TMT-Based Quantitative Proteomic Analysis of Intestinal Organoids Infected by *Listeria monocytogenes* with Different Virulence

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Abstract

Listeria monocytogenes (*Lm*) is an opportunistic food-borne pathogen that cause listeriosis. *L. monocytogenes* belonged to different serovars presents with different virulence in the host and caused different host reactions. To investigate the remodeling of host proteome by differently toxic strains, the cellular protein responses of intestinal organoids were analyzed using TMT labeling and high performance liquid chromatography-mass spectrometry. Quantitative proteomic analysis revealed 6564 differentially expressed proteins, of which 5591 proteins were quantified. The fold-change cutoff was set at 1.3 (*Lm* vs control), the virulent strain caused 102 up-regulated

proteins and 52 down-regulated proteins, while the low virulent strain caused 188 up-regulated proteins and 25 down-regulated proteins. These identified proteins were involved in the regulation of essential processes such as biological metabolism, energy metabolism, and immune system process. Some selected proteins were screened by Real-time PCR and Western blotting. These results revealed that differently toxic *L. monocytogenes* induced similar biological functions and immune responses while had different regulation on differential proteins in the pathway.

Keywords: *Listeria monocytogenes*; virulence; proteomic; host response; intestinal organoid.

Introduction

Listeria monocytogenes is a gram-positive foodborne pathogen, which can lead to listeriosis ^[1]. The infection causes a spectrum of illness, ranging from febrile gastroenteritis to invasive disease ^[2]. Listeriosis is a relatively rare foodborne disease, while has a high mortality rate ranging from 20% to 30% ^[3]. For example, the incidence of listeriosis was estimated to be 0.3 per 100000 persons in the United States, and the mortality was 21% in 2019 ^[4]. Therefore, it is the second most frequent cause of foodborne infection-related deaths in Europe and USA ^[5]. Moreover, listeriosis is mainly caused by contaminated food. In 2018, the listeriosis outbreak in South Africa was caused by ready-to-eat processed meat products, which resulted in 1034 cases of illness and 204 deaths ^[6, 7]. Despite many achievements in food safety and laboratory diagnostics methods in developed countries, *L. monocytogenes* remains a major

challenge in food industries and public health.

L. monocytogenes has 4 evolutionary lineages (I, II, III, and IV) based on multigene phylogenetic analyses, and was divided into 13 serotypes according to somatic O antigen [8]. Of the 13 serotypes, over 98% of isolates from human listeriosis belong to serotypes within lineages I and II (1/2a, 1/2c, 1/2b, and 4b) [9]. Food or food production environment was commonly contaminated with serotypes 1/2a and 1/2b, and clinical cases were mainly caused by virulent strain 4b, while low virulent strains were usually not pathogenic or weakly pathogenic, as serovar 4a [10]. Besides, strains belonged to different serovars presents with different levels of virulence in the host and causes different host reactions, which due to their differences in growth and movement characteristics or expression of virulence factors [11, 12]. In addition, *L. monocytogenes* will lose or weaken its toxicity due to the deletion of some virulence genes [13]. There were many studies on the comparison of different toxic *L. monocytogenes*, mainly focusing on the following aspects: the genetic relationship between virulent and low-virulence strains [14], survival ability under stressful environments [15, 16], biological characteristics and pathogenicity in cell and mouse models [17, 18], expression of important virulence genes [19, 20], and host immune response [21], etc. Studies have shown that virulent strains are generally more pathogenic and invasive than attenuated strains. The *L. monocytogenes* 10403s in the 1/2a serotype strain can colonize the spleen and liver of mice in large quantities, and well invade Caco2 cells and replicate intracellularly [22]. Moreover, strains of serotype 4a could cause infections, but could not establish long-term infections in macrophages [23]. Compared with the 1/2a serotype

strains, the 4a non-toxic strains had a shorter propagation time in the cells, but they all express similar metabolic related proteins.

L. monocytogenes is facultative intracellular pathogen that causes systemic infection by first invading the intestinal mucosal barrier [24]. In the stage of intestinal infection, *L. monocytogenes* invaded intestinal epithelium by *L. monocytogenes* invasion protein InlA and zipper mechanism, entering Peyer's patch through M cells, or leading the mislocalization of junctional proteins by Listeria adhesion protein [25, 26]. The infection affected the normal intestinal renewal, causing the increased crypt depth and excessive proliferation, which damages the activity of intestinal stem cells [27].

Moreover, *L. monocytogenes* can immediately activate the host's innate immune response when interacting with intestinal epithelial cells. Studies demonstrated that *L. monocytogenes* induced a dramatic innate inflammatory response in intestinal epithelial cells of germ-free, human E-cadherin transgenic mice [28]. The innate immune activation was induced by pattern recognition receptors (PRRs) on epithelial cells, such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). Many studies demonstrated the importance of TLR-mediated signaling in innate immune defense; however, the expression of TLR in the intestine was highly regulated and restricted to prevent exaggerated adaptive immunity to the intestinal microbiota [29, 30]. Besides, several studies implicated that cytosolic proteins of NLRs contributed to the defense against intestinal bacterial infection. For example, it was found that mice lacking Nod2 have greater susceptibility to intestinal infection with *L. monocytogenes* [31]. On the other hand, *L. monocytogenes* can employ strategies

to evade or modulate immune defences. For example, *L. monocytogenes* modified bacterial ligands to avoid detection, modulated host signalling pathways to alter host innate defences^[32]. The detailed understanding of *L. monocytogenes*-host interactions and infection and immunity is essential for elucidating these mechanisms. Therefore, investigating the changes in the overall protein abundance of host cells by *L. monocytogenes* can help to understand the relationship between the pathogenicity and toxicity of the bacteria.

The majority of studies on *L. monocytogenes* infection have been conducted in animals such as mice, and single cell line models such as colon adenocarcinoma cells or macrophage cells^[25, 33]. Most knowledge of the innate and adaptive immune responses has been learned from experimental *L. monocytogenes* infections of mice^[34]. In addition to immune cells, intestinal epithelial cells were also involved in the defense against *L. monocytogenes* infections. Studies explored that host responses caused by *L. monocytogenes* included innate immune responses first activated by PRRs of intestinal epithelial cells, immune cells recruited by downstream cytokines and adaptive immune responses subsequently stimulated^[32, 34]. However, a single immune model or intestinal cell model both cannot completely reflect the damage of *L. monocytogenes* to the entire intestinal epithelium. Recently, intestinal organoid was emerging as a more effective infection model that reproduced the differentiation of intestinal epithelial cells, and showed the greatest similarity to the intestinal epithelium with respect to cell composition and structure^[35]. It was verified that organoids had been used to study the interaction between pathogens and host cells at the intestinal interface^[36]. Therefore,

intestinal organoid is a suitable *L. monocytogenes* infection model for exploring the host response of non-immune cells.

Proteomics can provide protein information related to the biological metabolism and infection mechanism of the host or microorganism, which may be useful to further understand the interaction between the pathogenic microorganism and host [37]. Recently, tandem mass tags (TMT)-based proteomic platforms have been used as one of the most robust proteomics techniques due to high sensitivity [38]. It was shown that genomic and proteomics techniques were widely used to analyze the differences of strains in transcription and protein expression [39-41]. Studies have shown that *L. monocytogenes* infection may have a major impact on host transcription and translation, cytoskeleton and connections, mitochondrial fission, host immune response, and apoptosis pathway [42, 43]. However, researches on intestinal epithelial host responses caused by *L. monocytogenes* infection excluding the effects of immune cells is not yet comprehensive. Therefore, information on proteome changes in the infected intestinal organoids is necessary to understand host response of non-immune cells.

Here, intestinal organoids and two strains of *L. monocytogenes* (serotype 1/2a and 4a) were used, and the significant changes on global protein expression of infected intestinal organoids was described by a highly sensitive quantitative approach, including tandem mass tag (TMT) labeling and an LC-MS/MS platform combined with advanced bioinformatics analysis. Data revealed that major different proteins were involved in metabolic process, transcription and translation, and defense mechanisms (response to stimulus and immune system process). Furthermore, some significantly

differentially expressed proteins (DEPs) related to host defence were further analyzed and validated.

Materials and Methods

Bacterial strains, Animals and Intestinal Organoids

The *Listeria monocytogenes* 10403s and M7 was a gift of Prof. Weihuan Fang (Zhejiang University)^[44, 45]. Cryopreservation liquid of bacteria was transferred and scribed on PALCAM agar, and the plates were incubated at 37 °C for 48 h. Each single colony was picked out to 5 mL BHI broths supplemented with 5 µg/mL erythromycin and cultured with agitation at 37 °C for 16 h. The final concentration of the BHI broth was assessed by the plate count method.

4 weeks old, specific-pathogen-free (SPF) C57BL/6 mice were purchased from the Animal Research Centre of Yangzhou University. All the animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Agricultural University, and the National Institutes of Health guidelines for the performance of animal experiments were followed.

Intestinal organoids were cultured from intestines (mostly jejunum and ileal) of 4-week-old C57BL/6 mice, as described in Hou et al. After cervical dislocation, small intestine was removed and dissected immediately. Subsequently, intestine was flushed out with phosphate-buffered saline (PBS), and was cut into small pieces. Next, tissues were rocked in DPBS containing 2 mM EDTA for 30 min at 4 °C. After incubating, crypts were released by vigorously shaken, and cells were filtered through a 70-µm sterile cell strainer. Then, crypts were collected by centrifugation at 700 rpm for 5 min, mixed with

Matrigel (Corning, USA) and then seed into a 24-well tissue culture plate. The plate was incubated for at least 15 min at 37 °C to polymerize. Finally, 500 μ L of complete crypt culture medium was added to each well, which contained Advanced DMEM/F12 supplemented with penicillin–streptomycin, 10 mM HEPES, 2 mM glutamine, N2, B27 (Gibco, California, USA; Life Technologies, Carlsbad, California, USA), EGF (50 ng/mL, Peprotech, USA), R-spondin1 (500 ng/mL, Peprotech), Noggin (100 ng/mL, Peprotech), and Y-27632 (10 mM, Sigma, Germany). The medium was changed every 2–3 days, and organoids were passaged every 3–5 days.

Experimental Design and *L. monocytogenes* Infection

Thirty four-week-old mice were randomly divided into 3 groups, 10 mice in each group, and placed in different cages. One group was inoculated with 1×10^9 CFU of the virulent strain *L. monocytogenes* 10403s by oral gavage, the other group was also intragastrically inoculated with 1×10^9 CFU of the attenuated strain *L. monocytogenes* M7, and another group was mockinfected with sterile PBS in the same manner. At 24, 72, and 96 hs after infection, mice were sacrificed, and CFUs in the intestine, liver and spleen were determined by dilution coating on Brain Heart Infusion plates with 5- μ g ml⁻¹ erythromycin and PALCAM agar plates. The body weight and survival rate were recorded for a week after inoculation.

Organoids were cultured in complete crypt culture medium at 37 °C in a 5% CO₂-air atmosphere. To mimic the invasion of the intestine from the correct side, organoids were mechanical dissociated prior to infection, part of buds were fell off and thus exposed the lumen. After 3 days of passage, organoids were large enough to perform

mechanical dissociation. Organoids were divided into 3 groups: one group was infected with 1×10^7 CFU of the virulent strain *L. monocytogenes* 10403s; the other group was infected with 1×10^7 CFU of the attenuated strain *L. monocytogenes* M7; and another group was mockinfected with sterile culture medium in the same mechanical dissociation. These were done by gently pipetting up and down with 10 mL pipettes to make a suitable wound [46, 47].

The detailed methods of organoids infection are listed as follows. First, *L. monocytogenes* was grown as described above and pelleted at 5000 rpm for 5 min. Subsequently, they were re-suspended in complete crypt culture medium to 10^7 CFU/mL. Further, organoids were separated from Matrigel, and then re-suspended in complete crypt culture medium containing bacteria or not, and shook every 15 min at 37 °C. Specifically, infected organoids were incubated in complete culture medium with the indicated *L. monocytogenes* strain for 1 h while control organoids were only incubated with culture medium. After infection, organoids were centrifuged at 900 rpm for 5 min, and extracellular bacteria were removed by washing twice with DPBS. Finally, organoids were embedded into fresh Matrigel and cultured for 18 h, and the media was refreshed with penicillin-streptomycin media for the experiment.

Protein Sample Preparation

Organoids were infected in three groups (virulent strain infection, attenuated strain infection and control) at two time points (1h after incubation and 18h after culture), a total of six groups; and three-well organoids were used as a sample, with three replicate samples in each group. Samples were sonicated three times on ice using a high intensity

ultrasonic processor (Scientz) in lysis buffer (8 M urea, 1% Protease Inhibitor Cocktail). The remaining debris was removed by centrifugation at 12,000 g for 10 min. Finally, the supernatant was collected and the protein concentration was determined with BCA kit according to the manufacturer's instructions. The aliquots were stored at -80 °C for further proteomic and Western blotting studies. The pooling of individual samples is a cost-effective approach for proteomic studies; therefore, four samples with equal amounts of protein from infected organoids or controls were mixed to obtain three virulent strain infection, three attenuated strain infection and three control samples.

Trypsin Digestion

For trypsin digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C, and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted by adding 100 mM TEAB to urea concentration less than 2M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion. Approximately 100 µg of protein for each sample was digested with trypsin for the following experiments

TMT Labeling

After trypsin digestion, peptide was desalted by Strata X C18 SPE column (Phenomenex) and vacuum-dried. Peptide was reconstituted in 0.5 M TEAB and processed according to the manufacturer's protocol for TMT kit. Briefly, one unit of TMT reagent (defined as the amount of reagent required to label 100 µg of protein) were thawed and reconstituted in acetonitrile. The peptide mixtures were then incubated

for 2 h at room temperature and pooled, desalted and dried by vacuum centrifugation.

Prepared samples were stored at -80°C until liquid chromatography-mass spectrometry (LC–MS/MS) analysis.

HPLC Fractionation

The samples were fragmented into a series of fractions by high pH reverse-phase HPLC using Agilent 300Extend C18 column (5 μm particles, 4.6 mm ID, and 250 mm length). Briefly, peptides were first separated with a gradient of 8% to 32% acetonitrile (pH 9.0) over 60 min into 60 fractions. Then, the peptides were combined into 18 fractions and dried by vacuum centrifuging.

LC-MS/MS

The peptides were dissolved in liquid chromatography mobile phase A (0.1% formic acid and 2% acetonitrile) and separated using the EASY-nLC 1000 ultra-high performance liquid system. Mobile phase B is an aqueous solution containing 0.1% formic acid and 90% acetonitrile. Liquid phase gradient setting as follows: 0 ~ 42 min, 6% -22% B; 42 ~ 54 min, 22% -30% B; 54 ~ 57 min, 30% -80% B; 57 ~ 60 min, 80% B, all at a constant flow rate of 500 nL / min.

The peptides were subjected into the NSI ion source for ionization, and then analyzed by Orbitrap Fusion LumosTM mass spectrometry. The ion source voltage was set to 2.4 kV, the peptide precursor ions and their secondary fragments were detected and analyzed using high-resolution Orbitrap. The m/z scan range of full scan was 350 to 1550, and the resolution of the complete peptide detected in Orbitrap was 60,000; the scan range of the second-level mass spectrometer was fixed at 100 m/z , and the

resolution was set to 15,000. The data acquisition mode used a data-dependent scanning (DDA) program. In order to improve the effective utilization of the mass spectrum, the automatic gain control (AGC) was set to 5E4; the signal threshold was set to 10000 ions/ s; the maximum injection time was set to 60 ms; and the dynamic exclusion time of the tandem mass spectrometry scan was set to 30 seconds to avoid precursor ion Repeat the scan.

Data Analysis

The resulting MS/MS data were processed using Maxquant search engine (v.1.5.2.8). Tandem mass spectra were searched against SwissProt Mouse database concatenated with reverse decoy database. An anti-library was added to calculate the false positive rate (FDR) caused by random matching, and a common contamination library was added to the database to eliminate the impact of contaminated proteins in the identification results. Trypsin/P was specified as cleavage enzyme allowing up to 2 missing cleavages. The minimum length of the peptide was set to 7 amino acid residues; the maximum number of modifications of the peptide was set to 5; the mass error of the primary precursor ion of First search and Main search was respectively set to 20 ppm and 5 ppm; and the mass error of the secondary fragment ion was 0.02 Da.

The cysteine alkylation was set as a fixed modification, and the variable modification was the oxidation of methionine, acetylation at the N-terminus of the protein, and deamidation (NQ). The quantitative method was set to TMT-6plex, FDR for protein identification and PSM identification was set to 1%, and minimum score for peptides was set > 40.

Real-Time RT –PCR

Total RNA was respectively extracted from organoid sample using RNAiso Plus (Takara, Beijing, China). Following, reverse transcription of the RNA was performed. With the primers listed in Table 1, master mix was used to yield a final volume of 20 μ L (Takara). The thermal cycling conditions were 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 34 s at 60 °C using an Applied Biosystems 7500 real-time PCR system as described previously (Hou et al., 2018). The mRNA expression level of each target gene was normalized to the expression level of GAPDH, the expression levels of uninfected organoids comparing the expression levels of infected organoids were normalize as 1 which was analyzed by $\Delta\Delta$ Ct. All real-time PCR reactions were performed in triplicate. Primer sequence of target and reference genes were shown as Table 1.

Western Blot Analysis

Organoids were lysed in RIPA buffer (50mM Tris-HCl, pH 7.4, 1% NP-40, 150mM NaCl) containing a protease inhibitor cocktail (Thermo FisherScientific). Protein concentrations were detected using a BCA protein quantification kit (Thermo Fisher Scientific). Equal amounts of protein were separated by 10% SDS-PAGE, and then transferred to PVDF membranes (Millipore, China). After blocking with 5% non-fat milk in TBS containing 0.1% Tween-20, the membranes were probed with the appropriate antibody. The following antibodies were used for Western blot analysis as follows: rabbit anti-NOD2 (1:400), anti-GAPDH (1:1,000, SAB4300645, Sigma). After washing, the membranes were incubated with goat anti-rabbit secondary antibodies

(1:10000). Finally, Blots were developed using efficient chemiluminescence (ECL) kit and light emission was captured using the Versa DOC 4000 imaging system. Use Quantity one software to analyze the gray value of the band.

Results

Listeria monocytogenes infection and clinical signs

After 3 days of acclimation, the mice were in good condition with normal activity. After randomized intragastric administration, the control group was in a normal state, and both the group of virulent strain *L. monocytogenes* 10403s and low virulent strain *L. monocytogenes* M7 showed abnormal state and deaths with different degrees. As shown in Figure 1A, within 7 days after gavage, the overall weight of the control group showed an upward trend, with the largest increase in the 4th to 5th days, and there was no death during the entire process. The weight of the virulent group showed a trend of decreasing first and then increasing. It continued to decrease in the first 4 days and increased after the 5th day. Compared with the control group, the mice died on the first day after gavage, and the survival rate continued to decline, and did not change after the 5th day. The overall weight of the low virulent strain group fluctuated, and the change was large, which decreased on the 3rd day and increased on the 4th-6th days; the group only died on the 2nd day, and the final survival rate was lower than the control group but higher than the virulent group. 24 h after infection, two strains of *L. monocytogenes* can be respectively detected in the intestine, liver, and spleen (Figure 1B). The main lesions of infected mice comprised partial hemorrhage in small intestine, longer colon length and enlarged spleen, and lesions caused by the virulent strain were more serious.

The results showed that mice in the infected groups were successfully infected with two stains of *L. monocytogenes*, and they both induced short-term body weight changes and varying degrees of death. In contrast, highly toxic *L. monocytogenes* 10403s resulted in more serious infections and lower survival rates.

Quality validation of the proteomic data

To reveal the changes in the protein levels under *L. monocytogenes* infections with different toxicity, an integrated proteomic approach was performed using organoids (control and two infected group). The statistical information of the treatment groups and differential proteins were shown in Table 2 and Table 3. As shown in Figure 2A, the Pearson coefficient between all replicate samples was greater than 0.6, which met the biological repeat quantitative consistency standard; it indicated that the correlation coefficient of 18 experimental samples displayed good repeatabilities. Two important parameters, including peptide length and peptide mass, were analyzed to verify the quality of mass spectrometry data. The data showed that the sample preparation met the standard requirements, most of the peptides were distributed between 7-20 amino acids, which is in accordance with the general law based on trypsin digestion and HCD fragmentation, and the first-order mass error of most spectra is within 10 ppm (Figure 2B and C).

Analysis of the DEPs under *L. monocytogenes* 10403s and *L. monocytogenes* M7 infections

In this study, we performed a quantitative analysis of the overall proteome of small intestinal infected organoids. Altogether, 6564 proteins were identified, of which 5591

proteins were quantified. For the differentially expressed proteins (DEPs), the cutoff criteria considered was set with p value < 0.05 and the infection vs control group ratio >1.3-fold difference. Based on our previous experimental results, three groups of differential proteins cultured for 18 h after incubation were subsequently analyzed (B, D, F group). Among the quantitative proteins, 102 up-regulated and 52 down-regulated proteins were identified under *L. monocytogenes* 10403s infection, while 188 up-regulated and 25 down-regulated proteins were identified under *L. monocytogenes* M7 infection. The differential expressed proteins between *L. monocytogenes* 10403s and *L. monocytogenes* M7 infection were also calculated, resulting in 4 up-regulated and 58 down-regulated proteins (Figure 3). Table S1 (Supplementary Materials) presents relative expression of DEGs in *L. monocytogenes* 10403s vs Control (D/B) and *L. monocytogenes* M7 vs Control (F/B). The protein ratio were expressed as *L. monocytogenes* infection vs control.

Classification of the DEPs under *L. monocytogenes* 10403s and *L. monocytogenes* M7 infections

To further understand the DEPs in *L. monocytogenes*-infected intestinal organoids, functional classification was performed from the Gene Ontology (GO) and subcellular structure localization (Figure 4). GO was divided into three main categories: biological process, cellular component and molecular function, which can explain the biological role of proteins from different angles.

Under the *L. monocytogenes* 10403s infection, the main biological processes of DEPs included single-organism process, biological regulation and metabolism, defense

response from stimulation and immunity; cell composition mainly included membrane and macromolecular complex, and molecular function mainly included binding and catalytic activity (Figure 4A). Under *L. monocytogenes* M7 infection, the main biological processes of DEPs were the same as those of *L. monocytogenes* 10403s. The main conclusions of cell composition and molecular function were also similar to those of *L. monocytogenes* 10403s (Figure 4B). In the comparison between *L. monocytogenes* 10403s and *L. monocytogenes* M7, up-regulated proteins were grouped into single-organism process, membrane and binding; while down-regulated proteins were related to biological regulation and metabolism, immune system process, macromolecular complex and catalytic activity (Figure 4C and D).

In the subcellular localization of DEPs, the host cell proteins infected by *L. monocytogenes* 10403s were mainly distributed in the extracellular and cytoplasm, and the proteins of *L. monocytogenes* M7 infection were mainly distributed in the extracellular and nucleus. The DEPs of comparing two infections were mainly distributed in the nucleus (Figure 4E).

Enrichment analysis of the DEPs under *L. monocytogenes* 10403s and *L. monocytogenes* M7 infections

To find out whether DEPs had a significant enrichment trend in certain functional types, enrichment analysis of GO classification and protein domain were performed in each comparison group (Figure 5). Fisher's exact test p-value ($-\log_{10} [\text{p-value}]$) was used to evaluate the enrichment level of DEPs; the larger the p-value, the more differentially expressed proteins enriched in this category.

The first was the enrichment analysis of GO classification. Under *L. monocytogenes* 10403s infection, the significantly enriched biological process were mainly associated with defense response to bacterium, antimicrobial humoral response, extracellular matrix disassembly; the significantly enriched cellular component were related to extracellular matrix, basement membrane; the significantly enriched molecular function were mainly correlated with extracellular matrix structural constituent, oxidoreductase activity and various bindings, such as glycosphingolipid binding. Besides, the significantly enriched domain terms were Laminin EGF domain, Laminin and Fibrinogen, and Metallothionein domain.

Under *L. monocytogenes* M7 infection, the significantly enriched biological process were mainly associated with protein activation cascade, antimicrobial humoral response, extracellular matrix disassembly; the significantly enriched cellular component were basically the same as *L. monocytogenes* 10403s; the significantly enriched molecular function were mainly correlated with extracellular matrix structural constituent, iron ion binding and structural molecule activity. Moreover, the top three significantly enriched domain terms were same as *L. monocytogenes* 10403s, while the remaining domains included Histone.

In the comparison between *L. monocytogenes* 10403s and *L. monocytogenes* M7, the significantly enriched biological processes were mainly associated with negative regulation of gene expression and biosynthetic process, cellular macromolecular complex assembly. The significantly enriched cellular components were related to nucleosome and DNA packaging complex. The significantly enriched molecular

functions were mainly correlated with chromatin DNA binding and nucleosomal DNA binding. In addition, the significantly enriched domain term were Histone and Histone-fold.

KEGG analysis of the DEPs under *L. monocytogenes* 10403s and *L. monocytogenes* M7 infections

All DEPs in *L. monocytogenes* 10403s infection group and *L. monocytogenes* M7 infection group were put together for KEGG analysis. Figure 6A showed all the KEGG pathways enriched. Combining with Figure 3C, 301 differential proteins participated in 198 KEGG pathways, of which only 16 were significantly enriched. Figure 6B was the percentage of these enriched KEGG pathways. The larger the proportion, the more proteins involved in this pathway. The most significant enrichment was chemical carcinogenesis, which reflected the genotoxic and non-genotoxic effects of *L. monocytogenes* on the host. The main significant enrichments were some metabolic related pathways, such as Retinol metabolism, steroid hormone biosynthesis, and drug metabolism-cytochrome P450. In addition, fat digestion and absorption, glycolysis/gluconeogenesis and other metabolic pathways were also been enriched.

Besides, the DEPs reflected the ability of *L. monocytogenes* to adhere and damage during the invasion were significantly involved in the small cell lung cancer, Amoebiasis and some other host responses, such as the ECM-receptor interaction, Complement and coagulation cascades, and HIF-1 signaling pathway. Moreover, it could be seen from Figure 6B that some signaling pathways related to host defense and immune response were enriched, which didn't contain many differential proteins, such

as apoptosis and autophagy, Ferroptosis, and NOD-like receptor signaling pathway, etc.

Figure 7 showed that KEGG pathway of *L. monocytogenes* 10403s and *L. monocytogenes* M7 infection group were mainly related to invasion, host response and metabolism. Among the KEGG pathways to which up-regulated protein enriched in the *L. monocytogenes* 10403s infection group, the pathways related to bacterial adhesion and invasion included Amoebiasis, small cell lung cancer, chemical carcinogenesis and focal adhesion. In addition, it also included some host response, such as ECM-receptor interaction, complement and coagulation cascades and PI3K-Akt signaling pathway. Moreover, KEGG pathways related to metabolic processes contained steroid hormone biosynthesis, retinol metabolism and drug metabolism - cytochrome P450.

Similar results were found in the KEGG pathway enriched in the *L. monocytogenes* M7 infection group. Up-regulated proteins were enriched in the same three pathways related to bacterial adhesion and invasion as *L. monocytogenes* 10403s, involved in ECM-receptor interaction and complement and coagulation cascades, and enriched in three host lipid metabolism-related metabolic processes. However, unlike the virulent strain *L. monocytogenes* 10403s, the upregulated protein of *L. monocytogenes* M7 were contributed in systemic lupus erythematosus, and the downregulated proteins were only enriched in glycolysis/gluconeogenesis.

In our intestine organoid model, the innate immune response was the primary host defense response caused by *L. monocytogenes*. Therefore, we selected the DEPs of five pathways in the *L. monocytogenes* 10403s vs control group and *L. monocytogenes* M7 vs control group, which were related to the immune system process. Then these proteins

were accurately analyzed at 1.3-fold and 1.2-fold differential folds. The five pathways included ECM-receptor interaction, complement and coagulation cascade, HIF-1 signaling pathway, Ferroptosis and NOD-like receptor signaling pathway.

Among the ECM-receptor interactions, the significantly upregulated proteins which were mutual in the *L. monocytogenes* 10403s and M7 groups were Fn1, Lamc1, Lama1, Lamb1, Col4a2, Col4a1, Lamb2, Hspg2, and Agrn. Besides, Agrn was up-regulated but not significant in the *L. monocytogenes* 10403s group, while 1.3-fold significantly up-regulated in the *L. monocytogenes* M7 group, and 1.2-fold significant in the *L. monocytogenes* 10403s vs M7 comparison group. It showed that *L. monocytogenes* M7 could significantly increase Agrn 1.3-fold, and the degree of upregulation was 1.2-fold more significant than *L. monocytogenes* 10403s.

For the complement and coagulation cascades, six differential proteins were both identified in the *L. monocytogenes* 10403s and M7 infection groups, Plg, Fgb, Fga, Fgg, Clu, and C3. These differential proteins were significantly up-regulated in the *L. monocytogenes* 10403s and M7 infection groups, while not significantly changed in the comparison group. In addition, F10 was significantly changed in the *L. monocytogenes* 10403s vs *L. monocytogenes* M7 comparison group, which was significantly up-regulated 1.3-fold in the *L. monocytogenes* M7 group, while was down-regulated but not significant in the *L. monocytogenes* 10403s group. This showed that effect of *L. monocytogenes* M7 on F10 was opposite to *L. monocytogenes* 10403s, and the degree of activation was significantly higher than *L. monocytogenes* 10403s.

In the NOD-like receptor signaling pathway, the differential proteins shared by the *L.*

461 *monocytogenes* 10403s and M7 infection groups were significantly down-regulated.

462 Among them, Nod2 and Pycard were 1.3-fold significantly reduced in both *L.*

463 *monocytogenes* 10403s group and *L. monocytogenes* M7 group, and Nampt was 1.2-

464 fold significantly reduced in both group. In addition, the 1.2-fold significantly up-

465 regulated proteins in the *L. monocytogenes* 10403s group were Vdac1, Vdac2, and

466 Vdac3, which were also up-regulated in the *L. monocytogenes* M7 group but the

467 changes were not significant. This suggested that one of the differences between the

468 effects of *L. monocytogenes* 10403s and *L. monocytogenes* M7 on the NOD-like

469 signaling pathway was the activation of these proteins. In particular, Txn2 and Nek7

470 were significantly down-regulated in the *L. monocytogenes* 10403s vs *L.*

471 *monocytogenes* M7 comparison group, they were down-regulated in the *L.*

472 *monocytogenes* 10403s group and up-regulated in the *L. monocytogenes* M7 group,

473 which both were not significant. It indicated that the activation of *L. monocytogenes*

474 M7 on these proteins was significantly different from *L. monocytogenes* 10403s.

475 In the HIF-1 signaling pathway, the significant differentially upregulated differential

476 proteins shared by the *L. monocytogenes* 10403s and M7 infection groups were Tfrc,

477 Tf, Hkdc1 and Cdkn1b, and the significantly downregulated protein was Enol.

478 However, only Slc2a1 changed significantly in the *L. monocytogenes* 10403s vs *L.*

479 *monocytogenes* M7 comparison group, which was significantly increased.

480 For Ferroptosis, the significantly upregulated proteins shared by *L. monocytogenes*

481 10403s and M7 infection groups were Acs15, Tf, Acs11, Tfrc, and Lpcat3; and the

482 significantly down-regulated proteins were Fth1 and Ftl1, which both did not change

significantly in the comparison group. In addition, *L. monocytogenes* 10403s 1.2-fold significantly increased Gpx4, Vdac3 and Vdac2, which were up-regulated but not significant in the *L. monocytogenes* M7 group.

Confirmation of proteomic data by RT-PCR and western blot analysis

Among the five related pathways, only the differential proteins in the NOD-like receptor-signaling pathway were down-regulated. This showed that the effect of *L. monocytogenes* infection on the NOD-like receptor-signaling pathway is mainly to inhibit or reduce the differential proteins. Among them, Nod2 is expressed in Paneth cells and stem cells in the intestine, and very important for intestinal immunity. Therefore, the gene and protein level of the Nod2 pathway protein were verified.

The results were shown in Figure 8. *L. monocytogenes* with different toxicity significantly increased the mRNA expression level of Nod2, while it was down-regulated at the protein level, consistent with the proteome results. However, for other proteins in the Nod2 pathway, *L. monocytogenes* 10403s significantly down-regulated RIP2, TAK1, P38 and NF- κ B.

Discussion

Foodborne illness has been a major threat to human health and public health ^[48]. Although the incidence has been greatly reduced with the improvement of sanitary conditions, food poisoning is still a major problem today ^[49]. Listeriosis is one of the most serious foodborne diseases, and mainly caused by contaminated food. *Listeria monocytogenes* enters the digestive tract through contaminated food, and then crosses the intestinal barrier, which is a critical step in systemic infection ^[26]. In the researches

on the stage of intestinal infection, most analyzed virulence gene and survival mechanism of *L. monocytogenes*, and the host response [50, 51]. Furthermore, the infection may alter host intestinal microbiota to promote bacterial colonization [25]. In the proteomics analysis of *L. monocytogenes*, many studies focused on bacterial proteins, and explored the relationship between some proteins and bacterial virulence by comparing the different protein expression between different strains in stress, biological metabolism, and virulence genes [23, 39]. The others focused on host protein changes, and investigated the interaction between host response and bacterial virulence. [52].

However, researches on changes of intestinal epithelial host protein were not complete. Previous studies used either animal models or single intestinal cell line, which did not exclude the effects of immune cells or could not fully represent epithelial cells. As an emerging intestinal model, small intestine organoids have been used to study the interaction between bacteria and hosts [47, 53]. However, in the study of *L. monocytogenes* and small intestine organoids, the focus was only to verify whether the intestine organoids could be used as an invasion model for *L. monocytogenes* and the apparent damage effect of bacteria on organoids. On the other hand, there was no comprehensive analysis of infected intestine organoids using proteomics.

In the present study, tandem mass tag-based quantitative proteomic analysis was used to compare the total proteomes in organoids infected by different toxic *L. monocytogenes*. Quantitative analysis demonstrated 154 differentially expressed proteins in the virulent strain (*L. monocytogenes* 10403s)-infected organoids, 213

proteins in the low virulent strain (*L. monocytogenes* M7)-infected organoids, and 62

proteins in the *L. monocytogenes* 10403s vs *L. monocytogenes* M7 comparison group.

These proteins were found to be involved in cell transport, binding, biological

metabolism, energy metabolism, transcriptional regulation, signal transduction, and

defense response.

The infection of *L. monocytogenes* in intestinal organoids is a process that involves

many proteins and pathways. The results of analyzing *L. monocytogenes* 10403s and *L.*

monocytogenes M7 groups showed that the damage of *L. monocytogenes* infection was

mainly reflected in the destruction of intestinal barrier, affecting the disease-signaling

pathway, and changing the metabolic process of host cell. In addition, different toxic *L.*

monocytogenes led to the changes of five important pathways related to host immune

process.

ECM-receptor interaction is a micro-environmental pathway that maintains cell and

tissue structure and function, which leads to a direct or indirect control of cellular

activities such as adhesion, migration, differentiation, proliferation, and apoptosis.

Recent studies have identified that this pathway was possibly involved in the

development of breast cancer^[54]. Studies confirmed that these proteins were utilized

by pathogens to adhere to and invade host tissues, and can increase adherence of *L.*

monocytogenes to HEp-2 cells^[55, 56]. *L. monocytogenes* upregulated many ECMs

during the infection, such as fibronectin, Laminin, and collagen, indicating that *L.*

monocytogenes can improve the ability of adhesion and invasion of cells by adjusting

ECM.

The significantly different proteins in HIF-1 signaling pathway and Ferroptosis were Tfrc and Tf, which regulated intracellular iron. They were both upregulated in *L. monocytogenes* 10403s and *L. monocytogenes* M7 infection groups. It was found that transferrin (Tf), transferrin receptor (Tfrc) and ferroportin favored oxidative damage and Ferroptosis by increasing iron uptake and reducing iron export ^[57]. However, hypoxia inducible factor-1 (HIF-1) was not identified, which may be caused by the lack of immune cells in the small intestine organoids. Therefore, it indicated that the enriched HIF-1 signaling pathway was not caused by HIF-1. In addition, there was no significant difference in the key regulatory protein glutathione peroxidase 4 (Gpx4) in the Ferroptosis pathway. This indicated that the effect of different toxic *L. monocytogenes* on Ferroptosis was not critical.

Daniel G. et al. claimed that complement was an essential defense of *L. monocytogenes* infection ^[58]. Complement, coagulation and fibrinolytic systems can form serine protease system, which plays an essential role in the innate immune responses. The interplay between complement and coagulation contributed to strengthen innate immunity, and activate adaptive immunity to eliminate bacteria ^[59]. In our study, three fibrinogen chains (Fga, Fgb and Fgg) were upregulated in two *L. monocytogenes* infections, while coagulation factor X (F10) and C3 was upregulated only in *L. monocytogenes* M7 infection group. It indicated that the different toxic *L. monocytogenes* activated the complement system and coagulation cascade in the stage of intestinal infection, and low-virulence strains caused a more significant coagulation cascade.

The NOD-like receptor signaling pathway is mediated by Nod-like receptors in the host cell and is also an important innate immune response ^[60]. Nod2 in NOD-like receptors can be expressed in intestinal epithelial cells, such as Paneth cells and stem cells ^[61]. Besides, extensive studies have shown that Nod2 plays an important role in maintaining the balance between bacteria, epithelial cells and the innate immune response of the host ^[31]. Nod2 recruits downstream proteins by recognizing the muramyl dipeptide (MDP) in the cell wall of pathogens, and then induces the activation of NF- κ B, MAPK, and caspase-1 pathways^[62]. In general, bacteria will activate Nod2 during infection and increase the expression level of Nod2. Interestingly, Nod2 was down-regulated in both *L. monocytogenes* 10403 and *L. monocytogenes* M7 groups in our result.

Studies showed that under the stimulation of different concentrations of MDP, Nod2 in dental pulp stem cells was activated, but the expression level was reduced ^[63]. This suggested that Nod2 in stem cells could be inhibited by MDP. In addition, studies showed that the expression of Nod2 in the terminal ileum of sterile mice was lower, and the expression of Nod2 was increased after supplementing symbiotic bacteria in sterile mice ^[64]. This indicated that the expression level of Nod2 in the intestine was related to commensal bacteria. Therefore, the reason for the low expression of Nod2 in the small intestine organoid model could be related to the lack of symbiotic bacteria.

Combined with the mRNA expression results of Nod2 pathway proteins, it can be seen that the infection led to the activation of Nod2 at the mRNA level, while it caused a reduction in the protein expression. This indicated that the bactericidal effect of Nod2 pathway in intestine organoids was limited within 18h. It could not effectively eliminate

593 *L. monocytogenes*, and resulted in a significant reduction of Nod2 expression in
 594 damaged cells (such as intestinal stem cells). Therefore, the overall protein level of
 595 Nod2 and mRNA expression of other proteins in the Nod2 pathway were decreased in
 596 *L. monocytogenes* 10403s group.

597 Besides, NOD2 is highly expressed in Paneth cells, which defense intestinal pathogen
 598 by secreting antimicrobial compounds. Several studies highlighted the essential role
 599 that NOD2 played in maintaining the equilibrium between intestinal microbiome and
 600 host immune responses [61, 65]. In addition, recent studies showed that Nod had an
 601 important effect on intestinal stem cells. Nigro et al. reported that NOD2 provided
 602 cytoprotection to intestinal stem cells, and Levy et al. found that the mechanism of
 603 NOD2-mediated cytoprotection involved the clearance of the lethal excess of ROS
 604 molecules through mitophagy [66, 67]. Combined with our unpublished research results,
 605 it was found that the damage of *L. monocytogenes* to intestinal stem cells could be
 606 achieved by down-regulation of NOD2.

607 Moreover, the downstream of the NOD-like receptor-signaling pathway includes the
 608 formation of inflammatory corpuscle complexes and the activation of caspase-1. Pycard
 609 (also known as ASC) is a key adaptor protein of inflammatory bodies (such as NLRP3)
 610 and an essential protein that activates inflammatory responses and apoptosis signaling
 611 pathways [68]. In the *L. monocytogenes* 10403s and *L. monocytogenes* M7 groups,
 612 Pycard expression was down-regulated, indicating that *L. monocytogenes* may down-
 613 regulate Pycard to inhibit the formation of inflammatory corpuscle complexes. In
 614 addition, in the *L. monocytogenes* 10403 vs M7 comparison group, Txn2 and Nek7

related to NLRP3 inflammatory body assembly were significantly down-regulated.

Specifically, they were down-regulated by *L. monocytogenes* 10403s and up-regulated by *L. monocytogenes* M7, while their change difference did not reach 1.2 times significantly. This showed that that difference between the two strains on the Nod-like receptor-signaling pathway in the host was mainly in the regulation of inflammasomes assembly.

Overall, KEGG enrichment analysis showed that different toxic *L. monocytogenes* increased the expression of adhesion and invasion-related proteins, reduced energy metabolism of host and triggered various host defense responses. Besides, the virulent strain *L. monocytogenes* 10403s had a more significant activation effect on the Ferroptosis pathway, while the low virulent strain *L. monocytogenes* M7 has a more significant activation effect on the complement system. More importantly, *L. monocytogenes* with different toxicity could affect the proliferation and cell protection of intestinal stem cells by down-regulating Nod2. In addition, the down-regulated proteins of *L. monocytogenes* 10403s vs *L. monocytogenes* M7 comparison group were enriched into systemic lupus erythematosus and transcriptional misregulation in cancer. It suggested that the low virulent strain had a stronger interference effect on immunodeficiency disease and transcriptional regulation.

In summary, complex responses to virulent and low virulent *L. monocytogenes* infections were revealed by TMT-based quantitative proteomics analysis using intestinal organoids. The DEPs between the *L. monocytogenes* 10403s and *L. monocytogenes* M7 infected groups displayed similar biological functions and

subcellular localizations as previous analysis. The difference in their influence on the host biological function was mainly reflected in transcription regulation and metabolism. These different DEPs were mainly distributed in the nucleus, and their domains were related to histones. Furthermore, complement and coagulation cascade and NOD-like receptor-signaling pathway were detected as the innate immune responses caused by two strains. Our result revealed that the modulation of protein expression attributed to the strategy of *L. monocytogenes* to overcome host defense response, and the data may give a comprehensive resource for investigating the overall response of intestinal epithelial cells excluding immune cells to infection with different toxic *L. monocytogenes*.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest (financial, professional or personal).

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Figure legends:

Figure 1 *Listeria monocytogenes* infection in mice

A. The body weight change rate and survival rate of mice 7 days after gavage. B. Bacterial load after 24h and 72h infection

Figure 2 Experimental strategy for quantitative proteome analysis and QC validation.

A. Pearson's correlation of protein quantitation. B. Length distribution of all identified peptides. X-axis: No. of Peptide; Y-axis: Peptide length. C. Mass delta of all identified peptides. X- axis: Peptide Score; Y-axis: Peptides mass delta.

Figure 3 The numbers of DEPs in different comparisons.

A. The numbers of the up- and down-regulated proteins in each comparison. B. Venn diagram of DEPs in different comparisons. C. Statistical overview of the bioinformatics of DEPs obtained in the combination of *L. monocytogenes* 10403s infection group and *L. monocytogenes* M7 infection group.

Figure 4 GO analysis and subcellular locations of DEPs in different comparisons.

A. GO analysis of regulated DEPs in *L. monocytogenes* 10403s vs Control. B. GO analysis of regulated DEPs in *L. monocytogenes* M7 vs Control. C. GO analysis of the up-regulated DEPs in *L. monocytogenes* 10403s vs *L. monocytogenes* M7. D. GO analysis of the down-regulated DEPs in *L. monocytogenes* 10403s vs *L. monocytogenes* M7. All proteins were classified by GO terms. X-axis: Number of DEPs. E. Subcellular locations of the DAPs in different comparisons.

Figure 5 GO and protein domain enrichment analysis of the DEPs.

Heatmaps showed the enrichments of the DEPs in different comparisons with GO

annotation belonging to biological process (A), cellular component (B), and molecular function (C). D. Significantly enriched protein domains of the DEPs.

Figure 6 KEGG enrichment analysis of all DEPs obtained in the combination of *L. monocytogenes* 10403s infection group and *L. monocytogenes* M7 infection group

All differentially expressed proteins in *L. monocytogenes* 10403s infection group and *L. monocytogenes* M7 infection group were put together for KEGG analysis. A. Columnar Section of KEGG enrichment analysis results. B. Pie chart of KEGG enrichment analysis results.

Figure 7 KEGG enrichment analysis of the DEPs.

A. Significantly enriched KEGG terms of the DAPs in the *L. monocytogenes* 10403s vs control comparison. B. Significantly enriched KEGG terms of the DAPs in the *L. monocytogenes* M7 vs control comparison. C. Significantly enriched KEGG terms of the DAPs in the *L. monocytogenes* 10403s vs *L. monocytogenes* M7 comparison.

Figure 8 Expression levels of the DEPs were verified using RT-PCR and western blot analysis.

A. Verification results of Nod2 pathway genes (Nod2, RIP2, TAK1, P38, and NF- κ B) at the mRNA levels. B. Verification results of Nod2 at the protein levels.

Figure 1 *Listeria monocytogenes* infection in mice

Figure 2 Experimental strategy for quantitative proteome analysis and QC validation.

Figure 3 The numbers of DEPs in different comparisons.

Figure 4 GO analysis and subcellular locations of DEPs in different comparisons

Figure 5 GO and protein domain enrichment analysis of the DEPs

Figure 6 KEGG enrichment analysis of all DEPs obtained in the combination of *L.*

monocytogenes 10403s infection group and *L. monocytogenes* M7 infection group

Figure 7 KEGG enrichment analysis of the DEPs

A

Lm 10403s / Control

B

Lm M7 / Control

C

Lm 10403s / Lm M7

Figure 8 Expression levels of the DEPs were verified using RT-PCR and western blot analysis.

Table1 Primer sequence of target and reference genes

Gene	Forward Primer	Reverse Primer
GAPDH	ATGGTGAAGGTCGGTGTGAA	TGGAAGATGGTGATGGGCTT
NOD2	CAGGTCTCCGAGAGGGTACTG	GCTACGGATGAGCCAAATGAAG
RIP2	CCATCCCGTACCACAAGCTC	GCAGGATGCGGAATCTCAAT
TAK1	CCTGAGGTTCTGGCAAAGAT	CACTGCTGAGGTCCTTCTGG
P38	ATGAGGAGATGACCGGATATGTG	GCAGCAGTTCAGCCATGATG
NF-κB	ATGGCAGACGATGATCCCTAC	TGTTGACAGTGGTATTTCTGGTG

Table2 Treatment conditions of 6 treatment groups

Groups	1 h	18 h
Control	A	B
<i>L. monocytogenes 10403s</i>	C	D
<i>L. monocytogenes M7</i>	E	F

Table3 Summary of Differentially expressed proteins

Comparison group	up-regulated (>1.3)	down-regulated (<1/1.3)
B/A	103	146
C/A	37	2
E/A	6	12
D/B	102	52
F/B	188	25
C/D	486	388
C/E	107	12
D/F	19	121
E/F	386	595

TableS1 Relative expression of DEGs in *Lm 10403s* vs Control (D/B) and *Lm M7* vs Control (F/B)

Protein accession	Protein description	Gene name	MW [kDa]	D/B Ratio	F/B Ratio
A2A6A1	G patch domain-containing protein 8	Gpatch8	164.99	1.068	1.318
A2AFS3	UPF0577 protein KIAA1324	Kiaa1324	110.68	1.675	2.123
A2ASQ1	Agrin	Agrn	207.54	1.133	1.36
A4Q9F1	Protein monoglycylase TTLL8	Ttll8	94.914	0.65	0.59
A6BLY7	"Keratin, type I cytoskeletal	Krt28	50.346	1.137	1.457
B2RWS6	Histone acetyltransferase p300	Ep300	263.3	1.165	1.409
E9PV24	Fibrinogen alpha chain	Fga	87.428	1.404	1.492
E9Q414	Apolipoprotein B-100	Apob	509.43	1.302	1.204
E9Q4F7	Ankyrin repeat domain-containing protein 11	Ankrd11	296.18	1.451	1.415
F2YMG0	Serine protease 56	Prss56	65.133	1.136	2.246
O08739	AMP deaminase 3	Ampd3	88.651	1.227	1.304
O08811	General transcription and DNA repair factor IIH helicase subunit XPD	Ercc2	86.841	1.182	1.395
O35054	Claudin-4	Cldn4	22.338	2.314	2.539
O35215	D-dopachrome decarboxylase	Ddt	13.077	0.744	0.859
O35682	Myeloid-associated differentiation marker OS=Mus musculus	Myadm	35.284	1.354	1.17
O35685	Nuclear migration protein nudC	Nudc	38.358	0.765	0.789
O54750	Cytochrome P450 2J6	Cyp2j6	57.791	1.278	1.354
O55071	Cytochrome P450 2B19	Cyp2b19	55.996	1.309	1.532
O70303	Cell death activator CIDE-B	Cideb	24.8	1.398	1.214
O70422	General transcription factor IIH subunit 4	Gtf2h4	52.224	1.161	1.361
O70494	Transcription factor Sp3	Sp3	82.361	1.165	1.439
O70570	Polymeric immunoglobulin receptor	Pigr	84.998	0.767	0.863
O70572	Sphingomyelin phosphodiesterase 2	Smpd2	47.466	1.344	1.159
O88286	Protein Wiz	Wiz	184.29	0.644	0.818
O88322	Nidogen-2	Nid2	153.91	1.318	1.379
O88700	Bloom syndrome protein homolog	Blm	158.36	1.681	2.059
O88746	Target of Myb protein 1	Tom1	54.325	1.278	1.999
O88792	Junctional adhesion molecule A	F11r	32.423	1.308	1.325
O88844	Isocitrate dehydrogenase [NADP] cytoplasmic	Idh1	46.674	0.764	0.856
O88947	Coagulation factor X	F10	54.017	0.884	1.632
P01027	Complement C3	C3	186.48	1.242	1.479
P02089	Hemoglobin subunit beta-2	Hbb-b2	15.878	1.465	1.396
P02463	Collagen alpha-1(IV) chain	Col4a1	160.68	1.387	1.402
P02468	Laminin subunit gamma-1	Lamc1	177.3	1.452	1.544
P02469	Laminin subunit beta-1	Lamb1	197.09	1.405	1.47
P02798	Metallothionein-2	Mt2	6.1153	0.676	0.754
P02802	Metallothionein-1	Mt1	6.0181	0.678	0.81
P03899	NADH-ubiquinone oxidoreductase chain 3	Mtnd3	13.219	1.336	1.32

P04104	"Keratin, type II cytoskeletal 1	Krt1	65.605	1.579	1.969
P05064	Fructose-bisphosphate aldolase A	Aldoa	39.355	0.789	0.745
P06151	L-lactate dehydrogenase A chain	Ldha	36.498	0.828	0.76
P06745	Glucose-6-phosphate isomerase	Gpi	62.766	0.768	0.784
P07214	SPARC OS=Mus musculus	Sparc	34.45	1.288	1.303
P08043	Zinc finger protein 2	Zfp2	52.534	0.981	0.746
P08122	Collagen alpha-2(IV) chain	Col4a2	167.32	1.398	1.355
P08228	Superoxide dismutase [Cu-Zn]	Sod1	15.942	0.685	0.794
P09602	Non-histone chromosomal protein HMG-17	Hmgn2	9.4226	0.721	1.721
P09922	Interferon-induced GTP-binding protein Mx1	Mx1	72.037	1.198	2.178
P10107	Annexin A1 OS=Mus musculus	Anxa1	38.734	1.306	1.345
P10493	Nidogen-1 OS=Mus musculus	Nid1	136.54	1.403	1.512
P11276	Fibronectin OS=Mus musculus	Fn1	272.53	1.706	1.592
P12710	"Fatty acid-binding protein, liver	Fabp1	14.245	0.749	0.838
P14152	"Malate dehydrogenase, cytoplasmic	Mdh1	36.511	0.734	0.794
P15864	Histone H1.2 OS=Mus musculus	Hist1h1c	21.266	1.401	2.705
P15920	V-type proton ATPase 116 kDa subunit a isoform 2	Atp6v0a2	98.144	1.253	1.404
P17182	Alpha-enolase	Eno1	47.14	0.796	0.732
P17665	"Cytochrome c oxidase subunit 7C, mitochondrial	Cox7c	7.3325	1.367	1.115
P17717	UDP-glucuronosyltransferase 2B17	Ugt2b17	60.855	1.328	1.279
P17809	"Solute carrier family 2, facilitated glucose transporter member 1	Slc2a1	53.984	1.379	1.05
P17897	Lysozyme C-1	Lyz1	16.794	0.684	0.795
P19137	Laminin subunit alpha-1	Lama1	338.14	1.406	1.482
P19324	Serpin H1	Serpinh1	46.533	1.523	1.6
P20152	Vimentin	Vim	53.687	1.2	1.323
P20918	Plasminogen	Plg	90.807	1.616	1.592
P22315	"Ferrochelatase, mitochondrial	Fech	47.13	1.25	1.547
P26350	Prothymosin alpha	Ptma	12.254	0.756	0.737
P27661	Histone H2AX	H2afx	15.142	1.3	2.157
P28798	Granulins	Grn	63.458	1.297	1.46
P30115	Glutathione S-transferase A3	Gsta3	25.36	0.761	0.795
P31786	Acyl-CoA-binding protein	Dbi	10	0.732	0.84
P34022	Ran-specific GTPase-activating protein	Ranbp1	23.596	0.755	0.808
P36536	GTP-binding protein SAR1a	Sar1a	22.371	1.436	2.088
P39061	Collagen alpha-1(XVIII) chain	Col18a1	182.17	1.317	1.363
P43137	Lithostathine-1	Reg1	18.518	0.554	0.678
P46412	Glutathione peroxidase 3	Gpx3	25.424	1.496	1.588
P46414	Cyclin-dependent kinase inhibitor 1B	Cdkn1b	22.193	1.379	1.261
P47915	60S ribosomal protein L29	Rpl29	17.587	1.055	1.506
P48760	"Folypolyglutamate synthase, mitochondrial	Fpgs	64.955	1.184	1.343
P50543	Protein S100-A11	S100a11	11.083	1.286	1.318
P51670	C-C motif chemokine 9	Ccl9	13.871	0.957	2.014
P52800	Ephrin-B2	Efnb2	37.202	1.434	2.546

P54227	Stathmin	Stmn1	17.274	0.665	0.747
P54754	Ephrin type-B receptor 3	Ephb3	109.66	1.164	1.722
P55050	Fatty acid-binding protein	Fabp2	15.126	0.747	0.817
P55821	Stathmin-2 OS=Mus musculus	Stmn2	20.828	0.607	0.671
P55937	Golgin subfamily A member 3	Golga3	167.22	1.125	1.337
P58044	Isopentenyl-diphosphate Delta-isomerase 1	Idi1	26.289	0.756	0.74
P59326	YTH domain-containing family protein 1	Ythdf1	60.878	1.177	1.411
P60904	DnaJ homolog subfamily C member 5	Dnajc5	22.101	1.257	1.377
P61022	Calcineurin B homologous protein 1	Chp1	22.432	1.327	1.159
P61961	Ubiquitin-fold modifier 1	Ufm1	9.1175	0.77	0.734
P61965	WD repeat-containing protein 5	Wdr5	36.588	1.149	1.422
P61971	Nuclear transport factor 2	Nutf2	14.478	0.811	0.767
P62313	U6 snRNA-associated Sm-like protein LSM6	Lsm6	9.1275	0.828	0.701
P62342	Thioredoxin reductase-like selenoprotein T	Selenot	22.292	1.39	1.128
P62627	Dynein light chain roadblock-type 1	Dynlrb1	10.99	0.761	0.777
P62858	40S ribosomal protein S28	Rps28	7.8409	1.178	1.654
P62984	Ubiquitin-60S ribosomal protein L40	Uba52	14.728	1.075	1.347
P63166	Small ubiquitin-related modifier 1	Sumo1	11.557	1.073	1.855
P63254	Cysteine-rich protein 1	Crip1	8.5497	0.716	0.689
P68037	Ubiquitin-conjugating enzyme E2 L3	Ube2l3	17.861	0.767	0.822
P84228	Histone H3.2	Hist1h3b	15.388	1.096	1.721
P84244	Histone H3.3	H3f3a	15.328	1.305	1.869
P97466	Noggin	Nog	25.77	1.285	2.294
P97789	5'-3' exoribonuclease 1	Xrn1	194.31	1.284	1.763
P97872	Dimethylaniline monooxygenase [N-oxide-forming] 5	Fmo5	60	1.281	1.321
Q01237	3-hydroxy-3-methylglutaryl-coenzyme A reductase	Hmgcr	97.039	1.401	1.105
Q05793	Basement membrane-specific heparan sulfate proteoglycan core protein	Hspg2	398.29	1.332	1.434
Q08879	Fibulin-1	Fbln1	78.032	1.29	1.521
Q31125	Zinc transporter SLC39A7	Slc39a7	50.656	1.236	1.396
Q3TKY6	Spliceosome-associated protein CWC27 homolog	Cwc27	53.542	0.757	0.775
Q3TMQ6	Angiogenin-4	Ang4	16.425	0.63	0.717
Q3U1G5	Interferon-stimulated 20 kDa exonuclease-like 2	Isg20l2	41.019	1.047	1.325
Q3UMU9	Hepatoma-derived growth factor-related protein 2	Hdgfl2	74.29	0.977	1.325
Q3UUQ7	GPI inositol-deacylase	Pgap1	104.58	1.305	1.186
Q3UX10	Tubulin alpha chain-like 3	Tubal3	49.988	1.114	1.345
Q45VN2	Alpha-defensin 20	Defa20	10.601	0.713	0.854
Q4VAE3	Transmembrane protein 65	Tmem65	24.918	1.415	1.358
Q4ZJM7	Otolin-1 OS=Mus musculus	Otol1	49.6	1.284	2.128
Q5G865	Alpha-defensin 24	Defa24	10.327	1.449	2.702
Q5I012	Putative sodium-coupled neutral amino acid transporter 10	Slc38a10	117.19	1.113	1.374
Q5SVR0	TBC1 domain family member 9B	Tbc1d9b	141.78	0.729	0.945

Q60592	Microtubule-associated serine/threonine-protein kinase 2	Mast2	190.53	1.51	1.458
Q60722	Transcription factor 4	Tcf4	71.624	1.32	1.413
Q60829	Protein phosphatase 1 regulatory subunit 1B	Ppp1r1b	21.78	0.714	0.828
Q61152	Tyrosine-protein phosphatase non-receptor type 18	Ptpn18	50.201	1.034	1.315
Q61292	Laminin subunit beta-2	Lamb2	196.58	1.335	1.409
Q61382	TNF receptor-associated factor 4	Traf4	53.503	1.067	1.445
Q61598	Rab GDP dissociation inhibitor beta	Gdi2	50.537	0.754	0.804
Q61749	Translation initiation factor eIF-2B subunit delta	Eif2b4	57.624	1.149	1.378
Q62203	Splicing factor 3A subunit 2	Sf3a2	49.911	1.078	1.722
Q62241	U1 small nuclear ribonucleoprotein C	Snrpc	17.364	0.723	0.931
Q62266	Cornifin-A OS=Mus musculus	Sprr1a	15.765	1.498	1.214
Q62273	Sulfate transporter	Slc26a2	81.603	1.29	1.489
Q62313	Trans-Golgi network integral membrane protein 1	Tgoln1	37.848	1.32	1.398
Q62388	Serine-protein kinase ATM	Atm	349.41	1.068	1.427
Q62392	Pleckstrin homology-like domain family A member 1	Phlda1	45.582	1.403	1.203
Q62395	Trefoil factor 3 OS=Mus musculus	Tff3	8.8081	0.728	0.866
Q62452	UDP-glucuronosyltransferase 1-9	Ugt1a9	60.007	1.338	1.626
Q64435	UDP-glucuronosyltransferase 1-6	Ugt1a6	60.438	1.213	1.308
Q64458	Cytochrome P450 2C29	Cyp2c29	55.715	1.286	1.376
Q64459	Cytochrome P450 3A11	Cyp3a11	57.854	1.528	1.662
Q64464	Cytochrome P450 3A13	Cyp3a13	57.492	1.428	1.29
Q66JX5	FGFR1 oncogene partner	Fgfr1op	42.758	0.722	0.889
Q689Z5	Protein strawberry notch homolog 1	Sbno1	153.74	1.044	1.412
Q6NVG1	Lysophospholipid acyltransferase LPCAT4	Lpcat4	57.143	1.44	1.219
Q6PDH0	Pleckstrin homology-like domain family B member 1	Phldb1	150.07	0.621	0.558
Q6PGC1	ATP-dependent RNA helicase DHX29	Dhx29	153.97	1.247	1.635
Q6PHN9	Ras-related protein Rab-35 OS=Mus musculus	Rab35	23.025	1.236	1.347
Q6PIJ4	Nuclear factor related to kappa-B-binding protein	Nfrkb	138.76	1.125	1.673
Q6ZQ06	Centrosomal protein of 162 kDa	Cep162	160.85	1.088	2.187
Q6ZQF0	DNA topoisomerase 2-binding protein 1	Topbp1	168.86	1.271	1.31
Q6ZWY9	Histone H2B type 1-C/E/G	Hist1h2bc	13.906	1.383	4.136
Q71KT5	Delta(14)-sterol reductase	Tm7sf2	46.52	1.489	1.611
Q71LX4	Talin-2 OS=Mus musculus	Tln2	253.62	1.024	1.663
Q76KJ5	DNA-directed RNA polymerase I subunit RPA34 OS=Mus musculus	Cd3eap	43.082	0.977	1.393
Q78IK2	Up-regulated during skeletal muscle growth protein 5	Usmg5	6.3814	1.429	1.374
Q791T5	Mitochondrial carrier homolog 1	Mtch1	41.565	1.198	2.201
Q7TNS2	MICOS complex subunit Mic10	Minos1	8.5669	1.496	1.349
Q7TT45	Ras-related GTP-binding protein D	Rragd	51.232	1.206	1.545
Q80T69	Lysine-specific demethylase 9	Rsbm1	89.25	1.466	1.23

Q80TE0	RNA polymerase II-associated protein 1	Rpap1	155.27	1.275	1.806
Q80U49	Centrosomal protein of 170 kDa protein B	Cep170b	170.82	1.087	1.358
Q80WQ6	Inactive rhomboid protein 2	Rhbdf2	93.433	1.102	1.414
Q80X80	Phospholipid transfer protein C2CD2L	C2cd2l	76.328	1.376	1.385
Q80YT7	Myomegalin	Pde4dip	250.63	1.584	1.494
Q80ZM8	Cardiolipin synthase (CMP-forming)	Crsl1	32.502	1.219	1.308
Q80ZW2	Protein THEM6	Them6	23.802	1.4	1.385
Q8BG73	SH3 domain-binding glutamic acid-rich-like protein 2	Sh3bgrl2	12.255	0.86	0.753
Q8BH59	Calcium-binding mitochondrial carrier protein Aralar1	Slc25a12	74.569	1.297	1.343
Q8BHE8	"m-AAA protease-interacting protein 1, mitochondrial	Maip1	32.985	1.412	1.37
Q8BHG2	UPF0587 protein C1orf123 homolog	---	18.02	0.768	0.85
Q8BHL4	Retinoic acid-induced protein 3	Gprc5a	40.1	1.51	1.204
Q8BIG7	Catechol O-methyltransferase domain-containing protein 1	Comtd1	28.96	1.346	1.17
Q8BJ03	Cytochrome c oxidase assembly protein COX15 homolog	Cox15	45.852	1.317	1.144
Q8BMD8	Calcium-binding mitochondrial carrier protein SCA1	Slc25a24	52.901	1.367	1.24
Q8BND5	Sulfhydryl oxidase 1	Qsox1	82.784	0.757	0.844
Q8BNU0	Armado repeat-containing protein 6	Armc6	50.683	1.303	1.705
Q8BNW9	Kelch repeat and BTB domain-containing protein 11	Kbtbd11	67.945	1.227	1.331
Q8BP92	Reticulocalbin-2	Rcn2	37.27	1.26	1.407
Q8BQZ4	Ral GTPase-activating protein subunit beta	Ralgapb	165.2	1.232	1.738
Q8BTU1	Cilia- and flagella-associated protein 20	Cfap20	22.748	1.06	1.327
Q8BTW3	Exosome complex component MTR3	Exosc6	28.37	1.244	1.937
Q8BW75	Amine oxidase [flavin-containing] B OS=Mus musculus	Maob	58.557	1.377	1.287
Q8BWQ1	UDP-glucuronosyltransferase 2A3	Ugt2a3	61.119	1.337	1.306
Q8BZR9	Nuclear cap-binding protein subunit 3	Ncbp3	70.042	1.312	1.568
Q8C3B8	Protein RFT1 homolog	Rft1	60.303	1.24	1.368
Q8C4V1	Rho GTPase-activating protein 24	Arhgap24	84.099	0.809	1.663
Q8C561	LMBR1 domain-containing protein 2	Lmbrd2	81.1	1.293	1.335
Q8C5T8	Coiled-coil domain-containing protein 113	Ccdc113	44.214	0.876	0.761
Q8CD91	SPARC-related modular calcium-binding protein 2	Smoc2	49.891	0.873	1.421
Q8CGA0	Protein phosphatase 1F	Ppm1f	49.61	0.665	0.975
Q8CHP8	Glycerol-3-phosphate phosphatase	Pgp	34.54	0.757	0.835
Q8CIM7	Cytochrome P450 2D26	Cyp2d26	56.975	1.215	1.359
Q8JZL7	Ras-GEF domain-containing family member 1B	Rasgef1b	55.273	1.201	1.425
Q8JZR0	Long-chain-fatty-acid--CoA ligase 5	Acs15	76.205	1.316	1.306
Q8K072	Receptor expression-enhancing protein 4	Reep4	29.69	1.194	1.43
Q8K0C5	Zymogen granule membrane protein 16	Zgl16	18.209	1.48	1.314

Q8K0E3	Sodium/myo-inositol cotransporter 2	Slc5a11	73.797	1.165	1.305
Q8K0E8	Fibrinogen beta chain	Fgb	54.752	1.419	1.493
Q8K296	Myotubularin-related protein 3	Mtmr3	133.84	0.909	1.402
Q8K2C9	Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3	Hacd3	43.131	1.31	1.379
Q8K3Z0	Nucleotide-binding oligomerization domain-containing protein 2	Nod2	113.56	0.692	0.679
Q8R003	Muscleblind-like protein 3	Mbnl3	37.57	1.156	1.497
Q8R1M8	Mucosal pentraxin	Mptx1	24.538	1.307	1.236
Q8R2K1	Fucose mutarotase	Fuom	16.805	0.766	0.72
Q8R3P6	Integrator complex subunit 14	Ints14	57.236	1.292	1.384
Q8R4D5	Transient receptor potential cation channel subfamily M member 8	Trpm8	127.71	0.992	2.019
Q8VCM7	Fibrinogen gamma chain	Fgg	49.391	1.366	1.378
Q8VED9	Galectin-related protein	Lgalsl	18.955	1.274	1.718
Q8VHG0	Dimethylaniline monooxygenase [N-oxide-forming] 4	Fmo4	63.791	1.328	1.268
Q8VHK1	Caskin-2	Caskin2	126.78	1.109	1.786
Q91V04	Translocating chain-associated membrane protein 1	Tram1	43.039	1.249	1.349
Q91V76	Ester hydrolase C11orf54 homolog	---	34.995	0.8	0.763
Q91W97	Putative hexokinase HKDC1	Hkdc1	102.26	1.365	1.25
Q91WE4	UPF0729 protein C18orf32 homolog	---	8.0355	1.485	2.253
Q91WP6	Serine protease inhibitor A3N	Serpina3n	46.717	1.605	1.586
Q91XB7	Protein YIF1A	Yif1a	32.134	1.384	1.404
Q91Y74	"CMP-N-acetylneuraminase-beta-galactosamide-alpha-2,3-sialyltransferase 4	St3gal4	38.058	1.429	1.198
Q91ZF2	Cathepsin 7	Cts7	37.724	1.398	1.782
Q921I1	Serotransferrin	Tf	76.723	1.277	1.36
Q921U8	Smoothelin	Smtn	100.29	1.376	1.889
Q923S9	Ras-related protein Rab-30	Rab30	23.058	1.615	1.409
Q93092	Transaldolase	Taldo1	37.387	0.755	0.742
Q99JR5	Tubulointerstitial nephritis antigen-like	Tinagl1	52.664	1.455	1.4
Q99LH1	Nucleolar GTP-binding protein 2	Gnl2	83.345	1.006	1.419
Q99LJ0	CTTNBP2 N-terminal-like protein	Cttnbp2nl	69.84	1.085	1.339
Q99LX0	Protein/nucleic acid deglycase DJ-1	Park7	20.021	0.722	0.794
Q99MI1	ELKS/Rab6-interacting/CAST family member 1	Erc1	128.33	1.014	1.497
Q99PG0	Arylacetamide deacetylase	Aadac	45.25	1.303	1.19
Q9CQC2	Colipase OS=Mus musculus	Clps	12.444	0.608	0.747
Q9CQD1	Ras-related protein Rab-5A	Rab5a	23.598	1.329	1.5
Q9CQJ8	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9 OS=Mus musculus OX=10090 GN=Ndufb9 PE=1 SV=3	Ndufb9	21.984	1.317	1.27
Q9CQM2	ER lumen protein-retaining receptor 2	Kdelr2	24.454	1.353	3.241
Q9CQS5	Serine/threonine-protein kinase RIO2	Rio2	62.49	1.175	2.177

Q9CQT2	RNA-binding protein 7	Rbm7	30.148	1.059	1.405
Q9CR62	Mitochondrial 2-oxoglutarate/malate carrier protein	Slc25a11	34.155	1.352	1.277
Q9CWK3	CD2 antigen cytoplasmic tail-binding protein 2	Cd2bp2	37.694	0.995	1.419
Q9CWV6	PRKR-interacting protein 1	Prkrip1	21.491	1.159	1.654
Q9CWY9	RPA-interacting protein	Rpain	24.897	1.557	2.202
Q9CXY6	Interleukin enhancer-binding factor 2	Ilf2	43.062	0.755	0.789
Q9CY57	Chromatin target of PRMT1 protein	Chtop	26.585	0.753	0.842
Q9CYH2	Redox-regulatory protein FAM213A	Fam213a	24.394	1.404	1.414
Q9D032	Single-stranded DNA-binding protein 3	Ssbp3	40.421	1.225	1.333
Q9D136	2-oxoglutarate and iron-dependent oxygenase domain-containing protein 3	Ogfod3	35.384	1.273	1.559
Q9D1I2	Caspase recruitment domain-containing protein 19	Card19	20.936	1.348	1.349
Q9D1J1	Adaptin ear-binding coat-associated protein 2	Necap2	28.598	0.968	1.591
Q9D1N9	"39S ribosomal protein L21, mitochondrial	Mrpl21	23.366	1.019	1.38
Q9D2L9	Protein FAM111A	Fam111a	69.948	1.137	1.309
Q9D2Q2	Probable tRNA (uracil-O(2)-)-methyltransferase	Trmt44	79.835	1.257	1.399
Q9D379	Epoxide hydrolase 1	Ephx1	52.576	1.318	1.336
Q9D6M3	Mitochondrial glutamate carrier 1	Slc25a22	34.67	1.112	1.487
Q9D7S0	Ly6/PLAUR domain-containing protein 8	Lypd8	27.524	0.713	0.684
Q9DB90	Protein SMG9	Smg9	57.62	1.035	1.372
Q9DBC3	Cap-specific mRNA (nucleoside-2'-O)-methyltransferase 1	Cmtr1	95.675	0.667	0.933
Q9DBM1	G patch domain-containing protein 1	Gpatch1	103.01	1.045	1.308
Q9DBT3	Coiled-coil domain-containing protein 97	Ccdc97	38.724	1.235	1.477
Q9DBY1	E3 ubiquitin-protein ligase synoviolin	Syvn1	67.296	1.419	2.27
Q9DCF9	Translocon-associated protein subunit gamma	Ssr3	21.064	1.32	2.09
Q9DCS9	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	Ndufb10	21.024	1.43	1.319
Q9DCT5	Stromal cell-derived factor 2	Sdf2	23.159	1.715	2.419
Q9EP75	Leukotriene-B4 omega-hydroxylase 3	Cyp4f14	59.8	1.388	1.328
Q9EPB4	Apoptosis-associated speck-like protein containing a CARD	Pycard	21.458	0.72	0.728
Q9EPS3	D-glucuronyl C5-epimerase	Glce	70.088	1.303	1.298
Q9EQ06	Estradiol 17-beta-dehydrogenase 11	Hsd17b11	32.88	1.363	1.371
Q9JKP5	Muscleblind-like protein 1	Mbnl1	36.975	1.203	1.534
Q9JLJ1	Selenoprotein K	Selenok	10.642	1.426	1.302
Q9JLJ5	Elongation of very long chain fatty acids protein 1	Elov11	32.677	1.637	2.101
Q9JM52	Misshapen-like kinase 1	Mink1	147.29	1.213	1.663
Q9QXD8	LIM domain-containing protein 1	Limd1	71.421	1.248	1.831
Q9QY14	DnaJ homolog subfamily B member 12	Dnajb12	41.987	1.331	1.616
Q9QZI9	Serine incorporator 3	Serinc3	52.622	1.127	1.55
Q9QZR0	E3 ubiquitin-protein ligase RNF25	Rnf25	51.226	0.737	0.794
Q9R0B9	"Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	Plod2	84.487	1.421	1.469
Q9R0E1	"Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	Plod3	84.921	1.298	1.306

Q9R0Q3	Transmembrane emp24 domain-containing protein 2	Tmed2	22.705	1.259	1.333
Q9R0U0	Serine/arginine-rich splicing factor 10	Srsf10	31.3	1.127	1.345
Q9WTI7	Unconventional myosin-Ic	Myo1c	121.94	1.335	1.267
Q9WUZ9	Ectonucleoside triphosphate diphosphohydrolase 5	Entpd5	47.101	1.247	1.318
Q9WVQ5	Methylthioribulose-1-phosphate dehydratase	Apip	26.949	0.754	0.914
Q9Z1S5	Neuronal-specific septin-3	Sept3	40.037	0.75	1.118
Q9Z247	Peptidyl-prolyl cis-trans isomerase FKBP9	Fkbp9	62.995	1.388	1.373
Q9Z2A7	Diacylglycerol O-acyltransferase 1	Dgat1	56.789	1.253	1.406
Q9Z2G0	Protein fem-1 homolog B	Fem1b	70.222	1.49	1.777
Q9Z2Z6	Mitochondrial carnitine/acylcarnitine carrier protein	Slc25a20	33.026	1.318	1.194