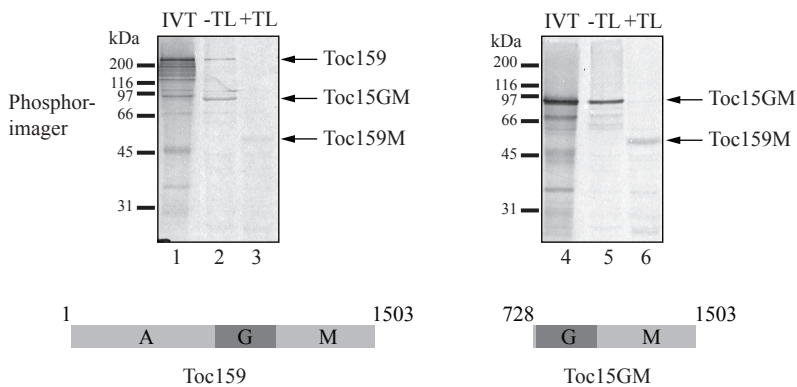
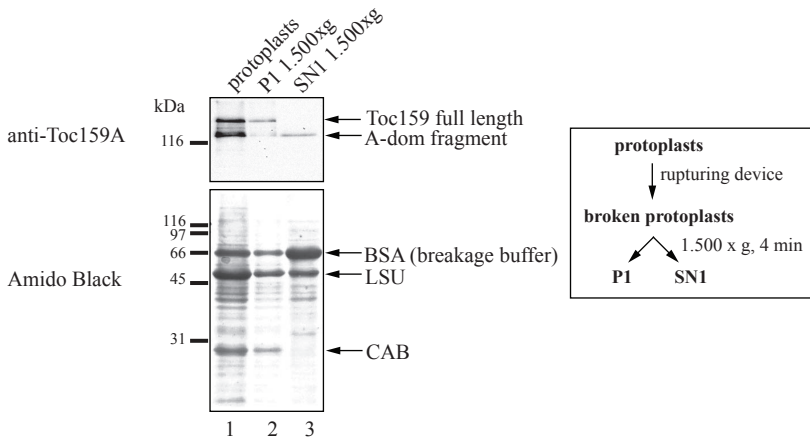


**Figure. S1. Prediction of CK2 phosphorylation sites.** NetPhosK 1.0 has been used to predict CK2 phosphorylation sites for the A-domains of Arabidopsis Toc120, Toc132, and Toc159, and the G-domains of Toc33 and Toc159. Serine phosphorylation sites are indicated in red and threonine phosphorylation sites in gray. Sequences used for the in silico prediction are identical to the sequences of the construction used in the in vitro phosphorylation assay, see Figure 7 B.

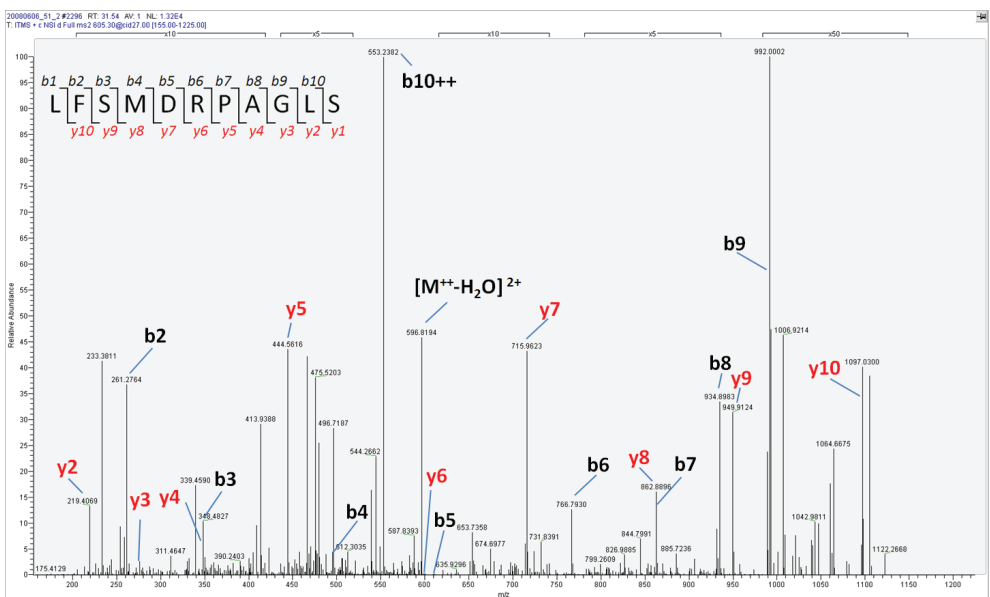
**A**



**B**



**C**



**Supplemental Figure S2. Toc159 cleavage.** (A) A Toc159 processing activity in isolated chloroplasts. [35S]-methionin-labelled, in vitro translated (IVT) Toc159 or Toc159GM were incubated with isolated chloroplasts. Chloroplasts were re-isolated and incubated with (+TL) or without (-TL) thermolysin. Samples were analyzed by SDS-PAGE and PhosphorImager. (B) Occurrence of the A-domain fragment after gentle cell disruption. Protoplasts of wild-type Arabidopsis plants were disrupted with a rupturing device employing 23 and 18  $\mu\text{m}$  nylon mesh in two layers (Smith et al., 2002). Organelles were separated by centrifugation. 50  $\mu\text{g}$  of protoplast, pellet and supernatant protein were analyzed by Western Blotting with anti-Toc159A. (C) An annotated semi-tryptic peptide identified in the SN100 soluble fraction. To identify the most C-terminal amino acid of the soluble A-domain, MS/MS spectra were searched by Mascot for peptides that show tryptic specificity at one terminus only, and where the other terminus may be the result of non-tryptic cleavage. The semi-tryptic peptide mapping to amino acids 733-744 of atToc159 was identified in the SN100 fraction of three independent purifications with ion scores of 37, 42 and 47.

locus	peptide sequence	title	query number
AT4G02510	VGADDLpSDSEK	F059194	q801_p1
AT4G02510	VVEGDpS AEEDENKLPVEDIVSSR	F075163	q1354_p1
AT4G02510	KVVEGDpSAEEDENKLPVEDIVSSR	F075163	q1360_p1
AT4G02510	FDQIGDDDpSGEFEPVSDK	F059194	q1585_p1
AT4G02510	VGADDLSDSEKEKPNLVGDGK	F050642	q1602_p1
AT4G02510	ASSGIEAHSDEANISNNMSDR	F059194	q1640_p1
AT4G02510	ASSGIEAHSDEAN ISNNMSDR	F059194	q1641_p1
AT4G02510	VVEGDpSAEEDENKLPVEDIVSSR	F059194	q1672_p1

**Supplemental Table S1:** List of phosphorylated peptides presented in the main text (Tab.I) and the respective query number as its spectra identifier.

### **Supplemental Spectra S1: Pages 3 to 10**

Spectra of phosphorylated peptides as listed before. Unambiguous phosphorylation sites are assigned by " p " prior to the phosphorylated aminoacid. The p-site assignment " \* " in the peptide sequence on each spectra page was automatically annotated without validation.

title: F059194  
query number: q832\_p1  
mascot Score: 85.73  
precursormass/charge: 608.242810,2+  
delta score; pep rank1 – pep rank2: 33.9  
peptide sequence: VGADDL\*SDSEK

Relative Intensity

100  
80  
60  
40  
20  
0

228

406

584

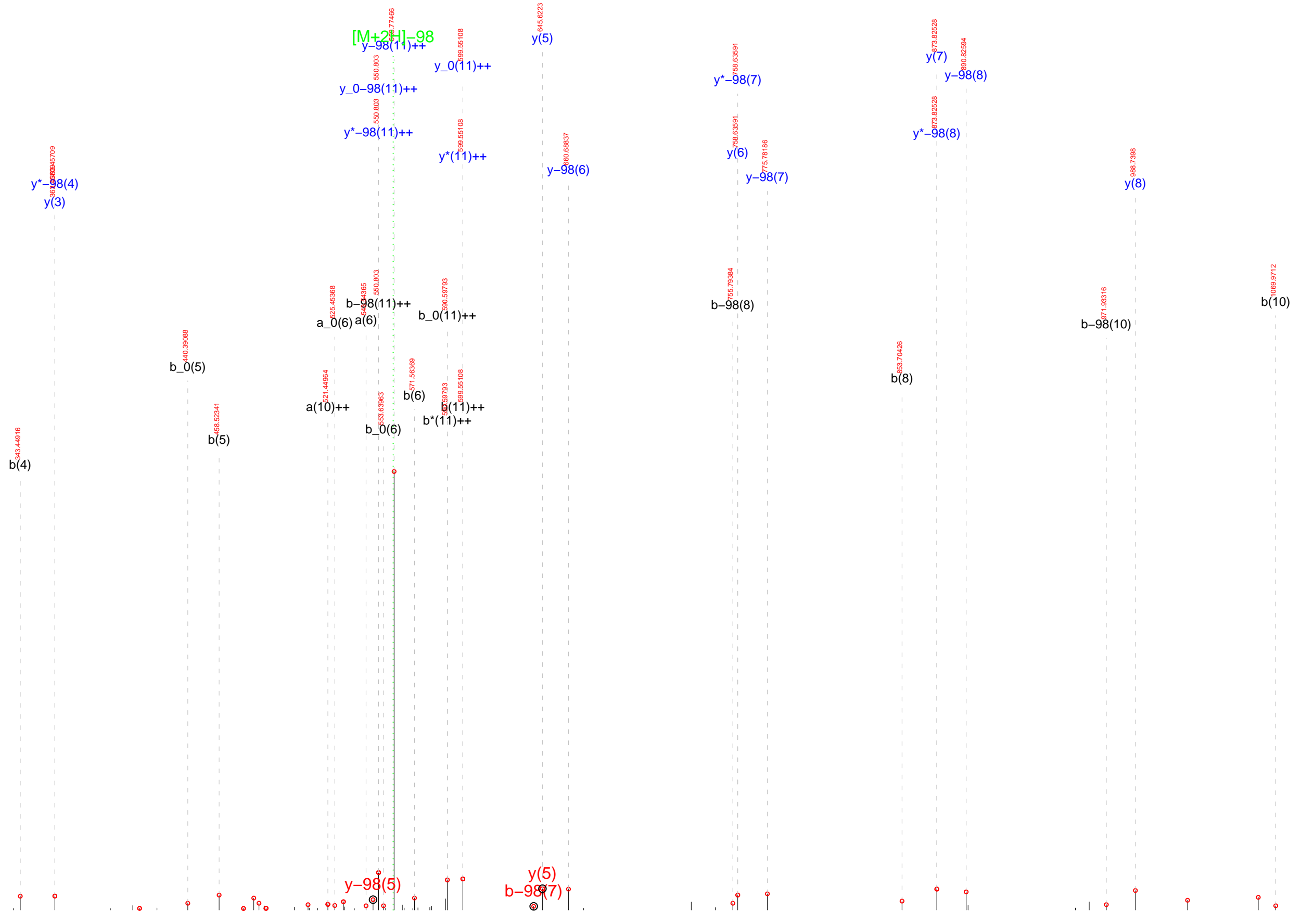
761

939

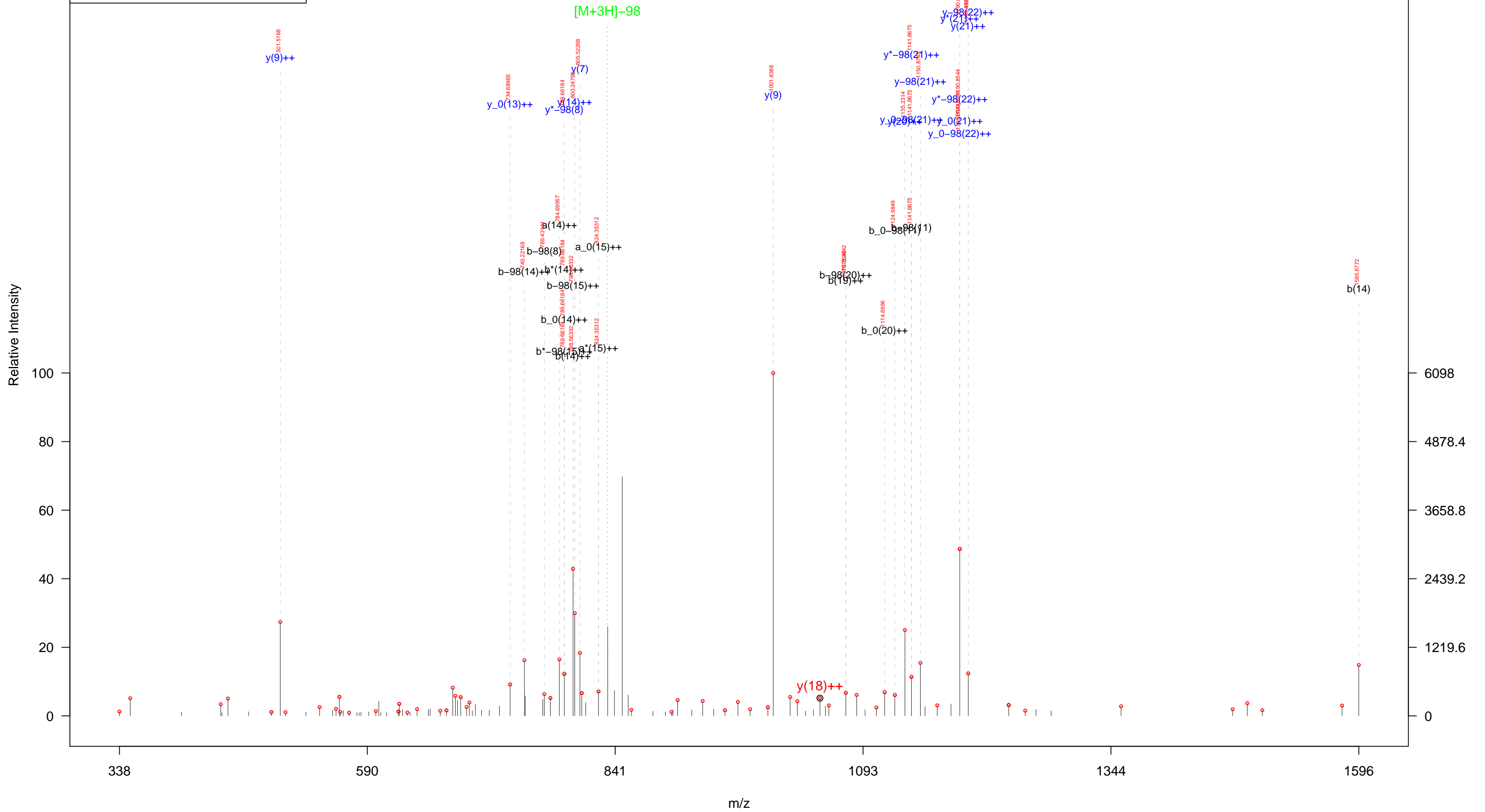
1117

m/z

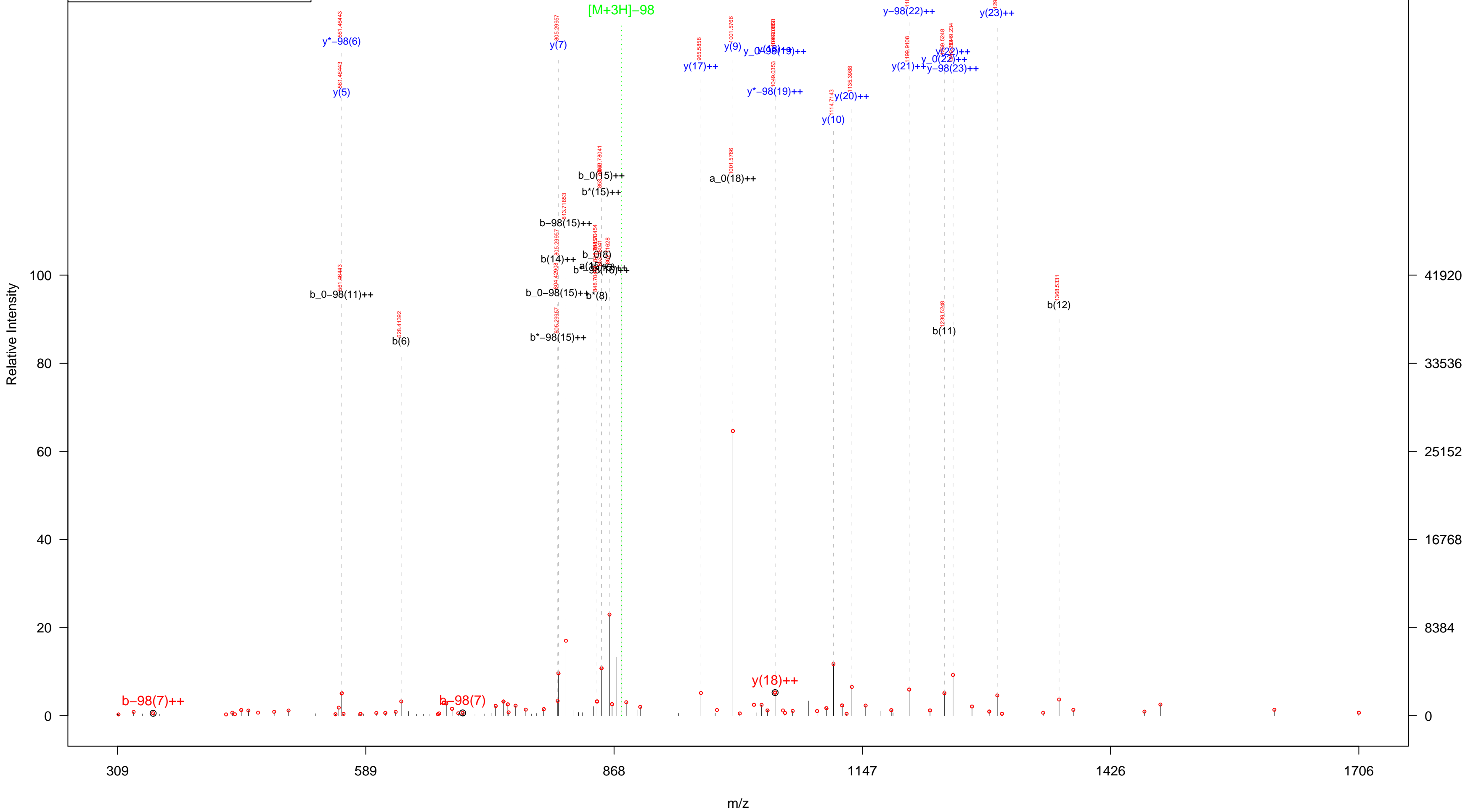
30780  
24624  
18468  
12312  
6156  
0



title: F075163  
query number: q1354\_p1  
mascot Score: 76.76  
precursormass/charge: 866.061710,3+  
delta score; pep rank1 - pep rank2: 63.39  
peptide sequence: VVEGD\*SAEEDENKLPVEDIVSSR

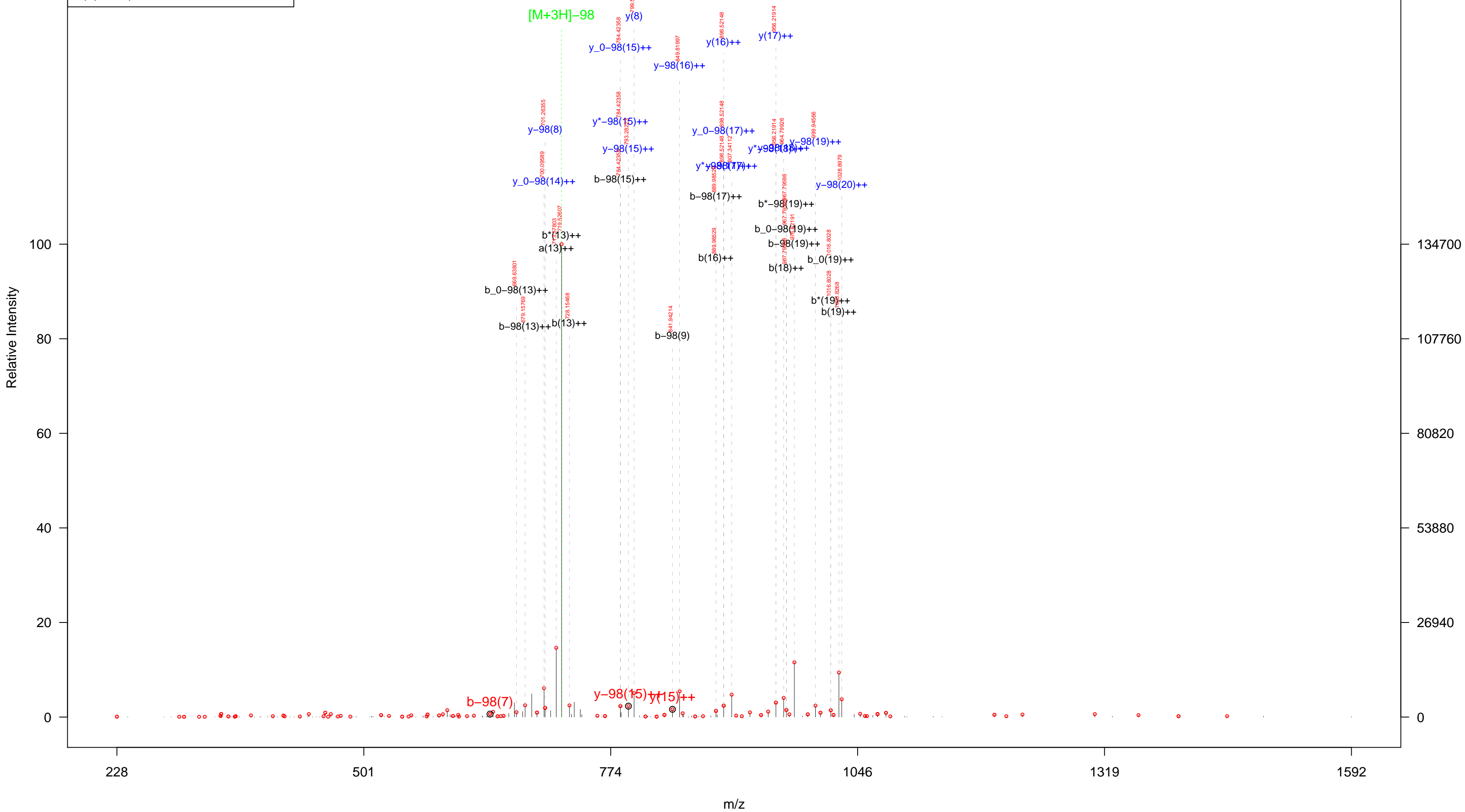


title: F075163  
query number: q1360\_p1  
mascot Score: 89.63  
precursormass/charge: 908.759280,3+  
delta score; pep rank1 – pep rank2: 72.73  
peptide sequence: KVEGD\*SAEEDENKLPVEDIVSSR



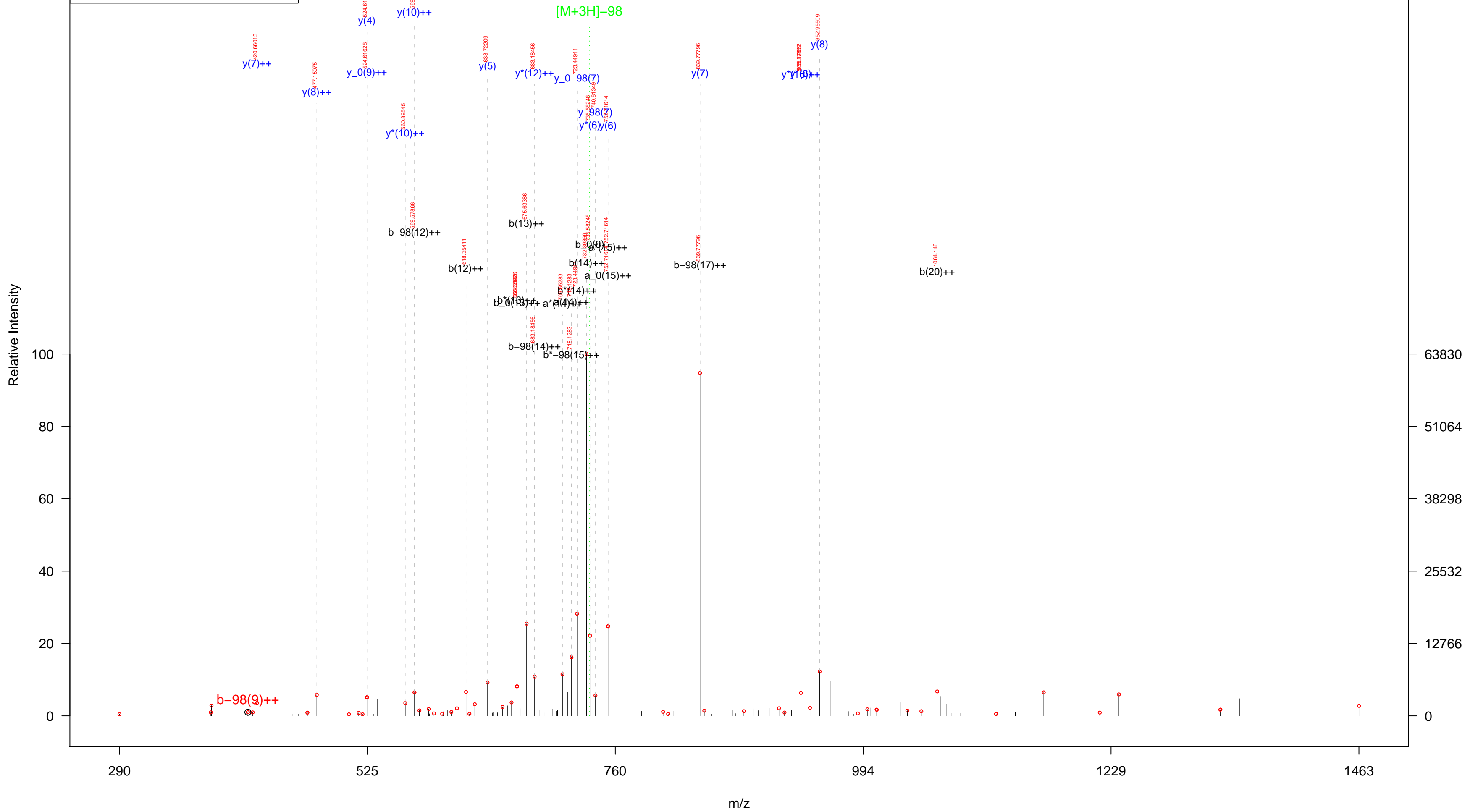


title: F050642  
query number: q1602\_p1  
mascot Score: 68.72  
precursormass/charge: 752.015930,3+  
delta score; pep rank1 – pep rank2: 6.05  
peptide sequence: VGADDL\*SDSEKEKPNLVGDGK





title: F059194  
query number: q1640\_p1  
mascot Score: 49.02  
precursormass/charge: 767.640560,3+  
delta score; pep rank1 – pep rank2: 7.88  
peptide sequence: ASSGIEAH\*SDEANISNN\*MSDR



title: F059194  
query number: q1641\_p1  
mascot Score: 52.90  
precursormass/charge: 767.642180,3+  
delta score; pep rank1 – pep rank2: 0  
peptide sequence: AS\*SGIEAHSDEANISNN\*MSDR

Relative Intensity

100  
80  
60  
40  
20  
0

326

554

781

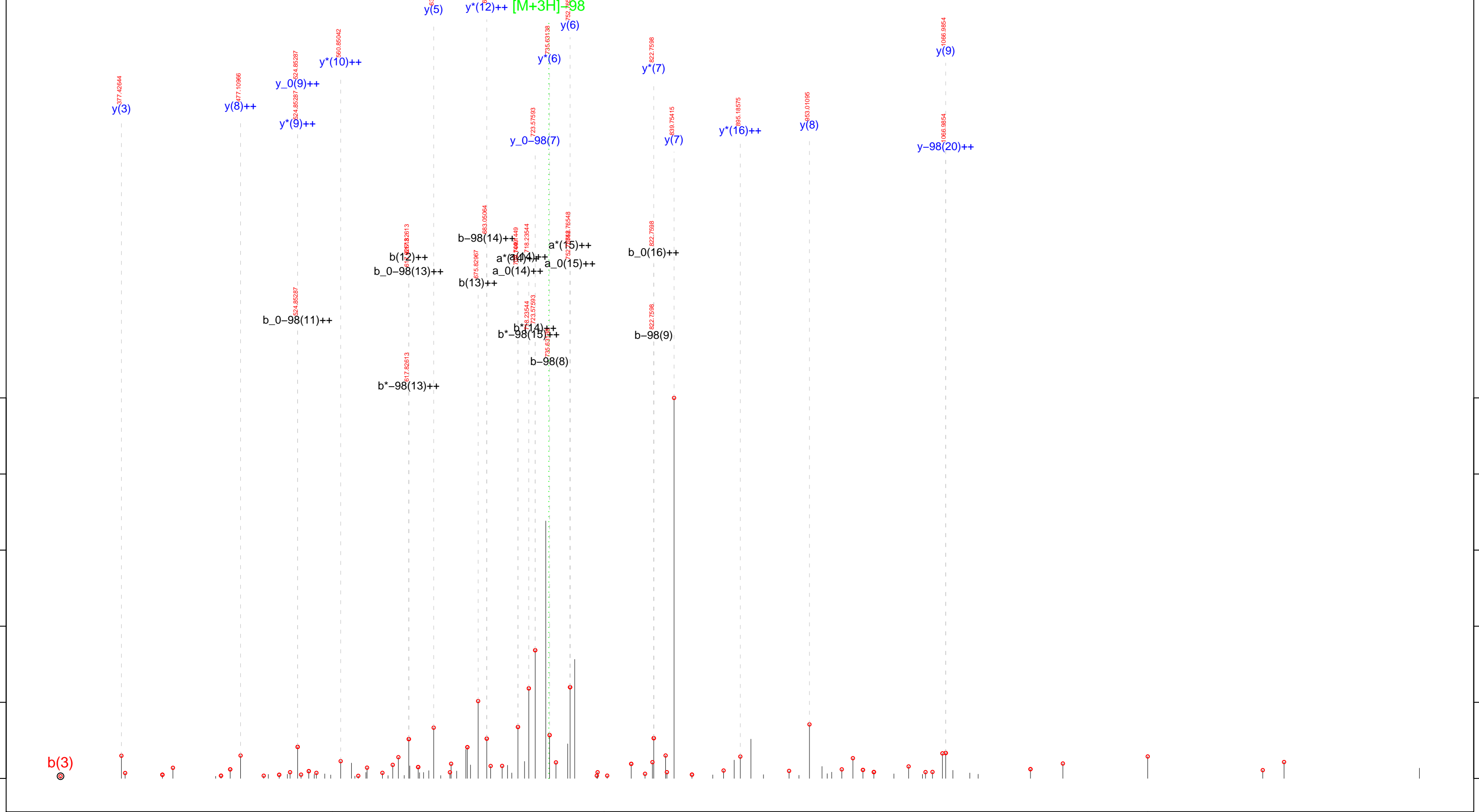
1009

1236

1463

m/z

46730  
37384  
28038  
18692  
9346  
0



y(3)

y(8)++

y\_0(9)++

y\*(9)++

y\*(10)++

y(5)

y\*(12)++ [M+3H] 98

y(6)

y\*(6)

y\*(7)

y(9)

y-98(20)++

b(12)++

b\_0-98(13)++

b\_0-98(11)++

b\*-98(13)++

b(13)++

b\*-98(15)++

b-98(8)

b\*-98(15)++

b-98(8)

b\*-98(15)++

b-98(8)

b-98(14)++

a\*(14)++

a\_0(14)++

a\*(15)++

a\_0(15)++

b\_0(16)++

b-98(9)

y\*(16)++

y(8)

y-98(20)++

b(3)

y(3)

y(8)++

y\_0(9)++

y\*(9)++

y\*(10)++

y(5)

y\*(12)++ [M+3H] 98

y(6)

y\*(6)

y\*(7)

y(9)

y-98(20)++

b(12)++

b\_0-98(13)++

b\_0-98(11)++

b\*-98(13)++

b(13)++

b\*-98(15)++

b-98(8)

b\*-98(15)++

b-98(8)

b-98(14)++

a\*(14)++

a\_0(14)++

a\*(15)++

a\_0(15)++

b\_0(16)++

b-98(9)

y\*(16)++

y(8)

y-98(20)++

b(3)

title: F059194  
query number: q1672\_p1  
mascot Score: 36.89  
precursormass/charge: 866.059720,3+  
delta score; pep rank1 – pep rank2: 25.44  
peptide sequence: VVEGD\*SAEEDENKLPVEDIVSSR

Relative Intensity

100  
80  
60  
40  
20  
0

328

582

835

1089

1343

1596

m/z

3282  
2625.6  
1969.2  
1312.8  
656.4  
0

