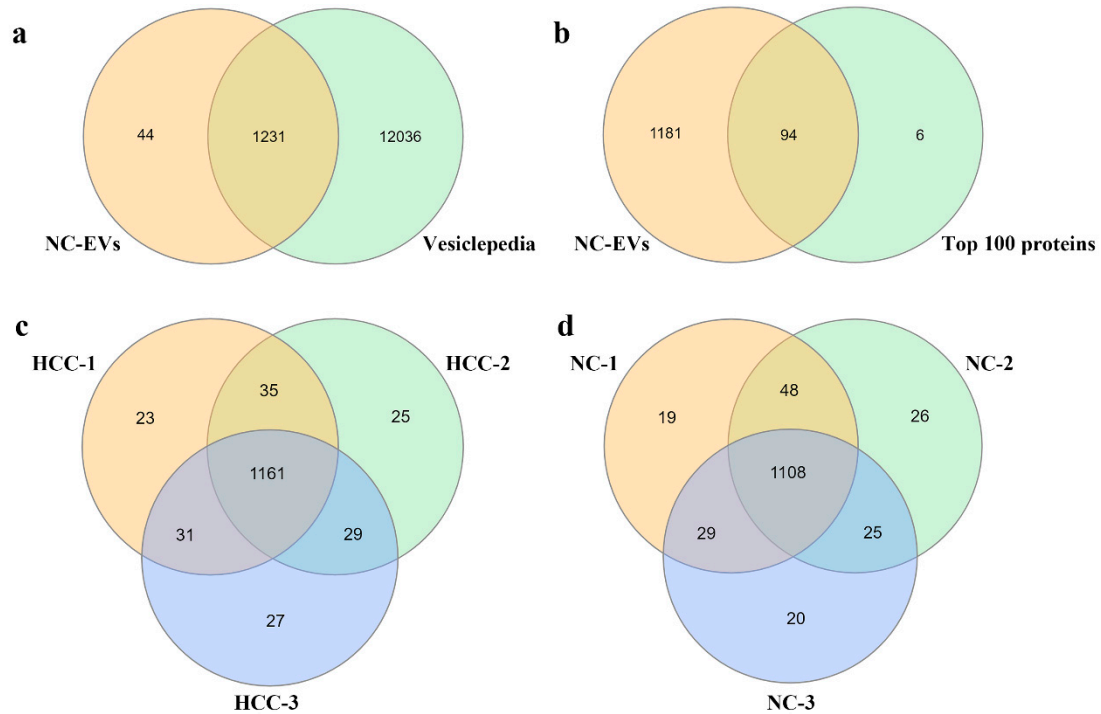
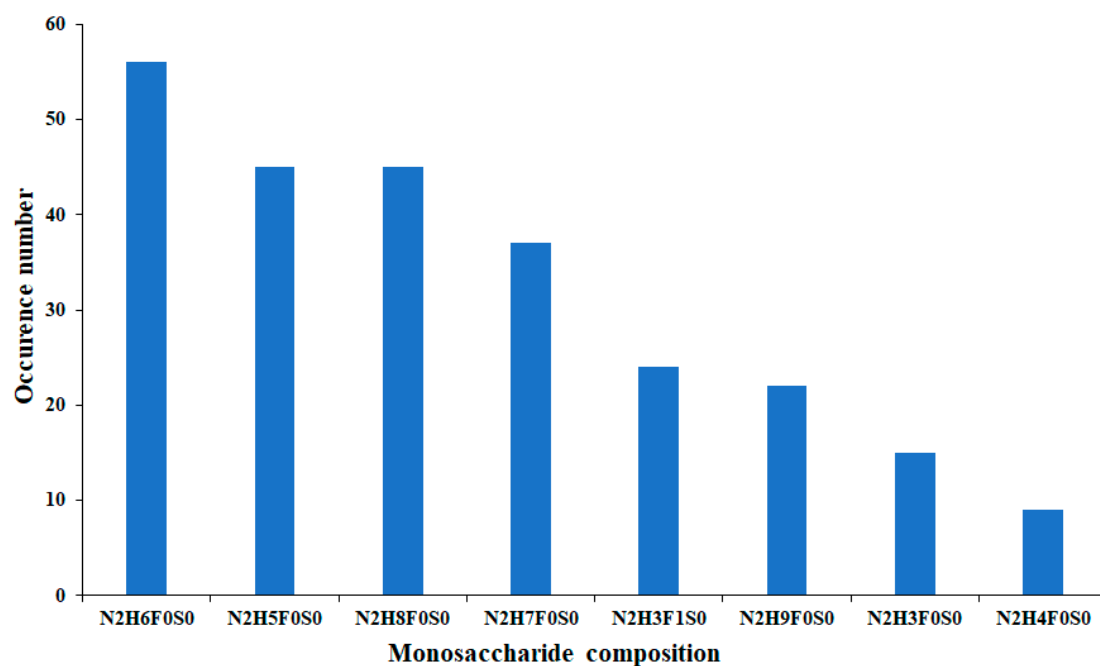


## Supplementary Materials for

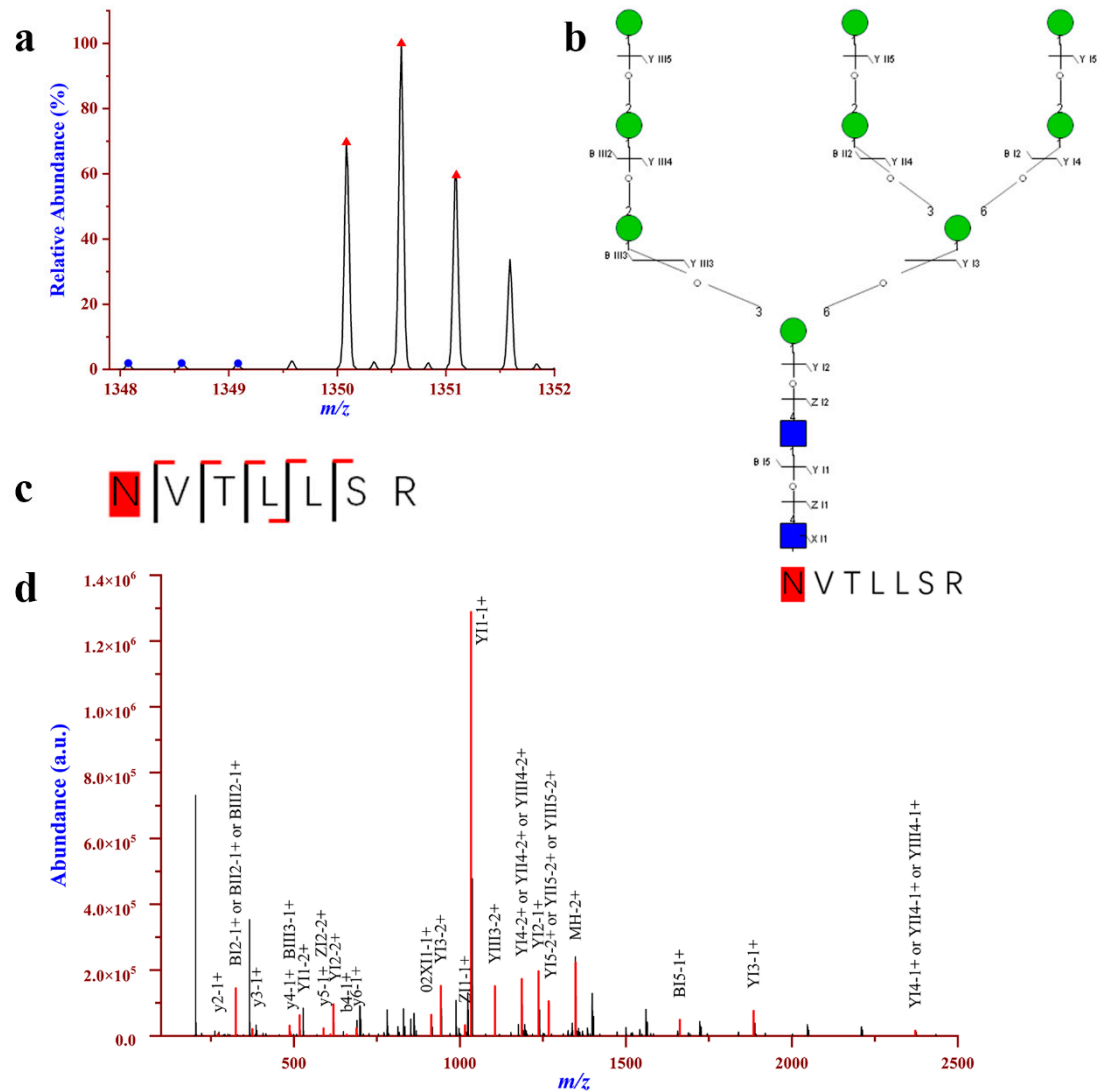
### *Glycoproteomic Analysis of Urinary Extracellular Vesicles for Biomarkers of Hepatocellular Carcinoma*



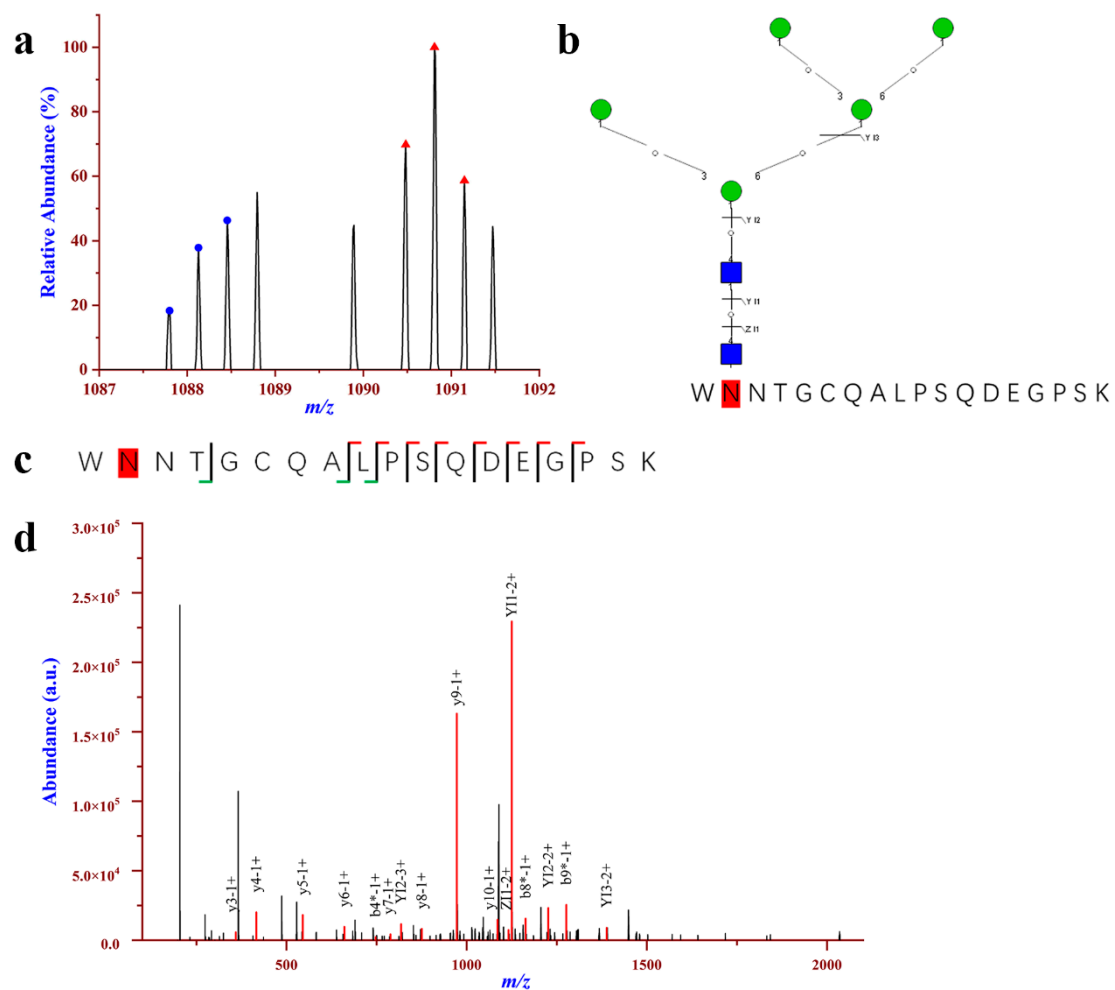
**Figure S1.** Evaluation of the urinary EVs isolated by EVTRAP method derived from normal controls (NC). (a) The Venn diagram depicted the overlap of proteins identified in urinary EVs derived from NC with those published in the Vesiclepedia database; (b) Overlapping with the top 100 proteins in the Vesiclepedia database; (c, d) Overlap with three needles in the proteomic analysis of urinary EVs from HCC and NC, respectively.



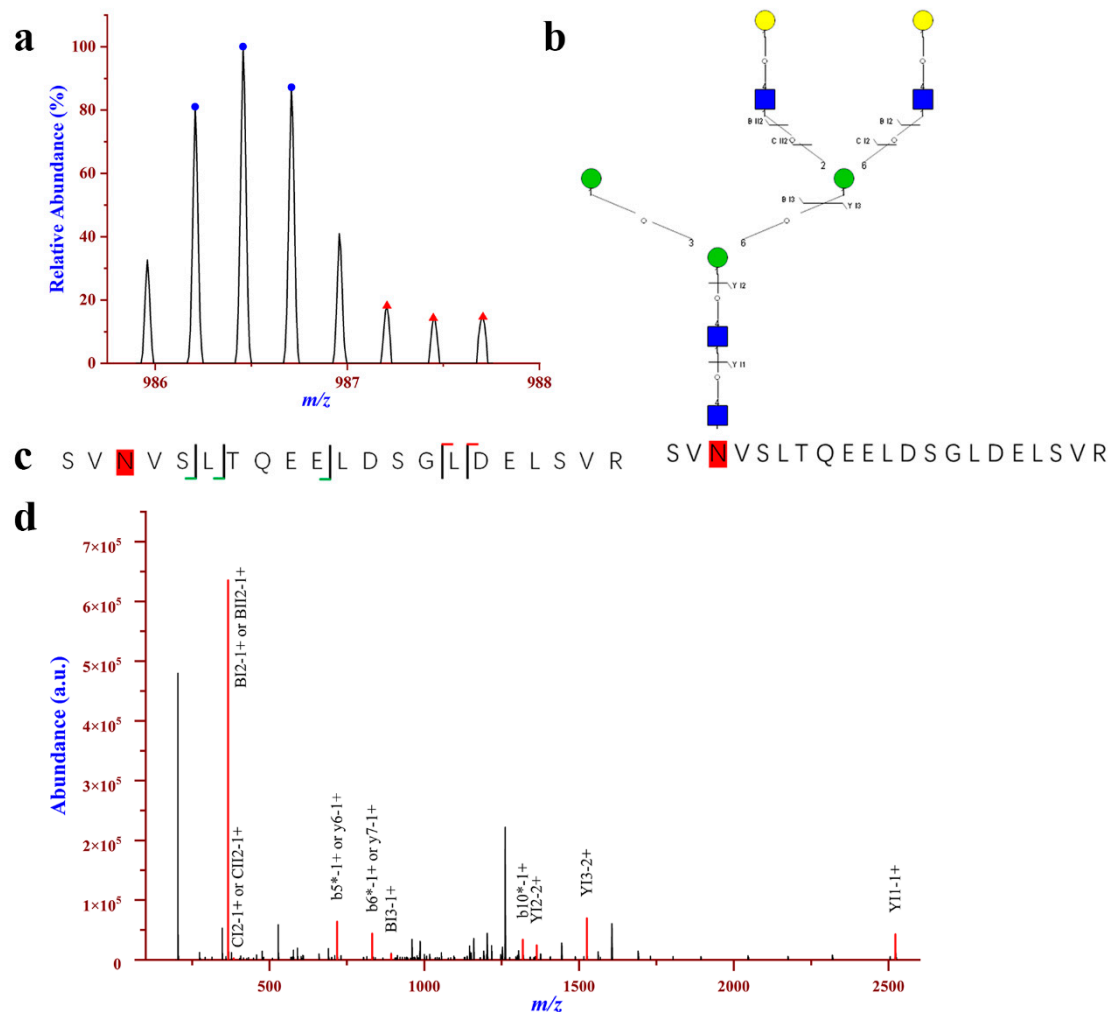
**Figure S2.** The eight most common N-glycan monosaccharide compositions vs the occurrence number of intact N-glycopeptide IDs identified in this study.



**Figure S3.** Upregulation of intact N-glycopeptide NVTLLSR\_N2H9F0S0 in urinary EVs from patients with HCC relative to NC; the N-glycosite is N828 on Glutamyl aminopeptidase (AMPE\_HUMAN, Q07075). (a) paired precursor ions. (b, c) N-glycan and peptide backbone graphical fragmentation maps annotated with the matched fragment ions. (d) The MS/MS spectrum with the matched fragment ions.



**Figure S4.** Upregulation of intact N-glycopeptide WNNTGCQALPSQDEGPSK\_N2H5F0S0 in urinary EVs from patients with HCC relative to NC; the N-glycosite is N499 on Polymeric immunoglobulin receptor (PIGR\_HUMAN, P01833). (a) paired precursor ions. (b, c) N-glycan and peptide backbone graphical fragmentation maps annotated with the matched fragment ions. (d) The MS/MS spectrum with the matched fragment ions.



**Figure S5.** Downregulation of intact N-glycopeptide SVNVSALTQEELDSGLDELSVR\_N4H5F0S0 in urinary EVs from patients with HCC relative to NC; the N-glycosite is N463 on A-kinase anchor protein 2 (AKAP2\_HUMAN, Q9Y2D5). (a) paired precursor ions. (b, c) N-glycan and peptide backbone graphical fragmentation maps annotated with the matched fragment ions. (d) The MS/MS spectrum with the matched fragment ions.

Table S1. Clinical characteristics of the HCC patient enrolled in this study.

| No.  | Gender | Age | Liver cirrhosis <sup>a</sup> | HBsAg (Hepatitis B surface antigen) <sup>b</sup> | HBcAb (Hepatitis B core antibody) <sup>b</sup> | HCV antibody <sup>b</sup> | NAS H <sup>b</sup> | Tumor number | Size of tumor (cm) <sup>c</sup> | Lymphatic metastasis <sup>a</sup> | AFP (ng/L) <sup>c</sup> | PIVK A II (ng/L) <sup>c</sup> | Child-Pugh Score | Differentiated degree <sup>c</sup> |
|------|--------|-----|------------------------------|--|--|---------------------------|--------------------|--------------|---------------------------------|-----------------------------------|-------------------------|-------------------------------|------------------|------------------------------------|
| HCC1 | male   | 64  | 1                            | N  | N  | P                         | 0                  | 3            | 2.1×1.7;<br>2.2×1.8;<br>1.6×1.4 | 1                                 | 804.34                  | 642.56                        | B                | III b/C                            |
| HCC2 | female | 75  | 1                            | N  | N  | N                         | 1                  | > 3          | the largest, 3.7×2.9            | 1                                 | 400.93                  | 3256.75                       | C                | IV/D                               |
| HCC3 | female | 65  | 0                            | P  | P  | N                         | 0                  | 2            | 5.0×4.7;<br>1.9×1.7             | 1                                 | 306.65                  | 87.34                         | A                | III b/C                            |
| HCC4 | male   | 55  | 1                            | N  | P  | N                         | 0                  | 1            | 4.8×6.7                         | 1                                 | 407.07                  | 36.12                         | B                | III b/C                            |
| HCC5 | male   | 65  | 1                            | N  | N  | N                         | 0                  | 2            | 2.9×2.6;<br>3.2×2.7             | 0                                 | 8.23                    | N/A                           | A                | II a/B                             |
| HCC6 | female | 58  | 1                            | N  | N  | P                         | 0                  | 2            | 2.4×1.8;<br>1.9×2.5             | 0                                 | 3.31                    | N/A                           | A                | I b/A                              |
| HCC7 | male   | 62  | 1                            | N  | N  | P                         | 0                  | 1            | 3.9×3.4                         | 1                                 | 2.24                    | 1829.91                       | B                | III b/C                            |
| HCC8 | female | 64  | 1                            | N  | N  | N                         | 0                  | > 3          | the largest, 1.9×1.5            | 1                                 | 468                     | 383.42                        | A                | III b/C                            |

|           |            |    |   |   |   |   |   |     |                            |   |             |            |   |         |
|-----------|------------|----|---|---|---|---|---|-----|----------------------------|---|-------------|------------|---|---------|
| HCC9      | male       | 52 | 0 | P | P | N | 0 | 1   | 5.3×3.4                    | 0 | ><br>3000   | N/A        | B | III a/C |
| HCC1<br>0 | male       | 54 | 1 | P | P | N | 0 | 2   | 4.8×5.6;3.9×2<br>.7        | 1 | 14.81       | 826.3<br>3 | A | III b/C |
| HCC1<br>1 | male       | 54 | 0 | P | P | N | 0 | 1   | 2×2.3                      | 0 | 2.99        | N/A        | A | I a/A   |
| HCC1<br>2 | male       | 53 | 1 | N | P | N | 0 | 2   | 2.6×1.9;<br>3.5×3.8        | 1 | 752         | 743.26     | C | IV/D    |
| HCC1<br>3 | femal<br>e | 70 | 1 | P | P | N | 0 | 1   | 3.2×3.3                    | 0 | 2823.1<br>5 | 66.57      | B | Ia/A    |
| HCC1<br>4 | male       | 53 | 1 | N | P | N | 0 | 1   | 3.5×3.4                    | 1 | 652.06      | 339.42     | B | III b/C |
| HCC1<br>5 | femal<br>e | 38 | 0 | P | P | N | 0 | 1   | 1.8×1.5                    | 0 | 387.42      | 66.88      | A | I a/0   |
| HCC1<br>6 | femal<br>e | 57 | 1 | N | N | P | 0 | 2   | 3.3×3.5;<br>2.2×2.5        | 0 | 10.58       | N/A        | A | II a/B  |
| HCC1<br>7 | male       | 44 | 0 | N | N | P | 0 | > 3 | the<br>largest,5.2×5.<br>5 | 0 | 201.61      | 468.34     | A | II b/B  |
| HCC1<br>8 | femal<br>e | 69 | 1 | N | N | N | 1 | 1   | 11.4×10..8                 | 1 | 1872        | N/A        | C | IV/D    |
| HCC1<br>9 | male       | 55 | 1 | N | N | N | 0 | > 3 | the<br>largest,3.5×2.<br>9 | 0 | 976.42      | 455.21     | A | II b/B  |
| HCC2<br>0 | male       | 34 | 0 | P | P | N | 0 | 1   | 5.5×4.8;                   | 0 | ><br>3000   | 23.56      | A | I b/A   |
| HCC2<br>1 | femal<br>e | 48 | 1 | P | P | N | 0 | 2   | 6.7×5.7;3.3×3<br>.7        | 0 | 2668.<br>45 | 7.67       | B | IIIa/C  |