

## Supplementary information

**Table S1** List of confirmed glycopeptides, glycosites and glycan compositions/structures of standard protein mixture.

| Protein | M+H                             | Glycosite   | Glycan                                      | Related TN-peptides                  |
|---------|---------------------------------|-------------|---------------------------------------------|--------------------------------------|
| HRP     | 3041.9                          | PNVSN       |                                             | DTIVNELR,<br>GFPVIDRMK               |
|         | 3894.7                          | DNTTS       |                                             | DTIVNELR,<br>GFPVIDRMK               |
|         | 1843.8                          | LNRSS       |                                             | SSDLVALSGGHTFGK                      |
|         | 4984.2 <sup>a</sup>             | YNFSN       | Hex <sub>3</sub> HexNAc <sub>2</sub> FucXyl | TPTIFDNK                             |
|         | 2591.2                          | LNTTY       |                                             | TPTIFDNK                             |
|         | 3604.6                          | GNLSA       |                                             | TPTIFDNK                             |
|         | 3671.7<br>3526.0<br>2850.4      | PNATD       |                                             | MGNITPLTGTQGQIR                      |
|         | 3378.9 <sup>b</sup><br>(3353.4) | ANSTQ       |                                             | MGNITPLTGTQGQIR                      |
| ASF     | 3102.3<br>2737.9                | ANCSV       |                                             | HTLNQIDSVKVVPR,<br>QQTQHAVEGDCDIHVLK |
|         | 3728.5                          | LNDSR       |                                             | QDGQFSVLFTK                          |
|         | 5004.3 <sup>c</sup>             | SNGSY       | Hex <sub>6</sub> HexNAc <sub>5</sub>        | QDGQFSVLFTK                          |
|         | 5659.5 <sup>d</sup>             | PSA...PTPSA | Hex <sub>4</sub> HexNAc <sub>3</sub>        | HTFSGVASVESSSGEAFHVGK                |
|         | 2205.1                          | PSI         | Hex <sub>2</sub> HexNAc <sub>2</sub>        | IRYFKI                               |

□, HexNAc; ○, Hex; ▲, Fucose (Fuc); ☆, Xylose (Xyl); Glycosites are bolded N, S or T.

<sup>a</sup>, for glycopeptide at m/z 4984.2, the glycan composition on NFS sequon was deduced under assumption that glycans on NTT sequon was the same as glycopeptides PTLNNTTYLQTLR (m/z 2591.2).

<sup>b</sup>, MS<sup>3</sup> analysis of its fragment (3353.4) was elucidated to be a glycopeptides bearing this glycan.

<sup>c</sup>, for glycopeptide [M+H] 5004.3: m/z 1668.77 (3+) , glycopeptide fragments were obtained partially due to the m/z 2000 cut-off. Plausible composition was deduced.

<sup>d</sup>, for glycopeptide [M+H] 5659.5, it has three potential O-glycosites.

**Table S2** List of glycoproteins and glycosites from two gel-bands of the SDS-PAGE gel (Figure 3a).

**Gel-band 1**

| Protein Name              | SwissProt ID | Matched Peptide |                         | Indicated Glycosite    |
|---------------------------|--------------|-----------------|-------------------------|------------------------|
|                           |              | Position        | Sequence                |                        |
| Alpha-1-antichymotrypsin  | P01011       | 145-159         | EQLSLLDRFTEDAKR         | FN <sub>106</sub> LTE  |
|                           |              | 178-185         | KLINDYVK                | LN <sub>127</sub> QSS* |
| Kininogen-1               | P01042       | 188-196         | QVVAGLNFR               | KN <sub>186</sub> GTR* |
|                           |              | 270-268         | DIPTNSPELEETLTHTITK     | NN <sub>169</sub> NTQ  |
| Ig alpha-1 chain C region | P01876       | 154-168         | DASGVTFWTPSSGK          | TN <sub>205</sub> CSK  |
| Clusterin                 | P10909       | 386-408         | VTTVASHTSDSDVPSGVTEVVVK | NN <sub>294</sub> ATF* |
|                           |              | 386-408         | VTTVASHTSDSDVPSGVTEVVVK | AN <sub>144</sub> LTC* |
| Ig alpha-2 chain C region | P01877       | 141-155         | DASGATFTWTPSSGK         | LN <sub>354</sub> TSS  |
|                           |              | 209-218         | SGNTFRPEVH              | AN <sub>374</sub> LTQ  |
|                           |              | 241-250         | GFSPKDLVLR              | AN <sub>131</sub> LTC  |
|                           |              | 251-262         | WLQGSQELPREK            | AN <sub>205</sub> ITK* |
| Endoplasmin               | P14625       | 117-135         | LISLTDENALSGNEELTVK     | SN <sub>107</sub> ASD  |

**Gel-band 2**

| Protein Name              | SwissProt ID | Matched Peptide |                                 | Indicated Glycosite    |
|---------------------------|--------------|-----------------|---------------------------------|------------------------|
|                           |              | Position        | Sequence (gel-band No.)         |                        |
| Alpha-1-antichymotrypsin  | P01011       | 145-158         | EQLSLLDRFTEDAK                  | FN <sub>106</sub> LTE  |
|                           |              | 160-177         | LYGSEAFATDFQDSAAAK              | LN <sub>127</sub> QSS  |
| Kininogen-1               | P01042       | 188-196         | QVVAGLNFR                       | KN <sub>186</sub> GTR* |
|                           |              | 188-196         | QVVAGLNFR                       | NN <sub>169</sub> NTQ  |
| Ig alpha-1 chain C region | P01876       | 154-168         | DASGVTFWTPSSGK                  | TN <sub>205</sub> CSK  |
| Clusterin                 | P10909       | 386-408         | VTTVASHTSDSDVPSGVTEVVVK         | AN <sub>144</sub> LTC* |
|                           |              | 386-408         | VTTVASHTSDSDVPSGVTEVVVK         | LN <sub>354</sub> TSS  |
| Ig alpha-2 chain C region | P01877       | 156-164         | SAVQGPPER                       | AN <sub>374</sub> LTQ  |
|                           |              | 241-250         | GFSPKDLVLR                      | AN <sub>131</sub> LTC  |
| Endoplasmin               | P14625       | 117-135         | LISLTDENALSGNEELTVK             | AN <sub>205</sub> ITK* |
|                           |              | 244-265         | GTTITLVLEKEASDYLELDTIK          | SN <sub>107</sub> ASD  |
| Alpha-2-macroglobulin     | P01023       | 897-912         | DTVIKPLLVEPEGLEK                | NN <sub>217</sub> DTQ  |
|                           |              | 935-945         | LPPNVVEESAR                     | VN <sub>869</sub> FTV* |
| Complement C3             | P01024       | 36-66           | LESEETMVLEAHDAQGDVPVTVTVHDFPGKK | LN <sub>991</sub> ETQ  |

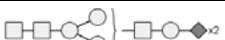
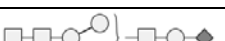
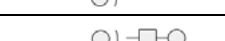
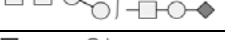
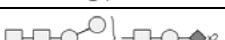
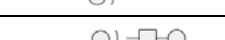
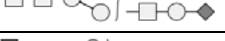
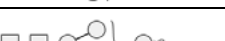
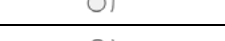
|                                              |        |         |                       |                                                                              |
|----------------------------------------------|--------|---------|-----------------------|------------------------------------------------------------------------------|
| Hemopexin                                    | P02790 | 32-49   | HGNVAEGETKPDVDVTER    | AT <sub>24</sub> P*                                                          |
|                                              |        |         |                       | DN <sub>64</sub> GTM                                                         |
|                                              |        | 333-343 | GGYTLVSGYPK           | GN <sub>187</sub> CSS*                                                       |
|                                              |        |         |                       | RN <sub>240</sub> GTG                                                        |
| Alpha-1B-glycoprotein                        | P04217 | 53-62   | LETPDFQLFK            | AN <sub>44</sub> VTL*                                                        |
|                                              |        | 107-114 | LLELTGPK              | GN <sub>179</sub> YSC                                                        |
|                                              |        | 407-417 | ATWSGAVLAGR           | HN <sub>363</sub> ISV                                                        |
|                                              |        |         |                       | AN <sub>371</sub> YSC                                                        |
| Complement C4-A                              | P0C0L4 | 235-244 | KYVLPNFVVK            | SN <sub>226</sub> SST*                                                       |
| Afamin                                       | P43652 | 89-97   | LPNNVLQEK             | HN <sub>109</sub> FSH*                                                       |
|                                              |        | 438-453 | IAPQLSTEELVSLGEK      | EN <sub>488</sub> RTI                                                        |
| Lumican                                      | P51884 | 171-181 | LKEDAVSAAFK           | VN <sub>160</sub> LTF                                                        |
| Inter-alpha-trypsin inhibitor heavy chain H4 | Q14624 | 48-56   | FAHTVVTSR             | TN <sub>81</sub> FSM                                                         |
|                                              |        | 61-75   | ANTVQEATFQMELPK       |                                                                              |
|                                              |        | 99-111  | AEAQAQYSAAVAK         |                                                                              |
| N-acetylmuramoyl-L-alanine amidase           | Q96PD5 | 175-194 | DGSPDVTADIGANTPDATK   | LN <sub>77</sub> ATE                                                         |
|                                              |        | 257-264 | TFTLLDPK              | AN <sub>367</sub> ATK                                                        |
| Complement C1r subcomponent-like protein     | Q9NZP8 | 73-80   | APEGFAVR              | EN <sub>147</sub> KTA                                                        |
|                                              |        | 177-190 | GSEAINAPGDNPAK        | VN <sub>166</sub> YSQ                                                        |
| Ig mu chain C region                         | P01871 | 65-76   | YAATSQVLLPSK          | KN <sub>46</sub> NSD*                                                        |
|                                              |        | 178-185 | VTSTLTIK              | QN <sub>209</sub> ASS                                                        |
|                                              |        | 323-332 | GVALHRPDVY            | TN <sub>272</sub> ISE...PN <sub>279</sub> ATF*                               |
|                                              |        | 377-391 | YVTSAPMPEPQAPGR       | YN <sub>439</sub> VSL*                                                       |
| Serotransferrin                              | P02787 | 400-420 | IMNGEADAMSLDGGFVYIAGK | YN <sub>432</sub> KSD*                                                       |
|                                              |        | 643-651 | SETKDLLFR             | SN <sub>630</sub> VTD*                                                       |
| Ig delta chain C region                      | P01880 | 210-222 | VPTGGVEEGLLER         | SN <sub>225</sub> GSQ                                                        |
|                                              |        |         |                       | VN <sub>316</sub> TSG                                                        |
|                                              |        |         |                       | GS <sub>331</sub> T <sub>332</sub> T <sub>333</sub> F...WS <sub>338</sub> V* |
| Vitronectin                                  | P04004 | 198-212 | DVWGIEGPIDAAFTR       | KN <sub>169</sub> GSL                                                        |
|                                              |        |         |                       | RN <sub>242</sub> ISD                                                        |
| Biotinidase                                  | P43251 | 145-156 | GDMFLVANLGTK          | FN <sub>119</sub> FTR                                                        |
|                                              |        |         |                       | FN <sub>150</sub> DTE                                                        |
|                                              |        |         |                       | NN <sub>203</sub> GTL                                                        |
| Heparin cofactor 2                           | P05546 | 368-376 | TLEAQLTPR             | TN <sub>387</sub> RTR                                                        |

|               |        |         |           |                       |
|---------------|--------|---------|-----------|-----------------------|
| Pantetheinase | O95497 | 115-123 | FGQTPVQER | PN <sub>38</sub> ATL  |
|               |        |         |           | KN <sub>130</sub> NSI |

Indicated N-glycosites are bolded letters with their position numbers as subscripts.  
 They are within two missed cleavage to TN-peptides during Lys-C digestion.

\*, glycosite confirmed by MS/MS spectra interpretation (details in Table S3).

**Table S3** List of glycoproteins/peptides and corresponding glycan compositions/structures from two gel-bands in Figure 3a.

| Spectrum No. | Protein ID | Gel-band No. | Glycopeptide Mol. Mass [M+H] <sup>+</sup> | Glycan Composition <sup>a</sup> | Accurate Glycan Mol. Mass [M] | Peptide Backbone                                    |                                                   |                                          |                              | Glycan Structure <sup>e</sup>                                                         |
|--------------|------------|--------------|-------------------------------------------|---------------------------------|-------------------------------|-----------------------------------------------------|---------------------------------------------------|------------------------------------------|------------------------------|---------------------------------------------------------------------------------------|
|              |            |              |                                           |                                 |                               | Practical Mol. Mass <sup>b</sup> [M+H] <sup>+</sup> | Matched Sequence <sup>c</sup>                     | Theoretical Mol. Mass [M+H] <sup>+</sup> | Mass Difference <sup>d</sup> |                                                                                       |
| 1            | P01011     | 1            | 4417.8351                                 | 5-4-2-0                         | 2204.7724                     | 2213.0627                                           | TLN <sub>127</sub> QSSDELQLSMGNAMFVK              | 2213.0582                                | 0.0045                       |    |
| 2            |            | 1            | 3497.4481                                 | 5-4-2-0                         | 2204.7724                     | 1292.6757                                           | LINDYVKN <sub>186</sub> GTR                       | 1292.6963                                | -0.0206                      |    |
| 3            | P01042     | 1            | 2980.1894                                 | 4-3-1-0                         | 1548.5448                     | 1431.6446                                           | LNAENN <sub>294</sub> ATFYFK                      | 1431.6909                                | -0.0463                      |    |
| 4            | P01876     | 1            | 4877.2472                                 | 5-4-1-0                         | 1913.677                      | 2963.5702                                           | LSLHRPALEDLLLGSEAN <sub>144</sub> LTCTL TGLR      | 2963.5988                                | -0.0286                      |    |
| 5            | P01877     | 1            | 2930.2667                                 | 5-5-0-1                         | 1971.7189                     | 958.5478                                            | TPLTAN <sub>205</sub> ITK                         | 958.5573                                 | -0.0095                      |    |
| 6            | P01011     | 2            | 3497.4551                                 | 5-4-2-0                         | 2204.7724                     | 1292.6827                                           | LINDYVKN <sub>186</sub> GTR                       | 1292.6963                                | -0.0136                      |    |
| 7            | P01876     | 2            | 4877.2540                                 | 5-4-1-0                         | 1913.677                      | 2963.5770                                           | LSLHRPALEDLLLGSEAN <sub>144</sub> LTCTL TGLR      | 2963.5988                                | -0.0218                      |    |
| 8            | P01877     | 2            | 2930.2696                                 | 5-5-0-1                         | 1971.7189                     | 958.5507                                            | TPLTAN <sub>205</sub> ITK                         | 958.5573                                 | -0.0066                      |    |
| 9            | P01023     | 2            | 4630.0082                                 | 5-2-0-0                         | 1216.4228                     | 3413.5854                                           | SLGNVN <sub>869</sub> FTVSAEALESQELCGT EVPSVPEHGR | 3413.6284                                | -0.0430                      |    |
| 10           | P01024     | 2            | 2836.1946                                 | 6-2-0-0                         | 1378.4756                     | 1457.7190                                           | HMGN <sub>85</sub> VTFTIPANR                      | 1457.7323                                | -0.0133                      |   |
| 11           |            |              | 2981.2701                                 |                                 |                               | 1602.7945                                           | TVLTPATNHMGN <sub>85</sub> VTF                    | 1602.7950                                | -0.0005                      |                                                                                       |
| 12           | P02790     | 2            | 3609.4211                                 | 5-4-2-0                         | 2204.7724                     | 1404.6487                                           | SWPAVGN <sub>187</sub> CSSALR                     | 1404.6694                                | -0.0207                      |  |
| 13           |            | 2            | 3651.409                                  | 5-4-1-0                         | 1913.677                      | 1737.7320                                           | GTGHGN <sub>246</sub> STHHGPEYMR                  | 1737.7516                                | -0.0196                      |  |

|                 |        |   |           |         |           |           |                                                                                                                                                                                                                                                                                                 |           |         |    |
|-----------------|--------|---|-----------|---------|-----------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|---------|----|
| 14              |        |   | 3942.5068 | 5-4-2-0 | 2204.7724 | 1737.7344 |                                                                                                                                                                                                                                                                                                 |           | -0.0172 |    |
| 15 <sup>f</sup> |        | 2 | 3371.5565 | 1-1-1-0 | 656.2276  | 2715.3289 | <b>T</b> <sub>24</sub> <b>PLP</b> <b>P</b> <b>T</b> <b>S</b> <b>A</b> <b>H</b> <b>G</b> <b>N</b> <b>V</b> <b>A</b> <b>E</b> <b>G</b> <b>E</b> <b>T</b> <b>K</b> <b>P</b> <b>D</b> <b>P</b> <b>D</b> <b>V</b> <b>T</b> <b>E</b> <b>R</b>                                                         | 2715.3320 | -0.0031 |    |
| 16              | P04217 | 2 | 4010.7791 | 4-5-0-1 | 1809.6661 | 2201.1130 | <b>A</b> <b>E</b> <b>S</b> <b>E</b> <b>S</b> <b>L</b> <b>L</b> <b>K</b> <b>P</b> <b>L</b> <b>A</b> <b>N</b> <sub>44</sub> <b>V</b> <b>T</b> <b>L</b> <b>T</b> <b>C</b> <b>Q</b> <b>A</b> <b>R</b>                                                                                               | 2201.1594 | -0.0464 |    |
| 17              | P0C0L4 | 2 | 3477.3655 | 8-2-0-0 | 1702.5812 | 1774.7843 | <b>F</b> <b>S</b> <b>D</b> <b>G</b> <b>L</b> <b>E</b> <b>S</b> <b>N</b> <sub>226</sub> <b>S</b> <b>S</b> <b>T</b> <b>Q</b> <b>F</b> <b>E</b> <b>V</b> <b>K</b>                                                                                                                                  | 1774.8135 | -0.0292 |    |
| 18              | P43652 | 2 | 3127.3326 | 3-4-0-0 | 1298.476  | 1828.8566 | <b>E</b> <b>G</b> <b>L</b> <b>P</b> <b>Q</b> <b>K</b> <b>H</b> <b>N</b> <sub>109</sub> <b>F</b> <b>S</b> <b>H</b> <b>C</b> <b>C</b> <b>S</b> <b>K</b>                                                                                                                                           | 1828.8224 | 0.0342  |    |
| 19              | P01871 | 2 | 2688.1111 | 4-3-0-1 | 1403.5073 | 1284.6038 | <b>Y</b> <b>K</b> <b>N</b> <sub>46</sub> <b>N</b> <b>S</b> <b>D</b> <b>I</b> <b>S</b> <b>S</b> <b>T</b> <b>R</b>                                                                                                                                                                                | 1284.6184 | -0.0146 |    |
| 20              |        | 2 | 2663.0782 | 6-2-0-0 | 1378.4756 | 1284.6026 |                                                                                                                                                                                                                                                                                                 |           | -0.0158 |    |
| 21              |        | 2 | 3158.3415 | 3-4-0-1 | 1444.5339 | 1713.8076 | <b>T</b> <b>H</b> <b>T</b> <b>N</b> <sub>272</sub> <b>I</b> <b>S</b> <b>E</b> <b>S</b> <b>H</b> <b>P</b> <b>N</b> <sub>279</sub> <b>A</b> <b>T</b> <b>F</b> <b>S</b> <b>A</b>                                                                                                                   | 1713.7832 | 0.0244  | NA |
| 22              |        | 2 | 3904.6182 | 8-2-0-0 | 1702.5812 | 2202.0370 | <b>S</b> <b>T</b> <b>G</b> <b>K</b> <b>P</b> <b>T</b> <b>L</b> <b>Y</b> <b>N</b> <sub>439</sub> <b>V</b> <b>S</b> <b>L</b> <b>V</b> <b>M</b> <b>S</b> <b>D</b> <b>T</b> <b>A</b> <b>G</b> <b>T</b> <b>C</b>                                                                                     | 2202.0423 | -0.0053 |    |
| 23              | P02787 | 2 | 3681.5112 | 5-4-2-0 | 2204.7724 | 1476.7388 | <b>C</b> <b>G</b> <b>L</b> <b>V</b> <b>P</b> <b>V</b> <b>L</b> <b>A</b> <b>E</b> <b>N</b> <b>Y</b> <b>N</b> <sub>432</sub> <b>K</b>                                                                                                                                                             | 1476.7521 | -0.0133 |    |
| 24              |        | 2 | 4143.5815 | 5-4-2-0 | 2204.7724 | 1938.8091 | <b>Q</b> <b>Q</b> <b>Q</b> <b>H</b> <b>L</b> <b>F</b> <b>G</b> <b>S</b> <b>N</b> <sub>630</sub> <b>V</b> <b>T</b> <b>D</b> <b>C</b> <b>S</b> <b>G</b> <b>N</b> <b>F</b>                                                                                                                         | 1938.8405 | -0.0314 |    |
| 25 <sup>f</sup> | P01880 | 2 | 4026.7826 | 2-2-2-0 | 1312.4552 | 2714.3274 | <b>T</b> <b>S</b> <b>G</b> <b>F</b> <b>A</b> <b>P</b> <b>A</b> <b>R</b> <b>P</b> <b>P</b> <b>P</b> <b>Q</b> <b>P</b> <b>G</b> <b>S</b> <sub>331</sub> <b>T</b> <sub>332</sub> <b>T</b> <sub>333</sub> <b>F</b> <b>W</b> <b>A</b><br><b>W</b> <b>S</b> <sub>338</sub> <b>V</b> <b>L</b> <b>R</b> | 2714.3837 | -0.0563 | NA |

<sup>a</sup>, numbers represent various kinds of monosaccharides in order of Hex-HexNAc-NeuAc-Fuc.

<sup>b</sup>, practical Mol. Mass values were calculated as Mol. Mass<sub>glycopeptide</sub> – Mol. Mass<sub>glycan</sub>, namely, they were the experimentally observed m/z values of multiply protonated molecular ions of peptides without their glycosylation modifications.

<sup>c</sup>, glycosites are bolded letters with their position numbers as subscripts.

<sup>d</sup>, mass differences of peptide backbones were calculated as  $\text{Mol. Mass}_{\text{practical}} - \text{Mol. Mass}_{\text{theoretical}}$ .

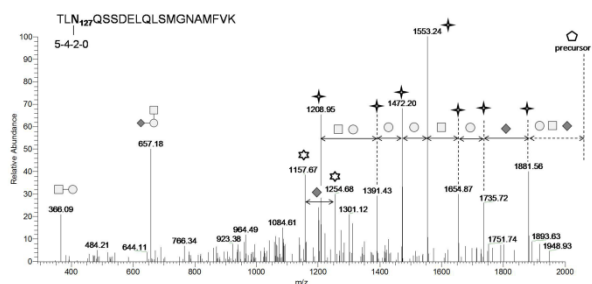
<sup>e</sup>, plausible glycan structures could be deduced from glycan-related ions (see also MS/MS spectra in Figure S1); NA, not available because of more than one potential glycosite in one peptide.

<sup>f</sup>, O-glycosites were predicted through <http://www.cbs.dtu.dk/services/NetOGlyc/>.

□, HexNAc; ○, Hex; ◆, NeuAc; ▲, Fucose (Fuc).

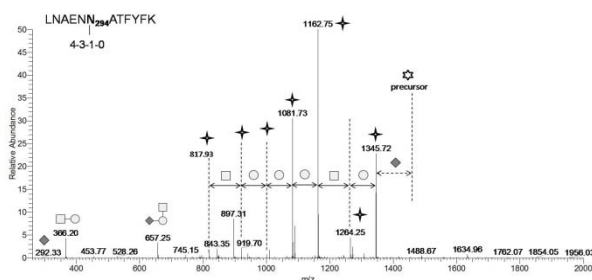
(1)

(2) see to Figure 3a



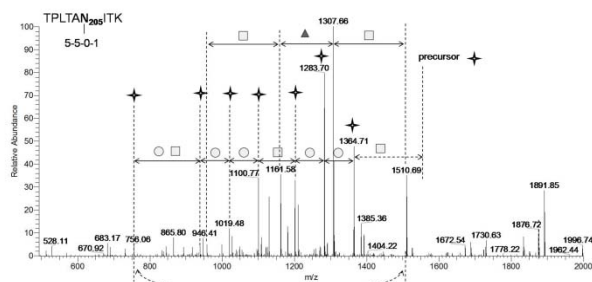
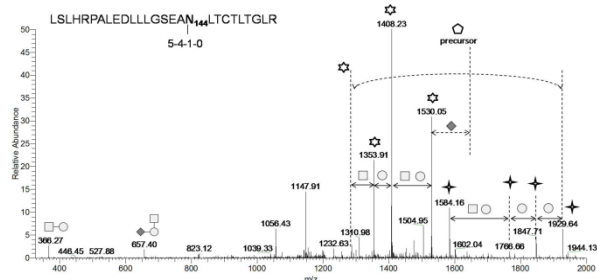
(3)

(4)



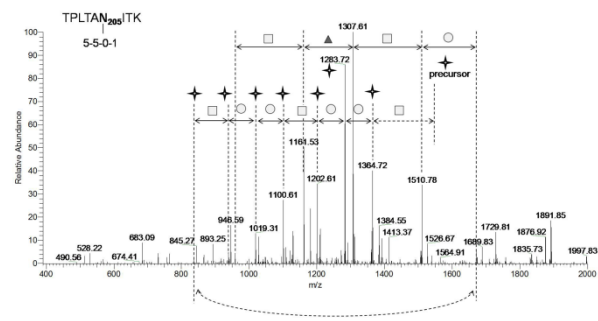
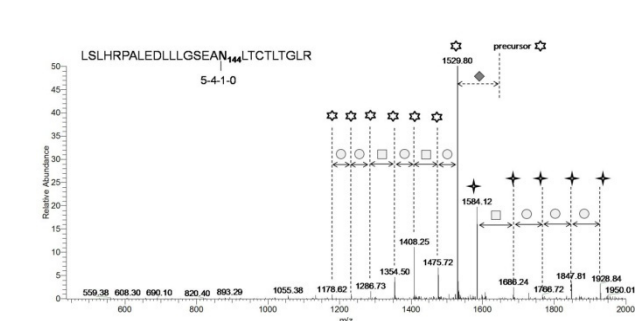
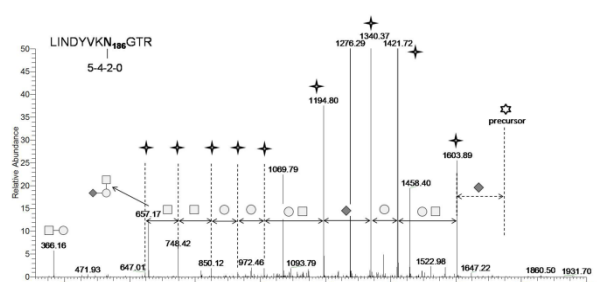
(5)

(6)



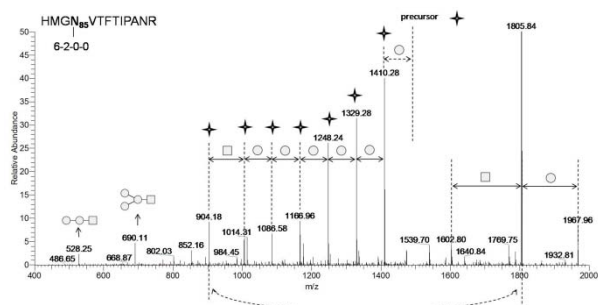
(7)

(8)

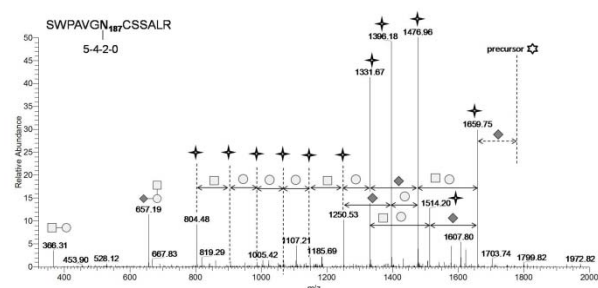




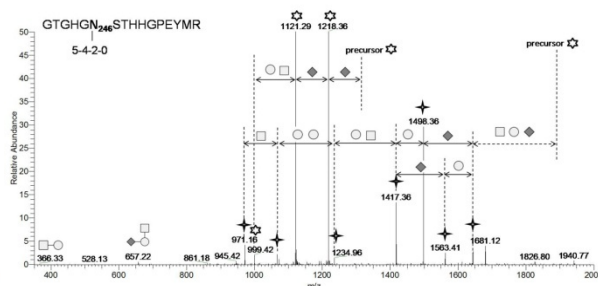
(10)



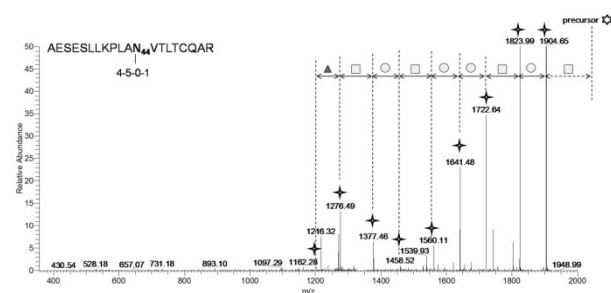
(12)



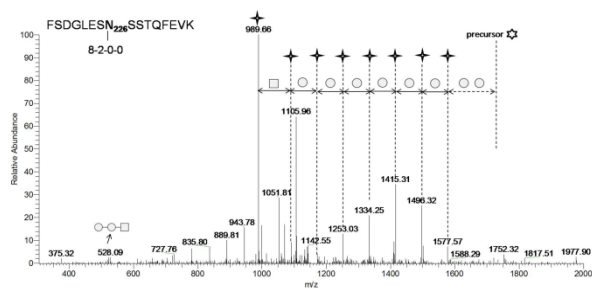
(14)



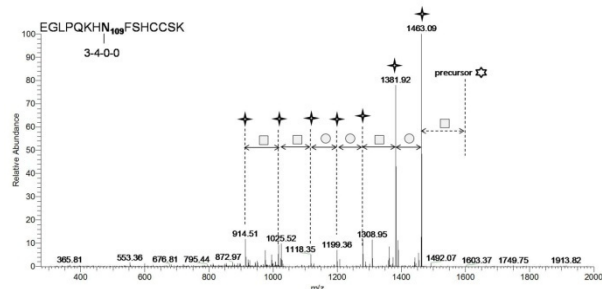
(16)



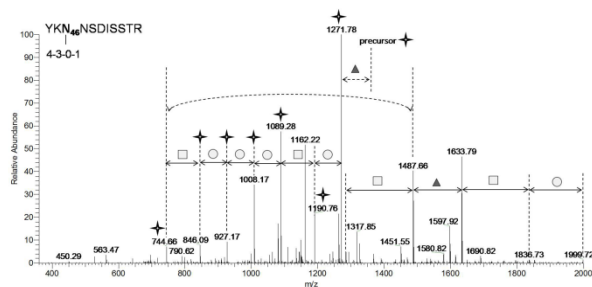
(17)



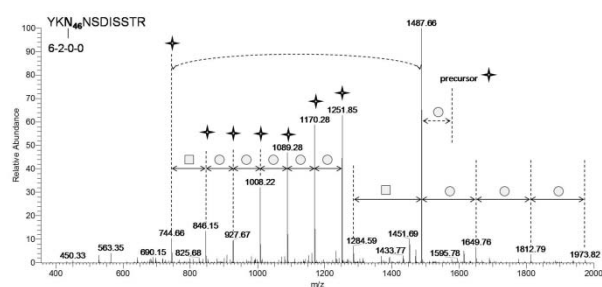
(18)



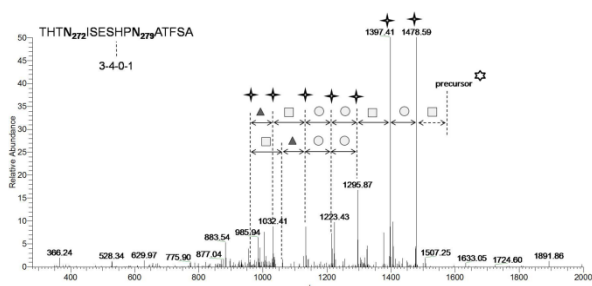
(19)



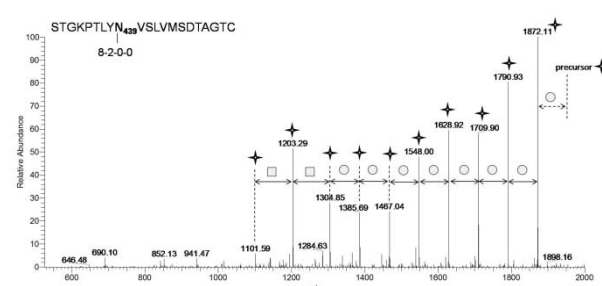
(20)



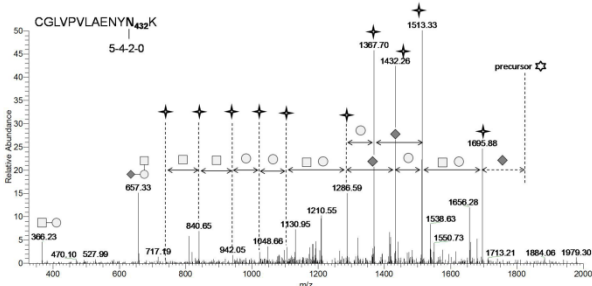
(21)



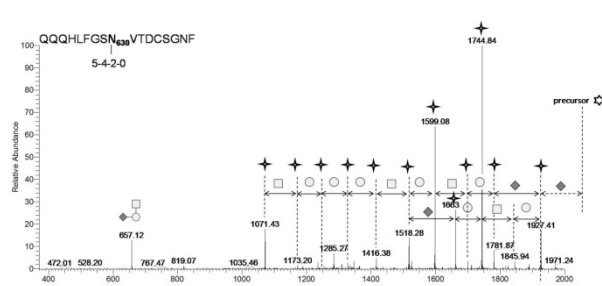
(22)



(23)



(24)



(25) see to Figure 3c

**Figure S1** ESI MS/MS Spectra of the glycopeptides in table S3.

Spectrum No. are consistent with Table S3. Precursors (drawn as impending) are only for the convenience of annotating their mass differences with glycopeptides-related peaks.

✦, ☆ and ⬡ represent ions charged 2+, 3+ and 4+, respectively.

□, HexNAc; ○, Hex; ◆, NeuAc; ▲, Fuc.