SUPPLEMENTAL DATA

Supplemental Data 1: Uniprot Histone H1 database.

Supplemental Data 2: Replicate total ion chromatograms and deconvoluted spectra of histone H1 isolated from the chromatin of asynchronous MCF-10A, MCF-7, MDA-MB-231 and confluent MCF-10A cells. Red bars indicate a change in 80Da corresponding to a phosphorylation event. MCF-10A and MCF-7 H1.2 show peaks with an increase of 30Da corresponding to an allelic variant (A142T) denoted by *.

Supplemental Data 3: Replicate peak abundances for histone variants H1.2 and H1.4 from the LC-MS data for each breast cell line. The calculated abundance ratios were used to construct the bar charts in **Figure 1B & 1C**.

Supplemental Data 4: Base peak chromatograms from RP-LC-MS/MS analysis. **A)** Shotgun proteomic analysis of MDA-MB-231 histone H1. **B)** Phospho peptide enrichment of MDA-MB-231 histone H1.

Supplemental Data 5: Representative MS/MS data for the additional phospho H1.2 and H1.4 sites identified by RP-LC-MS/MS. Peak indexes for the phosphorylated peptides identified in the MS/MS data.

Supplemental Data 6: Total ion chromatograms from cell cycle synchronization of MDA-MB-231 cells.

Supplemental Data 7: Replicate total ion chromatograms and deconvoluted spectra from the estradiol treated MCF-7 cells. Red bars indicate a change in 80Da corresponding to a phosphorylation event.

Supplemental Data 8: Replicate peak abundances for histone variants H1.2 and H1.4 from the LC-MS data for the estradiol treated MCF-7 cells.

Supplemental Data 9: Replicate total ion chromatograms from the LY294002 treated MDA-MB-231 cells.

Supplemental Data 10: Tumor microarray ID key and thumbnail images of the H&E and pT146 staining.

Supplemental Data 11: Pathological assessment of individual primary breast tumors and clinical biomarker data.

Supplemental Data 12: Statistical analyses for tumor staining independence.

SUPPLEMENTAL DATA 1

>sp|P07305|H10_HUMAN Histone H1.0 OS=Homo sapiens GN=H1F0 PE=1 SV=3
TENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSIQKYIKSHYKV
GENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKSDEPKKSVAFKKTKKEIKKVATP
KKASKPKKAASKAPTKKPKATPVKKAKKKLAATPKKAKKPKTVKAKPVKASKPKKAKPVK
PKAKSSAKRAGKKK
>sp|Q02539|H11_HUMAN Histone H1.1 OS=Homo sapiens GN=HIST1H1A PE=1 SV=3
SETVPPAPAASAAPEKPLAGKKAKKPAKAAAASKKKPAGPSVSELIVQAASSSKERGGV
SLAALKKALAAAGYDVEKNNSRIKLGIKSLVSKGTLVQTKGTGASGSFKLNKKASSVETK
PGASKVATKTKATGASKKLKKATGASKKSVKTPKKAKKPAATRKSSKNPKKPKTVKPKKV
AKSPAKAKAVKPKAAKARVTKPKTAKPKKAAPKKK
>sp|P16403|H12_HUMAN Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2
SETAPAAPAAAPPAEKAPVKKKAAKKAGGTPRKASGPPVSELITKAVAASKERSGVSLA
ALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKAASGEAKPKV
KKAGGTKPKKPVGAAKKPKKAAGGATPKKSAKKTPKKAKKPAAATVTKKVAKSPKKAKVA
KPKKAAKSAAKAVKPKAAKPKVVKPKKAAPKKK
>sp|P16402|H13_HUMAN Histone H1.3 OS=Homo sapiens GN=HIST1H1D PE=1 SV=2
SETAPLAPTIPAPAEKTPVKKKAKKAGATAGKRKASGPPVSELITKAVAASKERSGVSL
AALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKAASGEGKPK
AKKAGAAKPRKPAGAAKKPKKVAGAATPKKSIKKTPKKVKKPATAAGTKKVAKSAKKVKT
PQPKKAAKSPAKAKAPKPKAAKPKSGKPKVTKAKKAAPKKK
>sp|P10412|H14_HUMAN Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2
SETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLA
ALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKAASGEAKPKA
KKAGAAKAKKPAGAAKKPKKATGAATPKKSAKKTPKKAKKPAAAAGAKKAKSPKKAKAAK
PKKAPKSPAKAKAVKPKAAKPKTAKPKAAKPKKAAAKKK
>sp|P16401|H15_HUMAN Histone H1.5 OS=Homo sapiens GN=HIST1H1B PE=1 SV=3
SETAPAETATPAPVEKSPAKKKATKKAAGAGAAKRKATGPPVSELITKAVAASKERNGL
SLAALKKALAAGGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKAASGEAK
PKAKKAGAAKAKKPAGATPKKAKKAAGAKKAVKKTPKKAKKPAAAGVKKVAKSPKKAKAA
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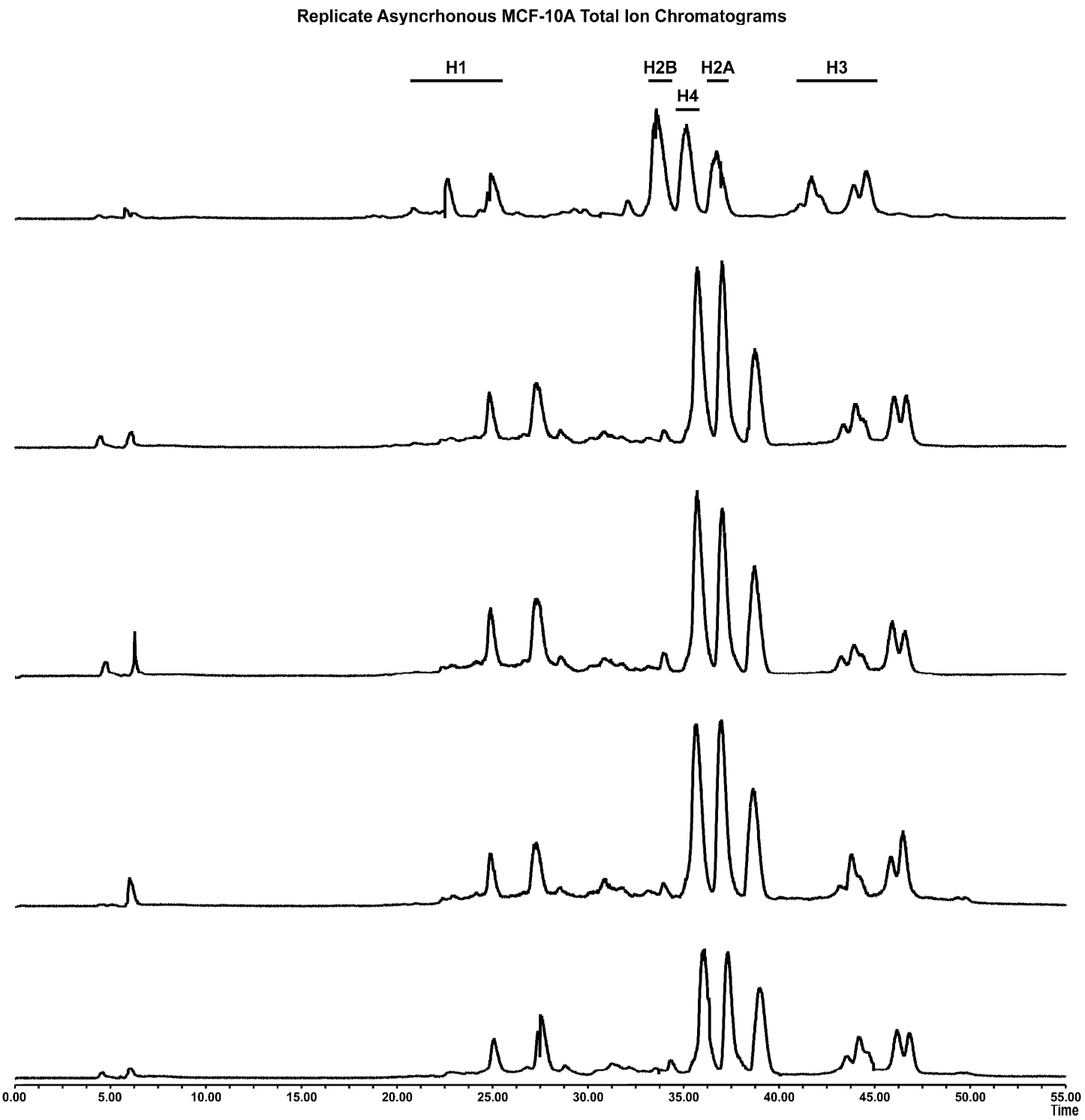
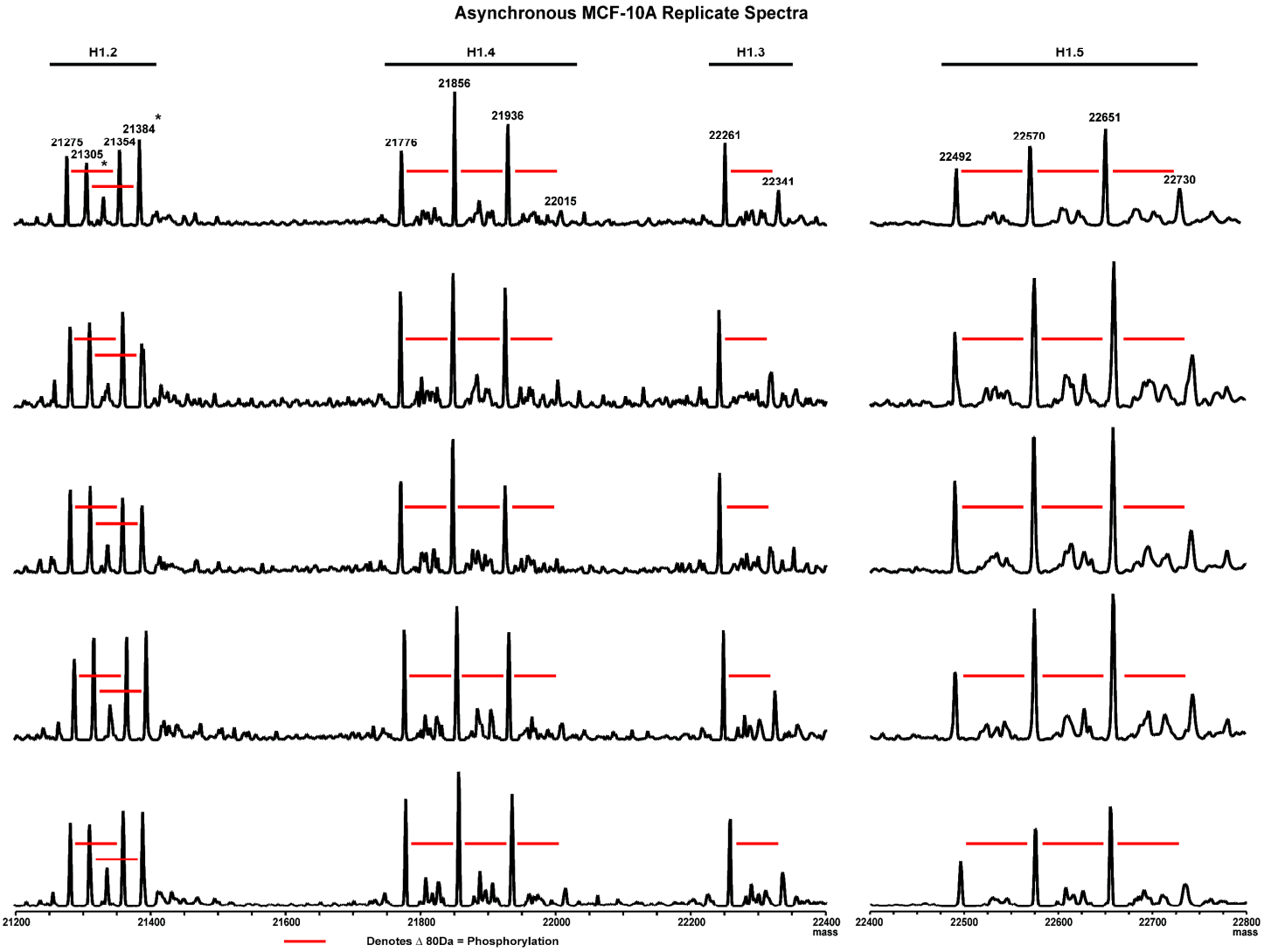
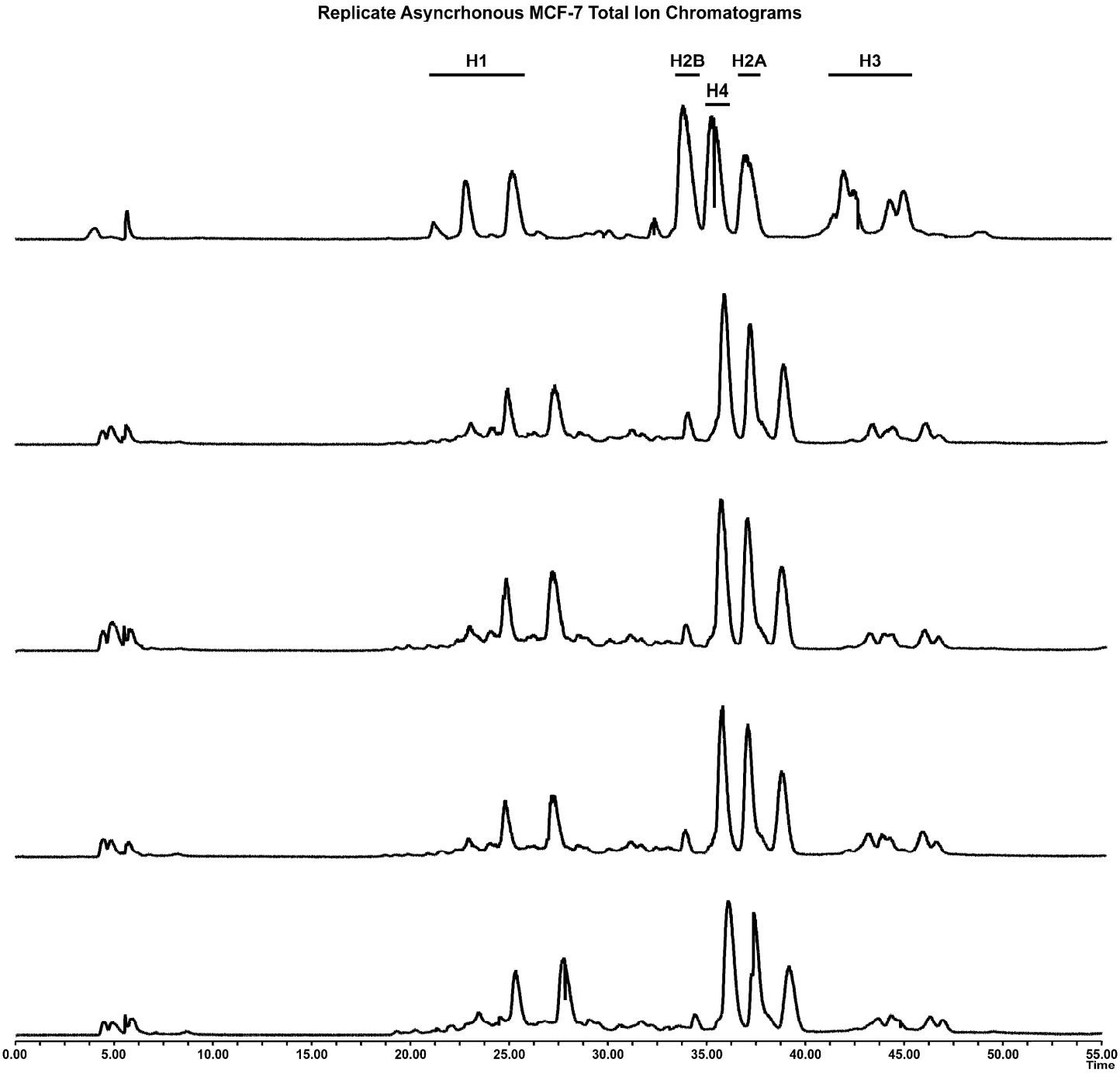
SUPPLEMENTAL DATA 2




Asynchronous MCF-10A Replicate Spectra
H1.2
H1.4
H1.3
H1.5
21275
21305
21354
21384
21776
21856
21936
22015
22261
22341
22492
22570
22651
22730
21200
21400
21600
21800
22000
22200
22400
mass
22400
22500
22600
22700
22800
mass
Denotes Δ 80Da = Phosphorylation


Replicate Asyncrhonous MCF-7 Total Ion Chromatograms
H1
H2B
H4
H2A
H3
0.00
5.00
10.00
15.00
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30.00
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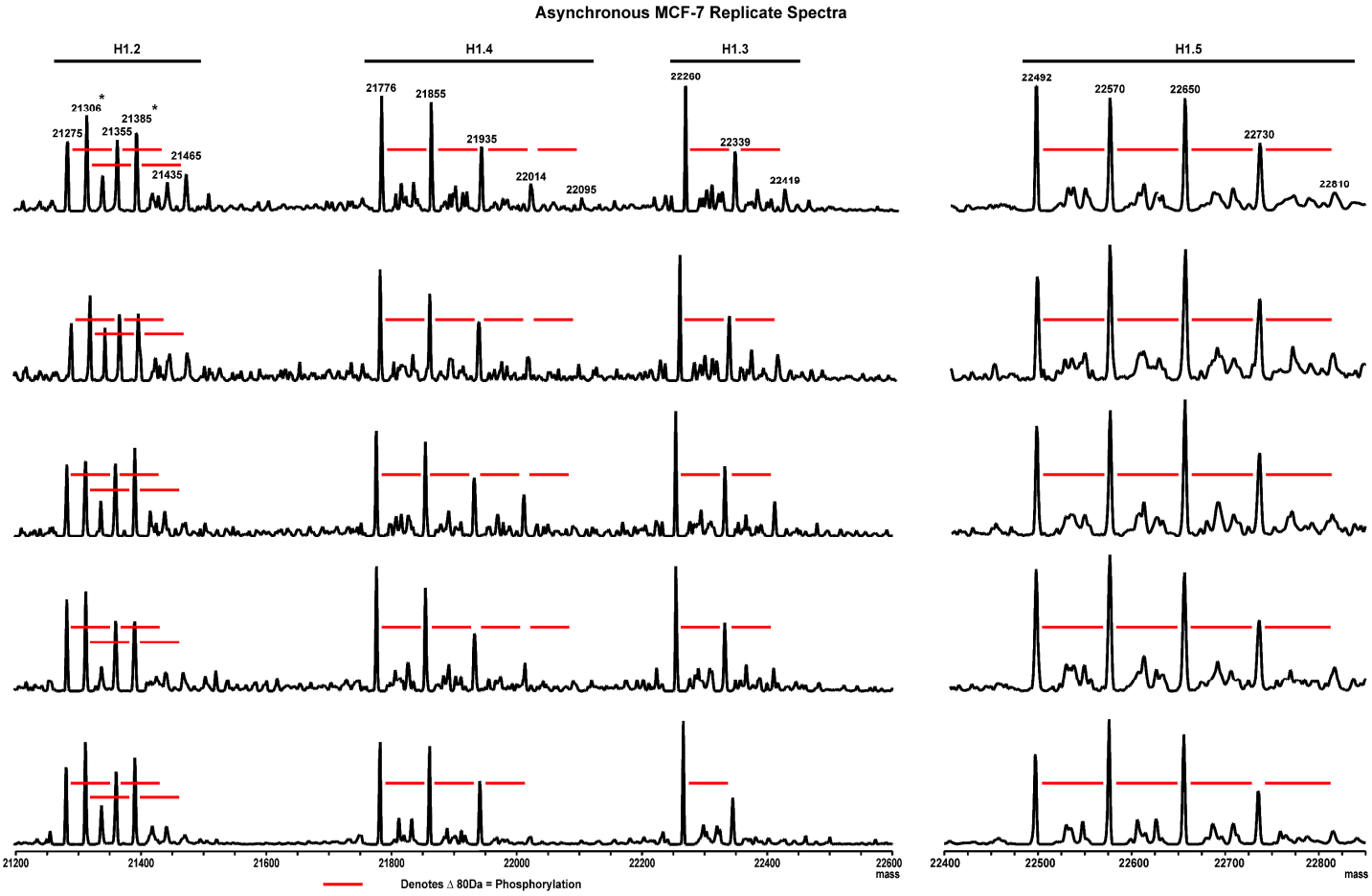
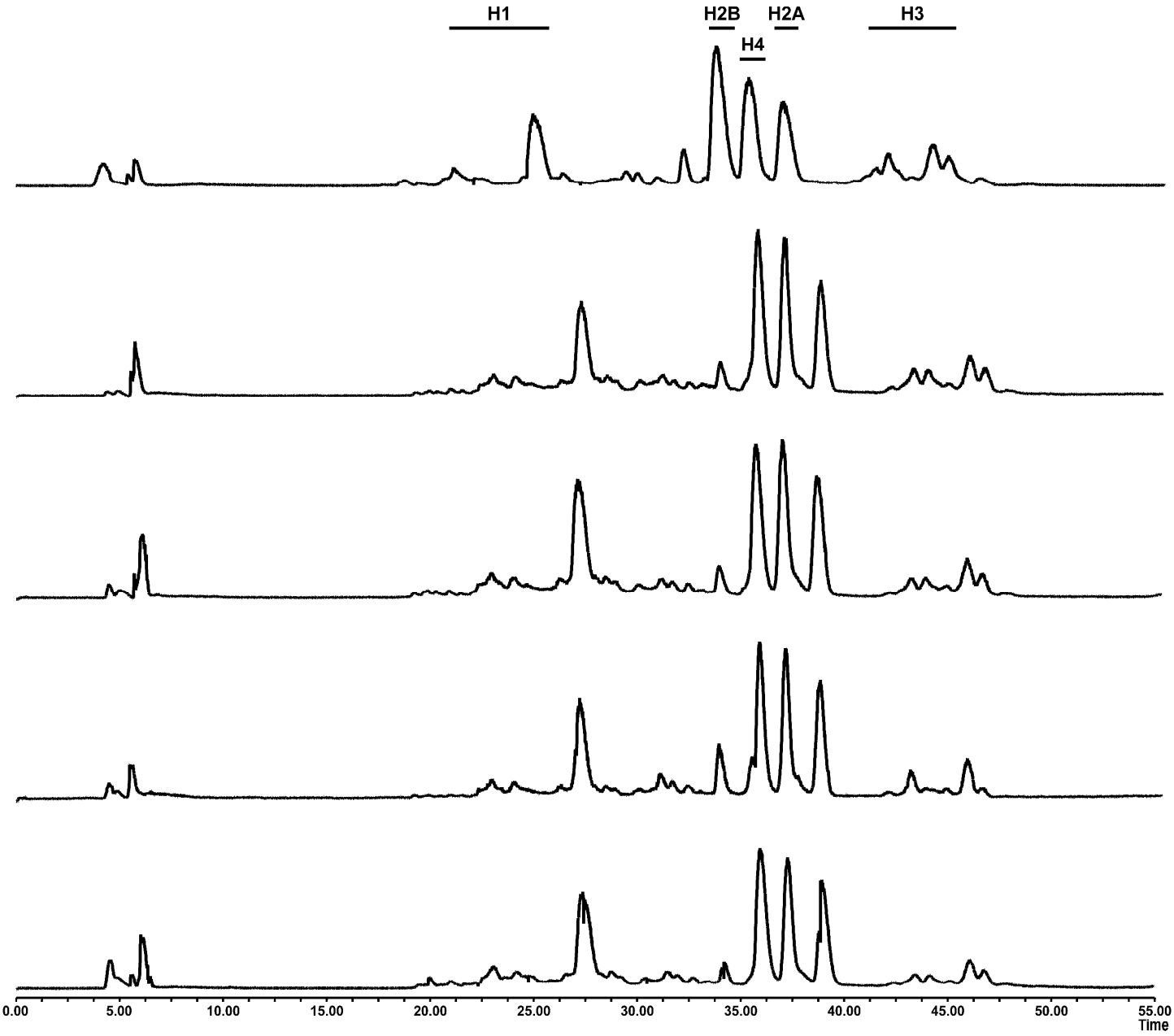
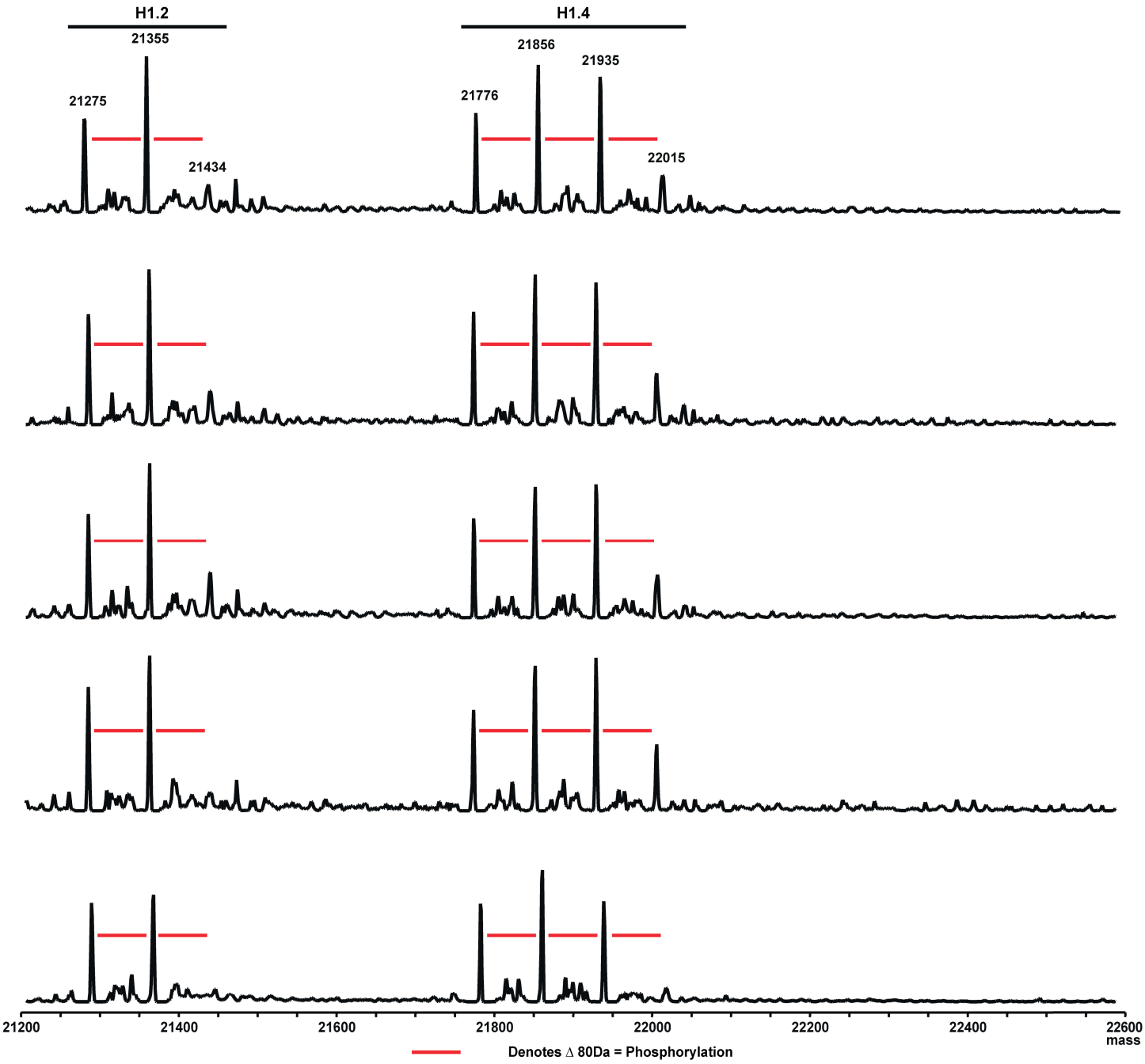
40.00
45.00
50.00
55.00
Time

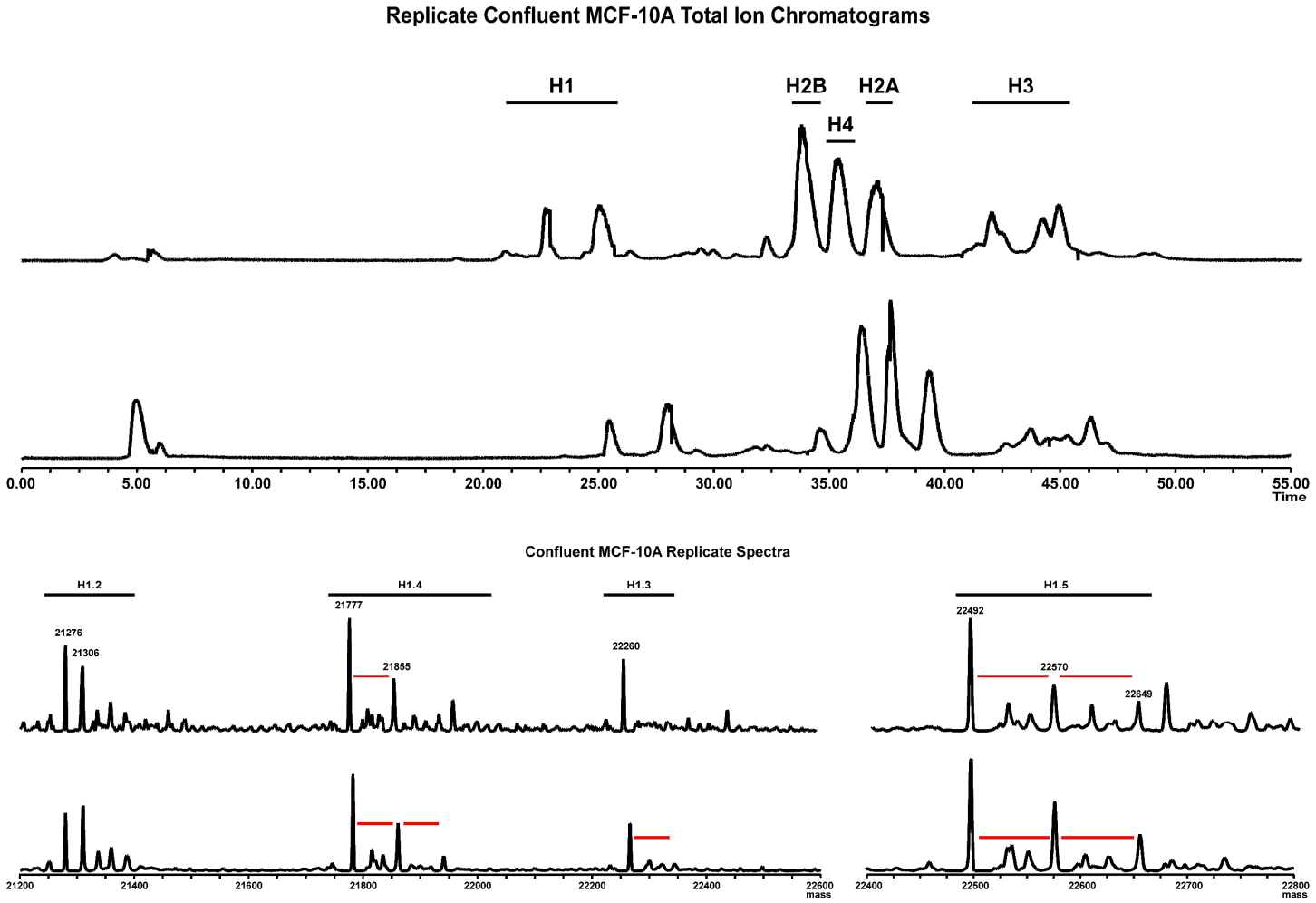

Asynchronous MCF-7 Replicate Spectra
H1.2
H1.4
H1.3
H1.5
21275
21306
21355
21385
21435
21465
21776
21855
21935
22014
22095
22260
22339
22419
22492
22570
22650
22730
22810
21200
21400
21600
21800
22000
22200
22400
22600
mass
22400
22500
22600
22700
22800
mass
Denotes Δ 80Da = Phosphorylation

Replicate Asyncrhonous MDA-MB-231 Total Ion Chromatograms



Asynchronous MDA-MB-231 Replicate Spectra




Replicate Confluent MCF-10A Total Ion Chromatograms
H1
H2B
H2A
H4
H3
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time
Confluent MCF-10A Replicate Spectra
H1.2
H1.4
H1.3
H1.5
21276
21306
21777
21855
22260
22492
22570
22649
21200
21400
21600
21800
22000
22200
22400
22600
mass
22400
22500
22600
22700
22800
mass

SUPPLEMENTAL DATA 3

Replcate Peak Abundances from Asynchronous Breast Cell Line LC-MS

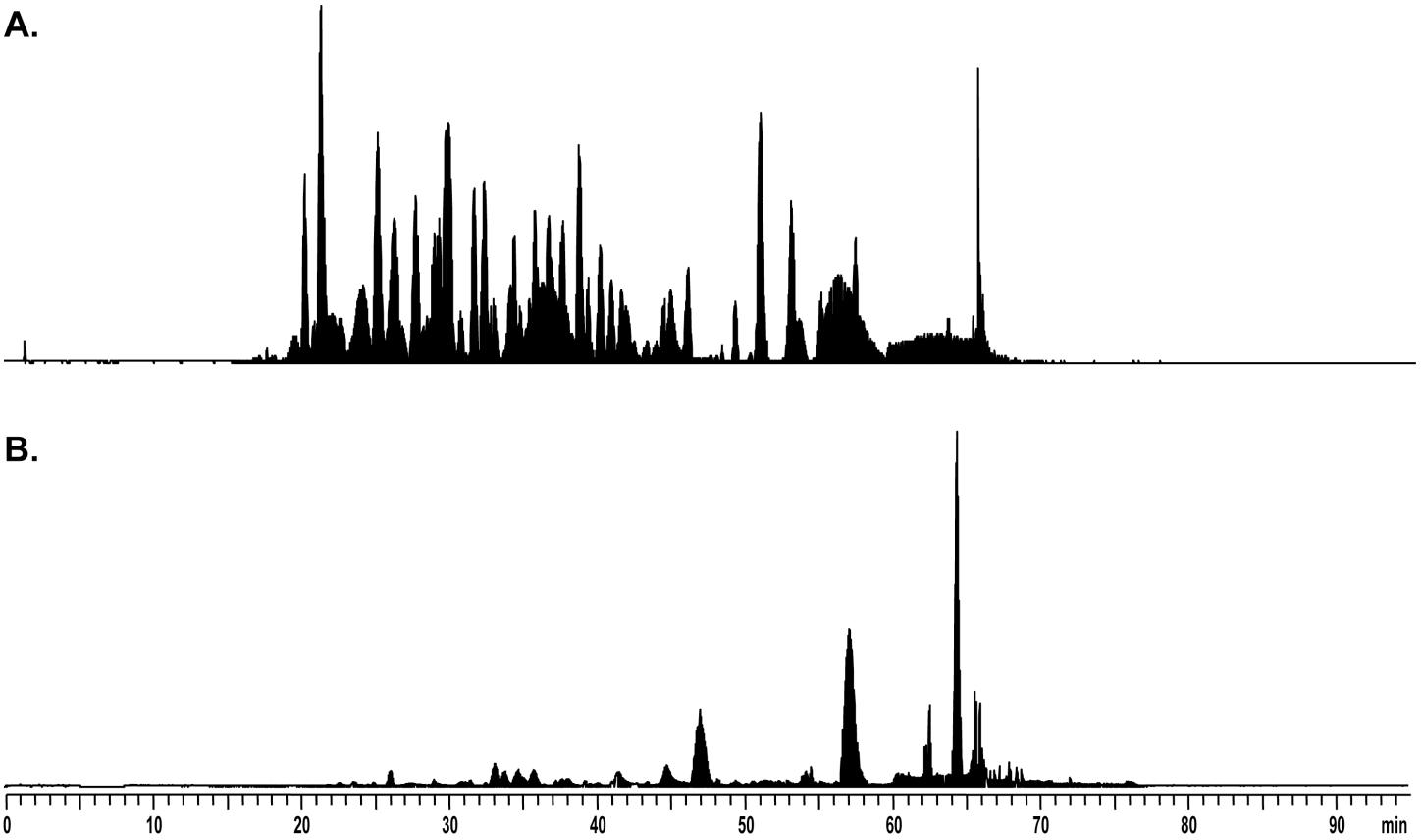
Replicate	H1.2	pH1.2	ppH1.2	Total pH1.2	Ratio pH1.2/H1.2	H1.4	pH1.4	ppH1.4	pppH1.4	ppppH1.4	Total pH1.4	Ratio pH1.4/H1.4
MCF-10A - 1	3428	3739	-	-	1.091	3695	6674	5042	717	-	12433	3.365
MCF-10A - 2	1488	1782	-	-	1.198	2154	2506	2224	596	-	5326	2.473
MCF-10A - 3	2379	2150	-	-	0.904	2611	3812	2496	408	-	6716	2.572
MCF-10A - 4	1742	2205	-	-	1.266	2374	2870	2331	378	-	5579	2.350
MCF-10A - 5	3921	4531	-	-	1.156	5071	6321	5300	859	-	12480	2.461

Replicate	H1.2	pH1.2	ppH1.2	Total pH1.2	Ratio pH1.2/H1.2	H1.4	pH1.4	ppH1.4	pppH1.4	ppppH1.4	Total pH1.4	Ratio pH1.4/H1.4
MCF-7 - 1	4863	4974	1953	6927	1.424	8064	7636	4474	1854	917	14881	1.845
MCF-7 - 2	944	1088	439	1527	1.618	1826	1417	966	388	258	3029	1.659
MCF-7 - 3	1996	2016	666	2682	1.344	2910	2605	1606	1151	286	5648	1.941
MCF-7 - 4	1676	1274	344	1618	0.965	2293	1890	1048	517	186	3641	1.588
MCF-7 - 5	6202	5860	1412	7272	1.173	8257	7939	5133	620	328	14020	1.698

Replicate	H1.2	pH1.2	ppH1.2	Total pH1.2	Ratio pH1.2/H1.2	H1.4	pH1.4	ppH1.4	pppH1.4	ppppH1.4	Total pH1.4	Ratio pH1.4/H1.4
MDA-MB-231 - 1	10020	16763	2963	19726	1.969	10635	15858	14582	3925	-	34365	3.231
MDA-MB-231 - 2	3167	4457	969	5426	1.713	3238	4318	4067	1475	-	9860	3.045
MDA-MB-231 - 3	5190	7710	2278	9988	1.924	4976	6518	6641	2161	-	15320	3.079
MDA-MB-231 - 4	1888	2373	290	2663	1.410	1542	2218	2340	1012	-	5570	3.612
MDA-MB-231 - 5	11020	11921	1443	13364	1.213	10929	14637	11195	1579	-	27411	2.508

Replicate	H1.2	pH1.2	ppH1.2	Total pH1.2	Ratio pH1.2/H1.2	H1.4	pH1.4	ppH1.4	pppH1.4	ppppH1.4	Total pH1.4	Ratio pH1.4/H1.4
MCF-10A Con - 1	3354	1136	-	-	0.339	4420	2022	705	-	-	2727	0.617
MCF-10A Con - 2	5577	2323	-	-	0.417	9308	4598	1513	-	-	6111	0.657

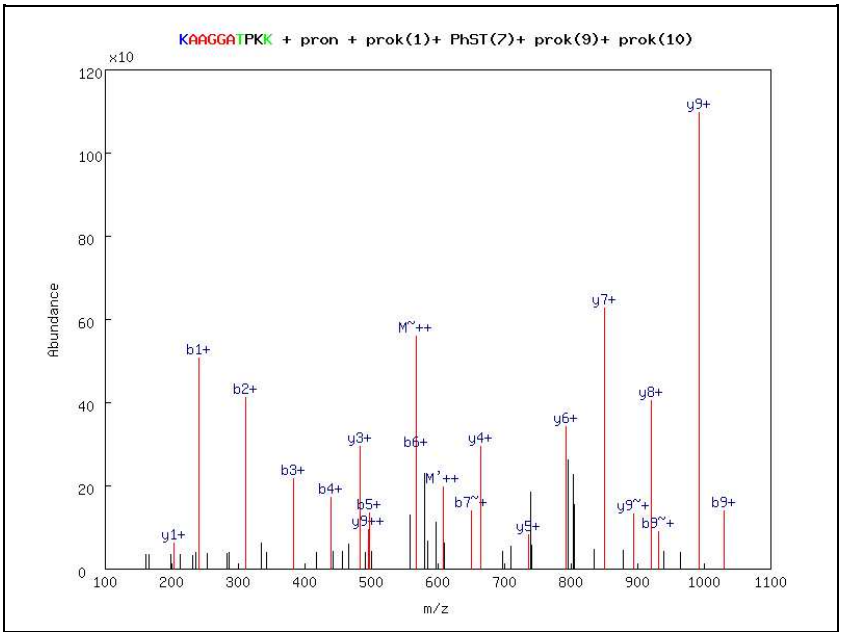
SUPPLEMENTAL DATA 4



SUPPLEMENTAL DATA 5
Data File for all MS/MS data: CH_SH_20130821_NTA_E_C_CID_NEUTRAL_LOSS (PPE) and CH_SH_20130814_PC_CID (SP)

H1.2 pT146

9/3/13 9:10 AM



Index scan# charge score pp pp_2 pp_{tag} m/z MW(obs) MW delta miss Unique sequence + modifications

2135 1981 +2 75 43.3 34.5 6.7 616.8177 1232.6282 1232.6286 -0.0004 2 √ KAAGGATPKK + pron + prok(1)

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	--	--	121.08	--	--	--	--	**241.15**	K	--	**567.83**	**607.81**	--	616.82	--	1134.65	1214.62	--	1232.63	M
2	--	--	--	--	156.60	--	--	--	--	**312.19**	A	--	447.76	487.74	--	**496.74**	--	**894.50**	974.47	--	**992.48**	9
3	--	--	--	--	192.12	--	--	--	--	**383.23**	A	--	412.24	452.22	--	461.23	--	823.47	903.43	--	**921.44**	8
4	--	--	--	--	220.63	--	--	--	--	**440.25**	G	--	376.72	416.70	--	425.71	--	752.43	832.40	--	**850.41**	7
5	--	--	--	--	249.14	--	--	--	--	**497.27**	G	--	348.21	388.19	--	397.20	--	695.41	775.37	--	**793.39**	6
6	--	--	--	--	284.66	--	--	--	--	**568.31**	A	--	319.70	359.68	--	368.69	--	638.39	718.35	--	**736.36**	5
7	--	326.18	--	--	375.17	--	**651.35**	--	--	749.32	T	--	284.18	324.16	--	333.17	--	567.35	647.32	--	**665.33**	4
8	--	374.70	--	--	423.69	--	748.40	--	--	846.38	P	--	--	233.65	--	242.66	--	--	466.30	--	**484.31**	3
9	--	466.76	--	--	515.75	--	**932.52**	--	--	**1030.50**	K	--	--	185.13	--	194.13	--	--	369.25	--	387.26	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	185.13	--	**203.14**	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
1981	34.87	0.00	0.00%	2393.53

All possible peptide matches for this spectrum

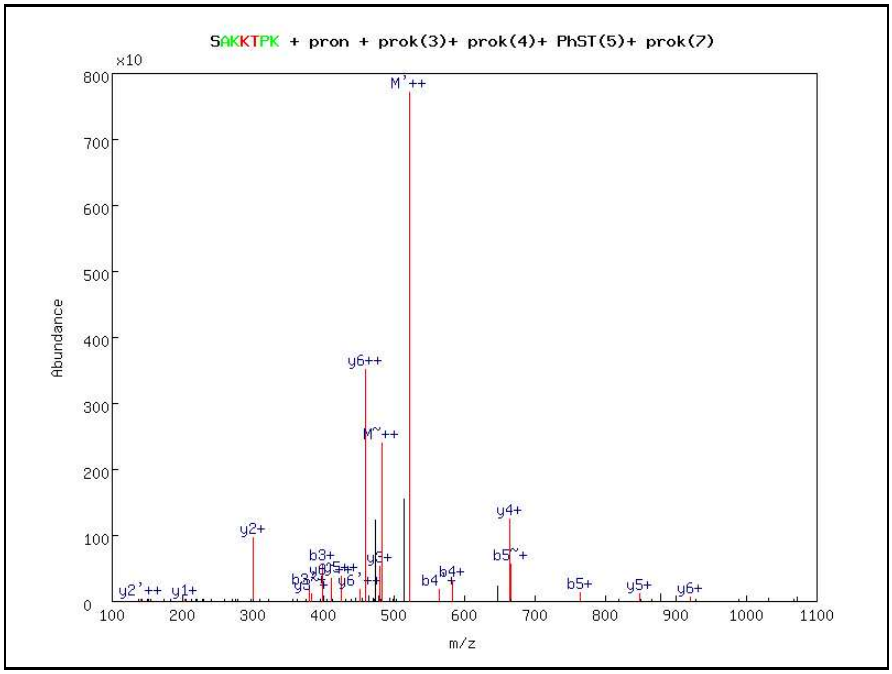
2135 1981 +2 75 43.3 34.5 6.7 616.8177 1232.6282 1232.6286 -0.0004 2 √ KAAGGATPKK + pron + prok(1)

The peptide is from:

hit2 sp|P16403|H12_HUMAN Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2

H1.2 pT154

9/3/13 9:10 AM



Index scan# charge score pp pp_2 pp_{tag} m/z MW(obs) MW delta miss Unique sequence + modifications

1589 1836 +2 50 39.5 21.8 4.4 532.2751 1063.5430 1063.5435 -0.0005 2 × SAKKTPK + pron + prok(3)+]

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	63.53	--	72.54	--	--	126.05	--	144.07	S	--	483.29	523.27	--	532.28	--	965.57	1045.53	--	1063.54	M
2	--	--	99.05	--	108.05	--	--	197.09	--	215.10	A	--	411.76	451.74	--	460.75	--	822.51	902.47	--	920.49	6
3	--	--	191.11	--	200.12	--	--	381.21	--	399.22	K	--	376.24	416.22	--	425.23	--	751.47	831.44	--	849.45	5
4	--	--	283.17	--	292.18	--	--	565.33	--	583.34	K	--	284.18	324.16	--	333.17	--	567.35	647.32	--	665.33	4
5	--	333.69	373.68	--	382.68	--	666.38	746.35	--	764.36	T	--	192.12	232.10	--	241.11	--	383.23	463.20	--	481.21	3
6	--	382.22	422.20	--	431.21	--	763.43	843.40	--	861.41	P	--	--	141.59	--	150.60	--	--	282.18	--	300.19	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	185.13	--	203.14	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
1836	33.16	0.00	0.00%	49208.29

All possible peptide matches for this spectrum

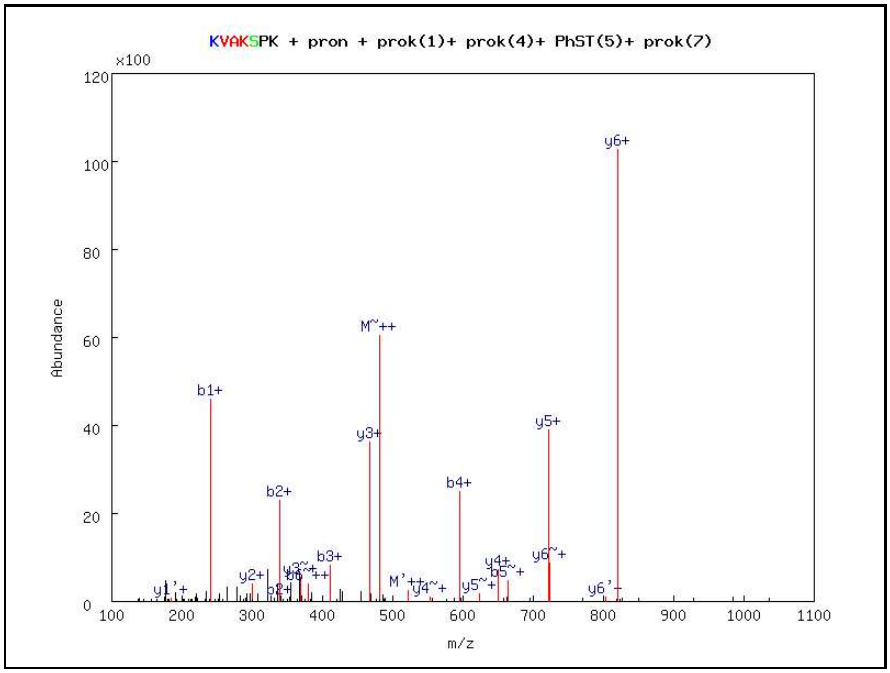
1589 1836 +2 50 39.5 21.8 4.4 532.2751 1063.5430 1063.5435 -0.0005 2 × SAKKTPK + pron + prok(3)+]

The peptide is from:

hit1 sp|P10412|H14_HUMAN Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2
hit2 sp|P16403|H12_HUMAN Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2

H1.2 pT173

9/3/13 9:09 AM



Index scan# charge score pp pp_2 pp_{tag} m/z MW(obs) MW delta miss Unique sequence + modifications

1519 2342 +2 72 38.1 32.2 3.7 531.2859 1061.5646 1061.5642 0.0004 2 × KVAKSPK + pron + prok(1)+ p:

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	--	--	121.08	--	--	--	--	241.15	K	--	482.30	522.28	--	531.29	--	963.59	1043.55	--	1061.56	M
2	--	--	--	--	170.62	--	--	--	--	340.22	V	--	362.22	402.21	--	411.21	--	723.44	803.41	--	821.42	6
3	--	--	--	--	206.13	--	--	--	--	411.26	A	--	312.69	352.67	--	361.68	--	624.37	704.34	--	722.35	5
4	--	--	--	--	298.19	--	--	--	--	595.38	K	--	277.17	317.15	--	326.16	--	553.33	633.30	--	651.31	4
5	--	332.71	--	--	381.69	--	664.40	--	--	762.38	S	--	185.11	225.09	--	234.10	--	369.21	449.18	--	467.19	3
6	--	381.23	--	--	430.22	--	761.46	--	--	859.43	P	--	--	141.59	--	150.60	--	--	282.18	--	300.19	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	185.13	--	203.14	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
2342	40.20	0.00	0.00%	90130.04

All possible peptide matches for this spectrum

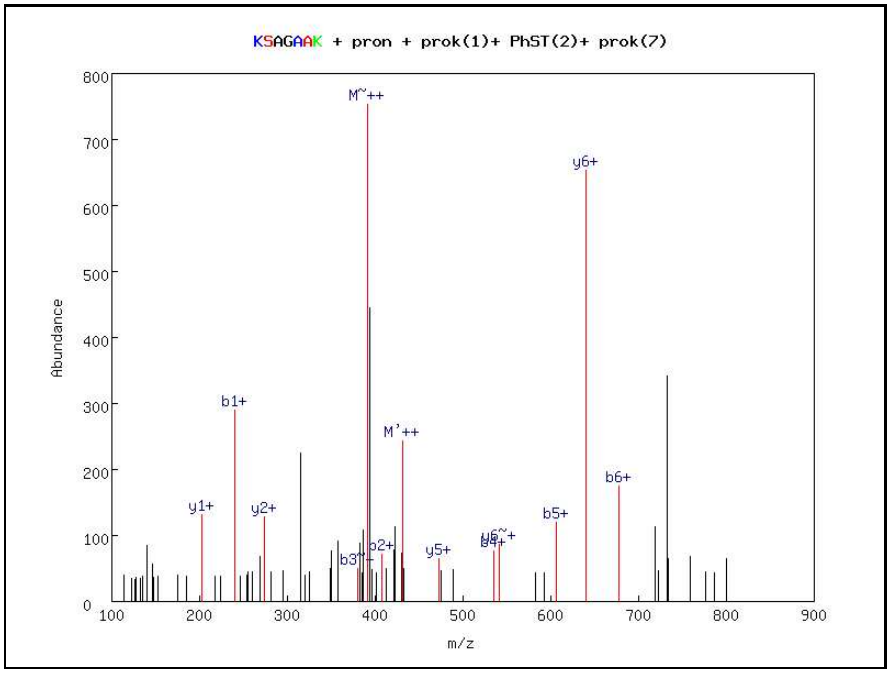
1519 2342 +2 72 38.1 32.2 3.7 531.2859 1061.5646 1061.5642 0.0004 2 × KVAKSPK + pron + prok(1)+ p:

The peptide is from:

hit2 sp|P16403|H12_HUMAN Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2
hit3 sp|P16401|H15_HUMAN Histone H1.5 OS=Homo sapiens GN=HIST1H1B PE=1 SV=3

H1.4 pS27

9/3/13 9:08 AM



Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
501	1645	+2	19	24.7	22.1	2.8	440.7123	880.4174	880.4176	-0.0002	1	√	KSAGAAK + pron + prok(1)+ 1

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	--	--	121.08	--	--	--	--	241.15	K	--	391.72	431.71	--	440.71	--	782.44	862.41	--	880.42	M
2	--	155.59	--	--	204.58	--	310.18	--	--	408.15	S	--	271.65	311.63	--	320.64	--	542.29	622.26	--	640.27	6
3	--	191.11	--	--	240.10	--	381.21	--	--	479.19	A	--	--	228.13	--	237.14	--	--	455.26	--	473.27	5
4	--	219.62	--	--	268.61	--	438.23	--	--	536.21	G	--	--	192.62	--	201.62	--	--	384.22	--	402.23	4
5	--	255.14	--	--	304.13	--	509.27	--	--	607.25	A	--	--	164.10	--	173.11	--	--	327.20	--	345.21	3
6	--	290.66	--	--	339.65	--	580.31	--	--	678.29	A	--	--	128.59	--	137.59	--	--	256.17	--	274.18	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	185.13	--	203.14	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
1645	30.43	0.00	0.00%	6229.79

All possible peptide matches for this spectrum

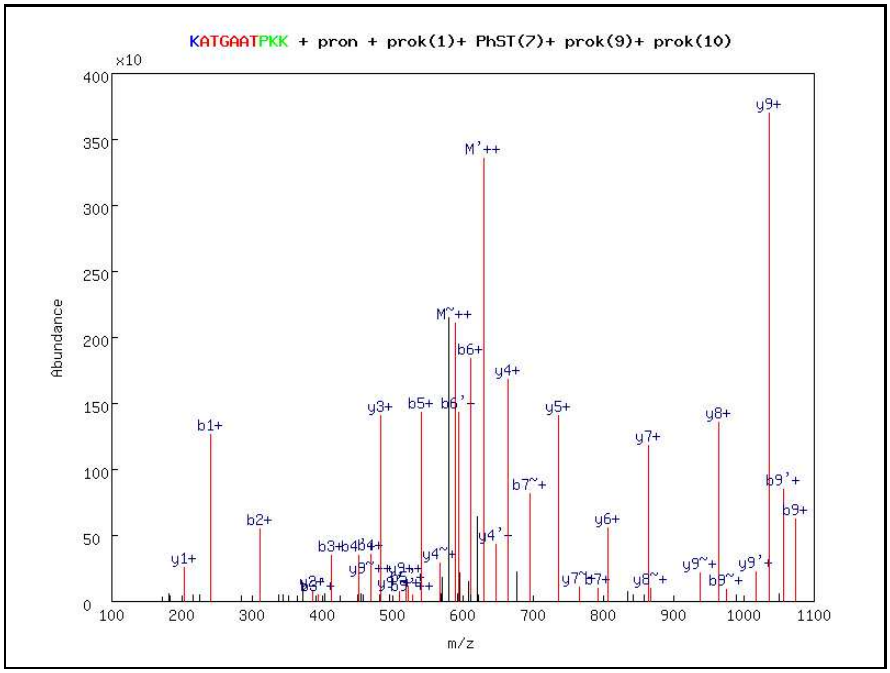
501 1645 +2 19 24.7 22.1 2.8 440.7123 880.4174 880.4176 -0.0002 1 √ KSAGAAK + pron + prok(1)+ 1

The peptide is from:

hit1 sp|P10412|H14_HUMAN Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2

H1.4 pT146

9/3/13 9:06 AM



Index scan# charge score pp pp_2 pp_{tag} m/z MW(obs) MW delta miss Unique sequence + modifications

2253 2005 +2 164 76.9 43.3 9.4 638.8310 1276.6547 1276.6548 -0.0001 2 √ KATGAATPKK + pron + prok(1

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	--	--	121.08	--	--	--	--	241.15	K	--	589.84	629.83	--	638.83	--	1178.68	1258.64	--	1276.65	M
2	--	--	--	--	156.60	--	--	--	--	312.19	A	--	469.77	509.75	--	518.76	--	938.53	1018.50	--	1036.51	9
3	--	--	198.12	--	207.12	--	--	395.23	--	413.24	T	--	434.25	474.23	--	483.24	--	867.49	947.46	--	965.47	8
4	--	--	226.63	--	235.63	--	--	452.25	--	470.26	G	--	383.73	423.71	--	432.71	--	766.45	846.41	--	864.42	7
5	--	--	262.15	--	271.15	--	--	523.29	--	541.30	A	--	355.22	395.20	--	404.20	--	709.42	789.39	--	807.40	6
6	--	--	297.67	--	306.67	--	--	594.32	--	612.34	A	--	319.70	359.68	--	368.69	--	638.39	718.35	--	736.36	5
7	--	348.19	388.17	--	397.18	--	695.37	775.34	--	793.35	T	--	284.18	324.16	--	333.17	--	567.35	647.32	--	665.33	4
8	--	396.72	436.70	--	445.70	--	792.43	872.39	--	890.40	P	--	--	233.65	--	242.66	--	--	466.30	--	484.31	3
9	--	488.78	528.76	--	537.77	--	976.55	1056.51	--	1074.52	K	--	--	185.13	--	194.13	--	--	369.25	--	387.26	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	185.13	--	203.14	1

Spectral Info:

Scan# t_R(min) t_R(Pred) Conf. t_R Peak Area

2005 35.69 0.00 0.00% 112530.66

All possible peptide matches for this spectrum

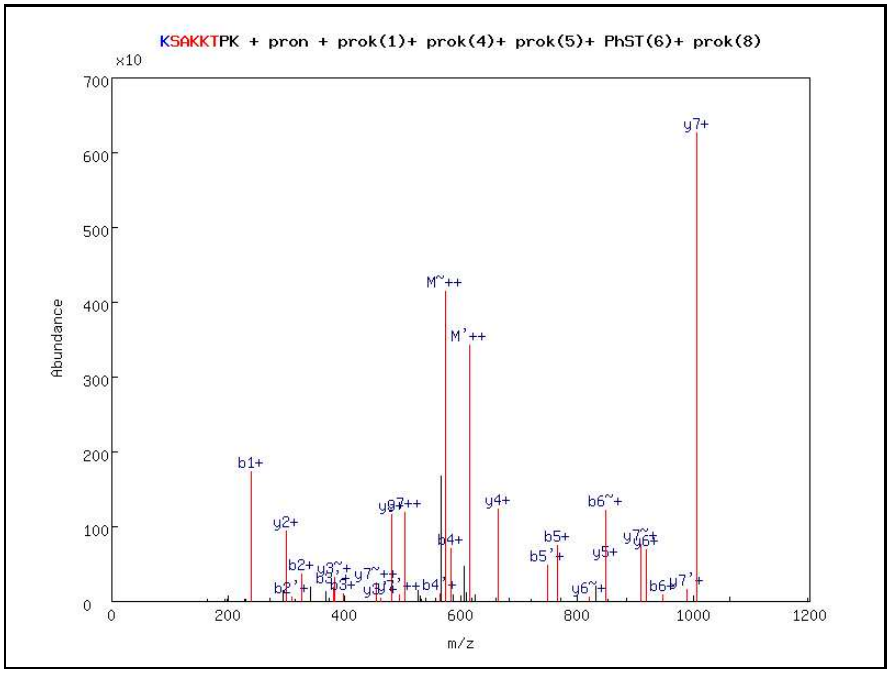
2253 2005 +2 164 76.9 43.3 9.4 638.8310 1276.6547 1276.6548 -0.0001 2 √ KATGAATPKK + pron + prok(1

The peptide is from:

hit1 sp|P10412|H14_HUMAN Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2

H1.4 pT154

9/3/13 9:08 AM



Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
2208	2247	+2	101	58.8	28.5	5.4	624.3358	1247.6644	1247.6647	-0.0003	3	×	KSAKKTPK + pron + prok(1)+

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	--	--	121.08	--	--	--	--	241.15	K	--	575.35	615.33	--	624.34	--	1149.69	1229.65	--	1247.66	M
2	--	--	155.59	--	164.60	--	--	310.18	--	328.19	S	--	455.27	495.26	--	504.26	--	909.54	989.51	--	1007.52	7
3	--	--	191.11	--	200.12	--	--	381.21	--	399.22	A	--	411.76	451.74	--	460.75	--	822.51	902.47	--	920.49	6
4	--	--	283.17	--	292.18	--	--	565.33	--	583.34	K	--	376.24	416.22	--	425.23	--	751.47	831.44	--	849.45	5
5	--	--	375.23	--	384.24	--	--	749.46	--	767.47	K	--	284.18	324.16	--	333.17	--	567.35	647.32	--	665.33	4
6	--	425.76	465.74	--	474.74	--	850.50	930.47	--	948.48	T	--	192.12	232.10	--	241.11	--	383.23	463.20	--	481.21	3
7	--	474.28	514.26	--	523.27	--	947.56	1027.52	--	1045.53	P	--	--	141.59	--	150.60	--	--	282.18	--	300.19	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	185.13	--	203.14	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
2247	38.74	0.00	0.00%	43078.40

All possible peptide matches for this spectrum

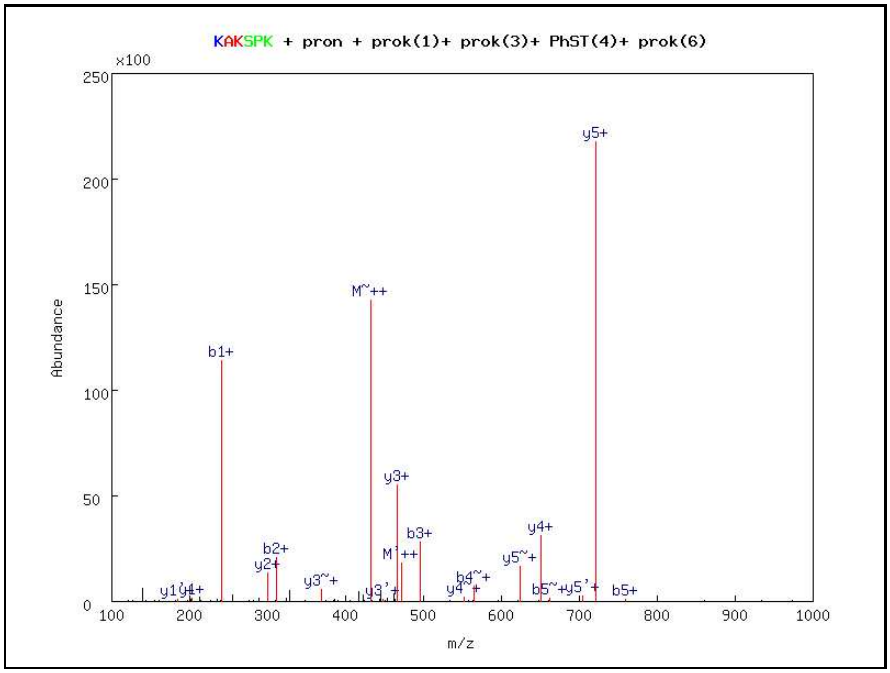
2208 2247 +2 101 58.8 28.5 5.4 624.3358 1247.6644 1247.6647 -0.0003 3 × KSAKKTPK + pron + prok(1)+

The peptide is from:

hit1 sp|P10412|H14_HUMAN Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2
hit2 sp|P16403|H12_HUMAN Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2

H1.4 pS172

9/3/13 9:07 AM



Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
1021	1922	+2	88	40.2	24.5	4.4	481.7510	962.4948	962.4958	-0.0010	2	√	KAKSPK + pron + prok(1)+ pr

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	--	--	121.08	--	--	--	--	241.15	K	--	432.76	472.75	--	481.75	--	864.52	944.49	--	962.50	M
2	--	--	--	--	156.60	--	--	--	--	312.19	A	--	312.69	352.67	--	361.68	--	624.37	704.34	--	722.35	5
3	--	--	--	--	248.66	--	--	--	--	496.31	K	--	277.17	317.15	--	326.16	--	553.33	633.30	--	651.31	4
4	--	283.17	--	--	332.16	--	565.33	--	--	663.31	S	--	185.11	225.09	--	234.10	--	369.21	449.18	--	467.19	3
5	--	331.70	--	--	380.69	--	662.39	--	--	760.36	P	--	--	141.59	--	150.60	--	--	282.18	--	300.19	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	185.13	--	203.14	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
1922	34.51	0.00	0.00%	248805.94

All possible peptide matches for this spectrum

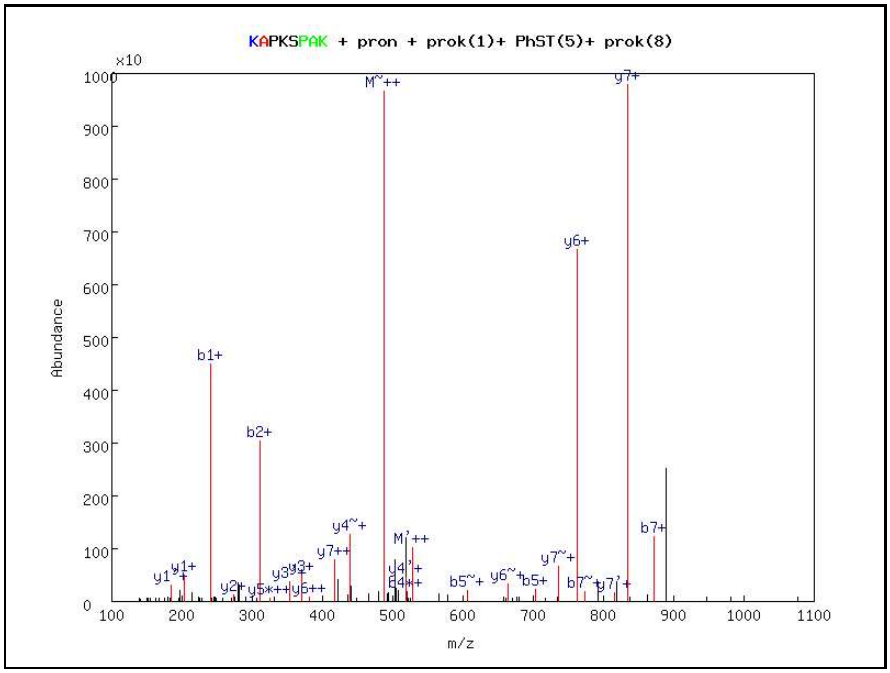
1021 1922 +2 88 40.2 24.5 4.4 481.7510 962.4948 962.4958 -0.0010 2 √ KAKSPK + pron + prok(1)+ pr

The peptide is from:

hit1 sp|P10412|H14_HUMAN Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2

H1.4 pS187

9/3/13 9:07 AM



Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications
1615	1342	+2	78	45.0	27.9	2.2	537.7831	1074.5589	1074.5595	-0.0006	2	√	KAPKSPAK + pron + prok(1)+

#	b^++	b~++	b'++	b*++	b++	b^+	b~+	b'+	b*+	b+	seq	y^++	y~++	y'++	y*++	y++	y^+	y~+	y'+	y*+	y+	#
1	--	--	--	--	121.08	--	--	--	--	**241.15**	K	--	**488.79**	**528.78**	529.27	537.78	--	976.58	1056.55	1057.53	1074.56	M
2	--	--	--	--	156.60	--	--	--	--	**312.19**	A	--	368.72	408.70	409.20	**417.71**	--	**736.44**	**816.40**	817.39	**834.41**	7
3	--	--	--	--	205.13	--	--	--	--	409.24	P	--	333.20	373.19	373.68	**382.19**	--	**665.40**	745.36	746.35	**763.37**	6
4	--	--	--	260.66	269.17	--	--	--	**520.31**	537.34	K	--	284.68	324.66	**325.15**	333.66	--	568.35	648.31	649.30	666.32	5
5	--	303.68	--	344.16	352.67	--	**606.36**	--	687.31	**704.34**	S	--	220.63	260.61	--	269.62	--	**440.25**	**520.22**	--	538.23	4
6	--	352.21	--	392.69	401.20	--	703.41	--	784.36	801.39	P	--	--	177.11	--	186.12	--	--	**353.22**	--	**371.23**	3
7	--	387.73	--	428.20	436.72	--	**774.45**	--	855.40	**872.43**	A	--	--	128.59	--	137.59	--	--	256.17	--	**274.18**	2
	--	--	--	--	--	--	--	--	--	--	K	--	--	93.07	--	102.07	--	--	**185.13**	--	**203.14**	1

Spectral Info:

Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
1342	26.24	0.00	0.00%	7457.63

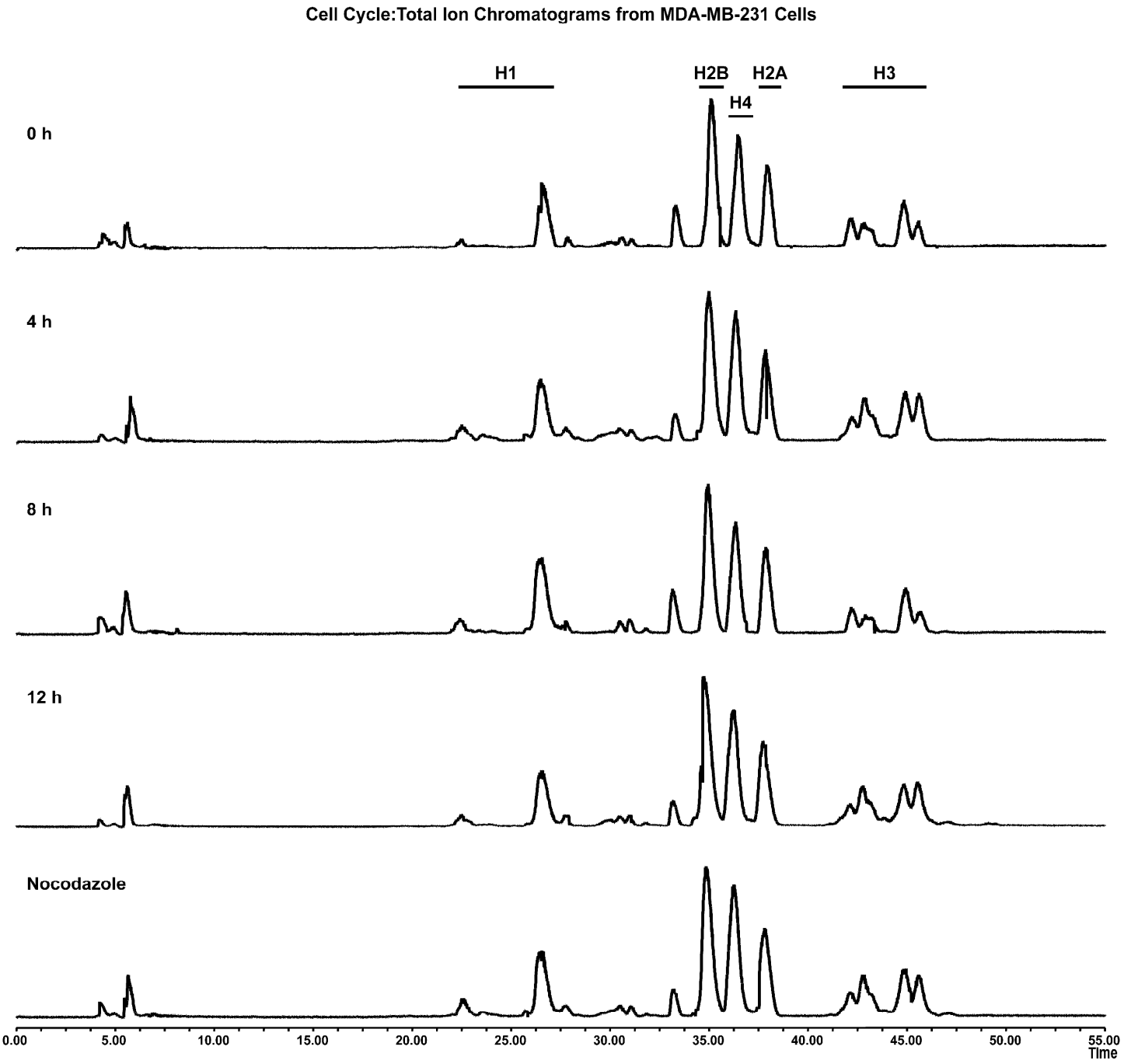
All possible peptide matches for this spectrum

1615 1342 +2 78 45.0 27.9 2.2 537.7831 1074.5589 1074.5595 -0.0006 2 √ KAPKSPAK + pron + prok(1)+

The peptide is from:

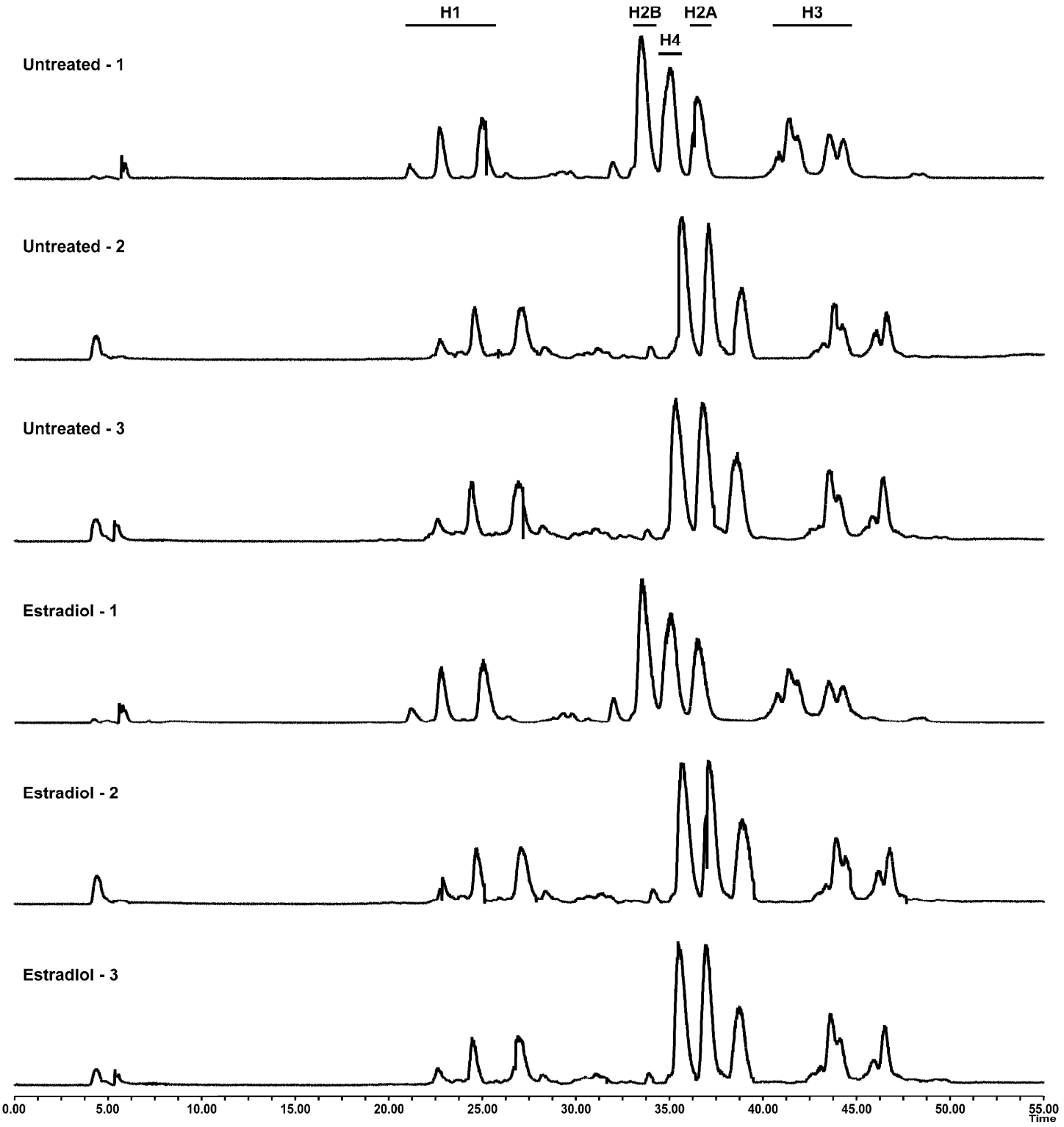
hit1 sp|P10412|H14_HUMAN Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2

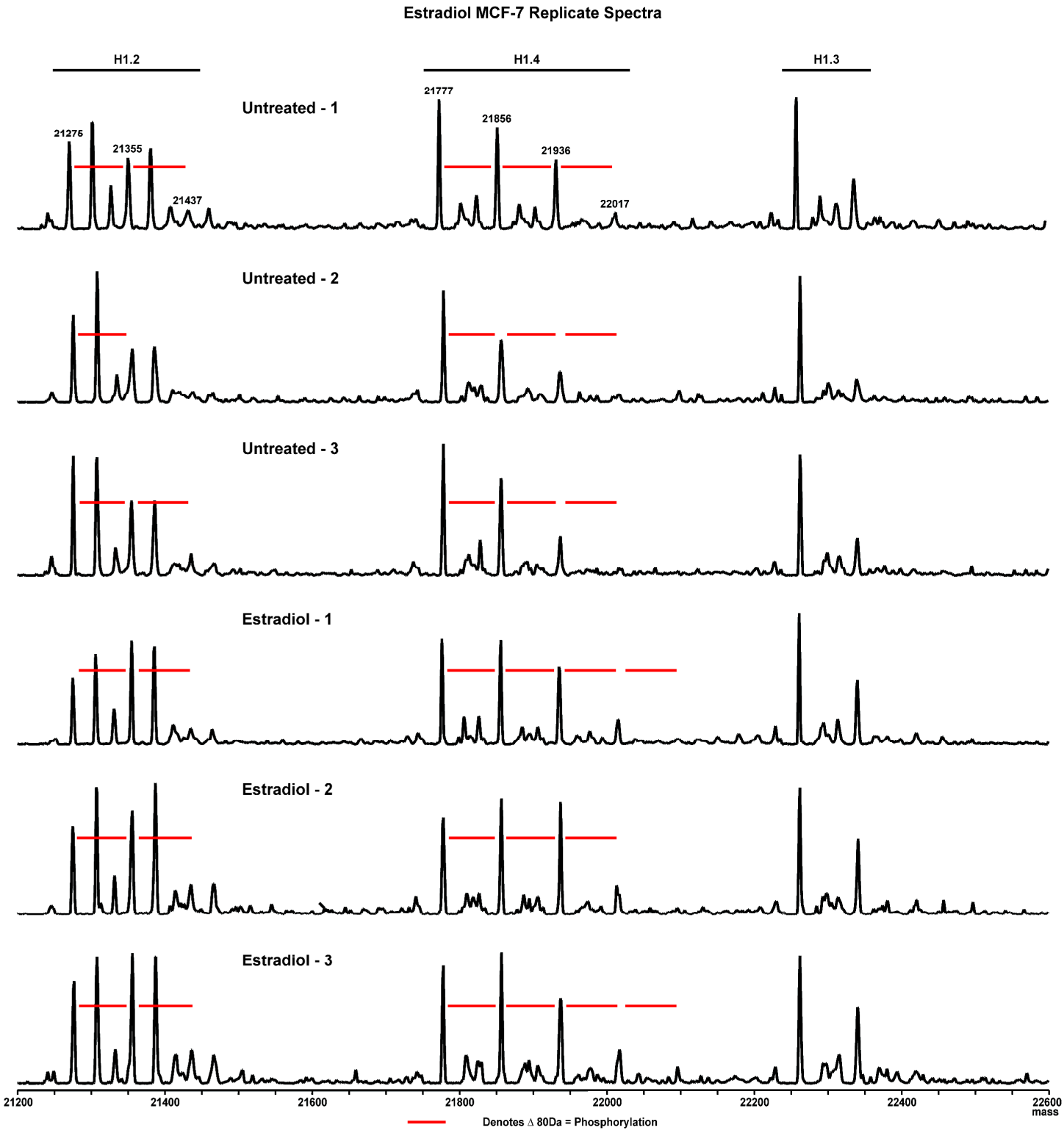
SUPPLEMENTAL DATA 6



SUPPLEMENTAL DATA 7

Estradiol:Total Ion Chromatograms from MCF-7 Treated Cells




Estradiol MCF-7 Replicate Spectra
H1.2
H1.4
H1.3
Untreated - 1
21275
21355
21437
21777
21856
21936
22017
Untreated - 2
Untreated - 3
Estradiol - 1
Estradiol - 2
Estradiol - 3
21200
21400
21600
21800
22000
22200
22400
22600
mass
Denotes Δ 80Da = Phosphorylation

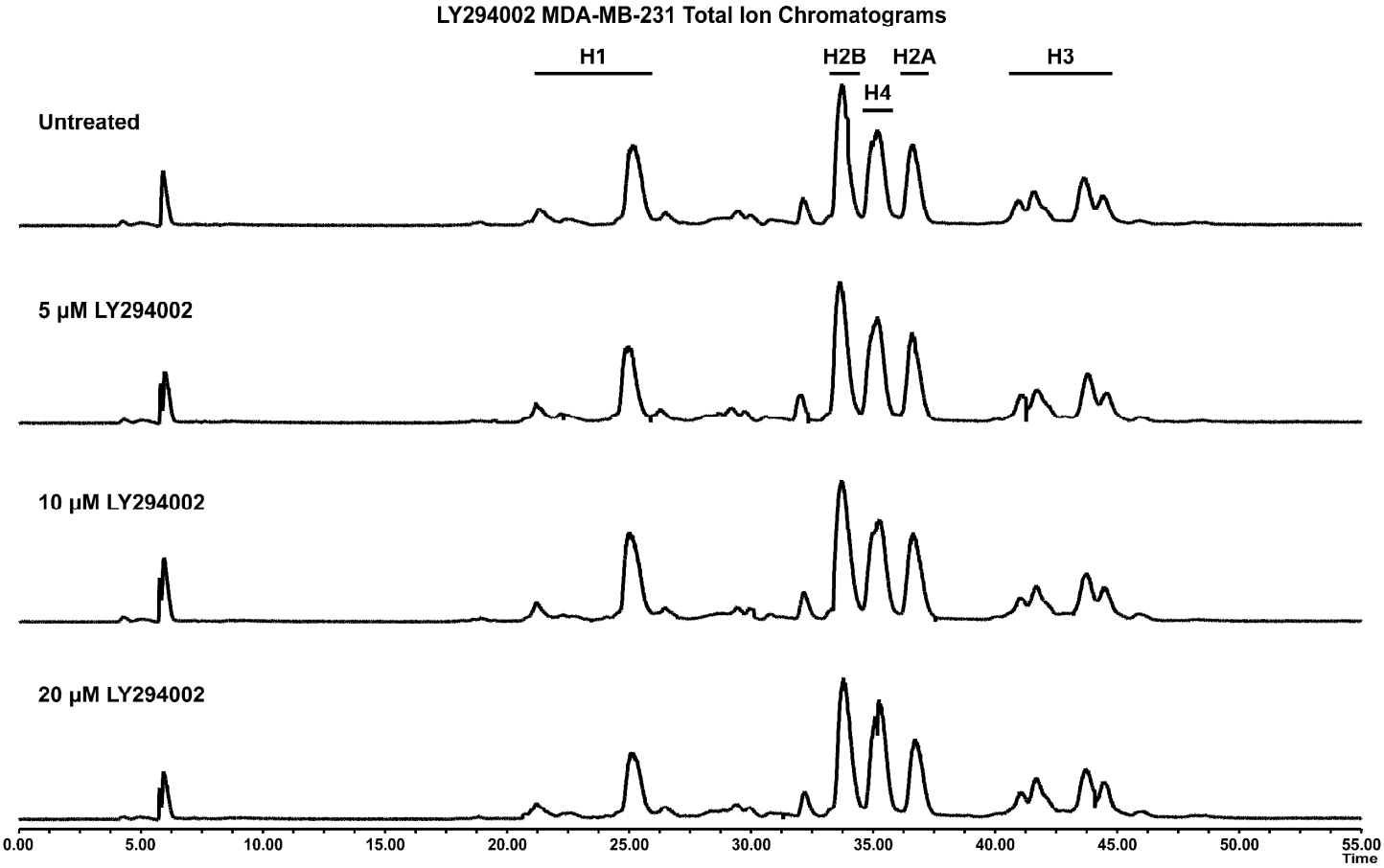
SUPPLEMENTAL DATA 8

Replcate LC-MS Peak Abundances from Estradiol Treated MCF-7 Cells

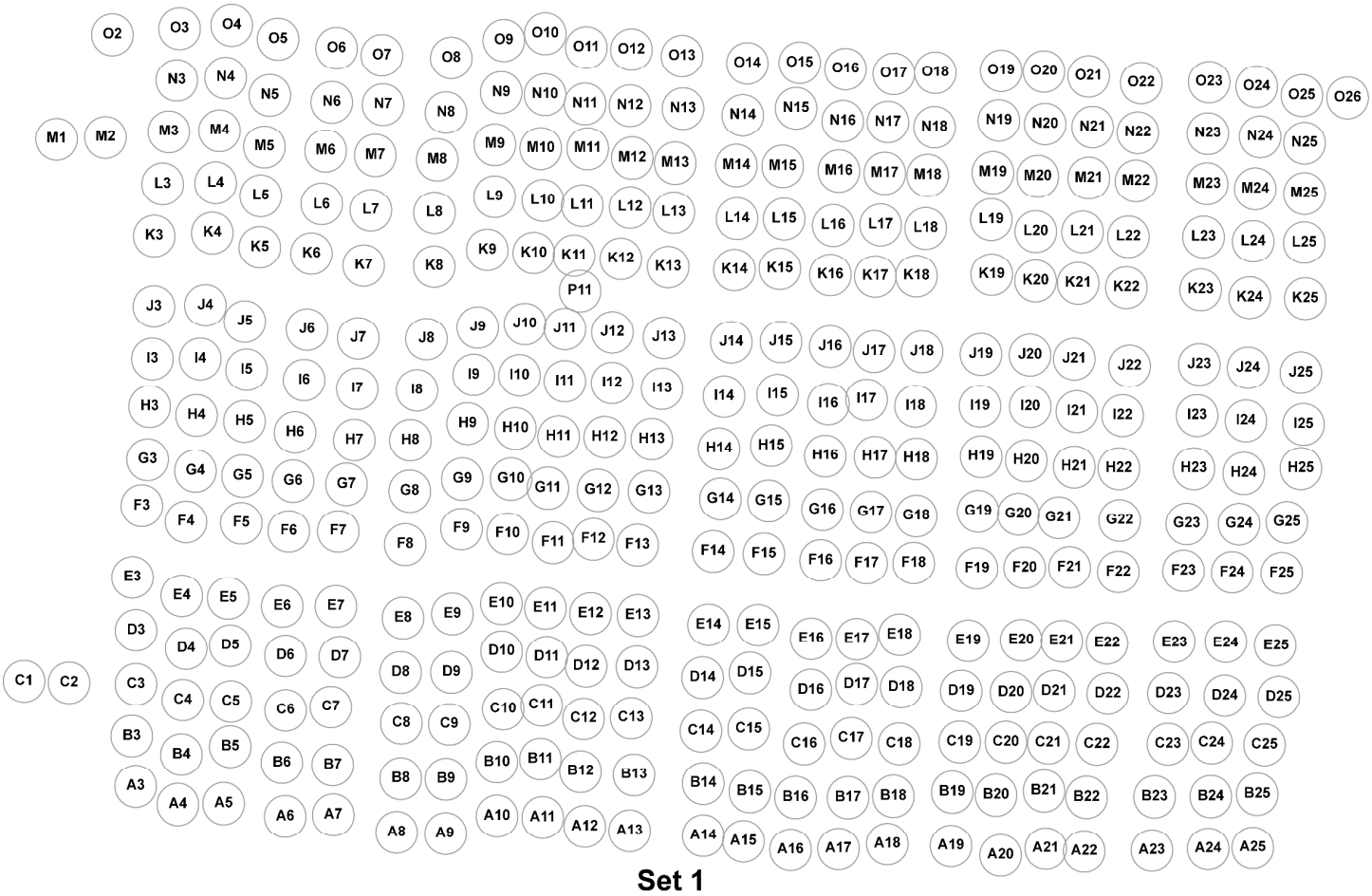
Untreated	H1.2	pH1.2	ppH1.2	Total pH1.2	Ratio pH1.2/H1.2	H1.4	pH1.4	ppH1.4	pppH1.4	ppppH1.4	Total pH1.4	Ratio pH1.4/H1.4
MCF-7 - 1	2229	1819	488	2307	0.816	3301	2598	1779	431	-	4808	1.457
MCF-7 - 2	1410	879	-	879	0.623	1816	1014	500	134	-	1648	0.907
MCF-7 - 3	2841	1783	532	2315	0.815	3118	2321	934	184	-	3439	1.103

70nM Estradiol	H1.2	pH1.2	ppH1.2	Total pH1.2	Ratio pH1.2/H1.2	H1.4	pH1.4	ppH1.4	pppH1.4	ppppH1.4	Total pH1.4	Ratio pH1.4/H1.4
MCF-7 - 1	3330	5157	807	5964	1.791	5248	5189	3859	1233	276	10557	2.012
MCF-7 - 2	1645	1929	549	2478	1.506	1800	2151	2088	531	-	4770	2.650
MCF-7 - 3	1126	1436	359	1795	1.594	1294	1443	944	364	181	2932	2.266

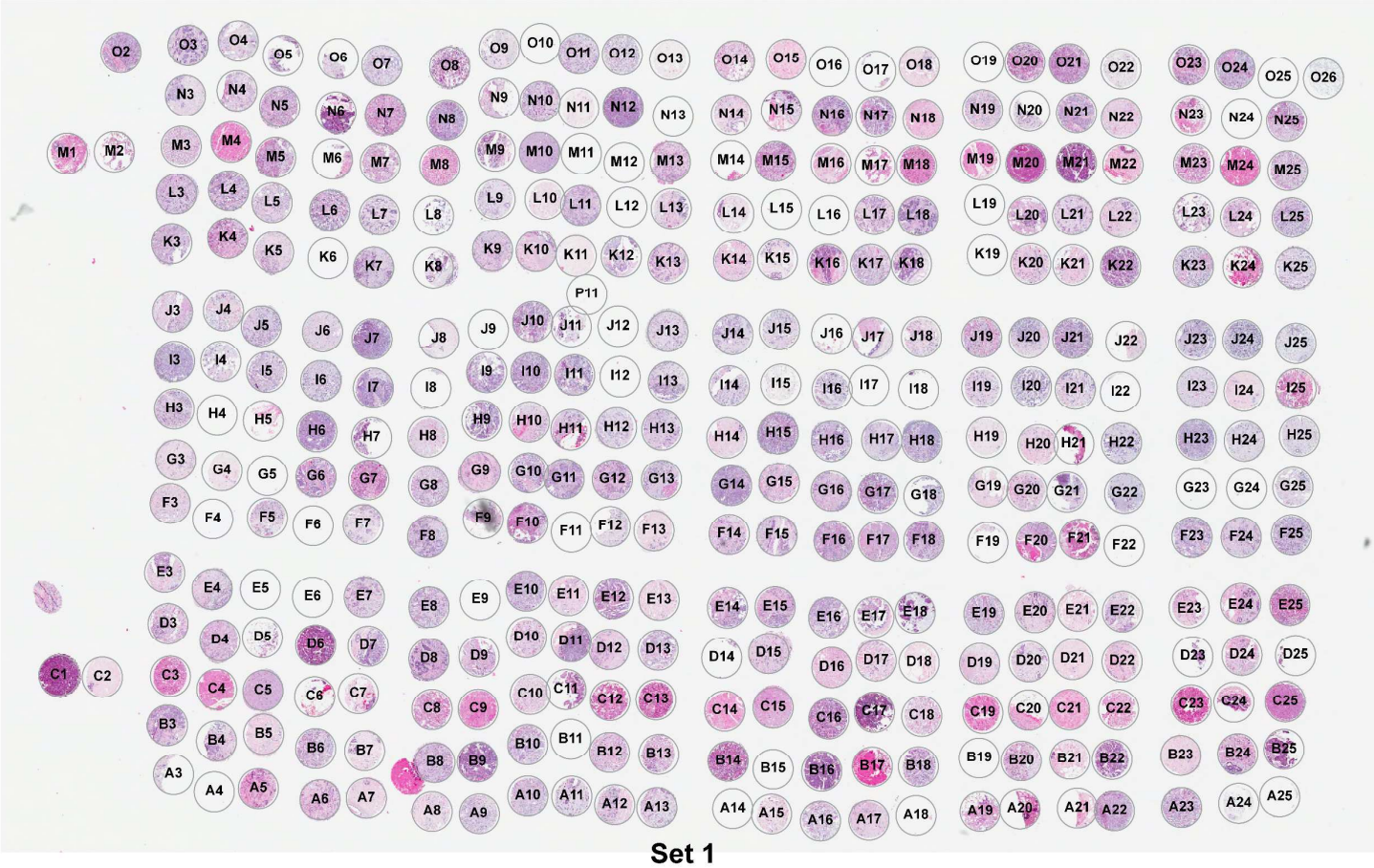
SUPPLEMENTAL DATA 9



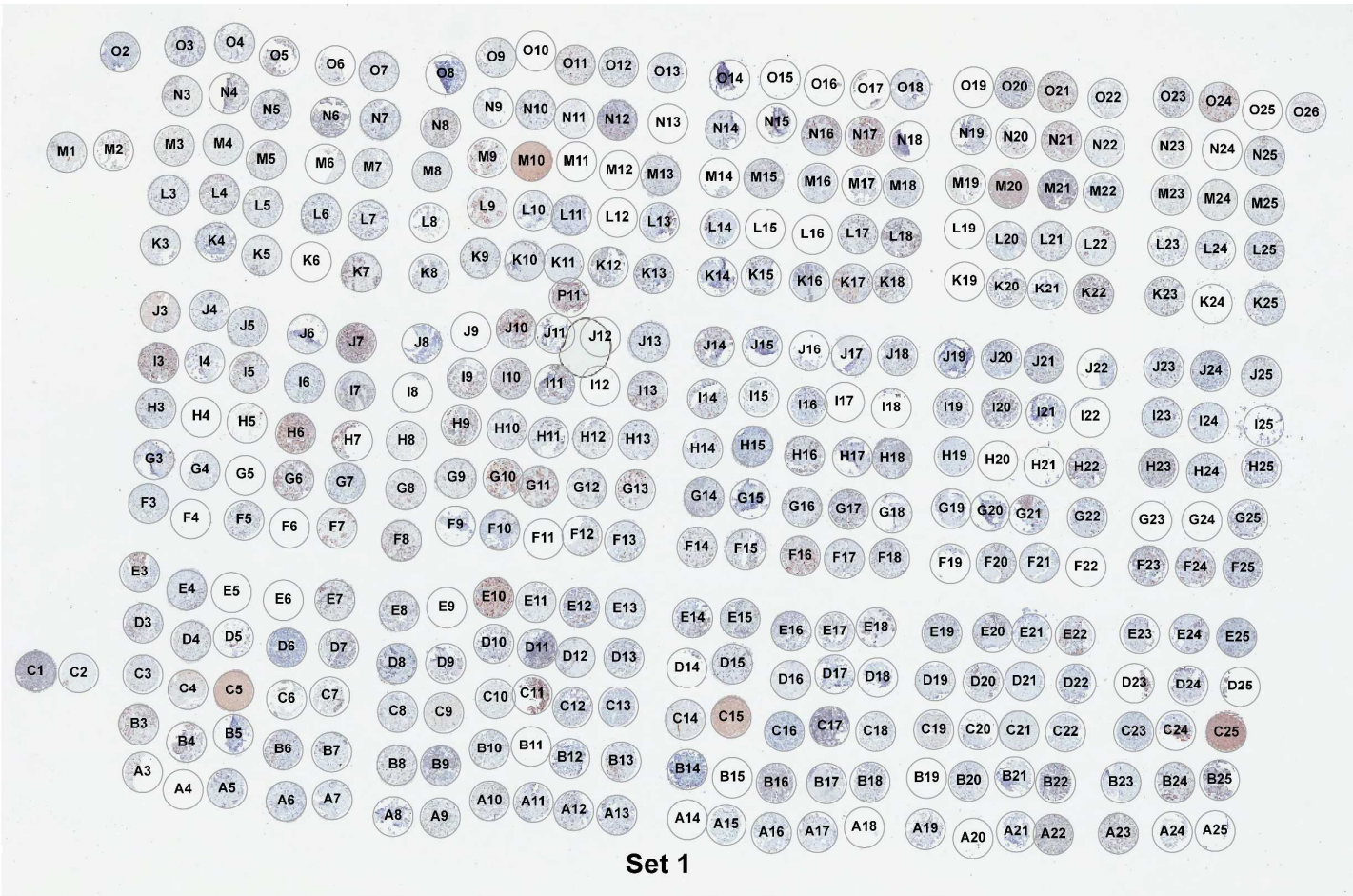
SUPPLEMENTAL DATA 10

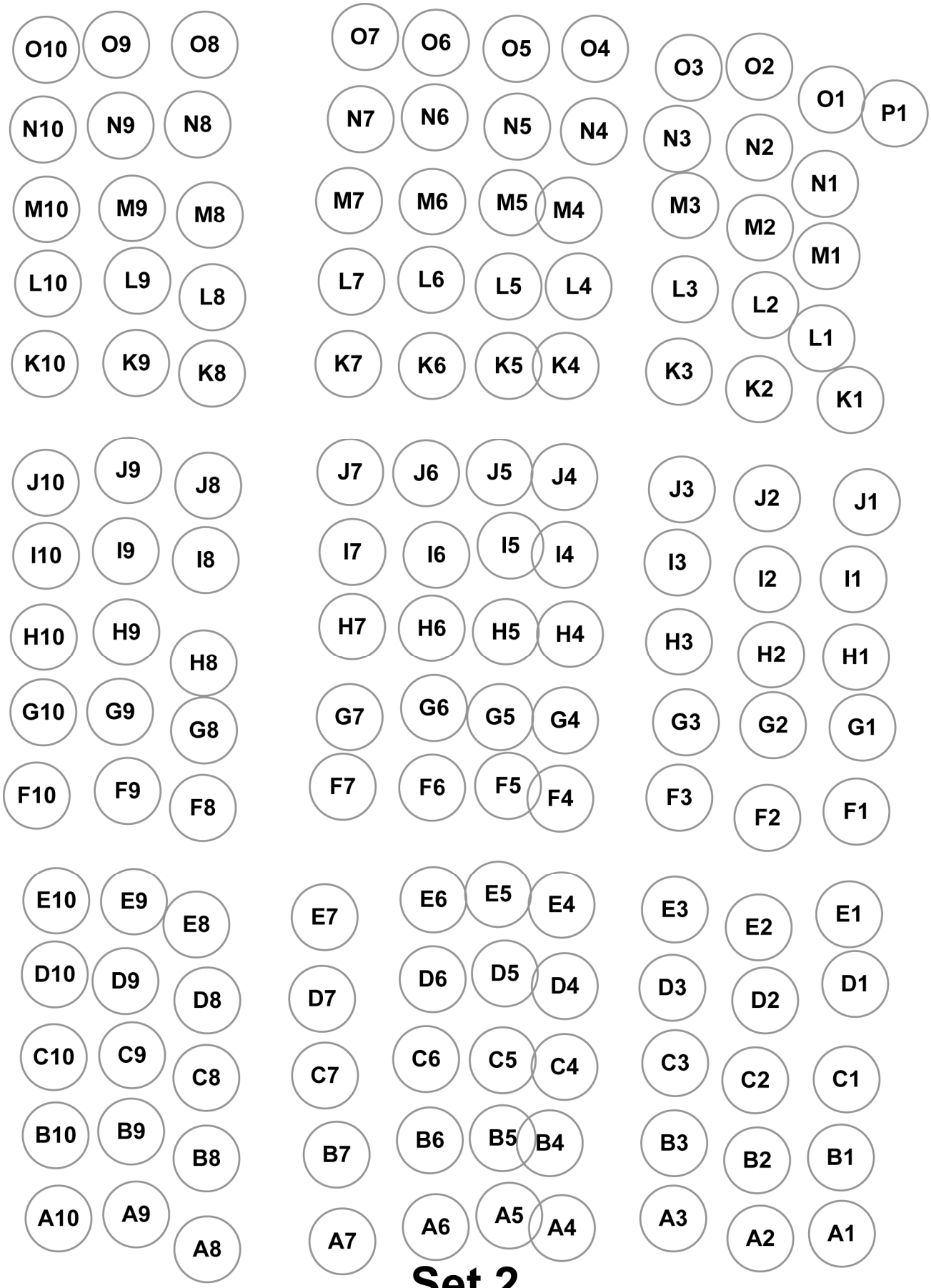


Set 1

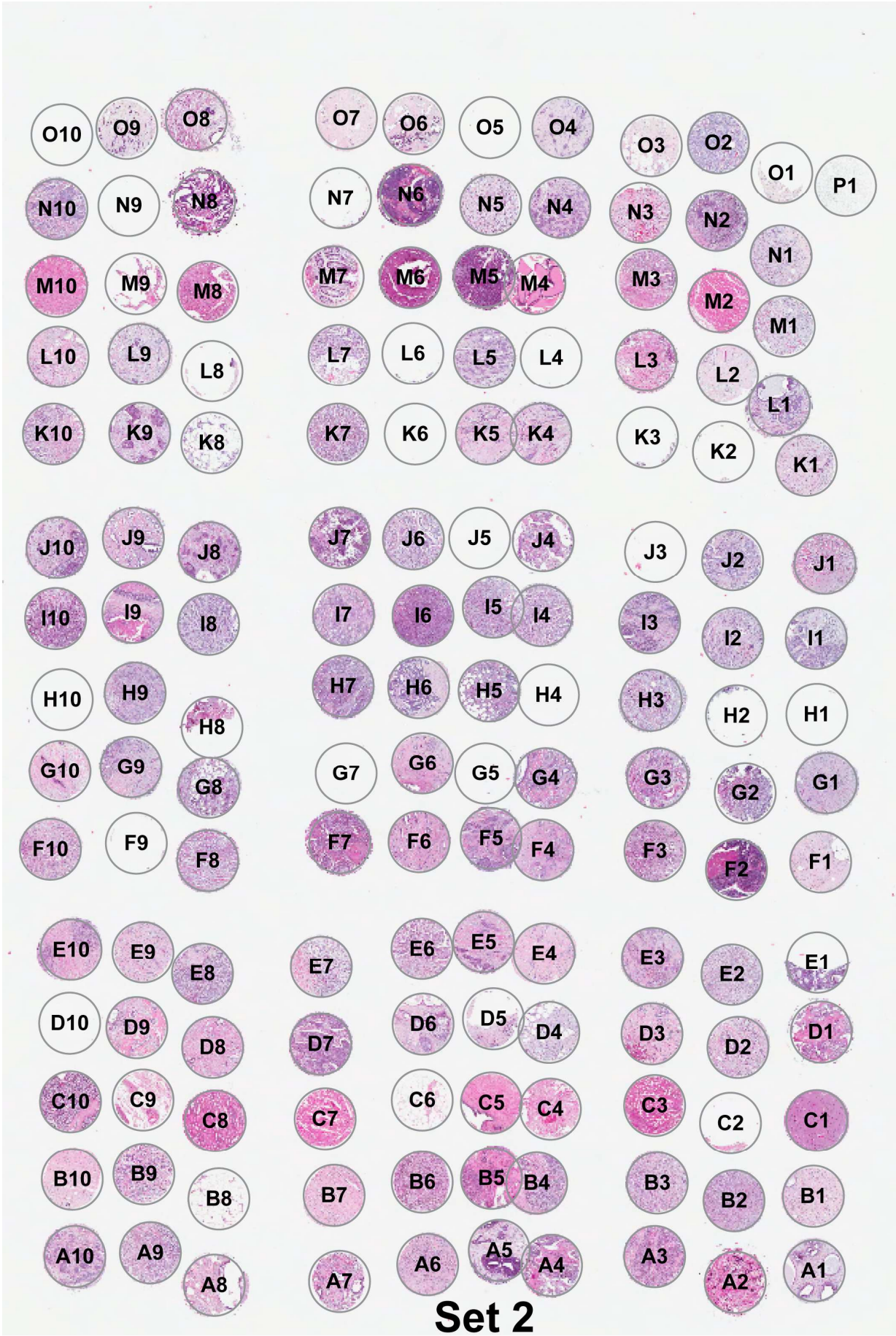


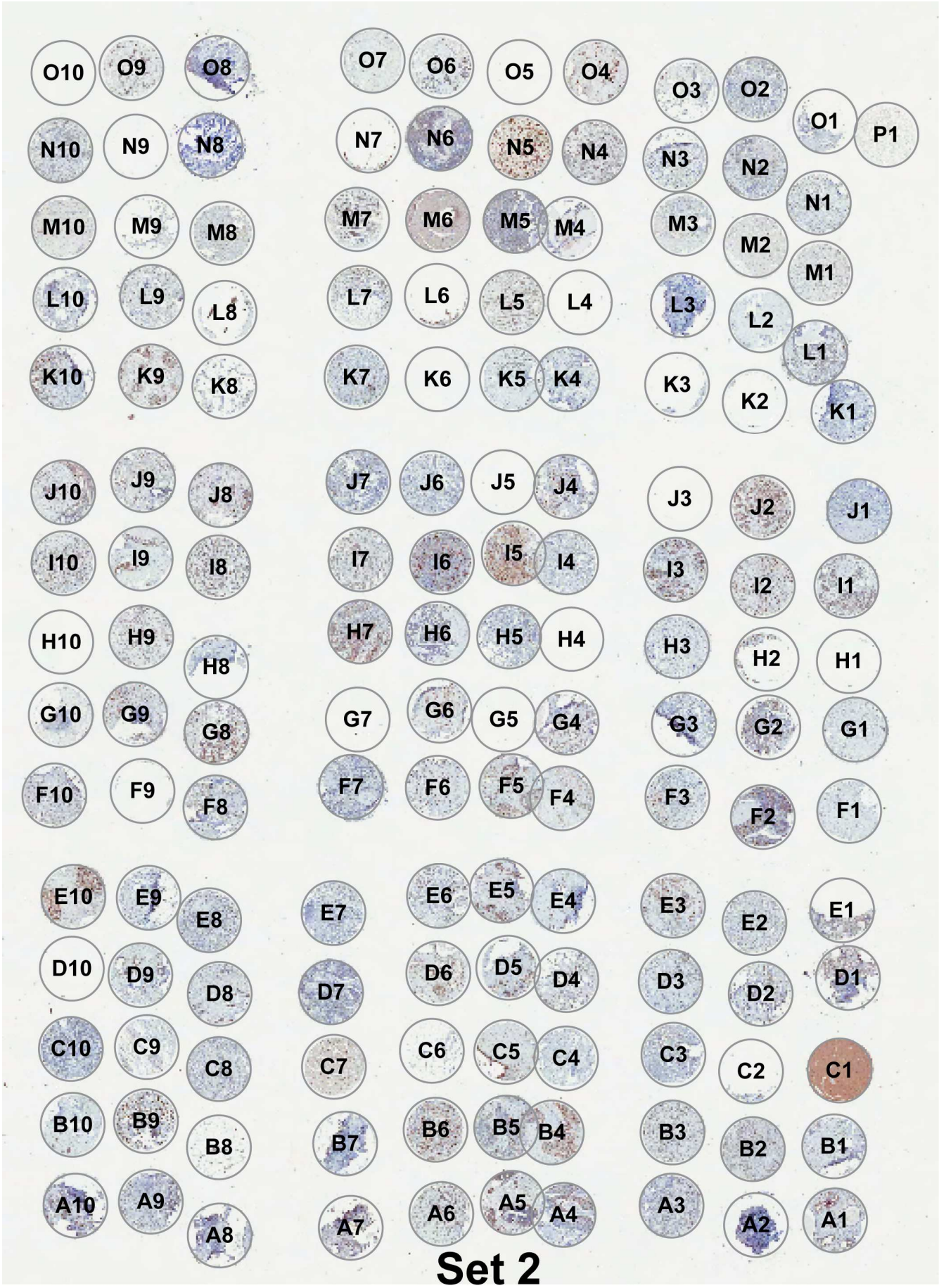
Set 1

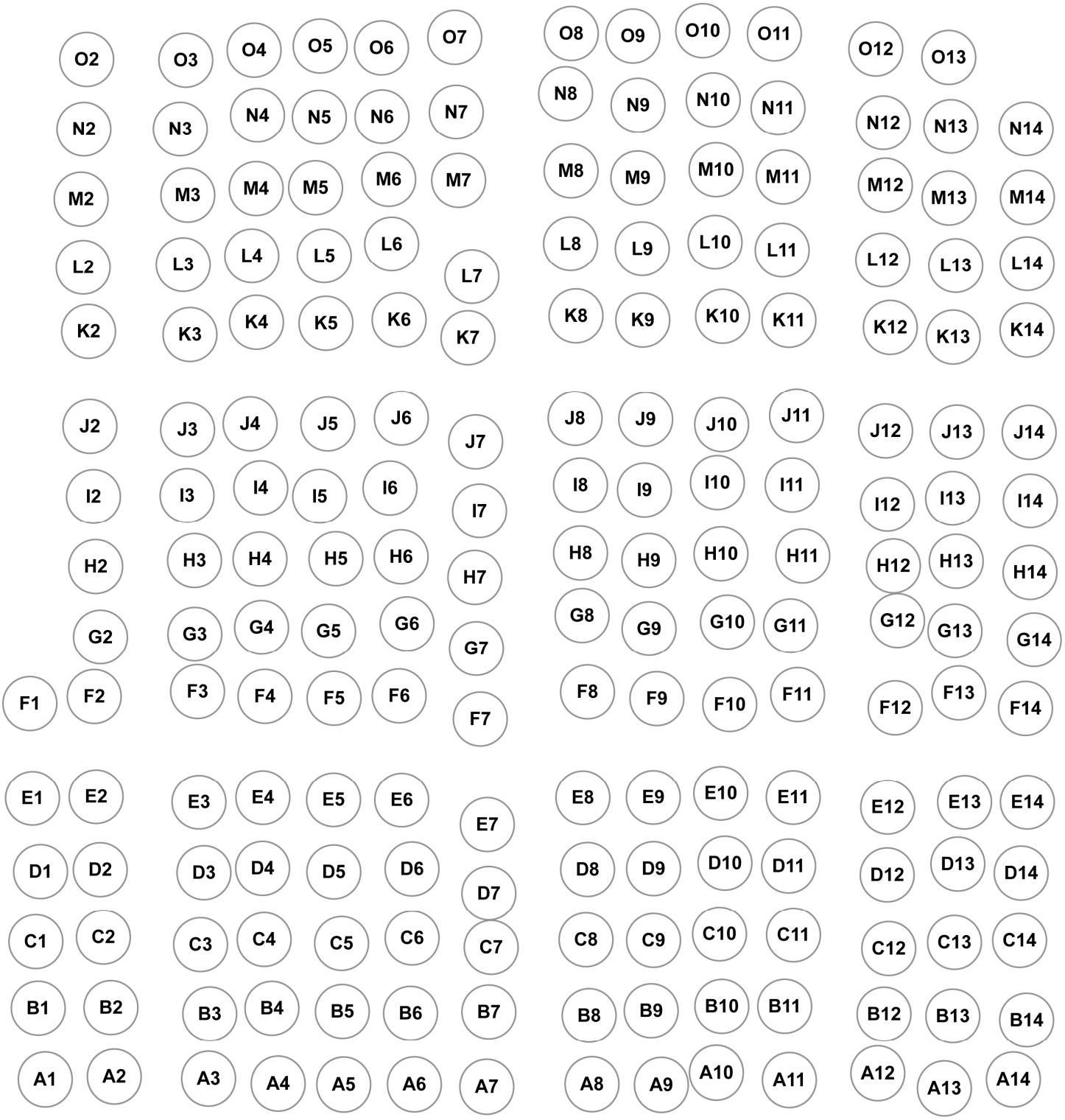

Set 1


O10 O9 O8
O7 O6 O5 O4
O3 O2 O1 P1
N10 N9 N8
N7 N6 N5 N4
N3 N2 N1
M10 M9 M8
M7 M6 M5 M4
M3 M2 M1
L10 L9 L8
L7 L6 L5 L4
L3 L2 L1
K10 K9 K8
K7 K6 K5 K4
K3 K2 K1
J10 J9 J8
J7 J6 J5 J4
J3 J2 J1
I10 I9 I8
I7 I6 I5 I4
I3 I2 I1
H10 H9 H8
H7 H6 H5 H4
H3 H2 H1
G10 G9 G8
G7 G6 G5 G4
G3 G2 G1
F10 F9 F8
F7 F6 F5 F4
F3 F2 F1
E10 E9 E8
E7
E6 E5 E4
E3 E2 E1
D10 D9 D8
D7
D6 D5 D4
D3 D2 D1
C10 C9 C8
C7
C6 C5 C4
C3 C2 C1
B10 B9 B8
B7
B6 B5 B4
B3 B2 B1
A10 A9 A8
A7
A6 A5 A4
A3 A2 A1

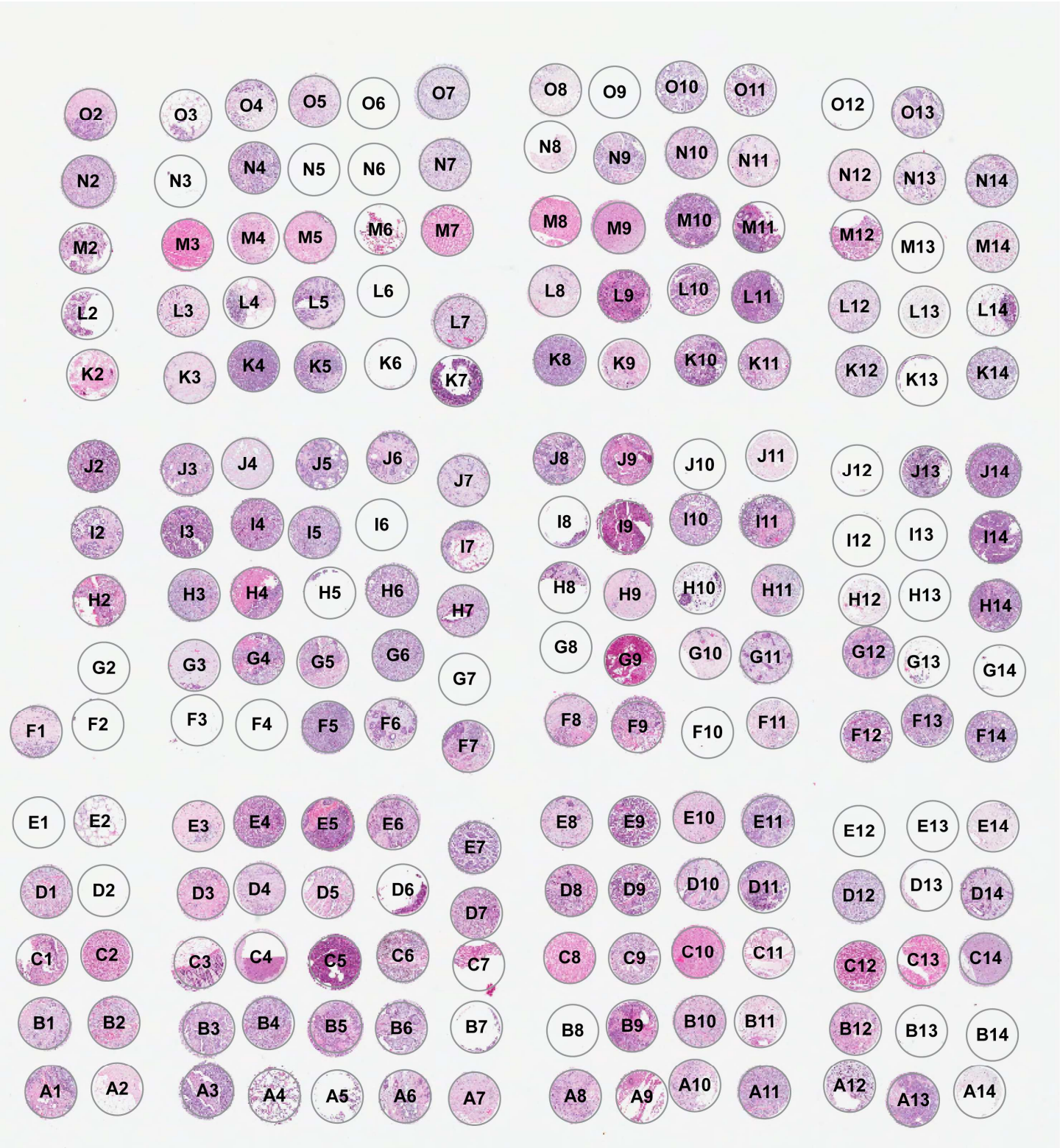
Set 2


O10 O9 O8 O7 O6 O5 O4 O3 O2 O1 P1
N10 N9 N8 N7 N6 N5 N4 N3 N2 N1
M10 M9 M8 M7 M6 M5 M4 M3 M2 M1
L10 L9 L8 L7 L6 L5 L4 L3 L2 L1
K10 K9 K8 K7 K6 K5 K4 K3 K2 K1
J10 J9 J8 J7 J6 J5 J4 J3 J2 J1
I10 I9 I8 I7 I6 I5 I4 I3 I2 I1
H10 H9 H8 H7 H6 H5 H4 H3 H2 H1
G10 G9 G8 G7 G6 G5 G4 G3 G2 G1
F10 F9 F8 F7 F6 F5 F4 F3 F2 F1
E10 E9 E8 E7 E6 E5 E4 E3 E2 E1
D10 D9 D8 D7 D6 D5 D4 D3 D2 D1
C10 C9 C8 C7 C6 C5 C4 C3 C2 C1
B10 B9 B8 B7 B6 B5 B4 B3 B2 B1
A10 A9 A8 A7 A6 A5 A4 A3 A2 A1
Set 2

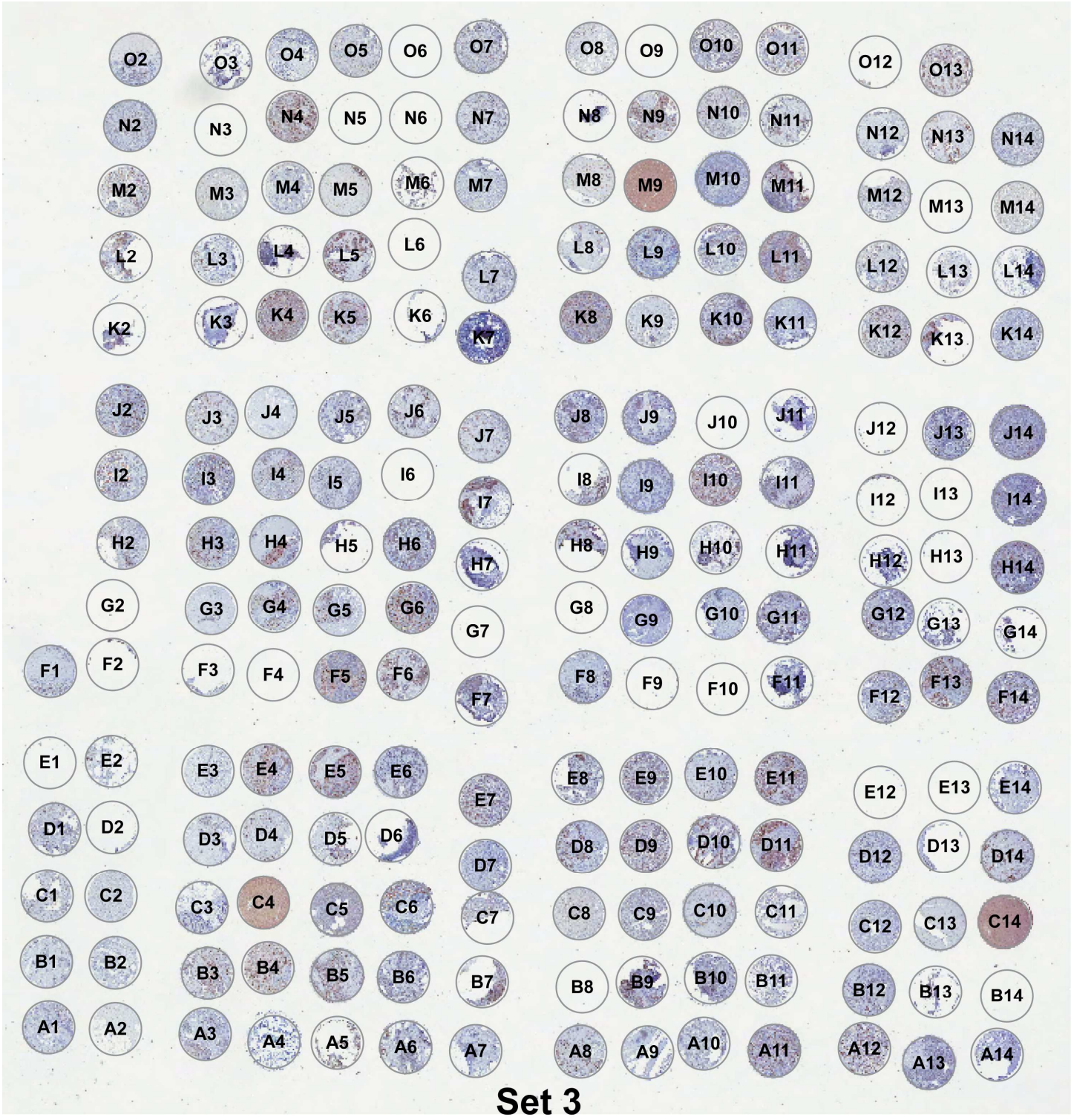

O10 O9 O8 O7 O6 O5 O4 O3 O2 O1 P1
N10 N9 N8 N7 N6 N5 N4 N3 N2 N1
M10 M9 M8 M7 M6 M5 M4 M3 M2 M1
L10 L9 L8 L7 L6 L5 L4 L3 L2 L1
K10 K9 K8 K7 K6 K5 K4 K3 K2 K1
J10 J9 J8 J7 J6 J5 J4 J3 J2 J1
I10 I9 I8 I7 I6 I5 I4 I3 I2 I1
H10 H9 H8 H7 H6 H5 H4 H3 H2 H1
G10 G9 G8 G7 G6 G5 G4 G3 G2 G1
F10 F9 F8 F7 F6 F5 F4 F3 F2 F1
E10 E9 E8 E7 E6 E5 E4 E3 E2 E1
D10 D9 D8 D7 D6 D5 D4 D3 D2 D1
C10 C9 C8 C7 C6 C5 C4 C3 C2 C1
B10 B9 B8 B7 B6 B5 B4 B3 B2 B1
A10 A9 A8 A7 A6 A5 A4 A3 A2 A1
Set 2


O2 O3 O4 O5 O6 O7 O8 O9 O10 O11 O12 O13
N2 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12 N13 N14
M2 M3 M4 M5 M6 M7 M8 M9 M10 M11 M12 M13 M14
L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12 L13 L14
K2 K3 K4 K5 K6 K7 K8 K9 K10 K11 K12 K13 K14
J2 J3 J4 J5 J6 J7 J8 J9 J10 J11 J12 J13 J14
I2 I3 I4 I5 I6 I7 I8 I9 I10 I11 I12 I13 I14
H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12 H13 H14
G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12 G13 G14
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 F13 F14
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12 E13 E14
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 B13 B14
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 A13 A14

Set 3


O2 O3 O4 O5 O6 O7 O8 O9 O10 O11 O12 O13
N2 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12 N13 N14
M2 M3 M4 M5 M6 M7 M8 M9 M10 M11 M12 M13 M14
L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12 L13 L14
K2 K3 K4 K5 K6 K7 K8 K9 K10 K11 K12 K13 K14
J2 J3 J4 J5 J6 J7 J8 J9 J10 J11 J12 J13 J14
I2 I3 I4 I5 I6 I7 I8 I9 I10 I11 I12 I13 I14
H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12 H13 H14
G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12 G13 G14
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 F13 F14
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12 E13 E14
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 B13 B14
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 A13 A14

Set 3


O2 O3 O4 O5 O6 O7 O8 O9 O10 O11 O12 O13
N2 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12 N13 N14
M2 M3 M4 M5 M6 M7 M8 M9 M10 M11 M12 M13 M14
L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12 L13 L14
K2 K3 K4 K5 K6 K7 K8 K9 K10 K11 K12 K13 K14
J2 J3 J4 J5 J6 J7 J8 J9 J10 J11 J12 J13 J14
I2 I3 I4 I5 I6 I7 I8 I9 I10 I11 I12 I13 I14
H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12 H13 H14
G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12 G13 G14
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 F13 F14
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12 E13 E14
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 B13 B14
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 A13 A14
Set 3

SUPPLEMENTAL DATA 11

See additional excel file for data.

SUPPLEMENTAL DATA 12

Chi-Squared Test for Independence: Subgroup, Stain Intensity and Tumor Grade

Subgroup	Stain Intensity	Grade	
		Low	High
Her2+	Low	1	7
	High	1	2
Luminal A	Low	54	18
	High	2	5
Luminal B	Low	8	7
	High	1	0
Triple Negative	Low	8	80
	High	4	44

Total Cases	242
Number of Factors	3
Chi-squared	541.7355
df	15
p-value =	2.20E-16