ABSTRACT

Title of Thesis: INVESTIGATION BY MASS SPECTROMETRY OF THE UBIQUITOME AND PROTEIN CARGO OF EXOSOMES DERIVED FROM MYELOID-DERIVED SUPPRESSOR CELLS

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Exosomes released by myeloid-derived suppressor cells (MDSC) are 30 nm in diameter extracellular vesicles that have been shown to carry biologically active proteins as well as ubiquitin molecules. Ubiquitin is known to have many functions, including involvement in the formation of exosomes, although the exact role is highly contested. In the study reported here, the proteome and ubiquitome of MDSC exosomes has been investigated by bottom-up proteomics techniques. This report identifies more than 1000 proteins contained in the MDSC exosome cargo and 489 sites of ubiquitination in more than 300 ubiquitinated proteins based on recognition of glycinylglycine tagged peptides without antibody enrichment. This has allowed extensive chemical and biological characterization of the ubiquitinated cohort compared to that of the entire protein cargo to support hypotheses on the role of ubiquitin in exosomes.

INVESTIGATION BY MASS SPECTROMETRY OF THE UBIQUITOME AND PROTEIN CARGO OF EXOSOMES DERIVED FROM MYELOID-DERIVED SUPPRESSOR CELLS

by

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Professor Nicole LaRonde

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Dedication

This work is dedicated to my parents, David and Mary Lou Adams, who have always supported me in all walks of life and taught me the value of hard work. To my older brother Matthew who taught me all of life's important lessons. And to my boyfriend and best friend Adam who was always available for a pep talk and food.

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Table of Contents

Dedication ii
Acknowledgements iii
Table of Contents iv
List of Tables v
List of Figures vi
List of Abbreviations viii
Chapter 1: Introduction 1
1.1 Proteomics 1
1.1.1 Overview 1
1.1.2 Orbitrap Fusion Lumos Tribrid 5
1.2 Biological question 7
1.2.1 Exosome biogenesis and function 7
1.2.2 Ubiquitin as a post-translational modification 8
1.2.3 The role of ubiquitin in exosome formation 11
1.3 Research objectives and significance 12
Chapter 2: Identification of proteins in the exosome lysate 14
2.1 Introduction 14
2.2 Materials and methods 16
2.2.1 Materials 16
2.2.2 Biological sample preparation 16
2.2.3 Protein analysis 16
2.2.4 LC-MS/MS and bioinformatics 17
2.3 Results and discussion 19
2.4 Conclusions 27
Chapter 3: The Ubiquitome 29
3.1 Introduction 29
3.2 Materials and methods 32
3.2.1 Materials 32
3.2.2 Biological sample preparation and protein analysis 32
3.2.3 Immunoprecipitations 33
3.2.4 Western blotting 34
3.3 Results and discussion 34
3.3.1 Confirmation of ubiquitinated proteins by immunoprecipitation 34
3.3.2 Bottom-up identification of ubiquitinated proteins 36
3.4 Conclusions 42
Appendices 45
Bibliography 86

List of Tables

Table 2.1 – Some biologically active proteins identified in the exosome lysate.

Table 2.2 – Proteasome components identified in the exosome lysate.

Table 2.3 – Ubiquitination pathway enzymes identified in the exosome lysate.

List of Figures

Figure 1.1. Diagram of the fragmentation and ion types that can occur along the peptide backbone.

Figure 1.2. Schematic of the orbitrap Fusion Lumos Tribrid mass spectrometer. (http://planetorbitrap.com/)

Figure 1.3. Illustration of the formation of multi-vesicular bodies and intraluminal vesicles, and their pathway to degradation in the lysosome or release as exosomes.

Figure 1.4. An overview of the components involved in the ubiquitin system in mammalian cells.

Figure 2.1. A flowchart summarizing sample preparation and this bottom-up workflow.

Figure 2.2a-b. GO annotations of the **(a)** molecular functions and **(b)** cellular components for the whole exosome lysate.

Figure 2.3a-b. (a) Isoelectric point, pI, distribution of the proteins identified in the exosome lysate and **(b)** a comparison of the isoelectric point distributions of the exosome lysate and mouse proteome (available from http://isoelectricpointdb.org/index.html).

Figure 2.4. MW distributions of the proteins identified in the MDSC exosomes; inset shows the 0 - 350,000 Da range.

Figure 3.1. A bottom-up proteomic workflow for ubiquitinated proteins utilizing digestion by trypsin and activation by CID. (Adapted from references 11 and 39).

Figure 3.2a-f. Western blots probing with anti-ubiquitin antibody #3933 of **(a)** exosome lysate, **(b)** eluate of #3933 immunoprecipitation, **(c)** non-bound fraction from #3933 immunoprecipitation, **(d)** negative control of #3933 immunoprecipitation, **(e)** eluate of #19271 immunoprecipitation, **(f)** negative control of #19271 immunoprecipitation.

Figure 3.3a-b. The distribution of the **(a)** grand average of hydropathy, GRAVY, scores and **(b)** isoelectric points, pI, for proteins identified in the whole exosome lysate, ubiquitinated proteins, and multi-ubiquitinated proteins.

Figure 3.4a-b. Molecular weight, MW, distributions of **(a)** the ubiquitinated cohort and **(b)** multi-ubiquitinated protein cohort.

Figure 3.5a-b. GO annotations comparisons of **(a)** molecular functions and **(b)** cellular components; green, multi-ubiquitinated protein cohort; orange, ubiquitinated protein cohort; blue, all proteins identified in the exosome lysate.

Figure 3.6. Manually annotated spectrum of a terminal K-GG peptide (residue 494-517) of myosin-9.

List of Abbreviations

Collision-induced dissociation……..CID

Disuccinimidyl suberate……...DSS

Dithiotheritol……DTT

Electrospray ionization……..ESI

Extracellular vesicle……...EV

False discovery rate……..FDR

Gene ontology……GO

Glycinylglycine…….....GG

Grand average of hydropathy……GRAVY

High pressure liquid chromatography……...HPLC

Iodoacetamide……...IAA

Immunoprecipitation……IP

Intraluminal vesicle..……ILV

Isoelectric point……pI

Liquid chromatography……...LC

Mass spectrometry…….MS

Methyl methanethiosulfonate……...MMTS

Molecular weight……..MW

Multivesicular body.....……..MVB

Myeloid-derived suppressor cells…….MDSC

Peptide spectrum match……...PSM

Phosphate buffered saline…….PBS

Poly acrylamide gel electrophoresis……..PAGE

Polyvinylidene difluoride……..PVDF

Post-translational modification……PTM

Protein information resource…….PIR

Reverse phase……...…..RP

Sodium dodecyl sulfate……SDS

Tandem mass spectrometry…….MS/MS

Western blot……...WB

Chapter 1: Introduction

1.1 Proteomics

1.1.1 Overview

The field of mass spectrometry began with JJ. Thomson, who constructed the first mass spectrometer and discovered two isotopes of neon at the beginning of the twentieth century.[1] Numerous advances and Nobel Prizes later, mass spectrometry is an invaluable technique utilized in all parts of science and technology, especially the analysis of biomolecules. There are many different biomolecules that utilize mass spectrometry for characterization including proteins, oligonucleotides, oligosaccharides, lipids, and metabolites. Proteomics is the study of proteins in a proteome, including identification and quantification.[2] Proteins and peptides are complex biopolymers made up of the 20 amino acids and may also undergo covalent modifications or post-translational modifications, PTMs. Common PTMs include glycosylation, phosphorylation, acetylation, and ubiquitination. In order to identify and characterize proteins and peptides in samples, precise determination of molecular mass is needed to determine sequences and mass additions for post-translational modifications.

The initiation step in mass spectral analysis is the ionization of the sample molecules of interest. The challenge with biological samples, including proteins and peptides for proteomic research, is converting large molecules to gas-phase ions

without degradation, loss of modifications, or unwanted fragmentation.[3] The earliest ionization techniques, electron ionization (EI) and chemical ionization (CI), required volatile analytes, and therefore could not be employed for heavy biomolecules. Electrospray ionization, ESI, was developed by John Fenn in 1988 and allowed nonvolatile molecules to be easily ionized so they could be analyzed by mass spectrometry.[3] Ions are created by flowing solution through a heated narrow metal capillary which has an electric potential. This generates a fine mist of ions that are sprayed into the inlet of the mass spectrometer.[4] Nanoelectrospray ionization, nESI, was developed from ESI in 1994 by M. Wilm and M. Mann.[1] The nESI technique uses a decreased flow rate to more efficiently create ions, improve sensitivity, and reduce sample size.[3]

Protein and peptide biological samples are usually complex and require separation techniques before mass spectral analysis. Pre-fractionation helps with more accurate mass determination and fragmentation; if samples are too complex entering into the mass analyzer, many important ions can be missed and not accurately measured.[1] Additionally, high concentrations of background molecules in biological samples can decrease ionization efficiency and effectively shield detection of ions of interest. Some common pre-fractionation techniques for proteins and peptides include SDS-Page, size exclusion chromatography, ion exchange chromatography, and reverse phase liquid chromatography (RP-HPLC).[5] RP-HPLC is one of the most popular pre-fractionation techniques and is used to concentrate, desalt, and simplify mixtures before mass spectral analysis. RP-HPLC can be coupled directly to a mass spectrometer in proteomic workflows, as most LC mobile

phases are volatile and compatible with ESI and nESI. LC-MS workflows decrease sample loss, as fractionated sample is eluted from the column directly into the mass spectrometer.

Tandem mass spectrometry (MS/MS) is commonly used in proteomic workflows as it gives important fragmentation and sequence information about proteins and peptides. A precursor ion is first isolated for activation and fragmentation, and the subsequent product ions are then measured. For proteins and peptides, fragmentation occurs mostly along the peptide backbone and yields information to sequence peptides and intact proteins. Collision-induced dissociation (CID) is the most common technique for fragmenting peptides and proteins.[6] Neutral gas molecules are introduced to the reaction cell and collide with the precursor ions. The energy transferred during collision cleaves along the weakest point in the peptide backbone, and creates b and y type ions, as shown in Figure 1.1 highlighted in blue.

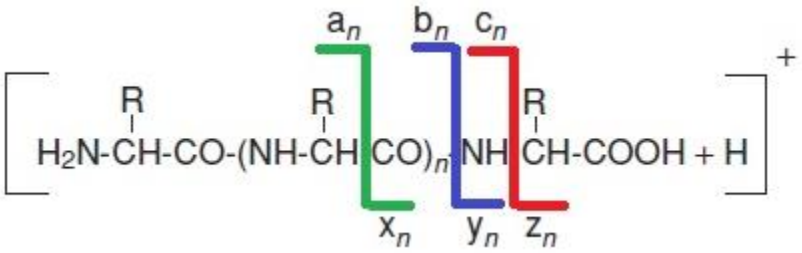

Figure 1.1. Representative of the fragmentation and ion types that can occur along the peptide backbone. Figure adapted from reference 1.

Other types of fragmentation are higher energy CID, commonly termed HCD, that utilizes higher energy collisions for fragmentation and can produce a/x type ions along with b/y ions (green in Figure 1.1).[1] Electron transfer dissociation, ETD, uses a negative reagent ion to effectively transfer an electron to the precursor ion and create c/z type ions (red in Figure 1.1). ETD requires higher charged precursor ions for efficient fragmentation to occur and is commonly used with large peptides or intact proteins.[7,8]

The measurement and analysis of intact proteins is referred to as top-down proteomics. This method is advantageous for identifying protein isoforms in biological samples and characterizing sites and types of PTMs.[1,9] Currently there are many limitations in top-down experiments. Analysis of intact proteins relies on accurate mass measurements and fragmentation information for sequencing. Large proteins have lesser ionization and transfer efficiency in mass spectrometers and extensive fragmentation is needed for sequencing and accurate PTM assignment. Additionally, many instruments are limited by the mass range they can separate and analyze. Top-down experiments require MS instruments with improvements in mass resolving power and fragmentation methods.[10]

Bottom-up proteomics is the most widely used method of characterizing proteins by analyzing their peptides. In these experiments, proteins of interest are first digested into peptides. There are many enzymes in nature that cleave along the peptide backbone at specific and reproducible residues to create smaller peptides. The most commonly used enzyme is trypsin, which cleaves along the peptide backbone at the C-terminus of arginine (R) and lysine (K) residues, producing

peptides commonly 7 to 35 amino acids in length.[11] Protein identifications are made by using fragmentation information to sequence and identify peptides, which are then searched against protein databases to identify the original protein.[2] This method is advantageous as the size of peptides is amenable to most available instruments and the bioinformatics search engines for analyzing peptide spectra are well developed.[12]

1.1.2 Orbitrap Fusion Lumos Tribrid

Development of the orbitrap mass analyzer has allowed for the field to progress rapidly in the past two decades. The first orbitrap based instrument was presented in 1999 at the American Society for Mass Spectrometry national conference, but would not be commercialized until 2005.[13] There have been seven instruments in the orbitrap family released by Thermo Scientific since 2005, featuring improvements in coupled ionization sources, activation abilities, and added speed and resolving power.[1,13] Based off the principle of orbital trapping designed by Kingdon in 1923, the orbitrap utilizes three distinct electrodes.[13] The outer two electrodes are positioned around a spindle-like central electrode and mass to charge measurements are based off the frequency of ions' movement along the length of the central electrode.

The orbitrap Fusion Lumos Tribrid mass spectrometer was released by Thermo Scientific in 2015. A schematic is shown in Figure 1.2. This new generation orbitrap mass spectrometer has increased sensitivity and acquisition speed with an ultra-high field orbitrap and improved vacuum systems. This improved orbitrap mass analyzer has a resolving power of up to 450,000 at 200 m/z and the vacuum system provides lower pressure in the ion routing multipole, orbitrap, and C-trap to allow for

more efficient ion transfer through the instrument.[13,14] Increases in acquisition speed and duty cycle also stem from the ion routing multipole (IRM) cell. The IRM cell controls where packets of ions are being sent for activation or for measurement, allowing ions to be isolated in one analyzer while separately detecting ions in the remaining analyzers of the dual-pressure linear ion trap.[14] This increases sensitivity for lower abundant ions by allocating analysis time for ion accumulation while parallel ion detection is occurring.

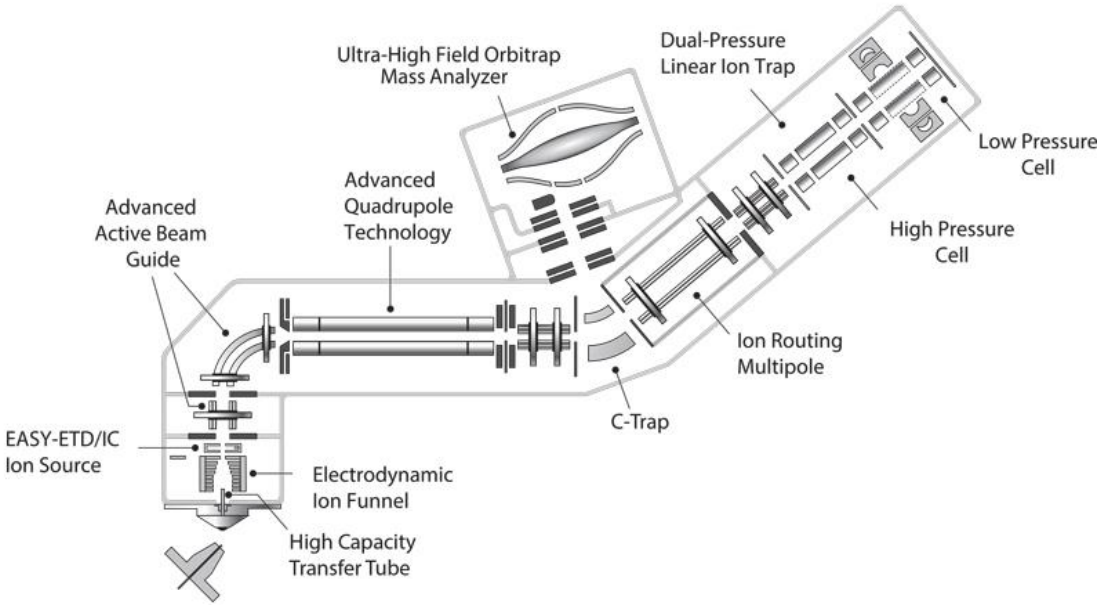

Figure 1.2. Schematic of the orbitrap Fusion Lumos Tribrid mass spectrometer. (http://planetorbitrap.com/)

1.2 Biological question

1.2.1 Exosome biogenesis and function

Exosomes, by definition, are 30-100 nm extracellular vesicles released by all cell types.[15–17] They are widely studied because they carry protein and small RNA cargo important to the biological function of the parental cells and convey information from the parental cell to a receiving cell or location.[18] They are under development as vehicles for drug delivery.[19] Exosomes are formed by the invagination of endosomal membranes forming multivesicular bodies (MVB) that form intraluminal vesicles (ILV) as shown in Figure 1.3.[18] There are two fates of these MVBs, fusion with lysosomes leading to the degradation of their protein cargo or fusion with the plasma membrane to release the ILVs as exosomes.[16,20–23] Exosome biogenesis may vary within a cell and between cell types, and investigations are ongoing to identify the signals that lead to the two fates of MVBs.[23]

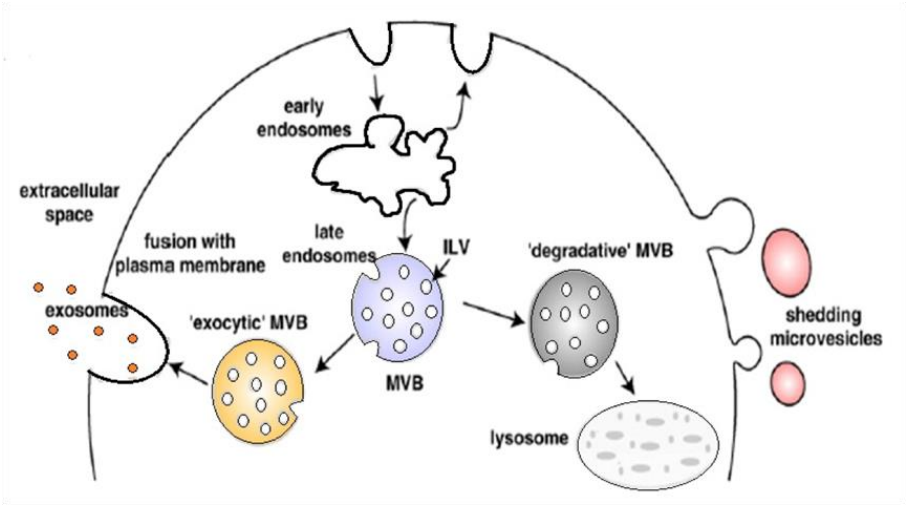

Figure 1.3. Illustration of the formation of MVBs and ILVs, and their pathway to degradation in the lysosome or release as exosomes.[18]

The protein cargo of exosomes is not random; incorporation of proteins into exosomes has been shown to be a regulated process, which usually reflects functions of the parent cell.[16,22] The endosomal sorting complex required for transport, or ESCRT complex, has been shown to mediate the formation of MVBs and protein cargo sorting. It has been shown to cluster ubiquitinated proteins into ILVs destined for degradation by the lysosome as ESCRT-0, -I, and -II proteins contain ubiquitin binding domains that interact with ubiquitinated cargo.[16,23–25] Ubiquitination is hypothesized to have an important role in MVB fate and exosome formation, and additionally it has been reported that there are ubiquitinated proteins contained in exosomes of different cell types.[21,26–28]

1.2.2 Ubiquitin as a post-translational modification

Ubiquitin is a small 8.5 kDa protein that can be found as mono- or polyubiquitin in cells either unanchored or conjugated to a protein substrate. Conjugation is formed through an isopeptide bond at its C-terminal glycine-76 with the ε-amine of a lysine (K) residue in another ubiquitin or a substrate protein. Ubiquitin conjugation occurs through the coordination of three families of enzymes and is regulated by hydrolase enzymes, shown in Figure 1.4.[29]

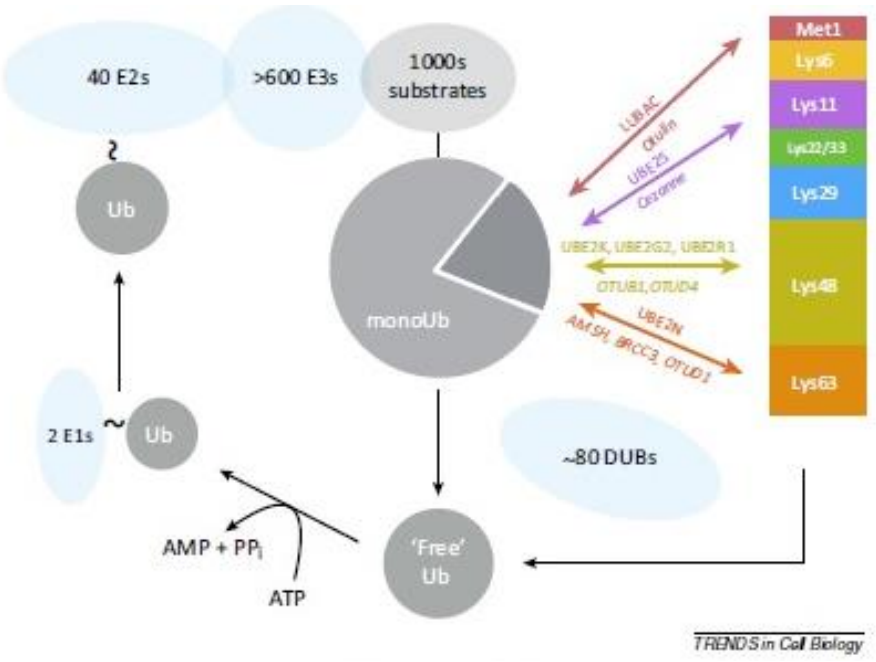

Figure 1.4. An overview of the components involved in the ubiquitin system in mammalian cells.[30]

E1-activating enzymes are the first step in ubiquitin conjugation. E1's contain an active-site cysteine to which the carboxy-terminal glycine of ubiquitin becomes attached through a reactive thioester bond for conjugation of ubiquitin to the enzyme.[31,32] The activated ubiquitin molecule is then transferred to an E2-conjugating enzyme that is recruited by the E1 enzyme.[31,32] Lastly, a specific E3 ligase catalyzes the attachment of the activated ubiquitin to the lysine of the substrate protein.[31] The enzymes increase in complexity and specificity with each additional step in the conjugation pathway; there are few E1 enzymes with low specificity that conjugate with many E2 enzymes, and hundreds of E3 ligases with high specificity for the target protein type.

Deubiquitinating enzymes, or ubiquitin hydrolases, reshape ubiquitin modifications by shortening polyubiquitin chains or by complete removal of the entire modification.[29,33] These enzymes hydrolyze at the C-terminal Gly76 of ubiquitin to remove it from the modified lysine. Humans have five different families of deubiquitinating enzymes; some have broad specificity while others can be selective for substrate or for linkage type.[34] These enzymes are important for regulating the production of monomeric ubiquitin by recycling ubiquitin from conjugates and the proteasome, and editing polymer chain lengths.[35]

Ubiquitin itself has seven lysine residues and therefore can form polyubiquitin chains at multiple sites and of various linkage types, including chains conjugated at the N-terminus of ubiquitin.[36] There are many different functions proposed for ubiquitin and ubiquitin polymers in the cell.[33,36] Monoubiquitin, ubiquitin conjugated to a single lysine, is thought to be involved in protein localization, transcriptional activation and chromatin structure.[29] Multi-monoubiquitin, ubiquitin conjugated to multiple individual lysines in a single protein, may assist with ubiquitin binding to low affinity ubiquitin-interacting domains and adapter proteins' recognition of substrate proteins.[28] There are seven "linear" polyubiquitins, in which each ubiquitin chain is attached to an analogous lysine of another chain, and hundreds of mixed and branched linkage polymers that could be formed. However, not all types have been isolated *in vivo* or *in vitro*, and not all have known functions.[16,29,37] The K48-linked tetramer polyubiquitin is well studied for its involvement in the 26S proteasome and the most well-known function of ubiquitination is as a protein tag for disposal.[28,38] There is also evidence of K-29 linked polyubiquitin involvement in

lysosomal degradation and K-11 linked polyubiquitin involvement in endoplasmic reticulum-associated degradation.[36] Ubiquitin may have signaling roles in other pathways besides degradation; many studies also suggest the importance of ubiquitin in kinase activation, DNA repair, ribosome function, and cell signaling.[16,29,31] Interestingly, there have been many recent studies on the importance of ubiquitinated proteins in the formation of exosomes.[20] For example, K-63 linked polyubiquitin is reported to be involved in cargo sorting and the trafficking of membrane proteins into exosomes.[16,31,33,39]

1.2.3 The role of ubiquitin in exosome formation

Ubiquitin is hypothesized to be an important player in determining the fate of MVBs, but the exact roles are still disputed. There is strong evidence that ubiquitination is involved in invagination, the first step in MVB formation.[20] Ubiquitination is also reported to be an important tag for sorting proteins into MVBs destined to be degraded by the lysosome.[25,40] The ESCRT complex, specifically ESCRT-I, -II, and –III, has been shown to deactivate proteins by monoubiquitin addition and to cluster these tagged proteins into ILVs destined for the lysosome.[23,25]

There are recently reported studies that show that ubiquitination may also influence formation of exosomes in MVBs, but there is still disagreement about how ubiquitin signaling occurs and whether there should be any ubiquitinated proteins present within MVBs and exosomes. One hypothesis is that protein sorting into exosomes happens through deubiquitination of the proteins before addition to the MVBs, thus preventing transport into the lysosomes.[33] Amerik *et al.* reported observations of ESCRT-III recruiting the deubiquitinase Doa4 and removing

ubiquitin tags from cargo prior to incorporation into newly forming MVBs.[41] Conversely, Buschow *et al.* hypothesize that deubiquitination occurs before incorporation into the MVB for the lysosome pathway, and it is the proteins that escape deubiquitination that are sorted into ILVs destined to be released as exosomes.[21] Huebner *et al.* also propose that deubiquitination is not essential for the protein cargo to be incorporated into MVBs destined to be released as exosomes.[28] It should be noted that ubiquitinated proteins have been identified in urinary exosomes and exosomes released from human B-cells, murine immature dendritic cells, and murine myeloid-derived suppressor cells.[21,26,28] Ubiquitination has been shown to be important for trafficking in some cell types but not others.[16] This indicates that different cell types have their own specific functions and regulators, and different cell types need individual study as not all assumptions will hold across cell types.

1.3 Research objectives and significance

Ubiquitin is widely studied as an important protein post-translational modification and it has many suggested functions in the cell. The aim of my research is to investigate the ubiquitome of exosomes secreted by myeloid-derived suppressor cells (MDSC) collected directly from tumor-bearing mice. MDSC-exosomes are interesting biological samples as the parental cells block innate and acquired immune responses in the tumor microenvironment.[42] The MDSCs are collected from animals with elevated inflammation and are under considerable cellular stress at the time of exosome release.[43] The role of ubiquitin in exosome formation and protein sorting is

highly contested, and identification of the ubiquitinated proteins in exosomes may provide insight into this role.

For this project, the first objective is to provide an in depth inventory of the proteome of the MDSC exosomes utilizing the orbitrap Fusion Lumos Tribrid mass spectrometer with tryptic digestion and HPLC separation. The second objective is to utilize the same sensitive tryptic and LC-MS/MS strategy to also identify the ubiquitinated proteins in the exosomes. By first inventorying the larger proteome of this biological sample, the ubiquitinated cohort can be compared to look for insights to the role of ubiquitin in exosome formation.

Chapter 2: Identification of proteins in the exosome lysate

2.1 Introduction

Myeloid-derived suppressor cells, MDSC, congregate in the tumor microenvironment of individuals and mice with cancer. Under inflammatory conditions, which are prevalent in many solid tumors, these cells increase tumor growth by a variety of mechanisms including preventing the activation of tumor-reactive T lymphocytes and polarizing macrophages towards a M2 tumor-promoting phenotype.[42,43] MDSCs produce reactive oxygen species and reactive nitrogen species in the tumor microenvironment, creating an environment of oxidative sress.[43] MDSC accumulation and function are driven by pro-inflammatory molecules and other tumor-secreted factors that are present in the microenvironment. Chemotaxis migration assays are used to study environmental effects to MDSC function, and it has been shown that both S100-A8 and S100-A9 activity is important for migration of MDSC.[44] MDSC also release exosomes that show significant chemotactic activity towards other MDSCs, catalyzing their migration into the tumor environment.[44–46]

Exosomes are small (30-100 nm) secreted extracellular microvesicles involved in the transport of bioactive molecules between cells.[15] Exosomes are secreted by all cell types and can be isolated from nearly all tissue samples and physiological fluids.[16,17] They have been shown to carry proteins, small RNAs, and lipids, and it has been reported that exosome content reflects the cellular stress of the

parental cells.[18,47] Exosomes are formed by invagination of the limiting membrane of specific endosomal compartments, multivesicular bodies (MVBs), that form intraluminal vesicles (ILVs) that once fused with the plasma membrane are released as exosomes.[16,21–23] Protein sorting into exosomes is not random. The endosomal sorting complex required for transport (ESCRT complex) has been shown to play a role in the formation of MVBs and protein sorting by clustering ubiquitinated proteins into ILVs.[16,23,25] However, the exact mechanisms for sorting cargo is not yet known. Furthermore, the cargo carried by exosomes has received considerable attention for its potential and demonstrated intercellular bioactivities.

MDSC-derived exosomes are 25-30 nm in diameter, as measured by Transmission electron microscopy by Burke *et al.,* and have been shown to contain bioactive proteins including the calcium- and zinc-binding pro-inflammatory proteins S100-A8 and -A9, histones, and other nucleic acid binding proteins.[44] A recent study by Burke *et al.* sought to identify proteins differentially expressed between two types of MDSC-derived exosomes. This work was successful in identifying over 400 proteins in the exosomes and differential expression of proteins involved in innate immune response, cytoskeletal proteins, chemotactic proteins, and nucleotide binding proteins.[44] Additionally, chemotaxis assays confirmed the hypothesis that MDSC-derived exosomes support MDSC functions in the tumor microenvironment.[44]

2.2 Materials and methods

2.2.1 Materials

All materials and chemicals were purchased from Sigma Aldrich (St. Louis, MI) unless otherwise stated.

2.2.2 Biological sample preparation

MDSC-derived exosomes were isolated and purified by methods described by Burke *et al.* (2014).[26] Exosomes were stored at -80 °C until use. All procedures with animals and animal-derived materials were approved by the UMBC and UMCP Institutional Animal Care and Use Committees.

2.2.3 Protein analysis

Exosomes were lysed in an optimized lysis buffer of 8 M urea in 50 mM ammonium bicarbonate with 50 uM deubiquitinase inhibitor PR-619 (LifeSensors, Malvern, PA) and 1% protease inhibitor cocktail. The solution was centrifuged at 13,000 g for 30 minutes at room temperature in 3 kDa molecular weight cut off filters (Millipore, Darmstadt, Germany), and the filtrate was discarded. This process was performed three times. After filtration, the protein was recovered in 0.8 M urea in 50 mM ammonium bicarbonate for subsequent analyses. Protein content was measured using the Pierce BCA Assay Kit (Thermo Scientific, Waltham, MA).

Tryptic digestion was performed in solution using 25 ug of protein lysate. Samples were reduced with 20 mM dithiothreitol (DTT) for 30 min at 56 °C and alkylated with 10 mM methyl methanethiosulfonate (MMTS) for 45 min. Trypsin (Promega, Madison, WI) was added for a final 1:50 enzyme:protein concentration

and digestion was performed overnight at 37 °C and stopped with the addition of 0.1% formic acid. Three technical replicates of 1 ug total protein were analyzed by LC-MS/MS for each of three biological replicates.

2.2.4 LC-MS/MS and bioinformatics

LC-MS/MS analyses were performed on an Ultimate 3000 nano-HPLC system (Dionex, Sunnyvale, CA) in-line with an orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific). A 2 uL aliquot of tryptic peptide solution was injected onto a C18 precolumn (Dionex, Sunnyvale, CA) followed by desalting with 90% solvent A (97.5% H_20, 2.5% ACN, and 0.1% formic acid) for 10 minutes. Peptides were fractionated on a C18 column (Dionex, Sunnyvale, CA) with a 2 hour linear gradient at a flow rate of 300 nL/min increasing from 5 to 55% solvent B (97.5% ACN, 2.5% H_2O, and 0.1% formic acid) in 90 minutes, followed by an increase from 55 to 90% solvent B in 5 minutes, and held at 90% solvent B for 5 minutes. Precursor scans were acquired in the orbitrap with a resolution of 120,000 at m/z 200. The most abundant ions, as many as possible, were selected for fragmentation by CID (35% collision energy) in the ion trap during each 3 second duty cycle, and product ion scans were acquired in the ion trap. A dynamic exclusion of 1 repeat count over 30 seconds was used.

Peptide and protein identifications were made by the PepArML[48,49] meta-search engine against the UniProt mouse database (01 2015). Peptide identifications, including decoy identifications resulting from searching a reversed protein sequence database, were filtered at 10% spectral FDR. Proteins with at least two unique peptides were retained. Decoy protein identifications were used to estimate target

protein FDR as ≤ 0.87%, after correction using the MAYU technique.[50] Fixed modifications listed methylthio modification of cysteine, and variable modifications included oxidation of methionine, Gln to pyro-Glu, Glu to pyro-Glu, pyro-carbamidomethyl, and glycinylglycine modification of lysine. Subcellular location and function assignments of the identified proteins were made using the Protein Information Resource GO Slim (http://pir.georgetown.edu) and UniProt Gene Ontology annotations (05 2016). Hydrophobicity and chemical characteristics were analyzed using the ExPASy Bioinformatics Resource Portal ProtParam.[51]

An overview of this bottom-up workflow is provided in Figure 2.1.

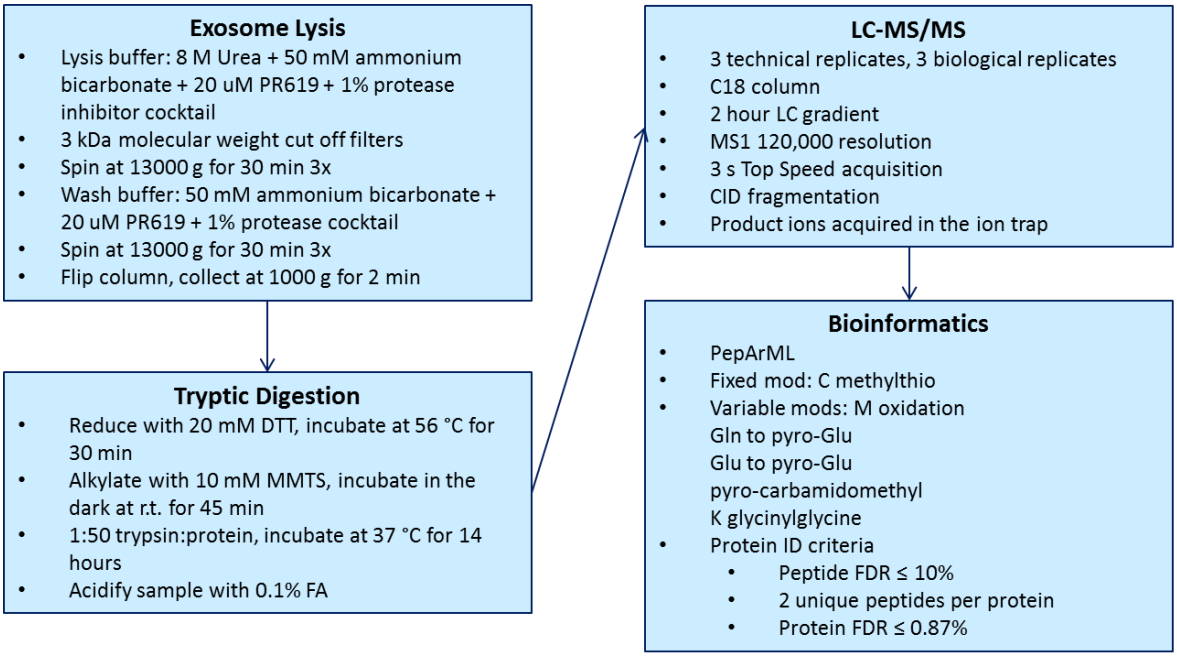

Figure 2.1. A flowchart summarizing the sample preparation and bottom-up methods used.

2.3 Results and discussion

One thousand sixteen proteins were identified in the exosome lysate by bottom-up analysis with tryptic digestion, and are listed in Appendix Table 1. Previously, whole exosome lysate analyses by bottom-up have identified 412 proteins contained in the MDSC exosomes.[44] This updated inventory holds many functionally important proteins and allowed for extensive chemical and biological characterization. Multiple proteins reported as being characteristic of exosomes[18] were identified including 7 annexins (A1, A2, A3, A, A7, A11), 5 tetraspanins, heat shock 70 kDa protein 4, heat shock cognate 71 kDa, and HSP 90 alpha and beta, listed in Table 2.1. Additionally, five S100 family proteins, also listed in Table 2.1, were identified in this sample: S100-A6, -A8, -A9, -A11, and -A13. S100-A8 and -A9 can form an active heterodimer and S100-A8 is present with numerous isoforms in MDSC exosomes.[52] S100-A8 and -A9 have previously been shown to be chemotactic.[44,53,54] The content of S100-A8 and –A9 in MDSC exosomes plays a role in exosome mediation of MDSC chemotaxis in the tumor microenvironment.[44]

Table 2.1. Some biologically active proteins identified in the exosome lysate.			
Accession Number	**Protein Name**	**No. of non-overlapping peptides**	**% Coverage**
P10107	Annexin A1	16	64.5%
P07356	Annexin A2	14	59.9%
O35639	Annexin A3	15	63.2%
P48036	Annexin A5	8	37.9%
P14824	Annexin A6	10	22.3%
Q07076	Annexin A7	7	23.3%
P97384	Annexin A11	12	29.8%
P10810	CD14	3	13.4%
P40237	CD82	2	8.7%
Q9Z0M6	CD97	4	8.1%
Q64277	CD157	2	7.1%
Q8R2S8	CD177	12	21.5%
Q3U2G2	Heat shock 70 kDa protein 4	17	33.1%
P63017	Heat shock cognate 71 kDa protein	30	73.4%
P07901	Heat shock protein HSP 90-alpha	24	48.3%
P11499	Heat shock protein HSP 90-beta	23	50.4%
P14069	Protein S100-A6	6	80.9%
P27005	Protein S100-A8	5	94.4%
P31725	Protein S100-A9	8	83.2%
P50543	Protein S100-A11	2	27.6%
P97352	Protein S100-A13	2	14.4%

Gene ontology annotations of the proteins identified in the exosomes indicate that 24% of the proteins are involved in protein binding and 16% in ion binding molecular functions, Figure 2.2a. The five S100 proteins identified contribute to the latter category. Three of the top 5 molecular function categories are related to nucleic acid binding (nucleic acid binding, nucleotide binding, and nucleoside binding). Additionally, GO cellular locations, Figure 2.2b, annotate 16% of the identified proteins to the nucleus, including 17 histones, 20 ribosomal proteins, and 11

transcription factors. Numerous other studies have identified many nucleic acid binding proteins in extracellular vesicles.[27,44]

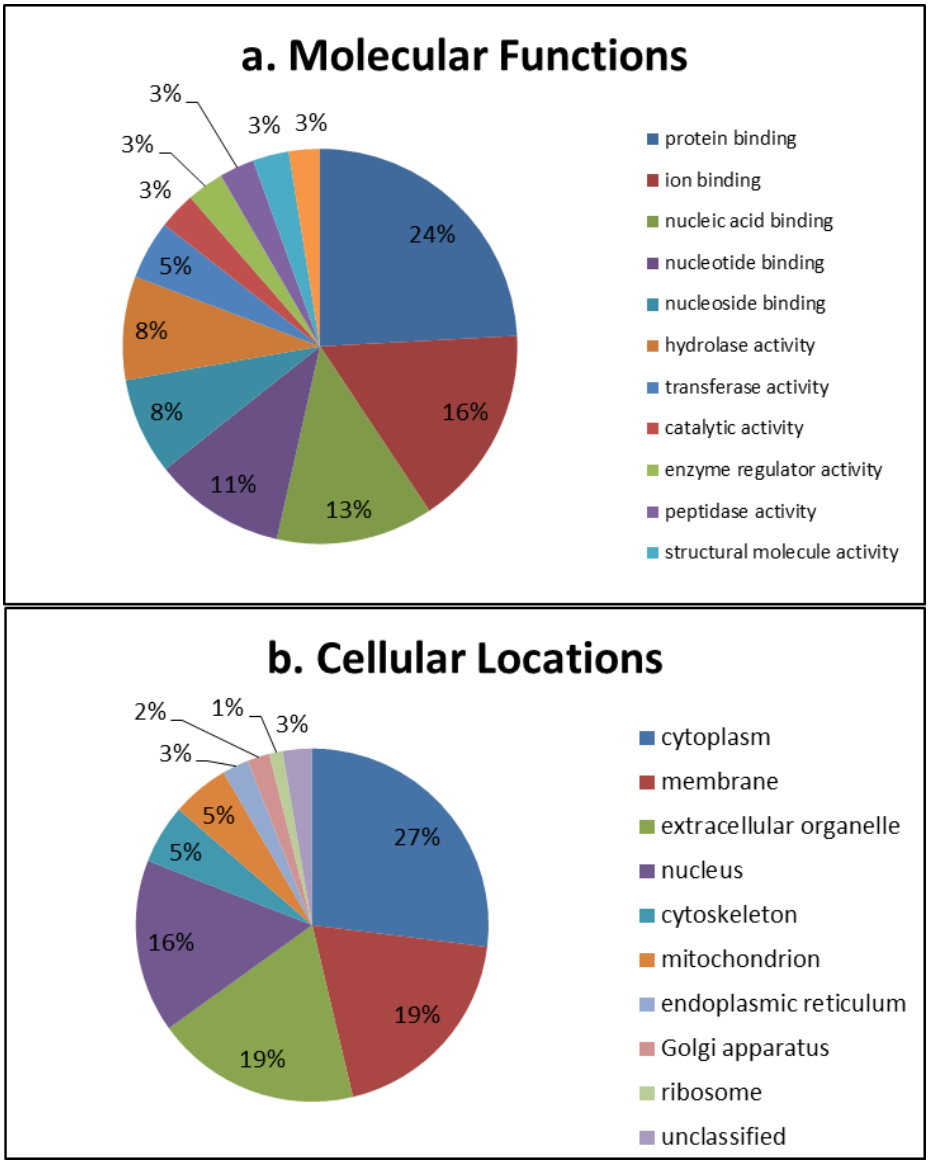

Figure 2.2a-b. Gene ontology annotations of the **(a)** molecular functions and **(b)** cellular locations for the 1016 proteins identified in MDSC exosomes. Proteins occur in multiple categories.

The compilation of isoelectric points, pI, shows a distribution through the range of pH 3 to 12 with the greatest abundance between pH 5 and 6.5, as seen in Figure 2.3a. The pI distribution of the MDSC exosome proteome was compared to that of the whole mouse proteome, and the pI distribution of the exosome proteome is slightly more acidic than that of the mouse proteome, as seen in Figure 2.3b. The molecular weight distribution of the identified proteins shows the highest frequency of proteins around 50 kDa, as seen in Figure 2.4. The four largest proteins identified are nesprin-1 (Acc. No. Q6ZWR6, MW=1,009,925.81 Da), E3 ubiquitin-protein ligase UBR4 (A2AN08, MW=572,289.85 Da), plectin (Q9QXS1, MW=534,187.76 Da), and cytoplasmic dynein 1 heavy chain 1 (Q9JHU4, MW=531,914.24 Da). The grand average of hydropathy, GRAVY, scores of the exosome lysate show an uneven distribution of more hydrophilic proteins, as seen in Figure 3.3a. These distributions will be compared with those of proteins conjugated with ubiquitin in the next chapter.

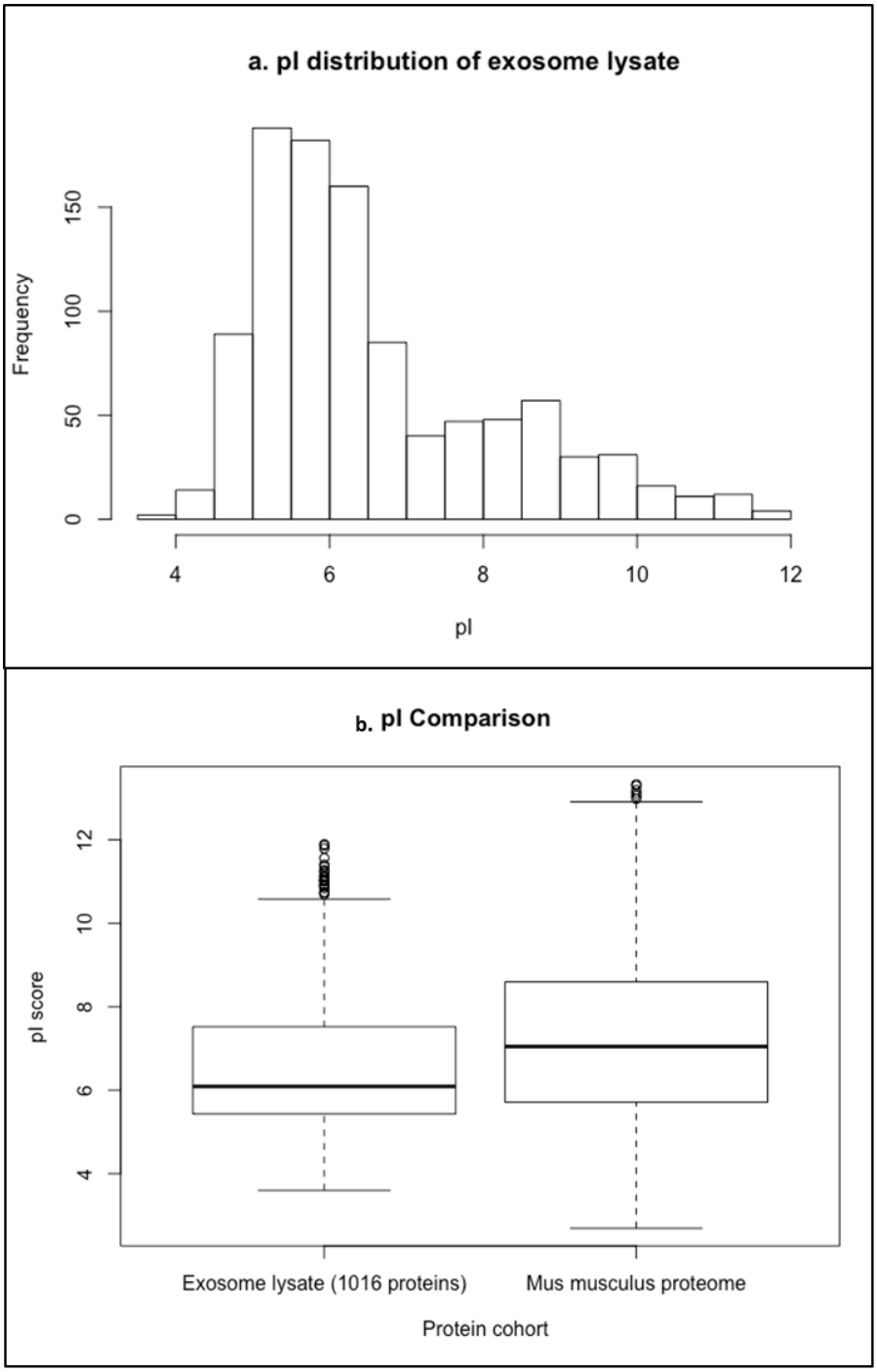

Figure 2.3a-b. Chemical characteristics analyses including **(a)** isoelectric point, pI, distribution of the proteins identified in the exosome lysate and **(b)** comparison of the pI distribution of the exosome proteome and the mouse proteome (available from http://isoelectricpointdb.org/index.html).

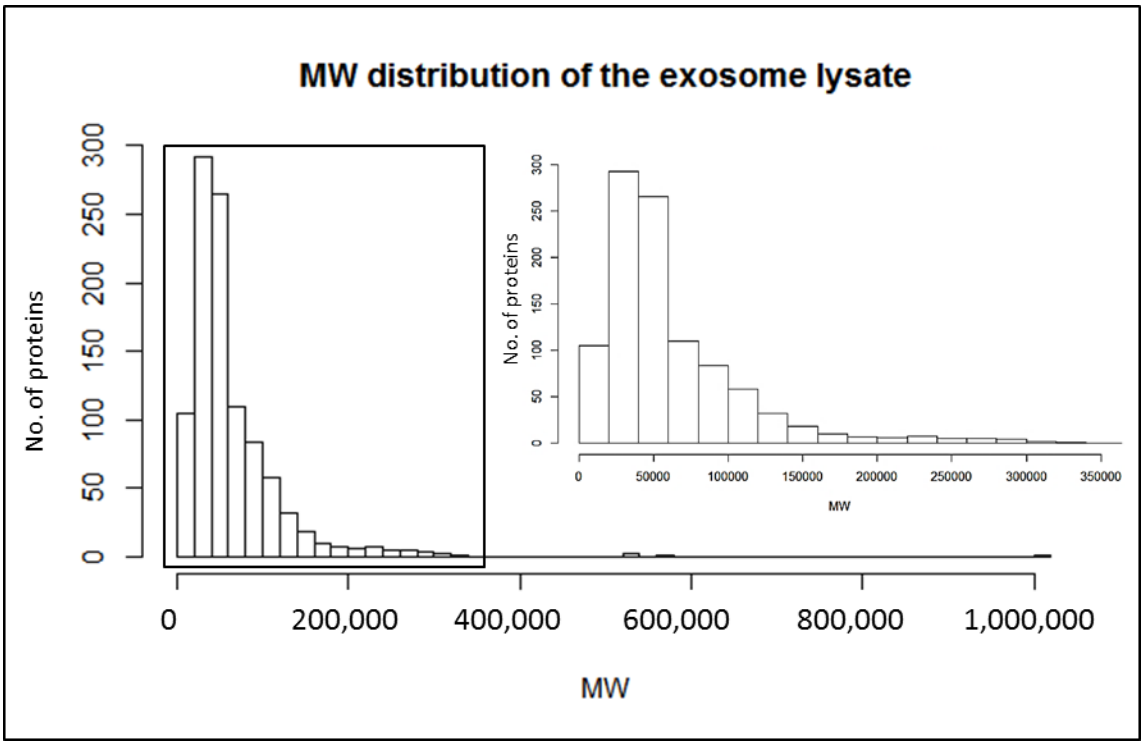

Figure 2.4. Molecular weight distribution of the proteins identified in the MDSC exosomes; inset shows the 0 - 350,000 Da range.

Thirty subunits of the 26S proteasome were identified in MDSC exosomes, all listed in Table 2.2, encompassing all but 4 of the 34 known components in mice.[55] Some components of the 26S proteasome have been previously identified in exosomes.[44,56] Recently, Huebner *et al.* identified 36 of the 37 human components[57] in urinary exosomes and Zhu *et al.* reported identifying 33 components in exosomes released from murine tumor-associated macrophages.[28,58] Zhu *et al.* also performed a 20S proteasome activity assay that indicated that the tumor-associated macrophage exosomes have proteolytic activity.[58]

Table 2.2. Proteasome components identified in the exosome lysate			
Accession Number	**Protein Name**	**No. of non-overlapping peptides**	**% Coverage**
Q3TXS7	26S proteasome non-ATPase regulatory subunit 1	14	29.9%
Q8VDM4	26S proteasome non-ATPase regulatory subunit 2	23	43.5%
P14685	26S proteasome non-ATPase regulatory subunit 3	11	36.2%
Q8BJY1	26S proteasome non-ATPase regulatory subunit 5	4	13.9%
Q99JI4	26S proteasome non-ATPase regulatory subunit 6	8	29.3%
P26516	26S proteasome non-ATPase regulatory subunit 7	8	53.0%
Q9CPS5	26S proteasome non-ATPase regulatory subunit 8	2	15.9%
Q8BG32	26S proteasome non-ATPase regulatory subunit 11	9	32.9%
Q9D8W5	26S proteasome non-ATPase regulatory subunit 12	7	18.0%
Q9WVJ2	26S proteasome non-ATPase regulatory subunit 13	10	47.9%
O35593	26S proteasome non-ATPase regulatory subunit 14	6	41.3%
P62192	26S protease regulatory subunit 4	6	23.0%
O88685	26S protease regulatory subunit 6A	10	40.1%
P54775	26S protease regulatory subunit 6B	9	42.3%
P46471	26S protease regulatory subunit 7	6	22.2%
Q8K1K2	26S protease regulatory subunit 8	5	23.3%
P62334	26S protease regulatory subunit 10B	6	26.0%
Q9R1P4	Proteasome subunit alpha type-1	10	56.3%
O70435	Proteasome subunit alpha type-3	5	23.1%
Q9R1P0	Proteasome subunit alpha type-4	8	57.5%
Q9Z2U1	Proteasome subunit alpha type-5	7	45.2%
Q9QUM9	Proteasome subunit alpha type-6	10	52.0%
Q9Z2U0	Proteasome subunit alpha type-7	8	47.2%
O09061	Proteasome subunit beta type-1	9	58.8%
Q9R1P3	Proteasome subunit beta type-2	7	64.2%
Q9R1P1	Proteasome subunit beta type-3	5	40.5%
P99026	Proteasome subunit beta type-4	8	55.7%
O55234	Proteasome subunit beta type-5	7	40.9%
Q60692	Proteasome subunit beta type-6	8	65.6%
P70195	Proteasome subunit beta type-7	9	55.2%

The present study has identified evidence for polyubiquitins in the exosome lysate, to be discussed in greater detail in Chapter 3. We have also identified multiple proteins involved in the ubiquitination pathway, listed in Table 2.3. Ubiquitin-

activating enzyme E1 (Q02053) is an enzyme that catalyzes the first step in ubiquitin conjugation marking cellular proteins for degradation by the proteasome and is a critical component of ubiquitination in DNA repair pathways.[59,60] Ubiquitin-conjugating enzyme E2 L3 (P68037) is an ubiquitin-conjugating E2 enzyme that is reported to catalyze K-11 linked polyubiquitination in vitro.[61] Four E3 type ligases were identified. E3 ubiquitin-protein ligase CBL (P22682), ubiquitin-protein ligase E3A (E9QKT1), and E3 ubiquitin-protein ligase UBR4 (A2AN08) promote proteins for degradation by the proteasome.[62–64] The latter is also reported to form meshwork structures with clathrin (also identified in MDSC exosomes) and may be involved in invagination.[22,65] Probable E3 ubiquitin-protein ligase HERC4 (Q6PAV2) is reported to be involved in protein trafficking.[66]

Table 2.3. Ubiquitination pathway enzymes identified in the exosome lysate

Accession Number	Protein Name	No. of non-overlapping peptides	% Coverage
Q02053	Ubiquitin-activating enzyme E1	32	53.8%
P68037	Ubiquitin-conjugating enzyme E2 L3	3	40.3%
P22682	E3 ubiquitin-protein ligase CBL	2	3.0%
A2AN08	E3 ubiquitin-protein ligase UBR4	2	0.8%
Q6PAV2	Probable E3 ubiquitin-protein ligase HERC4	2	3.5%
E9QKT1	Ubiquitin-protein ligase E3A	2	3.8%
P70398	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	5	4.1%
Q9JMA1	Ubiquitin carboxyl-terminal hydrolase 14	6	19.9%
P56399	Ubiquitin carboxyl-terminal hydrolase 5	6	13.4%
D3YWF6	Ubiquitin thioesterase OTUB1	4	30.7%
F8VPX1	Ubiquitin carboxyl-terminal hydrolase	12	17.4%

Five deubiquitinase enzymes were identified in the exosome lysate, also listed in Table 2.3. Probable ubiquitin carboxyl-terminal hydrolase (P70398) is a deubiquitinase thought to favor K-29 and K-33 polyubiquitin chains.[67] Ubiquitin carboxyl-terminal hydrolase 5 (P56399) is reported to have a preference for branched chains.[38] Ubiquitin carboxyl-terminal hydrolase 14 (Q9JMA1) is a proteasomal-associated deubiquitinase that releases ubiquitin from proteins tagged for the proteasome.[35,68,69] Ubiquitin thioesterase OTUB1 (D3YWF6) and ubiquitin carboxyl-terminal hydrolase (F8VPX1) are also identified.

2.4 Conclusions

This study provides an in depth inventory of proteins in MDSC-derived exosomes. Transwell migration studies have previously shown that members of the protein cargo of exosomes are biologically active and contribute to parental cell functions.[44] Gene ontology annotations characterize the majority of the lysate as protein binding and ion binding, including the pro-inflammatory S100-A8 and –A9 proteins. Additionally, nucleic acid binding functions contribute to three top molecular function categories, and families of histones, ribosomal proteins, and transcription factors have been identified. MDSC exosomes contain several hundred small RNAs (private communication from Lucia Geis Asteggiante) and it may be hypothesized that the highly abundant nucleic acid binding proteins act as chaperones for this genetic material.

The components of the 26S proteasome were identified in MDSC exosomes. Recently there have been two additional studies that have identified the 26S

proteasome components in exosomes of different cell type and origin.[28,58] Large numbers of proteasome subunits have not been identified in proteomic analyses of most exosomes. It is not clear if they are only present – and active – in a limited selection of exosomes, or if they were missed in many earlier proteomic analyses.

Three of the ubiquitination enzymes identified, ubiquitin-like modified-activating enzyme 1, E3 ubiquitin-protein ligase UBR4, and ubiquitin carboxyl-terminal hydrolase 14, are known to be associated with the 26S proteasome.[59,62,69] However, ubiquitin-like modified-activating enzyme 1 has additional roles in the cell.[60] Therefore these results suggest that the status of ubiquitination may be dynamic inside the exosomes, as all of the players are present.

Chapter 3: The Ubiquitome

3.1 Introduction

Ubiquitin is a small 8.5 kDa protein that can be found unanchored in cells or conjugated to protein substrates as mono- or poly-ubiquitin. Conjugation is formed through an isopeptide bond at its C-terminal glycine-76 with the ε-amine of a lysine residue. Ubiquitination as a post-translational modification is of great interest because it imposes a variety of biological functions on substrate proteins, including protein degradation, protein trafficking, DNA repair, and invagination.[20,33,37,39,40,70] There have been recent studies disagreeing on the role of ubiquitinated proteins in the formation of exosomes from MVBs[21,28,33,41] as discussed in Chapter 1, however ubiquitinated proteins have been identified in exosomes from multiple cell types.[21,26,28]

Ubiquitin as a protein post-translational modification is challenging to study, as it is a very large modification and ubiquitinated proteins are usually present at substoichiometric levels compared to unmodified proteins.[29,32,39] There are multiple approaches to targeting and identifying ubiquitin and ubiquitinated proteins. The use of cell strains expressing 6xHis-tagged or streptavidin-tagged ubiquitin has been successful for quick and efficient purification of ubiquitinated proteins. For example, Peng *et al.* utilized 6xHis-tagged ubiquitin to detect 110 precise ubiquitination sites in 72 ubiquitinated proteins from *Saccharomyces cerevisiae* lysate.[39] Danielson *et al.* purified streptavidin-tagged ubiquitin by affinity purification and reported 753 unique sites on 471 proteins from a human U2OS osteosarcoma cell line.[32] These protocols

require the use of transfected cell lines and are unsuitable for animal tissues or clinical samples.[29]

Anti-ubiquitin antibodies are commercially available to detect ubiquitin and ubiquitin-modified proteins in cell lysate samples by western blotting and more limitedly by immunoprecipitation and co-immunoprecipitation. Ubiquitin-remnant profiling K- ε -GG antibodies have been used successfully to enrich peptide samples after tryptic digestion and allowed Xu *et al.* to identify 374 sites on 236 proteins from HEK293 cells using this technique.[71] Both ubiquitin-specific antibody enrichment methods, general anti-ubiquitin antibody enrichment followed by tryptic digestion and subsequent ubiquitin remnant profiling, have been utilized by Burke *et al.* to compile a list of 50 ubiquitinated proteins in MDSC-derived exosomes.[26] It has been reported that most anti-ubiquitin antibodies have limited and differential affinities for ubiquitin-modified proteins as substrates, and therefore these enrichment methods may produce biased findings.[72]

Digestion of conjugated ubiquitin by trypsin occurs rapidly at its Arg74 residue, leaving two glycine residues attached to the substrate lysine by an isopeptide bond and adding a mass tag of 114.04 Da to the modified tryptic peptide.[37,39,73,74] This allows a searchable modification in bottom-up proteomic workflows, making trypsin digestion an effective approach for exploring ubiquitinated proteins, as outlined in Figure 3.1. It has been established in the literature that trypsin cleaves both unmodified and modified lysine residues in the substrate, the later producing a peptide with a terminal lysine modified with the GG tag.[26,28,32,71,73,75] Cleavage at GG-modified lysine residues was observed, for example, by Denis *et al.* in 39% of

tryptic peptides from an MCF7 lysate and Huebner *et al.* on 20% of the GG-peptides they identified in human urinary exosomes.[28,73] Burke *et al.* reported in a study of MDSC exosome lysate that 15 of 66 GG-modified tryptic peptides were identified with the terminal lysine of the peptide holding the modification.[26] A subsequent paper investigating this phenomenon with K48-linked diubiquitin confirmed that trypsin cleaves at the modified K48 about 16% of the time.[75] Crystal structures show that the binding pocket of trypsin is 10-12 Å deep[76,77] and the extended lysine chain with a GG-modification measures 10.9 Å and carries the requisite terminal basic residue.[75] Such studies bring to light the importance of considering peptide identifications with the GG-modification on the terminal lysine.

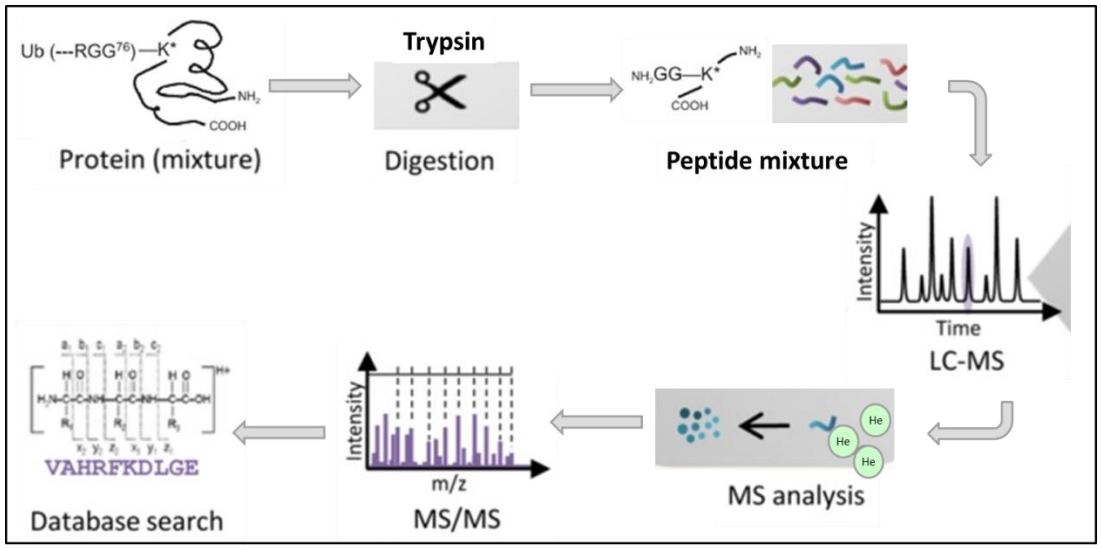

Figure 3.1. A bottom-up proteomic workflow for ubiquitinated proteins utilizing digestion by trypsin and LC-MS/MS with activation by CID. (Adapted from references 11 and 39).

Alkylation of cysteine residues after reduction and before digestion during bottom-up workflows is an important consideration when exploring ubiquitination as a PTM. Iodoacetamide (IAA) is a commonly used alkylating agent, adding a carbamidomethyl group, +57.0513 Da to cysteine residues. However, overalkylation has been reported, with IAA adding two carbamidomethyl groups to lysine residues.[7,8] The added mass is +114.04292 Da, exactly that of an added GG-tag, leading to possible false identifications. Chloroacetamide (ClAA) is an alternative alkylating agent as it does not overalkylate to the extent of IAA, but the same mass tag is still added and the problem could still arise.[7] To avoid this issue, methyl methanethiosulfonate (MMTS) has been used here, which does not readily alkylate lysine and adds a mass tag of +45.988 on cysteine residues to allow for no mass tag ambiguity.

3.2 Materials and methods

3.2.1 Materials

All materials and chemicals were purchased from Sigma Aldrich (St. Louis, MI) unless otherwise stated.

3.2.2 Biological sample preparation and protein analysis

MDSC-derived exosomes were isolated and purified by methods previously described.[26] Exosomes were stored at -80 °C until use. All procedures with animals and animal-derived materials were approved by the UMBC and UMCP Institutional Animal Care and Use Committees. Exosomes were first lysed and then digested by

trypsin, as previously described in Chapter 2, using MMTS as the alkylating agent. After LC-MS/MS analysis of three technical replicates for each of three biological replicates, spectra were searched with the fixed and variable modifications previously described in Chapter 2 and Figure 2.1, including the variable lysine modification of glycinylglycine to identify ubiquitinated peptides and proteins using PepArML. Subcellular location and function assignments of the identified proteins were made using the Protein Information Resource GO Slim (http://pir.georgetown.edu) using UniProt Gene Ontology annotations (05 2016). Hydrophobicity and chemical characteristic analyses were performed by ExPASy Bioinformatics Resource Portal ProtParam.[51] A summary of this bottom-up workflow is provided in Figure 2.1.

3.2.3 Immunoprecipitations

All IP experiments used protein A-functionalized sepharose beads added to snap cap spin columns and prepared by washing with PBS for 1 hour at 4 °C followed by additional washes of 0.1% BSA in PBS. Two IP procedures were evaluated to analyze the ubiquitinated proteins in the MDSC exosome lysate. In the first, the anti-ubiquitin antibody #3933S (Cell Signaling, 1:20) was incubated with the prepared beads for 4 hours at 4 °C followed by chemical crosslinking using DSS and incubation of 100 ug of exosome lysate with the antibody crosslinked beads overnight at 4 °C. In the second, the anti-ubiquitin antibody #19271 (Abcam, 5 ug) was incubated directly with 100 ug of exosome lysate for 1 hour at 4 °C followed by a 1 hour incubation with the prepared protein A-sepharose beads at 4 °C. For both antibodies, centrifugation at 1000 g for 5 minutes was performed directly after lysate incubation to collect the “non-bound” protein fraction. Low pH elution was then

performed twice with the addition of 0.2 M glycine solution (pH=2.6) incubated for 1 hour at 4 °C, collection by centrifugation at 13000 g for 5 minutes, and supernatant neutralization with 1 M Tris-base (pH=8.5). The eluate was buffer exchanged to 50 mM ammonium bicarbonate before further analysis.

3.2.4 Western blotting

Western blotting was performed by 1D SDS-Page with transfer onto PVDF membrane at 250 mA and 100 V for 50 minutes. Blocking was performed for 2 hours at 4 °C in 5% BSA TBS/T buffer. The primary antibody used was Cell Signaling rabbit anti-ubiquitin antibody #3933S, diluted 1:1000, and incubated at 4 °C for 14 hours. The secondary antibody used was Cell Signaling anti-mouse IgG HRP-linked antibody #7076, diluted 1:2000, and incubated at 4 °C for 2 hours. Imagining was performed using Super Signal West Dura Substrate Kit and a ChemiDoc Imager (Bio-Rad, Hercules, CA).

3.3 Results and discussion

3.3.1 Confirmation of ubiquitinated proteins by immunoprecipitation

It has previously been reported that MDSC exosomes contain ubiquitinated proteins.[26] This was confirmed by western blotting the exosome lysate for ubiquitin, shown in Figure 3.2a. Immunoprecipitation by two anti-ubiquitin antibodies was evaluated by western blotting to further confirm and visualize the presence of ubiquitinated proteins in the exosome lysate. Anti-ubiquitin antibody #3933 clearly showed an inefficient pull down when the eluate (Figure 3.2b) was compared to the

non-bound fraction (Figure 3.2c). Anti-ubiquitin antibody #19271 showed more efficient pull down of the higher MW ubiquitin band than the lower MW band, as seen in Figure 3.2e. Antibody bleeding during immunoprecipitation elution and subsequent western blot probing is an important consideration, as an eluted antibody would give false positive signals at 25 and 50 kDa for the light and heavy chains of the antibody. Negative controls were performed for each IP experiment of only antibody addition (#3933 and #19271 respectively) without any lysate protein addition. Blots are shown in Figures 3.2d and 3.2f respectively. Any antibody elution during the IP experiments is under the limit of detection by this western blot protocol.

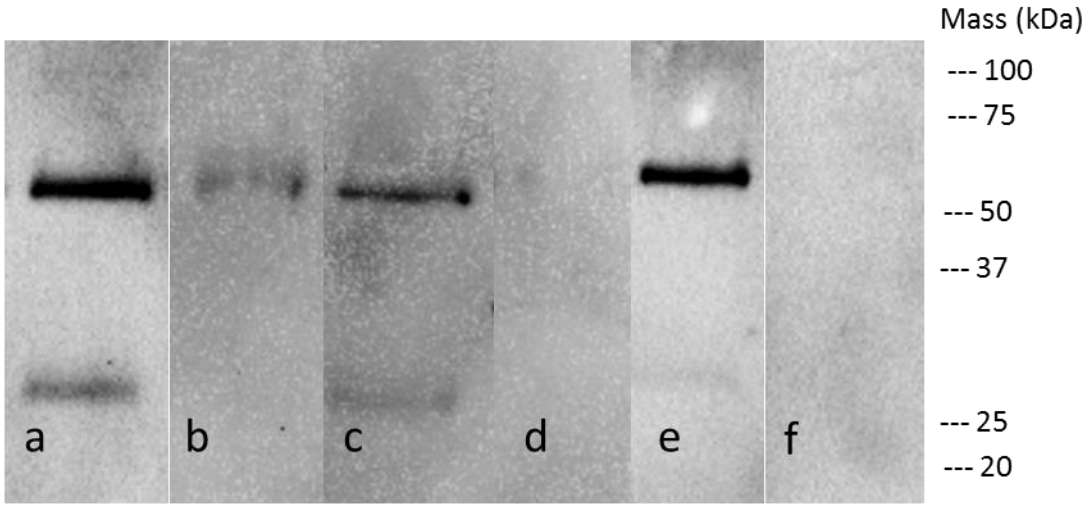

Figure 3.2a-f. Western blots probing with anti-ubiquitin antibody #3933 of **(a)** exosome lysate, **(b)** eluate of #3933 immunoprecipitation, **(c)** non-bound fraction from #3933 immunoprecipitation, **(d)** negative control of #3933 immunoprecipitation, **(e)** eluate of #19271 immunoprecipitation, **(f)** negative control of immunoprecipitation.

These three independent western blots for ubiquitin show discrete bands of ubiquitinated proteins. Similar discrete bands were also seen by Liu *et al*. and Buschow *et al.*, who hypothesized that these discrete bands were caused by dominantly monoubiquitinated proteins.[21,78] These results are consistent with the conclusion of Gilda *et al.* that there are differences in the affinities of anti-ubiquitin antibodies[72] and limits in their use for immunoprecipitation.

3.3.2 Bottom-up identification of ubiquitinated proteins

In order to carry out an unbiased study of the MDSC exosome ubiquitome, bottom-up proteomic strategies were employed directly on the lysate, without prior antibody enrichment. Utilizing tryptic digestion and the GG-tag variable modification in bioinformatic searches, 304 ubiquitinated proteins were identified in the MDSC exosomes based on 424 GG-peptides. A table of all the ubiquitinated proteins identified with the GG-modified peptide sequences is provided in Appendix Table 2. This indicates that a third of the protein cargo in these exosomes is ubiquitinated. A recent study looking for ubiquitinated proteins in human urinary exosomes also reported a high abundance, 13%, of ubiquitinated proteins.[28] Fifty percent of the GG sites identified experimentally are predicted as ubiquitination sites *in silico* by the ubiquitination prediction tool UbiProber[79] with probabilities greater than UbiProber's confidence level of 0.7. Based on the 424 ubiquitinated peptides and 489 GG-sites identified, 126 proteins were identified with multiple sites of ubiquitination. This multi-ubiquitinated protein cohort was also compared to the total ubiquitinated cohort and the total exosome lysate.

Unspecified polyubiquitins were identified based on three peptides that have a GG-modified lysine. The identified peptides indicate that polyubiquitins exist in the exosome lysate with branched points at K11, K48, and K63. The length and topology of the polyubiquitin chains, anchored or unanchored, cannot be identified from the peptides. Nine of the 30 identified 26S proteasome components were identified as ubiquitinated. One of these subunits, 26S proteasome regulatory subunit 4, has also been reported as ubiquitinated in urinary exosomes.[28] Seven of these ubiquitinated proteasome components are also identified as unconjugated at the same sites, including 26S proteasome regulatory subunit 4.

Chemical analyses including GRAVY scores (Figure 3.3a), isoelectric point (Figure 3.3b), and molecular weight distributions (Figure 3.4a-b) were carried out on the ubiquitinated and multi-ubiquitinated cohort and compared to those profiled for the whole exosome lysate. Three of the four largest proteins identified in the exosome lysate have multiple sites of ubiquitination; nesprin-1, E3 ubiquitin-protein ligase UBR4, and cytoplasmic dynein 1 heavy chain 1, seen in Figure 3.4b.

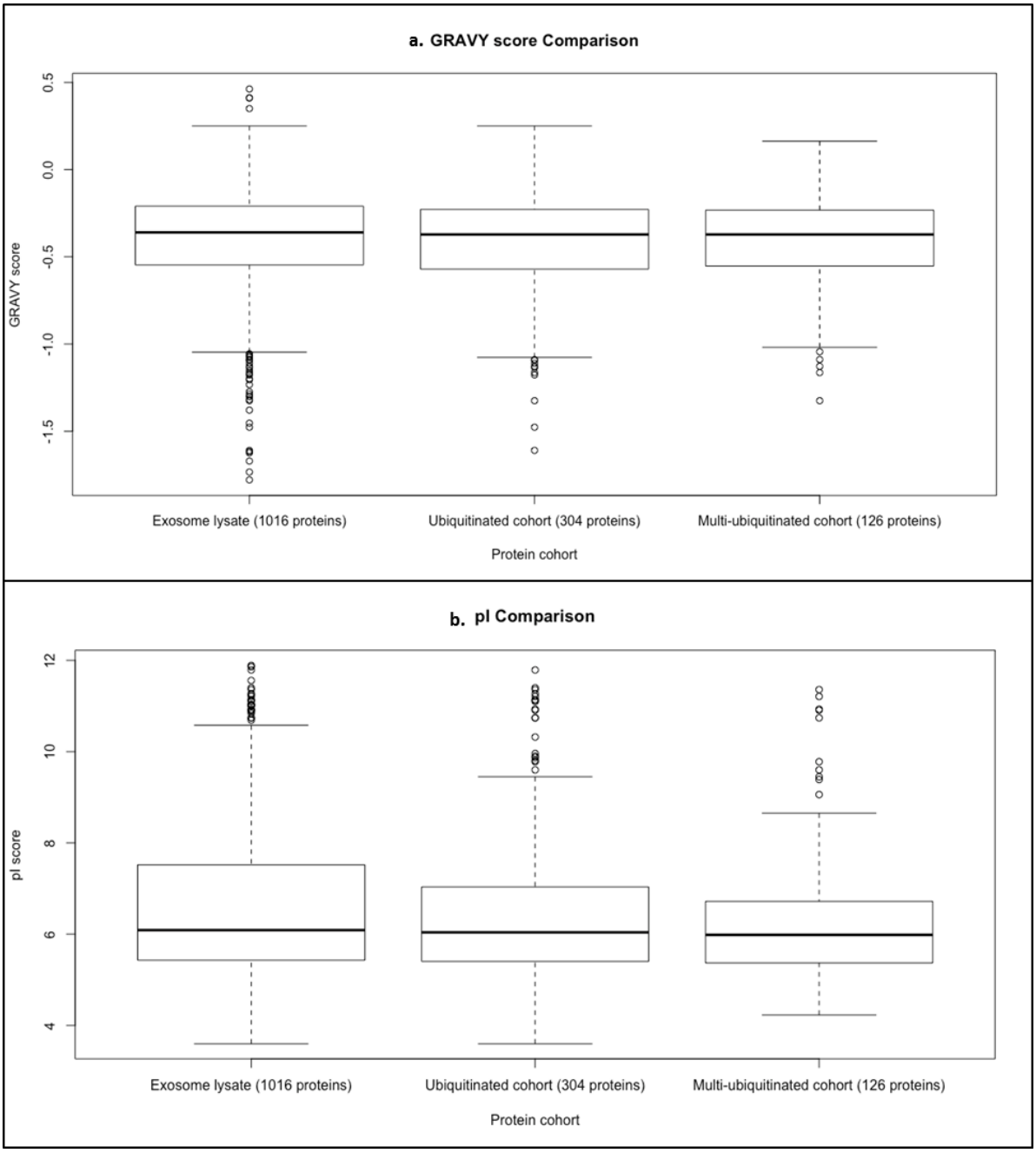

Figure 3.3a-b. The distribution of the **(a)** grand average for hydropathy, GRAVY, scores and the **(b)** isoelectric points, pI, of proteins identified in the exosome lysate, ubiquitinated cohort, and multi-ubiquitinated protein cohort. The bolded line is the data set median, the upper box is the top quartile, the whisker above it represents the maximum value, the lower box represents the lower quartile, the lower whisker represents the minimum value, and all dots are outliers.

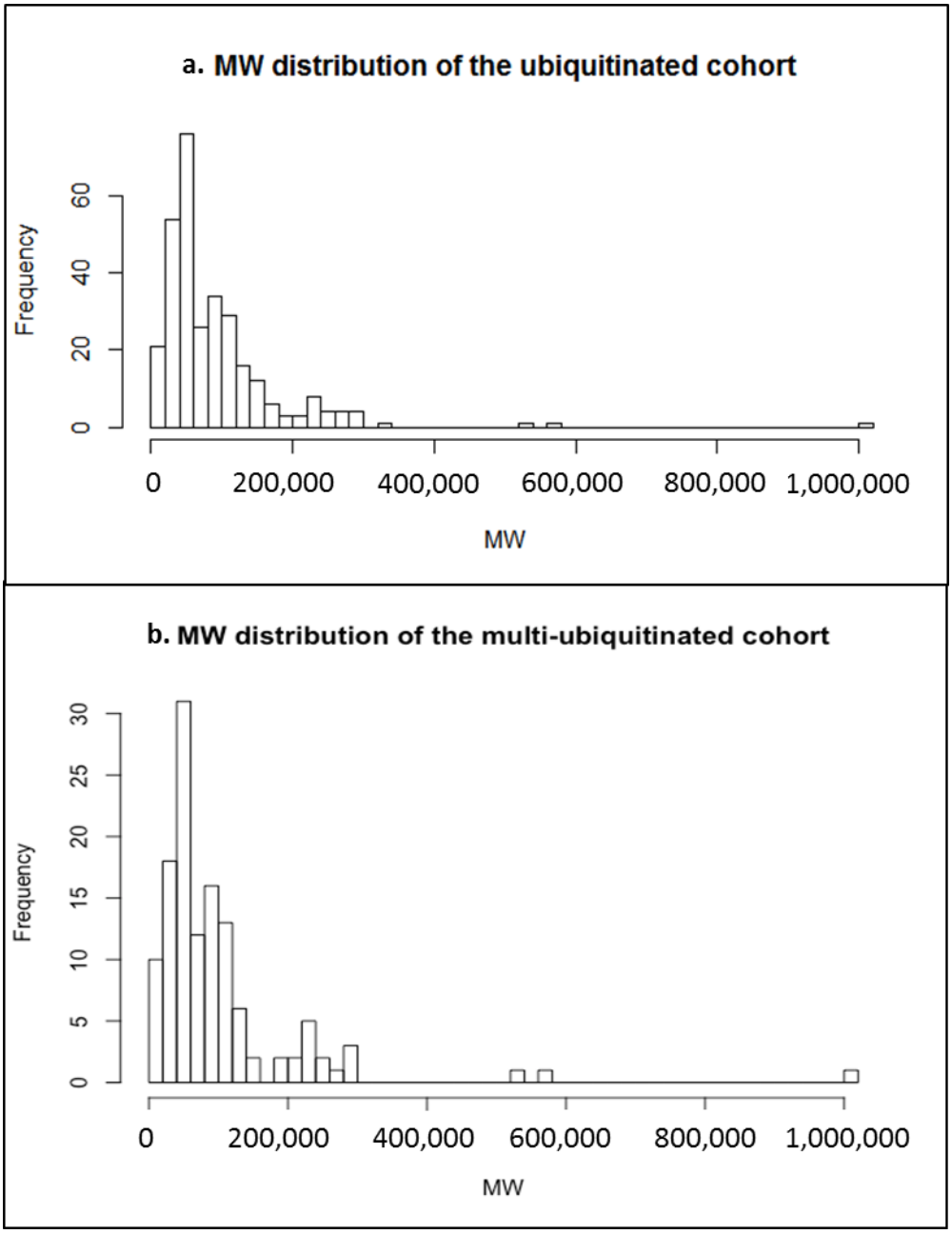

Figure 3.4a-b. MW distributions of substrate proteins in the **(a)** ubiquitinated cohort and **(b)** multi-ubiquitinated protein cohort.

Biological characteristics of three protein cohorts were compared; proteins identified as multi-ubiquitinated, all proteins identified as ubiquitinated (including those in the multi-ubiquitinated cohort), and all proteins identified in the exosome

proteome (including all proteins also identified as ubiquitinated). As shown in Figure 3.5a, the proteins conjugated by ubiquitin, orange bars, are enriched in nucleoside and nucleotide binding functions compared to the entire lysate, and comparable enrichment can be seen in these two nucleic acid-related categories in the multi-ubiquitinated protein subset, green bars. Eleven of 17 histones, 4 of 20 ribosomal proteins, and 4 of 11 transcription factors were found to be ubiquitinated which contribute to these categories. In Figure 3.5b, it can be seen that ubiquitinated proteins are more likely to originate from the membrane and cytoskeleton of the parent cell, consistent with the proven role of ubiquitination in invagination[20] and also from the nucleus.

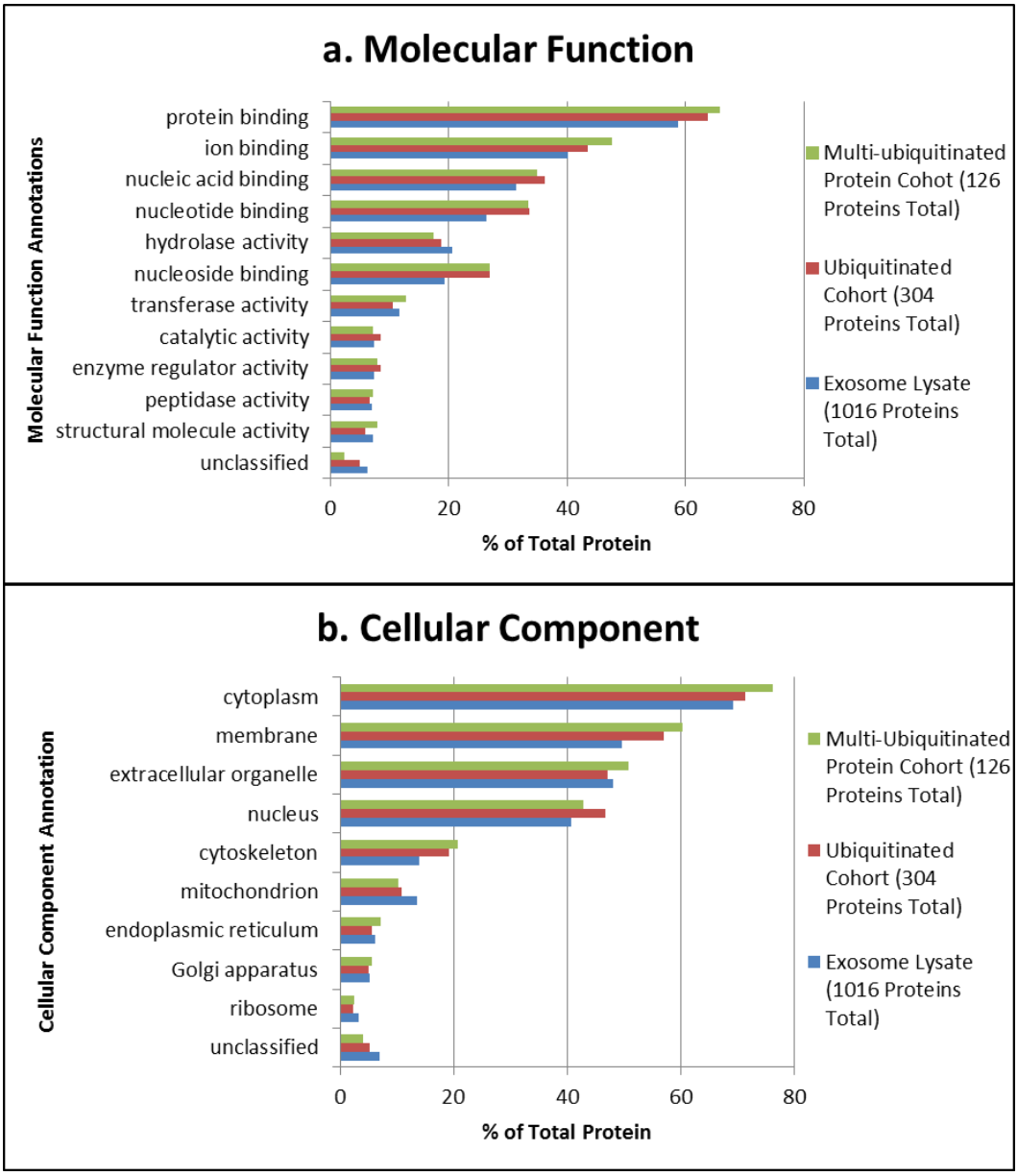

Figure 3.5a-b. Comparisons of GO annotations for the parent cells of **(a)** molecular functions and **(b)** intracellular protein locations of conjugated proteins in the multi-ubiquitinated protein cohort (green), all conjugated proteins in the ubiquitinated cohort (orange), and all proteins in the exosome lysate (blue). Proteins in the multi-ubiquitinated cohort are included in the total ubiquitinated cohort, and proteins identified in the total ubiquitinated cohort are included in the exosome lysate cohort. Proteins occur in multiple categories.

Three hundred seventeen of the 424 ubiquitinated tryptic peptides characterized carry the glycinylglycine modification on the lysine at the carboxyl terminal. These peptide identifications were manually confirmed, and an example of an annotated spectrum of a GG-modified tryptic peptide from myosin-9 is shown in Figure 3.6.

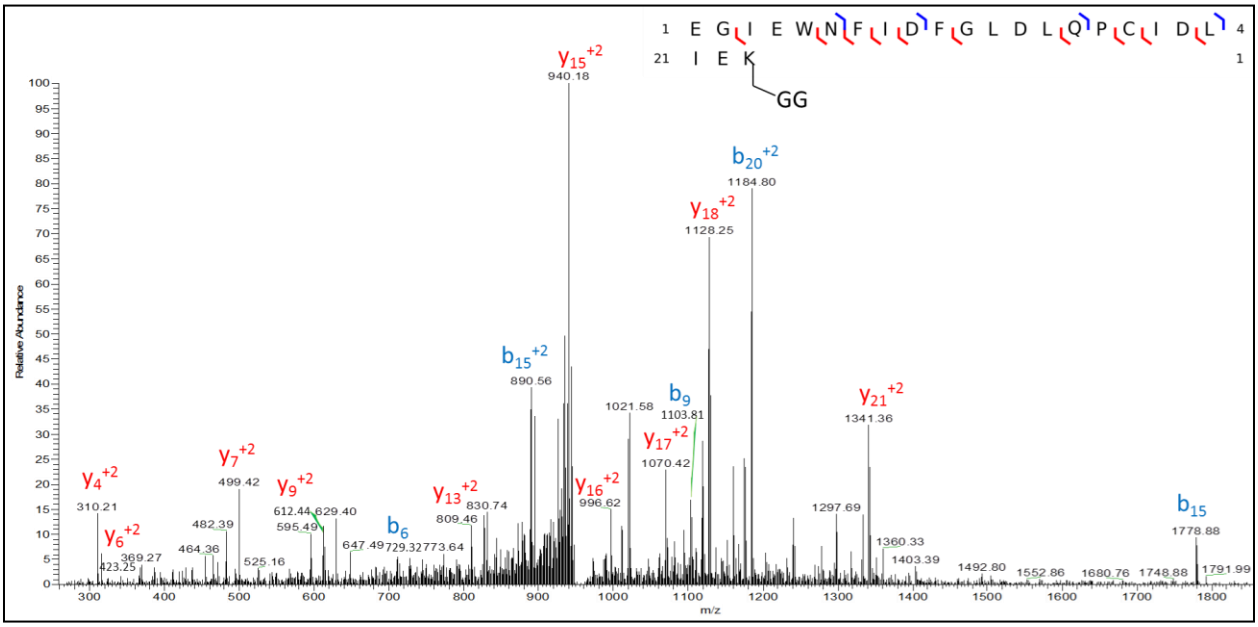

Figure 3.6. Manually annotated spectrum of a terminal K-GG peptide (residue 494-517) of myosin-9.

3.4 Conclusions

This study reports 304 ubiquitinated proteins identified in MDSC-derived exosomes. Ubiquitinated proteins must be high in abundance in MDSC exosomes, as they were identified and characterized without prior antibody enrichment. Taking into consideration previous antibody-based analyses of this biological sample,[26] a

total of 343 ubiquitinated proteins have been identified in MDSC exosomes, indicating that over 33% of the total exosome lysate is ubiquitinated. This fraction may be higher as there may be GG-peptides not selected during mass spectral analysis. This method utilized the superior sensitivity of state of the art mass spectrometry for this in depth analysis. These findings further support previous conclusions[26,28,73,75] that peptide identifications with modified terminal lysine(s) need to be included in lysine PTM analyses. Transwell migration assays (private communication from Professor Suzanne Ostrand-Rosenberg) have shown that some component(s) of the MDSC exosome ubiquitome are chemotactic.

Chemical characteristics of the proteins conjugated by ubiquitin were found to be similar to those of all the proteins in the exosome lysate. However, proteins that bind nucleosides and nucleotides in the parent cell appear to be favored for ubiquitination as exosome cargo. For example, 11 of 17 histones are identified as ubiquitinated, along with 4 ribosomal proteins and 4 transcription factors.

Although there is evidence for K-11, K-48, and K-63 polyubiquitins in the exosome lysate, top-down studies on intact proteins are needed to characterize the extent and nature of ubiquitination. One hundred twenty-six proteins were identified as multi-ubiquitinated. Top-down experiments could investigate whether multi-ubiquitination happens on the same proteoform.

Currently it is assumed that selective sorting takes place in proteins that end up in multivesicular bodies that are released as exosomes. The mechanism for such protein sorting into exosomes is highly contested. As was discussed in Chapter 1, there is recent experimental evidence for two roles of ubiquitin in cargo sorting in

MVBs destined to be released as exosomes: that deubiquitination must occur during MVB sorting,[33,41] and the contrary view that the ubiquitination tag is needed for protein sorting into MVBs.[21,28] The observations reported in Chapter 3 that a large and diverse fraction of the exosome protein cargo is ubiquitinated in MDSC exosomes appears to argue against the hypothesis that deubiquitination is necessary for proteins to be sorted into MVBs destined to be released as exosomes. However, the results present in Chapter 2 identified a full set of the enzymes required for ubiquitination and deubiquitination. This raises the possibility that conjugation of ubiquitin may be a dynamic process within exosomes that obscures the role of external ubiquitination.

Appendices

Appendix Table 1. Proteins identified in MDSC exosomes

Accession Number	Protein Name	No. of non-overlapping peptides	% Coverage
A0A087WQS2	Basic leucine zipper and W2 domain-containing protein 1	2	11.8%
A0A087WR50	Fibronectin	71	56.4%
A0A087WRZ5	TAR DNA-binding protein 43	3	20.4%
A0A0A0MQ90	Protein S100-A13	2	14.4%
A0A0A0MQG2	Spectrin beta chain, non-erythrocytic 1 (Fragment)	25	19.6%
A0JNY3	Gephyrin	3	9.6%
A2A4J1	Proteasome activator complex subunit 3 (Fragment)	2	11.8%
A2A513	Keratin, type I cytoskeletal 10	5	27.3%
A2A841	Protein 4.1	2	3.9%
A2ACG7	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	7	24.9%
A2AD84	Serine/threonine-protein kinase 26	2	7.9%
A2AE27	AMP deaminase 2	7	12.0%
A2AFF6	Cohesin subunit SA-2	2	2.8%
A2AFQ2	3-hydroxyacyl-CoA dehydrogenase type-2	5	39.5%
A2AH85	116 kDa U5 small nuclear ribonucleoprotein component	19	31.8%
A2AN08	E3 ubiquitin-protein ligase UBR4	2	0.8%
A2AP78	High mobility group protein B3 (Fragment)	3	24.5%
A2AQ07	Tubulin beta-1 chain	13	44.4%
A2ATU9	Histone acetyltransferase type B catalytic subunit	2	7.3%
A2AUR7	Ras suppressor protein 1	3	26.2%
A2AWT7	Nucleolar transcription factor 1	2	4.0%
A2BDW0	Tyrosine-protein kinase BTK	3	8.9%
A2CES4	U2 small nuclear ribonucleoprotein B" (Fragment)	2	18.9%
A2RTH5	Leucine carboxyl methyltransferase 1	2	9.0%
A6PWC3	Nardilysin	8	12.4%
B1AR28	Very long-chain-specific acyl-CoA dehydrogenase, mitochondrial	3	8.8%
B1ATU4	COP9 signalosome complex subunit 1	2	11.4%

B1ATY1	Actin, cytoplasmic 2	11	84.3%
B1AUY2	Protein Arhgap4	5	9.6%
B2M1R6	Heterogeneous nuclear ribonucleoprotein K	8	27.1%
B2RV77	MCG130182, isoform CRA_a	4	53.6%
B2RXR6	Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit B	3	4.4%
B5THE2	Maltase-glucoamylase	10	7.8%
B7FAU9	Filamin, alpha	81	51.5%
B7ZCL7	H/ACA ribonucleoprotein complex subunit 4 (Fragment)	2	13.1%
B9EJ54	MCG21756, isoform CRA_b	4	3.0%
D3YUQ9	Elongation factor 1-delta (Fragment)	4	31.4%
D3YW48	Calpain small subunit 1 (Fragment)	9	78.4%
D3YWF6	Ubiquitin thioesterase OTUB1	4	30.7%
D3YX27	Serine protease HTRA2, mitochondrial	2	10.3%
D3YYD0	Olfactomedin-4	10	24.2%
D3YYD5	Vacuolar protein sorting-associated protein 29 (Fragment)	2	26.6%
D3YYM6	40S ribosomal protein S5 (Fragment)	2	15.4%
D3Z315	Coatomer subunit epsilon (Fragment)	3	26.4%
D3Z5M4	Ankyrin-1	14	12.6%
D3Z627	Integrin alpha-L	9	13.7%
D3Z629	60S ribosomal protein L9	2	19.9%
D3Z6Q9	Bridging integrator 2	5	17.2%
D3Z712	40S ribosomal protein S15a (Fragment)	2	31.9%
D3Z7R7	Minor histocompatibility protein HA-1	7	11.8%
D4AFX7	Protein Dnajc13	5	4.6%
E0CXA0	Hepatoma-derived growth factor (Fragment)	1	8.4%
E9PUE7	Active breakpoint cluster region-related protein	4	9.2%
E9PUF7	Rho guanine nucleotide exchange factor 1	9	15.6%
E9PV24	Fibrinogen alpha chain	13	29.4%
E9PV60	Protein Wdfy4	2	1.2%
E9PVA8	Protein Gcn1l1	3	2.2%
E9PW39	Putative helicase MOV-10	3	5.0%
E9PWG6	Protein Ncapg	2	3.0%
E9PWY9	Phenylalanine--tRNA ligase alpha subunit	3	9.5%
E9PYD5	Transcription elongation factor A protein 1	2	13.1%
E9PYM7	Alpha-mannosidase (Fragment)	2	2.8%
E9PYV4	Protein Niban	7	24.7%
E9PZF0	Nucleoside diphosphate kinase	9	48.3%
E9Q070	Uncharacterized protein	6	32.8%
E9Q0K6	Deoxynucleoside triphosphate	22	53.2%

	triphosphohydrolase SAMHD1		
E9Q1N8	Uncharacterized protein	2	9.6%
E9Q1S3	Protein transport protein Sec23A	5	13.2%
E9Q397	Spectrin beta chain, erythrocytic	48	33.8%
E9Q3X0	Major vault protein	30	57.9%
E9Q450	Tropomyosin alpha-1 chain	7	31.7%
E9Q604	Integrin alpha-M	31	46.9%
E9Q800	MICOS complex subunit Mic60	2	5.7%
E9Q8H9	Complement factor H	2	3.1%
E9QAI5	CAD protein	5	3.7%
E9QKR0	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	4	16.8%
E9QKT1	Ubiquitin-protein ligase E3A	2	3.8%
E9QKZ2	Importin-9	6	13.8%
E9QNN1	ATP-dependent RNA helicase A	15	20.5%
F6QA74	DNA-(apurinic or apyrimidinic site) lyase (Fragment)	5	36.3%
F6RPJ9	Insulin-degrading enzyme (Fragment)	2	2.6%
F6RQA2	Ribosomal protein S6 kinase	4	7.7%
F6UND7	Tyrosine-protein kinase HCK	5	16.6%
F6W687	Non-histone chromosomal protein HMG-17 (Fragment)	3	41.1%
F6YVP7	Uncharacterized protein	4	24.3%
F6YY69	14-3-3 protein theta (Fragment)	9	40.4%
F7CHQ7	Nucleolar protein 56 (Fragment)	2	12.6%
F8VPN4	Protein Agl	2	2.3%
F8VPX1	Ubiquitin carboxyl-terminal hydrolase	12	17.4%
F8WGL3	Cofilin-1	6	42.3%
F8WGM5	Syntaxin-binding protein 2 (Fragment)	5	15.5%
F8WHL2	Coatomer subunit alpha	8	10.7%
F8WHZ9	Alpha-adducin	3	6.2%
F8WI35	Histone H3	5	60.0%
G3UVV6	MCG123217	3	3.8%
G3UXZ5	Proteasome activator complex subunit 1 (Fragment)	3	18.9%
G3UY93	Valine--tRNA ligase (Fragment)	12	18.6%
G3UYY1	Serine hydroxymethyltransferase (Fragment)	3	10.7%
G3UZG5	Kelch domain-containing protein 4	2	7.6%
G3X8U3	MCG6895	2	10.4%
G3X8Y3	N-alpha-acetyltransferase 15, NatA auxiliary subunit	3	7.4%
G3X925	Pyruvate kinase	3	9.1%

G3X956	FACT complex subunit SPT16	2	4.1%
G3X972	Protein Sec24c	5	9.1%
G3X9G4	Dynamin-2	13	30.0%
G3X9I4	Aly/REF export factor 2	3	25.7%
G3X9T8	Ceruloplasmin	9	15.1%
G5E866	Splicing factor 3B subunit 1	13	18.6%
G5E8N5	L-lactate dehydrogenase	13	60.4%
G5E924	Heterogeneous nuclear ribonucleoprotein L (Fragment)	9	33.2%
H3BKH6	S-formylglutathione hydrolase	5	39.0%
H3BKN2	Alkyldihydroxyacetonephosphate synthase, peroxisomal	2	6.0%
H3BLE7	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	4	11.9%
I7HPV9	Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 1 protein	2	2.8%
J3QMV5	Cytokine receptor-like factor 3	2	21.4%
J3QPG5	Prosaposin	2	4.7%
K3W4L0	Unconventional myosin-XVIIIa	3	3.1%
K3W4R2	Myosin-14	6	4.1%
O08553	Dihydropyrimidinase-related protein 2	5	19.6%
O08582	GTP-binding protein 1	3	8.1%
O08663	Methionine aminopeptidase 2	2	8.0%
O08692	Myeloid bactenecin (F1)	10	71.9%
O08709	Peroxiredoxin-6	9	64.7%
O08738	Caspase-6	3	13.4%
O08739	AMP deaminase 3	11	26.4%
O08749	Dihydrolipoyl dehydrogenase, mitochondrial	6	24.0%
O08795	Glucosidase 2 subunit beta	2	4.2%
O08992	Syntenin-1	7	58.5%
O09061	Proteasome subunit beta type-1	9	58.8%
O09106	Histone deacetylase 1	8	25.3%
O09159	Lysosomal alpha-mannosidase	14	25.7%
O09172	Glutamate--cysteine ligase regulatory subunit	2	11.3%
O35129	Prohibitin-2	2	10.4%
O35286	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	8	14.5%
O35295	Transcriptional activator protein Pur-beta	3	25.3%
O35343	Importin subunit alpha-3	4	15.6%
O35350	Calpain-1 catalytic subunit	14	24.3%
O35593	26S proteasome non-ATPase regulatory subunit 14	6	41.3%

O35639	Annexin A3	15	63.2%
O35643	AP-1 complex subunit beta-1	16	31.7%
O35737	Heterogeneous nuclear ribonucleoprotein H	4	13.4%
O35744	Chitinase-like protein 3	15	60.1%
O35841	Apoptosis inhibitor 5	5	16.9%
O35864	COP9 signalosome complex subunit 5	3	13.8%
O35930	Platelet glycoprotein Ib alpha chain	4	10.6%
O35955	Proteasome subunit beta type-10	2	14.7%
O54774	AP-3 complex subunit delta-1	2	2.8%
O54824	Pro-interleukin-16	3	4.0%
O54890	Integrin beta-3	3	6.1%
O54962	Barrier-to-autointegration factor	2	42.7%
O55013	Trafficking protein particle complex subunit 3	2	15.0%
O55029	Coatomer subunit beta'	8	14.1%
O55098	Serine/threonine-protein kinase 10	2	4.1%
O55131	Septin-7	3	13.1%
O55222	Integrin-linked protein kinase	8	25.0%
O55234	Proteasome subunit beta type-5	7	40.9%
O70138	Neutrophil collagenase	14	38.1%
O70145	Neutrophil cytosol factor 2	12	35.4%
O70194	Eukaryotic translation initiation factor 3 subunit D	9	27.6%
O70251	Elongation factor 1-beta	3	26.7%
O70310	Glycylpeptide N-tetradecanoyltransferase 1	4	15.9%
O70325	Phospholipid hydroperoxide glutathione peroxidase, mitochondrial	2	13.2%
O70435	Proteasome subunit alpha type-3	5	23.1%
O70497	Ficolin-2	2	15.6%
O70591	Prefoldin subunit 2	2	16.9%
O88307	Sortilin-related receptor	17	11.3%
O88342	WD repeat-containing protein 1	18	56.3%
O88456	Calpain small subunit 1	9	70.6%
O88487	Cytoplasmic dynein 1 intermediate chain 2	3	10.6%
O88543	COP9 signalosome complex subunit 3	2	7.3%
O88544	COP9 signalosome complex subunit 4	8	31.3%
O88545	COP9 signalosome complex subunit 6	3	21.6%
O88569	Heterogeneous nuclear ribonucleoproteins A2/B1	12	43.3%
O88593	Peptidoglycan recognition protein 1	5	50.0%
O88685	26S protease regulatory subunit 6A	10	40.1%
O88696	ATP-dependent Clp protease proteolytic subunit, mitochondrial	3	17.7%

O88783	Coagulation factor V	3	1.9%
O88842	FYVE, RhoGEF and PH domain-containing protein 3	2	5.5%
O88844	Isocitrate dehydrogenase [NADP] cytoplasmic	15	50.2%
O88879	Apoptotic protease-activating factor 1	2	2.3%
O88958	Glucosamine-6-phosphate isomerase 1	2	8.3%
O88968	Transcobalamin-2	4	19.5%
O89051	Integral membrane protein 2B	2	8.7%
O89053	Coronin-1A	15	52.7%
O89086	Putative RNA-binding protein 3	2	32.7%
P00493	Hypoxanthine-guanine phosphoribosyltransferase	6	41.3%
P00920	Carbonic anhydrase 2	4	30.0%
P01027	Complement C3	70	61.6%
P01325	Insulin-1	2	18.5%
P01837	Ig kappa chain C region	2	50.0%
P01872	Ig mu chain C region	12	39.4%
P01900	H-2 class I histocompatibility antigen, D-D alpha chain	2	6.9%
P01942	Hemoglobin subunit alpha	7	83.8%
P02088	Hemoglobin subunit beta-1	11	95.2%
P02089	Hemoglobin subunit beta-2	11	91.2%
P02301	Histone H3.3C	6	60.3%
P02535	Keratin, type I cytoskeletal 10	5	26.7%
P03958	Adenosine deaminase	2	8.5%
P04104	Keratin, type II cytoskeletal 1	3	13.0%
P04202	Transforming growth factor beta-1	2	8.2%
P04919	Band 3 anion transport protein	13	27.1%
P05064	Fructose-bisphosphate aldolase A	16	77.2%
P05201	Aspartate aminotransferase, cytoplasmic	5	23.2%
P05202	Aspartate aminotransferase, mitochondrial	7	30.0%
P06745	Glucose-6-phosphate isomerase	22	69.7%
P07356	Annexin A2	14	59.9%
P07724	Serum albumin	18	40.6%
P07742	Ribonucleoside-diphosphate reductase large subunit	8	20.0%
P07759	Serine protease inhibitor A3K	4	17.7%
P07901	Heat shock protein HSP 90-alpha	24	48.3%
P08003	Protein disulfide-isomerase A4	13	33.4%
P08030	Adenine phosphoribosyltransferase	7	61.1%
P08032	Spectrin alpha chain, erythrocytic 1	73	47.7%
P08071	Lactotransferrin	38	75.4%

P08113	Endoplasmin	9	18.2%
P08226	Apolipoprotein E	6	38.3%
P08228	Superoxide dismutase [Cu-Zn]	4	32.5%
P08249	Malate dehydrogenase, mitochondrial	8	35.8%
P08607	C4b-binding protein	5	13.2%
P08752	Guanine nucleotide-binding protein G(i) subunit alpha-2	9	35.5%
P08905	Lysozyme C-2	3	33.1%
P09103	Protein disulfide-isomerase	18	51.3%
P09405	Nucleolin	11	21.4%
P09411	Phosphoglycerate kinase 1	14	53.5%
P09528	Ferritin heavy chain	8	68.7%
P09542	Myosin light chain 3	2	12.3%
P09671	Superoxide dismutase [Mn], mitochondrial	2	12.6%
P0C0S6	Histone H2A.Z	5	58.6%
P0CG50	Polyubiquitin-C	6	9.8%
P10107	Annexin A1	16	64.5%
P10126	Elongation factor 1-alpha 1	13	48.1%
P10518	Delta-aminolevulinic acid dehydratase	6	38.5%
P10639	Thioredoxin	3	31.4%
P10649	Glutathione S-transferase Mu 1	7	42.2%
P10810	Monocyte differentiation antigen CD14	3	13.4%
P10852	4F2 cell-surface antigen heavy chain	3	12.7%
P11031	Activated RNA polymerase II transcriptional coactivator p15	4	37.8%
P11247	Myeloperoxidase	27	50.4%
P11352	Glutathione peroxidase 1	7	49.3%
P11499	Heat shock protein HSP 90-beta	23	50.4%
P11672	Neutrophil gelatinase-associated lipocalin	6	51.0%
P11835	Integrin beta-2	16	33.6%
P11983	T-complex protein 1 subunit alpha	12	39.6%
P12246	Serum amyloid P-component	2	14.3%
P12265	Beta-glucuronidase	7	19.1%
P12382	ATP-dependent 6-phosphofructokinase, liver type	3	6.3%
P12787	Cytochrome c oxidase subunit 5A, mitochondrial	4	51.4%
P12815	Programmed cell death protein 6	6	52.4%
P13020	Gelsolin	19	48.0%
P13439	Uridine 5'-monophosphate synthase	2	6.4%
P13864	DNA (cytosine-5)-methyltransferase 1	3	3.8%
P14069	Protein S100-A6	6	80.9%
P14131	40S ribosomal protein S16	4	30.1%

P14152	Malate dehydrogenase, cytoplasmic	6	26.4%
P14206	40S ribosomal protein SA	10	52.2%
P14211	Calreticulin	9	46.2%
P14234	Tyrosine-protein kinase Fgr	5	18.0%
P14685	26S proteasome non-ATPase regulatory subunit 3	11	36.2%
P14733	Lamin-B1	9	25.5%
P14824	Annexin A6	10	22.3%
P15532	Nucleoside diphosphate kinase A	7	65.8%
P15864	Histone H1.2	6	37.7%
P16110	Galectin-3	7	40.9%
P16125	L-lactate dehydrogenase B chain	3	12.9%
P16546	Spectrin alpha chain, non-erythrocytic 1	48	34.4%
P16858	Glyceraldehyde-3-phosphate dehydrogenase	13	67.0%
P16879	Tyrosine-protein kinase Fes/Fps	3	6.1%
P17095	High mobility group protein HMG-I/HMG-Y	1	23.4%
P17182	Alpha-enolase	19	70.1%
P17225	Polypyrimidine tract-binding protein 1	9	33.0%
P17426	AP-2 complex subunit alpha-1	9	15.2%
P17427	AP-2 complex subunit alpha-2	16	30.1%
P17433	Transcription factor PU.1	2	8.5%
P17710	Hexokinase-1	6	9.5%
P17742	Peptidyl-prolyl cis-trans isomerase A	8	70.7%
P17751	Triosephosphate isomerase	9	48.2%
P17879	Heat shock 70 kDa protein 1B	5	15.0%
P18242	Cathepsin D	5	23.2%
P19096	Fatty acid synthase	30	21.2%
P19157	Glutathione S-transferase P 1	3	20.5%
P19973	Lymphocyte-specific protein 1	6	34.9%
P20029	78 kDa glucose-regulated protein	21	49.6%
P20060	Beta-hexosaminidase subunit beta	5	17.7%
P20152	Vimentin	19	51.3%
P21107-2	Isoform 2 of Tropomyosin alpha-3 chain	8	41.1%
P21550	Beta-enolase	9	29.3%
P22682	E3 ubiquitin-protein ligase CBL	2	3.0%
P22892	AP-1 complex subunit gamma-1	10	20.2%
P23116	Eukaryotic translation initiation factor 3 subunit A	12	14.7%
P24270	Catalase	14	40.8%
P24369	Peptidyl-prolyl cis-trans isomerase B	7	38.9%
P24527	Leukotriene A-4 hydrolase	13	33.1%
P24547	Inosine-5'-monophosphate dehydrogenase 2	5	17.1%

P25206	DNA replication licensing factor MCM3	5	12.4%
P25911-2	Isoform 2 of Tyrosine-protein kinase Lyn	13	44.4%
P26039	Talin-1	72	50.3%
P26040	Ezrin	14	31.1%
P26041	Moesin	26	60.0%
P26043	Radixin	16	37.7%
P26369	Splicing factor U2AF 65 kDa subunit	6	25.1%
P26443	Glutamate dehydrogenase 1, mitochondrial	16	45.7%
P26516	26S proteasome non-ATPase regulatory subunit 7	8	53.0%
P26638	Serine--tRNA ligase, cytoplasmic	3	10.7%
P27005	Protein S100-A8	5	94.4%
P27048	Small nuclear ribonucleoprotein-associated protein B	2	12.6%
P27612	Phospholipase A-2-activating protein	6	13.7%
P27661	Histone H2AX	6	60.8%
P27773	Protein disulfide-isomerase A3	21	57.2%
P27870	Proto-oncogene vav	10	17.6%
P28063	Proteasome subunit beta type-8	8	40.9%
P28076	Proteasome subunit beta type-9	4	24.7%
P28293	Cathepsin G	9	50.2%
P28650	Adenylosuccinate synthetase isozyme 1	9	31.3%
P28656	Nucleosome assembly protein 1-like 1	4	14.6%
P28658	Ataxin-10	2	4.4%
P28665	Murinoglobulin-1	5	6.8%
P28867	Protein kinase C delta type	4	12.3%
P29341	Polyadenylate-binding protein 1	11	23.4%
P29351	Tyrosine-protein phosphatase non-receptor type 6	17	44.0%
P29391	Ferritin light chain 1	7	53.0%
P29416	Beta-hexosaminidase subunit alpha	4	11.4%
P29699	Alpha-2-HS-glycoprotein	2	15.4%
P29758	Ornithine aminotransferase, mitochondrial	4	24.2%
P30416	Peptidyl-prolyl cis-trans isomerase FKBP4	4	14.0%
P30681	High mobility group protein B2	6	37.1%
P31725	Protein S100-A9	8	83.2%
P31938	Dual specificity mitogen-activated protein kinase kinase 1	3	16.3%
P32067	Lupus La protein homolog	4	12.8%
P32921	Tryptophan--tRNA ligase, cytoplasmic	2	6.9%
P34884	Macrophage migration inhibitory factor	3	35.7%
P35174-2	Isoform 2 of Stefin-2	3	45.6%

P35505	Fumarylacetoacetase	7	31.0%
P35564	Calnexin	4	9.0%
P35585	AP-1 complex subunit mu-1	6	21.8%
P35700	Peroxiredoxin-1	6	32.2%
P35821	Tyrosine-protein phosphatase non-receptor type 1	2	5.6%
P35980	60S ribosomal protein L18	3	19.7%
P38060	Hydroxymethylglutaryl-CoA lyase, mitochondrial	4	24.0%
P38647	Stress-70 protein, mitochondrial	5	10.5%
P39655	Arachidonate 12-lipoxygenase, 12S-type	5	13.0%
P40124	Adenylyl cyclase-associated protein 1	16	57.4%
P40142	Transketolase	27	67.9%
P40237	CD82 antigen	2	8.7%
P40336	Vacuolar protein sorting-associated protein 26A	3	12.8%
P41241	Tyrosine-protein kinase CSK	3	9.8%
P41245	Matrix metalloproteinase-9	24	54.7%
P42227	Signal transducer and activator of transcription 3	8	20.9%
P42669	Transcriptional activator protein Pur-alpha	3	35.2%
P42932	T-complex protein 1 subunit theta	11	25.9%
P43274	Histone H1.4	6	33.8%
P43275	Histone H1.1	4	31.0%
P43276	Histone H1.5	6	30.9%
P43277	Histone H1.3	6	33.5%
P43430	Mast cell protease 8	1	6.1%
P45376	Aldose reductase	2	13.9%
P45591	Cofilin-2	2	16.9%
P45952	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	2	10.5%
P46062	Signal-induced proliferation-associated protein 1	2	3.5%
P46467	Vacuolar protein sorting-associated protein 4B	8	30.9%
P46471	26S protease regulatory subunit 7	6	22.2%
P46664	Adenylosuccinate synthetase isozyme 2	3	10.5%
P47738	Aldehyde dehydrogenase, mitochondrial	21	74.0%
P47754	F-actin-capping protein subunit alpha-2	7	42.3%
P47757-2	Isoform 2 of F-actin-capping protein subunit beta	12	67.7%
P47791	Glutathione reductase, mitochondrial	14	57.6%
P47809	Dual specificity mitogen-activated protein kinase kinase 4	2	8.6%
P47811	Mitogen-activated protein kinase 14	6	23.9%
P47856	Glutamine--fructose-6-phosphate	2	4.3%

	aminotransferase [isomerizing] 1		
P47911	60S ribosomal protein L6	3	13.9%
P47941	Crk-like protein	2	13.2%
P47955	60S acidic ribosomal protein P1	3	72.8%
P47962	60S ribosomal protein L5	5	21.2%
P47968	Ribose-5-phosphate isomerase	2	10.9%
P48025	Tyrosine-protein kinase SYK	18	49.4%
P48036	Annexin A5	8	37.9%
P49312	Heterogeneous nuclear ribonucleoprotein A1	8	35.9%
P49717	DNA replication licensing factor MCM4	3	5.2%
P49722	Proteasome subunit alpha type-2	8	60.3%
P49962	Signal recognition particle 9 kDa protein	2	25.6%
P50247	Adenosylhomocysteinase	8	30.3%
P50396	Rab GDP dissociation inhibitor alpha	7	30.9%
P50516	V-type proton ATPase catalytic subunit A	12	39.9%
P50518	V-type proton ATPase subunit E 1	3	20.8%
P50543	Protein S100-A11	2	27.6%
P50580	Proliferation-associated protein 2G4	16	50.3%
P51125	Calpastatin	3	9.1%
P51150	Ras-related protein Rab-7a	2	12.1%
P51174	Long-chain specific acyl-CoA dehydrogenase, mitochondrial	11	34.0%
P51437	Cathelin-related antimicrobial peptide	2	19.7%
P51863	V-type proton ATPase subunit d 1	2	11.4%
P52196	Thiosulfate sulfurtransferase	2	10.4%
P52480	Pyruvate kinase PKM	23	69.3%
P52624	Uridine phosphorylase 1	9	56.6%
P54071	Isocitrate dehydrogenase [NADP], mitochondrial	9	27.0%
P54775	26S protease regulatory subunit 6B	9	42.3%
P54822	Adenylosuccinate lyase	2	6.8%
P54823	Probable ATP-dependent RNA helicase DDX6	11	39.1%
P54923	Protein ADP-ribosylarginine hydrolase, [Protein ADP-ribosylarginine] hydrolase	2	12.7%
P54987	Cis-aconitate decarboxylase	2	10.5%
P55258	Ras-related protein Rab-8A	3	17.9%
P55264	Adenosine kinase	3	13.0%
P56399	Ubiquitin carboxyl-terminal hydrolase 5	6	13.4%
P56480	ATP synthase subunit beta, mitochondrial	15	52.4%
P57759	Endoplasmic reticulum resident protein 29	3	18.7%
P57780	Alpha-actinin-4	37	62.2%
P57784	U2 small nuclear ribonucleoprotein A'	5	38.4%
P58058	NAD kinase	6	19.6%

P58252	Elongation factor 2	28	51.2%
P58389	Serine/threonine-protein phosphatase 2A activator	2	12.1%
P59325	Eukaryotic translation initiation factor 5	2	5.6%
P59999	Actin-related protein 2/3 complex subunit 4	8	66.1%
P60122	RuvB-like 1	6	18.9%
P60229	Eukaryotic translation initiation factor 3 subunit E	13	37.8%
P60335	Poly(rC)-binding protein 1	5	25.8%
P60710	Actin, cytoplasmic 1	26	97.1%
P60766	Cell division control protein 42 homolog	8	68.6%
P60843	Eukaryotic initiation factor 4A-I	15	55.9%
P60867	40S ribosomal protein S20	2	19.3%
P61021	Ras-related protein Rab-5B	3	20.5%
P61161	Actin-related protein 2	14	48.7%
P61164	Alpha-centractin	8	39.1%
P61202	COP9 signalosome complex subunit 2	4	12.2%
P61222	ATP-binding cassette sub-family E member 1	4	11.2%
P61759	Prefoldin subunit 3	2	22.5%
P61967	AP-1 complex subunit sigma-1A	2	15.8%
P61982	14-3-3 protein gamma	8	50.6%
P62082	40S ribosomal protein S7	3	33.0%
P62137	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	7	29.7%
P62141	Serine/threonine-protein phosphatase PP1-beta catalytic subunit	6	27.2%
P62192	26S protease regulatory subunit 4	6	23.0%
P62242	40S ribosomal protein S8	3	18.8%
P62259	14-3-3 protein epsilon	10	57.7%
P62305	Small nuclear ribonucleoprotein E	2	25.0%
P62307	Small nuclear ribonucleoprotein F	2	55.8%
P62309	Small nuclear ribonucleoprotein G	2	26.3%
P62315	Small nuclear ribonucleoprotein Sm D1	4	54.6%
P62317	Small nuclear ribonucleoprotein Sm D2	5	47.5%
P62320	Small nuclear ribonucleoprotein Sm D3	3	31.8%
P62334	26S protease regulatory subunit 10B	6	26.0%
P62492	Ras-related protein Rab-11A	2	19.4%
P62702	40S ribosomal protein S4, X isoform	3	15.2%
P62746	Rho-related GTP-binding protein RhoB	5	48.0%
P62748	Hippocalcin-like protein 1	3	21.8%
P62806	Histone H4	5	61.2%
P62814	V-type proton ATPase subunit B, brain isoform	15	50.5%

P62821	Ras-related protein Rab-1A	4	30.7%
P62827	GTP-binding nuclear protein Ran	8	59.3%
P62830	60S ribosomal protein L23	2	25.0%
P62843	40S ribosomal protein S15	2	28.3%
P62855	40S ribosomal protein S26	2	20.9%
P62889	60S ribosomal protein L30	2	24.4%
P62908	40S ribosomal protein S3	4	25.5%
P62960	Nuclease-sensitive element-binding protein 1	5	31.4%
P62962	Profilin-1	7	65.0%
P62996	Transformer-2 protein homolog beta	2	10.4%
P63001	Ras-related C3 botulinum toxin substrate 1	5	36.5%
P63005	Platelet-activating factor acetylhydrolase IB subunit alpha	8	28.5%
P63017	Heat shock cognate 71 kDa protein	30	73.4%
P63028	Translationally-controlled tumor protein	2	35.5%
P63085	Mitogen-activated protein kinase 1	7	27.4%
P63101	14-3-3 protein zeta/delta	11	62.5%
P63158	High mobility group protein B1	7	40.0%
P63242	Eukaryotic translation initiation factor 5A-1	6	70.8%
P63276	40S ribosomal protein S17	2	32.6%
P63328-2	Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform Isoform 2 of	4	16.6%
P63330	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	6	31.7%
P67778	Prohibitin	4	24.6%
P67871	Casein kinase II subunit beta	2	15.8%
P68033	Actin, alpha cardiac muscle 1	18	68.4%
P68037	Ubiquitin-conjugating enzyme E2 L3	3	40.3%
P68040	Guanine nucleotide-binding protein subunit beta-2-like 1	9	45.7%
P68368	Tubulin alpha-4A chain	15	59.8%
P68369	Tubulin alpha-1A chain	15	59.4%
P68372	Tubulin beta-4B chain	21	69.2%
P68373	Tubulin alpha-1C chain	15	59.7%
P68433	Histone H3.1	6	60.3%
P68510	14-3-3 protein eta	4	27.2%
P70168	Importin subunit beta-1	21	44.1%
P70195	Proteasome subunit beta type-7	9	55.2%
P70268	Serine/threonine-protein kinase N1	2	5.3%
P70290	55 kDa erythrocyte membrane protein	4	13.7%
P70296	Phosphatidylethanolamine-binding protein 1	2	20.9%
P70336	Rho-associated protein kinase 2	3	3.8%

P70372	ELAV-like protein 1	3	12.9%
P70398	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	5	4.1%
P70404	Isocitrate dehydrogenase [NAD] subunit gamma 1, mitochondrial	2	13.7%
P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	2	7.0%
P70460	Vasodilator-stimulated phosphoprotein	8	31.7%
P70670	Nascent polypeptide-associated complex subunit alpha, muscle-specific form	3	1.9%
P70677	Caspase-3	2	11.9%
P70698	CTP synthase 1	4	11.5%
P80313	T-complex protein 1 subunit eta	7	23.4%
P80314	T-complex protein 1 subunit beta	8	29.2%
P80315	T-complex protein 1 subunit delta	8	25.1%
P80316	T-complex protein 1 subunit epsilon	6	25.5%
P80317	T-complex protein 1 subunit zeta	6	21.5%
P80318	T-complex protein 1 subunit gamma	14	38.4%
P82198	Transforming growth factor-beta-induced protein ig-h3	21	57.3%
P84078	ADP-ribosylation factor 1	6	49.7%
P84084	ADP-ribosylation factor 5	6	55.0%
P84089	Enhancer of rudimentary homolog	2	40.4%
P84091	AP-2 complex subunit mu	6	25.1%
P84096	Rho-related GTP-binding protein RhoG	4	31.4%
P84228	Histone H3.2	6	61.0%
P97310	DNA replication licensing factor MCM2	4	8.4%
P97369	Neutrophil cytosol factor 4	9	45.1%
P97372	Proteasome activator complex subunit 2	3	20.9%
P97384	Annexin A11	12	29.8%
P97450	ATP synthase-coupling factor 6, mitochondrial	2	34.3%
P97807	Fumarate hydratase, mitochondrial	8	30.6%
P97814	Proline-serine-threonine phosphatase-interacting protein 1	2	8.0%
P99024	Tubulin beta-5 chain	21	69.4%
P99026	Proteasome subunit beta type-4	8	55.7%
P99027	60S acidic ribosomal protein P2	4	69.6%
P99029	Peroxiredoxin-5, mitochondrial	7	49.1%
Q00519	Xanthine dehydrogenase/oxidase	23	33.4%
Q00612	Glucose-6-phosphate 1-dehydrogenase X	30	74.2%
Q00PI9	Heterogeneous nuclear ribonucleoprotein U-like protein 2	2	5.4%
Q01320	DNA topoisomerase 2-alpha	2	1.9%

Q01853	Transitional endoplasmic reticulum ATPase	34	70.7%
Q02053	Ubiquitin-activating enzyme EI (Ubiquitin-like modifier-activating enzyme 1)	32	53.8%
Q03265	ATP synthase subunit alpha, mitochondrial	8	21.2%
Q04750	DNA topoisomerase 1	17	27.8%
Q05144	Ras-related C3 botulinum toxin substrate 2	7	53.1%
Q06138	Calcium-binding protein 39	2	7.0%
Q07076	Annexin A7	7	23.3%
Q07417	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	2	10.4%
Q07797	Galectin-3-binding protein	3	9.9%
Q09014	Neutrophil cytosol factor 1	13	50.5%
Q09PK2	Retroviral-like aspartic protease 1	8	58.1%
Q11011	Puromycin-sensitive aminopeptidase	23	38.8%
Q14CH1	Molybdenum cofactor sulfurase	2	5.1%
Q3THE2	Myosin regulatory light chain 12B	9	79.7%
Q3THK7	GMP synthase [glutamine-hydrolyzing]	2	5.3%
Q3TKD0	Transportin-1 (Fragment)	8	15.6%
Q3TRM8	Hexokinase-3	25	43.1%
Q3TTY5	Keratin, type II cytoskeletal 2 epidermal	3	9.3%
Q3TVK3	Aspartyl aminopeptidase	10	34.7%
Q3TW96	UDP-N-acetylhexosamine pyrophosphorylase-like protein 1	4	11.4%
Q3TXS7	26S proteasome non-ATPase regulatory subunit 1	14	29.9%
Q3U0V1	Far upstream element-binding protein 2	2	3.6%
Q3U1J4	DNA damage-binding protein 1	7	10.1%
Q3U2G2	Heat shock 70 kDa protein 4	17	33.1%
Q3U7R1	Extended synaptotagmin-1	6	10.4%
Q3U898	Myeloma-overexpressed gene 2 protein homolog	2	71.9%
Q3UJB0	Protein Sf3b2	3	3.9%
Q3UKJ7	WD40 repeat-containing protein SMU1	2	4.9%
Q3UKW2	Calmodulin	6	60.4%
Q3UM45	Protein phosphatase 1 regulatory subunit 7	2	10.5%
Q3UMW7	MAP kinase-activated protein kinase 3	2	8.1%
Q3UND0	Src kinase-associated phosphoprotein 2	6	24.9%
Q3UP87	Neutrophil elastase	8	40.8%
Q3UPV6	Voltage-gated potassium channel subunit beta-2	4	23.8%
Q3UQ44	Ras GTPase-activating-like protein IQGAP2	4	4.6%
Q3UYV9	Nuclear cap-binding protein subunit 1	8	14.3%
Q3UZ39	Leucine-rich repeat flightless-interacting protein 1	5	11.7%

Q3UZG4	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1	4	21.3%
Q3V1G4	Olfactomedin-like protein 2B	4	8.5%
Q3V1L4	Cytosolic purine 5'-nucleotidase	10	28.4%
Q3V3R1	Monofunctional C1-tetrahydrofolate synthase, mitochondrial	2	3.0%
Q497J0	MCG130175, isoform CRA_b	3	37.1%
Q4LDD4	Arf-GAP with Rho-GAP domain, ANK repeat and PH domain-containing protein 1	2	2.8%
Q4QRL3	Coiled-coil domain-containing protein 88B	4	4.1%
Q501J6	Probable ATP-dependent RNA helicase DDX17	7	13.2%
Q543K9	Purine nucleoside phosphorylase	9	53.6%
Q571E4	N-acetylgalactosamine-6-sulfatase	3	10.2%
Q571I9	Aldehyde dehydrogenase family 16 member A1	8	14.5%
Q5FWK3	Rho GTPase-activating protein 1	2	10.0%
Q5RKN9	Capping protein (Actin filament) muscle Z-line, alpha 1	9	52.5%
Q5SQX6	Cytoplasmic FMR1-interacting protein 2	5	5.4%
Q5SSW2	Proteasome activator complex subunit 4	2	2.2%
Q5SUR0	Phosphoribosylformylglycinamidine synthase	10	14.4%
Q5SUS9	RNA-binding protein EWS	3	7.1%
Q5SXR6	Clathrin heavy chain	48	48.7%
Q5XJF6	Ribosomal protein	4	22.6%
Q60597	2-oxoglutarate dehydrogenase, mitochondrial	3	7.1%
Q60605-2	Isoform Smooth muscle of Myosin light polypeptide 6	8	77.5%
Q60631	Growth factor receptor-bound protein 2	3	22.1%
Q60668	Heterogeneous nuclear ribonucleoprotein D0	5	27.6%
Q60692	Proteasome subunit beta type-6	8	65.6%
Q60737	Casein kinase II subunit alpha	6	26.3%
Q60749	KH domain-containing, RNA-binding, signal transduction-associated protein 1	2	7.7%
Q60864	Stress-induced-phosphoprotein 1	7	19.2%
Q60865	Caprin-1	4	13.2%
Q60963	Platelet-activating factor acetylhydrolase	9	35.0%
Q60972	Histone-binding protein RBBP4	9	36.9%
Q61024	Asparagine synthetase [glutamine-hydrolyzing]	5	12.1%
Q61033	Lamina-associated polypeptide 2, isoforms alpha/zeta	7	18.9%
Q61035	Histidine--tRNA ligase, cytoplasmic	12	36.5%
Q61081	Hsp90 co-chaperone Cdc37	2	9.8%
Q61093	Cytochrome b-245 heavy chain	2	8.6%
Q61096	Myeloblastin	5	31.5%

Q61166	Microtubule-associated protein RP/EB family member 1	3	21.3%
Q61171	Peroxiredoxin-2	6	57.1%
Q61187	Tumor susceptibility gene 101 protein	2	11.8%
Q61206	Platelet-activating factor acetylhydrolase IB subunit beta	2	18.8%
Q61233	Plastin-2	36	82.9%
Q61362	Chitinase-3-like protein 1	5	24.2%
Q61400	Carcinoembryonic antigen-related cell adhesion molecule 10	2	19.6%
Q61598	Rab GDP dissociation inhibitor beta	17	60.2%
Q61599	Rho GDP-dissociation inhibitor 2	6	53.0%
Q61635	GTP-binding protein	3	9.8%
Q61646	Haptoglobin	12	53.3%
Q61753	D-3-phosphoglycerate dehydrogenase	6	14.6%
Q61768	Kinesin-1 heavy chain	3	4.5%
Q61792	LIM and SH3 domain protein 1	2	10.7%
Q61805	Lipopolysaccharide-binding protein	2	9.8%
Q61881	DNA replication licensing factor MCM7	3	6.4%
Q61937	Nucleophosmin	4	26.0%
Q61990	Poly(rC)-binding protein 2	4	16.6%
Q62093	Serine/arginine-rich splicing factor 2	2	11.3%
Q62167	ATP-dependent RNA helicase DDX3X	8	16.5%
Q62178	Semaphorin-4A	2	5.1%
Q62318	Transcription intermediary factor 1-beta	4	10.8%
Q62348	Translin	3	22.4%
Q62376	U1 small nuclear ribonucleoprotein 70 kDa	5	16.5%
Q62383	Transcription elongation factor SPT6	3	3.6%
Q62393	Tumor protein D52	2	22.3%
Q62418	Drebrin-like protein	6	22.0%
Q62422	Osteoclast-stimulating factor 1	5	37.2%
Q62431	AT-rich interactive domain-containing protein 3A	2	4.7%
Q62465	Synaptic vesicle membrane protein VAT-1 homolog	6	24.6%
Q62523	Zyxin	4	11.4%
Q63810	Calcineurin subunit B type 1	4	52.4%
Q63844	Mitogen-activated protein kinase 3	9	33.7%
Q63918	Serum deprivation-response protein	2	6.0%
Q64012	RNA-binding protein Raly	2	9.9%
Q64152	Transcription factor BTF3	3	33.8%
Q64277	ADP-ribosyl cyclase/cyclic ADP-ribose	2	7.1%

	hydrolase 2		
Q64442	Sorbitol dehydrogenase	2	9.2%
Q64511	DNA topoisomerase 2-beta	4	2.9%
Q64514	Tripeptidyl-peptidase 2	12	15.9%
Q64522	Histone H2A type 2-B	7	68.5%
Q64674	Spermidine synthase	3	15.6%
Q64727	Vinculin	32	47.0%
Q64737	Trifunctional purine biosynthetic protein adenosine-3	3	5.6%
Q6DTY7	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4	3	7.3%
Q6IRU2	Tropomyosin alpha-4 chain	6	35.5%
Q6NS79	Diap1 protein	7	11.3%
Q6NSR8	Probable aminopeptidase NPEPL1	2	6.7%
Q6NXH9	Keratin, type II cytoskeletal 73	3	6.5%
Q6P069	Sorcin	8	48.5%
Q6P2B1	Transportin-3	6	15.9%
Q6P4T2	U5 small nuclear ribonucleoprotein 200 kDa helicase	20	15.6%
Q6P5E4	UDP-glucose:glycoprotein glucosyltransferase 1	26	31.1%
Q6P5F9	Exportin-1	18	26.8%
Q6P6I8	Signal-regulatory protein alpha	2	10.0%
Q6PAV2	Probable E3 ubiquitin-protein ligase HERC4	2	3.5%
Q6PD03	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit alpha isoform	7	25.7%
Q6PDG5	SWI/SNF complex subunit SMARCC2	2	3.3%
Q6PDI5	Proteasome-associated protein ECM29 homolog	6	7.0%
Q6PDM2	Serine/arginine-rich splicing factor 1	4	23.8%
Q6PDQ2	Chromodomain-helicase-DNA-binding protein 4	6	6.2%
Q6PEB6	MOB-like protein phocein	2	23.1%
Q6PFA2	Clathrin light chain A	3	17.9%
Q6PHU5	Sortilin	2	2.9%
Q6Q899	Probable ATP-dependent RNA helicase DDX58	3	6.3%
Q6WVG3	BTB/POZ domain-containing protein KCTD12	6	24.2%
Q6ZQ38	Cullin-associated NEDD8-dissociated protein 1	9	13.2%
Q6ZQA0	Neurobeachin-like protein 2	8	5.5%
Q6ZQK5	Arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 2	4	8.8%
Q6ZWM4	U6 snRNA-associated Sm-like protein LSm8	2	41.7%
Q6ZWQ9	MCG5400	9	79.7%
Q6ZWR6	Nesprin-1	17	3.4%
Q6ZWX6	Eukaryotic translation initiation factor 2 subunit	3	10.5%

	1		
Q6ZWY9	Histone H2B type 1-C/E/G	8	65.9%
Q6ZWZ7	60S ribosomal protein L17	2	16.3%
Q76MZ3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	18	49.6%
Q78PY7	Staphylococcal nuclease domain-containing protein 1	11	21.7%
Q78ZA7	Nucleosome assembly protein 1-like 4	4	15.2%
Q792Z1	MCG140784	3	23.2%
Q7M6Y3	Phosphatidylinositol-binding clathrin assembly protein	3	6.8%
Q7TMB8	Cytoplasmic FMR1-interacting protein 1	4	4.2%
Q7TNG5	Echinoderm microtubule-associated protein-like 2	4	11.3%
Q7TNV0	Protein DEK	5	16.3%
Q7TPR4	Alpha-actinin-1	44	76.0%
Q7TPV4	Myb-binding protein 1A	6	6.9%
Q7TSI3	Serine/threonine-protein phosphatase 6 regulatory subunit 1	5	13.0%
Q7TSV4	Phosphoglucomutase-2	2	4.8%
Q80UI5	Phosphatidylinositol 3-kinase regulatory subunit alpha	4	13.7%
Q80UR4	Mast cell protease-11	3	12.9%
Q80X41	Serine/threonine-protein kinase VRK1	6	19.1%
Q80XR6	Heterogeneous nuclear ribonucleoprotein A/B	6	25.4%
Q80YQ1	Thrombospondin 1	25	29.5%
Q80ZJ2	Impa1 protein	4	21.0%
Q810B6	Rabankyrin-5	2	4.5%
Q8BFQ4	WD repeat-containing protein 82	2	10.9%
Q8BFY6	Peflin	4	24.7%
Q8BFZ3	Beta-actin-like protein 2	17	63.0%
Q8BG32	26S proteasome non-ATPase regulatory subunit 11	9	32.9%
Q8BGQ7	Alanine--tRNA ligase, cytoplasmic	8	16.3%
Q8BH59	Calcium-binding mitochondrial carrier protein Aralar1	2	7.2%
Q8BH61	Coagulation factor XIII A chain	9	19.5%
Q8BH69	Selenide, water dikinase 1	3	17.1%
Q8BHD7	Polypyrimidine tract-binding protein 3	3	12.4%
Q8BHN3	Neutral alpha-glucosidase AB	14	23.9%
Q8BJS4	SUN domain-containing protein 2	4	6.7%
Q8BJY1	26S proteasome non-ATPase regulatory subunit 5	4	13.9%

Q8BK67	Protein RCC2	5	15.8%
Q8BKC5	Importin-5	23	34.3%
Q8BL97	Serine/arginine-rich splicing factor 7	3	15.7%
Q8BMJ2	Leucine--tRNA ligase, cytoplasmic	3	4.4%
Q8BMS1	Trifunctional enzyme subunit alpha, mitochondrial	2	7.3%
Q8BND5	Sulfhydryl oxidase 1	6	11.6%
Q8BP47	Asparagine--tRNA ligase, cytoplasmic	9	24.7%
Q8BPU7	Engulfment and cell motility protein 1	6	22.7%
Q8BRF7	Sec1 family domain-containing protein 1	2	6.0%
Q8BT60	Copine-3	10	25.5%
Q8BTS0	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	7	15.1%
Q8BTZ7	Mannose-1-phosphate guanyltransferase beta	3	12.2%
Q8BU30	Isoleucine--tRNA ligase, cytoplasmic	4	5.6%
Q8BV13	COP9 signalosome complex subunit 7b	2	11.7%
Q8BVE3	V-type proton ATPase subunit H	4	15.3%
Q8BVG4	Dipeptidyl peptidase 9	3	6.2%
Q8BVQ0	WD repeat-containing protein 61	3	25.1%
Q8BWT1	3-ketoacyl-CoA thiolase, mitochondrial	6	30.5%
Q8BWY3	Eukaryotic peptide chain release factor subunit 1	4	15.1%
Q8BYA0	Tubulin-specific chaperone D	2	3.6%
Q8C0E2	Vacuolar protein sorting-associated protein 26B	7	29.5%
Q8C147	Dedicator of cytokinesis protein 8	11	9.0%
Q8C166	Copine-1	4	8.8%
Q8C1A5	Thimet oligopeptidase	2	6.4%
Q8C1B7	Septin-11	4	13.2%
Q8C266	Ras-related protein Rab-5C	4	24.8%
Q8C3J5	Dedicator of cytokinesis protein 2	7	7.2%
Q8C605	6-phosphofructokinase	3	7.3%
Q8CC86	Nicotinate phosphoribosyltransferase	6	20.5%
Q8CCK0	Core histone macro-H2A.2	3	14.8%
Q8CDN6	Thioredoxin-like protein 1	3	20.8%
Q8CFQ9	Fusion, derived from t(1216) malignant liposarcoma (Human)	2	8.9%
Q8CG29	Myosin IF	10	16.5%
Q8CG48	Structural maintenance of chromosomes protein 2	2	2.7%
Q8CGC7	Bifunctional glutamate/proline--tRNA ligase	12	13.6%
Q8CGK3	Lon protease homolog, mitochondrial	2	7.3%
Q8CGP0	Histone H2B type 3-B	7	56.4%
Q8CGP4	Histone H2A	5	60.5%
Q8CGP6	Histone H2A type 1-H	8	74.2%

Q8CGY8	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit	2	2.9%
Q8CHP8	Phosphoglycolate phosphatase	2	9.7%
Q8CI94	Glycogen phosphorylase, brain form	12	20.4%
Q8CIH5	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-2	28	34.6%
Q8CIN4	Serine/threonine-protein kinase PAK 2	5	15.7%
Q8CIZ8	von Willebrand factor	15	8.9%
Q8CJG0	Protein argonaute-2	3	5.7%
Q8JZQ9	Eukaryotic translation initiation factor 3 subunit B	11	26.5%
Q8K0E8	Fibrinogen beta chain	11	34.5%
Q8K1B8	Fermitin family homolog 3	24	54.4%
Q8K1K2	26S protease regulatory subunit 8	5	23.3%
Q8K1M6	Dynamin-1-like protein	4	10.1%
Q8K1X4	NCK associated protein 1 like	4	8.4%
Q8K2Q0	COMM domain-containing protein 9	2	16.7%
Q8K2Z4	Condensin complex subunit 1	4	5.8%
Q8K411	Presequence protease, mitochondrial	2	3.6%
Q8K426	Myeloid cysteine-rich protein	3	32.5%
Q8K482	EMILIN-2	12	18.3%
Q8K4Z5	Splicing factor 3A subunit 1	2	3.5%
Q8QZW8	Protein Arhgap9	2	7.4%
Q8QZY1	Eukaryotic translation initiation factor 3 subunit L	15	37.6%
Q8QZY6	Tetraspanin-14	2	10.4%
Q8QZY9	Splicing factor 3B subunit 4	6	30.2%
Q8R010	Aminoacyl tRNA synthase complex-interacting multifunctional protein 2	4	33.1%
Q8R016	Bleomycin hydrolase	9	42.2%
Q8R0J7	Vacuolar protein sorting-associated protein 37B	3	21.8%
Q8R146	Acylamino-acid-releasing enzyme	11	26.9%
Q8R180	ERO1-like protein alpha	5	13.6%
Q8R1B4	Eukaryotic translation initiation factor 3 subunit C	11	15.9%
Q8R1Q8	Cytoplasmic dynein 1 light intermediate chain 1	2	7.7%
Q8R1V9	Protein Qars	3	13.9%
Q8R2P8	Lysine--tRNA ligase	6	20.2%
Q8R2S8	CD177 antigen	12	21.5%
Q8R3C7	DEAD (Asp-Glu-Ala-Asp) box polypeptide 19b	2	7.7%
Q8R5L1	Complement component 1 Q subcomponent-binding protein, mitochondrial	4	32.3%
Q8VBV3	Exosome complex component RRP4	2	11.3%

Q8VBV7	COP9 signalosome complex subunit 8	3	23.9%
Q8VC88	Grancalcin	8	57.3%
Q8VCI0	Phospholipase B-like 1	5	16.2%
Q8VCM7	Fibrinogen gamma chain	12	43.8%
Q8VCT3	Aminopeptidase B	8	23.1%
Q8VDD5	Myosin-9	70	52.1%
Q8VDL4	ADP-dependent glucokinase	3	12.7%
Q8VDM4	26S proteasome non-ATPase regulatory subunit 2	23	43.5%
Q8VDM6	Heterogeneous nuclear ribonucleoprotein U-like protein 1	2	4.0%
Q8VDP3	Protein-methionine sulfoxide oxidase MICAL1	2	2.6%
Q8VDW0	ATP-dependent RNA helicase DDX39A	7	18.7%
Q8VE37	Regulator of chromosome condensation	3	12.1%
Q8VEK3	Heterogeneous nuclear ribonucleoprotein U	6	13.4%
Q8VIJ6	Splicing factor, proline- and glutamine-rich	5	10.9%
Q91V12	Cytosolic acyl coenzyme A thioester hydrolase	4	12.1%
Q91V41	Ras-related protein Rab-14	5	39.5%
Q91V89	Protein Ppp2r5d	2	5.9%
Q91V92	ATP-citrate synthase	22	32.7%
Q91VC3	Eukaryotic initiation factor 4A-III	7	25.6%
Q91VH6	Protein MEMO1	2	11.5%
Q91VI7	Ribonuclease inhibitor	8	30.3%
Q91VW3	SH3 domain-binding glutamic acid-rich-like protein 3	3	46.2%
Q91WK2	Eukaryotic translation initiation factor 3 subunit H	9	42.9%
Q91WQ3	Tyrosine--tRNA ligase, cytoplasmic	4	11.4%
Q91X72	Hemopexin	10	30.2%
Q91XL1	Leucine-rich HEV glycoprotein	4	17.8%
Q91YI4	Beta-arrestin-2	3	13.7%
Q91YP3	Deoxyribose-phosphate aldolase	9	41.5%
Q91YQ5	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	2	5.6%
Q91YR9	Prostaglandin reductase 1	8	42.6%
Q91Z50	Flap endonuclease 1	5	24.0%
Q91Z69	SLIT-ROBO Rho GTPase-activating protein 1	2	2.2%
Q91ZJ5	UTP--glucose-1-phosphate uridylyltransferase	13	41.3%
Q91ZR2	Sorting nexin-18	2	11.1%
Q91ZW3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5	2	2.6%
Q920E5	Farnesyl pyrophosphate synthase	10	60.1%

Q921H8	3-ketoacyl-CoA thiolase A, peroxisomal	3	14.9%
Q921I1	Serotransferrin	19	34.7%
Q921M3	Splicing factor 3B subunit 3	24	33.9%
Q921M7	Protein FAM49B	6	34.9%
Q922B2	Aspartate--tRNA ligase, cytoplasmic	9	22.8%
Q922D4	Serine/threonine-protein phosphatase 6 regulatory subunit 3	5	10.7%
Q922D8	C-1-tetrahydrofolate synthase, cytoplasmic	3	4.6%
Q922F4	Tubulin beta-6 chain	10	32.4%
Q922H4	Mannose-1-phosphate guanyltransferase alpha	3	12.9%
Q922R8	Protein disulfide-isomerase A6	8	28.4%
Q922S4	cGMP-dependent 3',5'-cyclic phosphodiesterase	8	17.7%
Q923D4	Splicing factor 3B subunit 5	3	43.0%
Q923L6	Eosinophil-associated ribonuclease 10	2	19.9%
Q924K8	Metastasis-associated protein MTA3	2	4.7%
Q93092	Transaldolase	9	31.8%
Q99J99	3-mercaptopyruvate sulfurtransferase	4	15.5%
Q99JI4	26S proteasome non-ATPase regulatory subunit 6	8	29.3%
Q99JI6	Ras-related protein Rap-1b	5	44.0%
Q99JX4	Eukaryotic translation initiation factor 3 subunit M	9	43.6%
Q99JY9	Actin-related protein 3	19	72.3%
Q99K48	Non-POU domain-containing octamer-binding protein	4	13.7%
Q99K85	Phosphoserine aminotransferase	2	8.9%
Q99K94	Signal transducer and activator of transcription	8	16.4%
Q99KE1	NAD-dependent malic enzyme, mitochondrial	9	27.8%
Q99KI0	Aconitate hydratase, mitochondrial	6	12.4%
Q99KJ8	Dynactin subunit 2	4	15.2%
Q99KK7	Dipeptidyl peptidase 3	6	17.8%
Q99KP6	Pre-mRNA-processing factor 19	6	28.2%
Q99KQ4	Nicotinamide phosphoribosyltransferase	4	12.6%
Q99KV1	DnaJ homolog subfamily B member 11	4	17.9%
Q99L45	Eukaryotic translation initiation factor 2 subunit 2	4	19.9%
Q99LB4	Capping protein (Actin filament), gelsolin-like	6	29.5%
Q99LC5	Electron transfer flavoprotein subunit alpha, mitochondrial	3	17.7%
Q99LI7	Cleavage stimulation factor subunit 3	2	4.0%
Q99LT0	Protein dpy-30 homolog	2	36.4%
Q99MK8	Beta-adrenergic receptor kinase 1	5	14.1%

Q99NF7	Ppm1b protein	2	6.3%
Q99P58	Ras-related protein Rab-27B	2	10.1%
Q99PT1	Rho GDP-dissociation inhibitor 1	4	29.9%
Q99PV0	Pre-mRNA-processing-splicing factor 8	13	10.5%
Q9CPS5	26S proteasome non-ATPase regulatory subunit 8	2	15.9%
Q9CPW4	Actin-related protein 2/3 complex subunit 5	5	61.6%
Q9CPX6	Ubiquitin-like-conjugating enzyme ATG3	2	7.3%
Q9CPY7	Cytosol aminopeptidase	3	10.6%
Q9CQ19	Myosin regulatory light polypeptide 9	7	50.6%
Q9CQ80	Vacuolar protein-sorting-associated protein 25	2	30.1%
Q9CQE1	Protein NipSnap homolog 3B	4	28.7%
Q9CQE8	UPF0568 protein C14orf166 homolog	3	20.5%
Q9CQH7	Transcription factor BTF3 homolog 4	2	31.7%
Q9CQI6	Coactosin-like protein	3	35.9%
Q9CQR6	Serine/threonine-protein phosphatase 6 catalytic subunit	4	24.3%
Q9CQV8	14-3-3 protein beta/alpha	10	53.3%
Q9CQW9	Interferon-induced transmembrane protein 3	2	20.4%
Q9CR16	Peptidyl-prolyl cis-trans isomerase D	4	11.6%
Q9CR57	60S ribosomal protein L14	2	11.5%
Q9CR86	Calcium-regulated heat stable protein 1	2	35.1%
Q9CRA5	Golgi phosphoprotein 3	2	12.4%
Q9CS42	Ribose-phosphate pyrophosphokinase 2	2	10.1%
Q9CU62	Structural maintenance of chromosomes protein 1A	4	4.3%
Q9CVB6	Actin-related protein 2/3 complex subunit 2	16	77.3%
Q9CW03	Structural maintenance of chromosomes protein 3	8	10.7%
Q9CWJ9	Bifunctional purine biosynthesis protein PURH	16	47.1%
Q9CXW3	Calcyclin-binding protein	2	13.1%
Q9CXW4	60S ribosomal protein L11	2	12.9%
Q9CXY6	Interleukin enhancer-binding factor 2	4	19.0%
Q9CY58	Plasminogen activator inhibitor 1 RNA-binding protein	2	7.9%
Q9CYL5	Golgi-associated plant pathogenesis-related protein 1	4	45.5%
Q9CZ13	Cytochrome b-c1 complex subunit 1, mitochondrial	3	15.6%
Q9CZ30	Obg-like ATPase 1	4	18.7%
Q9CZ44	NSFL1 cofactor p47	7	30.3%
Q9CZD3	Glycine--tRNA ligase	5	13.2%
Q9CZN7	Serine hydroxymethyltransferase	7	24.0%

Q9CZU6	Citrate synthase, mitochondrial	9	38.2%
Q9D020	Cytosolic 5'-nucleotidase 3A	6	30.2%
Q9D051	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	2	8.4%
Q9D0E1	Heterogeneous nuclear ribonucleoprotein M	4	9.2%
Q9D0I9	Arginine--tRNA ligase, cytoplasmic	9	20.3%
Q9D0R2	Threonine--tRNA ligase, cytoplasmic	13	24.4%
Q9D154	Leukocyte elastase inhibitor A	15	54.4%
Q9D1A2	Cytosolic non-specific dipeptidase	4	11.4%
Q9D1K2	V-type proton ATPase subunit F	3	40.3%
Q9D1Q6	Endoplasmic reticulum resident protein 44	5	23.2%
Q9D2V7	Coronin-7	12	29.8%
Q9D554	Splicing factor 3A subunit 3	5	20.8%
Q9D662	Protein transport protein Sec23B	5	13.8%
Q9D6J6	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	2	12.1%
Q9D6R2	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	5	21.0%
Q9D6Y7	Mitochondrial peptide methionine sulfoxide reductase	2	14.2%
Q9D7M1	Glucose-induced degradation protein 8 homolog	3	29.4%
Q9D819	Inorganic pyrophosphatase	3	15.6%
Q9D883	Splicing factor U2AF 35 kDa subunit	2	13.0%
Q9D8B3	Charged multivesicular body protein 4b	2	13.4%
Q9D8N0	Elongation factor 1-gamma	10	41.0%
Q9D8W5	26S proteasome non-ATPase regulatory subunit 12	7	18.0%
Q9D964	Glycine amidinotransferase, mitochondrial	5	20.3%
Q9DB05	Alpha-soluble NSF attachment protein	2	9.5%
Q9DBC7	cAMP-dependent protein kinase type I-alpha regulatory subunit	2	9.5%
Q9DBG3	AP-2 complex subunit beta	21	39.7%
Q9DBG5	Perilipin-3	2	8.5%
Q9DBJ1	Phosphoglycerate mutase 1	10	69.3%
Q9DBK7	MCG18845, isoform CRA_d	2	3.5%
Q9DBZ5	Eukaryotic translation initiation factor 3 subunit K	4	26.6%
Q9DCC5	Cbx3 protein	4	39.3%
Q9DCD0	6-phosphogluconate dehydrogenase, decarboxylating	22	66.9%
Q9DCH4	Eukaryotic translation initiation factor 3 subunit F	6	31.0%
Q9DCL9	Multifunctional protein ADE2	4	19.3%

Q9DCX2	ATP synthase subunit d, mitochondrial	2	21.7%
Q9EPK7	Exportin-7	2	3.3%
Q9EPL8	Importin-7	8	12.2%
Q9EQG9	Collagen type IV alpha-3-binding protein	3	11.2%
Q9EQH3	Vacuolar protein sorting-associated protein 35	19	37.9%
Q9EQU5	Protein SET	5	35.0%
Q9ER72	Cysteine--tRNA ligase, cytoplasmic	7	16.4%
Q9ERD8	Gamma-parvin	5	23.3%
Q9ERK4	Exportin-2	11	20.0%
Q9ERL7	Glia maturation factor gamma	2	23.9%
Q9ES46	Beta-parvin	2	8.8%
Q9ES52	Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1	2	2.9%
Q9ESP1	Stromal cell-derived factor 2-like protein 1	2	19.9%
Q9EST5	Acidic leucine-rich nuclear phosphoprotein 32 family member B	2	10.7%
Q9ET01	Glycogen phosphorylase, liver form	40	66.7%
Q9JHJ0	Tropomodulin-3	3	17.6%
Q9JHK5	Pleckstrin	6	35.7%
Q9JHU4	Cytoplasmic dynein 1 heavy chain 1	24	9.0%
Q9JI10	Serine/threonine-protein kinase 3	3	8.7%
Q9JI11	Serine/threonine-protein kinase 4	3	9.5%
Q9JIF0	Protein arginine N-methyltransferase 1	4	14.6%
Q9JIF7	Coatomer subunit beta	10	18.2%
Q9JIG7	Coiled-coil domain-containing protein 22	2	4.8%
Q9JJ28	Protein flightless-1 homolog	13	21.3%
Q9JKF1	Ras GTPase-activating-like protein IQGAP1	57	56.9%
Q9JKR6	Hypoxia up-regulated protein 1	9	18.6%
Q9JL26	Formin-like protein 1	7	10.2%
Q9JLJ2	4-trimethylaminobutyraldehyde dehydrogenase	6	14.6%
Q9JLV6	Bifunctional polynucleotide phosphatase/kinase	16	52.7%
Q9JM14	5'(3')-deoxyribonucleotidase, cytosolic type	2	23.0%
Q9JM76	Actin-related protein 2/3 complex subunit 3	6	50.0%
Q9JMA1	Ubiquitin carboxyl-terminal hydrolase 14	6	19.9%
Q9JMH6	Thioredoxin reductase 1, cytoplasmic	9	27.2%
Q9QUG9	RAS guanyl-releasing protein 2	2	6.7%
Q9QUH0	Glutaredoxin-1	2	38.3%
Q9QUI0	Transforming protein RhoA	8	61.1%
Q9QUM0	Integrin alpha-IIb	20	38.7%
Q9QUM9	Proteasome subunit alpha type-6	10	52.0%
Q9QUR6	Prolyl endopeptidase	7	16.1%
Q9QXK3	Coatomer subunit gamma-2	4	7.4%

Q9QXS1	Plectin	18	6.5%
Q9QYB5	Gamma-adducin	3	5.0%
Q9QZD9	Eukaryotic translation initiation factor 3 subunit I	5	27.1%
Q9QZE5	Coatomer subunit gamma-1	11	23.6%
Q9QZK7	Docking protein 3	2	5.9%
Q9QZQ8	Core histone macro-H2A.1	15	64.5%
Q9QZU3	Platelet glycoprotein V (Fragment)	3	12.9%
Q9R062	Glycogenin-1	10	63.7%
Q9R0N0	Galactokinase	3	14.3%
Q9R0P5	Destrin	7	55.2%
Q9R0Q7	Prostaglandin E synthase 3	2	18.1%
Q9R111	Guanine deaminase	20	72.7%
Q9R190	Metastasis-associated protein MTA2	4	7.6%
Q9R1P0	Proteasome subunit alpha type-4	8	57.5%
Q9R1P1	Proteasome subunit beta type-3	5	40.5%
Q9R1P3	Proteasome subunit beta type-2	7	64.2%
Q9R1P4	Proteasome subunit alpha type-1	10	56.3%
Q9R1T2	SUMO-activating enzyme subunit 1	2	9.4%
Q9WTM5	RuvB-like 2	8	23.3%
Q9WTX5	S-phase kinase-associated protein 1	3	27.6%
Q9WTX6	Cullin-1	2	3.2%
Q9WU00	Nuclear respiratory factor 1	2	10.1%
Q9WU28	Prefoldin subunit 5	3	33.8%
Q9WU78	Programmed cell death 6-interacting protein	15	31.1%
Q9WUA2	Phenylalanine--tRNA ligase beta subunit	3	7.5%
Q9WUM3	Coronin-1B	4	21.5%
Q9WUM4	Coronin-1C	5	17.5%
Q9WV32	Actin-related protein 2/3 complex subunit 1B	10	40.6%
Q9WVA3	Mitotic checkpoint protein BUB3	5	23.6%
Q9WVA4	Transgelin-2	8	56.8%
Q9WVG5	Endothelial lipase	6	19.8%
Q9WVJ2	26S proteasome non-ATPase regulatory subunit 13	10	47.9%
Q9WVK4	EH domain-containing protein 1	18	59.9%
Q9WVQ5	Methylthioribulose-1-phosphate dehydratase	3	19.1%
Q9Z0I7	Protein Slfn1	3	13.1%
Q9Z0M6	CD97 antigen	4	8.1%
Q9Z0N1	Eukaryotic translation initiation factor 2 subunit 3, X-linked	6	26.3%
Q9Z0P5	Twinfilin-2	8	41.3%
Q9Z126	Platelet factor 4	4	46.7%

Q9Z183	Protein-arginine deiminase type-4	14	37.1%
Q9Z1B7	Mitogen-activated protein kinase 13	3	13.9%
Q9Z1D1	Eukaryotic translation initiation factor 3 subunit G	3	16.9%
Q9Z1E4	Glycogen [starch] synthase, muscle	11	26.4%
Q9Z1F9	SUMO-activating enzyme subunit 2	5	16.1%
Q9Z1G3	V-type proton ATPase subunit C 1	2	8.9%
Q9Z1N5	Spliceosome RNA helicase Ddx39b	10	35.3%
Q9Z1Q5	Chloride intracellular channel protein 1	11	71.0%
Q9Z1T1	AP-3 complex subunit beta-1	2	2.4%
Q9Z1Z2	Serine-threonine kinase receptor-associated protein	3	13.4%
Q9Z204	Heterogeneous nuclear ribonucleoproteins C1/C2	3	10.5%
Q9Z2D6	Methyl-CpG-binding protein 2	2	8.1%
Q9Z2I9	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	2	9.5%
Q9Z2U0	Proteasome subunit alpha type-7	8	47.2%
Q9Z2U1	Proteasome subunit alpha type-5	7	45.2%
Q9Z2X1	Heterogeneous nuclear ribonucleoprotein F	6	24.6%
S4R1I3	Xaa-Pro aminopeptidase 1	4	13.7%
S4R1S4	Receptor-type tyrosine-protein phosphatase C	5	7.0%
S4R2A9	Protein transport protein Sec31A (Fragment)	4	7.5%
S4R2T5	Ubiquitin-like modifier-activating enzyme ATG7	5	10.7%
V9GX06	Uncharacterized protein (Fragment)	3	27.3%
Z4YKA3	Heterochromatin protein 1-binding protein 3	9	25.2%

Appendix Table 2. Ubiquitinated proteins in MDSC exosomes

Accession Number	No. of non-overlapping peptides	% Coverage	GG peptide sequence
A0A087WR50	71	56.4%	GFNCESKPEPEETCFDK(GG)
A2A841	2	3.9%	TLNINGQVPTGDGPPLVK(GG)
A2AE27	7	12.0%	NSVLMSGFSHK(GG)
A2AFF6	2	2.8%	DPEEDGLMK(GG)R
A2AH85	19	31.8%	K(GG)MYSEIDIK(GG)
A2AN08	2	0.8%	LLLFICGSK(GG)EK(GG)
A2AUR7	3	26.2%	NQPEVDMSDRGISSMLDVNGLSVPPNVAELK(GG)
A2AWT7	2	4.0%	PDEIMRDYIQK(GG)
A6PWC3	8	12.4%	EMLFGSLARPGHPMGK(GG) TYFNILIK(GG)PETLAK LNFAPLEREMPVQFQVVELPSGHHLCK(GG)
B2RV77	4	53.6%	AVEYKSQVVAGQNYFIK(GG)
B5THE2	10	7.8%	ARWYDYYK(GG) WYDYYKGVDINATGEWK(GG)
B7FAU9	81	51.5%	DLAEDAPWK(GG) LIALLEVLSQK(GG)
B9EJ54	4	3.0%	K(GG)HK(GG)PDFISLFK
D3YW48	9	78.4%	MFLVNSFLK(GG)GGGGGGGGGGLGGGLGNVLGGLISGAAGGGGGGGGGMGLGGGGGGGGTAMR FLVNSFLK(GG)GGGGGGGGGGLGGGLGNVLGGLISGAAGGGGGGGGGMGLGGGGGGGGTAMR
D3Z627	9	13.7%	EESGARPDATK(GG) MPVSCEELTEGSSLLTK(GG)TLK
D3Z6Q9	5	17.2%	MAEGKAGGAAGLFAK(GG)
D3Z7R7	7	11.8%	AEEEQQGTGPGAGTAASKALDK(GG)
D4AFX7	5	4.6%	LFQHPSMAIVK(GG) VEMALEALRNVIK(GG)
E9PV60	2	1.2%	ELTYVMLSTVVTLLQGSVRNAVVLK(GG)
E9PVA8	3	2.2%	CGLALALNK(GG)
E9PWG6	2	3.0%	INLLKETEQLEIK(GG)
E9PZF0	9	48.3%	PFFPGLVK(GG)
E9Q035	19	24.7%	GYYAVAVVKASDTSITWNNLK(GG)
E9Q397	48	33.8%	LMEADIAIQGDK(GG) FFWEMDEAESWIKEK(GG)

			VK(GG)EVSAQWDHLK(GG)
E9Q8H9	2	3.1%	VYVQGQSLK(GG)
E9QAI5	5	3.7%	MAALVLEDGSVLQGRPFGAAVSTAGEV VFQTGMVGYPEALTDPSYK(GG) EQGSLLGKLVQK(GG)
			NILLTIGSYK(GG) EDFASGTAAALAGGVTMVCAMPNTRPPI IDAPALALAQK(GG)
E9QNN1	15	20.5%	GMTLVTPLQLLLFASKK(GG)
F6UND7	5	16.6%	VAVKTMK(GG)PGSMSVEAFLAEANLMK (GG)
F6YVP7	4	24.3%	K(GG)IAFAITAIK
F8WGL3	6	42.3%	YALYDATYETK(GG)ESK EDLVFIFWAPENAPLK(GG) MIYASSK(GG)DAIK
F8WHL2	8	10.7%	QQPLFVSGGDDYK(GG) QELILSNSEDK(GG) NFDKLSFLYLITGNLEK(GG)
F8WHZ9	3	6.2%	QDFNMMEQK(GG)
F8WI35	5	60.0%	FQSAAIGALQASEAYLVGLFEDTNLCAIH AK(GG)
G3UY93	12	18.6%	SK(GG)DVVEPLLR
G3X9T8	9	15.1%	EEFFIGSK(GG)YK(GG) MHAINGKMFGNLQGLTMHVK(GG)
G5E924	9	33.2%	TENAGDQHGGGGGGGSGAAGGGGGEN YDDPHK(GG)
I7HPV9	2	2.8%	TFVHPK(GG)AGAAGSLGAGLIPVSSELCY R
K3W4L0	3	3.1%	DMLLAEAFSLKQQMEEK(GG)
K3W4R2	6	4.1%	INFDIAGYIVGANIETYLLEK(GG)
O08582	3	8.1%	PQQIKMQSTK(GG)
O08739	11	26.4%	ELQK(GG)ELAEQK(GG)
O08992	7	58.5%	MAPSIMK(GG)
O09106	8	25.3%	QQTDIAVNWAGGLHHAK(GG)K(GG)
O09159	14	25.7%	ATFDSGTGLLMK(GG)
O35343	4	15.6%	LLHSPHQNVCEQAVWALGNIIGDGPQCR DYVISLGVVK(GG)
O35955	2	14.7%	MLK(GG)QAVEPTGGFSFENCQR
O54774	2	2.8%	VTYDIQASLQK(GG)
O54824	3	4.0%	ESSSTSSKEK(GG)
O55029	8	14.1%	EEPAMSMDANGK(GG)IIWAK(GG) PTAQQEPDGK(GG)PASSPVIMASQTTHK(GG)

O55222	8	25.0%	DIVQKLLQYK(GG)
O88307	17	11.3%	STVFTIFGSNK(GG)ESVHSWLILQVNATD ALGVPCTENDYK
O88456	9	70.6%	MFLVNSFLK(GG)GGGGGGGGGGLGGGL GNVLGGLISGAAGGGGGGGGGGMGLGG GGGGGGTAMR FLVNSFLK(GG)GGGGGGGGGGLGGGLG NVLGGLISGAAGGGGGGGGGGMGLGGG GGGGGTAMR KLFVQLAGDDMEVSATELMNILNK(GG) LFVQLAGDDMEVSATELMNILNK(GG)
O88543	2	7.3%	QCLSSLYKK(GG) TFLTLSLQDMASRVQLSGPQEAEK(GG)
O88544	8	31.3%	QDLAQLMNSSGSHKDLAGK(GG) QDLAQLMNSSGSHK(GG)DLAGK(GG) GNQLQEFAAMLMPHQK(GG)
O88783	3	1.9%	NSYSGMK(GG)
O88844	15	50.2%	DIFQEIYDK(GG)K(GG)
O88879	2	2.3%	EDIKDYYTDLSILQK(GG)
O89053	15	52.7%	MSERQVALWDTK(GG)
P02088	11	95.2%	YFDSFGDLSSASAIMGNAK(GG)
P02301	6	60.3%	FQSAAIGALQEASEAYLVGLFEDTNLCAI HAK(GG) FQSAAIGALQEASEAYLVGLFEDTNLCAI HAK(GG)R
P04919	13	27.1%	LIK(GG)IFQDYPLQQTYAPVVMKPKPQGP VPNTALFSLVLMAGTFLLAMTLR
P05064	16	77.2%	PHPYPALTPEQK(GG)K
P07724	18	40.6%	GLVLIAFSQYLQKCSYDEHAK(GG)
P07742	8	20.0%	QGLK(GG)TGMYYLR
P07759	4	17.7%	AVLDVAETGTEAAAATGVIGGIRK(GG)
P07901	24	48.3%	AFMEALQAGADISMIGQFGVGFYSAYLV AEKVTVITK(GG) EMLQQSKILK(GG) FYEQFSK(GG)NIK(GG)
P08003	13	33.4%	EILTLK(GG)QVQEFLK(GG)
P08113	9	18.2%	SGTSEFLNK(GG)
P09405	11	21.4%	EAMEDGEIDGNKVTLDWAK(GG)
P09411	14	53.5%	VNEMIIGGGMAFTFLK(GG) TGQATVASGIPAGWMGLDCGTESSK(GG)
P09542	2	12.3%	AAAPK(GG)AAPAPAAAPAAAPAAAPEPE RPK(GG)
P0CG50	6	9.8%	TLTGK(GG)TITLEVEPSDTIENVK

			LIFAGK(GG)QLEDGR TLSDYNIQK(GG)ESTLHLVLR
P10126	13	48.1%	IGYNPDTVAFVPISGWNGDNMLEPSANM PWFK(GG)
P11031	4	37.8%	DDNMFQIGK(GG)MR EYWMDSEGEMKPGRK(GG)
P11499	23	50.4%	EEEDKEDEEK(GG)PK
P11835	16	33.6%	LK(GG)SQWNNDNPLFK(GG)
P13020	19	48.0%	SGRAQVHVSEEGGEPEAMLQVLGPKPAL PEGTEDTAK(GG) AVEVMPKSGALNSNDAFVLK(GG)
P13439	2	6.4%	KFADIGNTVK(GG)
P13864	3	3.8%	RVVDTESGAAAAVEK(GG)
P14152	6	26.4%	K(GG)LSSAMSAAK(GG) EKMDLTAK(GG)
P14211	9	46.2%	K(GG)PEDWDEEMDGEWEPPVIQNPEYK(GG)GEWK K(GG)PEDWDEEMDGEWEPPVIQNPEYK GEWK(GG)
P14824	10	22.3%	LMLAVVK(GG)
P16125	3	12.9%	GYTNWAIGLSVADLIESMLK(GG)
P16546	48	34.4%	LEAELAAHEPAIQGVLDTGK(GG) NTTGVTEEALK(GG)EFSMMFK(GG)
P17182	19	70.1%	SGK(GG)YDLDFK(GG)
P17427	16	30.1%	WK(GG)QLSNPQQEVQNIFK(GG)
P17710	6	9.5%	GK(GG)FTTSDVAAIETDK(GG)
P19096	30	21.2%	SGECPAALVGGINLLLKPNTSVQFMK(GG) PK(GG)EQTAHAFVNVLTR
P22682	2	3.0%	K(GG)SSGAGGGGSGGSGAGGLIGLMK(G G)
P24547	5	17.1%	QLLCGAAIGTHEDDK(GG) VAQGVSGAVQDK(GG)GSIHK(GG)
P25206	5	12.4%	DHQTITIQEMPEK(GG)APAGQLPR
P25911-2	13	44.4%	VAVK(GG)TLK(GG)PGTMSVQAFLEEAN LMK
P26039	72	50.3%	MVEAAK(GG)GAAAHPDSEEQQQR
P26040	14	31.1%	QLERQQLETEK(GG) EDEVEEWQHRAK(GG)
P27005	5	94.4%	ALSNLIDVYHNYSNIQGNHHALYK(GG) K(GG)MVTTECPQFVQNINIENLFR ELDINSDNAINFEEFLAMVIK(GG)
P27612	6	13.7%	EALTFDQANPTQILGKLK(GG)

			GK(GG)PANQLLALR
P27661	6	60.8%	LLGGVTIAQGGVLPNIQAVLLPK(GG)K LLGGVTIAQGGVLPNIQAVLLPKK(GG)
P28650	9	31.3%	GHEWGVTTGRK(GG)
P28665	5	6.8%	FALEIPVEFSMVPMAK(GG)
P29699	2	15.4%	QVLNQIDK(GG)VK QVLNQIDKVK(GG)
P30416	4	14.0%	VKAEVAAGDHPTDAEMK(GG)
P31725	8	83.2%	QMVEAQLATFMK(GG) RNEALINDIMEDLDTNQDNQLSFEECMM LMAK(GG) NEALINDIMEDLDTNQDNQLSFEECMML MAK(GG)
P32921	2	6.9%	DEIDSAVKMLLSLK(GG) IGHPK(GG)PALLHSTFFPALQGAQTKMSA SDPNSSIFLTDTAK
P35980	3	19.7%	ILEVPKLK(GG)
P38647	5	10.5%	EQQIVIQSSGGLSK(GG)
P40124	16	57.4%	VPTISINKTDGCHAYLSK(GG)
P42227	8	20.9%	FSESSK(GG)EGGVTFTWVEK(GG)
P42669	3	35.2%	MADRDSGSEQGGAALGSGGSLGHPGSGS GSGGGGGGGGGGGGSGGGGGAPGGLQH ETQELASK(GG)
P43274	6	33.8%	KALAAAGYDVEK(GG)
P43275	4	31.0%	AK(GG)ASGAAK(GG)
P43276	6	30.9%	K(GG)PAGATPKKPK(GG) ALAAGGYDVEK(GG)
P45591	2	16.9%	KEDLVFIFWAPESAPLK(GG) EDLVFIFWAPESAPLK(GG)
P45952	2	10.5%	ENVLIGEGAGFK(GG)
P47738	21	74.0%	EEIFGPVMQILKFK(GG)
P47791	14	57.6%	GHILVDEFQNTNVK(GG)GVYAVGDVCG K
P47809	2	8.6%	AAPSPSGGGGSGGGGGTPGPIGPPASGHP AVSSMQGK(GG)
P47955	3	72.8%	INALIK(GG)AAGVSVEPFWPGLFAK(GG)
P47962	5	21.2%	ENPVYEK(GG)K(GG)
P48025	18	49.4%	EYVKQTWNLQGQALEQAIISQKPQLEK(G G) TGK(GG)LSIPEGK(GG)
P51125	3	9.1%	EQK(GG)PFTPASPVQSTPSKPSDK EK(GG)STGEIFK(GG) EK(GG)IKPEHSEK(GG)

			EKIK(GG)PEHSEK(GG) PEKPPTKK(GG) PEKPPTK(GG)K(GG) PEK(GG)PPTK(GG)K
P51863	2	11.4%	LLFEGAGSNPGDK(GG)
P52480	23	69.3%	K(GG)GVNLPGAAVDLPAVSEK
P54987	2	10.5%	MILDSLGVGFLGTGTEVFHK(GG)
P57759	3	18.7%	FDTQYPYGEK(GG)QDEFK
P57780	37	62.2%	VHK(GG)PPKVQEK(GG) ETTDTDTADQVIASFKVLAGDK(GG)
P58252	28	51.2%	EGIPALDNFLDK(GG)
P60229	13	37.8%	QLQAETEPIVK(GG)
P60710	26	97.1%	DLYANTVLSGGTTMYPGIADRMQK(GG) K(GG)DLYANTVLSGGTTMYPGIADR
P61202	4	12.2%	SINSILDYISTSKQMDLLQEFYETTLEALK(GG)
P61222	4	11.2%	DETK(GG)TQAIVCQQLDLTHLK(GG) MLAGRLK(GG)PDEGGEVPVLNVSYK(GG)
P62192	6	23.0%	ESVELPLTHPEYYEEMGIK(GG)
P62307	2	55.8%	PFLNGLTGKPVMVK(GG)LK(GG)
P62746	5	48.0%	KLVVVGDGACGK(GG) HFCPNVPIILVANK(GG)
P62806	5	61.2%	DNIQGITK(GG)PAIR K(GG)TVTAMDVVYALK
			TVTAMDVVYALK(GG)
			TVTAMDVVYALK(GG)R
P62827	8	59.3%	DGYYIQAQCAIIMFDVTSRVTYK(GG)
P62960	5	31.4%	PGSTGSGAGSGGPGGLTSAAPAGGDK(GG)
P63005	8	28.5%	SPVTRVIFHPVFSVMVSASEDATIK(GG)
P63017	30	73.4%	LLQDFFNGK(GG)ELNK(GG) EEFEHQQK(GG)
P63158	7	40.0%	LGEMWNNTAADDK(GG)
P68033	18	68.4%	YPIEHGIITNWDDMEK(GG) LCYVALDFENEMATAASSSSLEK(GG)
P68433	6	60.3%	FQSSAVMALQEACEAYLVGLFEDTNLCAIHAK(GG)
P68510	4	27.2%	AVTELNEPLSNEDRNLLSVAYK(GG)
P70168	21	44.1%	DAAVMAFGSILEGPEPNQLKPLVIQAMPTLIELMK(GG)
P70195	9	55.2%	LVSEAIAAGIFNDLGSGSNIDLCVISK(GG)
P70268	2	5.3%	LLLTAQQMLQDSK(GG)TK(GG)

P70290	4	13.7%	QDK(GG)IAILDIEPQTLK(GG)
P70336	3	3.8%	DEEMSAAAIKAQFEK(GG)
P70398	5	4.1%	PRWVVPVLPK(GG) QIWK(GG)CLAENAVYLCDR WYKFDDGDVTECK(GG)
P70460	8	31.7%	K(GG)MQPDQQVVINCAIIR
P70670	3	1.9%	QIPTPEDAVTILAGSPLSPKK(GG) VPKTAAPEETSTTPSPQK(GG)
P80315	8	25.1%	SGAPTAGPGSRGK(GG) MLVELSK(GG)
P80318	14	38.4%	K(GG)GESQTDIEITR
P82198	21	57.3%	K(GG)YFTNCK(GG)
P84228	6	61.0%	FQSSAVMALQEASEAYLVGLFEDTNLCAI HAK(GG) FQSSAVMALQEASEAYLVGLFEDTNLCAI HAK(GG)R
P97310	4	8.4%	GLALALFGGEPK(GG) MYSDLRK(GG)
P97369	9	45.1%	LLSDEDVGLMVK(GG)
P99024	21	69.4%	ISVYYNEATGGK(GG)YVPR
P99027	4	69.6%	LASVPAGGAVAVSAAPGSAAPAAGSAPA AAEEK(GG) LASVPAGGAVAVSAAPGSAAPAAGSAPA AAEEK(GG)K(GG)
Q00612	30	74.2%	DVMQNHLLQMLCLVAMEK(GG)PATTGS DDVRDEK EK(GG)PQPIPYVYGSR
Q01853	34	70.7%	K(GG)MDLIDLEDETIDAEVMNSLAVTMD DFR QAAPCVLFFDELDSIAK(GG)AR
Q02053	32	53.8%	YFLVGAGAIGCELLK(GG)
Q03265	8	21.2%	EPMQTGIK(GG)
Q07076	7	23.3%	QMFTQMYQK(GG)TLSTMIASDTSGDYR
Q11011	23	38.8%	AQELDALDNSHPIEVSVGHPSEVDEIFDAI SYSK(GG)GASVIR
Q3TXS7	14	29.9%	TNLYQDDAVTGEAAGLALGLVMLGSK(G G)
Q3U898	2	71.9%	MK(GG)PAVDEMFPEGAGPYVDLDEAGG STGLLMDLAANEK
Q3UKJ7	2	4.9%	LIMQYLK(GG)
Q3UMW7	2	8.1%	EEMTSALATMRVDYDQVK(GG)
Q3UQ44	4	4.6%	ESK(GG)TDNESSEGSWVTLNVQEK
			LFK(GG)TALEEEIK(GG)

Q3UZ39	5	11.7%	PAEDSALSPGPLAGAK(GG)CEQQVQSQD QENTSDLK
Q3V1L4	10	28.4%	GK(GG)DILYIGDHIFGDILK(GG)
Q3V3R1	2	3.0%	QAQFDIAVASEIMAVLALTDSLTDMK(GG)ER
Q4LDD4	2	2.8%	QDARSVHLK(GG)
Q4QRL3	4	4.1%	EAPQGELVHK(GG)
Q5SQX6	5	5.4%	DK(GG)PANVQPYYLYGSK(GG)
Q5SUS9	3	7.1%	DFQGSKLK(GG)
Q5SXR6	48	48.7%	WISLNTVALVTDNAVYHWSMEGESQPV K(GG) K(GG)DPELWGSVLLESNPYR
Q60668	5	27.6%	K(GG)IFVGGLSPDTPEEK(GG)
Q61599	6	53.0%	LNYKPPPQK(GG)
Q61646	12	53.3%	LPECEAVCGK(GG)PK(GG) ATDLKDWVQETMAK(GG)
Q61768	3	4.5%	TK(GG)EYELLSDELNQK(GG) RGHSAQIAK(GG)PIR
Q61990	4	16.6%	QICVVMLESPPK(GG)GVTIPYRPK QMSGAQIK(GG)IANPVEGSTDR
Q64514	12	15.9%	RLNEIVDAANAVISHIDQTALAVYIAMK(GG)
Q64737	3	5.6%	MLNIHPSLLPSFK(GG)
Q6IRU2	6	35.5%	MEILEMQLK(GG) LLSDKLK(GG)
Q6NS79	7	11.3%	EKPNSAHRNSSASYGDDPTAQSLQDISDE QVLVLFEQMLVDMNLNEEK(GG)
Q6NXH9	3	6.5%	AEAEMVYQTK(GG)
Q6P2B1	6	15.9%	RTEIIEDLAFYSSTVVSLLMTCVEK(GG)
Q6P4T2	20	15.6%	LDLVHTAALMLDK(GG)
Q6P5F9	18	26.8%	EFMKDTDSINLYK(GG)
Q6PD03	7	25.7%	EHWNQTIVALVYNVLKTLMEMNGK(GG)
Q6PDG5	2	3.3%	DIGEGNLSTAAAAALAAAAVK(GG)AK(G G)
Q6PDQ2	6	6.2%	ILNHSVDK(GG) AHRIGQNK(GG) EIIK(GG)QEESVDPDYWEK(GG)
Q6Q899	3	6.3%	IMNESILRLQTWDEMK(GG)
Q6ZQK5	4	8.8%	LVVDAAK(GG)
Q6ZWR6	17	3.4%	WSDTSGDPSATQK(GG)K(GG) WSDTSGDPSATQKK(GG) DMWESLLSAAIRCK(GG) AHTKEEAEQLAVK(GG)

			MLFDEVQFK(GG) K(GG)DYQEEIAVAQENK(GG)
Q6ZWX6	3	10.5%	ENAEVDGDDDAEEMEAK(GG)
Q78ZA7	4	15.2%	VVIAEKEAATVEELNPK(GG)
Q7TNV0	5	16.3%	LLADANLEEVTMK(GG)QICK(GG)
Q7TPR4	44	76.0%	QYEKSIVNYKPK(GG)
Q7TPV4	6	6.9%	MLGVQRPK(GG)SEK
Q8BH69	3	17.1%	DAAEEAGTSVTGGQTVLNPWIVLGGVAT TVCQPNEFIMPDNAVPGDVLVLTK(GG) MAAVSK(GG)ACGNMFGLMHGTCPETSG GLLICLPR
Q8BKC5	23	34.3%	LVLEQVVTSIASVADTAEEK(GG)
Q8BMS1	2	7.3%	IIDAVK(GG)AGLEQGSDAGYLAESQK(GG)
Q8BPU7	6	22.7%	PSSLDQFK(GG)
Q8BT60	10	25.5%	IK(GG)NSLNPK(GG)
Q8BU30	4	5.6%	LFLNETQTQEITEDIPMK(GG)
Q8BVE3	4	15.3%	ELMEGSDLNYYFNWIK(GG)
Q8BVG4	3	6.2%	LVYFQGTK(GG)DTPLEHHLYVVSYESAG EIVR
Q8BWT1	6	30.5%	DGTVTAGNASGVSDGAGAVIIASEDAVK(GG) VVGYFVSGCDPTIMGIGPVPAINGALK(G G)K
Q8C147	11	9.0%	IEK(GG)VLQQGEIADCAEPYMIIK NCSRMSYYCSGNSDAPGSTAAPRPVSK(G G)
Q8C1B7	4	13.2%	KELEEEVSNFQK(GG)
Q8C3J5	7	7.2%	PQLLQENLEK(GG)LK
Q8CG48	2	2.7%	QVVIGGRNK(GG)
Q8CGC7	12	13.6%	EDIDAAVKQLLTLK(GG)
Q8CGK3	2	7.3%	LYK(GG)ALSLLK(GG)
Q8CGP0	7	56.4%	VLK(GG)QVHPDTGISSK
Q8CGP4	5	60.5%	NDEELNK(GG)LLGR VTIAQGGVLPNIQAVLLPK(GG) VTIAQGGVLPNIQAVLLPK(GG)K VTIAQGGVLPNIQAVLLPKK(GG)
Q8JZQ9	11	26.5%	DRPQEADGIDSVIVVDNVPQVGPDRLEK(GG)
Q8K1K2	5	23.3%	VDPLVSLMMVEK(GG)
Q8K1M6	4	10.1%	SK(GG)PIPIMPASPQK(GG)GHAVNLLDVP VPVAR
Q8K1X4	4	8.4%	MLVETSDLSTFCFHLRTFEK(GG)

			EFLVVASVSLLQLGQETDK(GG)LK
Q8K2Z4	4	5.8%	ATEK(GG)VPSSPLER
Q8K4Z5	2	3.5%	QSDDEVYAPGLDIESSLK(GG) TEWK(GG)LNGQGLVFTLPLTDQVSVIK(GG)
Q8QZY9	6	30.2%	VSEPLLWELFLQAGPVVNTHMPK(GG)
Q8R1Q8	2	7.7%	K(GG)TGSPGGPGVGGSPGGGAAGASPSLPPSAK(GG)
Q8R2P8	6	20.2%	MLSGMVK(GG)SITGSYK
Q8R3C7	2	7.7%	SFEELRLK(GG)PQLLQGVYAMGFNR NCQMLLFSATFEDSVWK(GG) QYYVLCNNREEK(GG)
Q8VCT3	8	23.1%	FLK(GG)AYVDEFK(GG) GVDSIPGFEFDRWLNTPGWPPYLPDLSPGDSLMK(GG)
Q8VDD5	70	52.1%	EGIEWNFIDFGLDLQPCIDLIEK(GG) MQQNIQELEEQLEEEESARQK(GG) RALEQQVEEMK(GG)
Q8VDM4	23	43.5%	SSTTSMTSVPK(GG)PLK CFAADIISVLAMTMSGERECLK(GG)
Q8VDM6	2	4.0%	YNILGTNAIMDK(GG)MR
Q8VIJ6	5	10.9%	EEYEGPNK(GG)K(GG)
Q91V92	22	32.7%	LK(GG)QGLYR
Q91VH6	2	11.5%	IDQK(GG)IYGELWK(GG)
Q91VI7	8	30.3%	ELDLSNNCMGGPGVLQLLESLK(GG)
Q91Z50	5	24.0%	LIADVAPSAIRENDIK(GG)
Q91Z69	2	2.2%	DKEIIAEYESQVK(GG)
Q91ZJ5	13	41.3%	IQRPPEDSIQPYEK(GG) TTSDLLLVMSNLYSLNAGSLTMSEK(GG)
Q91ZW3	2	2.6%	ENMELEEKEK(GG)
Q921H8	3	14.9%	QDDFALASQQK(GG)AASAQSR
Q922B2	9	22.8%	QQQFNVQALVAVGDHASK(GG) QMCICADFEK(GG)
Q922D8	3	4.6%	MVVASSK(GG)
Q922R8	8	28.4%	KAATALK(GG) ALDLFSDNAPPPELLEIINEDIAK(GG)K(GG)
Q93092	9	31.8%	K(GG)FAADAIK
Q99JI4	8	29.3%	YSVFFQSLAIVEQEMK(GG) NWQYQETIK(GG)K(GG)
Q99K94	8	16.4%	FLEQVHQLYDDSFPMEIRQYLAQWLEK(GG)
Q99KK7	6	17.8%	VVEHLEK(GG)

Q99KV1	4	17.9%	ITRPGAKLWK(GG)
Q99L45	4	19.9%	K(GG)TSFVNFTDICK(GG)
Q99LB4	6	29.5%	ESPIFK(GG)QFFK(GG)
Q99MK8	5	14.1%	QVPPDLFQPYIEEICQNLRGDVFQK(GG) GEVNAADAFDIGSFDEEDTK(GG)GIK
Q99MK8	5	14.1%	GEVNAADAFDIGSFDEEDTKGIK(GG)
Q99PV0	13	10.5%	PADTEPPPLLVYK(GG)WCQGINNLQDVW ETSEGECNVMLESR
Q9CQR6	4	24.3%	QCK(GG)YLPENDLK
Q9CRA5	2	12.4%	LLALIYLAHASDVLENAFAPLLDEQYDLA TK(GG)
Q9CS42	2	10.1%	MVLVGDVK(GG)
Q9CU62	4	4.3%	TVALDGTLFQKSGVISGGASDLK(GG) NQHLAK(GG)K(GG)
Q9CVB6	16	77.3%	IIEETLALK(GG)
Q9CW03	8	10.7%	DLQDELAGNSEQRK(GG)
Q9CWJ9	16	47.1%	GAVDIPAAASFK(GG)HVSPAGAAVGVPL SEDEAR
Q9CZ44	7	30.3%	SPGETSK(GG)PR
Q9CZD3	5	13.2%	DHPK(GG)FQSVADLCLYLYSAK(GG)
Q9D0E1	4	9.2%	GIGMGNLGPAGMGMEGIGFGINK(GG)IG GMEGPFGGGMENMGR
Q9D154	15	54.4%	EQLSSANTLFALELFQTLNESSPTGNIFFSP FSISSALAMVILGAKGSTAAQLSK(GG)
Q9D1Q6	5	23.2%	EITFENGEELTEEGLPFLILFHMK(GG) TPADCPVIAIDSFRHMYVFGDFK(GG)
Q9D8B3	2	13.4%	LFGAGGGK(GG)
Q9DBZ5	4	26.6%	WLLAEMLGDLTDNQLK(GG)VWMSK
Q9DCC5	4	39.3%	IIGATDSSGELMFLMK(GG)WK(GG)
Q9EQH3	19	37.9%	NKLMDALK(GG)
Q9ER72	7	16.4%	MK(GG)IPPSEMFLSEVNK
Q9ERD8	5	23.3%	ILYSLFQK(GG)
Q9ERK4	11	20.0%	GSSTIATAAADK(GG)
Q9ES52	2	2.9%	MPAMVPGWNHGNITRSK(GG) ILQLIKSQK(GG) SFTCSSSAEGRMTSGDK(GG) SEMSQQTTPIPAPRPPLPVK(GG)SPAVLQ LQHSK(GG)
Q9ESP1	2	19.9%	VAGPTLLGLLLALSVRSGGASK(GG) LLNTHHK(GG)
Q9JHU4	24	9.0%	DSAIQQQVANLQMK(GG) PVTGNLRPEEALQALTIYEGK(GG)FGR VFEEDALSWEDK(GG)

			EHSNPNYDK(GG)TSAPITCELLNK(GG) TSFLDDAFRK(GG) QLQNISQAAASGGAKELK(GG)
Q9JI10	3	8.7%	EISIMQQCDSPYVVKYYGSYFK(GG) PELWSDDFTDFVK(GG)K(GG) QPILDAMDAK(GG)K
Q9JI11	3	9.5%	EISIMQQCDSPHVVK(GG)YYGSYFK
Q9JIF0	4	14.6%	AAAEAANCIMENFVATLANGMSLQPPLE EVSCGQAESSEK(GG)
Q9JIF7	10	18.2%	FDNLRMLIVEK(GG) VLSECSPLMNDIFNK(GG)ECR
Q9JJ28	13	21.3%	CVYILDCWSDVFIWLGRK(GG)
Q9JKR6	9	18.6%	ESGDK(GG)PEAQK(GG)PNEK(GG)GQAG PEGAAPAPEEDK PNEKGQAGPEGAAPAPEEDK(GG)
Q9JMA1	6	19.9%	NGMTVLMMGSADALPEEPSAK(GG) FPLMLDVYELCTPELQEK(GG)
Q9QUM0	20	38.7%	LAEVGRVYLFLQPK(GG)
Q9QZD9	5	27.1%	LGTYMGHTGAVWCVDADWDTK(GG)
Q9QZE5	11	23.6%	YMKQAIVDK(GG) ALNAGYILNGLTVSIPGLEKALQQYTLEP SEKPFDLK(GG)
Q9R0P5	7	55.2%	MIYASSK(GG)DAIK
Q9R1P0	8	57.5%	QDYK(GG)EGEMTLK
Q9R1P1	5	40.5%	SIMSYNGGAVMAMK(GG)
Q9R1T2	2	9.4%	VLIVGMKGLGAEIAK(GG)
Q9WTM5	8	23.3%	AAGVVLEMIREGK(GG)
Q9WU78	15	31.1%	K(GG)QEGLLK(GG)
Q9WUM3	4	21.5%	EAYVPSK(GG)QR
Q9WUM4	5	17.5%	NADPILISLKHGYIPGK(GG)
Q9Z0N1	6	26.3%	QATINIGTIGHVAHGK(GG)
Q9Z183	14	37.1%	EDPQASGMDFEDDK(GG)ILDNK CKLTVCPEEENIDDQWMQDEMEIGYIQA PHK(GG)
Q9Z1F9	5	16.1%	SMAGNIIPAIATTNAVIAGLIVLEGLKILSG K(GG)
Q9Z1G3	2	8.9%	VAQYMADVLEDSK(GG)DK(GG)
Q9Z1N5	10	35.3%	HFILDECDK(GG)
Q9Z1Z2	3	13.4%	PMLRQGDTGDWIGTFLGHK(GG)
Q9Z2D6	2	8.1%	KPGSVVAAAAAEAK(GG)
Q9Z2U1	7	45.2%	LNATNIELATVQPGQNFHMFTK(GG)EEL EEVIK(GG)
Z4YKA3	9	25.2%	PKMDAILTEAIK(GG)

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