



Quantitative Label-Free Proteomic Analysis of Milk Fat Globule Membrane in Donkey and Human Milk

Xinhao Zhang^{1,2†}, Bo Jiang^{3†}, Chuanliang Ji^{2†}, Haijing Li², Li Yang², Guimiao Jiang², Yantao Wang², Guangyuan Liu², Guiqin Liu⁴, Lingjiang Min^{5*} and Fuwei Zhao^{2*}

¹ College of Animal Science and Technology, Shandong Agricultural University, Taian, China, ² National Engineering Research Center for Gelatin-Based Traditional Chinese Medicine, Dong-E E-Jiao Co., Ltd, Liaocheng, China, ³ Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agricultural and Forestry Sciences, Beijing, China, ⁴ Shandong Donkey Industry, Technology Collaborative Innovation Center, Liaocheng University, Liaocheng, China, ⁵ College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, China

OPEN ACCESS

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*Correspondence:

Fuwei Zhao
819899164@qq.com
Lingjiang Min
mlj2963@126.com

[†]These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Food Chemistry,
a section of the journal
Frontiers in Nutrition

Received: 20 February 2021

Accepted: 21 May 2021

Published: 22 June 2021

Citation:

Zhang X, Jiang B, Ji C, Li H, Yang L,
Jiang G, Wang Y, Liu G, Liu G, Min L
and Zhao F (2021) Quantitative
Label-Free Proteomic Analysis of Milk
Fat Globule Membrane in Donkey and
Human Milk. *Front. Nutr.* 8:670099.
doi: 10.3389/fnut.2021.670099

Previous studies have found donkey milk (DM) has the similar compositions with human milk (HM) and could be used as a potential hypoallergenic replacement diet for babies suffering from cow's milk allergy. Milk fat globule membrane (MFGM) proteins are involved in many biological functions, behaving as important indicators of the nutritional quality of milk. In this study, we used label-free proteomics to quantify the differentially expressed MFGM proteins (DEP) between DM (in 4–5 months of lactation) and HM (in 6–8 months of lactation). In total, 293 DEP were found in these two groups. Gene Ontology (GO) enrichment analysis revealed that the majority of DEP participated in regulation of immune system process, membrane invagination and lymphocyte activation. Several significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were determined for the DEP, such as lysosome, galactose metabolism and peroxisome proliferator-activated receptor (PPAR) signaling pathway. Our study may provide valuable information in the composition of MFGM proteins in DM and HM, and expand our knowledge of different biological functions between DM and HM.

Keywords: human, donkey, quantitative proteomic, comparison, milk fat globule membrane

INTRODUCTION

Donkey milk is more similar to human milk because of its total protein and lactose contents, similar fatty acid and protein profiles (1). It has been indicated to be more suitable for children and elderly people due to its remarkable nutritional value and less allergenic. Besides, milk fat globules in DM are smaller and more easily digested and absorbed by infants (2). With its obvious advantages, the demand of direct consumption for DM has increased.

Milk fat globule membrane (MFGM), a three-layer membrane, covers on the surface of the milk fat globule (3). MFGM proteins make up only 1–2% of the total milk proteins, but they are thought to play important roles in biological processes, including cell growth promotion, cell activity regulation and defense mechanisms against bacteria and viruses in infants (4). Investigations on MFGM proteome have primarily focused on profiling analyses of MFGM fractions from different mammals. For example, Yang et al. identified 232 differentially expressed MFGM proteins in HM

and CM across different lactation stages using the iTRAQ proteomic approach (5). To reveal the differences in the formation of MFGM in different mammals, the MFGM proteins of cow, yak, buffalo, goat, horse, camel and human were also compared by iTRAQ proteomics (6). Recently, Li et al. hoped to explore the changes in the regulation mechanism of different lactation stages by analyzing the differences of MFGM proteins between donkey colostrum and mature milk (2). However, studies of the DM MFGM proteome are relatively sparse and less comprehensive, especially fully comparative analyses of MFGM protein compositions and potential biological activities between DM and other species.

The aim of our study was to compare the expression of MFGM proteins between DM and HM by label-free quantification and to explore the biological processes they were involved in. The results are helpful for us to better understand the differences between DM and HM in the composition of MFGM and provide strong support for the future development of formula milk using donkey milk as nutritional provider.

MATERIALS AND METHODS

Sample Collection and Treatment

HM samples were donated by twelve healthy mothers with the lactation of 6–8 months with written informed consent which indicated that the milk would be used in research. Twelve DM samples were obtained from a local farm breeding Dezhou donkeys (6–9 years old) with the lactation of 4–5 months in Liaocheng City of Shandong province, China. All procedures involving donkeys were performed by the Shandong Agricultural University Animal Care and Use Committee (approval number, SDAUA-2020-053). Four samples of each group were randomly mixed and stored at -80°C .

MFGM proteins separation was done as described by previous report (2). All samples were centrifuged at 4°C and 10,000 g for 15 min to obtain the upper fat portion containing MFGM. The upper fat layer was washed with 10 ml cold phosphate-buffered saline (0.24 g KH_2PO_4 , 1.44 g Na_2HPO_4 , 8 g NaCl and 0.2 g KCl were dissolved in 800 ml deionized water, the PH was adjusted to 7.4 and the volume was fixed to 1,000 mL) and homogenized by ultrasound (80 W, 15 s) for 3 times. The mixture was centrifuged at 4°C and 10,000 g for 1 h. Then, acetone was added to the collected supernatant to segregate the fat globules overnight. After centrifugation at 15,000 g at 4°C for 30 min, the supernatant was poured away. Finally, lysate was added to the protein particle sample and ultrasonicated for 15 s at 80 W for 10 cycles.

MFGM Protein Digestion

Thirty microliters protein solution were taken from each sample and dithiothreitol (DTT) were added to the final concentration of 100 mM. The samples were incubated in boiling water for 5 min and cooled to room temperature. Then, 200 μL UA buffer (8 M urea, 150 mM TrisHCl, pH 8.0) was added and mixed thoroughly. After that, each sample was transferred into a 10 kDa ultrafiltration centrifuge tube (Sartorius, Germany) and centrifuged at 14,000g for 15 min, then discarded the filtrate (repeat this step once). 100 μL IAA buffer (100 mM IAA in UA)

was added to the protein mixture in the tube and shaken at 600 rpm for 1 min. The mixture was left at room temperature in dark for 30 min and centrifuged at 14,000 g for 15 min and the process was repeated twice. Hundred microliters 25mm NH_4HCO_3 solution was added to the tube and centrifuged at 14,000 g for 15min and repeated the procedure twice. Subsequently, 40 μL trypsin buffer (4 μg trypsin in 40 μL 100mM NH_4HCO_3) were added to the mixture and sample was shaken at 600 rpm for 1 min. The mixture was left at 37°C for 16–18 h and then transferred to a new collecting tube and centrifuged at 14,000 g for 15min. Then, 40 μL 25 mm NH_4HCO_3 was added in the mixture and sample was centrifuged at 14,000 g for 15 min to collect the filtrate. The peptide was desalted by C18 cartridge (Sigma, USA). After freeze-drying, the peptide was dissolved in 40 μL 0.1% formic acid solution.

LC- MS/MS Experiments

Each sample was separated by the Easy-nLCTM (Proxeon Biosystems, Thermo Fisher Scientific) orbitrap HPLC system with nano-flow. Buffer A was 0.1% formic acid, and buffer B was 84% acetonitrile in 0.1% formic acid. The chromatographic column was equilibrated with 95% buffer A. The sample was loaded by an automatic sampler to the sample column (Thermo Scientific Acclaim Pepmap 100, 100 $\mu\text{m} \times 2$ cm, nanoViper C18), and separated by the analytical column (Thermo scientific EASY column, 10 cm, ID75 μm , 3 μm , C18-A2). The flow rate was 300 nL/min. Two-hour gradient: 0–110 min, buffer B linear gradient from 0 to 55%; 110–115 min, buffer B linear gradient from 55 to 100%; 115–120 min, buffer B maintained at 100%.

The samples were analyzed on a Q ExactiveTM mass spectrometer (Thermo Fisher Scientific). Parameters were set as follows: analysis time 120 min; detection mode: positive ion; scanning range of parent ion: 300–1,800 m/z; first-order mass spectrometry resolution: 70,000 at 200 m/z; AGC (automatic gain control) target: 1e6; maximum IT (inject time): 50 ms; dynamic exclusion: 60 s. The mass charge ratio of polypeptide and polypeptide fragments was collected according to the following methods: after each full scan, 20 fragments (MS2 scan) were collected. The MS2 activation type was HCD, the isolation width was 2 m/z, the secondary mass spectrum resolution was 17,500 at 200 m/z. Normalized collision energy was 30 eV, and the underfill ratio was defined as 0.1%.

Sequence Database Searching

Relative intensity-based label-free quantification (LFQ) was analyzed by MaxQuant version 1.5.3.17 (Max Planck Institute of Biochemistry in Martinsried, Germany) and searched against the UniProtKB *Equus asinus* database (47,825 total entries, downloaded on August 12, 2019) and *Homo sapiens* (included 20,422 series, downloaded on May 22, 2019). Proteins with $p < 0.05$ and fold change >2 or <0.5 were deemed significantly expressed between groups by t -test. The false discovery rate (FDR) for peptide and protein identification was set to 1%.

Bioinformatics Analysis

Quantified MFGM samples were used to performing hierarchical clustering analysis. For this purpose, Cluster3.0 (<http://bonsai>)

hgc.jp/\$sim\$mdhooon/software/cluster/software.htm) and the Java Treeview software (<http://jtreeview.sourceforge.net>) were used. GO enrichment on three ontologies (biological process, molecular function, and cellular component) and KEGG pathway enrichment analyses were applied based on the Fisher's exact test, considering the whole quantified protein annotations as background dataset using DAVID (<https://david.ncifcrf.gov/>). Benjamini-Hochberg correction for multiple testing was further applied to adjust derived *P*-values, and only functional categories and pathways with *P*-values under a threshold of 0.05 were considered as significant.

RESULTS

Quantitative Overview of Identified MFGM Proteins in DM and HM

As shown in **Figures 1A**, 454 MFGM proteins were identified in DM and HM. Proteins having at least two replicates and fold changes >2.0 or <0.5 and $P < 0.05$ were defined as differentially expressed proteins (DEP). In addition, the proteins with at least two replicates in one group and in another group with null values were also defined as DEP. A volcano plot was used to show significant differences between the two groups based on the fold change and *P*-value (**Figure 1B**). Compared with MFGM proteins in HM, 204 MFGM proteins in DM were upregulated and 89 MFGM proteins were downregulated. DEP detailed information is shown in **Supplementary Table 1**.

Cluster Analysis

Meanwhile, a hierarchical cluster analysis of MEGM proteins in DM and HM group was shown in **Figure 2**. The upregulated MFGM proteins in DM compared with HM mainly included alpha-s2-casein, lysozyme, alpha-s1-casein and secreted

phosphoprotein 1 (SPP1), and the downregulated MFGM proteins were lipoprotein lipase, fatty acid-binding protein 3 and nucleobindin 1.

GO Analysis of DEP in DM Compared With HM and CM

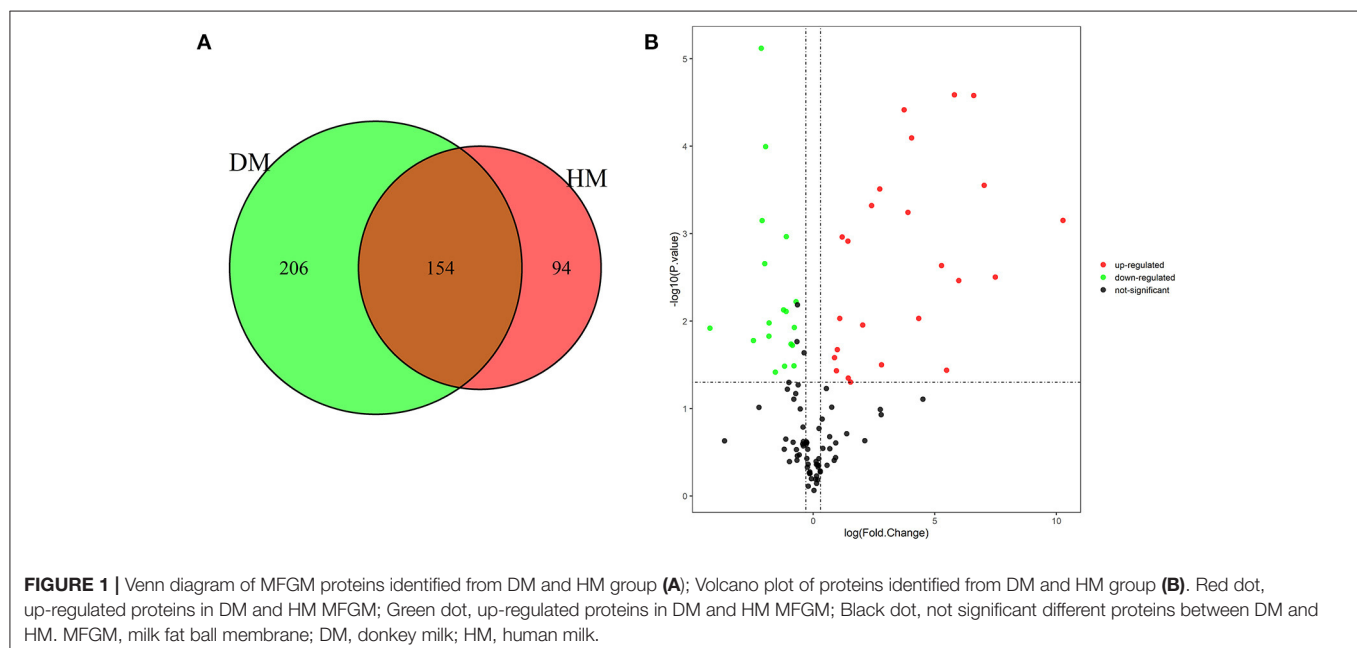
DEP were then classified into the GO enrichment analysis of three distinctive functional sets, cellular component, molecular function, and biological process. In terms of molecular function, DEP in DM and HM group were primarily related to immunoglobulin receptor binding, protein homodimerization activity and antigen binding (**Figure 3**). In the category of biological process, DEP in these two groups were mainly involved in regulation of immune system process, membrane invagination and lymphocyte activation. GO enrichment of DEP information is listed in **Supplementary Table 2**.

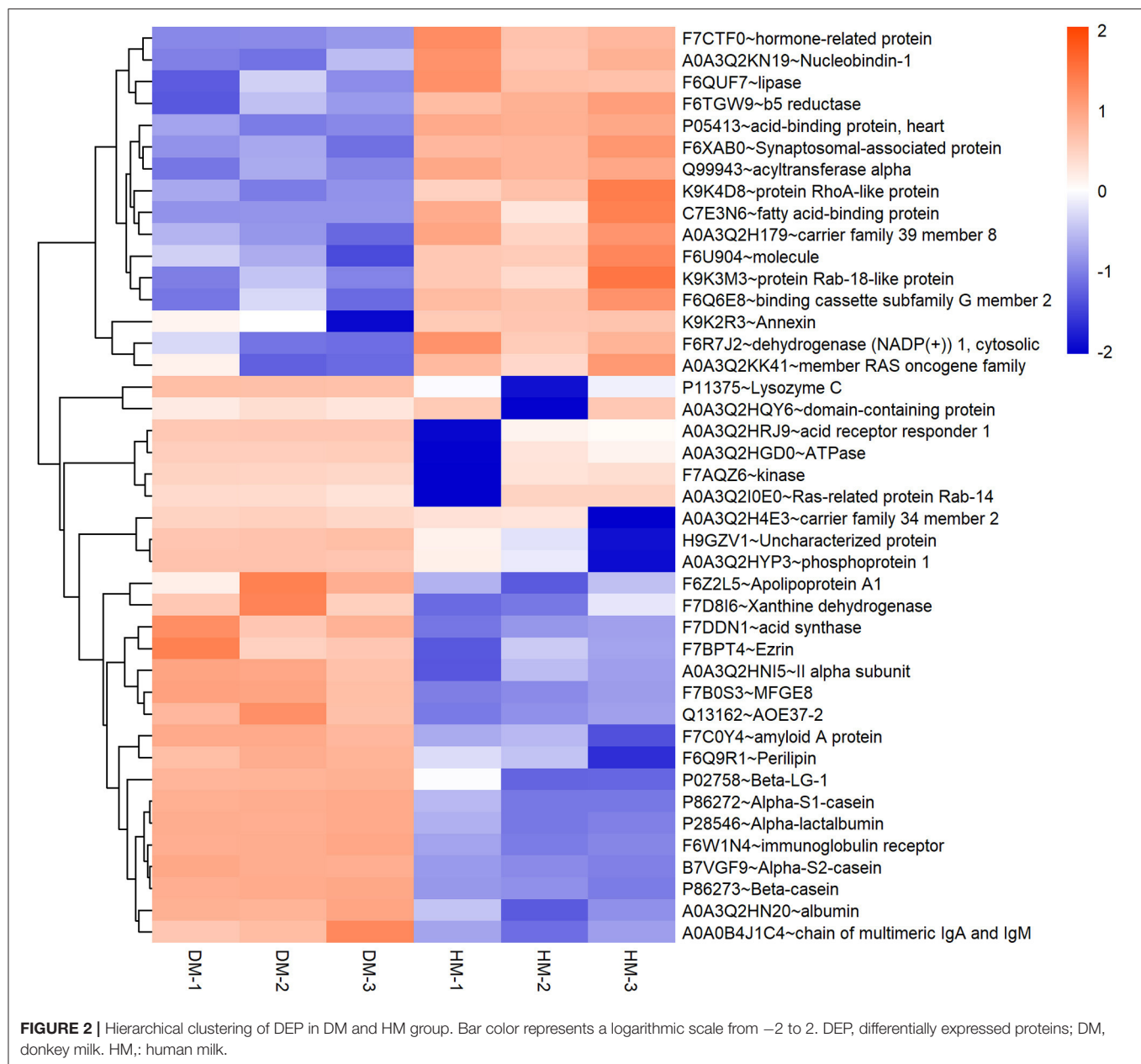
KEGG Pathway Analysis of DEP in DM Compared With HM and CM

In **Figure 4**, the DEP were mainly involved in lysosome, galactose metabolism and PPAR signaling pathway. KEGG pathway enrichment of DEP information was listed in **Table 1**.

DISCUSSION

In this study, 293 MFGM proteins were found to be significantly different between DM and HM. These DEP were involved in regulation of immune system process, complement activation and integral component of membrane. These results may provide valuable information in the MFGM composition of DM and HM, especially for low abundant components, and expand our knowledge of different biological functions between DM and HM.





Caseins in milk exert multifunctional effects including amino acid and calcium supply (5), cellular immune functions stimulation (7, 8), and chemotactic properties (9). Studies of human, bovine, goat, and camel MFGM proteomes identified milk caseins in the MFGM fractions (2, 6, 10). In our data, four caseins (α -s1-casein, α -s2-casein, β -casein, κ -casein) were detected in DM MFGM fractions and significantly higher than that in HM. The main role of human α -s1-casein is to serve as an amino acid source to the newborns. Beyond nutritional aspects, α -s1-casein could contribute to the development of immune system. Moreover, α -s1-casein in nursed individuals gives rise to sustained specific IgG production (8). Cocco et al. found that single amino acid substitution in the specific linear epitopes of

α -s1-casein can significantly reduce the binding ability of serum IgE in patients with CM allergy (11). Donkey and cow milk α -s1-casein share a low sequence homology, and particularly their IgE binding linear epitopes have remarkable differences in amino acid sequences (12). Moreover, Bertino et al. found that donkey α -s1-casein appears as both phosphorylated and glycosylated forms, but neither human nor bovine α -s1-caseins have been reported to be glycosylated (13). Studies have shown that in many cases milk from donkey represents a safe and alternative food in both IgE-mediated and non-IgE-mediated cow's milk protein allergy (14, 15). These differences in α -s1-casein amino acid sequence or post-translational modifications might be related to the low allergenic properties

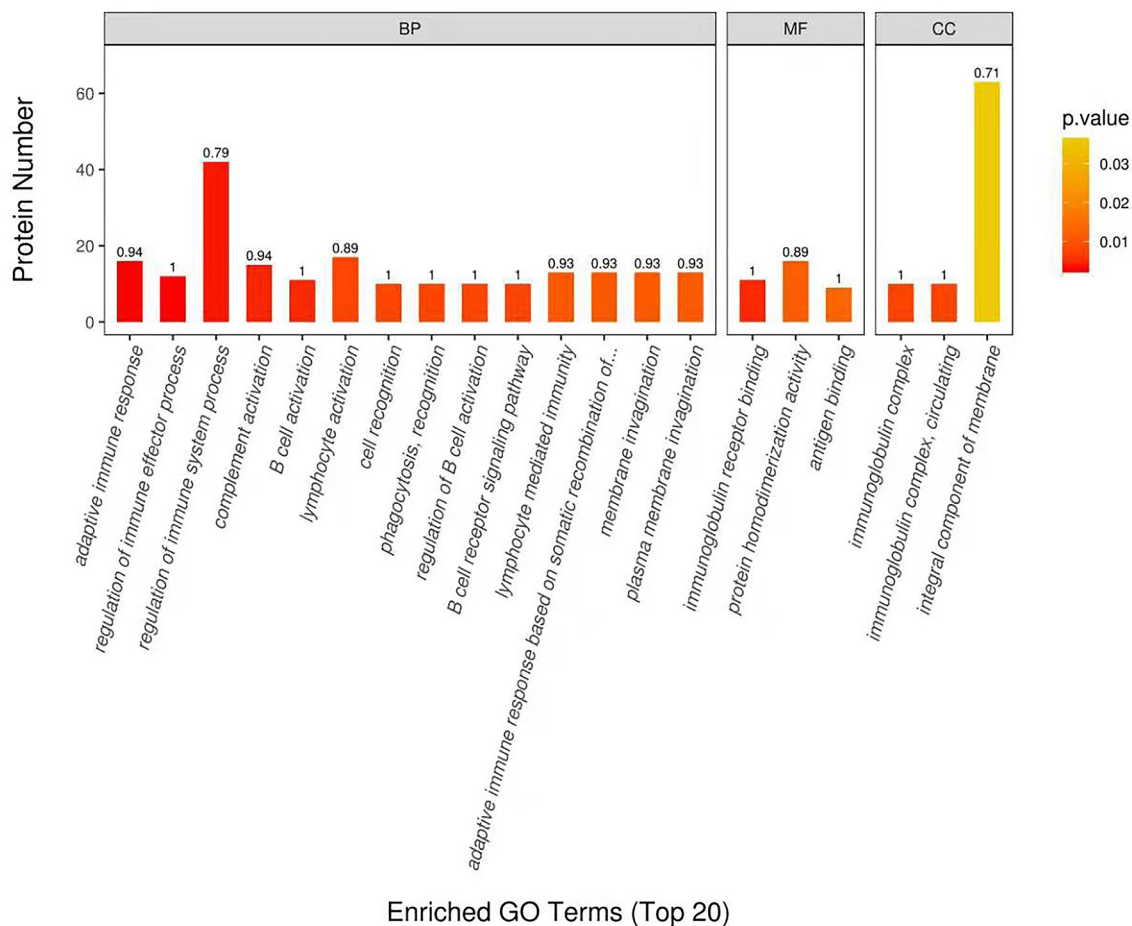


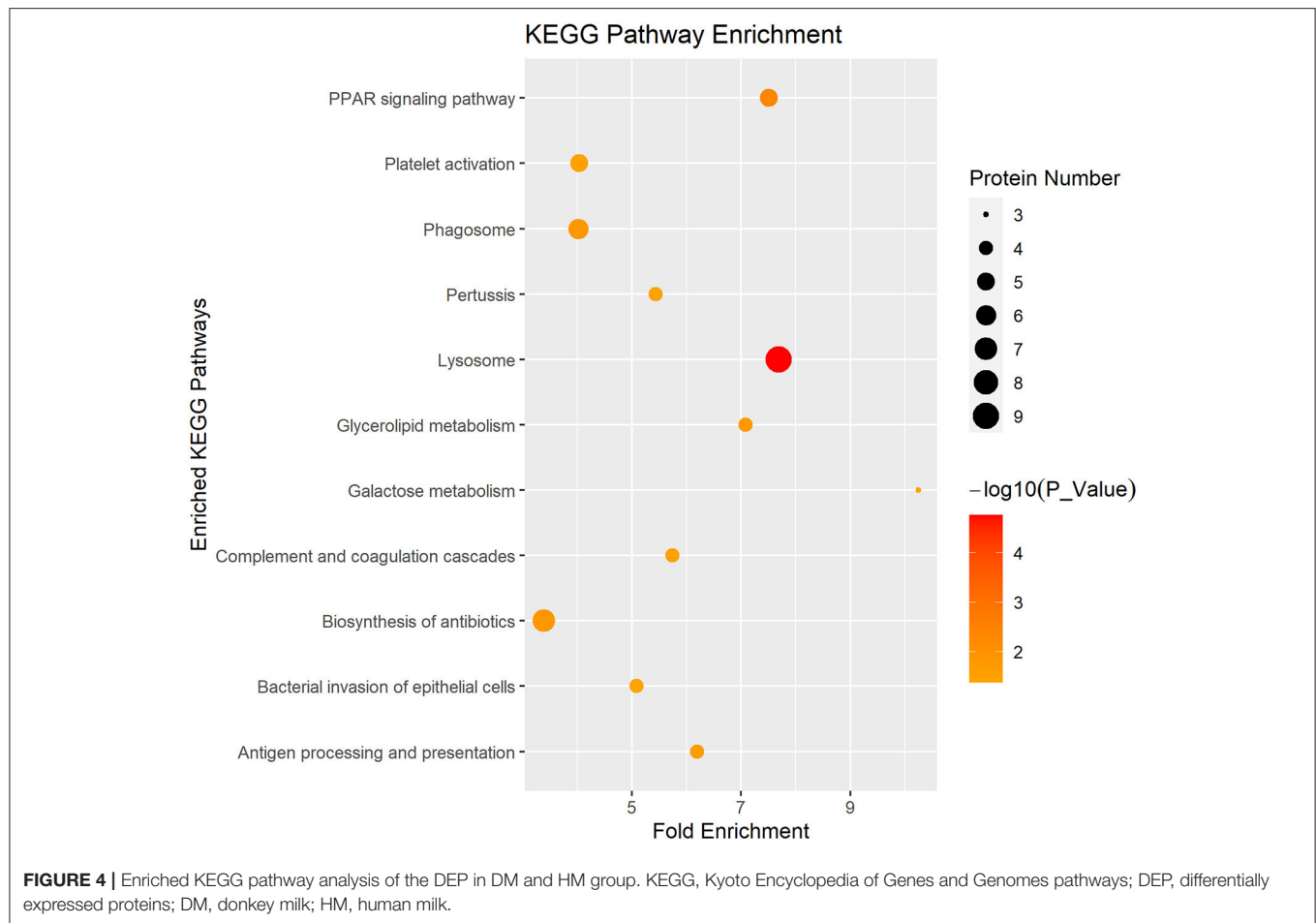
FIGURE 3 | Enriched Gene Ontology (GO) Terms of the DEP in DM and HM group. GO enrichment of DEP on three categories. BP, biological processes; MF, molecular functions; CC, cellular components; DEP, differentially expressed proteins; DM, donkey milk; HM, human milk.

of donkey milk. As for β -casein and kappa-casein, both of them from HM and DM are more closely related to each other than the cow counterparts (16). β -casein is the major casein constituent in human MFGM and generates smaller casein phosphopeptides upon digestion to aid the absorption of calcium (17). κ -casein is the only glycosylated in the four casein families. DM κ -casein carries a higher number of potential O-glycosylation than that cow milk κ -casein. Therefore, DM similar to HM may contribute to inhibit the adhesion of *Helicobacter pylori* to gastric mucosa in infant (18).

In general, β -lactoglobulin can be found in the majority of milks, but not in human's milk. However, in this study we detected β -lactoglobulin in HM. It has been found that beta-lactoglobulin can be detected in the human milk within 7 days after ingestion of milk (19). We hypothesized that the mothers who donated breast milk in this study were likely to have taken other milks before providing milk samples.

According to the GO enrichment analysis, DEP in DM vs. HM group were mainly involved immune response, such as complement activation, defense response or positive regulation of B cell activation (**Supplementary Table 2**). It is well-known

that milk could provide large amounts of bioactive components to the infants in the critical phase of immunological immaturity. Mature breast milk could enhance B cell proliferation and antibody secretion (20). Among the DEP involved in the positive regulation of B cell activation, 9 proteins were all upregulated in DM, including immunoglobulin heavy constant gamma 3 (IGHG3) and immunoglobulin kappa constant (IGKC), which all function in B cell selection or antigen recognition (21). In addition, 49 DEP were involved in defense response, among which 32 proteins were upregulated in the DM MFGM, namely semaphorin 7A, complement 3 (C3), joining (J) chain. Semaphorin 7A (also known as CD108) plays a key role in innate immune regulation. Semaphorin 7A could induce proinflammatory cytokines production (22). Semaphorin 7A-deficient mice are defective in T cell-mediated inflammatory responses, indicating the role of semaphorin 7A in evoking inflammatory immune reactions (23). In addition to its role in the immune response, semaphorin 7A also functions as a chemoattractant and stimulates neuronal migration, which is an essential process in central nervous system development (24). The defect of neuron migration may lead to nervous system



disorder (25). J chain is a small polypeptide contained in dimeric IgA and pentameric IgM, which plays an important role in the generation of secretory antibodies (26). This peptide can be produced by immunocytes of all Ig isotypes, but it becomes incorporated only into IgA and pentameric IgM (27). Moreover, the expression of J chain may be a marker of B cell clone in mucosa associated lymphoid tissue, as there is a positive correlation between the production of polymeric IgA, IgG or IgD-producing cells and J chain (28). C3 is an important part of the innate immune system. It combines with other complement proteins to form the main host mechanism for detecting and eliminating potential pathogens (29). Complement proteins contribute to the establishment of natural immune system in newborns (2). The presence of abundant immunological factors in DM MFGM proteins are helpful for the newborns to establish an immune system against microbial infection to adapt to the new environment to prevent diseases.

In this study, a solute carrier (SLC) superfamily, namely, SLC34A2, SLC36A1, SLC4A9, and SLC9A3R1, was more abundant in DM MFGM proteins. This superfamily is a major membrane transporter group that controls the uptake and excretion of nutrients, neurotransmitters, metabolites, drugs and toxins (30). SLC34A2 is a member of SLC34 family, a

group of phosphate transporters, which are responsible for transporting inorganic phosphate. Phosphate is an essential nutrient for life and a key component of bone formation (31). Recently, it was concluded that SLC34A2 was responsible for the sodium-dependent component of intestinal phosphate absorption (32). SLC36A1, an amino acids transporter in small intestinal enterocytes, could regulate cell growth and sense the availability of amino acids in other cell types. SLC36A1 also could be a target for rapamycin complex 1 (TORC1) activation (33). TORC1 regulates some metabolic pathways and adapts cells to applied bioenergy and anabolic conditions (34). SLC9A3R1 is a multifunctional scaffold protein, which is involved in cell activities and affects many protein functions, including ion channels, receptors, signaling and nuclear proteins. In addition, SLC9A3R1 has potential antitumor effects in breast cancer (35). SLCs also play an important role in the function of the central nervous system. A total of 287 SLC genes were identified in the brain, especially in the barrier cells. SLCs expressed in neurons and glial cells play irreplaceable roles in maintaining brain homeostasis (36). Moreover, we also found some other abundant proteins in DM MFGM that are expressed in the brain, such as neuro plastin (NP). NP is a cell adhesion molecule rich in synaptic membranes and belongs

TABLE 1 | Detailed information of KEGG pathway-based enrichment of the differentially expressed proteins between DM and HM.

Pathway_ID	KEGG pathways	Protein number	Genes	Fold enrichment	P-value
ecb04142	Lysosome	9	F6YQM5, F7BPX8, F6YNH6, F6V812, F7B6D0, F7DG10, F6V3X9, F7BV85, F6VUW2	7.69	1.72E-05
ecb03320	PPAR signaling pathway	5	F7AYT5, F6Z2L5, F6RM73, F6QUF7, F6U904	7.51	4.07E-03
ecb04145	Phagosome	6	Q95M34, Q5XWB8, F7BPX8, F7DG10, F6U904, F6VUW2	4.02	1.56E-02
ecb01130	Biosynthesis of antibiotics	7	F6X8Q2, F6TL52, F6SX98, F6Y688, F6RI26, L7MRN0, F6R7J2	3.38	1.58E-02
ecb00561	Glycerolipid metabolism	4	F6TN81, F6QUF7, F7B6D0, F6RI26	7.08	1.79E-02
ecb04612	Antigen processing and presentation	4	Q9GKX8, F7BPX8, F6YNH6, F6VUW2	6.19	2.55E-02
ecb04610	Complement and coagulation cascades	4	F6USP9, F6XSF7, F6PH38, F6XGE0	5.74	3.10E-02
ecb00052	Galactose metabolism	3	F6X8Q2, F7B6D0, F6SUZ2	10.25	3.33E-02
ecb04611	Platelet activation	5	F6QMB8, F6XAB0, F7AQZ6, K9K4D8, F6PH38	4.03	3.36E-02
ecb05133	Pertussis	4	Q6TGR2, Q5XWB8, F6XSF7, K9K4D8	5.43	3.57E-02
ecb05100	Bacterial invasion of epithelial cells	4	F6VZN7, F6Y0D9, K9K4D8, F7BV85	5.08	4.22E-02

to immunoglobulin superfamily. Np plays a role in synaptic plasticity and neurite growth (37, 38). In recent years, NP has attracted much attention because of its correlation with adolescent cortical thickness and intelligence (39). Therefore, the abovementioned functional components in DM MFGM may play a more important role in promoting the growth and learning of newborn infants.

Among these DEP, a family of apolipoproteins (Apos), namely, apolipoprotein A1 (ApoA1), ApoA2, ApoC3, ApoD, and ApoE, were significantly upregulated in DM MFGM. ApoA1 and ApoA2 are the major proteins in high-density lipoproteins (HDL) (40). ApoA1 has multiple beneficial functions, including potent antioxidant, anti-inflammatory, antiviral and antibacterial activities in blood (41, 42). Recently, ApoA1 was found in HM and DM. Kim et al. identified that ApoA1 interacts with cholesterol in HM, provides antioxidant activity and improves embryo survivability (43). In DM MFGM, ApoA1 was upregulated in colostrum compared with mature milk (2). ApoD is a glycosylated protein involved in lipid transport, food intake, inflammation, antioxidant response and development. In humans, ApoD levels rise considerably in association with aging possibly in response to accumulated damage (44). Overexpression of human ApoD could protect drosophila against various extrinsic stresses and extend its normal lifespan (45). ApoE is an important element in the lipoprotein metabolism and cholesterol transport (46). Cholesterol plays a key role in vitamin D and steroid hormones synthesis, which is critical to the development of the newborns (47). In our study, DM MFGM provides a higher level of cholesterol

transporters, which may help the newborns acquire a large amount of cholesterol.

In conclusion, 204 up-regulated proteins were identified in the lipid globules of donkey milk. Through GO functional annotation and KEGG pathway enrichment analysis, we found that these upregulated proteins not only have nutritional effects, but also promote the improvement of the immune system, cognitive learning and anti-oxidation of newborns. Therefore, donkey milk might be used as a nutritional provider in infant formula. In addition, the comparison of the fat globular membrane protein between donkey milk and cow milk was missing in this study, which was a limitation of our research and will be carried out in the future.

CONCLUSION

A quantitative proteomic method was used to investigate the MFGM proteins proteome in DM and HM. DEP were analyzed by multivariate statistical methods and found mainly involved in regulation of immune system process, membrane invagination and lymphocyte activation. Our findings also provided more in-depth reference for the dairy food industry and infant health.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Animal Care and Use Committee of Shangdong Agricultural University. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Institutional Animal Care and Use Committee of Shangdong Agricultural University. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

FZ designed the experiment and revised the manuscript. XZ completed the experiment and drafted the manuscript. BJ and CJ participated in improving the experiment. HL, LY, GJ, YW, Gual,

and GuiL participated in collecting and preparing the samples. LM helped to revise the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by The Open Project of Shandong Collaborative Innovation Center for Donkey Industry Technology (No. 3193308) and Agriculture Improved Varieties Project of Shandong Province, China (2017LZGC020). Beijing Agricultural Forestry Academy Youth Fund (No. QNJJ201930).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2021.670099/full#supplementary-material>

REFERENCES

- Medhammar E, Wijesinha-Bettoni R, Stadlmayr B, Nilsson E, Charrondiere UR, Burlingame B. Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective. *J Sci Food Agric.* (2012) 92:445–74. doi: 10.1002/jsfa.4690
- Li W, Li M, Cao X, Yang M, Han H, Kong F, et al. Quantitative proteomic analysis of milk fat globule membrane (MFGM) proteins from donkey colostrum and mature milk. *Food Funct.* (2019) 10:4256–68. doi: 10.1039/C9FO00386J
- Argov N, Lemay DG, German JB. Milk fat globule structure & function; nanoscience comes to milk production. *Trends Food Sci Technol.* (2008) 19:617–23. doi: 10.1016/j.tifs.2008.07.006
- Reinhardt TA, Lippolis JD. Bovine milk fat globule membrane proteome. *J Dairy Res.* (2006) 73:406–16. doi: 10.1017/S0022029906001889
- Yang M, Cong M, Peng X, Wu J, Wu R, Liu B, et al. Quantitative proteomic analysis of milk fat globule membrane (MFGM) proteins in human and bovine colostrum and mature milk samples through iTRAQ labeling. *Food Funct.* (2016) 7:2438–50. doi: 10.1039/C6FO00083E
- Yang Y, Zheng N, Zhao X, Zhang Y, Han R, Ma L, et al. Proteomic characterization and comparison of mammalian milk fat globule proteomes by iTRAQ analysis. *J Proteomics.* (2015) 116:34–43. doi: 10.1016/j.jprot.2014.12.017
- Wada Y, Lonnerdal B. Bioactive peptides derived from human milk proteins—mechanisms of action. *J Nutr Biochem.* (2014) 25:503–14. doi: 10.1016/j.jnutbio.2013.10.012
- Vordenbaumen S, Saenger T, Braukmann A, Tahan T, Bleck E, Jose J, et al. Human casein alpha s1 induces proinflammatory cytokine expression in monocytic cells by TLR4 signaling. *Mol Nutr Food Res.* (2016) 60:1079–89. doi: 10.1002/mnfr.201500792
- Vordenbaumen S, Braukmann A, Altendorfer I, Bleck E, Jose J, Schneider M. Human casein alpha s1 (CSN1S1) skews *in vitro* differentiation of monocytes towards macrophages. *BMC Immunol.* (2013) 14:46. doi: 10.1186/1471-2172-14-46
- Saadaoui B, Henry C, Khorchani T, Mars M, Martin P, Cebo C. Proteomics of the milk fat globule membrane from *Camelus dromedarius*. *Proteomics.* (2013) 13:1180–4. doi: 10.1002/pmic.201200113
- Cocco RR, Jarvinen KM, Sampson HA, Beyer K. Mutational analysis of major, sequential IgE-binding epitopes in alpha s1-casein, a major cow's milk allergen. *J Allergy Clin Immunol.* (2003) 112:433–7. doi: 10.1067/mai.2003.1617
- Cunsolo V, Cairone E, Fontanini D, Criscione A, Muccilli V, Saletti R, et al. Sequence determination of alphas1-casein isoforms from donkey by mass spectrometric methods. *J Mass Spectrom.* (2009) 44:1742–53. doi: 10.1002/jms.1683
- Bertino E, Gastaldi D, Monti G, Baro C, Fortunato D, Perono Garoffo L, et al. Detailed proteomic analysis on DM: insight into its hypoallergenicity. *Front Biosci.* (2010) 2:526–36. doi: 10.2741/e111
- Carroccio A, Cavataio F, Montalto G, D'Amico D, Alabrese L, Iacono G. Intolerance to hydrolysed cow's milk proteins in infants: clinical characteristics and dietary treatment. *Clin Exp Allergy.* (2000) 30:1597–603. doi: 10.1046/j.1365-2222.2000.00925.x
- Monti G, Bertino E, Muratore Cristina M, Coscia A, Cresi F, Silvestro L, et al. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an *in vivo* and *in vitro* study. *Pediatr Allergy Immunol.* (2008) 19:90–1. doi: 10.1111/j.1399-3038.2007.00655.x
- Cunsolo V, Saletti R, Muccilli V, Gallina S, Di Francesco A, Foti S. Proteins and bioactive peptides from donkey milk: the molecular basis for its reduced allergenic properties. *Food Res Int.* (2017) 99(Pt. 1):41–57. doi: 10.1016/j.foodres.2017.07.002
- Lonnerdal B. Bioactive proteins in breast milk. *J Paediatr Child Health.* (2013) 49(Suppl. 1):1–7. doi: 10.1111/jpc.12104
- Spada V, Ferranti P, Chianese L, Salimei E, Addeo F, Picariello G. Antibacterial potential of donkey's milk disclosed by untargeted proteomics. *J Proteomics.* (2021) 231:104007. doi: 10.1016/j.jprot.2020.104007
- Matangkasombut P, Padungpak S, Thaloengsok S, Kamchaisatian W, Sasisakulporn C, Jotikasthira W, et al. Detection of beta-lactoglobulin in human breast-milk 7 days after cow milk ingestion. *Paediatr Int Child Health.* (2017) 37:199–203. doi: 10.1080/20469047.2017.1289310
- Juto P. Human milk stimulates B cell function. *Arch Dis Child.* (1985) 60:610–3. doi: 10.1136/ad.60.7.610
- Steck AJ, Kinter J, Renaud S. Differential gene expression in nerve biopsies of inflammatory neuropathies. *J Peripher Nerv Syst.* (2011) 16 Suppl 1:30–3. doi: 10.1111/j.1529-8027.2011.00302.x
- Kang HR, Lee CG, Homer RJ, Elias JA. Semaphorin 7A plays a critical role in TGF-beta1-induced pulmonary fibrosis. *J Exp Med.* (2007) 204:1083–93. doi: 10.1084/jem.20061273
- Suzuki K, Okuno T, Yamamoto M, Pasterkamp RJ, Takegahara N, Takamatsu H, et al. Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. *Nature.* (2007) 446:680–4. doi: 10.1038/nature05652
- Iragavarapu-Charyulu V, Wojcikiewicz E, Urdaneta A. Semaphorins in angiogenesis and autoimmune diseases: therapeutic targets? *Front Immunol.* (2020) 11:346. doi: 10.3389/fimmu.2020.00346
- Rahimi-Balaei M, Bergen H, Kong J, Marzban H. Neuronal migration during development of the cerebellum. *Front Cell Neurosci.* (2018) 12:484. doi: 10.3389/fncel.2018.00484

26. Johansen FE, Braathen R, Brandtzaeg P. Role of J chain in secretory immunoglobulin formation. *Scand J Immunol.* (2000) 52:240–8. doi: 10.1046/j.1365-3083.2000.00790.x
27. Brandtzaeg P, Baekkevold ES, Farstad IN, Jahnsen FL, Johansen FE, Nilsen EM, et al. Regional specialization in the mucosal immune system: what happens in the microcompartments? *Immunol Today.* (1999) 20:141–51. doi: 10.1016/S0167-5699(98)01413-3
28. Erlandsson L, Akerblad P, Vingsbo-Lundberg C, Kallberg E, Lycke N, Leanderson T. Joining chain-expressing and -nonexpressing B cell populations in the mouse. *J Exp Med.* (2001) 194:557–70. doi: 10.1084/jem.194.5.557
29. Delanghe JR, Speckaert R, Speckaert MM. Complement C3 and its polymorphism: biological and clinical consequences. *Pathology.* (2014) 46:1–10. doi: 10.1097/PAT.000000000000042
30. Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. *Int Rev Physiol.* (2004) 44:465–8. doi: 10.1007/s00424-003-1192-y
31. Hernandez N, Gagnon K, Lederer E. Phosphate transport in epithelial and nonepithelial tissue. *Physiol Rev.* (2021) 101:1–35. doi: 10.1152/physrev.00008.2019
32. Marks J. The role of SLC34A2 in intestinal phosphate absorption and phosphate homeostasis. *Pflugers Arch.* (2019) 471:165–73. doi: 10.1007/s00424-018-2221-1
33. Jensen A, Figueiredo-Larsen M, Holm R, Broberg ML, Brodin B, Nielsen CU. PAT1 (SLC36A1) shows nuclear localization and affects growth of smooth muscle cells from rats. *Am J Physiol Endocrinol Metab.* (2014) 306:E65–74. doi: 10.1152/ajpendo.00322.2013
34. Proud CG. mTOR signalling in health and disease. *Biochem Soc Trans.* (2011) 39:431–6. doi: 10.1042/BST0390431
35. Liu H, Ma Y, He HW, Wang JP, Jiang JD, Shao RG. SLC9A3R1 stimulates autophagy via BECN1 stabilization in breast cancer cells. *Autophagy.* (2015) 11:2323–34. doi: 10.1080/15548627.2015.1074372
36. Hu C, Tao L, Cao X, Chen L. The solute carrier transporters and the brain: physiological and pharmacological implications. *Asian J Pharm Sci.* (2020) 15:131–44. doi: 10.1016/j.ajps.2019.09.002
37. Owczarek S, Soroka V, Kiryushko D, Larsen MH, Yuan Q, Sandi C, et al. Neuroplastin-65 and a mimetic peptide derived from its homophilic binding site modulate neurite outgrowth and neuronal plasticity. *J Neurochem.* (2011) 117:984–94. doi: 10.1111/j.1471-4159.2011.07269.x
38. Owczarek S, Kiryushko D, Larsen MH, Kastrup JS, Gajhede M, Sandi C, et al. Neuroplastin-55 binds to and signals through the fibroblast growth factor receptor. *FASEB J.* (2010) 24:1139–50. doi: 10.1096/fj.09-140509
39. Desrivieres S, Lourdasamy A, Tao C, Toro R, Jia T, Loth E, et al. Single nucleotide polymorphism in the neuroplastin locus associates with cortical thickness and intellectual ability in adolescents. *Mol Psychiatry.* (2015) 20:263–74. doi: 10.1038/mp.2013.197
40. Mineo C, Shaul PW. HDL stimulation of endothelial nitric oxide synthase: a novel mechanism of HDL action. *Trends Cardiovasc Med.* (2003) 13:226–31. doi: 10.1016/S1050-1738(03)00098-7
41. Biedzka-Sarek M, Metso J, Katefides A, Meri T, Jokiranta TS, Muszynski A, et al. Apolipoprotein A-I exerts bactericidal activity against *Yersinia enterocolitica* serotype O:3. *J Biol Chem.* (2011) 286:38211–9. doi: 10.1074/jbc.M111.249482
42. Suzuki M, Pritchard DK, Becker L, Hoofnagle AN, Tanimura N, Bammler TK, et al. High-density lipoprotein suppresses the type I interferon response, a family of potent antiviral immunoregulators, in macrophages challenged with lipopolysaccharide. *Circulation.* (2010) 122:1919–27. doi: 10.1161/CIRCULATIONAHA.110.961193
43. Kim SM, Kim SJ, Kim JY, Kim JR, Cho KH. Breast milk from smokers contains less cholesterol and protein and smaller size of apolipoprotein A-I resulting in lower zebrafish embryo survivability. *Breastfeed Med.* (2017) 12:365–72. doi: 10.1089/bfm.2016.0097
44. Kalman J, McConathy W, Araoz C, Kasa P, Lacko AG. Apolipoprotein D in the aging brain and in Alzheimer's dementia. *Neurol Res.* (2000) 22:330–6. doi: 10.1080/01616412.2000.11740678
45. Muffat J, Walker DW, Benzer S. Human ApoD, an apolipoprotein up-regulated in neurodegenerative diseases, extends lifespan and increases stress resistance in *Drosophila*. *Proc Natl Acad Sci USA.* (2008) 105:7088–93. doi: 10.1073/pnas.0800896105
46. Phie J, Krishna SM, Moxon JV, Omer SM, Kinobe R, Golledge J. Flavonols reduce aortic atherosclerosis lesion area in apolipoprotein E deficient mice: a systematic review and meta-analysis. *PLoS ONE.* (2017) 12:e0181832. doi: 10.1371/journal.pone.0181832
47. Zhang L, Boeren S, Hageman JA, van Hooijdonk T, Vervoort J, Hettinga K. Perspective on calf and mammary gland development through changes in the bovine milk proteome over a complete lactation. *J Dairy Sci.* (2015) 98:5362–73. doi: 10.3168/jds.2015-9342

Conflict of Interest: XZ, CJ, HL, LY, GJ, YW, Gual, and FZ were employed by the company Dong-E E-Jiao Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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1.Detailed information of the differentially expressed proteins

1.1 Information for each significantly up-regulated protein (P < 0.05) identified in DM vs HM group

UniProt Accession no.	Protein Name	Gene Name	LFQ intensity						Fold change	t test p value
			DM-1	DM-2	DM-3	HM-1	HM-2	HM-3		
B7VGF9	Alpha-S2-casein	CSN1S2	7.57E+11	5.82E+11	5.40E+11	3.68E+07	1.75E+07	1.07E+07	2.89E+04	7.05E-04
P11375	Lysozyme C	LYZ	2.14E+11	1.83E+11	1.58E+11	1.47E+08		5.98E+07	1.79E+03	3.14E-03
P86272	Alpha-S1-casein	CSN1S1	3.75E+11	4.32E+11	5.01E+11	9.40E+08	1.06E+08	1.08E+08	1.13E+03	2.80E-04
P28546	Alpha-lactalbumin	LALBA	3.42E+11	3.19E+11	3.74E+11	1.01E+09	1.78E+08	2.25E+08	7.34E+02	2.63E-05
F7C0Y4	amyloid A protein	LOC100056936	6.72E+10	6.67E+10	3.91E+10	1.66E+08	2.57E+08	1.12E+07	3.99E+02	3.45E-03
P86273	Beta-casein	CSN2	4.54E+11	4.91E+11	5.32E+11	1.95E+09	1.54E+09	9.51E+08	3.33E+02	2.59E-05
H9GZV1	Uncharacterized protein		1.81E+10	2.24E+10	3.63E+10	2.06E+08	6.74E+06		2.41E+02	3.65E-02
A0A3Q2HYP3	phosphoprotein 1	SPP1	7.30E+10	7.48E+10	5.77E+10	6.72E+08	2.75E+07		1.96E+02	2.32E-03
F7B0S3	Uncharacterized protein	MFGE8	1.40E+11	1.33E+11	6.57E+10	1.10E+09	1.38E+09	1.94E+09	7.67E+01	9.33E-03
P02758	Beta-LG-1	LGB1	3.15E+11	3.36E+11	3.82E+11	1.76E+10	2.13E+08	2.37E+08	5.73E+01	8.03E-05
A0A3Q2HRJ9	acid receptor responder 1	RARRES1	3.90E+08	4.57E+08	4.49E+08		1.18E+07	5.71E+06	4.92E+01	5.71E-04
F6W1N4	immunoglobulin receptor	PIGR	9.76E+09	1.02E+10	1.15E+10	3.46E+08	1.82E+08	2.17E+08	4.22E+01	3.84E-05
F7DDN1	acid synthase	FASN	6.53E+09	2.50E+09	3.50E+09	1.82E+08	2.69E+08	3.04E+08	1.66E+01	3.16E-02
A0A3Q2HN20	albumin	ALB	5.26E+10	5.02E+10	6.40E+10	6.14E+09	1.53E+09	3.12E+09	1.55E+01	3.08E-04
A0A3Q2HGD0	ATPase	ATP2B2	4.25E+08	3.99E+08	4.45E+08		5.91E+07	1.75E+07	1.10E+01	4.79E-04
Q13162	AOE37-2	PRDX4	2.77E+08	4.52E+08	2.49E+08	3.53E+07	4.30E+07	4.93E+07	7.68E+00	1.11E-02
A0A3Q2H4E3	carrier family 34 member 2	SLC34A2	1.97E+09	2.52E+09	1.26E+09	4.67E+08	3.59E+08		4.64E+00	4.99E-02
F7BPT4	Ezrin	EZR	1.07E+09	4.96E+08	5.51E+08	1.03E+08	2.26E+08	1.72E+08	4.23E+00	4.49E-02
F6Q9R1	Perilipin	PLIN2	8.01E+09	9.59E+09	8.82E+09	3.04E+09	2.49E+09	7.79E+08	4.19E+00	1.22E-03
A0A3Q2HNI5	II alpha subunit	GANAB	1.39E+09	1.35E+09	1.11E+09	2.75E+08	4.85E+08	4.10E+08	3.30E+00	1.09E-03
A0A0B4J1C4	chain of multimeric IgA and IgM	JCHAIN	3.72E+09	4.01E+09	5.72E+09	1.64E+09	1.26E+09	1.60E+09	2.99E+00	9.34E-03
F7AQZ6	kinase	LYN	2.22E+08	2.09E+08	1.57E+08		5.87E+07	8.67E+07	2.70E+00	2.12E-02
F6Z2L5	Apolipoprotein A1	APOA1	1.40E+09	2.82E+09	2.11E+09	8.93E+08	5.89E+08	9.45E+08	2.61E+00	3.70E-02
F7D8I6	Xanthine dehydrogenase	LOC100054601	5.62E+10	8.46E+10	5.39E+10	2.15E+10	2.27E+10	3.66E+10	2.41E+00	2.62E-02

A0A3Q2I8J7	lectin domain-containing protein		6.50E+06	8.10E+06						
F7CLE3	pyrophosphatase/phosphodiesterase 3	ENPP3	8.32E+06	1.57E+07						
K9KFF1	protein (Fragment)		8.69E+06	9.23E+06						
Q9TQV1	Transferrin(Fragment)		8.73E+06	2.58E+07	1.30E+07					
K9KB26	emp24 domain-containing protein 10-like protein (Fragment)		8.76E+06	7.46E+06						
F6TBM0	17-beta-dehydrogenase 11-like protein	HSD17B11	9.78E+06	6.47E+07						
F6TL52	kinase isoenzyme 1	AK1	1.01E+07	1.05E+07						
A0A3Q2I9V2	transport protein	SFT2D2	1.03E+07	1.43E+07	1.47E+07					
A0A3Q2HV56	protein	RPL11	1.06E+07	7.73E+06						
Q13228	oxidase	SELENBP1	1.17E+07	2.76E+07						
F6PNM1	cell derived factor 4	SDF4	1.20E+07	7.67E+06	1.49E+07					
Q861L3	class I antigen (Fragment)		1.20E+07	2.44E+07						
A0A3Q2HQH1	domain-containing protein		1.26E+07	1.35E+07						
F6Y426	Nucleobindin 2	NUCB2	1.27E+07	1.26E+07	1.23E+07					
P69905	subunit alpha	HBA1	1.30E+07	2.39E+07						
P62826	nuclear protein Ran	RAN	1.30E+07	5.19E+06	4.74E+06					
K9KEF8	reticulum resident protein ERp44-like protein (Fragment)		1.32E+07	1.43E+07	1.20E+07					
L7MRN0	aminotransferase (Fragment)	GOT1	1.47E+07	1.11E+07						
P14625	GRP-94	HSP90B1	1.54E+07	1.27E+07	3.33E+07					
F7B4C0	domain-containing protein	MAL2	1.60E+07	3.16E+07	3.28E+07					
P01834	kappa constant	IGKC	1.72E+07	6.29E+06						
K9K4A9	protein Rap-2c-like protein (Fragment)		1.74E+07	2.43E+07						
A0A3Q2HIU8	fragment of IgA and IgM receptor	FCAMR	1.76E+07	2.42E+07	2.23E+07					
A8HDQ5	disintegrin-metalloprotease		1.76E+07	2.14E+07						
F7A676	aminopeptidase 3	LAP3	1.78E+07	2.95E+06	8.64E+05					
F6XSG4	ATPase	ATP2B1	1.80E+07	4.83E+07	5.39E+07					
F6RCA8	Peroxiredoxin-5	PRDX5	1.92E+07	1.17E+07	1.09E+07					

F6RHD5	phosphoribosyltransferase	APRT	1.97E+07	1.80E+07	2.32E+07					
F6YSK7		MARCKS	2.00E+07		5.85E+06					
A0A3Q2H6L1	cofactor protein	LOC100057176	2.06E+07	2.26E+07	2.36E+07					
Q7L0Q8	GTP-binding protein RhoU	RHO	2.07E+07	3.67E+07	3.80E+07					
A0A3Q2IBB5	glycoprotein 1	GLG1	2.09E+07	2.53E+07						
F6U5V3	Transcobalamin 2	TCN2	2.13E+07	3.17E+07	3.53E+07					
H9GZL8	acidic ribosomal protein P2	RPLP2	2.15E+07	3.00E+07	3.94E+07					
A0A3Q2HWY7		LOC100060539	2.20E+07		4.66E+07					
F7B6D0	Alpha-galactosidase	GLA	2.23E+07	7.62E+07	4.19E+07					
F7C8R2	Tetraspanin	CD82	2.24E+07	2.65E+07	2.53E+07					
F6VBA3		SYTL1	2.36E+07	2.71E+07						
Q9GKX8	shock protein HSP 90-beta	HSP90AB1	2.42E+07	2.26E+07	1.57E+07					
Q15836	membrane protein 3	VAMP3	2.62E+07	1.46E+07						
F6SUZ2	UDP-glucose 4-epimerase	GALE	2.93E+07	2.88E+07						
F6Q3S6	associated domain containing 1	PRADC1	3.01E+07	3.16E+07	2.96E+07					
F6ZAQ9	trafficking 1	VTA1	3.07E+07	2.84E+07						
F6XSF7	C4a anaphylatoxin	LOC100059239	3.16E+07	2.40E+07						
A0A3Q2HSC6	Endoglin	ENG	3.19E+07	2.17E+07						
P01024	C3-beta-c	C3	3.19E+07	2.60E+07	5.79E+07					
F6SFY3	15, cell surface associated	MUC15	3.22E+07	5.12E+07	3.92E+07					
A0A3Q2H332	synthetase short chain family member 2	ACSS2	3.37E+07	2.43E+07	3.79E+07					
A0A3Q2IG74	Podocalyxin	PODXL	3.39E+07	3.07E+07	2.40E+07					
F6Q5L6	purinoceptor	P2RX7	3.42E+07	5.79E+07	3.19E+07					
F6WPD2	protein	IFI27	3.46E+07	4.31E+07	3.99E+07					
A0A3Q2IDD2	C3/C5 convertase	CFB	3.49E+07		4.72E+07					
F7BC79	Syntaxin 3	STX3	3.61E+07	3.69E+07	2.92E+07					
F6S8C6	PDZ domain containing family member 2	GIPC2	3.64E+07	3.22E+07	1.05E+07					
F6ZHQ5	intracellular channel protein	CLIC1	3.64E+07	2.28E+07	2.16E+07					
Q5T749	proline-rich protein	KPRP	3.67E+07	1.89E+07						
F6YCC0	Cystatin A	CSTA	3.77E+07	3.61E+07	3.56E+07					

A0A3Q2L2R4	domain-containing protein	LOC100051562	3.81E+07	4.64E+07	4.43E+07					
F6TLH4	carrier-associated membrane protein	SCAMP2	3.85E+07	2.99E+07	2.39E+07					
U3MI26	(Fragment)	CD105	3.87E+07	4.21E+07						
A0A3Q2GUH6	peroxidase	GPX4	3.88E+07	6.03E+07	2.35E+07					
F6YNH6		CTSB	3.97E+07	3.42E+07	3.72E+07					
K9K2D2	dehydrogenase		4.00E+07	2.95E+07						
F6XM13	Apolipoprotein D	APOD	4.06E+07	5.36E+07	2.97E+07					
F6W9B1	domain-containing protein	ST13	4.10E+07	4.80E+07	2.29E+07					
K9K4J7	chaperone 1-like protein (Fragment)		4.24E+07	4.42E+07	5.50E+07					
K9K410	phosphatase type IVA 2-like protein		4.24E+07	8.17E+07	7.15E+07					
A0A3Q2H3D5		CD81	4.25E+07	4.39E+07	4.14E+07					
F6Y3Z2	like proto-oncogene A	RALA	4.32E+07	9.83E+07	8.07E+07					
P01008	Antithrombin-III	SERPINC1	4.50E+07	1.67E+08						
A0A3Q2H198	protein	LOC111769297	4.61E+07	6.80E+07	2.33E+07					
F6TIR2	Lipocalin 2	LCN2	4.81E+07		2.72E+08					
F7BPX8	protein	CTSV	4.86E+07	6.71E+07	8.41E+07					
Q9TQW8	(Fragment)		4.87E+07	3.51E+07						
A0A3Q2LL78	S1 domain-containing protein	LOC100055297	5.20E+07	4.14E+07	1.15E+08					
F7C7V8	Semaphorin 7A	SEMA7A	5.62E+07	4.88E+07	5.02E+07					
F7AVT9	protein	LOC100071734	5.63E+07	4.89E+07	2.83E+07					
F6VUW2	Cathepsin S	CTSS	5.91E+07	8.46E+07	7.31E+07					
Q6TGR2	co-receptor MD-2		5.92E+07	5.57E+07	3.90E+07					
F6VJR6	Alpha-1B-glycoprotein	A1BG	6.05E+07	4.91E+07	7.41E+07					
F7AKG6	methylcytosine dioxygenase 3	TET3	6.05E+07	1.15E+08						
F6T0P6	vitamin D binding protein	GC	6.07E+07	6.09E+07	7.66E+07					
A0A3Q2I2H1	domain-containing protein		6.22E+07	1.02E+08	1.42E+08					
F7C450	2-HS glycoprotein	AHSG	6.42E+07	7.10E+07	9.47E+07					
A0A3Q2ICL4	protein	LOC111767442	6.48E+07	3.75E+07	3.03E+07					
F7CFK9	cell derived factor 2	SDF2	6.60E+07	5.78E+07						
Q14697	alpha-glucosidase AB	GANAB	7.27E+07		4.37E+07					
A0A3Q2HBP1	binding protein 1	HEBP1	7.50E+07	1.03E+08	8.62E+07					

F7DJZ1	domain-containing protein		7.83E+07	5.58E+07						
F6UJ33	Profilin	PFN1	7.83E+07	5.20E+07						
A0A3Q2H5J5	RAB10	RAB10	8.04E+07	6.82E+07	6.46E+07					
A0A3Q2L3I3	Clusterin	CLU	8.10E+07	8.78E+07	1.52E+08					
F7CSL8	domain-containing protein	SPI2	8.10E+07	8.47E+07	1.04E+08					
A0A3Q2KY85		APOH	8.33E+07	5.94E+07	6.92E+07					
F6TF18	BAX inhibitor motif containing 1	TMBIM1	8.85E+07	1.30E+08	1.06E+08					
F6VGG1	ribosomal protein S12	RPS12	8.97E+07	5.10E+07	2.88E+07					
A0A0A1E464	lambda light chain variable region (Fragment)	IGL	9.53E+07	1.05E+08	1.35E+08					
P0DN28	C-III	APOC3	9.87E+07	2.15E+08	2.24E+08					
F6V812	N-acetyl-alpha-glucosaminidase	NAGLU	1.00E+08	7.93E+07	5.70E+07					
F6QXW2	binding protein 1	PEBP1	1.00E+08		4.78E+07					
F6QK77	Deoxyribonuclease	DNASE1L1	1.02E+08	2.06E+08	1.49E+08					
F7DZ70	member RAS oncogene family	RAB3D	1.07E+08	1.60E+08	1.23E+08					
O97973	(Fragment)		1.11E+08	8.57E+07	1.27E+08					
A0A3Q2HRU9	binding protein	SDCBP	1.19E+08	1.05E+08	1.04E+08					
F7C603	phosphodiesterase acid like 3B	SMPDL3B	1.31E+08	2.51E+08	6.28E+07					
P01860	heavy constant gamma 3	IGHG3	1.32E+08	3.91E+07	3.20E+07					
A0A3Q2HQ25	protein	LOC100054641	1.34E+08	6.27E+07						
F6UL68	Transthyretin	TTR	1.35E+08	1.17E+08	1.08E+08					
F6SX98	aldolase	ALDOA	1.36E+08	3.42E+07	5.16E+07					
F7BV85	heavy chain	CLTC	1.36E+08	7.64E+07	7.87E+07					
A0A0A1E417	lambda light chain variable region (Fragment)	IGL	1.49E+08	1.14E+08	1.99E+08					
Q0R0D3	class I antigen	Eqca-16	1.51E+08	2.70E+08	1.24E+08					
P47710	Alpha-S1-casein	CSN1S1	1.57E+08	2.14E+07	2.32E+07					
Q5XWB8	receptor (Fragment)	CD14	1.62E+08	2.19E+08	1.84E+08					
F7C1X7	exchange regulatory cofactor NHE-RF	SLC9A3R1	1.63E+08	1.06E+08	8.58E+07					
A0A3Q2L6D8	cell death 6 interacting protein	PDCD6IP	1.65E+08	1.67E+08						

F7CXT0	b reductase 1	CYBRD1	1.68E+08	1.84E+08	2.25E+08					
F6Y688	cyclase/mutase family member	LSS	1.76E+08	2.84E+08	2.38E+08					
F6T835	Tetraspanin	TSPAN6	1.78E+08	1.10E+08	5.83E+07					
F6S3Y7	S100	S100G	1.84E+08	1.72E+08	1.61E+08					
A0A3Q2I2V3	A1 domain-containing protein	CTSD	1.92E+08	1.99E+08	2.48E+08					
A0A3Q2LRH1	carrier family 36 member 1	SLC36A1	1.94E+08	5.99E+08	3.33E+08					
A0A3Q2KP77	protein	DEFB1	2.04E+08	8.74E+07	2.84E+07					
A0A3Q2HM99	protein	LOC102148710	2.11E+08		1.50E+08					
Q863B0	Beta-2-microglobulin	b-2-m	2.12E+08	1.29E+08	9.24E+07					
F6VBP9	E	APOE	2.27E+08	3.06E+08	3.36E+08					
A0A3Q2LFM9	cytoplasmic 1 intermediate chain 1	DYNC111	2.44E+08	2.87E+08	2.96E+08					
A5YBL8	cis-trans isomerase	PPIB	2.59E+08	1.65E+08	1.76E+08					
F6TT70	protein	LOC100146423	2.60E+08	5.50E+08	6.84E+08					
A0A3Q2I5T2	domain-containing protein	CST3	2.66E+08	2.55E+08	3.11E+08					
F6RI26	dehydrogenase 9 family member A1	ALDH9A1	2.75E+08	1.56E+08						
P08334	A		2.84E+08	2.46E+08						
A0A3Q2ID55	carrier family 4 member 9	SLC4A9	2.97E+08	4.85E+08	6.17E+08					
F5CEP2	fat globule-EGF factor 8 splice variant (Fragment)	MFGE-8	3.19E+08	4.45E+08						
F6WG98		SLC6A14	3.21E+08	3.52E+08	3.43E+08					
M9ZUR8	S-transferase A1	GSTA1	3.28E+08	2.47E+08	2.06E+08					
A0A3Q2HWQ6	C3-beta-c	LOC100060505	3.60E+08	4.83E+07	2.51E+08					
A0A3Q2H3T6	protein	MFGE8	3.73E+08	3.13E+08	3.47E+08					
A0A3Q2IBR6	repeat containing protein X-linked	SRPX	3.93E+08	3.18E+08	2.97E+08					
C0HJR4	(Fragment)	MFGE8	3.94E+08	4.02E+08	2.81E+08					
F6USP9		PLG	3.95E+08	2.59E+08	2.24E+08					
A0A0A1E6E8	lambda light chain variable region (Fragment)	IGL	4.00E+08	1.27E+09	1.37E+09					
F7A684	protein	IFI27	4.13E+08	4.43E+08	5.55E+08					
F6X8Q2	phosphoglucomutase 1	PGM1	4.64E+08	1.48E+08	1.22E+08					
F6SQS6	like 7A	METTL7A	4.68E+08	7.11E+08	4.34E+08					

Q865P6	Beta-defensin 1	defb1	4.69E+08	1.18E+08	1.12E+08					
F6VKB4		CSN3	4.80E+08	4.36E+08	4.68E+08					
B5BV04	Alpha-1-antitrypsin	Spi2-5	5.16E+08	4.33E+08	7.69E+08					
H9GZR2	protein		5.49E+08	3.31E+08	3.04E+08					
F6Y0D9	Cadherin-1	CDH1	5.57E+08	6.51E+08	6.16E+08					
A0A3Q2IBE3	uridylyltransferase	UGP2	5.60E+08	2.40E+08	1.97E+08					
F6RM73	Apolipoprotein A-II	APOA2	5.61E+08	4.98E+08	6.39E+08					
H9GZQ9	protein		6.07E+08	5.34E+08	1.07E+09					
A0A3Q2GVQ5	precursor	GRN	7.74E+08	6.36E+08	6.30E+08					
P19857	amyloid A protein	SAA1	8.29E+08	1.17E+09	4.92E+08					
F6PH38	beta chain	FGB	9.01E+08	2.94E+09	3.02E+09					
Q6X9W5	amyloid A protein (Fragment)	SAA	1.08E+09	6.42E+08	9.11E+08					
F7AYT5	synthetase long chain family member 4	ACSL4	1.34E+09	1.78E+09	9.13E+08					
A0A3Q2GXW2	domain-containing protein	CIDEA	1.54E+09	2.26E+09	2.08E+09					
A0A0A1E4I0	lambda light chain variable region (Fragment)	IGL	1.55E+09	8.73E+08	8.89E+08					
F6VF11	like 2	CHRD12	1.69E+09	1.32E+09	1.48E+09					
F6W3F0	fibrocystin/polyductin	PKHD1	3.02E+09	1.12E+10	1.27E+10					
P08896	B/C		4.01E+09	3.17E+09	3.42E+09					
H9GZU8	protein		4.44E+09	2.49E+09	4.57E+09					
F7BL38	domain-containing protein	MUC1	4.54E+09	5.05E+09	3.48E+09					
F6XGE0	protein	CD55	4.86E+09	5.97E+09	3.94E+09					
F7CWC8	oxidase	LOC100147151	5.48E+09	8.88E+09	1.14E+10					
H9GZT5	protein		6.83E+09	5.38E+09	6.37E+09					
H9GZN9	protein		7.14E+09	9.70E+09	1.14E+10					
Q95M34	gamma 1 heavy chain constant region (Fragment)	IGHC1	8.81E+09	9.06E+09	8.53E+09					
P11376	C, milk isozyme	LYZ	2.03E+10	1.59E+10	1.48E+10					
A0A0A1E9A8	lambda light chain variable region (Fragment)	IGL	2.29E+10	2.38E+10	4.72E+09					
P82187		CSN3	3.74E+10	2.79E+10	1.18E+10					
K9K4B2	protein Rab-8B-like protein	RAB8B		6.38E+06	7.27E+06					
Q6UWP8		SBSN		9.15E+06	1.61E+07					

F6R942		A2M		1.15E+07	1.24E+07					
A0A3Q2L0G4	domain-containing protein	SRSF4		1.64E+07	1.20E+08					
A0A3Q2GV97		NPTN		1.98E+07	1.54E+07					
F7DG10	H ⁺ transporting accessory protein 1	ATP6AP1		4.10E+07	3.04E+07					
F7CAC5	domain-containing protein			5.21E+08	1.52E+08					

1.2 Information for each significantly down-regulated protein (P < 0.05) identified in DM vs HM group

UniProt Accession no.	Protein Name	Gene Name	LFQ intensity						Fold change	t test p value
			DM-1	DM-2	DM-3	HM-1	HM-2	HM-3		
F6R7J2	dehydrogenase (NADP(+)) 1, cytosolic	IDH1	1.02E+08	7.32E+07	7.22E+07	1.88E+08	1.46E+08	1.62E+08	4.97E-01	5.99E-03
A0A3Q2I0E0	member RAS oncogene family	RAB14	1.64E+07	1.44E+07	9.06E+06		2.86E+07	2.92E+07	4.60E-01	1.18E-02
K9K3M3	protein Rab-18-like protein		1.14E+09	1.48E+09	1.16E+09	2.43E+09	2.22E+09	3.69E+09	4.54E-01	3.25E-02
C7E3N6	fatty acid-binding protein	FABP3	1.69E+09	1.70E+09	1.70E+09	3.98E+09	2.93E+09	4.99E+09	4.27E-01	1.89E-02
K9K4D8	protein RhoA-like protein	RHOA	2.57E+08	2.14E+08	2.35E+08	4.70E+08	5.18E+08	7.59E+08	4.04E-01	1.83E-02
F6XAB0	protein	SNAP23	7.11E+07	7.91E+07	6.03E+07	1.94E+08	1.98E+08	2.44E+08	3.31E-01	1.08E-03
F6Q6E8	binding cassette subfamily G member 2	ABCG2	5.51E+08	9.29E+08	5.15E+08	1.84E+09	1.73E+09	2.50E+09	3.29E-01	7.77E-03
A0A3Q2KK41	member RAS oncogene family	RAB1A	6.44E+08	1.95E+08	2.08E+08	1.09E+09	8.17E+08	1.49E+09	3.09E-01	3.28E-02
A0A3Q2H179	carrier family 39 member 8	SLC39A8	9.38E+07	7.93E+07	6.12E+07	2.79E+08	1.95E+08	3.17E+08	2.96E-01	7.43E-03
F6U904	molecule	CD36	9.51E+08	7.20E+08	3.35E+08	2.39E+09	2.33E+09	4.76E+09	2.11E-01	3.84E-02
F7CTF0	hormone-related protein	PTHLH	5.39E+07	5.56E+07	6.17E+07	4.76E+08	2.67E+08	3.04E+08	1.63E-01	1.05E-02
F6QUF7	lipase	LPL	7.31E+08	1.98E+09	1.10E+09	1.09E+10	6.34E+09	6.18E+09	1.63E-01	1.49E-02
Q99943	acyltransferase alpha	AGPAT1	5.76E+07	8.99E+07	6.57E+07	5.21E+08	4.48E+08	5.31E+08	1.42E-01	1.01E-04
F6TGW9	b5 reductase	CYB5R3	7.37E+07	1.94E+08	1.37E+08	8.09E+08	9.42E+08	1.21E+09	1.37E-01	2.20E-03
A0A3Q2HQY6	domain-containing protein	FOLR2	1.20E+07	3.36E+07	1.78E+07	1.64E+08		1.81E+08	1.23E-01	7.09E-04
P05413	acid-binding protein, heart	FABP3	5.18E+08	3.63E+08	4.06E+08	3.66E+09	3.42E+09	3.75E+09	1.19E-01	7.60E-06
A0A3Q2KN19	protein	NUCB1	6.91E+07	5.93E+07	1.34E+08	1.47E+09	6.71E+08	9.23E+08	8.57E-02	1.67E-02

K9K2R3	(Fragment)		9.86E+06	4.05E+06		3.51E+08	5.36E+08	5.69E+08	1.43E-02	1.21E-02
Q14390	hydrolase light chain 2	GGTLC2				1.13E+08	1.24E+08	1.47E+08		
O00481	subfamily 3 member A1	BTN3A1				2.70E+07	3.86E+07	6.16E+07		
O00754	alpha-mannosidase	MAN2B1				6.82E+07	6.52E+07	4.90E+07		
O60543	death activator CIDE-A	CIDEA				5.69E+08	5.47E+08	1.21E+09		
O95084	protease 23	PRSS23				2.23E+08	3.55E+08	2.87E+08		
P00441	dismutase [Cu-Zn]	SOD1					3.56E+07	3.11E+07		
P01833	immunoglobulin receptor	PIGR				4.86E+08	3.67E+08	5.23E+08		
P05186	phosphatase, tissue-nonspecific isozyme	ALPL				1.11E+07	8.40E+06	3.05E+07		
P05423	RNA polymerase III subunit RPC4	POLR3D				4.93E+10	4.28E+10	3.27E+10		
P07339	D	CTSD					1.07E+08	9.81E+07		
F7CK20	kinase	FGR				4.25E+07	4.47E+07	4.83E+07		
P16403	H1.2	HIST1H1C				6.70E+07	1.01E+09	6.72E+07		
A0A3Q2HUK6	domain-containing protein	HIST1H1B					1.90E+08	1.25E+07		
P22079		LPO				7.07E+07	1.18E+08			
F6RA08		STOM				1.95E+08	7.54E+08	2.64E+08		
K9KD26	aldehyde dehydrogenase-like protein (Fragment)					2.79E+07	1.73E+07	3.34E+07		
P34096	4	RNASE4				1.57E+09	1.99E+09	2.52E+09		
F6WCS1	heavy chain 9	MYH9					1.10E+08	1.13E+07		
Q9TUL9	inhibitor 3	TIMP3				1.59E+09	1.20E+09	1.18E+09		
P47989	dehydrogenase/oxidase	XDH				1.66E+09	1.49E+09	1.45E+09		
P48449	synthase	LSS				1.40E+08	9.87E+07	2.43E+08		
A0A3Q2LC76	ESCRT-III associated factor	IST1				1.85E+07	1.86E+07	2.06E+07		
P55259	secretory granule membrane major glycoprotein GP2	GP2				8.18E+08	7.11E+08	1.06E+09		
P60660	light polypeptide 6	MYL6				4.92E+06	3.88E+07			
P80511	S100-A12	S100A12				7.46E+07	1.02E+08			
P81605		DCD				1.06E+08	2.08E+07	2.73E+07		
Q08431		MFGE8				1.57E+09	6.69E+09	1.07E+10		
F6QMB8	protein subunit alpha 13	GNA13				1.13E+08	1.19E+08	1.49E+08		
K9KD98	receptor (Fragment)					3.72E+07	3.64E+07	6.10E+07		

A0A3Q2HYJ5	acyltransferase 4	GPAT4				4.28E+07	2.65E+07	4.32E+07		
K9K4C9	(Fragment)					9.62E+07	8.46E+08	1.00E+08		
Q8TBF2	F synthase	PRXL2B					3.93E+07	5.24E+07		
K9KDJ7	transport protein 81-like protein-like protein (Fragment)					3.28E+08	1.51E+09	1.45E+08		
Q92743	protease HTRA1	HTRA1				7.39E+07	1.25E+08	1.19E+08		
Q96CE8	4 L6 family member 18	TM4SF18				7.20E+06	3.14E+06	3.57E+07		
Q99541		PLIN2				8.71E+09	9.11E+09	1.59E+10		
F7CUM5	and integrin binding 1	CIB1				8.90E+07		9.79E+07		
Q9BXJ0	C1q tumor necrosis factor-related protein 5	C1QTNF5				1.87E+07	7.88E+07	9.66E+07		
Q9H1C7	and transmembrane domain-containing protein 1	CYSTM1				9.20E+07	9.05E+07	1.62E+08		
Q9HCL2	acyltransferase 1, mitochondrial	GPAM					2.53E+07	2.71E+07		
Q9NZG7		NINJ2				1.99E+08	5.26E+07	2.87E+07		
F7B3U1	protein-coupled receptor class C group 5 member B	GPRC5B				1.10E+08	1.48E+08	2.37E+08		
Q9UBS3	homolog subfamily B member 9	DNAJB9				7.66E+06	1.53E+07	1.45E+08		
Q9UNQ0	cassette sub-family G member 2	ABCG2				2.18E+09	2.49E+09	3.64E+09		
Q9Y4Z0	snRNA-associated Sm-like protein LSM4	LSM4				3.16E+10	4.04E+10	6.12E+10		
K9K9N6	domain-containing protein 1-like protein (Fragment)					3.06E+08	2.83E+08	3.38E+08		
Q9Y5G8	gamma-A5	PCDHGA5				4.14E+07	2.86E+07	4.85E+07		
F6TN81	G, endothelial type	LIPG				1.36E+07	4.93E+07	5.99E+07		
A0A3Q2GU69	v-SNARE homolog	YKT6				6.70E+07	8.38E+07	1.12E+08		
A0A3Q2GYF5	phosphatase	ALPL				1.07E+08	8.24E+07	1.81E+08		
A0A3Q2KMN3	rich secretory protein LCCL domain containing 2	CRISPLD2				1.75E+08	1.63E+08	1.80E+08		
A0A3Q2H6M5	like 2	HHIPL2				1.67E+09	1.84E+09	1.47E+09		
A0A3Q2HG76	protein subunit alpha o1	GNAO1				3.03E+07	1.75E+07	4.17E+07		
A0A3Q2HSB3		LPO				2.46E+09	2.14E+09	2.43E+09		
F7B5C4		VIM				3.25E+07	7.43E+08	6.29E+07		

F7CAB8	1, zinc-binding	AZGP1				9.36E+08	6.82E+08	7.15E+08		
A0A3Q2KJE2	cotransporter	LOC111772073				5.41E+08	4.99E+08	5.77E+08		
F6HXH6	type XVIII alpha 1 chain	COL18A1				1.87E+07	2.10E+07	9.22E+06		
F6V3X9	protein	MAN2B1				4.34E+07	6.81E+07	1.73E+07		
A0A3Q2LEZ9	binding lectin serine peptidase 1	MASP1				1.53E+07		1.12E+07		
A0A3Q2LT54	binding protein 2	STXBP2				2.77E+07	3.48E+07	2.29E+07		
K9K2N5	protein subfamily B member 9-like protein (Fragment)					2.66E+08	1.57E+08	2.76E+08		
F6SZJ2	domain containing 4	EHD4					3.16E+07	4.31E+07		
F6VZN7	homolog family member G	RHOG				1.85E+07	4.16E+07	2.47E+07		
F6WR95	oxidase	QSOX1				1.32E+09	1.33E+09	1.15E+09		
F6YQM5		PSAP				1.41E+08	6.19E+07	8.05E+07		
F6ZBH7	nucleotide exchange factor	SIL1				8.81E+07	5.98E+07	6.84E+07		
K9KB03	GDP-dissociation inhibitor 2-like protein					6.55E+06	2.24E+07			
F7BKA2	kinase	PDXK				7.58E+07	8.48E+07	2.17E+08		
F7BYW1	2	ADM2				2.48E+07	2.75E+07	5.13E+07		
F7CLX6	like EF-hand protein 1	CHP1					4.73E+07	1.53E+08		

Detailed information of GO-based enrichment of the differentially expressed proteins

GO_ID	Term	Category	Test	Ref	TestAll	RefAll	Test_per	Ref_per	Over_Under	TestSeqs	RefSeqs	P value	FDR	richFactor
GO:0002250	adaptive immune response	P	16	17	293	473	0.05461	0.0359	over	P01024,F7CAB8,F6Q5L6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7,O00481,A0A0B4J1C4,F6VUW2	P01024,F7CAB8,F6Q5L6,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7,O00481,A0A0B4J1C4,F6VUW2	0.0029	0.99999	0.9411765
GO:0002697	regulation of immune effector process	P	12	12	293	473	0.04096	0.0254	over	P01024,F7CAB8,F6Q5L6,F6RM73,F6Z2L5,P01834,P01860,F6T835,F7AQZ6,F7CK20,Q92743,F7C7V8	P01024,F7CAB8,F6Q5L6,F6RM73,F6Z2L5,P01834,P01860,F6T835,F7AQZ6,F7CK20,Q92743,F7C7V8	0.0029	0.99999	1
GO:0002682	regulation of immune system process	P	42	53	293	473	0.14334	0.1121	over	P01024,A0A3Q2LEZ9,F7CAB8,F6Q5L6,P14625,F7AQZ6,F6RM73,F6Z2L5,K9KD98,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,O00481,F7CK20,P00441,F7C603,Q6TGR2,P01833,F6T835,Q9HCL2,F6VBP9,P05423,F7CUM5,K9K4D8,Q92743,F7BPT4,Q9GKX8,F7C7V8,F6VZN7,F6XM13,F7DG10,A0A3Q2L2R4	P01024,A0A3Q2LEZ9,F7CAB8,F6Q5L6,P06702,P14625,F7AQZ6,F6RM73,F6Z2L5,K9KD98,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,O00481,F6RQM5,P23396,P63000,F7CK20,P00441,F7C603,Q6TGR2,P02788,P01833,P59998,P60709,F6T835,Q9HCL2,F6VBP9,P05423,P26583,F7CUM5,K9K4D8,Q92743,F7BPT4,Q9GKX8,F7C7V8,F6VZN7,F6VWX6,F6XM13,O77811,F7DG10,A0A3Q2L2R4	0.0036	0.99999	0.7924528
GO:0006956	complement activation	P	15	16	293	473	0.05119	0.0338	over	A0A3Q2LEZ9,P01024,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7	A0A3Q2LEZ9,P01024,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7	0.0045	0.99999	0.9375
GO:0042113	B cell activation	P	11	11	293	473	0.03754	0.0233	over	Q9UBS3,F7AQZ6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	Q9UBS3,F7AQZ6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0048	0.99999	1
GO:0034987	immunoglobulin receptor binding	F	11	11	293	473	0.03754	0.0233	over	F7CK20,P01834,P01860,A0A0B4J1C4,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	F7CK20,P01834,P01860,A0A0B4J1C4,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0048	0.99999	1
GO:0046649	lymphocyte activation	P	17	19	293	473	0.05802	0.0402	over	K9K4D8,Q9UBS3,F7AQZ6,F7CK20,P00441,F6Q5L6,Q9HCL2,O00481,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	K9K4D8,Q9UBS3,F7AQZ6,P63000,F7CK20,P00441,F6Q5L6,P23396,Q9HCL2,O00481,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0076	0.99999	0.8947368

GO:0008037	cell recognition	P	10	10	293	473	0.03413	0.0211	over	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0078	0.99999	1
GO:0019814	immunoglobulin complex	C	10	10	293	473	0.03413	0.0211	over	P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,A0A0B4J1C4	P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,A0A0B4J1C4	0.0078	0.99999	1
GO:0050864	regulation of B cell activation	P	10	10	293	473	0.03413	0.0211	over	F7AQZ6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	F7AQZ6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0078	0.99999	1
GO:0006910	phagocytosis, recognition	P	10	10	293	473	0.03413	0.0211	over	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0078	0.99999	1
GO:0050853	B cell receptor signaling pathway	P	10	10	293	473	0.03413	0.0211	over	F7AQZ6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	F7AQZ6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0078	0.99999	1
GO:0042571	immunoglobulin complex, circulating	C	10	10	293	473	0.03413	0.0211	over	A0A0B4J1C4,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	A0A0B4J1C4,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0078	0.99999	1
GO:0010324	membrane invagination	P	13	14	293	473	0.04437	0.0296	over	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6VZN7,P01024,K9K4D8	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,P63000,F6VZN7,P01024,K9K4D8	0.0109	0.99999	0.9285714
GO:0099024	plasma membrane invagination	P	13	14	293	473	0.04437	0.0296	over	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6VZN7,P01024,K9K4D8	P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,P63000,F6VZN7,P01024,K9K4D8	0.0109	0.99999	0.9285714
GO:0002449	lymphocyte mediated immunity	P	13	14	293	473	0.04437	0.0296	over	P01024,F7CAB8,F6Q5L6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	P01024,F7CAB8,F6Q5L6,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	0.0109	0.99999	0.9285714
GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	P	13	14	293	473	0.04437	0.0296	over	P01024,F7CAB8,F6Q5L6,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	P01024,F7CAB8,F6Q5L6,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	0.0109	0.99999	0.9285714
GO:0042803	protein homodimerization activity	F	16	18	293	473	0.05461	0.0381	over	O60543,P00441,Q9GKX8,F6RA08,P47989,Q13162,Q9UNQ0,A0A0B4J1C4,A0A3Q2LEZ9,F6QUF7,F6RM73,F6VBP9,F6Y0D9,F6YQM5,F7B6D0,F7BKA2,F7E3Y7	O60543,P00441,Q9GKX8,F6RA08,P47989,P49327,Q13162,Q9UNQ0,A0A0B4J1C4,A0A3Q2LEZ9,F6QUF7,F6RM73,F6VBP9,F6Y0D9,F6YQM5,F7B6D0,F7BKA2,F7E3Y7	0.0113	0.99999	0.8888889
GO:0003823	antigen binding	F	9	9	293	473	0.03072	0.019	over	P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0128	0.99999	1

GO:0050871	positive regulation of B cell activation	P	9	9	293	473	0.03072	0.019	over	P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0128	0.99999	1
GO:0050776	regulation of immune response	P	33	42	293	473	0.11263	0.0888	over	P01024,A0A3Q2LEZ9,F7CAB8,F6Q5L6,P14625,F7AQZ6,F6RM73,F6Z2L5,K9KD98,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,O00481,F7C603,Q6TGR2,P01833,F6T835,F7CK20,F6VBP9,P05423,F7BPT4,Q9GKX8,F7C7V8	P01024,A0A3Q2LEZ9,F7CAB8,F6Q5L6,P06702,P14625,F7AQZ6,F6RM73,F6Z2L5,K9KD98,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,O00481,F6RQM5,P23396,F7C603,Q6TGR2,P02788,P01833,P63000,P59998,P60709,F6T835,F7CK20,F6VBP9,P05423,P26583,F7BPT4,Q9GKX8,F7C7V8	0.0133	0.99999	0.7857143
GO:0006911	phagocytosis, engulfment	P	12	13	293	473	0.04096	0.0275	over	F6VZN7,P01024,P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	P63000,F6VZN7,P01024,P01834,P01860,Q08431,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0166	0.99999	0.9230769
GO:0050865	regulation of cell activation	P	15	17	293	473	0.05119	0.0359	over	F6VBP9,F7AQZ6,F7CK20,P00441,Q9HCL2,K9K4D8,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	F6VBP9,F7AQZ6,P63000,F7CK20,P00441,P23396,Q9HCL2,K9K4D8,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0167	0.99999	0.8823529
GO:0072376	protein activation cascade	P	17	20	293	473	0.05802	0.0423	over	A0A3Q2LEZ9,P01024,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6PH38,P01008	A0A3Q2LEZ9,P01024,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,A0A3Q2HTG2,F6PH38,P01008,F7CYR1	0.022	0.99999	0.85
GO:0002684	positive regulation of immune system process	P	31	40	293	473	0.1058	0.0846	over	P01024,A0A3Q2LEZ9,F7CAB8,F6Q5L6,P14625,F7AQZ6,K9KD98,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,O00481,F7C603,Q6TGR2,F6T835,Q9HCL2,F7CK20,P05423,K9K4D8,F7BPT4,F7C7V8,F7DG10	P01024,A0A3Q2LEZ9,F7CAB8,F6Q5L6,P06702,P14625,F7AQZ6,K9KD98,P01834,P01860,A0A3Q2HWY7,A0A3Q2HWQ6,A0A3Q2IDD2,F6XSF7,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,O00481,F6RQM5,P23396,P63000,F7C603,Q6TGR2,P02788,P59998,P60709,F6T835,Q9HCL2,F7CK20,P05423,P26583,K9K4D8,F7BPT4,F7C7V8,F7DG10	0.0231	0.99999	0.775
GO:0043408	regulation of MAPK cascade	P	14	16	293	473	0.04778	0.0338	over	P00441,F6Q5L6,P80511,F6VBP9,F6QXW2,F6YQM5,Q9TUL9,F7BPT4,F7C1X7,F7CUM5,F6PH38,F7C7V8,F7DG10,P47989	P00441,F6Q5L6,P80511,F6VBP9,F6QXW2,F6YQM5,P23396,Q9TUL9,F7BPT4,F7C1X7,F7CUM5,A0A3Q2HTG2,F6PH38,F7C7V8,F7DG10,P47989	0.0245	0.99999	0.875

GO:0002694	regulation of leukocyte activation	P	14	16	293	473	0.04778	0.0338	over	F7AQZ6,F7CK20,P00441,Q9HCL2,K9K4D8,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	F7AQZ6,P63000,F7CK20,P00441,P23396,Q9HCL2,K9K4D8,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0245	0.99999	0.875
GO:0051249	regulation of lymphocyte activation	P	14	16	293	473	0.04778	0.0338	over	F7AQZ6,F7CK20,P00441,Q9HCL2,K9K4D8,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	F7AQZ6,P63000,F7CK20,P00441,P23396,Q9HCL2,K9K4D8,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0245	0.99999	0.875
GO:0016064	immunoglobulin mediated immune response	P	11	12	293	473	0.03754	0.0254	over	P01024,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	P01024,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	0.0253	0.99999	0.9166667
GO:0019724	B cell mediated immunity	P	11	12	293	473	0.03754	0.0254	over	P01024,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	P01024,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,F6XSF7	0.0253	0.99999	0.9166667
GO:0006952	defense response	P	49	67	293	473	0.16724	0.1416	over	P01024,A0A3Q2LEZ9,P14625,A0A3Q2KP77,P01008,F6XAB0,K9KD98,P19857,Q6X9W5,F7C0Y4,P01834,P01860,P11375,P22079,A0A3Q2I0E0,P80511,P81605,A0A3Q2HSB3,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,Q865P6,F6Q5L6,F6XSF7,P08334,P08896,F7CK20,P11376,A0A0B4J1C4,F7C603,Q6TGR2,F6T835,F6PH38,P05423,F6VBP9,Q92743,F7C7V8,P00441,F6Z2L5,F6QUF7,Q9HCL2,Q9GKX8,Q15836,F6XM13,A0A3Q2I5T2	P20160,P01024,A0A3Q2LEZ9,P06702,P14625,O77811,P02788,A0A3Q2KP77,P26583,P01008,F6XWM5,F6XAB0,K9KD98,P19857,Q6X9W5,Q9N0Y1,F7BFJ1,F7C0Y4,P00709,P01834,P01860,P11375,P22079,A0A3Q2I0E0,P80511,P81605,A0A3Q2HSB3,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1,Q865P6,P04406,P63000,F6Q5L6,F6XSF7,P08334,P08896,F7CK20,P11376,A0A0B4J1C4,F7C603,Q6TGR2,F6T835,A0A3Q2HTG2,F6PH38,A6NMB1,P05423,F6PZG1,F6ZIS4,F6VBP9,Q92743,F7C7V8,P00441,P21589,F6Z2L5,F6QUF7,Q9HCL2,Q9GKX8,P08670,Q15836,A0A3Q2IBQ7,F6XM13,A0A3Q2I5T2	0.027	0.99999	0.7313433
GO:1904950	negative regulation of establishment of protein localization	P	7	7	293	473	0.02389	0.0148	over	F6RM73,F6Z2L5,F6XM13,F7CLX6,F6VBP9,O60543,F7BPT4	F6RM73,F6Z2L5,F6XM13,F7CLX6,F6VBP9,O60543,F7BPT4	0.034	0.99999	1
GO:0051224	negative regulation of protein transport	P	7	7	293	473	0.02389	0.0148	over	F6RM73,F6Z2L5,F6XM13,F7CLX6,F6VBP9,O60543,F7BPT4	F6RM73,F6Z2L5,F6XM13,F7CLX6,F6VBP9,O60543,F7BPT4	0.034	0.99999	1

GO:0016021	integral component of membrane	C	63	89	293	473	0.21502	0.1882	over	P01833,F6RA08,Q9NZG7,Q9Y5G8,F7C8R2,A0A3Q2KJE2,F6Q5L6,F6T835,F7C7V8,Q6TGR2,F6WR95,Q15836,F6ZHQ5,O00481,A0A3Q2I9V2,F6XSG4,K9KB26,F7BC79,K9KD98,A0A3Q2HYJ5,A0A3Q2IBB5,F7B4C0,Q96CE8,Q99943,Q9H1C7,Q9HCL2,F7B3U1,Q9UNQ0,A0A3Q2GV97,F6Q6E8,A0A3Q2GU69,A0A3Q2HGD0,A0A3Q2H179,A0A3Q2H332,A0A3Q2H3D5,A0A3Q2H4E3,A0A3Q2H6L1,F6U904,A0A3Q2HIU8,A0A3Q2ID55,A0A3Q2I2V3,F6SQS6,A0A3Q2IG74,A0A3Q2LRH1,F6SFY3,F6TF18,F6TGW9,F6TLH4,F6W3F0,F6W1N4,F6WG98,F6WPD2,F6Y0D9,F7A684,F7BL38,F7CLE3,F7CXT0,F7DG10,H9GZQ9,H9GZR2,H9GZU8,Q0R0D3,Q861L3	P01833,F6RA08,F6VZD2,Q9HAS3,Q9NZG7,Q9Y5G8,F7C8R2,A0A3Q2I560,A0A3Q2KJE2,F6Q5L6,F6T835,F7C7V8,F6YG82,Q96G97,F7E293,Q6TGR2,A6NMB1,A0A3Q2H7N6,F6WR95,Q15836,F6ZHQ5,F6QHY8,O00481,O75911,A0A3Q2I9V2,O95573,F6XSG4,K9KB26,F6RNM2,F7BC79,A0A3Q2HIR5,Q53FP2,K9KD98,A0A3Q2HYJ5,A0A3Q2IBB5,F7B4C0,Q96CE8,Q99536,Q99943,Q9H1C7,Q9HCL2,F7B3U1,Q9UNQ0,A0A3Q2GV97,F6Q6E8,A0A3Q2GU69,A0A3Q2HGD0,A0A3Q2GWR2,A0A3Q2H179,F6RQM5,A0A3Q2H332,A0A3Q2H3D5,A0A3Q2H4E3,A0A3Q2H6L1,A0A3Q2H8Y8,F6U904,A0A3Q2HIU8,A0A3Q2I5A5,A0A3Q2ID55,A0A3Q2HWU4,A0A3Q2I2V3,F6SQS6,A0A3Q2IG74,A0A3Q2LRH1,F2VYZ0,F7CG05,F6SAH3,F6SFY3,F6SPH9,F6TF18,F6TGW9,F6TLH4,F6W3F0,F6W1N4,F6WG98,F6WPD2,F6Y0D9,F6ZH19,F7A684,F7BL38,F7CLE3,F7CXT0,F7DG10,H9GZQ9,H9GZR2,H9GZU8,Q0R0D3,Q861L3,Q9BDF6	0.0358	0.99999	0.7078652
GO:1902532	negative regulation of intracellular signal transduction	P	10	11	293	473	0.03413	0.0233	over	F7C1X7,F6T835,F6VBP9,F6Q5L6,F6QXW2,P47989,F7CUM5,Q9TUL9,F7BPT4,F7CLX6	F7C1X7,F6T835,F6VBP9,F6Q5L6,F6QXW2,P47989,F7CUM5,Q9TUL9,F7BPT4,F7CLX6,F7E3Y7	0.0383	0.99999	0.9090909
GO:0006958	complement activation, classical pathway	P	10	11	293	473	0.03413	0.0233	over	P01024,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	P01024,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0383	0.99999	0.9090909
GO:0002455	humoral immune response mediated by circulating immunoglobulin	P	10	11	293	473	0.03413	0.0233	over	P01024,P01834,P01860,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	P01024,P01834,P01860,F6PZG1,F7CAC5,H9GZN9,H9GZQ9,H9GZR2,H9GZT5,H9GZU8,H9GZV1	0.0383	0.99999	0.9090909