

Borner et al., <http://www.jcb.org/cgi/content/full/jcb.200607164/DC1>

## Supplemental materials and methods

### CCV preparations

CCV-enriched fractions were prepared essentially as described by Hirst et al. (2004). In brief, four to six confluent dishes (500 cm<sup>2</sup>) of HeLa cells were scraped into buffer A (0.1 M MES, pH 6.5, 0.2 mM EGTA, 0.5 mM MgCl<sub>2</sub>, and 0.02% NaN<sub>3</sub>) supplemented with 0.2 mM AEBSF. Cells were homogenized with a motorized Dounce homogenizer (20 strokes), and centrifuged at 4,147 *g* for 32 min. Supernatants were treated with ribonuclease A at 50 µg/ml for 60 min. Membranes were pelleted by centrifugation at 135,750 *g* for 30–45 min. Membranes were resuspended in buffer A and mixed with an equal volume of 12.5% Ficoll/12.5% sucrose in buffer A. Samples were spun at 21,720 *g* for 34 min to pellet most of the contaminating membranes. Supernatants were diluted with four volumes of buffer A, and CCV-enriched fractions were pelleted by centrifugation at 135,750 *g* for 30–45 min. All preparations were performed at 4°C. Mock CCV fractions were prepared likewise, but from 8 to 12 confluent dishes (15 cm) of HeLa cells that had been treated with siRNA against clathrin heavy chain.

### 1D and 2D gels

SDS-PAGE and Western blots were performed according to standard protocols. The antibodies used in this study are listed in Table S3. For analysis by 2D gel electrophoresis, CCV-enriched fractions were resuspended in 2.5% SDS/50 mM Tris, pH 8.0, heated to 65°C for 2 min, and spun at 16,000 *g* for 2 min. Proteins were precipitated from supernatants with 5 volumes of acetone at –20°C for a minimum of 16 h and resuspended in AUT sample buffer (10 mM Tris-HCl, pH 8.5, 7 M urea, 2 M thiourea, 2% ASB14, and 0.5% Triton X-100) at room temperature. Samples were labeled with CyDyes Cy3 and Cy5 as described by Borner et al. (2003). All labeling reactions were performed in reciprocal duplicate. Fluorescent 2D DIGE was performed as in Borner et al. (2003), based on the method first published by Unlu et al. (1997). Nonlinear IPG-strips (pH 3–10) were used for the first dimension and 10% polyacrylamide gels for the second dimension.

### Mass spectrometry

#### Proteins excised from 1D gels

Identification of proteins was achieved by the standard techniques of in-gel trypsin digestion and peptide mass fingerprinting and fragmentation. Gel bands were excised and digested with trypsin according to the method of Shevchenko et al. (1996). Samples of tryptic peptide mixtures were mixed with α-cyano-4-hydroxy-transcinnamic acid matrix and analyzed with a MicroMass ToF-Spec2E or an ABI 4700 proteomics analyzer with TOFTOF optics. For peptide mass fingerprints, mass calibration was performed with features internal to the spectrum, specifically the matrix-related ion peak at 1,060.048 D and the trypsin autolysis peaks at 2,163.057 and 2,273.160 D. For peptide fragment spectra, an “external” mass calibration was generated from the 2,163.057-D trypsin autolysis peak, using the y10, y14, and y16 fragment ions. Spectra were interpreted by the Mascot sequence database search engine configured with a mass tolerance of 40 ppm and the gel-derived variable modifications “propionamide cysteine” and “methionine sulfoxide” against the National Center for Biotechnology Information (NCBI) database. Probability-based MASCOT scores were used to evaluate identifications. Only matches with *P* < 0.05 for random occurrence were considered significant (further explanation of Mascot scores can be found at <http://www.matrixscience.com>).

#### Proteins excised from 2D gels

Proteins within the gel pieces were first reduced, carboxyamidomethylated, and digested to peptides using trypsin on a MassPrepStation (Waters). The resulting peptides were applied to a liquid-chromatography tandem mass spectrometer. The reverse-phase liquid chromatographic separation of peptides was achieved with a PepMap C18 reverse phase, 75 mm i.d., 15-cm column (LC Packings) on a capillary LC system (Waters) attached to a QToF2 (Waters) mass spectrometer or the same column attached to a Dionex Dual Gradient LC system attached to a QSTAR XL (Applied Biosystems). The MS/MS fragmentation data achieved was used to search the NCBI database using the MASCOT search engine. Probability-based MASCOT scores were used to evaluate identifications. Only matches with *P* < 0.05 for random occurrence were considered significant.

### iTRAQ analysis

#### Sample preparation, labeling, and fractionation

For analysis by iTRAQ (Ross et al., 2004), control and mock CCV preps were resuspended in 2.5% SDS/50 mM Tris, pH 8.0, heated to 65°C for 2 min and spun at 16,000 *g* for 2 min. Proteins were precipitated from supernatants with 5 volumes of acetone at –20°C for a minimum of 16 h. Precipitates were solubilized in ~100 µl labeling buffer (25 mM triethylammonium bicarbonate [TEAB], pH 8.5, 8 M Urea, 2% Triton X-100, and 0.1% SDS). Protein concentrations were determined using the BCA Protein Assay kit (Pierce Chemical Co.). Samples were adjusted to the same protein concentration with labeling buffer and split into two equal aliquots to ob-

tain two pairs of mock and control CCVs (technical replicates). Samples (~70 µg of protein) were reduced (4 mM Tris[2-carboxyethyl]phosphine, 20°C, 1 h) and cysteines blocked (8 mM methyl methanethiosulfonate, 20°C, 10 min). Samples were diluted with 50 mM TEAB, pH 8.5, such that the urea concentration was <1 M, digested with trypsin overnight at 37°C (5 µg; Promega), and lyophilized. Samples were resuspended in 100 µl 0.25 M TEAB, pH 8.5, 75% ethanol, added to one unit of the corresponding iTRAQ reagent and incubated for 1 h at 20°C. A four-plex iTRAQ labeling (Applied Biosystems) was performed as follows: mock CCVs technical repeat 1 labeled with iTRAQ reagent 114; mock CCVs technical repeat 2 with 115; control CCVs technical repeat 1 with 116; control CCVs technical repeat 2 with 117. Residual reagents were hydrolyzed with 100 µl water (20°C, 15 min). The labeled peptides were then pooled, lyophilized, and resuspended in 3 ml 20% vol/vol acetonitrile, 10 mM KH<sub>2</sub>PO<sub>4</sub>/H<sub>3</sub>PO<sub>4</sub>, pH 2.7. Peptides were fractionation by cation-exchange chromatography on a BioLC HPLC system (Dionex) using a PolySulfoethyl A column (PolyLC; 2.1 × 200 mm, 5 µm, 300 Å). Samples were loaded onto the column and washed for 60 min at 200 µl/min. Peptides were eluted using a 70-min linear gradient of 30–125 mM KCl (20% vol/vol acetonitrile and 10 mM KH<sub>2</sub>PO<sub>4</sub>/H<sub>3</sub>PO<sub>4</sub>, pH 2.7) at 200 µl/min. Fractions were collected at 2-min intervals, lyophilized, and resuspended in 140 µl 2% acetonitrile and 0.1% trifluoroacetic acid.

Samples were analyzed by LC-MS/MS. The reverse-phase liquid chromatographic separation of peptides was achieved with a PepMap C18 reverse phase, 75 mm i.d., 15-cm column (LC Packings) on a capillary LC system (Waters) attached to a Dionex Dual Gradient LC system attached to a QSTAR XL (Applied Biosystems). The obtained MS/MS fragmentation data were used to search the NCBI database using the MASCOT search engine. Probability-based MASCOT scores were used to evaluate identifications. Only matches with  $P < 0.05$  for random occurrence were considered significant (further explanation of MASCOT scores can be found at <http://www.matrixscience.com>).

#### **Generation of a nonredundant protein database**

An important feature of the iTRACKER software (Shadforth et al., 2005) used for the quantitation of iTRAQ-labeled peptides is that it only processes peptides that are unique in the protein database. This ensures that the peptides are assigned unambiguously to parent proteins and that the observed quantitation refers to only one protein. This rigorous approach absolutely requires a nonredundant protein database, as multiple entries for the same protein (partial sequences, splice variants, etc.) will lead to the exclusion of the corresponding peptides. However, all currently available public databases are partially redundant; trial analysis of our iTRAQ data with the UniProt and Ensembl databases was unsatisfactory, as many identified proteins were not quantified (unpublished data). We therefore created our own protein database specifically for the iTRAQ analysis.

Proteins that had been identified in the iTRAQ-labeled samples by LC-MS/MS and the MASCOT search engine were compiled into a nonredundant list. Only proteins that fulfilled the following requirements were included: (1) a MASCOT score >36 (i.e.,  $P < 0.05$  for random match) and (2) at least one unique peptide with a MASCOT score  $\geq 20$ . Protein sequences were downloaded from the NCBI protein database (<http://www.ncbi.nlm.nih.gov>). In cases of redundant entries for the same protein, a representative sequence was chosen. With these criteria, a list of 903 proteins in FastA format was assembled. This list was called CP database (CCV Protein database) and used for iTRAQ quantitation.

#### **MS data analysis and protein quantitation**

MS data files were processed using the wiff2DTA software, which converts QSTAR data files (.wiff) into text files that contain  $m/z$  and intensity information (peak lists) for each product ion spectra (Boehm et al., 2004). Each MS data file was processed twice in order to generate centroided peak lists, which contain a single  $m/z$  value for each ion corresponding to the center of the peak, and uncentroided peak lists, which contain  $m/z$  information for the entire peak. Mascot version 2.0.01 (Matrix Science), was used to search centroided peak lists against the CP database. The following modifications were used: fixed, iTRAQ (K), iTRAQ (N-term), MMTS (C), variable, oxidation (M), and iTRAQ (Y). The MS tolerance was 0.2 D and the MSMS tolerance 0.5 D. The Mascot search results comprise a list of peptide identifications, each of which has a score that indicates the quality of the result. To determine the minimum peptide Mascot score for accurate protein identification, each peak list was searched against a version of the CP database in which the protein sequences were reversed. The identifications that resulted from the reversed database search were used to calculate the false protein identification rate: false protein identification rate = number of proteins identified from the reversed search/number of proteins identified from the normal search  $\times 100$ . Mascot peptide scores of at least 25 for proteins identified with two or more matched peptides and scores of at least 44 for identifications based on single peptides resulted in false protein identification rates of <1%. Normalized iTRAQ reporter ion ratios were calculated from the uncentroided peak lists using the recently developed i-Tracker software (Shadforth et al., 2005). Normalized reporter ion areas were calculated as follows: normalized area  $A = \text{area } A / (\text{area } A + \text{area } B + \text{area } C + \text{area } D)$ . The Genome Annotating Proteome Pipeline (GAPP) system ([www.ccbitt.org/gapp](http://www.ccbitt.org/gapp)) was used to parse peptide identification and scoring information from the Mascot output files and link these to the quantitation data in a relational database (MySQL version 4.0, MySQL AB). Peptides were quantified if at least three of the reporter ion peaks were above a threshold of 10 counts and if they had a Mascot score of at least 20. In addition, only peptides that were unique to a single identified protein were quantified.

Averaged normalized areas were used to calculate the relative abundance of proteins in control and mock CCVs, which was expressed as an average ratio [con/mock], using the following formula:  $([A_{116}/A_{114}] + [A_{116}/A_{115}] + [A_{117}/A_{114}] + [A_{117}/A_{115}])/4$ . ( $A_{116}$  and  $A_{117}$  correspond to control CCVs and  $A_{114}$  and  $A_{115}$  to mock CCVs.) Proteins were ranked according to ratios. Ratios were

normalized by division by 2.5, to obtain a ratio of ~1 for the center point of the distribution (protein no. 261). These normalized ratios were used for the plot (ratio over rank) as shown in Fig. 3.

An inherent difficulty of iTRAQ is the nontrivial data analysis, as well as the considerable processing time required for the mass-spectrometric analysis. Hence, the iTRAQ data discussed in this study was derived from a single pair of control and mock CCV fractions, analyzed in duplicate. However, this particular pair of CCVs was probed by Western blot for >20 different marker proteins, including CCV and non-CCV proteins. All of them showed enrichment or depletion as expected (unpublished data). This as well as the consistency with the DIGE and Western blot data make it very likely that the iTRAQ data presented here are highly representative.

## References

- Advani, R.J., B. Yang, R. Prekeris, K.C. Lee, J. Klumperman, and R.H. Scheller. 1999. VAMP-7 mediates vesicular transport from endosomes to lysosomes. *J. Cell Biol.* 146:765–776.
- Ball, C.L., S.P. Hunt, and M.S. Robinson. 1995. Expression and localization of alpha-adaptin isoforms. *J. Cell Sci.* 108:2865–28675.
- Boehm, A., R. Galvin, and A. Sickmann. 2004. Extractor for ESI quadrupole TOF tandem MS data enabled for high throughput batch processing. *BMC Bioinformatics.* 5:162.
- Hay, J.C., D.S. Chao, C.S. Kuo, and R.H. Scheller. 1997. Protein interactions regulating vesicle transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells. *Cell.* 89:149–158.
- Hirst, J., A. Motley, K. Harasaki, S.Y. Peak Chew, and M.S. Robinson. 2003. EpsinR: an ENTH domain-containing protein that interacts with AP-1. *Mol. Biol. Cell.* 14:625–641. (published erratum appears in *Mol. Biol. Cell.* 2003. 14: 1743)
- Nazarian, R., M. Starcevic, M.J. Spencer, and E.C. Dell'Angelica. 2006. Reinvestigation of the dysbindin subunit of BLOC-1 (biogenesis of lysosome-related organelles complex-1) as a dystrobrevin-binding protein. *Biochem. J.* 395:587–598.
- Page, L.J., and M.S. Robinson. 1995. Targeting signals and subunit interactions in coated vesicle adaptor complexes. *J. Cell Biol.* 131:619–630.
- Seaman, M.N., P.J. Sowerby, and M.S. Robinson. 1996. Cytosolic and membrane-associated proteins involved in the recruitment of AP-1 adaptors onto the trans-Golgi network. *J. Biol. Chem.* 271:25446–25451.
- Shadforth, I.P., T.P. Dunkley, K.S. Lilley, and C. Bessant. 2005. i-Tracker: for quantitative proteomics using iTRAQ. *BMC Genomics.* 6:145.
- Shevchenko, A., M. Wilm, O. Vorm, and M. Mann. 1996. Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. *Anal. Chem.* 68:850–858.
- Steegmaier, M., J. Klumperman, D.L. Foletti, J.S. Yoo, and R.H. Scheller. 1999. Vesicle-associated membrane protein 4 is implicated in trans-Golgi network vesicle trafficking. *Mol. Biol. Cell.* 10:1957–1972.
- Steegmaier, M., V. Oorschot, J. Klumperman, and R.H. Scheller. 2000. Syntaxin 17 is abundant in steroidogenic cells and implicated in smooth endoplasmic reticulum membrane dynamics. *Mol. Biol. Cell.* 11:2719–2731.
- Unlu, M., M.E. Morgan, and J.S. Minden. 1997. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis.* 18: 2071–2077.

Table S1. Complete list of proteins quantified by iTRAQ

Rank	Protein ID	Protein name	Mascot score	Peptides quantified	Control/mock ratio	SD
1	gi 30582397	AP-1 $\sigma$ 1A	96	1	12.24	5.13
2	gi 32451593	Clathrin heavy chain (CHC17)	1826	30	6.27	1.46
3	gi 30582933	DNase II (lysosomal)	63	1	5.01	1.09
4	gi 1363934	Dynamin-2	569	11	4.83	1.31
5	gi 41016993	EpsinR/Enthoprotin	478	10	4.59	1.31
6	gi 17028334	AP-1 $\mu$ 1A	379	8	4.59	1.08
7	gi 56203491	TPD52L1	104	1	4.45	1.24
8	gi 12643391	AP-1 $\gamma$	540	18	4.37	1.05
9	gi 3294548	Cathepsin Z (lysosomal)	102	2	4.10	1.19
10	gi 38570101	Unknown protein (RAB-GAP domain)	45	3	4.05	0.98
11	gi 1335854	Clathrin heavy chain homologue (CHC22)	558	1	3.84	1.09
12	gi 17402231	Clathrin light chain a	231	9	3.81	1.02
13	gi 2143260	PI 3-kinase C2 $\alpha$	822	11	3.62	1.03
14	gi 55662275	Cl-Mannose 6-phosphate receptor	668	14	3.53	0.85
15	gi 70608172	TPD52	160	1	3.52	0.85
16	gi 25090897	CALM	258	5	3.43	0.94
17	gi 21903712	Carboxypeptidase D	406	7	3.41	0.93
18	gi 116505	Clathrin light chain b	171	9	3.39	0.77
19	gi 2226273	TGN46	163	1	3.30	0.75
20	gi 33150596	AP-1 $\sigma$ 1B	49	1	3.20	0.74
21	gi 55663531	Syntaxin 6	121	1	3.15	0.78
22	gi 13477131	Sorting nexin 9	260	6	3.07	0.79
23	gi 56205909	Rab4A	319	4	2.78	0.71
24	gi 62287155	NECAP-1	66	1	2.75	0.86
25	gi 182516	Ferritin light subunit	131	3	2.67	0.57
26	gi 14043007	AP-1 $\beta$ 1	1020	8	2.60	0.73
27	gi 2827434	Sorting nexin 2	403	4	2.58	0.54
28	gi 9716092	Sortilin	125	2	2.53	0.77
29	gi 66932909	<i>Pumilio 1</i>	105	1	2.46	0.74
30	gi 8546849	CD-Mannose 6-phosphate receptor	152	5	2.44	0.63
31	gi 67477390	Inositolpolyphosphate 5-phosphatase OCRL-1	581	8	2.40	0.61
32	gi 2337920	Syntaxin 7	220	3	2.38	0.61
33	gi 47125326	Ferritin heavy chain	84	5	2.37	0.77
34	gi 55958410	<i>Argininosuccinate synthetase</i>	176	5	2.37	0.62
35	gi 8922952	Cappuccino	73	1	2.36	0.79
36	gi 47086495	BLOC-1, subunit 3	96	3	2.35	0.58
37	gi 56205243	Auxilin	150	3	2.33	0.63
38	gi 7920147	<i>N</i> -ethylmaleimide-sensitive factor (NSF)	63	1	2.33	0.64
39	gi 4557469	AP-2 $\beta$ 2	831	7	2.32	0.66
40	gi 57162630	AP-3 $\mu$ 3A	90	1	2.31	0.46
41	gi 15489411	AP-2 $\mu$ 2	121	4	2.31	0.67
42	gi 54695838	Rab5C	311	5	2.29	0.55
43	gi 62751805	D19	88	2	2.29	0.47
44	gi 15214676	Unknown protein (putative Rab interactor)	62	1	2.28	0.50
45	gi 4433649	Syntaxin 8	122	2	2.21	0.52
46	gi 13543973	<i>IMP (inosine monophosphate dehydrogenase) 2</i>	510	15	2.17	0.55
47	gi 9557955	Sorting nexin 5	206	3	2.15	0.61
48	gi 17375734	GAK/auxilin2	288	3	2.12	0.64
49	gi 15214696	Glucosamine (N-acetyl)-6-sulfatase (lysosomal)	162	4	2.11	0.58
50	gi 13431563	Huntingtin interacting protein 1 related (Hip1R)	210	1	2.09	0.81
51	gi 1184699	<i>tyrosyl-tRNA synthetase</i>	47	1	2.06	0.61
52	gi 30582345	Snapin	48	1	2.05	0.53
53	gi 19913414	AP-2 $\alpha$	388	7	2.03	0.46
54	gi 3024589	BLOC-1, subunit 1	138	2	1.98	0.54
55	gi 16307067	Activated RNA polymerase II transcription cofactor 4	100	3	1.96	0.50
56	gi 55249561	Protein kinase Njmu-R1	72	2	1.92	0.55
57	gi 14714586	ADP-Ribosylation Factor 1 (ARF1)	218	3	1.90	0.46
58	gi 32965400	Dysbindin	146	4	1.90	0.44
59	gi 62896680	Sorting nexin 1	126	1	1.88	0.57
60	gi 11125673	TPD52L2	243	2	1.78	0.44
61	gi 18203690	Solute carrier family12 (potassium/chloride transporters), member 4	185	3	1.76	0.52
62	gi 18653904	Vacuolar-type H(+)-ATPase	115	1	1.76	0.41
63	gi 44890366	Coatomer protein complex, subunit $\gamma$ 1	305	4	1.75	0.45
64	gi 30583499	Phosphatidylinositol 4-kinase type II	240	5	1.74	0.58
65	gi 15679932	ETF1 protein	215	3	1.72	0.33
66	gi 15072481	LOH12CR1	78	2	1.70	0.50
67	gi 60593497	Bet3	103	2	1.69	0.42
68	gi 10443244	SH3-domain GRB2-like endophilin B1	195	4	1.68	0.43
69	gi 15741221	Unknown protein	129	3	1.67	0.42
70	gi 4689252	Sorting nexin 6	204	4	1.67	0.45
71	gi 46558847	BLOC-1, subunit 2	78	2	1.66	0.41
72	gi 7706441	VPS29	112	4	1.64	0.40
73	gi 30582953	RuvB-like 1	472	7	1.63	0.43
74	gi 55957331	Rab14	313	5	1.62	0.43
75	gi 3005742	Unknown protein	70	2	1.62	0.38
76	gi 1255188	Dynamin	122	3	1.61	0.41
77	gi 28422140	Rab4B	106	1	1.57	0.44
78	gi 13543574	Vesicle-associated membrane protein 3 (VAMP3; cellubrevin)	173	1	1.57	0.53
79	gi 563886	$\beta$ -Centractin	96	2	1.56	0.48
80	gi 57999523	Unknown protein	341	8	1.53	0.41

81	gi 55960369	Coatomer protein complex, subunit $\alpha$	264	6	1.53	0.35
82	gi 27503838	TRAPPC5 protein	117	2	1.51	0.35
83	gi 15011984	Bystin	92	3	1.48	0.43
84	gi 46854518	Proliferation-associated 2G4	159	4	1.48	0.41
85	gi 51094839	Coatomer protein complex, subunit $\gamma$ 2	94	1	1.48	0.44
86	gi 12653119	Coatomer protein complex, subunit $\beta$ 2	108	5	1.47	0.37
87	gi 542837	H <sup>+</sup> -exporting ATPase (EC3.6.3.6), chainD, vacuolar	185	5	1.47	0.39
88	gi 23512245	AP-3 $\beta$ 3A	438	9	1.47	0.34
89	gi 186629	Keratin 10	123	2	1.47	0.36
90	gi 55959755	OTTHUMP00000017090 (similar to ribosomal protein L29)	57	1	1.47	0.53
91	gi 307121	Low density lipoprotein receptor	99	2	1.47	0.47
92	gi 61212960	Proteasome-associated protein ECM29	1287	26	1.46	0.41
93	gi 55665502	VPS26	180	6	1.46	0.39
94	gi 12654697	Transferrin receptor	953	20	1.46	0.38
95	gi 15530265	Eukaryotic translation elongation factor 1 $\gamma$	198	5	1.45	0.30
96	gi 5326998	Erythrocyte cytosolic protein of 51kDa (ECP-51)	573	13	1.45	0.37
97	gi 3757822	IkappaB kinase complex associated protein (IKAP)	316	11	1.44	0.37
98	gi 62822514	Unknown protein	224	7	1.43	0.38
99	gi 33874734	GSPT1 protein	111	2	1.42	0.42
100	gi 21040277	Diacylglycerol lipase $\beta$	181	5	1.42	0.35
101	gi 40555890	Nin one binding protein	78	3	1.42	0.35
102	gi 55664716	WD repeat domain 11	103	4	1.42	0.40
103	gi 31545	Valyl-tRNA synthetase	281	5	1.42	0.35
104	gi 2996192	Vesicle-associated membrane protein (VAMP 8 or VAMP 5)	91	2	1.39	0.36
105	gi 51474392	Similar to 40S ribosomal protein S16	74	1	1.39	0.32
106	gi 6648106	Ubiquitin-protein ligase E3A	56	1	1.38	0.37
107	gi 337459	Small nuclear ribonucleoprotein particle	75	2	1.37	0.46
108	gi 12005633	NGT	168	5	1.36	0.45
109	gi 55661754	Tripeptidyl peptidase II	1587	62	1.36	0.32
110	gi 57209813	Tubulin, $\beta$ polypeptide	689	1	1.34	0.32
111	gi 307200	Brain glycogen phosphorylase	865	29	1.34	0.31
112	gi 1008856	Ribosomal protein L34	138	4	1.34	0.34
113	gi 17390260	Rho GTPase activating protein 1	80	1	1.33	0.53
114	gi 32880009	AP-3 $\sigma$ 3A	57	1	1.33	0.46
115	gi 9651706	HT014	135	2	1.32	0.31
116	gi 21619196	Trafficking protein particle complex 1	86	3	1.32	0.36
117	gi 34327966	KIAA0625 protein	66	2	1.32	0.29
<b>118</b>	<b>gi 83302113</b>	<b>DnaJ homolog subfamily C member 13 (RME8)</b>	<b>821</b>	<b>12</b>	<b>1.31</b>	<b>0.33</b>
119	gi 21753189	Unknown protein	148	4	1.31	0.38
120	gi 5311171	Csa-19	86	3	1.31	0.38
121	gi 55663551	Rho/vrac guanine nucleotide exchange factor (GEF) 2	138	3	1.31	0.25
122	gi 49456277	PSME1	387	8	1.31	0.30
123	gi 10438450	Unknown protein	371	7	1.30	0.29
124	gi 48428689	Leucyl-tRNA synthetase, cytoplasmic	91	2	1.30	0.39
125	gi 12653493	Brain abundant, membrane attached signal protein 1	283	3	1.30	0.35
126	gi 62822165	Unknown protein	183	3	1.30	0.31
127	gi 18089054	ZNF277 protein	47	1	1.29	0.31
128	gi 25304086	Rab5B	193	1	1.28	0.49
129	gi 38609748	Down syndrome critical region protein 2	119	3	1.28	0.39
130	gi 42659817	Similar to ribosomal protein S2	190	1	1.28	0.36
131	gi 55959864	Nicastrin	68	1	1.28	0.40
132	gi 1346343	Keratin, typeII cytoskeletal 1 (Cytokeratin1)	316	4	1.28	0.33
133	gi 47604944	CVAK104	213	2	1.27	0.37
134	gi 56204408	Transmembrane protein 9	65	1	1.27	0.48
135	gi 14583268	Cytoplasmic protein	104	1	1.27	0.28
136	gi 33150526	zeta-COP	84	2	1.27	0.11
137	gi 47940610	Poly(rC)-binding protein 2	264	2	1.27	0.29
<b>138</b>	<b>gi 106507164</b>	<b>Rab12</b>	<b>77</b>	<b>2</b>	<b>1.26</b>	<b>0.37</b>
139	gi 3164202	Ribosomal protein L10	70	1	1.26	0.54
140	gi 517222	Ribosomal protein S24	61	2	1.25	0.36
141	gi 41152237	Muted	52	2	1.25	0.28
142	gi 38571606	Ribosomal protein L23	127	2	1.25	0.39
143	gi 12654681	High-density lipoprotein binding protein	89	2	1.25	0.43
<b>144</b>	<b>gi 7243268</b>	<b>FENS1</b>	<b>86</b>	<b>2</b>	<b>1.25</b>	<b>0.31</b>
145	gi 55961080	Ribosomal protein S8	169	4	1.24	0.35
146	gi 40889023	Ribosomal protein L4	239	8	1.24	0.31
147	gi 55665494	PAI-1 mRNA-binding protein	68	2	1.22	0.27
148	gi 16924231	Ribosomal protein S19	93	2	1.22	0.35
149	gi 55961090	ADP-ribosylation factor-like 10B	74	3	1.22	0.32
150	gi 57284201	FilaminA, $\alpha$ (actin binding protein 280)	201	3	1.21	0.38
151	gi 404015	Ribosomal protein L23a	176	4	1.21	0.30
152	gi 288100	Initiation factor 4B	46	1	1.21	0.34
153	gi 12654637	Thioredoxin-like 1	345	11	1.20	0.32
154	gi 66392203	Nucleoside-diphosphate kinase 2	168	4	1.20	0.20
155	gi 5442368	AP-4 $\sigma$ 4	76	1	1.20	0.29
156	gi 17426453	Ubiquilin 2	92	1	1.20	0.39
157	gi 37492	$\alpha$ -Tubulin	490	7	1.19	0.22
158	gi 14044037	RPS11 protein	105	5	1.19	0.36
<b>159</b>	<b>gi 49457542</b>	<b>Rab8A</b>	<b>207</b>	<b>1</b>	<b>1.19</b>	<b>0.34</b>
160	gi 550019	Ribosomal protein L28	62	2	1.19	0.28
161	gi 407421	SEB4B	49	1	1.19	0.32
162	gi 7582292	BM-010	105	1	1.19	0.38
163	gi 18255173	RPL27 protein	85	4	1.18	0.29
164	gi 56181368	HSD48	58	1	1.18	0.31

165	gi 56203109	Ribosomal protein L22	152	4	1.18	0.35
166	gi 292435	Ribosomal protein L26	139	6	1.17	0.32
167	gi 28625486	Carbamoylphosphate synthetase	170	3	1.17	0.44
168	gi 34192133	PRPS2 protein	166	4	1.17	0.30
169	gi 39752567	Tetanus insensitive vesicle-associated membrane protein (Ti-VAMP)	93	1	1.17	0.33
170	gi 47678643	RANBP1	57	1	1.17	0.28
171	gi 66347408	Proteasome 26S subunit, non-ATPase, 5	90	2	1.16	0.34
172	gi 21708110	PHKB protein	385	10	1.16	0.29
173	gi 62896517	Ribosomal protein S4	189	4	1.15	0.27
174	gi 55959133	Unknown protein	158	4	1.15	0.30
175	gi 2102696	Karyopherin $\beta$ 3	62	1	1.15	0.34
176	gi 550023	Ribosomal protein S9	272	13	1.15	0.26
177	gi 55960870	Ubiquitination factor E4B	52	1	1.14	0.25
178	gi 558528	Proteasome subunit Y	174	8	1.14	0.27
179	gi 17444701	Similar to ribosomal protein L18a	77	3	1.14	0.30
180	gi 32880069	Endothelial differentiation-related factor 1	53	1	1.14	0.41
181	gi 15131403	Similar to testis-specific protein PBS13	73	1	1.14	0.26
182	gi 41150652	Similar to ribosomal protein S18	111	5	1.13	0.27
183	gi 182642	Rapamycin-binding protein	79	1	1.13	0.37
184	gi 2832753	Phosphorylase kinase $\gamma$ subunit	111	3	1.13	0.26
185	gi 41149143	Similar to ribosomal protein L13a	237	8	1.12	0.25
186	gi 55662798	Valosin-containing protein	598	14	1.12	0.28
187	gi 16877708	RPL35A protein	97	4	1.12	0.30
188	gi 30583113	Proteasome subunit, $\beta$ type, 2	266	14	1.12	0.31
189	gi 54696884	Stress-induced-phosphoprotein1 (Hsp70/Hsp90organizingprotein)	494	15	1.12	0.28
190	gi 81175191	60S ribosomal protein L5	133	6	1.11	0.27
191	gi 42657272	Similar to 40S ribosomal protein S25	122	5	1.11	0.28
192	gi 37675283	AP-4 $\epsilon$	90	3	1.11	0.22
193	gi 62088766	CD68 antigen	172	4	1.11	0.28
194	gi 7243183	KIAA1401 protein	119	3	1.11	0.28
195	gi 56202651	Microtubule-associated protein 7	188	4	1.11	0.30
196	gi 6580116	Glycogen-debranchingenzyme	1898	98	1.10	0.26
197	gi 56204938	AP-4 $\beta$ 4	69	2	1.10	0.26
198	gi 2323410	Skb1Hs	161	5	1.10	0.28
199	gi 13436332	RPL13 protein	251	10	1.10	0.23
200	gi 15559343	Phosphorylase kinase, $\alpha$ 2	283	7	1.10	0.28
201	gi 30583205	Ubiquitin specific protease 14	257	9	1.10	0.27
202	gi 435476	Cytokeratin 9	100	2	1.09	0.40
203	gi 4689134	60S ribosomal protein L36	100	4	1.09	0.27
204	gi 34532087	Unknown protein	109	4	1.09	0.25
205	gi 4432754	Ribosomal protein L27a	93	3	1.09	0.19
206	gi 181402	Epidermal cytokeatin 2	145	1	1.09	0.32
207	gi 22766819	RAVER1	55	1	1.09	0.20
208	gi 39645240	HNRPU protein	98	2	1.09	0.27
209	gi 12654583	Ribosomal protein P0	348	7	1.08	0.27
210	gi 27368062	Class IVb $\beta$ tubulin	561	3	1.08	0.33
211	gi 2232243	Secretory carrier membrane protein (SCAMP3)	173	3	1.08	0.29
212	gi 32879911	Proteasome subunit, $\alpha$ type, 4	390	25	1.08	0.29
213	gi 41201737	Similar to 40S ribosomal protein SA	192	6	1.08	0.31
214	gi 495126	Ribosomal protein L11	120	2	1.08	0.25
215	gi 2150046	26S proteasome subunit 9	714	38	1.08	0.27
216	gi 55958183	Ribosomal protein L7a	146	4	1.08	0.27
217	gi 2580552	Deadbox, X isoform	215	2	1.08	0.24
218	gi 71891792	KIAA0399 protein	116	3	1.08	0.41
219	gi 2134662	26S proteainase chain 5a	483	13	1.07	0.24
220	gi 62897773	Ribosomal protein S5 variant	144	2	1.07	0.29
221	gi 4092058	Proteasome subunit HSPC (PSMA7)	511	28	1.07	0.25
222	gi 49456343	<b>Rab11B</b>	<b>321</b>	<b>8</b>	<b>1.06</b>	<b>0.27</b>
223	gi 55665593	Eukaryotic translation elongation factor 1 $\alpha$ -like 3	313	17	1.06	0.25
224	gi 619444	Dihydrolipoamide acetyltransferase	148	3	1.06	0.30
225	gi 190447	Prosomeal protein P30-33K	390	1	1.06	0.24
226	gi 33329091	Phosphonoformate immunoassociated protein 4	457	14	1.05	0.23
227	gi 4105819	<b>Rab7</b>	<b>324</b>	<b>7</b>	<b>1.05</b>	<b>0.27</b>
228	gi 28374352	RPS15A protein	124	5	1.05	0.29
229	gi 3153908	Liver glycogen phosphorylase	1137	23	1.05	0.26
230	gi 48145757	PSMB4 proteasome subunit, $\beta$ type, 4	332	9	1.05	0.24
231	gi 37574023	Proteasome 26S non-ATPase subunit 8	256	15	1.05	0.27
232	gi 460771	hnRNP-E1	224	2	1.04	0.29
233	gi 30583143	Adenylyl cyclase-associated protein	136	3	1.04	0.26
234	gi 15278174	Proteasome $\beta$ 3 subunit	252	18	1.04	0.29
235	gi 15214948	Proteasome 26S non-ATPase subunit 7	282	17	1.03	0.29
236	gi 62286617	Aftiphilin	89	1	1.03	0.29
237	gi 1228049	Multifunctional protein CAD	1429	33	1.03	0.25
238	gi 3618343	26S proteasome subunit p40.5	689	32	1.03	0.25
239	gi 30410796	Proteasome activator subunit 3	239	5	1.03	0.24
240	gi 20810439	Proteasome $\alpha$ 3 subunit	365	13	1.03	0.27
241	gi 6731237	<b>Myoferlin</b>	<b>643</b>	<b>14</b>	<b>1.02</b>	<b>0.26</b>
242	gi 51095158	Synaptophysin-like protein	63	8	1.02	0.23
243	gi 31973	Unknown protein	45	1	1.02	0.34
244	gi 30582133	Proteasome subunit, $\alpha$ type, 1	415	2	1.02	0.25
245	gi 3197223	Acidic ribosomal phosphoprotein P1	104	2	1.02	0.22
246	gi 11610605	Testis-specific poly(A)-binding protein	87	1	1.02	0.20
247	gi 30583597	Ribosomal protein L8	54	1	1.02	0.28

248	gi 30583275	Proteasome subunit, $\beta$ type, 7 (PSMB7)	161	7	1.02	0.25
249	gi 12653473	Proteasome $\beta$ 1 subunit	239	14	1.02	0.24
250	gi 14714524	Proteasome 26S ATPase subunit 4	310	10	1.01	0.26
251	gi 17512153	Proteasome 26S non-ATPase subunit 12	804	27	1.01	0.24
252	gi 976227	26S proteasome subunit p45	905	28	1.01	0.24
253	gi 1808578	Proteasome subunit p112	757	6	1.01	0.19
254	gi 7023651	Unknown protein	52	1	1.01	0.20
255	gi 42490917	26S proteasome-associated pad1 homologue	324	15	1.01	0.26
256	gi 62241042	Glutamyl-prolyl tRNA synthetase	204	5	1.01	0.25
257	gi 20379122	Small GTP-binding protein RhoG	99	1	1.00	0.28
258	gi 18204442	Ribosomal protein L18	210	6	1.00	0.26
259	gi 52632425	Threonyl-tRNA synthetase	96	2	1.00	0.33
260	gi 12274840	ADRM1	70	1	1.00	0.21
261	gi 45594399	CTD-like phosphatase domain-containing protein	89	2	1.00	0.25
262	gi 4432748	Ribosomal protein S27	71	1	1.00	0.24
263	gi 33150538	<b>Rab10</b>	<b>180</b>	<b>3</b>	<b>1.00</b>	<b>0.31</b>
264	gi 296736	Macropain subunit iota	309	16	0.99	0.25
265	gi 28422545	UDP-glucose pyrophosphorylase 2	357	9	0.99	0.26
266	gi 13606056	DNA dependent protein kinase catalytic subunit	58	1	0.99	0.29
267	gi 6941766	Ubiquitin-conjugating enzyme E2N-like	109	2	0.99	0.24
268	gi 496895	Glycogenin	117	4	0.98	0.24
269	gi 345717	26S proteasome regulatory chain 4	493	13	0.98	0.28
270	gi 31108	EF-2	544	13	0.98	0.22
271	gi 55959947	Protein kinase, cAMP-dependent, catalytic, $\beta$	63	1	0.97	0.25
272	gi 115206	C-1-tetrahydrofolate synthase, cytoplasmic	287	4	0.97	0.22
273	gi 1526426	Proteasome subunit p42	592	17	0.97	0.22
274	gi 312803	Cdk2	80	1	0.97	0.21
275	gi 951338	CAS	202	6	0.97	0.21
276	gi 51493358	Similar to 40S ribosomal protein S16	152	4	0.96	0.24
277	gi 56205096	<b>Phosphoglycerate dehydrogenase</b>	<b>498</b>	<b>12</b>	<b>0.96</b>	<b>0.24</b>
278	gi 54696890	Tyrosine 3-mono-oxygenase $\theta$ subunit	237	2	0.95	0.26
279	gi 16306837	TCPI, subunit 5 ( $\epsilon$ )	689	26	0.95	0.21
280	gi 21619421	Ribosomal protein S2	168	1	0.95	0.30
281	gi 41472112	Unknown protein	824	32	0.95	0.24
282	gi 5726310	14-3-3 $\gamma$ protein	186	1	0.95	0.23
283	gi 56181387	STIP1 homology and U-box containing protein 1	71	3	0.95	0.21
284	gi 16741043	MRCL3 protein	264	9	0.95	0.24
285	gi 306891	90-kD heat shock protein	831	31	0.95	0.24
286	gi 38382891	Rab43	70	1	0.95	0.32
287	gi 28704029	Protein phosphatase 1, regulatory subunit 3D (PPP1R3D)	113	1	0.95	0.38
288	gi 13559060	MEP50 protein	103	2	0.94	0.22
289	gi 48146259	CCT2	756	35	0.94	0.23
290	gi 126376	LAMP-1	103	4	0.94	0.22
291	gi 13623199	ATP citrate lyase	742	19	0.94	0.24
292	gi 19070472	Retinoblastoma-associated factor 600	1845	50	0.94	0.22
293	gi 2648022	HLA classII region expressed gene KE2	104	2	0.94	0.30
294	gi 4929609	CGI-70 protein	491	20	0.93	0.21
295	gi 187387	Myristoylated alanine-rich C-kinase substrate	177	2	0.93	0.18
296	gi 37267	Transketolase	135	5	0.93	0.24
297	gi 23821872	Myosin-I (C/E)	173	2	0.93	0.28
298	gi 48257068	HSPA8 protein	565	2	0.92	0.26
299	gi 62896799	Eukaryotic translation initiation factor 3, subunit 3 $\gamma$	128	2	0.92	0.29
300	gi 62131678	14-3-3 protein $\epsilon$	205	2	0.92	0.23
301	gi 12803639	Tubulin, $\beta$ 6	349	2	0.92	0.30
302	gi 183355	Glycogen synthase	787	40	0.92	0.23
303	gi 62896535	Guanine nucleotide binding protein	209	6	0.92	0.23
304	gi 54673670	Twinfilin	69	3	0.91	0.25
305	gi 306890	Chaperonin (HSP60)	190	4	0.91	0.21
306	gi 12667788	Myosin, heavy polypeptide 9, nonmuscle	2054	36	0.91	0.22
307	gi 671527	Gamma subunit of CCT chaperonin	738	50	0.91	0.22
308	gi 337760	Cerebroside sulfate activator protein	83	2	0.91	0.28
309	gi 806854	eIF-2B $\epsilon$	59	1	0.91	0.35
310	gi 704416	Elongation factor Tu	72	2	0.91	0.26
311	gi 41350401	Migration-inducing gene 10 protein	100	3	0.91	0.25
312	gi 2370149	PDZ domain protein	97	4	0.90	0.22
313	gi 33438760	Myosin heavy chain	258	1	0.90	0.24
314	gi 62896539	Chaperonin containing TCP1, subunit 8	1234	59	0.90	0.21
315	gi 5670324	Gps1	226	5	0.89	0.19
316	gi 51094672	Ubiquitin protein ligase E3C	186	4	0.89	0.24
317	gi 337580	Ribosomal protein L3	140	4	0.89	0.22
318	gi 188590	Myosin light chain 3	167	5	0.89	0.17
319	gi 52001478	TCP1-delta	938	32	0.88	0.20
320	gi 1465751	VHL binding protein-1	199	4	0.88	0.20
321	gi 12275866	Tripartite motif protein TRIM29 $\beta$	66	1	0.88	0.22
322	gi 71891685	KIAA0829protein; TIP120	230	5	0.88	0.20
323	gi 33875694	Aspartyl-aminopeptidase	116	3	0.88	0.17
324	gi 683580	Ribonucleoprotein, 68 kD (U1)	78	2	0.88	0.23
325	gi 48145555	CCT7	839	29	0.87	0.21
326	gi 55962551	Heatshock protein 1A (70 kD)	510	7	0.87	0.21
327	gi 36796	T-complex polypeptide 1	841	56	0.87	0.20
328	gi 1336765	Glucosephosphate isomerase	118	3	0.87	0.22
329	gi 559330	KIAA0077	213	6	0.87	0.20
330	gi 11125348	Putative protein kinase	79	1	0.87	0.26
331	gi 50345286	Dynein, cytoplasmic, heavy polypeptide 1	366	10	0.87	0.24

332	gi 20147659	ADP-ribosylation factor protein 4	138	2	0.87	0.12
333	gi 1170683	Phosphorylase b kinase $\alpha$ regulatory chain	69	3	0.87	0.31
334	gi 14517632	Acute morphine dependence related protein 2 chaperone	928	40	0.86	0.24
335	gi 24234686	Heatshock 70-kD protein 8	676	2	0.86	0.22
336	gi 18307485	Myosin-1F	108	1	0.86	0.23
337	gi 499719	Mitochondrial dihydrolipoamide succinyl transferase	144	3	0.86	0.21
338	gi 2959876	Synaptogyrin 2	133	4	0.85	0.25
339	gi 30582627	Eukaryotic translation initiation factor 3, subunit 5 $\epsilon$	133	2	0.85	0.10
340	gi 49065418	BAG2	179	3	0.85	0.16
341	gi 62088644	Eukaryotic translation initiation factor 2B, subunit4 delta	170	3	0.85	0.24
342	gi 5410300	COP9 complex subunit 4	113	2	0.85	0.15
343	gi 1352618	2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor	77	1	0.84	0.23
344	gi 223582	Histone H4	76	1	0.84	0.20
345	gi 30582227	Programmed cell death 5	107	2	0.84	0.21
<b>346</b>	<b>gi 54695928</b>	<b>Rab1A</b>	<b>479</b>	<b>4</b>	<b>0.84</b>	<b>0.26</b>
347	gi 55662967	Golgi-specific brefeldin A resistance factor 1	131	1	0.83	0.21
<b>348</b>	<b>gi 13560109</b>	<b>ARF1 GAP</b>	<b>165</b>	<b>3</b>	<b>0.83</b>	<b>0.18</b>
349	gi 88974153	Unknown protein LOC23045	68	3	0.83	0.27
350	gi 55666249	Phosphofruktokinase	168	2	0.83	0.18
351	gi 940536	P1Cdc21 protein	47	2	0.83	0.21
352	gi 2791552	Tyrosine 3 mono-oxygenase $\beta$ subunit	257	3	0.83	0.23
353	gi 2209276	Oxytocinase	90	4	0.82	0.20
<b>354</b>	<b>gi 62913988</b>	<b>Vac14</b>	<b>199</b>	<b>3</b>	<b>0.82</b>	<b>0.21</b>
355	gi 62089410	Thrombospondin 1 precursor	147	3	0.81	0.23
356	gi 12803097	DnaJ (Hsp40) homologue, subfamilyB, member 1	119	3	0.81	0.21
357	gi 6634031	KIAA0406 protein	149	3	0.81	0.25
358	gi 36065	M1 subunit of ribonucleotide reductase	168	3	0.81	0.23
359	gi 5231133	Ubiquitin-specific protease homologue	79	2	0.81	0.23
360	gi 38648667	Fatty acid synthase	1870	46	0.81	0.20
361	gi 7662446	Unknown protein LOC22832	54	1	0.80	0.29
362	gi 40788956	Unknown protein KIAA0111	112	1	0.80	0.14
363	gi 5262359	C14orf3 protein	57	1	0.80	0.17
364	gi 31416724	DnaJ C7 protein	277	7	0.80	0.20
365	gi 4960030	Rab GDP dissociation inhibitor $\beta$	49	1	0.80	0.22
366	gi 5922017	AMP deaminase	117	2	0.80	0.25
367	gi 3212110	Prefoldin subunit 1	142	4	0.79	0.20
368	gi 31179	Enolase	121	2	0.79	0.14
369	gi 55959886	Peroxisredoxin 1	402	9	0.79	0.21
<b>370</b>	<b>gi 46577636</b>	<b>Rab-2A</b>	<b>119</b>	<b>2</b>	<b>0.79</b>	<b>0.22</b>
371	gi 57284174	Ubiquitin-like 4	82	2	0.79	0.17
372	gi 21903479	Vinculin	136	2	0.78	0.31
373	gi 17939425	PAICS protein	283	8	0.78	0.19
374	gi 30583309	TIP47	100	2	0.78	0.22
375	gi 532313	NF45 protein	80	1	0.78	0.15
376	gi 12654931	Protein disulfide isomerase associated protein 6	96	2	0.77	0.28
377	gi 55959870	Rab3 GTPase-activating protein, noncatalytic subunit	202	4	0.77	0.18
378	gi 19683948	TXNDC9 protein	91	4	0.77	0.19
379	gi 182890	Glucose-6-phosphate dehydrogenase	93	2	0.77	0.19
<b>380</b>	<b>gi 49065350</b>	<b>Rab1B</b>	<b>417</b>	<b>1</b>	<b>0.77</b>	<b>0.20</b>
381	gi 32425722	VAT1 protein	372	8	0.76	0.17
<b>382</b>	<b>gi 54696730</b>	<b>Annexin A11</b>	<b>104</b>	<b>2</b>	<b>0.76</b>	<b>0.22</b>
383	gi 45501322	Lactate dehydrogenase A	316	8	0.76	0.19
384	gi 30583423	DnaJ (Hsp40) homologue, subfamilyA, member 1	159	2	0.76	0.17
385	gi 54696396	Lactate dehydrogenase B	276	10	0.75	0.20
386	gi 21595720	RUFY1 protein	184	5	0.75	0.20
387	gi 44890424	MARCKS-like1	102	2	0.74	0.21
388	gi 662841	Heat shock protein 27	202	1	0.74	0.17
389	gi 7671655	Translocase of outer mitochondrial membrane 34	47	1	0.74	0.08
390	gi 34223697	Insulin receptor tyrosine kinase substrate	73	2	0.73	0.19
391	gi 55662983	Kinesin family member 5B	85	2	0.73	0.22
392	gi 55957740	RUN and SH3 domain containing 1	158	5	0.73	0.21
393	gi 47496659	PRDX2	108	1	0.72	0.18
394	gi 4758544	Heterogeneous nuclear ribonucleoprotein C	47	1	0.72	0.23
395	gi 55665263	G protein-coupled receptor 107	46	1	0.72	0.26
396	gi 18997185	NIMA-related kinase Nek8	56	2	0.71	0.21
397	gi 30354619	YWHAZ protein	303	4	0.71	0.18
398	gi 56203412	Phospholipase A2, group IVA	95	3	0.71	0.17
399	gi 12052969	Unknown protein	148	3	0.71	0.13
400	gi 71891760	Unknown protein KIAA0683	46	1	0.70	0.20
401	gi 24429617	RNA polymerase III subunit RPC2	50	1	0.70	0.19
402	gi 30582847	Cell division cycle 2, G1 to S and G2 to M	99	2	0.70	0.16
403	gi 54261593	Unknown protein	90	1	0.70	0.07
404	gi 39645106	Sec23-interacting protein p125	97	3	0.69	0.18
405	gi 89060144	Unknown protein LOC57481	54	1	0.69	0.28
406	gi 17384416	RAD23 homology B	100	2	0.68	0.15
407	gi 63102287	Rab13	141	2	0.67	0.19
408	gi 6120135	COP9 complex subunit 7a	69	1	0.67	0.16
409	gi 89059963	Unknown protein KIAA1817	58	2	0.67	0.17
410	gi 35830	Ubiquitin activating enzyme E1	137	3	0.67	0.15
411	gi 13445272	Rab32	54	1	0.64	0.16
412	gi 1477565	GEF p532	436	11	0.64	0.12
413	gi 1654346	Ubiquitin fusion-degradation 1-like protein	61	1	0.64	0.17
414	gi 1941923	YWHAH	254	1	0.63	0.10
415	gi 1666075	Ubiquitin hydrolase	131	2	0.63	0.13



416	gi 755746	p85 Mcm protein	105	2	0.63	0.16
417	gi 39645311	NIP30 protein	67	1	0.63	0.13
418	gi 1143492	BiP	169	4	0.63	0.20
419	gi 230868	Glyceraldehyde-3-Phosphate Dehydrogenase	143	5	0.62	0.15
420	gi 2465729	TFAR15	50	1	0.62	0.17
421	gi 1478503	SCG10	53	1	0.62	0.16
422	gi 5714634	Niemann-Pick C1 protein	67	1	0.61	0.15
423	gi 1136228	UV-damaged DNA binding factor	227	5	0.60	0.15
424	gi 30582841	Profilin 1	89	2	0.60	0.18
425	gi 52545677	Hypothetical protein	54	1	0.60	0.13
<b>426</b>	<b>gi 54696200</b>	<b>Rap1A</b>	<b>146</b>	<b>5</b>	<b>0.60</b>	<b>0.16</b>
427	gi 62702173	Unknown protein	94	2	0.60	0.09
428	gi 5419633	Moesin	331	5	0.59	0.16
429	gi 31419796	Discoidin domain receptor family, member 2 precursor	62	3	0.59	0.14
430	gi 1170586	Ras GTPase-activating protein IQGAP1 (p195)	302	5	0.59	0.18
431	gi 15079512	Protein kinase C, delta binding protein	95	2	0.59	0.10
<b>432</b>	<b>gi 999937</b>	<b>Annexin V</b>	<b>94</b>	<b>2</b>	<b>0.59</b>	<b>0.14</b>
433	gi 37595069	Neuroblastoma RAS viral (v-ras) oncogene homologue	107	2	0.58	0.14
434	gi 21707505	Bone marrow stromal cell antigen 2	53	1	0.58	0.13
435	gi 20379102	Rac1	80	1	0.58	0.17
436	gi 12803567	Transgelin 2	89	3	0.58	0.13
437	gi 25121972	Copine VIII	57	1	0.58	0.23
438	gi 1197636	p105 MCM	53	2	0.57	0.13
<b>439</b>	<b>gi 464541</b>	<b>Radixin</b>	<b>302</b>	<b>3</b>	<b>0.57</b>	<b>0.17</b>
440	gi 7959259	Unknown protein KIAA1499	65	3	0.57	0.11
441	gi 15029597	Progesterin induced protein	332	9	0.57	0.13
442	gi 31127073	RANBP9 protein	109	2	0.57	0.18
443	gi 187281	M4 protein	46	1	0.56	0.13
444	gi 181400	Cytokeratin 8	307	5	0.55	0.14
445	gi 42406425	VPS13C-1A protein	100	5	0.55	0.10
446	gi 68084446	Solute carrier family, member 2	683	29	0.55	0.14
447	gi 27227551	Class II $\beta$ tubulin isotype	495	1	0.55	0.24
448	gi 37181332	EMMPRN/other basigin variants	71	2	0.54	0.11
449	gi 3319931	Melanoma antigen family D, 2	74	1	0.54	0.10
450	gi 30311	Cytokeratin 18	115	2	0.54	0.13
451	gi 55665020	Prostaglandin F2 receptor negative regulator	124	4	0.53	0.11
452	gi 183225	G protein, $\alpha$ subunit	253	7	0.53	0.13
453	gi 12963885	Prostate antigen PARIS-1	55	1	0.52	0.18
454	gi 27469833	Armadillo repeat containing 8	115	3	0.52	0.13
455	gi 55749758	Unknown protein LOC57609	63	1	0.51	0.10
456	gi 416178	Desmoglein 2	88	2	0.51	0.11
457	gi 2653424	Inositol polyphosphate 5-phosphatase	64	1	0.51	0.12
458	gi 49456297	PRDX4	103	2	0.50	0.12
459	gi 40789065	Unknown protein KIAA0079	241	5	0.50	0.11
460	gi 27503713	Solute carrier family 7, member 5	143	1	0.50	0.10
461	gi 20381372	Solute carrier family 7, member6	61	1	0.49	0.17
462	gi 52632407	SEC13-like 1	180	7	0.48	0.11
463	gi 31657226	Galectin-3	76	4	0.48	0.11
<b>464</b>	<b>gi 1703319</b>	<b>AnnexinIV (LipocortinIV, EndonexinI)</b>	<b>105</b>	<b>2</b>	<b>0.47</b>	<b>0.15</b>
<b>465</b>	<b>gi 71773329</b>	<b>AnnexinVI</b>	<b>285</b>	<b>6</b>	<b>0.47</b>	<b>0.10</b>
466	gi 3282205	Prostate apoptosis response protein par-4	49	1	0.47	0.03
467	gi 62087720	Dynamin-like protein DYNIV-11	74	3	0.47	0.12
468	gi 37852	Vimentin	51	1	0.47	0.09
469	gi 30582531	Cofilin1	125	4	0.46	0.10
470	gi 190028	T-plastin polypeptide	121	2	0.45	0.16
471	gi 62089356	Ret finger protein	57	1	0.45	0.08
<b>472</b>	<b>gi 55959292</b>	<b>Annexin A1</b>	<b>79</b>	<b>3</b>	<b>0.45</b>	<b>0.14</b>
473	gi 46249758	Villin 2	513	10	0.45	0.10
474	gi 6424942	ALG-2 interacting protein 1	168	3	0.44	0.12
475	gi 29124496	Viral oncogene yes-1 homolog 1	147	3	0.44	0.10
<b>476</b>	<b>gi 306785</b>	<b>G protein <math>\beta</math> subunit</b>	<b>64</b>	<b>2</b>	<b>0.44</b>	<b>0.10</b>
477	gi 757924	Epidermal growth factor receptor	114	4	0.43	0.08
478	gi 47117499	Zinc finger CCCH type anti-viral protein 1	228	3	0.43	0.10
479	gi 14250401	Actin, $\beta$	502	20	0.43	0.09
480	gi 1174072	G $\alpha$ -q	71	2	0.42	0.10
481	gi 225451	Decay accelerating factor	85	3	0.42	0.11
482	gi 7715039	Sec31	752	39	0.41	0.10
483	gi 2627129	Polyubiquitin	328	22	0.40	0.09
484	gi 54695954	Myelin protein zero-like1	56	1	0.40	0.07
485	gi 21754049	Unknown protein	61	1	0.39	0.05
486	gi 125484	Hepatocyte growthfactor receptor precursor	74	1	0.38	0.06
487	gi 5457119	Junction adhesion molecule	56	2	0.37	0.06
488	gi 14248495	MEG3	64	3	0.37	0.11
489	gi 4588526	Nuclear chloride channel	54	2	0.37	0.03
490	gi 17389486	Phenylalanine-tRNA synthetase-like	167	3	0.37	0.09
491	gi 21070332	Lysosome-associated membrane protein-3 (LAMP-3)	60	5	0.36	0.10
492	gi 5730051	Solute carrier family 2, member 1	82	4	0.34	0.07
493	gi 190281	Protein phosphatase 1 $\alpha$ subunit (PPP1A)	44	1	0.34	0.08
494	gi 62089404	Coronin, actin binding protein	56	1	0.34	0.08
495	gi 35010	Neural cell adhesion molecule L1	140	3	0.33	0.08
496	gi 61743954	AHNAK nucleoprotein	391	8	0.33	0.09
497	gi 37999904	Scavenger receptor class B member 1	97	2	0.33	0.10
498	gi 62897641	Solute carrier family 16, member 3	198	5	0.33	0.09
499	gi 125333	Ephrin type-A receptor 2 precursor	80	2	0.32	0.04

500	gi 15217171	CD81 partner 3	101	2	0.32	0.08
501	gi 13633711	Rho-related GTP-binding protein RhoF	70	1	0.32	0.09
502	gi 15080812	MHC class I antigen precursor (several indistinguishable isoforms)	92	2	0.31	0.07
503	gi 12804857	SQSTM1 protein	94	1	0.31	0.08
504	gi 6996444	CTL2 protein	67	1	0.31	0.07
505	gi 30582653	CD47 antigen	58	1	0.31	0.07
506	gi 854189	Tropomyosin isoform	46	1	0.29	0.08
507	gi 28614	<b>Aldolase A/B/C</b>	<b>239</b>	<b>5</b>	<b>0.29</b>	<b>0.07</b>
508	gi 86988	Ca <sup>2+</sup> -transporting ATPase	154	2	0.28	0.06
509	gi 62089374	Integrin $\alpha$ -V precursor	177	5	0.28	0.07
510	gi 3694920	<b>Putative poly (ADP-ribosyl) transferase; VAULT protein</b>	<b>315</b>	<b>8</b>	<b>0.28</b>	<b>0.07</b>
511	gi 62898293	Programmed cell death 6	161	6	0.28	0.07
512	gi 2795863	Syntenin	69	2	0.27	0.07
513	gi 12803195	Galectin 3 binding protein	67	1	0.25	0.06
514	gi 15418734	ABC transporter ABCG2	62	1	0.25	0.06
515	gi 124963	Integrin $\beta$ -1 precursor	141	4	0.24	0.06
516	gi 34148711	Melanoma chondroitin sulfate proteoglycan	264	5	0.23	0.02
517	gi 68067956	Intercellular adhesion molecule 1 precursor (ICAM-1)	268	7	0.22	0.04
518	gi 13177796	G protein-coupled receptor, family C, group 5, member A	99	3	0.19	0.04
519	gi 15079985	Solute carrier family 9, isoform 3, regulator 1	111	1	0.16	0.05
520	gi 15990478	Major vault protein	838	20	0.15	0.03
521	gi 17865536	Podocalyxin-like protein 1 precursor	104	1	0.14	0.04
522	gi 20138067	LIM domain and actin-binding protein 1	48	1	0.08	0.03

Control and mock CCV fractions were prepared as in Fig.1 and analyzed by iTRAQ in conjunction with LC-MS/MS (Fig. 3). Identified proteins were ranked based on their relative abundance in mock and control CCVs; a high rank corresponds to a high ratio of control/mock CCVs and, thus, to candidate CCV proteins. The Mascot score reflects the confidence with which a protein was identified; a score >36 indicates >95% confidence of identification. "Peptides quantified" indicates how many iTRAQ-labeled peptides were used for the quantitation. The control/mock ratio corresponds to the relative abundance of a protein in control and mock CCVs; the ratio is the average of two technical replicates. Likely false positives among high ranking proteins are in italics. Proteins that were identified in CCV preparations by Blondeau et al. (2004) or Girard et al. (2005) but are <50% depleted from mock CCVs (and thus not predicted to be genuine CCV proteins) are in bold. Please note that ratios <1 indicate proteins that are enriched in the mock CCVs relative to the control CCVs, suggesting that either their expression is increased or their subcellular localization is changed by clathrin heavy chain knockdown.

Table S2. Summary of CCV proteins identified in this study by different methods							
Number	Protein ID	Protein name	Established CCV protein?	1D-PAGE	2D-DIGE	Western blot	iTRAQ
1	gi 14043007	AP-1 1	√	+	+	+	+
2	gi 12643391	AP-1	√	ND	ND	+	+
3	gi 17028334	AP-1 1A	√	ND	+	+	+
4	gi 30582397	AP-1 1A	√	ND	ND	ND	+
5	gi 33150596	AP-1 1B	√	ND	ND	ND	+
6	gi 19913414	AP-2	√	ND	ND	+	+
7	gi 4557469	AP-2 2	√	ND	+	+	+
8	gi 15489411	AP-2 2	√	ND	ND	+	+
9	gi 57162630	AP-3 3A	New	ND	ND	+	+
10	gi 56205243	Auxilin	√	ND	ND	ND	+
11	gi 47086495	BLOC-1, subunit 3	New	ND	ND	ND	+
12	gi 25090897	CALM	√	ND	ND	ND	+
13	gi 8922952	Cappuccino	New	ND	ND	ND	+
14	gi 21903712	Carboxypeptidase D	√	ND	ND	ND	+
15	gi 3294548	Cathepsin Z (lysosomal)	New	ND	ND	ND	+
16	gi 8546849	CD-Mannose 6-phosphate receptor	√	ND	ND	ND	+
17	gi 55662275	CI-Mannose 6-phosphate receptor	√	+	ND	+	+
18	gi 32451593	Clathrin heavy chain (CHC17)	√	+	+	+	+
19	gi 1335854	Clathrin heavy chain homologue (CHC22)	New	ND	ND	ND	+
20	gi 17402231	Clathrin light chain a	√	ND	+	ND	+
21	gi 116505	Clathrin light chain b	√	ND	ND	ND	+
22	gi 47604944	CVAK104	√	ND	+	+	+/=
23	gi 62751805	D19	New	ND	ND	ND	+
24	gi 30582933	DNase II (lysosomal)	New	ND	ND	ND	+
25	gi 1363934	Dynamin-2	√	ND	ND	ND	+
26	gi 41016993	EpsinR/Enthoprotin	√	ND	+	+	+
27	gi 47125326	Ferritin heavy chain	√	ND	ND	ND	+
28	gi 182516	Ferritin light subunit	√	ND	ND	ND	+
29	gi 17375734	GAK/auxilin2	√	ND	ND	ND	+
30	gi 15214696	Glucosamine ( <i>N</i> -acetyl)-6-sulfatase	New	ND	ND	ND	+
31	gi 13431563	Hip1R	√	ND	ND	ND	+
32	gi 67477390	OCRL-1	√	ND	ND	ND	+
33	gi 62287155	NECAP	√	ND	ND	ND	+
34	gi 7920147	<i>N</i> -ethylmaleimide-sensitive factor	√	ND	+	ND	+
35	gi 6912574	Pallidin	New	ND	ND	+	ND
36	gi 2143260	PI 3-kinase C2	√	ND	ND	ND	+
37	gi 56205909	Rab4A	√	ND	ND	ND	+
38	gi 54695838	Rab5C	√	ND	ND	ND	+
39	gi 47678695	SNAP29	New	ND	ND	+	ND
40	gi 30582345	Snapin	New	ND	ND	ND	+
41	gi 9716092	Sortilin	√	ND	ND	ND	+
42	gi 62896680	Sorting nexin 1	New	ND	+	+	+/=
43	gi 2827434	Sorting nexin 2	New	ND	+	ND	+
44	gi 9557955	Sorting nexin 5	New	ND	+	ND	+
45	gi 4689252	Sorting nexin 6	New	ND	+	ND	+/=
46	gi 13477131	Sorting nexin 9	√	ND	+	ND	+
47	gi 55663531	Syntaxin 6	New	ND	ND	+	+
48	gi 2337920	Syntaxin 7	New	ND	ND	+	+
49	gi 4433649	Syntaxin 8	New	ND	ND	+	+
50	gi 2961087	Syntaxin 16	New	ND	ND	+	ND
51	gi 2226273	TGN46	√	ND	ND	ND	+
52	gi 70608172	TPD52	New	ND	ND	ND	+
53	gi 56203491	TPD52L1	New	ND	ND	ND	+
54	gi 12654697	Transferrin receptor	√	+/=	+/=	+/=	+/=
55	gi 38570101	Unknown protein, RAB-GAP domain	New	ND	ND	ND	+
56	gi 15214676	Unknown protein, putative Rab interactor	New	ND	ND	ND	+
57	gi 13543574	VAMP3	New	ND	ND	+	+/=
58	gi 3248920	VAMP4	New	ND	ND	+	ND
59	gi 39752567	VAMP7	New	ND	ND	+	+/=
60	gi 9622852	VPS 26	New	ND	ND	+	+/=
61	gi 17999541	VPS 35	New	ND	+	ND	ND
62	gi 16877603	Vti1a	√	ND	ND	+	ND
63	gi 5454166	Vti1b	√	ND	ND	+	ND
=		63 total	35 established and 28 new CCV proteins	4	15	25	56

This table lists all CCV proteins identified in this study. Only proteins that showed clear depletion from mock CCVs by one or more techniques are listed. Four different methods were used to compare control and mock CCVs: 1D-PAGE, 2D-DIGE, Western blotting, and iTRAQ. In the corresponding columns, a plus sign indicates that a protein was identified and depleted from mock CCVs (bona fide CCV protein). +/= indicates that a protein was identified, but only a moderate level of depletion from mock CCVs was observed. ND indicates that a protein was not detected. Proteins whose association with CCVs had previously not been established are annotated as new.

Table S3. List of antibodies

Antibody against	Host	Mono/polyclonal	Name/source
Primary antibodies			
AP-1 $\beta$ 1	Rabbit	P	Page et al., 1995
AP-1 $\gamma$	Rabbit	P	Seaman et al., 1996
AP-2 $\alpha$	Rabbit	P	Ball et al., 1995
AP-2 $\beta$ 2	Rabbit	P	Page et al., 1995
AP-2 $\mu$ 2	Rabbit	P	Page et al., 1995
AP-3 $\mu$ 3	Rabbit	P	Simpson et al., 1996
AP-4 $\beta$ 4	Rabbit	P	Hirst et al., 1999
CI Mannose-6-phosphate receptor	Rabbit	P	1001/gift from P. Luzio, Cambridge Institute for Medical Research, University of Cambridge, UK
Clathrin heavy chain	Rabbit	P	Simpson et al., 1996
Elongation factor 2	Goat	P	C-14/Santa Cruz Biotechnology, Inc.
EpsinR	Rabbit	P	Hirst et al., 2003
Pallidin	Mouse	M	Nazarian et al., 2006
Sec 22	Rabbit	P	Hay et al., 1997
SNAP 23	Rabbit	P	Synaptic Systems
SNAP 29	Rabbit	P	Synaptic Systems
Sorting nexin 1	Rabbit	P	Seaman, 2004
Syntaxin 2	Rabbit	P	Calbiochem
Syntaxin 3	Rabbit	P	Calbiochem
Syntaxin 4	Mouse	M	Transduction Laboratories
Syntaxin 6	Rabbit	P	Gift from A. Peden, Genentech, Inc., South San Francisco, CA
Syntaxin 7	Rabbit	P	Gift from A. Peden
Syntaxin 8	Mouse	M	BD Biosciences
Syntaxin 16	Mouse	M	Gift from A. Peden
Syntaxin 17	Goat	P	Steegmaier et al., 2000
Transferrin receptor	Mouse	P	Zymed
VAMP3	Rabbit	P	Abcam
VAMP4	Hamster	P	Steegmaier et al., 1999
VAMP7	Mouse	M	Advani et al., 1999
VPS26	Rabbit	P	Seaman, 2004
Vti1a	Mouse	M	BD Biosciences
Vti1b	Mouse	M	BD Biosciences
Secondary antibodies			
Anti-goat HRP	Rabbit	P	Sigma-Aldrich
Anti-Hamster HRP	Goat	P	Jackson ImmunoResearch Laboratories
Anti-mouse HRP	Rabbit	P	Sigma-Aldrich
Anti-rabbit HRP	Goat	P	Sigma-Aldrich