

Supporting Information

Crosstalk of homocysteinylation, methylation and acetylation on histone H3

Leilei Xu^{a,b,1}, Jiajia Chen^{a,b,1}, Jun Gao^a, Hongxiu Yu^{a,b*}, Pengyuan Yang^{a,b*}

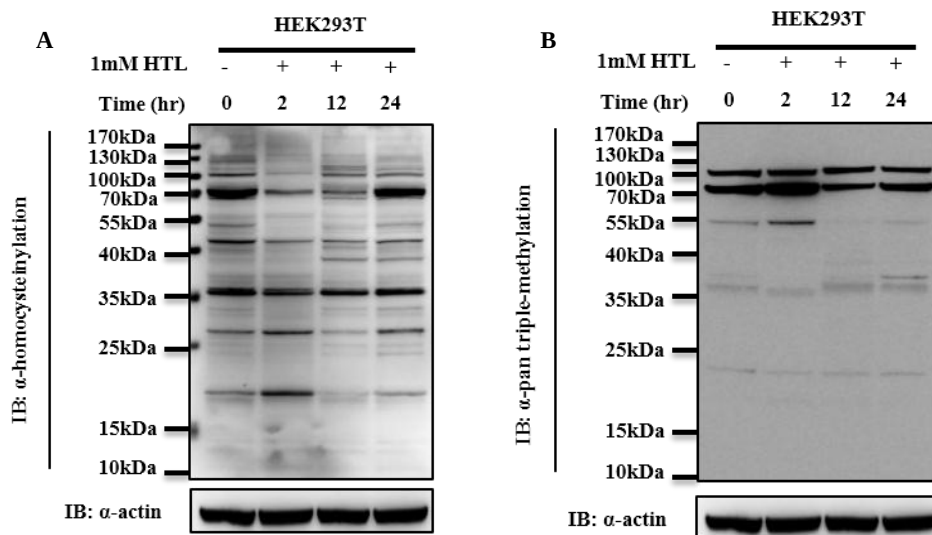


Fig. S1. Excess HTL promoted protein homocysteinylation in HEK293T cells.

HEK293T cells were cultured with or without 1mM HTL. (A) Homocysteinylation was increased by extending treated time. (B) Tri-methylation has been affected by excess HTL.

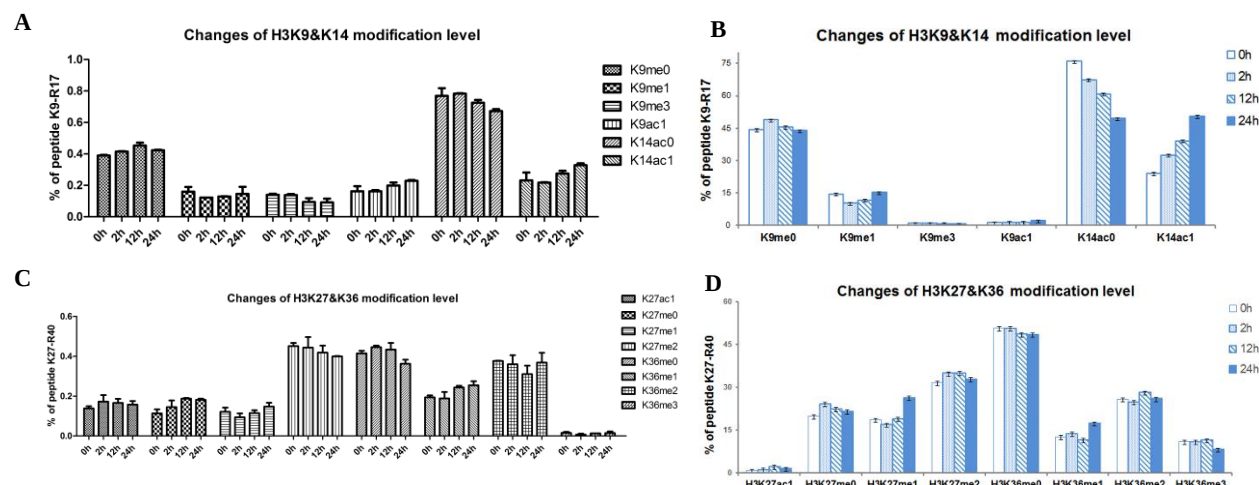


Fig. S2. Quantification results of multiple types of modifications on histone H3. (A)(B)(C)(D) Relative (gray) and absolute (color) quantification of multiple modifications of peptide K9-R17. (C)(D) Relative (gray) and absolute (color) quantification of multiple modifications of peptide K27-R40.

Table S1. List of histone H3 peptides and their post-translational modifications

Peptide sequence	Modification	[M+H]⁺
Peptide(3-8)	K ₄ (Me ₀)	816.45674
T ₃ KQTAR ₈	K ₄ (Me ₁)	830.4724
Peptide(9-17)	K ₉ (Me ₀)K ₁₄ (Ac ₀)	1069.59938
K ₉ STGGKAPR ₁₇	K ₉ (Me ₀)K ₁₄ (Ac ₁)	1055.58372
	K ₉ (Me ₁)K ₁₄ (Ac ₀)	1083.61504
	K ₉ (Me ₁)K ₁₄ (Ac ₁)	1069.59938
	K ₉ (Me ₂)K ₁₄ (Ac ₁)	1027.58882
	K ₉ (Me ₃)K ₁₄ (Ac ₀)	1055.62008
	K ₉ (Ac ₁)K ₁₄ (Ac ₀)	1055.5844
	K ₉ (Ac ₁)K ₁₄ (Ac ₁)	1041.56808
Peptide(18-26)	K ₁₈ (Me ₀)K ₂₃ (Me ₀)	1154.6892
K ₁₈ QLATKAAR ₂₆	K ₁₈ (Ac ₀)K ₂₃ (Ac ₁)	1140.6735
	K ₁₈ (Ac ₁)K ₂₃ (Ac ₁)	1126.6579
H3.1 Peptide(27-40)	K ₂₇ (Me ₀)K ₃₆ (Me ₀)	1657.93766
K ₂₇ SAPATGGVKKPHR ₄₀	K ₂₇ (Me ₀)K ₃₆ (Me ₁)	1671.95332
	K ₂₇ (Me ₁)K ₃₆ (Me ₀)	1671.95332
	K ₂₇ (Me ₀)K ₃₆ (Me ₂)	1629.94274
	K ₂₇ (Me ₂)K ₃₆ (Me ₀)	1629.94274
	K ₂₇ (Me ₁)K ₃₆ (Me ₂)	1643.9584
	K ₂₇ (Me ₂)K ₃₆ (Me ₁)	1643.9584
	K ₂₇ (Me ₃)K ₃₆ (Me ₀)	1643.9584
	K ₂₇ (Ac ₁)K ₃₆ (Me ₀)	1643.9288
	K ₂₇ (Me ₁)K ₃₆ (Me ₁)	1685.96898
	K ₂₇ (Me ₁)K ₃₆ (Me ₃)	1657.97358
	K ₂₇ (Me ₃)K ₃₆ (Me ₁)	1657.97358
	K ₂₇ (Ac ₁)K ₃₆ (Me ₁)	1657.9384
	K ₂₇ (Me ₂)K ₃₆ (Me ₂)	1601.94786
	K ₂₇ (Me ₃)K ₃₆ (Me ₂)	1615.9642
	K ₂₇ (Ac ₁)K ₃₆ (Me ₂)	1615.9275
Peptide(54-63)	K ₅₆ (Ac ₀)	1362.76274
Y ₅₄ QKSTELLIR ₆₃	K ₅₆ (Ac ₁)	1348.74709
Peptide(73-83)	K ₇₉ (Me ₀)	1447.74132
E ₇₃ IAQDFKTDLR ₈₃	K ₇₉ (Me ₁)	1461.75702
	K ₇₉ (Me ₂)	1419.74652
	K ₇₉ (ac ₁)	1433.7271

Table S2. MRM parameters of the 35 endogenous and isotope labeled histone PTMs peptides

Peptide sequence	Modification	Q1(endogenous)	Q3(endogenous)	Q1 (heavy)	Q3 (heavy)	DP(V)	CE(eV)	fragment ions	retention time (min)
Peptide(3-8) T ₃ KQTAR ₈	K ₄ (Me ₀)	408.7	475.5	414.2	485.5	100	22	y4+	14.4
		408.7	659.4	414.2	669.4	100	20	y5+	14.4
	K ₄ (Me ₁)	415.8	475.5	421.2	485.5	90	23	y4+	18.5
		415.8	673.4	421.2	683.4	90	23	y5+	18.5
Peptide(9-17) K ₉ STGGKAPR ₁₇	K ₉ (Me ₀)K ₁₄ (Ac ₀)	535.5	241.2	540.5	241.2	60	21	b1+	21.2
		535.5	829.6	540.5	839.5	60	21	y8+	21.2
	K ₉ (Me ₀)K ₁₄ (Ac ₁)	528.4	241	533.4	241	60	22	b1+	19.3
		528.4	815.5	533.4	825.5	60	22	y8+	19.3
	K ₉ (Me ₁)K ₁₄ (Ac ₀)	542.3	255.3	547.3	255.3	85	22	b1+	25.0
		542.3	829.5	547.3	839.5	85	24	y8+	25.0
	K ₉ (Me ₁)K ₁₄ (Ac ₁)	535.5	255.3	540.5	255.3	85	21	b1+	23.2
		535.5	815.7	540.5	825.5	85	21	y8+	23.2
	K ₉ (Me ₂)K ₁₄ (Ac ₁)	514.4	213.2	519.8	213.2	110	38	b1+	12.8
		514.4	815.4	519.8	815.4	100	35	y8+	12.8
	K ₉ (Me ₂)K ₁₄ (Ac ₀)	528.4	546.5	533.4	546.5	85	45	c5+	13.9
		528.4	140	533.4	264.2	85	40	y2-water	13.9
	K ₉ (Ac ₁)K ₁₄ (Ac ₀)	528.4	227	533.4	227	110	22	b1+	19.0
		528.4	829.5	533.4	839.5	110	25	y8+	19.0
	K ₉ (Ac ₁)K ₁₄ (Ac ₁)	521.5	815.5	526.5	825.5	70	22	y8+	16.9
		521.5	627.2	526.5	637.2	70	26	y6+	16.9
Peptide(18-26) K ₁₈ QLATKAAR ₂₆	K18(Me ₀)K23(Me ₀)	577.8	673.4	582.8	683.4	80	22	y6+	22.2
		577.8	241.2	582.8	241.2	80	22	b1+	22.2
	K18(Ac ₀)K23(Ac ₁)	570.8	687.4	575.8	697.4	90	19	y6+	20.5
		570.8	241.2	575.8	241.2	80	19	b1+	20.5
	K18(Ac ₁)K23(Ac ₁)	563.8	659.4	568.8	669.4	90	24	y6+	17.8
		563.8	227.1	568.8	227.1	90	24	b1+	17.8
H3.1 Peptide(27-40) K ₂₇ SAPATGGVKKPHR ₄₀	K ₂₇ (Me ₀)K ₃₆ (Me ₀)	553.5	630.9	557	635.9	80	18	y11++	26.4
		830	709.8	835	714.8	150	25	y13++	26.4
	K ₂₇ (Me ₀)K ₃₆ (Me ₁)	558.2	716.8	561.7	721.8	100	17	y13++	27.5
		558.2	637.8	561.7	642.8	100	24	y11++	27.5
	K ₂₇ (Me ₁)K ₃₆ (Me ₀)	558.2	709.8	561.7	714.8	80	18	y13++	27.9
		836.5	227.1	841.5	227.1	150	45	a1+	27.9
	K ₂₇ (Me ₀)K ₃₆ (Me ₂)	544.1	695.8	547.4	700.8	100	27	y13++	23.6
		544.1	616.8	547.4	621.8	100	27	y11++	23.6
	K ₂₇ (Me ₂)K ₃₆ (Me ₀)	544.1	371.3	547.5	371.3	80	37	b3+	21.9
		544.1	409.1	547.5	419.1	80	30	y3+	21.9
	K ₂₇ (Me ₁)K ₃₆ (Me ₂)	548.8	616.7	552	621.7	80	30	y11++	26.0
		548.8	695.5	552	700.5	80	28	y13++	26.0
	K ₂₇ (Me ₂)K ₃₆ (Me ₁)	548.8	371.3	552	371.3	80	32	b3+	23.8
		548.8	409.2	552	419.2	80	32	y3+	23.8
	K ₂₇ (Me ₃)K ₃₆ (Me ₀)	548.8	385.2	552	385.2	80	42	b3+	21.8
		548.8	777.6	552	787.6	80	30	y5+	21.8
	K ₂₇ (Ac ₁)K ₃₆ (Me ₀)	548.8	630.9	552	635.9	80	24	y11++	25.4
		548.8	709.8	552	714.8	80	24	y13++	25.4
	K ₂₇ (Me ₁)K ₃₆ (Me ₁)	562.8	716.7	566.2	721.7	80	24	y13++	28.8
		843.5	791.5	848.5	801.5	150	45	y5+	28.8
	K ₂₇ (Me ₁)K ₃₆ (Me ₃)	553.5	702.8	557	707.8	80	30	y13++	25.9
		553.5	255.2	557	255.2	80	45	b1+	25.9
	K ₂₇ (Me ₃)K ₃₆ (Me ₁)	553.5	385.3	557	285.3	80	42	b3+	23.8
		553.5	768.4	557	768.4	80	40	b8+	23.8
	K ₂₇ (Ac ₁)K ₃₆ (Me ₁)	553.5	637.9	557	642.8	80	23	y11++	26.7
		553.5	716.7	557	721.7	80	23	y13++	26.7
	K ₂₇ (Me ₂)K ₃₆ (Me ₂)	535	371.3	538	371.3	80	40	b3+	18.3
		535	616.6	538	621.4	80	35	y11++	18.3
	K ₂₇ (Me ₃)K ₃₆ (Me ₂)	405	385.5	407.5	385.5	80	25	b3+	18.3
		539.5	175	543	185	170	37	y1+	18.3
	K ₂₇ (Ac ₁)K ₃₆ (Me ₂)	539.5	695.8	543	700.9	60	25	y13++	22.2
		539.5	616.8	543	621.8	60	27	y11++	22.2
Peptide(73-83) E ₇₃ IAQDFKTDLR ₈₃	K ₇₉ (Me ₀)	724.8	288.1	730	298.3	160	33	y2+	32.4
		724.8	835.5	730	845.5	160	31	y6+	32.4
	K ₇₉ (Me ₁)	731.8	288.1	737	298.3	160	33	y2+	32.4
		731.8	702.8	737	712.8	160	31	y5+	32.4
	K ₇₉ (Me ₂)	710.2	288.1	715.5	298.1	80	34	y2+	32.2
		710.2	175.1	715.5	185.1	80	30	y1+	32.2