

Supplementary information

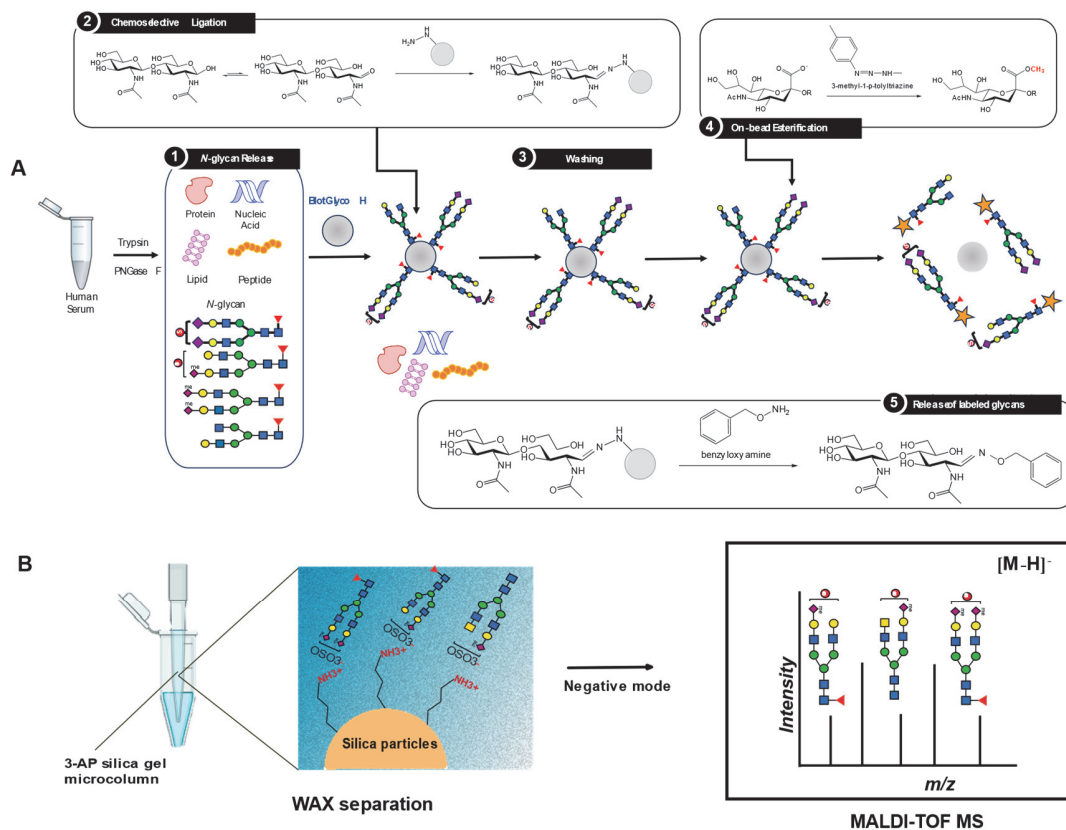


Figure S1. Schematic workflow of the glycoblotting-based sulphoglycomic approach for sulfated N-glycan analysis. A) Overall workflow of the glycoblotting-based MALDI-TOF M/S analysis of N-glycans derived from serum glycoproteins. 1) Enzymatic releasing of N-glycans from serum glycoproteins 2) Chemoselective ligations of N-glycans by capturing reducing sugars onto hydrazide-functionalized BlotGlyco H beads 3) Washing to remove impurities 4) On-bead methyl esterification of –COOH of terminal sialic acid residues 5) Recovery of Benzyloxyamine (BOA)-labeled glycans using trans-iminization reaction. B) Weak anion exchange (WAX) separation using a 3-aminopropyl (3-AP) silica gel microcolumn, followed by MALDI-TOF M/S analysis in negative mode.

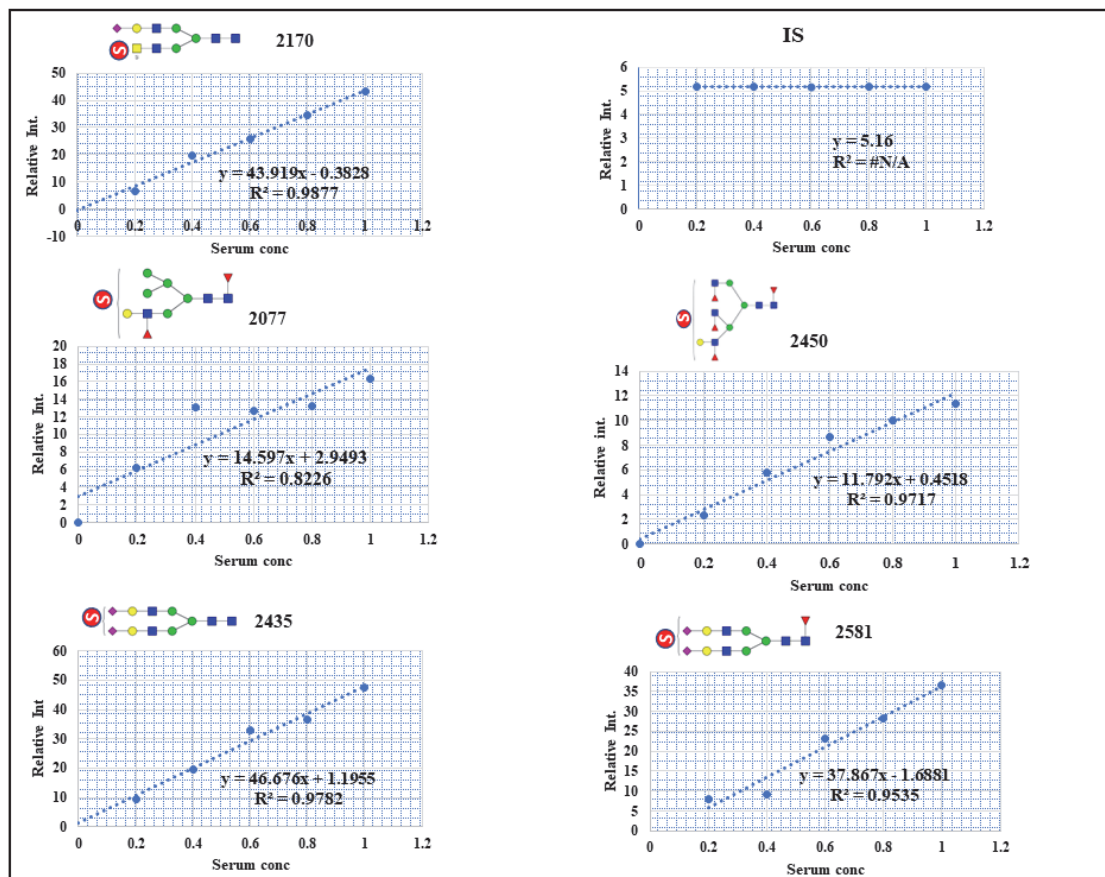


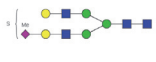
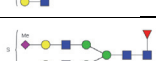
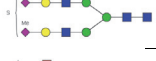
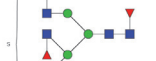
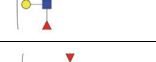
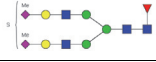
Figure S2: Human serum calibration curve showing the quantitative reproducibility of common sulfated glycans.

Table S1. Demographic characteristics of the study participants

		Mean $\pm$ SD		Number of subjects per age range				
Status	Number (n)	Age	BMI (kg/m ²)	20-30	31-40	41-50	51-60	≥ 61
NC	20	32.3 $\pm$ 7.54	22.33 $\pm$ 3.20	10	8	1	1	
BC-I	17	43.0 $\pm$ 13.88	22.37 $\pm$ 2.56	4	5	3	3	2
BC-II	20	42.8 $\pm$ 11.23	21.90 $\pm$ 3.10	3	7	5	2	3
BC-III	17	39.7 $\pm$ 11.02	22.90 $\pm$ 2.66	3	9	4		1
BC-IV	22	40.36 $\pm$ 12.29	22.86 $\pm$ 2.87	6	7	6	2	1
Total (N)	96			26	36	19	8	7

Table S2: Estimated compositions of sulfated N-glycans from human serum glycoproteins labeled with BOA.

Observed mass, m/z [M-H] ⁻	Calculated mass, m/z [M-H] ⁻	Error ppm	Glycan composition	Probable structure ¹⁻³	Glyconnect database links
1764.623	1764.589	25.06	(Hex) ₁ (HexNAc) ₁ (NeuAc) ₁ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		
1808.838	1808.615	120.09	(Hex) ₁ (HexNAc) ₂ (dHex) ₁ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		GlyConnect
1824.629	1824.610	7.79	(Hex) ₂ (HexNAc) ₂ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		GlyConnect
1913.718	1913.646	34.60	(Hex) ₂ (HexNAc) ₂ (dHex) ₂ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		
2034.750	2034.63	56.63	Hex) ₁ (HexNAc) ₂ (dHex) ₂ (Sulph) ₂ + (Man) ₃ (GlcNAc) ₂		
2076.558	2075.699	410.88	(Hex) ₃ (HexNAc) ₁ (dHex) ₂ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		

2130.004	2129.721	137.66	(Hex) ₂ (HexNAc) ₂ (NeuAc) ₁ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		GlyConnect
2137.073	2136.66	195.57	(Hex) ₁ (HexNAc) ₁ (dHex) ₂ (NeuAc) ₁ (Sulph) ₂ + (Man) ₃ (GlcNAc) ₂		
2170.980	2170.747	112.04	(Hex) ₁ (HexNAc) ₃ (NeuAc) ₁ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		GlyConnect
2271.621	2271.795	-79.13	(Hex) ₁ (HexNAc) ₅ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		
2275.807	2275.779	16.79	(Hex) ₂ (HexNAc) ₂ (dHex) ₁ (NeuAc) ₁ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		GlyConnect
2340.237	2339.741	216.31	(Hex) ₁ (HexNAc) ₂ (dHex) ₂ (NeuAc) ₁ (Sulph) ₂ + (Man) ₃ (GlcNAc) ₂		
2435.022	2434.832	88.8	(Hex) ₂ (HexNAc) ₂ (NeuAc) ₂ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		
2449.363	2449.868	208.53	(Hex) ₁ (HexNAc) ₃ (dHex) ₄ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		
2486.101	2485.798	125.59	(Hex) ₁ (HexNAc) ₂ (dHex) ₃ (NeuAc) ₁ (Sulph) ₂ + (Man) ₃ (GlcNAc) ₂		
2581.064	2580.890	77.57	(Hex) ₂ (HexNAc) ₂ (dHex) ₁ (NeuAc) ₂ (Sulph) ₁ + (Man) ₃ (GlcNAc) ₂		GlyConnect
3010.391	3009.984	143.91	(Hex) ₂ (HexNAc) ₃ (dHex) ₂ (NeuAc) ₂ (Sulph) ₂ + (Man) ₃ (GlcNAc) ₂		
3156.523	3156.042	160.69	(Hex) ₂ (HexNAc) ₃ (dHex) ₃ (NeuAc) ₂ (Sulph) ₂ + (Man) ₃ (GlcNAc) ₂		

The m/z 1824 was the internal standard (IS). The m/z values of 1764.623, 1913.718, 2130.004, and 3156.523 were not included in the quantitative comparison because they were not quantitatively reproducible for the entire study sample. Monosaccharide nomenclatures are based on SNFG: Hexose (Hex), *N*-acetyl hexosamine (HexNAc), mannose (Man), fucose (dHex), *N*-acetyl glucosamine (GlcNAc), sulfate (Sulph), and *N*-acetyl neuraminic acid (NeuAc).

Reference

1. Lee J, Ha S, Kim M, Kim S-W, Yun J, Ozcan S, et al. Spatial and temporal diversity of glycome expression in mammalian brain. *Proceedings of the National Academy of Sciences*. **2020**;117(46):28743-53.
2. Van Rooijen JJ, Kamerling JP, Vliegenthart JF. Sulfated di-, tri-and tetraantennary N-glycans in human Tamm-Horsfall glycoprotein. *European Journal of Biochemistry*. **1998**;256(2):471-87.
3. Hård K, Van Zadelhoff G, Moonen P, Kamerling JP, Vliegenthart JF. The Asn-linked carbohydrate chains of human Tamm-Horsfall glycoprotein of one male: Novel sulfated and novel N-acetylgalactosamine-containing N-linked carbohydrate chains. *European Journal of Biochemistry*. **1992**;209(3):895-915.