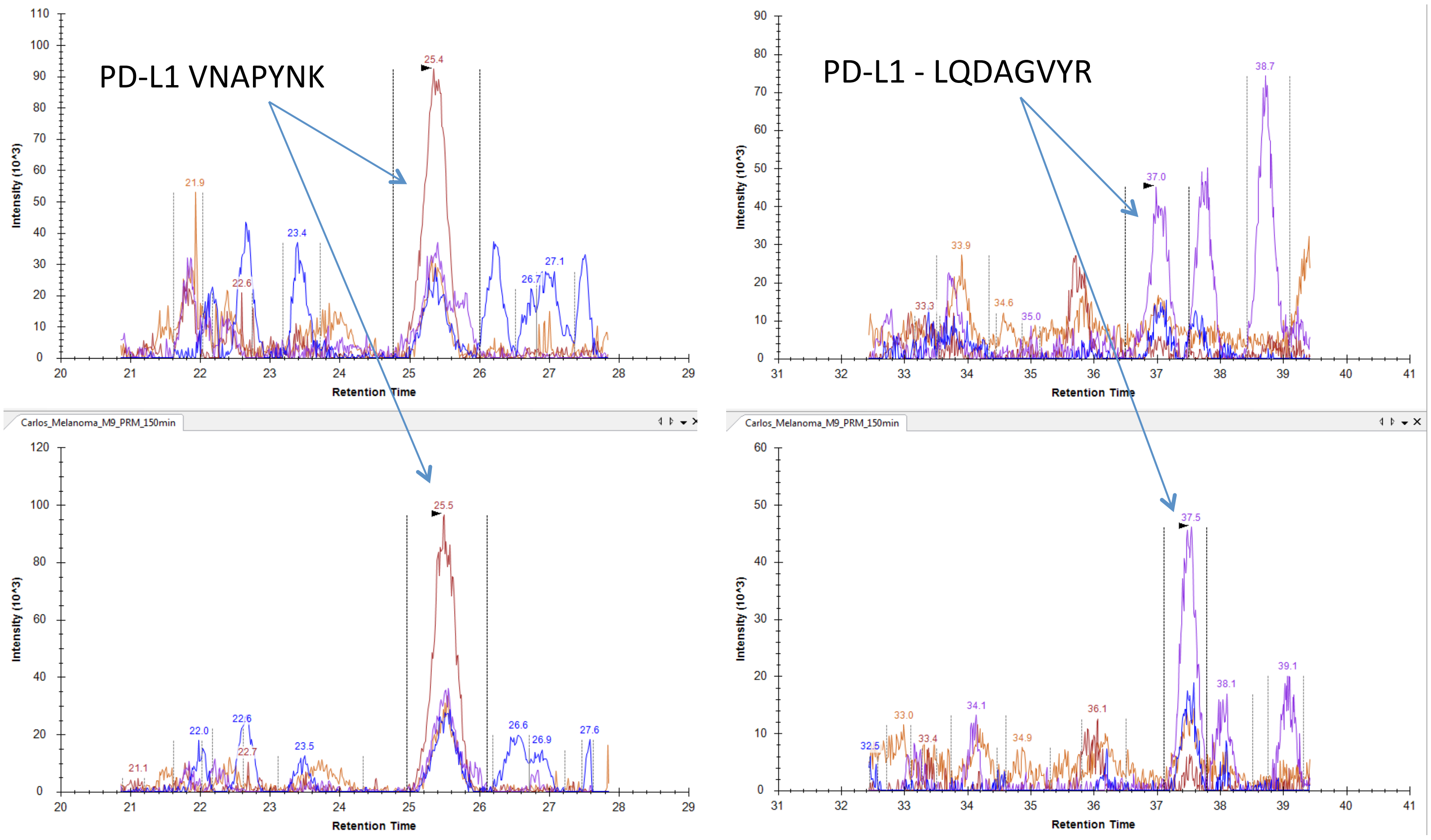
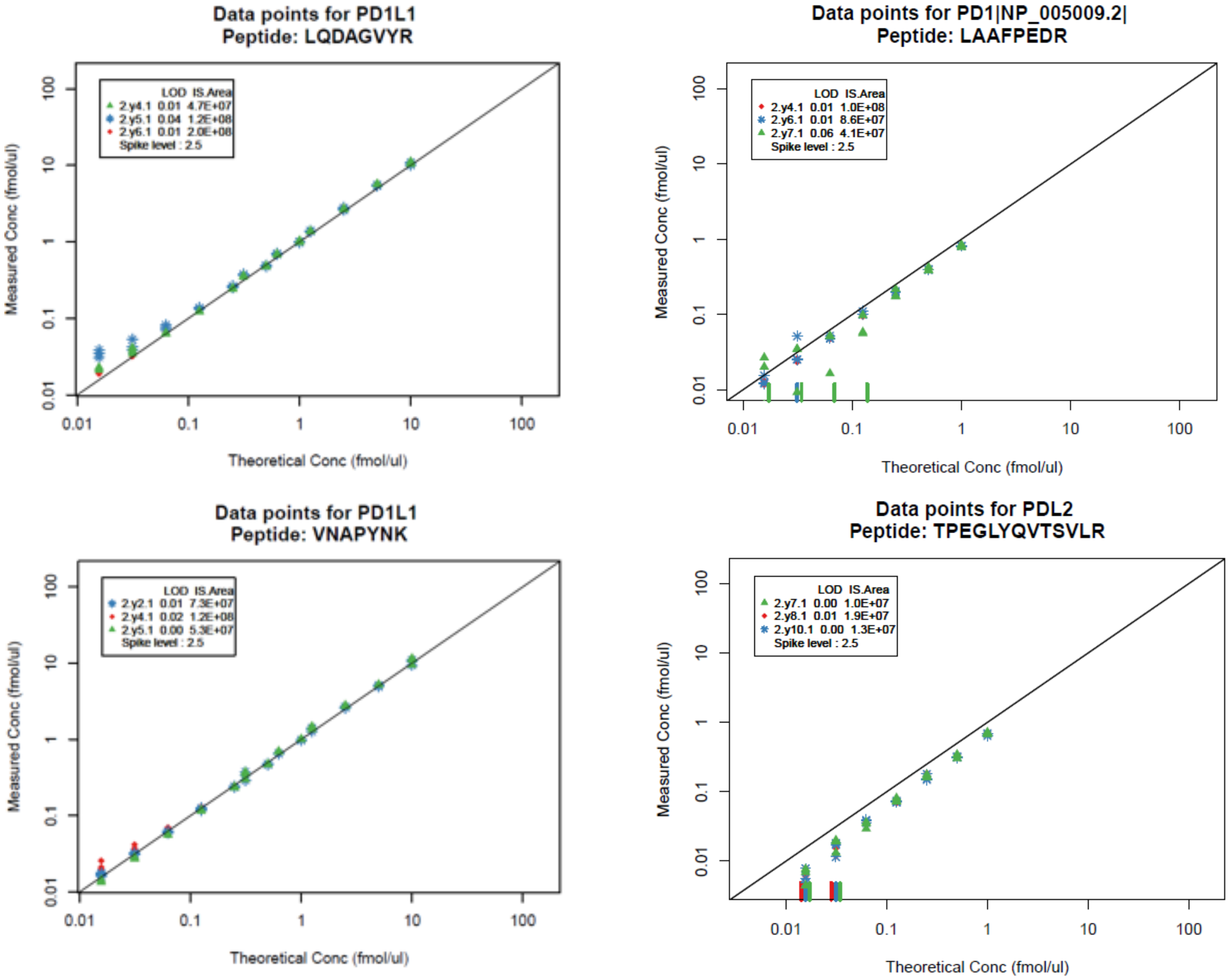


Figure S1. Detection of PD-L1 peptides in long gradient LC-PRM analyses of unfractionated digests from FFPE melanomas.


Data points for PD1L1
Peptide: LQDAGVYR

Data points for PD1|NP_005009.2|
Peptide: LAAFPEDR

Data points for PD1L1
Peptide: VNAPYNK

Data points for PDL2
Peptide: TPEGLYQVTSVLR

Figure S2. SID calibration curves

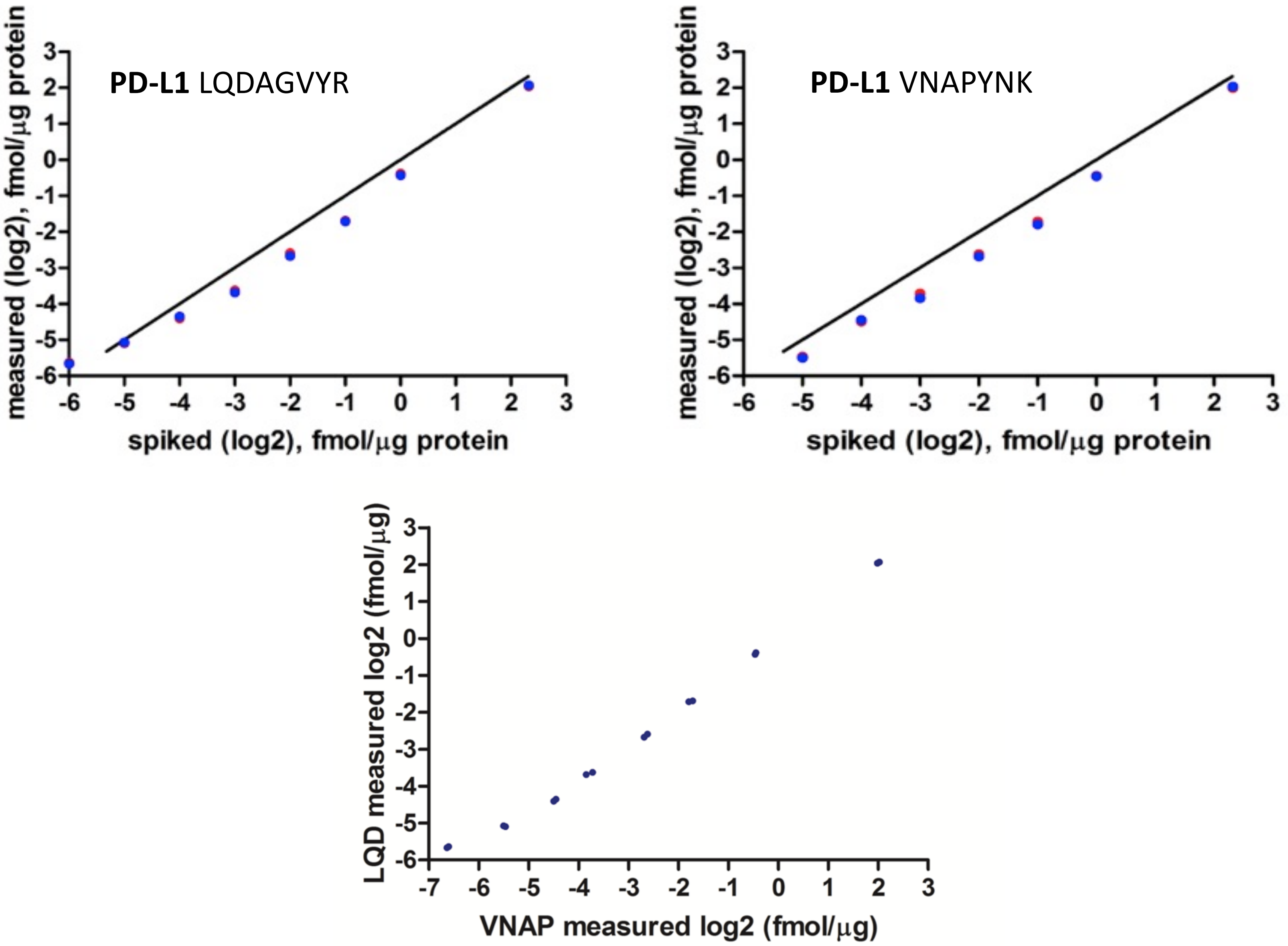
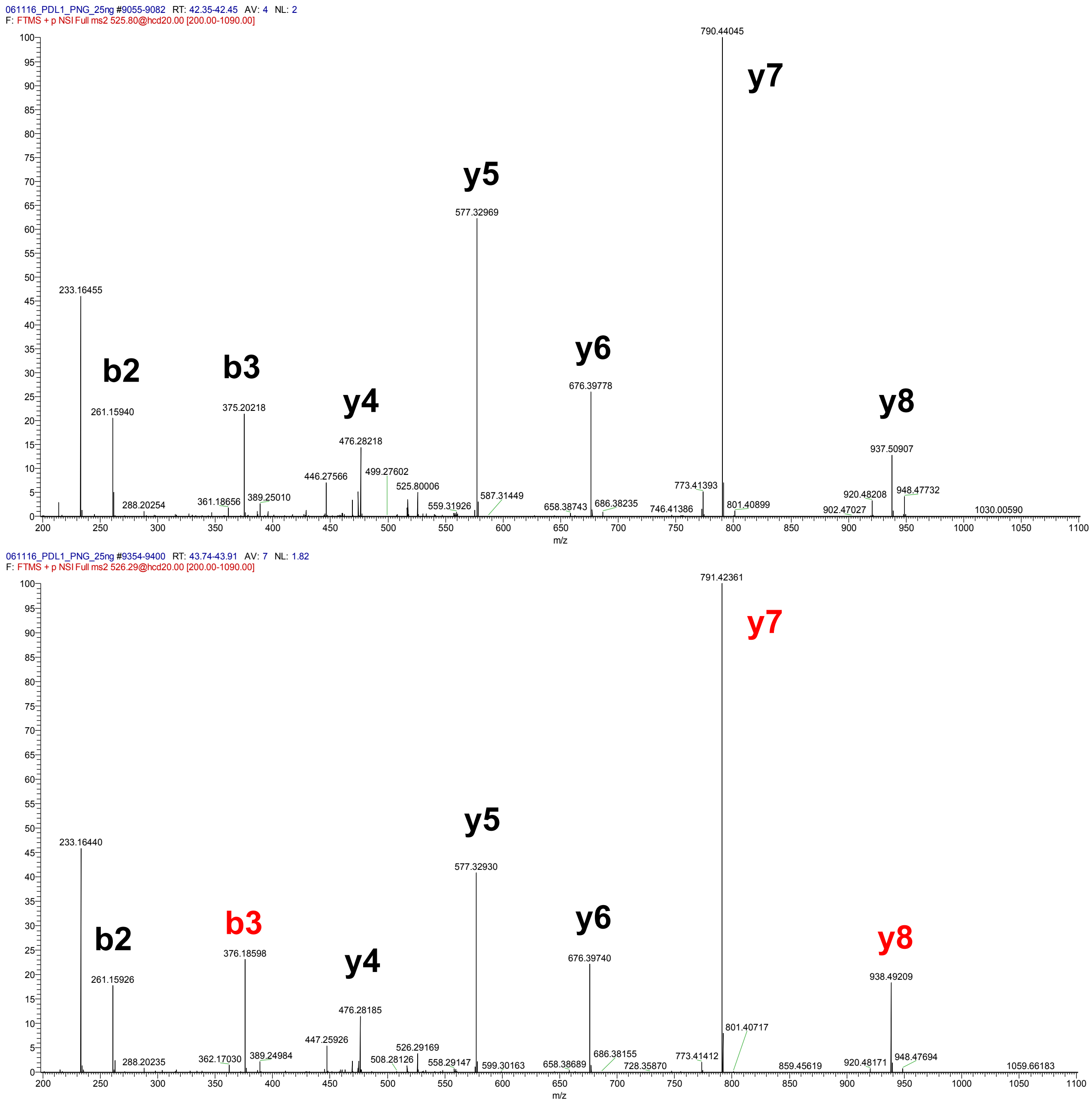


Figure S3. Spike recovery study for recombinant human PD-L1 100 µg HEK293 cell lysate 0.031, 0.063, 0.125, 0.25, 0.5, 1 or 5 fmol/microgram protein.

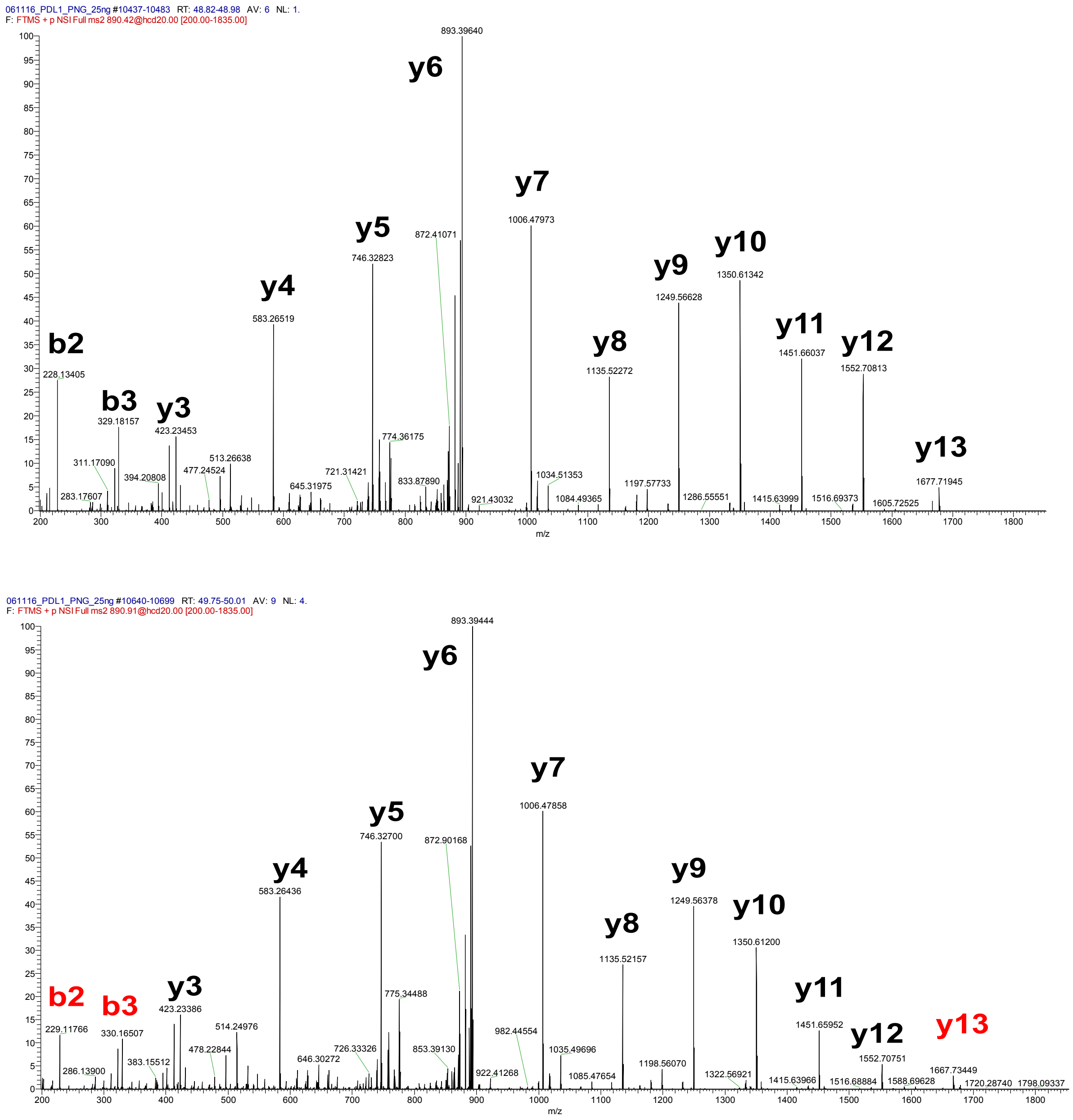


LFNVTSTLR

LFDVTSTLR

(fragments with m/z shift from N → D substitution labeled in red)

Figure S4. MS/MS spectra of PD-L1 LFNVTSTLR peptide and LFDVTSTLR peptide released by PNGase F treatment of recombinant human PD-L1 tryptic digest.

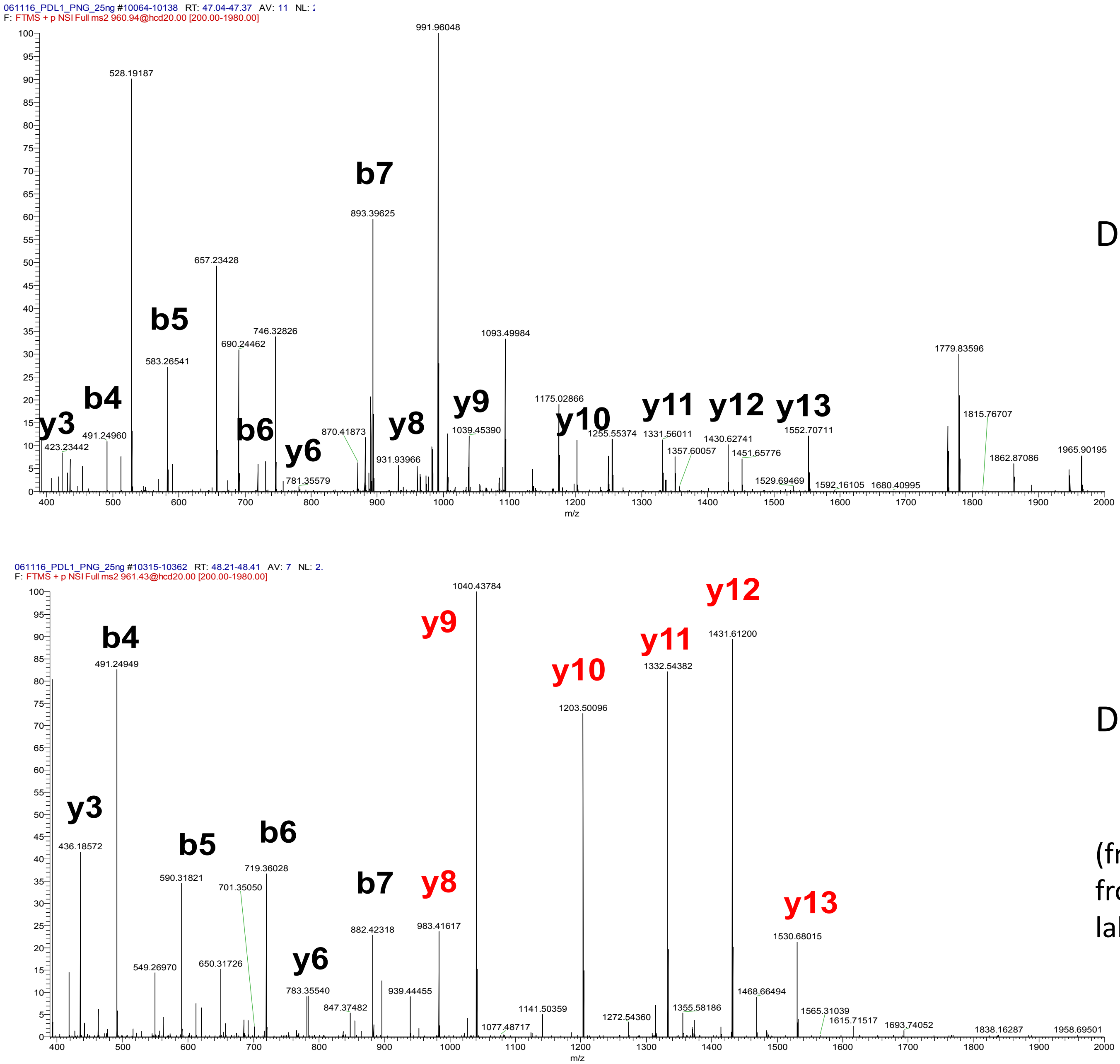


INTTTNEIFYCTFR

IDTTTNEIFYCTFR

(fragments with m/z shift from N → D substitution labeled in red)

Figure S5. MS/MS spectra of PD-L1 INTTTNEIFYCTFR peptide and L1 IDTTTNEIFYCTFR peptide released by PNGase F treatment of recombinant human PD-L1 tryptic digest.



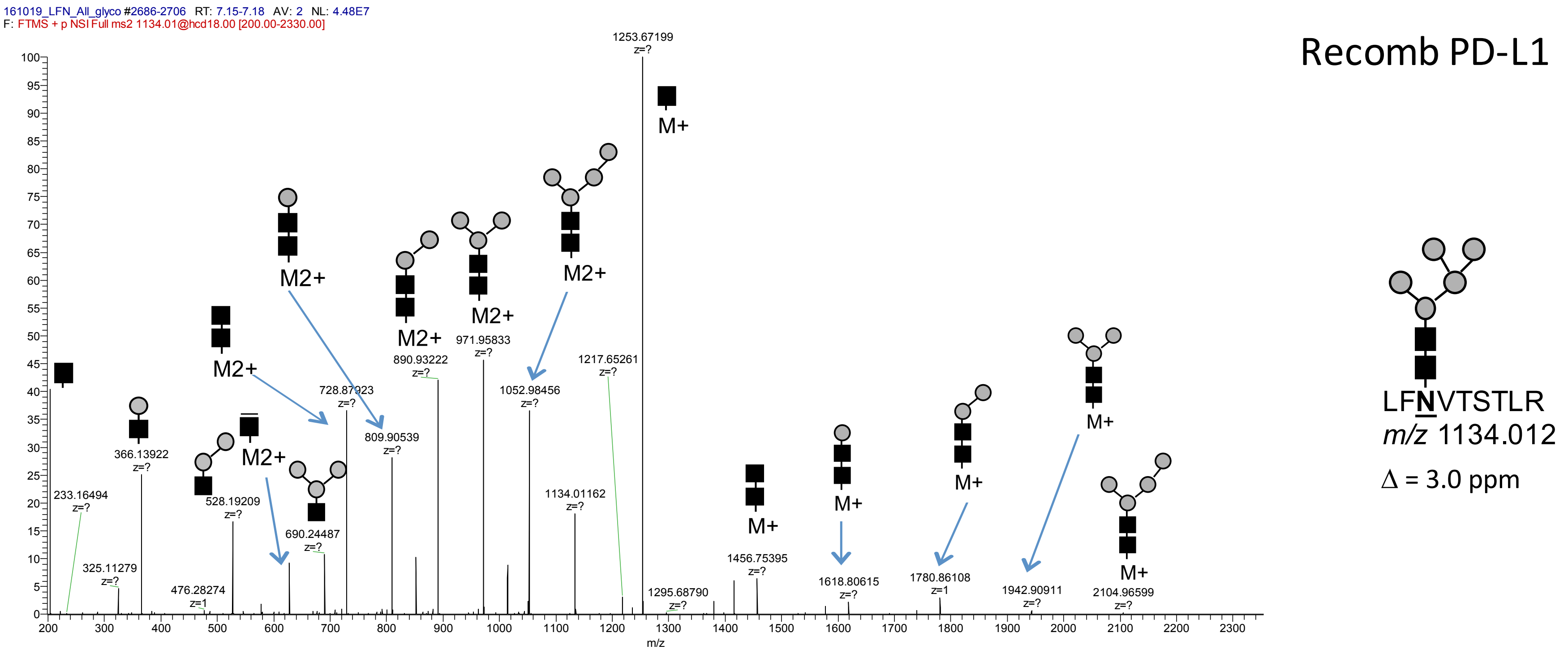
DLYVVEYGSNMEIECK

DLYVVEYGSDMEIECK

(fragments with m/z shift
from N → D substitution
labeled in red)

Figure S6. MS/MS spectra of PD-L1 DLYVVEYGSNMEIECK peptide and L1 DLYVVEYGSDMEIECK peptide released by PNGase F treatment of recombinant human PD-L1 tryptic digest.

Recomb PD-L1



LF**N**VTSTLR
m/z 1134.012
Δ = 3.0 ppm

Melanoma B14

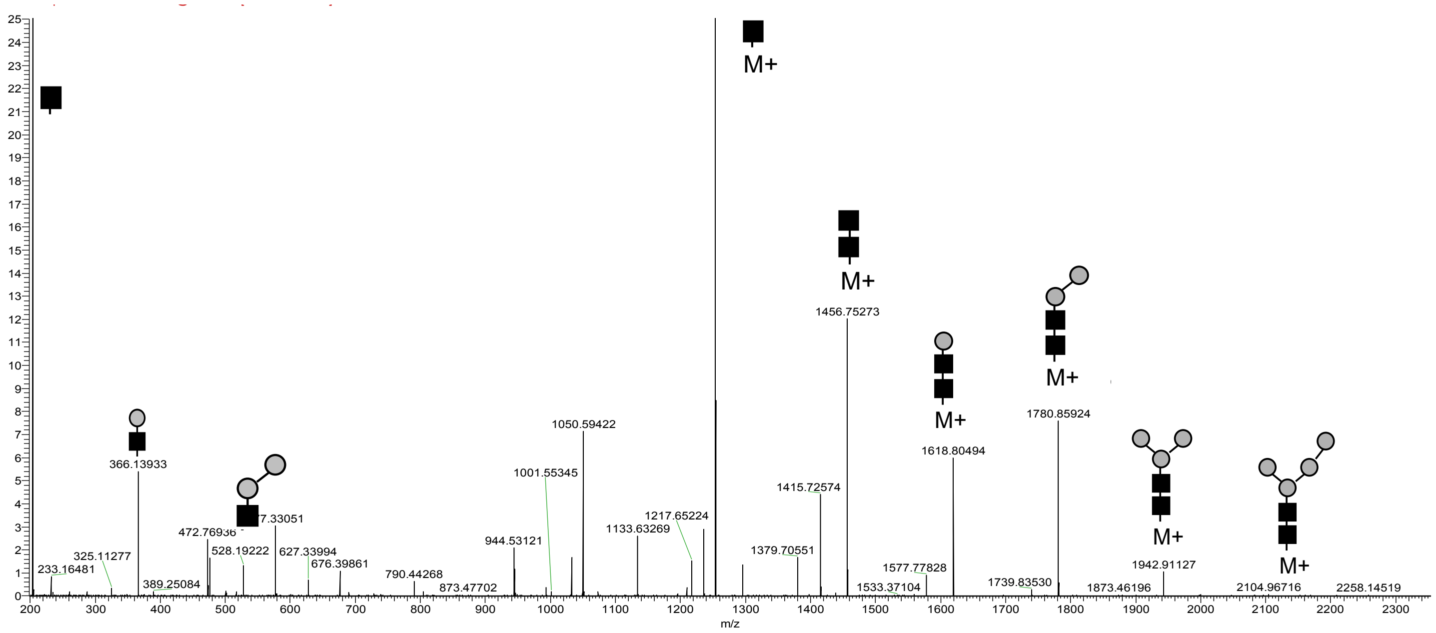
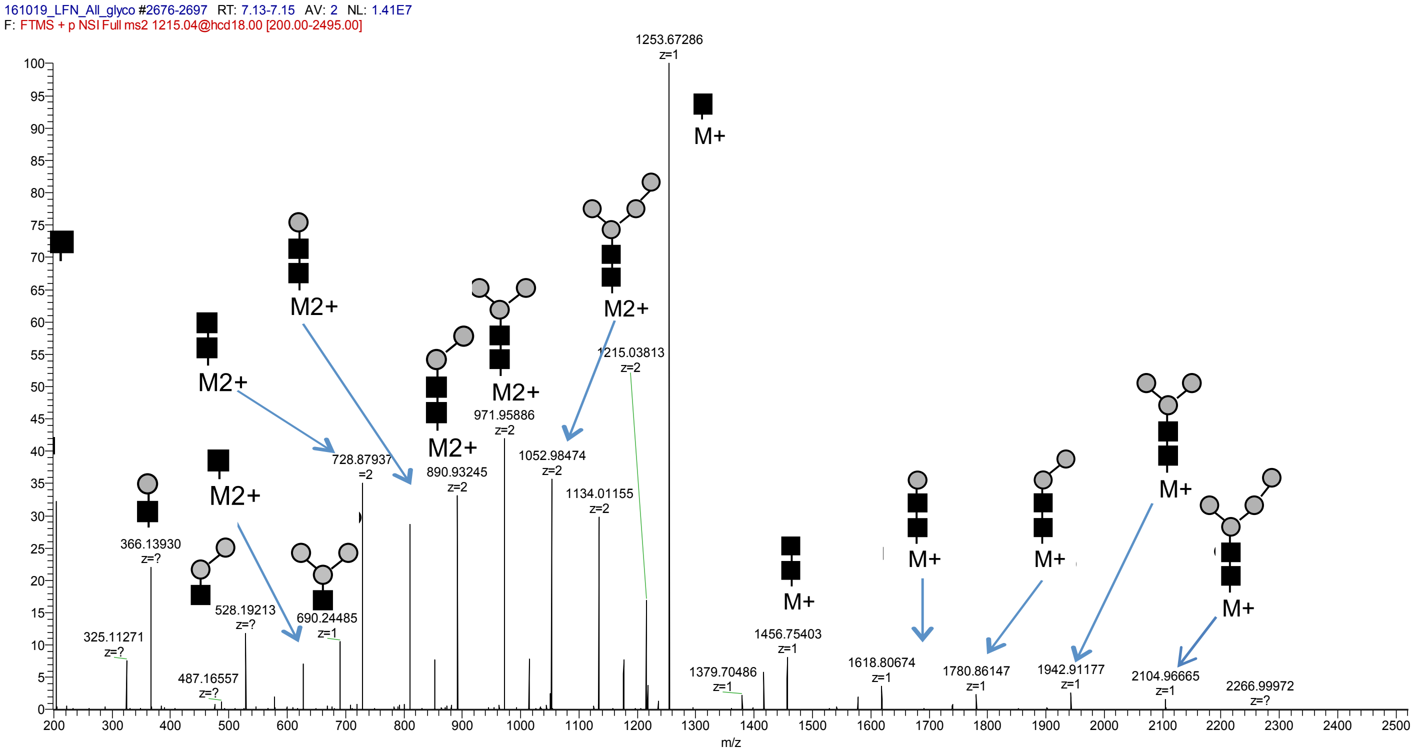
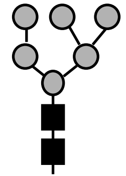


Figure S7



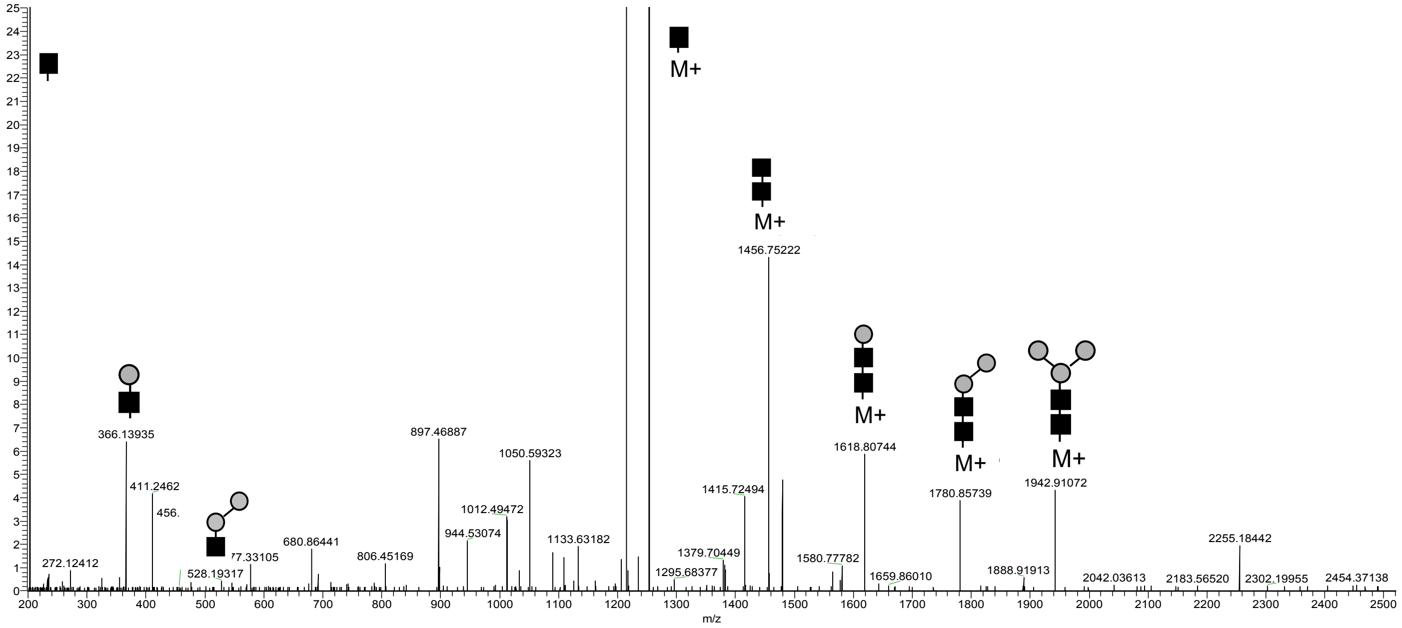
Recomb PD-L1



LFNVTSTLR

m/z 1215.0386 (+2)

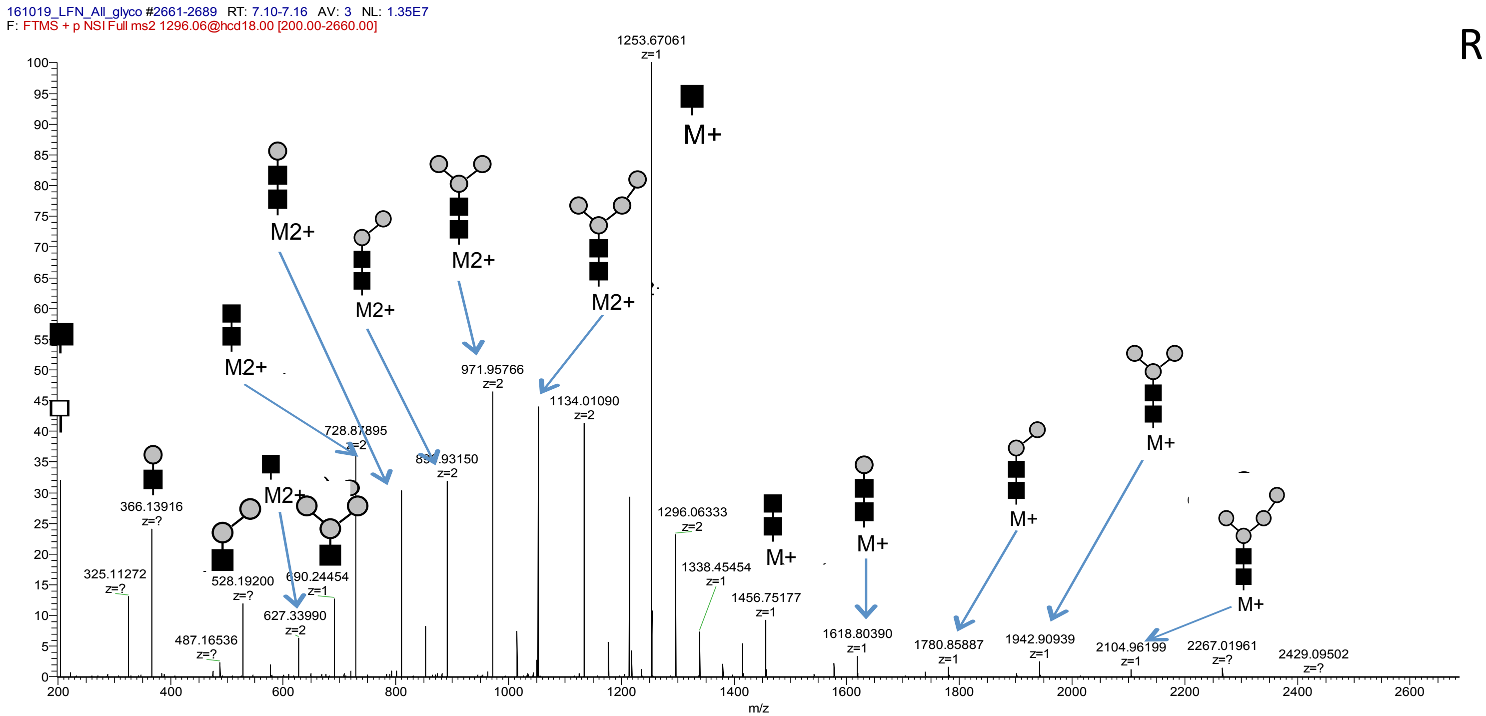
$\Delta = 2.9$ ppm

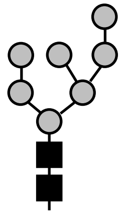


Melanoma B14

Figure S8

Recomb PD-L1





LF**N**VTSTLR

m/z 1296.065 (+2)

Δ = 3.0 ppm

Melanoma B14

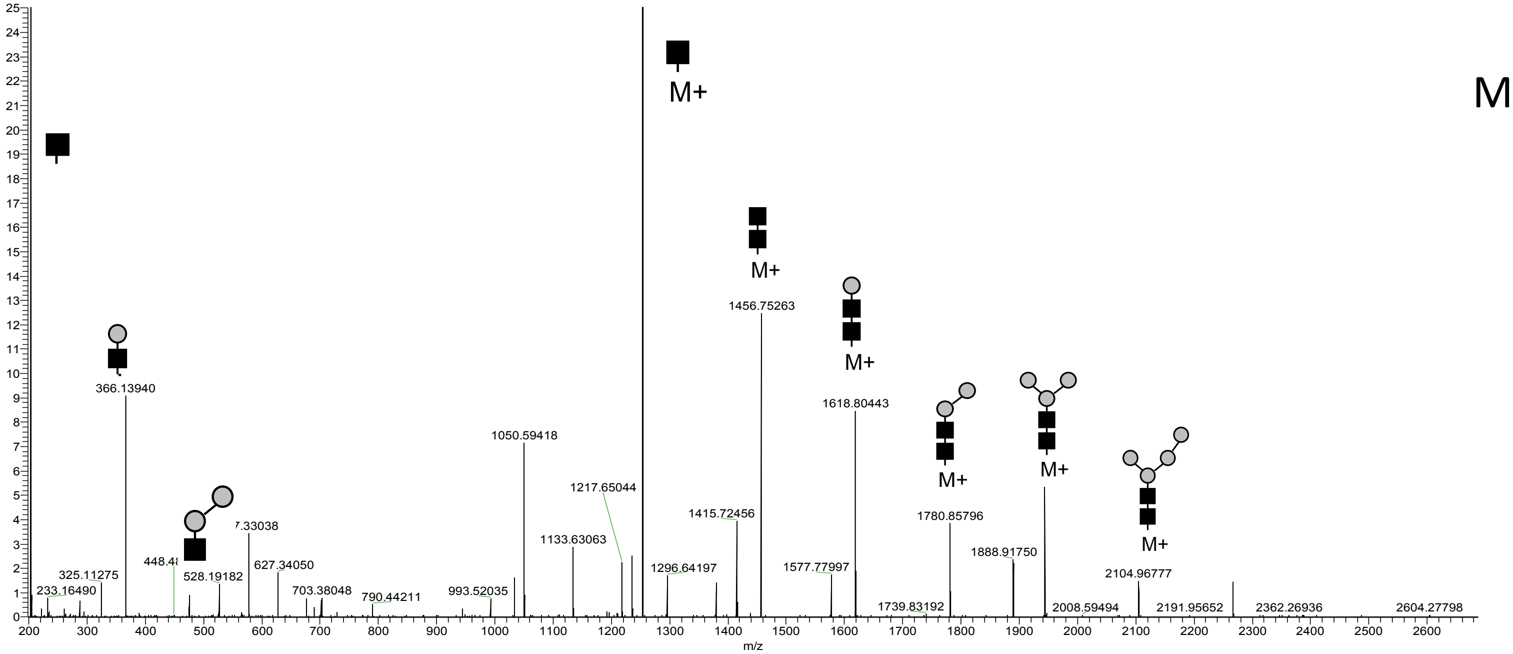


Figure S9

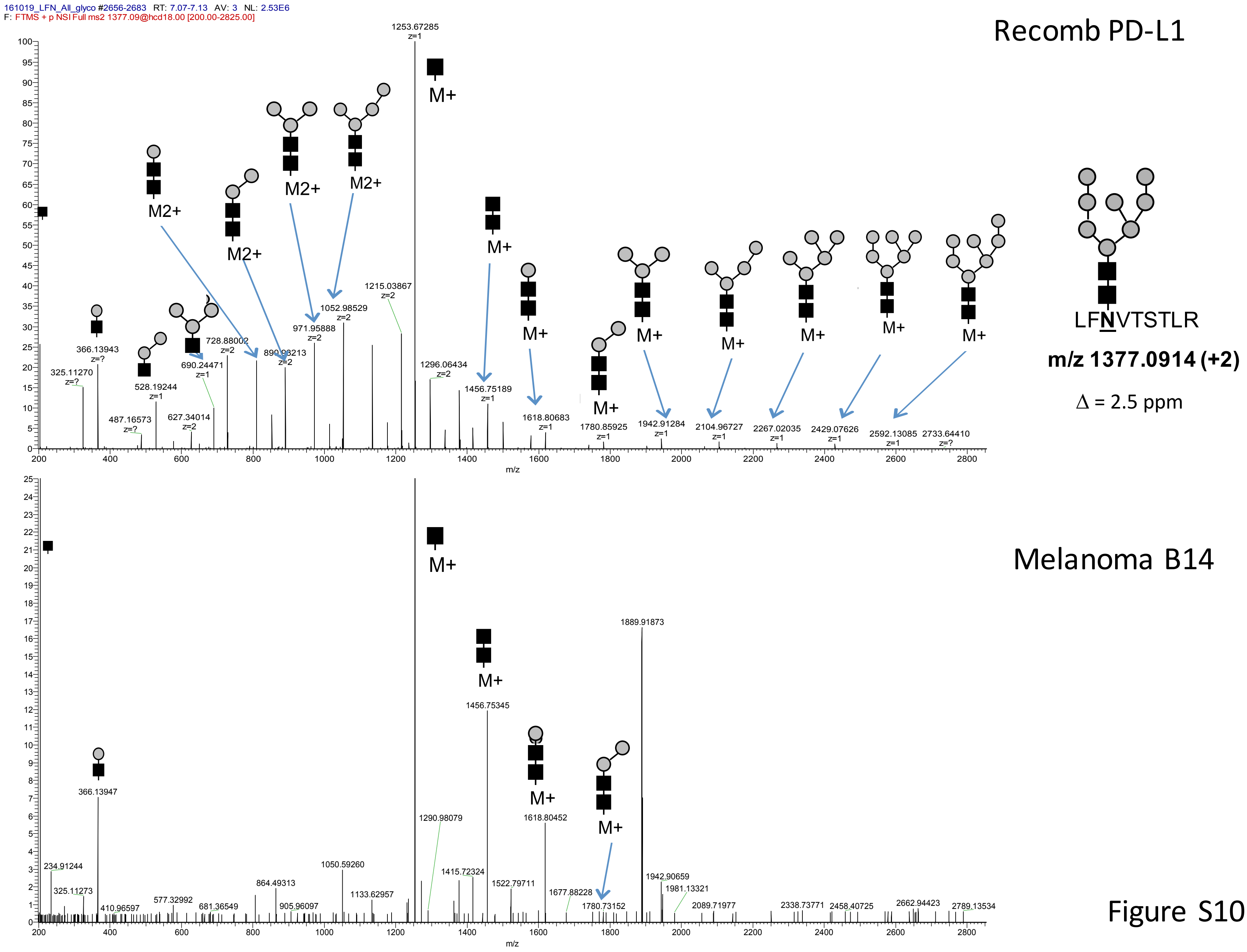
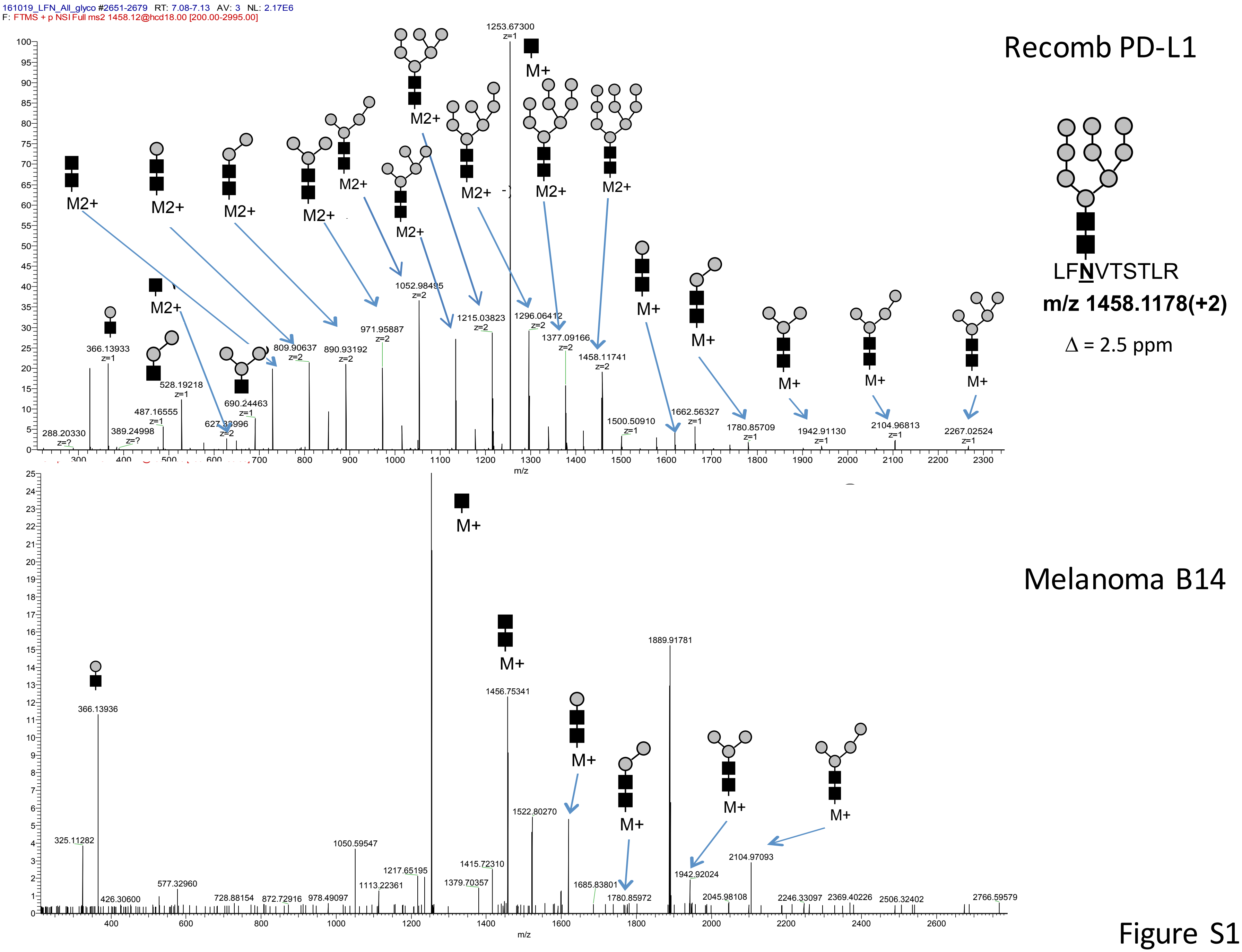

161019_LFN_All_glyco #2656-2683 RT: 7.07-7.13 AV: 3 NL: 2.53E6
F: FTMS + p NSI Full ms2 1377.09@hcd18.00 [200.00-2825.00]
Recomb PD-L1
1253.67285 z=1
M+
M2+
M2+
M2+
M2+
M+
M+
M+
M+
M+
M+
M+
M+
M+
366.13943 z=?
325.11270 z=?
528.19244 z=1
487.16573 z=?
690.24471 z=1
627.34014 z=2
728.88002 z=2
890.93213 z=2
971.95888 z=2
1052.98529 z=2
1215.03867 z=2
1296.06434 z=2
1456.75189 z=1
1618.80683 z=1
1780.85925 z=1
1942.91284 z=1
2104.96727 z=1
2267.02035 z=1
2429.07626 z=1
2592.13085 z=1
2733.64410 z=?
LFNVTSTLR
m/z 1377.0914 (+2)
Δ = 2.5 ppm
m/z
Melanoma B14
M+
M+
M+
M+
366.13947
234.91244
325.11273
410.96597
577.32992
681.36549
864.49313
905.96097
1050.59260
1133.62957
1290.98079
1415.72324
1456.75345
1522.79711
1618.80452
1677.88228
1780.73152
1889.91873
1942.90659
1981.13321
2089.71977
2338.73771
2458.40725
2662.94423
2789.13534
m/z

Figure S10



Recomb PD-L1

LF**N**VTSTLR

m/z 1458.1178(+2)

Δ = 2.5 ppm

Melanoma B14

Figure S11