Protein interactomics based on direct molecular fishing on paramagnetic particles: Practical realization and further SPR validation

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Abbreviations: CYB5A, human cytochrome b5 (microsomal); TTR, human transthyretin (prealbumin); CYP1B1, CYP3A5, CYP2C9, human cytochromes P450 types 1B1, 3A5 and 2C9, respectively; PMP, paramagnetic particles; PPI, protein–protein interactions

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ABSTRACT

There is increasing evidence that proteins function in the cell as integrated stable or temporally formed protein complexes, interactomes. Previously, using model systems we demonstrated applicability of direct molecular fishing on paramagnetic particles for protein interactomics (Ershov et al. Proteomics, 2012, 12, 3295). In the present study we have used a combination of affinity based molecular fishing and subsequent mass spectrometry for investigation of human liver proteins involved in interactions with immobilized microsomal cytochrome b5 (CYB5A), also transthyretin and BSA as alternative affinity ligands (baits). The LC−MS/MS identification of prey proteins fished on these baits revealed three sets of proteins: 98, 120, and 220, respectively. Comparison analysis of these sets revealed only three proteins common for all the baits. In the case of paired analysis, the number of common proteins varied from 2 to 9. The binding capacity of some identified proteins has been validated by a SPR-based biosensor. All the investigated proteins effectively interacted with the immobilized CYB5A (Kd values ranged from 0.07 μM to 1.1 μM). Results of this study suggest that direct molecular fishing is applicable for analysis of protein-protein interactions (PPI) under normal and pathological conditions, in which altered PPIs are especially important.

INTRODUCTION

The study of protein-protein interactions (PPI) provides new insights in our understanding of protein functions as integrated stable or temporally formed protein complexes, interactomes [1].

It employs various approaches including bioinformatic, genomic, and biochemical technologies [2-4]. Biochemical approaches use a common principle of molecular fishing of proteins and their subsequent mass spectrometry (MS) identification. Molecular fishing is a type of affinity purification based on specific interaction between an immobilized ligand (bait molecule) and its putative (one or more) functionally competent partners (prey molecules) present in natural biological systems [4-6]. Various compounds ranged from small organic molecules, to proteins or nuclear acids can be used as baits.

Although productive capacity of biochemical approaches is not as high as genomic ones (and so they are not applicable for serial screenings), identification of PPI under physiological conditions appears to be more reliable. The latter may be illustrated by the fact that proteomic profiling of rat and mouse brain proteins using a small nonpeptide endogenous substance isatin as an affinity ligand revealed more than sixty individual proteins and subsequent optical biosensor-based analysis of all selected purified proteins demonstrated specific interaction with the immobilized ligand [7].

Recently, using model protein mixtures and paramagnetic particles (PMP) we have demonstrated applicability of direct molecular fishing for protein interactomics [8] and its effectiveness for interactomic analysis of real biological objects [9]. In the present study this approach has been employed for PPI identification in the human liver preparation using microsomal cytochrome b5 (CYB5A), as well as transthyretin (TTR) and bovine serum albumin (BSA) as alternative baits.

CYB5A is a highly conservative hemoprotein [10] involved in various electron

transport reactions in endoplasmic reticulum (microsomes) [11], where it can potentially interact with various proteins of the cytochrome P450 superfamily [12]. However, direct interaction has been experimentally demonstrated only for limited number of P450 proteins.

Transthyretin (TTR) functions as a serum carrier of the thyroid hormone thyroxine (T4) and also retinol-binding protein with retinol [13]. It is produced in the liver and secreted into the blood. TTR plays a role in the development of several amyloid diseases [14] and Alzheimer's Disease [15].

BSA plays various functions unrelated to CYB5A and TTR and therefore it has been used as a control for nonspecific binding [16].

All this suggests existence of various protein partners, which may be potentially involved in the interaction with these proteins.

MATERIALS AND METHODS

A liver tissue sample from a male subject was obtained from the ILSbio, LLC (www.ilsbio.com).

High-purity samples of human recombinant cytochrome b5 (microsomal form, CYB5A) and cytochromes P450 (CYP1B1, CYP3A5 and CYP2C9) were obtained as previously described [8, 17]. All proteins were expressed in *E. coli* and purified using metal affinity chromatography on an NiHiTrap-chelating column followed by anion-exchange chromatography on a DE-agarose column (CYB5A) or hydroxylapatite chromatography (P450s). Rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was purified as described previously [18]. The human transthyretin (TTR) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (USA). All the individual proteins were highly purified (at least 95% purity by SDS-PAGE), and functionally competent.

Liver tissue samples were homogenized in 0.05 M potassium-phosphate buffer, pH 7.4,

using an Ultra-Turrax T8 homogenizer (IKA, Germany). The resultant homogenate was lyzed using the CelLytic MT reagent and Protease Inhibitor Cocktail (Sigma-Aldrich, USA) optimized for lysis of mammalian tissues and protein extraction.

All SPR measurements were carried out using an optical biosensor Biacore 3000 operated under Biacore Control v.3.1 and BiaEvaluation v.4.1 software, standard optical chips CM5 and special reagents purchased from GE Healthcare (USA). Protein immobilization on the optical chip was carried out by forming covalent bonds between protein amino groups and carboxyl groups on the chip surface in accordance with the standard Biacore protocol for amine coupling procedure [19]. Molecular interactions were registered as sensograms representing the real time records of the biosensor signal.

Functionality of CYB5A immobilized on the optical chip was tested as described earlier [8] by its interaction with added CYP3A5, a well documented protein partner of CYB5A (Fig. 1). The calculated Kd value ($4 \cdot 10^{-7}$ M) well corresponds to affinity interactions reported for protein partners of the cytochrome P450 monooxygenase systems [20].

Cleared liver lysate samples were tested using an SPR biosensor for presence of components interacting with CYB5A, TTR, or BSA immobilized on the surface of the optical chip as bait proteins and control, respectively.

Direct molecular fishing was performed using NHS Mag Sepharose micro-paramagnetic particles (PMP) with bait proteins (CYB5A, TTR or BSA) immobilized as described earlier [8]. Briefly, each PMP sample was mixed with 250 µL liver lysate (total protein concentration of 30 mg/mL) and incubated at room temperature for 1 h under mild stirring. PMP were collected and washed in 100 µL "HBS-EP+" buffer (GE Healthcare) for 1 min. Trapped pray proteins were eluted twice by 100 µL of regeneration solution (1 M NaCl, 0.2 % CHAPS) within 2 min. The pooled eluate was centrifuged at 10,000 rpm for 10 min in a Vivaspin 500 centrifugal concentrator (GE Healthcare) with MWCO 100 kDa to remove

PMP fragments.

All the procedures of tryptic cleavage of proteins were performed in the Vivaspin 500 (MWCO 10 kDa) using a modified FASP protocol [21]. Briefly, prey protein mixtures (total protein of about 200 µg) were placed into concentrator tubes and centrifuged at 20°C for 40 min at 4,000 rpm (Eppendorf centrifuge). The resultant samples were diluted with 200 µL of working buffer (8 M urea, 100 mM Tris-HCl, pH 8.5) and centrifuged again. Protein sulfhydryl groups were alkylated by adding 100 µL 50 mM iodoacetamide dissolved in working buffer. Samples were then incubated at 20°C for 30 min and centrifuged as above. The alkylating solution was washout using 200 µL of working buffer and centrifuged. Protein digestion was carried out at 37°C during overnight incubation with 40 µL of trypsin solution in 50 mM tetraethylammonium bicarbonate (pH 8.5) at the trypsin/protein ratio of 1:100. Digested proteins were mixed with 50 µL of aqueous 500 mM NaCl, centrifuged, and filtered peptide mixtures were used in the subsequent LC-MS/MS analysis.

Peptide samples were analyzed three times using an Ultimate 3000 nano-flow HPLC (Dionex, USA) connected to a hybrid linear ion trap LTQ Orbitrap Elite (Thermo Scientific), equipped with a nanoelectrospray ion source (Thermo Scientific). Peptide separation was carried out on a RP-HPLC column Zorbax 300SB-C18 (C18 particle size 3.5 µm, 75 µm inner diameter and 150 mm length) using a linear gradient from 95% solvent A (water, 0.1% formic acid) and 5% solvent B (water, 0.1% formic acid, and 80% acetonitrile) to 40% solvent A and 60% solvent B over 85 min at a flow rate of 0.3 µL/min.

Mass spectra were acquired in the positive ion mode. High resolution data was acquired in the Orbitrap analyzer with a resolution of 60,000 (m/z 400) for MS and 15,000 (m/z 400) for MS/MS scans. Survey MS scan was followed by MS/MS spectra of the five most abundant precursors. For peptide fragmentation higher energy collisional dissociation (HCD) was used, the signal threshold was set to 5,000 for an isolation window of 2 m/z and the first

mass of HCD spectra was set to 100 m/z. The collision energy was set to 35 eV. Fragmented precursors were dynamically excluded from targeting for 60 seconds. Singly charged ions and ion with not defined charge state were excluded from triggering MS/MS scans.

Protein identification was performed using the MASCOT software (www.matrixscience.com), and all tandem mass spectra were searched against the SwissProt database (www.uniprot.org). The following search parameters were used: trypsin was used as the cutting enzyme, mass tolerance for the monoisotopic peptide window was set to ± 2.6 Da, the MS/MS tolerance window was set to ± 0.6 Da and two missed cleavage was allowed. Oxidized methionine was chosen as variable modification. Carbamidomethyl was chosen as fixed modification. The criteria of positive identification were set as follows: minimum score of 50, at least two positive identifications from three different samples and FDR < 0.01.

All experiments were performed in triplicates.

RESULTS

The LC−MS/MS identification of prey proteins fished on three different baits (CYB5A, TTR and BSA) (Fig. 2A) revealed three sets of proteins: 98, 120, and 220, respectively (see the lists of proteins in Tables 1 - 3). Comparison analysis of these sets (Fig. 2B) revealed only three protein common for all the baits. In the case of paired analysis, the number of common proteins varied from 2 to 9 (thus representing less than 10% of total number of identified proteins).

Since validation of PPI, recognized in the affinity purification experiments, requires highly purified proteins for sequential testing we have used three of identified proteins (CYP1B1, CYP2C9, GAPDH) available in our Laboratories and CYB5A as the bait. Correctness of the use of highly purified rabbit muscle GAPDH for validation of the enzyme interaction with CYB5A was due to its availability and high sequence identity (91%) with the

human enzyme [18, 22].

All the investigated proteins effectively interacted with the immobilized CYB5A with Kd values 0.07, 1.1 and 0.1 μM for CYP1B1, CYP2C9 and GAPDH, respectively. First two values fall within the range of known PPI in the cytochrome P450 monooxygenase systems [20]. We did not find any indications on the direct interaction of GAPDH with CYB5A in available literature.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12), a classical glycolytic enzyme, exhibits various important non-glycolytic activities (see for review [23]). This redox-sensitive enzyme interacts with cytoskeleton components (actin, microtubules) [24, 25] and plays an essential role in endoplasmic reticulum to Golgi transport [26]. Specific GAPDH interaction with various microsomal membranes was reported in the literature [27], but particular microsomal components interacting with this protein remained poorly characterized. Our results suggest that interacting with CYB5A GAPDH may also participate in feeding of microsomal electron transport chain with reduced equivalents.

Although possible functional/biological importance of the interaction between CYB5 and the glycolytic enzyme GAPDH requires further investigation certain evidence exists that GAPDH and CYB5 are involved together with cytochrome b5 reductase into the process of methemoglobin reduction (see for example [28]). In addition, using search service of the FunCoup database [29] (http://funcoup.sbc.su.se), which contains information about functional couplings or functional associations of proteins in 11 model organisms, we have found indications for possible functional interaction between these proteins.

DISCUSSION

Analysis of PPI involving multifuncitonal proteins, which can potentially interact with numerous protein partners, represents the most difficult problem in protein interactomics.

In this context we have chosen a multifunctional protein CYB5A as the bait. CYB5A is involved in various reactions catalyzed by enzymes of the cytochrome P450 superfamily (e.g. 17,20 lyase reaction catalyzed by CYP17, testosterone 6β-hydroxylation, catalyzed by CYP3A4, etc.). It exhibits effector action in reactions catalyzed by 3β-hydroxysteroid dehydrogenases. It should be also noted that in various living systems various protein complexes of CYB5A are rather conservative as evidenced by formation of functionally competent chimeric complexes of CYB5A with proteins from different species. The latter implies its potential interaction with various actual and potential protein partners especially if one takes into consideration that involvement of CYB5A in the cytochrome P450 monooxygenase systems depends not only on particular enzyme of the cytochrome P450 superfamily but also on particular substrates. Specificity of direct fishing was validated using TTR and BSA as alternative baits. The former is a blood protein synthesized in the liver and secreted into circulation and therefore it can be potentially involved in PPI with sets of proteins, which obviously differ from corresponding sets of protein partners of CYB5A.

BSA binds numerous physiological peptide and non-peptide ligands in blood [30] and therefore may be considered as a good candidate for evaluation of specificity of PPI with particular baits.

Although low overlapping of identified preys fished on three different protein baits (Fig. 2B) suggests high specificity of direct fishing it seems unlikely that each bait does interact with all the fished preys *in vivo*. In this context it should be noted that the identified prey proteins may be “contaminated” by some proteins forming higher order complexes with preys as schematically shown in Fig. 3. It is also possible that the particular bait may interact with micellar/aggregate structures containing direct protein partners of the bait. Discrimination of primary and secondary partners among all fished components is the most difficult problem of all affinity-based approaches.

In our opinion, solution of this problem consists in the use of all currently available techniques (molecular biology, bioinformastics, textomics, etc) for rational ranking of prey proteins and subsequent SPR-based direct validation of PPI using putative protein partners from the top list.

CONCLUDING REMARKS

Molecular fishing is based on affinity interaction between a bait protein and putative one or more functionally competent partners (preys) in natural biological systems.

Direct molecular fishing appears to be the simplest method. It does not require gene engineered construction of bait proteins and additional affinity reagents (e.g. antibodies etc) for fishing of the bait/prey protein complex(s). It is applicable for identification of high affinity PPI (Kd < 10 μM) in any biological materials including human tissue specimens.

Although productive capacity of biochemical approaches is not as high as genomic ones (and so they are not applicable for serial screenings) identification of PPI under physiological conditions appears to be more reliable [4]. Comparative analysis using three different proteins as alternative baits revealed three absolutely different sets of prey proteins, which may be attributed to affinity limits of direct molecular fishing. This opens wide applicability of use direct molecular fishing for PPI analysis in normal and pathological conditions, in which altered PPIs are especially important (e.g. Alzheimer's, Parkinson's, and other diseases)

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The authors have declared no conflict of interest.

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Figure 1. Sensograms illustrating interaction between injected CYP3A5 and the immobilized CYB5A. Number indicate the following concentrations of injected CYP3A5 solutions: 5 nM – (1); 10 nM – (2); 100 – (3); 500 nM – (4); 2000 nM – (5). Other explanations are given in the text.

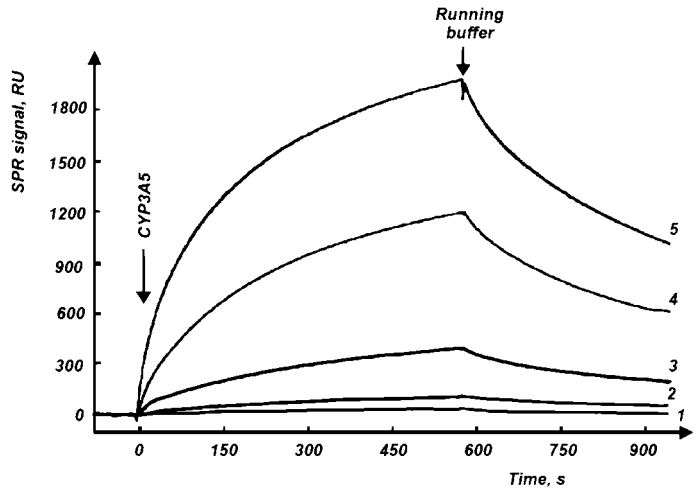

Figure 2. Fishing of human liver proteins on three different immobilized baits.
A - total number of prey proteins fished on three different baits; B - overlapping of prey proteins fished on these three baits (see tables 1 - 3).

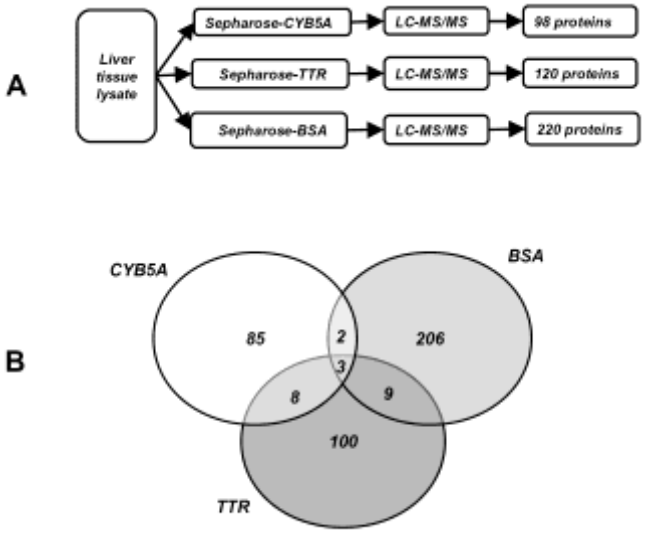

Figure 3. The scheme of possible -protein-protein interactions with the immobilized bait during affinity purification: (a) direct binding of a corresponding PPI partner the immobilized bait; (b) indirect binding of secondary partners of the protein bound to the immobilized bait. (c) binding of larger molecular aggregates containing the corresponding PPI partner of the immobilized bait and some (specific/nonspecific) proteins.

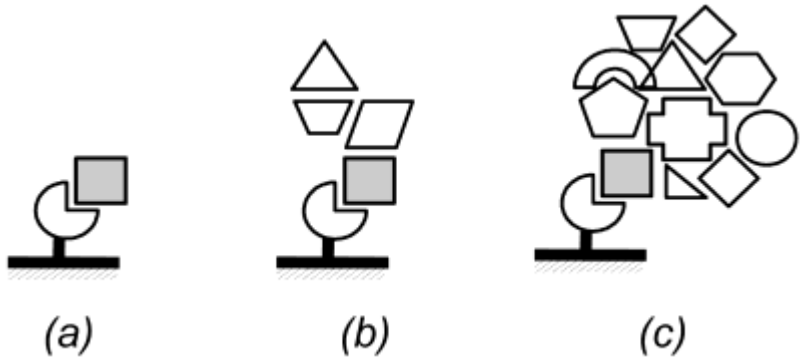

Table 1. Identification of pray proteins fished from human liver tissue lyzate using CYB5A as the bait.

№	Overlapping proteins	Gene name	Protein name	MASCOT		
				Acc. number[a]	Score[b]	Peptides[c]
1	I/II/III	MTCH1	Mitochondrial carrier homolog 1	Q9NZJ7	94	3
2		TMEM205	Transmembrane protein 205	Q6UW68	90	2
3		FAM120C	Constitutive coactivator of PPAR-gamma-like protein 2	Q9NX05	80	2
4		MNX1	Motor neuron and pancreas homeobox protein 1	P50219	74	2
5	I/II	DUSP7	Dual specificity protein phosphatase 7	Q16829	74	2
6	I/II	CACNG8	Voltage-dependent calcium channel gamma-8 subunit	Q8WXS5	74	1
7		GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	P04406	73	1
8		KY	Kyphoscoliosis peptidase	B4DGA7	71	1
9		TMEM221	Transmembrane protein 221	A6NGB7	71	2
10		C22orf26	Putative uncharacterized protein C22orf26	Q9NV39	70	2
11	I/II/III	KDM1A	Lysine-specific histone demethylase 1A	O60341	69	2
12		CTCF	Transcriptional repressor CTCF	P49711	67	1
13		NFRKB	Nuclear factor related to kappa-B-binding protein	Q6P4R8	66	1
14	I/II	PITPNM2	Membrane-associated phosphatidylinositol transfer protein 2	Q9BZ72	65	1
15		ACSM2A	Acyl-coenzyme A synthetase ACSM2A, mitochondrial	Q08AH3	64	1
16		CYP2C9	Cytochrome P450 2C9	P11712	64	2
17		ARNT2	Aryl hydrocarbon receptor nuclear translocator 2	Q86TN1	63	1
18		ITPR3	Inositol 1,4,5-trisphosphate receptor type 3	Q14573	63	1
19		LIN7A	Protein lin-7 homolog A	O14910	61	1
20		PPP1R12C	Protein phosphatase 1 regulatory subunit 12C	B4DME2	59	1
21		IRS2	Insulin receptor substrate 2	Q9Y4H2	59	1
22		ESPNL	Espin-like protein	Q6ZVH7	59	1
23		ALAS2	5-aminolevulinate synthase, erythroid-specific, mitochondrial	P22557	58	1
24		CUX2	Homeobox protein cut-like 2	O14529	58	1
25	I/II	COL5A1	Collagen alpha-1(V) chain	P20908	58	1
26		ZBTB3	Zinc finger and BTB domain-containing protein 3	Q9H5J0	57	1
27		GMPS	GMP synthase [glutamine-hydrolyzing]	P49915	56	1
28		SVIL	Supervillin	Q569J5	56	1
29		SPR	Sepiapterin reductase	P35270	56	1
30		CTSS	Cathepsin S	P25774	56	1
31		SH2B1	SH2B adapter protein 1	Q9NRF2	56	1
32		PURA	Transcriptional activator protein Pur-alpha	Q00577	56	1
33		EOMES	Eomesodermin homolog	B7ZA51	56	1
34		PRX	Periaxin	Q9BXM0	56	1
35		MSLNL	Mesothelin-like protein	Q96KJ4	55	1
36		GRIPAP1	GRIP1-associated protein 1	Q4V328	55	1
37		MICALL2	MICAL-like protein 2	Q8IY33	55	1
38		ABCC6	Multidrug resistance-associated protein 6	O95255	55	1
39		NTHL1	Endonuclease III-like protein 1	P78549	55	1

40	I/II	COL4A3	Collagen alpha-3(IV) chain	Q01955	55	1
41		NPC1L1	Niemann-Pick C1-like protein 1	B7ZLE6	54	1
42		MOCS3	Adenylyltransferase and sulfurtransferase MOCS3	O95396	54	1
43		ALDH3A2	Fatty aldehyde dehydrogenase	P51648	54	1
44		PI4K2A	Phosphatidylinositol 4-kinase type 2-alpha	Q9BTU6	54	1
45		MTMR10	Myotubularin-related protein 10	Q9NXD2	54	1
46		C16orf71	Uncharacterized protein C16orf71	K7EPW6	54	1
47		CPNE9	Copine-9	Q8IYJ1	54	1
48		BBC3	Bcl-2-binding component 3	Q96PG8	54	1
49		ZNF251	Zinc finger protein 251	Q9BRH9	54	1
50		IGFBP2	Insulin-like growth factor-binding protein 2	P18065	54	1
51		TRERF1	Transcriptional-regulating factor 1	Q96PN7	54	1
52		ASIC3	Acid-sensing ion channel 3	Q9UHC3	53	1
53		NECAB1	N-terminal EF-hand calcium-binding protein 1	Q8N987	53	1
54		ACAA1	3-ketoacyl-CoA thiolase, peroxisomal	P09110	53	1
55		OPA1	Dynamin-like 120 kDa protein, mitochondrial	O60313	53	1
56		GGT3P	Putative gamma-glutamyltranspeptidase 3	A6NGU5	53	1
57		ENG	Endoglin	P17813	53	1
58		DDX26B	Protein DDX26B	Q5JSJ4	53	1
59		AZI1	5-azacytidine-induced protein 1	Q9UPN4	53	1
60		JPH1	Junctophilin-1	Q9HDC5	53	1
61		CHTF8	Chromosome transmission fidelity protein 8 homolog isoform 2	P0CG12	52	1
62		ENOSF1	Mitochondrial enolase superfamily member 1	Q7L5Y1	52	1
63		F2	Prothrombin	P00734	52	1
64		SNX30	Sorting nexin-30	Q8WV41	52	1
65		FN1	Fibronectin	P02751	52	1
66		DOK3	Docking protein 3	Q7L591	52	1
67		C12orf68	Uncharacterized protein C12orf68	Q52MB2	52	1
68		C12orf65	Probable peptide chain release factor C12orf65, mitochondrial	Q9H3J6	52	1
69		EIF4G2	Eukaryotic translation initiation factor 4 gamma 2	P78344	52	1
70		TMEM63B	Transmembrane protein 63B	Q5T3F8	52	1
71		NBEA	Neurobeachin	Q8NFP9	52	1
72		CCT2	T-complex protein 1 subunit beta	P78371	52	1
73		DNMBP	Dynamin-binding protein	Q6XZF7	51	1
74		NHSL2	NHS-like protein 2	Q5HYW2	51	1
75	I/II/III	COL1A1	Collagen alpha-1(I) chain	P02452	51	1
76		ARNTL	Aryl hydrocarbon receptor nuclear translocator-like protein 1	O00327	51	1
78		UNC45A	Protein unc-45 homolog A	Q9H3U1	51	1
79		HOXB2	Homeobox protein Hox-B2	P14652	51	1
80	I/II	RGAG1	Retrotransposon gag domain-containing protein 1	Q8NET4	51	1
81		RNF214	RING finger protein 214	Q8ND24	51	1
82		SOLH	Calpain-15	O75808	51	1
83		RNPEP	Aminopeptidase B	Q9HAU8	51	1
84		NAPB	Beta-soluble NSF attachment protein	Q9H115	51	1
85		PASK	PAS domain-containing serine/threonine-protein kinase	Q96RG2	51	1

86		SLC6A5	Sodium- and chloride-dependent glycine transporter 2	Q9Y345	51	1
87		RAB3D	Ras-related protein Rab-3D	O95716	50	1
88	I/III	OTUD7B	OTU domain-containing protein 7B	Q6GQQ9	50	1
89		SMPD1	Sphingomyelin phosphodiesterase	P17405	50	1
90	I/III	SHC1	SHC-transforming protein 1	P29353	50	1
91		COL28A1	Collagen alpha-1(XXVIII) chain	Q2UY09	50	1
92	I/II	D3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3	Q6STE5	50	1
93		CYP1B1	Cytochrome P450 1B1	Q16678	50	1
94		COL15A1	Collagen alpha-1(XV) chain	P39059	50	1
95		RPL10	60S ribosomal protein L10	P27635	50	1
96	I/III	MUC16	Mucin-16	Q8WXI7	50	1
97		SAFB	Scaffold attachment factor B1	Q15424	50	1
98		FCGRT	IgG receptor FcRn large subunit p51	P55899	50	1

a - Accession number refers to the Uniprot database
b - The protein score in the result report from an MS/MS search is derived from the ions score
c - Number of unique peptides matched by MASCOT
Proteins were considered as reliably identified at the minimum Mascot score of 50 and at least two positive identifications from three different biological samples.
Overlapping proteins: I/II - overlapping proteins in tables 1 and 2; I/III - overlapping proteins in tables 1 and 3; II/III - overlapping proteins in tables 2 and 3; I/II/III - overlapping proteins in all three tables 1-3.

Table 2. Identification of pray proteins fished from human liver tissue lyzate using transthyretin as the bait.

№	Overlapping proteins	Gene name	Protein name	MASCOT		
				Acc. number[a]	Score[b]	Peptides[c]
1	I/II	DUSP7	Dual specificity protein phosphatase 7	Q16829	433	15
2		SKOR1	SKI family transcriptional corepressor 1	P84550	363	12
3		MYEF2	Myelin expression factor 2	Q9P2K5	263	42
4	II/III	COL27A1	Collagen alpha-1(XXVII) chain	Q8IZC6	212	68
5		ANKS1A	Ankyrin repeat and SAM domain-containing protein 1A	Q49AR9	204	17
6		HNRNPM	Heterogeneous nuclear ribonucleoprotein M	P52272	199	93
7		COL5A2	Collagen alpha-2(V) chain	P05997	197	50
8		CDV3	Protein CDV3	Q9UKY7	187	2
9		COL11A2	Collagen alpha-2(XI) chain	P13942	185	43
10		GSK3A	Glycogen synthase kinase-3 alpha	P49840	178	11
11		MARCO	Macrophage receptor MARCO	Q9UEW3	168	32
12		MAP2K4	Dual specificity mitogen-activated protein kinase kinase 4	P45985	167	20
13		COL2A1	Collagen alpha-1(II) chain	P02458	167	38
14		COL3A1	Collagen alpha-1(III)	P02461	161	58
15	I/II	RGAG1	Retrotransposon gag domain-containing protein 1	Q8NET4	158	72
16		SOX4	Transcription factor SOX-4	Q06945	157	9
17		HOXB3	Homeobox protein Hox-B3	P14651	154	10
18	II/III	COL4A2	Collagen alpha-2(IV) chain	P08572	153	55
19	II/III	TTN	Titin	Q8WZ42	148	359
20		COL17A1	Collagen alpha-1(XVII) chain	Q9UMD9	138	47
21		NRG3	Pro-neuregulin-3, membrane-bound isoform	P56975	134	10
22		ZNF282	Zinc finger protein 282	Q9UDV7	131	10
23		FTCD	Formimidoyltransferase-cyclodeaminase	O95954	130	17
24		FZD8	Frizzled-8	Q9H461	128	8
25		PACS1	Phosphofurin acidic cluster sorting protein 1	Q6VY07	125	21
26		COL4A5	Collagen alpha-5(IV) chain	P29400	123	20
27		MAML3	Mastermind-like protein 3	Q96JK9	122	17
28		RAPGEFL1	Rap guanine nucleotide exchange factor-like 1	Q9UHV5	122	9
29	I/II	COL4A3	Collagen alpha-3(IV) chain	Q01955	121	55
30		COL9A2	Collagen alpha-2(IX) chain	Q14055	119	26
31		DNAH14	Dynein heavy chain 14, axonemal	Q0VDD8	118	40
32		SFTPD	Pulmonary surfactant-associated protein D	P35247	115	23
33		HBG2	Hemoglobin subunit gamma-2	P69892	114	6
34	II/III	COL4A6	Collagen alpha-6(IV) chain	Q14031	112	50
35		MSX1	Homeobox protein MSX-1	P28360	112	5
36	II/III	RCOR1	REST corepressor 1	Q9UKL0	109	7
37		MPEG1	Macrophage-expressed gene 1 protein	Q2M385	108	13
38		FAM181B	Protein FAM181B	A6NEQ2	107	9
39	I/II/III	COL1A1	Collagen alpha-1(I) chain	P02452	106	46
40		HNRNPL	Heterogeneous nuclear ribonucleoprotein L	P14866	106	12
41		ALK	ALK tyrosine kinase receptor	Q9UM73	106	20

42		SEC31A	Protein transport protein Sec31A	O94979	104	8
43	I/II	CACNG8	Voltage-dependent calcium channel gamma-8 subunit	Q8WXS5	104	10
44	II/III	COL7A1	Collagen alpha-1(VII)	Q02388	102	60
45	II/III	SHOX2	Short stature homeobox protein 2	O60902	102	12
46		COL11A1	Collagen alpha-1(XI) chain	P12107	102	39
47	I/II/III	KDM1A	Lysine-specific histone demethylase 1A	O60341	102	18
48	I/II	SMARCD3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3	Q6STE5	102	15
49		BPTF	Nucleosome-remodeling factor subunit BPTF	Q12830	101	58
50		EP300	Histone acetyltransferase p300	Q09472	100	34
51		SNX27	Sorting nexin-27	Q96L92	100	5
52		TKTL1	Transketolase-like protein 1	P51854	100	8
53		FAM59B	Protein FAM59B	Q75VX8	99	10
54		NKX2-1	Homeobox protein Nkx-2.1	P43699	98	21
55		FAM73A	Protein FAM73A	Q8NAN2	98	6
56		FAM71A	Protein FAM71A	Q8IYT1	98	15
57		IRF2BP1	Interferon regulatory factor 2-binding protein 1	Q8IU81	98	31
58		HBA1	Hemoglobin subunit alpha	P69905	98	7
59		CTAG1A	Cancer/testis antigen 1	P78358	97	10
60		EN1	Homeobox protein engrailed-1	Q05925	97	1
61		MLLT4-AS1	Putative uncharacterized protein MLLT4-AS1	Q9Y6Z5	96	9
62		COL22A1	Collagen alpha-1(XXII) chain	Q8NFW1	94	32
63		SHANK1	SH3 and multiple ankyrin repeat domains protein 1	Q9Y566	94	34
64		FUBP1	Far upstream element-binding protein 1	Q96AE4	93	13
65		GATA6	Transcription factor GATA-6	Q92908	93	8
66		CADPS	Calcium-dependent secretion activator 1	B7ZM57	93	25
67		NYAP1	Neuronal tyrosine-phosphorylated phosphoinositide-3-kinase adapter 1	Q6ZVC0	92	25
68		ZNF609	Zinc finger protein 609	O15014	92	22
69		CACNA1G	Voltage-dependent T-type calcium channel subunit alpha-1G	O43497	91	28
70		COL4A1	Collagen alpha-1(IV) chain	P02462	91	41
71		TAF10	Transcription initiation factor TFIID subunit 10	Q12962	90	5
72		BCL9L	B-cell CLL/lymphoma 9-like protein	Q86UU0	90	32
73	I/II	MUC16	Mucin-16	Q8WXI7	90	98
74	I/II/III	MTCH1	Mitochondrial carrier homolog 1	Q9NZJ7	90	11
75		POM121L2	POM121-like protein 2	C9J1I7	89	7
76	II/III	COL1A2	Collagen alpha-2(I) chain	P08123	89	42
77		ELN	Elastin	P15502	88	9
78		SLC2A13	Proton myo-inositol cotransporter	Q96QE2	88	6
79	I/II	COL5A1	Collagen alpha-1(V) chain	P20908	88	44
80		PAN3	PAB-dependent poly(A)-specific ribonuclease subunit 3	-	88	16
81		SLC25A12	Calcium-binding mitochondrial carrier protein Aralar1	O75746	86	9
82		AHNAK	Neuroblast differentiation-associated protein AHNAK	Q09666	86	152

83		RAI1	Retinoic acid-induced protein 1	Q7Z5J4	86	26
84		HOXA2	Homeobox protein Hox-A2	O43364	85	3
85		CHD6	Chromodomain-helicase-DNA-binding protein 6	Q8TD26	85	33
86		KIAA1549L	UPF0606 protein KIAA1549L	E9PAT2	85	21
87		COL4A4	Collagen alpha-4(IV) chain	P53420	84	52
88	I/II	PITPNM2	Membrane-associated phosphatidylinositol transfer protein 2	Q9BZ72	84	17
89		ABCG4	ATP-binding cassette sub-family G member 4	Q9H172	84	22
90		WIZ	Protein Wiz	B7ZM82	82	21
91		GRM5	Metabotropic glutamate receptor 5	P41594	82	20
92		SMARCC1	SWI/SNF complex subunit SMARCC1	Q92922	82	17
93		BFSP2	Phakinin	Q13515	81	12
94		GLCCI1	Glucocorticoid-induced transcript 1 protein	Q86VQ1	81	12
95		COL19A1	Collagen alpha-1(XIX) chain	Q14993	81	16
96		XBP1	X-box-binding protein 1	P17861	81	4
97		RYR2	Ryanodine receptor 2	Q92736	81	53
98		COL5A3	Collagen alpha-3(V) chain	P25940	80	29
99		NLGN2	Neuroligin-2	Q8NFZ4	80	5
100		CUL4B	Cullin-4B	Q13620	80	14
101		DGKI	Diacylglycerol kinase iota	O75912	80	11
102		ARHGAP22	Rho GTPase-activating protein 22	Q7Z5H3	80	16
103		GLYCAM1	Putative glycosylation-dependent cell adhesion molecule 1	Q8IVK1	80	1
104		BSN	Protein bassoon	Q9UPA5	80	53
105		FOXF1	Forkhead box protein F1	Q12946	80	12
106		TTLL11	Tubulin polyglutamylase TTLL11	Q8NHH1	79	10
107		RNPS1	RNA-binding protein with serine-rich domain 1	Q15287	79	6
108		CELF5	CUGBP Elav-like family member 5	Q8N6W0	79	3
109		TTC28	Tetratricopeptide repeat protein 28	Q96AY4	79	49
110		FLI1	Friend leukemia integration 1 transcription factor	Q01543	79	9
111		FAM179B	Protein FAM179B	Q9Y4F4	78	14
112		C2orf44	WD repeat-containing protein C2orf44	Q9H6R7	78	8
113		SOCS7	Suppressor of cytokine signaling 7	O14512	78	10
114		ZNF423	Zinc finger protein 423	Q2M1K9	77	9
115		SEPT12	Septin-12	Q8IYM1	77	7
116		KIF26A	Kinesin-like protein KIF26A	Q9ULI4	77	38
117		PSD2	PH and SEC7 domain-containing protein 2	Q9BQI7	77	12
118		SUCLA2	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	Q9P2R7	76	8
119	II/III	KCNQ3	Potassium voltage-gated channel subfamily KQT member 3	O43525	76	9
120		C1QC	Complement C1q subcomponent subunit C	P02747	76	9

a - Accession number refers to the Uniprot database

b - The protein score in the result report from an MS/MS search is derived from the ions score

c - Number of unique peptides matched by MASCOT

Proteins were considered as reliably identified at the minimum Mascot score of 50 and at least two positive identifications from three different biological samples.

Overlapping proteins: I/II - overlapping proteins in tables 1 and 2; I/III - overlapping proteins in tables 1 and 3; II/III - overlapping proteins in tables 2 and 3; I/II/III - overlapping proteins in all three tables 1-3.

Table 3. Identification of protein partners of BSA fished from human liver lyzate.

№	Overlapping proteins	Gene name	Protein name	MASCOT		
				Acc. number[a]	Score[b]	Peptides[c]
1	II/III	RCOR1	REST corepressor 1	Q9UKL0	58	10
2	I/III	SHC1	SHC-transforming protein 1	P29353	57	5
3	II/III	COL1A2	Collagen alpha-2(I) chain	P08123	57	36
4		SP140	Nuclear body protein SP140	Q13342	57	11
5		EPHA8	Ephrin type-A receptor 8	P29322	57	16
6	I/II/III	COL1A1	Collagen alpha-1(I) chain	P02452	57	56
7	II/III	COL7A1	Collagen alpha-1(VII) chain	Q02388	56	79
8	II/III	TTN	Titin	Q8WZ42	56	472
9		SNCG	Gamma-synuclein	O76070	56	6
10		NOL4	Nucleolar protein 4	O94818	56	9
11	I/III	OTUD7B	OTU domain-containing protein 7B	Q6GQQ9	56	15
12	I/II/III	KDM1A	Lysine-specific histone demethylase 1A	O60341	56	12
13		ITPR1	Inositol 1,4,5-trisphosphate receptor type 1	Q14643	56	33
14		CNTNAP3B	Contactin-associated protein-like 3B	Q96NU0	56	14
15		CNTNAP3	Contactin-associated protein-like 3	Q9BZ76	55	19
16		HCFC1	Host cell factor 1	P51610	55	20
17		PARP8	Poly [ADP-ribose] polymerase 8	Q8N3A8	55	16
18		ZNF473	Zinc finger protein 473	Q8WTR7	55	5
19		FBN2	Fibrillin-2	P35556	55	24
20		IGHMBP2	DNA-binding protein SMUBP-2	P38935	55	17
21		GPR61	Probable G-protein coupled receptor 61	Q9BZJ8	55	2
22		EFNB3	Ephrin-B3	Q15768	55	6
23		STAB2	Stabilin-2	Q8WWQ8	55	20
24		MKL1	MKL/myocardin-like protein 1	Q969V6	54	9
25		MREG	Melanoregulin	Q8N565	54	3
26		KIAA1324	UPF0577 protein KIAA1324	Q6UXG2	53	9
27		OVCH1	Ovochymase-1	Q7RTY7	53	16
28	II/III	COL4A2	Collagen alpha-2(IV) chain	P08572	53	47
29	II/III	COL27A1	Collagen alpha-1(XXVII) chain	Q8IZC6	53	40
30		RABL6	Rab-like protein 6	Q3YEC7	53	11
31	II/III	COL4A6	Collagen alpha-6(IV) chain	Q14031	53	37
32		MACROD1	O-acetyl-ADP-ribose deacetylase MACROD1	Q9BQ69	53	21
33		RSC1A1	Regulatory solute carrier protein family 1 member 1	Q92681	52	6
34		ZFHX4	Zinc finger homeobox protein 4	Q86UP3	52	24
35		FAM21C	WASH complex subunit FAM21C	Q9Y4E1	52	14
36		GDF7	Growth/differentiation factor 7	Q7Z4P5	52	17
37		MSN	Moesin	P26038	51	9
38		RDX	Radixin	P35241	51	6
39		EZR	Ezrin	P15311	51	6
40		EBLN2	Endogenous Bornavirus-like nucleoprotein 2	Q6P2I7	51	5
41		ATP12A	Potassium-transporting ATPase alpha chain 2	P54707	51	19
42	II/III	SHOX2	Short stature homeobox protein 2	O60902	51	6

43		FES	Tyrosine-protein kinase Fes/Fps	P07332	51	10
44	II/III	KCNQ3	Potassium voltage-gated channel subfamily KQT member 3	O43525	51	16
45		DTL	Denticleless protein homolog	Q9NZJ0	51	8
46		NME3	Nucleoside diphosphate kinase 3	Q13232	51	4
47		SH3RF2	Putative E3 ubiquitin-protein ligase SH3RF2	Q8TEC5	51	10
48		PHF1	PHD finger protein 1	O43189	51	13
49		PRR14	Proline-rich protein 14	Q9BWN1	51	11
50		APMAP	Adipocyte plasma membrane-associated protein	Q9HDC9	51	6
51		SNRNP70	U1 small nuclear ribonucleoprotein 70 kDa	P08621	50	7
52		TRIM24	Transcription intermediary factor 1-alpha	O15164	50	14
53		DACT1	Dapper homolog 1	Q9NYF0	50	10
54		KCTD9	BTB/POZ domain-containing protein KCTD9	Q7L273	50	8
55		CLEC4G	C-type lectin domain family 4 member G	Q6UXB4	50	6
56		SLC36A4	Proton-coupled amino acid transporter 4	Q6YBV0	50	10
57		PLCL1	Inactive phospholipase C-like protein 1	Q15111	50	5
58		PCDHGC3	Protocadherin gamma-C3	Q9UN70	50	17
59		STAU2	Double-stranded RNA-binding protein Staufen homolog 2	Q9NUL3	50	12
60		C8orf33	UPF0488 protein C8orf33	Q9H7E9	50	4
61		CENPF	Centromere protein F	P49454	50	44
62		BRCA2	Breast cancer type 2 susceptibility protein	P51587	50	41
63		FCGBP	IgGFc-binding protein	Q9Y6R7	49	27
64		KHSRP	Far upstream element-binding protein 2	Q92945	49	9
65		PRDM1	PR domain zinc finger protein 1	O75626	49	4
66		C17orf78	Uncharacterized protein C17orf78	Q8N4C9	49	7
67		ZNF746	Zinc finger protein 746	Q6NUN9	48	13
68		NUDT16L1	Protein syndesmos	Q9BRJ7	48	5
69		SLC6A9	Sodium- and chloride-dependent glycine transporter 1	P48067	48	7
70		KANSL	KAT8 regulatory NSL complex subunit 1	Q7Z3B3	47	21
71		CCAR1	Cell division cycle and apoptosis regulator protein 1	Q8IX12	47	8
72		ARHGAP17	Rho GTPase-activating protein 17	Q68EM7	46	11
73		DGCR8	Microprocessor complex subunit DGCR8	Q8WYQ5	46	8
74		ANKRD28	Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A	O15084	46	8
75		ARL15	ADP-ribosylation factor-like protein 15	Q9NXU5	46	3
76	I/II/III	MTCH1	Mitochondrial carrier homolog 1	Q9NZJ7	46	7
77		RELA PE	Transcription factor p65	Q04206	46	9
78		WNT4	Protein Wnt-4	P56705	46	8
79		CNR2	Cannabinoid receptor 2	P34972	46	8
80		PRSS58	Serine protease 58	Q8IYP2	46	2
81		ACAD10	Acyl-CoA dehydrogenase family member 10	Q6JQN1	46	22
82		MFSD2A	Major facilitator superfamily domain-containing protein 2A	Q8NA29	46	2
83		FRY	Protein furry homolog	Q5TBA9	45	30
84		SAMD7	Sterile alpha motif domain-containing protein 7	Q7Z3H4	45	3
85		NKX2-4	Homeobox protein Nkx-2.4	Q9H2Z4	45	5

86		LOH12CR1	Loss of heterozygosity 12 chromosomal region 1 protein	Q969J3	45	4
87		TPD52L1	Tumor protein D53	Q16890	44	8
88		AADAT	Kynurenine/alpha-aminoadipate aminotransferase	Q8N5Z0	43	8
89		SPAG5	Sperm-associated antigen 5	Q96R06	42	20
90		ANXA8	Annexin A8	P13928	41	5
91		TP53I13	Tumor protein p53-inducible protein 13	Q8NBR0	41	7
92		MARCH5	E3 ubiquitin-protein ligase MARCH5	Q9NX47	41	11
93		C1orf114	Uncharacterized protein C1orf114	-	41	5
94		TMEM126A	Transmembrane protein 126A	Q9H061	41	18
95		TOPORS	E3 ubiquitin-protein ligase Topors	Q9NS56	41	24
96		TMC4	Transmembrane channel-like protein 4	Q7Z404	41	7
97		MCF2L2	Probable guanine nucleotide exchange factor MCF2L2	Q86YR7	41	9
98		KCNMA1	Calcium-activated potassium channel subunit alpha-1	Q12791	41	23
99		BMS1	Ribosome biogenesis protein BMS1 homolog	Q14692	41	22
100		ATAD1	ATPase family AAA domain-containing protein 1	Q8NBU5	41	3
101		MYZAP	Myocardial zonula adherens protein	P0CAP1	41	4
102		DYNC1LI1	Cytoplasmic dynein 1 light intermediate chain 1	Q9Y6G9	41	10
103		TCTEX1D4	Tctex1 domain-containing protein 4	Q5JR98	41	8
104		CHD3	Chromodomain-helicase-DNA-binding protein 3	Q12873	41	44
105		BICC1	Protein bicaudal C homolog 1	Q9H694	41	15
106		GLTPD1	Glycolipid transfer protein domain-containing protein 1	Q5TA50	41	8
107		ADAMTS16	A disintegrin and metalloproteinase with thrombospondin motifs 16	Q8TE57	41	19
108		TRNP1	TMF-regulated nuclear protein 1	Q6NT89	41	10
109		BRSK2	Serine/threonine-protein kinase BRSK2	Q8IWQ3	41	16
110		PGM5	Phosphoglucomutase-like protein 5	Q15124	41	18
111		MUC3A	Mucin-3A	Q02505	41	4
112		HERC1	Probable E3 ubiquitin-protein ligase HERC1	Q15751	41	54
113		DISC1	Disrupted in schizophrenia 1 protein	Q9NRI5	41	19
114		ASZ1	Ankyrin repeat, SAM and basic leucine zipper domain-containing protein 1	Q8WWH4	40	8
115		ASAP1	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 1	Q9ULH1	40	10
116		RPS2	40S ribosomal protein S2	P15880	40	11
117		AMOTL2	Angiomotin-like protein 2	Q9Y2J4	40	13
118		CXCL6	C-X-C motif chemokine 6	P80162	40	9
119		ANKRD61	Ankyrin repeat domain-containing protein 61	A6NGH8	40	3
120		CKAP2L	Cytoskeleton-associated protein 2-like	Q8IYA6	40	10
121		ZNF865	Zinc finger protein 865	P0CJ78	40	18
122		ARMC6	Armadillo repeat-containing protein 6	Q6NXE6	40	8
123		UNC45B	Protein unc-45 homolog B	Q8IWX7	40	19
124		TACC2	Transforming acidic coiled-coil-containing protein 2	O95359	40	49
125		H1FOO	Histone H1oo	Q8IZA3	40	28
126		SMARCD1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D	Q96GM5	40	7

			member 1			
127		FAT1	Protocadherin Fat 1	Q14517	40	43
128		CLEC3B	Tetranectin	P05452	39	3
129		SMCR8	Smith-Magenis syndrome chromosomal region candidate gene 8 protein	Q8TEV9	39	17
130		OR8G1	Olfactory receptor 8G1	Q15617	39	5
131		GIMAP5	GTPase IMAP family member 5	Q96F15	39	6
132		PIGW	Phosphatidylinositol-glycan biosynthesis class W protein	Q7Z7B1	39	7
133		IER2	Immediate early response gene 2 protein	Q9BTL4	39	7
134		LRP1	Prolow-density lipoprotein receptor-related protein 1	Q07954	39	54
135		DNAH5	Dynein heavy chain 5, axonemal	Q8TE73	38	59
136		VSX2	Visual system homeobox 2	P58304	38	10
137		POM121C	Nuclear envelope pore membrane protein POM 121C	A8CG34	38	18
138		GDF6	Growth/differentiation factor 6	Q6KF10	38	9
139		RNF183	RING finger protein 183	Q96D59	38	1
140		FLRT3	Leucine-rich repeat transmembrane protein FLRT3	Q9NZU0	38	12
141		GAL3ST3	Galactose-3-O-sulfotransferase 3	Q96A11	38	7
142		NCAM1	Neural cell adhesion molecule 1	P13591	38	9
143		LRRC7	Leucine-rich repeat-containing protein 7	Q96NW7	38	11
144		SPEN	Msx2-interacting protein	Q96T58	38	52
145		PABPN1	Polyadenylate-binding protein 2	Q86U42	38	10
146		TMEM131	Transmembrane protein 131	Q92545	38	26
147		PCDH7	Protocadherin-7	O60245	38	10
148		C1orf131	Uncharacterized protein C1orf131	Q8NDD1	38	7
149		PAX1	Paired box protein Pax-1	P15863	38	8
150		GDPD4	Glycerophosphodiester phosphodiesterase domain-containing protein 4	Q6W3E5	38	5
151		PMM1	Phosphomannomutase 1	Q92871	38	4
152		MCM4	DNA replication licensing factor MCM4	P33991	38	22
153		ZDBF2	DBF4-type zinc finger-containing protein 2	Q9HCK1	38	22
154		KLHL30-AS1	Putative uncharacterized protein KLHL30-AS1	Q8NEE0	38	3
155		SLCO1A2	Solute carrier organic anion transporter family member 1A2	P46721	38	5
156		ONECUT2	One cut domain family member 2	O95948	38	10
157		LRRC37A3	Leucine-rich repeat-containing protein 37A3	O60309	38	9
158		LRRC37A2	Leucine-rich repeat-containing protein 37A2	A6NM11	38	10
159		OR6Y1	Olfactory receptor 6Y1	Q8NGX8	38	3
160		PTPRE	Receptor-type tyrosine-protein phosphatase epsilon	P23469	38	7
161		NBEAL2	Neurobeachin-like protein 2	Q6ZNJ1	38	17
162		LTBP4	Latent-transforming growth factor beta-binding protein 4	Q8N2S1	38	17
163		TRPA1	Transient receptor potential cation channel subfamily A member 1	O75762	38	12
164		CPQ	Carboxypeptidase Q	Q9Y646	38	5

165		ZNF561	Zinc finger protein 561	Q8N587	38	6
166		INVS	Inversin	Q9Y283	38	23
167		ART5	Ecto-ADP-ribosyltransferase 5	Q96L15	38	4
168		ANK1	Ankyrin-1	P16157	38	23
169		CPSF1	Cleavage and polyadenylation specificity factor subunit 1	Q10570	38	18
170		LHX1	LIM/homeobox protein Lhx1	P48742	38	6
171		WDR45L	WD repeat domain phosphoinositide-interacting protein 3	Q5MNZ6	38	5
172		OTOP3	Otopetrin-3	Q7RTS5	37	5
173		LOC439951	Putative uncharacterized protein LOC439951	Q8NDZ9	37	9
174		KCNQ5	Potassium voltage-gated channel subfamily KQT member 5	Q9NR82	37	19
175	I/II/III	COL1A1	Collagen alpha-1(I) chain	P02452	37	56
176		POTEA	POTE ankyrin domain family member A	Q6S8J7	37	7
177		AHCTF1	Protein ELYS	Q8WYP5	37	22
178		KIAA1210	Uncharacterized protein KIAA1210	Q9ULL0	37	26
179		HILS1	Spermatid-specific linker histone H1-like protein	P60008	37	13
180		YRDC	YrdC domain-containing protein, mitochondria	Q86U90	37	4
181		C1QTNF7	Complement C1q tumor necrosis factor-related protein 7	Q9BXJ2	37	9
182		DMD	Dystrophin	P11532	37	39
183		DNAJC13	DnaJ homolog subfamily C member 13	O75165	36	21
184		TMEM5	Transmembrane protein 5	Q9Y2B1	36	8
185		MYBPC3	Myosin-binding protein C, cardiac-type	Q14896	36	16
186		EPHB4	Ephrin type-B receptor 4	P54760	36	14
187		NAPG	Gamma-soluble NSF attachment protein	Q99747	36	7
188		FAM8A1	Protein FAM8A1	Q9UBU6	36	3
189		MAD1L1	Mitotic spindle assembly checkpoint protein MAD1	Q9Y6D9	36	13
190		LOC388882	Putative uncharacterized protein LOC388882	Q8N402	36	3
191		RPRM	Protein reprimo	Q9NS64	36	1
192		TBKBP1	TANK-binding kinase 1-binding protein 1	A7MCY6	36	17
193		POU5F2	POU domain, class 5, transcription factor 2	Q8N7G0	36	6
194		SUCLG1	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	P53597	36	11
195		F8	Coagulation factor VIII	P00451	36	30
196		FOXN2	Forkhead box protein N2	P32314	36	8
197		FMO4	Dimethylaniline monooxygenase [N-oxide-forming] 4	P31512	36	6
198		ZNF613	Zinc finger protein 613	Q6PF04	36	16
199		PCBP1	Poly(rC)-binding protein 1	Q15365	36	8
200		GPANK1	G patch domain and ankyrin repeat-containing protein 1	O95872	36	6
201		ABCA10	ATP-binding cassette sub-family A member 10	Q8WWZ4	36	13
202		MYCL2	Protein L-Myc-2	P12525	36	4
203		ITK	Tyrosine-protein kinase ITK/TSK	Q08881	35	10
204		TAF11	Transcription initiation factor TFIID subunit 11	Q15544	35	4
205		FIBCD1	Fibrinogen C domain-containing protein 1	Q8N539	35	3

206		CHD1	Chromodomain-helicase-DNA-binding protein 1	O14646	35	28
207		PIGA PE	Phosphatidylinositol N-acetylglucosaminyltransferase subunit A	P37287	35	6
208		NPR2	Atrial natriuretic peptide receptor 2	P20594	34	11
209		PTGR1	Prostaglandin reductase 1	Q14914	34	4
210		BATF2	Basic leucine zipper transcriptional factor ATF-like 2	Q8N1L9	34	6
211		HRNR	Hornerin	Q86YZ3	34	31
212		BOLA2	BolA-like protein 2	Q9H3K6	34	1
213		CETP	Cholesteryl ester transfer protein	P11597	34	3
214		MESP2	Mesoderm posterior protein 2	Q0VG99	34	10
215		KRT82	Keratin, type II cuticular Hb2	Q9NSB4	33	5
216		PDE9A	High affinity cGMP-specific 3',5'-cyclic phosphodiesterase 9A	O76083	33	7
217		ADCY5	Adenylate cyclase type 5	O95622	33	15
218		ZMIZ1	Zinc finger MIZ domain-containing protein 1	Q9ULJ6	33	4
219		SDR9C7	Short-chain dehydrogenase/reductase family 9C member 7	Q8NEX9	33	1
220		PTPRD	Receptor-type tyrosine-protein phosphatase delta	P23468	33	23

a - Accession number refers to the Uniprot database
b - The protein score in the result report from an MS/MS search is derived from the ions score
c - Number of unique peptides matched by MASCOT
Proteins were considered as reliably identified at the minimum Mascot score of 50 and at least two positive identifications from three different biological samples.
Overlapping proteins: I/II - overlapping proteins in tables 1 and 2; I/III - overlapping proteins in tables 1 and 3; II/III - overlapping proteins in tables 2 and 3; I/II/III - overlapping proteins in all three tables 1-3.