

Supplement for

Site-specific N-glycan analysis of antibody-binding Fc γ receptors from primary human monocytes

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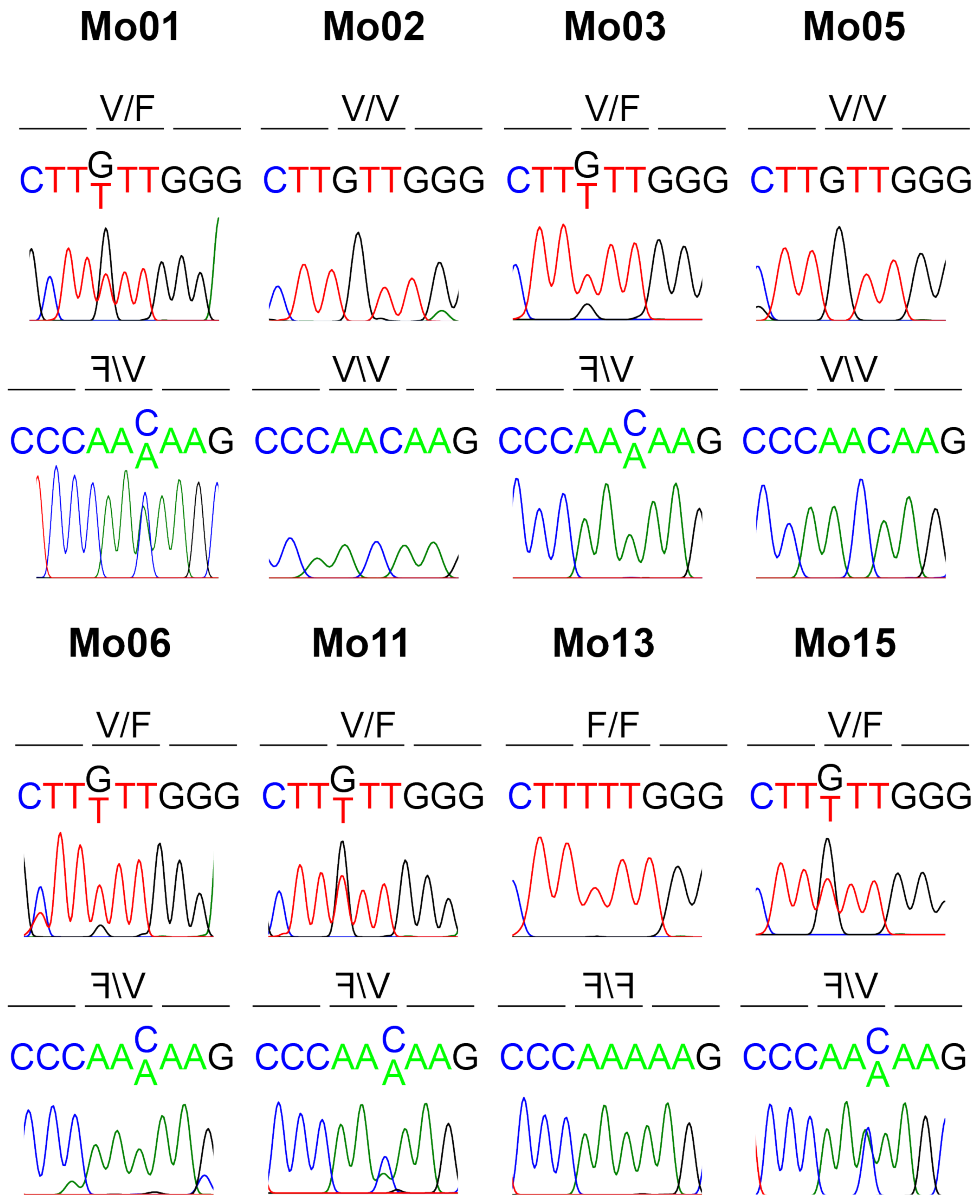


Figure S1. Donor genotypes for V/F158 on CD16a. The forward (top row) and reverse (bottom row) CD16a cDNA sequencing results. Donor genotype is labeled at the top of each chromatogram.

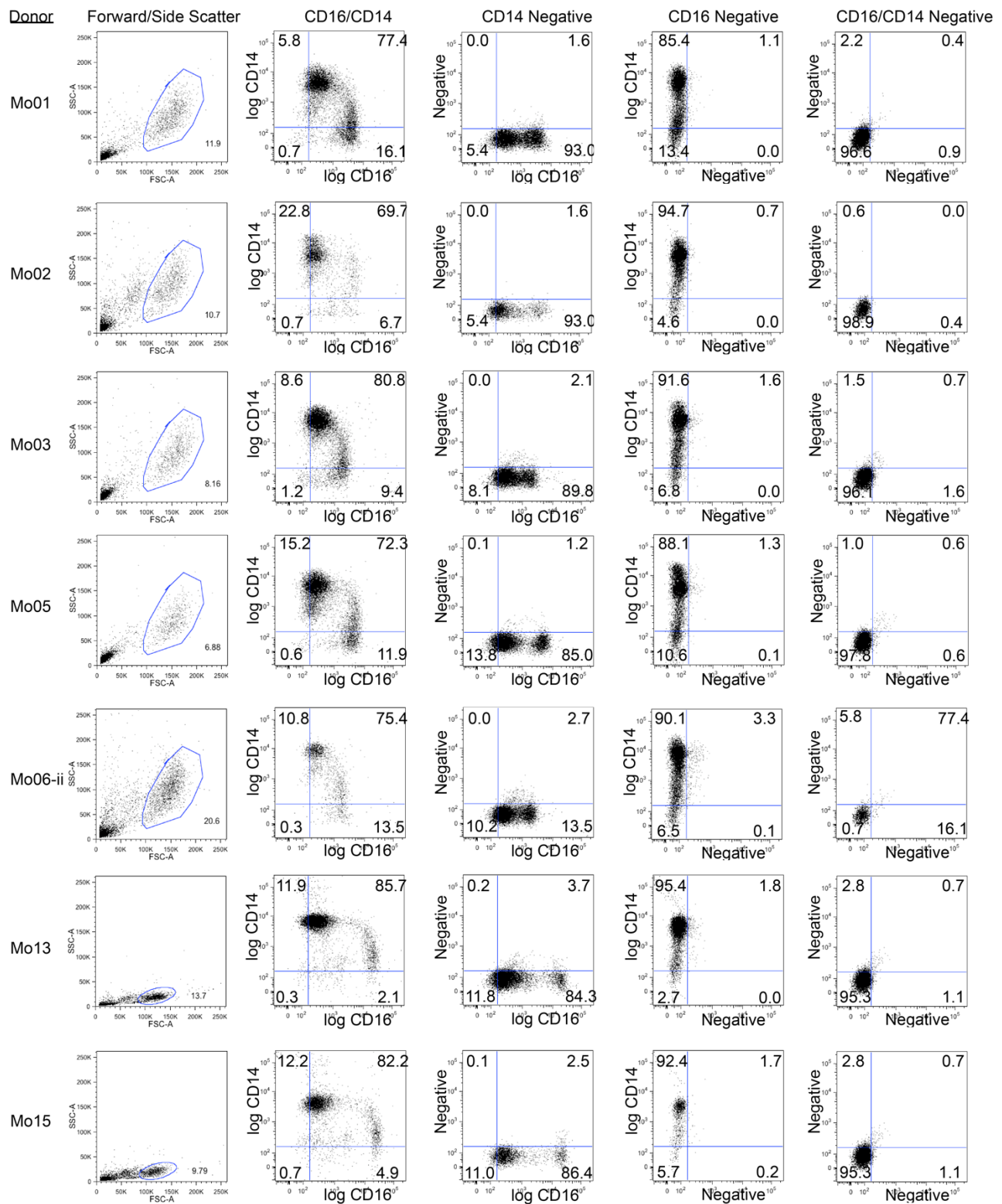


Figure S2. Flow Cytometry of CD16 and CD14 expression on monocytes. Rows represent different experiments from an individual donor labeled on the left side as “Mo##.” Experiments in the left column are side/forward scatter for each donor with gating of the monocyte population in blue. The next four columns are plots for CD16 and CD14 surface expression based on APC or PE fluorescence intensity with CD16 on the x-axis and CD14 on the y-axis with negative controls where indicated. Gated populations are displayed next to the gates for forward/side scatter or in the corresponding quadrants for CD16 and CD14 staining.

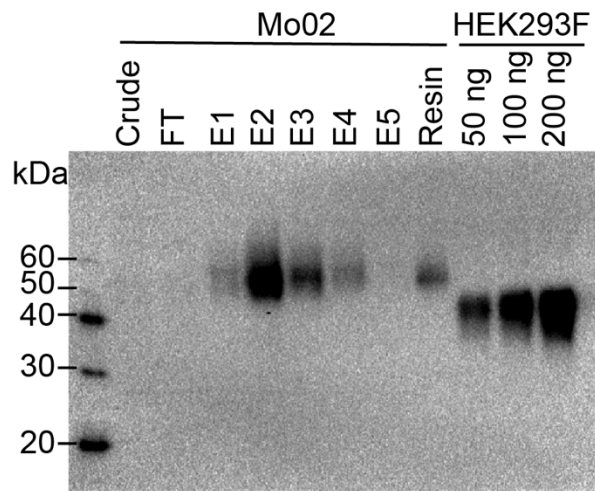


Figure S3. Immunoprecipitation of CD16a from monocytes. CD16a recovery was determined by comparing to recombinant CD16a standards expressed from HEK293F cells. Typical yields of CD16a from a single immunoprecipitation ranged between 100 - 1000 ng. Individual dataset estimates were: MoA = ~ 1 μ g, MoB = ~ 1 μ g, Mo2 = ~ 500 ng, Mo11 = 400 ng, Mo13 = 200 ng and Mo15 = 100 ng.

RT: 2.76 - 66.24

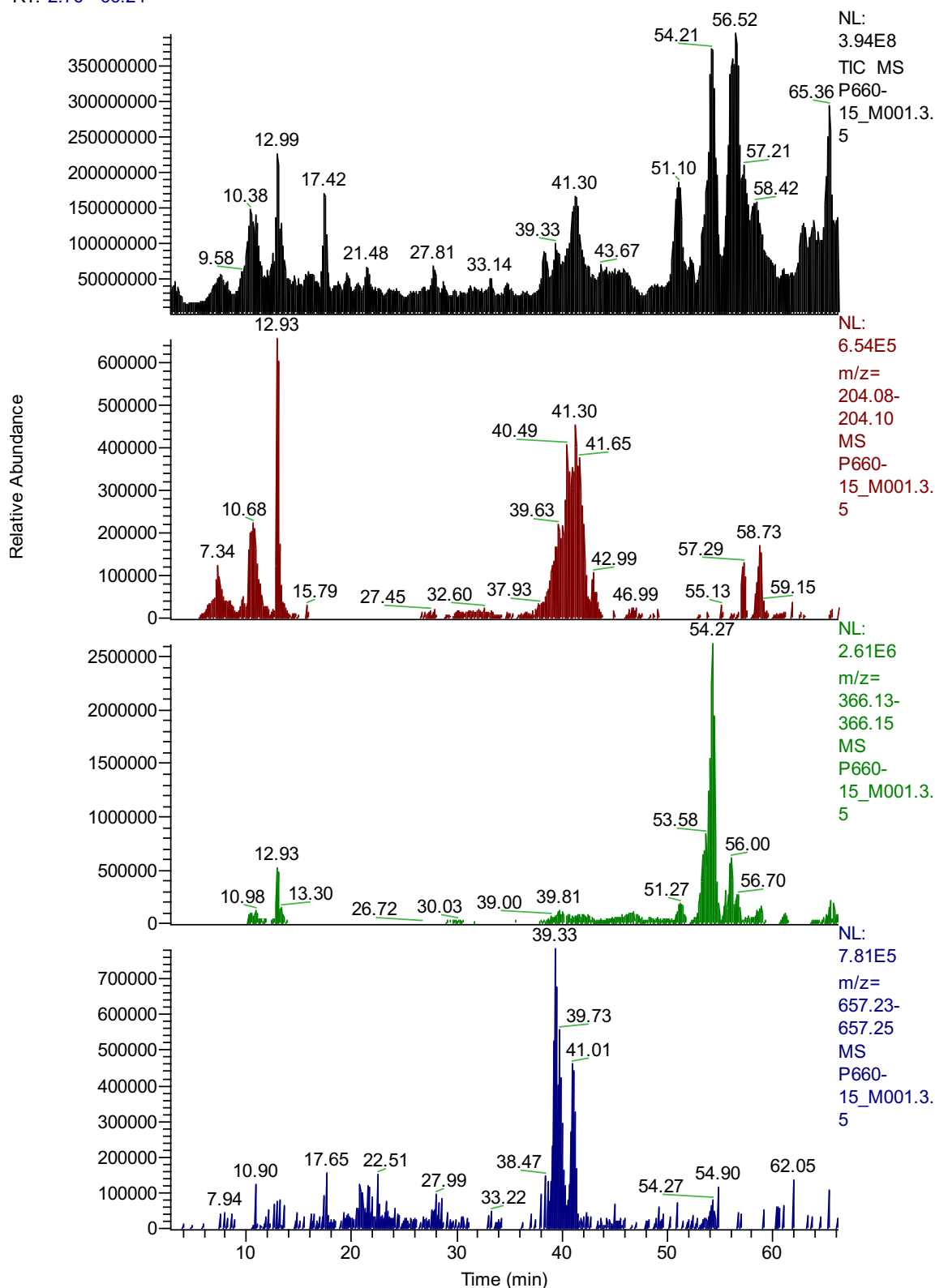


Figure S4. MoA TIC and XIC. The total ion chromatogram (TIC) for the dataset MoA is displayed with absolute intensities for relative intensities between 2.76 and 66.24 minutes. The extracted ion chromatograms (XIC) for the oxonium ions 204.09 (HexNAc), 366.14 (HexNAc + Hexose) and 657.24 (HexNAc + Hexose + NeuAc) are also displayed. The 657.24 peaks are only found in species which contain sialic acid.

RT: 2.76 - 66.24

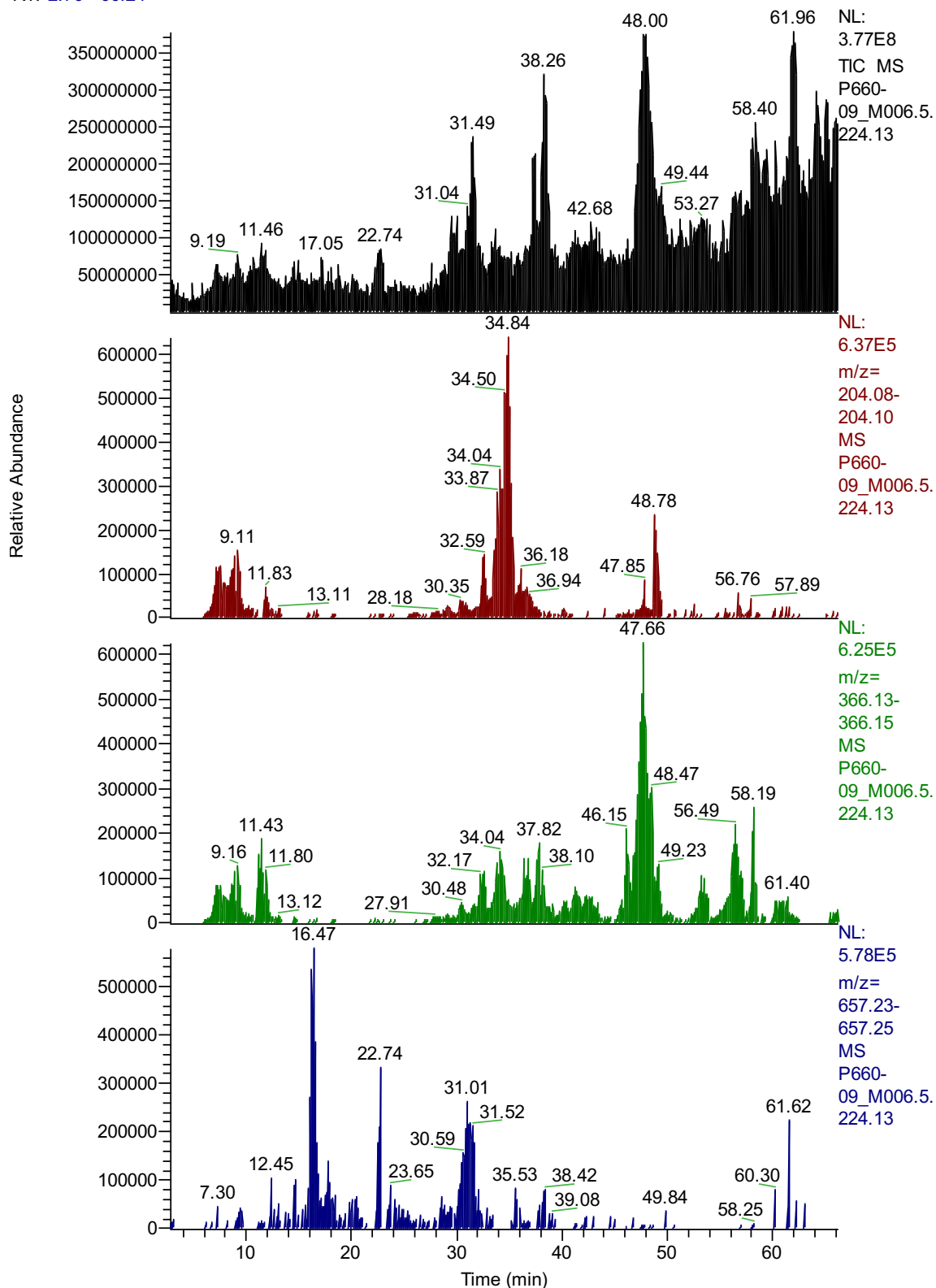


Figure S5. MoB TIC and XIC. The total ion chromatogram (TIC) is displayed with absolute intensities for relative intensities between 2.76 and 66.24 minutes. The extracted ion chromatograms (XIC) for the oxonium ions 204.09 (HexNAc), 366.14 (HexNAc + Hexose) and 657.24 (HexNAc + Hexose + NeuAc) are also displayed. The 657.24 peaks are only found in species which contain sialic acid.

RT: 2.76 - 66.24

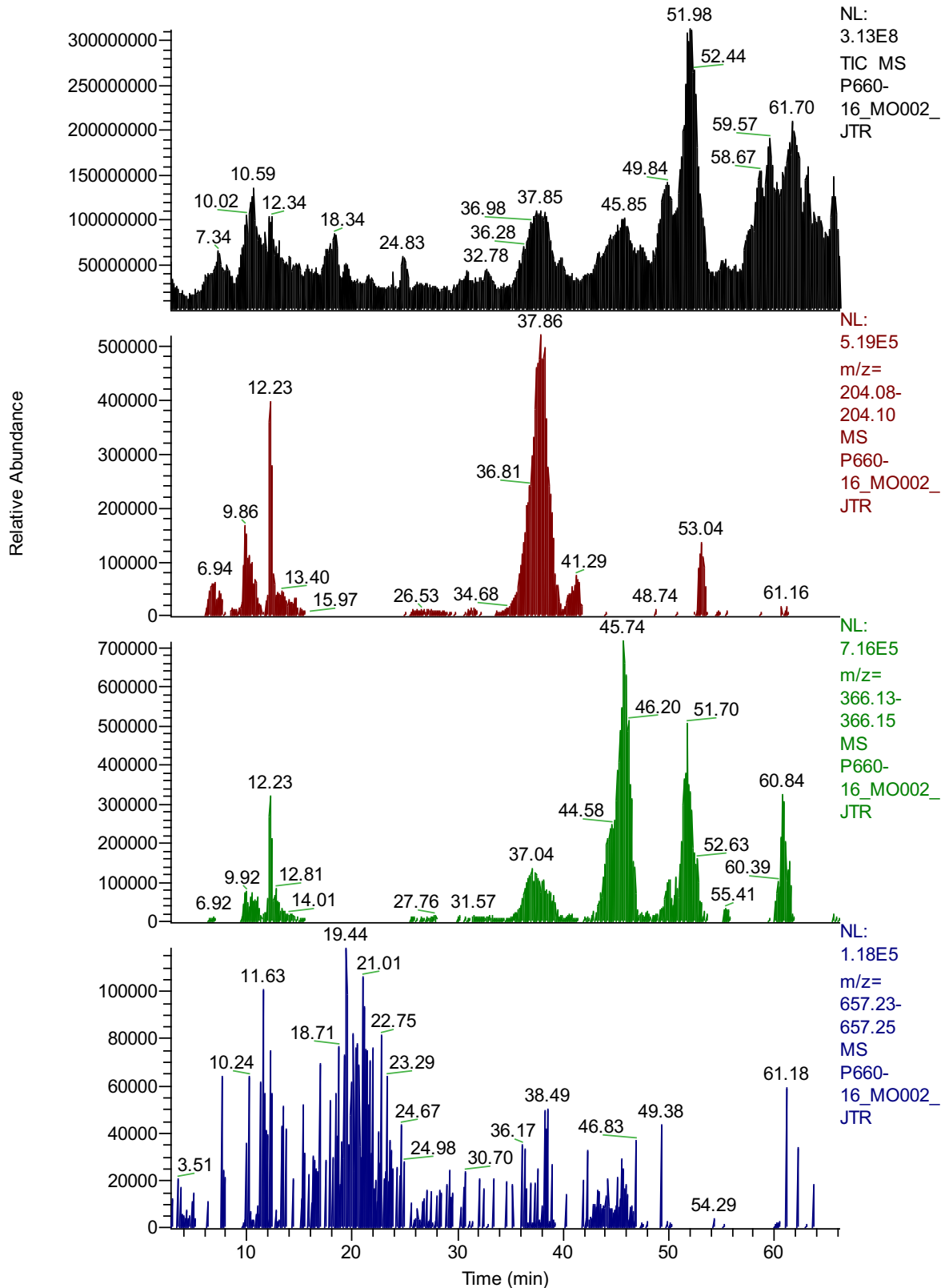


Figure S6. Mo02 TIC and XIC. The total ion chromatogram (TIC) is displayed with absolute intensities for relative intensities between 2.76 and 66.24 minutes. The extracted ion chromatograms (XIC) for the oxonium ions 204.09 (HexNAc), 366.14 (HexNAc + Hexose) and 657.24 (HexNAc + Hexose + NeuAc) are also displayed. The 657.24 peaks are only found in species which contain sialic acid.

RT: 2.76 - 66.24

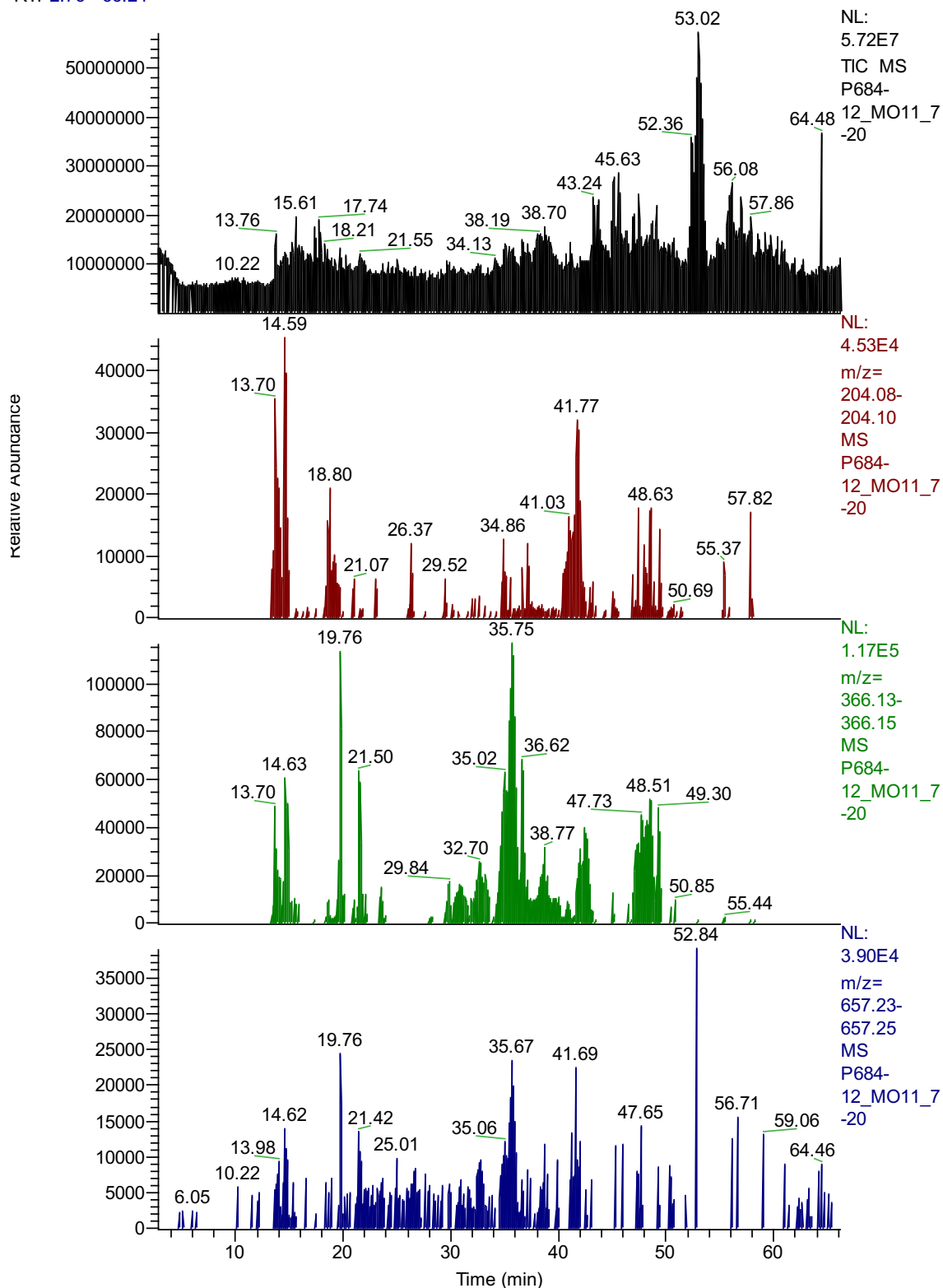


Figure S7. Mo11 TIC and XIC. The total ion chromatogram (TIC) is displayed with absolute intensities for relative intensities between 2.76 and 66.24 minutes. The extracted ion chromatograms (XIC) for the oxonium ions 204.09 (HexNAc), 366.14 (HexNAc + Hexose) and 657.24 (HexNAc + Hexose + NeuAc) are also displayed. The 657.24 peaks are only found in species which contain sialic acid.

RT: 2.76 - 66.24

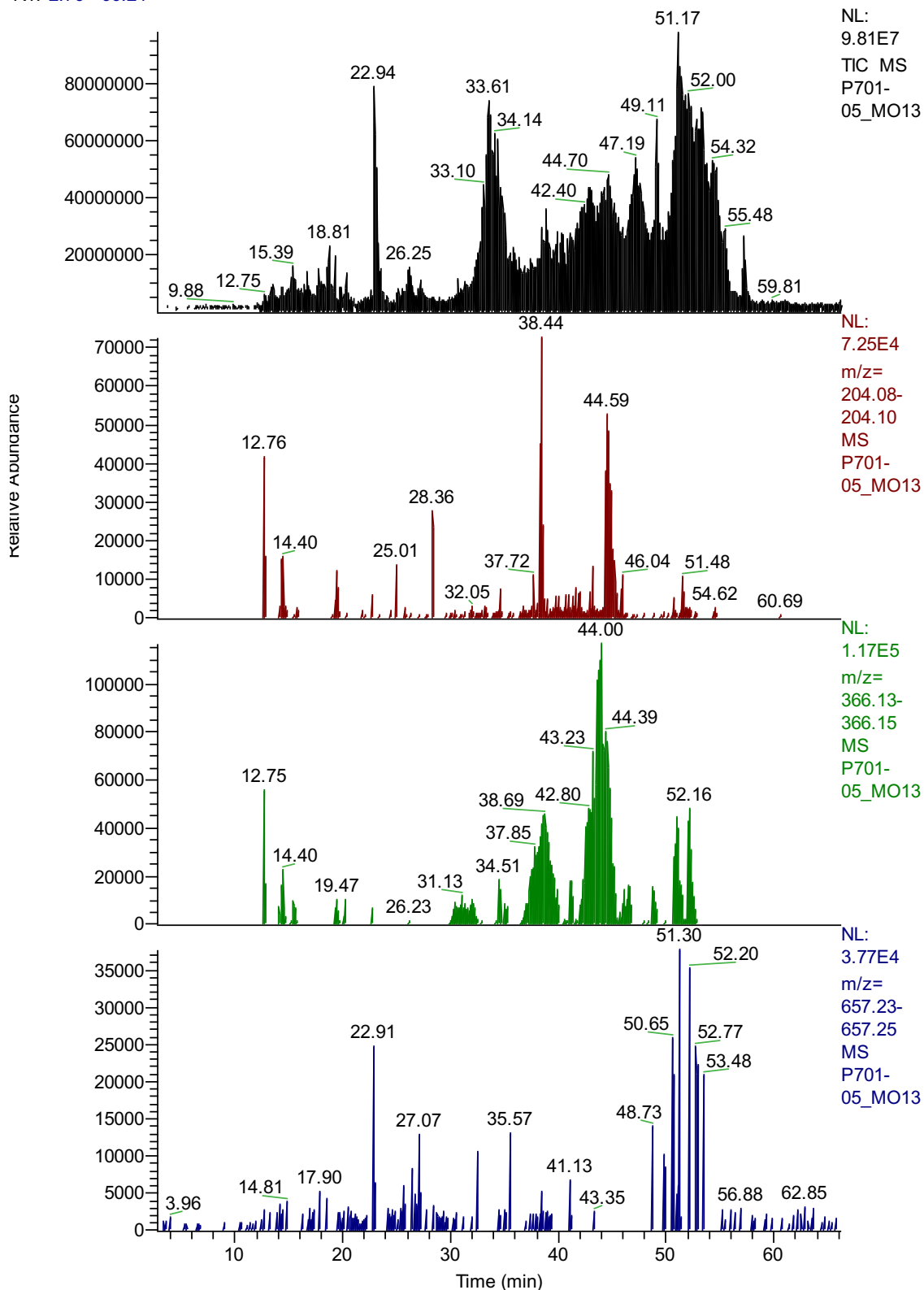


Figure S8. Mo13 TIC and XIC. The total ion chromatogram (TIC) is displayed with absolute intensities for relative intensities between 2.76 and 66.24 minutes. The extracted ion chromatograms (XIC) for the oxonium ions 204.09 (HexNAc), 366.14 (HexNAc + Hexose) and 657.24 (HexNAc + Hexose + NeuAc) are also displayed. The 657.24 peaks are only found in species which contain sialic acid.

RT: 2.76 - 66.24

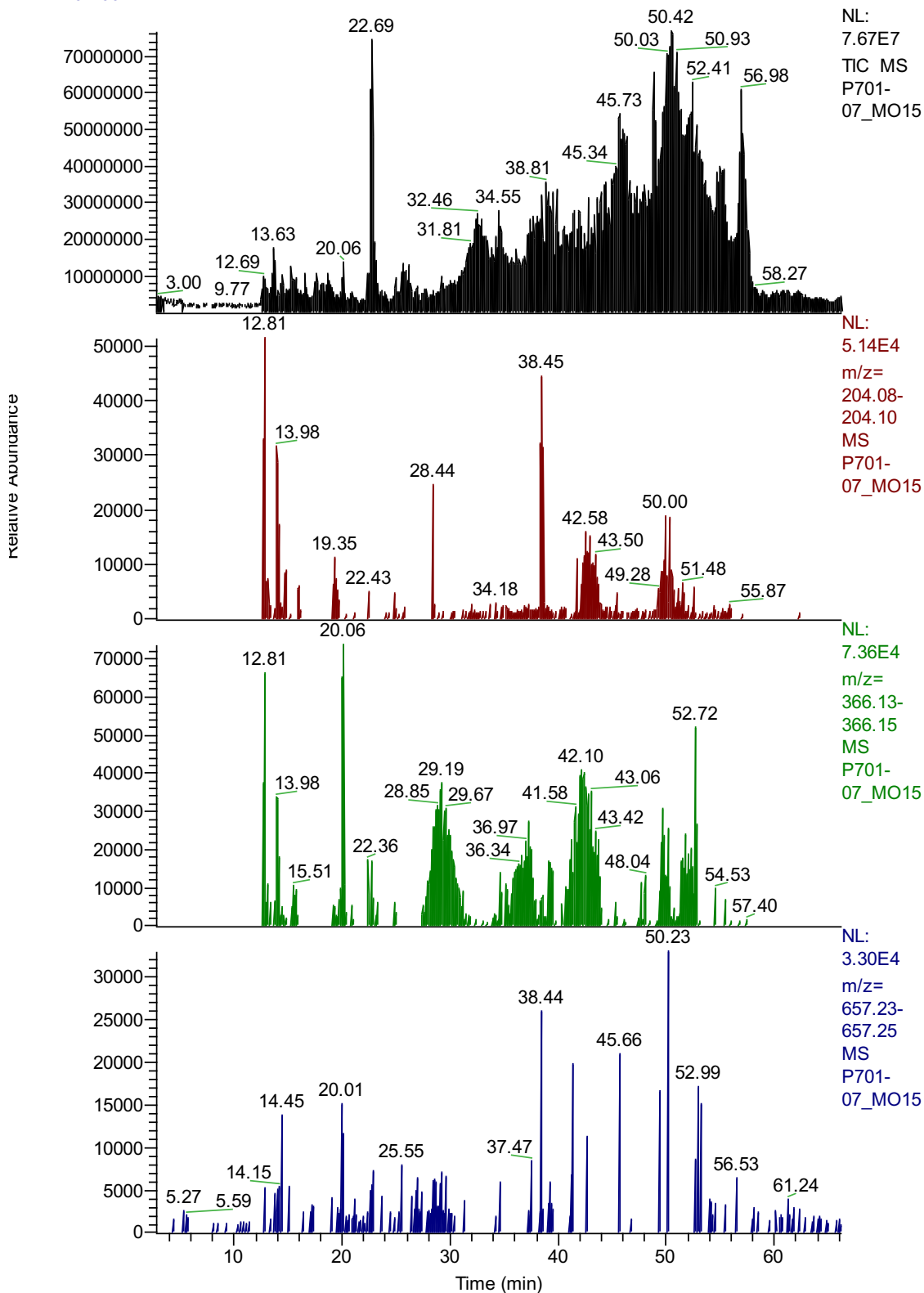


Figure S9. Mo15 TIC and XIC. The total ion chromatogram (TIC) is displayed with absolute intensities for relative intensities between 2.76 and 66.24 minutes. The extracted ion chromatograms (XIC) for the oxonium ions 204.09 (HexNAc), 366.14 (HexNAc + Hexose) and 657.24 (HexNAc + Hexose + NeuAc) are also displayed. The 657.24 peaks are only found in species which contain sialic acid.