A Survey of Membrane Proteins in Human Serum

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Abstract: Serum and membrane proteins are two of the most attractive targets for proteomic analysis. Previous membrane protein studies tend to focus on tissue sample, while membrane protein studies in serum are still limited. In this study, an analysis of membrane proteins in normal human serum was carried out. Nano-liquid chromatography-electrospray ionization mass spectrometry (NanoLC-ESI-MS/MS) and bioinformatics tools were used to identify membrane proteins. Two hundred and seventeen membrane proteins were detected in the human serum, of which 129 membrane proteins have at least one transmembrane domain (TMD). Further characterizations of identified membrane proteins including their subcellular distributions, molecular weights, post translational modifications, transmembrane domains and average of hydrophobicity, were also implemented. Our results showed the potential of membrane proteins in serum for diagnosis and treatment of diseases.

Keywords: membrane proteins, proteomics, serum, NanoLC-ESI-MS/MS
Introduction
As the most easily obtained sample from patients, blood plasma is the primary specimen used to diagnose and monitor many diseases. Because changes in certain components of the plasma are indicative of abnormalities in the body system, many researches have been carried out in search for disease biomarkers in the plasma since the early history of clinical study. After proteomics emerged as a major discipline at the end of the 20th century, plasma study was launched in this new research direction, which could potentially analyze hundreds of proteins at the same time, instead of the one-protein study allowed by traditional genetic/biochemical approach.

The plasma contains thousands of proteins, including those originating from most, if not all kinds of cells and tissues. The plasma is considered one of the most promising proteomes for proteomic research, and many proteomic techniques and methods have been utilized and modified to analyze the human plasma. In an early study of human plasma, Pieper et al could identify 325 proteins using two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS). Advancements in the field of proteomics in the past decade have assisted plasma studies in both breadth and depth, exemplified by the forming of a human plasma Peptide Atlas by researchers participating in the Plasma Proteome Project of the Human Proteome Organization (HUPO). Comparative studies of the plasma have revealed many potential markers and some insights into the mechanisms of tissue specific diseases, such as cancers, Alzheimer’s disease, and myocardial infarction.

Membrane proteomics is a highly focused branch of proteomics. Approximately 30% of proteins encoded by the mammalian genome are transmembrane proteins. Membrane proteins play an important part in many important cellular processes, including cross-membrane transportation, cell adhesion, signal transduction, immune response, etc. Due to their roles as transporters, receptors and structural proteins as well as their impacts on intracellular processes, they are the usual candidates for drug development: about 60% of approved drugs target membrane proteins. Many membrane proteins were also found to directly associate with various human diseases, such as Alzheimer’s disease, diabetes, Hodgkin’s disease and liver cirrhosis. Thus, researches on membrane proteins also hold promises for developments of diagnosis and disease treatment.

The recent studies on human and mammalian membrane proteins tend to focus on cell/tissue samples. Using carbonate extraction, trypsin digestion and NanoLC-MS/MS, a multilaboratory project has been found to profile membrane proteins from mouse liver. Another study based on similar approaches, more than 200 proteins were detected in pancreatic cancer cells. Furthermore, a large number (862) of membrane proteins were identified in the brain cortex. However, we have not found any independent research that focus only on membrane proteins in serum. As a subset of the plasma, serum contains thousands of proteins, including the proteins of membrane origin, even though their abundance can be low. In this study, some results of profiling and characterization of membrane proteins in human serum by using combination of NanoLC-ESI-MS/MS methods and bioinformatics tools are shown. The present study was approved by the Ethics Committee of the Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology (VAST).

Materials and Methods
Materials
Dithiothreitol (DTT), iodoacetamide (IAA), ammonium bicarbonate, ammonium acetate, trypsin (proteomics sequencing grade), sodium bicarbonate and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid (FA) and triflouracetate (TFA) were obtained from Fluka (Fluka Chemie GmbH, Buchs, Switzerland). Acetonitrile (ACN, chromatogram grade) and other chemicals (analytical grade) were obtained from Barker (Pittsburgh, USA). The Bradford assay kit, Aurum serum protein mini kit, acrylamide, bis-acrylamide, urea, glycine, Tris, CHAPS, and SDS were purchased from Bio-Rad (Hercules, CA, USA). All equipment and standard reagents used directly should be clean as necessary.

Sample preparation
Sera from healthy middle-aged individuals (20–40 years old) were supplied by Bach Mai Hospital, Hanoi, Vietnam, and stored at −80 °C until analysis. After that, albumin and IgG were depleted from the samples using the Aurum serum protein mini kit (Bio-Rad...
Laboratories, Hercules, CA, USA). Briefly, serum protein column was washed with 1 ml of serum protein binding buffer. For each sample, 60 µl of serum was diluted with 180 µl serum protein binding buffer and then, 200 µl of diluted serum was loaded onto the column. Subsequently, the depleted serum – unbound fraction was collected by centrifugation at 10,000 × g for 20 sec. The removal of albumin and IgG was evaluated by 12.6% SDS-PAGE.

**In-solution trypsin digestion**

The depleted sera containing approximately 30 µg of total protein were dried by vacuum centrifugation and then dissolved in 50 mM NH₄HCO₃. The samples were reduced by 10 mM dithiothreitol (DTT) at 56 °C for 30 min and alkylated by 5 mM iodoacetamide (IAA) at room temperature for 1 hr. Proteins in the samples were digested using trypsin (Sequencing grade, Sigma-Aldrich, St. Louis, MO, USA) at ratio of 1:50 (w/w) enzyme to protein at 37 °C for 12 hrs. The digestion was stopped by formic acid with final concentration of 0.1%.

**Nano two-dimensional chromatography and mass spectrometry**

Tryptic peptides were dissolved in 0.1% formic acid and loaded onto Strong Cation Exchange (SCX) Chromatography Column (LC Packing, Dionex, The Netherlands) for separation in the first dimension. The second dimension was performed using a C18 Reversed Phase (RP) Column (GraceVydac, Hesperia, CA, USA) with mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in 85% acetonitrile (B). Peptides were eluted in a linear gradient from 0% to 100% mobile phase B at a flow rate of 0.2 µl/min for 90 min.

Tandem mass spectrometry analysis were performed using an ABI QSTAR®XL hybrid quadrupole/TOF MS/MS instrument (Applied Biosystems/MDS Sciex, Ontario, Canada) equipped with a nanoelectrospray source (Protana XYZ manipulator). Positive mode nanoelectrospray was generated from fused-silica PicoTip emitters with a 10 µm aperture (New Objective, Woburn, MA) at 2.5 kV. MS and MS/MS spectra were recorded and processed in IDA mode (Information Dependent Acquisition) controlled by Analyst QS software. The range of the MS full scan was from 200 to 1500 amu followed by MS/MS fragmentation of the three most intense precursor ions. The dynamic ion selection threshold for MS/MS experiments was set to 45 counts.

**Identification of membrane proteins**

The obtained MS and MS/MS spectra were searched against the NCBInr and the Swiss-Prot protein sequence database using Mascot™ V1.8 software (Matrix Science Ltd., London, UK). The parameters for searching were set as following: enzymatic digestion with trypsin with one potential missed cleavage; a peptide and fragment mass tolerance of ±0.5; carbamidomethyl (cysteine) as fixed modification; oxidation (methionine) as variable modification. Protein identifications were performed using a Mowse scoring algorithm with a confidence level of 95% and with at least two matched peptides. For further verification, proteins were validated using the open-source software MSQuant v 1.5 (http://msquant.sourceforge.net/).

Membrane proteins were sorted from total identified serum proteins based on UniProt protein database (http://www.uniprot.org). SOSUI prediction algorithm was used to predict transmembrane domains and average values of hydrophobicity of those membrane proteins.20

**Results**

**Identification of membrane proteins in serum**

The major aim of this study is to detect and profile membrane proteins from human serum. With the strategy and methods described above, 217 membrane proteins were detected from 2778 matched peptides (see Table 1, Supplementary Data). It is interesting to note that more than 90% of the identifications were based on 3 or more matched peptides. From the total of 217 identified membrane proteins, 129 proteins have at least one transmembrane domain (TMD) based on SOSUI prediction algorithm (see Table 1, Supplementary Data).

**Evaluation of membrane protein molecular weight**

The distribution of molecular weights of membrane proteins from serum was shown in Figure 1. Membrane
proteins in serum have a wide range of molecular weights. Proteins with molecular weights from 100–200 kDa make up the largest group, accounting for 43% the total of 217 identified membrane proteins. The second most abundant is the group of proteins with molecular weights in the range of 200–300 kDa (26%). It is also notable that several membrane proteins (6%) have molecular weights above 500 kDa.

Transmembrane domains (TMDs) and average of hydrophobicity
TMD is one of the most distinguished features of membrane proteins. In our study, 129 (59.4%) proteins were predicted to have at least one TMD in the total of 217 membrane proteins according to SOSUI prediction algorithm. The majority of these 129 proteins have one TMD (25 proteins) and two TMDs (40 proteins), but a considerable number have more than 8 TMDs, which are often receptors, transporters or ion channels.

In this study, SOSUI prediction algorithm was also used to evaluate the hydrophobicity of membrane proteins from human serum. The average hydrophobicity values of all identified membrane proteins were calculated based on their amino acid sequences. It is notable that almost all proteins have average hydrophobicity values below zero. Only 17 proteins have positive average hydrophobicity values and most of them (16 proteins) are integral membrane proteins or transmembrane proteins. In addition, 7 UniProt annotated membrane proteins could not be analyzed by SOSUI prediction algorithm because their sizes have more than 5000 amino acids.

Subcellular distribution of membrane proteins
The subcellular distribution of 217 identified membrane proteins was determined using UniProt database according to their accession numbers. Analyzing the available information about the identified membrane proteins, we found that, these proteins have a certain distribution or are shuttled between organelles: 119 proteins are from plasma membrane and a range of proteins are from other cell components, such as nucleus (11 proteins), endoplasmic reticulum (16 proteins), Golgi apparatus (23 proteins), and mitochondrion (7 proteins).

Post-translational modifications of membrane proteins
In this study, 359 post-translational modifications (PTMs) of membrane proteins were found in 187 proteins based on UniProt database; the other 30 membrane proteins do not have post translational modifications. Among the total of membrane proteins having PTMs, proteins with one PTM (77 proteins – 41.2%) and two PTMs (66 proteins – 35.3%) are the majority, while proteins with 5 PTMs make up the smallest group (4 proteins – 2.1%).

Among 359 modifications found in the identified membrane proteins, phosphorylation was the most common modification, with 136 phosphoproteins. Follow-up was glycosylation, with 96 glycoproteins. The third and fourth common groups were proteins with disulfide bonds (48 proteins) and acetylation sites.
(34 proteins). Lipoproteins, palmitoylated proteins, nitrated proteins, ubiquitinated proteins... contributed to a small fraction of proteins with modifications (Fig. 3).

**Discussions**

In membrane proteomics, former studies are often inclined to use tissue/cell samples. Using membrane protein extraction by organic acid and NanoLC-MS/MS, Da Cruz et al found 182 membrane proteins from mouse liver mitochondrial inner membrane. Another study used membrane protein extraction from pancreatic cancer cells, SDS-PAGE, in-gel digestion and NanoLC-ESI-MS/MS and detected more than 200 proteins. In addition, a large number (862) of membrane proteins were found in the brain cortex. The difference in the number of detected membrane proteins from various tissue types can be due to the specificity of each tissue, as well as fractionation and enrichment methods of each study. Combining data from different sources, an early survey of the plasma by Anderson et al found 212 proteins with at least one TMD with multiple origins, which is similar to our results. Probably, there might be more membrane proteins in the serum that have not been detected yet because of their low abundance compared to other serum proteins.

Phosphorylation and glycosylation are the two most common modifications of protein in animal cells. Phosphorylation modifies the structure and function of many proteins. An upset balance between phosphorylation and dephosphorylation is the cause of many diseases. Glycosylation was estimated to be found in over 50% of human proteins, and also of interest because some diseases were found to be related to glycan structural alterations. Our results with the high proportion of phosphoproteins and glycoproteins showed the potential of membrane proteins in serum for diagnosis and treatment of disease.

**Conclusion**

By using NanoLC-ESI-MS/MS technologies and bioinformatics tools, a data set of 217 membrane proteins from normal human serum was identified. Some characterization such as subcellular distribution, molecular weight, post translational modification, transmembrane domain (TMD) and average of hydrophobicity of the identified proteins were also given. 129 proteins (59%) have at least one transmembrane domain, and 187 proteins (86%) have post-translational modifications.

**Author Contributions**

Conceived and designed the experiments: PVC. Analysed the data: NTD. Wrote the first draft of the manuscript: PVC, NTD. Contributed to the writing of the manuscript: PVC, NTD. Agree with manuscript results and conclusions: PVC, NTD. Jointly developed the structure and arguments for the paper: PVC, NTD. Made critical revisions and approved final version: PVC, NTD. All authors reviewed and approved of the final manuscript.

**Acknowledgments**

The work was carried out at the National Key Laboratory of Gene Technology (NKLGT), Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology (VAST).

**Funding**

The work was funded by National Foundation for Science and Technology Development (NAFOSTED).

**Competing Interests**

The author declares no conflicts of interest

**Disclosures and Ethics**

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References

1. Anderson NL, Polanski M, Peiper R, et al. The human plasma proteome: A nonredundant list developed by combination of four separate sources. Mol Cell Proteomics. 2004;3:311–26.
2. Pernemalm M, Lewensohn R, Lehtiö J. Affinity prefractionation for MS-based plasma proteomics. Proteomics. 2009;9(6):1420–7.
3. Pieper R, Gatlin CL, Makusky AJ, et al. The human serum proteome: Display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins. Proteomics. 2003;3(7):1345–64.
4. Farrah T, Deutsch EW, Omenn GS, et al. A high-confidence human plasma proteome reference set with estimated concentrations in Peptide Atlas. Mol Cell Proteomics. In press.
5. Hanash SM, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. Nature. 2008;452(7187):571–9.
6. Hye A, Lynham S, Thambisetty M, et al. Proteome-based plasma biomarkers for Alzheimer’s disease. Brain. 2006;129(11):3042–50.
7. Marshall J, Kapp EA, Fenyö D, et al. The Asia Oceania Human Proteome Organisation Membrane Proteomics Initiative. Preparation and characterisation of the carbonate-washed membrane standard. Proteomics. 2010;10(22):4142–8.
8. Sprenger RR, Jensen ON. Proteomics and the dynamics plasma membrane: Quo Vadis? Proteomics. 2010;10(22):3997–4011.
9. Santoni V, Molloy M, Rabilloud T. Membrane proteins and proteomics: Un amour impossible? Electrophoresis. 2000;21(6):1054–70.
10. Wu CC, Yates JR. The application of mass spectrometry to membrane proteomics. Nat Biotechnol. 2003;21(3):262–7.
11. Hopkins AL, Groom CR. The druggable genome. Nat Rev Drug Discov. 2002;1(9):727–30.
12. Josic D, Clifton JG. Mammalian plasma membrane proteomics. Proteomics. 2007;7(16):3010–29.
Supplementary Data
Table 1. List of identified membrane proteins in human serum.

| No. | Accession number | Protein name                                                                 | Score | Peptide match | Molecular weight |
|-----|------------------|------------------------------------------------------------------------------|-------|---------------|------------------|
| 1   | Q8TC27           | A disintegrin and metalloprotease domain 32                                  | 43    | 5             | 90328            |
| 2   | Q9H2U9           | A disintegrin and metalloproteinase 7                                         | 35    | 9             | 88036            |
| 3   | Q9B2C7           | ABC transporter ABCA2                                                         | 61    | 16            | 272140           |
| 4   | Q8IZY2           | ABC transporter ABCA7                                                         | 46    | 6             | 236328           |
| 5   | Q97S58           | ABC3                                                                         | 41    | 23            | 192862           |
| 6   | P00519           | Abl protein                                                                  | 46    | 3             | 123852           |
| 7   | O00763           | Acetyl-CoA carboxylase                                                        | 60    | 6             | 281569           |
| 8   | O00763           | Acetyl-CoA carboxylase 2                                                      | 54    | 6             | 273861           |
| 9   | Q14617           | Adapter-related protein complex 3 delta 1 subunit variant                    | 62    | 9             | 145094           |
| 10  | Q9S996           | Adenomatosis polyposis coli 2                                                | 49    | 24            | 245966           |
| 11  | Q13813           | Alpha II spectrin                                                            | 70    | 17            | 285689           |
| 12  | P02763           | Alpha-1-acid Glycoprotein 1 precursor                                        | 90    | 37            | 23579            |
| 13  | Q9UHC3           | Amiloride-sensitive cation channel 3 isoform c                               | 48    | 8             | 61376            |
| 14  | Q07837           | Amino acid transport protein                                                  | 56    | 11            | 79240            |
| 15  | Q07075           | Aminopeptidase A                                                             | 50    | 15            | 109689           |
| 16  | Q5Y190           | Anchor protein                                                                | 53    | 7             | 458487           |
| 17  | Q9S6Q91          | Anion exchanger AE4                                                           | 47    | 4             | 105149           |
| 18  | Q9P2R3           | ANKHZN                                                                      | 54    | 7             | 129534           |
| 19  | P02647           | Apolipoprotein A-I preproprotein                                             | 245   | 45            | 30759            |
| 20  | Q12797           | Aspartyl(asparaginyl) beta-hydroxylase; HAAH                                  | 42    | 3             | 86294            |
| 21  | Q86UQ4           | ATP binding cassette, sub-family A (ABC1), member 13                          | 50    | 12            | 580524           |
| 22  | Q9S6J66          | ATP-binding cassette protein C11 isoform A                                   | 40    | 18            | 151566           |
| 23  | Q9S6J66          | ATP-binding cassette transporter MRP8                                         | 47    | 5             | 155872           |
| 24  | Q9HC28           | ATP-binding cassette, sub-family A, member 2 isoform b                       | 77    | 39            | 275244           |
| 25  | Q9S2887          | ATP-binding cassette, sub-family C (CFTR/MRP), member 2                       | 36    | 68            | 175237           |
| 26  | Q9UMD9           | Autoantigen                                                                  | 55    | 8             | 154929           |
| 27  | Q13563           | Autosomal dominant polycystic kidney disease type II protein                 | 47    | 4             | 110462           |
| 28  | P50851           | Beige-like protein; CDC4L protein                                             | 57    | 18            | 320076           |
| 29  | Q76KP1           | Beta-1,4-N-acetyl-galactosaminyl transferase 4                               | 39    | 10            | 116954           |
| 30  | Q01082           | Beta-spectrin                                                                | 43    | 10            | 275259           |
| 31  | Q01082           | Beta-spectrin 2 isoform 2                                                    | 39    | 8             | 251948           |
| 32  | Q15413           | Brain ryanodine receptor                                                      | 73    | 24            | 556937           |
| 33  | Q14514           | Brain-specific angiogenesis inhibitor 1                                       | 51    | 14            | 176900           |
| 34  | O60241           | Brain-specific angiogenesis inhibitor 2                                       | 70    | 11            | 174738           |
| 35  | O60242           | Brain-specific angiogenesis inhibitor 3                                       | 59    | 7             | 176123           |
| 36  | Q9HCQ4           | Cadherin EGF LAG seven-pass G-type receptor 2                                | 44    | 7             | 322214           |
| 37  | Q9NYQ7           | Cadherin EGF LAG seven-pass G-type receptor 3                                | 51    | 10            | 362937           |
| 38  | Q9H251           | Cadherin-22                                                                  | 74    | 6             | 370095           |
| 39  | Q59FJ3           | Calcium channel, voltage-dependent, N type, alpha 1B subunit                 | 49    | 8             | 264553           |
| 40  | Q9HCF6           | Calcium-permeable store-operated channel TRPM3b                              | 72    | 11            | 180008           |
| 41  | Q08499           | cAMP-specific phosphodiesterase HPDE4D3 variant                              | 42    | 6             | 76872            |
| 42  | Q96P48           | Centaurin delta 2 isoform a variant                                          | 41    | 9             | 166159           |
| Subcellular location                              | PTM                                           | Hydrophobicity value | TMDs |
|--------------------------------------------------|------------------------------------------------|----------------------|------|
| Unclear                                          | Disulfide bond, Glycoprotein, Phosphoprotein   | −0.312452            | 2    |
| Unclear                                          | Disulfide bond, Glycoprotein                   | −0.414589            | 2    |
| Membrane                                         | Glycoprotein, Phosphoprotein                   | −0.065216            | 14   |
| Plasma membrane, Endosome membrane, Golgi apparatus membrane | Disulfide bond, Glycoprotein | 0.055080           | 12   |
| Plasma membrane                                  | Phosphoprotein                                 | 0.089026             | 13   |
| Nucleus membrane                                 | Lipoprotein, Myristate, Phosphoprotein         | −0.597169            | 0    |
| Endomembrane                                     | Phosphoprotein                                 | −0.227218            | 1    |
| Endomembrane                                     | Phosphoprotein                                 | −0.227218            | 1    |
| Golgi apparatus membrane                         | Phosphoprotein                                 | −0.491761            | 1    |
| Golgi apparatus membrane                         | Unknown                                        | −0.529135            | 0    |
| Extracellular space                              | Acetylation, Phosphoprotein                    | −0.790330            | 0    |
| Unclear                                          | Disulfide bond, Glycoprotein, Lipoprotein, Palmitate, Phosphoprotein | −0.535821 | 1 |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein   | −0.265348            | 2    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein                   | −0.441082            | 1    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein   | −0.390491            | 1    |
| Plasma membrane                                  | Unknown                                        | −0.188033            | 20   |
| Plasma membrane                                  | Glycoprotein, Phosphoprotein                   | 0.143947             | 11   |
| Endosome membrane                                | Acetylation                                    | −0.154490            | 0    |
| Plasma membrane                                  | Glycation, Glycoprotein, Phosphoprotein        | −0.717228            | 0    |
| Endoplasmic reticulum membrane                   | Glycoprotein, Phosphoprotein                   | −0.788128            | 1    |
| Plasma membrane                                  | Unknown                                        | −0.010508            | 14   |
| Plasma membrane                                  | Glycoprotein                                   | 0.191607             | 12   |
| Plasma membrane                                  | Glycoprotein                                   | 0.191607             | 12   |
| Lyososome membrane                               | Unknown                                        | −0.064204            | 14   |
| Plasma membrane                                  | Glycoprotein, Phosphoprotein                   | 0.091068             | 13   |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Hydroxylation, Phosphoprotein | −0.573080 | 1 |
| Endoplasmic reticulum membrane                   | Glycoprotein                                   | −0.328512            | 8    |
| Plasma membrane                                  | Acetylation, Phosphoprotein                    | −0.201817            | 0    |
| Golgi apparatus membrane                         | Glycoprotein                                   | −0.597691            | 0    |
| Plasma membrane                                  | Acetylation, Glycoprotein, Phosphoprotein      | −0.766116            | 0    |
| Plasma membrane                                  | Acetylation, Glycoprotein, Phosphoprotein      | −0.766116            | 0    |
| Endoplasmic reticulum                            | Glycoprotein                                   | −0.278727            | 5    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein   | −0.311806            | 8    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein   | −0.211924            | 9    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein   | −0.211863            | 9    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Hydroxylation, Phosphoprotein | −0.270751 | 8 |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Hydroxylation     | −0.271258            | 8    |
| Plasma membrane                                  | Glycoprotein                                   | −0.142278            | 2    |
| Unclear                                          | Unknown                                        | −0.264964            | 12   |
| Unclear                                          | Unknown                                        | −0.302195            | 6    |
| Unclear                                          | Phosphoprotein                                 | −0.674043            | 0    |
| Plasma membrane, Golgi apparatus, Membrane       | Phosphoprotein                                 | −0.422553            | 0    |

(Continued)
### Table 1. (Continued)

| No. | Accession number | Protein name                                                                 | Score | Peptide match | Molecular weight |
|-----|------------------|------------------------------------------------------------------------------|-------|---------------|------------------|
| 43  | Q15700           | Channel associated protein of synapse                                         | 35    | 8             | 97896            |
| 44  | Q96QT4           | Channel-kinase 1                                                             | 65    | 11            | 214583           |
| 45  | P51788           | Chloride channel protein 2                                                   | 41    | 8             | 99402            |
| 46  | P00751           | Complement factor B prepropeptide                                             | 175   | 13            | 86847            |
| 47  | P17927           | Complement receptor 1                                                        | 38    | 8             | 230417           |
| 48  | P10606           | COX5B                                                                        | 64    | 178           | 13914            |
| 49  | Q7Z407           | CUB and Sushi multiple domains 3 isoform 1                                   | 36    | 13            | 414007           |
| 50  | Q8IY37           | DEAH (Asp-Glu-Ala-His) box polypeptide 37                                    | 40    | 4             | 130547           |
| 51  | Q6IC98           | Death-inducing-protein                                                       | 49    | 14            | 66766            |
| 52  | Q14185           | Dedicator of cytokinesis 1                                                   | 52    | 6             | 216208           |
| 53  | Q5VWQ8           | Disabled homolog 2 interacting protein isoform 2                             | 37    | 8             | 118547           |
| 54  | Q75923           | Dysferlin isoform 8                                                          | 43    | 4             | 239254           |
| 55  | P22413           | Ectonucleotide pyrophosphatase/phosphodiesterase 1                           | 36    | 6             | 107024           |
| 56  | Q8WY5P           | ELYS transcription factor-like protein TMBS62                                 | 43    | 4             | 258191           |
| 57  | Q541P7           | EPH receptor B4 precursor                                                    | 35    | 4             | 109741           |
| 58  | Q15375           | Ephrin receptor EphA7                                                        | 55    | 10            | 113735           |
| 59  | Q15375           | Ephrin receptor EphA7 variant                                                | 61    | 10            | 113994           |
| 60  | P54762           | Ephrin receptor EphB1 precursor                                             | 45    | 20            | 111297           |
| 61  | P29323           | Ephrin type-B receptor 2                                                     | 36    | 11            | 119128           |
| 62  | Q9NZJ5           | Eukaryotic translation initiation factor 2-alpha kinase 3                    | 48    | 11            | 126095           |
| 63  | Q16099           | Excitatory amino acid receptor 1; kainate receptor subunit EAA1              | 41    | 9             | 108529           |
| 64  | Q86X4X           | Extracellular matrix protein FRAS1                                            | 45    | 10            | 453936           |
| 65  | Q9NYQ8           | FAT tumor suppressor 2 precursor                                             | 44    | 7             | 482097           |
| 66  | Q6VO17           | Fat-like cadherin FATJ protein                                               | 43    | 10            | 354108           |
| 67  | Q9NZM1           | Fer-1 like protein 3                                                          | 66    | 11            | 234737           |
| 68  | Q59F30           | Fibroblast growth factor receptor 4 variant                                  | 38    | 5             | 114772           |
| 69  | P42345           | FK506 binding protein 12-rapamycin associated protein 1                      | 40    | 10            | 290759           |
| 70  | Q9YZ27           | FYVE finger-containing phosphoinositide kinase                               | 66    | 18            | 239581           |
| 71  | Q86SQ6           | G protein-coupled receptor 123                                              | 35    | 6             | 139868           |
| 72  | QST848           | G protein-coupled receptor 158                                               | 53    | 8             | 136886           |
| 73  | Q75899           | GABBR2 protein                                                               | 42    | 5             | 100861           |
| 74  | Q14789           | Giantin                                                                     | 44    | 13            | 377273           |
| 75  | P42261           | Glutamate receptor type 1                                                    | 42    | 7             | 102270           |
| 76  | Q9Y3R0           | Glutamate receptor-interacting protein 1                                     | 46    | 5             | 123203           |
| 77  | Q14789           | Golgi antigen gcp372                                                         | 44    | 8             | 373440           |
| 78  | Q08378           | Golgi autoantigen, golgin subfamily a, 3                                     | 69    | 17            | 167765           |
| 79  | Q08378           | Golgin-160                                                                  | 57    | 16            | 167810           |
| 80  | Q6PRD1           | GPR158-like 1 receptor                                                       | 54    | 11            | 260635           |
| 81  | Q8WXG9           | G-protein coupled receptor 98                                                | 69    | 21            | 694181           |
| 82  | Q8IWJ2           | GRIP and coiled-coil domain-containing 2 isoform a                           | 53    | 15            | 196873           |
| 83  | O15068           | Guanine nucleotide exchange factor DBS                                        | 37    | 4             | 129340           |
| 84  | P52272           | Heterogeneous nuclear ribonucleoprotein M isoform a                         | 44    | 13            | 77749            |
| 85  | O43166           | High-risk human papilloma viruses E6 oncoproteins targeted protein E6TP1 alpha; putative GAP protei | 59    | 12            | 198570           |
| Subcellular location                           | PTM                                                                 | Hydrophobicity value | TMDs |
|-----------------------------------------------|----------------------------------------------------------------------|----------------------|------|
| Plasma membrane                               | Lipoprotein, Palmitate, Phosphoprotein                               | −0.519310            | 0    |
| Plasma membrane                               | Phosphoprotein                                                       | −0.270456            | 6    |
| Plasma membrane                               | Unknown                                                              | 0.132517             | 11   |
| Plasma membrane                               | Acetylation                                                          | −0.501047            | 0    |
| Plasma membrane                               | Disulfide bond, Glycoprotein, Pyrrolidone carboxylic acid            | −0.320010            | 3    |
| Mitochondrion membrane                        | Acetylation                                                          | −0.297674            | 0    |
| Plasma membrane                               | Disulfide bond, Glycoprotein                                         | −0.260239            | 1    |
| Unclear                                       | Unknown                                                              | −0.415385            | 0    |
| Mitochondrion membrane                        | Phosphoprotein                                                       | −0.431833            | 3    |
| Unclear                                       | Phosphoprotein                                                       | −0.430617            | 0    |
| Plasma membrane                               | Phosphoprotein                                                       | −0.578302            | 0    |
| Vesicle                                       | Phosphoprotein                                                       | −0.400145            | 1    |
| Plasma membrane                               | Disulfide bond, Glycoprotein                                         | −0.456000            | 1    |
| Nuclear membrane                              | Acetylation, Phosphoprotein                                          | −0.448765            | 0    |
| Plasma membrane                               | Unknown                                                              | −0.235562            | 2    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.284268            | 2    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.284268            | 2    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.214431            | 2    |
| Plasma membrane                               | Lipoprotein, Myristate, Palmitate                                    | −0.293460            | 2    |
| Endoplasmic reticulum membrane                | Glycoprotein, Phosphoprotein                                         | −0.450717            | 2    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.046653            | 4    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.295608            | 2    |
| Plasma membrane                               | Disulfide bond, Glycoprotein, Phosphoprotein                         | −0.249645            | 2    |
| Plasma membrane                               | Disulfide bond, Glycoprotein, Phosphoprotein                         | −0.262940            | 2    |
| Nuclear membrane, plasma membrane             | Acetylation, Phosphoprotein                                          | −0.456091            | 1    |
| Unclear                                       | Unknown                                                              | 0.017312             | 6    |
| Golgi membrane, endoplasmic reticulum membrane| Acetylation, Phosphoprotein                                          | −0.192586            | 0    |
| membrane, mitochondrial outer membrane         |                                                                     |                      |      |
| Endosome membrane                             | Acetylation, Phosphoprotein                                          | −0.534366            | 0    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.317749            | 6    |
| Plasma membrane                               | Glycoprotein, Isopeptide bond, Phosphoprotein, Ubl conjugation       | −0.480988            | 7    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.149734            | 8    |
| Golgi apparatus membrane                      | Disulfide bond, Phosphoprotein                                       | −0.936847            | 1    |
| Plasma membrane, endoplasmic reticulum membrane| Glycoprotein, Lipoprotein, Palmitate, Phosphoprotein                 | −0.199227            | 5    |
| Endoplasmic reticulum membrane                | Phosphoprotein                                                       | −0.362500            | 0    |
| Plasma membrane, endoplasmic reticulum membrane| Phosphoprotein                                                       | −0.362500            | 0    |
| Golgi apparatus membrane                      | Disulfide bond, Phosphoprotein                                       | −0.936847            | 1    |
| Golgi apparatus membrane                      | Acetylation, Phosphoprotein                                          | −0.834781            | 0    |
| Golgi apparatus membrane                      | Acetylation, Phosphoprotein                                          | −0.834781            | 0    |
| Plasma membrane                               | Glycoprotein, Phosphoprotein                                         | −0.583101            | 7    |
| Plasma membrane                               | Unknown                                                              | Too long             | 0    |
| Golgi apparatus membrane                      | Phosphoprotein                                                       | −0.918825            | 0    |
| Plasma membrane                               | Phosphoprotein                                                       | −0.552067            | 0    |
| Plasma membrane                               | Acetylation, Phosphoprotein, Ubl conjugation                         | −0.341643            | 0    |
| Plasma membrane                               | Phosphoprotein                                                       | −0.671840            | 0    |

(Continued)
| No. | Accession number | Protein name                                                | Score | Peptide match | Molecular weight |
|-----|------------------|-------------------------------------------------------------|-------|---------------|------------------|
| 86  | P35523           | Human CIC-1 muscle chloride channel                         | 37    | 7             | 109696           |
| 87  | Q14643           | Human type 1 inositol 1,4,5-trisphosphate receptor          | 43    | 8             | 309942           |
| 88  | Q9HCF6           | Hypothetical protein                                        | 72    | 10            | 175211           |
| 89  | Q9P1Z9           | Hypothetical protein                                        | 43    | 14            | 199652           |
| 90  | A6N173           | Immunoglobulin-like transcript 11 protein                  | 39    | 4             | 27186            |
| 91  | Q14571           | Inositol 1,4,5-trisphosphate receptor type 2               | 50    | 6             | 311074           |
| 92  | Q6P9B9           | Integrator complex subunit 5                               | 38    | 5             | 109239           |
| 93  | Q15811           | Integrin alpha long form                                   | 40    | 7             | 196293           |
| 94  | Q9H4E7           | IRF4-binding protein                                       | 48    | 7             | 74422            |
| 95  | Q25058           | KIAA0233                                                   | 50    | 17            | 234888           |
| 96  | Q13023           | KIAA0311                                                   | 40    | 7             | 259702           |
| 97  | Q6P48            | KIAA0782 protein                                           | 45    | 4             | 143525           |
| 98  | Q9Y2H9           | KIAA0973 protein                                           | 61    | 34            | 173192           |
| 99  | Q96RV3           | KIAA0995 protein                                           | 42    | 7             | 109401           |
| 100 | Q9BZ29           | KIAA1058 protein                                           | 42    | 7             | 241774           |
| 101 | Q5T848           | KIAA1136 protein                                           | 49    | 11            | 66642            |
| 102 | Q5T4S7           | KIAA1307 protein                                           | 53    | 7             | 188524           |
| 103 | Q9BZ72           | KIAA1457 protein                                           | 43    | 11            | 151395           |
| 104 | Q9P1Z9           | KIAA1529 protein                                           | 46    | 6             | 196058           |
| 105 | Q6KCM7           | KIAA1896 protein                                           | 45    | 7             | 63937            |
| 106 | Q16787           | Laminin alpha 3 subunit isoform 1                          | 45    | 7             | 375652           |
| 107 | P11047           | Laminin B2 chain                                           | 54    | 7             | 183195           |
| 108 | Q9Y6N6           | Laminin gamma 3 chain precursor                            | 40    | 6             | 177756           |
| 109 | Q13449           | LAMP                                                        | 41    | 13            | 37798            |
| 110 | P42704           | Leucine-rich PPR-motif containing protein                   | 52    | 7             | 146306           |
| 111 | Q86UK5           | Limbin                                                     | 41    | 5             | 148825           |
| 112 | P50851           | Lipopolysaccharide-responsive and beige-like anchor protein| 63    | 10            | 321639           |
| 113 | Q9HCF6           | Long transient receptor potential channel 3                | 75    | 10            | 196157           |
| 114 | Q9N2R2           | Low density lipoprotein receptor related protein-deleted in tumor | 51    | 9             | 534844           |
| 115 | Q5JRA6           | Melanoma inhibitory activity family, member 3              | 52    | 7             | 214255           |
| 116 | Q86UL8           | Membrane associated guanylate kinase, WW and PDZ domain containing 2 | 39    | 5             | 159454           |
| 117 | Q13421           | Mesothelin                                                  | 45    | 4             | 68652            |
| 118 | A1L467           | Met proto-oncogene isoform b precursor                     | 48    | 6             | 157779           |
| 119 | Q8IWA4           | Mitofusin 1 precursor                                      | 38    | 10            | 84892            |
| 120 | Q6UVY6           | Monooxygenase X                                             | 36    | 9             | 63474            |
| 121 | Q8WXI7           | Mucin 1                                                    | 38    | 7             | 747071           |
| 122 | O75970           | Multiple PDZ domain protein                                | 60    | 7             | 222792           |
| 123 | Q9N2M1           | Myoferlin                                                  | 42    | 6             | 231092           |
| 124 | B2RTY4           | Myosin-IXa                                                 | 54    | 9             | 294918           |
| 125 | Q8NFP9           | Neurobeachin                                               | 53    | 6             | 330066           |
| 126 | P21359           | Neurofilamin isoform 1                                     | 43    | 10            | 322760           |
| 127 | Q9HE23           | Neuropilin-2b(5)                                           | 53    | 6             | 102975           |
| 128 | Q13423           | Nicotinamide nucleotide transhydrogenase                  | 85    | 9             | 114564           |
| 129 | Q59GR1           | Niemann-Pick disease, type C1 variant                      | 36    | 5             | 145931           |
| Subcellular location | PTM                        | Hydrophobicity value | TMDs |
|----------------------|----------------------------|----------------------|------|
| Plasma membrane      | Glycoprotein, Phosphoprotein | 0.264919             | 11   |
| Endoplasmic reticulum membrane | Glycoprotein, Phosphoprotein | -0.315448          | 6    |
| Unclear              | Unknown                    | -0.300924            | 6    |
| Unclear              | Phosphoprotein             | -0.647996            | 2    |
| Unclear              | Disulfide bond, Glycoprotein | -0.267224           | 2    |
| Plasma membrane      | Acetylation, Phosphoprotein | -0.266755            | 7    |
| Nucleus membrane     | Phosphoprotein             | 0.190929             | 2    |
| Plasma membrane      | Acetylation, Phosphoprotein | -0.668043           | 0    |
| Plasma membrane      | Acetylation, Phosphoprotein | -0.946435           | 0    |
| Endoplasmic reticulum membrane | Glycoprotein    | -0.0002375          | 23   |
| Nucleus membrane     | Unknown                    | -0.675982            | 0    |
| Plasma membrane,     | Phosphoprotein             | -0.422553            | 0    |
| Golgi apparatus membrane |                      |                     |      |
| Plasma membrane      | Phosphoprotein             | -0.569363            | 0    |
| Plasma membrane      | Glycoprotein               | -0.284538            | 13   |
| Plasma membrane      | Phosphoprotein             | -0.349204            | 0    |
| Plasma membrane      | Glycoprotein, Isopeptide bond, | -0.480988         | 7    |
|                      | Phosphoprotein, Ub conjugation |                 |      |
| Unclear              | Acetylation, Phosphoprotein | Too long            | 0    |
| Plasma membrane      | Phosphoprotein             | -0.405561            | 0    |
| Unclear              | Phosphoprotein             | -0.647996            | 2    |
| Unclear              | Disulfide bond, Glycoprotein | -0.381368           | 0    |
| Unclear              | Disulfide bond, Glycoprotein | -0.615042           | 0    |
| Unclear              | Disulfide bond, Glycoprotein | -0.465015           | 0    |
| Plasma membrane      | Disulfide bond, GPI-anchor, Glycoprotein, Lipoprotein | -0.278106          | 2    |
| Nuclear inner membrane | Acetylation               | -0.206097            | 0    |
| Plasma membrane      | Glycoprotein               | -0.445185            | 2    |
| Plasma membrane      | Acetylation, Phosphoprotein | -0.201817           | 0    |
| Plasma membrane      | Unknown                    | -0.300924            | 6    |
| Plasma membrane      | Disulfide bond, Glycoprotein | -0.489888           | 2    |
| Endoplasmic reticulum membrane | Glycoprotein, Phosphoprotein | -0.887466         | 3    |
| Plasma membrane      | Phosphoprotein             | -0.668455            | 0    |
| Plasma membrane      | Cleavage on pair of basic residues, GPI-anchor, Glycoprotein, Lipoprotein | -0.105714          | 2    |
| Plasma membrane      | Unknown                    | -0.144388            | 2    |
| Mitochondrial membrane | Unknown                  | -0.267476            | 2    |
| Endoplasmic reticulum membrane | Disulfide bond, Glycoprotein | -0.260848          | 2    |
| Plasma membrane      | Disulfide bond, Glycoprotein, Phosphoprotein | Too long            | 1    |
| Plasma membrane      | Phosphoprotein             | -0.233203            | 0    |
| Vesicle membrane, nuclear membrane, plasma membrane | Acetylation, Phosphoprotein | -0.456091          | 1    |
| Unclear              | Phosphoprotein             | -0.623980            | 0    |
| Plasma membrane      | Phosphoprotein             | -0.188358            | 2    |
| Plasma membrane      | Disulfide bond, Glycoprotein, Lipoprotein | -0.136950          | 4    |
| Plasma membrane      | Unknown                    | -0.440066            | 2    |
| Mitochondrial membrane | Acetylation              | 0.299079             | 13   |
| Plasma membrane      | Unknown                    | 0.216447             | 13   |
| No. | Accession number | Protein name                                      | Score | Peptide match | Molecular weight |
|-----|------------------|---------------------------------------------------|-------|---------------|------------------|
| 130 | Q13224           | N-methyl-D-aspartate receptor subunit NR3          | 53    | 16            | 168067           |
| 131 | Q13813           | Nentrythroid alpha-spectrin                        | 59    | 7             | 284905           |
| 132 | Q9UM47           | Notch homolog 3                                   | 43    | 8             | 256640           |
| 133 | Q5STG5           | Notch homolog 4 (Drosophila)                      | 51    | 7             | 221594           |
| 134 | P46531           | Notch1 preproprotein                              | 38    | 11            | 286350           |
| 135 | Q92823           | NrCAM protein                                     | 37    | 11            | 144138           |
| 136 | Q8WXH0           | NUANCE                                            | 104   | 29            | 801683           |
| 137 | Q5VU65           | Nucleoporin 210 kDa-like isof orm 1               | 43    | 10            | 211668           |
| 138 | Q13017           | p190-B                                            | 45    | 6             | 173000           |
| 139 | Q5T4S7           | p600                                              | 121   | 21            | 580607           |
| 140 | O60245           | PCDH7 (BH-Pcdh)c                                  | 40    | 4             | 130999           |
| 141 | Q9BXM0           | Perixxin                                          | 59    | 8             | 155248           |
| 142 | O75747           | Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing gamma polypeptide | 38    | 9             | 167814           |
| 143 | O00443           | Phosphoinositide 3-kinase                         | 43    | 9             | 192271           |
| 144 | O00750           | PI-3 kinase                                       | 46    | 5             | 184101           |
| 145 | Q6T4P5           | Plasticity-related protein 2                      | 43    | 12            | 79523            |
| 146 | Q75051           | Plexin A2                                         | 37    | 13            | 214220           |
| 147 | Q9Y4D7           | Plexin-D1                                         | 48    | 9             | 215381           |
| 148 | Q15142           | Polycystic kidney disease-associated protein       | 57    | 5             | 396273           |
| 149 | Q8TDX9           | Polycystin-1 L1                                   | 35    | 4             | 319453           |
| 150 | P13942           | Pro-a2(XI)                                        | 51    | 10            | 160545           |
| 151 | P08123           | Pro-alpha 2(I) collagen                           | 51    | 9             | 129858           |
| 152 | P02647           | Proapolipoprotein                                 | 664   | 75            | 28944            |
| 153 | Q07954           | Prolow-density lipoprotein receptor-related protein 1 | 66    | 13            | 523119           |
| 154 | Q05655           | Protein kinase C-delta 13                         | 37    | 4             | 78624            |
| 155 | Q8TF72           | Protein Shroom 3                                  | 41    | 8             | 218125           |
| 156 | Q96QU1           | Protocadherin 15                                  | 59    | 13            | 217261           |
| 157 | Q96QU1           | Protocadherin 15 isof orm CD1-4 precursor         | 48    | 12            | 217303           |
| 158 | Q9UN70           | Protocadherin 43                                  | 45    | 5             | 98230            |
| 159 | Q14517           | Protocadherin Fat 1                               | 52    | 9             | 509384           |
| 160 | Q9NYQ8           | Protocadherin Fat 2                               | 59    | 7             | 482172           |
| 161 | Q86V60           | PTPRM protein                                     | 39    | 8             | 158434           |
| 162 | Q8WZA2           | RAPGEF4 protein                                   | 53    | 13            | 100339           |
| 163 | P23467           | Receptor-type tyrosine-protein phosphatase beta    | 50    | 26            | 225497           |
| 164 | Q13332           | Receptor-type tyrosine-protein phosphatase S       | 43    | 9             | 218155           |
| 165 | Q86UR5           | Regulating synaptic membrane exocytosis 1         | 40    | 6             | 190154           |
| 166 | Q5T4S7           | Retinoblastoma-associated factor 600              | 124   | 20            | 580547           |
| 167 | Q5T5U3           | Rho GTPase-activating protein 21                   | 41    | 8             | 218567           |
| 168 | Q13464           | Rho-associated, coiled-coil containing protein kinase 1 | 48    | 6             | 159102           |
| 169 | Q5T5U3           | Rho-GTPase activating protein 10                   | 41    | 5             | 218563           |
| 170 | Q5JTH9           | Ribosomal RNA processing 12 homolog (S. cerevisiae) | 56    | 9             | 145067           |
| 171 | Q86UR5           | RIM long form                                     | 48    | 7             | 164927           |
| 172 | P21817           | Ryanodine receptor 1                               | 61    | 11            | 55204            |
| Subcellular location                              | PTM                                                | Hydrophobicity value | TMDs |
|--------------------------------------------------|----------------------------------------------------|----------------------|------|
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein       | −0.388342            | 6    |
| Unclear                                          | Acetylation, Phosphoprotein                        | −0.790330            | 0    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein       | −0.304826            | 3    |
| Unclear                                          | Unknown                                            | −0.287183            | 3    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein       | −0.420119            | 3    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein       | −0.379601            | 2    |
| Nuclear membrane                                 | Acetylation, Phosphoprotein                        | Too long             | 0    |
| Unclear                                          | Glycoprotein                                      | 0.050265             | 3    |
| Unclear                                          | Nitration, Phosphoprotein                          | −0.592345            | 0    |
| Unclear                                          | Acetylation, Phosphoprotein                        | Too long             | 0    |
| Plasma membrane                                  | Glycoprotein, Phosphoprotein                       | −0.390365            | 2    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.225736            | 0    |
| Unclear                                          | Phosphoprotein                                     | −0.322423            | 1    |
| Plasma membrane                                  | Acetylation, Phosphoprotein                        | −0.302136            | 0    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.363037            | 0    |
| Membrane                                         | Glycoprotein                                      | −0.178273            | 6    |
| Cell membrane                                    | Glycoprotein, Phosphoprotein                       | −0.181468            | 3    |
| Cell membrane, Membrane                          | Glycoprotein                                      | −0.159013            | 2    |
| Integral to membrane                             | Unknown                                            | 0.022760             | 14   |
| Integral to membrane                             | Glycoprotein                                      | −0.136856            | 7    |
| Cell membrane                                    | Disulfide bond, Glycoprotein, Sulfation            | −0.805186            | 0    |
| Plasma membrane                                  | Glycoprotein, Hydroxylation, Pyrrolidine carboxylic acid | −0.648098            | 0    |
| Plasma membrane                                  | Glycation, Glycoprotein, Lipoprotein, Palmitate, Phosphoprotein | −0.717228            | 0    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein       | −0.510386            | 2    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.377958            | 0    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.801652            | 0    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.245473            | 2    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.245473            | 2    |
| Plasma membrane                                  | Glycoprotein                                       | −0.261670            | 2    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein       | −0.294946            | 2    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein, Phosphoprotein       | −0.249645            | 2    |
| Unclear                                          | Unknown                                            | −0.385540            | 1    |
| Unclear                                          | Phosphoprotein                                     | −0.333927            | 0    |
| Plasma membrane                                  | Glycoprotein, Phosphoprotein                       | −0.372159            | 2    |
| Plasma membrane                                  | Disulfide bond, Glycoprotein                       | −0.401798            | 2    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.922459            | 0    |
| Unclear                                          | Acetylation, Phosphoprotein                        | Too long             | 0    |
| Golgi apparatus membrane, vesicle membrane       | Phosphoprotein                                     | −0.827338            | 0    |
| Golgi apparatus membrane                          | Unknown                                            | −0.827338            | 0    |
| Golgi apparatus membrane, vesicle membrane       | Phosphoprotein                                     | −0.827338            | 0    |
| Nuclear membrane                                 | Phosphoprotein                                     | −0.225366            | 1    |
| Plasma membrane                                  | Phosphoprotein                                     | −0.922459            | 0    |
| Endoplasmic reticulum membrane                   | Glycoprotein, Phosphoprotein                       | −0.314300            | 7    |

(Continued)
| No. | Accession number | Protein name                                      | Score | match | Molecular weight |
|-----|------------------|---------------------------------------------------|-------|-------|------------------|
| 173 | Q92736           | Ryanodine receptor 2                              | 70    | 10    | 569626           |
| 174 | Q15413           | Ryanodine receptor 3                              | 71    | 24    | 557794           |
| 175 | O15027           | SEC16 homolog A                                  | 36    | 5     | 115891           |
| 176 | A4QN19           | SEC16A protein                                    | 40    | 2     | 116196           |
| 177 | Q9H2E6           | Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A | 36    | 4     | 115608           |
| 178 | O94921           | Serine/threonine protein kinase PFTAIRE-1        | 41    | 8     | 53609            |
| 179 | Q8NGB0           | Seven transmembrane helix receptor                | 49    | 10    | 157779           |
| 180 | Q9Y566           | SH3 and multiple ankyrin repeat domains protein 1 | 49    | 7     | 225738           |
| 181 | Q9UPX8           | SH3 and multiple ankyrin repeat domains protein 2 | 54    | 6     | 135115           |
| 182 | Q7Z5N4           | Sidekick-like protein 1                           | 39    | 4     | 242926           |
| 183 | P21817           | Skeletal muscle ryanodine receptor                | 78    | 9     | 570517           |
| 184 | Q94813           | Slit homolog 2                                    | 47    | 13    | 175803           |
| 185 | Q94813           | SLIT2                                             | 40    | 7     | 175329           |
| 186 | Q9C0H9           | SNAP-25-interacting protein                      | 42    | 6     | 127182           |
| 187 | Q9Y6M7           | Sodium bicarbonate cotransporter2                | 44    | 8     | 115215           |
| 188 | Q53ZR1           | Solute carrier family 12 (sodium/potassium/chloride transporters), member 2 | 64    | 13 | 132048 |
| 189 | Q9HBR0           | Solute carrier family 38, member 10 isoform b     | 40    | 5     | 84069            |
| 190 | Q9NRC6           | Spectrin beta chain, brain 4                     | 72    | 14    | 419259           |
| 191 | Q01082           | Spectrin, beta, non-erythrocytic 1 isoform 1 variant | 48    | 8     | 276797           |
| 192 | Q8WWQ8           | Stabilin 2 precursor                             | 57    | 9     | 288323           |
| 193 | Q8WWQ8           | Stabilin-2                                        | 46    | 9     | 288329           |
| 194 | Q8I6L0           | Steerin3 protein                                 | 37    | 5     | 256885           |
| 195 | Q4LDE5           | Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1 | 52    | 10    | 401915           |
| 196 | Q9Y4G6           | Talin-2                                           | 50    | 11    | 273723           |
| 197 | Q9H2K2           | Tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2 | 41    | 4     | 128492           |
| 198 | O60343           | TBC1 domain family, member 4                     | 36    | 5     | 148068           |
| 199 | Q15643           | Thyroid receptor-interacting protein 11          | 38    | 7     | 228184           |
| 200 | P12270           | Tpr                                              | 54    | 10    | 265840           |
| 201 | Q9BX84           | Transient receptor potential cation channel subfamily M member 6 variant c | 79    | 20    | 232975           |
| 202 | Q8N6Q1           | Transmembrane and coiled-coil domains 5A         | 47    | 5     | 34495            |
| 203 | Q95271           | TRF1-interacting ankyrin-related ADP-ribose polymerase | 47    | 12    | 143690           |
| 204 | Q15643           | Trip230                                          | 92    | 12    | 228116           |
| 205 | Q9BX84           | Truncated transient receptor potential cation channel subfamily M member 6 variant a | 57    | 13    | 224977           |
| 206 | Q8NF4A0          | Ubiquitin specific protease 32                   | 47    | 8     | 183821           |
| 207 | P55916           | Uncoupling protein 3 isoform UCP3S               | 36    | 15    | 30219            |
| 208 | P17927           | Unnamed protein product                           | 41    | 10    | 230401           |
| 209 | P53420           | Unnamed protein product                           | 65    | 10    | 126774           |
| 210 | P11717           | Unnamed protein product                           | 48    | 14    | 281089           |
| 211 | Q6ZUB1           | Unnamed protein product                           | 36    | 10    | 158870           |
| 212 | O75110           | Unnamed protein product                           | 39    | 10    | 108198           |
| Subcellular location                          | PTM                                                   | Hydrophobicity value | TMDs |
|----------------------------------------------|-------------------------------------------------------|----------------------|------|
| Plasma membrane                              | Glycoprotein, Phosphoprotein                          | −0.324300            | 7    |
| Endoplasmic reticulum membrane               | Glycoprotein                                          | −0.278727            | 5    |
| Endoplasmic reticulum membrane,              | Acetylation, Phosphoprotein                           | −0.606607            | 0    |
| Golgi apparatus membrane                     |                                                       |                      |      |
| Endoplasmic reticulum membrane,              | Acetylation, Phosphoprotein                           | −0.606607            | 0    |
| Golgi apparatus membrane                     |                                                       |                      |      |
| Plasma membrane                              | Disulfide bond, Glycoprotein                          | −0.448543            | 2    |
| Plasma membrane                              | Phosphoprotein                                        | −0.470362            | 0    |
| Unclear                                      | Unknown                                               | −0.275069            | 6    |
| Plasma membrane                              | Phosphoprotein                                        | −0.539239            | 0    |
| Plasma membrane                              | Glycoprotein, Phosphoprotein                          | −0.608572            | 0    |
| Plasma membrane                              | Disulfide bond, Glycoprotein                          | −0.314144            | 1    |
| Plasma membrane                              | Glycoprotein, Phosphoprotein, S-nitrosylation         | Too long             | 0    |
| Plasma membrane                              | Disulfide bond, Glycoprotein                          | −0.323414            | 0    |
| Plasma membrane                              | Disulfide bond, Glycoprotein                          | −0.323414            | 0    |
| Plasma membrane                              | Phosphoprotein                                        | −0.644456            | 0    |
| Plasma membrane                              | Glycoprotein, Phosphoprotein                          | −0.130725            | 10   |
| Plasma membrane                              | Unknown                                               | 0.069720             | 11   |
| Unclear                                      | Phosphoprotein                                        | −0.363718            | 11   |
| Unclear                                      | Unknown                                               | −0.576588            | 0    |
| Plasma membrane                              | Acetylation, Glycoprotein, Phosphoprotein             | −0.766116            | 0    |
| Plasma membrane                              | Disulfide bond, Glycoprotein, Phosphoprotein, Proteoglycan | −0.268563            | 2    |
| Plasma membrane                              | Disulfide bond, Glycoprotein, Phosphoprotein, Proteoglycan | −0.268563            | 2    |
| Nuclear membrane                             | Phosphoprotein                                        | −0.638784            | 0    |
| Unclear                                      | Disulfide bond, Glycoprotein                          | −0.320528            | 1    |
| Plasma membrane                              | Acetylation, Phosphoprotein                           | −0.218412            | 0    |
| Golgi apparatus membrane                     | ADP-ribosylation                                      | −0.317066            | 0    |
| Plasma membrane                              | Phosphoprotein                                        | −0.553083            | 1    |
| Golgi apparatus membrane                     | Phosphoprotein                                        | −0.843506            | 0    |
| Nuclear membrane                             | Acetylation, Phosphoprotein                           | −0.968726            | 0    |
| Unclear                                      | Phosphoprotein                                        | −0.369882            | 5    |
| Unclear                                      | Unknown                                               | −0.563888            | 1    |
| Golgi apparatus membrane                     | ADP-ribosylation, Phosphoprotein                      | −0.327506            | 0    |
| Unclear                                      | Phosphoprotein                                        | −0.843506            | 0    |
| Plasma membrane                              | Phosphoprotein                                        | −0.369882            | 5    |
| Unclear                                      | Lipoprotein, Phosphoprotein, Prenylation              | −0.474689            | 0    |
| Mitochondrion membrane                       | Unknown                                               | 0.074679             | 0    |
| Plasma membrane                              | Disulfide bond, Glycoprotein, Pyrrolidone carboxylic acid | −0.320010            | 3    |
| Vesicle membrane                             | Acetylation, Phosphoprotein                           | −0.659231            | 1    |
| Plasma membrane                              | Acetylation, Disulfide bond, Glycoprotein, Phosphoprotein | −0.368528            | 3    |
| Unclear                                      | Glycoprotein                                          | −0.709205            | 1    |
| Unclear                                      | Phosphoprotein                                        | 0.062178             | 9    |

(Continued)
Table 1. (Continued)

| No. | Accession number | Protein name                              | Score | Peptide match | Molecular weight |
|-----|------------------|-------------------------------------------|-------|---------------|------------------|
| 213 | Q9NRW7           | Vacuolar protein sorting                  | 41    | 6             | 65388            |
| 214 | Q86Y38           | Xylosyltransferase I                      | 41    | 12            | 108357           |
| 215 | Q13433           | Zinc transporter ZIP6                     | 41    | 5             | 84685            |
| 216 | Q13439           | 256 kD golgin                              | 59    | 9             | 256666           |
| 217 | Q8N110           | Dedicator of cytokinesis protein 4        | 49    | 11            | 225206           |
| Accession  | Protein Name                          | Score | Peptide Match | Molecular Weight | Subcellular Location                        | PTM                | Hydrophobicity Value | TMDs |
|------------|---------------------------------------|-------|---------------|------------------|--------------------------------------------|--------------------|----------------------|------|
| Q9nRW7     | Vacuolar protein sorting 41           | 6     | endosome mem., go. app. mem. | 65388           | endosome membrane, golgi apparatus mem.    | Unknown            | −0.310526            | 0    |
| Q86Y38     | Xylosyltransferase I 41               | 12    | Disulfide bond, Glycoprotein | 108357          | Endoplasmic reticulum mem., golgi app. mem.| Unknown            | −0.540564            | 0    |
| Q13433     | Zinc transporter ZIP6 41              | 5     | Phosphoprotein | 84685            | Plasma membrane                            | Disulfide bond, Glycoprotein | −0.421987  | 6    |
| Q13439     | 256 kD golgin 59                       | 9     | Phosphoprotein | 256666          | Golgi apparatus mem.                        | Unknown            | −1.052981            | 0    |
| Q8n1I0     | Dedicator of cytokinesis protein 4 49 | 11    | Phosphoprotein | 225206          | Endomembrane                                | Disulfide bond, Glycoprotein | −0.371464  | 0    |