

Mapping the phosphorylation of the human RNA polymerase II reveals that the identity and modification of the seventh residues of heptad repeats direct Tyr1 phosphorylation

Nathaniel T. Burkholder^{1*}, **Sarah N. Sipe**^{2*}, **Edwin E. Escobar**², **Mukesh Kumar**¹, **Seema Irani**¹, **Wanjie Yang**¹, **Haoyi Yu**¹, **Wendy M. Matthews**¹, **Jennifer S. Brodbelt**^{2,#} and **Yan Zhang**^{1,3#}

SUPPORTING INFORMATION

Table S1. CTD construct sequences.

Table S2. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated, trypsin digested 26xDistal CTD substrate.

Table S3. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated 3xWT, 3xS7E Mid and Sp, and 3xS2E Mid substrates.

Table S4. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated, GluC digested 26xS7E Sp CTD substrate.

Table S5. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated 3xS7R Sp, Arg1810, and Cit1810 substrates.

Table S6. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated 3xS7K Sp and 3xS7Q Mid/Sp substrates.

Table S1. CTD construct sequences. 6xHis-GST sequence up to the 3C cleavage site is included in the distal CTD sequence, but is written in short hand for all sequences thereafter as “6xHis-GST - “. 3C cleavage site is underlined and the cleavage site is indicated with a dash (LEVLFQ/GP). Ser7 mutations are highlighted based on residue character (**red** – negatively charged, **purple** – positively charged, **green** – polar, **yellow** – nonpolar).

CTD Protein/Peptide	Sequence
6xHis-GST-human distal 26x CTD	MHHHHHSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGD KWRNKKFELGLEFPNLPYYIDGDVKLTQSMARIYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLP EMLKMFEDRLC HKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKR IEAIP QIDKYLKSSKYIAWPLQG WQATFGGGDHPKSSS <u>LEVLFQ/GP</u> SGMS PTSP ^N YTPTSP ^N YSPTSPSYSPTSPSYSPTSPSYSPPSSPR ^R YTPQSP ^T Y TPSSPSYSPSSPSYSPTSP ^K YTPTSPSYSPPSSP ^R YTPTSP ^K YSPTSP ^K Y SPTSP ^K YSPTSP ^T YSPTTP ^K YSPTSP ^T YSPTSP ^V YTPTSP ^K YSPTSP ^T Y SPTSP ^K YSPTSP ^T YSPTSP ^K GSTYSPTSP ^G YSPTSP ^T YSLTSPAISPDD SDEEN
6xHis-GST-yeast 26x CTD	6xHis-GST - <u>GPGSGMGFGVSSPGFSPTSP</u> ^T YSPTSP ^A YSPTSPSYSPTSPSYSPTSP SYSPTSPSYSPTSPSYSPTSPSYSPTSPSYSPTSPSYSPTSPSYSPTSP SYSPTSPSYSPTSPSYSPTSPSYSPTSPSYSPTSP ^A YSPTSPSYSPTSP SYSPTSPSYSPTSPSYSPTSP ^N YSPTSPSYSPTSP ^G YSPGSP ^A YSPKQ DEQKHNEENSR
6xHis-GST-yeast 26x S7E CTD	6xHis-GST - <u>GPGSGMGFGVSSPGFSPTSP</u> ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTS P ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTS P ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTS P ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPTSP ^R YSPGSP ^R YSPK QDEQKHNEENSR
6xHis-GST-yeast 26x S7E Sp CTD	6xHis-GST - <u>GPGSGMGFGVSSPGFSPTSP</u> ^R YSPTSP ^A YSPTSP ^R YSPTSPSYSPTS P ^R YSPTSPSYSPTSP ^R YSPTSPSYSPTSP ^R YSPTSPSYSPTSP ^R YSPTS PSYSPTSP ^R YSPTSPSYSPTSP ^R YSPTSPSYSPTSP ^R YSPTSPSYSPTS P ^R YSPTSPSYSPTSP ^R YSPTSP ^N YSPTSP ^R YSPTSP ^G YSPGSP ^R YSPK QDEQKHNEENSR
6xHis-GST-3xWT	6xHis-GST - <u>GPGSGM</u> SPSYSPTSPSYSPTSPSYSPT
6xHis-GST-3xS7E Sp	6xHis-GST - <u>GPGSGM</u> SP ^R YSPTSPSYSPTSP ^R YSPT
6xHis-GST-3xS7E Mid	6xHis-GST - <u>GPGSGM</u> SPSYSPTSP ^R YSPTSPSYSPT

6xHis-GST-3xS7R Sp	6xHis-GST - <u>G</u> PGSGMSPRYSPTSPSYSPTSPRYSPT
6xHis-GST-3xS7R Mid	6xHis-GST - <u>G</u> PGSGMSPSYSPTSPRYSPTSPSYSPT
6xHis-GST-3xS7K Sp	6xHis-GST - <u>G</u> PGSGMSPKYSPTSPSYSPTSPKYSPT
6xHis-GST-3xS7K Mid	6xHis-GST - <u>G</u> PGSGMSPSYSPTSPKYSPTSPSYSPT
6xHis-GST-3xS7Q Sp	6xHis-GST - <u>G</u> PGSGMSPQYSPTSPSYSPTSPQYSPT
6xHis-GST-3xS7Q Mid	6xHis-GST - <u>G</u> PGSGMSPSYSPTSPQYSPTSPSYSPT
Distal CTD 1801-1822 Arg1810 peptide	H-SPSYSPSSPRYSPTQSPRYSPTSS-OH
Distal CTD 1801-1822 Cit1810 peptide	H-SPSYSPSSPCYSPTQSPRYSPTSS-OH

Table S2. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated, trypsin digested 26xDistal CTD substrate (Fig. 1B-G). The phosphorylated Tyr1 sites were shown in blue boxes.

A) Repeat 32 pTyr1 peak:

Y T P Q S L P T V I T L P S S L P S Y L S L P L S L P L S L P T 25
26 S P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y5	528.2908	528.2921	2.5
B4	569.1887	569.1895	1.4
Y6	615.3228	615.3235	1.2
B5	656.2207	656.2231	3.6
Y7	778.3861	778.3909	6.1
C7	871.3475	871.3498	2.7
X8	891.3974	891.3991	1.9
Y9	962.4709	962.4735	2.7
B8	1017.384	1017.386	1.6
Y10	1049.503	1049.504	1.2
B9	1118.432	1118.433	0.4
Y11	1136.535	1136.538	2.4
Y12	1233.588	1233.59	2.0
Y13	1320.62	1320.624	3.0
B12	1389.549	1389.551	1.5
Y14	1483.683	1483.684	0.3
C14	1590.66	1590.663	2.1
Y16	1667.768	1667.769	0.8
Y17	1754.8	1754.806	3.6
Y19	1938.885	1938.889	2.3
Y20	2039.932	2039.943	5.1
Y23	2401.096	2401.1	1.6
Y24	2488.128	2488.129	0.5

Repeat 33 pTyr1 peak:

Y T P Q S L P T V I T L P S S L P S Y L S L P L S L P L S L P T 25
26 S P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1587	1.7
Y5	528.2908	528.2912	0.9
B5	576.2544	576.2557	2.3
Y6	615.3228	615.324	2.0
Y7	778.3861	778.3874	1.7
Y8	865.4181	865.4201	2.3
Y9	962.4709	962.4729	2.0
B8	1017.384	1017.385	1.0
Y10	1049.503	1049.506	3.0
B9	1118.432	1118.431	-1.5
Y11	1136.535	1136.537	1.5
Y12	1233.588	1233.592	3.4
Y13	1320.62	1320.623	2.6
B12	1389.549	1389.553	3.0
C14	1590.66	1590.655	-3.1
Y16	1667.768	1667.77	1.4
Y17	1754.8	1754.802	0.9
B16	1823.729	1823.731	1.1
Y19	1938.885	1938.888	1.8
Y23	2481.062	2481.058	-2.0
Y24	2568.095	2568.098	1.5

Repeat 34 pTyr1 peak:

Y T P Q S L P T V I T L P S S L P S Y L S L P L S L P L S L P T 25
26 S P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1587	1.8
Y3	330.1903	330.1908	1.3
B4	489.2224	489.2242	3.8
Y5	528.2908	528.2923	2.8
B5	576.2544	576.2569	4.3
Y6	615.3228	615.3242	2.4
B7	774.3548	774.3569	2.6
Y7	778.3861	778.3898	4.7
C7	791.3811	791.3837	3.3
X8	891.3974	891.4025	5.7
B8	937.4182	937.4202	2.2
Y9	962.4709	962.4738	3.0
B9	1038.466	1038.47	3.9
Y10	1049.503	1049.505	1.9
Y11	1136.535	1136.539	3.2
B11	1222.551	1222.548	-2.4
Y12	1233.588	1233.591	2.6
B12	1309.583	1309.585	2.0
Y13	1320.62	1320.627	5.2
Y14	1563.649	1563.651	0.8
B15	1736.697	1736.692	-2.8
Y16	1747.734	1747.738	2.0
B16	1823.729	1823.731	1.2
Y17	1834.766	1834.771	2.8
Y19	2018.851	2018.857	3.0
Y20	2119.899	2119.906	3.4
Y21	2282.962	2282.966	1.9
Y23	2481.062	2481.073	4.4
Y24	2568.095	2568.102	2.9

Repeat 35 pTyr1 peak:

Y T P Q S L P T V I T L P S S L P S Y L S L P L S L P L S L P T 25
26 S P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1586	1.4
Y3	330.1903	330.1906	0.9
Y5	528.2908	528.2918	2.0
Y6	615.3228	615.3238	1.6
C7	791.3811	791.3823	1.4
Y7	858.3524	858.3566	4.9
B8	937.4182	937.4217	3.8
B9	1038.466	1038.469	3.2
Y9	1042.437	1042.439	1.3
Y10	1129.469	1129.471	1.4
Y11	1216.501	1216.496	-4.0
B12	1309.583	1309.583	0.5
Y12	1313.554	1313.557	2.1
Y13	1400.586	1400.588	1.2
B15	1656.731	1656.735	2.3
B16	1743.763	1743.765	1.4
Y16	1747.734	1747.737	1.8
Y17	1834.766	1834.768	0.9
Y19	2018.851	2018.852	0.7
Y20	2119.899	2119.9	0.8
Y23	2481.062	2481.069	2.7
Y24	2568.095	2568.099	1.7

B) Repeat 36 pTyr1 peak:

Y TLP T L S L P S L Y L S L P L S L S L P L E L V L T L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A1	215.0347	215.0341	-2.9
Y2	243.1583	243.1584	0.4
Y2	243.1583	243.1584	0.4
Z3	314.1716	314.1715	-0.1
A2	316.0824	316.0825	0.1
Y3	330.1903	330.1902	-0.4
B2	344.0773	344.0774	0.2
Z4	415.2192	415.2187	-1.4
X+4	458.2251	458.2257	1.3
Y5	528.2908	528.2911	0.6
B4	542.1778	542.1781	0.6
Z6	613.3197	613.3188	-1.4
B5	629.2098	629.2104	0.9
Y6	629.3384	629.3383	-0.3
Y7	792.4018	792.4029	1.4
X7	818.381	818.3819	1.0
C7	830.3209	830.3215	0.7
Y8	921.4444	921.4443	0.0
X8	947.4236	947.4241	0.5
B8	976.3579	976.3582	0.3
Y9	1018.497	1018.498	1.0
B9	1063.39	1063.39	0.1
Y10	1105.529	1105.531	1.4
Z11	1176.542	1176.543	0.8
Y11	1192.561	1192.562	0.4
X11	1218.54	1218.543	2.0
Y12	1289.614	1289.614	-0.2
B12	1334.507	1334.509	1.5
Y13	1376.646	1376.647	1.0
A+13	1404.572	1404.578	3.8
A+14	1533.615	1533.616	0.4
Y14	1539.709	1539.711	1.0
C14	1577.628	1577.629	0.2
Y16	1723.794	1723.798	2.4
Y17	1810.826	1810.831	2.9
Y19	2008.927	2008.926	-0.2

Repeat 37 pTyr1 peak:

Y TLP T L S L P S L Y L S L P L S L S L P L E L V L T L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1584	0.5
Y3	330.1903	330.1902	-0.3
Z4	415.2192	415.2191	-0.2
B4	462.2115	462.212	1.3
Y5	528.2908	528.2912	0.8
C5	566.2698	566.2709	1.9
Z6	613.3197	613.319	-1.0
Y6	629.3384	629.3391	1.0
B7	733.3283	733.3273	-1.4
C7	750.3546	750.3556	1.4
Y7	792.4018	792.4029	1.4
Y8	921.4444	921.4476	3.5
X8	947.4236	947.4185	-5.4
B8	976.3579	976.3582	0.3
Y9	1018.497	1018.498	1.1
B9	1063.39	1063.389	-1.2
Y10	1105.529	1105.53	1.0
Y11	1192.561	1192.564	2.0
X11	1218.54	1218.534	-5.3
B11	1247.475	1247.474	-0.3
Y12	1289.614	1289.614	-0.1
B12	1334.507	1334.51	2.8
Y13	1376.646	1376.648	1.6
B14	1560.602	1560.606	2.8
C14	1577.628	1577.627	-1.1
B15	1723.665	1723.666	0.6
Y16	1803.76	1803.763	1.6
Y17	1890.792	1890.797	2.7
Y19	2088.893	2088.897	2.0

Repeat 38 pTyr1 peak:

Y TLP T L S L P L S L Y L S L P L S L S L P L E L V L T L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1583	0.0
B2	264.111	264.111	0.0
Y3	330.1903	330.1904	0.3
Z4	415.2192	415.2191	-0.4
B4	462.2115	462.2116	0.4
Y5	528.2908	528.2908	0.1
Z6	613.3197	613.3191	-0.9
Y6	629.3384	629.3386	0.3
C7	750.3546	750.3554	1.1
Y7	872.3681	872.3687	0.7
B8	896.3916	896.392	0.4
B9	983.4236	983.421	-2.6
Y8	1001.411	1001.411	0.7
Y9	1098.463	1098.465	1.4
B11	1167.508	1167.506	-1.9
Y10	1185.495	1185.496	0.4
B12	1254.54	1254.541	0.5
Y11	1272.528	1272.526	-1.2
Y12	1369.58	1369.581	0.5
Y13	1456.612	1456.614	1.4
Y14	1619.676	1619.674	-1.1
Y15	1706.708	1706.705	-1.7
Y16	1803.76	1803.763	1.3
Y17	1890.792	1890.795	1.3
Y19	2088.893	2088.895	0.9

C) Repeat 41 pTyr1 peak:

N Y L S L P L T L S L P L T L Y L S L P L T L T L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1584	0.4
Z4	429.2349	429.235	0.3
X4	471.2329	471.2328	-0.2
B4	528.1621	528.1626	0.8
Y5	542.3064	542.3067	0.6
Z6	613.3197	613.3203	1.0
Y6	629.3384	629.3398	2.2
Y7	792.4018	792.4025	1.0
C7	830.3209	830.3131	-9.4
Z8	877.4307	877.4294	-1.5
Y8	893.4494	893.4509	1.7
X+8	920.4365	920.4333	-3.5
A8	948.363	948.3629	-0.1
B8	976.3579	976.3588	0.9
Y9	990.5022	990.5033	1.1
C8	993.3842	993.3832	-1.0
Y10	1077.534	1077.535	1.1
Y11	1178.582	1178.585	2.7
X11	1204.561	1204.561	-0.4
Y12	1275.635	1275.636	0.7
C11	1278.517	1278.515	-1.2
A12	1334.543	1334.541	-1.4
B12	1362.538	1362.538	-0.4
Y13	1362.667	1362.668	0.8

Repeat 42 pTyr1 peak:

N Y L S L P L T L S L P L T L Y L S L P L T L T L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1584	0.4
C2	267.1217	267.122	1.1
Z4	429.2349	429.2351	0.5
Y5	542.3064	542.3065	0.2
Y6	629.3384	629.3383	-0.2
Y7	872.3681	872.3679	-0.2
X7	898.3474	898.3471	-0.3
A8	948.363	948.3613	-1.8
Y8	973.4158	973.4163	0.5
X8	999.395	999.3945	-0.5
A+9	1036.403	1036.401	-2.0
Y9	1070.469	1070.464	-4.1
Y10	1157.501	1157.501	0.4
Y11	1258.548	1258.551	2.3
X11	1284.528	1284.526	-0.8
Y12	1355.601	1355.602	0.9
B12	1362.538	1362.539	0.5
Y13	1442.633	1442.636	2.0

D) Repeat 43 pTyr1 peak:

Y SLP T L S L P T L V L S L P L T L S L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1585	0.8
X+2	270.1454	270.1456	0.7
B2	330.0617	330.0619	0.7
Y3	330.1903	330.1903	-0.1
Z4	415.2192	415.219	-0.5
B4	528.1621	528.1626	0.9
Y5	528.2908	528.2907	0.0
Z6	613.3197	613.3201	0.7
B5	615.1942	615.1943	0.3
Y6	629.3384	629.3392	1.2
Y7	792.4018	792.402	0.3
B7	813.2946	813.296	1.7
X+7	819.3889	819.3881	-0.9
C7	830.3209	830.3202	-0.8
Z8	875.4514	875.4543	3.3
Y8	891.4702	891.4719	1.9
X8	917.4494	917.4498	0.4
A8	948.363	948.3627	-0.3
B8	976.3579	976.358	0.1
Y9	988.5229	988.5234	0.5
B9	1063.39	1063.391	0.9
Y10	1075.555	1075.555	0.4
Z11	1160.584	1160.587	3.0
Y11	1176.603	1176.606	3.1
X+11	1203.59	1203.59	-0.1
B11	1261.49	1261.493	2.4
Y12	1273.655	1273.656	0.6
B12	1348.522	1348.523	0.3
Y13	1360.687	1360.688	0.3
A+13	1418.588	1418.587	-0.5
A+14	1517.657	1517.657	0.5
Y14	1523.751	1523.751	0.3
B14	1544.644	1544.65	4.4
C14	1561.67	1561.666	-2.6
Y16	1721.851	1721.853	1.1
Y17	1808.883	1808.89	3.9
Y19	2006.984	2006.989	2.5

Repeat 44 pTyr1 peak:

Y SLP T L S L P L T L S L P L T L S L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1585	0.7
C2	267.1217	267.1221	1.6
X+2	270.1454	270.1454	0.0
Y3	330.1903	330.1904	0.2
Z4	415.2192	415.2184	-2.1
Y4	431.238	431.2382	0.5
B4	448.1958	448.1957	-0.2
C4	465.2221	465.2235	2.9
Y5	528.2908	528.2913	1.0
B5	535.2278	535.2282	0.6
X+5	555.2778	555.278	0.4
Z6	613.3197	613.3192	-0.8
Y6	629.3384	629.339	1.0
C6	649.3069	649.3078	1.4
B7	733.3283	733.326	-3.1
Y7	792.4018	792.403	1.6
X7	818.381	818.3817	0.8
Z8	875.4514	875.4531	1.9
X8	917.4494	917.4531	3.9
A8	948.363	948.3637	0.7
B8	976.3579	976.3601	2.3
Y9	988.5229	988.5238	0.8
C8	993.3842	993.3857	1.5
A+9	1036.403	1036.4	-2.8
Y10	1075.555	1075.557	1.7
C9	1080.416	1080.417	1.0
Z11	1160.584	1160.586	1.8
Y11	1176.603	1176.605	2.1
X+11	1203.59	1203.592	1.6
B11	1261.49	1261.49	0.1
Y12	1273.655	1273.658	2.2
B12	1348.522	1348.525	2.1
Y13	1360.687	1360.69	1.5
B13	1445.575	1445.58	3.4
A14	1516.649	1516.655	4.1
B14	1544.644	1544.648	2.9
C14	1561.67	1561.67	0.1
Y14	1603.717	1603.717	-0.1
A15	1679.712	1679.718	3.6
B15	1707.707	1707.706	-0.7
Y16	1801.818	1801.819	0.9
B16	1808.755	1808.757	1.2
Y17	1888.85	1888.843	-3.6
Y18	1989.897	1989.894	-1.7
Y19	2086.95	2086.951	0.6

Repeat 45 pTyr1 peak:

Y SLP T L S L P L T L S L P L T L S L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1583	0.2
B2	250.0954	250.0955	0.7
C2	267.1217	267.122	1.2
Z3	314.1716	314.1718	0.8
Y3	330.1903	330.1903	-0.2
Z4	415.2192	415.219	-0.7
B4	448.1958	448.196	0.4
Y5	528.2908	528.291	0.4
Z6	613.3197	613.3191	-1.0
Y6	629.3384	629.339	0.9
B7	733.3283	733.3289	0.9
C7	750.3546	750.3558	1.6
Y7	872.3681	872.3684	0.3
B8	896.3916	896.3917	0.1
Z8	955.4177	955.4227	5.2
A9	955.4287	955.4227	-6.3
Y8	971.4365	971.4369	0.4
B9	983.4236	983.4247	1.1
X8	997.4158	997.4153	-0.4
C9	1000.45	1000.449	-1.4
Y9	1068.489	1068.491	1.5
Y10	1155.521	1155.523	1.3
B11	1181.524	1181.524	-0.2
Z11	1240.55	1240.554	3.1
A12	1240.561	1240.554	-8.8
Y11	1256.569	1256.567	-1.7
B12	1268.556	1268.556	-0.1
Y12	1353.622	1353.623	0.6
Y13	1440.654	1440.656	1.7
Y14	1603.717	1603.719	1.2
B15	1707.707	1707.711	2.6
Y16	1801.818	1801.819	0.6
Y18	1989.897	1989.895	-1.3
Y19	2086.95	2086.951	0.3

E) Repeat 46/48 pTyr1 peak:

Y L S L P L T L S L P L T L S L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1584	0.4
X+2	270.1454	270.1459	2.0
Y3	330.1903	330.1904	0.4
A3	399.1195	399.1199	0.9
Z4	415.2192	415.2189	-0.7
B4	528.1621	528.1625	0.8
Y5	528.2908	528.2911	0.7
Z6	599.304	599.3043	0.5
Y6	615.3228	615.3231	0.5
X6	641.3021	641.3034	2.0
X7	804.3654	804.366	0.8
B7	813.2946	813.2967	2.6
Z8	863.415	863.4151	0.1
Y8	879.4338	879.4344	0.7
X8	905.4131	905.4146	1.7
A8	948.363	948.3628	-0.3
B8	976.3579	976.3642	6.4
Y9	976.4866	976.4871	0.5
B9	1063.39	1063.392	2.2
Y10	1063.519	1063.518	-0.6
Z11	1148.548	1148.549	1.3
Y11	1164.566	1164.566	-0.3
X+11	1191.553	1191.552	-1.5
Y12	1261.619	1261.62	0.6
Z13	1332.632	1332.637	3.3
B12	1348.522	1348.52	-1.8
Y13	1348.651	1348.651	-0.1

Repeat 47/49 pTyr1 peak:

Y L S L P L T L S L P L T L S L P L T L S L P K

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	243.1583	243.1582	-0.2
C2	267.1217	267.1219	1.1
Y5	528.2908	528.2907	0.0
Y6	615.3228	615.3231	0.6
Y7	858.3524	858.3531	0.8
X7	884.3317	884.3324	0.8
Z8	943.3814	943.3824	1.1
Y8	959.4001	959.4012	1.1
X8	985.3794	985.3803	0.9
C8	993.3842	993.3841	-0.2
A+9	1036.403	1036.404	0.9
Y9	1056.453	1056.454	1.2
Y10	1143.485	1143.486	0.5
Y11	1244.533	1244.535	1.7
X11	1270.512	1270.513	0.6
A12	1320.528	1320.532	3.1
Y12	1341.585	1341.587	0.9
Y13	1428.617	1428.618	0.6

F) Repeat 50 pTyr1 peak:

□ G S T V L S L P T L S L P G V L S L P T L S L P T V L S L L L T L S L P A L I 25
 26 L S L P D D L S D E E N .

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A4	460.1359	460.1405	10.0
B4	488.1308	488.1312	0.9
C4	505.1571	505.1614	8.4
Y4	505.1656	505.1614	-8.4
Y5	592.1976	592.1977	0.1
B7	773.2633	773.2644	1.4
B8	860.2953	860.2967	1.6
Y8	919.3043	919.305	0.8
Y9	1006.336	1006.337	0.7
C10	1031.396	1031.399	2.8
Y10	1119.42	1119.418	-1.9
B11	1177.433	1177.434	1.3
B12	1264.465	1264.465	0.1
Y12	1287.51	1287.512	1.1
Y13	1374.542	1374.545	1.9
B14	1462.565	1462.566	0.4
Y14	1475.59	1475.594	2.4
Y15	1588.674	1588.674	0.2
Y16	1675.706	1675.706	0.1
C17	1764.724	1764.726	1.0
B19	1997.793	1997.793	0.1
Y19	2036.87	2036.864	-2.8
Y20	2123.902	2123.902	0.1
B21	2211.925	2211.925	0.2
Y22	2322.002	2322.004	0.6
Y23	2409.034	2409.022	-5.2
B24	2467.047	2467.053	2.6
A25	2552.136	2552.137	0.4
B25	2580.131	2580.135	1.4
B26	2667.163	2667.182	7.1
Y26	2726.172	2726.174	0.6
Y-29	3010.297	3010.301	1.3
C30	3098.328	3098.349	7.0
Y30	3098.336	3098.349	4.2

Repeat 51 pTyr1 peak:

□ G S T V L S L P T L S L P G V L S L P T L S L P T V L S L L L T L S L P A L I 25
 26 L S L P D D L S D E E N .

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
B4	408.1645	408.1648	0.8
B5	495.1965	495.197	0.9
Y6	707.2246	707.225	0.5
B8	780.329	780.3304	1.8
A9	849.3868	849.3866	-0.3
Y8	919.3043	919.3054	1.3
C10	951.4295	951.4314	1.9
Y9	1006.336	1006.337	1.1
Y10	1119.42	1119.424	3.4
B11	1177.433	1177.434	0.6
B12	1264.465	1264.462	-2.5
Z12	1271.491	1271.503	8.9
Y12	1287.51	1287.512	1.7
Y13	1374.542	1374.545	1.9
B14	1462.565	1462.569	2.3
Y14	1475.59	1475.595	3.3
B15	1549.597	1549.6	1.4
Y15	1588.674	1588.675	0.5
Y16	1675.706	1675.709	1.9
C17	1764.724	1764.729	2.6
B18	1910.761	1910.762	0.7
Y19	2036.87	2036.87	0.2
B20	2110.877	2110.874	-1.6
Y20	2123.902	2123.915	6.2
B21	2211.925	2211.927	0.8
B22	2298.957	2298.949	-3.3
Y22	2322.002	2322.011	3.5
B24	2467.047	2467.049	0.9
B25	2580.131	2580.135	1.6
B26	2667.163	2667.16	-0.9
Y26	2806.138	2806.141	0.8
Y27	2893.17	2893.167	-1.2
Y29	3091.271	3091.279	2.6

Repeat 52 pTyr1 peak:

□ G S T V L S L P T L S L P G V L S L P T L S L P T V L S L L L T L S L P A L I 25
 26 L S L P D D L S D E E N .

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A4	380.1696	380.1695	-0.3
B4	408.1645	408.1647	0.4
B5	495.1965	495.1971	1.1
Y6	707.2246	707.2257	1.5
A+8	753.3419	753.338	-5.2
A9	849.3868	849.3858	-1.2
Y8	919.3043	919.3053	1.1
B10	934.4032	934.4089	6.0
C10	951.4295	951.4308	1.3
Y9	1006.336	1006.337	0.9
A+11	1070.479	1070.48	0.5
B11	1097.467	1097.466	-0.6
Y10	1119.42	1119.422	1.5
B12	1184.499	1184.499	0.3
A+13	1254.564	1254.561	-2.9
Y12	1287.51	1287.512	1.4
C13	1298.578	1298.579	1.3
Y13	1374.542	1374.546	2.5
B14	1382.599	1382.602	2.1
A+15	1442.644	1442.648	2.6
B15	1469.631	1469.634	1.8
Y14	1475.59	1475.595	3.3
Y15	1588.674	1588.674	0.3
Y16	1675.706	1675.71	2.5
B18	1910.761	1910.768	3.8
B20	2110.877	2110.881	1.9
Y19	2116.836	2116.836	0.2
Y20	2203.868	2203.867	-0.7
B21	2211.925	2211.928	1.2
B22	2298.957	2298.961	1.7
Y22	2401.969	2401.972	1.5
B24	2467.047	2467.052	2.1
Y23	2489.001	2489.008	2.8
B25	2580.131	2580.135	1.5
B26	2667.163	2667.168	1.9
Y26	2806.138	2806.141	1.0
Y27	2893.17	2893.178	2.6
Y29	3091.271	3091.276	1.6
Y30	3178.303	3178.314	3.6

Table S3. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated 3xWT, 3xS7E Mid and Sp, and 3xS2E Mid substrates (Fig. 3). The phosphorylated Tyr1 sites were shown in blue boxes.

A) 3xWT Repeat #1 pTyr1 peak:

11 G P G S G M L S P L S V S L P T L S L P S I V L S L P T L S L P S I V L S 25
26 P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.7
B6	486.19	486.189	-2.1
B7	573.222	573.222	0.2
Y6	650.291	650.29	-1.7
Y7	737.323	737.322	-1.1
Y9	935.424	935.423	-0.5
B10	1000.34	1000.33	-3.1
Y10	1022.46	1022.45	-1.6
B13	1285.47	1285.47	-0.4
Y13	1369.6	1369.6	-1.0
B14	1372.5	1372.5	-1.5
Y14	1456.64	1456.63	-3.3
C16	1573.61	1573.61	-0.5
Y16	1654.74	1654.73	-1.8
B17	1719.65	1719.65	-0.5
Y17	1741.77	1741.77	-1.7
B18	1806.68	1806.68	-2.2
B20	2004.78	2004.78	-1.4
Z19	2055.81	2055.79	-8.5
Y20	2168.88	2168.88	-0.8
Y21	2255.92	2255.9	-5.3
C23	2292.92	2292.91	-5.8
B24	2438.96	2438.96	-0.3
B25	2525.99	2525.99	-1.2

3xWT Repeat #2 pTyr1 peak:

11 G P G S G M L S P L S V L S P T L S L P S I V L S P T L S L P S I V L S 25
26 P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.5
B6	486.19	486.189	-1.8
B7	573.222	573.221	-0.5
Y6	650.291	650.29	-1.7
Y7	737.323	737.323	-0.7
C9	774.333	774.332	-0.9
B10	920.37	920.369	-1.5
Y9	935.424	935.424	-0.2
Y10	1022.46	1022.46	-0.7
B13	1205.5	1205.5	-2.1
B14	1292.53	1292.53	-0.5
Y13	1449.57	1449.57	-1.1
Y14	1536.6	1536.6	-2.6
B17	1719.65	1719.65	0.7
B18	1806.68	1806.69	3.1
Y17	1821.74	1821.73	-0.8
B20	2004.78	2004.79	1.8
B21	2091.81	2091.81	-1.5
Y21	2255.92	2255.91	-2.6
C23	2292.92	2292.9	-8.7
B24	2438.96	2438.97	2.3
B25	2525.99	2525.99	-2.7

3xWT Repeat #3 pTyr1 peak:

11 G L P G S G M L S P L S I V L S L P T L S L P S I V L S L P T L S L P S I V L S 25
26 P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
C6	503.216	503.214	-4.7
A+8	643.287	643.289	2.6
C8	687.301	687.301	0.0
Y6	730.258	730.256	-1.5
B9	757.307	757.306	-0.5
C9	774.333	774.332	-0.5
Y7	817.29	817.289	-0.4
Y8	918.337	918.334	-3.4
B10	920.37	920.368	-2.5
C10	937.396	937.402	6.4
A11	979.407	979.403	-3.6
B11	1007.4	1007.4	-2.5
Y9	1015.39	1015.39	-1.2
Y10	1102.42	1102.42	-0.7
B13	1205.5	1205.5	-1.5
C13	1222.53	1222.52	-7.7
Y11	1265.49	1265.5	8.0
B14	1292.53	1292.54	0.4
Y13	1449.57	1449.57	-1.2
B16	1476.62	1476.62	1.2
C16	1493.65	1493.65	0.0
Y14	1536.6	1536.6	-1.4
B17	1639.68	1639.68	-1.4
B18	1726.71	1726.71	-0.8
Y16	1734.7	1734.7	-1.2
Z17	1805.72	1805.72	0.4
Y17	1821.74	1821.73	-1.2
B20	1924.82	1924.82	0.8
Y18	1984.8	1984.81	6.8
B21	2011.85	2011.85	-1.2
Y21	2255.92	2255.91	-2.1
B24	2438.96	2438.96	-0.6
B25	2525.99	2526	1.0
Y-26	2684.08	2684.07	-1.4

B) 3xS7E Mid Rep. #2 pTyr1 peak: 3xS7E Mid Rep. #3 pTyr1 peak:

10 G P G S G M L S I P S I Y L S I P T L S I P E I Y L S I P T L S I P S I Y L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.2
B6	486.19	486.189	-1.0
B7	573.222	573.222	0.0
Y6	650.291	650.29	-1.1
Y7	737.323	737.326	3.5
C9	774.333	774.333	0.0
B10	920.37	920.369	-0.8
Y9	935.424	935.424	0.0
B11	1007.4	1007.4	-0.8
Y10	1022.46	1022.46	-0.6
B13	1205.5	1205.5	-0.2
Y11	1265.49	1265.49	6.0
B14	1292.53	1292.54	0.2
Y13	1491.58	1491.58	-0.9
B16	1518.63	1518.63	-1.9
Y14	1578.61	1578.61	-0.6
B17	1761.66	1761.66	0.6
Y16	1776.71	1776.71	-1.3
B18	1848.69	1848.69	-0.5
Y17	1863.75	1863.74	-1.3
B20	2046.79	2046.79	-3.1
B21	2133.82	2133.82	-2.1
Y20	2210.89	2210.89	-0.1
Y21	2297.93	2297.93	2.0
C23	2334.94	2334.94	0.2
B24	2480.97	2480.97	-1.5
B25	2568	2568.01	1.0
X+26	2754.08	2754.1	7.5

10 G P G S G M L S I P S I Y L S I P T L S I P E I Y L S I P T L S I P S I Y L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.0
B6	486.19	486.189	-1.5
B7	573.222	573.221	-0.9
Y6	730.258	730.257	-1.2
B9	757.307	757.307	0.8
C9	774.333	774.333	0.3
Y7	817.29	817.289	-0.4
B10	920.37	920.369	-0.6
A+11	980.415	980.407	-8.4
Y9	1015.39	1015.39	-0.9
Y10	1102.42	1102.42	-0.4
B13	1205.5	1205.5	0.6
B14	1292.53	1292.54	0.4
Y13	1491.58	1491.58	-1.2
B16	1518.63	1518.63	0.9
C16	1535.66	1535.66	-0.5
Y14	1578.61	1578.61	-0.9
B17	1681.69	1681.69	-0.4
B18	1768.73	1768.72	-3.1
Y16	1776.71	1776.71	-0.3
Z17	1847.73	1847.74	7.1
Y17	1863.75	1863.74	-0.8
B20	1966.83	1966.83	0.7
B21	2053.86	2053.86	-1.2
Y20	2210.89	2210.89	-1.3
Y21	2297.93	2297.93	-0.3
B24	2480.97	2480.97	-2.0
B25	2568	2568.01	1.0

C) 3xS7E Sp Rep. #1 pTyr1 peak: 3xS7E Sp Rep. #2 pTyr1 peak: 3xS7E Sp Rep. #3 pTyr1 peak:

10 G P G S G M L S I P E I Y L S I P T L S I P S I Y L S I P T L S I P E I Y L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.3
Y4	466.206	466.206	-1.3
B6	486.19	486.189	-1.4
B7	573.222	573.221	-0.5
Y6	692.302	692.301	-1.2
Y7	779.334	779.333	-0.8
Y9	977.434	977.433	-1.1
B10	1042.35	1042.34	-2.8
Y10	1064.47	1064.47	-1.1
B11	1129.38	1129.38	-1.3
Y11	1227.53	1227.53	-0.3
B13	1327.48	1327.48	-0.2
Y13	1411.61	1411.61	-0.4
B14	1414.51	1414.51	-3.5
Y14	1498.65	1498.65	-1.2
C16	1615.62	1615.62	-0.5
B17	1761.66	1761.66	-0.4
Y17	1783.78	1783.78	-1.3
B18	1848.69	1848.69	-0.7
B21	2133.82	2133.82	-0.9
Y20	2252.9	2252.9	-1.6
Y21	2339.94	2339.94	0.2
B23	2359.92	2359.92	0.4
C23	2376.95	2376.93	-6.3
B24	2522.98	2522.98	-0.4
B25	2610.02	2610.02	0.5

10 G P G S G M L S I P E I Y L S I P T L S I P S I Y L S I P T L S I P E I Y L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.1
B6	486.19	486.189	-1.1
Y6	692.302	692.301	-0.7
Y7	779.334	779.333	-0.8
A10	934.386	934.384	-1.5
B10	962.38	962.379	-1.2
Y9	977.434	977.434	-0.3
B11	1049.41	1049.41	-2.2
Y10	1064.47	1064.47	-0.4
B13	1247.51	1247.51	-0.5
Y11	1307.5	1307.51	9.0
B14	1334.55	1334.55	-0.1
Y-13	1490.57	1490.57	-0.1
Y13	1491.58	1491.58	-0.4
B16	1518.63	1518.63	2.5
C16	1535.66	1535.66	1.3
Y14	1578.61	1578.61	-1.6
B17	1761.66	1761.66	1.1
Y16	1776.71	1776.71	0.0
B18	1848.69	1848.7	2.6
Y17	1863.75	1863.74	-0.7
B20	2046.79	2046.79	-0.1
B21	2133.82	2133.82	-1.0
Y20	2252.9	2252.89	-5.8
Y21	2339.94	2339.93	-1.8
B23	2359.92	2359.93	3.0

10 G P G S G M L S I P E I Y L S I P T L S I P S I Y L S I P T L S I P E I Y L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.4
B6	486.19	486.189	-0.8
B7	573.222	573.222	-0.2
Y6	772.268	772.267	-1.5
B9	799.317	799.321	4.5
Y7	859.3	859.299	-0.9
A10	934.386	934.386	0.0
B10	962.38	962.378	-2.2
B11	1049.41	1049.41	-2.7
Y9	1057.4	1057.4	-1.2
Y10	1144.43	1144.43	-0.7
B13	1247.51	1247.51	-0.6
B14	1334.55	1334.54	-1.0
Y13	1491.58	1491.58	-1.4
B16	1518.63	1518.63	2.1
C16	1535.66	1535.66	0.5
Y14	1578.61	1578.61	-0.3
B17	1681.69	1681.69	-1.3
B18	1768.73	1768.72	-1.0
Y16	1776.71	1776.71	-1.1
Y17	1863.75	1863.74	-1.4
B20	1966.83	1966.83	2.0
B21	2053.86	2053.85	-1.8
Y20	2252.9	2252.89	-8.2
Y21	2339.94	2339.94	-0.5
B24	2522.98	2522.98	-0.4
B25	2610.02	2610.01	-0.3

D) 3xS2E Mid Rep. #1 pTyr1 peak:

GLP G S G M S I P S V S I P T L S I P S I V L E L P T L S I P S I V L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.2
B6	486.19	486.189	-0.4
Y6	650.291	650.291	-0.8
Y7	737.323	737.323	-0.7
Y9	935.424	935.423	-0.4
B10	1000.34	1000.33	-2.2
C10	1017.36	1017.36	-1.5
Y10	1064.47	1064.47	-1.2
B11	1087.37	1087.37	-2.3
B13	1285.47	1285.47	4.3
B14	1372.5	1372.5	-0.2
Y13	1411.61	1411.61	-0.5
Y14	1498.65	1498.65	0.0
C16	1573.61	1573.61	-0.4
B17	1719.65	1719.65	-0.4
Y17	1783.78	1783.78	-1.2
B18	1848.69	1848.69	-0.9
B20	2046.79	2046.79	-0.2
Z19	2097.82	2097.8	-9.7
B21	2133.82	2133.82	-1.3
Y20	2210.89	2210.89	-1.8
C23	2334.94	2334.93	-0.5
B24	2480.97	2480.97	-0.3
B25	2568	2568.01	0.8

3xS2E Mid Rep. #2 pTyr1 peak:

GLP G S G M S I P S S I V L S I P T L S I P S V L E L P T L S I P S I V L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.1
B6	486.19	486.189	-1.4
B7	573.222	573.221	-0.5
Y6	650.291	650.291	-1.1
Y7	737.323	737.328	6.4
C9	774.333	774.333	-0.1
B10	920.37	920.369	-1.0
Y9	935.424	935.424	0.1
B11	1007.4	1007.4	-1.0
Y10	1064.47	1064.46	-1.8
B13	1205.5	1205.5	0.3
B14	1292.53	1292.54	0.7
Y13	1491.58	1491.58	-0.2
Y14	1578.61	1578.61	-1.0
Y16	1776.71	1776.71	-0.4
B18	1848.69	1848.69	-0.5
Y17	1863.75	1863.74	-0.8
B20	2046.79	2046.79	0.1
Y20	2210.89	2210.89	-0.2
Y21	2297.93	2297.93	2.6
C23	2334.94	2334.93	-0.9
B24	2480.97	2480.97	-1.4
B25	2568	2568.01	0.5

3xS2E Mid Rep. #3 pTyr1 peak:

GLP G S G M S I P S S I V L S I P T L S I P S I V L E L P T L S I P S I V L S 25
20] P T

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.143	-1.2
B6	486.19	486.189	-0.6
Y6	730.258	730.257	-1.1
B9	757.307	757.306	-0.3
C9	774.333	774.333	0.3
Y7	817.29	817.289	-0.5
A10	892.375	892.368	-7.9
B10	920.37	920.368	-1.8
B11	1007.4	1007.4	-2.2
Y9	1015.39	1015.39	0.7
Y9	1015.39	1015.39	-0.9
Y10	1144.43	1144.43	-0.9
B13	1205.5	1205.5	-0.2
A14	1264.54	1264.53	-7.6
B14	1292.53	1292.54	0.5
Y11	1307.5	1307.5	-0.4
Y13	1491.58	1491.58	-0.1
Y14	1578.61	1578.61	-2.3
B17	1639.68	1639.68	-1.6
B18	1768.73	1768.72	-1.6
Y16	1776.71	1776.71	-1.4
Z17	1847.73	1847.74	6.5
Y17	1863.75	1863.74	-0.9
B20	1966.83	1966.83	1.7
Y18	2026.81	2026.8	-3.9
B21	2053.86	2053.86	-0.7
Y20	2210.89	2210.89	0.1
Y21	2297.93	2297.93	0.2
B24	2480.97	2480.97	-1.8
B25	2568	2568.01	0.4
X26	2753.07	2753.09	5.2

Table S4. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated, GluC digested 26xS7E Sp CTD substrate (Fig. 3B and 3E-F). The phosphorylated Tyr1 sites were shown in blue boxes.

A) 26xS7E Sp Glu7-pTyr1 peak:



Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	244.1059	244.1045	-5.6
Y3	331.1379	331.1363	-5.0
B4	528.1621	528.1595	-4.9
Y5	529.2384	529.2357	-5.0
B5	615.1942	615.1914	-4.5
Y6	616.2704	616.2675	-4.8
B7	799.279	799.2757	-4.1
C7	816.3053	816.3015	-4.5
Y9	963.4185	963.4164	-2.3
Y10	1050.451	1050.448	-2.9
Y12	1248.551	1248.54	-8.9
B12	1334.507	1334.5	-5.4

26xS7E Sp Ser7-pTyr1 peak:



Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	331.1379	331.1363	-5.0
B4	448.1958	448.1932	-5.7
Y5	529.2384	529.2358	-5.0
B5	535.2278	535.2252	-4.9
Y6	616.2704	616.2676	-4.5
Y-8	945.3243	945.329	5.0
B8	962.3423	962.3377	-4.8
C8	979.3686	979.3641	-4.6
A9	1021.379	1021.376	-3.1
Y9	1043.385	1043.38	-4.3
B9	1049.374	1049.37	-4.6
C9	1066.401	1066.396	-4.3
Y10	1130.417	1130.412	-4.3
B11	1247.475	1247.471	-3.2
Y12	1328.517	1328.512	-3.8
B12	1334.507	1334.502	-3.7

B) 26xS7E Sp Glu7-pTyr1 peak:



Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	244.1059	244.1045	-5.7
Y3	331.1379	331.1352	-8.4
B4	528.1621	528.1593	-5.3
Y5	529.2384	529.2361	-4.3
B5	615.1942	615.1912	-4.9
Y6	616.2704	616.2676	-4.6
Y7	779.3337	779.3303	-4.5
B7	783.284	783.2802	-4.8
C7	800.3103	800.3068	-4.4
Y9	947.4236	947.4262	2.7
Y10	1034.456	1034.453	-2.9
Y12	1232.556	1232.549	-6.1
B12	1318.512	1318.504	-6.1

26xS7E Sp Ala7-pTyr1 peak:



Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
C2	267.1217	267.1205	-4.3
Y3	331.1379	331.1363	-5.0
B4	448.1958	448.1934	-5.3
Y5	529.2384	529.2358	-4.8
B5	535.2278	535.2255	-4.3
Y6	616.2704	616.2677	-4.4
B7	703.3177	703.3148	-4.1
Y7	859.3001	859.2951	-5.8
B8	946.3474	946.3425	-5.1
C8	963.3737	963.3703	-3.5
Y9	1027.39	1027.386	-4.0
B9	1033.379	1033.375	-4.3
C9	1050.406	1050.4	-5.2
Y10	1114.422	1114.417	-4.9
B11	1231.48	1231.474	-5.1
Y12	1312.522	1312.515	-5.5
B12	1318.512	1318.507	-3.9

C) 26xS7E Sp Glu7-pTyr1 peak:

N Y S P T S P N Y S P T S P E C

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y2	244.1059	244.1045	-5.8
Y3	331.1379	331.1363	-5.1
B4	528.1621	528.1596	-4.8
Y5	529.2384	529.2355	-5.4
Y6	616.2704	616.2676	-4.6
B7	826.2899	826.2874	-2.9
C7	843.3162	843.3126	-4.3
Y9	990.4294	990.4281	-1.3
Y10	1077.461	1077.46	-1.5
Y12	1275.562	1275.567	3.7
B12	1361.518	1361.511	-4.6

26xS7E Sp Asn7-pTyr1 peak:

N Y S P T S P N Y S P T S P E C

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
C2	267.1217	267.1205	-4.3
Y3	331.1379	331.1363	-4.9
B4	448.1958	448.1935	-5.2
Y5	529.2384	529.2357	-5.1
B5	535.2278	535.2254	-4.5
Y6	616.2704	616.2677	-4.3
A7	718.3286	718.3263	-3.2
Y7	859.3001	859.2956	-5.2
Y-8	972.3352	972.3365	1.4
B8	989.3532	989.3492	-4.0
Y9	1070.396	1070.391	-4.7
B9	1076.385	1076.38	-4.4
C9	1093.412	1093.405	-5.9
Y10	1157.428	1157.423	-4.2
B11	1274.486	1274.48	-4.2
B12	1361.518	1361.512	-4.3

Table S5. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated 3xS7R Sp, Arg1810, and Cit1810 substrates (Fig. 5A and 5C/D). The phosphorylated Tyr1 sites were shown in blue boxes. Tables are split across pages due to size constraints.

A) 3xS7R Sp Rep. #1 peak:

11 G L P L G L S L G I M L S L P L R Y S L P L T L S L P L S L Y L S L P L T L S L P L R L Y S 25
26 P L T C

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A+6	459.2026	459.2029	0.7
C6	503.216	503.2158	-0.2
Z5	606.2887	606.2895	1.4
Y5	622.3075	622.3077	0.4
A+8	643.2874	643.2869	-0.7
X5	648.2867	648.2875	1.1
Y6	719.3602	719.3555	-6.5
A+9	799.3885	799.3857	-3.5
Y7	806.3923	806.3947	3.0
Z8	891.4212	891.4214	0.3
Y8	907.4399	907.4452	5.8
X8	933.4192	933.4204	1.2
Y9	1004.493	1004.495	2.3
X+9	1031.48	1031.475	-4.8
A+10	1042.418	1042.418	-0.2
Z10	1075.506	1075.508	1.7
C10	1086.432	1086.433	1.1
Y10	1091.525	1091.528	2.7
A+11	1129.45	1129.446	-3.6
A+12	1226.503	1226.505	1.3
Y11	1254.588	1254.59	1.5
C12	1270.516	1270.506	-8.4
X11	1280.567	1280.566	-1.2
A13	1326.543	1326.54	-1.8
B13	1354.538	1354.546	6.4
X12	1367.599	1367.599	-0.4
C13	1371.564	1371.566	1.2
A14	1413.575	1413.576	1.2
B14	1441.57	1441.569	-0.8
A+15	1511.635	1511.636	0.4
Y14	1525.705	1525.71	3.4
C15	1555.649	1555.648	-0.4
B16	1625.655	1625.66	3.3
Y15	1626.753	1626.745	-4.6
C16	1642.681	1642.683	1.2
X15	1652.732	1652.729	-1.9
Y-16	1722.797	1722.801	2.2
A17	1760.723	1760.727	2.5
B17	1788.718	1788.724	3.3
C17	1805.744	1805.749	2.5
Y17	1810.837	1810.833	-2.6
A18	1847.755	1847.759	2.2
B18	1875.75	1875.756	3.1
A+19	1945.816	1945.813	-1.3
C19	1989.829	1989.834	2.7
A20	2045.855	2045.862	3.1
B20	2073.85	2073.866	7.6
C20	2090.877	2090.874	-1.2
A21	2132.887	2132.895	3.8
B21	2160.882	2160.896	6.3
Z19	2193.949	2193.951	0.9

3xS7R Sp Rep. #2 peak:

11 G L P L G L S L G I M L S L P L R L Y S L P L T L S L P L S L Y S L P L T L S L P L R L Y S 25
26 P L T C

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A+6	459.2026	459.2033	1.6
B6	486.1897	486.1899	0.4
C6	503.216	503.2149	-2.2
A7	545.2268	545.2273	0.9
Z5	606.2887	606.2886	-0.1
Y-5	621.2996	621.2965	-5.1
A+8	643.2874	643.2878	0.6
X5	648.2867	648.2878	1.6
X+5	649.2946	649.2916	-4.6
Y6	719.3602	719.3615	1.8
A+9	799.3885	799.3899	1.8
Y7	806.3923	806.3943	2.5
B9	826.3756	826.3765	1.1
X7	832.3715	832.3755	4.8
Z8	891.4212	891.4209	-0.3
Y8	907.4399	907.4467	7.5
X8	933.4192	933.4192	-0.1
A10	961.444	961.4451	1.1
A+10	962.4518	962.4502	-1.7
Y-9	1003.485	1003.486	0.9
Y9	1004.493	1004.492	-0.3
X+9	1031.48	1031.471	-8.5
A+11	1049.484	1049.483	-0.4
B11	1076.471	1076.473	1.6
Y10	1091.525	1091.529	4.1
A+12	1146.537	1146.532	-3.6
C12	1190.55	1190.551	0.6
A13	1246.576	1246.586	7.9
B13	1274.571	1274.565	-4.8
A14	1333.608	1333.61	0.9
B14	1361.603	1361.606	2.2
Y12	1421.586	1421.587	0.2
A+15	1431.669	1431.669	-0.2
X12	1447.566	1447.571	3.5
C15	1475.682	1475.684	1.3
Y13	1518.639	1518.639	0.1
B16	1545.688	1545.691	1.6
Y14	1605.671	1605.675	2.3
Y15	1706.719	1706.727	4.7
A+17	1761.731	1761.733	1.1
B17	1788.718	1788.726	4.8
Y16	1803.772	1803.774	1.3
A18	1847.755	1847.765	5.4
Z17	1874.785	1874.793	4.4
A+19	1945.816	1945.817	0.8
C19	1989.829	1989.834	2.5
A+20	2046.863	2046.873	4.6
Y18	2053.867	2053.883	8.0
B20	2073.85	2073.865	6.9

Y19	2209.968	2209.972	1.9	C20	2090.877	2090.88	1.6
A+22	2230.948	2230.949	0.4	A21	2132.887	2132.895	3.7
X19	2235.947	2235.954	2.8	B21	2160.882	2160.897	6.6
Y-20	2306.013	2306.013	0.0	Z19	2193.949	2193.956	2.8
A23	2386.041	2386.041	-0.2	Y19	2209.968	2209.977	4.0
Y21	2394.053	2394.055	1.0	A+22	2230.948	2230.952	1.9
X+21	2421.04	2421.038	-0.7	X19	2235.947	2235.957	4.3
C23	2431.063	2431.059	-1.6	Y-20	2306.013	2306.015	0.9
Y22	2525.093	2525.088	-2.1	A23	2386.041	2386.047	2.4
A24	2549.105	2549.11	2.2	Y21	2394.053	2394.056	1.5
A+24	2550.112	2550.113	0.0	X+21	2421.04	2421.045	2.0
Y23	2582.115	2582.122	2.9	C23	2431.063	2431.051	-4.6
C24	2594.126	2594.133	2.8	Y22	2525.093	2525.1	2.5
X23	2608.094	2608.106	4.7	B24	2577.1	2577.115	6.2
A+25	2637.144	2637.142	-0.8	C24	2594.126	2594.132	2.5
B25	2664.132	2664.133	0.5	X23	2608.094	2608.107	5.1
Y-24	2668.139	2668.144	1.7	A+25	2637.144	2637.142	-0.9
Y25	2726.168	2726.183	5.5	B25	2664.132	2664.138	2.4
A+26	2734.197	2734.203	2.2	Y24	2669.147	2669.145	-0.5
X25	2752.148	2752.147	-0.2	Y25	2726.168	2726.18	4.4
Y-26	2822.213	2822.229	5.5	A+26	2734.197	2734.201	1.2

B) Arg1810 Peptide Rep. #1 peak: Arg1810 Peptide Rep. #3 peak:

S[P]L[S] Y[L]S[P]L[S]L[S]P[R]Y]T]P Q[S]L[P]T]Y]T]P]S]S

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y4	390.1751	390.175	-0.2
A+4	487.1594	487.1583	-2.3
B4	514.1465	514.1471	1.2
C4	531.1728	531.1736	1.5
Y6	654.2861	654.2883	3.5
A6	670.2364	670.2384	3.1
A+6	671.2442	671.2436	-0.8
Y7	751.3388	751.3395	0.8
B7	785.2633	785.2641	1.1
C7	802.2896	802.2883	-1.6
A+8	845.3082	845.3027	-6.5
B8	872.2953	872.2978	2.9
Y10	1063.482	1063.483	0.8
A10	1097.454	1097.453	-1.2
B10	1125.449	1125.45	1.1
C10	1142.476	1142.478	2.1
A+11	1261.525	1261.522	-2.9
B11	1288.513	1288.513	0.3
C11	1305.539	1305.539	-0.1
A12	1361.565	1361.569	2.9
B12	1389.56	1389.559	-0.9
Y13	1483.694	1483.693	-1.1
X13	1509.674	1509.672	-1.3
Y14	1580.747	1580.75	1.6
B14	1614.672	1614.672	0.0
C14	1631.698	1631.712	8.7
Y15	1667.779	1667.771	-4.6
B15	1701.704	1701.714	6.1
Y16	1754.811	1754.811	-0.1
A+16	1771.769	1771.77	0.2
X16	1780.79	1780.795	2.3
C16	1815.783	1815.785	1.2

S[P]L[S]Y]S]P]S]S]P]R]Y]T]P]Q]S]P]T] Y]T]P]S]S

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y4	390.1751	390.1752	0.2
A4	406.1852	406.1854	0.3
B4	434.1802	434.1804	0.5
C4	451.2065	451.2068	0.8
B5	521.2122	521.2127	1.0
A+6	591.2779	591.2794	2.5
B7	705.297	705.2969	-0.1
C7	722.3233	722.3232	-0.1
Y6	734.2524	734.2516	-1.1
A+8	765.3419	765.3366	-6.9
Y7	831.3052	831.3062	1.3
A+9	862.3947	862.394	-0.8
Y8	918.3372	918.3376	0.4
B10	1045.483	1045.482	-0.4
C10	1062.509	1062.507	-2.0
Y10	1143.449	1143.449	0.5
A+11	1181.559	1181.558	-0.8
Y11	1244.496	1244.493	-2.3
A+12	1282.607	1282.605	-1.1
B12	1309.594	1309.595	1.0
A13	1378.652	1378.646	-4.2
Y12	1407.56	1407.565	3.7
A14	1506.71	1506.712	1.3
B14	1534.705	1534.709	2.8
C14	1551.732	1551.733	0.8
Y13	1563.661	1563.669	5.6
X+13	1590.648	1590.655	4.5
A+15	1594.75	1594.747	-2.2
B15	1621.737	1621.739	1.1
Y14	1660.713	1660.713	-0.1
C16	1735.816	1735.817	0.5
Y15	1747.745	1747.753	4.6

Y17	1851.864	1851.861	-1.7	Y16	1834.777	1834.793	8.5
B17	1899.804	1899.809	2.8	Y17	1931.83	1931.832	1.0
C17	1916.83	1916.838	4.2	Y18	2018.862	2018.862	0.1
Y18	1938.896	1938.899	1.4	X18	2044.842	2044.861	9.5
A+18	2035.88	2035.878	-1.2	B18	2062.867	2062.876	4.2
B18	2062.867	2062.872	2.3	C18	2079.894	2079.896	0.9
C18	2079.894	2079.896	1.0	A+19	2136.928	2136.924	-1.8
A+19	2136.928	2136.923	-2.3	B19	2163.915	2163.918	1.2
B19	2163.915	2163.917	1.1	Y19	2181.926	2181.926	0.3
A20	2232.973	2232.97	-1.4	Y20	2268.958	2268.963	2.4
Y20	2268.958	2268.962	1.9	Y21	2366.01	2366.012	0.7
Y-21	2365.003	2365.004	0.6				
C21	2365.026	2365.004	-9.4				
Y4	390.1751	390.175	-0.2				
A+4	487.1594	487.1583	-2.3				
B4	514.1465	514.1471	1.2				

C) Cit1810 Peptide Rep. #1 peak:

□ S P S Y | S | P | S | S | P | R | Y | T | P | Q | S | P | T | Y | T | P | S | S

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A+4	487.1594	487.1579	-3.0
B4	514.1465	514.147	0.9
C4	531.1728	531.1735	1.3
Y5	553.2384	553.2387	0.6
B5	601.1785	601.1786	0.1
Y-6	653.2782	653.2822	6.1
C6	715.2576	715.2578	0.3
Y7	751.3388	751.3392	0.5
B7	785.2633	785.2633	0.0
C7	802.2896	802.2895	-0.1
Y8	838.3709	838.3701	-0.9
B8	872.2953	872.2961	0.9
A+9	942.361	942.3583	-2.9
Y9	966.4294	966.4292	-0.3
C9	986.3744	986.3706	-3.9
Y10	1063.482	1063.483	0.3
A+10	1099.446	1099.448	1.3
B10	1126.433	1126.434	0.3
C10	1143.46	1143.461	1.6
Y11	1164.53	1164.533	2.3
A+11	1262.509	1262.507	-1.6
B11	1289.497	1289.497	0.5
C11	1306.523	1306.522	-0.8
Y12	1327.593	1327.6	5.1
B12	1390.544	1390.545	0.3
Y14	1581.731	1581.738	4.3
B14	1615.656	1615.657	0.6
C14	1632.682	1632.677	-3.2
Y15	1668.763	1668.769	3.8
B15	1702.688	1702.687	-0.2
Y17	1852.848	1852.849	0.5
C17	1917.814	1917.814	-0.4
Y18	1939.88	1939.88	0.2
C18	2080.878	2080.87	-3.8
B19	2164.899	2164.893	-2.8

Cit1810 Peptide Rep. #2 peak:

□ S P S Y | S | P | S | S | P | R | Y | T | P | Q | S | P | T | Y | T | P | S | S

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A4	406.1852	406.1854	0.4
B4	434.1802	434.1803	0.4
C4	451.2065	451.2068	0.7
B5	521.2122	521.2125	0.6
Y5	553.2384	553.2389	0.9
A6	590.27	590.2737	6.3
B7	705.297	705.2984	2.0
C7	722.3233	722.3245	1.7
Y7	751.3388	751.3394	0.8
A+8	765.3419	765.3379	-5.3
B8	792.329	792.3292	0.2
Y8	838.3709	838.3715	0.7
A9	861.3868	861.3869	0.0
C9	906.4081	906.4084	0.3
Y9	966.4294	966.426	-3.6
A+10	1019.48	1019.471	-8.5
B10	1046.467	1046.472	4.8
Y10	1063.482	1063.483	1.1
C10	1063.493	1063.483	-9.2
Y11	1164.53	1164.535	4.3
A+11	1262.509	1262.508	-1.0
B11	1289.497	1289.499	1.6
C11	1306.523	1306.521	-1.6
A12	1362.549	1362.55	0.4
B12	1390.544	1390.545	0.8
Y12	1407.56	1407.562	1.4
C12	1407.571	1407.562	-6.4
A+14	1588.668	1588.665	-2.0
B14	1615.656	1615.657	0.7
C14	1632.682	1632.674	-5.0
Y14	1661.697	1661.697	-0.3
B15	1702.688	1702.69	1.6
Y15	1748.729	1748.733	1.8
B17	1900.788	1900.792	2.3
C17	1917.814	1917.813	-0.6
Y17	1932.814	1932.817	1.6
B19	2164.899	2164.901	1.1

Cit1810 Peptide Rep. #3 peak:

□ S P S Y | S | P | S | S | P | R | Y | T | P | Q | S | P | T | Y | T | P | S | S

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A4	406.1852	406.1853	0.2
B4	434.1802	434.1801	-0.1
C4	451.2065	451.2065	0.1
B7	705.297	705.2952	-2.6
C7	722.3233	722.3228	-0.6
B8	792.329	792.33	1.3
Y7	831.3052	831.3062	1.3
A9	861.3868	861.387	0.1
C9	906.4081	906.4087	0.7
Y8	918.3372	918.3375	0.3
A10	1018.472	1018.469	-3.4
B10	1046.467	1046.474	7.1
C10	1063.493	1063.494	0.8
Y10	1143.449	1143.45	1.0
A11	1181.535	1181.537	1.4
B11	1209.53	1209.525	-4.1
Y11	1244.496	1244.498	1.3
A+12	1283.591	1283.594	2.2
B12	1310.578	1310.581	2.1
Y12	1407.56	1407.557	-2.0
B14	1535.689	1535.692	1.8
C14	1552.716	1552.707	-5.7
A15	1594.726	1594.716	-6.2
B15	1622.721	1622.724	1.4
Y14	1661.697	1661.698	0.1
Y15	1748.729	1748.732	1.2
Y17	1932.814	1932.818	2.0
B19	2164.899	2164.885	-6.6
B21	2348.984	2348.99	2.5

Table S6. Identified ions in UVPD-MS analysis of singly phosphorylated peaks from ABL1 treated 3xS7K Sp and 3xS7Q Mid/Sp substrates (Fig. 6A and 6C/D). The phosphorylated Tyr1 sites were shown in blue boxes. Tables are split across pages due to size constraints.

A) 3xS7K Sp Rep. #1 peak:

11 G|P|G S|G|M|S|P|K|**T**|S|P|T|S|P|S|Y|S|P|T|S|P|K|Y|S 25
26]P]T C

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.1432	0.7
A+6	459.2026	459.2028	0.6
B6	486.1897	486.1896	-0.1
A+7	546.2346	546.2308	-6.9
Z5	578.2826	578.2824	-0.3
Y5	594.3013	594.3013	0.0
A+8	643.2874	643.2882	1.4
Y6	691.3541	691.355	1.3
A+9	771.3823	771.3842	2.4
Y7	778.3861	778.3885	3.1
B9	798.3694	798.3678	-2.1
Y-8	878.426	878.42	-6.9
Y9	976.4866	976.4875	0.9
A10	1013.404	1013.405	0.9
A+10	1014.412	1014.412	-0.3
C10	1058.425	1058.429	3.1
Y10	1063.519	1063.521	2.3
A+11	1101.444	1101.441	-2.5
B11	1128.431	1128.432	0.8
A+12	1198.497	1198.495	-1.4
Y11	1226.582	1226.586	3.7
C12	1242.51	1242.517	5.8
Y12	1313.614	1313.615	1.0
B13	1326.532	1326.536	3.2
X12	1339.593	1339.586	-5.3
C13	1343.558	1343.56	1.2
Y13	1410.667	1410.668	0.8
B14	1413.564	1413.563	-0.2
A+15	1483.629	1483.629	0.1
Y14	1497.699	1497.702	2.2
C15	1527.643	1527.645	1.5
A16	1569.653	1569.655	1.2
B16	1597.648	1597.654	3.4
C16	1614.675	1614.676	1.1
X+15	1625.734	1625.729	-2.6
Y-16	1694.791	1694.805	8.2
A17	1732.717	1732.719	1.2
B17	1760.712	1760.709	-1.6
C17	1777.738	1777.741	1.8
Y17	1782.831	1782.826	-3.2
A+18	1820.757	1820.769	6.9
B18	1847.744	1847.748	2.1
A+19	1917.809	1917.811	0.9
C19	1961.823	1961.826	1.5
A20	2017.849	2017.861	5.6
Y18	2025.861	2025.863	1.2
B20	2045.844	2045.852	3.6
A+21	2105.889	2105.892	1.3
Z19	2137.937	2137.931	-2.6
Y19	2153.956	2153.962	2.8

3xS7K Sp Rep. #2 peak:

11 G|P|G S|G|M|S|P|K|Y|S|P|T|S|P|S|**Y**|S|P|T|S|P|K|Y|S 25
26]P]T C

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.1433	0.8
A+6	459.2026	459.2029	0.8
B6	486.1897	486.1898	0.3
C6	503.216	503.2162	0.5
Z5	578.2826	578.2828	0.3
Y5	594.3013	594.3021	1.3
X5	620.2806	620.2812	0.9
C8	687.3008	687.3012	0.7
Y6	691.3541	691.3548	1.0
A+9	771.3823	771.3865	5.4
Y7	778.3861	778.3889	3.6
B9	798.3694	798.3668	-3.3
C9	815.3957	815.3983	3.2
Z8	863.415	863.4181	3.6
A10	933.4378	933.4384	0.6
A+10	934.4457	934.4445	-1.2
Y9	976.4866	976.4877	1.2
C10	978.459	978.4656	6.7
X+9	1003.474	1003.466	-7.9
A+11	1021.478	1021.477	-0.6
Y10	1063.519	1063.523	3.9
A12	1117.523	1117.523	0.2
C12	1162.544	1162.545	1.1
B13	1246.565	1246.556	-7.0
C13	1263.592	1263.594	2.1
A+14	1306.61	1306.61	-0.4
B14	1333.597	1333.598	0.6
Y12	1393.58	1393.585	3.1
A15	1402.655	1402.654	-0.8
A+15	1403.663	1403.664	0.6
X+12	1420.567	1420.571	2.6
C15	1447.676	1447.679	1.6
Y13	1490.633	1490.631	-1.6
B16	1517.682	1517.682	0.2
Y14	1577.665	1577.667	1.5
A+17	1733.725	1733.727	1.2
B17	1760.712	1760.714	1.1
Y16	1775.766	1775.766	0.3
X+16	1802.753	1802.752	-0.4
A+18	1820.757	1820.772	8.4
A19	1916.802	1916.783	-9.6
A20	2017.849	2017.867	8.6
C20	2062.87	2062.879	4.3
A+21	2105.889	2105.891	1.0
Z19	2137.937	2137.935	-0.8
X19	2179.935	2179.938	1.2
A+22	2202.942	2202.954	5.3
Y-20	2250.001	2249.999	-0.6
Y21	2338.041	2338.042	0.7
X+21	2365.028	2365.029	0.6

X+19	2180.943	2180.955	5.4	C23	2375.05	2375.035	-6.4
A22	2201.934	2201.934	-0.1	Z22	2453.062	2453.062	-0.1
Y-20	2250.001	2249.999	-0.8	Y22	2469.081	2469.09	3.4
A+23	2331.037	2331.042	2.4	A24	2493.092	2493.104	4.6
Y21	2338.041	2338.039	-0.6	Y-23	2525.095	2525.101	2.7
B23	2358.024	2358.03	2.4	C24	2538.114	2538.115	0.7
C23	2375.05	2375.045	-2.2	X+23	2553.09	2553.095	2.1
Y22	2469.081	2469.082	0.5	A+25	2581.132	2581.131	-0.6
A24	2493.092	2493.095	1.2	B25	2608.119	2608.123	1.4
B24	2521.087	2521.086	-0.6	Y25	2670.156	2670.171	5.5
Y23	2526.103	2526.109	2.6	A+26	2678.185	2678.189	1.5
C24	2538.114	2538.118	1.7	X25	2696.135	2696.14	1.7
X23	2552.082	2552.096	5.5	Y-26	2766.201	2766.213	4.4
A25	2580.124	2580.124	0.0				
B25	2608.119	2608.123	1.6				
Y25	2670.156	2670.166	3.9				
A+26	2678.185	2678.188	1.3				
X25	2696.135	2696.133	-1.0				
B26	2705.172	2705.175	1.0				
Y-26	2766.201	2766.214	4.6				

B) 3xS7Q Mid Rep. #2 peak:

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
A+6	459.2026	459.204	3.1
Y4	466.2064	466.2073	1.9
C6	503.216	503.2138	-4.3
A+8	643.2874	643.2895	3.4
Y6	650.2912	650.292	1.3
C8	687.3008	687.3024	2.4
A+9	730.3194	730.3194	0.0
Y7	737.3232	737.325	2.4
C9	774.3328	774.3337	1.2
Y8	838.3709	838.372	1.4
B10	920.3698	920.3707	0.9
Y9	935.4236	935.425	1.5
X+9	962.4107	962.411	0.3
A+11	980.4148	980.4127	-2.1
B11	1007.402	1007.402	0.5
A+12	1077.468	1077.464	-3.3
A13	1177.507	1177.5	-6.4
B13	1205.502	1205.504	1.1
Y11	1265.485	1265.497	9.2
B14	1292.534	1292.537	1.9
Y13	1490.597	1490.596	-0.1
B16	1517.646	1517.646	0.2
Y14	1577.629	1577.633	2.9
A17	1732.68	1732.694	8.1
B17	1760.675	1760.678	1.3
Y16	1775.729	1775.73	0.4
X+16	1802.716	1802.709	-4.0
B18	1847.707	1847.71	1.6
Y17	1862.761	1862.762	0.7
Z19	2096.838	2096.819	-8.7
A21	2104.845	2104.852	3.2
B21	2132.84	2132.845	2.4
Y20	2209.909	2209.911	0.6
Z21	2280.923	2280.917	-2.3
Y21	2296.941	2296.945	1.5

3xS7Q Mid Rep. #3 peak:

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
B5	355.1492	355.1504	3.4
B6	486.1897	486.1904	1.4
C6	503.216	503.2167	1.5
A+8	643.2874	643.288	1.0
C8	687.3008	687.3028	3.0
Y6	730.2575	730.2582	1.0
C9	774.3328	774.3339	1.5
Y7	817.2895	817.2904	1.1
A10	892.3749	892.3706	-4.8
B10	920.3698	920.3709	1.1
C10	937.3961	937.399	3.1
A11	979.4069	979.4063	-0.7
B11	1007.402	1007.403	1.2
Y9	1015.39	1015.391	1.2
A+12	1077.468	1077.468	0.3
Y10	1102.422	1102.424	1.8
A13	1177.507	1177.506	-1.4
B13	1205.502	1205.504	1.7
A14	1264.539	1264.538	-1.1
B14	1292.534	1292.537	2.0
Y13	1490.597	1490.598	1.0
B16	1517.646	1517.649	2.5
C16	1534.672	1534.674	1.6
Y14	1577.629	1577.63	0.8
A17	1652.714	1652.711	-1.9
B17	1680.709	1680.71	0.9
A18	1739.746	1739.735	-6.6
B18	1767.741	1767.742	0.8
Y16	1775.729	1775.73	0.6
Y17	1862.761	1862.763	1.1
B20	1965.841	1965.848	3.2
B21	2052.873	2052.879	2.8
B23	2236.958	2236.954	-2.1
Y21	2296.941	2296.947	2.5
A+24	2453.001	2453	-0.4

C23	2333.951	2333.954	1.1	B24	2479.988	2479.991	1.3
A24	2451.993	2451.998	2.0	A25	2539.025	2539.019	-2.4
B24	2479.988	2479.993	1.9	B25	2567.02	2567.027	2.7
C24	2497.014	2497.032	7.3				
A25	2539.025	2539.018	-2.6				
B25	2567.02	2567.026	2.3				
Y26	2726.11	2726.11	0.1				
X+26	2753.097	2753.12	8.4				

C) 3xS7Q Sp Rep. #1 peak:

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
B4	298.1277	298.1275	-0.7
Y3	303.143	303.1433	0.8
A+6	459.2026	459.2025	-0.1
Y4	466.2064	466.2066	0.6
C6	503.216	503.2165	1.1
Z5	578.2462	578.2459	-0.5
A+8	643.2874	643.2864	-1.4
Y6	691.3177	691.3185	1.1
A+9	771.3459	771.3408	-6.6
C9	815.3593	815.3591	-0.3
X8	905.3767	905.3791	2.7
Y9	976.4502	976.451	0.8
A+10	1014.376	1014.375	-0.4
B10	1041.363	1041.362	-1.1
Y10	1063.482	1063.484	1.5
A+11	1101.408	1101.402	-5.4
B11	1128.395	1128.396	1.2
Y11	1226.546	1226.552	5.0
A13	1298.5	1298.504	2.9
B13	1326.495	1326.498	1.9
C13	1343.521	1343.519	-1.9
A+14	1386.54	1386.532	-5.5
Y13	1410.63	1410.632	1.3
B14	1413.527	1413.527	-0.4
Y14	1497.662	1497.664	1.2
B16	1597.612	1597.615	2.2
C16	1614.638	1614.641	2.0
Y-16	1694.755	1694.748	-3.8
A17	1732.68	1732.681	0.2
B17	1760.675	1760.675	-0.1
Y17	1782.795	1782.799	2.3
A18	1819.712	1819.713	0.3
B18	1847.707	1847.711	1.8
B20	2045.808	2045.812	2.0
B21	2132.84	2132.83	-4.8
Y20	2250.936	2250.937	0.7
A+23	2330.964	2330.968	1.8
Y21	2337.968	2337.966	-0.6
B23	2357.951	2357.965	5.7
C23	2374.977	2374.975	-0.9
B24	2521.014	2521.017	1.1
A25	2580.052	2580.05	-0.4
B25	2608.047	2608.044	-0.8

3xS7Q Sp Rep. #2 peak:

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
B4	298.1277	298.1278	0.2
A+6	459.2026	459.2037	2.5
Y4	466.2064	466.2068	0.9
B6	486.1897	486.1902	1.1
C6	503.216	503.2172	2.5
B7	573.2217	573.2225	1.4
Z5	578.2462	578.2471	1.7
A+8	643.2874	643.2881	1.2
Y6	691.3177	691.3189	1.8
Y7	778.3497	778.3518	2.6
C9	815.3593	815.3616	2.8
A10	933.4014	933.4018	0.4
B10	961.3964	961.3984	2.1
Y9	976.4502	976.452	1.9
X9	1002.429	1002.425	-4.7
A+11	1021.441	1021.442	0.8
B11	1048.428	1048.429	0.7
Y10	1063.482	1063.485	2.8
A+12	1118.494	1118.491	-3.0
B13	1246.529	1246.529	-0.1
C13	1263.555	1263.548	-5.4
B14	1333.561	1333.564	2.4
Y12	1393.544	1393.548	2.7
A+15	1403.627	1403.626	-0.7
Y13	1490.597	1490.599	1.4
B16	1517.646	1517.644	-1.0
C16	1534.672	1534.671	-0.9
Y14	1577.629	1577.633	2.7
A17	1732.68	1732.693	7.2
B17	1760.675	1760.668	-3.9
Y16	1775.729	1775.73	0.7
X+16	1802.716	1802.711	-2.6
A+18	1820.72	1820.734	7.7
B18	1847.707	1847.714	3.4
Y17	1862.761	1862.764	1.5
Y18	2025.824	2025.83	2.8
B20	2045.808	2045.811	1.8
B21	2132.84	2132.822	-8.5
A+22	2202.905	2202.904	-0.8
Y20	2250.936	2250.936	0.2
A+23	2330.964	2330.968	1.6
B23	2357.951	2357.963	4.9
C23	2374.977	2374.97	-3.3
B24	2521.014	2521.019	2.0
X+23	2553.017	2552.994	-9.1
A25	2580.052	2580.054	1.0
B25	2608.047	2608.07	9.2
A26	2677.104	2677.085	-7.1
Z26	2751.117	2751.139	7.8

3xS7Q Sp Rep. #3 peak:

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
Y3	303.143	303.1433	0.9
C8	687.3008	687.3014	1.0
Y6	771.284	771.2842	0.2
B9	798.333	798.3345	1.8
C9	815.3593	815.3613	2.4
Y7	858.3161	858.3172	1.4
A10	933.4014	933.4004	-1.2
Y8	959.3637	959.3659	2.2
B10	961.3964	961.3957	-0.7
C10	978.4227	978.4238	1.1
A+11	1021.441	1021.438	-2.8
B11	1048.428	1048.429	0.3
Y9	1056.417	1056.419	2.0
Y10	1143.449	1143.449	0.7
B13	1246.529	1246.528	-0.5
C13	1263.555	1263.544	-8.8
B14	1333.561	1333.563	1.6
Y12	1393.544	1393.55	4.3
Y13	1490.597	1490.597	0.4
B16	1517.646	1517.648	1.9
C16	1534.672	1534.674	1.1
Y14	1577.629	1577.632	2.1
B17	1680.709	1680.708	-0.8
B18	1767.741	1767.742	0.8
Y16	1775.729	1775.73	0.7
Y17	1862.761	1862.764	1.6
B20	1965.841	1965.844	1.5
Y18	2025.824	2025.842	8.7
Y20	2250.936	2250.926	-4.2
B23	2277.985	2277.984	-0.2
Y21	2337.968	2337.968	-0.1
B24	2521.014	2521.017	0.9
B25	2608.047	2608.053	2.4