

## Supplementary Information

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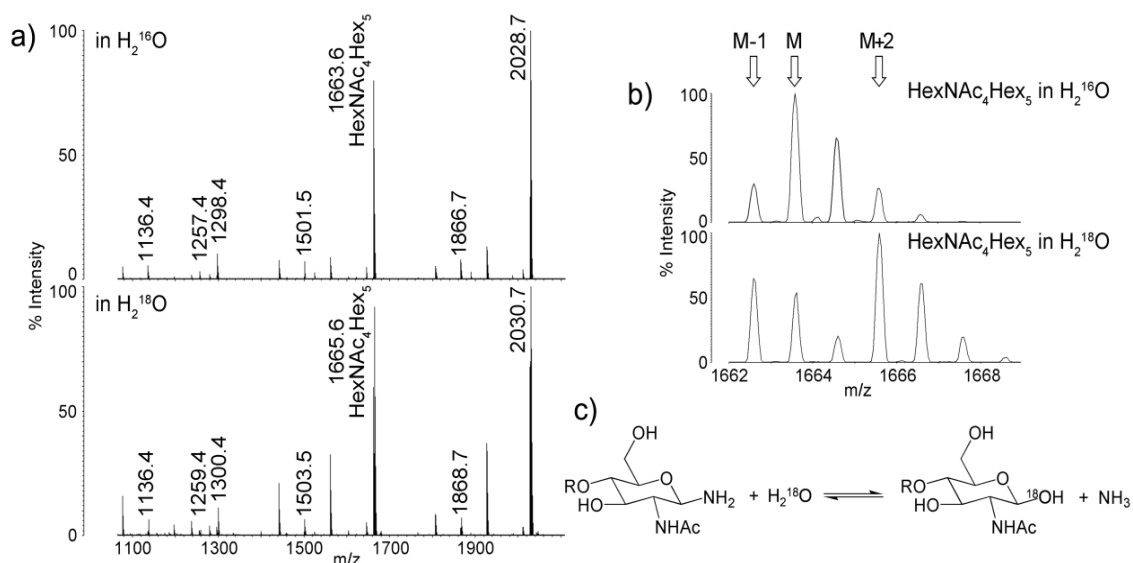
**Figure S3.** SDS-PAGE analysis of IgG from HCC/normal serum

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**Table S2.** N-glycan quantitative results of IgG from HCC/normal serum

**Table S3.** N-glycopeptide quantitative results of IgG from HCC/normal serum

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**Figure S1.** PNGase F-catalyzed  $^{18}\text{O}$ -labeling of N-glycans from asialofetuin in alkaline condition. (a) Spectra of glycans released in  $\text{H}_2^{16}\text{O}$  (top) and  $\text{H}_2^{18}\text{O}$  (bottom). (b) Enlarged spectra of the peaks at  $m/z$  1663 and 1665, sodium adduct ions of  $\text{HexNAc}_4\text{Hex}_5$  glycan. (c) The diagram of glycosamine hydrolysis equilibrium reaction.

### S-1. Overlapping-peak deconvolution method

The isotope interference was avoided by using our previously developed overlapping-peak deconvolution method (*Ref. W. Zhang, H. Wang, H. Tang and P. Yang, Anal. Chem., 2011, 83, 4975.*). The ratios of  $^{16}\text{O}$ -/ $^{18}\text{O}$ -labeled glycans were calculated using the following equation.

$$\text{ratio} \left( \frac{^{16}\text{O}}{^{18}\text{O}} \right) = \frac{I_A - \frac{I_{b-2}}{I_b} I_B}{I_B - \frac{I_{a+2}}{I_a} I_A}$$

$I_a$ : intensities of  $^{16}\text{O}$ -labeled monoisotope peaks in the  $^{16}\text{O}$ -labeled sample (Fig. 1b top)

$I_{a+2}$ : intensities of 2 Da higher peaks in the  $^{16}\text{O}$ -labeled sample (Fig. 1b top)

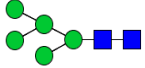

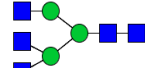
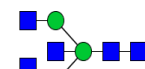


$I_b$ : intensities of  $^{18}\text{O}$ -labeled monoisotope peaks in the  $^{18}\text{O}$ -labeled sample (Fig. 1b middle)

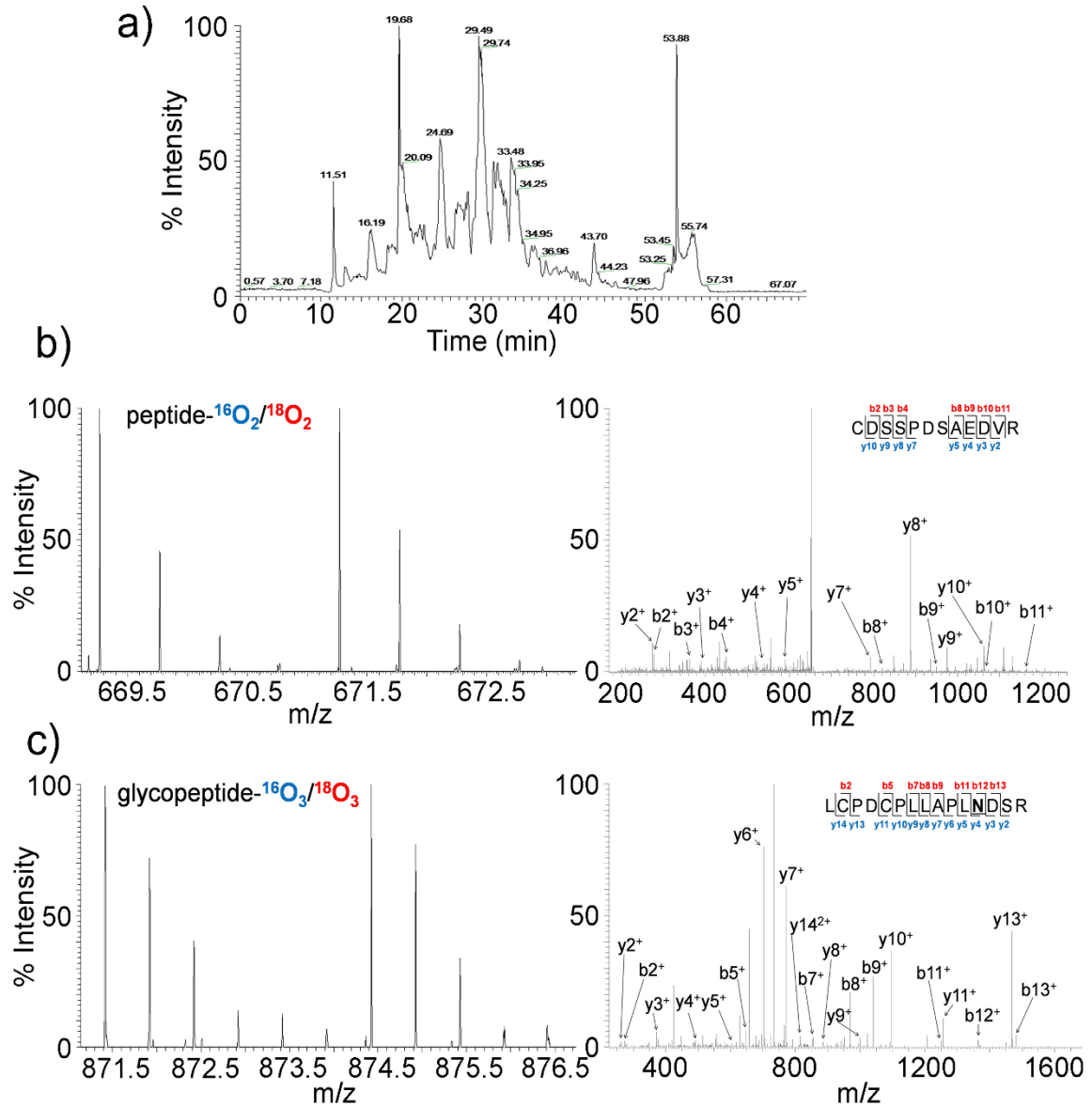
$I_{b+2}$ : intensities of 2 Da higher peaks in the  $^{18}\text{O}$ -labeled sample (Fig. 1b middle)

$I_A$ : intensities of  $^{16}\text{O}$ -labeled monoisotope peaks in mixture of  $^{16}\text{O}$ - and  $^{18}\text{O}$ -labeled samples (Fig. 1b bottom)

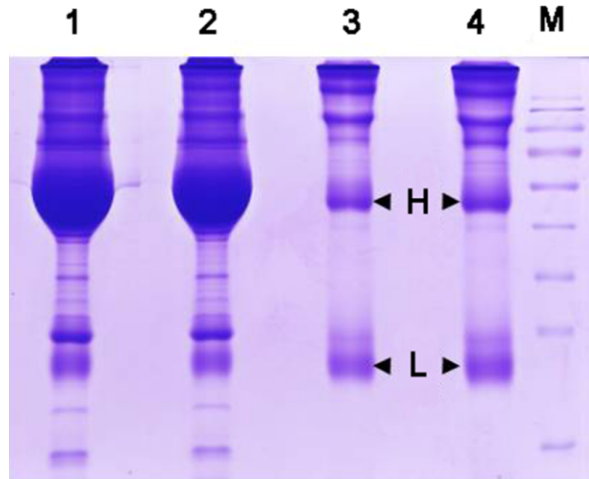
$I_B$ : intensities of 2 Da higher peaks in mixture  $^{16}\text{O}$ - and  $^{18}\text{O}$ -labeled samples (Fig. 1b bottom)

**Table S1.** Representative glycans quantitative results

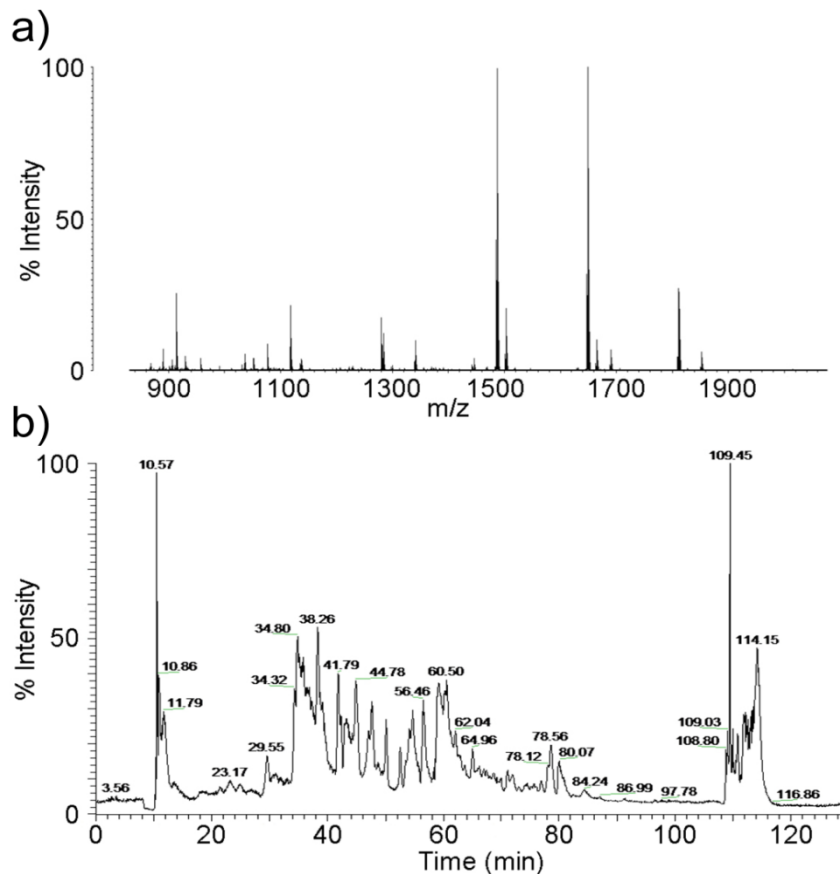
| <i>Glycan composition</i>            | <i>Main structure</i>  | <i>MW(M+Na<sup>+</sup>)</i> | <i>Protein</i> | <i>Minimum CV (n=6)</i> | <i>Max CV (n=6)</i> | <i>Slope</i> | <i>Y intercept (X=0)</i> | <i>X intercept (Y=0)</i> |
|--------------------------------------|--|-----------------------------|----------------|-------------------------|---------------------|--------------|--------------------------|--------------------------|
| HexNAc <sub>2</sub> Hex <sub>5</sub> |   | 1257.42                     | RNB            | 7.47%                   | 18.02%              | 0.97         | -0.014                   | 0.014                    |
| HexNAc <sub>2</sub> Hex <sub>6</sub> |   | 1419.48                     | RNB            | 4.51%                   | 19.50%              | 1.01         | -0.030                   | 0.030                    |
| HexNAc <sub>5</sub> Hex <sub>3</sub> |   | 1542.56                     | OVA            | 3.95%                   | 18.70%              | 0.95         | 0.074                    | -0.078                   |
| HexNAc <sub>6</sub> Hex <sub>3</sub> |   | 1745.63                     | OVA            | 4.34%                   | 14.19%              | 0.97         | 0.034                    | -0.035                   |
| HexNAc <sub>4</sub> Hex <sub>5</sub> |   | 1663.58                     | ASF            | 4.51%                   | 18.16%              | 1.08         | -0.014                   | 0.013                    |
| HexNAc <sub>5</sub> Hex <sub>6</sub> |  | 2028.71                     | ASF            | 4.92%                   | 17.53%              | 1.02         | -0.043                   | 0.042                    |



**Figure S2.** LC-ESI mass spectra of labeled peptides from asialofetuin. (a) Total ion chromatogram (TIC) of LC-ESI mass spectra of peptides. (b) MS and MS<sup>2</sup> of <sup>16</sup>O<sub>2</sub>/<sup>18</sup>O<sub>2</sub>-labeled non-glycopeptide CDSSPDSAEDVR with equal mixture, (c) MS and MS<sup>2</sup> of <sup>16</sup>O<sub>3</sub>/<sup>18</sup>O<sub>3</sub>-labeled glycopeptide LCPDCPLLAPL~~N~~DSR with equal mixture.

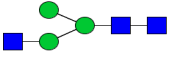
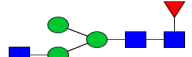
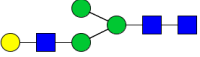
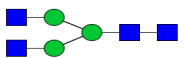
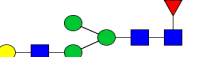
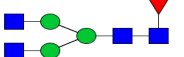
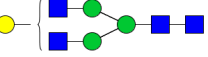

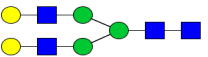
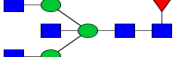
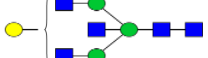
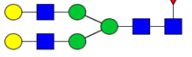
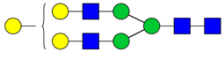
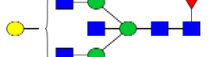
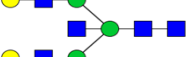



**Figure S3.** SDS-PAGE analysis of IgG from HCC/normal serum (1: normal serum, 2: HCC serum, 3: normal serum after purification, 4: HCC serum after purification ).



**Figure S4.** MS analysis of labeled IgG glycans and peptides associated with HCC based on enzyme-catalyzed  $^{18}\text{O}_4$  labeling strategy. (a) MALDI-TOF mass spectra of N-glycans, (b) Total ion chromatogram (TIC) of LC-ESI mass spectra of peptides.

**Table S2.** N-glycan quantitative results of IgG from HCC/normal serum

| <i>MW(M+Na)<sup>+</sup></i> | <i>Glycan composition</i>  | <i>Main structure</i>  | <i>Ratio(HCC/normal)</i> | <i>CV (n=6)</i> |
|-----------------------------|--|--|--------------------------|-----------------|
| 1136.4                      | GlcNAc <sub>3</sub> Man <sub>3</sub>                                   |     | 0.77                     | 17.80%          |
| 1282.5                      | GlcNAc <sub>3</sub> Man <sub>3</sub> Fuc <sub>1</sub>                  |    | 0.58                     | 6.10%           |
| 1298.4                      | GlcNAc <sub>3</sub> Man <sub>3</sub> Gal <sub>1</sub>                  |    | 0.71                     | 16.10%          |
| 1339.5                      | GlcNAc <sub>4</sub> Man <sub>3</sub>                                   |    | 1.74                     | 19.30%          |
| 1444.5                      | GlcNAc <sub>3</sub> Man <sub>3</sub> Gal <sub>1</sub> Fuc <sub>1</sub> |    | 0.45                     | 19.90%          |
| 1485.5                      | GlcNAc <sub>4</sub> Man <sub>3</sub> Fuc <sub>1</sub>                  |    | 0.81                     | 11.00%          |
| 1501.5                      | GlcNAc <sub>4</sub> Man <sub>3</sub> Gal <sub>1</sub>                  |    | 1.55                     | 13.00%          |
| 1647.6                      | GlcNAc <sub>4</sub> Man <sub>3</sub> Gal <sub>1</sub> Fuc <sub>1</sub> |   | 1.24                     | 13.90%          |
| 1663.6                      | GlcNAc <sub>4</sub> Man <sub>3</sub> Gal <sub>2</sub>                  |  | 2.45                     | 16.30%          |
| 1688.6                      | GlcNAc <sub>5</sub> Man <sub>3</sub> Fuc <sub>1</sub>                  |  | 1.69                     | 15.20%          |
| 1704.6                      | GlcNAc <sub>5</sub> Man <sub>3</sub> Gal <sub>1</sub>                  |  | only in normal           | N/A             |
| 1809.6                      | GlcNAc <sub>4</sub> Man <sub>3</sub> Gal <sub>2</sub> Fuc <sub>1</sub> |  | 2.68                     | 15.70%          |
| 1825.6                      | GlcNAc <sub>4</sub> Man <sub>3</sub> Gal <sub>3</sub>                  |  | only in normal           | N/A             |
| 1850.7                      | GlcNAc <sub>5</sub> Man <sub>3</sub> Gal <sub>1</sub> Fuc <sub>1</sub> |  | 2.71                     | 8.40%           |
| 1866.7                      | GlcNAc <sub>5</sub> Man <sub>3</sub> Gal <sub>2</sub>                  |  | only in normal           | N/A             |
| 2012.7                      | GlcNAc <sub>5</sub> Man <sub>3</sub> Gal <sub>2</sub> Fuc <sub>1</sub> |  | only in HCC              | N/A             |

**Table S3.** N-glycopeptide quantitative results of IgG from HCC/normal serum

| <i>No.</i> | <i>Glycopeptide sequence</i> | <i>IgG</i>       | <i>Ratio (HCC/normal)</i> | <i>CV</i> |
|------------|------------------------------|------------------|---------------------------|-----------|
| G1         | EEQFN <u>N</u> STYR          | IgG <sub>4</sub> | 0.92                      | 7.30%     |
| G2         | EEQFN <u>N</u> STFR          | IgG <sub>2</sub> | 0.53                      | 3.90%     |

**Table S4.** Non-glycopeptide quantitative results of IgG from HCC/normal serum

| <i>No.</i> | <i>Non-glycopeptide sequence</i> | <i>IgG</i>  | <i>Ratio(HCC/normal)</i> | <i>CV</i> |
|------------|----------------------------------|---|--------------------------|-----------|
| N1         | FNWYVDGVEVHNAK                   | IgG <sub>1</sub> , IgG <sub>2</sub> , IgG <sub>3</sub> , IgG <sub>4</sub> | 1.01                     | 2.30%     |
| N2         | TVLHQDWLNGK                      | IgG <sub>1</sub> , IgG <sub>2</sub> , IgG <sub>3</sub> , IgG <sub>4</sub> | 1.93                     | 1.30%     |
| N3         | SNKALPAPIEK                      | IgG <sub>1</sub> , IgG <sub>3</sub>                                       | 0.98                     | 5.10%     |
| N4         | GPSVFPLAPSSK                     | IgG <sub>1</sub>  | 0.97                     | 2.30%     |
| N5         | VVVDVSHEDPEVK                    | IgG <sub>1</sub>  | 0.98                     | 12.30%    |
| N6         | SLSSVVTVPSSSLGTK                 | IgG <sub>4</sub>  | 0.9                      | 2.30%     |