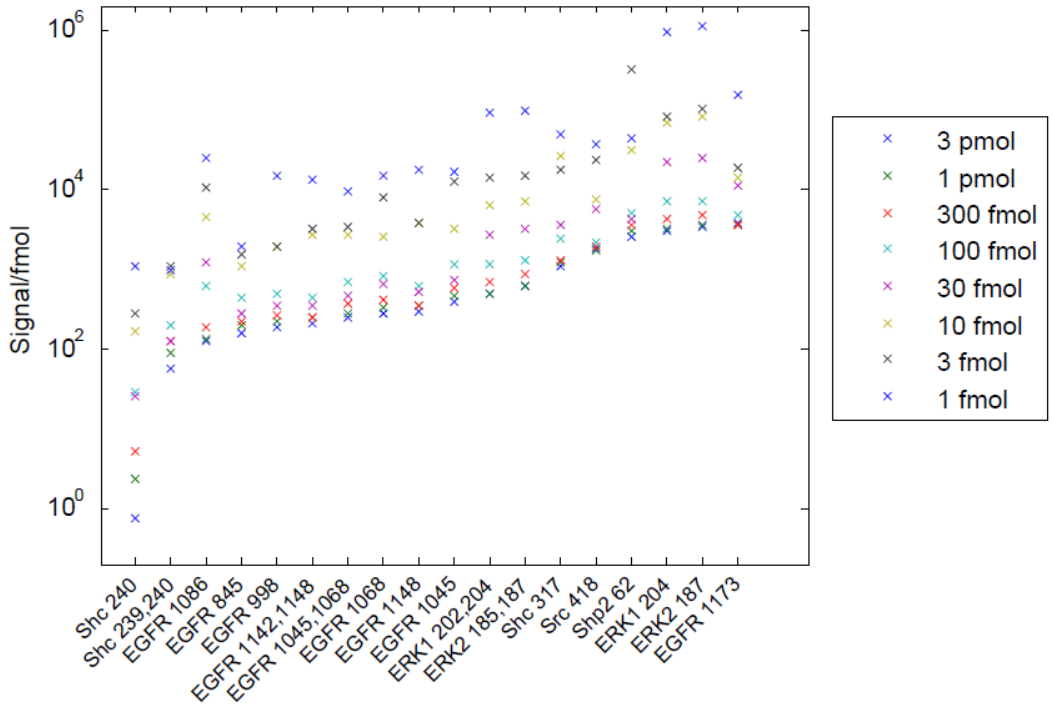
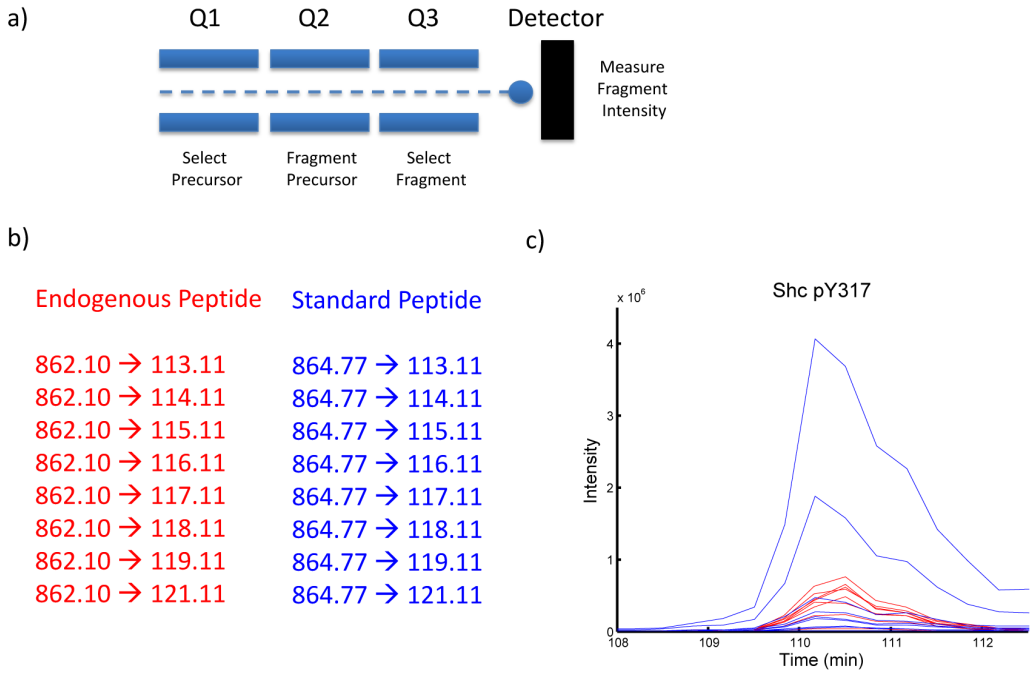
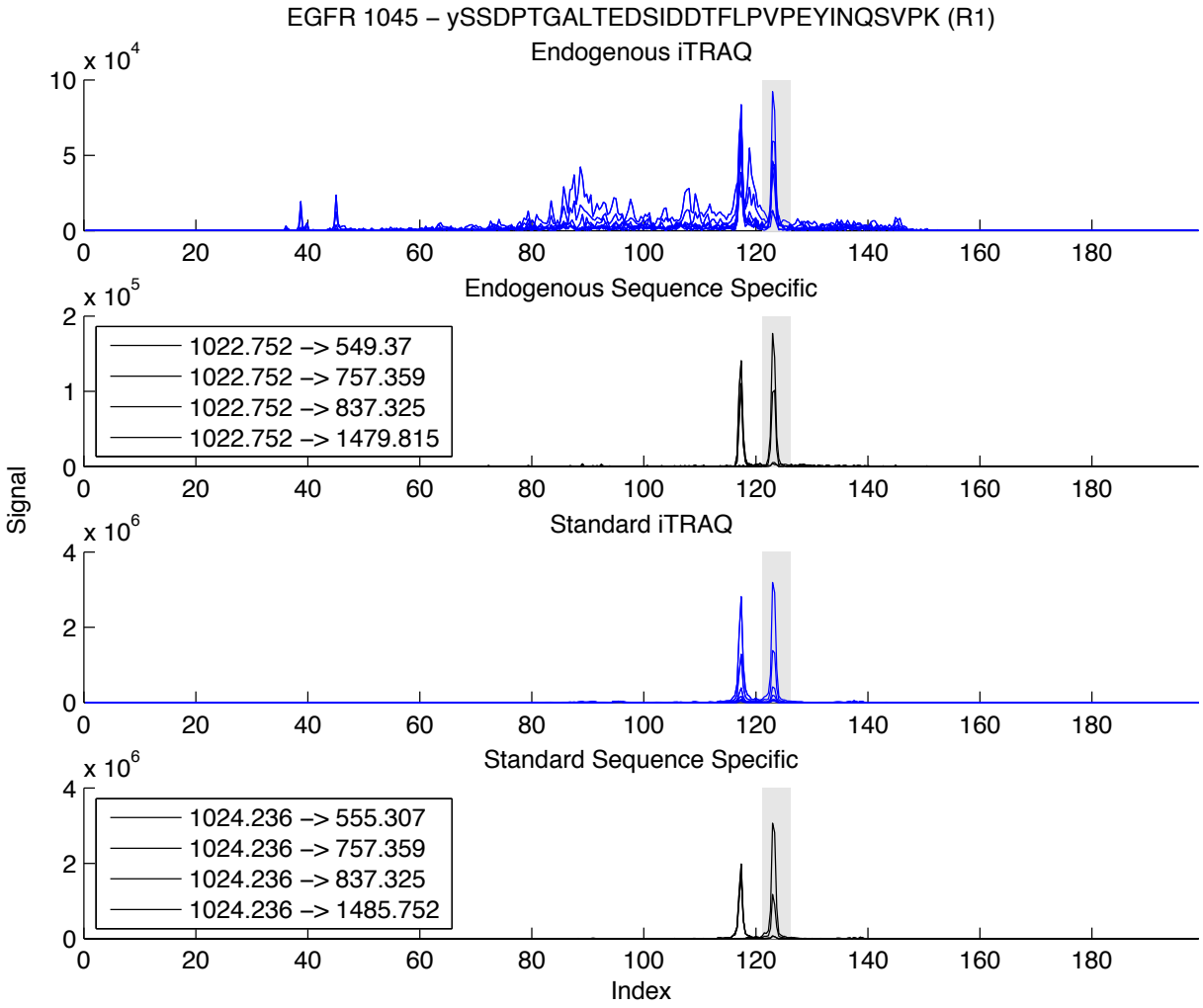
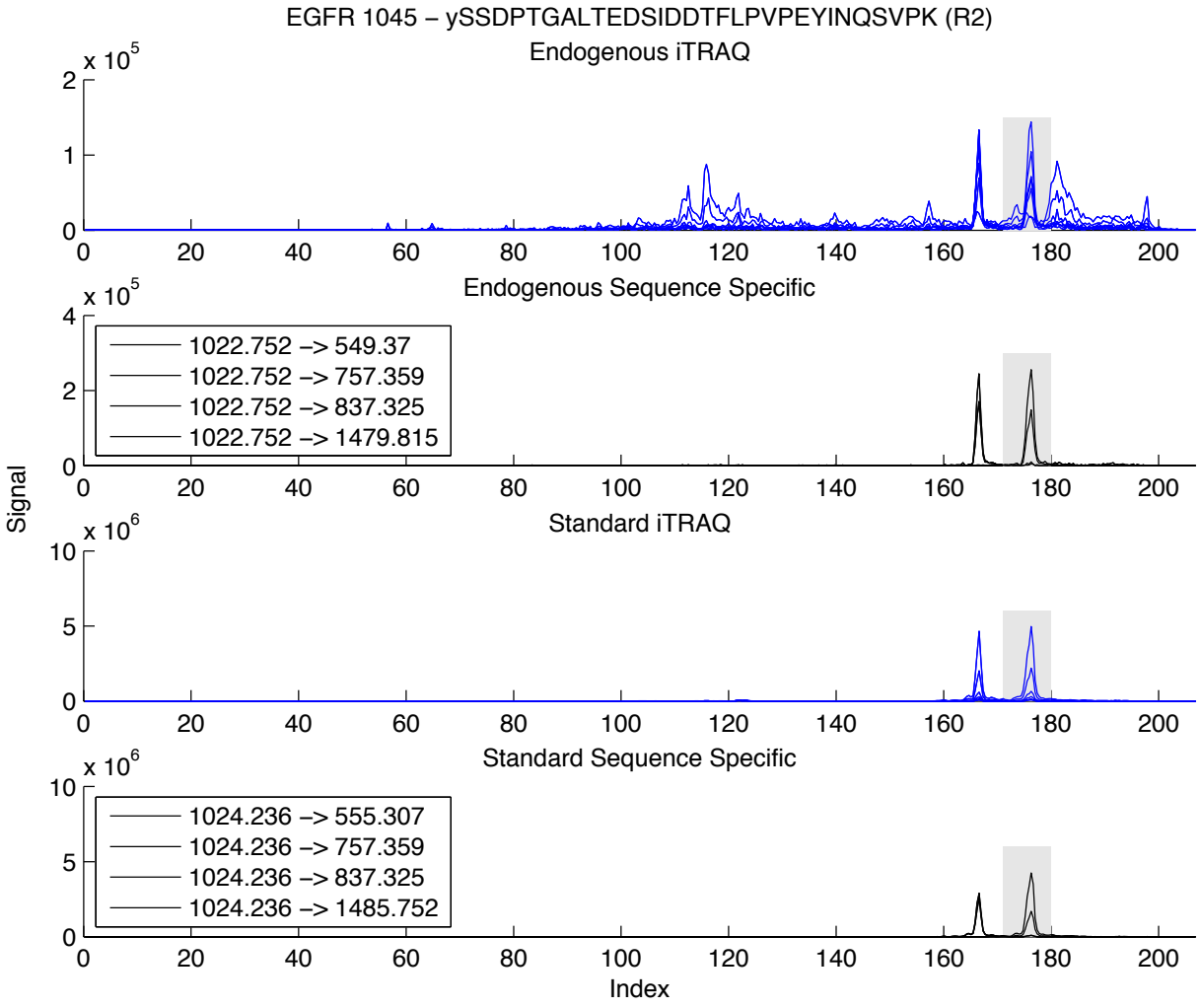


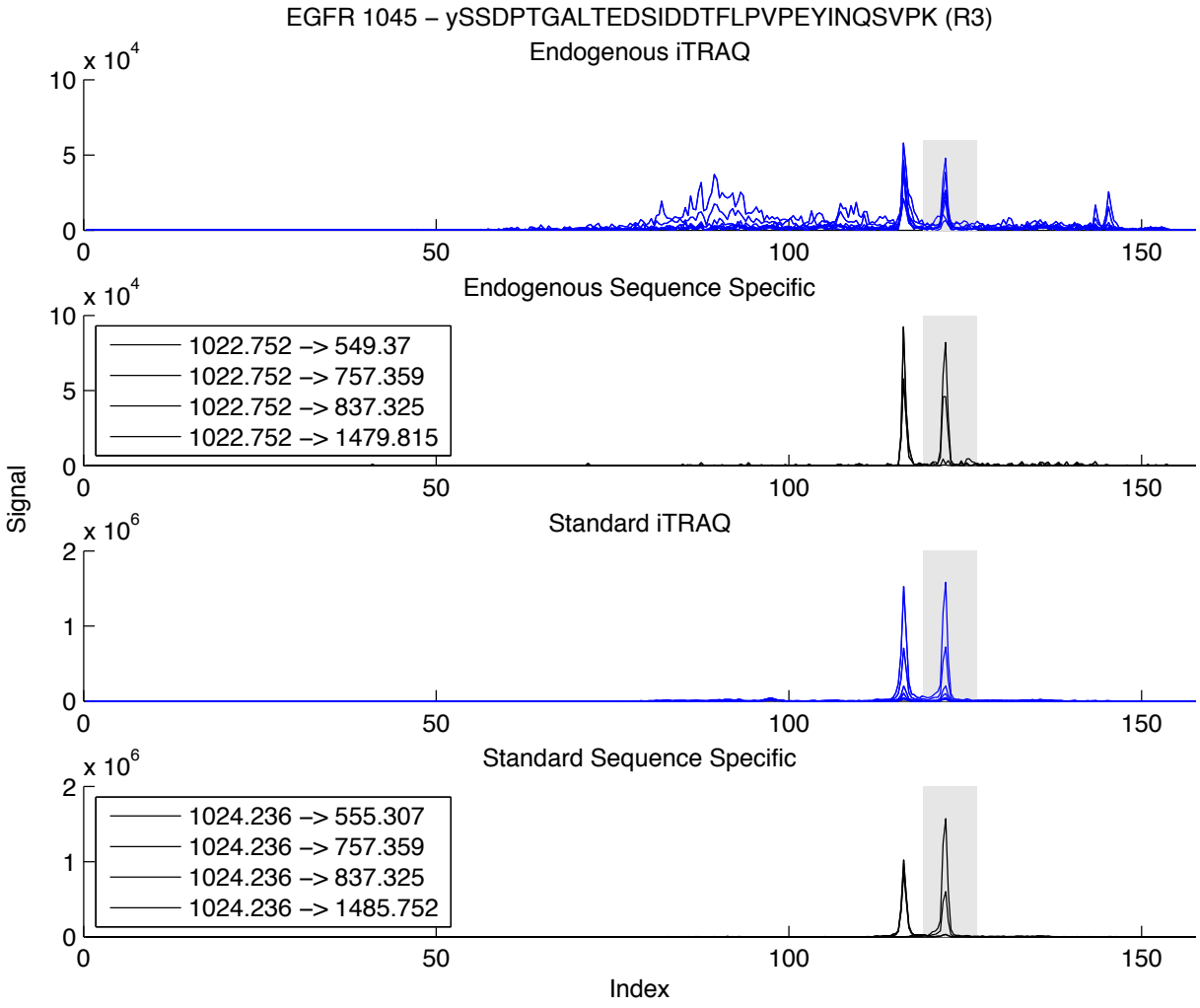
Supplementary Figure 1: Peptide Specific Signal Intensity. The average integrated intensity vs. amount of all heavy-labeled standard peptides. Each point represents a single measurement of the indicated peptide at the indicated dose. Inside the linear range, the points will overlay each other, because the slope is unchanged. Below the linear range, additive noise increases the observed intensity for a given dose raising the corresponding point above the grouping established by the linear range. Above the linear range, saturated signal intensity causes the corresponding point to drop below the grouping established by the linear range. The large range is due to sequence specific effects compiled throughout sample processing, liquid chromatography, and ionization.

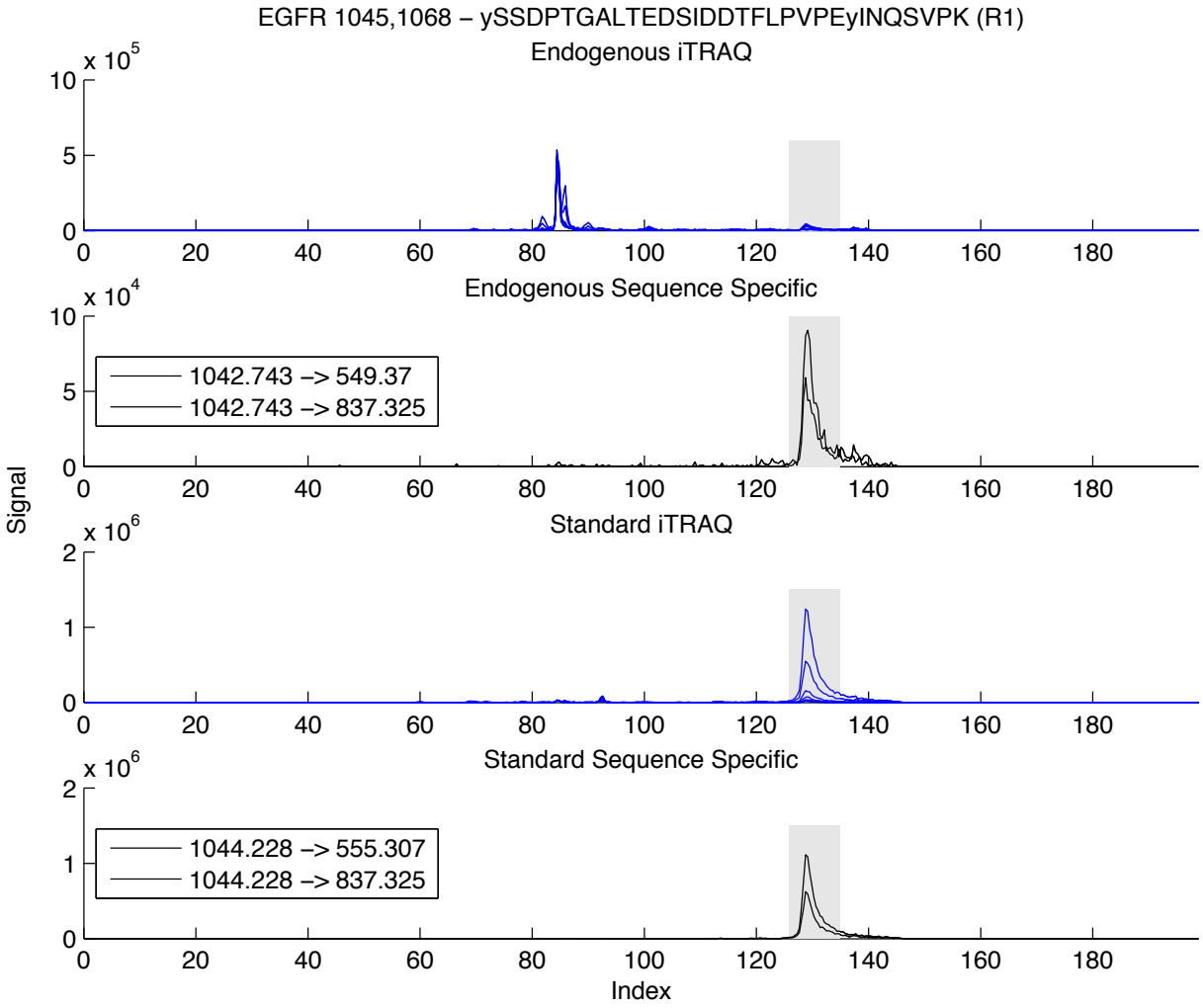


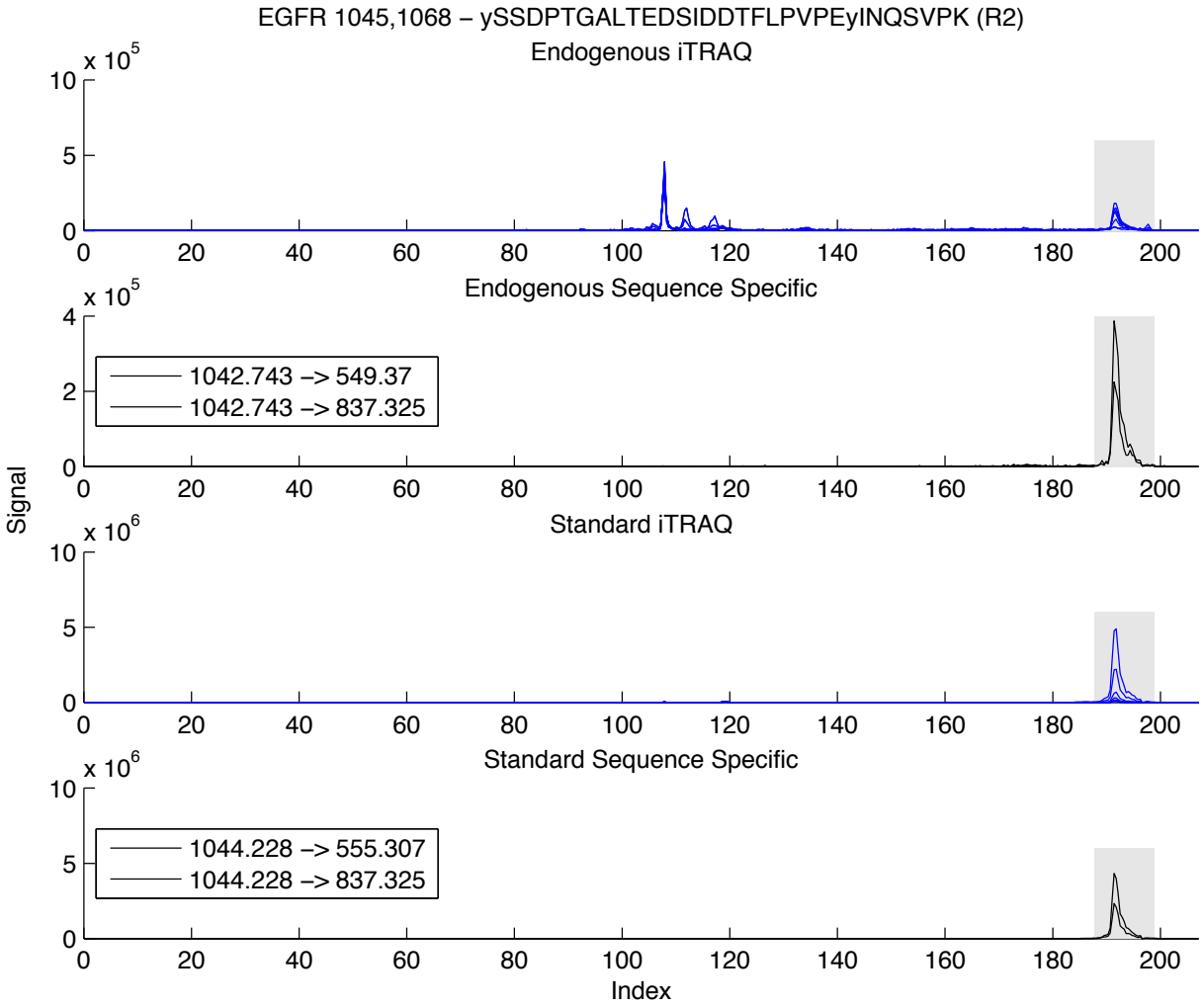
Supplementary Figure 2: Details of MRM Data Collection Method. a) The triple quadrupole is used to isolate and fragment peptides of interest. In the first quadrupole (Q1), the precursor mass is isolated and allowed to pass through into the second quadrupole (Q2) where it is fragmented with a prescribed collision energy. These fragments are passed into the third quadrupole (Q3), a desired fragment mass is isolated, and the intensity of this "transition" is recorded by the detector. b) Example iTRAQ transitions for the endogenous (red) and standard (blue) Shc pY317 peptide. c) The intensity vs. time of each Shc pY317 iTRAQ transition.

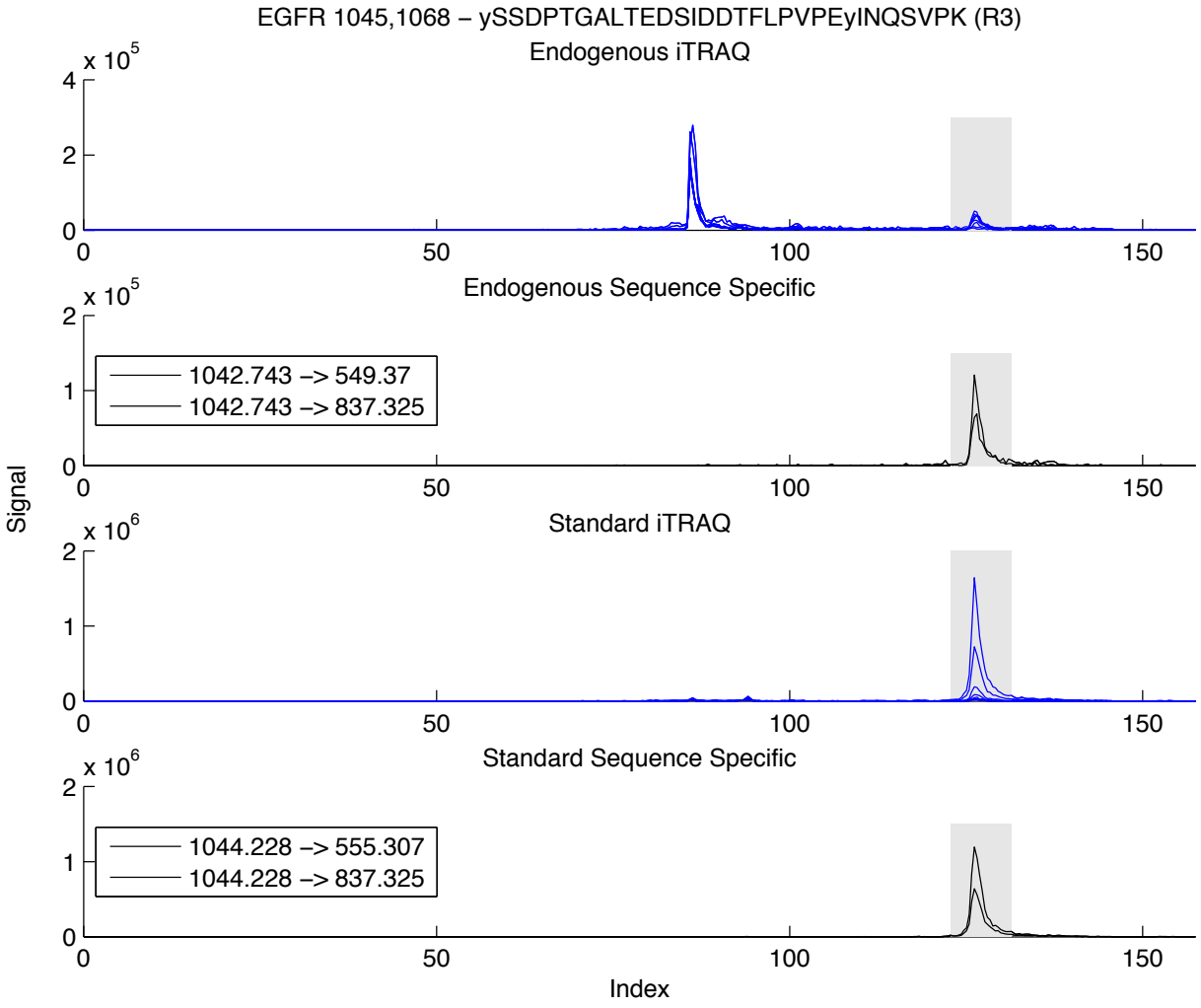

EGFR 1045 – ySSDPTGALTEDSIDDTFLPVPEYINQSVPK (R1)
Endogenous iTRAQ
x 10^4
Endogenous Sequence Specific
x 10^5
1022.752 –> 549.37
1022.752 –> 757.359
1022.752 –> 837.325
1022.752 –> 1479.815
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^6
1024.236 –> 555.307
1024.236 –> 757.359
1024.236 –> 837.325
1024.236 –> 1485.752
Signal
Index

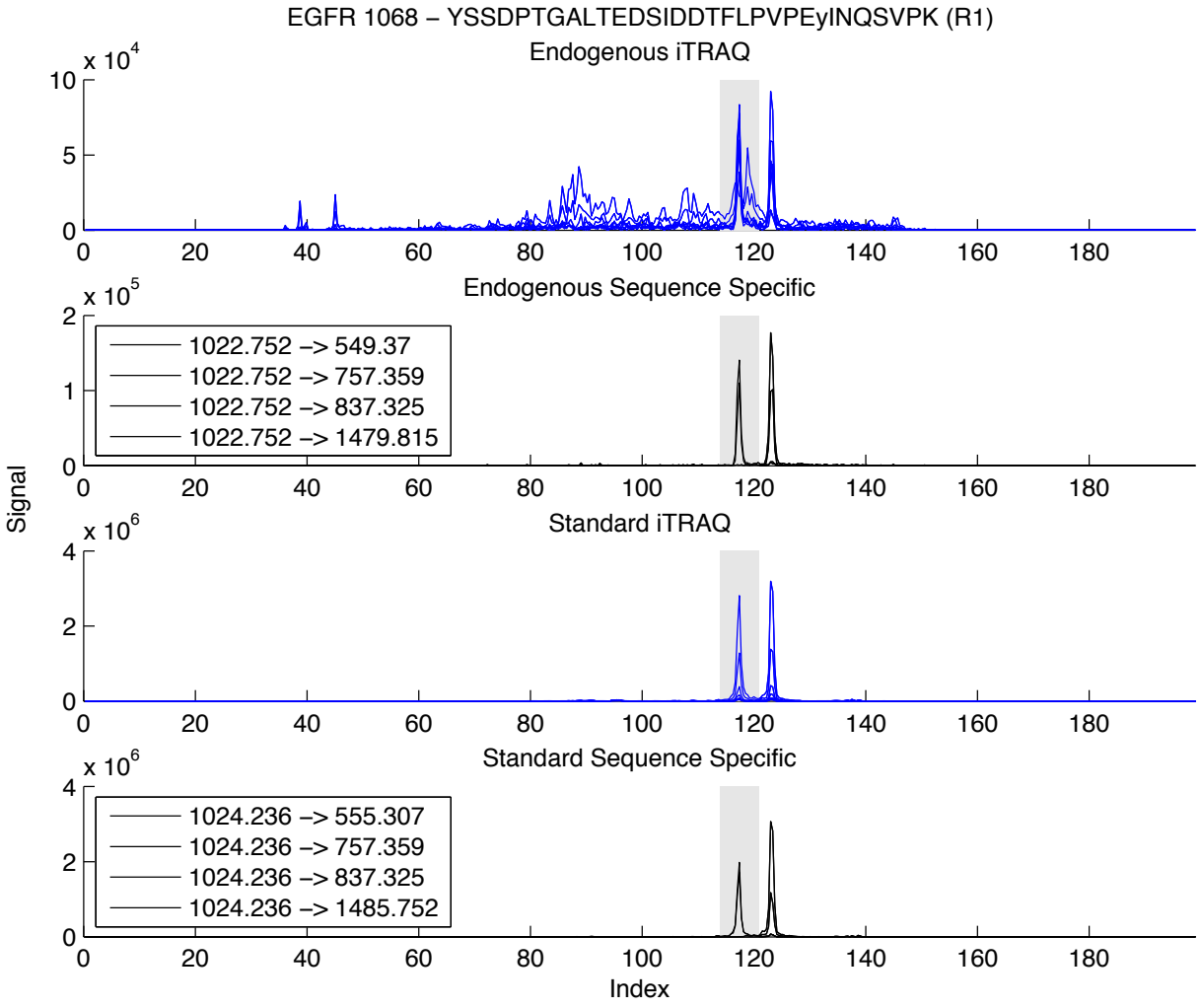

EGFR 1045 – ySSDPTGALTEDSIDDTFLPVPEYINQSVPK (R2)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
1022.752 –> 549.37
1022.752 –> 757.359
1022.752 –> 837.325
1022.752 –> 1479.815
Standard iTRAQ
x 10^6
Standard Sequence Specific
1024.236 –> 555.307
1024.236 –> 757.359
1024.236 –> 837.325
1024.236 –> 1485.752
Signal
Index

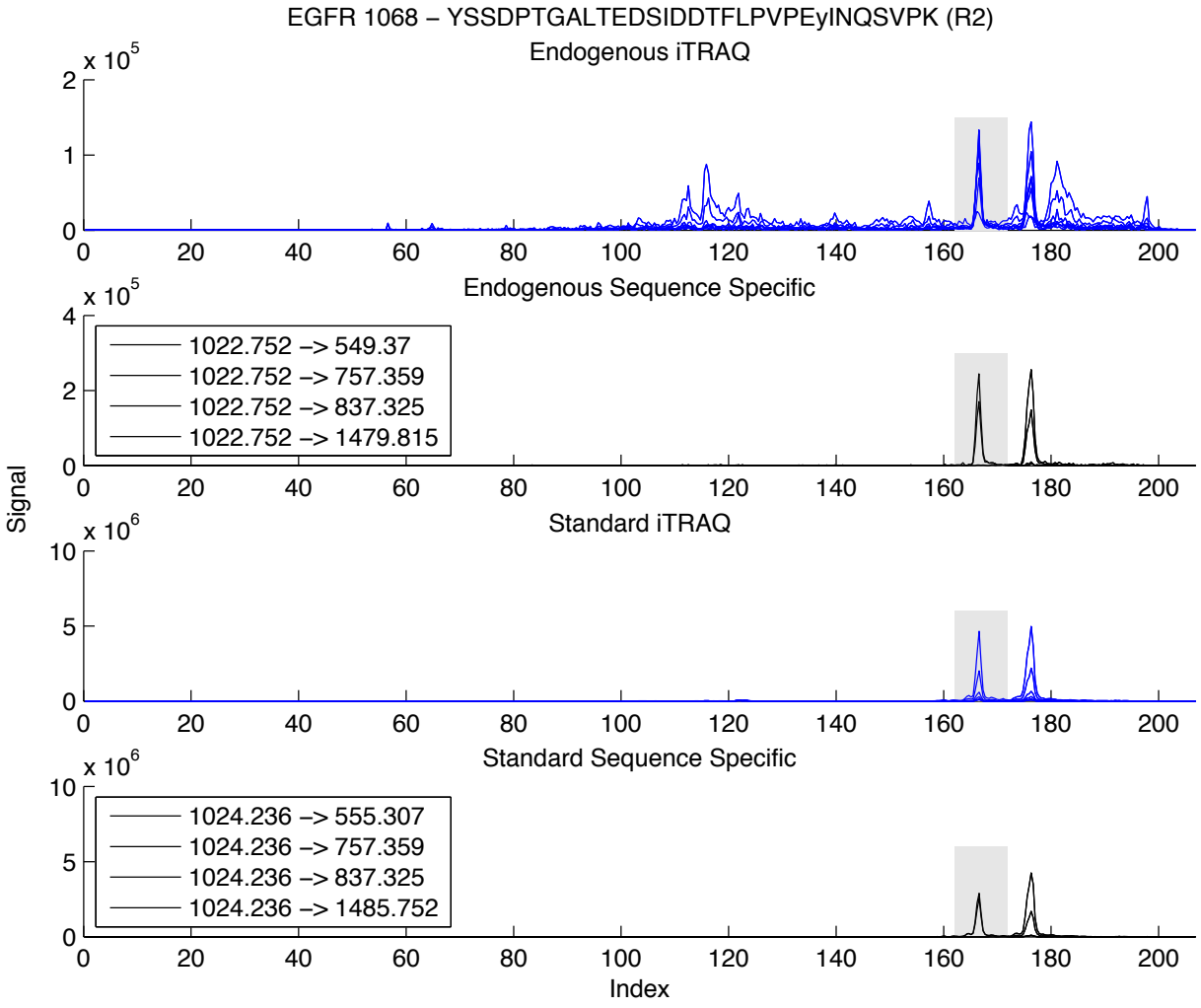

EGFR 1045 – ySSDPTGALTEDSIDDTFLPVPEYINQSVPK (R3)
Endogenous iTRAQ
x 10^4
Endogenous Sequence Specific
1022.752 –> 549.37
1022.752 –> 757.359
1022.752 –> 837.325
1022.752 –> 1479.815
Standard iTRAQ
x 10^6
Standard Sequence Specific
1024.236 –> 555.307
1024.236 –> 757.359
1024.236 –> 837.325
1024.236 –> 1485.752
Signal
Index

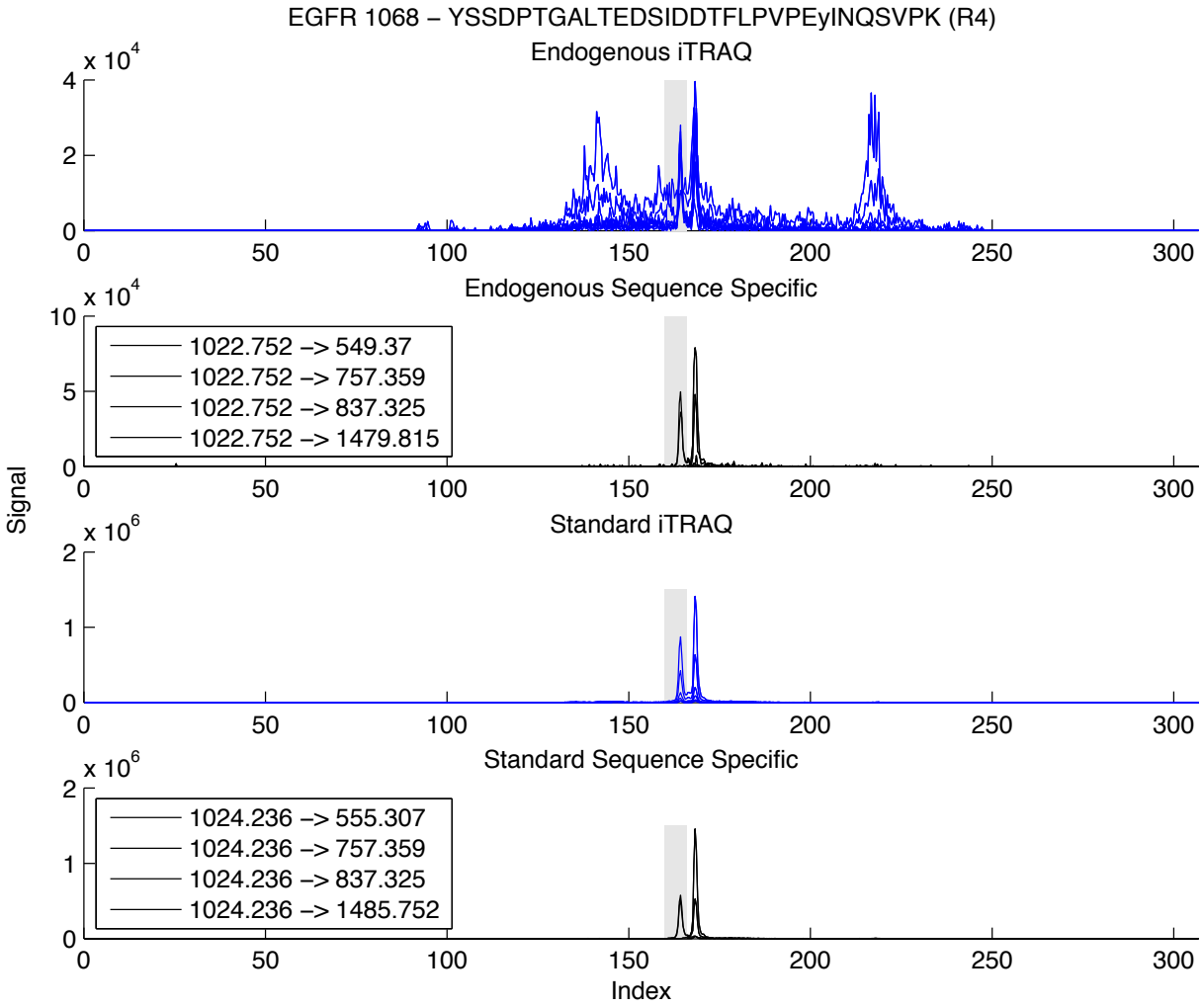

EGFR 1045,1068 – ySSDPTGALTEDSIDDTFLPVPEyINQSVPK (R1)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
x 10^4
1042.743 –> 549.37
1042.743 –> 837.325
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^6
1044.228 –> 555.307
1044.228 –> 837.325
Signal
Index

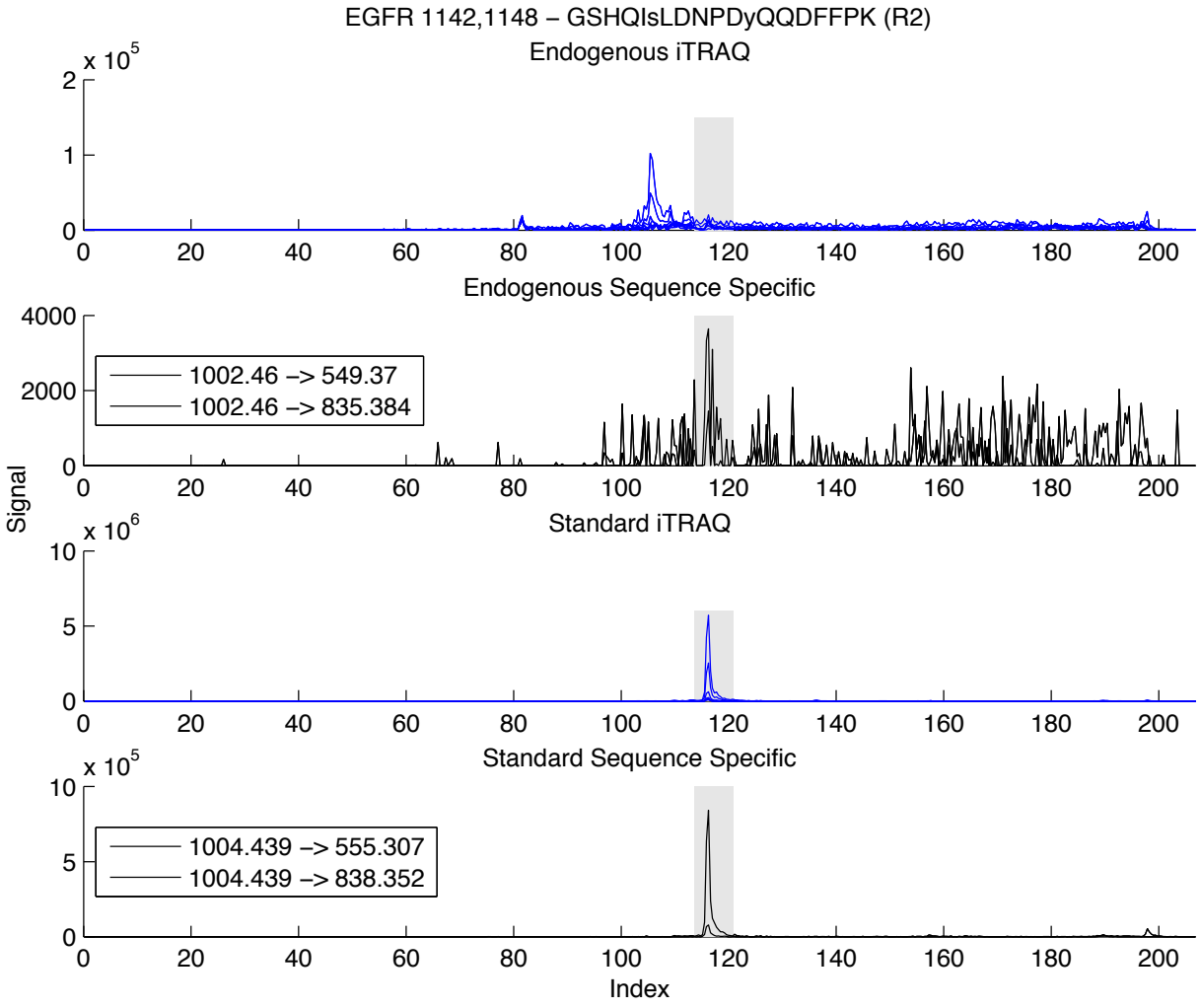

EGFR 1045,1068 – ySSDPTGALTEDSIDDTFLPVPEyINQSVPK (R2)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
1042.743 –> 549.37
1042.743 –> 837.325
Standard iTRAQ
x 10^6
Standard Sequence Specific
1044.228 –> 555.307
1044.228 –> 837.325
Signal
Index

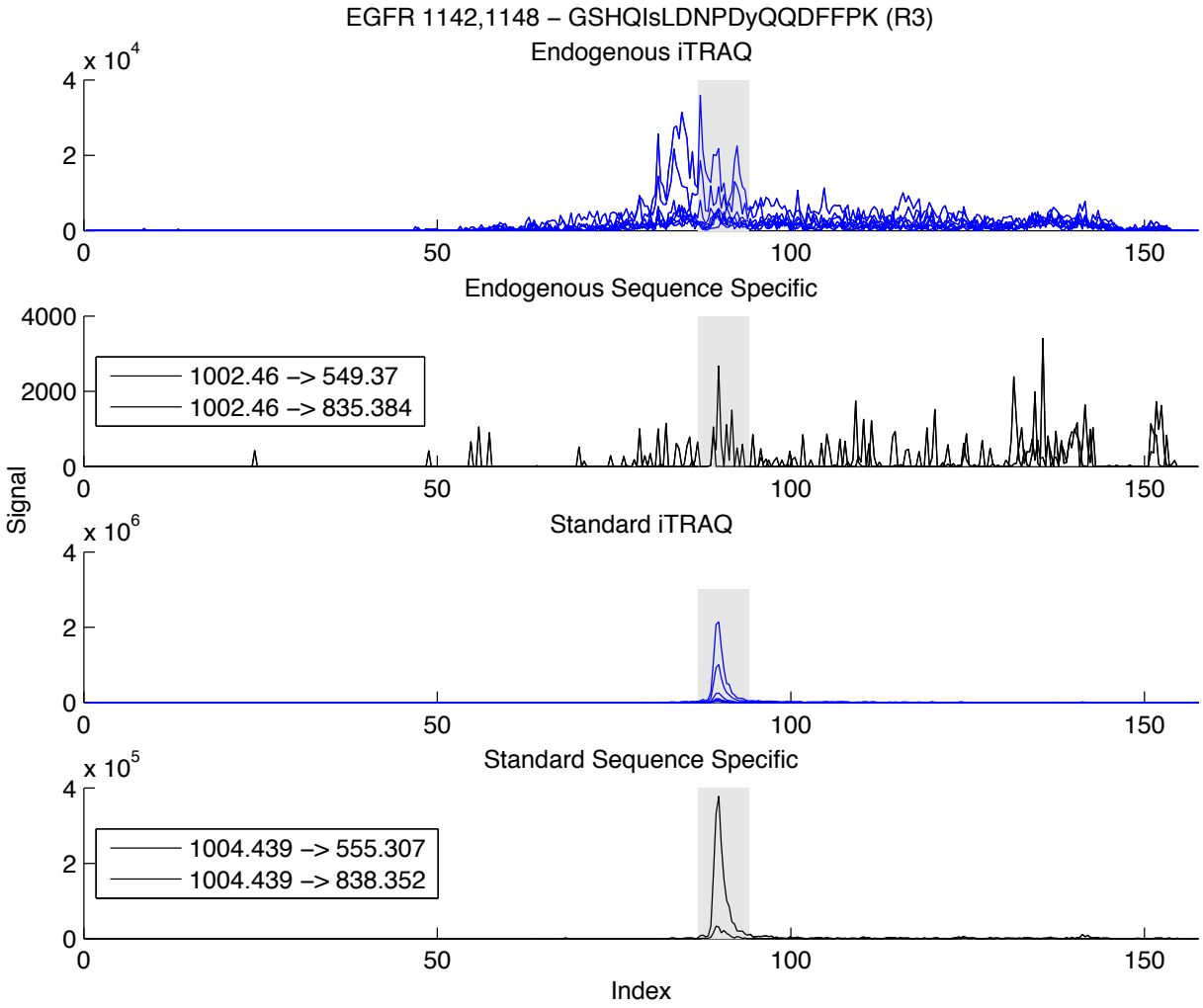

EGFR 1045,1068 – ySSDPTGALTEDSIDDTFLPVPEyINQSVPK (R3)
Endogenous iTRAQ
x 10^5
4
2
0
0
50
100
150
Endogenous Sequence Specific
x 10^5
2
1
0
1042.743 –> 549.37
1042.743 –> 837.325
Signal
Standard iTRAQ
x 10^6
2
1
0
Standard Sequence Specific
x 10^6
1044.228 –> 555.307
1044.228 –> 837.325
Index

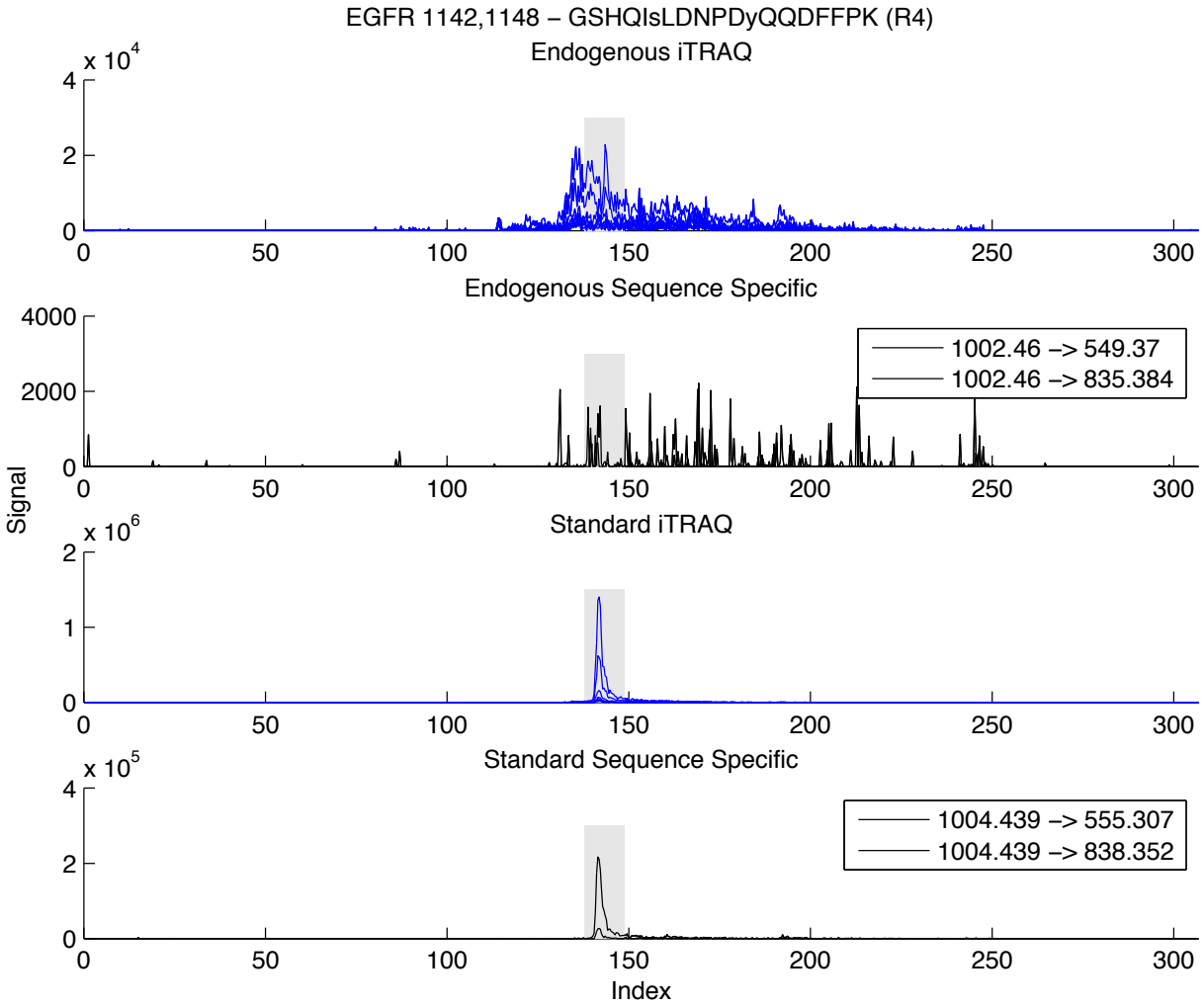

EGFR 1068 – YSSDPTGALTEDSIDDTFLPVPEyINQSVPK (R1)
Endogenous iTRAQ
x 10^4
Endogenous Sequence Specific
x 10^5
1022.752 –> 549.37
1022.752 –> 757.359
1022.752 –> 837.325
1022.752 –> 1479.815
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^6
1024.236 –> 555.307
1024.236 –> 757.359
1024.236 –> 837.325
1024.236 –> 1485.752
Signal
Index

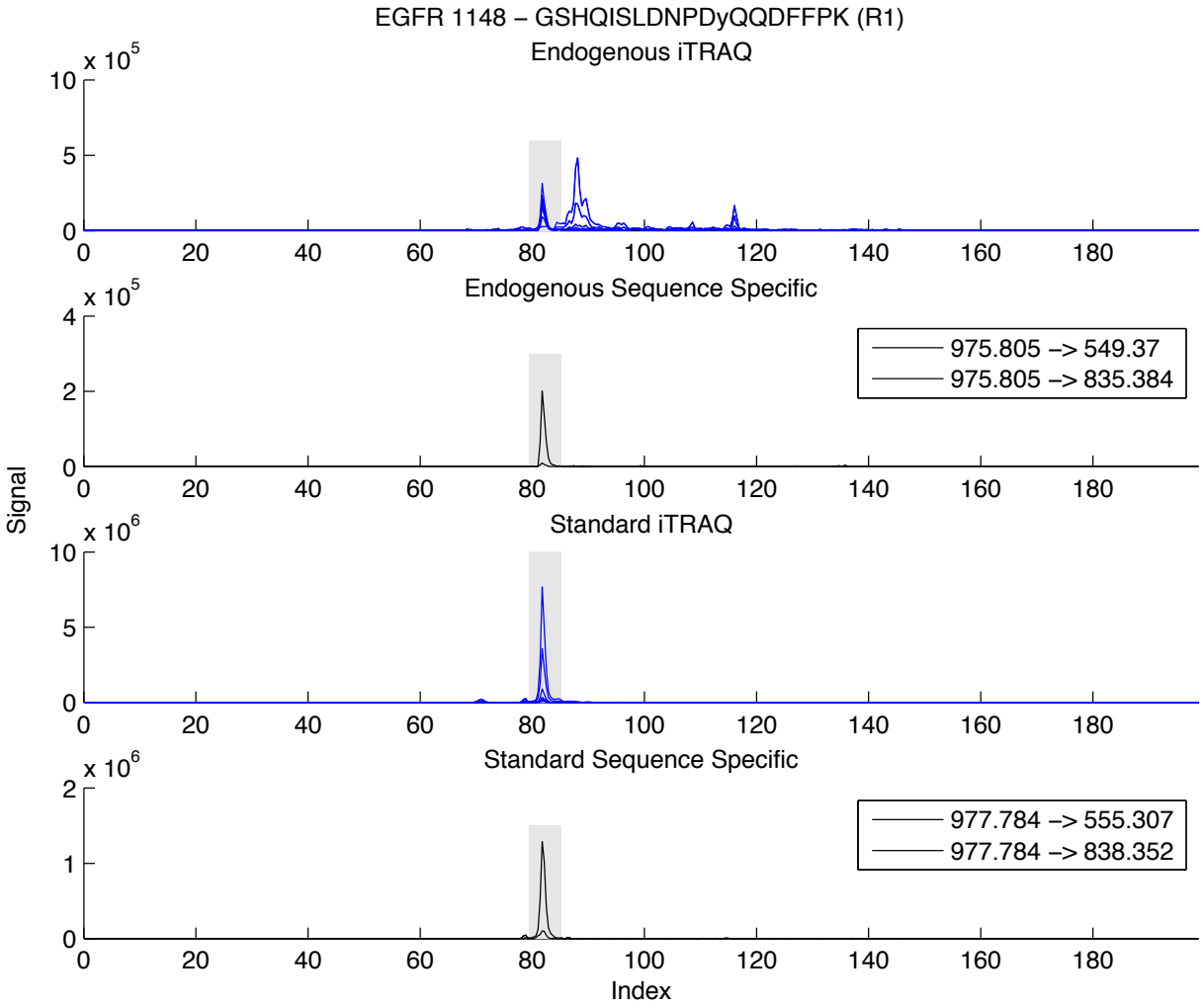

EGFR 1068 – YSSDPTGALTEDSIDDTFLPVPEyINQSVPK (R2)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
1022.752 –> 549.37
1022.752 –> 757.359
1022.752 –> 837.325
1022.752 –> 1479.815
Standard iTRAQ
x 10^6
Standard Sequence Specific
1024.236 –> 555.307
1024.236 –> 757.359
1024.236 –> 837.325
1024.236 –> 1485.752
Signal
Index

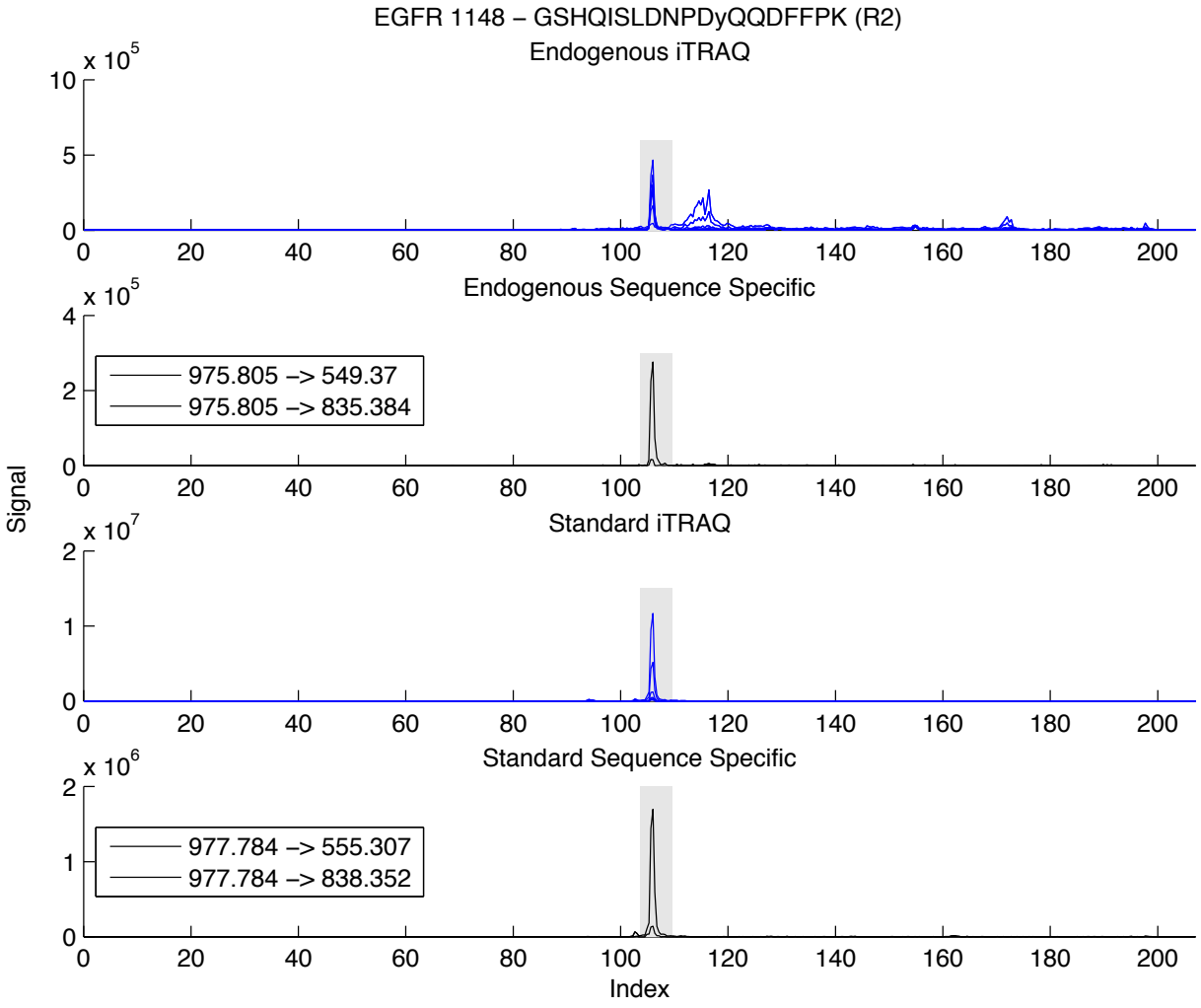

EGFR 1068 – YSSDPTGALTEDSIDDTFLPVPEyINQSVPK (R4)
Endogenous iTRAQ
x 10^4
Endogenous Sequence Specific
1022.752 –> 549.37
1022.752 –> 757.359
1022.752 –> 837.325
1022.752 –> 1479.815
Standard iTRAQ
x 10^6
Standard Sequence Specific
1024.236 –> 555.307
1024.236 –> 757.359
1024.236 –> 837.325
1024.236 –> 1485.752
Signal
Index

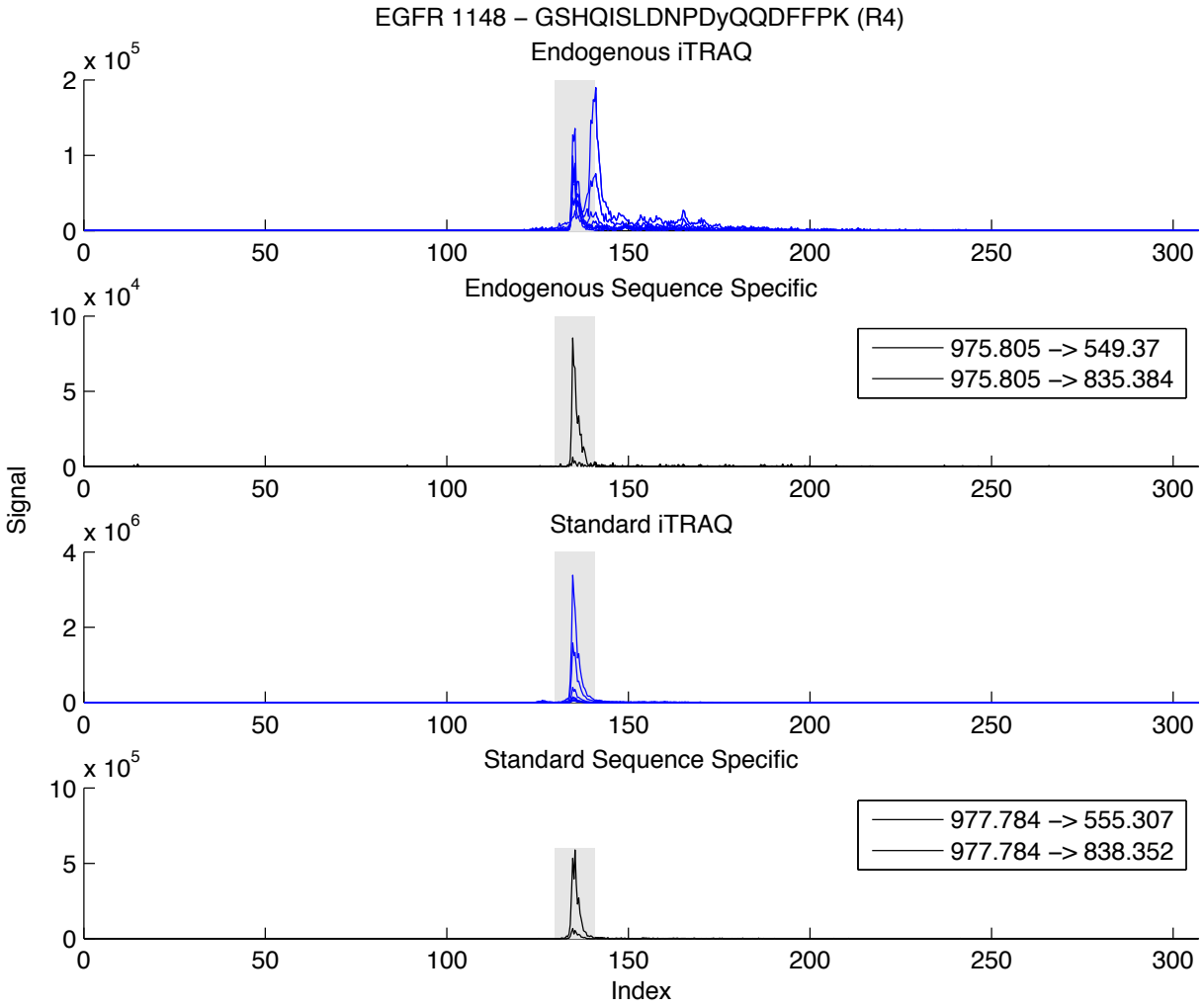

EGFR 1142,1148 – GSHQIsLDNPDyQQDFFPK (R2)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
1002.46 –> 549.37
1002.46 –> 835.384
Signal
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^5
1004.439 –> 555.307
1004.439 –> 838.352
Index

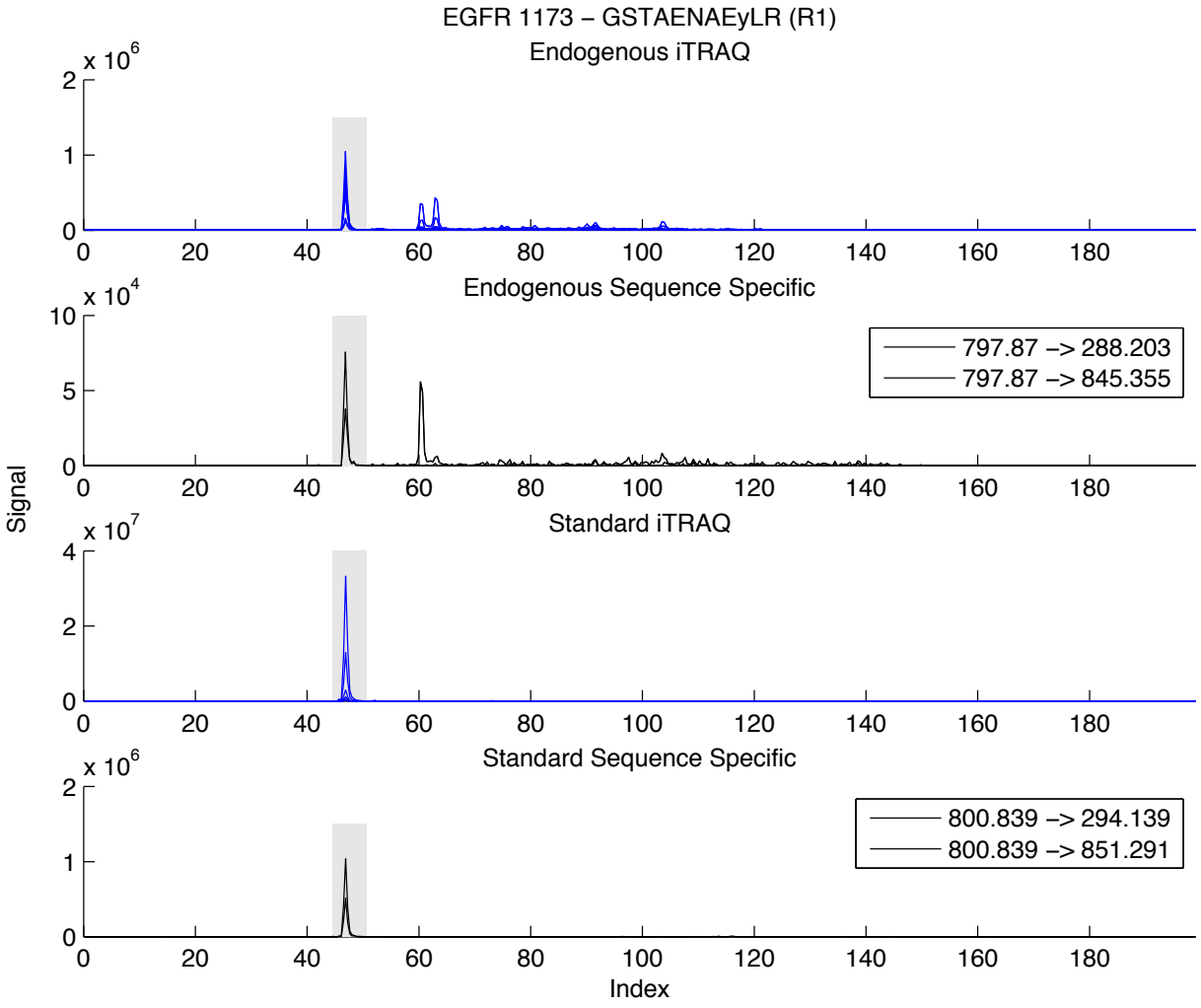

EGFR 1142,1148 – GSHQIsLDNPDyQQDFFPK (R3)
Endogenous iTRAQ
x 10^4
4
2
0
0
50
100
150
Endogenous Sequence Specific
4000
2000
0
1002.46 –> 549.37
1002.46 –> 835.384
Signal
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^5
1004.439 –> 555.307
1004.439 –> 838.352
Index

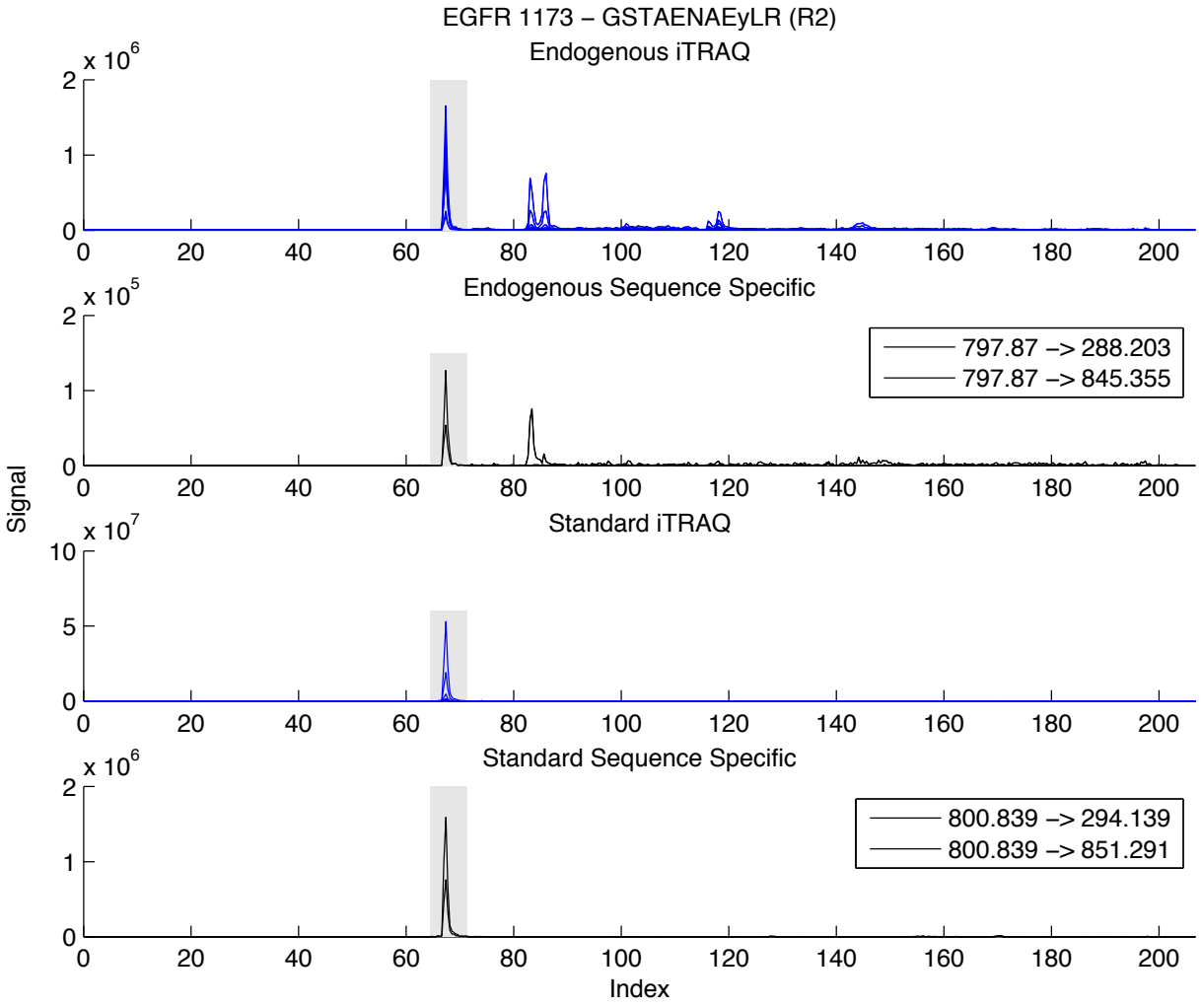

EGFR 1142,1148 – GSHQIsLDNPDyQQDFFPK (R4)
Endogenous iTRAQ
x 10^4
4
2
0
0
50
100
150
200
250
300
Endogenous Sequence Specific
4000
2000
0
1002.46 –> 549.37
1002.46 –> 835.384
Signal
Standard iTRAQ
x 10^6
2
1
0
Standard Sequence Specific
x 10^5
4
2
0
1004.439 –> 555.307
1004.439 –> 838.352
Index

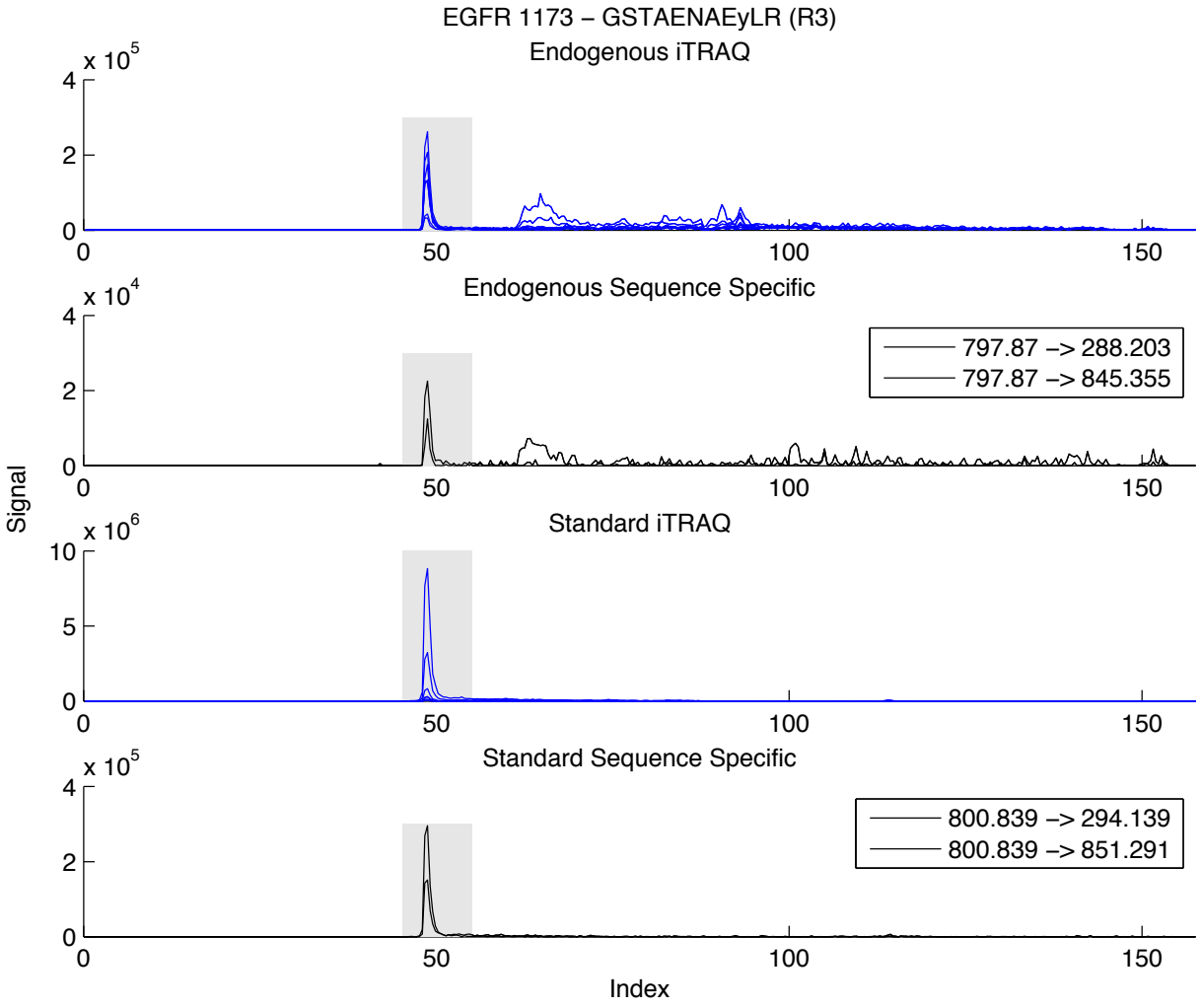

EGFR 1148 – GSHQISLDNPDyQQDFFPK (R1)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
x 10^5
975.805 –> 549.37
975.805 –> 835.384
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^6
977.784 –> 555.307
977.784 –> 838.352
Signal
Index

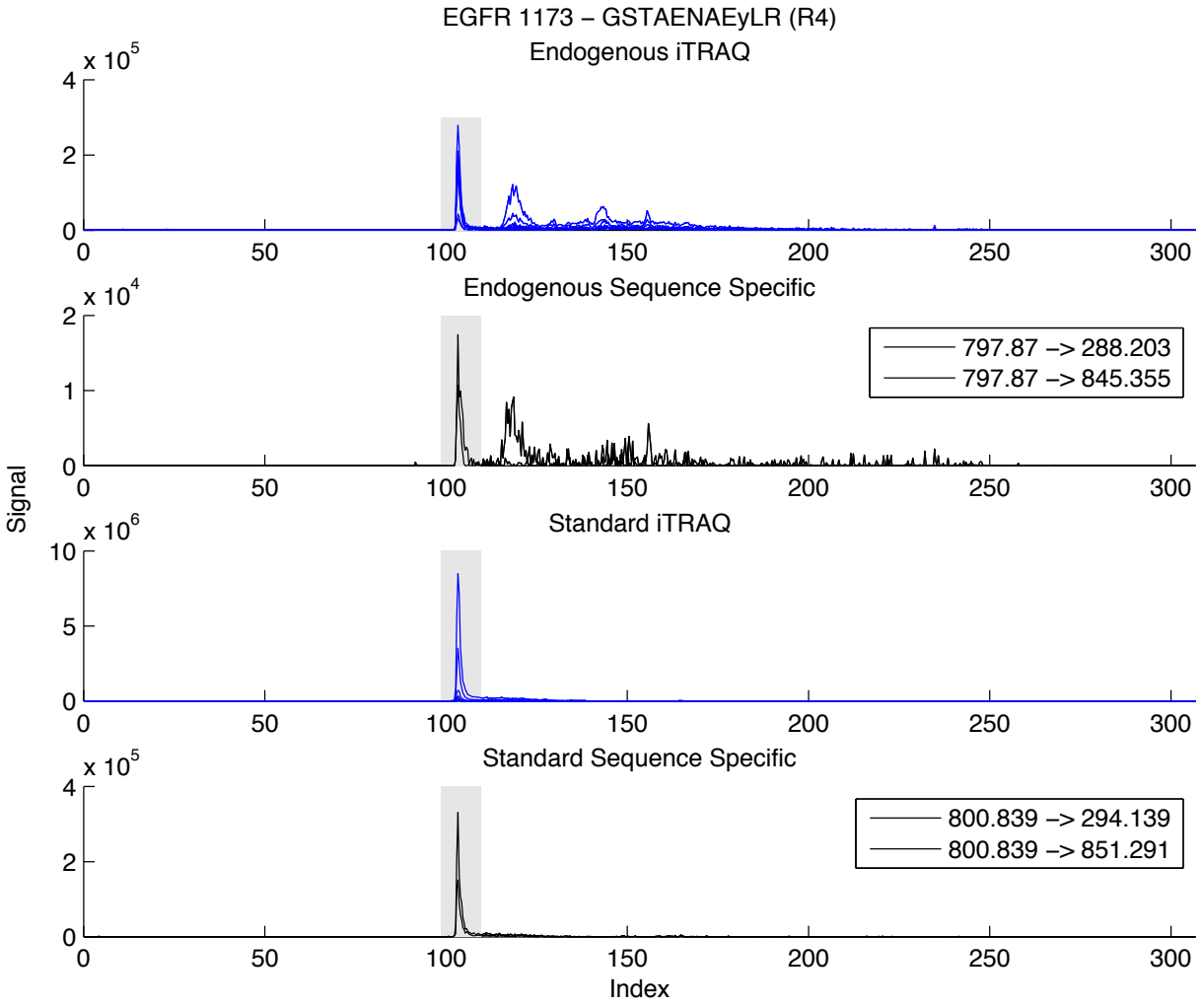

EGFR 1148 – GSHQISLDNPDyQQDFFPK (R2)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
x 10^5
975.805 –> 549.37
975.805 –> 835.384
Standard iTRAQ
x 10^7
Standard Sequence Specific
x 10^6
977.784 –> 555.307
977.784 –> 838.352
Signal
Index

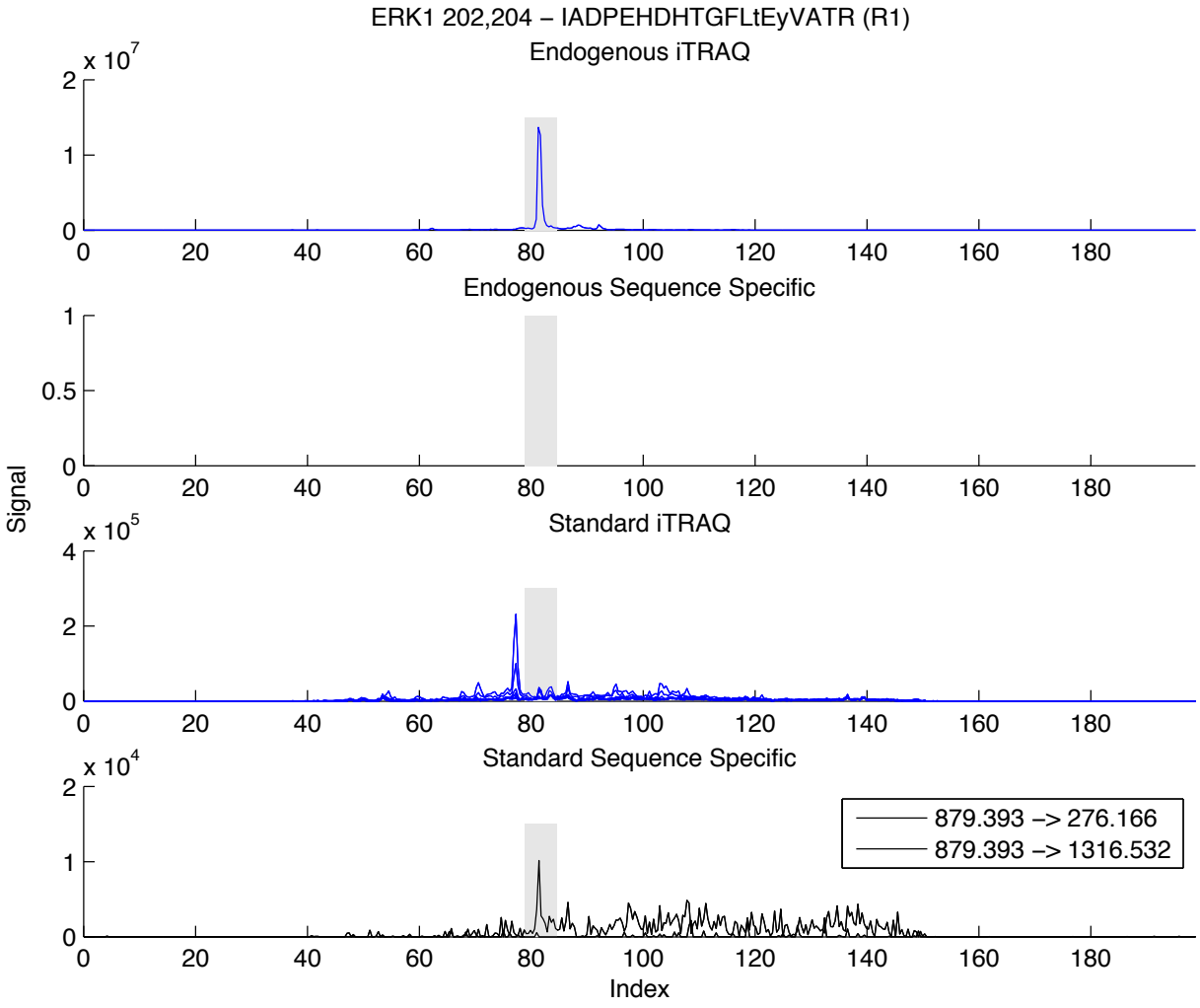

EGFR 1148 – GSHQISLDNPDyQQDFFPK (R4)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
x 10^4
975.805 –> 549.37
975.805 –> 835.384
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^5
977.784 –> 555.307
977.784 –> 838.352
Signal
Index

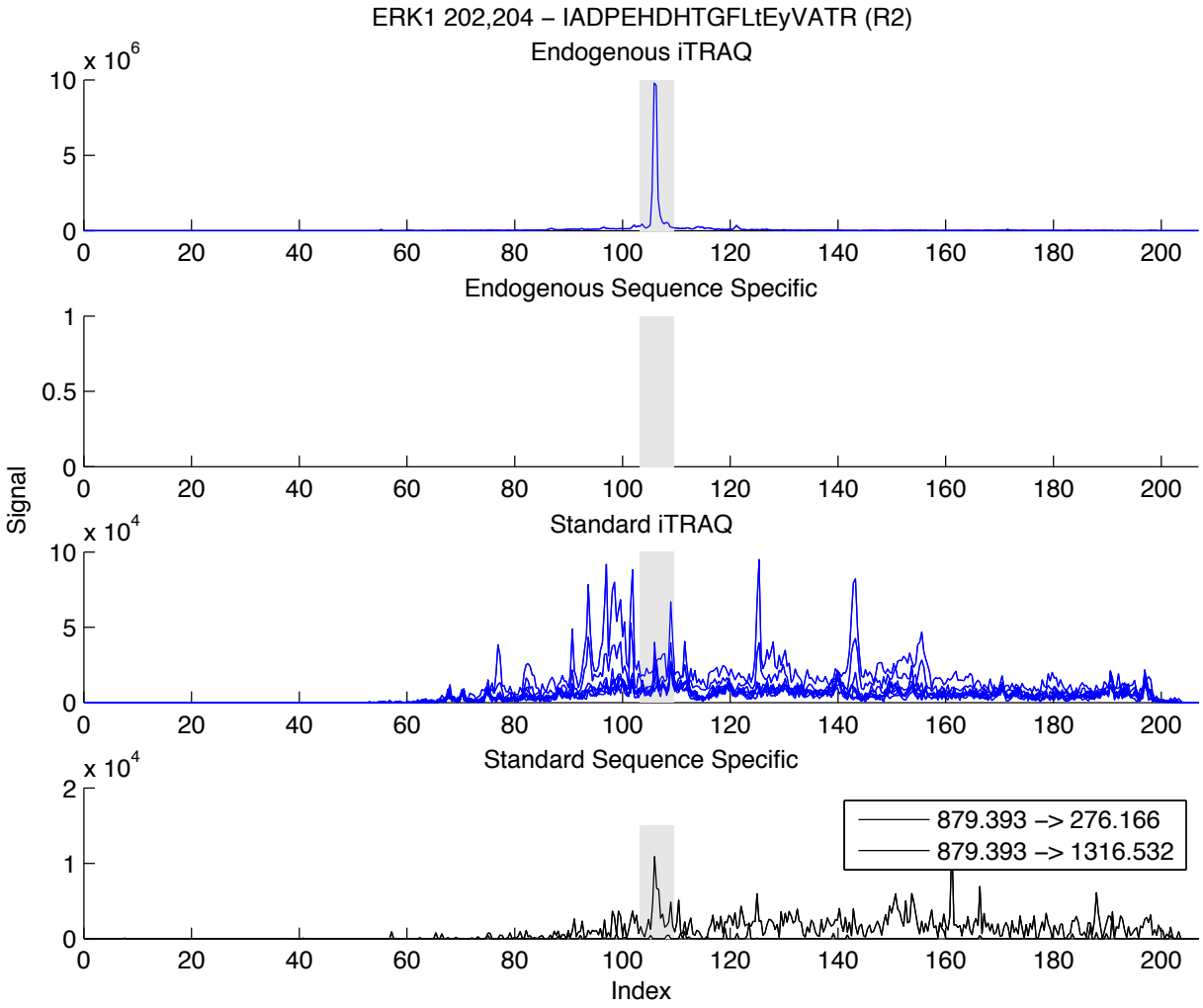

EGFR 1173 – GSTAENAEyLR (R1)
Endogenous iTRAQ
x 10^6
Endogenous Sequence Specific
x 10^4
797.87 –> 288.203
797.87 –> 845.355
Standard iTRAQ
x 10^7
Standard Sequence Specific
x 10^6
800.839 –> 294.139
800.839 –> 851.291
Signal
Index

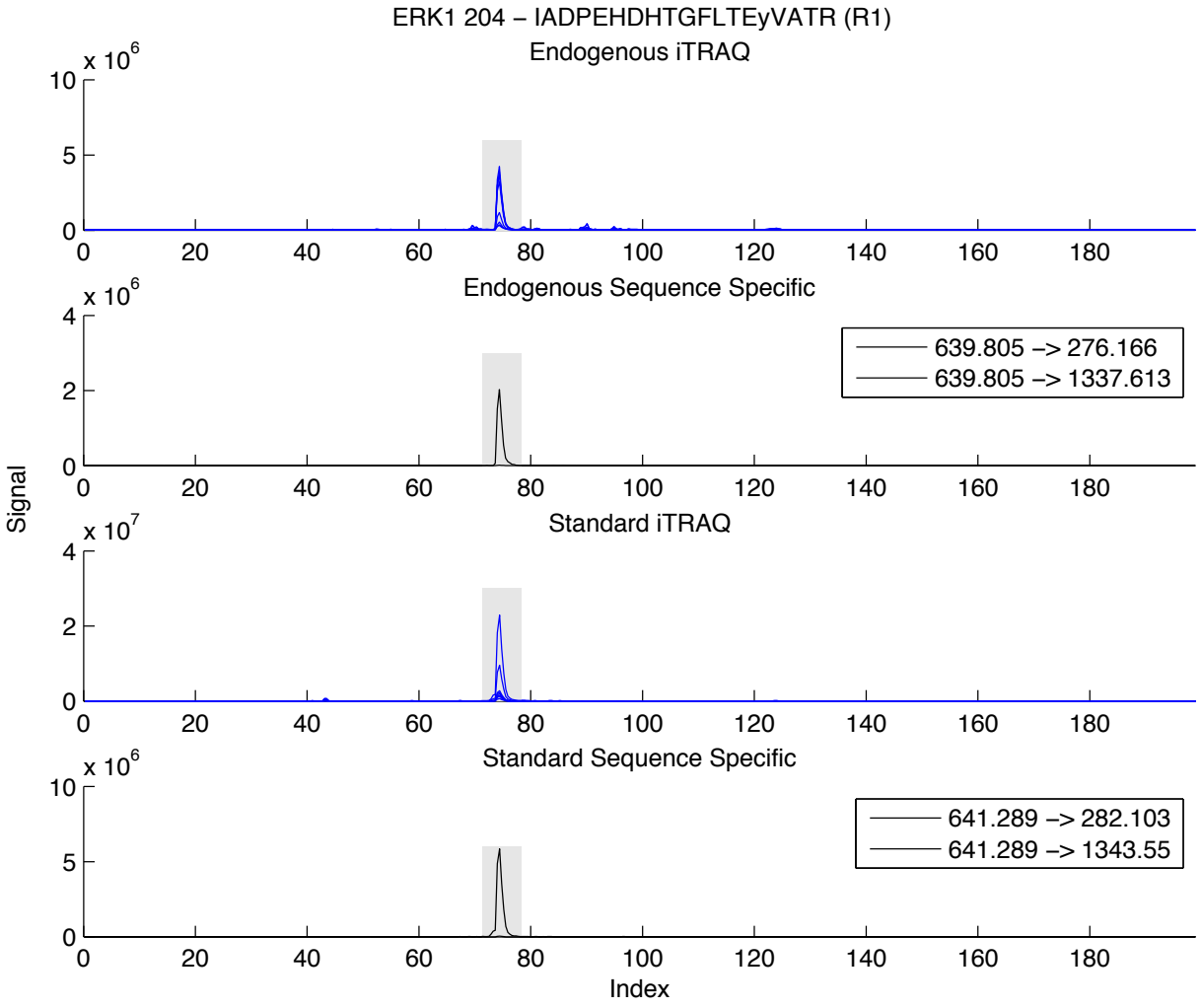

EGFR 1173 – GSTAENAEyLR (R2)
Endogenous iTRAQ
x 10^6
Endogenous Sequence Specific
x 10^5
797.87 –> 288.203
797.87 –> 845.355
Standard iTRAQ
x 10^7
Standard Sequence Specific
x 10^6
800.839 –> 294.139
800.839 –> 851.291
Signal
Index

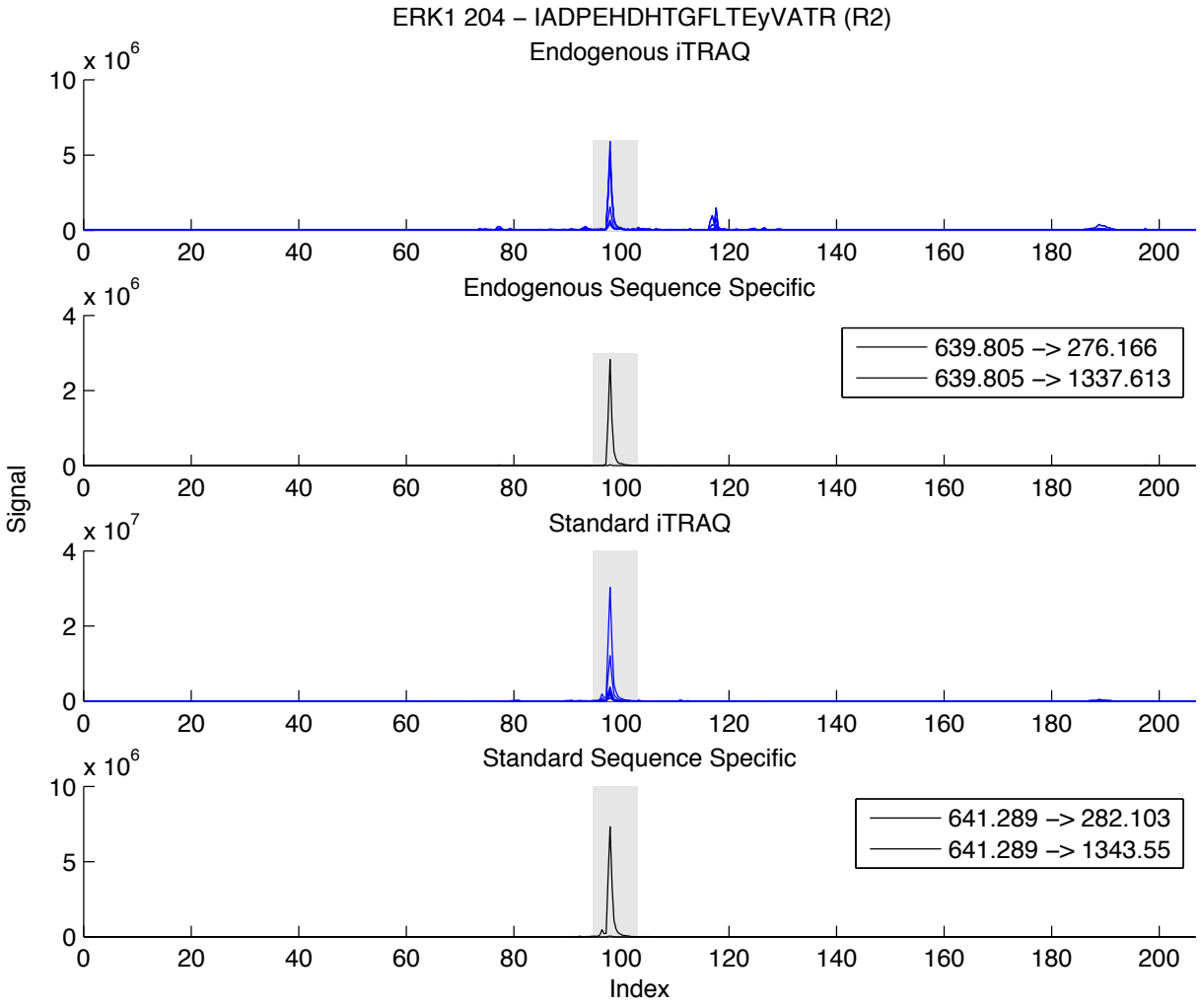

EGFR 1173 – GSTAENAEyLR (R3)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
x 10^4
797.87 –> 288.203
797.87 –> 845.355
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^5
800.839 –> 294.139
800.839 –> 851.291
Signal
Index

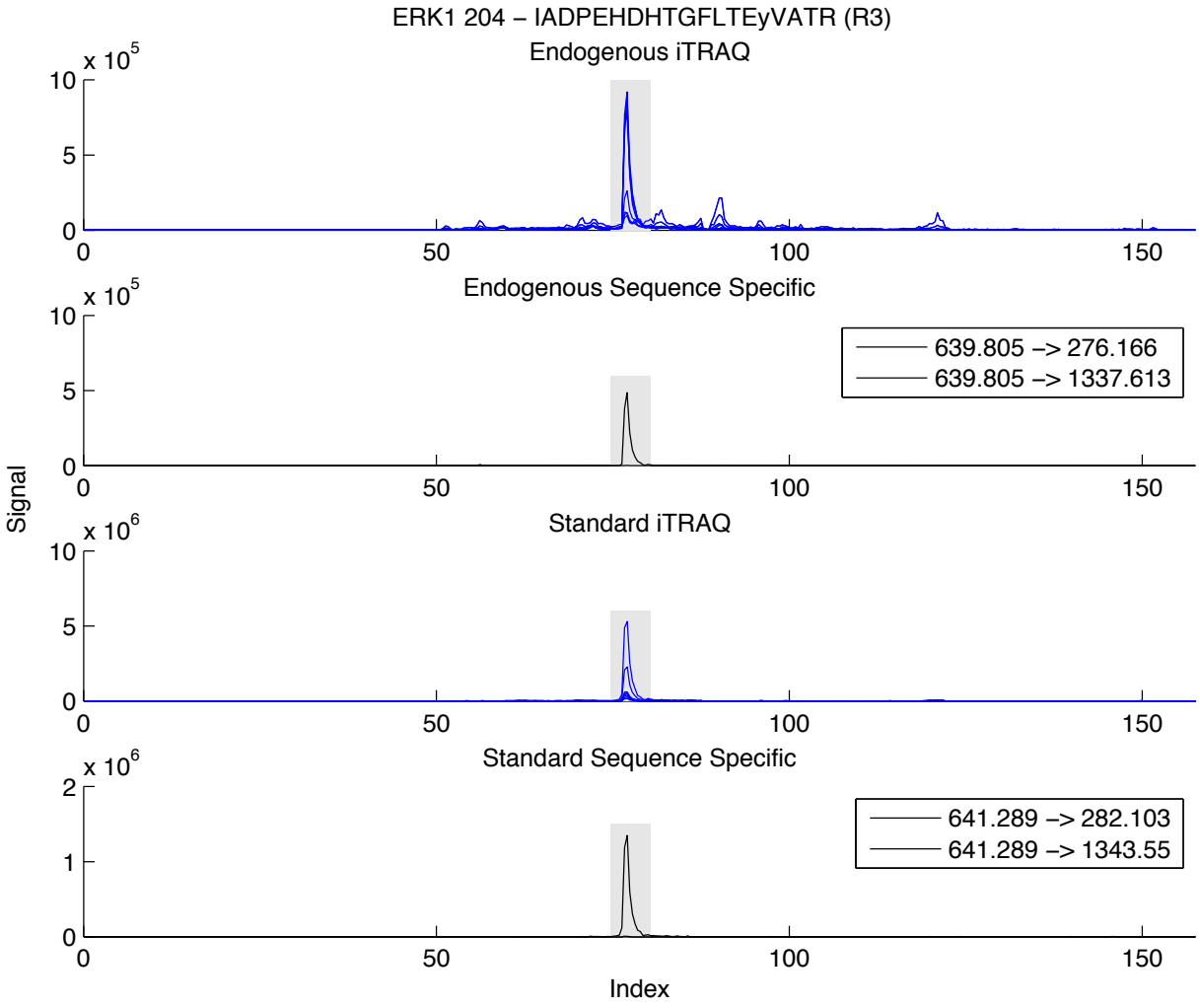

EGFR 1173 – GSTAENAEyLR (R4)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
x 10^4
797.87 –> 288.203
797.87 –> 845.355
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^5
800.839 –> 294.139
800.839 –> 851.291
Signal
Index

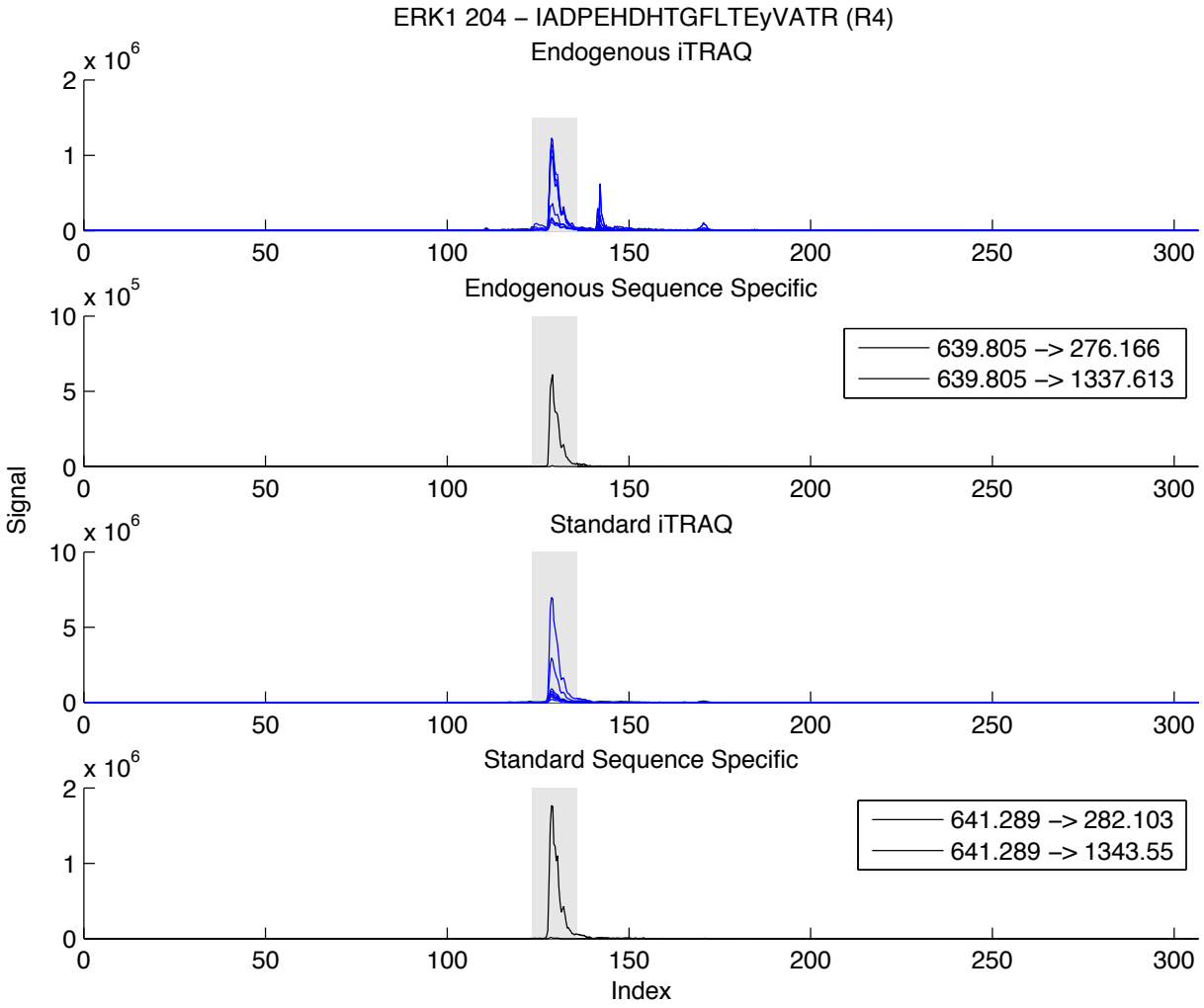

ERK1 202,204 – IADPEHDHTGFLtEyVATR (R1)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
Signal
Standard iTRAQ
x 10^5
Standard Sequence Specific
x 10^4
879.393 –> 276.166
879.393 –> 1316.532
Index

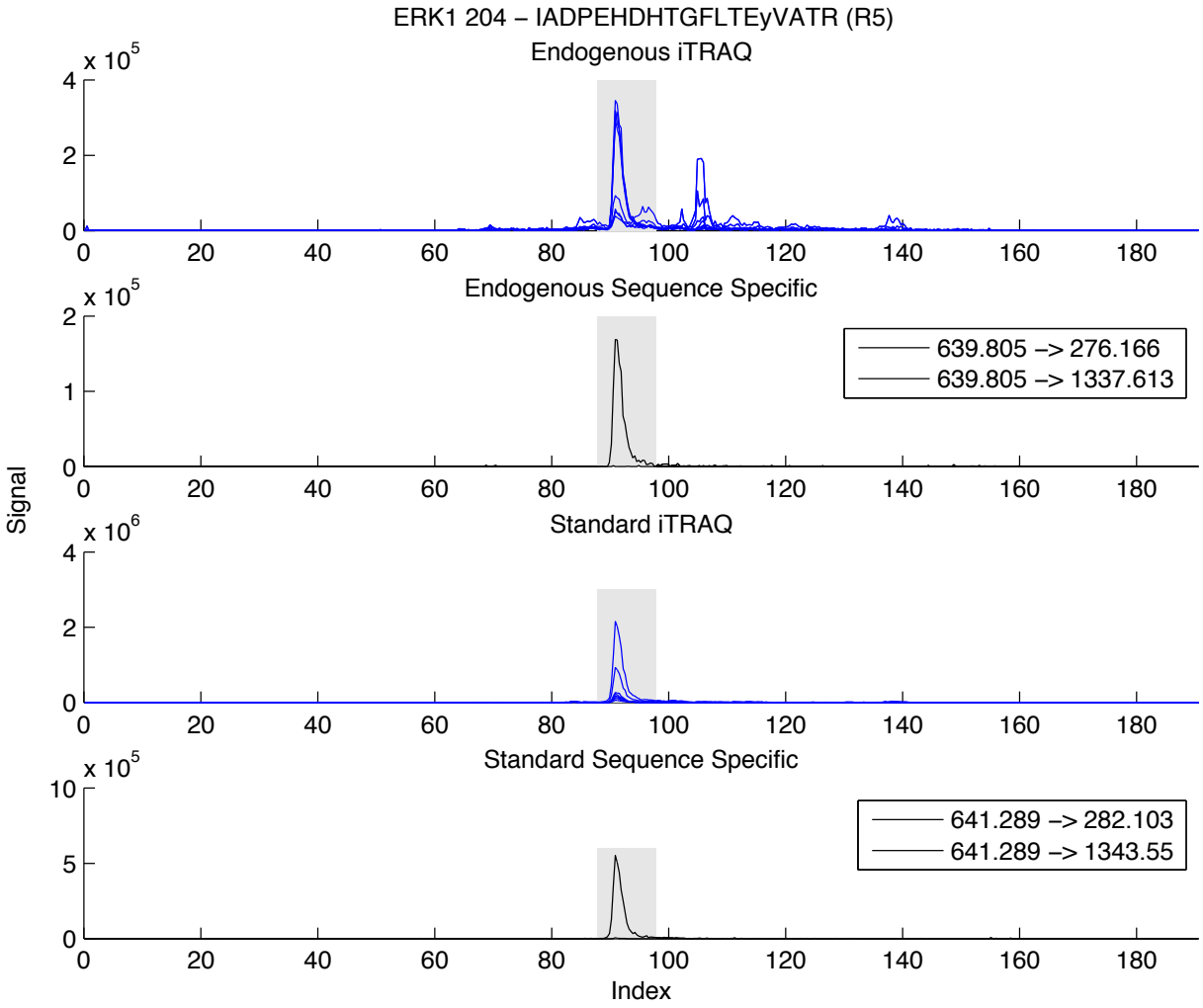

ERK1 202,204 – IADPEHDHTGFLtEyVATR (R2)
Endogenous iTRAQ
x 10^6
Endogenous Sequence Specific
Signal
Standard iTRAQ
x 10^4
Standard Sequence Specific
x 10^4
879.393 –> 276.166
879.393 –> 1316.532
Index

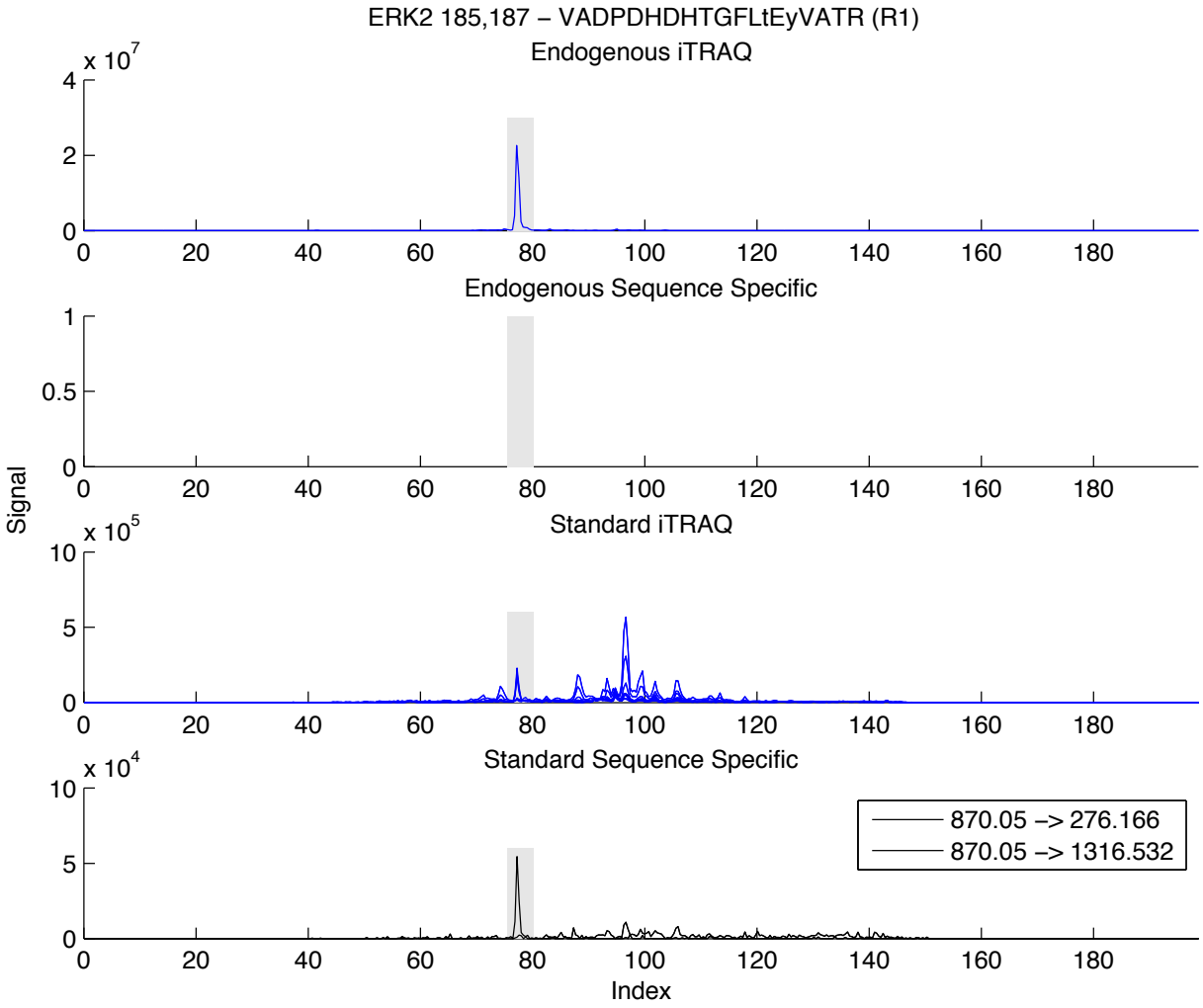

ERK1 204 – IADPEHDHTGFLTEyVATR (R1)
Endogenous iTRAQ
x 10^6
Endogenous Sequence Specific
639.805 –> 276.166
639.805 –> 1337.613
Standard iTRAQ
x 10^7
Standard Sequence Specific
641.289 –> 282.103
641.289 –> 1343.55
Signal
Index

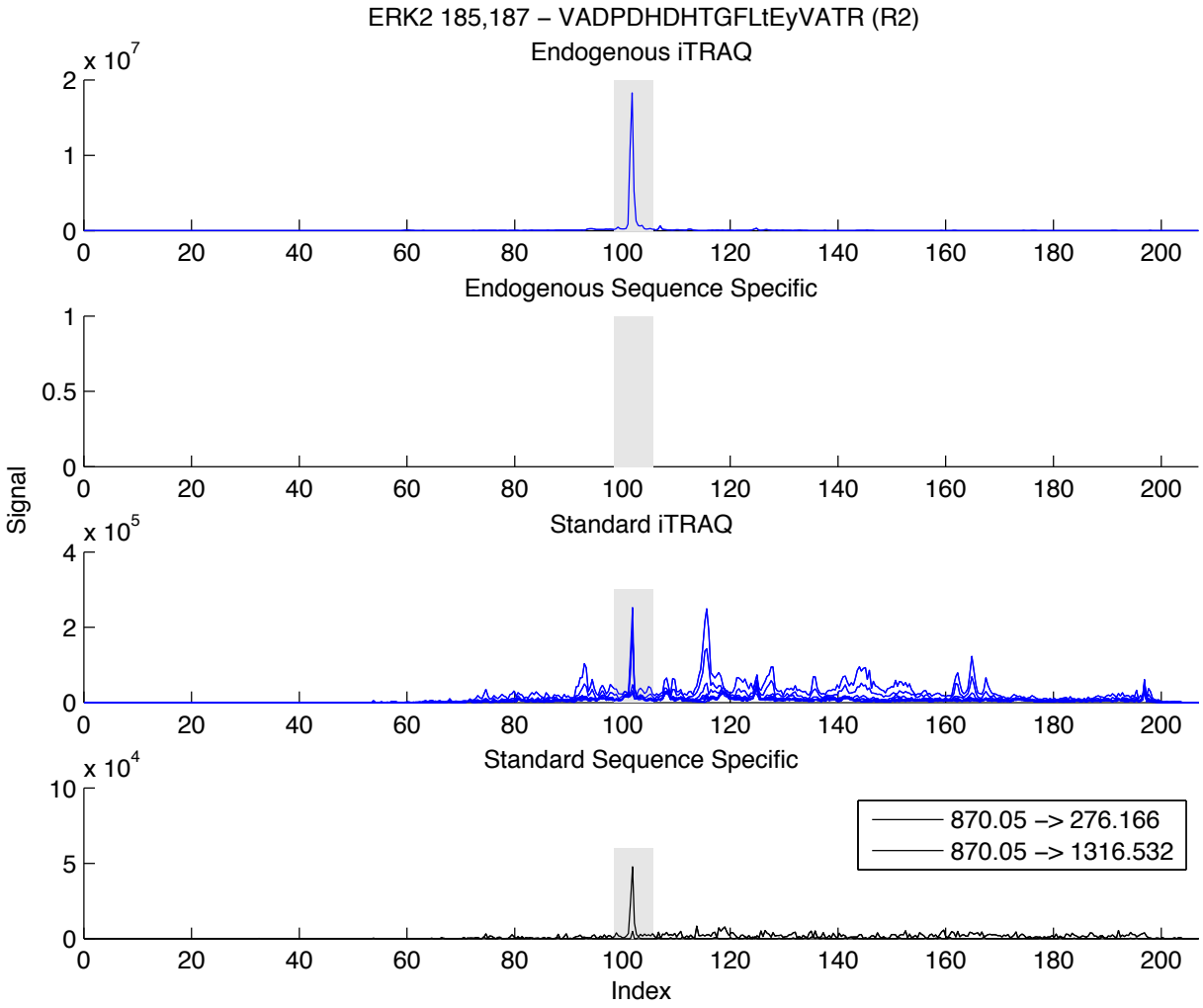

ERK1 204 – IADPEHDHTGFLTEyVATR (R2)
Endogenous iTRAQ
x 10^6
Endogenous Sequence Specific
639.805 –> 276.166
639.805 –> 1337.613
Standard iTRAQ
x 10^7
Standard Sequence Specific
641.289 –> 282.103
641.289 –> 1343.55
Signal
Index

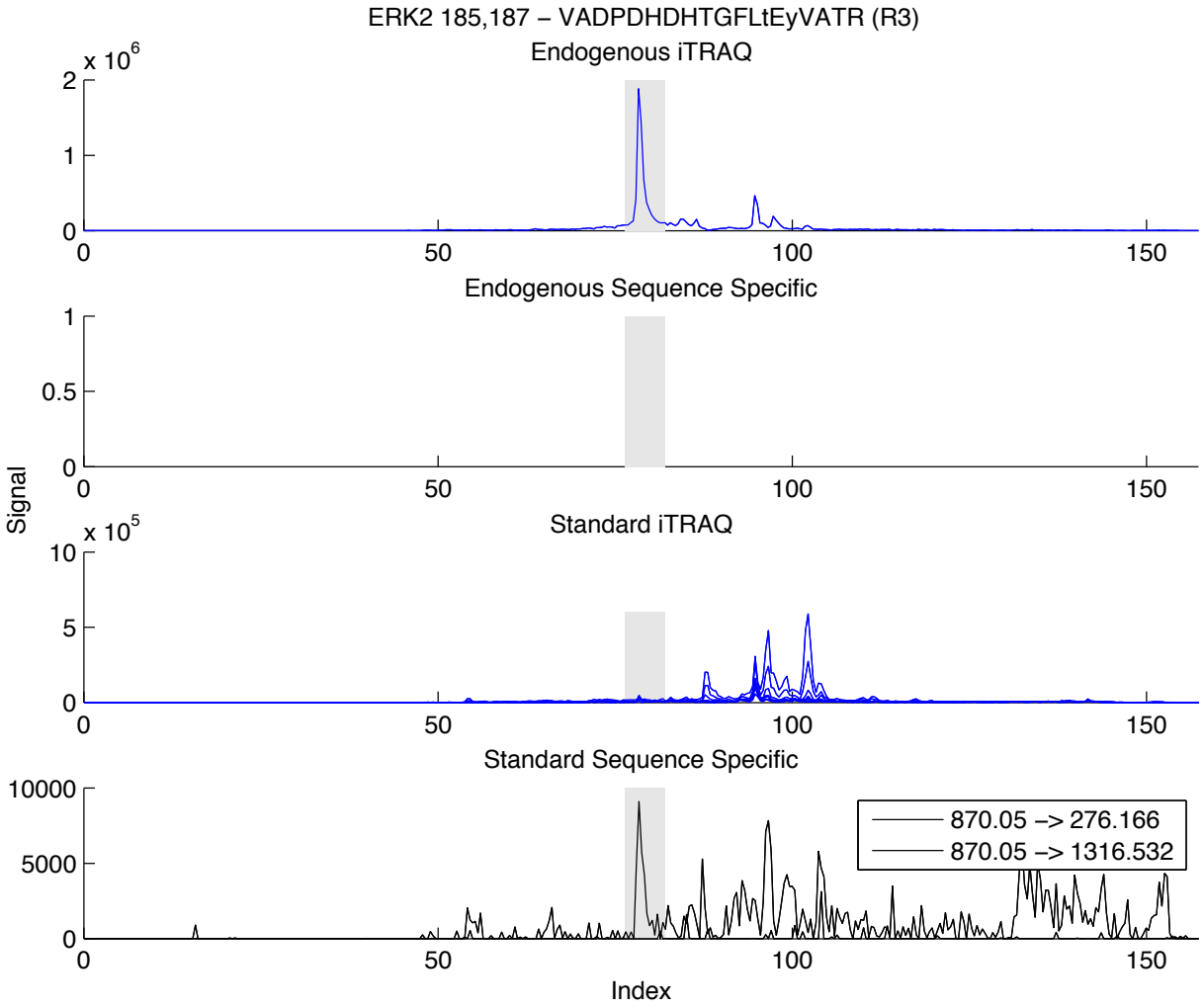

ERK1 204 – IADPEHDHTGFLTEyVATR (R3)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
639.805 –> 276.166
639.805 –> 1337.613
Standard iTRAQ
x 10^6
Standard Sequence Specific
641.289 –> 282.103
641.289 –> 1343.55
Signal
Index

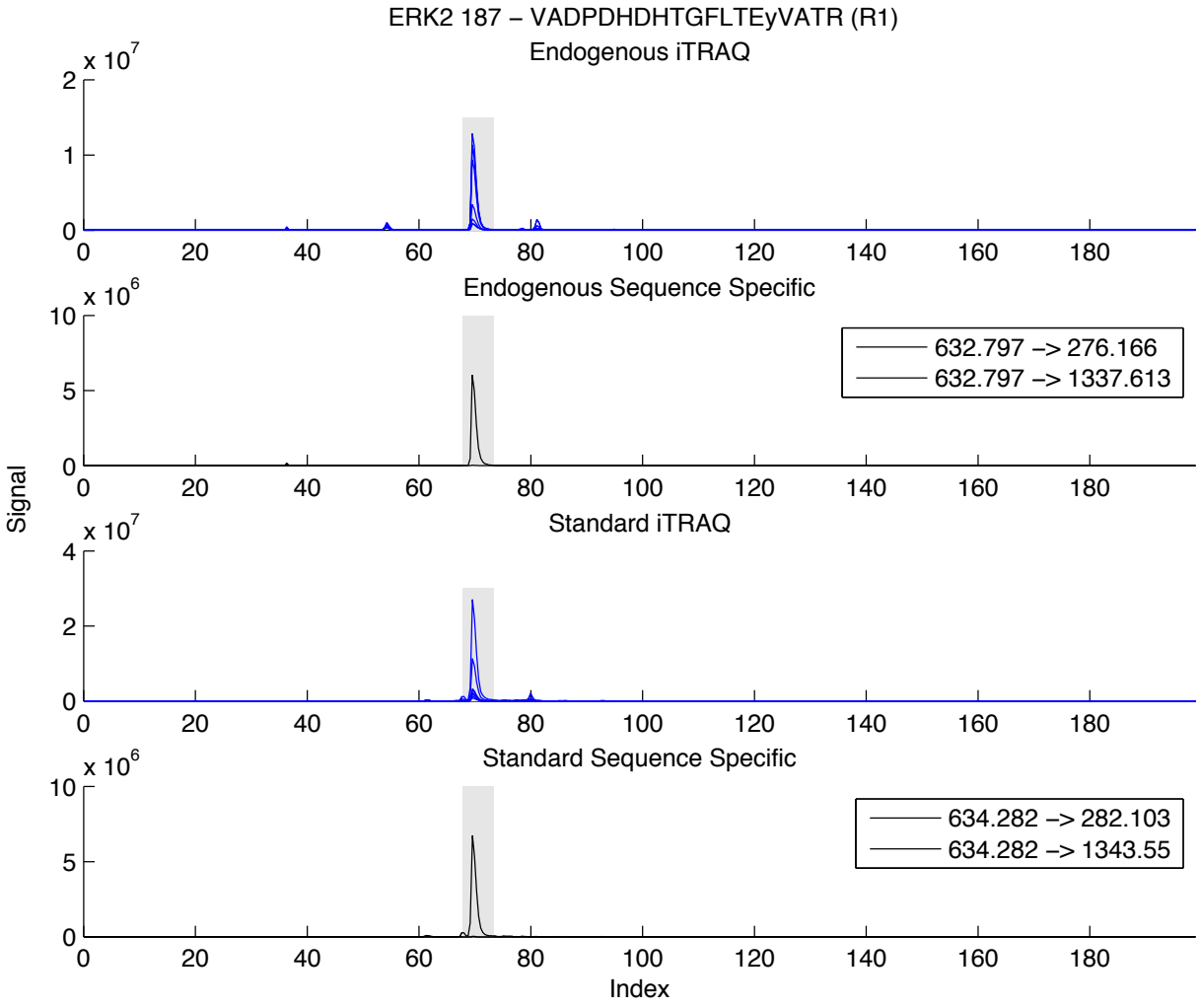

ERK1 204 – IADPEHDHTGFLTEyVATR (R4)
Endogenous iTRAQ
x 10^6
Endogenous Sequence Specific
x 10^5
639.805 –> 276.166
639.805 –> 1337.613
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^6
641.289 –> 282.103
641.289 –> 1343.55
Signal
Index

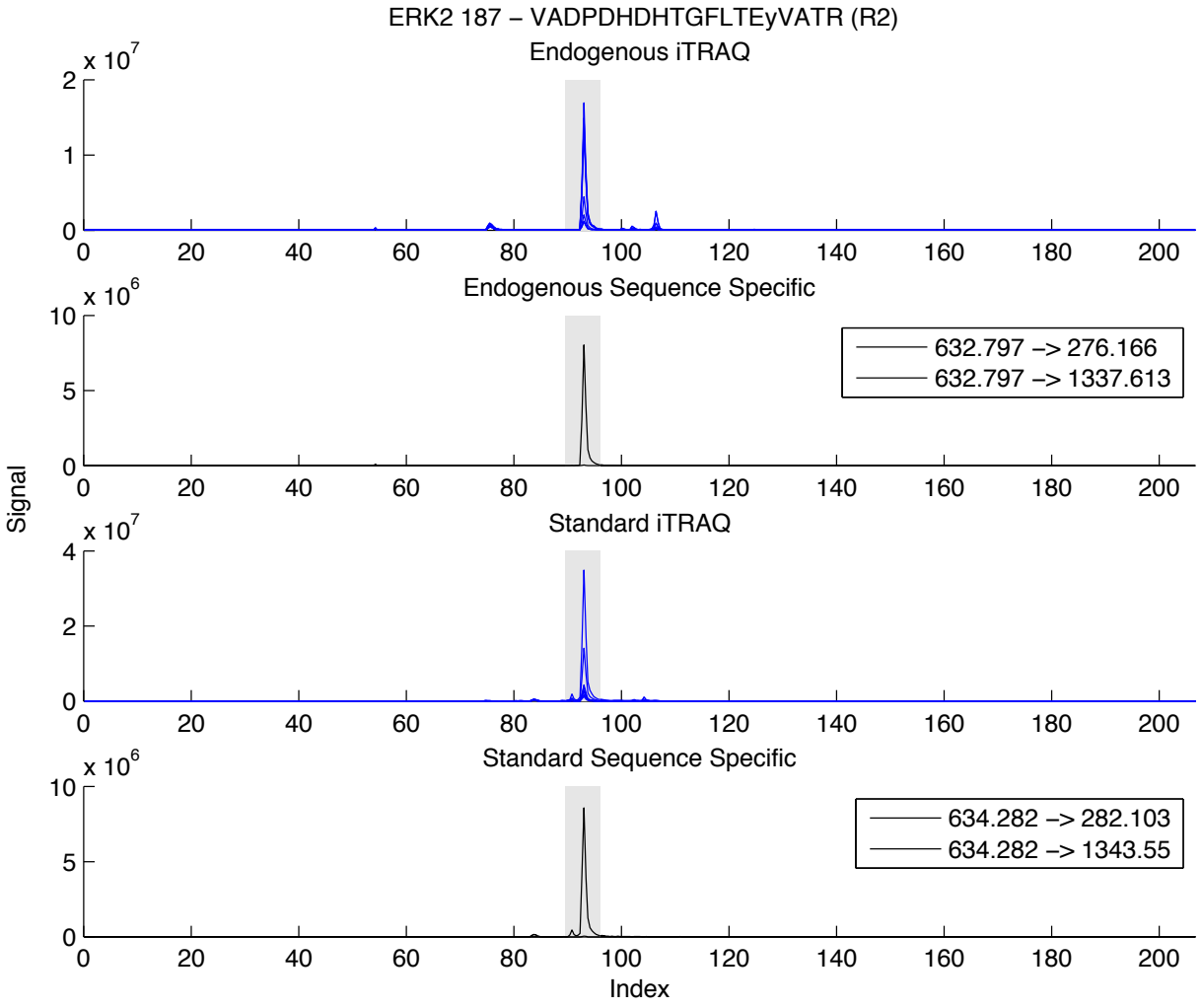

ERK1 204 – IADPEHDHTGFLTEyVATR (R5)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
639.805 –> 276.166
639.805 –> 1337.613
Signal
Standard iTRAQ
x 10^6
Standard Sequence Specific
641.289 –> 282.103
641.289 –> 1343.55
Index

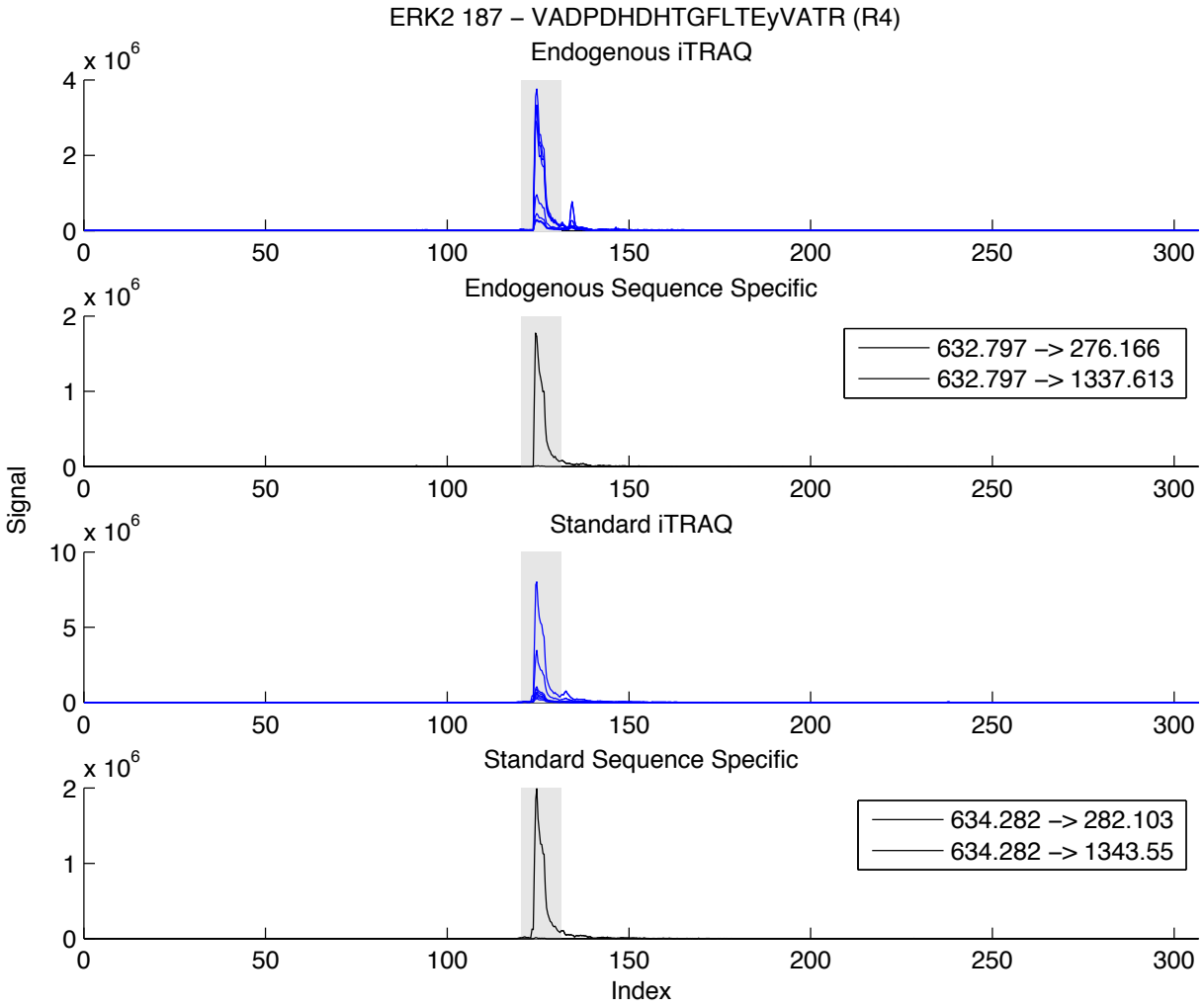

ERK2 185,187 – VADPDHDHTGFLtEyVATR (R1)
Endogenous iTRAQ
x 10^7
4
2
0
0 20 40 60 80 100 120 140 160 180
Endogenous Sequence Specific
1
0.5
0
0 20 40 60 80 100 120 140 160 180
Signal
Standard iTRAQ
x 10^5
10
5
0
0 20 40 60 80 100 120 140 160 180
Standard Sequence Specific
x 10^4
10
5
0
0 20 40 60 80 100 120 140 160 180
Index
870.05 –> 276.166
870.05 –> 1316.532

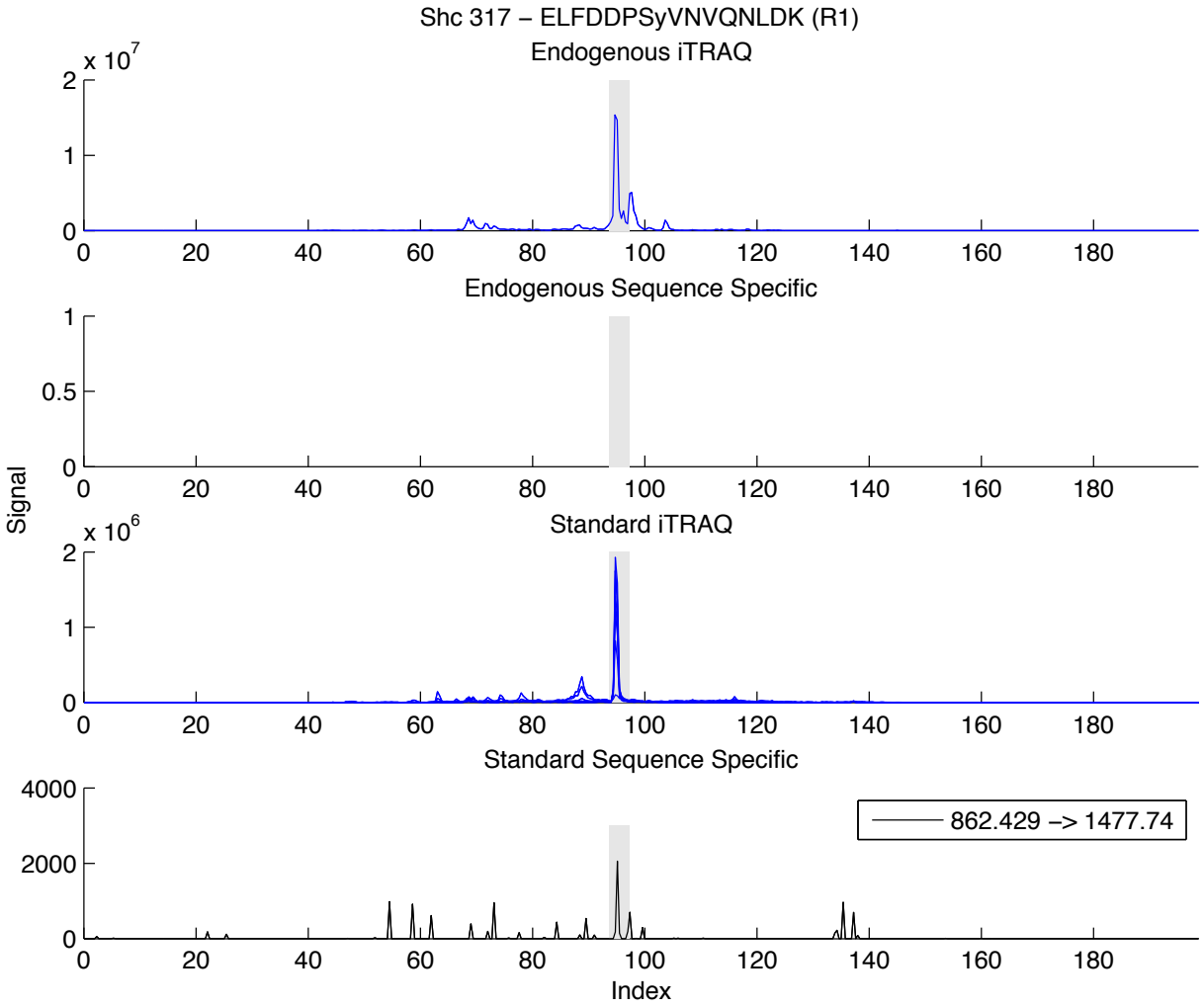

ERK2 185,187 – VADPDHDHTGFLtEyVATR (R2)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
Standard iTRAQ
x 10^5
Standard Sequence Specific
x 10^4
870.05 –> 276.166
870.05 –> 1316.532
Signal
Index

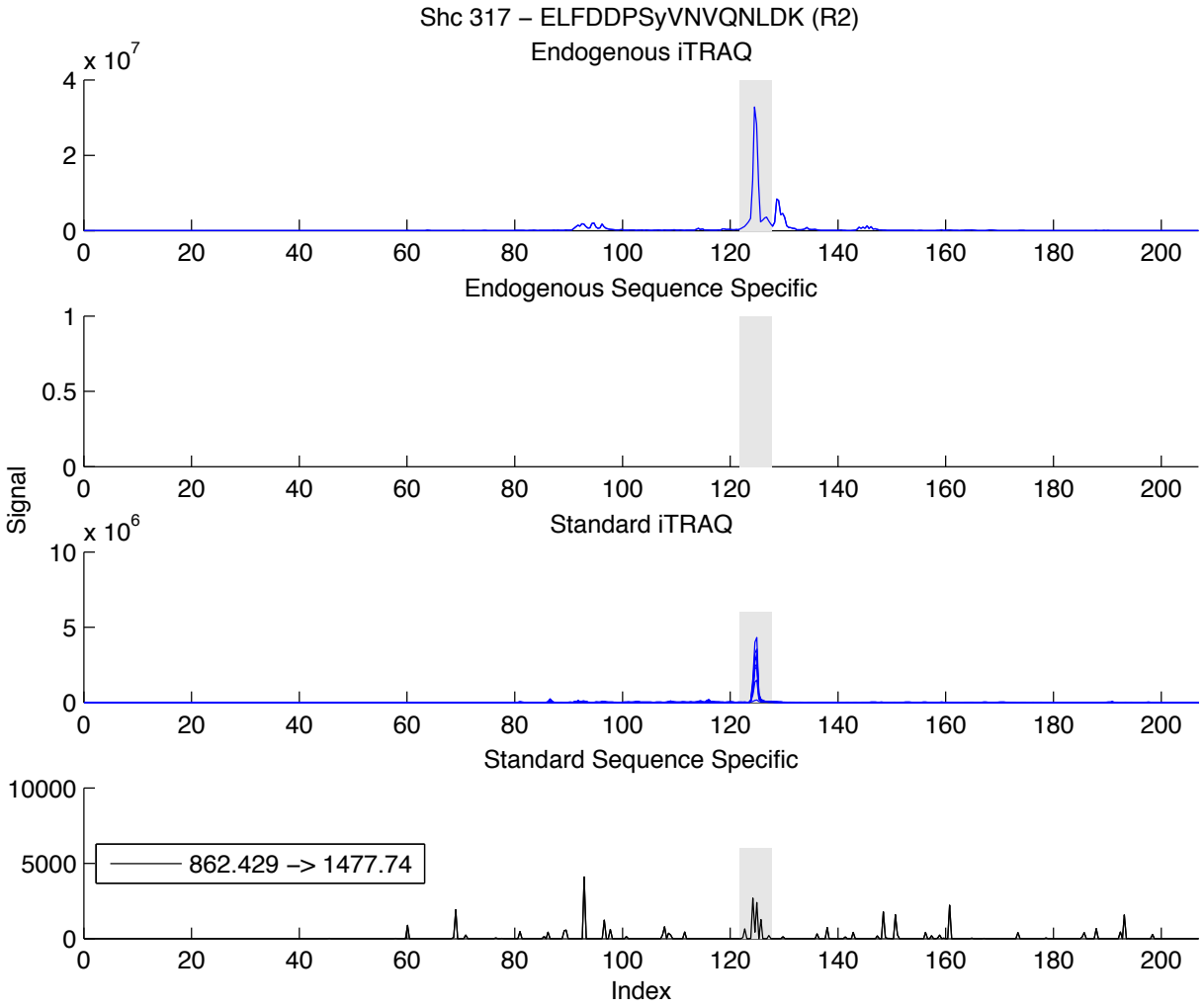

ERK2 185,187 – VADPDHDHTGFLtEyVATR (R3)
Endogenous iTRAQ
x 10^6
2
1
0
0
50
100
150
Endogenous Sequence Specific
1
0.5
0
Signal
Standard iTRAQ
x 10^5
10
5
0
Standard Sequence Specific
10000
5000
870.05 –> 276.166
870.05 –> 1316.532
Index

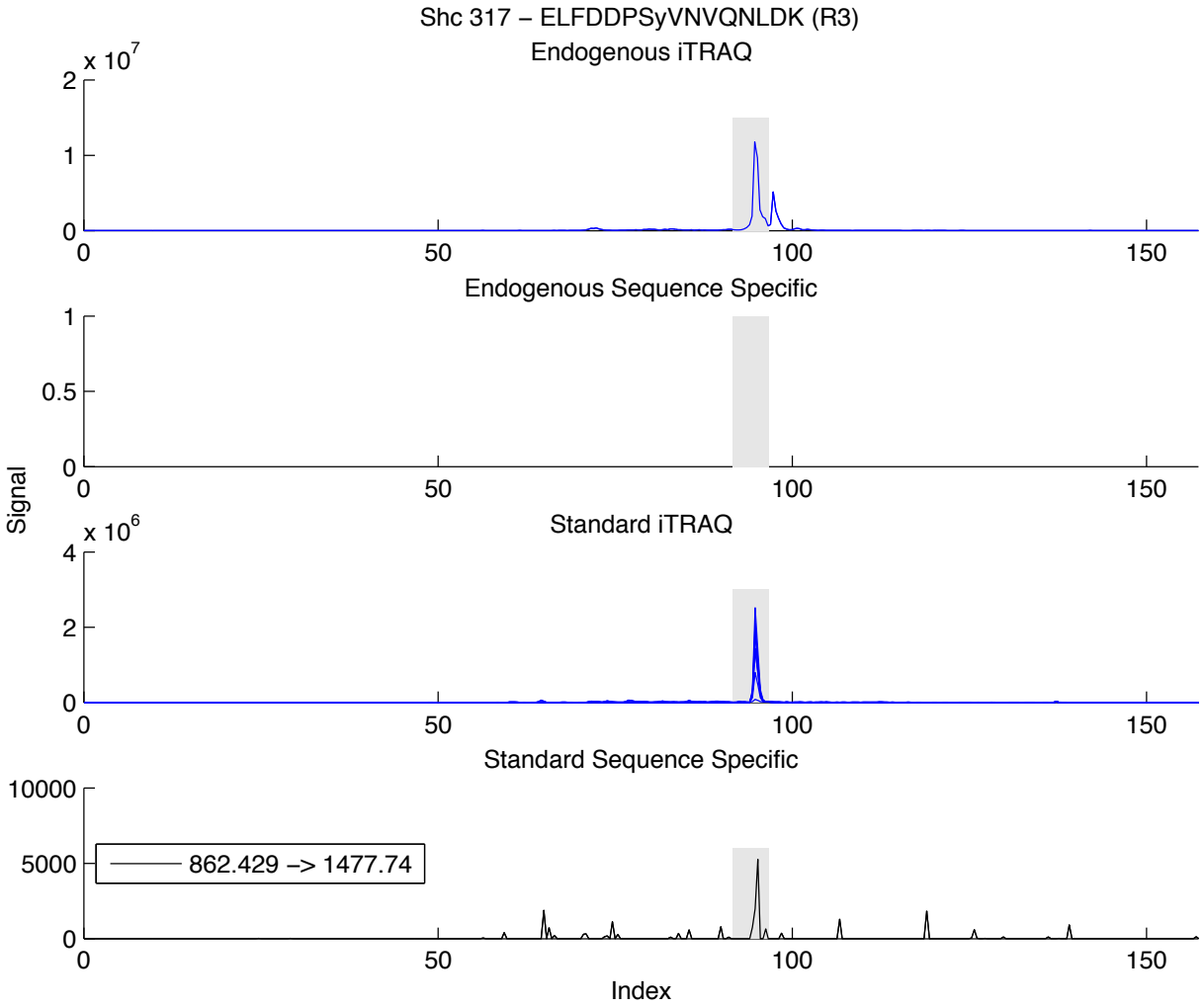

ERK2 187 – VADPDHDHTGFLTEyVATR (R1)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
x 10^6
632.797 –> 276.166
632.797 –> 1337.613
Standard iTRAQ
x 10^7
Standard Sequence Specific
x 10^6
634.282 –> 282.103
634.282 –> 1343.55
Signal
Index

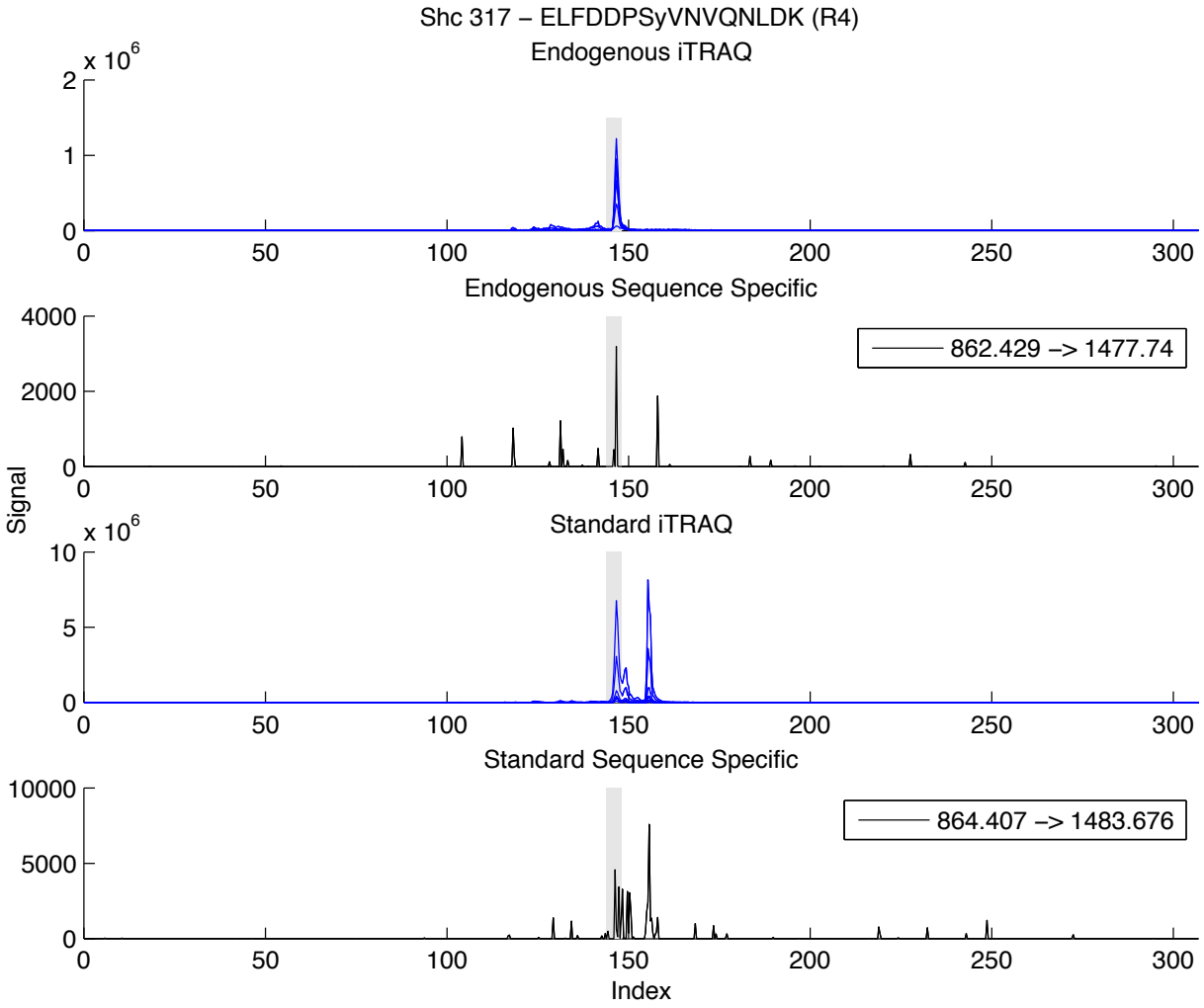

ERK2 187 – VADPDHDHTGFLTEyVATR (R2)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
x 10^6
632.797 –> 276.166
632.797 –> 1337.613
Standard iTRAQ
x 10^7
Standard Sequence Specific
x 10^6
634.282 –> 282.103
634.282 –> 1343.55
Signal
Index

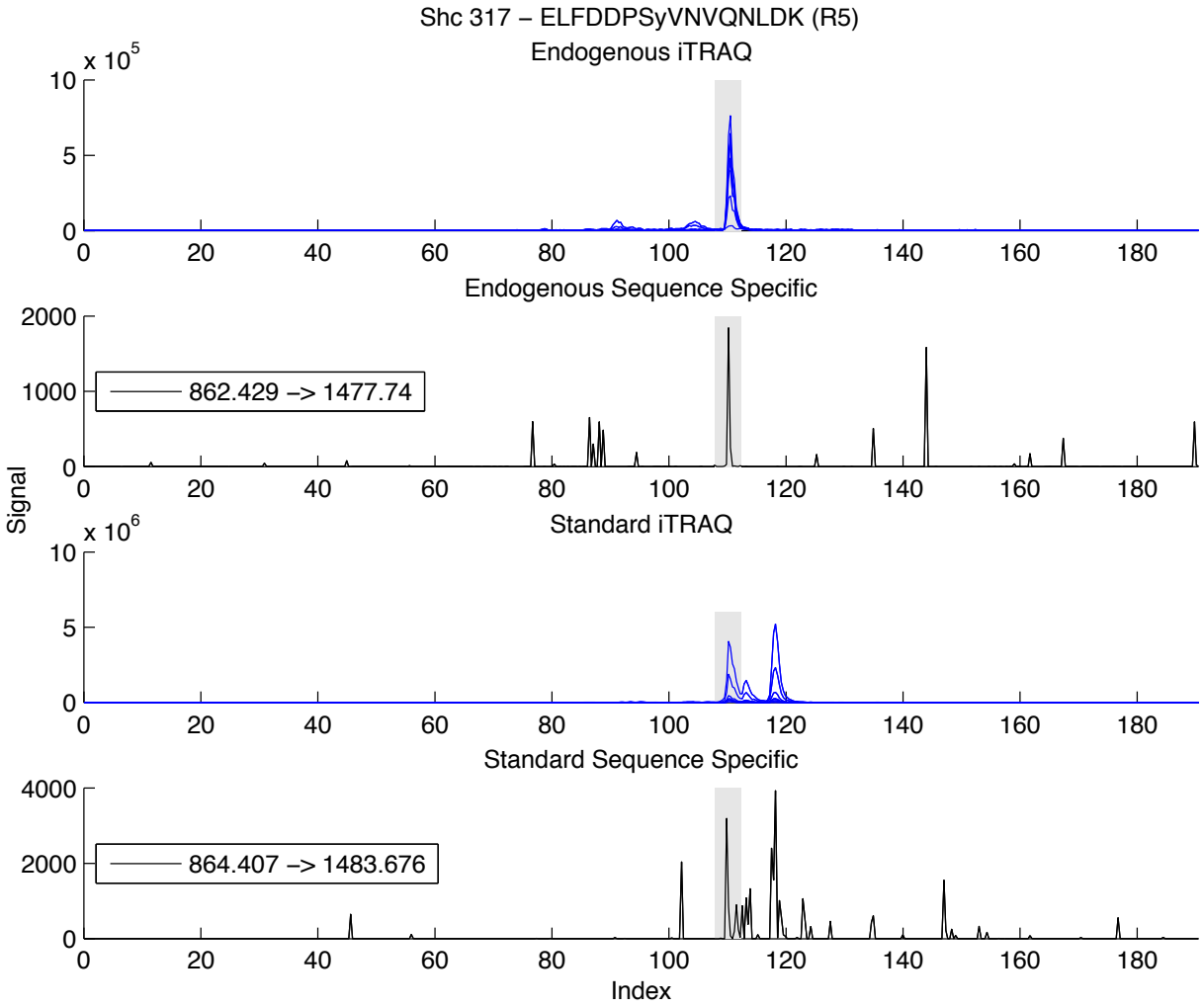

ERK2 187 – VADPDHDHTGFLTEyVATR (R4)
Endogenous iTRAQ
x 10^6
4
2
0
0
50
100
150
200
250
300
Endogenous Sequence Specific
x 10^6
2
1
0
632.797 –> 276.166
632.797 –> 1337.613
Signal
Standard iTRAQ
x 10^6
10
5
0
Standard Sequence Specific
x 10^6
634.282 –> 282.103
634.282 –> 1343.55
Index

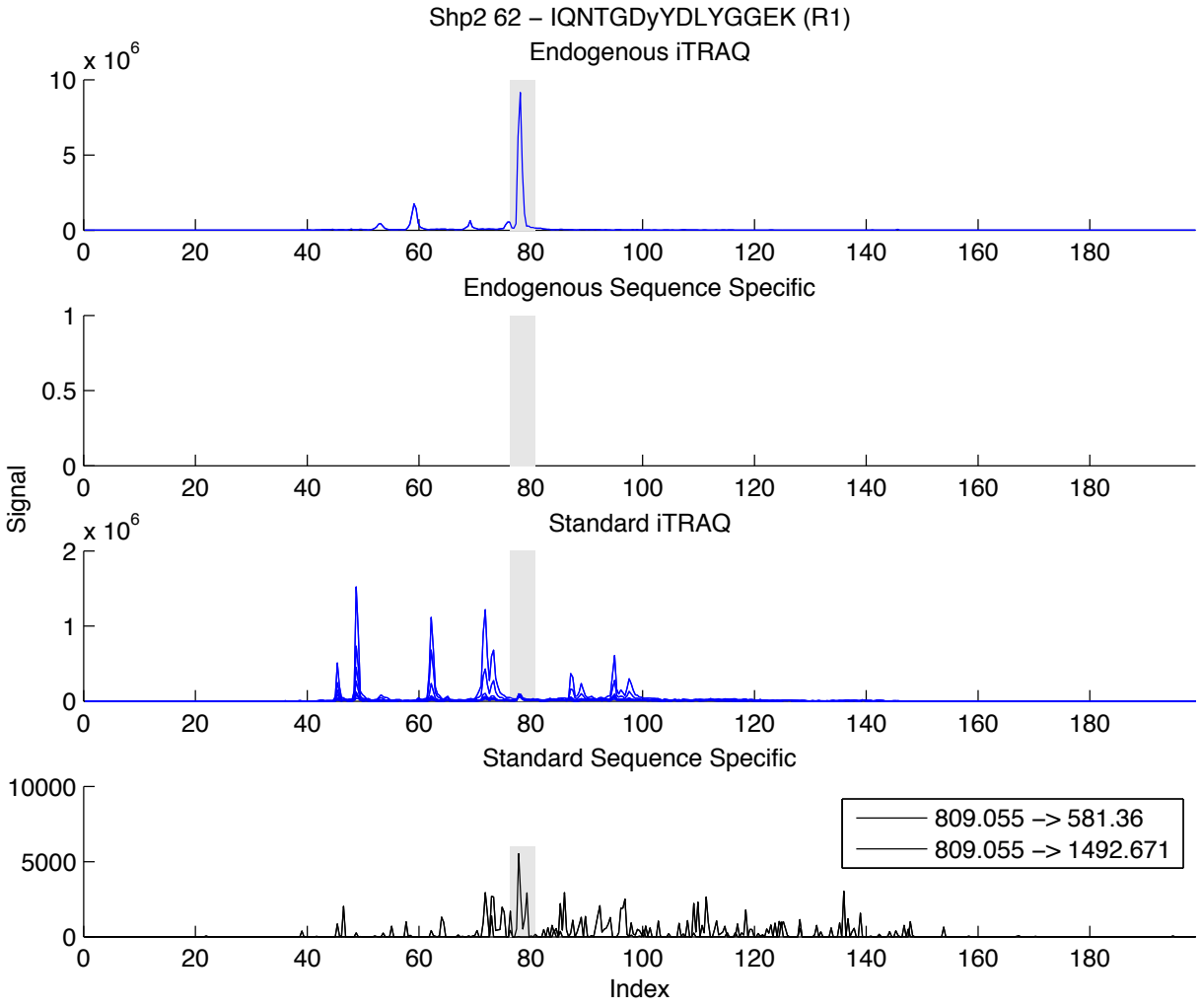

Shc 317 – ELFDDPSyVNVQNLDK (R1)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
Signal
Standard iTRAQ
x 10^6
Standard Sequence Specific
862.429 –> 1477.74
Index

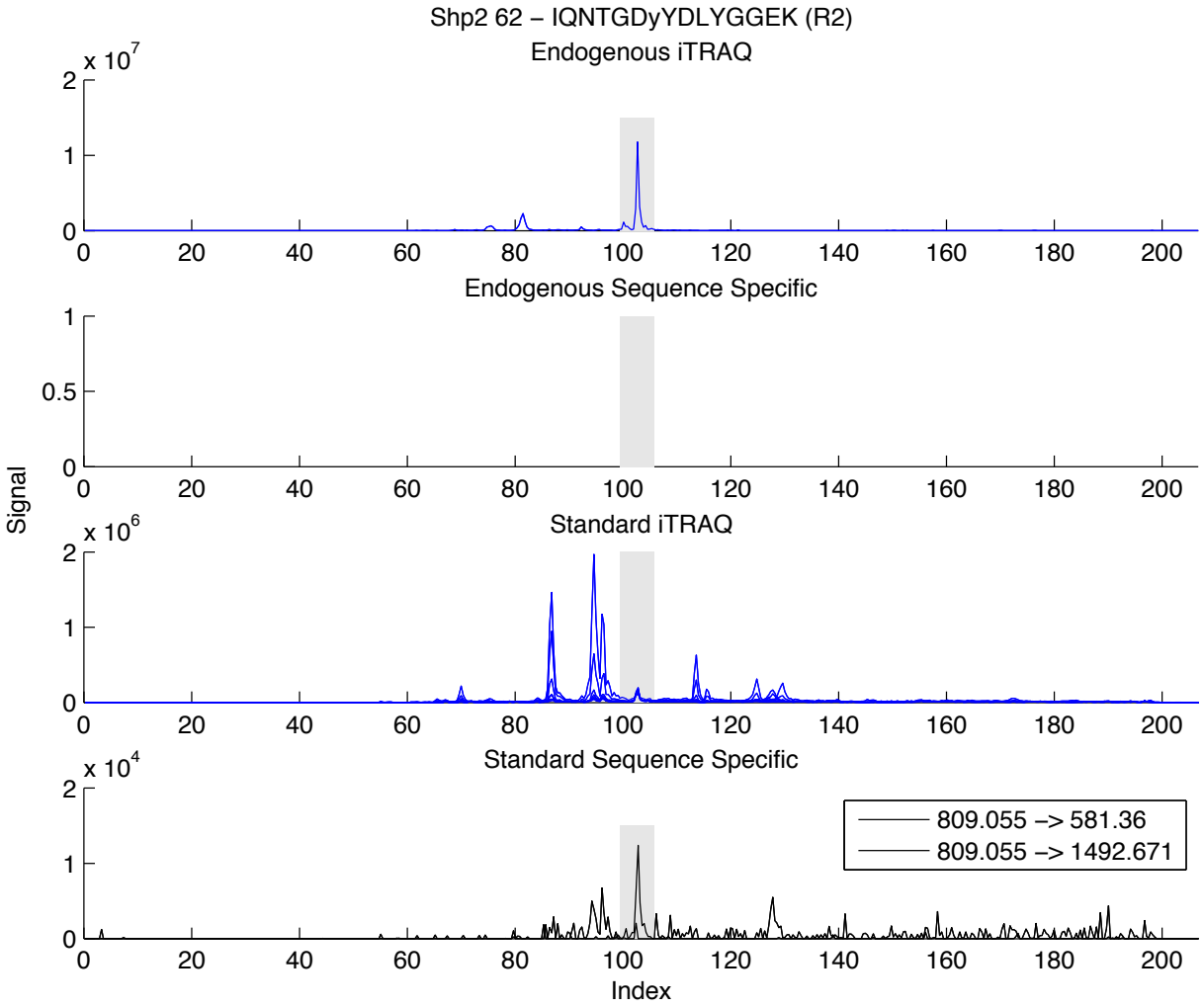

Shc 317 – ELFDDPSyVNVQNLDK (R2)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
Signal
Standard iTRAQ
x 10^6
Standard Sequence Specific
862.429 –> 1477.74
Index

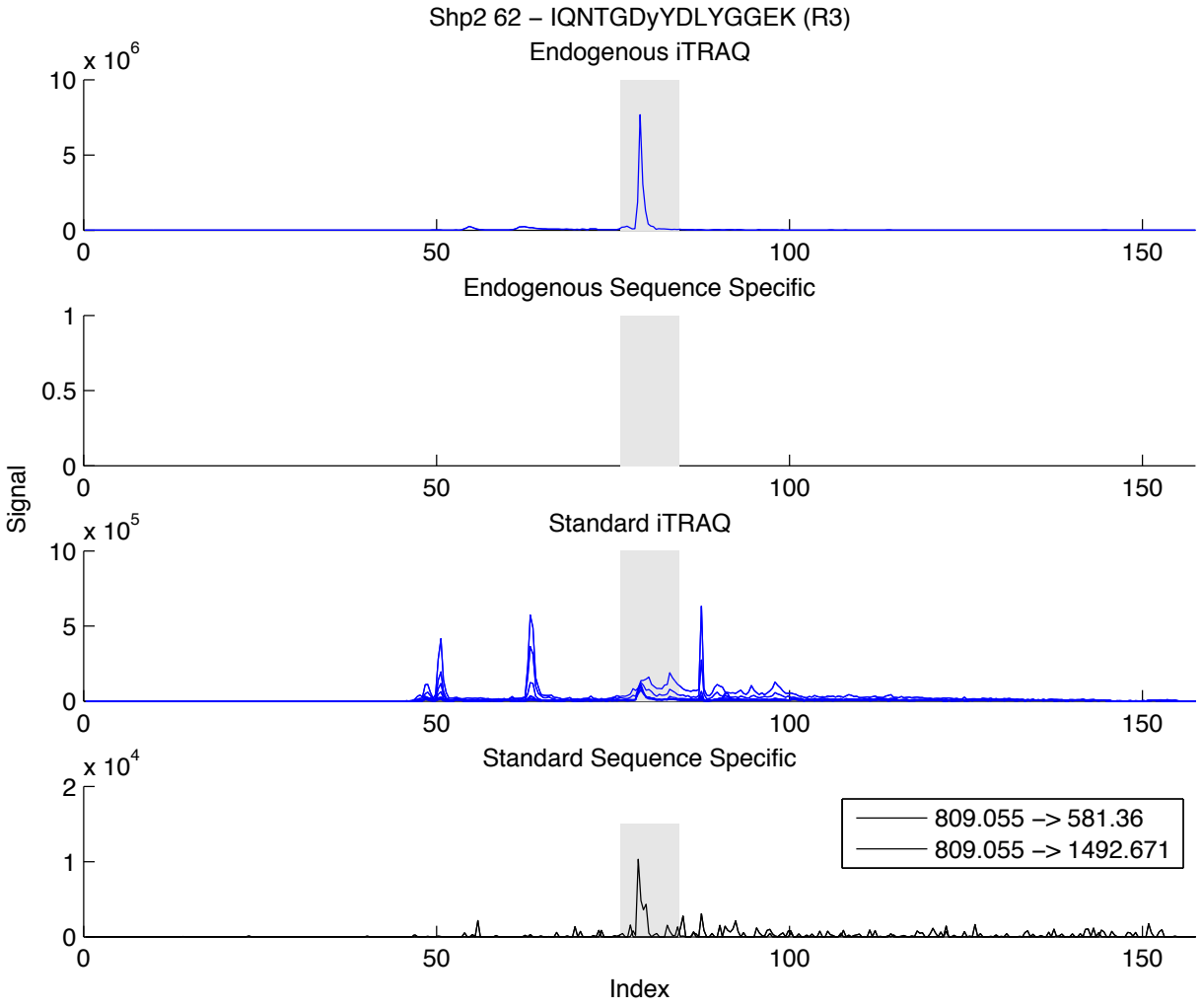

Shc 317 – ELFDDPSyVNVQNLDK (R3)
Endogenous iTRAQ
x 10^7
2
1
0
0
50
100
150
Endogenous Sequence Specific
1
0.5
0
Signal
Standard iTRAQ
x 10^6
4
2
Standard Sequence Specific
10000
5000
862.429 –> 1477.74
Index

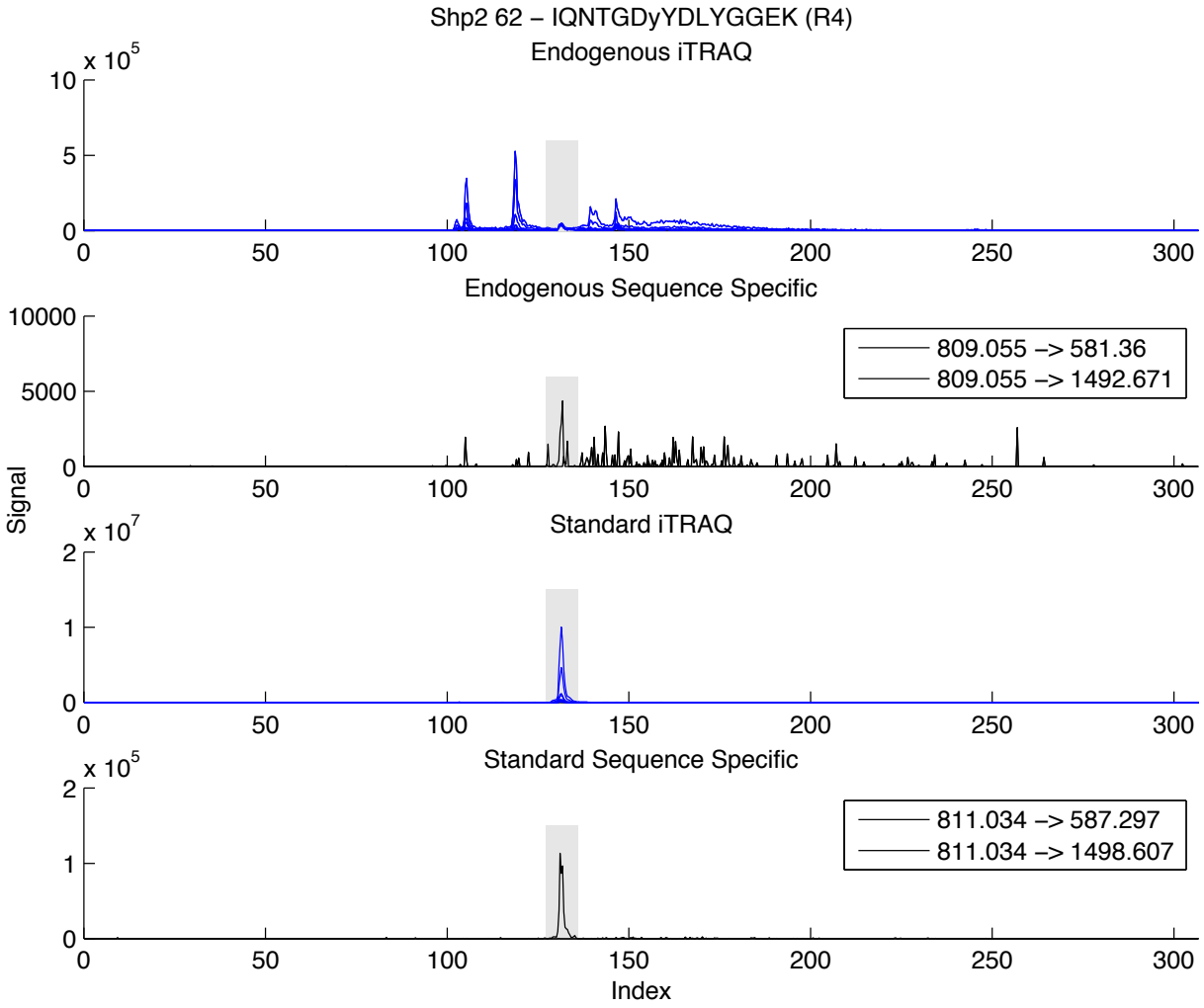

Shc 317 – ELFDDPSyVNVQNLDK (R4)
Endogenous iTRAQ
x 10^6
2
1
0
0
50
100
150
200
250
300
Endogenous Sequence Specific
4000
2000
0
862.429 –> 1477.74
Signal
Standard iTRAQ
x 10^6
10
5
0
Standard Sequence Specific
10000
5000
0
864.407 –> 1483.676
Index

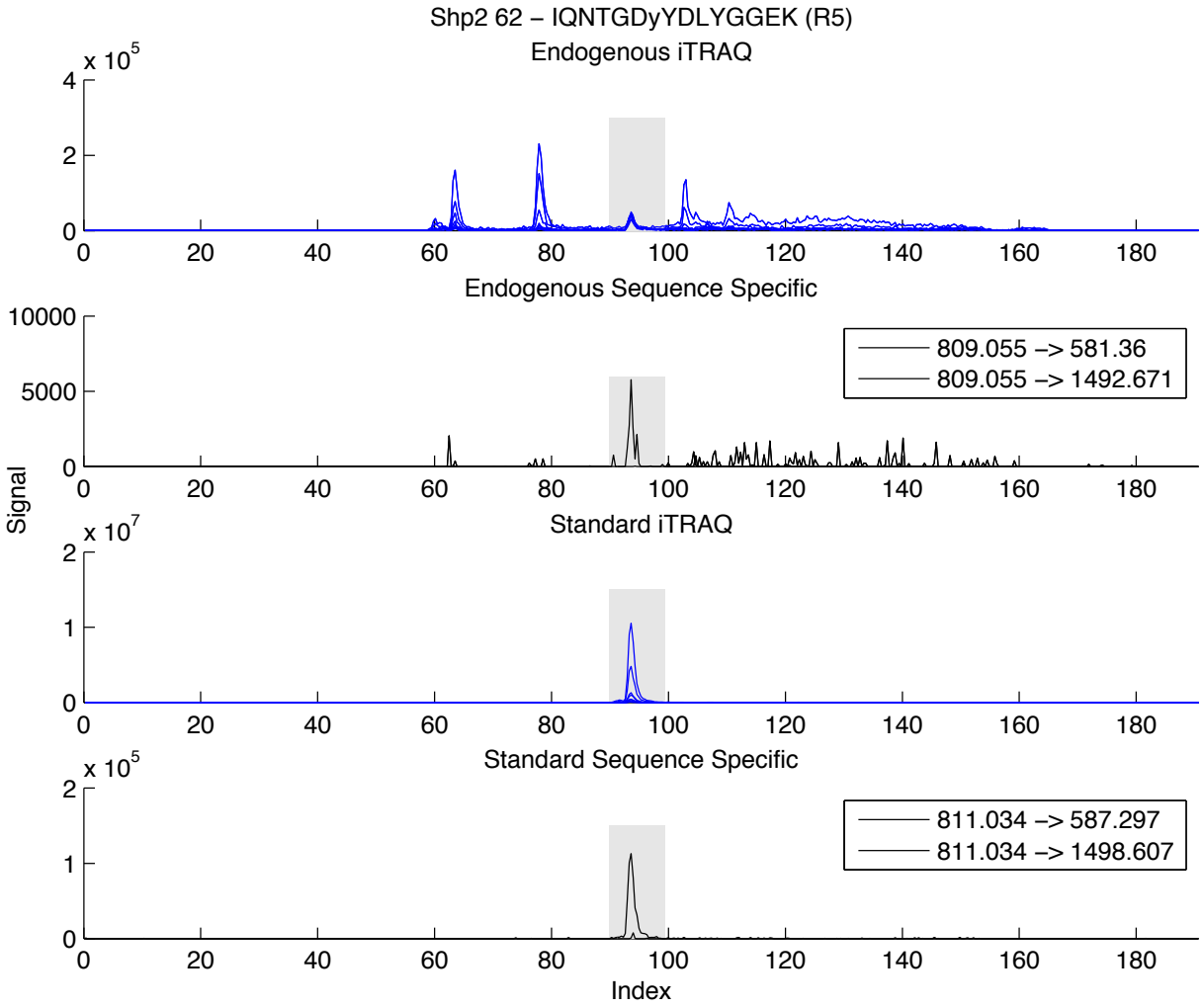

Shc 317 – ELFDDPSyVNVQNLDK (R5)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
862.429 –> 1477.74
Signal
Standard iTRAQ
x 10^6
Standard Sequence Specific
864.407 –> 1483.676
Index

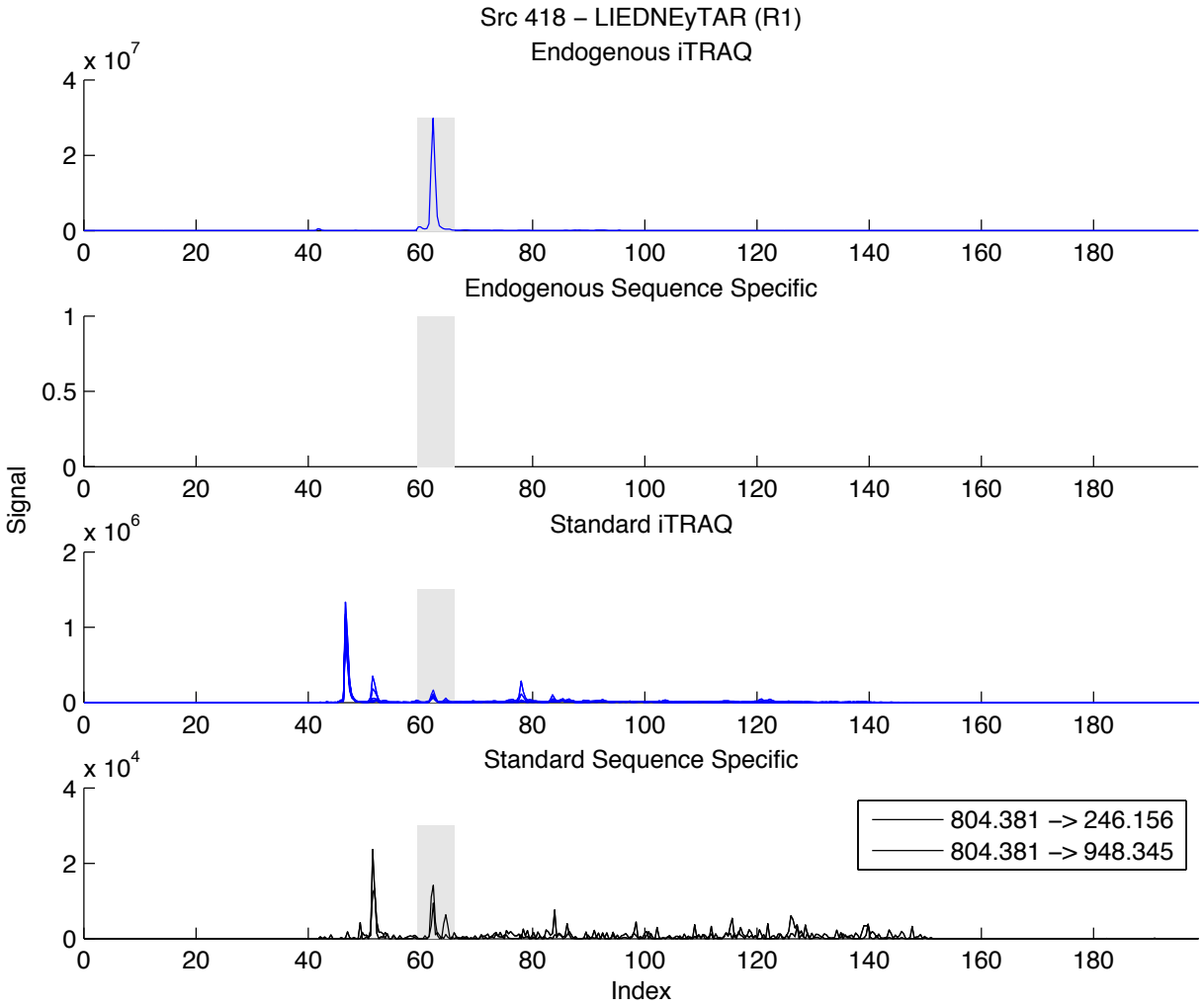

Shp2 62 – IQNTGDyYDLYGGEK (R1)
Endogenous iTRAQ
x 10^6
10
5
0
0 20 40 60 80 100 120 140 160 180
Endogenous Sequence Specific
1
0.5
0
0 20 40 60 80 100 120 140 160 180
Signal
Standard iTRAQ
x 10^6
2
1
0
0 20 40 60 80 100 120 140 160 180
Standard Sequence Specific
10000
5000
0
809.055 –> 581.36
809.055 –> 1492.671
0 20 40 60 80 100 120 140 160 180
Index

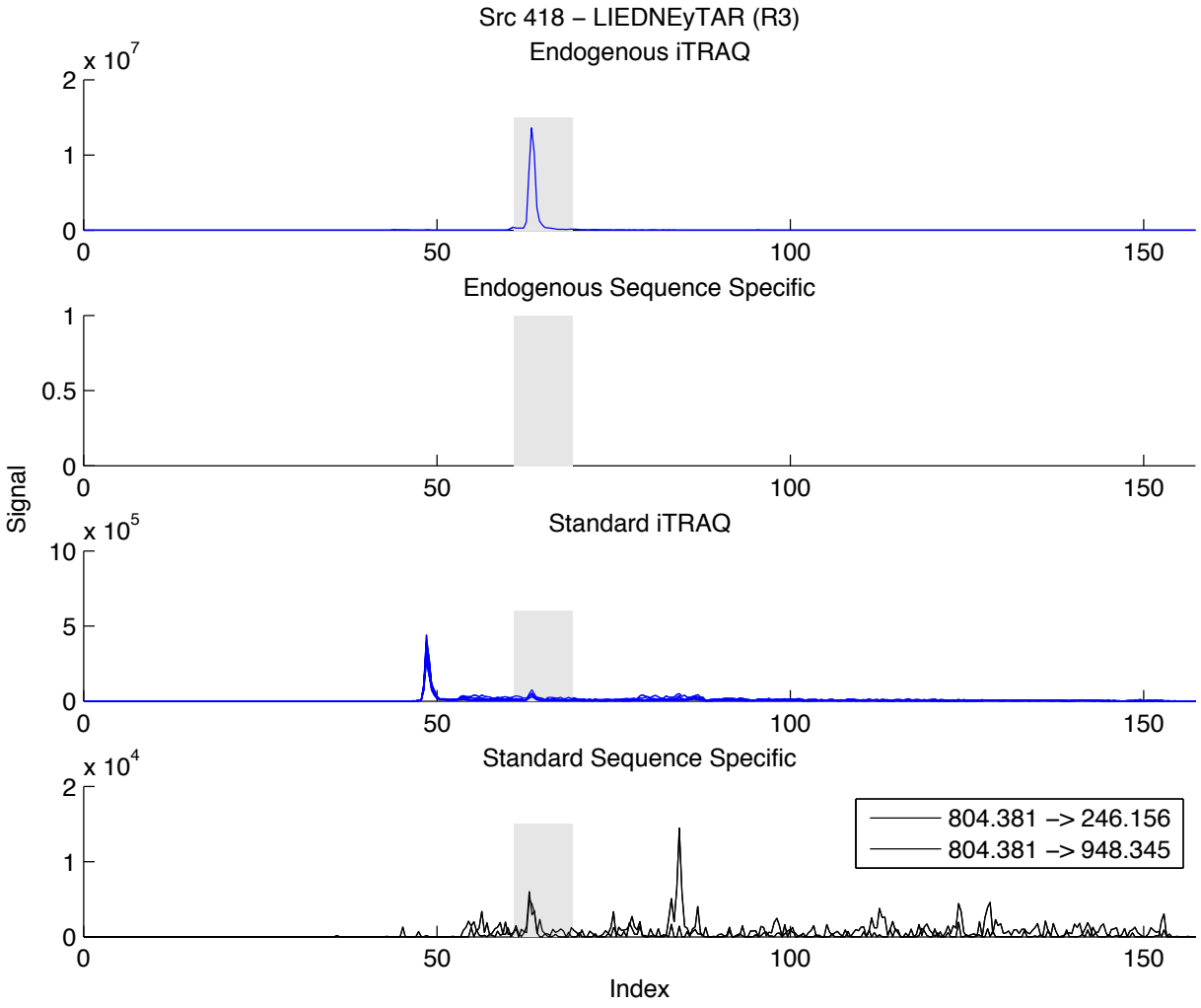

Shp2 62 – IQNTGDyYDLYGGEK (R2)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
Signal
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^4
809.055 –> 581.36
809.055 –> 1492.671
Index

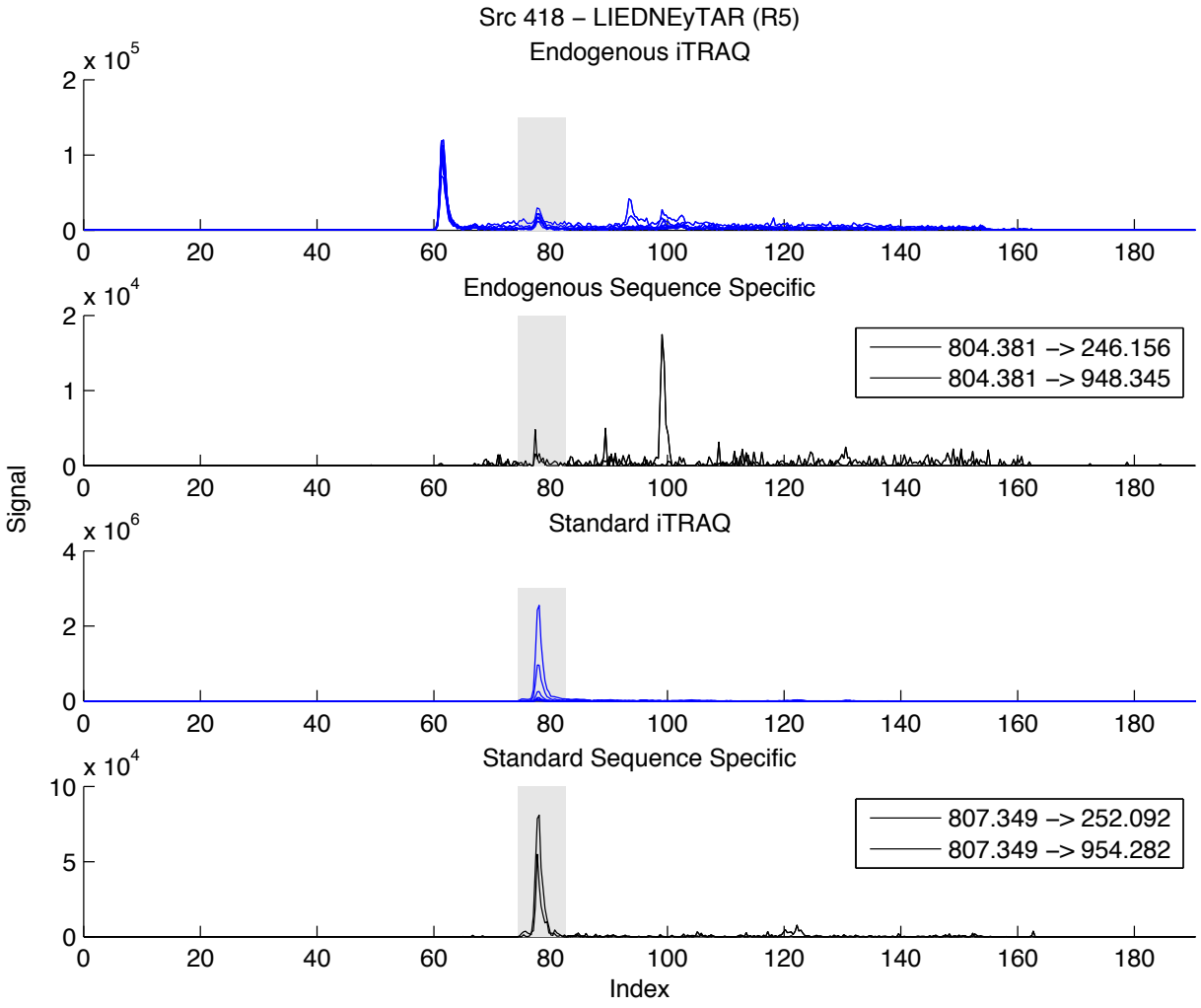

Shp2 62 – IQNTGDyYDLYGGEK (R3)
Endogenous iTRAQ
x 10^6
10
5
0
0
50
100
150
Endogenous Sequence Specific
1
0.5
0
0
50
100
150
Signal
Standard iTRAQ
x 10^5
10
5
0
0
50
100
150
Standard Sequence Specific
x 10^4
2
1
0
0
50
100
150
Index
809.055 –> 581.36
809.055 –> 1492.671


Shp2 62 – IQNTGDyYDLYGGEK (R4)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
809.055 –> 581.36
809.055 –> 1492.671
Signal
Standard iTRAQ
x 10^7
Standard Sequence Specific
x 10^5
811.034 –> 587.297
811.034 –> 1498.607
Index

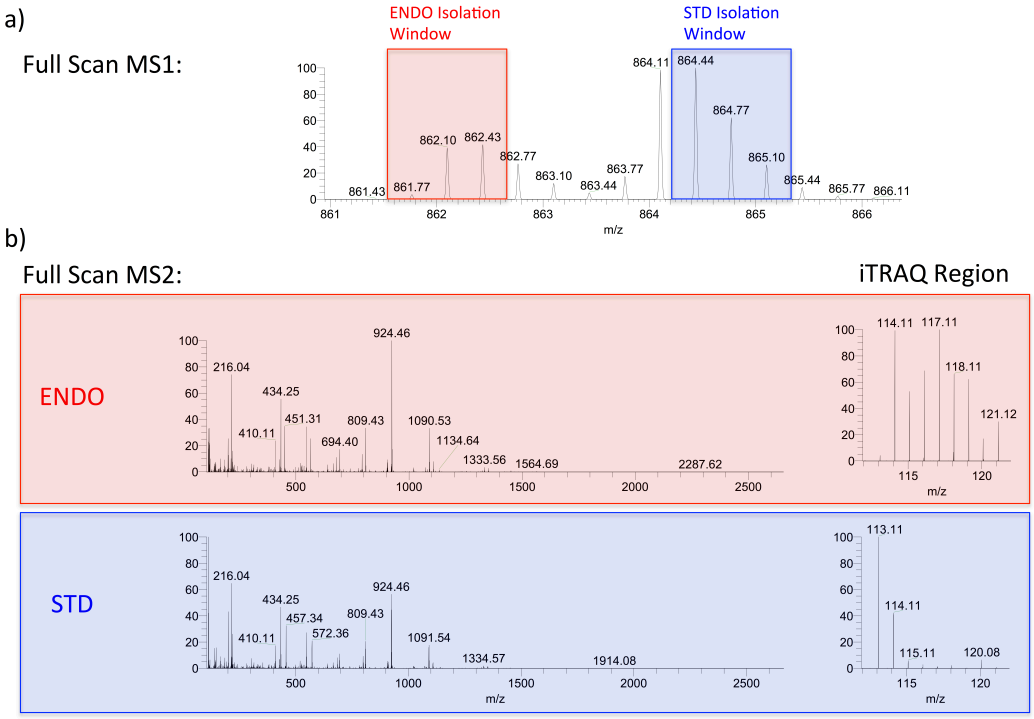

Shp2 62 – IQNTGDyYDLYGGEK (R5)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
809.055 –> 581.36
809.055 –> 1492.671
Signal
Standard iTRAQ
x 10^7
Standard Sequence Specific
x 10^5
811.034 –> 587.297
811.034 –> 1498.607
Index


Src 418 – LIEDNEyTAR (R1)
Endogenous iTRAQ
x 10^7
Endogenous Sequence Specific
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^4
804.381 –> 246.156
804.381 –> 948.345
Signal
Index

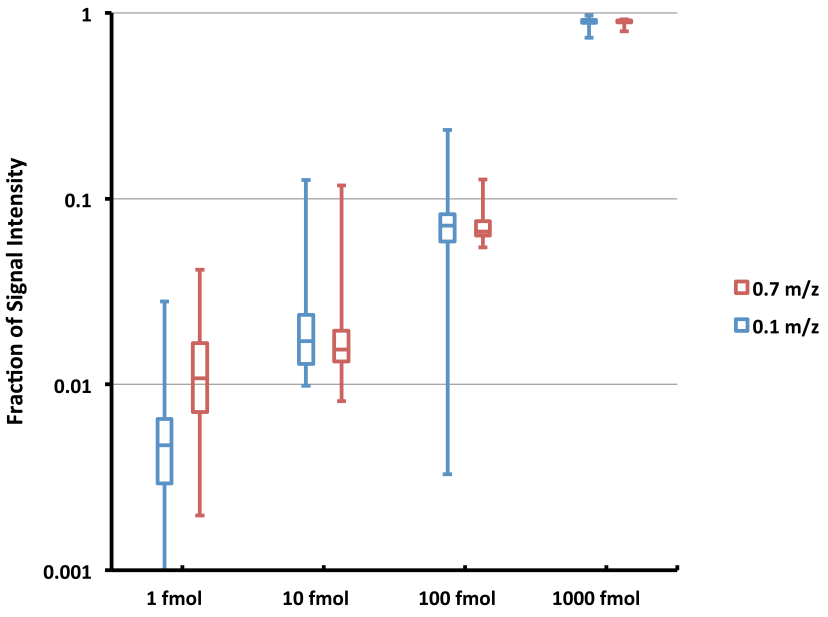

Src 418 – LIEDNEyTAR (R3)
Endogenous iTRAQ
x 10^7
2
1
0
0
50
100
150
Endogenous Sequence Specific
1
0.5
0
0
50
100
150
Signal
Standard iTRAQ
x 10^5
10
5
0
0
50
100
150
Standard Sequence Specific
x 10^4
2
1
0
0
50
100
150
804.381 –> 246.156
804.381 –> 948.345
Index


Src 418 – LIEDNEyTAR (R5)
Endogenous iTRAQ
x 10^5
Endogenous Sequence Specific
x 10^4
804.381 -> 246.156
804.381 -> 948.345
Standard iTRAQ
x 10^6
Standard Sequence Specific
x 10^4
807.349 -> 252.092
807.349 -> 954.282
Signal
Index

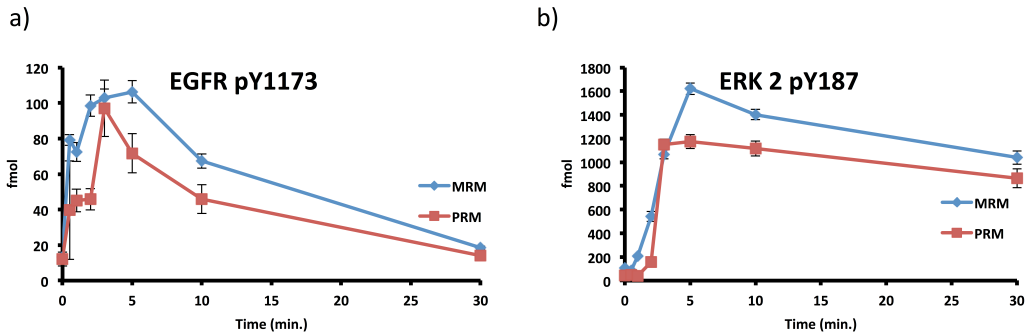
Supplementary Figure 3: MRM transition plots. The highlighted area is the preliminary search area specified by the user. Inside this time window, the algorithm finds the peak and half-max time window, which is then used to calculate the area for each transition.



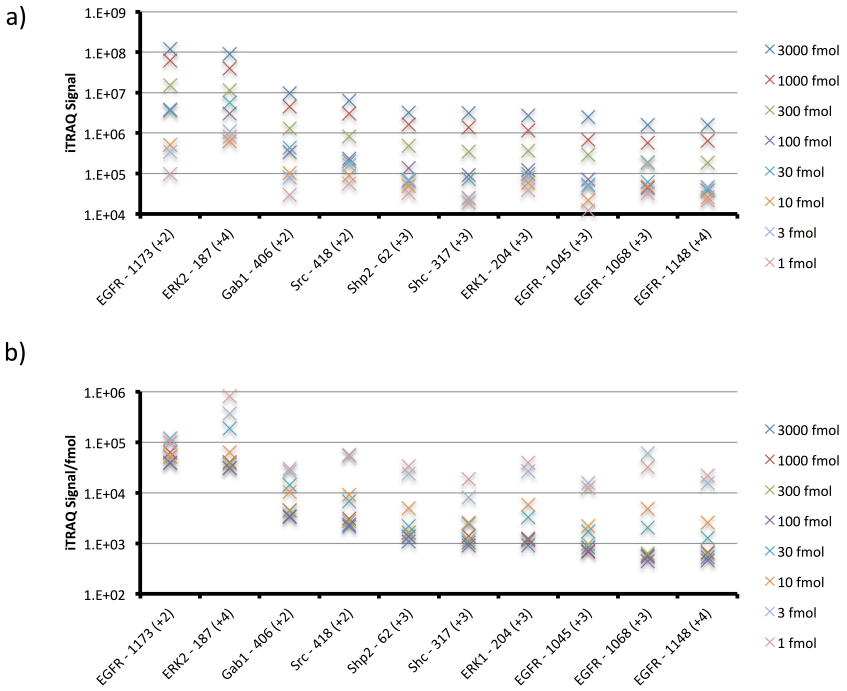
Supplementary Figure 4: Details of PRM Data Collection Method. a) Precursor masses for the endogenous (red) and standard (blue) Shc pY317 peptide can be individually isolated for subsequent fragmentation. b) The full scan MS2 result of individually isolated and fragmented peptides; endogenous (red), standard (blue). The iTRAQ reporter ion region has been magnified to illustrate the relative quantification data available from the full MS2 scan.



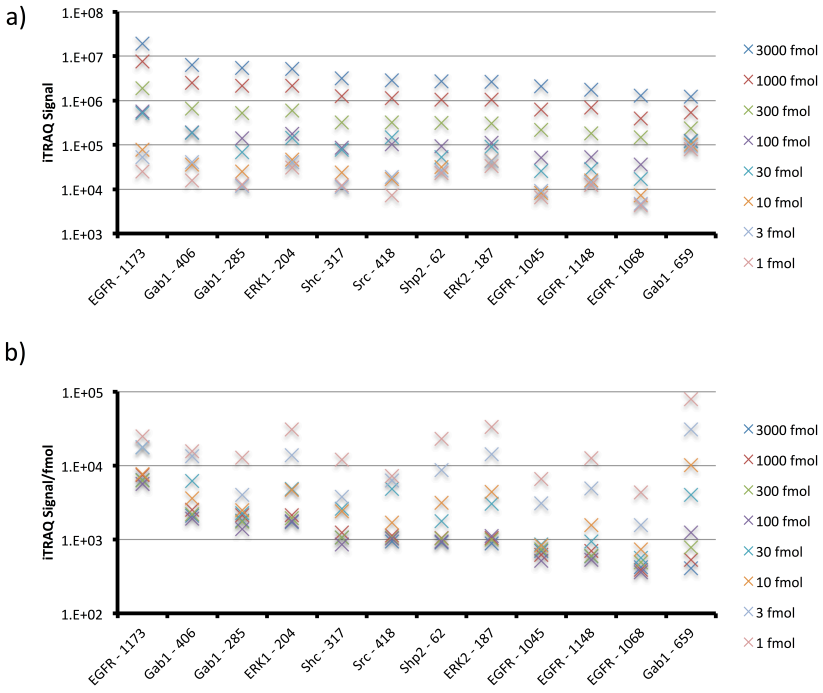
Supplementary Figure 5: Comparison between MRM and PRM for EGFR pY1173 and ERk2 pY187. Absolute quantified amount of phosphorylation compared between the two data collection methods. Data drawn from Supplementary Table 3.



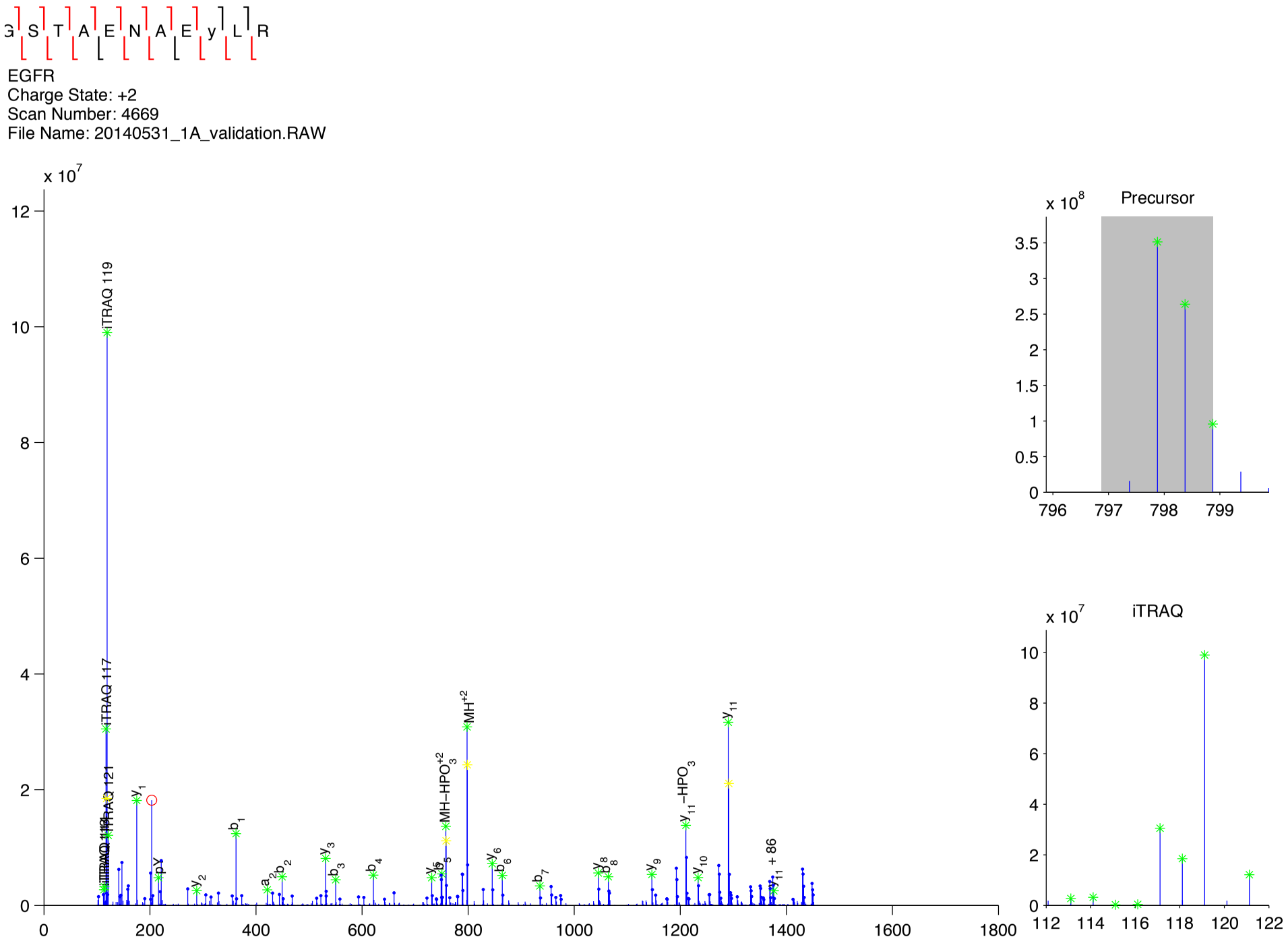
Supplementary Figure 6: Q1 Isolation Width Comparison. 0.7 m/z (red) and 0.1 m/z (blue). Data represented in this figure can be found in Supplementary Table 5.



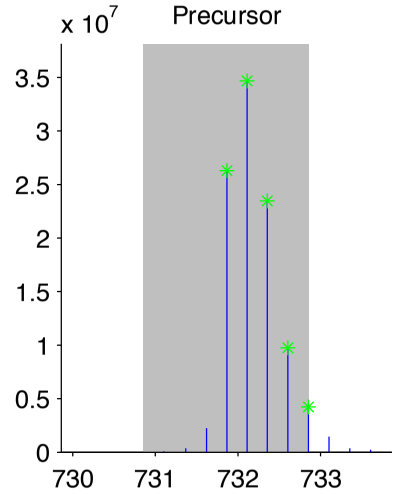
Supplementary Figure 7: iTRAQ Signal for range of peptide standard doses in GBM background. a) The iTRAQ signal intensity for each indicated dose of each indicated peptide standard. b) iTRAQ signal/fmol.

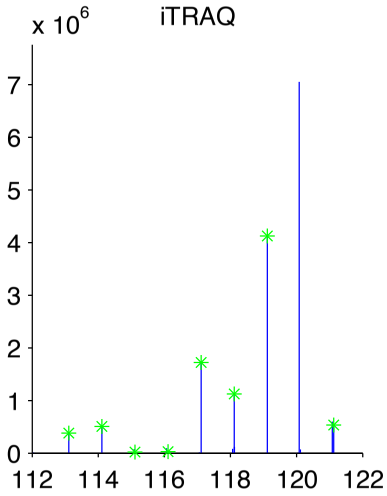


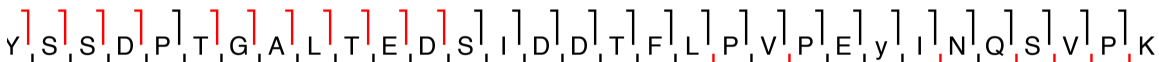
Supplementary Figure 8: iTRAQ Signal for range of peptide standard doses in GBM12 EGFR inhibitor panel background. a) The iTRAQ signal intensity for each indicated dose of each indicated peptide standard. b) iTRAQ signal/fmol.


pSTAENAEyLR
EGFR
Charge State: +2
Scan Number: 4669
File Name: 20140531_1A_validation.RAW
x 10^7
iTRAQ 119
iTRAQ 117
iTRAQ 121
MH+2
MH–HPO3+2
y11–HPO3
y11 + 86
Precursor
x 10^8
iTRAQ
x 10^7

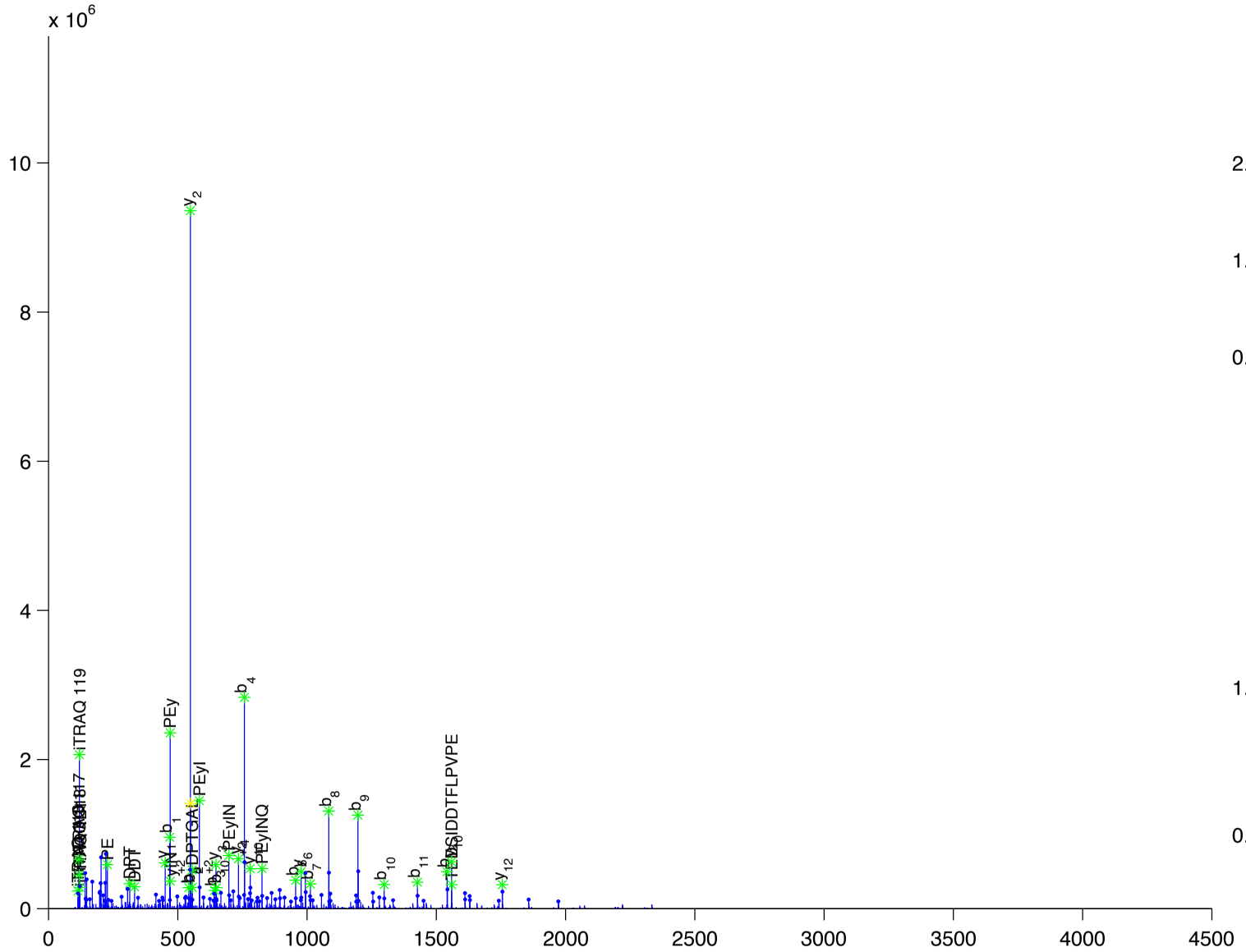
EGFR
Charge State: +4
Scan Number: 6889
File Name: 20140531_1A_validation.RAW

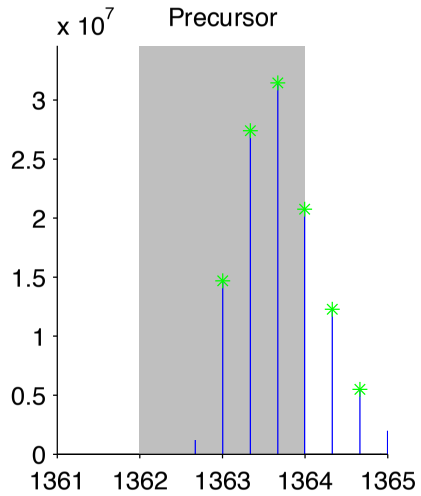


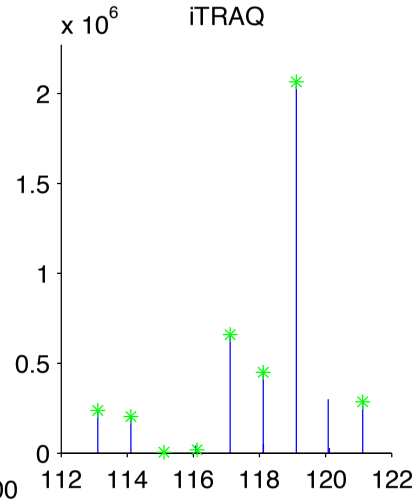


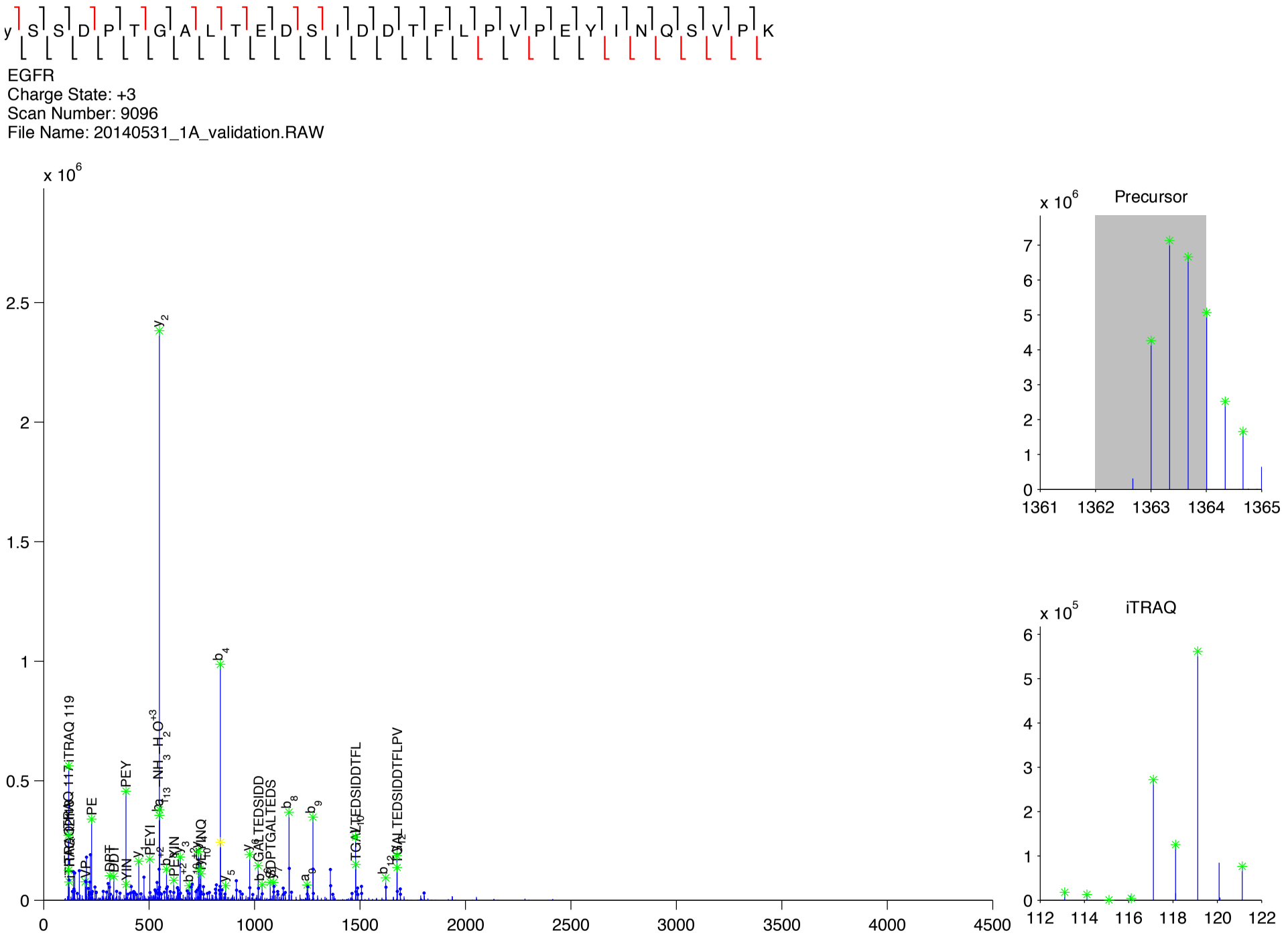


EGFR
Charge State: +3
Scan Number: 8719
File Name: 20140531_1A_validation.RAW

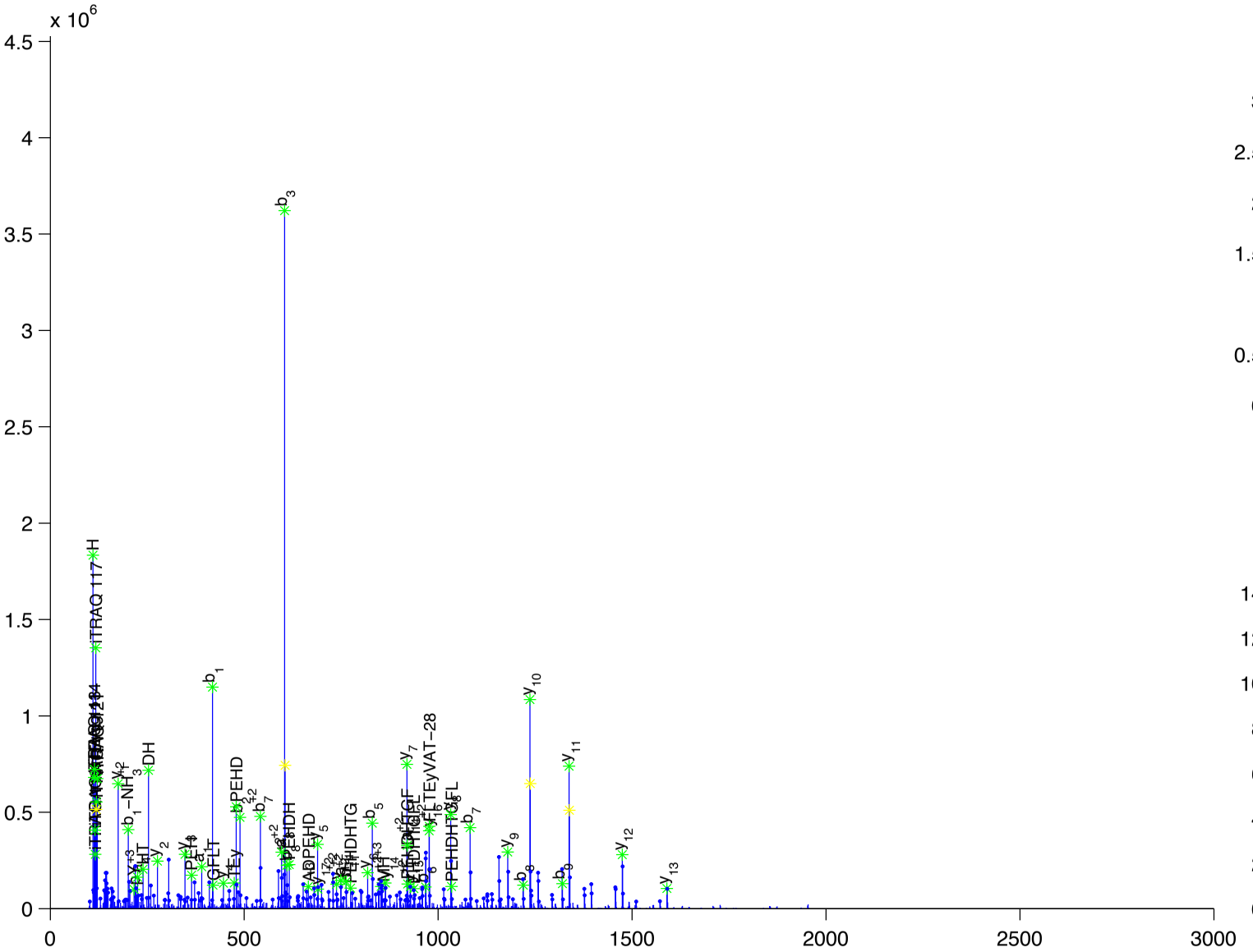


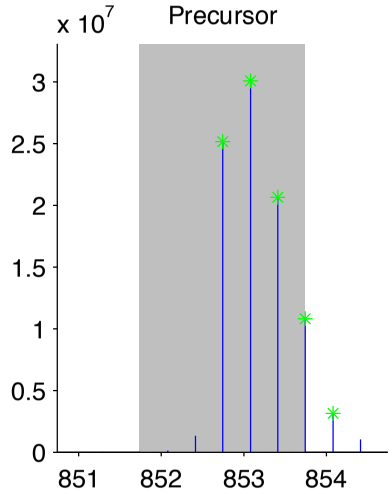


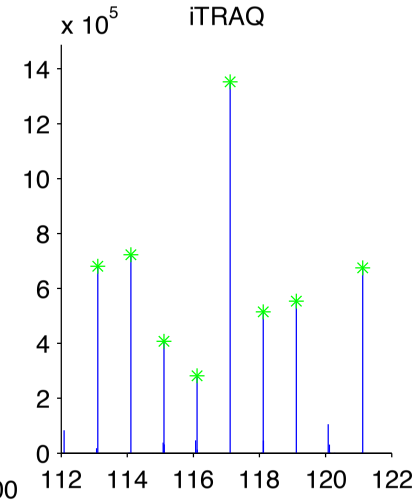



y S S D P T G A L T E D S I D D T F L P V P E Y I N Q S V P K
EGFR
Charge State: +3
Scan Number: 9096
File Name: 20140531_1A_validation.RAW
x 10^6
Precursor
iTRAQ
x 10^5

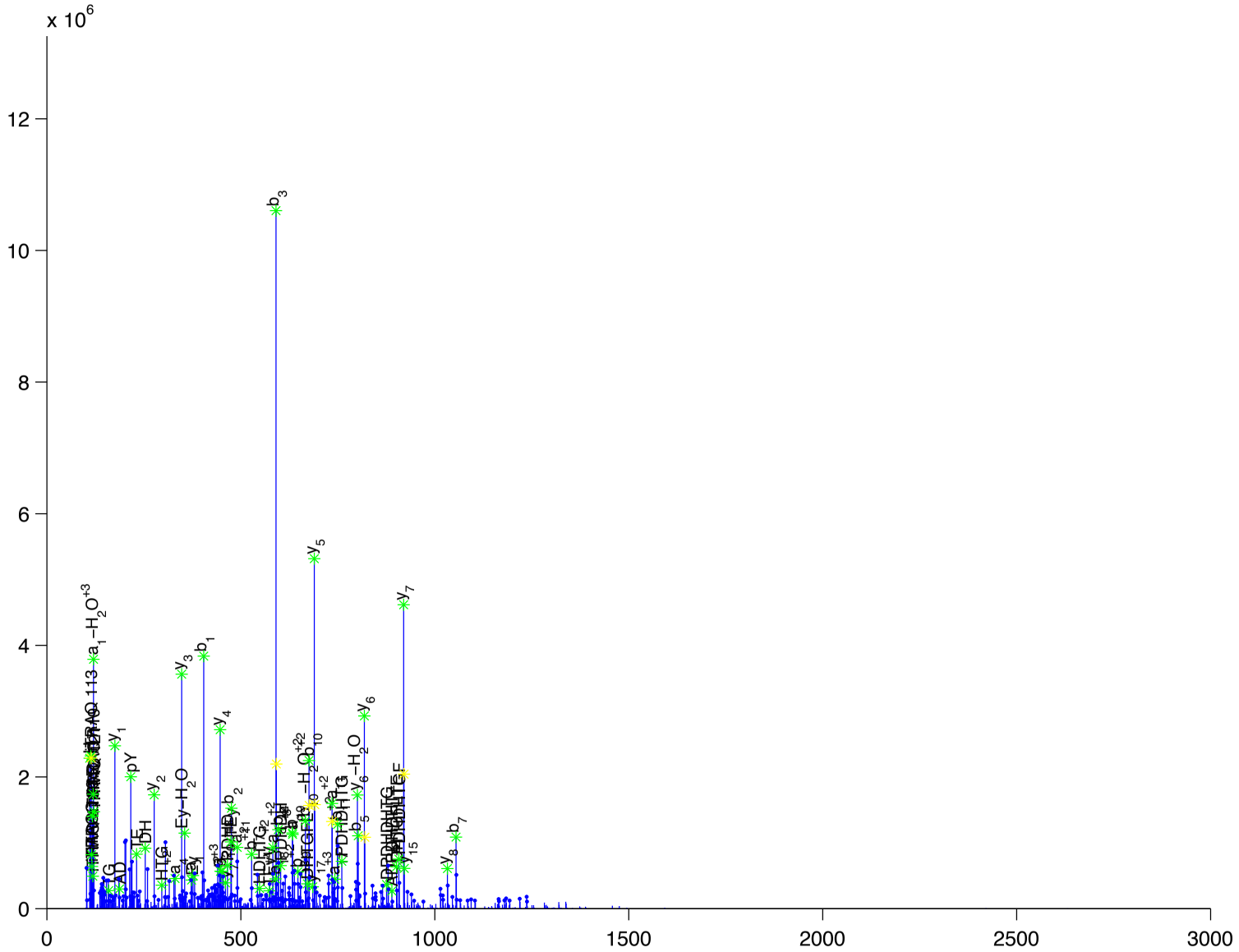
ERK1
Charge State: +3
Scan Number: 6421
File Name: 20140531_1A_validation.RAW

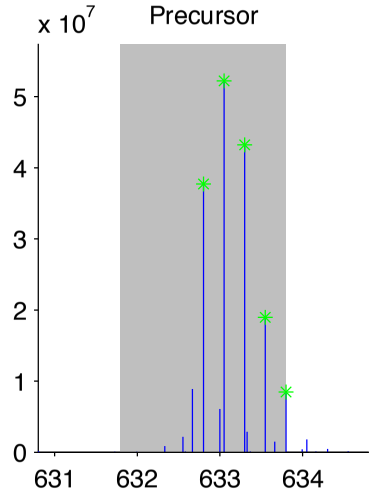


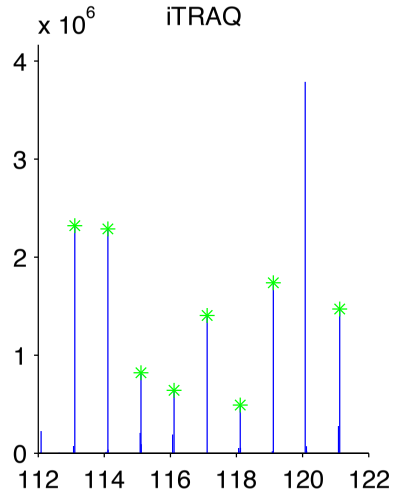




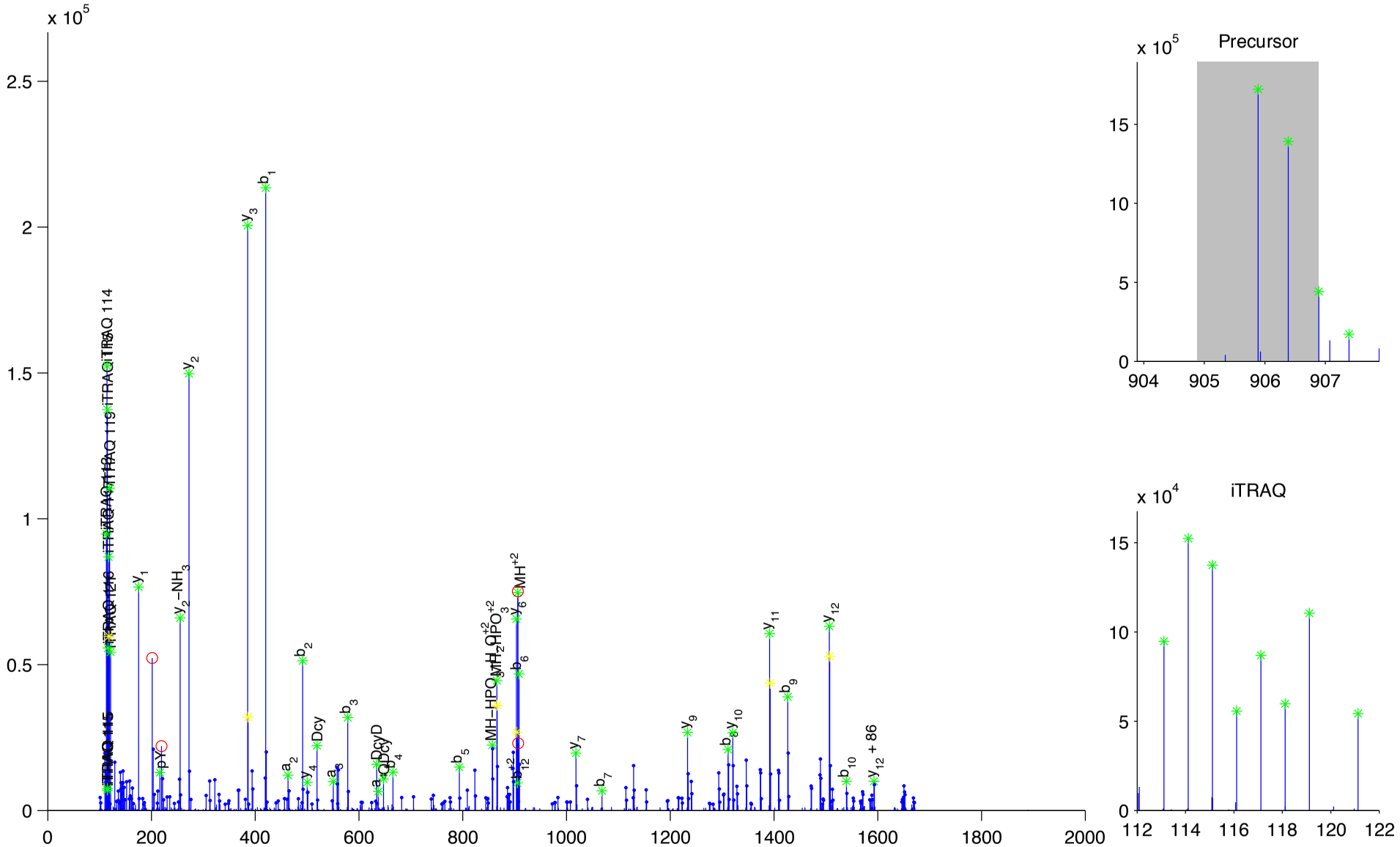
ERK2
Charge State: +4
Scan Number: 6139
File Name: 20140531_1A_validation.RAW

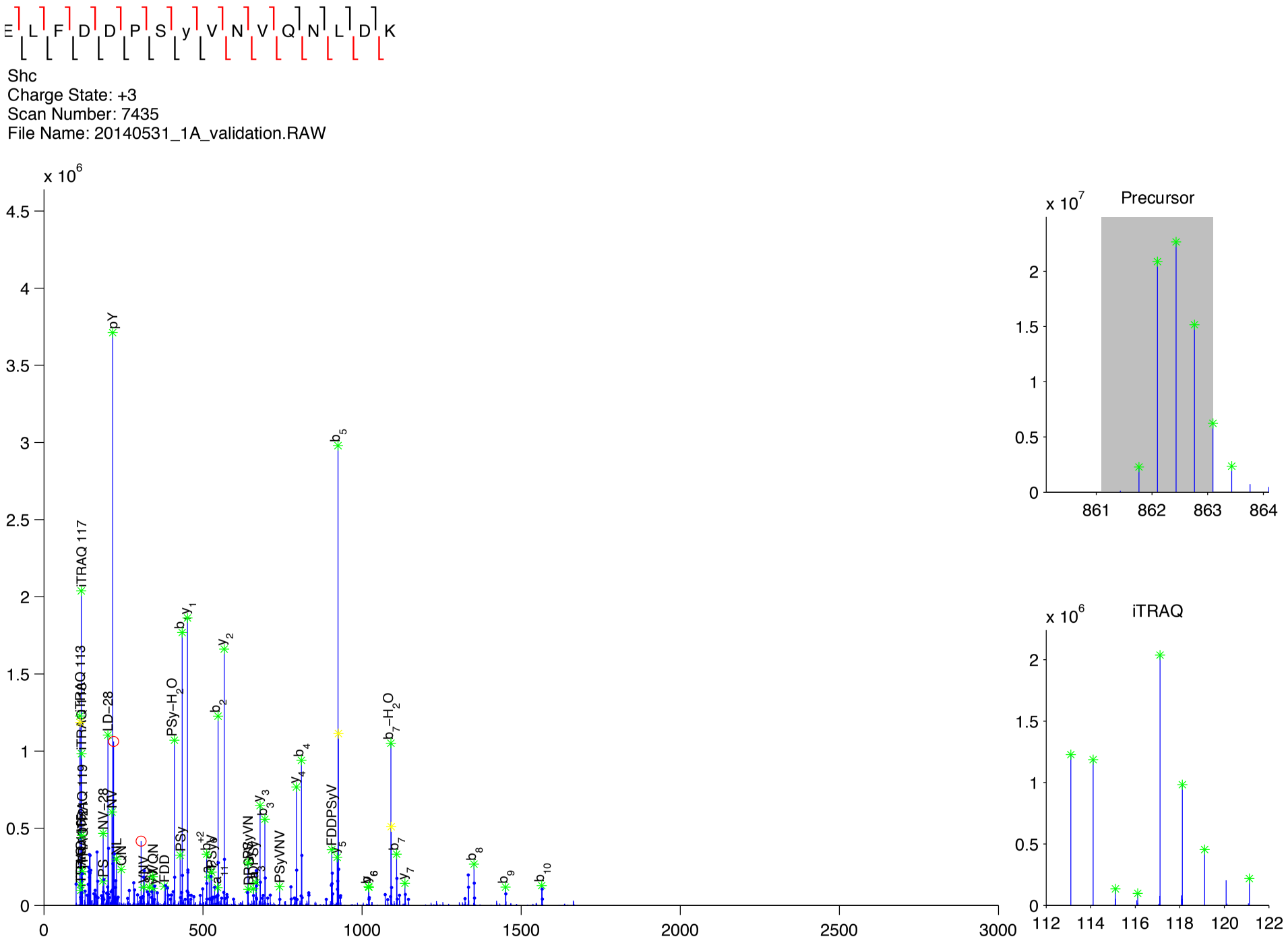


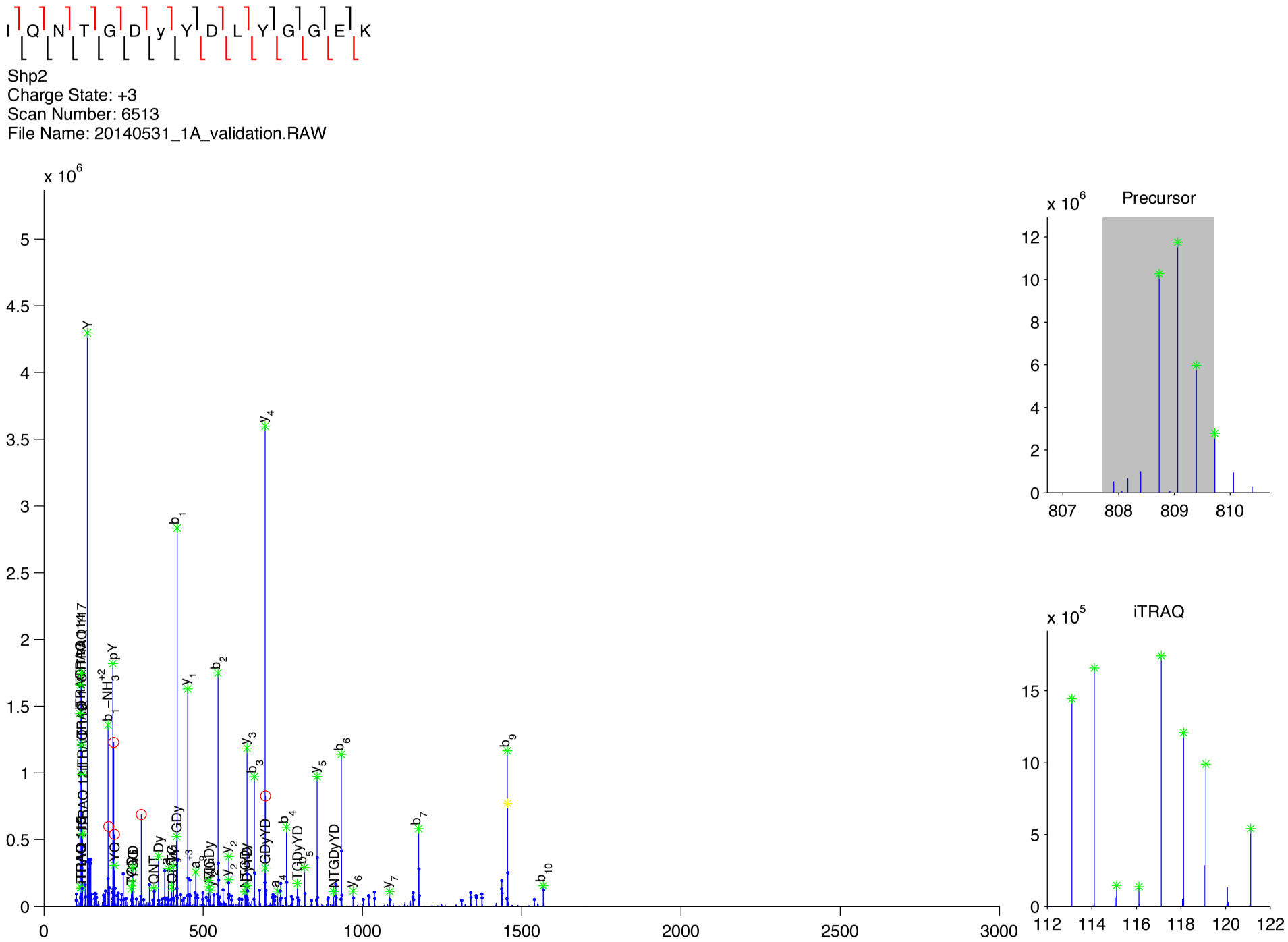




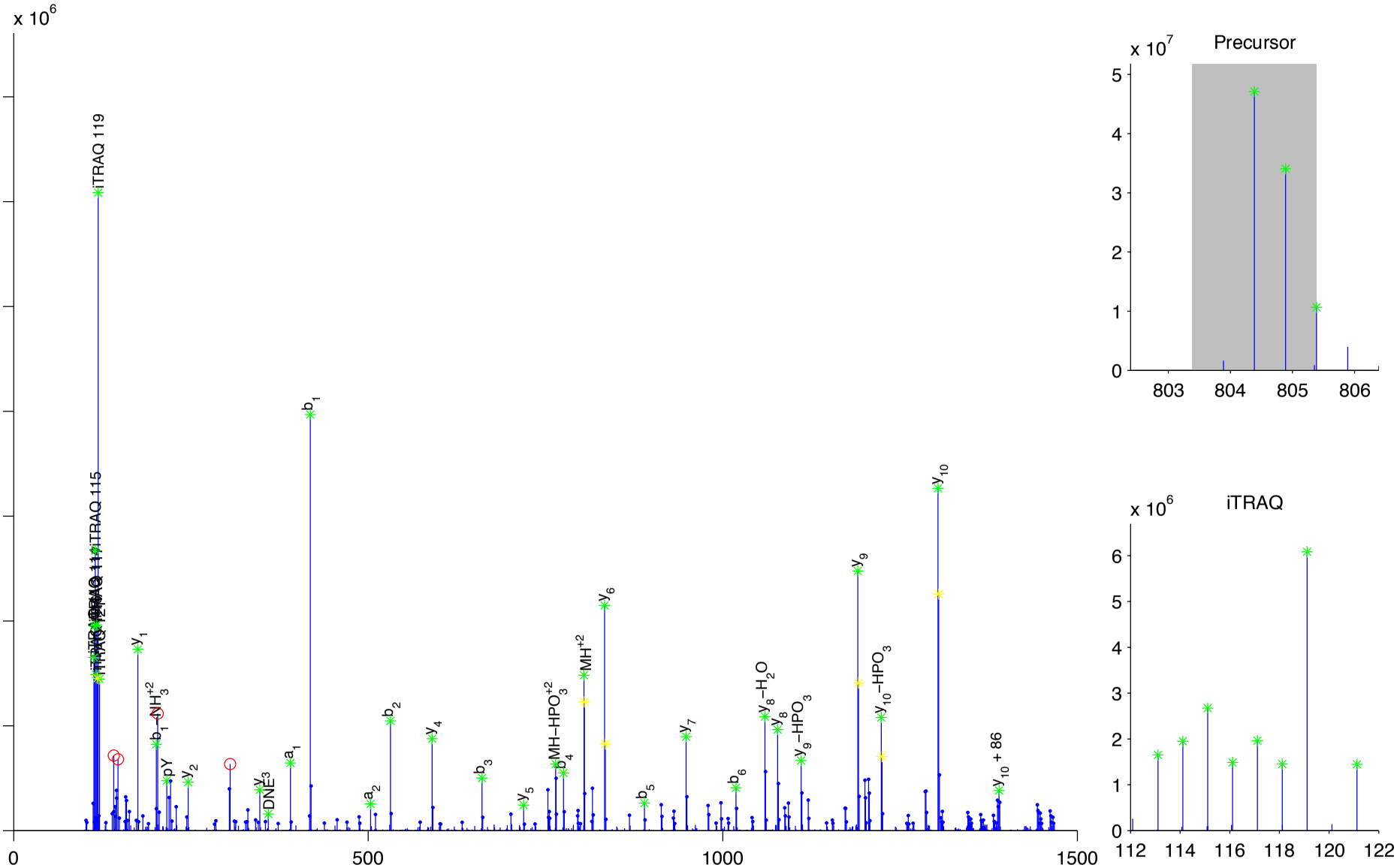
Gab1
Charge State: +2
Scan Number: 4797
File Name: 20140531_1A_validation.RAW



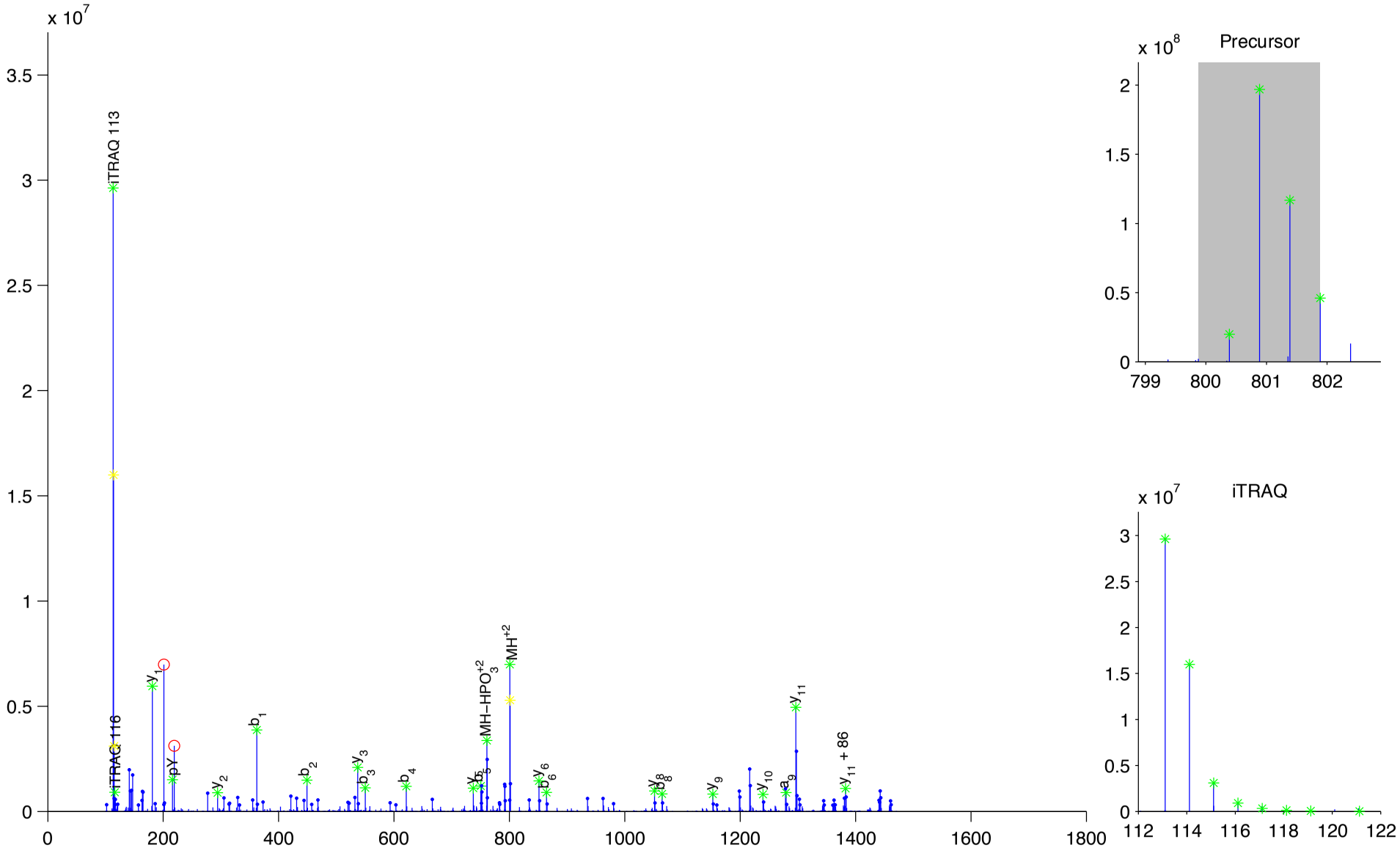

E L F D D P S y V N V Q N L D K
Shc
Charge State: +3
Scan Number: 7435
File Name: 20140531_1A_validation.RAW
x 10^6
iTRAQ 117
pY
PSy–H2O
b7–H2O
FDDPSyV
PSyVNV
x 10^7
Precursor
x 10^6
iTRAQ

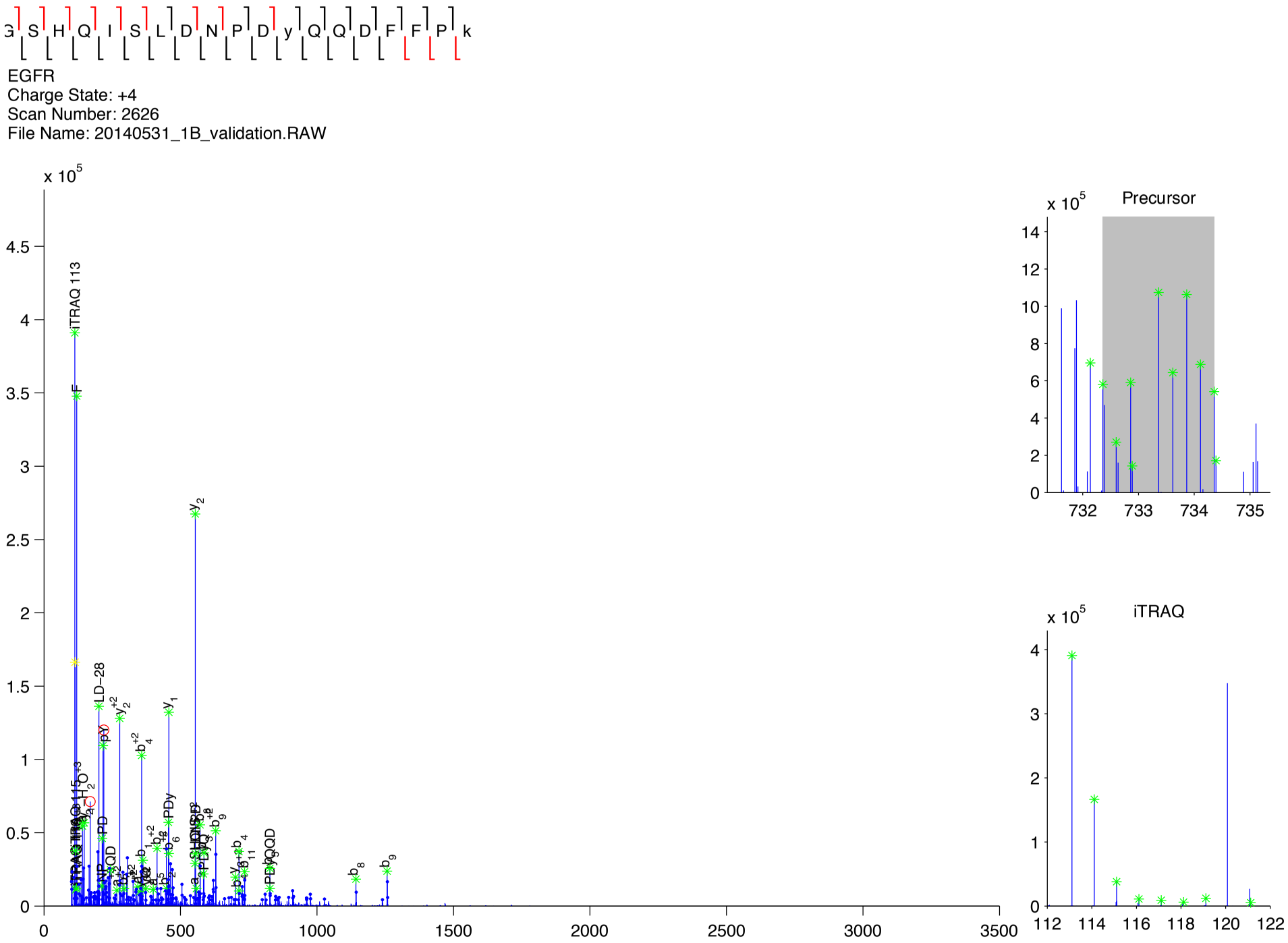

I Q N T G D y Y D L Y G G E K
Shp2
Charge State: +3
Scan Number: 6513
File Name: 20140531_1A_validation.RAW
x 10^6
Precursor
iTRAQ
x 10^5

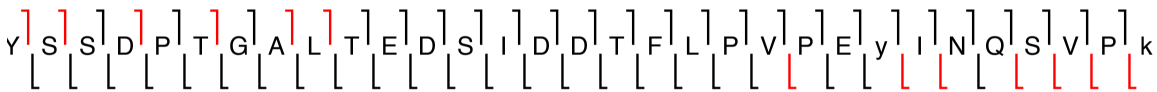
Src
Charge State: +2
Scan Number: 5613
File Name: 20140531_1A_validation.RAW



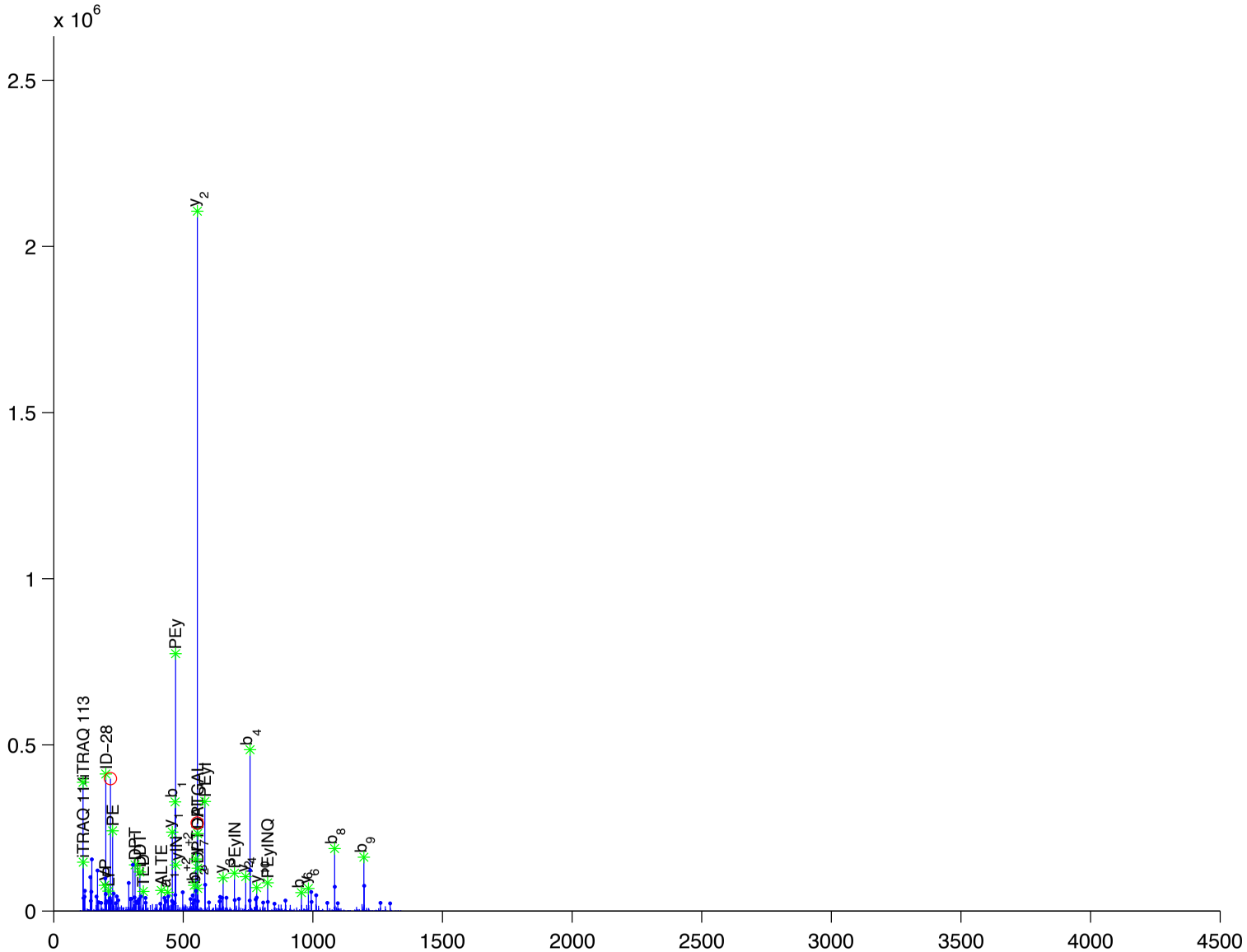
EGFR
Charge State: +2
Scan Number: 2259
File Name: 20140531_1B_validation.RAW

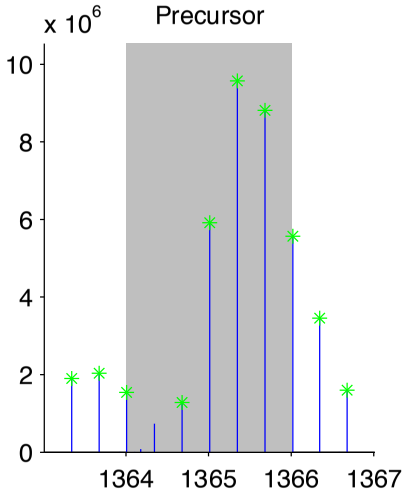


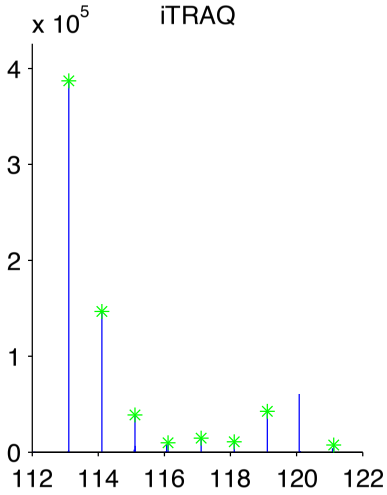

s S H Q I S L D N P D y Q Q D F F P k
EGFR
Charge State: +4
Scan Number: 2626
File Name: 20140531_1B_validation.RAW
x 10^5
iTRAQ 113
LD-28
PD
PDy
QQD
Precursor
iTRAQ

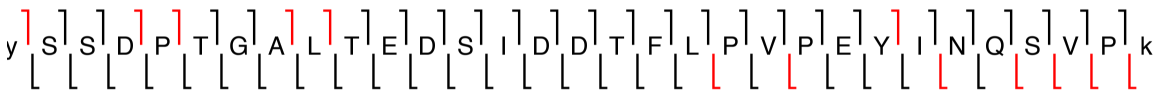


EGFR
Charge State: +3
Scan Number: 2857
File Name: 20140531_1B_validation.RAW

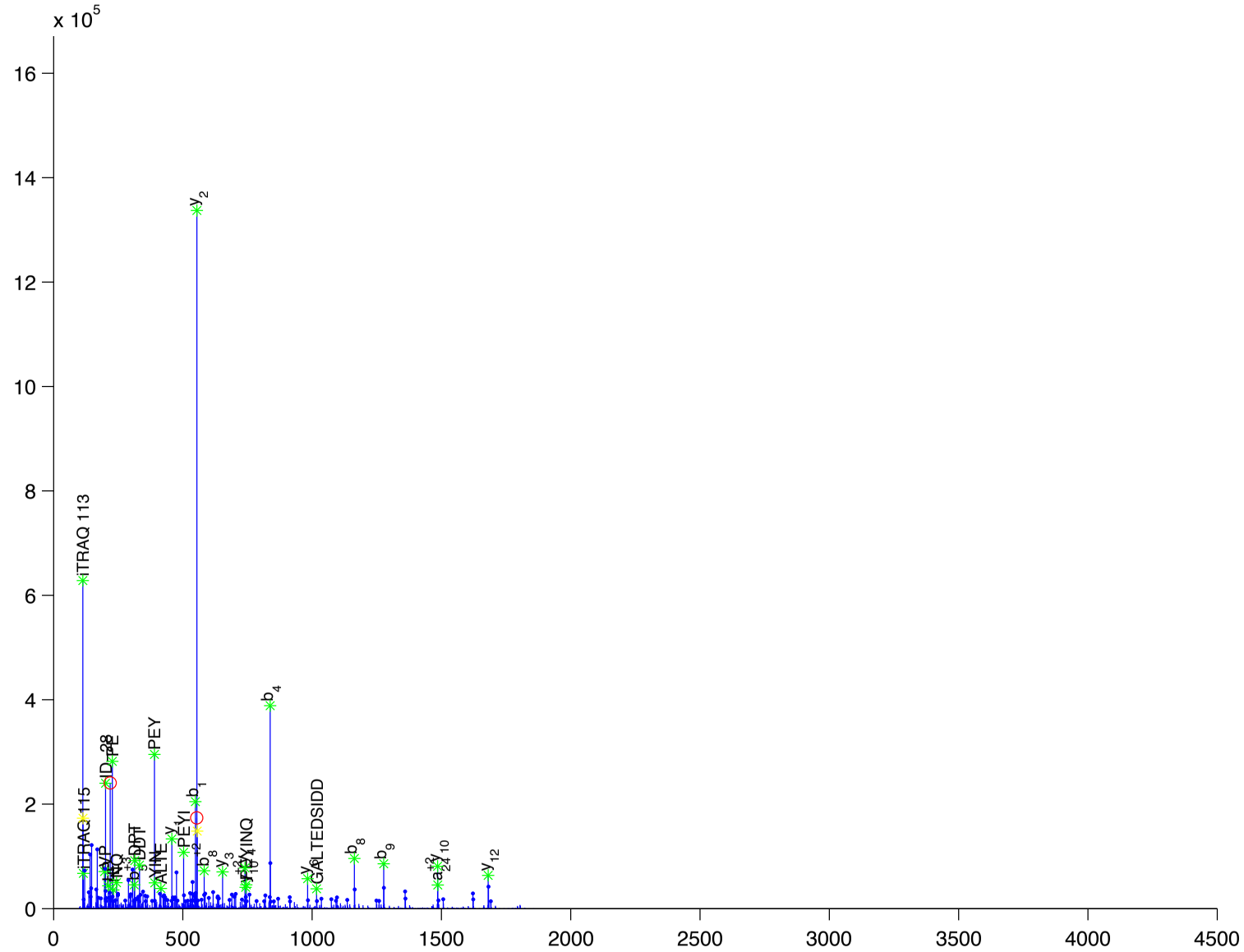


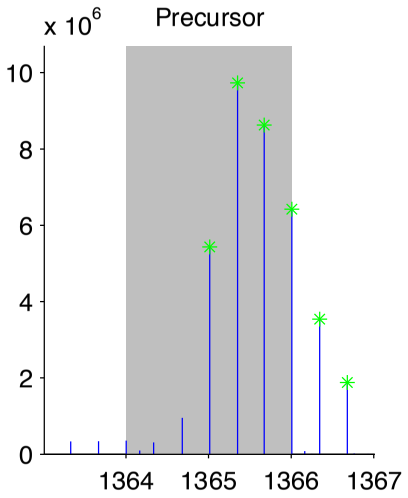


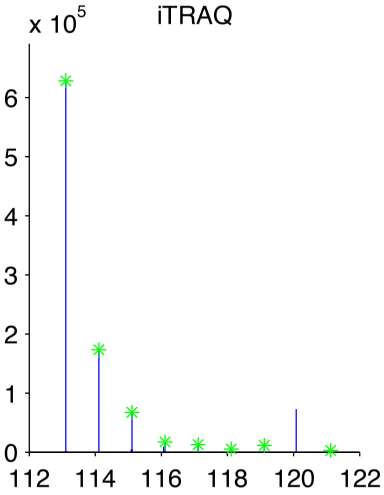




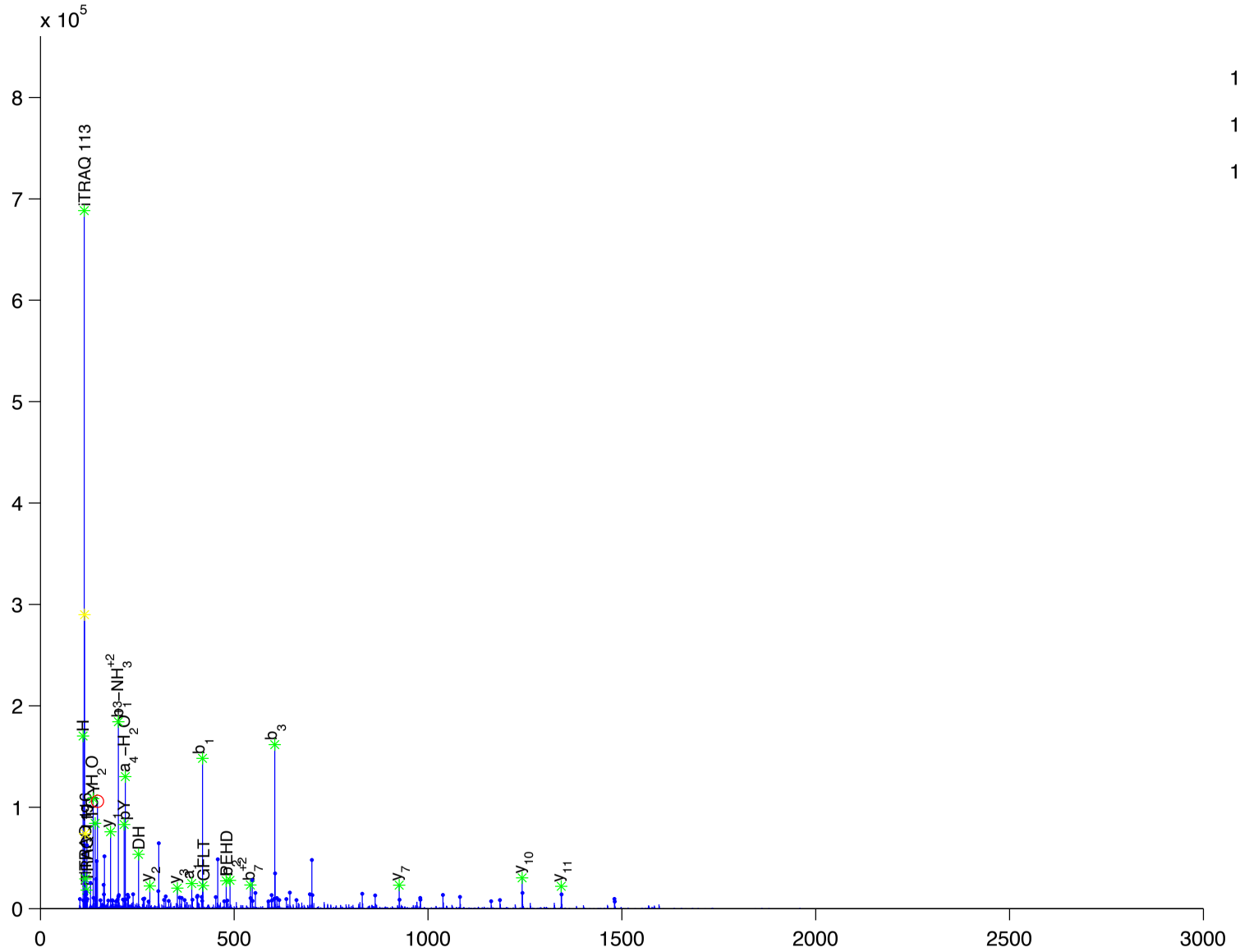
EGFR
Charge State: +3
Scan Number: 2922
File Name: 20140531_1B_validation.RAW

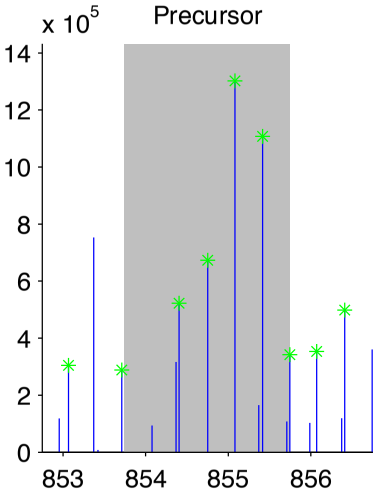


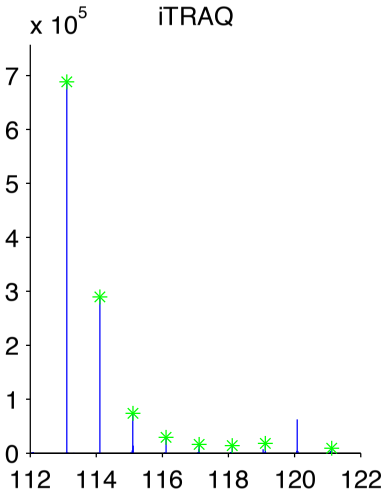




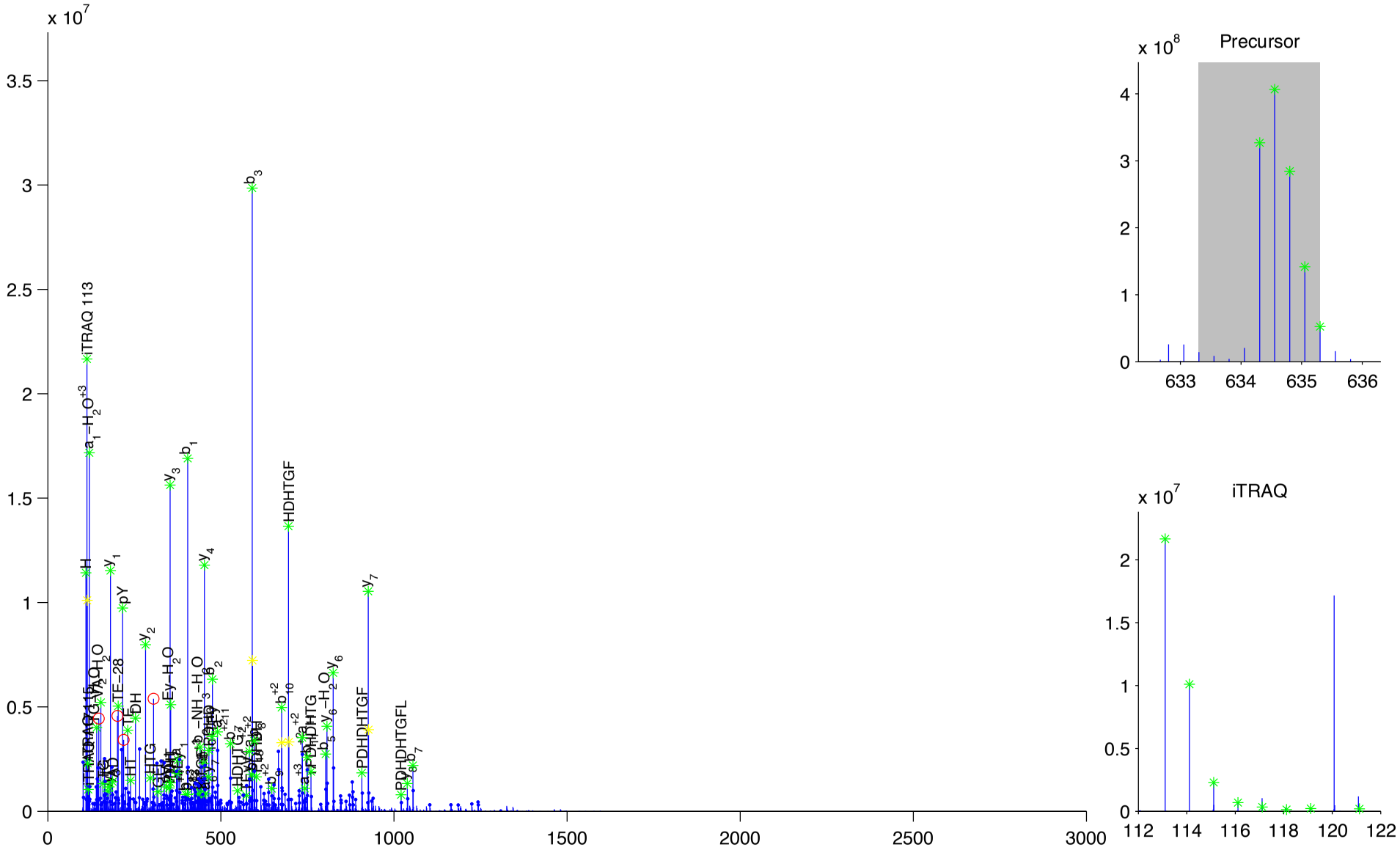
ERK1
Charge State: +3
Scan Number: 2505
File Name: 20140531_1B_validation.RAW

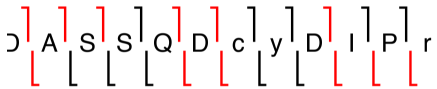




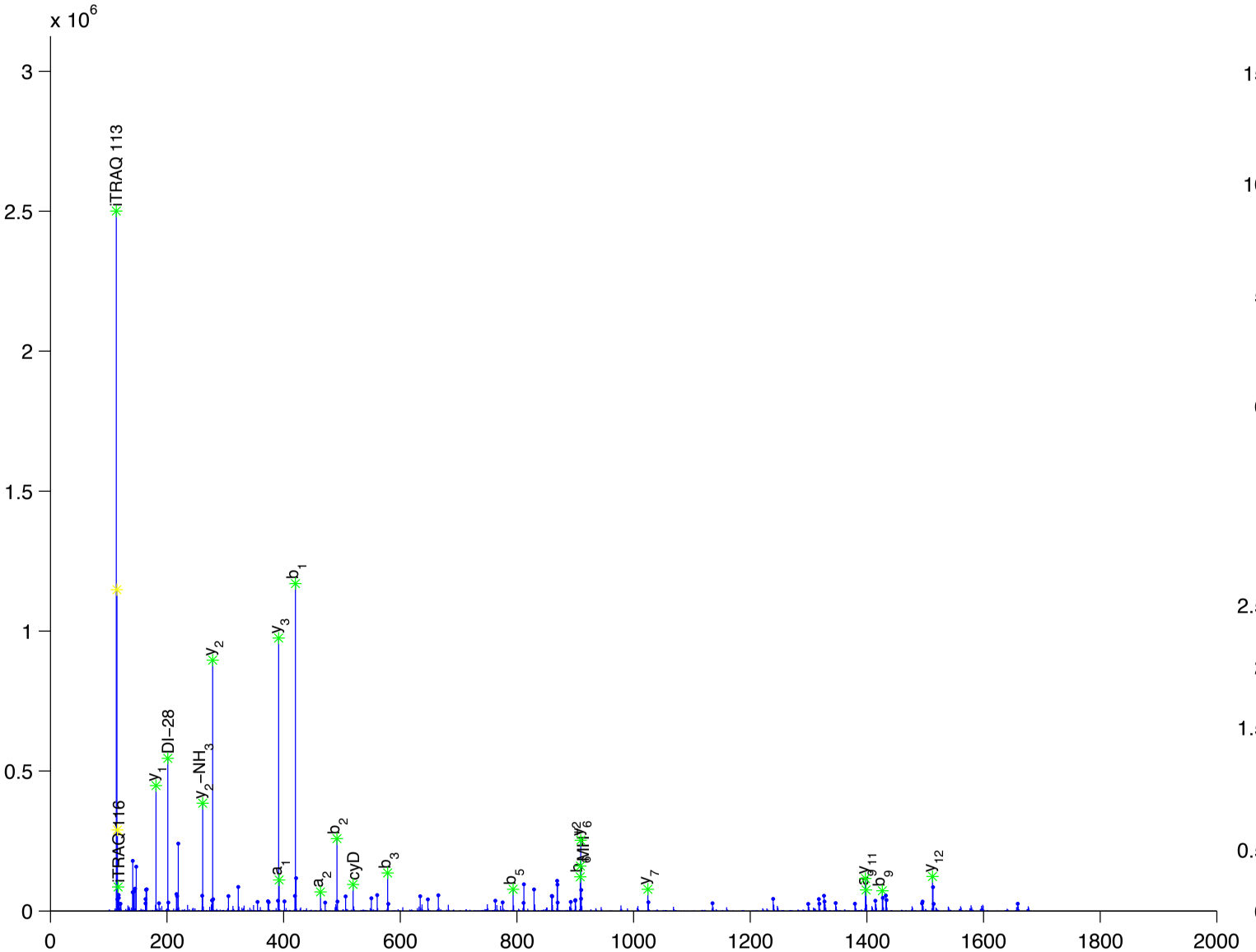


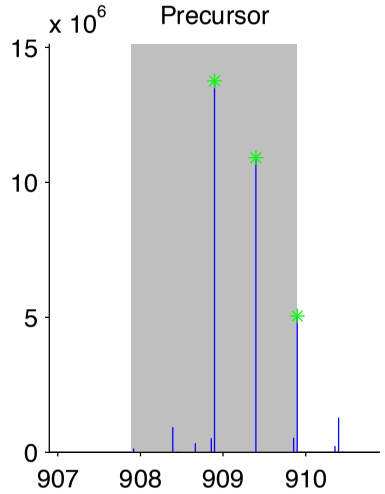
ERK2
Charge State: +4
Scan Number: 2475
File Name: 20140531_1B_validation.RAW

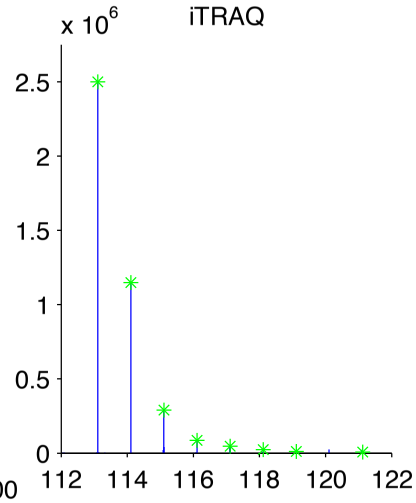




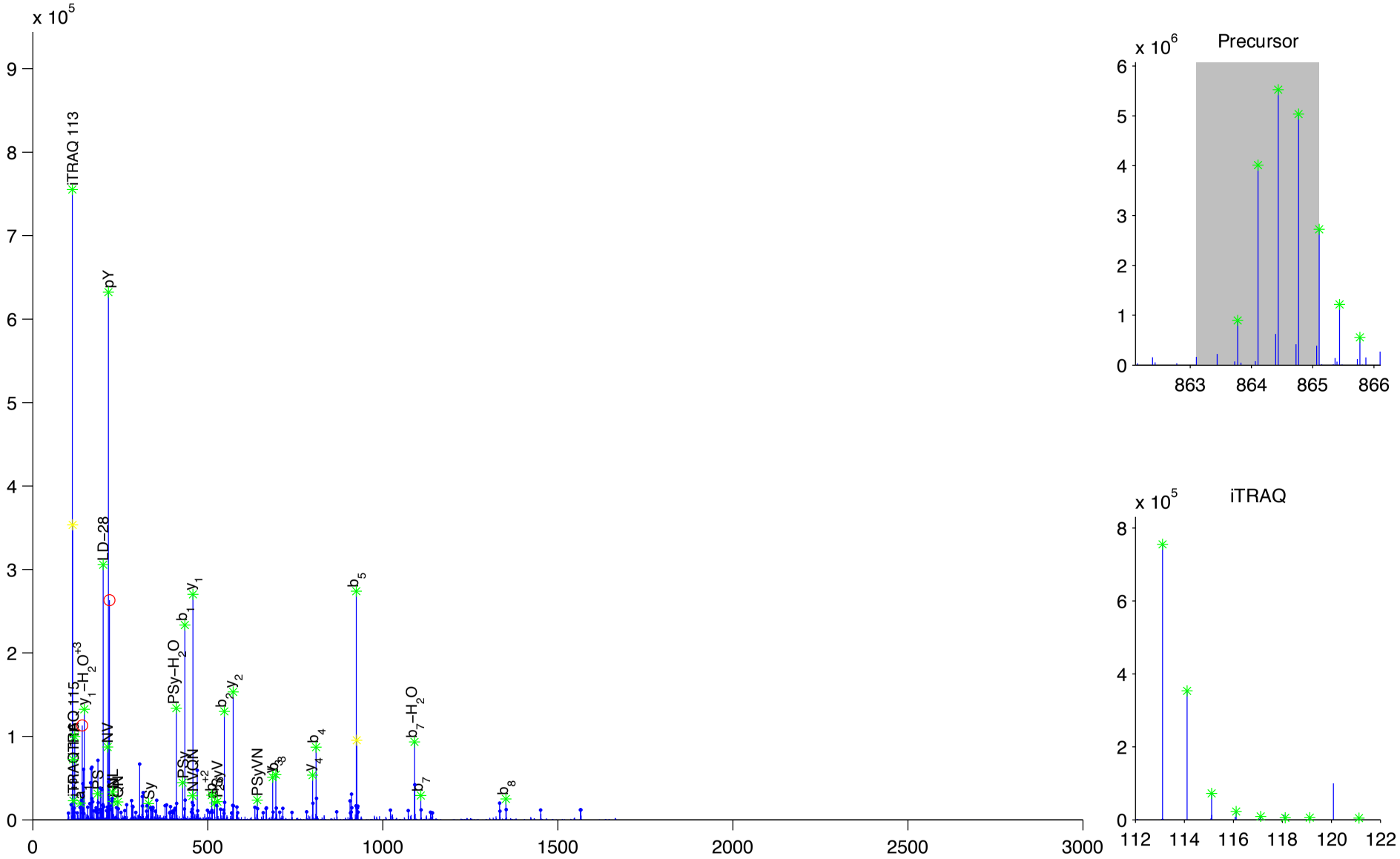
Gab1
Charge State: +2
Scan Number: 2268
File Name: 20140531_1B_validation.RAW

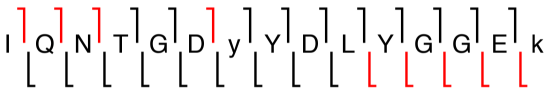




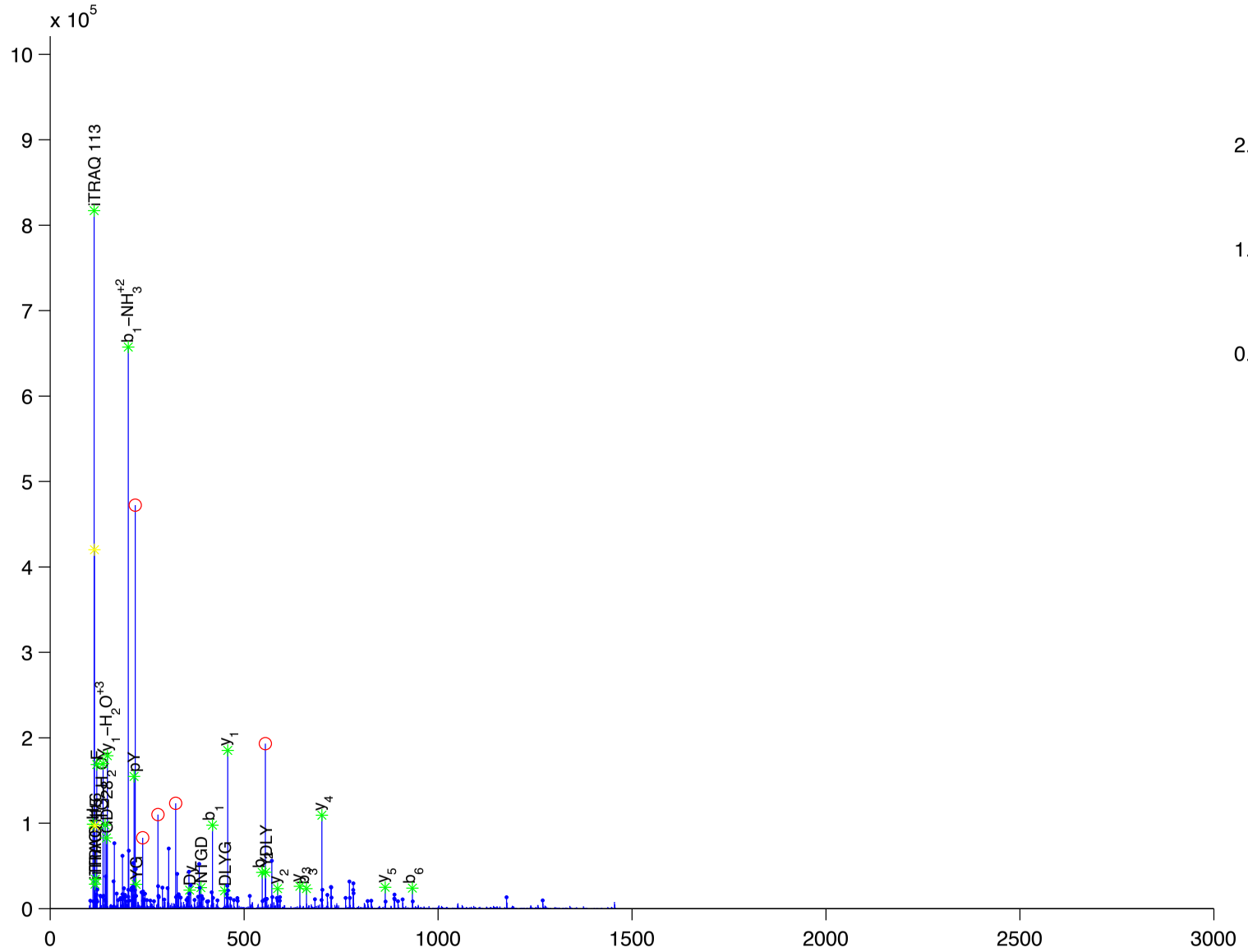


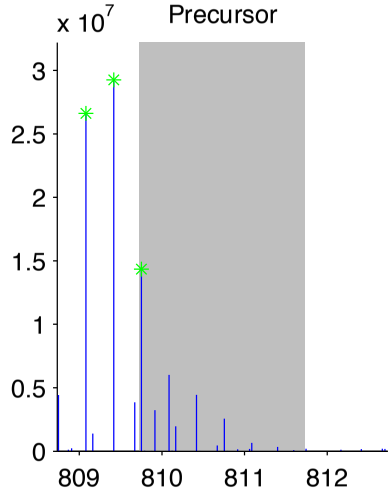
Shc
Charge State: +3
Scan Number: 2718
File Name: 20140531_1B_validation.RAW

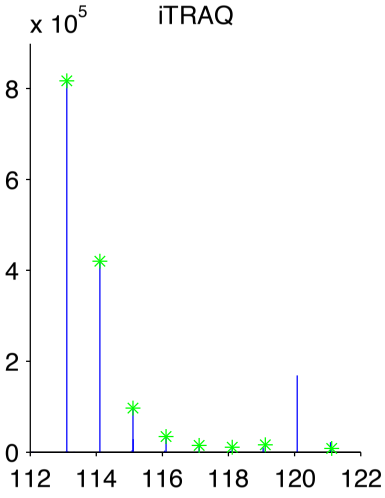




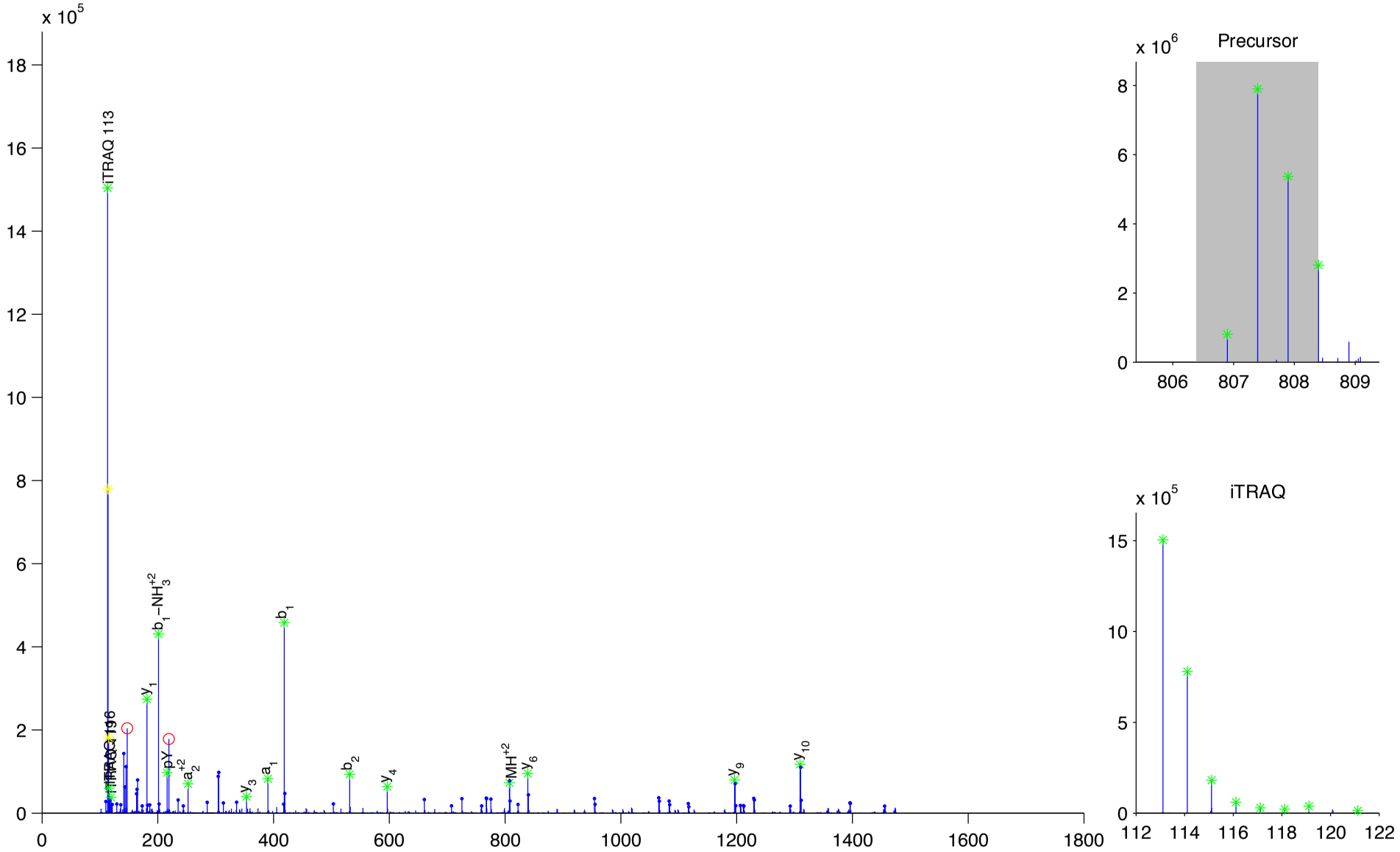
Shp2
Charge State: +3
Scan Number: 2499
File Name: 20140531_1B_validation.RAW



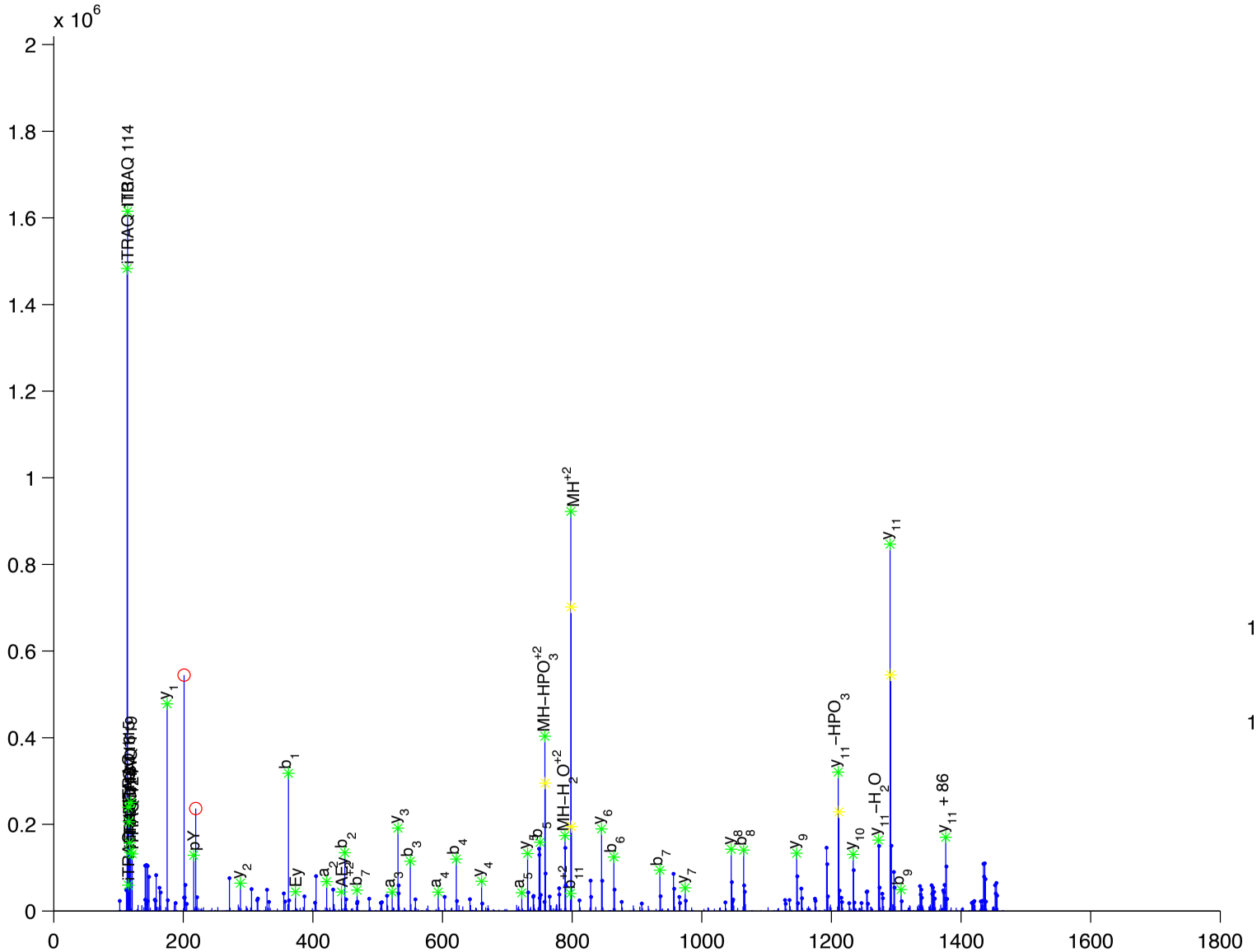


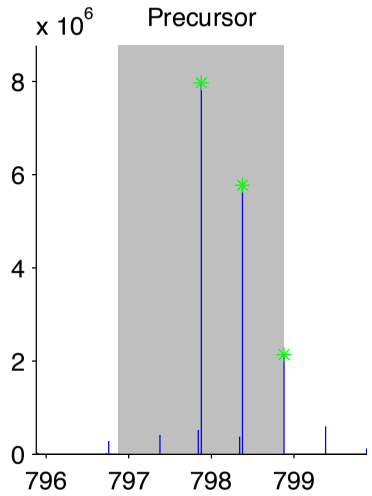


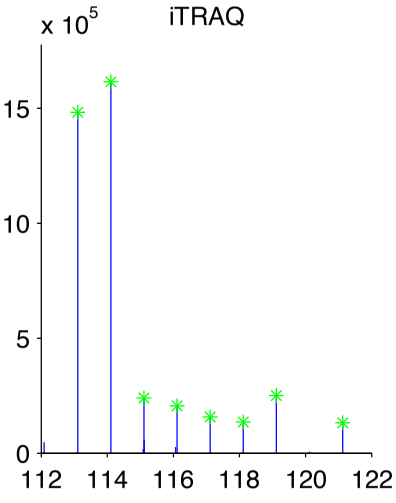
Src
Charge State: +2
Scan Number: 2427
File Name: 20140531_1B_validation.RAW



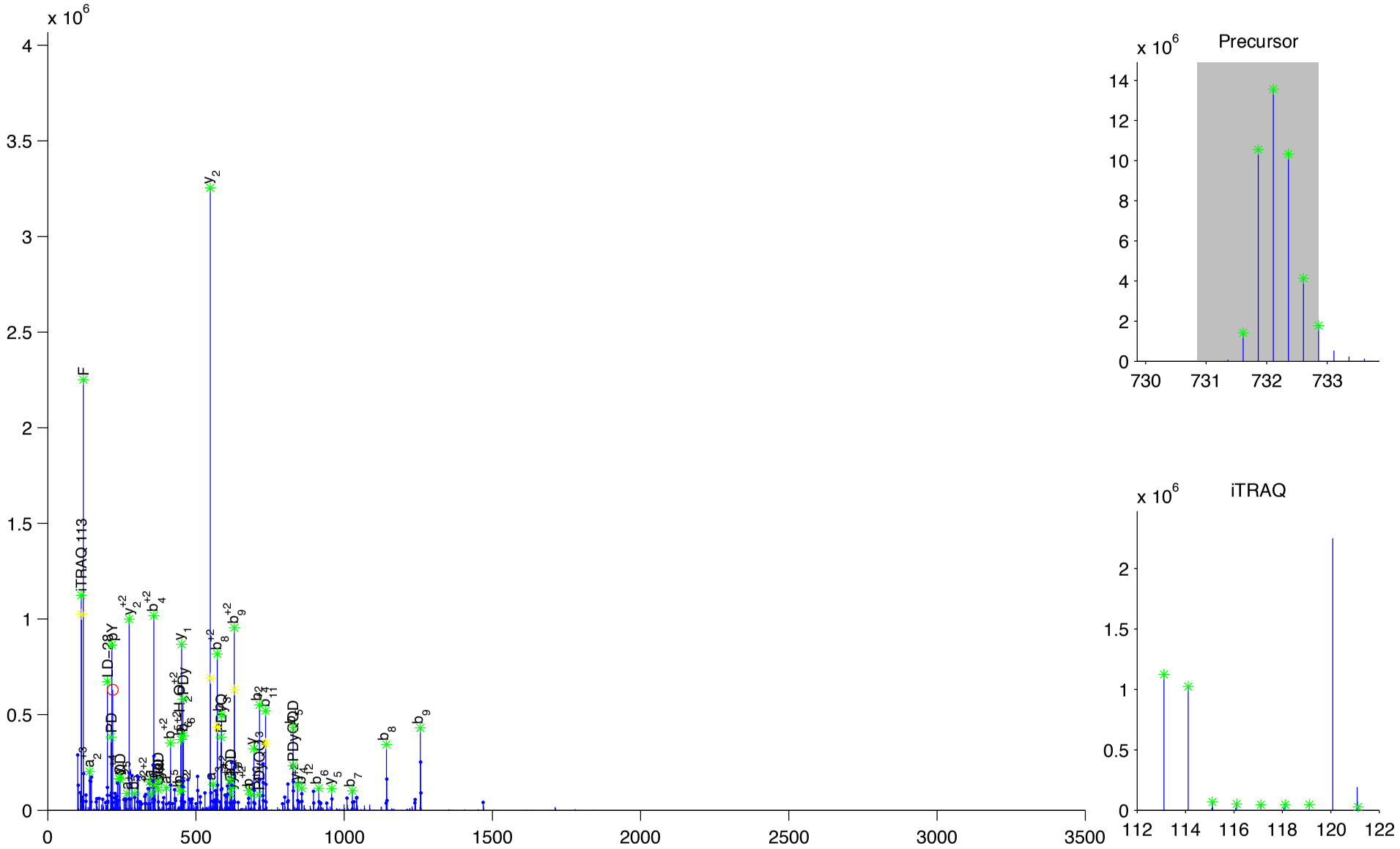
EGFR
Charge State: +2
Scan Number: 4506
File Name: 20140531_2A_validation.RAW

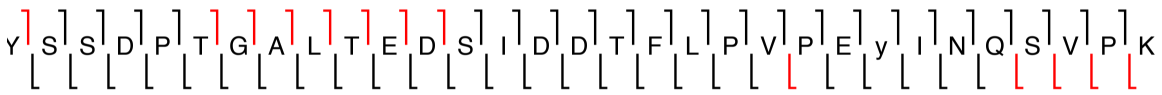




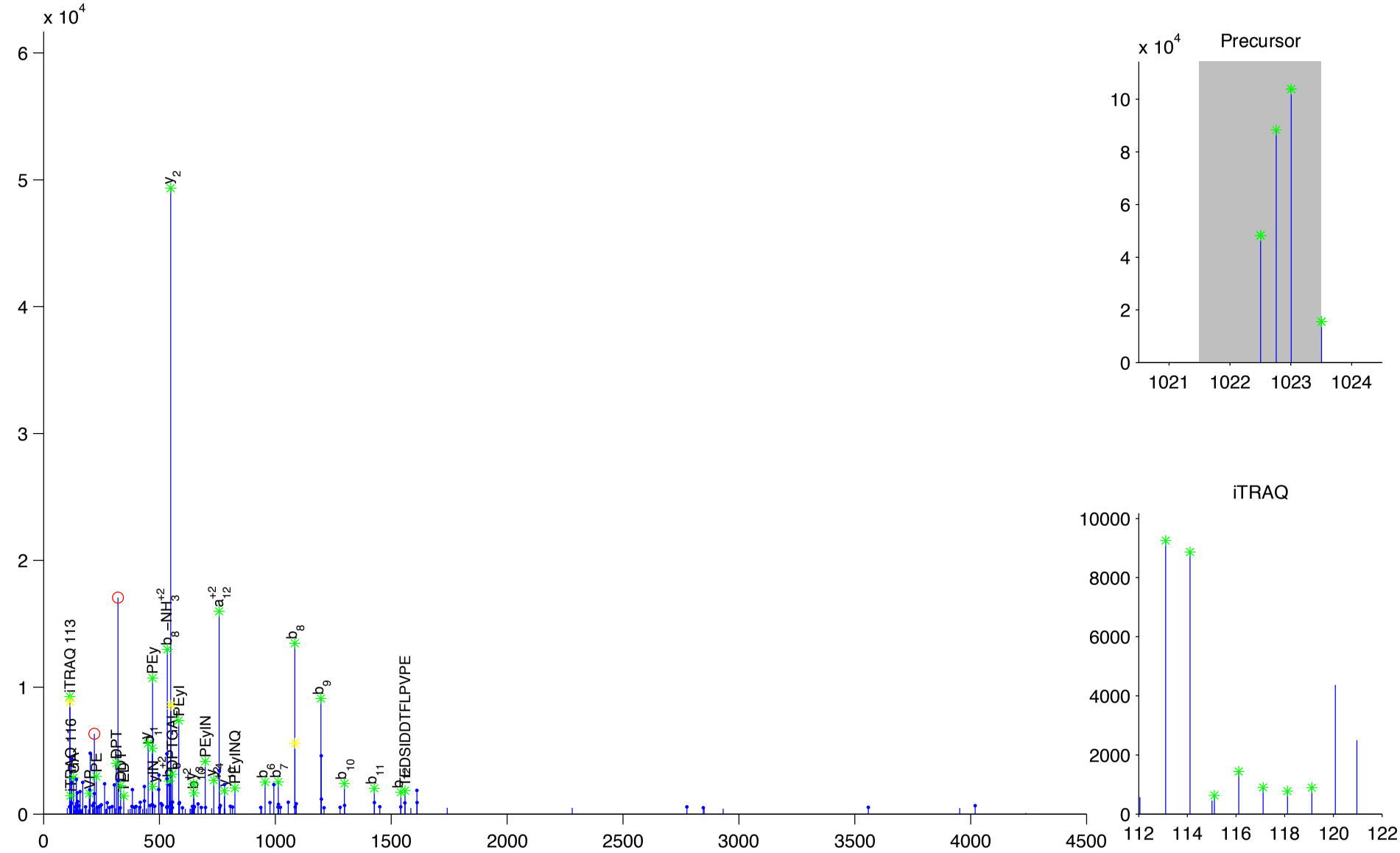


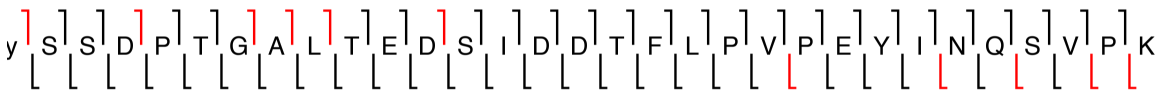
EGFR
Charge State: +4
Scan Number: 6774
File Name: 20140531_2A_validation.RAW



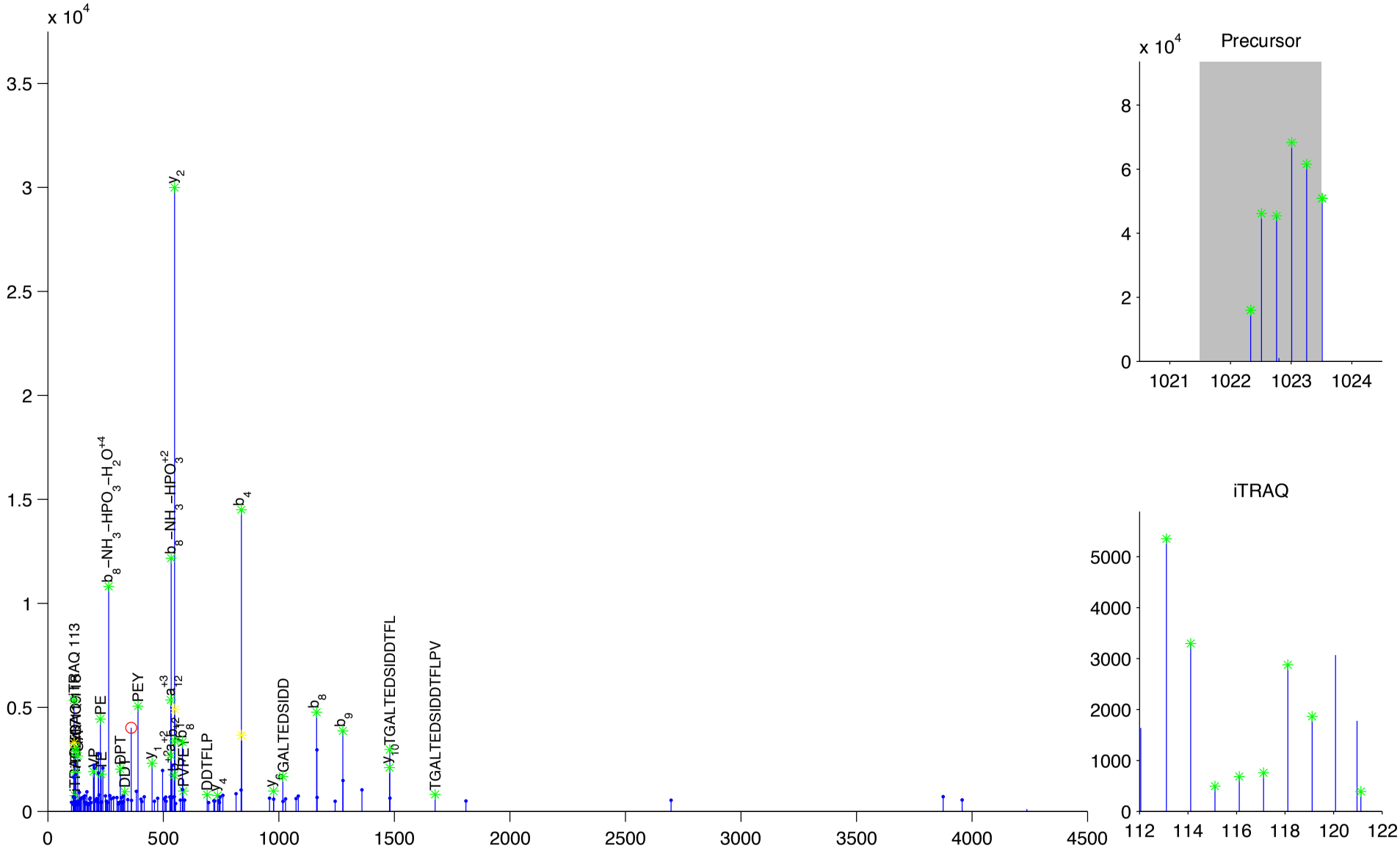


EGFR
Charge State: +4
Scan Number: 8664
File Name: 20140531_2A_validation.RAW

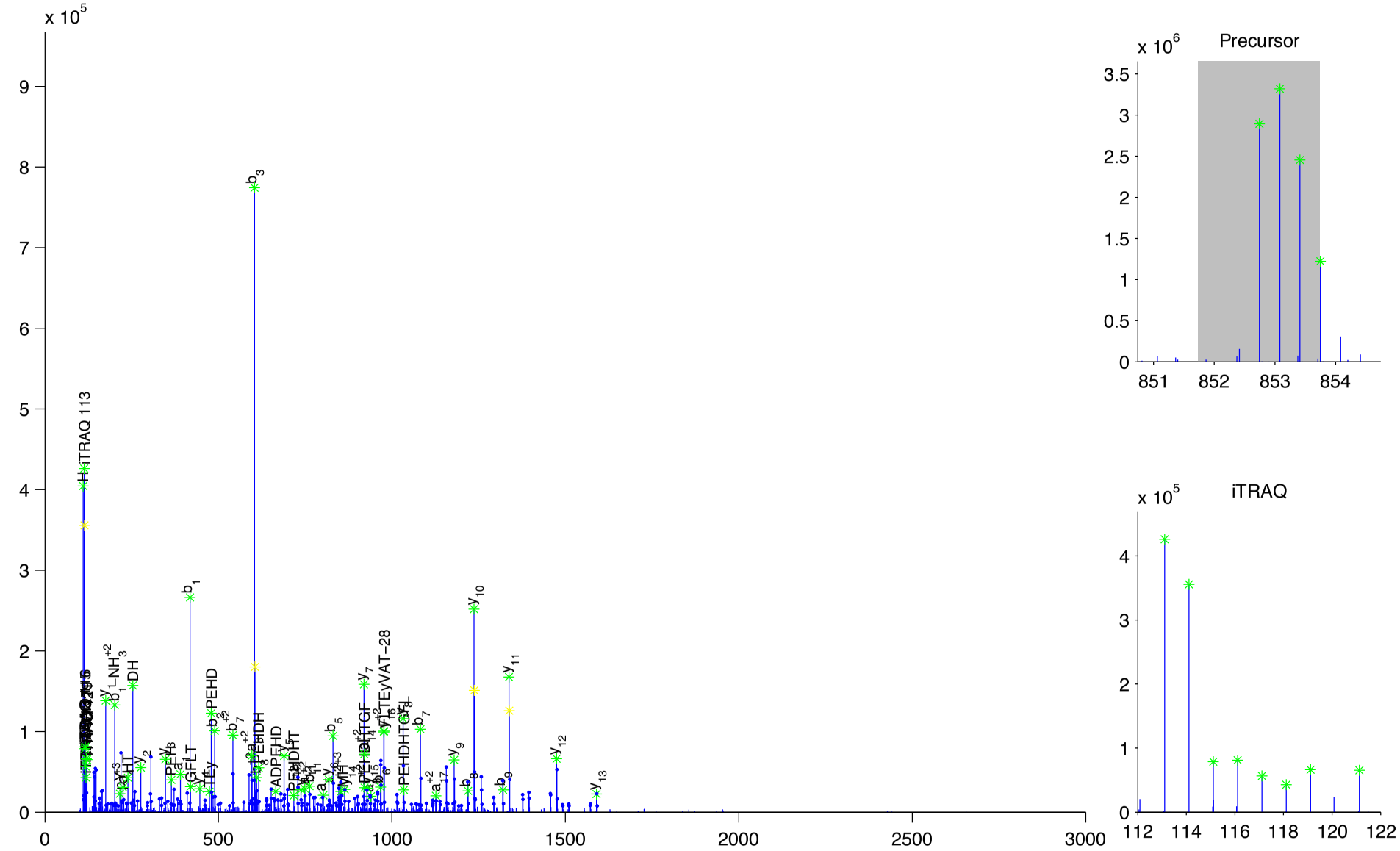




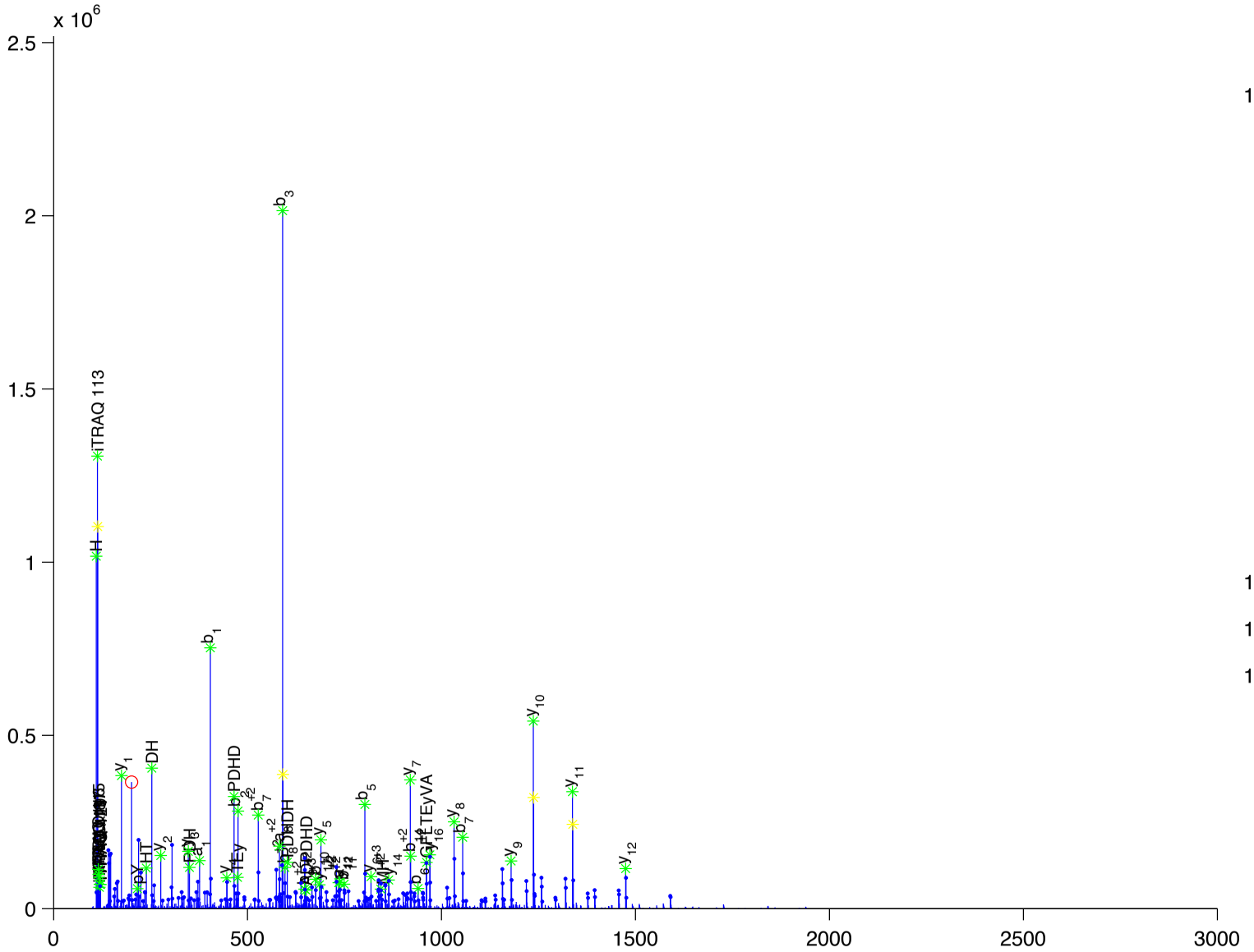
EGFR
Charge State: +4
Scan Number: 9064
File Name: 20140531_2A_validation.RAW

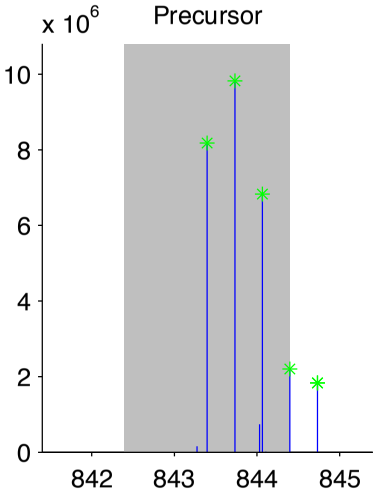


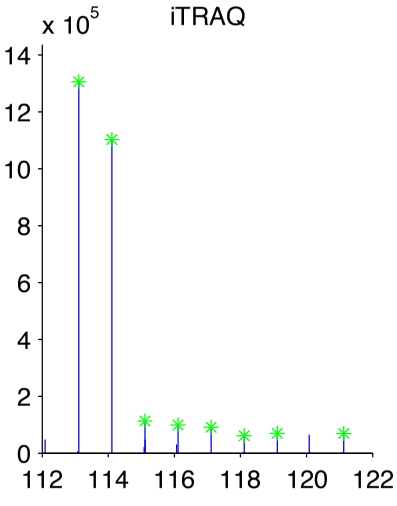
ERK1
Charge State: +3
Scan Number: 6329
File Name: 20140531_2A_validation.RAW

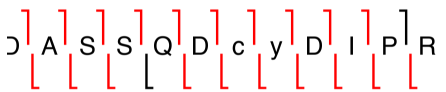


ERK2
Charge State: +3
Scan Number: 6053
File Name: 20140531_2A_validation.RAW

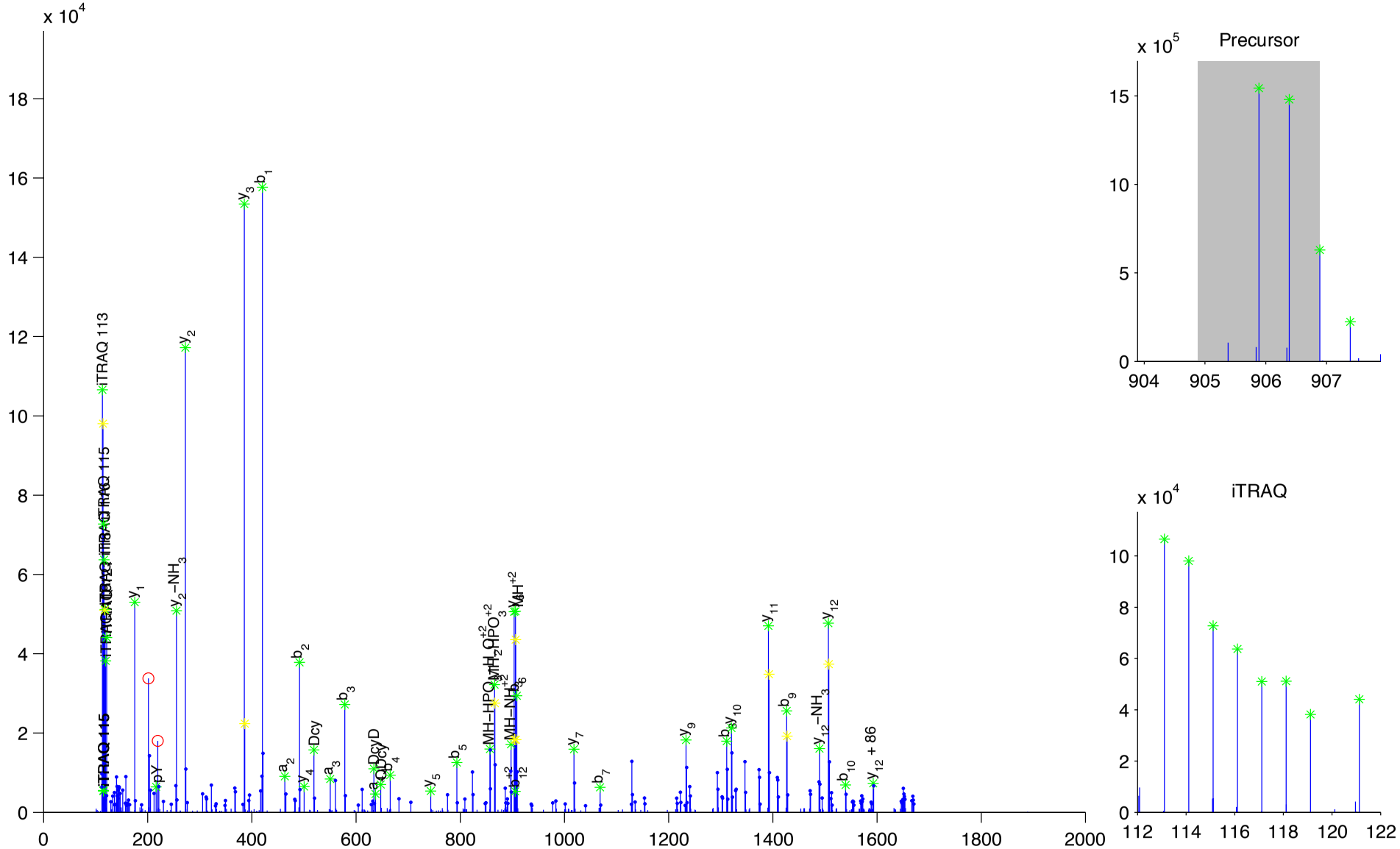




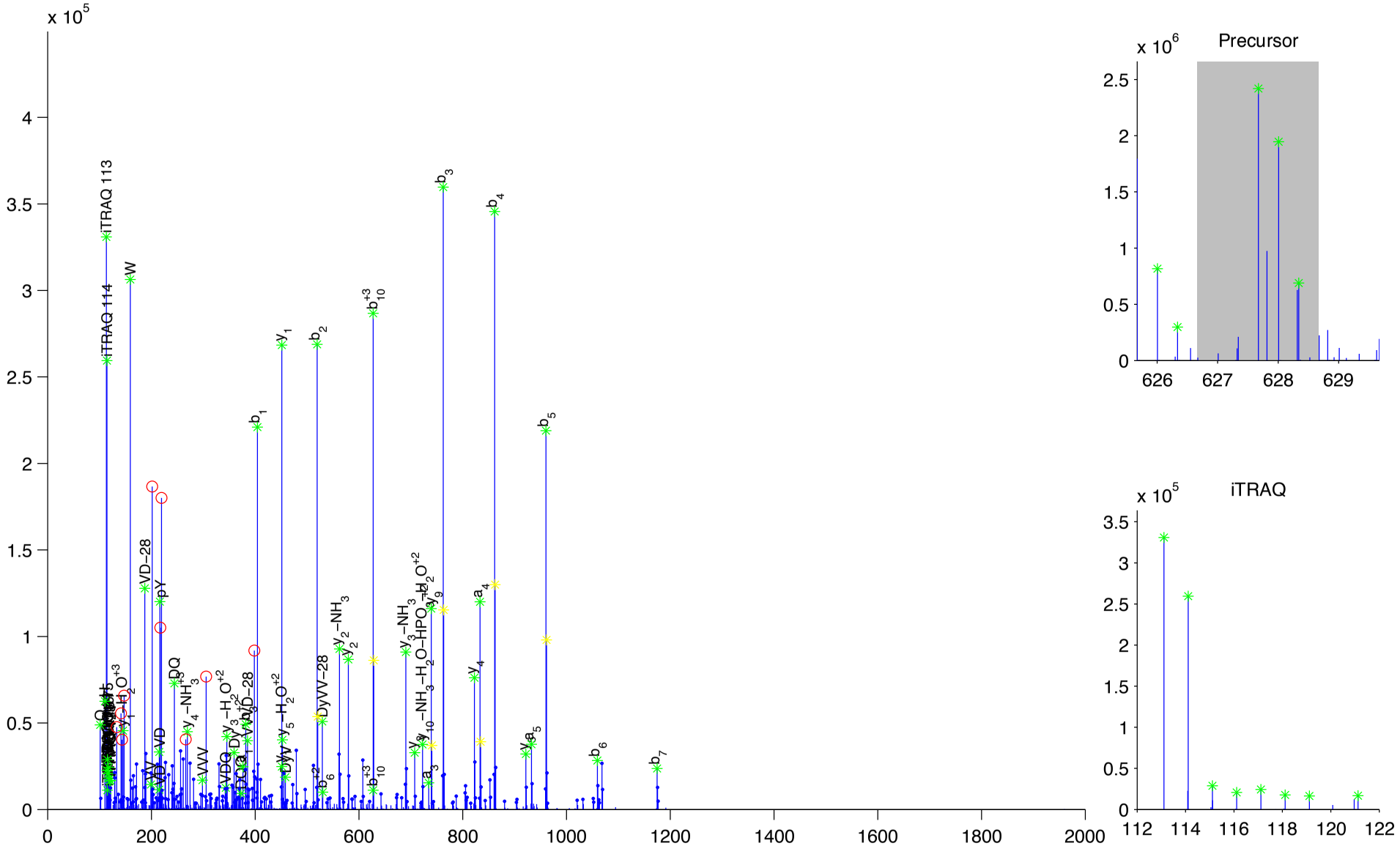


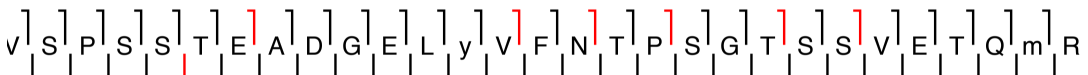


Gab1
Charge State: +2
Scan Number: 4655
File Name: 20140531_2A_validation.RAW

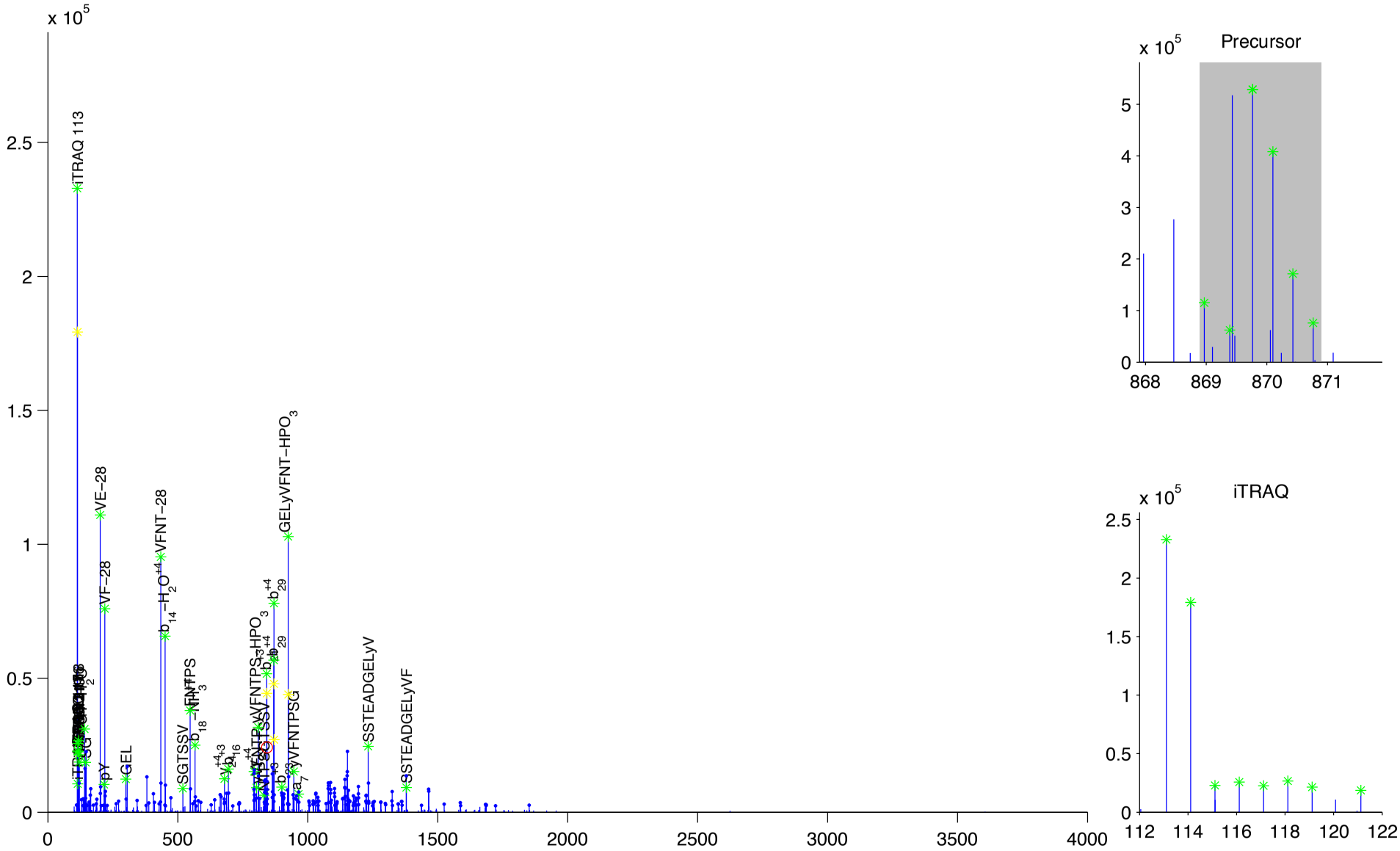


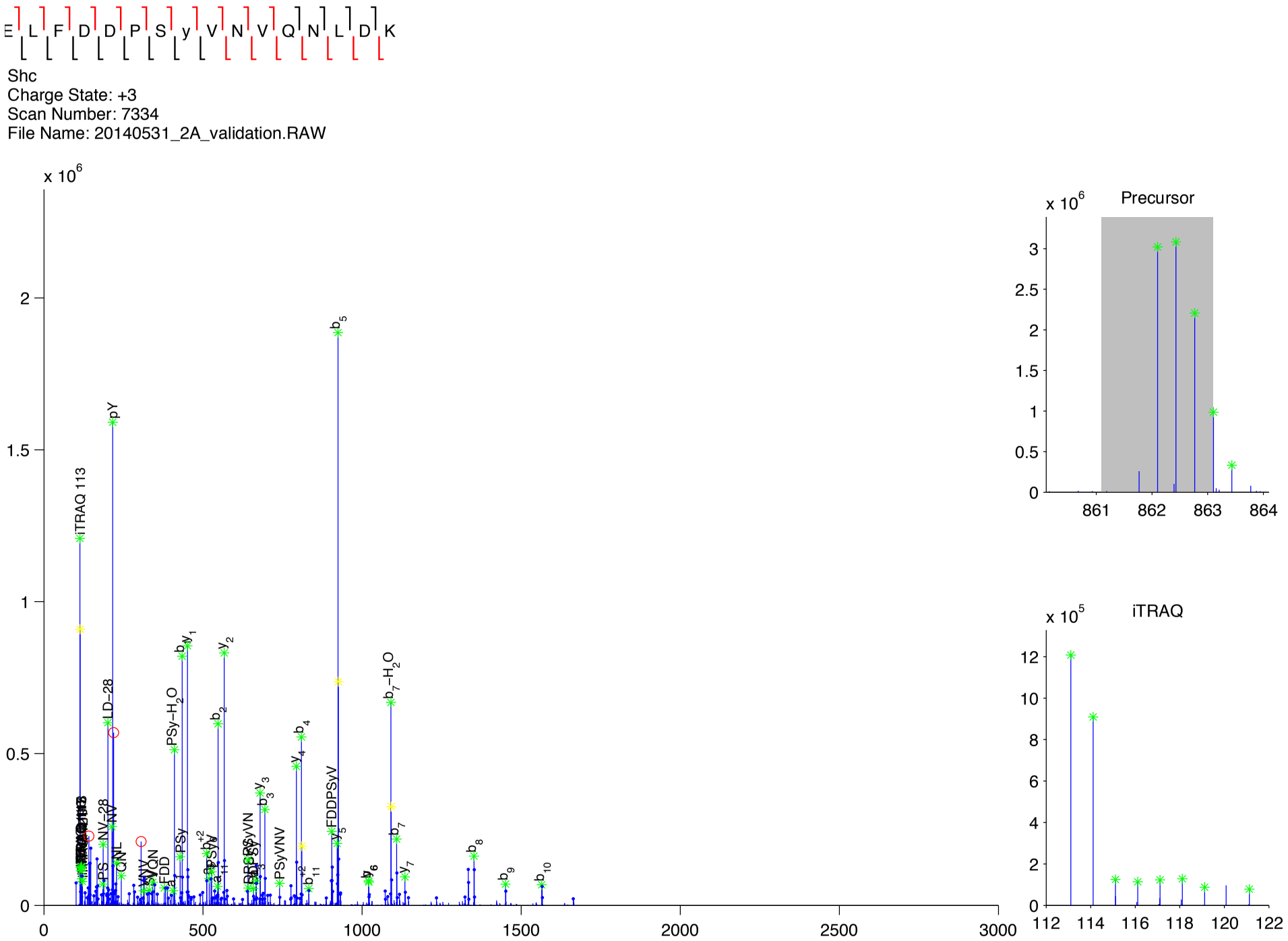
Gab1
Charge State: +3
Scan Number: 5811
File Name: 20140531_2A_validation.RAW

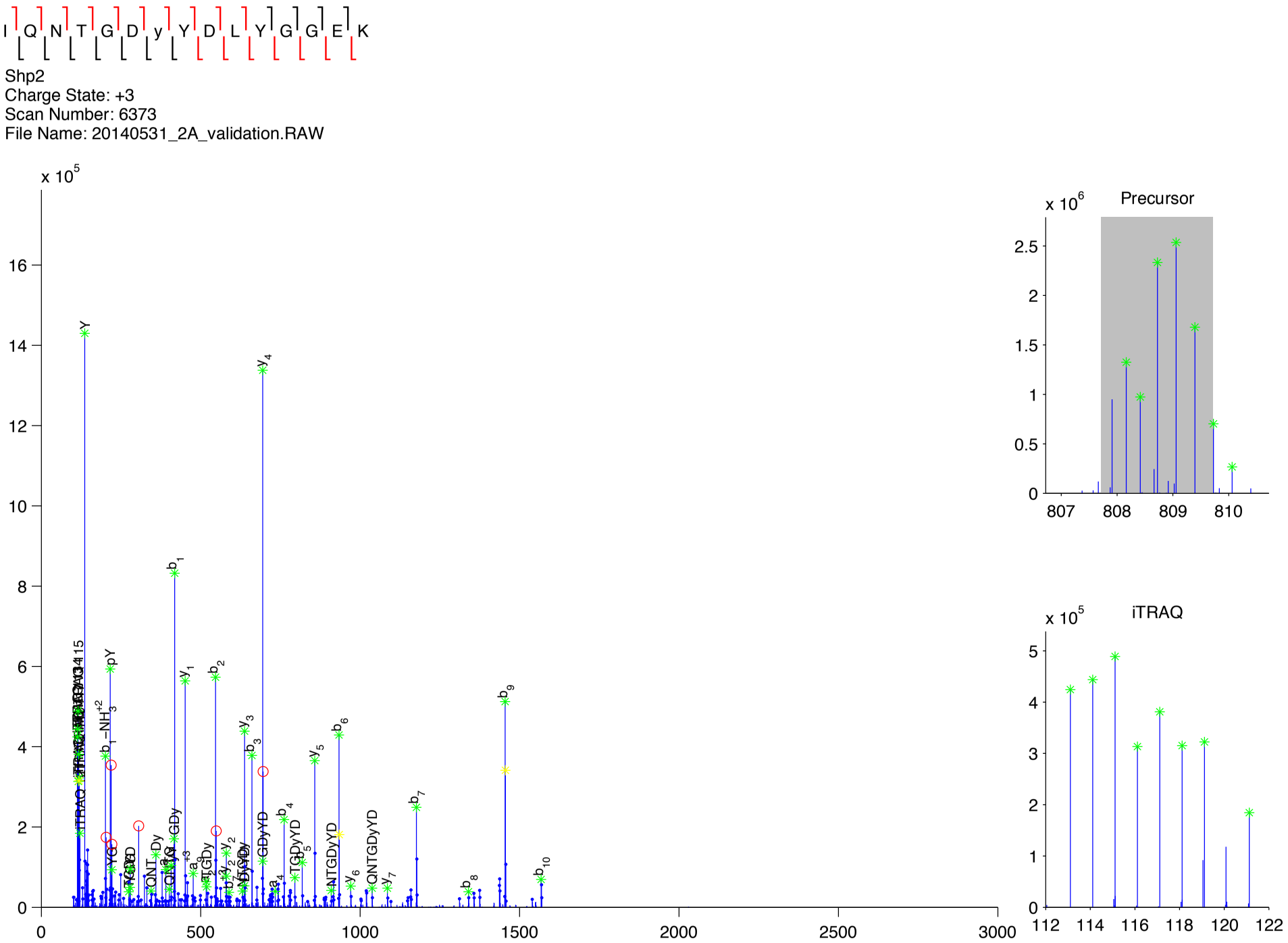




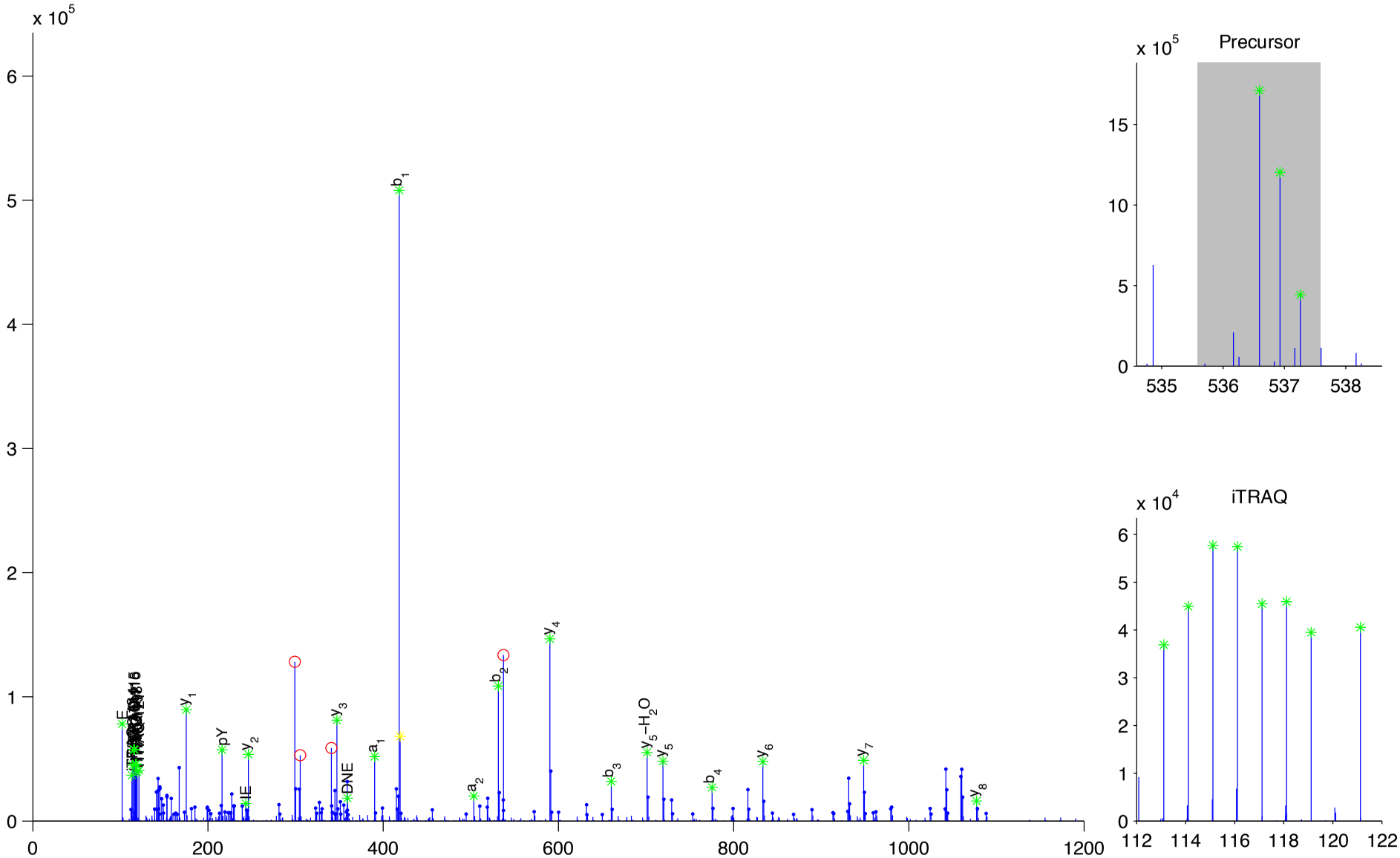
Gab1
Charge State: +4
Scan Number: 7335
File Name: 20140531_2A_validation.RAW



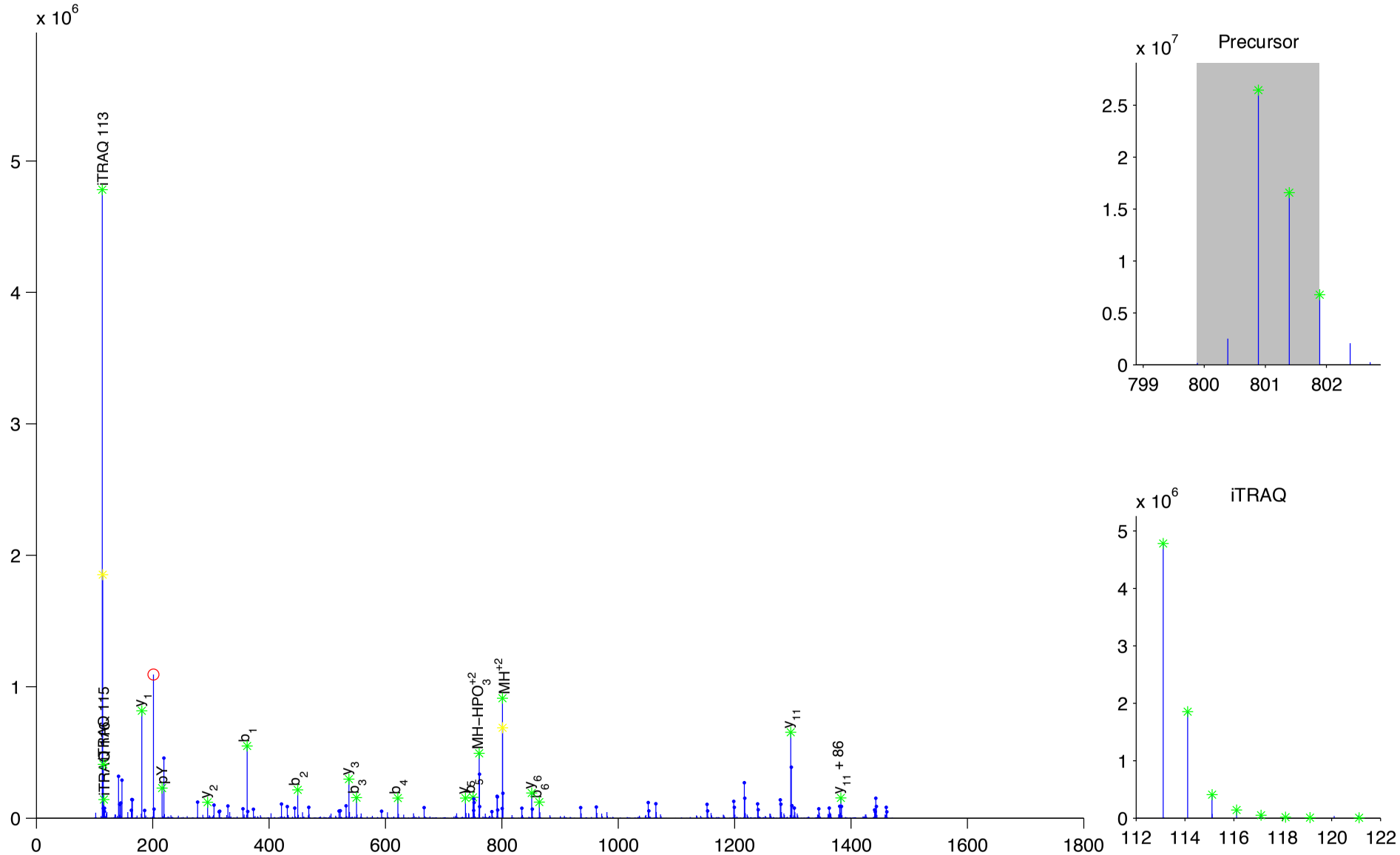

E L F D D P S y V N V Q N L D K
Shc
Charge State: +3
Scan Number: 7334
File Name: 20140531_2A_validation.RAW
x 10^6
iTRAQ 113
pY
LD−28
PSy−H2O
b7−H2O
FDDPSyV
PSyVNV
Precursor
iTRAQ
x 10^5

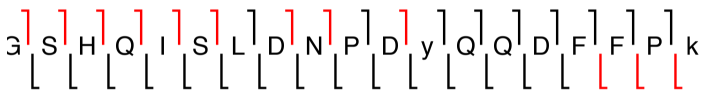

I Q N T G D y Y D L Y G G E K
Shp2
Charge State: +3
Scan Number: 6373
File Name: 20140531_2A_validation.RAW
x 10^5
Precursor
x 10^6
iTRAQ
x 10^5

Src
Charge State: +3
Scan Number: 5494
File Name: 20140531_2A_validation.RAW



EGFR
Charge State: +2
Scan Number: 2260
File Name: 20140531_2B_validation.RAW





EGFR
Charge State: +4
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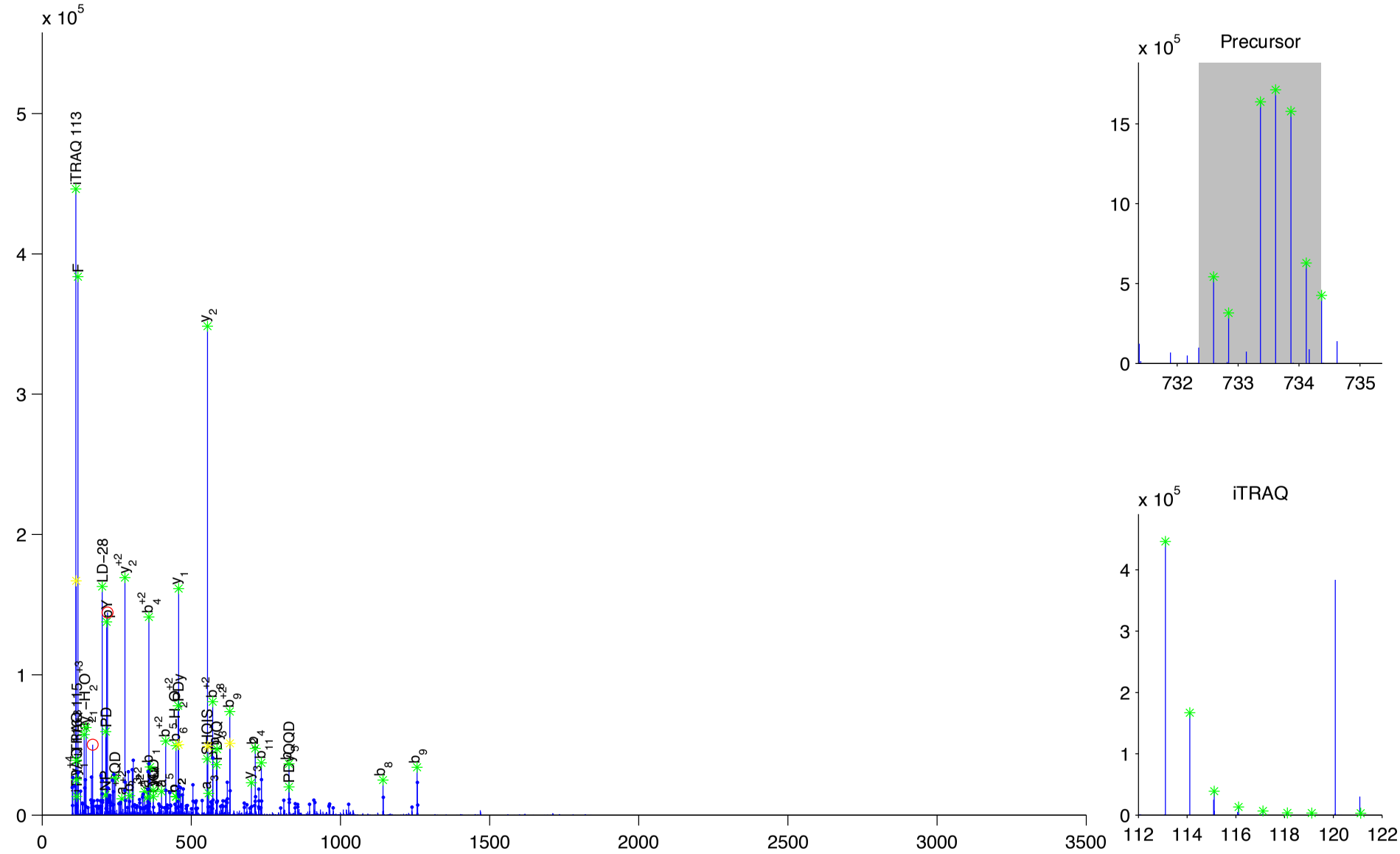

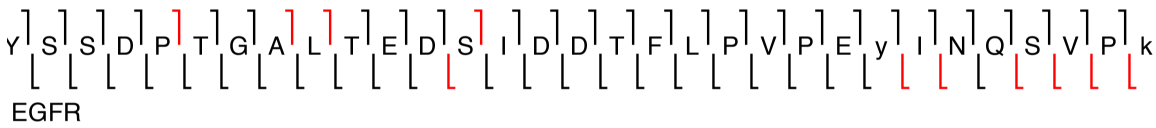

EGFR
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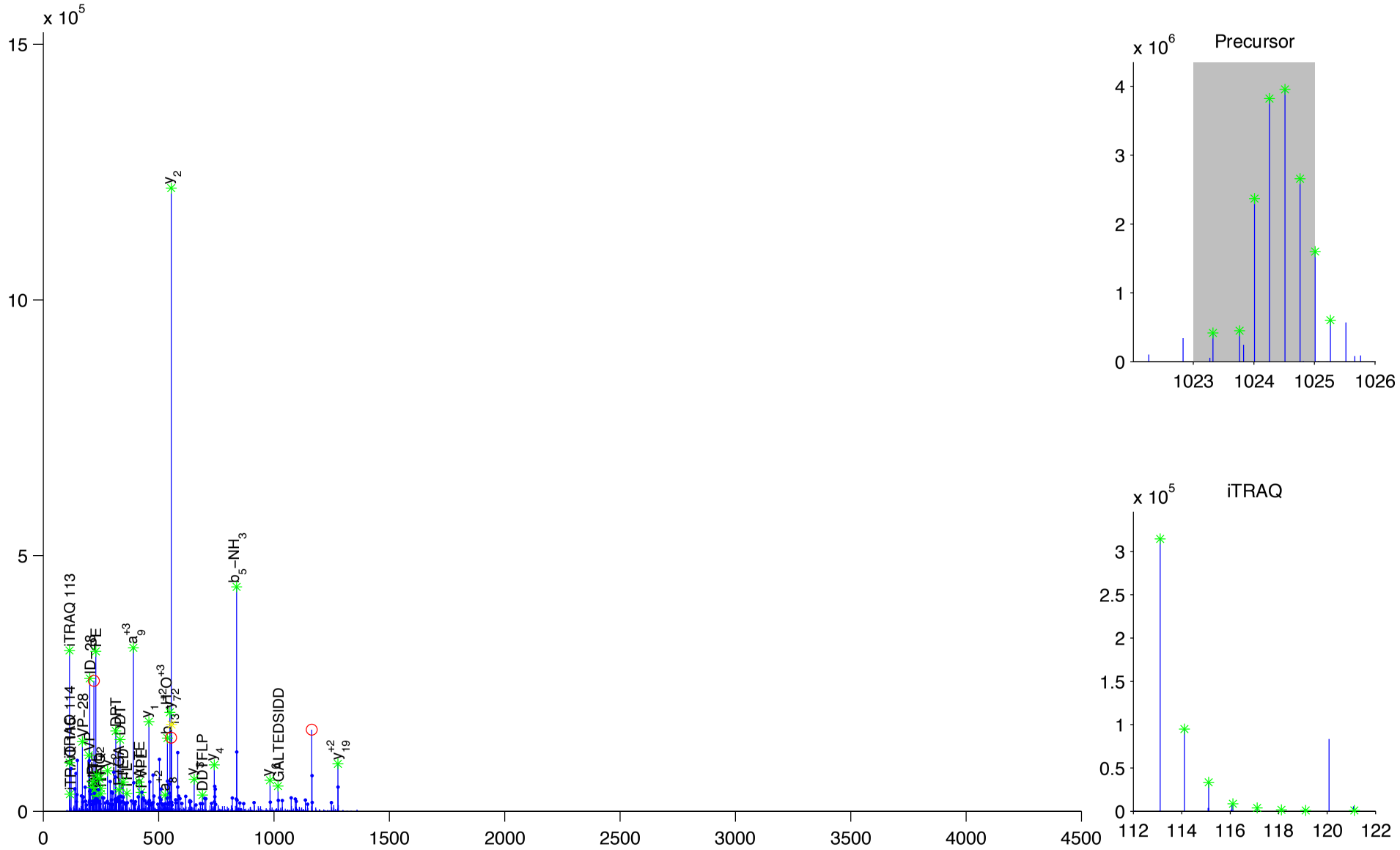

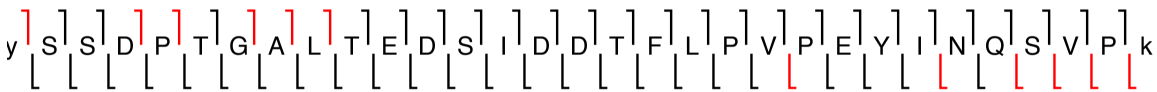

EGFR
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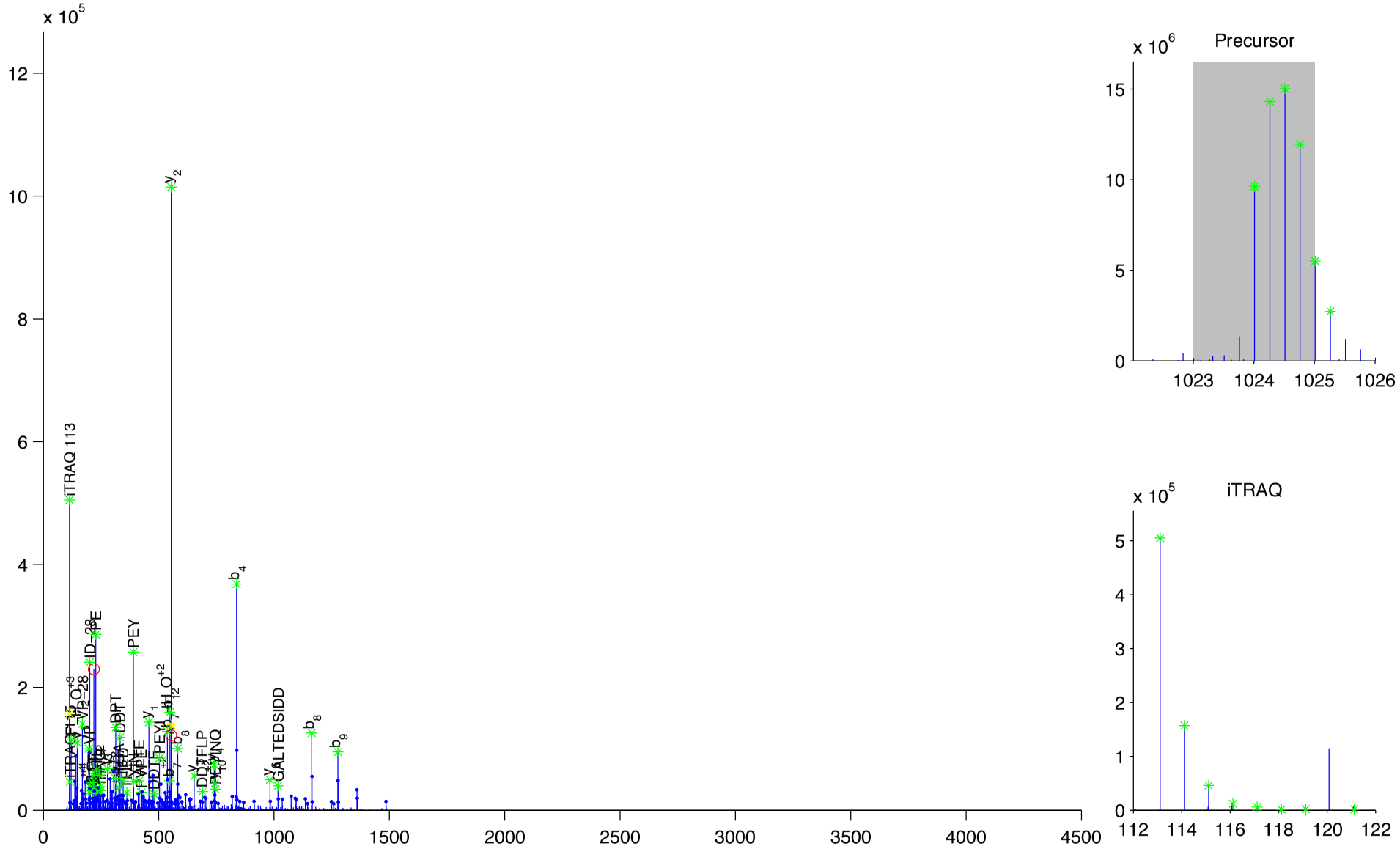

ERK1
Charge State: +3
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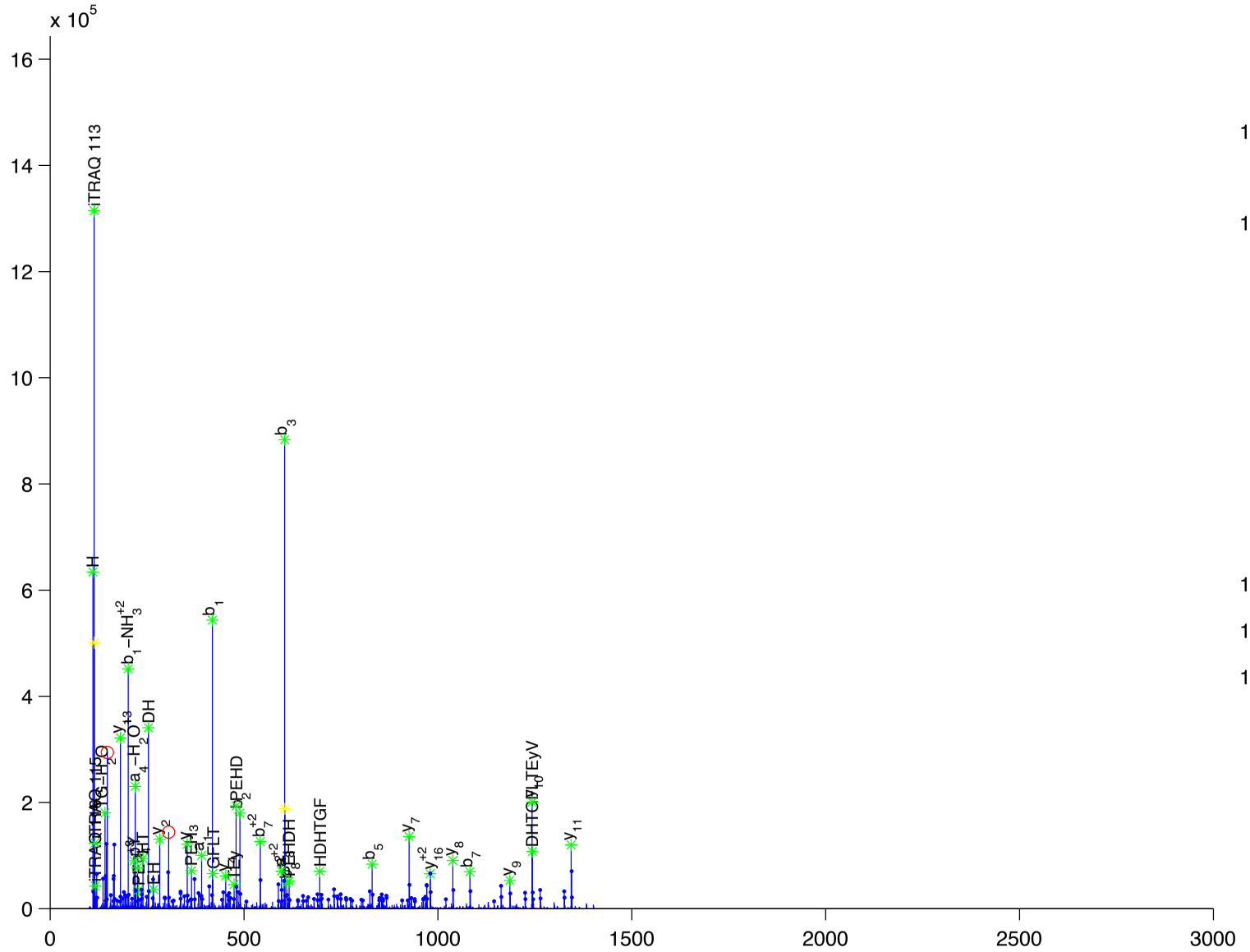

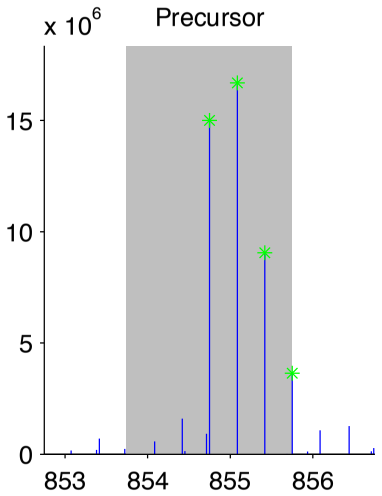

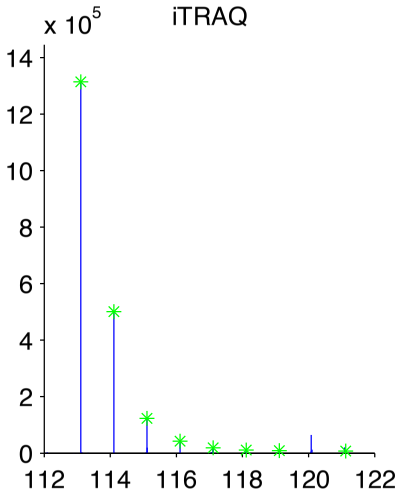

ERK2
Charge State: +3
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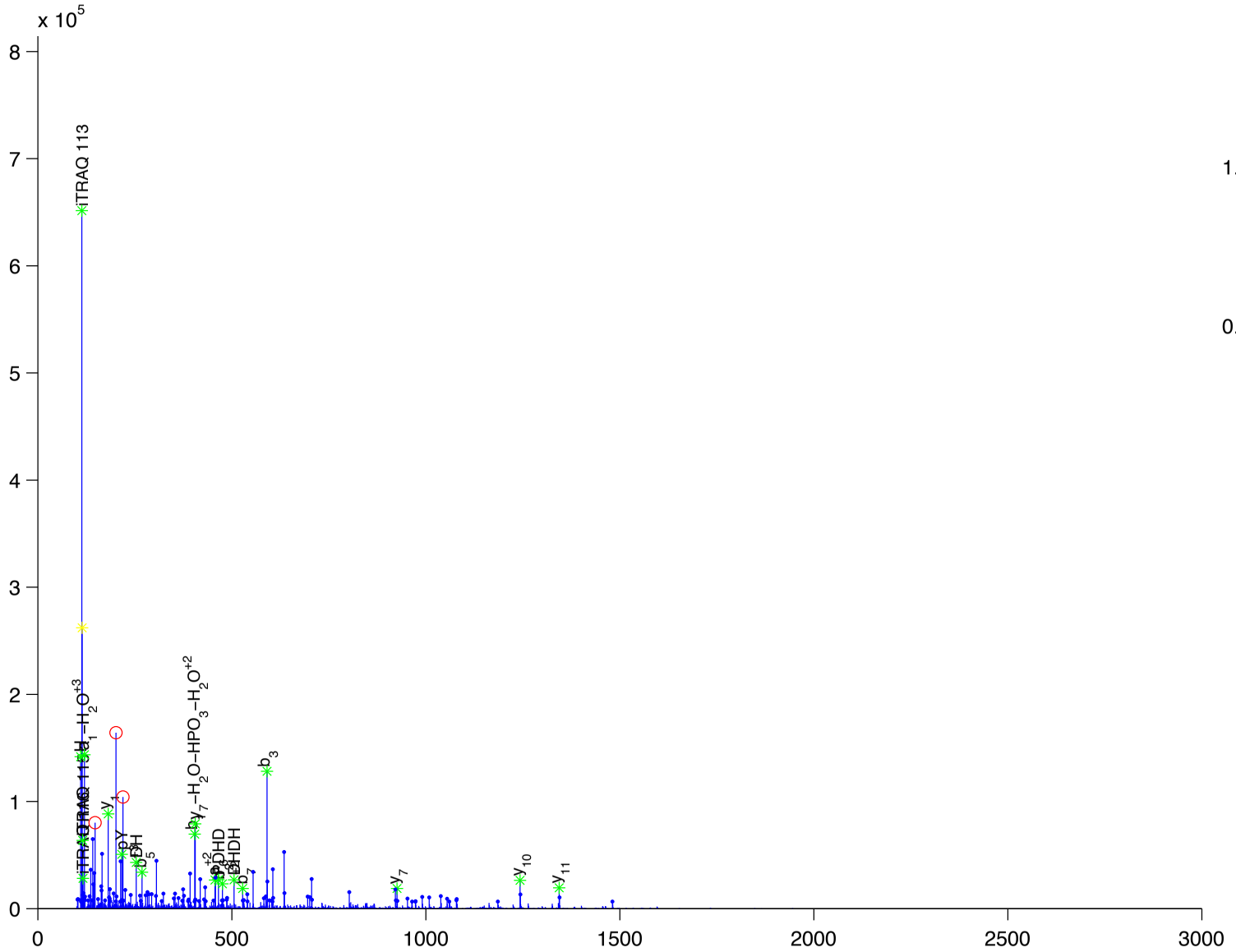
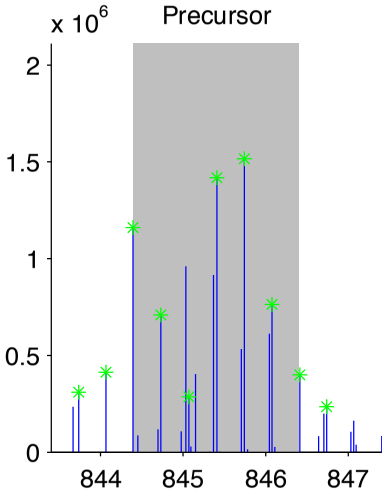
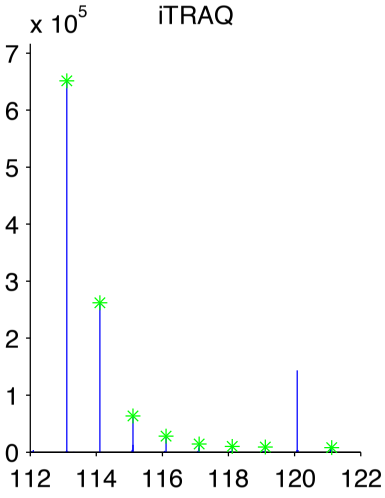
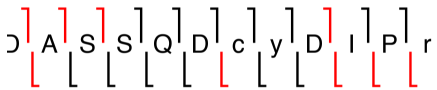
File Name: 20140531_2B_validation.RAW









Gab1
Charge State: +2
Scan Number: 2269
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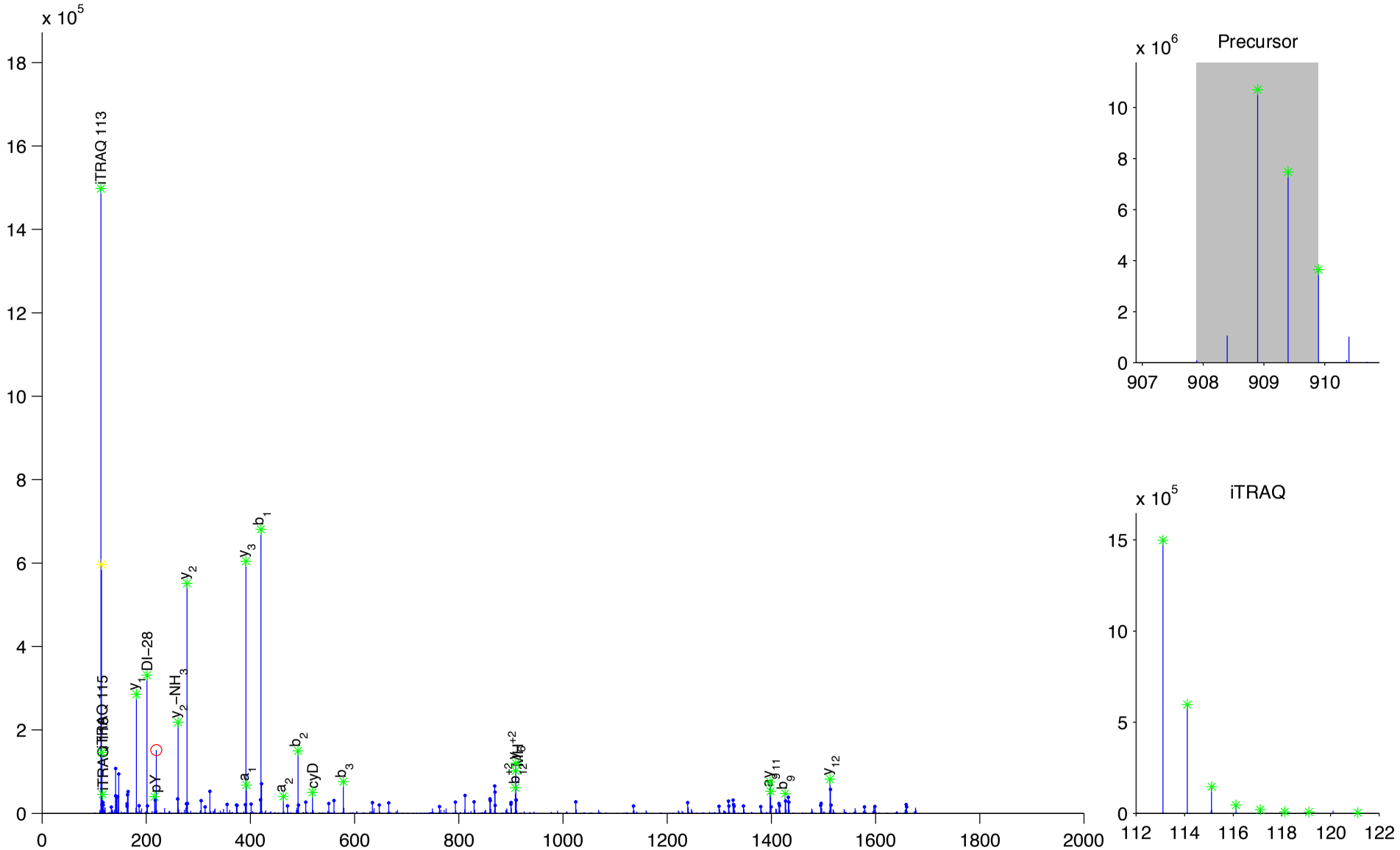
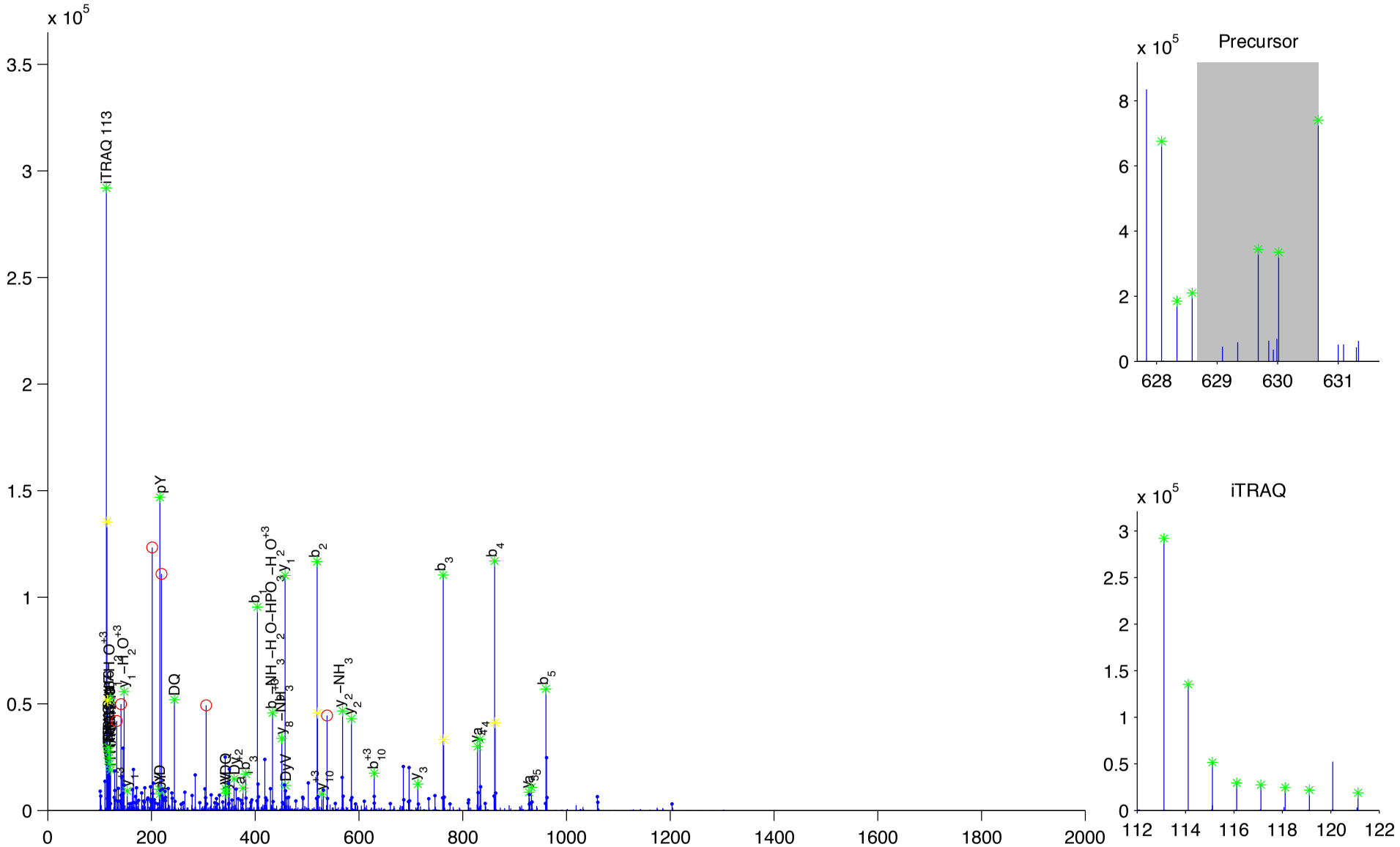
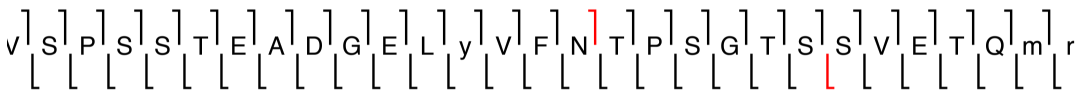


Gab1
Charge State: +3
Scan Number: 2402
File Name: 20140531_2B_validation.RAW





Gab1
Charge State: +4
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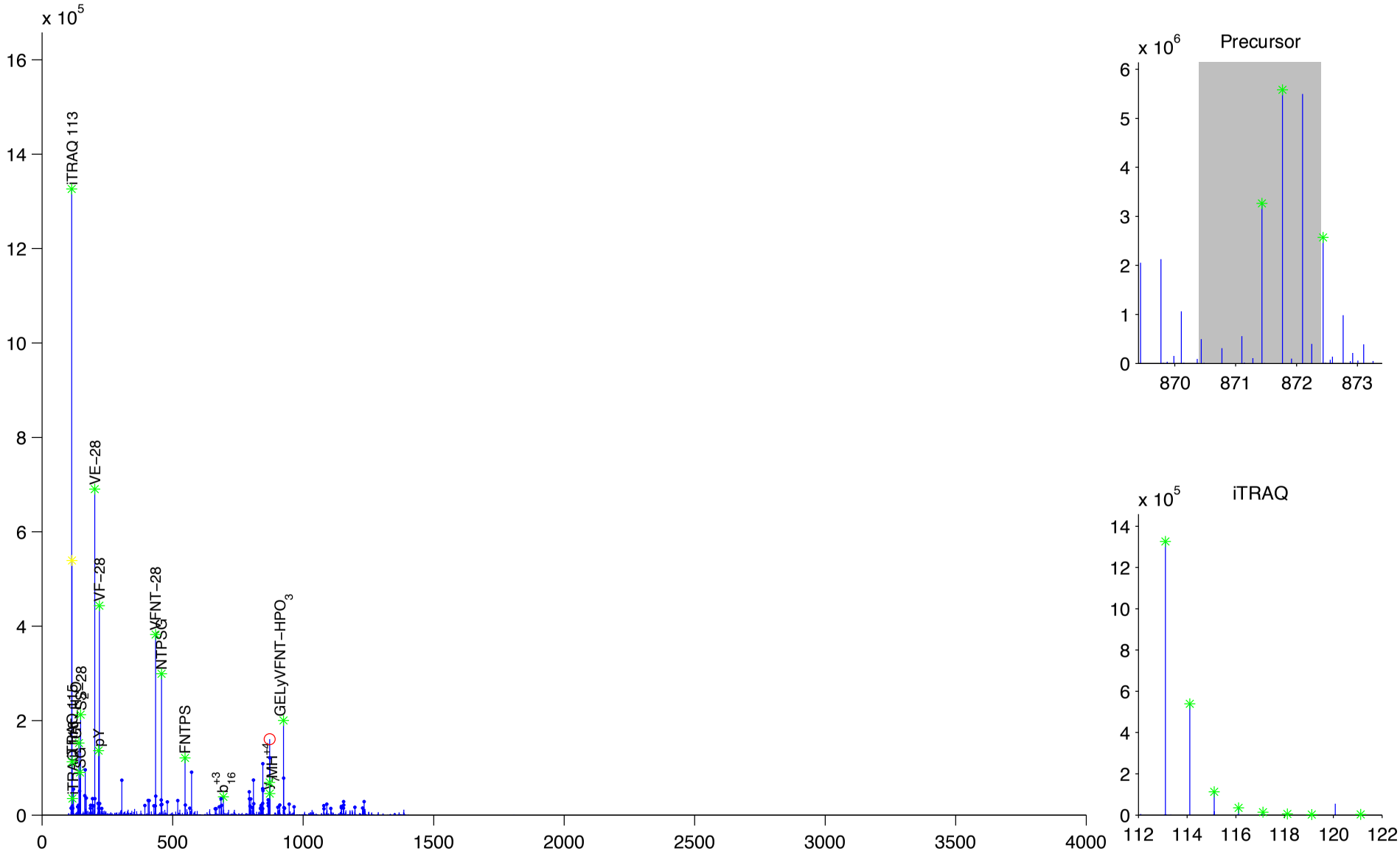

Shc
Charge State: +3
Scan Number: 2744
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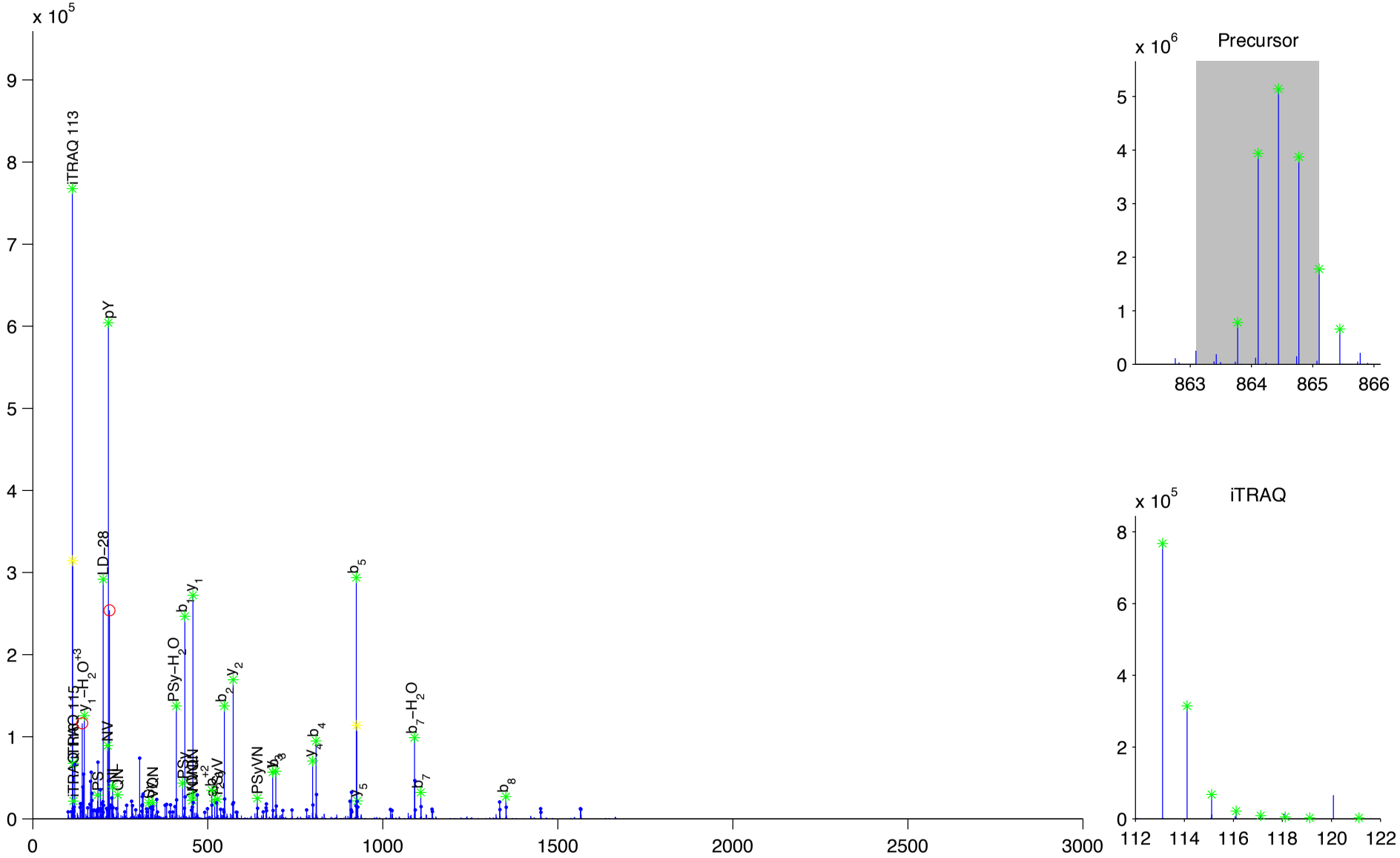
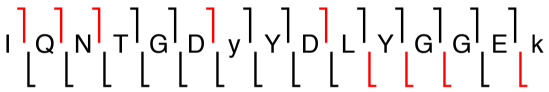
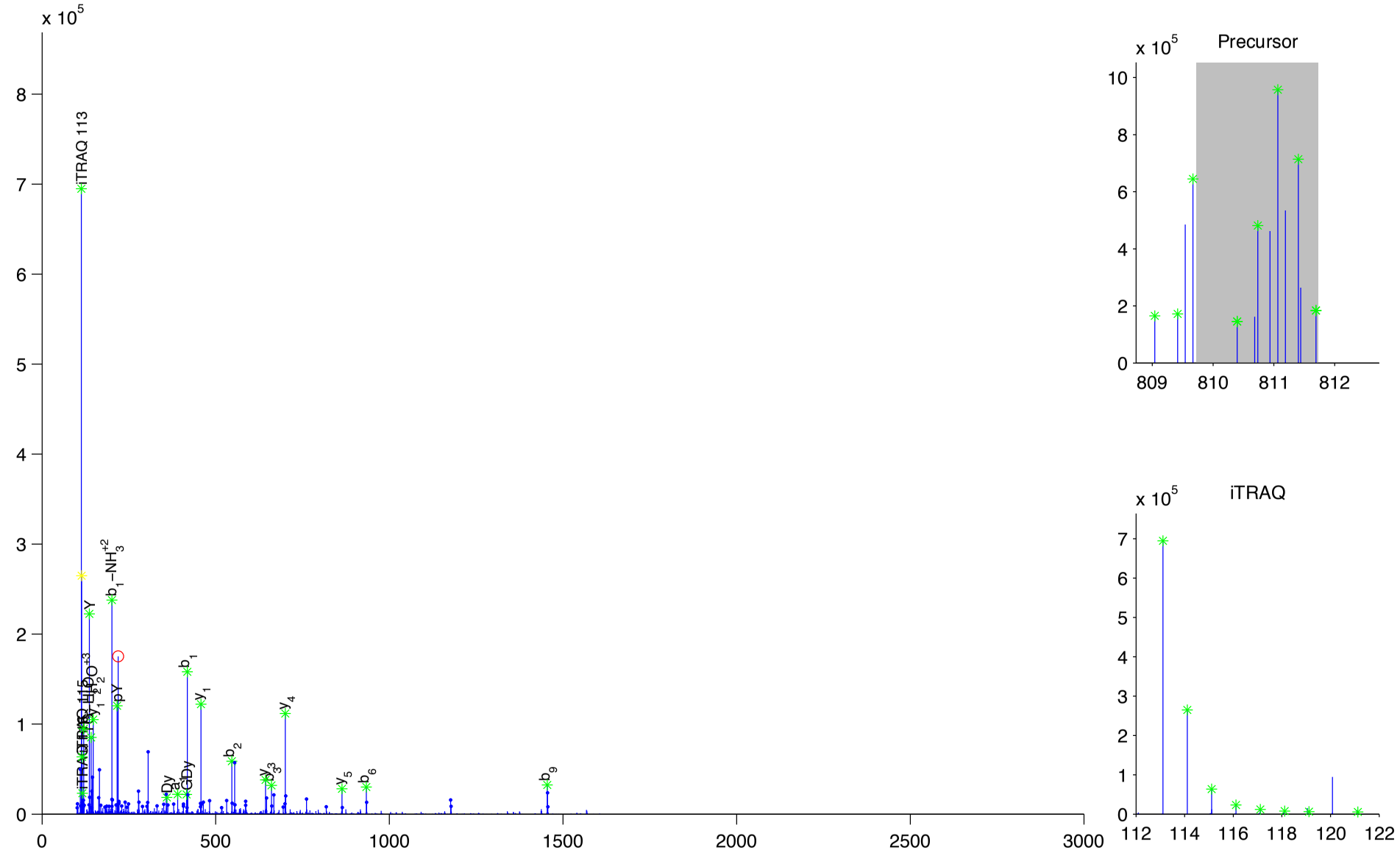
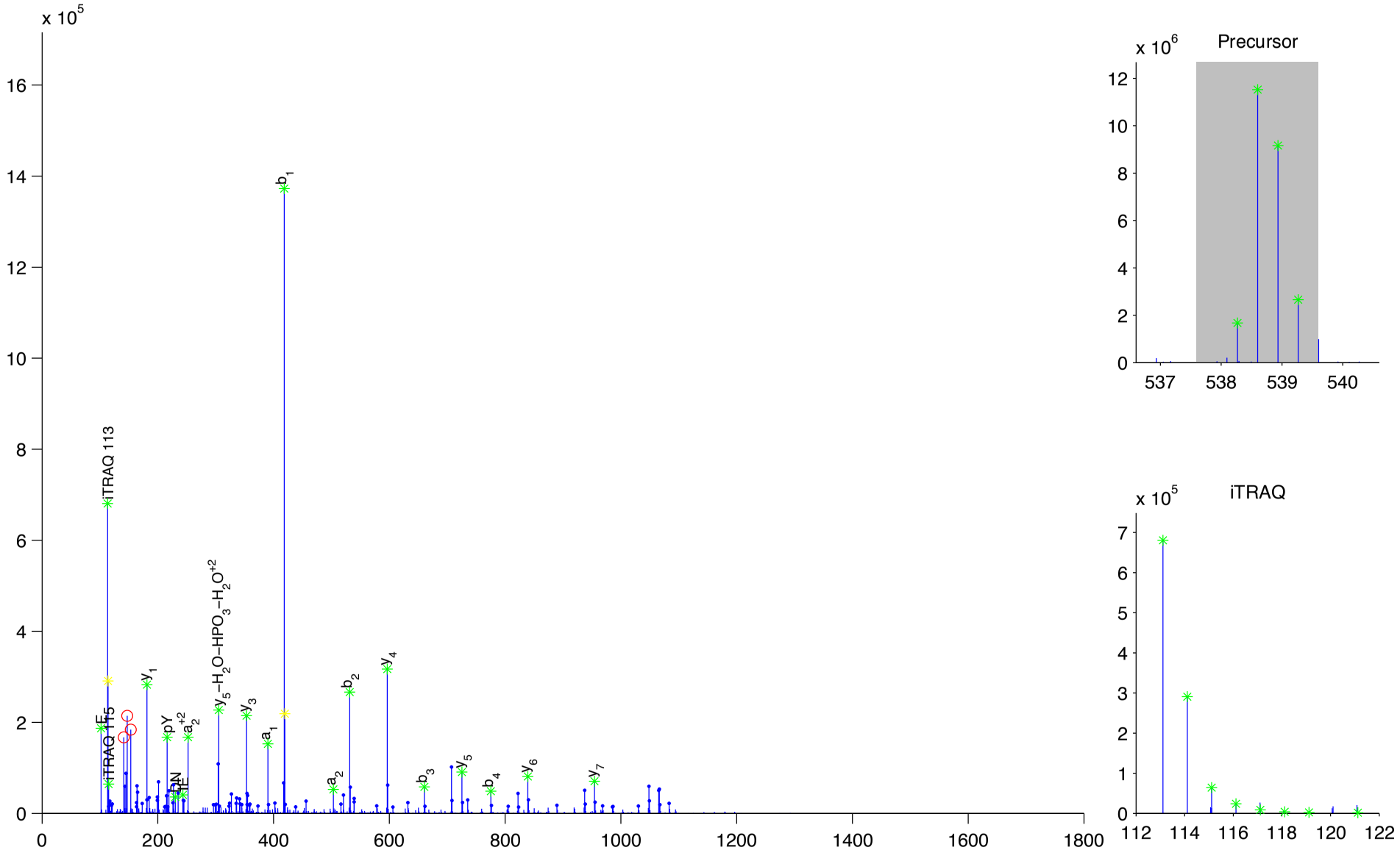




Shp2
Charge State: +3
Scan Number: 2567
File Name: 20140531_2B_validation.RAW



Src
Charge State: +3
Scan Number: 2399
File Name: 20140531_2B_validation.RAW



Supplementary Figure 9: Spectral validation of scans used for quantitation. MS2-level spectral validation was performed using CAMV[23]. a) Tumor samples without added standard, used for uncontaminated iTRAQ information of endogenous peptides (set 1A). b) Tumor samples with added standard, used for MS1 level comparison of standard and endogenous intensity and standard peptide iTRAQ information (set 1B). c) PDX inhibitor endogenous (set 2A). d) PDX inhibitor standard (set 2B).

ERK2	185,187	VADPDHDHTGFLtEyVATR*	654.273	1322.468

Supplementary Table 1. MRM Quantification of endogenous tyrosine phosphorylated peptides and Transition list for MRM. Raw iTRAQ intensities for endogenous peptides (columns E-L) and corresponding heavy-labeled standard peptides (columns M-T) across technical replicate analysis of EGF stimulated MCF10A cells. Calculated amounts of endogenous peptides based on regression to the standard curve are contained in columns U-AB. On the second tab, precursor and fragment ion mass-to-charge ratios for all transitions monitored for the endogenous and heavy-labeled standard peptides. ^{13}C labeled residues in the synthetic peptides are labeled with an asterisk.

Supplementary Table 2. PRM Quantification of endogenous tyrosine phosphorylated peptides. Raw MS1 intensities were collected for endogenous and heavy-labeled standard peptides in either full scan (columns E-J) or SIM mode (columns K-P) for either single scan (E-G, K-M) or averaged (H-J, N-P) across the chromatographic elution profile. These estimates were averaged to produce the final ratio (column Q). Amino acid analysis was performed for each standard peptide to provide a correction factor for the concentration (column R). Total amount of standard after correction (column S) was multiplied by the ratio in column Q to produce the total amount of endogenous (column T). iTRAQ intensities for endogenous peptides (columns U-AB) were used to calculate the fractional iTRAQ values (AC-AJ), which were then multiplied by the total endogenous peptide amount (column T) to produce the calculated amount of endogenous peptide in each sample (columns AK-AR).

Supplementary Table 3. Comparison of MRM and PRM quantification for 13 tyrosine phosphorylated peptides. Absolute quantification values from Supplementary Tables 1 and 2 are combined here for easy comparison of the two mass spectrometric platforms. Columns D-K contain the mean amount of the peptide as measured by MRM or PRM; columns L-S contain calculated standard deviations from the technical replicate data in the respective tables. Finally, columns T-AA contain the corresponding coefficient of variation for each peptide, as calculated by standard deviation divided by the mean.

Supplementary Table 4. Ligand-Specific EGFR Absolute Quantification. Integrated intensity for each iTRAQ reporter generated from endogenous transitions for key EGFR C-terminal phosphorylation sites (columns E-H). Integrated iTRAQ reporter ion intensity from standard peptide transitions (columns I-L). Standard peptide concentration was determined by amino acid analysis and a correction factor was applied to the nominal doses included (column M). Actual standard dose included into each channel following AAA correction (columns N-Q). The standard curve built from the standard amount (columns N-Q) and the standard intensities (columns I-L) was used to calculate the endogenous amount of the corresponding peptide in each sample (columns R-U) based on the endogenous intensities (columns E-H). Prior to MS analysis, the number of cells contained in the sample was calculated with a BCA assay; standard cell number stock lysate solutions were used to calibrate the unknown samples (columns V-Y). Total amount of endogenous peptide (columns R-U) was converted to moles and divided by the corresponding cell number (columns V-Y) to produce molecules/cell (columns Z-AC). The mean and range of two biological replicates is reported (columns AG-AK) for each ligand. To mitigate contamination from standard peptide iTRAQ signals, several precautions were taken. First, standard doses and iTRAQ channels were varied between replicates. Second, four time-zero replicates were analyzed separately from other samples, because this condition was anticipated to have low signal. In addition, the biological replicate in the channel containing the 1 pmol standard dose were

excluded, because significant residual signal from the standard peptide was visible.

Supplementary Table 5: Q1 Width Comparisons. Data for Supplementary Figure 5.

Supplementary Table 6: Tumor Sample Comparison Data: Intensity for each iTRAQ reporter ion generated from PRM analysis for key phosphorylation sites for GBM PDX tumors. Biological replicates of each tumor were analyzed in a single 8-plex iTRAQ analysis. iTRAQ intensity was converted to fmol amount by extrapolation against the standard curve on the second tab of the spreadsheet. Columns W-AD report the mean and the standard range of the phosphorylation levels for each site in the given tumor model.

Supplementary Table 7: PDX Inhibitor Panel Data: Intensity for each iTRAQ reporter ion generated from PRM analysis for key phosphorylation sites for GBM12 PDX tumor treated with placebo, erlotinib, dacomitinib, or NT-113. Biological replicates of each tumor were analyzed in a single 8-plex iTRAQ analysis. iTRAQ intensity was converted to fmol amount by extrapolation against the standard curve on the second tab of the spreadsheet. Columns W-AD report the mean and the standard range of the phosphorylation levels for each site in tumor under the appropriate treatment condition.