

# Supplementary Information File

## Mass spectrometry–based analysis of serum *N*-glycan biomarkers for rapid lung cancer detection

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**Supplementary Table 1.** Basic demographic and histopathological characteristics of patients whose blood was collected at the time of diagnosis, prior to undergoing therapy, and serum samples were used for *N*-glycan analysis.

| Patient | Age<br>Gender <sup>1</sup> | S/N <sup>2</sup> | Pathology               | Stage/Grade | Clinical state <sup>3</sup> |
|---------|----------------------------|------------------|-------------------------|-------------|-----------------------------|
| LAC-F1  | 38/F                       | S                | Adenocarcinoma          | pTx/Gx      | Progression                 |
| LAC-F2  | 78/F                       | N                | Adenocarcinoma          | pTx/Gx      | Progression                 |
| LAC-F3  | 62/F                       | S                | Adenocarcinoma          | pTx/Gx      | Rapid progression.          |
| LAC-F4  | 55/F                       | S                | Adenocarcinoma          | pTx/Gx      | Rapid progression.          |
| LAC-F5  | 66/F                       | S                | Adenocarcinoma          | pTx/Gx      | Progression.                |
| LAC-M1  | 79/M                       | ExS              | Adenocarcinoma          | pTx/G2      | Rapid progression           |
| LAC-M2  | 77/M                       | ExS              | Adenocarcinoma          | pTx/G2      | Rapid progression           |
| LAC-M3  | 69/M                       | S                | Adenocarcinoma          | pTx/G3      | Progression                 |
| LAC-M4  | 58/M                       | S                | Adenocarcinoma          | pTx/G3      | Progression                 |
| LAC-M5  | 72/M                       | ExS              | Adenocarcinoma          | pTx/Gx      | Progression                 |
| LAC-M6  | 58/M                       | ExS              | Adenocarcinoma          | pTx/Gx      | Rapid progression           |
| LAC-M7  | 57/M                       | ExS              | Adenocarcinoma          | pTx/Gx      | Progression                 |
| LAC-M8  | 82/M                       | ExS              | Adenocarcinoma          | pTx/Gx      | Rapid progression           |
| LAC-M9  | 66/M                       | S                | Adenocarcinoma          | pTx/Gx      | Rapid Progression           |
| LAC-M10 | 67/M                       | ExS              | Adenocarcinoma          | pTx/Gx      | Regression                  |
| LAC-M11 | 73/M                       | S                | Adenocarcinoma          | pTx/Gx      | Progression                 |
| LAC-M12 | 71/M                       | ExS              | Adenocarcinoma          | pTx/Gx      | Progression                 |
| Sq-F1   | 67/F                       | S                | Squamous cell carcinoma | pTx/G1      | Progression                 |
| Sq-F2   | 73/F                       | ExS              | Squamous cell carcinoma | pTx/G2      | Progression                 |
| Sq-F3   | 65/F                       | S                | Squamous cell carcinoma | pTx/G1      | Rapid progression           |
| Sq-F4   | 75/F                       | ExS              | Squamous cell carcinoma | pTx/G3      | Rapid progression           |
| Sq-F5   | 67/F                       | S                | Squamous cell carcinoma | pTx/Gx      | Progression                 |
| Sq-F6   | 74/F                       | ExS              | Squamous cell carcinoma | pTx/Gx      | Rapid progression           |
| Sq-F7   | 68/F                       | ExS              | Squamous cell carcinoma | pTx/Gx      | Rapid progression           |
| Sq-F8   | 65/F                       | S                | Squamous cell carcinoma | pTx/G3      | Rapid progression           |
| Sq-F9   | 50/F                       | S                | Squamous cell carcinoma | pTx/Gx      | Progression                 |
| Sq-M1   | 63/M                       | S                | Squamous cell carcinoma | pTx/G1      | Rapid progression           |
| Sq-M2   | 55/M                       | S                | Squamous cell carcinoma | pTx/G2      | Rapid progression           |
| Sq-M3   | 50/M                       | S                | Squamous cell carcinoma | pTx/G2      | Rapid progression           |
| Sq-M4   | 68/M                       | ExS              | Squamous cell carcinoma | pTx/G2      | Rapid progression           |
| Sq-M5   | 64/M                       | S                | Squamous cell carcinoma | pTx/G2      | Rapid progression           |
| Sq-M6   | 72/M                       | S                | Squamous cell carcinoma | pTx/G2      | Progression                 |
| Sq-M7   | 72/M                       | ExS              | Squamous cell carcinoma | pTx/G2      | Progression                 |
| Sq-M8   | 59/M                       | S                | Squamous cell carcinoma | pTx/G2      | Progression                 |
| Sq-M9   | 78/M                       | ExS              | Squamous cell carcinoma | pTx/G2      | Progression                 |
| Sq-M10  | 71/M                       | S                | Squamous cell carcinoma | pTx/G2      | Progression                 |

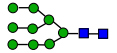
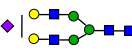
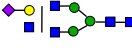
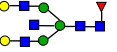
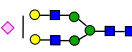
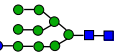
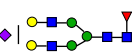
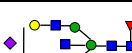
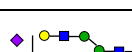
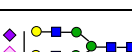
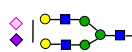
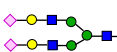
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|---------------|------|-----|-------------------------|-----------|-------------------|
| <b>Sq-M11</b> | 71/M | ExS | Squamous cell carcinoma | pTx/G2    | Progression       |
| <b>Sq-M12</b> | 78/M | ExS | Squamous cell carcinoma | pTx/G2    | Progression       |
| <b>Sq-M13</b> | 71/M | ExS | Squamous cell carcinoma | pTx/G2    | Remission         |
| <b>Sq-M14</b> | 71/M | ExS | Squamous cell carcinoma | pTx/G2    | Remission         |
| <b>Sq-M15</b> | 70/M | ExS | Squamous cell carcinoma | IIIB/G2-3 | Progression       |
| <b>Sq-M16</b> | 61/M | S   | Squamous cell carcinoma | pTx/G3    | Progression       |
| <b>Sq-M17</b> | 80/M | S   | Squamous cell carcinoma | pTx/G3    | Rapid progression |
| <b>Sq-M18</b> | 58/M | S   | Squamous cell carcinoma | pTx/G3    | Stabilized        |
| <b>Sq-M19</b> | 55/M | S   | Squamous cell carcinoma | pTx/G3    | Rapid progression |
| <b>Sq-M20</b> | 55/M | S   | Squamous cell carcinoma | pTx/G3    | Rapid progression |
| <b>Sq-M21</b> | 71/M | S   | Squamous cell carcinoma | pTx/G3    | Progression       |
| <b>Sq-M22</b> | 64/M | S   | Squamous cell carcinoma | pTx/G3    | Rapid progression |
| <b>Sq-M23</b> | 65/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M24</b> | 62/M | S   | Squamous cell carcinoma | pTx/Gx    | Rapid progression |
| <b>Sq-M25</b> | 84/M | S   | Squamous cell carcinoma | pTx/Gx    | Rapid progression |
| <b>Sq-M26</b> | 81/M | ExS | Squamous cell carcinoma | pTx/Gx    | Rapid progression |
| <b>Sq-M27</b> | 74/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M28</b> | 65/M | S   | Squamous cell carcinoma | pTx/Gx    | Advanced          |
| <b>Sq-M29</b> | 74/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M30</b> | 60/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M31</b> | 73/M | ExS | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M32</b> | 80/M | N   | Squamous cell carcinoma | pTx/Gx    | Rapid progression |
| <b>Sq-M33</b> | 76/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M34</b> | 67/M | ExS | Squamous cell carcinoma | pTx/Gx    | Remission         |
| <b>Sq-M35</b> | 60/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M36</b> | 76/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M37</b> | 60/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M38</b> | 64/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M39</b> | 71/M | ExS | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M40</b> | 71/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M41</b> | 69/M | ExS | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M42</b> | 69/M | ExS | Squamous cell carcinoma | pTx/Gx    | Stabilized        |
| <b>Sq-M43</b> | 80/M | ExS | Squamous cell carcinoma | pTx/Gx    | Regression        |
| <b>Sq-M44</b> | 80/M | ExS | Squamous cell carcinoma | pTx/Gx    | Stabilized        |
| <b>Sq-M45</b> | 62/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M46</b> | 73/M | ExS | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M47</b> | 74/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M48</b> | 66/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M49</b> | 69/M | ExS | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M50</b> | 71/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M51</b> | 73/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M52</b> | 56/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M53</b> | 60/M | S   | Squamous cell carcinoma | pTx/Gx    | Stabilized        |
| <b>Sq-M54</b> | 80/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M55</b> | 75/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>Sq-M56</b> | 66/M | S   | Squamous cell carcinoma | pTx/Gx    | Progression       |
| <b>SC-F1</b>  | 61/F | S   | Small cell carcinoma    | pTx/G3    | Progression       |

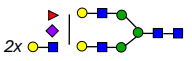
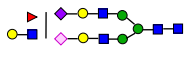
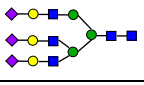
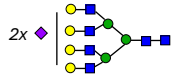
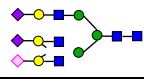
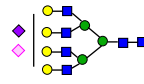
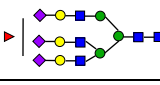
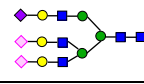
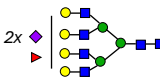
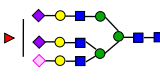
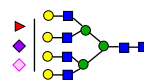
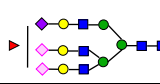
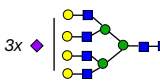
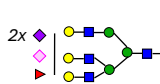
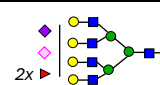
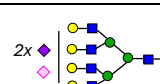
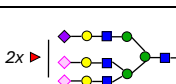
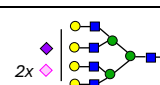
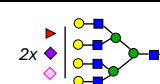
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|----------------|------|-----|----------------------|--------|--------------------|
| <b>SC-F2</b>   | 68/F | ExS | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-F3</b>   | 68/F | S   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-F4</b>   | 55/F | S   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-F5</b>   | 61/F | S   | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M1</b>   | 70/M | S   | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M2</b>   | 71/M | S   | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M3</b>   | 64/M | S   | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M4</b>   | 76/M | S   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-M5</b>   | 54/M | S   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-M6</b>   | 58/M | ExS | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-M7</b>   | 76/M | N   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-M8</b>   | 69/M | S   | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M9</b>   | 73/M | ExS | Small cell carcinoma | pTx/G3 | Stabilized         |
| <b>SC-M10</b>  | 71/M | S   | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M11</b>  | 61/M | S   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-M12</b>  | 75/M | S   | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M13</b>  | 65/M | S   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-M14</b>  | 64/M | S   | Small cell carcinoma | pTx/G3 | Progression        |
| <b>SC-M15</b>  | 74/M | ExS | Small cell carcinoma | pTx/G3 | Rapid progression  |
| <b>SC-M16</b>  | 68/M | ExS | Small cell carcinoma | pTx/G3 | Rapid progression. |
| <b>Inf-F1</b>  | 62/F | S   | Chronic Inflammation | -      | -                  |
| <b>Inf-F2</b>  | 82/F | S   | Chronic Inflammation | -      | -                  |
| <b>Inf-F3*</b> | 67/F | N   | Inflammation         | -      | -                  |
| <b>Inf-F4</b>  | 71/F | N   | Chronic Inflammation | -      | -                  |
| <b>Inf-F5</b>  | 74/F | N   | Chronic Inflammation | -      | -                  |
| <b>Inf-F6</b>  | 71/F | N   | Chronic Inflammation | -      | -                  |
| <b>Inf-M1</b>  | 73/M | N   | Active Inflammation  | -      | -                  |
| <b>Inf-M2</b>  | 74/M | ExS | Active Inflammation  | -      | -                  |

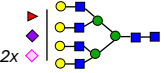
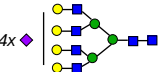
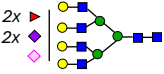
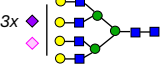
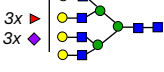
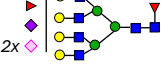
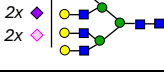
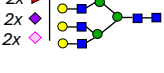
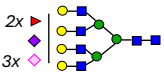
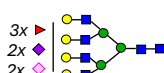
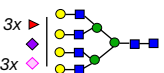
<sup>1</sup>F – female, **M** – male, <sup>2</sup>S - smoker, **N** - non-smoker, **ExS** - exsmoker. <sup>3</sup>Does not relate to TNM classification; refers state after therapy

**Supplementary Table 2.** *N*-glycans detected in serum samples after applying EST-PHN approach. Black arrow represents the significant increase or decrease of glycan in patients' sera; red arrow is assigned to structure not commonly observed in control tissues; dash designate no significant change; ND-not detected. <sup>1</sup>Glycan structures derived from molecular masses, tandem mass experiments; in case of more isomers for each glycan composition, only dominant or general structures are shown. <sup>2</sup>H-Hex, N-HexNAc, F-Fuc, Se - 2,6-linked NeuAc, Sp - 2,3-linked NeuAc. For more details see legend of Fig. 1.

| Glycan Structure <sup>1</sup> | Composition <sup>2</sup> | <i>m/z</i><br>[M+Na] <sup>+</sup> | LAC | SqCC | SCLC | Infl |
|-------------------------------|--------------------------|-----------------------------------|-----|------|------|------|
|                               | H5N2                     | 1347.481                          | ↓   | ↓    | ↓    | ↓    |
|                               | H3N3F1                   | 1372.513                          | ↓   | ↓    | ↓    | ↓    |
|                               | H4N3                     | 1388.508                          | ↓   | ↓    | –    | ↓    |
|                               | H3N4                     | 1429.535                          | –   | –    | –    | –    |
|                               | H6N2                     | 1509.534                          | ↓   | ↓    | ↓    | ↓    |
|                               | H5N3                     | 1550.561                          | –   | ↓    | ↓    | ↓    |
|                               | H3N4F1                   | 1575.593                          | ↑   | ↑    | ↑    | ↑    |
|                               | H4N4                     | 1591.587                          | –   | –    | –    | –    |
|                               | H3N5                     | 1632.614                          | ↑   | ↑    | ↑    | ↑    |
|                               | H7N2                     | 1671.587                          | ↓   | ↓    | ↓    | ↓    |
|                               | Se1H4N3                  | 1693.627                          | –   | –    | –    | –    |
|                               | H6N3                     | 1712.614                          | ↓   | ↓    | ↓    | ↓    |
|                               | H4N4F1                   | 1737.645                          | ↓   | ↓    | ↓    | ↓    |
|                               | H5N4                     | 1753.640                          | ↓   | ↓    | ↓    | ↓    |
|                               | H3N5F1                   | 1778.672                          | ↑   | ↑    | ↑    | –    |
|                               | H4N5                     | 1794.667                          | ↑   | ↑    | ↑    | ↑    |
|                               | H8N2                     | 1833.640                          | ↓   | ↓    | ↓    | ↓    |
|                               | Se1H4N3F1                | 1839.685                          | –   | –    | –    | –    |
|                               | Se1H5N3                  | 1855.680                          | ↑   | –    | –    | –    |
|                               | Se1H4N4                  | 1896.706                          | –   | –    | –    | ↑    |
|                               | H5N4F1                   | 1899.698                          | ↓   | ↓    | ↓    | ↓    |
|                               | H4N5F1                   | 1940.725                          | ↓   | ↓    | ↓    | ↓    |
|                               | H5N5                     | 1956.720                          | ↓   | –    | –    | ↓    |

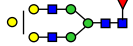
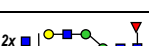
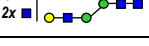
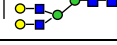
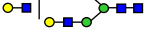
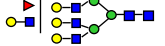
|                                                                                     |                      |          |   |   |   |   |
|-------------------------------------------------------------------------------------|----------------------|----------|---|---|---|---|
|    | <b>H9N2</b>          | 1995.693 | ↓ | ↓ | ↓ | ↓ |
|    | <b>Se1H6N3</b>       | 2017.733 | – | – | – | – |
|    | <b>Se1H4N4F1</b>     | 2042.764 | – | ↓ | ↓ | – |
|    | <b>Se1H5N4</b>       | 2058.759 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se1H4N5</b>       | 2099.785 | ↑ | ↑ | – | ↑ |
|    | <b>H5N5F1</b>        | 2102.777 | ↓ | ↓ | ↓ | ↓ |
|    | <b>Sp1H5N4</b>       | 2134.794 | – | ↓ | – | – |
|    | <b>H10N2</b>         | 2157.745 | ↑ | ↑ | – | ↑ |
|    | <b>Se1H5N4F1</b>     | 2204.817 | ↓ | ↓ | ↓ | ↓ |
|    | <b>Se1H4N5F1</b>     | 2245.844 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se1H5N5</b>       | 2261.838 | – | ↑ | ↑ | ↑ |
|    | <b>Sp1H5N4F1</b>     | 2280.852 | ↓ | ↓ | – | ↓ |
|   | <b>Se2H5N4</b>       | 2363.878 | – | ↑ | ↑ | – |
|  | <b>Se1H5N5F1</b>     | 2407.896 | – | – | – | – |
|  | <b>Se1H6N5</b>       | 2423.891 | – | ↑ | ↑ | – |
|  | <b>Se1Sp1H5N4</b>    | 2439.913 | – | – | – | – |
|  | <b>Sp1H5N5F1 (?)</b> | 2483.931 | ↑ | – | – | – |
|  | <b>Se2H5N4F1</b>     | 2509.936 | – | – | – | ↑ |
|  | <b>Se1Sp1H5N4F1</b>  | 2585.971 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Sp2H5N4F1</b>     | 2662.005 | – | – | ↑ | – |
|  | <b>Se2H5N5F1</b>     | 2713.015 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se2H6N5</b>       | 2729.010 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp1H5N5F1</b>  | 2789.050 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp1H6N5</b>    | 2805.045 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se2H6N5F1</b>     | 2875.068 | ↑ | ↑ | ↑ | ↑ |

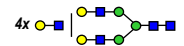
|                                                                                     |                     |          |   |   |   |   |
|-------------------------------------------------------------------------------------|---------------------|----------|---|---|---|---|
|    | <b>Se1H7N6F1</b>    | 2935.081 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se1Sp1H6N5F1</b> | 2951.103 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se3H6N5</b>      | 3034.123 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se2H7N6</b>      | 3094.142 | – | – | – | – |
|    | <b>Se2Sp1H6N5</b>   | 3110.164 | – | – | – | – |
|    | <b>Se1Sp1H7N6</b>   | 3170.177 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se3H6N5F1</b>    | 3180.187 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se1Sp2H6N5</b>   | 3186.198 | ↑ | ↑ | ↑ | – |
|    | <b>Se2H7N6F1</b>    | 3240.200 | ↑ | ↑ | ↑ | ↑ |
|   | <b>Se2Sp1H6N5F1</b> | 3256.222 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp1H7N6F1</b> | 3316.235 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp2H6N5F1</b> | 3332.258 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se3H7N6</b>      | 3399.261 | ↓ | ↓ | ↓ | ↓ |
|  | <b>Se2Sp1H6N5F2</b> | 3402.280 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Sp1Se1H7N6F2</b> | 3462.293 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se2Sp1H7N6</b>   | 3475.296 | ↓ | ↓ | ↓ | ↓ |
|  | <b>Se1Sp2H6N5F2</b> | 3478.314 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se3H7N6F1</b>    | 3545.319 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp2H7N6</b>   | 3551.331 | ↑ | – | – | ↑ |
|  | <b>Se2Sp1H7N6F1</b> | 3621.354 | ↑ | ↑ | ↑ | ↑ |

|                                                                                     |                     |          |   |   |   |   |
|-------------------------------------------------------------------------------------|---------------------|----------|---|---|---|---|
|    | <b>Se1Sp2H7N6F1</b> | 3697.389 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se3H7N6</b>      | 3704.382 | ↓ | ↓ | ↓ | ↓ |
|    | <b>Se2Sp1H7N6F2</b> | 3767.412 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se3Sp1H7N6</b>   | 3780.415 | — | — | — | — |
|    | <b>Se3H7N6F3</b>    | 3837.435 | ↑ | ↑ | ↑ | ↑ |
|    | <b>Se1Sp2H7N6F2</b> | 3843.446 | ↑ | ↑ | ↑ | — |
|    | <b>Se2Sp2H7N6</b>   | 3856.450 | — | — | — | — |
|    | <b>Se3Sp1H7N6F1</b> | 3926.473 | ↑ | ↑ | ↑ | ↑ |
|   | <b>Se1Sp3H7N6</b>   | 3932.484 | ↓ | ↓ | ↓ | ↓ |
|  | <b>Se1Sp2H7N6F3</b> | 3989.504 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se2Sp2H7N6F1</b> | 4002.507 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se3Sp1H7N6F2</b> | 4072.531 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp3H7N6F1</b> | 4078.542 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se2Sp2H7N6F2</b> | 4148.565 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp3H7N6F2</b> | 4224.600 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se2Sp2H7N6F3</b> | 4294.623 | ↑ | ↑ | ↑ | ↑ |
|  | <b>Se1Sp3H7N6F3</b> | 4370.658 | ↑ | ↑ | ↑ | ↑ |

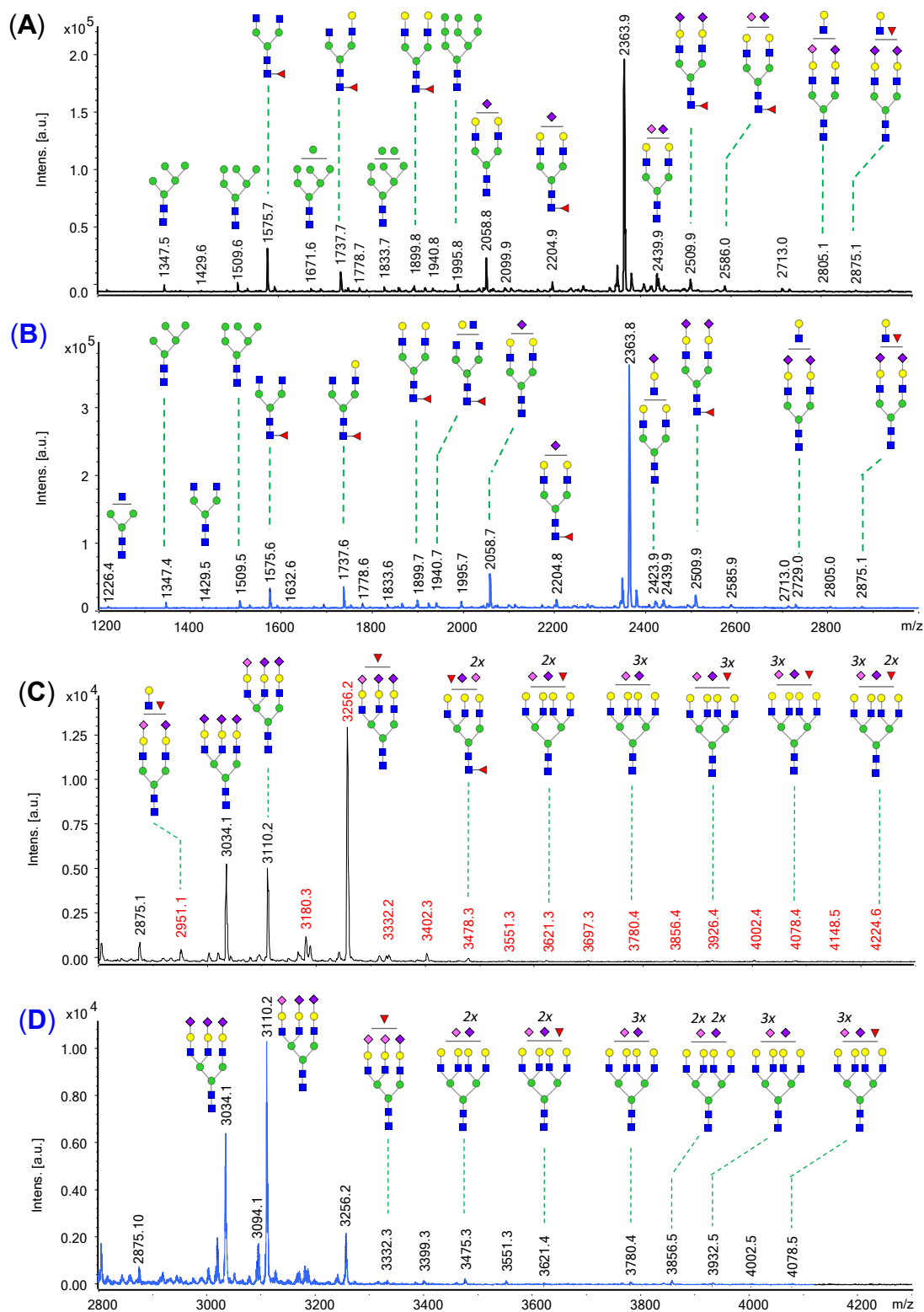
**Supplementary Table 3.** The list of *N*-glycans detected in desialylated serum samples of LC patients and their change when compare with control profiles. Black arrow represents an increase/decrease of glycan in patient sample; red arrow is assigned to structure not detected in control serum; double arrows indicate preferences with LC type; dash designate no significant change; *ND* - not detected.

| Glycan <sup>1</sup>                                                                 | Composition <sup>2</sup> | m/z <sup>3</sup><br>[M+Na] <sup>+</sup> | Glycan change |      |      |      |
|-------------------------------------------------------------------------------------|--------------------------|-----------------------------------------|---------------|------|------|------|
|                                                                                     |                          |                                         | LAC           | SqCC | SCLC | INFL |
|    | H3N3                     | 1226.455                                | -             | -    | ↓    | -    |
|    | H5N2                     | 1347.481                                | ↓             | ↓    | ↓    | -    |
|    | H3N3F1                   | 1372.513                                | ↓             | -    | ↓    | ↓    |
|    | H4N3                     | 1388.508                                | -             | -    | -    | -    |
|    | H3N4                     | 1429.535                                | -             | -    | -    | -    |
|    | H6N2                     | 1509.532                                | ↓             | ↓    | ↓    | ↑    |
|    | H4N3F1                   | 1534.566                                | ↓             | ↓    | ↓    | ↓    |
|    | H5N3                     | 1550.561                                | ↓             | ↓    | ↓    | -    |
|   | H3N4F1                   | 1575.593                                | ↑             | ↑    | ↑    | ↑    |
|  | H4N4                     | 1591.587                                | ↓             | -    | ↓    | -    |
|  | H7N2                     | 1671.587                                | -             | -    | -    | -    |
|  | H5N3F1                   | 1696.619                                | -             | -    | -    | -    |
|  | H6N3                     | 1712.614                                | -             | ↓    | ↓    | -    |
|  | H4N4F1                   | 1737.645                                | ↓             | ↓    | ↓    | ↓    |
|  | H5N4                     | 1753.640                                | -             | -    | -    | ↑    |
|  | H3N5F1                   | 1778.672                                | -             | -    | -    | -    |
|  | H4N5                     | 1794.667                                | ↑             | ↑    | ↑    | ↑    |
|  | H8N2                     | 1833.640                                | ↓             | ↓    | ↓    | -    |
|  | H5N4F1                   | 1899.698                                | ↓             | ↓    | ↓    | ↓    |
|  |                          |                                         | ↑             | ↑    | ↑    | ND   |
|  | H4N5F1                   | 1940.725                                | ↓             | ↓    | ↓    | ↓    |
|  | H5N5                     | 1956.720                                | -             | -    | ↑    | -    |
|  | H9N2                     | 1995.693                                | ↓             | -    | ↑    | ↓    |
|  | H5N4F2                   | 2045.756                                | ↑             | ↑    | ↑    | ↑    |

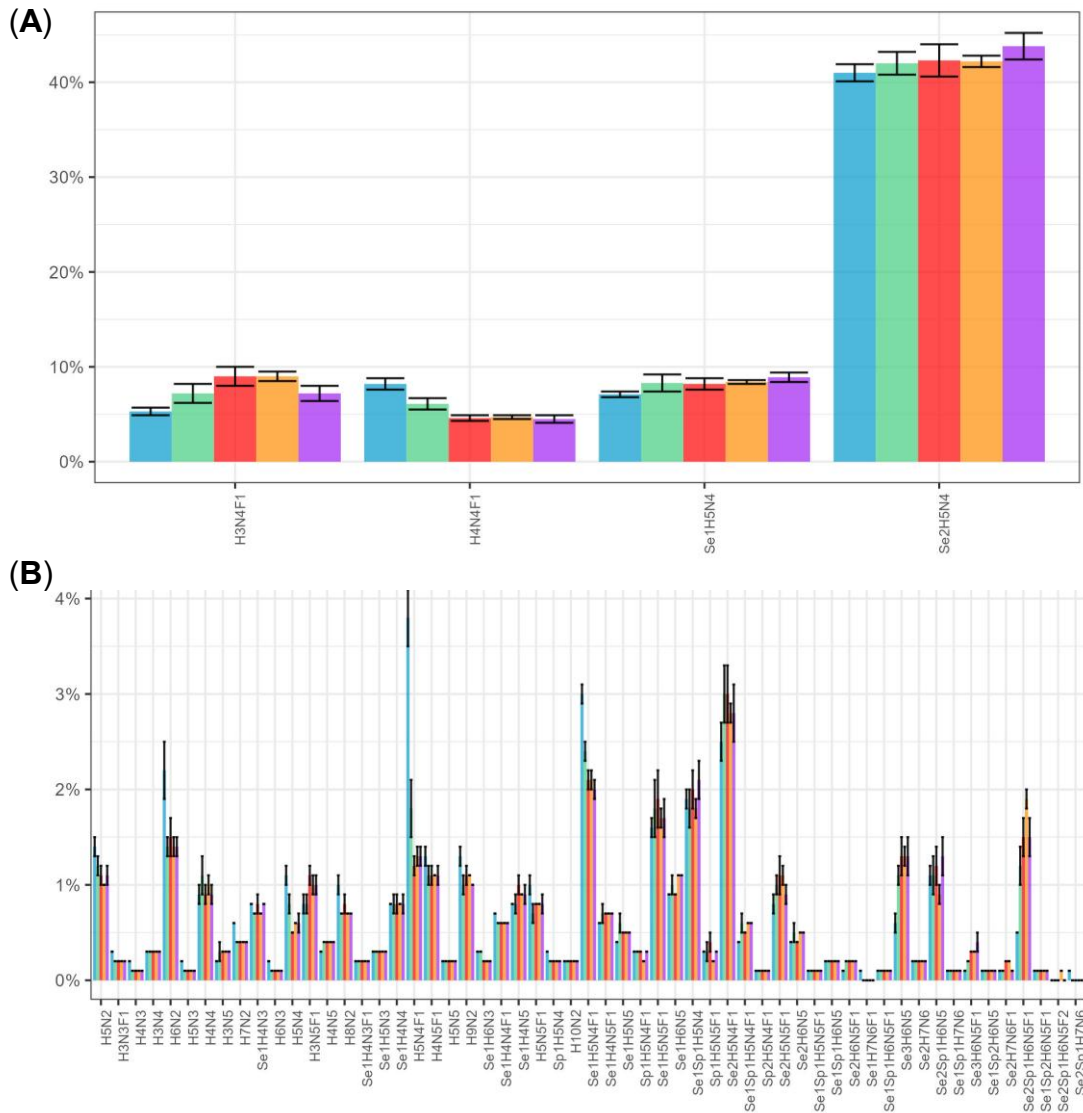
|                                                                                     |               |          |   |   |   |    |
|-------------------------------------------------------------------------------------|---------------|----------|---|---|---|----|
|    | <b>H6N4F1</b> | 2061.751 | ↑ | ↑ | ↑ | -  |
|    | <b>H5N5F1</b> | 2102.778 | - | - | ↑ | ↑  |
|    | <b>H6N5</b>   | 2118.772 | ↓ | ↓ | - | ↓  |
|    | <b>H5N5F2</b> | 2248.835 | - | - | - | -  |
|    | <b>H6N5F1</b> | 2264.830 | ↑ | ↑ | ↑ | ↑  |
|    | <b>H6N5F1</b> | 2264.830 | ↑ | ↑ | ↑ | ↑  |
|    | <b>H5N6F1</b> | 2305.860 | ↑ | ↑ | ↑ | ↑  |
|    | <b>H6N6</b>   | 2321.850 | ↑ | ↑ | ↑ | ↑  |
|    | <b>H6N5F2</b> | 2410.888 | ↑ | ↑ | ↑ | ↑  |
|    | <b>H5N6F2</b> | 2451.915 | ↑ | ↑ | ↑ | ND |
|    | <b>H6N6F1</b> | 2467.910 | ↑ | ↑ | ↑ | -  |
|   | <b>H7N6</b>   | 2483.905 | ↓ | ↓ | - | ↓  |
|  | <b>H6N5F3</b> | 2556.946 | ↑ | ↑ | ↑ | ↑  |
|  | <b>H5N6F3</b> | 2597.973 | ↑ | ↑ | ↑ | ND |
|  | <b>H6N6F2</b> | 2613.968 | ↑ | ↑ | ↑ | ↑  |
|  | <b>H7N6F1</b> | 2629.962 | ↑ | ↑ | ↑ | ↑  |
|  |               |          | ↑ | ↑ | ↑ | ↑  |
|  | <b>H6N7F1</b> | 2670.989 | ↑ | ↑ | ↑ | ND |
|  | <b>H7N6F2</b> | 2776.021 | ↑ | ↑ | ↑ | ↑  |
|  | <b>H7N7F1</b> | 2833.042 | ↑ | ↑ | ↑ | ↑  |
|  | <b>H8N7</b>   | 2849.037 | ↓ | ↓ | - | ↓  |
|  | <b>H6N6F4</b> | 2906.084 | ↑ | ↑ | ↑ | ND |
|  | <b>H7N6F3</b> | 2922.081 | ↑ | ↑ | ↑ | ↑  |
|  | <b>H8N7F1</b> | 2995.095 | - | - | ↑ | -  |
|  | <b>H7N6F4</b> | 3068.136 | ↑ | ↑ | ↑ | -  |
|  | <b>H8N7F2</b> | 3141.155 | ↑ | ↑ | ↑ | ↑  |

|                                                                                   |               |          |   |   |   |    |
|-----------------------------------------------------------------------------------|---------------|----------|---|---|---|----|
|  | <b>H9N8</b>   | 3214.169 | ↓ | ↓ | - | ↓  |
|  | <b>H8N7F3</b> | 3287.211 | ↑ | ↑ | ↑ | ↑  |
|  | <b>H9N8F1</b> | 3360.229 | - | - | ↑ | -  |
|  | <b>H8N7F4</b> | 3433.269 | ↑ | ↑ | ↑ | ND |
|  | <b>H9N8F2</b> | 3506.285 | ↑ | ↑ | ↑ | ↑  |
|  | <b>H10N9</b>  | 3579.301 | ↓ | ↓ | ↓ | ↓  |
|  | <b>H9N8F3</b> | 3652.343 | ↑ | ↑ | ↑ | ↑  |

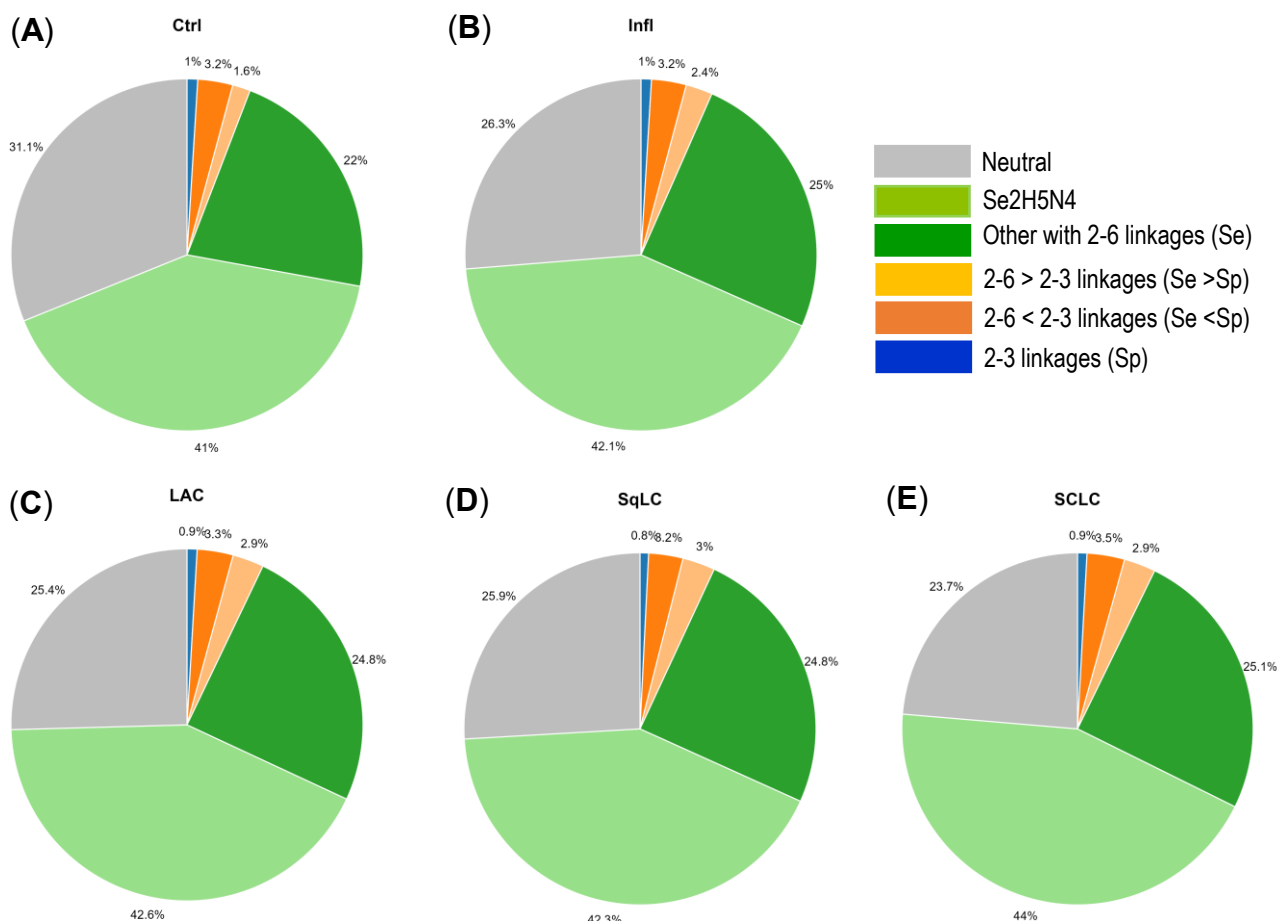
<sup>1</sup>Glycan structures were derived from molecular masses, tandem mass experiments; not all isomers are shown. In case of tri and more antennary glycans analyzed in patients, isomers with fucoses only on the antennae were more prevalent than core fucosylated analogs (see Scheme 1). <sup>2</sup>**H-*Hex***, **N-*HexNAc***, **F-*Fuc***. <sup>3</sup>Glycans are labeled with PHN at the reducing terminus (+90.058).



**Supplementary Figure 1.** MALDI-MS spectra of *N*-glycans obtained from male patient with SCLC (A,C) and control serum (B,D). The  $m/z$  values in red color were decreased or not detected in control serum samples. The 2,6-linked Neu5Ac is detected as methyl ester (violet diamond,  $291.095 + 14.026$  Da) and glycan with 2,3-linked NeuAc has additional PHN residue ( $291.095 + 90.058$  Da, pinky diamond).



**Supplementary Figure 2.** The relative abundances of serum *N*-glycans detected in controls (blue bars) versus patients with inflammation (green), LAC (red), SqLC (orange) and SCLC (purple bars): **(A)** *N*-glycans with relative abundances over 5% and **(B)** *N*-glycans with relative abundances under 5%. *N*-glycan compositions: H – *Hex*; N – *HexNAc*; F – *Fuc*; Se – *2,6-linked NeuAc*; Sp – *2,3-linked NeuAc*.



**Supplementary Figure 3.** Pie charts illustrating the relative abundances of serum *N*-glycans, grouped according to the presence of SA residues and categorized based on prevalence of SA linkage types: **(A)** controls and patients with **(B)** inflammation, **(C)** LAC, **(D)** SqLC and **(E)** SCLC. Glycans without any sialic acid (SA) residues are shown in grey. The most dominant glycan detected in all serum samples, carrying both 2,6-linked SA residues (Se2H5N4), is shown in green. Other sialylated glycans with only 2,6-linked SA are in dark green. Glycans with both 2,3- and 2,6-linked SA residues but a prevalence of 2,6-linkages are shown in orange. Those with equal numbers of both linkage types or with a prevalence of 2,3-linked SA residues are in dark orange. Glycans carrying only 2,3-linked SA residues are in blue.

PCA

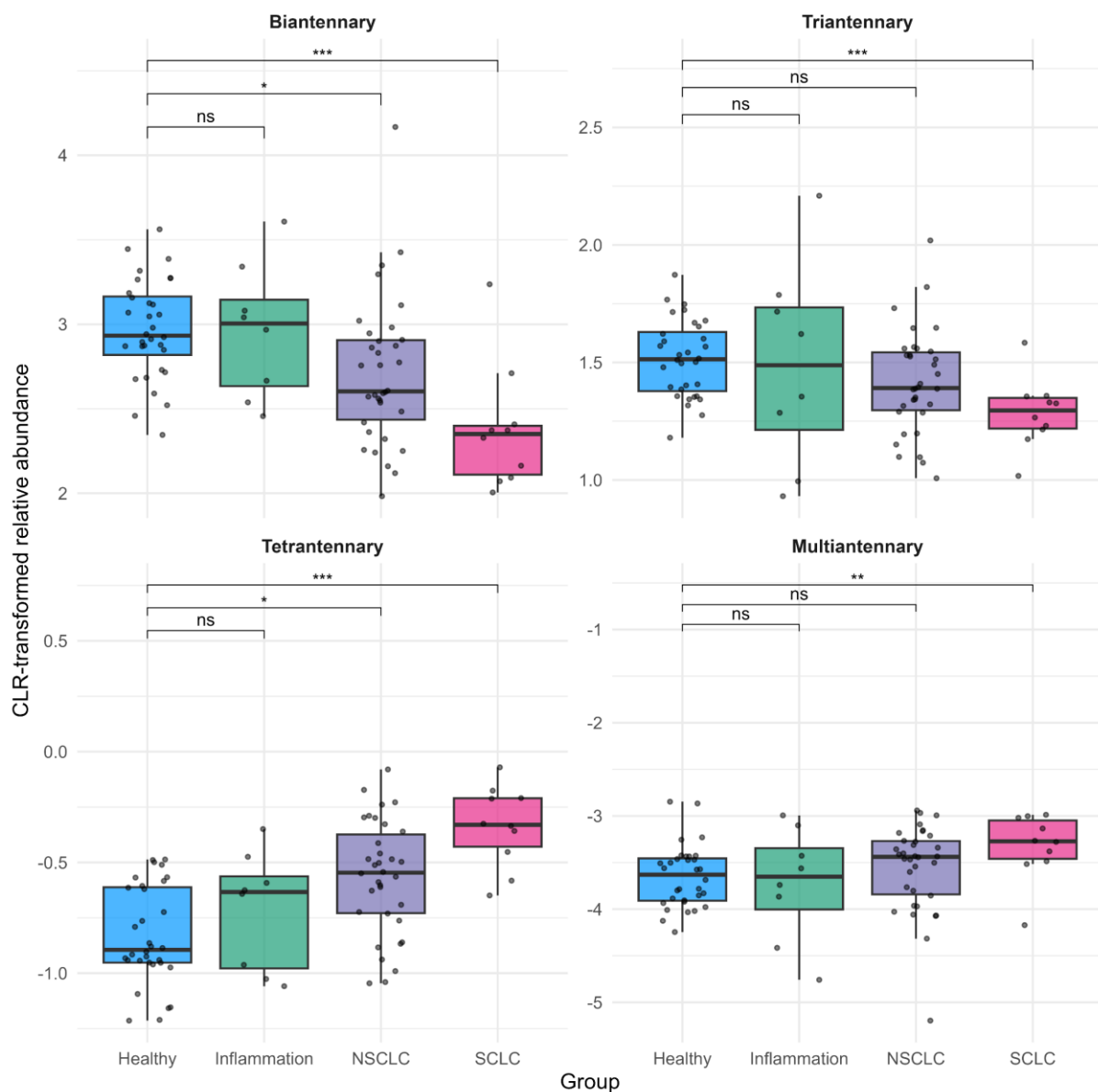
Dim1 (45.1%)

Dim2 (24.2%)

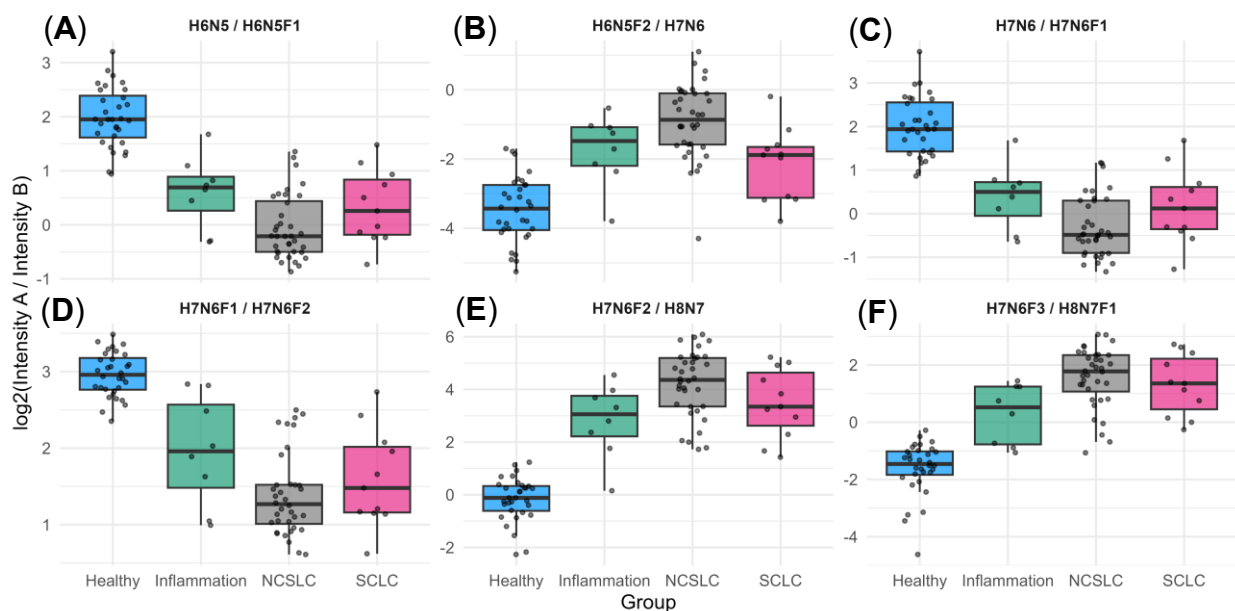
Groups

- Ctrl
- Infl
- LAC
- SqLC
- SCLC

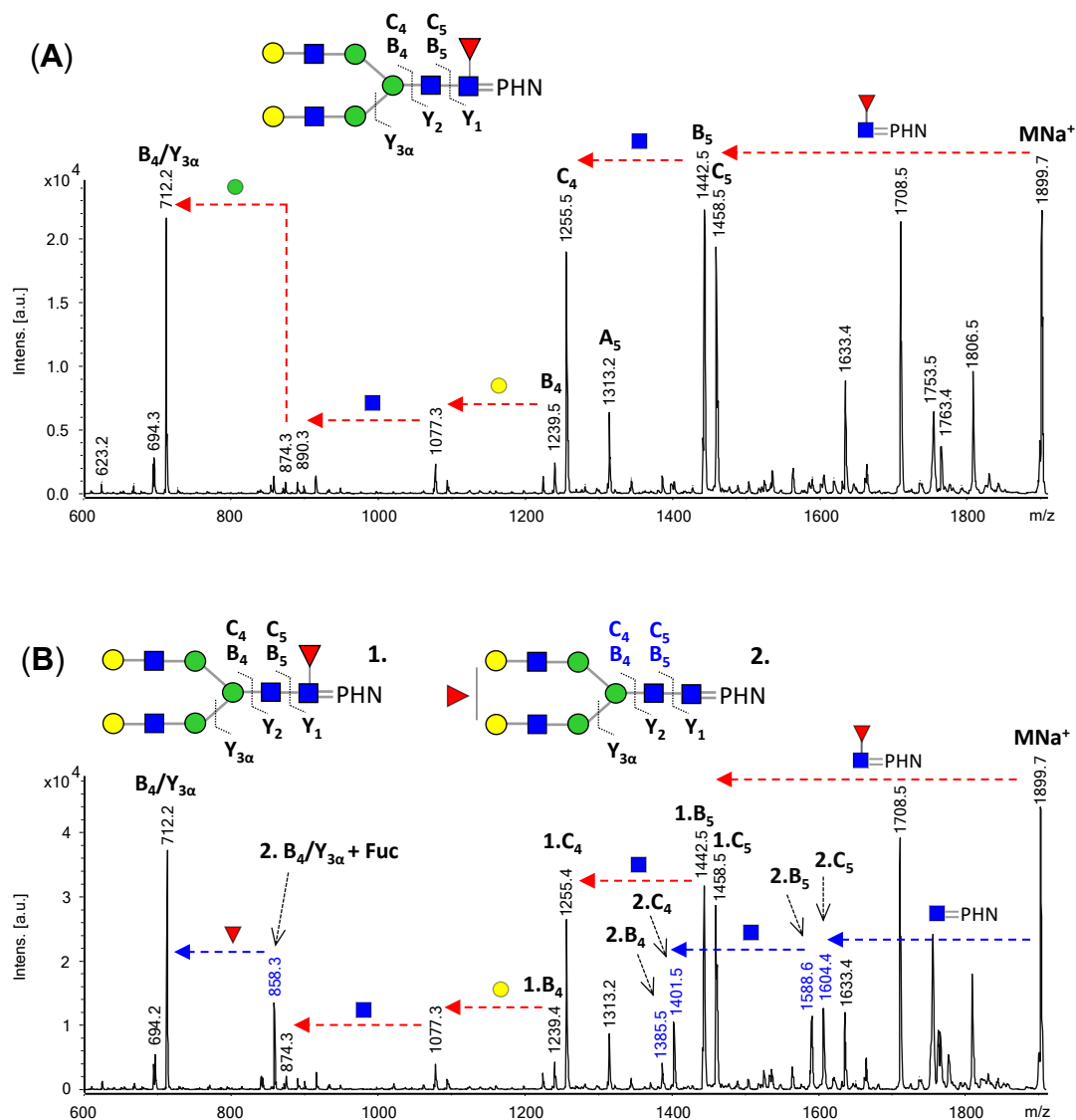
**Supplementary Figure 4.** Graphical illustration of differences between LC patients and controls based on analysis of desialylated serum samples: **(A)** Principal component analysis (PCA) of log<sub>2</sub>-transformed standardized glycan intensities showing separation of patients and controls along (PC1 = 45.1 %, PC2 = 11.8 %). **(B)** PCA biplot illustrating how individual glycan contribute to the overall variance captured by Dim1 and Dim2, with darker green arrows indicating stronger influence



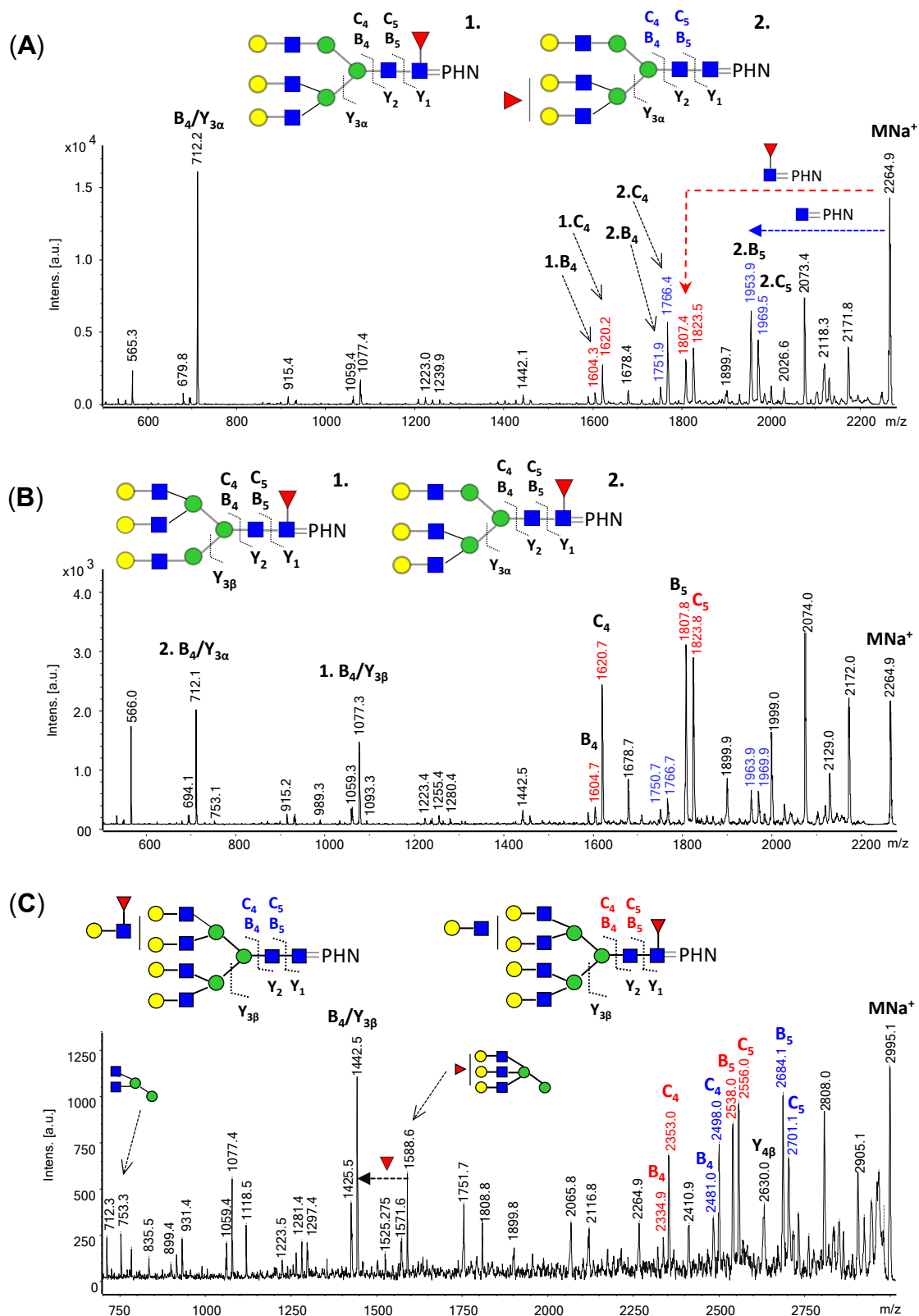
**Supplementary Figure 5.** Graphical illustration of differences between controls and patients when *N*-glycans were summarized according to antennary trait. Results are shown for centered log-ratio transformed relative abundances. Linear regression models were fitted to assess the effect of patient group (inflammation, NSCLC and SCLC) on glycosylation trait and adjusted for age and sex with the healthy group set as the reference. Results are presented as mean  $\pm$  SD. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .



**Supplementary Figure 6.** Graphical illustration of log<sub>2</sub> fold change in intensities for selected glycans detected in serum of healthy donors and patients: (A)  $R_{H6N5/H6N5F1}$  or  $R_{1/2}$ , (B)  $R_{H6N5F2/H7N6}$  or  $R_{3/4}$ , (C)  $R_{H7N6/H7N6F1}$  or  $R_{4/5}$ , (D)  $R_{H7N6F1/H7N6F2}$ , or  $R_{5/6}$ , (E)  $R_{H7N6F2/H8N7}$ , or  $R_{6/7}$ , and (F)  $R_{H7N6F3/H8N7F1}$  or  $R_{8/9}$ . For glycan composition and their numbering see Table 2.



**Supplementary Figure 7.** Tandem mass spectra and fragmentations schemes of glycans recorded for the precursor ions with  $m/z$  1899.7 from desialylated serum of: **(A)** cancer free man and **(B)** male patient with SqCC. Red lines indicate fragment ions associated with isomer having Fuc residue at the chitobiose core. Lines and fragment ions in blue color indicate a presence of second isomer with Fuc linked on one of the antennae (in this case preferably on the 6-arm).



**Supplementary Figure 8.** MALDI tandem mass spectra obtained from desialylated samples for the fucosylated complex *N*-glycans with precursor ions at: (A)  $m/z$  2264.8 from serum of a patient with SqLC (Sq-M16) and (B) lung biopsy of the same patient, and (C)  $m/z$  2995.1 analyzed in control serum from a 30-year-old non-smoking man.