

## Supplementary Information

### A trial proteomics fingerprint analysis of HepaRG cells by FD-LC-MS/MS

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## **Experimental**

### Preparation of HepaRG cell lysates

Frozen HepaRG cells were purchased from Biopredic International (Rennes, France). To prepare HepaRG cell lysates, the frozen cells ( $12 \times 10^6$  cells) were thawed and centrifuged. The pelleted cells were suspended in 1,200  $\mu\text{L}$  of cell lysis buffer (phosphate buffered saline, 0.50% Triton X-114, Halt Protease Inhibitor Cocktail (Pierce, Waltham, MA, USA), and incubated at 4.0°C for 1.0 h. The cells were homogenized by homogenizer (I.S.O. Inc., Kanagawa, Japan), left on ice for 30 min and then centrifuged at 13,000g for 15 min at 4.0°C. The supernatant was recovered. Total protein concentration was measured by BCA protein assay kit (Pierce) using bovine serum albumin (BSA) as a standard.

### Conditions for FD-LC-MS/MS method

HepaRG protein lysate (40  $\mu\text{g}/35 \mu\text{L}$ ) was mixed with 40  $\mu\text{L}$  of a mixture of 1.25 mM Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and 5.0 mM ethylenediamine-N, N, N', N'-tetraacetic acid sodium salt (Na<sub>2</sub>EDTA) in pH 8.7 guanidine buffer solution; 20  $\mu\text{L}$  of 2.5% Triton X-114 in pH 8.7 guanidine buffer solution; and 5.0  $\mu\text{L}$  of 140 mM DAABD-C1 (Tokyo Chemical Industry, Tokyo, Japan) in acetonitrile. After the reaction mixture had been placed in a 40°C water bath for 10 min, 3.0  $\mu\text{L}$  of 20% trifluoroacetic acid (TFA) was added to stop the derivatization reaction. Twenty microliters (equivalent to 8.0  $\mu\text{g}$  protein) of the reaction mixture was injected into the LC system. The LC system consisted of a pump (L-2130; Hitachi, Tokyo, Japan), a column oven (L-2350; Hitachi), and a fluorescence detector (L-2485; Hitachi). For the stationary phase to separate the derivatized proteins, we used columns of core

shell materials (WIDEPOR<sup>®</sup> XB-C8, 250 x 4.6 mm i.d., 3.6  $\mu$ m particle, Phenomenex, USA), either singly or as 3 connected columns with a column temperature of 30°C, 40°C, 50°C, 60°C, or 70°C. The excitation and emission wavelengths for detecting fluorescence were modified from those previously adopted and were set at 395 nm and 505 nm, respectively. The flow cell volume was 3.0  $\mu$ L. The gradient program for a single column was as follows: 0→5→20→25→40→120→450→510→585→600 min; B 0→0→30→30→30→40→60→70→100→100% ((A) acetonitrile:isopropanol:water:TFA=9.0:1.0:90:0.10, v/v/v/v; (B) acetonitrile:isopropanol:water:TFA=74:1.0:25:0.10, v/v/v/v). The flow rates were gradually changed from 0.10 to 0.60 mL/min. The gradient program for the three connected columns was similar to that for a single column except that the flow rate was changed to 0.40→0.40→0.40→0.20→0.20→0.20→0.20→0.20→0.20→0.20 mL/min and temperature was set to 60°C. The HPLC chromatograms were obtained by Hitachi EZChrom software (v. 3.1.7J).

#### Identification of the proteins

Each protein peak eluted from the column(s) (a single one, or the 3 connected) was concentrated to 5.0  $\mu$ L under reduced pressure. The residual material was diluted with 50  $\mu$ L of a 250 mM ammonium bicarbonate solution (pH 7.8) containing 0.50 U trypsin (Promega, Madison, WI, USA) and 10 mM calcium chloride, and the resultant mixture was incubated for 2.0 h at 37°C. The solution was subsequently concentrated to 20  $\mu$ L. The peptide mixture (6.0  $\mu$ L) was directly injected into a nano-LC-ESI-MS/MS system (UltiMate 3000, LTQ Orbitrap XL Thermo Fisher Scientific, Waltham, MA, USA), which combines a hybrid FT mass spectrometer with a linear ion trap MS and an Orbitrap mass

analyzer. Peptides were loaded on a pre column (LC Packings, CA, USA; phase: C18PM), followed by elution and separation on the spray tip column (3.0 µm particle, 75 µm i.d. × 100 mm, Nikkyo Technos, Tokyo, Japan) at a flow rate of 0.30 µL/min, employing a gradient from 0% to 40% for buffer B (0.10% formic acid in 80% acetonitrile) over a period of 35 min (buffer A: 0.10% formic acid in 2.0% acetonitrile). Peptides were eluted into the MS via a spray tip column, which was used with a spray voltage ranging from 1000 to 2000 V, depending on the state of the tip column, and the transfer tube temperature was set at 200°C. Data-dependent acquisition was controlled by the Tune Plus software (Thermo Fisher Scientific, Waltham, MA, USA). For database searching, the Mascot version 2.3.01 program was used and the data were searched against the IPI human database (version 3.74, 89,575 proteins). The variable modification was set as DAABD for cysteine residues. The peptide tolerance was set at 5.0ppm and the MS/MS tolerance was set at 0.70Da. Under these conditions, Mascot scores of more than 21 were given.

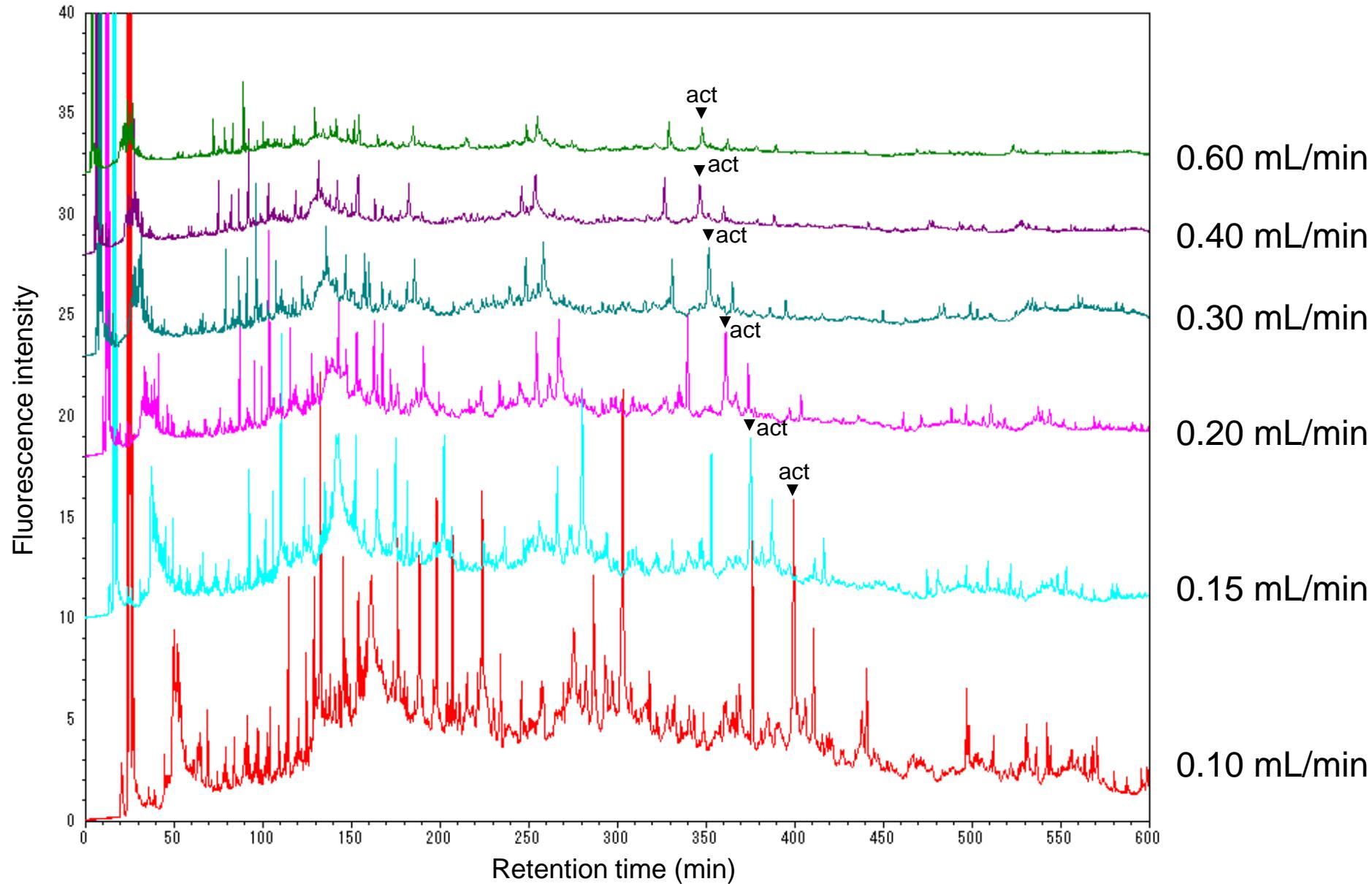
Protein identification corresponding to each peak was performed as described previously<sup>1,2</sup>; the protein name with the significantly largest score was assigned to the corresponding peak. The protein name(s) for the second or the third largest score(s) numbers but similar to those for the first assigned protein was (were) assigned to the same peak, since the peak might have overlapped on account of low efficient separation under the present HPLC conditions. The protein names derived from obviously recognizable contaminants, such as keratin, as well as those without cysteine residues were deleted. If the peak shape was not asymmetrical, the protein name(s) for the second or the third largest score(s) numbers was (were) added to the list, in addition to the protein name for the first largest score number.

## Bioinformatics analysis of identified proteins

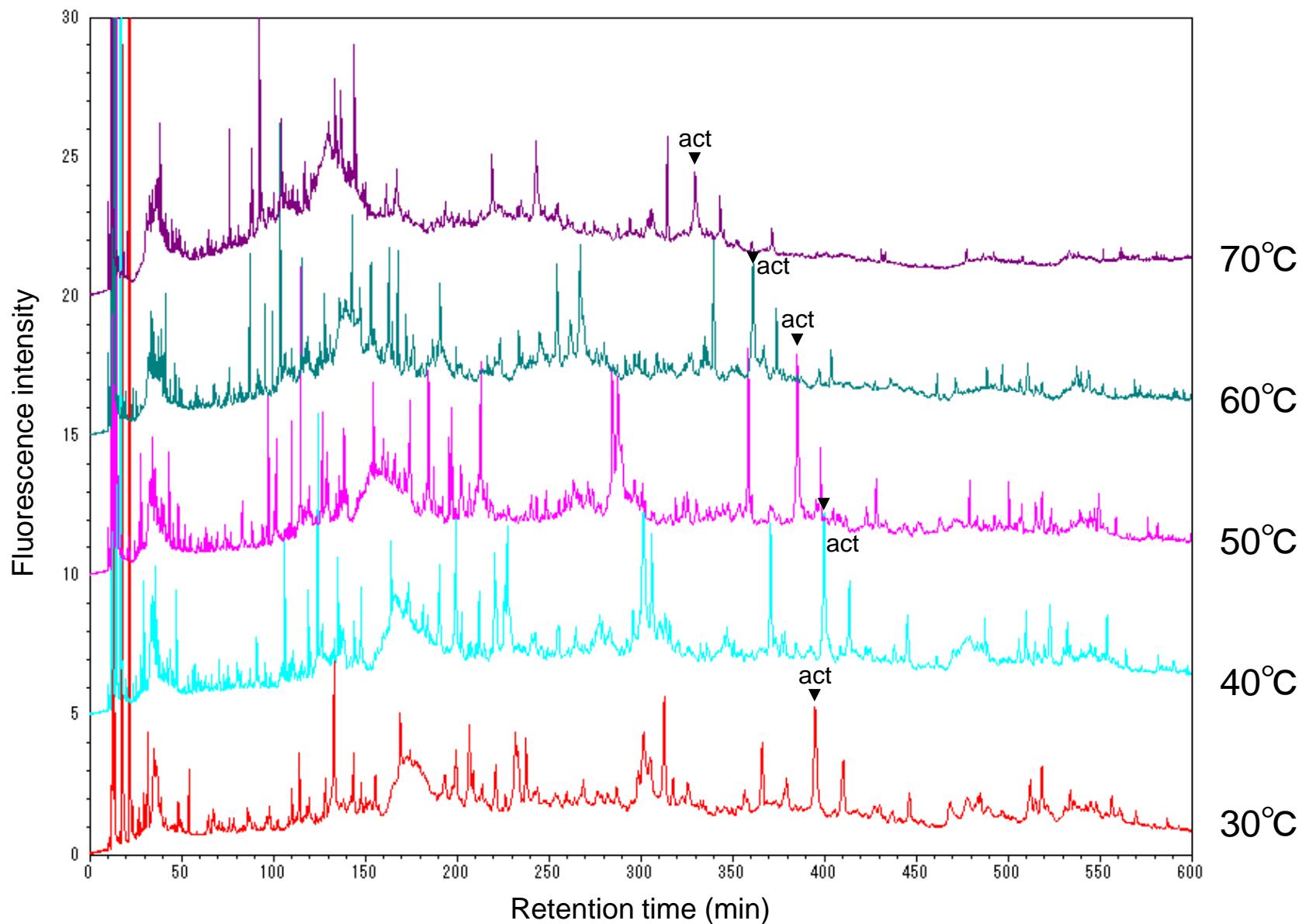
Protein annotations were obtained from DAVID (<http://david.abcc.ncifcrf.gov/>) as Gene Ontology (GO).<sup>3</sup>

## References

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2. K. Nakata, R. Saitoh, J. Amano, T. Ichibangase, M. Ishigai, and K. Imai, *Biomed. Chromatogr.*, 2014, **28**, 742-750.
3. D. W. Huang, B. T. Sherman, and R. A. Lempicki, *Nat. Protoc.*, 2009, **4**, 44–57.



Supplementary Figure 1. Chromatograms of the protein mixtures (8.0 ug protein/ injection) obtained from the HepaRG extract at flow rates of 0.10, 0.15, 0.20, 0.30, 0.40, and 0.60 mL/min at a column temperature of 60°C. Arrowheads indicate peaks identified as actin.



Supplementary Figure 2. Chromatograms of the protein mixtures (8.0 ug protein/ injection) obtained from the HepaRG extract at column temperatures of 30°C, 40°C, 50°C, 60°C, and 70°C mL/min and at a flow rate of 0.20 mL/min. Arrowheads indicate peaks identified as actin.

Supplementary Table 1. List of HepaRG proteins identified by the FD-LC-MS/MS method

Accession No.	Gene symbol	Protein name	Score	Mass (Da)	Peptides	Mitochondrion	Endoplasmic reticulum	Cytoskeleton	Ribosomes	Nucleolus	Cytosol	hepatocyte specific	HepaRG 2D-PAGE (Narayan, 2008)	HepaRG Shotgun (Sokolowska, 2012)	HepaRG Shotgun (Petraru, 2013)
IPI00296635	GBE1	1,4-alpha-glucan-branched enzyme	261	80409	17						x				
IPI00220362	HSPE1	10 kDa heat shock protein, mitochondrial	104	10925	2	x									
IPI00290553	ALDH1L1	10-formyltetrahydrofolate dehydrogenase	738	98767	49							x			
IPI00216318	YWHAB	14-3-3 protein beta/alpha	337	28065	15						x		x	x	
IPI0000816	YWHAE	14-3-3 protein epsilon	192	29155	10	x					x		x	x	
IPI00021263	YWHAZ	14-3-3 protein zeta/delta	395	27728	20	x		x				x	x		
IPI00003482	DEC1	2,4-dienoyl-CoA reductase, mitochondrial	172	36045	10	x								x	
IPI00016703	DHCR24	24-dehydrocholesterol reductase	117	60062	6	x									
IPI00023919	PSMC5	26S proteasome regulatory subunit 8	343	45597	20					x					
IPI00011603	PSMD3	26S proteasome non-ATPase regulatory subunit 3	248	60939	17					x					
IPI00019927	PSMD7	26S proteasome non-ATPase regulatory subunit 7	283	37002	11										
IPI00017726	HSD17B10	3-hydroxyacyl-CoA dehydrogenase type-2	756	26906	25	x	x								
IPI00001539	ACAA2	3-ketoacyl-CoA thiolase, mitochondrial	183	41898	12	x									
IPI00012828	ACAA1	3-ketoacyl-CoA thiolase, peroxisomal	271	44264	13										
IPI00221092	RPS16	40S ribosomal protein S16	283	16435	15			x	x			x			
IPI00017448	RPS21	40S ribosomal protein S21	301	9106	24			x	x						
IPI00011253	RPS3	40S ribosomal protein S3	462	26671	26			x	x						
IPI00021840	RPS6	40S ribosomal protein S6	109	28663	8			x	x	x					
IPI00216587	RPS8	40S ribosomal protein S8	392	24190	16			x	x	x			x		
IPI00413108	RPSA	40S ribosomal protein SA	266	33293	9			x	x	x					
IPI00784154	HSPD1	60 kDa heat shock protein, mitochondrial	694	61016	25	x				x			x		
IPI00024933	RPL12	60S ribosomal protein L12	378	17808	11			x	x					x	
IPI00465361	RPL13	60S ribosomal protein L13	157	24247	7			x	x					x	
IPI00419919	RPL29	60S ribosomal protein L29	96	17741	5			x	x						
IPI00215790	RPL38	60S ribosomal protein L38	230	8213	9			x	x						
IPI00003918	RPL4	60S ribosomal protein L4	257	47667	11			x	x						
IPI00790342	RPL6	60S ribosomal protein L6	402	32871	34			x	x						
IPI00299573	RPL7A	60S ribosomal protein L7a	407	29977	22			x	x				x		
IPI00012772	RPL8	60S ribosomal protein L8	108	28007	6			x	x						
IPI00031691	RPL9	60S ribosomal protein L9	231	21850	15			x	x	x				x	
IPI00219525	PGD	6-phosphogluconate dehydrogenase, decarboxylating	238	53106	12								x	x	
IPI00030363	ACAT1	Acetyl-CoA acetyltransferase, mitochondrial	301	45171	12	x									
IPI00017855	ACO2	Aconitate hydratase, mitochondrial	433	85372	27	x									
IPI00021439	ACTB	Actin, cytoplasmic 1	308	41710	19		x		x			x	x	x	
IPI00028031	ACADVL	Acyl-CoA dehydrogenase, mitochondrial	366	75162	16	x									
IPI00644771	ACSM2A	Acyl-coenzyme A synthetase ACSM2A, mitochondrial	156	64182	10	x									
IPI00329444	ACSM2B	Acyl-coenzyme A synthetase ACSM2B, mitochondrial	445	64230	18	x						x			
IPI00215914	ARF1	ADP-ribosylation factor 1	178	20684	7					x		x	x	x	
IPI00473031	ADH1B	Alcohol dehydrogenase 1B	394	39829	24					x					
IPI00296183	ALDH3A1	Aldehyde dehydrogenase, dimeric NADP-preferring	154	50347	21	x				x			x		
IPI00006663	ALDH2	Aldehyde dehydrogenase, mitochondrial	223	56346	13	x							x	x	
IPI00029715	AOX1	Aldehyde oxidase	358	147822	31										
IPI00105407	AKR1B10	Aldo-keto reductase family 1 member B10	344	35998	19										
IPI00291483	AKR1C3	Aldo-keto reductase family 1 member C3	500	36821	19										
IPI00013808	ACTN4	Alpha-actinin-4	268	104788	17		x		x					x	
IPI00221234	ALDH7A1	Alpha-amino adipic semialdehyde dehydrogenase	321	58450	22	x									
IPI00465248	ENO1	Alpha-enolase	351	47139	23					x		x	x	x	
IPI00008483	MAOA	Amine oxidase [flavin-containing] A	503	59644	28	x									
IPI00218918	ANXA1	Annexin A1	101	38690	5		x				x	x	x	x	
IPI00418169	ANXA2	Annexin A2	143	40386	8						x	x	x	x	
IPI00793199	ANXA4	Annexin A4	329	36062	13							x	x	x	
IPI00329801	ANXA5	Annexin A5	105	35914	6								x	x	
IPI00002459	ANXA6	Annexin A6 isoform 2	488	75229	26										
IPI00220007	APOL2	Apolipoprotein-L2	141	49735	5							x			
IPI00554742	API5	Apoptosis inhibitor 5	121	56735	4										
IPI00038356	ARG1	Arginase-1	38	25340	1						x				
IPI00220267	ASL	Argininosuccinate lyase	279	51625	18										
IPI00219029	GOT1	Aspartate aminotransferase, cytoplasmic	390	46219	19					x			x		
IPI00550523	ATL3	Atlastin-3	118	60503	8	x									
IPI00440493	ATP5A1	ATP synthase subunit alpha, mitochondrial	275	59714	13	x						x	x	x	
IPI00029133	ATP5F1	ATP synthase subunit b, mitochondrial	400	28890	26	x					x	x	x	x	

IPI00218200	BCAP31	B-cell receptor-associated protein 31	113	27974	10	x			x							
IPI00219575	BLMH	Bleomycin hydrolase	34	52528	1											
IPI00218342	MTHFD1	C-1-tetrahydrofolate synthase, cytoplasmic	278	101495	16	x										
IPI00218695	CALD1	Caldesmon	363	64218	22		x									
IPI00216682	CNN3	Calponin-3	170	36391	7		x				x					
IPI00020599	CALR	Calreticulin	192	48112	11	x			x			x		x	x	
IPI00011062	CPS1	Carbamoyl-phosphate synthase [ammonia], mitochondrial	553	164835	41	x					x					
IPI00295386	CBR1	Carboxyl reductase [NADPH] 1	231	30356	10											
IPI00383046	CMBL	Carboxymethylenebutenolidase homolog	225	28030	12											
IPI00465436	CAT	Catalase	34	59719	1	x	x		x			x				
IPI00295741	CTSB	Cathepsin B	480	37797	18	x								x		
IPI00012011	CFL1	Cofilin-1	291	18491	15		x					x		x	x	
IPI00015833	CHCHD3	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial	228	26136	13	x										
IPI00021828	CSTB	Cystatin-B	162	11133	7			x								
IPI00442073	CSRP1	Cysteine and glycine-rich protein 1	275	20554	14											
IPI00305383	UQCRC2	Cytchrome b-c1 complex subunit 2, mitochondrial	749	48413	22	x										
IPI00465315	CYCS	Cytochrome c	56	11741	2	x				x		x		x	x	
IPI00218144	COX17	Cytochrome c oxidase copper chaperone	85	6910	5	x										
IPI00006579	COX4I1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	265	19564	16	x										
IPI00021785	COX5B	Cytochrome c oxidase subunit 5B, mitochondrial	61	13687	6	x										
IPI00465138	CYP3A4	Cytochrome P450 3A4	179	57306	10	x										
IPI00011200	PHGDH	D-3-phosphoglycerate dehydrogenase	134	56614	5											
IPI00106913	DHRS4	Dehydrogenase/reductase SDR family member 4	130	29518	10	x										
IPI00006957	DHRS7	Dehydrogenase/reductase SDR family member 7	167	38274	9											
IPI00011416	ECH1	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	325	35793	14	x										
IPI00217871	ALDH4A1	Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial	345	61681	19	x				x		x				
IPI00014439	QDPR	Dihydropteridine reductase	351	25773	12											
IPI00257508	DPYSL2	Dihydropyrimidinase-related protein 2	148	62255	7	x	x									
IPI00002506	ALG5	Dolichyl-phosphate beta-glucosyltransferase	81	36922	2	x										
IPI00329536	EEA1	Early endosome antigen 1	381	162367	55				x							
IPI00010810	ETFA	Electron transfer flavoprotein subunit alpha, mitochondrial	192	35058	9	x						x				
IPI00004902	ETFB	Electron transfer flavoprotein subunit beta	692	27826	35	x										
IPI00396485	EEF1A1	Elongation factor 1-alpha 1	362	50109	12				x			x				
IPI00023048	EEF1D	Elongation factor 1-delta	272	31103	8				x			x		x	x	
IPI00024911	ERP29	Endoplasmic reticulum resident protein 29	565	28975	25	x					x		x			
IPI00027230	HSP90B1	Endoplasmic	237	92411	14	x			x		x	x	x	x	x	
IPI00256376	ECHDC3	Enoyl-CoA hydratase domain-containing protein 3, mitochondrial	329	32673	15	x										
IPI00009896	EPHX1	Epoxide hydrolase 1	223	52915	11	x										
IPI00025491	EIF4A1	Eukaryotic initiation factor 4A-I	121	46125	8				x			x		x	x	
IPI00022744	CSE1L	Exportin-2	88	110346	3											
IPI00010290	FABP1	FABP1 protein (Fragment)	465	15083	25				x							
IPI00375441	FUBP1	Far upstream element-binding protein 1	213	67518	17			x								
IPI00026781	FASN	Fatty acid synthase	392	273254	30				x		x	x	x	x	x	
IPI00007797	FABP5	Fatty acid-binding protein, epidermal	41	15155	1											
IPI00333619	ALDH3A2	Fatty aldehyde dehydrogenase	278	54813	22	x										
IPI00375676	FTL	Ferritin	285	21283	10		x		x							
IPI00021891	FGG	Fibrinogen gamma chain	149	51479	11											
IPI00289334	FLNB	Filamin-B	596	277990	44		x									
IPI00783862	BLVRB	Flavin reductase	608	22105	37											
IPI00465439	ALDOA	Fructose-bisphosphate aldolase A	668	39395	27	x						x	x			
IPI00218407	ALDOB	Fructose-bisphosphate aldolase B	502	39448	27	x			x							
IPI00019383	GALK1	Galactokinase	351	45329	13				x							
IPI00031583	USO1	General vesicular transport factor p115	301	109127	12				x							
IPI00216008	G6PD	Glucose-6-phosphate 1-dehydrogenase	110	63786	4	x		x			x		x			
IPI00027497	GPI	Glucose-6-phosphate isomerase	482	63107	22											
IPI00026154	PRKCSH	Glucosidase 2 subunit beta	99	60096	4	x										
IPI00016801	GLUD1	Glutamate dehydrogenase 1, mitochondrial	303	61359	15	x										
IPI00016862	GSR	Glutathione reductase, mitochondrial	295	56221	21	x			x			x		x	x	
IPI00219673	GSTK1	Glutathione S-transferase kappa 1	452	25480	19	x					x		x	x	x	
IPI00219018	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	443	36030	31								x	x	x	
IPI00216279	GATM	Glycine amidinotransferase, mitochondrial	697	44854	47	x										
IPI00037448	GRHPR	Glyoxylate reductase/hydroxypyruvate reductase	238	35646	13				x		x	x	x	x	x	
IPI00004962	GOLM4	Golgi integral membrane protein 4	197	81831	8											
IPI00002149	SAR1B	GTP-binding protein SAR1b	162	22396	8	x										
IPI00641737	HP	Haptoglobin	77	46693	3						x					
IPI00304925	HSPA1A;HSPA1B	Heat shock 70 kDa protein 1A/1B	417	70009	21	x					x	x	x	x	x	
IPI00003865	HSPA8	Heat shock cognate 71 kDa protein	245	70854	14				x		x	x	x	x	x	
IPI00025512	HSPB1	Heat shock protein beta-1	114	22768	5	x						x		x	x	

IPI00382470	HSP90AA1	Heat shock protein HSP 90-alpha	568	98099	31				x		x	x
IPI00414676	HSP90AB1	Heat shock protein HSP 90-beta	241	83212	16						x	x
IPI00215965	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	131	38723	12			x				
IPI00216049	HNRNPK	Heterogeneous nuclear ribonucleoprotein K	276	50944	14			x				
IPI00396378	HNRNPA2B1	Heterogeneous nuclear ribonucleoproteins A2/B1	114	37407	7			x		x	x	
IPI00239077	HINT1	Histidine triad nucleotide-binding protein 1	220	13793	14		x					x
IPI00003362	HSPA5	HSPA5 protein	284	72377	10	x			x			x
IPI00006934	HAO1	Hydroxyacid oxidase 1	323	40898	20							
IPI00217561	ITGB1	Integrin beta-1	198	91560	12							
IPI00027223	IDH1	Isocitrate dehydrogenase [NADP] cytoplasmic	131	46630	3			x				
IPI00011107	IDH2	Isocitrate dehydrogenase [NADP], mitochondrial	256	50877	14	x						
IPI00021405	LMNA	Lamin-A/C	100	74095	2		x					
IPI00783271	LRPPRC	Leucine-rich PPR motif-containing protein, mitochondrial	203	157805	22	x	x					
IPI00027444	SERPINB1	Leukocyte elastase inhibitor	442	42715	23					x	x	
IPI00010180	CES1	Liver carboxylesterase 1	81	62481	4	x			x	x	x	
IPI00012728	ACSL1	Long-chain-fatty-acid-CoA ligase 1	282	77893	15	x	x		x			
IPI00448095	DCXR	L-xylulose reductase	199	25897	10							x
IPI00916111	MDH1	Malate dehydrogenase	82	38631	7			x		x	x	x
IPI00291006	MDH2	Malate dehydrogenase, mitochondrial	184	35481	9	x						
IPI00005040	ACADM	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	149	46559	10	x						
IPI00168565	CNNM3	Metal transporter CNNM3	39	76072	1							
IPI00784044	MCCC2	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	528	61294	24	x						
IPI00024990	ALDH6A1	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	415	57803	21	x						
IPI00024934	MUT	Methylmalonyl-CoA mutase, mitochondrial	244	83068	9	x						
IPI00022300	METTL7A	Methyltransferase-like protein 7A	63	28301	4							
IPI00003842	MAP2	Microtubule-associated protein 2	73	199404	5		x					
IPI00043863	MAP4	Microtubule-associated protein 4	34	88222	2		x					
IPI00003833	MTCH2	Mitochondrial carrier homolog 2	207	33309	12	x						
IPI00554469	IMMT	Mitochondrial inner membrane protein	467	82574	27	x						
IPI00219365	MSN	Moesin	349	67778	37		x	x		x	x	
IPI00335168	MYL6	Myosin light polypeptide 6	354	16919	16		x					
IPI00033494	MYL12B	Myosin regulatory light chain 12B	257	19767	7		x				x	
IPI00019502	MYH9	Myosin-9	263	226392	19		x		x	x	x	x
IPI00219301	MARCKS	Myristoylated alanine-rich C-kinase substrate	33	31536	1		x					
IPI00003527	SLC9A3R1	Na(+)-H(+) exchange regulatory cofactor NHE-RF1	341	38845	21		x			x		
IPI00328415	CYB5R3	NADH-cytochrome b5 reductase 3	163	34213	11	x	x			x		
IPI00021812	AHNAK	Neuroblast differentiation-associated protein AHNAK	112	628699	2							
IPI00018873	NAMPT	Nicotinamide phosphoribosyltransferase	298	55487	12			x		x		
IPI00026105	SCP2	Non-specific lipid-transfer protein	300	58956	15	x						
IPI00444262	NCL	Nucleolin	244	65922	13			x			x	
IPI00010414	PDLIM1	PDZ and LIM domain protein 1	78	36049	7		x					
IPI00419585	PP1A	Peptidyl-prolyl cis-trans isomerase A	402	18001	38				x			
IPI00646304	PP1B	Peptidyl-prolyl cis-trans isomerase B	544	23728	32	x						
IPI00218775	FKBP5	Peptidyl-prolyl cis-trans isomerase FKBP5	893	51180	45							
IPI00006658	PIN4	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 4	118	16598	5	x	x	x				
IPI00000874	PRDX1	Peroxiredoxin-1	795	22096	69	x				x	x	
IPI00220301	PRDX6	Peroxiredoxin-6	289	25019	16			x				
IPI00216164	EHHADH	Peroxisomal bifunctional enzyme	392	79445	25	x						
IPI00019912	HSD17B4	Peroxisomal multifunctional enzyme type 2	334	79636	13	x						
IPI00219446	PEBP1	Phosphatidylethanolamine-binding protein 1	105	21044	2	x	x					
IPI00797038	PCK2	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	143	70685	12	x			x			
IPI00219526	PGM1	Phosphoglucomutase-1	272	61411	9			x				
IPI00169383	PGK1	Phosphoglycerate kinase 1	301	44586	21				x	x	x	
IPI00549725	PGAM1	Phosphoglycerate mutase 1	385	28786	20			x		x	x	
IPI00008524	PABPC1	Polyadenylate-binding protein 1	241	70626	21			x		x		
IPI00329600	SCCPDH	Probable saccharopine dehydrogenase	224	47121	8							
IPI00003964	USP9X	probable ubiquitin carboxyl-terminal hydrolase FAF-X isoform 4	31	290278	1							
IPI00216691	PFN1	Profilin-1	348	15045	16		x			x	x	
IPI00017334	PHB	Prohibitin	427	29786	12	x						
IPI00744115	PCCA	Propionyl-CoA carboxylase alpha chain, mitochondrial isoform a precursor	453	80008	22	x						
IPI00292657	PTGR1	Prostaglandin reductase 1	472	35847	23							
IPI00000783	PSMB8	Proteasome subunit beta type-8	247	30335	9							
IPI00010796	P4HB	Protein disulfide-isomerase	1050	57081	68	x			x	x	x	
IPI00025252	PDIA3	Protein disulfide-isomerase A3	1004	56747	45	x				x	x	x
IPI00009904	PDIA4	Protein disulfide-isomerase A4	627	72887	39	x				x	x	x
IPI00299571	PDIA6	Protein disulfide-isomerase A6	260	53867	5	x				x		
IPI00298547	PARK7	Protein DJ-1	300	19878	21	x			x			x
IPI00026530	LMAN1	Protein ERGIC-53	159	57513	9	x						

IPI ID	Protein Name	Accession	Molecular Weight	Number of Proteins	73	36	35	20	17	78
IPI00328715	MTDH	Protein LYRIC	48	63799	3	x		x		
IPI00007047	S100A8	Protein S100-A8	61	10828	4					
IPI00939362	S100A9	Putative uncharacterized protein S100A9	38	9377	2			x		
IPI00479186	PKM2	Pyruvate kinase isozymes M1/M2	414	57900	17			x		x x
IPI00000792	CRYZ	Quinone oxidoreductase	190	35185	13					
IPI00903145	RDX	Radixin	346	68521	32	x				x
IPI00299048	IQGAP2	Ras GTPase-activating-like protein IQGAP2	228	180465	8	x				
IPI00020436	RAB11B	Ras-related protein Rab-11B	183	24473	6					
IPI00291928	RAB14	Ras-related protein Rab-14	123	23882	7	x		x		
IPI00031169	RAB2A	Ras-related protein Rab-2A	193	23531	4	x				
IPI00218914	ALDH1A1	Retinal dehydrogenase 1	505	54827	36			x		x x
IPI00005038	HRSP12	Ribonuclease UK114	81	14485	2					
IPI00555744	RPL14	Ribosomal protein L14 variant	257	23772	9	x	x			
IPI00514399	RPS27	Ribosomal protein S27	66	7352	5		x	x		
IPI00215743	RRBP1	Ribosome-binding protein 1	325	152381	18	x	x			
IPI00012303	SELENBP1	Selenium binding protein 1	244	56830	13			x		
IPI00014177	SEPT_2	Septin-2	500	41461	27	x	x			
IPI00002519	SHMT1	Serine hydroxymethyltransferase, cytosolic	226	53049	14	x		x		
IPI00002520	SHMT2	Serine hydroxymethyltransferase, mitochondrial	210	55958	10	x				
IPI00022463	TF	Serotransferrin	335	77000	28			x		
IPI00745872	ALB	Serum albumin	443	69321	20					
IPI00004267	SIGMAR1	Sigma non-opioid intracellular receptor 1	90	21469	4	x				
IPI00303063	PDS5A	Sister chromatid cohesion protein PDS5 homolog A	143	146575	4					
IPI00003419	C11orf58	Small acidic protein	82	20320	1					
IPI00006482	ATP1A1	Sodium/potassium-transporting ATPase subunit alpha-1	189	112824	10					
IPI00029601	CTTN	Src substrate cortactin	229	61549	11	x				
IPI00007765	HSPA9	Stress-70 protein, mitochondrial	334	73635	14	x				x
IPI00013894	STIP1	Stress-induced-phosphoprotein 1	327	62599	27					
IPI00217232	SUCLA2	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	157	48009	11	x				
IPI00218733	SOD1	Superoxide dismutase [Cu-Zn]	108	15926	7	x			x	
IPI00298994	TLN1	Talin-1	223	269599	17	x	x			x
IPI00297779	CCT2	T-complex protein 1 subunit beta	224	57452	22		x	x		
IPI00784090	CCT8	T-complex protein 1 subunit theta	250	59583	13			x		
IPI00027626	CCT6A	T-complex protein 1 subunit zeta	314	57988	24			x		
IPI00024919	PRDX3	Thioredoxin-dependent peroxide reductase, mitochondrial	419	27675	11	x		x		x x
IPI00216293	TST	Thiosulfate sulfurtransferase	474	33408	22	x				
IPI00744692	TALDO1	Transaldolase	315	37516	15		x		x	x
IPI00022774	VCP	Transitional endoplasmic reticulum ATPase	446	89266	27	x	x		x	x
IPI00643920	TKT	Transketolase	391	68698	28		x		x	x
IPI00018768	TSN	Translin	206	26167	11					
IPI00031522	HADHA	Trifunctional enzyme subunit alpha, mitochondrial	335	82947	14	x				x
IPI00022793	HADHB	Trifunctional enzyme subunit beta, mitochondrial	314	51262	21	x				
IPI00465028	TPI1	Triosephosphate isomerase isoform 2	289	30772	11		x		x	x
IPI00218319	TPM3	Tropomyosin alpha-3 chain	702	29015	40	x			x	
IPI00027107	TUFM	Tu translation elongation factor, mitochondrial precursor	512	49843	29	x				
IPI00007752	TUBB2C	Tubulin beta-2C chain	133	49799	4	x	x			x
IPI00550917	TWF2	Twinfilin-2	119	39523	8					
IPI00179330	RPS27A	Ubiquitin and ribosomal protein S27a precursor	49	17953	1		x	x	x	x
IPI00552452	UBA1	Ubiquitin-like modifier activating enzyme 1	93	25036	1				x	
IPI00031420	UGDH	UDP-glucose 6-dehydrogenase	412	54989	27		x			x
IPI00451965	UGT1A6	UDP-glucuronosyltransferase 1-6	149	60711	8	x				
IPI00301491	UGT2B4	UDP-glucuronosyltransferase 2B4	59	60473	8	x				
IPI00219953	CMPK1	UMP-CMP kinase isoform a	566	25838	18					x
IPI00006211	VAPB	Vesicle-associated membrane protein-associated protein B/C	130	27211	7	x				
IPI00216308	VDAC1	Voltage-dependent anion-selective channel protein 1	394	30754	13	x				
IPI00024145	VDAC2	Voltage-dependent anion-selective channel protein 2	121	30393	3	x				
IPI00257882	PEPD	Xaa-Pro dipeptidase	322	54513	14					

Number of proteins 73 36 35 20 17 78

Supplementary Table 2. List of HepaRG proteins identified by the FD-LC-MS/MS method but not by 2D-PAGE or Shotgun proteomics

Accession No.	Gene symbol	Protein name	Score	Mass (Da)	Peptides	Cellular localization
IPI00296635	GBE1	1,4-alpha-glucan-branched enzyme	261	80409	17	C
IPI00220362	HSPE1	10 kDa heat shock protein, mitochondrial	104	10925	2	M
IPI00016703	DHCR24	24-dehydrocholesterol reductase	117	60062	6	E
IPI00023919	PSMC5	26S protease regulatory subunit 8	343	45597	20	N
IPI00011603	PSMD3	26S proteasome non-ATPase regulatory subunit 3	248	60939	17	C
IPI00019927	PSMD7	26S proteasome non-ATPase regulatory subunit 7	283	37002	11	
IPI00017726	HSD17B10	3-hydroxyacyl-CoA dehydrogenase type-2	756	26906	25	M/E
IPI00001539	ACAA2	3-ketoacyl-CoA thiolase, mitochondrial	183	41898	12	M
IPI00012828	ACAA1	3-ketoacyl-CoA thiolase, peroxisomal	271	44264	13	
IPI00017448	RPS21	40S ribosomal protein S21	301	9106	24	R/C
IPI00011253	RPS3	40S ribosomal protein S3	462	26671	26	R/C
IPI00021840	RPS6	40S ribosomal protein S6	109	28663	8	R/N/C
IPI00413108	RPSA	40S ribosomal protein SA	266	33293	9	R/C
IPI000419919	RPL29	60S ribosomal protein L29	96	17741	5	R/C
IPI00215790	RPL38	60S ribosomal protein L38	230	8213	9	R/C
IPI00003918	RPL4	60S ribosomal protein L4	257	47667	11	R/C
IPI00790342	RPL6	60S ribosomal protein L6	402	32871	34	R/C
IPI00012772	RPL8	60S ribosomal protein L8	108	28007	6	R/C
IPI00030363	ACAT1	Acetyl-CoA acetyltransferase, mitochondrial	301	45171	12	M
IPI00017855	ACO2	Aconitase hydratase, mitochondrial	433	85372	27	M
IPI00028031	ACADVL	Acyl-CoA dehydrogenase, mitochondrial	366	75162	16	M
IPI00644771	ACSM2A	Acyl-coenzyme A synthetase ACSM2A, mitochondrial	156	64182	10	M
IPI00329444	ACSM2B	Acyl-coenzyme A synthetase ACSM2B, mitochondrial	445	64230	18	M
IPI00473031	ADH1B	Alcohol dehydrogenase 1B	394	39829	24	C
IPI00029715	AOX1	Aldehyde oxidase	358	147822	31	
IPI00105407	AKR1B10	Aldo-keto reductase family 1 member B10	344	35998	19	
IPI00291483	AKR1C3	Aldo-keto reductase family 1 member C3	500	36821	19	
IPI00221234	ALDH7A1	Alpha-aminoacidic semialdehyde dehydrogenase	321	58450	22	M
IPI00008483	MAOA	Amine oxidase [flavin-containing] A	503	59644	28	M
IPI00793199	ANXA4	Annexin A4	329	36062	13	
IPI00002459	ANXA6	Annexin A6 isoform 2	488	75229	26	
IPI00220007	APOL2	Apolipoprotein-L2	141	49735	5	
IPI00554742	API5	Apoptosis inhibitor 5	121	56735	4	
IPI00038356	ARG1	Arginase-1	38	25340	1	
IPI00220267	ASL	Argininosuccinate lyase	279	51625	18	
IPI00550523	ATL3	Atlastin-3	118	60503	8	E
IPI00218200	BCAP31	B-cell receptor-associated protein 31	113	27974	10	E/C
IPI00219575	BLMH	Bleomycin hydrolase	34	52528	1	
IPI00218342	MTHFD1	C-1-tetrahydrofolate synthase, cytoplasmic	278	101495	16	M
IPI00218695	CALD1	Caldesmon	363	64218	22	CS
IPI00011062	CPS1	Carbamoyl-phosphate synthase [ammonia], mitochondrial	553	164835	41	M
IPI00295386	CBR1	Carbonyl reductase [NADPH] 1	231	30356	10	
IPI00383046	CMBL	Carboxymethylenebutenolidase homolog	225	28030	12	
IPI00015833	CHCHD3	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial	228	26136	13	M
IPI00021828	CSTB	Cystatin-B	162	11133	7	N
IPI00442073	CSRPA1	Cysteine and glycine-rich protein 1	275	20554	14	
IPI00305383	UQCRC2	Cytochrome b-c1 complex subunit 2, mitochondrial	749	48413	22	M
IPI00218144	COX17	Cytochrome c oxidase copper chaperone	85	6910	5	M
IPI00006579	COX41	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	265	19564	16	M
IPI00021785	COX5B	Cytochrome c oxidase subunit 5B, mitochondrial	61	13687	6	M
IPI00465138	CYP3A4	Cytochrome P450 3A4	179	57306	10	E
IPI00011200	PHGDH	D-3-phosphoglycerate dehydrogenase	134	56614	5	
IPI00106913	DHRS4	Dehydrogenase/reductase SDR family member 4	130	29518	10	M
IPI00006957	DHRS7	Dehydrogenase/reductase SDR family member 7	167	38274	9	
IPI00011416	ECH1	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	325	35793	14	M
IPI00014439	QDPR	Dihydropteridine reductase	351	25773	12	
IPI00257508	DPYSL2	Dihydropyrimidinase-related protein 2	148	62255	7	M/CS
IPI00002506	ALG5	Dolichol-phosphate beta-glucosyltransferase	81	36922	2	E
IPI00329536	EEA1	Early endosome antigen 1	381	162367	55	C
IPI00004902	ETFB	Electron transfer flavoprotein subunit beta	692	27826	35	M
IPI00256376	ECHDC3	Enoyl-CoA hydratase domain-containing protein 3,	329	32673	15	M
IPI00009896	EPHX1	Epoxide hydrolase 1	223	52915	11	E
IPI00022744	CSE1L	Exportin-2	88	110346	3	
IPI00010290	FABP1	FABP1 protein (Fragment)	465	15083	25	C
IPI00375441	FUBP1	Far upstream element-binding protein 1	213	67518	17	N
IPI00007797	FABP5	Fatty acid-binding protein, epidermal	41	15155	1	
IPI00375676	FTL	Ferritin	285	21283	10	R/C
IPI00021891	FGG	Fibrinogen gamma chain	149	51479	11	
IPI00218407	ALDOB	Fructose-bisphosphate aldolase B	502	39448	27	CS/C
IPI00019383	GALK1	Galactokinase	351	45329	13	C
IPI00031583	USO1	General vesicular transport factor p115	301	109127	12	C
IPI00027497	GPI	Glucose-6-phosphate isomerase	482	63107	22	
IPI0026154	PRKCSH	Glucosidase 2 subunit beta	99	60096	4	E
IPI00216279	GATM	Glycine amidinotransferase, mitochondrial	697	44854	47	M
IPI00004962	GOLM4	Golgi integral membrane protein 4	197	81831	8	
IPI00002149	SAR1B	GTP-binding protein SAR1b	162	22396	8	E
IPI00641737	HP	Haptoglobin	77	46693	3	
IPI00215965	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	131	38723	12	N
IPI00216049	HNRNPK	Heterogeneous nuclear ribonucleoprotein K	276	50944	14	N
IPI00006934	HAO1	Hydroxyacid oxidase 1	323	40898	20	
IPI00217561	ITGB1	Integrin beta-1	198	91560	12	
IPI00027223	IDH1	Isocitrate dehydrogenase [NADP] cytoplasmic	131	46630	3	C
IPI00011107	IDH2	Isocitrate dehydrogenase [NADP], mitochondrial	256	50877	14	M
IPI00021405	LMNA	Lamin A/C	100	74095	2	CS
IPI00783271	LRPPRC	Leucine-rich PPR motif-containing protein, mitochondrial	203	157805	22	M/CS
IPI00012728	ACSL1	Long-chain-fatty-acid-CoA ligase 1	282	77893	15	M/E/C
IPI00291006	MDH2	Malate dehydrogenase, mitochondrial	184	35481	9	C
IPI00005040	ACADM	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	149	46559	10	M
IPI00168565	CNNM3	Metal transporter CNNM3	39	76072	1	
IPI00784044	MCCC2	Methylcrotonyl-CoA carboxylase beta chain, mitochondrial	528	61294	24	M

IPI00024990	ALDH6A1	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	415	57803	21	M
IPI00024934	MUT	Methylmalonyl-CoA mutase, mitochondrial	244	83068	9	M
IPI00022300	METTL7A	Methyltransferase-like protein 7A	63	28301	4	
IPI00003842	MAP2	Microtubule-associated protein 2	73	199404	5	CS
IPI00043863	MAP4	Microtubule-associated protein 4	34	88222	2	CS
IPI0003833	MTCH2	Mitochondrial carrier homolog 2	207	33309	12	M
IPI00554469	IMMT	Mitochondrial inner membrane protein	467	82574	27	M
IPI00335168	MYL6	Myosin light polypeptide 6	354	16919	16	CS
IPI00219301	MARCKS	Myristoylated alanine-rich C-kinase substrate	33	31536	1	CS
IPI00328415	CYB5R3	NADH-cytochrome b5 reductase 3	163	34213	11	M/E/C
IPI00021812	AHNAK	Neuroblast differentiation-associated protein AHNAK	112	628699	2	
IPI00026105	SCP2	Non-specific lipid-transfer protein	300	58956	15	M
IPI00010414	PDLIM1	PDZ and LIM domain protein 1	78	36049	7	CS
IPI00646304	PPIB	Peptidyl-prolyl cis-trans isomerase B	544	23728	32	E
IPI00218775	FKBP5	Peptidyl-prolyl cis-trans isomerase FKBP5	893	51180	45	
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IPI00220301	PRDX6	Peroxiredoxin-6	289	25019	16	C
IPI00216164	EHHADH	Peroxisomal bifunctional enzyme	392	79445	25	M
IPI00219446	PEBP1	Phosphatidylethanolamine-binding protein 1	105	21044	2	M/E/CS
IPI00797038	PCK2	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	143	70685	12	M
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IPI00020436	RAB11B	Ras-related protein Rab-11B	183	24473	6	
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IPI00745872	ALB	Serum albumin	443	69321	20	
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IPI00303063	PDS5A	Sister chromatid cohesion protein PDS5 homolog A	143	146575	4	
IPI00003419	C11orf58	Small acidic protein	82	20320	1	
IPI00006482	ATP1A1	Sodium/potassium-transporting ATPase subunit alpha-1	189	112824	10	
IPI00029601	CTTN	Src substrate cortactin	229	61549	11	CS
IPI00013894	STIP1	Stress-induced-phosphoprotein 1	327	62599	27	
IPI00217232	SUCLA2	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	157	48009	11	M
IPI000218733	SOD1	Superoxide dismutase [Cu-Zn]	108	15926	7	M/C
IPI00297779	CCT2	T-complex protein 1 subunit beta	224	57452	22	N/C
IPI00784090	CCT8	T-complex protein 1 subunit theta	250	59583	13	C
IPI00027626	CCT6A	T-complex protein 1 subunit zeta	314	57988	24	C
IPI00216293	TST	Thiosulfate sulfurtransferase	474	33408	22	M
IPI00018768	TSN	Translin	206	26167	11	
IPI00022793	HADHB	Trifunctional enzyme subunit beta, mitochondrial	314	51262	21	M
IPI00027107	TUFM	Tu translation elongation factor, mitochondrial precursor	512	49843	29	M
IPI00550917	TWF2	Twinfilin-2	119	39523	8	
IPI00451965	UGT1A6	UDP-glucuronosyltransferase 1-6	149	60711	8	E
IPI00301491	UGT2B4	UDP-glucuronosyltransferase 2B4	59	60473	8	E
IPI00006211	VAPB	Vesicle-associated membrane protein-associated protein B/C	130	27211	7	E
IPI00216308	VDAC1	Voltage-dependent anion-selective channel protein 1	394	30754	13	M
IPI00024145	VDAC2	Voltage-dependent anion-selective channel protein 2	121	30393	3	M
IPI00257882	PEPD	Xaa-Pro dipeptidase	322	54513	14	

Cellular components: M; mitochondrion, E; endoplasmic reticulum, CS; cytoskeleton, R; ribosome and C; cytosol