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Subproteome analysis of the neutrophil cytoskeleton

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Abstract

Neutrophils play a key role in the early host-defense mechanisms due to their capacity to migrate into inflamed tissues and phagocytose microorganisms. The cytoskeleton has an essential role in these neutrophil functions, however, its composition is still poorly understood. We separately analyzed different cytoskeletal compartments: cytosolic skeleton, phagosome membrane skeleton, and plasma membrane skeleton. Using a proteomic approach, 138 nonredundant proteins were identified. Proteins not previously known to associate with the skeleton were: *n*-acetylglucosamine kinase, phosphoglycerate mutase 1, prohibitin, ficolin-1, phosphogluconate dehydrogenase, glucosidase, transketolase, major vault protein, valosin-containing protein, aldehyde dehydrogenase, and lung cancer-related protein-8 (LCRP8). The majority of these proteins can be classified as energy metabolism enzymes. Such a finding was interesting because neutrophil energy metabolism is unusual, mainly relying on glycolysis. The enrichment of phosphoglycerate mutase in cytosolic skeleton was additionally indicated by the use of Western blotting. This is the broadest subcellular investigation to date of the neutrophil cytoskeletal proteome and the first proteomic analysis in any cell type of the phagosome skeleton. The association of metabolic enzymes with cytoskeleton is suggestive of the importance of their localized enrichment and macromolecular organization in neutrophils.

Keywords

Cytoskeleton; Glycolysis; Neutrophil; Phagosome; Plasma membrane

1 Introduction

Neutrophils play a key role in early host-defense mechanisms against invading pathogens due to their capacity to penetrate the endothelium, migrate into inflamed tissues, and phagocytose microorganisms [1]. Actin in the cytoplasmic, membrane, and phagosome

skeleton is essential for all of these neutrophil functions [1, 2]. Despite its importance in early immune defense, the neutrophil cytoskeleton is still largely uncharacterized.

The cytoskeleton is a cellular “scaffolding” or “skeleton” and its dynamic structure maintains cell shape, enables cellular motion, intracellular transport, cellular division, phagocytosis, and membrane organization. Eukaryotic cells contain three main types of cytoskeletal filaments: microfilaments, intermediate filaments, and microtubules, composed mainly of actin, vimentin, and tubulin, respectively.

Phagocytosis is a process of elimination of foreign particles larger than 0.5 μm in diameter or host apoptotic cells, by phagocytic leukocytes [3]. It comprises multiple events: particle binding, actin assembly, membrane remodeling, pseudopod extension, and phagosome closure. This uptake mechanism is dependent on a complex rearrangement of the actin cytoskeleton. However, such nascent phagosomes lack the ability to kill pathogens or to degrade the ingested targets. These properties are acquired during the course of phagosomal maturation, a complex sequence of reactions that result in drastic remodeling of the phagosomal membrane and content. Actin assembly is essential for this process [4]. The molecules and mechanisms coordinating the various steps involved in phagolysosome biogenesis are still mostly unknown. A significant part of our limited understanding of this process originates from our incomplete knowledge of the protein composition of the phagosome. Furthermore, our knowledge is derived mainly from phagosome studies in macrophages [5, 6] and much less is known about neutrophils.

The plasma membrane skeleton is a specialized part of the cytoskeleton, situated in close proximity to the cell membrane with a protein composition and structure that differs from that of the bulk cytoskeleton in cytosol. Plasma membranes are organized into functional domains by attachment to cytoskeleton fibers and packing into “lipid rafts” [7]. Lipid rafts are to a variable extent connected to the underlying membrane skeleton [8]. Both cytoskeleton-stabilized membrane domains and lipid rafts are suggested to act as sites of cell signaling events [9]. Thus, it is of great interest to comprehensively study the protein composition of the plasma membrane skeleton.

The objective of the present study was to identify the constituents of the neutrophil cytoskeleton by proteomic profiling. To our knowledge, there have been no reports so far on any cell type regarding the proteomic analysis of the phagosome-associated skeleton. Because different subcellular compartments regulate distinctive neutrophil functions, we analyzed the components of cytosolic skeleton, phagosome membrane skeleton, and plasma membrane skeleton separately. Importantly, our profiling study resulted in the discovery of a series of proteins previously not known to associate with the cytoskeleton.

2 Materials and methods

2.1 Materials

Unless otherwise noted, all chemicals were purchased from Sigma Chemical (Poole, UK). IPG buffers and strips were purchased from GE Life Sciences (Chalfont, UK). Goat

polyclonal antibody to actin came from Santa Cruz Biotechnology (Santa Cruz, CA), mouse mAb to phosphoglycerate mutase was from Abcam (Cambridge, MA).

2.2 Isolation of human neutrophils

As described previously [10], human neutrophils were purified from buffy coats by sedimentation in 1% dextran (MP Biomedicals, Aurora, OH) for 45 min. The purity of the neutrophil fraction was analyzed by Giemsa staining, and was always higher than 90%.

2.3 Isolation of detergent-resistant phagosome

The experimental procedure is explained in detail by Segal et al. [11] and illustrated in Supporting Information Fig. S1. Briefly, latex beads (0.81 μm diameter) coated with human IgG were mixed with neutrophils at a beads/cell ratio of 100:1. Phagocytosis was allowed to proceed for 4 min and neutrophils were then disrupted by approximately 150 strokes in a glass potter homogenizer. This lysate was spun at $400\times g$ for 10 min in order to obtain the post-nuclear supernatant (PNS) to which 2.4 volume of 60% sucrose was added. The 33 and 11.2% sucrose was then layered over the PNS and centrifuged for 20 min at $25\,000\times g/4^{\circ}\text{C}$. Phagocytic vacuoles have floated up to the interface between the 33 and 11.2% sucrose. This fraction was collected and the sucrose concentration adjusted to 11.2%. Vacuoles were then centrifuged again for 20 min at $25\,000\times g, 4^{\circ}\text{C}$. This pellet was homogenized with a break buffer containing 1% Triton X-100 (TX-100) for 15 min in a Dounce homogenizer and pelleted again at $415\,000\times g$ for 30 min at 4°C in order to obtain the TX-100-insoluble phagosome fraction.

2.4 Isolation of cytoplasmic cytoskeleton

An overview of the purification procedure of different cell fractions is presented on Supporting Information Fig S1. Purified neutrophils (1×10^9) were disrupted by nitrogen cavitation in a break buffer (10 mM/KCl, 3 mM NaCl, 4 mM MgCl_2 , 10 mM PIPES, pH 7.2) containing protease inhibitors: leupeptin 10 $\mu\text{g}/\text{mL}$, pepstatin 10 $\mu\text{g}/\text{mL}$, tosyl-lysine-chloromethyl ketone 10 $\mu\text{g}/\text{mL}$, and 2 μM PMSF. PNS was prepared by centrifugation at $400\times g$ for 10 min at 4°C . The PNS was layered over a discontinuous gradient of 15, 34, and 55% sucrose w/w and centrifuged at $100\,000\times g$ for 20 min at 4°C . Under these conditions membranes settle at the interface of 15–34% sucrose while granules accumulate at the interface of 34–55% sucrose. Membrane fraction was used for the isolation of membrane skeleton explained below, while granules were discarded. The cytosol above 15% sucrose was recentrifuged at $420\,000\times g$ for 30 min in order to obtain the detergent-insoluble cytosol (Fig. 1A).

2.5 Isolation of detergent-resistant plasma membrane

Membranes from the 15 to 34% interface were centrifuged at $420\,000\times g$ for 30 min at 4°C (Fig. 1A). The pellet was extracted with 1% TX-100, 60 mM PIPES, 25 mM HEPES, 10 mM EGTA, and 2 mM MgCl_2 . After 15 min on ice, the suspension was layered over 6% sucrose containing 1% TX-100 and spun at $250\,000\times g$ for 30 min in order to obtain the TX-100-insoluble fraction of the membrane (Fig. 1A).

2.6 SDS-PAGE and immunoblotting

SDS-PAGE and immunoblotting were performed as described [12], except for visualization of immunoblots which was by ECL plus (GE Life Sciences). Densitometry was carried out in Photoshop CS2 software.

2.7 2-DE

2-DE was performed as previously described in detail [13]. Briefly, proteins from all fractions were solubilized in IEF buffer (7 M urea, 2 M thiourea, 65 mM DTT, 2% ASB-14, 4% CHAPS) and separated according to their *pI* using immobilized pH-gradient strips (pH 3–10), 7–13 cm in length (GE Life Sciences). The proteins were then additionally separated by SDS-PAGE and visualized by silver staining.

2.8 MS and database search

Tryptic digests (0.7 μ L) were applied to the target plate and allowed to air-dry, then 0.7 μ L of saturated solution of CHCA in 33% ACN and 0.1% TFA v/v was overlaid. Mass spectra of the tryptic digests were obtained on an Autoflex MALDI-TOF MS (Bruker Daltonics, Billerica, MA), calibrated with a mixture of seven standard peptides. Monoisotopic peptide masses were assigned and the mass lists were used for protein identification in the NCBI nonredundant (nr) protein database by MASCOTsearch algorithm (Matrix Science, London, UK). Additional criteria were considered in order to positively identify a protein from its tryptic peptide mass finger-print. Most important was the coverage of the full-length protein and number of matched peptides. When the coverage exceeded 20%, it was considered to be sufficient unless there were some obvious conflicts such as discrepancies between the experimental molecular weight and that of the identified protein. Between 15 and 20% of coverage, the identification was validated only if confirmed by three different search engines: MASCOT, PepIdent and MS-Fit. The minimal requirement for the number of peptides matched was five. The mass accuracy of our analysis was usually set at 100 ppm. Molecular function and subcellular localization of identified proteins was annotated by use of ProteinCenter database tool (www.proxeon.com).

3 Results and discussion

The aim of this study was to identify proteins associating with cytoskeleton. Analysis of the cytoskeleton proteome was carried out in different cellular compartments: cytosolic skeleton, phagosome membrane skeleton, and plasma membrane skeleton. Subproteome cytoskeleton analysis was done in order to reduce the complexity of the sample and obtain an insight into the distribution of cytoskeletal and cytoskeleton-associated proteins. Performing the cytoskeleton isolation on already highly purified subcellular compartments also helped to reduce the contamination by unrelated cellular proteins. Fractions were prepared as described in Section 2 and illustrated in Supporting Information Fig. S1. Proteins were resolved by 2-DE and the resulting patterns are shown in Supporting Information Fig. S2. Alternatively, samples were analyzed only by 1-D SDS-PAGE (not shown) in order to identify large and/or hydrophobic proteins (Table 1) as these cannot be resolved by 2-DE. Overall, 138 nonredundant proteins were identified (Table 1 and Supporting Information Fig. S2, A–C) of which 72 from the detergent-insoluble cytosol, 39

from the detergent-insoluble plasma membrane, and 27 associated with the detergent-insoluble phagosome. Only proteins identified in at least two out of three experiments were listed (Table 1). A majority of the 138 identified proteins are known to associate with cytoskeleton, thus confirming the validity of our cytoskeleton isolation procedures. It has been widely documented and accepted that cytoskeleton-associated proteins constitute a major part of detergent-insoluble pellets, as interactions between cytoskeletal proteins provide resistance against disruption by nonionic detergents [14]. In order to further validate the enrichment of cytoskeletal proteins in our preparations, we examined the enrichment of β -actin, the major cytoskeletal protein, in all three of our cytoskeletal fractions, matched for protein loading (Fig. 1A). On the basis of densitometry measurements, β -actin was enriched 17 ± 3 -fold in detergent-insoluble plasma membrane, 13.1 ± 2 -fold in detergent-insoluble cytosol, and 12.9 ± 3 -fold in detergent-insoluble phagosome, all in comparison to the total cellular extract, which is already known to contain a high amount of cytoskeletal proteins (Fig. 1A). Furthermore, analysis by use of ProteinCenter, a bioinformatics tool, revealed the enrichment of cytoskeletal proteins in all three preparations (Supporting Information Fig. S3). Namely, the content of cytoskeletal proteins varied between 32 and 38% which by far exceeded the percentage observed in total cellular lysates averaging 12% (unpublished observation). As expected, membrane proteins were found to be enriched in the plasma membrane skeleton fraction, while cytoplasmic and catalytic activity proteins were enriched in the cytoplasmic skeleton (Supporting Information Fig. S3). This analysis also indicated a notable enrichment of proteins participating in cell communication, transporter activity, signal transducer, and cell death proteins in detergent-insoluble phagosome in comparison to the other two fractions (Supporting Information Fig. S3).

Eleven out of the 138 identified proteins were to our knowledge not previously known to associate with cytoskeleton: *n*-acetylglucosamine kinase, phosphoglycerate mutase 1 (PGM), prohibitin, ficolin, phosphogluconate dehydrogenase, glucosidase, transketolase, major vault protein, valosin-containing protein, aldehyde dehydrogenase, and lung cancer-related protein-8 (LCRP8) [3, 8, 15, 16]. These proteins were mostly (8/11) identified in detergent-resistant cytosol (Table 1).

3.1 Detergent-insoluble phagosome fraction

Unlike most approaches relying on isolation of organelles based on their intrinsic density, isolation of latex bead-containing phagosomes is facilitated by the low buoyant density of the phagocytized latex (Supporting Information Fig. S1B). Thus, phagosomes are floated in the region of the sucrose gradient where other cellular organelles are not detected. Previous morphological and biochemical analysis of latex bead phagosome preparations indicated the virtual absence of contamination by mitochondria, Golgi vesicles, endosomes, and plasma membrane [17]. The function of phagosome skeleton proteins such as actin and tubulin is to give structure to a phagosome and to facilitate fusion between phagosomes and late endosomes [18]. The cytoskeleton networks around membranes are TX-100 insoluble and copellet with their tightly associated integral membrane proteins, forming assemblies called “membrane skeletons” [19]. In this study, the design of the sucrose gradient (Supporting Information Fig. S1) was tailored for the isolation of recently described high-density detergent-resistant membranes (DRM-H). Its increased density is due to the association of

membrane detergent-insoluble lipid rafts with the underlying membrane cytoskeleton [8]. Therefore, the coisolation of lipid rafts and membrane skeleton is not a contamination problem as it reflects the structural connection of these two compartments *in vivo* where membrane cytoskeleton fibers are attached to membrane lipid rafts [8, 20]. Detection of flotilin, the marker protein of lipid rafts, in the detergent-insoluble phagosome and plasma membrane fractions was consistent with the recent report demonstrating the presence of lipid rafts on phagosomes [21]. This result also confirms that our preparation mainly consisted of DRM-H, as flotilin is predominantly present in DRM-H fraction [20].

The first comprehensive proteomic analysis of the total human neutrophil phagosome proteome resulted in identification of 198 proteins [22]. Our study is the first analysis of the detergent-insoluble phagosome fraction, in any cell type. It identified fewer proteins because this fraction represents only a part of the total phagosome proteome. As expected, the representation of cytoskeletal proteins in our detergent-insoluble phagosome (48%) was much higher than in the previous [22] whole-phagosome preparation (15%). Burlak *et al.* [22] found all of the proteins identified here, except for: valosin-containing protein, p47phox, EF hand domain containing 2, microtubule associated protein-6, and VDAC1. In addition to these proteins, another proteomic study of phagosomes performed by Garin *et al.* [6] also did not identify: filamin, prohibitin, grancalcin, Cap Z alpha, l-plastin, heat shock protein 70, myosin, vimentin, flotilin, and IQGAP1. Such discrepancy may be due to our earlier time point of phagocytosis (4 min), compared to the study of Burlak *et al.* (30 min) or Garin *et al.* (120 min). Therefore, the above proteins may have dissociated from the phagosome skeleton during its maturation. Also, different results of Garin *et al.*, analyzing phagosomes from a mouse macrophage cell line, may be attributed to species and cell-type differences. Furthermore, it is plausible that the above proteins may be enriched in the detergent-insoluble phagosome fraction that was analyzed here, as opposed to the whole phagosome in both previous studies mentioned.

Nine of the proteins identified here in the phagosome skeleton fraction were not found in the previous study of the azurophil granule DRMs [23], presumably due to the fact that the origin of the phagosome membrane is different in comparison to the granular membrane. The early time point, when granule content is not yet delivered to phagosomes, is also the most plausible explanation for the fact that our detergent-insoluble phagosome preparation did not contain any of the typical granular proteins [24]. Phagosome proteome in macrophages has been studied more extensively than in neutrophils [6, 25]. Proteomes of the neutrophil and macrophage phagosomes mainly overlap, with the exception of proteins moderating the effects of ROS such as catalase, peroxiredoxins, and GST. These proteins are associated with neutrophil [22], but not macrophage phagosomes [6]. Also, a greater number of microbicidal proteins are found in neutrophil phagosomes. Overall, these findings reflect the known enhanced capacity of PMNs to kill bacteria and fungi in comparison to macrophages. Our study did not identify any of the microbicidal proteins or antioxidant enzymes, as these are detergent-soluble and mainly localized within the lumen of the phagosome.

Three of the proteins identified in the detergent-insoluble phagosome have not been previously reported to associate with cytoskeleton: prohibitin, ficolin, and valosin-

containing protein. Ficolins are lectins with collagen- and fibrinogen-like domains that function as opsonins by binding to the *n*-acetylglucosamine moiety found on bacterial cell walls, thereby promoting phagocytosis and activating the lectin pathway of complement. Human M-ficolin does not contain transmembrane domains but is present on the cell surface and is indirectly implicated as phagocytic receptor in phagocytosis of *E. coli* [26]. The presence of ficolin on the plasma membrane can explain its occurrence on phagosomes, as it is known that plasma membrane is the major source of membrane for phagosome formation [27].

Valosin-containing protein is required for the degradation of ubiquitinated-proteasome substrates and it copurifies with the 26S proteasome. In phagosomes, it may be involved in antigen presentation due to its possible role in the process of degradation [28] of ingested foreign particles. Additionally, it may be involved in phagosome maturation due to its known function as a chaperone in membrane fusion [29].

3.2 Cytosolic skeleton fraction

T complex protein-1 (TCP1) is a cytosolic molecular chaperone implicated in the biogenesis of cytoskeletal proteins by promoting the correct folding of the actin and tubulin [30]. This protein has been previously demonstrated to associate with erythrocyte actin fibers only following their exposure to elevated temperature [31]. Interestingly, in neutrophils, as shown here, TCP1 is associated with the cytoskeleton even without the prior heat shock, thus indicating a different role of this protein in neutrophils. Our results also indicate the association of the valosin-containing protein and major vault protein with cytoskeleton for the first time, thus supporting the previous observation of the colocalization of the major vault protein with filamentous actin in neurites [32].

The majority of the proteins that were not previously implicated to associate with the skeleton were metabolic enzymes (Table 1A): phosphoglycerate mutase (glycolysis), transketolase, phosphogluconate dehydrogenase (pentose phosphate pathway), *n*-acetylglucosamine kinase (glutamate and aminosugar metabolism), glucosidase (glycoprotein processing), and aldehyde dehydrogenase (oxidation of aldehydes). Several metabolic enzymes identified here have already been known to associate with actin fibers: glutamate dehydrogenase [33], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [34], pyruvate kinase [35], and enolase [36].

The enrichment of PGM in the detergent-insoluble cytosol was additionally indicated by Western blotting (Fig. 1). PGM was absent in the membrane and phagosome detergent-insoluble fractions (Fig. 1B), which is in agreement with the absence of any protein spots in the PGM location in these fractions (Supporting Information Fig. S2). A 2.8 ± 0.6 -fold enrichment of PGM in the detergent-insoluble cytosol in comparison to detergent soluble cytosol was surprising, because the previous study of epithelial cells did not indicate any enrichment of PGM in the cytoskeletal fraction [34]. This discrepancy may be due to the different cell type, or the fact that binding of PGM to cytoskeletal fibers in neutrophils is not constitutive. Consistent with this hypothesis is the earlier finding of the progressive association of GAPDH with cytoskeletal fibers during the process of epithelial morphogenesis [34]. In the same way, the observed association of PGM with cytoskeleton in

neutrophils may have been due to hereby employed experimental design which involved a phagocytic stimulus. Our result is consistent with the previous report indicating that glycolytic enzymes enolase, aldolase, creatine kinase, pyruvate kinase, and PGM form a complex in rabbit muscle [37]. Thereby, association of PGM with the cytoskeleton may be mediated through other members of this enzyme multiprotein complex such as pyruvate kinase and enolase, whose ability to associate with cytoskeleton is well known [35, 36]. PGM is an enzyme involved in glycolysis and is found in the cytoplasm of cells in all living organisms. Glycolysis is much less energy efficient than the oxidative metabolism occurring in mitochondria, however, unlike most other types of cells, neutrophils mainly rely on glycolysis for their energy needs [38]. Following phagocytosis, neutrophils kill the ingested microorganisms by use of oxidative burst, which is mediated by the NADPH oxidase. During phagocytosis, the glycolysis pathway increasingly branches into the pentose phosphate pathway in order to generate NADPH for the NADPH oxidase, thus increasing the oxidative and microbicidal response [39]. This switch from glycolysis to the pentose phosphate pathway is achieved by the p21-activated kinase (PAK)-mediated phosphorylation of PGM [38, 40]. Whether the association of PGM with the cytosolic skeleton is constitutive in neutrophils or is induced by phosphorylation, remains to be investigated. It has been previously indicated that associations of glycolytic enzymes with the cytoskeleton are of relatively low affinities and transient [41]. This is in agreement with our finding of PGM both within the detergent-soluble and -insoluble fractions, which indicated an equilibrium of free- and cytoskeleton-bound PGM (Fig. 1B). The binding of glycolytic enzymes to cytoskeleton has been suggested to regulate metabolism through changes in enzymatic activity and facilitation of localized enrichment of the enzymes [41].

3.3 Detergent-insoluble plasma membrane fraction

This fraction was prepared according to the standard procedure [8]. Of the proteins that were not previously reported to associate with cytoskeleton, this fraction contained ficolin, valosin-containing protein, and major vault protein, all discussed above, and LCRP8, the novel protein of unknown function (Table 1C). Twenty-one of the proteins identified in this fraction were not found in the previous major study of the corresponding DRM fraction in human HL-60 [42]. This was probably due to the use of different cells and the lack of phagocytic stimulus in the previous study [42].

4 Concluding remarks

We examined the composition of the cytoskeleton at three sites in neutrophils: cytosol, plasma membrane, and phagosome. A number of proteins were identified, mostly metabolic enzymes, which have not been reported to associate with the cytoskeleton. In addition, the enrichment of PGM in the cytosolic skeleton was demonstrated by use of Western blotting. This study provides a unique comprehensive inventory of neutrophil cytoskeleton proteome and associated lipid rafts. Further investigations are necessary to elucidate the precise role of all proteins that were here for the first time implicated to associate with cytoskeleton.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DRM	detergent-resistant membranes
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
PGM	phosphoglycerate mutase 1
PNS	post-nuclear supernatant
TCP1	Tcomplex protein-1
TX-100	Triton X-100

5 References

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Figure 1.

Western blotting analysis of β -actin and PGM enrichment among the isolated subcellular fractions. (A) β -Actin; (B) PGM. The lanes for (A): 1, total cellular extract; 2, detergent-insoluble plasma membrane; 3, detergent-insoluble cytosol; 4, detergent-insoluble phagosome. The lanes for (B): 1, detergent-soluble cytosol; 2, detergent-insoluble plasma membrane; 3, detergent-insoluble cytosol; 4, detergent-insoluble phagosome. Twenty micrograms of protein was run in each lane for (A) and 50 μ g for (B). Results are representative of three preparations.

Table 1

Proteins identified in neutrophil cytoskeletal fractions^{a)}

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
(A) Cytosol high-speed pellet													
Actin and associated proteins													
14-3-3 β	YWHA ^B	gi 4507949	3404.3	28 179	30 000	4.76	4.55	12	11	43	135	22	N/A
14-3-3 ε	YWHA ^Z	gi 4507953	3406.2	27 899	30 000	4.73	4.6	11	48	44	118	23	12551948
Actin β	ACTB1.	gi 14250401	N/A	41 321	43 000	5.56	5.9	7	90	25	82	15	12663865
Actin γ	ACTG1	gi 4501887	1614.2	42 108	43 000	5.31	5.4	7	45	23	96	13	12663865
Actin γ	ACTG1	gi 4501887	1614.2	42 108	43 000	5.31	5.3	8	21	26	74	12	12663865
Actin γ	ACTG1	gi 4501887	1614.2	42 108	43 000	5.31	5.2	11	26	33	124	11	12663865
Actin γ	ACTG1	gi 4501887	1614.2	42 108	43 000	5.31	5.1	11	21	33	91	10	12663865
α-Actinin	ACTN1	gi 4639781	N/A	103 480	100 000	5.22	7.1	16	26	20	170	123	2351691
Annexin I	ANXA1	gi 4502101	700.1	38 918	38 000	6.57	6.35	10	27	40	117	88	9187254
Annexin III	ANXA3	gi 4826643	5139.1	36 524	36 000	5.63	5.8	12	23	42	132	85	15260827
ARP1	ACTR1A	gi 5031569	5736.2	42 701	48 000	6.19	6.5	7	13	21	78	77	15157458
ARP 2	ACTR2	gi 5131571	5722.1	45 030	45 000	6.3	6	6	38	20	94	75	10322212
ARP 2/3 (subunit 2)	ARPC2	gi 5031599	5731	34 430	34 000	6.84	6	5	30	21	100	55	10322212
ARP 3	ACTR3	gi 5031573	5721.3	47 797	52 000	5.61	5.8	17	21	30	136	8	10322212
Cofilin 1	CFL1	gi 5031635	5507.2	18 719	21 000	8.22	9	5	6	40	76	40	14598374
Coronin 1A	CORO1A	gi 5902134	7074.1	51 678	67 000	6.25	6.4	18	18	38	185	111	10461187
eEF 1 g	EEFIG	gi 4503481	1404.3	50 429	52 000	6.25	6.6	13	17	27	150	72	N/A
Filamin	FLN	gi 1203969	N/A	283 460	250 000	5.7	N/A	39	29	18	175	+1-D	7929100
Gelsolin	GSN	gi 4504165	177.3	86 043	87 000	5.9	5.9	13	17	17	141	4	12663865
Gelsolin	GSN	gi 4504165	177.3	86 043	87 000	5.9	5.9	13	21	17	79	120	12663865
Grancalcin	GCA	gi 6912388	12198.2	24 223	30 000	5.02	5	10	117	34	80	32	12529388
L-Plastin	LCP1	gi 4504965	2298	70 814	72 000	5.29	5.7	25	17	40	273	118	12525560
L-Plastin	LCP1	gi 4504965	2298	70 814	72 000	5.29	5.6	32	19	54	394	117	12525560
Moessin	MSN	gi 4505257	2444.1	67 892	85 000	6.08	5.1	26	26	43	223	3	15147559
Profilin 1	1AW1-A	gi 3891601	N/A	15 014	16 000	8.46	8.55	12	23	75	157	46	9360613
S100 protein, A8	S100A8	gi 21614544	2964.3	10 870	12 000	6.5	7.4	5	12	44	78	110	9920411

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
S100 protein, A8	S100A8	gi 21614544	2964.3	10 870	12 000	6.5	7.7	8	18	57	125	48	9920411
S100 protein, A8	S100A8	gi 21614544	2964.3	10 870	12 000	6.5	7	6	16	46	109	47	9920411
S100 protein, A9	S100A9	gi 4506773	N/A	13 280	16 000	5.7	6	6	9	53	83	44	9920411
S100 protein, A9	S100A9	gi 4506773	N/A	13 280	18 000	5.7	5.8	8	47	78	111	106	9920411
Cytoskeletal tropomyosin	N/A	gi 37424	N/A	28 185	28 000	4.81	4.8	6	57	26	81	107	14722123
Vinculin	VCL	gi 24657579	N/A	117 234	110 000	5.83	7.1	26	29	28	244	148	14702644
Tubulin and associated proteins													
Bip protein	HSPA5	gi 6470150	N/A	71 002	70 000	5.23	5.45	12	41	25	129	5	8797085
Chaperonin containing TCP1, subunit 6A	CCT6A	gi 4502643	1762	58 444	60 000	6.23	5.45	8	43	18	120	51	11532003
Chaperonin containing TCP1, subunit 2	CCT2	gi 5453603	6431	58 444	60 000	6.01	6.5	24	19	49	278	57	11532003
Heat shock cognate protein	HSPA8	gi 24234686	153201.1	53 598	70 000	5.62	5.2	14	21	33	154	50	8404847
Heat shock protein 90	HSPCA	gi 123678	N/A	85 020	92 000	4.94	5.33	14	17	22	111	122	12522269
T complex protein 1	CCT1	gi 14755380	30752	60 819	60 000	5.8	6.85	13	22	23	109	58	10079067
β-Tubulin	TUBB	gi 18088719	N/A	50 096	52 000	4.75	5	22	16	43	128	7	12700769
Intermediate filaments and associated proteins													
Vimentin	VIM	gi 4507895	3380.1	53 710	50 000	5.06	5.1	24	23	55	254	121	14676269
Molecular motors and associated proteins													
Myosin light chain alkali	MLY6	gi 17986258	21019.2	17 090	16 000	4.56	4.2	9	27	52	101	42	2304459
Myosin light chain alkali	MLY6	gi 17986258	21019.2	17 090	16 000	4.56	4.2	10	12	58	139	43	2304460
Myosin, heavy polypeptide 9	MYH9	gi 12667788	2473.2	227 646	220 000	5.5	5	23	24	14	141	+1-D	14706930
Myosin regulatory light chain	MRCL3	gi 5453740	6471.1	19 500	19 000	4.67	4.5	6	5	35	94	41	3839239
Energy and metabolic enzymes													
Adenylyl cyclase-associated protein	CAP1	gi 5453595	6367	51 926	62 000	8.07	8	12	13	30	107	66	10391948
Aldolase A	1ALD	gi 229674	N/A	39 720	39 000	8.39	8.55	7	53	23	70	19	9244396
Aldolase A	1ALD	gi 229674	N/A	39 720	39 000	8.39	8.55	12	7	29	117	83	9244396
Catalase	CAT	gi 4557014	1752.1	59 947	60 000	6.9	7.1	21	24	42	212	6	15206463
Catalase	CAT	gi 4557014	1752.1	59 947	60 000	6.9	7.2	12	23	29	140	59	15206463
Catalase	CAT	gi 4557014	1752.1	59 947	60 000	6.9	7.5	9	14	24	114	61	15206463
Catalase	CAT	gi 4557014	1752.1	59 947	60 000	6.9	7	11	75	26	66	69	15206463
Enolase 1	EN01	gi 4503571	1428.2	47 481	49 000	7.01	7.1	16	18	43	163	9	2553125
Enolase	EN01	gi 4503571	1428.2	47 481	49 000	7.01	7.1	17	20	42	171	73	2553125

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
GAPDH	GAPD	gi 7669492	2046.2	36 201	35 000	8.57	8.8	14	24	46	141	20	9568479
GAPDH	GAPD	gi 7669492	2046.2	36 201	35 000	8.57	8.9	16	19	51	143	21	9568479
Glucosidase II ^{f)}	KIAA0088	gi 2274968	N/A	107 289	102 000	5.71	6.66	17	37	22	165	+1-D	15234963
Leukotriene A4 hydrolase	LTA4H	gi 4505029	895.1	69 868	70 000	5.8	5	18	12	32	178	49	11175901
N-acetylglucosamine kinase ^{f)}	NAGK	gi 24638065	N/A	37 694	38 000	5.81	5.9	9	22	29	78	86	10824116
Phosphoglycerate mutase ^{f)}	PGAM1	gi 4505753	2629.2	28 900	30 000	6.67	6.9	5	40	28	64	96	2553125
Phosphogluconate dehydrogenase ^{f)}	PGD	gi 12653201	N/A	53 619	51 000	6.8	6.4	18	22	43	150	71	15128828
Procollagen-proline, 2-oxoglutarate 4-dioxygenase	P4HB	gi 20070125	918.2	57 480	60 000	4.76	4.6	19	15	35	215	52	14985345
Protein disulfide-isomerase	N/A	gi 7437388	N/A	57 160	50 000	5.98	6.9	14	13	24	139	73	9826346
Pyruvate kinase	N/A	gi 478822	N/A	58 470	60 000	7.96	8.2	16	19	30	146	65	2553125
Transketolase ^{f)}	tk	gi 388891	N/A	68 528	68 000	7.89	7.8	8	23	16	84	60	7679932
Vacuolar ATP synthase catalytic subunit A	ATP6V1A	gi 22096378	N/A	68 677	70 000	5.35	5.2	15	25	28	136	123	12598655
Signal transduction molecules													
IQGAP ^{f)}	IQGAP1	gi 4506787	3870.2	189 761	180 000	6.08	N/A	24	63	18	107	+1-D	8798539
Rab GDP-dissociation inhibitor, beta	GDI2	gi 6598323	1494.2	51 087	55 000	6.11	8.3	14	44	32	96	64	10512627
Rac-Rho GDI complex, chain B	ARHGDIB	gi 1835002	1175.1	23 031	27 000	6.1	5	6	25	25	60	33	11513579
Proteins involved in ubiquitin-proteasome degradation													
Major vault protein ^{f)}	MVP	gi 19913410	17458.2	99 551	105 000	5.34	5.3	13	28	17	98	119	9331335
Proteasome, a1	PSMA1	gi 4506179	2786.2	29 822	31 000	6.15	6.4	9	11	34	77	26	12424373
Proteasome, a1	PSMA1	gi 4506179	2786.2	29 822	31 000	6.15	6.2	6	10	42	85	25	12424373
Proteasome, a1	PSMA1	gi 4506179	2786.2	29 822	31 000	6.15	6.6	9	11	34	79	27	12424373
Proteasome, a2	PSMA2	gi 4506181	2787.3	25 996	27 000	6.92	7	9	19	47	97	36	12424373
Proteasome, a3	PSMA3	gi 296736	152132.1	25 078	29 000	5.58	5.1	7	40	31	102	24	12424373
Proteasome, a4	PSMA4	gi 4506185	2789.3	29 750	30 000	7.57	7.1	5	22	21	69	97	12424373
Proteasome, a4	PSMA4	gi 4506185	2789.3	29 750	30 000	7.57	7.6	7	5	21	87	28	12424373
Proteasome, a6	PSMA6	gi 8394076	17283.2	27 840	30 000	6.3	6.5	10	76	39	130	29	12424373
Proteasome, a7	PSMA7	gi 4092058	2792.2	28 057	28 500	8.6	8.9	11	23	52	145	31	12424373
Proteasome, b1	PSMB1	gi 12653473	N/A	26 674	24 000	8.27	8.3	14	25	50	123	39	12424373
Proteasome, b2	PSMB2	gi 4506195	2794.3	22 993	24 000	6.51	6.8	8	14	38	144	37	12424373

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
Proteasome, b2	PSMB2	gi 4506195	2794.3	22 993	24 000	6.51	7.2	8	52	38	134	38	12424373
Proteasome, b3	PSMB3	gi 22538465	2795.2	23 219	25 000	6.14	6.25	9	8	42	93	34	12424373
Proteasome, b4	PSMB4	gi 631345	2796	25 950	25 000	5.7	6.4	5	44	25	64	124	12424373
Proteasome activator 1	PSME1	gi 5453990	6263.2	28 880	28 000	5.8	6.3	11	87	44	68	35	12424373
Proteasome LMP2.s	LMP-2	gi 2118154	2800	22 420	22 000	4.9	4.6	6	32	33	70	59	12424373
Valosin containing protein ^{f)}	VCP	gi 6005942	6005942	89 950	90 000	5.14	5.1	14	28	21	133	3	10364224
Others													
Chain A of constant regulatory domain of human Pp2a	N/A	gi 4558258	N/A	65 934	60 000	5	5.7	12	49	18	117	53	9989501
Cyclophilin A complexed with Dipeptide Gly-Pro	5CYH-A	gi 1633054	N/A	18 098	18 000	7.82	8	5	41	43	93	108	8652512
aldehyde dehydrogenase ^{f)}	N/A	gi 23503239	153329.2	86 086	90 000	6.35	6.8	15	15	20	178	114	15863807
PTK 9L protein tyrosine kinase 9-like	PTK9L	gi 6005846	7284.3	39 751	41 000	6.37	6.7	8	19	33	85	79	10406962
(B) Detergent-resistant plasma membrane													
Actin and associated proteins													
Actin β	ACTB1	gi 14250401	N/A	41 321	43 000	5.56	5.8	13	45	33	110	75	12663865
Actin β	ACTB1	gi 14250401	N/A	41 321	43 000	5.56	4.8	6	48	20	81	73	12663865
Actin β	ACTB1	gi 14250401	N/A	41 321	43 000	5.56	4.5	7	60	22	89	72	12663865
Actin β	ACTB1	gi 14250401	N/A	41 321	43 000	5.56	4.3	7	6	21	89	71	12663865
ARP 3	ACTR3	gi 5031573	5721.3	47 797	52 000	5.61	5.8	7	24	19	64	63	10322212
ARP 3	ACTR3	gi 5031573	5721.3	47 797	52 000	5.61	5.8	13	32	33	139	62	10322213
β-Contractin	HSBCENTR	gi 563888	N/A	37 187	45 000	6.19	6.3	5	24	17	77	76	15157458
Cap Z, a1	CAPZA1	gi 5453597	6135.1	33 073	38 000	5.45	5.2	12	61	57	131	82	12660160
Cap Z, a1	CAPZA1	gi 5453597	6135.1	33 073	38 000	5.45	5.5	9	15	40	85	83	12660161
Cap Z, a1	CAPZA1	gi 5453597	6135.1	33 073	32 000	5.45	5.3	9	15	40	85	86	12660162
Cap Z, a2	CAPZA2	gi 5453599	6136.1	33 157	37 000	5.57	6.2	7	34	38	88	85	8120062
Coronin 1A	COR01A	gi 5902134	7074.1	51 678	67 000	6.25	7.9	17	22	29	119	54	10461187
Coronin like protein	HCOR01	gi 1002923	N/A	51 722	60 000	6.12	7.1	12	29	22	136	53	8380174
Gelsolin	GSN	gi 4504165	177.3	86 043	87 000	5.9	5.8	12	36	15	78	39	12663865
Gelsolin	GSN	gi 4504165	177.3	86 043	87 000	5.9	5.8	13	17	17	128	40	12663866
Grancalcin	GCA	gi 6912388	12198.2	24 223	27 000	5.02	N/A	N/A	N/A	N/A	N/A	+WB	12529388
Laminin-binding protein	HSLAMBR	gi 34234	N/A	31 888	47 000	4.84	3.8	12	18	52	82	70	12950264

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
Moesin	MSN	gi 4505257	2444.2	67 892	85 000	6.08	6.8	11	38	20	133	42	15147559
Moesin	MSN	gi 4505257	2444.2	67 892	85 000	6.08	7.1	15	37	28	192	43	15147561
Villin 2	VIL2	gi 21614499	3379.3	69 484	90 000	5.94	6.8	14	49	21	85	41	1563281
Tubulin and associated proteins													
Heat shock 70kDa protein 8 isoform 1	HSPA8	gi 5729877	6597.3	71 082	80 000	5.37	4.8	14	44	25	154	44	14612456
Heat shock 70kDa protein 8 isoform 1	HSPA8	gi 5729878	6597.3	71 082	80 000	5.37	5	12	9	21	105	46	14612457
Intermediate filaments and associated proteins													
Keratin 10, cytoskeletal	KRT10	gi 71528	N/A	59 740	55 000	5.2	4.2	10	21	15	95	60	15274135
Keratin 10, cytoskeletal	KRT10	gi 18588130	49972.4	59 740	59 000	5.2	6.4	11	22	16	77	51	15274136
Vimentin	VIM	gi 2119204	N/A	53 676	55 000	5.06	4.1	23	25	47	223	58	14676269
vimentin	VIM	gi 21757045	N/A	52 463	52 000	4.99	4	12	29	28	138	59	14676269
Vimentin	VIM	gi 2119204	N/A	53 676	60 000	5.06	4.6	30	18	68	215	48	14676270
Energy and metabolic enzymes													
Carbonic anhydrase IV	N/A	gi 2554743	N/A	30 532	38 000	6.4	7.8	12	33	43	116	89	9054574
GAPDH	GAPD	gi 31645	N/A	36 201	35 000	8.57	9	6	43	19	76	90	9568479
GAPDH	GAPD	gi 31646	N/A	36 201	35 000	8.57	9.3	9	43	30	80	91	9568480
Glutamate dehydrogenase	glud1	gi 20151189	N/A	56 315	60 000	6.71	7.1	8	11	17	87	55	12125925
Glutamate dehydrogenase	glud1	gi 20151189	N/A	56 315	60 000	6.71	7.3	8	12	18	82	56	12125926
Protein disulfide-isomerase	N/A	gi 860986	N/A	57 160	60 000	5.98	5.8	8	22	15	78	49	9826346
Protein disulfide-isomerase	N/A	gi 860986	N/A	57 160	60 000	5.98	6	10	37	20	80	50	9826347
Protein disulfide-isomerase	N/A	gi 860986	N/A	57 160	61 000	5.98	6.3	10	37	20	80	52	9826348
Signal transduction molecules													
Ficolin 1 ^{f)}	FCN1	gi 1510127	2003.2	34 830	35 000	6.7	6.9	12	62	28	117	88	8947836
Flotillin	FLOT	gi 5114049	N/A	47 531	52 000	7.04	7.9	24	12	44	150	68	15128873
Flotillin 1	FLOT1	gi 5031699	5803.2	47 554	52 000	7.08	8	25	29	51	206	69	15128874
G protein, alpha inhibiting unit	N/A	gi 45044041	2070	40 995	42 000	5.34	4.6	13	16	43	136	78	15196565
G protein, alpha inhibiting unit	N/A	gi 45044041	2070	40 995	42 000	5.34	5.3	13	16	43	136	80	15196566
G1alpha-2 chain	WUGSC:H_L_gi 4218034	N/A	22 314	44 000	5.3	6.09	6	41	37	78	81	12153986	
G protein, beta 2	Gnb2	gi 13937391	10312.3	38 061	35 000	5.6	5.8	9	23	32	97	87	12887916
G protein, beta 2	Gnb2	gi 13937391	10312.3	38 061	37 000	5.6	5.8	7	27	22	77	84	12887917
Hpast (EHD1)	HPAST	gi 2529707	N/A	60 722	70 000	6.49	7.6	11	20	21	97	47	10673336

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
IQGAP1	IQGAP1	gi 4506787	3870.2	189 761		6.08	N/A	N/A	N/A	N/A	N/A	+WB	8798539
Lyn B	LynB	gi 2117805	N/A	56 454	55 000	6.1	7	10	17	23	123	67	8125304
Lyn B	LynB	gi 2117805	N/A	56 454	55 000	6.1	6.4	10	52	23	80	65	8125305
Lyn	Lyn	gi 37589566	N/A	56 454	55 000	6.1	6.8	13	51	24	82	66	8125306
Lyn B protein		gi 187271	N/A	56 454	55 000	6.1	6.3	9	27	23	65	64	8125307
Proteins involved in ubiquitin-proteasome degradation													
Major vault protein ^{f)}	MVP	gi 19913410	17458.2	99 551	115 000	5.34	4.5	22	33	29	192	37	9331335
Major vault protein ^{f)}	MVP	gi 19913410	17458.2	99 551	115 000	5.34	5	19	41	25	170	38	9331336
Valosin containing protein ^{f)}	VCP	gi 6005942	7126.2	89 950	90 000	5.14	N/A	N/A	N/A	N/A	N/A	+WB	10364224
Others													
eukaryotic translation initiation factor 3, subunit 2 beta	EIF3S2	gi 4503513	3757.1	36 878	42 000	5.38	4.9	10	48	40	92	79	10887144
Neutrophil adherence receptor alpha-M subunit	ITGAM	gi 386975	N/A	127 628	200 000	6.73	7	12	23	11	70	35	2454931
Neutrophil adherence receptor alpha-M subunit	ITGAM	gi 386975	N/A	127 628	200 000	6.73	7.3	20	63	19	178	36	2454932
Neutrophil adherence receptor alpha-M subunit	ITGAM	gi 386975	N/A	127 628	200 000	6.73	6.7	14	28	13	115	32	2454933
Integrin alpha M precursor	ITGAM	gi 1708572	N/A	128 470	180 000	6.9	6.5	27	69	26	186	33	2454931
Integrin alpha-IIb precursor	ITGA2B	gi 124951	N/A	114 460	180 000	5.21	6.7	20	10	22	136	34	3801670
PWP1 interacting protein 4	N/A	gi 14579002	N/A	40 760	45 000	5.81	5.8	12	20	34	124	77	15588312
Lung-related protein ^{g)}	N/A	gi 13477103	N/A	51 781	46 000	9.15	5.2	7	56	17	70	74	12477932
(C) Detergent-resistant phagosome													
Actin and associated proteins													
Actin β	ACTB1	gi 14250401	N/A	41 321	43 000	5.56	4.8	6	92	22	80	11	12663865
Actin β	ACTB1	gi 14250401	N/A	41 321	43 000	5.56	5	6	18	20	69	12	12663865
Actin β	ACTB1	gi 14250401	N/A	41 321	43 000	5.56	5.2	7	12	27	88	13	12663865
Actin γ	ACTG1	gi 4501887	1614.2	42 108	43 000	5.31	6.5	7	82	26	83	17	12663865
Actin β	ACTB1	gi 15077503	N/A	40 540	45 000	5.6	6.2	12	93	33	85	16	12663865
Annexin VI	ANXA6	gi 113962	N/A	76 168	70 000	5.42	6.2	11	23	19	89	4	9230932
Annexin VII	ANXA7	gi 4809279	4034.1	53 010	50 000	5.5	7	7	138	17	81	18	9230932
Annexin XI	ANXA11	gi 4557317	1157.2	54 710	55 000	7.7	8	8	102	20	68	7	12805373
Annexin XI	ANXA11	gi 4557317	1157.2	54 710	55 000	7.7	8.2	8	95	20	98	9	12805374
Cap Z alpha 1	CAZ1	gi 3453597	6135	33 073	32 000	5.45	5.1	10	104	39	121	22	12660160

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
Cap Z alpha 1	CAZ1	gi 3453597	6135	33 073	32 000	5.45	5.8	5	91	24	77	23	12660160
Filamin	FLN	gi 1203969	N/A	283 460	250 000	5.7	N/A	39	29	18	230	+ 1-D	7929100
Grancalcin	GCA	gi 6912388	12198.2	24 223	30 000	5.02	5	7	102	25	78	30	12529388
Grancalcin	GCA	gi 6912388	12198.2	24 223	30 000	5.02	5.2	8	88	21	81	31	12529388
L-Plastin	LCP1	gi 4504965	2298	70 814	72 000	5.29	4.9	32	26	52	396	3	12525560
Tubulin and associated proteins													
Heat shock 70kDa protein 9B precursor	HSPA9B	gi 24234688	4134.4	73 920	75 000	5.87	5	10	33	19	77	1	12729798
Heat shock 70kDa protein 9B precursor	HSPA9B	gi 24234688	4134.4	73 920	75 000	5.87	5.4	11	86	19	73	2	12729799
Intermediate filaments and assoc. proteins													
Cytokeratin 9	KRT9	gi 435476	N/A	62 320	65 000	5.19	5.8	14	46	24	98	5	2425683
Keratin 10, cytoskeletal	KRT10	gi 71528	N/A	59 740	59 000	5.2	6.4	11	24	18	88	+ 1-D	15274135
Vimentin	VIM	gi 4507895	3380.1	53 710	60 000	5.06	4.2	13	115	29	111	6	14676269
Molecular motors and assoc. proteins													
Myosin tail domain containing protein	DKFZp68610336	gi 361873968	N/A	37 980	33 000	5.7	4.9	9	125	27	68	25	N/A
Myosin heavy chain	N/A	gi 620305	N/A	226 602	220 000	5.5	6.5	28	33	17	124	+ 1-D	9013678
Energy and metabolic enzymes													
ATP synthase	ATP5B	gi 32189394	1686.3	56 540	52 000	5.3	4.3	13	109	33	172	10	15048984
Protein disulfide-isomerase	N/A	gi 7437388	N/A	57 160	50 000	5.98	N/A	7	29	13	79	+ 1-D	9826346
Signal transduction molecules													
Ficolin ^{f)}	FCN	gi 1510127	N/A	34 830	35 000	6.7	7.2	14	62	29	135	26	14642811
Ficolin ^{f)}	FCN	gi 1510127	N/A	34 830	35 000	6.7	7.5	12	108	28	112	27	14642812
Flotillin	FLOT	gi 5114049	N/A	47 531	52 000	7.04	8	11	116	31	122	19	15128873
Flotillin	FLOT	gi 5114049	N/A	47 531	52 000	7.04	8.1	7	82	23	89	20	15128873
IQGAP1	IQGAP1	gi 4506787	3870.2	189 761	180 000	6.08	N/A	N/A	N/A	N/A	N/A	+WB	8798539
Proteins involved in ubiquitin-proteasome degradation													
Valosin containing protein ^{f)}	VCP	gi 6005942	6005942	89 950	92 000	5.14	N/A	N/A	N/A	N/A	N/A	+WB	10364224
Others													
Adaptor protein p47phox	NCF1	gi 13345794	N/A	31 484	38 000	9.01	4.4	6	54	23	84	21	12716910
EF hand domain containing 2	EFHD2	gi 20149675	24329.4	26 790	33 000	5.2	4.8	15	118	36	142	24	10673336
Microtubule associated protein-6	N/A	gi 48375157	N/A	49 130	55 000	9.9	8.2	7	86	27	72	8	N/A

Proteins	Gene	Accession no. (gi:)	Closest RefSeq	Mass predicted	Mass actual	p/predicted	p/actual	No. of peptides	Error (ppm)	Coverage (%) ^{b)}	Score ^{c)}	Spot no. ^{d)}	PMID ^{e)}
VDAC1	N/A	gi 238427	N/A	30 740	35 000	8.8	9	8	71	38	74	28	11035728
Prohibitin ^{f)}	PHB	gi 4505773	2634.2	29 840	29 000	5.6	5.5	8	110	35	92	29	15378696

a) Only proteins identified in two out of three experiments are listed.

b) The sequence coverage represents the percentage of coverage of the whole sequence by tryptic peptides detected by MALDI-TOF MS.

c) Significance scores were obtained from Mascot. Pubmed Index number

d) Spot numbers are indicated in Fig. 2A. Spots marked +WB were identified by Western blot and presented on Fig. 4, while spots marked +1-D were identified by 1-D SDS-PAGE (not shown).

e) PMID, denotes the Medline reference describing the function of each protein.

f) Proteins previously not known to associate with cytoskeleton.