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Functional Proteomic Analysis of Corticosteroid Pharmacodynamics in Rat Liver: Relationship to Hepatic Stress, Signaling, Energy Regulation, and Drug Metabolism

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Abstract

Corticosteroids (CS) are anti-inflammatory agents that cause extensive pharmacogenomic and proteomic changes in multiple tissues. An understanding of the proteome-wide effects of CS in liver and its relationships to altered hepatic and systemic physiology remains incomplete. Here, we report the application of a functional pharmacoproteomic approach to gain integrated insight into the complex nature of CS responses in liver *in vivo*. An in-depth functional analysis was performed using rich pharmacodynamic (temporal-based) proteomic data measured over 66 hours in rat liver following a single dose of methylprednisolone (MPL). Data mining identified 451 differentially regulated proteins. These proteins were analyzed on the basis of temporal regulation, cellular localization, and literature-mined functional information. Of the 451 proteins, 378 were clustered into six functional groups based on major clinically-relevant effects of CS in liver. MPL–responsive proteins were highly localized in the mitochondria (20%) and cytosol (24%). Interestingly, several proteins were related to hepatic stress and signaling processes, which appear to be involved in secondary signaling cascades and in protecting the liver from CS-induced oxidative damage. Consistent with known adverse metabolic effects of CS, several rate-controlling enzymes involved in amino acid metabolism, gluconeogenesis, and fatty-acid metabolism were altered by MPL. In addition, proteins involved in the metabolism of endogenous compounds, xenobiotics, and therapeutic drugs including cytochrome P450 and Phase-II enzymes were differentially regulated. Proteins related to the inflammatory acute-phase response were up-regulated in response to MPL. Functionally-similar proteins showed large diversity in their temporal profiles, indicating complex mechanisms of regulation by CS.

Graphical abstract

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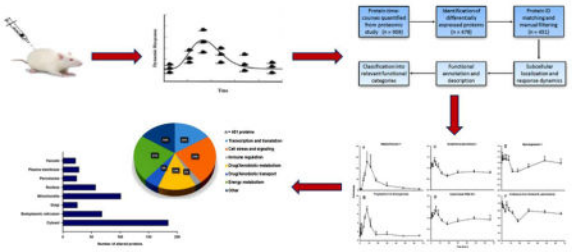
Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.



Keywords

methylprednisolone; liver; pharmacoproteomics; functional proteomics; pharmacodynamics

INTRODUCTION

Functional pharmacoproteomics is an emerging area that aims to elucidate relevant biological functions of proteins altered by drugs and define mechanisms of drug action at the molecular level. By understanding the functions of proteins altered by drug treatment, mechanistic connections between molecular-level perturbations and ultimate systemic responses can be clarified. Routinely performed static '-omics' studies assess a single moment of genomic or proteomic expression [1]. However, the regulation of protein expression by most drugs is not static but rather a dynamic (i.e. time-dependent) phenomenon. Alterations in protein expression may emerge in a delayed manner and persist for several hours after the drug has been cleared from the system. Thus, pharmacodynamic (i.e. temporal) changes following drug dosing must be assessed in order to more comprehensively analyze proteomic alterations as well as understand the mechanisms underlying the temporal nature of drug responses *in vivo*.

Corticosteroids (CS), the synthetic analogues of endogenous glucocorticoid (GC) hormones, are a class of potent anti-inflammatory drugs used extensively in treating various diseases such as rheumatoid arthritis [2], asthma [3], and some lymphomas [4]. However, high-dose or chronic CS therapy leads to a magnification of GC's normal metabolic effects, which are manifested adversely as diabetes, insulin resistance, osteoporosis, and obesity [5, 6]. Most of these effects are mediated through genomic mechanisms by the steroid binding to cytosolic glucocorticoid receptors (GR) which subsequently leads to activation, dimerization, and translocation of the drug-receptor complex into the nucleus, thereby producing widespread changes in mRNA and protein expression [7].

The liver plays a central role in maintaining systemic energy homeostasis and is one of the most sensitive organs to CS exposure [8]. The CS induce extensive biochemical changes in liver [9], altering the homeostasis of several biological processes. In addition to being the principal site for gluconeogenesis, liver stores glucose in the form of glycogen, which is released in response to hormonal signals to maintain systemic glucose concentrations. The liver also plays a critical role in lipid metabolism, which is altered upon CS treatment [10]. Hence, long-term CS use causes numerous side-effects that stem from a dysregulation of liver function. Since CS cause large-scale perturbations in mRNA and protein expression,

direct assessment of drug-induced proteomic changes *in vivo* combined with extensive data mining and functional analyses will provide molecular-level insights into the functional and mechanistic aspects of numerous proteins altered by CS.

We conducted a study whereby a population of animals received a single dose of the synthetic CS methylprednisolone (MPL), with multiple animals sacrificed at 11 time points following dosing, and livers from these animals harvested for proteomic analysis. The development of a robust and reproducible ion-current-based quantitative nano-LC/MS method that enabled assessment of drug-induced dynamic proteomic changes *in vivo*, and its application in examining the temporal proteomic response of liver from these animals was reported [11]. In addition, tandem changes in the temporal responses of mRNA from a similar set of animals [12, 13] and protein expression from this animal set were also analyzed [14]. Since proteins are better predictors of phenotypic change as compared to mRNA [15, 16], a natural extension to our studies was performing a proteome-wide functional analysis to better understand the liver-specific effects of CS.

The present report describes the functional analysis and classification of proteins that were differentially regulated in rat liver following MPL dosing. Systems-based ‘omics’ approaches coupled with intensive literature-based data mining were applied to the rich proteomic time-series data in order to functionally annotate, describe, and classify 451 differentially-regulated CS-responsive proteins. The rich functional information of the MPL-responsive proteins coupled with characterization of their temporal responses provides added insights into the physiological and pharmacological effects of CS in liver.

MATERIALS AND METHODS

Animals

Liver tissues for the proteomic analysis were obtained from a large, population-based animal study conducted in our laboratory. Fifty-five adrenalectomized (ADX) male Wistar rats were given methylprednisolone sodium succinate (Solu-Medrol) at 50 mg/kg by intramuscular injection, and killed at 11 different time points (0.5, 1, 2, 4, 5.5, 8, 12, 18, 30, 48 and 66 hr) after MPL dosing, with 5 replicate animals for each time point. After perfusion and sacrifice, livers were harvested, flash frozen in liquid nitrogen and stored at −80°C until further analysis. Each time point group was compared with five vehicle (saline)-dosed animals that were sacrificed at random times after injection. The ADX rats were used to abrogate the circadian rhythm of endogenous GC production and provide a stable pharmacodynamic baseline. Perfused liver tissue was used for the proteomic analyses in order to remove the high concentrations of blood proteins. A summary of the animal study design is provided in Supplementary Figure 1. All animal protocols adhered to “Principles of Laboratory Animal Care” (NIH publication 85-23, revised in 1985) and were approved by the University at Buffalo IACUC committee (PHC08128N). The institution’s animal welfare assurance number is D16-00231.

Experimental

Proteomics—Proteins from perfused and flash frozen livers were extracted, digested, and analyzed using a nano-LC/MS instrument. A total of 80 mg of powdered liver tissue was added to 800 μL of detergent-cocktail lysis buffer [150 mM sodium chloride, 1% sodium deoxycholate, 2% Nonidet P-40 (NP-40), and 2.5% sodium dodecyl sulfate and protease inhibitors (Complete tablets, EDTA-free, Roche, Inc.)] and homogenized using a Polytron homogenizer (Kinematica, Switzerland). The samples were then sonicated using a high-energy sonicator (Qsonica, Newtown, CT). The extract was centrifuged at 20,000 g for 60 min at 4 °C. Total protein concentrations in the supernatant was measured by the Bicinchoninic Acid Assay. 100 μg of protein was diluted with the lysis buffer to a final concentration of 2 mg/mL, which was then subjected to a precipitation/on-pellet-digestion procedure. The Nano Flow Ultra-High Pressure LC system (nano-UPLC) consisted of a Spark Endurance autosampler (Emmen, Holland) and an ultra-high pressure Eksigent (Dublin, CA) Nano-2D Ultra capillary/nano-LC system, with a LTQ/Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA) used for detection. Separation was performed on a long column [100 cm long and 50-μm inner diameter (ID)] with small particles (Pepmap 2-μm C18, 100 Å) under high pressure (~9000–11,000 psi with heating at 52 °C). The LC/MS raw data were searched against the Uniprot reviewed rat protein database (released October 2012) with 7,853 protein entries using SEQUEST-based Proteome Discoverer® (version 1.2.0.208, Thermo-Scientific). Mass tolerances for precursor and fragment ion masses were 15 ppm and 0.5 amu. Two missed cleavages were permitted for fully tryptic peptides. Carbamidomethylation of cysteines was set as a fixed modification and a variable modification of methionine oxidation was allowed. The false discovery rate was estimated by a target-decoy search strategy, using a concatenated database containing both forward and reversed sequences. Protein quantification was based on the area under the curve (AUC) of the ion-current peaks. Technical details on the ion-current based quantification method is described in a previous publication [17]. Strict criteria were applied for peak detection and frame generation, e.g., S/N > 10 for peptide precursor peaks and the elimination of peptides with ambiguous assignment, to ensure good quantitative accuracy and precision. The AUC data was interfaced to a PHP script, which transformed the quantitative data, followed by normalization for each individual sample. The protein ratios of time-course groups versus vehicle controls were computed by aggregating the AUC data on peptide levels to protein levels using a weighting model based on relative variances [11, 18]. A step-by-step summary of the proteomic profiling methodology is provided in Supplementary Figure 1 in the supporting information (SI). A more detailed description of our analytical methodology was published [11, 17]. The detailed quantitative data on peptide identification and protein levels across all the time-points are provided in Supplemental Tables 1 and 2 in the SI.

Measurements of plasma MPL and glucose concentrations—Plasma MPL concentrations were determined by a normal-phase high-performance liquid chromatography method [19]. The limit of quantitation was 10 ng/mL for MPL. The inter-day and intra-day coefficients of variation (CV) were less than 10%. A two-compartment pharmacokinetic model with dual absorption pathways as described previously [20] was used to fit the MPL plasma concentration-time profile. Plasma glucose concentrations were

measured by the glucose oxidase method (Sigma GAGO-20; Sigma-Aldrich, St. Louis, MO). The manufacturer's instructions were modified such that the assay was carried out in a 1-ml assay volume, and a standard curve consisting of seven concentrations over a 16-fold range was prepared from the glucose standard and run with each experimental set in triplicate.

Data Analysis

Analysis of Differentially Regulated Proteins—From approximately 3000 proteins identified in the LC/MS analysis, 1753 unique protein groups were quantified with sufficient quality (S/N, number of peptides, etc.) across the time points based on stringent cut-off criteria [11]. The 1753 quantified proteins were filtered to include only the protein groups that were quantified at all 11 time-points, yielding 959 proteins for further analysis. Next, the data set was filtered for differential expression over time using software for the Extraction and Analysis of Gene Expression (EDGE) [21]. Within-class differential expression was employed in order to identify proteins that showed a differential expression profile over time. Only proteins that varied significantly over time (p value < 0.05 and q-value < 0.01) were utilized in the subsequent analysis.

UniProt/Swiss-Prot ID Matching and Manual Filtering—The significantly altered proteins were listed according to their unique Universal Protein Resource (UniProt) accession number (AC) or identifier (ID) and fed into the UniProt database [22, 23]. Proteins were mapped based on their UniProt AC/ID into the UniProt knowledgebase to obtain information regarding corresponding gene and/or protein names. Redundancies in the final protein list were manually analyzed and filtered.

Characterization of Response Profiles—The pharmacodynamic response versus time profiles of each significantly altered protein was visually inspected. Based on all of the profiles observed in the dataset, each protein was classified into either up-regulated, down-regulated, or complex (biphasic) patterns of expression. Proteins showing biphasic behavior were annotated as either "up/down" or "down/up" to denote the dynamics of their temporal response.

Cellular Localization and Functional Clustering—Uniprot accession numbers corresponding to each of the significantly altered proteins were analyzed using various online tools and databases, including National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST), Gene Ontology Consortium - Gene Ontology Database [24], and GeneCards to confirm the identity and the annotations of the proteins provided in the UniProt database as well as check for alternate protein names. Preliminary gene ontology analysis of the significantly changing proteins was done using the functional annotation tool DAVID (Database for Annotation, Visualization and Integrated Discovery; NIH) [25], where functional classification was performed using medium-level stringency. Utilizing this information as a starting-point, extensive literature searches were performed to identify the liver-specific functions and other relevant information for the differentially expressed proteins. Cellular component information for the proteins were derived from the Gene Ontology Annotation (GOA) resource, which provides gene

annotation information to the UniProt database [26] and applied to identify the sub-cellular locations of steroid-induced proteomic changes. Direct use of results from DAVID and other currently available pathway analysis tools were avoided for final functional clustering, as the databases for these tools are not complete (i.e., they do not contain functional information on all the identified proteins), and do not take into account liver-specific physiological functions of the proteins. Therefore, six functional clusters were devised based on the clinically relevant effects of MPL in liver. The proteins were then allocated into a functional cluster based on literature-derived functional information. Additional sub-clustering was performed within the classes of energy regulation and drug metabolism. Proteins that did not fit any of the six functional categories were grouped into an additional cluster referred to as 'other MPL-regulated proteins'.

RESULTS

The main goal of this pharmacoproteomic analysis was to functionally annotate, describe, and classify the 451 hepatic proteins that were significantly altered by CS treatment *in vivo*. The major steps applied in the data analysis are summarized in Figure 1. Starting from the 1753 proteins that were quantified at one or more time-points in our proteomic study [11], proteins that were not quantified at each of the 11 time-points were filtered, which left 959 proteins for further analysis. Next, proteins with complete time-courses were analyzed using the EDGE software [21] in order to identify those which were differentially-regulated by MPL with respect to time. Using this method, 478 out of 959 proteins were found to show significantly varying temporal profiles (meeting the cut-offs p value < 0.05 and q-value < 0.01). The significantly altered proteins were then manually filtered in order to remove any redundancies (e.g. duplicate listings or identical protein subunits) that were present in the data set. However, if one or more subunits of a large protein complex (e.g. 60S ribosomal protein) possessed distinct identity and/or functionality, the protein subunits of the complex were retained for further analysis. After this step, a final group of 451 unique proteins were available for analyzing their regulation, localization, and function.

Figure 2 depicts the concentration-time profile of drug in the rats following a single 50 mg/kg intramuscular bolus of MPL. Following the absorption phase of about 30 minutes, the drug concentrations declined in a bi-exponential fashion and fell below the level of detection by 8 h. This profile in ADX rats is similar to that observed following intramuscular MPL dosing in intact (i.e. non-adrenalectomized) rats [20].

Direction of temporal regulation by CS

Tables 1–6 present identified regulated proteins based on function. It is evident based on previous temporal cluster analyses of our genomic and proteomic studies that multiple patterns of changes in mRNA and protein expression occur in response to MPL dosing [13, 14, 27]. The direction of regulation of each altered protein is listed under "Regulation" in these tables. Of the total 451 proteins, 77% were enhanced or up-regulated, 18% displayed biphasic behavior, and 5% were down-regulated. Figure 3 provides representative examples of the characteristic patterns of behavior of the proteins. Shown are the expression profiles of two proteins, metallothionein-1 (Figure 3A) and tryptophan 2,3-dioxygenase (Figure 3B),

where enhancement of expression is observed followed by a return to baseline. Both proteins display dynamic behavior that is similar to numerous mRNAs whose expression is enhanced by MPL in liver, including the prototypic CS-regulated gene, tyrosine aminotransferase (TAT) [7]. Mechanistically, such patterns of enhancement are often observed for proteins encoded by gene sequences containing glucocorticoid-response elements (GREs) in their promoter region [28]. While metallotheonein-1 indeed contains a pair of adjacent GREs in its promoter region [29], conflicting mechanistic explanations may exist for tryptophan 2,3-dioxygenase. Specifically, its regulation by MPL could occur either directly through the presence of a GRE-like sequence [30], or via a CS-regulated, short-lived transcription factor which indirectly alters tryptophan 2,3-dioxygenase expression [31]. Also depicted are the expression profiles of two proteins, glutathione peroxidase-1 (Figure 3C) and cytochrome P450 2A1 (Cyp2a1; Figure 3D), which display biphasic patterns of expression. While such patterns of expression are more difficult to decipher mechanistically, the involvement of secondary biosignals (e.g. transcription factors) has been postulated [7]. The temporal responses of two proteins, murinoglobulin-2 (Figure 3E) and peroxisomal 3-ketoacyl-CoA thiolase B (Figure 3F) illustrate down-regulation. While murinoglobulin-2 shows down-regulation only at 10 hours after dosing and returns to baseline by 48 hours, peroxisomal 3-ketoacyl-CoA thiolase B shows rapid down-regulation followed by a return to an ‘apparent’ baseline which is lower than in untreated animals. Together, the data presented in Figure 3 illustrate that changes in protein expression in response to CS are diverse in their dynamics and may involve multiple mechanisms of regulation.

Cellular localization of CS-responsive proteins

Gene ontology analysis was performed based on information available from the Gene Ontology Annotation (GOA) resource, which provides the latest evidence-based gene ontology annotations to proteins in the UniProt knowledgebase [26]. The sub-cellular distributions of the altered proteins are shown in Figure 4. There were 184 proteins localized to the cytosol, 101 to the mitochondria, and 68 to the endoplasmic reticulum. The fact that a large portion of MPL-regulated proteins were localized to the mitochondria is consistent with the effects of CS on hepatic energy metabolism [32–34]. Several cytosolic proteins were involved in either hepatic signaling processes (Table 2) or xenobiotic/drug metabolism (Table 4). In addition, 57 proteins localized to the nucleus were altered by MPL, which is indicative of the transcriptional mechanisms of CS effects. A few instances exist where a functional protein was found to be localized to more than one cellular component. Furthermore, less than 3% of the altered proteins were localized to other compartments (e.g. cell junction), which are not presented in the figure.

Functional clustering of CS-responsive proteins

From data mining and extensive literature searches, biological and/or pharmacological functions for the 451 altered proteins were identified. While direct use of functional gene ontology analysis tools such as DAVID were avoided for functional clustering (due to reasons cited in the “Discussion”), the UniProt knowledgebase was used to trace relevant literature information for some proteins that were functionally annotated on the database based on ‘evidence at the experimental-level’ (i.e. information derived from published reports). The proteins were further clustered into six functional groups. Proteins with

biological functions that did not fit into any of the six clusters were grouped into an additional cluster referred to as ‘other MPL-regulated proteins’ (Table 7). Tables list identifying criteria, brief functional descriptions, and temporal responses following acute MPL dosing. For each protein listed in the tables, the UniProt accession number (“UProt_ID”) and corresponding gene and protein names are provided. Further, the biological function(s) of each protein along with other information (important substrates, mechanistic interactions, etc.) where relevant and/or are available are presented. The responses of the protein to MPL dosing as up-regulated (up), down-regulated (down), or complex/biphasic (some combination of both) are listed. Quantitative values at each time-point for the 451 proteins included in the analysis are provided in Supplemental Table 3 in the SI.

Cellular Stress and Signal Transduction

Cell stress and signaling corresponds to the largest functional group, representing 107 proteins (Table 1). In general, proteins in this group include those which serve as intermediary components of various signal transduction pathways such as kinases, chaperone proteins, immunophilins, transcription factors, or enzymes involved in the termination (inactivation or catabolic breakdown) of hormone and neurotransmitter signaling. Figure 5 presents representative examples in this category. Creatine kinase (CK) isozymes catalyze the transfer of the phosphate group of phosphocreatine to ADP, to yield ATP and creatine in high-demand metabolic organs such as muscle, brain, and heart. However, conflicting results exist regarding its expression in liver [35–37]. Our results indicate that CK M-type is expressed in liver and strongly enhanced (~6-fold) in response to MPL (Figure 5A). Up-regulation of CK by GC has been reported previously in the developing rat skeletal muscle, but not in liver [38]. Interestingly, 11β-dehydrogenase type-1, the isozyme that catalyzes the conversion of inactive cortisone (11-dehydrocorticosterone in rodent) to active cortisol (corticosterone) [39], displayed a biphasic profile where expression showed a sustained increase (~1.4-fold) up to 30 hours followed by a decline below baseline at 48 and 66 hours post-dosing (Figure 5B). Since regulation of 11β-dehydrogenase type-1 is an important determinant of synthetic CS pharmacokinetics [40], alterations in this enzyme could influence CS pharmacodynamics. Two well-established molecular chaperones of cytoplasmic GR, heat-shock protein (hsp) 90-alpha [41] and FK506 binding protein 4 (encoded by *Fkbp52*), were up-regulated by MPL (data not shown). Since the FKBP-hsp90 chaperone complex functions to modulate steroid receptor activity [42], their enhancement in expression might serve as a protective mechanism against the presumably high intracellular steroid concentrations. This group is also represented by a number of enzymes which are responsive to cellular oxidative stress - suggestive of a protective mechanism against the oxidative stress-inducing effects of CS in tissues [43–45]. For instance, expression of aldehyde dehydrogenase 7 family member A1 (*Aldh7a1*; antiquitin), which plays a major role in the detoxification of aldehydes generated by alcohol metabolism, lipid peroxidation, and other cases of oxidative stress [46, 47] was altered over time. Antiquitin showed a complex time-profile where expression peaked at 2 hours followed by a decline below baseline by 12 hours and remained fairly steady up to 66 hours (Figure 5C). The expression of two major isoforms of the heavy-metal and free-radical binding proteins metallothionein (MT-I and MT-II) [48], were strongly enhanced by MPL. In

fact, of all the proteins quantified in the study, the strongest drug-induced change was observed for MT-II (~100-fold; Figure 5D). This observation is in agreement with previous findings at the transcriptional level [49]. Similar to MT-I (shown in Figure 3A), enhancement of MT-II by CS is regulated by a pair of adjacent GREs in its promoter region [29].

Energy Metabolism

Proteins involved in energy metabolism formed the second-largest group, consisting of 102 proteins (Table 2), and representative examples are presented in Figure 6. Since pathways controlling energy metabolism are highly complex and involve multiple biochemical processes for macromolecular breakdown to yield energy, this group was further sub-clustered into proteins involved in protein/amino acid metabolism, carbohydrate metabolism, lipid/fatty acid metabolism, and the Krebs cycle.

Drug-responsive proteins involved in hepatic amino acid metabolism are listed in Table 2A. Aminotransferase (or transaminase) enzymes catalyze the redistribution of nitrogen between amino acids and corresponding oxoacids participating in both protein metabolism and gluconeogenesis [50]. Significant MPL-induced up-regulation was observed for four aminotransferases: alanine aminotransferase (AAT), cytosolic aspartate aminotransferase (cASAT), ornithine aminotransferase (OAT), and tyrosine aminotransferase (TAT). Interestingly, although CS stimulate expression of all four aminotransferases through GRE-binding [51–53], the kinetics and dynamics (i.e. magnitude) of induction by MPL markedly differ among the enzymes. For instance, the induction of cASAT peaked near 30 h and continued until 48 h after drug dosing before returning to baseline, whereas TAT expression showed a sharp rise to peak by 12 hours and returned to baseline by 18 hours (Figure 6A). Such differences in response profiles may be attributed to the differential rates of protein turnover, viz compare the half-lives of TAT (~4 h) versus cASAT (5–11 days) [11]. In addition to the aminotransferases, phenylalanine hydroxylase (PAH), which catalyzes the rate-limiting step of phenylalanine catabolism into tyrosine, was also significantly up-regulated in response to MPL, peaking at 30 hours (Figure 6B). This observation is in line with previous findings that GC administration stimulates PAH enzyme activity in rat liver *in vivo* [54]. Furthermore, tryptophan 2,3-dioxygenase, a sensitive GC-inducible enzyme that catalyzes amino acid tryptophan metabolism [55], was also strongly up-regulated (~7-fold) by 8 hours after MPL dosing (Figure 3B). These examples, along with others listed in Table 2A illustrate that MPL induces a number of hepatic amino-acid metabolizing enzymes that in turn provide gluconeogenic substrates for glucose production [56].

Drug-responsive proteins involved in hepatic glucose metabolism are listed in Table 2B. Net utilization and/or production of hepatic glucose production is controlled by the relative expression and activity of specific enzymes involved in the glycolytic or gluconeogenic pathways. Hepatic gluconeogenesis, the production of glucose from non-carbohydrate sources, is, activated either under fasting-conditions [56] or upon CS treatment [57, 58]. Hepatocytes contain rate-controlling enzymes specific for gluconeogenesis [pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase (1,6-FBPase), and glucose-6-phosphatase (G-6-P)] [59]. The time-course of

PEPCK, the rate-limiting enzyme in gluconeogenesis, is shown in Figure 6C. It is evident that MPL strongly enhances the expression of PEPCK (~ 3.5-fold) by 5.5 hours, whereas PC and 1,6-FBPase are altered to much lower extents (data not shown). Mechanistically, stimulation of PEPCK transcription rate by CS occurs via GRE-binding in its promoter region [60]. The time-course of PEPCK enhancement correlated with plasma glucose concentrations measured in the same animals (Figure 6D).

MPL-responsive proteins involved in the processes of hepatic lipid and/or fatty acid metabolism are listed in Table 2C. The CS stimulate lipolysis in white adipose tissue producing free fatty acids for use by other tissues [61] and glycerol for gluconeogenesis [62]. In addition, CS also inhibit hepatic fatty acid β-oxidation [63], the process by which fatty acids are broken down in the mitochondria and/or in peroxisomes to generate acetyl-CoA which subsequently feeds into the Krebs cycle. Together, these effects trigger hepatic fat accumulation (steatosis) which is associated with the metabolic syndrome. Acyl-CoA dehydrogenases (ACAD) are mitochondrial enzymes that catalyze the initial rate-limiting step in the beta-oxidation of fatty acyl-CoA [64]. Multiple ACADs including long-chain specific acyl-CoA dehydrogenase (*Acadl*), medium-chain acyl-CoA dehydrogenase (*Acadm*), short/branched chain acyl-CoA dehydrogenase (*Acadsb*), and short-chain specific acyl-CoA dehydrogenase (*Acads*) were diversely altered by MPL, mostly displaying biphasic profiles (data not shown). In addition, as shown in Figure 3F, expression of peroxisomal 3-ketoacyl-CoA thiolase B, which catalyzes the final step in the peroxisomal β-oxidation of straight-chain acyl-CoA, was strongly down-regulated (~60%) by MPL by 18 hours.

Altered proteins related to the Krebs (tricarboxylic acid) cycle are listed in Table 2D. Requirement of a separate Krebs cycle group is justified by the fact that this process serves as key metabolic pathway that unifies carbohydrate, fat, and protein metabolism. Metabolic intermediates produced from all three energy sources feed into the Krebs cycle and undergo oxidative reactions to ultimately generate ATP. While metabolic stress as well as GC are known to influence the activity of the Krebs cycle [65], little information exists regarding the specific proteins altered by CS. The time-profiles of two well-known Krebs cycle-related proteins, succinate dehydrogenase (Figure 6E) and malate dehydrogenase (Figure 6F), are shown. Both proteins were modestly up-regulated (~1.5-fold), the former peaking around 5.5 hours whereas the latter peaked by 8 hours.

Transcription, Translation, and Protein-processing

Another highly populated group consisting of 73 proteins altered by MPL contains those related to transcription, translation, and post-translational protein processing (Table 3). Representative examples are presented in Figure 7. A majority of proteins in this group function as nuclear proteins regulating transcription, specialized ribosomal protein subunits involved in translation, and enzymes or chaperone proteins which facilitate post-translational protein folding and/or glycosylation. A number of heterogeneous nuclear ribonucleoproteins (hnRNPs), which are present as complexes of RNA and protein in the cell nucleus during gene transcription of the newly synthesized RNA (pre-mRNA) [66], were up-regulated in response to MPL. In particular, hnRNPU, which is reported to interact with nuclear GR

complex to regulate transcription *in vivo* [67], was modestly up-regulated by 2 hours before a slow return to baseline (Figure 7A). The hnRNPA2/B1, which is involved in DNA replication and repair, gene transcription, pre-mRNA splicing, and nucleo-cytoplasmic mRNA export [68], showed a sharp (12-fold) rise to peak at 4 hours and promptly returned to baseline by 5.5 hours (Figure 7B). In addition, nucleolin, a histone chaperone that regulates chromatin remodeling and gene transcription [69], and also interacts with GR [70] was up-regulated to peak by 18 hours and returned to baseline only at 66 hours (Figure 7C). In regard to protein translation, a number of ribosomal protein subunits of both 60S and 40S complexes were altered over time, mostly being up-regulated by MPL. While functional information on all the subunits is unavailable, specialized roles of some ribosomal proteins are listed in Table 3. Additionally, expression of isozymes involved in the post-translational modification (PTM) of nascent peptides such as dolichyl-diphosphooligosaccharide glycosyltransferases, protein disulfide-isomerases, and peptidyl-prolyl cis-trans isomerases were diversely altered in response to MPL (data not shown).

Metabolism and Transport of Small Molecules

The proteins classified under this group are involved in the production, processing, and degradation, or transport of endogenous compounds, xenobiotics, and therapeutic drugs. The liver is highly involved in the elimination of potentially toxic endogenous by-products as well as in the biotransformation of exogenous lipophilic chemicals (e.g. drugs and pesticides) into more water-soluble products that are excretable in urine. For the purpose of a more in-depth functional classification, hepatic metabolizing enzymes were further sub-clustered into groups comprising the cytochrome P450 (CYP) (Phase-I) enzymes (Table 4A), Phase-II enzymes (Table 4B), or other metabolic enzymes (Table 4C), while proteins involved in the active or facilitated transport of endogenous and/or drug compounds are listed in Table 5. A total of 62 hepatic metabolizing enzymes were found to be differentially regulated by MPL of which 20 were CYP enzymes, 14 Phase-II enzymes, and 28 other enzymes involved in small molecule metabolism.

While a number of CYPs were up-regulated by MPL, dynamic changes in protein abundance of three CYP enzymes, CYP2C13, CYP2D10, and CYP3A2 (Figures 8A–C) as measured by proteomic profiling are shown. In all three instances, hepatic CYP protein expression was increased by 2–3 fold, peaking at 5.5 hours (2C13 and 2D10) or at 12 hours (3A2). In addition, CYP2A1 showed complex biphasic regulation over time (Figure 3D). In rat, CYP3A2 is the main constitutive liver enzyme; sharing 73% homology in the amino acid sequence, some substrate preference and functional analogies to human CYP3A4 [71], which metabolizes a wide range of drugs and endogenous compounds. Our finding that MPL enhances CYP3A2 expression in the rat is in line with previous findings that dexamethasone (DEX) induces CYP3A2 mRNA and protein expression as well as enzyme activity in rat liver [72].

The expression profiles of two major Phase-II conjugation enzymes, sulfonyltransferase 1A1 (SULT1A1) and UDP-glucuronosyltransferase 1A1 (UGT1A1) are depicted in Figures 8D and 8E. Both enzymes showed distinct and interesting patterns of regulation. SULT1A1 was up-regulated to peak expression by 18 hours (~2 fold) and remained enhanced until 48

hours, before starting to decline to baseline at 66 hours. UGT1A1 displayed strong biphasic behavior; expression was down-regulated (~40%) by 2 hours, followed by a sharp rise to peak around 8 hours before eventually returning to baseline. SULT1A1 is involved in the conjugation of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to several endogenous and exogenous substrates such as hormones (e.g. thyroid hormone) [73], neurotransmitters (e.g. dopamine) [73], and phenolic compounds [74]; whereas UGT1A1 catalyzes the conjugation of UDP-glucuronic acid to small, hydrophobic molecules such as bilirubin [75], ethinylestradiol [76], opioids [77], and the active metabolite of irinotecan, SN-38 [78].

Other MPL-regulated enzymes related to small molecule metabolism are listed in Table 4C. Carboxylesterases are ubiquitously distributed in mammalian tissues, and show highest activity in liver microsomes [79]. They play an important role in the hydrolytic biotransformation of several drugs, especially those containing ester or amide bonds [79] such as methylphenidate and clopidogrel. Carboxylesterase 1D showed an unusual profile in response to MPL, where expression remained at baseline levels until 12 hours before sharply rising to peak (~6-fold) by 18 hours and eventually returning to baseline by 48 hours (Figure 8F). NADPH:CYP oxidoreductase (POR) is an essential component for all microsomal CYP monooxygenases, to which it transfers electrons originating from NADPH [80]. POR expression showed a delayed enhancement profile, where it increased in a time-dependent manner (~2.5-fold) for up to 30 hours, before declining to baseline (Figure 8G).

There were 25 proteins involved in the intracellular transport and/or excretion (i.e. influx or efflux) of endogenous compounds and xenobiotics that were significantly altered by MPL dosing (Table 5). Of note, protein expression of bile salt export pump (BSEP), an important liver-specific efflux transporter located on the canalicular membrane of hepatocytes involved in the biliary excretion of monovalent bile salts [81], was modestly up-regulated (~1.7-fold) by 8 hours after MPL dosing (Figure 8H). This observation is consistent with previous reports of DEX-mediated Bsep mRNA induction in cultured rat hepatocytes [82].

Immune Regulation

Table 6 lists and describes 11 proteins related to immune and/or inflammatory processes that were significantly altered by MPL, with representative examples presented in Figure 9. Over half of these proteins were found to be involved in the hepatic acute phase response, which plays a significant role in tissue and organ protection in response to diverse stimuli [83]. The serine anti-proteinases, α1-antitrypsin (serpin A1) and α 1-antichymotrypsin (serpin A3n), which play a prominent role in proteolytic cascades, including the mammalian coagulation pathways [84], were up-regulated in response to MPL. In addition, signal transducer and activator of transcription-3 (STAT3), an important upstream transcription factor that is regulated by CS and co-interacts with GR [85] to regulate several downstream inflammatory responses was also enhanced following MPL. In fact, STAT3 signaling is reported to play a role in downstream serpin activation [86], which might explain the observed increases in serpin A1 and A3n protein expression. The temporal protein expression profiles of STAT3, serpin A3n, and serpin A1 (Figures 9A–C) are shown. STAT3 is up-regulated (~3.3-fold) by 8 hours and shows a wide response window before returning to baseline after 48 hours. While both serpins peaked at ~12 hours, serpin A1 protein expression was enhanced to a

lesser extent compared to serpin A3n (2.5-fold vs. 5-fold). Our observation of the MPL-induced increase in STAT3 protein expression is consistent with previous work showing up-regulation of STAT3 mRNA by MPL in liver [87].

Other MPL-regulated proteins

Of the 451 differentially regulated proteins, 73 proteins that did not meet functional criteria for classification into any one of the six clusters were grouped separately as ‘other MPL-regulated proteins’. These proteins are listed along with their description in Table 7. Several proteins involved in cytoskeletal structure and membrane anchorage such as Titin, Talin-1, Vimetin, Tubulin, and Coronin-1B were diversely altered by MPL. Interestingly, kynurenine 3-monooxygenase (KMO), which metabolizes kynurenine (tryptophan metabolite) into kynurenic acid and quinolinic acid was modestly up-regulated by MPL (~ 2-fold; profile not shown). Increased production of kynurenine and quinolinic acid metabolites in brain by KMO has been suggested to play a role in the pathogenesis of multiple neurological disorders via modulation of N-methyl-D-aspartate receptor (NMDAR) activity and glutamatergic signaling [88]. While the role of inflammatory cytokines in mediating KMO-induced neurotoxicity has been reported [89], GC-regulation of KMO in brain and its potential role in CS-induced neurotoxicity [90] have not been previously documented.

DISCUSSION

This report describes the mining of a rich proteomic time-series dataset obtained from the analysis of liver tissues obtained from a population of 60 adrenalectomized rats given a single 50 mg/kg intramuscular bolus of MPL and killed at select times over the course of 66 hours after dosing. An in-depth functional analysis was performed to better elucidate the complex nature of CS effects in liver *in vivo*. Analysis of global changes in transcriptomic expression has been an integral part in studying mechanisms of actions of various pharmacological agents, and have been extensively performed in our laboratory to understand the tissue-specific effects of CS [12, 13, 27, 87, 91]. Although highly useful in understanding the mechanisms of pharmacogenomic regulation by CS, genomic approaches fall short in that changes in mRNA expression may not directly correlate with, and hence be reflective of drug effects occurring in the tissue ‘proteome’ [16, 92]. Proteomics can be viewed as being complementary to genomics as it focuses on the gene products which mediate cellular responses [93]. While measurement of dynamic proteomic changes is highly desirable, several challenges exist in accurately quantifying large-scale proteomic changes *in vivo*. We reported the development of a sensitive and robust label-free quantification strategy for large-scale quantitative proteomics [11]. The complexities in mRNA-protein correlation were highlighted in our previous report where tandem changes in MPL-induced mRNA and protein expression in liver were analyzed. The concordance between mRNA and protein dynamics were observed only for a small number of genes [14]. Since proteins better reflect drug-induced physiological changes, mining of functional information at the proteomic-level coupled with characterizing temporal responses of important proteins can provide mechanistic insight into the physiological and pharmacological effects of CS.

Significance analysis for differentially-regulated proteins was performed using the EDGE software, which analyzes time course differential expression using spline-based methods for longitudinal fitting of data [21, 94]. There were 478 out of 959 proteins that showed significantly varying temporal profiles (p value < 0.05; q-value < 0.01). Following further manual filtering for redundancies in the dataset, a final group of 451 proteins became the subject of intensive analysis of MPL-induced regulation, cellular localization, and biological function.

Upon exploratory data analysis of time-courses of protein expression following MPL, it was evident that great diversity exists in the shapes of protein response profiles over time. In general, this observation is consistent with mRNA response profiles observed at the transcriptomic level in liver as well as in other tissues [13, 14, 95]. Proteins were annotated on the basis of temporal regulation by simply visually inspecting all 451 profiles for “up” and “down”-regulation based on deviation from baseline. In many cases, temporal response profiles met both criteria, indicating complex biphasic regulation. Around 75% of all proteins showed up-regulation in response to MPL, while approximately 20% displayed biphasic behavior. A small number of proteins (~5%) were purely down-regulated by MPL dosing. However, it is possible that some other down-regulated proteins were not detected due to their low abundance following suppression by MPL. The widely accepted mechanism for CS-mediated enhancement of target gene expression involves binding of free steroid to its cytosolic receptor, translocation of drug-receptor complex into the nucleus, homo-dimerization followed by binding to GREs located in the 5′-promoter region of target genes, and consequent alteration of the rates of transcription of select genes. Alternatively, CS may also post-transcriptionally regulate the rate of mRNA expression by altering mRNA stability [96]. However, it is possible that these understandings of pharmacogenomic regulation by CS are too simplistic [27], especially in cases where complex patterns in mRNA and/or protein expression emerge over time. Indeed, mechanistic studies have revealed the involvement of transcription factors which serve as secondary biosignals or coactivators of GR signaling such as STAT3 [85] (Figure 9A) or CCAAT/enhancer-binding protein [97, 98]. It can be hypothesized that such secondary signals might synergize with or antagonize GR-mediated transcriptional or post-transcriptional effects, which in turn produce complex patterns of mRNA and protein expression. Based on work by Ramamoorthy and Cidlowski [99], CS-induced repression of the target genes may occur via GR-NCoR1-histone deacetylase 3 interactions with negative GREs.

It is known that CS mediate their pharmacologic effects by inducing molecular and cellular changes via genomic (receptor/gene-mediated) and non-genomic mechanisms [100, 101]. However, there is at present no integrated knowledge regarding the subcellular localization of genes or proteins that are affected by CS. We found that significant portions were localized to the cytosol (24%), mitochondria (20%), and endoplasmic reticulum (13%). The indication that a large number of mitochondrial proteins were altered by MPL is consistent with current knowledge regarding MPL-effects on metabolic pathways such as gluconeogenesis, fatty acid oxidation, and the Krebs cycle, which primarily occur within the mitochondria [5, 65]. Among the small number of proteins that were localized to the peroxisome (~5%), a few proteins were involved in fatty acid oxidation in that organelle as well (e.g. peroxisomal 3-ketoacyl-CoA thiolase B), which is reasonable since both

mitochondria and the peroxisome are known sites for lipid metabolism [102]. A number of drug metabolizing CYP450 enzymes were altered in response to MPL (Table 4A). There were 19 out of the 20 CYP enzymes regulated by MPL found to be microsomal; i.e. localized to endoplasmic reticulum *in vivo*. A significant number (57 proteins) were localized to the nucleus, of which a number of proteins were involved in the regulation of replication, gene transcription, and also interacting with GR. There were a few instances where an active protein was localized to more than one organelle. For example, programmed cell death-8 (PCD8), an apoptotic factor, is released from the mitochondrion intermembrane space into the cytosol and to the nucleus in response to cellular stress, where it functions as a proapoptotic factor [103]. In such instances, proteins were counted to be present in more than one organelle. Collectively, these results illustrate that CS mediate effects that are widespread at the scale of subcellular location.

We utilized a function-based approach to understand how CS-induced perturbations at the proteome-level related to adverse and/or therapeutic pharmacologic effects of CS therapy in the liver. The liver is a vital organ involved in regulating essential physiological processes in the body, with hepatocytes as the major cell type occupying close to 80% of the total liver volume [104]. The liver functions to detoxify and eliminate potentially toxic products such as endogenous metabolic waste-products or foreign compounds (e.g. drugs). It is also involved in the synthesis of plasma proteins (e.g. albumin), clotting factors (e.g. fibrinogen), and certain globulins which transport substances such as cholesterol, vitamin D, and iron [105, 106]. In addition, the liver produces acute-phase proteins in response to microbes or hepatic stressors, which are associated with inflammation, tissue repair, and immune cell activities [107]. Another crucial function of the liver is the regulation of systemic energy metabolism, which is under complex control by hormones such as insulin, glucagon, and importantly, GC [56].

A summary of the functional cluster analysis of proteins differentially regulated by MPL is shown in Figure 10. A number of proteins altered by CS belonged to biological processes that are critical to hepatic and whole-body function. The two largest functional clusters belonged to the processes of hepatic stress and signaling (24%) and energy regulation (22%) (Figure 10; left). With respect to cell signaling, a number of proteins such as annexin A6, regucalcin, profilin-1, calnexin, and calreticulin, which are involved in upstream- and/or downstream-regulation of intracellular calcium (Ca^{2+}) were diversely regulated upon MPL dosing. While this may or may not be a direct consequence of MPL effects on the proteins, alterations in the highly-sensitive Ca^{2+}-pathway are quite likely involved in mediating further downstream effects, suggestive of complex higher-order regulation of biological processes following MPL. In addition, a number of glutathione S-transferase isozymes, which are involved in the detoxification of electrophilic xenobiotics (e.g. chemical carcinogens, antitumor agents) as well as endogenous unsaturated aldehydes, quinones, epoxides, and hydroperoxides formed during oxidative stress, were up-regulated (refer to Table 1). This effect is suggestive of a protective mechanism against the oxidative stress-inducing effects of CS in tissues [43–45]. Approximately 100 proteins related to various aspects of energy metabolism (amino acid metabolism, carbohydrate metabolism, lipid/fatty acid metabolism, and the Krebs cycle) were identified as differentially regulated by MPL (Figure 10; bottom right). Our observations of MPL-induced up-regulation in amino acid

degrading enzymes such as TAT are consistent with previous findings at the mRNA level [13, 108]. Furthermore, the time-course of TAT protein expression strongly complemented that of TAT activity measured using a colorimetric assay [108] in livers obtained from the same animals (Supplementary Figure S2 in the SI). We found that expression of glutamine synthetase, an amino-acid metabolizing gene extensively studied in skeletal muscle [109, 110], was slightly up-regulated in liver as well. Interestingly, glutamine synthetase is reported to be expressed in a rim of hepatocytes surrounding hepatic veins [111], which provides a plausible explanation for its regulation by CS in liver. The temporal profile of PEPCK protein expression showed good correlation with previously reported PEPCK mRNA and activity profiles [112]. Furthermore, the profile of PEPCK, which is the rate-limiting enzyme of gluconeogenesis, showed good correspondence to systemic plasma glucose concentrations measured from the same rats, demonstrating the mechanisms through which CS induce their hyperglycemic effects *in vivo*. Peroxisomal 3-ketoacyl-CoA thiolase B, which catalyzes the final step in the peroxisomal β-oxidation of straight-chain acyl-CoA, was down-regulated by MPL. Interestingly, lack of peroxisomal 3-ketoacyl-CoA thiolase B is associated with decreased β-oxidation under conditions of metabolic stress in mice [113]. Together, these findings suggest that peroxisomal 3-ketoacyl-CoA thiolase B may play an important role in CS-induced dysregulation of hepatic fatty acid metabolism.

Another important group of proteins altered by MPL are those involved in the metabolism of endogenous substrates, xenobiotics, or therapeutic drugs. Of the metabolizing enzymes regulated by MPL, 32% were CYP enzymes, 23% Phase-II conjugating enzymes, and the remaining other enzymes (including non-CYP Phase-I enzymes). While it is evident from Figure 8 that MPL regulates the protein expression of important CYP enzymes (e.g. CYP3A2) and Phase-II enzymes (e.g. UGT1A1 and SULT1A1), these findings do not imply that MPL has a direct impact on the metabolism and pharmacokinetics of drug substrates for these enzymes. Instead, these findings serve to generate mechanistic hypotheses for further evaluating potential drug-interactions with MPL. Collectively, these results provide global insight into the effect of CS at the proteomic level and its relationship to physiological processes occurring in liver.

An important element that is often overlooked while investigating drug action is the time-dependent nature of pharmacological responses. Assessment of effects at a single point in time does not fully characterize the actions of the drug. This approach can be even more misleading for characterizing of the magnitude of expression change, since the magnitude of effect will be dependent on the particular time-point examined, as exemplified in this study. Through use of our “giant-rat” study paradigm, pharmacodynamic changes in the liver proteome was assessed across 66 hours after dosing. Although drug concentrations were dissipated by 8 hours, temporal responses of several proteins showed that many biological cascades remained active well after the drug was cleared from the system. Furthermore, the temporal proteomic data enabled us to more rigorously functionally characterize MPL-induced changes in liver; especially in cases where changes in protein expression began to occur several hours after dosing. Male ADX rats were used in our animal experiments in order to prevent the confounding effects of endogenous corticosterone on tissue gene expression, and hence create stable pharmacodynamic baselines. However, in reality,

expression of several GC-regulated mRNA and proteins occur in a circadian fashion in peripheral tissues [114], due to rhythmic corticosterone production by the adrenal cortex.

Our approach for functionally annotating and classifying significantly altered proteins is similar to one applied in our microarray studies [27, 87, 115]; literature-based functional information for each protein was mined and annotated. Each protein was then assigned to a functional cluster devised based on the physiologically and pharmacologically relevant effects of CS. Direct use of DAVID and other currently available gene ontology and pathway analysis tools were avoided for final functional clustering for several reasons. First, although highly comprehensive in terms of genomic and proteomic coverage [116], the functional information on the proteins contained in the databases for these tools are not complete. Second, as noted by us and others [115, 116], these tools do not take into account tissue-specific or context-specific physiological functions of proteins. Finally, gene ontology analyses based on “biological process” and “molecular function” yielded overly broad or specific functional clusters. For instance, functional clustering based on gene ontology tools would cluster MPL-responsive CYP enzymes which are involved in drug metabolism into either “iron-binding” (biological process) or as an “oxidoreductase” (molecular function). While both these classifications are in technical terms not incorrect, they do not serve in identifying any further implications for CS action. For instance, information regarding drug substrates of the CYP enzyme could provide insight into potential drug interactions with MPL. However, this is not to imply that our approach is devoid of limitations. Our stringent criteria for selecting differentially-regulated protein may have led to the exclusion of potentially useful information on some down-regulated proteins, which are consistently detected in controls but detected at fewer times in the treated animals. However, the stringent selection criteria set in this study was tailored towards i) ensuring that protein fold-changes were reported with high confidence across each time-point, and ii) meeting the goals of subsequent data analyses. Therefore, proteins which are undetectable in the treatment group at one or two time-points could still warrant consideration for further analyses, depending on the downstream application of the results. An inherent difficulty in manually clustering based on functionality is that a protein could (and often does) mediate multiple biological effects, and hence can be clustered into more than one functional group. In instances where a protein functionally met criteria for classification into more than one cluster, the protein was allocated to the cluster with greater relevance to MPL-induced effects in liver. Although this system may bear some caveats, these groupings together with the proteomic expression profiles provide insight into the global impact of CS on liver physiology.

For over a decade, our laboratories have utilized genomic microarray data in conjunction with mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) modeling [117] to better understand the receptor/gene-mediated effects of steroids in multiple organ systems [7, 118, 119]. In order to identify common mechanisms underlying MPL-induced mRNA expression of genes, unifying mathematical models were developed to describe temporally-clustered groups of genes. While this approach is extremely useful in understanding mechanisms of CS-induced gene regulation at the level of mRNA, a functional clustering approach is required at the protein level as it provides insight into designing systems PK/PD models based on the biological function of proteins as opposed to temporal patterns alone. An initial question that was addressed during the analysis was whether proteins clustered on

the basis of function would show similar trends in their temporal responses. However, to the contrary, it was interesting to note that the time-course of proteins are highly varied within functional groups–reflecting diversity in response times of proteins mediating similar functions. Future work involves developing mechanistic PK/PD models that quantitatively integrate and describe the large-scale changes observed in our transcriptomic and proteomic datasets to gain a more holistic understanding of CS pharmacodynamics. To our knowledge, this is the first effort that merges pharmacodynamics with large-scale functional pharmacoproteomics to gain an understanding of drug-induced changes *in vivo*. The time profiles of proteins mediating important biological functions provided new insights into the diverse temporal changes and their implications associated with gene transcription and protein translation, cell stress and signaling, energy regulation (amino acid metabolism, gluconeogenesis, fatty acid oxidation), hepatic drug metabolism, and inflammatory responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- An approach for function-based clustering of pharmacoproteomic data is described.
- Cytosolic and mitochondrial proteins are highly altered by corticosteroid in liver.
- Relationship of altered hepatic proteomics to systemic steroid effects is described.
- Functionally similar steroid-regulated proteins display diverse temporal patterns.

SIGNIFICANCE

Clinical use of corticosteroid (CS) therapy is frequent and chronic. However, current knowledge on the proteome-level effects of CS in liver and other tissues is sparse. While transcriptomic regulation following methylprednisolone (MPL) dosing has been temporally examined in rat liver, proteomic assessments are needed to better characterize the tissue-specific functional aspects of MPL actions. This study describes a functional pharmacoproteomic analysis of dynamic changes in MPL-regulated proteins in liver and provides biological insight into how steroid-induced perturbations on a molecular level may relate to both adverse and therapeutic responses presented clinically.

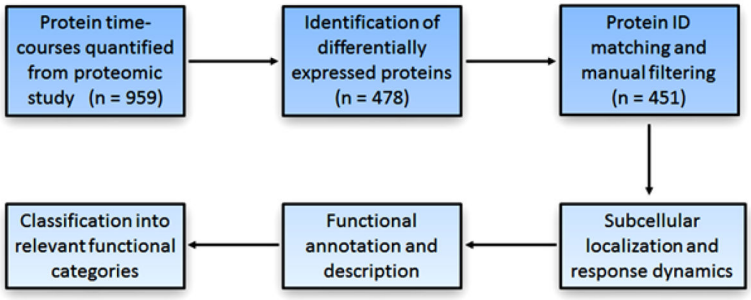

Figure 1.
Workflow for the functional analysis and clustering of the proteomic data.

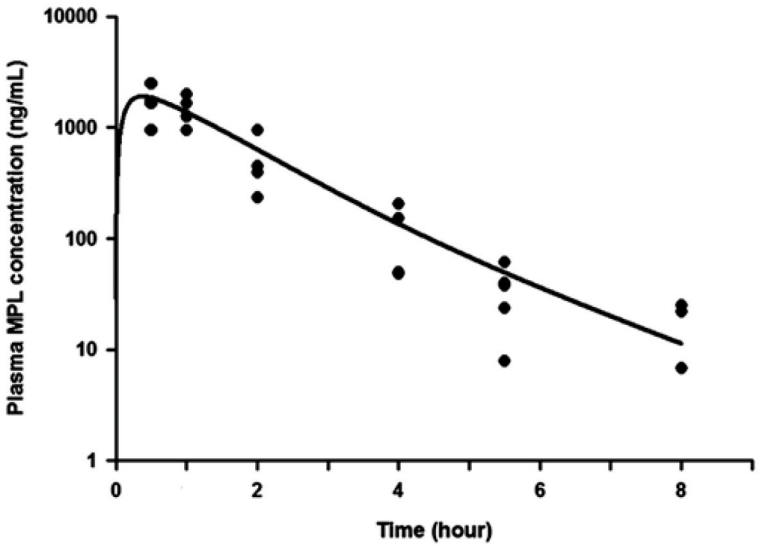

Figure 2.

MPL concentrations in rat plasma following a single 50 mg/kg intramuscular dose of drug. MPL concentrations were determined by normal-phase HPLC analysis of plasma samples obtained from individual animals. Closed circles depict observed measurements from each animal and the solid line model fitting results.

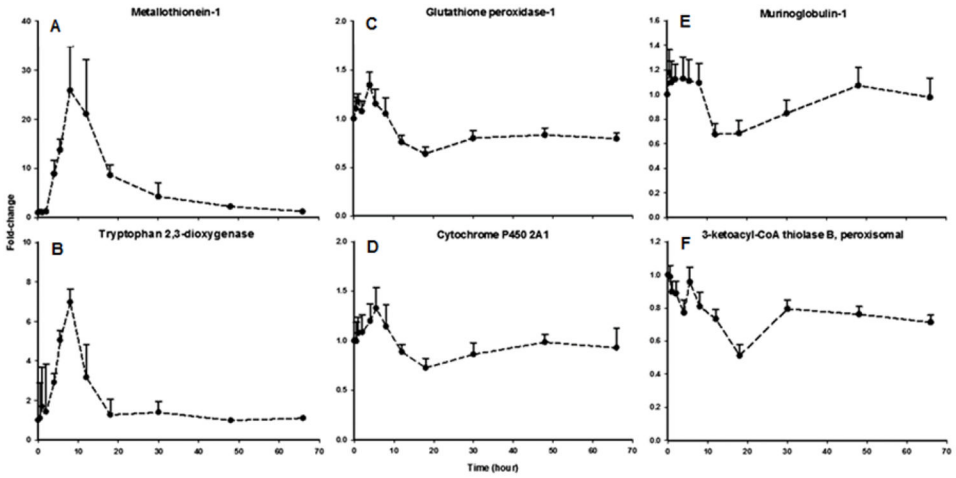

Figure 3.

Temporal response profiles of representative proteins showing (A and B) enhanced expression, (C and D) biphasic expression, and (E and F) down-regulation. Closed circles represent the mean and the error bars one standard deviation.

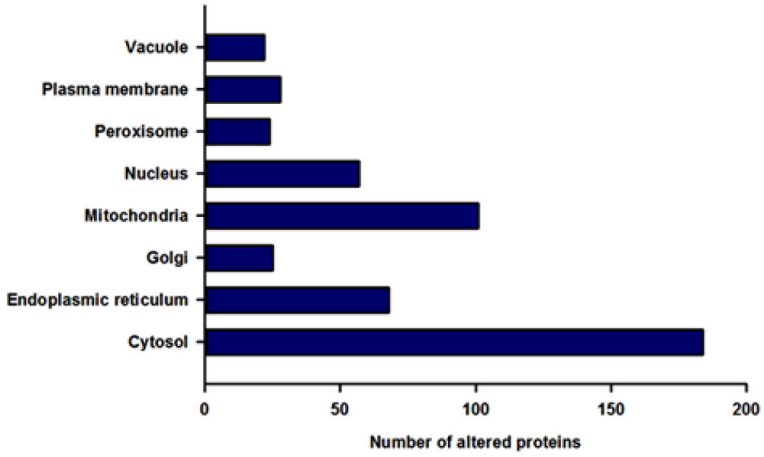

Figure 4.

Gene Ontology-based annotation of cellular distribution of significantly altered proteins based on sub-cellular organelles. Solid bars represent the number of proteins altered in each cellular compartment.

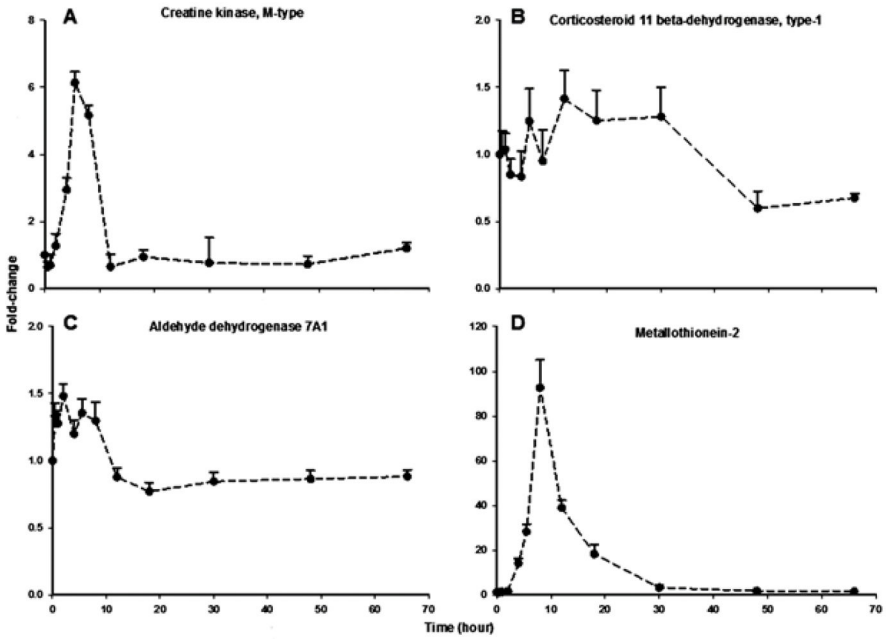

Figure 5.

Temporal response profiles of four proteins involved in hepatic stress and/or cellular signal transduction. Closed circles depict the mean and the error bars one standard deviation.

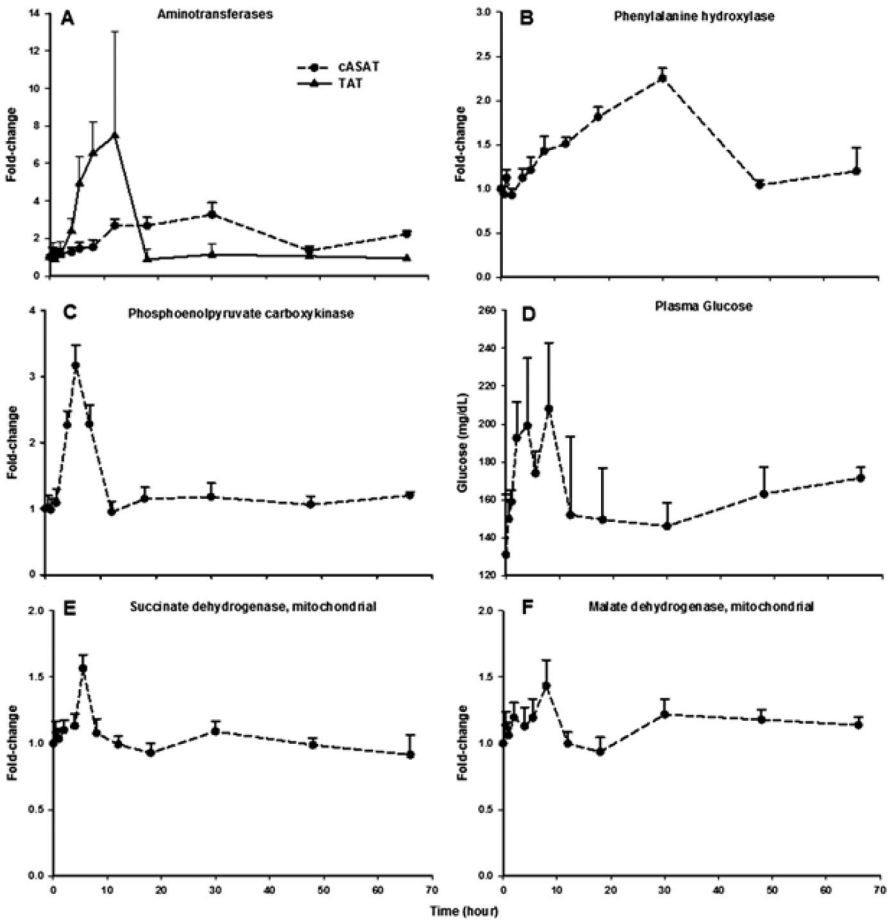

Figure 6.

Temporal response profiles of representative proteins involved in the regulation of hepatic energy metabolism. Closed circles represent the mean and the error bars one standard deviation.

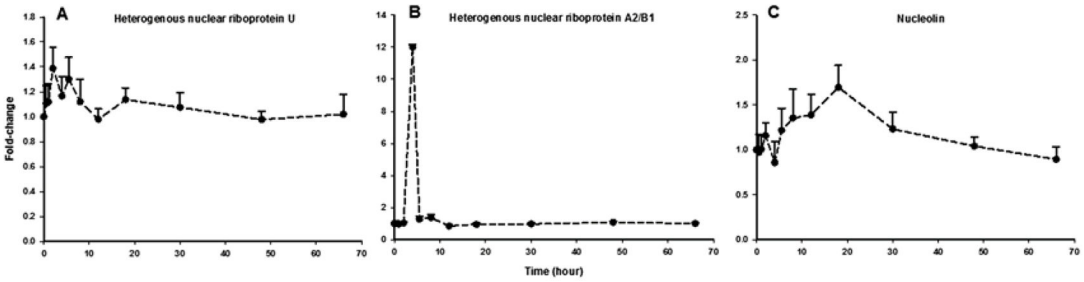

Figure 7.

Temporal response profiles of three proteins involved in the regulation of transcription, translation, and protein processing. Closed circles represent the mean and the error bars one standard deviation.

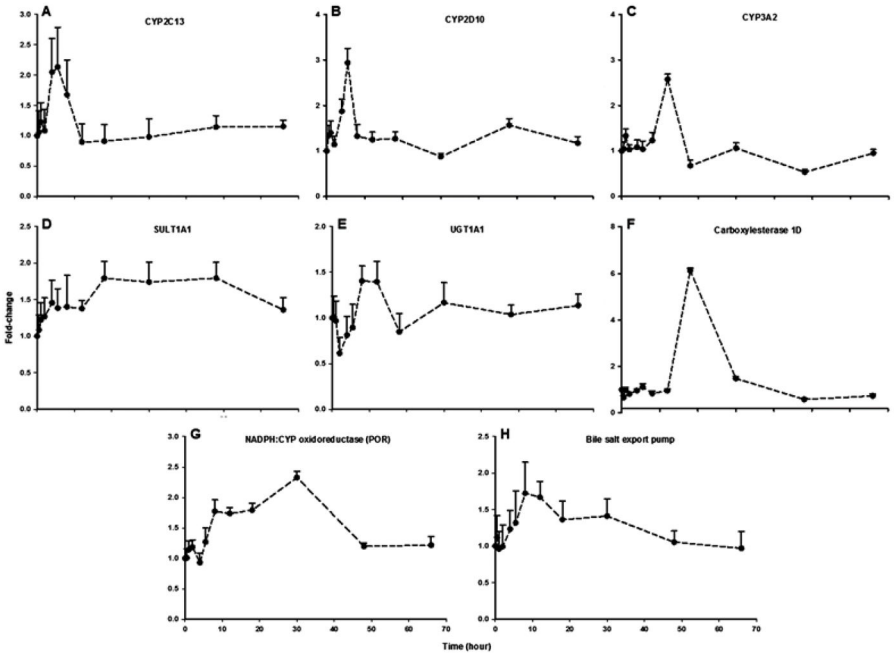

Figure 8.

Temporal response profiles of representative proteins involved in transport and metabolism of endogenous substrates, xenobiotics, and/or drugs. Closed circles represent the mean and the error bars one standard deviation.

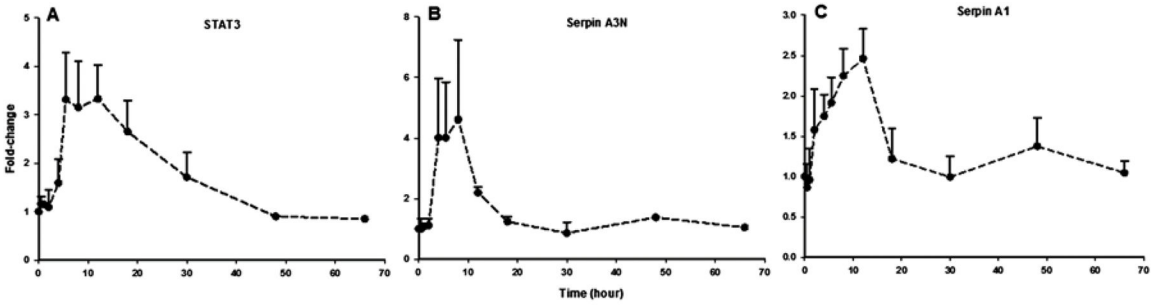

Figure 9.

Temporal response profile of representative immune-related proteins involved in the acute phase response. Closed circles represent the mean and the error bars one standard deviation.

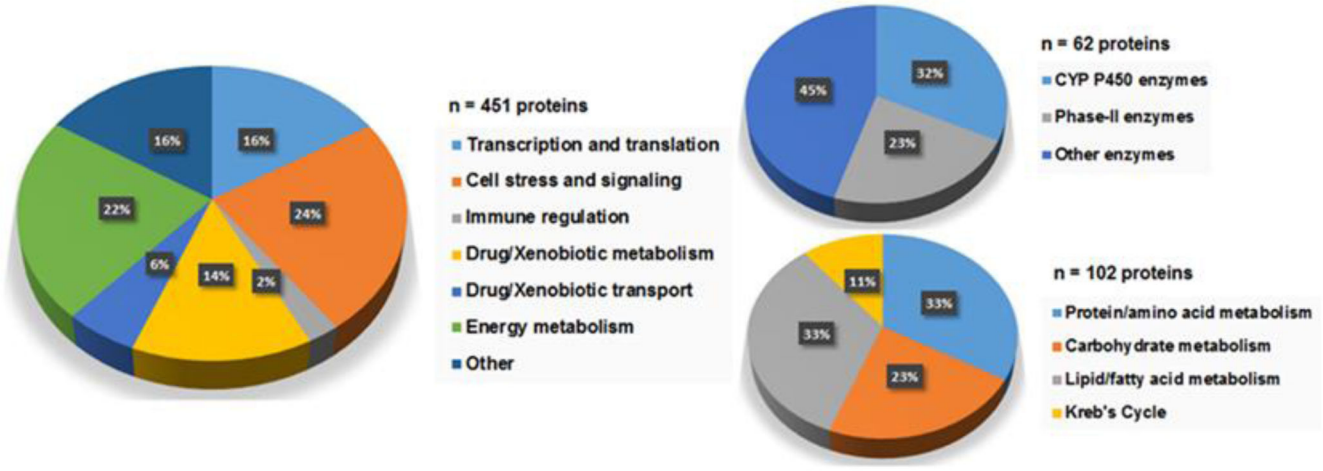

Figure 10.

Summary of functional clustering results for MPL-altered proteins involved in diverse biological processes. The pie-chart on the left summarizes the percentage of proteins involved in each of the functional categories. The chart on the top-right summarizes the percentage of proteins in the sub-clusters related to hepatic endogenous substrate, xenobiotic, and/or drug metabolism. The chart on the bottom-right summarizes the percentage of proteins in the sub-clusters related to hepatic energy regulation.

Table 1

MPL-regulated proteins related to cell stress and signal transduction.

UProt_ID	Gene Name	Protein Name	Function	Regulation
P63102	Ywhaz	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)	Adapter protein which modulates partner activity	UP
O70351	Hsd17b10	17-beta-hydroxysteroid dehydrogenase 10	Catalyzes 20-beta-OH and 21-OH dehydrogenase activities with C21 steroids	UP
Q62730	Hsd17b2	17-beta-hydroxysteroid dehydrogenase type 2	Oxidation of estradiol and testosterone	UP/DOWN
P62334	Psmc6	26S protease regulatory subunit	ATP-dependent degradation of ubiquinated proteins	UP
P13437	Acaa2	3-ketoacyl-CoA thiolase, mitochondrial	Inhibits BNIP3-mediated apoptosis and mitochondrial damage	UP
P50554	Abat	4-aminobutyrate aminotransferase, mitochondrial	Catabolism of inhibitory neurotransmitter gamma-aminobutyric acid (GABA)	UP
Q64640	Adk	Adenosine kinase	ATP dependent phosphorylation of adenosine	DOWN/UP
P10760	Ahcy	Adenosylhomocysteinase	Control of methylations via regulation of the intracellular concentration of adenosylhomocysteine	UP
P29410	Ak2	Adenylate kinase 2, mitochondrial	Reversible transfer of the terminal phosphate group between ATP and AMP	UP
Q08163	Cap1	Adenylyl cyclase-associated protein 1	Directly regulates filament dynamics	UP
Q64057	Aldh7a1	Aldehyde dehydrogenase family 7 member A1 (antiquitin)	Protects cells from oxidative stress	UP
P14669	Anxa3	Annexin A3	Inhibitor of phospholipase A2; also possesses anti-coagulant properties	UP
P48037	Anxa6	Annexin A6	Release of Ca^{2+} from intracellular stores	UP/DOWN
P18484	Ap2a2	AP-2 complex subunit alpha-2	Involved in clathrin-dependent endocytosis	UP
Q7TMA5	Apob	Apolipoprotein B-100	Recognition signal for the binding and internalization of LDL particles by the apoB/E receptor	DOWN
P09034	Ass1	Argininosuccinate synthase	Blood pressure regulation; catalyzes the penultimate step of the arginine biosynthetic pathway	UP
P14173	Ddc	Aromatic-L-amino-acid decarboxylase	Decarboxylation of L-DOPA to dopamine, and L-5-hydroxytryptophan to serotonin	UP
P26453	Bsg	Basigin (CD antigen CD147)	Stimulates hepatic fibroblasts to produce matrix metalloproteinases	UP/DOWN
O88428	Papss2	Bifunctional PAPS synthase 2	ATP sulfurylase and APS kinase activity - mediates two steps in the sulfate activation pathway	UP
P35565	Canx	Calnexin	Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum	UP
P18418	Calr	Calreticulin	Protein folding and export from E.R. to Golgi; also involved in regulating intracellular free calcium levels	UP
P07756	Cps1	Carbamoyl-phosphate synthase	Role in removing excess ammonia from the cell	UP
B0BNN3	Ca1	Carbonic anhydrase 1	Reversible hydration of carbon dioxide	UP
P14141	Ca3	Carbonic anhydrase 3	Reversible hydration of carbon dioxide; major participant in the liver response to oxidative stress	UP

UProt_ID	Gene Name	Protein Name	Function	Regulation
P04762	Cat	Catalase	Protects cells against oxidative damage	UP
Q61301	Ctnna2	Catenin alpha-2	Essential for actin cytoskeletal organization and canalicular membrane formation in liver	UP
P24268	Ctsd	Cathepsin D	Acid protease active in intracellular protein breakdown	UP
P16232	Hsd11b1	Corticosteroid 11-beta-dehydrogenase isozyme 1	Reversible conversion of corticosterone to 11-dehydrocorticosterone	UP
P00564	Ckm	Creatine kinase M-type	Energy metabolism - transfer of phosphate between ATP and various phosphogens (e.g. creatine phosphate)	UP
P09605	Ckmt2	Creatine kinase S-type, mitochondrial	Energy metabolism - transfer of phosphate between ATP and various phosphogens (e.g. creatine phosphate)	UP
Q63270	Aco1	Cytoplasmic aconitate hydratase	Iron sensor	UP
Q68FS4	Lap3	Cytosol aminopeptidase	Processing and regulation of turnover of intracellular proteins	UP
Q6Q0N1	Cndp2	Cytosolic non-specific dipeptidase	Hydrolyzes a variety of dipeptides	DOWN/UP
P80254	Ddt	D-dopachrome decarboxylase	Tautomerization of D-dopachrome	UP/DOWN
O08557	Ddah1	Dimethylarginine dimethylaminohydrolase 1	Nitric oxide generation by regulating cellular concentrations of methylarginines	UP/DOWN
Q63342	Dmgdh	Dimethylglycine dehydrogenase, mitochondrial	Catabolism of choline	UP
P80067	Ctsc	Dipeptidyl peptidase 1 (Cathepsin C)	Thiol protease; has dipeptidylpeptidase activity	UP/DOWN
Q924C3	Enpp1	Ectonucleotide pyrophosphatase/phosphodiesterase member 1	Regulation of purinergic signaling	UP
P02692	Fabp1	Fatty acid-binding protein, liver	Lipoprotein-mediated cholesterol uptake in hepatocytes	UP/DOWN
Q80X90	Flnb	Filamin-B (FLN-B)	Connects cell membrane constituents to the actin cytoskeleton	UP
Q9QVC8	Fkbp52	FK506-binding protein 4	Immunophilin protein with PPIase and co-chaperone activities (e.g. GR)	UP
Q07071	Gckr	Glucokinase regulatory protein	Role in glucose homeostasis; inhibits glucokinase (GCK) by forming an inactive complex with this enzyme	UP
P04041	Gpx1	Glutathione peroxidase 1	Protects hemoglobin in erythrocytes from oxidative breakdown	UP/DOWN
P00502	Gsta1	Glutathione S-transferase alpha-1	Glutathione peroxidase activity - protect cells from oxidative stress	DOWN
P04903	Gsta2	Glutathione S-transferase alpha-2	Glutathione peroxidase activity - protect cells from oxidative stress	DOWN/UP
P04904	Gsta3	Glutathione S-transferase alpha-3	Glutathione peroxidase activity - protect cells from oxidative stress	UP
P24473	Gstk1	Glutathione S-transferase kappa 1	Protection against genotoxic and cytotoxic electrophiles in the mitochondrial compartment	UP
P04905	Gstm1	Glutathione S-transferase Mu 1	Conjugation of glutathione to exogenous and endogenous compounds	UP
P08010	Gstm2	Glutathione S-transferase Mu 2	Conjugation of glutathione to exogenous and endogenous compounds	UP
Q80W21	Gstm7	Glutathione S-transferase Mu 7	Conjugation of reduced glutathione to exogenous and endogenous hydrophobic electrophiles	UP
P30713	Gstt2	Glutathione S-transferase theta-2	Inactivation of reactive sulfate esters in carcinogenic arylmethanols	UP
P08009	Gstm3	Glutathione S-transferase Yb-3	Conjugation of glutathione to exogenous and endogenous compounds	UP

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UProt_ID	Gene Name	Protein Name	Function	Regulation
Q63060	Gk	Glycerol kinase	Regulation of glycerol uptake and metabolism; also an ATP-stimulated GR translocation promoter	UP
O88600	Hspa4	Heat shock 70 kDa protein 4	Transcription factor-binding protein	UP
P48721	Grp75	Heat shock 70 kDa protein, mitochondrial (Mortalin)	Cell proliferation and cellular aging	UP
P63018	Hsc70	Heat shock cognate 71 kDa protein	Binds bacterial lipopolysaccharide (LPS) and mediates LPS-induced inflammatory response	UP
P82995	Hsp90aa1	Heat shock protein HSP 90-alpha	Molecular chaperone - maturation, structural maintenance and regulation of specific - proteins (e.g. GR)	UP
P34058	Hsp90ab1	Heat shock protein HSP 90-beta	Molecular chaperone - maturation, structural maintenance and proper regulation of specific target proteins	UP
P01946	Hba1	Hemoglobin subunit alpha-1/2	Involved in oxygen transport from the lung to the various peripheral tissues	DOWN
P02091	Hbb	Hemoglobin subunit beta-1	Involved in oxygen transport from the lung to the various peripheral tissues	DOWN
P20059	Hpx	Hemopexin	Circulating heme-binding proterin; transports to the liver for breakdown and iron recovery	UP
P52296	Kpnb1	Importin subunit beta-1	Nuclear protein import	UP
D3ZW55	Itpa	Inosine triphosphate pyrophosphatase	Hydrolyzes purine nucleotides such as inosine triphosphate (ITP)	UP
P70615	Lmnb1	Lamin-B1	Components of the nuclear lamina; provide a framework for the nuclear envelope	UP
P38983	Lamr1	Laminin receptor 1	Cell adhesion, differentiation, migration, signaling, and metastasis	UP
Q5SGE0	Lrpprc	Leucine-rich PPR motif-containing protein, mitochondrial	Role in RNA metabolism in both nuclei and mitochondria	UP
Q63108	Ces3	Liver carboxylesterase 3	Detoxification of xenobiotics and in the activation of ester and amide prodrugs	UP
P02803	Mt1	Metallothionein-1	Heavy-metal binding protein; transcriptionally regulated by both heavy metals and glucocorticoids	UP
P04355	Mt2	Metallothionein-2	Heavy-metal binding protein; transcriptionally regulated by both heavy metals and glucocorticoids	UP
P62260	Ywhae	Mitochondrial import stimulation factor L subunit	Adapter protein which modulates partner activity	UP
P21396	Maoa	Monoamine oxidase type A	Oxidative deamination of biogenic and xenobiotic amines such as serotonin, norepinephrine and epinephrine	UP
Q1HCL7	Nadk2	NAD kinase 2, mitochondrial	Mitochondrial NAD^+ kinase that phosphorylates NAD^+ to yield $NADP^+$	UP
P19804	Nme2	Nucleoside diphosphate kinase B	Synthesis of nucleoside triphosphates other than ATP	UP
P02625	Pvalb	Parvalbumin alpha	Calcium-binding albumin protein involved in calcium signaling	UP
Q63716	Prdx1	Peroxiredoxin-1	Cellular redox regulation	UP
O35244	Prdx6	Peroxiredoxin-6	Regulation of phospholipid turnover and protection against oxidative injury	UP
P31044	Pebp1	Phosphatidylethanolamine-binding protein 1	Binds ATP, opioids and phosphatidylethanolamine; also acts as an inhibitor of MEK phosphorylation	UP
Q9EPH8	Pabpc1	Polyadenylate-binding protein 1	Cytoplasmic regulatory processes of mRNA metabolism	UP
P62963	Pfn1	Profilin-1	Inhibits formation of IP_3 and DAG by inhibiting PIP_2	UP/DOWN

UProt_ID	Gene Name	Protein Name	Function	Regulation
Q9JM53	Pdcd8	Programmed cell death protein 8	Regulator of cellular apoptosis	UP
P67779	Phb	Prohibitin	Inhibits DNA synthesis; role in regulating cell proliferation	UP
Q63347	Psmc2	Proteasome 26S subunit ATPase 2	ATP-dependent degradation of ubiquinated proteins	UP
O88767	Park7	Protein deglycase DJ-1	Cell protection against oxidative stress and cell death	UP
Q8VBU2	Ndrg2	Protein NDRG2	Regulation of the Wnt signaling pathway	UP
P85973	Pnp	Purine nucleoside phosphorylase (PNP)	Breakdown of beta-(deoxy)-ribonucleoside molecules	UP
Q3UQ44	Iqgap2	Ras GTPase-activating-like protein IQGAP2	GTPase-activating protein; known to associate with calmodulin and Nrf-2	UP
Q03336	Rgn	Regucalcin	Calcium binding protein; also involved in ascorbic acid (vitamin C) biosynthesis	UP/DOWN
Q920A6	Scpep1	Retinoid-inducible serine carboxypeptidase	Carboxyl-terminal proteolytic activity at acidic pH	UP
Q64578	Atp2a1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	Reuptake of cytosolic Ca^{2+} into the sarcoplasmic reticulum	UP
P18596	Atp2a3	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	Hydrolysis of ATP coupled with the transport of the calcium from cytosol to E.R. lumen	UP
Q8VIF7	Selenbp1	Selenium-binding protein 1	Sensing of reactive xenobiotics in the cytoplasm; also involved in intra-Golgi protein transport	UP
Q63836	Selenbp2	Selenium-binding protein 2 (acetaminophen-binding protein)	Sensing of reactive xenobiotics in the cytoplasm	UP
P12346	Tf	Serotransferrin (Transferrin)	Transport of iron from sites of absorption and heme degradation to those of storage and utilization	UP
Q4FZX7	Srprb	Signal recognition particle receptor subunit beta (SR-beta)	Targeting of the nascent secretory proteins to the endoplasmic reticulum membrane system	UP
Q66X93	Snd1	Staphylococcal nuclease domain-containing protein 1	Transcriptional coactivator for STAT5	UP
Q4FZT0	Stoml2	Stomatin-like protein 2, mitochondrial (SLP-2)	Regulates the biogenesis and the activity of mitochondria; regulates cardiolipin biosynthesis	UP
O89049	Txnrd1	Thioredoxin reductase 1, cytoplasmic	Role in selenium metabolism and protection against oxidative stress	UP/DOWN
P24329	Tst	Thiosulfate sulfurtransferase	Mitochondrial import factor for the cytosolic 5S rRNA	DOWN
P63029	Tpt1	Translationally-controlled tumor protein	Involved in calcium binding and microtubule stabilization	UP
Q4KLZ6	Tkfc	Triokinase/FMN cyclase	Phosphorylation of dihydroxyacetone and of glyceraldehyde	UP
Q5XHZ0	Trap1	Tumor necrosis factor type 1 receptor-associated protein	Chaperone that expresses ATPase activity	UP
P68037	Ube2l3	Ubiquitin-conjugating enzyme E2 L3	Ubiquitin conjugation to target proteins	UP/DOWN
Q5U300	Uba1	Ubiquitin-like modifier-activating enzyme 1	Ubiquitin conjugation to mark cellular proteins for degradation	UP
Q9Z1A6	Hdlbp	Vigilin (High density lipoprotein-binding protein)	Sterol metabolism; protects cells from over-accumulation of cholesterol	UP
Q5RKI0	Wdr1	WD repeat-containing protein 1	Induces disassembly of actin filaments	UP
P22985	Xdh	Xanthine dehydrogenase/oxidase	Purine metabolism - oxidation of hypoxanthine to xanthine, and xanthine to uric acid	UP/DOWN
Q3MIF4	Xylb	Xylulose kinase (Xylulokinase)	Phosphorylates D-xylulose; plays an important role in regulation of glucose metabolism and lipogenesis	UP

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Table 2A

MPL-regulated proteins related to amino acid metabolism.

UProt_ID	Gene Name	Protein Name	Function	Regulation
P20673	Asl	Argininosuccinate lyase	Reversible breakdown of argininosuccinate producing arginine and dicarboxylic acid fumarate	UP
P13221	Got1	Aspartate aminotransferase, cytoplasmic	Biosynthesis of L-glutamate from L-aspartate or L-cysteine	UP
P00507	Got2	Aspartate aminotransferase, mitochondrial	Catalyzes interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate	UP
Q03248	Bup1	Beta-ureidopropionase	Converts N-carbamoyl-beta-alanine (3-ureidopropanoate) to beta-alanine	UP
P25093	Fah	Fumarylacetoacetate hydrolase	Synthesizes acetoacetate and fumarate from L-phenylalanine degradation	UP
P10860	Glud1	Glutamate dehydrogenase 1, mitochondrial	Converts L-glutamate into alpha-ketoglutarate	DOWN
P09606	Glul	Glutamine synthetase	Production of glutamine and 4-aminobutanoate	UP
P21213	Hal	Histidine ammonia-lyase	First reaction in histidine catabolism	UP/DOWN
Q07523	Hao2	Hydroxyacid oxidase 2	Oxidation of L-alpha-hydroxy acids as well as L-alpha-amino acids	DOWN
Q58FK9	Kat3	Kynurenine-oxoglutarate transaminase 3	L-kynurenine to kynurenic acid	UP
Q5XIT9	Mccc2	Methylcrotonoyl-CoA carboxylase beta chain	Amino acid (L-leucine) metabolism	UP
P04182	Oat	Ornithine aminotransferase, mitochondrial	Formation of proline from acid ornithine	UP
P04176	Pah	Phenylalanine-4-hydroxylase (PAH)	Hydroxylation of the aromatic side-chain of phenylalanine to generate tyrosine	UP
Q2V057	Prodh2	Proline dehydrogenase 2	Converts proline to delta-1-pyrroline-5-carboxylate	UP
Q5I0J9	Aspdh	Putative L-aspartate dehydrogenase	Dehydrogenation of L-aspartate to iminoaspartate	UP
P50431	Shmt1	Serine hydroxymethyltransferase, cytosolic	Interconversion of serine and glycine	DOWN
Q68FT5	Bhmt2	S-methylmethionine-homocysteine S-methyltransferase	Regulation of homocysteine metabolism	UP
P21643	Tdo2	Tryptophan 2,3-dioxygenase	Amino acid (tryptophan) metabolism	UP
P04694	Tat	Tyrosine aminotransferase (TAT)	Conversion of tyrosine to 4-hydroxyphenylpyruvate (liver-specific)	UP
P29266	Hibadh	3-hydroxyisobutyrate dehydrogenase, mitochondrial	Reversible oxidation of 3-hydroxy-isobutanoate to (S)-methylmalonate-semialdehyde	UP
P32755	Hpd	4-hydroxyphenylpyruvate dioxygenase	Involved in tyrosine degradation	UP
A2VCW9	Aass	Alpha-aminoadipic semialdehyde synthase	Catalyzes first two steps in lysine degradation pathway	UP
O09171	Bhmt	Betaine-homocysteine S-methyltransferase 1	Conversion of betaine and homocysteine to dimethylglycine and methionine	UP
P0C2X9	Aldh4a1	Delta-1-pyrroline-5-carboxylate dehydrogenase	Conversion of delta-1-pyrroline-5-carboxylate (P5C), derived either from proline or ornithine, to glutamate	UP
Q60759	Gcdh	Glutaryl-CoA dehydrogenase, mitochondrial	Amino acid (L-lysine, L-hydroxylysine, and L-tryptophan) metabolism	UP
P13255	Gnmt	Glycine N-methyltransferase	Methylation of glycine by using S-adenosylmethionine to form N-methylglycine	UP
P12007	Ivd	Isovaleryl-CoA dehydrogenase, mitochondrial	Third step in leucine catabolism	UP/DOWN
P13444	Mat1a	S-adenosylmethionine synthase isoform type-1	Formation of S-adenosylmethionine from methionine and ATP	UP

UProt_ID	Gene Name	Protein Name	Function	Regulation
P18298	Mat2a	S-adenosylmethionine synthase isoform type-2	Formation of S-adenosylmethionine from methionine and ATP	UP
P53395	Dbt	Dihydrolipoamide branched chain transacylase	Mitochondrial breakdown of the branched-chain amino acids isoleucine, leucine, and valine	UP

Table 2B

MPL-regulated proteins related to carbohydrate metabolism.

UProt_ID	Gene Name	Protein Name	Function	Regulation
P85968	Pgd	6-phosphogluconate dehydrogenase	Pentose phosphate pathway - decarboxylation of 6-phosphogluconate to ribulose 5-phosphate and CO_2	UP
P85971	Pgls	6-phosphogluconolactonase (6PGL)	Pentose phosphate pathway - hydrolysis of 6-phosphogluconolactone to 6-phosphogluconate	UP
P04764	Eno1	Alpha-enolase	Glycolysis - conversion of 2-phosphoglycerate to phosphoenolpyruvate	UP/DOWN
P15429	Eno3	Beta-enolase-3	Glycolysis - conversion of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP)	DOWN/UP
P19112	Fbp1	Fructose-1,6-bisphosphatase 1	Gluconeogenesis - catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate	UP/DOWN
P05065	Aldoa	Fructose-bisphosphate aldolase A	Catalyzes reversible reactions in both glycolysis and gluconeogenesis	UP
P00884	Aldob	Fructose-bisphosphate aldolase B	Glycolytic subpathway - synthesizes D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose	UP
Q6P6V0	Gpi	Glucose-6-phosphate isomerase	Glycolysis - interconverts glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P)	UP
P04797	Gapdh	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Conversion of glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate	UP
P09811	Pygl	Glycogen phosphorylase, liver form	Release glucose-1-phosphate from liver glycogen stores (rate-limiting)	UP
Q02974	Khk	Ketohexokinase (Hepatic fructokinase)	Phosphorylation of fructose to fructose-1-phosphate	UP
P09367	Sds	L-serine dehydratase/L-threonine deaminase	Gluconeogenesis - conversion of L-serine to pyruvate and L-threonine to 2-oxobutanoate	UP
P04636	Mdh2	Malate dehydrogenase, mitochondrial	Gluconeogenesis - reversibly catalyzes reduction of oxaloacetate to malate	UP
P07379	Pck1	Phosphoenolpyruvate carboxykinase, cytosolic	Gluconeogenesis - conversion of oxaloacetate to phosphoenolpyruvate (rate-limiting step)	UP
P38652	Pgm1	Phosphoglucomutase-1	Breakdown and synthesis of glucose	UP/DOWN
P16617	Pgk1	Phosphoglycerate kinase 1	Glycolysis - reversible reaction of 1,3-bisphosphoglycerate to 3-phosphoglycerate	UP
P16290	Pgam2	Phosphoglycerate mutase 2	Glycolysis - reversible reaction of 3-phosphoglycerate to 2-phosphoglycerate	UP
P52873	Pc	Pyruvate carboxylase, mitochondrial	Gluconeogenesis - irreversible carboxylation of pyruvate to form oxaloacetate	UP
P12928	Pklr	Pyruvate kinase	Glycolysis - transfer of phosphate from phosphoenolpyruvate to ADP	UP/DOWN
P27867	Sord	Sorbitol dehydrogenase	Carbohydrate metabolism - converts sorbitol to fructose	UP
P50137	Tkt	Transketolase	Pentose phosphate pathway - transfers glycoaldehyde from ketose-donor to aldose-acceptor sugars	UP
P48500	Tpi1	Triosephosphate isomerase	Reversible interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate	DOWN/UP

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Table 2C

MPL-regulated proteins related to lipid/fatty acid metabolism.

UProt_ID	Gene Name	Protein Name	Function	Regulation
Q8CHM7	Hacl1	2-hydroxyacyl-CoA lyase 1	Fatty acid alpha oxidation - carbon-carbon cleavage reaction	UP
P21775	Acaa1a	3-ketoacyl-CoA thiolase A, peroxisomal	Fatty acid beta oxidation - convert two units of acetyl-CoA to acetoacetyl CoA	UP/DOWN
P07871	Acaa1b	3-ketoacyl-CoA thiolase B, peroxisomal	Fatty acid beta oxidation - catalyzes the final step of beta-oxidation	DOWN
Q5XI22	Acat2	Acetyl-CoA acetyltransferase, cytosolic	Degradative pathways such as fatty acid beta-oxidation	UP
P11497	Acaca	Acetyl-CoA carboxylase 1	Rate-limiting reaction in the biogenesis of long-chain fatty acids	UP
Q9QXG4	Acss2	Acetyl-coenzyme A synthetase, cytoplasmic	Activates acetate for use in lipid synthesis or energy generation	UP
Q14DH7	Acss3	Acyl-CoA synthetase short-chain family member 3, mitochondrial	Activates acetate for use in lipid synthesis or energy generation	UP
P70473	Amacr	Alpha-methylacyl-CoA racemase	Bile acid biosynthesis; racemization of 2-methyl-branched fatty acid CoA esters	UP/DOWN
P23965	Eci1	Enoyl-CoA delta isomerase 1, mitochondrial	Fatty acid metabolism - isomerizes both 3-cis and trans double bonds into enoyl-CoA species	UP
P14604	Echs1	Enoyl-CoA hydratase, mitochondrial	Fatty acid beta oxidation - Hydration of 2-trans-enoyl-coenzyme A (CoA) intermediates	DOWN/UP
P12785	Fasn	Fatty acid synthase	Formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH	UP
P55053	Fabp5	Fatty acid-binding protein, epidermal	High specificity for fatty acids	UP/DOWN
P97612	Faah1	Fatty-acid amide hydrolase 1	Degrades bioactive fatty acid amides like oleamide, and the endogenous cannabinoid, anandamide	UP
O35077	Gpd1	Glycerol-3-phosphate dehydrogenase	Reversible conversion of dihydroxyacetone phosphate to glycerol-3-phosphate	UP
Q9WVK7	Hadh	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Fatty acid beta oxidation - short chain fatty acids	UP
P97519	Hmgcl	HMG-CoA lyase, mitochondrial	Key enzyme in ketogenesis	UP
P17425	Hmgcs1	HMG-CoA synthase, cytoplasmic	Condenses acetyl-CoA with acetoacetyl-CoA to form HMG-CoA	UP/DOWN
P22791	Hmgcs2	HMG-CoA synthase, mitochondrial	Condenses acetyl-CoA with acetoacetyl-CoA to form HMG-CoA	UP
O35760	Idi1	Isopentenyl-diphosphate Delta-isomerase 1	Regulation of farnesyl diphosphate and cholesterol synthesis	DOWN/UP
P15650	Acadl	Long-chain specific acyl-CoA dehydrogenase (LCAD)	Fatty acid beta oxidation - catalyze the initial step of mitochondrial beta-oxidation of straight- chain fatty acid	UP/DOWN
P18163	Acsl1	Long-chain-fatty-acid-CoA ligase 1	Activates long-chain fatty acids for synthesis of cellular lipids and degradation via beta-oxidation	UP
O88813	Acsl5	Long-chain-fatty-acid-CoA ligase 5	Activates long-chain fatty acids for synthesis of cellular lipids, and degradation via beta-oxidation	UP
P08503	Acadm	Medium-chain acyl-CoA dehydrogenase, mitochondrial	Fatty acid metabolism - catalyzes the initial step of fatty acid beta-oxidation	UP
Q9Z2M4	Decr2	Peroxisomal 2,4-dienoyl-CoA reductase	Degradation of unsaturated fatty enoyl-CoA esters in peroxisome	DOWN
P07872	Acox1	Peroxisomal acyl-coenzyme A oxidase 1	Fatty acid beta-oxidation - desaturation of acyl-CoAs to 2-trans-enoyl-CoAs	UP/DOWN

UProt_ID	Gene Name	Protein Name	Function	Regulation
P97562	Acox2	Peroxisomal acyl-coenzyme A oxidase 2	Fatty acid beta-oxidation - oxidizes the CoA esters of bile acid intermediates	UP
Q63448	Acox3	Peroxisomal acyl-coenzyme A oxidase 3	Fatty acid beta-oxidation	UP/DOWN
P07896	Ehhadh	Peroxisomal bifunctional enzyme	Fatty acid beta oxidation - contains enoyl-CoA hydratase as well as 3-hydroxyacyl-CoA dehydrogenase activity	DOWN
P97852	Hsd17b4	Peroxisomal multifunctional enzyme type 2	Fatty acid beta oxidation - formation of 3-ketoacyl-CoA intermediates from straight-chain fatty acids	DOWN
Q9WVK3	Pecr	Peroxisomal trans-2-enoyl-CoA reductase	Chain elongation of fatty acids	UP
P57093	Phyh	Phytanoyl-CoA dioxygenase, peroxisomal	Fatty acid metabolism - converts phytanoyl-CoA to 2-hydroxyphytanoyl-CoA	UP
P14882	Pcca	Propionyl-CoA carboxylase alpha chain, mitochondrial	Fatty acid beta oxidation - catalyses the carboxylation reaction of propionyl CoA to form (S)-methylmalonyl CoA	UP
P70584	Acadsb	Short/branched chain acyl-CoA dehydrogenase, mitochondrial	Fatty acid beta oxidation - greatest activity toward short branched chain acyl-CoA derivatives	UP/DOWN
P15651	Acads	Short-chain specific acyl-CoA dehydrogenase (SCAD)	Fatty acid beta oxidation - catalyze the initial step of mitochondrial beta-oxidation of straight-chain fatty acid	UP/DOWN
Q5PPL3	Nsdhl	Sterol-4-alpha-carboxylate 3-dehydrogenase	Cholesterol biosynthesis	UP
Q64428	Hadha	Trifunctional enzyme subunit alpha, mitochondrial	Catalyzes the final step of beta-oxidation	UP
Q60587	Hadhb	Trifunctional enzyme subunit beta, mitochondrial	Catalyzes the final step of beta-oxidation	UP
P45953	Vlcad	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	Fatty-acid metabolism - esters of very long chain fatty acids such as palmitoyl-CoA	UP

Table 2D

MPL-regulated proteins related to the Krebs (Tricarboxylic acid) cycle.

UProt_ID	Gene Name	Protein Name	Function	Regulation
Q4KLP0	Dhtkd1	2-oxoglutarate dehydrogenase E1	Conversion of 2-oxoglutarate to succinyl-CoA and CO2	UP
Q9ER34	Aco2	Aconitate hydratase, mitochondrial	Isomerization of citrate to isocitrate via cis-aconitate	UP/DOWN
P41562	Idh1	NADP-isocitrate dehydrogenase	Reversible oxidative decarboxylation of isocitrate to yield α-ketoglutarate (α-KG)	UP/DOWN
P14408	Fh	Fumarate hydratase, mitochondrial	Formation of L-malate from fumarate	UP/DOWN
O88989	Mdh1	Malate dehydrogenase	Reversible oxidation of malate to oxaloacetate	UP
P16332	Mut	Methylmalonyl-CoA mutase, mitochondrial	Degradation of amino acids, odd-chain fatty acids and cholesterol via propionyl-CoA	UP
Q920L2	Sdha	Succinate dehydrogenase, mitochondrial	Electron transfer from succinate to ubiquinone (coenzyme Q)	UP/DOWN
Q9Z2I9	Sucla2	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	ATP-dependent ligation of succinate and CoA to form succinyl-CoA	UP/DOWN
Q9Z2I8	Suclg2	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial	GTP-dependent ligation of succinate and CoA to form succinyl-CoA	UP
P00481	Otc	Ornithine carbamoyltransferase, mitochondrial	Catalyzes the second step of the urea cycle	UP/DOWN
P13697	Mod-1	NADP-dependent malic enzyme	Reversible oxidative decarboxylation of malate; links the glycolytic and citric acid cycles.	UP

Table 3

MPL-regulated proteins related to transcription, translation, and (post-translational) protein processing.

UProt_ID	Gene Name	Protein Name	Function	Regulation
			TRANSCRIPTION	
P13383	Ncl	Nucleolin (Protein C23)	Induces chromatin decondensation by binding to histone H1	UP
Q99020	Hnrnpab	Heterogeneous nuclear ribonucleoprotein A/B	Transcriptional repressor	UP
Q794E4	Hnrnpf	Heterogeneous nuclear ribonucleoprotein F	Regulation of alternative splicing events	UP
P61980	Hnrnpk	Heterogeneous nuclear ribonucleoprotein K	pre-mRNA-binding protein; role in p53/TP53 response to DNA damage	UP
Q8VEK3	Hnrnpu	Heterogeneous nuclear ribonucleoprotein U	Circadian regulation of the core clock component ARNTL/BMAL1 transcription	UP
A7VJC2	hnRNPA2/B1	Heterogeneous nuclear ribonucleoproteins A2/B1	pre-mRNA processing and mRNA metabolism and transport	UP
P60843	Eif4a1	Eukaryotic initiation factor 4A-I	RNA helicase - cap recognition and is required for mRNA binding to ribosome	UP
Q5RKI1	Eif4a2	Eukaryotic initiation factor 4A-II (eIF-4A-II)	RNA helicase required for mRNA binding to ribosome	UP
Q6NZJ6	Eif4g1	Eukaryotic translation initiation factor 4 gamma 1	Recognition of the mRNA cap and recruitment of mRNA to the ribosome	UP
P43274	H1f4	Histone H1.4	Condense nucleosome chains into chromatin fibers; regulator of gene transcription by chromatin remodeling	UP/DOWN
Q9D2U9	Hist3h2ba	Histone H2B type 3-A	Component of nucleosome; role in transcription , DNA repair, DNA replication and chromosomal stability	UP
P84245	H3.3	Histone H3.3	Incorporated into chromatin independently of DNA synthesis	UP
P62804	Hist4	Histone H4	Component of nucleosome; role in transcription , DNA repair, DNA replication and chromosomal stability	UP
P62961	Nsep1	Nuclease-sensitive element-binding protein 1	pre-mRNA alternative splicing, binds and stabilizes cytoplasmic mRNA	DOWN/UP
Q00438	Ptbp1	Polypyrimidine tract-binding protein 1	Role in pre-mRNA splicing and in the regulation of alternative splicing events	UP
P48679	Lmna	Prelamin-A/C	Role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics	UP/DOWN
			TRANSLATION	
P02401	Rplp2	60S acidic ribosomal protein P2	Component protein of the 60S ribosomal protein; role in the elongation step of protein synthesis	UP
P04644	Rps17	40S ribosomal protein S17	Component protein of the 40S ribosomal protein	UP
P05426	Rpl7	60S ribosomal protein L7	Component protein of the 60S ribosomal protein; inhibitor of cell-free translation of mRNAs	UP
P12001	Rpl18	60S ribosomal protein L18	Component protein of the 60S ribosomal protein	UP
P17077	Rpl9	60S ribosomal protein L9	Component protein of the 60S ribosomal protein	UP
P86048	Rpl10l	60S ribosomal protein L10	Component of the 60S ribosomal subunit	UP
P19944	Rplp1	60S acidic ribosomal protein P1	Component protein of the 60S ribosomal protein; elongation step of protein synthesis	DOWN/UP
P21531	Rpl3	60S ribosomal protein L3 (L4)	Component protein of the 60S ribosomal protein	UP

UProt_ID	Gene Name	Protein Name	Function	Regulation
P21533	Rpl6	60S ribosomal protein L6	Component protein of the 60S ribosomal protein	UP
P23358	Rpl12	60S ribosomal protein L12	Component protein of the 60S ribosomal protein	UP
P24049	Rpl17	60S ribosomal protein L17	Component protein of the 60S ribosomal protein	UP
P29314	Rps9	40S ribosomal protein S9	Component protein of the 40S ribosomal protein	UP
P47198	Rpl22	60S ribosomal protein L22	Component protein of the 60S ribosomal protein	UP
P62909	Rps3	40S ribosomal protein S3	Component of the 40S ribosomal subunit; role in repair of damaged DNA	UP
P49242	Rps3a	40S ribosomal protein S3a	Component of the 40S ribosomal subunit; erythropoiesis through regulation of transcription factor DDIT3	UP
P50878	Rpl4 Rpl1	60S ribosomal protein L4 (60S ribosomal protein L1)	Component protein of the 60S ribosomal protein	UP
P62083	Rps7	40S ribosomal protein S7	Component of the 40S ribosomal subunit; rRNA maturation	UP
P62250	Rps16	40S ribosomal protein S16	Component of the 40S ribosomal subunit	UP
P62271	Rps18	40S ribosomal protein S18	Component of the 40S ribosomal subunit; contacts several helices of the 18S rRNA	UP/DOWN
P62425	Rpl7a	60S ribosomal protein L7a	Interaction with nuclear hormone receptors, including thyroid hormone receptor	UP
P62703	Rps4x	40S ribosomal protein S4, X isoform	Component of the 40S ribosomal subunit	UP/DOWN
P62752	Rpl23a	60S ribosomal protein L23a	Component protein of the 60S ribosomal protein	UP
P62856	Rps26	40S ribosomal protein S26	Component of the 40S ribosomal subunit	UP
P62902	Rpl31	60S ribosomal protein L31	Component protein of the 60S ribosomal protein	UP
P15178	Dars	Aspartate-tRNA ligase, cytoplasmic	Specific attachment of an amino acid to its cognate tRNA during translation	UP
Q8CGC7	Eprs	Bifunctional glutamate/proline-tRNA ligase	Attachment of the cognate amino acid to the corresponding tRNA	UP
Q5XHY5	Tars	Threonine--tRNA ligase, cytoplasmic	Aminoacyl-tRNA biosynthesis	UP
P62630	Eef1a1	Elongation factor 1-alpha 1	Promotes the GTP-dependent binding of aminoacyl-tRNA to ribosomes during protein biosynthesis	UP
P62632	Eef1a2	Elongation factor 1-alpha 2	Promotes the GTP-dependent binding of aminoacyl-tRNA to ribosomes during protein biosynthesis	UP
O70251	Eef1b	Elongation factor 1-beta (EF-1-beta)	Stimulate the exchange of GDP bound to EF-1-alpha to GTP	UP
Q68FR9	Eef1d	Elongation factor 1-delta	Enzymatic delivery of aminoacyl tRNAs to the ribosome	UP
Q68FR6	Eef1g	Elongation factor 1-gamma	Enzymatic delivery of aminoacyl tRNAs to the ribosome	UP
P05197	Eef2	Elongation factor 2	GTP-dependent ribosomal translocation step during translation elongation	UP
P52759	Psp1	Ribonuclease UK114	Inhibition of translation by cleaving mRNA	UP
		POST-TRANSLATIONAL PROCESSING		
P07153	Rpn1	Dolichyl-diphosphooligosaccharide glycosyltransferase 1	Protein glycosylation	UP
P25235	Rpn2	Dolichyl-diphosphooligosaccharide glycosyltransferase 2	Protein glycosylation	UP

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UProt_ID	Gene Name	Protein Name	Function	Regulation
Q641Y0	Ddost	Dolichyl-diphosphooligosaccharide glycosyltransferase 48	Protein glycosylation	UP
Q66HD0	Hsp90b1	Heat shock protein 90 kDa beta member 1 (Endoplasmin)	Molecular chaperone - processing and transport of secreted proteins	UP
Q9D0S9	Hint2	Histidine triad nucleotide-binding protein 2, mitochondrial	Control of oxidative protein folding in the endoplasmic reticulum	UP/DOWN
P28480	Tcp1	T-complex protein 1 subunit alpha	Molecular chaperone - folding of proteins upon ATP hydrolysis	UP
Q5XIM9	Cctb	T-complex protein 1 subunit beta (TCP-1-beta)	Molecular chaperone - assists the folding of proteins upon ATP hydrolysis	UP
Q68FQ0	Cct5	T-complex protein 1 subunit epsilon (TCP-1-epsilon)	Molecular chaperone - folding of proteins upon ATP hydrolysis	UP
Q6P502	Cct3	T-complex protein 1 subunit gamma	Molecular chaperone - folding of proteins upon ATP hydrolysis	UP
Q9D1Q6	Txndc4	Thioredoxin domain-containing protein 4	Post-translational modifications of proteins	UP
Q9JLA3	Uggt1	UDP-glucose:glycoprotein glucosyltransferase 1	Quality control for protein folding in the endoplasmic reticulum	UP
P04785	P4hb	Protein disulfide-isomerase	Formation, breakage and rearrangement of disulfide bonds	UP
P11598	Pdia3	Protein disulfide-isomerase A3	Rearrangement of disulfide (-S-S-) bonds in proteins	UP
P38659	Pdia4	Protein disulfide-isomerase A4	Rearrangement of disulfide (-S-S-) bonds in proteins	UP
Q63081	Pdia6	Protein disulfide-isomerase A6	Rearrangement of disulfide (-S-S-) bonds in proteins; may also inhibit aggregation of misfolded proteins	UP
P17879	Hspa1b	Heat shock 70 kDa protein 1B	Folding of newly translated polypeptides	UP
P06761	Hspa5	Heat shock 70 kDa protein 5	Assembly of multimeric protein complexes inside the endoplasmic reticulum	UP
P26772	Hspe1	10 kDa heat shock protein, mitochondrial	Protein folding; also a molecular chaperone	DOWN/UP
P10111	Ppia	Peptidyl-prolyl cis-trans isomerase A	Protein folding - cis-trans isomerization of proline imidic peptide bonds in oligopeptides	DOWN/UP
P24368	Ppib	Peptidyl-prolyl cis-trans isomerase B (PPIase B)	Protein folding - cis-trans isomerization of proline imidic peptide bonds in oligopeptides	UP
P10111	Ppia	Peptidyl-prolyl cis-trans isomerase A	Protein folding - cis-trans isomerization of proline imidic peptide bonds in oligopeptides	DOWN/UP
P24368	Ppib	Peptidyl-prolyl cis-trans isomerase B (PPIase B)	Protein folding - cis-trans isomerization of proline imidic peptide bonds in oligopeptides	UP
Q923V8	15-Sep	15-kDa selenoprotein	Protein folding - redox reactions associated with the formation of disulfide bonds	UP

Table 4A

MPL-regulated cytochrome P450 enzymes related to drug/xenobiotic/endogenous substrate metabolism.

UProt_ID	Gene Name	Protein Name	Function	Regulation
P04799	Cyp1a2	Cytochrome P450 1A2	Endogenous substrates = steroids, arachidonic acid; Drugs = caffeine, clozapine; benzo-a-pyrene (procarcinogen)	UP
P17178	Cyp27a1	Cytochrome P450 27A1	Endogenous substrates = sterol intermediates; Vitamin D_3 (25-hydroxylase activity)	UP
P11711	Cyp2a1	Cytochrome P450 2A1	Endogenous substrates = testosterone, progesterone and androstenedione (steroid hydroxylase)	UP/DOWN
P15149	Cyp2a2	Cytochrome P450 2A2	Endogenous substrate = testosterone (adult male-specific steroid hydroxylase)	UP/DOWN
P04167	Cyp2b2	Cytochrome P450 2B2	Transcriptionally induced by phenobarbital (CAR-C/EBP dependent); substrate = Polychlorinated biphenyls	UP
P13107	Cyp2b3	Cytochrome P450 2B3	Transcriptionally induced by phenobarbital (CAR-C/EBP dependent); substrate = Polychlorinated biphenyls	UP
P08683	Cyp2c11	Cytochrome P450 2C11	Endogenous substrate = testosterone; Drugs = bufuralol, sildenafil; Vitamin D_3 / D_2 (25- and 24-hydroxylase activity)	UP
P20814	Cyp2c13	Cytochrome P450 2C13	Endogenous substrate = testosterone (male-specific 6-beta-hydroxylase activity) and arachidonic acid (low activity)	UP
P24470	Cyp2c23	Cytochrome P450 2C23	Endogenous substrate = arachidonic acid	DOWN
Q64458	Cyp2c29	Cytochrome P450 2C29	Endogenous substrate = arachidonic acid; Drug = tolbutamide	UP
Q91X77	Cyp2c50	Cytochrome P450 2C50	Endogenous substrates = arachidonic acid and linoleic acid	DOWN
P05179	Cyp2c7	Cytochrome P450 2C7	Endogenous substrates = testosterone and retinoic acid	UP
P19225	Cyp2c70	Cytochrome P450 2C70	Endogenous substrate = testosterone	UP/DOWN
P12939	Cyp2d10	Cytochrome P450 2D10	*No literature information found regarding substrates*	UP
P10634	Cyp2d26	Cytochrome P450 2D26	*No literature information found regarding substrates*	UP/DOWN
P05182	Cyp2e1	Cytochrome P450 2E1	Procarcinogens = acrylamide, alcohol, benzene, 4-nitrophenol; Drugs = acetaminophen, isoniazid	UP
P05183	Cyp3a2	Cytochrome P450 3A2	Cyp3a4 homolog in rat; numerous drugs, xenobiotics, and endogenous substrates	UP
P20817	Cyp4a14	Cytochrome P450 4A14	Endogenous substrate = arachidonic acid (omega-hydroxylase)	UP/DOWN
P51869	Cyp4f4	Cytochrome P450 4F4	Endogenous substrates = prostaglandin A1, prostaglandin E1, and leukotrienes (omega-hydroxylase)	UP
Q64654	Cyp51a1	Cytochrome P450 51A1	Catalyzes a step in zymosterol biosynthesis from lanosterol	DOWN

Table 4B

MPL-regulated Phase-II enzymes related to drug/xenobiotic/endogenous substrate metabolism.

UProt_ID	Gene Name	Protein Name	Function	Regulation
Q64550	Ugt1a1	UDP-glucuronosyltransferase 1A1	Drugs = opioids, SN-38 (irinotecan); endogenous substrates = bilirubin, ethinylestradiol; polymorphic enzyme	DOWN/UP
Q62452	Ugt1a9	UDP-glucuronosyltransferase 1A9	Drugs = R-oxepam, mycophenolic acid, SN-38 (irinotecan); halogenated phenols; polymorphic enzyme	DOWN
P09875	Ugt2b1	UDP-glucuronosyltransferase 2B1	Drug = diclofenac ; bisphenol A (environmental chemical)	UP
P36511	Ugt2b15	UDP-glucuronosyltransferase 2B15	Drug = S-oxazepam, paracetamol, (+)-menthol, and eugenol; polymorphic enzyme	UP
P08542	Ugt2b17	UDP-glucuronosyltransferase 2B17	Drug = MK-7246; endogenous substrates = steroid hormones; polymorphic enzyme	UP
P08541	Ugt2b2	UDP-glucuronosyltransferase 2B2	Endogenous substrates = triiodothyronine, androsterone	UP
P17988	Sult1a1	Sulfotransferase 1A1	Sulfate conjugation of catecholamines, phenolic drugs and neurotransmitters	UP
P50237	Sult1c1	Sulfotransferase 1C1	Sulfonation of p-nitrophenol and N-hydroxy-2-acetylaminofluorene	UP/DOWN
P22789	St2a2	Sulfotransferase 2A2	Sulfonation of hydroxysteroids and xenobiotics	UP
P52847	Sult1b1	Sulfotransferase 1B1	Major SULT in liver; sulfonation of 4-methyllumbellferone, p-nitrophenol, and 1-naphthol	UP
P08011	Gst1	Glutathione S-transferase 1 (microsomal)	Drugs = chlorambucil, BCNU	UP
Q5PQT3	Glyat	Glycine N-acyltransferase	Detoxification of salicylic acid and benzoic acid by glycine conjugation	UP
P22734	Comt	Catechol O-methyltransferase	Inactivation of catecholamine neurotransmitters and hormones	UP
P49889	Ste	Estrogen sulfotransferase, isoform 3	Sulfate conjugation of estradiol and estrone	UP/DOWN

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Table 4C

Other MPL-regulated enzymes related to drug/xenobiotic/endogenous substrate metabolism.

UProt_ID	Gene Name	Protein Name	Function	Regulation
Q64563	Adh4	Alcohol dehydrogenase 4	Reduction of benzoquinones	UP
P12711	Adh5	Alcohol dehydrogenase class-3	Ineffective in oxidizing ethanol, but it readily catalyzes the oxidation of long-chain primary alcohols	UP/DOWN
P06757	Adh1	Alcohol dehydrogenase 1	Conversion of primary unbranched alcohols to their corresponding aldehydes	UP
P30839	Aldh3a2	Aldehyde dehydrogenase 4, microsomal	Oxidation of long-chain aliphatic aldehydes to fatty acids	UP/DOWN
Q8BH00	Aldh8a1	Aldehyde dehydrogenase family 8 member A1	Converts 9-cis-retinal to 9-cis-retinoic acid	UP
Q66HF8	Aldh1b1	Aldehyde dehydrogenase 1B1, mitochondrial	Ethanol degradation - transformation from acetaldehyde to acetic acid	UP
Q8K009	Aldh1l2	Aldehyde dehydrogenase 1L2, mitochondrial	Formate oxidation (homolog of 10-formyltetrahydrofolate dehydrogenase)	UP
Q9JLJ3	Aldh9a1	Aldehyde dehydrogenase 9A1	Carnitine biosynthesis	UP
P13601	Aldh1a7	Aldehyde dehydrogenase 1A7, cytosolic	Oxidizes benzaldehyde, propionaldehyde and acetaldehyde	UP
P11884	Aldh2	Aldehyde dehydrogenase, mitochondrial	Ethanol degradation - catalyzes the transformation from acetaldehyde to acetic acid	UP
Q9Z0U5	Aox1	Aldehyde oxidase 1	Metabolizes aldehyde compounds and drugs containing aromatic azaheterocyclic substituents	UP
P31210	Akr1d1	Aldo-keto reductase 1D1	Reduction of progesterone, androstenedione, and testosterone to 5-beta-reduced metabolites	UP
Q63276	Baat	Bile acid-CoA:amino acid N-acyltransferase	Glycine and/or taurine conjugation for biliary excretion	UP/DOWN
Q9ES38	Slc27a5	Bile acyl-CoA synthetase	Glycine and/or taurine conjugation for biliary excretion	UP/DOWN
P16303	Ces1d	Carboxylesterase 1D	Metabolism of xenobiotics and of natural substrates; hydrolyzes triacylglycerols and monoacylglycerols	UP
P28037	Aldh1l1	Cytosolic 10-formyltetrahydrofolate dehydrogenase	Folate metabolism - interconversion of tetrahydrofolate molecules	UP
P07687	Ephx1	Epoxide hydrolase 1, microsomal	Hydrolysis of arene and aliphatic epoxides to less reactive and more water soluble dihydrodiols	UP
Q923D2	Blvrb	Flavin reductase	NADPH-dependent reduction of flavins, such as riboflavin, FAD or FMN, and biliverdins	UP
P00388	Por	NADPH-cytochrome P450 reductase	Required for electron transfer from NADP to cytochrome P450 in microsomes	UP
P97524	Slc27a2	Solute carrier family 27 member 2 (THCA-CoA ligase)	Bile acid metabolism	UP
P23457	Akr1c9	Dihydrodiol dehydrogenase	Inactivates several endogenous steroid hormones	UP
Q9EQ76	Fmo3	Dimethylaniline monooxygenase	Oxidative metabolism of a variety of xenobiotics such as drugs and pesticides	UP
P36365	Fmo1	Hepatic flavin-containing monooxygenase 1	Oxidative metabolism of a variety of xenobiotics such as drugs and pesticides	UP
P20070	Cyb5r3	NADH-cytochrome b5 reductase 3	Desaturation and elongation of fatty acids, cholesterol biosynthesis, and drug metabolism	UP
P55159	Pon1	Serum paraoxonase/arylesterase 1	Hydrolyzes the toxic metabolites of a variety of organophosphorus insecticides	UP
Q6AXM8	Pon2	Serum paraoxonase/arylesterase 2 (PON 2)	Hydrolysis of lactones and aromatic carboxylic acid esters	UP
Q68FP2	Pon3	Serum paraoxonase/lactonase 3	Hydrolysis of lactones such as statin prodrugs	UP

UProt_ID	Gene Name	Protein Name	Function	Regulation
P17764	Acat1	Acetyl-CoA acetyltransferase, mitochondrial	Ketone body metabolism	UP

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Table 5

MPL-regulated proteins involved in active/facilitated transport of endogenous and drug compounds.

UProt_ID	Gene Name	Protein Name	Function	Regulation
P63039	Hsp60	60 kDa heat shock protein, mitochondrial	Mitochondrial protein import and macromolecular assembly	UP
P11030	Dbi	Acyl-CoA-binding protein	Intracellular carrier of acyl-CoA esters	UP
P61751	Arf4	ADP-ribosylation factor 4	Protein trafficking; may modulate vesicle budding and uncoating within the Golgi apparatus	UP
P62332	Arf6	ADP-ribosylation factor 6	Endocytic recycling and cytoskeleton remodeling	UP
P41034	Ttpa	Alpha-tocopherol transfer protein	Binds alpha-tocopherol, enhances its transfer between separate membranes, and stimulates release from liver	UP
P02650	Apoe	Apolipoprotein E (Apo-E)	Binding, internalization, and catabolism of lipoprotein particles; ligand for hepatic LDL-receptor	UP/DOWN
P15999	Atp5a1	ATP synthase subunit alpha, mitochondrial	Mitochondrial membrane ATP synthase	UP
P10719	Atp5b	ATP synthase subunit beta, mitochondrial	Mitochondrial membrane ATP synthase	UP
P31399	Atp5h	ATP synthase subunit d, mitochondrial (ATPase subunit d)	Mitochondrial membrane ATP synthase	UP
P29419	Atp5i	ATP synthase subunit e, mitochondrial	Mitochondrial membrane ATP synthase	UP/DOWN
P35435	Atp5c1	ATP synthase subunit gamma, mitochondrial	Mitochondrial membrane ATP synthase	UP
Q06647	Atp5o	ATP synthase subunit O, mitochondrial	Mitochondrial membrane ATP synthase	UP/DOWN
Q8K442	Abca8a	ATP-binding cassette sub-family A member 8-A	ATP-dependent lipophilic drug transporter (e.g. substrate = digoxin)	UP
O70127	Bsep	Bile salt export pump	ATP-dependent secretion of bile salts into the canaliculus of hepatocytes	UP
P11915	Scp2	Non-specific lipid-transfer protein	Transfer of common phospholipids, cholesterol and gangliosides between membranes	UP
P61107	Rab14	Ras-related protein Rab-14	Endocytic transport of ADAM10, N-cadherin/CDH2 shedding and cell-cell adhesion	UP
P10536	Rab1b	Ras-related protein Rab-1B	Regulates vesicular transport between the endoplasmic reticulum and successive Golgi compartments	UP
P09527	Rab7a	Ras-related protein Rab-7a	Endo-lysosomal maturation and trafficking; also involved in growth-factor cell signaling	UP
Q9Z1J8	Sec14l3	SEC14-like protein 3	Transport of hydrophobic ligands like tocopherol, squalene and phospholipids	UP
Q9QXX4	Slc25a13	Solute carrier family 25 member 13	Calcium-dependent exchange of cytoplasmic glutamate with mitochondrial aspartate	UP
Q09073	Slc25a5	Solute carrier family 25 member 5	Exchange of cytoplasmic ADP with mitochondrial ATP across the mitochondrial inner membrane	DOWN/UP
O35913	Oatp1a4	Solute carrier organic anion transporter family member 1A4	Sodium-independent transport of organic anions	DOWN
Q9JMD3	Stard10	StAR-related lipid transfer protein 10	Phospholipid transfer protein	UP
Q9Z2L0	Vdac1	Voltage-dependent anion-selective channel protein 1	Facilitated diffusion of small hydrophilic molecules across mitochondrial outer membrane	UP
P81155	Vdac2	Voltage-dependent anion-selective channel protein 2	Facilitated diffusion of small hydrophilic molecules across mitochondrial outer membrane	DOWN

Table 6

Immune-related proteins regulated by MPL.

UProt_ID	Gene Name	Protein Name	Function	Regulation
P17475	Serpina1	Alpha-1-antiproteinase (Serpin A1)	Inhibitor of serine proteases	UP
Q63041	A1m	Alpha-1-macroglobulin	Inhibitor of proteinases	UP
P14480	Fgb	Fibrinogen beta chain	Fibrin precursor; antibacterial immune response via both innate and T-cell mediated pathways	UP
Q03626	Mug1	Murinoglobulin-1	Proteinase inhibitor involved in acute phase inflammatory response	UP/DOWN
Q6IE52	Mug2	Murinoglobulin-2	Inhibitor of proteinases	DOWN
P97584	Ptgr1	Prostaglandin reductase 1 (PRG-1)	Conversion of leukotriene B4 into its biologically less active metabolite, 12-oxo-leukotriene B4	UP/DOWN
Q63797	Psme1	Proteasome activator 28 subunit alpha	Immunoproteasome subunit that processes class I MHC peptides	UP
P09006	Serpina3N	Serine protease inhibitor A3N	Irreversible, suicide inhibition of protease	UP
P05544	Serpina3l	Serine protease inhibitor A3L	Irreversible, suicide inhibition of protease	UP
P52631	Stat3	Signal transducer and activator of transcription-3	Transcription-factor involved in gene regulation of multiple biological processes	UP
P01026	C3	Complement C3	Acute phase protein - central role in the activation of the complement system	UP
P06866	HpBa1	Haptoglobin	Hepatic recycling of heme iron; also involved in acute phase response	UP

Table 7

Other MPL-regulated proteins.

UProt_ID	Gene Name	Protein Name	Function	Regulation
P46953	Haao	3-hydroxyanthranilate oxygenase	Biosynthesis of NAD^+	UP/DOWN
P63259	Actg	Actin, cytoplasmic	Cell motility	UP
P46664	Adss2	Adenylosuccinate synthetase isozyme 2	De novo and salvage pathways of purine nucleotide biosynthesis	UP
Q0D2L3	Agmat	Agmatinase, mitochondrial	Putrescine biosynthesis via agmatine pathway	UP
Q8VHE9	Retsat	All-trans-retinol 13,14-reductase	Retinol metabolism	UP
Q6DGG1	Abhd14b	Alpha/beta hydrolase domain-containing protein 14B	Hydrolase activity towards p-nitrophenyl butyrate	UP
P24090	Ahsg	Alpha-2-HS-glycoprotein	Serum glycoprotein synthesized by hepatocytes; exact function unclear	UP
Q9JI91	Actn2	Alpha-actinin-2	Anchor actin to intracellular structures	UP
Q9QXQ0	Actn4	Alpha-actinin-4	Anchors actin to a variety of intracellular structures	UP
P97384	Anx11	Annexin A11	Calcium-dependent phospholipid-binding protein	UP
P07824	Arg1	Arginase-1	Subpathway of the urea cycle that synthesizes L-ornithine and urea from L-arginine	UP
P16638	Acly	ATP-citrate synthase	Synthesis of cytosolic acetyl-CoA	UP
Q62095	Ddx3y	ATP-dependent RNA helicase DDX3Y	Important for spermatogenesis; role in liver unclear	UP
Q8BFZ3	Actbl2	Beta-actin-like protein 2	Cell motility	UP
O35826	Gne	Bifunctional UDP-N-acetylglucosamine 2-epimerase	Biosynthesis of N-acetylneuraminic acid (NeuAc), a precursor of sialic acids	UP
P27653	Mthfd1	C-1-tetrahydrofolate synthase, cytoplasmic	Folate metabolism - interconversion of tetrahydrofolate molecules	UP
P26231	Ctnna1	Catenin alpha-1	Linkage of cadherins to the actin cytoskeleton	UP
P11442	Cltc	Clathrin heavy chain 1	Major protein of the polyhedral coat of coated pits and vesicles	UP
Q66H80	Copd	Coatomer subunit delta (Archain)	Required for vesicle budding from Golgi membrane	UP
O89046	Coro1b	Coronin-1B (Coronin-2)	Regulates cell motility	DOWN/UP
P32232	Cbs	Cystathionine beta-synthase	Transsulfuration pathway - mediates elimination of L-methionine and the toxic metabolite L-homocysteine	UP
P18757	Cth	Cystathionine gamma-lyase	Last step in the trans-sulfuration pathway from methionine to cysteine	DOWN
P00173	Cyb5a	Cytochrome b5	Hemoprotein which functions as an electron carrier for several membrane bound oxygenases	DOWN
P11240	Cox5a	Cytochrome c oxidase subunit 5A, mitochondrial	Terminal oxidase in mitochondrial electron transport	UP
Q9D0M3	Cyc1	Cytochrome c1, heme protein, mitochondrial	Steroid biosynthesis	UP
Q8VID1	Dhrs4	Dehydrogenase/reductase SDR family member 4	Retinal metabolism	UP
P06214	Alad	Delta-aminolevulinic acid dehydratase	Catalyzes an early step in the biosynthesis of tetrapyrroles	UP/DOWN

UProt_ID	Gene Name	Protein Name	Function	Regulation
P11348	Qdpr	Dihydropteridine reductase	Production of tetrahydrobiopterin, an essential cofactor for phenylalanine, tyrosine, and tryptophan hydroxylases	UP/DOWN
P13803	Etfa	Electron transfer flavoprotein subunit alpha, mitochondrial	Electron acceptor for several dehydrogenase enzymes	UP/DOWN
Q6UPE1	Etfdh	Electron transfer flavoprotein-ubiquinone oxidoreductase, mito	Electron transport - reduces ubiquinone	DOWN/UP
P05369	Fdps	Farnesyl pyrophosphate synthase	Isoprenoid biosynthesis - formation of farnesyl diphosphate	UP
O88618	Ftcd	Formimidoyltransferase-cyclodeaminase	Channels 1-carbon units from formiminoglutamate to the folate pool	UP
Q9QZU7	Bbox1	Gamma-butyrobetaine dioxygenase	Carnitine biosynthesis - formation of L-carnitine from gamma-butyrobetaine	UP
O08795	Prkcsh	Glucosidase II subunit beta	N-linked glycan-processing enzyme in the endoplasmic reticulum	UP
Q91Z53	Grhpr	Glyoxylate reductase/hydroxypyruvate reductase	Dicarboxylic acid metabolism	UP/DOWN
Q9WU19	Hao1	Hydroxyacid oxidase 1	Glycolate degradation	UP
O35952	Hagh	Hydroxyacylglutathione hydrolase, mitochondrial	Hydrolysis of S-D-lactoyl-glutathione to form glutathione	UP
Q5BJY9	Krt18	Keratin, type I cytoskeletal 18	Role in filament reorganization	UP
Q10758	Krt8	Keratin, type II cytoskeletal 8	Together with KRT19, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle	UP
O88867	Kmo	Kynurenine 3-monooxygenase	Synthesis of kynurenic acid and quinolinic acid - neurotoxic NMDA receptor antagonists	UP
P04642	Ldha	L-lactate dehydrogenase A chain	Inter-conversion of pyruvate and L-lactate	UP
Q5M7W5	Map4	Microtubule-associated protein 4	Promotes microtubule assembly	UP
Q791V5	Mtch2	Mitochondrial carrier homolog 2	Mitochondrial depolarization	UP
Q9QZ76	Mb	Myoglobin	Carrier of reserve supply of oxygen	UP
A2AQP0	Myh7b	Myosin heavy chain 7B	Muscle contraction - ‘slow’ ATPase	UP
P02600	Myl1	Myosin light chain 1/3, skeletal muscle isoform	Regulatory light chain of myosin; non-calcium binding	UP
P16409	Myl3	Myosin light chain 3	Regulatory light chain of myosin; non calcium binding	UP/DOWN
Q64122	Myl9	Myosin regulatory light polypeptide 9	Regulation of both smooth muscle and nonmuscle cell contractile activity	UP
Q62812	Myh9	Myosin-9	Role in cytoskeleton reorganization, focal contacts formation	UP
Q5BK63	Ndufa9	NADH dehydrogenase 1 alpha subcomplex 9, mitochondrial	Subunit of the mitochondrial membrane respiratory chain	UP
Q9DCT2	Ndufs3	NADH dehydrogenase iron-sulfur protein 3, mitochondrial	Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase	UP
Q62736	Cald1	Non-muscle caldesmon	Regulation of actomyosin	UP
Q68G31	Pbld	Phenazine biosynthesis-like domain-containing protein	*Function unclear*	UP
Q63598	Pls3	Plastin-3 (T-plastin)	Actin-bundling protein	UP
P07633	Pccb	Propionyl-CoA carboxylase, mitochondrial	Synthesizes succinyl-CoA from propanoyl-CoA	UP
Q9Z2Q1	Sec31a	Protein transport protein Sec31A	Formation of transport vesicles from the endoplasmic reticulum	UP/DOWN
P26043	Rdx	Radixin (ESP10)	Binding of the barbed end of actin filaments to the plasma membrane	UP

UProt_ID	Gene Name	Protein Name	Function	Regulation
Q99PL5	Rrbp1	Ribosome-binding protein 1	Electron chain transport - accepts electrons from Rieske protein and transfers to cytochrome c	UP
Q64380	Sardh	Sarcosine dehydrogenase, mitochondrial	Amine (sarcosine) degradation	UP/DOWN
Q9EP89	Lactb	Serine beta-lactamase-like protein LACTB, mitochondrial	Function unclear in liver	UP/DOWN
Q76MZ3	Ppp2r1a	Serine/threonine-protein phosphatase 2A	Assembly of the catalytic subunit of serine/threonine-protein phosphatase	UP
Q62261	Sptbn1	Spectrin beta chain, non-erythrocytic 1	Actin crosslinking and molecular scaffold protein	UP
P26039	Tln1	Talin-1	Anchors major cytoskeletal structures to the plasma membrane	UP
A2ASS6	Ttn	Titin (Connectin)	Assembly of contractile machinery in muscle cells	DOWN
P04692	Tpm1	Tropomyosin alpha-1 chain	Stabilizes cytoskeleton actin filaments in non-muscle cells	UP/DOWN
P09495	Tpm4	Tropomyosin alpha-4 chain	Stabilizes cytoskeleton actin filaments in non-muscle cells	UP/DOWN
P58775	Tpm2	Tropomyosin beta chain (Tropomyosin-2)	Stabilizes cytoskeleton actin filaments in non-muscle cells	UP
Q6AYZ1	Tuba1c	Tubulin alpha-1C chain	Major constituent of microtubules	UP
Q4QRB4	Tubb3	Tubulin beta-3 chain (Neuron-specific class III beta-tubulin)	Major constituent of microtubules	UP
Q6P9T8	Tubb4b	Tubulin beta-4B chain	Major constituent of microtubules	UP
Q6PEC1	Tbca	Tubulin-specific chaperone A	Tubulin folding pathway	UP
Q91ZJ5	Ugp2	UTP-glucose-1-phosphate uridylyltransferase	Glucosyl donor in cellular metabolic pathways	UP/DOWN
P31000	Vim	Vimentin	Anchors the position of the organelles in the cytosol	UP/DOWN