

## 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 H1.2SNPA18VN $\alpha$ -ac

	<b>H12ac (<i>m/z</i>)</b>	<b>H12SNPac (<i>m/z</i>)</b>	<b><math>\Delta m/z</math></b>	<b><math>\Delta</math> mass (Da)</b>
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  $\Delta m/z$  is  $28.03742 \pm 0.01178$ ,  $0.528 \sigma$  for H12SNP and dimethylation, and  $3.611 \sigma$  for formylation, given the theoretical mass of H12SNP and dimethylation is 28.03132, whereas formylation is 27.9949.

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

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

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

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

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

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

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

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

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

<b>Histone Variants</b>	<b>Asynchronous</b>	<b>S phase</b>	<b>M phase</b>
H12	$5.70 \times 10^{-9}$	$8.35 \times 10^{-8}$	$7.89 \times 10^{-9}$
H13	$1.59 \times 10^{-6}$	$4.70 \times 10^{-9}$	$1.61 \times 10^{-9}$
H14	$5.43 \times 10^{-10}$	$5.99 \times 10^{-8}$	$3.40 \times 10^{-7}$

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

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

## **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.

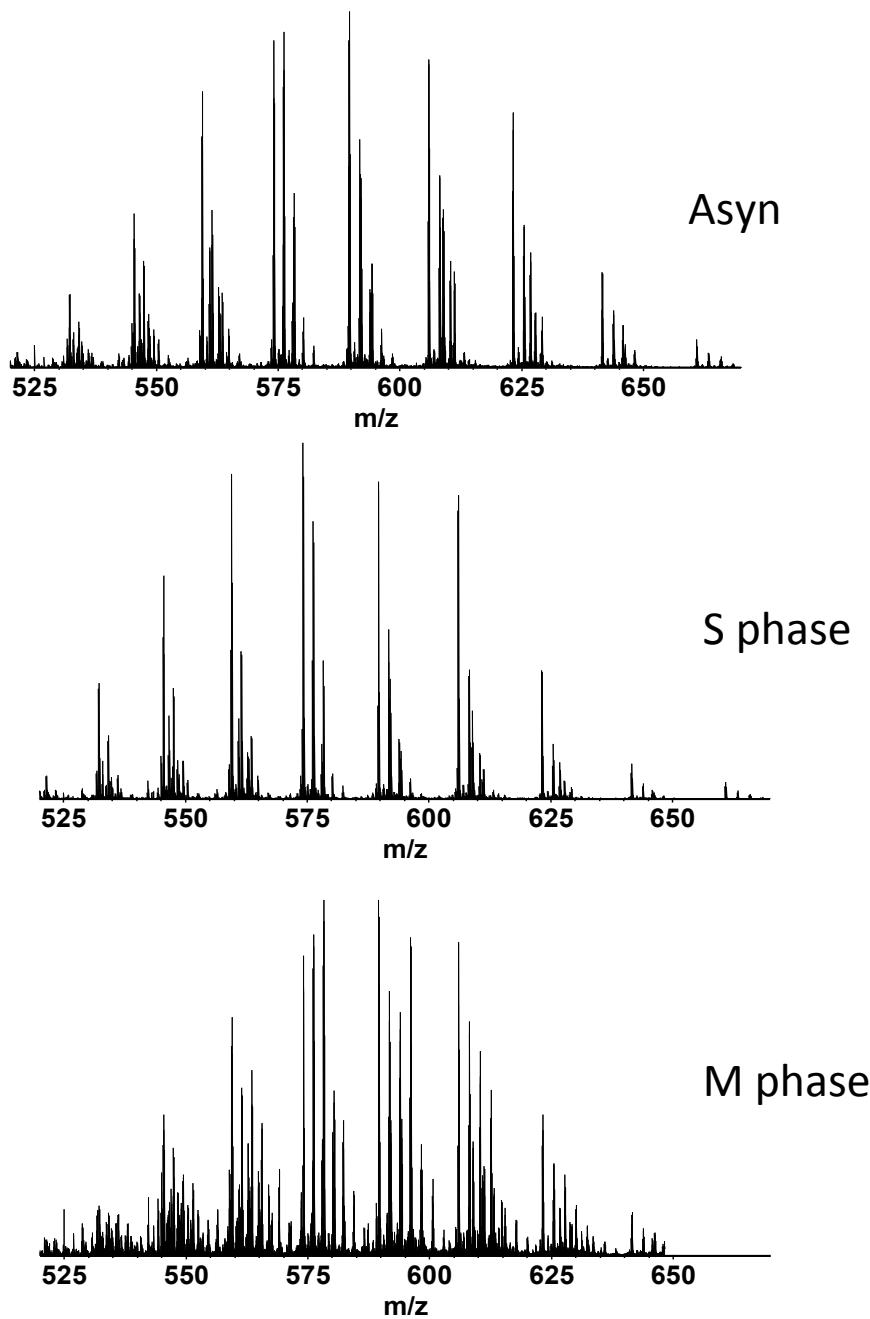
**Figure S5.** Peak annotations for the ECD product ion mass spectrum of asynchronous histone H1.2 SNP A18V from cell line MCF-10A. .

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

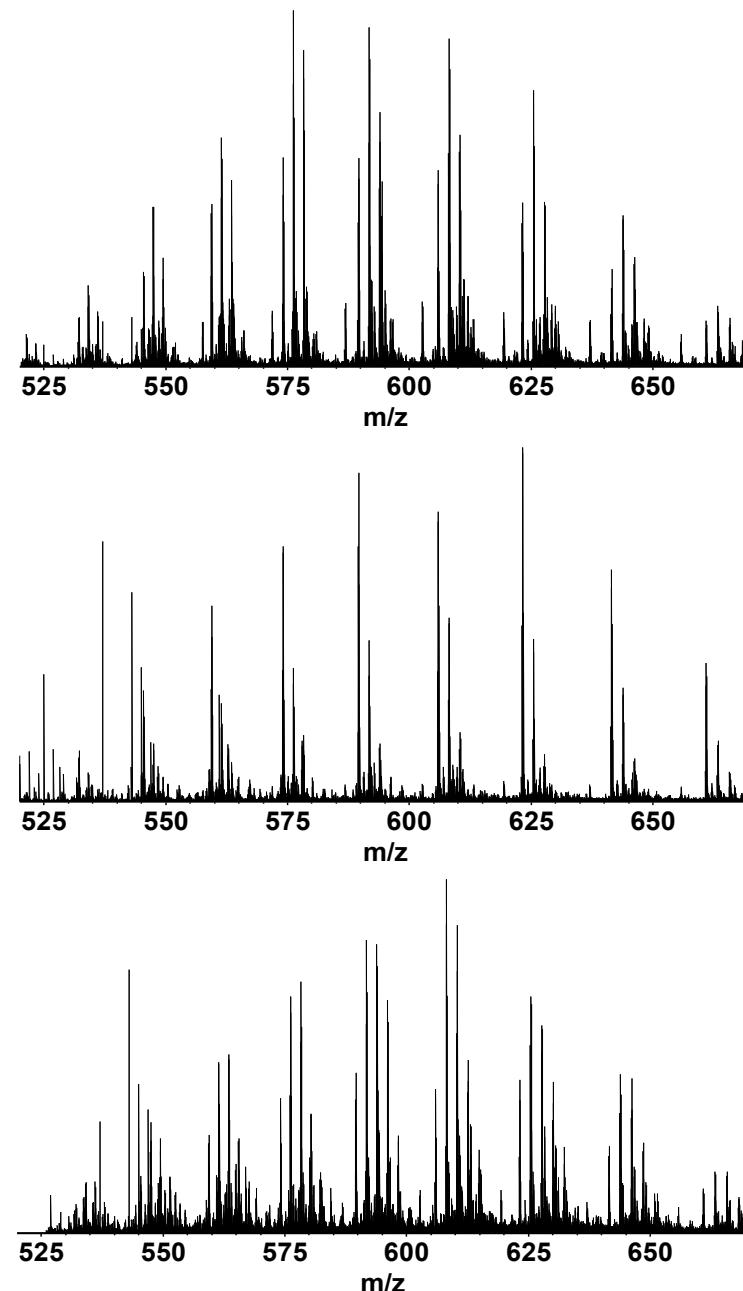
**Figure S7.** Peak annotations for the ECD product ion mass spectrum of asynchronous phosphorylated histone H1.2 SNP A18V from cell line MCF-10A.

Figure S1

MDA-MB-231



MCF-10A



**H12ac\_asyn**

ac-S E T A P A A P A A P A E K A P V K K K A A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K L  
 A K K P A A A T V T K K V A K S P K K A K V A K P K L  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

**H12acph\_asyn**

ac-S E T A P A A P A A P A E K A P V K K K A A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K L  
 A K K P A A A T V T K K V A K S P K K A K V A K P K L  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

**H120acT146ph\_asyn**

ac-S E T A P A A P A A P A E K A P V K K K A A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K L  
 A K K P A A A T V T K K V A K S P K K A K V A K P K L  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

**H14ph\_asyn**

<sup>ph</sup>S E T A P A A P A A P A P A E K T P V K K K A R K S  
 A G A A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K L  
 A K K P A A A A G A K K A K S P K K A K A K P K L  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K

**H14ac\_asyn**

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S  
 A G A A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K L  
 A K K P A A A A G A K K A K S P K K A K A K P K L  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K

**H14acph\_asyn**

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S  
 A G A A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K L  
 A K K P A A A A G A K K A K S P K K A K A K P K L  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K

## H14acphx2\_asyn

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S  
 A G G A L K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 A G A A K K P K A T G A A T P K K S A K K T P K K L  
 A K K P A A A L G A L K K A K S P K L K A L K A A L K P K K  
 A P K S P A K A K A V K P K A A L K P K T A K P K A A L  
 K P K K A A L K K K

## H12ac\_s phase

ac-S E T A P A A P A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K L  
 K A L A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

## H12acph\_s phase

ac-S E T A P A A P A A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K L  
 K A L A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

## H12acT146ph\_s phase

ac-S E T A P A A P A A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K L  
 K A L A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

# MDA-MB-231

# Figure S2

## H14ph\_s phase

<sup>ph</sup>  
S E T A P A A P A A P A P A E K<sup>T</sup> P V K<sup>K</sup> K A R K S  
A G A A K<sup>R</sup> K A S G P P V<sup>S</sup> E L I T K A V A A S K E  
R S G V S L<sup>A</sup> A L K K A L A A A G Y<sup>D</sup> V E K N N S R  
I K<sup>L</sup> G L K S L V S K G T L V Q<sup>T</sup> K G T G A S G S F  
K L N K K A A S G E A K P K A K K A G A A K A K K P  
A G A A K<sup>L</sup> K P K K A T G A A T P K K S L A K K T P K L  
A K<sup>L</sup> K P A A A A G A K K A K S P K K A K A K P K K  
A P K S P A K A K A V K P K A A K P K T A K P K A A L  
K P K K A A L A K K K

## H14ac\_s phase

ac-S E T A P A A P A A P A P A E K<sup>T</sup> P V K<sup>K</sup> K A R K S  
A<sup>G</sup> A A K<sup>R</sup> K A S G P P V<sup>S</sup> E L I T K A V A A S K E  
R<sup>S</sup> G V<sup>S</sup> L<sup>A</sup> A L K K A L A A A G Y<sup>D</sup> V E K N N S R  
I K<sup>L</sup> G L K S L V S K G T L V Q<sup>T</sup> K G T G A S G S F  
K L N K K A A S G E A K P K A K K A G A A K A K K P  
A G A A K<sup>L</sup> K P K K A T G A A T P K K S L A K K T P K L  
A K<sup>L</sup> K P A A A A G A K K A K S P K K A K A K A K P K K  
A P K S P A K A K A V K P K A A K P K T A K P K A A L  
K P K K A A L A K K K

## H14acph\_s phase

ac-S E T A P A A P A A P A P A E K<sup>T</sup> P V K K K A R K S  
A G A A K<sup>R</sup> K A S G P P V<sup>S</sup> E L I T K A V A A S K E  
R<sup>S</sup> G V<sup>S</sup> L<sup>A</sup> A L K K A L A A A G Y<sup>D</sup> V E K N N S R  
I K<sup>L</sup> G L K S L V S K G T L V Q<sup>T</sup> K G T G A S G S F  
K L N K K A A S G E A K P K A K K A G A A K A K K P  
A G A A K<sup>L</sup> K P K K A T G A A T P K K S L A K K T P K L  
A K<sup>L</sup> K P A A A A G A K K A K S P K K A K A K A K P K K  
A P K S P A K A K A V K P K A A K P K T A K P K A A L  
K P K K A A L A K K K

## H14acph2\_s phase

ac-S E T A P A A P A A P A P A E K<sup>T</sup> P V K<sup>K</sup> K A R K S  
A<sup>G</sup> A A K<sup>R</sup> K A S G P P V<sup>S</sup> E L I T K A V A A S K E  
R<sup>S</sup> G V<sup>S</sup> L<sup>A</sup> A L K K A L A A A G Y<sup>D</sup> V E K N N S R  
I K<sup>L</sup> G L K S L V S K G T L V Q<sup>T</sup> K G T G A S G S F  
K L N K K A A S G E A K P K A K K A G A A K A K K P  
A G A A K<sup>L</sup> K P K K A T G A A T P K K S L A K K T P K L  
A K<sup>L</sup> K P A A A A G A K K A K S P K K A K A K A K P K K  
A P K S P A K A K A V K P K A A K P K T A K P K A A L  
K P K K A A L A K K K

## H12ac\_m phase

ac-S E T A P A A P A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

## H12acph\_m phase

ac-S E T A P A A P A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

## H12acT146ph\_m phase

ac-S E T A P A A P A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

## H14ph\_m phase

<sup>ph</sup>S E T A P A A P A A P A P A E K T P V K K K A R K S  
 A G A A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

## H14ac\_m phase

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S  
 A G A A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

## H14acph\_m phase

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S  
 A G A A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

**H14acph2\_m phase**

ac-S E T A P A A P A P A E K T P V K K K A R K S I  
 A G A L A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K L  
 A K K P A A A A L G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

**H14acph3\_m phase**

ac-S E T A P A A P A P A E K T P V K K K A R K S I  
<sup>ph</sup>  
 A G A L A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S P K K A K A A K P K K  
<sup>ph</sup>  
 A K K P A A A A L G A K K A K S P K K A K A A K P K K  
<sup>ph</sup>  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

**H14acph4\_m phase**

ac-S E T A P A A P A P A E K T P V K K K A R K S I  
<sup>ph</sup>  
 A G A L A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
<sup>ph</sup>  
 A G A A K K P K K A T G A A T P K K S P K K A K A A K P K K  
<sup>ph</sup>  
 A K K P A A A A L G A K K A K S P K K A K A A K P K K  
<sup>ph</sup>  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

**H14acph5\_m phase**

ac-S E T A P A A P A P A E K T P V K K K A R K S I  
 A G A L A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S P K K A K A A K P K K  
<sup>ph</sup>  
 A K K P A A A A L G A K K A K S P K K A K A A K P K K  
<sup>ph</sup>  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

**H12 ac\_asyn**

ac-S E T A P A A P A A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

**H12 acph\_asyn**

ac-S E T A P A A P A A A P P A E K A P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

**H12SNPac\_asyn**

ac-S E T A P A A P A A A P P A E K V P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

**H12SNPacph\_asyn**

ac-S E T A P A A P A A A P P A E K V P V K K K A A K K  
 A G G T P R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A L K A V K P K A A K P K V V K P K K A A  
 P K K K

**H13 ac\_asyn**

ac-S E T A P I P A P A E K T P V K K K A A K K  
 G A T A G K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 F K L N K K A A S G E G K P K A K K A G A A K P R K  
 P A G A A K K P K K V A G A A T P K K S I K K T P K  
 K V K K P A T A A G T K K V A K S A K K V K T P Q P  
 K K A A K S P A K A K A P K P K A A K P K S G K P K  
 V T K A K K A A P K K K

**H14ac\_asyn**

ac-S E T A P A A P A A P A E K T P V K K K A A K K  
 A G A A L K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K  
 A K K P A A A L A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A L K K K

# MCF-10A

## H14acph\_asyn

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S I  
 A G A A K R K A S G P P V S E L I T K A V A A S K E I  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K A T G A A T P K K S A K K T P K K L  
 A K K P A A A A G A K K A K S P K K A K A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K

## H14acph2\_asyn

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S I  
 A G A A K R K A S G P P V S E L I T K A V A A S K E I  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K A T G A A T P K K S A K K T P K K L  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K

## Figure S3

## H14acph3\_asyn

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S I  
 A G A A K R K A S G P P V S E L I T K A V A A S K E I  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K A T G A A T P K K S A K K T P K K L  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K

## MCF-10A s phase

### H12ac\_s phase

ac-S E T A P A A P A A A P P A E K A P V K K K A A K K I  
 A G G T P R K A S G P P V S E L I T K A V A A S K E I  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A K A V K P K A A K P K V V K P K K A A  
 P K K K

### H12acph\_s phase

ac-S E T A P A A P A A A P P A E K A P V K K K A A K K I  
 A G G T P R K A S G P P V S E L I T K A V A A S K E I  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A K A V K P K A A K P K V V K P K K A A  
 P K K K

### H12SNPac\_s phase

ac-S E T A P A A P A A A P P A E K V P V K K K A A K K I  
 A G G T P R K A S G P P V S E L I T K A V A A S K E I  
 R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K V K K A G G T K P K K P  
 V G A A K K P K K A A G G A T P K K S A K K T P K K  
 A K K P A A A T V T K K V A K S P K K A K V A K P K  
 K A A K S A A K A V K P K A A K P K V V K P K K A A  
 P K K K

# Figure S3

## H14acph\_s phase

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S I  
 A G A A L A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K K

## H14ac\_s phase

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S I  
 A G A A L A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K K

## MCF-10A

## H13ac\_s phase

ac-S E T A P L A P T I P A P A E K T P V K K K A R K A  
 G A T A G K R K A S G P P V S E L I T K A V A A S K E  
 E R S G V S L A A L K K A L A A A G Y D V E K N N S R  
 R I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 F K L N K K A A S G E A K P K A K K A G A A K P R K  
 P A G A A K K P K K V A G A A T P K K S I K K T P K L  
 K V K K P A T A A G T K K V A K S A K K V K T P Q P  
 K K A K S P A K A K A P K P K A A K P K S G K P K  
 V T K A K K A A P K K K

## H14acph2\_s phase

ac-S E T A P A A P A A P A P A E K T P V K K K A R K S I  
 A G A A L A K R K A S G P P V S E L I T K A V A A S K E  
 R S G V S L A A L K A L A A A G Y D V E K N N S R  
 I K L G L K S L V S K G T L V Q T K G T G A S G S F  
 K L N K K A A S G E A K P K A K K A G A A K A K K P  
 A G A A K K P K K A T G A A T P K K S A K K T P K K  
 A K K P A A A A G A K K A K S P K K A K A A K P K K  
 A P K S P A K A K A V K P K A A K P K T A K P K A A L  
 K P K K A A A K K K K

# Figure S3

MCF-10A m phase

H14ac\_m phase

```
ac-S E T A P A A P A A P A P A E K1T P V K1K1K1A R1K1S
A1G1A1A K1R1K1A S1G P P V1S1E1L I T K1A V A1A1S K E1
R S G V S L1A A L K K A L A A A G Y1D V E K N N S R
I K L G L K S L V S K G T L V Q T K G T G A S G S F
K L N K K A A S G E A K P K A K K A G A A K A K K P
A G A A K K P K K A T G A A T P K K S A K K T P K K
A K K P A A A A G A K K A KphS P K1K1A1K1A A K P K K
A P K S P A L1K A K A V1K P K A A L1K P K T A L1K P K A A L
K P K1K A A A L K K K
```

H14acph\_m phase

```
ac-S E T A P A A P A A P A P A E K1T P V K1K1K1A R1K1S
A1G1A1A K1R1K1A S G P P V1S1E1L I T K A V A A S K E1
R S G V S L1A A L K K A L A A A G Y1D V E K N N S R
I K L G L K S L V S K G T L V Q T K G T G A S G S F
K L N K K A A S G E A K P K A K K A G A A K A K K P
A G A A K K P K K A T G A A T P K K S A K K T P K K
A K K P A A A A G A K K A KphS P K1K1A1K1A A K P K K
A P K S P A L1K A K A V1K P K A A L1K P K T A L1K P K A A L
K P K1K A A A L K K K
```

H14acph2\_m phase

```
ac-S E T1A P A A P A A P A P A E K1T P V K1K1K1A R1K1S
A1G1A1A K1R1K1A S1G P P V1S1E1L I T K A V A A S K E1
R S G V S L1A A L K K A L A A A G Y1D V E K N N S R
I K L G L K S L V S K G T L V Q T K G T G A S G S F
K L N K K A A S G E A K P K A K K A G A A K A K K P
A G A A K K P K K A T G A A T P K K S A K K T P K K
A K K P A A A A G A K K A KphS P K1K1A1K1A A K P K K
A P K S P A L1K A K A V1K P K A A L1K P K T A L1K P K A A L
K P K1K A A A L K K K
```

H14acph3\_m phase

```
ac-S E T1A P A A P A A P A P A E K1Tph P V K1K1K1A R1K1S
A1G1A1A K1R1K1A S1G P P V1S1E1L I T K A V A A S K E1
R S G V S L1A A L K K A L A A A G Y1D V E K N N S R
I K L G L K S L V S K G T L V Q T K G T G A S G S F
K L N K K A A S G E A K P K A K K A G A A K A K K P
A G A A K K P K K A T G A A T P K K S A K K T P K K
A K K P A A A A G A K K A KphS P K1K1A1K1A A K P K K
A P K S P A L1K A K A V1K P K A A L1K P K T A L1K P K A A L
K P K1K A A A L K K K
```

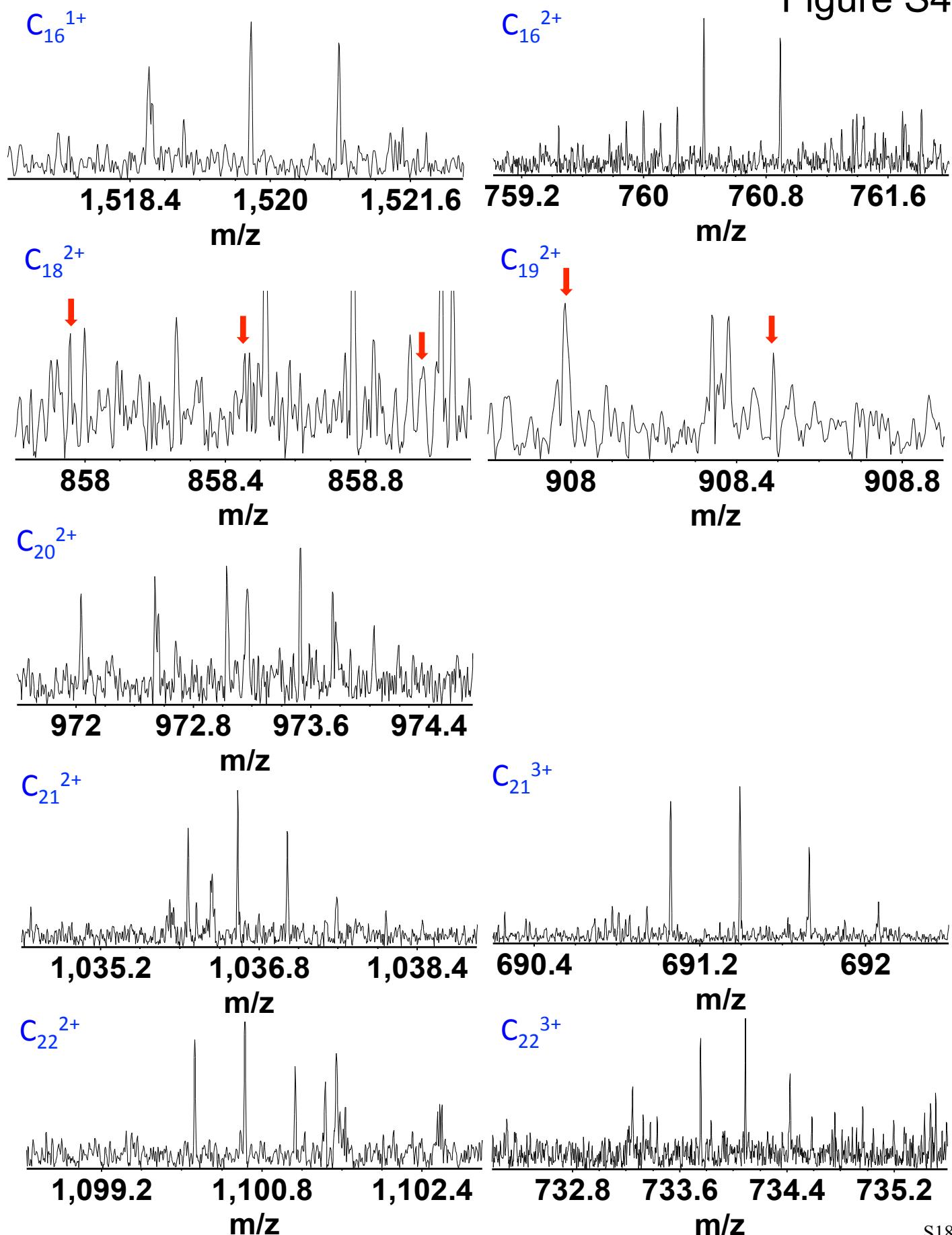
H14acph4\_m phase

```
ac-S E T1A P A A P A A P A P A E K1Tph P V K1K1K1A R1K1S
A1G1A1A K1R1K1A S1G P P V1S1E1L I T K A V A A S K E1
R S G V S L1A A L K K A L A A A G Y1D V E K N N S R
I K L G L K S L V S K G T L V Q T K G T G A S G S F
K L N K K A A S G E A K P K A K K A G A A K A K K P
A G A A K K P K K A T G A A T P K K S A K K T P Kph
A K K P A A A A G A K K A KphS P K1K1A1K1A A K P K K
A P K S P A L1K A K A V1K P K A A L1K P K T A L1K P K A A L
K P K1K A A A L K K K
```

H14acph5\_m phase

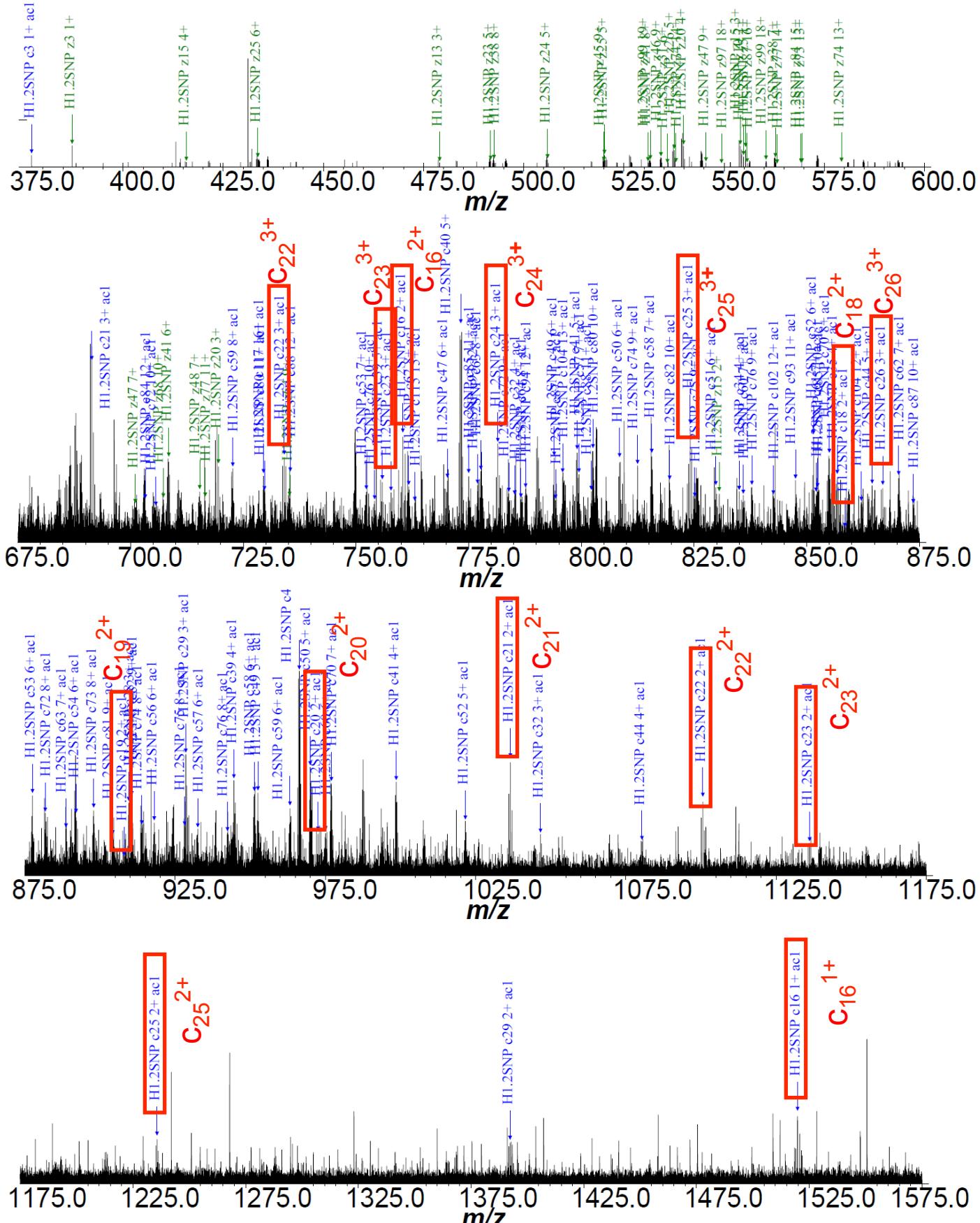
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ac-S E T1A P A A P A A P A P A E K1Tph P V K1K1K1A R1K1S
A1G1A A K1R1K1A S G P P V1S1E1L I T K A V A A S K E1
R S G V S L A A L K K A L A A A G Y D V E K N N S R
I K L G L K S L V S K G T L V Q T K G T G A S G S F
K L N K K A A S G E A K P K A K K A G A A K A K K P
A G A A K K P K K A T G A A T P K K S A K K T P Kph
A K K P A A A A G A K K A KphS P K1K1A1K1A A K P K K
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K P K1K A A A L K K K
```

Figure S4



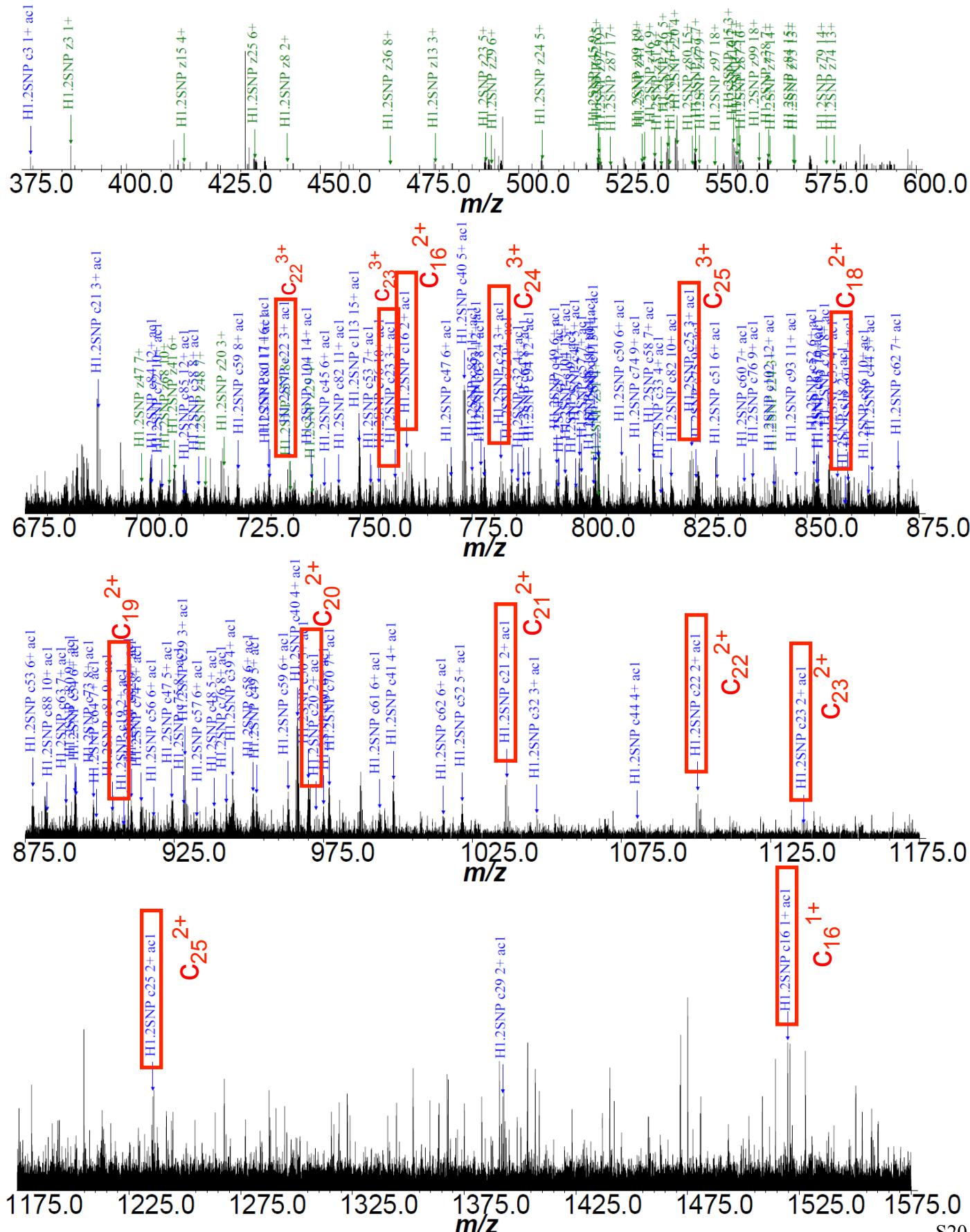
## Asynchronous H1.2SNP A18V N<sub>α</sub>-ac

# Figure S5



## S phase H1.2SNP A18V N<sub>α</sub>-ac

# Figure S6



## Figure S7

## Asynchronous H1.2SNP A18V N<sub>α</sub>-ac

