

Development of Serum Glycoproteomic Profiling Technique; Simultaneous Identification of Glycosylation Sites and Site-Specific Quantification of Glycan Structure Changes*[§]

Koji Ueda*, Sachiko Takami†‡, Naomi Saichi†‡, Yataro Daigo‡, Nobuhisa Ishikawa§, Nobuoki Kohno§, Masaru Katsumata¶, Akio Yamane¶, Minoru Ota¶, Taka-Aki Sato||, Yusuke Nakamura‡, and Hidewaki Nakagawa*

Characterization and interpretation of disease-associated alterations of protein glycosylation are the central aims of the emerging glycoproteomics projects, which are expected to lead to more sensitive and specific diagnosis and improve therapeutic outcomes for various diseases. Here we report a new approach to identify carbohydrate-targeting serum biomarkers, termed isotopic glycosidase elution and labeling on lectin-column chromatography (IGEL). This technology is based on glycan structure-specific enrichment of glycopeptides by lectin-column chromatography and site-directed tagging of N-glycosylation sites by ¹⁸O during the elution with N-glycosidase. The combination of IGEL with 8-plex isobaric tag for relative and absolute quantitation (iTRAQ) stable isotope labeling enabled us not only to identify N-glycosylation sites effectively but also to compare glycan structures on each glycosylation site quantitatively in a single LC/MS/MS analysis. We applied this method to eight sera from lung cancer patients and controls, and finally identified 107 glycopeptides in their sera, including A2GL_Asn151, A2GL_Asn290, CD14_Asn132, CO8A_Asn417, C163A_Asn64, TIMP1_Asn30, and TSP1_Asn1049 which showed the significant change of the affinity to Concanavalin A (ConA) lectin between the lung cancer samples and the controls ($p < 0.05$ and more than twofold change). These screening results were further confirmed by the conventional lectin-column chromatography and immunoblot analysis using additional serum samples. Our novel methodology, which should be valuable for diverse biomarker discoveries, can provide high-throughput and quantitative profiling of glycan structure alterations. *Molecular & Cellular Proteomics* 9:1819–1828, 2010.

Glycan structure variations often show highly organ-specific manners (1, 2), as well as those manners that correlate with diverse disease states, (3, 4) e.g., cancer and inflammation. Thus, the carbohydrates are currently attracting a great deal of attention as specific targets of cancer biomarkers and therapy (5). In fact, certain changes of glycan structures are already in clinical use as serum biomarkers, such as AFP-L3 (6), and glycosylation at the specific site of therapeutic antibody proved to be essential for its therapeutic effect (7). Advances in proteomic technologies and analysis have stimulated a great interest in application of MS to identify glycosylation sites (8, 9) or analyze glycan structures (10, 11) from various biological specimens, but the comprehensive techniques which allow quantitative profiling of glycan structures on each glycosylation site have not been developed.

The two major issues facing recent glycoproteomic studies are the difficulties in glycopeptide-specific enrichment tools involving lectin-column chromatography and the detection of glycopeptides in mass spectrometers. In the conventional lectin-column chromatography experiments, glycoprotein enrichment from complicated protein mixtures, such as human sera, resulted in a heavy contamination of hapten sugar, salts, and nonspecific proteins caused by protein-protein interactions of serum proteins (12). Even when the digested peptide mixture was subjected to the lectin-column chromatography, salt contamination and the eluting sugar-dependent biases of elution efficiency were inevitable. Moreover, the straightforward analysis of the eluted glycopeptides by MS was hardly possible without further deglycosylation and desalting steps.

In this study we report our new approach for the identification of carbohydrate-targeting biomarkers, termed isotopic glycosidase elution and labeling on lectin-column chromatography (IGEL),¹ which is based on glycan structure-specific enrichment of glycopeptides by lectin-column chromatography and site-directed labeling of N-glycosylation sites by

From the *Laboratory for Biomarker Development, Center for Genomic Medicine, RIKEN, Tokyo, Japan; †CSK Institute for Sustainability, Ltd., Tokyo, Japan; ‡Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; §Department of Molecular and Internal Medicine, Hiroshima University, Hiroshima, Japan; ¶Toppan Printing Co., Ltd., Tokyo, Japan; and ||Shimadzu Corporation, Kyoto, Japan

Received, May 18, 2010

Published, MCP Papers in Press, June 1, 2010, DOI 10.1074/mcp.M110.000893

¹ The abbreviations used are: IGEL, isotopic glycosidase elution and labeling on lectin-column chromatography; iTRAQ, isobaric tag for relative and absolute quantitation; COPD, chronic obstructive pulmonary disease; Ig, immunoglobulin; SCX, strong cation exchange.

water- ^{18}O during the elution with N-glycosidase. We combined this method with 8-plex isotopic tag for relative and absolute quantitation (iTRAQ) labeling for relative quantification of glycopeptides and applied them to search for carbohydrate-targeting serum biomarkers in lung cancer patient sera.

EXPERIMENTAL PROCEDURES

Serum Samples—Archived human serum samples were obtained with informed consent from 12 patients who had lung adenocarcinoma and six patients who had chronic obstructive pulmonary disease (COPD) at Hiroshima University Hospital. Serum samples as normal controls were also obtained with informed consent from six healthy volunteers who received medical examinations at Hiroshima University Hospital. Serum was collected using standard protocol from whole blood by centrifugation at $1,500 \times g$ for 10 minutes and stored at -150°C . This study was approved by individual institutional ethical committees.

Removal of Serum Abundant Proteins—Fourteen abundant proteins in serum (albumin, immunoglobulinG [IgG], antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein A1, apolipoprotein AII, complement C3, and transthyretin) were removed from 40 μL of each serum sample using 4.6×100 mm Multiple Affinity Removal System (MARS) LC Column - Human 14 (Agilent Technologies, Santa Clara, CA) coupled with Prominence HPLC system (Shimadzu, Tokyo, Japan). The unbound fractions were directly loaded to 4.6×50 mm mRP-C18 column (Agilent) and desalted with the protocols recommended by the manufacturer. The eluted proteins were vacuum-dried and subjected to trypsin digestion.

Digestion of Serum Proteins—The dried proteins were resuspended in 50 μL of [8 mol/L Urea, 50 mmol/L ammonium bicarbonate (Sigma, St. Louis, MO)] and reduced with 10 mmol/L tris(2-carboxyethyl)phosphine (Sigma) at 37°C for 30 minutes and alkylated in 50 mmol/L iodoacetamide (Sigma) with 50 mmol/L ammonium bicarbonate for 45 minutes in the dark at 25°C . Porcine trypsin (Promega, Madison, WI) was added for a final enzyme to protein ratio of 1:20. The digestion was conducted at 37°C for 16 hours, followed by quenching the reaction with the addition of trifluoroacetic acid at the final concentration 0.4%. The digested peptides were then desalted with Oasis HLB cartridges (Waters, Milford, MA) according to the manufacturer's instructions except for the use of binding solution (0.1% trifluoroacetic acid with 2% acetonitrile) and elution solution (0.1% trifluoroacetic acid with 70% acetonitrile). The eluted peptides were vacuum-dried and subjected to 8-plex iTRAQ labeling.

8-plex iTRAQ Labeling—The dried peptides were resuspended in 30 μL of [500 mmol/L triethylammonium bicarbonate (Sigma), 50% acetonitrile, pH 8.5] and combined with a vial of 8-plex iTRAQ reagent (Applied Biosystems, Foster City, CA) that was reconstructed with 70 μL of ethanol. After incubation for two hours with gentle shaking at ambient temperature, eight samples were mixed and vacuum-dried.

Capturing Glycopeptides and Glycosylation Site-Directed Stable Isotope Labeling in the Lectin-Column Chromatography—The iTRAQ-labeled peptides were resuspended in 500 μL of [100 mmol/L ammonium bicarbonate, 10% acetonitrile] and applied to the 1 mL polypropylene column (Qiagen, Valencia, CA) packed with 500 μL of ConA-agarose beads (Seikagaku Biobusiness Corporation, Tokyo, Japan). After 60 minutes of incubation with gentle rotation at ambient temperature, the column was washed with 1 mL of [100 mmol/L ammonium bicarbonate, 10% acetonitrile] five times and subsequently 500 μL of [100 mmol/L ammonium bicarbonate, 10% acetonitrile, 90% water (^{18}O 98%, Wako Pure Chemical Industries, Osaka, Japan)] three times. The column was then incubated with 5 units (5

μL) of N-glycosidase F (Roche Applied Science) in 500 μL of [100 mmol/L ammonium bicarbonate, 10% acetonitrile, 90% water (^{18}O 98%)] at 37°C for 12 hours. The eluate and additional five rinses with 500 μL of [100 mmol/L ammonium bicarbonate, 10% acetonitrile] were combined and vacuum-dried.

Strong Cation Exchange (SCX) Chromatography—The peptides were resuspended in 20 μL of [0.1% trifluoroacetic acid, 70% acetonitrile] and loaded to 0.2×200 mm MonoCap SCX column (GL Science, Tokyo, Japan) with the use of solvent A [10 mmol/L ammonium formate, 25% acetonitrile] and solvent B [1 mol/L ammonium formate, 25% acetonitrile] at a flow rate of 5 $\mu\text{L}/\text{min}$. During the multistep linear gradient of solvent B 0 to 15% for 15 minutes and 15 to 100% for 5 minutes, the eluted fraction was separately collected each minute, followed by vacuum-drying.

LC/MS/MS Analysis and the Peptide Identification—The fractions of SCX chromatography were individually analyzed with QSTAR Elite MS (Applied Biosystems) combined with UltiMate 3000 nano-flow HPLC system (DIONEX Corporation, Sunnyvale, CA). The peptides were separated on $75 \mu\text{m} \times 150$ mm L-Column (Chemicals Evaluation and Research Institute) using solvent A [0.1% formic acid, 2% acetonitrile] and solvent B [0.1% formic acid, 70% acetonitrile] with the multistep linear gradient of solvent B 5 to 45% for 65 minutes and 45 to 95% for 10 minutes at a flow rate 200 nL/min. QSTAR Elite mass spectrometer was used in standard MS/MS data-dependent acquisition mode. The 1 s survey MS spectra were collected (m/z 400–1800) followed by three MS/MS measurements on the most intense parent ions (30 counts/sec threshold, +2–+4 charge state, m/z 500–2000 mass range for MS/MS), using the manufacturer's "smart exit" setting (SIDA = 3.0). Previously targeted parent ions were excluded from repetitive MS/MS acquisition for 40 seconds (100 mDa mass tolerance). The reporter region was enhanced and collision energy was adjusted by the manufacturer's setting "Using iTRAQ Reagent".

Identification of Glycosylation Sites—ProteinPilot 2.0 software (Applied Biosystems) with the Paragon Algorithm was used for the identification and relative abundance quantification of proteins. Tandem MS data were searched against the human protein database from SwissProt 57.4 (20,400 sequences). The search parameters were trypsin as enzyme, carbamidomethylation at cysteines as fixed modification, and thorough IDAs search method. We report only peptide identifications with Conf. > 95 and protein identifications with ProtScore > 1.3, which represents > 95% statistical confidence in ProteinPilot. Particularly, we used protein identifications with more than two peptide identifications. Whereas common modifications such as oxidation of methionine and deamidation of asparagine were included automatically as variable modifications in Paragon Algorithm, we additionally entered a sentence in "ProteinPilot. DataDictionary.xml" file to define the IGEL-tag as follows;

```
<Mod rKey = "0">
<Nme>User modH on Asn</Nme>
<TLC>UNH</TLC>
<Tgt>Asparagine</Tgt>
<TS>255</TS>
<Fma>Ob</Fma>
<RpF>NH</RpF>
<NLF></NLF>
<IIF></IIF>
<Chg>0</Chg>
</Mod>
```

The peptide identification data including iTRAQ-based quantification results was exported into the XML-formatted file from ProteinPilot software. We manually excluded peptides with "low confidence", "no iTRAQ", "no quant", and "weak signal" and used peptides with the value "Used = 1" for the further quantification analysis. Furthermore, we accepted only the following modifications; iTRAQ8plex@N-term,

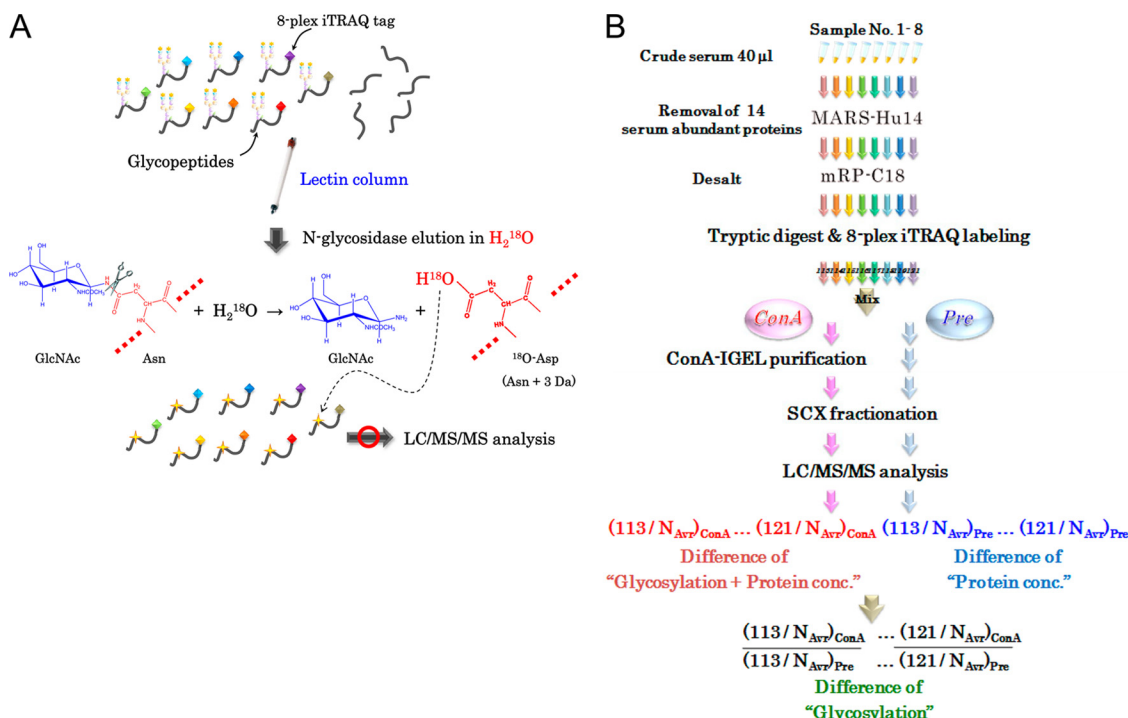


FIG. 1. Concept of IGEL technology and schematic representation of IGEL-based biomarker screening. (A) Tryptic digests from eight clinical samples were labeled with 8-plex iTRAQ tags and loaded to lectin-column chromatography. After replacement of the solvent with $H_2^{18}O$, glycopeptides which bind to lectin-column specifically by glycan-lectin interaction were eluted with N-glycosidase. As the eluate includes ^{18}O -tagged "peptides" without any involatile salts, it can be directly subjected to LC/MS/MS analysis and usual database search, which results in simultaneous identification of N-glycosylation sites and quantification of site-specific affinity changes to the lectin. (B) Following immuno-depletion of 14 abundant proteins, eight serum samples were processed with or without IGEL purification (termed ConA or Pre, respectively). The relative difference from the normal level (e.g., $113/N_{Avr}$) was used to compare the affinity variations to ConA lectin among eight samples (see Method for procedural details).

iTRAQ8plex(K), Carbamidomethyl(C), Oxidation(M), and User modH on Asn(N). After removing the redundancy and selecting the peptides with unique amino acid sequence and modifications, peptides possessing "User modH on Asn(N)" modification (described above) were extracted, which were expected to be derived from N-glycosylated peptides. Next, the existence of N-glycosylation consensus sequence N x S/T was checked.

Glycosylation Site-Specific Quantification Analysis—The peak area of eight reporter ions from each identified peptide was used for the following quantification analysis. We converted the absolute value of the area into the relative difference from the normal levels by dividing eight reporter values by the average of the area from three healthy controls labeled with iTRAQ-113, 114, and 115 (N_{Avr}). The ratios obtained from the sample with ConA lectin purification were described here as $[(113/N_{Avr})_{ConA}, \dots, (121/N_{Avr})_{ConA}]$. We further analyzed samples without ConA lectin purification to examine relative protein concentrations among eight samples, termed as $[(113/N_{Avr})_{Pre}, \dots, (121/N_{Avr})_{Pre}]$. These ratios for a typical protein were calculated from the average of all peptide quantification data assigned to that protein. Finally, comparative glycan structure changes among eight samples were calculated as follows.

$$\frac{(113/N_{Avr})_{ConA}}{(113/N_{Avr})_{Pre}} \dots \frac{(121/N_{Avr})_{ConA}}{(121/N_{Avr})_{Pre}} \quad (\text{Eq. 1})$$

ConA Spin Column Purification and Immunoblot Analysis—Twenty microliters each of 24 serum samples were purified with MARS Human-14 column and mRP-C18 column as described above. The immunodepleted protein samples were resuspended with 10 μ L of

[8 mmol/L Urea, 50 mmol/L Tris-HCl (8.0)] thoroughly and diluted with 90 μ L of the Binding/Wash Buffer (Glycoprotein Isolation Kit ConA, Thermo Scientific, Waltham, MA). The ConA-bound fractions were collected in accordance with the manufacturer's instructions, excepting three times repeated elution with 50 μ L of the elution buffer (Glycoprotein Isolation Kit ConA, Thermo Scientific). The 20 μ L aliquots of the eluate were run on 10% SDS-polyacrylamide gel and electroblotted onto a PVDF membrane. The blots were probed with anti-TIMP1 antibody (Abcam, Cambridge, MA). After incubation of the membrane with horseradish peroxidase-conjugated secondary antibody (GE Healthcare), the reactivity was visualized on x-ray films (Kodak) using a chemiluminescent detection kit (PerkinElmer Life Sciences).

RESULTS

Isotopic Glycosidase Elution and Labeling on Lectin-Column Chromatography (IGEL)—Our IGEL technology clearly resolved major problems for lectin-column chromatography and mass spectrometric analysis of glycosylated peptides. Since the eluted product of the IGEL method was completely deglycosylated peptides containing ^{18}O -tagged N-glycosylation sites dissolved in volatile solution, it was directly analyzable by MS (Fig. 1A). Although 1-Da mass shift by deamidation of asparagine residues (Asn to Asp) and glutamine residues (Gln to Glu) could be detected in mass spectrometric analysis, the 3-Da increase of the asparagine residue was

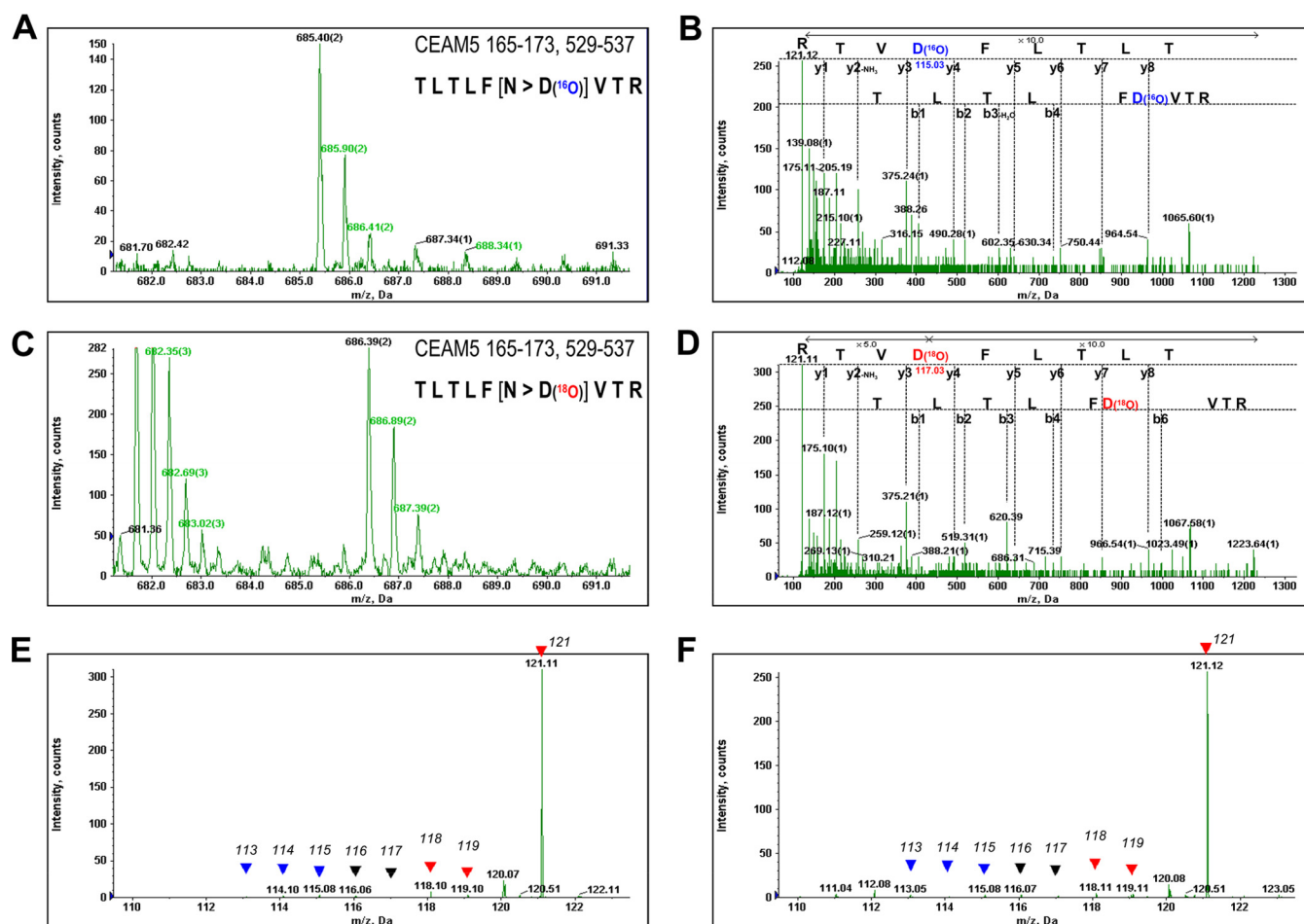


FIG. 2. Representative mass spectra of carcinoembryonic antigen-related cell adhesion molecule 5 (CEAM5, well known as CEA) peptides processed with IGEL. The MS spectra of CEAM5 165–173 or 521–529 peptides eluted with glycopeptidase-F in H_2^{16}O (A) or H_2^{18}O (C) were shown. The MS/MS spectrum of the same peptide in each condition and identified fragment ions were shown in (B) or (D), respectively. The reporter ions of 8-plex iTRAQ tags were magnified from the spectrum (B) or (D) and indicated by arrow heads in (E) or (F), respectively. Reporters from three healthy controls and three lung cancer samples were indicated with blue or red arrow heads.

more specific to the IGEL process, which enabled precise identification of glycosylation sites. Furthermore, the combination with 8-plex iTRAQ labeling of peptides diminished potential experimental errors of lectin-column chromatography and allowed quantitative comparison of glycan structures on serum proteins from eight individuals. To identify carbohydrate-targeting serum markers for lung cancer, we applied this IGEL method to eight human serum samples, consisting of sera from three healthy controls, two chronic obstructive pulmonary disease (COPD) patients, and three lung cancer patients (Fig. 1B, supplemental Table 1).

Because serum abundant proteins, particularly abundant glycosylated proteins, critically interfere with the association between serum minor components and lectins, the 14 most abundant serum proteins were removed from sera using the MARS Human 14 column before IGEL. In this step, 92.8% w/w of serum proteins on the average was immunodepleted (supplemental Fig. 1). The protein recovery rate of the desalting process by mRP- C_{18} column chromatography was 98%

(supplemental Fig. 2). After tryptic digestion, peptides were labeled with 8-plex iTRAQ stable isotope tags. We optimized the peptide labeling protocol by reacting one vial of 8-plex iTRAQ reagent to 25 μg of serum peptide mixture so that 94.2% of lysine residues and N-terminal amino acids of peptides were labeled by iTRAQ (supplemental Fig. 3). The vacuum-dried iTRAQ-tagged peptides were applied to the ConA lectin-column chromatography.

Evaluation of the Accuracy and Efficiency of the IGEL Tagging—Preliminarily, we evaluated the transduction efficiency of ^{18}O in the IGEL-purified peptides using QSTAR Elite LC/MS/MS system. The typical MS or MS/MS spectra of the identified peptide in our screening, $\text{T}_{165}\text{LTLFNVTR}_{173}$ or $\text{T}_{521}\text{LTLFNVTR}_{529}$ from the CEAM5 protein (well-known tumor marker CEA), were shown in Figure 2. The TLTFLNVTR sequence was present in the two locations in the CEAM5 protein. When PNGase F reaction was conducted in H_2^{16}O as a control experiment, CEAM5 165–173 or 521–529 peptides were detected at $m/z = 685.40$ with doubly charged state in

MS analysis (Fig. 2A). The MS/MS experiment and the subsequent database search showed an amino acid conversion of asparagine to aspartic acid in this sequence, resulting in 1-Da increase of the molecular weight (Fig. 2B), whereas a 3-Da increase of the same peptide ($m/z = 686.39$) was observed from the sample eluted in the presence of $H_2^{18}O$ by IGEL method (Figs. 2C and 2D). The peak of m/z 685.40 corresponding to the CEAM5 peptide with ^{16}O -incorporated asparagine residue was hardly detectable in Figure 2C, indicating that $H_2^{16}O$ contamination in PNGase F reaction with $H_2^{18}O$ could be almost negligible for the mass spectrometric analysis. Additionally, the MS/MS spectrum acquired from the precursor ion m/z 686.39 clearly showed the 3-Da increase of the asparagine residue (Fig. 2D). The iTRAQ reporter ions detected in the MS/MS spectrum of m/z 685.40 (Fig. 2E) and m/z 686.39 (Fig. 2F) indicated that CEAM5 165–173 or 521–529 peptide was dominantly detected in one lung cancer serum, which was labeled with iTRAQ-121. These data implicated that the relative abundance of eight reporter ions was not affected by the use of elution buffer containing $H_2^{18}O$, and the IGEL method could have a great potential for the reliable identification of glycosylation sites on even small quantity of proteins, such as serum CEA (ng/mL range). Moreover, the use of 8-plex iTRAQ tags enabled the relative quantification of glycan structures on each glycosylation site.

Quantitative Profiling of Lung Cancer Specific Glycosylations by IGEL—We applied this method to the wide screening for the cancer-associated glycan structure alterations in lung cancer patients' sera. When we undertake the quantification analysis of glycosylations, it is important to be aware of the fact that the quantification analysis using ConA-purified peptides can be reflected both by their affinity changes to the ConA lectin column and by the difference of their protein concentrations. To eliminate the effect of protein concentrations and accurately quantify the glycan structure alterations, we simultaneously performed LC/MS/MS analyses of iTRAQ-tagged peptides without ConA lectin-column purification (termed pre-ConA purified sample), as well as the same sample after ConA lectin-column purification (termed ConA-purified sample) (Fig. 1B). For the identification of glycopeptides from ConA-purified sample, 10,709 peptides were automatically identified with ProteinPilot software from 61,216 MS/MS analyses. By removing the redundancy and peptides with unreliable identification or very weak signals under the criteria described under "Experimental Procedures," we identified 127 unique peptides including 119 peptides possessing ^{18}O -incorporated asparagine residues (termed IGEL-tagged asparagine residues). The enrichment yield of glycopeptides using the ConA-IGEL procedure was 93.7%. Although ProteinPilot had great advantages in that the selection of variable modifications was completely automated and the peptide quantification were performed simultaneously with the peptide identification, the search results by ProteinPilot included a large number of improper modifications, such as

dehydration of serine + phosphorylation of threonine + de-thiomethylation of methionine on a peptide. Therefore, we excluded the unreliable modification data using the criteria described under "Experimental Procedures." Among the 119 peptides, all of the IGEL-tagged asparagine residues on 107 peptides coincided with the N-glycosylation consensus sequences "N x S/T (x is one of any amino-acids)," while the other 12 peptides had at least one IGEL-tagged asparagine residue that did not match the consensus sequences above (supplemental Table 2). Finally, 101 unique N-glycosylation sites were found on 119 glycopeptides, which were assigned to 56 serum proteins (supplemental Table 3). However, LC/MS/MS analysis on pre-ConA purified samples identified 274 serum proteins (supplemental Table 4), and 50 proteins among them were commonly observed in the list of the glycopeptides identified from ConA-purified samples, which were corresponding to 97 glycopeptides out of 119 glycopeptides that IGEL identified confidently from only ConA-purified samples. Although six proteins which were identified from only ConA-purified samples were not able to be applied to the quantification analysis because of the lack of the protein concentration information from pre-ConA lectin purified samples, they included a serum minor component integrin alpha-1 (> 3.4 ng/mL) (13) (supplemental Table 5). These findings indicated significant improvement of the detection limit of mass spectrometric analysis by the enrichment of IGEL method.

Next we quantified site-directed variations of the glycan structures on each of these 97 glycopeptides. For relative quantification using 8-plex iTRAQ tags, the area of the reporter ions derived from eight samples ($m/z = 113, 114, 115, 116, 117, 118, 119$, and 121) was divided by the averaged peak area (N_{Avr}) of three controls ($m/z = 113, 114$, and 115), and the comparative affinity variations to ConA lectin among eight samples were calculated as described under "Experimental Procedures" (supplemental Table 6). The distributions of the relative changes of the 97 identified glycopeptides among eight serum samples were plotted in Figure 3A. For instance, glycopeptides corresponding to CEAM5 165–173 and 521–529 showed 46.7-fold affinity change to ConA lectin in lung cancer patients' sera. To identify glycosylation sites on which the glycan structures varied in lung cancer-specific manner, we extracted peptides satisfying the following two criteria: (1) the affinity to ConA lectin showed more than twofold change in all three lung cancer patients' sera, compared with the normal level (N_{Avr}), and (2) showing the p value in the Student's t test between the control group (113, 114, 115, 116, and 117) and the lung cancer group (118, 119, and 121) was less than 0.05. This selection identified eight glycopeptides as candidates for serum glycan biomarkers of lung cancer (Fig. 3B and Table 1). Among them, six peptides showed more than twofold higher affinity to ConA lectin in all lung cancer sera compared with normal levels, whereas two peptides showed less than 0.5-fold lower affinity to ConA

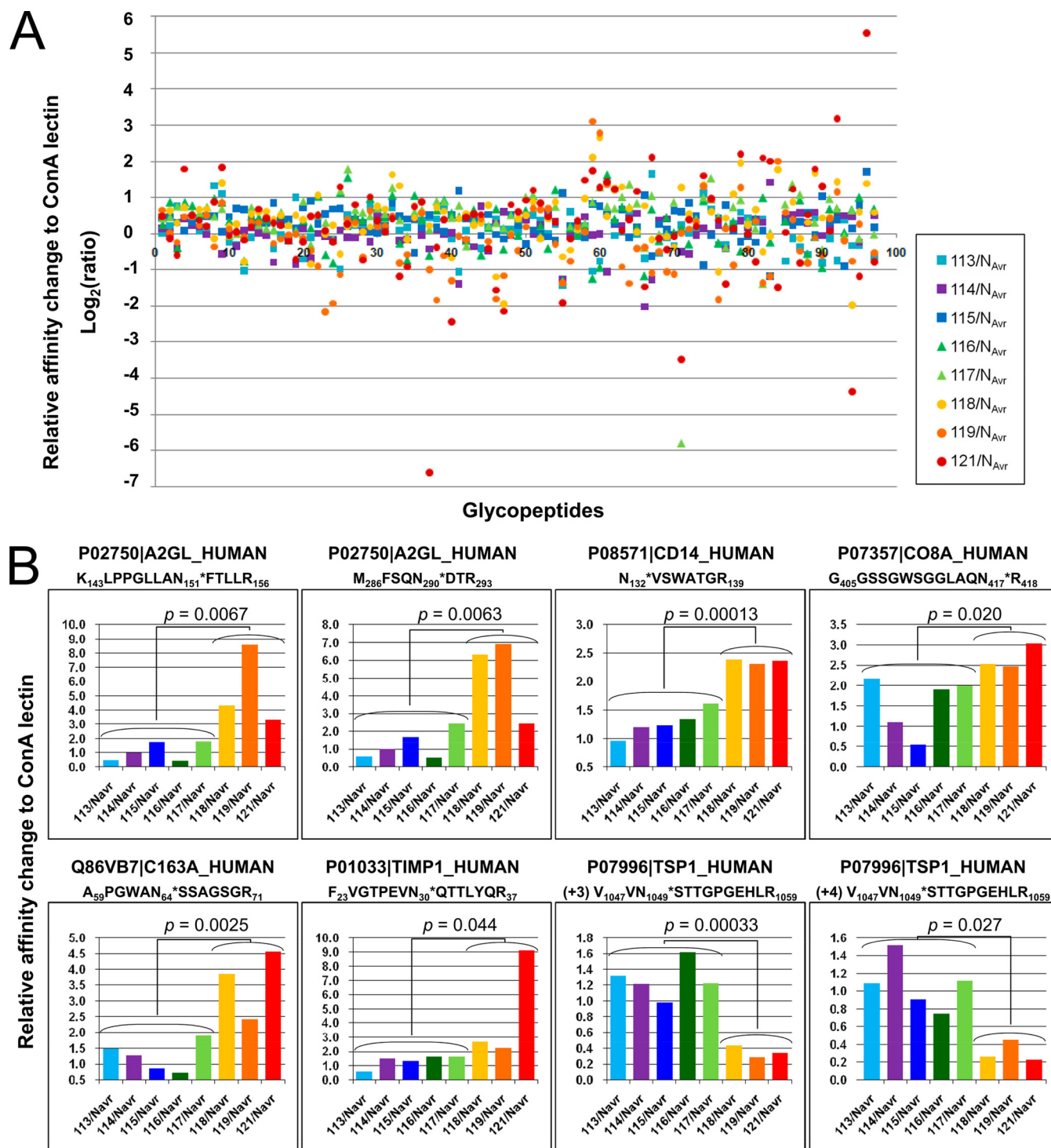


FIG. 3. IGEL-based screening of lung cancer-associated glycan structure alterations. A, Relative affinity variations to ConA lectin for 97 glycopeptides were plotted. The bold line, $\text{log}_2(\text{ratio}) = 0$, indicates the average of three normal controls (N_{Avr}) labeled with iTRAQ-113, 114, and 115. Detailed information of peptide sequences and ratios were available in [supplemental Table 6](#). Two COPD samples and three lung cancer samples were labeled with iTRAQ-116, 117 and iTRAQ-118, 119, 121, respectively. B, 8 glycopeptides whose affinity to ConA lectin showed more than twofold or less than 0.5-fold change in all three lung cancer patient samples compared with normal level (N_{Avr}) and $p < 0.05$ in the t test between the control group (113 to 117) and the lung cancer group (118, 119, and 121) were extracted from A and displayed in the bar charts. Accession number and the sequence of each peptide were shown. Identified N-glycosylation sites were indicated with asterisks. TSP1 1047–1059 peptide was observed at charge state +3 and +4.

TABLE I
List of 8 glycopeptides showing more than 2-fold affinity change to ConA lectin in lung cancer patients' sera

Accessions	m/z (z)	^a Conf.	^b Sequence	Modifications	^c 113/Navr	^c 114/Navr	^c 115/Navr	^c 116/Navr	^c 117/Navr	^c 118/Navr	^c 119/Navr	^c 121/Navr
P02750/A2GL_HUMAN	722.12 (+3)	99	K ₁₄₃ LPPLGLLAN ₁₅₁ *FTLLR ₁₅₆	iTRAQ8plex@N-term iTRAQ8plex(K)@1	0.487	0.977	1.771	0.430	1.818	4.298	8.601	3.339
P02750/A2GL_HUMAN	653.32 (+2)	98	M ₂₈₆ FSQN ₂₉₀ *DTR ₂₉₃	User modH on Asn(N)@9 iTRAQ8plex@N-term	0.588	0.996	1.686	0.530	2.433	6.324	6.919	2.442
P08571/CD14_HUMAN	599.32 (+2)	98	N ₁₃₂ *VSWATGR ₁₃₉	User modH on Asn(N)@5 iTRAQ8plex@N-term	0.964	1.200	1.227	1.338	1.611	2.396	2.305	2.358
P07357/CO8A_HUMAN	547.61 (+3)	99	G ₄₀₅ GSSGWSGGLAQN ₄₁₇ *R ₄₁₈	User modH on Asn(N)@1 iTRAQ8plex@N-term	2.173	1.101	0.544	1.903	1.996	2.523	2.470	3.033
Q86VB7/C163A_HUMAN	762.89 (+2)	99	A ₅₉ PGWAN ₆₄ *SSAGSGR ₇₁	User modH on Asn(N)@13 iTRAQ8plex@N-term	1.503	1.276	0.861	0.722	1.903	3.862	2.412	4.575
P01033/TIMP1_HUMAN	687.37 (+3)	99	F ₂₃ VGTPENV ₃₀ *QTLLYQR ₃₇	User modH on Asn(N)@6 iTRAQ8plex@N-term	0.601	1.492	1.316	1.614	1.626	2.695	2.238	9.126
P07996/TSP1_HUMAN	558.64 (+3)	99	V ₁₀₄₇ VN ₁₀₄₉ *STTGPGEHLR ₁₀₅₉	User modH on Asn(N)@8 iTRAQ8plex@N-term	1.318	1.214	0.982	1.623	1.225	0.440	0.287	0.339
P07996/TSP1_HUMAN	495.28 (+4)	98	V ₁₀₄₇ VN ₁₀₄₉ *STTGPGEHLR ₁₀₅₉	User modH on Asn(N)@3 iTRAQ8plex@N-term iTRAQ8plex(T)@5	1.088	1.517	0.904	0.748	1.113	0.260	0.449	0.226

^a The Confidence values in ProteinPilot software were shown. We set the peptide identification threshold as Conf. > 95. ^b The identified N-glycosylation sites were indicated by asterisks and their amino acid numbers were also shown. ^c Fold change values of the affinity to ConA lectin were calculated as relative differences from the average of three normal samples (N_{Navr}). The ratio of more than 2-fold or less than 0.5-fold change was highlighted in red or blue, respectively.

lectin in all lung cancer sera. We selected four glycopeptides, Monocyte differentiation antigen CD14 (CD14) 132–139, Scavenger receptor cysteine-rich type 1 protein M130 (C163A) 59–71, Metalloproteinase inhibitor 1 (TIMP1) 23–37 and Thrombospondin-1 (TSP1) 1047–1059, as potential carbohydrate-targeting tumor markers whose glycan structure changes were specific to three lung cancer patients' sera. The representative MS/MS spectra corresponding to the four candidates were shown in Figure 4A–4D. We found drastic changes in the relative intensities of each reporter ion between the samples before ConA lectin-column purification (Pre-ConA) and after purification (ConA-purified) for all of four peptides. Here, the relative difference of the reporter ion intensities in Pre-ConA samples indicates the difference of the identified protein concentration. By subtracting the protein concentration ratios (left side panels in Fig. 4) from the peptide quantification results of ConA-purified samples (right side panels in Fig. 4), accurate glycosylation status changes could be assessed. Thus, lung cancer[n dash]associated enhancement of the affinity to ConA lectin was observed for three glycopeptides CD14 132–139, C163A 59–71, and TIMP1 23–37, whereas significant attenuation was observed for TSP1 1047–1059 glycopeptide.

Validation for the Lung Cancer-Associated Aberrant Glycosylation on TIMP1—Because TIMP1 appeared to undergo remarkably upregulated modification of high-mannose[n dash]type oligosaccharides in the sera of advanced stage lung cancer patients (Fig. 3B), it warranted further examination in the increased number of human sera using a different experimental procedure. Therefore, sera from age- and sex-matched group of healthy volunteers ($n = 6$), COPD patients ($n = 6$), or lung cancer patients (stage-I or -II: $n = 6$, stage-IV: $n = 6$) were resolved by 1D gel electrophoresis, and the amount of TIMP1 in the sera was determined by immunoblotting, as described above. As Figure 5A shows, there was a minor difference of TIMP1 level detected in the immune-depleted sera between the control sera and the lung cancer patients' sera. However, TIMP1 was specifically enriched in the lung cancer patients' sera by ConA lectin column (Fig. 5B). Indeed, TIMP1 signals were detectable in one of the COPD samples, two of the stage-I or -II lung cancer samples, and all of the stage-IV lung cancer samples. Importantly, we included the same eight samples used in the iTRAQ-based screening (indicated by the number 113 to 121 in Fig. 5) for this validation study and found that the quantitative tendency of ConA-affinity among these eight samples was absolutely matched with the result of iTRAQ quantification (Fig. 3B). Both the IGEL-iTRAQ screening result and this validation experiment showed drastically enhanced affinity of TIMP1 to ConA lectin on the iTRAQ 121-labeled lung cancer sample. These integrated results strongly suggested that TIMP1 might undergo aberrant high-mannose[n dash]-type glycosylations in advanced stage lung cancer patients' sera. Although the sample scale was still small, the results

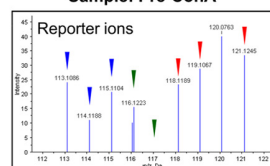
A

Peptide ID : P08571|CD14

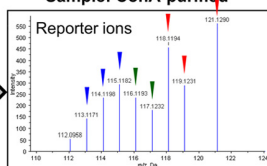
a.a.202-211

Precursor : m/z = 482.96 (+3)

Sample: Pre-ConA



Sample: ConA-purified



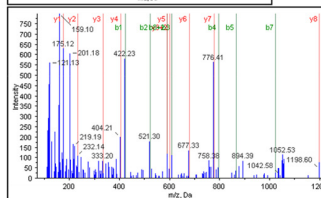
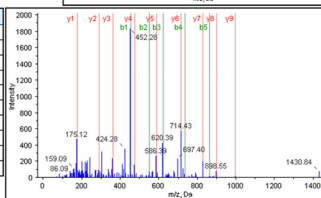
Peptide ID : P08571|CD14

a.a.132-139

Glycosylation : Asn132

Precursor : m/z = 599.32 (+3)

Residue	b	b-2	y	y-2
P	462.2819	226.6442	1446.8734	723.9403
V	549.3338	275.1705	995.5996	498.3035
A	628.3769	310.6891	998.5483	449.7771
I	733.4558	367.2311	927.5890	414.2205
Q	861.5136	431.2604	714.4257	357.7165
N	975.5565	488.2819	586.3671	293.6872
L	1088.6406	544.8239	472.3242	236.6657
A	1159.6777	580.3425	359.2401	180.1237
L	1272.7617	636.8845	288.2830	144.6051
R	1420.8628	714.9351	175.1198	88.0631



Residue	b	b-2	y	y-2
A	422.2438	211.6259	1197.6415	598.3244
V	521.3122	261.1599	776.4658	388.7061
S	668.3443	304.6738	677.3365	339.1719
W	784.4236	397.7154	598.3945	295.6559
A	865.4681	432.2340	494.2352	252.6163
T	969.5094	483.7579	333.1881	167.0977
O	1023.5290	512.2686	232.1484	116.5738
R	1179.6309	596.3191	175.1198	88.0631

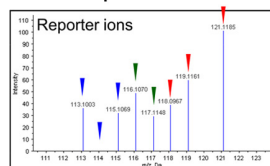
B

Peptide ID : Q86VB7|C163A

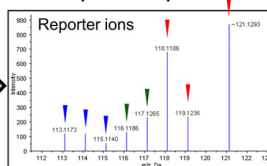
a.a.531-538

Precursor : m/z = 821.92 (+2)

Sample: Pre-ConA



Sample: ConA-purified



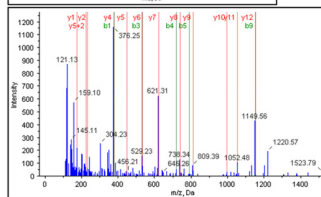
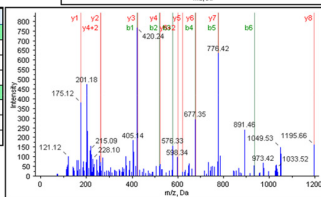
Peptide ID : Q86VB7|C163A

a.a.59-71

Glycosylation : Asn64

Precursor : m/z = 762.89 (+2)

Residue	b	b-2	y	y-2
D	426.2396	210.6234	1195.6496	596.3248
V	519.3889	260.1576	776.4683	388.7078
Q	676.3295	289.6584	677.3399	339.1736
V	675.3979	338.2028	626.3185	316.6529
V	774.4663	387.7368	524.2500	265.1287
C[AM]	934.4863	487.7521	422.1816	211.5945
S	1021.5290	511.2681	262.1618	131.5791
R	1177.6301	589.3187	175.1198	88.0631



Residue	b	b-2	y	y-2
A	376.2487	188.6295	1524.7594	762.8833
P	473.3025	237.1549	1149.6169	575.2617
G	538.3248	265.6656	1002.4642	526.7357
W	716.4833	358.7053	995.4427	498.2250
A	787.4884	394.2238	899.3634	445.1853
N[LRH]	904.4714	452.7394	738.3383	369.6665
S	991.5036	496.2504	625.2901	311.1512
S	1076.5356	539.7715	534.2631	267.6352
A	1148.5728	575.2900	447.2318	224.1191
Q	1208.5942	603.8008	376.1939	188.6009
S	1293.6282	647.3168	318.1724	160.0899
Q	1390.6477	695.8275	232.1484	116.5738
R	1506.7489	753.8781	175.1198	88.0631

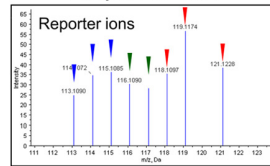
C

Peptide ID : P01033|TIMP1

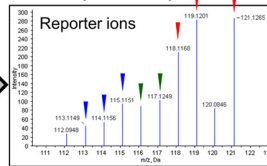
a.a.170-180

Precursor : m/z = 550.96 (+3)

Sample: Pre-ConA



Sample: ConA-purified



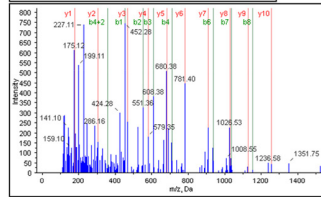
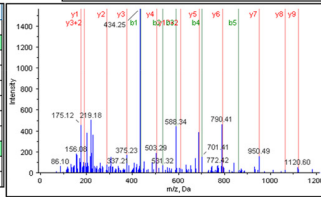
Peptide ID : P01033|TIMP1

a.a.23-37

Glycosylation : Asn30

Precursor : m/z = 687.37 (+3)

Residue	b	b-2	y	y-2
E	434.2552	217.6313	1650.8575	825.9324
P	531.3888	266.1576	1217.6096	609.3884
G	588.3295	294.6684	1120.5568	560.7820
L	781.4135	391.2104	1063.5353	532.2713
C[AM]	861.4442	431.2257	958.4513	475.7293
T	962.4918	481.7496	798.4206	395.7139
W	1148.5712	574.7892	689.3729	345.1901
Q	1276.6297	638.8185	583.2936	292.1504
S	1363.6818	682.3345	375.2359	188.4212
L	1476.7458	738.8766	288.2830	144.6051
R	1632.8469	816.5271	175.1198	88.0631



Residue	b	b-2	y	y-2
F	462.2819	226.6442	2060.0851	1030.5462
V	551.3485	276.1784	1608.8114	804.4093
Q	686.3769	343.6787	1509.7430	755.3781
T	789.4186	395.2078	1452.7218	726.8644
P	906.4714	453.7393	1351.6738	676.3405
E	935.5148	468.2608	1254.6211	627.8142
V	1034.5824	517.7943	1125.5785	563.2929
N[LRH]	1151.6138	576.3104	1026.5180	513.7587
Q	1219.6271	603.3387	968.4789	485.2427
T	1360.7188	680.8635	791.4263	395.2138
T	1481.7675	741.3874	686.3726	340.8889
L	1594.8516	797.9294	579.3249	290.1661
Y	1757.9149	879.4611	468.2489	233.6241
S	1885.9735	943.4934	383.1775	192.0924
R	2042.0746	1021.5403	175.1198	88.0631

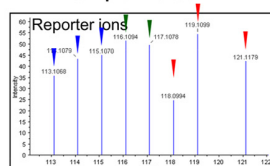
D

Peptide ID : P07996|TSP1

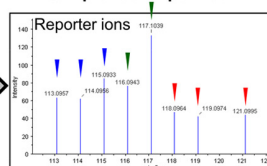
a.a.1037-1046

Precursor : m/z = 539.99 (+3)

Sample: Pre-ConA



Sample: ConA-purified



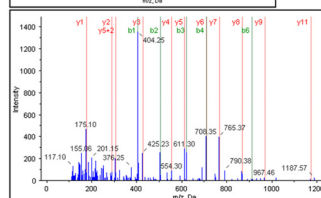
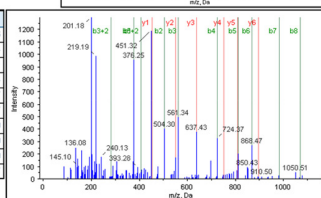
Peptide ID : P07996|TSP1

a.a.1047-1059

Glycosylation : Asn1049

Precursor : m/z = 558.64 (+3)

Residue	b	b-2	y	y-2
A	376.2487	188.6285	1617.9420	809.4746
Q	584.3883	292.6578	1242.6995	621.8534
Q	581.3298	281.6485	1114.6410	557.8241
Y	724.3931	362.7002	1057.6195	529.3134
S	811.4251	406.2162	894.5562	447.7817
G	868.4466	434.7269	887.5241	404.2657
L	981.5387	491.2690	758.5827	375.7550
S	1068.5627	534.7850	637.4186	319.2129
V	1167.6311	584.3192	568.3866	275.6969
Q[TH]	1599.9314	800.4694	451.3182	226.1627



Residue	b	b-2	y	y-2
V	464.2818	232.6442	1673.9040	837.4541
V	583.3486	292.1784	1270.6272	636.8172
N[LRH]	626.3887	310.6940	1175.5588	588.2830
S	787.4837	394.2450	1054.5278	527.7674
T	908.4934	454.7338	967.4856	484.2574
T	989.5089	495.2577	868.4479	433.7276
Q	968.5295	483.7684	765.4682	383.2057
P	1063.5823	532.2948	708.3781	354.6930
Q	1128.6037	560.8055	611.3254	306.7888
F	1249.6463	625.3269	543.3415	271.6555
H	1396.7052	693.8563	425.2619	213.1346
L	1499.7983	750.3983	288.2830	144.6051
R	1658.8804	829.4468	175.1198	88.0631

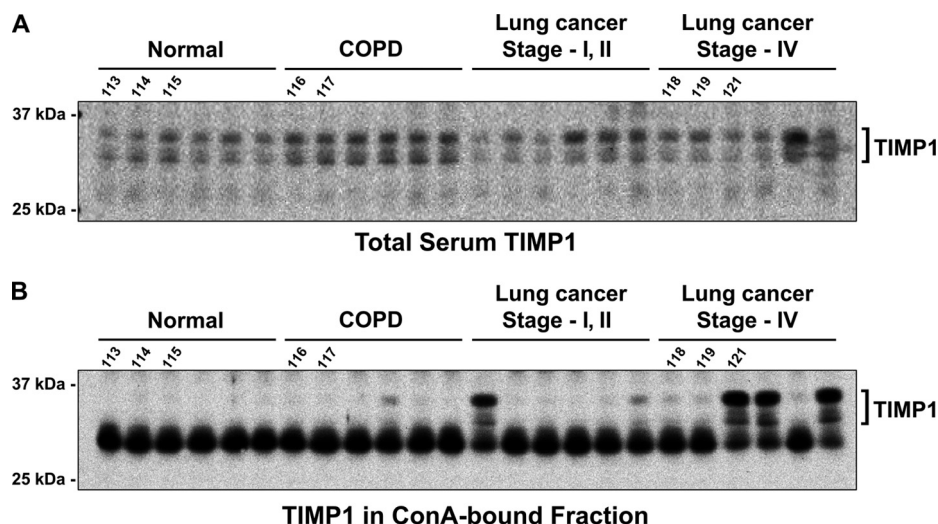


FIG. 5. Immunoblot analysis of TIMP1 levels in lung cancer patients' sera before or after ConA lectin-column chromatography. (A) Serum levels of TIMP1 were analyzed by immunoblot analysis using anti-TIMP1 antibody. 20 μ g each of the immunodepleted serum samples were prepared from six each of healthy volunteers (Normal), COPD patients, stage-I/II lung cancer patients, or stage-IV lung cancer patients and used for the blotting. Samples used in the IGEL-iTRAQ screening experiments were indicated by the iTRAQ reporter numbers (113 to 121). (B) Immunoblot of TIMP1 in the ConA-bound fractions prepared from the same sample set in (A). The entire ConA-bound fraction from 75 μ g of total protein was loaded for each lane. Samples used in the IGEL-iTRAQ screening experiments were indicated by the iTRAQ reporter numbers (113 to 121).

were consistent and highlight the potential of this methodology and marker.

DISCUSSION

Although modern MS instruments allow the measurements of unseparated mixtures at high sensitivity (14), glycoprotein or glycopeptide enrichment is still indispensable to achieving detection and identification of glycosylated components in low and precious amounts of biological materials. We have only few ways to enrich glycoproteins or glycopeptides so far, such as lectin affinity, chemicals covalently bound to specific glycan structures (15, 16), and oligosaccharide-recognizing antibodies (17). However, because of their specificity to a wide variety of substrates and their simple availability, lectins are most frequently used for the purpose of the enrichment of glycans and carbohydrate biomarker discovery. Our IGEL methodology can contribute a great deal to the simplification of these complicated experimental processes, improvement of quantitative reproducibility, and enhancement of throughput for the lectin-based glycopeptide enrichment. As we identified sub- μ g/mL serum proteins from the IGEL-purified sample (shown in supplemental Table 5), this method diminished a loss of scarce serum components and brought about increasing sensitivity. Regarding the glycopeptidase-based elution from the lectin column, highly specific release of

glycopeptides was achieved (93.7%) with little elution of non-specifically bound peptides, and the elution efficiency depended on neither the glycan structures nor the number of sugar chains on a peptide. Moreover, it is critical to maintain the reproducibility of lectin-column chromatography because lectin-glycan interactions are relatively weak ($K_d = 10^{-4}$ – 10^{-7} mol/L) compared with antigen-antibody interactions ($K_d = 10^{-8}$ – 10^{-12} mol/L) and the purification efficiency can vary by washing or elution conditions. The combination of the simple procedure of IGEL and the use of 8-plex iTRAQ tags could minimize quantitative errors caused by these technical biases. Actually, our validation experiment using conventional lectin-column chromatography and immunoblot analysis confirmed the quantitative reliability and reproducibility of IGEL-iTRAQ technology. These facts further emphasize the potential of IGEL technology, whose advantages in enrichment efficiency, throughput, consistency, and variety could contribute not only to proteomics studies but to diverse kinds of glycobiological studies and maybe even clinical practices.

ConA lectin strongly recognizes "core oligosaccharide" structure including Man (α 1–6) [Man (α 1–3)] Man components, which widely expresses in the N-linked sugar chains (18). It is also known that the existence of bisecting GlcNAc structure Man (α 1–6) [Man (α 1–3)] [GlcNAc (β 1–4)] Man sig-

FIG. 4. Representative MS/MS spectra of candidate biomarker glycopeptides. The whole view of MS/MS spectra, extracted area of reporter ions, fragment ion identification Tables in ProteinPilot and peptide identification information are shown for CD14 (A), C163A (B), TIMP1 (C), and TSP1 (D). The left or right side panels show peptide identification data from before or after ConA lectin-column purified samples (Pre-ConA or ConA-purified, respectively). Reporter ions corresponding to three healthy controls, two COPD samples and three lung cancer samples were indicated with blue, green, and red arrow heads.

nificantly reduces its affinity to ConA. Therefore, concerning for sugar chains on the seven glycosylation sites identified in our screening (Fig. 3), it would be conceivable that the frequency of N-glycosylation itself or addition of bisecting GlcNAc structure was significantly altered in lung cancer patients' sera. The same strategy can be expanded to various combinations of lectins and eluting glycosidases, which could achieve more comprehensive analysis of glycan structure alterations. Our preliminary experiments successfully allowed quantification and identification of serum glycopeptides using *Lens culinaris* agglutinin (LCA) column and N-glycosidase F elution. In addition, the higher throughput is also achievable by increasing the sample scale to $8 \times n$ sets with a universal control in each set, which could provide some potential for high-throughput quantitative analysis for glycan structures.

According to our data and previous studies, the advantages of 8-plex iTRAQ labeling system were clear, however, we should note that this system appeared to affect the number of peptide identifications negatively. Our 2D-LC/MS/MS experiment of pre-ConA column purification samples identified 274 proteins, while our previous experiment using the same conditions except for the use of 8-plex iTRAQ labeling identified more than 700 serum proteins (data not shown). This diminished number of identified proteins might be caused by the expanded scale of peptide concentrations after mixing eight samples together, by which considerable numbers of serum minor components should be obscured. Although the introduction of such multiplexed isotope labels can bring in quantitative reliability and high throughput, much heavier fractionation of peptides might be needed to improve its analytical dynamic range.

Furthermore, to pursue the clinical application of glycosylation-targeted disease biomarkers, we should consider that we have only few techniques so far to validate the glycan structure changes accurately. In particular, there is no established method by which glycan structure variations on a specific glycosylation site can be validated quickly, sensitively, and quantitatively for hundreds of samples. We here show that our sophisticated screening technique could contribute to the discovery of diverse disease-related glycan alterations, but new validation technologies are urgently required so that the glycoproteomic profiling results could lead to

clinical applications as diagnostic or prognostic biomarkers in the future.

Acknowledgments—We thank Ms. Mari Kikuchi and Ms. Miki Yamato at Applied Biosystems Japan for their technical assistances.

* This study was supported in part by the grants from Toppan Printing Co., Ltd. and Shimadzu Corporation.

[S] This article contains [supplemental material](#).

To whom correspondence should be addressed: Laboratory for Biomarker Development, Center for Genomic Medicine, RIKEN, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.

REFERENCES

- Wollscheid, B., Bausch-Fluck, D., Henderson, C., O'Brien, R., Bibel, M., Schiess, R., Aebersold, R., and Watts, J. D. (2009) *Nat. Biotechnol.* **27**, 378–386
- Brown, S. M., Smith, D. M., Alt, N., Thorpe, S. R., and Baynes, J. W. (2005) *Ann. N.Y. Acad. Sci.* **1043**, 817–823
- Tissot, B., North, S. J., Ceroni, A., Pang, P. C., Panico, M., Rosati, F., Capone, A., Haslam, S. M., Dell, A., and Morris, H. R. (2009) *FEBS Lett.* **583**, 1728–1735
- Bos, P. D., Zhang, X. H., Nadal, C., Shu, W., Gomis, R. R., Nguyen, D. X., Minn, A. J., van de Vijver, M. J., Gerald, W. L., Foekens, J. A., and Massague, J. (2009) *Nature* **459**, 1005–1009
- Kawasaki, N., Itoh, S., Hashii, N., Takakura, D., Qin, Y., Huang, X., and Yamaguchi, T. (2009) *Biol. Pharm. Bull.* **32**, 796–800
- Ludwig, J. A., and Weinstein, J. N. (2005) *Nat. Rev. Cancer* **5**, 845–856
- Suzuki, E., Niwa, R., Saji, S., Muta, M., Hirose, M., Iida, S., Shitsui, Y., Satoh, M., Shitara, K., Kondo, M., and Toi, M. (2007) *Clin. Cancer Res.* **13**, 1875–1882
- Kaji, H., Saito, H., Yamauchi, Y., Shinkawa, T., Taoka, M., Hirabayashi, J., Kasai, K., Takahashi, N., and Isobe, T. (2003) *Nat. Biotechnol.* **21**, 667–672
- Kaji, H., Yamauchi, Y., Takahashi, N., and Isobe, T. (2006) *Nat. Protoc.* **1**, 3019–3027
- Morelle, W., Faid, V., Chirat, F., and Michalski, J. C. (2009) *Methods Mol. Biol.* **534**, 5–21
- Li, B., An, H. J., Hedrick, J. L., and Lebrilla, C. B. (2009) *Methods Mol. Biol.* **534**, 133–145
- Jin, S., Cheng, Y., Reid, S., Li, M., and Wang, B. (2009) *Med. Res. Rev.* **30**, 171–257
- Bank, I., Weiss, P., Doolman, R., Book, M., and Sela, B. A. (1999) *J. Lab. Clin. Med.* **134**, 599–604
- Anderson, N. L., Anderson, N. G., Pearson, T. W., Borchers, C. H., Paulovich, A. G., Patterson, S. D., Gillette, M., Aebersold, R., and Carr, S. A. (2009) *Mol. Cell. Proteomics* **8**, 883–886
- Tian, Y., Zhou, Y., Elliott, S., Aebersold, R., and Zhang, H. (2007) *Nat. Protoc.* **2**, 334–339
- Gontarev, S., Shmanai, V., Frey, S. K., Kvach, M., and Schweigert, F. J. (2007) *Rapid Commun. Mass. Spectrom.* **21**, 1–6
- Heimburg-Molinaro, J., and Rittenhouse-Olson, K. (2009) *Methods Mol. Biol.* **534**, 341–357
- Ohyama, Y., Kasai, K., Nomoto, H., and Inoue, Y. (1985) *J. Biol. Chem.* **260**, 6882–6887

Supplementary Table 1

The iTRAQ labeling information and clinical characteristics of the 8 serum samples

iTRAQ	Sample	Age	Gender
113	Normal 1	55	Male
114	Normal 2	59	Male
115	Normal 3	61	Male
116	COPD 1	62	Male
117	COPD 2	59	Male
118	Lung adenocarcinoma stage-IV 1	61	Male
119	Lung adenocarcinoma stage-IV 2	62	Male
121	Lung adenocarcinoma stage-IV 3	60	Male

Supplementary Table 2

List of 12 identified N-glycosylation sites that did not match the N-glycosylation consensus sequences

Accessions	m/z (z)	^a Conf.	^b Sequence	Modifications	^c 113/Navr	^c 114/Navr	^c 115/Navr	^c 116/Navr	^c 117/Navr	^c 118/Navr	^c 119/Navr	^c 121/Navr
P04217 A1BG_HUMAN	614.32 (+4)	99	I ₂₄₁ FFHLN ₂₄₆ **AVALGDGGHY TCR ₂₅₉	iTRAQ8plex@N-term User modH on Asn(N)@6 Carbamidomethyl(C)@18	1.327	0.731	0.943	3.131	1.967	1.399	1.768	4.491
P04217 A1BG_HUMAN	572.33 (+4)	99	N ₄₂ **GVAQEPVHLDSPAIIK ₅₇	iTRAQ8plex@N-term User modH on Asn(N)@1 iTRAQ8plex(K)@16	1.014	0.926	1.059	2.315	1.574	1.591	1.886	2.108
P08603 CFAH_HUMAN	867.41 (+2)	99	M ₁₀₀₆ DGASN ₁₀₁₁ *VTCIN ₁₀₁₆ **S R ₁₀₁₈	iTRAQ8plex@N-term User modH on Asn(N)@6 Carbamidomethyl(C)@9 User modH on Asn(N)@11	0.868	0.712	1.420	1.548	1.215	1.348	1.710	1.398
P00450 CERU_HUMAN	955.54 (+4)	99	E ₇₃₅ LHHLQEQN ₇₄₃ *VSN ₇₄₆ **A FLDKGEFYIGSK ₇₅₉	iTRAQ8plex@N-term User modH on Asn(N)@9 User modH on Asn(N)@12 iTRAQ8plex(K)@17 iTRAQ8plex(K)@25	1.245	0.815	0.940	2.584	2.083	2.978	2.053	1.945
O75882 ATRN_HUMAN	657 (+3)	98	A ₈₉₁ ATCIN ₈₉₆ **PLN ₈₉₉ *GSVCE R ₉₀₅	iTRAQ8plex@N-term Carbamidomethyl(C)@4 User modH on Asn(N)@6 User modH on Asn(N)@9 Carbamidomethyl(C)@13	0.628	0.960	1.411	1.237	0.941	0.742	0.377	1.510
P02765 FETUA_HUMAN	891.45 (+3)	96	A ₁₄₆ ALAAFN ₁₅₄ **AQN ₁₅₇ **N ₁₅₈ * GSN ₁₆₁ **FQLEEISR ₁₆₉	iTRAQ8plex@N-term User modH on Asn(N)@7 User modH on Asn(N)@10 User modH on Asn(N)@11 User modH on Asn(N)@14	1.020	0.856	1.124	2.102	0.774	1.663	2.301	1.342
P01011 AACT_HUMAN	942.16 (+3)	95	T ₁₀₂ LN ₁₀₄ *QSSDELQLSMGN ₁₁₆ **AMFVK ₁₂₁	iTRAQ8plex@N-term User modH on Asn(N)@3 User modH on Asn(N)@15 iTRAQ8plex(K)@20	0.989	0.684	1.327	1.348	1.305	2.097	2.387	0.809
P02751 FINC_HUMAN	847.12 (+3)	96	L ₉₆₆ DAPT ₉₇₁ **LQFVN ₉₇₆ **ETD STVLVR ₉₈₅	iTRAQ8plex@N-term User modH on Asn(N)@6 User modH on Asn(N)@11	0.000	1.475	1.525	2.370	1.202	1.410	0.842	1.228
P04004 VTNC_HUMAN	1027.18 (+3)	99	N ₂₂₃ *ISDGF ₂₃₄ GIPDN ₂₃₄ **VDAA LALPAHSYSGR ₂₄₉	iTRAQ8plex@N-term User modH on Asn(N)@1 User modH on Asn(N)@12	1.079	0.590	1.331	0.636	1.576	1.831	0.808	0.785
P04004 VTNC_HUMAN	748.9 (+4)	98	N ₆₆ **NATVHEQVGGPSLTSDL QAQSK ₈₈	iTRAQ8plex@N-term User modH on Asn(N)@1 No User modH on Asn(N)@2 iTRAQ8plex(K)@23	0.647	0.618	1.736	1.067	1.103	1.519	0.978	0.517
P07996 TSP1_HUMAN	952.51 (+3)	99	G ₂₁₃ CSSTSVLLTL ₂₂₆ **N ₂₂₇ γ**VVN ₂₃₀ *GSSPAIR ₂₃₇	iTRAQ8plex@N-term Carbamidomethyl(C)@2 User modH on Asn(N)@14 User modH on Asn(N)@15 User modH on Asn(N)@18	0.744	1.783	0.473	2.048	2.303	0.000	0.867	0.664
P49908 SEPP1_HUMAN	811.09 (+3)	99	E ₅₀ GYSN ₆₄ *ISYIVVN ₇₁ **HQGIS SR ₇₈	iTRAQ8plex@N-term User modH on Asn(N)@5 User modH on Asn(N)@12	1.139	0.752	1.109	3.198	1.332	1.423	1.223	0.848

^a The Confidence values in ProteinPilot software were shown. We set the peptide identification threshold as Conf. > 95. ^b The identified N-glycosylation sites were indicated by asterisks and their amino acid numbers were also shown. ^c Fold change values of the affinity to ConA lectin

were calculated as relative differences from the average of three normal samples (N_{Avr}). The ratio of more than 2-fold or less than 0.5-fold change was highlighted in red or blue, respectively.

Supplementary Table 3

List of 107 glycopeptides identified by ConA - IGEL purification

Accessions	m/z (z)	^a Conf.	^b Sequence	Modifications	^c 113/ Navr	^c 114/ Navr	^c 115/ Navr	^c 116/ Navr	^c 117/ Navr	^c 118/ Navr	^c 119/ Navr	^c 121/ Navr
O75882 ATRN_HUMAN	813.44 (+2)	99	I ₃₉₆ DSTGN ₄₀₁ *VTNELR ₄₀₇	iTRAQ8plex@N-term User modH on Asn(N)@6 No User modH on Asn(N)@9	0.818	0.850	1.332	1.760	1.870	1.438	1.216	2.431
O75882 ATRN_HUMAN	614.64 (+3)	99	N ₇₁₆ *HSCSEQISIFR ₇₂₈	iTRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@4	1.169	0.787	1.044	2.499	1.664	1.360	1.525	2.818
O75882 ATRN_HUMAN	657.83 (+2)	98	G ₂₈₂ ICN ₂₈₅ *SSDVR ₂₉₀	iTRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@4	0.989	0.858	1.153	3.747	4.074	1.365	1.291	1.667
O75882 ATRN_HUMAN	886.11 (+3)	98	I ₂₄₇ SN ₂₄₉ *SSDVECECSSENWK ₂₆₃	iTRAQ8plex@N-term User modH on Asn(N)@3 Carbamidomethyl(C)@10 Carbamidomethyl(C)@12 No User modH on Asn(N)@15 iTRAQ8plex(K)@17	0.793	0.961	1.245	1.654	1.780	1.239	0.626	0.841
O95445 APOM_HUMAN	952.47 (+3)	99	T ₁₂₁ ELFSSSCPGGIMLN ₁₃₅ *ETGQ GYQR ₁₄₃	iTRAQ8plex@N-term Carbamidomethyl(C)@8 Oxidation(M)@13 User modH on Asn(N)@15	1.082	1.499	0.419	0.989	0.615	0.000	0.493	0.000
O95445 APOM_HUMAN	947.16 (+3)	99	T ₁₂₁ ELFSSSCPGGIMLN ₁₃₅ *ETGQ GYQR ₁₄₃	iTRAQ8plex@N-term Carbamidomethyl(C)@8 User modH on Asn(N)@15	0.658	1.478	0.864	1.520	0.999	0.149	0.332	0.059
P00450 CERU_HUMAN	734.03 (+3)	99	E ₁₁₀ HEGAIYPDN ₁₁₉ *TTDFQR ₁₂₅	iTRAQ8plex@N-term User modH on Asn(N)@10	0.945	0.760	1.295	1.020	1.200	1.618	1.622	1.337
P00450 CERU_HUMAN	812.08 (+3)	99	E ₃₇₇ N ₃₇₈ *LTAPGSDSAVFEEQGT TR ₃₈₆	iTRAQ8plex@N-term User modH on Asn(N)@2	1.428	0.486	1.086	1.189	1.070	1.972	1.655	0.938
P00450 CERU_HUMAN	626.81 (+4)	98	E ₁₁₀ HEGAIYPDN ₁₁₉ *TTDFQR ₁₂₅	iTRAQ8plex@N-term iTRAQ8plex(Y)@7 User modH on Asn(N)@10	0.697	1.133	1.169	1.631	1.515	1.978	1.503	1.827
P00450 CERU_HUMAN	699.38 (+4)	97	Y ₁₀₈ KEHEGAIYPDN ₁₁₉ *TTDFQR ₁₂₅	iTRAQ8plex@N-term iTRAQ8plex(K)@2 User modH on Asn(N)@12	0.442	0.949	1.609	1.467	1.865	1.814	1.531	1.321
P00734 THRB_HUMAN	892.22 (+4)	99	S ₁₁₀ RYPHKPEIN ₁₁₉ *STTHPGADL QENFCR ₁₃₄	iTRAQ8plex@N-term iTRAQ8plex(K)@6 User modH on Asn(N)@10 No User modH on Asn(N)@22 Carbamidomethyl(C)@24	0.936	1.423	0.641	2.705	1.625	1.053	1.670	1.472
P00734 THRB_HUMAN	1108.21 (+3)	99	Y ₁₁₂ PHKPEIN ₁₁₉ *STTHPGADLQE NFCR ₁₃₄	iTRAQ8plex@N-term iTRAQ8plex(K)@4 User modH on Asn(N)@8 No User modH on Asn(N)@20 Carbamidomethyl(C)@22	0.916	1.050	1.033	1.721	1.693	1.172	1.444	1.626
P00734 THRB_HUMAN	764.41 (+2)	97	N ₃₉₂ *FTENDLLVR ₄₀₁	iTRAQ8plex@N-term User modH on Asn(N)@1 No User modH on Asn(N)@5	0.812	0.959	1.229	0.785	1.020	0.697	0.653	0.469
P00748 FA12_HUMAN	626.97 (+3)	99	N ₄₁₄ *HSCEPCQLAVR ₄₂₆	iTRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@4 Carbamidomethyl(C)@7	0.938	1.131	0.930	2.549	2.548	2.234	0.828	1.380
P00748 FA12_HUMAN	509.51 (+4)	99	R ₄₁₃ N ₄₁₄ *HSCEPCQLAVR ₄₂₆	iTRAQ8plex@N-term User modH on Asn(N)@2 Carbamidomethyl(C)@5 Carbamidomethyl(C)@8	0.297	0.381	2.322	0.451	0.612	0.780	0.394	0.292
P00751 CFAB_HUMAN	788.43 (+3)	99	L ₂₄₈ VLDGSDSIGASN ₂₆₀ *FTGAK ₂₆₅	iTRAQ8plex@N-term User modH on Asn(N)@13 iTRAQ8plex(K)@18	0.628	0.694	1.678	1.136	0.938	1.930	2.411	1.846
P00751 CFAB_HUMAN	965.11 (+3)	99	S ₉₃ PYYN ₉₇ *VSDEISFHCYDGYTL R ₁₁₃	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@14	0.736	0.619	1.645	2.032	1.541	1.557	3.955	1.967
P00751 CFAB_HUMAN	800.15 (+4)	96	S ₉₃ PYYN ₉₇ *VSDEISFHCYDGYTL R ₁₁₃	iTRAQ8plex@N-term User modH on Asn(N)@5 iTRAQ8plex(S)@7 Carbamidomethyl(C)@14	0.451	1.334	1.215	0.855	1.603	1.612	3.598	1.942
P01008 ANT3_HUMAN	826.77 (+3)	99	L ₉₂ GACN ₉₆ *DTLQQLMEVFK ₁₀₇	iTRAQ8plex@N-term Carbamidomethyl(C)@4 User modH on Asn(N)@5 iTRAQ8plex(K)@16	0.690	1.028	1.282	1.563	1.408	0.938	1.039	2.069
P01008 ANT3_HUMAN	930.48 (+3)	99	S ₁₅₁ LTFN ₁₅₅ *ETYQDISELVYGA ₁₆₉	iTRAQ8plex@N-term User modH on Asn(N)@5 iTRAQ8plex(K)@19	0.883	1.385	0.733	2.314	1.984	1.323	1.224	2.254

P01008 ANT3_HUMAN	832.12 (+3)	98	L ₃₂ GACN ₉₆ *DTLQQLMEVFK ₁₀₇	iTRAQ8plex@N-term Carbamidomethyl(C)@4 User modH on Asn(N)@5 Oxidation(M)@12 iTRAQ8plex(K)@16	0.659	1.307	1.034	1.439	1.317	1.248	0.778	1.802
P01009 A1AT_HUMAN	789.78 (+3)	99	Y ₂₄₄ LGN ₂₄₇ *ATAIFFLPDEGK ₂₅₉	iTRAQ8plex@N-term User modH on Asn(N)@4 iTRAQ8plex(K)@16	0.000	0.000	0.000	0.000	0.000	16.190	#####	0.810
P01011 AACT_HUMAN	947.53 (+3)	99	T ₁₀₂ LN ₁₀₄ *QSSDELQLSMGNAMF VK ₁₂₁	iTRAQ8plex@N-term User modH on Asn(N)@3 Oxidation(M)@13 No User modH on Asn(N)@15 iTRAQ8plex(K)@20	0.938	0.000	2.062	2.286	2.242	3.851	2.817	1.545
P01011 AACT_HUMAN	788.79 (+3)	99	Y ₂₄₅ TGN ₂₄₈ *ASALFILPDQDK ₂₆₀	iTRAQ8plex@N-term User modH on Asn(N)@4 iTRAQ8plex(K)@16	0.611	0.798	1.591	0.916	1.212	3.127	1.140	0.511
P01033 TIMP1_HUMAN	687.37 (+3)	99	F ₂₃ VGTPENV ₃₀ *QTTLYQR ₃₇	iTRAQ8plex@N-term User modH on Asn(N)@8	0.404	1.188	1.408	2.327	1.978	3.890	3.111	6.016
P01042 KNG1_HUMAN	675.71 (+3)	96	I ₁₇₉ TYSIVQTN ₁₈₇ *CSK ₁₉₀	iTRAQ8plex@N-term User modH on Asn(N)@9 Carbamidomethyl(C)@10 iTRAQ8plex(K)@12	0.402	1.350	1.248	2.261	0.981	1.320	0.799	0.676
P01859 IGHG2_HUMAN	732.87 (+2)	98	E ₁₇₂ EQFN ₁₇₆ *STFR ₁₈₀	iTRAQ8plex@N-term User modH on Asn(N)@5	0.816	0.780	1.404	0.911	1.524	1.943	1.368	1.532
P02750 A2GL_HUMAN	722.12 (+3)	99	K ₁₄₃ LPPGLLAN ₁₅₁ *FTLLR ₁₅₆	iTRAQ8plex@N-term iTRAQ8plex(K)@1 User modH on Asn(N)@9	0.353	0.786	1.861	0.552	1.520	6.818	23.959	4.472
P02750 A2GL_HUMAN	653.32 (+2)	98	M ₂₈₆ FSQN ₂₉₀ *DTR ₂₉₃	iTRAQ8plex@N-term User modH on Asn(N)@5	0.427	0.801	1.771	0.681	2.034	10.032	19.273	3.270
P02751 FINC_HUMAN	564.75 (+4)	99	H ₅₀₄ EEGHMLN ₅₁₁ *CTCFGQGR ₅₁₉	iTRAQ8plex@N-term Oxidation(M)@6 User modH on Asn(N)@8 Carbamidomethyl(C)@9 Carbamidomethyl(C)@11	0.758	0.755	1.487	0.932	1.388	1.134	0.701	0.009
P02751 FINC_HUMAN	747.36 (+3)	99	H ₅₀₄ EEGHMLN ₅₁₁ *CTCFGQGR ₅₁₉	iTRAQ8plex@N-term User modH on Asn(N)@8 Carbamidomethyl(C)@9 Carbamidomethyl(C)@11	0.735	1.093	1.172	2.211	2.372	1.600	0.390	0.672
P02751 FINC_HUMAN	847.13 (+3)	99	L ₉₆₆ DAPTNLQFVN ₉₇₆ *ETDSTVLV R ₉₈₅	iTRAQ8plex@N-term No User modH on Asn(N)@6 User modH on Asn(N)@11	0.939	0.992	1.069	2.681	2.374	1.489	0.910	1.139
P02751 FINC_HUMAN	603.78 (+4)	99	R ₅₀₃ HEEGHMLN ₅₁₁ *CTCFGQGR ₅₁₉	iTRAQ8plex@N-term Oxidation(M)@7 User modH on Asn(N)@9 Carbamidomethyl(C)@10 Carbamidomethyl(C)@12	0.504	1.024	1.472	2.092	1.735	0.615	0.562	0.161
P02751 FINC_HUMAN	599.78 (+4)	99	R ₅₀₃ HEEGHMLN ₅₁₁ *CTCFGQGR ₅₁₉	iTRAQ8plex@N-term User modH on Asn(N)@9 Carbamidomethyl(C)@10 Carbamidomethyl(C)@12	0.267	0.384	2.349	1.948	2.189	1.291	1.324	0.000
P02765 FETUA_HUMAN	628.6 (+4)	99	K ₁₂₆ VCQDCPLLAPLN ₁₃₈ *DTR ₁₄₁	iTRAQ8plex@N-term iTRAQ8plex(K)@1 Carbamidomethyl(C)@3 Carbamidomethyl(C)@6 User modH on Asn(N)@13	0.765	1.251	0.984	2.170	1.696	1.635	0.895	2.435
P02765 FETUA_HUMAN	1040 (+2)	99	V ₁₂₇ CQDCPLLAPLN ₁₃₈ *DTR ₁₄₁	iTRAQ8plex@N-term Carbamidomethyl(C)@2 Carbamidomethyl(C)@5 User modH on Asn(N)@12	0.650	1.213	1.137	1.771	1.520	1.556	0.915	1.965
P02790 HEMO_HUMAN	1022.03 (+2)	99	A ₄₂₄ LPQPQN ₄₃₀ *VTSLLGCTH ₄₃₉	iTRAQ8plex@N-term User modH on Asn(N)@7 Carbamidomethyl(C)@14	0.607	0.836	1.558	1.283	1.287	1.452	1.777	1.801
P02790 HEMO_HUMAN	541.24 (+4)	99	N ₂₁₇ *GTGHGN ₂₂₃ *STHHGPEYM R ₂₃₃	iTRAQ8plex@N-term User modH on Asn(N)@1 User modH on Asn(N)@7	0.689	0.758	1.553	1.973	1.307	1.593	1.148	1.169
P02790 HEMO_HUMAN	617.05 (+4)	99	N ₂₁₇ *GTGHGN ₂₂₃ *STHHGPEYM R ₂₃₃	iTRAQ8plex@N-term User modH on Asn(N)@1 User modH on Asn(N)@7 iTRAQ8plex(T)@9	1.017	0.571	1.411	0.823	1.609	1.256	0.954	0.855
P02790 HEMO_HUMAN	719.88 (+2)	99	P ₁₆₀ AVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@6	0.796	1.002	1.202	1.964	1.608	1.704	1.557	4.439
P02790 HEMO_HUMAN	672.68 (+3)	99	S ₁₅₈ WPAVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term iTRAQ8plex(S)@1 User modH on Asn(N)@7 Carbamidomethyl(C)@8	0.708	0.888	1.403	1.845	1.422	1.422	1.554	1.842
P02790 HEMO_HUMAN	571.29 (+3)	99	S ₁₅₈ WPAVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term User modH on Asn(N)@7 Carbamidomethyl(C)@8	0.631	0.856	1.514	1.575	1.414	1.637	1.332	1.667
P02790 HEMO_HUMAN	672.69 (+3)	99	S ₁₅₈ WPAVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term User modH on Asn(N)@7 Carbamidomethyl(C)@8 iTRAQ8plex(S)@10	0.673	0.737	1.590	1.570	1.584	1.641	1.611	1.478

P03951 FA11_HUMAN	654.71 (+3)	99	V ₄₂₆ YSGILN ₄₃₂ *QSEIK ₄₃₇	iTRAQ8plex@N-term User modH on Asn(N)@7 iTRAQ8plex(K)@12	0.459	1.192	1.349	1.971	1.639	0.000	1.562	0.834
P04004 VTNC_HUMAN	609.83 (+2)	99	N ₁₅₀ *GSLFAFR ₁₅₇	iTRAQ8plex@N-term User modH on Asn(N)@1	0.669	0.659	1.672	1.209	1.170	1.744	1.138	1.099
P04114 APOB_HUMAN	665.02 (+3)	99	F ₁₄₉₅ N ₁₄₉₆ *SSYLQGTNQTGR ₁₅₀₉	iTRAQ8plex@N-term User modH on Asn(N)@2 No User modH on Asn(N)@10	0.487	1.405	1.108	1.051	0.980	0.953	0.861	0.764
P04217 A1BG_HUMAN	1036.51 (+4)	99	E ₁₃₂ GDHEFLVPEAQEDVEATFP VHQPGN ₁₅₈ *YSCSYR ₁₆₄	iTRAQ8plex@N-term User modH on Asn(N)@27 Carbamidomethyl(C)@30	1.487	0.000	1.513	2.333	1.318	1.175	1.054	1.980
P04217 A1BG_HUMAN	874.62 (+6)	97	F ₃₂₈ QSPAGTEALFELHN ₃₄₂ *ISVA DSAN ₃₅₀ *YSCVYVDLKPPFGGSA PSER ₃₇₀	iTRAQ8plex@N-term User modH on Asn(N)@15 User modH on Asn(N)@23 Carbamidomethyl(C)@26 iTRAQ8plex(K)@32	1.288	0.600	1.111	2.169	1.523	3.384	2.424	3.856
P04275 VWF_HUMAN	745.38 (+3)	99	G ₆₃₃ QVYLQCGTPCN ₆₄₄ *LTCR ₆₄₈	iTRAQ8plex@N-term Carbamidomethyl(C)@7 Carbamidomethyl(C)@11 User modH on Asn(N)@12 Carbamidomethyl(C)@15	0.642	0.562	1.796	0.982	1.647	1.931	1.529	1.793
P04278 SHBG_HUMAN	675.86 (+2)	97	L ₃₄₄ DVDQALN ₃₅₁ *R ₃₅₂	iTRAQ8plex@N-term User modH on Asn(N)@8	0.565	1.282	1.152	1.492	2.652	2.551	1.403	5.308
P05155 IC1_HUMAN	608.82 (+2)	99	D ₂₁₂ TFVN ₂₁₆ *ASR ₂₁₉	iTRAQ8plex@N-term User modH on Asn(N)@5	0.435	0.763	1.803	3.051	2.262	1.821	1.667	1.417
P05156 CFAI_HUMAN	686.35 (+3)	99	L ₄₄₂ FQPN ₄₄₆ *DTCIVSGWGR ₄₅₆	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@8	0.420	0.173	2.407	0.673	0.951	2.133	1.717	0.550
P05362 ICAM1_HUMAN	653.41 (+2)	99	A ₁₁₇ N ₁₁₈ *LTVVLLR ₁₂₅	iTRAQ8plex@N-term User modH on Asn(N)@2	1.046	1.105	0.849	1.182	1.401	8.133	2.401	5.041
P06681 CO2_HUMAN	822.39 (+3)	99	L ₄₃₈ TDITCGVGN ₄₄₇ *MSAN ₄₅₁ *AS DQER ₄₅₇	iTRAQ8plex@N-term Carbamidomethyl(C)@6 User modH on Asn(N)@10 Oxidation(M)@11 User modH on Asn(N)@14	0.411	0.914	1.675	2.609	2.214	0.847	0.946	3.721
P06731 CEAM5_HUMAN	686.4 (+2)	98	T ₁₆₅ LTLFN ₁₇₀ *VTR ₁₇₃ T ₂₂₁ LTLFN ₂₂₆ *VTR ₂₂₉	iTRAQ8plex@N-term User modH on Asn(N)@6	0.000	0.000	3.000	0.000	0.000	3.400	0.000	#####
P07357 CO8A_HUMAN	547.61 (+3)	99	G ₄₀₅ GSSGWSGGLAQN ₄₁₇ *R ₄₁₈	iTRAQ8plex@N-term User modH on Asn(N)@13	1.483	0.972	0.544	2.757	2.135	2.424	3.010	3.586
P07357 CO8A_HUMAN	649.01 (+3)	98	G ₄₀₅ GSSGWSGGLAQN ₄₁₇ *R ₄₁₈	iTRAQ8plex@N-term iTRAQ8plex(S)@4 User modH on Asn(N)@13	0.725	0.861	1.414	1.748	1.423	1.173	1.543	1.370
P07996 TSP1_HUMAN	558.64 (+3)	99	V ₁₀₄₇ VN ₁₀₄₉ *STTGPEHLR ₁₀₅₉	iTRAQ8plex@N-term User modH on Asn(N)@3	1.042	1.025	0.933	1.571	1.373	0.519	0.511	0.464
P07996 TSP1_HUMAN	495.28 (+4)	98	V ₁₀₄₇ VN ₁₀₄₉ *STTGPEHLR ₁₀₅₉	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(T)@5	0.860	1.281	0.859	0.724	1.247	0.307	0.798	0.310
P08571 CD14_HUMAN	489.61 (+3)	99	L ₁₃₀ RN ₁₃₂ *VSWATGR ₁₃₉	iTRAQ8plex@N-term User modH on Asn(N)@3	1.088	0.408	1.504	2.451	1.434	1.248	2.553	2.973
P08571 CD14_HUMAN	599.32 (+2)	98	N ₁₃₂ *VSWATGR ₁₃₉	iTRAQ8plex@N-term User modH on Asn(N)@1	0.704	1.014	1.282	1.052	1.377	2.131	2.259	2.604
P08603 CFAH_HUMAN	706.68 (+3)	99	C ₈₅₂ SQPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term Carbamidomethyl(C)@1 User modH on Asn(N)@13	0.551	0.886	1.563	1.782	1.126	1.363	1.879	1.139
P08603 CFAH_HUMAN	776.73 (+3)	99	I ₈₅₀ PCSQPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@15	0.518	0.649	1.833	1.498	1.171	1.434	1.733	1.230
P08603 CFAH_HUMAN	658.86 (+4)	99	I ₈₅₀ PCSQPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@15 iTRAQ8plex(S)@17	0.316	0.877	1.807	0.829	1.093	0.694	1.617	1.046
P08603 CFAH_HUMAN	725.02 (+3)	99	I ₈₈₉ SEEN ₈₉₃ *ETTCYMGK ₉₀₁	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@9 iTRAQ8plex(K)@13	0.592	0.928	1.480	1.954	1.938	1.719	2.157	1.353
P08603 CFAH_HUMAN	874.4 (+2)	99	M ₁₀₀₆ DGASN ₁₀₁₁ *VTCINSR ₁₀₁₈	iTRAQ8plex@N-term Oxidation(M)@1 User modH on Asn(N)@6 Carbamidomethyl(C)@9 No User modH on Asn(N)@11	0.749	0.841	1.410	1.912	1.387	1.793	2.137	1.893
P08603 CFAH_HUMAN	866.4 (+2)	99	M ₁₀₀₆ DGASN ₁₀₁₁ *VTCINSR ₁₀₁₈	iTRAQ8plex@N-term User modH on Asn(N)@6 Carbamidomethyl(C)@9 No User modH on Asn(N)@11	0.815	0.799	1.386	1.837	1.295	1.820	2.369	1.580

P08603 CFAH_HUMAN	653.34 (+3)	99	S ₈₅₃ QPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term User modH on Asn(N)@12	0.485	0.477	2.038	1.498	1.251	1.564	1.425	1.451
P09172 DOPO_HUMAN	661.04 (+3)	99	S ₁₄₀ LEAIN ₁₄₅ *GSGLQMGQR ₁₅₅	iTRAQ8plex@N-term User modH on Asn(N)@6	0.655	1.165	1.180	2.482	0.418	0.896	0.625	7.244
P09172 DOPO_HUMAN	666.36 (+3)	99	S ₁₄₀ LEAIN ₁₄₅ *GSGLQMGQR ₁₅₅	iTRAQ8plex@N-term User modH on Asn(N)@6 Oxidation(M)@12	0.000	2.507	0.493	1.064	1.108	1.213	0.715	6.801
P0C0L4 CO4A_HUMAN	796.08 (+3)	99	F ₂₀₀ SDGLESN ₂₀₇ *SSTQFEVK ₂₁₅	iTRAQ8plex@N-term User modH on Asn(N)@8 iTRAQ8plex(K)@16	0.619	0.830	1.551	1.088	1.390	1.332	2.165	0.817
P0C0L4 CO4A_HUMAN	706.4 (+2)	99	G ₁₃₀₇ LN ₁₃₀₉ *VTLSTGR ₁₃₁₇	iTRAQ8plex@N-term User modH on Asn(N)@3	0.502	0.730	1.768	1.362	1.152	1.082	1.829	0.903
P0C0L4 CO4A_HUMAN	572.67 (+3)	99	G ₁₃₀₇ LN ₁₃₀₉ *VTLSTGR ₁₃₁₇	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(S)@7	0.429	0.769	1.802	1.240	1.053	1.018	2.008	0.796
P0C0L4 CO4A_HUMAN	754.08 (+3)	99	N ₁₃₇₂ *TTCQDLQIEVTVK ₁₃₈₅	iTRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@4 iTRAQ8plex(K)@14	0.808	0.839	1.352	1.157	1.560	1.724	1.845	1.077
P0C0L4 CO4A_HUMAN	572.67 (+3)	98	G ₁₃₀₇ LN ₁₃₀₉ *VTLSTGR ₁₃₁₇	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(S)@8	0.457	0.737	1.806	1.041	1.087	0.940	1.159	0.857
P10909 CLUS_HUMAN	996.01 (+2)	99	L ₃₅₀ AN ₃₅₂ *LTQGEDQYYLR ₃₆₃	iTRAQ8plex@N-term User modH on Asn(N)@3	0.929	1.060	1.011	1.713	1.525	1.812	0.880	1.426
P12259 FA5_HUMAN	698.39 (+2)	99	T ₂₁₇₅ WN ₂₁₈₁ *QSIALR ₂₁₈₇	iTRAQ8plex@N-term User modH on Asn(N)@3	0.656	1.104	1.240	1.432	1.448	1.397	1.881	1.674
P12259 FA5_HUMAN	716.42 (+4)	98	V ₂₅₅ SAITLVSATSTAN ₂₆₉ *MTVG PEGK ₂₇₇	iTRAQ8plex@N-term User modH on Asn(N)@15 Oxidation(M)@16 iTRAQ8plex(K)@23	0.794	1.265	0.940	1.628	1.474	2.529	1.677	3.665
P17936 IBP3_HUMAN	720.88 (+2)	99	G ₈₅ LCVN ₈₉ *ASAVSR ₉₅	iTRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@5	0.958	0.818	1.224	1.301	1.266	0.871	0.838	1.550
P19823 ITIH2_HUMAN	701.05 (+3)	99	G ₉₅ AFISN ₁₀₀ *FSMTVDGK ₁₀₈	iTRAQ8plex@N-term User modH on Asn(N)@6 Oxidation(M)@9 iTRAQ8plex(K)@14	0.760	1.303	0.937	1.704	1.438	1.627	1.421	0.523
P19823 ITIH2_HUMAN	695.71 (+3)	96	G ₉₅ AFISN ₁₀₀ *FSMTVDGK ₁₀₈	iTRAQ8plex@N-term User modH on Asn(N)@6 iTRAQ8plex(K)@14	0.820	1.124	1.056	1.558	1.688	1.205	0.827	0.892
P20851 C4BB_HUMAN	809.88 (+2)	99	E ₅₁ WDN ₅₄ *TTTECR ₆₀	iTRAQ8plex@N-term User modH on Asn(N)@4 Carbamidomethyl(C)@9	0.729	1.140	1.131	2.658	2.183	1.578	2.651	1.264
P26927 HGFL_HUMAN	580.97 (+3)	99	G ₂₇₅ TAN ₂₇₈ *TTAGVPCQR ₂₈₈	iTRAQ8plex@N-term User modH on Asn(N)@4 Carbamidomethyl(C)@12	0.584	0.693	1.723	1.457	0.854	1.499	1.686	0.530
P29622 KAIN_HUMAN	833.43 (+2)	99	D ₂₁₂ FYVDEN ₂₁₈ *TTVR ₂₂₂	iTRAQ8plex@N-term User modH on Asn(N)@7	0.650	1.125	1.225	2.059	1.929	1.626	0.704	1.119
P29622 KAIN_HUMAN	671.71 (+3)	97	F ₁₃₅ LN ₁₃₇ *DTMAVYEAK ₁₄₆	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(K)@12	0.962	1.322	0.716	2.020	2.449	1.079	0.513	1.378
P41222 PTGDS_HUMAN	742.77 (+3)	99	S ₄₅ VVAPATDGGLN ₅₆ *LTSTFLR ₆₃	iTRAQ8plex@N-term User modH on Asn(N)@12	1.922	0.436	0.641	2.090	2.189	1.596	1.722	5.210
P41271 NBL1_HUMAN	532.04 (+4)	96	N ₂₃ *ITQIVGHSGCEAK ₃₆	iTRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@11 iTRAQ8plex(K)@14	0.000	0.000	3.000	8.502	3.879	7.788	7.225	13.264
P43251 BTD_HUMAN	714.88 (+2)	97	F ₁₀₈ N ₁₀₉ *DTEVLQR ₁₁₆	iTRAQ8plex@N-term User modH on Asn(N)@2	0.794	1.203	1.003	1.656	1.087	1.206	0.627	0.876
P43251 BTD_HUMAN	516.03 (+4)	95	L ₁₀₃ EPHRFN ₁₀₉ *DTEVLQR ₁₁₆	iTRAQ8plex@N-term User modH on Asn(N)@7	0.679	0.889	1.432	1.245	1.982	0.991	0.599	1.132
P49908 SEPP1_HUMAN	755.65 (+4)	99	C ₁₅₃ GN ₁₅₅ *CSLTTLKDEDFCKR ₁₆₉	iTRAQ8plex@N-term Carbamidomethyl(C)@1 User modH on Asn(N)@3 Carbamidomethyl(C)@4 iTRAQ8plex(K)@10 Carbamidomethyl(C)@15 iTRAQ8plex(K)@16	0.910	0.853	1.237	3.786	2.537	1.729	1.241	1.348

P49908 SEPP1_HUMAN	810.76 (+3)	99	E ₂₀ GYSN ₆₄ *ISYIVVNHQGISSR ₇₈	iTRAQ8plex@N-term User modH on Asn(N)@5 No User modH on Asn(N)@12	0.821	1.022	1.157	2.145	1.151	0.854	1.220	1.052
P49908 SEPP1_HUMAN	684.37 (+4)	99	E ₂₀ GYSN ₆₄ *ISYIVVNHQGISSR ₇₈	iTRAQ8plex@N-term User modH on Asn(N)@5 No User modH on Asn(N)@12 iTRAQ8plex(S)@17	0.630	1.282	1.088	1.386	1.727	1.250	1.251	0.813
P56199 ITA1_HUMAN	557.64 (+3)	97	Y ₄₃₁ N ₄₃₂ *HTGQVIYR ₄₄₁	iTRAQ8plex@N-term User modH on Asn(N)@2	0.777	0.784	1.439	2.467	1.531	1.953	1.342	2.176
P80108 PHLD_HUMAN	790.43 (+2)	99	L ₅₃₉ NVEAAN ₅₄₅ *WTVR ₅₄₉	iTRAQ8plex@N-term No User modH on Asn(N)@2 User modH on Asn(N)@7	0.583	1.060	1.356	1.219	1.435	0.539	0.427	1.716
Q04756 HGFA_HUMAN	726.36 (+2)	99	C ₂₅₁ FLGN ₂₅₅ *GTGYR ₂₆₀	iTRAQ8plex@N-term Carbamidomethyl(C)@1 User modH on Asn(N)@5	0.400	1.314	1.286	2.704	2.692	1.031	0.887	0.547
Q07954 LRP1_HUMAN	776.45 (+2)	99	I ₇₀₄ ETILLN ₇₁₀ *GTDR ₇₁₄	iTRAQ8plex@N-term User modH on Asn(N)@7	0.970	0.000	2.030	1.464	2.006	2.621	1.177	3.030
Q08380 LG3BP_HUMAN	552.31 (+3)	99	A ₆ LGFEEN ₅₁ *ATQALGR ₅₈	iTRAQ8plex@N-term User modH on Asn(N)@6	0.934	0.653	1.414	1.401	0.021	20.517	4.066	0.265
Q16610 ECM1_HUMAN	556.31 (+3)	99	H ₄₁₉ IPGLIHN ₄₂₅ *MTAR ₄₂₉	iTRAQ8plex@N-term User modH on Asn(N)@8	0.360	1.123	1.517	0.898	0.698	2.001	1.567	1.769
Q16620 NTRK2_HUMAN	557.67 (+3)	97	N ₆₄ *LTIVDSGLK ₇₃	iTRAQ8plex@N-term User modH on Asn(N)@1 iTRAQ8plex(K)@10	0.890	1.197	0.913	1.515	1.438	0.990	1.146	2.450
Q7Z7M0 MEGF8_HUMAN	557.6 (+3)	99	G ₁₀₇₈ YQGDGISHCN ₁₀₈₈ *R ₁₀₈₉	iTRAQ8plex@N-term Carbamidomethyl(C)@10 User modH on Asn(N)@11	0.610	1.173	1.217	3.370	3.158	2.085	2.272	3.006
Q86VB7 C163A_HUMAN	762.89 (+2)	99	A ₉ PGWAN ₆₄ *SSAGSGR ₇₁	iTRAQ8plex@N-term User modH on Asn(N)@6	1.047	1.094	0.859	1.133	2.172	6.332	3.750	8.565
Q92954 PRG4_HUMAN	592.83 (+2)	98	N ₁₁₃₅ *GTLVAFR ₁₁₄₂	iTRAQ8plex@N-term User modH on Asn(N)@1	0.551	0.666	1.783	2.643	0.980	1.873	2.154	0.459
Q96PD5 PGRP2_HUMAN	800.11 (+3)	99	G ₄₅₆ FGVAIVGN ₄₆₄ *YTAALPTEAA LR ₄₇₆	iTRAQ8plex@N-term User modH on Asn(N)@9	0.652	0.634	1.713	1.186	0.875	0.504	1.058	1.439
Q96PD5 PGRP2_HUMAN	855.46 (+4)	99	L ₃₂₅ EPVHLQLQCMSQEQLAQVAA N ₃₄₆ *ATK ₃₄₉	iTRAQ8plex@N-term Carbamidomethyl(C)@10 User modH on Asn(N)@22 iTRAQ8plex(K)@25	0.868	1.021	1.111	2.178	1.560	1.881	0.785	1.459
Q96PD5 PGRP2_HUMAN	734.42 (+3)	99	M ₃₃₅ SQEQLAQVAAN ₃₄₆ *ATK ₃₄₉	iTRAQ8plex@N-term User modH on Asn(N)@12 iTRAQ8plex(K)@15	0.691	0.972	1.336	0.711	1.343	0.892	0.328	1.084
Q96PD5 PGRP2_HUMAN	690.72 (+3)	98	S ₃₃₆ QEQLAQVAAN ₃₄₆ *ATK ₃₄₉	iTRAQ8plex@N-term User modH on Asn(N)@11 iTRAQ8plex(K)@14	0.637	0.890	1.474	0.611	0.970	1.056	0.384	0.874
Q9UJJ9 GNPTG_HUMAN	637.56 (+4)	99	Y ₅₇ EFCPFHN ₆₄ *VTQHEQTFR ₇₃	iTRAQ8plex@N-term Carbamidomethyl(C)@4 User modH on Asn(N)@8	0.362	1.205	1.433	0.933	1.452	2.662	1.422	4.179

^a The Confidence values in ProteinPilot software were shown. We set the peptide identification threshold as Conf. > 95. ^b The identified N-glycosylation sites were indicated by asterisks and their amino acid numbers were also shown. ^c Fold change values of the affinity to ConA lectin were calculated as relative differences from the average of three normal samples (N_{Avr}). The ratio of more than 2-fold or less than 0.5-fold change was highlighted in red or blue, respectively.

Supplementary Table 4

List of 274 proteins identified by the analysis of pre-ConA lectin purified samples and their fold change of the affinity to ConA lectin

Accessions	^a ProtScore	Name	^b 113/ Navr	^b 114/ Navr	^b 115/ Navr	^b 116/ Navr	^b 117/ Navr	^b 118/ Navr	^b 119/ Navr	^b 121/ Navr	^c Num. of Peptides
P0C0L5 CO4B_HUMAN	206.62	Complement C4-B precursor	0.590	0.766	1.215	1.137	0.939	1.046	1.616	0.845	840
P04114 APOB_HUMAN	177.48	Apolipoprotein B-100 precursor	0.710	1.095	0.931	1.190	0.997	1.034	1.214	0.840	920
P02751 FNC_HUMAN	162.64	Fibronectin precursor	0.557	1.010	1.038	1.395	1.280	0.971	1.389	0.875	674
P08603 CFAH_HUMAN	144.37	Complement factor H precursor	0.639	0.826	1.132	1.388	1.179	1.186	1.715	1.184	751
P02790 HEMO_HUMAN	137.49	Hemopexin precursor	0.594	0.751	1.132	1.068	0.954	1.025	1.128	1.285	701
P00450 CERU_HUMAN	133.31	Ceruloplasmin precursor	0.717	0.863	0.979	1.133	1.009	1.384	1.664	1.542	599
P00747 PLMN_HUMAN	105.78	Plasminogen precursor	0.628	1.008	1.104	1.402	1.147	0.756	1.342	1.183	416
P00734 THRB_HUMAN	96.29	Prothrombin precursor	0.717	1.074	0.927	1.291	1.075	0.777	0.976	0.882	363
Q14624 ITI4_HUMAN	92.85	Inter-alpha-trypsin inhibitor heavychain H4 precursor	0.660	0.971	0.998	1.255	0.994	1.230	1.317	1.289	429
P00751 CFAB_HUMAN	88.22	Complement factor B precursor	0.595	0.793	1.263	1.278	1.196	1.314	2.703	1.344	421
P02774 VTDB_HUMAN	83.45	Vitamin D-binding protein precursor	0.676	0.938	1.027	1.487	1.243	1.168	1.380	1.253	385
P10643 CO7_HUMAN	67.09	Complement component C7 precursor	0.607	0.901	0.899	1.359	1.560	2.279	1.705	4.228	258
P04003 C4BP_HUMAN	66.46	C4b-binding protein alpha chain precursor	0.564	1.005	1.056	1.099	1.146	1.014	2.605	1.079	256
P01024 CO3_HUMAN	63.79	Complement C3 precursor	0.733	0.757	0.910	0.919	0.980	2.574	1.426	4.599	405
P06396 GELS_HUMAN	62.19	Gelsolin precursor	0.654	0.991	1.005	1.220	1.179	1.154	1.324	1.002	335
P02647 APOA1_HUMAN	59.89	Apolipoprotein A-I precursor	0.854	1.131	0.943	0.756	0.483	0.273	0.260	0.367	333
P01042 KNG1_HUMAN	59.84	Kininogen-1 precursor	0.622	0.955	1.104	1.390	0.998	0.877	1.157	1.150	320
P04217 A1BG_HUMAN	59.79	Alpha-1B-glycoprotein precursor	0.595	0.887	1.071	1.180	0.950	1.288	1.352	1.082	315
P01031 CO5_HUMAN	59.37	Complement C5 precursor	0.620	0.981	1.062	1.115	1.011	1.275	1.484	1.469	284
P19827 ITI1_HUMAN	59.22	Inter-alpha-trypsin inhibitor heavychain H1 precursor	0.659	0.936	0.951	1.203	0.987	0.904	1.174	1.005	278
P06727 APOA4_HUMAN	59.06	Apolipoprotein A-IV precursor	0.655	0.940	1.083	1.370	1.057	0.904	0.972	0.934	288
P02765 FETUA_HUMAN	58.82	Alpha-2-HS-glycoprotein precursor	0.625	0.881	0.976	1.284	1.045	0.970	0.782	1.902	278
P01008 ANT3_HUMAN	56.02	Antithrombin-III precursor	0.633	1.069	0.888	1.470	0.986	0.932	1.071	1.185	359
P19823 ITI2_HUMAN	55.85	Inter-alpha-trypsin inhibitor heavychain H2 precursor	0.607	0.995	1.106	1.266	1.311	1.026	1.123	0.902	318
P02748 CO9_HUMAN	54.64	Complement component C9 precursor	0.560	0.835	1.194	1.155	0.873	1.366	2.577	1.313	168
P43652 AFAM_HUMAN	52.85	Afamin precursor	0.736	0.904	1.150	1.215	1.302	1.095	1.247	1.950	200
P02749 APOH_HUMAN	52.09	Beta-2-glycoprotein 1 precursor	0.620	0.921	1.170	1.270	0.971	0.953	1.090	0.754	228
O75882 ATRN_HUMAN	51.74	Attractin precursor	0.695	0.921	1.003	1.262	1.191	1.189	0.982	1.390	244
P01011 AACT_HUMAN	50.71	Alpha-1-antichymotrypsin precursor	0.572	0.913	1.136	1.088	0.933	1.243	1.999	1.149	285
P04275 VWF_HUMAN	50.57	von Willebrand factor precursor	0.638	0.826	1.000	1.185	1.186	1.322	1.401	1.366	314
P02671 FIBA_HUMAN	50.41	Fibrinogen alpha chain precursor	0.652	0.775	0.955	1.533	1.963	2.028	1.776	2.425	199
P00748 FA12_HUMAN	48.65	Coagulation factor XII precursor	0.790	0.907	1.059	1.087	1.520	1.217	1.009	1.099	160
P04004 VTNC_HUMAN	47.82	Vitronectin precursor	0.595	0.913	1.157	1.059	1.010	1.014	1.311	1.463	287
P13671 CO6_HUMAN	47.78	Complement component C6 precursor	0.631	0.862	1.134	1.318	1.068	1.243	1.393	1.002	193
P07996 TSP1_HUMAN	45.1	Thrombospondin-1 precursor	0.790	0.844	0.950	0.968	1.121	1.181	1.778	1.369	188
P04196 HRG_HUMAN	44.81	Histidine-rich glycoprotein precursor	0.449	1.028	1.048	1.347	1.177	1.072	1.014	0.785	164
P00736 C1R_HUMAN	44.44	Complement C1r subcomponent precursor	0.643	0.870	1.058	1.218	1.258	1.056	1.339	0.763	182
P10909 CLUS_HUMAN	43.74	Clusterin precursor	0.770	0.940	0.960	1.348	0.919	0.850	1.599	1.289	191
P09871 C1S_HUMAN	40.81	Complement C1s subcomponent precursor	0.584	0.950	1.033	1.374	1.278	1.202	1.385	0.935	166
P07358 CO8B_HUMAN	40.76	Complement component C8 beta chain precursor	0.761	0.876	0.982	1.396	1.259	0.863	1.240	0.949	126
P02649 APOE_HUMAN	38.75	Apolipoprotein E precursor	0.729	0.870	0.927	1.429	1.238	1.130	1.141	1.154	234
P03952 KLKB1_HUMAN	37.83	Plasma kallikrein precursor	0.589	0.820	1.199	1.474	0.784	0.650	1.076	1.173	138

P07357 CO8A_HUMAN	37.27	Complement component C8 alpha chain precursor	0.683	0.882	1.000	1.449	1.070	0.961	1.219	1.182	131
P06681 CO2_HUMAN	35.64	Complement C2 precursor	0.810	0.853	1.039	1.170	0.983	1.253	2.073	1.518	181
P05156 CFAI_HUMAN	34.47	Complement factor I precursor	0.672	0.702	1.157	1.491	1.245	1.248	2.241	1.522	147
P12259 FA5_HUMAN	34.15	Coagulation factor V precursor	0.688	0.914	1.016	1.436	1.209	1.286	1.628	1.311	306
Q96PD5 PGRP2_HUMAN	33.69	N-acetylmuramoyl-L-alanine amidase precursor	0.703	1.016	0.921	1.055	1.088	0.895	1.457	1.026	142
P02760 AMBIP_HUMAN	32.77	AMBIP protein precursor [Contains: Alpha-1-microglobulin	0.514	0.816	0.996	1.432	1.098	1.634	1.608	1.127	160
P05160 F13B_HUMAN	31.7	Coagulation factor XIII B chain precursor	0.684	0.879	1.153	1.290	1.413	1.079	1.312	1.338	95
P07225 PROS_HUMAN	30.35	Vitamin K-dependent protein S precursor	0.612	0.959	1.051	1.084	1.053	1.079	1.848	1.004	151
P05546 HEP2_HUMAN	29.22	Heparin cofactor 2 precursor	0.646	0.957	1.109	1.311	0.983	1.009	1.269	0.964	177
P26927 HGFL_HUMAN	28.69	Hepatocyte growth factor-like protein precursor	0.720	0.929	0.991	1.244	1.114	1.108	1.517	1.396	150
Q06033 ITIH3_HUMAN	27.69	Inter-alpha-trypsin inhibitor heavy chain H3 precursor	0.641	1.078	0.999	1.068	1.023	1.464	1.663	1.478	170
P01019 ANGT_HUMAN	27.55	Angiotensinogen precursor	0.568	0.855	1.143	1.057	0.987	0.707	0.973	1.135	97
P23142 FBLN1_HUMAN	26.59	Fibulin-1 precursor	0.795	0.846	1.009	1.104	1.186	0.976	1.061	1.185	103
P35858 ALS_HUMAN	26.02	Insulin-like growth factor-binding protein complex acid labile chain precursor	0.620	0.908	1.048	0.916	0.919	1.035	1.249	1.107	170
P08697 A2AP_HUMAN	23.98	Alpha-2-antiplasmin precursor	0.697	0.910	1.077	1.208	0.984	1.120	1.519	1.241	152
O75636 FCN3_HUMAN	23.44	Ficolin-3 precursor	0.585	1.001	1.135	1.111	1.002	1.012	1.068	1.267	86
P05155 IC1_HUMAN	22.65	Plasma protease C1 inhibitor precursor	0.476	0.700	1.432	1.887	1.167	1.332	1.241	1.349	138
P00738 HPT_HUMAN	22.63	Haptoglobin precursor	0.927	0.681	0.886	0.898	0.803	0.628	15.886	5.410	89
Q04756 HGFA_HUMAN	22.28	Hepatocyte growth factor activator precursor	0.518	0.884	1.029	1.406	1.277	1.124	1.244	0.952	116
P03951 FA11_HUMAN	21.99	Coagulation factor XI precursor	0.780	0.888	1.068	1.531	0.940	0.916	1.035	0.812	91
P01009 A1AT_HUMAN	21.23	Alpha-1-antitrypsin precursor	0.435	0.556	0.617	0.704	0.648	4.740	26.214	2.271	166
P22792 CPN2_HUMAN	21.12	Carboxypeptidase N subunit 2 precursor	0.689	0.840	1.203	1.088	1.151	1.053	1.176	1.199	101
P00742 FA10_HUMAN	20.7	Coagulation factor X precursor	0.656	0.773	1.204	1.161	1.171	1.727	2.263	0.928	90
P51884 LUM_HUMAN	20.18	Lumican precursor	0.808	0.963	0.914	1.332	1.277	1.330	1.391	1.355	95
P02743 SAMP_HUMAN	19.8	Serum amyloid P-component precursor	0.533	0.785	1.352	1.025	1.110	0.942	2.150	0.876	82
Q16610 ECM1_HUMAN	19.59	Extracellular matrix protein 1 precursor	0.539	0.918	1.024	1.304	1.175	1.607	2.748	1.227	104
Q14520 HABP2_HUMAN	19.32	Hyaluronan-binding protein 2 precursor	0.709	1.028	0.913	1.562	1.481	1.018	1.292	1.982	60
P36955 PEDF_HUMAN	19.29	Pigment epithelium-derived factor precursor	0.648	0.850	1.198	1.180	0.861	1.030	1.131	1.358	79
P07360 CO8G_HUMAN	18.58	Complement component C8 gamma chain precursor	0.711	0.818	1.003	1.072	1.005	0.593	1.079	1.078	77
P04278 SHBG_HUMAN	18.31	Sex hormone-binding globulin precursor	0.573	0.870	1.008	1.311	1.486	2.446	1.866	2.354	93
P02753 RETBIP_HUMAN	18.05	Plasma retinol-binding protein precursor	0.717	0.915	1.123	1.311	1.037	0.741	0.814	0.637	110
P00740 FA9_HUMAN	17.44	Coagulation factor IX precursor	0.643	0.981	1.192	1.124	1.175	1.104	1.394	1.373	82
P05543 THBG_HUMAN	17.39	Thyroxine-binding globulin precursor	0.771	1.005	0.952	1.011	1.272	1.454	1.027	1.936	77
P05452 TETN_HUMAN	17.21	Tetranectin precursor	0.586	0.735	0.929	0.785	1.191	0.643	0.992	0.627	52
P05154 PSP_HUMAN	16.67	Plasma serine protease inhibitor precursor	0.714	0.851	1.094	1.208	0.792	1.007	0.952	0.797	88
P00746 CFAD_HUMAN	16.14	Complement factor D precursor	0.622	1.035	1.109	1.140	1.101	1.217	1.905	1.239	64
Q86VB7 C163A_HUMAN	15.47	Scavenger receptor cysteine-rich type 1 protein M130 precursor	0.697	0.857	0.997	1.570	1.141	1.639	1.555	1.872	127
P48740 MASP1_HUMAN	15.33	Complement-activating component of Ra-reactive factor precursor	0.703	0.979	1.141	1.040	0.872	0.995	0.866	1.321	68
P22105 TENX_HUMAN	15.24	Tenascin-X precursor	0.672	0.956	0.982	1.200	0.998	1.010	1.329	1.549	585
Q96IY4 CBP2_HUMAN	15.24	Carboxypeptidase B2 precursor	0.790	0.978	0.936	1.296	0.999	0.892	1.478	1.253	71
P08571 CD14_HUMAN	14.94	Monocyte differentiation antigen CD14 precursor	0.730	0.845	1.045	0.786	0.855	0.889	0.980	1.104	79
P49747 COMP_HUMAN	14.92	Cartilage oligomeric matrix protein precursor	0.614	0.935	0.969	1.344	1.403	1.105	1.447	1.462	106
O00391 QSOX1_HUMAN	14.76	Sulfhydryl oxidase 1 precursor	0.631	0.998	0.990	1.570	1.365	1.202	1.181	1.269	181
P17936 IBP3_HUMAN	14.75	Insulin-like growth factor-binding protein 3 precursor	0.634	0.853	1.063	1.158	1.100	1.190	1.417	1.717	60
Q08380 LG3BP_HUMAN	14.29	Galectin-3-binding protein precursor	0.789	0.923	0.930	1.235	1.171	8.497	3.992	2.933	67
Q03591 FHR1_HUMAN	14.09	Complement factor H-related protein 1 precursor	0.661	0.943	1.075	1.288	0.949	1.248	1.846	1.152	131
P22891 PROZ_HUMAN	14.06	Vitamin K-dependent protein Z precursor	0.640	1.056	0.875	1.435	1.457	2.328	1.386	1.938	49
P29622 KAIN_HUMAN	14.05	Kallistatin precursor	0.624	0.976	0.772	1.218	0.955	0.662	1.330	1.051	86
P05362 ICAM1_HUMAN	13.82	Intercellular adhesion molecule 1 precursor	0.770	0.733	0.861	1.236	1.132	2.558	2.611	1.466	99
Q15113 PCOC1_HUMAN	13.72	Procollagen C-endopeptidase enhancer 1 precursor	0.679	0.987	0.992	1.220	1.201	1.208	2.037	1.359	79

P02747[C1QC_HUMAN	13.71	Complement C1q subcomponent subunit C precursor	0.687	0.872	1.200	0.877	0.963	1.385	3.356	1.563	50
Q9NQ79[CRAC1_HUMAN	13.46	Cartilage acidic protein 1 precursor	0.592	0.942	0.970	1.257	1.283	1.300	2.048	1.781	115
P01023[A2MG_HUMAN	13.22	Alpha-2-macroglobulin precursor	0.699	0.793	0.976	1.802	1.409	2.147	1.854	2.222	141
P13796[PLSL_HUMAN	12.85	Plastin-2	0.681	1.020	0.923	1.166	1.043	1.090	1.828	1.321	96
P80108[PHLD_HUMAN	12.8	Phosphatidylinositol-glycan-specific phospholipase D precursor	0.718	0.894	1.019	1.438	1.046	1.115	1.509	1.327	138
P98160[PGBM_HUMAN	12.65	Basement membrane-specific heparan sulfate proteoglycan core protein precursor	0.671	0.894	1.080	1.191	1.032	1.305	1.641	1.255	641
O43866[CD5L_HUMAN	12.5	CD5 antigen-like precursor	0.783	0.874	0.938	1.485	1.222	1.583	3.267	0.888	37
P02746[C1QB_HUMAN	12.4	Complement C1q subcomponent subunit B precursor	0.657	0.980	0.889	0.942	1.509	0.736	1.003	1.555	76
Q96KN2[CNDP1_HUMAN	12.31	Beta-Ala-His dipeptidase precursor	0.766	1.001	1.011	1.210	1.169	0.934	1.351	1.218	81
Q9UGM5[FETUB_HUMAN	12.26	Fetuin-B precursor	0.771	0.966	0.930	1.335	1.129	1.106	1.198	1.190	63
Q9Y6R7[FCGBP_HUMAN	12.04	IgGfC-binding protein precursor	0.638	0.878	1.004	1.140	1.064	0.946	1.215	1.283	262
P02652[APOA2_HUMAN	12.04	Apolipoprotein A-II precursor	0.737	1.109	0.815	0.641	0.441	0.639	2.200	0.674	62
P02675[FIBB_HUMAN	11.91	Fibrinogen beta chain precursor	0.612	0.969	0.856	1.112	1.715	3.117	0.882	1.195	64
P15169[CBPN_HUMAN	11.84	Carboxypeptidase N catalytic chain precursor	0.651	0.937	0.955	1.465	1.541	1.501	1.466	1.223	92
P02775[SCYB7_HUMAN	11.68	Platelet basic protein precursor	0.946	0.981	0.854	1.378	1.154	1.041	1.569	0.478	41
P27918[PROP_HUMAN	11.65	Properdin precursor	0.791	0.895	0.908	1.332	1.091	1.064	0.990	1.277	59
P11226[MBL2_HUMAN	11.53	Mannose-binding protein C precursor	0.808	0.867	0.959	1.936	0.817	1.260	1.272	1.389	41
P22352[GPX3_HUMAN	11.03	Glutathione peroxidase 3 precursor	0.923	0.829	0.955	1.192	1.311	1.306	1.516	1.118	41
P43251[BTD_HUMAN	10.84	Biotinidase precursor	0.852	0.910	0.922	1.203	1.090	1.280	1.305	1.181	57
P02750[A2GL_HUMAN	10.81	Leucine-rich alpha-2-glycoprotein precursor	0.726	0.804	1.051	1.284	0.836	1.586	2.786	1.339	93
P25311[ZA2G_HUMAN	10.33	Zinc-alpha-2-glycoprotein precursor	0.493	0.953	1.329	1.067	0.868	0.794	0.952	0.490	40
P01871[MUC_HUMAN	10.32	Ig mu chain C region	0.737	1.026	1.063	1.285	1.682	1.435	2.299	2.016	69
P09172[DOPD_HUMAN	10.25	Dopamine beta-hydroxylase	0.502	0.940	1.109	1.234	1.097	1.266	1.608	1.699	87
P02656[APOC3_HUMAN	9.82	Apolipoprotein C-III precursor	0.646	0.735	1.163	0.798	0.870	0.359	0.586	3.506	46
P22692[IBP4_HUMAN	9.73	Insulin-like growth factor-binding protein 4 precursor	0.581	0.932	1.160	1.126	1.769	1.322	2.361	0.855	46
P18065[IBP2_HUMAN	9.7	Insulin-like growth factor-binding protein 2 precursor	0.870	0.999	0.876	1.458	1.419	1.548	1.481	1.552	47
P00915[CAH1_HUMAN	9.44	Carbonic anhydrase 1	0.875	0.803	1.050	1.068	0.825	0.917	1.470	2.410	33
Q15582[BGH3_HUMAN	9.42	Transforming growth factor-beta-induced protein ig-h3 precursor	0.651	1.026	1.061	1.242	1.156	1.280	1.271	1.322	149
P63261[ACTG_HUMAN	9.42	Actin, cytoplasmic 2	0.754	0.840	1.054	1.070	0.915	1.179	1.551	0.834	68
P40197[GPV_HUMAN	9.4	Platelet glycoprotein V precursor	0.637	0.882	1.106	1.464	1.160	1.423	1.715	1.671	144
P33151[CADH5_HUMAN	9.13	Cadherin-5 precursor	0.614	1.091	0.949	1.539	1.441	1.138	1.365	1.690	78
P11021[GRP78_HUMAN	9.06	78 kDa glucose-regulated protein precursor	0.520	0.925	1.179	1.279	1.063	1.621	1.961	1.430	80
Q12805[FBLN3_HUMAN	8.55	EGF-containing fibulin-like extracellular matrix protein 1 precursor	0.659	0.946	1.126	1.738	1.470	1.015	2.108	1.571	78
P19320[VCAM1_HUMAN	8.32	Vascular cell adhesion protein 1 precursor	0.556	0.944	1.081	1.938	1.406	1.396	1.205	1.698	86
P09486[SPRC_HUMAN	8.08	SPARC precursor	0.789	0.847	1.114	1.295	1.103	0.757	1.550	1.679	24
Q92954[PRG4_HUMAN	7.9	Proteoglycan-4 precursor	0.691	0.787	1.034	1.304	1.077	1.236	1.783	1.035	129
P04180[LCAT_HUMAN	7.75	Phosphatidylcholine-sterol acyltransferase precursor	0.726	0.987	1.121	1.340	0.645	1.309	1.412	1.074	36
P06731[CEAM5_HUMAN	7.72	Carcinoembryonic antigen-related cell adhesion molecule 5 precursor	0.600	0.724	0.915	1.251	1.885	1.304	1.675	4.214	75
Q6EMK4[VASN_HUMAN	7.66	Vasorin precursor	0.676	0.904	1.078	1.302	1.186	1.805	1.928	1.417	114
P01034[CYTC_HUMAN	7.62	Cystatin-C precursor	0.614	0.918	1.125	1.420	1.154	1.149	1.554	1.424	35
Q15485[FCN2_HUMAN	7.51	Ficolin-2 precursor	0.670	0.952	1.214	1.571	1.429	0.837	1.202	1.006	55
Q76LX8[ATS13_HUMAN	7.43	ADAMTS-13 precursor	0.594	0.894	1.002	1.048	0.954	0.987	1.384	1.382	200
P15144[AMPN_HUMAN	7.36	Aminopeptidase N	0.674	0.986	1.144	1.118	0.849	0.974	1.033	1.036	92
Q9UK55[ZPI_HUMAN	7.26	Protein Z-dependent protease inhibitor precursor	0.675	0.937	1.047	1.405	1.144	1.355	1.433	1.370	88
P02741[CRP_HUMAN	7.25	C-reactive protein precursor	0.327	0.595	1.372	1.001	0.617	1.050	9.573	1.756	34

P04070 PROC_HUMAN	7.13	Vitamin K-dependent protein C precursor	0.496	1.076	1.018	1.382	1.201	0.961	0.886	1.043	44
O14791 APOL1_HUMAN	7.12	Apolipoprotein-L1 precursor	0.821	0.857	1.045	0.885	0.998	1.111	1.083	0.793	65
P27169 PON1_HUMAN	7.02	Serum paraoxonase/arylesterase 1	0.788	1.104	1.031	1.437	0.738	0.319	0.593	0.671	39
P02745 C1QA_HUMAN	7	Complement C1q subcomponent subunit A precursor	0.641	0.899	1.066	0.803	1.115	1.052	1.459	1.137	61
P32119 PRDX2_HUMAN	6.88	Peroxiredoxin-2	0.829	0.866	1.024	1.356	0.657	0.982	0.561	0.689	32
Q8NBP7 PCSK9_HUMAN	6.48	Proprotein convertase subtilisin/kexin type 9 precursor	0.668	0.876	0.929	0.937	0.823	0.981	1.819	2.226	91
P35542 SAA4_HUMAN	6.46	Serum amyloid A-4 protein precursor	0.705	0.915	1.014	0.714	0.737	0.583	0.528	1.465	41
P49908 SEPP1_HUMAN	6.44	Selenoprotein P precursor	0.634	0.865	1.158	1.817	1.220	0.939	0.770	0.587	35
P05062 ALDOB_HUMAN	6.42	Fructose-bisphosphate aldolase B	0.677	0.825	1.157	1.584	0.993	0.889	1.200	1.175	44
P02776 PLF4_HUMAN	6.32	Platelet factor 4 precursor	1.069	1.037	0.519	1.039	2.002	2.777	1.103	1.608	16
P08185 CBG_HUMAN	6.3	Corticosteroid-binding globulin precursor	0.633	0.975	1.039	1.260	1.372	0.905	1.228	1.072	51
P20851 C4B_HUMAN	6.2	C4b-binding protein beta chain precursor	0.461	1.142	1.157	1.349	0.759	1.039	1.393	0.641	25
P08709 FAT_HUMAN	6.16	Coagulation factor VII precursor	0.741	0.769	1.104	1.243	1.231	0.674	1.690	0.621	70
P02788 TRFL_HUMAN	6.01	Lactotransferrin precursor	0.629	0.996	1.024	1.101	1.054	1.207	2.522	1.126	77
P02792 FRIL_HUMAN	6.01	Ferritin light chain	0.576	0.855	1.124	0.864	1.028	1.909	3.421	1.160	27
O00533 CHL1_HUMAN	5.96	Neural cell adhesion molecule L1-like protein precursor	0.697	0.953	0.906	1.101	1.013	1.790	1.109	1.177	115
Q9UNW1 MINP1_HUMAN	5.9	Multiple inositol polyphosphate phosphatase 1 precursor	0.678	0.961	0.964	1.349	1.273	1.160	1.715	1.179	68
P36980 FHR2_HUMAN	5.8	Complement factor H-related protein 2 precursor	0.668	0.883	1.116	1.468	0.979	1.983	2.623	1.779	54
P68871 HBB_HUMAN	5.53	Hemoglobin subunit beta	1.014	1.173	0.813	0.788	0.439	0.485	1.711	0.631	16
P10124 SRGN_HUMAN	5.4	Serglycin precursor	0.785	0.881	0.734	0.616	1.229	0.476	1.410	1.513	15
Q9BXR6 FHR5_HUMAN	5.32	Complement factor H-related protein 5 precursor	0.775	0.871	1.081	1.148	1.084	1.061	2.239	0.836	88
P43121 MUC18_HUMAN	5.16	Cell surface glycoprotein MUC18 precursor	0.603	0.952	1.017	1.128	0.717	1.131	2.059	1.407	77
Q92820 GGH_HUMAN	5.1	Gamma-glutamyl hydrolase precursor	0.639	0.844	0.824	1.613	0.886	0.685	1.414	1.384	26
P07339 CATD_HUMAN	5.07	Cathepsin D precursor	0.438	0.879	1.392	1.325	1.030	1.744	1.428	1.939	31
P01033 TIMP1_HUMAN	5.05	Metalloproteinase inhibitor 1 precursor	0.673	0.796	1.070	1.442	1.216	1.443	1.390	0.659	26
P12830 CADH1_HUMAN	5	Epithelial cadherin precursor	0.617	1.027	0.996	1.297	0.997	1.074	1.108	1.659	75
P12955 PEPD_HUMAN	4.87	Xaa-Pro dipeptidase	0.646	0.934	0.944	1.152	1.039	1.066	1.997	2.799	63
Q9BW P8 COL11_HUMAN	4.75	Collectin-11 precursor	0.529	0.995	1.123	1.582	1.038	1.731	1.544	1.456	34
P06276 CHLE_HUMAN	4.73	Cholinesterase precursor	0.831	0.957	0.837	1.285	0.946	1.255	1.323	1.057	56
Q99784 NOE1_HUMAN	4.68	Noelin precursor	0.460	0.853	0.995	0.767	0.863	1.678	3.663	1.329	39
P05164 PERM_HUMAN	4.61	Myeloperoxidase precursor	0.790	0.906	0.977	1.224	1.063	0.888	1.540	1.308	156
O95810 SDPR_HUMAN	4.52	Serum deprivation-response protein	0.755	0.820	1.082	1.163	1.375	0.951	1.438	1.485	35
P41222 PTGDS_HUMAN	4.5	Prostaglandin-H2 D-isomerase precursor	0.617	1.044	0.993	1.296	1.060	1.784	3.631	1.201	26
P55058 PLTP_HUMAN	4.49	Phospholipid transfer protein precursor	0.768	1.067	1.032	1.025	1.225	2.014	1.258	2.013	90
Q7Z7G0 TARSH_HUMAN	4.46	Target of Nesh-SH3 precursor	0.863	0.869	0.833	1.208	1.206	1.964	1.625	2.009	69
P63104 I433Z_HUMAN	4.39	I4-3-3 protein zeta/delta	0.754	0.859	0.731	0.765	1.703	2.025	1.925	1.486	32
P54108 CRIS3_HUMAN	4.36	Cysteine-rich secretory protein 3 precursor	0.716	0.750	0.810	1.651	2.078	1.362	1.523	2.184	29
O00187 MASP2_HUMAN	4.29	Mannan-binding lectin serine protease 2 precursor	0.744	0.913	1.048	1.338	0.875	1.146	0.976	1.111	61
P55290 CAD13_HUMAN	4.28	Cadherin-13 precursor	0.721	0.892	1.074	1.078	1.006	1.150	1.187	1.011	67
P13592 NCA12_HUMAN	4.27	Neural cell adhesion molecule 1, 120 kDa isoform precursor	0.642	0.844	1.099	1.428	1.292	1.555	2.983	1.264	65
P15151 PVR_HUMAN	4.22	Poliovirus receptor precursor	0.643	0.856	1.283	1.285	1.120	1.025	2.029	1.869	55
P61626 LYSC_HUMAN	4.22	Lysozyme C precursor	0.952	0.721	0.861	1.094	0.992	1.743	0.974	1.569	45
P33908 MA1A1_HUMAN	4.19	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	0.524	0.927	0.989	1.165	1.059	0.907	1.967	1.177	75
P04264 K2C1_HUMAN	4.15	Keratin, type II cytoskeletal 1	0.572	0.887	0.955	1.022	0.850	1.204	1.111	1.506	133
P05067 A4_HUMAN	4.13	Amyloid beta A4 protein precursor	0.614	0.744	1.006	1.393	1.154	1.340	2.696	1.423	66

P02766 TTHY_HUMAN	4.12	Transthyretin precursor	0.708	0.669	1.270	1.918	1.228	0.519	1.061	1.030	17
Q12860 CNTN1_HUMAN	4.11	Contactin-1 precursor	0.647	0.860	0.921	0.969	1.038	1.412	1.363	1.792	84
P05109 S10A8_HUMAN	4.08	Protein S100-A8	0.652	0.839	1.279	1.007	1.232	1.818	2.711	1.546	13
P37802 TAGL2_HUMAN	4.05	Transgelin-2	0.634	0.895	0.989	1.296	1.668	0.992	0.974	1.504	56
Q9UJJ9 GNPTG_HUMAN	4.02	N-acetylglucosamine-1-phosphotransferase subunit gamma precursor	0.636	1.252	0.703	1.771	1.981	1.973	2.131	1.676	22
O75144 ICOSL_HUMAN	4.01	ICOS ligand precursor	0.486	0.988	1.065	1.131	0.732	0.980	1.528	0.974	39
P30043 BLVRB_HUMAN	4	Flavin reductase	0.390	0.819	1.191	1.160	1.051	1.397	1.809	1.151	25
P02763 A1AG1_HUMAN	4	Alpha-1-acid glycoprotein 1 precursor	0.621	0.818	0.879	1.115	0.625	1.785	4.068	1.836	22
Q99969 RARR2_HUMAN	4	Retinoic acid receptor responder protein 2 precursor	0.749	0.630	1.221	1.907	1.013	1.084	1.581	2.005	15
P14151 LYAM1_HUMAN	3.86	L-selectin precursor	0.589	1.402	1.008	1.378	1.071	0.659	0.854	1.140	19
Q13790 APOF_HUMAN	3.73	Apolipoprotein F precursor	0.617	0.979	1.063	1.104	0.983	1.728	1.310	1.022	44
P23470 PTPRG_HUMAN	3.7	Receptor-type tyrosine-protein phosphatase gamma precursor	0.637	0.822	1.083	0.932	0.869	0.972	1.672	1.265	144
P02679 FIBG_HUMAN	3.64	Fibrinogen gamma chain precursor	0.458	0.656	1.286	1.548	2.563	6.980	5.335	2.929	20
Q9UNN8 EPCR_HUMAN	3.6	Endothelial protein C receptor precursor	0.758	0.900	1.217	1.397	1.234	0.604	0.844	1.526	24
Q6UXB8 PI16_HUMAN	3.57	Peptidase inhibitor 16 precursor	0.664	0.812	1.095	1.002	0.925	1.381	1.814	1.793	49
P01344 IGF2_HUMAN	3.56	Insulin-like growth factor II precursor	0.851	1.132	0.910	0.897	1.118	0.536	0.652	1.052	28
Q86UD1 OAF_HUMAN	3.56	Out at first protein homolog precursor	0.981	0.799	0.886	0.898	1.103	1.180	2.397	4.797	18
P08195 4F2_HUMAN	3.53	4F2 cell-surface antigen heavy chain	0.642	0.893	0.897	1.621	1.318	1.121	1.164	1.079	74
P35443 TSP4_HUMAN	3.52	Thrombospondin-4 precursor	0.649	0.828	1.153	1.523	1.025	1.193	1.251	1.160	81
P50552 VASP_HUMAN	3.52	Vasodilator-stimulated phosphoprotein	0.595	0.715	1.202	1.419	0.900	2.142	2.043	0.589	43
Q13103 SPP24_HUMAN	3.52	Secreted phosphoprotein 24 precursor	0.397	0.936	1.047	0.914	1.131	1.382	3.892	1.091	29
P18428 LBP_HUMAN	3.41	Lipopolysaccharide-binding protein precursor	0.511	1.065	0.987	1.266	0.672	0.984	1.942	2.251	55
Q9Y6Z7 COL10_HUMAN	3.37	Collectin-10 precursor	0.498	0.921	1.307	1.081	1.309	1.430	1.112	1.626	33
P00338 LDHA_HUMAN	3.26	L-lactate dehydrogenase A chain	0.772	0.871	1.100	1.092	0.989	1.101	1.024	1.303	35
Q13822 ENPP2_HUMAN	3.2	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2 precursor	0.591	1.024	1.003	1.081	1.173	1.011	1.371	1.606	55
P04066 FUCO_HUMAN	3.11	Tissue alpha-L-fucosidase precursor	0.624	0.960	1.070	0.826	1.747	1.457	3.145	1.180	26
P08294 SODE_HUMAN	3.02	Extracellular superoxide dismutase [Cu-Zn] precursor	0.565	0.693	0.947	1.267	1.130	2.281	3.595	2.222	34
P02768 ALBU_HUMAN	2.95	Serum albumin precursor	0.838	0.980	0.982	2.245	1.362	1.030	1.551	1.517	60
P07737 PROF1_HUMAN	2.89	Profilin-1	1.008	0.666	1.326	1.782	1.341	0.924	0.480	0.812	14
P24592 IBP6_HUMAN	2.83	Insulin-like growth factor-binding protein 6 precursor	0.451	0.883	1.148	0.911	0.595	1.162	0.897	1.643	29
O95445 APOM_HUMAN	2.8	Apolipoprotein M	0.830	1.059	0.778	0.955	0.638	0.581	0.561	1.209	27
P00488 F13A_HUMAN	2.79	Coagulation factor XIII A chain precursor	0.628	0.874	0.939	1.324	0.797	1.156	1.534	0.941	75
P18206 VINC_HUMAN	2.78	Vinculin	0.636	0.953	1.071	1.203	0.973	1.133	1.074	1.276	150
P04746 AMYP_HUMAN	2.78	Pancreatic alpha-amylase precursor	0.746	1.019	1.039	1.310	0.768	1.065	1.197	0.944	46
P07333 CSF1R_HUMAN	2.77	Macrophage colony-stimulating factor 1 receptor precursor	0.717	0.881	0.968	1.186	1.103	1.345	1.513	1.240	76
Q15848 ADIPO_HUMAN	2.74	Adiponectin precursor	0.618	1.122	1.053	0.739	1.398	1.139	0.700	2.801	29
P02735 SAA_HUMAN	2.73	Serum amyloid A protein precursor	0.512	0.367	1.350	0.545	0.824	0.672	7.080	0.425	35
P12111 CO6A3_HUMAN	2.66	Collagen alpha-3(VI) chain precursor	0.660	0.888	1.054	1.112	1.059	1.094	1.598	1.136	551
Q9Y5C1 ANG13_HUMAN	2.44	Angiotensinogen-related protein 3 precursor	0.607	1.035	1.059	1.051	1.172	0.677	1.032	1.032	30
P14625 ENPL_HUMAN	2.41	Endoplasmic precursor	0.654	0.979	1.018	1.105	0.900	1.079	2.086	1.348	86
P36222 CH3L1_HUMAN	2.38	Chitinase-3-like protein 1 precursor	0.791	1.200	0.878	1.070	0.793	0.976	1.328	2.037	46
Q01459 DIAC_HUMAN	2.37	Di-N-acetylchitinase precursor	0.739	0.841	1.074	0.950	1.169	2.027	2.493	2.258	26
P08519 APOA_HUMAN	2.33	Apolipoprotein(a) precursor	0.614	0.951	0.980	1.247	1.210	0.742	1.503	1.596	165
P13688 CEAM1_HUMAN	2.33	Carcinoembryonic antigen-related cell adhesion molecule 1 precursor	0.656	0.790	1.042	1.414	2.641	1.984	1.802	3.551	41
P02654 APOC1_HUMAN	2.29	Apolipoprotein C-I precursor	1.024	1.202	0.514	1.379	0.685	0.420	0.622	0.428	23

Q13201 MMRN1_HUMAN	2.29	Multimerin-1 precursor	0.692	0.888	1.059	1.305	1.013	1.354	1.658	1.061	108
P01860 IGHG3_HUMAN	2.26	Ig gamma-3 chain C region	0.721	0.699	1.280	1.330	2.091	1.669	2.705	1.364	20
Q9NY97 B3GN2_HUMAN	2.18	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2	0.639	0.964	1.022	0.970	1.173	1.189	1.214	1.012	40
P52701 MSH6_HUMAN	2.15	DNA mismatch repair protein MSH6	0.710	0.901	1.044	1.109	0.935	1.437	2.123	1.283	130
P55103 INHBC_HUMAN	2.15	Inhibin beta C chain precursor	0.632	0.961	1.163	1.130	1.230	0.960	1.100	1.458	37
Q6UY14 ATL4_HUMAN	2.12	ADAMTS-like protein 4 precursor	0.685	0.846	1.019	1.187	1.028	1.008	1.709	1.154	200
P34096 RNAS4_HUMAN	2.1	Ribonuclease 4 precursor	0.693	1.175	0.982	1.580	1.539	0.726	2.280	1.984	20
Q8IW L1 SFTA2_HUMAN	2.04	Pulmonary surfactant-associated protein A2 precursor	0.747	0.714	0.939	0.824	1.144	5.029	2.145	0.968	45
P21333 FLNA_HUMAN	2.03	Filamin-A	0.618	0.920	1.044	1.313	1.146	1.163	1.685	1.369	194
P39060 COIA1_HUMAN	2.03	Collagen alpha-1(XVIII) chain precursor [Contains: Endostatin]	0.584	1.015	1.016	1.132	1.199	1.143	1.165	1.335	272
P01876 IGHA1_HUMAN	2.03	Ig alpha-1 chain C region	0.841	0.718	1.041	1.061	0.885	0.906	2.961	1.571	15
P35527 K1C9_HUMAN	2.02	Keratin, type I cytoskeletal 9	0.707	0.803	0.981	1.207	1.048	1.209	1.596	1.839	118
Q12913 PTPRJ_HUMAN	2.02	Receptor-type tyrosine-protein phosphatase eta precursor	0.544	0.914	1.007	1.165	1.064	1.159	1.931	1.183	101
Q10588 BST1_HUMAN	2.02	ADP-ribosyl cyclase 2 precursor	0.818	0.997	0.861	1.170	0.785	1.984	1.274	1.151	37
Q14515 SPRL1_HUMAN	2.02	SPARC-like protein 1 precursor	0.842	0.738	0.898	1.167	1.003	0.993	1.409	1.067	46
P62937 PIPIA_HUMAN	2.02	Peptidyl-prolyl cis-trans isomerase A	0.823	0.777	1.185	1.571	1.254	1.251	1.829	1.117	28
P69905 HBA_HUMAN	2.02	Hemoglobin subunit alpha	0.757	0.964	1.029	1.252	1.055	0.946	1.268	2.223	12
Q14118 DAG1_HUMAN	2.01	Dystroglycan precursor	0.696	0.946	1.047	1.400	1.076	1.070	1.350	0.890	87
Q5VY43 PEAR1_HUMAN	2.01	Platelet endothelial aggregation receptor 1 precursor	0.590	0.884	0.860	1.161	1.009	1.495	1.924	1.931	63
Q9BY67 CADM1_HUMAN	2.01	Cell adhesion molecule 1 precursor	0.707	0.956	0.988	1.071	2.302	1.492	1.371	1.515	43
Q16270 IBP7_HUMAN	2.01	Insulin-like growth factor-binding protein 7 precursor	0.487	1.031	1.251	1.220	1.321	0.948	0.899	0.546	26
Q86T10 TBCD1_HUMAN	2	TBC1 domain family member 1	0.685	0.982	1.062	1.176	1.002	1.287	2.287	2.058	133
Q9HDC9 APMAP_HUMAN	2	Adipocyte plasma membrane-associated protein	0.527	0.911	1.171	1.512	1.119	1.119	1.113	1.078	69
P31146 COR1A_HUMAN	2	Coronin-1A	0.851	0.906	0.823	1.271	0.974	2.045	1.448	1.480	50
O95497 VNN1_HUMAN	2	Pantetheinase precursor	0.737	0.655	0.761	1.013	0.781	0.760	1.552	1.659	39
Q9BTY2 FUCO2_HUMAN	2	Plasma alpha-L-fucosidase precursor	0.756	0.931	1.082	1.051	0.769	0.946	0.829	1.357	39
Q13740 CD166_HUMAN	2	CD166 antigen precursor	0.739	0.953	1.072	1.005	1.183	1.274	1.016	1.051	38
P04406 G3P_HUMAN	2	Glyceraldehyde-3-phosphate dehydrogenase	0.606	1.071	0.843	1.061	0.835	1.347	1.565	1.104	25
Q86YVW TRML1_HUMAN	2	Trem-like transcript 1 protein precursor	0.604	0.821	0.686	2.176	0.940	1.026	1.768	1.464	27
P08833 IBP1_HUMAN	2	Insulin-like growth factor-binding protein 1 precursor	0.731	0.742	0.955	0.619	1.011	1.231	0.977	1.764	21
P26992 CNTFR_HUMAN	2	Ciliary neurotrophic factor receptor alpha precursor	0.744	1.046	1.022	1.374	0.793	1.041	1.389	0.661	16
P10451 OSTP_HUMAN	2	Osteopontin precursor	0.777	0.954	0.740	1.453	0.964	1.941	1.887	1.740	17
Q14508 WFDC2_HUMAN	2	WAP four-disulfide core domain protein 2 precursor	0.248	0.859	0.892	1.057	0.330	9.133	3.720	1.114	6
P01834 KAC_HUMAN	2	Ig kappa chain C region	1.252	1.151	0.597	0.751	1.546	1.768	3.106	1.426	7
Q9Y566 SHAN1_HUMAN	1.96	SH3 and multiple ankyrin repeat domains protein 1	0.643	0.899	1.054	1.090	1.002	1.045	1.360	1.386	349
P02655 APOC2_HUMAN	1.95	Apolipoprotein C-II precursor	0.883	1.404	0.714	0.926	0.757	0.461	0.292	0.349	14
Q727M0 MEGF8_HUMAN	1.93	Multiple epidermal growth factor-like domains 8	0.670	0.816	1.039	1.361	1.233	1.139	1.726	1.278	221
P05090 APOD_HUMAN	1.9	Apolipoprotein D precursor	0.867	0.799	1.085	1.202	1.169	1.429	1.057	2.985	12
P08637 FCG3A_HUMAN	1.71	Low affinity immunoglobulin gamma Fc region receptor III-A precursor	0.824	0.906	1.009	0.670	1.136	1.421	1.416	2.238	23
P59665 DEF1_HUMAN	1.71	Neutrophil defensin 1 precursor	0.852	0.748	1.224	2.076	0.894	1.891	1.969	0.790	17
P11717 MPRI_HUMAN	1.68	Cation-independent mannose-6-phosphate receptor precursor	0.793	0.882	0.977	1.284	0.985	1.206	1.346	1.209	164
P14780 MMP9_HUMAN	1.63	Matrix metalloproteinase-9 precursor	0.634	0.785	1.141	0.907	0.870	1.433	1.668	2.002	75
P14543 NID1_HUMAN	1.54	Nidogen-1 precursor	0.733	0.924	0.977	1.111	1.019	1.440	1.343	1.079	131
Q9UBP4 DKK3_HUMAN	1.53	Dickkopf-related protein 3 precursor	0.737	1.083	0.895	1.468	1.072	1.947	1.053	0.988	21
Q14956 GPNMB_HUMAN	1.52	Transmembrane glycoprotein NMB precursor	0.761	0.972	1.002	1.641	1.134	2.035	1.343	1.115	34
P13987 CD59_HUMAN	1.41	CD59 glycoprotein precursor	0.923	0.729	1.348	1.339	1.022	0.641	0.810	0.738	3
P08253 MMP2_HUMAN	1.34	72 kDa type IV collagenase precursor	0.810	0.758	1.088	1.501	1.044	1.283	1.119	1.058	61

^a The ProtScore values in ProteinPilot software were shown. We set the protein identification threshold as ProtScore > 1.3 (the statistical confidence > 95%) and more than two peptide identifications for each protein. ^b The identified N-glycosylation sites were indicated by asterisks and their amino acid numbers were also shown. ^c Fold change values of the affinity to ConA lectin were calculated as relative differences from the average of three normal samples (N_{Avr}). The ratio of more than 2-fold or less than 0.5-fold change was highlighted in red or blue, respectively.

Supplementary Table 5

The list of 6 serum proteins identified from ConA purified samples but not from pre-ConA purified samples.

Accessions	Name	Concentration	^a Conf	m/z (z)	^b Sequence	Modifications
P0C0L4 CO4A	Complement C4-A precursor	145 ± 67 µg/ml (samples which expressed two or more C4A alleles), 40 ± 17 µg/ml (only one expressed allele), No C4A was detected (plasma samples which apparently did not express any C4A allele)(1)	99	796.08 (+3)	F ₂₀₀ SDGLESN ₂₀₇ *SSTQFEVK ₂₁₅	iTRAQ8plex@N-term User modH on Asn(N)@8 iTRAQ8plex(K)@16
			99	706.4 (+2)	G ₁₃₀₇ LN ₁₃₀₉ *VTLSTGR ₁₃₁₇	iTRAQ8plex@N-term User modH on Asn(N)@3
			99	572.67 (+3)	G ₁₃₀₇ LN ₁₃₀₉ *VTLSTGR ₁₃₁₇	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(S)@7
			99	572.67 (+3)	G ₁₃₀₇ LN ₁₃₀₉ *VTLSTGR ₁₃₁₇	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(S)@8
			99	754.08 (+3)	N ₁₃₇₂ *TTCQDLQIEVTVK ₁₃₈₅	iTRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@4 iTRAQ8plex(K)@14
Q07954 LRP1	Pro-low-density lipoprotein receptor-related protein 1 precursor	Healthy subjects: 6.1 ± 1.2 µg/ml, Patients with abnormal liver function: 7.1 ± 3.44 µg/ml, Patients with NIDDM: 6.5 ± 0.9 µg/ml(2)	99	776.45 (+2)	I ₇₀₄ ETILLN ₇₁₀ *GTDR ₇₁₄	iTRAQ8plex@N-term User modH on Asn(N)@7
P01859 IGHG2	Ig gamma-2 chain C region	IgG concentration Crohn's Disease:10.6 (4.3-25.9) g/l, Ulcerative Colitis:11.1 (4.8-25.6) g/l, Controls:10.3 (6.5-16.2)g/l (Mean values ± 2 SD)(3)	98	732.87 (+2)	E ₁₇₂ EQFN ₁₇₆ *STFR ₁₈₀	iTRAQ8plex@N-term User modH on Asn(N)@5
P56199 ITA1	Integrin alpha-1 precursor	>3.4 ng/mL (Normal individuals:5 of 8 serum samples, Patients with connective tissue diseases (CTD):3 of 8 serum samples, Patients with liver diseases (LD): 6 of 9 serum samples)(4)	97	557.64 (+3)	Y ₄₃₁ N ₄₃₂ *HTGQVVIYR ₄₄₁	iTRAQ8plex@N-term User modH on Asn(N)@2
Q16620 NTRK2	BDNF/NT-3 growth factors receptor precursor	N.A.	97	557.67 (+3)	N ₆₄ *LTIVDSGLK ₇₃	iTRAQ8plex@N-term User modH on Asn(N)@1 iTRAQ8plex(K)@10
P41271 NBL1	Neuroblastoma suppressor of tumorigenicity 1 precursor	N.A.	96	532.04 (+4)	N ₂₃ *ITQIVGHSGCEAK ₃₆	iTRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@11 iTRAQ8plex(K)@14

^a The Confidence values in ProteinPilot software were shown. We set the peptide identification threshold as Conf. > 95. ^b The identified N-glycosylation sites were indicated by asterisks and their amino acid numbers were also shown.

1.Chrispeels, J., Bank, S., Rittner, C., and Bitter-Suermann, D. (1989) *J Immunol Methods* **125**, 5-12

2.Quinn, K. A., Grimsley, P. G., Dai, Y. P., Tapner, M., Chesterman, C. N., and Owensby, D. A. (1997) *J Biol Chem* **272**, 23946-51

3.Weeke, B., and Jarnum, S. (1971) *Gut* **12**, 297-302

4.Bank, I., Weiss, P., Doolman, R., Book, M., and Sela, B. A. (1999) *J Lab Clin Med* **134**, 599-604

Supplementary Table 6

Relative affinity changes to ConA lectin column for 97 glycopeptides

Accessions	m/z (z)	*Conf.	^b Sequence	Modifications	^c 113/N avr	^c 114/N avr	^c 115/N avr	^c 116/N avr	^c 117/N avr	^c 118/N avr	^c 119/N avr	^c 121/N avr
P02790 HEMO_HUMAN	1022.03 (+2)	99	A ₄₂₄ LPQPQN ₄₃₀ *VTSLLGCTH ₄₃₉	iTRAQ8plex@N-term User modH on Asn(N)@7 Carbamidomethyl(C)@14	1.021	1.113	1.376	1.201	1.349	1.416	1.575	1.401
P02790 HEMO_HUMAN	541.24 (+4)	99	N ₂₁₇ *GTGHGN ₂₂₃ *STHHGPEYM R ₂₃₃	iTRAQ8plex@N-term User modH on Asn(N)@1 User modH on Asn(N)@7	1.159	1.009	1.371	1.847	1.370	1.554	1.017	0.910
P02790 HEMO_HUMAN	617.05 (+4)	99	N ₂₁₇ *GTGHGN ₂₂₃ *STHHGPEYM R ₂₃₃	iTRAQ8plex@N-term User modH on Asn(N)@1 User modH on Asn(N)@7 iTRAQ8plex(T)@9	1.711	0.760	1.246	0.770	1.686	1.225	0.845	0.665
P02790 HEMO_HUMAN	719.88 (+2)	99	P ₁₆₀ AVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@6	1.339	1.334	1.062	1.838	1.685	1.662	1.380	3.454
P02790 HEMO_HUMAN	672.68 (+3)	99	S ₁₅₈ W PAVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term iTRAQ8plex(S)@1 User modH on Asn(N)@7 Carbamidomethyl(C)@8	1.191	1.182	1.239	1.727	1.490	1.387	1.377	1.433
P02790 HEMO_HUMAN	571.29 (+3)	99	S ₁₅₈ W PAVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term User modH on Asn(N)@7 Carbamidomethyl(C)@8	1.062	1.140	1.337	1.474	1.482	1.597	1.180	1.297
P02790 HEMO_HUMAN	672.69 (+3)	99	S ₁₅₈ W PAVGN ₁₆₄ *CSSALR ₁₇₀	iTRAQ8plex@N-term User modH on Asn(N)@7 Carbamidomethyl(C)@8 iTRAQ8plex(S)@10	1.132	0.981	1.404	1.469	1.660	1.601	1.428	1.150
P04217 A1BG_HUMAN	1036.51 (+4)	99	E ₁₃₂ GDHEFLVPEAQEDVEATF PVHQPGN ₁₅₈ *YSCSYR ₁₆₄	iTRAQ8plex@N-term User modH on Asn(N)@27 Carbamidomethyl(C)@30	2.500	0.000	1.413	1.976	1.387	0.913	0.779	1.829
P04217 A1BG_HUMAN	874.62 (+6)	97	F ₃₂₈ QSPAGTEALFELHN ₃₄₂ *ISVA DSAN ₃₅₀ *YSCVVDLKPPFGGS APSER ₃₇₀	iTRAQ8plex@N-term User modH on Asn(N)@15 User modH on Asn(N)@23 Carbamidomethyl(C)@26 iTRAQ8plex(K)@32	2.166	0.677	1.037	1.837	1.602	2.628	1.793	3.563
P08603 CFAH_HUMAN	706.68 (+3)	99	C ₈₅₂ SQPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term Carbamidomethyl(C)@1 User modH on Asn(N)@13	0.862	1.073	1.381	1.284	0.955	1.149	1.096	0.962
P08603 CFAH_HUMAN	776.73 (+3)	99	I ₈₅₀ PCSQPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@15	0.811	0.786	1.619	1.079	0.993	1.209	1.011	1.039
P08603 CFAH_HUMAN	658.86 (+4)	99	I ₈₅₀ PCSQPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@15 iTRAQ8plex(S)@17	0.495	1.062	1.596	0.597	0.927	0.585	0.943	0.883
P08603 CFAH_HUMAN	725.02 (+3)	99	I ₈₅₉ SEEN ₈₉₃ *ETTCYMGK ₉₀₁	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@9 iTRAQ8plex(K)@13	0.926	1.124	1.308	1.408	1.644	1.449	1.258	1.143
P08603 CFAH_HUMAN	874.4 (+2)	99	M ₁₀₀₆ DGASN ₁₀₁₁ *VTCINSR ₁₀₁₈	iTRAQ8plex@N-term Oxidation(M)@1 User modH on Asn(N)@6 Carbamidomethyl(C)@9 No User modH on Asn(N)@11	1.172	1.019	1.246	1.377	1.177	1.511	1.246	1.599
P08603 CFAH_HUMAN	866.4 (+2)	99	M ₁₀₀₆ DGASN ₁₀₁₁ *VTCINSR ₁₀₁₈	iTRAQ8plex@N-term User modH on Asn(N)@6 Carbamidomethyl(C)@9 No User modH on Asn(N)@11	1.275	0.968	1.225	1.323	1.099	1.534	1.381	1.334
P08603 CFAH_HUMAN	653.34 (+3)	99	S ₈₅₃ QPPQIEHGTIN ₈₆₄ *SSR ₈₆₇	iTRAQ8plex@N-term User modH on Asn(N)@12	0.759	0.578	1.801	1.079	1.061	1.318	0.831	1.226
P00450 CERU_HUMAN	626.81 (+4)	98	E ₁₁₀ HEGAIYPDN ₁₁₉ *TTDFQR ₁₂₅	iTRAQ8plex@N-term iTRAQ8plex(Y)@7 User modH on Asn(N)@10	0.972	1.313	1.194	1.439	1.501	1.430	0.903	1.185
P00450 CERU_HUMAN	734.03 (+3)	99	E ₁₁₀ HEGAIYPDN ₁₁₉ *TTDFQR ₁₂₅	iTRAQ8plex@N-term User modH on Asn(N)@10	1.317	0.880	1.323	0.900	1.189	1.169	0.975	0.867
P00450 CERU_HUMAN	812.08 (+3)	99	E ₃₇₇ N ₃₇₈ *LTAPGSDSAVFEEQT TR ₃₉₆	iTRAQ8plex@N-term User modH on Asn(N)@2	1.991	0.563	1.110	1.049	1.060	1.425	0.994	0.608
P00450 CERU_HUMAN	699.38 (+4)	97	Y ₁₀₈ KEHEGAIYPDN ₁₁₉ *TTDFQR ₁₂₅	iTRAQ8plex@N-term iTRAQ8plex(K)@2 User modH on Asn(N)@12	0.616	1.099	1.644	1.294	1.848	1.311	0.920	0.857
Q96PD5 IPGR2_HUMAN	800.11 (+3)	99	G ₄₅₆ FGVAIVGN ₄₆₄ *YTAALPTEAA LR ₄₇₆	iTRAQ8plex@N-term User modH on Asn(N)@9	0.927	0.624	1.859	1.124	0.804	0.563	0.726	1.403

Q96PD5 PGRP2_HUMAN	855.46 (+4)	99	L ₃₂₅ EPVHLQLQCMSQEQLAQV AAN ₃₄₆ *ATK ₃₄₉	ITRAQ8plex@N-term Carbamidomethyl(C)@10 User modH on Asn(N)@22 ITRAQ8plex(K)@25	1.235	1.005	1.206	2.064	1.434	2.101	0.539	1.423
Q96PD5 PGRP2_HUMAN	734.42 (+3)	99	M ₃₃₅ SQEQLAQVAAN ₃₄₆ *ATK ₃₄₉	ITRAQ8plex@N-term User modH on Asn(N)@12 ITRAQ8plex(K)@15	0.983	0.956	1.450	0.674	1.234	0.996	0.225	1.057
Q96PD5 PGRP2_HUMAN	690.72 (+3)	98	S ₃₃₆ QEQLAQVAAN ₃₄₆ *ATK ₃₄₉	ITRAQ8plex@N-term User modH on Asn(N)@11 ITRAQ8plex(K)@14	0.906	0.876	1.600	0.579	0.891	1.179	0.264	0.852
P06681 CO2_HUMAN	822.39 (+3)	99	L ₄₃₈ TDTCGVGN ₄₄₇ *MSAN ₄₅₁ *A SDQER ₄₅₇	ITRAQ8plex@N-term Carbamidomethyl(C)@6 User modH on Asn(N)@10 Oxidation(M)@11 User modH on Asn(N)@14	0.508	1.071	1.613	2.231	2.253	0.676	0.456	2.452
O75882 ATRN_HUMAN	657.83 (+2)	98	G ₂₈₂ ICN ₂₈₅ *SSDVR ₂₉₀	ITRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@4	1.423	0.932	1.150	2.968	3.420	1.148	1.315	1.199
O75882 ATRN_HUMAN	813.44 (+2)	99	I ₅₉₆ DSTGN ₄₀₁ *VTNELR ₄₀₇	ITRAQ8plex@N-term User modH on Asn(N)@6 No User modH on Asn(N)@9	1.177	0.923	1.329	1.394	1.570	1.209	1.238	1.749
O75882 ATRN_HUMAN	886.11 (+3)	98	I ₂₄₇ SN ₂₄₉ *SSDTECECSENW _{K2} 63	ITRAQ8plex@N-term User modH on Asn(N)@3 Carbamidomethyl(C)@10 Carbamidomethyl(C)@12 No User modH on Asn(N)@15 ITRAQ8plex(K)@17	1.141	1.043	1.242	1.310	1.494	1.042	0.638	0.605
O75882 ATRN_HUMAN	614.64 (+3)	99	N ₇₁₆ *HSCSEQSISIFR ₇₂₈	ITRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@4	1.681	0.854	1.041	1.980	1.397	1.144	1.553	2.027
P02765 FETUA_HUMAN	628.6 (+4)	99	K ₁₂₆ VCQDCPLLAPLN ₁₃₈ *DTR ₁₄ 1	ITRAQ8plex@N-term ITRAQ8plex(K)@1 Carbamidomethyl(C)@3 Carbamidomethyl(C)@6 User modH on Asn(N)@13	1.225	1.420	1.008	1.691	1.623	1.685	1.144	1.280
P02765 FETUA_HUMAN	1040 (+2)	99	V ₁₂₇ CQDCPLLAPLN ₁₃₈ *DTR ₁₄₁	ITRAQ8plex@N-term Carbamidomethyl(C)@2 Carbamidomethyl(C)@5 User modH on Asn(N)@12	1.041	1.377	1.165	1.380	1.455	1.604	1.170	1.033
P01011 AACT_HUMAN	947.53 (+3)	99	T ₁₀₂ LN ₁₀₄ *QSSDELQLSMGNAM FVK ₁₂₁	ITRAQ8plex@N-term User modH on Asn(N)@3 Oxidation(M)@13 No User modH on Asn(N)@15 ITRAQ8plex(K)@20	1.639	0.000	1.815	2.100	2.403	3.097	1.409	1.345
P01011 AACT_HUMAN	788.79 (+3)	99	Y ₂₄₅ TGN ₂₄₈ *ASALFILPDQDK ₂₆₀	ITRAQ8plex@N-term User modH on Asn(N)@4 ITRAQ8plex(K)@16	1.067	0.874	1.401	0.842	1.299	2.515	0.570	0.445
P00734 THRB_HUMAN	764.41 (+2)	97	N ₃₉₂ *FTENDLLVR ₄₀₁	ITRAQ8plex@N-term User modH on Asn(N)@1 No User modH on Asn(N)@5	1.132	0.893	1.325	0.608	0.949	0.897	0.669	0.532
P00734 THRB_HUMAN	892.22 (+4)	99	S ₁₁₀ RYPHKPEIN ₁₁₉ *STTHPGAD LQENFCR ₁₃₄	ITRAQ8plex@N-term ITRAQ8plex(K)@6 User modH on Asn(N)@10 No User modH on Asn(N)@22 Carbamidomethyl(C)@24	1.305	1.325	0.691	2.095	1.512	1.355	1.712	1.669
P00734 THRB_HUMAN	1108.21 (+3)	99	Y ₁₁₂ PHKPEIN ₁₁₉ *STTHPGADLQ ENFCR ₁₃₄	ITRAQ8plex@N-term ITRAQ8plex(K)@4 User modH on Asn(N)@8 No User modH on Asn(N)@20 Carbamidomethyl(C)@22	1.277	0.977	1.114	1.333	1.575	1.509	1.480	1.843
P02751 FINC_HUMAN	564.75 (+4)	99	H ₅₀₄ EEGHMLN ₅₁₁ *CTCFGQGR ₅ 19	ITRAQ8plex@N-term Oxidation(M)@6 User modH on Asn(N)@8 Carbamidomethyl(C)@9 Carbamidomethyl(C)@11	1.362	0.748	1.433	0.668	1.084	1.168	0.505	0.010
P02751 FINC_HUMAN	747.36 (+3)	99	H ₅₀₄ EEGHMLN ₅₁₁ *CTCFGQGR ₅ 19	ITRAQ8plex@N-term User modH on Asn(N)@8 Carbamidomethyl(C)@9 Carbamidomethyl(C)@11	1.320	1.083	1.129	1.584	1.853	1.648	0.281	0.768
P02751 FINC_HUMAN	847.13 (+3)	99	L ₉₆₆ DAPTNLQFVN ₉₇₆ *ETDSTVL VR ₉₈₅	ITRAQ8plex@N-term No User modH on Asn(N)@6 User modH on Asn(N)@11	1.687	0.983	1.030	1.921	1.854	1.534	0.655	1.302

P02751 FINC_HUMAN	603.78 (+4)	99	R ₅₀₃ HEEGHMLN ₅₁₁ *CTCFGQG R ₅₁₉	iTRAQ8plex@N-term Oxidation(M)@7 User modH on Asn(N)@9 Carbamidomethyl(C)@10 Carbamidomethyl(C)@12	0.905	1.014	1.419	1.499	1.355	0.633	0.405	0.184
P02751 FINC_HUMAN	599.78 (+4)	99	R ₅₀₃ HEEGHMLN ₅₁₁ *CTCFGQG R ₅₁₉	iTRAQ8plex@N-term User modH on Asn(N)@9 Carbamidomethyl(C)@10 Carbamidomethyl(C)@12	0.480	0.380	2.264	1.396	1.710	1.330	0.953	0.000
P00751 CFAB_HUMAN	788.43 (+3)	99	L ₂₄₈ VLDGSDSIGASN ₂₆₀ *FTGAK 265	iTRAQ8plex@N-term User modH on Asn(N)@13 iTRAQ8plex(K)@18	1.056	0.875	1.329	0.889	0.785	1.468	0.892	1.374
P00751 CFAB_HUMAN	965.11 (+3)	99	S ₉₃ PYYN ₉₇ *VSDEISFHCYDGYT LR ₁₁₃	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@14	1.237	0.780	1.303	1.590	1.289	1.185	1.463	1.464
P00751 CFAB_HUMAN	800.15 (+4)	96	S ₉₃ PYYN ₉₇ *VSDEISFHCYDGYT LR ₁₁₃	iTRAQ8plex@N-term User modH on Asn(N)@5 iTRAQ8plex(S)@7 Carbamidomethyl(C)@14	0.758	1.681	0.962	0.669	1.341	1.226	1.331	1.445
P04004 VTNC_HUMAN	609.83 (+2)	99	N ₁₅₀ *GSLFAFR ₁₅₇	iTRAQ8plex@N-term User modH on Asn(N)@1	1.123	0.721	1.446	1.141	1.158	1.719	0.868	0.751
P07996 TSP1_HUMAN	558.64 (+3)	99	V ₁₀₄₇ VN ₁₀₄₉ *STTGPGEHLR ₁₀₅₉	iTRAQ8plex@N-term User modH on Asn(N)@3	1.318	1.214	0.982	1.623	1.225	0.440	0.287	0.339
P07996 TSP1_HUMAN	495.28 (+4)	98	V ₁₀₄₇ VN ₁₀₄₉ *STTGPGEHLR ₁₀₅₉	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(T)@5	1.088	1.517	0.904	0.748	1.113	0.260	0.449	0.226
P01008 ANT3_HUMAN	826.77 (+3)	99	L ₉₂ GACN ₉₆ *DTLQQLMEVFK ₁₀₇	iTRAQ8plex@N-term Carbamidomethyl(C)@4 User modH on Asn(N)@5 iTRAQ8plex(K)@16	1.089	0.962	1.443	1.063	1.427	1.006	0.970	1.746
P01008 ANT3_HUMAN	832.12 (+3)	98	L ₉₂ GACN ₉₆ *DTLQQLMEVFK ₁₀₇	iTRAQ8plex@N-term Carbamidomethyl(C)@4 User modH on Asn(N)@5 Oxidation(M)@12 iTRAQ8plex(K)@16	1.040	1.223	1.164	0.979	1.335	1.339	0.726	1.521
P01008 ANT3_HUMAN	930.48 (+3)	99	S ₁₅₁ LTFN ₁₅₅ *ETYQDISELVYGA K ₁₆₉	iTRAQ8plex@N-term User modH on Asn(N)@5 iTRAQ8plex(K)@19	1.394	1.296	0.825	1.574	2.011	1.419	1.143	1.902
P49908 SEPP1_HUMAN	755.65 (+4)	99	C ₁₅₃ GN ₁₅₅ *CSLTTLKDEDFCKR 169	iTRAQ8plex@N-term Carbamidomethyl(C)@1 User modH on Asn(N)@3 Carbamidomethyl(C)@4 iTRAQ8plex(K)@10 Carbamidomethyl(C)@15 iTRAQ8plex(K)@16	1.435	0.986	1.068	2.084	2.079	1.841	1.612	2.296
P49908 SEPP1_HUMAN	810.76 (+3)	99	E ₆₀ GYSN ₆₄ *ISYIVVNHQGISSR ₇₈	iTRAQ8plex@N-term User modH on Asn(N)@5 No User modH on Asn(N)@12	1.295	1.181	0.999	1.181	0.943	0.909	1.585	1.791
P49908 SEPP1_HUMAN	684.37 (+4)	99	E ₆₀ GYSN ₆₄ *ISYIVVNHQGISSR ₇₈	iTRAQ8plex@N-term User modH on Asn(N)@5 No User modH on Asn(N)@12 iTRAQ8plex(S)@17	0.994	1.482	0.939	0.763	1.415	1.331	1.625	1.384
P00748 FA12_HUMAN	626.97 (+3)	99	N ₄₁₄ *HSCEPCQTLAVR ₄₂₆	iTRAQ8plex@N-term User modH on Asn(N)@1 Carbamidomethyl(C)@4 Carbamidomethyl(C)@7	1.187	1.247	0.878	2.344	1.676	1.836	0.820	1.256
P00748 FA12_HUMAN	509.51 (+4)	99	R ₄₁₃ N ₄₁₄ *HSCEPCQTLAVR ₄₂₆	iTRAQ8plex@N-term User modH on Asn(N)@2 Carbamidomethyl(C)@5 Carbamidomethyl(C)@8	0.376	0.420	2.192	0.415	0.403	0.641	0.390	0.266
P17936 IBP3_HUMAN	720.88 (+2)	99	G ₈₅ LCVN ₈₉ *ASAVSR ₉₅	iTRAQ8plex@N-term Carbamidomethyl(C)@3 User modH on Asn(N)@5	1.512	0.959	1.152	1.123	1.150	0.732	0.591	0.903
P12259 FA5_HUMAN	698.39 (+2)	99	T ₂₁₇₉ WN ₂₁₈₁ *QSIALR ₂₁₈₇	iTRAQ8plex@N-term User modH on Asn(N)@3	0.953	1.208	1.221	0.997	1.198	1.087	1.155	1.277
P12259 FA5_HUMAN	716.42 (+4)	98	V ₂₅₅ SAITLVSATSTTAN ₂₆₉ *MTVG PEGK ₂₇₇	iTRAQ8plex@N-term User modH on Asn(N)@15 Oxidation(M)@16 iTRAQ8plex(K)@23	1.153	1.385	0.926	1.133	1.219	1.967	1.030	2.796
P02750 A2GL_HUMAN	722.12 (+3)	99	K ₁₄₃ LPPGLLAN ₁₅₁ *FTLLR ₁₅₆	iTRAQ8plex@N-term iTRAQ8plex(K)@1 User modH on Asn(N)@9	0.487	0.977	1.771	0.430	1.818	4.298	8.601	3.339

P02750 A2GL_HUMAN	653.32 (+2)	98	M ₂₈₆ FSQN ₂₉₀ *DTR ₂₉₃	iTRAQ8plex@N-term User modH on Asn(N)@5	0.588	0.996	1.686	0.530	2.433	6.324	6.919	2.442
P08571 CD14_HUMAN	489.61 (+3)	99	L ₁₃₀ RN ₁₃₂ *VSW ATGR ₁₃₉	iTRAQ8plex@N-term User modH on Asn(N)@3	1.490	0.483	1.439	3.118	1.677	1.403	2.605	2.692
P08571 CD14_HUMAN	599.32 (+2)	98	N ₁₃₂ *VSW ATGR ₁₃₉	iTRAQ8plex@N-term User modH on Asn(N)@1	0.964	1.200	1.227	1.338	1.611	2.396	2.305	2.358
P29622 KAIN_HUMAN	833.43 (+2)	99	D ₂₁₂ FYVDEN ₂₁₈ *TTVR ₂₂₂	iTRAQ8plex@N-term User modH on Asn(N)@7	1.041	1.153	1.586	1.691	2.021	2.455	0.529	1.064
P29622 KAIN_HUMAN	671.71 (+3)	97	F ₁₃₅ LN ₁₃₇ *DTMAVYEAK ₁₄₆	iTRAQ8plex@N-term User modH on Asn(N)@3 iTRAQ8plex(K)@12	1.541	1.355	0.927	1.659	2.565	1.629	0.386	1.311
P04278 SHBG_HUMAN	675.86 (+2)	97	L ₃₄₄ DVDQALN ₃₅₁ *R ₃₅₂	iTRAQ8plex@N-term User modH on Asn(N)@8	0.986	1.473	1.142	1.138	1.785	1.043	0.752	2.254
P05156 CFAI_HUMAN	686.35 (+3)	99	L ₄₄₂ FQPN ₄₄₆ *DTCIVSGW GR ₄₅₆	iTRAQ8plex@N-term User modH on Asn(N)@5 Carbamidomethyl(C)@8	0.625	0.247	2.080	0.451	0.764	1.710	0.766	0.361
P41222 PTGDS_HUMAN	742.77 (+3)	99	S ₆₃ VVAPATDGGGLN ₅₆ *LTSTFLR ₆₃	iTRAQ8plex@N-term User modH on Asn(N)@12	3.114	0.418	0.646	1.612	2.064	0.894	0.474	4.337
P04114 APOB_HUMAN	665.02 (+3)	99	F ₁₄₉₆ N ₁₄₉₆ *SSYLQGTNQITGR ₁₅₀	iTRAQ8plex@N-term User modH on Asn(N)@2 No User modH on Asn(N)@10	0.686	1.283	1.190	0.883	0.983	0.922	0.709	0.910
P43251 BTD_HUMAN	714.88 (+2)	97	F ₁₀₈ N ₁₀₉ *DTEVLQR ₁₁₆	iTRAQ8plex@N-term User modH on Asn(N)@2	0.932	1.322	1.088	1.376	0.997	0.942	0.480	0.742
P43251 BTD_HUMAN	516.03 (+4)	95	L ₁₀₃ EPHRFN ₁₀₉ *DTEVLQR ₁₁₆	iTRAQ8plex@N-term User modH on Asn(N)@7	0.797	0.977	1.553	1.035	1.818	0.774	0.459	0.958
Q08380 LG3BP_HUMAN	552.31 (+3)	99	A ₁₆ LGFEN ₅₁ *ATQALGR ₅₈	iTRAQ8plex@N-term User modH on Asn(N)@6	1.183	0.708	1.521	1.134	0.018	2.415	1.018	0.090
P04275 VWF_HUMAN	745.38 (+3)	99	G ₆₃₃ QVYLQCGTPCN ₆₄₄ *LTCR ₆₄₈	iTRAQ8plex@N-term Carbamidomethyl(C)@7 Carbamidomethyl(C)@11 User modH on Asn(N)@12 Carbamidomethyl(C)@15	1.006	0.680	1.795	0.829	1.388	1.461	1.091	1.313
P07357 CO8A_HUMAN	649.01 (+3)	98	G ₄₀₅ GSSGW SGGLAQN ₄₁₇ *R ₄₁₈	iTRAQ8plex@N-term iTRAQ8plex(S)@4 User modH on Asn(N)@13	1.062	0.976	1.414	1.206	1.330	1.221	1.266	1.159
P07357 CO8A_HUMAN	547.61 (+3)	99	G ₄₀₅ GSSGW SGGLAQN ₄₁₇ *R ₄₁₈	iTRAQ8plex@N-term User modH on Asn(N)@13	2.173	1.101	0.544	1.903	1.996	2.523	2.470	3.033
P20851 C4BB_HUMAN	809.88 (+2)	99	E ₅₁ WDN ₅₄ *TTTECR ₆₀	iTRAQ8plex@N-term User modH on Asn(N)@4 Carbamidomethyl(C)@9	1.580	0.999	0.978	1.970	2.876	1.519	1.903	1.971
P80108 PHLD_HUMAN	790.43 (+2)	99	L ₅₃₉ NVEAAN ₅₄₅ *W TVR ₅₄₉	iTRAQ8plex@N-term No User modH on Asn(N)@2 User modH on Asn(N)@7	0.812	1.186	1.331	0.848	1.372	0.484	0.283	1.293
P26927 HGFL_HUMAN	580.97 (+3)	99	G ₂₇₅ TAN ₂₇₈ *TTTAGVPCQR ₂₈₈	iTRAQ8plex@N-term User modH on Asn(N)@4 Carbamidomethyl(C)@12	0.811	0.746	1.738	1.171	0.767	1.353	1.111	0.380
P10909 CLUS_HUMAN	996.01 (+2)	99	L ₃₅₀ AN ₃₅₂ *LTQGEDQYYLR ₃₆₃	iTRAQ8plex@N-term User modH on Asn(N)@3	1.207	1.128	1.053	1.271	1.660	2.131	0.550	1.107
Q86VB7 C163A_HUMAN	762.89 (+2)	99	A ₅₉ PGW AN ₆₄ *SSAGSGR ₇₁	iTRAQ8plex@N-term User modH on Asn(N)@6	1.503	1.276	0.861	0.722	1.903	3.862	2.412	4.575
P19823 ITI2_HUMAN	695.71 (+3)	96	G ₉₅ AFISN ₁₀₀ *FSMTVDGK ₁₀₈	iTRAQ8plex@N-term User modH on Asn(N)@6 iTRAQ8plex(K)@14	1.351	1.130	0.955	1.230	1.287	1.174	0.737	0.989
P19823 ITI2_HUMAN	701.05 (+3)	99	G ₉₅ AFISN ₁₀₀ *FSMTVDGK ₁₀₈	iTRAQ8plex@N-term User modH on Asn(N)@6 Oxidation(M)@9 iTRAQ8plex(K)@14	1.252	1.310	0.847	1.346	1.097	1.585	1.266	0.580
P09172 DOPO_HUMAN	661.04 (+3)	99	S ₁₄₀ LEAIN ₁₄₅ *GSGLQMG LQR ₁₅₅	iTRAQ8plex@N-term User modH on Asn(N)@6	1.304	1.239	1.064	2.011	0.381	0.708	0.389	4.264
P09172 DOPO_HUMAN	666.36 (+3)	99	S ₁₄₀ LEAIN ₁₄₅ *GSGLQMG LQR ₁₅₅	iTRAQ8plex@N-term User modH on Asn(N)@6 Oxidation(M)@12	0.000	2.666	0.444	0.862	1.010	0.958	0.445	4.003
P01009 A1AT_HUMAN	789.78 (+3)	99	Y ₂₄₄ LGN ₂₄₇ *ATAIFFLPDEGK ₂₅₉	iTRAQ8plex@N-term User modH on Asn(N)@4 iTRAQ8plex(K)@16	0.000	0.000	0.000	0.000	0.000	3.416	3.993	0.357
P03951 FA11_HUMAN	654.71 (+3)	99	V ₄₂₆ YSGILN ₄₃₂ *QSEIK ₄₃₇	iTRAQ8plex@N-term User modH on Asn(N)@7 iTRAQ8plex(K)@12	0.588	1.343	1.263	1.287	1.744	0.000	1.509	1.027
Q727M0 MEGF8_HUMAN	557.6 (+3)	99	G ₁₀₇₈ YQGDGISHCN ₁₀₈₈ *R ₁₀₈₉	iTRAQ8plex@N-term Carbamidomethyl(C)@10 User modH on Asn(N)@11	0.910	1.438	1.171	2.477	2.561	1.830	1.317	2.352
Q04756 HGFA_HUMAN	726.36 (+2)	99	C ₂₅₁ FLGN ₂₅₅ *GTGYR ₂₆₀	iTRAQ8plex@N-term Carbamidomethyl(C)@1 User modH on Asn(N)@5	0.772	1.487	1.249	1.923	2.108	0.917	0.713	0.575

Q16610 ECM1_HUMAN	556.31 (+3)	99	H ₄₁₈ IPGLIHN ₄₂₅ *MTAR ₄₂₉	iTRAQ8plex@N-term User modH on Asn(N)@8	0.668	1.223	1.481	0.689	0.594	1.245	0.570	1.441
P05362 ICAM1_HUMAN	653.41 (+2)	99	A ₁₁₇ N ₁₁₈ *LTWLLR ₁₂₅	iTRAQ8plex@N-term User modH on Asn(N)@2	1.359	1.507	0.987	0.957	1.237	3.180	0.919	3.440
Q9UJJ9 GNPTG_HUMAN	637.56 (+4)	99	Y ₅₇ EFCPFHN ₆₄ *VTQHEQTFR ₇₃	iTRAQ8plex@N-term Carbamidomethyl(C)@4 User modH on Asn(N)@8	0.570	0.962	2.038	0.527	0.733	1.349	0.667	2.494
P05155 IC1_HUMAN	608.82 (+2)	99	D ₂₁₂ TFVN ₂₁₆ *ASR ₂₁₉	iTRAQ8plex@N-term User modH on Asn(N)@5	0.913	1.090	1.259	1.617	1.938	1.368	1.343	1.050
P01033 TIMP1_HUMAN	687.37 (+3)	99	F ₂₃ VGTPEVN ₃₀ *QTTLYQR ₃₇	iTRAQ8plex@N-term User modH on Asn(N)@8	0.601	1.492	1.316	1.614	1.626	2.695	2.238	9.126
O95445 APOM_HUMAN	952.47 (+3)	99	T ₁₂₁ ELFSSSCPGGIMLN ₁₃₅ *ETG QGYQR ₁₄₃	iTRAQ8plex@N-term Carbamidomethyl(C)@8 Oxidation(M)@13 User modH on Asn(N)@15	1.304	1.416	0.539	1.036	0.963	0.000	0.879	0.000
O95445 APOM_HUMAN	947.16 (+3)	99	T ₁₂₁ ELFSSSCPGGIMLN ₁₃₅ *ETG QGYQR ₁₄₃	iTRAQ8plex@N-term Carbamidomethyl(C)@8 User modH on Asn(N)@15	0.793	1.396	1.111	1.592	1.565	0.256	0.592	0.049
Q92954 PRG4_HUMAN	592.83 (+2)	98	N ₁₁₃₅ *GTLVAFR ₁₁₄₂	iTRAQ8plex@N-term User modH on Asn(N)@1	0.798	0.846	1.724	2.027	0.910	1.516	1.208	0.444
P06731 CEAM5_HUMAN	686.4 (+2)	98	T ₁₆₅ LTLFN ₁₇₀ *VTR ₁₇₃ T ₅₂₁ LTLFN ₅₂₆ *VTR ₅₂₉	iTRAQ8plex@N-term User modH on Asn(N)@6	0.000	0.000	3.277	0.000	0.000	2.607	0.000	46.724
P01042 KNG1_HUMAN	675.71 (+3)	96	I ₁₇₉ TYSIVQTN ₁₈₇ *CSK ₁₉₀	iTRAQ8plex@N-term User modH on Asn(N)@9 Carbamidomethyl(C)@10 iTRAQ8plex(K)@12	0.646	1.414	1.130	1.627	0.983	1.505	0.691	0.588

^a The Confidence values in ProteinPilot software were shown. We set the peptide identification threshold as Conf. > 95. ^b The identified N-glycosylation sites were indicated by asterisks and their amino acid numbers were also shown. ^c Fold change values of the affinity to ConA lectin were calculated as relative differences from the average of three normal samples (N_{Avr}). The ratio of more than 2-fold or less than 0.5-fold change was highlighted in red or blue, respectively.