Urinary proteome of dogs with kidney injury during babesiosis

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Dagmara Winiarczyk
University of Life Sciences
ORCiD: 0000-0002-1257-869X

Katarzyna Michalak
University of Life Sciences in Lublin Poland

Łukasz Adaszek
Uniwersytet Przyrodniczy w Lublinie

Mateusz Winiarczyk
Uniwersytet Medyczny w Lublinie

Stanisław Winiarczyk genp53@interia.pl
Corresponding Author
ORCiD: 0000-0002-8468-2154

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Abstract

Background

Acute kidney injury is the most frequent complication of babesiosis in dogs and may provide a natural model for identifying early and specific markers of kidney injury in this species. There are limited data on urine proteomics in dogs, and none of the effect of babesiosis on the urine proteome. This study aimed to identify urinary proteins of dogs with kidney injury during the natural course of babesiosis caused by Babesia canis, and to compare them with proteins in a control group to reveal any potential biomarkers predicting renal injury before the presence of azotemia.

Urine samples were collected from 10 dogs of various breeds and sex with naturally occurring babesiosis, and 10 healthy dogs. Pooled urine samples from both groups were separated by 2D (two-dimensional) electrophoresis, followed by protein identification using MALDI-TOF (matrix-assisted laser desorption ionization time of flight) mass spectrometry.

Results

In total, 176 proteins were identified in the urine samples from healthy dogs, and 403 proteins were identified in the urine samples from dogs with babesiosis. Of the 176 proteins, 146 were assigned exclusively to healthy dogs, and 373 of the 403 proteins were assigned exclusively to dogs with babesiosis; 30 proteins were common for both groups. Characteristic analysis of 373 proteins found in dogs with babesiosis led to the isolation of 8 proteins associated with 10 metabolic pathways involved in immune and inflammatory responses.

Conclusions

It was hypothesized that epithelial-mesenchymal transition might play an important role in the mechanisms underlying pathological changes in renal tissue during babesiosis, as
indicated by a causal relationship network built by combining 5 of the 10 selected metabolic pathways, and 4 of the 8 proteins associated with these pathways; this network included cadherins, gonadotropin releasing hormone receptors, inflammatory responses mediated by chemokine and cytokine signalling pathways, integrins, interleukins, and TGF-β (transforming growth factor β) pathways. Those pathways were linked by interleukin-13, bone morphogenetic protein 7, α2(1) collagen, and tyrosine protein kinase Fer, which are potential biomarkers of damage during babesiosis in dogs, that might indicate early renal injury.

Background

After heart failure, kidney disease is the most frequent cause of reduced quality of life and shortened survival of people and dogs [1–4]. Two different forms of kidney disease, acute kidney injury (AKI) and chronic kidney disease (CKD), are caused by various factors. In humans, 7.8% of patients with AKI develop CKD, and 4.9% of patients progress to end-stage renal disease [1]. Most AKI cases in medicine and veterinary science are diagnosed based on serum or plasma concentrations of non-protein nitrogenous creatinine (Cr) and urea compounds. This method has limited sensitivity, and is not suitable for early AKI detection [2], thus it is necessary to identify markers and methods adequate for the early detection of glomeruli, and/or tubule injury before the decreased glomerular filtration rate (GFR) is signalled by increased Crea concentration [3–6]. One such method is proteomic analysis, which compares the protein profile in normal urine with that typical for a given disease to select potential diagnostic, therapeutic, and prognostic biomarkers [7, 8]. With the decreased GFR and subsequent azotemia and uremia, AKI is among the most frequent complications of babesiosis in dogs, and may provide a natural model for identifying early and specific markers of kidney injury in this species [9,10,11,12]. Evaluation of urinary proteins is a promising strategy for detecting kidney injury. Normal urine should contain
only a small amount of protein, because of the mechanical barrier of the glomerulus, and the reabsorptive capacity of the proximal tubules. Urinary total protein is a mixture of filtered plasma proteins, kidney-derived proteins, and proteins originating from the lower urinary tract. The discovery of candidate urinary protein biomarkers for kidney injury is essentially a hypothesis-generating process. Altered functioning of the nephron can result in the presence of large amounts of proteins in urine. The glomerular filtration barrier normally excludes most proteins as large as, or larger than albumin (ie. MW > 69kDa). The charge of a proteins also influence its filtration: positively charged proteins pass the glomerular barrier more easily than negatively charged proteins. Changes in the structure or composition of this barrier, or in the heamodynamic state of the patient can lead to decreased glomerulal perselectivity. This primary glomerulal dysfunction results in the presence of high amounts of proteins with intermediate, or high molecular weight (MW) in the ultrafiltrate. Proteins smaller than albumin are freely filtered by the glomerulus. However, these proteins subsequently are efficiently reabsorbed by the proximal tubules in a normally functioning kidney. Both primary and secondary tubular dysfunction represent an inability of the tubules to completely reabsorb filtered proteins, and can result in proteinuria [11].

Possible causes of acute kidney injury in dogs with babesiosis include anaemic hypoxia, hypovolemia, haemoglobinuric nephropathy, and myoglobinuric nephropathy secondary to rhabdomyolysis [13,14,15]. Anoxia, reduced renal blood flow, hypotension, and renal ischaemia probably play more important roles in the development of AKI than haemoglobinuria. Hypoxia results in greater renal tubular injury than haemoglobin, and the nephrotoxic effects of haemoglobin are highly individual [14]. Additionally, as shown by Zygner et al. [16], the increase in serum TNF-α concentration in dogs with canine babesiosis influences the development of hypotension and renal failure.
Moreover, as AKI naturally occurs during babesiosis in dogs, this situation could serve as a good model for select studies on AKI in humans. This hypothesis is supported by comparative analysis of urine proteomes in humans and dogs; many proteins related to human diseases, including kidney diseases, have been identified in canine urine [17–19]. In addition, domestic dogs (Canis lupus familiaris) are increasingly perceived as an excellent animal model for studying complex human diseases [20]. Canine DNA and protein sequences are much closer than mouse sequences to human sequences, suggesting that canine biology is more similar in many aspects to human biology than is mouse biology [21–22]. It is also worth a mention that babesiosis is a zoonotic parasitic infection, and has similar clinical presentation to canine babesiosis [23].

This study aimed to identify proteins in the urine of dogs with subclinical kidney injury during the natural course of babesiosis, and to compare them with proteins in the control group to reveal potential biomarkers predicting renal injury before the presence of azotemia.

Results

Based on the clinicopathological variables, all dogs with babesiosis met the criteria for early phase AKI [24]. They had proteinuria with UPC>0.5, decreased urine specific gravity (average, 1.015) and significantly elevated uIgG/uCr, uTHP/uCr, and uRBP/uCr values, which indicated glomerular and tubular damage.

In this study, 176 proteins were identified in pooled urine samples collected from healthy dogs, and 403 proteins were identified in pooled urine samples collected from dogs with babesiosis. Tables 1 and 2 contain lists of the proteins, along with their names, scores, molecular weights, number of matches, UniProt base accession numbers and hyperlinks (see Appendix: Supplementary Table 1.,2.). With Venn diagram software, which shows logical correlations between groups (http://bioinfogp.cnb.csic.es), 146 of the 176 proteins
were assigned exclusively to healthy dogs, and 373 of the 403 proteins were exclusively assigned to dogs with babesiosis; 30 proteins were common for both groups. List of 30 common proteins between two groups has been presented in Supplementary data (see Appendix: Supplementary Table 3). From 146 proteins found exclusively in healthy dogs, 128 were identified by Pantherdb software. According to molecular pathways analysis, those were listed in categories as follows: binding, catalytic activity, molecular function regulator, molecular transducer activity, structural molecule activity, transcription regulator activity, and transporter activity. Two most prominent molecular functions of those proteins were binding, and catalytic activity, consisting of 40 and 27 proteins, respectively.

To further evaluate the 373 proteins found in only the dogs with babesiosis, the Panther programme (http://www.pantherdb.org) was used to isolate 21 proteins from the Canis familiaris species, which were used to form a collection of potential diagnostic and pathophysiological biomarkers for this disease (Table 1). Further analysis of these 21 proteins led to the isolation of 8 proteins associated with 10 metabolic pathways, that were attributed to immune and inflammatory response development (Table 2). Further analysis indicated that a causal relationship network could be built by combining 5 of the 10 selected metabolic pathways and 4 of the 8 proteins with which the pathways were associated. These pathways included cadherins, gonadotropin releasing hormone receptors, inflammatory responses mediated by chemokine and cytokine signalling pathways, integrins, and TGF-β pathways and were linked by interleukin (IL)-13, bone morphogenetic protein 7, α2(1) collagen, and FER tyrosine kinase.

[Insert Fig. 1. Here]

[Insert Fig. 2. Here]

Table 1. List of *Canis familiaris* proteins identified in the urine of dogs with babesiosis by
MALDI-TOF/TOF.

| Nr | Accessiona | Protein name | GO molecular function |
|----|-------------|--------------|-----------------------|
| 1  | P06596      | Phospholipase A2 | phospholipase |
| 2  | A4Z944      | Zinc finger BED domain-containing protein 5 | transcription factor |
| 3  | O46392      | Collagen alpha-2(I) chain | extracellular matrix structural constituent |
| 4  | Q9XSU4      | 40S ribosomal protein S11 | structural constituent of ribosome |
| 5  | O97556      | Rab GDP dissociation inhibitor beta | G-protein modulator acyltransferase |
| 6  | Q9TTY2      | Tyrosine-protein kinase Fer | tyrosine kinase activit |
| 7  | P19006      | Haptoglobin | hemoglobin binding |
| 8  | Q75V93      | Calcitonin receptor-stimulating peptide 2 | peptide hormone |
| 9  | Q2KNA0      | Cytospin-A | structural component |
| 10 | P01321      | Insulin | hormone |
| 11 | Q9N219      | Mitochondrial uncoupling protein 3 | oxidative phosphorylation |
| 12 | E2RK33      | Glutamyl-tRNA(Gln) amidotransferase subunit C, mitochondrial | ligase |
| 13 | Q32KH5      | N-acetylgalactosamine-6-sulfatase | hydrolase |
| 14 | P27597      | T-cell surface glycoprotein CD3 epsilon chain | transmembrane signalling receptor |
| 15 | P24408      | Ras-related protein Rab-9A | GTPase |
| 16 | P34819      | Bone morphogenetic protein 7 (fragment) | growth factor |
| 17 | Q5TJE5      | Ras guanine nucleotide dissociation stimulator-like 2 | guanyl-nucleotide exchange factor |
| 18 | Q9N0W9      | Interleukin-13 | cytokine |
| 19 | Q8WMX5      | Solute carrier family 15 member 1 | transporter |
| 20 | O97578      | Dipeptidyl peptidase 1 (fragment) | endopeptidase |
| 21 | Q861Y6      | Nicolin-1 | structural component |

Table 2. List of metabolic pathways and associated urinary proteins in dogs with babesiosis.

| No. | Pathway                                                                 | Protein                                           |
|-----|-------------------------------------------------------------------------|---------------------------------------------------|
| 1.  | CCKR signalling pathway                                                 | Calcitonin receptor-stimulating peptide 2         |
| 2.  | Cadherin signalling pathway                                             | Tyrosine-protein kinase Fer                       |
| 3.  | Gonadotropin-releasing hormone receptor pathway                          | Insulin/Bone morphogenetic protein 7 (fragment)   |
| 4.  | Inflammatory response mediated by chemokines and cytokines              | Interleukin 13                                    |
| 5.  | Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade | Insulin/Insulin-like growth factor                 |
| 6.  | Insulin/IGF pathway-protein kinase B signaling cascade                   | Insulin/Insulin-like growth factor                 |
| 7.  | Integrin signalling pathway                                             | Collagen alpha-2(I) chain                         |
| 8.  | Ras pathway                                                             | Ral guanine nucleotide dissociation stimulator-like 2 |
| 9.  | T cell activation                                                       | T cell surface glycoprotein CD3 epsilon chain      |
| 10. | TGF-beta signaling pathway                                              | Bone morphogenetic protein 7 (fragment)           |

Discussion

The final bioinformatic analysis of the urine proteome of dogs with babesiosis indicated...
that at least eight proteins (IL-13, bone morphogenetic protein 7, α2(1) collagen, tyrosine-protein kinase Fer, calcitonin receptor-stimulating peptide 2, insulin/insulin-like growth factor, ral guanine nucleotide dissociation stimulator-like 2 and T cell surface glycoprotein CD3 epsilon chain) are related to parasitic invasions and renal inflammatory responses. Non-specific immune responses are activated to limit the initial phase of parasitic invasion, or infection by pathogenic micro-organisms. Parasitic invasion initiates type Th2 immune response, characterised by the activation of Th2 lymphocytes, eosinophilia, basophilia, mast cells, and alternatively activated macrophages (AAM). This process is accompanied by the secretion of IgE antibodies and numerous cytokines, such as IL-3, IL-4, IL-5, IL-9, IL-10, IL-13 and TGF-β. It is well known that IL-13 plays a key role in regulating the anti-parasitic response [25] and is a primary factor that induces fibrosis in many chronic contagious and autoimmune diseases [26]. IL-13 increases the concentration of TGF-β, which leads to collagen deposition in lung and kidney tissues [27], by stimulating its production by macrophages via IL-13Rα2 [28, 29]. Fibrosis is considered the final stage in CKD development regardless of the primary cause, and the effector cells of this process include myofibroblasts generated from renal tubule epithelial cells by epithelial-mesenchymal transition (EMT) [30–33]. During this transition, cells lose polarity upon losing certain communication abilities, and degrading the basement membrane. Adhesive molecules that bind both epithelial cells and the basement membrane, such as E-cadherin and integrins, are replaced by mesenchymal cell markers, such as N-cadherin, nonstriated muscle α-actin, vimentin, fibronectin and collagen I. In an early inflammatory environment, EMT maintains renal tissue homoeostasis by inducing structural regeneration and reconstruction after harmful stress. Long-term support of EMT leads to fibrous degeneration as well as structural and functional tissue and organ disorders [34,35]. Pleiotropic TGF-β molecules and BMPs belonging to the transforming growth factor-β
superfamily (TGF-βSF) participate in one of the most well-known signalling pathways in EMT [36–38]. Increased TGF-β levels lead to loss of the epithelial phenotype, acquisition of the mesenchymal phenotype and collagen accumulation. Serine-threonine kinase receptors and cytoplasmic proteins (Smads) participate in transmitting TGF-β/BMP pathway signals. Smad3, which is induced by TGF-β stimulation, can bind the Col1A2 gene promoter to activate the expression of type 1α2 collagen, which may accumulate in interstitial tissue and contribute to extracellular matrix (ECM) accumulation, leading to fibrous degeneration of the organ [39]. On the other hand, BMP-7 inhibits fibrosis, exerts anti-inflammatory effects and stimulates the regeneration of damaged kidney tissues [40]. In experimental systems, BMP-7 recombinant protein expression or overexpression inhibits fibrosis in diabetic nephropathy or AKI, TGF-β-initiated EMT and E-cadherin suppression [41]. BMP-7 exerts an anti-inflammatory effect by inhibiting neutrophil, monocyte and macrophage infiltration and activity, as well as by repressing the expression of the proinflammatory cytokines IL-6 and IL-1β, and the proinflammatory chemokines MCP1 and IL-8 [41].

The epsilon chain is one of the four subunits of the CD3 protein complex that combines participation in the activation of T cells after antigen binding. The CD3 epsilon protein is also expressed in proximal and distal tubules, and Henle loops. The presence of this protein in renal tubules results from the participation in the active transport of sodium or hydrogen ions as sodium or proton pumps [42]. It is also believed that CD3 epsilon protein may be involved in communication of signal transduction, similar to T lymphocytes. Insulin-like growth factor-1 (IGF-1) is a peptide growth factor produced by the collecting duct of the adult kidney, and its receptors are present in glomeruli and on the basolateral membrane of renal proximal tubular cells. The IGF-1R signaling pathway initiates with binding of IGF-1 to its cell-surface receptor IGF-1R to activate phosphatidylinositol-3
kinase (PI3K)/Akt, or extracellular signal-regulated kinase (ERK)/mitogen-Activated Protein Kinase (MAPK) signaling pathway, to stimulate cell growth and proliferation, and to inhibit programmed cell death [43]. Following ischemic injury, renal IGF-1 has been shown to decrease. The administration of exogenous IGF-1 has been shown to accelerate recovery from ischemic acute renal failure, possible through enhanced proliferation and reduced apoptosis of tubular epithelial cells [44]. The presence of insulin in association with the IGF-I pathway demonstrated in our studies may indicate with some probability, that IGFBP-7 could be a potential biomarker of acute kidney injury in dogs with babesiosis. However, the exact mechanisms underlying this process are not completely understood.

Haptoglobin, and acute phase protein, also appeared among 21 proteins identified in the urine of dogs with babesiosis. Although it was not included in the final network of close connections selected by the Panther system, haptoglobin is considered to be one of the valuable markers used in the assessment of the course of babesiosis in dogs. In the other study, dogs with babesiosis had reduced serum level of haptoglobin in relation to normal values [45,46]. In this context, it is worth to ask a question about the correlation between a decreased serum level of haptoglobin and its level in excreted urine.

In this study, protein analysis was performed by mass spectrometry without any pre-treatment of urine. Looking at the gene ontology map derived from the dataset, it appears that urine proteome in dogs with babesiosis is composed of several clusters of proteins. Although our study has a major limitation of using a pooled samples, we believe that our findings complete in some way the description of urinary signs of a clinical conditions of renal tissue in dogs with babesiosis. Verifying their significance in the diagnosis and prognosis of the disease requires further study.

Conclusions

In summary, to the best of our knowledge, this study is the first to comprehensively
analyse the urinary proteome of dogs with babesiosis, demonstrating the association of the identified proteins with this disease, and kidney injury. Urine interleukin-13, bone morphogenetic protein 7, α2(1) collagen and tyrosine-protein kinase Fer are potential biomarkers of kidney injury during babesiosis in dogs that might indicate early renal injury; however, further studies are needed to verify their significance in the diagnosis and prognosis of the disease. Functional analysis of these four proteins indicates that epithelial-mesenchymal transition (EMT) might play an important role in the mechanisms underlying pathological changes in renal tissues during the course of babesiosis.

Methods

5.1 Animals and sample collection

Dogs were enrolled during routine admission to Faculty of Veterinary Medicine clinics at the University of Life Sciences in Lublin. Informed consent was obtained from the owners prior to clinical investigations and sample collection. The studies were reviewed and approved by the Ethics Committee of the University of Life Sciences in Lublin (Poland) No 70/2018. All relevant data for inclusion criteria for dogs and values of urine parameters and urinary biomarkers used in the study have already been published [47]. Briefly, the study involved 20 mixed-breed dogs (10 males, 10 females) weighing 5–8 kg (median, 6.2 kg) and aged 2–7 years (median, 4.35 years) that were divided into two groups. All dogs underwent individual clinical and laboratory tests to determine their health status, and to identify signs of kidney damage, particularly in the diseased group. Group 1 (study group, n = 10; five males and five females) consisted of dogs naturally infected with B. canis, while group 2 (control group, n = 10; five males and five females) consisted of healthy dogs [47]. All dogs in group 1 showed symptoms of babesiosis (apathy, anorexia, changes in urine colour, and pale mucous membranes), and haematology analysis revealed
thrombocytopenia (platelets 12–88 × 10⁹/l) and anaemia (erythrocytes 3.5–5.3 × 10¹²/l.)

All dogs were nonazotemic, and the serum creatinine concentration remained within the reference range. All dogs in this group had *Babesia*-positive blood smears, and infection was additionally confirmed by PCR according to the protocol described in other studies [12,16]. Possible co-infections (borreliosis, anaplasmosis, ehrlichiosis) were excluded in all dogs based on PCR and ELISA results [48]. All dogs in group 1 were successfully treated with imidocarb (5 mg/kg s.c.). Dogs in group 2 were clinically healthy and were referred to the clinic for vaccination purposes. Blood smear analysis and PCR for *B. canis* gave negative results for all animals in group 2. Voided midstream urine samples were collected in the morning before the treatment with imidocarb, and each sample was centrifuged on the day of collection at 500 × g for 10 minutes at 4°C. The supernatants were removed, and protease inhibitors were added (Protease Inhibitor Cocktail, Roche Diagnostic Corp.).

Urine protein and Cr concentration were measured by the enzymatic colorimetric method (BS-130 analyser, Mindray), and basic urinalysis with microscopic sediment analysis was performed on fresh urine samples. Urine specific gravity (USG) was measured using a refractometer. The remaining urine was frozen at −80°C for further analysis. Macroscopic evaluation of urine in group 1 showed yellow to dark brown samples, while all group 2 samples were yellow. Urine protein analysis revealed proteinuria in eight of the 10 group 1 dogs, and eight dogs in this group also had a urine protein/creatinine ratio > 0.5. Urine dipstick analysis showed haemoglobinuria in seven of the 10 group 1 dogs, which was severe (++++) in two dogs. Urine specific gravity was decreased in all diseased dogs, with an average value of 1.015 (Table 3). No dogs in the control group had proteinuria or haemoglobinuria. Statistically higher concentrations of urinary biomarkers (ulG/UCr, uTHP/UCr, and uRBP/UCr) were found in the urine samples from all dogs with babesiosis compared to those from the control animals (p < 0.05), indicating dysfunctional
glomerular and tubular kidney regions (Table 3). For proteomic analysis, 10 individual urine samples (0.5 ml each) from groups 1 (affected dogs) and 2 (healthy dogs) were collected and pooled. Pooled sample was made by mixing the same amount of protein of each tear fluid sample. Each pooled urine sample was subjected to desalting on the filter to enable quick ultrafiltration with a high-density coefficient (Amicon Ultra Merck). Protein concentrations were measured with a microlitre spectrophotometer (NANO), and the urine samples were then prepared and subjected to 2D electrophoresis. Each individual gel spot was then analysed by mass spectrometry with the MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) technique.

Table 3. Urinary parameters of renal function and concentration of urinary markers in dogs with babesiosis (group 1) and healthy dogs (group 2) (expressed as the median and range).

| Variable             | Group 1 (n = 10) | Group 2 (n = 10) | P    |
|----------------------|------------------|------------------|------|
| uCr [mg/dl]          | 58.68 (3.24–120.58) | 135.64 (53.91–212.82) | 0.02 |
| Specific gravity     | 1.015 (1.010–1.030) | 1.030 (1.015–1.045) | 0.02 |
| UPC [mg/mg]          | 2.3 (0.48–6.24) | 0.2 (0.05–0.40) | 0.02 |
| uRBP/uCr [mg/g]      | 24.65 (0.06–76.21) | 0.2 (0.09–0.3) | 0.02 |
| uTHP/uCr [mg/g]      | 1.50 (0.13–7.80) | 0.2 (0.09–0.3) | 0.02 |
| uIgG/uCr [mg/g]      | 120.78 (0-394.31) | 0 | 0.02 |

* Measured using the sulfosalicylic acid (SSA) method

UPC = urine protein-to-creatinine ratio, uRBP = urinary retinol binding protein, uTHP = urinary Tamm-Horsfal protein, uIgG = urinary immunoglobulin G, uCrea = urinary creatinine

5.2 2D electrophoresis

Two-dimensional electrophoresis was used to separate the proteins in the tested urine samples [49]. Preliminary tests showed that the optimum amount of protein for 2D electrophoresis is 85 µg; thus, this amount of protein was broken down via a precipitation and purification kit (ReadyPrep™ 2-D Cleanup Kit, Bio-Rad, Warsaw, Poland). The obtained protein pellets were then dissolved in rehydration buffer, and the resulting solutions were applied to a rehydration plate and covered with 17-cm immobilized pH gradient (IPG)
linear strips for isoelectric focusing (pH 3–10, Bio-Rad). The gel on the strips was soaked with the protein sample, and the strips were removed after a 12-hour rehydration period and then subjected to electrophoresis in the first dimension (IEF-100 Hoefer; 250 V/30 min; 10 000 V/3 hr; 60 kV/hr, with a current limit of 50 µA/strip hr). Under the influence of the electric field, proteins in the strips migrated to the location corresponding to their isoelectric point. After separation, the IPG strips were prepared for electrophoresis in the second dimension to separate the proteins by molecular mass. Vertical electrophoretic separation was performed in 12.5% polyacrylamide gels with the following current parameters: 600 V/30 mA/100 W in an electrophoretic chamber (PROTEAN® II xi, Bio-Rad). The obtained gels were subjected to a standard colouring procedure with silver in the presence of formaldehyde as a regulator. The protein spots were cut out of the gels, decolourized, reduced and alkylated using dithiothreitol and iodoacetamide [50]. Gel pieces containing proteins were subjected to digestion to obtain shorter peptide fragments. Trypsin digestion occurred in 50 mM ammonium bicarbonate buffer at 37°C for 12 hours (Promega, Trypsin Gold, Mass Spectrometry Grade, Technical Bulletin) [51]. The obtained peptides were subsequently eluted from the gel with a water/acetonitrile/TFA solution (v:v 450:500:50). The extracted peptides were purified using C18 Zip-TIP pipette tips according to the manufacturer’s instructions (Merck Chemicals, Billerica, MA, USA, PR 02358, Technical Note) and applied to the MTP AnchorChip 384 plate (Bruker, Bremen, Germany).

5.3 Mass spectrometry

After the protein samples were dried on the MTP AnchorChip 384 plate, the surface was covered with a super-saturated solution of α-cyano-4-hydroxycinnamic acid (HCCA, Bruker), functioning as a matrix mediating energy transmission to the sample. Simultaneously, 0.5 µl of a peptide standard was applied to the calibration fields (Peptide
Calibration Standard II, Bruker), which were also covered with the matrix solution. Spectrometric analysis was performed using an UltrafleXtreme III MALDI-TOF/TOF (Bruker), and flexControl 3.3 (Bruker) software was applied for mass spectra collection. The obtained peptides were subjected to mild ionization using the MALDI-TOF instrument in linear mode within the 900–4000 Da mass scope in reflectron mode. The obtained mass spectra were analysed with flexAnalysis 3.4 (Bruker) software as follows: smoothing (Savitsky-Golay method), baseline subtraction (Top Hat baseline algorithm), and peak geometry (Stanford Network Analysis Platform (SNAP) algorithm). All peaks with a signal to noise ratio > 3 qualified for further analysis. Experimental data were analysed using the abovementioned software to exclude peaks originating from trypsin or environmental pollution. To ensure correct identification, the selection of possible post-translational modifications using BioTools 3.2 (Bruker) was essential. Post-translational modifications are derived from both the methodology and metabolic processes in the patients. The obtained spectra were compared to the Swiss-Prot database restricted to “bony vertebrate” taxa using Mascot 2.2 software with a maximum error of 0.3 Da. The results with Mascot scores above 62 were considered statistically significant (p ≤ 0.05). If this threshold was not reached, the fragment ion spectra of chosen peptides were subjected to fragmentation in tandem spectrometry mode [52,53].

5.4 Bioinformatic analysis

Venn diagrams were used to show differences between gene lists of healthy and diseased dogs where the UniProt accession numbers were used.[54]. By means of this software it was possible to obtain subset of proteins assigned exclusively to healthy dogs, subset of proteins common to both groups and subset of proteins assigned exclusively to dogs with babesiosis.

Then to study the biological pathway networks and functional classification the UniProt
accession numbers of the protein subset assigned to dogs with babesiosis were entered into Panther Classification System [55]. Analysis was carried out selecting Canis lupus familiaris database.

**Abbreviations**

2D: two-dimensional, **MALDI-TOF**: Matrix-assisted laser desorption ionization time of flight, **TGF-β**: transforming growth factor, **AKI**: acute kidney injury, **CKD**: chronic kidney disease, **Cr**: creatinine, **GFR**: glomerular filtration rate, **ulgG**: urinary immunoglobulin G, **uTHP**: urinary TammHorsfall Protein, **uRBP**: urinary retinol-binding protein, **Il13**: interleukin 13, **EMT** epithelial-mesenchymal transition, **ECM**: extracellular matrix, **IGF-I** insulin-like growth factor, **CD3**: cluster of differentiation 3, **USG**: urine specific gravity, **UPC**: urine protein to creatinine ratio

**Declarations**

**Ethics approval and consent to participate**

The protocol was approved by the Animal Ethics Board of the Department an Clinic of Animal Internal Diseases, University of Life Sciences in Lublin.

The dog owners were informed about the methods and purpose of the study and gave their written informed consent.

**Consent for publication**

Not applicable

**Availability of data and material**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors have declared that no competing interests exist.

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Authors’ contributions
Conceived and designed the experiments: DW, MW, SW. DW, MW contributed to sample collection and laboratory analysis. ŁA performed PCR analysis. KM performed electrophoresis procedure and mass spectrometry analysis. All authors participated in the interpretation of results and the preparation of the manuscript.

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Supplementary Tables

Table 1. Proteins identified in urine from healthy dogs.

| Protein name                                      | Score | Mass | Matches | Access no. | Hyperlink                              |
|---------------------------------------------------|-------|------|---------|------------|----------------------------------------|
| Endophilin-A2                                      | 64    | 41.7 | 9       | Q2KJA1     | http://www.uniprot.org/uniprot/Q2KJA1  |
| BTB/POZ domain-containing protein KCTD1            | 66    | 29.7 | 7       | Q719H9     | http://www.uniprot.org/uniprot/Q719H9  |
| Prolyl 3-hydroxylase 3                            | 52    | 82.6 | 11      | Q8IVL6     | http://www.uniprot.org/uniprot/Q8IVL6  |
| Protein Name                                      | Score | Identity | Accession | Link                                      |
|--------------------------------------------------|-------|----------|-----------|-------------------------------------------|
| Essential MCU regulator                          | 54    | 11.5     | Q2M2S2    | http://www.uniprot.org/uniprot/Q2M2S2    |
| C-X-C motif chemokine 3                          | 43    | 11.3     | Q10746    | http://www.uniprot.org/uniprot/Q10746    |
| Desmin                                           | 65    | 53.3     | Q5XFN2    | http://www.uniprot.org/uniprot/Q5XFN2    |
| Uromodulin                                       | 65    | 72.9     | Q862Z3    | http://www.uniprot.org/uniprot/Q862Z3    |
| Heat shock factor-binding protein 1              | 51    | 8.5      | O75506    | http://www.uniprot.org/uniprot/O75506    |
| Phosphoglucomutase-2                             | 45    | 69.9     | Q7TSV4    | http://www.uniprot.org/uniprot/Q7TSV4    |
| Methylmalonyl-CoA mutase, mitochondrial          | 50    | 83.6     | Q9GK13    | http://www.uniprot.org/uniprot/Q9GK13    |
| Histone H1t                                      | 51    | 22.1     | P40286    | http://www.uniprot.org/uniprot/P40286    |
| General transcription factor II-I                | 50    | 110.6    | A7MB80    | http://www.uniprot.org/uniprot/A7MB80    |
| Zinc finger protein 106                          | 68    | 210.8    | O88466    | http://www.uniprot.org/uniprot/O88466    |
| Protein CutA                                     | 76    | 19.2     | O60888    | http://www.uniprot.org/uniprot/O60888    |
| Protein Lines homolog 1                          | 58    | 87.5     | Q8NG48    | http://www.uniprot.org/uniprot/Q8NG48    |
| Dihydropyrimidinase-related protein 1            | 48    | 62.5     | Q14194    | http://www.uniprot.org/uniprot/Q14194    |
| Interleukin-22                                   | 43    | 20.3     | Q9GZX6    | http://www.uniprot.org/uniprot/Q9GZX6    |
| BTB/POZ domain-containing protein KCDT1          | 46    | 29.7     | Q719H9    | http://www.uniprot.org/uniprot/Q719H9    |
| Ribosome-binding protein 1                       | 48    | 164.8    | Q28298    | http://www.uniprot.org/uniprot/Q28298    |
| Glycogen debranching enzyme                      | 43    | 176.9    | Q2PQH8    | http://www.uniprot.org/uniprot/Q2PQH8    |
| 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 | 67    | 54.2     | Q28901    | http://www.uniprot.org/uniprot/Q28901    |
| 60S ribosomal protein L37                        | 58    | 11.3     | P79244    | http://www.uniprot.org/uniprot/P79244    |
| Interleukin-11                                   | 46    | 21.6     | P47873    | http://www.uniprot.org/uniprot/P47873    |
| Vascular cell adhesion protein 1                 | 63    | 82.3     | P19320    | http://www.uniprot.org/uniprot/P19320    |
| Ig heavy chain V region AC38 205.12              | 66    | 13       | P06330    | http://www.uniprot.org/uniprot/P06330    |
| Collagen alpha-1(XXV) chain                     | 88    | 65.1     | Q98X50    | http://www.uniprot.org/uniprot/Q98X50    |
| Sphingosine 1-phosphate receptor 3               | 51    | 43       | Q99500    | http://www.uniprot.org/uniprot/Q99500    |
| Vascular cell adhesion protein 1                 | 51    | 82.3     | P19320    | http://www.uniprot.org/uniprot/P19320    |
| Vacuolar protein sorting-associated protein 4B   | 41    | 49.6     | P46467    | http://www.uniprot.org/uniprot/P46467    |
| Protein Name                                           | Accession | Score | p-value | Uniprot ID                                      |
|--------------------------------------------------------|-----------|-------|---------|-----------------------------------------------|
| SPRY domain-containing protein 7                       | 51        | 22.2  | 5       | Q2T9X3                                        |
| Myoglobin                                              | 48        | 17.3  | 7       | P02185                                        |
| Retinol-binding protein 2                              | 43        | 15.8  | 5       | Q08652                                        |
| Mesenteric estrogen-dependent adipogenesis protein      | 53        | 34.6  | 6       | A4IFN2                                        |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial | 49        | 40.9  | 6       | Q0MQB6                                        |
| Nucleoside diphosphate kinase A                        | 51        | 17.3  | 5       | Q05982                                        |
| PR domain zinc finger protein 12                       | 49        | 40.7  | 6       | A2AJ77                                        |
| Gastric inhibitory polypeptide receptor                | 53        | 54    | 8       | P48546                                        |
| Carbohydrate sulfotransferase 1                        | 54        | 47.5  | 9       | Q9EQC0                                        |
| Ras-specific guanine nucleotide-releasing factor 1      | 50        | 146.3 | 13      | Q13972                                        |
| Coiled-coil domain-containing protein 184              | 39        | 20.7  | 3       | Q52MB2                                        |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4-like 2 | 42        | 10.1  | 3       | Q9NRX3                                        |
| Elongation factor 1-beta                               | 59        | 25    | 7       | Q5E983                                        |
| Uncharacterized protein C12orf60 homolog               | 52        | 28.3  | 10      | Q810N5                                        |
| Serum albumin                                          | 44        | 70.6  | 10      | P49822                                        |
| DNA-binding protein RFX5                               | 52        | 65.7  | 11      | P48382                                        |
| C2 domain-containing protein 3                         | 50        | 262.6 | 15      | Q4AC94                                        |
| Protein deglycase DJ-1                                 | 55        | 20.1  | 6       | Q95LI9                                        |
| Calmodulin-regulated spectrin-associated protein 1      | 54        | 179.9 | 12      | D3Z8E6                                        |
| Zinc finger protein 101                                 | 49        | 51.9  | 7       | Q8IZC7                                        |
| Mini-chromosome maintenance complex-binding protein     | 51        | 73.8  | 7       | Q9BTE3                                        |
| Protein SOX-16 (Fragment)                              | 46        | 6.9   | 5       | Q62247                                        |
| Essential MCU regulator, mitochondrial                 | 45        | 11.5  | 4       | Q2M2S2                                        |
| G protein-coupled                                      | 52        | 62.6  | 7       | Q9Z2G7                                        |
| Protein Name                                      | Accession | Score | Number of Peptides | Uniprot Link                                      |
|--------------------------------------------------|-----------|-------|--------------------|--------------------------------------------------|
| Golgi SNAP receptor complex member 1             | O88441    | 5     | 4                  | http://www.uniprot.org/uniprot/O88441            |
| Metaxin-2                                        | P10649    | 9     | 7                  | http://www.uniprot.org/uniprot/P10649            |
| Glutathione S-transferase Mu 1                   | Q2TBU3    | 7     | 11                 | http://www.uniprot.org/uniprot/Q2TBU3            |
| Calpain-2 catalytic subunit                      | Q27971    | 1     | 9                  | http://www.uniprot.org/uniprot/Q27971            |
| Pleckstrin homology domain-containing family G member 4B | Q96PX9    | 9     | 15                 | http://www.uniprot.org/uniprot/Q96PX9            |
| Proflin-4                                        | Q9D6i3    | 3     | 6                  | http://www.uniprot.org/uniprot/Q9D6i3            |
| EH domain-containing protein 4                   | Q9H223    | 2     | 12                 | http://www.uniprot.org/uniprot/Q9H223            |
| Hepcidin                                         | Q8MJ80    | 3     | 5                  | http://www.uniprot.org/uniprot/Q8MJ80            |
| Glycine receptor subunit beta                    | P48167    | 7     | 5                  | http://www.uniprot.org/uniprot/P48167            |
| Sulfotransferase 4A1                             | P63046    | 6     | 8                  | http://www.uniprot.org/uniprot/P63046            |
| Unconventional myosin-IId                        | O94832    | 2     | 10                 | http://www.uniprot.org/uniprot/O94832            |
| Actin-related protein 2/3 complex subunit 3      | Q3T035    | 5     | 6                  | http://www.uniprot.org/uniprot/Q3T035            |
| Actin-related protein 2/3 complex subunit 3      | Q3T035    | 5     | 6                  | http://www.uniprot.org/uniprot/Q3T035            |
| Autophagy-related protein 16-1                   | Q676U5    | 5     | 7                  | http://www.uniprot.org/uniprot/Q676U5            |
| Carbonic anhydrase 5B, mitochondrial              | Q9Y2D0    | 0     | 5                  | http://www.uniprot.org/uniprot/Q9Y2D0            |
| Putative olfactory receptor 2B3                  | Q76000    | 0     | 4                  | http://www.uniprot.org/uniprot/Q76000            |
| Zinc finger protein 75D                          | P51815    | 5     | 10                 | http://www.uniprot.org/uniprot/P51815            |
| Trafficking protein particle complex subunit 1   | Q17Qi1    | 1     | 6                  | http://www.uniprot.org/uniprot/Q17Qi1            |
| Golgi SNAP receptor complex member 1             | Q2TBU3    | 3     | 9                  | http://www.uniprot.org/uniprot/Q2TBU3            |
| Zinc finger protein 491                          | Q8N8L2    | 2     | 9                  | http://www.uniprot.org/uniprot/Q8N8L2            |
| Cytoskeleton-associated protein 2-like            | A5PK21    | 1     | 12                 | http://www.uniprot.org/uniprot/A5PK21            |
| Retinoic acid receptor RXR-beta (fragment)       | P49743    | 3     | 9                  | http://www.uniprot.org/uniprot/P49743            |
| Apolipoprotein A-II                              | E2RAK7    | 7     | 4                  | http://www.uniprot.org/uniprot/E2RAK7            |
| Tubulin polymerization-promoting protein family member 2 | Q4R3A0 | 0     | 6                  | http://www.uniprot.org/uniprot/Q4R3A0            |
| Bcl-2-like protein 2                             | Q1RMX3    | 3     | 6                  | http://www.uniprot.org/uniprot/Q1RMX3            |
| Mini-chromosome maintenance complex              | A5PJM5    | 5     | 16                 | http://www.uniprot.org/uniprot/A5PJM5            |
| Protein Description | Accession | Mw | pI | Gene ID | URL |
|---------------------|-----------|----|-----|---------|-----|
| Alpha-2,8-sialyltransferase 8F | P61647 | 45.4 | 8 | http://www.uniprot.org/uniprot/P61647 |
| H-2 class I histocompatibility antigen, K-B alpha chain | P01901 | 41.7 | 11 | http://www.uniprot.org/uniprot/P01901 |
| Fibroleukin | Q14314 | 50.8 | 9 | http://www.uniprot.org/uniprot/Q14314 |
| Phosphatidylethanolamine-binding protein 2 | Q8VIN1 | 21.7 | 6 | http://www.uniprot.org/uniprot/Q8VIN1 |
| Zinc finger and SCAN domain-containing protein 5A | Q9BUG6 | 56.9 | 8 | http://www.uniprot.org/uniprot/Q9BUG6 |
| Fructose-1,6-bisphosphatase 1 | Q3SZB7 | 37 | 5 | http://www.uniprot.org/uniprot/Q3SZB7 |
| Beta-defensin 107A | A4H217 | 7.9 | 3 | http://www.uniprot.org/uniprot/A4H217 |
| Golgi SNAP receptor complex member 1 | Q62931 | 28.6 | 10 | http://www.uniprot.org/uniprot/Q62931 |
| Zinc finger protein 624 | Q9P2J8 | 102.5 | 13 | http://www.uniprot.org/uniprot/Q9P2J8 |
| Prelamin-A/C | P48679 | 74.6 | 12 | http://www.uniprot.org/uniprot/P48679 |
| Aspartate-tRNA ligase, cytoplasmic | P15178 | 57.5 | 18 | http://www.uniprot.org/uniprot/P15178 |
| Beta-lactoglobulin | Q29146 | 20.6 | 6 | http://www.uniprot.org/uniprot/Q29146 |
| ATP synthase subunit alpha, mitochondrial | P25705 | 59.8 | 16 | http://www.uniprot.org/uniprot/P25705 |
| RUN and FYVE domain-containing protein 2 | Q8R4C2 | 70.8 | 18 | http://www.uniprot.org/uniprot/Q8R4C2 |
| Pyridine nucleotide-disulfide oxidoreductase domain-containing protein 2 | Q3U4I7 | 63.5 | 10 | http://www.uniprot.org/uniprot/Q3U4I7 |
| Profilin-3 | Q8R4C2 | 15 | 7 | http://www.uniprot.org/uniprot/Q8R4C2 |
| Prolyl 3-hydroxylase 3 | Q8IVL6 | 82.6 | 10 | http://www.uniprot.org/uniprot/Q8IVL6 |
| Tumor susceptibility gene 101 protein | Q99816 | 44.1 | 7 | http://www.uniprot.org/uniprot/Q99816 |
| Vascular cell adhesion protein 1 | P29533 | 82.4 | 10 | http://www.uniprot.org/uniprot/P29533 |
| Ataxin-7 | Q8R4I1 | 93.8 | 8 | http://www.uniprot.org/uniprot/Q8R4I1 |
| Gamma-aminobutyric acid receptor subunit alpha-1 | P08219 | 52.1 | 6 | http://www.uniprot.org/uniprot/P08219 |
| 43 kDa Receptor-associated protein of the synapse | P12672 | 47.6 | 10 | http://www.uniprot.org/uniprot/P12672 |
| Protein-arginine deiminase type-2 | P20717 | 76 | 9 | http://www.uniprot.org/uniprot/P20717 |
| Protein Name                                      | Score | Coverage | Identity | Uniprot ID | UniProt Link                                      |
|--------------------------------------------------|-------|----------|----------|------------|--------------------------------------------------|
| Heat shock factor-binding protein 1              | 53    | 8.6      | 6        | Q9CQZ1     | http://www.uniprot.org/uniprot/Q9CQZ1             |
| Non-homologous end-joining factor 1              | 55    | 34.1     | 7        | Q6AYI4     | http://www.uniprot.org/uniprot/Q6AYI4             |
| Microtubule-associated protein RP/EB family member 1 | 51    | 30.1     | 10       | Q5R7Z5     | http://www.uniprot.org/uniprot/Q5R7Z5             |
| Protein kish-A                                    | 56    | 8.4      | 6        | Q9CR64     | http://www.uniprot.org/uniprot/Q9CR64             |
| Ubiquitin carboxyl-terminal hydrolase 14         | 56    | 56.3     | 9        | P40826     | http://www.uniprot.org/uniprot/P40826             |
| Cap-specific mRNA (nucleoside-2'-O-)-methyltransferase 1 | 64    | 96.6     | 12       | Q9DBC3     | http://www.uniprot.org/uniprot/Q9DBC3             |
| DNA dC->dU-editing enzyme APOBEC-3G              | 65    | 45.9     | 8        | Q694B9     | http://www.uniprot.org/uniprot/Q694B9             |
| Annexin A10                                      | 63    | 37.8     | 8        | Q9UJ72     | http://www.uniprot.org/uniprot/Q9UJ72             |
| Cysteine and glycine-rich protein 2              | 57    | 21.8     | 6        | P97314     | http://www.uniprot.org/uniprot/P97314             |
| Calmodulin-regulated spectrin-associated protein 1 | 50    | 179.9    | 15       | D3Z8E6     | http://www.uniprot.org/uniprot/D3Z8E6             |
| Tyrosine-protein phosphatase non-receptor type 12 | 58    | 87.2     | 7        | P35831     | http://www.uniprot.org/uniprot/P35831             |
| Dual specificity phosphatase DUPD1              | 55    | 24.3     | 6        | P0C595     | http://www.uniprot.org/uniprot/P0C595             |
| Protein cereblon                                 | 53    | 50.1     | 10       | Q5R6Y2     | http://www.uniprot.org/uniprot/Q5R6Y2             |
| Testis-expressed sequence 33 protein            | 53    | 30.8     | 5        | O43247     | http://www.uniprot.org/uniprot/O43247             |
| Complexin-3                                      | 51    | 17.6     | 7        | Q8WVH0     | http://www.uniprot.org/uniprot/Q8WVH0             |
| Plasmalemma vesicle-associated protein           | 61    | 50.6     | 12       | Q9WV78     | http://www.uniprot.org/uniprot/Q9WV78             |
| Calcium/calmodulin-dependent protein kinase II inhibitor 1 | 53    | 8.6      | 4        | A7MBG3     | http://www.uniprot.org/uniprot/A7MBG3             |
| BTB/POZ domain-containing protein KCTD1          | 56    | 29.7     | 6        | Q719H9     | http://www.uniprot.org/uniprot/Q719H9             |
| Threonine synthase-like 2                        | 62    | 54.8     | 6        | Q86YJ6     | http://www.uniprot.org/uniprot/Q86YJ6             |
| Probable tRNA pseudouridine synthase 1           | 65    | 36.6     | 7        | Q5M934     | http://www.uniprot.org/uniprot/Q5M934             |
| Ras-related protein Rab-7a                       | 51    | 23.8     | 8        | P51149     | http://www.uniprot.org/uniprot/P51149             |
| Essential MCU regulator, mitochondrial           | 52    | 11.5     | 4        | Q2M2S2     | http://www.uniprot.org/uniprot/Q2M2S2             |
| Golgi SNAP receptor complex member 1             | 51    | 28.6     | 5        | O88630     | http://www.uniprot.org/uniprot/O88630             |
| Dual specificity                                 | 54    | 25.5     | 8        | Q68J44     | http://www.uniprot.org/uniprot/Q68J44             |
| Protein Name                                          | Accession | Score | E-value | ID       | Link                                                                 |
|-------------------------------------------------------|-----------|-------|---------|----------|----------------------------------------------------------------------|
| Phosphatase DUPD1                                      | 53        | 20.5  | 5       | Q921J2   | http://www.uniprot.org/uniprot/Q921J2                                |
| GTP-binding protein Rheb                               | 60        | 42.4  | 10      | Q9MZU4   | http://www.uniprot.org/uniprot/Q9MZU4                                |
| Radical S-adenosyl methionine domain-containing protein 2 | 50        | 102.5 | 11      | Q9P2J8   | http://www.uniprot.org/uniprot/Q9P2J8                                |
| Zinc finger protein 624                                 | 56        | 320.5 | 16      | P97526   | http://www.uniprot.org/uniprot/P97526                                |
| Neurofibromin                                          | 54        | 121.9 | 13      | Q9ULE4   | http://www.uniprot.org/uniprot/Q9ULE4                                |
| Phosphomannomutase 2                                   | 58        | 28.4  | 6       | Q3SZJ9   | http://www.uniprot.org/uniprot/Q3SZJ9                                |
| Isocitrate dehydrogenase [NADP] cytoplasmic            | 53        | 47    | 6       | P41562   | http://www.uniprot.org/uniprot/P41562                                |
| Beta-1,3-galactosyltransferase 4                        | 44        | 42.9  | 7       | Q5TJE8   | http://www.uniprot.org/uniprot/Q5TJE8                                |
| Transmembrane protein 238                              | 51        | 18.1  | 4       | C9JJ98   | http://www.uniprot.org/uniprot/C9JJ98                                |
| Protein FAM184B                                        | 51        | 25.0  | 7       | Q810F4   | http://www.uniprot.org/uniprot/Q810F4                                |
| Coiled-coil domain-containing protein 136               | 51        | 133.7 | 9       | Q3TVA9   | http://www.uniprot.org/uniprot/Q3TVA9                                |
| Protein KHNYN                                         | 50        | 75.1  | 9       | Q80U38   | http://www.uniprot.org/uniprot/Q80U38                                |
| Retinol-binding protein 2                              | 50        | 15.8  | 4       | Q08652   | http://www.uniprot.org/uniprot/Q08652                                |
| Tetratricopeptide repeat protein 36                     | 72        | 20.7  | 7       | Q3SZV0   | http://www.uniprot.org/uniprot/Q3SZV0                                |
| E3 ubiquitin-protein ligase RNF152                      | 62        | 23.1  | 6       | D2H6Z0   | http://www.uniprot.org/uniprot/D2H6Z0                                |
| Protein RCC2                                           | 50        | 56.8  | 10      | Q9P258   | http://www.uniprot.org/uniprot/Q9P258                                |
| Signal peptidase complex subunit 2                     | 54        | 25.3  | 6       | Q5RAY6   | http://www.uniprot.org/uniprot/Q5RAY6                                |
| Protein myomaker                                       | 50        | 25.1  | 4       | A6NI61   | http://www.uniprot.org/uniprot/A6NI61                                |
| Apoptosis-enhancing nuclease                           | 57        | 37.6  | 9       | Q9CZI9   | http://www.uniprot.org/uniprot/Q9CZI9                                |
| Short-chain specific acyl-CoA dehydrogenase, mitochondrial | 53      | 44.6  | 9       | P16219   | http://www.uniprot.org/uniprot/P16219                                |
| Fanconi anemia group B protein                         | 61        | 99.4  | 12      | Q8NB91   | http://www.uniprot.org/uniprot/Q8NB91                                |
| Dual specificity phosphatase 28                        | 70        | 18.7  | 6       | Q4G0W2   | http://www.uniprot.org/uniprot/Q4G0W2                                |
| Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial | 65    | 50.3  | 12      | Q4R517   | http://www.uniprot.org/uniprot/Q4R517                                |
| Zinc finger and SCAN domain-containing protein 5A      | 51        | 56.9  | 9       | Q9BUG6   | http://www.uniprot.org/uniprot/Q9BUG6                                |
| Protein Name                                                                 | ID   | Score | BLAST | UniProt ID                                      | Link                                      |
|------------------------------------------------------------------------------|------|-------|-------|------------------------------------------------|-------------------------------------------|
| Eukaryotic translation initiation factor 4E-binding protein 1                | 50   | 12.7  | 5     | Q0P5A7                                         | http://www.uniprot.org/uniprot/Q0P5A7     |
| 39S ribosomal protein L30, mitochondrial                                      | 57   | 18.7  | 6     | Q58DV5                                         | http://www.uniprot.org/uniprot/Q58DV5     |
| WAP four-disulfide core domain protein 12                                    | 57   | 12.7  | 4     | A4K2P0                                         | http://www.uniprot.org/uniprot/A4K2P0     |
| UV-stimulated scaffold protein A                                             | 55   | 82.6  | 12    | Q9D479                                         | http://www.uniprot.org/uniprot/Q9D479     |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12               | 52   | 17.1  | 4     | Q9UI09                                         | http://www.uniprot.org/uniprot/Q9UI09     |
| Tribosomal protein L30, mitochondrial                                         | 57   | 18.7  | 6     | Q58DV5                                         | http://www.uniprot.org/uniprot/Q58DV5     |
| WAP four-disulfide core domain protein 12                                    | 57   | 12.7  | 4     | A4K2P0                                         | http://www.uniprot.org/uniprot/A4K2P0     |
| UV-stimulated scaffold protein A                                             | 55   | 82.6  | 12    | Q9D479                                         | http://www.uniprot.org/uniprot/Q9D479     |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12               | 52   | 17.1  | 4     | Q9UI09                                         | http://www.uniprot.org/uniprot/Q9UI09     |
| Tribosomal protein L30, mitochondrial                                         | 57   | 18.7  | 6     | Q58DV5                                         | http://www.uniprot.org/uniprot/Q58DV5     |
| WAP four-disulfide core domain protein 12                                    | 57   | 12.7  | 4     | A4K2P0                                         | http://www.uniprot.org/uniprot/A4K2P0     |
| UV-stimulated scaffold protein A                                             | 55   | 82.6  | 12    | Q9D479                                         | http://www.uniprot.org/uniprot/Q9D479     |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12               | 52   | 17.1  | 4     | Q9UI09                                         | http://www.uniprot.org/uniprot/Q9UI09     |
| Tribosomal protein L30, mitochondrial                                         | 57   | 18.7  | 6     | Q58DV5                                         | http://www.uniprot.org/uniprot/Q58DV5     |
| WAP four-disulfide core domain protein 12                                    | 57   | 12.7  | 4     | A4K2P0                                         | http://www.uniprot.org/uniprot/A4K2P0     |
| UV-stimulated scaffold protein A                                             | 55   | 82.6  | 12    | Q9D479                                         | http://www.uniprot.org/uniprot/Q9D479     |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12               | 52   | 17.1  | 4     | Q9UI09                                         | http://www.uniprot.org/uniprot/Q9UI09     |
| Tribosomal protein L30, mitochondrial                                         | 57   | 18.7  | 6     | Q58DV5                                         | http://www.uniprot.org/uniprot/Q58DV5     |
| WAP four-disulfide core domain protein 12                                    | 57   | 12.7  | 4     | A4K2P0                                         | http://www.uniprot.org/uniprot/A4K2P0     |
| UV-stimulated scaffold protein A                                             | 55   | 82.6  | 12    | Q9D479                                         | http://www.uniprot.org/uniprot/Q9D479     |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12               | 52   | 17.1  | 4     | Q9UI09                                         | http://www.uniprot.org/uniprot/Q9UI09     |
| Protein Name                        | Value1 | Value2 | Value3 | Accession | URL                              |
|------------------------------------|--------|--------|--------|-----------|----------------------------------|
| Retinol-binding protein 4          | 44     | 23.4   | 6      | P27485    | http://www.uniprot.org/uniprot/P27485 |
| BTB/POZ domain-containing protein KCTD1 | 49     | 29.7   | 6      | Q719H9    | http://www.uniprot.org/uniprot/Q719H9 |
| Collagen alpha-2(I) chain          | 45     | 80.9   | 11     | C0HJP6    | http://www.uniprot.org/uniprot/C0HJP6 |
| L-gulonolactone oxidase            | 55     | 51     | 13     | Q8HXW0    | http://www.uniprot.org/uniprot/Q8HXW0 |
| Zinc finger protein 624            | 58     | 102.5  | 12     | Q9P2J8    | http://www.uniprot.org/uniprot/Q9P2J8 |
| Cilia- and flagella-associated protein 52 | 57     | 69.2   | 11     | Q8N1V2    | http://www.uniprot.org/uniprot/Q8N1V2 |
| Autophagy-related protein 16-1      | 53     | 68.9   | 12     | Q676U5    | http://www.uniprot.org/uniprot/Q676U5 |
| IQ domain-containing protein D      | 55     | 51.8   | 13     | Q17QH9    | http://www.uniprot.org/uniprot/Q17QH9 |
| Fibroblast growth factor 12         | 55     | 27.6   | 6      | P61328    | http://www.uniprot.org/uniprot/P61328 |
| Interferon-induced protein with tetratricopeptide repeats 1 | 52     | 52.8   | 12     | Q4R5F5    | http://www.uniprot.org/uniprot/Q4R5F5 |
| Fanconi anemia group B protein      | 50     | 99.4   | 13     | Q8NB91    | http://www.uniprot.org/uniprot/Q8NB91 |
| Protein TMEM155                     | 55     | 14.4   | 5      | Q5R4Y3    | http://www.uniprot.org/uniprot/Q5R4Y3 |
| Transmembrane protein 225           | 58     | 26.3   | 5      | Q6GV28    | http://www.uniprot.org/uniprot/Q6GV28 |
| Telomerase reverse transcriptase    | 65     | 128.6  | 10     | Q14746    | http://www.uniprot.org/uniprot/Q14746 |
| Tetratricopeptide repeat protein 36 | 64     | 20.7   | 9      | Q3SZV0    | http://www.uniprot.org/uniprot/Q3SZV0 |
| Arginine/serine-rich protein 1      | 52     | 33.7   | 8      | Q9BUV0    | http://www.uniprot.org/uniprot/Q9BUV0 |
| V-set and transmembrane domain-containing protein 2B | 61     | 30.4   | 6      | A6NLU5    | http://www.uniprot.org/uniprot/A6NLU5 |
| Ninein                             | 52     | 245.2  | 20     | Q8N4C6    | http://www.uniprot.org/uniprot/Q8N4C6 |
| Electron transfer flavoprotein subunit beta | 51     | 27.9   | 7      | Q68FU3    | http://www.uniprot.org/uniprot/Q68FU3 |
| Coiled-coil domain-containing protein 25 | 53     | 24.6   | 6      | Q86WR0    | http://www.uniprot.org/uniprot/Q86WR0 |
| Protein-arginine deiminase type-2   | 49     | 76     | 8      | P20717    | http://www.uniprot.org/uniprot/P20717 |
| Radical S-adenosyl methionine domain-containing protein 2 | 55     | 42.4   | 8      | Q9MZU4    | http://www.uniprot.org/uniprot/Q9MZU4 |
| Golgi SNAP receptor complex member 1 | 61     | 28.6   | 7      | O88630    | http://www.uniprot.org/uniprot/O88630 |
| BTB/POZ domain-containing protein KCTD1 | 55     | 29.7   | 5      | Q719H9    | http://www.uniprot.org/uniprot/Q719H9 |
| Protein name                                                                 | Score | Mass  | Matches | Access no.   | Hyperlink                        |
|------------------------------------------------------------------------------|-------|-------|---------|--------------|----------------------------------|
| Zinc finger and SCAN domain-containing protein 5A                           | 57    | 56.9  | 9       | Q9BUG6       | http://www.uniprot.org/uniprot/Q9BUG6 |
| Calcium-binding mitochondrial carrier protein SCaMC-3                       | 51    | 52.7  | 9       | Q6GQS1       | http://www.uniprot.org/uniprot/Q6GQS1 |

Table 2. Proteins identified in urine from dogs with babesiosis.

| Protein name                                                                 | Score | Mass  | Matches | Access no.   | Hyperlink                        |
|------------------------------------------------------------------------------|-------|-------|---------|--------------|----------------------------------|
| Lipase member N                                                             | 54    | 45.7  | 8       | Q5VXI9       | http://www.uniprot.org/uniprot/Q5VXI9 |
| Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial               | 57    | 50.3  | 9       | Q4R517       | http://www.uniprot.org/uniprot/Q4R517 |
| TATA box-binding protein-like protein 2                                     | 51    | 39.3  | 5       | Q6SJ95       | http://www.uniprot.org/uniprot/Q6SJ95 |
| Interferon gamma                                                            | 53    | 18.1  | 5       | P01581       | http://www.uniprot.org/uniprot/P01581 |
| m7GpppX diphosphatase                                                       | 57    | 38.8  | 9       | Q96C86       | http://www.uniprot.org/uniprot/Q96C86 |
| Peptidyl-prolyl cis-trans isomerase F, mitochondrial                        | 52    | 22.6  | 6       | P30404       | http://www.uniprot.org/uniprot/P30404 |
| Mast cell carboxypeptidase A                                                | 52    | 48.9  | 6       | P15088       | http://www.uniprot.org/uniprot/P15088 |
| SprT-like domain-containing protein Spartan                                  | 54    | 56.1  | 14      | G3X912       | http://www.uniprot.org/uniprot/G3X912 |
| 39S ribosomal protein L50, mitochondrial                                     | 54    | 18.3  | 9       | Q8VDT9       | http://www.uniprot.org/uniprot/Q8VDT9 |
| Trafficking protein particle complex subunit 1                              | 55    | 17    | 6       | Q5NCF2       | http://www.uniprot.org/uniprot/Q5NCF2 |
| Desmin                                                                       | 36    | 53.3  | 9       | Q5XFN2       | http://www.uniprot.org/uniprot/Q5XFN2 |
| Dysferlin                                                                   | 57    | 240   | 17      | Q9ESD7       | http://www.uniprot.org/uniprot/Q9ESD7 |
| Leucine-rich repeat-containing protein 14 O                                   | 54    | 55.7  | 6       | A5Pjj5       | http://www.uniprot.org/uniprot/A5Pjj5 |
| Coenzyme Q-binding protein COQ10 homolog B, mitochondrial                  | 58    | 28    | 10      | Q5IOI9       | http://www.uniprot.org/uniprot/Q5IOI9 |
| Bactericidal permeability-increasing protein (Fragment)                      | 54    | 49    | 6       | Q28739       | http://www.uniprot.org/uniprot/Q28739 |
| G protein-activated inward                                                   | 51    | 48.3  | 6       | P48548       | http://www.uniprot.org/uniprot/P48548 |
| Protein Name                                      | Accession | EC Value | UniProt ID       | Link                                      |
|--------------------------------------------------|-----------|----------|------------------|-------------------------------------------|
| rectifier potassium channel 4                    | 4         | 8        | P47870           | http://www.uniprot.org/uniprot/P47870     |
| Gamma-aminobutyric acid receptor subunit beta-2  | 56        | 59.3     | Q8N9B4           | http://www.uniprot.org/uniprot/Q8N9B4     |
| Ankyrin repeat domain-containing protein 42      | 53        | 43.6     | Q5T0W9           | http://www.uniprot.org/uniprot/Q5T0W9     |
| Protein FAM83B                                   | 62        | 115.2    | Q9D6F4           | http://www.uniprot.org/uniprot/Q9D6F4     |
| Zinc finger protein 624                          | 61        | 102.5    | Q9P2J8           | http://www.uniprot.org/uniprot/Q9P2J8     |
| Gamma-aminobutyric acid receptor subunit alpha-4 | 62        | 61.3     | Q9P2J8           | http://www.uniprot.org/uniprot/Q9P2J8     |
| Hyaluronan and proteoglycan link protein 2       | 54        | 38.6     | Q9ESM3           | http://www.uniprot.org/uniprot/Q9ESM3     |
| HAUS augmin-like complex subunit 2               | 55        | 23.4     | Q5RE16           | http://www.uniprot.org/uniprot/Q5RE16     |
| Thromboxane-A synthase                           | 54        | 60.7     | P49430           | http://www.uniprot.org/uniprot/P49430     |
| Vimentin                                         | 54        | 53.7     | P20152           | http://www.uniprot.org/uniprot/P20152     |
| TD and POZ domain-containing protein 2           | 51        | 42.2     | Q717B2           | http://www.uniprot.org/uniprot/Q717B2     |
| Kinase suppressor of Ras 2                       | 62        | 110      | Q3UVC0           | http://www.uniprot.org/uniprot/Q3UVC0     |
| Cortxin-2                                        | 55        | 9.2      | Q3URE8           | http://www.uniprot.org/uniprot/Q3URE8     |
| Transmembrane protein 240                        | 53        | 20.3     | Q5SV17           | http://www.uniprot.org/uniprot/Q5SV17     |
| Protein FAM71C                                   | 50        | 27.9     | Q8NEG0           | http://www.uniprot.org/uniprot/Q8NEG0     |
| Carboxylesterase 1E                              | 58        | 61.8     | Q64176           | http://www.uniprot.org/uniprot/Q64176     |
| Cytochrome P450 3A31                             | 67        | 58       | Q70537           | http://www.uniprot.org/uniprot/Q70537     |
| Leucine-rich repeat-containing protein 14        | 61        | 55.3     | Q15048           | http://www.uniprot.org/uniprot/Q15048     |
| Protein phosphatase Slingshot homolog 1          | 66        | 116.5    | Q8WYL5           | http://www.uniprot.org/uniprot/Q8WYL5     |
| Twinkle protein, mitochondrial                   | 53        | 77.6     | Q96RR1           | http://www.uniprot.org/uniprot/Q96RR1     |
| Ubiquitin-conjugating enzyme E2 N                | 53        | 17.2     | Q0P5K3           | http://www.uniprot.org/uniprot/Q0P5K3     |
| Delta-1-pyrroline-5-carboxylate synthase         | 55        | 87.8     | Q9Z110           | http://www.uniprot.org/uniprot/Q9Z110     |
| 40S ribosomal protein S11                        | 35        | 18.6     | Q9XSU4           | http://www.uniprot.org/uniprot/Q9XSU4     |
| Synaptic vesicle membrane protein VAT-1 homolog-like | 61    | 46.2     | Q9HCJ6           | http://www.uniprot.org/uniprot/Q9HCJ6     |
| Glutamyl-tRNA(Gln) amidotransferase subunit C, mitochondrial | 40    | 18       | E2RK33           | http://www.uniprot.org/uniprot/E2RK33     |
| Trafficking protein                              | 63        | 17       | Q5NCF2           | http://www.uniprot.org/uniprot/Q5NCF2     |
| Protein Name                                      | Accession | % MASCOT Score | Database | Link                              |
|--------------------------------------------------|-----------|----------------|----------|-----------------------------------|
| Rab GDP dissociation inhibitor beta              | O97556    | 50.8           | 8        | http://www.uniprot.org/uniprot/O97556 |
| Parvalbumin alpha                                | P20472    | 12.1           | 10       | http://www.uniprot.org/uniprot/P20472 |
| B-cell lymphoma 6 protein homolog                | P41183    | 58.8           | 11       | http://www.uniprot.org/uniprot/P41183 |
| Probable tubulin polyglutamylase TTLL1           | Q5PPI9    | 49.5           | 9        | http://www.uniprot.org/uniprot/Q5PPI9 |
| Neurofilament medium polypeptide                 | P12839    | 95.8           | 7        | http://www.uniprot.org/uniprot/P12839 |
| Suppressor of tumorigenicity 7 protein           | Q07E08    | 67.7           | 9        | http://www.uniprot.org/uniprot/Q07E08 |
| Vesicle transport protein USE1                   | Q9CQ56    | 30.8           | 7        | http://www.uniprot.org/uniprot/Q9CQ56 |
| Protein C12orf4 homolog                          | D4A770    | 54.3           | 8        | http://www.uniprot.org/uniprot/D4A770 |
| Cell death activator CIDE-A                      | O70302    | 24.8           | 6        | http://www.uniprot.org/uniprot/O70302 |
| Tryptophan 5-hydroxylase 2                       | Q2HZ26    | 56.8           | 9        | http://www.uniprot.org/uniprot/Q2HZ26 |
| Kelch-like protein                               | Q2T9Z7    | 70.2           | 8        | http://www.uniprot.org/uniprot/Q2T9Z7 |
| Ubiquitin carboxyl-terminal hydrolase 37         | Q86T82    | 111            | 12       | http://www.uniprot.org/uniprot/Q86T82 |
| Coatamer subunit beta'                           | P35605    | 103.2          | 6        | http://www.uniprot.org/uniprot/P35605 |
| Centrosomal protein of 170 kDa protein B         | Q80U49    | 171.2          | 11       | http://www.uniprot.org/uniprot/Q80U49 |
| Eukaryotic translation initiation factor 4 gamma 2| Q62448    | 102.6          | 15       | http://www.uniprot.org/uniprot/Q62448 |
| Trifunctional enzyme subunit alpha, mitochondrial| Q64428    | 83.3           | 6        | http://www.uniprot.org/uniprot/Q64428 |
| Isovaleryl-CoA dehydrogenase, mitochondrial      | P12007    | 46.9           | 6        | http://www.uniprot.org/uniprot/P12007 |
| Pericentrin                                      | O95613    | 380.6          | 32       | http://www.uniprot.org/uniprot/O95613 |
| Hydroxysteroid dehydrogenase-like protein 2      | A4FUZ6    | 45.5           | 5        | http://www.uniprot.org/uniprot/A4FUZ6 |
| Ras-related protein Rab-34, isoform NARR         | P0DI83    | 21.1           | 4        | http://www.uniprot.org/uniprot/P0DI83 |
| Interferon-induced protein with tetratricopeptide repeats 1| Q4R5F5   | 55.8           | 11       | http://www.uniprot.org/uniprot/Q4R5F5 |
| Cysteine--tRNA ligase, mitochondrial             | Q2KIF8    | 62             | 12       | http://www.uniprot.org/uniprot/Q2KIF8 |
| Ubiquitin carboxyl-terminal hydrolase 37         | F1N5V1    | 111.2          | 13       | http://www.uniprot.org/uniprot/F1N5V1 |
| Interferon regulatory factor 2-binding protein    | Q8IU81    | 62.6           | 8        | http://www.uniprot.org/uniprot/Q8IU81 |
| Protein Name                                                                 | ID  | Mw    | Unit | Uniprot ID                          | Link                                  |
|-----------------------------------------------------------------------------|-----|-------|------|-------------------------------------|---------------------------------------|
| Ubiquitin carboxyl-terminal hydrolase 37                                    | 84  | 111.2 | 14   | F1N5V1                              | http://www.uniprot.org/uniprot/F1N5V1 |
| Centrosomal protein of 152 kDa                                              | 55  | 197.9 | 12   | O94986                              | http://www.uniprot.org/uniprot/O94986 |
| E3 SUMO-protein ligase PIAS2                                                 | 57  | 64.3  | 9    | Q6AZ28                              | http://www.uniprot.org/uniprot/Q6AZ28 |
| ATR-interacting protein                                                     | 61  | 72.4  | 11   | Q9N077                              | http://www.uniprot.org/uniprot/Q9N077 |
| T-cell surface glycoprotein CD3 epsilon chain                               | 54  | 23    | 4    | P27597                              | http://www.uniprot.org/uniprot/P27597 |
| Rab GDP dissociation inhibitor beta                                          | 55  | 50.8  | 10   | O97556                              | http://www.uniprot.org/uniprot/O97556 |
| Elongation factor Tu GTP-binding domain-containing protein 1                | 64  | 127.1 | 16   | Q8C0D5                              | http://www.uniprot.org/uniprot/Q8C0D5 |
| HIV Tat-specific factor 1 homolog                                           | 57  | 86.6  | 12   | Q8BG0C0                             | http://www.uniprot.org/uniprot/Q8BG0C0 |
| Peptidyl-prolyl cis-trans isomerase A                                       | 58  | 18.1  | 5    | Q9TTC6                               | http://www.uniprot.org/uniprot/Q9TTC6 |
| Tektin-4                                                                   | 58  | 51.3  | 7    | Q8WW24                               | http://www.uniprot.org/uniprot/Q8WW24 |
| Sp110 nuclear body protein                                                  | 58  | 79.6  | 9    | Q9HB58                               | http://www.uniprot.org/uniprot/Q9HB58 |
| Rab11 family-interacting protein 5                                           | 57  | 69.9  | 10   | Q8R361                               | http://www.uniprot.org/uniprot/Q8R361 |
| Alkylidihydroxyacetonephosphate synthase, peroxisomal                      | 53  | 73.7  | 8    | O00116                               | http://www.uniprot.org/uniprot/O00116 |
| Fibroblast growth factor 9                                                  | 57  | 23.5  | 4    | P31371                               | http://www.uniprot.org/uniprot/P31371 |
| Lysozyme C                                                                 | 52  | 16.9  | 5    | Q659U0                               | http://www.uniprot.org/uniprot/Q659U0 |
| Phosphoribosyl pyrophosphate synthase-associated protein 2                  | 55  | 41.2  | 7    | O08618                               | http://www.uniprot.org/uniprot/O08618 |
| Zona pellucida sperm-binding protein 3                                       | 60  | 47.1  | 6    | P42098                               | http://www.uniprot.org/uniprot/P42098 |
| Galactoside 2-alpha-L-fucosyltransferase 2                                   | 62  | 39.1  | 8    | O77485                               | http://www.uniprot.org/uniprot/O77485 |
| 39S ribosomal protein L30, mitochondrial                                    | 56  | 18.7  | 7    | Q58DV5                               | http://www.uniprot.org/uniprot/Q58DV5 |
| Desmin                                                                      | 47  | 53.3  | 6    | Q5XFN2                               | http://www.uniprot.org/uniprot/Q5XFN2 |
| Dipeptidyl peptidase 1 (Fragment)                                           | 43  | 50.1  | 4    | O97578                               | http://www.uniprot.org/uniprot/O97578 |
| Vimentin                                                                    | 50  | 53.7  | 8    | P20152                               | http://www.uniprot.org/uniprot/P20152 |
| Serine/threonine-protein kinase 3                                           | 53  | 57.1  | 9    | Q9J10                                | http://www.uniprot.org/uniprot/Q9J10  |
| Neurofilament medium polypeptide                                            | 62  | 95.8  | 12   | P12839                               | http://www.uniprot.org/uniprot/P12839 |
| Rab GDP dissociation                                                        | 62  | 50.8  | 8    | O97556                               | http://www.uniprot.org/uniprot/O97556 |
| Protein Name                                                                 | P | Mw  | Ref  | UniProt ID          | Link                          |
|----------------------------------------------------------------------------|---|------|------|--------------------|-------------------------------|
| Zinc finger protein 624                                                    | 65| 102.5| 10   | Q9P2J8             | http://www.uniprot.org/uniprot/Q9P2J8 |
| Uncharacterized protein KIAA1683 homolog                                  | 64| 87   | 6    | Q8WNU4             | http://www.uniprot.org/uniprot/Q8WNU4 |
| Phosphatidylserine decarboxylase proenzyme                                | 56| 47.7 | 5    | Q58DH2             | http://www.uniprot.org/uniprot/Q58DH2 |
| Probable tubulin polyglutamylase TTLL1                                    | 66| 49.4 | 9    | Q0VC71             | http://www.uniprot.org/uniprot/Q0VC71 |
| ATP synthase subunit d, mitochondrial                                      | 63| 18.7 | 5    | P13620             | http://www.uniprot.org/uniprot/P13620 |
| Breast cancer anti-estrogen resistance protein 3                           | 61| 93.5 | 8    | Q58DL5             | http://www.uniprot.org/uniprot/Q58DL5 |
| Sperm surface protein Sp17                                                 | 61| 17.4 | 4    | Q15506             | http://www.uniprot.org/uniprot/Q15506 |
| Actin-related protein T1                                                   | 55| 42.1 | 6    | Q4R821             | http://www.uniprot.org/uniprot/Q4R821 |
| Inhibitor of nuclear factor kappa-B kinase subunit beta                    | 54| 87.8 | 7    | O88351             | http://www.uniprot.org/uniprot/O88351 |
| Glucosamine-6-phosphate isomerase 2                                        | 53| 31.3 | 5    | Q9CRC9             | http://www.uniprot.org/uniprot/Q9CRC9 |
| E3 ubiquitin-protein ligase TRIM32                                         | 55| 73.5 | 9    | Q13049             | http://www.uniprot.org/uniprot/Q13049 |
| Protein FAM98B                                                             | 56| 45.9 | 6    | Q80VD1             | http://www.uniprot.org/uniprot/Q80VD1 |
| DNA topoisomerase 2-alpha                                                  | 56| 173.5| 11   | Q01320             | http://www.uniprot.org/uniprot/Q01320 |
| Interferon alpha-1/13                                                     | 50| 22.1 | 4    | P01562             | http://www.uniprot.org/uniprot/P01562 |
| Bone morphogenetic protein 7                                               | 56| 21.5 | 6    | P34819             | http://www.uniprot.org/uniprot/P34819 |
| Protein phosphatase Slingshot homolog 1                                   | 60| 116.5| 16   | Q8WYL5             | http://www.uniprot.org/uniprot/Q8WYL5 |
| Serum albumin                                                             | 42| 70.6 | 14   | P49822             | http://www.uniprot.org/uniprot/P49822 |
| Peroxiredoxin-1                                                           | 61| 22.3 | 9    | Q6B4U9             | http://www.uniprot.org/uniprot/Q6B4U9 |
| Zinc finger BED domain-containing protein 5                                | 48| 80.2 | 11   | A4Z944             | http://www.uniprot.org/uniprot/A4Z944 |
| Zinc finger and SCAN domain-containing protein 9                           | 57| 47   | 9    | O15535             | http://www.uniprot.org/uniprot/O15535 |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2             | 69| 11.1 | 7    | Q4R5E2             | http://www.uniprot.org/uniprot/Q4R5E2 |
| Tektin-4                                                                  | 67| 51.3 | 11   | Q8WW24             | http://www.uniprot.org/uniprot/Q8WW24 |
| Elongation factor Tu GTP-binding domain-containing protein 1              | 70| 127.1| 16   | Q8C0D5             | http://www.uniprot.org/uniprot/Q8C0D5 |
| DCC-interacting protein 13-beta                                            | 68| 75   | 7    | Q8NEU8             | http://www.uniprot.org/uniprot/Q8NEU8 |
| Protein Name                                      | Accession No. | Score | Value | PDB ID | Link                        |
|--------------------------------------------------|---------------|-------|-------|--------|-----------------------------|
| Ras-related protein Rab-28                       | Q3SWY9        | 55    | 25    | Q3SWY9 | http://www.uniprot.org/uniprot/Q3SWY9 |
| Translation initiation factor elf-2B subunit delta| Q63186        | 55    | 58.4  | Q63186 | http://www.uniprot.org/uniprot/Q63186 |
| D-beta-hydroxybutyrate dehydrogenase, mitochondrial| P86198        | 62    | 15.2  | P86198 | http://www.uniprot.org/uniprot/P86198 |
| Gap junction alpha-8 protein                      | Q8K4Q9        | 69    | 49.9  | Q8K4Q9 | http://www.uniprot.org/uniprot/Q8K4Q9 |
| Dual specificity phosphatase 28                   | Q4G0W2        | 54    | 18.7  | Q4G0W2 | http://www.uniprot.org/uniprot/Q4G0W2 |
| 39S ribosomal protein L30, mitochondrial           | Q58DV5        | 53    | 18.7  | Q58DV5 | http://www.uniprot.org/uniprot/Q58DV5 |
| Ras-related protein Rab-36                        | O95755        | 61    | 36.8  | O95755 | http://www.uniprot.org/uniprot/O95755 |
| tRNA-dihydrouridine(20) synthase [NAD(P)+]-like   | Q9NX74        | 50    | 55.8  | Q9NX74 | http://www.uniprot.org/uniprot/Q9NX74 |
| AP-3 complex subunit mu-2                         | P53677        | 55    | 47.2  | P53677 | http://www.uniprot.org/uniprot/P53677 |
| OTU domain-containing protein 6B                  | Q8N6M0        | 52    | 34    | Q8N6M0 | http://www.uniprot.org/uniprot/Q8N6M0 |
| A-kinase anchor protein 10, mitochondrial          | O88845        | 57    | 74.1  | O88845 | http://www.uniprot.org/uniprot/O88845 |
| Hemoglobin subunit beta                           | P02073        | 56    | 16.3  | P02073 | http://www.uniprot.org/uniprot/P02073 |
| Single-pass membrane and coiled-coil domain-      | Q95JR4        | 55    | 34.1  | Q95JR4 | http://www.uniprot.org/uniprot/Q95JR4 |
| containing protein 2                              |               |       |       |        |                             |
| Myotrophin                                        | Q3T0F7        | 56    | 13.1  | Q3T0F7 | http://www.uniprot.org/uniprot/Q3T0F7 |
| Zinc finger protein 622                           | Q969S3        | 77    | 54.8  | Q969S3 | http://www.uniprot.org/uniprot/Q969S3 |
| Protein POF1B                                     | Q8WVV4        | 63    | 68.9  | Q8WVV4 | http://www.uniprot.org/uniprot/Q8WVV4 |
| HORMA domain-containing protein 1                 | D3ZWE7        | 57    | 45.4  | D3ZWE7 | http://www.uniprot.org/uniprot/D3ZWE7 |
| Sperm surface protein Sp17                        | Q15506        | 58    | 17.4  | Q15506 | http://www.uniprot.org/uniprot/Q15506 |
| Tyrosine-protein kinase BAZ1B                     | Q9Z277        | 56    | 172.2 | Q9Z277 | http://www.uniprot.org/uniprot/Q9Z277 |
| Nicolin-1                                         | Q861Y6        | 43    | 24.6  | Q861Y6 | http://www.uniprot.org/uniprot/Q861Y6 |
| Parvalbumin alpha                                 | P20472        | 63    | 12.1  | P20472 | http://www.uniprot.org/uniprot/P20472 |
| Vimentin (Fragment)                               | P48670        | 58    | 51.9  | P48670 | http://www.uniprot.org/uniprot/P48670 |
| Protein FAM3C                                     | Q92520        | 52    | 24.9  | Q92520 | http://www.uniprot.org/uniprot/Q92520 |
| Uncharacterized aarF domain-containing protein kinase 5| Q3MIX3  | 53    | 66.3  | Q3MIX3 | http://www.uniprot.org/uniprot/Q3MIX3 |
| Interferon-induced protein with                   | P09914        | 51    | 55.8  | P09914 | http://www.uniprot.org/uniprot/P09914 |
| Protein Name | Accession | pI | MW  | m | Uniprot ID       |
|--------------|-----------|----|-----|---|------------------|
| tetratricopeptide repeats 1 | 50 | 27.4 | 10 | A2T7G9 | http://www.uniprot.org/uniprot/A2T7G9 |
| Oxidoreductase HTATIP2 | 52 | 25.4 | 10 | GSTA1_PIG | http://www.uniprot.org/uniprot/P5178 |
| Glutathione S-transferase alpha M14 | 50 | 18.8 | 8 | B6VH75 | http://www.uniprot.org/uniprot/B6VH75 |
| Sperm acrosome membrane-associated protein 3 | 53 | 27.1 | 6 | Q8C6C7 | http://www.uniprot.org/uniprot/Q8C6C7 |
| Protein FAM204A | 55 | 27.4 | 6 | A2T7G9 | http://www.uniprot.org/uniprot/A2T7G9 |
| Ras-related protein Rab-36 | 55 | 29.4 | 6 | O95755 | http://www.uniprot.org/uniprot/O95755 |
| Neurofibromin | 50 | 323.1 | 24 | Q04690 | http://www.uniprot.org/uniprot/Q04690 |
| Oxidoreductase HTATIP2 | 52 | 27.4 | 6 | A2T7G9 | http://www.uniprot.org/uniprot/A2T7G9 |
| Transmembrane emp24 domain-containing protein 9 | 61 | 27.5 | 6 | Q3T133 | http://www.uniprot.org/uniprot/Q3T133 |
| Centromere protein H | 61 | 28 | 8 | Q3T0L1 | http://www.uniprot.org/uniprot/Q3T0L1 |
| Centrosomal protein of 104 kDa | 72 | 105 | 14 | Q80V31 | http://www.uniprot.org/uniprot/Q80V31 |
| Nicolin-1 | 55 | 24.5 | 6 | Q9BSH3 | http://www.uniprot.org/uniprot/Q9BSH3 |
| Acylphosphatase-2 | 57 | 10.9 | 5 | P35745 | http://www.uniprot.org/uniprot/P35745 |
| Mitofusin-1 | 58 | 84.5 | 9 | Q811U4 | http://www.uniprot.org/uniprot/Q811U4 |
| Ras-related protein Rab-36 | 54 | 36.8 | 9 | O95755 | http://www.uniprot.org/uniprot/O95755 |
| Hippocalcin-like protein 1 | 51 | 22.4 | 7 | P62748 | http://www.uniprot.org/uniprot/P62748 |
| Stanniocalcin-2 | 47 | 34.1 | 9 | Q5RAT2 | http://www.uniprot.org/uniprot/Q5RAT2 |
| Major vault protein | 51 | 96.2 | 14 | Q9EQK5 | http://www.uniprot.org/uniprot/Q9EQK5 |
| Peptidyl-prolyl cis-trans isomerase FKBP1A | 59 | 12 | 4 | Q62658 | http://www.uniprot.org/uniprot/Q62658 |
| Tyrosine-protein phosphatase non-receptor type 12 | 54 | 87.2 | 17 | P35831 | http://www.uniprot.org/uniprot/P35831 |
| Kinase suppressor of Ras 2 | 52 | 108.9 | 16 | Q6VAB6 | http://www.uniprot.org/uniprot/Q6VAB6 |
| Putative ATP-dependent RNA helicase DHX30 | 54 | 136.9 | 10 | Q2NKY8 | http://www.uniprot.org/uniprot/Q2NKY8 |
| U3 small nucleolar RNA-associated protein 14 homolog A | 53 | 88.2 | 10 | Q3T0Q8 | http://www.uniprot.org/uniprot/Q3T0Q8 |
| 395 ribosomal protein L30, mitochondrial | 64 | 18.7 | 7 | Q58DV5 | http://www.uniprot.org/uniprot/Q58DV5 |
| Growth arrest and DNA damage-inducible proteins-interacting protein 1 | 63 | 25.9 | 8 | Q9CR59 | http://www.uniprot.org/uniprot/Q9CR59 |
| Protein FAM57B | 66 | 31.2 | 7 | Q71RH2 | http://www.uniprot.org/uniprot/Q71RH2 |
| Gene Name                                  | Accession Number | Description                                           | Score | E Value | Uniprot ID       |
|--------------------------------------------|------------------|-------------------------------------------------------|-------|----------|------------------|
| Ras-related protein Rab-9A                 | P24408           | Probable tubulin polycystatamylase TTLL1             | 2     |          | http://www.uniprot.org/uniprot/P24408 |
| Adenyl cyclase-associated protein 2         | Q9CYT6           | Collagen alpha-1(I) chain (Fragments)                | 4     | 8        | http://www.uniprot.org/uniprot/Q9CYT6 |
| NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial | Q0MQI4           | Rho GTPase-activating protein 18                      | 9     | 5        | http://www.uniprot.org/uniprot/Q0MQI4 |
| Unconventional myosin-Ie                   | Q6NSZ9           | Zinc finger and SCAN domain-containing protein 25    | 5     | 8        | http://www.uniprot.org/uniprot/Q6NSZ9 |
| Four and a half LIM domains protein 2       | O35115           | Putative uncharacterized protein MYH16              | 5     |          | http://www.uniprot.org/uniprot/O35115 |
| EGF domain-specific O-linked N-acetylglucosamine transferase | A0JND3           | Annexin A10                                          | 6     |          | http://www.uniprot.org/uniprot/A0JND3 |
| Tripartite motif-containing protein 42      | Q8IWZ5           | Beta-crystallin B2                                   | 4     |          | http://www.uniprot.org/uniprot/Q8IWZ5 |
| Syndecan-4                                 | O35988           | Sorting nexin-3                                      | 6     |          | http://www.uniprot.org/uniprot/O35988 |
| OTU domain-containing protein 6B            | Q8N6M0           | Desmin                                               | 7     |          | http://www.uniprot.org/uniprot/Q8N6M0 |
| Cytospin-A                                 | Q2KNA0           | Ribosome-binding protein 1                            | 10    |          | http://www.uniprot.org/uniprot/Q2KNA0 |
| OTU domain-containing protein 6B            | Q8N6M0           | Glutathione S-transferase alpha M14                  | 6     |          | http://www.uniprot.org/uniprot/Q8N6M0 |
| Ribosome-binding protein 1                 | Q28298           | Zinc finger protein castor homolog 1                 | 13    |          | http://www.uniprot.org/uniprot/Q28298 |
| Succinate                                  | Q9CQA3           | Annexin A10                                          | 6     |          | http://www.uniprot.org/uniprot/Q9CQA3 |
| Protein Name                                                                 | pI   | MW  | %I  | UniProt ID                  | Link                                   |
|------------------------------------------------------------------------------|------|-----|-----|----------------------------|----------------------------------------|
| Dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial               | A3   |     |     |                            |                                        |
| Coiled-coil domain-containing protein 122                                    | 60   | 32.4| 7   | Q5T0U0                     | http://www.uniprot.org/uniprot/Q5T0U0  |
| Zinc finger C2HC domain-containing protein 1C                                 | 58   | 61.1| 15  | Q9BGW4                     | http://www.uniprot.org/uniprot/Q9BGW4  |
| Ceramide synthase 3                                                          | 58   | 61.1| 8   | Q8IU89                     | http://www.uniprot.org/uniprot/Q8IU89  |
| Tetratricopeptide repeat protein 36                                          | 55   | 20.7| 8   | Q3SZV0                     | http://www.uniprot.org/uniprot/Q3SZV0  |
| Ras-related protein Rab-17                                                   | 51   | 23.7| 9   | Q9H0T7                     | http://www.uniprot.org/uniprot/Q9H0T7  |
| Transmembrane and coiled-coil domain-containing protein 5A                   | 52   | 34.5| 5   | Q8N6Q1                     | http://www.uniprot.org/uniprot/Q8N6Q1  |
| Inosine triphosphate pyrophosphatase                                         | 56   | 21.8| 6   | Q9BY32                     | http://www.uniprot.org/uniprot/Q9BY32  |
| Vacuolar protein sorting-associated protein 29                               | 51   | 20.7| 6   | Q9UBQ0                     | http://www.uniprot.org/uniprot/Q9UBQ0  |
| AP-3 complex subunit mu-2                                                    | 66   | 47.2| 8   | P53677                     | http://www.uniprot.org/uniprot/P53677  |
| Ras and EF-hand domain-containing protein homolog                            | 60   | 71.3| 10  | Q5RI75                     | http://www.uniprot.org/uniprot/Q5RI75  |
| Protein C19orf12 homolog                                                     | 65   | 15.1| 6   | Q8WUR0                     | http://www.uniprot.org/uniprot/Q8WUR0  |
| Serine palmitoyltransferase 2                                                | 54   | 63.6| 9   | O15270                     | http://www.uniprot.org/uniprot/O15270  |
| Thioredoxin, mitochondrial                                                  | 53   | 18.4| 4   | P97493                     | http://www.uniprot.org/uniprot/P97493  |
| Vesicle transport protein USE1                                              | 57   | 30.8| 9   | Q9CQ56                     | http://www.uniprot.org/uniprot/Q9CQ56  |
| Far upstream element-binding protein 3 O                                     | 55   | 61.9| 7   | Q96I24                     | http://www.uniprot.org/uniprot/Q96I24  |
| Nesprin-3                                                                    | 51   | 112.3| 20 | Q4FZC9                     | http://www.uniprot.org/uniprot/Q4FZC9  |
| Zinc finger protein 532                                                       | 50   | 112.2| 15 | Q6NXK2                     | http://www.uniprot.org/uniprot/Q6NXK2  |
| Hemoglobin subunit epsilon (Fragment)                                        | 50   | 15.6| 6   | O13071                     | http://www.uniprot.org/uniprot/O13071  |
| Vimentin                                                                     | 60   | 53.7| 15  | P20152                     | http://www.uniprot.org/uniprot/P20152  |
| Centromere protein H                                                         | 61   | 28  | 7   | Q3T0L1                     | http://www.uniprot.org/uniprot/Q3T0L1  |
| Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1 | 60   | 93.4| 11  | Q9MZS1                     | http://www.uniprot.org/uniprot/Q9MZS1  |
| Pleckstrin homology-like domain family A member 3                             | 59   | 13.9| 5   | Q9WV95                     | http://www.uniprot.org/uniprot/Q9WV95  |
| Zinc finger and SCAN                                                         | 71   | 56.9| 11  | Q98UG6                     | http://www.uniprot.org/uniprot/Q98UG6  |
| Domain-Containing Protein                  | pI     | Mw   | Description                                | UniProt ID          |
|------------------------------------------|--------|------|--------------------------------------------|---------------------|
| Signal recognition particle receptor subunit beta | 29.7   | 7    | Q4FZX7                                    | http://www.uniprot.org/uniprot/Q4FZX7 |
| Tetratricopeptide repeat protein 6       | 60     | 8    | Q86TZ1                                    | http://www.uniprot.org/uniprot/Q86TZ1  |
| Protein phosphatase 1 regulatory subunit beta | 47.7   | 8    | D3Z0R2                                    | http://www.uniprot.org/uniprot/D3Z0R2 |
| Oculomedin                               | 5.3    | 4    | Q9Y5M6                                    | http://www.uniprot.org/uniprot/Q9Y5M6 |
| Interleukin-2                            | 17.8   | 6    | Q95KP3                                    | http://www.uniprot.org/uniprot/Q95KP3  |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2 | 11     | 5    | O43678                                    | http://www.uniprot.org/uniprot/O43678  |
| Ras-related protein Rab-36              | 36.8   | 7    | O95755                                    | http://www.uniprot.org/uniprot/O95755  |
| ATP-dependent 6-phosphofructokinase, liver type | 58.8   | 10   | P17858                                    | http://www.uniprot.org/uniprot/P17858  |
| AarF domain-containing protein kinase 4  | 59.6   | 6    | Q6AY19                                    | http://www.uniprot.org/uniprot/Q6AY19  |
| Fidgetin-like protein 1                  | 74.8   | 12   | Q6PIW4                                    | http://www.uniprot.org/uniprot/Q6PIW4  |
| Neutrophil cytosol factor 4              | 39.1   | 8    | Q15080                                    | http://www.uniprot.org/uniprot/Q15080  |
| Glycine amidinotransferase, mitochondrial | 48.8   | 4    | Q9D964                                    | http://www.uniprot.org/uniprot/Q9D964  |
| Uncharacterized protein C1orf168 homolog | 82.8   | 9    | A2A995                                    | http://www.uniprot.org/uniprot/A2A995  |
| Haptoglobin                              | 36.9   | 5    | P19006                                    | http://www.uniprot.org/uniprot/P19006  |
| F-box/LRR-repeat protein 8               | 41.4   | 5    | Q96CD0                                    | http://www.uniprot.org/uniprot/Q96CD0  |
| Desmin                                   | 53.3   | 9    | Q5XFN2                                    | http://www.uniprot.org/uniprot/Q5XFN2  |
| Torsin-4A                                | 47.3   | 6    | Q9NXH8                                    | http://www.uniprot.org/uniprot/Q9NXH8  |
| OTU domain-containing protein 6B         | 34     | 6    | Q8N6M0                                    | http://www.uniprot.org/uniprot/Q8N6M0  |
| 39S ribosomal protein L2, mitochondrial   | 33.5   | 9    | Q2TA12                                    | http://www.uniprot.org/uniprot/Q2TA12  |
| Telomerase reverse transcriptase         | 128.6  | 8    | O14746                                    | http://www.uniprot.org/uniprot/O14746  |
| Solute carrier family 15 member 1        | 79.3   | 5    | Q8WMX5                                    | http://www.uniprot.org/uniprot/Q8WMX5  |
| Tripartite motif-containing protein 75    | 54.4   | 7    | Q3UWZ0                                    | http://www.uniprot.org/uniprot/Q3UWZ0  |
| Probable ATP-dependent RNA helicase DDX28 | 59.8   | 6    | Q9NUL7                                    | http://www.uniprot.org/uniprot/Q9NUL7  |
| Glutamyl-tRNA(Gln) amidotransferase      | 17.6   | 4    | E2RK33                                    | http://www.uniprot.org/uniprot/E2RK33  |
| Protein                          | Accession | Fold | Domain |
|---------------------------------|-----------|------|--------|
| Cap-specific mRNA (nucleoside-2'-O-)-methyltransferase 1 | Q9DBC3    | 96.6 | 12     |
| Protein MB21D2                  | Q8C525    | 49   | 8      |
| Desmin                          | Q5XFN2    | 53.3 | 3      |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2 | O43678    | 11   | 5      |
| Oculomedin                      | Q9Y5M6    | 5.3  | 4      |
| Glycogen phosphorylase, brain form | Q5MIB6    | 96.9 | 12     |
| V-set and transmembrane domain-containing protein 2B | Q9JME9    | 30.3 | 5      |
| Microsomal triglyceride transfer protein large subunit | P55156    | 99.6 | 11     |
| E3 ubiquitin-protein ligase ARHI2 | Q9Z1K6    | 59.3 | 8      |
| Ubiquitin carboxyl-terminal hydrolase 37 | Q86T82    | 111  | 6      |
| Putative homeodomain transcription factor 1 | Q9UMS5    | 88   | 9      |
| Junction plakoglobin            | P14923    | 82.4 | 6      |
| Pericentriolar material 1 protein | Q15154    | 230  | 10     |
| 43 kDa receptor-associated protein of the synapse | P12672    | 47.6 | 13     |
| Desmin                          | Q5XFN2    | 53.3 | 6      |
| Probable tubulin polyglutamylase TTLL1 | O95922    | 49.4 | 10     |
| Ubiquitin carboxyl-terminal hydrolase 48 | Q86UV5    | 121.1| 8      |
| Parvalbumin alpha               | P20472    | 12.1 | 7      |
| Putative fatty acid-binding protein 5-like protein 3 | A8MUU1    | 11.5 | 6      |
| Tyrosine-protein phosphatase non-receptor type 6 | P81718    | 70   | 11     |
| Zinc finger protein 624         | Q9P2J8    | 102.5| 13     |
| Zinc finger and SCAN domain-containing protein 5A | Q9BUG6    | 56.9 | 11     |
| Actin-related protein 2/3       | Q3T035    | 20.8 | 7      |
| Complex Subunit | UniProt ID | Cytoskeleton | UniProt ID | Cytoskeleton |
|-----------------|------------|--------------|------------|--------------|
| FSD1-like protein | P06830 | 61 | 22.3 | 7 |
| Transcriptional repressor NF-X1 | Q12986 | 53 | 130 | 10 |
| Phosphatidylinositol 4-phosphate 5-kinase type-1 alpha | P70182 | 62 | 60.8 | 12 |
| Thiamine-triphosphatase | Q9BU02 | 54 | 25.7 | 9 |
| Dual specificity phosphatase 28 | Q4G0W2 | 52 | 18.7 | 7 |
| Peptidyl-prolyl cis-trans isomerase D | Q9CR16 | 57 | 41.1 | 8 |
| Artemin | Q6AYE8 | 51 | 24.2 | 6 |
| Transcriptional repressor NF-X1 | P70182 | 53 | 130 | 10 |
| Tyrosine-protein kinase receptor TYRO3 | P55144 | 52 | 97.2 | 8 |
| Ras-related protein Rab-17 | Q9HOT7 | 53 | 23.7 | 5 |
| Zinc finger and SCAN domain-containing protein 5A | Q9BUG6 | 77 | 56.9 | 7 |
| SLAIN motif-containing protein 2 | Q8CI08 | 50 | 62.6 | 7 |
| Adenylyl cyclase-associated protein 2 | P52481 | 52 | 53.2 | 7 |
| Acylphosphatase-2 | P00818 | 51 | 11.2 | 6 |
| Protein Name                                                                 | Mw   | PPI | Uniprot ID            | Link                                              |
|------------------------------------------------------------------------------|------|-----|-----------------------|---------------------------------------------------|
| Centriolar coiled-coil protein of 110 kDa                                    | 51   | 11.9| Q7TSH4                | http://www.uniprot.org/uniprot/Q7TSH4             |
| Zinc finger and SCAN domain-containing protein 5A                            | 51   | 56.9| Q9BUG6                | http://www.uniprot.org/uniprot/Q9BUG6             |
| Fibronectin type 3 and ankyrin repeat domains protein 1                       | 54   | 38.6| Q6B858                | http://www.uniprot.org/uniprot/Q6B858             |
| Hemoglobin subunit beta                                                       | 60   | 16.3| Q6B858                | http://www.uniprot.org/uniprot/Q6B858             |
| Ankyrin repeat and death domain-containing protein 1A                        | 51   | 57.9| Q495B1                | http://www.uniprot.org/uniprot/Q495B1             |
| Protein-arginine deiminase type-4                                             | 63   | 75.1| Q9Z183                | http://www.uniprot.org/uniprot/Q9Z183             |
| Actin-binding Rho-activating protein                                          | 60   | 43  | Q8BUZ1                | http://www.uniprot.org/uniprot/Q8BUZ1             |
| Myotrophin                                                                   | 51   | 13.1| Q3T0F7                | http://www.uniprot.org/uniprot/Q3T0F7             |
| Ras GTPase-activating protein 2                                               | 52   | 97.8| P58069                | http://www.uniprot.org/uniprot/P58069             |
| Cleavage and polyadenylation specificity factor subunit 1                    | 54   | 162.4| Q10569               | http://www.uniprot.org/uniprot/Q10569             |
| Cholecystokinin                                                              | 55   | 12.9| P41520                | http://www.uniprot.org/uniprot/P41520             |
| Cortexin-2                                                                   | 55   | 9.2  | Q3URE8                | http://www.uniprot.org/uniprot/Q3URE8             |
| Uncharacterized protein C12orf60                                             | 53   | 27.7| Q5U649                | http://www.uniprot.org/uniprot/Q5U649             |
| Tettraticopeptide repeat protein 36                                           | 66   | 20.7| Q3SZV0                | http://www.uniprot.org/uniprot/Q3SZV0             |
| Coiled-coil domain-containing protein 81                                      | 64   | 76.8| Q6ZN84                | http://www.uniprot.org/uniprot/Q6ZN84             |
| Tyrosine-protein kinase Fer                                                   | 63   | 95.1| Q9TTY2                | http://www.uniprot.org/uniprot/Q9TTY2             |
| Tettraticopeptide repeat protein 36                                           | 59   | 20.7| Q3SZV0                | http://www.uniprot.org/uniprot/Q3SZV0             |
| Ras-related protein Rab-36                                                    | 64   | 36.8| O95755                | http://www.uniprot.org/uniprot/O95755             |
| Rab GDP dissociation inhibitor beta                                           | 66   | 50.8| O97556                | http://www.uniprot.org/uniprot/O97556             |
| Centromere/kinetochore protein zw10 homolog                                  | 63   | 89.6| O43264                | http://www.uniprot.org/uniprot/O43264             |
| Parvalbumin alpha                                                            | 62   | 12.1| P20472                | http://www.uniprot.org/uniprot/P20472             |
| Complement C1q subcomponent subunit B                                         | 62   | 26.6| Q2KIV9                | http://www.uniprot.org/uniprot/Q2KIV9             |
| Double-stranded RNA-binding protein Staufen homolog 2                        | 68   | 62.8| Q9NUL3                | http://www.uniprot.org/uniprot/Q9NUL3             |
| CD209 antigen-like protein B                                                  | 66   | 37.7| Q8CJ91                | http://www.uniprot.org/uniprot/Q8CJ91             |
| SRC kinase signaling inhibitor 1 | 66 | 112.7 | 11 | Q9C0H9 | [http://www.uniprot.org/uniprot/Q9C0H9](http://www.uniprot.org/uniprot/Q9C0H9) |
| Oxidoreductase HTATIP2 | 72 | 27.4 | 7 | A2T7G9 | [http://www.uniprot.org/uniprot/A2T7G9](http://www.uniprot.org/uniprot/A2T7G9) |
| Tyrosine--tRNA ligase, cytoplasmic | 51 | 59.4 | 12 | Q4KM49 | [http://www.uniprot.org/uniprot/Q4KM49](http://www.uniprot.org/uniprot/Q4KM49) |
| Zinc finger protein 101 | 50 | 51.9 | 10 | Q8IZC7 | [http://www.uniprot.org/uniprot/Q8IZC7](http://www.uniprot.org/uniprot/Q8IZC7) |
| 60S ribosomal protein L37 | 53 | 11.3 | 7 | P79244 | [http://www.uniprot.org/uniprot/P79244](http://www.uniprot.org/uniprot/P79244) |
| Zinc finger protein 621 | 52 | 50.2 | 6 | Q6ZSS3 | [http://www.uniprot.org/uniprot/Q6ZSS3](http://www.uniprot.org/uniprot/Q6ZSS3) |
| E3 ubiquitin-protein ligase RNF152 | 50 | 23.1 | 5 | D2H6Z0 | [http://www.uniprot.org/uniprot/D2H6Z0](http://www.uniprot.org/uniprot/D2H6Z0) |
| Ras-related protein Rab-36 | 68 | 36.8 | 9 | Q95755 | [http://www.uniprot.org/uniprot/Q95755](http://www.uniprot.org/uniprot/Q95755) |
| Prolactin | 64 | 26.1 | 7 | Q9QZL1 | [http://www.uniprot.org/uniprot/Q9QZL1](http://www.uniprot.org/uniprot/Q9QZL1) |
| Vacuolar ATPase assembly integral membrane protein Vma21 | 50 | 44.4 | 4 | Q78T54 | [http://www.uniprot.org/uniprot/Q78T54](http://www.uniprot.org/uniprot/Q78T54) |
| Hemoglobin subunit beta | 69 | 16.3 | 10 | P02073 | [http://www.uniprot.org/uniprot/P02073](http://www.uniprot.org/uniprot/P02073) |
| Sterile alpha and TIR motif-containing protein 1 | 60 | 80.8 | 12 | I3L5V6 | [http://www.uniprot.org/uniprot/I3L5V6](http://www.uniprot.org/uniprot/I3L5V6) |
| 39S ribosomal protein L50, mitochondrial | 62 | 18.3 | 6 | Q8VDT9 | [http://www.uniprot.org/uniprot/Q8VDT9](http://www.uniprot.org/uniprot/Q8VDT9) |
| Interferon regulatory factor 2-binding protein 1 | 66 | 62.6 | 14 | Q8IU81 | [http://www.uniprot.org/uniprot/Q8IU81](http://www.uniprot.org/uniprot/Q8IU81) |
| FUN14 domain-containing protein 2 | 50 | 16.6 | 5 | Q9D6K8 | [http://www.uniprot.org/uniprot/Q9D6K8](http://www.uniprot.org/uniprot/Q9D6K8) |
| Potassium voltage-gated channel subfamily B member 1 | 62 | 96.7 | 13 | Q14721 | [http://www.uniprot.org/uniprot/Q14721](http://www.uniprot.org/uniprot/Q14721) |
| Collagen alpha-2(I) chain | 64 | 129.8 | 9 | O46392 | [http://www.uniprot.org/uniprot/O46392](http://www.uniprot.org/uniprot/O46392) |
| Protein AAR2 homolog | 61 | 43.9 | 9 | Q08DJ7 | [http://www.uniprot.org/uniprot/Q08DJ7](http://www.uniprot.org/uniprot/Q08DJ7) |
| Aurora kinase C | 65 | 35.9 | 13 | Q9UQB9 | [http://www.uniprot.org/uniprot/Q9UQB9](http://www.uniprot.org/uniprot/Q9UQB9) |
| NAD-dependent protein deacetylase sirtuin-7 | 58 | 45.7 | 14 | Q8BKJ9 | [http://www.uniprot.org/uniprot/Q8BKJ9](http://www.uniprot.org/uniprot/Q8BKJ9) |
| Peroxiredoxin-1 | 56 | 22.3 | 9 | Q06830 | [http://www.uniprot.org/uniprot/Q06830](http://www.uniprot.org/uniprot/Q06830) |
| Perilipin-3 | 52 | 47.1 | 7 | Q5RAV8 | [http://www.uniprot.org/uniprot/Q5RAV8](http://www.uniprot.org/uniprot/Q5RAV8) |
| Protein kish-A | 52 | 8.3 | 4 | Q148I3 | [http://www.uniprot.org/uniprot/Q148I3](http://www.uniprot.org/uniprot/Q148I3) |
| Ras-related protein Rab-36 | 50 | 36.8 | 6 | Q95755 | [http://www.uniprot.org/uniprot/Q95755](http://www.uniprot.org/uniprot/Q95755) |
| Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial | 62 | 50.3 | 12 | Q4R517 | [http://www.uniprot.org/uniprot/Q4R517](http://www.uniprot.org/uniprot/Q4R517) |
| Protein Name                                      | PDB  | MW (kDa) | ID   | Link                                           |
|--------------------------------------------------|------|----------|------|------------------------------------------------|
| Glycogen phosphorylase, liver form               | 51   | 97.9     | 12   | P09811 http://www.uniprot.org/uniprot/P09811   |
| Integrin alpha-11                                | 50   | 134.1    | 13   | P61622 http://www.uniprot.org/uniprot/P61622   |
| Neuropathy target esterase                       | 63   | 150.9    | 17   | Q3TRM4 http://www.uniprot.org/uniprot/Q3TRM4   |
| Oncostatin-M                                     | 61   | 28.8     | 9    | P13725 http://www.uniprot.org/uniprot/P13725   |
| Vesicle transport protein USE1                   | 63   | 30.8     | 9    | Q9CQ56 http://www.uniprot.org/uniprot/Q9CQ56   |
| Testicular haploid expressed gene protein        | 50   | 43.8     | 8    | Q5XHX8 http://www.uniprot.org/uniprot/Q5XHX8   |
| Serine/threonine-protein phosphatase 6 catalytic subunit | 55   | 35.8     | 7    | O00743 http://www.uniprot.org/uniprot/O00743   |
| E3 ubiquitin-protein ligase MARCH8               | 53   | 33.7     | 5    | Q0VD59 http://www.uniprot.org/uniprot/Q0VD59   |
| Phospholipase A2                                 | 25   | 17.0     | 3    | P06596 http://www.uniprot.org/uniprot/P06596   |
| Protein polyglycylase TERTL10                    | 55   | 80.2     | 13   | A4Q9F3 http://www.uniprot.org/uniprot/A4Q9F3   |
| T-complex protein 1 subunit gamma                | 58   | 61.1     | 13   | Q3TOK2 http://www.uniprot.org/uniprot/Q3TOK2   |
| Vimentin (Fragment)                              | 57   | 51.9     | 14   | P48670 http://www.uniprot.org/uniprot/P48670   |
| Ras-related protein Rab-36                       | 61   | 36.8     | 9    | O95755 http://www.uniprot.org/uniprot/O95755   |
| Interferon-induced protein with tetratricopeptide repeats 1 | 54   | 55.8     | 10   | Q4R5F5 http://www.uniprot.org/uniprot/Q4R5F5   |
| Uncharacterized protein C1orf186                 | 52   | 19.6     | 4    | Q6ZWK4 http://www.uniprot.org/uniprot/Q6ZWK4   |
| Isocitrate dehydrogenase [NADP] cytoplasmic      | 51   | 47.1     | 7    | Q9XSG3 http://www.uniprot.org/uniprot/Q9XSG3   |
| Dual specificity phosphatase                     | 51   | 18.7     | 5    | Q4G0W2 http://www.uniprot.org/uniprot/Q4G0W2   |
| Methylmalonic aciduria type A protein, mitochondrial | 50   | 46.9     | 6    | Q8IVH4 http://www.uniprot.org/uniprot/Q8IVH4   |
| Rab GDP dissociation inhibitor beta              | 47   | 50.8     | 8    | O97556 http://www.uniprot.org/uniprot/O97556   |
| Vacuolar protein sorting-associated protein 4B   | 62   | 49.5     | 14   | Q5R658 http://www.uniprot.org/uniprot/Q5R658   |
| C-type natriuretic peptide                      | 66   | 13.5     | 9    | P56283 http://www.uniprot.org/uniprot/P56283   |
| T-complex protein 1 subunit alpha                | 64   | 60.8     | 14   | P18279 http://www.uniprot.org/uniprot/P18279   |
| Carboxylesterase 1E                              | 67   | 61.8     | 11   | Q64176 http://www.uniprot.org/uniprot/Q64176   |
| Stefin-2                                         | 52   | 11.9     | 6    | P35174 http://www.uniprot.org/uniprot/P35174   |
| Ras-related protein Rab-5                       | 55   | 24.5     | 6    | P59279 http://www.uniprot.org/uniprot/P59279   |
| Protein Name | Protein ID | M.wt | Spec. | SwissProt ID | Link |
|--------------|------------|------|-------|--------------|------|
| Hemoglobin subunit epsilon (Fragment) | 53 | 15.6 | 5 | O13071 | http://www.uniprot.org/uniprot/O13071 |
| Adenylosuccinate synthetase lisozyme 1 | 53 | 50.5 | 8 | Q8N142 | http://www.uniprot.org/uniprot/Q8N142 |
| Fragile X mental retardation protein 1 homolog | 52 | 67.3 | 7 | Q5R9B4 | http://www.uniprot.org/uniprot/Q5R9B4 |
| Calponin-1 | 50 | 33.4 | 8 | Q2HJ38 | http://www.uniprot.org/uniprot/Q2HJ38 |
| Calponin-1 | 84 | 33.4 | 10 | Q9GK38 | http://www.uniprot.org/uniprot/Q9GK38 |
| Tyrosine-tRNA ligase, cytoplasmic | 58 | 59.5 | 9 | Q5R8T5 | http://www.uniprot.org/uniprot/Q5R8T5 |
| 14 kDa phosphohistidine phosphatase | 50 | 14 | 4 | Q9NRX4 | http://www.uniprot.org/uniprot/Q9NRX4 |
| N-acetylgalactosamine-6-sulfatase | 45 | 58.4 | 4 | Q32KH5 | http://www.uniprot.org/uniprot/Q32KH5 |
| Norrin | 58 | 15.6 | 6 | Q2K178 | http://www.uniprot.org/uniprot/Q2K178 |
| Ribosome biogenesis protein BOP1 | 50 | 83.2 | 8 | P97452 | http://www.uniprot.org/uniprot/P97452 |
| Sp110 nuclear body protein | 51 | 79.6 | 7 | Q9HB58 | http://www.uniprot.org/uniprot/Q9HB58 |
| Endophilin-A2 | 52 | 41.7 | 7 | Q62419 | http://www.uniprot.org/uniprot/Q62419 |
| Ras-related protein Rab-36 | 54 | 36.8 | 5 | O95755 | http://www.uniprot.org/uniprot/O95755 |
| Putative uncharacterized protein C6orf50 | 53 | 12.6 | 5 | Q9HD87 | http://www.uniprot.org/uniprot/Q9HD87 |
| Cytochrome P450 2C23 | 52 | 57 | 8 | P24470 | http://www.uniprot.org/uniprot/P24470 |
| Protein MB21D2 | 52 | 49 | 9 | Q8C52 | http://www.uniprot.org/uniprot/Q8C52 |
| Heat shock 70 kDa protein 4L (Fragments) | 52 | 23.8 | 9 | P86265 | http://www.uniprot.org/uniprot/P86265 |
| Oxidoreductase HTATIP2 | 53 | 27.4 | 6 | A2T7G9 | http://www.uniprot.org/uniprot/A2T7G9 |
| Ras-related protein Rab-36 | 56 | 36.8 | 6 | O95755 | http://www.uniprot.org/uniprot/O95755 |
| OTU domain-containing protein 6B | 54 | 34 | 7 | Q8N6M0 | http://www.uniprot.org/uniprot/Q8N6M0 |
| Suppressor of IKBKE 1 | 53 | 23.7 | 8 | Q9CPR7 | http://www.uniprot.org/uniprot/Q9CPR7 |
| Myb/SANT-like DNA-binding domain-containing protein 3 | 64 | 32.7 | 8 | Q0III0 | http://www.uniprot.org/uniprot/Q0III0 |
| Ras-related protein Rab-36 | 52 | 36.8 | 8 | O95755 | http://www.uniprot.org/uniprot/O95755 |
| Calpastatin | 50 | 77.6 | 11 | P27321 | http://www.uniprot.org/uniprot/P27321 |
| NACHT, LRR and PYD domains-containing protein 5 | 62 | 123.4 | 21 | Q647I9 | http://www.uniprot.org/uniprot/Q647I9 |
| Protein Name | Protein ID | Event | Fraction | aa | UniProt ID | UniProt Link |
|-------------|------------|-------|----------|----|------------|-------------|
| Mitochondrial uncoupling protein 3 | 38 | 34.6 | 5 | Q9N2I9 | http://www.uniprot.org/uniprot/Q9N2I9 |
| Hemoglobin subunit beta | 62 | 16.3 | 8 | P02073 | http://www.uniprot.org/uniprot/P02073 |
| Diacylglycerol kinase theta | 50 | 104 | 11 | Q6P5E8 | http://www.uniprot.org/uniprot/Q6P5E8 |
| C-type natriuretic peptide | 65 | 13.5 | 8 | P56283 | http://www.uniprot.org/uniprot/P56283 |
| Interleukin-12 receptor subunit beta-2 | 55 | 98.5 | 17 | Q99665 | http://www.uniprot.org/uniprot/Q99665 |
| Tumor necrosis factor receptor superfamily member 5 | 58 | 33.4 | 7 | P27512 | http://www.uniprot.org/uniprot/P27512 |
| Tetra-tricopeptide repeat protein 34 | 53 | 61.8 | 10 | A8MYJ7 | http://www.uniprot.org/uniprot/A8MYJ7 |
| TATA box-binding protein-associated factor RNA polymerase I subunit D | 52 | 33 | 4 | Q5M948 | http://www.uniprot.org/uniprot/Q5M948 |
| Tetra-tricopeptide repeat protein 36 | 65 | 20.7 | 9 | Q3SZV0 | http://www.uniprot.org/uniprot/Q3SZV0 |
| Nicolin-1 | 62 | 24.5 | 9 | Q9B9SH3 | http://www.uniprot.org/uniprot/Q9B9SH3 |
| Gamma-tubulin complex component 2 | 51 | 103.8 | 22 | Q921G8 | http://www.uniprot.org/uniprot/Q921G8 |
| Calcium/calmodulin-dependent protein kinase II inhibitor 1 | 52 | 8.6 | 6 | A7MBG3 | http://www.uniprot.org/uniprot/A7MBG3 |
| Tropomodulin-4 | 53 | 39.5 | 7 | Q0VC48 | http://www.uniprot.org/uniprot/Q0VC48 |
| Protein THEM6 | 51 | 24 | 6 | Q5XIE1 | http://www.uniprot.org/uniprot/Q5XIE1 |
| F-box/SPRY domain-containing protein 1 | 55 | 31.1 | 7 | Q8K3B1 | http://www.uniprot.org/uniprot/Q8K3B1 |
| Actin-like protein 7B | 52 | 45.9 | 9 | Q9Y614 | http://www.uniprot.org/uniprot/Q9Y614 |
| Kinase suppressor of Ras 2 | 56 | 110 | 20 | Q3UVCO | http://www.uniprot.org/uniprot/Q3UVCO |
| Cytospin-A | 50 | 125 | 17 | Q2KNA0 | http://www.uniprot.org/uniprot/Q2KNA0 |
| Uridine 5’-monophosphate synthase | 51 | 52.6 | 8 | P11172 | http://www.uniprot.org/uniprot/P11172 |
| Coiled-coil domain-containing protein 25 | 50 | 24.6 | 8 | Q78PG9 | http://www.uniprot.org/uniprot/Q78PG9 |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12 | 64 | 17.1 | 6 | Q9UI09 | http://www.uniprot.org/uniprot/Q9UI09 |
| Ras-related protein Rab-36 | 62 | 36.8 | 9 | Q95755 | http://www.uniprot.org/uniprot/Q95755 |
| Epididymal-specific lipocalin-5 | 65 | 20.8 | 8 | P06911 | http://www.uniprot.org/uniprot/P06911 |
| NADH dehydrogenase [ubiquinone] 1 alpha | 57 | 11.1 | 7 | Q4R5E2 | http://www.uniprot.org/uniprot/Q4R5E2 |
| Subcomplex/Subunit | M.W. | Isoform | UniProt ID | Link |
|--------------------|------|---------|------------|------|
| Zinc finger protein cas tor homolog 1 | 72 | 193.3 | Q86V15 | http://www.uniprot.org/uniprot/Q86V15 |
| Hippocalcin-like protein 1 | 62 | 22.4 | P62748 | http://www.uniprot.org/uniprot/P62748 |
| Uncharacterized protein C12orf60 | 56 | 27.7 | Q5U649 | http://www.uniprot.org/uniprot/Q5U649 |
| Mini-chromosome maintenance complex-binding protein | 56 | 73.6 | Q8R3C0 | http://www.uniprot.org/uniprot/Q8R3C0 |
| Transmembrane protein 132C | 59 | 122.6 | Q8N3T6 | http://www.uniprot.org/uniprot/Q8N3T6 |
| Ubiquitin carboxyl-terminal hydrolase isozyme 1 | 56 | 25.2 | P50103 | http://www.uniprot.org/uniprot/P50103 |
| Apolipoprotein A-II | 54 | 11.2 | P0DM93 | http://www.uniprot.org/uniprot/P0DM93 |
| Kinesin light chain 2 | 52 | 69.3 | Q9H0B6 | http://www.uniprot.org/uniprot/Q9H0B6 |
| Glutathione peroxidase 3 | 50 | 25.6 | P23764 | http://www.uniprot.org/uniprot/P23764 |
| Prolactin | 55 | 26.6 | P12420 | http://www.uniprot.org/uniprot/P12420 |
| Arginine/serine-rich protein 1 | 51 | 33.7 | Q9BUV0 | http://www.uniprot.org/uniprot/Q9BUV0 |
| Amyloid beta A4 protein (Fragment) | 58 | 6.2 | Q29149 | http://www.uniprot.org/uniprot/Q29149 |
| Leucine-rich repeat-containing protein 49 | 61 | 79.4 | Q91YK0 | http://www.uniprot.org/uniprot/Q91YK0 |
| PH and SEC7 domain-containing protein 3 | 50 | 115.3 | Q2PFD7 | http://www.uniprot.org/uniprot/Q2PFD7 |
| Insulin | 54 | 12.5 | P01321 | http://www.uniprot.org/uniprot/P01321 |
| Dual specificity phosphatase 28 | 54 | 18.7 | Q4G0W2 | http://www.uniprot.org/uniprot/Q4G0W2 |
| Calpain-2 catalytic subunit | 50 | 80.8 | P17655 | http://www.uniprot.org/uniprot/P17655 |
| Peroxiredoxin-1 | 52 | 22.3 | Q06830 | http://www.uniprot.org/uniprot/Q06830 |
| Ras-related protein Rab-25 | 53 | 23.5 | P46629 | http://www.uniprot.org/uniprot/P46629 |
| tRNA-dihydouridine(47) synthase [NAD(P)(+)]-like | 53 | 72.4 | Q91X11 | http://www.uniprot.org/uniprot/Q91X11 |
| AarF domain-containing protein kinase 4 | 56 | 59.6 | Q6AY19 | http://www.uniprot.org/uniprot/Q6AY19 |
| RING finger protein 10 | 52 | 90.7 | Q08E13 | http://www.uniprot.org/uniprot/Q08E13 |
| E3 ubiquitin-protein ligase RNF152 | 60 | 23.1 | D2H6Z0 | http://www.uniprot.org/uniprot/D2H6Z0 |
| Protein-arginine deiminase type-3 | 53 | 76.3 | Q9Z184 | http://www.uniprot.org/uniprot/Q9Z184 |
| Sentan | 65 | 16.6 | A6NMZ2 | http://www.uniprot.org/uniprot/A6NMZ2 |
| Plakophilin-4 | 53 | 132.3 | Q68FH0 | http://www.uniprot.org/uniprot/Q68FH0 |
| Protein Name                        | ID   | E-Value | Length | Name                                                                 | Score | Link                                      | E-Value | Length | Name                                                                 | Score | Link                                      |
|------------------------------------|------|---------|--------|----------------------------------------------------------------------|-------|-------------------------------------------|---------|--------|----------------------------------------------------------------------|-------|-------------------------------------------|
| Urotensin-2B                       | 65   | 13.0    | 8      | Q765I1                                                              | http://www.uniprot.org/uniprot/Q765I     |                                           | 1       |        |                                                                      |       |                                           |
| Signal peptidase complex subunit 2 | 53   | 25.3    | 8      | Q15005                                                              | http://www.uniprot.org/uniprot/Q15005    |                                           | 5       |        |                                                                      |       |                                           |
| Tektin-4                           | 54   | 51.3    | 12     | Q8WW24                                                              | http://www.uniprot.org/uniprot/Q8WW24    |                                           | 24      |        |                                                                      |       |                                           |
| Cytochrome P450 2j3                | 53   | 58.4    | 10     | P51590                                                              | http://www.uniprot.org/uniprot/P51590    |                                           | 0       |        |                                                                      |       |                                           |
| Ras-related protein Rab-36         | 64   | 36.8    | 9      | O95755                                                              | http://www.uniprot.org/uniprot/O95755    |                                           | 5       |        |                                                                      |       |                                           |
| Isocitrate dehydrogenase [NADP] cytoplasmic | 50   | 47.2    | 6      | Q6XUZ5                                                              | http://www.uniprot.org/uniprot/Q6XUZ5    |                                           | 5       |        |                                                                      |       |                                           |
| E3 ubiquitin-protein ligase RNF169  | 55   | 77.1    | 8      | E9Q7F2                                                              | http://www.uniprot.org/uniprot/E9Q7F2    |                                           | 2       |        |                                                                      |       |                                           |
| Class E basic helix-loop-helix protein 40 | 51   | 45.9    | 6      | O14503                                                              | http://www.uniprot.org/uniprot/O14503    |                                           | 3       |        |                                                                      |       |                                           |
| Interleukin-13                     | 55   | 15.5    | 6      | Q9N0W9                                                              | http://www.uniprot.org/uniprot/Q9N0W9    |                                           | 6       |        |                                                                      |       |                                           |
| Rab GDP dissociation inhibitor beta | 67   | 50.8    | 7      | O97556                                                              | http://www.uniprot.org/uniprot/O97556    |                                           | 6       |        |                                                                      |       |                                           |
| Isocitrate dehydrogenase [NADP], mitochondrial | 60   | 51.3    | 8      | P54071                                                              | http://www.uniprot.org/uniprot/P54071    |                                           | 1       |        |                                                                      |       |                                           |
| Stanniocalcin-2                    | 63   | 34.1    | 8      | O97561                                                              | http://www.uniprot.org/uniprot/O97561    |                                           | 1       |        |                                                                      |       |                                           |
| Ephrin type-B receptor 4           | 50   | 110.3   | 11     | P54761                                                              | http://www.uniprot.org/uniprot/P54761    |                                           | 1       |        |                                                                      |       |                                           |
| NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12 | 60   | 17.1    | 7      | Q9UL09                                                              | http://www.uniprot.org/uniprot/Q9UL09    |                                           | 9       |        |                                                                      |       |                                           |
| Dual specificity phosphatase 28    | 54   | 18.7    | 7      | Q4G0W2                                                              | http://www.uniprot.org/uniprot/Q4G0W2    |                                           | 2       |        |                                                                      |       |                                           |
| Parafibromin                       | 54   | 60.7    | 13     | Q6P1J9                                                              | http://www.uniprot.org/uniprot/Q6P1J9    |                                           | 9       |        |                                                                      |       |                                           |
| Bactericidal permeability-increasing protein | 59   | 54      | 10     | Q6AXU0                                                              | http://www.uniprot.org/uniprot/Q6AXU0    |                                           | 0       |        |                                                                      |       |                                           |
| Zinc finger protein castor homolog 1 | 61   | 193.3   | 17     | Q86V15                                                              | http://www.uniprot.org/uniprot/Q86V15    |                                           | 5       |        |                                                                      |       |                                           |
| Calcitonin receptor-stimulating peptide 2 | 44   | 14.3    | 7      | Q75V93                                                              | http://www.uniprot.org/uniprot/Q75V93    |                                           | 3       |        |                                                                      |       |                                           |
| Corticoliberin                     | 58   | 20.8    | 9      | Q95MI6                                                              | http://www.uniprot.org/uniprot/Q95MI6    |                                           | 6       |        |                                                                      |       |                                           |
| Urotensin-2B                       | 50   | 13      | 6      | Q765I1                                                              | http://www.uniprot.org/uniprot/Q765I1    |                                           | 1       |        |                                                                      |       |                                           |
| Pumilio homolog 3                  | 53   | 73.9    | 16     | Q15397                                                              | http://www.uniprot.org/uniprot/Q15397    |                                           | 7       |        |                                                                      |       |                                           |
| Protein FAM162B                     | 55   | 18.1    | 9      | A6QPI4                                                              | http://www.uniprot.org/uniprot/A6QPI4    |                                           | 4       |        |                                                                      |       |                                           |
| Cytochrome c oxidase subunit 6C    | 55   | 8.6     | 7      | Q7YRK2                                                              | http://www.uniprot.org/uniprot/Q7YRK2    |                                           | 2       |        |                                                                      |       |                                           |
| Aromatase                          | 56   | 58.6    | 13     | P46194                                                              | http://www.uniprot.org/uniprot/P46194    |                                           | 4       |        |                                                                      |       |                                           |
| Protein Name                                                                 | ID | E价值 | 位置 | UniProt ID       | Link                                      |
|------------------------------------------------------------------------------|----|-------|------|------------------|-------------------------------------------|
| Zinc finger protein 101                                                      | 50 | 51.9  | 9    | Q8IZC7           | http://www.uniprot.org/uniprot/Q8IZC7    |
| Phosphatidylethanolamine-binding protein 1                                   | 52 | 21.2  | 5    | Q8MK67           | http://www.uniprot.org/uniprot/Q8MK67    |
| Leucine-tRNA ligase, cytoplasmian                                              | 57 | 135.7 | 11   | Q5R614           | http://www.uniprot.org/uniprot/Q5R614    |
| NACHT, LRR and PYD domains-containing protein 10                              | 76 | 77.3  | 14   | Q8CCN1           | http://www.uniprot.org/uniprot/Q8CCN1    |
| Urotensin-2B                                                                 | 70 | 13    | 7    | Q7651I           | http://www.uniprot.org/uniprot/Q7651I    |
| Mortality factor 4-like protein 2                                              | 82 | 32.2  | 8    | Q9R0Q4           | http://www.uniprot.org/uniprot/Q9R0Q4    |
| Nucleolar pre-ribosomal-associated protein 1                                  | 59 | 256.5 | 15   | O60287           | http://www.uniprot.org/uniprot/O60287    |
| Serine/threonine-protein phosphatase 6 catalytic subunit                     | 74 | 35.8  | 10   | O00743           | http://www.uniprot.org/uniprot/O00743    |
| Hemopexin                                                                    | 63 | 52    | 7    | Q91X72           | http://www.uniprot.org/uniprot/Q91X72    |
| Rab GDP dissociation inhibitor beta                                           | 52 | 51    | 8    | P50397           | http://www.uniprot.org/uniprot/P50397    |
| Sperm surface protein Sp17                                                    | 55 | 17.3  | 5    | Q62252           | http://www.uniprot.org/uniprot/Q62252    |
| Natriuretic peptides B                                                        | 46 | 15.1  | 4    | P16859           | http://www.uniprot.org/uniprot/P16859    |
| Iron-responsive element-binding protein 2                                     | 55 | 106.1 | 8    | B3VKQ2           | http://www.uniprot.org/uniprot/B3VKQ2    |
| STE20-related kinase adapter protein alpha                                   | 53 | 41.9  | 5    | Q5E9J9           | http://www.uniprot.org/uniprot/Q5E9J9    |
| E3 ubiquitin-protein ligase RNF169                                            | 50 | 77.1  | 9    | E9Q7F2           | http://www.uniprot.org/uniprot/E9Q7F2    |
| Acyl-coenzyme A synthetase ACSM2B, mitochondrial                              | 57 | 64.8  | 8    | Q68CK6           | http://www.uniprot.org/uniprot/Q68CK6    |
| Survival motor neuron protein                                                 | 63 | 32.2  | 9    | Q4R4F8           | http://www.uniprot.org/uniprot/Q4R4F8    |
| Tekitn-4                                                                     | 54 | 51.3  | 8    | Q8WW24           | http://www.uniprot.org/uniprot/Q8WW24    |
| Anaphase-promoting complex subunit CDC26                                      | 61 | 9.8   | 4    | Q3SZT7           | http://www.uniprot.org/uniprot/Q3SZT7    |
| 39S ribosomal protein L30, mitochondrial                                      | 71 | 18.7  | 5    | Q58DV5           | http://www.uniprot.org/uniprot/Q58DV5    |
| Interleukin-4                                                                | 64 | 15.5  | 6    | P55030           | http://www.uniprot.org/uniprot/P55030    |
| 39S ribosomal protein L10, mitochondrial                                      | 51 | 29.6  | 8    | Q3TBW2           | http://www.uniprot.org/uniprot/Q3TBW2    |
| 39S ribosomal protein L30, mitochondrial                                      | 50 | 18.7  | 5    | Q58DV5           | http://www.uniprot.org/uniprot/Q58DV5    |
| Acylphosphatase-2                                                             | 61 | 11.1  | 7    | P35744           | http://www.uniprot.org/uniprot/P35744    |
| Tekitn-4                                                                     | 51 | 51.3  | 8    | Q8WW24           | http://www.uniprot.org/uniprot/Q8WW24    |
| Potassium/sodium                                                             | 68 | 95.7  | 17   | Q9JKA9           | http://www.uniprot.org/uniprot/Q9JKA9    |
|                              | ID  | Value | Scale | PDB | Link                                      |
|------------------------------|-----|-------|-------|-----|-------------------------------------------|
| hyperpolarization-activated cyclic nucleotide-gated channel 2 | 51  | 18.7  | 6     | Q4G0W2 | http://www.uniprot.org/uniprot/Q4G0W2  |
| Dual specificity phosphatase 28 | 63  | 45.5  | 10    | A4FUZ6 | http://www.uniprot.org/uniprot/A4FUZ6   |
| Hydroxysteroid dehydrogenase-like protein 2 | 52  | 10    | 5     | P17716 | http://www.uniprot.org/uniprot/P17716   |
| Islet amyloid polypeptide | 9   | 152.8 | 13    | Q5U4C1 | http://www.uniprot.org/uniprot/Q5U4C1   |
| G-protein coupled receptor-associated sorting protein 1 | 51  | 47.9  | 7     | Q9UK05 | http://www.uniprot.org/uniprot/Q9UK05   |
| Cytosolic purine 5'-nucleotidase | 52  | 65.3  | 10    | O46411 | http://www.uniprot.org/uniprot/O46411   |
| Vesicle transport protein USE1 | 59  | 30.8  | 16    | Q9CQ56 | http://www.uniprot.org/uniprot/Q9CQ56   |
| Putative zinc finger protein 137 | 61  | 24.7  | 8     | P52743 | http://www.uniprot.org/uniprot/P52743   |
| Elongation factor 1-beta | 52  | 25    | 5     | Q5E983 | http://www.uniprot.org/uniprot/Q5E983   |
| Tektin-4 | 64  | 51.3  | 12    | Q8WW24 | http://www.uniprot.org/uniprot/Q8WW24   |
| Parathyroid hormone/parathyroid hormone-related peptide receptor | 67  | 66.7  | 9     | Q1LZC7 | http://www.uniprot.org/uniprot/Q1LZC7   |
| Chorionic somatomammotropin hormone 2 | 50  | 28.2  | 6     | P19159 | http://www.uniprot.org/uniprot/P19159   |
| Probable tubulin polyglutamylase TLL1 | 51  | 49.5  | 8     | Q5PPI9 | http://www.uniprot.org/uniprot/Q5PPI9   |
| Collagen alpha-1(XI) chain (Fragment) | 62  | 89.3  | 10    | Q28083 | http://www.uniprot.org/uniprot/Q28083   |
| G kinase-anchoring protein 1 | 68  | 42.2  | 10    | Q5XIG5 | http://www.uniprot.org/uniprot/Q5XIG5   |
| Protein phosphatase Slingshot homolog 1 | 67  | 116.5 | 14    | Q8WYI5 | http://www.uniprot.org/uniprot/Q8WYI5   |
| Arginine-serine-rich protein 1 | 58  | 33.7  | 8     | Q9BUV0 | http://www.uniprot.org/uniprot/Q9BUV0   |
| Sorting and assembly machinery component 50 homolog | 50  | 52.2  | 6     | Q8BGH2 | http://www.uniprot.org/uniprot/Q8BGH2   |
| Cystatin-B | 57  | 11.2  | 4     | P25417 | http://www.uniprot.org/uniprot/P25417   |
| Desmin | 61  | 53.6  | 13    | P17661 | http://www.uniprot.org/uniprot/P17661   |
| Transmembrane inner ear expressed protein | 52  | 53.6  | 6     | Q8K467 | http://www.uniprot.org/uniprot/Q8K467   |
| Putative uncharacterized protein encoded by CRHR1-IT1 | 50  | 17.2  | 6     | Q96LR1 | http://www.uniprot.org/uniprot/Q96LR1   |
| Oculomedin | 58  | 5.3   | 5     | Q9Y5M6 | http://www.uniprot.org/uniprot/Q9Y5M6   |
| Protein kish-A            | 62 | 8.3 | 5 | Q148I3 | http://www.uniprot.org/uniprot/Q148I3 |
|--------------------------|----|-----|---|--------|-------------------------------------|
| Rab GDP dissociation     | 51 | 50.8| 6 | O97556 | http://www.uniprot.org/uniprot/O97556|
| inhibitor beta           |    |     |   |        |                                     |
| Glutathione S- transferase Mu 6 | 52 | 25.8| 8 | O35660 | http://www.uniprot.org/uniprot/O35660|
| Hemoglobin subunit beta  | 68 | 16.3| 8 | P02073 | http://www.uniprot.org/uniprot/P02073|
| Zinc finger and SCAN    | 62 | 56.9| 11| Q9BUG6 | http://www.uniprot.org/uniprot/Q9BUG6|
| domain-containing protein 5A |    |     |   |        |                                     |
| Ras-related protein Rab-36 | 53 | 36.8| 7 | O95755 | http://www.uniprot.org/uniprot/O95755|
| 39S ribosomal protein    | 54 | 18.3| 9 | Q8VDT9 | http://www.uniprot.org/uniprot/Q8VDT9|
| L50, mitochondrial      |    |     |   |        |                                     |
| NAD-dependent protein    | 51 | 45.7| 9 | Q8BKJ9 | http://www.uniprot.org/uniprot/Q8BKJ9|
| deacetylase sirtuin-7    |    |     |   |        |                                     |
| Ras-related protein Rab-36 | 57 | 36.8| 12| O95755 | http://www.uniprot.org/uniprot/O95755|
| Putative uncharacterized | 62 | 7.3 | 5 | A6NGU7 | http://www.uniprot.org/uniprot/A6NGU7|
| protein encoded by LINC01546|    |     |   |        |                                     |
| Hemoglobin subunit beta  | 51 | 16.3| 7 | P02073 | http://www.uniprot.org/uniprot/P02073|
| 3-Oxo-5-beta-sterol 4- | 50 | 37.7| 8 | P51857 | http://www.uniprot.org/uniprot/P51857|
| dehydrogenase            |    |     |   |        |                                     |
| Synaptosomal-associated | 56 | 29.1| 7 | Q9Z2P6 | http://www.uniprot.org/uniprot/Q9Z2P6|
| protein 29               |    |     |   |        |                                     |
| Zinc finger protein 624  | 52 | 102.5|10| Q9P2J8 | http://www.uniprot.org/uniprot/Q9P2J8|
| Ubiquitin carboxyl-terminal hydroxylase 48 | 51 | 120.7|11| Q76LT8 | http://www.uniprot.org/uniprot/Q76LT8|
| Cholecystokinin          | 56 | 12.7| 4 | P23362 | http://www.uniprot.org/uniprot/P23362|

**Figures**
Figure 1

Image of 2DE gel from dogs with babesiosis
Figure 2

Image of 2DE gel from healthy dogs