Supplemental Data

Quantitative Mass Spectrometry Reveals that Intact Histone H1 Phosphorylations are Variant Specific and Exhibit Single Molecule Hierarchical Dependence

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Table S1. Relative abundance of proteoforms at different cell cycle stages of two cell lines(%)

Proteoforms	MDA-MB-231			MCF-10A		
	Asyn	S phase	M phase	Asyn	S phase	M phase
H12ac	16.9 ± 0.9	13.7 ± 1.0	10.5 ± 1.1	4.9 ± 0.6	4.5 ± 0.2	1.2 ± 0.2
H12acph	8.1 ± 1.2	4.3 ± 0.4	6.3 ± 0.6	7.9 ± 1.1	2.3 ± 0.3	2.6 ± 0.5
H12SNPac	0	0	0	4.4 ± 0.3	4.1 ± 0.3	2.2 ± 0.5
H12SNPacph	0	0	0	6.1 ± 0.7	1.8 ± 0.2	2.6 ± 0.8
H12SNPacph2	0	0	0	0	0	2.1 ± 0.2
H13ac	0	0	0	4.6 ± 0.4	2.2 ± 0.2	2.4 ± 0.3
H13acph	0	0	0	1.7 ± 0.5	1.2 ± 0.2	1.1 ± 0.1
H14ac	35.6 ± 0.8	48.2 ± 1.8	27.5 ± 2.8	11.8 ± 2.4	38.9 ± 1.4	10.2 ± 2.2
H14ph	2.3 ± 0.2	2.2 ± 0.1	1.6 ± 0.2	1.8 ± 0.4	3.8 ± 0.4	1.5 ± 0.4
H14acph	24.5 ± 1.4	22.2 ± 1.0	20.1 ± 0.5	26.5 ± 2.1	24.7 ± 1.7	25.6 ± 2.1
H14acph2	11.5 ± 0.8	8.7 ± 0.9	14.7 ± 2.0	22.3 ± 1.2	13.7 ± 0.7	26.6 ± 3.5
H14acph3	1.0 ± 0.5	0.7 ± 0.2	11.3 ± 2.4	8.2 ± 1.9	2.8 ± 0.5	15.3 ± 3.7
H14acph4	0	0	5.8 ± 0.7	0	0	5.4 ± 3.4
H14acph5	0	0	2.1 ± 0.2	0	0	1.3 ± 0.2

Table S2. PTM identification on histone H1.2SNPA18VNα-ac

	H12ac (*m/z*)	**H12SNPac (*m/z*)**	**Δ *m/z***	**Δ mass (Da)**
Asyn 1-1	608.86689	609.66750	0.80061	28.02135
Asyn 1-2	608.86739	609.66838	0.80099	28.03465
Asyn 1-3	608.86733	609.66820	0.80087	28.03045
Asyn 2-1	608.86640	609.66762	0.80122	28.04270
Asyn 2-2	608.86673	609.66782	0.80109	28.03815
Asyn 2-3	608.86734	609.66785	0.80051	28.01785
S phase 1-1	608.86597	609.66711	0.80114	28.03990
S phase 1-2	608.86624	609.66726	0.80102	28.03570
S phase 1-3	608.86637	609.66762	0.80125	28.04375
S phase 2-1	608.86607	609.66784	0.80177	28.06195
S phase 2-2	608.86656	609.66796	0.80140	28.04900
S phase 2-3	608.86678	609.66774	0.80096	28.03360

Note, charge state 35^{+}, average Δ *m/z* is 28.03742 ± 0.01178, 0.528 σ for H12SNP and dimethylation, and 3.611 σ for formylation, given the theoretical mass of H12SNP and dimethylationis 28.03132, whereas formylation is 27.9949.

Table S3. P-values ofANOVA and t-tests for proteoforms relative abundance at different cell cycle stages

	MDA-MB231		MCF10A	
Proteoforms	**ANOVA-test**	**t-test**	**ANOVA-test**	**t-test**
H12ac	4.95×10^{-8}	3.72×10^{-4}	8.15×10^{-11}	1.78×10^{-10}
H12acph	3.71×10^{-6}	7.64×10^{-5}	4.64×10^{-10}	***0.13***
H12SNPac	N/A	N/A	3.70×10^{-8}	6.64×10^{-6}
H12SNPacph	N/A	N/A	1.15×10^{-8}	***0.05***
H12SNPacph2	N/A	N/A	6.28×10^{-15}	3.48×10^{-10}
H13ac	N/A	N/A	8.30×10^{-10}	***0.18***
H13acph	N/A	N/A	***0.01***	***0.25***
H14ph	8.9×10^{-6}	4.97×10^{-5}	2.03×10^{-7}	2.71×10^{-6}
H14ac	5.92×10^{-11}	3.04×10^{-8}	1.57×10^{-13}	1.22×10^{-10}
H14acph	1.3×10^{-5}	1.15×10^{-3}	***0.32***	***0.47***
H14acph2	6.8×10^{-6}	5.92×10^{-5}	1.23×10^{-7}	4.52×10^{-6}
H14acph3	1.5×10^{-9}	9.39×10^{-7}	8.96×10^{-7}	8.86×10^{-6}
H14acph4	1.33×10^{-13}	2.65×10^{-9}	2.92×10^{-4}	3.29×10^{-3}
H14acph5	1.02×10^{-14}	4.81×10^{-10}	1.48×10^{-12}	1.31×10^{-8}

Table S4. P-values ofANOVA and t-tests for histone variants total phosphorylations at different cell cycle stages

	MDA-MB231		MCF10A	
Proteoforms	**ANOVA-test**	**t-test**	**ANOVA-test**	**t-test**
H12	2.31×10^{-6}	7.79×10^{-6}	2.26×10^{-15}	1.41×10^{-12}
H13	N/A	N/A	***0.02***	***0.17***
H14	7.07×10^{-11}	2.12×10^{-8}	1.49×10^{-13}	5.23×10^{-11}

Table S5. P-values ofANOVA and t-tests for total phosphorylations on different sites at different cell cycle stages

	MDA-MB231		MCF10A	
Proteoforms	**ANOVA-test**	**t-test**	**ANOVA-test**	**t-test**
H12S173	2.31×10^{-6}	7.79×10^{-6}	2.26×10^{-15}	1.41×10^{-12}
H14S2	1.25×10^{-6}	6.13×10^{-5}	1.94×10^{-6}	2.80×10^{-6}
H14S172	9.10×10^{-11}	2.45×10^{-8}	1.62×10^{-14}	3.72×10^{-11}
H14S187	7.74×10^{-12}	1.03×10^{-8}	7.20×10^{-11}	6.99×10^{-9}
H14T18	1.08×10^{-15}	5.94×10^{-11}	9.01×10^{-6}	7.64×10^{-5}
H14T146	6.48×10^{-14}	1.64×10^{-9}	5.35×10^{-5}	1.13×10^{-3}
H14T153	8.51×10^{-15}	4.26×10^{-10}	2.67×10^{-12}	1.93×10^{-8}

Table S6. P-values of t-tests for proteoformrelative abundance between two cell lines

Proteoforms	**Asynchronous**	**S phase**	**M phase**
H12ac	8.01×10^{-11}	5.14×10^{-10}	2.42×10^{-9}
H12acph	***0.71***	2.58×10^{-6}	5.87×10^{-7}
H12SNPac	5.06×10^{-12}	5.66×10^{-12}	3.49×10^{-7}
H12SNPacph	1.05×10^{-9}	1.80×10^{-9}	1.48×10^{-5}
H12SNPacph2	N/A	N/A	3.48×10^{-10}
H13ac	3.24×10^{-11}	5.18×10^{-11}	6.55×10^{-9}
H13acph	6.48×10^{-6}	4.75×10^{-9}	1.96×10^{-11}
H14ph	0.04	3.23×10^{-6}	***0.67***
H14ac	5.02×10^{-10}	1.31×10^{-6}	3.39×10^{-7}
H14acph	***0.08***	0.01	1.14×10^{-4}
H14acph2	6.07×10^{-9}	9.62×10^{-7}	2.84×10^{-5}
H14acph3	4.69×10^{-6}	1.58×10^{-6}	***0.05***
H14acph4	N/A	N/A	***0.77***
H14acph5	N/A	N/A	5.99×10^{-5}

Table S7. P-values for histone variants total phosphorylations between two cell lines

Histone Variants	Asynchronous	S phase	M phase
H12	5.70×10^{-9}	8.35×10^{-8}	7.89×10^{-9}
H13	1.59×10^{-6}	4.70×10^{-9}	1.61×10^{-9}
H14	5.43×10^{-10}	5.99×10^{-8}	3.40×10^{-7}

Table S8. P-values for total phosphorylations on different sites between two cell lines

Proteoforms	Asynchronous	S phase	M phase
H12S173	5.7×10^{-9}	8.35×10^{-8}	7.89×10^{-9}
H14S2	***0.19***	6.89×10^{-6}	***0.51***
H14S172	1.15×10^{-10}	8.79×10^{-8}	6.58×10^{-7}
H14S187	1.26×10^{-8}	3.89×10^{-7}	1.21×10^{-4}
H14T18	1.22×10^{-5}	1.40×10^{-6}	***0.49***
H14T146	N/A	N/A	***0.29***
H14T154	N/A	N/A	3.94×10^{-5}

Legends for Supporting Information Figures

Figure S1. Broadband ESI positive ion 9.4 T FT-ICR mass spectra of histone H1 from asynchronous, s phase, and m phase cells from cell lines MDA-MB-231 and MCF-10A.

Figure S2. ECD fragmentation maps for different histone proteoforms from cell line MDA-MB-231.

Figure S3. ECD fragmentation maps for different proteoforms from cell line MCF-10A.

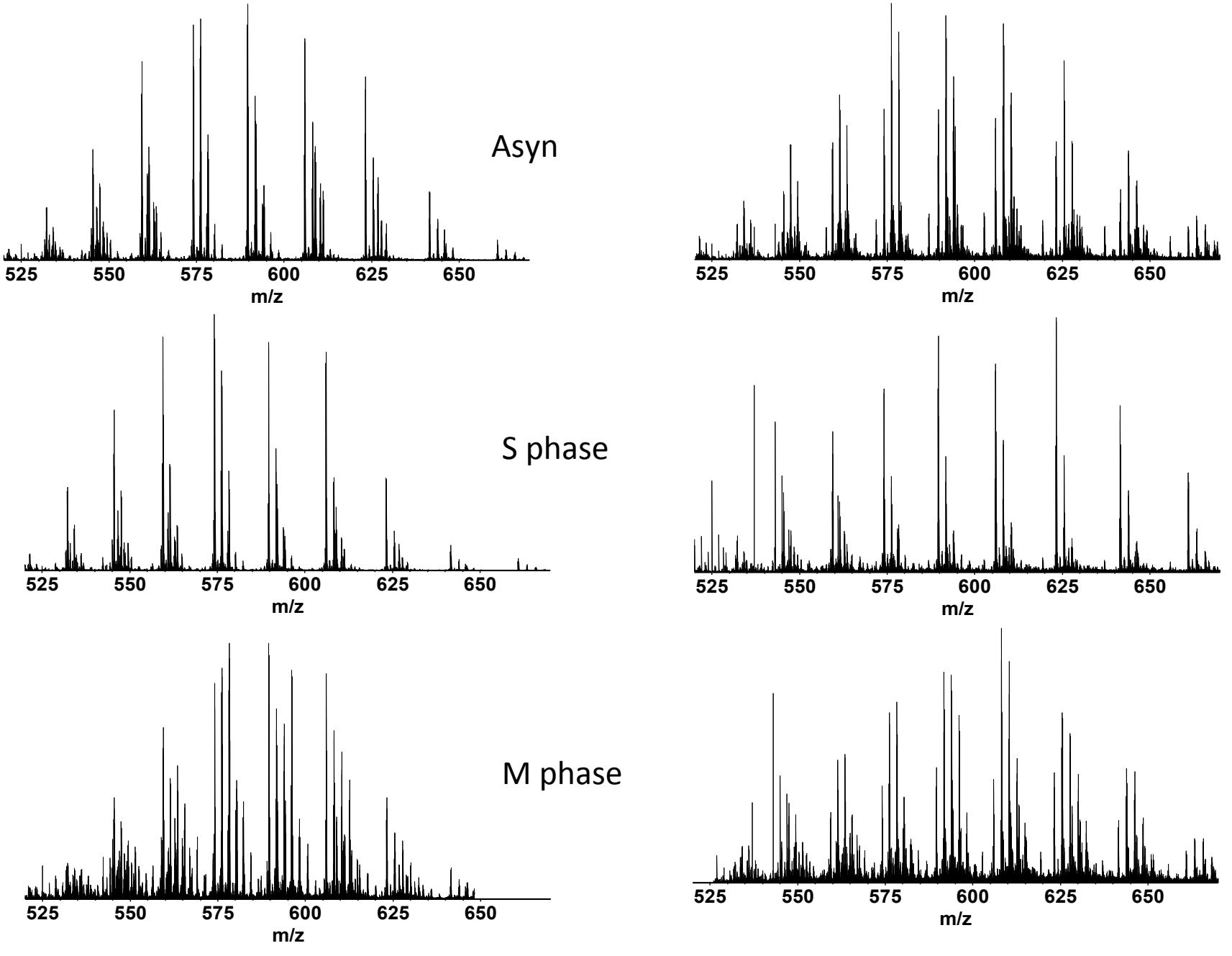
Figure S4. Mass scale-expanded segment of ECD product ion mass spectra of histone H1.2 SNP A18V from asynchronous cells from cell line MCF-10A, showing identification of c_{16}^{2+}, c_{18}^{2+}, c_{19}^{2+}, c_{20}^{2+}, c_{21}^{2+}, c_{21}^{3+}, c_{22}^{2+}, and c_{22}^{3+} ions.

Figure S5. Peak annotations for the ECD product ion mass spectrum of asynchronous histone H1.2 SNP A18V from cell line MCF-10A. .

Figure S6. Peak annotations for the ECD product ion mass spectrum of S phase histone H1.2 SNP A18V from cell line MCF-10A. .

Figure S7. Peak annotations for the ECD product ion mass spectrum of asynchronous phosphorylated histone H1.2 SNP A18V from cell line MCF-10A.

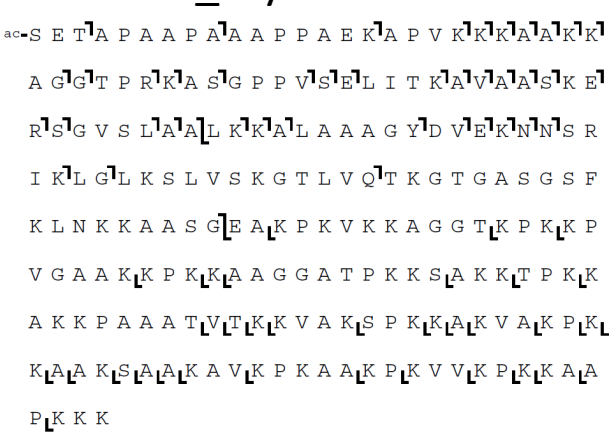
Figure S1


MDA-MB-231
MCF-10A
Asyn
S phase
M phase
525
550
575
600
625
650
m/z

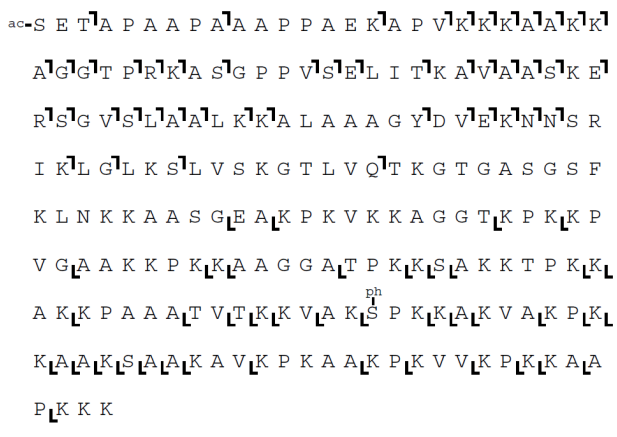
MDA-MB-231

Figure S2

H12ac_asyn



H12acph_asyn



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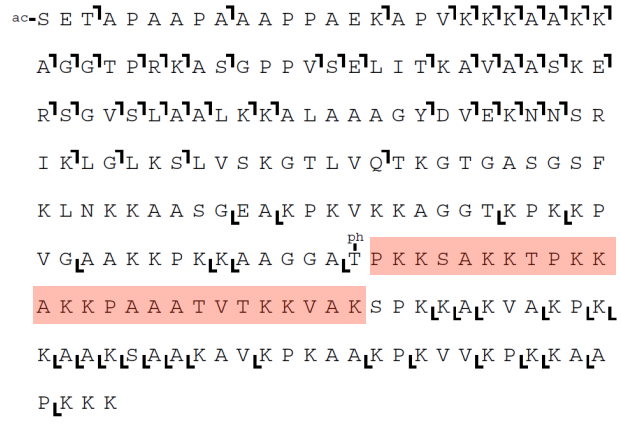

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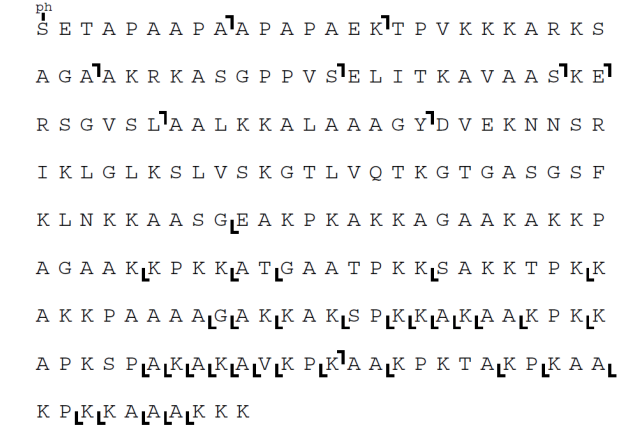

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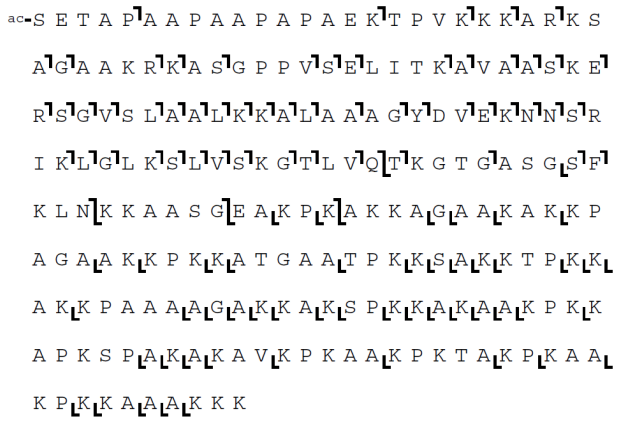

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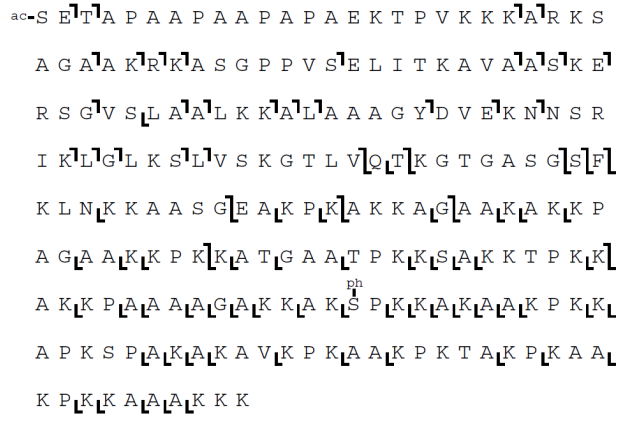
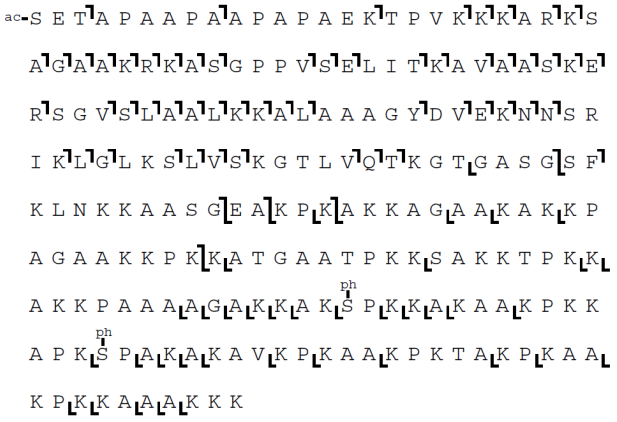


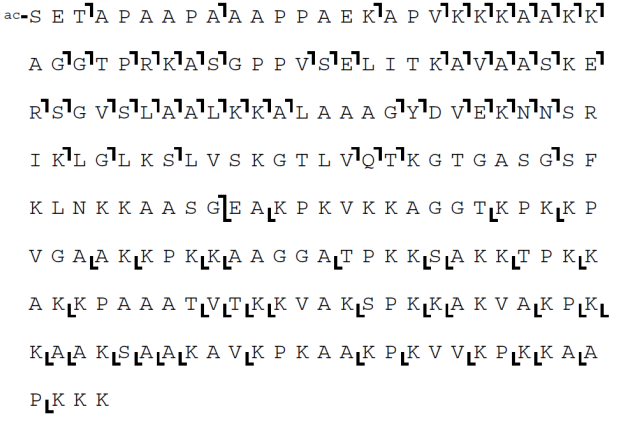
MDA-MB-231

Figure S2

H14acphx2_asyn



H12ac_s phase



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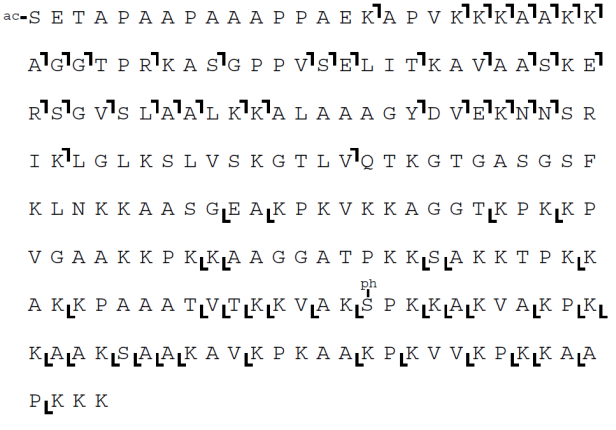

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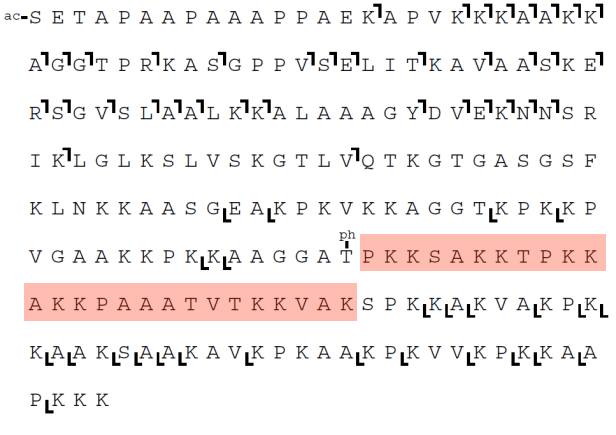
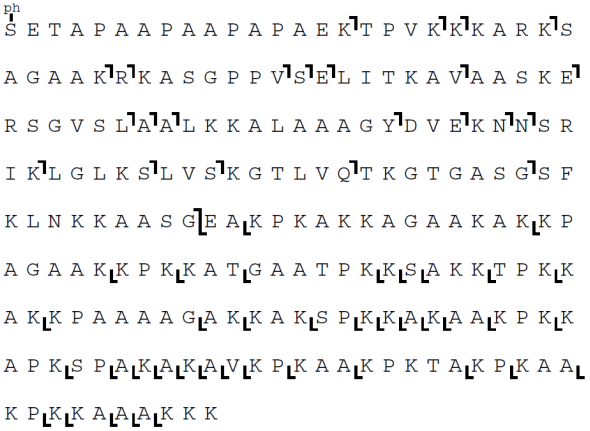


MDA-MB-231

Figure S2

H14ph_s phase



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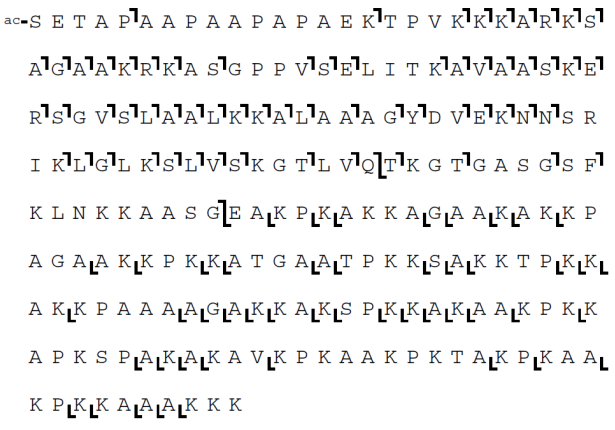

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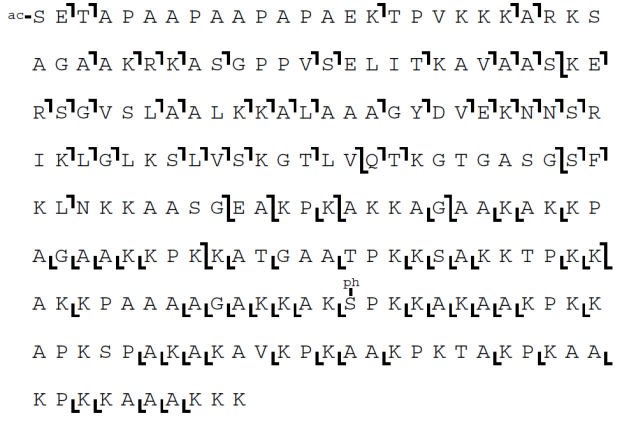

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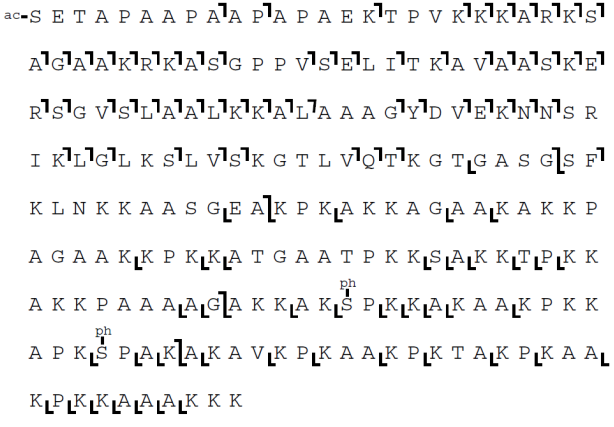
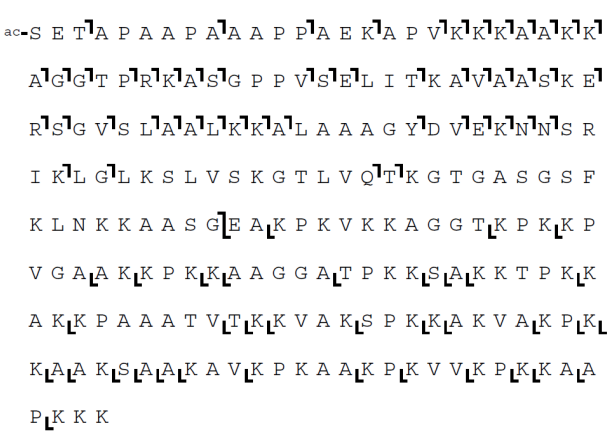


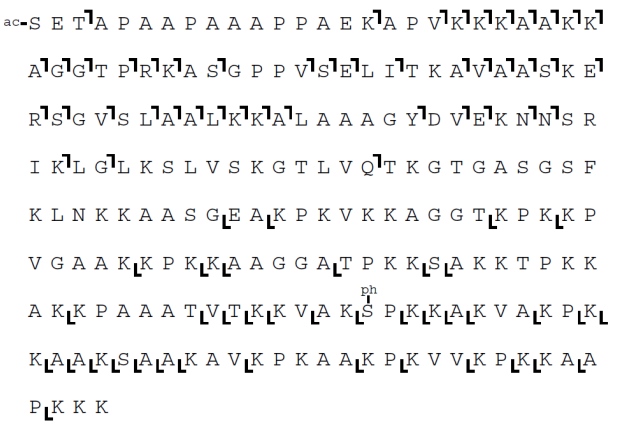
MDA-MB-231

Figure S2

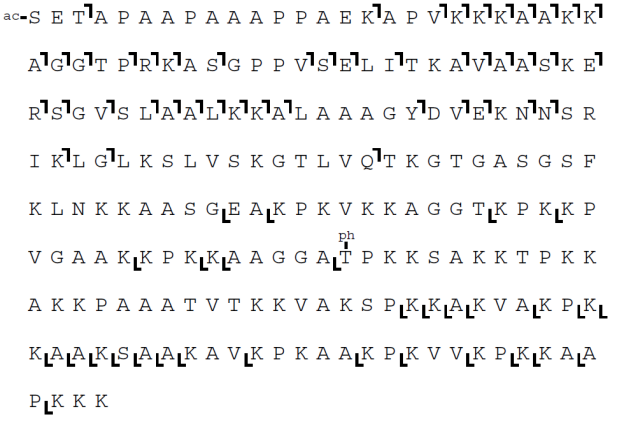
H12ac_m phase



H12acph_m phase



H12acT146ph_m phase



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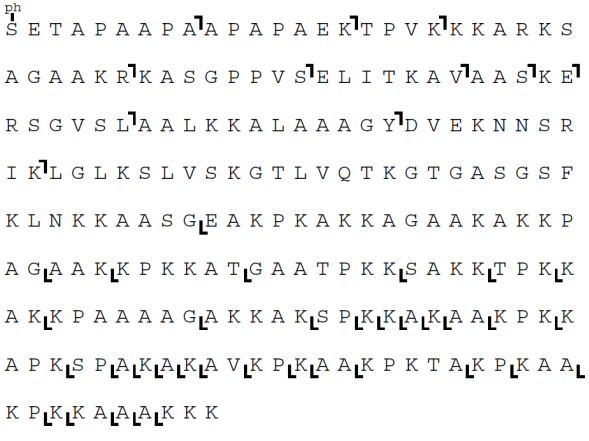

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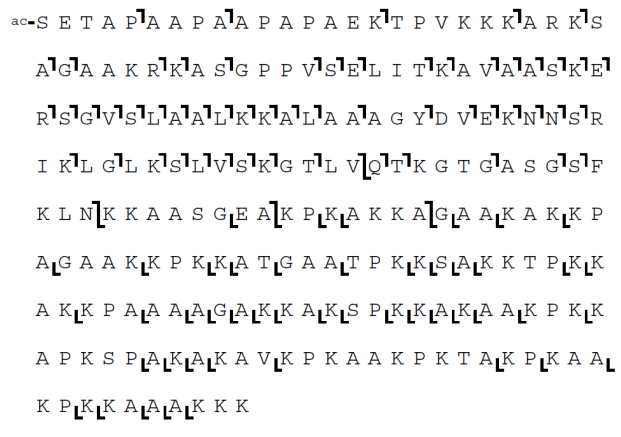

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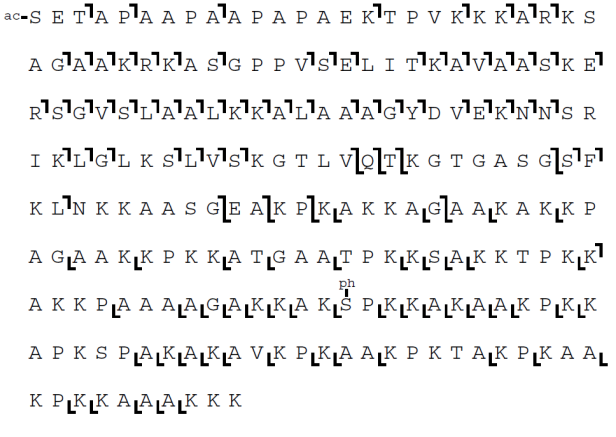
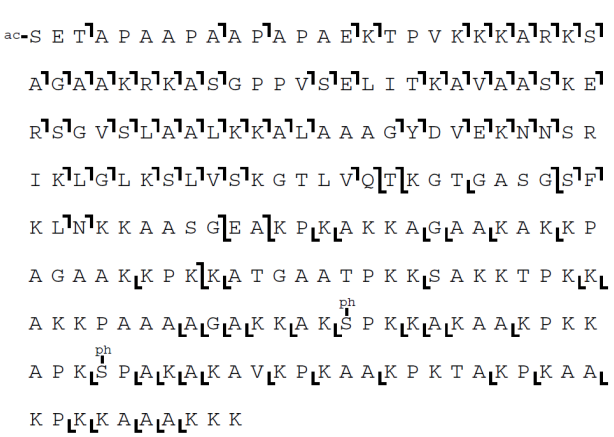


Figure S2

H14acph2_m phase



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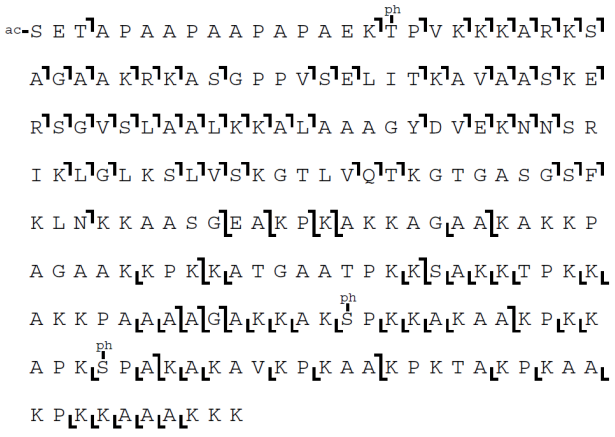

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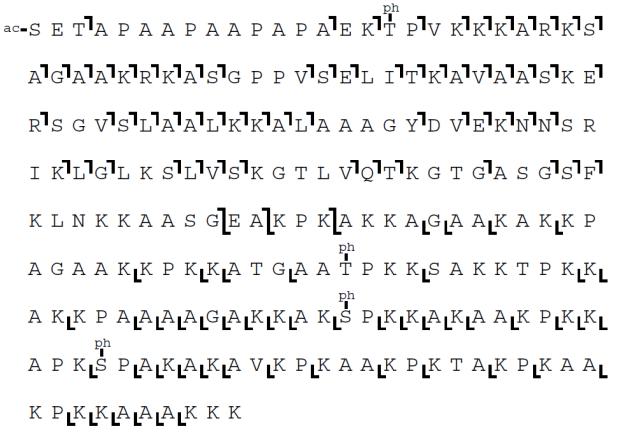

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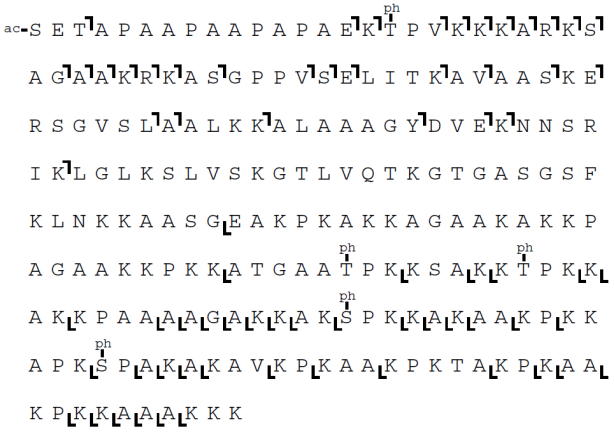

MCF-10A

Figure S3

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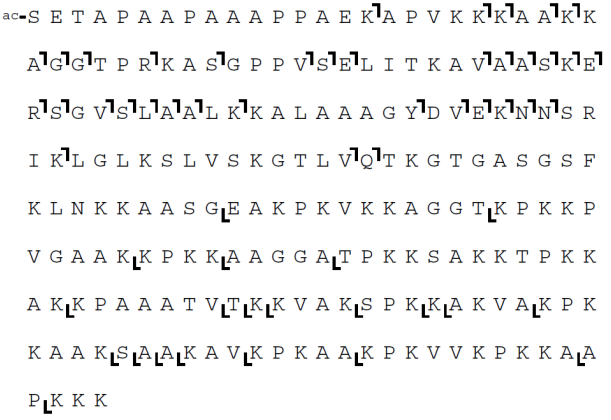

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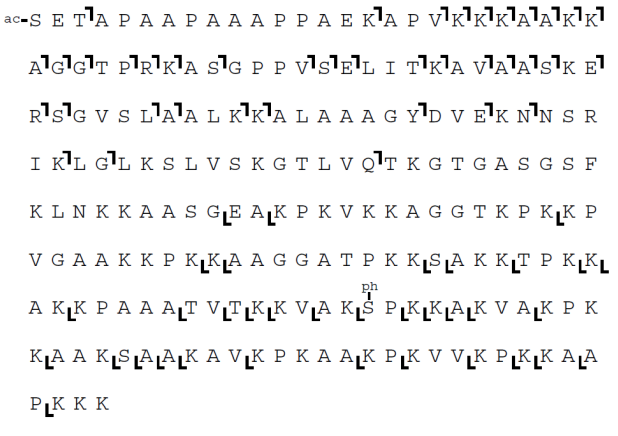

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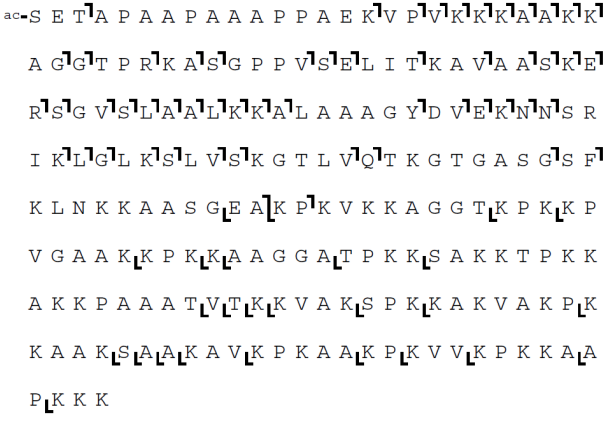

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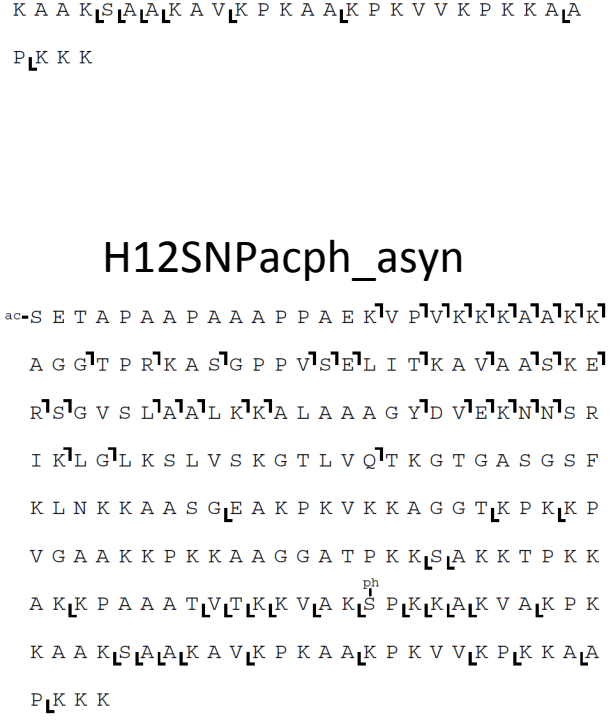

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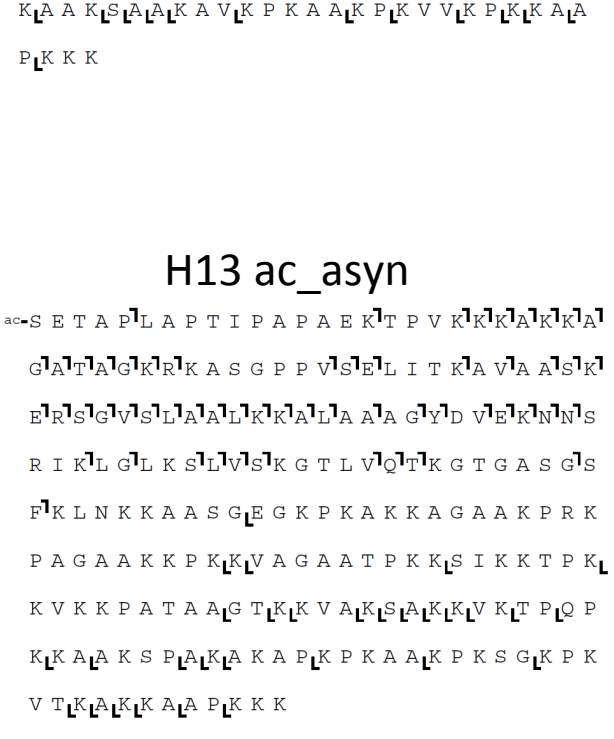
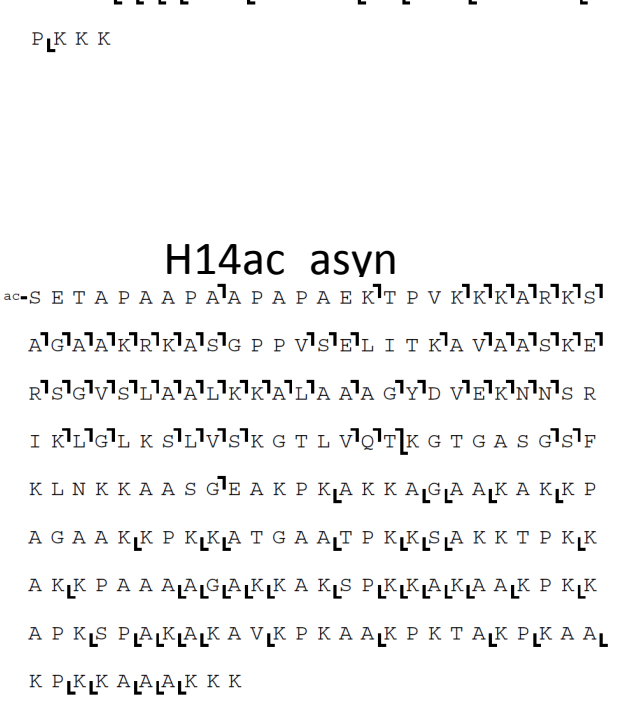
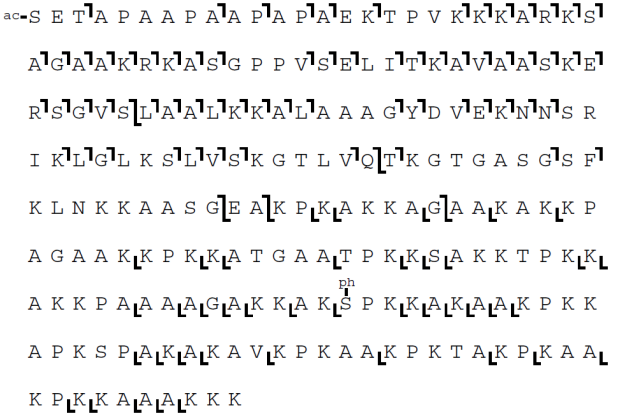


H14ac asyn



MCF-10A

H14acph_asyn



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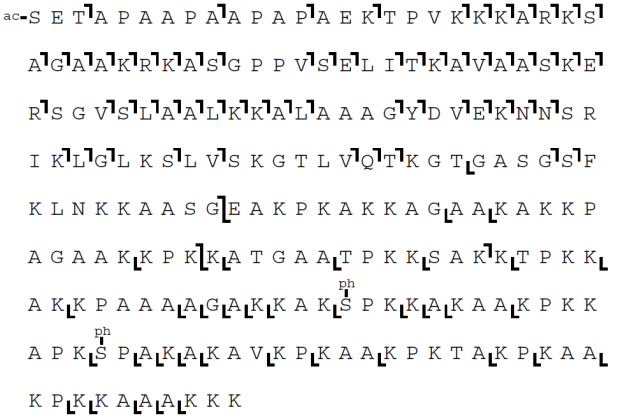
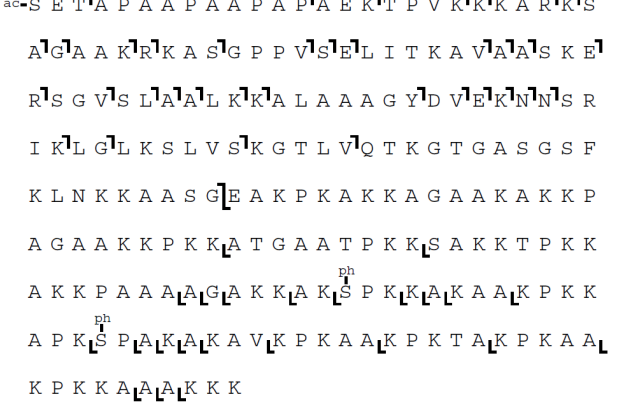
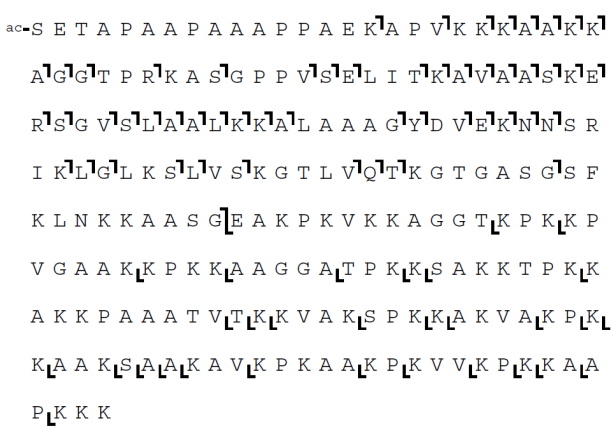


H14acph3_asyn

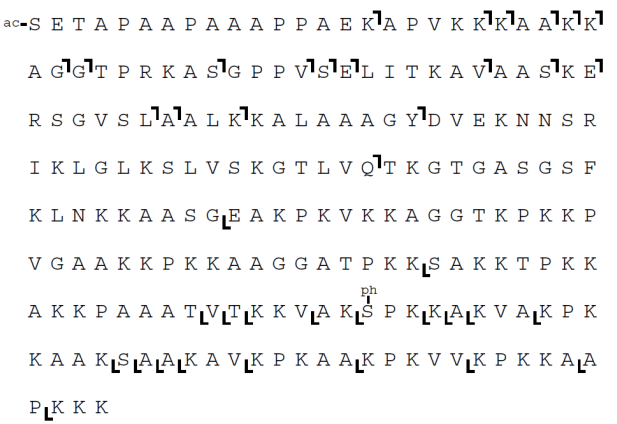


MCF-10A s phase

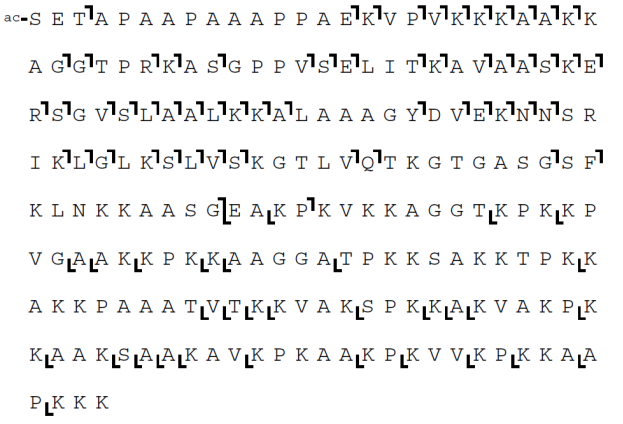
H12ac_s phase



H12acph_s phase



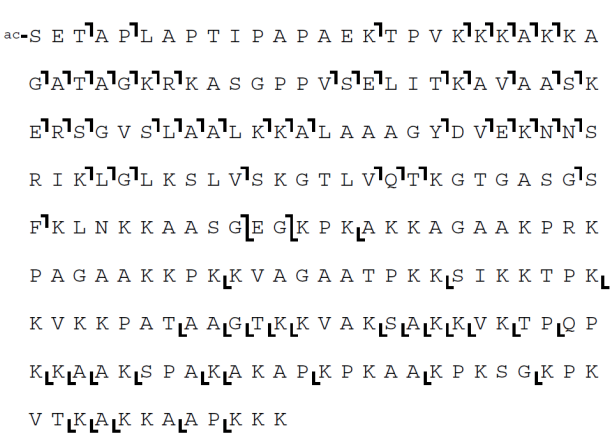
H12SNPac_s phase



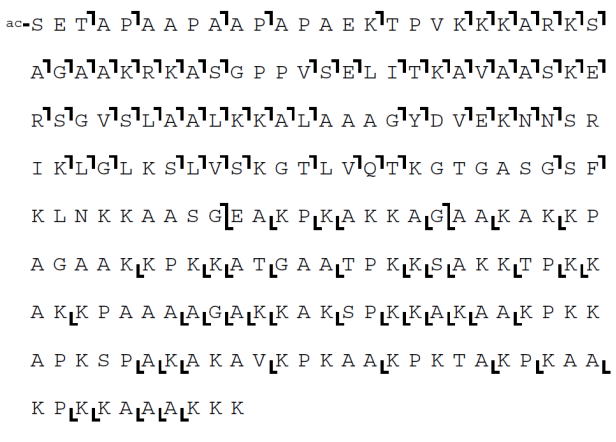
MCF-10A

Figure S3

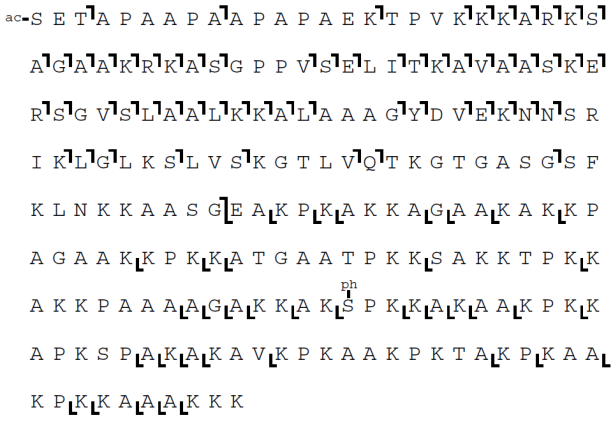
H13ac_s phase



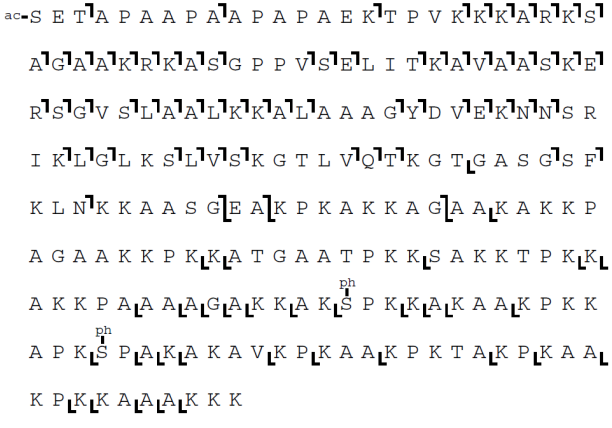
H14ac_s phase



H14acph_ s phase



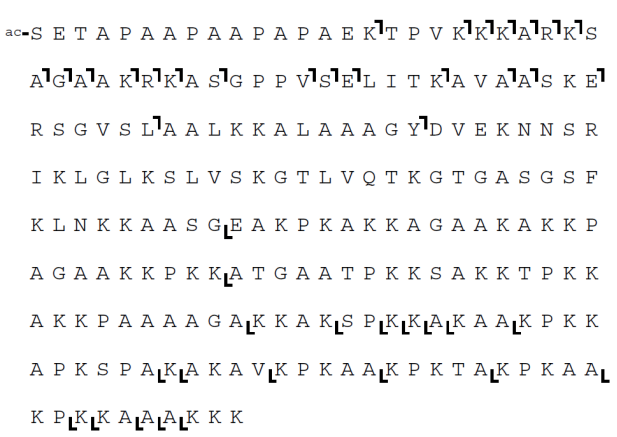
H14acph2_ s phase



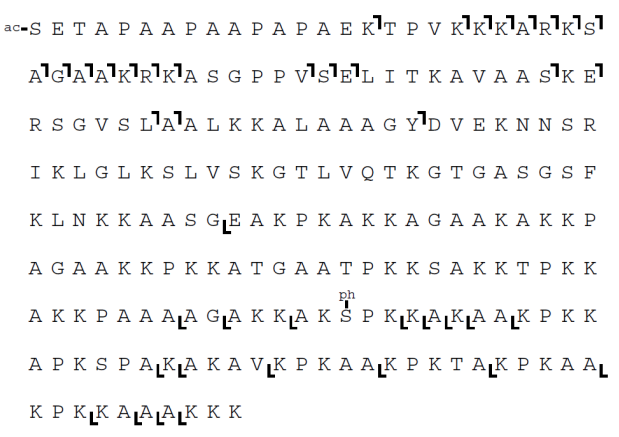
MCF-10A m phase

Figure S3

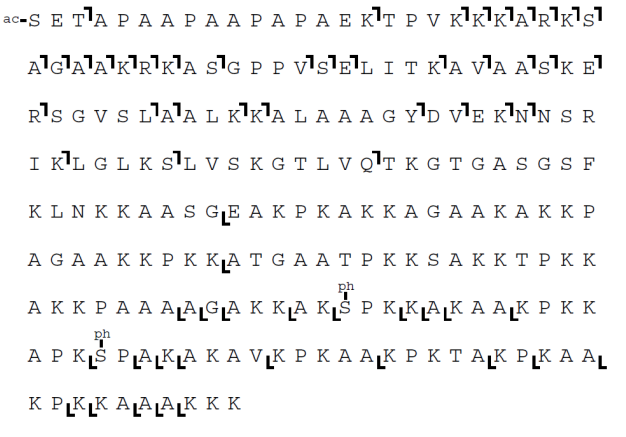
H14ac_m phase



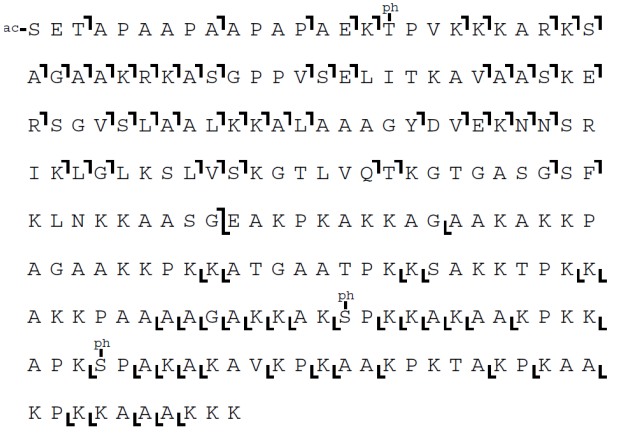
H14acph _m phase



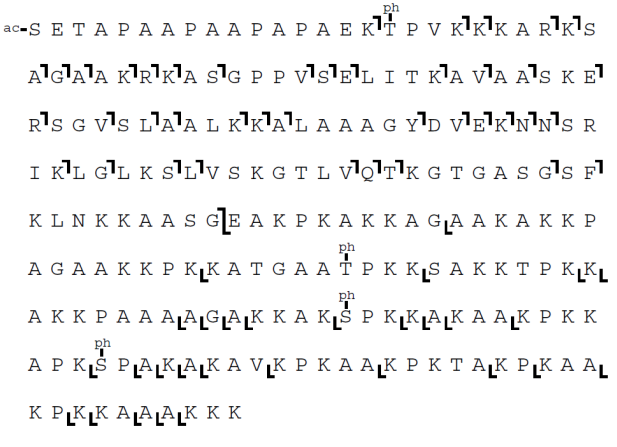
H14acph2 _m phase



H14acph3 _m phase



H14acph4 _m phase



H14acph5 _m phase

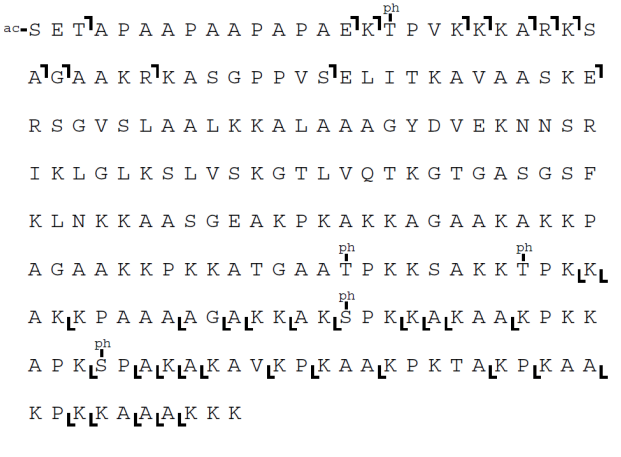
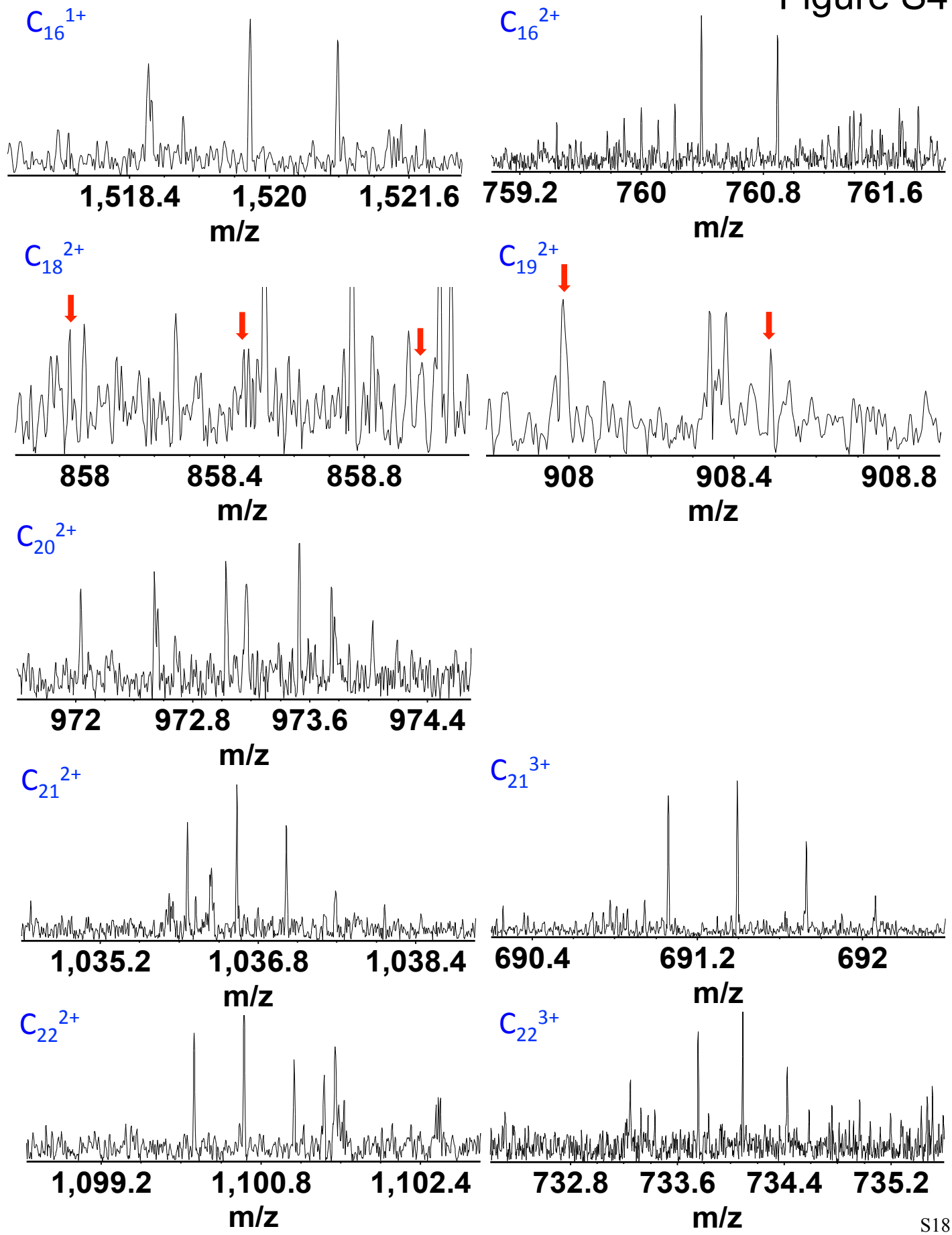


Figure S4


C16 1+
1,518.4
1,520
1,521.6
m/z
C16 2+
759.2
760
760.8
761.6
m/z
C18 2+
858
858.4
858.8
m/z
C19 2+
908
908.4
908.8
m/z
C20 2+
972
972.8
973.6
974.4
m/z
C21 2+
1,035.2
1,036.8
1,038.4
m/z
C21 3+
690.4
691.2
692
m/z
C22 2+
1,099.2
1,100.8
1,102.4
m/z
C22 3+
732.8
733.6
734.4
735.2
m/z

Asynchronous H1.2SNP A18V N_{α}-ac

Figure S5

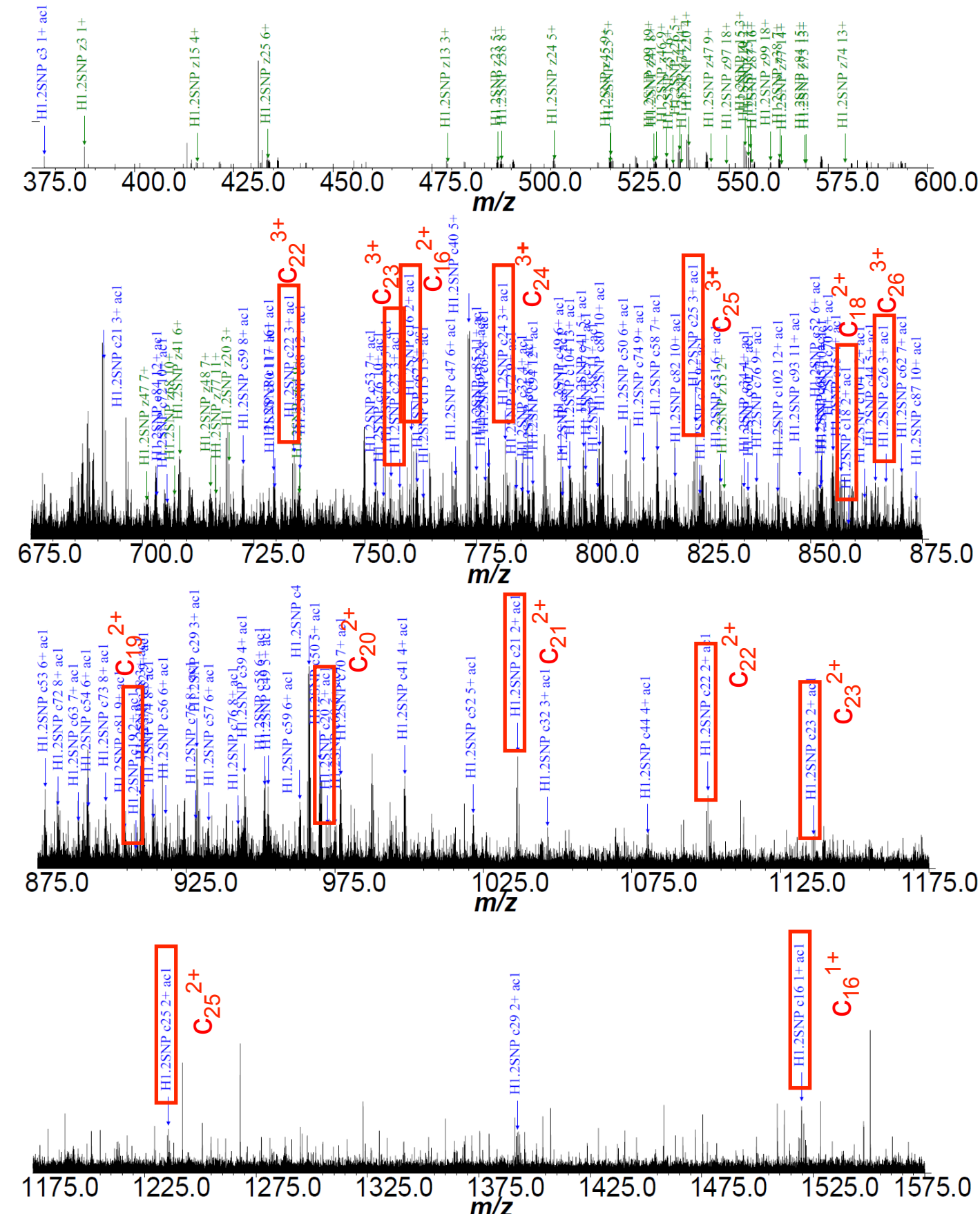
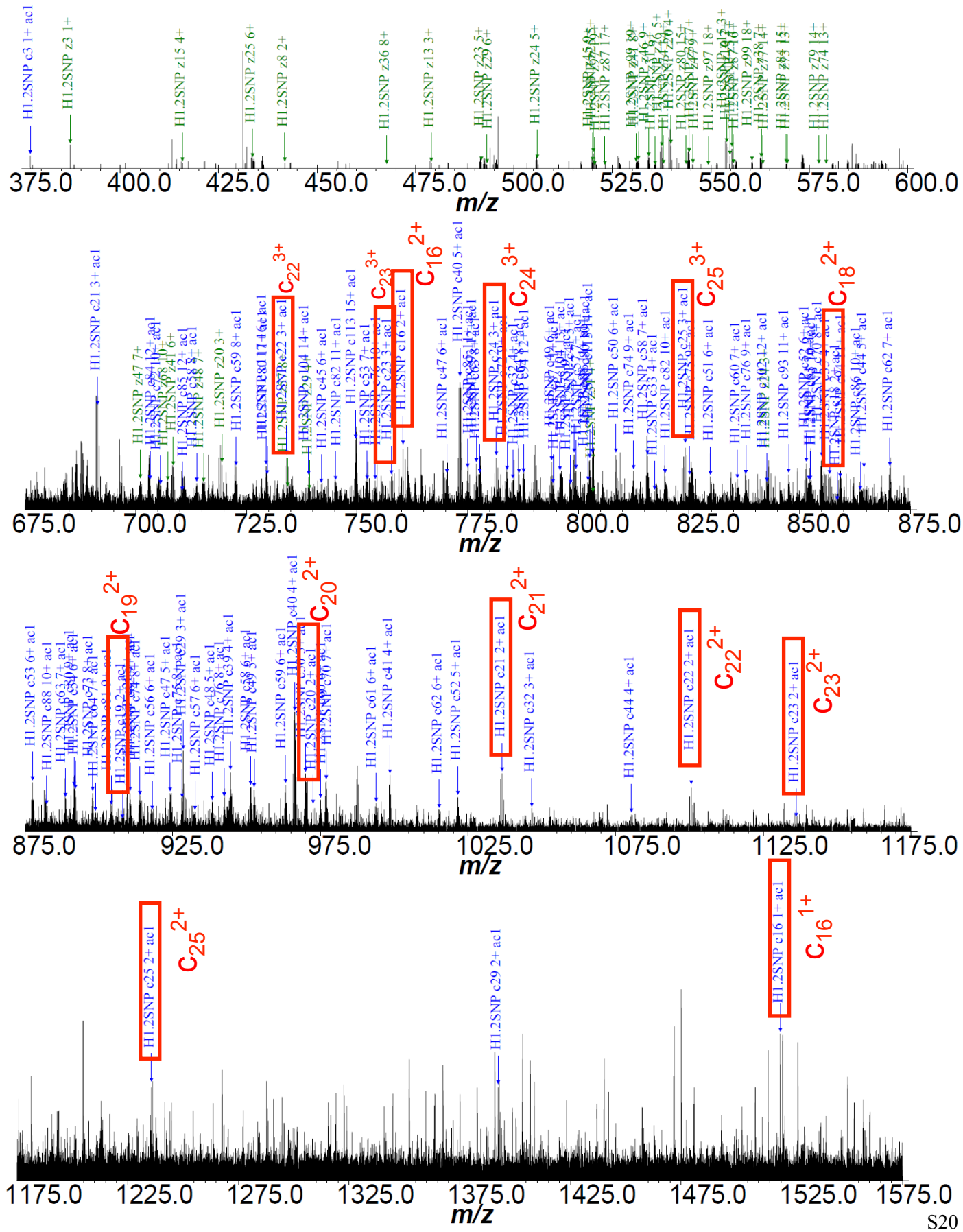


Figure S6



Asynchronous H1.2SNP A18V N_{α}-ac

Figure S7