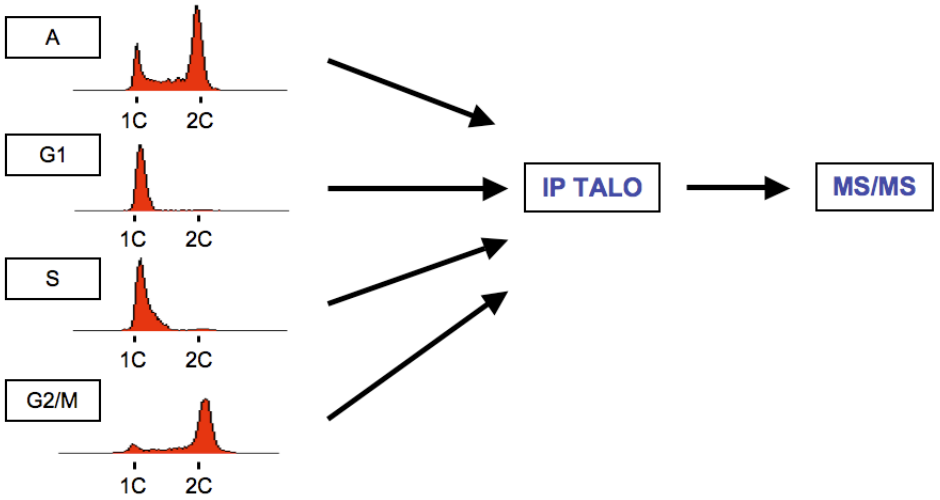
a



b

H2A (85%)

SGGKGGKAGS AAKASQSRSA KAGLTFPVG RV HRLLRRGNY AQ RIGSGAPV
YLTAVLEYLA AEILELAGNA A R DNKKTRII PRHLQLAIRN DDELNKLLGN
VTIAQGGVLP NIHQNLLPKK SAKATKASQEL

H2B (94%)

SAKAEKKPAS KAPAEKKPAA KKTSTSTDGK K RSKARKETY SSYIYKVLKQ
THPDTGISQK SMSILNSFVN DIFERIATEA SKLAAYNKKS TISA REIQTA
VRLILPGELA KHAVSEGT RA VTKYSSSTQ A

H3 (86%)

ARTKQTARKS TGGKAPRKQL ASKAA RKSAP STGGVKKPHR YKPGTVAL RE
IRRFQKSTEL LIRKLPFQRL V REIAQDFKT DLRFQSSAIG ALQESVEAYL
VSLFEDTNLA AIHAK RVTIQ KKDIKLARRL RGERS

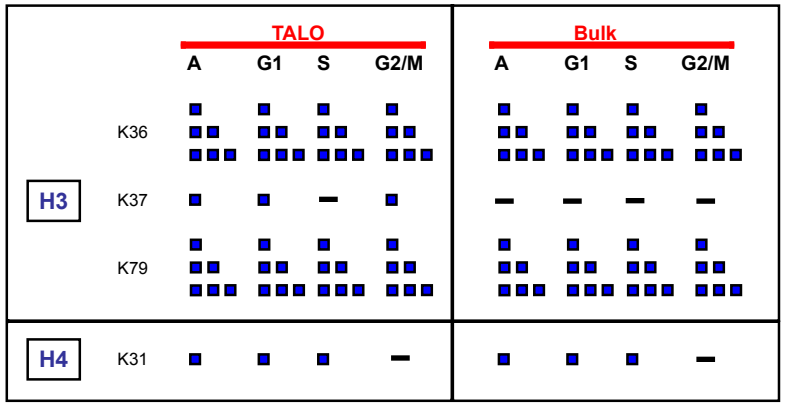
H4 (84%)

SGRGKGGKGL GKGGAKRHRK ILRDNIQGIT KPAI RRLARR GGVKRISGLI
YEEVRAVLKS FLESVI RDSV TYTEHAKRKT VTSLDVVYAL KRQGRTLYGF GG

c

		TALO				Bulk			
		A	G1	S	G2/M	A	G1	S	G2/M
H2A	S1	–	–	△	△	–	–	–	–
	S15	–	–	–	△	–	–	–	–
H2B	K111	–	–	–	–	■	–	■	■
	K123	◆	–	–	◆	◆	–	◆	◆
H3	K9	●	●	●	●	●	●	●	●
	K14	●	●	●	●	●	●	●	●
	K18	●	●	●	●	●	●	●	●
	K23	●	●	●	●	●	●	●	●
	K27	●	●	●	●	●	●	●	●
	K36	■	■	■	■	■	■	■	■
	K37	■	■	–	■	–	–	–	–
	K56	●	●	●	●	●	●	●	●
	K79	■	■	■	■	■	■	■	■
H4	K5	●	●	●	●	●	●	●	●
	K8	●	●	●	●	●	●	●	●
	K12	●	●	●	●	●	●	●	●
	K16	●	●	●	●	●	●	●	●
	K31	■	■	■	–	■	■	■	–
	K79	●	–	●	●	–	–	–	–

Methylation States

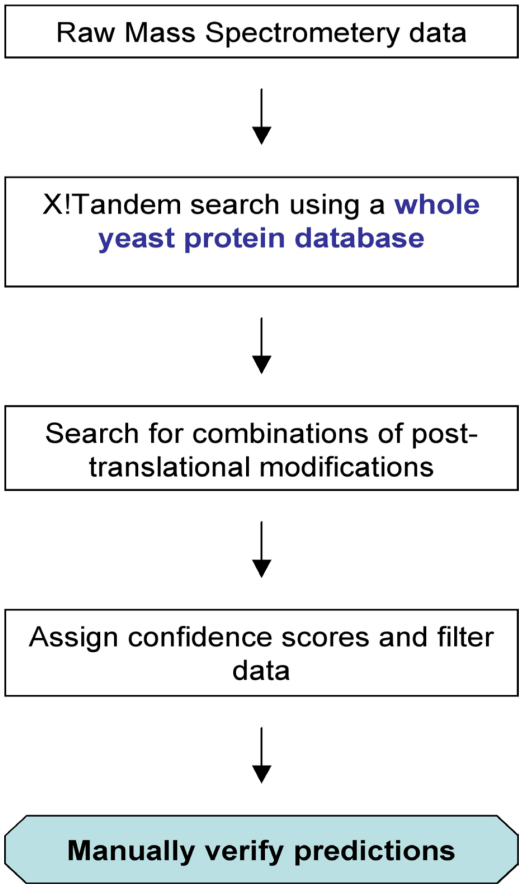


- ● Acetylation
- ■ Methylation
- ◆ Ubiquitination
- △ Phosphorylation
- – No modification observed

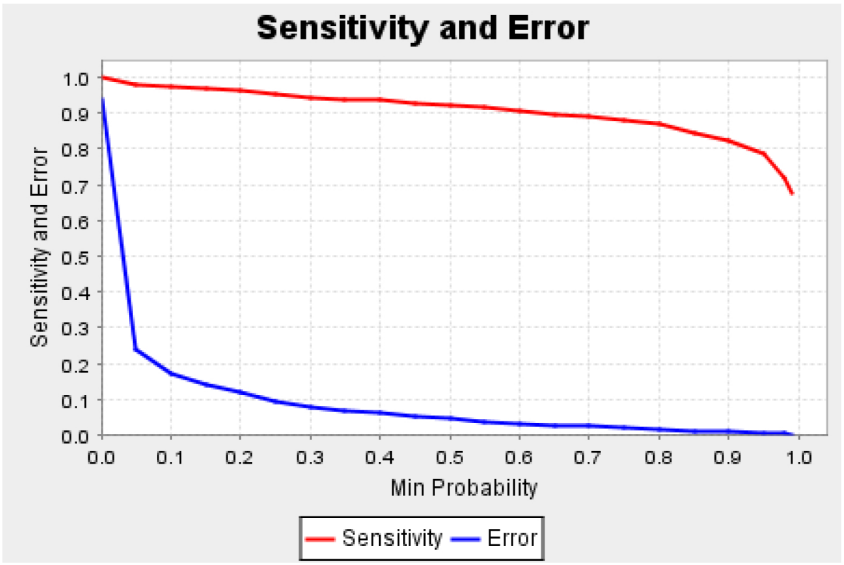
Supplementary Figure 1

Supplementary Figure 1. Qualitative detection of histone modifications over the cell cycle by mass spectrometry. (**a**) FACS profiles of TALO8-containing cells at harvest time points, growing asynchronously (A), in G1- phase, S-phase (17 minutes after release from G1) or G2/M-phase. DNA content is noted in red. As a control, isogenic cells lacking TALO-8 were also harvested from each of the stages and bulk, chromatin-bound histones were prepared and analyzed by mass spectrometry. (**b**) Peptides from the four core histones detected by mass spectrometry. Proteins were digested with ArgC, which cleaves at the carboxy-terminal of arginine residues (highlighted in blue). All the peptides detected by MS are shown in red while peptides that were not detected are in black. The coverage for each histone is indicated within parantheses. (**c**) The histone modifications detected by MS. The left half depicts histone modifications found on histones purified from TALO8, while the right half shows histone modifications found on bulk histones. In each half, each vertical column indicates all the histone modifications found in a particular cell cycle stage. Modified residues that were previously unreported in yeast are shown in purple. Lysine residues can be mono-, di- and tri-methylated and the inset box depicts all the methylation states that were detected.

a



b

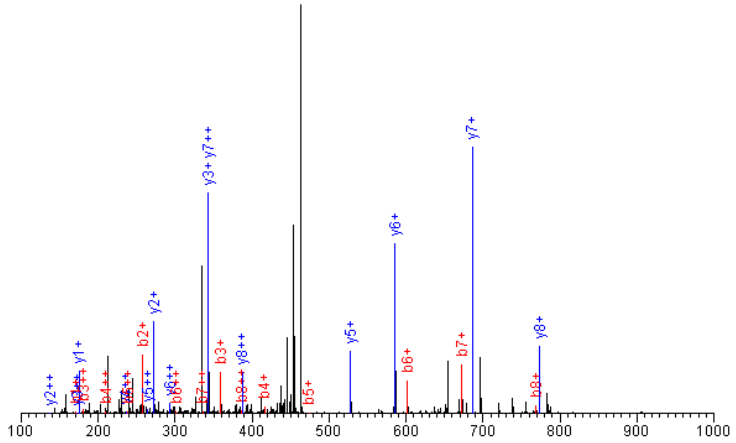


Minimum probability	Sensitivity	Error rate
0.00	1.0000	0.9368
0.05	0.9819	0.2378
0.10	0.9739	0.1704
0.15	0.9681	0.1419
0.20	0.9625	0.1217
0.25	0.9522	0.0932
0.30	0.9455	0.0778
0.35	0.9401	0.0675
0.40	0.9358	0.0610
0.45	0.9277	0.0511
0.50	0.9215	0.0444
0.55	0.9145	0.0384
0.60	0.9067	0.0330
0.65	0.8973	0.0272
0.70	0.8909	0.0240
0.75	0.8799	0.0197
0.80	0.8695	0.0167
0.85	0.8460	0.0113
0.90	0.8224	0.0079
0.95	0.7870	0.0049
0.98	0.7187	0.0022
0.99	0.6756	0.0014

Supplementray Figure 2

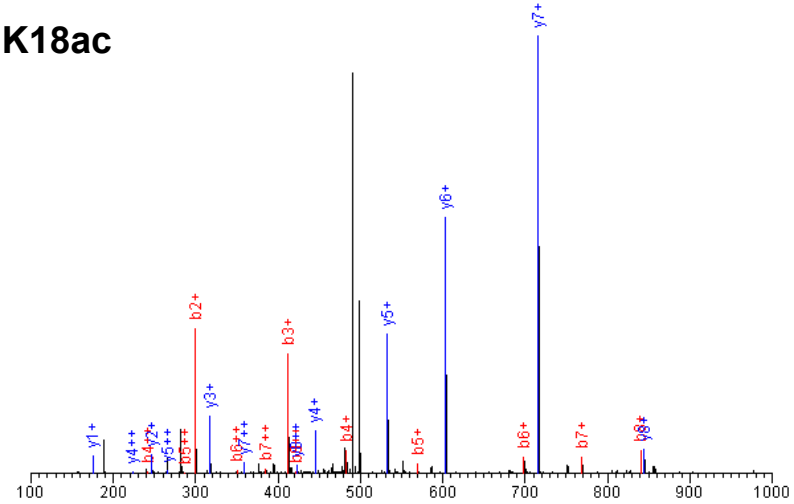
Supplementary Figure 2. Analysis of the MS data. (**a**) Flowchart description of the histone modification validation scheme used in this manuscript. The MS data was first searched using X!Tandem against the SGD whole protein database. The data was then filtered and the top hits were manually verified. (**b**) A typical false positive error rate for Peptide Prophet results from our MS data. The output from Peptide Prophet is used to create sensitivity and error curves from which a minimum Peptide Prophet probability can be selected to yield the desired error rate for filtering results. For this research, a minimum probability to produce an error of 5% was used as an initial cut-off. Peptide Prophet was used alongside other filtering methods to analyze data.

H3 K9ac



b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}
86.1185	171.2290	1	K-	9		
129.6576	258.3072	2	S	8	773.8674	387.4377
180.2101	359.4123	3	T	7	686.7892	343.8986
208.7361	416.4642	4	G	6	585.6841	293.3460
237.2620	473.5161	5	G	5	528.6322	264.8201
301.3491	601.6902	6	K	4	471.5803	236.2941
336.8885	672.7690	7	A	3	343.4062	172.2071
385.4468	769.8857	8	P	2	272.3274	136.6677
		9	R	1	175.2107	88.1093

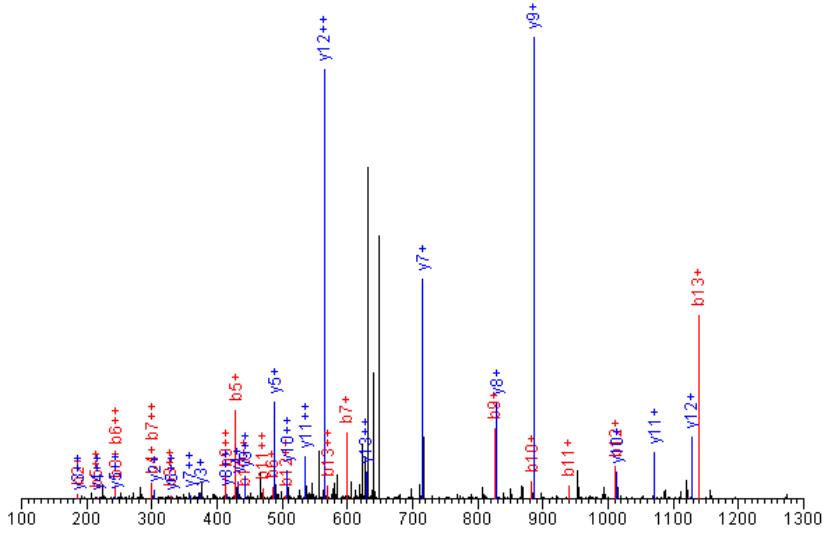
H3 K18ac



b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}
86.1185	171.2290	1	K-	9		
150.1838	299.3597	2	Q	8	844.9895	422.9987
206.7636	412.5192	3	L	7	716.8588	358.9334
242.3030	483.5980	4	A	6	603.6994	302.3537
285.8421	570.6762	5	S	5	532.6206	266.8143
349.9291	698.8503	6	K	4	445.5424	223.2752
385.4685	769.9291	7	A	3	317.3683	159.1881
421.0079	841.0079	8	A	2	246.2895	123.6487
		9	R	1	175.2107	88.1093

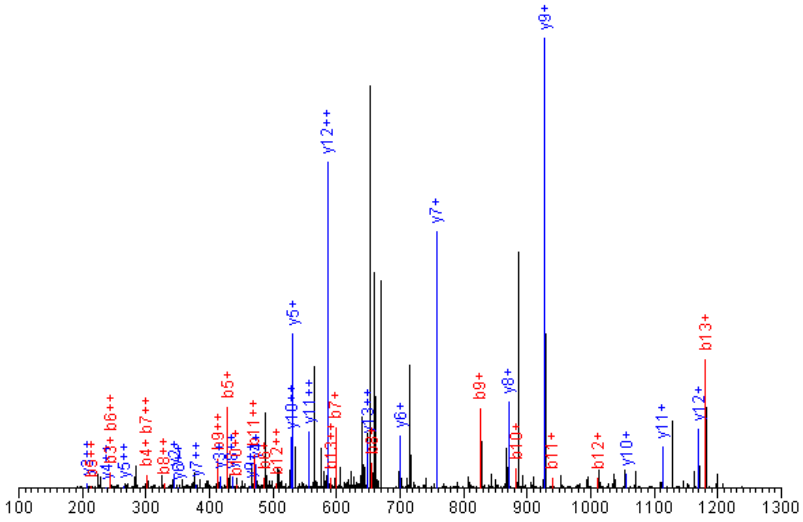
Supplementary Figure 3

H4 mono-ac (K12)



b2+	b+	#	AA	#	y+	y2+
29.5339	58.0599	1	G	14		
93.6209	186.2339	2	K	13	1256.5038	628.7559
122.1469	243.2859	3	G	12	1128.3297	564.6688
150.6729	300.3378	4	G	11	1071.2778	536.1429
214.7599	428.5119	5	K	10	1014.2259	507.6169
243.2859	485.5638	6	G	9	886.0518	443.5299
299.8656	598.7232	7	L	8	828.9999	415.0039
328.3915	655.7751	8	G	7	715.8404	358.4242
413.5021	825.9962	9	K-	6	658.7885	329.8982
442.0280	883.0481	10	G	5	488.5674	244.7877
470.5540	940.1001	11	G	4	431.5155	216.2617
506.0934	1011.1789	12	A	3	374.4636	187.7358
570.1804	1139.3529	13	K	2	303.3848	152.1964
		14	R	1	175.2107	88.1093

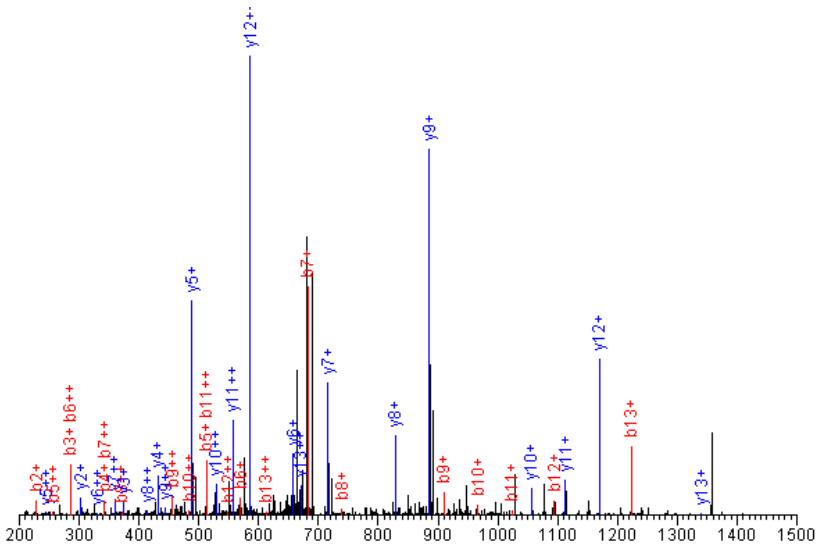
H4 di-ac (K12,16)



b2+	b+	#	AA	#	y+	y2+
29.5339	58.0599	1	G	14		
93.6209	186.2339	2	K	13	1298.5508	649.7794
122.1469	243.2859	3	G	12	1170.3767	585.6923
150.6729	300.3378	4	G	11	1113.3248	557.1664
214.7599	428.5119	5	K	10	1056.2729	528.6404
243.2859	485.5638	6	G	9	928.0988	464.5534
299.8656	598.7232	7	L	8	871.0469	436.0274
328.3915	655.7751	8	G	7	757.8874	379.4477
413.5021	825.9962	9	K-	6	700.8355	350.9217
442.0280	883.0481	10	G	5	530.6144	265.8112
470.5540	940.1001	11	G	4	473.5625	237.2852
506.0934	1011.1789	12	A	3	416.5106	208.7593
591.2039	1181.3999	13	K-	2	345.4318	173.2199
		14	R	1	175.2107	88.1093

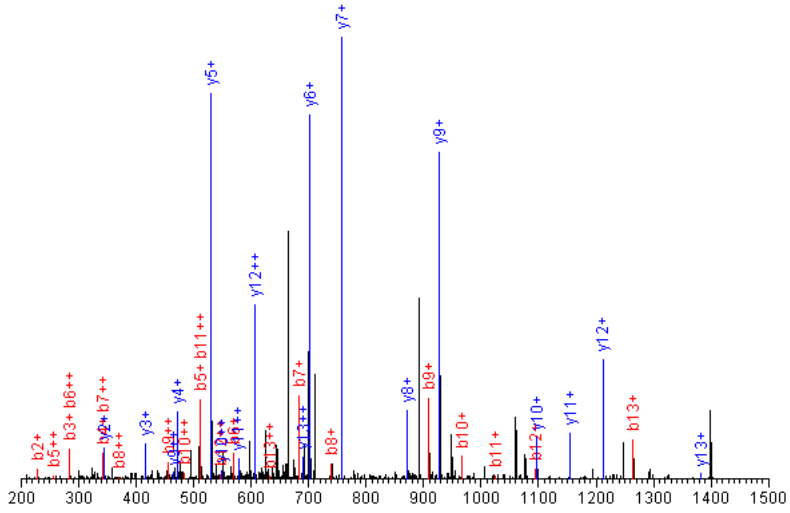
Supplementary Figure 3

H4 tri-ac (K5,8,12)



b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}
29.5339	58.0599	1	G	14		
114.6444	228.2809	2	K~	13	1340.5978	670.8029
143.1704	285.3329	3	G	12	1170.3767	585.6923
171.6964	342.3848	4	G	11	1113.3248	557.1664
256.8069	512.6059	5	K~	10	1056.2729	528.6404
285.3329	569.6578	6	G	9	886.0518	443.5299
341.9126	682.8172	7	L	8	828.9999	415.0039
370.4385	739.8691	8	G	7	715.8404	358.4242
455.5491	910.0902	9	K~	6	658.7885	329.8982
484.0750	967.1421	10	G	5	488.5674	244.7877
512.6010	1024.1941	11	G	4	431.5155	216.2617
548.1404	1095.2729	12	A	3	374.4636	187.7358
612.2274	1223.4469	13	K	2	303.3848	152.1964
		14	R	1	175.2107	88.1093

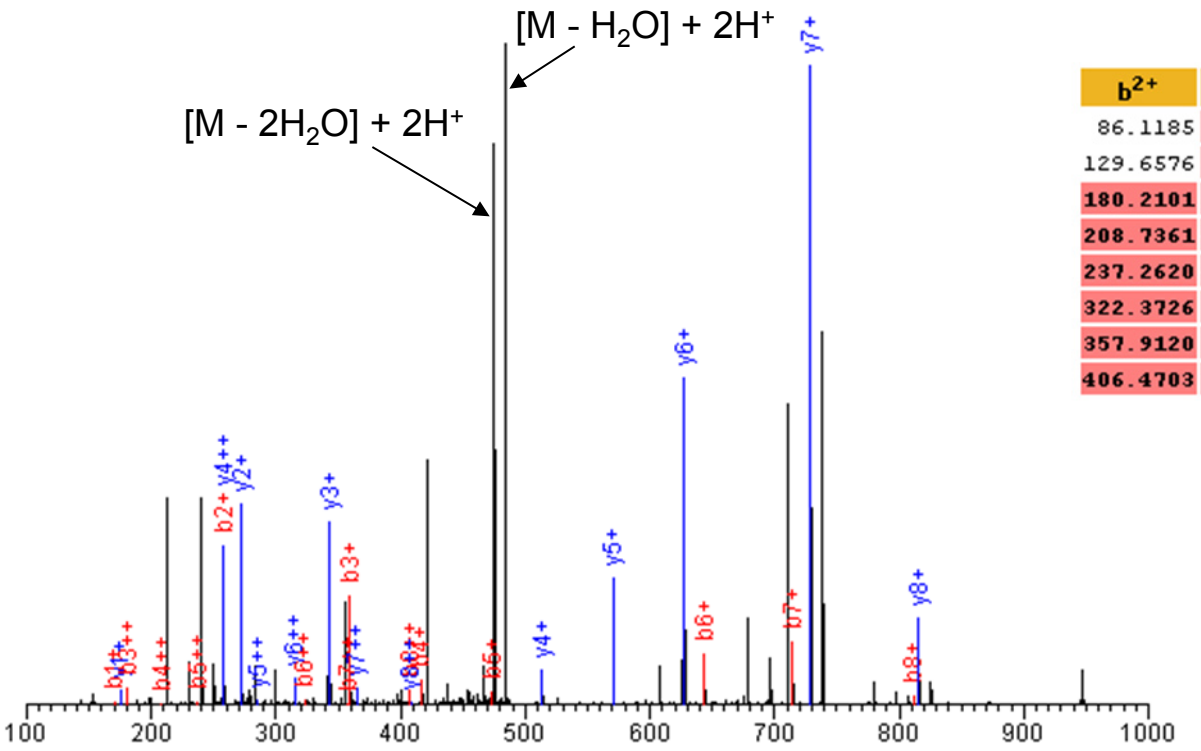
H4 tetra-ac (K5,8,12,16)



b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}
29.5339	58.0599	1	G	14		
114.6444	228.2809	2	K~	13	1382.6448	691.8264
143.1704	285.3329	3	G	12	1212.4237	606.7158
171.6964	342.3848	4	G	11	1155.3718	578.1899
256.8069	512.6059	5	K~	10	1098.3199	549.6639
285.3329	569.6578	6	G	9	928.0988	464.5534
341.9126	682.8172	7	L	8	871.0469	436.0274
370.4385	739.8691	8	G	7	757.8874	379.4477
455.5491	910.0902	9	K~	6	700.8355	350.9217
484.0750	967.1421	10	G	5	530.6144	265.8112
512.6010	1024.1941	11	G	4	473.5625	237.2852
548.1404	1095.2729	12	A	3	416.5106	208.7593
633.2509	1265.4939	13	K~	2	345.4318	173.2199
		14	R	1	175.2107	88.1093

Supplementary Figure 3

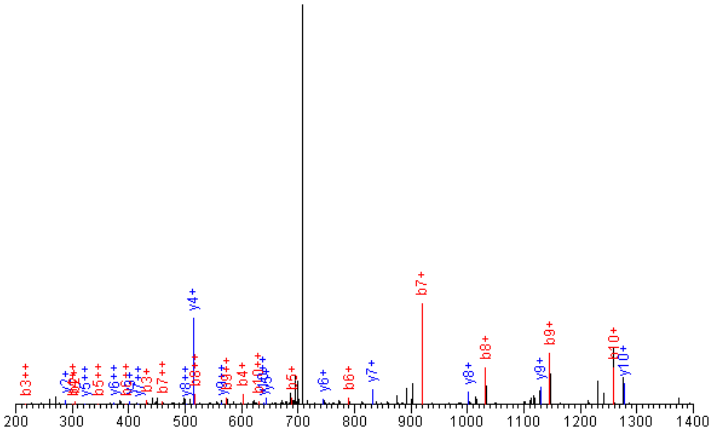
H3 K9,14ac



b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}
86.1185	171.2290	1	K~	9		
129.6576	258.3072	2	S	8	815.9144	408.4612
180.2101	359.4123	3	T	7	728.8362	364.9221
208.7361	416.4642	4	G	6	627.7311	314.3695
237.2620	473.5161	5	G	5	570.6792	285.8436
322.3726	643.7372	6	K~	4	513.6273	257.3176
357.9120	714.8160	7	R	3	343.4062	172.2071
406.4703	811.9327	8	P	2	272.3274	136.6677
		9	R	1	175.2107	88.1093

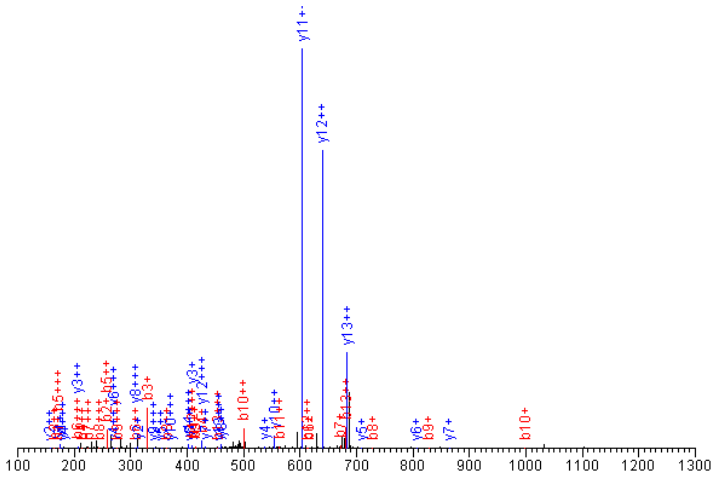
Supplementary Figure 3

H3 K56ac



b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}
79.1017	157.1954	1	R	11		
152.6900	304.3720	2	F	10	1277.5161	639.2620
216.7553	432.5027	3	Q	9	1130.3396	565.6738
301.8659	602.7238	4	K-	8	1002.2089	501.6084
345.4050	689.8020	5	S	7	831.9878	416.4979
395.9575	790.9071	6	T	6	744.9096	372.9588
460.5152	920.0225	7	E	5	643.8045	322.4062
517.0950	1033.1820	8	L	4	514.6890	257.8485
573.6747	1146.3414	9	L	3	401.5296	201.2688
630.2544	1259.5009	10	I	2	288.3701	144.6890
		11	R	1	175.2107	88.1093

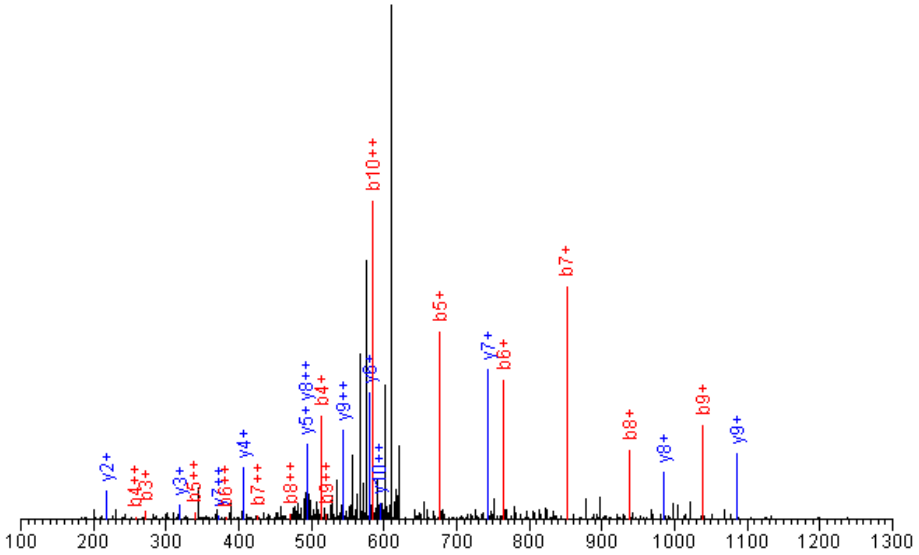
H3 K27acK36me3



b^{3+}	b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}	y^{3+}
57.7483	86.1185	171.2290	1	K-	14			
86.7744	129.6576	258.3072	2	S	13	1364.5570	682.7825	455.5243
110.4673	165.1970	329.3860	3	A	12	1277.4788	639.2434	426.4982
142.8395	213.7553	426.5027	4	P	11	1206.4000	603.7040	402.8053
171.8656	257.2944	513.5809	5	S	10	1109.2833	555.1456	370.4331
205.5673	307.8470	614.6860	6	T	9	1022.2051	511.6065	341.4070
224.5846	336.3729	671.7379	7	G	8	921.1000	461.0540	307.7053
243.6019	364.8989	728.7898	8	G	7	864.0481	432.5280	288.6880
276.6461	414.4652	827.9224	9	V	6	806.9962	404.0021	269.6707
333.3864	499.5757	998.1435	10	K-	5	707.8636	354.4358	236.6265
376.1111	563.6627	1126.3175	11	K	4	537.6425	269.3252	179.8861
408.4834	612.2211	1223.4342	12	P	3	409.4685	205.2382	137.1614
454.1971	680.7916	1360.5753	13	H	2	312.3518	156.6799	104.7892
			14	R	1	175.2107	88.1093	59.0755

Supplementary Figure 3

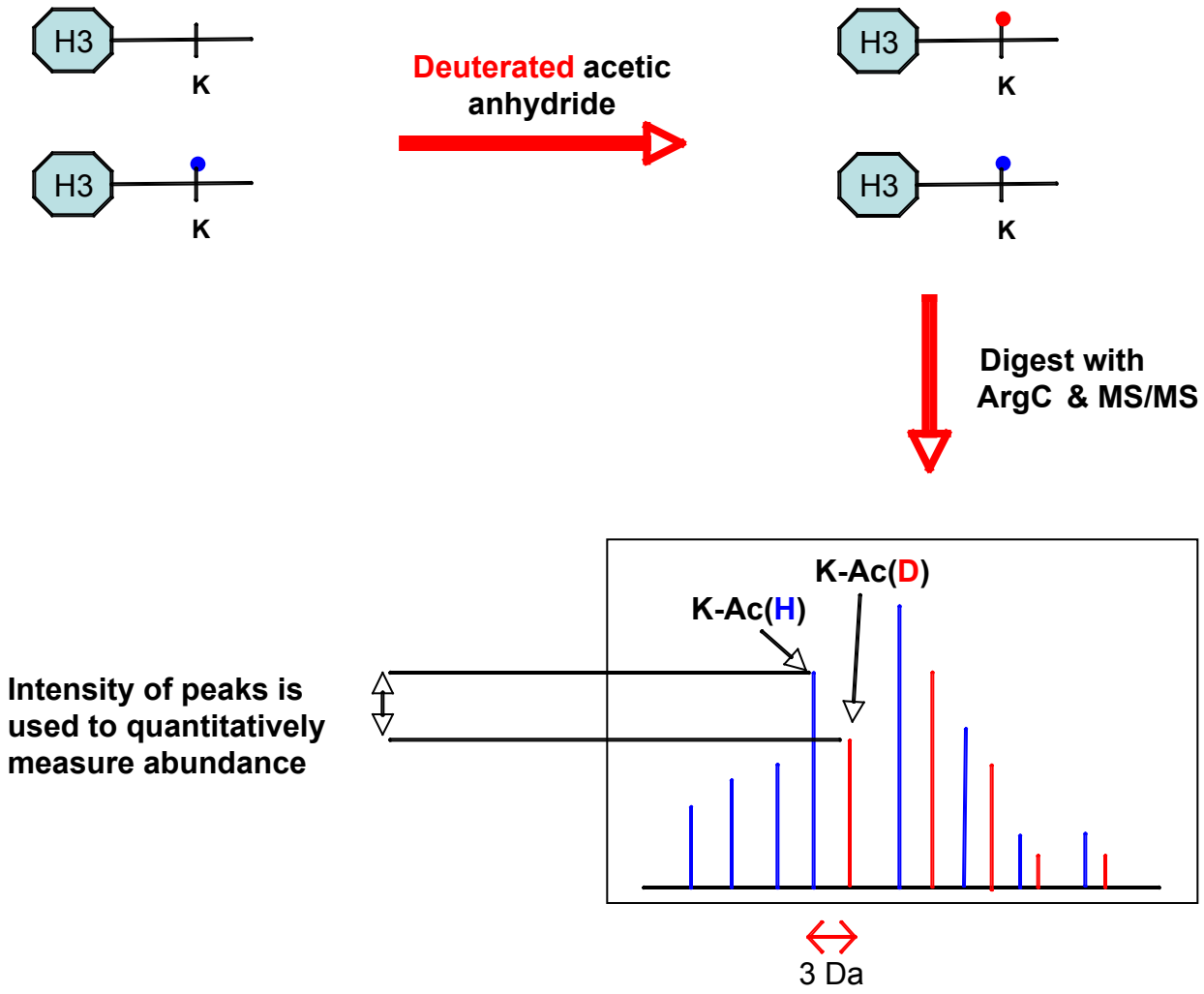
H2B K123ub1



b^{2+}	b^{+}	#	AA	#	y^{+}	y^{2+}
36.5473	72.0867	1	A	11		
86.1136	171.2193	2	V	10	1186.2030	593.6055
136.6662	272.3244	3	T	9	1087.0704	544.0392
257.7747	514.5414	4	K#	8	985.9654	493.4867
339.3626	677.7173	5	Y	7	743.7484	372.3782
382.9017	764.7955	6	S	6	580.5724	290.7902
426.4408	851.8737	7	S	5	493.4942	247.2511
469.9799	938.9519	8	S	4	406.4160	203.7120
520.5325	1040.0570	9	T	3	319.3378	160.1729
584.5978	1168.1877	10	Q	2	218.2327	109.6203
		11	A	1	90.1020	45.5550

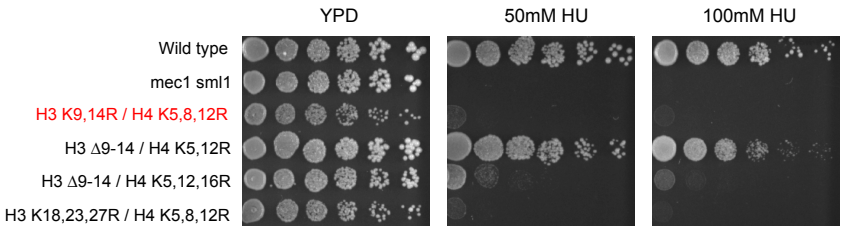
Supplementary Figure 3. The quality of MS data after filtering is very high.
Representative fragmentation spectra from MS of several modified histone peptides relevant to this manuscript, detected after filtering the mass spectrometry data and being manually verified. Predicted m/z values for b- and y-ions are indicated in the box on the right, and every b- and y-ion that was detected in the actual data is color-coded (red for b-ions and blue for y-ions). Almost all of the detectable ions were detected. Those not detected were typically in very low m/z ranges. The MS spectra for other histone peptides are available upon request.

Supplementary Figure 3



Supplementary Figure 4. A description of the scheme for quantitating acetylations by MS.
A schematic description of the histone acetylation quantitation method is shown for a hypothetical histone H3 protein containing a single lysine. When the histones are treated with deuterated acetic anhydride, unacetylated lysines are converted to deuterated acetyllysines (represented with a red dot). Histones where the lysine had been acetylated in vivo remain unchanged as protiated acetyllysines (depicted with a blue dot). The proteins are then digested and analyzed on a mass spectrometer as before. Fragments containing a deuterated acetyllysine are chemically identical to those with a protiated acetyllysine, except that they are mass shifted by 3 Da. The intensity difference in the peak heights is then used as a quantitative measure of abundance of each form.

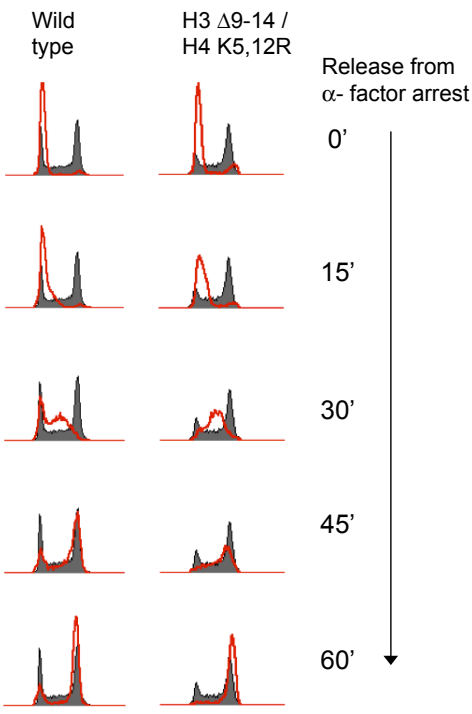
a



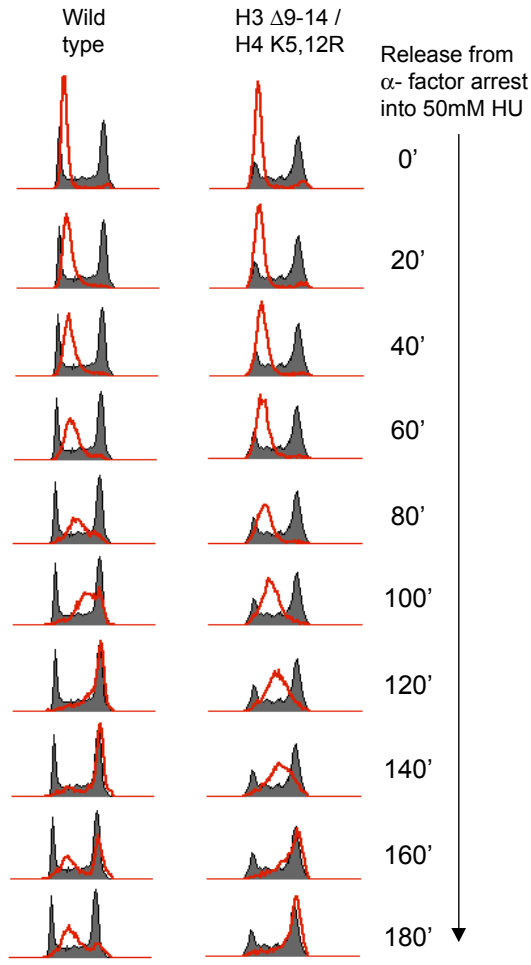
H3	H4	growth	HU	MMS
wild type	wild type	++++	++++	++++
H3 K9,14R	**H4 K5,8,12R**	**+++ /TS**	**+**	**+**
H3 K9,14R	wild type	++++	++++	++++
H3 K14,23R	wild type	++++	++++	++++
H3 K14,23,27R	wild type	++++	++++	++++
H3 K18,23,27R	wild type	++++	++++	++++
H3 K9,18,27R	wild type	++++	++++	++++
H3 del9-14	wild type	++++	++++	++++
wild type	H4 K5,12R	++++	++++	++++
wild type	H4 K5,8,12R	++++	++++	++++
wild type	H4 K5,12,16R	++++	++++	++++
H3 del9-14	H4 K5,8,12R	++ /TS	+	+
H3 del9-14	H4 K5,12R	++++	++++	++++
H3 del9-14	H4 K5,12,16R	+++ /TS	++	++
H3 K18,23,27R	H4 K5,8,12R	+++ /TS	+	+

mec1	sml1	++++	+	+

b



c



Supplementary Figure 5

Supplementary Figure 5. Multiple acetylations of H3 and H4 tails are required for efficient DNA replication. (**a**) Spot test of histone mutants.Five-fold serial dilution of cells were spotted onto rich media (YPD) with or without HU. A summary of the growth assays is shown as a table on the right. (**b**) FACS analysis of H3Δ9-14/H4 K5,12R mutant. Cells were arrested in G1, then released into YPD. Red lines show the kinetics of FACS profile, and the grey patterns denote the FACS profiles of log phase cells. (**c**) FACS analysis of H3Δ9-14/H4 K5,12R mutant in 50 mM HU. Cells were arrested in G1, then released into YPD containing 50 mMHU.

Protein	Sequence Mass	Peptides	Unique Peptides	AA Coverage
HTB1	14,252	28	17	93.9%
HHT1	15,356	62	42	86.2%
HTA2	13,989	19	12	84.8%
HHF1	11,368	42	27	83.7%
HTB2	14,237	24	11	61.1%
LacI	38,590	41	31	57.2%
HTZ1	14,283	7	5	54.5%
MCM3	107,518	60	39	45.5%
MCM5	86,411	35	28	40.9%
SSA2	69,470	19	16	39.6%
NHP6B	11,575	4	4	38.4%
MCM4	105,003	44	28	35.8%
RIM1	15,386	5	4	34.8%
SSB2	66,595	19	15	34.7%
NHP6A	10,802	2	2	28.0%
TIF2	44,697	6	6	26.8%
SPT15	27,003	3	3	26.7%
POB3	62,994	12	11	26.6%
SAR1	21,451	2	2	25.8%
MCM6	112,978	21	18	25.2%
ASC1	34,805	4	4	24.5%
RPB3	35,298	6	4	23.3%
YRA1	24,956	4	4	22.6%
ISW1	131,102	23	19	22.2%
RVB2	51,611	5	5	22.1%
RPB8	16,511	3	2	21.9%
DED1	65,553	8	8	21.9%
RPO26	17,910	3	2	21.3%
DIG1	49,356	6	6	21.2%
MCM7	94,875	15	12	21.2%
TEF2	50,033	9	8	21.0%
NOP58	56,956	6	6	20.2%
YKT6	22,707	3	3	20.0%
RPB10	8,278	2	2	20.0%
CDC19	54,545	8	7	19.8%
SEC65	31,170	3	3	19.8%
RPB2	138,751	19	19	19.7%
SUI3	31,574	3	3	19.6%
VMA2	57,749	7	7	19.3%
SRP54	59,624	7	7	18.7%
PDC1	61,495	9	8	18.5%

TSA1	21,590	2	2	18.4%
HSP12	11,693	2	2	18.3%
SSC1	70,628	13	11	18.2%
SPT16	118,630	17	12	17.7%
PGK1	44,738	8	6	17.5%
TDH3	35,747	4	4	17.5%
GPM1	27,609	4	3	17.4%
HSP82	81,406	8	8	16.9%
YHR121W	21,314	3	2	16.6%
CBF5	54,705	4	4	16.6%
GAL4	99,403	15	13	16.3%
YOP1	20,269	2	2	16.1%
ENO2	46,914	6	5	16.0%
SIK1	56,864	6	6	15.9%
RVB1	50,453	5	4	15.8%
ADK1	24,255	2	2	15.3%
RPO21	191,612	26	21	15.2%
SIS1	37,590	3	3	15.1%
YTA7	157,407	24	21	14.9%
SUM1	118,201	7	7	14.8%
GAL80	48,323	3	3	13.8%
IOC4	55,427	6	5	13.7%
OYE2	45,011	2	2	13.0%
SSA4	69,651	5	5	12.8%
SPT5	115,650	9	9	12.5%
PRE5	25,604	2	2	11.5%
ARO4	39,749	2	2	11.4%
IOC2	93,445	9	7	11.1%
TOP2	164,215	10	9	9.8%
RPB4	25,414	2	2	9.5%
RPB5	25,079	2	2	9.3%
GFA1	80,047	4	4	9.2%
IOC3	90,897	4	3	9.1%
SMC6	128,009	6	6	9.1%
SAM1	41,818	2	2	8.9%
ORC4	60,705	4	3	8.9%
RFA1	70,348	4	4	8.5%
TOP2	164,215	10	9	9.8%

Supplementary Table 1

A table of some of the proteins identified by mass spectrometry from TALO8 eluates prepared from asynchronously growing mid log-phase cells. The proteins are arranged in descending order of amino acid coverage, as determined from filtered mass spectrometry data. The data was first filtered in CPAS using ProteinProphet such that the false positive discovery rate was less than 0.05 and the top 80 hits were selected. As a result, all the peptide shown in this table have the false discovery rate of less than 0.002.

Protein	Sequence Mass	Peptides	Unique Peptides	AA Coverage
MCM3	107,518	60	39	45.5%
MCM5	86,411	35	28	40.9%
MCM4	105,003	44	28	35.8%
MCM6	112,978	21	18	25.2%
MCM7	94,875	15	12	21.2%
ORC4	60,705	4	3	8.9%
ORC1	104,400	4	4	6.1%
ORC6	50,296	1	1	6.0%
ORC3	72,077	3	2	5.7%
ORC5	55,289	1	1	2.9%
SLD2	52,272	1	1	2.6%
SLD3	77,298	1	1	1.5%
RFA3	13,816	1	1	12.3%
POB3	62,994	12	11	26.6%
SPT16	118,630	17	12	17.7%

Supplementary Table 2

A table of some replication-associated factors identified in TALO8 eluates from S-phase. The data was filtered as in Supplementary Table 1.

Name	Genetic Background	Notes
W1588-4c	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+*	RAD5+ version of W303-1 (Thomas & Rothstein, 1989(
YTT 2606	*MAT***a** *ura3-1*:pRS406-CMV-LacI-3FLAG-URA3	All YTT strains are derived from W1588-4C
YTT 3675	*MAT***a** *ura3-1*::pRS406-CMV-LacI-3FLAG-URA3 TALO8	
YTT 2455	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2::*NatMX *(*pRM200U-HHT2-HHF2*)*	
YTT 3515	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1::pRS404-HHT2-HHF2	
YTT 3690	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1::pRS404- HHT2 – hhf2K5,8,12R	
YTT 3716	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1::pRS404- hht2K9,14R -HHF2	
YTT 3846	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1::pRS404-hht2K9,14R-hhf2K5,8,12R	
YTT 4240	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1::pRS404-HHT2-HHF2 *bar1*::LEU2	
YTT 4238	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404-hht2K9,14R-hhf2K5,8,12R *bar1*::LEU2	
YTT 3722	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404-hht2K14,23R-HHF2	
YTT 3718	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404-hht2K14,23,27R-HHF2	

YTT 3773	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404-hht2K9,18,27R-HHF2	
YTT 3720	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404- hht2Δ9-14-HHF2	
YTT 2237	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX (pMP101- HHT2-hhf2K5,12R)	
YTT 3688	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404- HHT2-hhf2K5,12,16R	
YTT 3725	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404- hht2Δ9-14-hhf2K5,8,12R	
YTT 3898	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404- hht2Δ9-14-hhf2K5,12R	
YTT 3900	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404- hht2Δ9-14-hhf2K5,12,16R	
YTT 3848	*MAT***a** *hht1-hhf1*::HphMX *hht2-hhf2*::NatMX trp1-1:: pRS404-hht2K18,23,27R-hhf2K5,8,12R	

Supplementary Table 3

A table of all the yeast strains used in this study.

Supplementary Methods

In order to achieve necessary improvement in mini-chromosome purification and histone MS analyses, we modified the purification and MS protocols as the follwing:

1. Systems utilizing lac repressor (LacI) - lac operator (LacO), tet repressor - tet operator or LexA - LexA binding site were tested in parallel for minichromosome purification and the LacI – LacO system was chosen for the best yield and purity (data not shown).

2. The LacO sequence was placed in a wide nucleosome-free region upstream of *TRP1* [29] to facilitate binding of LacI to the minichromosome template (Fig 1a).

3. Minichromosomes containing one (1x), three (3x) or eight (8x) tandem copies of LacO were tested and minichromosomes with 8xLacO (henceforth referred to as TALO8) were chosen based on high yield and purity.

4. Since free LacI is highly susceptible to protease degradation *in vitro*, triple FLAG-epitope tagged LacI was expressed in yeast cells containing TALO8 such that the LacI – LacO interaction takes place *in vivo*.

5. Anti-FLAG M2 agarose beads (Sigma) and protein-G magnetic beads cross-linked with anti-FLAG M2 antibodies were tested in parallel to affinity purify

TALO8, and the latter was found to significantly reduce background of contaminating genomic DNA and non-histone proteins.

6. The optimal concentration of potassium chloride (KCl) in the wash solutions was systematically tested and it was determined that 300mM KCl yielded the cleanest sample without significant loss of minichromosome.

7. The relative abundance of LacI to TALO8 was crucial for the purity of the sample, since excess LacI brings down non-specifically bound genomic chromatin and associated proteins. LacI expressed off single and high-copy vectors were tested and it was determined that a single-copy vector, integrated into the genome, yielded the cleanest sample.

8. Elutions using Rapigest (Waters), a MS-compatible anionic surfactant, rather than competitive elution with FLAG-peptides, were found to significantly decrease the amounts of LacI that were eluted, while still resulting in complete elution of all histone proteins (Fig 1c). This eliminated the need for further purification of histones away from LacI, excess amounts of which interferes with subsequent MS analysis.
9. To detect the highly hydrophilic peptides corresponding to the histone H3 amino terminal without any additional chromatographic separations, solution-digested peptides from the elutions were directly fed from an in-line chromatographic column into the mass spectrometer.