Proteomic discovery of 21 proteins expressed in human plasma-derived but not platelet-derived microparticles

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Summary

Microparticles (MPs) are small membrane vesicles generated by essentially all cell types. In the plasma, most MPs are derived from platelets, but those from other sources, particularly leukocytes (macrophages, lymphocytes, and neutrophils), endothelial cells, and even smooth muscle cells can be detected and appear to play an important role in normal physiology and various diseases. In previous work we analyzed the proteome of MPs generated from isolated platelets (platelet MPs). Here, we report on a comparative analysis of microparticles isolated from plasma (plasma MPs) versus platelet MP using two complementary methods of comparative analysis. The first method, spectral count analysis, yielded 21 proteins detected in plasma MPs (with a total spectral count of 10 or greater) that were essentially absent in platelet MPs (with a total spectral count of 1 or 0). An additional two proteins (von Willebrand Factor, albumin) were present in both types of MPs but enriched in the plasma MPs. The second method, isotope-coded affinity tag (ICAT) labeling of proteins, supported the spectral count results for the more abundant proteins and provided better relative quantitation of differentially expressed proteins. Proteins present only in the plasma MPs include several associated with apoptosis (CD5-like antigen, galectin 3 binding protein, several complement components), iron transport (transferrin, transferrin receptor, haptoglobin), immune response (complement components, immunoglobulin J and kappa chains), and the coagulation process (protein S, coagulation factor VIII).

Keywords

Secretion / exocytosis, platelet activation markers, vascular cell markers

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Introduction

Microparticles (MPs) are small subcellular membranous vesicles released by essentially all cell types, especially when activated or under stress. They include ectosomes, generated from the ectocytosis (or blebbing) of the plasma membrane and exosomes, released by fusion of intracellular multivesicular endosomes with the cell surface (1, 2). In plasma, MPs were discovered as a component of the blood that promotes coagulation due to the presence of anionic phospholipids on their outer surface (3). These anionic phospholipids, later determined to be mostly phosphatidylserine, are now widely used to detect MPs from blood samples using flow cytometry based on their affinity for fluorescently labeled annexin V. The cellular source of the microparticles is determined by cell-specific markers detected by flow cytometry (4). For example, MPs with CD41 (glycoprotein IIb) expression are believed to be generated by platelets. Using this method, microparticles from platelets, erythrocytes, endothelial cells, neutrophils, lymphocytes and even smooth muscle cells have been detected in the plasma. In healthy individuals, over 90% of these MPs originate from platelets (4), and under a wide range of pathological conditions the total number of microparticles and the number originating from various cell population are altered (5, 6).

Traditionally, the presence of microparticles in the plasma is considered a sign of cellular activation and/or damage generated by the random blebbing of cell membranes. However, significant evidence has accumulated to indicate that MPs contain a unique set of proteins (7, 8) and inflammatory factors (9) that have important biological functions. For example, rapid secretion of

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IL-1β from THP-1 monocytes occurs via MP release in ectosomes, and this could be a general mechanism of release for secreted proteins that lack the conventional signal peptide for secretory proteins (10). MPs isolated either from plasma or the supernatants of stimulated cells have a wide variety of effects on cellular function (11). These effects include increased expression of adhesion molecules on endothelial cells and monocytes (12), stimulation of cytokine release (13, 14), altering vascular reactivity (15), inducing angiogenesis (16), decreasing the response to inflammatory mediators (17), and fibrin formation (18, 19). The effects vary depending on the cellular source of the microparticles, the method to generate them and the cells (or tissue) affected (11).

While many groups have studied the effects of these MPs on various cells, the protein composition of plasma MPs is largely unknown except for the presence of a few cell-surface markers. Recently, we analyzed the proteome of platelet-derived microparticles and identified 578 proteins (20). This current study examines the proteomic composition of plasma MPs with a focus on proteins not found in platelet-derived MP. Microparticles were isolated from plasma by gel filtration and ultracentrifugation. Two methods were used to determine the relative protein abundance; (1) MS-MS spectral count and (2) ICAT-based comparative analysis. For the MS-MS spectral count, microparticle proteins from plasma and platelet MPs were prepared and analyzed using a method consisting of electrophoresis, trypsin digestion, and online liquid chromatography-mass spectrometry (LC/MS) using a linear ion trap mass spectrometer. We identified peptides from 21 proteins in plasma MPs (with a total spectral count of 10 or greater) that were essentially absent in platelet MPs (with a total spectral count of 0 or 1). Ten of the 21 proteins were determined to be enriched in the plasma MPs to a statistically significant extent. Two additional proteins were present in both types of MPs but were more abundant in the plasma MPs. For ICAT-based comparative analysis, proteins from these two sources were differentially labeled with isotope-coded affinity tag (ICAT) reagents, mixed, delipidated by PAGE, trypsin digested, enriched for ICAT-labeled peptides, and analyzed by LC/MS. This method supported the results from spectral count analysis and provided additional quantitative information about the more abundant proteins. It also yielded detection of hemoglobin as an additional protein enriched in plasma MPs.

Materials and methods

Isolation of platelets, platelet-derived MPs, and plasma-derived MPs

Platelets and platelet-derived MPs were isolated as described (20). Briefly, human blood was collected by venipuncture into 1/10 volume of an acid-citrate-dextrose (85 mM trisodium citrate, 83 mM dextrose, and 21 mM citric acid) solution. Platelet-rich plasma (PRP) was obtained by centrifugation at 110 × *g* for 15 minutes (min). Platelets were pelleted by centrifugation at 710 × *g* for 15 min and the supernatant, platelet-poor plasma (PPP), was retained for isolation of plasma MPs (see below). The platelet pellet was washed three times, re-suspended in 10 ml of Tyrode's buffer, and centrifuged one additional time at 110 × *g* to remove remaining red blood cells and dead cells. To generate platelet-derived microparticles, ADP (10 μM final concentration) was added to the platelet suspension for 10 min (20). Platelets were removed by centrifugation (710 x *g* for 15 min) and platelet-derived MPs were pelleted by centrifugation at 150,000 × *g* for 90 min at 10°C.

Plasma-derived MPs were isolated by gel filtration chromatography followed by ultracentrifugation. Briefly, platelet-poor plasma (PPP) was centrifuged twice to remove residual cells and cell debris at 710 x *g* and 25°C for 15 min. This plasma was then applied to a Sephacryl® S-500 HR (GE Healthcare, Piscataway, NJ, USA) gel filtration column and MP-containing fractions were concentrated by ultracentrifugation at 150,000 x *g* for 90 min at 10°C.

Sample preparation for unlabeled protein analysis

Platelet- and plasma-derived microparticle pellets were processed as depicted in Figure 1A. MPs were re-suspended in a minimal volume of PBS (phosphate buffered saline, pH 7.4) and a small aliquot was taken for protein analysis using the Micro BCA Protein Assay (Pierce Biotechnology, Inc., Rockford, IL, USA). Forty microliters of plasma microparticles and an equivalent amount of protein from the platelet MPs, re-suspended with PBS to 40 μl, were mixed with 10 μl of 5X SDS-PAGE loading buffer (0.5 M Tris, pH 6.8, 10% SDS, 38% glycerol, 0.1% bromophenol blue). The separate samples (50 μl each) were heated to 95°C for 5 min, allowed to cool down to room temperature, and centrifuged for 2 min at 14,000 rpm prior to loading onto the gel. Microparticle proteins were electrophoresed approximately 1 cm into a 7.5 % acrylamide SDS-PAGE using a Mini-gel system (Biorad, Hercules, CA, USA) at 150 V. The acrylamide gel section containing the proteins was cut out and placed in fixative (50% methanol, 12% acetic acid, 0.05% formalin) for 2 hours (h). The in-gel tryptic digestion of the lanes and the peptide extraction were performed as described (21). The extracted peptide solutions were lyophilized and reconstituted to 20 μl with 0.1% acetic acid for mass spectrometry analysis. A total of three sets of platelet- and plasma-derived MP peptides were generated from three donors, and each of these samples was analyzed by LC/MS twice.

Western immunoblot analysis

Plasma MPs and platelet MPs were isolated and proteins were separated by SDS-PAGE as described above with the following modifications. The sample was divided into two equal aliquots and each was applied to a separate gel. The gels were stopped when the bromophenol blue band neared the bottom of the gel. One gel was silver stained to verify equal loading of the samples. The proteins on the other gel were transferred to an Immobilon P membrane (Millipore, Billerica, MA, USA) and the membrane was blocked with Sea-Block (Pierce Biotechnology) for 30 min. Immunoblotting was carried out to determine the presence of complement component 3, vWF, and galectin 3-binding protein. Primary antibodies against C3 (clone 2898, Cell Science, Canton, MA, USA), vWF (clone GMA-022, Upstate, Lake Placid, NY, USA), or galectin 3-binding protein (polyclonal rabbit anti-human, Protein Tech Group, Inc., Chicago, IL, USA) were used. Immunoreactive bands were visualized using horseradish peroxidase-conjugated anti-rabbit (Sigma, St. Louis, MO, USA) or anti-mouse IgG (Pierce) and Immun-star HRP Chemiluminescent Kit (Biorad).

ICAT-labeling, electrophoresis, digestion, and peptide enrichment

Comparative quantitation of proteins following ICAT-labeling was performed as depicted in Figure 1B. Briefly, platelet- and plasma-derived microparticle pellets were re-suspended in PBS (Phosphate buffered saline, pH 7.4), and protein concentration was determined. Solutions containing equivalent protein amounts of paired samples (plasma MP and platelet-derived MP) were lyophilized and re-suspended in a 1% SDS denaturing buffer. Samples were labeled and processed using the ICAT labeling kit (Applied Biosystems, Foster City, CA, USA) as instructed with the following modifications. The initial labeling reaction was performed at half of the recommended volume, protein amount, and ICAT reagent because of the low amount of

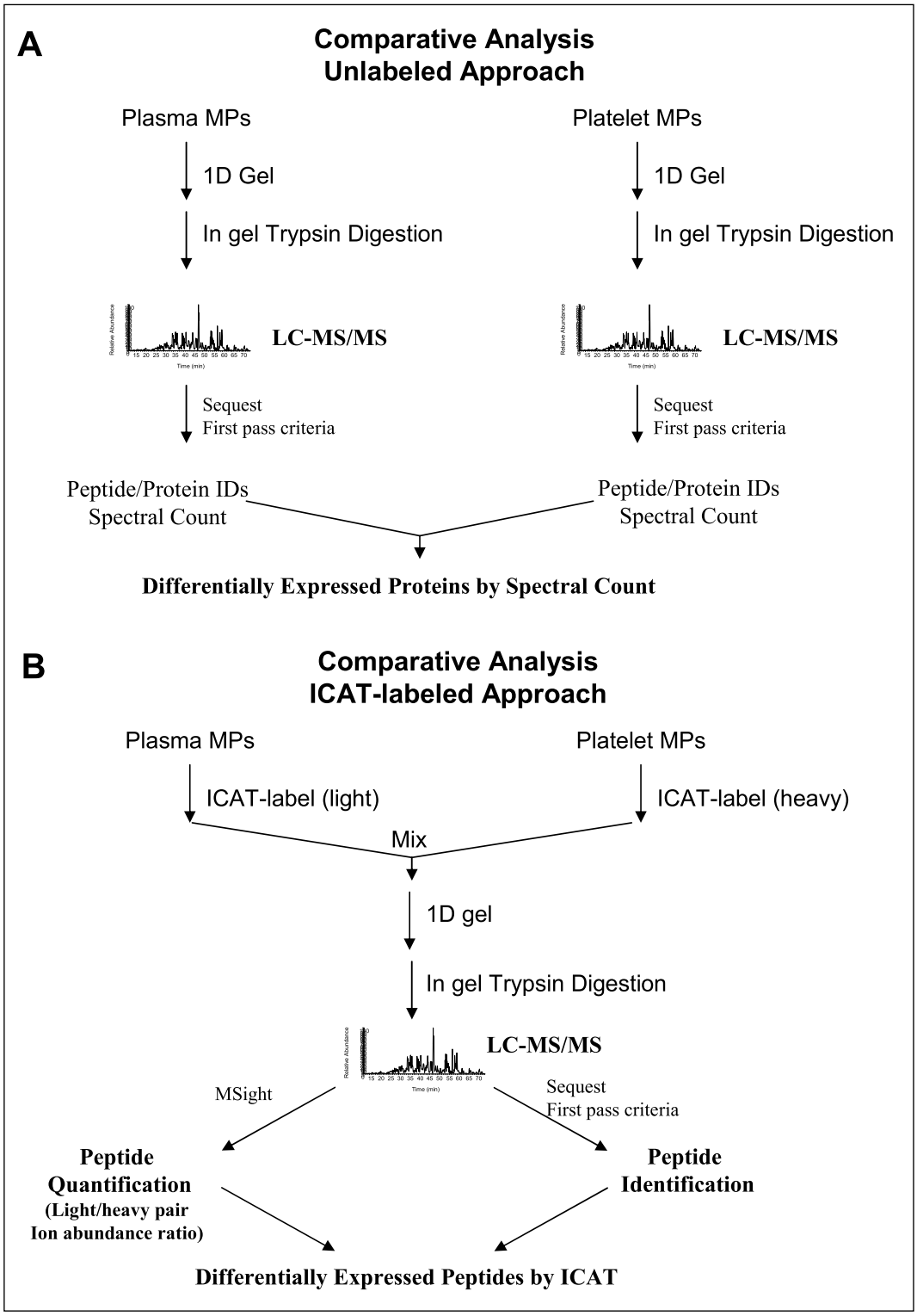

Figure 1: Comparative proteomic analysis of plasma MPs and platelet MPs. Two complementary approaches to compare the proteome of plasma MPs and platelet MPs are depicted. A) Comparative analysis of unlabeled proteins based on MS-MS spectral count. B) Comparative analysis of ICAT-labeled proteins detected by MSight followed by manual quantitation of labeled peptide ion intensities.

protein obtained from each plasma MP preparation. The differentially-labeled proteins were mixed, applied to the gel, electrophoresed, and cut from the gel as described above except that the loading buffer did not contain the reducing agent or SDS. The proteins were digested with trypsin, extracted from the gel and processed through the avidin column as recommended by manufacturer. The samples were lyophilized and reconstituted to 20 µl with 0.1% acetic acid for mass spectrometry analysis. This procedure was repeated three times with plasma MPs labeled with the light ICAT reagent for two of these samples and labeled with the heavy ICAT reagent in the third.

Liquid chromatography/mass spectrometry (LC/MS) and protein identification

Samples were loaded onto a 360 µm o.d. × 75 µm i.d. microcapillary fused silica precolumn packed with irregular 5–20 µm C18 resin. After sample loading, the precolumn was washed with 0.1% acetic acid for 15 min to remove any buffer salts or gel contaminants. The precolumn was then connected to a 360 µm o.d. × 50 µm i.d. analytical column packed with regular 5 µm C18 resin constructed with an integrated electrospray emitter tip. Samples were gradient eluted at a flow rate of 60 nL/min with an 1100 series binary HPLC solvent delivery system (Agilent, Palo Alto, CA, USA) directly through an electrospray ionization source interfaced to a Finnigan LTQ ion trap mass spectrometer (Thermo Electron Corp, San Jose, CA, USA). The HPLC gradient used was initially 100%A, 5% B at 5 min, 50% B at 220 min, 100% B at 240 min, and restored to 100% A at 280 min (solvent A = 0.1 M acetic acid, solvent B = 70% acetonitrile in 0.1 M acetic acid). The LTQ mass spectrometer was operated in the data-dependent mode in which first an initial MS scan recorded the mass to charge (*m*/*z*) ratios of ions over the mass range

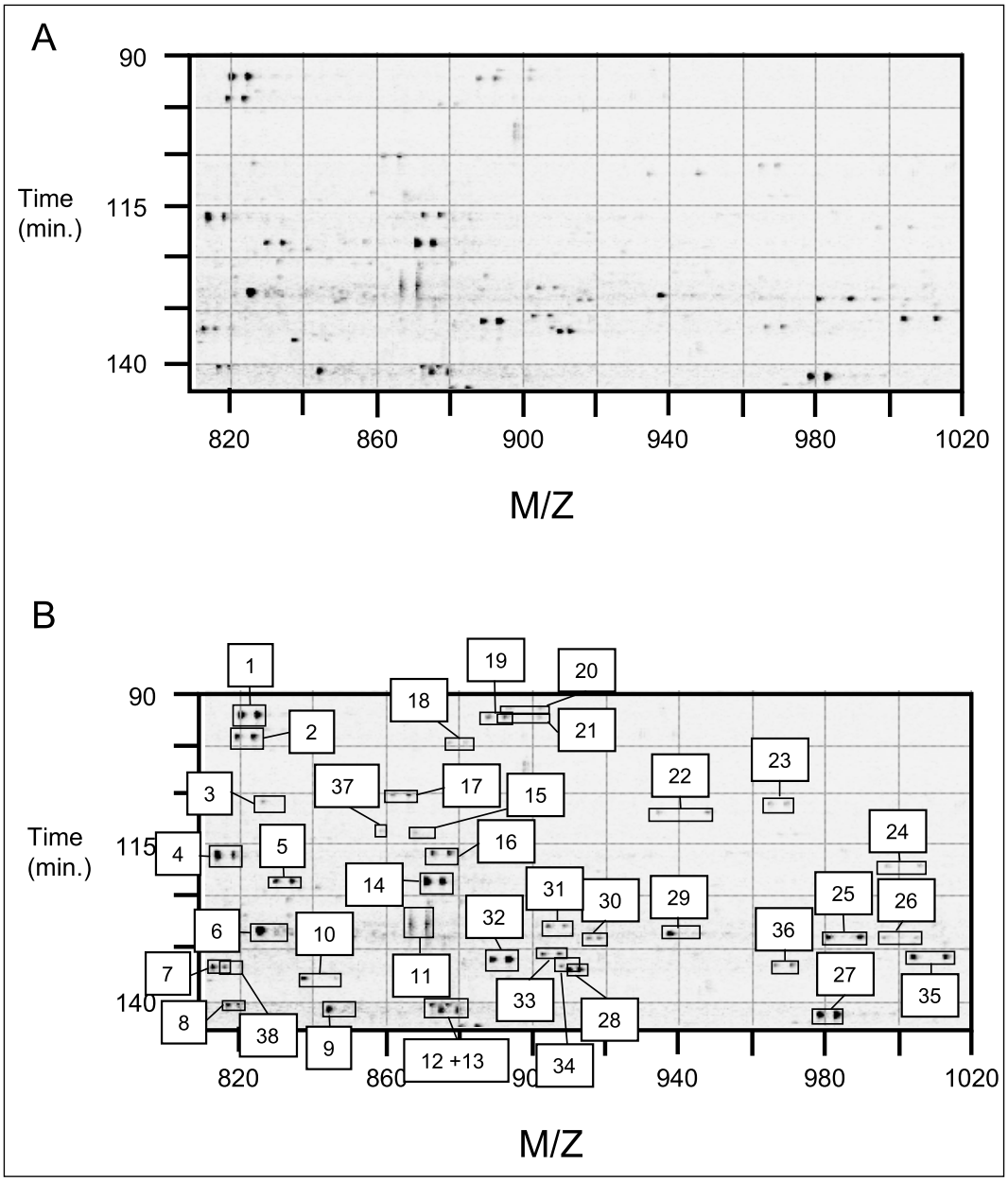

Figure 2: Sample section of 2D representation of data presented by MSight®. MS generated files (.raw) were converted into .mzXML files and imported into MSight. A) Sample portion of the plot of intensities of m/z 810 to 1020 ions that eluted between 90 and 145 min. B) Peptides were annotated and relative quantitation and identification (see Supplementary Table 1 available online at www.thrombosis-online.com). While most spots appear as pairs of equal intensity, there are several with different intensities (6, 9, 29, etc.)

300–2,000 Dalton (Da), and then the 10 most abundant ions were automatically selected for subsequent collisionally-activated dissociation and MS/MS spectrum recorded. All MS/MS data were searched against a human protein database downloaded from the NCBI database (www. ncbi.nlm.nih.gov) on August 24, 2004 using the SEQUEST® program (Thermo Electron Corp.). For unlabeled peptides, a static modification of 57 Da for cysteine residues was employed in the search parameters. For ICAT-labeled peptides, a static modification of 227.127 was used for the light isotope label and an additional 9 Da for the heavy ICAT-labeled peptides. Peptide identifications were made based on fully tryptic peptides, using a first-pass filtering of standard criteria as previously described (22), including cross correlation values ≥ 2.0 (+1 charge), 2.2 (+2 charge) and 3.5 (+3 charge). Protein assignments required at least two MS/MS spectra matches that passed the above criteria. Manual validation of at least one MS/MS spectrum –peptide sequence match per protein was performed for all proteins that were determined to be differentially expressed.

Comparative analysis of unlabeled peptides using spectral count

For the unlabeled scheme (Fig. 1A), all search results not passing the first-pass filter were eliminated. The remaining number of spectra for each peptide was determined, and the number of total proteins detected was calculated. Only proteins with an overall spectral count of 10 or greater were analyzed further by this method, and all with 1 or no MS-MS scan were considered essentially absent. The ratio of the number of spectra from the plasma MPs versus the platelet MPs was calculated, log 2 transformed, and then adjusted for an overall ratio score of 1 ($\log_2 = 0.00$) excluding the vWF-containing peptides. Statistical analysis was performed using the source statistical software package R (http://www.r-project.org/). The data from the unlabeled experiments were normalized so all experiments have the same interquartile ranges. In addition, log2-transformation was taken to reduce the skewed distributions. Some spectral counts were zero, so 0.5 was added to all values in order to allow log2-transformation. The two-sample t-test was performed to examine the significant difference in protein spectral counts between plasma and platelet MPs. $P < 0.05$ was used to determine differentially expressed proteins.

Comparative analysis of unlabelled peptides by ion intensity measurements

Ion intensities for peptides from each of the putative differentially expressed protein were determined using Qual Browser (Thermo Electron Corp.). Briefly, the twelve LC-MS chromatographs were aligned using 25 peptides that were detected in most, if not all, of the runs. The ion intensities for the five most abundant peptides (with a total spectral count of at least six) from each the putatively differentially expressed protein (based on spectral count results) were determined using a m/z range of ± 0.3 Da. For each peptide, a standard two-tailed t-test was performed. In addition, the overall significance of each protein was determined by normalizing the ion intensities for all peptides of a protein to have equal variances; hence, each peptide can have an equal weight when pooled for examining the overall significance. Treating peptides as a block effect, a randomized block design ANOVA was applied to the normalized data to determine the overall significance for a given protein.

Comparative analysis of ICAT-labeled peptides using MSight®

Comparative quantitation was performed using MSight, freely available from the Swiss Institute of Bioinformatics (www.expasy.org/Msight) (23). Data files (.RAW) generated using XCalibur Software (Thermo Electron Corp.) were converted to mzXML files using ReAdW (Institute for Systems Biology, Seattle, WA, USA). These files were imported into MSight, and the peptides were manually quantified. The peptides were identified using SEQUEST as described above. A sample representation of the MSight display for a small portion of one of these runs is shown in Figure 2 and ion intensities can be seen in Supplementary Table 1 available online at www.thrombosis-online.com. Initially, peptides that were detected in all three ICAT analyses based on SEQUEST results were quantified, if possible. Then, peptides with high ion intensities were quantified and linked backed to SEQUEST results. Attempts to quantify peaks sometimes failed due to poor signal-to-noise ratios, overlapping peptides, and ambiguous identification of a given peak. Unless otherwise noted, ICAT quantitation results are reported if good peak quantitation was possible in at least two of the three ICAT runs and labeled alternatively in these two runs. This led to the quantitation of 94 peptides. Utilizing the differential labeling, the ratios of plasma MPs to platelet MPs ICAT-peptide abundances were calculated, a log2 transformation of these ratios was performed, and adjusted to generate an overall log2 score of 0.00, excluding vWF. VWF was excluded because it was evident that there was a significant enrichment in the plasma MPs. Due to the large number of peptides detected for this protein and the extent of enrichment, not omitting it would make it appear that most other proteins were enriched in the platelet MPs. To determine significance, the paired t-test was used to examine differences in peptide expression intensities between plasma MPs and platelet MPs. $P < 0.05$ was used to determine differentially expressed peptides.

Results

While a large majority of the plasma MPs are platelet-derived, MPs from other sources have been implicated in normal physiology and under pathological conditions (4, 11). Previously, we have examined the platelet MP proteome and detected 578 proteins. In the present study, we isolated MP from plasma and compared them to platelet MP using two complementary techniques: i) spectral count analysis of unlabeled proteins/peptides and ii) manual relative quantitation of ion intensities of ICAT-labeled proteins/peptides.

Relative quantitation of plasma MPs and platelet MPs using spectral counts

Plasma MPs and platelet MPs were isolated from three individuals, the proteins processed and digested into tryptic peptides which were analyzed in duplicate (Fig. 1A). The MS/MS spectra was searched using SEQUEST and spectra not passing first-pass

Table 1: Proteins with altered expressions in plasma MP versus platelet MPs as determined by spectral count. Only proteins with a total spectal count of 10 or more are listed.

		Plasma MPs		Platelet MPs		Total	(Plasma MP/ Platelet MP)			
Significantly enriched in plasma MPs (p < 0.05)	**GI No.**	**Total scans**	**Different Peptides**	**Total scans**	**Different peptides**	**Spectral count**	**Ratio**	**Log2**	**Norm. log 2 ***	**P-value**
Complement component 4 binding protein, alpha	4502503	114	18	1	1	115	114.0	6.83	7.37	0.000
Fc fragment of IgG binding protein	4503681	167	46	0	0	167	--			0.000
Galectin 3 binding protein	5031863	68	16	0	0	68	--			0.001
Haptoglobin-related protein	45580723	86	16	0	0	86	--			0.002
CD5 antigen-like protein	5174411	24	10	0	0	24	--			0.006
Complement component 4 binding	4502505	14	5	0	0	14	--			0.018
Protein S, alpha	4506117	24	13	1	1	25	24.00	4.58	5.12	0.019
Immunoglobulin J chain	21489959	15	4	0	0	15	--			0.028
Ig kappa chain	51475407	10	2	0	0	10	--			0.032
Transferrin receptor	4507457	20	14	0	0	20	--			0.048
von Willebrand factor	4507907	745	85	23	15	768	32.39	5.02	5.56	0.000
Albumin	4502027	85	28	45	16	130	1.89	0.92	1.46	0.008
Present in plasma MPs but essentially absent in platelet MP (1 or less MS MS) of this study										
Complement component 3	4557385	11	10	0	0	11	--			0.052
Apolipoprotein C-III	4557323	10	2	0	0	10	--			0.052
Complement component 4B	4502501	86	37	1	1	87	86.00	6.43	6.97	0.060
Haptoglobin	4826762	32	10	0	0	32	--			0.071
Transferrin	4557871	11	8	1	1	12	11.00	3.46	4.00	0.073
Apolipoprotein E3	4557325	17	10	0	0	17	--			0.110
Tenascin C	4504549	10	9	0	0	10	--			0.121
Apolipoprotein B-100	4502153	37	25	0	0	37	--			0.144
Apolipoprotein A-I	4557321	28	10	0	0	28	--			0.203
Alpha-2-macroglobulin	4557225	36	21	0	0	36	--			0.204
Coagulation factor VIII	4503647	10	10	1	1	11	10.00	3.32	3.86	0.339
Enriched in Platelet MPs										
actin, beta	4501885	107	9	237	10	344	0.45	–1.15	–0.61	0.037
Pleckstrin	4505879	22	11	53	13	75	0.42	–1.27	–0.73	0.047
Myosin regulatory light chain MRCL2	15809016	17	7	43	8	60	0.40	–1.34	–0.80	0.022
Integrin alpha 2b	4504745	76	26	204	30	280	0.37	–1.42	–0.88	0.025
Transgelin 2	4507357	24	12	65	11	89	0.37	–1.44	–0.90	0.022
Glyceraldehyde 3-phosphate dehydrogenase	51465474	9	2	27	3	36	0.33	–1.58	–1.04	0.029
ATP synthase, H+ transporting, mitochondrial F1 complex beta	32189394	10	7	31	12	41	0.32	–1.63	–1.09	0.033
Profilin 1	4826898	35	9	112	7	147	0.31	–1.68	–1.14	0.012
Parvin, beta	20127528	12	6	43	8	55	0.28	–1.84	–1.30	0.009
SH3 domain binding glutamic acid-rich protein like	13775198	3	2	12	2	15	0.25	–2.00	–1.46	0.023
Ras suppressor protein 1 isoform 2	34577083	4	3	29	6	33	0.14	-2.86	-2.32	0.001

(*Normalized log2 of ratio of spectral count. The ratio of the spectral count for plasma MPs to platelet MPs is determine. The log2 of this ratio for each protein is calculated and than normalized to generate an overall log score of 0.00. See text for details.)

filtering were discarded. From the plasma MP samples, we obtained 1,343 ± 589 spectra per run (total of 8,058 spectra in six runs), identifying 3,726 unique peptides and 203 unique proteins with five or more spectra (see Supplementary Table 2 available online at www.thrombosis-online.com). From platelet MPs, we obtained 2,087 ± 812 spectra per run (total of 12,522 spectra in six runs), identifying 6,975 unique peptides and 229 unique proteins with five or more spectra.

For proteins with a cumulative spectral count of 10 or more, the relative spectral counts were compared between plasma MPs and platelet MPs (see Supplementary Table 3 available online at www.thrombosis-online.com and Table 1). The log 2 of the ratio of the spectral counts was normalized to an average of 0.00 (excluding vWF) which represents the null hypothesis of equal representation of proteins in plasma and platelet MPs. There were 21 proteins for which peptides were detected in plasma MPs but not platelet MPs (spectral count of 1 or 0); however, only 10 of these differences were statistically significant (Table 1). In addition, two other proteins (vWF, albumin) were present in both sets of MPs but enriched in the plasma MPs. There were also 11 proteins enriched in the platelet MPs. This apparent enrichment of proteins in the platelet MPs may simply be due to the overwhelming spectral count for vWF in the plasma MPs, accounting for roughly 10% of all spectra. This would tend to dilute the peptides for all other proteins on relative spectral count basis. Complete description of the proteins and the peptide sequences enriched in the plasma MPs and/or present only in the plasma MPs are in Table 2, and supplementary Table 4 at www.thrombosis-online.com), respectively. Peptides from three of these proteins, complement component 4-binding protein, Fc fragment of IgG-binding protein, Galectin 3-binding protein, and vWF, were confirmed to be overexpressed in plasma-derived microparticles by analyzing ion intensities (see Supplementary Table 5 available online at www.thrombosis-online.com).

Comparative analysis of ICAT-labeled proteins by manual quantitation of peptide ion intensities

As a complementary approach to examine differences in the protein composition of platelet MPs versus plasma MPs, a differential analysis using isotope labeling was performed. MP were differentially labeled and mixed prior to PAGE, trypsin digested, extracted, and analyzed by LC/MS (Fig. 1B). This was performed on three different samples, and 94 of the more abundant peptides were quantified (see Supplementary Table 6 available online at www.thrombosis-online.com). The log2 of the ratios of the peptide abundances in plasma MPs to platelet MPs was calculated, and the average adjusted to 0.00, excluding the peptides from vWF (Fig. 3). Peptides from three proteins (albumin, beta globin, and vWF) were consistently enriched in plasma MPs versus platelet MPs. The results for vWF and albumin are consistent with those obtained from spectral count analysis. While the spectral counts for beta globin (and several other hemoglobin proteins) were all about two-fold higher in the plasma MPs versus the platelet MPs using spectral count, that method was unable to demonstrate significant enrichment. Several peptides from three proteins, thrombospondin, and integrins α_{IIb} and β_3, appear to be enriched in the platelet MP.

ICAT quantitation of differentially expressed peptides based on scan number

In order to confirm the results of the spectral count analysis of unlabelled proteins, the peptides from all proteins which were present in the plasma MPs (spectral count of 10 or more) and essentially not present in the platelet MPs (spectral count of 0 or 1) were examined by ICAT methodology (Table 3). Spectra for peptides for 16 of the 27 proteins were detected in at least one of the three ICAT analyses. Similar to the spectral count of the unlabelled samples, there was a dramatic difference in spectral counts of the ICAT-labeled proteins between the two samples for these "enriched proteins" with 63 spectra for peptides from these proteins in the plasma MPs while only 2 were detected in the platelet MPs (Table 3). Of the 63 peptides identified by SEQUEST, the ion intensities of 33 (representing 27 different) peptides could be quantified in at least one analysis. It is important to note that in many cases the peptide from the platelet microparticle sample was not visually detected and the background ion intensity was used to calculate the ratios, thus yielding a lower bound. This likely led to an underestimation of the fold enrichment in plasma MPs. This is particularly true for several peptides that had poor signal-to-noise ratios due to low abundance. Peptides from all of the proteins detected only in the plasma MP had an adjusted log 2 score of greater than 1.58, representing at least a three-fold increase in concentration.

Peptides from the proteins present in both the plasma MPs and platelet MPs but enriched in the plasma MPs were also examined using ICAT. As described above, vWF and albumin appeared enriched by this method (see Supplementary Table 6 available online at www.thrombosis-online.com and Fig. 3).

Comparing the results from the two approaches (spectral counts versus ICAT-label) provides us with a wealth of information about the relative strengths and weaknesses of each. The most obvious strength of the spectral count approach is its ability to generate a large number of protein differences without extensive and time-consuming manual quantitation. The main strength of the ICAT-based approach is its superior quantitation; allowing determination of as little as two-fold differences in abundance. While it is currently extremely time consuming to detect these differences, the development and availability of better software should alleviate this problem. It is interesting to note that spectral count is able to detect differences in lower abundant peptides/proteins even when the ion current does not appear above the background. These features demonstrate the complementary nature of the two approaches.

Verification of mass spectrometric analysis by immunoblots

To verify the relative quantitation of the differentially expressed proteins, immunoblotting for three proteins overexperessed in the plasma MPs was performed (Fig. 4), plasma MPs were isolated from plasma and Platelet MPs were isolated following activation of platelets. The proteins were separated by PAGE, transferred onto a membrane and probed for complement component 3, galectin 3-binding protein, or vWF. Complement component 3 and galectin 3-binding protein were only detectable in the plasma MPs. vWF was greatly enriched in the plasma MPs, but was faintly visible in the platelet MP following overexposure of the film (data not shown).

Table 2: Brief description of proteins enriched or only present in the plasma MP. Only proteins with a total spectal count of 10 or more are listed. For type, P are plasma proteins, C are proteins involved with coagulation, A are proteins involved with apoptosis, I are proteins associated with the immune response, and Fe are those assocated with iron transport.

Type	Protein	Gene	GI	Swiss Pro #	Function	Refr.
	Fc fragment of IgG binding protein	FCGBP	4503681	Q9Y6R7	Unknown	52–54
P, A, I	Complement component 4 binding protein, alpha	C4BPA	4502503	P04003	Controls the classical pathway of complement activation	35
P, Fe	Haptoglobin-related protein	HPR	45580723	P00739	Unknown; Homologous to serine protease, but has no enzymatic activity	
A	Galectin 3 binding protein	LGALS3BP	5031863	Q08380	Promotes integrin-mediated cell adhesion and may stimulate host defense against viruses.	39–40
A	CD5 antigen-like protein	CD5L	5174411	O43866	May play a role in the regulation of the immune system and as an inhibitor of apoptosis.	37–38
P, C, A	Protein S, alpha	PROS1	4506117	P07225	Anticoagulant plasma protein; it is a cofactor to activated protein C	36,46
Fe	Transferrin receptor	TFRC	4507457	P02786	Cellular uptake of iron occurs via receptor-mediated endocytosis of ligand-occupied transferrin receptor	41
P, I	Immunoglobulin J chain	IGJ	21489959	P01591	Links two monomer units of either IgM or IgA and also helps bind these Igs to secretory component.	
P, A, I	Complement component 4 binding protein, beta	C4BPB	4502505	P20851	Controls the classical pathway of complement activation	35
P, I	Ig kappa chain	IGKC	51475407	P01834	Immunoglobulin Component	
P	Albumin	ALB	7959791	P02768	Most abundent plasma protein, binds fatty acids and hormones; regulates the colloidal osmotic pressure	
P, C	von Willebrand factor	VWF	4507907	P04275	Promotes adhesion of platelets to sites of injury by forming bridge between matrix and GPIb-IX-V; also binding Gp IIb/IIIa	47–49
P, A, I	Complement component 4B	C4B	4502501	Q6U2E7	Involved in regulation of complement (classical pathway); essential cofactor for C3b inactivator	31–32
P, L	Apolipoprotein B-100	APOB	4502153	P04114	Major protein constituent of chylomicrons and LDL	57
P	Alpha-2-macroglobulin	A2M	4557225	P01023	Inhibits all four classes of proteinases by a unique 'trapping' mechanism.	
P, Fe	Haptoglobin	HP	4826762	P00738	Combines with free plasma hemoglobin, preventing loss of iron through the kidneys and protects from Hg toxicity	
P, L	Apolipoprotein A-I	APOA1	4557321	P02647	Major protein of HDL; Participates in the reverse transport of cholesterol from tissues to the liver	58
P, L	Apolipoprotein E3	APOE	4557325	P02649	Mediates the binding, internalization, and catabolism of lipoprotein particles.	58
P, Fe	Transferrin	TF	4557871	P02787	Responsible for the transport of iron from sites of absorption and heme degradation to those of use	41
P, A, I	Complement component 3	C3	4557385	P01024	Plays a central role in the activation of the complement system by all pathways.	31–32
P, L	Apolipoprotein C-III	APOC3	4557323	P02656	Inhibits lipoprotein lipase and hepatic lipase and decreases uptake of chylomicrons by hepatic cells	57
	Tenascin C	TNC	4504549	P24821	Substrate-adhesion molecule that appears to inhibit cell migration.	55
P, C	Coagulation factor VIII	F8	4503647	P00451	Coagulation Factor, acts as a cofactor for factor IXa when it converts factor X to Xa	
Fe	Hemoglobin, beta chain	HBB	4504349	P68871	One of the chains of hemoglobin, involved in oxygen transport from the lung to the various peripheral tissues	

Table 3: ICAT analysis of proteins differentially expressed as determined by spectral count. All proteins which are differerntially expressed based on spectral count analysis were examined in the ICAT-labeled Experiment. Spectral count refers to number of times spectrum for ICAT-labeled peptide was detected in the three experiments. Whenever peaks were not visible, background ion intensity was used to caculate ratios which would underestimate sample differences. If neither peak visible, no quanitition was performed. Only five peptides for albumin and hemoglobin were examined. Additional peptides for vWF were not examined (Nine can be seen in Supplementary Table 5 available online at www.thrombosis-online.com).

				Spectral count		Peak detected (visual inspection)		Peptide intensity (normalized log2)	
GI No.	**Protein Name**	**z**	**Peptide**	**Plasma MP**	**Platelet MP**	**Plasma MP**	**Platelet MP**	**Average**	**sd**
4503681	Fc fragment of IgG binding protein	2	R.VVAEVQICHGK.T	1	0	1	0	2.48	
		3	R.VPAAYAGSLCGLCGNYNQDPADDLK.A	1	0	1	0	1.60	
		3	R.NEVTYDPYLVLIPDVAAYCPAYVVK.S	1	0	0	0		
		2	R.DKQSCPAGER.C	2	0	2	1	1.38	
		2	R.AQDFSPCYG.-	1	0	0	0		
		3	K.EEFCGLLSSPTGPLSSCHK.L	1	0	1	0	2.45	
		2	K.CPPELEKK.Y	1	0	0	0		
		2	K.AIGYATAADCGR.T	2	0	1	0	3.03	
		2	K.AGCVAESTAVCR.A	1	0	0	0		
4502503	Complement component 4 binding protein, alpha	2	K.YTCLPGYVR.S	2	0	2	0	2.50	0.23
		2	R.GSSVIHCDADSK.W	3	0	2	0	4.10	0.21
		2	R.LMQCLPNPEDVK.M	2	0	1	0	2.91	
		2	K.EEIIYECDK.G	1	0	1	0	3.11	
45580723	Haptoglobin-related protein	2	K.LPECEAVCGK.P	1	0	0	0		
		3	K.LPECEAVCGKPK.N	1	0	1	0	2.82	
		2	K.SCAVAEYGVYVK.V	2	0	2	0	4.29	3.29
4502501	Complement component 4B	2	R.VDVQAGACEGK.L	3	0	2	9	3.21	0.74
		2	R.CSVFYGAPSK.S	1	0	1	0	2.82	
		2	K.SCGLHQLLR.G	1	0	1	0	4.45	
		2	K.GLCVATPVQLR.V	2	0	1	0	1.87	
		2	K.FACYYPR.V	1	0	0	0		
5031863	Galectin 3 binding protein	2	K.STSSFPCPAGHFNGFR.T	1	0	1	0	2.55	
4502153	Apolipoprotein B-100		None						
4557225	Alpha-2-macroglobulin	2	K.YDVENCLANK.V	1	0	1	0	2.24	
4826762	Haptoglobin	3	K.SPVGVQPILNEHTFCAGMSK.Y	1	0	0	0		
		2	K.YVMLPVADQDQCIR.H	2	0	1	0	2.24	
4557321	Apolipoprotein A-I		None						
5174411	CD5 antigen-like protein	2	R.LVGGDNLCSGR.L	1	0	1	0	3.68	
		3	R.ELGCGAASGTPSGILYEPPAEK.E	2	0	2	0	3.18	
		2	K.CYGPGVGR.I	1	0	1	0	4.50	
4506117	Protein S, alpha	2	K.HCLVTVEK.G	1	0	0	0		
4507457	Transferrin receptor		None						
4557325	Apolipoprotein E3	2	R.LGADMEDVCGR.L	2	0	1	0	2.38	
21489959	Immunoglobulin J chain	2	R.FVYHLSDLCK.K	2	1	2	1	3.05	2.39
		2	K.MVETALTPDAC*YPD.-	1	0	1	0	2.44	
		2	K.C*YTAVVPLVYGGETK.M	2	0	1	0	2.60	
4502505	Complement component 4-binding protein, beta		None						

Table 3: Continued

				Spectral count		Peak detected (visual inspection)		Peptide Intensity (normalized log2)	
GI No.	**Protein Name**	**z**	**Peptide**	**Plasma MP**	**Platelet MP**	**Plasma MP**	**Platelet MP**	**Average**	**sd**
4557871	Transferrin	2	R.FDEFFSEGCAPGSK.K	1	0	0	0		
		2	R.DDTVCLAK.L	1	0	0	0		
		2	K.SVIPSDGPSVACVK.K	2	0	2	0	1.95	0.11
		2	K.CSTSSLLEACTFR.R	1	0	1	0	1.58	
4557385	Complement component 3		None						
51475407	Ig kappa chain		None						
4557323	Apolipoprotein C-III		None						
4504549	Tenascin C		None						
4503647	Coagulation factor VIII	2	R.DLASGLIGPLLICYK.E	1	0	0	0		
		3	K.DLNSGLIGALLVCR.E	1	0	1	0	2.34	

Discussion

Analysis of microparticles isolated from healthy volunteers shows 21 gene products detected predominantly in the plasma MPs and not in the platelet MPs isolated from the same individuals. Of these, 10 were significantly enriched while the other 11 proteins showed a trend to enrichment but did not reach statistical significance. Many of these probably represent proteins that are present during the initial formation of the microparticles and bind to the microparticle complex. Beyond statistical analysis, confidence in the detected differences is further enhanced by confirmation using direct ICAT-based comparison. In addition, there were two proteins detected in both sets of MPs but significantly enriched in plasma MPs based on spectral counts.

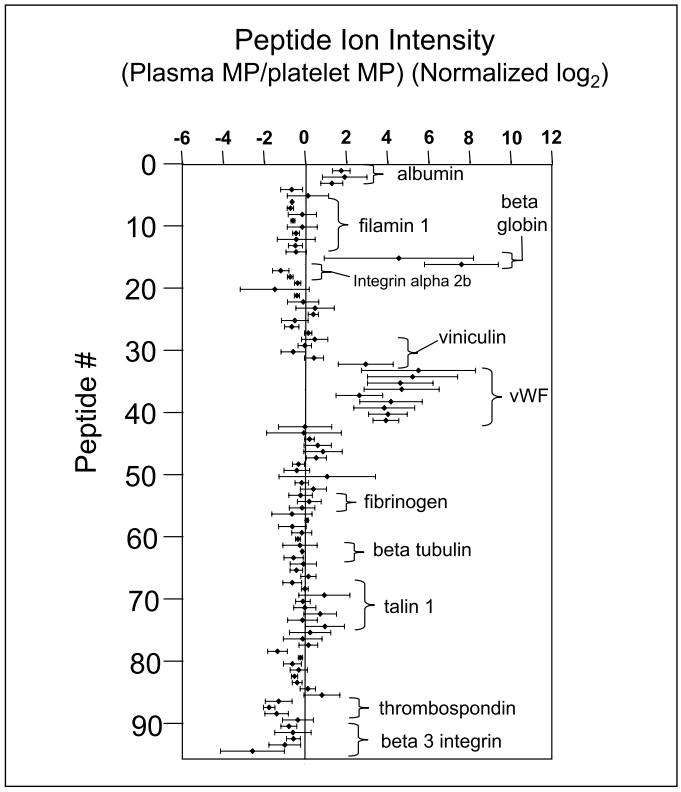

Figure 3: Relative ion intensities for 94 peptides from plasma-derived MPs and platelet MPs following ICAT labeling. Normalized log2 of adjusted ion intensities for 94 peptides. Peptides from selected proteins are noted. See Supplementary Table 6 available online at www.thrombosis-online.com for the complete list of peptides and ion abundance ratios.

In our previous report, we identified 578 proteins from platelet microparticles(20), while in this study, 229 proteins were detected. The reason for this disparity is explained by the goals and methods. In the previous study, the gel was sectioned into 26 equal parts and each was analyzed separately by LC-MS allowing detection of low-abundance peptides. In the present report, we were interested in a differential analysis and did not section the gel lane. The previous report was based on one sample from a single individual. Here, we analyzed samples from three donors, each in duplicate.

Seventy-five percent of the proteins detected in platelet MPs in this study were also detected in our previous study (Fig. 5). The 87 most abundant proteins detected in our previous study (based on spectral count) were also detected in the current analysis of platelet MPs. While approximately 25% of the platelet MP proteins from the previous work were not detected in this study, a significant fraction of these were hypothetical proteins and many may simply reflect differences in their annotation within the different databases searched. Comparing plasma MPs to the platelet MPs, 75% of the proteins detected are in common. Only 27 proteins (13%) were unique to the plasma MPs (Fig. 5).

Microparticles are produced by several cellular processes including ectocytosis (blebbing of the cell membrane) and release of exosomes. Ectocytosis can occur in essentially all cell types, particularly when they are activated or are undergoing apoptosis. Release of exosomes can occur in most cell types, particularly in culture, but is often associated with cells of the immune system.

The physiologic functions of plasma microparticles include delivery of tissue factor to promote intravascular fibrin generation (18), pro-inflammatory functions (9, 24, 25), alterations in vascular reactivity (15), induction of angiogenesis (16), intracellular communication (26), and perhaps certain anti-inflammatory functions (17). Plasma microparticles are increased in many disease states (5), and their composition is changed as determined by flow cytometry (5, 27). The present study, for the first time, defines the protein composition of plasma microparticles as compared with platelet-derived microparticles using LC/MS as an unbiased approach. As expected, most gene products identified in plasma MPs are also found in platelet microparticles, confirming that most plasma MPs are derived from platelets (4). However, we found significant and potentially biologically important differences. For all proteins detected with a spectral count of 10 or more, 21 were present almost exclusively in plasma MPs. In addition, two proteins were enriched in the protein MPs by spectral count and/or ICAT analysis.

Of these 23 proteins, some may be considered to be contamination by plasma proteins (marked P in Table 2). However, several lines of reasoning refute this interpretation. First, of the five most abundant plasma proteins, albumin, IgG, transferrin, fibrinogen, and IgA (28), only two, transferrin and albumin, were enriched in the plasma MPs versus the platelet MPs based on spectral counts. Albumin was present in relatively low amounts (45th most abundant protein) and some of this may represent protein associated with platelets (29), or platelet MPs (20). Transferrin also had a low spectral count (109th most abundant protein) and its receptor, a membrane protein, presumably in exosomes (30) (see below) had almost double the spectral counts. Second, the plasma-derived proteins detected in the plasma MPs are not a random collection as would be expected of a contaminant. For example, seven of these proteins are associated with apoptosis of cells, suggesting the clearance of these apoptotic cells or possibly the prevention of apoptosis in cells that have complement components attached (2, 31). Four of the proteins in plasma MPs, complement 3, complement 4B, complement 4-binding proteins α and β, are from the complement pathway. One mechanism by which nucleated cells prevent complement-induced cell death is by shedding the complement components off of the membrane (2). The complement components detected in our study could represent MPs generated by sub-clinical autoimmune processes. Complement C3 is the most important complement component that can be activated by any of the three distinct pathways, classical (antibody-initiated), lectin (microbe-initiated) and alternative pathways (32). The most likely explanation for the presence of complement proteins is sub-clinical activation of the classical pathway, which includes C3 and C4, by autoantibodies. Sub-clinical levels of autoantibodies are widely found in healthy individuals (33). Some of them include so-called natural antibodies, for example, to blood group antigens (33). Others are against certain pathogens such as *Streptococcus pneumoniae* and cross-react with endogenous oxidized lipids such as oxLDL (34). However, C4 is involved in both the lectin and the classical pathways, so we cannot exclude that intestinal or other commensal flora may provide a stimulus for sub-clinical complement activation that leads to expulsion of complement-bound membranes into microparticles. C4BP (C4-binding protein) is a potent circulating complement inhibitor that inhibits both the classical and alternative pathway with a role in localizing complement regulatory activity to the surface of apoptotic cells, which undergo ectocytosis to release MPs (35). Part of this complex includes Protein S, another protein enriched in the plasma MPs and independently shown to stimulate the phagocytosis of apoptotic cells (36).

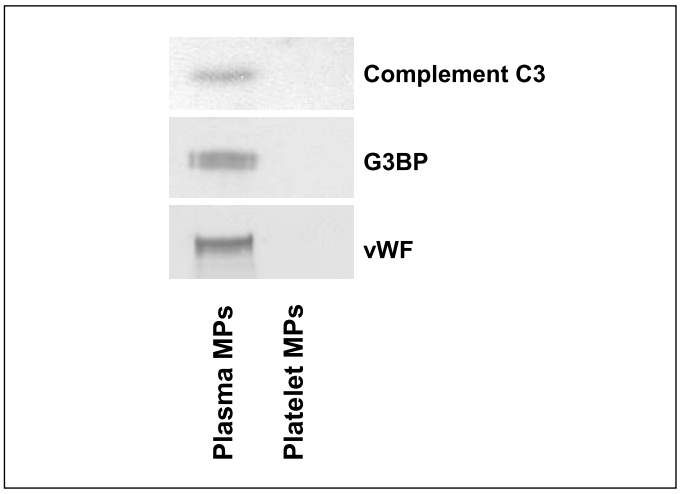

Figure 4: vWF, galectin 3-binding protein (G3BP), complement component 3 are enriched in plama MP versus platelet MPs. Platelet MPs were generated from human platelets and plasma MPs were isolated human plasma. Differences in the presence of vWF, galectin 3-binding protein, and complement component 3 were determined by western blot analysis. Complement compent 3 and galectin 3-binding protein were only detected in plasma MPs. vWF was greatly enriched in plasma MPs but was visible in platelet MP following overexposure of the film (data not shown).

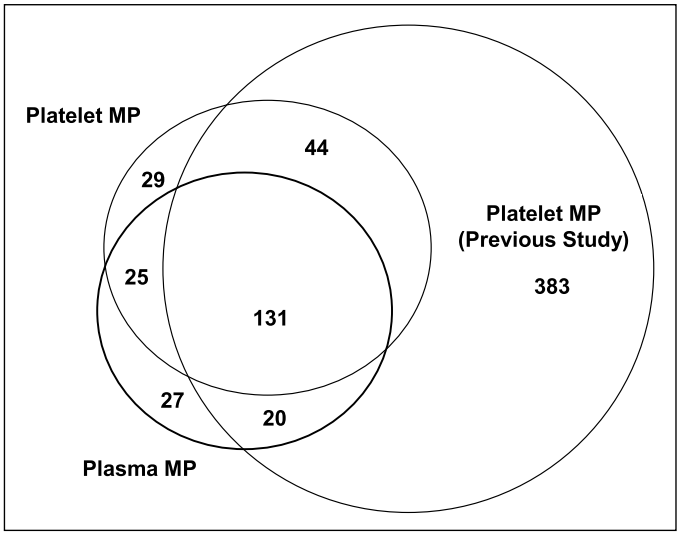

Figure 5: Venn diagram of proteins detected in plasma MPs and platelet MPs from this study and platelet MPs from our previous study (20). There is a significant overlap between the proteins detected in this study and those detected in our previous study. Approximately 13% of the proteins detected in the plasma MPs were not detected in a platelet MP preparation.

Two other proteins enriched in plasma MPs, CD5-like antigen and galectin 3-binding protein also are associated with inhibition of apoptosis. CD5-like antigen, also called SP-α, has not been extensively studied, but its mouse homolog, AIM (apoptosis inhibitor in macrophages), has several informative studies (37, 38). Macrophages of mice deficient in liver X receptors (LXRs) undergo accelerated apoptosis when challenged with *Listeria monocytogenes*, which is highly dependent on AIM, a direct target for regulation by LXRα (37). Similarly, thrombocytes show increased susceptibility to apoptosis in mice lacking AIM (38). Galectin 3-binding protein, also called Mac-2-binding protein, associates with galectin 3, which increases cellular resistance to apoptosis (39, 40). The presence of all these proteins in plasma MPs suggests that some of these particles are either derived from apoptotic cells or an indication that microparticles are shed to prevent apoptosis. These two mechanisms of production cannot be distinguished in the current study.

Another and more surprising set of proteins associated with plasma MPs is related to iron transport and hemoglobin clearance: transferrin, transferrin receptor, haptoglobin, haptoglobin-related protein, and hemoglobin. While transferrin is a very abundant plasma protein, it most likely does not represent a plasma contaminant in plasma MPs, because its receptor, a membrane protein, is also present, with almost double the spectral count. The transferrin receptor regulates the uptake of iron by most cells (41). During the maturing of reticulocytes, iron uptake decreases due to decreased transferrin receptor on their cell surface (30). Investigators examining the loss of this receptor from the cell surface were some of the first to describe the release of exosomes (42, 43). Therefore, transferrin receptor in plasma MPs may represent shedding of material during reticulocyte maturation. The transferrin present in microparticles could simply result from its binding to free microparticles. Haptoglobin is an abundant protein that binds free hemoglobin which would otherwise be toxic to cells, especially in the kidney (44). The haptoglobin knockout mouse did not impair clearance of free plasma hemoglobin but was more susceptible to oxidative damage and failed to repair or regenerate damaged renal tissues (45). It is possible that haptoglobin removes hemoglobin by associating with plasma MPs. Indeed, hemoglobin was detected by our analysis in both plasma MPs and platelet MPs, suggesting that haptoglobin-hemoglobin complexes may associate with MPs.

The third category of plasma proteins overexpressed in plasma MPs are immunoglobulins, represented by immunoglobulin (Ig) J chain and kappa chain. This is not a random selection of immunoglobulins, again making plasma contamination a less likely explanation. IgJ is associated with IgM, which includes all natural antibodies. Also enriched are several complement components, as discussed above. These findings are consistent with our proposal that microparticles reflect the sub-clinical activation of the classical complement pathway at a low level through IgM auto-antibodies, leading to the formation of microparticles from unknown cells (not platelets) that remove IgJ and associated complement components from the affected cell membrane.

Four proteins overexpressed in plasma MPs compared to platelet MPs are associated with lipoproteins (marked L in Table 2). Unlike the others described above, these most likely represent contamination of plasma MPs with the larger plasma lipoproteins of the chylomicrons (apo A-I and C-III), chylomicron remnants (apo E), VLDL (apo A-I, B100, and C-III) and IDL (apo B100 and E) fractions. Plasma lipoproteins appear in overlapping fractions with microparticles, because their sizes and therefore, their gel filtration properties are similar to MPs (data not shown). While they should remain in the supernatant after the final centrifugation step, their contamination with the pellet is difficult to avoid. In addition to the four lipoproteins found overexpressed in plasma microparticles, apolipoprotiens A-II, C-II, L-I, and Lp(a), were found only in the plasma MPs but the number of spectra for each was below 10, and were not examined further.

Three plasma proteins associated with blood coagulation, protein S, factor VIII, and vWF, (denoted P, C in Table 2) were also enriched in plasma MPs compared to platelet MPs. While the best known role of protein S is as an anti-coagulant, it also is associated with apoptosis, as described above, and this likely explains its presence in plasma MPs. Factor VIII is pro-coagulant, and the reason for its presence is unknown. While it has been reported that factor VIII binds directly to Protein S (46), the significance of this to its presence in plasma MPs is unclear.

vWF is a platelet and endothelial cell product that binds to P-selectin (47), GP1b (48) and GP IIb/IIIa (49). Since we previously detected vWF in platelet MPs (20), its presence in both plasma and platelet MPs in this study was no surprise. However, its enrichment in plasma MP was unexpected and suggests that endothelial vWF associates with plasma MPs or even promotes their formation. A recent study has demonstrated that microparticles are indeed formed from platelets bound to immobilized vWF under high shear stress, *in vitro* (50). Endothelial vWF is secreted from Weibel-Palade bodies and has been shown to be present in endothelial-derived MPs under disease conditions (51). It exists as a very large multimer and is cleaved by ADAMTS-13 (52). These data are consistent with our observation that plasma-derived MPs are enriched for vWF. Our proteomic analysis does not distinguish between monomers and multimers, so further work will be needed to identify the nature of the vWF associated with plasma MPs. The association of coagulation factors and platelet-binding factors is entirely consistent with the idea that plasma MPs are a pro-coagulant surface (11) and associate with nascent thrombi (18, 19).

FCGBP is a protein with unknown function produced by intestinal and colonic goblet cells (53, 54). Due to its cellular source and its ability to bind IgG, it has been hypothesized to assist in preventing antigen invasion into the mucosa (53, 55). Kobayashi et al. found that FCGBP could inhibit a complement-mediated reaction and suggested that it may play an important role in immunological defense of mucosal surfaces (55). While this follows our theories of ectosome production previously discussed, there is little evidence to support the connection between FCGBP and the prevention of cell death in vascular tissue. This is particularly true considering the location of FCGBP production. However, the detection of this protein in plasma MPs is very intriguing especially when one considers its prevalence (spectral count of 167 including 54 unique peptides).

The remaining protein, tenascin C is an extracellular matrix protein associated with provisional and tumor matrices but not

found in most mature tissues (56). It is possible that tenascin C is secreted at sites of small injuries and wound healing, and cells with rapid turnover such as leukocytes and wound fibroblasts may shed tenascin C associated with MPs. Alternatively, tenascin C may form aggregates that migrate in the MP fraction. Its presence was unexpected in the plasma MP fraction and further studies are needed to examine its implications.

Equally as interesting as the overexpressed gene products in plasmas MPs is the absence of detection of known components. Tissue factor is in plasma-derived microparticles (18), as shown by Western blot (57) and intravital microscopy (18), yet we could not detect this initiator of coagulation. This could be due to its low abundance, small cytoplasmic tail or to glycosylation of the extracellular domains (58). Glycosylated proteins are difficult to identify by standard proteomic analysis, as conducted here. A second interesting point is that no cell-specific proteins were detected as clearly overexpressed in plasma MPs. This reinforces the notion that plasma MPs in healthy people are mostly platelet-derived (4) and thus not very different from platelet MPs. However, we did detect proteins specific for endothelial cells (endothelial cell adhesion molecule), smooth muscle cell proteins (enolase-3, hexokinase 2), and erythrocytes (beta spectrin, erythrocyte membrane protein band 4.9 [dematin]) but we could not establish statistically significant overexpression in plasma MPs compared to platelet MPs due to their low spectral counts or low ion intensities by ICAT. This may reflect their minor contribution to the overall population of plasma MPs and emphasizes the need of complementing unbiased mass spectrometry analysis with a candidate-based flow cytometry or mass spectrometry approach (6, 18).

In conclusion, the present report establishes significant differences between plasma- and platelet-derived microparticle proteins. A large majority of these differences are proteins involved with apoptosis or iron transport. It is reassuring that very few proteins were detected in plasma MPs that could be classified as plasma contaminants. While it is likely that most of these proteins are present during the formation of microparticles, other proteins could bind following release into the plasma. These studies do not distinguish between these two mechanisms. In studying the biology of complement, coagulation and other processes, microparticles will have to be considered as important contributors for removing such components from cells. Sources of the proteins found in plasma-derived MPs only may be useful biomarkers for various disease processes.

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Abbreviations

AIM, Apoptosis Inhibitor in Macrophages; C4BP, Complement Component C4 Binding Protein; FCGBP,Fc fragment of IgG binding protein; ICAT, Isotope-Coded Affinity Tag; MPs, Microparticles; PAGE, Polyacrylamide gel electrophoresis; PBS, Phosphate buffered saline; PPP, Platelet-Poor Plasma; PRP, Platelet-Rich Plasma; vWF, von Willebrand Factor.

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Supplementary tables for TH-06-02-0066, "Proteomic discovery of 21 proteins expressed in human plasma-derived but not platelet-derived microparticles" by David M. Smalley et al.

Supplementary Table 1: Sample identifying ICAT-labeled peptides with their ion intensities for region shown in Figure 2. Ratios of ion intentities of light lable to heavy label, log 2 transformed and adjusted for equivalent protein levels as described in text (nd = no visible peak deleted over background).

					MH+	
Box	**GI number**	**Reference**	**Peptide**	**z**	**light**	**heavy**
1	16753233	talin 1	K.AGALQCSPSDAYTK.K	2	1639.68	1648.68
2	4505879	pleckstrin	R.GCVVTSVESNSNGR.K	2	1636.64	1645.64
3	4504349	beta globin	K.GTFATLSELHCDK.L	2	1649.72	1658.72
4	16753233	talin 1	K.ASAGPQPLLVQSCK.A	2	1626.77	1635.77
5	28626504	UNC-112 related protein 2 short form	K.GCEVVPDVNVSGQK.F	2	1658.72	1667.72
6	4504349	beta globin	K.GTFATLSELHCDK.L	2	1649.72	1658.72
7	---	No ID				
8	4503745	filamin 1	K.VHSPSGALEECY VTEIDQDK.Y	3	2448.51	2457.51
9	4507907	von Willebrand factor	K.LTGSCSYVLFQNK.E	2	1687.81	1696.81
10	4507907	von Willebrand factor	R.CLPTACTIQLR.G	2	1673.76	1691.76
11	12025678	actinin, alpha 4	R.ELPPDQAEYCIAR.M	2	1732.80	1741.80
12	4505733	platelet factor 4	K.AGPHCPTAQLIATLK.N	2	1748.94	1757.94
13	16753233	talin 1	R.QELAVFCSPEPPAK.T	2	1743.87	1752.87
14	4505981	pro-platelet basic protein	K.GTHCNQVEVIATLK.D	2	1740.87	1749.87
15	4507907	von Willebrand factor	K.SEVEVDIHYCQGK.C	2	1734.78	1743.78
16	40317626	thrombospondin 1 precursor	R.DNCQYVYNVDQR.D	2	1744.73	1753.73
17	12667788	myosin, heavy polypeptide 9	R.EDQSILCTGESGAGK.T	2	1722.72	1731.72
18	4758638	peroxiredoxin 6	K.DINAYNCEEPTEK.L	2	1753.73	1762.73
19	---	No ID				
20	4503745	filamin 1	K.MDCQECPEGYR.V	2	1785.74	1803.74
21	46249412	latent transforming growth factor beta binding protein	R.CTCGQGYQLSAAK.D	2	1784.77	1802.77
22	47078292	integrin beta chain, beta 3	K.CPTCPDACTFK.K	2	1867.79	1894.79
23	5453595	adenylyl cyclase-associated protein	R.ALLVTASQCQQPAENK.L	2	1929.05	1938.05
24	40317626	thrombospondin 1	K.DCVGDVTENQICNK.Q	2	1992.94	2010.94
25	11761631	fibrinogen, beta chain	R.TPCTVSCNIPVVSGK.E	2	1960.04	1978.04
26	---	No ID				
27	4503745	filamin 1	R.VQVQDNEGCPVEALVK.D	2	1956.08	1965.08
28	16753233	talin 1	R.MVAAATNNLCEAANA AVQGHASQEK.L	3	2727.88	2736.88
29	4507907	von Willebrand factor	R.CLPSACEVVTGSPR.G	2	1873.91	1891.91
30	----	No ID				
31	4503745	filamin 1	K.VDINTEDLEDGTCR.V	**2**	1807.78	1816.78
32	4504745	integrin alpha 2b	K.TPVGSCFLAQPESGR.R	2	1776.86	1785.86
33	16554592	enolase 3	K.VNQIGSVTESIQACK.L	2	1804.91	1813.91
34	5031571	actin-related protein 2	K.LCYVGYNIEQEQK.L	2	1814.91	1823.91
35	---	No ID		2		
36	12667788	myosin, heavy polypeptide 9	K.LEEEQIILEDQNCK.L	2	1932.01	1941.01
37	---	No ID				
38	16753233	talin 1	K.QVAASTAQLLVACK.V	2	1630.80	1639.80

M/Z (calc.)		M/Z (measured)		Ion Intensity (rel.)		ratio (L/H)	log(2)	ratio
light	heavy	light	heavy	light	heavy	ion int.		(adjusted**)
820.34	824.84	820.40	824.40	14.500	12.800	1.13	0.18	-0.20
818.82	823.32	819.40	823.40	7.110	10.560	0.67	-0.57	-0.95
825.36	829.86	826.40	nd	3.680	0.137	26.86	4.75	4.37
813.89	818.39	813.40	818.00	9.480	2.530	3.75	1.91	1.52
829.86	834.36	829.40	834.40	9.960	12.200	0.82	-0.29	-0.67
825.36	829.86	825.40	nd	48.800	0.900	54.22	5.76	5.38
		812.40	815.40	6.980	Overlap			
816.84	819.84	816.60	819.40	8.450	6.560	1.29	0.37	-0.02
844.41	848.91	844.40	nd	11.400	1.960	5.82	2.54	2.16
837.38	846.38	837.40	846.00	6.580	1.310	5.02	2.33	1.95
866.90	871.40	866.40	870.80	3.460	3.230	1.07	0.10	-0.28
874.97	879.47	875.00	879.00	7.010	5.700	1.23	0.30	-0.08
872.44	876.94	872.00	876.60	8.150	4.810	1.69	0.76	0.38
870.94	875.44	870.40	875.00	18.760	15.300	1.23	0.29	-0.09
867.89	872.39	868.00	nd	1.100	0.215	5.12	2.36	1.97
872.87	877.37	872.40	877.40	54.900	48.400	1.13	0.18	-0.20
861.86	866.36	861.80	865.80	3.290	3.190	1.03	0.04	-0.34
877.37	881.87	876.80	881.40	1.850	1.350	1.37	0.45	0.07
		887.40	892.40	2.92	overlap			
893.37	902.37	893.40	902.00	1.260	1.890	0.67	-0.58	-0.97
892.89	901.89	covered	901.40	covered	1.440			
934.40	947.90	934.40	948.00	0.500	1.390	0.36	-1.48	-1.86
965.03	969.53	643.40	545.80	1.43	2.450	0.58	-0.78	-1.16
996.97	1005.97	997.00	1006.00	0.669	1.6	0.42	-1.26	-1.64
980.52	989.52	980.00	989.00	9.720	9.240	1.05	0.07	-0.31
		996.00	1005.00	2.260	1.430	1.58	0.66	0.28
978.54	983.04	978.00	983.00	7.900	8.280	0.95	-0.07	-0.45
909.96	912.96	909.80	912.80	10.5	10.5	1.00	0.00	-0.38
937.46	946.46	947.00	nd	10.200	1.130	9.03	3.17	2.79
		915.20	918.61	3.260	3.170	1.03	0.04	-0.34
904.39	908.89	903.80	908.40	5.080	4.430	1.15	0.20	-0.18
888.93	893.43	889.00	893.00	11.2	32.8	0.34	-1.55	-1.93
902.96	907.46	903	907.4	5.88	5.38	1.09	0.13	-0.25
907.96	912.46	907.40	912.40	4.360	1.270	3.43	1.78	1.40
		1004.00	1013.00	8.490	6.980	1.22	0.28	-0.10
966.51	971.01	966.00	970.40	2.770	4.590	0.60	-0.73	-1.11
		859.40	Unknown	1.160	Unknown			
815.90	820.40			covered	nd			

Supplementary Table 2: Proteins detected in plasma MPs, platelet MPs, and our previous publication (20). Unique proteins from our previous study are not included.

A. Proteins common to plasma MPs, platelet MP (this study), and platelet MP (Garcia, et al.)

		Plasma MPs		Platelet MPs		Garcia, et al. Platelet MPs	
GI Number	Reference	Spectral Count	Unique Peptides	Spectral Count	Unique Peptides	Spectral Count	Unique Peptides
16753233	talin 1	346	106	612	109	3102	92
4503745	filamin 1 (actin-binding protein-280); filamin A	329	100	485	113	2383	87
4501885	beta actin	107	9	237	10	1826	4
12667788	myosin, heavy polypeptide 9, non-muscle	291	103	526	101	1111	41
40317626	thrombospondin 1	91	29	181	27	1522	44
4507877	vinculin isoform VCL	125	43	197	49	1461	43
4501891	actinin, alpha 1	92	32	166	41	1382	25
4507907	von Willebrand factor	745	85	23	15	854	82
28626504	UNC-112 related protein 2 short form	75	26	95	24	745	45
4504745	integrin alpha 2b	76	26	204	30	590	20
47078292	integrin beta chain, beta 3	59	21	102	24	592	34
13562114	beta tubulin 1, class VI	60	18	60	16	614	24
4503631	coagulation factor XIII A1 subunit	47	22	93	21	560	23
11761631	fibrinogen, beta chain	71	16	76	18	492	32
11761629	fibrinogen, alpha chain	57	19	47	17	534	33
33286418	pyruvate kinase 3 isoform 1	42	22	87	24	460	26
17921989	tubulin, alpha 1	13	5	20	6	544	19
38044288	gelsolin	52	14	85	19	392	25
11761633	fibrinogen, gamma chain	63	17	59	25	369	19
4501881	alpha 1 actin	109	11	123	12	200	6
4502027	albumin	85	28	45	16	277	10
4826898	profilin 1	35	9	112	7	210	20
7669492	glyceraldehyde-3-phosphate dehydrogenase	31	11	73	11	246	19
4827056	WD repeat-containing protein 1	18	10	23	9	283	24
21735625	tyrosine 3/tryptophan 5 -monooxygenase activation, Zeta	24	10	59	9	239	4
4504349	beta globin; hemoglobin beta chain	105	12	70	10	142	10
12025678	actinin, alpha 4	60	23	114	27	115	8
24234686	heat shock 70kDa protein 8 isoform 2	30	46	20	9	229	15
4507357	transgelin 2	24	12	65	11	172	15
24119203	tropomyosin 3	28	16	42	18	181	14
7661678	RAP1B, member of RAS oncogene family	12	3	24	3	205	11

5453595	adenylyl cyclase-associated protein	22	11	33	14	185	28
45269141	glycoprotein Ia	29	15	35	15	171	19
14251209	chloride intracellular channel 1	16	6	29	10	173	12
4503571	enolase 1	17	12	28	12	165	16
4505981	pro-platelet basic protein	35	7	55	7	119	6
34577110	aldolase A	18	11	28	12	163	16
4507677	tumor rejection antigen (gp96) 1	18	10	24	10	166	16
4759082	serum deprivation response protein	11	7	23	6	171	15
16507237	heat shock 70kDa protein 5	23	10	32	15	148	23
4505879	pleckstrin	22	11	53	13	122	16
13994151	PDZ and LIM domain 1 (elfin); carboxy terminal LI	16	11	10	6	171	18
38016911	stomatin isoform a	19	10	31	11	142	13
10863927	peptidylprolyl isomerase A isoform 1; cyclophilin	14	7	22	7	146	8
4507651	tropomyosin 4	23	8	33	12	123	8
12408656	calpain 1, large subunit	10	7	26	17	140	25
14389309	tubulin alpha 6	18	10	37	8	113	7
5031635	cofilin 1 (non-muscle)	15	8	30	9	122	8
4505257	moesin	26	15	22	10	118	24
4504183	glutathione transferase	7	6	6	3	151	11
4758606	integrin-linked kinase	17	11	24	10	116	22
28302131	hemoglobin gamma-a chain	14	3	10	2	132	13
4757810	ATP synthase, H+ transporting, mitochondrial F1	13	7	29	14	110	16
40254816	heat shock 90kDa protein 1, alpha	6	6	11	7	128	11
20127528	parvin, beta isoform b	12	6	43	8	89	11
4557032	lactate dehydrogenase B	19	8	23	10	100	15
13518026	LIM and senescent cell antigen-like domains 1	12	4	10	5	120	13
28373103	sarco/endoplasmic reticulum Ca2+ -ATPase isoform	8	5	17	10	114	22
29788768	tubulin, beta polypeptide	10	4	10	4	119	7
6005942	valosin-containing protein	14	9	26	15	98	4
5031573	ARP3 actin-related protein 3 homolog	13	7	16	11	107	15
21614499	Ezrin	8	4	15	6	107	10
29788785	beta 5-tubulin; beta Ib tubulin	9	3	11	3	109	12
4758012	clathrin heavy chain 1	18	13	13	12	95	22
7656991	coronin, actin binding protein, 1C	11	8	16	8	95	18
4826659	F-actin capping protein beta subunit	7	5	15	8	100	10
4557801	purine nucleoside phosphorylase	8	5	10	6	98	16
4505733	platelet factor 4 (chemokine (C-X-C motif) ligand	15	4	46	4	53	6
4508047	zyxin	14	8	12	7	88	17
15809016	myosin regulatory light chain MRCL2	17	7	43	8	52	7

4758950	peptidylprolyl isomerase B	10	5	10	6	88	15
21464101	tyrosine 3-monooxygenase/tryptophan 5-monooxygenagenase activation protein, gamma	7	4	8	4	93	13
4758638	peroxiredoxin 6	14	8	24	12	66	12
5729887	IQ motif containing GTPase activating protein 2	12	10	9	7	80	20
4557395	carbonic anhydrase II	6	3	5	2	88	10
4758460	glycoprotein V (platelet)	6	4	7	6	82	14
21314617	platelet/endothelial cell adhesion molecule (CD31)	9	5	15	7	68	13
4505763	phosphoglycerate kinase 1	7	6	13	8	70	16
7657056	EH-domain containing 3; EH domain containing 3	5	3	6	5	78	15
15991827	hexokinase 1 isoform HKI-R	5	5	7	5	77	21
17986258	smooth muscle and non-muscle myosin alkali light chain	18	6	23	7	48	10
4507869	vasodilator-stimulated phosphoprotein	5	4	9	6	74	14
25188179	voltage-dependent anion channel 3	5	5	9	4	73	7
15812212	phosphodiesterase 5A	5	4	6	5	75	19
23238211	actin related protein 2/3 complex subunit 2	5	5	9	7	72	10
10716563	calnexin	13	6	14	9	57	10
4503971	GDP dissociation inhibitor 1	6	4	7	5	71	18
5031571	actin-related protein 2; ARP2	12	7	18	10	52	17
13489087	serine (or cysteine) proteinase inhibitor	6	5	9	7	67	16
28178832	isocitrate dehydrogenase 2 (NADP+), mitochondrial	9	6	20	11	52	14
5453597	F-actin capping protein alpha-1 subunit	5	4	9	4	67	10
47419932	platelet glycoprotein Ib alpha	13	7	25	7	40	7
21361370	brain glycogen phosphorylase	8	6	10	8	60	19
4557014	catalase	12	6	8	6	57	16
4757900	calreticulin; Sicca syndrome antigen A	9	6	20	7	48	7
5902134	coronin, actin binding protein, 1A	6	5	11	5	60	12
41058276	Triosephosphate isomerase	8	5	16	7	53	18
5729997	RAB27B, member RAS oncogene family	10	4	12	7	54	8
5031857	lactate dehydrogenase A	6	4	17	7	53	11
20149594	heat shock 90kDa protein 1, beta; Heat-shock 90kD	9	6	11	8	55	12
42476296	tropomyosin 2 (beta)	13	5	18	6	43	8
7705296	bridging integrator 2; bridging integrator-2	8	5	6	4	60	14
4503327	cytochrome b5 reductase membrane-bound isoform	7	4	6	3	60	11
4502517	carbonic anhydrase I; carbonic dehydratase	11	5	5	2	53	9
42716297	clusterin isoform 1	18	6	14	8	35	6
31542292	chaperonin containing TCP1, subunit 3 (gamma)	13	4	9	4	44	11
10835187	superoxide dismutase 2, mitochondrial	7	3	8	2	51	7
30240932	EH-domain containing 1; testilin	13	8	7	4	41	10
7019375	formin homology 2 domain containing 1	7	4	7	4	47	9

21328448	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta = 14-3-3 protein beta/alpha	13	8	16	6	30	9
16950601	myosin light chain kinase	6	4	7	5	41	16
4503643	coagulation factor V	7	6	8	7	39	8
32189394	ATP synthase, H+ transporting, mitochondrial F1 c	10	7	31	12	12	4
4507951	tyrosine 3/tryptophan 5 -monooxygenase activation protein, eta	12	5	14	5	26	7
4507171	secreted protein, acidic, cysteine-rich (osteonectin)	10	5	9	4	26	5
40068518	phosphogluconate dehydrogenase	9	6	9	5	27	12
5031973	protein disulfide isomerase-related protein	6	4	16	5	22	8
13569962	RAB1B, member RAS oncogene family	8	4	7	4	28	3
4504041	guanine nucleotide binding protein (G protein)	8	4	8	4	27	7
4557419	CD36 antigen (collagen type I receptor)	8	3	9	4	25	3
24638454	ATPase, Ca++ transporting, cardiac muscle	7	4	7	2	24	5
5031987	peptidylprolyl isomerase F	5	3	5	3	26	6
4502903	clathrin, heavy polypeptide-like 1 isoform a	8	4	7	6	21	7
38202217	proto-oncogene tyrosine-protein kinase SRC	5	5	5	3	25	10
4557675	integrin alpha chain, alpha 6	6	6	9	7	19	6
4502695	cell division cycle 10	6	5	8	6	18	5
4502693	CD9 antigen	6	3	7	4	18	3
21956645	myotrophin; granule cell differentiation protein	5	1	6	1	20	2
5803227	tyrosine 3/tryptophan 5 -monooxygenase activation protein, theta polypeptide = 14-3-3 protein tau	5	3	8	4	17	5
4507745	thioredoxin	6	3	6	3	15	5
31542947	chaperonin; mitochondrial matrix protein P1	5	4	11	7	10	2

B. Proteins present in platelet MPs from this study and Garcia, et al. but not detected in plasma MPs (this study).

		Plasma MPs		Platelet MPs		Garcia, et al. Platelet MPs	
GI Number	**Reference**	**Spectral Count**	**Unique Peptides**	**Spectral Count**	**Unique Peptides**	**Spectral Count**	**Unique Peptides**
11321601	phosphofructokinase, platelet			6	5	102	25
5031677	dynamin 1-like protein isoform 3			14	6	88	17
18104989	protein tyrosine phosphatase, non-receptor type 6			6	4	93	17
16554592	enolase 3; enolase-3, beta, muscle			13	3	85	3
21735621	mitochondrial malate dehydrogenase			9	4	74	16
10835121	pyruvate kinase, liver and RBC isoform 1			6	1	69	4
5902128	syntaxin binding protein 2			9	6	64	25
10346135	microtubule-associated protein, RP/EB family			6	2	66	8
4504073	glycoprotein Ib beta polypeptide			13	3	55	4

34419635	heat shock 70kDa protein 6 (HSP70B')			8	4	59	8
13699840	thromboxane A synthase 1			10	7	50	15
42476281	voltage-dependent anion channel 2			5	1	55	9
34147513	RAB7, member RAS oncogene family			8	4	50	9
4557377	Bruton agammaglobulinemia tyrosine kinase			4	3	52	16
41406082	glutathione peroxidase 1			5	1	50	11
41406064	myosin, heavy polypeptide 10			8	6	47	11
31742532	diaphanous 1			7	6	47	12
18201905	glucose phosphate isomerase			8	4	41	12
4504077	glycoprotein IX (platelet)			8	3	40	5
41322908	plectin 1			6	6	39	12
27597085	tropomyosin 1 (alpha)			14	5	29	5
38202257	alpha glucosidase II alpha subunit			4	4	35	12
20070125	prolyl 4-hydroxylase, beta subunit			8	6	30	11
32455260	peroxiredoxin 5, isoform b			6	3	26	7
5031595	actin related protein 2/3 complex subunit 4			7	3	23	4
10863873	transforming growth factor, beta 1			6	5	24	7
4504085	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)			8	7	21	8
6005846	twinfilin-like protein			6	4	22	5
47933379	N-ethylmaleimide-sensitive factor attachment protein			5	4	23	9
46195765	unc-13 homolog D			5	5	21	6
4501867	aconitase 2; aconitate hydratase			3	3	23	10
4757766	Rho GTPase activating protein 1			7	4	17	8
22035665	talin 2			5	5	19	3
51470760	KIAA0830 protein			6	2	16	7
21327708	nucleosome assembly protein 1-like 1			5	2	15	3
38569421	ATP citrate lyase			4	3	15	4
29568111	myosin regulatory light polypeptide 9 isoform a			7	2	11	3
13775198	SH3 domain binding glutamic acid-rich protein			12	2	6	3
4506003	protein phosphatase 1, catalytic subunit, alpha			5	4	13	3
24234688	heat shock 70kDa protein 9B			6	4	11	4
5802966	destrin (actin depolymerizing factor)			5	4	12	2
23510338	ubiquitin-activating enzyme E1			6	4	11	3
7706190	spectrin, beta, non-erythrocytic 5			5	5	11	3
10645195	H2A histone family, member A			8	3	7	3

C. Proteins detected in plasma MPs from present study and platelet MPs from Garcia, et al. but not platelet MPs in this study*.

		Plasma MPs		Platelet MPs		Garcia, et al. Platelet MPs	
GI Number	Reference	Spectral Count	Unique Peptides	Spectral Count	Unique Peptides	Spectral Count	Unique Peptides
4502153	apolipoprotein B; apoB-100	37	25			73	17
4506117	protein S (alpha)	24	13			22	4
10835002	Rho GDP dissociation inhibitor (GDI) beta	8	5			52	9
4503529	eukaryotic translation initiation factor 4A	5	2			65	9
4502051	arachidonate 12-lipoxygenase; 12(S)-lipoxygenase	5	5			53	16
27436929	heat shock 70kDa protein 1-like; heat shock 70kD	5	3			39	9
4504517	heat shock 27kDa protein 1	5	3			30	3
5031601	actin related protein 2/3 complex subunit 1B; ARP2	5	3			24	10
4557325	apolipoprotein E ; apolipoprotein E3	17	10			7	5
15149463	caldesmon 1 isoform 4	7	3			39	14
19743813	integrin beta 1 isoform 1A; integrin V	6	6			44	8
4758304	protein disulfide isomerase related protein	6	5			27	10
21624607	coactosin-like 1	6	5			16	10
51458015	solute carrier family 25 m	6	1			6	4
51475407	Ig kappa chain	10	2			21	4
24111250	guanine nucleotide binding protein (G protein). Alpha 13	5	4			17	5
38570111	Threonyl-tRNA synthetase	5	1			25	8
21361122	four and a half LIM domains 1	11	4			93	11
4557871	transferrin	11	8			14	5
4557385	complement component 3	11	10			13	4

Proteins detected in both Plasma MPs and Platelet MP from this study but not in previous study.

		Plasma MPs		Platelet MPs		Previous Study Platelet MPs	
GI Number	Reference	Spectral Count	Unique Peptides	Spectral Count	Unique Peptides	Spectral Count	Unique Peptides
4504345	alpha 2 globin	85	7	50	6		
29736622	RIKEN cDNA 4732495G21 gene product	30	3	64	5		
4506413	RAP1A, member of RAS oncogene family; RAS-related	23	5	51	4		
21361657	glucose regulated protein, 58kDa	20	13	34	14		
37588853	vasoactive intestinal peptide isoform 2	25	1	20	1		

13676857	heat shock 70kDa protein 2	17	7	25	6		
14719392	cofilin 2	9	4	21	4		
21361322	tubulin, beta, 5	13	7	15	7		
19747267	titin isoform N2-A	7	7	21	20		
4758484	glutathione-S-transferase omega 1	10	5	16	7		
4502201	ADP-ribosylation factor 1	10	4	16	8		
14210536	tubulin beta 6	7	3	11	3		
4758984	Ras-related protein Rab-11A	7	4	11	5		
11024714	ubiquitin B	8	3	9	3		
4507021	solute carrier family 4, anion exchanger, member 1	12	7	3	3		
5803225	tyrosine 3/tryptophan 5 -monooxygenase activation protein, epsilon	7	5	8	3		
40254462	guanine nucleotide binding protein (G protein)	5	4	10	6		
4502781	centromere protein E	8	2	6	3		
28872786	CDK5 regulatory subunit associated protein 2	5	2	4	3		
21614544	S100 calcium-binding protein A8	6	4	2	1		
19923844	hypothetical protein FLJ23518	6	1	2	1		
4757768	Rho GDP dissociation inhibitor (GDI) alpha	5	3	3	2		
51493118	eukaryotic translation inititation factor	5	1	3	1		
21361684	G patch domain containing 1	5	2	2	2		
5032161	elongin C	5	1	2	1		

E. Proteins detected only in plasma MP from this study but not in any platelet MPs.**

		Plasma MPs		Platelet MPs		Previous Study Platelet MPs	
GI Number	**Reference**	**Spectral Count**	**Unique Peptides**	**Spectral Count**	**Unique Peptides**	**Spectral Count**	**Unique Peptides**
4503681	Fc fragment of IgG binding protein; IgG Fc binding	167	46				
4502503	complement component 4 binding protein, alpha	114	18				
45580723	haptoglobin-related protein	86	19				
4502501	complement component 4B proprotein	86	37				
5031863	galectin 3 binding protein	68	16				
4557225	alpha-2-macroglobulin	36	21				
4826762	haptoglobin	32	10				
4557321	apolipoprotein A-I	28	10				
5174411	CD5 antigen-like antigen	24	10				
4507457	transferrin receptor	20	14				
21489959	immunoglobulin J chain	15	4				
4502505	complement component 4 binding protein, beta	14	5				

4504549	tenascin C (hexabrachion)	10	9				
4503647	coagulation factor VIII isoform a	10	10				
4557323	apolipoprotein C-III	10	2				
22749297	oncoprotein-induced transcript 3; liver-specific	8	6				
4506355	pregnancy-zone protein	7	6				
21735614	apolipoprotein L1	7	4				
38016932	PTPL1-associated RhoGAP 1	6	2				
4504893	kininogen 1	6	3				
5730051	solute carrier family 2	5	3				
32171241	phosphodiesterase 4B, cAMP-specific	5	1				
27894373	nuclear DNA-binding protein	5	1				
31652251	hypothetical protein LOC51244	5	1				
19923578	hypothetical protein FLJ21839	5	1				
21361670	drebrin-like; src homology 3 domain-containing protein	5	5				
15149476	arginyl-tRNA synthetase	5	3				

F. Proteins detected only in platelet MP of this study.

		Plasma MPs		Platelet MPs		Platelet MPs	
		Spectral Count		Spectral Count		Spectral Count	
GI Number	Reference	Unique Peptides		Unique Peptides		Unique Peptides	
34577083	ras suppressor protein 1 isoform 2			29	6		
13376539	tubulin, alpha 4			14	2		
17986001	major histocompatibility complex, class I, B			10	6		
40255314	obscurin, cytoskeletal calmodulin			9	7		
23592238	glucose transporter 14			8	4		
11415030	H4 histone family, member E			7	4		
13376181	tubulin, alpha-like 3			7	3		
51460943	protein XP_498918			6	6		
23097308	nesprin 1 isoform longer			6	6		
4757952	cell division cycle 42 isoform 1			6	2		
19557677	major histocompatibility complex, class I, C			6	2		
22748825	protein FLJ31438			6	2		
16751921	dermcidin; AIDD protein			6	2		
31542770	protein FLJ33718			6	1		
13430854	nuclear RNA export factor 2			6	1		
4507191	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)			5	5		
51461034	KIAA0445 protein			5	5		
51493205	chromosome 14 open reading frame 78			5	5		

33563340	myosin, heavy polypeptide 14			5	3		
28626521	KIAA1404 protein			5	3		
11055998	guanine nucleotide-binding protein, beta-4 subunit			5	3		
20544189	tenascin XB isoform 1; tenascin XB1			5	3		
40548415	dishevelled associated activator of morphogenesis			5	3		
4502549	calmodulin 2			5	3		
10835035	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3			5	3		
51465886	protein CBG176			5	3		
4826730	FK506 binding protein 12-rapamycin associated protein			5	2		
51458868	osteotesticular protein tyrosine kinase			5	1		
41208756	protein BC282485_1			5	1		

*These protein are not truly unique to plasma MPs

** These are newly discovered proteins in microparticles

Supplementary Table 3: Spectral Count of all Proteins with a total of 10 or greater peptide spectral counts. Significant differences indicated in bold.

		Plasma MP		Platelet MP		all	Scan Ratio			
GI number	**Reference**	Spectral Count	uniq peptides	Spectral Count	uniq peptides	Spectral Count	**plasma MP/ platelet MP**	**log 2**	**Adj log 2**	**p-value**
4503681	**Fc fragment of IgG binding protein**	167	46	0	0	167	**-------**			**0.000***
4502503	**complement component 4 binding protein, alpha**	114	18	1	1	115	114.00	6.83	7.37	**0.000***
45580723	**haptoglobin-related protein**	86	16	0	0	86	**-------**			**0.002***
4502501	**complement component 4B protein**	86	37	1	1	87	86.00	6.43	6.97	0.060
5031863	**galectin 3 binding protein**	68	16	0	0	68	**-------**			**0.001***
4502153	**apolipoprotein B**	37	25	0	0	37	**-------**			0.114
4557225	**alpha-2-macroglobulin**	36	21	0	0	36	**-------**			0.204
4826762	**haptoglobin**	32	10	0	0	32	**-------**			0.071
4557321	**apolipoprotein A-I**	28	10	0	0	28	**-------**			0.203
5174411	**CD5 antigen-like protein**	24	10	0	0	24	**-------**			**0.006***
4506117	**protein S (alpha)**	24	13	1	1	25	24.00	4.58	5.12	**0.019***
4507457	**transferrin receptor**	20	14	0	0	20	**-------**			**0.048***
4557325	**apolipoprotein E; apolipoprotein E3**	17	10	0	0	17	**-------**			0.110
21489959	**immunoglobulin J chain**	15	4	0	0	15	**-------**			**0.028***
4502505	**complement component 4 binding protein, beta**	14	5	0	0	14	**-------**			**0.018***
4557871	**transferrin**	11	8	1	1	12	11.00	3.46	4.00	0.073
4557385	**complement component 3**	11	10	0	0	11	**-------**			0.052
51475407	**Ig kappa chain**	10	2	0	0	10	**-------**			**0.032***
4557323	**apolipoprotein C-III**	10	2	0	0	10	**-------**			0.052
4504549	**tenascin C (hexabrachion)**	10	9	0	0	10	**-------**			0.121
4503647	**coagulation factor VIII**	10	10	1	1	11	10.00	3.32	3.86	0.339
4507907	von Willebrand factor	745	85	23	15	768	32.39	5.02	5.56	**0.000***
4507021	solute carrier family 4, anion exchanger, member 1	12	7	3	3	15	4.00	2.00	2.54	0.174
21361122	four and a half LIM domains 1	11	4	3	1	14	3.67	1.87	2.41	0.192
15149463	caldesmon 1 isoform 4	7	3	3	3	10	2.33	1.22	1.76	0.490
4502517	carbonic anhydrase I	11	5	5	2	16	2.20	1.14	1.68	0.363
10835002	Rho GDP dissociation inhibitor (GDI) beta	8	5	4	3	12	2.00	1.00	1.54	0.063
4502027	albumin	85	28	45	16	130	1.89	0.92	1.46	**0.008***
30240932	EH-domain containing 1; testilin	13	8	7	4	20	1.86	0.89	1.43	0.112
4504345	alpha 2 globin; alpha globin	85	7	50	6	135	1.70	0.77	1.31	0.239
4504351	delta globin	13	6	8	4	21	1.63	0.70	1.24	0.403
13994151	PDZ and LIM domain 1 (elfin)	16	11	10	6	26	1.60	0.68	1.22	0.533
4504349	beta globin; hemoglobin beta chain	105	12	70	10	175	1.50	0.58	1.12	0.213
24234686	heat shock 70kDa protein 8 isoform 2	30	46	20	9	50	1.50	0.58	1.12	0.967

4557014	catalase	12	6	8	6	20	1.50	0.58	1.12	0.325
19743813	integrin beta 1 isoform 1A	6	6	4	4	10	1.50	0.58	1.12	0.102
31542292	chaperonin containing TCP1, subunit 3 (gamma)	13	4	9	4	22	1.44	0.53	1.07	0.358
28302131	A-gamma globin; hemoglobin gamma-a chain	14	3	10	2	24	1.40	0.49	1.03	0.443
4758012	clathrin heavy chain 1	18	13	13	12	31	1.38	0.47	1.01	0.494
5729887	IQ motif containing GTPase activating protein 2	12	10	9	7	21	1.33	0.42	0.96	0.635
7705296	bridging integrator 2	8	5	6	4	14	1.33	0.42	0.96	0.561
4502781	centromere protein E	8	2	6	3	14	1.33	0.42	0.96	0.371
42716297	clusterin isoform 1	18	6	14	8	32	1.29	0.36	0.90	0.193
37588853	vasoactive intestinal peptide isoform 2	25	1	20	1	45	1.25	0.32	0.86	0.831
11761629	fibrinogen, alpha chain isoform alpha	57	19	47	17	104	1.21	0.28	0.82	0.091
13518026	LIM and senescent cell antigen-like domains 1	12	4	10	5	22	1.20	0.26	0.80	0.677
4557395	carbonic anhydrase II; carbonate dehydratase II	6	3	5	2	11	1.20	0.26	0.80	0.503
4505257	moesin	26	15	22	10	48	1.18	0.24	0.78	0.171
4508047	zyxin	14	8	12	7	26	1.17	0.22	0.76	0.914
4504183	glutathione transferase	7	6	6	3	13	1.17	0.22	0.76	0.845
4503327	cytochrome b5 reductase membrane-bound isoform	7	4	6	3	13	1.17	0.22	0.76	0.270
13569962	RAB1B, member RAS oncogene family	8	4	7	4	15	1.14	0.19	0.73	0.685
4502903	clathrin, heavy polypeptide-like 1 isoform a	8	4	7	6	15	1.14	0.19	0.73	0.377
4507171	secreted protein, acidic, cysteine-rich (osteonectin)	10	5	9	4	19	1.11	0.15	0.69	0.494
10716563	calnexin	14	9	13	36	27	1.08	0.11	0.65	0.706
11761633	fibrinogen, gamma	63	17	59	25	122	1.07	0.09	0.63	----
13562114	beta tubulin 1, class VI	60	18	60	16	120	1.00	0.00	0.54	0.651
29788768	tubulin, beta polypeptide	10	4	10	4	20	1.00	0.00	0.54	0.494
4758950	peptidylprolyl isomerase B	10	5	10	6	20	1.00	0.00	0.54	0.658
40068518	phosphogluconate dehydrogenase	9	6	9	5	18	1.00	0.00	0.54	0.649
4504041	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	8	4	8	4	16	1.00	0.00	0.54	0.447
24638454	ATPase, Ca++ transporting, cardiac muscle, slow twitch	7	4	7	2	14	1.00	0.00	0.54	0.889
7019375	formin homology 2 domain containing 1	7	4	7	4	14	1.00	0.00	0.54	0.641
4507745	thioredoxin	6	3	6	3	12	1.00	0.00	0.54	0.379
38202217	proto-oncogene tyrosine-protein kinase SRC	5	5	5	3	10	1.00	0.00	0.54	0.749
5031987	peptidylprolyl isomerase F	5	3	5	3	10	1.00	0.00	0.54	0.655
11761631	fibrinogen, beta	71	16	76	18	147	0.93	-0.10	0.44	0.052
11024714	ubiquitin B; polyubiquitin B	8	3	9	3	17	0.89	-0.17	0.37	0.292
4557419	CD36 antigen	8	3	9	4	17	0.89	-0.17	0.37	0.352
4501881	alpha 1 actin; alpha skeletal muscle actin	109	11	123	12	232	0.89	-0.17	0.37	0.974
21464101	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma	7	4	8	4	15	0.88	-0.19	0.35	0.677
10835187	superoxide dismutase 2, mitochondrial	7	3	8	2	15	0.88	-0.19	0.35	0.807
5803225	tyrosine 3/tryptophan 5 -monooxygenase activation protein, epsilon	7	5	8	3	15	0.88	-0.19	0.35	0.867
4503643	coagulation factor V	7	6	8	7	15	0.88	-0.19	0.35	0.858

21361322	tubulin, beta, 5	13	7	15	7	28	0.87	-0.21	0.33	0.728
4507951	tyrosine 3/tryptophan 5 -monooxygenase activation protein, eta	12	5	14	5	26	0.86	-0.22	0.32	0.920
16950601	myosin light chain kinase isoform 6	6	4	7	5	13	0.86	-0.22	0.32	0.836
4758460	glycoprotein V (platelet)	6	4	7	6	13	0.86	-0.22	0.32	0.840
4503971	GDP dissociation inhibitor 1	6	4	7	5	13	0.86	-0.22	0.32	0.869
4502693	CD9 antigen; motility related protein	6	3	7	4	13	0.86	-0.22	0.32	0.826
5729997	RAB27B, member RAS oncogene family	10	4	12	7	22	0.83	-0.26	0.28	0.960
21956645	myotrophin; granule cell differentiation protein	5	1	6	1	11	0.83	-0.26	0.28	0.954
15812212	phosphodiesterase 5A	5	4	6	5	11	0.83	-0.26	0.28	0.367
7657056	EH-domain containing 3	5	3	6	5	11	0.83	-0.26	0.28	0.859
45269141	multimerin 1; glycoprotein Ia*	29	15	35	15	64	0.83	-0.27	0.27	0.513
4557032	lactate dehydrogenase B	19	8	23	10	42	0.83	-0.28	0.26	0.840
24119203	tropomyosin 3	23	8	28	8	51	0.82	-0.28	0.26	0.709
29788785	beta 5-tubulin; beta Ib tubulin	9	3	11	3	20	0.82	-0.29	0.25	0.579
20149594	heat shock 90kDa protein 1, beta	9	6	11	8	20	0.82	-0.29	0.25	0.676
5031573	ARP3 actin-related protein 3 homolog	13	7	16	11	29	0.81	-0.30	0.24	0.676
21328448	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase ctivation protein, beta	13	8	16	6	29	0.81	-0.30	0.24	0.972
21361370	brain glycogen phosphorylase	8	6	10	8	18	0.80	-0.32	0.22	0.859
4557801	purine nucleoside phosphorylase	8	5	10	6	18	0.80	-0.32	0.22	0.733
28626504	UNC-112 related protein 2 short form; kindlin 3	75	26	95	24	170	0.79	-0.34	0.20	0.286
17986258	smooth muscle and non-muscle myosin alkali light chain	18	6	23	7	41	0.78	-0.35	0.19	0.922
4827056	WD repeat-containing protein 1 isoform 2	18	10	23	9	41	0.78	-0.35	0.19	0.667
16507237	heat shock 70kDa protein 5 (glucose-regulated protein)	23	10	30	13	53	0.77	-0.38	0.16	0.300
4507677	tumor rejection antigen (gp96) 1	18	10	24	10	42	0.75	-0.42	0.12	0.489
4502695	cell division cycle 10; CDC10 protein homolog	6	5	8	6	14	0.75	-0.42	0.12	0.679
42476296	tropomyosin 2 (beta) isoform 1	13	5	18	6	31	0.72	-0.47	0.07	0.593
15991827	hexokinase 1 isoform HKI-R; brain form hexokinase	5	5	7	5	12	0.71	-0.49	0.05	0.972
4758606	integrin-linked kinase	17	11	24	10	41	0.71	-0.50	0.04	0.921
4507651	tropomyosin 4	23	8	33	12	56	0.70	-0.52	0.02	0.734
38016911	stomatin isoform a; erythrocyte membrane protein	11	7	16	6	27	0.69	-0.54	0.00	0.908
7656991	coronin, actin binding protein, 1C	11	8	16	8	27	0.69	-0.54	0.00	0.431
13676857	heat shock 70kDa protein 2; Heat-shock 70kD protein	17	7	25	6	42	0.68	-0.56	-0.02	0.928
4503745	filamin 1 (actin-binding protein-280)	329	100	484	113	813	0.68	-0.56	-0.02	0.188
5453595	adenylyl cyclase-associated protein	22	11	33	14	55	0.67	-0.58	-0.04	0.416
5031571	actin-related protein 2; ARP2 (actin-related protein)	12	7	18	10	30	0.67	-0.58	-0.04	0.869
13489087	serine (or cysteine) proteinase inhibitor	6	5	9	7	15	0.67	-0.58	-0.04	0.908
4557675	integrin alpha chain, alpha 6	6	6	9	7	15	0.67	-0.58	-0.04	0.708
23510338	ubiquitin-activating enzyme E1	4	4	6	4	10	0.67	-0.58	-0.04	0.558
17921989	tubulin, alpha 1; testis-specific alpha tubulin	13	5	20	6	33	0.65	-0.62	-0.08	0.721
34577110	aldolase A; Aldolase A, fructose-bisphosphatase	18	11	28	12	46	0.64	-0.64	-0.10	0.300

4505981	pro-platelet basic protein	35	7	55	7	90	0.64	-0.65	-0.11	0.329
10863927	peptidylprolyl isomerase A isoform 1	14	7	22	7	36	0.64	-0.65	-0.11	0.896
14210536	tubulin beta MGC4083	7	3	11	3	18	0.64	-0.65	-0.11	0.787
4758984	Ras-related protein Rab-11A	7	4	11	5	18	0.64	-0.65	-0.11	0.626
4507877	vinculin isoform VCL	125	43	197	49	322	0.63	-0.66	-0.12	0.181
4758484	glutathione-S-transferase omega 1	10	5	16	7	26	0.63	-0.68	-0.14	0.364
4502201	ADP-ribosylation factor 1	10	4	16	8	26	0.63	-0.68	-0.14	0.795
5803227	tyrosine 3/tryptophan 5 -monooxygenase protein, theta	5	3	8	4	13	0.63	-0.68	-0.14	0.642
38044288	gelsolin isoform b	52	14	85	19	137	0.61	-0.71	-0.17	0.235
4503571	enolase 1; MYC promoter-binding protein 1	17	12	28	12	45	0.61	-0.72	-0.18	0.522
21314617	platelet/endothelial cell adhesion molecule (CD31)	9	5	15	7	24	0.60	-0.74	-0.20	0.545
21361657	glucose regulated protein, 58kDa	20	13	34	14	54	0.59	-0.77	-0.23	0.189
4758638	peroxiredoxin 6; antioxidant protein 2	14	8	24	12	38	0.58	-0.78	-0.24	0.308
47078292	integrin beta chain, beta 3	59	21	102	24	161	0.58	-0.79	-0.25	0.214
16753233	talin 1	346	106	612	109	958	0.57	-0.82	-0.28	0.131
25188179	voltage-dependent anion channel 3	5	5	9	4	14	0.56	-0.85	-0.31	0.733
23238211	actin related protein 2/3 complex subunit 2	5	5	9	7	14	0.56	-0.85	-0.31	0.638
5453597	F-actin capping protein alpha-1 subunit	5	4	9	4	14	0.56	-0.85	-0.31	0.467
4507869	vasodilator-stimulated phosphoprotein	5	4	9	6	14	0.56	-0.85	-0.31	0.947
4501891	actinin, alpha 1	92	32	166	41	258	0.55	-0.85	-0.31	0.135
12667788	myosin, heavy polypeptide 9, non-muscle	291	103	526	101	817	0.55	-0.85	-0.31	0.139
14251209	chloride intracellular channel 1	16	6	29	10	45	0.55	-0.86	-0.32	0.295
40254816	heat shock 90kDa protein 1, alpha; heat shock 90k	6	6	11	7	17	0.55	-0.87	-0.33	0.541
5902134	coronin, actin binding protein, 1A; coronin, actin	6	5	11	5	17	0.55	-0.87	-0.33	0.313
6005942	valosin-containing protein	14	9	26	15	40	0.54	-0.89	-0.35	0.150
4505763	phosphoglycerate kinase 1	7	6	13	8	20	0.54	-0.89	-0.35	0.334
38016907	stomatin isoform b	8	3	15	5	23	0.53	-0.91	-0.37	0.529
21614499	villin 2; cytovillin; ezrin	8	4	15	6	23	0.53	-0.91	-0.37	0.304
12025678	actinin, alpha 4	60	23	114	27	174	0.53	-0.93	-0.39	0.162
47419932	platelet glycoprotein Ib alpha	13	7	25	7	38	0.52	-0.94	-0.40	0.226
4503631	coagulation factor XIII A1 subunit	47	22	93	21	140	0.51	-0.98	-0.44	0.192
40317626	thrombospondin 1	91	29	181	27	272	0.50	-0.99	-0.45	0.111
5031635	cofilin 1 (non-muscle)	15	8	30	9	45	0.50	-1.00	-0.46	0.284
7661678	RAP1B, member of RAS oncogene family	12	3	24	3	36	0.50	-1.00	-0.46	0.189
41058276	Triosephosphate isomerase	8	5	16	7	24	0.50	-1.00	-0.46	0.834
40254462	guanine nucleotide binding protein (G protein), q polypeptide	5	4	10	6	15	0.50	-1.00	-0.46	0.221
4504077	glycoprotein IX (platelet)	4	2	8	3	12	0.50	-1.00	-0.46	0.596
14389309	tubulin alpha 6	18	10	37	8	55	0.49	-1.04	-0.50	0.175
33286418	pyruvate kinase 3 isoform 1	42	22	87	24	129	0.48	-1.05	-0.51	0.116
7669492	glyceraldehyde-3-phosphate dehydrogenase	22	9	46	8	68	0.48	-1.06	-0.52	0.289
4759082	serum deprivation response protein	11	7	23	6	34	0.48	-1.06	-0.52	0.079

28373103	sarco/endoplasmic reticulum Ca2+ -ATPase isoform	8	5	17	10	25	0.47	-1.09	-0.55	0.127
29736622	RIKEN cDNA 4732495G21 gene product	30	3	64	5	94	0.47	-1.09	-0.55	0.086
4826659	F-actin capping protein beta subunit	7	5	15	8	22	0.47	-1.10	-0.56	0.489
31542947	chaperonin; mitochondrial matrix protein P1	5	4	11	7	16	0.45	-1.14	-0.60	0.235
4501885	beta actin; beta cytoskeletal actin	107	9	237	10	344	0.45	-1.15	-0.61	**0.037***
4506413	RAP1A, member of RAS oncogene family	23	5	51	4	74	0.45	-1.15	-0.61	0.597
28178832	isocitrate dehydrogenase 2 (NADP+), mitochondrial	9	6	20	11	29	0.45	-1.15	-0.61	0.190
4757900	calreticulin	9	6	20	7	29	0.45	-1.15	-0.61	0.117
4757810	ATP synthase, H+ transporting, mitochondrial F1 complex alpha	13	7	29	14	42	0.45	-1.16	-0.62	0.166
14719392	cofilin 2	9	4	21	4	30	0.43	-1.22	-0.68	0.235
13376181	tubulin, alpha-like 3	3	3	7	3	10	0.43	-1.22	-0.68	0.480
22748619	tropomyosin 3	5	4	12	10	17	0.42	-1.26	-0.72	0.797
4505879	pleckstrin	22	11	53	13	75	0.42	-1.27	-0.73	**0.047***
21735625	tyrosine 3/tryptophan 5 -monooxygenase activation protein, zeta	24	10	59	9	83	0.41	-1.30	-0.76	0.173
17986001	major histocompatibility complex, class I, B	4	4	10	6	14	0.40	-1.32	-0.78	0.405
15809016	myosin regulatory light chain MRCL2	17	7	43	8	60	0.40	-1.34	-0.80	**0.022***
12408656	calpain 1, large subunit	10	7	26	17	36	0.38	-1.38	-0.84	0.119
5031973	protein disulfide isomerase-related protein	6	4	16	5	22	0.38	-1.42	-0.88	0.454
23592238	glucose transporter 14	3	2	8	4	11	0.38	-1.42	-0.88	0.431
20070125	prolyl 4-hydroxylase, beta subunit	3	3	8	6	11	0.38	-1.42	-0.88	0.767
18201905	glucose phosphate isomerase; neuroleukin	3	2	8	4	11	0.38	-1.42	-0.88	0.305
10645195	H2A histone family, member A; histone H2AE	3	1	8	3	11	0.38	-1.42	-0.88	0.535
4504745	integrin alpha 2b; platelet fibrinogen receptor	76	26	204	30	280	0.37	-1.42	-0.88	**0.025***
4507357	transgelin 2; SM22-alpha homolog	24	12	65	11	89	0.37	-1.44	-0.90	**0.022***
5031857	lactate dehydrogenase A	6	4	17	7	23	0.35	-1.50	-0.96	0.167
51465474	Glyceraldehyde 3-phosphate dehydrogenase	9	2	27	3	36	0.33	-1.58	-1.04	**0.029***
19747267	titin isoform N2-A; connectin	7	7	21	20	28	0.33	-1.58	-1.04	0.930
21735621	mitochondrial malate dehydrogenase	3	1	9	4	12	0.33	-1.58	-1.04	0.201
5902128	syntaxin binding protein 2	3	3	9	6	12	0.33	-1.58	-1.04	0.169
4505733	platelet factor 4 (chemokine (C-X-C motif) ligand	15	4	46	4	61	0.33	-1.62	-1.08	0.112
32189394	ATP synthase, H+ transporting, mitochondrial F1 complex beta	10	7	31	12	41	0.32	-1.63	-1.09	**0.033***
4826898	profilin 1	35	9	112	7	147	0.31	-1.68	-1.14	**0.012***
16554592	enolase 3	4	2	13	3	17	0.31	-1.70	-1.16	0.375
4504073	glycoprotein Ib beta	4	3	13	3	17	0.31	-1.70	-1.16	0.277
13376539	tubulin, alpha 4	4	2	14	2	18	0.29	-1.81	-1.27	0.151
20127528	parvin, beta isoform b	12	6	43	8	55	0.28	-1.84	-1.30	**0.009***
13775198	SH3 domain binding glutamic acid-rich protein like	3	2	12	2	15	0.25	-2.00	-1.46	**0.023***
34419635	heat shock 70kDa protein 6 (HSP70B')	2	2	8	4	10	0.25	-2.00	-1.46	0.232
34147513	RAB7, member RAS oncogene family	2	2	8	4	10	0.25	-2.00	-1.46	0.184
27597085	tropomyosin 1 (alpha)	3	8	14	5	17	0.21	-2.22	-1.68	0.811
13699840	thromboxane A synthase 1	2	2	10	7	12	0.20	-2.32	-1.78	0.283

5031677	dynamin 1-like protein isoform 3	2	2	14	6	16	0.14	-2.81	-2.27	0.233
34577083	ras suppressor protein 1 isoform 2	4	3	29	6	33	0.14	-2.86	-2.32	**0.001***

Supplementary Table 4: Peptides from Proteins enriched in Plasma MPs.

GI Number	Reference	z	Peptide	Scan Count: Plasma MP	Scan Count: Platelet MP
4502503	Complement component	2	R.TPSCGDICNFPPK.I	18	
	4 binding protein, alpha	2	K.EDVYVVGTVLR.Y	13	1
		2	K.GYILVGQAK.L	12	
		2	R.FSAICQGDGTWSPR.T	12	
		2	K.LNNGEITQHR.K	9	
		2	K.LSLEIEQLELQR.D	9	
		3	R.KPDVSHGEMVSGFGPIYNYK.D	7	
		2	R.LMQCLPNPEDVK.M	7	
		2	K.CEWETPEGCEQVLTGK.R	5	
		2	K.YTCLPGYVR.S	5	
		2	R.GVGWSHPLPQCEIVK.C	5	
		2	K.MALEVYK.L	4	
		2	R.TWYPEVPK.C	3	
		1	K.EEIIYECDK.G	1	
		2	K.EEIIYECDK.G	1	
		2	K.IAHGHYK.Q	1	
		2	R.GSSVIHCDADSK.W	1	
		1	R.NGQVEIK.T	1	
4503681	Fc fragment of IgG	2	R.VNGVLTALPVSVADGR.I	13	
	binding protein	2	R.FAVLQENVAWGNGR.V	12	
		2	R.GEVGFVLVDNQR.S	11	
		2	R.ASQHGSDVVIETDFGLR.V	11	
		2	R.VAYDLVYYVR.V	7	
		2	R.YDLAFVVASQATK.L	6	
		2	R.TDFGLTVTYDWNAR.V	6	
		2	K.LDSLVAQQLQSK.N	6	
		1	R.GSQAVSYTR.S	5	
		2	R.GATTSPGVYELSSR.C	5	
		2	K.LPVVLANGQIR.A	5	
		3	K.ALASYVAACQAAGVVIEDWR.A	5	
		2	R.ISVTQGASK.A	4	
		2	R.APGWDPLCWDECR.G	4	
		2	K.VAVIVSNDHAGK.L	4	
		2	K.LVDPQGPLK.D	4	

		2	K.GCVLDVCMGGGDR.D	4
		2	K.AISGLTIDGHAVGAK.L	4
		2	K.AIGYATAADCGR.T	4
		2	R.YYPLGEVFYPGPECER.R	3
		2	R.VSYVGLVTVR.A	3
		2	R.LPVSLSEGR.L	3
		2	R.AYSHSVSLTR.G	3
		3	R.VPAAYAASLCGLCGNYNQDPADDLK.A	2
		2	R.TPDGSLLVR.Q	2
		3	R.SLAAYTAACQAAGVAVKPWR.T	2
		2	R.QCVYDLCAQK.G	2
		2	R.PFLEQCVYDLCVVGGER.L	2
		2	R.NMVLQTTK.G	2
		2	R.GSQTVSYTR.A	2
		2	R.GNPAVSYVR.V	2
		2	K.SVPGCEGVALVVAQTK.A	2
		2	K.PGDEDFSIVLEK.N	2
		2	K.LTYNHGGITGSR.G	2
		2	K.EFAVVAGAAGASVSVTLK.G	2
		2	R.SPANCPLSCPANSR.Y	1
		3	R.LLISSLSESPASVSILSQADNTSK.K	1
		2	R.FDFMGTCVYVLAQTCGTR.P	1
		2	R.EEFLTAFLQNYQLAYSK.A	1
		2	K.VTVNGVDMK.L	1
		2	K.VTASSPVAVLSGHSCAQK.H	1
		2	K.PGQVCQPSGGILSCVTK.D	1
		2	K.LASVSVSR.T	1
		2	K.GCVLDVCMGGGDHDILCK.A	1
		3	K.EEFCGLLSSPTGPLSSCHK.L	1
		2	K.AGCVAESTAVCR.A	1
5031863	Galectin 3 binding protein	2	R.ELSEALGQIFDSQR.G	11
		2	R.RIDITLSSVK.C	8
		2	R.IYTSPTWSAFVTDSSWSAR.K	8
		2	R.SDLAVPSELALLK.A	6
		2	R.ASHEEVEGLVEK.I	6
		2	K.YSSDYFQAPSDYR.Y	5
		3	R.GQWGTVCDNLWDLTDASVVCR.A	4
		2	R.KSQLVYQSR.R	3
		3	K.TLQALEFHTVPFQLLAR.Y	3
		2	K.AVDTWSWGER.A	3

		2	K.ALMLCEGLFVADVTDFEGWK.A	3
		1	R.STHTLDLSR.E	2
		2	R.LADGGATNQGR.V	2
		2	K.SQLVYQSR.R	2
		2	R.TIAYENK.A	1
		2	R.IDITLSSVK.C	1
45580723	Haptoglobin-related protein	2	K.SCAVAEYGVYVK.V	30
		2	R.TEGDGVYTLNDK.K	10
		2	R.TEGDGVYTLNDKK.Q	7
		1	K.DIAPTLTLYVGK.K	7
		2	R.VGYVSGWGQSDNFK.L	4
		2	R.LRTEGDGVYTLNDK.K	4
		2	K.GSFPWQAK.M	4
		1	K.QLVEIEK.V	3
		2	K.NPANPVQR.I	3
		2	K.LPECEAVCGKPK.N	3
		2	K.LPECEAVCGK.P	3
		2	K.KQLVEIEK.V	3
		2	K.AVGDKLPECEAVCGK.P	2
		2	R.ILGGHLDAK.G	1
		3	K.VVLHPNYHQVDIGLIK.L	1
		2	K.VTSIQHWVQK.T	1
5174411	CD5 antigen-like Antigen	2	R.EATLQDCPSGPWGK.N	5
		2	K.GQWGTVCDDGWDIK.D	5
		2	R.LVGGDNLCSGR.L	3
		2	K.GVWGSVCDDNWGEK.E	3
		2	R.ELGCGAASGTPSGILYEPPAEK.E	2
		1	K.DVAVLCR.E	2
		2	R.KPIWLSQMSCSGR.E	1
		2	R.IWLDNVR.C	1
		3	K.HQNQWYTVCQTGWSLR.A	1
		2	K.DVAVLCR.E	1
4502505	Complement component 4 binding protein, beta	2	K.EVEGQILGTYVCIK.G	5
		2	K.ALLAFQESK.N	4
		2	K.NLCEAMENFMQQLK.E	3
		2	K.LIQEAPKPECEK.A	2
4506117	Protein S, alpha	2	R.SFQTGLFTAAR.Q	4

		2	K.IETISHEDLQR.Q	3	1
		2	K.HCLVTVEK.G	3	
		2	R.QSTNAYPDLR.S	2	
		2	R.AHSCPSVWK.K	2	
		2	K.YELLYLAEQFAGVVLYLK.F	2	
		2	K.SCEVVSVCLPLNLDTK.Y	2	
		3	R.TYDSEGVILYAESIDHSAWLLIALR.G	1	
		2	R.NNLELSTPLK.I	1	
		2	R.KVESELIKPINPR.L	1	
		2	K.SQDILLSVENTVIYR.I	1	
		2	K.NGFVMLSNK.K	1	
		3	K.EAVMDINKPGPLFKPENGLLETK.V	1	
21489959	Immunoglobulin J chain	1	R.IVLVDNK.C	5	
		2	R.SSEDPNEDIVER.N	5	
		2	R.FVYHLSDLCK.K	4	
		2	K.CYTAVVPLVYGGETK.M	1	
51475407	Ig kappa chain	2	R.DIQMTQSPSSLSASVGDR.V	5	
		2	R.FSGSGSGTDFTLK.I	5	
4507457	Transferrin receptor	2	K.LLNENSYVPR.E	3	
		2	K.LAQMFSDMVLK.D	2	
		2	K.LAVDEEENADNNTK.A	2	
		2	K.LDSTDFTSTIK.L	2	
		2	R.SAFSNLFGGEPLSYTR.F	2	
		2	K.AFTYINLDK.A	1	
		2	K.DENLALYVENQFR.E	1	
		2	K.DSAQNSVIIVDK.N	1	
		2	K.GFVEPDHYVVVGAQR.D	1	
		2	K.LFGNMEGDCPSDWK.T	1	
		2	K.VSASPLLYTLIEK.T	1	
		2	R.SSGLPNIPVQTISR.A	1	
		3	R.VEYHFLSPYVSPK.E	1	
		2	R.YNSQLLSFVR.D	1	
4507907	Von Willebrand factor	2	K.AVVILVTDVSVDSVDAAADAAR.S	58	3
		2	R.NSMVLDVAFVLEGSDK.I	51	
		2	R.LLDLVFLLDGSSR.L	39	2
		2	K.SEVEVDIHYCQGK.C	33	
		2	K.TVMIDVCTTCR.C	25	

2	R.IGWPNAPILIQDFETLPR.E	23	
2	K.AHLLSLVDVMQR.E	21	
2	K.AFVLSSVDELEQQR.D	21	1
2	R.YLSDHSFLVSQGDR.E	20	
3	R.VKEEVFIQQR.N	20	
2	K.YAGSQVASTSEVLK.Y	18	1
2	K.LTGSCSYVLFQNK.E	17	
2	R.LSEAEFEVLK.A	16	3
3	R.SKEFMEEVIQR.M	15	
2	R.EGGPSQIGDALGFAVR.Y	14	1
2	R.HIVTFDGQNFK.L	13	
2	K.EQDLEVILHNGACSPGAR.Q	13	
2	R.EQAPNLVYMVTGNPASDEIK.R	12	
3	R.LTQVSVLQYGSITTIDVPWNVVPEK.A	11	
2	R.ILAGPAGDSNVVK.L	11	3
2	R.SGFTYVLHEGECCGR.C	10	
2	R.ILTSDVFQDCNK.L	10	1
2	K.TYGLCGICDENGANDFMLR.D	10	
2	K.TLVQEWTVQR.P	10	
3	K.RLPGDIQVVPIGVGPNANVQELER.I	10	
3	K.HSALSVELHSDMEVTVNGR.L	10	
2	R.IEDLPTMVTLGNSFLHK.L	9	
2	R.CMVQVGVISGFK.L	9	
2	K.YTLFQIFSK.I	9	
1	R.VTVFPIGIGDR.Y	8	
3	R.LPGDIQVVPIGVGPNANVQELER.I	8	
2	R.ICMDEDGNEK.R	8	
2	R.IALLLMASQEPQR.M	8	
2	K.IGCNTCVCR.D	8	
2	R.GGQIMTLK.R	7	
2	K.EFMEEVIQR.M	7	
2	R.TNGVCVDWR.T	6	
2	R.TATLCPQSCEER.N	6	
1	R.EAPDLVLQR.C	6	
2	R.DGTVTTDWK.T	6	
3	K.RDETLQDGCDTHFCK.V	6	
3	K.KVIVIPVGIGPHANLK.Q	6	
2	R.YLTSEMHGAR.P	5	
2	R.YIILLLGK.A	5	
2	R.VTGCPPFDEHK.C	5	
2	R.GLRPSCPNSQSPVK.V	5	

3	R.FNHLGHIFTFTPQNNEFQLQLSPK.T	5	
2	K.YLFPGECQYVLVQDYCGSNPGTFR.I	5	
3	K.ILDELLQTCVDPEDCPVCEVAGR.R	5	
2	K.APTCGLCEVAR.L	5	
2	K.AFVVDMMER.L	5	
2	R.WTCPCVCTGSSTR.H	4	
2	R.SLSCRPPMVK.L	4	
2	R.GEYFWEK.R	4	
2	R.CLPSACEVVTGSPR.G	4	
2	K.VIVIPVGIGPHANLK.Q	4	
2	K.RVTGCPPFDEHK.C	4	
2	K.DETHFEVVESGR.Y	4	
2	K.ALSVVWDR.H	4	
2	R.DETLQDGCDTHFCK.V	3	
2	R.CLPTACTIQLR.G	3	
2	K.LVCPADNLR.A	3	
2	R.YLTSEMHGARPGASK.A	2	
2	R.VTILVEGGEIELFDGEVNVK.R	2	2
3	R.KVPLDSSPATCHNNIMK.Q	2	
2	R.KTTCNPCPLGYK.E	2	
2	K.TTCNPCPLGYK.E	2	
3	K.SVGSQWASPENPCLINECVR.V	2	
2	K.LSGEAYGFVAR.I	2	1
2	K.IDRPEASR.I	2	
2	R.YDAAQLR.I	1	
2	R.VAVVEYHDGSHAYIGLK.D	1	
2	R.TNTGLALR.Y	1	
2	R.PSCPNSQSPVK.V	1	
2	R.MEACMLNGTVIGPGK.T	1	1
2	R.HLSISVVLK.Q	1	
2	R.GDSQSSWK.S	1	
2	R.ENGYECEWR.Y	1	
1	R.CVALER.C	1	
2	R.CPCFHQGK.E	1	
2	R.AEGLECTK.T	1	
2	K.VSSQCADTR.K	1	
2	K.TSACCPSCR.C	1	
2	K.QTMVDSSCR.I	1	
2	K.EENNTGECCGR.C	1	
2	R.YDVCSCSDGR.E		1
3	R.PPMVKLVCPADNLR.A		1

		2	R.IDGSGNFQVLLSDR.Y		1
		2	K.EYAPGETVK.I		1
4502027	Albumin	2	K.KVPQVSTPTLVEVSR.N	10	4
		3	R.FKDLGEENFK.A	6	2
		3	K.VFDEFKPLVEEPQNLIK.Q	6	5
		2	K.FQNALLVR.Y	5	3
		2	K.AVMDDFAAFVEK.C	5	2
		2	K.AAFTECCQAADK.A	5	4
		3	K.SHCIAEVENDEMPADLPSLAADFVESK.D	4	
		2	K.QNCELFEQLGEYK.F	4	4
		2	K.LVNEVTEFAK.T	4	3
		3	R.RHPDYSVVLLLR.L	3	2
		2	K.YLYEIAR.R	3	
		2	K.YICENQDSISSK.L	3	1
		2	K.VPQVSTPTLVEVSR.N	3	4
		2	K.QTALVELVK.H	3	1
		3	K.ALVLIAFAQYLQQCPFEDHVK.L	3	
		3	R.MPCAEDYLSVVLNQLCVLHEK.T	2	2
		2	K.TCVADESAENCDK.S	2	
		2	K.LDELRDEGK.A	2	
		2	K.CCTESLVNR.R	2	
		2	K.AEFAEVSK.L	2	
		3	R.RHPYFYAPELLFFAK.R	1	1
		2	R.HPDYSVVLLLR.L	1	1
		2	R.ETYGEMADCCAK.Q	1	
		2	K.TYETTLEK.C	1	
		3	K.RMPCAEDYLSVVLNQLCVLHEK.T	1	
		2	K.DDNPNLPR.L	1	
		3	K.ADDKETCFAEEGKK.L	1	1
		3	K.ADDKETCFAEEGK.K	1	
		2	K.DVFLGMFLYEYAR.R		5
11761633	Fibrinogen, gamma	2	R.YLQEIYNSNNQK.I	14	
		2	R.VELEDWNGR.T	4	
		2	R.TSTADYAMFK.V	2	
		2	R.LTIGEGQQHHLGGAK.Q	2	
		2	R.LDGSVDFKK.N	1	
		2	R.LDGSVDFK.K	2	
		2	R.DNCCILDER.F	7	
		2	K.YEASILTHDSSIR.Y	6	

		2	K.VGPEADKYR.L	3
		3	K.VAQLEAQCQEPCKDTVQIHDITGK.D	1
		2	K.VAQLEAQCQEPCK.D	1
		2	K.QSGLYFIKPLK.A	2
		2	K.IHLISTQSAIPYALR.V	1
		3	K.EGFGHLSPTGTTEFWLGNEK.I	3
		2	K.DTVQIHDITGK.D	3
		2	K.ASTPNGYDNGIIWATWK.T	9
		3	K.AIQLTYNPDESSKPNMIDAATLK.S	2
4557385	Complement component 3	2	K.SGSDEVQVGQQR.T	2
		2	K.AGDFLEANYMNLQR.S	1
		2	K.EYVLPSFEVIVEPTEK.F	1
		2	K.QELSEAEQATR.T	1
		2	K.SSLSVPYVIVPLK.T	1
		2	K.TGLQEVEVK.A	1
		2	K.VQLSNDFDEYIMAIEQTIK.S	1
		2	R.EVVADSVWVDVK.D	1
		2	R.IPIEDGSGEVVLSR.K	1
		2	R.TVMVNIENPEGIPVK.Q	1
32130518	Apolipoprotein C-III	2	K.ESLSSYWESAK.T	1
		2	K.TAAQNLYEK.T	1
4502501	Complement component 4B	2	K.VLSLAQEQVGGSPEK.L	8
		2	K.AEMADQAAAWLTR.Q	5
		2	K.LNMGITDLQGLR.L	5
		2	R.TTNIQGINLLFSSR.R	5
		2	K.VDFTLSSER.D	4
		2	R.ALEILQEEDLIDEDDIPVR.S	4
		2	R.DFALLSLQVPLK.D	4
		2	R.GLEEELQFSLGSK.I	4
		2	R.GSFEFPVGDAVSK.V	4
		2	K.DHAVDLIQK.G	3
		2	R.EMSGSPASGIPVK.V	3
		3	R.HLVPGAPFLLQALVR.E	3
		2	R.VGDTLNLNLR.A	3
		3	R.VTASDPLDTLGSEGALSPGGVASLLR.L	3
		2	K.ITQVLHFTK.D	2
		2	K.KYVLPNFEVK.I	2
		2	K.TEQWSTLPPETK.D	2

		2	R.GPEVQLVAHSPWLK.D	2	
		2	R.SFFPENWLWR.V	2	
		2	K.ADGSYAAWLSR.G	1	
		2	K.AEFQDALEK.L	1	
		3	K.LHLETDSLALVALGALDTALYAAGSK.S	1	
		2	K.PVQGVAYVR.F	1	
		2	K.SHALQLNNR.Q	1	
		3	K.VGLSGMAIADVTLLSGFHALR.A	1	
		2	K.YVLPNFEVK.I	1	
		2	R.EELVYELNPLDHR.G	1	
		2	R.FGLLDEDGK.K	1	
		2	R.FGLLDEDGKK.T	1	
		2	R.GQIVFMNR.E	1	
		2	R.LLATLCSAEVCQCAEGK.C	1	
		3	R.LLLFSPSVVHLGVPLSVGVQLQDVPR.G	1	
		2	R.PVAFSVVPTAAAAVSLK.V	1	
		3	R.RGHLFLQTDQPIYNPGQR.V	1	
		3	R.STQDTVIALDALSAYWIASHTTEER.G	1	
		2	R.TYNVLDMK.N	1	
		2	R.VEYGFQVK.V	1	
		2	R.GHLFLQTDQPIYNPGQRVR.Y	0	1
4826762	Haptoglobin	1	K.VTSIQDWVQK.T	7	
		2	K.YVMLPVADQDQCIR.H	4	
		2	R.TEGDGVYTLNNEK.Q	4	
		2	R.VGYVSGWGR.N	4	
		1	K.DYAEVGR.V	3	
		3	K.SPVGVQPILNEHTFCAGMSK.Y	3	
		2	R.HYEGSTVPEK.K	3	
		2	K.VVLHPNYSQVDIGLIK.L	2	
		2	K.LRTEGDGVYTLNNEK.Q	1	
		2	R.HYEGSTVPEKK.T	1	
4557871	Transferrin	2	R.FDEFFSEGCAPGSK.K	3	
		2	K.EDLIWELLNQAQEHFGK.D	2	
		2	K.DGAGDVAFVK.H	1	
		2	K.EDPQTFYYAVAVVK.K	1	
		2	K.HSTIFENLANK.A	1	
		2	K.SASDLTWDNLK.G	1	
		2	K.SVIPSDGPSVACVK.K	1	
		2	R.TAGWNIPMGLLYNK.I	1	

		2	R.DDTVCLAK.L	0	1
4557325	Apolipoprotein E	2	K.SELEEQLTPVAEETR.A	4	
		2	R.AATVGSLAGQPLQER.A	3	
		2	R.GEVQAMLGQSTEELR.V	3	
		2	K.LEEQAQQIR.L	1	
		2	K.SWFEPLVEDMQR.Q	1	
		2	K.VEQAVETEPEPELR.Q	1	
		2	R.AKLEEQAQQIR.L	1	
		2	R.LAVYQAGAR.E	1	
		2	R.LGPLVEQGR.V	1	
		3	R.WVQTLSEQVQEELLSSQVTQELR.A	1	
4504549	Tenascin C	2	R.GLEPGQEYNVLLTAEK.G	2	
		3	K.DVTDTTALITWFKPLAEIDGIELTYGIK.D	1	
		2	R.AVDIPGLEAATPYR.V	1	
		2	R.EEFWLGLDNLNK.I	1	
		2	R.LEELENLVSSLR.E	1	
		2	R.LSWTADEGVFDNFVLK.I	1	
		2	R.NMNKEDEGEITK.S	1	
		2	R.TPVLSAEASTAK.E	1	
		2	R.VSQTDNSITLEWR.N	1	
4502153	Apolipoprotein B	2	K.IAIANIIDEIIEK.L	4	
		2	K.IEGNLIFDPNNYLPK.E	3	
		2	K.VPLLLSEPINIIDALEMR.D	3	
		2	K.ATFQTPDFIVPLTDLR.I	2	
		2	K.IVQILPWEQNEQVK.N	2	
		2	K.LQDFSDQLSDYYEK.F	2	
		2	K.NLTDFAEQYSIQDWAK.R	2	
		2	K.VNWEEEAASGLLTSLK.D	2	
		2	K.AGHIAWTSSGK.G	1	
		2	K.ALVEQGFTVPEIK.T	1	
		2	K.ATGVLYDYVNK.Y	1	
		2	K.DSYDLHDLK.I	1	
		2	K.FPEVDVLTK.Y	1	
		2	K.IDDIWNLEVK.E	1	
		2	K.LDFSSQADLR.N	1	
		2	K.LPQQANDYLNSFNWER.Q	1	
		2	K.MTSNFPVDLSDYPK.S	1	
		2	K.VSALLTPAEQTGTWK.L	1	

		2	K.YENYELTLK.S	1
		2	R.ILGEELGFASLHDLQLLGK.L	1
		2	R.ITENDIQIALDDAK.I	1
		2	R.LPYTIITTPPLK.D	1
		2	R.SEYQADYESLR.F	1
		2	R.TLADLTLLDSPIK.V	1
		3	R.VPSYTLILPSLELPVLHVPR.N	1
4557321	Apolipoprotein A-I	2	R.EQLGPVTQEFWDNLEK.E	6
		2	K.VSFLSALEEYTK.K	5
		2	K.DLATVYVDVLK.D	4
		2	K.ATEHLSTLSEK.A	3
		2	K.LLDNWDSVTSTFSK.L	3
		2	R.DYVSQFEGSALGK.Q	3
		3	K.LREQLGPVTQEFWDNLEK.E	1
		2	K.LSPLGEEMR.D	1
		2	K.WQEEMELYR.Q	1
		2	R.THLAPYSDELR.Q	1
4557225	Alpha-2-macroglobulin	2	R.LLIYAVLPTGDVIGDSAK.Y	4
		2	K.DTVIKPLLVEPEGLEK.E	3
		2	R.TEHPFTVEEFVLPK.F	3
		2	K.FEVQVTVPK.I	2
		2	K.NEDSLVFVQTDK.S	2
		2	K.QFSFPLSSEPFQGSYK.V	2
		2	R.IAQWQSFQLEGGLK.Q	2
		2	R.LLLQQVSLPELPGEYSMK.V	2
		2	R.NALFCLESAWK.T	2
		2	R.SASNMAIVDVK.M	2
		2	R.VSVQLEASPAFLAVPVEK.E	2
		2	K.AIGYLNTGYQR.Q	1
		3	K.GGVEDEVTLSAYITIALLEIPLTVTHPVVR.N	1
		2	K.HYDGSYSTFGER.Y	1
		2	K.LPPNVVEESAR.A	1
		2	K.LSFYYLIMAK.G	1
		2	K.SLNEEAVK.K	1
		2	K.YDVENCLANK.V	1
		3	R.AVDQSVLLMKPDAELSASSVYNLLPEK.D	1
		2	R.TEVSSNHVLIYLDK.V	1
		2	R.VTAAPQSVCALR.A	1

4503647	Coagulation factor VIII	2	K.ASEGAEYDDQTSQR.E	1	
		2	K.DFPILPGEIFK.Y	1	
		2	K.DLNSGLIGALLVCR.E	1	
		2	K.MALYNLYPGVFETVEMLPSK.A	1	
		2	K.VVFQEFTDGSFTQPLYR.G	1	
		2	K.WTVTVEDGPTK.S	1	
		2	R.AEVEDNIMVTFR.N	1	
		2	R.DLASGLIGPLLICYK.E	1	
		2	R.FDDDNSPSFIQIR.S	1	
		2	R.PYSFYSSLISYEEDQR.Q	1	
		3	K.YETFSDDPSPGAIDSNNSLSEMTHFR.P	0	1

Supplementary Table 5: Ion Intensitites of Unlabelled Peptides for Proteins enriched in Plasma MPs based on Spectral Count. The Ion intensities for up to five peptides with an overall spectral count of 6 or greater from each protein enriched in the plasma MPs was determined. Background ion intensities were used when no peak was evident. In addition to the P-value for each peptide an overall P-value for each protein was determined as described in methods.

GI Number:	Protein	Peptide	M	Z	Ion Intensity of plasma MP ($X10^5$)	Ion Intensity of platelet MP ($X10^5$)	P value	Protein P value
4502503	Complement component 4 binding Protein, alpha	R.TPSCGDICNFPPK.I	1493.6	2	5.87 ± 3.49	3.52 ± 2.30	0.199	0.0001*
4502503	Complement component 4 binding Protein, alpha	K.EDVYVVGTVLR.Y	1250.4	2	13.61 ± 11.23	2.83 ± 1.72	0.042*	
4502503	Complement component 4 binding Protein, alpha	K.GYILVGQAK.L	949.13	2	16.29 ± 20.36	3.22 ± 2.10	0.149	
4502503	Complement component 4 binding Protein, alpha	R.FSAICQGDGTWSPR.T	1582.7	2	17.38 ± 22.78	2.20 ± 1.15	0.134	
4502503	Complement component 4 binding Protein, alpha	K.LNNGEITQHR.K	1182.3	2	17.72 ± 16.43	3.34 ± 1.55	0.059	
4503681	Fc fragment of IgG binding protein	R.VNGVLTALPVSVADGR.I	1568.8	2	5.65 ± 4.30	3.64 ± 1.55	0.313	0.6514
4503681	Fc fragment of IgG binding protein	R.FAVLQENVAWGNGR.V	1561.7	2	11.88 ± 8.72	60.2 ± 1.43	0.135	
4503681	Fc fragment of IgG binding protein	R.GEVGFVLVDNQR.S	1333.5	2	5.20 ± 2.03	10.36 ± 9.88	0.238	
4503681	Fc fragment of IgG binding protein	R.ASQHGSDVVIETDFGLR.V	1832	2	2.07 ± 1.56	5.06 ± 5.95	0.260	
4503681	Fc fragment of IgG binding protein	R.VAYDLVYYVR.V	1261.5	2	6.82 ± 5.51	4.77 ± 2.41	0.424	
5031863	Galectin 3 binding protein	R.ELSEALGQIFDSQR.G	1593.7	2	164.59 ± 288.27	38.76 ± 76.93	0.327	0.003*
5031863	Galectin 3 binding protein	R.RIDITLSSVK.C	1132.3	2	7.53 ± 3.10	4.86 ± 2.55	0.133	
5031863	Galectin 3 binding protein	R.IYTSPTWSAFVTDSSWSAR.K	2163.3	2	7.55 ± 6.63	1.32 ± 0.79	0.045*	
5031863	Galectin 3 binding protein	R.SDLAVPSELALLK.A	1356.6	2	7.87 ± 6.51	4.39 ± 3.93	0.288	
5031863	Galectin 3 binding protein	K.LNNGEITQHR.K	1182.3	2	9.63 ± 13.64	0.69 ± 0.84	0.140	
45580723	Haptoglobin-related protein	K.SCAVAEYGVYVK.V	1346.5	2	7.47 ± 5.76	6.49 ± 7.12	0.800	0.2651
45580723	Haptoglobin-related protein	R.TEGDGVYTLNDK.K	1312.4	2	19.72 ± 25.01	5.22 ± 8.36	0.208	

4502027	Albumin	K.KVPQVSTPTLVEVSR.N	1346.5	2	14.69 ± 14.74	16.14 ± 20.45	0.891	0.0625
4502027	Albumin	R.FKDLGEENFK.A	1227.4	3	14.37 ± 14.62	1.73 ± 2.09	0.063	
4502027	Albumin	K.VFDEFKPLVEEPQNLIK.Q	1182.3	2	2.94 ± 3.17	1.64 ± 0.83	0.357	
11761633	Fibrinogen, gamma	R.YLQEIYNSNNQK.I	1514.6	2	5.88 ± 5.93	3.88 ± 1.82	0.516	
4502501	Complement component 4B	K.VLSLAQEQVGGSPEK.L	1542.7	2	3.46 ± 2.05	3.66 ± 6.19	0.953	
4826762	Haptoglobin	K.VTSIQDWVQK.T	1204.4	1	1.42 ± 1.62	0.41 ± 0.18	0.160	
4557321	Apolipoprotein A-I	R.EQLGPVTQEFWDNLEK.E	1934.1	2	2.55 ± 1.60	3.51 ± 2.00	0.384	
4507907	Von Willebrand factor	K.AVVILVTDVSVDSV DAAADAAR.S	2158.4	2	1.33 ± 0.76	1.80 ± 1.00	0.365	0.1137
4507907	Von Willebrand factor	R.NSMVLDVAFVLEGSDK.I	1725	2	1.23 ± 0.53	1.52 ± 1.24	0.644	
4507907	Von Willebrand factor	R.LLDLVFLLDGSSR.L	1448.7	2	9.71 ± 8.80	1.51 ± 0.55	0.069	
4507907	Von Willebrand factor	K.SEVEVDIHYCQGK.C	1564.7	2	3.10 ± 1.78	1.97 ± 2.01	0.329	
4507907	Von Willebrand factor	K.TVMIDVCTTCR.C	1356.5	2	6.81 ± 3.46	2.84 ± 1.74	0.031 *	

Supplementary Table 6: Quanitified ICAT-labeled peptides. Peptide ID number corresponds to those of Figure 3.

Peptide ID number	GI No.	Protein	Peptide	Z	Log2 of ratio	sd	P value
1	4502027	albumin	K.ALVLIAFAQYLQQCPFEDHVK.L	3	1.72	0.43	0.072
2	4502027	albumin	K.ADDKETCFAEEGKK.L	3	1.89	1.08	0.109
3	4502027	albumin	K.CCTESLVNR.R	2	1.26	0.54	0.082
4	4503631	coagulation factor XIII	R.CGPASVQAIK.H	2	-0.69	0.54	0.427
5	4503631	coagulation factor XIII	R.CLGIPAR.I	2	0.11	1.01	0.856
6	4503745	filamin 1	K.IECDDKGDGSCDVR.Y	3	-0.67	0.02	0.097
7	4503745	filamin 1	K.IVGPSGAAVPCK.V	2	-0.75	0.16	0.068
8	4503745	filamin 1	K.CSGPGLSPGMVR.A	2	-0.17	0.69	0.828
9	4503745	filamin 1	K.AHVVPCFDASK.V	2	-0.63	0.10	0.192
10	4503745	filamin 1	K.VGTECGNQK.V	2	-0.17	0.73	0.857
11	4503745	filamin 1	K.CAPGVVGPAEADIDFDIIR.N	2	-0.47	0.16	0.192
12	4503745	filamin 1	R.VTYCPTEPGNYIINIK.F	2	-0.47	0.92	0.685
13	4503745	filamin 1	R.VQVQDNEGCPVEALVK.D	2	-0.51	0.34	0.207
14	4503745	filamin 1	R.TPCEEILVK.H	2	-0.47	0.49	0.526
15	4504349	beta globin	R.LLGNVLVCVLAHHFGK.E	2	4.53	3.63	0.285
16	4504349	beta globin	K.GTFATLSELHCDK.L	2	7.58	1.80	**0.021**
17	4504745	integrin alpha 2b	K.TPVGSCFLAQPESGR.R	2	-1.22	0.40	0.097
18	4504745	integrin alpha 2b	R.AEGGQCPSLLFDLR.D	2	-0.76	0.14	**0.032**
19	4504973	lactate dehydrogenase C	R.VIGSGCNLDSAR.F	2	-0.40	0.15	0.362
20	4505733	platelet factor 4	K.AGPHCPTAQLIATLK.N	2	-1.51	1.68	0.340
21	4505879	pleckstrin	K.GSTLTSPCQDFGK.R	2	-0.43	0.12	0.452
22	4505879	pleckstrin	R.GCVVTSVESNSNGR.K	2	-0.13	0.76	0.809
23	4505981	pro-platelet basic protein	R.KICLDPDAPR.I	2	0.45	0.93	0.368
24	4505981	pro-platelet basic protein	K.GTHCNQVEVIATLK.D	2	0.37	0.25	0.112
25	4506413	RAP1A	K.CDLEDER.V	2	-0.54	0.65	0.431
26	4507295	syntaxin 7	K.ITQCSVEIQR.T	2	-0.68	0.35	0.299
27	4507357	transgelin 2	K.NMACVQR.T	2	0.12	0.18	0.284
28	4507357	transgelin 2	K.DGTVLCELINALYPEGQAPVKK.I	3	0.43	0.64	0.115
29	4507877	vinculin isoform VCL	R.ALIQCAK.D	2	-0.05	0.33	0.494
30	4507877	vinculin isoform VCL	K.VGELCAGK.E	2	-0.61	0.60	0.512
31	4507877	vinculin isoform VCL	R.TNLLQVCER.I	2	0.40	0.47	0.265
32	4507877	vinculin isoform VCL	R.KIAELCDDPK.E	2	2.92	1.34	0.210
33	4507907	von Willebrand factor	K.APTCGLCEVAR.L	2	5.49	2.78	0.076

34	4507907	von Willebrand factor	K.VEETCGCR.W	2	5.20	2.19	**0.042**
35	4507907	von Willebrand factor	K.CLAEGGK.I	2	4.60	1.59	**0.046**
36	4507907	von Willebrand factor	R.VTGCPPFDEHK.C	2	4.67	1.83	**0.050**
37	4507907	von Willebrand factor	K.SEVEVDIHYCQGK.C	2	2.61	1.14	0.069
38	4507907	von Willebrand factor	R.CLPSACEVVTGSPR.G	2	4.15	1.52	0.173
39	4507907	von Willebrand factor	R.TATLCPQSCEER.N	2	3.82	1.48	**0.043**
40	4507907	von Willebrand factor	R.SGFTYVLHEGECCGR.C	3	4.00	0.94	**0.021**
41	4507907	von Willebrand factor	R.ICMDEDGNEK.R	2	3.90	0.63	**0.011**
42	4508047	zyxin	R.CHQPLAR.A	2	-0.03	1.30	0.811
43	4557890	keratin 5	R.VSLAGACGVGGYGSR.S	2	-0.09	1.82	0.888
44	4757952	cell division cycle 42 isoform 1	K.YVECSALTQK.G	2	0.18	0.23	0.278
45	4758086	cysteine and glycine-rich protein 1	R.CSQAVYAAEK.V	2	0.58	0.66	0.384
46	5031601	actin related protein 2/3 complex subunit 1B	R.IVTCGTDR.N	2	0.84	0.93	0.389
47	5031635	cofilin 1	K.HELQANCYEEVKDR.C	3	0.51	0.49	0.107
48	5031635	cofilin 1	K.MLPDKDCR.Y	2	-0.36	0.29	0.687
49	5453595	adenylyl cyclase-associated protein	R.ALLVTASQCQQPAENK.L	2	-0.44	0.63	0.912
50	5453595	adenylyl cyclase-associated protein	K.INSITVDNCKK.L	2	1.04	2.35	0.521
51	7669492	glyceraldehyde-3-phosphate dehydrogenase	K.IISNASCTTNCLAPLAK.V	3	-0.19	0.33	0.615
52	10835049	ras homolog gene family, member A	K.LVIVGDGACGK.T	2	0.37	0.64	0.202
53	10863927	peptidylprolyl isomerase A	K.KITIADCGQLE.-	2	-0.26	0.57	0.952
54	11761631	fibrinogen, beta	R.TPCVSCNIPVVSGK.E	3	0.17	0.58	0.938
55	11761631	fibrinogen, beta	R.TPCTVSCNIPVVSGK.E	2	-0.18	0.62	0.504
56	11761633	fibrinogen, gamma	K.DCQDIANK.G	2	-0.67	0.98	0.597
57	12025678	actinin, alpha 4	R.ELPPDQAEYCIAR.M	2	0.05	0.08	0.329
58	12667788	myosin, heavy polypeptide 9, non-muscle	R.CQHLQAEK.K	2	-0.65	0.67	0.724
59	12667788	myosin, heavy polypeptide 9, non-muscle	R.EDQSILCTGESGAGK.T	2	-0.20	0.49	0.814
60	13518026	LIM and senescent cell antigen-like domains 1	R.CDLCQEVLADIGFVK.N	2	-0.38	0.12	0.921
61	13562114	beta tubulin 1, class VI	R.NTMAACDLR.R	2	-0.28	0.84	0.944
62	13562114	beta tubulin 1, class VI	R.EIVHIQIGQCGNQIGAK.F	3	-0.18	0.03	0.427
63	13562114	beta tubulin 1, class VI	R.YLTVACIFR.G	2	-0.59	0.47	0.482
64	13994151	PDZ and LIM domain 1	K.CGTGIVGVFVK.L	2	-0.12	0.64	0.834
65	16357472	cell division cycle 42 isoform 2	K.CVVVGDGAVGK.T	2	-0.46	0.31	0.257
66	16554592	enolase 3	K.VNQIGSVTESIQACK.L	2	0.13	0.37	0.304
67	16753233	talin 1	K.CTQDLGNSTK.A	2	-0.66	0.46	0.097
68	16753233	talin 1	K.AVAAGNSCR.Q	2	-0.04	0.16	0.202
69	16753233	talin 1	K.ELIECAR.R	2	0.90	1.25	0.256
70	16753233	talin 1	K.AQEACGPLEMD SALSVVQNLEK.D	3	-0.14	0.35	0.709

71	16753233	talin 1	R.MVAAATNNLCEAANAA VQGHASQEK.L	3	-0.05	0.54	0.386
72	16753233	talin 1	K.ACEFAGFQCQIQFGPH NEQK.H	3	0.70	0.79	0.388
73	16753233	talin 1	K.NCGQMSEIEAK.V	2	-0.16	0.72	0.914
74	16753233	talin 1	K.ASAGPQPLLVQSCK.A	2	0.93	0.95	0.211
75	17986258	smooth muscle and non-muscle myosin alkali light	K.ILYSQCGDVMR.A	2	0.21	1.01	0.570
76	18491010	protein tyrosine phosphatase, receptor type, B	R.CQGRTVPLAVLQLRVK.H	2	-0.15	0.94	0.903
77	21464101	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase	K.NCSETQYESK.V	2	0.13	0.46	0.446
78	24497453	nucleoporin 88kDa; nuclear pore complex protein	K.LLCDQK.K	2	-1.38	0.48	0.119
79	28626504	UNC-112 related protein 2	R.CQDEQQYAR.W	2	-0.26	0.11	0.073
80	28626504	UNC-112 related protein 2	K.GCEVVPDVNVSGQK.F	2	-0.65	0.44	0.071
81	29736622	RIKEN cDNA 4732495G21 gene	K.CDVDIR.K	2	-0.34	0.42	0.800
82	33286418	pyruvate kinase 3	R.NTGIICTIGPASR.S	2	-0.55	0.15	0.222
83	33624848	synaptic nuclei expressed gene 2	R.VEKTRPEPTEVLHACK.T	2	-0.42	0.24	0.871
84	34577110	aldolase A	R.ALANSLACQGK.Y	2	0.10	0.37	0.267
85	38044288	gelsolin isoform b	R.LFACSNK.I	2	0.79	0.87	0.237
86	40317626	thrombospondin 1	R.LCNNPTPQFGGK.D	2	-1.32	0.65	0.167
87	40317626	thrombospondin 1	R.SCDSLNNR.C	2	-1.78	0.28	**0.006**
88	40317626	thrombospondin 1	K.DCVGDVTENQICNK.Q	2	-1.42	0.57	**0.021**
89	41058276	Triosephosphate isomerase	R.IIYGGSVTGATCK.E	2	-0.39	0.75	0.653
90	47078292	integrin beta chain, beta 3	R.EGQPVCSQR.G	2	-0.82	0.38	0.100
91	47078292	integrin beta chain, beta 3	K.NEDDCVVR.F	2	-0.63	0.88	0.650
92	47078292	integrin beta chain, beta 3	R.YCRDEIESVK.E	2	-0.60	0.34	**0.024**
93	47078292	integrin beta chain, beta 3	K.DNCAPESIEFPVSEAR.V	2	-1.02	0.77	0.153
94	47078292	integrin beta chain, beta 3	K.CPTCPDACTFK.K	2	-2.60	1.56	0.089