

## **Supporting information:**

### **A Novel Mass Spectrometry Platform for Multiplexed N-glycoprotein Biomarker Discovery from Patient Biofluids by Antibody Panel Based N-glycan Imaging**

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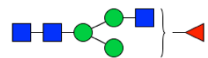
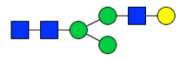


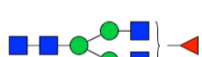






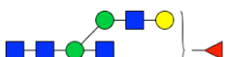
**Figure S1: HPLC and lectin profiles of A1AT and IgG N-glycans.** Orthogonal confirmation of N-glycan profiles of the 2 glycoproteins analyzed in this study.

**Figure S2. Quantifications of N-glycan peaks shown in Figure 4.**

**Figure S3. Capture of A1AT and IgG from normal serum samples.**

**Figure S4. Quantifications of N-glycan peaks observed in normal and cirrhotic serum.**

**Table S1. All observed N-glycans on A1AT and IgG.** This table describes the m/z values of all N-glycans observed on A1AT and IgG protein standards.

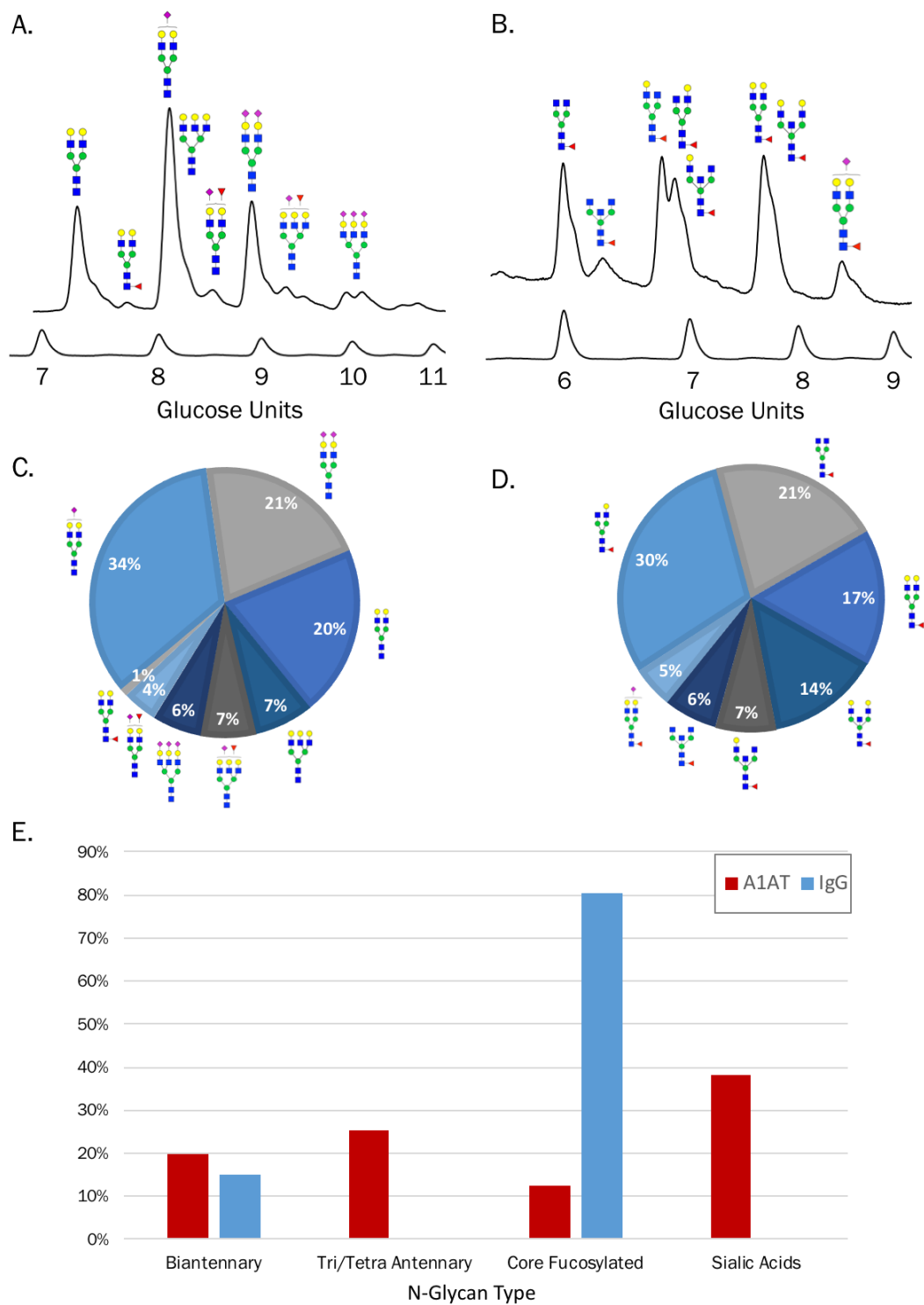
Theoretical mass	Observed mass	Error (ppm)	N-Glycan Composition	Proposed N-glycan Structure
1282.4543	1282.4562	1.4815	Hex3dHex1HexNAc3 + 1Na	
1298.4492	1298.4471	1.6019	Hex4HexNAc3 + 1Na	
1339.4757	1339.4731	1.9635	Hex3HexNAc4 + 1Na	
1444.5071	1444.5097	1.7861	Hex4dHex1HexNAc3 + 1Na	
1485.5337	1485.5362	1.7098	Hex3dHex1HexNAc4 + 1Na	
1501.5286	1501.5315	1.9380	Hex4HexNAc4 + 1Na	
1647.5865	1647.5825	2.4278	Hex4dHex1HexNAc4 + 1Na	
1663.5814	1663.5781	1.9837	Hex5HexNAc4 + 1Na	
1688.6130	1688.6143	0.7521	Hex3dHex1HexNAc5 + 1Na	
1704.6069	1704.6170	5.9486	Hex4HexNAc5 + 1Na	
1809.6393	1809.6397	0.2321	Hex5dHex1HexNAc4 + 1Na	
1850.6710	1850.6682	1.5400	Hex4dHex1HexNAc5 + 1Na	

1866.6608	1866.6468	7.5000	Hex5HexNAc5 + 1Na	
1976.6666	1976.6618	2.4182	Hex5HexNAc4NeuAc1 + 2Na	
2012.7187	2012.7201	0.7105	Hex5dHex1HexNAc5 + 1Na	
2028.7136	2028.7196	2.9773	Hex6HexNAc5 + 1Na	
2122.7245	2122.7206	1.8467	Hex5dHex1HexNAc4NeuAc1 + 2Na	
2174.7715	2174.7813	4.5062	Hex6dHex1HexNAc5 + 1Na	
2289.7722	2289.7638	3.6248	Hex5HexNAc4NeuAc2 + 3Na	
2341.8090	2341.7940	6.4138	Hex6HexNAc5NeuAc1 + 2Na	
2487.8669	2487.84282	9.6790	Hex6dHex1HexNAc5NeuAc1 + 2Na	

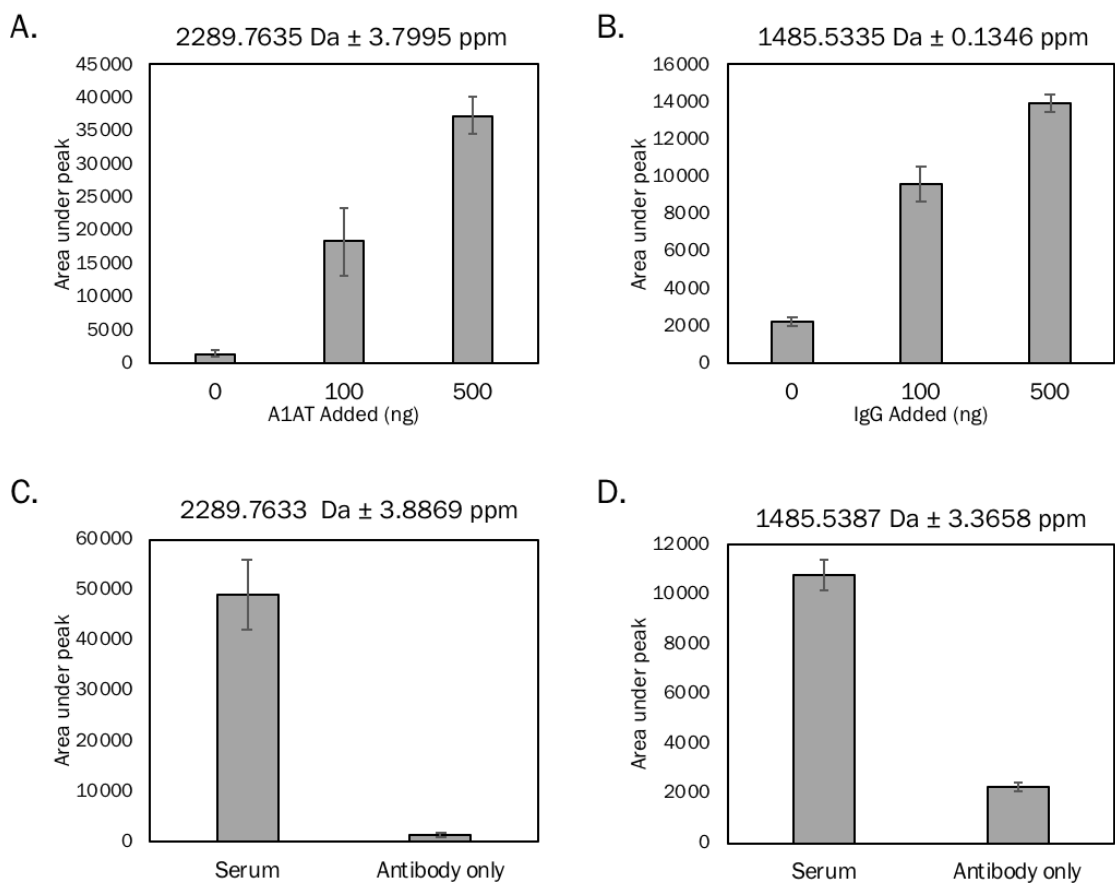
**Table S2. All N-glycans observed in serum protein capture.** This table describes the m/z values of all N-glycans observed on proteins captured from human serum. Those marked with an asterisk were only seen in cirrhotic patient serum.

Theoretical mass	Observed mass	Error (ppm)	N-Glycan Composition	Proposed N-glycan Structure
*1257.4226	1257.4245	1.5110	Hex5HexNAc2 + 1Na	
1282.4543	1282.4531	0.9357	Hex3dHex1HexNAc3 + 1Na	
1298.4492	1298.4504	0.9242	Hex4HexNAc3 + 1Na	
1339.4757	1339.4799	3.1356	Hex3HexNAc4 + 1Na	
*1419.4755	1419.4737	1.2681	Hex6HexNAc2 + 1Na	
1444.5071	1444.5082	0.7615	Hex4dHex1HexNAc3 + 1Na	
1485.5337	1485.5356	1.2790	Hex3dHex1HexNAc4 + 1Na	
*1581.5283	1581.5338	3.4776	Hex7HexNAc2 + 1Na	
1647.5865	1647.5885	1.2139	Hex4dHex1HexNAc4 + 1Na	
1663.5814	1663.5885	1.9837	Hex5HexNAc4 + 1Na	
1688.6130	1688.6127	0.1777	Hex3dHex1HexNAc5 + 1Na	
*1743.5811	1743.5828	0.9750	Hex8HexNAc2 + 1Na	

1809.6393	1809.6344	2.7077	Hex5dHex1HexNAc4 + 1Na	
1850.6710	1850.6658	2.8098	Hex4dHex1HexNAc5 + 1Na	
1976.6666	1976.6577	4.5023	Hex5HexNAc4NeuAc1 + 2Na	
2012.7187	2012.7190	0.1491	Hex5dHex1HexNAc5 + 1Na	
2028.7136	2028.7241	5.1757	Hex6HexNAc5 + 1Na	
2122.7245	2122.7057	8.8565	Hex5dHex1HexNAc4NeuAc1 + 2Na	
2174.7715	2174.7776	2.8049	Hex6dHex1HexNAc5 + 1Na	
2289.7722	2289.7623	4.3236	Hex5HexNAc4NeuAc2 + 3Na	
2325.8141	2325.7925	9.2871	Hex5dHex1HexNAc5NeuAc1 + 2Na	
2341.8090	2341.7946	6.1491	Hex6HexNAc5NeuAc1 + 2Na	
2487.8669	2487.8506	6.5518	Hex6dHex1HexNAc5NeuAc1 + 2Na	

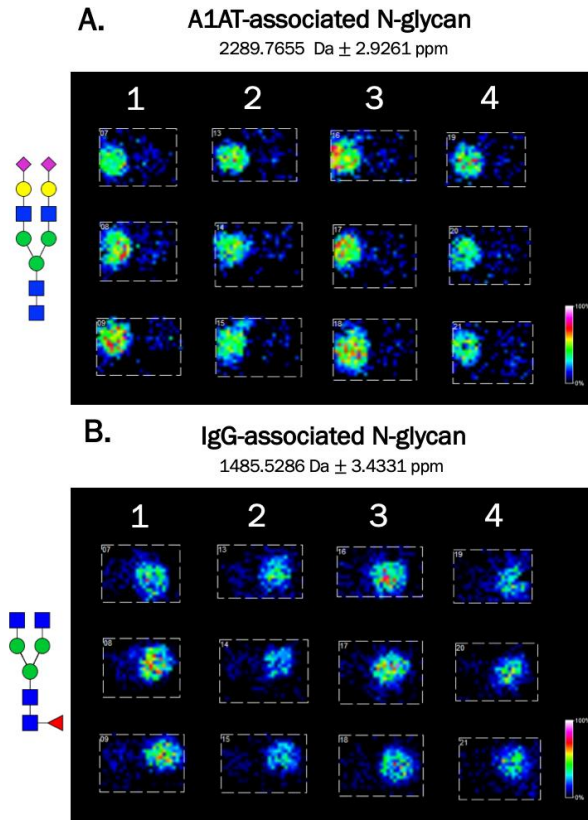


**Figure S1: HPLC and lectin profiles of A1AT and IgG N-glycans.** A-B) For orthogonal comparison of N-glycan profiles, A1AT and IgG were digested in-solution with PNGase F followed by HPLC analysis. C-D) Percentage of each N-glycan specie was calculated by the peak area divided by the total of all N-glycan peak areas. E) Prediction of N-glycans on A1AT and IgG by lectin profiling.

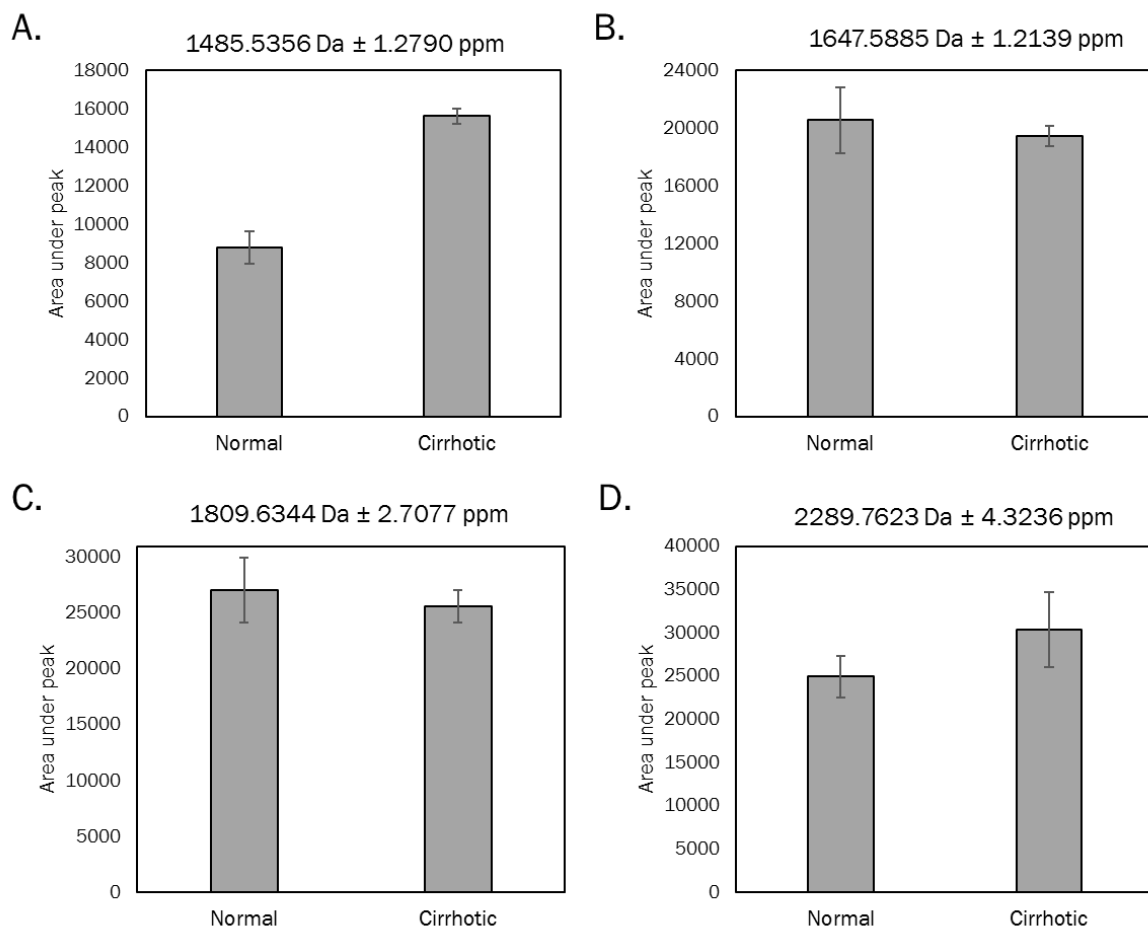


**Figure S2. Quantifications of N-glycan peaks observed in Figure 4.** A-D) Area under peak values were obtained for each region. Bars represent the mean  $\pm$  standard deviation of 3 samples and highlight that significant N-glycan signal was observed from each sample beyond that of the antibody background signal.





**Figure S3. Capture of A1AT and IgG from normal serum samples.** Normal human serum samples from 4 individual donors (SeraCare) were added to the array in triplicate. Donors were confirmed negative for HIV, HBV, and HCV. Samples were diluted 1:100 in PBS for a total of  $1\mu\text{L}$  serum added per well. Each well contained antibodies for anti-A1AT (left) and anti-IgG (right) as shown previously, spotted at  $200\text{ng}$  per  $1.5\mu\text{L}$  antibody spot. A) An A1AT-associated N-glycan was reproducibly observed localized to the left of each well. B) An IgG-associated N-glycan was reproducibly observed localized to the right of each well.



**Figure S4. Quantifications of N-glycan peaks observed in normal and cirrhotic serum.** A-C) IgG N-glycan intensities were compared by the area under peak values for each spot. Bars represent the mean  $\pm$  standard deviation of 3 samples and illustrate that not all IgG N-glycans are increased in cirrhotic serum as would be expected if the protein levels were varying. D) Comparison of an A1AT N-glycan by area under the peak values. Bars represent the mean  $\pm$  standard deviation of 3 samples.