Imaging Mass Spectrometry Reveals Alterations in N-linked glycosylation that are Associated with Histopathological Changes in Non-alcoholic Steatohepatitis in Mouse and Human

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Supplemental Figure 1: Histological system for components of NAFLD activity score (NAS), fibrosis staining and *col1a1* gene expression.
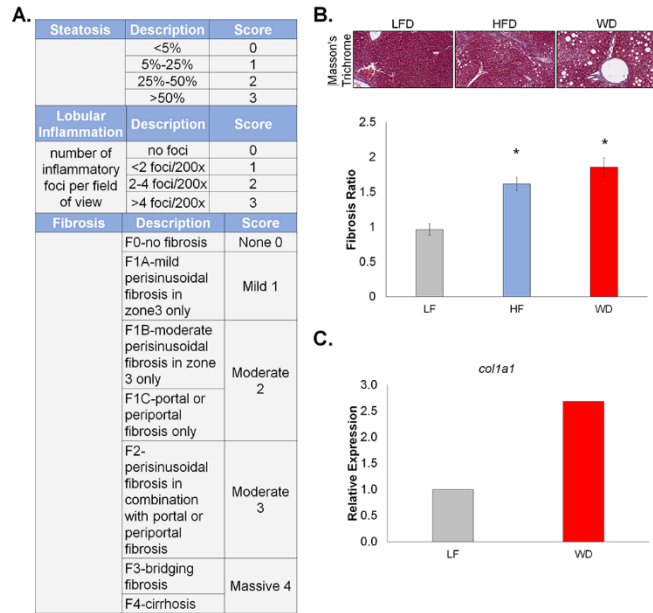
Supplemental Figure 2: NASH CRN fibrosis scoring system, clinical patient information and glycans observed in biopsies.

Supplemental Figure 3: Picrosirius Red staining quantification for NASH biopsies, representative images of core fucosylated glycans with Endo F3 Prime™ treatment and tissue selection before imaging.

Supplemental Table 1: Master list of N-Linked glycans of mouse analysis

Supplemental Table 2: Master List of N-Linked Glycans of human NASH biopsies analysis

Supplemental Table 3: Order in which MALDI-IMS data was acquired

A.

Steatosis	Description	Score
	<5%	0
	5%-25%	1
	25%-50%	2
	>50%	3
Lobular Inflammation	**Description**	**Score**
number of inflammatory foci per field of view	no foci	0
	<2 foci/200x	1
	2-4 foci/200x	2
	>4 foci/200x	3
Fibrosis	**Description**	**Score**
	F0-no fibrosis	None 0
	F1A-mild perisinusoidal fibrosis in zone3 only	Mild 1
	F1B-moderate perisinusoidal fibrosis in zone 3 only	Moderate 2
	F1C-portal or periportal fibrosis only	
	F2-perisinusoidal fibrosis in combination with portal or periportal fibrosis	Moderate 3
	F3-bridging fibrosis	Massive 4
	F4-cirrhosis	

B.

C.

Supplemental Figure 1. (**A**) University of Pittsburgh Medical Center (UPMC) histological system for components of NAFLD activity score (NAS) and fibrosis staging. (**B**) Masson's Trichrome staining quantification in paraffin embedded tissue. Scale bars: 100μm. n=2-3 per diet. (**C**) mRNA expression by qPCR for *cola1* (n=2 mice per diet).

A.

Group	Fibrosis Score	Description	Number of samples
FS 0	0	No fibrosis	8
FS1	1 (1a)	Mild perisinusoidal fibrosis	8
	1 (1b)	Moderate perisinusoidal fibrosis	
	1 (1c)	Portal/periportal fibrosis	
FS2	2	Perisinusoidal and portal/periportal fibrosis	9
FS3	3	Bridging fibrosis	15
FS4	4	Cirrhosis	11

B.

Fibrosis Score	0	1	2	3	4
Number of patients	8	8	10	12	11
Age (mean, SD)	60(6.4)	53(12.6)	58.5(8.0)	63(6.6)	58(19.5)
Gender (M/F)	0/7	5/3	2/8	6/5	4/6
BMI (kg/m²) (mean)	35	32	32	33	32
Biluribin total (mg/dL) (mean)	0.41	0.62	0.54	0.69	0.73
AST (U/L) (mean)	28	38	51	51	32
ALT (U/L) (mean)	37	63	64	48	48
Alk Phos (U/L) (mean)	90	83	81	85	85
INR (mean)	0.97	0.96	1.02	1.04	1.03

C.
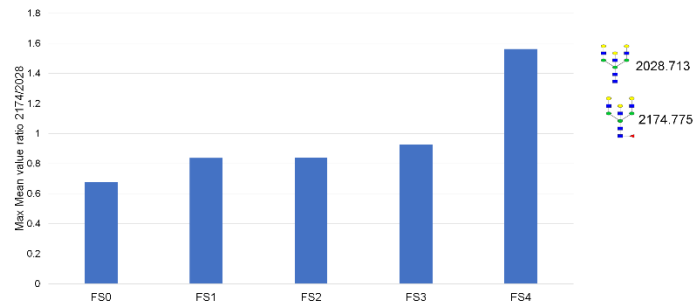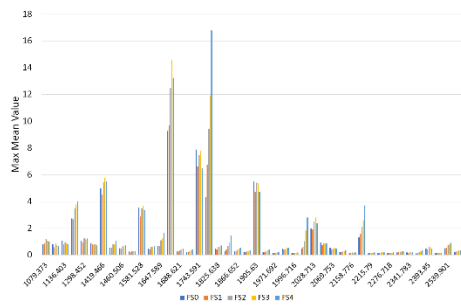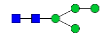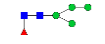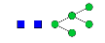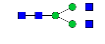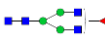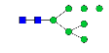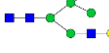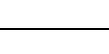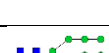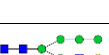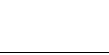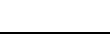
Supplemental Figure 2. (**A**) NASH CRN system for fibrosis scoring with description and number of samples in each group. Some patients had more than one biopsy (sample) collected (**B**) Patient clinical information from NASH biopsies. Some clinical information was not obtained for all patients. **(C)** all glycans observed in NASH biopsies analysis (left) ratio maximum mean value of bisected glycan to fucosylated bisected glycan based on fibrosis scores (right)
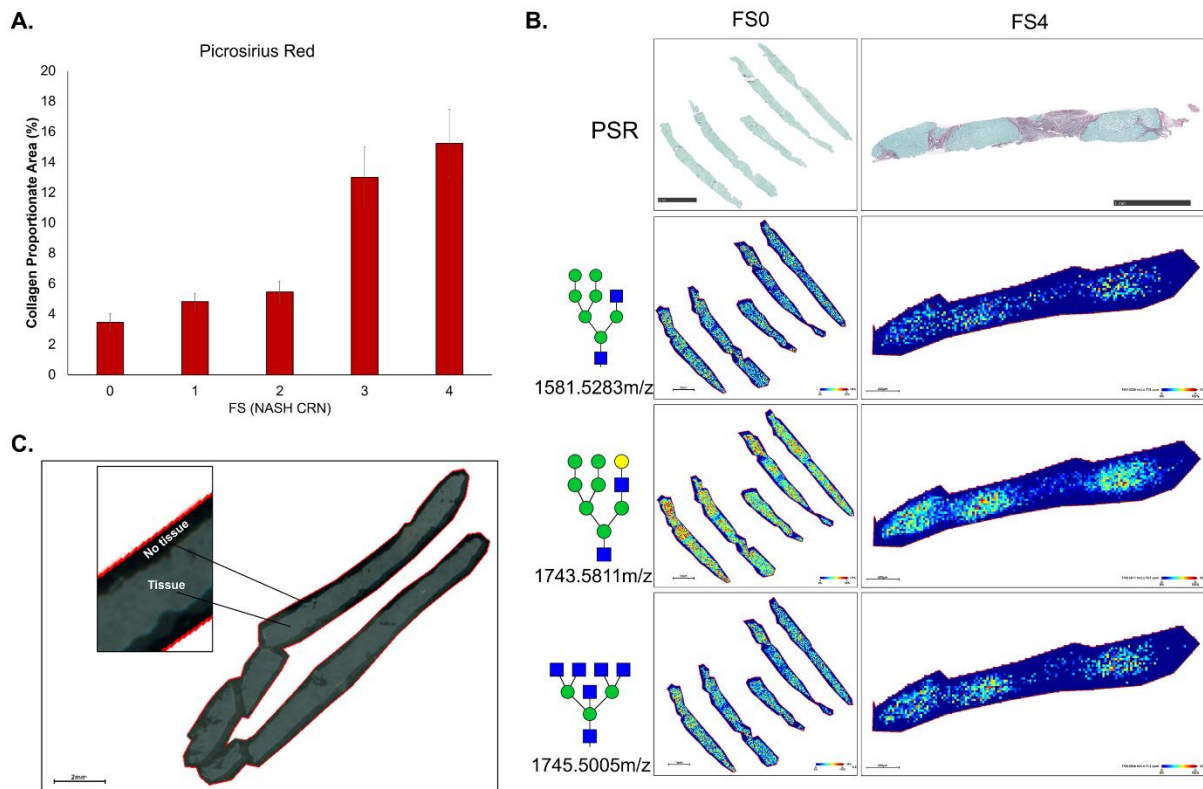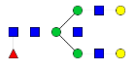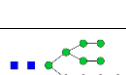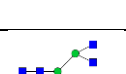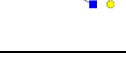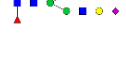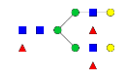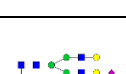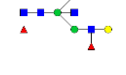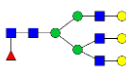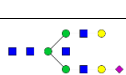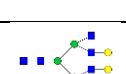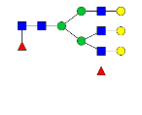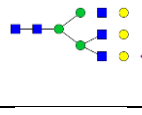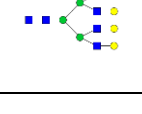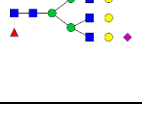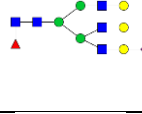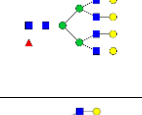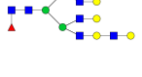
Supplemental Figure 3. (A) Collagen Proportionate area percent quantification of Picrosirius Red (PSR) staining by fibrosis score (FS) following the NASH CRN scoring system in NASH biopsies. Bars represent the mean ±SEM **(B)** Representative Picrosirius red (PSR) staining (top) and 2D heatmap images of core fucosylated glycans with EndoF3 treatment. PNGaseF mass shift: 1930.6656m/z, 2092.7184m/z, and 2094.7718m/z. (**C**) NASH biopsy with no 2D heatmap intensity (from Figure 2A) of any glycan showing how the tissue is selected for imaging. Zoom in image shows as space between the tissue and where the tissue was selected for imaging.

Supplementary Table 1- *Observed mass (m/z values) with the theoretical values, hexose compositions, and proposed glycan structure for the 18-month-old mouse cohort. Observed mass values are representative from low fat diet.*

Observed Mass (m/z)	Theoretical Mass (m/z)	Mass Error	Glycan Structure	Proposed Glycan
933.319	933.317	2.678	Hex3HexNAc2+ 1Na	
1079.374	1079.374	-0.278	Hex3dHex1HexNAc2 + 1Na	
1095.370	1095.369	0.730	Hex4HexNAc2 + 1Na	
1136.402	1136.396	5.279	Hex3HexNAc3 + 1Na	
1241.415	1241.427	-10.069	Hex4dHex1HexNAc2+ 1Na	
1257.422	1257.422	-0.477	Hex5HexNAc2 + 1Na	
1282.452	1282.454	-1.637	Hex3dHex1HexNAc3+ 1Na	
1298.448	1298.449	-0.847	Hex4HexNAc3 + 1Na	
1339.474	1339.475	-0.671	Hex3HexNAc4 + 1Na	
1403.474	1403.480	-3.990	Hex5dHex1HexNAc2+ 1Na	
1419.473	1419.475	-1.197	Hex6HexNAc2+ 1Na	
1444.502	1444.507	-2.907	Hex4dHex1HexNAc3 + 1Na	
1460.501	1460.502	-0.273	Hex5HexNAc3+ 1Na	
1485.532	1485.533	-0.673	Hex3dHex1HexNAc4+ 1Na	
1501.526	1501.528	-1.465	Hex4HexNAc4+ 1Na	
1542.555	1542.555	0.259	Hex3HexNAc5 + 1Na	
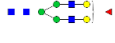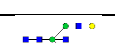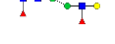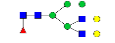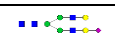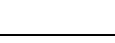
1581.527	1581.528	-0.316	Hex7HexNac2 + 1Na	
1606.537	1606.559	-13.818	Hex5dHex1HexNAc3+ 1Na	
1622.557	1622.554	1.170	Hex6HexNac3 + 1Na	
1647.586	1647.586	-0.182	Hex4dHex1HexNAc4 + 1Na	
1663.582	1663.581	0.601	Hex5HexNAc4+ 1Na	
1688.611	1688.613	-1.125	Hex3dHex1HexNAc5 + 1Na	
1704.606	1704.607	-0.762	Hex4HexNAc5+ 1Na	
1743.582	1743.581	0.860	Hex8HexNAc2 + 1Na	
1773.578	1773.579	0.563	Hex4dHex1HexNAc3NeuGc1 + 2Na	
1809.635	1809.639	-2.210	Hex5dHex1HexNAc4 + 1Na	
1825.634	1825.634	0.000	Hex6HexNAc4+ 1Na	
1850.677	1850.665	6.322	Hex4dHex1HexNAc5 + 1Na	
1866.674	1866.660	7.232	Hex5HexNAc5+ 1Na	
1905.635	1905.633	0.997	Hex9HexNAc2 + 1Na	
1928.688	1928.645	22.295	Hex6HexNAc3NeuGc1 + 1Na	
1953.647	1953.676	14.843	Hex4dHex1HexNAc4NeuGc1 + 1Na	
1971.676	1971.692	-8.013	Hex6dHex1HexNAc4 + 1Na	
1992.650	1992.653	1.505	Hex5HexNAc4NeuGc1 + 2Na	
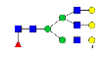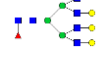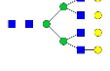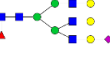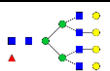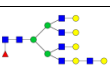
1995.656	1995.703	-23.249	Hex4HexNAc5NeuAc1+ 1Na	
2012.705	2012.718	-6.359	Hex5dHex1HexNAc5 + 1Na	
2028.706	2028.713	-3.351	Hex6HexNAc5 + 1Na	
2067.681	2067.686	-2.466	Hex10HexNAc2 + 1Na	
2069.695	2069.740	-21.451	Hex5HexNAc6 + 1Na	
2100.732	2100.734	-1.237	Hex5dHex1HexNAc4NeuAc1+ 1Na	
2101.738	2101.755	-7.945	Hex5dHex3HexNAc4+ 1Na	
2122.706	2122.724	-8.526	Hex5dHex1HexNAc4NeuAc1 + 2Na	
2157.755	2157.756	-0.509	Hex5HexNAc5NeuAc1+ 1Na	
2158.752	2158.776	-11.024	Hex5dHex2HexNAc5+ 1Na	
2174.769	2174.771	-0.965	Hex6dHex1HexNAc5+ 1Na	
2179.723	2179.745	-10.505	Hex5HexNAc5NeuAc1 + 2Na	
2231.769	2231.793	-10.708	Hex6HexNAc6+ 1Na	
2277.767	2277.782	6.585	Hex6dHex1HexNAc4NeuGc1 + 1Na	
2319.809	2319.809	0.301	Hex6HexNAc5NeuAc1+ 1Na	

2320.802	2320.829	-11.461	Hex6dHex2HexNAc5+ 1Na	
2341.768	2341.798	-12.767	Hex6HexNAc5NeuAc1 + 2Na	
2393.840	2393.845	-2.339	Hex7HexNAc6 + 1Na	
2465.858	2465.866	-3.568	Hex6dHex1HexNAc5NeuAc1+ 1Na	
2487.848	2487.856	-3.496	Hex6dHex1HexNAc5NeuAc1 + 2Na	
2539.912	2539.903	3.582	Hex7dHex1HexNAc6+ 1Na	
2905.013	2905.035	-7.814	Hex8dHex1HexNAc7+ 1Na	

Supplementary Table 2-_Observed mass (m/z values) with the theoretical values, hexose compositions, and proposed glycan structure from analysis of NASH patient biopsies._

Observed Mass (m/z)	Theoretical Mass (m/z)	Mass Error	Glycan Structure	Proposed Glycan
1079.373	1079.374	1.759	Hex3dHex1HexNAc2 + 1Na	
1095.378	1095.369	7.487	Hex4HexNAc2 + 1Na	
1136.403	1136.396	5.808	Hex3HexNAc3 + 1Na	
1257.417	1257.422	4.293	Hex5HexNAc2 + 1Na	
1298.452	1298.449	2.773	Hex4HexNAc3 + 1Na	
1339.466	1339.475	6.643	Hex3HexNAc4 + 1Na	
1419.466	1419.475	6.622	Hex6HexNAc2 + 1Na	
1444.508	1444.507	0.900	Hex4dHex1HexNAc3 + 1Na	
1460.506	1460.502	2.738	Hex5HexNAc3 + 1Na	
1542.553	1542.555	0.906	Hex3HexNAc5 + 1Na	
1581.528	1581.528	0.063	Hex7HexNac2 + 1Na	
1622.565	1622.554	2.896	Hex6HexNac3 + 1Na	
1647.589	1647.586	1.760	Hex4dHex1HexNAc4 + 1Na	
1663.575	1663.581	3.847	Hex5HexNAc4+ 1Na	
1688.621	1688.613	4.915	Hex3dHex1HexNAc5 + 1Na	
1704.603	1704.608	2.932	Hex4HexNAc5+ 1Na	
1743.591	1743.581	5.735	Hex8HexNAc2 + 1Na	

1809.638	1809.639	0.331	Hex5dHex1HexNAc4 + 1Na	
1825.632	1825.634	1.149	Hex6HexNAc4+ 1Na	
1850.667	1850.665	0.811	Hex4dHex1HexNAc5 + 1Na	
1866.652	1866.660	4.392	Hex5HexNAc5+ 1Na	
1891.692	1891.692	0.000	Hex3dHex1HexNAc6+ 1Na	
1905.630	1905.633	1.626	Hex9HexNAc2 + 1Na	
1955.694	1955.697	1.380	Hex5dHex2HexNAc4+ 1Na	
1971.692	1971.692	0.000	Hex6dHex1HexNAc4 + 1Na	
1976.662	1976.666	2.276	Hex5HexNAc4NeuAc1 + 2Na	
1996.716	1996.723	3.856	Hex4dHex2HexNAc5+ 1Na	
2012.713	2012.718	2.583	Hex5dHex1HexNAc5 + 1Na	
2028.713	2028.713	0.295	Hex6HexNAc5 + 1Na	
2067.683	2067.686	1.305	Hex10HexNAc2 + 1Na	
2069.753	2069.740	6.281	Hex5HexNAc6 + 1Na	
2122.714	2122.724	4.522	Hex5dHex1HexNAc4NeuAc1 + 2Na	
2158.776	2158.776	0.277	Hex5dHex2HexNAc5+ 1Na	
2174.775	2174.771	1.655	Hex6dHex1HexNAc5+ 1Na	
2215.790	2215.798	3.384	Hex5dHex1HexNAc6+ 1Na	

2231.793	2231.793	0.000	Hex6HexNAc6+ 1Na	
2276.718	2276.718	0.000	Hex6dHex1HexNAc5 + 1SO4 + 2Na	
2320.824	2320.829	2.240	Hex6dHex2HexNAc5+ 1Na	
2341.783	2341.798	6.477	Hex6HexNAc5NeuAc1 + 2Na	
2377.838	2377.850	5.046	Hex6dHex1HexNAc6+ 1Na	
2393.850	2393.845	1.754	Hex7HexNAc6 + 1Na	
2487.845	2487.856	4.542	Hex6dHex1HexNAc5NeuAc1 + 2Na	
2539.901	2539.903	0.708	Hex7dHex1HexNAc6+ 1Na	
2905.003	2905.035	2.788	Hex8dHex1HexNAc7+ 1Na	

Supplementary Table 3. *Order in which MALDI-IMS data was acquired, samples were prepared and ran for MALDI-IMS experiments randomly. Samples are organized by groups in this table for a better description of the order (i.e., samples in Group 1 were prepared and ran first, followed by samples in group 2, etc.). ID: identification number given to each sample based on the order they were registered in our laboratory database. Fibrosis score: biopsies were assessed for fibrosis by an independent pathologist using the NASH Clinical Research Network (CRN) scoring system.*

Fibrosis Score	ID	Group	Fibrosis Score	ID	Group
0	20	1	3	12	2
0	21	1	3	14	2
0	22	1	3	15	3
0	23	1	3	26	3
0	24	3	3	27	3
0	40	3	3	28	2
0	41	3	3	29	2
0	42	3	3	30	2
1	18	2	3	31	1
1	19	1	3	44	3
1	36	3	3	52	2
1	37	3	3	53	2
1	38	3	3	54	2
1	39	1	3	55	2
1	47	1	3	58	1
1	48	1	4	1	1
2	8	2	4	2	1
2	16	3	4	3	1
2	17	2	4	4	2
2	35	2	4	5	2
2	34	2	4	12	2
2	45	1	4	25	2

2	46	2	4	60	2
2	50	2	4	61	2
2	51	2	4	62	2
			4	63	2