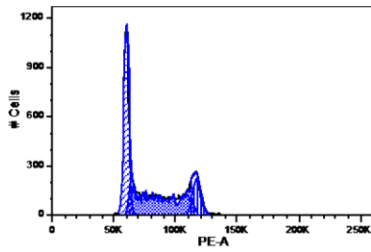
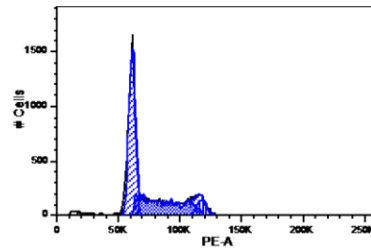


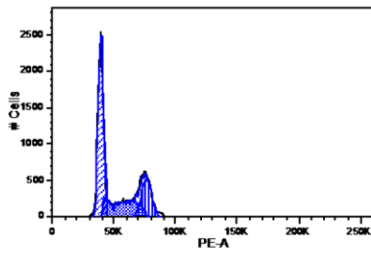
## SUPPLEMENTARY FIGURES



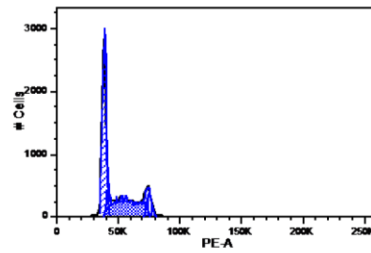
Watson Pragmatic: %G1 = 40.9; %S = 45.5; %G2 = 14.3.  
Cell Cycle  
MCF7\_60%\_001.fcs  
Event Count: 14145



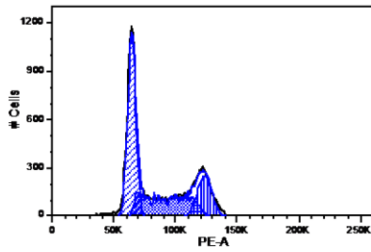
Watson Pragmatic: %G1 = 51.6; %S = 36.1; %G2 = 8.29.  
Cell Cycle  
MCF7\_100%\_002.fcs  
Event Count: 18637



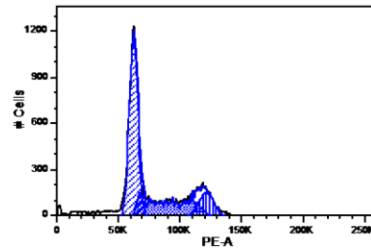
Watson Pragmatic: %G1 = 49.7; %S = 27.7; %G2 = 21.9.  
Cell Cycle  
MCF10\_60%\_003.fcs  
Event Count: 25998



Watson Pragmatic: %G1 = 46.9; %S = 41.2; %G2 = 9.65.  
Cell Cycle  
MCF10\_100%\_004.fcs  
Event Count: 23090

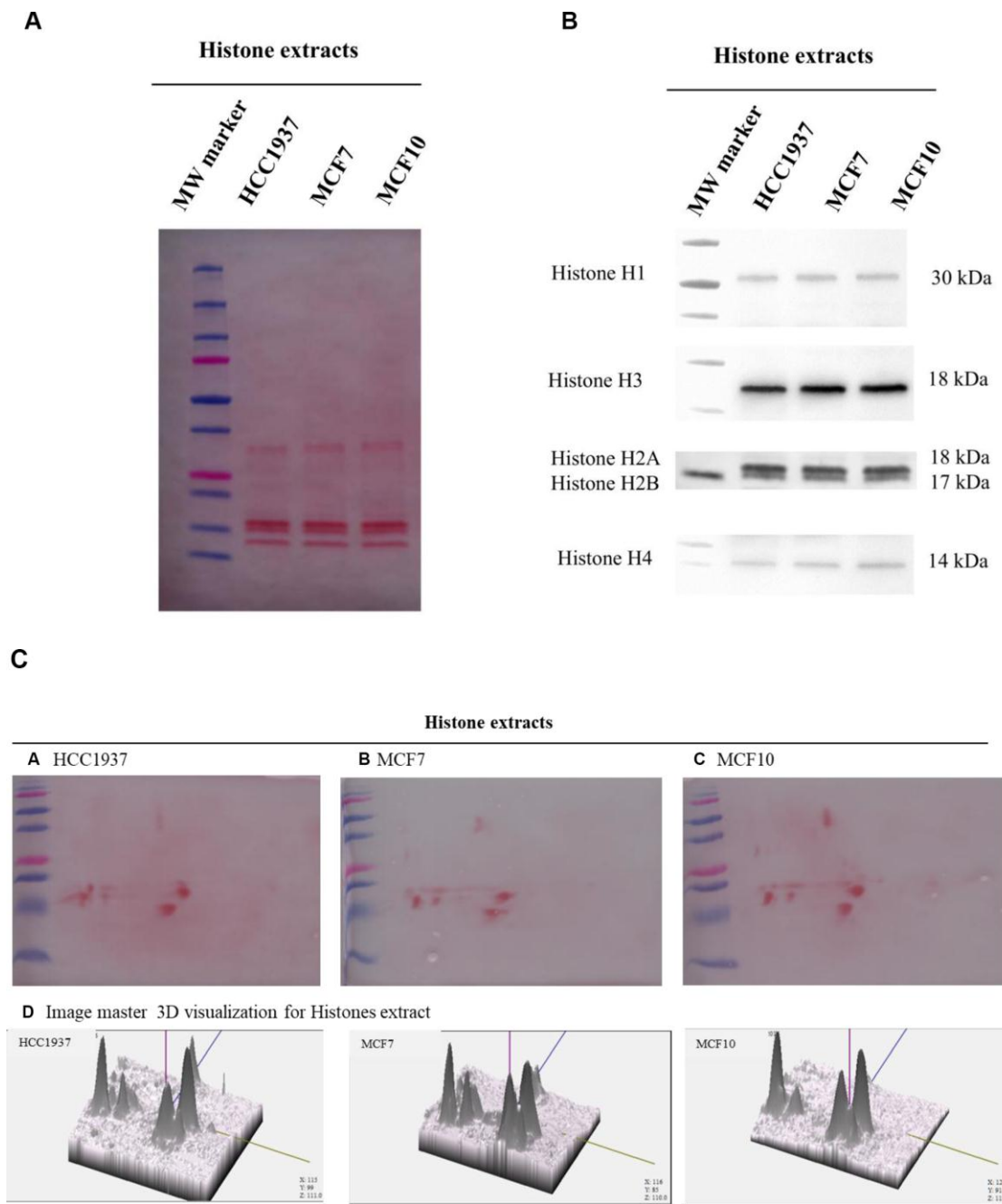


Watson Pragmatic: %G1 = 46.4; %S = 31.9; %G2 = 19.5.  
Cell Cycle  
HCC1937\_60%\_005.fcs  
Event Count: 19561



Watson Pragmatic: %G1 = 53; %S = 28.4; %G2 = 12.6.  
Cell Cycle  
HCC1937\_100%\_006.fcs  
Event Count: 19004

**Supplementary Figure 1. Cell cycle analysis.**  $1 \times 10^6$  cell, were fixed by resuspension in 500  $\mu$ l of cold 70% ethanol under continuous gentle vortexing and incubated at 4°C 30 minutes or kept overnight at -20°C. Cells were then recovered by centrifugation, washed twice in PBS and incubated for 1h at room temperature in 1 ml PBS containing propidium iodide (20  $\mu$ g/ml), NP40 0.1% and ribonuclease (40  $\mu$ /ml). Samples were analysed by a BD™ LSRFortessa™ X-20 Flow Cytometer with 488-nm excitation and a 610/20nm bandpass emission filter. FlowJo™ software was used for data analysis.



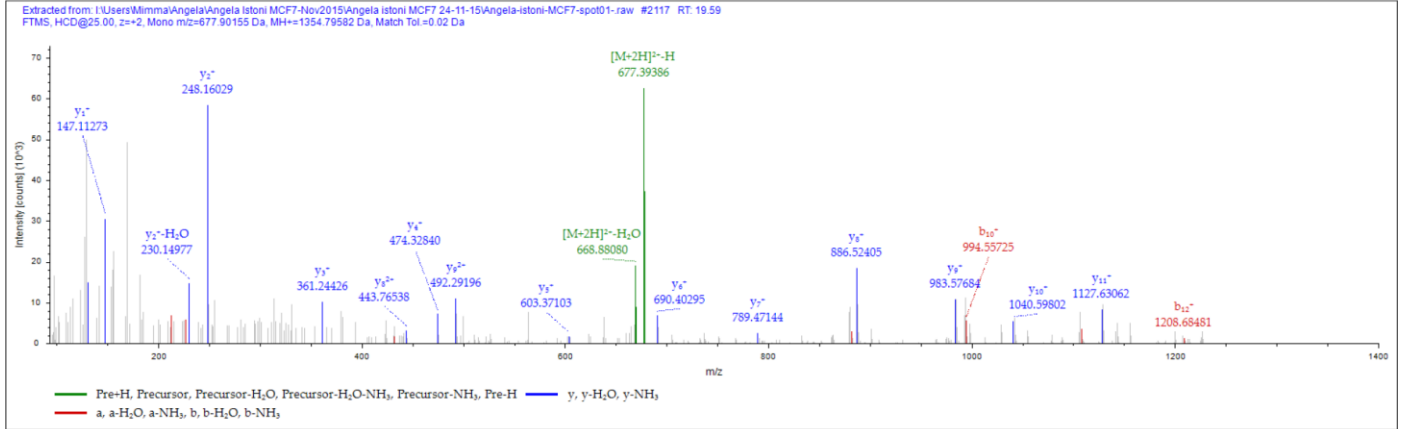
**Supplementary Figure 2. Western blot detection of Histone proteins.** (A) Red ponceau Staining of membrane. Histones extracts (5 micrograms) were resolved using 15% SDS-PAGE and electrotransferred to a nitrocellulose membrane with a Trans-blot turbo system (Biorad). Membranes were stained using red ponceau solution (P7170 Sigma-Aldrich). Nitrocellulose membrane was destained using PBS 1X and used for immunologically detection. (B) Immunoblotting. Membranes were incubated using the following primary antibodies: Histone H1 (1:1000; Abcam ab71594), Histone H2B (D2H6) (1:1000; Cell signaling 112364), Histone H2A (1:1000; Cell signaling 2578), Histone H3 Antibody (1:1000; Cell signaling 9715), Histone H4 (1:1000; Cell signaling 2592). (C) 2D Western Blot. Red ponceau Staining of membranes and image master analysis. For 2D Western blot analysis, equal amounts of histone extracts were resolved by 2D TAU/SDS gel. To minimize gel to gel variation and ensure a reliable comparison among analyzed samples second dimension for each sample was run on Mini-PROTEAN® TGX™ Precast Gels, IPG Well (15%). Resulting gel were transferred to nitrocellulose membranes with a Trans-blot turbo system (Biorad) using Trans-Blot® Turbo™ Mini Nitrocellulose Transfer Packs. To ensure equal protein loading, membranes were incubated with red ponceau solution (P7170 Sigma-Aldrich). Images were acquired using Image scanner II (GE Healthcare). Membrane image were visualized using image master 2D platinum. 3D visualization tool testify the loading of equal amount for each Histone extract (panel D).

Sequence: KASGPPVSELITK, K1-Dimethyl (28.03130 Da)

Charge: +2, Monoisotopic m/z: 677.90155 Da (+0.86 mmu/+1.26 ppm), MH<sup>+</sup>: 1354.79582 Da, RT: 19.59 min,

Identified with: Sequest HT (v1.3); XCorr:3.93, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



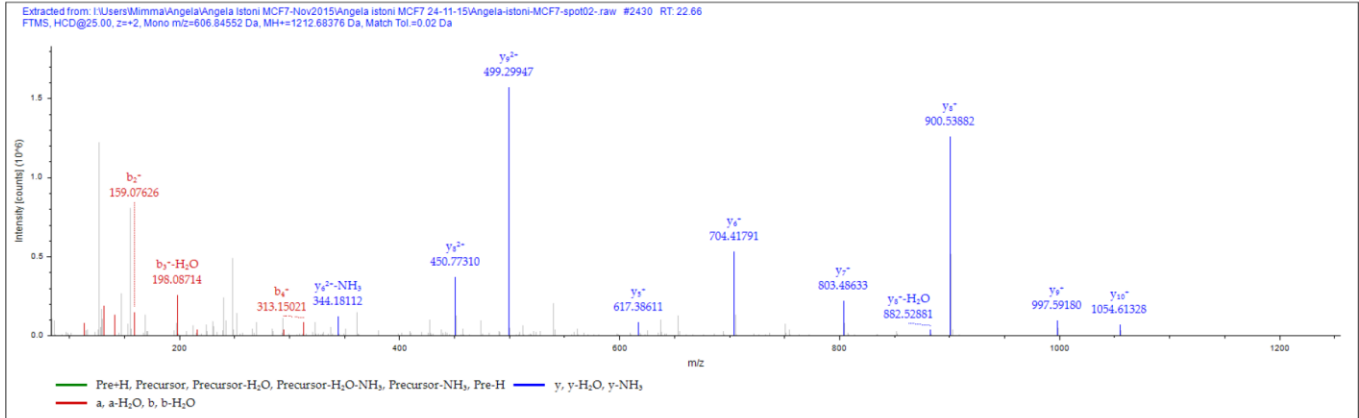
#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	12.913.863	6.507.295	15.713.355	7.907.041	K-Dimethyl			13
2	20.017.575	10.059.151	22.817.067	11.458.897	A	119.866.784	59.983.756	12
3	28.720.778	14.410.753	31.520.270	15.810.499	S	112.763.072	56.431.900	11
4	34.422.925	17.261.826	37.222.417	18.661.572	G	104.059.869	52.080.298	10
5	44.128.202	22.114.465	46.927.694	23.514.211	P	98.357.722	49.229.225	9
6	53.833.479	26.967.103	56.632.971	28.366.849	P	88.652.445	44.376.586	8
7	63.740.321	31.920.524	66.539.813	33.320.270	V	78.947.168	39.523.948	7
8	72.443.524	36.272.126	75.243.016	37.671.872	S	69.040.326	34.570.527	6
9	85.347.784	42.724.256	88.147.276	44.124.002	E	60.337.123	30.218.925	5
10	96.656.191	48.378.459	99.455.683	49.778.205	L	47.432.863	23.766.795	4
11	107.964.598	54.032.663	110.764.090	55.432.409	I	36.124.456	18.112.592	3
12	118.069.366	59.085.047	120.868.858	60.484.793	T	24.816.049	12.458.388	2
13					K	14.711.281	7.406.004	1

Sequence: ASGPPVSELITK, K12-Methyl (14.01565 Da)

Charge: +2, Monoisotopic m/z: 606.84552 Da (+0.14 mmu/+0.22 ppm), MH<sup>+</sup>: 1212.68376 Da, RT: 22.66 min,

Identified with: Sequest HT (v1.3); XCorr:2.25, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	4.404.948	2.252.838	7.204.440	3.652.584	A			12
2	13.108.151	6.604.439	15.907.643	8.004.185	S	114.164.637	57.132.682	11
3	18.810.298	9.455.513	21.609.790	10.855.259	G	105.461.434	52.781.081	10
4	28.515.575	14.308.151	31.315.067	15.707.897	P	99.759.287	49.930.007	9
5	38.220.852	19.160.790	41.020.344	20.560.536	P	90.054.010	45.077.369	8
6	48.127.694	24.114.211	50.927.186	25.513.957	V	80.348.733	40.224.730	7
7	56.830.897	28.465.812	59.630.389	29.865.558	S	70.441.891	35.271.309	6
8	69.735.157	34.917.942	72.534.649	36.317.688	E	61.738.688	30.919.708	5
9	81.043.564	40.572.146	83.843.056	41.971.892	L	48.834.428	24.467.578	4
10	92.351.971	46.226.349	95.151.463	47.626.095	I	37.526.021	18.813.374	3
11	102.456.739	51.278.733	105.256.231	52.678.479	T	26.217.614	13.159.171	2
12					K-Methyl	16.112.846	8.106.787	1

**ALAAAGYDVEK<sub>Methyl</sub>**

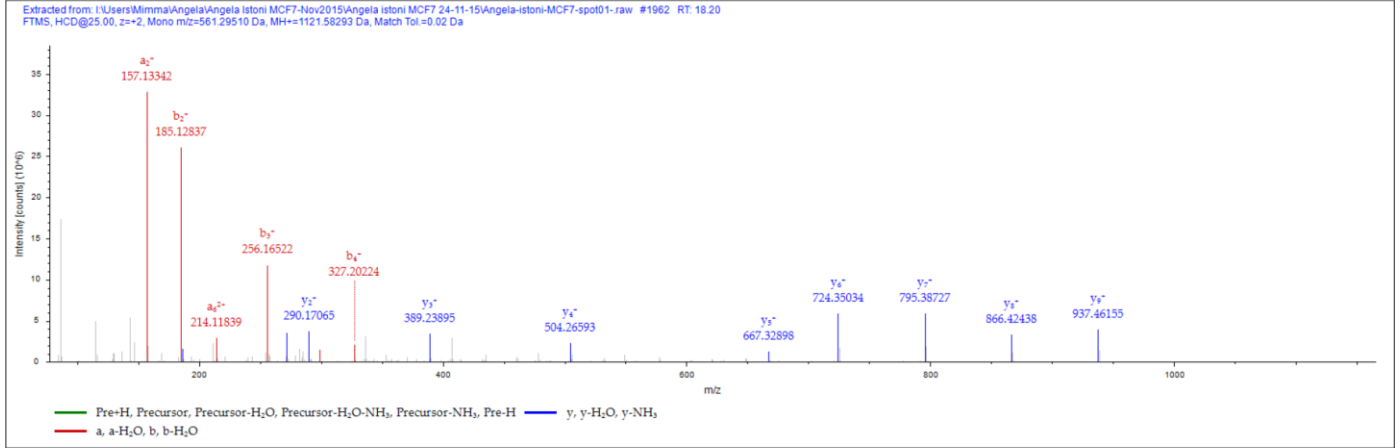
**H1.2K74 – H1.3K75**

Sequence: ALAAAGYDVEK, K11-Methyl (14.01565 Da)

Charge: +2, Monoisotopic m/z: 561.29510 Da (-0.42 mmu/-0.75 ppm), MH+: 1121.58293 Da, RT: 18.20 min,

Identified with: Sequest HT (v1.3); XCorr:3.13, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	4.404.948	2.252.838	7.204.440	3.652.584	A			11
2	15.713.355	7.907.041	18.512.847	9.306.787	L	105.054.665	52.577.696	10
3	22.817.067	11.458.897	25.616.559	12.858.643	A	93.746.258	46.923.493	9
4	29.920.779	15.010.753	32.720.271	16.410.499	A	86.642.546	43.371.637	8
5	37.024.491	18.562.609	39.823.983	19.962.355	A	79.538.834	39.819.781	7
6	42.726.638	21.413.683	45.526.130	22.813.429	G	72.435.122	36.267.925	6
7	59.032.970	29.566.849	61.832.462	30.966.595	Y	66.732.975	33.416.851	5
8	70.535.665	35.318.196	73.335.157	36.717.942	D	50.426.643	25.263.685	4
9	80.442.507	40.271.617	83.241.999	41.671.363	V	38.923.948	19.512.338	3
10	93.346.767	46.723.747	96.146.259	48.123.493	E	29.017.106	14.558.917	2
11					K-Methyl	16.112.846	8.106.787	1

**ALAAAGYDVEK**<sub>Dimethyl</sub>

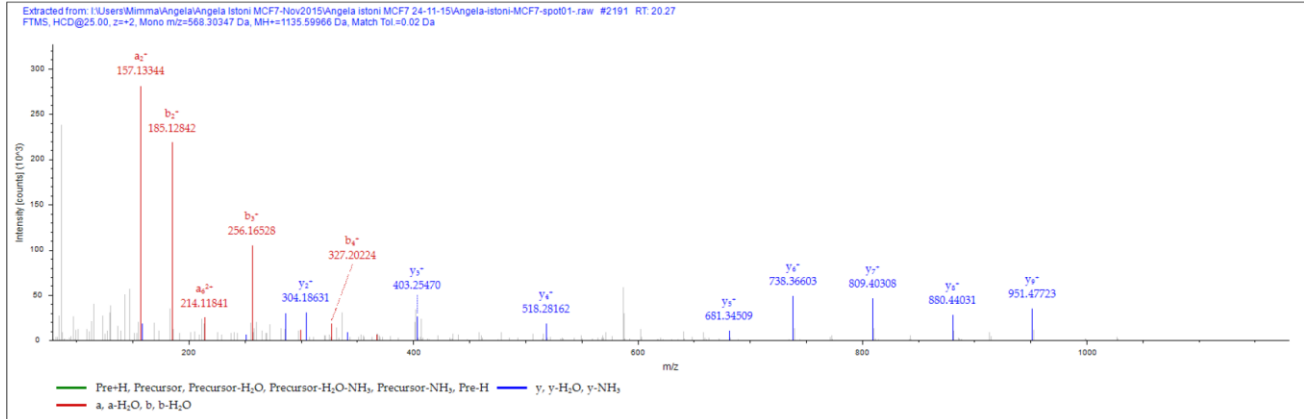
**H1.2K74 – H1.3K75**

Sequence: ALAAAGYDVEK, K11-Dimethyl (28.03130 Da)

Charge: +2, Monoisotopic m/z: 568.30347 Da (+0.12 mmu/+0.21 ppm), MH+: 1135.59966 Da, RT: 20.27 min,

Identified with: Sequest HT (v1.3); XCorr:2.87, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	4.404.948	2.252.838	7.204.440	3.652.584	A			11
2	15.713.355	7.907.041	18.512.847	9.306.787	L	106.456.230	53.278.479	10
3	22.817.067	11.458.897	25.616.559	12.858.643	A	95.147.823	47.624.275	9
4	29.920.779	15.010.753	32.720.271	16.410.499	A	88.044.111	44.072.419	8
5	37.024.491	18.562.609	39.823.983	19.962.355	A	80.940.399	40.520.563	7
6	42.726.638	21.413.683	45.526.130	22.813.429	G	73.836.687	36.968.707	6
7	59.032.970	29.566.849	61.832.462	30.966.595	Y	68.134.540	34.117.634	5
8	70.535.665	35.318.196	73.335.157	36.717.942	D	51.828.208	25.964.468	4
9	80.442.507	40.271.617	83.241.999	41.671.363	V	40.325.513	20.213.120	3
10	93.346.767	46.723.747	96.146.259	48.123.493	E	30.418.671	15.259.699	2
11					K-Dimethyl	17.514.411	8.807.569	1

**ALAAAGYDVEK**<sub>Trimethyl</sub>

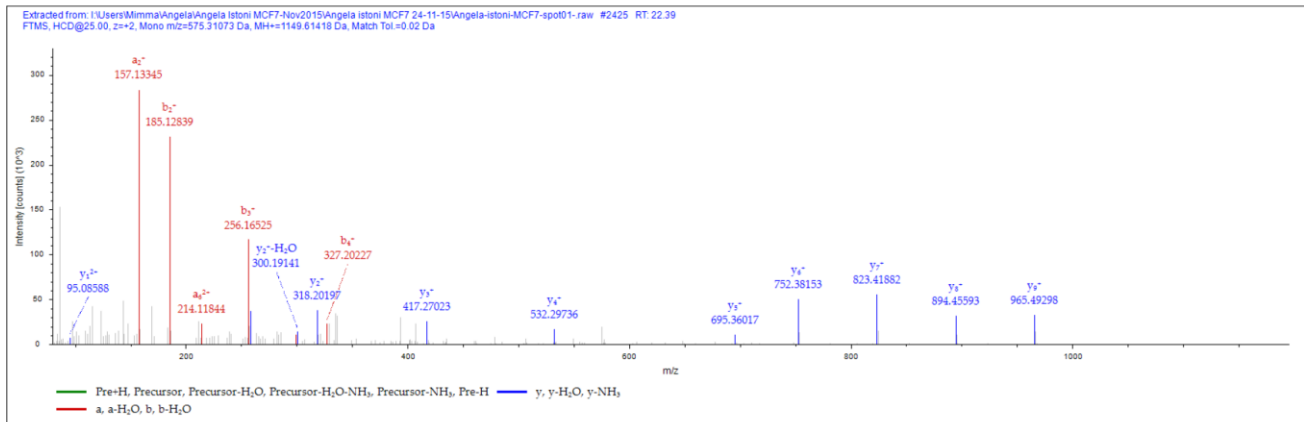
**H1.3K75**

Sequence: ALAAAGYDVEK, K11-Trimethyl (42.04695 Da)

Charge: +2, Monoisotopic m/z: 575.31073 Da (-0.44 mmu/-0.77 ppm), MH+: 1149.61418 Da, RT: 22.39 min,

Identified with: Sequest HT (v1.3); XCorr:2.95, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



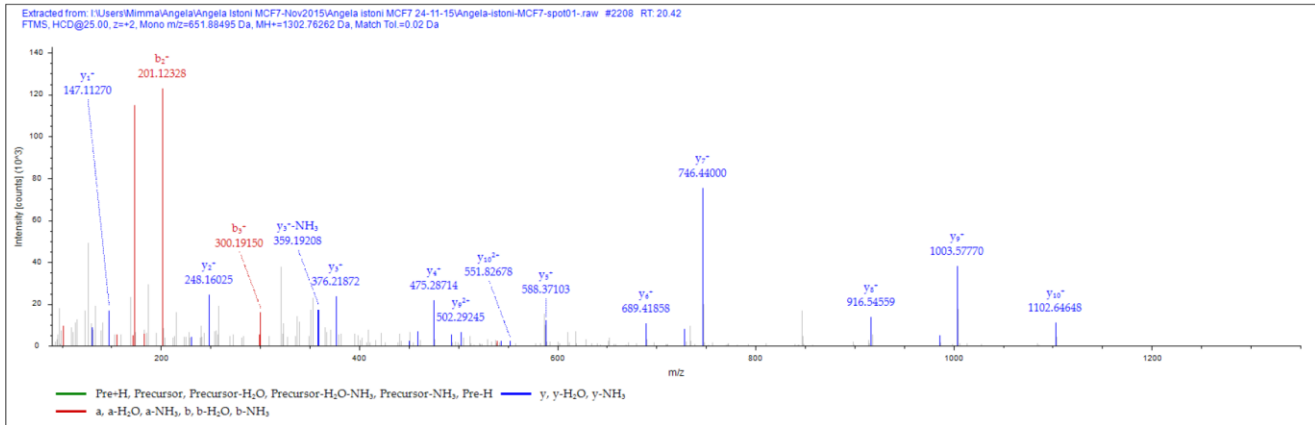
#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	4.404.948	2.252.838	7.204.440	3.652.584	A			11
2	15.713.355	7.907.041	18.512.847	9.306.787	L	107.857.795	53.979.261	10
3	22.817.067	11.458.897	25.616.559	12.858.643	A	96.549.388	48.325.058	9
4	29.920.779	15.010.753	32.720.271	16.410.499	A	89.445.676	44.773.202	8
5	37.024.491	18.562.609	39.823.983	19.962.355	A	82.341.964	41.221.346	7
6	42.726.638	21.413.683	45.526.130	22.813.429	G	75.238.252	37.669.490	6
7	59.032.970	29.566.849	61.832.462	30.966.595	Y	69.536.105	34.818.416	5
8	70.535.665	35.318.196	73.335.157	36.717.942	D	53.229.773	26.665.250	4
9	80.442.507	40.271.617	83.241.999	41.671.363	V	41.727.078	20.913.903	3
10	93.346.767	46.723.747	96.146.259	48.123.493	E	31.820.236	15.960.482	2
11					K-Trimethyl	18.915.976	9.508.352	1

Sequence: SLVSKGTLVQTK, K5-Acetyl (42.01057 Da)

Charge: +2, Monoisotopic m/z: 651.88495 Da (-0.09 mmu/-0.13 ppm), MH+: 1302.76262 Da, RT: 20.42 min,

Identified with: Sequest HT (v1.3); XCorr:3.03, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	6.004.439	3.052.583	8.803.931	4.452.329	S			12
2	17.312.846	8.706.787	20.112.338	10.106.533	L	121.573.077	60.836.902	11
3	27.219.688	13.660.208	30.019.180	15.059.954	V	110.264.670	55.182.699	10
4	35.922.891	18.011.809	38.722.383	19.411.555	S	100.357.828	50.229.278	9
5	52.933.445	26.517.086	55.732.936	27.916.832	K-Acetyl	91.654.625	45.877.676	8
6	58.635.592	29.368.160	61.435.083	30.767.905	G	74.644.071	37.372.399	7
7	68.740.360	34.420.544	71.539.851	35.820.289	T	68.941.924	34.521.326	6
8	80.048.767	40.074.747	82.848.258	41.474.493	L	58.837.156	29.468.942	5
9	89.955.609	45.028.168	92.755.100	46.427.914	V	47.528.749	23.814.738	4
10	102.761.467	51.431.097	105.560.958	52.830.843	Q	37.621.907	18.861.317	3
11	112.866.235	56.483.481	115.665.726	57.883.227	T	24.816.049	12.458.388	2
12					K	14.711.281	7.406.004	1

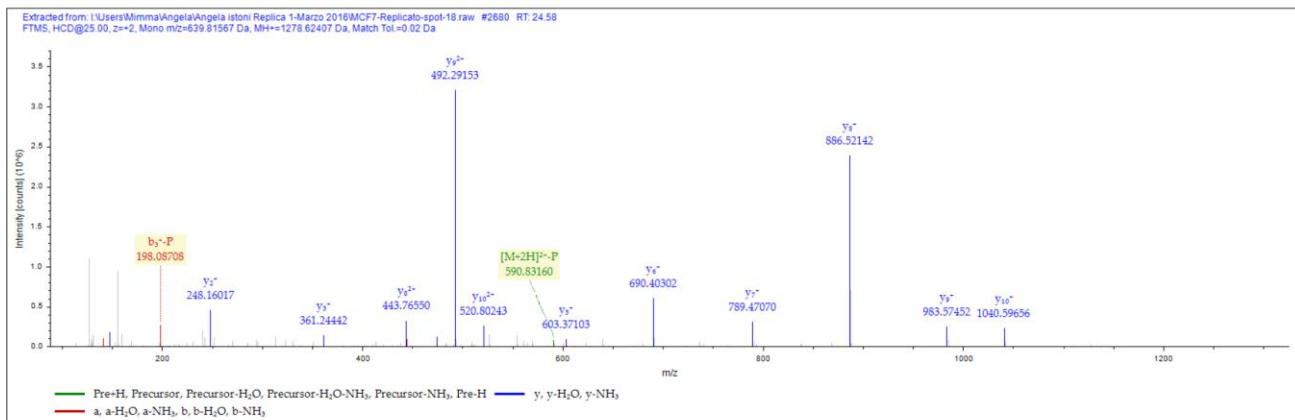


Sequence: ASGPPVSELITK, S2-Phospho (79.96633 Da)

Charge: +2, Monoisotopic m/z: 639.81567 Da (-5.05 mmu/-7.9 ppm), MH+: 1278.62407 Da, RT: 24.58 min,

Identified with: Sequest HT (v1.3); XCorr:2.86, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



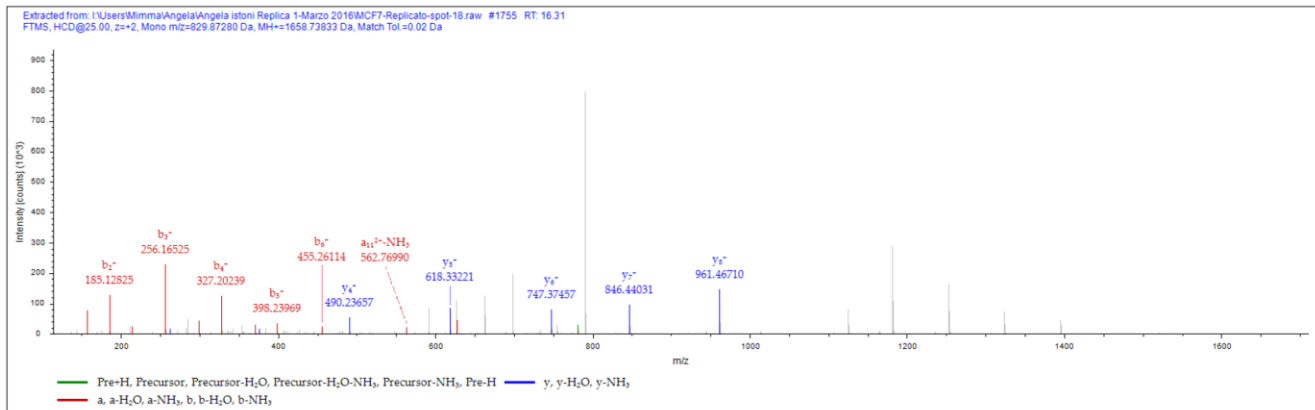
#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	4.404.948	2.252.838	7.204.440	3.652.584	A			12
2	21.104.784	10.602.756	23.904.276	12.002.502	S-Phospho	120.759.705	60.430.216	11
3	26.806.931	13.453.829	29.606.423	14.853.575	G	104.059.869	52.080.298	10
4	36.512.208	18.306.468	39.311.700	19.706.214	P	98.357.722	49.229.225	9
5	46.217.485	23.159.106	49.016.977	24.558.852	P	88.652.445	44.376.586	8
6	56.124.327	28.112.527	58.923.819	29.512.273	V	78.947.168	39.523.948	7
7	64.827.530	32.464.129	67.627.022	33.863.875	S	69.040.326	34.570.527	6
8	77.731.790	38.916.259	80.531.282	40.316.005	E	60.337.123	30.218.925	5
9	89.040.197	44.570.462	91.839.689	45.970.208	L	47.432.863	23.766.795	4
10	100.348.604	50.224.666	103.148.096	51.624.412	I	36.124.456	18.112.592	3
11	110.453.372	55.277.050	113.252.864	56.676.796	T	24.816.049	12.458.388	2
12					K	14.711.281	7.406.004	1

Sequence: ALAAAGYDVEKNNSR, Y7-Phospho (79.96633 Da)

Charge: +2, Monoisotopic m/z: 829.87280 Da (-7.57 mmu/-9.12 ppm), MH+: 1658.73833 Da, RT: 16.31 min,

Identified with: Sequest HT (v1.3); XCorr:2.90, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da



#1	a <sup>+</sup>	a <sup>2+</sup>	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	44.04948	22.52838	72.04440	36.52584	A			15
2	157.13355	79.07041	185.12847	93.06787	L	1587.71634	794.36181	14
3	228.17067	114.58897	256.16559	128.58643	A	1474.63227	737.81977	13
4	299.20779	150.10753	327.20271	164.10499	A	1403.59515	702.30121	12
5	370.24491	185.62609	398.23983	199.62355	A	1332.55803	666.78265	11
6	427.26638	214.13683	455.26130	228.13429	G	1261.52091	631.26409	10
7	670.29603	335.65165	698.29095	349.64911	Y-Phospho	1204.49944	602.75336	9
8	785.32298	393.16513	813.31790	407.16259	D	961.46979	481.23853	8
9	884.39140	442.69934	912.38632	456.69680	V	846.44284	423.72506	7
10	1013.43400	507.22064	1041.42892	521.21810	E	747.37442	374.19085	6
11	1141.52897	571.26812	1169.52389	585.26558	K	618.33182	309.66955	5
12	1255.57190	628.28959	1283.56682	642.28705	N	490.23685	245.62206	4
13	1369.61483	685.31105	1397.60975	699.30851	N	376.19392	188.60060	3
14	1456.64686	728.82707	1484.64178	742.82453	S	262.15099	131.57913	2
15					R	175.11896	88.06312	1

**Supplementary Figure 3. Fragmentation MS/MS spectra of the modified peptide carrying identified PTMs.** MS/MS data are referred to all analyzed histones. Data were analyzed by Proteome Discoverer 1.4 software. MS/MS data were searched on the Human UniProt database. For each PTM we reported: Sequence, type of modification, Charge, m/z, and RT.