Supporting Information

Glycomic analysis reveals a conserved response to bacterial sepsis induced by different bacterial pathogens.

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Sample Preparation for Mass Spectrometry
The excised gel band was cut into approximately 1mm³ pieces and destained by adding a 1:1 v/v destaining solution of methanol and 100 mM ammonium bicarbonate to the gel pieces and incubated at 37 °C for 15 minutes. The destaining solution was removed and fresh destaining solution added. This process was repeated until the blue stain was removed. The gel pieces were washed in 100% acetonitrile to partially dehydrate the band and further dehydrated in a SpeedVac concentrator. The proteins were reduced by adding 100 µL of 20 mM dithiothreitol and incubating for 1 hr at 57°C. Subsequently free cysteines were alkylated by adding 100 µL of 50 mM iodoacetamide and letting the solution incubate in the dark for 45 minutes. The alkylating solution was discarded and the gel pieces dehydrated again with a 100% acetonitrile wash and SpeedVac concentrator. The samples were digested overnight on a shaker with 250 ng trypsin in 100 mM ammonium bicarbonate at room temperature. The solution was removed and placed into a clean Eppendorf tube. An extraction buffer consisting of 1:2 v/v 5% formic acid/acetonitrile was added to the gel pieces and incubated on a shaker at 37 °C for 15 minutes. The solution extracted and combined with the previous extracted solution and this procedure repeated two more times. The combined solutions were concentrated using a SpeedVac concentrator to remove the organic solvent. The remaining solution was loaded onto equilibrated C18 microspin columns (Harvard apparatus) using a microcentrifuge for 30 sec at 6000 rpm. Sample tube was rinsed three times with 0.1%TFA and the solution passed through the microspin columns. Peptides were eluted into an Eppendorf tube using a solution of 40% acetonitrile in 0.5% acetic acid followed by the addition of 80% acetonitrile in 0.5% acetic acid. The samples were dried in a SpeedVac concentrator, reconstituted in 10 µL 0.5% acetic acid and stored at -80°C until analysis.

Mass Spectrometry Analysis
An aliquot of the sample was loaded onto a Acclaim PepMap trap column (2 cm x 75 μm) in line with an EASY-Spray analytical column (50 cm x 75 μm ID PepMap C18, 2 μm bead size) using the auto sampler of an EASY-nLC 1200 HPLC (Thermo Fisher Scientific). Solvent A consisting of 2% acetonitrile in 0.5% acetic acid and solvent B consisting of 80% acetonitrile in 0.5% acetic acid. Peptides were gradient eluted into a Thermo Fisher Scientific Orbitrap Eclipse mass spectrometer using the following gradient for Solvent B: 0-5% in 5 minutes, 5 - 35% in 60 min, 35 - 45% in 10 min, 45 - 100% in 10 min and 100 - 100% in 10 min. High resolution full MS spectra were acquired with a resolution of 120,000, scan range from 400 to 1500m/z, with a maximum ion time of 50ms, an AGC target of 4e5 and a dynamic exclusion of 30 seconds. The top 20 MS/MS spectra were collected with an AGC target of 2e5, maximum ion time of 200ms, one microscan, 2 m/z isolation window, and Normalized Collision Energy (NCE) of 27.

Data Processing
The MS/MS spectra were searched against the UniProt Mus musculus reference proteome database (downloaded 05/2019) containing common contaminant proteins using Sequest HT within Proteome Discoverer 1.4. The search parameters used were:
precursor mass tolerance ±10 ppm, fragment mass tolerance ±0.02 Da, enzyme trypsin (full) with two maximum missed cleavages, dynamic modification of oxidation on methionine and deamidation on glutamine and asparagine, static modification of carbamidomethyl on cysteine. The data was filtered using a 1% peptide and protein FDR cut off searched against a decoy database. Results were further filtered to only include only proteins identified by at least two unique peptides. The raw files are accessible at https://massive.ucsd.edu under MassIVE accession MSV000088518.
Supplementary Figure S1. Heat map of lectin microarray data for sera from SPN infected mice. Median normalized log₂ ratios (Sample (S)/Reference (R)) of mouse sera samples were ordered by uninfected, early, and late sepsis.
Supplementary Figure S2. Heat map of lectin microarray data for sera from MRSA infected mice. Median normalized log$_2$ ratios (Sample (S)/Reference (R)) of mouse sera samples were ordered by uninfected, early, and late sepsis.
Supplementary Figure S3. Volcano plot depicting changes in glycan abundances between septic and uninfected animals infected with SPN. Statistically significant lectins specific for core 1/3 O-glycans (blue) and bisecting GlcNAc (pink) are labeled. The dashed red line indicates $p < 0.05$ by the Students t-test.
Supplementary Figure S4. Volcano plot depicting changes in glycan abundances between septic and uninfected animals infected with MRSA. Statistically significant lectins specific for core 1/3 O-glycans (blue) and bisecting GlcNAc (pink) are labeled. The dashed red line indicates $p < 0.05$ by the Students t-test.
Supplementary Figure S5. Bisecting GlcNAc levels decrease during sepsis (SPN and MRSA). Box plot analysis of bisecting GlcNAc lectin binding by PHA-E. *P*-values derive from Student’s t-test (* p < 0.05, ** < 0.01, *** < 0.001). For consistency, the same probe of PHA-E is shown.
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Supplementary Figure S11. MPA Lectin Blots and Corresponding Ponceaus. The entire lectin blots for data shown in Figure 4 are shown along with Ponceau analysis that confirms even loading.
Supplementary Figure S12. Western Blot of Fibronectin and ITIH2 before and after MPA pulldown, and Corresponding Ponceaus. The entire Western Blot for data present in Figure 5c are shown along with Ponceau.
Supplementary Figure S13. Western Blot of Fibronectin and ITIH2, and Lectin Blot of MPA for sera from MRSA and ST infected mice with Corresponding Ponceaus. The entire Western Blot for data present in Figure 5d-e are shown along with Ponceau.
## Supplementary Table 1. Lectins used in microarrays

| Lectin       | Species/Origin                     | Print Conc. (µg/mL) | Rough Specificity /Inhibitory monosaccharide | Vendor/Source       |
|--------------|-----------------------------------|---------------------|---------------------------------------------|---------------------|
| AAL ¹        | *Aleuria aurantia*                | 1000                | Fucose                                      | Vector             |
| ACA ¹        | *Amaranthus Caudatus*             | 1000                | Gal-β1,3-GalNAc / Lac                       | Vector             |
| AIA ¹        | *Artocarpus integrifolia*        | 500                 | β1,3-GalNAc                                 | Vector/EY          |
| AMA ¹        | *Allium moly*                    | 500                 | Oligo mannose                               | EY                 |
| Anti-B.G.H2 ¹| MAb mouse IgM [A46-B/B10]         | undiluted           | Blood group H2 antigen                      | Santa Cruz Biotechnology |
| Anti-CD15 ¹  | MAb mouse IgM [MY-1]              | undiluted           | Lewis X                                    | Abcam              |
| Anti-Forssman ¹ | MAb Rat IgM [117C9]       | undiluted           | Forssman Antigen                           | Abcam              |
| Anti-Lewis B | IgM [T218]                        | undiluted           | Lewis B                                    | Sigma              |
| Anti-Lewis X ¹ | MAb mouse IgM [P12]         | undiluted           | Lewis X                                    | Abcam              |
| Anti-Lewis Y ¹ | MAb mouse IgM [F3]          | undiluted           | Lewis Y                                    | Abcam              |
| Anti-MUC5AC human ¹ | MAb mouse IgG1 [CLH2] | undiluted           | human MUC5AC                               | Sigma              |
| Anti-MUC5AC mouse | Goat polyclonal to mouse MUC5AC | undiluted           | mouse MUC5AC                               | LSBio              |
| Anti-Mucin 15 ¹ | MAb mouse IgG1 [H-5]        | undiluted           | Mucin 15                                   | Santa Cruz Biotechnology |
| Anti-Sialyl Lewis A ¹ | MAb mouse IgG1 | undiluted           | Sialyl Lewis A                             | Abcam              |
| Anti-Sialyl Lewis X ¹ | MAb mouse IgM | undiluted           | Sialyl Lewis X                            | Abcam              |
| AOL ¹        | *Aspergillus oryzae*            | 1000                | Fucose                                     | TCI America        |
| APA ¹        | *Abras precatorius*             | 500                 | Gal-β1,3-GalNAc / Lac                      | EY                 |
| ASA ¹        | *Allium sativum*                | 1000                | Mannose                                    | EY                 |
| Blackbean ¹  | *Blackbean crude*               | 1000                | GalNAc                                     | EY                 |
| BPA ¹        | *Bauhinia purpurea*             | 500                 | β-Gal / β-GalNAc                           | Vector             |
| BR6          | unknown (from unpublished work) | 480                 | under investigation                        | Gift from Dr. Barbara Bensing |
| CA           | *Colchicum autumnale*           | 1200                | Bi-antennary N-linked glycans              | EY                 |
| CAA ¹        | *Caragana arborescens*          | 1000                | Bi-antennary N-linked glycans              | EY                 |
| Calsepa ¹    | *Calystegia sepium*             | 1000                | Bisecting N-linked glycans                 | EY                 |
| CCA ¹        | *Cancer antennarius*            | 1000                | 9-O-Acetylated sialylation / 4-O-Acetyl sialylation | EY                 |
| Cholera Toxin | Vibrio cholerae | 1000 | GM1 ganglioside | Sigma |
| Con A | Canavalia ensiformis | 1000 | Tri-mannose core | EY/Vector |
| CSA † | Cystisus scoparius | 1000 | Terminal GalNAc | EY |
| DBA † | Dolichos Biflorus | 1000 | GalNAc | Vector |
| diCBM40 † | engineered Nani from Clostridium perfringens | 1000 | α Sialylation | Generated in house |
| DSA † | Datura stramonium | 500 | LacNAc | EY/Vector |
| ECA † | Erythrina cristagalli | 1000 | LacNAc | Vector |
| EEL/EEA † | Eunonymus europaeus | 1000 | Blood Group B | Vector/EY |
| GafD † | recombinant GafD from Escherichia coli | 1000 | GlcNAc | Generated in house |
| GHA † | Glechoma hederacea | 500 | GalNAc | EY |
| GNA/GNL † | Galanthus nivalis | 1500 | Oligo mannose | Vector/EY |
| GS-I † | Griffonia simplicifoia-I | 1000 | α-Gal / Lac | Vector/EY |
| GS-II † | Griffonia simplicifoia-II | 1000 | GlcNAc | Vector |
| GS-IB4 † | Griffonia simplicifoia-I, isolectin B4 | 2000 | Gal | Vector |
| H84T † | Banana lectin | 1000 | High mannose | Gift from Dr. David Markovitz |
| HAA † | Homarus americanus | 1000 | Terminal GalNAc | EY |
| HHL † | Hippeastrum Hybrid | 1500 | Oligo/High mannose | Vector |
| HPA † | Helix pomatia | 1000 | Blood Group A | Sigma/EY |
| IRA † | Iris Hybrid | 1000 | GalNAc / Lac | EY |
| LAA | Laburnum alpinum | 900 | GlcNAc | EY |
| LBA † | Phaseolus lunatus | 1000 | Blood Group A | EY |
| LcH † | Lens Culinaris | 1000 | Core Fucose | Vector |
| LEA/LEL † | Lycopersicon esculentum | 1000 | GlcNAc | Vector/EY |
| LFA † | Limax flavus | 500 | α Sialylation | EY |
| Lotus † | Lotus tetragonolobus | 1000 | Fucose | Vector |
| MAA † | Maackia amurensis | 500 | Sialylation/Sulfation | EY |
| MAL-I † | Maackia amurensis-I | 2000 | Sialylation/Sulfation | Vector |
| Source  | Species/Type                  | ohio2 | Sialylation/Sulfation          | Vector       |
|---------|------------------------------|-------|-------------------------------|--------------|
| MAL-II  | *Maackia amurensis-II*       | 2000  | Sialylation/Sulfation          | Vector       |
| MNA-G   | *Morus nigra Morniga G*      | 1000  | GalNAc                        | EY           |
| MNA-M   | *Morus nigra Morniga M*      | 1000  | Oligo mannose / Gal           | EY           |
| MPA/MPL | *Maclura pomifera*           | 1000  | β1,3-GalNAc                    | Vector       |
| NPA     | *Narcissus pseudonarcissus*  | 1000  | Oligo mannose                 | Vector       |
| PA-I    | *Pseudomonas aeruginosa*     | 1000  | Gal                           | Sigma        |
| PA-IL   | bacteria                     | 1000  | GalNAc                        | Generated in house |
| PHA-E   | *Phaseolus vulgaris*         | 1000  | Bisecting GlcNAc              | Vector/EY/Sigma |
| PHA-L   | *Phaseolus vulgaris*         | 1000  | β1,6 Branching N-Link glycans | Vector/EY/Roche |
| PMA     | *Polygonatum multiflorum*    | 500   | Oligo mannose                 | EY           |
| PNA     | *Arachis hyogaea*            | 1000  | Gal-β1,3-GalNAc               | Vector/EY    |
| PSA     | *Pisum sativum*              | 1000  | Core Fucose                    | Vector       |
| PSL     | *Polyporus squamosus*        | 1000  | α2,6 sialylation              | EY           |
| PTA     | *Psophocarpus tetragonolobus*| 500   | Blood Groups                  | EY           |
| PTL-I   | *Psophocarpus tetragonolobus-I*| 1500  | Blood Group A                 | Vector       |
| PTL-II  | *Psophocarpus tetragonolobus-II*| 1000  | α2 Fucose                     | Vector       |
| RCA120  | *Ricinus Communis Agglutinin I*| 1000  | Gal / Lac                     | Vector       |
| rCVN    | Recombinant Cyanovirin       | 1000  | High mannose                  | Gift from Dr. Barry O'Keefe |
| rGRFT   | Recombinant Griffithsin      | 1000  | High mannose                  | Gift from Dr. Barry O'Keefe |
| Ricin B Chain | *Ricinus communis* | 1000  | Gal                           | Vector       |
| RPA     | *Robinia pseudoacacia*       | 500   | Complex N-link glycans        | EY           |
| rSVN    | Recombinant Scytovirin       | 1000  | High mannose                  | Gift from Dr. Barry O'Keefe |
| SBA     | *Glycine max*                | 1000  | LacdiNAc                      | Vector       |
| SJA     | *Sophora japonica*           | 1000  | LacdiNAc                      | Vector       |
| SK1     | *Streptococcus sanguinis SK1*| 1800  | α2,3 sialylation              | Gift from Dr. Barbara Bensing |
| SK678   | *Streptococcus sanguinis SK678*| 450   | α2,3 sialylation              | Gift from Dr. Barbara Bensing |
| SLBR-B  | *Streptococcus gordonii M99* | 1000  | α2,3 sialylation              | Gift from Dr. Barbara Bensing |
| SLBR-H  | *Streptococcus gordonii DL1* | 2000  | α2,3 sialylation              | Gift from Dr. Barbara Bensing |
| SLBR-N  | *Streptococcus gordonii UB10712*| 1000  | α2,3 sialylation              | Gift from Dr. Barbara Bensing |
| SNA     | *Sambucus nigra*             | 500/1000 | α2,6 sialylation              | Vector/Sigma |
| Lectin  | Species / Genus         | Concentration | Carbohydrate Pattern | Supplier          |
|---------|-------------------------|---------------|----------------------|-------------------|
| SNA-II  | *Sambucus nigra*-II     | 1000          | α2 Fucose / oligo mannose | EY                |
| STA/STL | *Solanus tuberosum*     | 500           | GlcNAc               | Vector            |
| TJA-I   | *Trichosanthes japonica*-I | 1000         | α2,6 sialylation     | TCI               |
| TJA-II  | *Trichosanthes japonica*-II | 1000      | α2 Fucose            | NorthStar Bioproducts/Aniara Diagnostica |
| TL      | *Tulipa sp.*            | 700           | GlcNAc               | EY                |
| UDA     | *Urtica dioica*         | 1000          | GlcNAc / Oligo mannose | EY                |
| UEA-I   | *Ulex europaeus*-I      | 1000          | α2 Fucose            | Vector            |
| UEA-II  | *Ulex europaeus*-II     | 2000          | GlcNAc               | Vector            |
| VFA     | *Vicia faba*            | 1000          | GlcNAc               | EY                |
| VVA     | *Vicia villosa*         | 1000          | Terminal GalNAc      | Vector/EY         |
| VVA(man) | *Vicia villosa*         | 500           | Mannose              | Vector/EY         |
| X408    | unknown (from unpublished work) | 1000   | under investigation | Gift from Dr. Barbara Bensing |
| WFA     | *Wisteria floribunda*   | 1000          | GalNAc-β1,4          | Vector            |
| WGA     | *Triticum vulgare*      | 1000          | GlcNAc               | Vector/EY         |

1: lectins printed in the first set of lectin microarrays
2: lectins printed in the second set of lectin microarrays
Supplementary Table 2. Lectin Microarray Information

| **Description*** |  |
|------------------|---|
| **1. Sample: Glycan-containing sample (e.g. glycan, glycoprotein, cell lysate etc.)** |  |
| **Description of Sample** | Sera was collected from uninfected mice as well as mice infected with different types of bacteria (EC, ST, MRSA, SPN). Sera was collected at two different time points for the infected mice that correspond to early and late sepsis that are defined by c.f.u in the blood. All samples were collected in the Marth Laboratory at UCSD. |
| **Sample preparation protocol** | Sera samples were collected in the Marth Laboratory at UCSD and shipped to NYU and supplemented with protease inhibitor cocktails. |
| **Labelling protocol for sample detection** | Samples were labelled with Alexa Fluor 555-NHS (Thermo Fisher). Serum protein concentrations were determined using the DC assay. 50 µg of protein were labelled for each individual sample following the manufacturers protocol. |
| **Two-color reference (if used)** | Reference samples were created for each bacterial experiment and labeled with Alexa Fluor 647-NHS. For SPN, MRSA and ST, a bacteria-specific reference sample was prepared by mixing equal amounts of sera from all 48 animals used in each study. For EC, a master reference was created from the sera samples from the EC, ST and SPN experiments. |
| **Assay protocol** | Lectin microarrays are blocked with blocking buffer for one hour at room temperature. Slides are rinsed twice with PBST (0.005%) and once with PBS, then dry the slide using a slide spinner. Each slide was mounted on a 24-well format hybridization cassette (Arrayit), in which each well contains a subarray. To each well, add equal amounts of samples and universal reference, and dilute with PBS and PBST (0.2%) to reach the final volume (150µL). Incubate the slides on an orbital shaker for two hours at room temperature in the dark. After hybridization, wash the arrays with PBST (0.005%) twice for ten minutes, and twice for five minutes. Once finished, remove the slides from the cassette, and immerse the slides in ultrapure water, and dry the slides using a slide spinner. |
2. Lectin Library

| General description of the lectin library used in the array | Lectin microarrays are generated in house. |
|------------------------------------------------------------|--------------------------------------------|
| List of lectins and glycan binding proteins, source, concentration and buffer | Please see Supplementary Table 1. |
| Modification of lectins (e.g. biotin) if any. | N/A |

3. Immobilization Surface; e.g., Microarray Slide

| Immobilization surface | Nexterion Slide H Barcoded 3D Hydrogel Coated |
|------------------------|---------------------------------------------|
| Manufacturer           | Shott North America                          |
| Custom preparation of surface | N/A |

4. Array Production

| Description of Arrayer | Nano-Plotter 2.1 piezoelectric printer (GeSim, Germany) with cooled microwell plate holder and cooled printing deck |
|------------------------|-------------------------------------------------------------------------------------------------------------|
| Lectin deposition      | Three replicates of each lectin are printed onto each subarray. |
| Printing conditions    | Dilute lectins to the pre-determined concentrations in the print buffer (final concentration of print buffer: 0.01% Tween-20, 1mM monosaccharide in PBS; Please see Supplementary Table 1 for the concentrations of lectins). Load the mixed solution to the microplate. Before printing, check the humidity of the print chamber. The humidity should be kept around 50% during the entire printing. Ensure both microwell plate holder and printing deck are cooled. Adjust the cooling temperature based on ambient temperature and the temperature of the cooled slide deck surface, preventing moisture building up inside the print chamber. Once printing is complete, allow the slides to dry for at least one hour. |
| Array layout           | For each microarray, it contains 24 subarrays (3 columns and 8 rows). In each subarray, triplicates of a lectin are printed, and for a row with five lectins, the spot layout should be 15 columns. The row number depends on how many lectin probes are printed on the arrays (i.e., 110 lectins require 22 rows). |
**Quality control**  
Well-characterized glycoproteins including fetuin, asialofetuin and RNase B are used for quality assurances of the printed microarrays.

| 5. Detector and Data Processing |
|---------------------------------|
| **Instrument (scanner, flow cytometer)** | Fluorescent Slide Scanner Genepix 4300A (Molecular Devices) |
| **Instrument settings** | Preview the slide to adjust photomultiplier gain (PMT) for each channel (Alexa Fluor-555: 532nm, Alexa Fluor-647: 635nm) so that the signals are not saturated and within the linear detection range. |
| **Image analysis software** | GenePix Pro 7 (Molecular Devices) |
| **Data processing and statistical analysis** | Extracted data is processed for quality checks using Grubbs outlier test with $\alpha = 0.05$. Log$_2$ values of the average signals are median-normalized over the individual subarray in each channel. |

| 6. Lectin Microarray Data Presentation |
|----------------------------------------|
| **Data presentation and interpretation** | Hierarchical clustering of the processed data is performed using Pearson Correlation coefficient, and visualized with Multiexperiment Viewer (MeV, v4.8, TM4 Microarray Software Suite). If a lectin’s SNR (signal-to-noise ratio) $< 3$ for more than one third of the total samples, then this lectin is considered as inactive and excluded from the list. $P$-values are calculated using nonparametric statistical tests, which are generated by R (v3.6.1). |

| 7. Data Location |
|------------------|
| **Data Location** | doi: [10.7303/syn26387481](10.7303/syn26387481) |
Supplementary Table 3. Mass spectrometric analysis of MPA enriched proteins present in mouse septic samples

| Accession | Description                                                                 | Score | # PSMs | # AAs | MW [kDa] | calc. pl |
|-----------|-----------------------------------------------------------------------------|-------|--------|-------|----------|----------|
| Q9CQT7    | Desumoylating isopeptidase 1 (Desi1)                                        | 282.18| 156    | 168   | 18.4     | 4.94     |
| P63017    | Heat shock cognate 71kDa protein (Hspa8)                                     | 129.48| 36     | 646   | 70.8     | 5.52     |
| Q61696    | Heat shock 70kDa protein 1A (Hspa1a)                                        | 127.00| 37     | 641   | 70.0     | 5.72     |
| Q61703    | Inter-alpha-trypsin inhibitor heavy chain H2 (Itih2)                         | 64.54 | 30     | 946   | 105.9    | 7.27     |
| P63268    | Actin, gamma-enteric smooth muscle (Actg2)                                  | 33.47 | 10     | 376   | 41.8     | 5.48     |
| P68373    | Tubulin alpha-1C chain (Tuba1c)                                             | 28.91 | 11     | 449   | 49.9     | 5.10     |
| P11276    | Fibronectin (Fn1)                                                           | 23.16 | 10     | 2477  | 272.4    | 5.59     |
| Q61702    | Inter-alpha-trypsin inhibitor heavy chain H1 (Itih1)                         | 16.87 | 7      | 907   | 101.0    | 6.96     |
| Q61781    | Keratin, type I cytoskeletal 14 (Krt14)                                      | 15.03 | 6      | 484   | 52.8     | 5.17     |
| P16627    | Heat shock 70 kDa protein 1-like (Hspa1)                                    | 11.80 | 10     | 641   | 70.6     | 6.24     |
| Q61786    | Keratin, type II cytoskeletal 1b (Krt77)                                     | 10.72 | 5      | 572   | 61.3     | 8.02     |
| Q3UV17    | Keratin, type II cytoskeletal 2 oral (Krt76)                                 | 9.48  | 4      | 594   | 62.8     | 8.43     |
| P50446    | Keratin, type II cytoskeletal 6A (Krt6a)                                    | 6.17  | 3      | 553   | 59.3     | 7.94     |
| Q922U2    | Keratin, type II cytoskeletal 5 (Krt5)                                       | 6.09  | 3      | 580   | 61.7     | 7.75     |
| Q91X72    | Hemopexin (Hpx)                                                             | 5.41  | 2      | 460   | 51.3     | 7.80     |
| P28666    | Murinoglobulin-2 (Mug2)                                                     | 3.67  | 2      | 1451  | 162.3    | 6.74     |

Proteins labeled in red were removed from our final analysis as they were either a) unglycosylated or b) proteins that were clearly due to contamination (e.g. keratins).