Supplemental Material to:
Igor Prudovsky, Calvin P.H. Vary, Yolanda Maraki, Ada L. Olins and Donald E. Olins. Phosphatidylserine colocalizes with epichromatin in interphase nuclei and mitotic chromosomes. Nucleus 2012; 3(2):
http://dx.doi.org/10.4161/nucl.3.2.19662
http://www.landesbioscience.com/journals/nucleus/article/19662

Supplementary Material

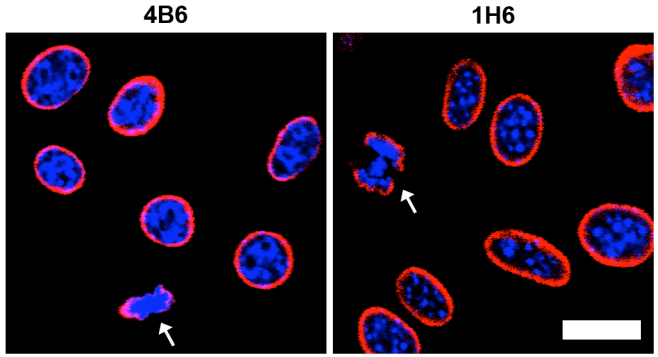


Fig. S1. Comparative confocal images of two mouse monoclonal anti-phosphatidylserine antibodies (Abcam #4B6 and Millipore #1H6) staining NIH 3T3 cells, following ethanol fixation. Color scheme: antibody staining (red); TOPRO-3 (blue). The arrows point to mitotic chromosomes. Scale bar: 20 μm.

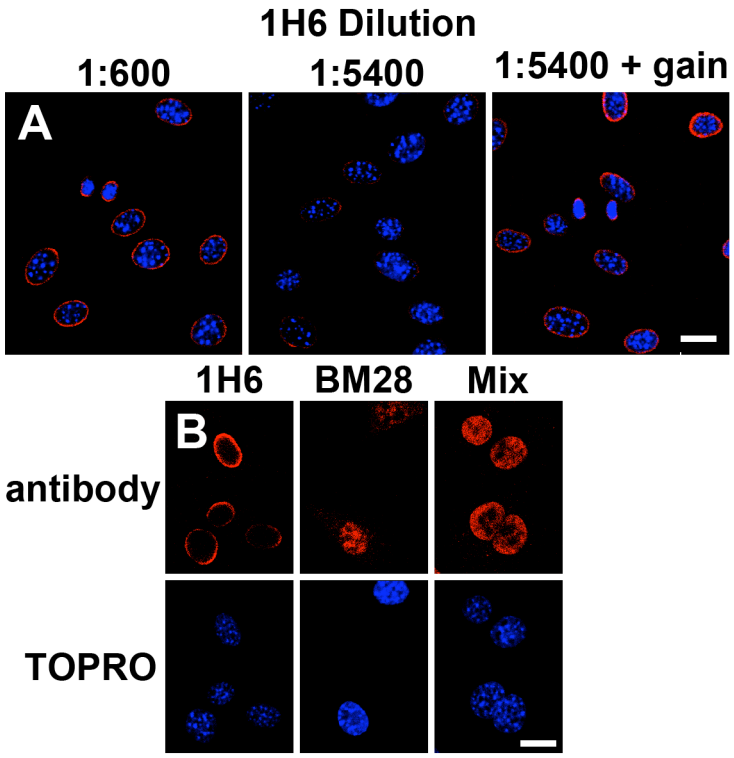


Fig. S2. Tests for possible immunostaining artifacts with anti-phosphatidylserine (1H6). (**A**) Image consistency during antibody dilution. Antibody 1H6 (red) gives peripheral staining at high dilutions (1:600 and 1:5400, with increased gain), compared to the routinely employed dilution (1:200). DNA (blue) stained with TOPRO-3. This experiment shows that a high concentration of 1H6 (1:200) does not form a physical barrier preventing 1H6 from diffusing into the nuclear interior, since a very high dilution (1:5400) yields an identical antibody distribution. (**B**) Absence of optical attenuation. Peripheral staining by 1H6 does not interfere with central nuclear staining by another mouse monoclonal anti-nuclear antibody (anti-BM28, an unrelated MCM nuclear protein). Both primary antibodies were reacted simultaneously, followed by a simultaneous reaction with the secondary anti-mouse IgG (red). DNA (blue) stained with TOPRO-3. Scale bars: 20 μm.

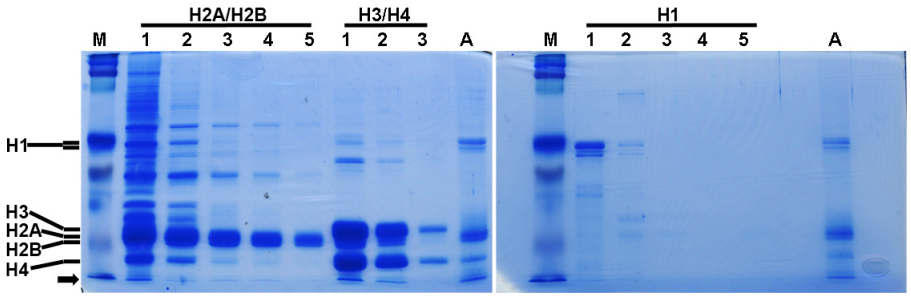
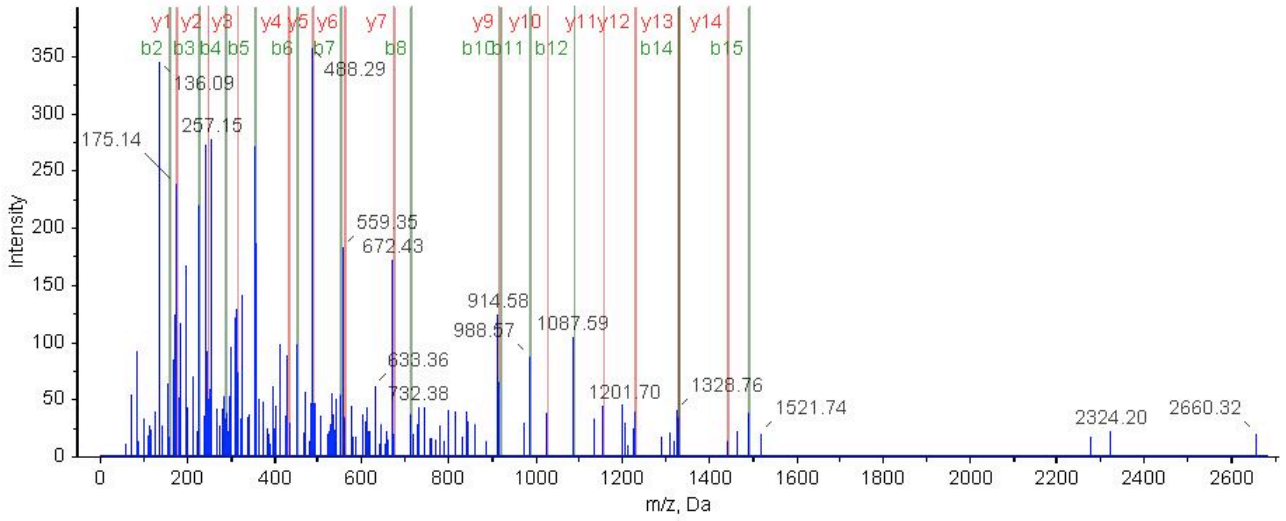


Fig. S3. Coomassie Blue stained 15% SDS-PAGE of NIH 3T3 histone subfractions (Active Motif kit) and HL-60/S4 total acid extracted histones (A). Arrow: dye front. M: MW markers. Loads: 5 µl of each histone subfraction (plus 5 µl of 2x SDS sample buffer); H2A+H2B, 5 sequential fractions; H3+H4, 3 sequential fractions; H1, 5 sequential fractions. The H1 fraction revealed no contamination by inner histones; H2A+H2B contained negligible amounts of H3+H4; H3+H4 showed <10% (by stain intensity) of contaminating H2A+H2B.

Antibody 1H6

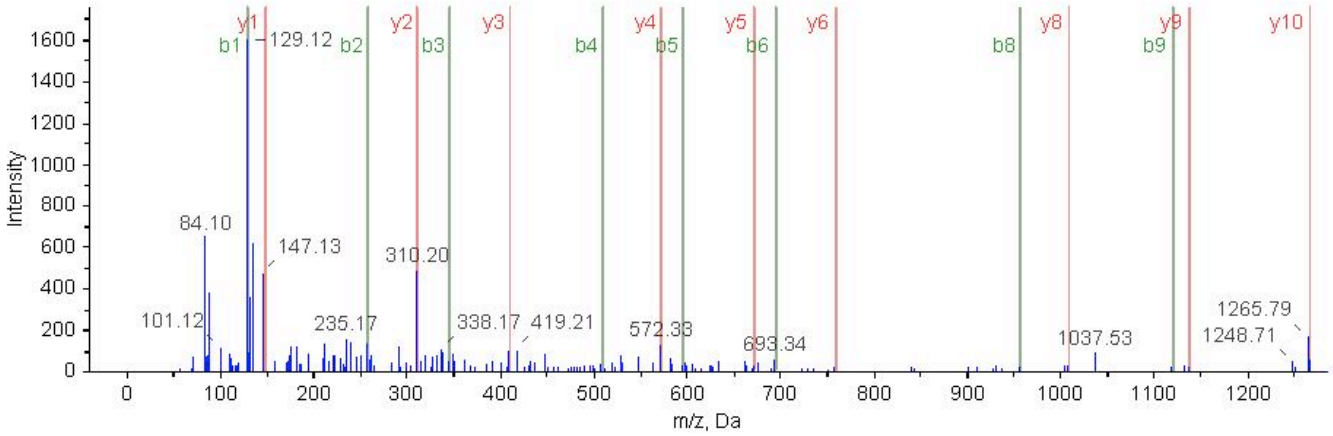
Representative peptide, histone H2A: **VGAGAPVYMAAVLEYLTAEILELAGNAAR**							
N	Unused	Total	% Cov	Accession #	Name	Species	Peptides(95%)
2	2.72	2.72	52.6	Q64426_MOUSE	Histone H2A	MOUSE	2



Histone H2A		
VGAGAPVYMAAVLEYLTAEILELAGNAAR		
Residue	b	y
V	100.0757	2933.5441
G	157.0972	2834.4757
A	228.1343	2777.4542
G	285.1557	2706.4171
A	356.1928	2649.3957
P	453.2456	2578.3585
V	552.3140	2481.3058
Y	715.3774	2382.2374
M	846.4178	2219.1740
A	917.4550	2088.1335
A	988.4921	2017.0964
V	1087.5605	1946.0593
L	1200.6445	1846.9909
E	1329.6871	1733.9068
Y	1492.7505	1604.8642
L	1605.8345	1441.8009
T	1706.8822	1328.7169
A	1777.9193	1227.6692
E	1906.9619	1156.6321
I	2020.0460	1027.5895
L	2133.1300	914.5054
E	2262.1726	801.4213
L	2375.2567	672.3787
A	2446.2938	559.2947
G	2503.3153	488.2576
N	2617.3582	431.2361
A	2688.3953	317.1932
A	2759.4324	246.1561
R	2915.5335	175.1190

Antibody 1H6

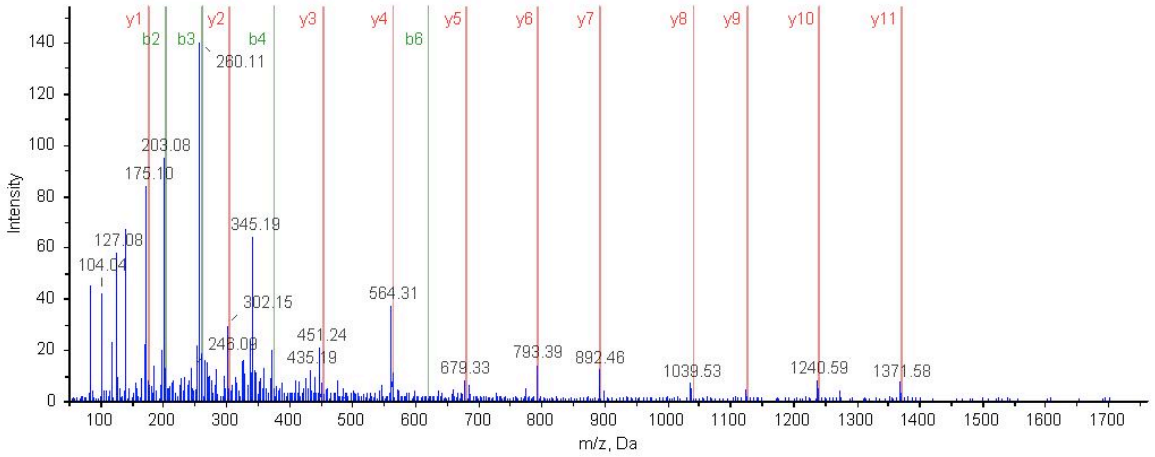
Representative peptide, histone H2B: **KESYSVYVYK**							
N	Unused	Total	% Cov	Accession #	Name	Species	Peptides(95%)
3	2.48	2.48	44.4	H2B_MOUSE	Histone H2B	MOUSE	2



Histone H2B		
KESYSVYVYK		
Residue	b	y
K	129.1022	1265.6412
E	258.1448	1137.5463
S	345.1769	1008.5037
Y	508.2402	921.4716
S	595.2722	758.4083
V	694.3406	671.3763
Y	857.4040	572.3079
V	956.4724	409.2445
Y	1119.5357	310.1761
K	1247.6307	147.1128

Antibody PL2-6

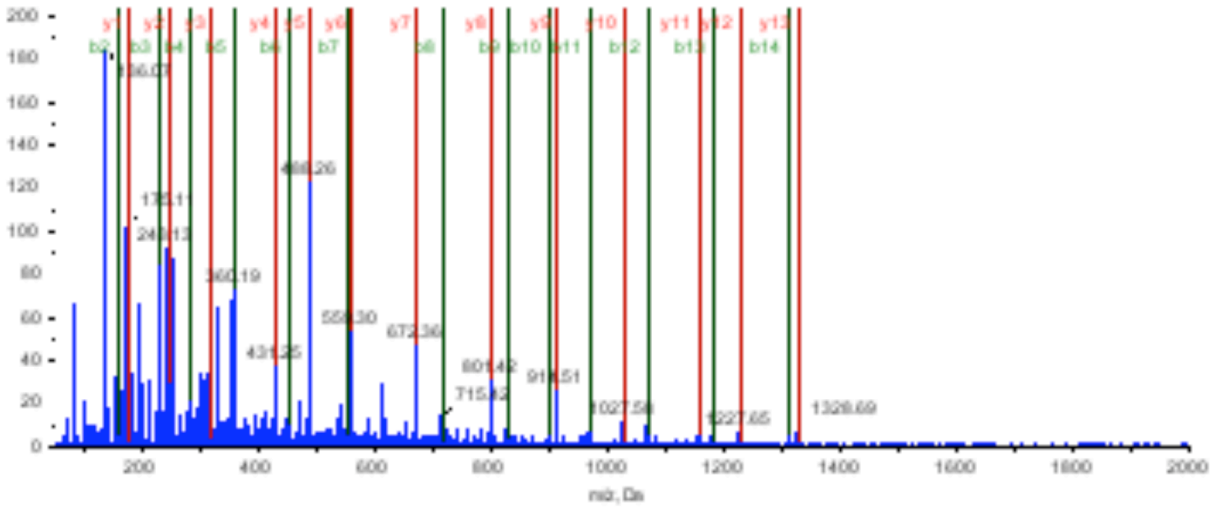
Representative peptide, Histone H2B: **AMGIMNSFVNDIFER**							
N	Unused	Total	% Cov	Accession #	Name	Species	Peptides(95%)
6	6.20	6.20	64.3	B2RVD5_MOUSE	Histone H2B	MOUSE	6



Histone H2B		
AMGIMNSFVNDIFER		
Residue	b	y
A	72.0444	1743.8193
M	203.0849	1672.7822
G	260.1063	1541.7417
I	373.1904	1484.7202
M	504.2309	1371.6362
N		1240.5957
S	705.3058	1126.5527
F	852.3743	1039.5207
V	951.4427	892.4523
N	1065.4856	793.3839
D	1180.5125	679.3410
I	1293.5966	564.3140
F	1440.6650	451.2300
E	1569.7076	304.1615
R	1725.8087	175.1190

Antibody PL2-6

Representative peptide, Histone H2A: **VGAGAPVYLAAVLEYLTAEILELAGNAAR**							
N	Unused	Total	% Cov	Accession #	Name	Species	Peptides(95%)
12	2.92	3.67	53.9	B2RVP5_MOUSE	Histone H2A	MOUSE	4



Histone H2A		
VGAGAPVYLAAVLEYLTAEILELAGNAAR		
Residue	b	y
V	100.0757	2915.5877
G	157.0972	2816.5193
A	228.1343	2759.4978
G	285.1557	2688.4607
A	356.1928	2631.4392
P	453.2456	2560.4021
V	552.3140	2463.3494
Y	715.3774	2364.2809
L	828.4614	2201.2176
A	899.4985	2088.1335
A	970.5356	2017.0964
V	1069.6041	1946.0593
L	1182.6881	1846.9909
E	1311.7307	1733.9068
Y	1474.7940	1604.8642
L	1587.8781	1441.8009
T	1688.9258	1328.7169
A	1759.9629	1227.6692
E	1889.0055	1156.6321
I	2002.0896	1027.5895
L	2115.1736	914.5054
E	2244.2162	801.4213
L	2357.3003	672.3787
A	2428.3374	559.2947
G	2485.3589	488.2576
N	2599.4018	431.2361
A	2670.4389	317.1932
A	2741.4760	246.1561
R	2897.5771	175.1190

Fig. S4. Representative time of flight mass spectra (MS, left panels) and collision-induced sequence data for selected high confidence (>99%) MS peptides (right tables), for the two most prevalent peptides in the 1H6 and PL6-2 immunoprecipitation reactions. The MS/MS-associated ion tables list the sequence of the identified peptide. For clarity of reference to MS/MS data, only selected critical y+1 ion and b+1 ion series are shown (shaded cells). y+2 and b+2 ions were also used for peptide identification (data not shown). Also presented above each spectrum is the percent coverage of H2A or H2B, for all unique peptides identified in at least 3 independent mass spectrometry experiments. MS data: "m/z", theoretical mass of the identified peptide; "z", observed charge state for the identified peptide.