

Association of UHRF1 with H3K9 methylation directs the maintenance of DNA methylation

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SUPPLEMENTARY INFORMATION

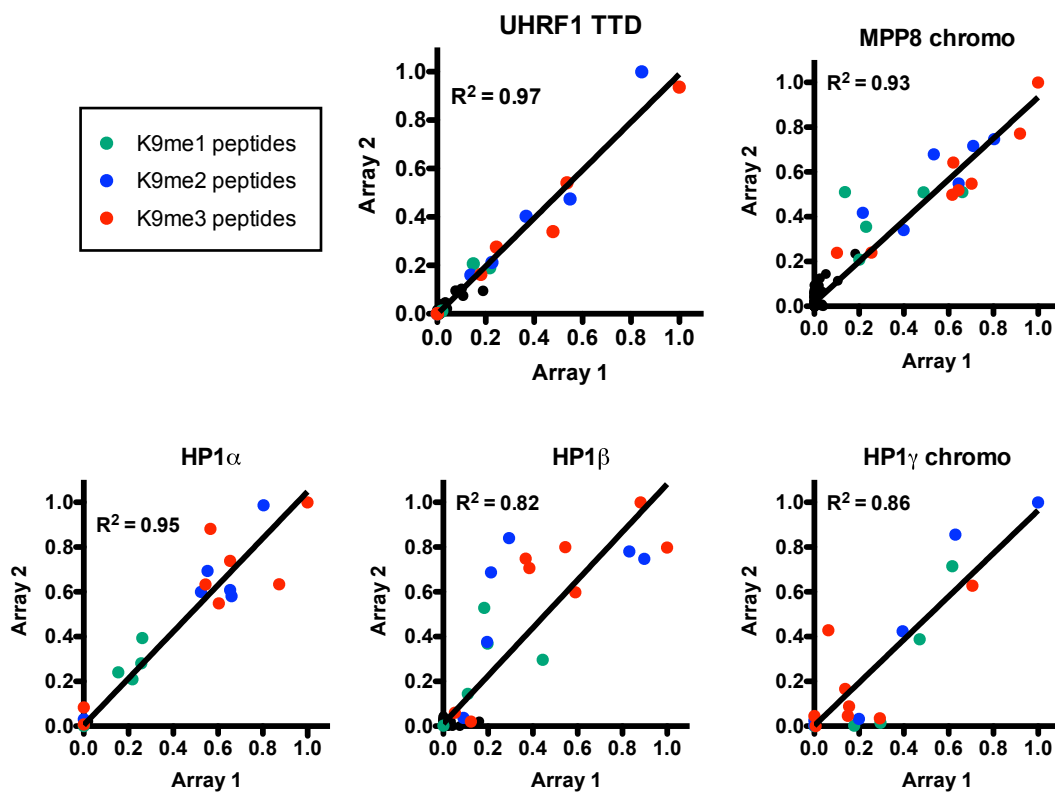
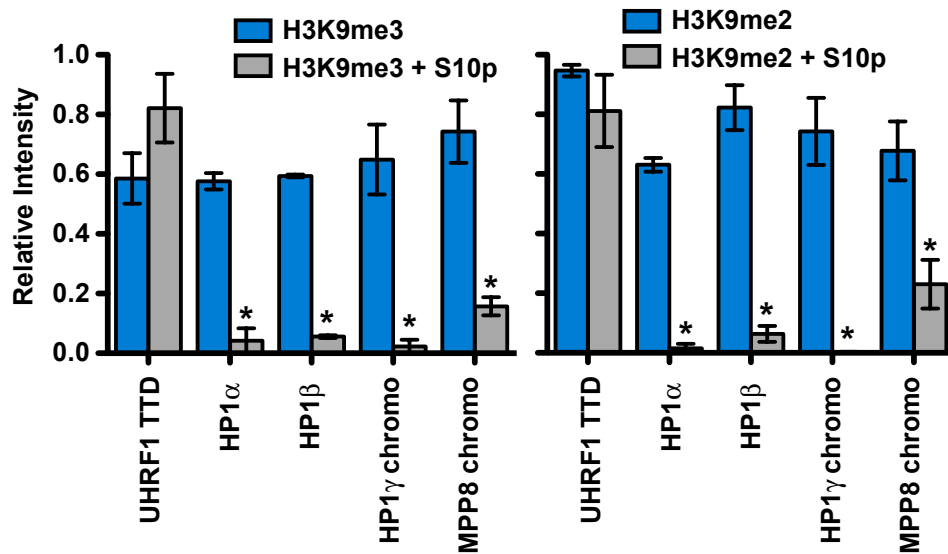
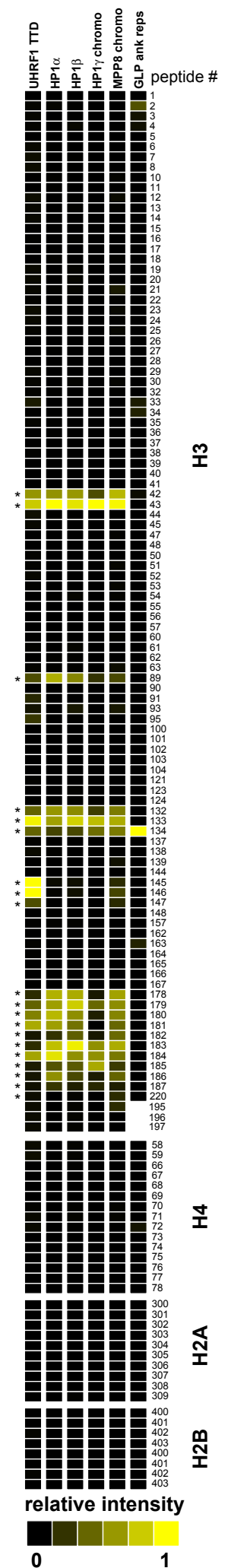
Supplementary Figure 1 – Peptide array binding profiles for H3K9 methyl effectors.

Supplementary Figure 2 – Cell synchronization, antibody characterization, and validation of UHRF1 knockdown.

Supplementary Figure 3 – Nucleotide sequence of the IGS-rDNA locus amplicon for bisulfite sequencing.

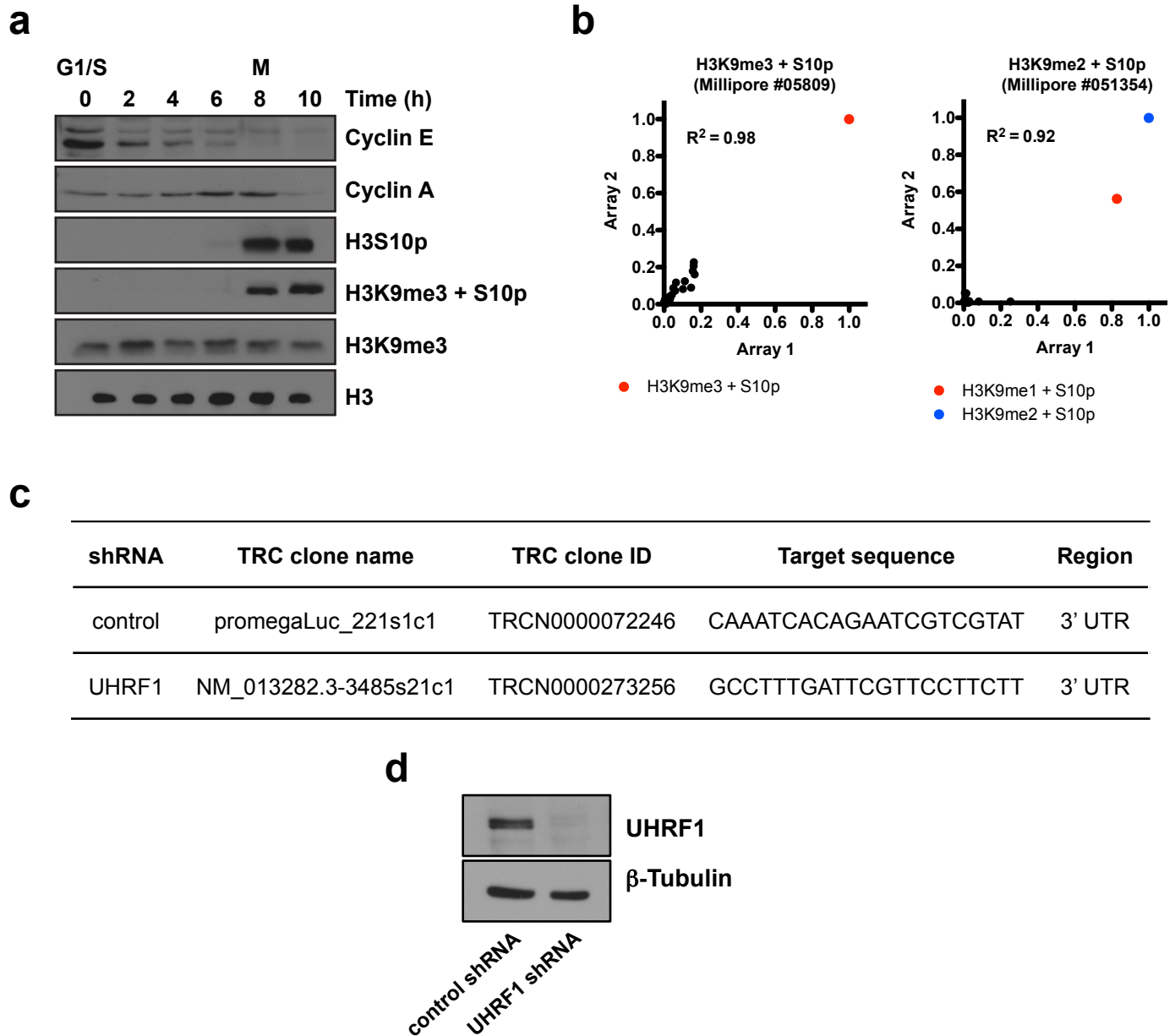
Supplementary Figure 4 – DNMT1 stability is dependent on mitotic binding of UHRF1 to H3K9 methylation and is independent of ubiquitin ligase function.

Supplementary Table 1 – List of Peptides

a**b****c**

Supplementary Figure 1: Peptide array binding profiles for H3K9 methyl effectors.

(a) Scatter plots comparing binding profiles of the indicated H3K9 methyl effector proteins or domains on two arrays. H3K9me1-containing peptides are in green, K9me2-containing peptides in blue, H3K9me3-containing peptides in red, and all other peptides on the array are in black. Correlation coefficients were determined by linear regression analysis using GraphPad Prism v5. (b) Bar graph depicting array results for H3K9me2 and me3 phospho/methyl switch sensitivity. Error is represented as \pm s.e.m. and significance within a sample group was determined by two-way ANOVA (* $p < 0.05$). (c) Full array results for H3K9 methyl effectors. Peptide numbers correspond to Supplementary Table 1. * deliniates H3K9 methylated peptides.



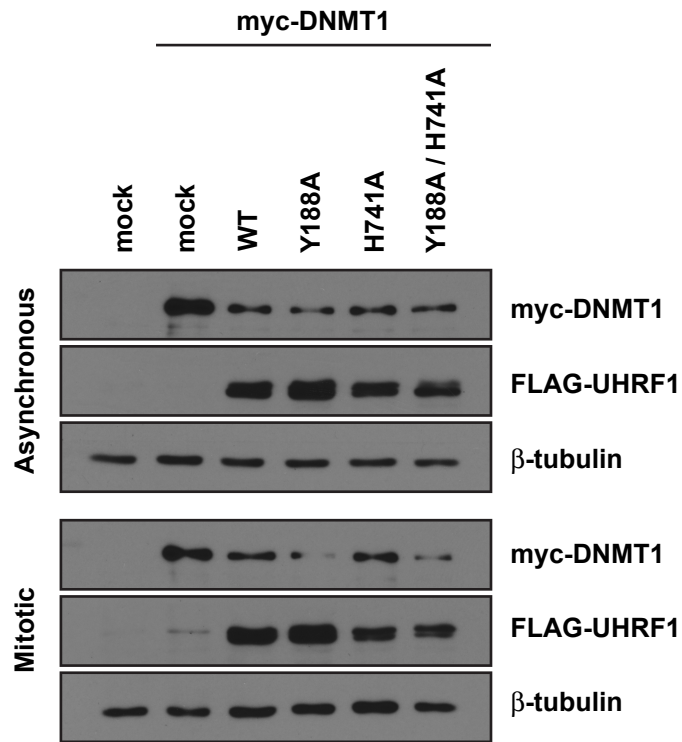
Supplementary Figure 2: Cell synchronization, antibody characterization, and validation of UHRF1 knockdown. (a) Western blot analysis of whole-cell protein extracts from HeLa cells at the indicated time points following release from double thymidine block. (b) Scatter plots comparing binding profiles of the indicated dual histone PTM antibodies on two arrays. Correlation coefficients were determined by linear regression analysis using GraphPad Prism v5. (c) shRNA clone information. (d) Western blot validating shRNA knockdown of UHRF1 in HeLa cells.

Intergenic Spacer of the rDNA locus (IGS-rDNA):

GenBank: U13369.1

225 **GAGGGGCATTTTCAGACTTTTCTCT**CGGTCA**CGTGTGGCGT**
CCGTACTTCTCCTATTTCC**CGATAAGTCTCCTCGACTTCAACATAAACTGTTAAGGC**CG
GA**CGCCAACA**CG**CGAAACCCCGTCTCTACTAAAAATACAAAGCTGAGT**CGGGAG**CGGTG**
GGCAGGCCCTGTAATGCCAGCTCCT**CGGGAGGCTGAGGCGGGAGAATCGCTTGAACCAG**
GGAAG**CGGAGGCTGCAGGGAGCGGAGATCGCG**CCACTGCACTA**CGGCCAGGCTGTAGAG**
TGAGTGAGA

Supplementary Figure 3: Nucleotide sequence of the IGS-rDNA locus amplicon for bisulfite sequencing.
CpG dinucleotides are highlighted in red, and annealing sites for sense and antisense primers are in bold.



Supplementary Figure 4: DNMT1 stability is dependent on mitotic binding of UHRF1 to H3K9 methylation and is independent of ubiquitin ligase function. Western blot on UHRF1 KD HeLa soluble protein extracts transfected with DNMT1 and the indicated UHRF1 construct for 48 hours. DMSO (asynchronous) or nocodazole (mitotic) was added as described in Methods.

Supplementary Table 1. List of Peptides

Peptide #	Sequence	annotation
1	H3 1-20 ARTKQTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3 (1-20)
2	H3 1-20 ARTKQTARKSTGGK(Ac)APRKQL-K(Biot)-NH ₂	H3K14ac
3	H3 1-20 ARTKQTARK(Ac)STGGKAPRKQL-K(Biot)-NH ₂	H3K9ac
4	H3 1-20 ARTK(Ac)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3K4ac
5	H3 1-20 ARTK(Ac)QTARKSTGGK(Ac)APRKQL-K(Biot)-NH ₂	H3K4ac + K14ac
6	H3 1-20 ARTKQTARK(Ac)STGGK(Ac)APRKQL-K(Biot)-NH ₂	H3K9ac + K14ac
7	H3 1-20 ARTK(Ac)QTARK(Ac)STGGKAPRKQL-K(Biot)-NH ₂	H3K4ac + K9ac
8	H3 1-20 ARTK(Ac)QTARK(Ac)STGGK(Ac)APRKQL-K(Biot)-NH ₂	H3K4ac + K9ac + K14ac
10	H3 1-20 ARTKQTARKSTGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K18ac
11	H3 1-20 ARTKQTARKSTGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K14ac + K18ac
12	H3 1-20 ARTKQTARK(Ac)STGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K9ac + K18ac
13	H3 1-20 ARTK(Ac)QTARKSTGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K18ac
14	H3 1-20 ARTKQTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K9ac + K14ac + K18ac
15	H3 1-20 ARTK(Ac)QTARKSTGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K14ac + K18ac
16	H3 1-20 ARTK(Ac)QTARK(Ac)STGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K9ac + K18ac
17	H3 1-20 ARTK(Ac)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K9ac + K14ac + K18ac
18	H3 1-20 ARTK(Me ₃)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3K4me3
19	H3 1-20 ARTK(Me ₃)QTARK(Ac)STGGKAPRKQL-K(Biot)-NH ₂	H3K4me3 + K9ac
20	H3 1-20 ARTK(Me ₃)QTARKSTGGK(Ac)APRKQL-K(Biot)-NH ₂	H3K4me3 + K14ac
21	H3 1-20 ARTK(Me ₃)QTARKSTGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K4me3 + K18ac
22	H3 1-20 ARTK(Me ₃)QTARK(Ac)STGGK(Ac)APRKQL-K(Biot)-NH ₂	H3K4me3 + K9ac + K14ac
23	H3 1-20 ARTK(Me ₃)QTARK(Ac)STGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K4me3 + K9ac + K18ac
24	H3 1-20 ARTK(Me ₃)QTARKSTGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4me3 + K14ac + K18ac
25	H3 1-20 ARTK(Me ₃)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4me3 + K9ac + K14ac + K18ac
26	H3 1-20 ARpTK(Me ₃)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3T3p + K4me3 + K9ac + K14ac + K18ac
27	H3 1-20 ARpTK(Me ₃)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3T3p + K4me3
28	H3 1-20 AR(Me ₂ a)pTK(Me ₃)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3R2me2a + T3p + K4me3 + K9ac + K14ac + K18ac
29	H3 1-20 AR(Me ₂ a)pTK(Me ₃)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me2a + T3p + K4me3
30	H3 1-20 AR(Me ₂ a)TK(Me ₃)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me2a + K4me3
32	H3 1-20 ARTK(Me ₂)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3K4me2
33	H3 1-20 ARTK(Me ₂)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4me2 + K9ac + K14ac + K18ac
34	H3 1-20 ARTK(Me)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3K4me1
35	H3 1-20 ARTK(Me)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4me1 + K9ac + K14ac + K18ac
36	H3 1-20 ARTKQTARKpSTGGKAPRKQL-K(Biot)-NH ₂	H3S10p
37	H3 1-20 ARTK(Ac)QTARK(Ac)pSTGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K9ac + S10p + K14ac + K18ac
38	H3 1-20 ARTK(Me ₃)QTARKpSTGGKAPRKQL-K(Biot)-NH ₂	H3K4me3 + S10p
39	H3 1-20 ARTK(Me ₃)QTARK(Ac)pSTGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4me3 + K9ac + S10p + K14ac + K18ac
40	H3 1-20 AR(Me ₂ a)TK(Me ₃)QTARKpSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me2a + K4me3 + S10p
41	H3 1-20 AR(Me ₂ a)TK(Me ₃)QTARK(Ac)pSTGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3R2me2a + K4me3 + K9ac + S10p + K14ac + K18ac
42	H3 1-20 ARTKQTARK(Me ₃)STGGKAPRKQL-K(Biot)-NH ₂	H3K9me3
43	H3 1-20 ARTK(Ac)QTARK(Me ₃)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K9me3 + K14ac + K18ac
44	H3 1-20 ARTK(Me ₂)QTARK(Ac)STGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K4me2 + K9ac + K18ac

45	H3 1-20	ARTK(Me)QTARK(Ac)STGGKAPRK(Ac)QL-K(Biot)-NH ₂	H3K4me1 + K9ac + K18ac
47	H3 1-20	AR(Me ₂ a)TKQTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me2a
48	H3 1-20	AR(Me ₂ a)TK(Ac)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3R2me2a + K4ac + K9ac + K14ac + K18ac
50	H3 1-20	AR(Me ₂ a)TK(Me ₃)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3R2me2a + K4me3 + K9ac + K14ac + K18ac
51	H3 1-20	AR(Me)TK(Me ₃)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me1 + K4me3
52	H3 1-20	AR(Me)TK(Me ₃)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3R2me1 + K4me3 + K9ac + K14ac + K18ac
53	H3 1-20	ACitTKQTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3Cit2
54	H3 1-20	ACitTK(Me ₃)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3Cit2 + K4me3
55	H3 1-20	ACitTK(Me ₃)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3Cit2 + K4me3 + K9ac + K14ac + K18ac
56	H3 1-20	ACitTK(Ac)QTARK(Ac)STGGK(Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3Cit2 + K4ac + K9ac + K14ac + K18ac
57	H3 1-20	ARpTKQTARKSTGGKAPRKQL-Peg-K(Biot)-NH ₂	H3T3p
58	H4 1-23	Ac-SGRGKGGKGLGKGGAKRHRKVLR-Peg-Biot	H4 (1-23)
59	H4 1-23	Ac-SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRKVLR-Peg-Biot	H4K5ac + K8ac + K12ac + K16ac
60	H3 1-20	AR(Me ₂ a)TK(Me ₂)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me2a + K4me2
61	H3 1-20	AR(Me ₂ s)TK(Me ₂)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me2s + K4me2
62	H3 1-20	AR(Me)TK(Me ₂)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3R2me1 + K4me2
63	H3 1-20	ACitTK(Me ₂)QTARKSTGGKAPRKQL-K(Biot)-NH ₂	H3Cit2 + K4me2
66	H4 1-23	Ac-SGRGK(Ac)GGKGLGKGGAKRHRKVLR-Peg-Biot	H4K5ac
67	H4 1-23	Ac-SGRGKGGK(Ac)GLGKGGAKRHRKVLR-Peg-Biot	H4K8ac
68	H4 1-23	Ac-SGRGKGGKGLGK(Ac)GGAKRHRKVLR-Peg-Biot	H4K12ac
69	H4 1-23	Ac-SGRGKGGKGLGKGGAK(Ac)RHRKVLR-Peg-Biot	H4K16ac
70	H4 1-23	Ac-SGRGK(Ac)GGKGLGK(Ac)GGAKRHRKVLR-Peg-Biot	H4K5ac + K12ac
71	H4 1-23	Ac-SGRGKGGK(Ac)GLGKGGAK(Ac)RHRKVLR-Peg-Biot	H4K8ac + K16ac
72	H4 1-23	Ac-SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAKRHRKVLR-Peg-Biot	H4K5ac + K8ac + K12ac
73	H4 1-23	Ac-SGR(Me ₂ a)GKGGKGLGKGGAKRHRKVLR-K(Biot)-NH ₂	H4R3me2a
74	H4 1-23	Ac-SGR(Me ₂ s)GKGGKGLGKGGAKRHRKVLR-K(Biot)-NH ₂	H4R3me2s
75	H4 1-23	Ac-SGR(Me)GKGGKGLGKGGAKRHRKVLR-K(Biot)-NH ₂	H4R3me1
76	H4 1-23	Ac-pSGR(Me ₂ a)GKGGKGLGKGGAKRHRKVLR-K(Biot)-NH ₂	H4S1p + R3me2a
77	H4 1-23	Ac-pSGR(Me ₂ s)GKGGKGLGKGGAKRHRKVLR-K(Biot)-NH ₂	H4S1p + R3me2s
78	H4 1-23	Ac-pSGR(Me)GKGGKGLGKGGAKRHRKVLR-K(Biot)-NH ₂	H4S1p + R3me1
89	H3 1-20	ARTK(Me ₃)QTAR(Me ₂ s)K(Me ₃)STGGKAPRKQL-K(Biot)-NH ₂	H3K4me3 + R8me2s + K9me3
90	H3 15-43	Ac-APRKQLATKAARKSAPSTGGVKKPHRYGGK(Biot)-NH ₂	H3 (15-41)
91	H3 15-43	Ac-APRK(Me ₃)QLATKAARKSAPSTGGVKKPHRY-GG-K(Biot)-NH ₂	H3K18me3
93	H3 15-43	Ac-APRKQLATKAARKSAPSTGGVK(Me ₃)KPHRY-GG-K(Biot)-NH ₂	H3K36me3
95	H3 15-43	Ac-APRK(Me ₃)QLATKAARKSAPSTGGVK(Me ₃)KPHRY-GG-K(Biot)-NH ₂	H3K18me3 + K36me3
100	H3 74-84	Ac-IAQDFKTDLRF-Peg-K(Biot)-NH ₂	H3 (74-84) N-ac
101	H3 74-84	Ac-IAQDFK(Me ₃)TDLRF-Peg-K(Biot)-NH ₂	H3K79me3
102	H3 74-84	Ac-IAQDFK(Me ₂)TDLRF-Peg-K(Biot)-NH ₂	H3K79me2
103	H3 74-84	Ac-IAQDFK(Me)TDLRF-Peg-K(Biot)-NH ₂	H3K79me1
104	H3 74-84	IAQDFKTDLRF-Peg-K(Biot)-NH ₂	H3 (74-84)
121	H3 27-45	KSAPSTGGVK(Me ₂)KPHRYKPGT-GG-K(Biot)-NH ₂	H3K36me2
123	H3 27-45	KSAPSTGGVK(Ac)KPHRYKPGT-GG-K(Biot)-NH ₂	H3K36ac
124	H3 27-45	KSAPSTGGVKKPHRYKPGT-GG-K(Biot)-NH ₂	H3 (27-45)
132	H3 1-20	ARTK(Me ₃)QTARK(Me ₃)STGGKAPRKQL-K(Biot)-NH ₂	H3K4me3 + K9me3
133	H3 1-20	ARTKQTARK(Me ₂)STGGKAPRKQL-K(Biot)-NH ₂	H3K9me2
134	H3 1-20	ARTKQTARK(Me)STGGKAPRKQL-K(Biot)-NH ₂	H3K9me1
137	H3 1-20	ARTKQTARKSTGGKAPRK(Me ₃)QL-K(Biot)-NH ₂	H3K18me3
138	H3 1-20	ARTKQTARKSTGGKAPRK(Me ₂)QL-K(Biot)-NH ₂	H3K18me2

139	H3 1-20	ARTKQTARK STGGK APRK(Me)QL-K(Biot)-NH ₂	H3K18me1
144	H3 1-20	ARTKQTARK(Ac)p STGGK APRKQL-K(Biot)-NH ₂	H3K9ac + S10p
145	H3 1-20	ARTKQTARK(Me₃)p STGGK APRKQL-K(Biot)-NH ₂	H3K9me3 + S10p
146	H3 1-20	ARTKQTARK(Me₂)p STGGK APRKQL-K(Biot)-NH ₂	H3K9me2 + S10p
147	H3 1-20	ARTKQTARK(Me)p STGGK APRKQL-K(Biot)-NH ₂	H3K9me1 + S10p
148	H3 1-20	ARTK(Me₃)QTARK(Ac)p STGGK APRKQL-K(Biot)-NH ₂	H3K4me3 + K9ac + S10p
157	H3 1-20	AR(Me₂s)TK(Me₃)QTARK STGGK APRKQL-K(Biot)-NH ₂	H3R2me2s + K4me3
162	H3 1-20	ARTKQpTARK STGGK APRKQL-K(Biot)-NH ₂	H3T6p
163	H3 1-20	ARTK(Me₃)QpTARK STGGK APRKQL-K(Biot)-NH ₂	H3K4me3 + T6p
164	H3 1-20	ARTK(Me₂)QpTARK STGGK APRKQL-K(Biot)-NH ₂	H3K4me2 + T6p
165	H3 1-20	ARTKQpTARK(Ac) STGGK (Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3T6p + K9ac + K14ac + K18ac
166	H3 1-20	ARTK(Me₃)QpTARK(Ac) STGGK (Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4me3 + T6p + K9ac + K14ac + K18ac
167	H3 1-20	ARTK(Me₂)QpTARK(Ac) STGGK (Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4me2 + T6p + K9ac + K14ac + K18ac
178	H3 1-20	ARTKQTAR(Me)K(Me₃) STGGK APRKQL-K(Biot)-NH ₂	H3R8me1 + K9me3
179	H3 1-20	ARTKQTAR(Me)K(Me₂) STGGK APRKQL-K(Biot)-NH ₂	H3R8me1 + K9me2
180	H3 1-20	ARTKQTAR(Me₂a)K(Me₃) STGGK APRKQL-K(Biot)-NH ₂	H3R8me2a + K9me3
181	H3 1-20	ARTKQTAR(Me₂a)K(Me₂) STGGK APRKQL-K(Biot)-NH ₂	H3R8me2a + K9me2
182	H3 1-20	ARTKQTAR(Me₂a)K(Me) STGGK APRKQL-K(Biot)-NH ₂	H3R8me2a + K9me1
183	H3 1-20	ARTKQTAR(Me₂s)K(Me₃) STGGK APRKQL-K(Biot)-NH ₂	H3R8me2s + K9me3
184	H3 1-20	ARTKQTAR(Me₂s)K(Me₂) STGGK APRKQL-K(Biot)-NH ₂	H3R8me2s + K9me2
185	H3 1-20	ARTKQTAR(Me₂s)K(Me) STGGK APRKQL-K(Biot)-NH ₂	H3R8me2s + K9me1
186	H3 1-20	ARTK(Ac)QTARK(Me₂) STGGK (Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K9me2 + K14ac + K18ac
187	H3 1-20	ARTK(Ac)QTARK(Me) STGGK (Ac)APRK(Ac)QL-K(Biot)-NH ₂	H3K4ac + K9me1 + K14ac + K18ac
195	H3 15-34	Ac-APRKQLATKAARK(Me₃)SAPSTGG-Peg-Biot	H3K27me3
196	H3 15-34	Ac-APRKQLATKAARK(Me₂)SAPSTGG-Peg-Biot	H3K27me2
197	H3 15-34	Ac-APRKQLATKAARK(Me)SAPSTGG-Peg-Biot	H3K27me1
220	H3 1-20	ARTKQpTARK(Me₃) STGGK APRKQL-K(Biot)-NH ₂	H3T6p + K9me3
300	H2A 1-17	Ac-SGRGKQGGKARAKAKTR-Peg-Biot	H2A (1-17)
301	H2A 1-17	Ac-SGRGK(Ac)QGGK(Ac)ARAK(Ac)AK(Ac)TR-Peg-Biot	H2AK5ac + K9ac + K13ac + K15ac
302	H2A 1-17	Ac-SGRGK(Ac)QGGKARAKAKTR-Peg-Biot	H2AK5ac
303	H2A 1-17	Ac-pSGRGK(Ac)QGGKARAKAKTR-Peg-Biot	H2AS1p + K5ac
304	H2A 1-17	Ac-SGR(Me₂a)GK(Ac)QGGKARAKAKTR-Peg-Biot	H2AR3me2a + K5ac
305	H2A 1-17	Ac-pSGR(Me₂a)GK(Ac)QGGKARAKAKTR-Peg-Biot	H2AS1p + R3me2a + K5ac
306	H2A 1-17	Ac-SGCitGK(Ac)QGGKARAKAKTR-Peg-Biot	H2ACit3 + K5ac
307	H2A 1-17	Ac-pSGCitGK(Ac)QGGKARAKAKTR-Peg-Biot	H2AS1p + Cit3 + K5ac
308	H2A 1-17	Ac-pSGRGK(Ac)QGGK(Ac)ARAK(Ac)AK(Ac)TR-Peg-Biot	H2AS1p + K5ac + K9ac + K13ac + K15ac
309	H2A 1-17	SGRGK(Ac)QGGK(Ac)ARAK(Ac)AK(Ac)TR-Peg-Biot	H2AK5ac + K9ac + K13ac + K15ac (no N-ac)
400	H2B 1-24	PEPAKSAPAPKKGSKKAVTKAQKK-Peg-Biot	H2B (1-24)
401	H2B 1-24	PEPAK(Me₃)SAPAPKKGSKKAVTKAQKK-Peg-Biot	H2BK5me3
402	H2B 1-24	PEPAK(Me₂)SAPAPKKGSKKAVTKAQKK-Peg-Biot	H2BK5me2
403	H2B 1-24	PEPAK(Me)SAPAPKKGSKKAVTKAQKK-Peg-Biot	H2BK5me1