Protein Identification of Mixture Samples
By Liquid Chromatography—Tandem Mass Spectrometry

Introduction

The combination of high-performance liquid chromatography and high-resolution tandem mass spectrometry (LC-MS/MS) emerged as a powerful and universal method for the global measurement of proteins. Unlike traditional western-blot or ELISA methods, MS-based technology can identify and quantify the protein mixtures simultaneously at large scale. Particularly, for interested proteins without available antibodies, MS-based protein identification is the fastest and most reliable approach.

PROMICFINDER, developed at Poochon, is a mass spectrometry-based protein analysis technology capable of identifying and measuring rapidly (high throughput), broadly (thousands of proteins simultaneously), and deeply (high-and low-abundance proteins). It is a highly sensitive, quantitative, and reproducible proteomic tool for profiling up to 4000 different proteins from a single protein gel band sample, one IP sample, or a single un-fractioned cell/tissue lysate sample by a 110-minute LC-MS/MS run. It is also the most cost-effective method for post-translational modifications identification and analysis. Our standardized SOP and report format ensures quality, reproducible data and generates publishable results.
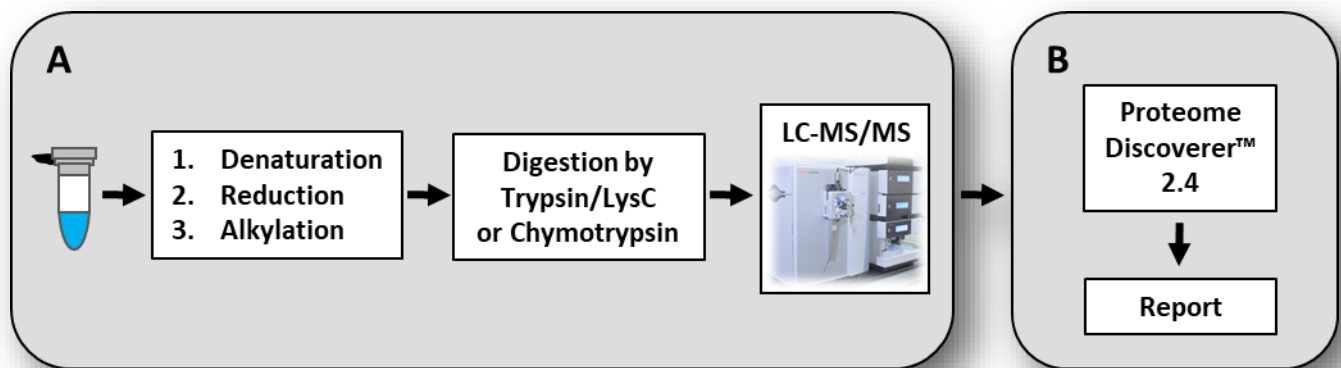
Specifications

→ **Method** – Protease digestion and LC-MS/MS

→ **Key Instruments** – Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer, Thermo Scientific™ Vanquish™ Neo UHPLC System

→ **Detection Limit** – Individual protein: ≥1ng, cell/tissue lysate: ≥5 µg

→ **Acceptable Samples** – Partially purified samples, e.g., IP, cellular organelle (≥5 µg/sample), tissue/cell lysate (≥100 µg/sample), cell pellet (>1 million cells/sample), solid tissue (≥50 mg/sample)

→ **Turnaround Time** – Typically, reports are available within 5 business days of sample receipt

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Schematic of Procedure Workflow

Figure 1:

A) Workflow for Protein ID analysis. *Note: LC-MS/MS = liquid chromatography and tandem mass spectrometry*
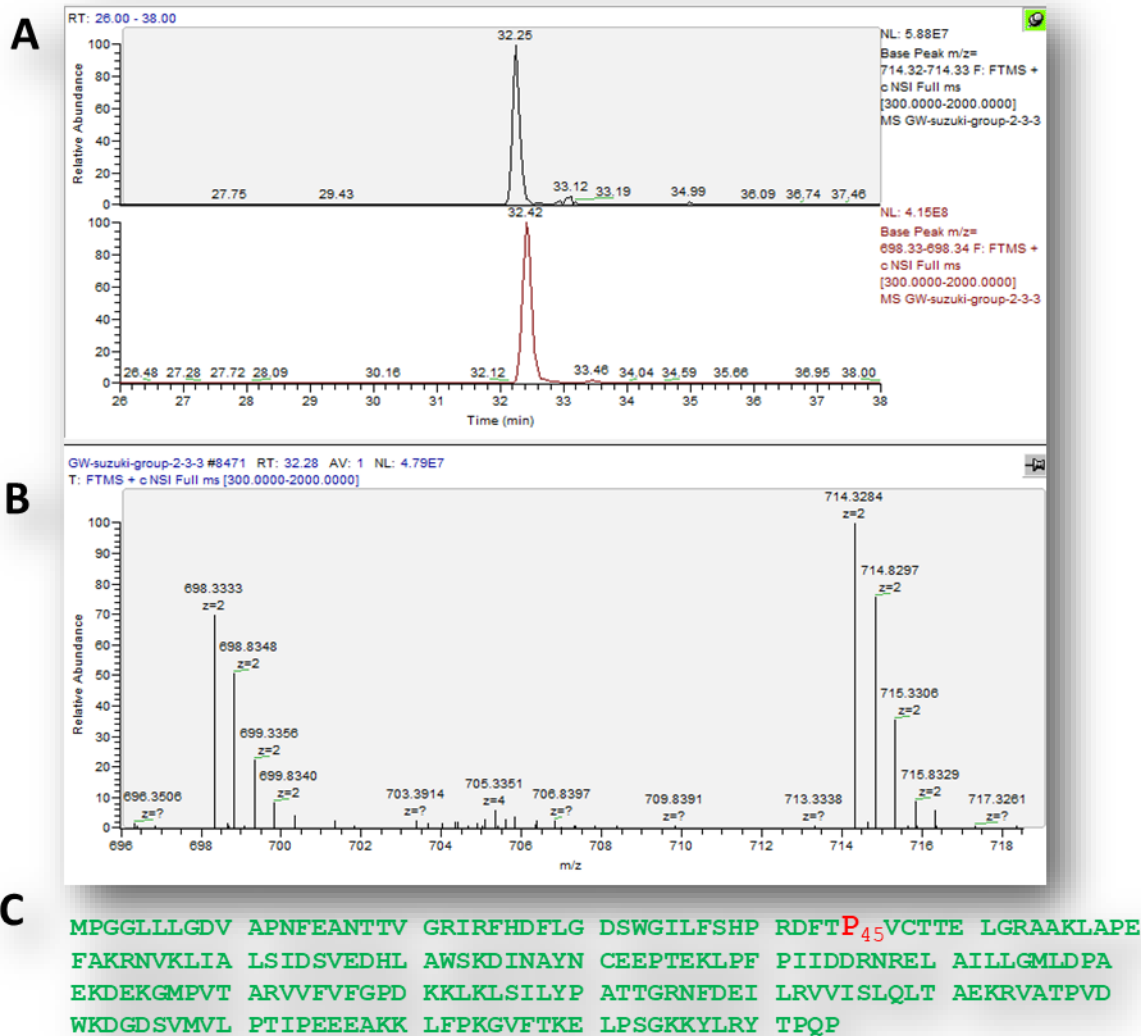
B) Bioinformatic analysis approach used for protein peptide identification of datasets and label-free quantification.

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Example One

Identification of conversion of the proline (P) residue at amino acid 45 to glutamic acid (E) in human protein Peroxiredoxin-6

Figure 2: Nano LC-MS/MS verification of conversion of Pro 45 to Glu at PRDX6 (DFT$P_{+31.990}$VCTTELGR). **A)** Extracted ion chromatograms of PRDX6 peptide (DFT$P_{+31.990}$VCTTELGR, +2 charge, m/z=714.33) *(top)*, and its non-conversion counterpart (DFTPVCTTELGR, +2 charge, m/z=698.33) *(bottom)*. Both peptides were eluted at the same retention time and are from affinity-enriched cultured human cell extract using anti-PRDX6 antibody. **B)** High resolution MS spectra of co-elution of peptides (DFT$P_{+31.990}$VCTTELGR, +2 charge, m/z=714.33) *(right)*, and its non-conversion counterpart (DFTPVCTTELGR, +2 charge, m/z=698.33) *(left)*. **C)** Protein sequence

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Example One

Identification of conversion of the proline (P) residue at amino acid 45 to glutamic acid (E) in human protein Peroxiredoxin-6
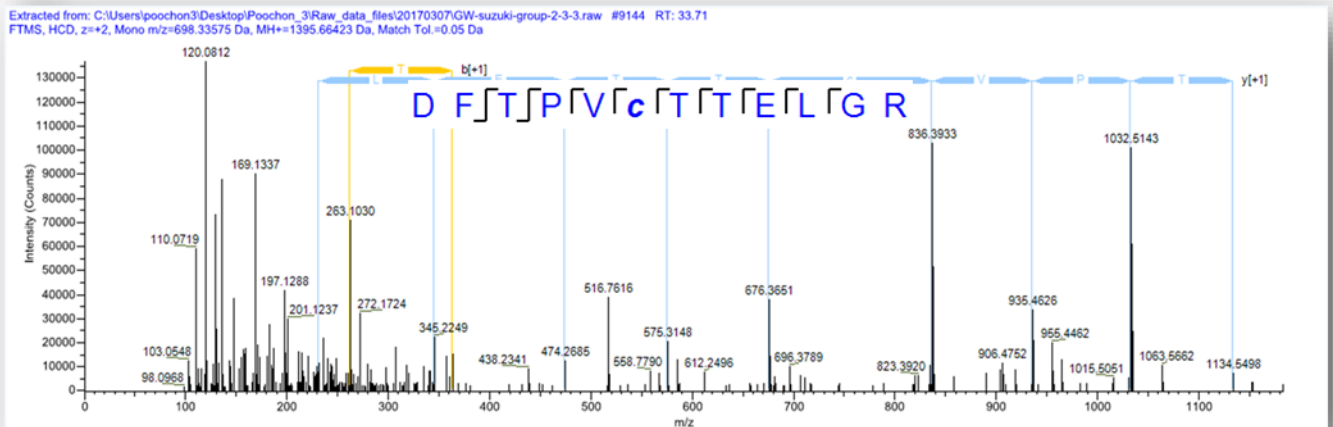
Figure 3: Nano LC-MS/MS verification of conversion of proline 45 to glutamic acid at PRDX6 (DFTP$_{+31.990}$VCTTELGR). **A)** High resolution MS/MS spectra of PRDX6 Glu conversion peptide (DFTP$_{+31.990}$VCTTELGR). **B,** High resolution MS/MS spectra of PRDX6 Pro 45 peptide (DFTPVCTTELGR). Spectrum was obtained by LC-MS/MS analysis using Thermo UltiMate 3000 RSLCnano System and Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer.

Example Two

Identification of Proteins from Exosome Vesicles Samples
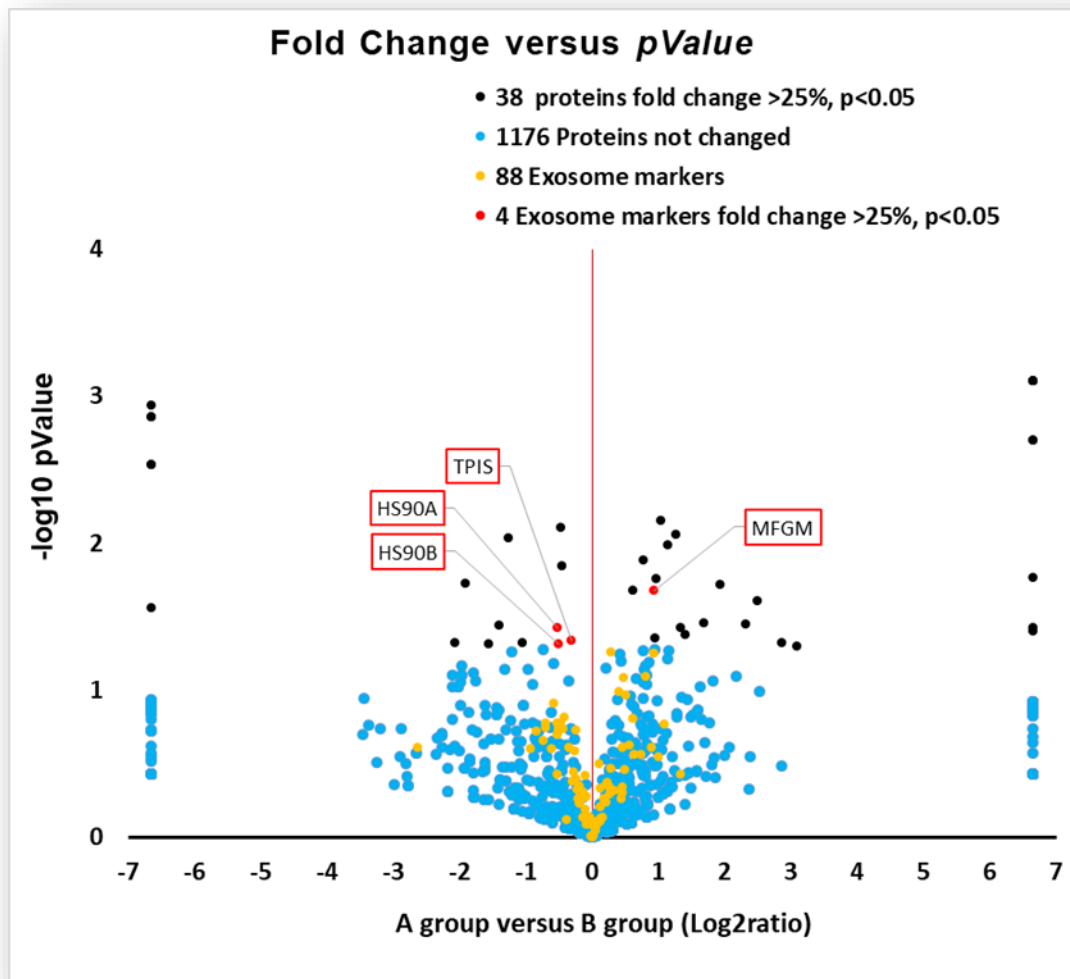
Figure 4: Volcano plot demonstrating the fold change of 1306 protein abundance between A group and B group (n=3). Two groups of 6 exosome samples were analyzed in this study by LC-MS/MS. 92 of 99 known human exosome markers annotated by Exocarta.org website were detected in this study (**Table 1**, *next page*). The x-axis represents the log2 of fold changes (A versus B), and the y-axis represents the statistically significant p-value (-log10 of p-value, n=3). Black dots represent 38 proteins fold change >25%, p<0.05, and red dots represent 4 exosome markers fold change >25%, p<0.05, yellow and blue dots represent 88 exosome markers and 1176 proteins. If any proteins are not detected in one group, the ratio (fold change) is assigned as 100 or 0.01.
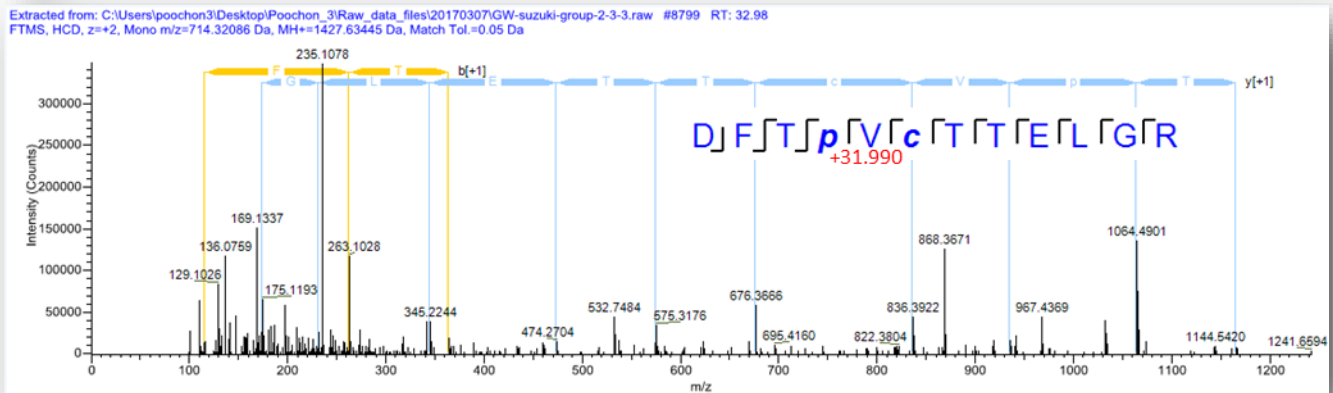
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Example Two

Identification of Proteins from Exosome Vesicles Samples

GeneSymbol	Note	Short Name	# PSM A1	# PSM B1	# PSM A2	# PSM B2	# PSM A3	# PSM B3
CD81	exosome marker (Exocarta)	CD81 antigen	7	4	19	4	4	6
CD63	exosome marker (Exocarta)	CD63 antigen	3	2	3	2	3	4
CD9	exosome marker (Exocarta)	CD9 antigen	1	1	10	2	2	3
SDCB1	exosome marker (Exocarta)	Syntenin-1	20	7	46	14	12	22
MFGM	exosome marker (Exocarta)	Lactadherin	166	100	346	97	177	127
HS90A	exosome marker (Exocarta)	Heat shock protein HSP 90-alpha	22	28	33	52	26	32
HS90B	exosome marker (Exocarta)	Heat shock protein HSP 90-beta	18	30	32	43	26	28
TKT	exosome marker (Exocarta)	Transketolase	1	2	1	15	2	2
HSP7C	exosome marker (Exocarta)	Heat shock cognate 71 kDa protein	43	44	70	61	41	48
1433B	exosome marker (Exocarta)	14-3-3 protein beta/alpha	12	13	15	14	15	15
1433E	exosome marker (Exocarta)	14-3-3 protein epsilon	27	30	31	34	36	39
1433F	exosome marker (Exocarta)	14-3-3 protein eta	6	10	10	10	10	9
1433G	exosome marker (Exocarta)	14-3-3 protein gamma	8	11	9	12	11	13
1433T	exosome marker (Exocarta)	14-3-3 protein theta	13	11	19	17	16	17
1433Z	exosome marker (Exocarta)	14-3-3 protein zeta/delta	16	23	31	20	24	24
4F2	exosome marker (Exocarta)	4F2 cell-surface antigen heavy chain	13	18	17	22	19	15
GRP78	exosome marker (Exocarta)	78 kDa glucose-regulated protein	6	9	7	13	7	6
ACTB	exosome marker (Exocarta)	Actin, cytoplasmic 1	110	103	128	110	128	108
SAHH	exosome marker (Exocarta)	Adenosylhomocysteinase	32	22	44	29	27	17
A2MG	exosome marker (Exocarta)	Alpha-2-macroglobulin	38	24	40	14	30	30
ACTN4	exosome marker (Exocarta)	Alpha-actinin-4	17	23	23	25	35	28
ENOA	exosome marker (Exocarta)	Alpha-enolase	34	43	55	76	45	30
ANXA1	exosome marker (Exocarta)	Annexin A1	4	10	8	23	12	9
ANX11	exosome marker (Exocarta)	Annexin A11	16	21	13	29	19	18
ANXA2	exosome marker (Exocarta)	Annexin A2	14	18	10	25	18	18
ANXA4	exosome marker (Exocarta)	Annexin A4	5	7	7	12	7	6
ANXA5	exosome marker (Exocarta)	Annexin A5	21	23	18	31	23	24
ANXA6	exosome marker (Exocarta)	Annexin A6	12	19	4	31	24	20
ACLY	exosome marker (Exocarta)	ATP-citrate synthase	11	9	10	12	9	6
BASI	exosome marker (Exocarta)	Basigin	9	12	23	13	16	12
CDC42	exosome marker (Exocarta)	Cell division control protein 42 homolog	7	5	15	4	5	8
CLIC1	exosome marker (Exocarta)	Chloride intracellular channel protein 1	4	6	10	5	6	6
CLH1	exosome marker (Exocarta)	Clathrin heavy chain 1	82	72	141	125	90	75
COF1	exosome marker (Exocarta)	Cofilin-1	8	6	11	8	9	8
EHD4	exosome marker (Exocarta)	EH domain-containing protein 4	4	4	9	5	4	4
EF1A1	exosome marker (Exocarta)	Elongation factor 1-alpha 1	23	29	37	38	22	25
EF2	exosome marker (Exocarta)	Elongation factor 2	28	26	48	38	20	25
STOM	exosome marker (Exocarta)	Erythrocyte band 7 integral membrane pr	10	13	13	9	10	11
EZRI	exosome marker (Exocarta)	Ezrin	18	22	24	16	20	19
FAS	exosome marker (Exocarta)	Fatty acid synthase	33	39	60	50	40	37
FLNA	exosome marker (Exocarta)	Filamin-A	34	34	40	35	48	33
FLOT1	exosome marker (Exocarta)	Flotillin-1	10	3	26	7	6	8
ALDOA	exosome marker (Exocarta)	Fructose-bisphosphate aldolase A	14	12	19	21	14	16
LG3BP	exosome marker (Exocarta)	Galectin-3-binding protein	51	18	76	24	37	39
G3P	exosome marker (Exocarta)	Glyceraldehyde-3-phosphate dehydroger	38	37	67	46	45	46
RAN	exosome marker (Exocarta)	GTP-binding nuclear protein Ran	4	3	11	7	5	7
GNAI2	exosome marker (Exocarta)	Guanine nucleotide-binding protein G(i) su	25	24	31	18	24	19
GBB1	exosome marker (Exocarta)	Guanine nucleotide-binding protein G(I)/C	11	15	32	11	13	17
GBB2	exosome marker (Exocarta)	Guanine nucleotide-binding protein G(I)/C	11	16	24	10	14	16
GNAS2	exosome marker (Exocarta)	Guanine nucleotide-binding protein G(s) s	16	17	16	17	12	14

Table 1: A list of 92 exosome protein markers identified from exosome samples

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GeneSymbol	Note	Short Name	# PSM A1	# PSM B1	# PSM A2	# PSM B2	# PSM A3	# PSM B3
IMB1	exosome marker (Exocarta)	Importin subunit beta-1	5	5	9	10	6	4
ITA6	exosome marker (Exocarta)	Integrin alpha-6	6	6	6	6	7	9
ITB1	exosome marker (Exocarta)	Integrin beta-1	8	10	20	10	14	11
LDHA	exosome marker (Exocarta)	L-lactate dehydrogenase A chain	14	12	22	19	16	20
LDHB	exosome marker (Exocarta)	L-lactate dehydrogenase B chain	17	22	26	25	23	18
LAMP2	exosome marker (Exocarta)	Lysosome-associated membrane glycopro	2	1	1	1	1	1
MOES	exosome marker (Exocarta)	Moesin	16	18	21	18	23	23
MOT1	exosome marker (Exocarta)	Monocarboxylate transporter 1	10	9	16	9	12	7
MYH9	exosome marker (Exocarta)	Myosin-9	42	37	61	36	53	37
PPIA	exosome marker (Exocarta)	Peptidyl-prolyl cis-trans isomerase A	5	7	10	8	7	9
PRDX1	exosome marker (Exocarta)	Peroxiredoxin-1	11	18	14	17	15	17
PRDX2	exosome marker (Exocarta)	Peroxiredoxin-2	7	7	7	15	10	8
PGK1	exosome marker (Exocarta)	Phosphoglycerate kinase 1	19	25	44	20	23	20
PROF1	exosome marker (Exocarta)	Profilin-1	5	2	11	14	6	6
PDC6I	exosome marker (Exocarta)	Programmed cell death 6-interacting prot	22	14	40	18	17	29
FPRP	exosome marker (Exocarta)	Prostaglandin F2 receptor negative regul	19	8	35	10	18	20
KPYM	exosome marker (Exocarta)	Pyruvate kinase PKM	29	29	66	49	30	33
GDIB	exosome marker (Exocarta)	Rab GDP dissociation inhibitor beta	17	17	34	31	18	16
RAC1	exosome marker (Exocarta)	Ras-related C3 botulinum toxin substrate	4	4	17	2	4	4
RAB14	exosome marker (Exocarta)	Ras-related protein Rab-14	5	4	3	4	6	2
RAB1A	exosome marker (Exocarta)	Ras-related protein Rab-1A	7	5	10	4	5	4
RAB5A	exosome marker (Exocarta)	Ras-related protein Rab-5A	3	4	7	5	3	9
RAB5B	exosome marker (Exocarta)	Ras-related protein Rab-5B	4	5	6	4	4	6
RAB5C	exosome marker (Exocarta)	Ras-related protein Rab-5C	8	14	11	12	10	14
RAB7A	exosome marker (Exocarta)	Ras-related protein Rab-7a	10	11	12	11	14	11
RAB8A	exosome marker (Exocarta)	Ras-related protein Rab-8A	7	6	8	6	11	6
RAP1B	exosome marker (Exocarta)	Ras-related protein Rap-1b	19	22	31	17	23	24
ALBU	exosome marker (Exocarta)	Serum albumin	17	17	21	14	14	14
AT1A1	exosome marker (Exocarta)	Sodium/potassium-transporting ATPase s	50	50	63	58	59	51
TCPA	exosome marker (Exocarta)	T-complex protein 1 subunit alpha	5	6	12	18	5	10
TCPB	exosome marker (Exocarta)	T-complex protein 1 subunit beta	8	9	17	26	7	9
TCPE	exosome marker (Exocarta)	T-complex protein 1 subunit epsilon	2	4	6	15	5	5
TCPG	exosome marker (Exocarta)	T-complex protein 1 subunit gamma	4	9	8	13	6	5
TSP1	exosome marker (Exocarta)	Thrombospondin-1	2	4	1	2	2	
TFR1	exosome marker (Exocarta)	Transferrin receptor protein 1	1	1	4	2	2	2
RHOA	exosome marker (Exocarta)	Transforming protein RhoA	6	4	18	5	6	8
TERA	exosome marker (Exocarta)	Transitional endoplasmic reticulum ATPas	26	20	26	34	23	26
TPIS	exosome marker (Exocarta)	Triosephosphate isomerase	11	13	16	21	15	16
TBA1A	exosome marker (Exocarta)	Tubulin alpha-1A chain	38	37	59	65	38	38
TBA1C	exosome marker (Exocarta)	Tubulin alpha-1C chain	43	43	63	70	42	42
TS101	exosome marker (Exocarta)	Tumor susceptibility gene 101 protein	5	4	9	2	2	4
UBA1	exosome marker (Exocarta)	Ubiquitin-like modifier-activating enzyme	14	15	16	25	15	11

Table 1: *(continued)*