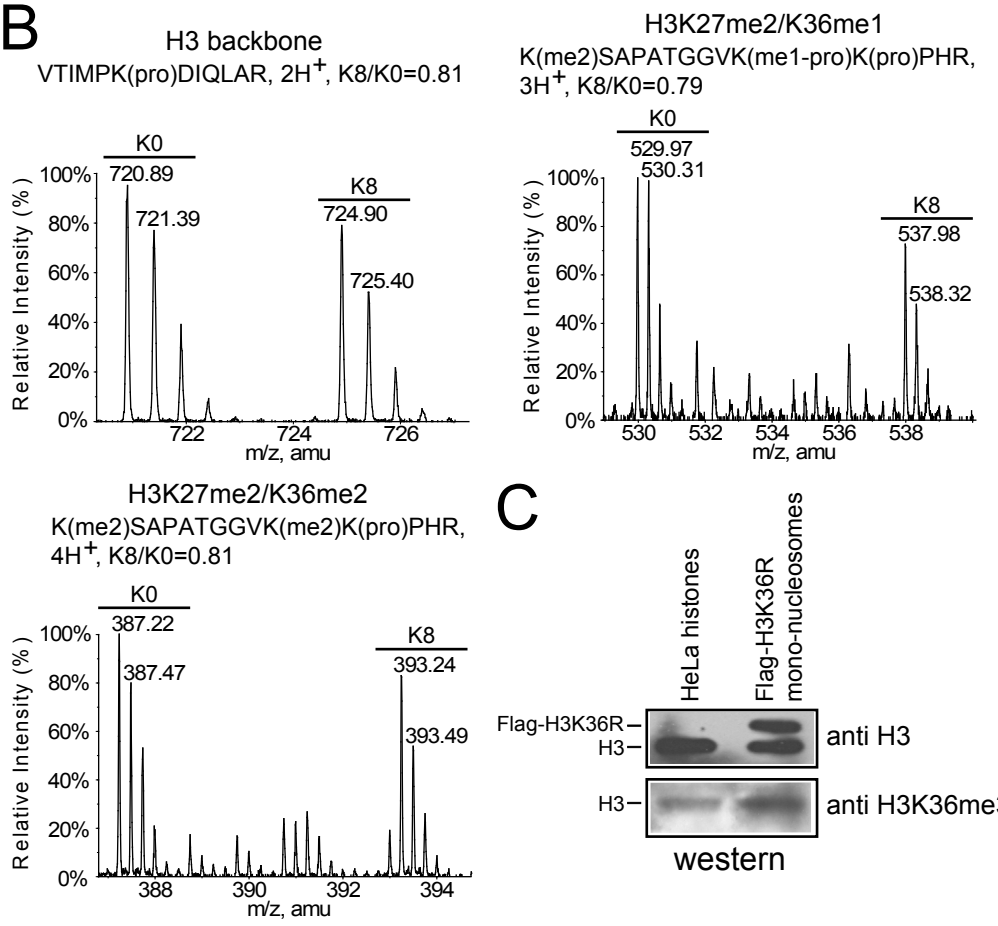
Figure S5

A

	K8 labeled HeLa H3 / K0 labeled partner H3 in Flag-H3K36R mono-nucleosomes	
	raw ratio	normalized ratio
H3 backbone		
VTIMPK(pro)DIQLAR	0.81	
YQK(pro)STELLIR	0.81	
H3K27me2/K36me1		
K(me2)SAPATGGVK(me1-pro)K(pro)PHR	0.79	0.98
H3K27me2/K36me2		
K(me2)SAPATGGVK(me2)K(pro)PHR	0.81	1.00

pro: propionylation

B



C

Figure S6