Supplementary data

Site-specific methylation and acetylation of lysine residues in the C-terminal domain (CTD) of RNA polymerase II

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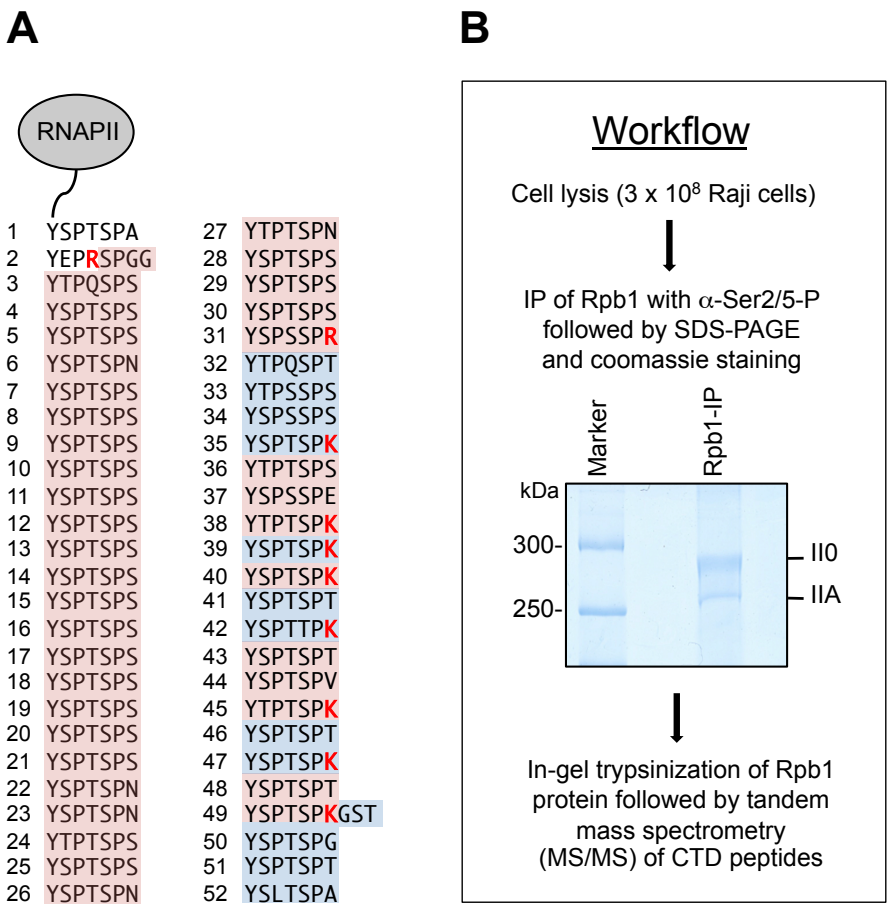


Amino acid composition of human RNAPII CTD and purification of RNAPII.
(A) Human CTD is composed of 52 consensus and non-consensus heptad repeats. Peptides of CTD after tryptic digestion are marked by red and blue boxes, basic amino acids by red letters.
(B) Purification scheme of RNAPII large subunit Rpb1.

Figure S1

YSPTSpPKacYSPTSPK

Peptide Summary

Fragment Matches

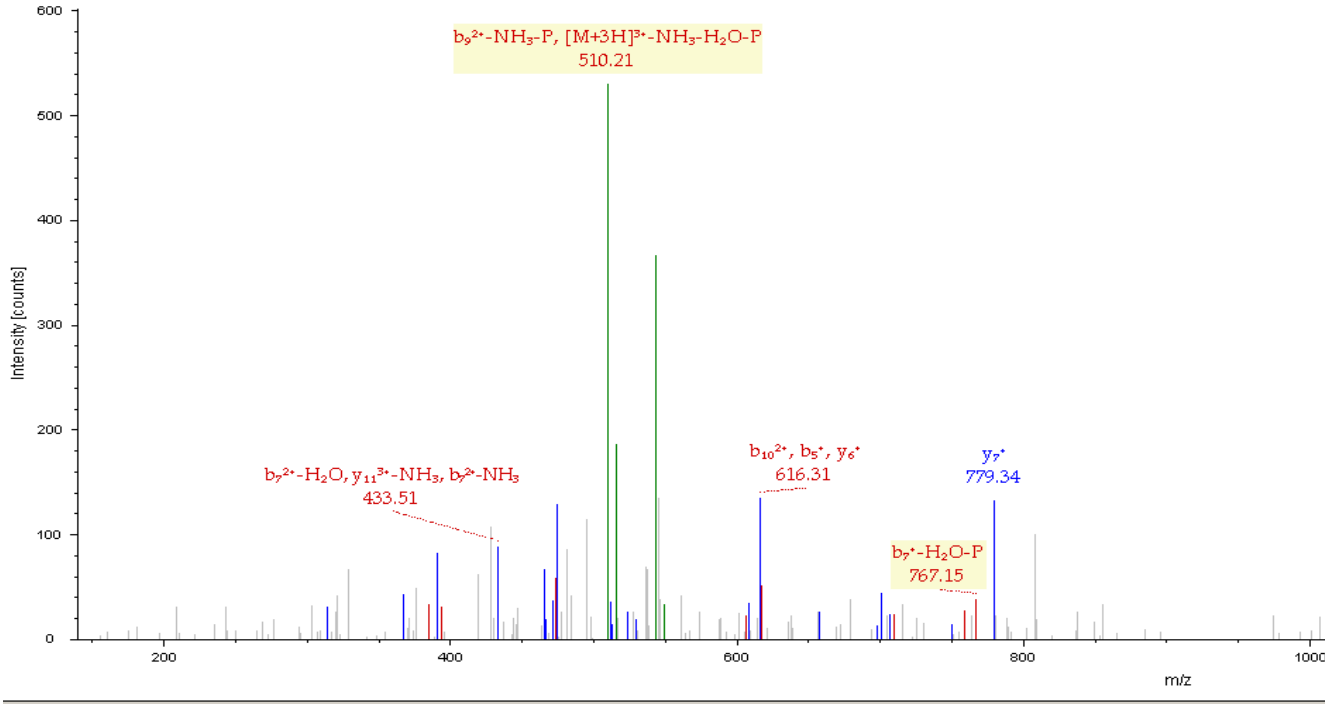
Value Type: Theo. Mass [Da]

Ion Series | Phosphorylation Losses | Neutral Losses | Multiple Neutral Losses | Precursor Ions

#1	b^{+}	b^{2+}	b^{3+}	Seq.	y^{+}	y^{2+}	y^{3+}	#2
1	164.07060	82.53894	55.36172	Y				14
2	251.10263	126.05495	84.37239	S	1498.68256	749.84492	500.23237	13
3	348.15540	174.58134	116.72332	P	1411.65053	706.32890	471.22169	12
4	449.20308	225.10518	150.40588	T	1314.59776	657.80252	438.87077	11
5	616.20144	308.60436	206.07200	S-Phospho	1213.55008	607.27868	405.18821	10
6	713.25421	357.13074	238.42292	P	1046.55172	523.77950	349.52209	9
7	883.35974	442.18351	295.12477	K-Acetyl	949.49895	475.25311	317.17117	8
8	1046.42306	523.71517	349.47921	Y	779.39341	390.20034	260.46932	7
9	1133.45509	567.23118	378.48988	S	616.33009	308.66868	206.11488	6
10	1230.50786	615.75757	410.84081	P	529.29806	265.15267	177.10420	5
11	1331.55554	666.28141	444.52337	T	432.24529	216.62628	144.75328	4
12	1418.58757	709.79742	473.53404	S	331.19761	166.10244	111.07072	3
13	1515.64034	758.32381	505.88497	P	244.16558	122.58643	82.06004	2
14				K	147.11281	74.06004	49.70912	1

Fragment Spectrum

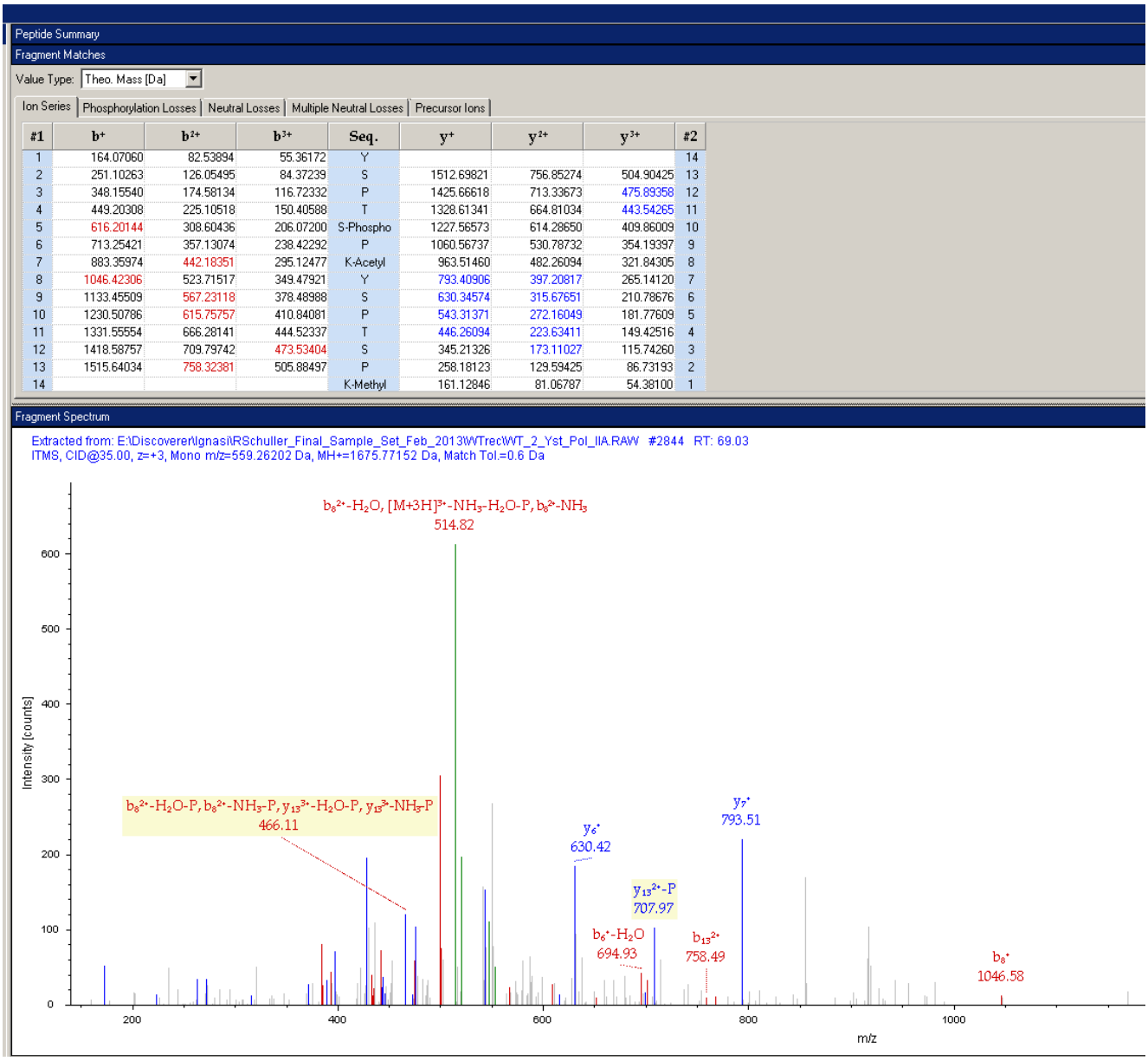
Extracted from: E:\Discoverer\Ignasi\RSchuller_Final_Sample_Set_Feb_2013\WTrecWT_2_Yst_Pol_IIA.RAW #2735 RT: 67.56
ITMS, CID@35.00, z=+3, Mono m/z=554.59113 Da, MH+=1661.75882 Da, Match Tol.=0.6 Da



MS/MS spectrum of peptide with heptad-repeats 39-40

Figure S2

YSPTSpPKacYSPTSPKme$_1$

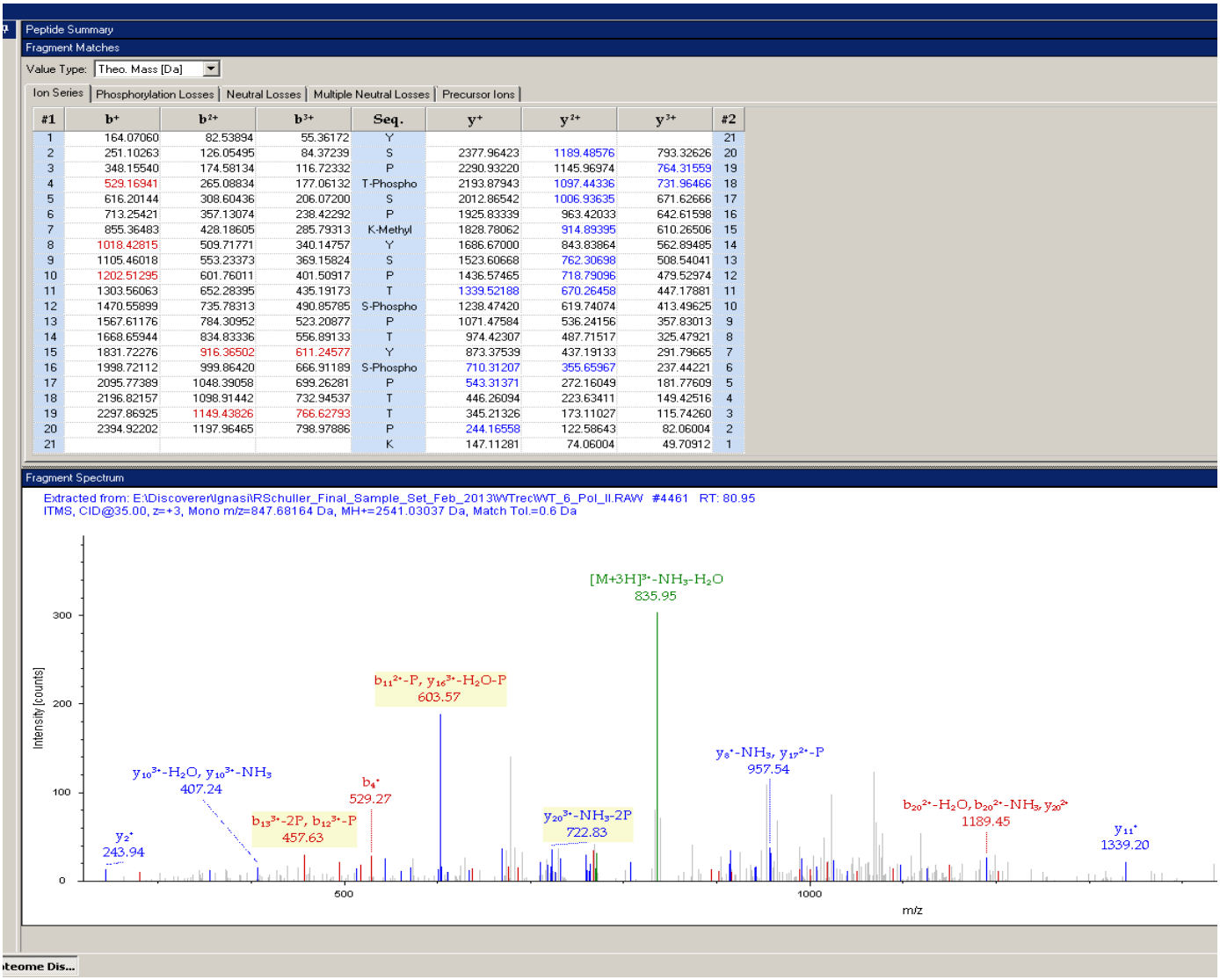


#1	b+	b2+	b3+	Seq.	y+	y2+	y3+	#2
1	164.07060	82.53894	55.36172	Y				14
2	251.10263	126.05495	84.37239	S	1512.69821	756.85274	504.90425	13
3	348.15540	174.58134	116.72332	P	1425.66618	713.33673	475.89358	12
4	449.20308	225.10518	150.40588	T	1328.61341	664.81034	443.54265	11
5	616.20144	308.60436	206.07200	S-Phospho	1227.56573	614.28650	409.86009	10
6	713.25421	357.13074	238.42292	P	1060.56737	530.78732	354.19397	9
7	883.35974	442.18351	295.12477	K-Acetyl	963.51460	482.26094	321.84305	8
8	1046.42306	523.71517	349.47921	Y	793.40906	397.20817	265.14120	7
9	1133.45509	567.23118	378.48988	S	630.34574	315.67651	210.78676	6
10	1230.50786	615.75757	410.84081	P	543.31371	272.16049	181.77609	5
11	1331.55554	666.28141	444.52337	T	446.26094	223.63411	149.42516	4
12	1418.58757	709.79742	473.53404	S	345.21326	173.11027	115.74260	3
13	1515.64034	758.32381	505.88497	P	258.18123	129.59425	86.73193	2
14				K-Methyl	161.12846	81.06787	54.38100	1

MS/MS spectrum of peptide with heptad-repeats 39-40

Figure S3

YSPTpSPKme$_1$YSPTSpPTYSpPTTPK

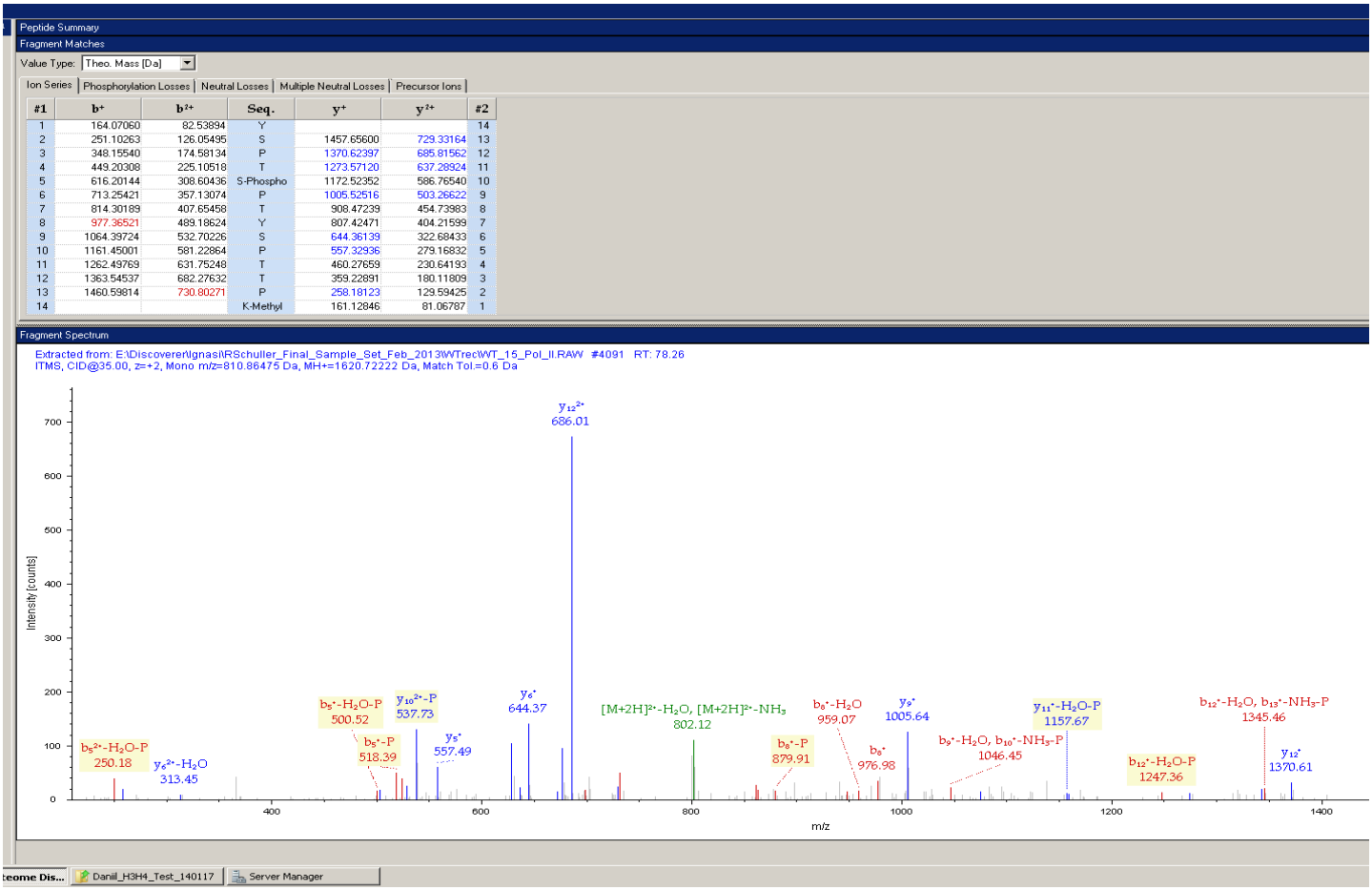


#1	b+	b2+	b3+	Seq.	y+	y2+	y3+	#2
1	164.07060	82.53894	55.36172	Y				21
2	251.10263	126.05495	84.37239	S	2377.96423	1189.48576	793.32626	20
3	348.15540	174.58134	116.72332	P	2290.93220	1145.96974	764.31559	19
4	529.16941	265.08834	177.06132	T-Phospho	2193.87943	1097.44336	731.96466	18
5	616.20144	308.60436	206.07200	S	2012.86542	1006.93635	671.62666	17
6	713.25421	357.13074	238.42292	P	1925.83339	963.42033	642.61598	16
7	855.36483	428.18605	285.79313	K-Methyl	1828.78062	914.89395	610.26506	15
8	1018.42815	509.71771	340.14757	Y	1686.67000	843.83864	562.89485	14
9	1105.46018	553.23373	369.15824	S	1523.60668	762.30698	508.54041	13
10	1202.51295	601.76011	401.50917	P	1436.57465	718.79096	479.52974	12
11	1303.56063	652.28395	435.19173	T	1339.52188	670.26458	447.17881	11
12	1470.55899	735.78313	490.85785	S-Phospho	1238.47420	619.74074	413.49625	10
13	1567.61176	784.30952	523.20877	P	1071.47584	536.24156	357.83013	9
14	1668.65944	834.83336	556.89133	T	974.42307	487.71517	325.47921	8
15	1831.72276	916.36502	611.24577	Y	873.37539	437.19133	291.79665	7
16	1998.72112	999.86420	666.91189	S-Phospho	710.31207	355.65967	237.44221	6
17	2095.77389	1048.39058	699.26281	P	543.31371	272.16049	181.77609	5
18	2196.82157	1098.91442	732.94537	T	446.26094	223.63411	149.42516	4
19	2297.86925	1149.43826	766.62793	T	345.21326	173.11027	115.74260	3
20	2394.92202	1197.96465	798.97886	P	244.16558	122.58643	82.06004	2
21				K	147.11281	74.06004	49.70912	1

MS/MS spectrum of peptide with heptad-repeats 40-42

Figure S4

YSPTpSPTYSPTTPKme$_1$

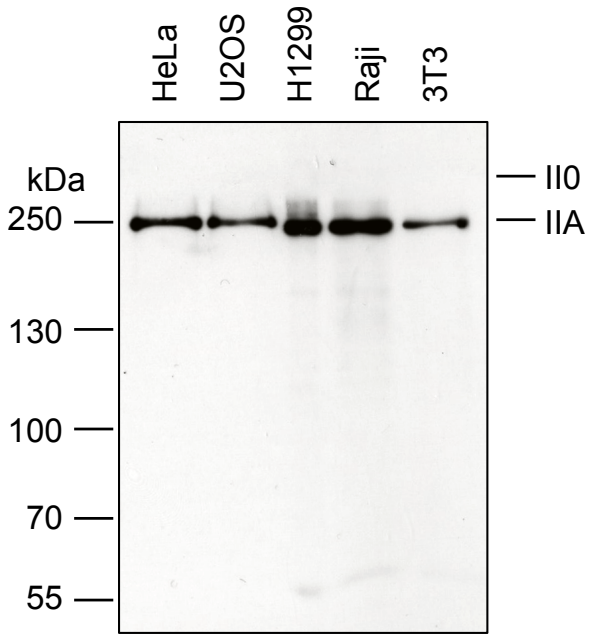


#1	b+	b2+	Seq.	y+	y2+	#2
1	164.07060	82.53894	Y			14
2	251.10263	126.05495	S	1457.65600	729.33164	13
3	348.15540	174.58134	P	1370.62397	685.81562	12
4	449.20308	225.10518	T	1273.57120	637.28924	11
5	616.20144	308.60436	S-Phospho	1172.52352	586.76540	10
6	713.25421	357.13074	P	1005.52516	503.26622	9
7	814.30189	407.65458	T	908.47239	454.73983	8
8	977.36521	489.18624	Y	807.42471	404.21599	7
9	1064.39724	532.70226	S	644.36139	322.68433	6
10	1161.45001	581.22864	P	557.32936	279.16832	5
11	1262.49769	631.75248	T	460.27659	230.64193	4
12	1363.54537	682.27632	T	359.22891	180.11809	3
13	1460.59814	730.80271	P	258.18123	129.59425	2
14			K-Methyl	161.12846	81.06787	1

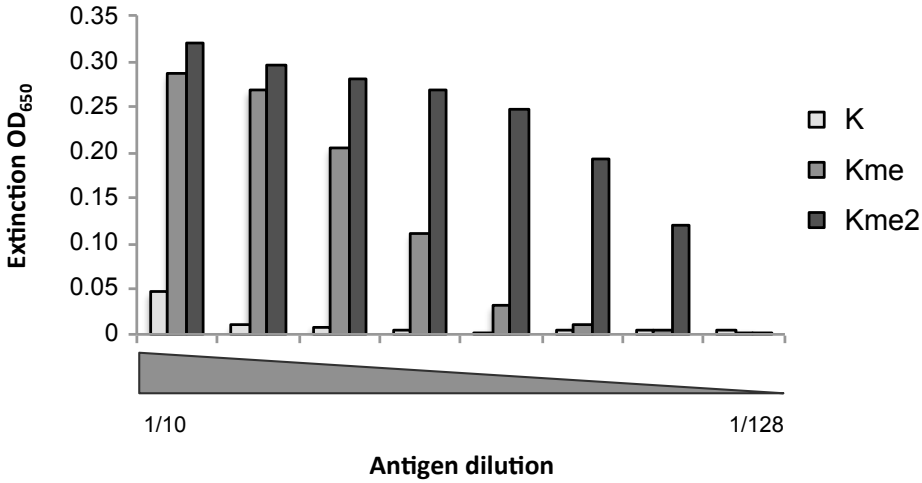
MS/MS spectrum of peptide with heptad-repeats 41-42

Figure S5

A



B

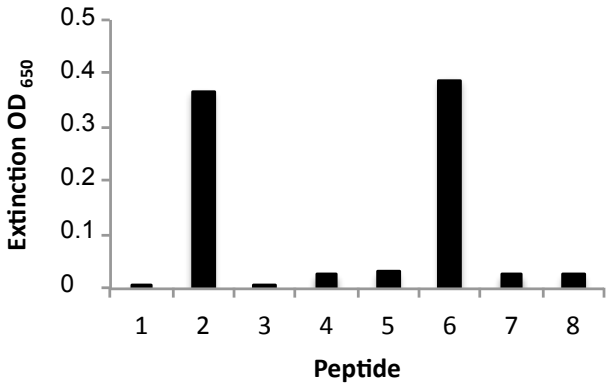


Peptide	Sequence	Modification
1	Y S P T S P K Y S P T S P K C	-
2	Y S P T S P Kme1 Y S P T S P K C	Kme
3	Y S P T S P Kme2 Y S P T S P K C	Kme2

(A) Reactivity of mAb 1F5 for RNAPII in cellular extracts of human cell lines HeLa, U2OS, H1299, and Raji, and mouse cell line NIH3T3.
(B) Titration of the specificity of mAb 1F5 for K7me1 and K7me2 epitopes in ELISA using synthetic di-heptad peptides with the corresponding modification.

Figure S6

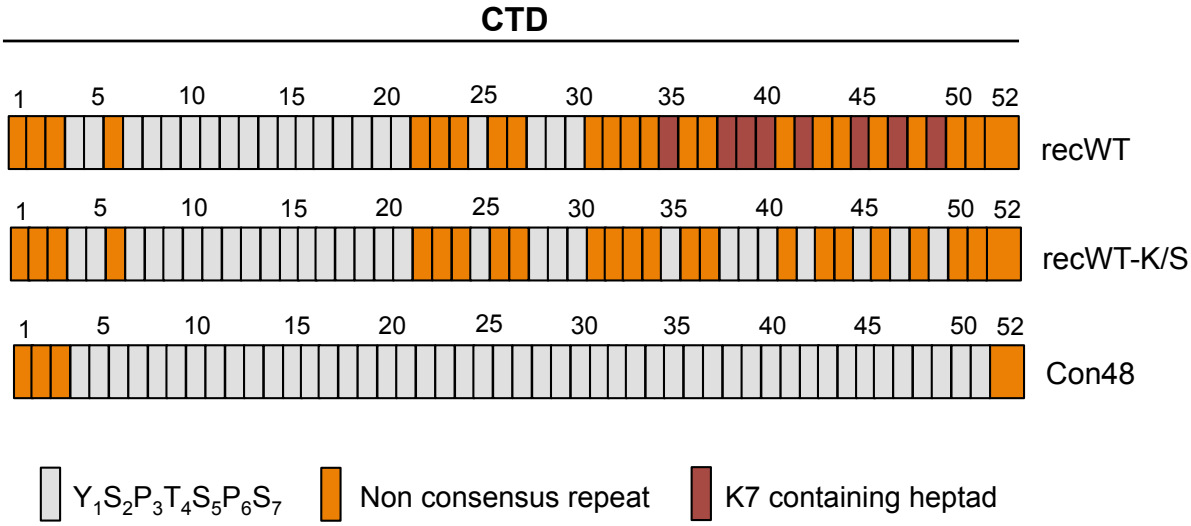
Peptide	Sequence	Modification
1	T S P S Y S P T S P K Y S P T S P S-PEG-Biotin	-
2	T S P S Y S P T S P K_{me2} Y S P T S P S-PEG-Biotin	Kme2
3	T S P S Y S P T S_P P K Y S P T S P S-PEG-Biotin	Ser5P
4	T S P S Y S P T S_P P K_{me2} Y S P T S P S-PEG-Biotin	Ser5P, Kme2
5	T S P S Y S P T S P K Y S_P P T S P S-PEG-Biotin	Ser2P
6	T S P S Y S P T S P K_{me2} Y S_P P T S P S-PEG-Biotin	Kme2, Ser2P
7	T S P S Y S P T S_P P K Y S_P P T S P S-PEG-Biotin	Ser2P/Ser5P
8	T S P S Y S P T S_P P K_{me2} Y S_P P T S P S-PEG-Biotin	Ser2P/Ser5P, Kme2



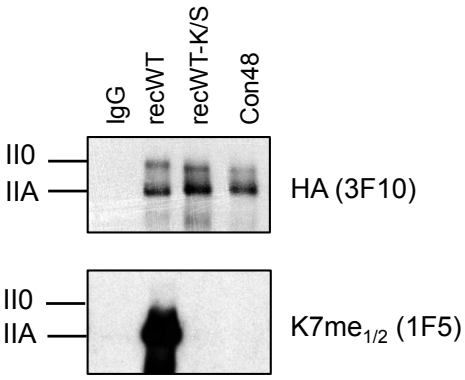
Phosphorylation of adjacent amino acids (red) can inhibit reactivity of mAb 1F5. ELISA experiment.

Figure S7

A



B



Recognition of RNAPII by mAb 1F5 requires CTD K7 residues.
(A) recWT: wild-type CTD; recWT-K/S: K7 containing heptads were replaced by consensus repeats; Con48, CTD with 48 consensus repeats.
(B) Western analysis of RNAPII mutants with mAb 1F5.

Figure S8