Analysis of peptides by mass spectrometry. Peptides derived from avidin were analyzed by a MALDI-TOF/TOF tandem mass spectrometer (ABI 4700 Proteomics Analyzer, Applied Biosystems, Foster City, CA) and peptides from serum samples were analyzed by a nanoLC-LTQ-orbitrap (ThermoFisher Scientific, Waltham, MA). All LC-MS runs were carried out in triplicate and the results were combined in the data processing step.

For nanoLC-LTQ-orbitrap analysis, an Agilent 1200 solvent delivery system was coupled to the LTQ-orbitrap. Peptides from mouse sera were reconstituted with 1% acetonitrile and 0.1% formic acid and applied to a fused and fritted silica capillary pre-column of 100 μm internal diameter (New Objectives, Woburn, MA) packed with 2 cm of 200 Å pore-size C18 resin. Samples were subsequently washed with solvent A (5 % acetonitrile in 0.1 % formic acid) on the pre-column, eluted with a gradient of 10-35% solvent B (100% acetonitrile) over 30 minutes to a 75 μm x 10 cm fused silica capillary column packed with 100 Å pore-size Magic C18AQ™ (Michrom Bioresources, Auburn, CA), and then injected into the LTQ-orbitrap with a nano-ESI source. Eluting peptides were analyzed in MS by data-dependent acquisition, in which the 5 most abundant precursor ions were selected for MS/MS fragmentation with a dynamic exclusion setting of 1 repeat count in 30 sec for 180-sec exclusion, and the size of exclusion list is 50 [1].

MS/MS database search, inference and quantitation of sample proteins. The Trans-Proteomic Pipeline (TPP) software suite [2] was used to analyze the RAW data files. In brief, the raw files were converted to mzXML format with ReAdW software (version 4.0.2), and the spectra having fewer than 6 ions with intensity less than 100 were discarded [3, 4]. The converted mzXML files were searched against the mouse IPI database (v3.82) appended with common contaminants by SEQUEST version 27 (revision 0, copyright 2003). The parameters used for searching were following: minimum 1 tryptic terminus and up to two miscleavages [5];

monoisotopic mass for precursor search, and average mass for MS/MS search; mass tolerance of ±3.0 amu for precursor mass, and 0.5 amu for MS/MS;[6] static modification of carbamidomethylated cysteine, and variable modifications of oxidized methionines. For labeled N-termini and lysines, a static modification of +113.08406 and a variable modification of +7.01717 were used; and for glycofraction, a variable modification of converting the asparagine in the consensus sequence to aspartic acid was applied.[7] Peptide quantitation was achieved by XPRESS.[8] PeptideProphet™ [9] and Protein Prophet [10] were used to infer protein identification and quantification.

Evaluating the labeling efficiency of the N-isotag. We tested the labeling efficiency of our N-isotag by labeling a commercial protein, chicken avidin, with both heavy and light formed N-isotags. The labeled peptides were mixed at 1:1 ratio and the MS analyses were carried out by a MALDI-TOF/TOF instrument. All the detected peptide peaks were modified by the tag and exhibited the expected ratio with a standard deviation around 15% (Figure S1).

Two global methods to assess the accuracy and precision of the relative protein quantity obtained by XPRESS. In the first method, the glycoprotein XPRESS ratios are calculated separately from both glycan-bearing peptides and the non-glycopeptides. In such case, the same protein's quantities from two separated MS analyses were compared to assess the precision of our proteomics measurement. The result is shown as a scatter plot in Figure S2. Evenly distributed data points around the theoretical line (y=x) indicates a good agreement between the two quantitation approaches. A few exceptions do observed, may contributed to the spectra counting error as glycopeptides tend to be low abundance with few spectra counts.

In the second global validation approach, the spectral counting is used as an orthogonal approach to assess the accuracy of XPRESS analysis. First, spectral counts of the light- and

heavy-isotope-labeled proteins were collected separately. Then, the ratios of light/heavy-protein counts were computed and plotted against the XPRESS ratio as shown in Figure S3 below. Again, a consistency is observed by the tightness of measured data points around the theoretical line (y=x). Collectively, the protein XPRESS ratios we obtained here were reliable.

Pathway analysis. We used Genego, a commercial pathway analysis software, to analyze the enriched pathways and molecular functions in our dataset. The enriched pathways and molecular functions are compared between total identified proteins and proteins with more than 2-fold quantity changes in Figure S4. Metabolic and catabolic pathways and molecular functions that are representative in organs such as the liver, kidney brain, heart and muscle were highlighted in our serum proteome, indicating many tissue proteins were leaked or excreted into blood. What accompanied with these metabolic and catabolic pathways was the decrease of the complement system, which may be exhausted in blood by interacting with a large number of tissue proteins and/or may be contributed by the damaged replenishing source, e.g. liver. These results suggest a suppressed immunoresponse in blood during a catastrophic xenobiotic insult. The tissue remodeling and wound-healing pathways of the early recovery had been listed as shown in Figure S4C. The increased and decreased protein categories showed differential responses in these pathways, in which a decrease of the cell mobility and an increase of the tissue extracellular matrix development were denoted.

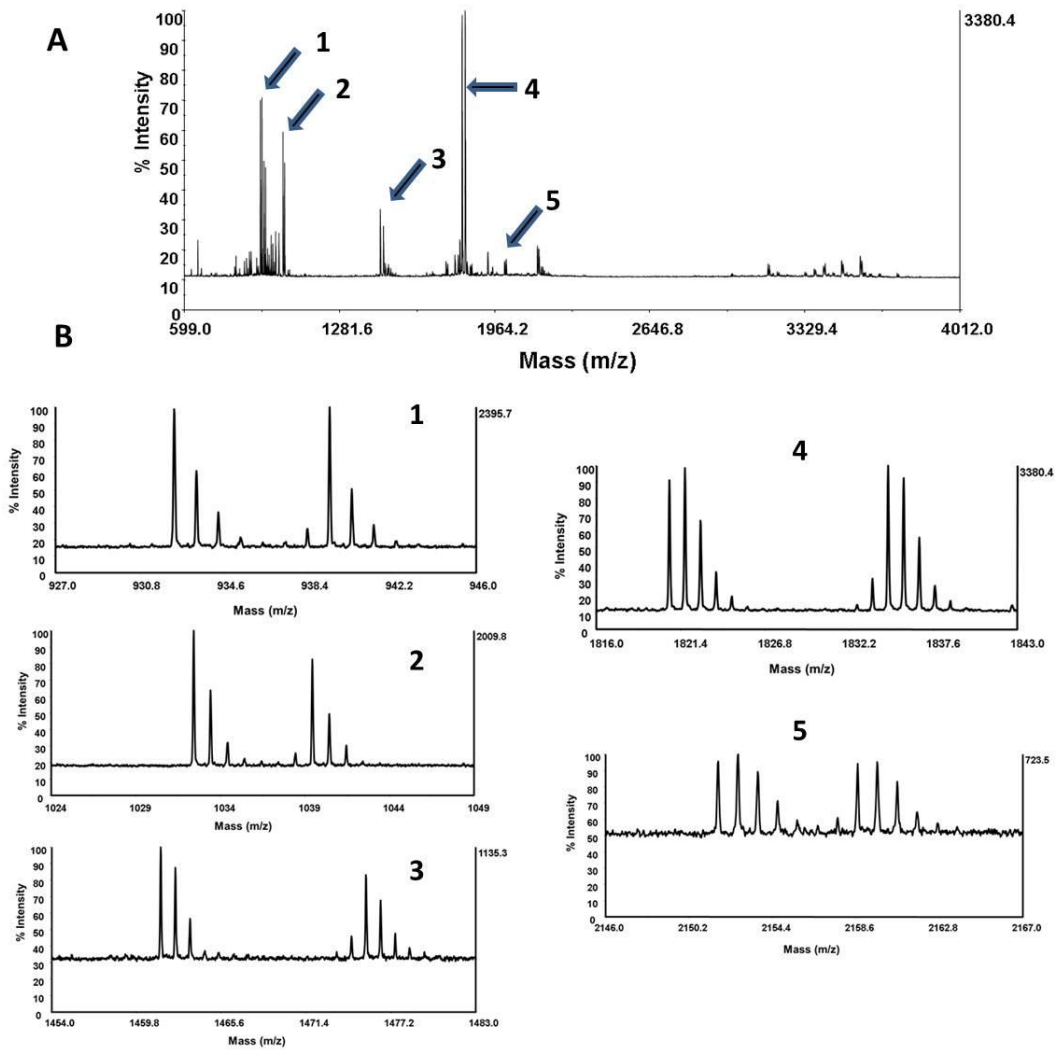

Figure S1. A, MALDI-TOF/TOF characterization of the N-isotag labeled tryptic peptides derived from chicken avidin at 1:1 (heavy:light) ratio; B, enlarged views arrow-pointed regions in panel A.

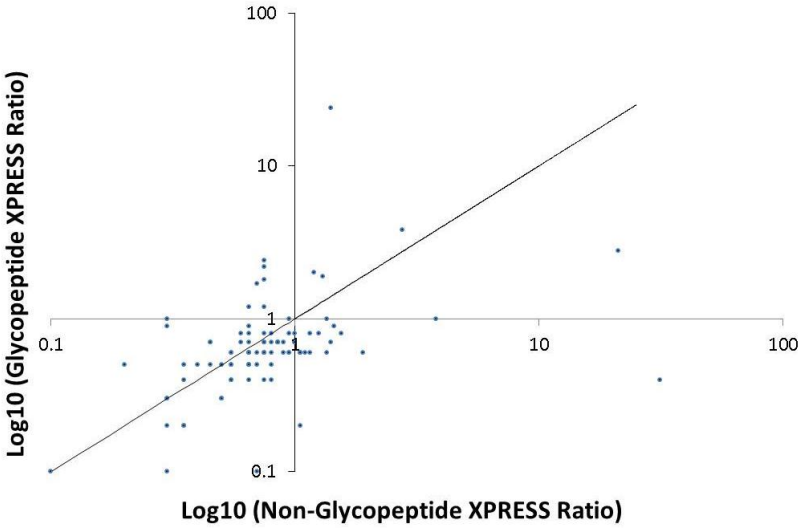

Figure S2. Logarithmic scatter plot of glycoprotein XPRESS ratios obtained from glycopeptides and non-glycopeptides of the same protein. The straight line is theoretical, y=x.

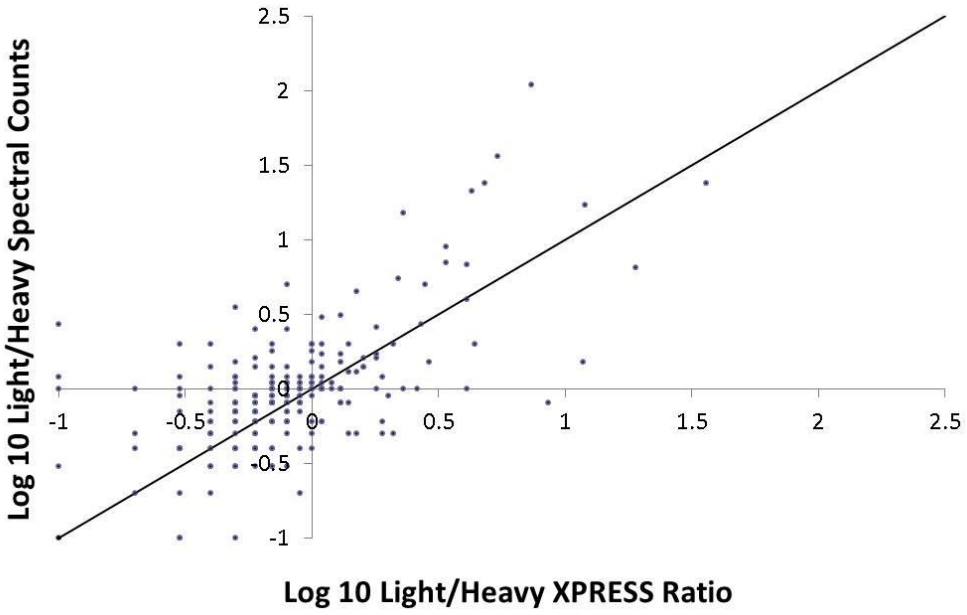

Figure S3. Logarithmic scatter plot of light/heavy-labeled sample ratios obtained through spectra counting and XPRESS quantitation. The straight line is theoretical, y=x.

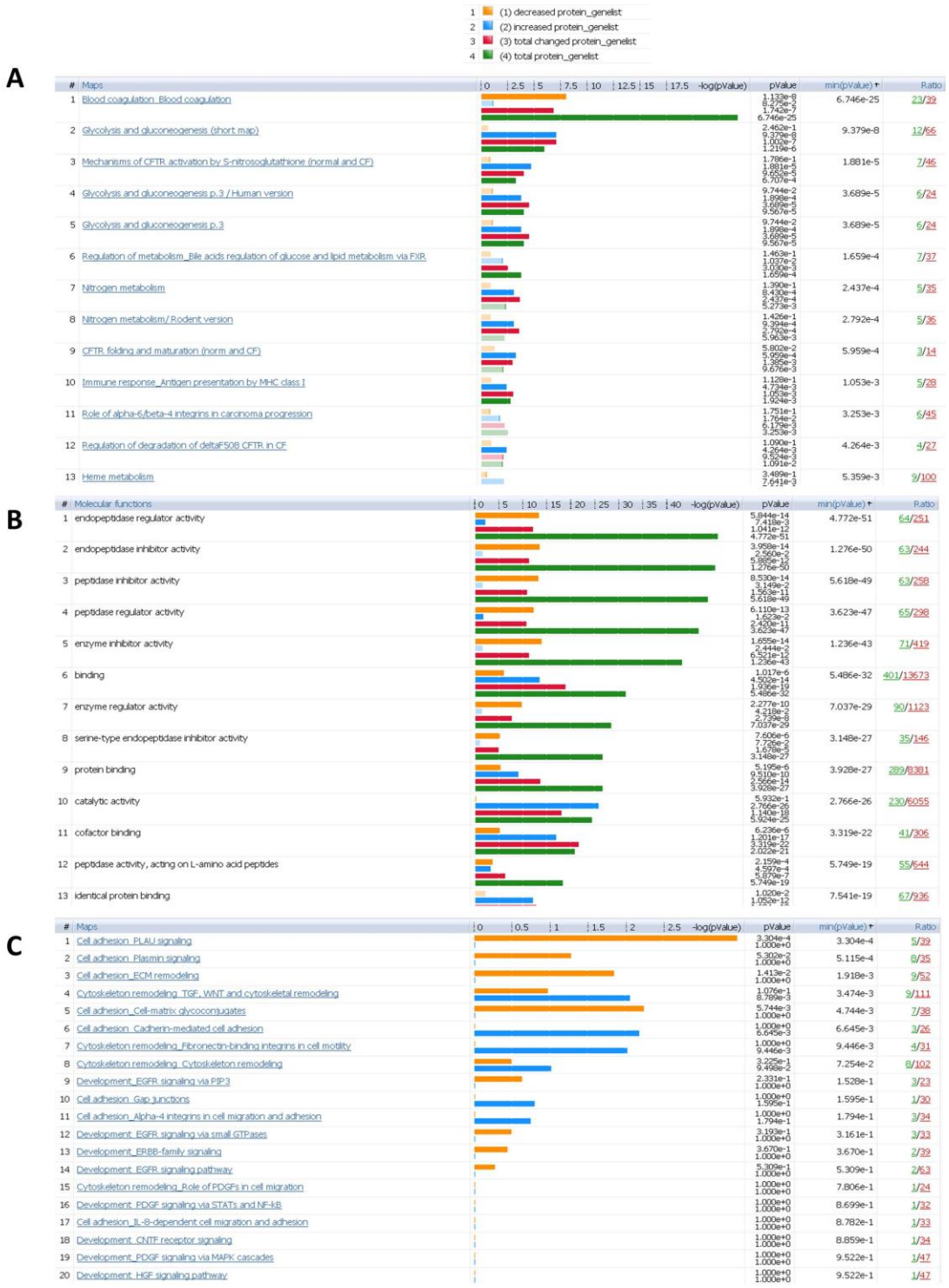

Figure S4. Genego pathway enrichment analysis of serum toxicoproteome. A, total enriched pathways; B, total enriched GO molecular functions; C, pathways enriched in tissue-repair and wound-healing categories. Ratios denote the number of proteins from our dataset over the number of proteins curated in the Genego pathway.

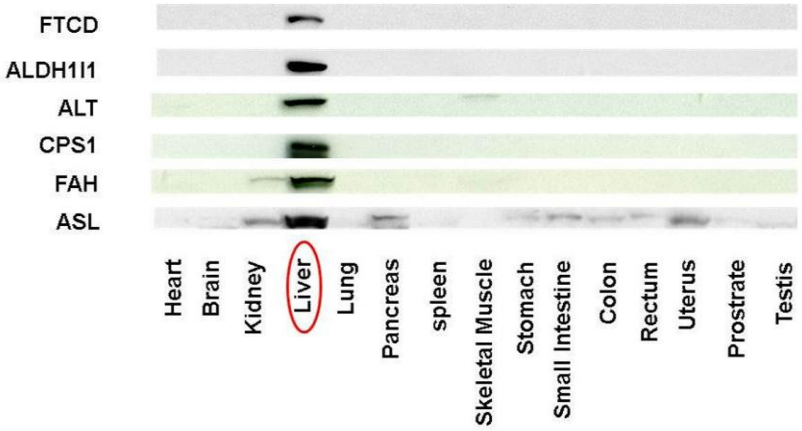

Figure S5. Western blot validation of the human-organ specificity of the selected proteins.

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