Purification of rod and cone outer segment from carp retina and proteomic analysis of their membrane proteins.

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Abstract

Rods and cones are both photoreceptors in the retina, but they are different in many aspects including the light response characteristics and, for example, cell morphology and metabolism. These differences would be caused by differences in proteins expressed in rods and cones. To understand the molecular bases of these differences between rods and cones, one of the ways is to compare proteins expressed in rods and cones, and to find those expressed specifically or dominantly. In the present study, I am interested in proteins in the outer segment (OS), the site responsible for generation of rod- or cone-characteristic light responses and also the site showing different morphology between rods and cones. For this, I established a method to purify the OS and the inner segment (IS) of rods and also of cones from purified carp rods and cones, respectively, using sucrose density gradient.

In this study, I was interested in proteins tightly bound to the membranes of rod OS or cone OS. To identify these proteins, I analyzed proteins in some selected regions of an SDS-gel of washed membranes of the OS and the IS obtained from both rods and cones, with Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) using a protein database constructed from carp retina. By comparing the lists of the proteins found in the OS and the IS of both rods and cones, I found some proteins present in rod OS membranes or cone OS membranes specifically or dominantly, in addition to the proteins already known to be present specifically in rod OS or cone OS.
Introduction

In the vertebrate retina, there are two types of photoreceptors, rods and cones. Both photoreceptors have a common structure with four regions: outer segment (OS), inner segment (IS), cell body and synaptic terminal (Fig 1). The OS is the specialized region to convert captured light signals into electrical signals. For this, OS contains molecular machineries to generate a light response. The molecular machinery, called phototransduction cascade, has been well studied in rods, and now is one of the well-known trimeric G-protein coupled signaling pathways (Pugh and Lamb, 1993, Kawamura and Tachibanaki, 2008, Fu and Yau, 2007).

For cones, it is known that proteins homologous or identical to those found in rods are expressed, and thus homologous signaling pathways are present in cones.

Rods and cones show different characteristics in light responses (Kawamura and Tachibanaki, 2008, Fu and Yau, 2007). The light responses in cones are much briefer than in rods (Fig 2A and B), and this enables cones to pick up the changes of light signals with higher time resolution than rods. In addition, the light sensitivity is higher in rods than in cones. Fig 2C shows flash intensity-response amplitude relations in carp rods and red-sensitive cones. The light sensitivity of rods is >100 fold higher than that of cones. Because of the difference in sensitivity, rods and cones mediate night vision and daylight vision, respectively. One possibility of these differences is that activities and/or expression levels of phototransduction proteins are different between rods and cones. Carp (Cyprinus carpio) is so far the only animal from which we can obtain purified cones in a quantity large enough to do biochemical studies (Tachibanaki et al., 2001). In previous studies, using purified rods and cones from the retina of carp, it was found that the signal amplification is lower, and termination of each reaction is much faster in cones than rods (Tachibanaki et al., 2005, Takemoto et al., 2009, Tachibanaki et al., 2012, Koshitani et al., 2014). These findings so far explain qualitatively the differences in light responses between rods and cones.
In addition, there is morphological differences in the OS shape and OS structure between rods and cones. As shown in Fig 1, the basic structure of the rod and the cone are similar but the OS shape is a rod-like shape in rods, whereas it is conical shape in cones. Also, the detailed membrane structure is different. In rod OS (ROS), about 1000 disk membranes are stacked and surrounded by a plasma membrane, while in cone OS (COS), the plasma membrane invaginates repeatedly to form a tightly stacked lamellae structure. Such differences seem to be caused by the differences in proteins involved in morphogenesis and preservation of OS, but little is known about such proteins.

As described above, phototransduction mechanisms have been studied already. However, it is still possible that proteins other than known ones are present in ROS or COS and that they contribute to the differences in the light responses between rods and cones. In addition, there could be ROS- or COS-specific protein(s) that contribute to the differences in morphology between rods and cones. To examine these possibilities, it would be most effective to compare proteins expressed in ROS and those in COS.
method to obtain purified ROS has been known for many years, but for COS, its purification method has not been known, mainly because of difficulties in obtaining purified cones in a quantity large enough to manipulate. In the present study, first using sucrose density gradient, I prepared purified COS and ROS from purified carp cones and rods, respectively. In the purified rods and cones in carp, the IS consisting of ellipsoid plus myoid and the OS are both preserved, so that I can also obtain purified IS.

In the present study, I focused on the proteins tightly bound to ROS or COS membranes, so that I prepared ROS and COS membranes washed intensively (washed ROS or washed COS membranes). I also obtained rod inner segment (RIS) membranes and cone inner segment (CIS) membranes similarly. To identify proteins tightly bound to ROS or COS membranes, I analyzed proteins in the regions outside of visual pigment bands in the SDS-PAGE gel of the washed ROS, RIS, COS and CIS membranes with Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) using a protein database constructed from carp retina. By comparing the lists of the proteins found in the above four kinds of membranes, I tried to find proteins expressed specifically or dominantly in ROS or COS membranes (ROS- or COS-specific/dominant proteins), and listed such candidate proteins in this study.
Materials and Methods

Chemicals and antibodies

All chemical reagents were purchased from Nacalai Tesque (Kyoto, Japan). Monoclonal anti-chicken Na+/K+ ATPase alpha subunit antibody (a5) was obtained from Developmental Studies Hybridoma Bank (Iowa, USA). Anti-human TOM20 antibody (sc-11415) and anti-human neurocalcin delta antibody (NBP2-15037) were obtained from Santa Cruz Biotechnology (Texas, USA) and Novus Biologicals (Colorado, USA), respectively. Polyclonal anti-carp mitochondrial aspartate aminotransferase 2 (mAAT) antiserum was raised against its peptide fragment (Ser28-Lys428; AB793727) in mice by Mr. Komatsu in the lab which I belonged to. Peroxidase-conjugated anti-mouse IgG antibody and anti-rabbit IgG antibody were obtained from Kirkegaard & Perry Laboratories (Maryland, USA). Alexa488-conjugated anti-rabbit IgG antibody and Alexa568-conjugated anti-mouse IgG antibody were obtained from Thermo Fisher Scientific (Massachusetts, USA).

Preparation of purified carp rod and cone photoreceptors

Carp (Cyprinus carpio: 25-30 cm in length) were purchased from Hirose Carp (Fukushima, Japan). Animal care was conducted according to the institutional guidelines.

Rod and cone photoreceptors were purified from carp retina as described in Tachibanaki et al. (2001) (Fig 3). Briefly, rods and cones were mechanically dissociated from the retina by tapping the retina using a paintbrush in Ringer’s solution (119.9 mM NaCl, 2.6 mM KCl, 0.5 mM CaCl₂, 0.5 mM MgCl₂, 0.5 mM MgSO₄, 1 mM NaHCO₃, 16 mM glucose, 0.5 mM NaH₂PO₄, 4 mM HEPES, pH 7.5). Detached cells retained the OS and the IS containing of ellipsoid plus myoid, but lacked the cell body and the synaptic terminal (Kawamura and Tachibanaki, 2008). Then, the rods and cones were separated using Percoll stepwise density gradients. Purified rods and cones were washed twice with a potassium gluconate buffer (115 mM potassium gluconate, 2.5 mM KCl, 2 mM MgCl₂, 0.1 mM CaCl₂, 0.2 mM EGTA, 1 mM dithiothreitol, 10 mM HEPES, pH 7.5) because by this wash, most of contaminated red blood cells were blasted and their soluble proteins were mostly removed in the supernatant while rods and cones remained intact. In previous studies, contamination of cones in the purified rods and that of rods in purified cones were both estimated < 1 % (Tachibanaki et al., 2001). Purified cells were suspended in Ringer’s solution.
Separation of OS membranes from IS membranes in purified cells.

Purified rods were suspended in Ringer’s solution and purified cones were suspended in 2 x Ringer’s solution (For unknown reasons, separation of OS and IS in cones was more effective when 2 x Ringer’s solution was used). Then, each suspension (~800 µL) was passed through a 27-gauge needle 15 times using a 1 mL syringe to mechanically dissociated OS and IS from purified rods or cones. During this procedure, cytoplasmic proteins were probably eluted out from the OS or the IS. The resultant suspension of a mixture of separated OS and IS membranes of rods or cones were then placed on top of a stepwise sucrose density gradient in Ringer's solution in a test tube, and centrifuged for an hour at 190,000 x g at 4 °C. With the above procedure, I obtained a fraction rich of ROS membranes and that of RIS membranes from purified rods, and those rich of COS and CIS membranes from purified cones (see Results and Discussion).

Preparation of polyclonal antiserum against calnexin

To raise antiserum against calnexin, partial peptide corresponding to Ile506 - Lys593 of carp calnexin (AB894402.1) was expressed as a GST-fusion protein. The
The coding region of calnexin was amplified from a carp retinal cDNA library (Shimauchi-Matsukawa et al., 2005) by PCR with a pair of primers, each of which contained a site for a restriction enzyme. The sequences of the primers were: 5’-TCCTCTTCTGCTGCACTG-3’ (forward) and 5’-TCATTTCCTGTCTGAGA-3’ (reverse). The BamHI- and XhoI-digested amplified region was inserted into BamHI/XhoI sites of expression vector, pGEX-5X-1 (GE Healthcare, USA). The recombinant plasmid was introduced into E.coli BL21 DE3 (Novagen). The expression and purification of the recombinant protein was carried out according to the manufacture’s instruction. The fusion protein was used to raise anti-calnexin antiserum in mice. The antisera obtained from mice were confirmed to react specifically to calnexin peptide, Ile506 - Lys593, in its N-terminal maltose-binding protein (MBP) fusion form.

**Quantitative evaluation of Contamination of IS or OS membranes**

To evaluate the purity of membranes separated with sucrose density gradient centrifugation described above, OS- or IS-specific marker proteins were quantified in each separated membrane fraction. As a marker of OS membranes, visual pigments were quantified spectrophotometrically as described Tachibanaki et al. (2001), Okano et al. (1989) and Yamaoka et al. (2015). As markers of IS membranes, F1 ATPase beta subunit, TOM20, calnexin, and Na\(^+\)/K\(^+\) ATPase alpha subunit were quantified. F1 ATPase beta subunit and TOM20 localize to the inner and outer membranes of mitochondria contained in IS, respectively. Calnexin and Na\(^+\)/K\(^+\) ATPase alpha subunit localize to the endoplasmic reticulum membranes and plasma membranes in IS. The amount of F1 ATPase beta subunit was quantified with Oriole (Bio-Rad Laboratories, California, USA) staining after SDS-PAGE using bovine serum albumin (BSA) as a molar standard. The amounts of Na\(^+\)/K\(^+\) ATPase alpha subunit, calnexin and TOM20 were measured by quantitative immunoblot analysis as described Tachibanaki et al. (2005). Briefly, the obtained fractions were transferred onto PVDF membranes (Immobilon-P Transfer Membrane, MERCKMILLIPORE, Darmstadt, Germany) after SDS-PAGE. The membranes were washed in TBS (100 mM Tris, 0.9 % NaCl, pH 7.5) and incubated in TBS containing anti-chicken Na\(^+\)/K\(^+\) ATPase alpha subunit antibody (at 1:250 dilution), anti-carp calnexin antiserum (at 1:200 dilution) or anti-human TOM20 antibody (at 1:100 dilution) at 4 °C overnight. After the membranes were washed in TBS, incubation was continued in TBS containing the secondary antibody at room temperature for an hour. After the membranes were washed in TBS, visualization of the immunoreactive protein bands was carried out according to the manufacturer’s protocol.
(Chemi-Lumi One L, Nacalai Tesque, Kyoto, Japan). The amounts of marker proteins were measured with the amount of visual pigments.

**Construction of a database of proteins expressed in carp retina**

For the comparative proteomic analysis of rod and cone proteins in the OS and the IS membranes, a database of proteins expressed in carp retina was constructed as described below. Retinal total RNA was extracted from carp retina using TRI reagent (Sigma-Aldrich, California, USA) according to the manufacturer’s protocol. Subsequent RNA quality check, RNA sequence library preparation and RNA sequencing were performed at Hokkaido System Science (Hokkaido, Japan. Raw sequence data were submitted to DDBJ Sequence Read Archive (DRA) under accession number DRA004555 (Bioproject ID: PRJDB4664)). Then, the acquired sequences were assembled as contigs by a de novo RNA-seq data assembly method at Hokkaido System Science using Trinity (version r20130225, k-mer 25). The assembled 258,867 contigs were BLASTX searched against NCBI vertebrate_other protein database (release 69, E-value 1e-5) using BLAST+ (version 2.2.29+). A total of 124,947 contigs hit with this search was each annotated with the top hit NCBI gene name and the species name. The contigs that were not identified by the BLAST search were removed from the analysis hereafter. Next, the annotated contigs were translated from DNA sequences to amino acid sequences using TransDecoder (version r20140704). As a result, 125,175 amino acid sequences were obtained. The increase of the number of sequences from the number of contigs was caused by the presence of contigs that have candidate of multiple start codons. The number of amino acid sequences was more than that of all carp genes, 52,610, reported previously (Xu et al., 2014), due to the presence of the splicing variants and the polymorphism in UTR regions and partially in coding regions. The DNA sequences coding same amino acid sequences but having different DNA sequences in UTR regions were not merged at this stage. Then, using acquired data of the sequences, a sequence database of carp retinal proteins was constructed (Fukagawa et al., 2016), and used for the following proteomic analysis.

**Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis**

In this study, I focused on proteins tightly bound to membranes. Therefore, in the LC-MS/MS analysis, I used washed membranes prepared from ROS, RIS, COS and CIS membranes. ROS, RIS, COS and CIS membranes were washed intensively by centrifugation (190,000 x g, 10 min, 4 °C) with a low salt buffer (4 mM HEPES, 1 mM EDTA, pH 7.5) twice and subsequently with a high pH buffer (100 mM Na₂CO₃,
(pH 11.5) four times to remove soluble and membrane-associated peripheral proteins. The washed membranes were dissolved in SDS-PAGE sample buffer (62.5 mM Tris, 10 % (w/v) glycerol, 100 mM dithiothreitol, 2.3 % SDS, 0.01 % (w/v) bromophenol blue), and kept on ice for more than 1 hour. After solubilization, membranes were subjected to SDS-PAGE, and gels were silver-stained. Then, four regions of each lane (Fig 8E) were subjected to in-gel digestion for subsequent LC-MS/MS analysis. The regions corresponding to rhodopsin monomer (30–40 kDa), dimer (55–65 kDa), and trimer (75–100 kDa) were omitted from the regions for the analysis in ROS-rich fractions to avoid the masking effect caused by massive mount of peptide derived from rhodopsin (about 95 % of total protein amount in ROS-rich fraction). Also in the analyses of other membranes, the same regions were omitted to compare the localized proteins impartially between rod and cone membranes, and also between the OS and IS membranes. Then the proteins in gels were digested by in-gel tryptic cleavage, and resultant peptides were extracted from the gel (Rosenfeld et al., 1992). The peptides were subjected to shotgun proteomic analysis using QTRAP®5500 LC-MS/MS System (AB SCIEX) and Mascot software (version 2.4, Matrix Science, Boston, USA).

Identification of proteins specifically/dominantly expressed in ROS or COS

The amount of a protein analyzed with LC-MS/MS can be quantified with the value of emPAI (Ishihama et al., 2005), which is calculated from the number of the observed peptides divided by the number of the observable peptides per protein in the MS analysis and is an index of the amount of each protein in a sample analyzed with LC-MS/MS. Because OS- and IS-rich fractions were contaminated with IS and OS membranes, respectively, I calculated the portion of a protein detected in the ROS-rich fraction by dividing the emPAI value of a protein in the ROS-rich fraction with the sum of its value in the ROS-rich and the RIS-rich fractions (ROS/(ROS+RIS) in Table 2). Proteins with ROS/(ROS+RIS) < 0.5, which indicates that the protein is detected more in the RIS-rich fraction, are not listed in Table 2. In addition, proteins also found in COS-rich fraction are eliminated under the criteria that their emPAI values in COS-rich fraction, obtained from the same number of cones, i. e., 5 × 10^4 cones, were more than 1/10 of those in ROS-rich fraction. In Table 2, proteins showing ROS/(ROS+RIS) > 0.8 are shown, based on the fact that many of the known ROS-specific proteins are present with ROS/(ROS+RIS) > 0.8 (see proteins indicated in red bold). In addition, those proteins showing emPAI values lower than 1/100 of that of a protein most abundantly detected, i. e., rhodopsin showing 35.41 emPAI value in the ROS-rich fraction, are eliminated in Table 2. It should be mentioned that the regions corresponding to
rhodopsin bands were omitted in the LC-MS/MS analysis (Fig 8E), but that was not completely removed and detected because of its massive expression. In the above process, ROS and COS volume differences (Yamaoka et al., 2015) were taken into account to compare the emPAI values in the equal volume. Proteins in Table 2 are listed in descending order of ROS/(ROS+RIS).

Similarly, I calculated the portion of a protein detected in the COS-rich fraction in Table 3. In this case, guanine nucleotide-binding protein G(t) subunit alpha-2 or cone transducin alpha subunit showing 28.3 emPAI value in the COS-rich fraction, was used as the most abundantly detected protein. It should be mentioned that cone transducin alpha subunit is a ~40 kDa protein. Proteins of this molecular mass may have been quantified only partially in my LC-MS/MS analysis on the proteins in the limited regions (Fig 8E). In other words, the emPAI value of cone transducin alpha subunit could be much higher when all of them were analyzed.

Immunological analysis on the localization of neurocalcin-delta B in cones

To investigate the subcellular localization of Neurocalcin-delta B (NCALD) in cones, immunoblot analysis was applied to separated ROS, RIS, COS and CIS membranes as described in Tachibanaki et al. (2005) using anti-human Neurocalcin-delta (NCALD) antibody (at 1:100 dilution), and immunocytochemical analysis was applied to isolated cones as described in Arinobu et al. (2010) with some modifications. Briefly, mechanically isolated carp photoreceptors were suspended in a carp Ringer’s solution supplemented with 4% (w/v) paraformaldehyde and then incubated for 12 hours at 4 °C in dark. Then they were mounted on a silane-coated slide glass and permeabilized with PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM NaH2PO4, pH 7.4) containing 1% (w/v) Triton X-100 for 5 min, air-dried and blocked with PBS containing 0.2% (w/v) Triton X-100 and 1.5 % (v/v) normal goat serum. Mounted cells were then washed with PBS for three times, and incubated for an hour at room temperature with a solution containing anti-human NCALD antibody (at 1:75 dilution) and anti-mitochondrial aspartate aminotransferase 2 (mAAT) antiserum (at 1:300 dilution). Then the cells were then washed with PBS for three times, and incubated at room temperature for 30 min with mixture of Alexa488-conjugated anti-rabbit IgG (at 1:200 dilution) to detect NCALD and Alexa568-conjugated anti-mouse IgG (at 1:300 dilution) to detect mAAT.
Results and Discussion

Purify of each of OS and IS membranes

In this study, I originally intended to identify soluble and membrane-bound proteins expressed specifically or dominantly in cone OS. It would be ideal to detect proteins expressed in purified cone OS and compare them with proteins expressed in cone IS, rod OS and rod IS. For this purpose, I first tried to isolate intact OS containing soluble and membrane-bound proteins from purified carp rods mechanically with the mechanical method reported previously for frog rod OS showing typical rod-shape (Woodruff et al., 1979). However, the attempt was not successful because I observed only deteriorated rod OS in carp after mechanical separation (see Fig 4E, for example). Thus, in this study, I tried to obtain OS membranes, instead of intact OS, from purified carp rods and cones. The procedure is shown in Fig 4. Purified rods (Fig 4A) and cones (Fig 4B) were mechanically disrupted to separate the OS and the IS (Fig 4C and D). A mixture of deteriorated and separated OS and IS was placed on top of a stepwise sucrose density gradient, 36/50 % (w/v) for rods and 36/70 % (w/v) for cones, in a test tube, and then centrifuged to separate them (Fig 4E and G for rods, and Fig 4F and H for cones). At this stage, OS and IS were disrupted and they were practically in the form of membranes. These membranes in each interface were collected and suspended in Ringer's solution.

Fig 4. Purification of OS and IS membranes from purified rods and cones.
Differential interference contrast microscopic (DIC) images of the cell fractions in each step of the purification are shown. Purified carp rods (A) and cones (B) were passed through a 27-gauge needle to dissociate the OS from the IS, and the resultant broken rods (C) and cones (D) were layered on a sucrose density gradient made in a test tube (drawings in the left of E/G and F/H) to centrifuge. The number in the drawings shows the density of sucrose (% w/v). Separated membranes at upper (E, F) and lower (G, H) interfaces were collected. Scale bar, 20 \( \mu m \) throughout.
To evaluate how effectively the separation of OS and IS membranes was achieved with this procedure, membranes obtained at upper interface (Fig 4E for rods and F for cones) and those at the lower interface (Fig 4G for rods and H for cones) were probed quantitatively with five membrane protein markers: visual pigments for the OS, F1 ATPase beta subunit for IS mitochondrial inner membranes (Schwaiger et al., 1987), TOM20 for IS mitochondrial outer membranes (Ramage et al., 1993, Xie et al., 2005), Na+/K+ ATPase alpha subunit for IS plasma membranes (Wetzel et al., 1999, Kwok et al., 2008), and calnexin for IS ER membranes (Hammond and Helenius, 1994, Lakkaraju et al., 2012).

Visual pigments in the membranes of starting purified rods and cones, and in the obtained fractions from upper interfaces (Fig 4E for rods and F for cones) and lower interfaces (Fig 4G for rods and H for cones) were quantified spectrophotometrically (Fig 5). Membranes obtained at each interface were suspended in Ringer’s solution of the volume same as that of the initial total membranes obtained from purified rods and cones. In the followings, I describe total membranes obtained from starting purified rods or cones as just initial rod or cone membranes, and indicate the content of a protein in each upper or lower fraction as the % of the content in the initial membranes. In the case of rods (Fig 5A), the rod visual pigment, rhodopsin, was mainly distributed at upper interface (Upper fraction in Fig 5A, middle panel) after the separation. Distribution of rhodopsin at the upper interface was 86.2 % (n = 3, Table 1) of the initial rod membranes (Initial in Fig 5A, left panel). In contrast, visual pigments in the lower interfaces were negligible (Lower fraction in Fig 5A, right panel). When the initial rod membranes and membranes both the upper and the lower fractions of rods were subjected to SDS-PAGE (Fig 6A), two or three thick bands of rhodopsin (arrowheads in Fig 6A) were detected in the initial rod membranes (Fig 6A, Initial) and in rod membranes in the upper fraction (Fig 6A, Upper). The lower (~35 kDa) and higher (~70 kDa and ~150 kDa) molecular masses of rhodopsin band are those of monomer, dimer and tetramer, respectively. In contrast, in the lower fraction (Fig 6A, Lower), rhodopsin bands were practically not detected. All of the results shown in Fig 5A and Fig 6A showed that ROS membranes were collected in the upper fraction almost exclusively.
Fig 5. Quantification of visual pigments.

Quantity of visual pigments was measured spectrophotometrically in three types of rod (A) and cone (B) preparations: membranes from purified cells as initial materials (Initial), membranes in the upper (Upper fraction) and lower (Lower fraction) fraction. (A) Rhodopsin content was measured in the initial rod membranes (left panels), in the upper and lower fraction (middle and right panels, respectively), all obtained from the same number of cells and suspended in the same volume of Ringer's solution. In each of upper panels, curve 1 (black) shows the absorption spectrum before bleach, and curve 2 (blue) shows the spectrum after complete bleach of rhodopsin with illumination of >440 nm light. Curve 2 was subtracted from curve 1 in each of the upper panel to obtain a difference spectrum, which is shown in the corresponding lower panel. From positive absorption by rhodopsin ($\lambda_{\text{max}} = 522$ nm), relative rhodopsin content was determined. (B) Contents of red-, green-, and blue-sensitive pigments were measured in the initial purified cone membranes (left panels), in the upper and lower fraction (middle and right panels, respectively). In each of upper panels, curve 1 (black) shows absorption spectrum before bleach. Red-sensitive pigment was first bleached with >675 nm light (curve 2), and then green-sensitive pigment with >600 nm light (curve 3) and finally blue-sensitive pigment with >440 nm light (curve 4). Curve 2 was subtracted from curve 1 to obtain a difference spectrum of red-sensitive pigment, which is shown in the corresponding lower panel (red curve 1', $\lambda_{\text{max}} = 622$ nm). Similarly, difference spectra were obtained for green-sensitive pigment (green curve 2', i.e., $\lambda_{\text{max}} = 535$ nm) and for blue-sensitive pigment (blue curve 3', i.e., $\lambda_{\text{max}} = 460$ nm) to determine the relative contents of these pigments.
Table 1. Recovery of membranes after separation of OS and IS.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Outer segment membrane</th>
<th>Inner segment, Mitochondrial membranes</th>
<th>Inner segment, Plasma membranes</th>
<th>Inner segment, ER membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>F1 ATPase beta subunit</td>
<td>TOM20</td>
<td>Na’/K’ ATPase alpha subunit</td>
</tr>
<tr>
<td>Rod upper fraction</td>
<td>86.2 ± 13.4 %</td>
<td>N.D.</td>
<td>9.1 ± 0.4 %</td>
<td>65.7 ± 9.5 %</td>
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<tr>
<td>Rod lower fraction</td>
<td>0.9 ± 0.2 %</td>
<td>84.8 ± 4.3 %</td>
<td>42.5 ± 7.0 %</td>
<td>40.2 ± 1.2 %</td>
</tr>
<tr>
<td>Cone upper fraction</td>
<td>54.5 ± 3.2 %</td>
<td>3.5 ± 0.9 %</td>
<td>6.86 ± 1.5 %</td>
<td>49.0 ± 8.0 %</td>
</tr>
<tr>
<td>Cone lower fraction</td>
<td>3.5 ± 0.2 %</td>
<td>54.6 ± 4.4 %</td>
<td>58.2 ± 12.3 %</td>
<td>10.9 ± 1.6 %</td>
</tr>
</tbody>
</table>

Each value is shown as mean ±S.E in 3 independent measurements. N.D., signals not detected.

Fig 6. Quantification of F1 ATPase beta subunit.
Quantities of F1 ATPase beta subunit were determined by SDS-PAGE in four rod (A) and four cone (B) membrane preparations. (A) Rod membranes as initial materials (Initial), and rod upper and rod lower fractions (Upper and Lower, respectively) were subjected to SDS-PAGE. The gels were stained with a fluorescent dye, Oriole. In the lane labeled as 4×Upper, 4 times volume of the upper fraction was applied to quantify the amount of F1 ATPase beta subunit precisely. Arrowheads indicate the monomer, dimer and tetramer bands of rhodopsin, and arrows indicate the band of F1 ATPase beta subunit. (B) Similar SDS-PAGE pattern using four cone membrane preparations. (C) An example of quantification of F1 ATPase beta subunit with Oriole staining in four rod membrane preparations. A calibration curve was obtained with Oriole staining using known amounts of BSA (open rectangles and filled line), which was performed in parallel with SDS-PAGE of the rod membrane preparations. From the signal intensity of F1 ATPase beta subunit in (A), the amount of F1 ATPase beta subunit was quantified in four rod membrane preparations using the calibration curve (downward arrows). Note that F1 ATPase beta subunit was not detected in upper fractions in (A). (D) Similar quantification in four cone membrane preparations.
In the case of cones, red-, green-, and blue-sensitive cone visual pigments were also dominantly detected in the membranes at the upper interface (Fig 5B, left and middle panels) as in the case of rods, but distribution of the cone pigments in the upper fraction was lower than that of rods: it was 54.5 % in cones (n = 3, Table 1) while it was 86.2 % in rods as mentioned above. The amount of the cone visual pigments in the lower fraction was almost negligible similarly as in rods (Fig 5B, right panel). These results clearly show that both the rod and cone OS membranes were collected effectively at the upper interface (Ringer’s solution/36 % sucrose interface) and therefore in the upper fraction, and there were little contaminations of OS membranes in the lower fractions.

Next, separation of IS membrane was examined. First, separation of inner mitochondrial membranes was examined by quantifying its marker protein, F1 ATPase beta subunit, in the upper and the lower fractions of both rods and cones. When the initial rod membranes and membranes in the upper and lower fractions of rods (Fig 6A), or those of cones (Fig 6B) were subjected to SDS-PAGE, a band was detected at 55 kDa (arrows) in the lane of initial rod membranes (Fig 6A, Initial) and that of initial cone membranes (Fig 6B, Initial). This band was identified as carp F1 ATPase beta subunit (AB023582.1) by mass spectrometry, and was also clearly detected in the lower fractions of both rods and cones (Upper in Fig 6A and B, respectively). The amount of F1 ATPase beta subunit in each of fractions (Initial, Upper, 4×Upper and Lower) was quantified by measuring the Oriole fluorescent signals of the band in each lane using BSA as a molar standard (Fig 6C and D) and the results showed that the mitochondrial inner membrane marker was detected minimally in the upper fraction (not detected in rods and 3.5 % in cones) and mainly in the lower fraction (84.8 % in rods and 54.6 % in cones) (Table 1, F1 ATPase beta subunit).
Fig 7. Estimation of separation of OS and IS membranes using TOM20, Na⁺/K⁺ ATPase alpha subunit and calnexin as marker proteins.

(A) Specificity of antibodies used to detect TOM20, Na⁺/K⁺ ATPase alpha subunit and that of anti-calnexin antiserum. Purified rod membranes containing 200 pmol of rhodopsin and cone membranes containing 6 pmol of cone total pigments were subjected to SDS-PAGE and were stained with Coomassie Brilliant Blue (left panel) or probed with antibodies or antiserum against each protein (right three panels). (B-D) Quantitative immunoblot analyses of TOM20 (B), Na⁺/K⁺ ATPase alpha subunit (C) and calnexin (D). In the upper panels in each of (B) - (D), purified rod membranes containing 200 pmol of rhodopsin or purified cone membranes containing 6 pmol of total cone pigments (Initial), upper and lower membrane fractions obtained from the same number of the purified cells (Upper and Lower, respectively), and a diluted series of initial rod and cone membranes were subjected to SDS-PAGE. These membranes were probed with antibodies or antiserum against each marker protein. To detect the amounts of target proteins precisely, 4 times volume of samples were applied when necessary (4×). The amount of a target protein in each of the membranes was determined with a calibration line obtained from immunoblot signals obtained in a diluted series of initial rod or cone membranes. In the lower panels in each of (B) - (D), examples of quantification are shown. The quantity of a target protein in each fraction is indicated by an arrow in lower panels. With this estimation, one can determine how much % of the target protein is present in each of the membranes as compared with the amount in the initial rod or cone membranes of the same cell number.
Then, separation of other membranes in the cellular organelles in the IS, IS plasma membranes, mitochondrial outer membranes and ER membranes, were examined. Those membranes in these fractions were probed with quantitative immunoblot analyses using marker proteins: TOM20 for mitochondrial outer membranes, Na\(^+/K^+\) ATPase alpha subunit for IS plasma membranes and calnexin for ER membranes (Fig 7). As shown in Fig 7A, each of antibodies or antiserum used in the analysis detected a single band in purified rod and cone membranes, and the molecular mass of each of the detected band corresponded to the known value of the target protein. These results indicated that used antibodies and antiserum reacted to target proteins selectively. Next, the three marker proteins were quantified in initial rod and cone membranes and in the upper and the lower fractions (Fig 7B-D and Table 1). Fig 7B shows an example of the quantification of TOM20, a marker of mitochondrial outer membrane. This marker was detected in the upper fraction to some extent (9.1 % in rods and 6.9 % in cones), but much more in the lower fraction (42.5 % in rods and 58.2 % in cones) similarly as mitochondrial inner membrane marker, F1 ATPase beta subunit.

The IS plasma membrane marker, Na\(^+/K^+\) ATPase alpha subunit, was detected in both the upper and the lower fractions, but the marker was present slightly more in the upper fraction: the percentage of the marker present in the upper fractions was 65.7 % in rods and 49.0 % in cones, and in the lower fractions, it was 40.2 % in rods and 10.9 % in cones (Fig 7C and Table 1). Calnexin, an ER membrane marker, was also present in both the upper and the lower fraction as IS plasma membranes. This marker was slightly enriched in the upper fraction in rods (26.5 % in the upper fraction and 9.9 % in the lower fraction) but detected almost equally in both fractions in cones (35.5 % in the upper fraction and 38.4 % in the lower fraction) (Fig 7D and Table 1).

From these results, it is concluded that upper fraction exclusively contains OS membranes together with some of IS membranes: IS plasma membranes, ER membranes and less abundantly mitochondrial inner and outer membranes. The membranes used are all from
purified rods and cones. These purified cells contain OS and also IS consisting of the ellipsoid and the myoid but lacking the nucleus and the terminal (Kawamura and Tachibanaki, 2008). From consideration of the structural basis of purified rods and cones, IS plasma membranes and ER membranes are probably present much less than mitochondrial membranes and also much less than membranes of highly membranous OS. For this reason, contamination of IS plasma membranes and that of ER membranes would be limited in OS-rich fraction of both rods and cones.

In the present study, I focused on the proteins expressed specifically/dominantly in ROS or COS. Estimation of the amount of a marker protein shown above was based on the percentage of the proteins found in each of the membrane fractions comparing with that in the initial rod or cone membranes. In the COS-rich fraction, 3.5-6.9 % contamination of CIS proteins was observed (Table 1). Note that this contamination is the percentage of the total CIS proteins, and therefore, the actual contamination of CIS proteins is dependent on the total CIS proteins and total COS proteins. Previous estimation of the volumes of COS and CIS in carp showed that the CIS volume is ~6 times higher than the COS volume (Yamaoka et al., 2015). Based on this volume ratio, I estimated the actual contamination of CIS proteins in COS-rich fraction. I assume an extreme case that the membrane density and protein density in the membranes are the same in COS and CIS. In this case, based on the distribution of visual pigment (~55 % of pigments in the initial COS membranes) and those of CIS proteins (TOM20 and F1 ATPase beta subunit; 3.5-6.9 % in the initial CIS membranes) in COS-rich fraction shown in Table 1, the calculation showed that the amount of the contaminated CIS proteins, mainly those in the mitochondrial inner and outer membranes, could be equivalent to the amount of the COS proteins in the COS-rich fraction (COS proteins: CIS proteins = ~55 % × 1: 3.5-6.9 % × 6). On the contrary, COS protein contamination in the CIS-rich fraction could be negligible (COS proteins: CIS proteins = 3.5 % × 1: 54.6-58.2 % × 6). Because contamination of CIS proteins in the COS-rich fraction cannot be ignored, I tried to identify proteins specifically/dominantly present in COS by excluding the proteins found in CIS-rich fraction which was contaminated with COS membranes minimally. Similar volume consideration was made for the amount of RIS proteins contaminated in ROS-rich fraction. In the ROS-rich fraction, 86.2 % of visual pigment, a ROS specific marker protein, was recovered, while the most abundant proteins in the RIS, mitochondrial inner and outer membrane proteins (F1 ATPase beta subunit and TOM20, respectively) were present in ROS-rich fraction at the amount of 9.1 % at the maximum (see Table 1). When the volume difference (ROS volume: RIS volume = 4:1, (Yamaoka et al., 2015)) is taken into consideration, contamination of
mitochondrial proteins in ROS-rich fraction is negligible (ROS proteins: mitochondrial proteins = 86.2 % × 4: 9.1 % × 1). The amount of other IS proteins such as IS plasma membrane proteins and inner segment ER membrane proteins are also little for the amount of ROS proteins in ROS-rich fraction. However, the amount of IS plasma membrane proteins and ER membrane proteins in ROS-rich fraction are equal to or larger than the amount of those in RIS-rich fraction (see Table 1). Therefore, because ROS proteins contamination in RIS-rich fraction are little (ROS proteins: RIS proteins = 0.9 % × 4: 42.5-84.8 % × 1), it is possible to efficiently identify proteins specifically/dominantly present in ROS by excluding the proteins which found and are rich in RIS-rich fraction.

**Identification of proteins specifically/dominantly expressed in ROS or COS**

To identify ROS- or COS-specific/dominant proteins, I made a list of proteins detected in each of the ROS-rich, RIS-rich, COS-rich, and CIS-rich fractions using shotgun proteomic analysis by LC-MS/MS. ROS-rich fraction contains contaminated RIS membranes to some extent. It is also contaminated with COS and CIS membranes, which are potentially present in purified rods in a small amount (Tachibanaki et al., 2001). Similarly, COS-rich fraction contains contaminated CIS membranes significantly, and also contaminated with ROS and RIS membranes and red blood cell membranes, which are potentially present in purified cones in a small amount. Then, to find proteins present specifically/dominantly in ROS, the proteins found in RIS-rich and COS-rich fractions were excluded from the list of the proteins detected in the ROS-rich fraction. With this comparison, not only the common proteins found in both ROS-rich and COS-rich fractions but also the RIS proteins contaminated or commonly present in ROS-rich fraction can be excluded. To find proteins present specifically/dominantly in COS, the same treatment was also carried out for COS-rich fraction.

In this study, I focused on the proteins tightly bound to membranes as the first step to identify proteins specifically/dominantly expressed in ROS or COS because only membranes were obtained (see above). For this purpose, membranes in each of the ROS-rich, RIS-rich, COS-rich, and CIS-rich fractions were washed to remove soluble and membrane-associated peripheral proteins as much as possible. SDS-PAGE patterns of the membranes in each of the fractions before wash (Fig 8A-D, Initial) and the final washed membranes (Fig 8A-D, Washed) are shown in Fig 8A-D. SDS-PAGE pattern of supernatants after washes with low salt buffer twice and with high pH buffer four times (Fig 8A-D, Low salt wash sup and High pH wash sup, respectively) showed that most of the removable proteins were washed away from the membranes.
Fig 8. Preparation of washed membranes for LC-MS/MS. Membranes in ROS-rich (A), RIS-rich (B), COS-rich (C) and CIS-rich (D) fractions were intensively washed with a low salt buffer and a high pH buffer to eliminate soluble and peripheral membrane proteins as much as possible. In (A) - (D), SDS-PAGE patterns of the membranes prepared from initial membranes (Initial), the membranes finally obtained after intensive washes...
These washed membranes in each of fractions shown in Fig 8A-D were then subjected to SDS-PAGE to separate proteins for the analysis with LC-MS/MS. In the ROS-rich fraction, visual pigment, rhodopsin, is the most abundant protein (approx. 95%). Proteins separated in the gel were digested for LC-MS/MS analysis, but massive amount of rhodopsin probably impedes the LC-MS/MS analysis by masking other proteolytic products when it is included. For this reason, rhodopsin band, monomer at 30–40 kDa, dimer at 55–65 kDa and trimer at 80–90 kDa, were excluded in LC-MS/MS analysis. In the analysis of proteins in other fractions, the same regions were omitted in order to compare the proteins equally. The regions indicated by four rectangles in each lane in Fig 8E were subjected to proteolysis and analyzed with LC-MS/MS. As a result, 648, 971, 1,064 and 629 proteins were identified from washed ROS-rich, RIS-rich, CIS-rich and COS-rich fraction, respectively (Supplemental Table S1-4).

Then, I identified proteins specifically/dominantly present in the ROS-rich or COS-rich fraction (Table 2 and 3, respectively). For these purposes, I used the emPAI (exponentially modified protein abundance index) value, which has been reported to be proportional to the amount of a protein present in a sample used for LC-MS/MS analysis (Ishihama et al., 2005). Briefly, I used emPAI values obtained from the same number of the cells as an index of the amount of a protein present in the washed membranes. First, I calculated the portion of a protein in the ROS-rich fraction in a total amount of that protein in ROS-rich and RIS-rich fractions (shown as ROS/(ROS+RIS) in Table 2). With this procedure, I excluded proteins present more in RIS-rich fraction (i.e., those proteins showing ROS/(ROS+RIS)<0.5). The same treatment was applied to the proteins in COS-rich and CIS-rich fractions to exclude CIS proteins. In addition, proteins found in both ROS-rich and COS-rich fractions to similar extent were excluded: for example, when a protein in COS-rich fraction shows the emPAI value of >1/10 of that in ROS-rich fraction, this protein was regarded as the protein expressed in both ROS and COS. Furthermore, in Tables 2 and 3, I excluded the proteins showing...
the emPAI value <1/100 of the maximum value of the most abundant protein in ROS or COS (see Materials and Methods). After this manipulation, 132 proteins and 48 proteins remained in the list of proteins specifically/dominantly found in ROS-rich and COS-rich fraction, respectively. Note that ideally, but not actually, proteins present in the rhodopsin bands or visual pigments bands were not analyzed, and those present at the region close to these bands were not analyzed correctly.
### Table 2. List of probable proteins present almost exclusively in ROS-rich fraction.

<table>
<thead>
<tr>
<th>Protein*</th>
<th>Molecular Mass</th>
<th>emPAI (ROS-rich)</th>
<th>RIS-rich</th>
<th>(ROS+RIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*peripherin-2 (Travis et al., 1991)</td>
<td>21 kDa</td>
<td>37.89</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>rod outer segment membrane protein 1 (Moritz and Molday, 1996)</td>
<td>25 kDa</td>
<td>11.87</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>retinal guanylyl cyclase 2 (Dizhoor et al., 1994, Lowe et al., 1995, Yang et al., 1995)</td>
<td>124 kDa</td>
<td>11.27</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: olfactory guanylyl cyclase GC-D isoform X3 (Dizhoor et al., 1994, Lowe et al., 1995, Yang et al., 1995)</td>
<td>88 kDa</td>
<td>9</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>retinal guanylyl cyclase 2 (Dizhoor et al., 1994, Lowe et al., 1995, Yang et al., 1995)</td>
<td>125 kDa</td>
<td>7.78</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>uncharacterized protein LOC100005305 (Azadi et al., 2010)</td>
<td>11 kDa</td>
<td>5.06</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ADP-ribosylation factor-like protein 8B-A</td>
<td>21 kDa</td>
<td>4.15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>*PREDICTED: pleckstrin homology domain-containing family B member 1 isoform X1 (Xu et al., 1999)</td>
<td>21 kDa</td>
<td>4.15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: uncharacterized protein LOC571872 isoform X1 (Kwok et al., 2008)</td>
<td>23 kDa</td>
<td>3.63</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>olfactory guanylyl cyclase GC-D (Dizhoor et al., 1994, Lowe et al., 1995, Yang et al., 1995)</td>
<td>34 kDa</td>
<td>3.26</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ras-related protein Rab-18 (Kwok et al., 2008)</td>
<td>23 kDa</td>
<td>3.05</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ADP-ribosylation factor-like protein 8A</td>
<td>25 kDa</td>
<td>3.04</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: olfactory guanylyl cyclase GC-D isoform X2 (Dizhoor et al., 1994, Lowe et al., 1995, Yang et al., 1995)</td>
<td>26 kDa</td>
<td>2.9</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: ADP-ribosylation factor-like protein 13B-like isoform X5</td>
<td>27 kDa</td>
<td>2.67</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ras-related protein Rab-11A (Kwok et al., 2008, Ying et al., 2016)</td>
<td>22 kDa</td>
<td>2.57</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>dnu1 homolog subfamily C member 5G</td>
<td>22 kDa</td>
<td>2.53</td>
<td>0</td>
<td>1</td>
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<tr>
<td>PREDICTED: protein RD3-like (Azadi et al., 2010)</td>
<td>24 kDa</td>
<td>2.24</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>small GTPase RhoA</td>
<td>22 kDa</td>
<td>1.64</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>synaptobrevin homolog YKT6 (Kwok et al., 2008)</td>
<td>22 kDa</td>
<td>1.57</td>
<td>0</td>
<td>1</td>
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<tr>
<td>uncharacterized protein LOC49549 (Gordiyenko et al., 2010)</td>
<td>23 kDa</td>
<td>1.49</td>
<td>0</td>
<td>1</td>
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<td>ras-related protein Ral-A</td>
<td>23 kDa</td>
<td>1.48</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: tubulin beta-4B chain-like, partial</td>
<td>16 kDa</td>
<td>1.42</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: ras-related protein Rab-28 isoform X2 (Roosing et al., 2013)</td>
<td>25 kDa</td>
<td>1.35</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>*peripherin 2b (retinal degeneration, slow) (Travis et al., 1991)</td>
<td>39 kDa</td>
<td>1.29</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>guanylyl cyclase 3 (Fu and Yau, 2007, Kawamura and Tachibana, 2008)</td>
<td>123 kDa</td>
<td>1.22</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>*PREDICTED: GTP-binding protein SAR1b-like (Bar-Peled and Raikhel, 1997)</td>
<td>13 kDa</td>
<td>1.19</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>guanylyl cyclase 3 (Fu and Yau, 2007, Kawamura and Tachibana, 2008)</td>
<td>128 kDa</td>
<td>1.15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: alpha/beta hydrolase domain-containing protein 17A</td>
<td>28 kDa</td>
<td>1.12</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Molecular Weight</td>
<td>pI</td>
<td>Tryptic Peptides</td>
<td># Modifications</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------</td>
<td>----</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Predicted: protein RD3-like (Azadi et al., 2010)</td>
<td>24 kDa</td>
<td>1.09</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: retinol dehydrogenase 8a (Miyazono et al., 2008)</td>
<td>35 kDa</td>
<td>1.02</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: josephin-2 isoform X1</td>
<td>21 kDa</td>
<td>0.96</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: ADP-ribosylation factor 2</td>
<td>16 kDa</td>
<td>0.93</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: uncharacterized protein sidkey-182g1.3</td>
<td>16 kDa</td>
<td>0.93</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: periherin-2 (Travis et al., 1991)</td>
<td>39 kDa</td>
<td>0.9</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: vesicle transport through interaction with t-SNAREs homolog 1A isoform X1</td>
<td>26 kDa</td>
<td>0.72</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: phospholipid-transporting ATPase IB isoform X6</td>
<td>134 kDa</td>
<td>0.67</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: regulator of G-protein signaling 9 isoform X1 (Tachibanaki et al., 2012)</td>
<td>57 kDa</td>
<td>0.66</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: cGMP-gated cation channel alpha-1 (Weitz et al., 2002, Zheng et al., 2002)</td>
<td>36 kDa</td>
<td>0.65</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: guanine nucleotide-binding protein subunit beta-5 (Hu et al., 2003)</td>
<td>43 kDa</td>
<td>0.64</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: rho-related GTP-binding protein rhoC</td>
<td>22 kDa</td>
<td>0.62</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: ras homolog gene family, member A</td>
<td>22 kDa</td>
<td>0.61</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: ras-related protein Rab-30</td>
<td>15 kDa</td>
<td>0.61</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: ras-related protein Rab-8A (Deretic et al., 1995, Kwok et al., 2008, Ying et al., 2016)</td>
<td>23 kDa</td>
<td>0.59</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Predicted: CSC1-like protein 2 isoform X2</td>
<td>87 kDa</td>
<td>0.58</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: sodium/potassium/calcium exchanger 1 isoform X1 (Kim et al., 1998)</td>
<td>80 kDa</td>
<td>0.57</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: choline transporter-like protein 1 (Michel and Bakovic, 2009)</td>
<td>72 kDa</td>
<td>0.49</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: protein-L-isooaspartate(D-aspartate) O-methyltransferase isoform X1</td>
<td>26 kDa</td>
<td>0.49</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: ragulator complex protein LAMTOR1</td>
<td>18 kDa</td>
<td>0.49</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Predicted: CSC1-like protein 2</td>
<td>47 kDa</td>
<td>0.47</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Predicted: uncharacterized protein si:rp71-36a1.2</td>
<td>28 kDa</td>
<td>0.46</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: uncharacterized protein LOC100001558 (Weitz et al., 2002, Zheng et al., 2002)</td>
<td>19 kDa</td>
<td>0.46</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: tetrastricopeptide repeat protein 8-like isoform X1</td>
<td>58 kDa</td>
<td>0.45</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Predicted: protein tyrosine phosphatase type IVA 2-like isoform X3</td>
<td>19 kDa</td>
<td>0.44</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Predicted: E3 ubiquitin-protein ligase RNF170-like</td>
<td>29 kDa</td>
<td>0.44</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Predicted: solute carrier family 41 member 1</td>
<td>40 kDa</td>
<td>0.42</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Predicted: ADP-ribosylation factor-like protein 1 (Van et al., 2001)</td>
<td>20 kDa</td>
<td>0.41</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: palmitoyltransferase ZDHHC2</td>
<td>42 kDa</td>
<td>0.41</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: ADP-ribosylation factor-like protein 3</td>
<td>20 kDa</td>
<td>0.41</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: signal recognition particle receptor subunit beta</td>
<td>31 kDa</td>
<td>0.41</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: ras-related protein Rap-2c</td>
<td>21 kDa</td>
<td>0.4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Predicted: casein kinase I isoform gamma-1-like isoform X2</td>
<td>53 kDa</td>
<td>0.4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Mass (kDa)</td>
<td>Value</td>
<td>p-value</td>
<td>q-value</td>
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<td>alpha/beta hydrolase domain-containing protein 17C</td>
<td>32</td>
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<td>1</td>
</tr>
<tr>
<td>cell division control protein 42 homolog</td>
<td>21</td>
<td>0.39</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PREDICTED: F-box/LRR-repeat protein 20</td>
<td>45</td>
<td>0.38</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>syntaxin 5</td>
<td>34</td>
<td>0.36</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ras-related protein Rab-24</td>
<td>23</td>
<td>0.36</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>^3 receptor expression-enhancing protein 6 (Arno et al., 2016)</td>
<td>23</td>
<td>0.35</td>
<td>0</td>
<td>1</td>
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<tr>
<td>uncharacterized protein LOC101884052</td>
<td>11</td>
<td>0.35</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>*peripherin-2 (Travis et al., 1991)</td>
<td>18</td>
<td>13.19</td>
<td>0.06</td>
<td>0.996</td>
</tr>
<tr>
<td>PREDICTED: pyruvate kinase PKM isoform X1</td>
<td>60</td>
<td>9.33</td>
<td>0.07</td>
<td>0.992</td>
</tr>
<tr>
<td>neurotrimin isoform 1 precursor</td>
<td>38</td>
<td>3.08</td>
<td>0.03</td>
<td>0.992</td>
</tr>
<tr>
<td>rod outer segment membrane protein 1 (Moritz and Molday, 1996)</td>
<td>40</td>
<td>9.09</td>
<td>0.08</td>
<td>0.991</td>
</tr>
<tr>
<td>PREDICTED: cadherin-related family member 1-like isoform X2</td>
<td>99</td>
<td>0.61</td>
<td>0.01</td>
<td>0.984</td>
</tr>
<tr>
<td>uncharacterized protein LOC492355</td>
<td>23</td>
<td>2.41</td>
<td>0.04</td>
<td>0.982</td>
</tr>
<tr>
<td>*peripherin-2 (Travis et al., 1991)</td>
<td>20</td>
<td>6.1</td>
<td>0.11</td>
<td>0.982</td>
</tr>
<tr>
<td>PREDICTED: cGMP-gated cation channel alpha-1 (Weitz et al., 2002, Zheng et al., 2002)</td>
<td>63</td>
<td>2.31</td>
<td>0.05</td>
<td>0.979</td>
</tr>
<tr>
<td>lysophosphatidylcholine acyltransferase 2</td>
<td>60</td>
<td>0.62</td>
<td>0.02</td>
<td>0.974</td>
</tr>
<tr>
<td>rhodopsin</td>
<td>40</td>
<td>35.41</td>
<td>0.96</td>
<td>0.974</td>
</tr>
<tr>
<td>*peripherin-2 (Travis et al., 1991)</td>
<td>20</td>
<td>1.9</td>
<td>0.05</td>
<td>0.973</td>
</tr>
<tr>
<td>PREDICTED: ADP-ribosylation factor-like protein 13B-like isoform X5</td>
<td>42</td>
<td>1.77</td>
<td>0.05</td>
<td>0.973</td>
</tr>
<tr>
<td>PREDICTED: red-sensitive opsin-1 isoform X1 (Weitz et al., 2002)</td>
<td>41</td>
<td>0.85</td>
<td>0.02</td>
<td>0.972</td>
</tr>
<tr>
<td>ras-related protein Rab-18-B (Kwok et al., 2008)</td>
<td>23</td>
<td>1.5</td>
<td>0.04</td>
<td>0.971</td>
</tr>
<tr>
<td>ras-related protein Rap-1b-like (Kwok et al., 2008)</td>
<td>21</td>
<td>3.51</td>
<td>0.11</td>
<td>0.971</td>
</tr>
<tr>
<td>PREDICTED: cGMP-gated cation channel alpha-1 (Weitz et al., 2002, Zheng et al., 2002)</td>
<td>63</td>
<td>2.13</td>
<td>0.07</td>
<td>0.969</td>
</tr>
<tr>
<td>putative Ras-related protein Rab-42</td>
<td>18</td>
<td>1.7</td>
<td>0.06</td>
<td>0.967</td>
</tr>
<tr>
<td>PREDICTED: ras-related protein Rab-7a isoform X1 (Gordienko et al., 2010)</td>
<td>24</td>
<td>1.11</td>
<td>0.04</td>
<td>0.963</td>
</tr>
<tr>
<td>PREDICTED: ras-related protein Rab-10</td>
<td>23</td>
<td>3.73</td>
<td>0.16</td>
<td>0.959</td>
</tr>
<tr>
<td>ras-related protein Rab-2A</td>
<td>24</td>
<td>16.19</td>
<td>0.76</td>
<td>0.955</td>
</tr>
<tr>
<td>PREDICTED: ras-related protein Rab-11B (Kwok et al., 2008)</td>
<td>25</td>
<td>5.48</td>
<td>0.28</td>
<td>0.951</td>
</tr>
<tr>
<td>PREDICTED: phospholipid scramblase 2</td>
<td>26</td>
<td>1.55</td>
<td>0.08</td>
<td>0.95</td>
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<tr>
<td>^2 transmembrane protein 33 (Urade et al., 2014)</td>
<td>29</td>
<td>0.64</td>
<td>0.04</td>
<td>0.948</td>
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<td>epidermal retinol dehydrogenase 2</td>
<td>34</td>
<td>1.09</td>
<td>0.06</td>
<td>0.946</td>
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<tr>
<td>ADP-ribosylation factor-like protein 6</td>
<td>21</td>
<td>1.73</td>
<td>0.11</td>
<td>0.942</td>
</tr>
<tr>
<td>ras-related protein Rap-1b precursor (Kwok et al., 2008)</td>
<td>21</td>
<td>2.86</td>
<td>0.18</td>
<td>0.942</td>
</tr>
<tr>
<td>^5 ubiquitin-60S ribosomal protein L40 (Perrelet, 1972)</td>
<td>12</td>
<td>1.37</td>
<td>0.09</td>
<td>0.939</td>
</tr>
<tr>
<td>PREDICTED: cadherin-related family member 5-like isoform X2</td>
<td>17</td>
<td>0.83</td>
<td>0.06</td>
<td>0.933</td>
</tr>
<tr>
<td>^13 PREPARED: GTP-binding protein SAR1b (Bar-Peled and Raikhel, 1997)</td>
<td>22</td>
<td>0.6</td>
<td>0.05</td>
<td>0.93</td>
</tr>
<tr>
<td>ADP-ribosylation factor-like 15a</td>
<td>23</td>
<td>0.59</td>
<td>0.04</td>
<td>0.93</td>
</tr>
<tr>
<td>Protein Description</td>
<td>MW</td>
<td>Fold Change</td>
<td>p-Value</td>
<td>q-Value</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------------</td>
<td>-----</td>
<td>-------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>ras-related protein Rab-35</td>
<td>20 kDa</td>
<td>2.35</td>
<td>0.18</td>
<td>0.928</td>
</tr>
<tr>
<td>dolichol-phosphate mannosyltransferase subunit 1</td>
<td>28 kDa</td>
<td>0.46</td>
<td>0.04</td>
<td>0.928</td>
</tr>
<tr>
<td><strong>PREDICTED: uncharacterized protein LOC571872 isoform X1</strong> (Kwok et al., 2008)</td>
<td>32 kDa</td>
<td>7.97</td>
<td>0.62</td>
<td>0.927</td>
</tr>
<tr>
<td>140S ribosomal protein S3a (Perrelet, 1972)</td>
<td>33 kDa</td>
<td>0.39</td>
<td>0.03</td>
<td>0.926</td>
</tr>
<tr>
<td>ER membrane protein complex subunit 3 (Christianson et al., 2010)</td>
<td>30 kDa</td>
<td>1.3</td>
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<td>0.919</td>
</tr>
<tr>
<td>uncharacterized protein LOC393228</td>
<td>21 kDa</td>
<td>0.93</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>ras-related protein Rab-5B (Shelby et al., 2015)</td>
<td>24 kDa</td>
<td>0.82</td>
<td>0.09</td>
<td>0.898</td>
</tr>
<tr>
<td>peroxisomal membrane protein 11B</td>
<td>25 kDa</td>
<td>0.76</td>
<td>0.09</td>
<td>0.897</td>
</tr>
<tr>
<td><strong>PREDICTED: 40S ribosomal protein S3-like isoform X1 (Perrelet, 1972)</strong></td>
<td>28 kDa</td>
<td>0.65</td>
<td>0.08</td>
<td>0.895</td>
</tr>
<tr>
<td>ras-related protein Rab-14</td>
<td>24 kDa</td>
<td>1.81</td>
<td>0.21</td>
<td>0.894</td>
</tr>
<tr>
<td><strong>PREDICTED: ras-related protein Rab-1A-like isoform X1</strong></td>
<td>22 kDa</td>
<td>5.63</td>
<td>0.67</td>
<td>0.893</td>
</tr>
<tr>
<td>ras-related protein Rab-1B (Kwok et al., 2008)</td>
<td>22 kDa</td>
<td>5.57</td>
<td>0.67</td>
<td>0.893</td>
</tr>
<tr>
<td>140S ribosomal protein S10 (Perrelet, 1972)</td>
<td>20 kDa</td>
<td>0.41</td>
<td>0.05</td>
<td>0.891</td>
</tr>
<tr>
<td><strong>PREDICTED: cadherin-related family member 5-like isoform X2</strong></td>
<td>44 kDa</td>
<td>1.08</td>
<td>0.13</td>
<td>0.89</td>
</tr>
<tr>
<td>ras-related protein Rab-5C (Shelby et al., 2015)</td>
<td>37 kDa</td>
<td>1.86</td>
<td>0.25</td>
<td>0.88</td>
</tr>
<tr>
<td>ras-related protein Rab-1B (Kwok et al., 2008)</td>
<td>19 kDa</td>
<td>2.06</td>
<td>0.29</td>
<td>0.875</td>
</tr>
<tr>
<td>RAB11a, member RAS oncogene family, like (Kwok et al., 2008, Ying et al., 2016)</td>
<td>20 kDa</td>
<td>1.86</td>
<td>0.27</td>
<td>0.873</td>
</tr>
<tr>
<td>14alpha-1,3/1,6-mannosyltransferase ALG2 (Thiel et al., 2003)</td>
<td>46 kDa</td>
<td>0.48</td>
<td>0.07</td>
<td>0.872</td>
</tr>
<tr>
<td><strong>PREDICTED: sphingomyelin phosphodiesterase 2 isoform X1</strong></td>
<td>48 kDa</td>
<td>0.45</td>
<td>0.07</td>
<td>0.871</td>
</tr>
<tr>
<td>rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha (Catty et al., 1992)</td>
<td>99 kDa</td>
<td>0.44</td>
<td>0.06</td>
<td>0.871</td>
</tr>
<tr>
<td>1460S ribosomal protein L9 (Perrelet, 1972)</td>
<td>22 kDa</td>
<td>0.62</td>
<td>0.1</td>
<td>0.86</td>
</tr>
<tr>
<td>N-acetyltransferase 14</td>
<td>32 kDa</td>
<td>0.4</td>
<td>0.07</td>
<td>0.856</td>
</tr>
<tr>
<td>RAB5A, member RAS oncogene family, a (Shelby et al., 2015)</td>
<td>24 kDa</td>
<td>2.31</td>
<td>0.39</td>
<td>0.856</td>
</tr>
<tr>
<td>CDP-diacylglycerol--inositol 3-phosphatidytransferase</td>
<td>24 kDa</td>
<td>0.8</td>
<td>0.15</td>
<td>0.844</td>
</tr>
<tr>
<td>1440S ribosomal protein S5 (Perrelet, 1972)</td>
<td>25 kDa</td>
<td>0.77</td>
<td>0.14</td>
<td>0.844</td>
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<tr>
<td>14nicastrin precursor (Baulac et al., 2003)</td>
<td>42 kDa</td>
<td>0.41</td>
<td>0.08</td>
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</tr>
<tr>
<td>immunity-related GTPase family, q2</td>
<td>42 kDa</td>
<td>1.35</td>
<td>0.26</td>
<td>0.838</td>
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<tr>
<td>14vesicle-trafficking protein SEC22b-A (Nakajima et al., 2004)</td>
<td>24 kDa</td>
<td>1.07</td>
<td>0.21</td>
<td>0.836</td>
</tr>
<tr>
<td>14uncharacterized protein LOC100127828 (Moon and Horton, 2003)</td>
<td>34 kDa</td>
<td>0.86</td>
<td>0.18</td>
<td>0.826</td>
</tr>
<tr>
<td>RAB1A, member RAS oncogene family</td>
<td>28 kDa</td>
<td>1.44</td>
<td>0.31</td>
<td>0.825</td>
</tr>
<tr>
<td>guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 (Lerea et al., 1986)</td>
<td>37 kDa</td>
<td>16.62</td>
<td>3.61</td>
<td>0.821</td>
</tr>
<tr>
<td>14protein NDRG1 isoform 1 (Takita et al., 2016)</td>
<td>42 kDa</td>
<td>3.23</td>
<td>0.77</td>
<td>0.808</td>
</tr>
</tbody>
</table>

*Proteins already known to be specifically expressed in ROS are indicated in red bold, and those known to be expressed in other portions of a rod or in other cells are indicated with color background. Yellow background: proteins expressed in both ROS and COS, including those expressed in ROS, but their expression in COS not confirmed.
Yellow background: proteins abundantly expressed in ROS and less abundantly in COS (°), or those abundantly expressed in COS and less abundantly in ROS (°).

Green background: proteins detected in purified ROS disk membranes.

Gray background: COS-specific proteins (blue bold), ER specific proteins (ER), mitochondrial protein (Mt) and IS proteins (IS).

The values of emPAI shown are those obtained in the washed membranes of 30 × 10⁴ rods.
Table 3. List of probable proteins present almost exclusively in COS-rich fraction.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Mass</th>
<th>emPAI</th>
<th>COS-rich</th>
<th>CIS-rich</th>
<th>(COS+CIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>creatine kinase b-type (Sistermans et al., 1995)</td>
<td>16 kDa</td>
<td>1.92</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ADP-ribosylation factor 1</td>
<td>21 kDa</td>
<td>1.34</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>protein RD3 (Azadi et al., 2010)</td>
<td>15 kDa</td>
<td>1.04</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>retinoschisin precursor (Molday et al., 2007)</td>
<td>22 kDa</td>
<td>0.62</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP-ribosylation factor 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein RD3 (Azadi et al., 2010)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>retinoschisin precursor (Molday et al., 2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PREDICTED: guanine nucleotide-binding protein G(i) subunit alpha-1</td>
<td>40 kDa</td>
<td>0.56</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: guanine nucleotide-binding protein subunit alpha-13-like</td>
<td>34 kDa</td>
<td>0.52</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: gamma-glutamyltransferase 5 isoform X1</td>
<td>62 kDa</td>
<td>0.5</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: cyclic nucleotide-gated cation channel beta-3-like isoform X2 (Biel et al., 1999)</td>
<td>28 kDa</td>
<td>0.47</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>carboxylic anhydrase (Hageman et al., 1991)</td>
<td>29 kDa</td>
<td>0.45</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: cadherin-related family member 5-like isoform X2</td>
<td>20 kDa</td>
<td>0.41</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>neurocalcin-delta B</td>
<td>22 kDa</td>
<td>0.38</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: cyclic nucleotide-gated channel cone photoreceptor subunit alpha isoform X2 (Biel et al., 1999)</td>
<td>81 kDa</td>
<td>0.36</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: flotillin 1a isoform X1</td>
<td>47 kDa</td>
<td>0.35</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ras-related protein Rab-43</td>
<td>23 kDa</td>
<td>0.35</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>ER vitamin K epoxide reductase complex subunit 1-like protein 1 (Westhofen et al., 2011)</td>
<td>12 kDa</td>
<td>0.34</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>PREDICTED: ras-related protein Rab-35-like</td>
<td>24 kDa</td>
<td>0.34</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: Fc receptor-like protein 5 isoform X2</td>
<td>12 kDa</td>
<td>0.33</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: SH3-containing GRB2-like protein 3-interacting protein 1 isoform X2</td>
<td>12 kDa</td>
<td>0.32</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>synaptic vesicle glycoprotein 2B</td>
<td>12 kDa</td>
<td>0.32</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: uncharacterized protein LOC100334801 isoform X2</td>
<td>26 kDa</td>
<td>0.31</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>guanine nucleotide-binding protein G(i) subunit alpha-2</td>
<td>41 kDa</td>
<td>0.3</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>ubiquitin-like 3b</td>
<td>13 kDa</td>
<td>0.3</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ras-related GTP-binding protein C</td>
<td>13 kDa</td>
<td>0.29</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>band 3 anion transport protein (211V-519E)</td>
<td>35 kDa</td>
<td>3.68</td>
<td>0.08</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>(Schlüter and Drenckhahn, 1986)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyclic nucleotide-gated channel cone photoreceptor subunit alpha-like (Biel et al., 1999)</td>
<td>82 kDa</td>
<td>0.56</td>
<td>0.02</td>
<td>0.974</td>
<td></td>
</tr>
<tr>
<td>PREDICTED: ammonium transporter Rh type A isoform X1 (Iwamoto et al., 1998)</td>
<td>12 kDa</td>
<td>3.2</td>
<td>0.11</td>
<td>0.967</td>
<td></td>
</tr>
<tr>
<td>flotillin-1</td>
<td>41 kDa</td>
<td>2.14</td>
<td>0.1</td>
<td>0.955</td>
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</tr>
<tr>
<td>band 3 anion transport protein (92M-191C)</td>
<td>11 kDa</td>
<td>5.06</td>
<td>0.27</td>
<td>0.949</td>
<td></td>
</tr>
<tr>
<td>(Schlüter and Drenckhahn, 1986)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>flotillin-2a</td>
<td>47 kDa</td>
<td>2.12</td>
<td>0.12</td>
<td>0.947</td>
<td></td>
</tr>
<tr>
<td>guanine nucleotide-binding protein G(t) subunit alpha-2 (Lerea et al., 1986)</td>
<td>40 kDa</td>
<td>28.3</td>
<td>2.02</td>
<td>0.933</td>
<td></td>
</tr>
</tbody>
</table>

29
<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight (kDa)</th>
<th>emPAI</th>
<th>Relative Abundance</th>
<th>emPAI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opsin-1, short-wave-sensitive 1</td>
<td>39</td>
<td>0.44</td>
<td>0.03</td>
<td>0.933</td>
</tr>
<tr>
<td>Band 3 anion transport protein (562G-810W)</td>
<td>27</td>
<td>1.17</td>
<td>0.1</td>
<td>0.923</td>
</tr>
<tr>
<td>Opsin-4</td>
<td>39</td>
<td>2.04</td>
<td>0.25</td>
<td>0.892</td>
</tr>
<tr>
<td>Band 3 anion transport protein (562G-810W)</td>
<td>27</td>
<td>6.33</td>
<td>0.63</td>
<td>0.909</td>
</tr>
<tr>
<td>Band 3 anion transport protein (562G-810W)</td>
<td>27</td>
<td>0.44</td>
<td>0.03</td>
<td>0.933</td>
</tr>
<tr>
<td>PREDICTED: regulator of G-protein signaling 9-binding protein-like</td>
<td>27</td>
<td>0.44</td>
<td>0.03</td>
<td>0.933</td>
</tr>
<tr>
<td>(Schlüter and Drenckhahn, 1986)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PREDICTED: regulator of G-protein signaling 9-binding protein-like</td>
<td>27</td>
<td>0.44</td>
<td>0.03</td>
<td>0.933</td>
</tr>
<tr>
<td>(Tachibanaki et al., 2012, Hu et al., 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Signal peptidase complex subunit 3 (Kalies and Hartmann, 1996)</td>
<td>20</td>
<td>1.39</td>
<td>0.14</td>
<td>0.909</td>
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<tr>
<td>PREDICTED: metal transporter CNNM4</td>
<td>35</td>
<td>0.66</td>
<td>0.08</td>
<td>0.898</td>
</tr>
<tr>
<td>(Parry et al., 2009, Polok et al., 2009)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Brain creatine kinase b (Sistermans et al., 1995)</td>
<td>43</td>
<td>0.52</td>
<td>0.06</td>
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</tr>
<tr>
<td>Adipocyte plasma membrane-associated protein</td>
<td>47</td>
<td>1</td>
<td>0.12</td>
<td>0.893</td>
</tr>
<tr>
<td>Green-sensitive opsin-4</td>
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<td>2.04</td>
<td>0.25</td>
<td>0.892</td>
</tr>
<tr>
<td>Protein kinase, cAMP-dependent, regulatory, type II, alpha A</td>
<td>45</td>
<td>0.62</td>
<td>0.09</td>
<td>0.872</td>
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<tr>
<td>Sympotagmin II</td>
<td>47</td>
<td>0.36</td>
<td>0.06</td>
<td>0.867</td>
</tr>
<tr>
<td>G-protein-coupled receptor kinase 7A (Rinner et al., 2005)</td>
<td>62</td>
<td>0.9</td>
<td>0.11</td>
<td>0.889</td>
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<td>Guanine nucleotide-binding protein G(o) subunit alpha</td>
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<td>0.885</td>
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<td>Protein kinase, cAMP-dependent, regulatory, type II, alpha A</td>
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<td>0.62</td>
<td>0.09</td>
<td>0.872</td>
</tr>
<tr>
<td>Solute carrier family 2, facilitated glucose transporter member 1</td>
<td>53</td>
<td>0.61</td>
<td>0.1</td>
<td>0.854</td>
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<td>Peroxisredoxin-2</td>
<td>22</td>
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<td>0.13</td>
<td>0.831</td>
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<tr>
<td>PREDICTED: sodium/potassium/calcium exchanger 2-like isoform X2</td>
<td>68</td>
<td>0.45</td>
<td>0.1</td>
<td>0.816</td>
</tr>
<tr>
<td>(Li et al., 2006)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha'</td>
<td>98</td>
<td>0.39</td>
<td>0.1</td>
<td>0.8</td>
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<tr>
<td>(Stearns et al., 2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Proteins already known to be specifically expressed in COS are indicated in blue bold, and those known to be expressed in other portions of a cone or in other cells are indicated with color background.

Gray background: IS proteins (IS) and ER specific proteins (ER).

Yellow background: proteins expressed in both ROS and COS, or those abundantly expressed in COS and less abundantly in ROS (e).

Blue background: cone specific protein present both in OS and IS.

Pink background: red blood cell specific proteins.

The values of emPAI shown are those obtained in the washed membranes of $5 \times 10^4$ cones.
Among the 132 proteins found in ROS-rich fraction, 13 proteins are already known to be present only in ROS (shown in red bold in Table 2) and 23 proteins are known to be expressed abundantly in ROS although they are also known to be expressed in COS (proteins with yellow background in Table 2). Additionally, the other 13 proteins are known to be detected in highly purified ROS disk membranes (proteins with green background in Table 2) (Kwok et al., 2008). These facts ensure that the proteins with higher expression in ROS than COS remained in the list and the strategy to identify ROS-specific/dominant proteins is valid. On the one hand, 21 proteins that are known not to be localized at ROS also remained in the list (proteins with gray background in Table 2). With lowering the (ROS/ROS+RIS) value, number of proteins expressed not in the OS seems to be increased. The remaining 62 proteins without special indication in Table 2 could be specifically/dominantly separated into the ROS-rich fraction during the membrane separation. In general, with increasing the value of (ROS/ROS+RIS), the possibility that the protein is expressed specifically/dominantly in ROS would be higher.

Similarly, 48 proteins are listed in Table 3. Among them, 9 proteins are already known to be present only in COS (shown in blue bold in Table 3). The other three proteins are known to be present in both ROS and COS (with yellow background), and two of them (%) showing more than 20 times higher expression levels in COS than in ROS (Tachibanaki et al., 2012) are also listed in Table 3. These facts ensure that the strategy to identify COS-specific/dominant proteins is valid. However, 11 proteins that are known not to be localized in COS also remained in the list (proteins with gray and pink background in Table 3). Unlike ROS-rich fraction, there was little relation between these proteins and (COS/COS+CIS) values. The remaining 24 proteins without special indication in Table 3 could be specifically/dominantly separated into the COS-rich fraction during the membrane separation.

These results that proteins known to be localized outside of ROS or COS were found in Tables 2 and 3 may suggest that there is a local density difference in membranes to induce membrane contamination in my membrane separation using density gradient. In any event, the results indicate that these lists (Tables 2 and 3) contain ROS- or COS-specific/dominant proteins but that their exact localization should be confirmed with alternative ways such as immunocytochemistry.

**Localization of NCALD in cone**

Validation of my separation of membranes was tested by using one of the candidate proteins, neurocalcin delta B (NCALD), which was detected specifically in
the COS-rich fraction in my study. NCALD has been reported to be expressed in amacrine cells and ganglion cells (Nakano et al., 1992), and its localization is also recognized in CIS (Krishnan et al., 2004) but not in COS. As shown in Tables 3, S3 and S4, NCALD was detected in COS but not in CIS, and not in ROS or RIS (Tables S1 and S2).

Localization of NCALD was examined in isolated cones. As has been reported previously (Krishnan et al., 2004), NCALD was present in IS (anti-NCALD in Fig 9A) similarly as mitochondrial aspartate aminotransferase 2 (anti-mAAT in Fig 9A), a marker protein of mitochondrial inner membranes in IS (Masuda et al., 2016). In addition, some punctate NCALD signals were detected also in COS (Fig 9A, anti-NCALD) in the apical region of a COS as well as at the base of a COS, possibly the calycal process. The apparent discrepancy between this immunocytochemistry (mainly present in CIS and slightly in COS) and the result of LC-MS/MS analysis shown in Table 3 (only present in COS membranes) could be due to the difference in the membranes probed in these studies: in immunocytochemistry, native membranes in living cells were probed while in the LC-MS/MS analysis, membranes intensively washed were used. This possibility was examined with immunoblot.

In immunoblot analysis in non-washed membranes of ROS-rich and RIS-rich fractions (ROS-rich and RIS-rich in Fig 9C), NCALD signals was not observed (arrowhead), while positive signals in non-washed COS-rich and CIS-rich fractions were detected (COS-rich and CIS-rich in Fig 9C). The intensity of the signal was higher in the CIS-rich fraction (the number of purified cones used in Fig 9C was smaller for CIS-rich fraction, see legend to Fig 9C), which is in agreement with the immunocytochemical study shown in Fig 9A. However, in membranes intensively washed (washed membranes), which were used for LC-MS/MS analysis, NCALD signal was not detected (Fig 9D, arrowhead) even when the membranes applied for SDS-PAGE increased by 12.5 times for washed COS membrane and 50 times for washed CIS membranes (Washed COS and Washed CIS, respectively in Fig 9D) than those used in Fig 9C.
Neurocalcin is a member of the neuronal calcium sensor family (Burgoyne, 2007, Ames and Lim, 2012), and is thought to be soluble under low Ca\(^{2+}\) conditions. Therefore, it is possible that most of NCALD were removed from membranes during the wash of membranes used for LC-MS/MS analysis, because Ca\(^{2+}\) concentration in washing buffers were low. This would be the reason why NCALD was not detected in washed CIS-rich membranes. Previous studies showed that NCALD is able to interact...
directly with retinal guanylyl cyclase 1 (ROS-GC1) (Krishnan et al., 2004), which is known to be localized to ROS membranes. In carp cones, cone guanylate cyclase (GC-C (Takemoto et al., 2009) or guanylyl cyclase 3) is expressed as the homolog of bovine ROS-GC1. GC-C was detected in COS-rich fraction in my LC-MS/MS analysis (Table S3). It is possible that a few amount of NCALD, which is bound to GC-C in washed COS-rich membranes and is enough to be detected in the LC-MS/MS analysis (Tables 3 and S4) but not in immunoblot analysis (Fig 9D). GC-C was actually detected in the LC-MS/MS analysis in the washed COS-rich fraction but not in the washed CIS-rich fraction (Tables S3 and S4, respectively). However, GC-C was also detected in ROS-rich fraction (Tables 2 and S1) probably because of contamination of cones in purified rod preparation (Tachibanaki et al., 2001). It is the reason why GC-C is not listed in Table 3.

Proteomic analysis on proteins expressed in OS and IS in rods and cones

It has been known that rods and cones are different in many aspects. For example, phototransduction in OS is different (Pugh and Lamb, 1993, Kawamura and Tachibanaki, 2008, Fu and Yau, 2007), OS morphology is different (Pugh and Lamb, 1993, Kawamura and Tachibanaki, 2008, Fu and Yau, 2007), energy metabolism is different (Okawa et al., 2008), and so on. In previous studies, by transcriptomic analysis of purified rods and cones, proteins or genes that are specifically or dominantly expressed in ether rods or cones were found (Akimoto et al., 2006, Tsuji et al., 2007, Shimauchi-Matukawa et al., 2008). These proteins or genes are potentially the candidates participating in the differences between rods and cones. However, it is not easy to identify their functional roles generally. On this point, it is very probable that those proteins are expressed at the site where the difference is observed. In this study, a method to obtain OS and IS membranes effectively from purified rods and cones was established and the quantity was large enough to perform biochemical studies. Then, I actually succeeded in identifying a protein that localized to COS membranes but not in CIS, ROS and RIS membranes (Fig 9), although these membranes were intensively washed. Therefore, these results could contribute to the study aiming at finding proteins specifically expressed at appropriate site, OS or IS in rods or cones, to exert their specific functions.
Future perspectives

In this study, I established a method to obtain OS and IS membranes effectively from purified rods and cones, and found some candidates of ROS- or COS-specific/dominant proteins. I believe that functional analysis of these proteins will elucidate the mechanisms of the differences in function and structure between rods and cones.

By the way, in my study, LC-MS/MS analysis was performed except for rhodopsin bands in ROS-rich fraction and the same regions in RIS-, COS- and CIS-rich fractions because I thought that massive amount of rhodopsin probably impedes the LC-MS/MS analysis by masking other proteolytic products when it is included. However, LC-MS/MS analysis of all proteins in purified ROS membrane were performed (Skiba et al., 2013) and their result suggested that massive amount of rhodopsin had little impedance in LC-MS/MS analysis. From this, LC-MS/MS analysis in all regions of all fractions I prepared is potentially possible, and it is expected that more new ROS- or COS-specific/dominant proteins can be found.

I focused on the proteins tightly bound to membranes as the first step and found some candidates of ROS- or COS-specific/dominant proteins. Unfortunately, soluble proteins and peripheral membrane proteins were not included in my study, but ROS- or COS-specific/dominant proteins should be found in such a group of proteins. In particular, analysis of soluble proteins is difficult because soluble proteins were lost during purification of OS and IS membranes. On the one hand, analysis of peripheral membrane proteins is possible when one uses ROS, RIS, COS and CIS membranes before washing or the supernatants obtained during washes. It is possible to extend my study by using these preparations in elucidating the molecular mechanism of the differences between rods and cones. I hope that that my current study will contribute to those studies.

So far, I focused on ROS- or COS-specific/dominant proteins because phototransduction cascade exists in OS, but it is possible to focus on RIS- or CIS-specific/dominant proteins. The IS has a function mainly to produce energy, and IS size are greatly different between rods and cones (Fig 1). Also, it is known that cones have more energy requirements than rods (Okawa et al., 2008). From this, it is possible that qualitative and/or quantitative functional differences, and structural differences are present between RIS and CIS. On this point, it will be interesting to focus on IS with finding RIS- or CIS-specific/dominant proteins, which I believe practically possible.

To elucidate the molecular mechanisms of the differences between rods and cones, I believe that knockout study is very effective. Unfortunately, knockout study in carp is very difficult because of its slow growth rate and large size of breeding facility.
However, in zebrafish (*Danio rerio*) which is a kind of model animal and the most closely related to carp, knockout analysis can be rather easily performed by genome editing. First of all, using knockout zebrafish of NCALD newly found as a protein not localized in ROS but localized in COS in this study, I would like to investigate whether light response is changed. As already mentioned, NCALD is able to interact directly with ROS-GC1 (see Results and Discussion) and active ROS-GC1 (Krishnan *et al.*, 2004). High activation of guanylate cyclase (GC) in light response is known to be related to briefer response (Takemoto *et al.*, 2009). If light response is changed in NCALD knockout zebrafish, it is possible that NCALD contributes to the characteristic light response of cones including briefer response by interacting directly with and activating GC-C. I believe that to investigate the function of NCALD in COS will elucidate one of the differences of molecular bases between rods and cones.
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Acknowledgment

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I express gratitude to Professor Satoru Kawamura and Associate Professor Shuji Tachibanaki for invaluable discussions and suggestions throughout this study. I thank Professor Nobuhiko Yamamoto, Professor Seiji Takashima and Professor Yasushi Hiraoka for critical readings of this thesis. I thank all members of Kawamura laboratory for various discussions and supports.
Table S1. Identified proteins in washed ROS-rich fraction.
Proteins in washed ROS-rich fraction were identified with LC–MS/MS analysis and are listed in descending order of emPAI values for 5 × 10^5 rods.

<table>
<thead>
<tr>
<th>Identified proteins in washed ROS-rich fraction</th>
<th>Molecular mass</th>
<th>emPAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>uncharacterized protein LOC100145214</td>
<td>33 kDa</td>
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<td>peripherin-2</td>
<td>21 kDa</td>
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<tr>
<td>rhodopsin</td>
<td>40 kDa</td>
<td>5.7215</td>
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<td>37 kDa</td>
<td>2.6851</td>
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<tr>
<td>ras-related protein Rab-2A</td>
<td>24 kDa</td>
<td>2.6161</td>
</tr>
<tr>
<td>peripherin-2</td>
<td>18 kDa</td>
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<tr>
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<td>25 kDa</td>
<td>1.9179</td>
</tr>
<tr>
<td>retinal guanylyl cyclase 2 (guanylate cyclase retinal rod2 [Cyprinus carpio])</td>
<td>124 kDa</td>
<td>1.8202</td>
</tr>
<tr>
<td>guanine nucleotide–binding protein G(I)/G(S)/G(T) subunit beta-1</td>
<td>37 kDa</td>
<td>1.7943</td>
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<td>88 kDa</td>
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<td>ATP synthase F(0) complex subunit B1, mitochondrial</td>
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<td>prohibitin isoform X2</td>
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<td>E-value</td>
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<td>long-chain fatty acid transport protein 4</td>
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<td>ras-related protein Rab-1B</td>
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<td>ATP synthase subunit O, mitochondrial</td>
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<td>peripherin-2</td>
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<td>PREDICTED: prohibitin-2</td>
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<td>ras-related protein Rab-5C</td>
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<td>RAB11a, member RAS oncogene family, like</td>
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<td>PREDICTED: tubulin alpha chain</td>
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<td>ras-related protein Rab-14</td>
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<td>ADP-ribosylation factor-like protein 6</td>
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<td>creatine kinase S-type, mitochondrial</td>
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<td>PREDICTED: creatine kinase S-type, mitochondrial isoform X2</td>
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<tr>
<td>putative Ras-related protein Rab-42</td>
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<td>cytochrome c-1</td>
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<td>small GTPase RhoA</td>
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<td>synaptobrevin homolog YKT6</td>
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<td>ATPase, Na+/K+ transporting, beta 2b polypeptide</td>
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<tr>
<td>PREDICTED: phospholipid scramblase 2</td>
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<tr>
<td>PREDICTED: phosphate carrier protein, mitochondrial isoform X1</td>
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<td>PREDICTED: tubulin beta-2B chain-like isoform 1</td>
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<tr>
<td>PREDICTED: voltage-dependent anion-selective channel protein 2</td>
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<td>uncharacterized protein LOC449549</td>
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<td>ATP synthase subunit g, mitochondrial</td>
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<td>ras-related protein Ral-A</td>
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<td>PREDICTED: ATPase, Na+/K+ transporting, beta 2b polypeptide isoform X1</td>
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<td>RAB1A, member RAS oncogene family</td>
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<td>PREDICTED: tubulin beta-4B chain-like, partial</td>
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<tr>
<td>PREDICTED: LOW QUALITY PROTEIN: actin, gamma 1</td>
<td>40</td>
<td>0.2296</td>
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<tr>
<td>voltage-dependent anion-selective channel protein 2</td>
<td>20</td>
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<tr>
<td>ubiquitin-60S ribosomal protein L40</td>
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<td>0.2209</td>
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<tr>
<td>NAD(P) transhydrogenase, mitochondrial</td>
<td>114</td>
<td>0.2185</td>
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<tr>
<td>PREDICTED: ras-related protein Rab-28 isoform X2</td>
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<td>immunity-related GTPase family, q2</td>
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<td>PREDICTED: vesicle-associated membrane protein 2-like</td>
<td>12</td>
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<td>catechol-O-methyltransferase a</td>
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<td>ER membrane protein complex subunit 3</td>
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<tr>
<td>peripherin 2b (retinal degeneration, slow)</td>
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<td>60S ribosomal protein L10</td>
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<td>PREDICTED: tubulin alpha-1C chain</td>
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<td>guanylyl cyclase 3 (guanylate cyclase retinal cone [Cyprinus carpio])</td>
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<td>0.1967</td>
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<td>PREDICTED: GTP-binding protein SAR1b-like</td>
<td>13</td>
<td>0.193</td>
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<tr>
<td>regulator of G-protein signaling 9-binding protein B</td>
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<td>0.1869</td>
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<td>guanylyl cyclase 3 (guanylate cyclase retinal cone [Cyprinus carpio])</td>
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<tr>
<td>PREDICTED: alpha/beta hydrolase domain-containing protein 17A</td>
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<td>0.1808</td>
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<tr>
<td>PREDICTED: ras-related protein Rab-7a isoform X1</td>
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<td>0.18</td>
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<tr>
<td>PREDICTED: protein RD3-like</td>
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<td>epidermal retinol dehydrogenase 2</td>
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<td>0.1765</td>
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<td>PREDICTED: cadherin-related family member 5-like isoform X2</td>
<td>44</td>
<td>0.1752</td>
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<tr>
<td>Protein Description</td>
<td>MW</td>
<td>Purity</td>
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<td>vesicle-trafficking protein SEC22b-A</td>
<td>24 kDa</td>
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<tr>
<td>PREDICTED: L-lactate dehydrogenase B-B chain isoform X3</td>
<td>19 kDa</td>
<td>0.1705</td>
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<tr>
<td>PREDICTED: NADH dehydrogenase</td>
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<td>0.1692</td>
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<tr>
<td>PREDICTED: tubulin alpha chain-like</td>
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<td>0.1692</td>
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<tr>
<td>retinol dehydrogenase 8a</td>
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<td>mitochondrial inner membrane protein</td>
<td>83 kDa</td>
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<td>PREDICTED: josephin-2 isoform X1</td>
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<td>uncharacterized protein LOC393228</td>
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<tr>
<td>rhodopsin kinase</td>
<td>27 kDa</td>
<td>0.1481</td>
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<td>PREDICTED: transmembrane emp24 domain-containing protein 7 isoform X2</td>
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<td>reticulon-4</td>
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<td>0.1447</td>
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<tr>
<td>PREDICTED: peripherin-2</td>
<td>39 kDa</td>
<td>0.1447</td>
</tr>
<tr>
<td>NADH-cytochrome b5 reductase 1</td>
<td>28 kDa</td>
<td>0.1442</td>
</tr>
<tr>
<td>reticulon-4</td>
<td>22 kDa</td>
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<tr>
<td>ras-related protein Rab-3A</td>
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<td>erlin-2 precursor</td>
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<td>0.14</td>
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<tr>
<td>uncharacterized protein LOC100127828</td>
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<tr>
<td>guanine nucleotide-binding protein G(t) subunit alpha-2</td>
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<td>PREDICTED: mitochondrial glutamate carrier 1</td>
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<td>PREDICTED: red-sensitive opsin-1 isoform X1</td>
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<td>PREDICTED: cadherin-related family member 5-like isoform X2</td>
<td>17 kDa</td>
<td>0.1338</td>
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<td>vesicle-associated membrane protein-associated protein A</td>
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<td>ras-related protein Rab-6A isoform X2</td>
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<tr>
<td>ras-related protein Rab-5B</td>
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<tr>
<td>60S ribosomal protein L24</td>
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<tr>
<td>CDP-diacylglycerol-inositol 3-phosphatidytransferase</td>
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<td>PREDICTED: mitochondrial glutamate carrier 1</td>
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<tr>
<td>peroxisomal membrane protein 11B</td>
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<tr>
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<td>PREDICTED: ADP-dependent glucokinase isoform X2</td>
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<tr>
<td>PREDICTED: ER membrane protein complex subunit 1 isoform X1</td>
<td>111 kDa</td>
<td>0.1193</td>
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<tr>
<td>sorting and assembly machinery component 50 homolog A</td>
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<tr>
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<tr>
<td>ADP-ribosylation factor-like protein 9</td>
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<td>0.1162</td>
</tr>
<tr>
<td>PREDICTED: vesicle transport through interaction with t-SNAREs homolog 1A isoform X1</td>
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<td>0.1162</td>
</tr>
<tr>
<td>cytochrome c oxidase subunit 4 isoform 1, mitochondrial</td>
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<td>PREDICTED: phospholipid-transporting ATPase IB isoform X6</td>
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<td>PREDICTED: regulator of G-protein signaling 9 isoform X1</td>
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<td>0.1066</td>
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<tr>
<td>PREDICTED: ER membrane protein complex subunit 1 isoform X1</td>
<td>114 kDa</td>
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<tr>
<td>rhodopsin kinase</td>
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<tr>
<td>histone 2, H2a</td>
<td>14 kDa</td>
<td>0.1048</td>
</tr>
<tr>
<td>40S ribosomal protein S5</td>
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<tr>
<td>PREDICTED: adipocyte-derived adipocyte-secreted protein</td>
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<tr>
<td>PREDICTED: cGMP-gated cation channel alpha-1</td>
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<td>transmembrane protein 33</td>
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<tr>
<td>guanine nucleotide-binding protein subunit beta-5</td>
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<tr>
<td>very-long-chain enoyl-CoA reductase</td>
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<td>0.1028</td>
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<tr>
<td>retinol dehydrogenase-like</td>
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<td>lysophosphatidylcholine acyltransferase 2</td>
<td>60 kDa</td>
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<tr>
<td>creatine kinase U-type, mitochondrial</td>
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<td>0.1001</td>
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<td>60S ribosomal protein L9</td>
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<td>0.1001</td>
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<td>rho-related gtp-binding protein rho</td>
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<tr>
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<tr>
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<td>Description</td>
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<td>PREDICTED: 60S ribosomal protein L6</td>
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<td>172</td>
<td>PREDICTED: GTP-binding protein SAR1b</td>
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<td>173</td>
<td>PREDICTED: 40S ribosomal protein S9-like</td>
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<td>174</td>
<td>hexokinase-1</td>
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<td>175</td>
<td>ADP-ribosylation factor-like 15a</td>
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<td>ras-related protein Rab-8A</td>
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<td>NADH dehydrogenase 1 beta subcomplex subunit 6</td>
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<td>178</td>
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<td>181</td>
<td>surflet gene 4, like</td>
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<tr>
<td>182</td>
<td>Beta-centractin</td>
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<td>PREDICTED: sodium/potassium/calcium exchanger 1 isoform X1</td>
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<td>solute carrier family 3 (amino acid transporter heavy chain), member 2b</td>
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<td>185</td>
<td>NADH dehydrogenase</td>
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<td>187</td>
<td>isocitrate dehydrogenase</td>
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<td>PREDICTED: threonine dehydratase, mitochondrial–like isoform X2</td>
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<td>189</td>
<td>LETM1 and EF-hand–domain–containing protein 1, mitochondrial</td>
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<td>dihydrolipooylsine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial</td>
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<td>sideroflexin-4</td>
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<td>acyl–CoA synthetase long-chain family member 3b</td>
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<td>DDRGK domain–containing protein 1 precursor</td>
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<td>194</td>
<td>malate dehydrogenase, mitochondrial</td>
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<td>PREDICTED: choline transporter–like protein 1</td>
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<td>197</td>
<td>PREDICTED: protein–L–isoaspartate(D–aspartate) O–methyltransferase isoform X1</td>
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<td>PREDICTED: 40S ribosomal protein S24</td>
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<td>199</td>
<td>PREDICTED: regulator of G–protein signaling 9–binding protein–like</td>
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<tr>
<td>200</td>
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<td>201</td>
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<td>202</td>
<td>epimerase family protein SDR9U1</td>
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<td>203</td>
<td>alpha-1,3/1,6–mannosyltransferase ALG2</td>
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<td>204</td>
<td>PREDICTED: actin, aortic smooth muscle</td>
<td>18 kDa</td>
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<td>PREDICTED: arylacetamide deacetylase isoform X1</td>
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<td>206</td>
<td>PREDICTED: CSC1–like protein 2</td>
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<tr>
<td>207</td>
<td>dolichol–phosphate mannosyltransferase subunit 1</td>
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<td>208</td>
<td>PREDICTED: uncharacterized protein sirp71–36a1.2</td>
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<td>209</td>
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<td>212</td>
<td>green–sensitive opsin–4</td>
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<td>PREDICTED: protein tyrosine phosphatase type IVA 2–like isoform X3</td>
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<td>214</td>
<td>rod cGMP–specific 3’.5”–cyclic phosphodiesterase subunit alpha</td>
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<td>215</td>
<td>PREDICTED: E3 ubiquitin–protein ligase RNF170–like</td>
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<td>216</td>
<td>plasminogen receptor (KT)</td>
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<td>217</td>
<td>PREDICTED: syntaxin–12 isoform X1</td>
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<td>PREDICTED: solute carrier family 41 member 1</td>
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<td>219</td>
<td>PREDICTED: potassium voltage–gated channel subfamily B member 2</td>
<td>93 kDa</td>
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<td>220</td>
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<tr>
<td>221</td>
<td>mitochondrial carrier homolog 2</td>
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<tr>
<td>222</td>
<td>ADP–ribosylation factor–like protein 1</td>
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<td>guanine nucleotide–binding protein G(o) subunit alpha</td>
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<td>PREDICTED: exportin-1-like, partial</td>
<td>21</td>
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<td>398</td>
<td>guanine nucleotide-binding protein G0/G1/G2/G3/G4/G5/G6 subunit beta-3</td>
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<td>399</td>
<td>brain creatine kinase b</td>
<td>43</td>
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<td>400</td>
<td>PREDICTED: translocon-associated associated protein subunit gamma</td>
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<td>401</td>
<td>PREDICTED: cell division control protein 42 homolog isoform X1</td>
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<td>402</td>
<td>aspartate aminotransferase 2</td>
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<td>403</td>
<td>PREDICTED: glutaminase a isoform X1</td>
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<td>Protein</td>
<td>Molecular Weight (kDa)</td>
<td>P-value</td>
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<tr>
<td>Predicted: transmembrane and coiled-coil domains protein 1-like</td>
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<tr>
<td>peripherin 2, like</td>
<td>21</td>
<td>0.0288</td>
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<tr>
<td>outer dense fiber of sperm tails 2b</td>
<td>22</td>
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<tr>
<td>ADP-ribosylation-like factor 6 interacting protein 5</td>
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<tr>
<td>Predicted: ras-related C3 botulinum toxin substrate 1</td>
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<td>AFG3-like protein 2</td>
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<tr>
<td>Predicted: guanine nucleotide-binding protein G(a) subunit alpha isoform X2</td>
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<td>phosphatidylinositide phosphatase SAC1-B</td>
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<tr>
<td>protein kinase, cAMP-dependent, regulatory, type II, alpha A</td>
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<tr>
<td>ras-related protein R-Ras</td>
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<tr>
<td>Predicted: glucose-induced degradation protein 8 homolog</td>
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<td>Predicted: charged multisvesicular body protein 6-like isoform X1</td>
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<tr>
<td>Predicted: transmembrane protein 126A isoform X1</td>
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<td>protein phosphatase 2, regulatory subunit B, epsilon isoform a</td>
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<td>synaptotagmin II</td>
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<td>Predicted: cadherin-related family member 5-like isoform X2</td>
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<td>adipocyte plasma membrane-associated protein</td>
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<td>membrane-associated progesterone receptor component 2</td>
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<td>Predicted: metal transporter CNNM4</td>
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<td>protein THEM6 precursor</td>
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<td>HD domain-containing protein 2</td>
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<td>Predicted: neural cell adhesion molecule 1 isoform X1</td>
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<td>Predicted: dephospho-CoA kinase domain-containing protein X2</td>
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<td>transmembrane emp24 domain-containing protein X1</td>
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<td>rho GTPase-activating protein 1</td>
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<td>motile sperm domain-containing protein 1</td>
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<td>Predicted: testis-expressed sequence 264 protein-like</td>
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<td>cytochrome b-c1 complex subunit 2, mitochondrial</td>
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<td>rab GDP dissociation inhibitor beta</td>
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<td>tubulin gamma-1 chain</td>
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<td>Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit precursor</td>
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<td>Predicted: ras-related protein Rab-9B</td>
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<td>Predicted: phosphatidate cytidylyltransferase 1</td>
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<td>Probable saccharopine dehydrogenase</td>
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<td>Solute carrier family 2, facilitated glucose transporter 1</td>
<td>53</td>
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<tr>
<td>Squalene synthase</td>
<td>53</td>
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<tr>
<td>Oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipaomide)</td>
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<td>Predicted: coiled-coil domain-containing protein 136-like isoform X1</td>
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<td>CAAX prenyl protease 1 homolog</td>
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<td>BR13--binding protein precursor</td>
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<td>Uncharacterized protein LOC619200</td>
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<td>ATP synthase subunit s, mitochondrial</td>
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<tr>
<td>Predicted: probable ATP--dependent RNA helicase ddx6</td>
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<tr>
<td>Predicted: fatty aldehyde dehydrogenase--like</td>
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<td>Predicted: sterol 26-hydroxylase, mitochondrial</td>
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<td>Predicted: ribosomal protein S6 kinase alpha-3 isoform X1</td>
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<td>Protein Description</td>
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<tr>
<td>Sorting and assembly machinery component 50 homolog B</td>
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<td>PREDICTED: ATP synthase subunit beta, mitochondrial-like</td>
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<td>PREDICTED: sodium-coupled neutral amino acid transporter 3-like isofrom X1</td>
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<tr>
<td>PREDICTED: ankyrin repeat domain-containing protein 33B</td>
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<td>Thioredoxin-dependent peroxide reductase, mitochondrial</td>
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<td>V-type proton ATPase subunit D</td>
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<td>T-complex protein subunit beta</td>
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<td>PREDICTED: importin-5 isofrom X1</td>
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<td>Prenylcysteine oxidase 1 precursor</td>
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<td>Neuronal membrane glycoprotein M6-b</td>
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<tr>
<td>Catechol O-methyltransferase</td>
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<td>PREDICTED: protein phosphatase 1 regulatory subunit 16A</td>
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<td>PREDICTED: ATPase, Ca++ transporting, cardiac muscle, slow twitch 2b isofrom X1</td>
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<td>60S ribosomal protein L7a</td>
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<td>PREDICTED: calcium signal-modulating cyclophilin ligand isofrom X1</td>
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<td>0.0203</td>
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<td>Microtubule-associated protein RP/EB family member 3</td>
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<td>Aquaporin 1</td>
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<tr>
<td>PREDICTED: tetraspanin-5/EB family member 3</td>
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<td>E3 ubiquitin-protein ligase MARCH5</td>
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<td>Acyl-CoA-binding domain-containing protein 5-B</td>
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<td>Surfet locus protein 1</td>
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<td>B-cell receptor-associated protein 31</td>
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<td>Thioredoxin-related transmembrane protein 1 precursor</td>
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<td>Uncharacterized protein C18orF19 homolog B</td>
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<td>PREDICTED: rap1 GTPase-GDP dissociation stimulator 1-like isofrom X1</td>
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<td>PREDICTED: protein FAM57A-like</td>
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<td>PREDICTED: cyclic nucleotide-gated cation channel beta-3 isofrom X4</td>
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<td>Trimeric intracellular cation channel type B</td>
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<tr>
<td>PREDICTED: serine protease 33-like</td>
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<tr>
<td>Phosphatidylinositol N-acetylglucosaminyltransferase subunit Q</td>
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<td>PREDICTED: E3 ubiquitin-protein ligase CHIP isofrom X2</td>
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<td>0.0184</td>
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<td>N-ethylmaleimide-sensitive factor attachment protein, beta</td>
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<tr>
<td>PREDICTED: uncharacterized protein LOC323326 isofrom X1</td>
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<tr>
<td>PREDICTED: synembryn-A-like isofrom X2</td>
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<td>0.0183</td>
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<tr>
<td>RPE-retinal G protein-coupled receptor</td>
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<td>Pyrrolidine-5-carboxylate reductase 1a</td>
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<td>PREDICTED: protein CLN8 isofrom X1</td>
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<td>Mitochondrial carnitine/acetylcarbine carrier protein ACACL</td>
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<td>0.018</td>
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<td>PREDICTED: protein SCO1 homolog, mitochondrial</td>
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<td>Solute carrier family 7, member 3</td>
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<td>Mitochondrial Rho GTPase 2</td>
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<td>Retinal G protein coupled receptor b</td>
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<td>Cyclin-dependent kinase 5 activator 2</td>
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<td>PREDICTED: CAAX prenyl protease 2</td>
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<td>PREDICTED: heme oxygenase 2</td>
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<td>Glycerophosphodiester phosphodiesterase domain-containing protein 1</td>
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<td>Protein SCO2 homolog, mitochondrial</td>
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<td>PREDICTED: meckelin isofrom X2</td>
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<td>PREDICTED: transmembrane protein 43-like</td>
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<td>PREDICTED: xyloside xylosyltransferase 1 isofrom X1</td>
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<tr>
<td>Limbic system-associated membrane protein precursor</td>
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<td>Solute carrier family 35 member B1</td>
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<td>Inositol monophosphatase 3</td>
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<td>PREDICTED: epoxide hydrolase 1, partial</td>
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<td>Gene Name</td>
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<td>metal transporter CNNM2</td>
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<td>abhydrolase domain-containing protein 16A</td>
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<td>monoacylglycerol lipase abhd6-A</td>
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<td>dynamin-1-like protein</td>
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<td>ATP-dependent 6-phosphofructokinase, liver type-like</td>
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<td>eukaryotic translation initiation factor 4A, isoform 1A</td>
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<td>ATP-dependent RNA helicase DDX3-isoform X6</td>
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<td>ATP-dependent zinc metalloprotease YME1L1 isoform X1</td>
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<tr>
<td>opsins-1, short-wave-sensitive 2</td>
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<tr>
<td>opsins-1, short-wave-sensitive 1</td>
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<tr>
<td>NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial</td>
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<td>cyclic nucleotide-gated channel rod photoreceptor subunit alpha-like</td>
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<td>electrogenic sodium bicarbonate cotransporter 1 isoform X2</td>
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<td>presenilins-associated rhomboid-like protein, mitochondrial</td>
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<td>mitochondrial trifunctional protein, alpha subunit</td>
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<td>reticulon-1 isoform 1</td>
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<td>choline transporter-like protein 1 isoform X2</td>
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<td>thromboxane-A synthase</td>
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<td>reticulon-1 isoform 1</td>
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<td>monoacylglycerol lipase ABHD12</td>
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<td>protein EFR3 homolog B isoform X2</td>
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<td>26S protease regulatory subunit 8</td>
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<td>dyslexia-associated protein KIAA0319-like protein homolog isoform X1</td>
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<td>epoxide hydrolase 1</td>
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mitochondrial dynamics protein MID49 52 kDa 0.0115

ectonucleotide pyrophosphatase/phosphodiesterase family member 5 precursor 53 kDa 0.0114

PREDICTED: sodium-coupled neutral amino acid transporter 3 53 kDa 0.0113

NAD dehydrogenase 53 kDa 0.0113

PREDICTED: S-arrestin 53 kDa 0.0112

PREDICTED: abhydrolase domain-containing protein 8 53 kDa 0.0112

PREDICTED: protein LYRIC isoform X1 53 kDa 0.0112

PREDICTED: microtubule-associated protein futsch-like 217 kDa 0.0112

PREDICTED: sodium-coupled neutral amino acid transporter 3 53 kDa 0.0113

PREDICTED: NADH dehydrogenase 53 kDa 0.0113

PREDICTED: S-arrestin 53 kDa 0.0112

PREDICTED: abhydrolase domain-containing protein 8 53 kDa 0.0112

PREDICTED: protein LYRIC isoform X1 53 kDa 0.0112

PREDICTED: microtubule-associated protein futsch-like 217 kDa 0.0112

zinc transporter 1 54 kDa 0.0112

dihydrolipoyl dehydrogenase, mitochondrial 54 kDa 0.0112

PREDICTED: probable ATP-dependent RNA helicase ddx6-like isoform X2 54 kDa 0.0111

V-type proton ATPase subunit H isoform 1 55 kDa 0.0109

T-complex protein 11-like protein 1 56 kDa 0.0107

6-phosphogluconate dehydrogenase, decarboxylating isoform 1 56 kDa 0.0107

dol-P-Man:Man(7)GlcNAc(2)-PP-Dol alpha-1,6-mannosyltransferase precursor 56 kDa 0.0106

GDP-Man:Man(3)GlcNAc(2)-PP-Dol alpha-1,2-mannosyltransferase isoform 1 57 kDa 0.0106

PREDICTED: protein phosphatase 3, catalytic subunit, gamma isoform-like isoform X1 57 kDa 0.0104

T-complex protein 1 subunit zeta 58 kDa 0.0104

PREDICTED: protein phosphatase 3, catalytic subunit, gamma isoform isoform X1 58 kDa 0.0104

PREDICTED: cell adhesion molecule 3 isoform X1 58 kDa 0.0102

PREDICTED: glycerol kinase isoform X4 59 kDa 0.0102

PREDICTED: serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit alpha isoform 59 kDa 0.0102

amine oxidase 59 kDa 0.0102

PREDICTED: lysophosphatidylcholine acyltransferase 1-like 59 kDa 0.0102

PREDICTED: peroxisomal N(1)-acetyl-spermine/spermidine oxidase 59 kDa 0.0101

T-complex protein 1 subunit epsilon 59 kDa 0.0101

PREDICTED: voltage-dependent calcium channel subunit alpha-2/delta-1-like 120 kDa 0.01

T-complex protein 1 subunit epsilon 59 kDa 0.0101

PREDICTED: voltage-dependent calcium channel subunit alpha-2/delta-1-like 120 kDa 0.01

uncharacterized protein LOC619266 precursor 60 kDa 0.01

PREDICTED: calcium-activated potassium channel subunit alpha-1 isoform X15 60 kDa 0.01

PREDICTED: cyclin-dependent kinase 17 isoform X1 60 kDa 0.01

EH-domain containing 1a 60 kDa 0.01

non-specific lipid-transfer protein 60 kDa 0.0099

methylmalonate-semialdehyde dehydrogenase 61 kDa 0.0099

PREDICTED: brain-specific angiogenesis inhibitor 1-associated protein 2 isoform X1 61 kDa 0.0098

delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial precursor 62 kDa 0.0097

zinc transporter 9 64 kDa 0.0094

PREDICTED: acetoacetate synthase-like protein isoform X1 67 kDa 0.0089

PREDICTED: sodium/potassium/calcium exchanger 2-like isoform X2 68 kDa 0.0088

chaperone activity of bc1 complex-like, mitochondrial 70 kDa 0.0086

cleft lip and palate transmembrane protein 1 homolog 72 kDa 0.0083

78 kDa glucose-regulated protein precursor 72 kDa 0.0083

PREDICTED: LOW QUALITY PROTEIN: long-chain-fatty-acid--CoA ligase 6 75 kDa 0.0079

transmembrane 9 superfamily member 4 precursor 75 kDa 0.0079

PREDICTED: protein PTHB1 isoform X1 76 kDa 0.0079

sphingomyelin phosphodiesterase 3 76 kDa 0.0078

ATP-binding cassette sub-family B member 8, mitochondrial 77 kDa 0.0078

PREDICTED: potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 3-like 79 kDa 0.0075

dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A 81 kDa 0.0074

disintegrin and metalloproteinase domain-containing protein 10 precursor 85 kDa 0.007

aconitate hydratase, mitochondrial 86 kDa 0.007

PREDICTED: ATP-dependent 6-phosphofructokinase, platelet type isoform X2 86 kDa 0.0069

PREDICTED: retinal-specific ATP-binding cassette transporter isoform X2 261 kDa 0.0069

heat shock protein HSP 90-beta 89 kDa 0.0067

PREDICTED: paraplegin 92 kDa 0.0065

PREDICTED: 2-oxoglutarate dehydrogenase, mitochondrial 93 kDa 0.0064

PREDICTED: prominin-1-A isoform X10 94 kDa 0.0064

PREDICTED: oxysterol-binding protein-related protein 8-like 98 kDa 0.0061
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<tr>
<th></th>
<th>Name</th>
<th>Molecular Weight (kDa)</th>
<th>p-value</th>
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<tr>
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<td>cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha'</td>
<td>98</td>
<td>0.006</td>
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<tr>
<td>640</td>
<td>Predicted: cleavage and polyadenylation specificity factor subunit 1 isoform X1</td>
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<td>Predicted: bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 1</td>
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<td>642</td>
<td>Predicted: transmembrane and TPR repeat-containing protein 3</td>
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<td>Predicted: uncharacterized protein KIAA1614 homolog isoform X1</td>
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<td>647</td>
<td>Predicted: solute carrier family 12 member 7 isoform X5</td>
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<td>648</td>
<td>Predicted: oxygen-regulated protein 1</td>
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Table S2. Identified proteins in washed RIS-rich fraction.

Proteins in washed RIS-rich fraction were identified with LC–MS/MS analysis and are listed in descending order of emPAI values for $5 \times 10^5$ rods.

<table>
<thead>
<tr>
<th>Identified proteins in washed RIS-rich fraction</th>
<th>Molecular mass</th>
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<td>uncharacterized protein LOC100145214</td>
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<td>PREDICTED: ATP synthase subunit alpha, mitochondrial</td>
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<td>creatine kinase S–type, mitochondrial</td>
<td>47 kDa</td>
<td>8.4939</td>
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<td>PREDICTED: creatine kinase S–type, mitochondrial isoform X2</td>
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<td>8.4926</td>
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<td>PREDICTED: uncharacterized protein LOC100707031 isoform X1</td>
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<td>96</td>
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<td>97</td>
<td>PREDICTED: calcium-binding mitochondrial carrier protein Aralar2 isoform X1</td>
<td>48</td>
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<tr>
<td>98</td>
<td>NADH dehydrogenase</td>
<td>58</td>
</tr>
<tr>
<td>99</td>
<td>solute carrier family 25, member 23</td>
<td>31</td>
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<tr>
<td>100</td>
<td>NAD(P) transhydrogenase, mitochondrial</td>
<td>114</td>
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<td>101</td>
<td>PREDICTED: vesicle–associated membrane protein–associated protein A-like</td>
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<td>102</td>
<td>PREDICTED: vesicle–associated membrane protein–associated protein A-like</td>
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<tr>
<td>103</td>
<td>stomatin–like protein 2, mitochondrial</td>
<td>39</td>
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<td>104</td>
<td>PREDICTED: sodium/potassium–transporting ATPase subunit alpha–3–like</td>
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<td>105</td>
<td>stomatin–like protein 2, mitochondrial</td>
<td>39</td>
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<td>106</td>
<td>PREDICTED: pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial isoform X1</td>
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<td>107</td>
<td>PREDICTED: ubiquinol–cytochrome c reductase core protein II isoform X1</td>
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<td>108</td>
<td>LETM1 and EF–hand domain–containing protein 1, mitochondrial</td>
<td>86</td>
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<td>109</td>
<td>protein disulfide–isomerase TMX3 precursor</td>
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<td>mitochondrial inner membrane protein</td>
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<td>reticulon-4-interacting protein 1 homolog, mitochondrial</td>
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<td>18 kDa</td>
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<td>NipSnap homolog 2</td>
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<td>voltage-dependent anion-selective channel protein 2</td>
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<td>solute carrier family 25, member 23</td>
<td>23 kDa</td>
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<td>52 kDa</td>
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<td>apolipoprotein O</td>
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<td>cytochrome c oxidase subunit VIIa polypeptide 3</td>
<td>16 kDa</td>
<td>0.2071</td>
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<tr>
<td>PREDICTED: mitochondrial glutamate carrier 1</td>
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<td>16 kDa</td>
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<td>PREDICTED: tubulin alpha chain-like</td>
<td>12 kDa</td>
<td>0.2021</td>
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<tr>
<td>dihydrolipoyl dehydrogenase, mitochondrial</td>
<td>54 kDa</td>
<td>0.2018</td>
</tr>
<tr>
<td>hexokinase-1</td>
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<td>0.2009</td>
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<td>EST1 protein, mitochondrial precursor</td>
<td>31 kDa</td>
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<td>transmembrane protein 256 precursor</td>
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<td>PREDICTED: uncharacterized protein LOC571872 isoform X1</td>
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<td>alpha-enolase</td>
<td>47 kDa</td>
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<td>0.1825</td>
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<td>PREDICTED: apoptosis-inducing factor 1, mitochondrial isoform X3</td>
<td>68 kDa</td>
<td>0.181</td>
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<tr>
<td>succinyl-CoA ligase</td>
<td>51 kDa</td>
<td>0.1776</td>
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<td>PREDICTED: glutaryl-CoA dehydrogenase, mitochondrial-like</td>
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<td>0.1759</td>
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<tr>
<td>PREDICTED: apoptosis-inducing factor 1, mitochondrial isoform X1</td>
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<td>0.1759</td>
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<td>mitochondrial carrier homolog 2</td>
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<td>heat shock cognate 71 kDa protein</td>
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<tr>
<td>isocitrate dehydrogenase</td>
<td>40 kDa</td>
<td>0.1722</td>
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<td>PREDICTED: NADH dehydrogenase</td>
<td>21 kDa</td>
<td>0.1713</td>
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<td>enoyl-CoA hydratase, mitochondrial</td>
<td>31 kDa</td>
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<td>solute carrier family 25, member 23</td>
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<td>PREDICTED: LOW QUALITY PROTEIN: long-chain-fatty-acid-CoA ligase 6</td>
<td>50 kDa</td>
<td>0.1667</td>
</tr>
<tr>
<td>isocitrate dehydrogenase</td>
<td>43 kDa</td>
<td>0.1665</td>
</tr>
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<td>PREDICTED: NADH dehydrogenase</td>
<td>43 kDa</td>
<td>0.1648</td>
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<tr>
<td>L-lactate dehydrogenase B-A chain</td>
<td>36 kDa</td>
<td>0.1643</td>
</tr>
<tr>
<td>PREDICTED: isocitrate dehydrogenase</td>
<td>44 kDa</td>
<td>0.1624</td>
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<tr>
<td>PREDICTED: cytochrome b-c1 complex subunit 7-like</td>
<td>13 kDa</td>
<td>0.1619</td>
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<tr>
<td>pyruvate dehydrogenase E1 component subunit beta, mitochondrial</td>
<td>39 kDa</td>
<td>0.1589</td>
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<td>translocase of outer mitochondrial membrane 40 homolog, like</td>
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<td>0.1584</td>
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<td>methylmalonate-semialdehyde dehydrogenase</td>
<td>61 kDa</td>
<td>0.1579</td>
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<td>PREDICTED: uncharacterized protein LOC556653</td>
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<td>28 kDa</td>
<td>0.1561</td>
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<tr>
<td>rhodopsin</td>
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<td>PREDICTED: tubulin alpha-1C chain</td>
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<td>hexokinase-1</td>
<td>71 kDa</td>
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<td>uncharacterized protein LOC556781</td>
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<td>isocitrate dehydrogenase</td>
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<td>3-hydroxyacyl-CoA dehydrogenase type-2</td>
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<td>0.1504</td>
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<tr>
<td>mitochondrial import inner membrane translocase subunit tim16</td>
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<td>0.1501</td>
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<tr>
<td>PREDICTED: gap junction delta-2 protein-like</td>
<td>21 kDa</td>
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<td>retinol dehydrogenase-like</td>
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<td>0.1449</td>
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<tr>
<td>PREDICTED: tubulin alpha-4A chain-like, partial</td>
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<td>0.1426</td>
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<td>NADH dehydrogenase</td>
<td>24 kDa</td>
<td>0.1419</td>
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<tr>
<td>protein QIL1</td>
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<td>0.1404</td>
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<tr>
<td>creatine kinase U-type, mitochondrial</td>
<td>22 kDa</td>
<td>0.1399</td>
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<tr>
<td>PREDICTED: tubulin beta-2B chain-like isoform 1</td>
<td>55 kDa</td>
<td>0.1394</td>
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<tr>
<td>PREDICTED: tubulin alpha chain</td>
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<td>Score</td>
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<td>trifunctional enzyme subunit beta, mitochondrial</td>
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<td>reticulon=4</td>
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<td>PREDICTED: mitochondrial import inner membrane translocase subunit Tim23</td>
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<td>PREDICTED: protein FAM162B isoform X1</td>
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<tr>
<td>PREDICTED: complex I assembly factor TIMMDC1, mitochondrial isoform X1</td>
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<td>0.1305</td>
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<td>translocase of outer mitochondrial membrane 40 homolog, like</td>
<td>36 kDa</td>
<td>0.1302</td>
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<tr>
<td>ATPase family AAA domain-containing protein 3</td>
<td>69 kDa</td>
<td>0.1242</td>
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<tr>
<td>protein NDRG1 isoform 1</td>
<td>42 kDa</td>
<td>0.1242</td>
</tr>
<tr>
<td>succinate dehydrogenase</td>
<td>61 kDa</td>
<td>0.1235</td>
</tr>
<tr>
<td>ras-related protein Rab-2A</td>
<td>24 kDa</td>
<td>0.1227</td>
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<tr>
<td>uncharacterized protein LOC556781</td>
<td>13 kDa</td>
<td>0.1218</td>
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<td>succinate dehydrogenase</td>
<td>73 kDa</td>
<td>0.1212</td>
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<td>PREDICTED: FAS-associated factor 2-like isoform X1</td>
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<td>0.1196</td>
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<tr>
<td>PREDICTED: presenilins-associated rhomboid-like protein, mitochondrial</td>
<td>41 kDa</td>
<td>0.1181</td>
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<tr>
<td>PREDICTED: LOW QUALITY PROTEIN: long-chain-fatty-acid—CoA ligase 6</td>
<td>75 kDa</td>
<td>0.1152</td>
</tr>
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<td>mitochondrial import inner membrane translocase subunit Tim23</td>
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<td>0.1127</td>
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<tr>
<td>PREDICTED: arylacetamide deacetylase isoform X2</td>
<td>16 kDa</td>
<td>0.1101</td>
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<tr>
<td>PREDICTED: ras-related protein Rab-1A—like isoform X1</td>
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<td>0.1089</td>
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<tr>
<td>ATPase family AAA domain-containing protein 3</td>
<td>69 kDa</td>
<td>0.1088</td>
</tr>
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<td>coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial</td>
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<td>0.1085</td>
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<tr>
<td>ras-related protein Rab-1B</td>
<td>22 kDa</td>
<td>0.108</td>
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<tr>
<td>Tubulin beta-20 chain</td>
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<td>retinol dehydrogenase 13</td>
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<tr>
<td>PREDICTED: calcium-binding mitochondrial carrier protein SCaMC-2-B isoform X2</td>
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<td>0.1065</td>
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<tr>
<td>NADH dehydrogenase</td>
<td>17 kDa</td>
<td>0.1065</td>
</tr>
<tr>
<td>synaptosomal-associated protein 25–B</td>
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<td>aconitate hydratase, mitochondrial</td>
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<td>PREDICTED: tubulin alpha chain–like</td>
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<td>PREDICTED: uncharacterized protein LOC571872 isoform X1</td>
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<td>PREDICTED: uncharacterized protein LOC101885612</td>
<td>11 kDa</td>
<td>0.0971</td>
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<td>PREDICTED: actin, aortic smooth muscle</td>
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<td>mitochondria–eating protein</td>
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<td>28S ribosomal protein S36, mitochondrial</td>
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<td>brain creatine kinase b</td>
<td>43 kDa</td>
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<td>sorting and assembly machinery component 50 homolog B</td>
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<td>0.0953</td>
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<td>PREDICTED: NADH dehydrogenase</td>
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<tr>
<td>elongation factor Tu, mitochondrial</td>
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<td>0.0942</td>
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<td>PREDICTED: mitochondrial fission process protein 1–like</td>
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<tr>
<td>transmembrane protein 256 precursor</td>
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<td>0.0928</td>
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<td>glutaryl-CoA dehydrogenase a</td>
<td>18 kDa</td>
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<td>sideroflexin–4</td>
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<td>PREDICTED: vesicle–associated membrane protein 2–like</td>
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<td>acylglycerol kinase, mitochondrial precursor</td>
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<td>ras-related protein Rab–3A</td>
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<td>long-chain fatty acid transport protein 4</td>
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<td>0.0897</td>
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<td>histone 1, H4, like</td>
<td>12 kDa</td>
<td>0.0889</td>
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<tr>
<td>mitochondrial ubiquitin ligase activator of nfkb 1–A</td>
<td>12 kDa</td>
<td>0.0889</td>
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<tr>
<td>PREDICTED: succinate dehydrogenase cytochrome b560 subunit, mitochondrial isoform X1</td>
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<td>PREDICTED: ornithine aminotransferase, mitochondrial</td>
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<td>erlin–1 precursor</td>
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<td>PREDICTED: S–arrestin</td>
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<td>PREDICTED: arylacetamide deacetylase isoform X1</td>
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<td>PREDICTED: mitochondrial ubiquitin ligase activator of nfkb 1–A</td>
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<td>cadherin-related family member 5-like isoform X2</td>
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<td>inactive hydroxysteroid dehydrogenase-like protein 1</td>
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<td>growth hormone-inducible transmembrane protein</td>
<td>35 kDa</td>
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<td>solute carrier family 25 (mitochondrial carrier: glutamate), member 22</td>
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<td>malate dehydrogenase 1Aa, NAD (soluble) isoform X1</td>
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<td>0.0781</td>
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<td>0.0708</td>
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<td>NADH dehydrogenase (ubiquinone) Fe-S protein 8b</td>
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<td>probable D-lactate dehydrogenase, mitochondrial</td>
<td>53 kDa</td>
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<td>mitochondrial carnitine/acylcarnitine carrier protein CACL</td>
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<td>mitochondrial import inner membrane translocase subunit Tim21</td>
<td>27 kDa</td>
<td>0.0656</td>
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<td>Na+/K+ -ATPase alpha 1 subunit</td>
<td>90 kDa</td>
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<td>transmembrane emp24 domain-containing protein 9 isoform X1</td>
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<td>acetyl-CoA acetyltransferase, mitochondrial precursor</td>
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<td>RAB5A, member RAS oncogene family, a</td>
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<td>stress-70 protein, mitochondrial</td>
<td>73 kDa</td>
<td>0.0615</td>
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<tr>
<td>very-long-chain enoyl-CoA reductase</td>
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<td>0.0615</td>
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<td>cytochrome c</td>
<td>11 kDa</td>
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<td>metaxin 1</td>
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<td>cytochrome c oxidase subunit IV isoform 2</td>
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<td>coiled-coil domain-containing protein 136-like isoform X1</td>
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<td>protein QIL1</td>
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<td>ATPase, Na+/K+ transporting, beta 2 polypeptide</td>
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<td>synaptojanin-2-binding protein</td>
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<td>pyrroline-5-carboxylate reductase 1a</td>
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<td>transmembrane protein 11, mitochondrial</td>
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<td>reticulon-4</td>
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<td>glyceraldehyde-3-phosphate dehydrogenase 2</td>
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<td>60S ribosomal protein L24</td>
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<td>60 kDa heat shock protein, mitochondrial</td>
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<td>Acyl-CoA synthetase long-chain family member 3b</td>
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<td>PREDICTED: nuclease EXOG, mitochondrial</td>
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<td>Mitochondrial Rho GTPase</td>
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<td>Uncharacterized protein LOC619266 precursor</td>
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<td>DDDRK domain-containing protein 1 precursor</td>
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<td>PREDICTED: ras-related protein Rab-1B</td>
<td>25 kDa</td>
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<td>Predicted: ER membrane protein complex subunit 1 isoform X1</td>
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<td>Guanine nucleotide-binding protein G0j subunit alpha</td>
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<td>PREDICTED: syntaxin-12 isoform X1</td>
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<tr>
<td>RAB11a, member RAS oncogene family, like</td>
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<tr>
<td>Predicted: NADH dehydrogenase</td>
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<tr>
<td>Mitochondrial trifunctional protein, alpha subunit</td>
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<td>Phosphatidate cytidylyltransferase, mitochondrial precursor</td>
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<td>Solute carrier family 3 (amino acid transporter heavy chain), member 2b</td>
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<td>AFG3-like protein 2</td>
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<td>Glycerophosphodiester phosphodiesterase domain-containing protein 1</td>
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<td>Immunity-related GTPase family q2</td>
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<td>40S ribosomal protein S25</td>
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<td>Beta-centractin</td>
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<tr>
<td>Predicted: OCIA domain-containing protein 1 isoform X1</td>
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<tr>
<td>Predicted: mitochondrial fission factor-like isoform X3</td>
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<td>Mitochondrial dicarboxylate carrier</td>
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<td>Predicted: kynurenine/alpha-aminoadipate aminotransferase, mitochondrial isoform X1</td>
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<td>Oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)</td>
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<td>Ras-related protein Rab-5C</td>
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<td>Surfeit locus protein 1</td>
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<td>Predicted: succinate dehydrogenase</td>
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<td>Predicted: oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide) isoform X3</td>
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<td>Predicted: mitochondrial import inner membrane translocase subunit Tim22</td>
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<td>Predicted: 2-oxoglutarate dehydrogenase, mitochondrial</td>
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<tr>
<td>Predicted: potassium voltage-gated channel subfamily B member 2</td>
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<td>C3orf33 homolog</td>
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<td>Rhodopsin kinase</td>
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<td>Predicted: NADH dehydrogenase (ubiquinone) complex I, assembly factor 6</td>
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<td>Predicted: transmembrane emp24 domain-containing protein 7 isoform X2</td>
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<td>Predicted: FUN14 domain-containing protein 2 isoform X1</td>
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<td>Eukaryotic translation elongation factor 1 alpha 1-like</td>
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<td>ATPase family AAA domain-containing protein 1-B</td>
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<td>Immunoglobulin superfamily member 8 precursor</td>
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<td>Mitochondrial import receptor subunit TOM20 homolog B</td>
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<td>Dolichyl-diphosphooligosaccharide—protein glycosyltransferase 48 kDa subunit precursor</td>
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<td>Guanine nucleotide-binding protein G(1) subunit alpha-2</td>
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<td>ATP synthase subunit delta, mitochondrial</td>
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<td>Predicted: mitochondrial pyruvate carrier 2-like</td>
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<td>Protein disulfide-isomerase TMX3 precursor</td>
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<td>Ubiquinone biosynthesis monooxygenase COQ6</td>
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<td>Protein Description</td>
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<td>Phosphatidylglycerophosphatase and protein-tyrosine phosphatase</td>
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<td>Mitochondrial folate transporter/carrier</td>
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<td>Phosphatidate cytidylyltransferase, mitochondrial precursor</td>
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<td>PREDICTED: sodium/potassium-transporting ATPase subunit alpha-1-like, partial</td>
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<td>Surfeit gene 4, like</td>
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<td>PREDICTED: heme oxygenase 2</td>
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<td>PREDICTED: ras-related protein Rab-6A isoform X2</td>
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<td>Cytochrome b–c1 complex subunit Rieske, mitochondrial</td>
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<td>PREDICTED: ATP–dependent zinc metalloprotease YME1L1 isoform X1</td>
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<td>40S ribosomal protein S19</td>
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<td>Metaxin 1a</td>
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<td>60S ribosomal protein L27a</td>
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<td>Vesicle–trafficking protein SEC22b–A</td>
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<td>PREDICTED: sacrolemmal membrane-associated protein isoform X4</td>
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<td>Acyl-CoA dehydrogenase-like</td>
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<td>PREDICTED: 40S ribosomal protein S8</td>
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<td>60S ribosomal protein L27a</td>
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<td>Vesicle–trafficking protein SEC22b–A</td>
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<td>PREDICTED: saccharopine dehydrogenase-like oxidoreductase-likes</td>
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<td>Delta-1–pyrroline–5–carboxylate synthase</td>
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<td>oxidase (cytochrome c) assembly 1-like</td>
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<td>ubiquinol-cytochrome c reductase complex assembly factor 1</td>
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<td>fatty-acid amide hydrolase 1</td>
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<td>435</td>
<td>PREDICTED: glycerol kinase isoform X4</td>
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<td>translocase of outer mitochondrial membrane 20 homolog a</td>
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<td>40S ribosomal protein S5</td>
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<td>PREDICTED: calnexin isoform X1</td>
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<td>1-acyl-sn-glycerol-3-phosphate acyltransferase epsilon</td>
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<td>PREDICTED: probable palmitoyltransferase ZDHHC14 isoform X1</td>
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<td>441</td>
<td>PREDICTED: protein CCSMST1</td>
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<td>glutathione peroxidase 1</td>
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<td>bcl-2-modifying factor</td>
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<td>PREDICTED: cadherin-related family member 5-like isoform X2</td>
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<td>protein SCO2 homolog, mitochondrial</td>
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<td>isovaleryl-CoA dehydrogenase, mitochondrial</td>
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<td>predicted: neutral alpha-glucosidase AB</td>
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<td>solute carrier family 25 member 40</td>
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<td>mitofusin-2</td>
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<td>60S ribosomal protein L7a</td>
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<td>predicted: uncharacterized protein sich211–11k18.4</td>
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<td>hydroxysteroid dehydrogenase–like protein 2</td>
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<td>predicted: parapleegi</td>
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<td>predicted: calcium–binding mitochondrial carrier protein SCaMC–3–like isoform X2</td>
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<td>predicted: 40S ribosomal protein S2</td>
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<td>NADH dehydrogenase 1 beta subcomplex subunit 10</td>
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<td>long–chain fatty acid transport protein 4</td>
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<td>mitochondrial dynamics protein MID49</td>
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<td>ADP–ribosylation factor–like protein 6</td>
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<td>translocon–associated protein subunit delta precursor</td>
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<td>predicted: bcl10–interacting CARD protein isoform X2</td>
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<td>predicted: cytochrome c oxidase subunit 4 isoform 1, mitochondrial</td>
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<td>predicted: fatty aldehyde dehydrogenase–like</td>
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<td>outer dense fiber of sperm tails 2b</td>
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<td>PRA1 family protein 3</td>
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<td>60S ribosomal protein L9</td>
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<tr>
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<td>eukaryotic translation elongation factor 1 alpha 1–like</td>
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<td>Gene Name</td>
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<tr>
<td>PREDICTED: 10 kDa heat shock protein, mitochondrial isoform X2</td>
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<td>PREDICTED: aarF domain-containing protein kinase 4</td>
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<tr>
<td>PREDICTED: saccharopine dehydrogenase-like oxidoreductase</td>
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<tr>
<td>succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial</td>
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<tr>
<td>acyl-CoA dehydrogenase family member 9, mitochondrial</td>
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<tr>
<td>succinyl-CoA ligase</td>
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<td>dihydroorotate dehydrogenase (quinone), mitochondrial</td>
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<td>60S acidic ribosomal protein P0</td>
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<tr>
<td>mitochondrial ubiquitin ligase activator of nfkb 1-A</td>
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<tr>
<td>methylglutaconyl-CoA hydratase, mitochondrial</td>
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<tr>
<td>PREDICTED: transmembrane protein C9orf123 homolog</td>
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<td>PREDICTED: flotillin 1a isoform X1</td>
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<tr>
<td>PREDICTED: serine protease HTRA2, mitochondrial</td>
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<td>electron transfer flavoprotein subunit alpha, mitochondrial</td>
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<td>ADP-ribosylation factor-like protein 6-interacting protein 1</td>
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<td>PREDICTED: UPF0962 protein C7orf55 homolog</td>
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<td>oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)</td>
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<td>Protein Name</td>
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<td>flotillin-1</td>
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<td>nicastrin precursor</td>
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<td>aquaporin-9</td>
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<td>dehydrogenase/reductase SDR family member 4</td>
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<td>637</td>
<td>NADH dehydrogenase</td>
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<td>succinate dehydrogenase</td>
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<td>PREDICTED: 2–oxoglutarate dehydrogenase–like, mitochondrial</td>
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<td>687</td>
<td>peripherin–2</td>
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<td>688</td>
<td>PREDICTED: T–cell activation inhibitor, mitochondrial</td>
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<td>NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex 1–like</td>
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<td>1-acyl-sn-glycerol-3-phosphate acyltransferase gamma</td>
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<tr>
<td>28S ribosomal protein S27, mitochondrial</td>
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<td>P-Value</td>
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<td>serum/glucocorticoid regulated kinase 1-like</td>
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<td>keratin, type I cytoskeletal 18</td>
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<td>nucleoside diphosphate kinase 7</td>
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<td>ELMO domain-containing protein 2 precursor</td>
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<td>maleylacetate isomerase isoform 2</td>
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<td>prolactin regulatory element-binding protein</td>
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<td>hypoxanthine–guanine phosphoribosyltransferase</td>
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<td>60S ribosomal protein L10a</td>
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<td>bcl-2–like protein 13</td>
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<td>N-acetylneuraminate cytidylyltransferase</td>
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<td>polyribonucleotide nucleotidylyltransferase 1, mitochondrial</td>
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<td>methylmalonyl-CoA mutase, mitochondrial</td>
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<td>PREDICTED: dihydrofolate reductase, mitochondrial</td>
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<tr>
<td>PREDICTED: HCLS1–associated protein X-1</td>
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<td>ER membrane protein complex subunit 10 isoform 1 precursor</td>
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<td>Protein Description</td>
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<td>PREDICTED: G-protein coupled receptor 98 isoform X2</td>
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<td>leucine-rich PPR motif-containing protein, mitochondrial</td>
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<td>B-cell receptor–associated protein 31</td>
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<td>uncharacterized protein C18orf19 homolog B</td>
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<td>40S ribosomal protein S3a</td>
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<td>3-mercaptopruvate sulfurtransferase</td>
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<td>PREDICTED: protein FAM134C</td>
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<td>40S ribosomal protein SA</td>
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<td>PREDICTED: zinc finger protein–like 1 isoform X1</td>
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<td>PREDICTED: syntaxin–18 isoform X1</td>
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<td>PREDICTED: trans–Golgi network integral membrane protein 2</td>
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<td>amine oxidase</td>
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<td>neurotamin isoform 1 precursor</td>
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<td>2-methoxy-6-polyprenyl-1,4-benzoquinol methylase, mitochondrial precursor</td>
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<td>PREDICTED: serine/threonine–protein phosphatase PP1–beta catalytic subunit</td>
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<td>PREDICTED: red–sensitive opsin–1 isoform X1</td>
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<td>cAMP–dependent protein kinase catalytic subunit beta</td>
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<td>PREDICTED: protein phosphatase 1K, mitochondrial isoform X1</td>
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<td>PREDICTED: peroxisomal membrane protein PEX14</td>
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<td>polymerase delta–interacting protein 2</td>
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<td>acyl–CoA:lysophatidylglycerol acyltransferase 1</td>
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<td>PREDICTED: dolichyl–diphosphooligosaccharide–protein glycosyltransferase subunit STT3B</td>
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<td>PREDICTED: centrosomal protein POC5 isoform X1</td>
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<td>casein kinase 2, alpha 1 polypeptide</td>
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<td>PREDICTED: neutral alpha–glucosidase AB</td>
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<td>glycogen synthase kinase–3 alpha</td>
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<td>puromycin–sensitive aminopeptidase</td>
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<td>elongation factor 1–gamma</td>
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<td>epoxide hydrolase 1</td>
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<td>squalene synthase</td>
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<td>PREDICTED: sodium–coupled neutral amino acid transporter 3</td>
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<td>PREDICTED: AP–2 complex subunit alpha–1 isoform X1</td>
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<td>PREDICTED: succinate–semialdehyde dehydrogenase, mitochondrial isoform X1</td>
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<td>fatty–acid amide hydrolase 2–A</td>
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<td>PREDICTED: GPI transamidase component PIG–S isoform X2</td>
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<td>phosphatidylinositol phosphatase SAC1–B</td>
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<td>PREDICTED: V–type proton ATPase catalytic subunit A</td>
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<td>942</td>
<td>electron transfer flavoprotein–ubiquinone oxidoreductase, mitochondrial</td>
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<td>943</td>
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<td>PREDICTED: long–chain fatty acid transport protein 6–like</td>
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<td>PREDICTED: epidermal growth factor receptor kinase substrate 8 isoform X1</td>
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<td>946</td>
<td>polyadenylate–binding protein 1</td>
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<td>947</td>
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<td>PREDICTED: synembryn–A–like</td>
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<td>PREDICTED: oxysterol–binding protein–related protein 8–like</td>
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<td>PREDICTED: PDZ domain–containing protein 8</td>
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<td>PREDICTED: 26S proteasome non–ATPase regulatory subunit 1–like</td>
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<td>PREDICTED: protein fantom isoform X1</td>
<td>215 kDa</td>
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<tr>
<td>971</td>
<td>PREDICTED: rootletin isoform X1</td>
<td>226 kDa</td>
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</tbody>
</table>
Table S3. Identified proteins in washed COS-rich fraction.
Proteins in washed COS-rich fraction were identified with LC–MS/MS analysis and are listed in descending order of emPAI values for 5 × 10^5 cones.

<table>
<thead>
<tr>
<th>Identified proteins in washed COS-rich fraction</th>
<th>Molecular mass</th>
<th>emPAI</th>
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<tr>
<td>uncharacterized protein LOC100145214</td>
<td>33 kDa</td>
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<td>ba1 globin, like</td>
<td>16 kDa</td>
<td>36.229</td>
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<tr>
<td>guanine nucleotide-binding protein G(t) subunit alpha-2</td>
<td>40 kDa</td>
<td>28.315</td>
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<tr>
<td>voltage-dependent anion-selective channel protein 1</td>
<td>31 kDa</td>
<td>22.103</td>
</tr>
<tr>
<td>mitochondrial 2-oxoglutarate/malate carrier protein</td>
<td>38 kDa</td>
<td>19.592</td>
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<td>ATP synthase F(0) complex subunit B1, mitochondrial</td>
<td>31 kDa</td>
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<td>arrestin-C</td>
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<td>11 kDa</td>
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<td>PREDICTED: prohibitin</td>
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<td>7.3326</td>
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<td>NADH dehydrogenase</td>
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<td>102</td>
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<td>ras-related protein Rab-8A</td>
<td>23 kDa</td>
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<tr>
<td><strong>114</strong></td>
<td>serine/threonine-protein kinase MAK</td>
<td>11 kDa</td>
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<tr>
<td><strong>115</strong></td>
<td>DDRGK domain-containing protein 1 precursor</td>
<td>35 kDa</td>
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<td><strong>116</strong></td>
<td>mitochondrial import receptor subunit TOM70</td>
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<td><strong>117</strong></td>
<td>sorting and assembly machinery component 50 homolog B</td>
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<td>PREDICTED: 60S ribosomal protein L9 isoform X2</td>
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<td>HIG1 domain family member 1A</td>
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<td>ras-related protein Rab-5B</td>
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<td><strong>122</strong></td>
<td>protein NDRG1 isoform 1</td>
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<td><strong>123</strong></td>
<td>very-long-chain enoyl-CoA reductase</td>
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<td>protein THEM6 precursor</td>
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<td><strong>127</strong></td>
<td>PREDICTED: 40S ribosomal protein S8</td>
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<td><strong>128</strong></td>
<td>PREDICTED: tubulin alpha chain-like</td>
<td>12 kDa</td>
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<td><strong>129</strong></td>
<td>PREDICTED: mitochondrial fission process protein 1-like</td>
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<td><strong>130</strong></td>
<td>transmembrane protein 256 precursor</td>
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<td><strong>131</strong></td>
<td>transmembrane protein 256 precursor</td>
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<td><strong>132</strong></td>
<td>cytochrome b-c1 complex subunit 2, mitochondrial</td>
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<td><strong>133</strong></td>
<td>PREDICTED: reticulon-3-B-like isoform X2</td>
<td>25 kDa</td>
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<td><strong>134</strong></td>
<td>PREDICTED: Saccharopine dehydrogenase-like oxidoreductase-like</td>
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<td><strong>135</strong></td>
<td>solute carrier family 3 (amino acid transporter heavy chain), member 2b</td>
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<td><strong>136</strong></td>
<td>PREDICTED: tubulin alpha chain</td>
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<td>dihydriolipoyllysine--residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial</td>
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<td>calmodulin precursor</td>
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<td>mitochondrial ATP synthase subunit f</td>
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<td>PREDICTED: ubiquinol-cytochrome c reductase core protein II isoform X1</td>
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<td>protein NpSnaph homolog 2</td>
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<td>NADH dehydrogenase (ubiquinone) 1 subunit c2</td>
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<td><strong>143</strong></td>
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<td>PREDICTED: red-sensitive opsin-1 isoform X1</td>
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<td>peroxiredoxin-2</td>
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<td>ADP-dependent glucokinase isoform X2</td>
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<td>sodium/potassium/calcium exchanger 2-like isoform X2</td>
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<td>ops in-1, short-wave-sensitive 2</td>
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<td>ops in-1, short-wave-sensitive 1</td>
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<td>plasminogen receptor (KT)</td>
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<td>phosphatidyglycerophosphatase and protein-tyrosine phosphatase 1</td>
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<td>PREDICTED: retinol dehydrogenase--like</td>
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<td>CDP--diacylglycerol--inositol 3-phosphatidyltransferase</td>
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<td>protein THEM6 precursor</td>
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<td>PREDICTED: dephospho--CoA kinase domain--containing protein--like isoform X2</td>
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<td>PREDICTED: ras-related protein Rab-35--like</td>
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<td>phosphatidyglycerophosphatase and protein-tyrosine phosphatase 1</td>
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<td>PREDICTED: Fc receptor--like protein 5 isoform X2</td>
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<td>guanyl cyclase 3 (guanylate cyclase retinal cone [Cyprinus carpio])</td>
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<td>erin--1 precursor</td>
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<td>pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial</td>
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<td>PREDICTED: phospholipid scramblase 2</td>
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<td>cytochrome c oxidase subunit 6A1, mitochondrial</td>
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<td>guanine nucleotide--binding protein G(i) subunit alpha--2</td>
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<td>mannose-P-dolichol utilization defect 1 protein</td>
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<td>PREDICTED: prostate stem cell antigen-like</td>
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<td>60S ribosomal protein L7a</td>
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<td>PREDICTED: 60S ribosomal protein L23</td>
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<td>dehydrogenase complex, mitochondrial</td>
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<td>60S ribosomal protein L26</td>
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<td>neurotomin isoform 1 precursor</td>
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<td>2–methoxy–6–polyphenyl–1,4–benzoquinol methylase, mitochondrial precursor</td>
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<td>EF–hand calcium–binding domain–containing protein 4A</td>
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<td>PREDICTED: ceroid–lipofuscinosis, neuronal 6a isoform X1</td>
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<td>cytochrome P450, family 27, subfamily C, polypeptide 1</td>
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<td>phosphatidylinositol N-acetylglycosaminyltransferase subunit H</td>
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<td>PREDICTED: protein XRP2 isoform X1</td>
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<td>PREDICTED: protein TsetseEP–like</td>
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<td>PREDICTED: vesicle–fusing ATPase isoform X1</td>
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<td>PREDICTED: translocon–associated protein subunit gamma</td>
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<td>PREDICTED: cell division control protein 42 homolog isoform X1</td>
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aspartate aminotransferase 2 43 kDa 0.1787

PREDICTED: glutaminase a isoform X1 66 kDa 0.1786

PREDICTED: transmembrane and coiled-coil domains protein 1-like 21 kDa 0.1781

ADP-ribosylation factor-like protein 8B-A 21 kDa 0.1781

phosphatidylerosine synthase 1 21 kDa 0.1781

PREDICTED: cadherin-related family member 5-like isoform X2 44 kDa 0.1773

ADP-ribosylation-like factor 6 interacting protein 5 22 kDa 0.1771

outer dense fiber of sperm tails 2b 22 kDa 0.1771

peptidyl-prolyl cis-trans isomerase FKBP8 44 kDa 0.1768

mitochondrial NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 5 22 kDa 0.1753

Bcl-2/adenovirus E1B 19kD interaction protein XR 22 kDa 0.1753

ADP-ribosylation factor-like 3, like 1 22 kDa 0.1753

PREDICTED: guanine nucleotide-binding protein (G) subunit alpha isoform X2 44 kDa 0.1749

recoverin-like 22 kDa 0.1743

lactation elevated protein 1 homolog B 22 kDa 0.1743

ubiquinone biosynthesis protein COQ7 homolog 22 kDa 0.1734

PREDICTED: protein RER1 isoform X2 22 kDa 0.1734

rho-related gtp-binding protein rhoc 22 kDa 0.1734

synaptoprevin homolog YKT6 22 kDa 0.1707

PREDICTED: transmembrane protein 126A isoform X1 22 kDa 0.1698

PREDICTED: paraplegin 92 kDa 0.1696

F-box/LRR-repeat protein 2 46 kDa 0.1691

PREDICTED: flavin reductase (NADPH)-like 23 kDa 0.169

ras-related protein Rab-18 23 kDa 0.1681

PREDICTED: protein NDRG3 isoform X1 46 kDa 0.1678

bcl2-associated X protein, b 23 kDa 0.1672

PREDICTED: pyruvate dehydrogenase kinase, isozyme 3 isoform X2 46 kDa 0.1669

PREDICTED: regulator of microtubule dynamics protein 2 isoform X1 47 kDa 0.1665

PREDICTED: cadherin-related family member 5-like isoform X2 23 kDa 0.1664

doled-coil domain-containing protein 51 47 kDa 0.1649

saccharopine dehydrogenase b 47 kDa 0.1649

PREDICTED: prominin-1 isoform X1 95 kDa 0.1637

PREDICTED: ADP-ribosylation factor-like protein 3 23 kDa 0.1631

apolipoprotein O 24 kDa 0.1615

PREDICTED: sphingomyelin phosphodiesterase 2 isoform X1 48 kDa 0.1608

PREDICTED: NADP(--cytochrome P450 reductase isoform X1 48 kDa 0.1608

NADH dehydrogenase 48 kDa 0.1604

[3-methyl-2-oxobutanoate dehydrogenase 48 kDa 0.16

60S ribosomal protein L15 24 kDa 0.1584

PREDICTED: metal transporter CNNM4 74 kDa 0.1578

ceramide-1-phosphate transfer protein 24 kDa 0.1576

60S ribosomal protein L10 25 kDa 0.1547

PREDICTED: ras-related protein Rab-28 isoform X2 25 kDa 0.1532

60S ribosomal protein L10a 25 kDa 0.1525

PREDICTED: von Willebrand factor A domain-containing protein 1 25 kDa 0.1525

peroxisomal membrane protein 11B 25 kDa 0.1518

hydroxysteroid dehydrogenase-like protein 2 51 kDa 0.1516

PREDICTED: V-type proton ATPase subunit S1 52 kDa 0.1492

protein disulfide-isomerase TMX3 precursor 52 kDa 0.1481

epoxide hydrolase 1 52 kDa 0.1478

Probable saccharopine dehydrogenase 26 kDa 0.1464

PREDICTED: OCIA domain-containing protein 1 isoform X1 26 kDa 0.1451

PREDICTED: mitochondrial fission factor-like isoform X3 26 kDa 0.1451

PREDICTED: sodium-coupled neutral amino acid transporter 3 53 kDa 0.1449

PREDICTED: sarcoplasmic/endoplasmic reticulum calcium ATPase 3 isoform X2 53 kDa 0.1446

PREDICTED: abhydrolase domain-containing protein 8 53 kDa 0.1439

bcl-2-like protein 1 26 kDa 0.1432

NADH dehydrogenase 27 kDa 0.1426

PREDICTED: ATP synthase subunit s-like protein isoform X1 27 kDa 0.142.
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<th>Protein Description</th>
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<td>ER membrane protein complex subunit 7 precursor</td>
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<td>G protein–coupled receptor kinase 1 b</td>
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<td>Sorting and assembly machinery component 50 homolog B</td>
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<td>Transmembrane emp24 domain–containing protein 4 precursor</td>
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<td>PREDICTED: sodium–coupled neutral amino acid transporter 3–like isofrom X1</td>
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<td>Large neutral amino acids transporter small subunit 1</td>
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<td>Uncharacterized protein C2orf47 homolog, mitochondrial</td>
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<td>ER membrane protein complex subunit 10 isoform 1 precursor</td>
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<td>Serine hydroxymethyltransferase, mitochondrial</td>
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<td>3-hydroxyacyl-CoA dehydrogenase type-2</td>
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<td>Thioredoxin–dependent peroxide reductase, mitochondrial</td>
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<td>PREDICTED: 40S ribosomal protein S3–like isofrom X1</td>
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<td>PREDICTED: solute carrier family 1 (glial high affinity glutamate transporter), member 2a isofrom X2</td>
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<td>Aquaporin-9</td>
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<td>Calpain–5</td>
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<td>Surfeit locus protein 1</td>
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<td>Integral membrane protein 2B</td>
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<td>Disintegrin and metalloproteinase domain–containing protein 10 precursor</td>
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<td>Protein Name</td>
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<td>Bardet–Biedl syndrome 5 protein homolog</td>
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<td>PREDICTED: apoptosis–inducing factor 1, mitochondrial isoform X1</td>
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<td>keratin, type I cytoskeletal 18</td>
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<td>PREDICTED: calcium–binding mitochondrial carrier protein ScaMC–2–B isoform X2</td>
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<td>V-type proton ATPase subunit H isoform 1</td>
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<td>6-phosphogluconate dehydrogenase, decarboxylating isoform 1</td>
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<td>dol-P-Man:Man(7)GlcNAc(2)--&gt;PP-Dol alpha-1,6-mannosyltransferase precursor</td>
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<td>fatty-acid amide hydrolase 2-A</td>
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<td>leucine-rich repeat, immunoglobulin-like and transmembrane domains 1 precursor</td>
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<td>polyadenylate-binding protein 1</td>
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<td>PREDICTED: signal recognition particle receptor subunit alpha</td>
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<td>cleft lip and palate transmembrane protein 1 homolog</td>
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<td>long-chain fatty acid transport protein 1</td>
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<td>PREDICTED: dynamin 1a isoform X5</td>
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<td>PREDICTED: solute carrier family 12 member 7 isoform X5</td>
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<tr>
<td>628</td>
<td>PREDICTED: electrogenic sodium bicarbonate cotransporter 1 isoform X2</td>
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<td>629</td>
<td>PREDICTED: uncharacterized protein LOC102079324</td>
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Table S4. Identified proteins in washed CIS–rich fraction.
Proteins in washed CIS–rich fraction were identified with LC–MS/MS analysis and are listed in descending order of emPAI values for 5 × 10^5 cones.

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<thead>
<tr>
<th>#</th>
<th>Identified proteins in washed CIS–rich fraction</th>
<th>Molecular mass</th>
<th>emPAI</th>
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<td>1</td>
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<td>ATP synthase F(0) complex subunit B1, mitochondrial</td>
<td>31 kDa</td>
<td>2258</td>
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<tr>
<td>3</td>
<td>PREDICTED: prohibitin isoform X2</td>
<td>22 kDa</td>
<td>314.23</td>
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<td>4</td>
<td>voltage–dependent anion–selective channel protein 1</td>
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<tr>
<td>5</td>
<td>PREDICTED: prohibitin</td>
<td>22 kDa</td>
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<tr>
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<td>1.6335</td>
</tr>
<tr>
<td>152</td>
<td>PREDICTED: LOW QUALITY PROTEIN: actin, gamma 1</td>
<td>40 kDa</td>
<td>1.6204</td>
</tr>
<tr>
<td>153</td>
<td>creatine kinase U-type, mitochondrial</td>
<td>22 kDa</td>
<td>1.619</td>
</tr>
<tr>
<td>154</td>
<td>isocitrate dehydrogenase</td>
<td>43 kDa</td>
<td>1.6078</td>
</tr>
<tr>
<td>155</td>
<td>PREDICTED: calcium-binding mitochondrial carrier protein SCaMC-2-B isoform X2</td>
<td>49 kDa</td>
<td>1.5994</td>
</tr>
<tr>
<td>156</td>
<td>PREDICTED: vesicle-associated membrane protein-associated protein A-like</td>
<td>26 kDa</td>
<td>1.5929</td>
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<tr>
<td>157</td>
<td>PREDICTED: vesicle-associated membrane protein-associated protein A-like</td>
<td>26 kDa</td>
<td>1.5929</td>
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<tr>
<td>158</td>
<td>thioredoxin-dependent peroxide reductase, mitochondrial</td>
<td>28 kDa</td>
<td>1.5892</td>
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<td>159</td>
<td>mitochondrial import inner membrane translocase subunit Tim17-B</td>
<td>18 kDa</td>
<td>1.5758</td>
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<td>160</td>
<td>protein QIL1</td>
<td>12 kDa</td>
<td>1.5631</td>
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<tr>
<td>161</td>
<td>PREDICTED: nuclelease EXOG, mitochondrial</td>
<td>39 kDa</td>
<td>1.5583</td>
</tr>
<tr>
<td>162</td>
<td>transmembrane protein 256 precursor</td>
<td>12 kDa</td>
<td>1.5345</td>
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<tr>
<td>163</td>
<td>PREDICTED: very long-chain specific acyl-CoA dehydrogenase, mitochondrial isoform</td>
<td>71 kDa</td>
<td>1.5216</td>
</tr>
<tr>
<td>164</td>
<td>PREDICTED: dehydrogenase/reductase SDR family member 7B isoform X1</td>
<td>31 kDa</td>
<td>1.5171</td>
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<td>165</td>
<td>glutaryl-CoA dehydrogenase a</td>
<td>18 kDa</td>
<td>1.5004</td>
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<td>166</td>
<td>isocitrate dehydrogenase</td>
<td>40 kDa</td>
<td>1.4881</td>
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<td>167</td>
<td>ATP-binding cassette sub-family B member 8, mitochondrial</td>
<td>77 kDa</td>
<td>1.4777</td>
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<td>168</td>
<td>sorting and assembly machinery component 50 homolog B</td>
<td>27 kDa</td>
<td>1.464</td>
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<td>169</td>
<td>NAD(P) transhydrogenase, mitochondrial</td>
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<td>Molecular Weight</td>
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<td>170</td>
<td>PREDICTED: calcium/calmodulin-dependent protein kinase type II delta 1 chain isoform X3</td>
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<td>171</td>
<td>dihydrolipoyl dehydrogenase, mitochondrial</td>
<td>54 kDa</td>
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<td>172</td>
<td>metastin 1</td>
<td>36 kDa</td>
<td>1.4365</td>
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<td>173</td>
<td>PREDICTED: LOW QUALITY PROTEIN: long-chain-fatty-acid--CoA ligase 6</td>
<td>50 kDa</td>
<td>1.4107</td>
</tr>
<tr>
<td>174</td>
<td>PREDICTED: mitochondrial fission factor-like isoform X4</td>
<td>24 kDa</td>
<td>1.3838</td>
</tr>
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<td>175</td>
<td>COX16 cytochrome c oxidase assembly homolog</td>
<td>13 kDa</td>
<td>1.3807</td>
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<td>176</td>
<td>mitochondrial carrier homolog 2</td>
<td>31 kDa</td>
<td>1.3454</td>
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<td>177</td>
<td>PREDICTED: tubulin beta-4B chain-like</td>
<td>31 kDa</td>
<td>1.3266</td>
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<td>178</td>
<td>reticulon-4</td>
<td>22 kDa</td>
<td>1.3162</td>
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<td>179</td>
<td>PREDICTED: choline dehydrogenase, mitochondrial</td>
<td>25 kDa</td>
<td>1.2768</td>
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<tr>
<td>180</td>
<td>ATPase, Na+/K+ transporting, beta 2b polypeptide</td>
<td>34 kDa</td>
<td>1.2638</td>
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<td>181</td>
<td>[3-methyl-2-oxobutanoate dehydrogenase</td>
<td>48 kDa</td>
<td>1.2509</td>
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<td>182</td>
<td>PREDICTED: retinol dehydrogenase 12-like</td>
<td>34 kDa</td>
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<td>183</td>
<td>AFG3-like protein 2</td>
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<td>1.2328</td>
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<td>184</td>
<td>cytochrome b-c1 complex subunit Rieske, mitochondrial</td>
<td>30 kDa</td>
<td>1.2288</td>
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<td>185</td>
<td>PREDICTED: probable UDP--sugar transporter protein SLC35A4 isoform X1</td>
<td>11 kDa</td>
<td>1.2058</td>
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<tr>
<td>186</td>
<td>PREDICTED: presenilins-associated rhombid-like protein, mitochondrial-like isoform X2</td>
<td>11 kDa</td>
<td>1.2058</td>
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<tr>
<td>187</td>
<td>elongation factor Tu, mitochondrial</td>
<td>49 kDa</td>
<td>1.1921</td>
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<td>188</td>
<td>erlin-2 precursor</td>
<td>40 kDa</td>
<td>1.1837</td>
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<td>189</td>
<td>ADP ribosylation factor--like protein 6</td>
<td>21 kDa</td>
<td>1.1684</td>
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<td>190</td>
<td>acetyl-CoA acetyltransferase, mitochondrial precursor</td>
<td>48 kDa</td>
<td>1.1577</td>
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<td>191</td>
<td>PREDICTED: mitochondrial import inner membrane translocase subunit Tim22</td>
<td>21 kDa</td>
<td>1.1569</td>
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<tr>
<td>192</td>
<td>ras--related protein Rab--2A</td>
<td>24 kDa</td>
<td>1.1561</td>
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<tr>
<td>193</td>
<td>solute carrier family 25 (mitochondrial carrier: glutamate), member 22</td>
<td>36 kDa</td>
<td>1.1538</td>
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<td>194</td>
<td>PREDICTED: mitochondrial fission factor-like isoform X3</td>
<td>26 kDa</td>
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<td>mitochondrial import receptor subunit TOM20 homolog B</td>
<td>16 kDa</td>
<td>1.1448</td>
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<td>PREDICTED: glutaryl-CoA dehydrogenase, mitochondrial-like</td>
<td>49 kDa</td>
<td>1.1278</td>
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<td>197</td>
<td>zinc transporter 9</td>
<td>64 kDa</td>
<td>1.1104</td>
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<tr>
<td>198</td>
<td>citrate synthase, mitochondrial precursor</td>
<td>52 kDa</td>
<td>1.0972</td>
</tr>
<tr>
<td>199</td>
<td>PREDICTED: stress--70 protein, mitochondrial--like, partial</td>
<td>17 kDa</td>
<td>1.0902</td>
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<tr>
<td>200</td>
<td>PREDICTED: tubulin alpha chain-like</td>
<td>12 kDa</td>
<td>1.0861</td>
</tr>
<tr>
<td>201</td>
<td>mitochondrial trifunctional protein, alpha subunit</td>
<td>83 kDa</td>
<td>1.071</td>
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<tr>
<td>202</td>
<td>transmembrane protein 256 precursor</td>
<td>12 kDa</td>
<td>1.0682</td>
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<td>203</td>
<td>PREDICTED: coiled-coil domain--containing protein 136-like isoform X1</td>
<td>30 kDa</td>
<td>1.0538</td>
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<tr>
<td>204</td>
<td>PREDICTED: kynurenine-alpha--aminoadipate aminotransferase, mitochondrial isoform X1</td>
<td>48 kDa</td>
<td>1.0501</td>
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<tr>
<td>205</td>
<td>PREDICTED: ras--related protein Rab--1A--like isoform X1</td>
<td>22 kDa</td>
<td>1.0436</td>
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<tr>
<td>206</td>
<td>PREDICTED: nucleoside diphosphate kinase B isoform X1</td>
<td>17 kDa</td>
<td>1.0402</td>
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<tr>
<td>207</td>
<td>ras--related protein Rab--1B</td>
<td>22 kDa</td>
<td>1.0343</td>
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<tr>
<td>208</td>
<td>succinate dehydrogenase</td>
<td>61 kDa</td>
<td>1.0337</td>
</tr>
<tr>
<td>209</td>
<td>mitochondrial import inner membrane translocase subunit Tim17--A</td>
<td>17 kDa</td>
<td>1.0283</td>
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<td>210</td>
<td>regulator of microtubule dynamics protein 3</td>
<td>51 kDa</td>
<td>1.0172</td>
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<tr>
<td>211</td>
<td>PREDICTED: protein FAM162B isoform X1</td>
<td>17 kDa</td>
<td>1.0167</td>
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<tr>
<td>212</td>
<td>PREDICTED: peroxiredoxin--5, mitochondrial--like</td>
<td>20 kDa</td>
<td>1.0114</td>
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<td>213</td>
<td>succinate dehydrogenase</td>
<td>73 kDa</td>
<td>1.0022</td>
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<td>214</td>
<td>pyrroline-5-carboxylate reductase 1a</td>
<td>34 kDa</td>
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<td>215</td>
<td>uncharacterized protein LOC100135302</td>
<td>23 kDa</td>
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<tr>
<td>216</td>
<td>histone H2AX</td>
<td>15 kDa</td>
<td>0.972</td>
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<tr>
<td>217</td>
<td>dnaJ homolog subfamily C member 11</td>
<td>64 kDa</td>
<td>0.9565</td>
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<tr>
<td>218</td>
<td>methylglutaconyl-CoA hydratase, mitochondrial</td>
<td>35 kDa</td>
<td>0.9354</td>
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<tr>
<td>219</td>
<td>PREDICTED: FAS--associated factor 2--like isoform X1</td>
<td>54 kDa</td>
<td>0.9249</td>
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<td>220</td>
<td>dihydrolipoylleucine--residue succinytransferase component of 2--oxoglutarate dehydrogenase complex, mitochondrial</td>
<td>51 kDa</td>
<td>0.9224</td>
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<td>221</td>
<td>PREDICTED: dephospho-CoA kinase domain--containing protein--like isoform X2</td>
<td>24 kDa</td>
<td>0.9039</td>
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<td>222</td>
<td>PREDICTED: LOW QUALITY PROTEIN: long-chain-fatty-acid--CoA ligase 6</td>
<td>75 kDa</td>
<td>0.891</td>
</tr>
<tr>
<td>223</td>
<td>ubiquinone biosynthesis monooxygenase COQ6</td>
<td>52 kDa</td>
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<tr>
<td>224</td>
<td>metastin 1a</td>
<td>36 kDa</td>
<td>0.8861</td>
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<tr>
<td>225</td>
<td>PREDICTED: tubulin alpha chain</td>
<td>45 kDa</td>
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</tr>
<tr>
<td>#</td>
<td>Description</td>
<td>Molecular weight</td>
<td>M-Score</td>
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<tr>
<td>226</td>
<td>NADH dehydrogenase (ubiquinone) Fe-S protein 8b</td>
<td>22 kDa</td>
<td>0.8573</td>
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<tr>
<td>227</td>
<td>hexokinase-1</td>
<td>54 kDa</td>
<td>0.8501</td>
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<tr>
<td>228</td>
<td>ATP synthase F(0) complex subunit C3, mitochondrial</td>
<td>14 kDa</td>
<td>0.8416</td>
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<tr>
<td>229</td>
<td>PREDICTED: transmembrane protein 126A isoform X1</td>
<td>22 kDa</td>
<td>0.8358</td>
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<tr>
<td>230</td>
<td>histone 2, H2a</td>
<td>14 kDa</td>
<td>0.8304</td>
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<tr>
<td>231</td>
<td>uncharacterized protein C18orf19 homolog A</td>
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<td>232</td>
<td>histone 2, H2a</td>
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<td>0.8087</td>
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<td>233</td>
<td>hexokinase-1</td>
<td>11 kDa</td>
<td>0.8001</td>
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<tr>
<td>234</td>
<td>PREDICTED: apoptosis-inducing factor 1, mitochondrial isoform X3</td>
<td>68 kDa</td>
<td>0.7994</td>
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<tr>
<td>235</td>
<td>regulator of microtubule dynamics protein 3</td>
<td>35 kDa</td>
<td>0.7984</td>
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<td>236</td>
<td>cysteine desulfurase, mitochondrial</td>
<td>50 kDa</td>
<td>0.7902</td>
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<td>hexokinase-1</td>
<td>71 kDa</td>
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<td>238</td>
<td>protein disulfide-isomerase TMX3 precursor</td>
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<td>0.7839</td>
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<td>PREDICTED: coiled-coil-helix-coiled-coil-helix domain-containing protein 2, mitochondrial</td>
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<td>240</td>
<td>PREDICTED: tubulin alpha-1C chain</td>
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<td>241</td>
<td>PREDICTED: mitochondrial import inner membrane translocase subunit Tim21</td>
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<td>0.7623</td>
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<tr>
<td>242</td>
<td>protein NDRG1 isoform 1</td>
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<tr>
<td>243</td>
<td>ubiquinone biosynthesis monoxygenase COQ6</td>
<td>52 kDa</td>
<td>0.7533</td>
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<td>244</td>
<td>PREDICTED: uncharacterized protein LOC101885612</td>
<td>11 kDa</td>
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<td>245</td>
<td>cytochrome c</td>
<td>11 kDa</td>
<td>0.7513</td>
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<tr>
<td>246</td>
<td>optic atrophy 3 protein homolog</td>
<td>18 kDa</td>
<td>0.7487</td>
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<tr>
<td>247</td>
<td>vesicle–associated membrane protein–associated protein A</td>
<td>30 kDa</td>
<td>0.7465</td>
</tr>
<tr>
<td>248</td>
<td>PREDICTED: gap junction delta-2 protein–like</td>
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<td>0.7351</td>
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<td>249</td>
<td>PREDICTED: dnaJ homolog subfamily C member 11</td>
<td>65 kDa</td>
<td>0.7321</td>
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<tr>
<td>250</td>
<td>PREDICTED: protein SC01 homolog, mitochondrial</td>
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<td>0.7261</td>
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<tr>
<td>251</td>
<td>PREDICTED: hydroxyacylglutathione hydrolase, mitochondrial isoform X1</td>
<td>34 kDa</td>
<td>0.7261</td>
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<tr>
<td>252</td>
<td>uncharacterized protein LOC100127838</td>
<td>18 kDa</td>
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<td>253</td>
<td>PREDICTED: ATP–binding cassette sub–family B member 7, mitochondrial</td>
<td>82 kDa</td>
<td>0.716</td>
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<td>254</td>
<td>L–2-hydroxyglutarate dehydrogenase, mitochondrial</td>
<td>31 kDa</td>
<td>0.7117</td>
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<td>PREDICTED: uncharacterized protein LOC571872 isoform X1</td>
<td>12 kDa</td>
<td>0.7078</td>
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<td>256</td>
<td>PREDICTED: mitochondrial fission factor homolog B isoform X1</td>
<td>34 kDa</td>
<td>0.707</td>
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<tr>
<td>257</td>
<td>stress–70 protein, mitochondrial</td>
<td>73 kDa</td>
<td>0.7019</td>
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<td>258</td>
<td>mitochondrial trifunctional protein, alpha subunit</td>
<td>38 kDa</td>
<td>0.6997</td>
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<td>259</td>
<td>PREDICTED: protein MGARP isoform X2</td>
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<td>260</td>
<td>mitochondrial import inner membrane translocase subunit Tim23</td>
<td>22 kDa</td>
<td>0.6933</td>
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<tr>
<td>261</td>
<td>PREDICTED: uncharacterized protein LOC556653</td>
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<td>0.6888</td>
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<tr>
<td>262</td>
<td>mitochondrial ubiquitin ligase activator of nfkb 1–A</td>
<td>12 kDa</td>
<td>0.6878</td>
</tr>
<tr>
<td>263</td>
<td>COX16 cytochrome c oxidase assembly homolog</td>
<td>12 kDa</td>
<td>0.6878</td>
</tr>
<tr>
<td>264</td>
<td>phosphatidate cytidylyltransferase, mitochondrial precursor</td>
<td>12 kDa</td>
<td>0.6878</td>
</tr>
<tr>
<td>265</td>
<td>uncharacterized protein LOC100145220</td>
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<td>0.6777</td>
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<tr>
<td>266</td>
<td>PREDICTED: apoptosis–inducing factor 1, mitochondrial isoform X1</td>
<td>48 kDa</td>
<td>0.6765</td>
</tr>
<tr>
<td>267</td>
<td>PREDICTED: mitochondrial import inner membrane translocase subunit Tim23</td>
<td>22 kDa</td>
<td>0.6713</td>
</tr>
<tr>
<td>268</td>
<td>PREDICTED: pyrroline–5-carboxylate reductase 2–like</td>
<td>16 kDa</td>
<td>0.6675</td>
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<tr>
<td>269</td>
<td>calcium/calmodulin–dependent protein kinase type II delta 1 chain isoform 1</td>
<td>56 kDa</td>
<td>0.6639</td>
</tr>
<tr>
<td>270</td>
<td>aconitate hydratase, mitochondrial</td>
<td>86 kDa</td>
<td>0.6634</td>
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<tr>
<td>271</td>
<td>mitochondrial ATP synthase subunit f</td>
<td>13 kDa</td>
<td>0.6508</td>
</tr>
<tr>
<td>272</td>
<td>PREDICTED: choline dehydrogenase, mitochondrial</td>
<td>20 kDa</td>
<td>0.643</td>
</tr>
<tr>
<td>273</td>
<td>translocase of outer mitochondrial membrane 20 homolog a</td>
<td>16 kDa</td>
<td>0.6392</td>
</tr>
<tr>
<td>274</td>
<td>PREDICTED: cytochrome b–c1 complex subunit Rieske, mitochondrial–like</td>
<td>26 kDa</td>
<td>0.6391</td>
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<tr>
<td>275</td>
<td>PREDICTED: [Pyruvate dehydrogenase (acetyl–transferring)] kinase isozyme 1, mitochondrial</td>
<td>47 kDa</td>
<td>0.6342</td>
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<tr>
<td>276</td>
<td>arrestin–C</td>
<td>33 kDa</td>
<td>0.6335</td>
</tr>
<tr>
<td>277</td>
<td>PREDICTED: regulator of G–protein signaling 9–binding protein–like</td>
<td>27 kDa</td>
<td>0.6307</td>
</tr>
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<td>278</td>
<td>uncharacterized protein LOC541537</td>
<td>27 kDa</td>
<td>0.6307</td>
</tr>
<tr>
<td>279</td>
<td>PREDICTED: ATP–binding cassette sub–family B member 10, mitochondrial</td>
<td>30 kDa</td>
<td>0.6231</td>
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<tr>
<td>280</td>
<td>acylglycerol kinase, mitochondrial precursor</td>
<td>48 kDa</td>
<td>0.6225</td>
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<td>281</td>
<td>delta–1–pyrroline–5–carboxylate dehydrogenase, mitochondrial precursor</td>
<td>62 kDa</td>
<td>0.6219</td>
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<td>PREDICTED: ATPase, Na+/K+ transporting, beta 2b polypeptide isoform X1</td>
<td>24 kDa</td>
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<td>FUN14 domain–containing protein 2 isoform X1</td>
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<td>mitochondrial ubiquitin ligase activator of nfkb 1–A</td>
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<td>saccharopine dehydrogenase–like oxido-reductase–like</td>
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<td>LIM domain and actin–binding protein 1</td>
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<td>solute carrier family 25 member 51–like</td>
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<td>acyl–CoA dehydrogenase family member 9, mitochondrial</td>
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<td>outer dense fiber of sperm tails 2b</td>
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<td>metaxin-2</td>
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<td>PREDICTED: LETM1 domain-containing protein LETM2, mitochondrial</td>
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<td>345</td>
<td>PREDICTED: ADP-dependent glucokinase isoform X2</td>
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<td>heat shock protein 75 kDa, mitochondrial</td>
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<td>PREDICTED: mitochondrial glutamate carrier 1-like</td>
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<td>PREDICTED: 40S ribosomal protein S9-like</td>
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<td>surfet locus protein 1</td>
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<td>353</td>
<td>PREDICTED: succinate dehydrogenase</td>
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<td>354</td>
<td>PREDICTED: multiple PDZ domain protein isoform X1</td>
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<td>hydroxysteroid dehydrogenase-like protein 2</td>
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<td>COX15 homolog</td>
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<td>mpv17-like protein 2</td>
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<td>1-acyl-sn-glycerol-3-phosphate acyltransferase epsilon</td>
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<td>PREDICTED: 60S ribosomal protein L23a</td>
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<td>very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 2</td>
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<td>delta-1-pyruvole-5-carboxylate synthase</td>
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<td>fatty aldehyde dehydrogenase</td>
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<td>367</td>
<td>PREDICTED: LOW QUALITY PROTEIN: 40S ribosomal protein SID3-like</td>
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<td>PREDICTED: 40S ribosomal protein S14 isoform X1</td>
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<td>RAB5A, member RAS oncogene family, a</td>
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<td>ras-related protein Rab-1B</td>
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<td>PREDICTED: paraplegin</td>
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<td>PREDICTED: 40S ribosomal protein S8</td>
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<td>alpha/beta hydrolase domain-containing protein 11</td>
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<td>chaperone activity of bcl complex-like, mitochondrial</td>
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<td>PREDICTED: reticulon-3-B-like isoform X2</td>
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<td>dynamin-like 120 kDa protein, mitochondrial precursor</td>
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<td>electron transfer flavoprotein subunit alpha, mitochondrial</td>
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<td>383</td>
<td>retinol dehydrogenase 8a</td>
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<td>384</td>
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<td>PREDICTED: 40S ribosomal protein S15a isoform X1</td>
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<td>PREDICTED: regulator of microtubule dynamics protein 2 isoform X1</td>
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<td>acyl-CoA synthetase long-chain family member 3b</td>
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<td>389</td>
<td>succinate dehydrogenase</td>
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<td>arginase-1</td>
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<td>calcium-binding mitochondrial carrier protein SCA1C-1</td>
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<td>retinol dehydrogenase 14</td>
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<td>peptidyl-prolyl cis-trans isomerase FKBP8</td>
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<td>404</td>
<td>rho-related gtp-binding protein rhoc</td>
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<td>0.2986</td>
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<tr>
<td>406</td>
<td>carnitine O-palmitoyltransferase 2, mitochondrial</td>
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<td>407</td>
<td>PREDICTED: enoyl-CoA delta isomerase 1, mitochondrial</td>
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<td>408</td>
<td>L-lactate dehydrogenase A chain</td>
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<td>409</td>
<td>G-protein–coupled receptor kinase 7A</td>
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<td>mitochondrial ubiquitin ligase activator of nfkb 1–A</td>
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<td>DDRGK domain–containing protein 1 precursor</td>
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<td>419</td>
<td>renalasin</td>
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<tr>
<td>420</td>
<td>phosphatidyglycerophosphatase and protein–tyrosine phosphatase 1</td>
<td>11 kDa</td>
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<td>40S ribosomal protein S7</td>
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<td>40S ribosomal protein S4, X isofrom</td>
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<td>phosphatidate cytidylyltransferase, mitochondrial precursor</td>
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<td>band 3 anion transport protein</td>
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<td>429</td>
<td>NAD–dependent protein deacetylase sirtuin–3, mitochondrial</td>
<td>18 kDa</td>
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<td>lipoamide acyltransferase component of branched–chain alpha–keto acid</td>
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<td>60S ribosomal protein L14</td>
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<td>40S ribosomal protein S19</td>
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<td>433</td>
<td>60S ribosomal protein L7a</td>
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<td>0.2692</td>
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<td>434</td>
<td>PREDICTED: glycerol kinase–like isofrom X4</td>
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<td>435</td>
<td>glycerophosphodiester phosphodiesterase domain–containing protein 1</td>
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<td>mitofusin–2</td>
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<td>PREDICTED: syntaxin–12 isofrom X1</td>
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<td>protein THEM6 precursor</td>
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<td>solute carrier family 25 member 47–B</td>
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<td>PREDICTED: calnexin isofrom X1</td>
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<td>protein THEM6 precursor</td>
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<td>60S ribosomal protein L27a</td>
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<td>guanine nucleotide binding protein (G protein), beta polypeptide 3b</td>
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<td>447</td>
<td>probable 28S ribosomal protein S10, mitochondrial</td>
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<td>0.2617</td>
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<td>448</td>
<td>electron transfer flavoprotein–ubiquinone oxidoreductase, mitochondrial</td>
<td>69 kDa</td>
<td>0.2605</td>
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<td>449</td>
<td>G–protein–coupled receptor kinase 7A</td>
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<td>phosphatidyglycerophosphatase and protein–tyrosine phosphatase 1</td>
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<td>mitochondrial</td>
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<td>40S ribosomal protein S5</td>
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<td>peptidyl–prolyl cis–trans isomerase FKBP8</td>
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<td>eukaryotic translation elongation factor 1 alpha 1–like</td>
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<td>456</td>
<td>PREDICTED: epoxide hydrolase 1, partial</td>
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<td>457</td>
<td>PREDICTED: von Willebrand factor A domain–containing protein 1</td>
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<td>NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex 1–like</td>
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<td>459</td>
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<td>Protein ID</td>
<td>Description</td>
<td>M.W.</td>
<td>p-value</td>
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<td>460</td>
<td>PREDICTED: vesicle-associated membrane protein 2-like</td>
<td>12 kDa</td>
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<td>461</td>
<td>long-chain fatty acid transport protein 4</td>
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<td>0.2519</td>
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<td>462</td>
<td>dynamin-1-like protein</td>
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<td>0.2503</td>
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<td>463</td>
<td>PREDICTED: 60S ribosomal protein L36</td>
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<td>464</td>
<td>PREDICTED: 60 kDa heat shock protein, mitochondrial-like, partial</td>
<td>12 kDa</td>
<td>0.2501</td>
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<td>465</td>
<td>2-methoxy-6-polyprenyl-1,4-benzoquinol methylase, mitochondrial precursor</td>
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<td>0.2497</td>
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<td>PREDICTED: lipoyldeacyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial-like</td>
<td>45 kDa</td>
<td>0.2495</td>
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<tr>
<td>467</td>
<td>NADH dehydrogenase</td>
<td>19 kDa</td>
<td>0.2494</td>
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<td>468</td>
<td>PRELI domain containing 1b</td>
<td>25 kDa</td>
<td>0.2491</td>
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<tr>
<td>469</td>
<td>green-sensitive opsin-4</td>
<td>39 kDa</td>
<td>0.2479</td>
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<tr>
<td>470</td>
<td>mimtin, mitochondrial</td>
<td>19 kDa</td>
<td>0.2476</td>
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<td>471</td>
<td>solute carrier family 25 member 40</td>
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<td>PREDICTED: glutaminase kidney isofrom, mitochondrial isofrom X3</td>
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<td>473</td>
<td>PREDICTED: NADPH:adrenodoxin oxidoreductase, mitochondrial</td>
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<td>0.2446</td>
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<td>474</td>
<td>erlin-1 precursor</td>
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<td>475</td>
<td>bcl-2-like protein 13</td>
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<td>476</td>
<td>isovaleryl-CoA dehydrogenase, mitochondrial</td>
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<td>uncharacterized protein LOC100127834</td>
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<td>PREDICTED: NCK-interacting protein with SH3 domain-like</td>
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<td>479</td>
<td>PREDICTED: UPF0562 protein C7orf55 homolog</td>
<td>13 kDa</td>
<td>0.242</td>
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<td>pancreatic progenitor cell differentiation and proliferation factor A</td>
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<td>481</td>
<td>PREDICTED: OCIA domain-containing protein 1 isoform X1</td>
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<td>482</td>
<td>PREDICTED: dnaJ homolog subfamily C member 30-like isoform X1</td>
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<tr>
<td>483</td>
<td>probable glutamate-tRNA ligase, mitochondrial precursor</td>
<td>40 kDa</td>
<td>0.2374</td>
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<td>484</td>
<td>PREDICTED: apoptosis-inducing factor 1, mitochondrial isoform X1</td>
<td>13 kDa</td>
<td>0.2369</td>
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<td>PREDICTED: red-sensitive opsin-1 isoform X1</td>
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<td>PREDICTED: NADH dehydrogenase</td>
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<td>PREDICTED: protein phosphatase 1K, mitochondrial isoform X1</td>
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<td>0.2293</td>
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<td>PREDICTED: 40S ribosomal protein S2</td>
<td>20 kDa</td>
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<td>489</td>
<td>PREDICTED: cytochrome c oxidase assembly factor 1 homolog isoform X3</td>
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<td>PREDICTED: transmembrane emp24 domain-containing protein 9 isoform X1</td>
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<td>491</td>
<td>NADH dehydrogenase</td>
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<td>PREDICTED: epoxide hydrolase 1-like, partial</td>
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<td>493</td>
<td>40S ribosomal protein S10</td>
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<td>494</td>
<td>carnitine O-acetyltransferase b</td>
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<td>495</td>
<td>acyl-CoA dehydrogenase-like</td>
<td>49 kDa</td>
<td>0.2232</td>
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<td>496</td>
<td>PREDICTED: proteoglycan 4-like isoform X1</td>
<td>13 kDa</td>
<td>0.2228</td>
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<td>497</td>
<td>PREDICTED: phosphatidylinositol decarboxylase proenzyme-like isoform X2</td>
<td>42 kDa</td>
<td>0.2225</td>
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<td>498</td>
<td>mitochondrial folate transporter/carrier</td>
<td>35 kDa</td>
<td>0.2205</td>
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<td>499</td>
<td>ras-related protein Rap-1b precursor</td>
<td>21 kDa</td>
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<tr>
<td>500</td>
<td>probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial</td>
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<td>501</td>
<td>PREDICTED: T-cell activation inhibitor, mitochondrial</td>
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<td>502</td>
<td>ras-related protein Rap-1b-like</td>
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<td>60S ribosomal protein L7</td>
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<td>PREDICTED: coiled-coil domain containing 127a isoform X1</td>
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<td>NADH dehydrogenase</td>
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<td>0.2131</td>
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<td>60S ribosomal protein L30</td>
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<td>508</td>
<td>S-adenosylmethionine mitochondrial carrier protein</td>
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<td>0.2095</td>
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<td>509</td>
<td>protoporphyrinogen oxidase</td>
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<td>0.2089</td>
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<tr>
<td>510</td>
<td>ADP-ribosylation factor-like 3, like 1</td>
<td>22 kDa</td>
<td>0.2078</td>
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<td>511</td>
<td>PREDICTED: mitochondrial calcium uniporter regulator 1</td>
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<td>512</td>
<td>PREDICTED: choline dehydrogenase, mitochondrial</td>
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<td>513</td>
<td>recoverin-like</td>
<td>22 kDa</td>
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<tr>
<td>514</td>
<td>28S ribosomal protein S23, mitochondrial</td>
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<td>515</td>
<td>28S ribosomal protein S5, mitochondrial precursor</td>
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<td>0.2054</td>
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<td>516</td>
<td>calcium-binding mitochondrial carrier protein ScAMC-2-A</td>
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<tr>
<td>517</td>
<td>PREDICTED: uncharacterized aarF domain-containing protein kinase 2</td>
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<td>518</td>
<td>PREDICTED: cytochrome c oxidase subunit 5B, mitochondrial-like</td>
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<td>0.2044</td>
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<td>Protein Length</td>
<td>KDa</td>
<td>Score</td>
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<tr>
<td>Predicted: transmembrane protein 70, mitochondrial</td>
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<td>Predicted: 60S ribosomal protein L6</td>
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<td>synaptosomal-associated protein 25-B</td>
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<td>Predicted: transmembrane emp24 domain-containing protein 2</td>
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<td>opsin-1, short-wave-sensitive 2</td>
<td>39</td>
<td>0.1924</td>
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<td>elongation factor 1-alpha</td>
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<td>HIG1 domain family member 2A, mitochondrial</td>
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<td>39S ribosomal protein L14, mitochondrial precursor</td>
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<tr>
<td>guanine nucleotide-binding protein G(o) subunit alpha</td>
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<td>Predicted: multiple PDZ domain protein isoform X3</td>
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<td>Predicted: peptidyl-prolyl cis–trans isomerase FKBP8 isoform X1</td>
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<td>Predicted: ADP-ribosylation factor 2</td>
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<td>mitochondrial folate transporter/carrier</td>
<td>33</td>
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<td>Predicted: 60S ribosomal domain protein 10 precursor</td>
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<tr>
<td>Predicted: protein CCSMST1</td>
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<td>60S ribosomal protein L10a</td>
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<tr>
<td>UMP—CMP kinase</td>
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<td>0.1769</td>
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<td>Predicted: probable palmitoyltransferase ZDHHC14 isoform X1</td>
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<tr>
<td>Predicted: protein CCSMST1</td>
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<tr>
<td>ATP-dependent Clp protease ATP—binding subunit clpX-like, mitochondrial</td>
<td>68</td>
<td>0.175</td>
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<tr>
<td>Enoyl-CoA delta isomerase 2, mitochondrial</td>
<td>42</td>
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<tr>
<td>Predicted: carnitine palmitoyltransferase 1A isoform X2</td>
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<td>Predicted: AFG3-like protein 1</td>
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<td>Succinyl-CoA ligase</td>
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<td>polymerase delta-interacting protein 2</td>
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<td>60S acidic ribosomal protein P0</td>
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<td>ATP synthase subunit delta, mitochondrial</td>
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<td>Solute carrier family 25 member 33</td>
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<td>mitochondrial dynamics protein MID49</td>
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<td>Predicted: mitochondrial fission 1 protein</td>
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<tr>
<td>Probable saccharopine dehydrogenase</td>
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<tr>
<td>Probable D-lactate dehydrogenase, mitochondrial</td>
<td>53</td>
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<td>Probable asparagine—tRNA ligase, mitochondrial</td>
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<td>Predicted: 40S ribosomal protein S24</td>
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<td>28S ribosomal protein S14, mitochondrial</td>
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<tr>
<td>Flavin containing monoxygenase 5</td>
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<td>Microsomal glutathione S-transferase 1.1</td>
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<td>Mitochondrial peptide methionine sulfoxide reductase</td>
<td>27</td>
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<td>Predicted: l-isoaspartyl protein carboxyl methyltransferase, like isoform X2</td>
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<td>Protein SCO2 homolog, mitochondrial</td>
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<td>DnaJ (Hsp40) homolog, subfamily A, member 3B</td>
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<td>Dihydroorotate dehydrogenase (quinone), mitochondrial</td>
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<td>Enzyme/Molecule</td>
<td>Molec. Wt.</td>
<td>Confidence Score</td>
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<td>39S ribosomal protein L20, mitochondrial</td>
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<td>PREDICTED: biotin—protein ligase isoform X1</td>
<td>93 kDa</td>
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<td>cytosol aminopeptidase</td>
<td>37 kDa</td>
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<td>PREDICTED: 60S ribosomal protein L27-like</td>
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<td>succinate dehydrogenase</td>
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<td>PREDICTED: dnaJ homolog subfamily C member 30</td>
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<td>aminomethyltransferase, mitochondrial</td>
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<td>40S ribosomal protein S16</td>
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<tr>
<td>solute carrier family 3 (amino acid transporter heavy chain), member 2b</td>
<td>57 kDa</td>
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<tr>
<td>PREDICTED: uncharacterized protein At5g50100, mitochondrial-like isoform X1</td>
<td>19 kDa</td>
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<td>ras-related protein Rab-3A</td>
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<td>uncharacterized protein LOC794398</td>
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<td>phosphatidylinositide phosphatase SAC1-B</td>
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<td>PREDICTED: 40S ribosomal protein S3-like isoform X1</td>
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<td>mitoferrin-1</td>
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<td>dehydrogenase/reductase SDR family member 4</td>
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<td>dolichyl-diphosphooligosaccharide—protein glycosyltransferase subunit 2 precursor</td>
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<td>ras-related protein Rab-8B</td>
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<td>ATPase family AAA domain-containing protein 1–B</td>
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<td>peripherin-2</td>
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<td>complement component 1 Q subcomponent–binding protein, mitochondrial</td>
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<td>mitochondrial ubiquitin ligase activator of NFkB 1</td>
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<td>PREDICTED: NADH dehydrogenase</td>
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<td>signal peptidase complex subunit 3</td>
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<td>actin–like protein 6A</td>
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<td>PREDICTED: 60S ribosomal protein L11 isoform X2</td>
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<tr>
<td>2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial</td>
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<tr>
<td>3-hydroxyisobutyryl-CoA hydrolase, mitochondrial</td>
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<td>PREDICTED: protein TsetseEP–like</td>
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<td>ADP–ribosylation factor–like protein 2</td>
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<tr>
<td>oxoglutarate (alpha–ketoglutarate) dehydrogenase (lipoamide)</td>
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<td>39S ribosomal protein L12, mitochondrial</td>
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<td>uncharacterized protein LOC393228</td>
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<td>PREDICTED: ras–related G3 botulinum toxin substrate 1</td>
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<td>dnaJ homolog subfamily A member 3, mitochondrial</td>
<td>44 kDa</td>
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<td>peroxyredoxin–2</td>
<td>22 kDa</td>
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<td>Bcl-2/adenovirus E1B 19kD interaction protein XR</td>
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<td>PREDICTED: uncharacterized aarF domain–containing protein kinase 1 isoform X1</td>
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<td>60S ribosomal protein L9</td>
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<td>abhydrolase domain–containing protein 4</td>
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<tr>
<td>RPE–retinal G protein–coupled receptor</td>
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<td>trifunctional enzyme subunit alpha, mitochondrial</td>
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<td>elongation factor Ts, mitochondrial</td>
<td>34 kDa</td>
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<td>transmembrane protein 14A</td>
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<td>protein–S–isoprenylcysteine O–methyltransferase</td>
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<td>PREDICTED: aarF domain–containing protein kinase 4</td>
<td>11 kDa</td>
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<td>28S ribosomal protein S27, mitochondrial</td>
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<tr>
<td>40S ribosomal protein SA</td>
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<tr>
<td>succinyl–CoA:3–ketoacid coenzyme A transferase 1, mitochondrial</td>
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<td>Description</td>
<td>MW (kDa)</td>
<td>p-value</td>
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<td>637</td>
<td>PREDICTED: 2-oxoglutarate dehydrogenase, mitochondrial</td>
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<td>638</td>
<td>mitochondrial-processing peptidase subunit alpha</td>
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<td>0.1223</td>
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<td>639</td>
<td>PREDICTED: cytochrome b–c1 complex subunit 6, mitochondrial-like</td>
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<td>640</td>
<td>PREDICTED: potassium voltage–gated channel subfamily B member 2</td>
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<td>641</td>
<td>PREDICTED: glycerol-3-phosphate acyltransferase 1, mitochondrial</td>
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<td>642</td>
<td>electron transfer flavoprotein–ubiquinone oxidoreductase, mitochondrial</td>
<td>23</td>
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<td>PREDICTED: mitochondrial thiamine pyrophosphate carrier isoform X1</td>
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<td>flotillin 2</td>
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<td>ras-related protein Rab-18-B</td>
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<td>flotillin-2a</td>
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<td>surfeit gene 4, like</td>
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<td>ras-related protein Rab-5B</td>
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<td>653</td>
<td>long-chain fatty acid transport protein 4</td>
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<tr>
<td>654</td>
<td>lysine–tRNA ligase</td>
<td>60</td>
<td>0.1156</td>
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<td>655</td>
<td>L-2-hydroxyglutarate dehydrogenase, mitochondrial</td>
<td>12</td>
<td>0.1144</td>
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<td>656</td>
<td>coiled-coil domain containing 56-like</td>
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<td>PREDICTED: mitochondrial coenzyme A transporter SLC25A42 isoform X1</td>
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<td>PREDICTED: 3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial-like isoform X2</td>
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<td>660</td>
<td>PREDICTED: regulator of G-protein signaling 9 isoform X2</td>
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<td>661</td>
<td>PREDICTED: multiple PDZ domain protein isoform X1</td>
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<td>PREDICTED: sialic acid–binding Ig-like lectin 6</td>
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<td>protein FAM173A</td>
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<td>adenylyl cyclase–associated protein 1</td>
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<td>PREDICTED: peptidyl–prolyl cis–trans isomerase FKBP8 isoform X1</td>
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<td>PREDICTED: ammonium transporter Rh type A isoform X1</td>
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<td>ubiquitin–60S ribosomal protein L4</td>
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<td>0.1109</td>
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<td>669</td>
<td>PREDICTED: SRA stem–loop–interacting RNA–binding protein, mitochondrial</td>
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<td>PREDICTED: uncharacterized protein NCBP2–AS2–like</td>
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<td>671</td>
<td>superoxide dismutase</td>
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<td>672</td>
<td>guanulate kinase 1b</td>
<td>25</td>
<td>0.1094</td>
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<tr>
<td>673</td>
<td>GTP:AMP phosphotransferase AK3, mitochondrial</td>
<td>25</td>
<td>0.1089</td>
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<tr>
<td>674</td>
<td>PREDICTED: ER membrane protein complex subunit 6</td>
<td>12</td>
<td>0.1087</td>
</tr>
<tr>
<td>675</td>
<td>PREDICTED: 39S ribosomal protein L53, mitochondrial–like</td>
<td>12</td>
<td>0.1087</td>
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<td>676</td>
<td>PREDICTED: glutaminase kidney isoform, mitochondrial isoform X2</td>
<td>38</td>
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<td>677</td>
<td>serine/threonine–protein kinase DCLK2 isoform 1</td>
<td>90</td>
<td>0.1084</td>
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<td>678</td>
<td>cytochrome c oxidase assembly factor 3 homolog, mitochondrial</td>
<td>12</td>
<td>0.1077</td>
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<td>679</td>
<td>dolichyl–diphosphooligosaccharide–protein glycosyltransferase 48 kDa subunit precursor</td>
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<td>680</td>
<td>28S ribosomal protein S34, mitochondrial</td>
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<td>0.1073</td>
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<td>681</td>
<td>apolipoprotein O</td>
<td>25</td>
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<tr>
<td>682</td>
<td>propionyl–CoA carboxylase alpha chain, mitochondrial</td>
<td>79</td>
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<tr>
<td>683</td>
<td>PREDICTED: 40S ribosomal protein S2</td>
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<td>0.1056</td>
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<td>684</td>
<td>PREDICTED: cytochrome c oxidase subunit 7A–related protein, mitochondrial–like</td>
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<td>60S ribosomal protein L35a</td>
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<td>686</td>
<td>NADH dehydrogenase (ubiquinone) iron–sulfur protein 5</td>
<td>13</td>
<td>0.1046</td>
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<tr>
<td>687</td>
<td>28S ribosomal protein S33, mitochondrial</td>
<td>13</td>
<td>0.1046</td>
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<td>688</td>
<td>solute carrier family 2, facilitated glucose transporter member 1</td>
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<td>PREDICTED: fatty aldehyde dehydrogenase isoform X2</td>
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<tr>
<td>690</td>
<td>oxoglutarate (alpha–ketoglutarate) dehydrogenase (lipoamide)</td>
<td>26</td>
<td>0.1038</td>
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<tr>
<td>691</td>
<td>PREDICTED: LOW QUALITY PROTEIN: lysosome membrane protein 2</td>
<td>26</td>
<td>0.1038</td>
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<td>692</td>
<td>pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial</td>
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<td>693</td>
<td>uncharacterized aarF domain–containing protein kinase 1</td>
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<td>694</td>
<td>PREDICTED: LOW QUALITY PROTEIN: lysosome membrane protein 2</td>
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<td>Protein Name</td>
<td>M.W.</td>
<td>Description</td>
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<tr>
<td>diablo, IAP-binding mitochondrial protein a</td>
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<td>bcl-2-like protein 1</td>
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<td>PREDICTED: oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoyl) isomeric form X3</td>
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<td>PREDICTED: peroxisomal biogenesis factor 3</td>
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<tr>
<td>BRI3-binding protein precursor</td>
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<td>coenzyme Q-binding protein COQ10 homolog, mitochondrial</td>
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<td>39S ribosomal protein L17, mitochondrial</td>
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<td>flotillin-1</td>
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<td>PREDICTED: sodium/potassium/calcium exchanger 2-like isoform X2</td>
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<td>PREDICTED: fatty aldehyde dehydrogenase-like</td>
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<td>PREDICTED: aarF domain-containing protein kinase 4</td>
<td>70 kDa</td>
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<td>PREDICTED: transmembrane protein 186 isoform X1</td>
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<td>cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha'</td>
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<td>phosphofructokinase, muscle b</td>
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<td>PREDICTED: prostate stem cell antigen-like</td>
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<td>PREDICTED: small integral membrane protein 8 isoform X2</td>
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<td>UPF0545 protein C22orf39 homolog</td>
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<td>PREDICTED: sideroflexin-2 isoform X3</td>
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<td>PREDICTED: renin receptor isoform X1</td>
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<td>PREDICTED: cytochrome c oxidase subunit 5B, mitochondrial-like</td>
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<td>transmembrane protein 33</td>
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<td>heat shock cognate 71 kDa protein</td>
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<td>tyrosine--tRNA ligase, mitochondrial</td>
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<td>acyl-coenzyme A thiosterase THEM4</td>
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<td>ATP-dependent Clp protease proteolytic subunit, mitochondrial</td>
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<td>peroxisomal biogenesis factor 3</td>
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<td>28S ribosomal protein S2, mitochondrial</td>
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<td>PREDICTED: anoctamin-8</td>
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<td>PREDICTED: 40S ribosomal protein S26-like</td>
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<td>protein kinase, cAMP-dependent, regulatory, type II, alpha A</td>
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<td>glutamate dehydrogenase 1b</td>
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<td>methylmalonic aciduria type A protein, mitochondrial</td>
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<td>1-acyl-sn-glycerol-3-phosphate acyltransferase gamma</td>
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<td>alpha-1,3/1,6-mannosyltransferase ALG2</td>
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<td>PREDICTED: ATP synthase-coupling factor 6, mitochondrial isomeric form X1</td>
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<td>F-box/LRR-repeat protein 2</td>
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<td>membrane magnesium transporter 1 precursor</td>
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<td>cytochrome P450, family 27, subfamily C, polypeptide 1</td>
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<td>PREDICTED: histidine triad nucleotide-binding protein 2, mitochondrial-like isoform X1</td>
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<td>PREDICTED: serine/threonine-protein phosphatase PGAM5, mitochondrial isoform X1</td>
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<tr>
<td>PREDICTED: carnitine O-palmitoyltransferase 1, liver isoform isoform X2</td>
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<tr>
<td>protein phosphatase PTC7 homolog</td>
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<tr>
<td>3-hydroxyisobutyrate dehydrogenase, mitochondrial</td>
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<td>60S ribosomal protein L28</td>
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<tr>
<td>40S ribosomal protein S3a</td>
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<tr>
<td>protein phosphatase PTC7 homolog</td>
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<td>3-hydroxyisobutyrate dehydrogenase, mitochondrial</td>
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<tr>
<td>40S ribosomal protein S3a</td>
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<td>protein phosphatase PTC7 homolog</td>
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<td>3-hydroxyisobutyrate dehydrogenase, mitochondrial</td>
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<td>keratin, type II cytoskeletal 8</td>
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<td>reticulon-1 isoform 1</td>
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<td>synembryn-A</td>
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<td>62 kDa</td>
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<tr>
<td>PREICTED: coenzyme Q-binding protein COQ10 homolog A, mitochondrial</td>
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<td>minor histocompatibility antigen H13</td>
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<td>ADP-ribosylation factor-like protein 1</td>
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<td>PREICTED: synembryn-A isoform X1</td>
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<td>63 kDa</td>
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<td>L-threonine 3-dehydrogenase, mitochondrial</td>
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<td>leucine-rich repeat, immunoglobulin-like and transmembrane domains 1 precursor</td>
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<td>valacyclovir hydrolase</td>
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<td>PREICTED: transmembrane and coiled-coil domains protein 1-like</td>
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<td>ADP-ribosylation factor-like 7</td>
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<td>PREICTED: BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 isoform X1</td>
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<td>lactation elevated protein 1 homolog B</td>
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<td>immunoglobulin superfamily member 8 precursor</td>
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<td>PREICTED: tyrosine-protein phosphatase non-receptor type 1</td>
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<tr>
<td>GTP-binding protein SAR1b</td>
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<td>28S ribosomal protein S29, mitochondrial</td>
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<td>PREICTED: 60S ribosomal protein L18a-like</td>
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<td>bcl2-associated X protein, b</td>
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<td>transmembrane protein 160</td>
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<td>ras-related protein Rab-24</td>
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<td>NADPH-cytochrome P450 reductase isoform X1</td>
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<td>acylpyruvase FAH1, mitochondrial</td>
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<td>0.0536</td>
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<tr>
<td>keratin, type I cytoskeletal 18</td>
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<td>PREICTED: neural cell adhesion molecule 1 isoform X1</td>
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<td>PREICTED: protein TBRG4</td>
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<td>putative hexokinase HKDC1</td>
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<td>PREDICTED: phospholipid scramblase 2</td>
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<td>PREDICTED: succinate–semialdehyde dehydrogenase, mitochondrial isoform X1</td>
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<td>PREDICTED: tudor and KH domain–containing protein isoform X1</td>
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<td>4–aminobutyrate aminotransferase, mitochondrial</td>
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<td>PREDICTED: reticulon–1 isoform X1</td>
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<td>HCLS1–associated protein X–1</td>
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<td>apoptogenic protein 1, mitochondrial</td>
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<td>melanoregulin</td>
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<td>acyl–CoA thioesterase 9, tandem duplicate 1</td>
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<td>PREDICTED: Golgi SNAP receptor complex member 1 isoform X1</td>
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<td>39S ribosomal protein L9, mitochondrial</td>
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<td>phosducin 2</td>
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<td>internexin neuronal intermediate filament protein, alpha</td>
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<td>probable tRNA N6–adenosine threonylcarbamoyltransferase, mitochondrial</td>
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<td>39S ribosomal protein L10, mitochondrial precursor</td>
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<td>ES1 protein homolog, mitochondrial</td>
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<td>PREDICTED: E3 ubiquitin–protein ligase RNF170–like</td>
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<td>PREDICTED: calcium signal–modulating cyclophilin ligand isoform X1</td>
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<td>PREDICTED: ethylmalonyl–CoA decarboxylase isoform X1</td>
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<td>PREDICTED: trimethyllysine dioxygenase, mitochondrial</td>
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<td>PREDICTED: proline–rich protein 18–like</td>
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<td>endoplasmic reticulum precursor</td>
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<td>casein kinase 2, alpha 1 polypeptide</td>
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<td>glycosaminoglycan xylosylkinase</td>
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<tr>
<td>tapasin-like</td>
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<td>PREDICTED: ubiquitin associated protein 2b isomorph X1</td>
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<td>26S proteasome non--ATPase regulatory subunit 3</td>
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<td>PREDICTED: poly(A) RNA polymerase, mitochondrial</td>
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<tr>
<td>uncharacterized protein C6orf136 homolog</td>
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<td>sarcolemma associated protein b</td>
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<td>uncharacterized protein LOC405617</td>
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<td>PREDICTED: uncharacterized protein LOC557028 isomorph X2</td>
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<td>PREDICTED: ATP--binding cassette sub--family B member 6, mitochondrial isomorph X1</td>
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<td>PREDICTED: mannosyl--oligosaccharide glucosidase isomorph X1</td>
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<td>39S ribosomal protein L37, mitochondrial</td>
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<td>TLD--domain-containing protein 1</td>
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<td>PREDICTED: uncharacterized aaf--domain--containing protein kinase 2</td>
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<td>PREDICTED: drebrin isomorph X2</td>
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<td>PREDICTED: uncharacterized protein sich211--11k18.4</td>
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<td>elongation factor 1--gamma</td>
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<td>UBX--domain--containing protein 4</td>
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<td>protein disulfide--isomerase TMX3 precursor</td>
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<tr>
<td>N--acylneuraminate cytidylyltransferase</td>
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<td>F--box/LRR--repeat protein 4</td>
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<td>PREDICTED: protein LYRIC isomorph X1</td>
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<td>protein crumbs homolog 2</td>
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<td>0.0229</td>
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<tr>
<td>phosphatidate cytidylyltransferase 2</td>
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<td>coiled--coil domain--containing protein 47 precursor</td>
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<td>aldehyde dehydrogenase 2 family (mitochondrial), tandem duplicate 2</td>
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<tr>
<td>pyruvate dehydrogenase phosphatase catalytic subunit 1</td>
<td>57 kDa</td>
<td>0.0218</td>
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<td>PREDICTED: lysophosphatidylcholine acyltransferase 1--like</td>
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<td>amine oxidase</td>
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<td>prenylcoenzyme oxidase 1 precursor</td>
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<td>PREDICTED: pyruvate kinase PKM isomorph X1</td>
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<td>retinoid isomerohydrolase</td>
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<td>PREDICTED: GPI transamidase component PI--S isomorph X2</td>
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<td>T--complex protein 1 subunit gamma</td>
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<td>PREDICTED: serine beta--lactamase--like protein LACTB, mitochondrial isomorph X1</td>
<td>64 kDa</td>
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<tr>
<td>transmembrane anterior posterior transformation protein 1 homolog</td>
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<tr>
<td>protein FAM73B</td>
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<td>PREDICTED: long--chain fatty acid transport protein 6--isomorph X1</td>
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<td>PREDICTED: proline dehydrogenase 1, mitochondrial--like</td>
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<td>polyadenylate--binding protein 1</td>
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<td>membrane protein, palmitoylated 5b (MAGUK p55 subfamily member 5)</td>
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<td>PREDICTED: gamma--glutamyltransferase 7 isomorph X1</td>
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<td>PREDICTED: TBC1 domain family member 17 isomorph X1</td>
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<td>PREDICTED: caseinolytic peptidase B protein homolog</td>
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<td>lipase maturation factor 2</td>
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<td>transferrin receptor protein 1</td>
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<td>PREDICTED: probable threonine—tRNA ligase 2, cytoplasmic-like</td>
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<td>PREDICTED: reticulon-4 isoform X1</td>
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<td>protein crumbs homolog 2</td>
<td>162 kDa</td>
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<td>PREDICTED: cyclic nucleotide-gated channel rod photoreceptor subunit alpha-like</td>
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<td>PREDICTED: amyloid beta A4 protein-like isoform X2</td>
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<td>importin subunit beta-1</td>
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<td>PREDICTED: dynamin 1a isoform X5</td>
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<td>PREDICTED: bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 1</td>
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<td>alpha-aminoadipic semialdehyde synthase, mitochondrial</td>
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<td>PREDICTED: sarcoplasmic/endoplasmic reticulum calcium ATPase 2</td>
<td>116 kDa</td>
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<tr>
<td>von Willebrand factor A domain-containing protein 8</td>
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<td>PREDICTED: pyruvate carboxylase, mitochondrial-like</td>
<td>130 kDa</td>
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<td>insulin-like growth factor 1b receptor precursor</td>
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<td>PREDICTED: crumbs family member 2b isoform X1</td>
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<td>PREDICTED: rootletin isoform X1</td>
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<tr>
<td>PREDICTED: midasin</td>
<td>512 kDa</td>
<td>0.0024</td>
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Achievements

<投稿論文>
"Purification of cone outer segment for proteomic analysis on its membrane proteins in carp retina"
Takashi Fukagawa, Kazuaki Takafuji, Shuji Tachibanaki and Satoru Kawamura

"Substrate Specificity and Subcellular Localization of Aldehyde-Alcohol Redox (AL-OL)-Coupling Reaction in Carp Cones"
Shinya Sato, Takashi Fukagawa, Shuji Tachibanaki, Yumiko Yamano, Akimori Wada and Satoru Kawamura

＜学会発表＞
ポスター発表
「Substrate Specificity and Localization of AL-OL Coupling Reaction in Carp Cones」
Satoru Kawamura, Shinya Sato, Shuji Tachibanaki and Takashi Fukagawa
The Association for Research in Vision and Ophthalmology 2013 Annual Meeting
2013 年 5 月 6 日

口頭発表
「桿体・錐体視細胞の外節膜に発現している蛋白質の比較」
深川 貴志、橘木 修志、河村 悟
2014 年 日本動物学会近畿支部研究発表会、2014 年 5 月 10 日

口頭発表
「桿体・錐体視細胞外節に発現している蛋白質の比較解析の試み (Proteomic analysis of proteins expressed in the outer segment in carp rods and cones)」
深川 貴志、橘木 修志、河村 悟
第 86 回大会日本動物学会 新潟大会、2015 年 9 月 19 日