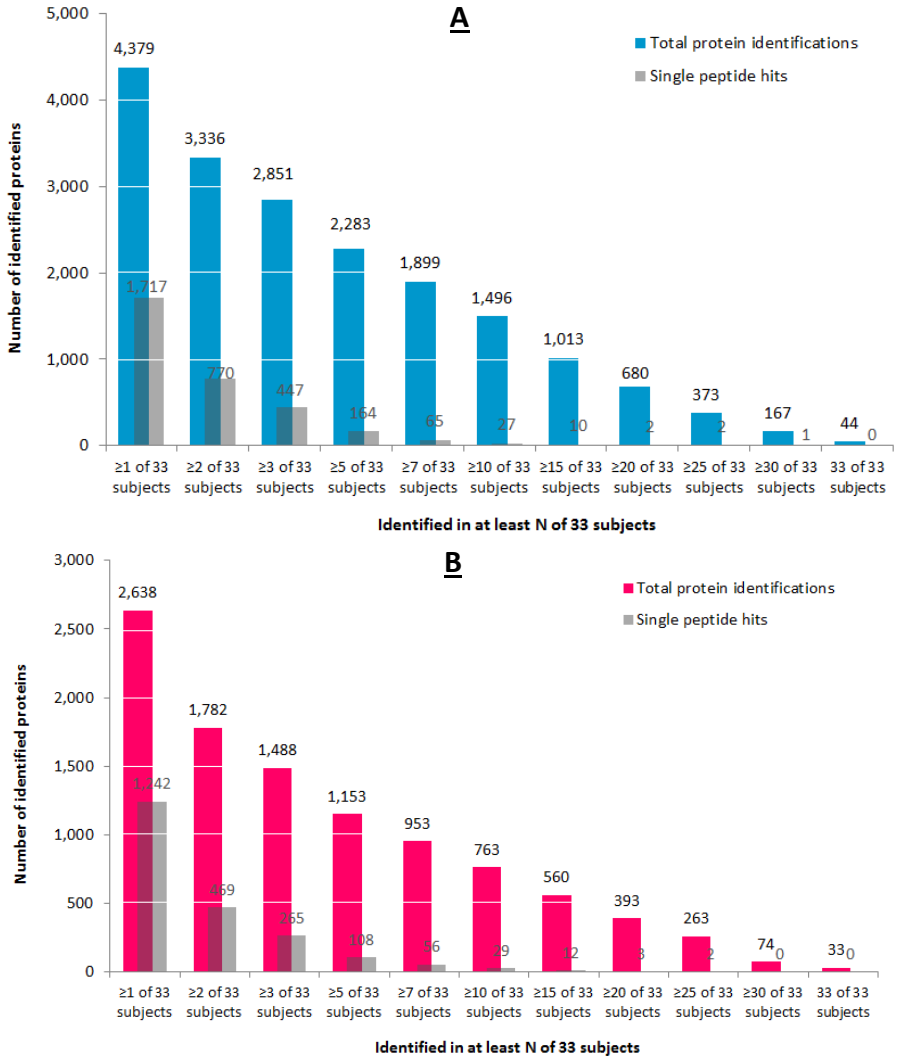
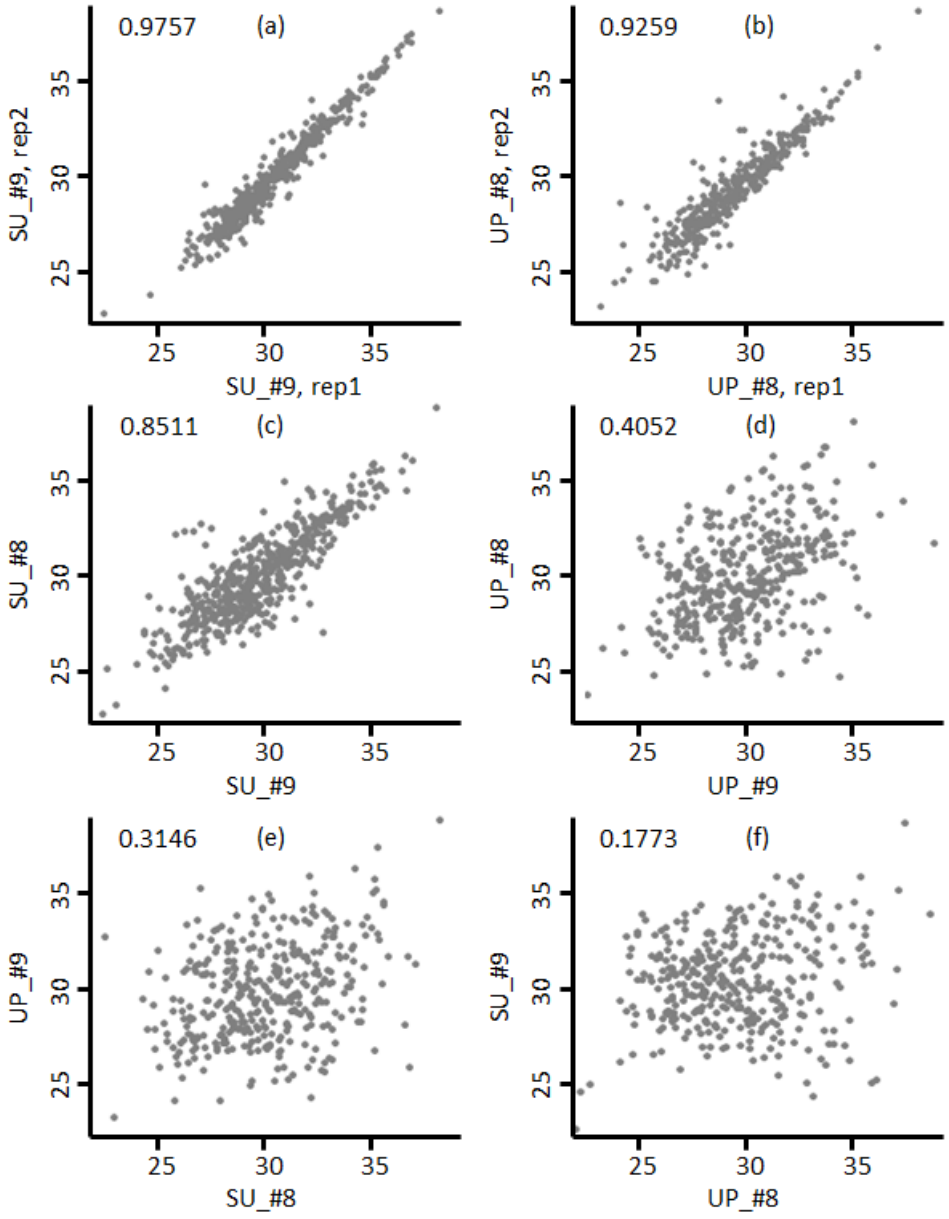
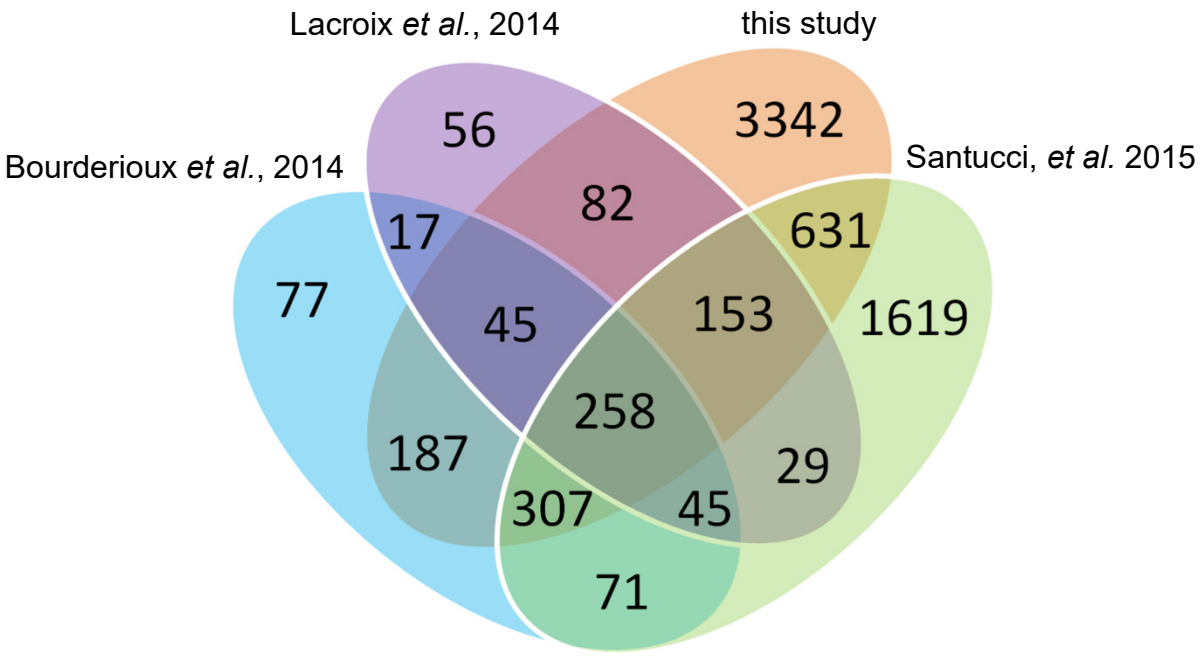
Supplemental Figure S1. The urinary proteome profiled in urinary pellet and soluble urine fractions from 33 clean-catch specimens of human subjects. (**A**) Distribution of protein IDs in urinary pellets (UP). In total, 4,379 proteins were identified from 33 human subjects, which included 1,717 proteins that feature a single peptide. 1,013 proteins were detected from at least 15 of the 33 subjects with less than 1% originating from a single peptide hit. (**B**) Distribution of protein IDs in soluble urine fractions (SU). In total 2,638 proteins were detected from all 33 subjects, and 1,242 (47%) proteins were limited to a single peptide ID. 560 proteins were identified from at least 15 of the 33 subjects, including 548 (98%) proteins that featured two or more unique peptides.



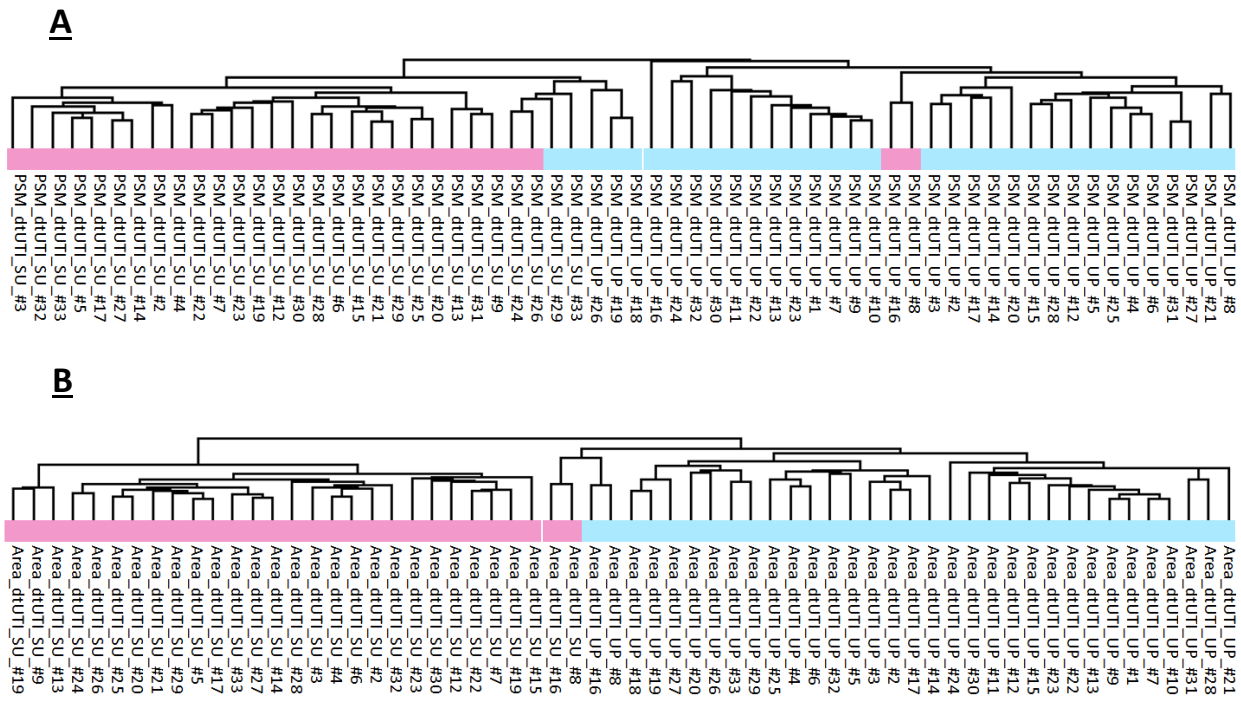
Supplemental Figure S2. Repeatability of LC-MS analyses for urine samples. The LFQ intensities between different technical replicates (rep1 *vs.* rep2) from the same soluble urine concentrate (a) or urinary pellet (b), and LFQ intensities between different samples (SU *vs*. SU from different subjects, c; UP *vs*. UP from different subjects, d; SU *vs*. UP from different subjects, e and f) are plotted. The Pearson correlation values are denoted in the plot. SU and UP samples #8 and #9 were analyzed as representative samples.



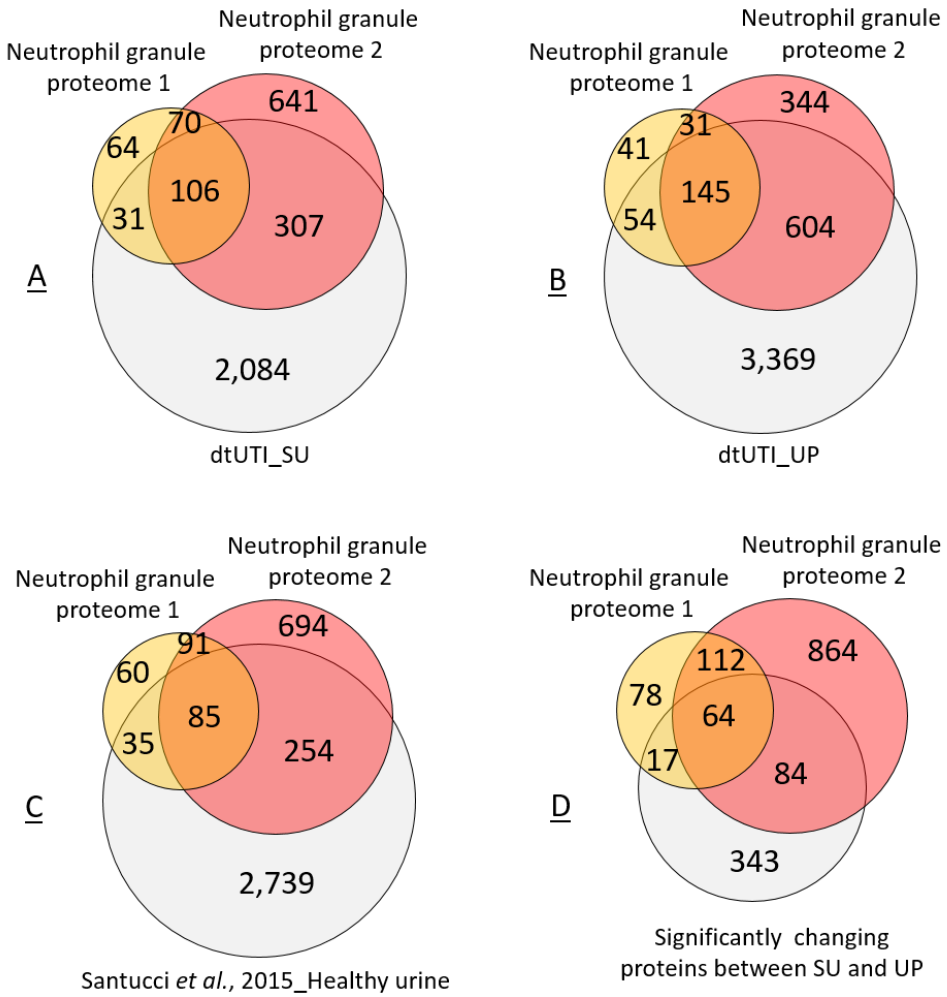
Supplemental Figure S3. Urinary proteome coverage in a comparison of four studies. Three recent LC-MS/MS studies with publicly accessible raw data generated using similar shotgun proteomics methods were compared with the present study. Santucci *et al.* applied multiple fractionation procedures (for instance, vesicle isolation, combinatorial peptide ligand libraries) and used FASP as well as an LTQ Orbitrap Velos Pro instrument for protein ID. The team obtained 3,429 proteins in total from healthy human urine [1]. Lacroix *et al.* employed FASP and used the LTQ Orbitrap Velos instrument [2]. This team reported 970 protein quantifications from urine supernatant of the newborns with obstructive nephropathy. Bourderioux *et al.* focused on the analysis of urine exosomes in cystinuria patients using OFFGel IEF for fractionation and the LTQ Orbitrap Velos instrument [3]. Overall, this group identified 1,794 proteins. In order to perform the comparison, different protein identifiers and accession numbers were converted to the same UniProt accessions using the publicly available Retrieve/ID mapping function in UniProt website (http://www.uniprot.org/). The total numbers of proteins are slightly different from the reported ones due to the conversion of protein annotations and database updates. Supplemental Table S4 contains detailed information of all protein IDs.



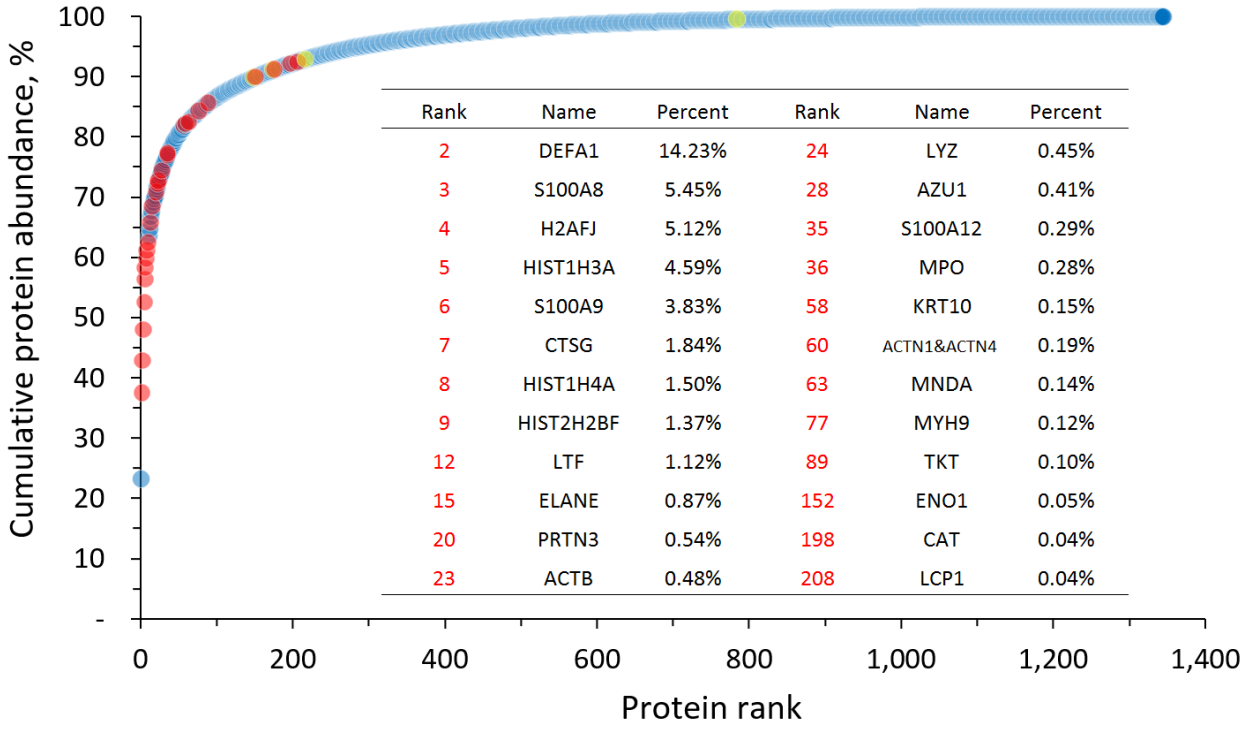
Supplemental Figure S4. Label-free quantitation of urinary proteome datasets using three different approaches (the LFQ method, spectral counting, and a peak area-based approach). The LFQ values were derived from MaxQuant software. The PSM values derived from the Proteome Discoverer software tool were used for spectral counting. PSMs of individual proteins from LC-MS/MS replicate experiments were averaged, and then divided by the sum of PSMs for all the proteins identified in the sample. Peak area values were also acquired from the Proteome Discoverer software tool, where the precursor Ion area detector module was included in the database search workflow. The resulting peak area values of individual proteins were divided by the overall peak area values. Data from the three methods were processed using Perseus software identically: transform with log2, exclude values that were found in less than three subjects in each group and subjects that contained less than 200 values, impute based on tuned parameters, and then normalize with Z-Score. Both PSM and peak area-based quantitation methods (**A** and **B**) revealed separate clusters for UP and SU datasets with few exceptions, similar to the results using LFQ-based quantification (Figure 3).



Supplemental Figure S5. Comparison of UP and SU urinary proteome profiles from this study with published datasets on the neutrophil granule proteome. Lominadze *et al.* [4] reported the first proteomic study of neutrophil granules by employing two-dimensional gels and MALDI-TOF-MS as well as 2D-LC-MS/MS analyses, and identified 286 proteins (shown as neutrophil granule proteome 1 in the diagrams). Rorvig *et al*. [5] performed subcellular fractionation of neutrophil granules followed by SDS PAGE and LC-MS/MS and identified 1,292 proteins using Orbitrap-based LC-MS/MS (shown as neutrophil granule proteome 2 in the diagrams). We compared the SU and UP proteomes (A, and B, each summed from the 33 datasets) separately with the published data. The number of granule proteins accounted for 17.6% of all SU proteins and 19.2% of all UP proteins. The urinary proteome with IDs of 3,429 proteins from healthy donor urine [1] is also included as a control Venn diagram (C), which resulted in 12% granule protein content. This data supports the notion that proteomes associated with UTIs (in our study) increased the relative neutrophil protein content due to a larger contribution of this cell type to the total proteome. Comparing the 508 proteins significantly changing (SU vs. UP; Figure 5) with the granule proteomes (D), 165 of these proteins (32.5%) were also identified in at least one of the previous granule proteome datasets. We used the method described in Figure S3 and Supplemental Table S4 for protein ID conversions.



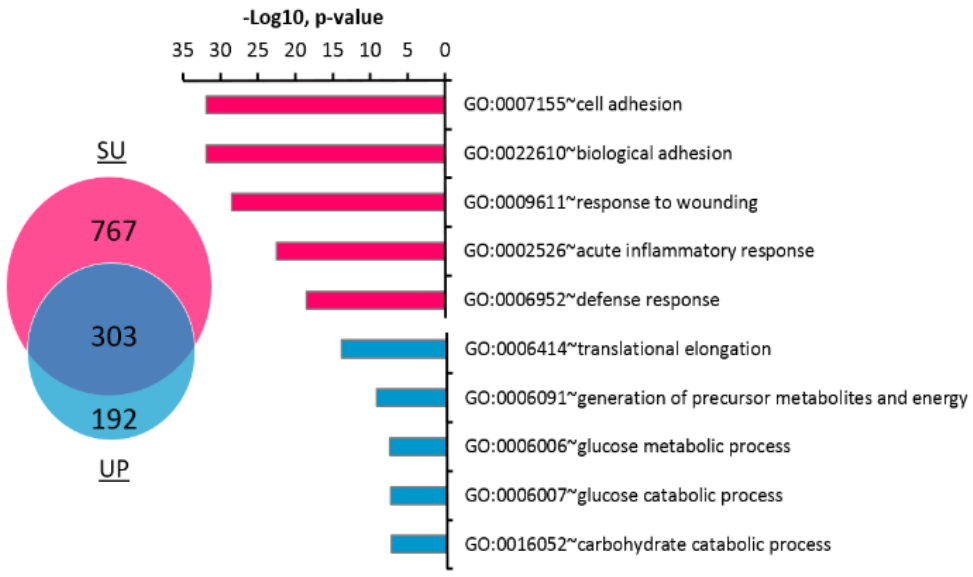
Supplemental Figure S6. Cumulative protein abundance from the highest to the lowest abundant proteins. The 1,321 human proteins identified in subject #6 were ranked based on their iBAQ intensity values. Their individual contribution to the total protein mass was calculated. The plot shows the cumulative contributions. The 24 neutrophil extracellular traps (NETs) associated proteins (red circles in the plot) with the detailed ranking and abundance information were indicated in the table. In addition, four other NETs proteins (H1, rank 149; BPI, rank 174; CAMP, rank 216; PTX3, rank 786; all have ≥2 unique peptides; yellow circles in the plot) that Urban *et al*. did not identified with their proteomic approach were also detected in our study.



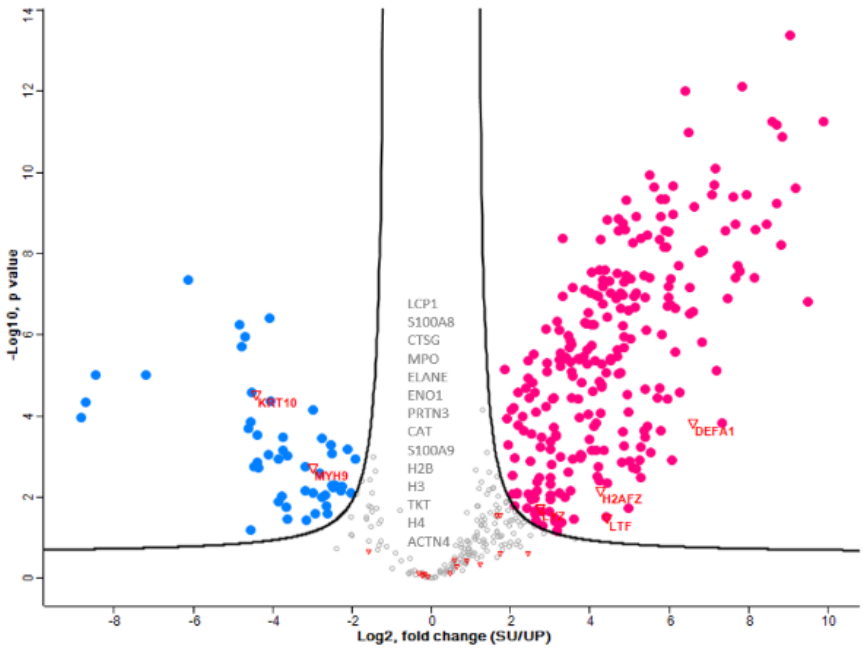
Rank	Name	Percent	Rank	Name	Percent
2	DEFA1	14.23%	24	LYZ	0.45%
3	S100A8	5.45%	28	AZU1	0.41%
4	H2AFJ	5.12%	35	S100A12	0.29%
5	HIST1H3A	4.59%	36	MPO	0.28%
6	S100A9	3.83%	58	KRT10	0.15%
7	CTSG	1.84%	60	ACTN1&ACTN4	0.19%
8	HIST1H4A	1.50%	63	MNDA	0.14%
9	HIST2H2BF	1.37%	77	MYH9	0.12%
12	LTF	1.12%	89	TKT	0.10%
15	ELANE	0.87%	152	ENO1	0.05%
20	PRTN3	0.54%	198	CAT	0.04%
23	ACTB	0.48%	208	LCP1	0.04%

Supplemental Figure S7. Proteomic study of healthy urine. (A) Comparison of SU and UP proteomes in terms of their proteome coverages and Gene Ontology biological processes. The top5 over-represented categories of each fraction were listed according to their significance. (B) Volcano plot shows the differential expression of the urinary proteins in the two fractions. The same type of t-test (as described in the Methods section and in Figure 6) was applied. Twenty of neutrophil associated proteins (such as MPO, LTF, S100A8/A9, LCP1, and histones) are highlighted in red triangle with labels.

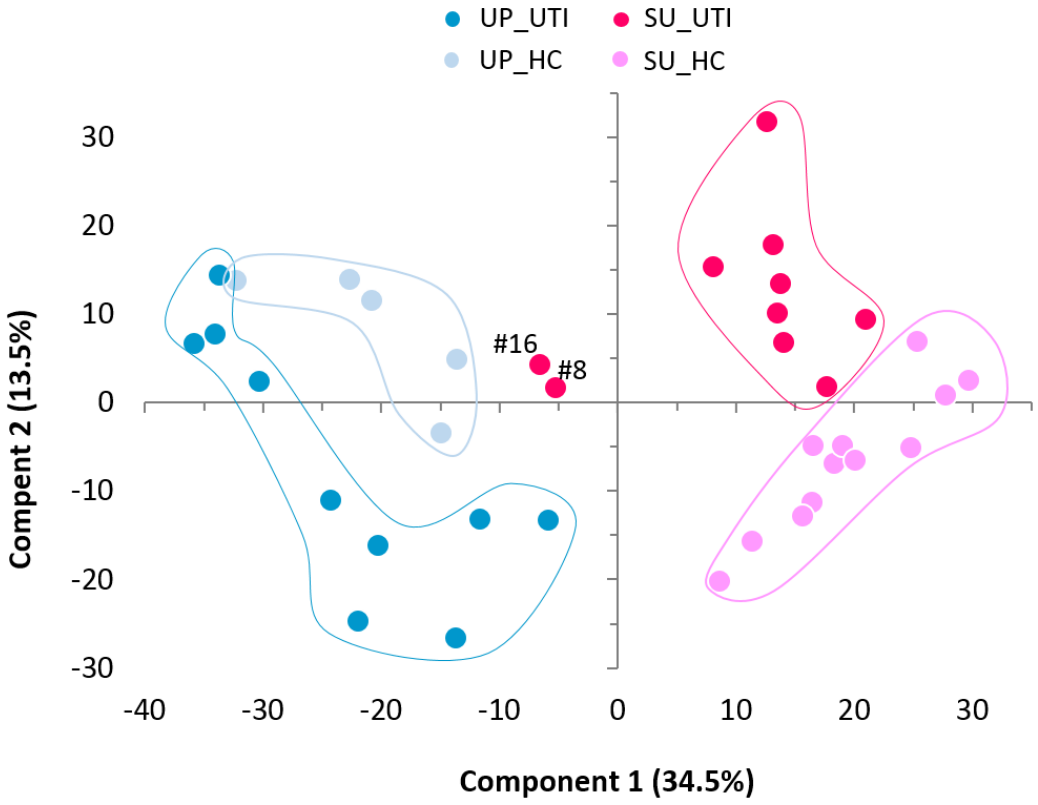
A



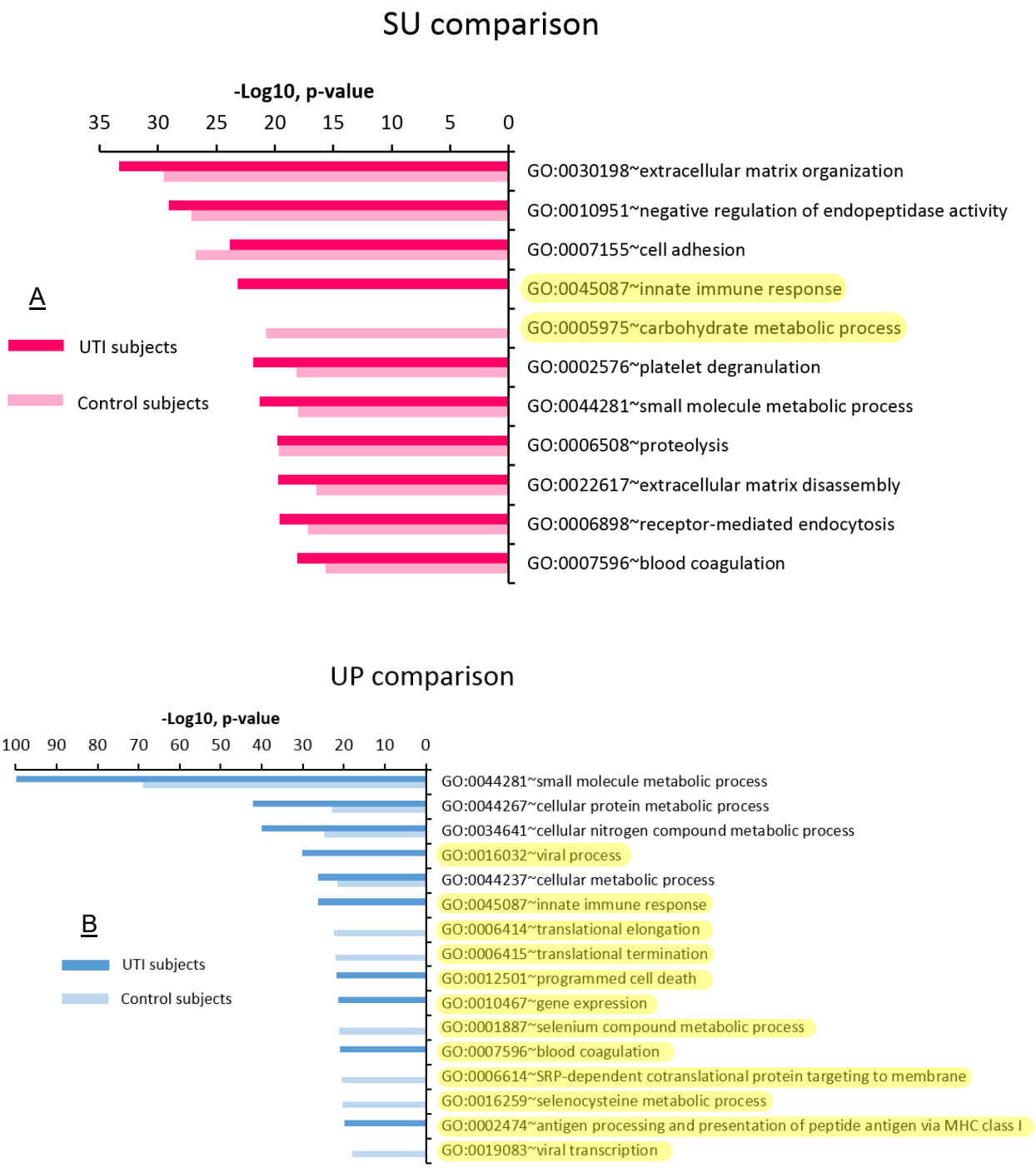
B



Supplemental Figure S8. Principle Component Analysis of UTI subjects and healthy controls. The logarithmic (Log2) LFQ values derived from the MaxQuant were used. Similar to what have processed in Figure 4, the subjects were filtered and missing valuses were imputed by default settings in Perseus software.



Supplemental Figure S9. Significantly over-represented GO biological process terms for the UTI and Control subjects. Top10 most significant terms of each group were listed according to their significance. (A) Comparison of the SU proteins. (B) Comparison of the UP proteins. The term that is unique to either of the groups were highlighted with yellow shade.



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