

Mass Spectrometry Analysis Reveals Dynamic Changes of Protein Components in Lipid Rafts from Rat Liver after Partial Hepatectomy

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1. Abstract

Lipid raft, as scaffolding platform for signal transduction, plays important role in the liver regeneration. But the lipid raft protein expression pattern during liver regeneration has been not reported. In this study, lipid raft proteins from the liver of 72 h post partial hepatectomy and sham-operated group were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with label-free semi-quantitative analysis. Totally 458 lipid raft proteins were identified, and most of identified lipid raft proteins were mainly involved in transporter, signal transduction, and metabolism. Moreover, label-free quantification analysis suggested that the level of 46 plasma membrane-related proteins have changed obviously (with ratio \geq 2) after 72 h hepatectomy. Several differently expressed proteins, including caveolin-1 and flotillin-1, were validated by western blotting. Further immuno fluorescence and quantitative real-time polymerase chain reaction data indicated the enhanced signal of flotillin-1 and caveolin-1 around the newly formed blood vessel. Here, the expression pattern of rat lipid raft protein after partial hepatectomy 72 h was investigated, and the differential expressed protein induced by partial hepatectomy was also indicated. Our results suggested that the increased expression of flotillin-1 and caveolin-1 could be involved in the liver regeneration.

2. Keywords: Liver regeneration; Lipid raft; Mass spectrometry; Proteomics; Flotillin-1

3. Introduction

Liver is unique in its ability to regenerate even in the mature stage [1, 2]. Liver regeneration is of great clinical significance in various liver-associated diseases [1]. It has been reported that liver regeneration undergoes three stages, including priming stage, proliferation stage and termination stage [3]. Angiogenesis is a key process in the third phase of liver regeneration. The key factor, Vascular Endothelial Growth Factor (VEGF), reached the peak of expression in 72 hours after Partial Hepatectomy (PH), which implicated that 72 h is critical node of angiogenesis during liver regeneration [4]. Besides transforming growth factor beta1 (TGF β 1), as one of the regeneration terminators, remains elevated till 72 h to maintain appropriate size of liver [5]. Thus we focused on the time point of 72 h post Partial Hepatectomy (PH).

Lipid raft as thickened sections of the cell membrane plays an important role in cell signaling and cell-cell interaction [6, 7]. Caveolae, as a specified structure in the lipid raft, perform transport and signaling functions influencing cell growth, angiogenesis and transvascular exchange [8, 9].

Caveolin-rich lipid rafts are involved in cholesterol trafficking, molecular transport and cell signaling [10-12]. It has been identified in the membranes of all liver cells, including parenchymal and non-parenchymal cells. More importantly, lipid rafts are active participants in multiple physiological and pathological conditions in individual types of liver cells [13]. Currently at least 300 proteins of lipid rafts in normal liver from human or rodents have been identified [14-17]. However, the protein functions of lipid raft in liver regeneration are not so clear. It is interesting to reveal the proteomic profile of lipid raft during liver regeneration after PH.

In this study, gel LC-mass spectrometry (MS) /MS-based proteomic techniques have been applied to explore the protein composition of lipid rafts in rat liver. Furthermore, semi-quantitative analysis indicated the difference of proteins between the PH 72 h group and the sham-operated group (PH 0 h group). Among them, five proteins were verified by western blot and the distribution of flotillin-1 and caveolin-1 were proved by immunofluorescence.

4. Materials and Methods

4.1. Ethics Statement

This study was carried out in strict accordance with the guidelines established by the Committee on the Use and Care of Animals at the Hunan Province, P. R. China. The protocol was approved by the Ethics Committee of the University of Hunan normal university (Permit number: 010008). All efforts were made to minimize suffering. All the experiments were approved by and were conducted in the Committee.

4.2. Materials

Electrophoresis grade chemicals and Hyper film ECL reagent were obtained from GE Healthcare (Uppsala, Sweden). Colloidal Coomassie Brilliant Blue G-250, dithiothreitol and iodoacetamide were from Sigma Aldrich (St Louis, MO, USA). RC-DC™ kit was from Bio-Rad Laboratories (Hercules, CA, USA). Complete protease inhibitors cocktail was from Roche (Mannheim, Germany). Horseradish peroxidase conjugated anti-mouse IgG were obtained from BD Bioscience (San Jose, CA, USA). Anti-annexin A2, anti-Na⁺/K⁺ ATPase monoclonal antibody, anti-CD73 monoclonal antibody, anti-flotillin 1, anti-caveolin-1 were from Abcam (Cambridge, UK). Proteomics sequencing grade trypsin, and sucrose were obtained from Promega (Madison, WI, USA). Male Sprague-Dawley rats (averaging 200-250 g and 6-8 weeks old) were purchased from the Experimental Animal House at Centre South University (Changsha, China).

4.3. 2/3 Partial Hepatectomy (2/3 PH) Model

2/3 PH model was performed mainly as described by Higgins and

Anderson [2]. Briefly, rats were allowed provided with rat chow and water ad libitum and maintained under a 12/12 hours light/dark cycle before surgery. Ten rats were divided into PH 72 h group and a sham-operated group randomly so that each group included five rats. All the surgeries were performed between 8 am to 11 am. The median and left lateral lobes of livers from the PH group were ligated and resected. The sham-operated group was also subjected to midline incision with liver manipulation but without removal. All rats were maintained in a temperature-controlled room during the surgeries.

4.4. Isolation of Detergent-Resistant Lipid Raft Fraction from Rat Liver

Rat livers were dissected out from PH 72 h group and control group. After removed the gall bladder and blood vessels, the liver pieces (3 g) were homogenized in eight times their weight (ml/g) of cold solution A containing 50 mM HEPES, 1 mM CaCl₂ and protease inhibitors with a tissue-tearor (Biospec products, CE 2000, Mexico) until completely liquefied. After homogenization, samples were centrifuged at 600 × g for 10 min at 4 °C firstly and supernatant were centrifuged at 100,000 × g for 30 min at 4 °C. Then sediments from sham-operated and 72 h group were resuspended in 1 mL buffered saline (25 mM MES, 150 mM NaCl, pH 6.5, 1% Triton X-100) and mixed with equal volume of buffered 80% (w/v) sucrose. The membranes were transferred to a SW41 centrifuge tube and overlaid with 7.5 ml of buffered 30% (w/v) sucrose and about 2.5 ml of buffered 5% (w/v) sucrose. The discontinuous gradient was centrifuged at 200,000 × g for 20 h in a SW41 rotor at 4°C. Twelve fractions were taken, starting from the bottom of the tube and the pellet at the bottom of the tube ("P" as the 13th fraction). All fractions were washed with buffered saline and centrifuged at 100,000 × g for 90 min at 4 °C, with the pellet being resuspended in buffered saline [18].

4.5. Western Blotting

Protein fractions from rat liver were dissolved with 2 × SDS sample loading buffer and total 50 µg separated on 10% separation gel and 5% stacking gel in each lane. Proteins were then transferred to nitrocellulose membrane and incubated with antibody at concentrations recommended by manufacturers. Images were taken by and Molecular Imager Gel Doc™ XRS⁺ System and analyzed using Quantity One 1D-Analysis software (Bio-Rad, Hercules, USA).

4.6. In-Gel Digestion and Protein Identification

The entire lane was cut into 16 strips according to the visible bands. Each fragment was cut into 1-2 mm³ gel pieces. In-gel digestion was performed exactly as described before [19]. The trypsinized mixtures were analyzed on a Q-TOF microhybrid Mass Spectrometer (Micro-

TOFQ-II, Bruker Daltonics, USA) equipped with an ESI nanospray source. The digested peptides were injected on an Ultimate 3000 LC system (Dionex Ultimate3000) and first desalted and pre-concentrated on a C18 PepMap™ pre-column (2 cm, ID 100 μm, 5 μm, C18 LC Packings). Peptides were separated on a C18 column (15 cm, ID 75 μm, 3 μm, C18) with a linear gradient of 5-40% solvent B (99.9 % acetonitrile with 0.1% formic acid) over 60 minutes with a constant flow of 300 nL/min. The peptides were detected in the positive ion MS mode and the data-dependent MS/MS mode. The data-dependent mode was used for survey scans (m/z 300 to 1500) in order to choose up to three most intense precursor ions. For collision-induced dissociation (CID) in MS/MS analysis, collision energies were chosen automatically as a function of m/z and charge. The collision gas was argon. The temperature of the heated sample source was 150 °C and the electro spray voltage was 1200 V. There were three technical replicates for each LC-MS run. The MS/MS data were acquired with the software compass control 1.3 (Bruker Daltonics, USA) that identified compounds and de-convoluted the spectra (Bruker compass Data Analysis 4.0 software) to “mascot generic files” (mgf), which include the mass values, the intensity (at least 5 counts/seconds) and the charge of the precursor ions. Background subtraction was set at 10%. Smoothing was done three times with “smooth window” (channels) 2.0 in the Savitzky Golay mode. The mgf. Files were analyzed using an online version of the Mascot 2.0 program (Matrix Science Ltd., London). Search parameters allowed for carbamido methylation of cysteine, oxidation (variable modification), one missed trypsin cleavage, and 0.5 Da mass accuracy for MS and MS/MS. To ensure the accuracy of protein identification, we selected the candidate proteins with individual scores greater than threshold according to the Peptide Summary Report of Mascot Search Results at a 95% confidence level ($p < 0.05$).

4.7. Label-Free Semi-Quantitative Analysis

Around 2 μg sample from sham-operated and PH 72 group to perform a semi-quantitative analysis, respectively. A semi-quantitative analysis was performed for lipid raft proteomic data, as described previously [20]. A normalization protocol was used to normalize the spectral counts so that the total counts for each group were similar. The average spectral counts for each protein were generated for sham-operated and PH 72 group. The total spectrum number of peptides for each protein was detected to estimate the relative abundance of each protein. We chose protein with peptides no fewer than 2 as the confidence protein, and obtain differences of proteins between PH 72 and sham-operated group according to the exponentially modified protein abundance index (emPAI). Only those proteins with ratio ≥ 2 are considered to be differential expressed proteins.

4.8. Bioinformatics Analysis

The Mascot score and number of unique peptides used to identify a protein as well as the sequence of each peptide were retrieved from Mascot searching output files. The mappings of putative trans membrane domains were carried out using the trans membrane hidden Markov model (TMHMM V 2.0) algorithm. The sub cellular location and function of the identified proteins were elucidated by literatures and gene ontology (GO) cellular component and function terms, respectively. The networks and pathways of the differentially expressed proteins were generated through STRING (Version 10.5, <https://string-db.org>).

4.9. Fluorescence Immunohistochemistry

Immunohistochemistry was performed as described before²¹. Liver tissue was cut into around 0.5 cm³ pieces and fixed in 4% paraformaldehyde. After fixation, the tissue block was embedded in paraffin and sectioned in the sagittal plane. The mounted sections were de-paraffinized in xylene and rehydrated in a graded series of ethanol solutions. Epitopes were retrieved by heating sections in 10 mM sodium citrate buffer (pH 6.0). Sections were incubated in primary antibodies (diluted in phosphate-buffered saline plus 5% goat serum) overnight and then treated with a species-specific Alexa-Fluor 488 dye conjugated secondary antibody (Molecular Probes, Invitrogen) diluted in phosphate-buffered saline plus 5% goat serum at room temperature for 2 h. The immune labeled sections were examined under a Leica DM6000B microscope.

4.10. Quantitative Real-Time Polymerase Chain Reaction

Total RNA was harvested from about 100 mg of rat liver using TRIzol (Invitrogen, USA). First-stand complementary DNA (cDNA) were synthesized from 0.2 μg of total RNA for quantitative real-time polymerase chain reaction (qRT-PCR) (Invitrogen, Carlsbad, CA, USA). Quantitative real-time polymerase chain reaction (qRT-PCR) analyses were performed using the Light-Cycler system (Roche, Indianapolis, IN). PCR was performed using 12.5 μl of 2 X Master Mix SYBR Green I (Takara, Japan), 0.5 μl of each primer, and 1 μl of sample or H₂O to a final volume of 25 μL. The amplification conditions were as follows: 95 °C (5 minutes); 40 cycles at 95 °C (15 seconds) and 65 °C (35 seconds); and extension. Detection of SYBR Green fluorescence at the end of the extension reflects the amount of double-stranded DNA. The expression of the target genes (Flotillin-1 and Caveolin-1) was calculated based on the ratio of the gene of interest to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene. The primer set sense and antisense sequences for the genes were as follows: GAPDH forward, 5'-TGG AGT CTA CTG GCG TCT T-3'; GAPDH reverse, 5'-TGT CAT ATT TCT

CGT GGT TCA-3'; Flotillin-1 forward, 5'-AAG TTC TCA GAG CAG GTT TTC AAG-3'; Flotillin-1 reverse, 5'-TTC CCC AAA GAG TGT AAG TAA TCC-3'; Caveolin-1 forward, 5'-CCC CAA GCA TCT CAA CGA C-3'; Caveolin-1 reverse, 5'-GGT AGA CAG CAA GCG GTA AAA C-3'.

5. Results

5.1. Subcellular Fractionation and Identification of Lipid Rafts

An enriched lipid raft fraction was isolated from plasma membrane preparations using Triton X-100 solubilization and discontinuous

sucrose density gradient centrifugation (Figure 1A). This approach allowed the insoluble lipid rafts (detergent-resistant micro domains, DRMs) to float to the 5%/30% sucrose boundary, where they were observed as a light-scattering band (Figure 1B). Proteins of plasma membrane and soluble fractions from rat liver were compared with DRMs proteins using immuno blotting. As shown in (Figure 1C), lipid raft marker proteins flotillin-1 and caveolin-1 [22, 23] have been enriched within the DRM fraction. This clearly demonstrated the enrichment of lipid raft fraction relative to plasma membrane was enriched by such methods.

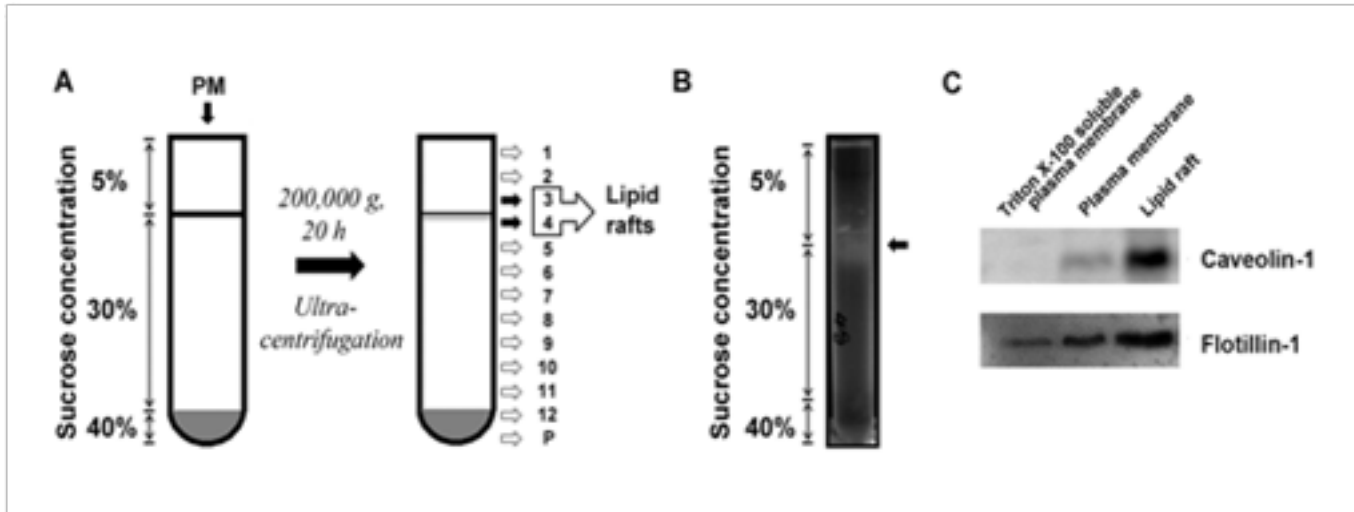


Figure 1: Sucrose density gradient enrichment of detergent-resistant membranes from rat liver. (A) Diagrammatic representation of sucrose density gradient fractionation of plasma membranes. (B) Photograph of tube showing the presence of a light-scattering band of DRMs at the interface of the 5% and 30% sucrose layers, corresponding to fractions 3 and 4. (C) Western blots of isolated protein fractions from rat liver. Blot developed with Caveolin-1 antibody and flotillin-1 antibody.

DRM proteins from sham-operated group and PH 72 h group were separated on SDS-PAGE. As shown in (Figure 2), compared with PH 0 h group, there were 7 obviously different bands in PH 72 h

group. Sixteen strips on the gel were cut in parallel and subjected into enzyme digestion and mass spectrometry.

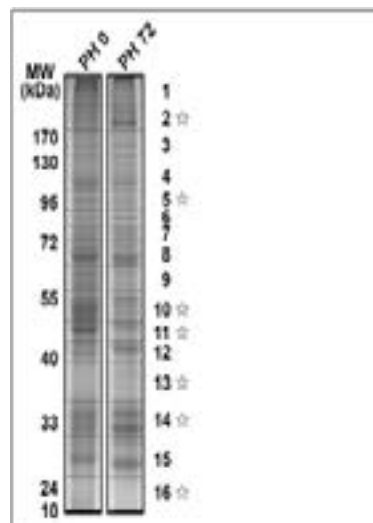


Figure 2: Representative SDS-PAGE profile of lipid rafts proteins in rat liver after 2/3 hepatectomy. LRs proteins from sham-operated and PH 72 groups were separated on SDS-PAGE. Intensity of bands indicated by pentagram exhibited obvious differences between PH72 group and sham group after partial hepatectomy. 16 strips on the gel were cut in parallel and subjected into enzyme digestion and mass spectrometry.

5.2. Proteomic Analysis of Lipid Rafts From Rat Liver; Were Present in All the Secretome Preparations

In total, 458 proteins were identified in lipid raft from PH 72 h group and control group (Supplementary (Table 1), and totally 316 proteins were present in both groups (Figure 3A). The identified proteins were characterized on the basis of their sub cellular localization, as predicted from the Gene Ontology (GO) terms or descriptions provided in Rattuse entries. Among the 456 identified proteins, 430 had descriptions for their sub cellular location. The identified proteins were associated with plasma membrane (27.5%), mitochondria

(26.6%), cytoplasm (11.6%), and ribosome (11.4%). Besides, there were proteins located in endoplasmic reticulum (11.1%), nucleus (3.1%), and peroxisome (1.5%). And still a relatively small proportion (7%) was unclassified (Figure 3B). We also classified the 458 proteins according to their GO function description. 28.2% were annotated as ion channel and transporters, 22.7% as catalytic proteins, 19.9% as structural proteins, 11.8% as regulatory proteins, 4.8% as signal proteins and receptors, 2.4 as cell adhesion proteins. Other annotated proteins (10.9%) included some unknown proteins (Figure 3C).

Table 1: Total Proteins

Location	IPI	Gene name	Protein name	MW	Score	Coverage	Matches peptides
Plasma membrane	IPI00762951	Abca6	Similar to ATP-binding cassette, sub-family A (ABC1), member 6	185449	62	3.01%	2
	IPI00361512	Abca8a	Similar to ATP-binding cassette, sub-family A (ABC1), member 8a	192706	57	1.85%	3
	IPI00195615	Abcb11	Bile salt export pump	147249	613	16.88%	17
	IPI00198519	Abcb4	Multidrug resistance protein 2	141080	152	8.61%	6
	IPI00205806	Abcc2	Canalicular multispecific organic anion transporter 1	174414	488	21.87%	21
	IPI00214263	Abcc5	Multidrug resistance-associated protein 5	160,857	33	0.91%	3
	IPI00207513	Abcc6	Multidrug resistance-associated protein 6	166771	281	13.72%	12
	IPI00231860	Abcd3	ATP-binding cassette sub-family D member 3	75780	549	41.27%	23
	IPI00327093	Abcg2	ATP-binding cassette sub-family G member 2	73427	64	4.72%	2
	IPI00364948	Aldh3a2	Fatty aldehyde dehydrogenase	54503	202	18.80%	8
	IPI00230862	Anpep	Aminopeptidase N	109779	719	39.88%	19
	IPI00421888	Anxa6	Annexin A6	76106	152	6.24%	3
	IPI00558343	Atp1a1	Na,K-ATPase alpha-1 subunit (Fragment)	27339	200	8.02%	7
	IPI00326305	Atp1a1	Sodium/potassium-transporting ATPase subunit alpha-1	114293	790	39.88%	28
	IPI00390795	Atp1a4	ATPase, Na ⁺ /K ⁺ transporting, alpha 4 polypeptide	115260	70	5.64%	6
	IPI00781766	Atp1a4	ATPase, Na ⁺ /K ⁺ Transporting, Alpha 4 Polypeptide	114882	46	6.71%	5
	IPI00339124	Atp1b1	Sodium/potassium-transporting ATPase subunit beta-1	35762	61	11.18%	3
	IPI00365705	Atp4a	Similar to Potassium-transporting ATPase alpha chain 1	115745	73	4.45%	2
	IPI00215580	Atp6v0c	V-type proton ATPase 16 kDa proteolipid subunit	15798	45	14.84%	4
	IPI00476086	Atp6v0d1	RCG51062, isoform CRA_a	40731	87	19.94%	5
	IPI00373076	Atp6v1a	ATPase, H ⁺ transporting, lysosomal V1 subunit A	68564	184	16.53%	6
	IPI00365106	Atp6v1b1	ATPase, H transporting, lysosomal V1 subunit B1	57214	78	9%	3
	IPI00199305	Atp6v1b2	V-type proton ATPase subunit B, brain isoform	56857	301	33.07%	9
	IPI00400615	Atp6v1e1	V-type proton ATPase subunit E 1	26169	89	16.37%	3
	IPI00193425	Bsg	Isoform 2 of Basigin	29852	44	3.61%	2
	IPI00391769	Cacna2d1	Voltage-gated calcium channel alpha2/delta-1 subunit	124530	35	2.29%	2
	IPI00231949	Cd14	Monocyte differentiation antigen CD14	40485	77	24.19%	5
	IPI00231197	Cd36	Platelet glycoprotein 4	53268	81	8.89%	4
	IPI00195173	Cd59	CD59 glycoprotein	14465	74	21.43%	2
	IPI00193983	Cltc	Clathrin heavy chain 1	193187	79	2.81%	3
	IPI00476292	Cp	Ceruloplasmin (Ferroxidase)	121458	178	14.64%	9
IPI00231013	Cyb5a	Isoform Short of Cytochrome b5	11400	121	67.16%	8	
IPI00324309	Cybb	Endothelial type gp91-phox	66163	76	16.67%	2	

	IPI00393883	Cyp2c22	56 kDa protein	56989	42	6.95%	2
	IPI00212694	Egfr	Epidermal growth factor receptor	138225	62	1.24%	3
	IPI00339118	Enpp1	Isoform 2 of Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	104929	86	9.71%	4
	IPI00326462	Enpp3	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3	100946	43	5.71%	3
	IPI00208784	Flot1	Flotillin-1	47755	168	41.82%	12
	IPI00210226	Flot2	Flotillin-2	47408	143	26.87%	7
	IPI00207292	Flot2	Isoform 4 of Flotillin-2	41953	36	11.64%	3
	IPI00207191	Gjb1	Gap junction beta-1 protein	32553	90	19.08%	3
	IPI00767363	Glipr2	Similar to GLI pathogenesis-related 2	25665	68	10.59%	2
	IPI00200437	Gna11	Guanine nucleotide-binding protein subunit alpha-11	42285	60	9.75%	2
	IPI00422053	Gna13	Guanine nucleotide binding protein alpha 13	44326	71	8.22%	2
	IPI00231925	Gnai2	Guanine nucleotide-binding protein G(i), alpha-2 subunit	41043	94	19.09%	4
	IPI00231726	Gnai3	Guanine nucleotide-binding protein G(k) subunit alpha	41066	57	13.84%	3
	IPI00210346	Gnal	Guanine nucleotide-binding protein G(olf) subunit alpha		52	3.94%	2
	IPI00199872	Gnas	Isoform Gnas-1 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	46091	77	9.89%	2
	IPI00212655	Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	38151	39	9.12%	2
	IPI00212658	Gnb2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	38048	279	52.94%	11
	IPI00204239	Gnb3	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3	38125	74	7.35%	3
	IPI00464668	Irgm	Immunity-related GTPase family M protein	46935	105	16.55%	5
	IPI00212499	Iyd	Iodotyrosine dehalogenase 1	32996	66	13.68%	2
	IPI00561286	LOC367858	Similar to GTPase HRas precursor	21607	35	19.50%	2
	IPI00195871	Lyn	Isoform LYN B of Tyrosine-protein kinase Lyn	56553	155	13.87%	5
	IPI00212376	Magt1	Magnesium transporter protein 1	38367	53	8.36%	2
	IPI00421625	Mrlc2	Myosin regulatory light chain 12B	19883	78	18.02%	3
	IPI00212314	Msn	Moesin	67868	49	5.02%	2
	IPI00339007	Myadm	Myeloid-associated differentiation marker	35866	111	12.89%	3
	IPI00211813	Myh10	Myosin-10	233515	58	2.22%	6
	IPI00367479	Myh14	Myosin, heavy chain 14	229819	63	2.50%	5
	IPI00209113	Myh9	Myosin-9	227566	951	30.09%	43
	IPI00189925	Napa	Alpha-soluble NSF attachment protein	33627	38	12.54%	2
	IPI00325189	Nme2	Nucleoside diphosphate kinase B	17386	101	35.52%	4
	IPI00204348	Nt5e	5'-nucleotidase	64384	411	42.01%	26
	IPI00480820	Pgrmc1	Membrane-associated progesterone receptor component 1	21699	50	20%	2
	IPI00209000	Plec1	Plectin 6	535379	49	1.32%	3
	IPI00205490	Ppap2b	Lipid phosphate phosphohydrolase 3	35922	60	15.71%	3
	IPI00382300	Rab1	Ras-related protein Rab-1A	33985	56	22.93%	3
	IPI00555185	Rab10	RAB10, member RAS oncogene family	22755	75	23.50%	3
	IPI00196795	Rab15	Ras-related protein Rab-15	24610	56	15.09%	2
	IPI00198316	Rab18	Ras-related protein Rab-18	23247	66	24.76%	3
	IPI00371187	Rab35	Ras-related protein Rab-35	23296	56	15.92%	2
	IPI00215564	Rab7a	Ras-related protein Rab-7a	23774	72	15.94%	2
	IPI00363395	Rap1b	Ras-related protein Rap-1b	21013	166	46.20%	6
	IPI00369635	Rdx	Radixin	68672	51	8.75%	3
	IPI00551693	RT1-EC2	Mature alpha chain of major histocompatibility complex class I antigen (Fragment)	39342	42	14.08%	4
	IPI00213735	Sfxn1	Sideroflexin-1	35808	150	21.12%	4
	IPI00371684	Sfxn2	Sideroflexin 2	36590	56	11.18%	2
	IPI00200069	Sfxn3	Sideroflexin-3	35696	98	15.26%	4
	IPI00364477	Siglec1	Sialic acid binding Ig-like lectin 1, sialoadhesin	184900	260	12.62%	10
	IPI00358860	Slc12a7	Similar to Solute carrier family 12 member 7	121268	32	2.86%	3

	IPI00205029	Slc22a1	Isoform 1 of Solute carrier family 22 member 1	62413	87	6.12%	2
	IPI00464615	Slc22a18	Solute carrier family 22, member 18	44245	67	14.11%	3
	IPI00562527	Slc25a	33 kDa protein	33346	160	22.73%	8
	IPI00382270	Slc25a15	Ab1-114	37524	224	30.77%	8
	IPI00558425	Slc25a5	Solute Carrier Family 25	27561	112	15.44%	5
	IPI00207298	Slc26a1	Sulfate anion transporter 1	76482	147	9.39%	3
	IPI00208247	Slc28a2	Sodium/nucleoside cotransporter 2	73765	101	6.53%	4
	IPI00196643	Slc2a2	Solute carrier family 2 (Facilitated glucose transporter), member 2	57590	87	6.13%	2
	IPI00200122	Slc38a3	Sodium-coupled neutral amino acid transporter 3	56113	67	5.36%	2
	IPI00189469	Slc38a4	Sodium-coupled neutral amino acid transporter 4	60915	63	4.75%	2
	IPI00199985	Slc4a4	Isoform 1 of Electrogenic sodium bicarbonate cotransporter 1	122178	59	2.78%	2
	IPI00214674	Slco1a1	Solute carrier organic anion transporter family member 1A1	75782	43	9.40%	4
	IPI00214031	Slco1a4	Solute carrier organic anion transporter family member 1A4	74857	228	29.50%	13
	IPI00208799	Slco1a5	Solute carrier organic anion transporter family member 1A5	76066	42	3.58%	4
	IPI00215390	Slco1b2	Isoform 1 of Solute carrier organic anion transporter family member 1B2	73859	177	12.08%	6
	IPI00209258	Spna2	Spectrin alpha chain, brain	285336	343	11.65%	17
	IPI00327662	Sptbn2	Spectrin beta chain, brain 2	272151	86	1.47%	3
	IPI00480706	Steap3	Metalloreductase STEAP3	55095	45	7.38%	2
	IPI00382258	Steap4	Metalloreductase STEAP4	52516	109	37.45%	4
	IPI00203528	Stoml2	Stomatin-like protein 2	38504	90	11.05%	3
	IPI00325618	Tap1	Antigen peptide transporter 1	79556	218	12.89%	5
	IPI00876611	Tap2	Transporter 2, ATP-binding cassette, sub-family B	78157	151	9.67%	4
	IPI00195019	Tapbp	TAP-binding protein	50298	122	22.63%	8
	IPI00734729	Tcirg1	V-H+ATPase subunit a3	93850	82	8.03%	3
	IPI00373155	Tm9sf4	Transmembrane 9 superfamily member 4	75424	48	6.53%	2
	IPI00231659	Tmed10	Transmembrane emp24 domain-containing protein 10	25013	54	21.46%	5
	IPI00187967	Tmem205	Transmembrane protein 205	21456	144	34.92%	4
	IPI00202688	UST4r	Putative integral membrane transport protein	62685	43	2.90%	2
	IPI00202689	Ust5r	Integral membrane transport protein UST5r	62989	69	5.62%	3
	IPI00209290	Vapa	Vesicle-associated membrane protein-associated protein A	28051	96	30.12%	5
	IPI00209283	Vapb	Vesicle-associated membrane protein-associated protein B	27127	52	6.58%	2
	IPI00421874	Vdac1	Voltage-dependent anion-selective channel protein 1	30851	1016	82.33%	32
	IPI00198327	Vdac2	Voltage-dependent anion-selective channel protein 2	32353	323	58.64%	13
	IPI00556929	Vdac3	Isoform 1 of Voltage-dependent anion-selective channel protein 3	31178	409	79.13%	17
	IPI00371710	Vnn1	Pantetheinase	57718	101	6.44%	2
Mitochondrial	IPI00188989	Acs1l	Long-chain-fatty-acid--CoA ligase 1	79155	218	38.91%	18
	IPI00213231	Acs15	Long-chain-fatty-acid--CoA ligase 5	77211	161	14.93%	7
	IPI00778252	ALDH2	Aldh2 Protein	53791	119	3.28%	7
	IPI00365481	Atad3a	ATPase family AAA domain-containing protein 3	66889	86	17.77%	8
	IPI00396910	Atp5a1	ATP synthase subunit alpha, mitochondrial	59831	490	59.31%	23
	IPI00551812	Atp5b	ATP synthase subunit beta, mitochondrial	56318	772	58.41%	29
	IPI00454288	Atp5c1	ATP synthase gamma chain	68590	158	10.59%	5
	IPI00196107	Atp5f1	ATP synthase subunit b, mitochondrial	28965	48	16.02%	4
	IPI00390086	Atp5j2	Similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2	10503	90	35.23%	3
	IPI00195123	Atp5o	ATP synthase subunit O, mitochondrial	23440	82	33.80%	5

IPI00480620	Bdh1	3-hydroxybutyrate dehydrogenase, type 1	38708	186	24.49%	6
IPI00764098	Ccdc109a	Coiled-coil domain containing 109A	40075	131	18.46%	5
IPI00869949	Chchd3	Coiled-coil-helix-coiled-coil-helix domain containing 3	26653	157	51.54%	9
IPI00358005	Chdh	Choline dehydrogenase	67088	86	10.85%	3
IPI00870183	Cisd1	CDGSH iron sulfur domain-containing protein 1	12260	57	39.81%	3
IPI00365293	Comtd1	Catechol-O-methyltransferase domain containing 1	29254	44	13.79%	3
IPI00194222	Cox4i1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	19559	344	75.15%	15
IPI00192246	Cox5a	Cytochrome c oxidase subunit 5A, mitochondrial	16347	82	13.70%	2
IPI00193918	Cox5b	Cytochrome c oxidase subunit 5B, mitochondrial	14191	56	41.09%	7
IPI00389152	Cox6b1-ps1	Cox6b1 Similar to cytochrome c oxidase, subunit VIb polypeptide 1	10293	36	35.16%	3
IPI00230832	Cox6c	Cytochrome c oxidase subunit 6C-2	8449	88	38.16%	5
IPI00195860	Cox7a1-ps1	Cox7a2 Cytochrome c oxidase polypeptide 7A2, mitochondrial	9347	66	19.35%	3
IPI00365505	Cox7a2l	Cytochrome c oxidase subunit VIIa polypeptide 2 like (Predicted), isoform CRA_d	12700	95	46.46%	3
IPI00564570	Cox7c	Cytochrome c oxidase, subunit VIIc	7370	77	23.81%	2
IPI00210644	Cps1	Carbamoyl-phosphate synthase [ammonia], mitochondrial	165673	1177	20.87%	50
IPI00193233	Cyb5b	Cytochrome b5 type B	16312	54	26.03%	2
IPI00231662	Cyb5r3	Isoform 1 of NADH-cytochrome b5 reductase 3	34381	254	26.25%	6
IPI00366416	Cyc1	Cytochrome c-1	35640	223	18.40%	7
IPI00366417	Cyc2	Cytochrome c-2	35641	224	19.05%	8
IPI00198947	Cyp1a2	Cytochrome P450 1A2	58621	179	14.62%	4
IPI00198324	Cyp2b3	Cytochrome P450 2B3	56861	70	9.98%	3
IPI00196748	Cyp2c7	Cytochrome P450 2C7	56187	2	5.71%	5
IPI00231473	Cyp2d2	Cytochrome P450 2D26	57620	62	9.80%	3
IPI00202584	Cyp2d5	Cytochrome P450 2D10	57495	85	10.32%	3
IPI00327623	Cyp4a1	Cytochrome P450 4A10	58520	179	17.86%	8
IPI00203317	Cyp4a2	Cytochrome P450 4A2	58274	251	40.67%	17
IPI00203318	Cyp4a3	Cytochrome P450 4A14	58537	190	24.85%	11
IPI00214540	Cyp4a8	Cytochrome P450 4A12	58807	54	8.46%	5
IPI00215574	Dei	3,2-trans-enoyl-CoA isomerase, mitochondrial	32348	68	12.80%	3
IPI00213659	Deer1	2,4-dienoyl-CoA reductase, mitochondrial	36395	41	9.85%	3
IPI00187918	Faah	Fatty acid amide hydrolase	81815	38	6.56%	2
IPI00199347	Ghitm	Growth hormone-inducible transmembrane protein	37324	51	8.67%	2
IPI00324633	Glud1	Glutamate dehydrogenase 1, mitochondrial	61719	131	33.69%	11
IPI00205157	Hadh	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	34540	58	5.09%	2
IPI00212622	Hadha	Trifunctional enzyme subunit alpha, mitochondrial	83297	127	14.55%	9
IPI00210444	Hmgcs2	Hydroxymethylglutaryl-CoA synthase, mitochondrial	57332	68	9.65%	2
IPI00454251	Hsd11b1	11 β -hydroxysteroid dehydrogenase type 1	128542	69	3.26%	4
IPI00231253	Hsd17b10	3-hydroxyacyl-CoA dehydrogenase	27343	52	17.24%	3
IPI00363696	Hsd17b13	17-beta hydroxysteroid dehydrogenase 13	33815	84	15%	3
IPI00363265	Hspa9	Stress-70 protein, mitochondrial	74097	84	5.01%	2
IPI00364895	Immt	MICOS complex subunit MIC60	82654	569	52.71%	26
IPI00373331	Lactb	lactamase, beta	60638	49	2.91%	2
IPI00202370	Maoa	Similar to Amine oxidase [flavin-containing] A	98754	415	40.49%	19
IPI00192043	Mccc2	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	62470	102	13.32%	4
IPI00197696	Mdh2	Malate dehydrogenase, mitochondrial	36117	69	8.88%	2
IPI00393599	MOCO1	Similar to MOCO sulphurase C-terminal domain containing 1	38508	137	25.79%	6

	IPI00214398	Mosc2	MOSC domain-containing protein 2, mitochondrial	38851	72	31.32%	13
	IPI00764690	Mtch2	Mitochondrial carrier homolog 2	35075	283	35.94%	13
	IPI00200472	Mt-co1	Cytochrome c oxidase subunit 1	56956	78	10.51%	5
	IPI00209908	Mt-co2	Cytochrome c oxidase subunit 2	26096	216	40.09%	12
	IPI00734781	Mt-nd1	NADH dehydrogenase subunit 1	36178	63	23.58%	5
	IPI00679192	Mt-nd2	NADH-ubiquinone oxidoreductase chain 2	38574	62	10.12%	3
	IPI00200487	Mt-nd4	NADH-ubiquinone oxidoreductase chain 4	51937	78	8.28%	3
	IPI00734731	Mt-nd5	NADH dehydrogenase subunit 5	68971	202	10.51%	4
	IPI00368621	Mtx1	Mtx1 protein (Fragment)	49211	69	4.50%	2
	IPI00370745	Mtx2	Metaxin 2	30155	74	23.57%	4
	IPI00189759	Ndufa10l1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10-like	40804	190	45.63%	12
	IPI00366206	Ndufa12	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12	21714	209	72.41%	10
	IPI00359981	Ndufa13	Similar to NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13	16950	114	30.56%	4
	IPI00191103	Ndufa2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2	10952	84	26.61%	2
	IPI00213436	Ndufa4	NDUFA4, mitochondrial complex associated	9321	53	37.80%	2
	IPI00231997	Ndufa5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	13460	58	17.24%	2
	IPI00198897	Ndufa6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6	15214	46	22.67%	3
	IPI00191312	Ndufa7	NADH Dehydrogenase (Ubiquinone) 1 Alpha Subcomplex, 7	12550	68	27.50%	3
	IPI00557327	Ndufa8	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	20408	87	28.72%	4
	IPI00382248	Ndufa8	Aa2-258	22408	70	15.35%	3
	IPI00358441	Ndufa9	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9	42646	540	90.45%	30
	IPI00202238	Ndufb10	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	21131	121	67.53%	8
	IPI00358015	Ndufb3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 3	11260	44	16.49%	2
	IPI00199169	Ndufb4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 4	15055	42	35.66%	4
	IPI00372179	Ndufb5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5	21650	116	35.45%	6
	IPI00364850	Ndufb6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6	15628	49	36.72%	3
	IPI00365170	Ndufb7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7	16785	79	31.25%	4
	IPI00370372	Ndufb8	NADH dehydrogenase (Ubiquinone) 1 beta subcomplex 8 (Predicted), isoform CRA_a	22058	60	27.42%	4
	IPI00190161	Ndufc2	NADH dehydrogenase [ubiquinone] 1 subunit C2	14406	111	42.50%	4
	IPI00358033	Ndufs1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	80331	933	86.38%	40
	IPI00202616	Ndufs3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	30379	448	48.86%	13
	IPI00212052	Ndufs4	NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial	19785	86	25.71%	4
	IPI00365962	Ndufs7	NADH dehydrogenase (Ubiquinone) Fe-S protein 7, isoform CRA_d	24215	119	27.12%	8
	IPI00188330	Ndufs8	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial	24411	121	14.15%	4
	IPI00191913	Ndufv1	NADH dehydrogenase (Ubiquinone) flavo-protein 1	51383	86	24.78%	10
	IPI00367152	Ndufv2	NADH dehydrogenase [ubiquinone] flavo-protein 2, mitochondrial	27703	106	25.81%	4
	IPI00364134	Nipsnap1	4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1	33610	142	25%	5
	IPI00555265	Nnt	Nicotinamide nucleotide transhydrogenase	114537	499	28.27%	19
	IPI00210435	Pc	Pyruvate carboxylase, mitochondrial	130436	104	4.16%	3

	IPI00194324	Pdhb	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	39299	37	5.85%	3
	IPI00211756	Phb	Prohibitin	29859	1286	76.69%	34
	IPI00190557	Phb2	Prohibitin-2	33292	469	62.57%	25
	IPI00198129	RGD1309362	Similar to interferon-inducible GTPase	48469	47	8.15%	2
	IPI00363182	RGD1560896	Similar to solute carrier family 25, member 5	33326	521	56.46%	19
	IPI00388687	RGD1561141	Similar to solute carrier family 25 (mitochondrial carrier, Aralar), member 12	51130	160	10.56%	5
	IPI00212980	RGD1563422	Isoform Long of Brain protein 44	14306	65	50.71%	4
	IPI00568360	RGD1564131	Similar to solute carrier family 25, member 5	29168	102	21.38%	7
	IPI00764502	Rmnd1	Similar to CG11679-PA	50831	86	11.11%	7
	IPI00372941	Samm50	Sorting and assembly machinery component 50 homolog	52384	373	51.81%	19
	IPI00200659	Sdha	Succinate dehydrogenase [ubiquinone] flavo-protein subunit, mitochondrial	72596	246	20.73%	10
	IPI00785564	Sdhb	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	32607	44	15.25%	4
	IPI00327694	Slc25a1	Tricarboxylate transport protein, mitochondrial	34156	220	40.19%	14
	IPI00209363	Slc25a10	Solute carrier family 25 (Mitochondrial carrier)	31890	227	30.07%	7
	IPI00231261	Slc25a11	Mitochondrial 2-oxoglutarate/malate carrier protein	34393	218	53.50%	11
	IPI00358163	Slc25a13	Similar to Calcium-binding mitochondrial carrier protein Aralar2	74751	1539	32.75%	61
	IPI00205413	Slc25a20	Mitochondrial carnitine/acylcarnitine carrier protein	33474	156	55.48%	13
	IPI00189072	Slc25a27	Uncoupling protein UCP-4, isoform a	35986	43	10.42%	2
	IPI00562259	Slc25a3	Phosphate carrier protein, mitochondrial	39876	143	30.06%	9
	IPI00209115	Slc25a3	Solute carrier family 25 (Mitochondrial carrier)	40052	107	27.53%	5
	IPI00231927	Slc25a4	ADP/ATP translocase 1	33196	349	48.66%	20
	IPI00200466	Slc25a5	ADP/ATP translocase 2	33108	238	57.38%	16
	IPI00210851	Srd5a1	Isoform Long of 3-oxo-5-alpha-steroid 4-dehydrogenase 1	29989	57	10.81%	2
	IPI00364390	Tmem126a	Transmembrane protein 126A	21758	69	13.27%	2
	IPI00914293	Tmem14c	Transmembrane protein 14C	11650	49	21.83%	3
	IPI00417749	Tomm22	Mitochondrial import receptor subunit TOM22 homolog	15481	116	29.58%	3
	IPI00421376	Tomm40	Mitochondrial import receptor subunit TOM40 homolog	38294	75	4.43%	3
	IPI00417750	Tomm70a	Mitochondrial import receptor subunit TOM70	68143	84	8.20%	4
	IPI00471577	Uqcrc1	Cytochrome b-c1 complex subunit 1, mitochondrial	53500	218	22.29%	15
	IPI00188924	Uqcrc2	Cytochrome b-c1 complex subunit 2, mitochondrial	48423	189	29.65%	12
	IPI00362949	Uqcrf1	Cytochrome b-c1 complex subunit Rieske, mitochondrial	29712	103	14.96%	5
	IPI00382312	Uqcrq	Cytochrome b-c1 complex subunit 8	9843	87	24.39%	2
	IPI00202111	Usmg5	Up-regulated during skeletal muscle growth protein 5	6460	78	30.91%	3
	IPI00197684	Vdac2	X-prolyl aminopeptidase (Aminopeptidase P) 2, membrane-bound	76545	359	58.64%	20
	IPI00207891	Vdac3	Voltage-dependent anion-selective channel protein 3	31292	449	69.26%	13
Cytoplasm	IPI00189819	Actb	Actin, cytoplasmic 1	42052	246	28.27%	14
	IPI00360356	Actb2	Actin, beta-like 2	42278	126	18.09%	8
	IPI00896224	Actg1	Actin, cytoplasmic 2	42108	337	65.87%	22
	IPI00209082	Actn1	Alpha-actinin-1	103466	55	3.59%	3
	IPI00471911	Aldob	Fructose-bisphosphate aldolase B	40049	67	21.15%	4
	IPI00231967	Arf6	ADP-ribosylation factor 6	20183	33	17.71%	3
	IPI00327518	Arg1	Arginase-1	36652	178	22.91%	4
	IPI00372040	Arpc4	Actin-related protein 2/3 complex subunit 4	19768	42	26.19%	4

	IPI00382241	Bco2	Ab2-079	55131	53	3.12%	2
	IPI00332027	Bhmt	Betaine--homocysteine S-methyltransferase 1	45404	311	53.81%	16
	IPI00370681	Capza2	F-actin-capping protein subunit alpha-2	33118	60	19.93%	3
	IPI00230788	Car3	Carbonic anhydrase 3	29698	34	14.83%	2
	IPI00362131	Cdh2	Cadherin-2	100081	34	6.18%	2
	IPI00214859	Coll18a1	Similar to Collagen alpha-1(XVIII) chain precursor	185965	47	13.82%	4
	IPI00194550	Cth	Cystathionine gamma-lyase	44262	49	8.29%	2
	IPI00358406	Ctnna1	Catenin (Cadherin-associated protein), alpha 1, isoform CRA_b	100858	62	3.75%	2
	IPI00366081	Dsp	Similar to desmoplakin isoform I isoform 2	334582	64	0.79%	2
	IPI00767363	Glipr2	Similar to GLI pathogenesis-related 2	25665	68	10.59%	2
	IPI00230897	Hbb	Hemoglobin subunit beta-1	16083	63	10.20%	2
	IPI00421857	Krt1	Keratin, type II cytoskeletal 1	65190	164	8%	6
	IPI00390975	Krt10	Keratin 10, Type I	57633	135	10.83%	5
	IPI00421805	Krt12	Keratin 12, Type I	51539	45	3.07%	2
	IPI00421855	Krt14	Keratin, type I cytoskeletal 14	52936	71	11.34%	4
	IPI00370090	Krt15	Keratin, type I cytoskeletal 15	49011	61	6.04%	2
	IPI00421793	Krt16	Type I keratin KA16	51088	53	5.40%	2
	IPI00480679	Krt18	Keratin, type I cytoskeletal 18	47732	72	12.29%	3
	IPI00188666	Krt27	Keratin, type I cytoskeletal 27	49649	65	3.12%	2
	IPI00421778	Krt4	Keratin, type II cytoskeletal 4	57973	55	4.85%	2
	IPI00382153	Krt5	Keratin, type II cytoskeletal 5	61959	95	7.64%	4
	IPI00421788	Krt7	Keratin, type II cytoskeletal 7	50678	52	6.13%	3
	IPI00421780	Krt73	Keratin, type II cytoskeletal 73	60977	72	11.03%	4
	IPI00421782	Krt75	keratin 75	62472	59	5.35%	4
	IPI00421812	Krt76	Type II keratin Kb9	62291	65	2.41%	2
	IPI00389571	Krt8	Keratin, type II cytoskeletal 8	53985	135	14.08%	4
	IPI00768167	LOC679312	Similar to beta tubulin 1, class VI	51068	51	3.31%	2
	IPI00764184	LOC683295	Similar to keratin complex 2, basic, gene 6a isoform 1	59735	45	5.51%	3
	IPI00393340	LOC683313	Keratin, type II cytoskeletal 6A	59555	107	8.15%	5
	IPI00231045	Myo1b	Isoform C of Myosin-Ib	125868	97	7.04%	4
	IPI00365929	Pdia6	Protein disulfide isomerase A6	49129	107	17.95%	4
	IPI00554039	RGD1565368	Similar to glyceraldehyde-3-phosphate dehydrogenase	36045	141	16.57%	3
	IPI00555287	Sptbn1	Non-erythroid spectrin beta	274328	49	1.35%	2
	IPI00189795	Tuba1a	Tubulin alpha-1A chain	50788	122	0.17%	4
	IPI00364046	Tuba1c	Tubulin alpha-1C chain	50590	125	17.14%	4
	IPI00400573	Tubb2c	Tubulin beta-2C chain	50225	84	14.83%	6
	IPI00197579	Tubb5	Isoform 1 of Tubulin beta-5 chain	50095	75	20.49%	5
	IPI00213569	Ugt1a1	UDP-glucuronosyltransferase 1-1	60422	198	23.36%	9
	IPI00742454	Ugt1a5	UDP glycosyltransferase 1 family, polypeptide A5 precursor	60453	32	3.01%	2
	IPI00475707	Ugt1a6	Ugt1a1 UDP glycosyltransferase 1 family, polypeptide A6	60823	118	12.10%	5
	IPI00476793	Ugt1a8	Ugt1a1 UDP glycosyltransferase 1 family, polypeptide A8	60751	56	0.39%	2
	IPI00554206	Ugt2b	UDP glycosyltransferase 2 family, polypeptide B	61416	109	12.17%	7
	IPI00212110	Ugt2b	UDP-glucuronosyltransferase 2B2	61459	109		7
	IPI00190402	Ugt2b17	UDP-glucuronosyltransferase 2B1	61072	61	17.36%	6
	IPI00327626	Ugt2b5	UDP-glucuronosyltransferase 2B3	61055	111	15.53%	7
Ribosome	IPI00200147	Arbp	60S acidic ribosomal protein P0	34365	120	31.86%	5
	IPI00327697	Dpep1	Dipeptidase 1	45949	60	9.51%	2
	IPI00208422	Dpp4	Dipeptidyl peptidase 4	88774	270	24.77%	17
	IPI00195372	Eef1a1	Elongation factor 1-alpha 1	50424	72	2.81%	2
	IPI00766254	LOC300481	Similar to 40S ribosomal protein S19	14597	34	8.37%	2
	IPI00196808	LOC688684	Similar to 60S ribosomal protein L32	15126	68	29.13%	4
	IPI00762931	LOC690152	LOC686749 Similar to acidic ribosomal phosphoprotein P0	7941	48	23.29%	2

	IPI00768386	LOC690384	Similar to ribosomal protein L31	14593	47	15.20%	3
	IPI00203481	LOC691716	LOC687680 Similar to ribosomal protein S15a	14562	54	20.59%	3
	IPI00206020	Lrrc59	Leucine-rich repeat-containing protein 59	35304	56	10.10%	2
	IPI00358826	RGD1561928	Similar to ribosomal protein L9	26253	55	12.67%	3
	IPI00361429	RGD1562055	Similar to ribosomal protein L31	13875	57	11.97%	2
	IPI00358026	RGD1562399	Similar to 40S ribosomal protein S2	31251	48	11.34%	5
	IPI00208158	RGD1562601	Similar to ribosomal protein L21	18754	52	11.43%	3
	IPI00368848	RGD1563899	Similar to 60S ribosomal protein L27a	13024	67	25.62%	2
	IPI00210164	Rpl12	Similar to 60S ribosomal protein L12	18007	50	46.67%	4
	IPI00230916	Rpl13	60S ribosomal protein L13	24351	100	28.91%	4
	IPI00655314	Rpl13a	60S ribosomal protein L13a	23575	91	37.43%	5
	IPI00475722	Rpl14	Rpl14 protein	23879	106	15.89%	3
	IPI00231445	Rpl15	60S ribosomal protein L15	24245	78	23.04%	4
	IPI00230917	Rpl18	60S ribosomal protein L18	21702	169	27.65%	4
	IPI00192257	Rpl18a	60S ribosomal protein L18a	21004	133	62.50%	8
	IPI00202214	Rpl19	60S ribosomal protein L19	23565	90	18.37%	3
	IPI00207980	Rpl23	60S ribosomal protein L23	14970	62	32.14%	2
	IPI00203523	Rpl23a	60S ribosomal protein L23a	17684	82	21.15%	2
	IPI00230939	Rpl24	60S ribosomal protein L24	17882	101	30.57%	4
	IPI00200552	Rpl26	60S ribosomal protein L26	17267	43	17.24%	2
	IPI00371209	Rpl27a	60S ribosomal protein L27a	16722	91	43.92%	4
	IPI00395285	Rpl3	60S ribosomal protein L3	46392	49	3.97%	2
	IPI00231346	Rpl30	60S ribosomal protein L30	12947	86	32.17%	2
	IPI00202512	Rpl4	60S ribosomal protein L4	47569	218	43.47%	12
	IPI00230914	Rpl5	60S ribosomal protein L5	34665	80	32.32%	6
	IPI00231609	Rpl6-ps1	Similar to 60S ribosomal protein L6	33582	90	23.15%	6
	IPI00199543	Rpl7	60S ribosomal protein L7	30367	186	40.38%	10
	IPI00363949	Rpl7a	60S ribosomal protein L7a	30148	207	35.71%	10
	IPI00215208	Rpl8	60S ribosomal protein L8	28235	138	21.01%	6
	IPI00188804	Rplp2	60S acidic ribosomal protein P2	11685	81	20%	2
	IPI00204365	Rpn1	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	68473	564	56.70%	25
	IPI00197713	Rps11	40S ribosomal protein S11	18590	48	34.81%	4
	IPI00366014	Rps13	LOC684988 40S ribosomal protein S13	17212	52	34.43%	4
	IPI00201500	Rps14	40S ribosomal protein S14	16420	117	23.18%	3
	IPI00421451	Rps16	Similar to 40S ribosomal protein S16	22231	77	32.88%	5
	IPI00392390	Rps2	40S ribosomal protein S2	31497	112	26.96%	8
	IPI00214654	Rps24	Isoform 1 of 40S ribosomal protein S24	15413	125	27.82%	3
	IPI00215184	Rps25	40S ribosomal protein S25	13791	56	23.20%	2
	IPI00212776	Rps3	40S ribosomal protein S3	26828	129	36.21%	6
	IPI00475474	Rps4x	40S ribosomal protein S4, X isoform	29807	47	28.90%	6
	IPI00886474	Rps5	Rps5 protein	23063	97	18.14%	2
	IPI00192486	Rps6	40S ribosomal protein S6	28834	87	16.47%	2
	IPI00231202	Rps8	40S ribosomal protein S8	24475	58	16.34%	2
	IPI00421626	Rps9	40S ribosomal protein S9	22635	40	14.95%	2
Endoplasmic reticulum	IPI00372341	Armc10	Armadillo repeat-containing protein 10	33743	51	11.11%	2
	IPI00190020	Atp2a2	Isoform SERCA2B of Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	116913	49	1.73%	2
	IPI00371634	Bcap31	B-cell receptor-associated protein 31	27951	41	15.92%	2
	IPI00199324	Cisd2	Similar to CG1458-PA	27441	100	26.67%	2
	IPI00324912	Cyp2e1	Cytochrome P450 2E1	56990	47	3.85%	2
	IPI00471645	Ddost	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	49093	127	24.04%	8
	IPI00366256	Der11	Derlin	28930	37	13.15%	2
	IPI00210431	Dher7	7-dehydrocholesterol reductase	54861	121	13.38%	4
	IPI00359162	Dnajc11	DnaJ (Hsp40) homolog, subfamily C, member 11	63508	114	21.65%	7
	IPI00209690	Ephx1	Epoxide hydrolase 1	52719	141	22.64%	9
	IPI00765365	Erlin1	SPFH domain family, member 1	39412	146	27.01%	6

	IPI00201494	Erlin2	Erlin-2	37915	120	13.86%	3
	IPI00565267	Fmo3	Dimethylaniline monooxygenase [N-oxide-forming] 3	60606	159	13.18%	4
	IPI00203106	Fmo5	Dimethylaniline monooxygenase [N-oxide-forming] 5	60530	96	11.63%	3
	IPI00372377	Fmo6	Similar to Putative dimethylaniline monooxygenase [N-oxide-forming] 6	60761	74	3.38%	2
	IPI00200360	G6pc	Glucose-6-phosphatase	41042	100	16.24%	5
	IPI00210874	Gpsn2	Synaptic glycoprotein SC2	36498	79	10.06%	2
	IPI00193277	Hsd17b11	Estradiol 17-beta-dehydrogenase 11	33316	68	19.13%	4
	IPI00197982	Hsd17b6	Hydroxysteroid (17-beta) dehydrogenase 6	37629	122	14.19%	4
	IPI00206624	Hspa5	78 kDa glucose-regulated protein	72473	166	22.17%	7
	IPI00208205	Hspa8	Heat shock cognate 71 kDa protein	71055	435	35.45%	19
	IPI00210116	Lman1	Protein ERGIC-53	58194	134	17.60%	6
	IPI00766280	LOC680782	Similar to signal peptidase complex subunit 3 homolog	21388	45	22.67%	3
	IPI00189361	Lpcat3	Lysophospholipid acyltransferase 5	56494	32	6.37%	2
	IPI00231774	Maob	Amine oxidase [flavin-containing] B	59049	474	50%	28
	IPI00230889	Mgst1	Microsomal glutathione S-transferase 1	17517	445	53.55%	11
	IPI00371173	Mlec	Malectin	32569	44	11.68%	2
	IPI00324741	Pdia3	Protein disulfide-isomerase A3	57499	356	52.08%	19
	IPI00231200	Por	NADPH--cytochrome P450 reductase	77313	141	21.09%	8
	IPI00213644	Ppib	Peptidyl-prolyl cis-trans isomerase B	23845	74	16.20%	2
	IPI00195614	Rdh2	Retinol dehydrogenase 2	35973	33	15.14%	3
	IPI00204128	RGD1561381	Similar to microsomal glutathione S-transferase 3	16924	154	23.87%	3
	IPI00188059	Rpn2	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	69149	142	21.24%	9
	IPI00198643	Sec11a	Signal peptidase complex catalytic subunit SEC11A	20586	88	16.76%	4
	IPI00364983	Sec61b	Sec61 beta subunit	10039	54	32.29%	3
	IPI00205417	Slc27a2	Very long-chain acyl-CoA synthetase	71447	161	13.71%	5
	IPI00191416	Slc27a5	Bile acyl-CoA synthetase	77129	397	40%	20
	IPI00396902	Soat2	Sterol O-acyltransferase 2	61427	65	11.07%	4
	IPI00364884	Ssr1	Translocon-associated protein subunit alpha	35778	64	15.05%	3
	IPI00201932	Ssr4	Translocon-associated protein subunit delta	19323	148	36.99%	6
	IPI00914170	Stt3a	Integral membrane protein 1	86332	74	3.41%	2
	IPI00213458	Surf4	Surfeit 4	30589	182	28.62%	5
	IPI00203757	Tmco1	Transmembrane and coiled-coil domains protein 1	21389	106	20.21%	3
	IPI00369540	Tmem11	Transmembrane protein 11	21784	70	10.53%	2
	IPI00206743	Tmem111	Transmembrane protein 111	29960	120	26.82%	4
	IPI00365607	Tmem195	Transmembrane protein 195	52064	97	10.07%	3
	IPI00766458	Tmem85	Transmembrane protein 85	20274	38	30.05%	3
	IPI00421330	Tram1	Translocation Associated Membrane Protein 1	58898	62	5.72%	3
	IPI00366247	Ttc35	Tetratricopeptide repeat protein 35	35018	194	21.89%	4
	IPI00214436	Ugt1a1	Ugt1a7c UGT1A7	60111	53	10.28%	2
	IPI00195585	Vdac2	Voltage-dependent anion-selective channel protein 2	35982	33	10.51%	2
Nucleus	IPI00767665	Acin1	Similar to Apoptotic chromatin condensation inducer in the nucleus		34	1.19%	3
	IPI00208091	Fbl	rRNA 2'-O-methyltransferase fibrillar	34315	90	16.82%	3
	IPI00367782	Fbl11	Fibrillar-like 1	33890	90	12.35%	2
	IPI00208098	Gpx4	Phospholipid hydroperoxide glutathione peroxidase, nuclear	29953	53	13.83%	2
	IPI00231475	H3f3b	Histone H3.3	15376	63	38.24%	4
	IPI00231650	Hist1h1d	Histone H1.2	21974	46	14.09%	2
	IPI00324991	Lbr	Lamin-B receptor	71192	129	6.94%	3
	IPI00764082	LOC680063	Similar to histone 2a	11129	33	30.59%	2
	IPI00190348	LOC680312	histone H2B	13882	98	25.83%	2
	IPI00368613	Mex3a	Similar to ring finger and KH domain containing 3	48986	36	2.98%	2

	IPI00368391	RGD1309696	Similar to AHNAK nucleoprotein isoform 1	904271	55	0.53%	2
	IPI00188688	RGD1564767	Histone H2A	14180	60	28.46%	3
	IPI00368249	Selt	Selenoprotein T	22459	46	16.41%	2
	IPI00372055	Sfrs18	Sfrs18 protein	51362	58	3.42%	7
Peroxisome	IPI00339123	Acox3	Peroxisomal acyl-coenzyme A oxidase 3	79080	40	2.71%	2
	IPI00231742	Cat	Catalase	60062	106	9.30%	3
	IPI00207933	Dhrs4	Peroxisomal short-chain alcohol dehydrogenase	30031	62	14.34%	2
	IPI00232011	Ehhadh	Peroxisomal bifunctional enzyme	79179	75	5.40%	2
	IPI00211779	Prdx1	Peroxiredoxin-1	22323	72	16.08%	2
	IPI00231204	Pxmp2	Peroxisomal membrane protein 2	22562	78	36.60%	6
	IPI00231365	Uox	Uricase	35140	325	60.07%	18
Others	IPI00454276	LOC499136	LRRGT00021	27775	33	6.01%	2
	IPI00569279	LOC690102	Hypothetical protein LOC690102	13991	33	11.67%	2
	IPI00764950	RCG36127	Isoform CRA_c	7457	45	18.75%	2
	IPI00567497	RGD1564406	Hypothetical protein	13978	54	6.21%	7
	IPI00359123	RGD1310427	Hypothetical protein LOC362643	111940	66	4.83%	3
	IPI00365595		107 kDa protein	108099	35	1.31%	2
	IPI00231340		11 kDa protein	11474	160	43.83%	8
	IPI00387709		12 kDa protein	12459	44	20.09%	2
	IPI00561016		127 kDa protein	128083	79	3.89%	3
	IPI00370178		128 kDa protein	129513	55	3.12%	2
	IPI00192280		14 kDa protein	13745	41	2.94%	3
	IPI00207486		14 kDa protein	14311	76	25.57%	3
	IPI00392340		14 kDa protein	14416	57	10.37%	2
	IPI00204925		15 kDa protein	15208	39	33.10%	5
	IPI00551563		15 kDa protein	14794	81	25.93%	3
	IPI00198439		17 kDa protein	17369	80	11.25%	2
	IPI00781734		18 kDa protein	18413	32	21.71%	3
	IPI00400550		19 kDa protein	18629	52	35.44%	4
	IPI00196783		19 kDa protein	18780	36	19.41%	3
	IPI00195147		25 kDa protein	25405	43	19.58%	4
	IPI00565717		27 kDa protein	27562	54	17.65%	4
	IPI00568876		27 kDa protein	27324	63	11.15%	5
	IPI00777725		28 kDa protein	28443	55	15.19%	3
	IPI00188066		28 kDa protein	28214	58	11.31%	2
	IPI00553900		28 kDa protein		40	5.83%	4
	IPI00777931		29 kDa protein	29022	252	25.86%	6
	IPI00391901		30 kDa protein	30363	109	30.47%	8
	IPI00566615		30 kDa protein	29924	75	11.64%	4
	IPI00776737		31 kDa protein		48	4.86%	2
	IPI00563424		31 kDa protein	31649	88	4.62%	4
	IPI00208495		33 kDa protein	32980	59	10.79%	2
	IPI00778270		68 kDa protein	68677	71	27.92%	4

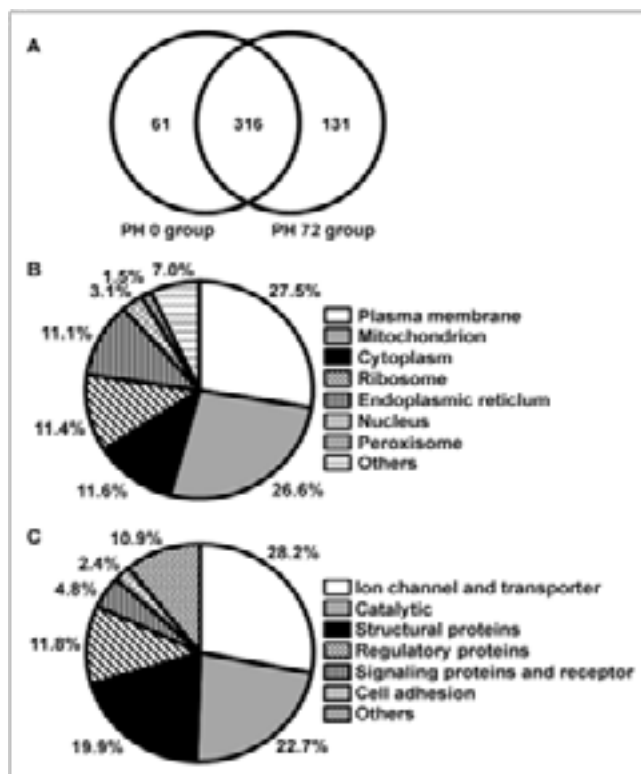


Figure 3: Classification of the total lipid raft proteins identified in rat liver after partial hepatectomy. (A) A Venn diagram of based on the comparison of lipid raft proteins from PH 72 h group and control group. (B) Classification of the identified lipid rafts proteins based on subcellular localization information. (C) Functional classification of the identified lipid rafts proteins.

Furthermore, the molecular weight, isoelectric point, hydrophobicity value and trans membrane region were analyzed for all proteins (Figure 4). Most of them were less than 60 kDa but there was nearly 7% molecular weight higher than 100 kDa (Figure 4A). The isoelectric points of these proteins ranged from 4 to 10 and fell into

normal distribution pattern (Figure 4B). The proportion of “neutral proteins” ($6 < pI < 8$) is abundant. Meanwhile, 277 trans membrane proteins were found and 30 proteins have at least 10 trans membrane domains (Figure 4C) and the hydrophobic value of near 3.2% protein is more than 0.5 (Figure 4D).

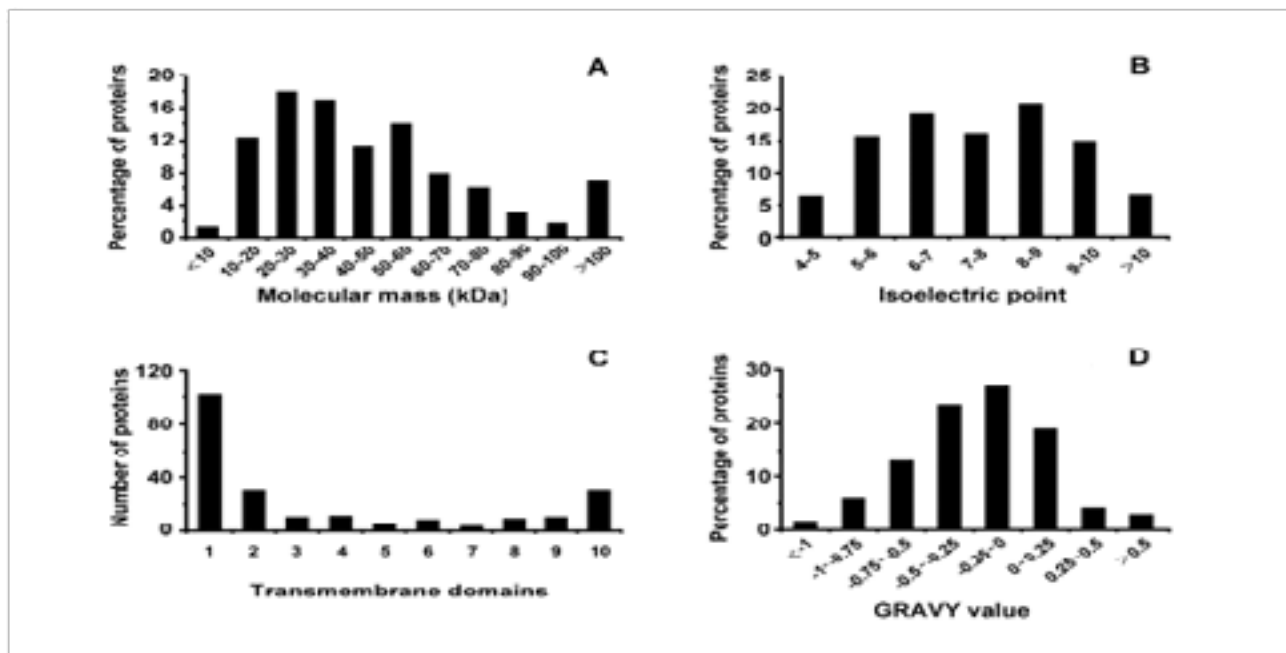


Figure 4: Physicochemical characterization of proteins identified in lipid rafts of rat liver. Molecular mass (A), isoelectric point (B), number of trans-membrane domains (TMD) (C), and GRAVY value (D).

5.3. Differentially Expressed Lipid Raft Proteins

In total, 377 and 447 proteins were identified in the sham-operated and group PH 72 group, respectively. And 46 plasma membrane-associated proteins were found to be differentially expressed (with change more than 2 folds) (Supplementary (Table 2)). Compared with the sham operation group, 34 proteins were up-regulated whereas 12 proteins were down-regulated at PH 72 h group. All the 46 differentially-expressed proteins were pooled together for pathway

analysis using STRING. Among them, up to 36 proteins could be clustered into cav-1 protein-mediated pathway (Figure 5). Obviously, cav-1 protein was one of the important nodes at all the networks and modulated the diverse physiological processes, such as material transport, signal transduction and cytoskeleton and so on. Also as a mark protein of lipid raft, flot-1 has a close interaction with cav-1. According to result of pathway analysis, besides cav-1 protein, we speculate that the flot-1 also is an important factor involved in the events at PH 72 h.

Table 2: Changes of Protein Expression

	Gene name	Protein name	MW
Up-regulated proteins			
	Abcb4	Multidrug resistance protein 2	141080
	Abcg2	ATP-binding cassette sub-family G member 2	73427
	Anpep	Aminopeptidase N	109779
	Atp1a3	Sodium/potassium-transporting ATPase subunit alpha-3	113045
	Atp1b1	Sodium/potassium-transporting ATPase subunit beta-1	35762
	Atp1b3	Sodium/potassium-transporting ATPase subunit beta-3	32151
	Atp5a1	ATP synthase subunit alpha, mitochondrial	59831
	Atp6v1b2	V-type proton ATPase subunit B, brain isoform	56857
	Cav1	Caveolin 1	20553
	Cd14	Monocyte differentiation antigen CD14	40485
	Cp	Ceruloplasmin	124393
	Ctnna1	Catenin (Cadherin associated protein), alpha 1	100858
	Cybb	Endothelial type gp91-phox	66163
	Dpp4	Dipeptidyl peptidase 4	88730
	Enpp1	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	101449
	Flot1	Flotillin-1	47755
	Flot2	Flotillin 2, isoform CRA_d	41804
	Gnas	Isoform Gnas-1 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	46091
	Hspa8	Heat shock cognate 71 kDa protein	71055
	Krt1	Keratin, type II cytoskeletal 1	65190
	Krt4	Keratin, type II cytoskeletal 4	56866
	Krt8	Keratin, type II cytoskeletal 8	53985
	Krt18	Keratin, type I cytoskeletal 18	47732
	Myh10	Myosin, heavy polypeptide 10, non-muscle, isoform CRA_b	229826
	Rap1b	Ras-related protein Rap-1b	21013
	Scarb1	Scavenger receptor class B member 1	56734
	Slco1a4	Solute carrier organic anion transporter family member 1A4	74857
	Slc22a1	Isoform 1 of Solute carrier family 22 member 1	62413
	Slc22a18	Solute carrier family 22, member 18	44245
	Slc26a1	Sulfate anion transporter 1	76540

	Slc28a2	Sodium/nucleoside cotransporter 2	73765
	Tuba1b	Tubulin alpha-1B chain	50804
	Vdac1	Voltage-dependent anion-selective channel protein 1	30851
	Xpnpep2	Membrane-bound aminopeptidase P	76545
Down-regulated proteins			
	Abcb11	Bile salt export pump	147120
	Abcc6	Multidrug resistance-associated protein 6	166771
	Anxa2	Annexin A2	38939
	Atp1a1	Sodium/potassium-transporting ATPase subunit alpha-1	113054
	Cat	Catalase	60062
	Cd59	CD59 glycoprotein	14465
	Esyt1	Extended synaptotagmin-1	121389
	Fn1	Fibronectin	275925
	Gnaq	Guanine nucleotide binding protein, alpha q polypeptide, isoform CRA_a	42416
	Napa	Alpha-soluble NSF attachment protein	33627
	Nt5e	5~ nucleotidase, ecto	64366
	Slc2a2	Solute carrier family 2, facilitated glucose transporter member 2	57370

Differentially expressed proteins of the first label-free semi-quantification analysis

	Gene name	Protein name	MW	PH72	SHam	PH72/Sham
	Abca8a	Protein Abca8	186486	0.13	0.02	6.5
	Abcb11	Bile salt export pump	147120	0.09	0.23	0.39
	Abcb1a	Multidrug resistance protein 1a	140695	0.16	0.04	4
	Abcb4	Multidrug resistance protein 2	141080	1.76	0.36	4.89
	Abcc6	Multidrug resistance-associated protein 6	166771	0.11	0.28	0.39
	Acad9	Acad9 protein	69256	0.11	0.05	2.2
	Acs11	Long-chain-fatty-acid--CoA ligase 1	79155	0.11	0.33	0.33
	Acs15	Long-chain-fatty-acid--CoA ligase 5	77211	0.37	1.11	0.33
	Actb	Actin, cytoplasmic 1	42052	0.83	0.14	5.93
	Actn4	Alpha-actinin-4	103364	0.27	0.06	4.5
	Aifm1	Apoptosis-inducing factor 1, mitochondrial	66435	0.24	0.96	0.25
	Anpep	Aminopeptidase N	109765	0.82	0.12	6.83
	Anxa2	Annexin A2	38939	0.08	0.28	0.29
	Armc1	Armadillo repeat-containing protein 1	31413	0.11	0.22	0.5
	Atad3	ATPase family AAA domain-containing protein 3	66889	0.19	0.69	0.28
	Atp1b3	Sodium/potassium-transporting ATPase subunit beta-3	32151	0.55	0.1	5.5
	Atp5c1	ATP synthase subunit gamma	33089	0.33	1.18	0.28
	Atp5f1	ATP synthase F(0) complex subunit B1, mitochondrial	28965	0.24	0.55	0.44
	Atp6v0a1	V-type proton ATPase subunit a	96321	0.16	0.03	5.33
	Atp6v1b2	V-type proton ATPase subunit B, brain isoform	56857	0.56	0.06	9.33
	Cat	Catalase	60062	0.15	0.44	0.34
	Cav1	Caveolin 1	20553	0.56	0.17	3.29
	Ccdc51	Coiled-coil domain-containing protein 51	48235	0.07	0.14	0.5
	Cers2	Longevity assurance homolog 2 (S. cerevisiae)	45093	0.24	0.11	2.18
	Cp	Ceruloplasmin	124393	0.3	0.03	10

Cpt1a	Carnitine O-palmitoyltransferase 1, liver isoform	88810	0.04	0.11	0.36
Ctnna1	Catenin (Cadherin associated protein), alpha 1	100858	1.16	0.53	2.19
Cyp1a2	Cytochrome P450 1A2	58621	1.09	0.44	2.48
Cyp2a1	Cytochrome P450 2A1	56073	0.21	0.78	0.27
Cyp2a3	Cytochrome P450 2A3	56325	0.07	0.14	0.5
Cyp2c11	Cytochrome P450 2C11	57658	0.72	0.23	3.13
Cyp2e1	Cytochrome P450 2E1	55785	0.07	0.19	0.37
Cyp4a1	Cytochrome P450 4A10	56340	0.25	0.12	2.08
Decr1	2,4-dienoyl CoA reductase 1, mitochondrial, isoform CRA_a	36395	0.1	0.34	0.29
Dpp4	Dipeptidyl peptidase 4	88730	0.04	0.13	0.31
Emc1	Protein Emc1	111628	0.22	0.54	0.41
Enpp1	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	101449	0.06	0.03	2
Ephx1	Epoxide hydrolase 1	52719	0.38	1.93	0.2
Esyt1	Extended synaptotagmin-1	121389	0.03	0.06	0.5
Exog	Endonuclease G-like 1 (Predicted), isoform CRA_d	41529	0.08	0.26	0.31
Fbp1	Fructose-1,6-bisphosphatase 1	40033	0.28	0.08	3.5
Flot1	Flotillin-1	47755	0.16	0.08	2
Flot2	Flotillin 2, isoform CRA_d	41804	0.82	0.09	9.11
Fn1	Fibronectin	275925	0.01	0.04	0.25
G6pc	Glucose-6-phosphatase	41042	0.17	0.02	8.5
Glud1	Glutamate dehydrogenase 1, mitochondrial	61719	0.16	1.18	0.14
Gm5414	Protein Gm5414	57880	0.07	0.25	0.28
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide, isoform CRA_a	42416	0.16	0.35	0.46
Gpam	Glycerol-3-phosphate acyltransferase 1, mitochondrial	94581	0.14	0.03	4.67
H2afy	Core histone macro-H2A.1	39650	0.09	0.49	0.18
Hadhb	Trifunctional enzyme subunit beta, mitochondrial	51667	0.19	0.07	2.71
Hsd17b13	17-beta-hydroxysteroid dehydrogenase 13	33815	0.11	0.37	0.3
Hspa1a	Heat shock 70 kDa protein 1A	70427	0.08	0.2	0.4
Hspa8	Heat shock cognate 71 kDa protein	72992	1	2.71	0.37
Immt	MIC complex subunit Mic60	86689	0.64	0.18	3.56
Kb15	Protein Kb15	58459	0.09	0.18	0.5
Krt10	Keratin, type I cytoskeletal 10	56950	0.06	0.12	0.5
Krt18	Keratin, type I cytoskeletal 18	47732	0.28	0.94	0.3
Krt5	Keratin, type II cytoskeletal 5	61959	0.05	0.13	0.38
Krt73	Keratin, type II cytoskeletal 73	59372	0.08	0.18	0.44
Krt8	Keratin, type II cytoskeletal 8	53985	0.29	0.85	0.34
Lman2	Lman2 protein	40709	0.23	0.49	0.47
LOC100361457	Actin, cytoplasmic 2	42108	0.1	5.28	0.02
LOC103692171	Glucosidase 1	92077	0.23	0.5	0.46
LOC684681	Protein LOC684681	21304	0.16	0.41	0.39
LOC685186	Glyceraldehyde-3-phosphate dehydrogenase	36076	0.19	0.43	0.44
Lrp1	Protein Lrp1	523489	0.04	0.01	4
Lrrc59	Leucine-rich repeat-containing protein 59	35304	0.1	0.67	0.15
Man2a1	Alpha-mannosidase 2	131786	0.37	0.1	3.7
Maoa	Amine oxidase [flavin-containing] A	60110	0.29	1.14	0.25
Maob	Amine oxidase [flavin-containing] B	59049	0.54	1.21	0.45

Mcu	Protein Mcu	36206	0.09	0.19	0.47
Mtco2	Cytochrome c oxidase subunit 2	26082	0.31	0.62	0.5
Myh10	Myosin, heavy polypeptide 10, non-muscle, isoform CRA_b	229826	0.06	0.01	6
Myh11	Myosin-11	228762	0.03	0.01	3
Myh9	Myosin-9	227566	0.58	0.24	2.42
Ncstn	Ncstn protein	67641	0.17	0.08	2.13
Ndufa10	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	40753	0.52	1.04	0.5
Ndufa9	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	42646	1.07	3.45	0.31
Ndufs1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	80331	0.22	0.65	0.34
Ndufs2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	52927	1.6	0.5	3.2
Ndufs8	NADH dehydrogenase (Ubiquinone) Fe-S protein 8 (Predicted), isoform CRA_a	24411	0.67	0.14	4.79
Ndufv1	NADH dehydrogenase (Ubiquinone) flavoprotein 1	51383	0.2	0.02	10
Ndufv2	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	27703	1.11	0.35	3.17
Nsdhl	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating	40671	0.08	0.17	0.47
Nt5e	5~ nucleotidase, ecto	64366	0.51	1.17	0.44
Opa1	Dynamin-like 120 kDa protein, mitochondrial	111751	0.07	0.03	2.33
Pdia3	Protein disulfide-isomerase A3	57044	0.32	2.07	0.15
Pecr	Peroxisomal trans-2-enoyl-CoA reductase	33423	0.1	0.25	0.4
Pgrmc1	Membrane-associated progesterone receptor component 1	21699	0.18	0.46	0.39
Phb	Prohibitin	29859	0.91	3.12	0.29
Phb2	Prohibitin-2	33148	2.51	8.48	0.3
Por	NADPH--cytochrome P450 reductase	77313	0.18	0.37	0.49
Rab1a	Protein Rab1a	36733	0.09	0.41	0.22
Rap1b	Ras-related protein Rap-1b	21013	1.36	0.58	2.34
Rpl4	60S ribosomal protein L4	47556	0.08	0.16	0.5
Scarb1	Scavenger receptor class B member 1	56734	0.14	0.06	2.33
Sdha	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	72596	0.16	0.8	0.2
Sfxn1	Sideroflexin	35850	0.86	1.95	0.44
Sfxn3	Sideroflexin	35714	0.27	0.11	2.45
Slc25a11	Mitochondrial 2-oxoglutarate/malate carrier protein	34286	1.3	0.59	2.2
Slc25a12	Protein Slc25a12	72270	0.65	0.17	3.82
Slc25a15	Protein Slc25a15	33346	1.2	0.16	7.5
Slc25a20	Mitochondrial carnitine/acylcarnitine carrier protein	33474	0.21	0.41	0.51
Slc25a42	Mitochondrial coenzyme A transporter SLC25A42	35134	0.23	0.11	2.09
Slc25a5	ADP/ATP translocase 2	33108	0.21	0.73	0.29
Slc27a5	Bile acyl-CoA synthetase	77129	0.12	0.34	0.35
Slco1a4	Solute carrier organic anion transporter family member 1A4	74857	0.2	0.09	2.22
Tap2	Antigen peptide transporter 2	78063	0.18	0.04	4.5
Tapbp	Protein Tapbp	50298	0.24	0.11	2.18
Tgm1	Protein-glutamine gamma-glutamyltransferase K	91625	0.1	0.04	2.5
Tm7sf2	Protein Tm7sf2	46700	0.15	0.61	0.25
Tmtc3	Protein Tmtc3	104852	0.04	0.14	0.29
Tomm70a	Mitochondrial import receptor subunit TOM70	62583	0.09	0.53	0.17

	Tuba1b	Tubulin alpha-1B chain	50804	0.43	0.21	2.05
	Tubb4b	Tubulin beta-4B chain	61832	0.34	0.17	2
	Ugt1a1	UDP-glucuronosyltransferase 1-1	60422	0.41	0.19	2.16
	Ugt2b	UDP-glucuronosyltransferase	61469	0.14	0.3	0.47
	Ugt2b10	UDP-glucuronosyltransferase	61568	0.12	0.05	2.4
	Ugt2b35	UDP-glucuronosyltransferase	61557	0.17	0.05	3.4
	Ugt2b7	UDP-glucuronosyltransferase 2B7	60620	0.13	0.05	2.6
	Vcp	Transitional endoplasmic reticulum ATPase	89977	0.36	0.15	2.4
	Vdac1	Voltage-dependent anion-selective channel protein 1	30828	0.48	1.51	0.32
	Xpnpep2	Membrane-bound aminopeptidase P	76545	0.33	0.04	8.25

Note : The value of PHx72 and Sham means emPAI according to the label free quantitation.

Differentially expressed proteins of the second label-free semi-quantification analysis

	Gene name	Protein name	MW	PH72	SHam	PH SHam 72/
	Abcb11	Bile salt export pump	147120	0.23	0.59	0.39
	Abcb4	Multidrug resistance protein 2	141080	0.15	0.02	7.5
	Abcc6	Multidrug resistance-associated protein 6	166771	0.06	0.17	0.35
	Abcg2	ATP-binding cassette sub-family G member 2	73427	0.09	0.04	2.25
	Acsl5	Long-chain-fatty-acid--CoA ligase 5	77211	0.34	0.09	3.78
	Actb	Actin, cytoplasmic 1	42052	0.83	0.08	10.38
	Actb12	actin, beta-like 2	42278	0.35	0.08	4.38
	Actg1	Actin, cytoplasmic 2	42108	1.88	0.08	23.5
	Anpep	Aminopeptidase N	109779	0.75	0.16	4.69
	Anxa2	Annexin A2	38939	0.05	0.27	0.19
	Anxa6	Annexin A6	76106	0.04	0.13	0.31
	Atad3a	ATPase family AAA domain-containing protein 3	66889	0.27	0.05	5.4
	Atp1a1	Sodium/potassium-transporting ATPase subunit alpha-1	113054	0.81	3.13	0.26
	Atp1b1	Sodium/potassium-transporting ATPase subunit beta-1	35762	0.19	0.09	2.11
	Atp5a1	ATP synthase subunit alpha, mitochondrial	59831	1.23	0.38	3.24
	Atp5b	ATP synthase subunit beta, mitochondrial	56318	2.91	0.71	4.1
	Atp5o	ATP synthase subunit O, mitochondrial	23440	0.31	0.14	2.21
	Atp6v1a	ATPase, H+ transporting, lysosomal V1 subunit A	68564	0.21	0.1	2.1
	Bhmt	Betaine--homocysteine S-methyltransferase 1	45404	1.67	0.37	4.51
	Capza2	F-actin-capping protein subunit alpha-2	33118	0.1	0.33	0.3
	Cat	Catalase	60062	0.11	0.05	2.2
	Cat	Catalase	60062	0.02	0.11	0.18
	Cav1	Caveolin 1	20553	1.84	0.59	3.12
	Cd14	Monocyte differentiation antigen CD14	40485	0.37	0.17	2.18
	Cd59	CD59 glycoprotein	14465	0.53	0.24	2.21
	Chdh	Choline dehydrogenase	67088	0.1	0.05	2
	Cisd2	similar to CG1458-PA	27441	0.12	0.26	0.46
	Cox4i1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	19559	8.26	2.57	3.21
	Cp	Ceruloplasmin	124393	0.57	0.08	7.13
	Cth	Cystathionine gamma-lyase	44262	0.15	0.07	2.14
	Cybb	Endothelial type gp91-phox	66163	0.1	0.05	2
	Cyp1a2	Cytochrome P450 1A2	58621	0.06	0.24	0.25
	Cyp4a2	Cytochrome P450 4A2	58274	0.83	0.25	3.32
	Dci	3,2-trans-enoyl-CoA isomerase, mitochondrial	32348	0.22	0.1	2.2
	Der1l	RCG59470, isoform CRA_a	28930	0.24	0.11	2.18
	Dpp4	Dipeptidyl peptidase 4	88774	0.39	0.08	4.88
	Ephx1	Epoxide hydrolase 1	52719	0.35	0.13	2.69
	Flot1	Flotillin-1	47755	0.23	0.09	2.56

Fmo5	Dimethylaniline monooxygenase [N-oxide-forming] 5	60530	0.11	0.05	2.2
Fn1	Fibronectin	275925	0.24	0.75	0.32
G6pc	Glucose-6-phosphatase	41042	0.36	0.08	4.5
Glud1	Glutamate dehydrogenase 1, mitochondrial	61719	0.37	0.05	7.4
Gnas	Isoform Gnas-1 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	46091	0.15	0.07	2.14
Gpsn2	Synaptic glycoprotein SC2	36498	0.09	0.3	0.3
Hadha	Trifunctional enzyme subunit alpha, mitochondrial	83297	0.21	0.08	2.63
Hist1h1d	Histone H1.2	21974	0.33	0.15	2.2
Hsd17b11	Estradiol 17-beta-dehydrogenase 11	33316	0.1	0.21	0.48
Hsd17b13	17-beta hydroxysteroid dehydrogenase 13	33815	0.32	0.1	3.2
Hspa8	Heat shock cognate 71 kDa protein	71055	0.88	0.05	17.6
Immt	82 kDa protein	82654	0.17	1.26	0.13
Krt1	Keratin, type II cytoskeletal 1	65190	0.28	0.1	2.8
Krt10	57 kDa protein	57633	0.18	0.06	3
Krt18	Keratin, type I cytoskeletal 18	47732	0.22	0.07	3.14
Krt4	Keratin, type II cytoskeletal 4	57973	0.12	0.06	2
Krt5	Keratin, type II cytoskeletal 5	61959	0.23	0.05	4.6
Krt75	keratin 75	62472	0.17	0.05	3.4
Lactb	lactamase, beta	60638	0.05	0.99	0.05
Lman1	Protein ERGIC-53	58194	0.32	0.06	5.33
LOC683313	Keratin, type II cytoskeletal 6A	59555	0.31	0.11	2.82
LOC688684	similar to 60S ribosomal protein L32	15126	0.23	0.5	0.46
Maoa	similar to Amine oxidase [flavin-containing] A	98754	0.63	0.14	4.5
Maob	Amine oxidase [flavin-containing] B	59049	1.51	0.06	25.17
Mlec	Malectin	32569	0.1	0.21	0.48
Mtch2	mitochondrial carrier homolog 2	35075	1.7	0.57	2.98
Mt-nd4	NADH-ubiquinone oxidoreductase chain 4	51937	0.13	0.06	2.17
Mtx2	Metaxin 2	30155	0.23	0.37	0.62
Myh9	Myosin-9	227566	0.6	0.29	2.07
Myo1b	Isoform C of Myosin-Ib	125868	0.03	0.08	0.38
Napa	Alpha-soluble NSF attachment protein	33627	0.1	0.21	0.48
Ndufa12	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12	21714	0.77	1.73	0.45
Ndufa4	RCG28086, isoform CRA_a	9321	0.37	1.06	0.35
Ndufa7	RCG37550, isoform CRA_a	12550	1.06	0.27	3.93
Ndufa8	Aa2-258	22408	0.15	0.32	0.47
Ndufb10	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	21131	1.81	0.34	5.32
Ndufb4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 4	15055	0.23	0.84	0.27
Ndufb5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5	21650	1.37	0.33	4.15
Ndufb6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6	15628	0.22	0.48	0.46
Ndufs1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	80331	2.32	0.04	58
Ndufs3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	30379	2.13	0.23	9.26
Nt5e	5~ nucleotidase, ecto	64366	0.41	0.97	0.42
Pdia3	Protein disulfide-isomerase A3	57499	0.95	0.32	2.97
Phb	Prohibitin	29859	16.3	7.27	2.24
Phb2	Prohibitin-2	33292	4.53	2.13	2.13
Rab18	Ras-related protein Rab-18	23247	0.5	0.14	3.57
Rap1b	Ras-related protein Rap-1b	21013	0.85	0.31	2.74

RGD1561141	similar to solute carrier family 25 (mitochondrial carrier, Aralar), member 12	51130	0.28	0.13	2.15
RGD1563422	Isoform Long of Brain protein 44	14306	0.53	0.24	2.21
RGD1563899	similar to 60S ribosomal protein L27a	13024	0.26	0.56	0.46
RGD1564131	similar to solute carrier family 25, member 5	29168	0.54	0.24	2.25
RGD1564767	Histone H2A	14180	0.25	0.54	0.46
Rmnd1	similar to CG11679-PA	50831	0.06	0.29	0.21
Rpl13	60S ribosomal protein L13	24351	0.67	0.14	4.79
Rpl19	60S ribosomal protein L19	23565	0.14	0.49	0.29
Rpl23a	60S ribosomal protein L23a	17684	0.19	0.39	0.49
Rpl24	60S ribosomal protein L24	17882	0.41	0.19	2.16
Rpl30	60S ribosomal protein L30	12947	0.6	0.26	2.31
Rpl6-ps1	similar to 60S ribosomal protein L6	33582	0.46	0.1	4.6
Rpl7	60S ribosomal protein L7	30367	1.07	0.23	4.65
Rpl8	60S ribosomal protein L8	28235	0.75	0.12	6.25
Rpn2	Dolichyl-diphosphooligosaccharide--protein glycosyl-transferase subunit 2	69149	0.2	0.05	4
Rps13	LOC684988 40S ribosomal protein S13	17212	0.43	0.2	2.15
Rps24	Isoform 1 of 40S ribosomal protein S24	15413	0.22	0.82	0.27
Rps3	40S ribosomal protein S3	26828	0.6	0.26	2.31
Rps4x	40S ribosomal protein S4, X isoform	29807	0.24	0.11	2.18
Rps6	40S ribosomal protein S6	28834	0.12	0.24	0.5
Samm50	Sorting and assembly machinery component 50 homolog	52384	1.35	0.06	22.5
Sdha	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	72596	0.3	0.14	2.14
Sec11a	Signal peptidase complex catalytic subunit SEC11A	20586	0.16	0.35	0.46
Sec61b	Sec61 beta subunit	10039	0.35	0.82	0.43
Slc22a1	Isoform 1 of Solute carrier family 22 member 1	62413	0.11	0.05	2.2
Slc22a18	solute carrier family 22, member 18	44245	0.15	0.07	2.14
Slc25a11	Mitochondrial 2-oxoglutarate/malate carrier protein	34393	0.9	0.32	2.81
Slc25a13	similar to Calcium-binding mitochondrial carrier protein Aralar2	74751	6.54	0.35	18.69
Slc25a20	Mitochondrial carnitine/acylcarnitine carrier protein	33474	1.13	0.1	11.3
Slc26a1	Sulfate anion transporter 1	76482	0.13	0.04	3.25
Slc27a2	Very long-chain acyl-CoA synthetase	71447	0.25	0.09	2.78
Slc27a5	Bile acyl-CoA synthetase	77129	0.79	0.13	6.08
Slc28a2	Sodium/nucleoside cotransporter 2	73765	0.14	0.04	3.5
Sptbn1	Non-erythrocyte beta-spectrin	251733	0.04	0.14	0.29
Sptbn2	Spectrin beta chain, brain 2	272151	0.01	0.02	0.5
Srd5a1	Isoform Long of 3-oxo-5-alpha-steroid 4-dehydrogenase 1	29989	0.23	0.11	2.09
Ssr1	Translocon-associated protein subunit alpha	35778	0.3	0.09	3.33
Tap1	Antigen peptide transporter 1	79556	0.18	0.04	4.5
Tmco1	Transmembrane and coiled-coil domains protein 1	21389	0.55	0.16	3.44
Tmed10	Transmembrane emp24 domain-containing protein 10	25013	0.29	0.65	0.45
Tmem195	Transmembrane protein 195	52064	0.2	0.06	3.33
Tomm40	Mitochondrial import receptor subunit TOM40 homolog	38294	0.09	0.18	0.5
Ugt2b	UDP-glucuronosyltransferase 2B2	61459	0.37	0.16	2.31
Uqcrc1	Cytochrome b-c1 complex subunit 1, mitochondrial	53500	0.61	0.06	10.17
Uqcrcf1	Cytochrome b-c1 complex subunit Rieske, mitochondrial	29712	0.53	0.11	4.82
Usmg5	Up-regulated during skeletal muscle growth protein 5	6460	2.77	1.74	1.59
Vdac1	Voltage-dependent anion-selective channel protein 1	30851	9.52	3.19	2.98
Xpnpep2	Membrane-bound aminopeptidase P	76545	0.19	0.07	2.71
Slco1a4	Solute carrier organic anion transporter family member 1A4	74857	0.63	0.11	5.73

	Note: The value of PHx72 and Sham means empAI according to the label free quantitation.					
Differentially expressed proteins of the third label-free semi-quantification analysis						
	Gene name	Protein name	MW	PH72	SHam	PH 72/Sham
	3 SV	Ribosomal protein L15	19471	0.38	0.07	5.43
	Abca8a	Protein Abca8	186486	0.02	0.06	0.33
	Abcb11	Bile salt export pump	147120	0.12	0.25	0.48
	Abcb4	Multidrug resistance protein 2	141080	0.67	0.07	9.57
	Abcc6	Multidrug resistance-associated protein 6	166771	0.02	0.17	0.12
	Abcd3	ATP-binding cassette sub-family D member 3	75780	0.4	0.09	4.44
	Abcg2	ATP-binding cassette sub-family G member 2	73427	0.16	0.05	3.2
	Abhd16a	Abhydrolase domain-containing protein 16A (Fragment)	48511	0.07	0.27	0.26
	Acs15	Long-chain-fatty-acid--CoA ligase 5	77211	0.13	0.04	3.25
	Agmo	Alkylglycerol monooxygenase	52064	0.06	0.15	0.4
	Aldob	Fructose-bisphosphate aldolase	39956	0.17	0.08	2.13
	Anpep	Aminopeptidase N	109779	0.06	0.03	2
	Anxa2	Annexin A2	38939	0.15	0.43	0.35
	Atp1a3	Sodium/potassium-transporting ATPase subunit alpha-3	113045	0.19	0.03	6.33
	Atp1b1	Sodium/potassium-transporting ATPase subunit beta-1	35762	0.28	0.11	2.55
	Atp1b3	Sodium/potassium-transporting ATPase subunit beta-3	32151	0.85	0.14	6.07
	Atp5a1	ATP synthase subunit alpha, mitochondrial	59831	0.89	0.32	2.78
	Atp5b	ATP synthase subunit beta	56309	1.16	0.58	2
	Atp5c1	ATP synthase subunit gamma	33089	0.16	0.38	0.42
	Atp6v1a	Protein Atp6v1a	68564	0.1	0.05	2
	Atp6v1e1	ATPase, H ⁺ transporting, V1 subunit E isoform 1, isoform CRA_a	26184	0.13	0.27	0.48
	Bdh1	3-hydroxybutyrate dehydrogenase, type 1, isoform CRA_a	38708	0.42	0.2	2.1
	Bhmt2	S-methylmethionine--homocysteine S-methyltransferase BHMT2	40261	0.37	0.09	4.11
	Cat	Catalase	60062	0.05	0.11	0.45
	Cav1	Caveolin 1	20553	0.83	0.36	2.31
	Cd14	Monocyte differentiation antigen CD14	40485	1.71	0.54	3.17
	Cd59	CD59 glycoprotein	14465	2.31	0.88	2.63
	Chchd3	Coiled-coil-helix-coiled-coil-helix domain containing 3 (Predicted), isoform CRA_a	26646	0.6	0.27	2.22
	Cox4i1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	19559	2.04	0.89	2.29
	Cox5a	Cytochrome c oxidase subunit 5A, mitochondrial	16347	0.24	0.54	0.44
	Cox6c2	Cytochrome c oxidase subunit 6C-2	8449	0.42	1	0.42
	Cox7c	Cytochrome c oxidase subunit 7C, mitochondrial	7370	1.2	0.48	2.5
	Cp	Ceruloplasmin	124393	0.85	0.13	6.54
	Ctnna1	Catenin (Cadherin associated protein), alpha 1	100858	1.66	0.67	2.48
	Cyb5r3	NADH-cytochrome b5 reductase 3	34381	0.45	0.2	2.25
	Cyb5r3	NADH-cytochrome b5 reductase 3	34381	0.45	0.22	2.05
	Cybb	Endothelial type gp91-phox	66163	0.29	0.13	2.23
	Cyp2b3	Cytochrome P450 2B3	56861	0.05	0.12	0.42
	Cyp2d26	Cytochrome P450 2D26	56876	0.06	0.17	0.35
	Cyp4b1	Cytochrome P450 4A2	58195	0.06	0.28	0.21
	Ddost	Dolichyl-diphosphooligosaccharide--protein glycosyl-transferase 48 kDa subunit	49093	0.3	0.14	2.14
	Dpp4	Dipeptidyl peptidase 4	88730	0.24	0.11	2.18
	Emc3	ER membrane protein complex subunit 3	29960	0.13	0.27	0.48
	Enpp1	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	101449	0.09	0.02	4.5

Esyt1	Extended synaptotagmin-1	121389	0.05	1.19	0.04
Flot1	Flotillin-1	47755	0.17	0.06	2.83
Fn1	Fibronectin	275925	0.63	2.08	0.3
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide, isoform CRA_a	42416	0.21	0.49	0.43
Gnas	Isoform Gnas-1 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	46091	0.38	0.13	2.92
Hist1h4b	Histone H4	11360	3.92	1.89	2.07
Hsd17b13	17-beta-hydroxysteroid dehydrogenase 13	33815	0.24	0.11	2.18
Hspa5	78 kDa glucose-regulated protein	72473	0.19	0.05	3.8
Hspa8	Heat shock cognate 71 kDa protein	71055	1.15	0.31	3.71
Hspa9	Stress-70 protein, mitochondrial	73984	0.09	0.04	2.25
Kb15	Protein Kb15	58459	0.12	0.06	2
Krt1	Keratin, type II cytoskeletal 1	65059	0.28	0.1	2.8
Krt14	Keratin, type I cytoskeletal 14	47553	0.16	0.08	2
Krt18	Keratin, type I cytoskeletal 18	47732	0.19	0.03	6.33
Krt4	Keratin, type II cytoskeletal 4	56866	0.18	0.06	3
Krt6a	Keratin, type II cytoskeletal 6A	59555	0.24	0.11	2.18
Krt6a	Keratin, type II cytoskeletal 6A	59555	0.06	0.15	0.4
Krt73	Keratin, type II cytoskeletal 73	59372	0.13	0.06	2.17
Krt8	Keratin, type II cytoskeletal 8	53985	0.13	0.06	2.17
Lman2	Lman2 protein	40709	0.29	0.75	0.39
LOC100361457	Actin, cytoplasmic 2	42108	0.57	0.19	3
LOC100910109	Protein LOC100910109	30017	0.37	0.11	3.36
Maoa	Amine oxidase [flavin-containing] A	60110	0.2	0.06	3.33
Maob	Amine oxidase [flavin-containing] B	59049	0.84	0.06	14
Mcu	Protein Mcu	36206	0.19	0.09	2.11
Mgst1	Microsomal glutathione S-transferase 1	17517	1.25	0.37	3.38
Myadm	Myadm protein	35836	0.19	0.09	2.11
Myh10	Myosin, heavy polypeptide 10, non-muscle, isoform CRA_b	229826	0.15	0.02	7.5
Napa	Alpha-soluble NSF attachment protein	33627	0.23	0.55	0.42
Ndufa2	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2	10952	0.86	0.36	2.39
Ndufs1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	80331	0.76	0.05	15.2
Ndufs3	NADH dehydrogenase (Ubiquinone) Fe-S protein 3 (Predicted), isoform CRA_c	30379	1.54	0.68	2.26
Ndufs7	NADH dehydrogenase (Ubiquinone) Fe-S protein 7	24215	0.14	0.47	0.3
Nnt	Nicotinamide nucleotide transhydrogenase	114537	0.33	0.12	2.75
Nt5e	5~ nucleotidase, ecto	64366	0.35	0.82	0.43
Phb	Prohibitin	29859	6.44	2.55	2.53
Rap1b	Ras-related protein Rap-1b	21013	0.65	0.18	3.61
Rpl4	60S ribosomal protein L4	47556	0.26	0.11	2.36
Rpl6	60S ribosomal protein L6	33582	0.46	0.21	2.19
Rpl7	60S ribosomal protein L7	30351	0.52	0.23	2.26
Rpn1	Dolichyl-diphosphooligosaccharide--protein glycosyl-transferase subunit 1	68473	0.52	0.15	3.47
Scarb1	Scavenger receptor class B member 1	56734	0.34	0.13	2.62
Sdha	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	72596	0.28	0.05	5.6
Slc22a1	Isoform 1 of Solute carrier family 22 member 1	62413	0.23	0.09	2.56
Slc25a11	Mitochondrial 2-oxoglutarate/malate carrier protein	34286	0.1	0.87	0.11
Slc25a13	Protein Slc25a13	54293	1.84	0.07	26.29

Slc25a4	ADP/ATP translocase 1	33111	1.15	0.11	10.45
Slc25a5	ADP/ATP translocase 2	33108	3.2	1.15	2.78
Slc26a1	Sulfate anion transporter 1	76540	0.04	0.1	0.4
Slc27a2	Very long-chain acyl-CoA synthetase	71447	0.14	0.06	2.33
Slc27a5	Bile acyl-CoA synthetase	77129	0.33	0.05	6.6
Slc2a2	Solute carrier family 2, facilitated glucose transporter member 2	57370	0.06	0.13	0.46
Slco1a4	Solute carrier organic anion transporter family member 1A4	74857	0.04	0.19	0.21
Tmed10	Transmembrane emp24 domain-containing protein 10	25013	0.15	0.33	0.45
Tmem205	Protein Tmem205	21456	0.55	0.16	3.44
Tomm22	Mitochondrial import receptor subunit TOM22 homolog	15481	0.49	0.22	2.23
Tomm70a	Mitochondrial import receptor subunit TOM70	62583	0.05	0.11	0.45
Tuba1b	Tubulin alpha-1B chain	50804	0.36	0.17	2.12
Uox	Uricase	35140	1.46	0.72	2.03
Vdac1	Voltage-dependent anion-selective channel protein 1	30828	7.38	1.44	5.13
Vdac3	Voltage-dependent anion-selective channel protein 3	31292	1.48	0.35	4.23
Xpnpep2	Membrane-bound aminopeptidase P	76545	0.29	0.13	2.23
Note : The value of PHx72 and Sham means empPAI according to the label free quantitation.					

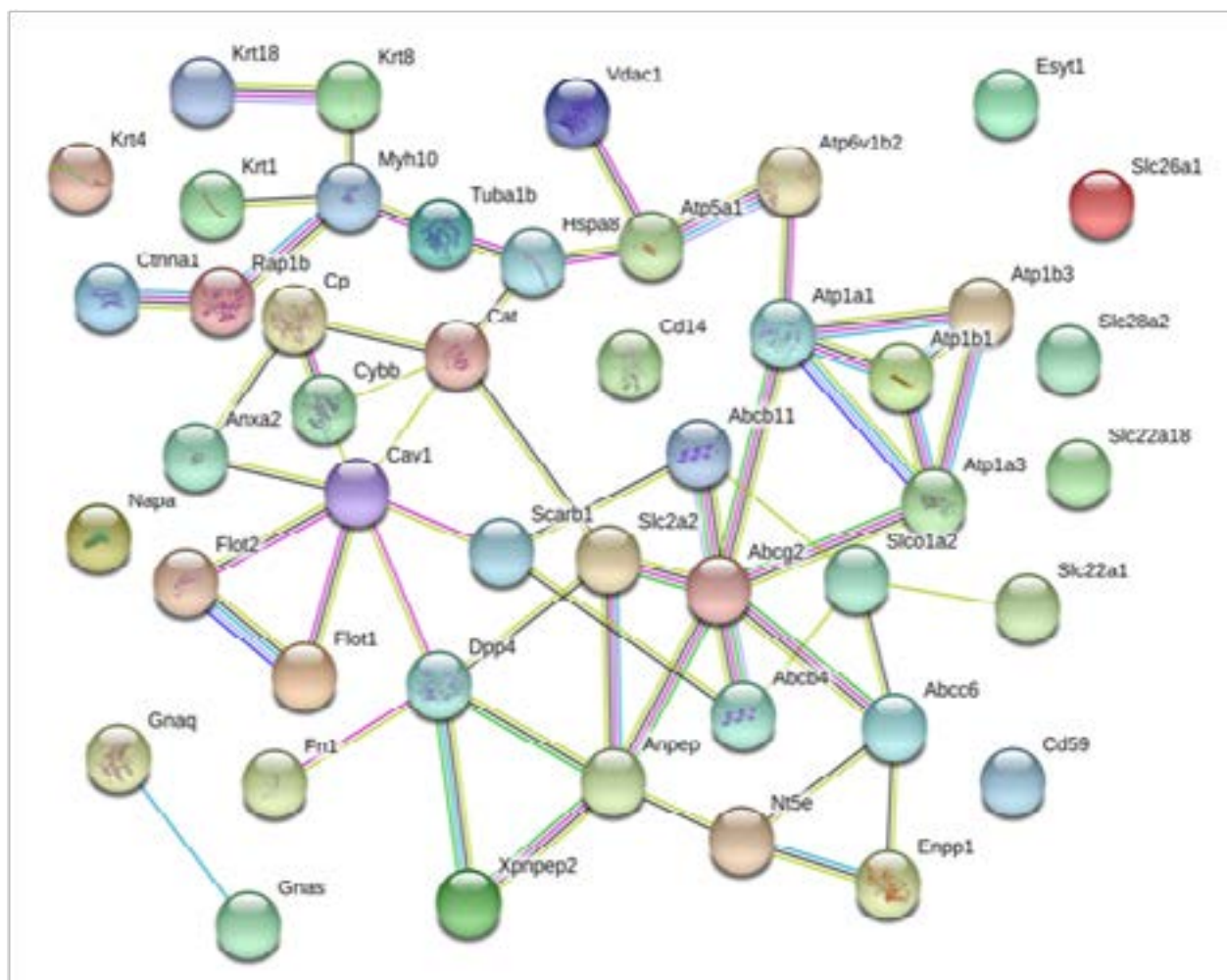


Figure 5: Construction of integrated signaling networks. Data sets obtained from proteomic analysis were uploaded to the web-based tool STRING to identify functional signaling networks. The protein-protein interaction network is presented by Gene Ontology classification. The corresponding proteins for the genes are indicated in supplemental tables 2.

5.4. Validation of Differential Protein Expression

Western blot was conducted to validate the differential expression and five differential proteins were included in present study, such as Nt5e, ATP1A-1, annexin A2, caveolin-1 and flotillin-1. As shown in (Figure 6), the expression level of Nt5e, Annexin A2, caveolin-1 and flotillin-1 in lipid raft increased in the PH 72 h when compared with that of control ($P<0.01$ for Nt5e and Caveolin-1; $P<0.05$ for Annexin A2 and Flotillin-1). In contrast, the abundance of ATP1A-1 in lipid raft after PH 72 h decreased ($P<0.05$ vs. PH 0 group) from western blotting. Western blot results of caveolin-1 and flotillin-1 were in accordance with the data from label free quantitative assay. But results of Nt5e, ATP1A-1, annexin A2 is different to label free quantitative assay which might link to that label free quantitative as-

say is semi-quantitative research. In addition, reproducibility of proteins might also be affected by physiological factors. Therefore, there will be difference between western blotting and quantitative results.

To further validate of these differential proteins expression, the messenger RNA (mRNA) levels of those molecules were determined by qRT-PCR at several time points (Figure 7A-E). These results were in accordance with the data from western blot assay. Besides, the mRNA level of some plasma membrane proteins, which expressed more than 5 times in PH72 /SHAM group, was determined by qRT-PCR, such as flotillin-2, abcb4 and xpnpep2. These results were in accordance with the data from label free quantitative assay (Figure 7F-H).

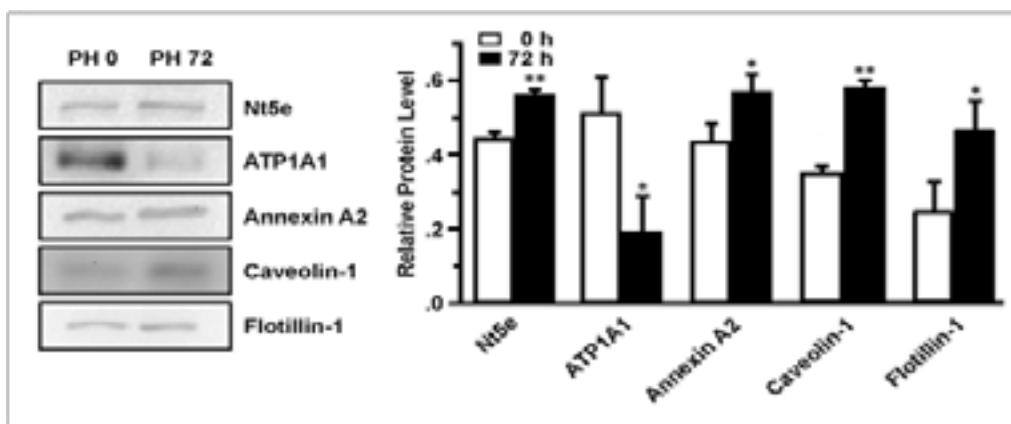


Figure 6: Western blotting and qRT-PCR analysis for Caveolin-1 and Flotillin-1. (A, B) The Western blotting results for Nt5e, Atp1a1, Annexin A2, Caveolin-1 and Flotillin-1. Sham-operation control groups and after PH groups. Each panel is the representative result of three independent experiments.

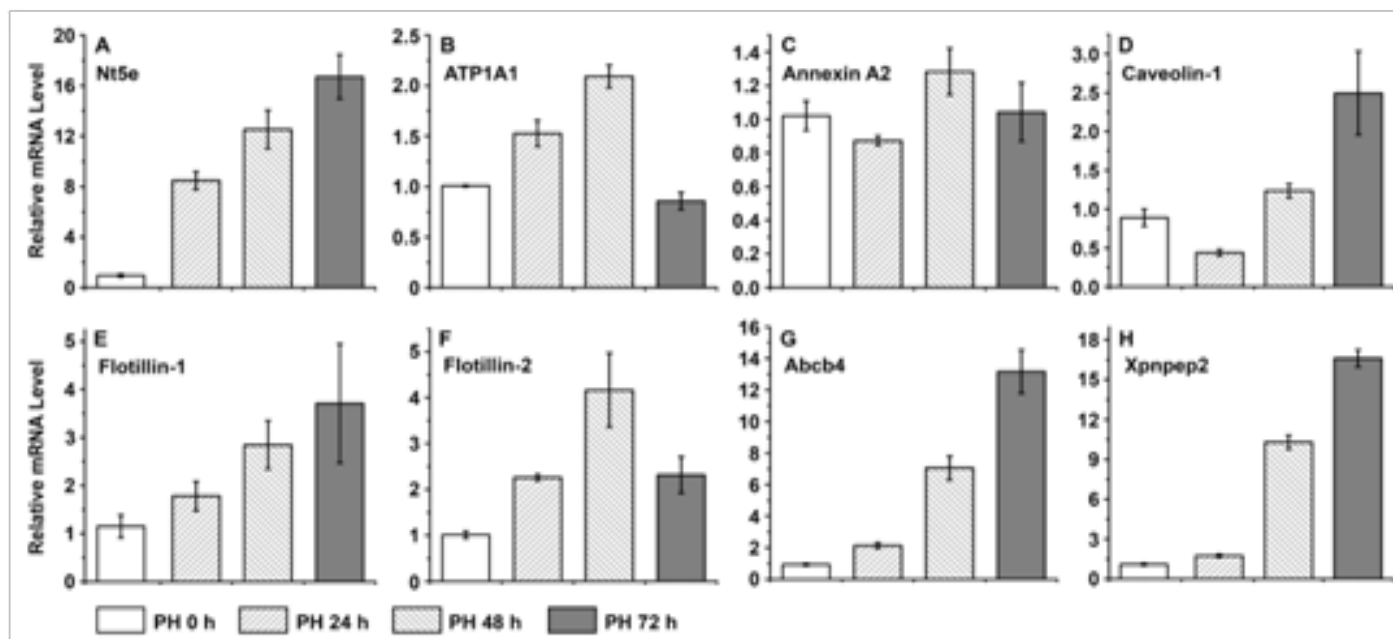


Figure 7: qRT-PCR analysis. (A-H) Nt5e, Atp1a1, Annexin A2, Caveolin-1, Flotillin-1, Flotillin-2, Abcb4 and Xpnpep2 gene expression in rat liver of PH 24 h, 48 h, 72 h and sham groups by qRT-PCR.

5.5. Illustration of Immunofluorescence and Qrt-PCR Analysis of Caveolin-1 and Flotillin-1.

We speculated that differentially expressed lipid raft protein flotillin-1 and caveolin-1 play important roles in liver regeneration. Then immunofluorescence was performed to observe the localization of flotillin-1 and caveolin-1 during liver regeneration. As shown in (Figure 8), the signal of flotillin-1 and caveolin-1 is very weak at sham-operated group whereas the scattered bright signals emerged in PH 24 h, especially around the small blood vessel liked structure. From PH 48 h, the signals become stronger and widely distributed and PH 72 h have brightest field under microscope. All above data suggested the increased expression of flotillin-1 and caveolin-1 with the progression

of live recovery after PH (Figure 8). Besides, we can also find caveolin-1 and flotillin-1 expressed on endothelial cells of new vessels at PH 24 h and PH 36 h and then they mainly expressed on hepatocyte.

To further study the effect of caveolin-1 and flotillin-1 on liver regeneration, the messenger RNA (mRNA) levels of those molecules were determined by qRT-PCR at several time points (Figure 7D, E). Compared with sham operation group, the mRNA expression level of flotillin-1 was gradually raising and caveolin-1 also had significantly higher levels after a short down-regulation at PH 24 h, which showed a consistent trend with caveolin-1 and flotillin-1 on protein level. These results indicated that caveolin-1 and flotillin-1 might play important roles during liver regeneration.

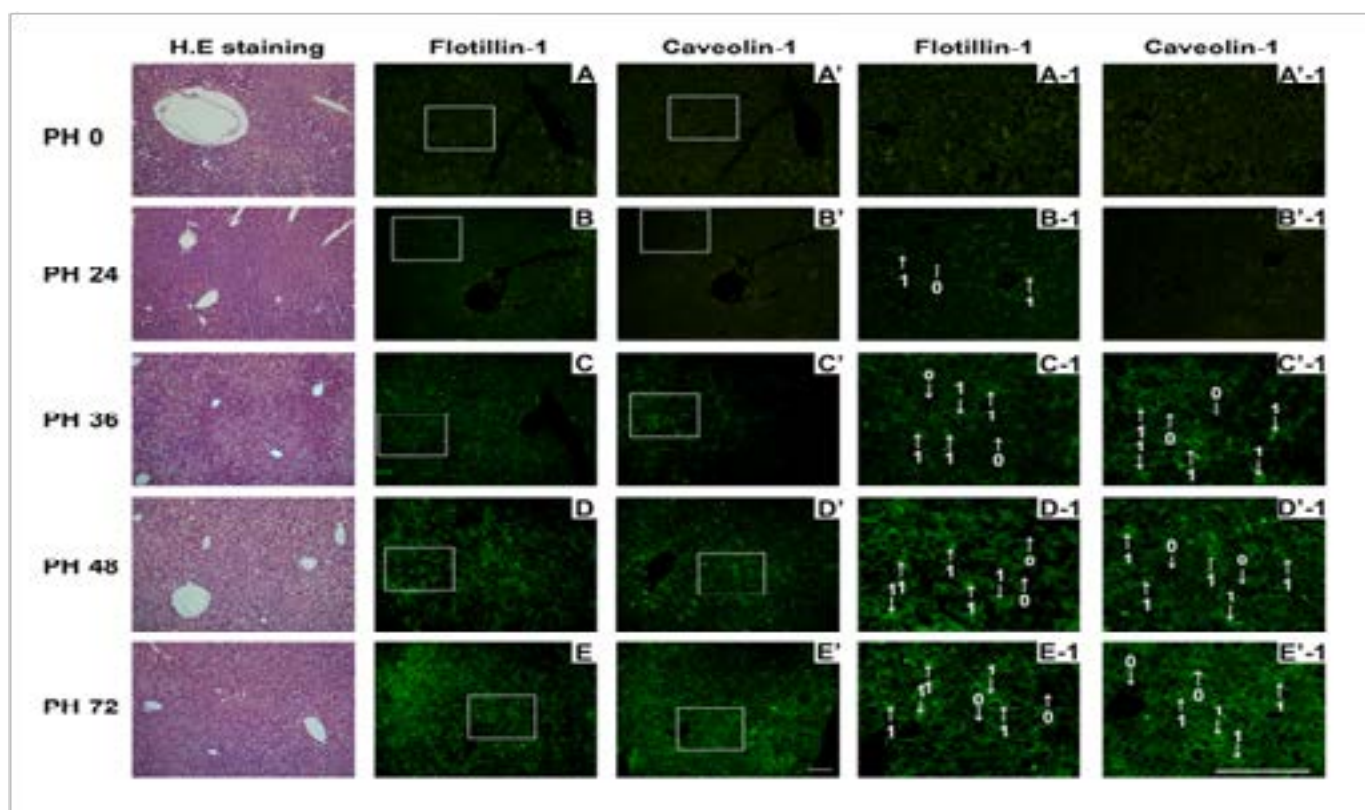


Figure 8: Expression of flotillin-1 and caveolin-1 in liver after after PH. The signals of flotillin-1 and caveolin-1 in sham-operated group (PH 0) and after PH groups (PH 24, PH 36, PH 48, PH 72) were illustrated by immunofluorescence. The nucleus were stained with Hoechst as shown in blue. Fluorescence was observed using a Nikon EclipseE600 microscope. The right two columns of immune fluorescence spectrum is local enlarged drawing of the corresponding white box in second and third column. The arrows "1" indicate hepatic sinusoid within green fluorescence, and arrows "0" indicate hepatic sinusoid without green fluorescence. Scale bars, 100 μ m.

6. Discussion

Recently, the progress of liver regeneration has been widely studied through proteomics and transcriptomics [24-27]. Among them, most of the studies focus on the change of molecules at early stage, i.e., 24 h after PH. In this period, liver cell proliferation is the major event and cell cycle-related proteins were identified frequently. However, only a few reports were focused on the late stage of liver recovery, i.e., 72 hours after PH [19, 28-29].

Our previous study has been revealed the proteomic profile of liver sinusoidal endothelial cells (LSECs) in rat liver [19]. Present study was concentrated on the variation of lipid raft proteins during liver regeneration. The lipid raft proteins were separated on 1D SDS-PAGE and identified by LC-MS/MS. According to available published data, this lipid raft proteome is the largest one in rat liver. It need to be mentioned that it still have contamination from cytosolic proteins (around 11.6%), nuclear proteins (3.1%) and ribosomal

proteins (11.4%) in this work. Given the known sub cellular localization, those proteins did not affect analysis of lipid raft proteins. By using the same method, there were 26.52% cytosolic proteins included in lipid raft data [30]. And 28.72% cytosolic proteins in the lipid raft data [31]. It might indicated that the contamination from cytosolic proteins or other organells is difficult to remove by sucrose gradient ultracentrifugation and further modification is worth to be explored for pure preparation of lipid raft.

Our previous work on the profile of plasma membrane covered partial information of lipid rafts [19]. Among them, 98 proteins were identified repeatedly in this study. In addition, the profile was identified by including 196 proteins in total and 90 proteins were also detected in this study [14]. Proteins expression level between PH 0 h and PH 72 h were analyzed by label free quantification strategies, and 46 proteins changed dramatically during partial hepatectomy and were involved in liver regeneration in this study, especially angiogenesis. For molecules especially expressed at after PH 72 h, [28] identified 218 proteins in total, 83 of which presented over 2-fold differentially expressing level. Among them, 20 proteins were included in our profile of 72 hours after surgery. In addition, the transcriptomics data after PH 72 h identified 767 transcripts, which might be corresponding to 767 proteins and 30 proteins of them were also presented in our study. Among 30 transcripts, the corresponding proteins of one mRNAs, i.e., monocyte differentiation antigen CD14 showed similar pattern with this study [29].

As key molecules of lipid rafts, flotillin-1 and caveolin-1 play important roles in different physiological and pathological conditions. Caveolin-1 is essential molecular in cancer occurrence, development, invasive metastatic and prognosis [32, 33]. It has been reported that caveolin-1 is essential for liver regeneration, which mainly functions in the formation of the lipid droplets and new membrane, and also energy supply [34, 35]. The expression intensities of caveolin-1 was mainly localized at LSECs and hepatic stellate in liver cancers and para-cancer liver tissues [36]. In this work, the expression of caveolin-1 was upregulated slightly on plasma membrane of LSECs after PH, which confirmed the importance of caveolin-1 and also illustrate the reliability of our data.

Flotillin-1 could provide a platform for the interaction between proteins in lipid raft and involve in vesicular trafficking and signal transduction [23, 37-38]. Besides roles in the biomembrane, flotillin-1 might participate in development and progression of tumor genesis and angiogenesis [39-41]. The study has confirmed flotillin-1 was over expressed in Hepatocellular Carcinoma (HCC) and indicated flotillin-1 played an essential role in HCC [42]. Furthermore, other report indicated that the hyper expression of caveolin-1 and

flotillin-1 could contribute to development of HCC though active Toll-like receptors signaling [43], but it was not clear whether parenchymal cells proliferation was dependent on flotillin-1 in liver. Here, our data suggested that flotillin-1 might play a role in liver regeneration. It could be also speculated from the interaction network that flotillin-1 may provide signal transduction platform for angiogenesis factor such as integrin β 1, vascular endothelial growth factor-A and fibronectin-1. But it has not been reported that flotillin has relation with LSECs. And molecular mechanisms of flotillin-1 in the angiogenesis, especially liver regeneration, need to be further studied.

In addition, The trends of flotillin-1 expression was consistent with caveolin-1 in liver cancers and paracancer liver tissues [43] and had slightly up-regulated in this work. We speculate that the differential expression of caveolin-1 and flotillin-1 might be one of the differences between normal liver regeneration and liver cancer.

In summary, our data presented a large lipid raft proteome database. Furthermore, differential expression profile after PH 72 h and sham-operated group in liver were also revealed. Among them, flotillin-1 and caveolin-1 as scaffold proteins could play important roles in angiogenesis, which may provide further insight into the mechanism of angiogenesis and the regulation of liver regeneration.

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