

Supporting Information

Disease-specific haptoglobin- β chain N-glycosylation as biomarker to differentiate non-small cell lung cancer from benign lung diseases

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Table S1. Patterns of serum IIRPCs of patients with NSCLC and BLDs

| | a | b | c | d | e | f | g |
|------------|-------|-------|-------|------|------|------|------|
| BLDs | 122 | 88 | 64 | 3 | 4 | 11 | 8 |
| NSCLC | 147 | 111 | 28 | 4 | 3 | 5 | 2 |
| Total | 269 | 199 | 92 | 7 | 7 | 16 | 10 |
| Percentage | 44.8% | 33.2% | 15.3% | 1.2% | 1.2% | 2.7% | 1.7% |

Percentage of cases (x)=number(x)/number(a + b + c + d + e + f + g); BLDs, benign lung diseases; NSCLC, non-small cell lung cancer.

Table S2. Comparison of the N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features of DSHp- β between patterns a and b in BLDs or NSCLC patients.

| N-glycopeptides, N-glycopeptide ratios and N-glycosylation features | BLDs | | NSCLC | |
|---|----------------|-------------------------------|----------------|-------------------------------|
| | <i>p</i> value | <i>p</i> _{adj} value | <i>p</i> value | <i>p</i> _{adj} value |
| VVLHPN₂₄₁YSQVDIGLIK | | | | |
| G2 | 0.019 | 0.147 | 0.789 | 0.891 |
| G2S | 0.965 | 1.015 | 0.278 | 0.679 |
| G2S2 | 0.028 | 0.188 | 0.305 | 0.715 |
| G2/G2S | 0.004 | 0.113 | 0.153 | 0.585 |
| G2/G2S2 | 0.005 | 0.096 | 0.575 | 0.856 |
| G2S/G2S2 | 0.049 | 0.211 | 0.850 | 0.894 |
| NLFLN₂₀₇HSEN₂₁₁ATAK | | | | |
| G2G2 | 0.349 | 0.626 | 0.093 | 0.434 |
| G2G2S | 0.455 | 0.711 | 0.210 | 0.581 |
| G2G3 | 0.764 | 0.863 | 0.019 | 0.288 |
| G2G2S2 | 0.228 | 0.479 | 0.593 | 0.861 |
| G2G3S | 0.133 | 0.368 | 0.209 | 0.608 |
| G2G4 | 1.000 | 1.017 | 0.042 | 0.423 |
| G2G3FS | 0.412 | 0.680 | 0.112 | 0.456 |
| G2G2S3 | 0.531 | 0.705 | 0.568 | 0.888 |
| G2G3S2 | 0.089 | 0.300 | 0.328 | 0.689 |
| G2G4S | 0.414 | 0.664 | 0.188 | 0.572 |
| G2G3FS2 | 0.092 | 0.295 | 0.001 | 0.035 |
| G2G2S4 | 0.486 | 0.722 | 0.391 | 0.746 |
| G2G3S3 | 0.179 | 0.455 | 0.867 | 0.881 |
| G2G4S2 | 0.958 | 1.025 | 0.370 | 0.728 |
| G2G3FS3 | 0.872 | 0.967 | 0.176 | 0.564 |
| G2G3S4 | 0.610 | 0.715 | 0.811 | 0.900 |
| G2G4S3 | 0.048 | 0.224 | 0.064 | 0.392 |
| G2G2/G2G2S2 | 0.009 | 0.107 | 0.820 | 0.893 |
| G2G2S2/G2G2S3 | 0.050 | 0.204 | 0.484 | 0.819 |
| G2G2/G2G2S | 0.036 | 0.200 | 0.613 | 0.814 |
| G2G2/G2G2S3 | 0.206 | 0.465 | 0.707 | 0.845 |
| G2G2/G2G2S4 | 0.309 | 0.589 | 0.659 | 0.856 |
| G2G3/G2G3S | 0.599 | 0.730 | 0.820 | 0.878 |
| G2G3/G2G3S2 | 0.037 | 0.189 | 0.367 | 0.746 |
| G2G3/G2G3S3 | 0.515 | 0.698 | 0.780 | 0.898 |
| G2G3/G2G3S4 | 0.554 | 0.704 | 0.042 | 0.363 |
| G2G3S/G2G3S2 | 0.498 | 0.723 | 0.855 | 0.884 |
| G2G3S/G2G3S3 | 0.285 | 0.580 | 0.166 | 0.597 |

| | | | | |
|-----------------|--------------|-------|--------------|-------|
| G2G3S/G2G3S4 | 0.130 | 0.378 | 0.233 | 0.618 |
| G2G3S2/G2G3S3 | 0.189 | 0.443 | 0.448 | 0.805 |
| G2G3S2/G2G3S4 | 0.183 | 0.448 | 0.700 | 0.853 |
| G2G3S3/G2G3S4 | 0.168 | 0.445 | 0.400 | 0.739 |
| G2G2S/G2G2S2 | 0.966 | 0.999 | 0.171 | 0.579 |
| G2G2S/G2G2S3 | 0.208 | 0.453 | 0.272 | 0.692 |
| G2G2S/G2G2S4 | 0.109 | 0.333 | 0.713 | 0.837 |
| G2G2S2/G2G2S4 | 0.003 | 0.186 | 0.603 | 0.836 |
| G2G2S3/G2G2S4 | 0.053 | 0.204 | 0.096 | 0.417 |
| G2G3FS/G2G3FS2 | 0.009 | 0.081 | 0.941 | 0.941 |
| G2G3FS/G2G3FS3 | 0.079 | 0.284 | 0.601 | 0.853 |
| G2G3FS2/G2G3FS3 | 0.499 | 0.708 | 0.456 | 0.795 |
| G2G4/G2G4S | 0.564 | 0.702 | 0.016 | 0.320 |
| G2G4/G2G4S2 | 1.000 | 1.000 | 0.044 | 0.297 |
| G2G4/G2G4S3 | 0.380 | 0.662 | 0.025 | 0.307 |
| G2G4S/G2G4S2 | 0.759 | 0.874 | 0.487 | 0.803 |
| G2G4S/G2G4S3 | 0.553 | 0.717 | 0.305 | 0.689 |
| G2G4S2/G2G4S3 | 0.505 | 0.700 | 0.611 | 0.829 |
| G2G3FS/G2G3S | 0.009 | 0.089 | 0.571 | 0.871 |
| G2G3FS2/G2G3S2 | 0.408 | 0.691 | 0.067 | 0.374 |
| G2G3FS3/G2G3S3 | 0.299 | 0.588 | 0.015 | 0.471 |
| S1 | 0.602 | 0.720 | 0.666 | 0.846 |
| S2 | 0.009 | 0.131 | 0.314 | 0.685 |
| S3 | 0.457 | 0.696 | 0.526 | 0.844 |
| S4 | 0.909 | 0.990 | 0.697 | 0.868 |
| S | 0.034 | 0.210 | 0.088 | 0.448 |
| F | 0.346 | 0.639 | 0.043 | 0.324 |

A *p* value of <0.05 was considered to be statistically significant.

BLDs patients:

pattern a: sex(M/F), 24/24; age(mean, range), 55.0,30-70 (pulmonary sarcoidosis (n=32), pneumonia (n=8), and interstitial lung disease(n=8)).

pattern b: sex(M/F):24/25, age(mean, range):56.9,31-73 (pulmonary sarcoidosis (n=33), pneumonia(n=7), and interstitial lung disease(n=9)).

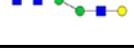
NSCLC patients:

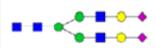
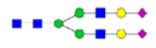
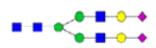
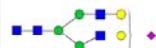
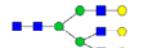
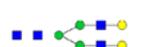
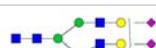
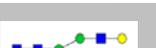
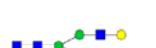
pattern a: sex(M/F), 73/74; age(mean, range), 57.3,33-77.

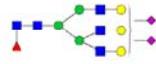
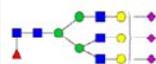
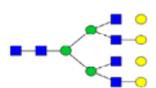
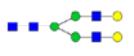
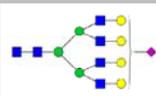
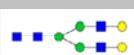
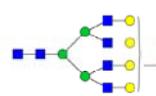
Pattern b: sex(M/F), 54/56; age(mean, range), 58.1,35-74.

M: male; F: female.

Table S3. The detected N-glycopeptides of DSHp- β and their potential glycan structures

| Glycopeptides | Experimental <i>m/z</i> | Theoretical <i>m/z</i> | Error (Da) | Accuracy (ppm) | potential glycan structure/mass | Glycan formula |
|--|----------------------------|---------------------------|---------------|-------------------|---|--|
| VVLHPN₂₄₁YSQVDIGLIK | | | | | | |
| G2 | 3417.5018 | 3418.6958 | -1.194 | -349.26 |  1640.5922 Da | (Hex) ₂ (HexNAc) ₂ + (Man) ₃ (GlcNAc) ₂ |
| G2S | 3708.6629 | 3709.7912 | -1.1283 | -304.14 |  1931.6875 Da | (Hex) ₂ (HexNAc) ₂ (NeuAc) ₁ + (Man) ₃ (GlcNAc) ₂ |
| G2S2 | 3999.8862 | 4000.8866 | -1.0004 | -250.04 |  2222.7829 Da | (Hex) ₂ (HexNAc) ₂ (NeuAc) ₂ + (Man) ₃ (GlcNAc) ₂ |
| NLFLN₂₀₇HSEN₂₁₁ATAK | | | | | | |
| G2G2 | 4704.0852 | 4704.7606 | -0.6754 | -143.56 |   1640.5922 Da | [(Hex) ₂ (HexNAc) ₂ +(Man) ₃ (GlcNAc) ₂] + [(Hex) ₂ (HexNAc) ₂ +(Man) ₃ (GlcNAc) ₂] |
| G2G2S | 4995.8742 | 4995.856 | 0.0182 | 3.64 |   1640.5922 Da | [(Hex) ₂ (HexNAc) ₂ (NeuAc) ₁ +(Man) ₃ (GlcNAc) ₂] + [(Hex) ₂ (HexNAc) ₂ +(Man) ₃ (GlcNAc) ₂] |
| G2G2S2 | 5287.2131 | 5286.9514 | 0.2617 | 49.50 |   1640.5922 Da | [(Hex) ₂ (HexNAc) ₂ (NeuAc) ₂ +(Man) ₃ (GlcNAc) ₂] + [(Hex) ₂ (HexNAc) ₂ +(Man) ₃ (GlcNAc) ₂] |

| | | | | | | | |
|---------------|-----------|-----------|--------|--------|---|--------------|---|
| G2G2S3 | 5578.4733 | 5578.0468 | 0.4265 | 76.46 |  | 2222.7829 Da | [(Hex)2 (HexNAc)2 (NeuAc)2 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 (NeuAc)1 +(Man)3 (GlcNAc)2] |
| | | | | |  | 1931.6875 Da | |
| G2G2S4 | 5869.7643 | 5869.1422 | 0.6221 | 106.00 |  | 2222.7829 Da | [(Hex)2 (HexNAc)2 (NeuAc)2 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 (NeuAc)2 +(Man)3 (GlcNAc)2] |
| | | | | |  | 2222.7829 Da | |
| G2G3S | 5361.2831 | 5360.9882 | 0.2949 | 55.01 |  | 2296.8198 Da | [(Hex)3 (HexNAc)3 (NeuAc)1 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 +(Man)3 (GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |
| G2G3 | 5070.9386 | 5069.8928 | 1.0458 | 206.28 |  | 2005.7244 Da | [(Hex)3 (HexNAc)3 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 +(Man)3 (GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |
| G2G3S3 | 5943.8685 | 5943.179 | 0.6895 | 116.02 |  | 2879.0106 Da | [(Hex)3 (HexNAc)3 (NeuAc)3 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 +(Man)3 (GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |
| G2G3S4 | 6235.3923 | 6234.2744 | 1.1179 | 179.32 |  | 2879.0106 Da | [(Hex)3 (HexNAc)3 (NeuAc)3 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 (NeuAc)1+(Man)3 (GlcNAc)2] |
| | | | | |  | 1931.6875 Da | |
| G2G3FS | 5507.3909 | 5507.0461 | 0.3448 | 62.61 |  | 2442.8777Da | [(Hex)3 (HexNAc)3 (NeuAc)1 (Fuc)1 + (Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 + (Man)3(GlcNAc)2] |

| | | | | | | | |
|----------------|-----------|-----------|--------|--------|---|--------------|--|
| | | | | |  | 1640.5922 Da | |
| G2G3FS2 | 5798.4513 | 5798.1415 | 0.3098 | 53.43 |  | 2733.9731 Da | [(Hex)3 (HexNAc)3 (NeuAc)2 (Fuc)1 + (Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 + (Man)3(GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |
| G2G3FS3 | 6090.1032 | 6089.2369 | 0.8663 | 142.27 |  | 3025.0685 Da | [(Hex)3 (HexNAc)3 (NeuAc)3 (Fuc)1 + (Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 + (Man)3(GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |
| G2G4 | 5435.4077 | 5435.025 | 0.3827 | 70.41 |  | 2370.8566 Da | [(Hex)4 (HexNAc)4 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 +(Man)3 (GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |
| G2G4S | 5726.7593 | 5726.1204 | 0.6389 | 111.58 |  | 2661.9520 Da | [(Hex)4 (HexNAc)4 (NeuAc)1 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 +(Man)3 (GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |
| G2G4S2 | 6017.8359 | 6017.2158 | 0.6201 | 103.05 |  | 2953.0474 Da | [(Hex)4 (HexNAc)4 (NeuAc)2 +(Man)3 (GlcNAc)2] + [(Hex)2 (HexNAc)2 +(Man)3 (GlcNAc)2] |
| | | | | |  | 1640.5922 Da | |

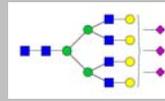
G2G4S3

6310.4691

6308.3112

2.1579

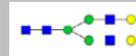
342.07



3244.1428 Da

[(Hex)4 (HexNAc)4 (NeuAc)3 +(Man)3 (GlcNAc)2] +

[(Hex)2 (HexNAc)2 +(Man)3 (GlcNAc)2]



1640.5922 Da

Table S4. The relative standard deviations (RSDs) of 9 DSHp- β N-glycopeptides of the QC sample as external reference.

| Glycosylation site | Asn241 | | Asn207/211 | | | | | | |
|---------------------------|---------------|-----------|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Glycopeptide | G2S2 | G2G2S | G2G2S2 | G2G2S3 | G2G3S | G2G3S2 | G2G3S3 | G2G4S2 | G2G4S3 |
| <i>m/z</i> | 3999.8862 | 4995.8742 | 5287.2131 | 5578.4733 | 5361.2831 | 5652.6081 | 5943.8685 | 6017.8359 | 6310.4691 |
| RSD (%) | 19.46 | 19.92 | 15.41 | 18.28 | 19.5 | 16.58 | 16.53 | 19.49 | 19.53 |

Table S5. Comparison of the N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features of DSHp- β between three different pathological states of BLDs patients with patterns a, b, d, e, f, and g.

| N-glycopeptides, N-glycopeptide ratios and N-glycosylation features | <i>p</i> value |
|--|-----------------------|
| VVLHPN₂₄₁YSQVDIGLIK | |
| G2 | 0.892 |
| G2S | 0.973 |
| G2S2 | 0.340 |
| G2/G2S | 0.973 |
| G2/G2S2 | 0.943 |
| G2S/G2S2 | 0.999 |
| NLFLN₂₀₇HSEN₂₁₁ATAK | |
| G2G2 | 0.698 |
| G2G2S | 0.143 |
| G2G3 | 0.710 |
| G2G2S2 | 0.858 |
| G2G3S | 0.487 |
| G2G4 | 0.644 |
| G2G3FS | 0.102 |
| G2G2S3 | 0.871 |
| G2G3S2 | 0.355 |
| G2G4S | 0.814 |
| G2G3FS2 | 0.361 |
| G2G2S4 | 0.859 |
| G2G3S3 | 0.789 |
| G2G4S2 | 0.755 |
| G2G3FS3 | 0.460 |
| G2G3S4 | 0.161 |
| G2G4S3 | 0.239 |
| G2G2/G2G2S2 | 0.313 |
| G2G2S2/G2G2S3 | 0.060 |
| G2G2/G2G2S | 0.821 |
| G2G2/G2G2S3 | 0.120 |
| G2G2/G2G2S4 | 0.186 |
| G2G3/G2G3S | 0.248 |
| G2G3/G2G3S2 | 0.802 |
| G2G3/G2G3S3 | 0.928 |
| G2G3/G2G3S4 | 0.540 |
| G2G3S/G2G3S2 | 0.729 |
| G2G3S/G2G3S3 | 0.370 |
| G2G3S/G2G3S4 | 0.745 |
| G2G3S2/G2G3S3 | 0.138 |

| N-glycopeptides, N-glycopeptide ratios and N-glycosylation features | <i>p</i> value |
|--|-----------------------|
| G2G3S2/G2G3S4 | 0.242 |
| G2G3S3/G2G3S4 | 0.252 |
| G2G2S/G2G2S2 | 0.285 |
| G2G2S/G2G2S3 | 0.142 |
| G2G2S/G2G2S4 | 0.431 |
| G2G2S2/G2G2S4 | 0.458 |
| G2G2S3/G2G2S4 | 0.933 |
| G2G3FS/G2G3FS2 | 0.101 |
| G2G3FS/G2G3FS3 | 0.206 |
| G2G3FS2/G2G3FS3 | 0.981 |
| G2G4/G2G4S | 0.222 |
| G2G4/G2G4S2 | 0.258 |
| G2G4/G2G4S3 | 0.936 |
| G2G4S/G2G4S2 | 0.980 |
| G2G4S/G2G4S3 | 0.690 |
| G2G4S2/G2G4S3 | 0.876 |
| G2G3FS/G2G3S | 0.959 |
| G2G3FS2/G2G3S2 | 0.251 |
| G2G3FS3/G2G3S3 | 0.169 |
| S1 | 0.508 |
| S2 | 0.719 |
| S3 | 0.856 |
| S4 | 0.250 |
| S | 0.671 |
| F | 0.650 |

Three different pathological states of BLDs:

pulmonary sarcoidosis, sex (M/F): 76/85, age (mean, range):56.4, 31-80;

Pneumonia: sex (M/F):16/19, age (mean, range):56.6, 37-77;

Interstitial lung disease: sex (M/F):21/19, age (mean, range):58.8, 30-74.

Statistical analysis was performed by Kruskal-Wallis test ($\alpha=0.05$).

M: male; F: female.

Table S6. Comparison of N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features of DSHp- β between different staging of NSCLC patients.

| N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features | <i>p</i> value | <i>p</i>_{adj} value |
|---|-----------------------|-------------------------------------|
| VVLHPN₂₄₁YSQVDIGLIK | | |
| G2 | 0.910 | 0.994 |
| G2S | 0.942 | 0.992 |
| G2S2 | 0.114 | 1.677 |
| G2/G2S | 0.533 | 0.767 |
| G2/G2S2 | 0.421 | 0.856 |
| G2S/G2S2 | 0.708 | 0.853 |
| NLFLN₂₀₇HSEN₂₁₁ATAK | | |
| G2G2 | 0.934 | 1.002 |
| G2G2S | 0.191 | 1.409 |
| G2G2S2 | 0.294 | 1.444 |
| G2G2S3 | 0.517 | 0.762 |
| G2G2S4 | 0.727 | 0.858 |
| G2G3 | 0.692 | 0.851 |
| G2G3S | 0.322 | 1.056 |
| G2G3S2 | 0.003 | 0.183 |
| G2G3S3 | 0.267 | 1.431 |
| G2G3S4 | 0.435 | 0.754 |
| G2G4 | 0.168 | 1.649 |
| G2G4S | 0.229 | 1.351 |
| G2G4S2 | 0.318 | 1.250 |
| G2G4S3 | 0.430 | 0.818 |
| G2G3FS | 0.646 | 0.847 |
| G2G3FS2 | 0.443 | 0.688 |
| G2G3FS3 | 0.322 | 1.186 |
| G2G2/G2G2S | 0.331 | 1.027 |
| G2G2/G2G2S2 | 0.635 | 0.851 |
| G2G2/G2G2S3 | 0.311 | 1.312 |
| G2G2S/G2G2S2 | 0.657 | 0.843 |
| G2G2S/G2G2S3 | 0.362 | 1.018 |
| G2G2S/G2G2S4 | 0.442 | 0.705 |
| G2G2S2/G2G2S3 | 0.302 | 1.368 |
| G2G2S2/G2G2S4 | 0.378 | 0.970 |
| G2G2S3/G2G2S4 | 0.091 | 1.796 |
| G2G3/G2G3S | 0.901 | 1.003 |
| G2G3/G2G3S2 | 0.405 | 0.920 |
| G2G3/G2G3S3 | 0.506 | 0.766 |
| G2G3/G2G3S4 | 0.439 | 0.739 |
| G2G3FS/G2G3FS2 | 0.751 | 0.869 |

| N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features | <i>p</i> value | <i>p_{adj}</i> value |
|---|-----------------------|-------------------------------------|
| G2G3FS/G2G3FS3 | 0.607 | 0.852 |
| G2G3FS/G2G3S | 0.633 | 0.868 |
| G2G3FS2/G2G3FS3 | 0.228 | 1.491 |
| G2G3FS2/G2G3S2 | 0.134 | 1.577 |
| G2G3FS3/G2G3S3 | 0.965 | 0.982 |
| G2G3S/G2G3S2 | 0.431 | 0.794 |
| G2G3S/G2G3S3 | 0.322 | 1.117 |
| G2G3S/G2G3S4 | 0.351 | 1.035 |
| G2G3S2/G2G3S3 | 0.071 | 2.080 |
| G2G3S2/G2G3S4 | 0.184 | 1.547 |
| G2G3S3/G2G3S4 | 0.402 | 0.948 |
| G2G4/G2G4S2 | 0.407 | 0.889 |
| G2G4/G2G4S3 | 0.368 | 0.987 |
| G2G4S/G2G4S2 | 0.678 | 0.851 |
| G2G4S/G2G4S3 | 0.407 | 0.857 |
| G2G4S2/G2G4S3 | 0.427 | 0.841 |
| S1 | 0.991 | 0.991 |
| S2 | 0.395 | 0.970 |
| S3 | 0.433 | 0.774 |
| S4 | 0.814 | 0.924 |
| S | 0.943 | 0.976 |
| F | 0.441 | 0.722 |

Stage I, sex (M/F):3/3, age (mean, range):62, 50-74;

Stage II, 0;

Stage III: sex (M/F): 6/6, age (mean, range):59.5,51-69;

Stage IV: sex (M/F):15/15, age (mean, range):58.5, 49-69.

Statistical analysis was performed by Kruskal-Wallis test ($\alpha=0.05$).

M: male; F: female.

Table S7. Comparison of N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features of DSHp- β with sex in BLDs or NSCLC patients.

| N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features | BLDs | | NSCLC | |
|--|----------------|------------------------------|----------------|------------------------------|
| | <i>p</i> value | <i>p_{adj}</i> value | <i>p</i> value | <i>p_{adj}</i> value |
| VVLHPN₂₄₁YSQVDIGLIK | | | | |
| G2 | 0.488 | 0.902 | 0.829 | 1.032 |
| G2S | 0.440 | 0.994 | 0.428 | 0.768 |
| G2S2 | 0.207 | 0.840 | 0.114 | 0.463 |
| G2/G2S | 0.344 | 0.954 | 0.228 | 0.662 |
| G2/G2S2 | 0.927 | 0.943 | 0.323 | 0.680 |
| G2S/G2S2 | 0.474 | 0.933 | 0.860 | 0.990 |
| NLFLN₂₀₇HSEN₂₁₁ATAK | | | | |
| G2G2 | 0.972 | 0.972 | 0.710 | 0.963 |
| G2G2S | 0.490 | 0.880 | 0.577 | 0.903 |
| G2G3 | 0.836 | 0.911 | 0.659 | 0.957 |
| G2G2S2 | 0.791 | 0.911 | 0.053 | 0.464 |
| G2G3S | 0.096 | 0.734 | 0.887 | 0.967 |
| G2G4 | 0.701 | 0.873 | 0.138 | 0.496 |
| G2G3FS | 0.791 | 0.928 | 0.231 | 0.639 |
| G2G2S3 | 0.678 | 0.919 | 0.137 | 0.523 |
| G2G3S2 | 0.230 | 0.780 | 0.851 | 1.018 |
| G2G4S | 0.064 | 0.645 | 0.891 | 0.954 |
| G2G3FS2 | 0.167 | 0.847 | 0.579 | 0.883 |
| G2G2S4 | 0.507 | 0.883 | 0.312 | 0.679 |
| G2G3S3 | 0.121 | 0.673 | 0.610 | 0.907 |
| G2G4S2 | 0.538 | 0.864 | 0.923 | 0.970 |
| G2G3FS3 | 0.027 | 0.404 | 0.025 | 0.508 |
| G2G3S4 | 0.483 | 0.921 | 0.280 | 0.682 |
| G2G4S3 | 0.913 | 0.944 | 0.923 | 0.954 |
| G2G2/G2G2S2 | 0.454 | 0.924 | 0.156 | 0.527 |
| G2G2S2/G2G2S3 | 0.831 | 0.922 | 0.727 | 0.965 |
| G2G2/G2G2S | 0.517 | 0.875 | 0.859 | 1.008 |
| G2G2/G2G2S3 | 0.704 | 0.859 | 0.028 | 0.420 |
| G2G2/G2G2S4 | 0.735 | 0.880 | 0.698 | 0.968 |
| G2G3/G2G3S | 0.358 | 0.909 | 0.570 | 0.914 |
| G2G3/G2G3S2 | 0.104 | 0.702 | 0.743 | 0.964 |
| G2G3/G2G3S3 | 0.454 | 0.954 | 0.404 | 0.771 |
| G2G3/G2G3S4 | 0.597 | 0.868 | 0.045 | 0.458 |
| G2G3S/G2G3S2 | 0.846 | 0.905 | 0.067 | 0.508 |
| G2G3S/G2G3S3 | 0.817 | 0.923 | 0.105 | 0.491 |

| | | | | |
|-----------------|------------------|--------------|--------------|-------|
| G2G3S/G2G3S4 | 0.211 | 0.806 | 0.218 | 0.664 |
| G2G3S2/G2G3S3 | 0.700 | 0.890 | 0.298 | 0.673 |
| G2G3S2/G2G3S4 | 0.581 | 0.887 | 0.676 | 0.959 |
| G2G3S3/G2G3S4 | 0.584 | 0.868 | 0.821 | 1.043 |
| G2G2S/G2G2S2 | 0.261 | 0.838 | 0.019 | 0.565 |
| G2G2S/G2G2S3 | 0.658 | 0.912 | 0.042 | 0.515 |
| G2G2S/G2G2S4 | 0.655 | 0.929 | 0.841 | 1.026 |
| G2G2S2/G2G2S4 | 0.859 | 0.903 | 0.472 | 0.800 |
| G2G2S3/G2G2S4 | 0.577 | 0.902 | 0.243 | 0.644 |
| G2G3FS/G2G3FS2 | 0.302 | 0.878 | 0.986 | 0.986 |
| G2G3FS/G2G3FS3 | 0.023 | 0.474 | 0.280 | 0.657 |
| G2G3FS2/G2G3FS3 | 0.347 | 0.921 | 0.190 | 0.608 |
| G2G4/G2G4S | 0.450 | 0.980 | 0.974 | 0.990 |
| G2G4/G2G4S2 | 0.688 | 0.912 | 0.500 | 0.824 |
| G2G4/G2G4S3 | 0.186 | 0.872 | 0.352 | 0.694 |
| G2G4S/G2G4S2 | 0.391 | 0.916 | 0.875 | 0.970 |
| G2G4S/G2G4S3 | 0.112 | 0.685 | 0.086 | 0.476 |
| G2G4S2/G2G4S3 | 0.389 | 0.949 | 0.071 | 0.433 |
| G2G3FS/G2G3S | 0.224 | 0.805 | 0.012 | 0.743 |
| G2G3FS2/G2G3S2 | 0.005 | 0.148 | 0.067 | 0.454 |
| G2G3FS3/G2G3S3 | <0.001 | 0.003 | 0.093 | 0.470 |
| S1 | 0.689 | 0.894 | 0.419 | 0.775 |
| S2 | 0.080 | 0.694 | 0.462 | 0.805 |
| S3 | 0.197 | 0.858 | 0.255 | 0.648 |
| S4 | 0.528 | 0.871 | 0.350 | 0.712 |
| S | 0.296 | 0.903 | 0.871 | 0.984 |
| F | 0.056 | 0.681 | 0.107 | 0.464 |

A $p(p_{adj})$ value of < 0.05 was considered to be statistically significant.

For BLDs patients:

Male (n=111), age (mean, range):57.7, 37-74; female (n=115), age (mean, range):57.5, 37-73.

For NSCLC patients:

Male (n=136), age (mean, range):57.9, 37-73; female (n=127), age (mean, range):57.0, 37-73.

Table S8. Comparison of the N-glycopeptides, N-glycopeptide ratios, or N-glycosylation features of DSHp- β with age in BLDs or NSCLC patients.

| N-glycopeptides, N-glycopeptide ratios, and N-glycosylation features | BLDs | | NSCLC | |
|---|----------------|-------------------------------|----------------|-------------------------------|
| | <i>p</i> value | <i>p</i> _{adj} value | <i>p</i> value | <i>p</i> _{adj} value |
| VVLHPN₂₄₁YSQVDIGLIK | | | | |
| G2 | 0.714 | 0.947 | 0.982 | 0.998 |
| G2S | 0.444 | 0.933 | 0.988 | 0.988 |
| G2S2 | 0.113 | 0.861 | 0.654 | 1.050 |
| G2/G2S | 0.211 | 1.070 | 0.962 | 1.030 |
| G2/G2S2 | 0.312 | 1.002 | 0.978 | 1.028 |
| G2S/G2S2 | 0.471 | 0.927 | 0.332 | 0.920 |
| NLFLN₂₀₇HSEN₂₁₁ATAK | | | | |
| G2G2 | 0.231 | 0.941 | 0.028 | 1.721 |
| G2G2S | 0.027 | 0.811 | 0.670 | 1.022 |
| G2G3 | 0.061 | 0.739 | 0.660 | 1.032 |
| G2G2S2 | 0.085 | 0.868 | 0.980 | 1.014 |
| G2G3S | 0.191 | 1.058 | 0.760 | 1.053 |
| G2G4 | 0.697 | 0.966 | 0.126 | 0.769 |
| G2G3FS | 0.365 | 1.011 | 0.509 | 1.036 |
| G2G2S3 | 0.274 | 0.985 | 0.142 | 0.722 |
| G2G3S2 | 0.431 | 0.973 | 0.790 | 1.048 |
| G2G4S | 0.793 | 0.987 | 0.896 | 1.012 |
| G2G3FS2 | 0.701 | 0.950 | 0.652 | 1.074 |
| G2G2S4 | 0.629 | 1.010 | 0.373 | 0.949 |
| G2G3S3 | 0.386 | 0.980 | 0.851 | 1.039 |
| G2G4S2 | 0.976 | 1.009 | 0.293 | 0.892 |
| G2G3FS3 | 0.855 | 1.023 | 0.931 | 1.032 |
| G2G3S4 | 0.361 | 1.048 | 0.112 | 0.850 |
| G2G4S3 | 0.620 | 1.022 | 0.210 | 0.754 |
| G2G2/G2G2S2 | 0.037 | 0.752 | 0.125 | 0.846 |
| G2G2S2/G2G2S3 | 0.975 | 1.025 | 0.083 | 1.271 |
| G2G2/G2G2S | 0.017 | 1.021 | 0.090 | 1.093 |
| G2G2/G2G2S3 | 0.524 | 0.969 | 0.204 | 0.889 |
| G2G2/G2G2S4 | 0.655 | 0.999 | 0.229 | 0.777 |
| G2G3/G2G3S | 0.598 | 1.042 | 0.599 | 1.044 |
| G2G3/G2G3S2 | 0.549 | 0.985 | 0.816 | 1.060 |
| G2G3/G2G3S3 | 0.680 | 0.965 | 0.627 | 1.062 |
| G2G3/G2G3S4 | 0.230 | 1.001 | 0.042 | 1.284 |
| G2G3S/G2G3S2 | 0.973 | 1.041 | 0.512 | 1.007 |
| G2G3S/G2G3S3 | 0.900 | 0.998 | 0.586 | 1.052 |
| G2G3S/G2G3S4 | 0.997 | 0.997 | 0.207 | 0.841 |

| | | | | |
|-----------------|-------|-------|-------|-------|
| G2G3S2/G2G3S3 | 0.992 | 1.009 | 0.375 | 0.915 |
| G2G3S2/G2G3S4 | 0.916 | 0.998 | 0.097 | 0.982 |
| G2G3S3/G2G3S4 | 0.892 | 1.007 | 0.058 | 1.178 |
| G2G2S/G2G2S2 | 0.486 | 0.927 | 0.888 | 1.022 |
| G2G2S/G2G2S3 | 0.666 | 0.991 | 0.128 | 0.712 |
| G2G2S/G2G2S4 | 0.218 | 1.025 | 0.501 | 1.053 |
| G2G2S2/G2G2S4 | 0.715 | 0.928 | 0.188 | 0.881 |
| G2G2S3/G2G2S4 | 0.311 | 1.054 | 0.460 | 1.002 |
| G2G3FS/G2G3FS2 | 0.811 | 0.989 | 0.459 | 1.037 |
| G2G3FS/G2G3FS3 | 0.670 | 0.974 | 0.541 | 0.999 |
| G2G3FS2/G2G3FS3 | 0.455 | 0.924 | 0.837 | 1.064 |
| G2G4/G2G4S | 0.431 | 0.939 | 0.737 | 1.046 |
| G2G4/G2G4S2 | 0.606 | 1.026 | 0.885 | 1.038 |
| G2G4/G2G4S3 | 0.864 | 1.014 | 0.316 | 0.917 |
| G2G4S/G2G4S2 | 0.881 | 1.014 | 0.111 | 0.968 |
| G2G4S/G2G4S3 | 0.648 | 1.014 | 0.781 | 1.059 |
| G2G4S2/G2G4S3 | 0.353 | 1.075 | 0.680 | 1.012 |
| G2G3FS/G2G3S | 0.128 | 0.864 | 0.531 | 1.011 |
| G2G3FS2/G2G3S2 | 0.131 | 0.800 | 0.856 | 1.024 |
| G2G3FS3/G2G3S3 | 0.378 | 1.003 | 0.208 | 0.793 |
| S1 | 0.421 | 0.987 | 0.248 | 0.795 |
| S2 | 0.060 | 0.917 | 0.716 | 1.039 |
| S3 | 0.102 | 0.891 | 0.932 | 1.015 |
| S4 | 0.418 | 1.021 | 0.406 | 0.951 |
| S | 0.240 | 0.915 | 0.846 | 1.053 |
| F | 0.738 | 0.938 | 0.369 | 0.978 |

BLDs, n=236; NSCLC, n=272.

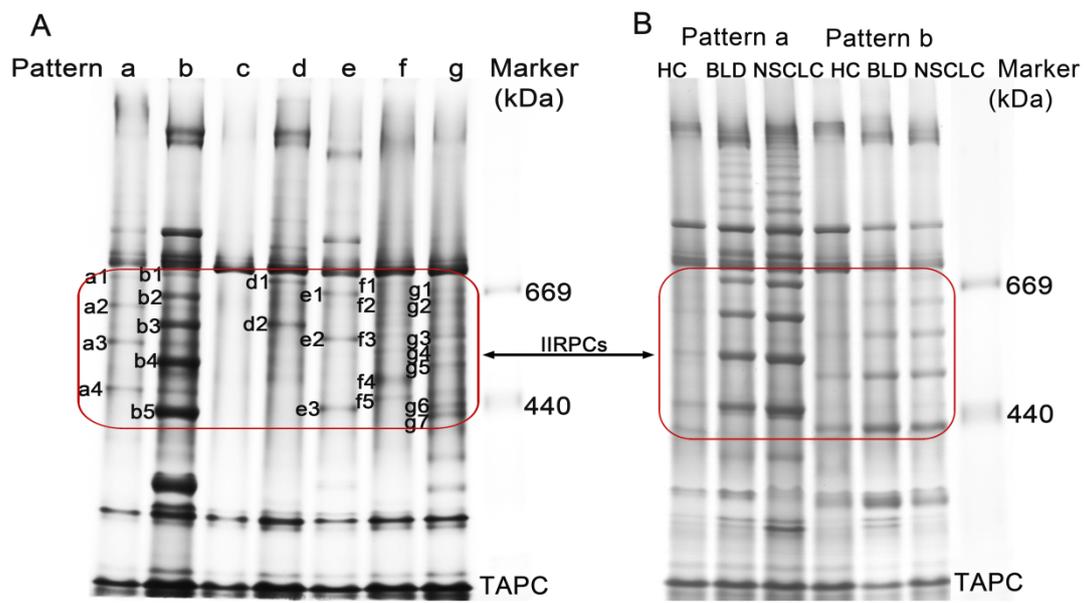


Figure S1. Serum IIRPCs isolated by native-PAGE. HC, healthy control; IIRPCs, immunoinflammation-related protein complexes; TAPC, transferrin-related protein complex.

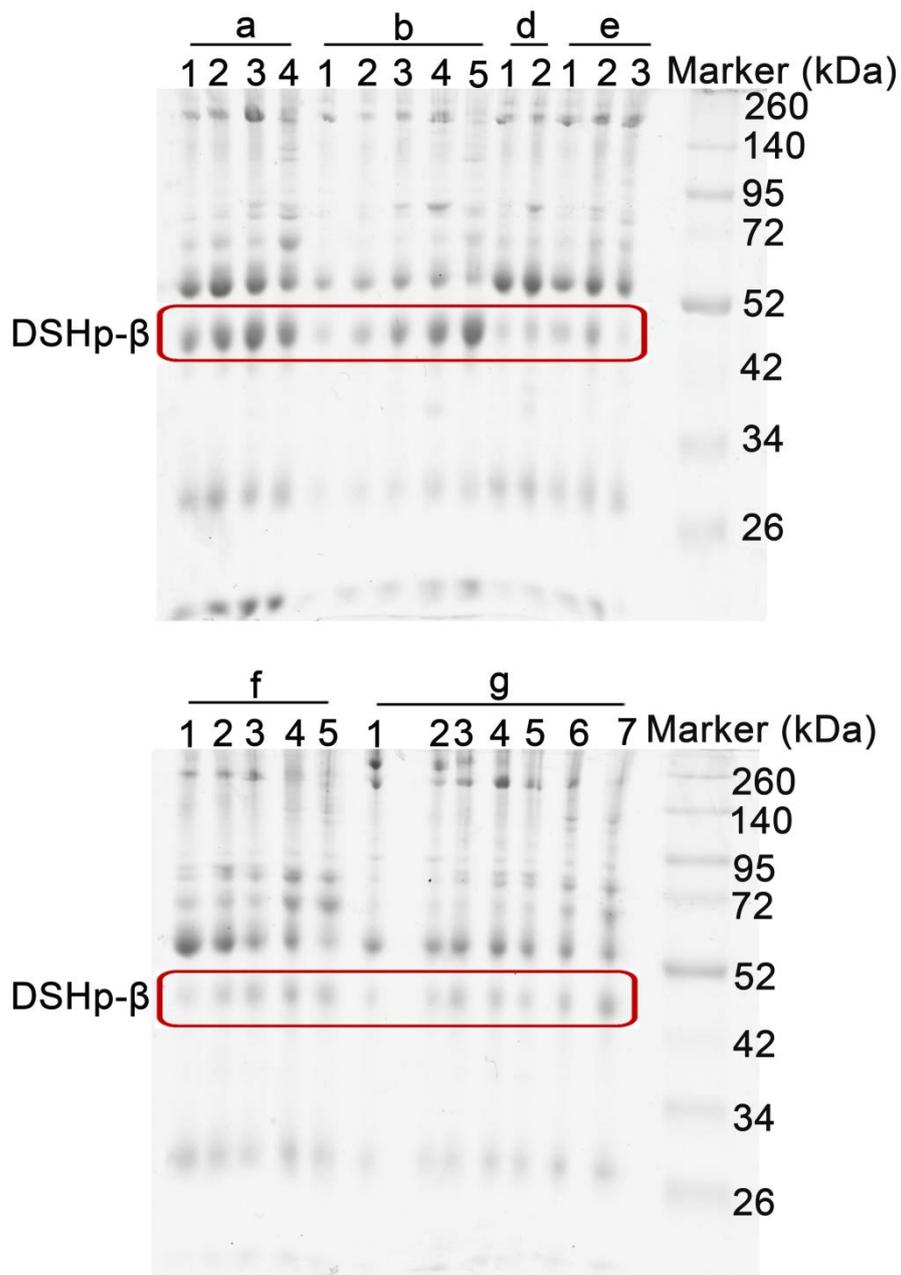


Figure S2. Isolation of DSHp- β from serum IIRPCs by SDS-PAGE. DSHp- β , disease specific Hp- β .

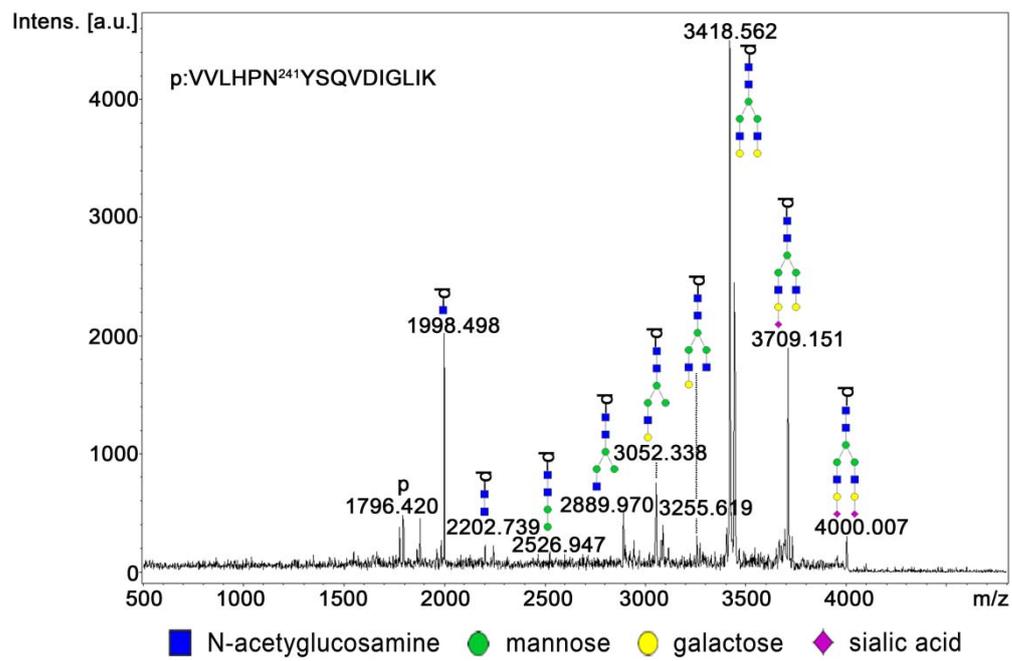


Figure S3. Tandem mass spectrum of G2S2 at m/z 4000.007 obtained using an UltrafleXtreme MALDI-TOF/TOF mass spectrometry.