**Table S1.** Determined N- and O-linked glycosylation sites within glycoprotein E ($gE_{HP}$)

| Start | End | Sequence | Glycan modification(s) |
|---|---|---|---|
| 73 | 85 | KAYDHN**S**PYIWPR | None; **HexHexNAcNeuAc(2)** |
| 74 | 85 | AYDHN**S**PYIWPR | None; **HexHexNAcNeuAc** |
| 114 | 145 | LMQPTQMSAQEDLGDDTGIHVIPTLNGDDRHK | 2 HexHexNAcNeuAc; 2 HexHexNAcNeuAc(2) |
| 114 | 145 | LMQPTQMSAQEDLGDDTGIHVIPTLNGDDRHK | HexHexNAcNeuAc and HexHexNAcNeuAc(2) |
| 114 | 145 | LMQPTQMSAQEDLGDDTGIHVIPTLNGDDRHK | HexHexNAcNeuAc(2)Ac and HexHexNAcNeuAc(2) |
| 114 | 145 | LMQPTQMSAQEDLGDDTGIHVIPTLNGDDRHK | HexHexNAcNeuGc and HexHexNAcNeuAc(2) |
| 153 | 159 | QYGDVFK | None |
| 153 | 165 | QYGDVFKGDLNPK | None |
| 153 | 170 | QYGDVFKGDLNPKPQGQR | None |
| 160 | 170 | GDLNPKPQGQR | None |
| 249 | 260 | MDSPHEYGTWVR | None |
| 305 | 320 | GSDGTSTYATFLVTWK | None |
| 305 | 324 | GSDGTSTYATFLVTWKGDEK | None |
| 321 | 337 | GDEKTRNP**T**PAV**T**PQPR | **2 HexHexNAcNeuAc;** HexHexNAc and HexHexNAcNeuAc |
| 325 | 337 | TRNPTPAV**T**PQPR | **HexHexNAc; HexHexNAcNeuAc; HexHexNAcNeuAc(2)** |
| 325 | 337 | TRNP**T**PAV**T**PQPR | **2 HexHexNAc**; **2 HexHexNAcNeuAc** |
| 325 | 337 | TRNP**T**PAV**T**PQPR | **HexHexNAc**[a] and **HexHexNAcNeuAc**[a] |
| 325 | 337 | TRNP**T**PAV**T**PQPR | **HexHexNAcNeuAc**[a] and **HexHexNAcNeuAc(2)**[a] |
| 430 | 440 | QNCEHAD**N**YTA | dHex(1)Hex(5)HexNAc(4) |
| 430 | 441 | QNCEHAD**N**YTAY | dHex(1)Hex(5)HexNAc(4) |
| 433 | 441 | EHAD**N**YTAY | dHex(1)Hex(5)HexNAc(4) |
| 433 | 440 | EHAD**N**YTA | dHex(1)Hex(5)HexNAc(4) |
| 430 | 439 | QNCEHAD**N**YT | dHex(1)Hex(5)HexNAc(4)NeuAc(1) |
| 430 | 440 | QNCEHAD**N**YTA | dHex(1)Hex(5)HexNAc(4)NeuAc(1) |
| 433 | 439 | EHAD**N**YT | dHex(1)Hex(5)HexNAc(4)NeuAc(1) |
| 433 | 441 | EHAD**N**YTAY | dHex(1)Hex(5)HexNAc(4)NeuAc(1) |
| 433 | 440 | EHAD**N**YTA | dHex(1)Hex(5)HexNAc(4)NeuAc(1) |
| 508 | 523 | GFPPTAGQPPA**T**TKPK | **HexHexNAcNeuAc; HexHexNAcNeuAc(2)** |
| 508 | 523 | GFPP**T**AGQPPA**T**TKPK | **2 HexHexNAcNeuAc** |
| 508 | 537 | GFPPTAGQPPATTKPKEITPVNPGTSPLLR | 2 HexHexNAcNeuAc(2) |
| 508 | 537 | GFPP**T**AGQPPA**TT**KPKEITPVNPGTSPLLR | **3 HexHexNAcNeuAc** |
| 508 | 537 | GFPPTAGQPPATTKPKEITPVNPGTSPLLR | HexHexNAcNeuAc; HexHexNAcNeuAc(2) |
| 508 | 537 | GFPPTAGQPPATTKPKEITPVNPGTSPLLR | HexHexNAcNeuAc and HexHexNAcNeuAc(2) |
| 508 | 537 | GFPPTAGQPPATTKPKEITPVNPGTSPLLR | 4-6 HexHexNAcNeuAc |
| 524 | 537 | EI**T**PVNPGTSPLLR | None; **HexHexNAcNeuAc**; HexHexNAcNeuAc(2) |
| 524 | 537 | EITPVNPGTSPLLR | HexHexNAcNeuAc and HexHexNAcNeuAc(2) |

Bold and underlined Ser/Thr residues were identified as glycosylation sites from the ETD spectra analysis. The corresponding structures in bold were assigned to those sites. Non-bold structures were only identified in the HCD spectra analysis. [a] denotes that both glycans were pinpointed to each site. N-glycopeptides were identified from the pronase sample.

**Table S2.** Reactivity of VZV-positive and VZV-negative sera towards a panel of synthetic peptides/glycopeptides representing VZV gE[1].

| Range of sequence | Complete peptide sequence | Number of VZV+ sera showing reactivity[2] | Number of VZV- sera showing reactivity[3] | Glycan modification verified in $gE_{HP}$ |
|---|---|---|---|---|
| W64 – Y81 | WVNRGESSRKAYDHNSPY | 3 | 1 | n.a. |
| W64 – Y81 | WVNRGES*S*RKAYDHNSPY | 0 | 0 | - |
| W64 – Y81 | WVNRGES*S*RKAY*DHNSPY | 0 | 1 | - |
| W64 – Y81 | WVNRGESS*RKAYDHNSPY | 0 | 0 | - |
| W64 – Y81 | WVNRGESSRKAYDHNS*PY | 0 | 1 | + |
| W64 – Y81 | WVNRGESSRKAY*DHNSPY | 0 | 2 | - |
| W64 – Y81 | WVNRGES*SRKAYDHNSPY | 2 | 2 | - |
| K73 – G90 | KAYDHNSPYIWPRNDYDG | 10 | 4 | n.a. |
| K73 – G90 | KAY*DHNSPYIWPRNDYDG | 2 | 1 | - |
| K73 – G90 | KAYDHNS*PYIWPRNDYDG | 1 | 0 | + |
| K73 – G90 | KAYDHNSPYIWPRNDY*DG | 1 | 1 | - |
| K73 – G90 | KAYDHNSPY*IWPRNDYDG | 1 | 1 | - |
| V101 – M120 | VYNQGRGIDSGERLMQPTQM | 4 | 0 | n.a. |
| V101 – M120 | VYNQGRGIDSGERLMQPT*QM | 0 | 0 | +[#] |
| G111 – T130 | GERLMQPTQMSAQEDLGDDT | 0 | 0 | n.a. |
| G111 – T130 | GERLMQPT*QMSAQEDLGDDT | 0 | 0 | +[#] |
| G111 – T130 | GERLMQPT*QMS*AQEDLGDDT | 0 | 1 | +[#] |
| G111 – T130 | GERLMQPTQMS*AQEDLGDDT | 2 | 0 | +[#] |
| S121 - G140 | SAQEDLGDDTGIHVIPT*LNG | 3 | 0 | + |
| S121 - G140 | S*AQEDLGDDTGIHVIPT*LNG | 3 | 2 | +[#] |
| S121 - G140 | S*AQEDLGDDTGIHVIPTLNG | 3 | 0 | +[#] |
| S121 - G140 | SAQEDLGDDTGIHVIPTLNG | 3 | 0 | n.a. |

| | | | | |
|---|---|---|---|---|
| G131 – D150 | GIHVIPT*LNGDDRHKIVNVD | 0 | 2 | + |
| G131 – D150 | GIHVIPTLNGDDRHKIVNVD | 0 | 0 | n.a. |
| R190 – T207 | RIYGVRYTETWSFLPS*LT | 0 | 0 | - |
| T199 – I216 | TWSFLPS*LTCTGDAAPAI | 1 | 1 | - |
| L316 – T333 | LVTWKGDEKTRNPT*PAVT | 0 | 1 | + |
| T329 – W344 | TPAVT*PQPRGAEFHMW | 0 | 0 | + |
| T325 – H342 | TRNPTPAVTPQPRGAEFH | 0 | 0 | n.a. |
| T325 – H342 | TRNPT*PAVTPQPRGAEFH | 0 | 1 | + |
| T325 – H342 | TRNPTPAVT*PQPRGAEFH | 0 | 0 | + |
| L379 – C396[‡] | LYVPIDPT*CQPMRLYSTC | 8 | 9 | - |
| L406 – R423 | LSHMNSGCTFTS*PHLAQR | 1 | 0 | - |
| L406 – R423 | LSHMNSGCTFTSPHLAQR | 0 | 0 | n.a. |
| L406 – R423 | LSHMNSGCTFT*SPHLAQR | 0 | 0 | - |
| A421 – A440 | AQRVASTVYQNCEHADNYTA | 1 | 0 | n.a. |
| N431 – P450 | NCEHADNYTAYCLGISHMEP | 0 | 0 | n.a. |
| C442 – G459 | CLGISHMEPS*FGLILHDG | 0 | 0 | - |
| G460 – V477 | GTTLKFVDTPESLS*GLYV | 4 | 1 | - |
| G460 – V477 | GTTLKFVDTPESLSGLYV | 0 | 0 | n.a. |
| G460 – V477 | GTTLKFVDTPES*LSGLYV | 0 | 0 | - |
| G460 – V477 | GTTLKFVDT*PESLSGLYV | 0 | 0 | - |
| E487 – I504 | EAVAYTVVSTVDHFVNAI | 0 | 2 | n.a. |
| E487 – I504 | EAVAYTVVS*TVDHFVNAI | 0 | 0 | - |
| E487 – I504 | EAVAYTVVST*VDHFVNAI | 0 | 4 | - |
| H499 – G514 | HFVNAIEERGFPPT*AG | 0 | 0 | + |
| E505 – P522 | EERGFPPTAGQPPATT*KP | 1 | 1 | + |
| E505 – P522 | EERGFPPTAGQPPATTKP | 0 | 0 | n.a. |
| E505 – P522 | EERGFPPT*AGQPPATTKP | 0 | 2 | + |
| E505 – P522 | EERGFPPTAGQPPAT*TKP | 4 | 3 | + |

| | | | | |
|---|---|---|---|---|
| R507 – P522 | RGFPPTAGQPPAT*TKP | 1 | 0 | + |
| R507 – P522 | RGFPPTAGQPPATT*KP | 0 | 0 | + |
| R507 – P522 | RGFPPT*AGQPPATTKP | 1 | 1 | + |
| R507 – P522 | RGFPPTAGQPPATT*KP | 0 | 1 | + |
| G514 – G531 | GQPPAT*TKPKEITPVNPG | 3 | 2 | + |
| G514 – G531 | GQPPATTKPKEITPVNPG | 4 | 3 | n.a. |
| G514 – G531 | GQPPATT*KPKEITPVNPG | 0 | 2 | + |
| G514 – G531 | GQPPATTKPKEIT*PVNPG | 4 | 4 | + |
| Q515 – G531 | QPPATT*KPKEITPVNP | 0 | 0 | + |
| Q515 – G531 | QPPAT*TKPKEITPVNP | 0 | 0 | + |
| Q515 – G531 | QPPATTKPKEIT*PVNP | 1 | 1 | + |
| K523 – Y538 | KEIT*PVNPGTSPLLRY | 3 | 1 | + |
| K523 – A540 | KEITPVNPGTS*PLLRYAA | 3 | 2 | + |
| K523 – A540 | KEITPVNPGT*SPLLRYAA | 0 | 2 | + |
| K523 – A540 | KEITPVNPGTSPLLRYAA | 3 | 2 | n.a. |

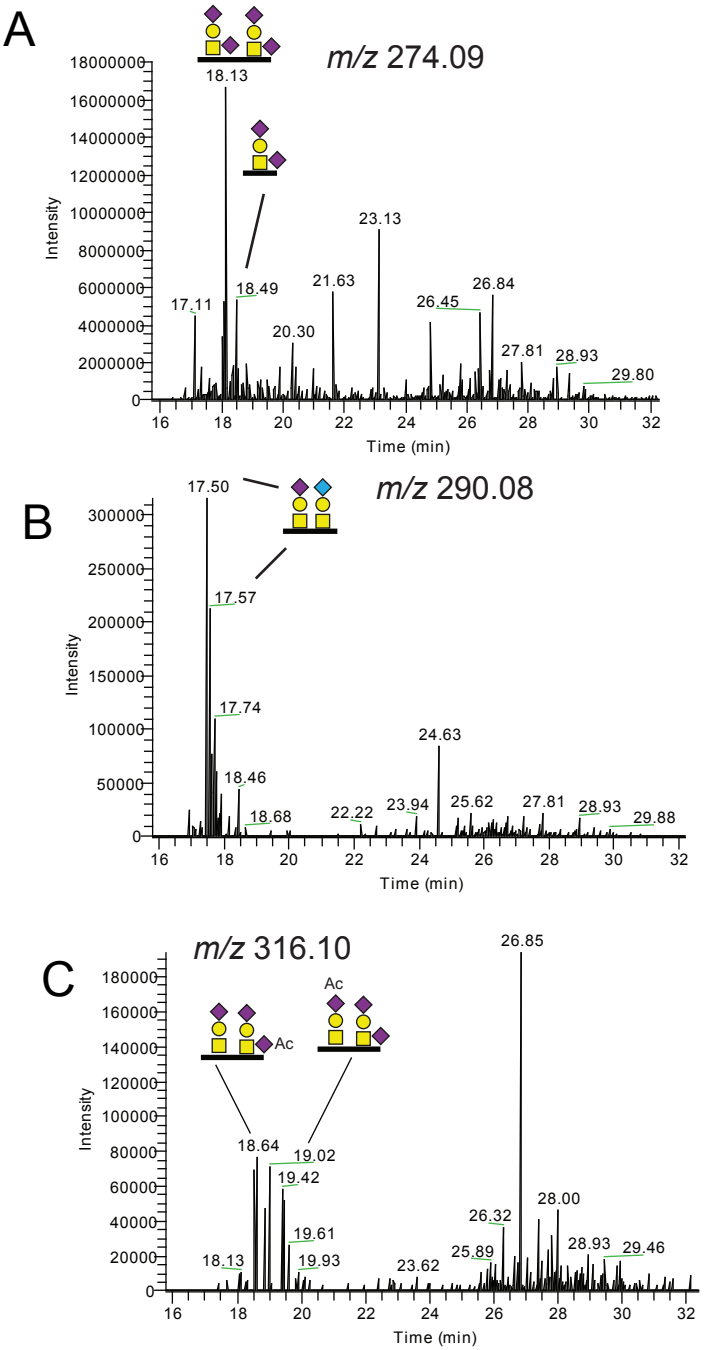
[1] The synthetic peptides were immobilized on a glass slide and incubated with sera from VZV positive (individual serum samples n=11 and pooled serum samples n=2) and negative (individual serum samples n=11 and pooled serum samples n=2) individuals and the intensity of fluorescence determined. Serum samples with a relative fluorescence value higher than two standard deviations over the mean of the control group were designated positive. The portion of gE spanning W64–D135 was previously shown to constitute a linear B cell epitope [30]. [2] Serum samples were assigned as VZV-positive using ELISA and immunofluorescence microscopy. [3] Serum samples that did not show reactivity with ELISA nor immunofluorescent microscopy were determined as VZV-negative. n.a. = not assigned, * indicates addition of a GalNAc residue to Ser, Thr or Tyr, # indicate that the glycosylation sites could not be unambiguously assigned, ‡ the peptide was synthesized with a GalNAc residue present at Thr-386 but this modification was not observed in $gE_{HP}$ or $gE_{L}$ as determined by LC-MS/MS indicating that the observed reactivity could represent cross reactivity with other viral or human proteins

Fig. S1

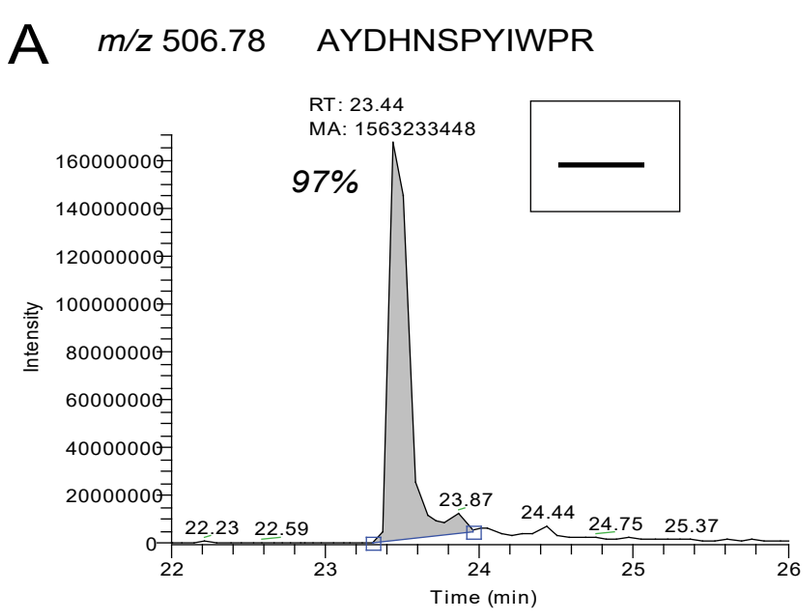
| | | | | | |
|---|---|---|---|---|---|
| 1 | MGTVNKPVVG | VLMGFGIITG | TLRITNPVRA | SVLRYDDFHI | DEDKLDTNSV |
| 51 | YEPYYHSDHA | ESSWVNRGES | SRKAYDHNSP | YIWPRNDYDG | FLENAHEHHG |
| 101 | VYNQGRGIDS | GERLMQPT*QM | S*AQEDLGDDT | GIHVIPTLNG | DDRHKIVNVD |
| 151 | QRQYGDVFKG | DLNPKPQGQR | LIEVSVEENH | PFTLRAPIQR | IYGVRYTETW |
| 201 | SFLPSLTCTG | DAAPAIQHIC | LKHTTCFQDV | VVDVDCAENT | KEDQLAEISY |
| 251 | RFQGKKEADQ | PWIVVN**TSTL | FDELELDPPE | IEPGVLKVLR | TEKQYLGVYI |
| 301 | WNMRGSDGTS | TYATFLVTWK | GDEKTRNPTP | AVTPQPRGAE | FHMWNYHSHV |
| 351 | FSVGDTFSLA | MHLQYKIHEA | PFDLLLEWLY | VPIDPTCQPM | RLYSTCLYHP |
| 401 | NAPQCLSHMN | SGCTFTSPHL | AQRVASTVYQ | NCEHADNYTA | YCLGISHMEP |
| 451 | SFGLILHDGG | TTLKFVDTPE | SLSGLYVFVV | YFNGHVEAVA | YTVVSTVDHF |
| 501 | VNAIEERGFP | PTAGQPPATT | KPKEITPVNP | GTSPLLRYA | |

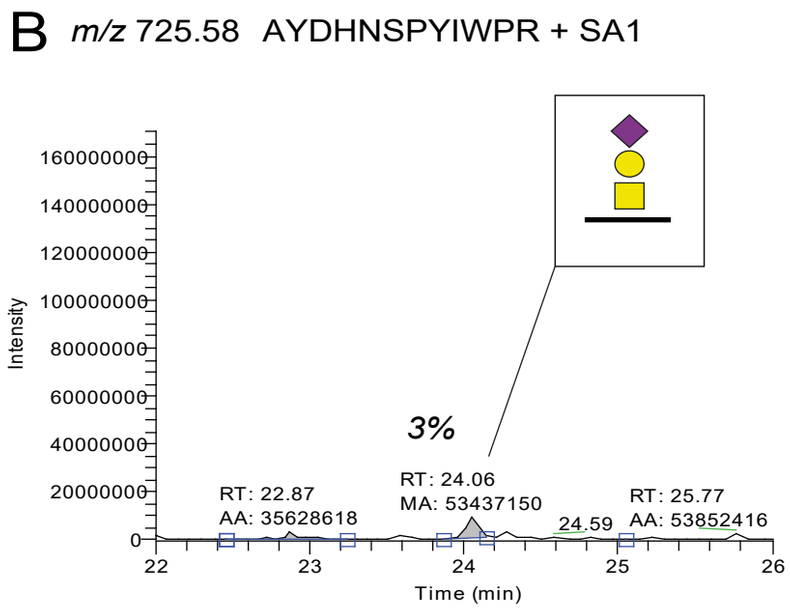
**TRYPSIN**, **PRONASE**, **BOTH.** *One of these is glycosylated. ** Not observed but possible N-glycosylation site.

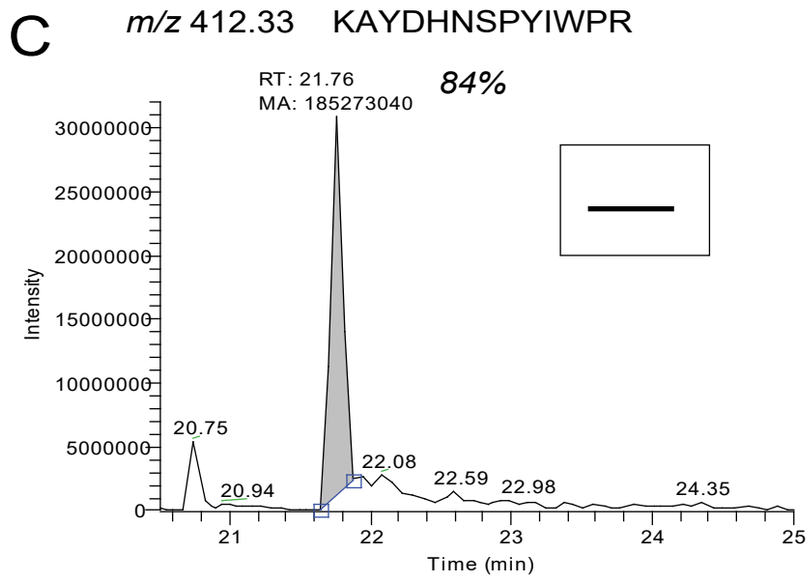
Fig. S2

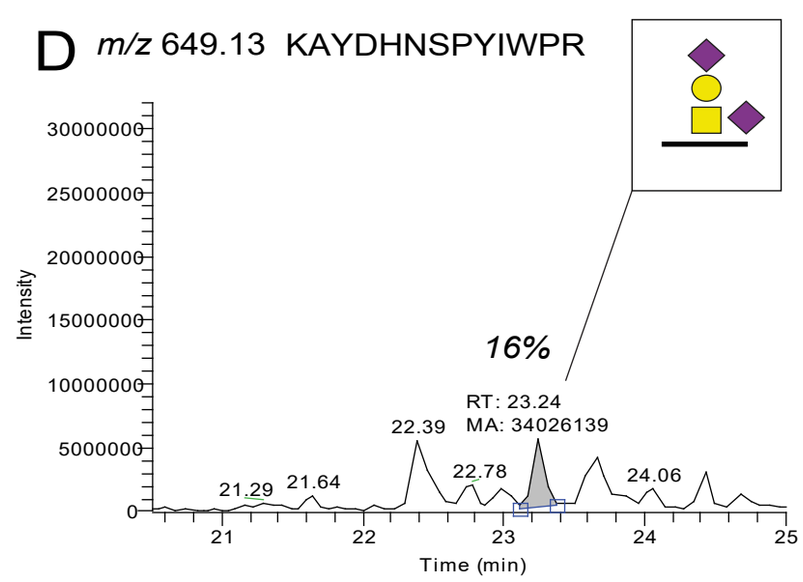

A
m/z 274.09
18.13
18.49
17.11
20.30
21.63
23.13
26.45
26.84
27.81
28.93
29.80
Intensity
Time (min)
B
m/z 290.08
17.50
17.57
17.74
18.46
18.68
22.22
23.94
24.63
25.62
27.81
28.93
29.88
Intensity
Time (min)
C
m/z 316.10
Ac
Ac
18.64
19.02
19.42
19.61
19.93
18.13
23.62
25.89
26.32
26.85
28.00
28.93
29.46
Intensity
Time (min)

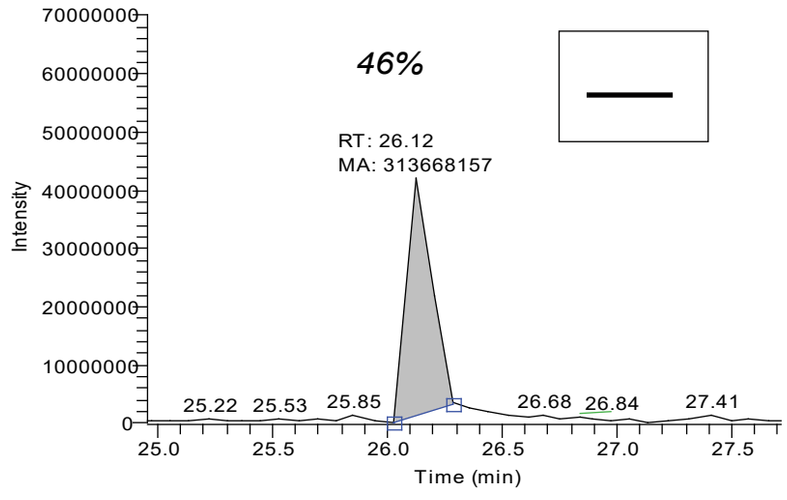
# Fig. S3

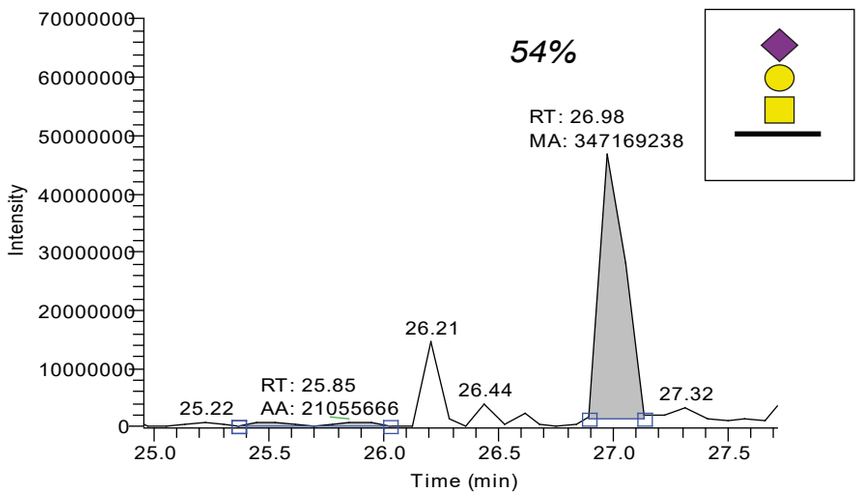

A
m/z 506.78 AYDHNSPYIWPR
RT: 23.44
MA: 1563233448
97%
Intensity
Time (min)
22.23
22.59
23.87
24.44
24.75
25.37


B
m/z 725.58 AYDHNSPYIWPR + SA1
3%
RT: 22.87
AA: 35628618
RT: 24.06
MA: 53437150
24.59
RT: 25.77
AA: 53852416
Intensity
Time (min)

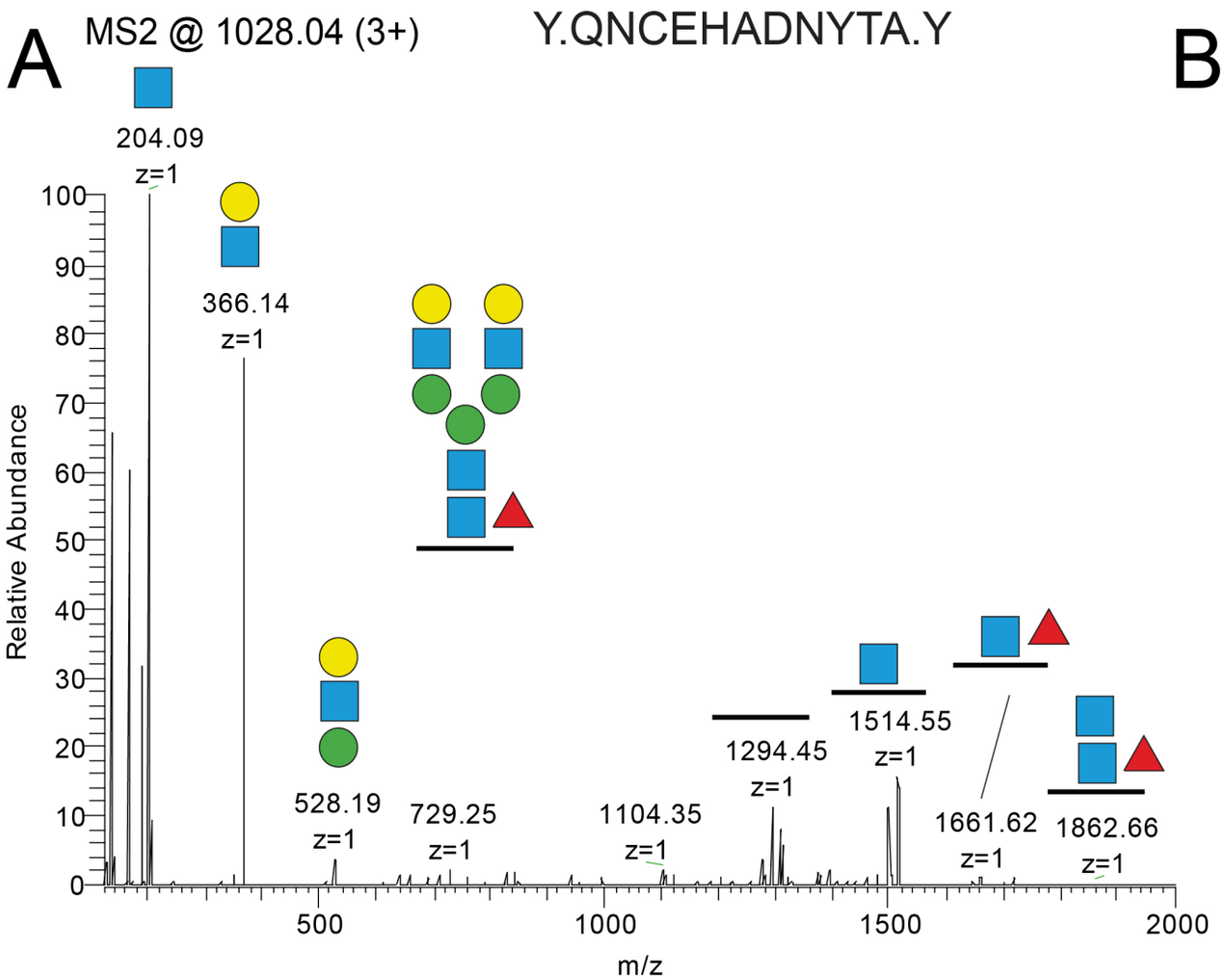

C
m/z 412.33 KAYDHNSPYIWPR
RT: 21.76
MA: 185273040
84%
Intensity
Time (min)
20.75
20.94
22.08
22.59
22.98
24.35

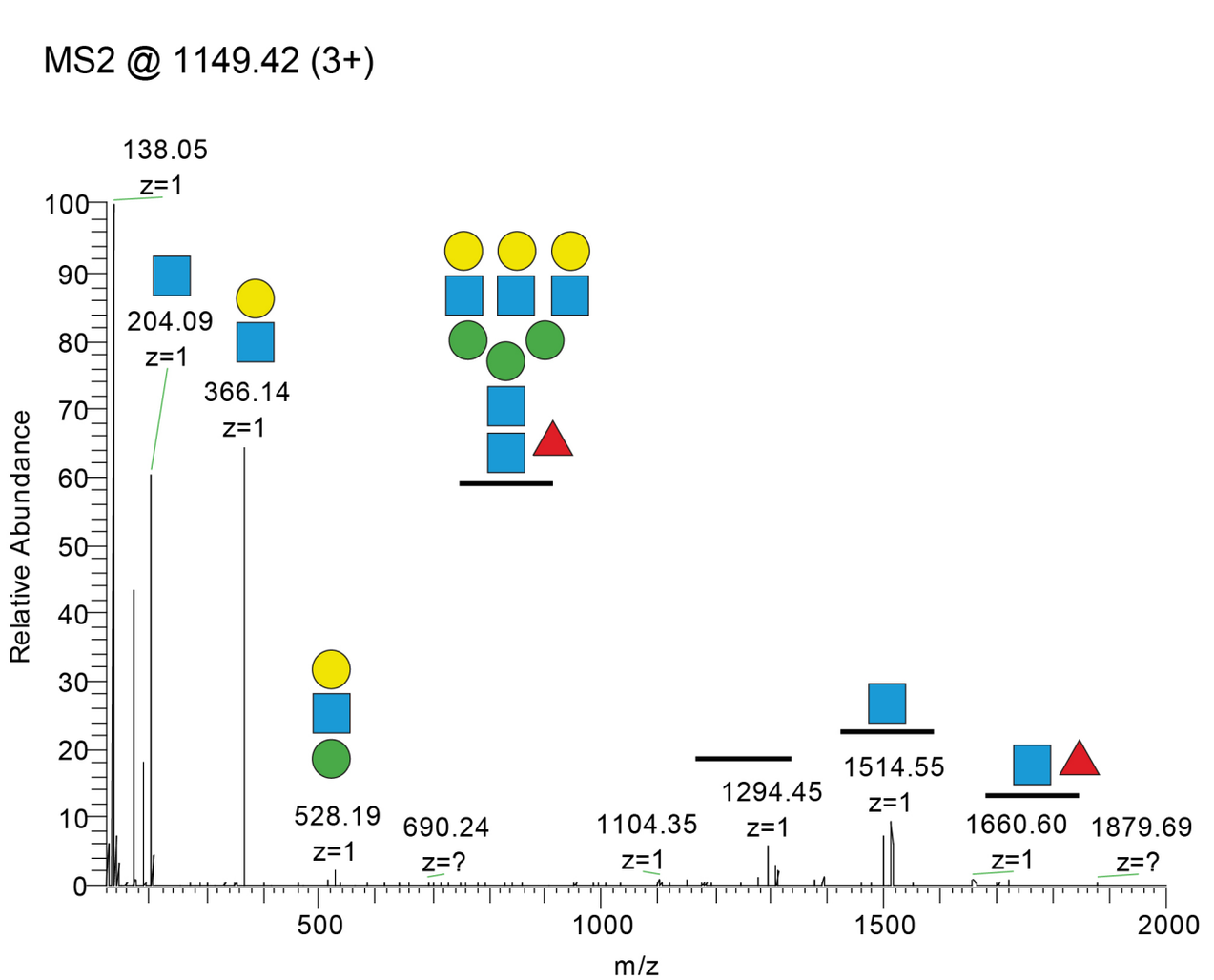

D
m/z 649.13 KAYDHNSPYIWPR
16%
RT: 23.24
MA: 34026139
Intensity
Time (min)
21.29
21.64
22.39
22.78
24.06

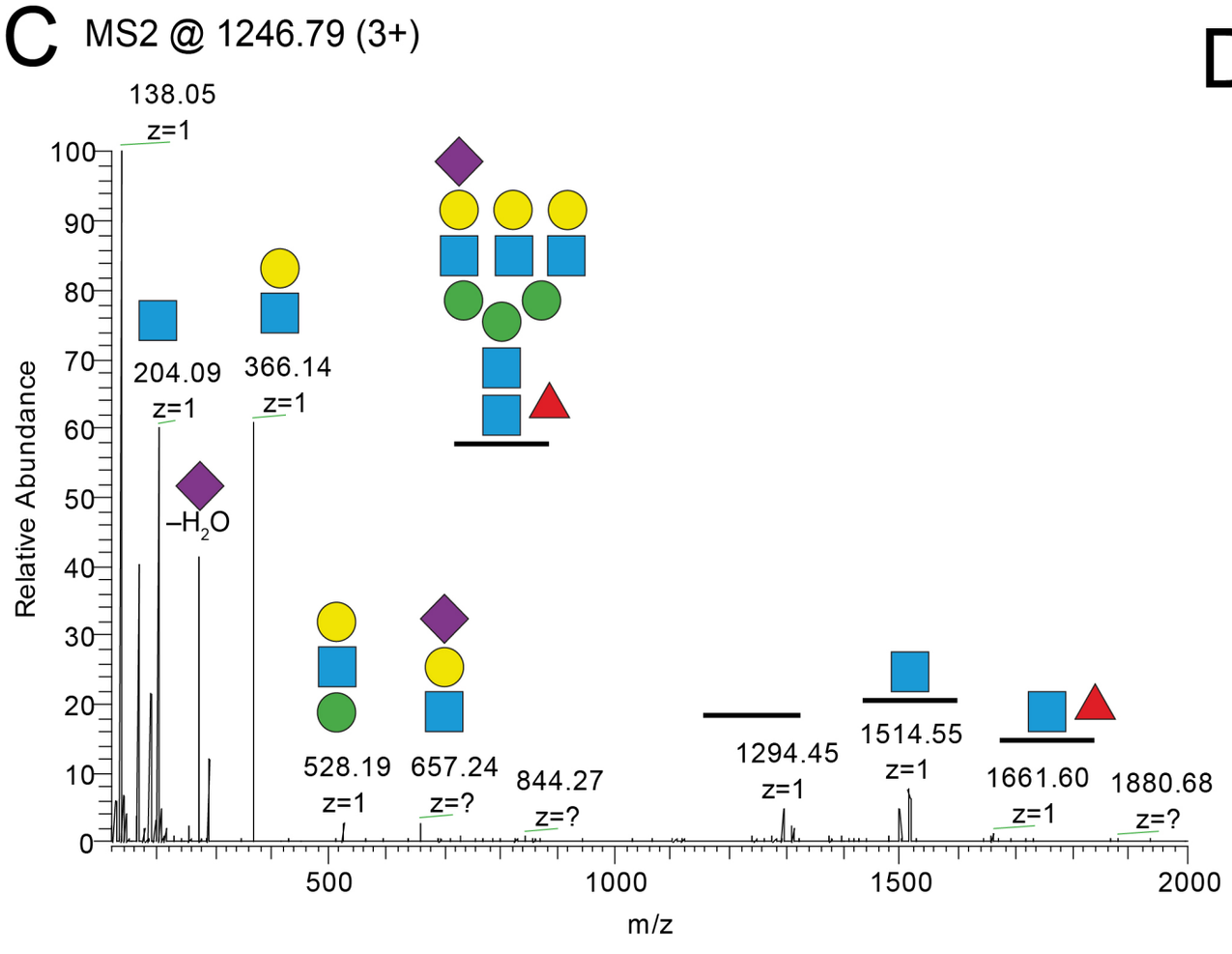

E
m/z 498.51 EITPVNPGTSPLLR
46%
RT: 26.12
MA: 313668157
Intensity
Time (min)
25.22
25.53
25.85
26.68
26.84
27.41


F
m/z 717.28 EITPVNPGTSPLLR
54%
RT: 26.98
MA: 347169238
Intensity
Time (min)
25.22
RT: 25.85
AA: 21055666
26.21
26.44
27.32

# Fig. S4


A
MS2 @ 1028.04 (3+)
Y.QNCEHADNYTA.Y
204.09
z=1
366.14
z=1
528.19
z=1
729.25
z=1
1104.35
z=1
1294.45
z=1
1514.55
z=1
1661.62
z=1
1862.66
z=1
Relative Abundance
m/z


B
MS2 @ 1149.42 (3+)
138.05
z=1
204.09
z=1
366.14
z=1
528.19
z=1
690.24
z=?
1104.35
z=1
1294.45
z=1
1514.55
z=1
1660.60
z=1
1879.69
z=?
Relative Abundance
m/z


C
MS2 @ 1246.79 (3+)
138.05
z=1
204.09
z=1
366.14
z=1
–H2O
528.19
z=1
657.24
z=?
844.27
z=?
1294.45
z=1
1514.55
z=1
1661.60
z=1
1880.68
z=?
Relative Abundance
m/z

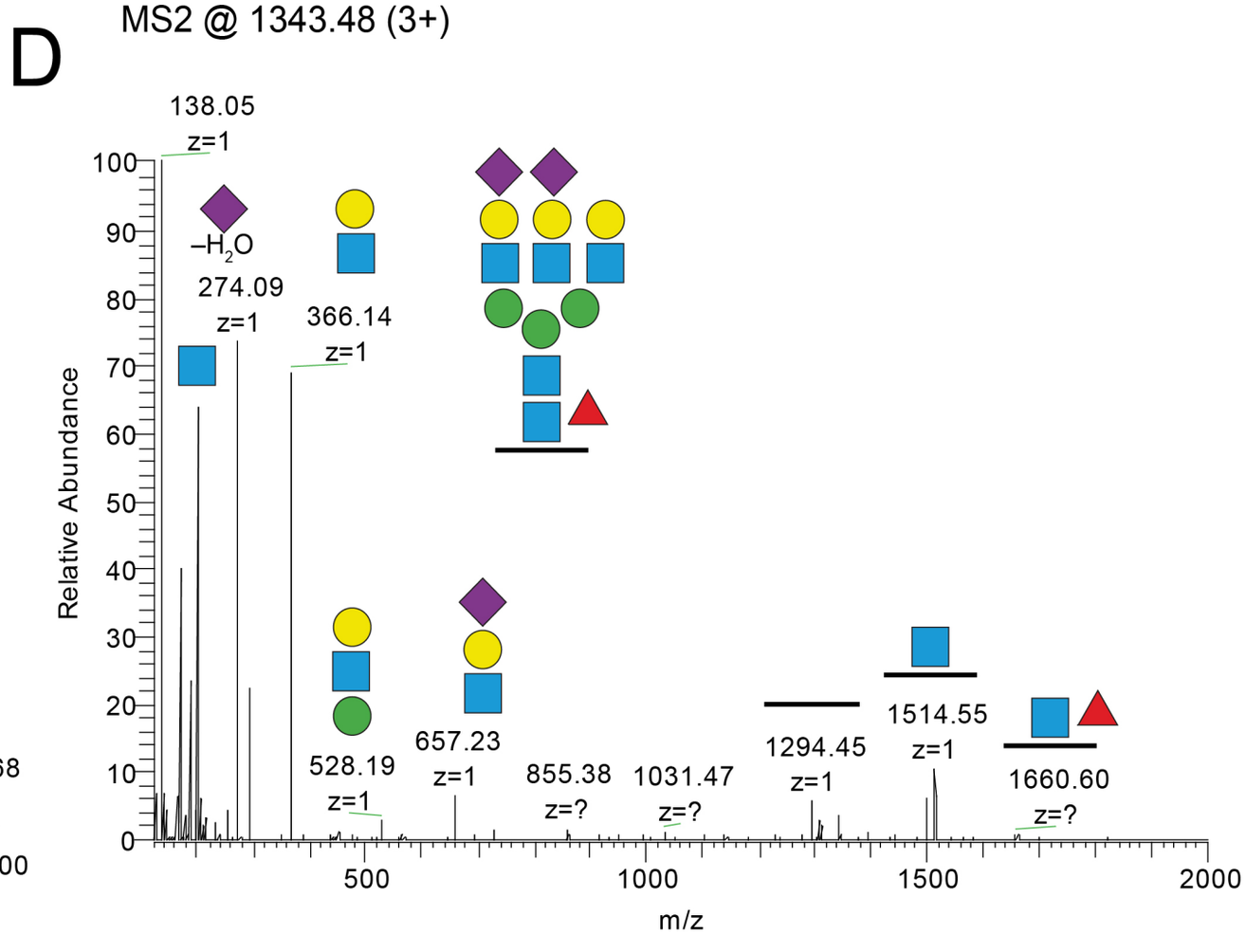
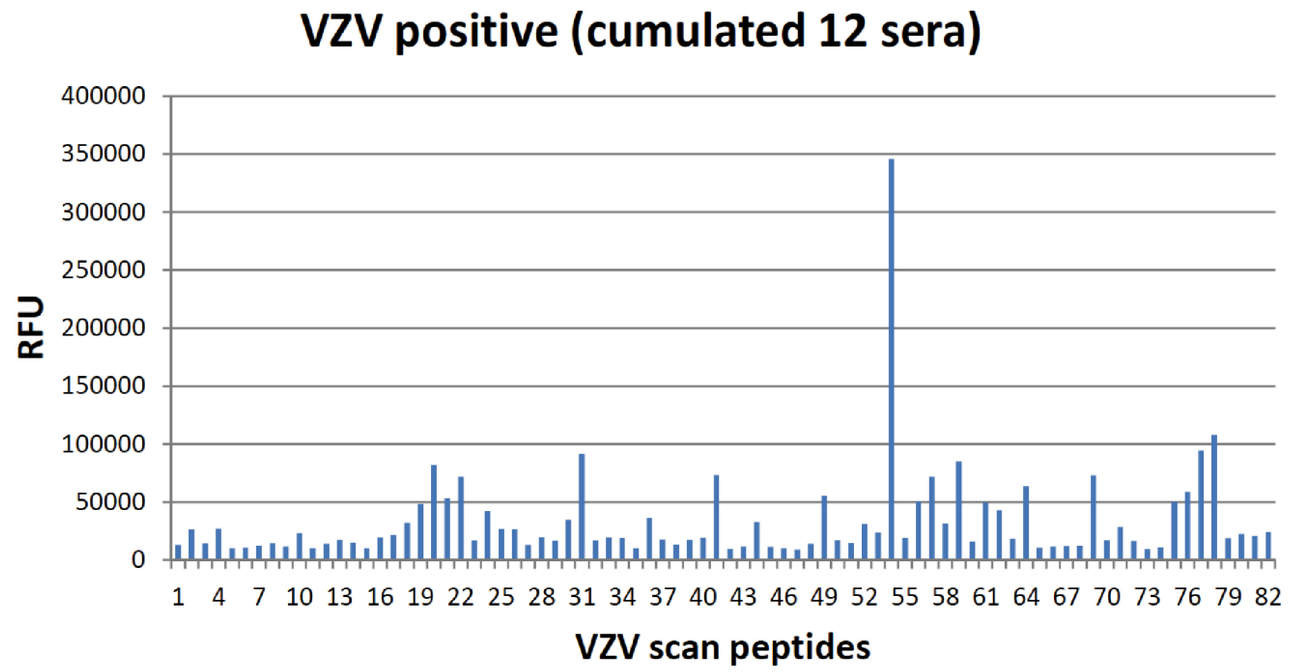
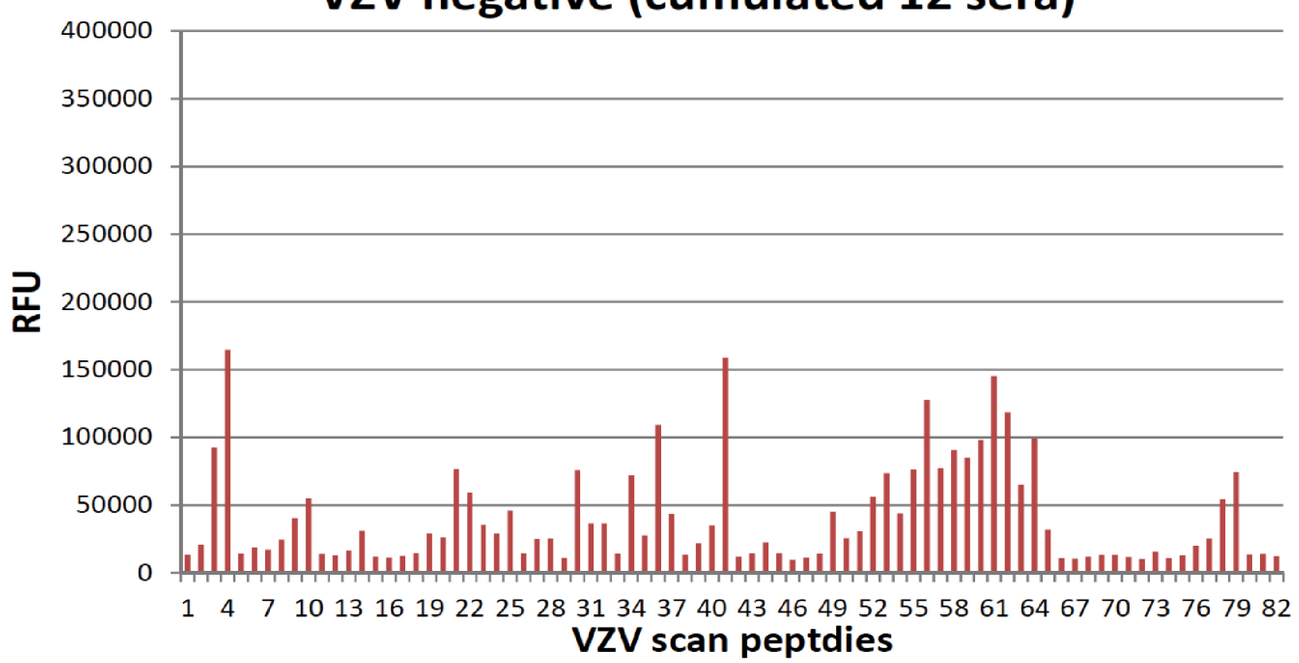

D
MS2 @ 1343.48 (3+)
138.05
z=1
–H2O
274.09
z=1
366.14
z=1
528.19
z=1
657.23
z=1
855.38
z=?
1031.47
z=?
1294.45
z=1
1514.55
z=1
1660.60
z=?
Relative Abundance
m/z

Fig. S5

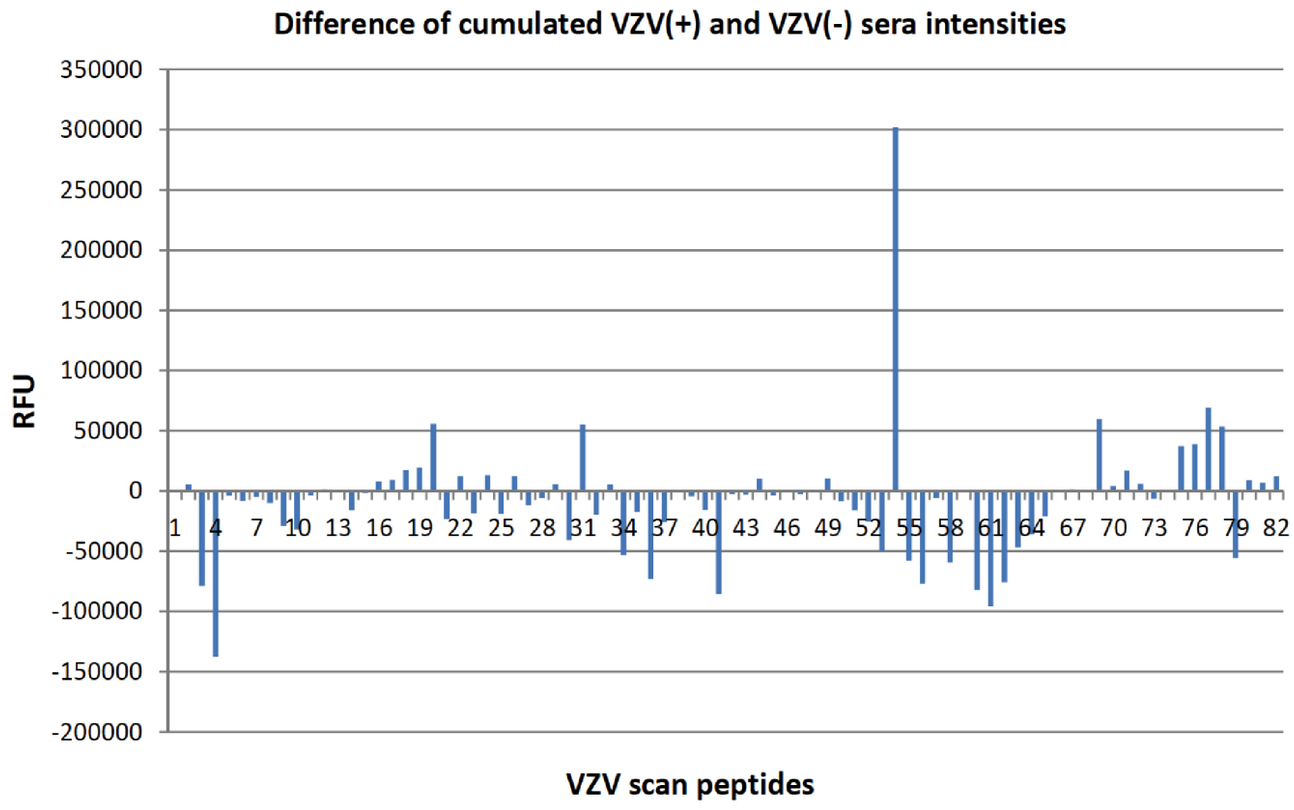
A


VZV positive (cumulated 12 sera)
RFU
400000
350000
300000
250000
200000
150000
100000
50000
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82
VZV scan peptides

B


VZV negative (cumulated 12 sera)
RFU
400000
350000
300000
250000
200000
150000
100000
50000
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82
VZV scan peptdies

C


Difference of cumulated VZV(+) and VZV(-) sera intensities
RFU
350000
300000
250000
200000
150000
100000
50000
0
-50000
-100000
-150000
-200000
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82
VZV scan peptides