Published in final edited form as:

Cell Immunol. 2011 ; 271(2): 239–255. doi:10.1016/j.cellimm.2011.07.002.

Proteomic Biosignatures for Monocyte-Macrophage Differentiation+

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Abstract

We used pulsed stable isotope labeling of amino acids in cell culture (pSILAC) to assess protein dynamics during monocyte-macrophage differentiation. pSILAC allows metabolic labeling of newly synthesized proteins. Such *de novo* protein production was evaluated from 3 to 7 days in culture. Proteins were identified by liquid chromatography tandem mass spectrometry then quantified by MaxQuant. Protein-protein linkages were then assessed by Ingenuity Pathway Analysis. Proteins identified were linked to cell homeostasis, free radical scavenging, molecular protein transport, carbohydrate metabolism, small molecule chemistry, and cell morphology. The data demonstrates specific biologic events that are linked to monocyte transformation in a defined biologic system.

Keywords

monocyte differentiation; monocyte-derived macrophages; proteomic biosignatures; pulsed stable isotope labeling with amino acids in cell culture; liquid chromatography tandem mass spectrometry; MaxQuant

1. Introduction

Hematopoietic stem cells differentiate through myeloid progenitor intermediates in the bone marrow, to circulating blood monocytes, then to tissue macrophages. The end organ locale defines the tissue macrophage as Kupffer cells in liver, histiocytes in connective tissue, osteoclasts in bone, alveolar macrophages in lung, microglia in brain and spinal cord, and dendritic cells and macrophages in lymph nodes and spleen [1]. While differences in cell morphology and function serve to define the biology of monocytes and macrophages [2–5], much is unresolved as to the dynamic molecular, biochemical, and cellular events that underlie monocyte differentiation. Defining the steps of such differentiation pathways are of particular interest as these determine common secretory, phagocytic, and antigen presentation functions as well as the abilities to destroy cancer cells, repair tissue injuries,

⁺Grant support: Support for research was provided by National Institutes of Health Grants P20 DA026146, 5P01 DA028555-02, R01 NS36126, P01 NS31492, 2R01 NS034239, P20 RR15635, P01 MH64570, and P01 NS43985 (to H.E.G)

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and restrict or allow microbial infection [6,7]. Indeed, mononuclear phagocytes (MP; monocytes, macrophages, and dendritic cells) define innate immune-mediated processes that include clearance of debris and microbial infections, control of cancer progression, and secretion of immunoregulatory bioactive factors including interferons, interleukins, growth factors and chemokines [8–10].

Morphologic changes also result from cell differentiation linked to tissue repair, cell remodeling and monocyte-macrophage physiology [5]. In recent years, research efforts have focused on biochemical and genetic changes that occur during cell differentiation [11,12]. These included the transcriptional regulation of the macrophage colony stimulating factor receptor [13,14]. Moreover, protein kinase C signaling was shown to engage Toll-like receptor (TLR) expression during monocyte differentiation [10]. However, each of these studies represented static cell assessments. Recent technology development seen for proteomics biomarker discovery has made dynamic assessments of the monocyte-macrophage proteome possible. To this end, we examined changes in *de novo* protein synthesis in primary human monocytes during differentiation using the technique of pulsed stable isotope labeling of amino acids in cell culture (pSILAC). pSILAC allows the incorporation of modified amino acids for identification of new protein synthesis through mass spectrometry, where protein ratios that have incorporated modified amino acids are observed and quantified [15]. Using this assay, we identified changes in protein expression essential to the control of transcription, surface markers specific for macrophage homeostasis, and proteins involved in the inflammatory response. This delineation of monocyte differentiation uncovered new pathways linked to a host of cellular functions critical to innate immunity and host immune surveillance.

2. Materials and Methods

2.1. Cell recovery and cultivation

Primary human monocytes were obtained through leukaphoresis from donors seronegative for HIV-1, HIV-2, and hepatitis and were purified by countercurrent centrifugal elutriation [16]. Cells were plated in complete medium consisting of Dulbecco's Modified Eagle's Media (DMEM) supplemented with 10% dialyzed fetal bovine serum (FBS), 2 mM L-glutamine, 2 µg/ml macrophage colony stimulating factor (MCSF, a generous gift from Pfizer, Inc., Cambridge, MA), and 50 µg gentamicin). Three million cells/well were plated in 6 well plates at a density of 1×10^6 cells/mL.

2.2. SILAC procedures, cell lysis and sample preparation

SILAC medium was prepared using two different combinations of isotopically-labeled L-Arginine and L-Lysine, “Medium” complete media where L-Arginine and L-Lysine were replaced with [$^{13}\text{C}_6$]-L-Arginine and [D_4]-L-Lysine (SIGMA-Isotec, St. Louis, MO) and “Heavy” complete media where L-Arginine and L-Lysine were replaced with [$^{13}\text{C}_6$, $^{15}\text{N}_4$]-L-Arginine and [$^{13}\text{C}_6$, $^{15}\text{N}_2$]-L-Lysine (SIGMA-Isotec, St. Louis, MO) [17]. SILAC media was applied to monocytes for a 48 hour period, followed immediately by cell lysis. “Medium” complete media was applied at days 1 and 5 following plating, and “Heavy” media was applied at day 3. The procedure was repeated with 3 separate monocyte donors. After 48 hours of incubation in SILAC medium, all media was removed, cells were washed with PBS and lysed in lysis buffer (4% sodium dodecyl sulfate, 0.1 M dithiothreitol, 0.1 M Tris-HCl, pH 7.6). After lysis buffer was applied, cells were scraped and collected. Cell lysates were boiled. Fifty micrograms of protein was aliquoted from each day of collection and combined with 50 µg of its uninfected control from the same day of collection. Lysates were diluted in UA (8M Urea, 0.1 M Tris-HCl, pH 8.5), applied to a 30 kDa molecular weight cutoff centrifugal filter (Millipore, Bellerica, MA), and centrifuged at $14,000 \times g$ for

15 minutes. Lysates were washed with UA and centrifugation was repeated. IAA (0.5M iodoacetamide) was added to the filter and incubated at room temperature, followed by centrifugation at $14,000 \times g$ for 10 minutes. The filter unit was washed with UA, and centrifugation was repeated. ABC (0.05 M NH_4HCO_3) was added to the filter, centrifuged and repeated once. Two micrograms of trypsin (Promega, Madison, WI) in ABC was applied to the filter, mixed for 1 minute and incubated 18 hours at 37°C in a humidified chamber. The filter containing digested peptides was transferred to a new collection tube and centrifuged at $14,000 \times g$ for 10 minutes. The filter was washed with 0.5M NaCl and centrifuged at $14,000 \times g$ for 10 minutes [adapted from [18]]. The digested sample was brought to 1 ml total volume with 0.2% formic acid. The acidified protein was added to a wet MXC 30 mg extraction cartridge (Waters-Oasis, Milford, MA) in 1 mL 50% methanol, and the cartridge was washed with 1 ml of 5% methanol/0.1% formic acid, then with 100% methanol. The proteins were eluted with 1 mL elution buffer (1.4% NH_3OH in methanol) and samples dried using a SpeedVac.

2.3. Isoelectric focusing/fractionation

Dried samples were resuspended and separated by isoelectric focusing through OFFGEL electrophoresis (Agilent, Santa Clara, CA) following the manufacturer's suggested protocol for separating peptides [19]. Samples were separated on a pH 3–10 strip and collected from all 12 wells. Wells were washed with 200 μl 50% methanol, 1% formic acid. Wash was collected, dried with a SpeedVac, and previously collected samples from the corresponding wells were combined with the dried wash [20]. Fractions collected from OFFGEL electrophoresis were mixed 3 parts sample to 1 part 2% TFA and 20% ACN. C18 spin columns (Thermo Fisher, Rockford, IL) were placed in collection tubes and activated by the addition 100% ACN, followed by being washed with 0.5% TFA and 5% ACN. Diluted samples were applied to the pre-wet column twice and washed with 0.5% TFA and 5% ACN. The column was placed in a new receiver tube, and peptides were eluted with the addition of 70% ACN. Samples were dried using a SpeedVac and resuspended in 6 μl 0.1% formic acid for liquid chromatograph-tandem mass spectrometry (LC-MS/MS) analysis.

2.4. Mass spectrometry

An LTQ Orbitrap XL with Eksigent nano-LC system was used, equipped with two alternating peptide traps and a PicoFrit C18 column-emitter (New Objective, Woburn, MA). Samples were loaded onto the peptide trap with 98:2 HPLC water with 1% formic acid: ACN with 1% formic acid and eluted using a 60 minute linear gradient of 0–60% ACN with 1% formic acid. The instrument was tuned using direct infusion of angiotensin and calibrated every 2–3 days using standards provided by the manufacturer without Lock Mass. The acquisition method was created in data-dependent mode with one precursor scan in the Orbitrap, followed by fragmentation of the 5 most abundant peaks by CID and detected in the LTQ. Resolution of the precursor scan was set to 60,000, scanning from 300–2000 m/z . Precursor peaks were dynamically excluded after two selections for 60 seconds. No charge state rejection was used, but previously-detected background peaks were included in a mass rejection list. Collision energy was set to 35 using an isolation width of 2 and an activation Q of 0.250.

2.5. Peptide identification and quantification

Files obtained from LTQ-Orbitrap were submitted to MaxQuant (version 1.0.13.8) for peak list generation. The peak list files were searched using Mascot (Matrix Science, Boston, MA) against the International Protein Index (IPI) human database for protein identifications. The search parameters were set as 2 maximum missed cleavages; Carbamidomethyl (C) as fixed modification; N-acetyl (Protein) and Oxidation (M) as variable modifications; top 6 MS/MS peaks per 100 Da; and MS/MS mass tolerance of 0.5 Da. Output files from Mascot

were submitted back to MaxQuant for final identifications and validation. MaxQuant was used because of its abilities to provide quantitative analysis of large mass spectrometric data sets acquired when performing pSILAC for high-resolution peptide pair detection. Different mass measurements were corrected for achieving mass accuracy. One unique peptide with a minimum length of 6 amino acids was required. A false discovery rate of 0.01 was applied for both protein and peptide identification, ensuring that at most only 1% of proteins would be falsely identified. Ratios were obtained for days 5 (“Heavy”) to 3 (“Medium”) and days 7 (“Medium”) to 5 (“Heavy”) and then inversed. Significant protein ratio cutoff was set at a Significance B value ≤ 0.05 as calculated by MaxQuant [21,22]. Since proteins of high abundance have high-intense signals and can be measured more accurately than ones with low abundance, we used the B-value because it groups the proteins of similar intensities into bins of equal occupancy and estimates the standard deviation of the protein ratios using the protein subset grouped into the same intensity bin. Contaminants and reverse hits identified by MaxQuant were excluded from further analysis. False discovery rate (FDR) control via the Benjamini Hochberg method was applied to protein groups with a significance B value ≤ 0.05 . Remaining proteins following application of statistical filtering were utilized for bioinformatic analysis.

2.6. Bioinformatic Analysis

Ingenuity Pathway Analysis (IPA) version 8.8 (Ingenuity Systems, Redwood City, CA) was used for biological function characterization and protein linkages.

3. Results

3.1. pSILAC

This work used pSILAC technologies to measure dynamic protein synthesis during monocyte differentiation. The assay allows protein identification as a result of dynamic incorporation of modified amino acids by mass spectrometric analysis {Schwanhauser, 2009 #563}. Proteins isolated on days 3, 5 and 7 were combined to compare changes on days 3 to 5 and on days 5 to 7. The heavy to medium ratios obtained were $\log_{10}$ transformed (Table 2). Thus a positive ratio indicated rates of protein synthesis were greater in samples collected on day 5 and a negative ratio implied production rates decreased by day 5 and were therefore higher on day 3. The same designations are made for the comparisons made from days 7 to 5.

3.2. Rates of protein synthesis

The greatest increases in rates of protein synthesis were those seen on day 5 compared to day 3 (Figure 1a). Therefore, the greatest rate of change occurred from day 3 to 5. When comparing day 7 to 5, decreasing synthesis rates were evident on day 7. Nonetheless, some rates increased, but less so (Figure 1b). Therefore, initial increases in translational rates from days 3 to 5 were maintained throughout the remaining time periods for examination.

3.3. Proteins Identified and Quantified by MaxQuant

MaxQuant identified a total of 138 proteins that were considered to be statistically significant following exclusion. A FDR was applied, combined with filtering out those ratios with a significance B ≥ 0.05 (Table 1). This included 91 significant ratios when days 5 to 3 were compared (Table 1) and 64 significant ratios when comparing days 7 to 5 (Table 2). Seventeen protein identifications were similar between days 5 to 3 and days 7 to 5 comparisons. To elucidate the key functions in which significant proteins were involved and how these proteins affect each other, we submitted to Ingenuity Pathway Analysis (IPA) lists of identified proteins considered significant.

3.4. IPA Yields Distinct Functional Groupings

IPA categorized the top functional groupings of identified proteins. The top 5 Molecular and Cellular functions affected comparing days 5 to 3 were related to cell-to-cell signaling and interaction, cellular function and maintenance, free radical scavenging, molecular transport and carbohydrate metabolism (Figure 2, Table 3). Comparing days 7 to 5 the top molecular and cellular functions affected were molecular transport, followed by small molecule biochemistry, carbohydrate metabolism, cellular function and maintenance, and cell morphology (Figure 2, Table 3). The top 5 Physiological and System Development Classifications proteins comparing days 5 to 3 were hematological system development and function, tissue development, hematopoiesis, immune cell trafficking, and digestive system development and function (Figure 3, Table 4). Comparison of days 7 to 5 found the 5 most significant groupings were linked to cardiovascular system development and function, organ morphology, the hematological system and tissue, and tumor morphologies (Figure 3, Table 4). To understand specific interactions of proteins that showed significant changes within functional groupings, we examined the top interaction networks generated by IPA for both days 5 to 3 and days 7 to 5 comparisons.

3.5. Phagocytic and Free Radical Scavenging Proteins Synthesis Rates Increase by Day 5

Proteins were grouped under the functional classifications of cell-to-cell signaling and interaction, and cellular function and maintenance contained groupings of proteins related to phagocytosis (Table 3). These proteins had increased synthesis rates from days 5 to 3 (Table 1), but no change in synthesis rates were noted when comparing the rate of synthesis on days 7 to 5 (Table 2). Proteins grouped under free radical function were also found to have increased synthesis rates on days 5 to 3, which were not detected to be altered on day 7. This signifies increased rate of protein synthesis on day 5, which is maintained throughout the differentiation process for proteins involved in phagocytosis and free radical scavenging, two major functions of the macrophage.

3.6. Hemoglobin Synthesis Rates Decrease as Differentiation Progresses

Hemoglobin and proteins within the hemoglobin family were synthesized early in the differentiation process with a steady decrease in the rate of synthesis as differentiation progressed to day 5 (Figure 4) and more dramatically by day 7 (Figure 5). These proteins cluster within both networks (Figures 4 and 5) and demonstrate a distinct change that is occurring universally during differentiation in relation to hemoglobin transcription. Hemoglobin synthesis is most beneficial when monocytes are in circulation, therefore the highest translation rates of hemoglobin synthesis should occur prior to macrophage maturation. This is evident with our findings that hemoglobin translation rates decrease as monocytes differentiate.

3.7. Proteins Responsible for NF- κ B Suppression are Increased During Differentiation

IPA was utilized to identify known interactions between identified proteins. By day 5, a significant increase was observed in the synthesis rates of proteins that are known to suppress NF- κ B activation. These include superoxide dismutase 2 (SOD2) [23], thioredoxin (TXN) [24,25], sodium pump subunit alpha-1 (ATP1A1) [26] and proteins in the vacuolar H⁺ + ATPase family [27] (Figure 4). By day 7 of differentiation suppression of NF- κ B activation was variable, evident by the further increased translation of activation suppressor ATP1A1, but down-regulation of TXN synthesis rates (Figure 5). The rate of NF- κ B stimulated translation of both fatty acid binding protein 5 (FABP5) [28,29] and serpin peptidase inhibitor, clade B member 2 (SERPINB2) [30,31] are decreased from day 5 to day 7, further substantiating that NF- κ B activation is suppressed throughout the differentiation

process. Figure 6 provides a summary of the information gleaned from monocyte-differentiation based on the current works compared to that performed previously.

4. Discussion

Monocyte differentiation is an essential biological process affecting cell and tissue homeostasis and immune surveillance during microbial infection. The latter is linked to the inherent abilities of macrophages for environmental sensing, clearance of pathogens and tumor cells, and mobilization of adaptive immune responses [32]. Such functional processes are linked to specific sets of cellular functions regulated as a result of cell differentiation [33]. Using combinations of “state of the art” proteomic and bioinformatic analyses, protein groups were uncovered that were strongly linked to hematologic and immune cell function. Unexpectedly, associations between monocyte differentiation were also found with cancer, dermatologic and gastrointestinal diseases. Such proteins, nonetheless, are strongly associated with a range of immune, differentiation and cell trafficking functions that include the adhesive protein CD44, mitochondrial ATP1A, SOD2 and hemoglobin-associated proteins (Table 3).

Links between erythroid and monocyte differentiation are likely operative. Indeed, transmembrane and skeletal proteins present on red cell membranes that are seen during terminal erythroid differentiation and reticulocyte maturation were found linked to monocyte differentiation. Specifically, a 30-fold modulation in CD44 expression is known to occur during differentiation from proerythroblasts to orthochromatic erythroblasts and can enable erythroblasts to be distinguished at various stages of development [34]. In addition, CD44 is a well-known multifunctional cell surface adhesion protein involved in intercellular and cell-matrix interactions and tumor growth [35]. Such activities clearly parallel a differentiating monocyte phenotype. ATP1A is linked to maintenance of electrochemical gradients across the plasma membrane and has been implicated in the formation of multinucleated giant cell formation during HIV-1 infection [36]. ATP-binding cassette transporters are also highly conserved cell transmembrane transport proteins involved in a broad range of immune homeostatic responses [36]. Because of the generic nature of such proteins, their dysfunctions are involved in the progression and metastatic potential of a range of cancers [37,38]. SOD2 catalyzes the production of hydrogen peroxide from superoxide and arguably the most important anti-oxidant enzymes in preserving cellular homeostasis [39]. Interestingly, this and other free radical scavengers were also seen by day 5, indicating increased protein production during cell differentiation. Interestingly, most proteins of the hematologic system showed increased production by day 5 without change by day 7. These findings together, represent potential *in vivo* mechanisms with noted limitations in the experimental approach. Indeed, any host cell differentiation is governed by monocyte migratory behavior and is also affected by the tissue environment, endothelial barriers, and intra- and inter- cellular signaling events [40,41]. These events can affect terminal differentiation of monocyte-macrophages, but were not recapitulated in the current laboratory system. Nonetheless, our focus on the monocyte and the abilities to correlate differentiation to regulation of microbial infections remains a viable means to correlate the dynamics of protein synthesis with cellular immune functions.

It is also worth noting that the most significant changes in protein synthesis rates occurred early in cell cultivation at days 3 to 5 of differentiation. The majority of the proteins involved in cell-to-cell signaling had increased synthesis rates by day 5 indicative of increased cellular communication. Homeostatic regulatory proteins grouped under cellular function and maintenance proteins were also increased. Proteins critical for phagocytosis, a function well linked to macrophage biology, were identified with increased rates of

synthesis by day 5, implying the rate of phagocytic-related protein synthesis is determined early during monocyte differentiation.

The top network for both days 5 to 3 and days 7 to 5 comparisons contained a concentration of hemoglobin proteins that indicated a significant down-regulation in synthesis rates over time. We identified hemoglobin synthesis in monocytes as an early event in differentiation. While mature monocyte-derived macrophages (MDM) remain a source of this protein, its rate of synthesis was slowed over time. Monocyte-macrophages are known sources of hemoglobin within rat granular tissues [42]. In fetal erythroid cells, hemoglobin synthesis decreases with maturation [43]. This parallels human monocyte differentiation. The other conserved signaling interactions between comparisons were those linked to NF- κ B. While we did not detect altered translation rates of NF- κ B, proteins that are known to interact with it and affect its activation were altered. NF- κ B is an essential transcription factor for a variety of proteins essential to cellular homeostasis, complete silencing of expression would not be expected. Because differentiating monocytes are not activated, a suppression of NF- κ B prevents transcription of inflammatory cytokines and other factors involved in an active immune response. Expression rates of NF- κ B suppressors by day 5 are increased compared to day 3 and altered again by day 7. These findings imply that monocytes and MDM have increased NF- κ B activity due to decreased levels of suppressors, while intermediate states of differentiation do not.

We acknowledge the challenges faced when working with pure cultures of primary human monocytes. While time-matched, undifferentiated monocytes would have served as ideal controls, primary human monocytes cannot be maintained in culture in an undifferentiated state. To control for variability, we assessed monocyte proteins manufactured for the defined time periods. Moreover, we chose to work with primary human monocytes because they readily differentiate in culture and possess divergent properties in cell biology than those seen with transformed cell lines. MCSF elicits an M2 macrophage phenotype [44] known to be anti-inflammatory [45] and as such contribute to the suppression of NF- κ B identified. Because a majority of macrophages reflect an M2 phenotype, this can model monocyte differentiation.

NF- κ B plays a large role in activation of immune cells, so it would be expected that in the absence of inflammatory stimulators such as cytokines, NF- κ B levels would be suppressed. The HIV-1 long terminal repeat contains two NF- κ B binding sites. Binding of NF- κ B is therefore required for successful transcription of HIV-1 [46]. Partial suppression of NF- κ B activation during the early stages of monocyte differentiation may contribute to viral restriction.

Prior works from others and other laboratories demonstrated that restriction of HIV-1 and other lentiviral infection in monocytes results in a non-productive, latent infection that becomes productive after monocyte differentiation [47–58]. This begs the question of what is any proteins identified in this report are virus life cycle enabling. Certainly cellular proteins acquired through monocyte differentiation helps to complete the HIV-1 life cycle and those linked to a more immature cell phenotype present in monocytes assists in viral evasion from immune surveillance. This is contained, within the monocyte proteome, and upon passage of the cell into final end organ destination such as the CNS viral propagation occurs. This notion is commonly referred to as the Trojan Horse model of viral host dissemination [59,60]. Monocyte population subtypes are phenotype-defined in relation to their ability to harbor latent HIV-1 infection [61] and are known to be CD14+, CD16+, CD11b+, and Mac387+ [62]. Thus, the inter- and intra-relationships between the monocyte-macrophage proteome and the virus is linked one to the other. Finally, we fully accept that the system developed in this report is quite artificial. Indeed, a variety of cellular and tissue

signals affect monocyte-macrophage differentiation and are not contained in this model system. Moreover, the addition of MCSF which serves to speed differentiation is likely present at different levels *in vivo*. Nonetheless, the data presented in the current report and taken together demonstrate that monocyte differentiation is a complex process involving a wide variety of cellular signals. The current study does provides insights into critical protein changes essential to fully-differentiated macrophage function, including those involved in free radical scavenging and phagocytosis. Increased synthesis rates of NF- κ B suppressors result in decreased NF- κ B activation, therefore decreasing inflammatory activation in differentiating cells. This may contribute to microbial resistance during cell maturation stages.

Acknowledgments

We would like to thank Babu Guda, Rufina Dominic Savio and Melinda Wojtkiewicz for technical assistance, Drs. Howard Fox and Pawel Ciborowski for lively discussion and Dr. R. Lee Mosley for critical reading of the manuscript.

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Figure 1.

Distribution of ratios generated by MaxQuant. Primary human monocytes were differentiated *in vitro* in medium containing MCSF. SILAC media was added for 48 hour intervals starting on days 1, 3, or 5. Protein collected at days 3, 5 or 7 was combined and new protein synthesis was quantitated using MaxQuant. This was repeated with monocytes from 3 separate donors. Log_2 (ratio) for all proteins identified compared with their intensity for the comparison of (a) day 5 to day 3 and (b) day 7 to day 5.

Figure 2.

Top 5 Molecular and Cellular Functions identified by IPA. Proteins with significant ratios ($p \leq 0.05$) were submitted with their corresponding ratios to IPA for functional classification. The top 5 functional groupings under the classification Molecular and Cellular Functions, determined by the number of proteins corresponding to that function, are represented for the two different comparisons. Day 5 to 3 comparisons are colored in blue and day 7 to day 5 are colored in red.

Figure 3.

Top 5 Physiological System Development and Function groupings generated by IPA. Proteins with significant ratios ($p \leq 0.05$) were submitted with their corresponding ratios to IPA for functional classification. The top 5 functional groupings under the classification Physiological System Development and Function as determined by the number of proteins corresponding to that function, are represented for the two different comparisons. Day 5 to day 3 comparisons are colored in blue and day 7 to 5 are colored in red.

Figure 4.

Top scoring protein interaction network for day 5 to 3 comparisons. Significant proteins were submitted to IPA for analysis of known protein-protein interactions. These interactions are labeled with a connecting line. Red coloration indicates an increase in expression at day 5 when compared to day 3, green indicates a decrease in expression at day 5 compared to day 3. Lack of color indicates a protein involved in bridging interactions, but was not identified as differentially-regulated.

Figure 5.

Top scoring protein interaction network for day 7 to 5 comparisons. Significant proteins were submitted to IPA for analysis of known protein-protein interactions. These interactions are labeled with a connecting line. Red coloration indicates an increase in expression at day 7 when compared to day 5, green indicates a decrease in expression at day 7 compared to day 5. Lack of color indicates a protein involved in bridging interactions, but was not identified as differentially regulated.

Figure 6.

Summary of changes in the rate of protein synthesis during monocyte differentiation. Protein and morphological changes observed at days 5 and 7 of monocyte differentiation are illustrated. Known cell surface and cytoplasmic proteins with accompanying organelle changes, which include mitochondria and lysosomes, are illustrated with black labels. Our pSILAC studies are indicated in red and green labels. Red labels indicate proteins where synthesis rates were increased. These include: ATP1A1–4, RAB21, CD44, TXN, SOD2, ATP6V1, PLAUR and CD68 on day 5; and ATP1A1, PLAUR, and CD68 on day 7. Green labels indicate those synthesis rates that were decreased on day 7 and include RAB21, TXN, ATP1A2–4.

Table 1

List of significant proteins by MaxQuant for 5 to 3 day comparison.

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
-21.03	6.40E-24	RPL22	ribosomal protein L22	IP00219153	Nucleus
-16.991	2.96E-13	ANP32E	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	IP00165393	Nucleus
				IP00640833	
				IP00910423	
				IP00514333	
-13.025	8.02E-18	VCL	vinculin	IP00307162	Plasma Membrane
				IP00291175	
				IP00909711	
-12.732	1.48E-17	LOC729317	voltage-dependent anion channel 2 pseudogene	IP00737171	unknown
				IP00888783	
	VDAC2	voltage-dependent anion channel 2		IP00902560	Cytoplasm
				IP00216026	
				IP00024145	
-10.45	8.74E-10	H3F3C	H3 histone, family 3C	IP00917420	Nucleus
				IP00419884	
-10.446	8.74E-10	HIST3H3 (includes EG:8290)	histone cluster 3, H3	IP00216402	Nucleus
-8.191	2.84E-08	NDUFB10	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22kDa	IP00479905	Cytoplasm
				IP00074489	
				IP00433834	
-8.07	3.47E-08	RPL26	ribosomal protein L26	IP00027270	Cytoplasm
				IP00789514	
				IP00792420	
				IP00007144	
-7.155	1.70E-07	KRT9	keratin 9	IP00019359	Cytoplasm

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
-6.875	2.82E-07	NOP58	NOP58 ribonucleoprotein homolog (yeast)	IP100006379 IP100924866	Nucleus
-6.813	3.16E-07	NUP210	nucleoporin 210kDa	IP100291755	Nucleus
-6.726	3.71E-07	AIFM1	apoptosis-inducing factor, mitochondrion-associated, 1	IP100000690 IP100157908 IP100300018 IP100910786 IP100910617	Cytoplasm
-5.335	5.75E-06	RAB1A	RAB1A, member RAS oncogene family	IP100005719 IP100937805 IP100917079 IP100334174 IP100185217	Cytoplasm
-4.928	1.37E-05	PLAC8	placenta-specific 8	IP100410649	Nucleus
-4.308	2.09E-07	ITIH2	inter-alpha (globulin) inhibitor H2	IP100305461 IP100645038 IP100910636 IP100514159	Extracellular Space
-4.103	4.60E-07	SERPINB10 SERPINB2	serpin peptidase inhibitor, clade B (ovalbumin), member 10 serpin peptidase inhibitor, clade B (ovalbumin), member 2	IP100792070 IP100791734 IP100007117 IP100797177 IP100797920 IP100789721	Cytoplasm Extracellular Space
-3.958	1.26E-04	PPP2R4	protein phosphatase 2A activator, regulatory subunit 4	IP100893197 IP100293102 IP100935879 IP100217296	Cytoplasm

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
				IP100910771	
				IP100217297	
				IP100941236	
				IP100640101	
				IP100853351	
				IP100853207	
				IP100470813	
				IP100880122	
				IP100925818	
-3.913	1.40E-04	BTF3	basic transcription factor 3	IP100221035	Nucleus
				IP100419473	
-3.908	9.90E-07	SERPIND1	serpin peptidase inhibitor, clade D (heparin cofactor), member 1	IP100292950	Extracellular Space
				IP100879573	
				IP100013877	
				IP100216492	
				IP100216493	
-3.81	1.79E-04	HNRNPH3	heterogeneous nuclear ribonucleoprotein H3 (2H9)	IP100908651	Nucleus
				IP100216494	
				IP100216495	
				IP100216496	
				IP100017763	
				IP100941463	
-3.648	2.67E-04	NAPIL4	nucleosome assembly protein 1-like 4	IP100798071	Nucleus
				IP100788840	
				IP100789437	
				IP100220855	
-3.535	4.46E-06	H2AFJ	H2A histone family, member J	IP100102165	unknown
				IP100930144	
		HIST1H2AC	histone cluster 1, H2ac	IP100216456	Nucleus

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
-3.384	8.34E-06	HIST1H2AD	histone cluster 1, H2ad	IP100255316	unknown
		HIST2H2AC	histone cluster 2, H2ac	IP100339274	
		HIST3H2A	histone cluster 3, H2a	IP100031562	
-3.32	6.11E-04	SNRPF	group-specific component (vitamin D binding protein)	IP100555812	Extracellular Space
				IP100954102	
-3.307	6.31E-04	NDRG1	small nuclear ribonucleoprotein polypeptide F	IP100220528	Nucleus
				IP100022078	
-3.177	1.99E-05	SERPINF2	N-myc downstream regulated 1	IP100923597	Nucleus
				IP100879231	
-3.168	2.07E-05	CRIP1	serpin peptidase inhibitor, clade F	IP100879608	Extracellular Space
				IP100879937	
-2.785	2.50E-03	RPS17 (includes EG:6218)	(alpha-2 antipiasmin, pigment epithelium derived factor), member 2	IP100215611	Cytoplasm
				IP100221093	
-2.76	1.22E-04	PNP	cysteine-rich protein 1 (intestinal)	IP100017672	Nucleus
				IP100217468	
-2.65	1.99E-04	HIST1H1B	histone cluster 1, H1b	IP100815867	Nucleus
				IP100746251	
-2.539	3.28E-04	FABP5	histone cluster 2, H2bd	IP100007797	Cytoplasm
				IP100788781	
-2.376	6.87E-04	PRSS1 (includes EG:5644)	fatty acid binding protein 5 (psoriasis-associated)	IP100869087	unknown
				IP100935215	
-2.376	6.87E-04	PRSS1 (includes EG:5644)	fatty acid binding protein 5-like 3 (pseudogene)	IP100945846	Extracellular Space
				IP100169276	
-2.376	6.87E-04	PRSS1 (includes EG:5644)	protease, serine, 1 (trypsin 1)	IP100011694	Extracellular Space
				IP100941783	
-2.376	6.87E-04	PRSS1 (includes EG:5644)	protease, serine, 1 (trypsin 1)	IP100946754	Extracellular Space
				IP100946754	

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
		PRSS3 (includes EG:5646)	protease, serine, 3	IP100954434 IP100011695	
		HBB (includes EG:3043)	hemoglobin, beta	IP100884107 IP100654755 IP100796636 IP100815947 IP100473011 IP100657660 IP100829896 IP100791558	
		HBD	hemoglobin, delta	IP100930351 IP100217471	Cytoplasm
-2.117	2.26E-03	HBE1	hemoglobin, epsilon 1	IP100853641 IP100744503 IP100939544 IP100220706 IP100749035 IP100939160 IP100554676 IP100657911	
		HBG1	hemoglobin, gamma A		
		HBG2	hemoglobin, gamma G		
		ENO3	enolase 3 (beta, muscle)	IP100218474 IP100909949 IP100942494	Cytoplasm
		ASAHI	N-acylsphingosine amidohydrolase (acid ceramidase) 1	IP100418446 IP100013698 IP100940046 IP100059685	Cytoplasm
		MTCH2	mitochondrial carrier homolog 2 (C. elegans)	IP100003833	Cytoplasm
		TXN	thioredoxin	IP100216298	Cytoplasm

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
				IP100552768	
4.465	1.15E-03	RAB1B	RAB1B (includes EG:81876)	IP100008964	Cytoplasm
				IP100896425	
				IP100022134	
		RAB1C	RAB1C, member RAS oncogene family pseudogene	IP100374519	unknown
4.772	6.90E-04	SASH3	SAM and SH3 domain containing 3	IP100909545	unknown
				IP100004504	
		CSDA	cold shock domain protein A	IP100555698	
				IP100643351	
				IP100031812	Nucleus
4.818	6.40E-04	YBX1	Y box binding protein 1	IP100450235	
				IP100643807	
				IP100479509	
		YBX2	Y box binding protein 2	IP100250153	Cytoplasm
				IP100556023	
5.026	4.56E-04	ALDH1A1	aldehyde dehydrogenase 1 family, member A1	IP100218914	
				IP100910463	
				IP100644628	Cytoplasm
				IP100644077	
				IP100642144	
5.597	1.84E-04	CAP1	CAP, adenylate cyclase-associated protein 1 (yeast)	IP100939159	Plasma Membrane
5.737	1.48E-04	SDCBP	syndecan binding protein (syntenin)	IP100479018	
				IP100299086	
				IP100478874	Plasma Membrane
				IP100910606	
				IP100794699	
5.815	1.32E-04	KYNU	kynureninase (L-kynurenine hydrolase)	IP100003818	Cytoplasm
				IP100651624	

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
				IP100916732	
				IP100915775	
		ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	IP100008161	
				IP100872242	
		ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	IP100646182	
				IP100006482	
				IP100414005	
				IP100003021	
6.198	7.41E-05	ATP1A2	ATPase, Na+/K+ transporting, alpha 2 polypeptide	IP100640401	Plasma Membrane
				IP100908591	
				IP100647102	
		ATP1A3	ATPase, Na+/K+ transporting, alpha 3 polypeptide	IP100788782	
				IP100302840	
		ATP1A4	ATPase, Na+/K+ transporting, alpha 4 polypeptide	IP100375339	
				IP100647099	
		ATP4A	ATPase, H+/K+ exchanging, alpha polypeptide	IP100218919	
				IP100018873	
				IP100337370	
				IP100922378	
6.205	7.34E-05	NAMPT	nicotinamide phosphoribosyltransferase	IP100853098	Extracellular Space
				IP100853507	
				IP100853047	
				IP100853387	
				IP100021766	
				IP100478442	
				IP100298289	
6.394	5.56E-05	RTN4	reticulon 4	IP100894213	Cytoplasm
				IP100477663	
				IP100894099	
				IP100873380	

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
				IP100219207	
6.458	5.07E-05	NCF2	neutrophil cytosolic factor 2	IP100910100 IP100815773 IP100021070 IP100641875	Cytoplasm
7.244	1.67E-05	EFHD1	EF-hand domain family, member D1	IP100031091 IP100916914 IP100915909 IP100917888	unknown
		EFHD2	EF-hand domain family, member D2	IP100060181 IP100552365	
7.345	1.45E-05	RAB21	RAB21, member RAS oncogene family	IP100007755	Cytoplasm
7.987	6.17E-06	TRAP1	TNF receptor-associated protein 1	IP100030275 IP100646055	Cytoplasm
8.188	4.75E-06	ACP5	acid phosphatase 5, tartrate resistant	IP100419240	Cytoplasm
8.788	2.23E-06	CYBA	cytochrome b-245, alpha polypeptide	IP100218433	Cytoplasm
9.489	9.50E-07	ATP6V1B1	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1	IP100304911 IP100926751 IP100925534 IP100925827	Cytoplasm
		ATP6V1B2	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	IP100007812 IP100848320 IP100792597	
9.864	3.28E-03	UFM1	ubiquitin-fold modifier 1	IP100010207 IP100645510	Cytoplasm
10.278	2.80E-03	C5ORF51	chromosome 5 open reading frame 51	IP100374272	unknown

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
10.432	2.64E-03	ASNS	asparagine synthetase (glutamine-hydrolyzing)	IP100554777	
				IP100925572	
				IP100926480	
				IP100925298	unknown
				IP100926959	
				IP100926760	
				IP100925986	
11.282	1.94E-03	SLC27A3	solute carrier family 27 (fatty acid transporter), member 3	IP100013623	
				IP100645636	Cytoplasm
				IP100922217	
11.414	1.08E-07	AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)	IP100413641	
				IP100556258	Cytoplasm
				IP100926520	
12.275	1.38E-03	CNBP	CCHC-type zinc finger, nucleic acid binding protein	IP100895806	
				IP100895911	
				IP100430812	Nucleus
				IP100895852	
				IP100430813	
				IP100430814	
13.246	1.66E-08	GRB2	growth factor receptor-bound protein 2	IP100021327	
				IP100218070	Cytoplasm
				IP100792746	
				IP100790124	
14.548	6.68E-04	BIN1	bridging integrator 1	IP100186966	
				IP100395680	
				IP100220996	Nucleus
				IP100220997	
				IP100220587	
				IP100220998	

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
14.859	3.62E-09	NPC2	Niemann-Pick disease, type C2	IP1003329733	Extracellular Space
				IP1002221000	
				IP1002220999	
				IP1002220586	
				IP1002221001	
14.95	3.34E-09	ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	IP100291792	Plasma Membrane
				IP100103356	
				IP100795567	
17.072	3.25E-04	SIRT2	sirtuin 2	IP100179109	Nucleus
				IP100640953	
				IP100472047	
				IP100643990	
				IP100382551	
17.834	2.66E-04	ARRB1	arrestin, beta 1	IP100940031	Cytoplasm
				IP100293857	
				IP100943289	
22.731	8.16E-05	SIRPA	signal-regulatory protein alpha	IP100336017	Plasma Membrane
				IP100656113	
				IP100848309	
				IP100332887	
				IP100656087	
23.886	3.30E-12	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isozyme	IP100375705	Cytoplasm
				IP100902512	
				IP100027423	
				IP100550451	
				IP100410128	

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
		PPP1CB	protein phosphatase 1, catalytic subunit, beta isozyme	IP100218236	
				IP100930380	
				IP100894274	
				IP100892668	
				IP100894333	
		PPP1CC	protein phosphatase 1, catalytic subunit, gamma isozyme	IP100894160	
				IP100218187	
				IP100005705	
				IP100022314	
24.085	2.89E-12	SOD2	superoxide dismutase 2, mitochondrial	IP100847322	Cytoplasm
				IP100607577	
				IP100911015	
				IP100305064	
				IP100828117	
				IP100827658	
				IP100827650	
				IP100418465	
				IP100828064	
				IP100828192	
				IP100827795	
				IP100828056	
				IP100419219	Plasma Membrane
			CD44 molecule (Indian blood group)	IP100827893	
				IP100827982	
				IP100827937	
				IP100854669	
				IP100297160	
				IP100922478	
				IP100827555	
				IP100828108	
34.682	6.04E-15	CD44			

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
				IP100954073	
38.457	4.66E-06	PSMC6	proteasome (prosome, macropain) 26S subunit, ATPase, 6	IP100926977 IP100021926	Nucleus
42.961	1.18E-16	TUBA4B	tubulin, alpha 4b (pseudogene)	IP100017454	Cytoplasm
				IP100003348	
				IP100790302	
		GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2	IP100790896	
57.279	4.09E-19			IP100797969	Plasma Membrane
				IP100925530	
				IP100924758	
		GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	IP100012451	
				IP100946384	
60.473	2.83E-07	DYNLT3	dynein, light chain, Tctex-type 3	IP100639982 IP100020885	Cytoplasm
				IP100171438	
71.233	9.50E-08	TXNDC5	thioredoxin domain containing 5 (endoplasmic reticulum)	IP100946258 IP100395646 IP100939560	Cytoplasm
75.058	6.65E-08	THBS1	thrombospondin 1	IP100296099	Extracellular Space
82.06	1.90E-22	PLAUR	plasminogen activator, urokinase receptor	IP100010676 IP100215707	Plasma Membrane
				IP1000002252	
96.038	1.17E-08	CD68	CD68 molecule	IP100942678 IP100744995 IP100217167	Plasma Membrane
99.828	8.81E-09	CD93	CD93 molecule	IP100299485 IP100909029	Plasma Membrane

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
108.83	4.66E-09	SLC4A2	solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)	IP100383597 IP100946699 IP100477139	Plasma Membrane
109.55	4.43E-09	ATP6V1H	ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H	IP100296191 IP100414079 IP100909198	Cytoplasm
110.48	4.16E-09	AP3B1	adaptor-related protein complex 3, beta 1 subunit	IP100021129	Cytoplasm

Table 2

List of significant proteins by MaxQuant for 7 to 5 day comparison.

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
-395.601	1.24E-128	UFM1	ubiquitin-fold modifier 1	IP100010207	Cytoplasm
-250.094	6.29E-110	HBD	hemoglobin, delta	IP100791558	Cytoplasm
-33.372	3.83E-28	CD68	CD68 molecule	IP100744995	Plasma Membrane
		GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2	IP100003348	
-22.695	2.35E-36	SERPINF2	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2	IP100879231	Extracellular Space
				IP100879608	
				IP100879937	
-22.261	6.28E-36	HIST1H1B	histone cluster 1, H1b	IP100217468	Nucleus
				IP100815867	
-19.798	9.71E-21	H3F3C	H3 histone, family 3C	IP100419884	Nucleus
		HIST3H3 (includes EG:8290)	histone cluster 3, H3	IP100216402	
-18.054	1.81E-31	LOC729317	voltage-dependent anion channel 2 pseudogene	IP100737171	unknown
				IP100888783	
				IP100902560	
		VDAC2	voltage-dependent anion channel 2	IP100216026	Cytoplasm
				IP100024145	
				IP100917420	
-8.696	1.66E-11	KYNU	kynureninase (L-kynurenine hydrolase)	IP100916732	Cytoplasm
-6.856	2.17E-09	PPP1CB	protein phosphatase 1, catalytic subunit, beta isozyme	IP100218236	Cytoplasm
		PPP1CC	protein phosphatase 1, catalytic subunit, gamma isozyme	IP100005705	
-6.305	1.06E-08	HBD	hemoglobin, delta	IP100473011	Cytoplasm
-5.614	8.45E-08	ATP4A	ATPase, H ⁺ /K ⁺ + exchanging, alpha polypeptide	IP100218919	Plasma Membrane
		RPL26	ribosomal protein L26	IP100433834	Cytoplasm

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
		RPL26L1	ribosomal protein L26-like 1	IP100027270	unknown
-5.357	1.88E-07	ATP6V1B2	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	IP100792597	Cytoplasm
-5.346	1.95E-07	ATP6V1B1	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1	IP100925534 IP100925827	Cytoplasm
-5.324	1.27E-11	GC	group-specific component (vitamin D binding protein)	IP100555812 IP100954102	Extracellular Space
-5.09	4.40E-07	ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1	IP100418446 IP100013698 IP100940046	Cytoplasm
-4.96	6.69E-07	RAB21	RAB21, member RAS oncogene family	IP100007755	
		RPL26	ribosomal protein L26	IP100789514 IP100792420	Cytoplasm
-4.891	8.38E-07	HBG2	hemoglobin, gamma G	IP100657911	Cytoplasm
		SERP1B10	serpin peptidase inhibitor, clade B (ovalbumin), member 10	IP100792070 IP100791734	Cytoplasm
-4.857	1.49E-10	SERP1B2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	IP100007117 IP100797177 IP100797920 IP100789721	Extracellular Space
-4.726	1.44E-06	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isozyme	IP100550451	Cytoplasm
				IP100000690 IP100157908	
-4.027	1.54E-05	AIFM1	apoptosis-inducing factor, mitochondrion-associated, 1	IP100300018 IP100910786 IP100910617	Cytoplasm

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
-4.007	1.66E-05	RAB1B (includes EG:81876)	RAB1B, member RAS oncogene family	IP100022134	Cytoplasm
-3.95	2.02E-05	ATP1A4	ATPase, Na ⁺ /K ⁺ transporting, alpha 4 polypeptide	IP100647099	Plasma Membrane
-3.817	5.06E-08	ITIH2	inter-alpha (globulin) inhibitor H2	IP100305461 IP100645038 IP100910636 IP100514159	Extracellular Space
-3.664	5.56E-05	PPP1CB	protein phosphatase 1, catalytic subunit, beta isozyme	IP100894274 IP100892668 IP100894333 IP100894160	Cytoplasm
		RAB1A	RAB1A, member RAS oncogene family	IP100005719 IP100937805 IP100917079 IP100334174 IP100185217	
-3.562	2.28E-07	PRSS1 (includes EG:5644)	protease, serine, 1 (trypsin 1)	IP100935215 IP100945846 IP100169276 IP100011694 IP100941783 IP100946754 IP100954434 IP100011695	Extracellular Space
-3.206	2.95E-04	ATP1A2	ATPase, Na ⁺ /K ⁺ transporting, alpha 2 polypeptide	IP100008161 IP100872242 IP100908591 IP100647102 IP100375339	Plasma Membrane

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
-3.127	3.94E-04	KYNU	kynureninase (L-kynurenine hydrolase)	IP100915775	Cytoplasm
		MTCH2	mitochondrial carrier homolog 2 (C. elegans)	IP1000003833	
-2.978	7.80E-06	HBB (includes EG:3043)	hemoglobin, beta	IP100884107	Cytoplasm
-2.884	9.73E-04	GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2	IP100790302	Plasma Membrane
-2.831	1.19E-03	FABP5	fatty acid binding protein 5 (psoriasis-associated)	IP100007797	Cytoplasm
		FABP5L3	fatty acid binding protein 5-like 3 (pseudogene)	IP100788781	
				IP100869087	
-2.804	2.28E-05	KRT9	keratin 9	IP100019359	Cytoplasm
-2.698	4.41E-05	HBB (includes EG:3043)	hemoglobin, beta	IP100815947	Cytoplasm
		HBD		IP100930351	
		HBE1	hemoglobin, epsilon 1	IP100853641	
-2.685	2.06E-03	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isozyme	IP100410128	Cytoplasm
		PPP1CB	protein phosphatase 1, catalytic subunit, beta isozyme	IP100930380	
-2.648	2.37E-03	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isozyme	IP100902512	Cytoplasm
		SASH3	SAM and SH3 domain containing 3	IP100027423	
		SASH3	SAM and SH3 domain containing 3	IP100909545	
				IP100004504	
-2.626	6.93E-05	SERPND1	serpin peptidase inhibitor, clade D (heparin cofactor), member 1	IP100292950	Extracellular Space
				IP100879573	
-2.618	7.26E-05	AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)	IP100413641	Cytoplasm
				IP100556258	
				IP100926520	
-2.61	2.73E-03	GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2	IP100925530	Plasma Membrane
		GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	IP100924758	
				IP100012451	
				IP100946384	

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
		KYNU	kynureninase (L-kynurenine hydrolase)	IP100003818 IP100651624	Cytoplasm
-2.429	2.36E-04	HBG1	hemoglobin, gamma A	IP100744503	
		HBG2	hemoglobin, gamma G	IP100939544 IP100749035 IP100939160	Cytoplasm
-2.301	5.24E-04	ATP1A2	ATPase, Na+/K+ transporting, alpha 2 polypeptide	IP100003021	
		ATP1A3	ATPase, Na+/K+ transporting, alpha 3 polypeptide	IP100640401 IP100302840	Plasma Membrane
-2.201	9.78E-04	HBG2	hemoglobin, beta	IP100654755	
		HBG2	hemoglobin, gamma G	IP1005554676	Cytoplasm
-2.124	1.58E-03	HBD	hemoglobin, delta	IP100829896	Cytoplasm
-2.102	1.81E-03	TXN	thioredoxin	IP100216298 IP100552768	Cytoplasm
1.536	3.44E-03	PLAUR	plasminogen activator, urokinase receptor	IP100010676 IP100215707	Plasma Membrane
		SLC27A3	solute carrier family 27 (fatty acid transporter), member 3	IP100013623 IP100645636 IP100922217	Cytoplasm
		UFM1	ubiquitin-fold modifier 1	IP100645510	
1.595	1.72E-03	ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1	IP100059685 IP100013877 IP100216492 IP100216493	Cytoplasm
		HNRNP3	heterogeneous nuclear ribonucleoprotein H3 (2H9)	IP100908651 IP100216494 IP100216495 IP100216496	Nucleus

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
1.762	3.08E-03	NAPIL4	nucleosome assembly protein 1-like 4	IP100017763	
		PNP	purine nucleoside phosphorylase	IP100017672	
		TUBA4B	tubulin, alpha 4b (pseudogene)	IP100017454	Cytoplasm
1.775	2.81E-03	ATPIA1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	IP100414005	
		ATPIA3	ATPase, Na+/K+ transporting, alpha 3 polypeptide	IP100788782	Plasma Membrane
1.796	2.41E-03	CD68	CD68 molecule	IP100002252	Plasma Membrane
				IP100942678	
1.814	2.12E-03	RAB1B (includes EG:81876)	RAB1B, member RAS oncogene family	IP100008964	Cytoplasm
		RAB1C	RAB1C, member RAS oncogene family pseudogene	IP100896425	
				IP100374519	unknown
1.916	2.91E-05	ATP6V1B2	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	IP100007812	Cytoplasm
1.977	1.28E-05	PPP1CC	protein phosphatase 1, catalytic subunit, gamma isozyme	IP100218187	Cytoplasm
2.246	3.34E-07	HBD	hemoglobin, delta	IP100657660	
		HBE1	hemoglobin, epsilon 1	IP100217471	Cytoplasm
		HBG1	hemoglobin, gamma A	IP100220706	
2.263	2.64E-07	H2BFO	histone cluster 2, H2bd	IP100746251	unknown
2.428	2.72E-08	ATP6V1B1	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1	IP100304911	Cytoplasm
				IP100926751	
2.473	1.66E-05	ATP6V1B2	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	IP100848320	Cytoplasm
2.481	1.56E-05	ATPIA1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	IP100646182	Plasma Membrane
		ATPIA1		IP100006482	
		NOP58	NOP58 ribonucleoprotein homolog (yeast)	IP100006379	Nucleus
				IP100924866	
3.473	1.31E-08	GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2	IP100790896	Plasma Membrane

Fold Change	p-value	Symbol	Entrez Gene Name	ID	Location
				IP100797969	

Table 3

Classification of proteins grouped for their role in Molecular and Cellular Function by IPA.*

Category	Dates	p-value (range)	Molecules
Cell-To-Cell Signaling and Interaction	Day 3–5	1.69E-06-1.93E-02	AP3B1, GRB2, CD68, SERPINF2, TXN, CD93, RAB21, VCL, THBS1, GC, NAMPT, SIRPA, ITGB2, NDRG1, SIRPB1, SERPINBA2, SLC4A2, SOD2, PLAUR, CD44
	Day 5–7	3.4E-03-4.66E-02	TXN, SERPINF2, SERPINB2, RAB21, PRSS3 (includes EG:5646), PLAUR
Cellular Function and Maintenance	Day 3–5	1.69E-06-1.93E-02	AP3B1, GRB2, CAPI, ATP1A4, TXN, CD93, BIN1, VCL, THBS1, ATP6V1H, SIRPA, ARRB1, ITGB2, SIRPB1, AIFM1, PLAUR, CD44, ATP6V1B2, PNP
	Day 5–7	1.12E-04-4E-02	ATP1A4, TXN, PLAUR, ATP6V1B2, PNP, KRT9
Free Radical Scavenging	Day 3–5	2.09E-06-1.92E-02	ITGB2, TXN, CYBA, SOD2, AIFM1, TRAP1, PLAUR, NCF2, CD44, AKR1B1
Molecular Transport	Day 3–5	2.09E-06-1.93E-02	PPP1CB, TRAP1, GNB2, ATP1A1, NPC2, HBD, PPP1CA, CYBA, SLC27A3, NCF2, PLAUR, ALDH1A1, CD44, ATP1A3, ATP1A4, TXN, HBB (includes EG:3043), ATP1A2, THBS1, ATP6V1B1, GC, FABP5, SIRPA, ATP12A, ITGB2, SOD2, PPP1CC, PNP, ATP6V1B2
	Day 5–7	1.11E-07-4.33E-02	ATP1A3, ATP1A4, HBB (includes EG:3043), GNB4, ATP1A2, PPP1CB, ASAH1, ATP6V1B1, GC, FABP5, HBD, ATP1A1, PPP1CA, ATP12A, SLC27A3, PPP1CC, PLAUR, AKR1B1, PNP, ATP6V1B2
Carbohydrate Metabolism	Day 3–5	2.98E-05-1.93E-02	PPP1CA, ARRB1, PPP1CB, THBS1, PPP1CC, CD44, ITIH2
	Day 5–7	2.91E-05-4.98E-02	PPP1CA, PPP1CB, PPP1CC, FABP5, AKR1B1
Small Molecule Biochemistry	Day 3–5	4.13E-05-1.93E-02	PPP1CB, ASAH1, CNBP, ATP1A1, NPC2, HBD, PPP1CA, CYBA, SLC27A3, PLAUR, NCF2, ALDH1A1, CD44, ITIH2, ATP1A4, TXN, HBB (includes EG:3043), ATP1A2, SIRT2, THBS1, GC, FABP5, SIRPA, ITGB2, SOD2, PPP1CC, AKR1B1, PNP, ATP6V1B2
	Day 5–7	1.13E-05-4.98E-02	ATP1A4, TXN, HBB (includes EG:3043), ATP1A2, PPP1CB, ASAH1, GC, FABP5, HBD, ATP1A1, PPP1CA, KYNU, AIFM1, SLC27A3, PLAUR, PPP1CC, AKR1B1, ATP6V1B2, PNP
Cell Morphology	Day 3–5	6.93E-05-1.93E-02	CAPI, HBB (includes EG:3043), VCL, HBE1, THBS1, ASAH1, HBD, SIRPA, ITGB2, RAB1A, SLC4A2, ATP4A, SOD2, AIFM1, RTN4, PLAUR, CD44
	Day 5–7	2.35E-04-4.33E-02	HBD, PPP1CA, RAB1A, HBB (includes EG:3043), ATP4A, AIFM1, HBE1, ASAH1, PLAUR

* Green indicates decreased synthesis rates and red indicates increased synthesis rates.

Table 4

Classification of proteins by their roles in Physiological System Development and Function as grouped by IPA.*

Category	Dates	p-value	Molecules
Hematological System Development and Function	Day 3–5	5.16E-06-1.93E-02	NDRG1, GRB2, THBS1, CD93, SIRPB1, PLAUR, SERPINF2, ITGB2, SOD2, ARRB1, HBB (includes EG:3043), CD44, PNP, AP3B1, TXN, HBD, AKR1B1, GC, SIRPA, AIFM1
	Day 5–7	1.13E-05-4.66E-02	HBB (includes EG:3043), FABP5, PNP, PLAUR, TXN, HBD, SERPINF2, SERPINB2
Tissue Development	Day 3–5	5.52E-05-1.93E-02	RPL22, RAB21, THBS1, PLAUR, SERPINF2, SERPINB2, ITGB2, ALDH1A1, CD44, RTN4, TXN, VCL, AKR1B1, GC, CNBP, SIRPA
	Day 5–7	3.4E-03-4E-02	RAB21, PLAUR, TXN, SERPINF2, SERPINB2
Hematopoiesis	Day 3–5	8.24E-05-1.93E-02	GRB2, THBS1, PLAUR, ITGB2, SOD2, HBB (includes EG:3043), ARRB1, CD44, PNP, AP3B1, HBD, TXN, SIRPA, AIFM1
	Day 5–7	8.62E-04-3.35E-02	HBB (includes EG:3043), PNP, PLAUR, TXN, HBD
Immune Cell Trafficking	Day 3–5	8.24E-05-1.93E-02	ITGB2, NDRG1, THBS1, CD93, AP3B1, CD44, PLAUR, TXN, GC, SERPINF2, SIRPA
	Day 5–7	6.62E-03-4.66E-02	PLAUR, TXN, SERPINF2
Digestive System Development and Function	Day 3–5	1.23E-04-1.29E-02	SLC4A2, THBS1, ATP4A
	Day 5–7	1.02E-02-1.02E-02	ATP4A
Cardiovascular System Development and Function	Day 3–5	1.25E-04-1.93E-02	RAB1A, THBS1, PLAUR, SERPINF2, ITGB2, ATP1A1, SOD2, CYBA, ATP1A2, RTN4, CD44, VCL, TXN, PPP1CA, AIFM1
	Day 5–7	9.87E-06-3.68E-02	ATP1A1, RAB1A, ATP1A2, PLAUR, PPP1CA, SERPINF2, AIFM1
Organ Morphology	Day 3–5	1.25E-04-1.93E-02	ACP5, PLAUR, CSDA, SERPINF2, SASH3, ATP1A1, SOD2, HBB (includes EG:3043), ATP1A2, PNP, CD44, HBD, TXN, PPP1CA, AIFM1
	Day 5–7	9.87E-06-2.02E-02	ATP1A1, HBB (includes EG:3043), ATP1A2, PNP, PLAUR, HBD, TXN, PPP1CA, SERPINF2, SASH3, AIFM1
Tissue Morphology	Day 3–5	8.5E-04-1.93E-02	SLC4A2, NDRG1, THBS1, CSDA, PLAUR, ATP4A, SERPINF2, YBX2, ITGB2, ATP1A1, ALDH1A1, SOD2, HBB (includes EG:3043), FABP5, ATP1A2, PNP, CD44, AP3B1, VCL, TXN, HBD, GC, SIRPA
	Day 5–7	2.35E-04-3.68E-02	ATP1A1, HBB (includes EG:3043), FABP5, ATP1A2, TXN, HBD
Tumor Morphology	Day 3–5	1.13E-03-1.29E-02	ITGB2, THBS1, CD44, PLAUR, AKR1B1, SIRPA, SERPINB2
	Day 5–7	3.13E-04-1.69E-02	PLAUR, AKR1B1, SERPINB2

* Green indicates decreased synthesis rates and red indicates increased synthesis rates.