Supplementary Information (SI) for Molecular Omics.
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# Supporting document for

Integrating *N*-glycan and CODEX imaging reveal cell-specific protein glycosylation in healthy human lung

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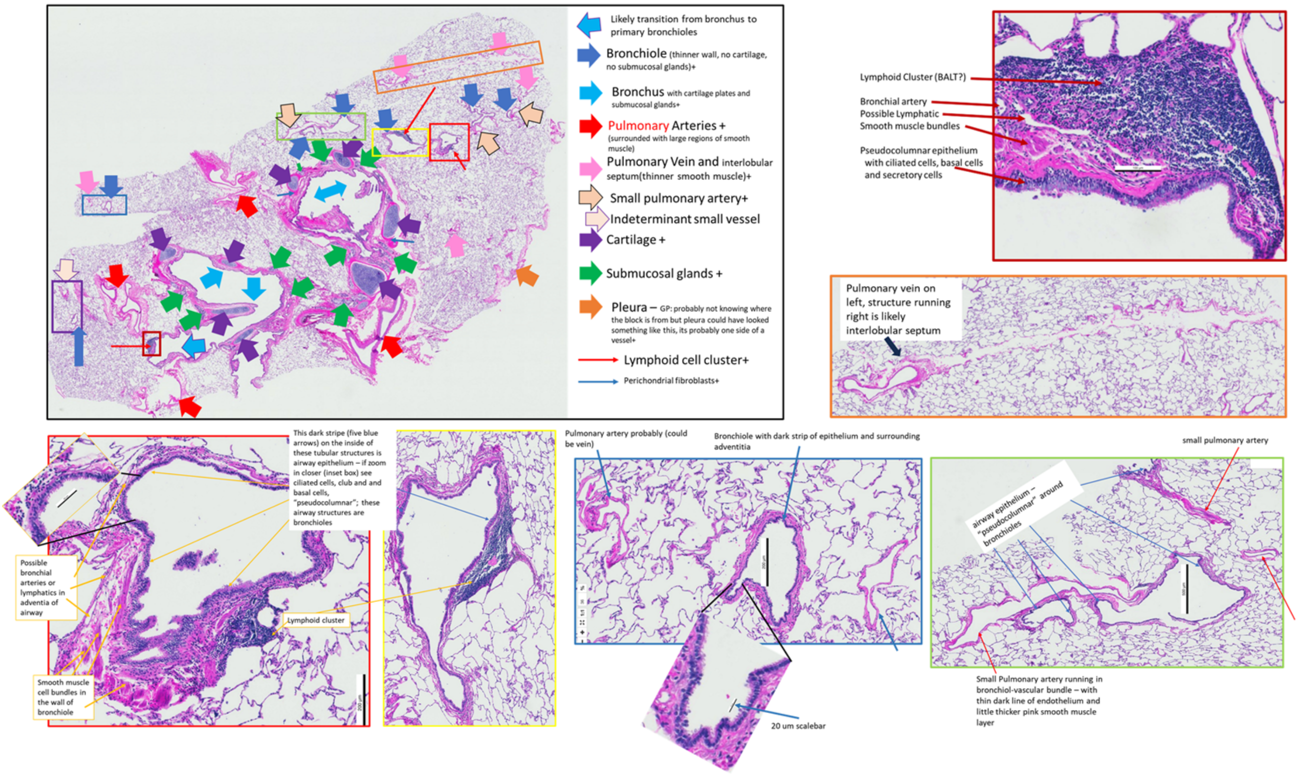
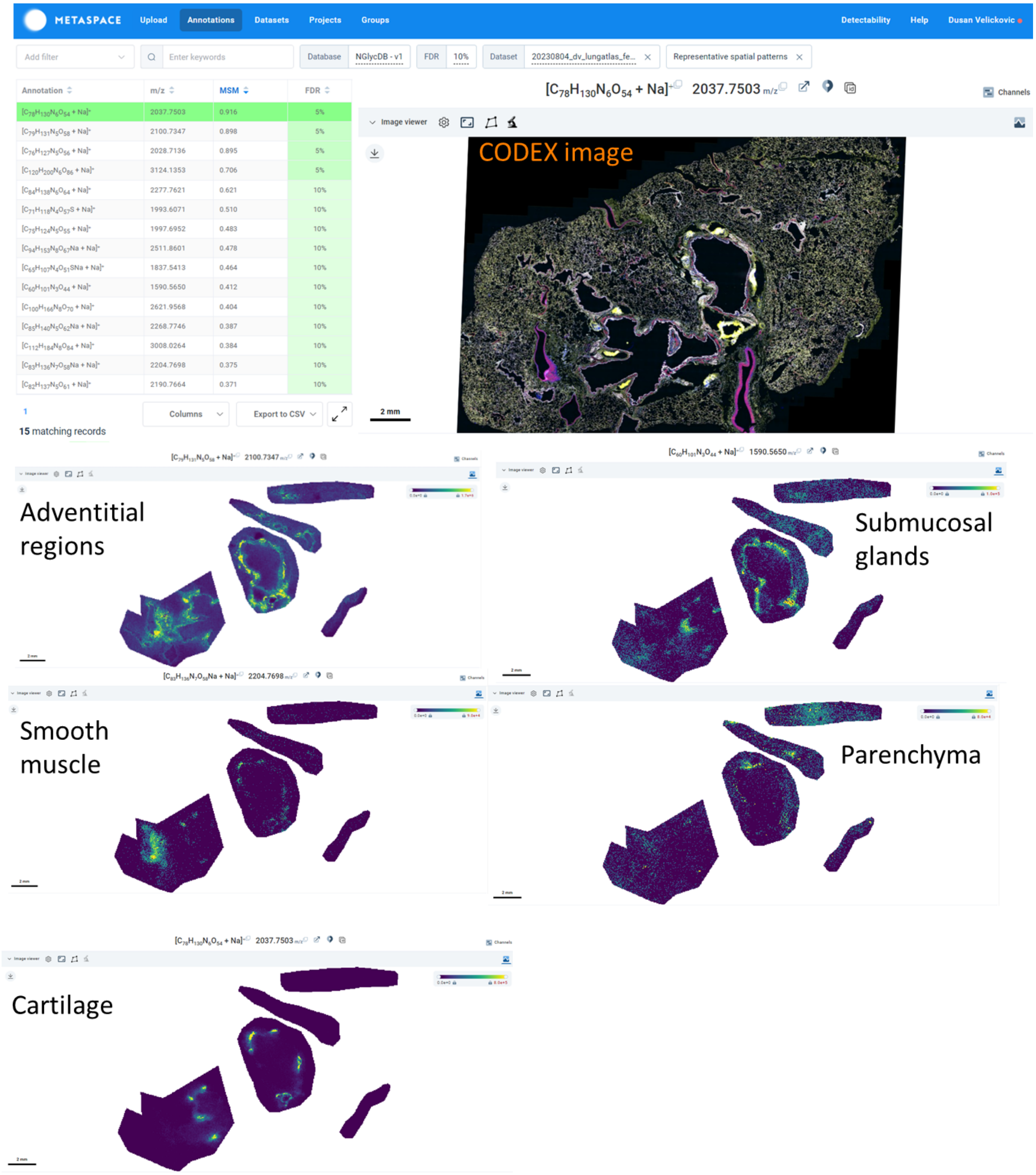


**Figure S1**.Annotated H&E microscopy image of the serial lung tissue section used in the study. Several regions of interest were zoomed in for better visualization.



| Annotation | m/z | MSM | FDR |
|---|---|---|---|
| $[C_{78}H_{130}N_6O_{54} + Na]^+$ | 2037.7503 | 0.916 | 5% |
| $[C_{79}H_{131}N_5O_{58} + Na]^+$ | 2100.7347 | 0.898 | 5% |
| $[C_{76}H_{127}N_5O_{56} + Na]^+$ | 2028.7136 | 0.895 | 5% |
| $[C_{120}H_{200}N_6O_{86} + Na]^+$ | 3124.1353 | 0.706 | 5% |
| $[C_{84}H_{138}N_6O_{64} + Na]^+$ | 2277.7621 | 0.621 | 10% |
| $[C_{71}H_{118}N_4O_{57}S + Na]^+$ | 1993.6071 | 0.510 | 10% |
| $[C_{75}H_{124}N_5O_{55} + Na]^+$ | 1997.6952 | 0.483 | 10% |
| $[C_{94}H_{153}N_8O_{67}Na + Na]^+$ | 2511.8601 | 0.478 | 10% |
| $[C_{65}H_{107}N_4O_{51}SNa + Na]^+$ | 1837.5413 | 0.464 | 10% |
| $[C_{60}H_{101}N_3O_{44} + Na]^+$ | 1590.5650 | 0.412 | 10% |
| $[C_{100}H_{166}N_8O_{70} + Na]^+$ | 2621.9568 | 0.404 | 10% |
| $[C_{85}H_{140}N_5O_{62}Na + Na]^+$ | 2268.7746 | 0.387 | 10% |
| $[C_{112}H_{184}N_8O_{84} + Na]^+$ | 3008.0264 | 0.384 | 10% |
| $[C_{83}H_{136}N_7O_{58}Na + Na]^+$ | 2204.7698 | 0.375 | 10% |
| $[C_{82}H_{137}N_5O_{61} + Na]^+$ | 2190.7664 | 0.371 | 10% |

**Figure S2.** Representative spatial patterns of *N*-glycans from METASPACE. 15 spatial patterns were detected (https://metaspace2020.eu/annotations?db_id=353&ds=2023-08-10_00h34m44s&locs=1), with the five most distinct displayed. A list of glycans that co-localize with those features is presented in **Supporting Table S1**.

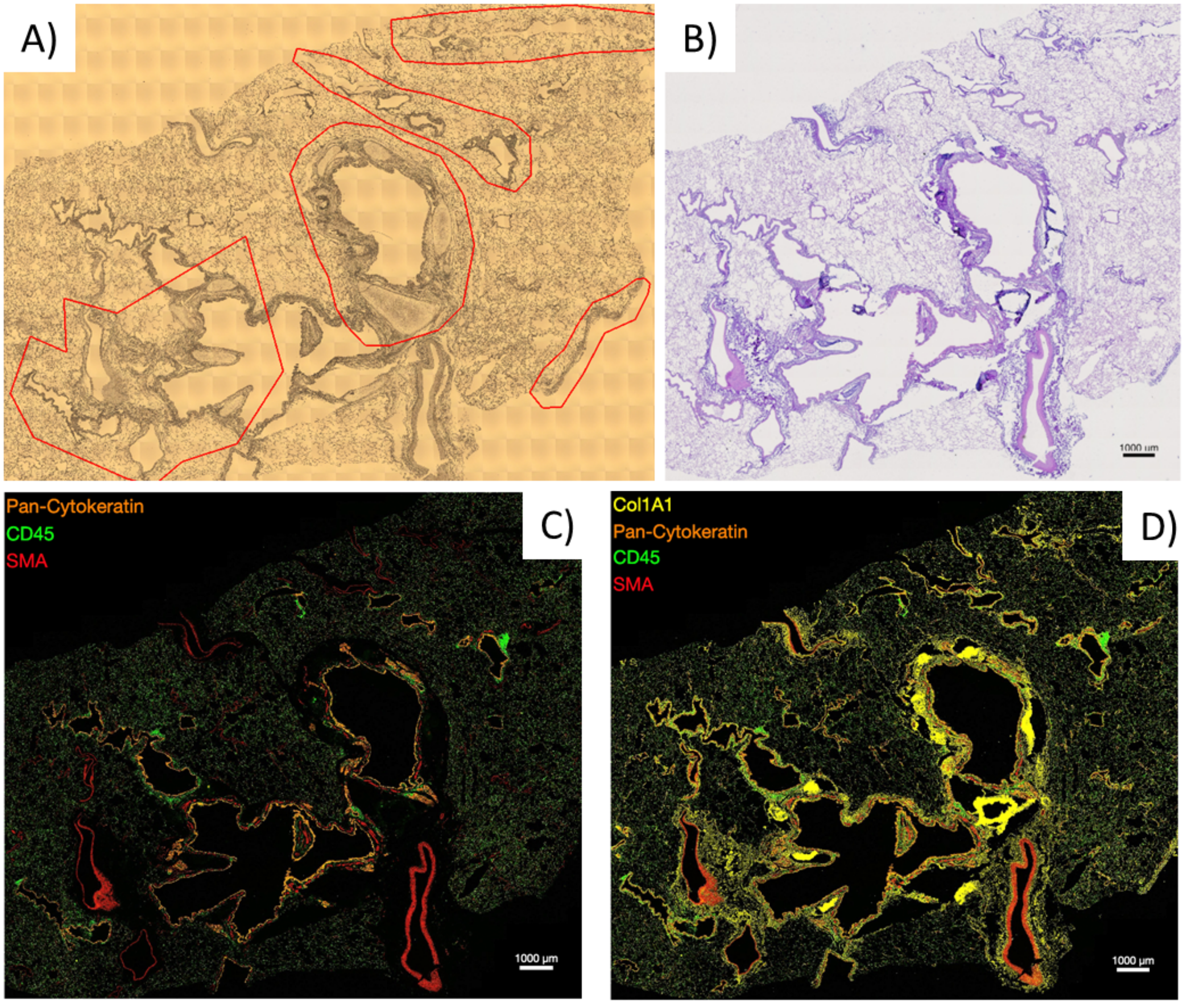


**Figure S3.** MALDI-MSI *N*-glycans protocol followed by CODEX highly multiplexed immunofluorescence. A) Scout image of lung section with regions of interest to be imaged for N-glycans (red objects) and intact cartilaginous plates. B) H&E performed after N-glycan and CODEX assays demonstrate loss and condensation of cartilaginous plates. C) CODEX image of tissue section following *N*-glycans assay showing antibodies for C) pan-cytokeratin, CD45, and SMA and D) with the addition of Col1A1. Collagen is most notable around broncho-vascular structures and particularly dense in folded cartilaginous plates.

**Table S1.** List of N-glycans with different spatial patterns observed in METASPACE. All ions were found as [M+Na] or, in the case of some sialic acid N-glycans, as [M-xH+ (x+1)Na] adducts.

| **Adventitial regions** | | |
|---|---|---|
| **m/z** | **N-glycan composition** | **Adduct** |
| 892.2905 | Hex:4 HexNAc:1 | [M+Na] |
| 917.3221 | Hex:2 HexNAc:2 dHex:1 | [M+Na] |
| 933.317 | Hex:3 HexNAc:2 | [M+Na] |
| 974.3435 | Hex:2 HexNAc:3 | [M+Na] |
| 1079.375 | Hex:3 HexNAC:2 dHex:1 | [M+Na] |
| 1095.37 | Hex:4 HexNAc:2 | [M+Na] |
| 1120.402 | Hex:2 HexNAc:3 dHex:1 | [M+Na] |
| 1136.396 | Hex:3 HexNAc:3 | [M+Na] |
| 1257.423 | Hex:5 HexNAc:2 | [M+Na] |
| 1282.454 | Hex:3 HexNAc:3 dHex:1 | [M+Na] |
| 1298.449 | Hex:4 HexNAc:3 | [M+Na] |
| 1339.476 | Hex:3 HexNAc:4 | [M+Na] |
| 1419.476 | Hex:6 HexNAc:2 | [M+Na] |
| 1444.507 | Hex:4 HexNAc:3 dHex:1 | [M+Na] |
| 1460.502 | Hex:5 HexNAc:3 | [M+Na] |
| 1485.534 | Hex:3 HexNAc:4 dHex:1 | [M+Na] |
| 1501.529 | Hex:4 HexNAc:4 | [M+Na] |
| 1542.555 | Hex:3 HexNAc:5 | [M+Na] |
| 1581.528 | Hex:7 HexNAc:2 | [M+Na] |
| 1605.54 | Hex:4 HexNAc:3 NeuGc:1 | [M+Na] |
| 1606.56 | Hex:5 HexNAc:3 dHex:1 | [M+Na] |
| 1622.555 | Hex:6 HexNAc:3 | [M+Na] |
| 1630.571 | Hex:3 HexNAc:4 NeuAc:1 | [M+Na] |
| 1647.587 | Hex:4 HexNAc:4 dHex:1 | [M+Na] |
| 1663.581 | Hex:5 HexNAc:4 | [M+Na] |
| 1688.613 | Hex:3 HexNAc:5 dHex:1 | [M+Na] |
| 1704.608 | Hex:4 HexNAc:5 | [M+Na] |
| 1743.581 | Hex:8 HexNAc:2 | [M+Na] |
| 1792.624 | Hex:4 HexNAc:4 NeuAc:1 | [M+Na] |
| 1793.644 | Hex:4 HexNAc:4 dHex:2 | [M+Na] |
| 1809.639 | Hex:5 HexNAc:4 dHex:1 | [M+Na] |
| 1850.666 | Hex:4 HexNAc:5 dHex:1 | [M+Na] |
| 1866.661 | Hex:5 HexNAc:5 | [M+Na] |
| 1905.634 | Hex:9 HexNAc:2 | [M+Na] |
| 1938.682 | Hex:4 HexNAc:4 dHex:1 NeuAc:1 | [M+Na] |
| 1954.677 | Hex:5 HexNAc:4 NeuAc:1 | [M+Na] |

|  |  |  |
|---|---|---|
| 1976.6588 |  | [M-H+2Na] |
| **Adventitial regions (cont.)** |  |  |
| **m/z** | **N-glycan composition** | **Adduct** |
| 1955.697 | Hex:5 HexNAc:4 dHex:2 | [M+Na] |
| 1971.692 | Hex:6 HexNAc:4 dHex:1 | [M+Na] |
| 1995.703 | Hex:4 HexNAc:5 NeuAc:1 | [M+Na] |
| 2012.719 | Hex:5 HexNAc:5 dHex:1 | [M+Na] |
| 2028.714 | Hex:6 HexNAc:5 | [M+Na] |
| 2067.687 | Hex:10 HexNAc:2 | [M+Na] |
| 2100.735 | Hex:5 HexNac:4 dHex:1 NeuAc:1 | [M+Na] |
| 2122.7167 |  | [M-H+2Na] |
| 2101.755 | Hex:5 HexNAc:4 dHex:3 | [M+Na] |
| 2157.756 | Hex:5 HexNAc:5 NeuAc:1 | [M+Na] |
| 2158.777 | Hex:5 HexNAc:5 dHex:2 | [M+Na] |
| 2174.772 | Hex:6 HexNAc:5 dHex:1 | [M+Na] |
| 2245.772 | Hex:5 HexNAc:4 NeuAc:2 | [M+Na] |
| 2303.814 | Hex:5 HexNAc:5 dHex:1 NeuAc:1 | [M+Na] |
| 2325.796 |  | [M-H+2Na] |
| 2304.835 | Hex:5 HexNAc:5 dHex:3 | [M+Na] |
| 2320.829 | Hex:6 HexNAc:5 dHex:2 | [M+Na] |
| 2336.824 | Hex:7 HexNAc:5 dHex:1 | [M+Na] |
| 2341.791 | Hex:6 HexNAc:5 NeuAc:1 | [M-H+2Na] |
| 2377.851 | Hex:6 HexNAc:6 dHex:1 | [M+Na] |
| 2391.83 | Hex:5 HexNAc:4 dHex:1 NeuAc:2 | [M+Na] |
| 2413.8121 |  | [M-H+2Na] |
| 2392.851 | Hex:5 HexNAc:4 dHex:3 NeuAc:1 | [M+Na] |
| 2414.8325 |  | [M-H+2Na] |
| 2393.846 | Hex:7 HexNAc:6 | [M+Na] |
| 2466.887 | Hex:6 HexNAc:5 dHex:3 | [M+Na] |
| 2487.849 | Hex:6 HexNAc:5 dHex:1 NeuAc:1 | [M-H+2Na] |
| 2522.888 | Hex:6 HexNAc:6 NeuAc:1 | [M+Na] |
| 2523.909 | Hex:6 HexNAc:6 dHex:2 | [M+Na] |
| 2537.888 | Hex:5 HexNAc:4 dHex:2 NeuAc:2 | [M+Na] |
| 2539.904 | Hex:7 HexNAc:6 dHex:1 | [M+Na] |
| 2668.946 | Hex:6 HexNAc:6 dHex:1 NeuAc:1 | [M+Na] |
| 2690.928 |  | [M-H+2Na] |
| 2669.967 | Hex:6 HexNAc:6 dHex:3 | [M+Na] |
| 2756.962 | Hex:6 HexNAc:5 dHex:1 NeuAc:2 | [M+Na] |
| 2778.9443 |  | [M-H+2Na] |
| 2757.983 | Hex:6 HexNAc:5 dHex:3 NeuAc:1 | [M+Na] |
| 2779.9647 |  | [M-H+2Na] |

| | | |
|---|---|---|
| 2758.978 | Hex:8 HexNAc:7 | [M+Na] |
| 2816.025 | Hex:6 HexNAc:6 dHex:4 | [M+Na] |
| 2830.999 | Hex:7 HexNAc:6 dHex:1 NeuAc:1 | [M+Na] |
| 2852.9811 | | [M-H+2Na] |
| 2832.02 | Hex:7 HexNAc:6 dHex:3 | [M+Na] |
| 2887.025 | Hex:5 HexNAc:5 dHex:3 NeuAc:2 | [M+Na] |
| 2889.041 | Hex:7 HexNAc:7 dHex:2 | [M+Na] |
| 2903.02 | Hex:6 HexNAc:5 dHex:2 NeuAc:2 | [M+Na] |
| 2905.036 | Hex:8 HexNAc:7 dHex:1 | [M+Na] |
| **Adventitial regions (cont.)** | | |
| **m/z** | **N-glycan composition** | **Adduct** |
| 3035.099 | Hex:7 HexNAc:7 dHex:3 | [M+Na] |
| 3050.073 | Hex:8 HexNAc:7 NeuAc:1 | [M+Na] |
| 3122.095 | Hex:7 HexNAc:6 dHex:1 NeuAc:2 | [M+Na] |
| 3254.173 | Hex:8 HexNAc:8 dHex:2 | [M+Na] |
| 3269.173 | Hex:7 HexNAc:6 dHex:4 NeuAc:1 | [M+Na] |
| **Submucosal glands** | | |
| **m/z** | **N-glycan composition** | **Adduct** |
| 1225.433 | Hex:3 HexNAc:2 dHex:2 | [M+Na] |
| 1428.512 | Hex:3 HexNAc:3 dHex:2 | [M+Na] |
| 1589.545 | Hex:4 HexNAc:3 NeuAc:1 | [M+Na] |
| 1590.565 | Hex:4 HexNAc:3 dHex:2 | [M+Na] |
| 1768.613 | Hex:6 HexNAc:3 dHex:1 | [M+Na] |
| 1776.629 | Hex:3 HexNAc:4 dHex:1 NeuAc:1 | [M+Na] |
| 2246.793 | Hex:5 HexNAc:4 dHex:2 NeuAc:1 | [M+Na] |
| 2268.7746 | | [M-H+2Na] |
| 2247.813 | Hex:5 HexNAc:4 dHex:4 | [M+Na] |
| 2612.945 | Hex:6 HexNAc:5 dHex:4 | [M+Na] |
| 2978.077 | Hex:7 HexNAc:6 dHex:4 | [M+Na] |
| **Cartilage** | | |
| **m/z** | **N-glycan composition** | **Adduct** |
| 1323.4808 | Hex:2 HexNAc:4 dHex:1 | [M+Na] |
| 1631.5916 | Hex:3 HexNAc:4 dHex:2 | [M+Na] |
| 1833.6505 | Hex:3 HexNAc:5 NeuAc:1 | [M+Na] |
| 1745.6345 | Hex:3 HexNAc:6 | [M+Na] |
| 1891.6924 | Hex:3 HexNAc:6 dHex:1 | [M+Na] |
| 2037.7503 | Hex:3 HexNAc:6 dHex:2 | [M+Na] |
| 2183.8082 | Hex:3 HexNAc:6 dHex:3 | [M+Na] |
| 2297.8511 | Hex:3 HexNAc:8 dHex:1 | [M+Na] |
| 1996.7238 | Hex:4 HexNAc:5 dHex:2 | [M+Na] |
| 2053.7452 | Hex:4 HexNAc:6 dHex:1 | [M+Na] |

| | | |
|---|---|---|
| 2110.7667 | Hex:4 HexNAc:7 | [M+Na] |
| 2256.8246 | Hex:4 HexNAc:7 dHex:1 | [M+Na] |
| 2069.7401 | Hex:5 HexNAc:6 | [M+Na] |
| 2215.798 | Hex:5 HexNAc:6 dHex:1 | [M+Na] |
| 2361.856 | Hex:5 HexNAc:6 dHex:2 | [M+Na] |
| 2621.9568 | Hex:5 HexNAc:8 dHex:1 | [M+Na] |
| 2580.9302 | Hex:6 HexNAc:7 dHex:1 | [M+Na] |
| 3311.1946 | Hex:8 HexNAc:9 dHex:1 | [M+Na] |

| **Smooth muscle** | | |
|---|---|---|
| **m/z** | **N-glycan composition** | **Adduct** |
| 1938.6819 | Hex:4 HexNAc:4 dHex:1 NeuAc:1 | [M+Na] |
| 1960.6638 | | [M-H+2Na] |
| 2141.7613 | Hex:4 HexNAc:5 dHex:1 NeuAc:1 | [M+Na] |
| 2163.7432 | | [M-H+2Na] |
| 2204.7698 | Hex:3 HexNAc:6 dHex:1 NeuAc:1 | [M-H+2Na] |
| 3124.1102 | Hex:9 HexNAc:8 | [M+Na] |
| 3196.1313 | Hex:8 HexNAc:7 dHex:1 NeuAc:1 | [M+Na] |
| 3218.1132 | | [M-H+2Na] |
| 3270.1681 | Hex:9 HexNAc:8 dHex:1 | [M+Na] |
| 3583.2454 | Hex:9 HexNAc:8 dHex:1 NeuAc:1 | [M-H+2Na] |
| 3635.3003 | Hex:10 HexNAc:9 dHex:1 | [M+Na] |
| **Parenchyma** | | |
| **m/z** | **N-glycan composition** | |
| 1825.6342 | Hex:6 HexNAc:4 | [M+Na] |
| 1987.687 | Hex:7 HexNAc:4 | [M+Na] |
| 2157.7562 | Hex:5 HexNAc:5 NeuAc:1 | [M+Na] |
| 2179.7381 | | [M-H+2Na] |
| 2231.793 | Hex:6 HexNAc:6 | [M+Na] |
| 2596.9252 | Hex:7 HexNAc:7 | [M+Na] |
| 2685.9616 | Hex:7 HexNAc:6 dHex:2 | [M+Na] |
| 2742.9831 | Hex:7 HexNAc:7 dHex:1 | [M+Na] |
| 3051.0938 | Hex:8 HexNAc:7 dHex:2 | [M+Na] |
| 3108.1153 | Hex:8 HexNAc:8 dHex:1 | [M+Na] |