



A Proteomic View of the Cross-Talk Between Early Intestinal Microbiota and Poultry Immune System

D. R. Rodrigues¹, K. M. Wilson¹, M. Trombetta¹, W. N. Briggs¹, A. F. Duff¹, K. M. Chasser¹, W. G. Bottje² and L. Bielke^{1*}

¹ Department of Animal Sciences, The Ohio State University, Columbus, OH, United States, ² Department of Poultry Science, University of Arkansas, Fayetteville, AR, United States

OPEN ACCESS

Edited by:

Krystyna Pierzchala-Koziec, University of Agriculture in Krakow, Poland

Reviewed by:

Kent M. Reed, University of Minnesota Twin Cities, United States Pawel Tomasz Maćkowiak, Poznań University of Life Sciences, Poland Michael Kogut, Agricultural Research Service, United States Department of Agriculture, United States

> *Correspondence: L. Bielke bielke.1@osu.edu

Specialty section:

This article was submitted to Avian Physiology, a section of the journal Frontiers in Physiology

Received: 22 October 2019 Accepted: 13 January 2020 Published: 13 February 2020

Citation:

Rodrigues DR, Wilson KM, Trombetta M, Briggs WN, Duff AF, Chasser KM, Bottje WG and Bielke L (2020) A Proteomic View of the Cross-Talk Between Early Intestinal Microbiota and Poultry Immune System. Front. Physiol. 11:20. doi: 10.3389/fphys.2020.00020 Proteomics has been used to investigate cross-talk between the intestinal microbiome and host biological processes. In this study, an in ovo technique and a proteomics approach was used to address how early bacterial colonization in the gastrointestinal tract (GIT) could modulate inflammatory and immune responses in young broilers. Embryos at 18 embryogenic days were inoculated with saline (S), 10² CFU of Citrobacter freundii (CF), Citrobacter species (C2), or lactic acid bacteria mixture (L) into the amnion. At 10 days posthatch, ileum samples from 12 birds per treatment were selected for tandem mass spectrometry analysis. Our further findings indicated that treatment-specific influences on early GIT microbiota resulted in different immune responses in mature broilers. Predicted functional analyses revealed activation of inflammation pathways in broilers treated in ovo with L and CF. Exposure to L enhanced functional annotation related to activation, trafficking of immune cells, and skeletal growth based-network, while CF inhibited biological functions associated with immune cell migration and inflammatory response. These results highlighted that proper immune function was dependent on specific GIT microbiota profiles, in which early-life exposure to L-based probiotic may have modulated the immune functions, whereas neonatal colonization of Enterobacteriaceae strains may have led to immune dysregulation associated with chronic inflammation.

Keywords: segmented filamentous bacteria, probiotic *in ovo*, immunity, inflammation, *Enterobacteriaceae*, pioneer colonizers, Ingenuity Pathway Analysis

INTRODUCTION

Pioneer colonization of intestinal microbiota has a major effect on driving the maturation and composition course of microbial communities over time (Juricova et al., 2013; Rodrigues et al., 2019; Wilson et al., 2019). Later, the cross-talk between microbiota composition and immune cells has been highly associated with the establishment of immune competence (Crhanova et al., 2011; Chung et al., 2012; Schokker et al., 2017; Duan, 2018). Germ-free mouse models have been essential to reveal a strong influence of intestinal microbial communities on the proper immune function. The lack of intestinal microbiota in these mice caused extensive deficits in the development of the gut-associated lymphoid tissues, abnormal production of immune cells, and other immunological deficiencies (Round and Mazmanian, 2009). In this context, a recent study with broilers has

1

shown that the use of antibiotics during early life perturbed microbiota colonization, subsequently triggering an alteration in systemic immune programming (Schokker et al., 2017). Nevertheless, the specific microbial populations involved in immune-modulatory functions are beginning to be deciphered with the advancement of metagenomic analyses.

It has been reported by Kogut (2019) that the avian neonatal phase is an important window of opportunity to manipulate the intestinal microbiome toward beneficial bacterial growth. In fact, our previous studies showed that early exposure of embryos to lactic acid bacteria or *Enterobacteriaceae* strains resulted in different microbiome profiles at day of hatch and 10 days of age, suggesting that neonatal exposure to beneficial bacteria may be critical for influencing gastrointestinal tract (GIT) populations throughout the maturation of the poultry microbiota (Wilson et al., 2019). However, whether this pioneer intestinal microbiome modulation can affect the host immunological functions remain unclear.

In this study, the influence of early intestinal bacterial colonization on the inflammatory and immune response of young broilers was investigated. For this purpose, two non-pathogenic *Enterobacteriaceae* isolates and a lactic acid bacteria-based probiotics were introduced *in ovo*, and mass spectrometry-based proteome analysis was performed on ileum tissue. To test our hypothesis, we focused on intestinal inflammatory and immune-related proteins, screened the biological functions predicted by Ingenuity Pathway Analysis (IPA), and linked inflammation biomarkers to intestinal microbial signatures established in the ileal microbiome of 10-day-old broiler chickens.

MATERIALS AND METHODS

Experimental Design

A total of 400 eggs from commercial Ross 708 broiler breeder flocks were obtained from a local hatchery. Per standard operating procedures, the eggs were sanitized before storage and incubation. All eggs were incubated under standard conditions at the Ohio Agricultural Research and Development Center's poultry research farm. Once eggs were confirmed fertile, at embryonic day 18, the air-cell end of each egg was treated with iodine (povidone-iodine 10% topical solution, Drug Mart, Medina, OH, United States) before a small hole was punched into the shell with an inoculation needle. In ovo inoculations contained one of the following: 0.2 ml of 0.9% sterile saline (S), which served as the control group, or approximately 10^2 cells of Citrobacter freundii (CF), Citrobacter spp. (C2), or lactic acid bacteria mixture (L) administered into the amnion (Figure 1). After inoculation, up to 30 eggs were allocated by treatments into three separate benchtop hatchers (Hova-Bator model 1602N, Savannah, GA, United States) for a total of 12 hatchers. All hatchers were disinfected with 10% bleach before use. Strains CF and C2 were selected from our previous study as non-pathogenic bacteria from the gut of healthy birds (Bielke et al., 2003), and the homology of strains was confirmed by next-generation sequencing. The L culture was composed of a mixed inoculum of *Lactobacillus salivarius* and *Pediococcus* sp. Bacterial inoculations were prepared as described by Wilson et al. (2019). Preliminary experimental observations concluded that the inclusion of isolates at $\sim 10^2$ CFU did not affect hatchability compared to the S control treatment (data not published). All experimental procedures were approved by the Ohio State University's Institutional Animal Care and Use Committee.

Sample Collection

Immediately posthatch, chicks were comingled on a treatment basis, and 128 chicks were placed into treatment-separated brooder battery cages with *ad libitum* access to a standard cornsoy diet and water (Nutrient Requirements of Poultry, 1994). At 10 days posthatch, 12 chicks per treatment were randomly selected for ileal proteome analysis, however, only nine birds were sampled from CF. Chicks were euthanized via cervical dislocation, and the region proximal to the ileocecal junction and distal to Meckel's diverticulum, designated as lower ileum, was aseptically collected *post mortem* (**Figure 1**). Ileum tissue was placed into 1.5-ml tubes, flash frozen in liquid nitrogen at the time of collection, and stored at -80° C until further use.

Once thawed, 0.1 g of ileal tissue from each sample was individually placed in 5 ml of buffer (8 M urea/2 M thiourea, 2 mM dithiothreitol, 50 mM Tris, 5% sodium dodecyl sulfate). The extraction protocol was a modified version previously described by Iqbal et al. (2004) and Kong et al. (2016). In brief, samples were homogenized for 5 s (PRO250 Homogenizer, Pro Scientific, Oxford, CT, United States), then 500 µl of homogenate was added to 2-ml tubes containing 0.1 g stainless steel beads (SSB14B Next Advance, Averill Park, NY, United States). Samples were homogenized for a total of 3 min in 30-second intervals (MiniBeadbeater-16, Model 607, BioSpec Products, Bartlesville, OK, United States) and centrifuged at 4°C at 14,000 rpm (21,952 × g) for 20 min. The supernatant was collected, aliquoted, and placed into -80° C until further use.

To ensure proper extraction, concentration of total protein was quantified with the Bradford assay (Bradford reagent, VWR, Suwanee, GA, United States) and a standard bovine serum albumin curve (VWR, Suwanee, GA, United States) on a Synergy HTX multimode plate reader (BioTek U.S., Winooski, VT, United States). Samples were mixed to create pooled samples of two birds per treatment (n = 6 samples for L and C2; n = 5 samples CF; **Figure 1**) and sent to the Ohio State University Proteomics Core lab for in-solution digestion and mass spectrometry.

Proteomics Analyses

Samples were precipitated with trichloroacetic acid and then resuspended in 50 mM ammonium bicarbonate. A total of 5 ml of dithiothreitol (5 μ g/ μ l in 50 mM ammonium bicarbonate) was added, and the samples were incubated at 56°C for 15 min. After incubation, 5 μ l of iodoacetamide (15 mg/ml in 50 mM ammonium bicarbonate) was added, and the samples were kept in the dark at room temperature for 30 min. Sequencing grade-modified trypsin (Promega; Madison, WI, United States) prepared in 50 mM ammonium bicarbonate was added to each sample at an estimated 1:20/1:100 enzyme/substrate ratio and incubated at 37°C overnight. The reaction was quenched the



chicks were selected for ileum sample collection (pooled samples of two birds per treatment) to perform proteome analyses.

following day by adding acetic acid for acidification. Once samples were quenched, the peptide concentration was measured by Nanodrop (Thermo Scientific Nanodrop 2000; Waltham, MA, United States).

Capillary-liquid chromatography-nanospray tandem mass spectrometry (capillary-LC/MS/MS) of global protein identification was performed on a Thermo Fisher Fusion mass spectrometer (Thermo Scientific, Waltham, MA, United States). Samples were separated on a Thermo Nano C18 column (UltiMateTM 3000 HPLC system, Thermo Scientific; Waltham, MA, United States). The MS/MS data sequences were scanned and based on the preview mode data-dependent TopSpeedTM method with collision-induced dissociation and electrontransfer dissociation as fragmentation methods. The raw data were searched on Sequest via Proteome Discoverer (Proteome DiscovererTM software, Thermo Scientific, Waltham, MA, United States). The data were searched against the most recent Uniprot Gallus gallus database for the identification of proteins. Only proteins with <0.05 false discovery rate were reported. Proteins with a Mascot score of 50 or higher with a minimum of two unique peptides from one protein having a -b or -y ion sequence tag of five residues or better were accepted. Any

modifications or low-score peptide/protein identifications were manually checked for validation.

Biological Interpretation

Label-free quantitation was performed using the spectral count approach, in which the relative protein quantitation is measured by comparing the number of MS/MS spectra identified from the same protein in each of the multiple LC/MS/MS datasets. Comparisons between in ovo bacterial treatments and S control group were performed in Scaffold (Scaffold 4.8.4, Proteome Software, Portland, OR, United States). Student's *t* test (p < 0.05) was performed to identify significance across the fold-change values. Differentially expressed proteins (DEPs) at the level of $p \leq 0.10$ were uploaded into IPA system¹ to retrieve further inflammatory and immune information in terms of gene ontology, upstream regulators, and causal networks. The IPA functionalities for differentially expressed genes in chicken are based primarily on mammalian biological mechanisms (Kong et al., 2011). The statistical measure Z score was displayed to make predictions about potential activation ($Z \text{ score} \ge 2.00$) or

¹http://www.ingenuity.com

inhibition ($Z \operatorname{score} \leq -2.00$) of regulators using the information of the protein regulation direction. Qualified predictions were also made for high ($Z \operatorname{score} \geq | 1.90 |$ or more) medium ($Z \operatorname{score} = | 1.70-1.90 |$) or low ($Z \operatorname{score} = | 1.50-1.70 |$), respectively (Kong et al., 2016). Casual networks were performed to show mechanistic hypotheses to explain the expression changes observed in the datasets based on cause–effect relationships reported in the literature (Krämer et al., 2014). The p value of the overlap, which measures any significant statistical overlap between the samples in the dataset and the genes that are regulated by the corresponding transcriptional regulator, was also determined and recorded. Fisher's exact test calculated the value at a significance of p < 0.05.

Correlation Analyses

To further identify the specific bacterial groups that primarily accounted for the differences observed in the expression of inflammation-related proteins in the ileum of broilers, the microbiome data of same samples were added into the analysis, from BioProject ID PRJNA552855, previously published by Rodrigues et al. (2019). The most dominant genus-level operational taxonomic units detected in the four treatments were identified (Supplementary Table S1). Based on the inflammatory annotation generated by the IPA system, the DEPs involved in the inflammation signaling also were determined. Given the considerable number of variables, only overexpressed proteins or those with at least one upregulated DEP within each treatment dataset were selected. Owing to the unlikelihood of perfectly linear relationships and the presence of significant variation between relative bacterial abundance and protein log2-fold change, we applied Spearman's correlation coefficient (R) using RStudio software.

RESULTS

From the proteomic datasets, we identified 617 proteins in L, 613 proteins in CF, and 625 proteins in C2 (**Supplementary Tables S2–S4**, respectively). Accordingly, 608 proteins were common across all three treatment conditions (**Figure 2**).

Subsequently, DEPs significantly lower or equal to 0.1 were identified. A total of 61 DEPs were displayed in L (**Table 1**), 44 DEPs in CF (**Table 2**), and 63 in C2 (**Table 3**). Furthermore, we only evaluated the biological interactions associated with inflammatory and immune response signaling.

Biological Functions

To assess the functional annotation associated with DEPs, the disease processes and cellular functions were predicted by the IPA approach. **Figure 3** summarizes the major predicted effects on functional annotation coordinated to the inflammatory and immune responses. In L treatment, inflammation of organ (Z score = 1.80) and inflammation of absolute region (Z score = 1.65) were predicted to be activated in relation to S control group (**Figure 3A**). In CF, a predicted high activation Z score = 2.32). Biological functions associated with inflammatory



FIGURE 2 Venn diagram depicting unique and shared proteins identified in the ileum of 10-day-old broilers treated *in ovo* with lactic acid bacteria mixture (L), *Citrobacter freundii* (CF), or *Citrobacter* spp. (C2).

response (Z score = -1.96), cell movement of granulocytes (Z score = -1.95) and leukocytes (Z score = -1.60) were predicted to be inhibited when compared to S control group (**Figure 3B**). There was no qualified prediction of downstream functional effects in C2.

To better understand the differential regulation of inflammatory and immune signaling response across treatments, the L protein expression metadata generated by IPA were further compared to CF and C2 profiles (IPA comparison analyses). As illustrated in **Figure 3C**, when L was compared to CF, the biological functional analysis predicted the activation of leukocyte migration (Z score = 1.54), immune response of leukocytes (Z score = 1.56), cellular infiltration by leukocytes (Z score = 1.64), cell movement of leukocytes (Z score = 2.19), and inflammatory response (Z score = 2.44). When examining L versus C2, there were predicted activation of inflammation of absolute anatomical region (Z score = 1.57), inflammation of organ (Z score = 1.62), and cell movement of neutrophils (Z score = 1.63; **Figure 3D**).

Inflammatory-Related Proteins

Based on the IPA diseases and function annotation, the proteomic signatures associated with inflammatory downstream effects were identified (**Figure 4** and **Supplementary Table S5**). Venn diagrams show the ileal DEPs related to inflammatory molecular functions (**Supplementary Figure S1**) in each *in ovo* treatment. The exposure to L *in ovo* downregulated the expression of eleven proteins (PRSS2, XDH, ADA, SCP2, SPINK5, ATP1A2, GLG1, PHB, MYH9, OVT, and ACE) and overexpressed eight (HMGB1, ITGA1, BSG, ARF6, MYL9, TUBB3, CKB, and LECT2). Similarly, there were nine downregulated (ACE, GLG1, HDAC1, PSMD1, PARP1, BLMH, PRDX1, TGM2, and PKM) and three upregulated DEPs (TPI1, ITGA1, and CTSD) found in CF versus S control. In the C2 treatment, the expression

TABLE 1 | Differentially expressed proteins (DEPs) from ileal samples of broilers treated with lactic acid bacteria mixture (L) in ovo.

UniProt ID	Proteins	Protein name	Fold change	p Value
Q10751	ACE	Angiotensin I converting enzyme	-1.000	0.003
P02612	MYL9	Myosin light chain 9	2.400	0.004
Q5ZIV5	PDCD10	Programmed cell death 10	6.800	0.005
P20678	Cyp2c23	Cytochrome P450, family 2, subfamily c, polypeptide 23	-10.000	0.007
Q9PW72	PDLIM4	PDZ and LIM domain 4	-2.000	0.013
P24797	ATP1A2	ATPase Na + /K + transporting subunit alpha 2	-1.600	0.014
P50594	MAGOH	Mago homolog, exon junction complex subunit	4.400	0.015
Q90597	MX2	MX dynamin like GTPase 2	-1.600	0.016
P08940	LECT2	Leukocvte cell derived chemotaxin 2	3.500	0.017
P67883	RPL30	Ribosomal protein L30	-1.660	0.019
Q5ZMU6	PPP4R2	Protein phosphatase 4 regulatory subunit 2	∞	0.021
P31696-10	AGRN	Agrin	-5.000	0.024
P02701	AVD	Avidin	6.700	0.027
E1C2P3	HSPA14	Heat shock protein family A (Hsp70) member 14	5.000	0.029
P05180	Cvp2c23	Cytochrome P450 family 2 subfamily c polypeptide 23	-3.300	0.031
P47990	XDH	Xanthine dehydrogenase	-2.500	0.031
0.571W/2	CNOT10	CCB4-NOT transcription complex subunit 10	-3.300	0.032
P05122-5	CKB	Creatine kinase B	3,300	0.033
P02789	TE	Transferrin	-1.250	0.035
057535	NIME2	NME/NM23 nucleoside dinhosnhate kinase 2	-1.420	0.037
061/44	LIBAS	Libiquitin like modifier activating enzyme 5	3 500	0.038
057KG5		Acid phoephataea 1	-2.000	0.000
007598	SCP2	Sterol carrier protein 2	-2.000	0.042
Q07390			-2.000	0.042
Q021(1 0			-2.000	0.045
CE71 NE		TAR DNA binding protein	2,000	0.045
DOREGE		Pilosomal protein \$17	-2.000	0.040
P61160		APP2 actin related protein 2 homolog	1.300	0.040
057 11 0		SAM and HD domain deaviru closeide tripheenhate tripheenhabydroleee 1	- 1.230	0.057
057/29		Paraspackla component 1	2.000	0.052
000620		Paraspeckie component i	- 1.000	0.054
002301	CL C1		-2.500	0.055
Q02391			-1.000	0.055
P84173	PHB	Prohibitin	-1.420	0.000
P26000	ARE6	ADP ribosvlation factor 6	- 1:420	0.003
P47807		Abi hibosylation lactor o	2.400	0.005
057LN1			-1.000	0.000
			-1.420	0.008
		Uniovinal autophama a raduataga. Disaka iran, autor nalupantida 1	-2.300	0.072
QOZERO D17700	DQCRFST	Decision (Ok black group)	1.400	0.074
P17790	DOG	Basigin (Ok blood group)	2.100	0.075
		High mobility group box 1	1.700	0.078
Q5ZLIU		exportin /	-2.000	0.084
Q5ZKD7	MOV10	Mov10 RISC complex RINA helicase	-1.420	0.084
P27003	S100A10	S100 calcium binding protein A10	∞	0.084
P10184	SPINK5	Serine peptidase inhibitor, Kazai type 5	-2.000	0.085
P05083	ASL	Argininosuccinate lyase	2.200	0.085
P23228	HMGCS1	3-Hydroxy-3-methylglutaryl-CoA synthase 1	-2.500	0.086
Q5ZM33	HP1BP3	Heterochromatin protein 1 binding protein 3	1.500	0.086
Q52K62	ACAP2	ArtiGAP with colled coll, ankyrin repeat PH domains 2	-1.000	0.093
P16924	P4HA1	Prolyl 4-hydroxylase subunit alpha 1	-1.420	0.095
Q52KV8	KIF2A	Kinesin family member 2A	3.500	0.095
042265	PSMA1	Proteasome subunit alpha 1	-1.660	0.097
P68139	ACIA1	Actin, alpha 1, skeletal muscle	3.200	0.098
Q90615	IIGA1	Integrin subunit alpha 1	1.800	0.099

(Continued)

TABLE 1 | Continued

UniProt ID	Proteins	Protein name	Fold change	p Value
Q5ZHP5	CHMP4B	Charged multivesicular body protein 4B	-1.420	0.100
P70079	CKMT1	Creatine kinase U-type, mitochondrial	-1.250	0.100
P09652	TUBB3	Tubulin beta 3 class III	2.500	0.100
P24802	PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	3.000	0.100
Q5ZKC1	EIF2A	Eukaryotic translation initiation factor 2A	∞	0.100

TABLE 2 | Summary of differentially expressed proteins (DEPs) identified in the ileum of broilers exposed to Citrobacter freundii (CF) in ovo.

UniProt ID	Proteins	Protein name	Fold change	p Value
Q6JHU8	P3H1	Prolyl 3-hydroxylase 1	3.200	0.003
Q5ZKC9	YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta	1.500	0.003
Q5Z189	TRAPPC11	Trafficking protein particle complex 11	-1.000	0.007
Q01841	TGM2	Transglutaminase 2	-1.420	0.010
P00940	TPI1	Triosephosphate isomerase 1	1.500	0.015
Q10751	ACE	Angiotensin I converting enzyme	-10.000	0.016
P55080	MFAP1	Microfibril-associated protein 1	7.500	0.018
Q5ZIV5	PDCD10	Programmed cell death protein 10	5.800	0.021
Q9PW72	PDLIM4	PDZ and LIM domain 4	-5.000	0.021
Q5ZM35	TWF2	Twinfilin actin binding protein 2	7.100	0.022
Q90615	ITGA1	Integrin subunit alpha 1	1.600	0.026
Q02391	GLG1	Golgi glycoprotein 1	-4.000	0.028
Q5ZLN0	LRRC40	Leucine rich repeat containing 40	2.200	0.031
Q5ZJ08	YARS	Tyrosyl-tRNA synthetase	-4.000	0.031
Q02020	FGB	Fibrinogen beta chain	-1.600	0.032
P26446	PARP1	Poly(ADP-ribose) polymerase 1	-3.300	0.036
Q9W6H0	OGN	Osteoglycin	1.600	0.037
Q5ZKU5	RAB14	RAB14, member RAS oncogene family	-4.000	0.038
P51890	LUM	Lumican	-2.500	0.042
Q5ZHN4	RP2	RP2, ARL3 GTPase activating protein	-3.300	0.051
P00548	PKM	Pyruvate kinase M1/2	-1.250	0.052
P0CB50	PRDX1	Peroxiredoxin 1	-1.600	0.052
P19966	TAGLN	Transgelin	2.100	0.057
P87362	BLMH	Bleomycin hydrolase	-2.500	0.059
Q08392	Gsta1	Glutathione S-transferase alpha 1	1.700	0.059
P13914	NAT1	N-acetyltransferase 1	2.600	0.059
Q5ZIY5	PPP2R2D	Protein phosphatase 2 regulatory subunit B delta	2.000	0.062
P05083	ASL	Argininosuccinate lyase	2.200	0.064
P23228	HMGCS1	3-Hydroxy-3-methylglutaryl-CoA synthase 1	-3.300	0.064
Q90YH9	TES	Testin LIM domain protein	-2.500	0.067
Q5F381	EPCAM	Epithelial cell adhesion molecule	-1.420	0.069
P56517	HDAC1	Histone deacetylase 1	-4.000	0.070
A0A1N8W591	HDAC1	Histone deacetylase	1.200	0.075
Q90611	MMP2	Matrix metallopeptidase 2	-1.000	0.076
Q5F418	PSMD1	Proteasome 26S subunit, non-ATPase 1	-4.000	0.076
Q5ZIL2	VPS29	VPS29, retromer complex component	-1.000	0.076
Q5F464	LPP	LIM domain containing preferred translocation partner in lipoma	1.300	0.079
Q5ZJ27	HOOK1	Hook microtubule tethering protein 1	-1.000	0.090
P02701	AVD	Avidin	6.200	0.099
Q05744	CTSD	Cathepsin D	1.800	0.100
P12902	HMG-14A	Non-histone chromosomal protein HMG-14A	-3.300	0.100
Q98TF8	RPL22	Ribosomal protein L22	2.000	0.100

TABLE 3 | Differentially expressed proteins (DEPs) in ileum of 10-day-old broilers exposed to Citrobacter spp. (C2) in ovo.

Ph912 ORS Orderine kerses B -1.450 0.002 P147366 P1954X R1000000000000000000000000000000000000	UniProt ID	Proteins	Protein name	Fold change	p Value
PH788 PH780 PH780 Control 0.002 PM7802 CATHL2 Cathlinic/n2 4.000 0.003 PM8122- CATHL2 Cathlinic/n2 4.000 0.003 PM8122- TUBER Tuber interpretation and congation factor: hota 2.2 1.000 0.016 OSZMUB PPP4/B2 Protein phrophatase 4 regulatory stork 3/congaturase 1 3.000 0.020 OSZMUB PP04/B2 Protein phrophatase 4 regulatory stork 3/congaturase 1 3.000 0.021 OSZMUE PE4/EE Congaturation 5/congaturase 1 3.000 0.021 OSZMUE CONTIO CORFA-FOT Transcription configurase storum 10 -5.000 0.021 OSZMUE FEA FEA FEA -7.000 0.032 OSZMUE FEA FEA FEA -7.000 0.032 OSZMUE FEA FEA Argeneroscolarite base -7.000 0.032 OSZMUE FEA FEA Argeneroscolarite base -7.000 0.032 OSZMUE FEA FEA andatand p	P05122	CKB	Creatine kinase B	-1.250	0.002
CAHL7 CAPLB2 Capter Simse Bindform 4 -10.000 0.003 POSIB2.5 CPC B2 Capter Simse Bindform 4 -10.000 0.008 POVMO1 EEF B2 Exacyon transition is compatin factor 1 bata 2 18.00 0.008 OXMO1 EEF B2 Exacyon transition is compatin factor 1 bata 2 18.00 0.008 OXMO1 Poster property transition informatine - chonganome 1 3.400 0.001 OXZMA RAOT1 DORTA NOT transcription compets statumt 10 -1.600 0.023 OXZMA EFF3 Exacyon transition infoliant factor 3 statumt 31 1.600 0.032 OXZMA EFF3 Exacyon transition infoliant factor 3 statumt 31 1.600 0.035 OXDMA RHP First Site Transition infoliant factor 3 statumt 31 1.600 0.035 OXDMA RHP3 First Site Transition infoliant factor 3 statumt 31 1.600 0.035 OXDMA RHP3 First Site Transition infoliant factor 3 statumt 31 1.600 0.035 OXDMA RHP3 Response montant 31 1.600 0.044	P47836	RPS4X	Ribosomal protein S4 X-linked	-1.600	0.002
PB152:5 CPK-B Costativ kinase B lockorm 4 -1.0000 0.008 P06662 TUBBS Takan to a Sauss III 2.000 0.008 CAVID1 EFF182 Extrapolation takan to thata 2 n.001 P24802 P1001 P0colagen-ysins2-cologitation to thata 2 n.001 P24802 CM0110 Costand to moly solution to thata 4 n.001 0.022 OX2/V2 CM0110 COStand to thata 4 n.001 0.023 OX2/V2 CM0110 COStand to thata 4 n.001 0.023 OX2/V2 CM0110 COStand to thata 4 n.001 0.023 OX2/V2 CM0110 CM4400 to thata 4 0.003 0.033 OX2/V3 ITGA1 Integration takan 1 1.600 0.032 OX2/V4 IFP2 FIR3 4 CTFFase advanting protein 13	Q2IAL7	CATHL2	Cathelicidin-2	4.500	0.003
P6662 TUB8 TUb/in beta 3 class III 2,000 0.006 0079G1 EFF182 Ekayopic translation inspirator factor 1 budy 1,800 0.008 0227U0 PF.001 Potoal party-spirator 4 regulatory suburit 2 n 0.008 0227H5 CHMP4R2 Charged multivesicular body protein 4B -1,603 0.028 0227H2 CN0110 COMPATT Faranceptica compare suburit 10 1,600 0.002 026204 EFS1 Ekarged translation failout factor 3 suburit 3Ph 1 1,600 0.003 026005 ITGA1 Infigrin suburit 4ph 1 1,600 0.003 026005 ITGA1 Infigrin suburit 4ph 1 -1,420 0.003 026201 EFP3 Exarged translation infigrin factor 2A -50 0.003 026201 EFP3 Ekayopic translation infigrin factor 3 suburit 4ph 1 1,600 0.003 026201 EFP3 Ekayopic translation infigrin factor 3 suburit 4ph 2 -1,420 0.003 026201 EFP3 Ekayopic translation infigrin factor 3 -1,420 0.003 0262	P05122-5	CPK-B	Creatine kinase B Isoform 4	-10.000	0.006
GDYGO1 EFF182 Extenyoto transition elongation factor 1 bota 2 1.800 0.008 GZXUUS PPPPPPP Protologan-sjama 2 acoglutarels 5-diopanaes 1 3.400 0.002 GZXPPS CAMPAB Chegan and antibacia dar bota dar protein 4B -1.800 0.028 GZXPPS CAMPAB Chegan antibacia dar bota dar protein 4B -1.800 0.028 GZXPAS EFS1 Extenyoto transition instator 3 shount 10 -8.000 0.038 GQXDAS HGA1 Integrator transcription complex subunit 10 -8.000 0.038 GQXDAS HGA1 Integrator transcription complex subunit 10 -8.000 0.038 GQXDAS HGA1 Extenyoto transcription factor 2A -8.000 0.038 GZXDAS HGA1 Extenyoto transcription conductase, Freese consultur polypotof 1 1.800 0.0041 GZXDAS UGXGRFS1 UGUNARS-Consume conductase, Freese consultur polypotof 1 1.800 0.0401 GZXDAS HMACG1 Baryotary sambalia distation conductase, Freese consultur polypotof 1 1.800 0.0401 GZXDAS HMACG1 Baryota	P09652	TUBB3	Tubulin beta 3 class III	2.900	0.006
Op/2010 PP074P/2 PP0760 protein properties 4-regulatory submit 2 ** 0.016 PR4000 PR0001 Processpecifies 34.00 0.000 Op/2014 CNOT10 CORD4-NOT1 manocryptic complex submit 10 -1.000 0.021 Op/2014 Elf-301 Elf-301 1.800 0.032 Op/2014 Elf-301 Elf-301 0.032 Op/2014 Elf-301 Elf-301 0.032 Op/2014 Elf-301 Elf-301 0.032 Op/2014 Elf-301 Condentaria 0.030 Op/2014 Elf-301 Condentaria 0.030 Op/2014 Elf-301 Propering protein 13 1.700 0.031 Op/2014 TITA Transfityperine 1.700 0.031 Op/2014 TITA Transfityperine 1.700 0.031 Op/2014 TITA Transfityperine 1.700 0.035 Op/2014 TTA Transfityperine 1.700 0.035 Op/2014 Propering/1.4467.01 memore 2	Q9YGQ1	EEF1B2	Eukaryotic translation elongation factor 1 beta 2	1.800	0.008
P4402 PLOD Procellagen-sense2-cooglutantes-clooxygenes 1 3.400 0.020 OR2PHPS CHW94B Churgen Linkesicable bypy presin 48 -1.600 0.021 0521W2 CNOT10 CCPA+NOT transcription complex subunt 10 -5.000 0.028 05001 HSP Falvapskem internit-binding presin 39 subunt 10 1.600 0.039 050010 HSP Par upatesm internit-binding presin 29 -1.420 0.039 0521M1 PP2 PR2_ARL3 GTPae activating protein -3.300 0.039 0527K1 EP2 RP2_ARL3 GTPae activating protein -3.400 0.044 0521K1 Displand-synchrome creatures and transcreature polypeptide 1 1.600 0.044 0521K1 RP411 Transcreature presin 1.600 0.044 0521K1 PR40M1 Polypolycaster mutates 1.600 0.044 0521K1 RP40M1 Polypolycaster mutates 1.600 0.051 0521K1 PR40M1 Polypolycaster mutates 1.600 0.051 052228 HW6059 Street mutates	Q5ZMU6	PPP4R2	Protein phosphatase 4 regulatory subunit 2	∞	0.016
CA2HPS CHMP4B Charged multihesical body protein 4B -1.600 0.028 CASTM2 Ch0110 CCR4-NOT transcription complex subunit 10 -5.000 0.028 CASTM2 Elkasyntc translation inflation factor 3 suburit 10 -1.600 0.029 CASM5 ITGA1 Integrin suburit fagina 1 -1.600 0.039 CASTM4 PEP2 PP2.AR13.GTTRese achieving protein -3.300 0.037 CASTM4 RP2 PP2.AR13.GTTRese achieving protein -3.300 0.037 CASZM5 PEP1.3 Bibascharl protein reductase, Rieske ron-suftur polypoptde1 1.000 0.044 PE77.31 TTR Translyrukin -1.420 0.0064 CASZM5 HMCCS1 3-Hydroxy-3-methylutas/-COA synthase 1 -3.300 0.051 PE328 HMCCS1 3-Hydroxy-3-methylutas/-COA synthase 1 -1.420 0.051 PC3248 HMCCS1 3-Hydroxy-3-methylutas/-COA synthase 1 -1.420 0.051 PC3248 HMCCS1 A-Hydroxy-3-methylutas/-COA synthase 1 -1.420 0.051 PC3248 HMCCS	P24802	PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	3.400	0.020
0x70v/v CR74-NOT insertioption complex suburit 10 -6.000 0.002 0x72vA4 EP3.1 Euterprint transition initian factor 3 suburit 30 1.600 0.002 0x8015 ITGA1 Integrin suburit 3pha 1 1.600 0.0032 0x8010 KHSIP Far usetnem enternt-bring pretein 2 -1.420 0.0035 0x2XrM4 RP2 RP2, ARLS GTPase activating protein 2 -3.300 0.037 0x2XrM1 RP2 RP2, ARLS GTPase activating protein 2 -3.300 0.044 0x2XrM1 RP13 Resonal protein L13 1.700 0.044 0x2XrM1 RQM1 Prosphoglycentine mutate 1 -3.300 0.051 0x2XrM1 RQM1 Prosphoglycentine mutate 1 -3.300 0.051 0x2XrM1 RQM1 Prosphoglycentine mutate 1 -1.420 0.051 0x2XrM2 RAM1 Lassinger and transporting V1 suburit 801 -1.420 0.051 0x2XrM1 RQM1 Lassinger and transporting V1 suburit 81b2 -1.420 0.051 0x2XrM2 ARPM1 Arparagine synthetises (y	Q5ZHP5	CHMP4B	Charged multivesicular body protein 4B	-1.600	0.021
QE2/A4 EFAJ Exkery/orb translation intestion factor 3 subunit J 1.600 0.032 QB0B15 ITGA1 Integrin subunit alpha 1 1.600 0.032 QB0B30 KH-SPP Fur gathmen element-trinding protein 2 -1.420 0.033 QC2MVA RP2 RP2.ARLS GTPBase activating protein 2 -0.030 0.037 QC2KC1 BPL3 Buckary/orb translation infation factor 2A no 0.030 QC2KC1 BPL3 Buckary/orb translation infation factor 2A no 0.030 QC2KC1 BPL3 Buckary/orb translation infation factor 2A no 0.031 QC2KC1 BPL3 Buckary/orb translation infation factor 2A subtary 1.600 0.041 QC2K1 TR Translation infation factor 3A subtary of translation infation factor 3A subtary	Q5ZIW2	CNOT10	CCR4-NOT transcription complex subunit 10	-5.000	0.028
C00615 ITGA1 Integrin subunt laphen 1 1.000 0.032 C08UN09 KHSRP For upstream element-binding protein 2 -1.420 0.033 C62HN4 RP2 RP2, AFL3 CTPase activating protein 2 -3.300 0.037 C62HV1 ElF2A Elkayolic branistion initiation factor 2A ∞ 0.039 PA1125 ElF2A Elkayolic branistion initiation factor 2A ∞ 0.034 C52LN1 CQCRFS1 Ubicuino-hytochrome creductase, Reske icon-sulfur polypeptide 1 1.600 0.044 C52LN1 PGAM1 Phosphoolycentate mutase 1 -1.420 0.051 C52LN1 PGAM1 Phosphoolycentate mutase 1 -1.420 0.051 C52LN3 ASN Apparagine synthetase (glutamine-hydrohydrohydrohydrohydrohydrohydrohydro	Q5ZKA4	EIF3J	Eukaryotic translation initiation factor 3 subunit J	1.600	0.032
QBUMD HSBRP Fur upstream dement-binding protein 2 -1.420 0.035 P65083 ASL Arginnosucontate lyase 2.300 0.035 QSZ-MA RP2 RP2.AFL3 GTPase activating protein -3.300 0.037 QSZ-MST EFPA Eukaryotic transition initiation factor 2A n 0.037 QSZ-RS UQCRFST Ubicquinol-cytochrome o reductase, Riseke iron-sulfur polypeptide 1 1.600 0.041 QSZ-RS UQCRFST Ubicquinol-cytochrome o reductase, Riseke iron-sulfur polypeptide 1 1.600 0.041 P2723 TTR Transtyrerin 1.620 0.051 P3050 HSPA2 Heat shock protein family A (Hsp70) member 2 1.420 0.051 P26233 LETM1 Lautone zipper and E-hand containing transmembrane protein 1 2.400 0.053 P264797 APPV1B2 ATPase N + remaporting VI subunit B2 1.600 0.053 P26497 APP1A2 APPase N + rK + transporting subunit alpha 2 1.420 0.053 P26497 APP1A2 APPase N + rK + transporting subunit alpha 2 1.600 <t< td=""><td>Q90615</td><td>ITGA1</td><td>Integrin subunit alpha 1</td><td>1.600</td><td>0.032</td></t<>	Q90615	ITGA1	Integrin subunit alpha 1	1.600	0.032
PK958 AEL Argininsuscionate lyses 2,200 0.037 GSZ-NM PR2 PR2, ARLS GTPase activating protein -3,300 0.037 GSZ-NM EF2.A Eukaryotic transition initiation factor 2A ~ 0.0404 PX1125 RPL13 Bioannal protein L13 1.700 0.0414 P27731 TTR Transchryetin 1.800 0.049 P27274 TTR Transchryetin 1.800 0.049 P27275 TTR Transchryetin 1.800 0.051 P32282 HMGCS1 Hytory-3-metriphytikytor/CxA synthase 1 -1.420 0.051 P32328 HMGCS1 Arbory-3-metriphytikytor/CxA synthase 1 .0003 0.052 P32328 HMGCS1 Arbory-3-metriphytikytor/CxA synthase 1 .0003 0.052 P32328 HMGVS1 Arbory 3-metriphytikytor/CxA synthase 1 .0003 0.052 P32328 LEFM1 Lanport pripher 3-metriphytikytor/CxA synthase 1 .0003 0.052 P32477 AFP6 Arba shock transporting protein famb 2 .1200	Q8UVD9	KHSRP	Far upstream element-binding protein 2	-1.420	0.034
Qa2HM PP2 PP2, PR12, GPT and activating protein -3.300 0.337 Qa5XC1 EP2A Eudaryotic translation initiation factor 2A ∞ 0.039 P1125 FPL13 Blocanal protein 113 1.700 0.041 Qa5ZLN1 PGAM1 Phosphoglocartis mutase 1 1.400 0.049 Qa5ZLN1 PGAM1 Phosphoglocartis mutase 1 1.420 0.051 P22228 HMGOS1 3-Hydroxysmethyglutary/CoA synthase 1 2.030 0.051 P22228 HMGOS1 2-Hydroxysmethyglutary/CoA synthase 1 2.000 0.051 P22228 HMGOS1 2-Hydroxysmethyglutary/CoA synthase 1 2.000 0.051 P22328 LETM1 Leudrox proper and EF-hand containing transmethrane protein 1 2.000 0.051 P4712 ATBave N+ /K + transporting V1 suburit Bta 2 1.420 0.053 P24377 ATF1A2 ATBave N+ /K + transporting suburit alpha 2 1.420 0.0561 P2449 PP515 F1050 0.053 0.053 0.053 P26840 MP515 <	P05083	ASL	Argininosuccinate lyase	2.300	0.035
CR2XC1 EFA Edwayotic translation initiation factor 2A ∞ 0.039 P41125 PFL13 Filoscermal protein L13 1.700 0.041 CSZLP5 UOCRFS1 Ubiquinc-lycotomotor esdudtase, Fileske ion-sulfur polypeotide 1 1.600 0.044 P27731 TR Transitywetin 1.800 0.0631 P28228 HMGC51 3-hydroxy-3-methylguitug/-CoA synthase 1 -3.300 0.0651 P08106 HSPA2 Heat shock protein family A (Hsp70) member 2 -1.420 0.0651 P49712 ATP6V1B2 ATPase H + transporting V1 suburit B2 1.600 0.053 P24707 ATP14.2 ATPase N + A'+ transporting v1 suburit alpha 2 -2.500 0.053 P24707 ATP14.2 ATPase N + A'+ transporting v1 suburit alpha 2 -1.420 0.053 Q0629 PRSS2 Series protease 2 -2.500 0.053 Q2409 AFF6 ADP toosylaton factor 6 2.000 0.060 Q0629 PRSS2 Series protease 2 -2.500 0.053 Q26050 AFF6 <t< td=""><td>Q5ZHN4</td><td>RP2</td><td>RP2, ARL3 GTPase activating protein</td><td>-3.300</td><td>0.037</td></t<>	Q5ZHN4	RP2	RP2, ARL3 GTPase activating protein	-3.300	0.037
PA1125 PRU13 Bibesonal protein L13 1.700 0.041 Q652LR6 UQCRFS1 Ubiquinol-cytochrome c reductase, Riesk iron-sulfur polypaptide 1 1.600 0.044 Q652LN1 PGAM1 Phosphoglycente mutase 1 -1.420 0.050 Q63228 HMGCS1 31-hydroxy-3-methydlytanyl-CoA synthase 1 -3.300 0.051 Q63248 LETM1 Leucine zipper and EF-hand containing transmembrane protein 1 2.400 0.052 Q63243 LETM1 Leucine zipper and EF-hand containing transmembrane protein 1 2.400 0.052 Q63243 ASNS Asparagine synthetase (glutamine-hydrokying) 1.700 0.053 Q63059 PRSS2 Serien protease 2 -1.420 0.054 Q63069 PRSS2 Relat shock protein family A (Hsp70) member 8 -1.420 0.054 Q63069 PRSS2 Relat shock protein family A (Hsp70) member 6 -1.250 0.056 Q63069 PRSS2 Relat shock protein family A (Hsp70) member 6 -1.250 0.056 Q63069 PRSS2 Relat shock protein family A (Hsp70) member 6 -1.2	Q5ZKC1	EIF2A	Eukaryotic translation initiation factor 2A	∞	0.039
QSZR5 UGQRFS1 Ubiquinol-cytochrome c reductase, Rieske iron-sultur polypeptide 1 1.600 0.044 P27731 TR Transtrynetin 1.800 0.045 P2228 HMGCS1 3-Hydroxy-3-methylgittary/CoA synthase 1 -3.300 0.051 P08106 HSPA2 Heat shock protein family A (Hsp70) member 2 -1.420 0.051 P08107 ATPRV1B2 ATPRV1B2 1.600 0.052 P24797 ATPLA2 ATPase Na + /K + transporting V1 subunit B2 -1.420 0.053 P24797 ATPLA2 ATPase Na + /K + transporting subunit alpha 2 -1.420 0.053 P26790 ATFLA2 ATPase Na + /K + transporting subunit alpha 2 -1.420 0.054 P26790 ATFLA2 ATPase Na + /K + transporting subunit alpha 2 -1.420 0.054 P26840 RPS16 Bibosomal protein family A (Hsp70) member 8 -1.420 0.056 P26840 RPS16 Bibosomal protein family A (Hsp70) member 8 -1.420 0.056 P26840 RPS16 Bibosomal protein family A (Hsp70) member 8 -1.620 0.066	P41125	RPL13	Ribosomal protein L13	1.700	0.041
P2731 TR Transityetin 1.800 0.049 GZLN1 PGAM1 Phosphoglycerate mutase 1 -1.420 0.050 P32228 HM0GS1 3-Hydrox/-3-methydjultanyl-CoA synthase 1 -3.300 0.051 P08106 HSPA2 Heat shock protein family A (Hsp70) member 2 -1.420 0.051 GZX33 LETM1 Leucine zipper and EF-hend containing transmembrane protein 1 2.400 0.052 062/U3 ASNS Asparagine synthetase (glutamine-hydrolyzing) 1.700 0.053 073885 HSPA8 Heat shock protein family A (Hsp70) member 8 -1.420 0.053 073885 HSPA8 Heat shock protein family A (Hsp70) member 8 -1.420 0.0661 020629 PRS52 Serine protease 2 -2.500 0.0561 026890 AFF6 AP hobsylton factor 6 2.600 0.061 026929 CLP1 CAP-Gy domain containing linker protein 1 & 0.062 026920 CLP1 CAP-Gy domain containing linker protein 1 & 0.062 0269540 MVL4	Q5ZLR5	UQCRFS1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	1.600	0.044
QAZLN1 PGAM1 Phosphogyoerate mutase 1 -1.420 0.0651 P32228 HMGCS1 3-Hydroxy-3-methylgilusry-CoA synthase 1 -3.300 0.0651 P32328 LETM1 Leusine zipper and EF-hand containing transmembrane protein 1 2.400 0.051 QAZX33 LETM1 Leucine zipper and EF-hand containing transmembrane protein 1 2.400 0.053 QAZV37 ATP6VTB2 ATPase Na + /K + transporting v1 subunit E2 -1.420 0.053 QAG029 PRSS2 Brine protease 2 -2.500 0.053 QAG038 HSPA Heat shock protein family A (Hps70) member 8 -1.420 0.054 QAG039 PRSS2 Brine protease 2 -2.500 0.056 P26990 ARF6 ADP robosylation factor 6 2.000 0.060 P26990 ARF6 Heat shock protein family A (Hps70) member 5 -1.50 0.061 P00540 MYL4 Mycain light chain 4 0.000 0.663 0.000 0.663 P01944 SPINK5 Serie perplicase inhibitor, Kazal type 5 1.500 0.065	P27731	TTR	Transthyretin	1.800	0.049
P23228 HMGCS1 3-Hydrox-3-methylgutayl-CoA synthase 1 -3.300 0.051 P06106 HSPA2 Heat shock protein family (Hsp70) member 2 -1.420 0.051 P06106 HSPA2 ATP6V1B2 ATP6V1B2 ATP6V1B2 ATP6V1B2 1.600 0.052 QG2L03 ASNS Asparagine synthetase (gutamine-hydrolyging) 1.700 0.053 QG629 PRSS2 Serine protease 2 -2.500 0.053 QG629 PRSS2 Serine protease 2 -2.500 0.053 QG6629 PRSS2 Serine protease 2 -2.500 0.050 P08900 ARF6 ADP rhosylation factor 6 2.600 0.061 P08902 QLP1 CAP-Gly domains (protein 1 ∞ 0.062 P09904 MYL4 Mycain ight chain 4 1.400 0.068 P09910 MYL4 Mycain ight chain 4 1.400 0.068 P099240 MYL4 Mycain ight chain 4 1.600 0.062 P099350 MSRA Serine peritase inhibitor. Kazal type 5	Q5ZLN1	PGAM1	Phosphoglycerate mutase 1	-1.420	0.050
P08106 HSPA2 Heat shock protein family A (Hsp70) member 2 -1.420 0.051 GG2X33 LETM1 Leucine zipper and EF-hand containing transmembrane protein 1 2.400 0.051 P49712 AT66V1B2 ATFase H + transporting V1 subunit B2 1.600 0.053 QG2U3 ASNS Asparagine synthetase (juttamine-hydrolyzing) 1.700 0.053 P4797 ATF1A2 ATFase Na + /K + transporting subunit alpha 2 -1.420 0.053 Q90629 PRS2 Serine protease 2 -2.500 0.051 703885 HSPA8 Heat shock protein family A (Hsp70) member 8 2.000 0.061 703895 HSPA5 Heat shock protein family A (Hsp70) member 5 1.620 0.061 703895 HSPA5 Heat shock protein family A (Hsp70) member 5 1.500 0.066 705083 WDR1 WD orspeat domin 1 ∞ 0.062 705083 WDR1 WD orspeat domin 1 ∞ 0.066 057275 FGB FDinogen beta chain 0.001 0.062 0522.77 RICSA	P23228	HMGCS1	3-Hydroxy-3-methylglutaryl-CoA synthase 1	-3.300	0.051
Q2K33 LETM1 Leucine zipper and EF-hand containing transmembrane protein 1 2.400 0.051 P49712 ATP6V1B2 ATP6V1B2 1.600 0.052 Q5ZJU3 ASNS Asparagine synthetase (jutamine-hydrokyzing) 1.700 0.053 P24797 ATP1A2 ATPase Na + /K + transporting subunit alpha 2 -1.420 0.053 Q30269 PRS2 Serie protease 2 -2.500 0.0561 Q3285 HSPA8 Heat shock protein family A (Hsp70) member 8 -1.420 0.0561 P26960 ARF6 ADP rhosylation factor 6 2.600 0.0661 Q90583 HSPA5 Heat shock protein family A (Hsp70) member 5 -1.250 0.061 Q90583 HSPA5 Beat shock protein family A (Hsp70) member 5 -1.250 0.066 P095840 MYL 4 Myo repeat domain 1 .000 0.066 Q90583 HSPA5 Serine perildase inhibitor, Kazal type 5 1.500 0.066 Q9217 RiC8 Hick ayotic translation elongation factor 1 alpha 1 1.400 0.068 Q9214	P08106	HSPA2	Heat shock protein family A (Hsp70) member 2	-1.420	0.051
P49712 ATP6v1B2 ATPase H + transporting V1 subunit B2 1.600 0.052 GZ.U3 ASNS Asparagine synthetase (glutamine-hydrokyzing) 1.700 0.053 P24797 ATP1A2 ATPase Na + /K + transporting subunit alpha 2 -1.420 0.053 020529 PRSS2 Senie proteese 2 -0.2500 0.053 073885 HSPA8 Heat shock protein family A (Hsp70) member 8 -1.420 0.064 P62846 RPS15 AL000 0.061 0.062 P26990 ARF6 ADP robosylation factor 6 2.600 0.061 P30522 CLIP1 CAP-G/Q domain containing linker protein 1 ∞ 0.062 P09540 MYL4 Myosin light chain 4 1.400 0.063 075083 WDR1 WD repeat domain 1 3.000 0.066 059725 mic60 MICOS complex subunit Mic60 8.000 0.066 02675 FGB Florinogen beta chain 1.600 0.072 02767 FGB Florinogen beta chain 1.600 0.072	Q5ZK33	LETM1	Leucine zipper and EF-hand containing transmembrane protein 1	2.400	0.051
Q5ZJU3 ASNS Asparagine synthetase (jutamine-hydrolyzing) 1.700 0.053 P24797 ATP1A2 ATPase Na + /K + transporting subunt alpha 2 -1.420 0.053 090629 PRSS2 Serine protease 2 -2.500 0.053 073885 HSPA8 Heat shock protein family A (Hsp70) member 8 -1.420 0.054 P2690 ARF6 APP ribosylation factor 6 2.600 0.061 P30593 HSPA5 Heat shock protein family A (Hsp70) member 5 -1.250 0.061 P30622 CLP1 CAP-Gly domain containing linker protein 1 ∞ 0.062 P09540 MVL4 Myosin light chain 4 1.400 0.063 P10184 SPINK5 Serine petidase inhibitor, Kazal type 5 1.500 0.066 059725 mic60 MICOS complex subunit Mic60 8.000 0.068 052L77 RIC8A RIS guarine nucleotide exchange factor A ∞ 0.072 052L7 FGB Fibrinogen beta chain 1.800 0.068 052L07 FQS3 VPS53, GAPP comp	P49712	ATP6V1B2	ATPase H + transporting V1 subunit B2	1.600	0.052
P24797 ATP 1A2 ATP ase Na + /K + transporting subunit alpha 2 -1.420 0.053 Q30629 PRSS2 Serine protease 2 -2.500 0.063 Q73885 HSPA8 Heat shock protein family A (Hsp70) member 8 -1.420 0.056 P26940 ARF6 ADP ribosylation factor 6 2.000 0.061 Q30523 HSPA5 Heat shock protein family A (Hsp70) member 5 -1.250 0.061 P30622 CLP1 CAP-Gy domain containing linker protein 1 ∞ 0.062 P30623 MVL4 Myosin light chain 4 1.400 0.063 P0550 MicK5 Serine peridase inhibitor, Kazal type 5 1.500 0.066 C59725 mic60 MICCS complex subunit Mic60 8.000 0.066 C52L77 RIC8A RIC8 guanine nucleotide exchange factor A ∞ 0.071 C22675 FGB Fibrinogen beta chain 1.600 0.072 C32177 RIC8A Rick synthase 1.600 0.072 C32100 LPF1A1 Eukayotic translation elongation factor 1 al	Q5ZJU3	ASNS	Asparagine synthetase (glutamine-hydrolyzing)	1.700	0.053
Q90629 PRSS2 Serine protease 2 -2.500 0.053 073885 HSPA5 Heat shock protein family A (Hsp70) member 8 -1.420 0.064 P62846 RPS 15 Ribosomal protein S15 2.000 0.060 P62890 ARF6 ADP ribosylation factor 6 2.600 0.061 Q90593 HSPA5 Heat shock protein family A (Hsp70) member 5 -1.250 0.061 P30622 CLIP1 CAP-Gly domain containing linker protein 1 ∞ 0.062 P09540 MYL4 Myosin light chain 4 1.400 0.063 P075083 WDR1 WD repeat domain 1 3.000 0.066 C97275 mic60 MICOS complex subunit Mic60 8.000 0.066 C92675 FGB Florinogen beta chain 1.800 0.068 Q9835 EEF1A1 Eukaryotic translation elongation factor 1 alpha 1 1.400 0.069 Q52L77 NCS3 VPS53, GAPP complex subunit ∞ 0.071 P12276 FASN Fatty acid synthase 1.600 0.072<	P24797	ATP1A2	ATPase Na $+$ /K $+$ transporting subunit alpha 2	-1.420	0.053
073885 HSPA8 Heat hock protein family A (Hsp70) member 8 -1.420 0.054 P62846 RPS15 Ribosomal protein S15 2.000 0.060 P26990 ARF6 AD Pribosylation factor 6 2.600 0.061 90593 HSPA5 Heat shock protein family A (Hsp70) member 5 -1.250 0.061 90622 CLIP1 CAP-CkJ vomain containing linker protein 1 ∞ 0.062 P09540 MYL4 Myosin light chain 4 1.400 0.063 075083 WDR1 WD repeat domain 1 3.000 0.066 055725 mi60 MICOS complex subunit Mic60 8.000 0.066 052L77 RIC8A RIC8 guanine nucleotide exchange factor A ∞ 0.061 052L07 VPS53 VPS53, GARP complex subunit ∞ 0.071 12276 FGB Folynophic translation elongation factor 1 alpha 1 1.400 0.072 052L07 VPS53 VPS53, GARP complex subunit ∞ 0.071 12276 FASN Fatjleaci synthase 1.600	Q90629	PRSS2	Serine protease 2	-2.500	0.053
P62846 RPS15 Ribosomal protein S15 2.000 0.060 P26990 ARF6 ADP ribosylation factor 6 2.600 0.061 Q90593 HSPA5 Heat shock protein family A (Hsp70) member 5 -1.250 0.061 Q90592 CLIP1 CAP-Gly domain containing linker protein 1 ∞ 0.062 P09540 MYL4 Myosin light chain 4 1.400 0.068 P0563 WDR1 WD repeat domain 1 3.000 0.068 C55725 mic60 MICOS complex subunit Mic60 8.000 0.066 C52L77 RIC8A RIC8 guanine nucleotide exchange factor A ∞ 0.066 C52L75 FGB Fibrinogen beta chain 1.800 0.068 Q3025 EEF1A1 Eukaryotic translation elongaton factor 1 alpha 1 1.400 0.069 Q52LD7 VPS53 VPS53, GARP complex subunit ∞ 0.072 P12276 FASN Fatty acid synthase 1.600 0.072 Q52L9 RPA1 Replication protein A1 -2.500 0.075	O73885	HSPA8	Heat shock protein family A (Hsp70) member 8	-1.420	0.054
P26990ARF6ADP ribosylation factor 62.6000.061Q90593HSPA5Heat shock protein family A (Hsp70) member 5-1.2500.061P30622CLIP1CAP-Gly domain containing linker protein 1 ∞ 0.062P09540MYL4Myosin light chain 41.4000.063P075083WDR1WD repeat domain 13.0000.066C95725mic60MICOS complex subunit Mic608.0000.066C9277RIC8ARIC8 guanine nucleotide exchange factor A ∞ 0.068P02675FGBEibrinogen beta chain1.8000.068Q90835EEF1A1Eukryotic translation elongation factor 1 alpha 11.4000.069Q90835EEF1A1Eukryotic translation elongation factor 1 alpha 11.4000.0672P12276FASNFatty acid synthase1.6000.072E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.073Q52JL0RPA1Replication protein AI-2.5000.073Q52JL0LRRC40Leucine rich repeat containing 402.0000.077Q52LN0LRRC40Leucine rich repeat containing 402.0000.077Q52LSCOQ5Cocarzyme C5, methyltransferase1.8000.078Q52LSCOQ5Cocarzyme C5, methyltransferase1.8000.078Q52LSHNRNPDLHetorgeneous nuclear ribonucleoprotein D like1.8000.088P0251HBADHetorgeneous nuclear ribonucleoprotein D like1.800	P62846	RPS15	Ribosomal protein S15	2.000	0.060
Q90593HSPA5Heat shock protein family A (Hsp70) member 5-1.2500.061P30622CLIP1CAP-Gly domain containing linker protein 1∞0.062P09540MYL4Myosin light chain 41.4000.063075083WDR1WD repeat domain 13.0000.063010144SPINK5Serine peptidase inhibitor, Kazal type 51.5000.0665059725mic60MICOS complex subunit Mic608.0000.066026775FGBFibrinogen beta chain1.8000.068090835EFF1A1Eukaryotic translation elongation factor 1 alpha 11.4000.0680262L77VPS53VPS53, GARP complex subunit∞0.071P12276FASNFatty acid synthase1.6000.072E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.072052L10LRRC40Leucine rich repeat containing 402.0000.076052LN0LRRC40Leucine rich repeat containing 402.0000.07705415COC5Coerzyme C5, methyltransferase1.8000.088052L50LUMLumican-4.0000.07805434CALB1Calbindin 1-1.6000.08605217HNRNPDLHetrogeneous nuclear ribonucleoprotein D like1.8000.086052180LUMLumican-1.6000.06805454CALB1Calbindin 1-1.6000.06805215HBADHetrogeneous nuclear ribonucleoprotein D like1	P26990	ARF6	ADP ribosylation factor 6	2.600	0.061
P30622CLIP1CAP-Gly domain containing linker protein 1∞0.062P09540MYL4Myosin light chain 41.4000.063075083WDR1WD repeat domain 13.0000.063P10184SPINK5Serine peptidase inhibitor, Kazal type 51.5000.066C59725mic60MICOS complex subunit Mic608.0000.066C62L77RICBARICB quarine nucleotide exchange factor A∞0.068C90855EEF 1A1Eukaryotic translation elongation factor 1 alpha 11.4000.069C62L77VPS53VPS53, GARP complex subunit∞0.071P12276FASNFatty acid synthase1.6000.072C52L92HSPA14Heat shock protein family A (Hsp70) member 143.2000.072C52L92RPA1Replication protein A1-2.5000.075C52LN0LRRC40Leucine rich repeat containing 402.0000.077C9214HMGN2Succinate dehydrogenase complex iron sulfur subunit B1.9000.076C92L5CO05Coenzyme Q5, methyltransferase1.8000.084P04354CALB1Calbindin 1-1.6000.086C52L72HNRNPDLHerogeneous nuclear ribonucleoprotein D like1.8000.086C92L14HNRDQLLeleingeneous nuclear ribonucleoprotein D like1.8000.086C92L15CO05Coenzyme Q5, methyltransferase1.8000.086C92L14HNRNPDLHerogeneous nuclear ribonucleoprotein D like1.800 </td <td>Q90593</td> <td>HSPA5</td> <td>Heat shock protein family A (Hsp70) member 5</td> <td>-1.250</td> <td>0.061</td>	Q90593	HSPA5	Heat shock protein family A (Hsp70) member 5	-1.250	0.061
P09540 MYL4 Myosin light chain 4 1.400 0.063 075083 WDR1 WD repeat domain 1 3.000 0.063 P10184 SPINK5 Serine peptidase inhibitor, Kazal type 5 1.500 0.0665 059725 mic60 MICOS complex subunit Mic60 8.000 0.0666 052L77 RIC8A RIC8 guanine nucleotide exchange factor A ∞ 0.0668 090825 FGB Fibriogen beta chain 1.800 0.0669 092275 FGB Fibriogen beta chain 1.400 0.069 052LD7 VPS53 VPS53, GARP complex subunit ∞ 0.071 09226 FASN Fatty acid synthase 1.600 0.072 E1C2P3 HSPA14 Heat shock protein family A (Hsp70) member 14 3.200 0.072 052JJ2 RPA1 Replication protein A1 -2.500 0.073 052LN0 LRRC40 Leucine rich repeat containing 40 2.000 0.076 052LN0 LRRC40 Leucine rich repeat containing 40 2.000 0.077 </td <td>P30622</td> <td>CLIP1</td> <td>CAP-Gly domain containing linker protein 1</td> <td>∞</td> <td>0.062</td>	P30622	CLIP1	CAP-Gly domain containing linker protein 1	∞	0.062
O75083 WDR1 WD repeat domain 1 3.000 0.063 P10184 SPINK5 Serine peptidase inhibitor, Kazal type 5 1.500 0.065 O59725 mic60 MICOS complex subunit Mic60 8.000 0.066 O25L77 RIC8A RIC8 guanine nucleotide exchange factor A ∞ 0.066 P02675 FGB Fibrinogen beta chain 1.800 0.068 Q52LD7 VPS53 VPS53, GARP complex subunit ∞ 0.071 P12276 FASN Fatty acid synthase 1.600 0.072 G52LD7 NPS14 Heat shock protein family A (Hsp70) member 14 3.200 0.072 G52LJ2 RPA1 Replication protein A1 -2.500 0.073 G52LJ2 RPA1 Replication protein A1 -2.500 0.076 G52LS0 LRRC40 Leuice rich rich repeat containing 40 2.000 0.076 G52LS1 SDHB Succinate dehydrogenase complex iron sulfur subunit B 1.900 0.078 G52LS2 COQ5 Coenzyme Q5, methyltransferase 1.80	P09540	MYL4	Myosin light chain 4	1.400	0.063
P10184SPINK5Serine peptidase inhibitor, Kazal type 51.5000.0665059725mic60MICOS complex subunit Mic608.0000.0666Q5ZL77RIC8ARIC8 guanine nucleotide exchange factor A ∞ 0.066P02675FGBFibrinogen beta chain1.8000.06809835EEF1A1Eukaryotic translation elongation factor 1 alpha 11.4000.06905ZLD7VPS53, GARP complex subunit ∞ 0.071P12276FASNFatty acid synthase1.6000.072E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.07205ZJL7RPA1Replication protein A1-2.5000.07505ZJL7RPA1Replication protein A1-2.5000.07505ZJL7BPA1Non-histone chromosomal protein HMG-17-2.5000.07505ZLN0LRRC40Leucine rich repeat containing 402.0000.076093256KRT19keratin 199.7000.07709YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.07805ZLSCOQ5Coenzyme Q5, methyltransferase1.8000.08605ZI72HNRNPDLHetrogeneous nuclear ribonucleoprotein D like1.8000.08605ZL5CALB1Calbindi 1-1.6000.08605ZL5HSRDHetrogeneous nuclear ribonucleoprotein D like1.8000.086052L5HSRDHetrogeneous nuclear ribonucleoprotein D like1.8000.086052	075083	WDR1	WD repeat domain 1	3.000	0.063
OS9725mic60MICOS complex subunit Mic608.0000.066Q5ZL77RIC8ARIC8 guanine nucleotide exchange factor A ∞ 0.066P02675FGBFibrinogen beta chain1.8000.068Q9835EEF1A1Eukaryotic translation elongation factor 1 alpha 11.4000.069Q5ZL77VPS53VPS53, GARP complex subunit ∞ 0.071P12276FASNFatty acid synthase1.6000.072E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.072Q5ZJL7VPS53Non-histone chromosomal protein HMG-17-2.5000.073Q5ZJ4HMGN2Non-histone chromosomal protein HMG-17-2.5000.075Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.077Q9YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P13890LUMLumican-4.0000.079Q5ZL5COQ5Coenzyme Q5, methyltransferase1.8000.088Q52172HNRNPDLHeterogeneous nuclear ribonucleoportein D like1.8000.088P02010HEADHongobin subunit alpha-D1.8000.088P02021ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.078P09102P4HBProlyl 4-hydroxylase subunit beta1.2500.098	P10184	SPINK5	Serine peptidase inhibitor, Kazal type 5	1.500	0.065
QSZL77 RIC8A RIC8 guanine nucleotide exchange factor A ∞ 0.066 P02675 FGB Fibrinogen beta chain 1.800 0.068 Q09835 EEF1A1 Eukaryotic translation elongation factor 1 alpha 1 1.400 0.069 Q5ZL07 VPS53 VPS53, GARP complex subunit ∞ 0.071 P12276 FASN Fatty acid synthase 1.600 0.072 E1C2P3 HSPA14 Heat shock protein family A (Hsp70) member 14 3.200 0.073 Q5ZJJ2 RPA1 Replication protein A1 -2.500 0.073 P02314 HMGN2 Non-histone chromosomal protein HMG-17 -2.500 0.075 Q5ZLN0 LRRC40 Leuine rich repeat containing 40 2.000 0.076 Q93256 KRT19 keratin 19 9.700 0.077 Q9YH72 SDHB Succinate dehydrogenase complex iron suffur subunit B 1.900 0.078 Q52L5 COQ5 Coenzyme Q5, methyltransferase 1.800 0.084 P04354 CALB1 Calbincin 1	O59725	mic60	MICOS complex subunit Mic60	8.000	0.066
P02675FGBFibrinogen beta chain1.8000.068Q90835EEF1A1Eukaryotic translation elongation factor 1 alpha 11.4000.069Q5ZLD7VPS53VPS53, GARP complex subunit ∞ 0.071P12276FASNFatty acid synthase1.6000.072E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.073Q5ZJJ2RPA1Replication protein A1-2.5000.073P02314HMGN2Non-histone chronosomal protein HMG-17-2.5000.076Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.076O93256KRT19keratin 199.7000.077Q9YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLumican-4.0000.079Q5ZL5COQ5Coenzyme Q5, methyltransferase1.8000.084P04354CALB1Calbindin 1-1.6000.086P02011HBADHerogeneous nuclear ribonucleoprotein D like1.8000.088P02051ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.093	Q5ZL77	RIC8A	RIC8 guanine nucleotide exchange factor A	∞	0.066
Q90835EEF1A1Eukaryotic translation elongation factor 1 alpha 11.4000.069Q5ZLD7VPS53VPS53, GARP complex subunit ∞ 0.071P12276FASNFatty acid synthase1.6000.072E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.073Q5ZJJ2RPA1Replication protein A1-2.5000.073P02314HMGN2Non-histone chromosomal protein HMG-17-2.5000.075Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.076Q93256KRT19keratin 199.7000.077Q9YH72SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLumican-4.0000.078Q5ZL5COQ5Coenzyme Q5, methyltransferase1.8000.086Q52172HNRNPDLHeterogeneous nuclear ribonucleoprotein D like1.8000.088P02011HBADHemoglobin subunit alpha-D1.8000.088P08251ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.090	P02675	FGB	Fibrinogen beta chain	1.800	0.068
QSZLD7 VPS53 VPS53, GARP complex subunit	Q90835	EEF1A1	Eukaryotic translation elongation factor 1 alpha 1	1.400	0.069
P12276FASNFatty acid synthase1.6000.072E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.072Q5ZJJ2RPA1Replication protein A1-2.5000.073P02314HMGN2Non-histone chromosomal protein HMG-17-2.5000.075Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.076093256KRT19keratin 199.7000.077Q9YH72SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLumican-4.0000.078Q5ZLL5COQ5Coenzyme Q5, methyltransferase1.8000.084P04354CALB1Calbindin 1-1.6000.086P02001HBADHerogeneous nuclear ribonucleoprotein D like1.8000.088P08251ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.098	Q5ZLD7	VPS53	VPS53, GARP complex subunit	∞	0.071
E1C2P3HSPA14Heat shock protein family A (Hsp70) member 143.2000.072Q5ZJJ2RPA1Replication protein A1-2.5000.073P02314HMGN2Non-histone chromosomal protein HMG-17-2.5000.075Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.076093256KRT19keratin 199.7000.077Q9YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLumican-4.0000.079Q5ZLL5COQ5Coenzyme Q5, methyltransferase1.8000.086P04354CALB1Calbindin 1-1.6000.086P02001HBADHenoglobin subunit alpha-D1.8000.088P08251ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.090	P12276	FASN	Fatty acid synthase	1.600	0.072
Q5ZJJ2RPA1Replication protein A1-2.5000.073P02314HMGN2Non-histone chromosomal protein HMG-17-2.5000.075Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.076093256KRT19keratin 199.7000.077Q9YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLumican-4.0000.079Q5ZLL5COQ5Coenzyme Q5, methyltransferase1.8000.084P04354CALB1Calbindin 10.086P02001HBADHerogeneous nuclear ribonucleoprotein Dike1.8000.088P08251ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.090	E1C2P3	HSPA14	Heat shock protein family A (Hsp70) member 14	3.200	0.072
P02314HMGN2Non-histone chromosomal protein HMG-17-2.5000.075Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.076093256KRT19keratin 199.7000.077Q9YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLumican-4.0000.079Q5ZLL5COQ5Coenzyme Q5, methyltransferase1.8000.086P04354CALB1Calbinin 1-1.6000.086P02001HBADHerogeneous nuclear ribonucleoprotein D like1.8000.088P08251ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.090	Q5ZJJ2	RPA1	Replication protein A1	-2.500	0.073
Q5ZLN0LRRC40Leucine rich repeat containing 402.0000.076093256KRT19keratin 190.077Q9YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLunican-4.0000.079Q5ZLL5COQ5Coenzyme Q5, methyltransferase1.8000.084P04354CALB1Calbinin 1-1.6000.086Q5Z172HNRNPDLHetrogeneous nuclear ribonucleoprotein D like1.8000.088P02001HEADHemoglobin subunit alpha-D1.8000.088P08251ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.090	P02314	HMGN2	Non-histone chromosomal protein HMG-17	-2.500	0.075
Q93256 KRT19 keratin 19 9.700 0.077 Q9YHT2 SDHB Succinate dehydrogenase complex iron sulfur subunit B 1.900 0.078 P51890 LUM Lumican -4.000 0.079 Q5ZLL5 COQ5 Coenzyme Q5, methyltransferase 1.800 0.084 P04354 CALB1 Calbindin 1 -1.600 0.086 Q5ZI/2 HNRNPDL Heterogeneous nuclear ribonucleoprotein D like 1.800 0.088 P02001 HBAD Hemoglobin subunit alpha-D 1.800 0.088 P08251 ATP1B1 ATPase Na+/K+transporting subunit beta 1 -2.500 0.089 P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.099	Q5ZLN0	LRRC40	Leucine rich repeat containing 40	2.000	0.076
Q9YHT2SDHBSuccinate dehydrogenase complex iron sulfur subunit B1.9000.078P51890LUMLumican-4.0000.079Q5ZLL5COQ5Coenzyme Q5, methyltransferase1.8000.084P04354CALB1Calbindin 1-1.6000.086Q5ZI72HNRNPDLHetrogeneous nuclear ribonucleoprotein D like1.8000.086P02001HBADHemoglobin subunit alpha-D1.8000.088P08251ATP1B1ATPase Na+/K+transporting subunit beta 1-2.5000.089P09102P4HBProlyl 4-hydroxylase subunit beta-1.2500.090	O93256	KRT19	keratin 19	9.700	0.077
P51890 LUM Lumican -4.00 0.079 Q5ZLL5 COQ5 Coenzyme Q5, methyltransferase 1.800 0.084 P04354 CALB1 Calbindin 1 -1.600 0.086 Q5Z172 HNRNPDL Heterogeneous nuclear ribonucleoprotein D like 1.800 0.086 P02001 HBAD Hemoglobin subunit alpha-D 1.800 0.088 P08251 ATP 1B1 ATPase Na+/K+transporting subunit beta 1 -2.500 0.089 P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.090	Q9YHT2	SDHB	Succinate dehydrogenase complex iron sulfur subunit B	1.900	0.078
CSZLL5 COQ5 Coenzyme Q5, methyltransferase 1.800 0.084 P04354 CALB1 Calbindin 1 -1.600 0.086 Q5Z172 HNRNPDL Heterogeneous nuclear ribonucleoprotein D like 1.800 0.086 P02001 HBAD Hemoglobin subunit alpha-D 1.800 0.088 P08251 ATP1B1 ATPase Na+/K+transporting subunit beta 1 -2.500 0.089 P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.090	P51890	LUM	Lumican	-4.000	0.079
P04354 CALB1 Calbindin 1 -1.600 0.086 Q5Z172 HNRNPDL Heterogeneous nuclear ribonucleoprotein D like 1.800 0.086 P02001 HBAD Hemoglobin subunit alpha-D 1.800 0.088 P08251 ATP1B1 ATPase Na+/K+transporting subunit beta 1 -2.500 0.089 P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.090	Q5ZLL5	COQ5	Coenzyme Q5, methyltransferase	1.800	0.084
Q5Z172 HNRNPDL Heterogeneous nuclear ribonucleoprotein D like 1.800 0.086 P02001 HBAD Hemoglobin subunit alpha-D 1.800 0.088 P08251 ATP1B1 ATPase Na+/K+transporting subunit beta 1 -2.500 0.089 P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.090	P04354	CALB1	Calbindin 1	-1.600	0.086
P02001 HBAD Hemoglobin subunit alpha-D 1.800 0.088 P08251 ATP1B1 ATPase Na+/K+transporting subunit beta 1 -2.500 0.089 P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.090	Q5ZI72	HNRNPDL	Heterogeneous nuclear ribonucleoprotein D like	1.800	0.086
P08251 ATP1B1 ATPase Na+/K+transporting subunit beta 1 -2.500 0.089 P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.090	P02001	HBAD	Hemoglobin subunit alpha-D	1.800	0.088
P09102 P4HB Prolyl 4-hydroxylase subunit beta -1.250 0.090	P08251	ATP1B1	ATPase Na+/K+transporting subunit beta 1	-2.500	0.089
	P09102	P4HB	Prolyl 4-hydroxylase subunit beta	-1.250	0.090

(Continued)

TABLE 3 | Continued

UniProt ID	Proteins	Protein name	Fold change	p Value
P12957	Cald1	Caldesmon 1	-1.250	0.094
P68139	ACTA1	Actin, alpha 1, skeletal muscle	3.200	0.096
P02612	MYL9	Myosin light chain 9	1.700	0.096
P26583	HMGB2	High mobility group box 2	-2.500	0.097
P08940	LECT2	Leukocyte cell derived chemotaxin 2	5.100	0.097
Q5ZKQ5	CASC4	Cancer susceptibility 4	-1.600	0.100
O13268	PSMA7	Proteasome subunit alpha 7	-1.420	0.100
Q5ZHX1	RAP1B	RAP1B, member of RAS oncogene family	1.400	0.100
P27003	S100A10	S100 calcium binding protein A10	∞	0.100



FIGURE 3 | Downstream effects analysis based on differentially expressed proteins from ileal samples of broilers early exposed to lactic acid bacteria mixture (L), *Citrobacter freundii* (CF), or *Citrobacter* spp. (C2). Biological functions involved with inflammation and immune response processes of (A) L vs. S, following (B) CF vs. S, (C) L vs. CF, and (D) L vs. C2. The vertical axis shows the predicted biological function, while the horizontal axis shows the *Z* score calculated for the particular effect.

of eight proteins (CKB, ATP1B1, PRSS2, CALB1, CHMP4B, ATP1A2, HSPA8, and HSPA5) related to the inflammatory mechanism was downregulated. Likewise, seven DEPs (RAP1B, SPINK5, ATP6V1B2, SDHB, LECT2, TUBB3, MYL9, LETM1, ARF6, ITGA1, and EIF2A) were found to be upregulated in the ileum of C2-treated chicks. The Venn diagram showed that nine proteins (ATP1A2, TUBB3, LECT2, MYL9, PRSS2, CKB, ARF6, SPINK5, and CHMP4B) were present throughout L and C2 treatments, and only ITGA1 was shared across L, CF, and C2 (**Supplementary Figure S1**). It is also important to

note that some of the above proteins were implicated in more than one process.

Causal Network

Network analysis, derived from Ingenuity Knowledge Base, was drawn to determine the likely relevant causal relationships for changes in DEPs within the *in ovo* datasets. The top-enriched network in L treatment contained proteins associated with nucleic acid metabolism, skeletal and muscular development, and small molecule biochemistry (score, 44; 19 molecules,



Supplementary Figure S2A). The DEPs identified in this network included ACP1, ACTA1, ACTR2, ADA, AGRN, ATP1A2, BSG, CKB, KIF2A, MX2, MYH9, MYL4, MYL9, P4HA1, PGAM1, RPS17, TARDBP, UBA5, and XDH.

Interestingly, only in the CF dataset, the top-enriched network was composed of several proteins related to immunological and inflammatory functions, as well as the hub regulators Hsp70, nuclear factor- κ B (complex), Vegf, and CD3. This network complex was characterized as cancer, organismal injury, abnormalities, and respiratory disease (42 score, 17 molecules, **Figure 5**), and the determined DEPs were CTSD, EPCAM, HDAC1, LPP, NAT1, OGN, PARP1, PKM, PRDX1, PSMD1, RAB14, RPL22, TES, TPI1, TWF2, YARS, and YWHAZ.

In C2, the most enriched network was linked to cellular function and maintenance, endocrine system disorders, and small molecule biochemistry (score 47, 20 molecules, **Supplementary Figure S2B**). This network consists of 20 proteins in our proteomic data set (ATP1A2, ATP1B1, ATP6V1B2, Cald1, CLIP1, HMGCS1, HSPA5, ITGA1, KRT19, LRRC40, LUM, MYL4, P4HB, PGAM1, PRSS2, RAP1B, RPS15, RPS4X, S100A10, and SDHB).

Correlation Analysis

Spearman's rank correlation was performed to search for potential positive relationships between the microbial

composition and the intestinal inflammatory related-proteins (**Figure 6** and **Supplementary Table S6**). Correlation analysis of the broilers' intestinal proteome and bacterial abundance revealed a positive relationship between *Candidatus Savagella*, also known as segmented filamentous bacteria (SFB), and myosin light chain 9 (MYL9; R = 0.95, p = 0.051) and Integrin alpha-1 (ITGA1; R = 0.95, p = 0.051). In addition, *Enterococcus* was positively associated with serine peptidase inhibitor Kazal type 5 (SPINK5; R = 0.95, p = 0.051).

DISCUSSION

Given the magnitude of understanding the cross-talk between intestinal microbiota and host physiology, the impact of the early intestinal colonization on the microbiota in young broilers has previously been addressed by our lab (Rodrigues et al., 2019; Wilson et al., 2019). To comprehensively complement these previous studies, we used a proteomic approach to examine the ileal protein composition in response to the early exposure to L-based probiotics or *Enterobacteriaceae* strains. The findings presented here indicate that the intestinal pioneer colonization may modulate the immunological functions of young broilers.

Predicted function analyses showed enhanced annotation related to inflammation signaling in broilers *in ovo* treated



with L (*Z* score = 1.80; **Figure 3A**) and CF (*Z* score = 2.32; **Figure 3B**). The prediction of inflammatory signaling found in L treatment was supported by the upregulation of a marker of inflammation, such as high mobility group box 1 (HMGB1; Yang and Tracey, 2010). Furthermore, treatment-specific biological functions found in L were associated with inflammatory response (**Figure 3C**). In general, the inflammatory response may be beneficial for the host as an acute and transient mechanism to mediate clearance of inciting agents in the GIT. Alternatively, the failure to control inflammation could inflict chronic and severe tissue damage (Xiao, 2017; Ptaschinski and Lukacs, 2018).

Currently, the predominant knowledge of intestinal inflammation in poultry is based on dysbiosis and mucosal barrier leakage studies (Kuttappan et al., 2015; Bielke et al., 2017; Ducatelle et al., 2018). These physiological conditions are primarily caused by the proliferation and colonization of pathogens in the intestine. However, the recent multiomics pipelines have identified commensal bacteria not only influencing metabolic and immune function but also triggering a state of tolerance with an inflammation-like response (Buffie and Pamer, 2013; Kogut et al., 2018). It has been proposed that SFB, a Clostridiaceae member, have a dominant effect on the mucosal immune system via stimulation of T cells, such as Th17, and increased proinflammatory cytokines and immunoglobulin A (IgA) production. Owing to the fact that colonization of SFB stimulates IgA release, it has been postulated as one of the mechanisms by which SFB might control pathogen overgrowth (Chen et al., 2018). On the other hand, the excessive immune reactions driven by SFB may also be accompanied by a physiological inflammation status (Ivanov et al., 2009; Ivanov and Littman, 2010; Chung et al., 2012; Buffie and Pamer, 2013). Our microbiome results previously published by Wilson et al. (2019) and Rodrigues et al. (2019) showed the succession of microbial communities colonization through the maturation of microbiota in chicks treated with L in ovo, with no evidence of potential pathogens overgrowth. Nonetheless, the previous findings showed a reduction in Enterococcus abundance and a particular prominent population of SFB in lower ileum of 10-day-old broilers in L treatment (Supplementary Table S1; Rodrigues et al., 2019). In this context, further action was taken to identify a potential microbial signature, which could be highly associated with the observed ileal inflammatory



signaling. Spearman's coefficient analyses revealed a strong positive correlation between SFB population and ITGA1 and MYL9. The protein MYL9 plays a crucial role in the complex system that regulates the contraction of smooth muscle. Recently, Hayashizaki et al. (2016) reported that MYL9 and MYL12 are functional ligands for CD69, suggesting that CD69-MYL9/12 interaction is also involved in recruiting activated cells to inflamed tissues. Indeed, high expression of MYL9/12 was related to sepsis-induced acute kidney injury, inflamed mouse airways, and patients with eosinophilic chronic rhinosinusitis (Wu et al., 2015; Hayashizaki et al., 2016). Similarly, Zhang et al. (2018) identified ITGA1 as an inflammatory-associated gene. Notably, we speculate that the high SFB population may be driving the predicted ileal mucosa proinflammatory status found in broilers early exposed to L. Nevertheless, the mechanism underlying SFB-mediated inflammation is yet unknown and must be further explored. Other groups have hypothesized that the interaction of commensal microbiota and young broilers during the development of the immune system could result in a transient inflammation without tissue damage. To the best of our knowledge, the link between SFB colonization and intestinal inflammation in broilers has not been proposed before. Future research is warranted to validate the SFB-associated proteomic signature generated from mass spectrometry. Such studies have the potential to develop markers of colonization and physiological effects mediated by SFB in human medicine and livestock.

Further support of early-life microbiota in programming immunological functions was shown by the biological annotation and causal network analyses. Treatment with L promoted a differential regulation of systemic immune processes in molecular profiles compared to CF and C2. The exposure of embryos to L enhanced the immune response function annotation associated with activation and trafficking of immune cells (Figures 3C,D), and the top-enriched network was related to skeletal growth (Supplementary Figure S2A). Citrobacter treatments, particularly CF, promoted inhibition of functions linked to immune cell migration and inflammatory response (Figures 3C,D, 5). Besides, based on the up- and downexpression pattern of proteins and the cause-effect relationships existing in Ingenuity Knowledge Base, a network related to injury and abnormalities in the organ was predicted in CF. This network is concerned primarily with the canonical nuclear factor-KB pathway, which is activated during the onset of inflammation (Lawrence, 2009). Our recent research has shown that the neonatal Enterobacteriaceae colonization mediated intestinal proteomic changes accompanied by inflammation in newly hatched chicks (Wilson et al., 2020). Chronic inflammation develops when the immune system is unable to clear a persisting insult, which generates a harmful environment and results in tissue impair (Meirow and Baniyash, 2017). Under all conditions, long-term inflammation can suppress immunity by decreasing immune cell numbers and function and/or increasing active immunosuppressive mechanisms (Dhabhar, 2009). Based on the cumulative findings, it is thought that the introduction of CF *in ovo* produced specific host–microbe interactions, which may have led to dysregulated immunity and immunosuppression by inducing long-term inflammation in 10-day-old broilers.

The maturation of the immune system starts in the first week of life in broilers (Crhanova et al., 2011), and although the immune maturation process can be driven by genetics and environmental conditions, the intestinal microbial composition has been identified to play a significant role in modulating immune responses (Crhanova et al., 2011; Chung et al., 2012; Arsenault et al., 2014; Gollwitzer and Marsland, 2015). Given these pieces of evidence, it is believed that manipulating the intestinal bacteria colonization with early-life exposure to L-based probiotics may modulate the development and maturation of immune functions of broilers. Madej and Bednarczyk (2016) and Pender et al. (2017) have reported that the modification of early-life intestinal microbiota through Lactobacillus-based probiotics in ovo stimulated an immunomodulatory effect in broiler chickens. In addition, the supplementation of host-tailored probiotics has shown to enhance colonization of ileal SFB, which may have contributed to confer immunostimulatory benefits for turkeys (Ward et al., 2019). Our analyses here show that the high colonization of SFB (Rodrigues et al., 2019) may be potentially associated with the enhancement of immune response in L-treated broilers at 10 days of age. These results increased the evidence that host-specific microbiota may drive the intestinal immune maturation (Ivanov et al., 2009; Crhanova et al., 2011; Chung et al., 2012; Buffie and Pamer, 2013; Hedblom et al., 2018).

In summary, the proteomics and bioinformatics analyses presented here suggest that despite shared predicted inflammation pathways by 10 days of age, triggers and inflammatory response were treatment specific in L and CF birds. Based on the microbiome profile previously published by Rodrigues et al. (2019), it is speculated that the high population of SFB in the ileum may be associated with the inflammation-like response found in L treatment, while the early intestinal colonization by Enterobacteriaceae may have caused a low-grade chronic inflammation in the intestine of CF birds. Supporting this, it was highlighted that proper immune function was dependent on specific intestinal microbiota. For instance, exposure of L-based probiotics may have shaped the development of immune functions, whereas the complexity of intestinal microbiota caused by early colonization of Enterobacteriaceae strains may have dysregulated the immunological response in 10-day-old broilers.

REFERENCES

- Arsenault, R. J., Trost, B., and Kogut, M. H. (2014). A comparison of the chicken and turkey proteomes and phosphoproteomes in the development of poultryspecific immuno-metabolism kinome peptide arrays. *Front. Vet. Sci.* 1:22. doi: 10.3389/fvets.2014.00022
- Bielke, L. R., Elwood, A. L., Donoghue, D. J., Donoghue, A. M., Newberry, L. A., Neighbor, N. K., et al. (2003). Approach for selection of individual enteric bacteria for competitive exclusion in turkey poults. *Poult. Sci.* 82, 1378–1382. doi: 10.1093/ps/82.9.1378
- Bielke, L. R., Hargis, B. M., and Latorre, J. D. (2017). "Impact of enteric health and mucosal permeability on skeletal health and lameness in poultry," in

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD015504.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

AUTHOR CONTRIBUTIONS

DR, MT, KW, AD, KC, and WB carried out the project. DR performed analyses, interpreted the results, and wrote the manuscript in consultation with LB and WB. All authors contributed to experimental design, discussed the results, and commented on the manuscript.

FUNDING

This research was supported by the OARDC Research Enhancement Competitive Grants Program (SEEDS) grant no. 2016035, and Arkansas Biosciences Institute (ABI: Little Rock, AR).

ACKNOWLEDGMENTS

The authors would like to thank the Proteomics Lab and Dr. Wilbur Ouma for their bioinformatics assistance. The authors would also like to thank the OARDC Poultry Research Farm for providing eggs and participating in the animal husbandry portion of the study. Lastly, the authors would like to acknowledge and thank the OARDC Research Enhancement Committee Grants Program (SEEDS) and Arkansas Biosciences Institute for providing the funding of this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys. 2020.00020/full#supplementary-material

Understanding the Gut-Bone Signaling Axis: Mechanisms and Therapeutic Implications Advances in Experimental Medicine and Biology, eds L. R. McCabe, and N. Parameswaran (Cham: Springer), 185–197. doi: 10.1007/978-3-319-666 53-2_9

- Buffie, C. G., and Pamer, E. G. (2013). Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* 13, 790–801. doi: 10.1038/ nri3535
- Chen, B., Chen, H., Shu, X., Yin, Y., Li, J., Qin, J., et al. (2018). Presence of segmented filamentous bacteria in human children and its potential role in the modulation of human gut immunity. *Front. Microbiol.* 9:1403. doi: 10.3389/fmicb.2018. 01403

- Chung, H., Pamp, S. J., Hill, J. A., Surana, N. K., Edelman, S. M., Troy, E. B., et al. (2012). Gut immune maturation depends on colonization with a host-specific microbiota. *Cell* 149, 1578–1593. doi: 10.1016/j.cell.2012.04.037
- Crhanova, M., Hradecka, H., Faldynova, M., Matulova, M., Havlickova, H., Sisak, F., et al. (2011). Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar enteritidis infection. *Infect. Immun.* 79, 2755–2763. doi: 10.1128/IAI.01375-10
- Dhabhar, F. S. (2009). Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation* 16, 300–317. doi: 10.1159/000216188
- Duan, M. (2018). Microbiota and immune cell crosstalk: dialogues driving health and disease. *Clin. Transl. Immunol.* 7:e1020. doi: 10.1002/cti2. 1020
- Ducatelle, R., Goossens, E., De Meyer, F., Eeckhaut, V., Antonissen, G., Haesebrouck, F., et al. (2018). Biomarkers for monitoring intestinal health in poultry: present status and future perspectives. *Vet. Res.* 49:43. doi: 10.1186/ s13567-018-0538-6
- Gollwitzer, E. S., and Marsland, B. J. (2015). Impact of early-life exposures on immune maturation and susceptibility to disease. *Trends Immunol.* 36, 684– 696. doi: 10.1016/j.it.2015.09.009
- Hayashizaki, K., Kimura, M. Y., Tokoyoda, K., Hosokawa, H., Shinoda, K., Hirahara, K., et al. (2016). Myosin light chains 9 and 12 are functional ligands for CD69 that regulate airway inflammation. *Sci. Immunol.* 1:eaaf9154. doi: 10.1126/sciimmunol.aaf9154
- Hedblom, G. A., Reiland, H. A., Sylte, M. J., Johnson, T. J., and Baumler, D. J. (2018). Segmented filamentous bacteria – metabolism meets immunity. *Front. Microbiol.* 9:1991. doi: 10.3389/fmicb.2018.01991
- Iqbal, M., Pumford, N. R., Tang, Z. X., Lassiter, K., Wing, T., Cooper, M., et al. (2004). Low feed efficient broilers within a single genetic line exhibit higher oxidative stress and protein expression in breast muscle with lower mitochondrial complex activity. *Poult. Sci.* 83, 474–484. doi: 10.1093/ps/83. 3.474
- Ivanov, I. I., Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498. doi: 10.1016/j.cell.2009.09.033
- Ivanov, I. I., and Littman, D. R. (2010). Segmented filamentous bacteria take the stage. *Mucosal Immunol.* 3, 209–212. doi: 10.1038/mi.2010.3
- Juricova, H., Videnska, P., Lukac, M., Faldynova, M., Babak, V., Havlickova, H., et al. (2013). Influence of *Salmonella enterica* serovar enteritidis infection on the development of the cecum microbiota in newly hatched chicks. *Appl. Env. Microbiol.* 79, 745–747. doi: 10.1128/AEM.02628-12
- Kogut, M. H. (2019). The effect of microbiome modulation on the intestinal health of poultry. Anim. Feed Sci. Technol. 250, 32–40. doi: 10.1016/j.anifeedsci.2018. 10.008
- Kogut, M. H., Genovese, K. J., Swaggerty, C. L., He, H., and Broom, L. (2018). Inflammatory phenotypes in the intestine of poultry: not all inflammation is created equal. *Poult. Sci.* 97, 2339–2346. doi: 10.3382/ps/pey087
- Kong, B., Song, J., Lee, J. Y., Hargis, B. M., Wing, T., Lassiter, K., et al. (2011). Gene expression in breast muscle associated feed efficiency in a single male broiler line using a chicken 44k microarray. I. Top differentially expressed genes. *Poult. Sci.* 90, 2535–2547. doi: 10.3382/ps.2011-01435
- Kong, B.-W., Lassiter, K., Piekarski-Welsher, A., Dridi, S., Reverter-Gomez, A., Hudson, N. J., et al. (2016). Proteomics of breast muscle tissue associated with the phenotypic expression of feed efficiency within a pedigree male broiler line: I. highlight on mitochondria. *PLoS One* 11:e0155679. doi: 10.1371/journal.pone. 0155679
- Krämer, A., Green, J., Pollard, J., and Tugendreich, S. (2014). Causal analysis approaches in ingenuity pathway analysis. *Bioinformatics* 30, 523–530. doi: 10.1093/bioinformatics/btt703
- Kuttappan, V. A., Vicuña, E. A., Latorre, J. D., Wolfenden, A. D., Téllez, G. I., Hargis, B. M., et al. (2015). Evaluation of gastrointestinal leakage in multiple enteric inflammation models in chickens. *Front. Vet. Sci.* 2:66. doi: 10.3389/ fvets.2015.00066
- Lawrence, T. (2009). The Nuclear Factor NF-κB Pathway in Inflammation. *Cold Spring Harb. Perspect. Biol.* 1:a001651. doi: 10.1101/cshperspect.a001651

- Madej, J. P., and Bednarczyk, M. (2016). Effect of in ovo-delivered prebiotics and synbiotics on the morphology and specific immune cell composition in the gut-associated lymphoid tissue. *Poult. Sci.* 95, 19–29. doi: 10.3382/ps/pev291
- Meirow, Y., and Baniyash, M. (2017). Immune biomarkers for chronic inflammation related complications in non-cancerous and cancerous diseases. *Cancer Immunol. Immunother.* 66, 1089–1101. doi: 10.1007/s00262-017-2 035-6
- Nutrient Requirements of Poultry, (1994). Nutrient Requirements of Poultry. Washington, DC: The National Academies Press.
- Pender, C. M., Kim, S., Potter, T. D., Ritzi, M. M., Young, M., and Dalloul, R. A. (2017). In ovo supplementation of probiotics and its effects on performance and immune-related gene expression in broiler chicks. *Poult. Sci.* 96, 1052–1062. doi: 10.3382/ps/pew381
- Ptaschinski, C., and Lukacs, N. W. (2018). "Chapter 2 Acute and Chronic Inflammation Induces Disease Pathogenesis," in *Molecular Pathology (Second Edition)*, eds W. B. Coleman, and G. J. Tsongalis (Cambridge, MA: Academic Press), 25–43. doi: 10.1016/b978-0-12-802761-5.00002-x
- Rodrigues, D. R., Wilson, K., Brings, W., Duff, A., Chasser, K., and Bielke, L. R. (2019). Intestinal pioneer colonizers as drivers of ileal microbial composition and diversity of broiler chickens. *Front. Microbiol.* 10:2858. doi: 10.3389/fmicb. 2019.02858
- Round, J. L., and Mazmanian, S. K. (2009). The gut microbiome shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9, 313–323. doi: 10.1038/nri2515
- Schokker, D., Jansman, A. J. M., Veninga, G., de Bruin, N., Vastenhouw, S. A., de Bree, F. M., et al. (2017). Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. *BMC Genomics* 18:241. doi: 10.1186/s12864-017-3625-6
- Ward, T. L., Weber, B. P., Mendoza, K. M., Danzeisen, J. L., Llop, K., Lang, K., et al. (2019). Antibiotics and host-tailored probiotics similarly modulate effects on the developing avian microbiome, mycobiome, and host gene expression. *mBio* 10:e2171-19. doi: 10.1128/mBio.02171-19
- Wilson, K. M., Rodrigues, D. R., Briggs, W. N., Duff, A. F., Chasser, K. M., and Bielke, L. R. (2019). Evaluation of the impact of in ovo administered bacteria on microbiome of chicks through 10 days of age. *Poult. Sci.* 98, 5949–5960. doi: 10.3382/ps/pez388
- Wilson, K. M., Rodrigues, D. R., Briggs, W. N., Duff, A. F., Chasser, K. M., Bottje, W. G., et al. (2020). Impact of *in ovo* administered pioneer colonizers on intestinal proteome in day of hatch chicks. *Poult. Sci.* (in press). doi: 10.1016/j. psj.2019.10.017
- Wu, F., Dong, X.-J., Li, Y.-Y., Zhao, Y., Xu, Q.-L., and Su, L. (2015). Identification of phosphorylated MYL12B as a potential plasma biomarker for septic acute kidney injury using a quantitative proteomic approach. 8. *Int. J. Clin. Exp. Pathol.* 8, 14409–14416.
- Xiao, T. S. (2017). Innate immunity and inflammation. *Cell. Mol. Immunol.* 14, 1–3. doi: 10.1038/cmi.2016.45
- Yang, H., and Tracey, K. J. (2010). Targeting HMGB1 in inflammation. *Biochim. Biophys. Acta BBA Gene Regul. Mech.* 1799, 149–156. doi: 10.1016/j.bbagrm. 2009.11.019
- Zhang, J., Wang, N., and Xu, A. (2018). Screening of genes associated with inflammatory responses in the endolymphatic sac reveals underlying mechanisms for autoimmune inner ear diseases. *Exp. Ther. Med.* 16, 2460–2470. doi: 10.3892/etm.2018.6479

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

Copyright © 2020 Rodrigues, Wilson, Trombetta, Briggs, Duff, Chasser, Bottje and Bielke. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.