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Comparative proteomic analysis of PAI-1 and TNF-alpha-derived endothelial microparticles

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Abstract

Endothelium-derived microparticles (EMPs) are small vesicles released from endothelial cells in response to cell injury, apoptosis, or activation. Elevated concentrations of EMPs have been associated with many inflammatory and vascular diseases. EMPs also mediate long range signaling and alter downstream cell function. Unfortunately, the molecular and cellular basis of microparticle production and downstream cell function is poorly understood. We hypothesize that EMPs generated by different agonists will produce distinct populations of EMPs with unique protein compositions. To test this hypothesis, different EMP populations were generated from human umbilical vein endothelial cells by stimulation with plasminogen activator inhibitor type 1 (PAI-1) or tumor necrosis factor-alpha (TNF-α) and subjected to proteomic analysis by LC/MS. We identified 432 common proteins in all EMP populations studied. Also identified were 231 proteins unique to control EMPs, 104 proteins unique to PAI-1 EMPs and 70 proteins unique to TNF-α EMPs. Interestingly, variations in protein abundance were found among many of the common EMP proteins, suggesting that differences exist between EMPs on a relative scale. Finally, gene ontology (GO) and KEGG pathway analysis revealed many functional similarities and few differences between the EMP populations studied. In summary, our results clearly indicate that EMPs generated by PAI-1 and TNF-α produce EMPs with overlapping but distinct protein compositions. These observations provide fundamental insight into the mechanisms regulating the production of these particles and their physiological role in numerous diseases.

Keywords

EMP; Microparticles; PAI-1; TNF-alpha

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1 Introduction

Endothelial microparticles (EMPs) contain fragments of the endothelial cell plasma membrane ranging in size from 0.1 to 3 μm [1]. They are released into the blood stream by endothelial cells upon activation, injury, or apoptosis [2] and have been described in a number of human disease states. Other components of the vascular system, including erythrocytes [3, 4], leukocytes [3, 5], lymphocytes [6, 7], platelets [8, 9], and vascular smooth muscle cells [10, 11], release microparticles (MP) into the circulation. All MP are composed of a phospholipid bilayer and cell surface proteins that reflect their cell of origin. For example, MP derived from endothelial cells have cell surface markers consistent with those found on endothelial cells such as CD 31/platelet-endothelial cell adhesion molecule-1 (PECAM-1) or CD 62 (E-selectin) [1, 12]. Another common feature of MP is that they are composed of a phospholipid bilayer that has an asymmetrical distribution of negatively charged particles such as phosphatidylserine. MP were originally described by Wolf in 1967 [13] and initially thought of as “cell dust” or debris. However, recent reports by our group and others suggest that their generation is an active process that involves the directed packaging of proteins from numerous cellular compartments [14, 15]. Cells stimulated by agonists such as plasminogen activator inhibitor type 1 (PAI-1) [16] or tumor necrosis factor-alpha (TNF-α) [1] yield an increase in the number of MP produced by endothelial cells, although the mechanism by which MP are released from the cell into the blood stream has yet to be fully elucidated. Understanding the protein composition of EMPs generated by different agonists will provide insight into the biological processes mediating EMP formation and release into the circulation, as well as their downstream effects.

It is clear from the literature that EMPs can serve not only as markers of disease, but also that these MP are effectors of cellular function. EMPs have been found to initiate coagulation [1, 14], regulate angiogenesis [17, 18], alter endothelial cell proliferation and migration [19], impair endothelial cell function [20, 21] as well as induce acute lung injury (ALI) in a Brown Norway rat model [21]. MP are known to be present at low levels in the plasma of healthy individuals. Elevated concentrations of EMPs have been linked to many inflammatory and vascular diseases including diabetes [22], renal failure [23], acute myocardial infarction [24], cancer [25], vasculitis [26], and sickle cell disease [27]. In addition, the EMPs of patients with systemic lupus erythematosus and antiphospholipid syndrome have a significant increase in quantity as well as procoagulant activity as compared to the EMPs of healthy individuals [28]. This suggests that EMP function may be different in diseased patients as compared to healthy controls.

In order to gain insight into the possible mechanisms by which EMPs can alter downstream cell function, it is important to determine their protein composition. Of course, *in vivo* EMP generation does not occur in the presence of a simple agonist, however the initial phase of inquiry into this complex issue is to comparatively analyze the protein composition of EMPs generated *in vitro* by different agonists. The proteome of TNF-α [14] or PAI-1 [15] generated EMPs has been reported independently by our group and others, but the EMP populations from these different stimuli have never been comparatively analyzed. While the previously published EMP proteomes provide initial insight into the potential functions of

EMPs, they are by no means comprehensive, list secondary to the limitations and differences in the methodologies employed by these studies. The goal of this current study is to comprehensively compare the proteome of EMPs from unstimulated endothelial cells and endothelial cells stimulated by PAI-1 or TNF-α using the sensitive and state-of-the-art method of LC/MS. This will afford additional perspective into the mechanism by which EMPs are produced by different agonists with potentially distinct functions and downstream effects. Such understanding is fundamental toward designing and developing diagnostic tools and potential therapies for the treatment of diseases associated with elevated levels of EMPs.

2 Materials and methods

2.1 EMP generation

Endothelial MP were generated *in vitro* from human umbilical vein endothelial cells (HUVECs) (Clonetics) as described previously [15, 21]. Briefly, cells were grown in gelatin coated T75 flasks (passage 4–6) in M199 media (Invitrogen) supplemented with 20% FBS (Lonza), 0.01% Heparin (Sigma), 0.05% Endothelial Mitogen (Biomedical Technologies), and 1% Penstrep:Glutamine (Invitrogen). At 100% confluence, the cells were washed with Hank's balanced salt solution (HBSS without Ca^{2+} and Mg^{2+}) and incubated in EBM-2 base media (Lonza), without any additives, for 2 h. Cultured flasks were divided into three groups. The media was discarded and replaced with fresh EBM-2 base media. One group was treated as control (no agonist). The other two groups were supplemented with either 10 ng/mL plasminogen activator inhibitor-1 (PAI-1) or 10 ng/ mL TNF-α. All flasks were maintained for 3 h at 37°C in an incubator with 5% CO_2. The HUVEC conditioned media was then collected for isolation of EMPs by serial centrifugation.

The media containing EMPs was collected in 50 mL conical tubes and centrifuged at room temperature for 4 min at 200 × *g* to remove cell debris. The supernatant was then transferred into 90 mL polycarbonate bottles (Kendro) and ultracentrifuged (Sorval) at 4°C for 1 h at 100 000 × *g*. The EMP pellet was resuspended in HBSS (20 μL/T75 flask) and stored at 4°C for further use (not more than 72 h).

2.2 EMP disruption and protein identification

EMPs are suspended in MOPS disruption buffer with protease (1X) and phosphatase inhibitors (1X) (Sigma). Disruption was completed by sonicating the pellet twice for 30 s each at 4°C using a dismembranator (Fisher). The sample was then centrifuged for 10 min at 20 000 × *g* to pellet the insoluble protein fraction. The supernatant containing soluble proteins was used for the protein analysis. An aliquot of the sample (25 μL) was used for protein estimation using a BCA-protein assay kit (Pierce). The protein sample (50 μg) from each group was electrophoresed into a 10% Criterion gel (BioRad) for 10–15 min at 150 V. The gel was then washed with water and silver stained. The stained protein area of the gel was excised and washed twice in deionized water for 10 min each. This was followed by two additional 15 min washes in water containing 50 mM sodium thiosulfate (Sigma) and 15 mM potassium ferricyanide (Sigma). The gel piece was then washed repeatedly with water to completely remove the color. A final wash was performed in 50% ACN with 10 mM

ammonium bicarbonate. The washed and destained gel piece was dried then soaked in 100 μL of 50 mM ammonium bicarbonate (Fisher) containing 1 μg trypsin (Promega). Digestion of proteins in the gel was carried out overnight at 37°C. The digested proteins were extracted by sonicating the gel piece for 15 min in 70% ACN (Fisher) in MS water and 0.1% formic acid (Fisher). This extraction procedure was repeated three times. The extracts were pooled together, dried, and reconstituted to 20 μL in 6 M guanidine-HCl (Pierce), 5 mM potassium phosphate, pH 6.5 (Sigma). The sample was further purified using a C_{18} ZipTip (Millipore) in preparation for the LC/MS analysis. Nano-HPLC-MS was performed using an LTQ mass spectrometer (Thermo Fisher) in line with a Surveyor HPLC system (Thermo Fisher) equipped with a Finnigan Micro AS autosampler. An Aquasil, C_{18} PicoFrit capillary column (75 μm × 9.8 cm; New Objective) was used in these experiments. Samples were applied to the column in the presence of solvent A (5% ACN in MS water and 0.1% formic acid). Peptides were resolved using a linear gradient from 100% solvent A to 80% solvent B (5% MS water and 95% ACN containing 0.1% formic acid) over 180 min at a flow rate of 0.2 μL/ min. After each sample the column was washed for 1 h with solvent A. Ions eluted from the column were electrosprayed into the ion transfer tube of the mass spectrometer at a voltage of 1.75 kV. The capillary voltage was 15 V and the temperature was kept at 200°C. A full mass spectrum (400–2000 *m*/*z*) was followed by fragmentation of the 7 most abundant peaks from the full scan spectrum, using 35% of the normalized collision energy for obtaining MS/MS spectra. The MS/MS data were searched using the SEQUEST (Version 27; Thermo Fisher) algorithm against the human subset of the UniProt database (Version 49.1; www.uniprot.org/). The search was limited to tryptic peptides and protein identifications were filtered from the search results using the *Epitomize* Program, which can be licensed with *Visualize* software as part of the *ZoomQuant* package for no fee through the Medical College of Wisconsin (http://proteomics.mcw.edu/zoomquant/) [29].

2.3 Data analysis

The proteins identified by LC/MS-MS were analyzed using *Visualize* software (Medical College of Wisconsin) as described above [29]. Proteins with a combined Protein Probability value of ≥0.95 were considered for further analysis as this indicates these proteins to be a true positive. The false discovery rate (FDR) for proteins with a Protein Probability value of ≥0.95 was 2.5% as determined by searching against a decoy database as previously described [30]. Protein TIC value (total TIC) for a given protein represents the summation of the TICs for all of the spectra above the probability threshold that were assigned to that protein.

The Uniprot IDs of the proteins identified *via* the MS/ MS analysis were used to annotate the proteins with their corresponding gene ontology (GO) annotations using *Apropos* software (available through the Medical College of Wisconsin, http://apropos.mcw.edu). The annotations were obtained from the GOA Human gene association file downloaded from the Gene Ontology Consortium in October 2007. For the purposes of this analysis, all evidence codes were included. The enrichment of the specific GO annotations was calculated using the hypergeometric distribution with the whole human genome used as the reference annotation set. A Bonferroni multiple testing correction was applied to the resulting *p*-values and values less than or equal to 0.01 are considered significant. A similar

analysis strategy was applied to the KEGG pathway annotations. Pathway analysis was also done using Ingenuity Pathway Analysis (IPA) version 5.5 (Ingenuity Systems, Redwood City, CA). UniProt accession numbers for all proteins with probability scores of >0.9 for all three samples were mapped to gene names and used in both Biological Function and Canonical Pathway comparisons of the three samples.

MS and data analysis parameters as well as all software programs are summarized in Table 1.

3 Results and discussion

3.1 Protein composition of EMP populations

In order to determine if the protein compositions of EMPs change as a result of the stimulus used to generate EMPs, LC/MS-MS was used to comprehensively identify the protein composition of EMPs generated using no agonist (control EMPs), PAI-1 generated EMPs, and TNF-α generated EMPs. A total of 783 proteins were identified in the control EMP proteome, 679 proteins from PAI-1 generated EMPs, and 643 proteins from TNF-α generated EMPs (see Fig. 1). All MS and data analysis parameters are provided in Table 1. In addition, Tables S1–S3 of Supporting Information provide a complete list of all identified proteins with their respective peptide counts, scan counts, and percent coverage. We identified significantly more proteins than have been identified in previous proteomic reports [14, 15]. This study identified 23 (39.7%) of the proteins previously identified from the PAI-1 generated EMP proteome (see Table S2 of Supporting Information) [15]. Similarly, 38 (49.4%) of the proteins previously identified in TNF-α generated EMPs were identified using LC/MS-MS (see Table S3 of Supporting Information) [14]. The most likely explanation for the increase in total protein identification reported here is that the more sensitive technique of LC/MS-MS was employed in these studies. Heterogeneity among the EMP populations and inherent limitations of MS sampling likely explains why the entire EMP proteome identified in previous studies [14, 15] are not represented here.

All three EMP populations contain proteins, ranging from 25 to 30% of the proteome, that are actually known to be expressed in endothelial cells (see Tables S1–S3 of Supporting Information) [31]. Each EMP population generated with a different agonist has a unique protein composition. Twenty-two percent, or 231 of the proteins identified are unique to the control group of EMPs. In comparison, 10% or 104 of the proteins identified are unique to PAI-1 generated EMPs, and 6.7% or 70 proteins identified are unique to TNF-α generated EMPs. All of the unique proteins identified in control EMPs, PAI-1 EMPs, and TNF-α EMPs are listed in Tables 2-4. Given our recent report that EMPs induce ALI in a rat model [21], analysis identified two proteins of interest: transferrin receptor protein 1 from control EMPs and heat-shock 70 kDa protein 1 from TNF-α EMPs. These proteins have been linked to ALI. Specifically, the expression of transferrin receptor proteins is known to be increased in the bronchoalveolar lavage fluid in patients with acute respiratory distress syndrome, and is thought to diminish oxidative stress which is a known contributing mechanism to ALI [32]. Similarly, heat-shock 70 kDa protein has been shown to play a protective role in models of acute respiratory distress syndrome [33, 34].

Altogether, these data clearly show that the protein composition of EMPs is altered when generated by different stimuli. This suggests that when the endothelial cell is stimulated with an agonist, such as PAI-1 or TNF-α, the spectrum of proteins that are packaged into the EMP is modified to rid the endothelial cell of specific proteins and that these may mediate downstream signals. Figure 1 clearly demonstrates that PAI-1 and TNF-α generate EMP populations contain overlapping but distinct protein compositions.

3.2 Common proteins of EMP populations

Nearly one-half, or 432 of the proteins we identified (see Fig. 1), are common to all EMPs regardless of stimulus. Because there are a large number of shared proteins in the EMP proteome, we examined the relative concentrations of many common proteins. We specifically compared the shared proteome of PAI-1 and TNF-α generated EMPs. As seen in Fig. 2A, 82 of the proteins common to PAI-1-and TNF-α generated EMPs were plotted against one another based on TIC. We found that there are significant differences in the protein abundance among the shared proteins of TNF-α and PAI-1 generated EMPs. Proteins that showed the greatest difference in relative abundance between PAI-1 and TNF-α generated EMPs are labeled in Fig. 2A. To further illustrate the difference in relative abundance based on TIC, the most abundant 11 proteins are graphed in Fig. 2B.

Uromodulin precursor protein (UROM), while expressed by both PAI-1 and TNF-α generated EMPs, is 157 times more abundant in TNF-α generated EMPs than in PAI-1 generated EMPs. Uromodulin, also known as Tamm-Horsfall urinary glycoprotein (THP), has been implicated as playing a protective role in the urothelium [35]. Another report has identified THP as a specific antigen recognized by a novel mAb which positively immunostained the pharynx, trachea, and mesothelial lining of the lung in human tissue [36]. This relationship suggests the possibility that uromodulin may also play a protective role in the respiratory system as it does in the urothelium. If this is the case, then EMPs carrying uromodulin in the bloodstream may act as downstream signals or effectors of function not only of endothelial cells but also potentially of other cells in the respiratory system.

Another protein commonly expressed by both PAI-1 and TNF-α EMPs is keratin, type II cytoskeletal 5 protein. It is more abundantly expressed in PAI-1 generated EMPs, at a ratio of 9.1 times that of the same protein expressed by TNF-α generated EMPs. Of note, no cytokeratin proteins were identified in control EMPs. The cytokeratin family, specifically cytokeratin 19 (CK19), has been linked to ALI [37]. CK19 is expressed in type I and type II alveolar epithelial cells and CK19 fragments are released during cell injury or cell death. Increased CK19 fragment concentrations have been noted in the bronchoalveolar lavage fluid of patients with ALI and associated with a poor prognosis [37]. Here too the data suggest a protein by which EMPs may be altering downstream cell signaling.

Another protein of note is glutathione peroxidase. This protein is differentially expressed in all three EMP populations studied and has also been linked to ALI. Glutathione peroxidase appears to play a role in the endogenous anti-oxidant system of the lung. It has been postulated that in order to recover from ALI, a patient must regain oxidative balance and regenerate reduced glutathione [38-41]. Our results suggest a possible mechanism by which

EMPs and their protein components may contribute to the imbalance of the antioxidant system by shuttling glutathione peroxidase outside the endothelial cell and thus overwhelming the redox potential in the extracellular fluid.

Taken together, these data indicate that in addition to the obvious differences in protein composition between the EMP populations (Fig. 1), there are more subtle differences even among the shared proteins of the different EMP populations with respect to relative abundance (Fig. 2).

3.3 Gene ontology (GO) and KEGG pathway analysis of proteins identified from EMP populations

Using the Uniprot IDs and Apropos software, we searched the GOA Human gene association file downloaded from the Gene Ontology Consortium to determine the known annotations of cellular component, molecular function, biological process, and KEGG pathway for the proteins identified from control, PAI-1, and TNF-α generated EMPs. Statistical analysis was used to determine which GO terms or KEGG pathways were significantly enriched in the EMP population as compared to the human genome. A number of GO categories and KEGG pathways were identified that are significantly (Bonferroni corrected p-value ≤0.01) enriched in the different EMP populations. These enriched categories and pathways along with the number of proteins identified in each category or pathway are shown in Fig. 3 for cellular component (Fig. 3A), molecular function (Fig. 3B), biological process (Fig. 3C), and KEGG pathway (Fig. 3D). While proteins were found that are significantly over-represented in the EMP populations, we observed that the enriched GO categories or KEGG pathways are not strikingly different between the different EMP populations. For example, many of the proteins identified in the control, TNF-α, and PAI-1 EMP populations derive from the cytoplasm, ER, mitochondrion, and ribosomes (Fig. 3A). In addition, several of the proteins in all three EMP populations are associated with nucleotide binding, protein binding, protein folding, protein transport, and translation processes (Figs. 3B and 3C), as well as oxidative phosphorylation, glycolysis, and ribosome pathways (Fig. 3D). However, we also observed more subtle, but distinct differences in categories and pathways enriched between the EMP populations. In the control EMPs, proteins from the cellular membrane components showed significant enrichment in the population (p = 2.68E − 11). However, while proteins were identified in PAI-1 and TNF-α EMPs that derive from the cell membrane, these proteins were not significantly over-represented in the population, compared to the control EMPs (data not shown). In contrast, PAI-1 and TNF-α EMPs, but not control EMPs, show significant enrichment in proteins from the proteasome complex and proteasome core complex (Fig. 3A).

With regard to molecular function, NADH dehydrogenase activity was significantly over-represented in control EMPs (p = 3.15E − 07) but not in PAI-1 EMPs (p = 2.6) or TNF-α EMPs (p = 190.3), whereas threonine endopeptidase activity showed significant enrichment in PAI-1 EMPs (p = 0.01) and TNF-α EMPs (p = 8.8E − 08), but not in control EMPs (p = 17) (Fig. 3B). The biological process GO annotations also reveal distinct differences with respect to control EMPs as compared to PAI-1 EMPs and TNF-α EMPs. Control EMPs have an increased number of proteins involved in electron transport (p = 0.005) as compared with

PAI-1 EMPs and TNF-α EMPs (Fig. 3C). Similarly, small GTPase-mediated signal transduction was enriched in control EMPs ($p = 0.003$) but not in the other EMP populations studied (Fig. 3C).

Finally, KEGG pathway analysis revealed many potential similarities and differences between the three populations of EMPs studied. Specifically, two disease pathways, *Escherichia coli* infection and cholera, were different between the EMP populations. With *E. coli* significant in more proteins were identified in both PAI-1 EMPs ($p = 0.001$) and TNF-α EMPs ($p = 0.0096$) (Fig. 3D). In the pathway analysis for cholera, only PAI-1 EMP proteins were significantly over-represented (Fig. 3D). Analysis using the commercially available software system, Ingenuity Pathway Analysis (version 5.5; Ingenuity Systems) gave similar results (data not shown).

Altogether, GO annotation and KEGG pathway analysis data revealed that there may be functional differences between the EMP populations. However, for the majority of proteins identified in control EMPs, PAI-1 EMPs, and TNF-α EMPs there is no apparent significant enrichment or over-representation of proteins with regard to cellular components, molecular function, biological processes, or KEGG pathway. It may be extrapolated that additional functional differences are due to variations in protein abundance among the proteins common to all three populations (Fig. 2).

4 Concluding remarks

In summary, we have provided evidence that the EMP proteome contains overlapping yet distinct proteins when different stimuli are used to generate EMPs. Furthermore, among the proteins common to all EMP populations there are significant differences in the relative abundance. Finally, GO and KEGG pathway analysis reveals that EMPs generated under different conditions may contain proteins derived from similar yet distinct cellular compartments, and perform molecular functions and biological processes that participate in many of the same KEGG pathways. From these observations, one can speculate that EMPs may then have several overlapping functions, regardless of the EMP generating stimulus. While the proteome of various EMPs are similar and overlap, each population is clearly distinct with variations in protein abundance and composition. These unique aspects may confer functional differences. Clearly, more mechanistic studies are indicated to pursue the functional aspects of EMPs generated by different stimuli. This is currently the focus of ongoing studies in our laboratory. Our current findings that EMPs stimulated by different agonists generate distinct populations of EMPs with unique protein compositions provide fundamental insight into the mechanisms regulating the production of these particles and their physiological role in different diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ALI	acute lung injury
CK19	cytokeratin 19
EMP	endothelial microparticle
GO	gene ontology
HBSS	Hank’s balanced salt solution
HUVEC	human umbilical vein endothelial cell
MP	microparticles
PAI-1	plasminogen activator inhibitor type 1
TNF-α	tumor necrosis factor-alpha

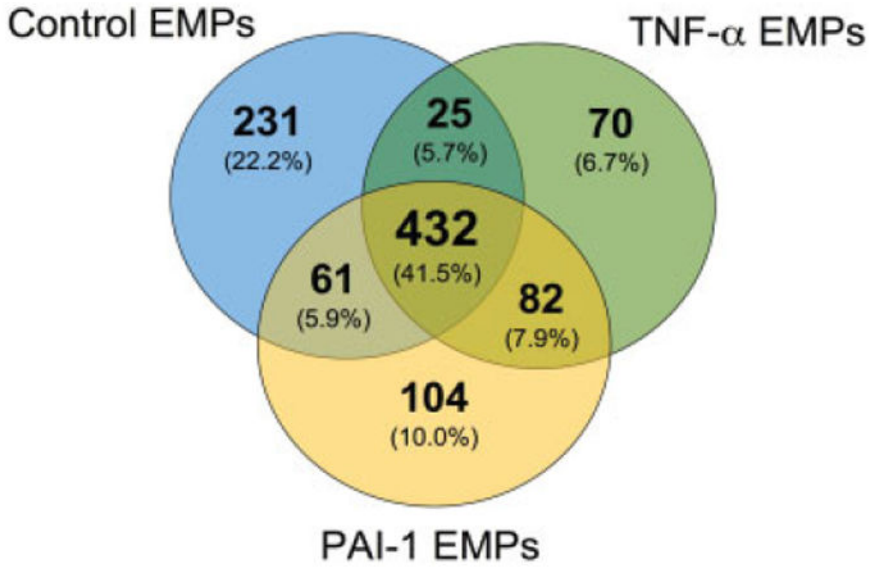
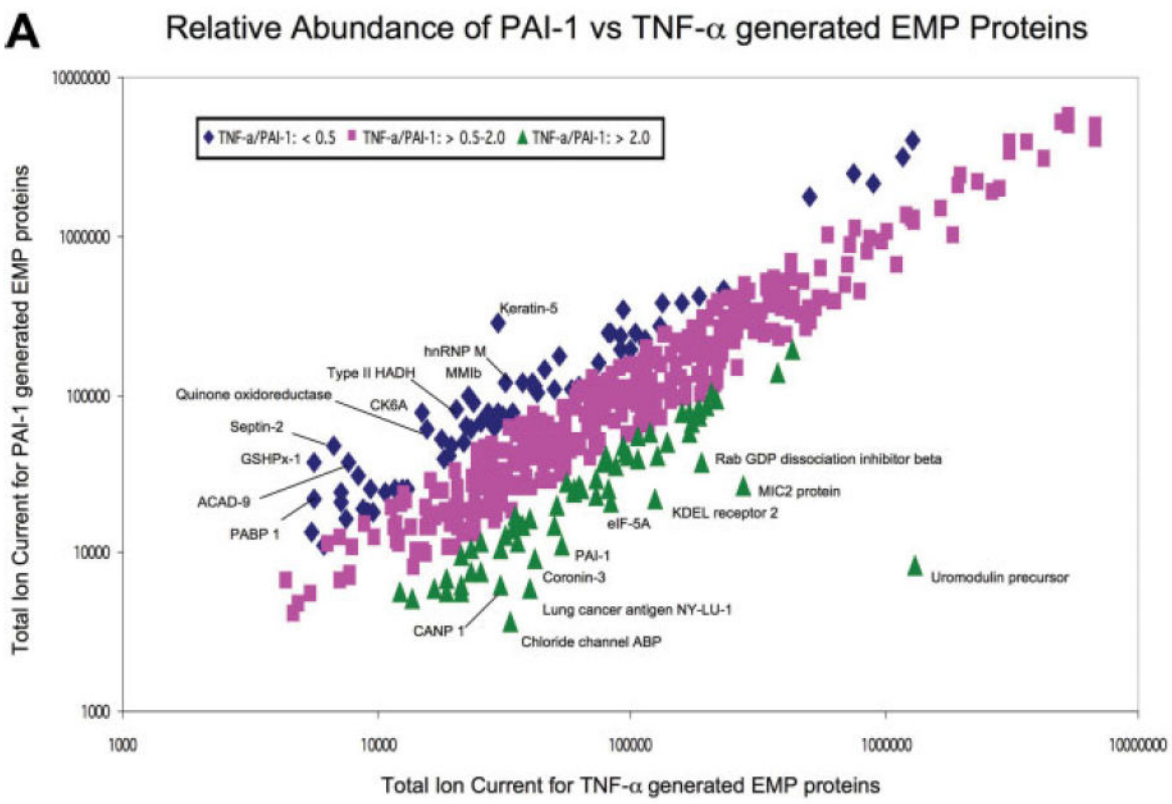


Figure 1.

Venn diagram of number of proteins identified in control EMPs, TNF-α generated EMPs, and PAI-1 generated EMPs. EMPs were generated from HUVECs *via* stimulation with no agonist (control), PAI-1, or TNF-α. Proteins were identified in each EMP population by LTQ nanospray-LC/MS-MS (n = 4 control EMPs; n = 5 PAI-1 generated EMPs; n = 5 TNF-α generated EMP). The diagram is labeled with the number of proteins identified as well as the percentage of the proteins identified based on the total number of proteins within each respective section of the diagram.



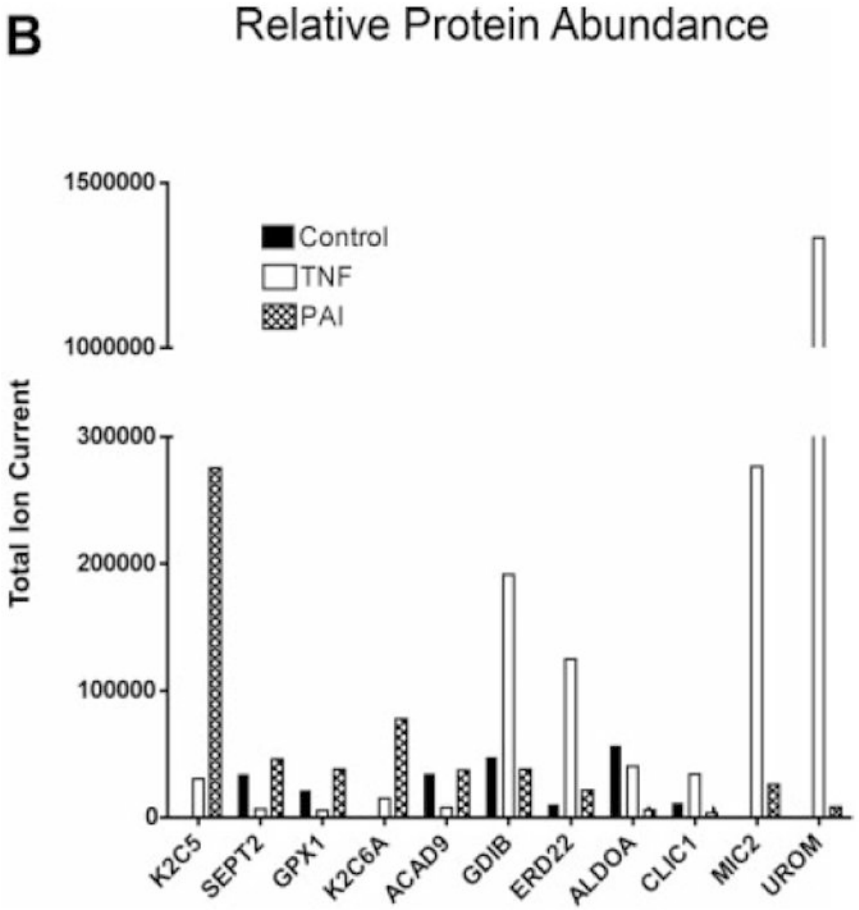
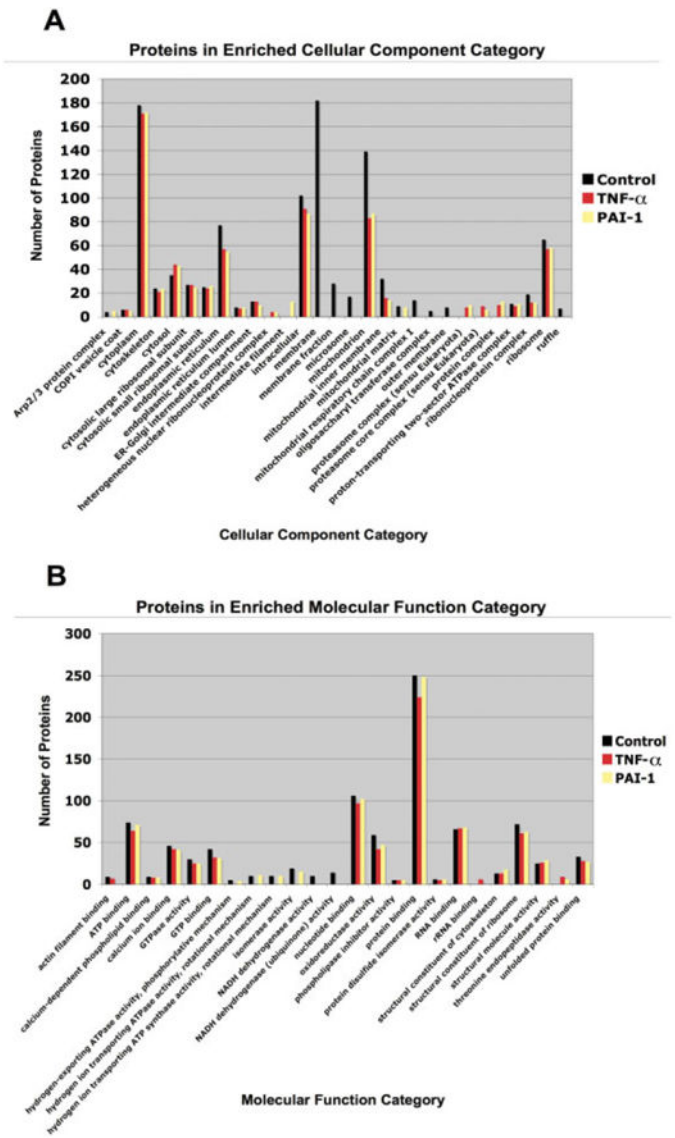


Figure 2.

Relative abundance of proteins common to PAI-1- and TNF-α generated EMPs. (A) The relative abundance of proteins common to PAI-1- and TNF-α generated EMPs was plotted based upon TIC using *Microsoft Excel*. Twenty proteins with the largest difference in abundance were identified between PAI-1 and TNF-α generated EMPs using *Petroplot*. (B) The relative abundance, as represented by TIC, of the top 11 differentially expressed proteins was used to generate a bar graph in *Microsoft Excel*. The proteins included in the graph are keratin, type II cytoskeletal 5 (K2C5), septin-2 (SEPT2), glutathione peroxidase 1 (GPX1), keratin, type II cytoskeletal 6A (K2C6A), acyl-CoA dehydrogenase family member 9 (ACAD9), rab GDP dissociation inhibitor β (GDIB), ER lumen protein retaining receptor 2 (ERD22), fructose-bisphosphate aldolase A (ALDOA), chloride intracellular channel protein 1 (CLIC1), T-cell surface glycoprotein E2 precursor (MIC2), UROM.



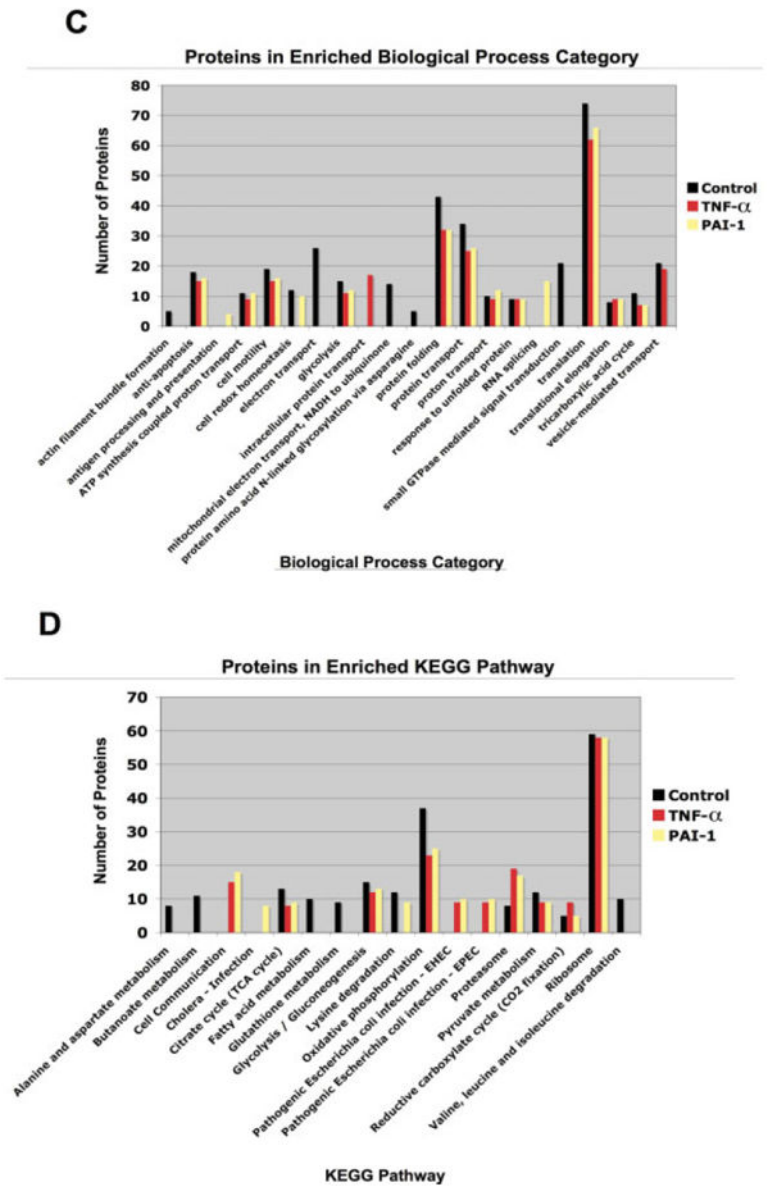


Figure 3.

GO annotation and KEGG pathway analysis for EMP proteins. The Uniprot IDs of the total proteins identified in control, TNF-α, and PAI-1 generated EMPs *via* the MS/MS analysis were used to annotate the proteins with their corresponding GO annotations using *Apropos* software. Annotations with regard to cellular component (A), molecular function (B), biological process (C), and KEGG pathway (D) were obtained from the GOA Human gene association file downloaded from the Gene Ontology Consortium. The enrichment of the specific GO and KEGG pathway annotations were calculated using the hypergeometric distribution with the whole human genome used as the reference annotation set. A Bonferroni multiple testing correction was applied to the resulting *p*-values and values less than or equal to 0.01 were considered significant. For the GO categories or KEGG pathways

that were significantly enriched, the number of proteins identified that belong to each category are shown.

Table 1

MS and Data analysis parameters

Parameter	Value
Program used to collect data	Xcalibur 4 (Thermo Fisher)
Program used to create peak lists	Extract_ms (Thermo Fisher)
Parameters used to generate peak lists	-B600 -T3500 -M1.4 -S1 -G1 -I15 -E250
Program used for Database searches	Sequest V 27 (Thermo Fisher)
Parameters used for Database searches	Enzyme: Trypsin (KR)
	Peptide mass tolerance: 2.5 Da
	Fragment mass tolerance: 0.0 Da
	Differential search options M16, C57
	Max missed cleavage sites: 3
Database Searched	Uniprot Human V49.1 (www.uniprot.org/)
Program used to filter data and assign protein probability scores	Epitomize V 2 (Medical College of Wisconsin, http://proteomics.mcw.edu/zoomquant/)
Program used to combine and compare data from multiple mass spectrometer runs	Visualize V0.5 (Medical College of Wisconsin, http://proteomics.mcw.edu/zoomquant/)
Program used for GO annotation and KEGG pathway analysis	Apropos (Medical College of Wisconsin, http://apropos.mcw.edu)
Program used for biological function and canonical pathway analysis	Ingenuity Pathway Analysis (IPA) version 5.5 (Ingenuity Systems).

Table 2

Proteins identified unique to control EMPs

Reference	Accession no.	Protein name	TIC
1A02_HUMAN	P01892	HLA class I histocompatibility antigen	30 994.6
2ABA_HUMAN	P63151	Serine/threonine-protein phosphatase	18 880.1
AAAT_HUMAN	Q15758	Neutral amino acid transporter B	31 927.8
ACOT9_HUMAN	Q9Y305	Acyl-coenzyme A thioesterase 9	36 741.1
ADPGK_HUMAN	Q9BRR6	ADP-dependent glucokinase	7808
AKIP_HUMAN	Q9NWT8	Aurora kinase A-interacting protein	9668.9
AL7A1_HUMAN	P49419	α-Aminoadipic semialdehyde dehydrogenase	38 385
ARLY_HUMAN	P04424	Argininosuccinate lyase	12 616.5
ASNS_HUMAN	P08243	Asparagine synthetase	7732.1
AT2A2_HUMAN	P16615	ER calcium ATPase 2	48 719.7
AVEN_HUMAN	Q9NQS1	Cell death regulator Aven	51 921.3
BCAT2_HUMAN	O15382	Branched-chain amino acid aminotransferase, mitochondrial precursor	15 250.3
BCL9_HUMAN	O00512	B-cell lymphoma 9 protein	11 352.1
BID_HUMAN	P55957	BH3-interacting domain death agonist	18 864
CAPS1_HUMAN	Q9ULU8	Calcium-dependent secretion activator 1	18 330.1
CAPZB_HUMAN	P47756	F-actin-capping protein subunit-β	34 806.6
CATC_HUMAN	P53634	Dipeptidyl-peptidase 1 precursor	32 905.7
CD81_HUMAN	P60033	CD81 antigen	7444.4
CDC37_HUMAN	Q16543	Hsp90 co-chaperone Cdc37	6736.4
CDIPT_HUMAN	O14735	CDP-diacylglycerol–inositol 3-phosphatidyltransferase	8319.7
CHD2_HUMAN	O14647	Chromodomain-helicase DNA-binding protein 2	10 725.7
CILP2_HUMAN	Q8IUL8	Cartilage intermediate layer protein 2 precursor	4985.9
CJ070_HUMAN	Q9NZ45	CDGSH iron sulfur domain-containing protein 1	7602.6
CKLF6_HUMAN	Q9NX76	CKLF-like MARVEL transmembrane domain-containing protein 6	9380.8
CLPX_HUMAN	O76031	ATP-dependent Clp protease ATP-binding subunit clpX-like, mitochondrial precursor	14 317
CMC1_HUMAN	O75746	Calcium-binding mitochondrial carrier protein Aralar1	21 042.2
CNN2_HUMAN	Q99439	Calponin-2	9245.1
COMT_HUMAN	P21964	Catechol *O*-methyltransferase	11 550.3
COTL1_HUMAN	Q14019	Coactosin-like protein	8445.7
COX6C_HUMAN	P09669	Cytochrome *c* oxidase polypeptide VIc precursor	14 907.7
CPNE3_HUMAN	O75131	Copine-3	63 379
CPT1A_HUMAN	P50416	Carnitine *O*-palmitoyltransferase I	11 394
CPT2_HUMAN	P23786	Carnitine *O*-palmitoyltransferase 2	8983.7
CRIP2_HUMAN	P52943	Cysteine-rich protein 2	14 039.6
CT022_HUMAN	Q8N2K0	Abhydrolase domain-containing protein 12	8523.1
CTL2_HUMAN	Q8IWA5	Choline transporter-like protein 2	16 668.2
CUL4A_HUMAN	Q13619	Cullin-4A	10 036.2
CX4NB_HUMAN	O43402	Neighbor of COX4	15 031.5
CY1_HUMAN	P08574	Cytochrome *c*1 heme protein, mitochondrial precursor	30 523.1

Reference	Accession no.	Protein name	TIC
CYB_HUMAN	P00156	Cytochrome *b*	8721.9
D3D2_HUMAN	P42126	3,2-*trans*-Enoyl-CoA isomerase, mitochondrial precursor	9947.5
DC1L1_HUMAN	Q9Y6G9	Cytoplasmic dynein 1 light intermediate chain 1	8099.9
DCXR_HUMAN	Q7Z4W1	L-xylulose reductase	21 826.4
DNJCD_HUMAN	O75165	DnaJ homolog subfamily C member 13	7074.2
DPM1_HUMAN	O60762	Dolichol-phosphate mannosyltransferase	31 085.1
DPYL2_HUMAN	Q16555	Dihydropyrimidinase-related protein 2	7181.2
EDG1_HUMAN	P21453	Sphingosine 1-phosphate receptor Edg-1	18 866.8
EFHD1_HUMAN	Q9BUP0	EF-hand domain-containing protein 1	8327.1
EHD3_HUMAN	Q9NZN3	EH domain-containing protein 3	10 396.4
ERD21_HUMAN	P24390	ER lumen protein retaining receptor 1	8209.9
ERG7_HUMAN	P48449	Lanosterol synthase	9864.7
ERO1A_HUMAN	Q96HE7	ERO1-like protein α-precursor	50 453.4
F10A1_HUMAN	P50502	Hsc70-interacting protein	6322.6
FALZ_HUMAN	Q12830	Nucleosome-remodeling factor subunit BPTF	4769.7
FKB11_HUMAN	Q9NYL4	FK506-binding protein 11 precursor	6478.2
FKBP2_HUMAN	P26885	FK506-binding protein 2 precursor	7629.6
FOXE1_HUMAN	O00358	Forkhead box protein E1	5485.3
FSHP1_HUMAN	Q92674	Centromere protein I	34 948.6
FUBP2_HUMAN	Q92945	Far upstream element-binding protein 2	12 317.2
G6PD_HUMAN	P11413	Glucose-6-phosphate 1-dehydrogenase	6507.7
G6PE_HUMAN	O95479	GDH/6PGL endoplasmic bifunctional protein precursor	9999.1
GALT1_HUMAN	Q10472	Polypeptide *N*-acetylgalactosaminyltransferase	53 048.7
GALT2_HUMAN	Q10471	Polypeptide *N*-acetylgalactosaminyltransferase 2	9890.4
GAN_HUMAN	Q9H2C0	Gigaxonin	10 382.9
GIMA1_HUMAN	Q8WWP7	GTPase IMAP family member 1	17 434.6
GIMA4_HUMAN	Q9NUV9	GTPase IMAP family member 4	30 626.4
GNA13_HUMAN	Q14344	Guanine nucleotide-binding protein α-13 subunit	18 203.6
GNAQ_HUMAN	P50148	Guanine nucleotide-binding protein G(q) subunit-α	12 849.4
GNPAT_HUMAN	O15228	Dihydroxyacetone phosphate acyltransferase	17 857.3
GOT1B_HUMAN	Q9Y3E0	Vesicle transport protein GOT1B	22 845.7
GPI8_HUMAN	Q92643	GPI-anchor transamidase precursor	8474.6
GSLG1_HUMAN	Q92896	Golgi apparatus protein 1 precursor	8259.1
GSTK1_HUMAN	Q9Y2Q3	GSTκ 1	13 196.9
GTR1_HUMAN	P11166	Solute carrier family 2, facilitated glucose transporter member 1	9697.9
H12_HUMAN	P16403	Histone H1.2	10 648.9
H13_HUMAN	P16402	Histone H1.3	10 588.7
HEXA_HUMAN	P06865	β-Hexosaminidase α-chain precursor	4790.5
HEXB_HUMAN	P07686	β-Hexosaminidase β-chain precursor	44 696.5
HPCA_HUMAN	P84074	Neuron-specific calcium-binding protein hippocalcin	5485.2
HS105_HUMAN	Q92598	Heat-shock protein 105 kDa	31 625.6
HYEP_HUMAN	P07099	Epoxide hydrolase 1	15 510.7

Reference	Accession no.	Protein name	TIC
IF2B_HUMAN	P20042	Eukaryotic translation initiation factor 2 subunit 2	6551.9
IF3I_HUMAN	Q9Y262	Eukaryotic translation initiation factor 3 subunit 6-interacting protein	17 844.5
IMA4_HUMAN	O00629	Importin subunit-α-4	18 660.5
IPYR2_HUMAN	Q9H2U2	Inorganic pyrophosphatase 2, mitochondrial precursor	32 618.6
ITA6_HUMAN	P23229	Integrin α-6 precursor	9834.6
ITB3_HUMAN	P05106	Integrin β-3 precursor	37 183.4
JAM1_HUMAN	Q9Y624	Junctional adhesion molecule A precursor	23 813.4
KCD12_HUMAN	Q96CX2	BTB/POZ domain-containing protein KCTD12	5322.3
KCY_HUMAN	P30085	UMP-CMP kinase	21 553
LAMC1_HUMAN	P11047	Laminin subunit-γ-1 precursor	8384.3
LCB1_HUMAN	O15269	Serine palmitoyltransferase 1	44 771.1
LCB2_HUMAN	O15270	Serine palmitoyltransferase 2	13 951.1
LETM1_HUMAN	O95202	Leucine zipper-EF-hand-containing transmembrane protein 1	102 800.4
LRC8A_HUMAN	Q8IWT6	Leucine-rich repeat-containing protein 8A	5524.6
LYRIC_HUMAN	Q86UE4	Protein LYRIC	14 411.6
M6PBP_HUMAN	O60664	Mannose-6-phosphate receptor-binding protein 1	17 737.2
MAGBA_HUMAN	Q96LZ2	Melanoma-associated antigen B10	13 155.4
MARCS_HUMAN	P29966	Myristoylated alanine-rich C-kinase substrate	6761.5
MCCC2_HUMAN	Q9HCC0	Methylcrotonoyl-CoA carboxylase β-chain, mitochondrial precursor	16 404
MDHC_HUMAN	P40925	Malate dehydrogenase, cytoplasmic	3436.2
MESD2_HUMAN	Q14696	Mesoderm development candidate 2	16 093.2
MGST1_HUMAN	P10620	Microsomal GST 1	14 198.8
MGST2_HUMAN	Q99735	Microsomal GST 2	4816.8
MINP1_HUMAN	Q9UNW1	Multiple inositol polyphosphate phosphatase 1 precursor	9538.3
MMP1_HUMAN	P03956	Interstitial collagenase precursor	141 867.5
MPPA_HUMAN	Q10713	Mitochondrial-processing peptidase α subunit	28 196.5
MRP5_HUMAN	O15440	Multidrug resistance-associated protein 5	16 717.4
MTX1_HUMAN	Q13505	Metaxin-1	7487.1
MYO7A_HUMAN	Q13402	Myosin-VIIa	17 899
MYO9B_HUMAN	Q13459	Myosin-IXb	24 284.9
NB5M_HUMAN			42 319.2
NCLN_HUMAN	Q969V3	Nicalin precursor	40 879.5
NI2M_HUMAN	Q9Y6M9	NADH dehydrogenase (ubiquinone) 1-β-subcomplex subunit 9	6197.3
NICA_HUMAN	Q92542	Nicastrin precursor	60 611.1
NIDM_HUMAN	O96000	NADH dehydrogenase (ubiquinone) 1-β-subcomplex subunit 10	34 170.2
NIPS1_HUMAN	Q9BPW8	Protein NipSnap1	36 764.3
NIPS2_HUMAN	O75323	Protein NipSnap2	7204.1
NLTP_HUMAN	P22307	Nonspecific lipid-transfer protein	19 383.6
NMDZ1_HUMAN	Q05586	Glutamate (NMDA) receptor subunit-ζ-1 precursor	18 973.7
NPC1_HUMAN	O15118	Niemann-Pick C1 protein precursor	32 284.3
NRDC_HUMAN	O43847	Nardilysin precursor	15 906.1
NU2M_HUMAN	P03891	NADH-ubiquinone oxidoreductase chain 2	11 552.3

Reference	Accession no.	Protein name	TIC
NUCM_HUMAN	O75306	NADH dehydrogenase (ubiquinone) iron-sulfur protein 2	17 946.8
NUDM_HUMAN	O95299	NADH dehydrogenase (ubiquinone) 1-α-subcomplex subunit 10	34 228.4
NUKM_HUMAN	O75251	NADH dehydrogenase (ubiquinone) iron-sulfur protein 7	6697.2
NUYM_HUMAN	O43181	NADH dehydrogenase (ubiquinone) iron-sulfur protein 4	10 494.6
OAT_HUMAN	P04181	Ornithine aminotransferase	21 921.7
ODO2_HUMAN	P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	172 525.6
ODPA_HUMAN	P08559	Pyruvate dehydrogenase E1 component α-subunit, somatic form	41 851.8
OFUT1_HUMAN	Q9H488	GDP-fucose protein *O*-fucosyltransferase 1 precursor	49 430.7
OFUT2_HUMAN	Q9Y2G5	GDP-fucose protein *O*-fucosyltransferase 2 precursor	8774.8
OSTF1_HUMAN	Q92882	Osteoclast-stimulating factor 1	9496.5
P4HA2_HUMAN	O15460	Prolyl 4-hydroxylase subunit-α-2 precursor	20 283.1
PCDGM_HUMAN	Q9Y5F6	Protocadherin-γ-C5 precursor	26 803.1
PCYOX_HUMAN	Q9UHG3	Prenylcysteine oxidase 1 precursor	44 449.7
PDCD7_HUMAN	Q8N8D1	Programmed cell death protein 7	16 635.4
PDCD8_HUMAN	O95831	Apoptosis-inducing factor 1	48 808.6
PEBP_HUMAN	P30086	Phosphatidylethanolamine-binding protein 1	18 351.4
PERT_HUMAN	P07202	Thyroid peroxidase precursor	9268.4
PIGU_HUMAN	Q9H490	GPI transamidase component PIG-U	6885.6
PLSL_HUMAN	P13796	Plastin-2	4736.4
PPA5_HUMAN	P13686	Tartrate-resistant acid phosphatase type 5 precursor	7165.2
PPAL_HUMAN	P11117	Lysosomal acid phosphatase precursor	22 810.8
PPIC_HUMAN	P45877	Peptidyl-prolyl *cis–trans* isomerase C	7521.7
PRDX5_HUMAN	P30044	Peroxiredoxin-5, mitochondrial precursor	27 545
PSA7_HUMAN	O14818	Proteasome subunit-α-type-7	7698.1
PTN1_HUMAN	P18031	Tyrosine-protein phosphatase nonreceptor type 1	9163.5
RAB14_HUMAN	P61106	Ras-related protein Rab-14	51 245.1
RAB5A_HUMAN	P20339	Ras-related protein Rab-5A	47 974.5
RAB6A_HUMAN	P20340	Ras-related protein Rab-6A	19 901
RAB8B_HUMAN	Q92930	Ras-related protein Rab-8B	16 517.6
RAC1_HUMAN	P63000	Ras-related C3 botulinum toxin substrate 1 precursor	21 333
RAC2_HUMAN	P15153	Ras-related C3 botulinum toxin substrate 2 precursor	25 760.3
RAD51_HUMAN	Q06609	DNA repair protein RAD51 homolog 1	26 360
RDH11_HUMAN	Q8TC12	Retinol dehydrogenase 11	9905.1
RER1_HUMAN	O15258	Protein RER1	51 282.1
RHG01_HUMAN	Q07960	Rho GTPase-activating protein 1	5484.1
RHG05_HUMAN	Q13017	Rho GTPase-activating protein 5	29 811.6
RL26L_HUMAN	Q9UNX3	60S ribosomal protein L26-like 1	13 508.4
RL35A_HUMAN	P18077	60S ribosomal protein L35a	14 556.4
RM03_HUMAN	P09001	Mitochondrial 39S ribosomal protein L3	7947.6
RM19_HUMAN	P49406	39S ribosomal protein L19,	18 941.2
RM23_HUMAN	Q16540	Mitochondrial 39S ribosomal protein L23	7021.3

Reference	Accession no.	Protein name	TIC
RM45_HUMAN	Q9BRJ2	39S ribosomal protein L45	4865.1
RM47_HUMAN	Q9HD33	39S ribosomal protein L47	8733.4
RM49_HUMAN	Q13405	Mitochondrial 39S ribosomal protein L49	9348.2
ROAA_HUMAN	Q99729	Heterogeneous nuclear ribonucleoprotein A/B	11 786.2
RS29_HUMAN	P23368	40S ribosomal protein S29	28 316.9
RS30_HUMAN	P62861	40S ribosomal protein S30	28 780
RT05_HUMAN	P82675	Mitochondrial 28S ribosomal protein S5	22 731.3
RT21_HUMAN	P82921	Mitochondrial 28S ribosomal protein S21	5830.9
RT22_HUMAN	P82650	Mitochondrial 28S ribosomal protein S22	19 168
RT23_HUMAN	Q9Y3D9	Mitochondrial ribosomal protein S23	11 273.1
RT27_HUMAN	Q92552	Mitochondrial 28S ribosomal protein S27	9109.5
RT29_HUMAN	P51398	Mitochondrial 28S ribosomal protein S29	16 080.6
RT31_HUMAN	Q92665	28S ribosomal protein S31	9180.6
SAHH_HUMAN	P23526	Adenosylhomocysteinase	6693.1
SAM50_HUMAN	Q9Y512	Sorting and assembly machinery component 50 homolog	20 417.2
SC61B_HUMAN	P60468	Protein transport protein Sec61 subunit-β	4296.1
SCOT2_HUMAN	Q9BYC2	Succinyl-CoA:3-ketoacid-coenzyme A transferase 2	5226
SEC63_HUMAN	Q9UGP8	Translocation protein SEC63 homolog	13 869.8
SELT_HUMAN	P62341	Selenoprotein T precursor	6084.9
SNP23_HUMAN	O00161	Synaptosomal-associated protein 23	6975
SPB9_HUMAN	P50453	Serpin B9	12 587.1
SPC18_HUMAN	P67812	Signal peptidase complex catalytic subunit SEC11A	57 989.1
SPCS2_HUMAN	Q15005	Signal peptidase complex subunit 2	109 085.2
SPCS3_HUMAN	P61009	Signal peptidase complex subunit 3	42 697.3
SPEE_HUMAN	P19623	Spermidine synthase	6974.5
SPFH2_HUMAN	O94905	Erlin-2 precursor	10 735.2
SRP14_HUMAN	P37108	Signal recognition particle 14 kDa protein	13 486.8
SRPRB_HUMAN	Q9Y5M8	Signal recognition particle receptor subunit-β	40 101.7
STAB1_HUMAN	Q9NY15	Stabilin-1 precursor	9749.5
STIP1_HUMAN	P31948	Stress-induced-phosphoprotein 1	12 685.4
STT3_HUMAN	P46977	Dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit STT3A	92 851.8
STXB3_HUMAN	O00186	Syntaxin-binding protein 3	14 934.9
SUCA_HUMAN	P53597	Succinyl-CoA ligase (GDP-forming) subunit-α	13 559.2
SUCB2_HUMAN	Q96I99	Succinyl-CoA ligase (GDP-forming) β-chain	22 082.8
SUMF2_HUMAN	Q8NBJ7	Sulfatase-modifying factor 2 precursor	25 979.5
SYFA_HUMAN	Q9Y285	Phenylalanyl-tRNA synthetase α-chain	8374.5
SYHH_HUMAN	P49590	Probable histidyl-tRNA synthetase	7355.6
SYJ2B_HUMAN	P57105	Synaptojanin-2-binding protein	11 689.6
SYNC_HUMAN	O43776	Asparaginyl-tRNA synthetase, cytoplasmic	9428.2
SYTC_HUMAN	P26639	Threonyl-tRNA synthetase, cytoplasmic	13 743
SYYM_HUMAN	Q9Y2Z4	Tyrosyl-tRNA synthetase	9239
TCTP_HUMAN	P13693	Translationally controlled tumor protein	14 280.2

Reference	Accession no.	Protein name	TIC
TFAM_HUMAN	Q00059	Transcription factor A	13 117.6
TFR1_HUMAN	P02786	Transferrin receptor protein 1	102 292.9
THIM_HUMAN	P42765	3-Ketoacyl-CoA thiolase, mitochondrial	55 188
TIM44_HUMAN	O43615	Import inner membrane translocase subunit TIM44	18 388.5
TINAL_HUMAN	Q9GZM7	Tubulointerstitial nephritis antigen-like precursor	7084.2
TM9S2_HUMAN	Q99805	Transmembrane 9 superfamily protein member 2 precursor	15 776
TOM70_HUMAN	O94826	Mitochondrial precursor proteins import receptor	21 430.9
TOR1A_HUMAN	O14656	Torsin-1A precursor	4789.9
TPM3_HUMAN	P06753	Tropomyosin α-3 chain	19 764.1
TRA2A_HUMAN	Q13595	Transformer-2 protein homolog	9704.9
TRS85_HUMAN	Q9Y2L5	Protein TRS85 homolog	4569.6
TSN14_HUMAN	Q8NG11	Tetraspanin-14	21 012.1
TXD10_HUMAN	Q96JJ7	Protein disulfide-isomerase TXNDC10 precursor	59 406.2
TXD12_HUMAN	O95881	Thioredoxin domain-containing protein 12 precursor	22 843.6
TXK_HUMAN	P42681	Tyrosine-protein kinase TXK	6124.9
TXTP_HUMAN	P53007	Tricarboxylate transport protein	35 465
UCHL1_HUMAN	P09936	Ubiquitin carboxyl-terminal hydrolase isozyme L1	24 672.3
UN84B_HUMAN	Q9UH99	Sad1/unc-84-like protein 2	16 362.3
VAMP2_HUMAN	P63027	Vesicle-associated membrane protein 2	2904.5
VATB1_HUMAN	P15313	Vacuolar ATP synthase subunit B, kidney isoform	25 398.4
VKORL_HUMAN	Q8N0U8	Vitamin K epoxide reductase complex subunit 1-like protein 1	5392.5
VPP1_HUMAN	Q93050	Vacuolar proton translocating ATPase 116 kDa subunit a isoform 1	39 169.7
XRP2_HUMAN	O75695	Protein XRP2	21 357.6
ZBTB5_HUMAN	O15062	Zinc finger and BTB domain-containing protein 5	10 611.5
ZN157_HUMAN	P51786	Zinc finger protein 157	19 137.7

Table 3

Proteins identified unique to PAI-1 EMPs

Reference	Accession no.	Protein name	TIC
A2MG_HUMAN	P01023	α-2-Macroglobulin precursor	47 140
ACOX2_HUMAN	Q99424	Acyl-coenzyme A oxidase 2	30 595.3
AKA11_HUMAN	Q9UKA4	A-kinase anchor protein 11	25 373.4
AL2S3_HUMAN	O60296	Trafficking kinesin-binding protein 2	17 423.5
AL4A1_HUMAN	P30038	δ-1-pyrroline-5-carboxylate dehydrogenase	11 675.2
ALP_HUMAN	Q9H0A0	*N*-acetyltransferase 10	14 821.5
ANM1_HUMAN	Q99873	Protein arginine *N*-methyltransferase 1	11 491.5
APC_HUMAN	P25054	Adenomatous polyposis coli protein	30 515.7
APOA1_HUMAN	P02647	Apolipoprotein A-I precursor	7573.1
ARPC2_HUMAN	O15144	Actin-related protein 2/3 complex subunit 2	4460.5
ASXL1_HUMAN	Q8IXJ9	Putative polycomb group protein ASXL1	16 073.1
AT5G1_HUMAN	P05496	ATP synthase lipid-binding protein	79 498.2
ATD3B_HUMAN	Q5T9A4	ATPase family AAA domain-containing protein 3B	9533.6
B2MG_HUMAN	P61769	β-2-Microglobulin precursor	4474
BINCA_HUMAN	Q96LW7	Bcl10-interacting CARD protein	25 791.4
CAR14_HUMAN	Q9BXL6	Caspase recruitment domain-containing protein 14	13 620
CD5L_HUMAN	O43866	CD5 antigen-like precursor	13 869.5
CEP4_HUMAN	Q66GS9	Centrosomal protein 4	8373.1
CN021_HUMAN	Q86U38	Pumilio domain-containing protein C14orf21	5057.9
CPSF5_HUMAN	O43809	Cleavage and polyadenylation specificity factor 5	22 770.2
CSK21_HUMAN	P68400	Casein kinase II subunit-α	6560.2
CUL1_HUMAN	Q13616	Cullin-1	18 137.6
DMBT1_HUMAN	Q9UGM3	Deleted in malignant brain tumors 1 protein precursor	29 815
DYN2_HUMAN	P50570	Dynamin-2	10 084.5
ECH1_HUMAN	Q13011	δ(3,5)-δ(2,4)-dienoyl-CoA isomerase	32 274.5
EHD1_HUMAN	Q9H4M9	EH domain-containing protein 1	31 397
EHD4_HUMAN	Q9H223	EH domain-containing protein 4	17 203.3
EVI2B_HUMAN	P34910	EVI2B protein precursor	6844.5
FETA_HUMAN	P02771	α-Fetoprotein precursor	7866.1
FINC_HUMAN	P02751	Fibronectin precursor	14 674.5
FLNC_HUMAN	Q14315	Filamin-C	9765.5
FSTL1_HUMAN	Q12841	Follistatin-related protein 1 precursor	121 026.8
FUSIP_HUMAN	O75494	FUS-interacting serine-arginine-rich protein 1	28 150.1
G3PT_HUMAN	O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific	8769.1
GGT5_HUMAN	P36269	γ-Glutamyltransferase 5 precursor	47 995.4
GRB10_HUMAN	Q13322	Growth factor receptor-bound protein 10	156 086.1
HBA_HUMAN	P69905	Hemoglobin subunit-α	120 457.8
HNRPR_HUMAN	O43390	Heterogeneous nuclear ribonucleoprotein R	29 275.2
HXB4_HUMAN	P17483	Homeobox protein Hox-B4	6668

Reference	Accession no.	Protein name	TIC
IDHC_HUMAN	O75874	Isocitrate dehydrogenase (NADP) cytoplasmic	4740.8
IF39_HUMAN	P55884	Eukaryotic translation initiation factor 3 subunit 9	10 199.1
ITB5_HUMAN	P18084	Integrin β-5 precursor	19 295.3
K0196_HUMAN	Q12768	Strumpellin	22 066.1
K1C14_HUMAN	P02533	Keratin, type I cytoskeletal 14	241 145.9
K1C16_HUMAN	P08779	Keratin, type I cytoskeletal 16	191 445.2
K22O_HUMAN	Q01546	Keratin, type II cytoskeletal 2 oral	28 104.7
KIFC1_HUMAN	Q9BW19	Kinesin-like protein KIFC1	23 906.5
LACTB_HUMAN	P83111	Serine β-lactamase-like protein LACTB, mitochondrial precursor	29 817.8
LNX2_HUMAN	Q8N448	Ligand of Numb protein X 2	19 453.1
M3K3_HUMAN	Q99759	Mitogen-activated protein kinase kinase kinase 3	13 983.5
MAGB1_HUMAN	P43366	Melanoma-associated antigen B1	4812.8
MLEY_HUMAN	P14649	Myosin light polypeptide 6B	5508.2
MMRN2_HUMAN	Q9H8L6	Multimerin-2 precursor	15 402.4
MYH14_HUMAN	Q7Z406	Myosin-14	33 589.1
NB6M_HUMAN	Q9P0J0	NADH dehydrogenase (ubiquinone) 1-α-subcomplex subunit 13	2925
NEDD8_HUMAN	Q15843	NEDD8 precursor	6134.5
NEUL_HUMAN	Q9BYT8	Neurolysin, mitochondrial precursor	62 238.3
NIPBL_HUMAN	Q6KC79	Nipped-B-like protein	18 925.7
NP1L4_HUMAN	Q99733	Nucleosome assembly protein 1-like 4	10 005.6
NPT2B_HUMAN	O95436	Sodium-dependent phosphate transport protein 2B	16 003
NR1H4_HUMAN	Q96RI1	Bile acid receptor	109 977.5
NSF_HUMAN	P46459	Vesicle-fusing ATPase	15 351.3
NUPM_HUMAN	P51970	NADH dehydrogenase (ubiquinone) 1-α-subcomplex subunit 8	7865.2
O13C3_HUMAN	Q8NGS6	Olfactory receptor 13C3	38 066.4
OTUB1_HUMAN	Q96FW1	Ubiquitin thioesterase OTUB1	5642.2
PAIRB_HUMAN	Q8NC51	Plasminogen activator inhibitor 1 RNA-binding protein	9677.4
PARP1_HUMAN	P09874	Poly(ADP-ribose) polymerase 1	15 303.6
PML_HUMAN	P29590	Probable transcription factor PML	7979.4
PRP8_HUMAN	Q6P2Q9	Pre-mRNA-processing-splicing factor 8	27 736.9
PRS6A_HUMAN	P17980	26S protease regulatory subunit 6A	12 072.7
PRS6B_HUMAN	P43686	26S protease regulatory subunit 6B	16 682.9
PRS8_HUMAN	P62195	26S protease regulatory subunit 8	26 249.4
PSA3_HUMAN	P25788	Proteasome subunit-α-type 3	6300.6
PSB5_HUMAN	P28074	Proteasome subunit-β-type 5 precursor	18 972.3
PSD1_HUMAN	Q99460	26S proteasome non-ATPase regulatory subunit	39 869.6
PSME1_HUMAN	Q06323	Proteasome activator complex subunit 1	12 661.9
PX11B_HUMAN	O96011	Peroxisomal membrane protein 11B	12 671.3
RANG_HUMAN	P43487	Ran-specific GTPase-activating protein	5664.9
RO60_HUMAN	P10155	60 kDa SS-A/Ro ribonucleoprotein	5432.3
RT30_HUMAN	Q9NP92	Mitochondrial 28S ribosomal protein S30	6654.8
SC23A_HUMAN	Q15436	Protein transport protein Sec23A	20 929

Reference	Accession no.	Protein name	TIC
SCN8A_HUMAN	Q9UQD0	Sodium channel protein type 8 subunit-α	24 593.9
SF3B1_HUMAN	O75533	Splicing factor 3B subunit 1	14 944.1
SF3B3_HUMAN	Q15393	Splicing factor 3B subunit 3	21 675.6
SH3L3_HUMAN	Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3	8768.1
SODC_HUMAN	P00441	Superoxide dismutase (Cu-Zn)	7680.4
SPRC_HUMAN	P09486	SPARC precursor	30 380.7
STX5_HUMAN	Q13190	Syntaxin-5	10 522.4
SYD_HUMAN	P14868	Aspartyl-tRNA synthetase, cytoplasmic	7720
SYFB_HUMAN	Q9NSD9	Phenylalanyl-tRNA synthetase β-chain	12 248.2
SYNE1_HUMAN	Q8NF91	Nesprin-1	15 490
TALDO_HUMAN	P37837	Transaldolase	36 482.2
TLL1_HUMAN	O43897	Tolloid-like protein 1 precursor	24 774
TPR_HUMAN	P12270	Nucleoprotein TPR	3998.5
TRA2B_HUMAN	P62995	Splicing factor, arginine/serine-rich 10	38 082.7
TRY1_HUMAN	P07477	Trypsin-1 precursor	14 681
UBP14_HUMAN	P54578	Ubiquitin carboxyl-terminal hydrolase 14	14 597.9
VATC_HUMAN	P21283	Vacuolar ATP synthase subunit C 1	9337.2
VATL_HUMAN	P27449	Vacuolar ATP synthase 16 kDa proteolipid subunit	107 533.9
VPS26_HUMAN	O75436	Vacuolar protein sorting-associated protein 26A	23 112.4
VTNC_HUMAN	P04004	Vitronectin precursor	36 645
WNT6_HUMAN	Q9Y6F9	Protein Wnt-6 precursor	12 273.1
XYLT2_HUMAN	Q9H1B5	Xylosyltransferase 2	35 817
ZN443_HUMAN	Q9Y2A4	Zinc finger protein 443	9180.1

Table 4

Proteins identified unique to TNF-α EMPs

Reference	Accession no.	Protein name	TIC
3HIDH_HUMAN	P31937	3-Hydroxyisobutyrate dehydrogenase	132 06.7
ABCE1_HUMAN	P61221	ATP-binding cassette subfamily E member	4498.5
AP2B1_HUMAN	P63010	AP-2 complex subunit-β-1	7398.7
AP3M1_HUMAN	Q9Y2T2	AP-3 complex subunit-μ-1	12 842.9
APEX1_HUMAN	P27695	DNA-(apurinic or apyrimidinic site) lyase	7705.5
BRSK1_HUMAN	Q8TDC3	BR serine/threonine-protein kinase 1	23 559.7
BTF3_HUMAN	P20290	Transcription factor BTF3	4593.6
BZRP_HUMAN	P30536	Translocator protein	23 303.5
CD276_HUMAN	Q5ZPR3	CD276 antigen precursor	16 043.7
CDS2_HUMAN	O95674	Phosphatidate cytidylyltransferase 2	4957.3
COA1_HUMAN	Q13085	Acetyl-CoA carboxylase 1	22 689.5
CRTAP_HUMAN	O75718	Cartilage-associated protein precursor	8178.9
CTNL1_HUMAN	Q9UBT7	α-Catulin	36 690.7
CXCC1_HUMAN	Q9P0U4	CpG-binding protein	20 920
DESP_HUMAN	P15924	Desmoplakin	22 369.4
DNJC7_HUMAN	Q99615	DnaJ homolog subfamily C member 7	21 279.4
DRIM_HUMAN	O75691	Small subunit processome component 20 homolog	21 872.6
DYNA_HUMAN	Q14203	Dynactin subunit 1	16 194.7
FKBP7_HUMAN	Q9Y680	FK506-binding protein 7 precursor	23 947.3
GNPI_HUMAN	P46926	Glucosamine-6-phosphate isomerase	18 263.3
HNRPG_HUMAN	P38159	Heterogeneous nuclear ribonucleoprotein G	6693
HSP71_HUMAN	P08107	Heat-shock 70 kDa protein 1	47 387.8
I23O_HUMAN	P14902	Indoleamine 2,3-dioxygenase	15 442.5
ICAM1_HUMAN	P05362	Intercellular adhesion molecule 1 precursor	26 540.7
IMA2_HUMAN	P52292	Importin subunit-α-2	27 202.6
IMB3_HUMAN	O00410	Importin-β-3	15 359
ITPR3_HUMAN	Q14573	Inositol 1,4,5-trisphosphate receptor type 3	15 484.8
K2C4_HUMAN	P19013	Keratin, type II cytoskeletal 4	48 652
KAP3_HUMAN	P31323	cAMP-dependent protein kinase type II-β regulatory subunit	16 233.4
KIF4A_HUMAN	O95239	Chromosome-associated kinesin KIF4A	26 392
LAMA4_HUMAN	Q16363	Laminin subunit-α-4 precursor	23 825.6
LAMB1_HUMAN	P07942	Laminin subunit-β-1 precursor	21 492.5
LIPA3_HUMAN	O75145	Liprin-α-3	2729.6
LRFN2_HUMAN	Q9ULH4	Leucine-rich repeat and fibronectin type III domain-containing protein 2 precursor	62 612.5
MACF1_HUMAN	Q9UPN3	Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5	26 409.1
MCM6_HUMAN	Q14566	DNA replication licensing factor MCM6	14 861.6
MIS_HUMAN	P03971	Muellerian-inhibiting factor precursor	11 582.3
NEP_HUMAN	P08473	Neprilysin	8834.7
NEUA_HUMAN	Q8NFW8	*N*-acylneuraminate cytidylyltransferase	26 264.8

Reference	Accession no.	Protein name	TIC
NMT1_HUMAN	P30419	Glycylpeptide *N*-tetradecanoyltransferase 1	9662
NQO1_HUMAN	P15559	NAD(P)H dehydrogenase (quinone) 1	15 878.3
NTHL1_HUMAN	P78549	Endonuclease III-like protein 1	6149.2
NU4M_HUMAN	P03905	NADH-ubiquinone oxidoreductase chain 4	5422
ORN_HUMAN	Q9Y3B8	Oligoribonuclease, mitochondrial precursor	11 393.8
PAPPA_HUMAN	Q13219	Pappalysin-1 precursor	6338.7
PLXD1_HUMAN	Q9Y4D7	Plexin-D1 precursor	19 437.3
PSB2_HUMAN	P49721	Proteasome subunit-β-type 2	53 641.3
PSB6_HUMAN	P28072	Proteasome subunit-β-type 6 precursor	5379.3
PSB7_HUMAN	Q99436	Proteasome subunitβ-type 7 precursor	7635.4
PSDE_HUMAN	O00487	26S proteasome nonATPase regulatory subunit 14	14 168.5
PUR2_HUMAN	P22102	Trifunctional purine biosynthetic protein adenosine-3	5535
RBL2_HUMAN	Q08999	Retinoblastoma-like protein 2	4621.4
RINI_HUMAN	P13489	Ribonuclease inhibitor	15 180.7
RL13A_HUMAN	P40429	60S ribosomal protein L13a	8885.7
RL35_HUMAN	P42766	60S ribosomal protein L35	10 179.2
S10AG_HUMAN	Q96FQ6	Protein S100-A16	7285.6
SC10A_HUMAN	Q9Y5Y9	Sodium channel protein type 10 subunit-α	44 255.4
SC24C_HUMAN	P53992	Protein transport protein Sec24C	8599.8
SF3A3_HUMAN	Q12874	Splicing factor 3A subunit 3	8704.3
SNUT1_HUMAN	O43290	U4/U6.U5 tri-snRNP-associated protein 1	76 618.9
SPRE_HUMAN	P35270	Sepiapterin reductase	11 487.8
STX16_HUMAN	O14662	Syntaxin-16	12 549.9
SYI_HUMAN	P41252	Isoleucyl-tRNA synthetase, cytoplasmic	39 914.8
SYS_HUMAN	P49591	Seryl-tRNA synthetase, cytoplasmic	29 871
SYW_HUMAN	P23381	Tryptophanyl-tRNA synthetase, cytoplasmic	5227
TENX_HUMAN	P22105	Tenascin-X precursor	9015.2
TF3A_HUMAN	Q92664	Transcription factor IIIA	14 060.6
THIO_HUMAN	P10599	Thioredoxin	24 809.3
TIM50_HUMAN	Q3ZCQ8	Import inner membrane translocase subunit TIM50, mitochondrial precursor	18 674.5
ZN225_HUMAN	Q9UK10	Zinc finger protein 225	17 042.8