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RAFAELA FURIOSO FERREIRA, DR. MED. VET.

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INTERNATIONAL DUAL DOCTORATE

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Supervisors:

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RAFAELA FURIOSO FERREIRA

**KARAKTERIZACIJA EGZOSOMA
MLIJEKA KRMAČA U RAZLIČITIM
RAZDOBLJIMA LAKTACIJE**

MEĐUNARODNI DVOJNI DOKTORAT

Mentori:
Prof. Vladimir Mrljak
Prof. Helga Sauerwein

Zagreb, 2022



University of Zagreb
Faculty of Veterinary Medicine



UNIVERSITÄT **BONN**
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Characterization of porcine milk exosomes from different stages of lactation

Dissertation
for the degree of
Doctor of Philosophy (Ph.D.)

Faculty of Veterinary Medicine of the University of Zagreb
&
Agricultural Faculty of the Rheinische Friedrich Wilhelms-Universität Bonn

By

Rafaela Furioso Ferreira

From

Ribeirão Preto, Brazil

Bonn, 2022

VETERINARSKI FAKULTET

IZJAVA / STATEMENT

Ja, RAFAELA FURIOSO FERREIRA, potvrđujem da je moj doktorski rad izvorni rezultat mojega rada te da se u njegovoj izradi nisam koristila drugim izvorima do onih navedenih u radu. /

I, RAFAELA FURIOSO FERREIRA confirm that my doctoral work is the original result of my work and that I did not use it in its work to other sources than those mentioned in the paper.



(potpis studenta)

SIGNATURE OF STUDENT

Zagreb, 2022

“Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.”

Marie Curie

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ENGLISH ABSTRACT

CHARACTERIZATION OF PORCINE MILK EXOSOMES FROM DIFFERENT STAGES OF LACTATION

Exosomes are membranous vesicles considered major players in cell-cell communication. Milk provides adequate nutrition, transfers immunity, and promotes neonate development, and milk exosomes are suggested to have a key role in these processes. The ratio of dietary omega-6:omega-3 (ω -6: ω -3) polyunsaturated fatty acids (PUFA) affects health and inflammation, and can induce changes in milk fatty acid composition, but no reports have included the proteome and the lipidome of the exosomal fraction. Milk samples were obtained on days 0, 7, and 14 after parturition from sows receiving either a standard diet (ω -6: ω -3 = 13:1), or a test diet enriched in ω -3 (ω -6: ω -3 = 4:1), and exosomes were isolated using ultracentrifugation coupled with size exclusion chromatography, and characterized by nanoparticle tracking analysis, transmission electron microscopy, and assessment of exosomal markers via Western blotting. The proteome was explored using a Tandem Mass Tag-based high-resolution quantitative approach and the lipidome was assessed using untargeted metabolomics by a liquid chromatography-quadrupole time-of-flight mass spectrometry approach. A total of 319 proteins in each timepoint were identified in milk exosomes. Exosomes from colostrum presented 162 differentially abundant proteins (DAP) as compared to exosomes from milk on day 7, and 170 DAP from milk on day 14. The DAP revealed to be related to biological functions such as uptake of metabolites, regulation of homeostasis, and cellular development. A total of 947 lipids from sixteen subclasses were identified in both colostrum and milk exosomes. When compared to colostrum exosomes, we identified 734 differentially abundant lipids (DAL) in milk exosomes on day 7 and 779 DAL on day 14. Dietary treatments exerted an effect on the milk exosome proteome but not on their lipidome. Results unveil a distinct proteomic and lipidomic profile in porcine milk exosomes in different stages of lactation, with implications for their functional biology, and are relevant for potential strategies using milk exosomes as vehicles for drug or additive delivery.

Keywords: milk, colostrum, exosomes, extracellular vesicles, nutrition,

ABSTRACT AUF DEUTSCH

CHARAKTERISIERUNG VON PORCINEN MILCH-EXOSOMEN AUS UNTERSCHIEDLICHEN LAKTATIONSSTADIEN

Exosomen sind membranhaltige Vesikel, denen eine Hauptrolle in der interzellulären Kommunikation zugesprochen wird. Milch sichert die Nährstoffversorgung, überträgt Immunität und fördert die optimale Entwicklung von Neugeborenen, wobei Exosomen wahrscheinlich zentrale Funktionen haben. Das Verhältnis von ω -6 zu ω -3 mehrfach ungesättigte Fettsäuren (PUFA) in der Nahrung nimmt Einfluss auf die Gesundheit und auf Entzündungsprozesse, und kann auch die Fettsäurezusammensetzung des Milchfetts verändern. Ob diese Veränderungen auch das Proteom und Lipidom der in der Milch enthaltenen Exosomen betreffen, ist bisher unzureichend untersucht. Milchproben von Sauen wurden 0, 7 und 14 Tage post partum von Sauen gewonnen, welche entweder einen erhöhten ω -3 (ω -6: ω -3 = 4:1) Anteil in Futter oder eine Standardration (ω -6: ω -3 = 13:1) erhielten. Im Weiteren wurden die Exosomen aus der Milch mittels Ultrazentrifugation und Größenausschlusschromatographie isoliert und anschließend mit Nanopartikel-Tracking-Analyse, Transmissionselektronenmikroskopie und dem Nachweis von Markerproteinen mittels Western Blot charakterisiert. Das Proteom wurde mittels Tandem-Massenspektrometrie, das Lipidom mit einem ungerichteten Metabolomics-Verfahren (*liquid chromatography-quadrupole time-of-flight mass spectrometry*) analysiert. Insgesamt wurden 319 Proteine/Zeitpunkt bei den aus der Milch isolierten Exosomen identifiziert. Im Vergleich zu den Isolaten von Tag 7 post partum wurden bei den aus Kolostrum isolierten Exosomen 162 in unterschiedlicher Menge vorliegende Proteine (*differentially abundant proteins; DAP*) gefunden, im Vergleich zu Tag 14 wurden 170 DAP identifiziert. Die DAP weisen auf eine veränderte biologische Funktion wie z.B. die Aufnahme von Metaboliten, die Regulierung der Homöostase oder eine veränderte Zellentwicklung hin. Insgesamt wurden in Kolostrum- und Milhexosomen 947 Lipide aus sechzehn Subklassen identifiziert. Im Vergleich zwischen Kolostrum- und Milhexosomen wurden an Tag 7 post partum 734 unterschiedlicher Menge vorliegende Lipide (*differentially abundant lipids; DAL*) identifiziert und 779 DAL an Tag 14. Die unterschiedlichen Futterrationen wirkten sich auf das Proteom der Milhexosomen aus, nicht aber auf ihr Lipidom. Die Ergebnisse zeigen ein unterschiedliches Proteom- und Lipidom-Profil in Milhexosomen von Sauen in verschiedenen Laktationsstadien, was Auswirkungen auf ihre funktionelle Biologie hat. Zudem sind die Resultate für mögliche Ansätze zur

Verwendung von Milhexosomen als Vesikel für die Verabreichung von Medikamenten oder Zusatzstoffen von Bedeutung.

Keywords: Milch, Kolostrum, Exosomen, Extrazelluläre Vesikel, Ernährung, Schwein

PROŠIRENI SAŽETAK / EXTENDED ABSTRACT

KARAKTERIZACIJA EGZOSOMA MLIJEKA KRMAČA U RAZLIČITIM RAZDOBLJIMA LAKTACIJE

UVOD

Egzosomi su membranske vezikule endocitnog podrijetla, koje se smatraju važnim čimbenicima u međustaničnoj komunikaciji. Mlijeko je vrlo složenog sastava koji osigurava adekvatnu prehranu, prijenos imuniteta i potiče adekvatan razvoj odojka. Smatra se da egzosomi mlijeka imaju ključnu ulogu u tim procesima, ali ih tek treba dodatno istražiti, kao i prijenosne mehanizme u kojima sudjeluju egzosomi u različitim fazama laktacije, te njihovu ulogu koja je važna je za razumijevanje različitih čimbenika tijekom laktacije, kao i za poboljšanje mliječnih nadomjestaka za ljude i životinje. Proteom i lipidom izvanstaničnih vezikula (EV) sadrži proteinske sastojke i različite druge aktivne tvari; promjene u tim odjeljcima mogle bi utjecati na njihovu ulogu u komunikaciji između stanica. Poznato je da omjer ω -6 i ω -3 višestruko nezasićenih masnih kiselina (PUFA) u prehrani utječe na zdravlje i upalu te izaziva promjene u sastavu masnih kiselina u mlijeku, ali do sada nijedno istraživanje nije uključivalo frakciju EV u mlijeku. Cilj ovog istraživanja bio je istražiti kako se mijenja sastav egzosoma mlijeka tijekom prijelaza s kolostruma u fazu odbića odojaka, te kako dva različita omjera ω -6 i ω -3 mliječnih kiselina utječu na lipidom i proteom egzosoma mlijeka i kolostruma krmača.

MATERIJALI I METODE

Šesnaest krmača, plotkinja koje su se više puta prasile, te su nasumično raspoređene u dva dijetalna tretmana: kontrolna skupina (Skupina S - GS) primala je standardnu prehranu s omjerom ω -6: ω -3 od 13:1 od 28. dana gravidnosti do prasnjenja, te u omjeru od 10:1 tijekom laktacije); a druga (tretirana) skupina (Grupa L - GL) hranjena je obrokom s niskim omjerom ω -6: ω -3 (4:1 od 28. dana gravidnosti do kraja laktacije, tj. 24 dana nakon prasnjenja). Uzorci mlijeka prikupljeni su 0, 7 i 14 dana nakon partusa tijekom prirodnog izlučivanja mlijeka, zamrznuti odmah nakon prikupljanja i čuvani na -80 °C do izolacije egzosoma. Egzosomi su izolirani ultracentrifugiranjem u kombinaciji s kromatografijom isključenja po veličini čestica

(SEC), karakterizirani analizom praćenja nanočestica (NTA) pomoću NanoSight NS300 instrumenta, zatim transmisijskom elektronskom mikroskopijom (TEM) i metodom Western blot. Izolirani egzosomi pripremljeni su za digestiju u gelu, zatim za obilježavanje peptida izobarnim privjescima (TMT) te analizirani pomoću spektrometra masa spregnutog s tekućinskom kromatografijom (LC-MS/MS). Statističke analize podataka na razini podudaranja peptidnog spektra (PSM) provedene su u "R" programu, verzija 3.6, korištenjem „in house” razvijenog protokola. Analiza genske ontologije (GO) i analiza bioloških puteva provedeni su u STRING softverskoj verziji 11.0 i Cytoscapeu v3.8.2. pomoću ClueGO aplikacije. Lipidi su ekstrahirani Folch metodom, a lipidom izoliranih egzosoma određen je LC-MS/MS spektrometrijom masa. Analiza podataka o lipidima provedena je u MS-DIAL programu primjenom baze podataka LipidBlast, a statistička analiza provedena je na programu MetaboAnalyst 5.0 (<http://www.metaboanalyst.ca>).

REZULTATI I RASPRAVA

Egzosomi mlijeka krmača karakterizirani su analizom praćenja nanočestica, transmisijskim elektronskim mikroskopom i metodom Western blot. Promjene u hranidbi i odnos između vremena i tretmana nisu dali razlike u veličini EV, dok su različite faze laktacije (kolostrum naspram mlijeka) dale razlike. Prosječni EV promjer nije se razlikovao ($p = 0,6$) između dvije skupine (GL = $151 \pm 18,5$; GS = $154 \pm 19,3$; (\pm SD u nm), za izolaciju korištenu u proteomskoj analizi; i GL = $162 \pm 14,7$ GS = $156 \pm 20,6$, za izolaciju korištenu u analizi lipida). Populacija egzosoma bila je nešto manja u kolostrumu (139 ± 19 nm u izolaciji korištenoj u analizi proteoma; i $147 \pm 21,3$ u izolaciji korištenoj u analizi lipida) od mlijeka ($162 \pm 12,5$ u izolaciji primijenjenoj u proteomskoj analizi; i 162 ± 18 u izolaciji koja se koristi u analizi lipida). Ispitivanje TEM metodom pokazalo je okrugle i poput čaše, konkavne sfere s morfologijom kompatibilnom s egzosomima. TSG101, proteinski marker egzosoma, detektiran Western blot metodom, bio je prisutan u mlijeku i egzosomima kolostruma u obje skupine. Rezultati analize metodom spektrometrije masa (LC-MS/MS) također su mapirani prema bazi podataka ExoCarta za oznake egzosoma, a svih 10 najboljih markera bilo je prisutno u uzorcima.

LC-MS/MS analiza mapirala je 3989 peptida koji predstavljaju 637 proteina. Nakon što su primijenjeni kriteriji isključenja ($FDR \leq 0,5\%$ i najmanje dva jedinstvena peptida, uklanjanje suvišnih peptidno-spektralnih podudaranja, single-shot proteina i peptida s visokom interferencijom izolacije), ukupno je 319 proteina u svakoj vremenskoj točki korišteno za statističku analizu. Kvantitativna proteomska analiza otkrila je različite profile proteoma za

egzosome kolostruma i egzosome mlijeka. Egzosomi iz kolostruma imali su 162 različito zastupljena proteina (DAP) (82 povećana i 80 smanjena) u usporedbi s egzosomima iz mlijeka 7. dana, a 170 DAP (81 povećan i 89 smanjen) iz mlijeka 14. dana. Varijacije u proteomu unutar skupina s različitom hranidbom ustanovljene su 7. dana, s razlikama u zastupljenosti proteina spondina-2 i glukozom regulirani protein molekularne težine 78 kDa. Nisu pronađene razlike u egzosomima kolostruma između promatranih skupina. Proteini s funkcijama u razvoju imunološkog odgovora, regulaciji staničnih procesa i staničnom razvoju različito su eksprimirani između kolostruma i egzosoma mlijeka. Funkcionalna analiza istaknula je povećano eksprimirane biološke puteve povezane s regulacijom homeostaze u egzosomima kolostruma, te povećano eksprimirane biološke puteve kao što su razvoj endotelne stanice i metabolizam lipida u egzosomima mlijeka. Funkcionalna analiza različito eksprimiranih proteina između promatranih skupina istaknula je molekule važne za vezikulom posredovani transport (GO:0016192) i transport (GO:0006810), koji bi mogao biti pod utjecajem promjena u sastavu PUFA.

Lipidomska analiza primjenom spektrometrije masa uspjela je identificirati ukupno 947 lipida u uzorcima egzosoma mlijeka i kolostruma. Identificirani lipidi uključivali su 25 glavnih klasa i 47 potklasa lipida. Najzastupljenije klase lipida bili su diacilgliceroli, triacilgliceroli i fosfosfingolipidi, koji se sastoje od sfingolipida, uglavnom sfingomijelina, koji uključuju fosforne skupine. U usporedbi s egzosomima kolostruma, identificirali smo 734 različito zastupljena lipida u egzosomima mlijeka 7. dana i 779 DAL 14. dana. U usporedbi s egzosomima iz mlijeka 7. dana, egzosomi kolostruma imali su niži diacilglicerol (DG) ($n = 176$), triacilglicerol (TG) ($n = 70$), fosfatidilkolin (PC) ($n = 62$), sfingomijelin (SM) ($n = 50$), masne kiseline (FA9 ($n = 48$) i fosfatidiletanolamin (PE) ($n = 48$), a za razliku između dana 0 i dana 14, egzosomi kolostruma imali su niži DG ($n = 193$), TG ($n = 81$), PC ($n = 61$), Cer ($n = 61$), PE ($n = 60$), SM ($n = 58$) i FA ($n = 48$). Nisu uočene značajne razlike u lipidnom sastavu egzosoma mlijeka između 7. i 14. dana, a uglavnom nema razlika u sastavu lipida između tretmana s različitim omjerima hranjenja ω -6 i ω -3 masnih kiselina. Samo jedan lipid (PA 17:0_28:6) bio je smanjen u usporedbi između egzosoma mlijeka iz GS i GL 14. dana. Rezultati otkrivaju specifičan profil lipida u egzosomima mlijeka krmača u različitim fazama laktacije, s mogućim implikacijama na njihovu funkcionalnu biologiju, uključujući razvoj strategija koje koriste upotrebu mliječnih egzosoma kao nosača za isporuku lijekova ili aditiva.

ZAKLJUČCI

Ova studija potvrđuje važnost egzosoma kao aktivnih biokomponenti mlijeka i daje nove temelje za buduća istraživanja uloge egzosoma u regulaciji imuniteta i razvoju odojaka. Identificirani funkcionalni proteom i dobivene mreže interakcija među proteinima u našoj studiji pomažu razjasniti ulogu egzosoma mlijeka u različitim razdobljima laktacije. Buduće studije istražiti će daljnje učinke prehrane na sastav izvanstaničnih vezikula (EV) u mlijeku i njihov odnos prema razvoju mladunčadi. Karakterizacija lipida pruža znanja o strukturi, funkciji i stabilnosti egzosoma mlijeka. Dobiveni rezultati su relevantni za osnovno razumijevanje njihovog utjecaja na razvoj odojka, ali i za strategije koje uključuju upotrebu egzosoma mlijeka kao sredstva za prijenos lijekova ili aditiva te unaprijeđenje znanja za proizvodnju zamjenskih mliječnih proizvoda.

Ključne riječi: mlijeko; kolostrum; egzosomi; izvanstanične vezikule; prehrana; svinja

LIST OF ABBREVIATIONS

| Abbreviation | Meaning |
|---------------------|--------------------------------------|
| AB | Apoptotic bodies |
| ACN | Acetonitrile |
| ACTB | Actin, cytoplasmic 1 |
| AFM | Atomic force microscopy |
| AKT | Protein kinase B |
| ALA | α -linolenic acid |
| ALDOA | Fructose-bisphosphate aldolase A |
| ALIX | Alix ALG-2 interacting protein X |
| ANXA2 | Annexin A2 |
| ANXA5 | Annexin A5 |
| APCS | Serum amyloid P-component |
| ARF | ADP ribosylation factor |
| ARRDC1 | Arrestin domain containing protein 1 |
| A-SMase | Acid sphingomyelinase |
| ATP | Adenosine triphosphate |
| BP | Biological Process |
| BCS | Body condition score |
| BW | Body weight |
| C19orf12 | Protein C19orf12 |
| C2 | Complement component protein 2 |
| C3 | Complement component protein 3 |
| C4orf19 | Chromosome 8 C4orf19 homolog |
| C6 | Complement component protein 6 |
| C7 | Complement component protein 7 |
| CANX | Calnexin |
| CC | Cellular Component |
| CD25 | Interleukin-2 receptor alpha chain |
| CD4 | T-cell surface glycoprotein |

| | |
|------------------|--|
| CD63 | CD63 antigen |
| CD9 | CD9 antigen |
| CD69 | CD69 antigen |
| CD81 | CD81 antigen |
| CD82 | CD82 antigen |
| CE | Cholesterol esters |
| Cer | Ceramides |
| CHMP4A | Charged multivesicular body protein 4a |
| Chol | Cholesterol |
| CR | Control ratio |
| DAP | Differentially abundant proteins |
| DG | Diacylglycerol |
| DG/UC | Density gradient ultracentrifugation |
| DLS | Dynamic light scattering |
| DNA | Deoxyribonucleic acid |
| EEF1A1 | Eukaryotic translation elongation factor 1 alpha 1 |
| EEF2 | Elongation factor 2 |
| EM | Electron microscopy |
| ENO1 | Alpha-enolase |
| ER | Endoplasmic reticulum |
| ERM | Proteins Moesin/ezrin/radixin |
| ESCRT | Endosomal sorting complex required for transport |
| ESCRT-0 | Endosomal sorting complex required for transport 0 |
| ESCRT-I | Endosomal sorting complex required for transport I |
| ESCRT-II | Endosomal sorting complex required for transport II |
| ESCRT-III | Endosomal sorting complex required for transport III |
| EV | Extracellular vesicle |
| EZR | Ezrin |
| FA | Fatty acid |
| FABP3 | Fatty acid-binding protein |
| FASN | Fatty acid synthase |

| | |
|--------------------------------|--|
| FC | Fold change |
| FDR | False discovery rates |
| Foxp3 | Forkhead-Box-Protein |
| FPLC | Fast protein liquid chromatography |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| GB1/RHD3-type G | GB1/RHD3-type G domain-containing protein |
| GO | Gene Ontology |
| HDL | High-density lipoprotein |
| Hp | Haptoglobin |
| HPLC | High Performance Liquid Chromatography |
| HRS | Hepatocyte growth factor-regulated tyrosine kinase substrate |
| HSP | Heat shock protein |
| HSP70 | Heat shock 70 kDa protein 1A |
| HSP90 | Heat shock protein HSP 90 |
| HSP90AA1 | Heat shock protein HSP 90-alpha |
| HSP90AB1 | Heat shock protein HSP 90-beta |
| HSP90B1 | Endoplasmic reticulum chaperone |
| HSPA5 | 78 kDa glucose-regulated protein |
| HSPA8 | Heat shock cognate 71 kDa protein |
| IAC | Immunoaffinity capture |
| IB | Immuno-magnetic beads |
| IFN-γ | Interferon- γ |
| IL-2 | Interleukin-2 |
| ILV | Intraluminal vesicles |
| ISEV | International Society for Extracellular Vesicles |
| ITGAV | Integrin alpha-V |
| IU | Unit |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| LC-ESI-MS/MS | Liquid chromatography electrospray ionization tandem mass spectrometry |
| LA | Linoleic acid |

| | |
|-----------------|--|
| LC-MS/MS | Liquid chromatography coupled with mass spectrometry |
| LDHA | L-lactate dehydrogenase A chain |
| LR | Low ratio |
| MALDI | Matrix assisted laser desorption/ionization |
| MF | Molecular Function |
| MFG | Milk fat globules |
| miRNA | MicroRNAs |
| MISEV | Minimal experimental requirements for definition of extracellular vesicles |
| mRNA | Messenger RNA |
| MS/MS | Tandem mass spectrometry |
| MV | Microvesicles |
| MVB | Multivesicular bodies |
| nSMase2 | Neutral sphingomyelinase 2 |
| NTA | Nanoparticle tracking analysis |
| PC | Phosphatidylcholine |
| PDCD6IP | Programmed cell death 6-interacting protein |
| PE | Phosphatidylethanolamine |
| PGK1 | Phosphoglycerate kinase 1 |
| PI3K | Phosphatidylinositol 3-kinase |
| PKM | Pyruvate kinase PKM |
| PPI | Protein-protein interaction |
| PS | Phosphatidylserine |
| PSM | Peptide-spectrum match |
| PUFA | Polyunsaturated fatty acids |
| QTOF | Quadrupole time-of-flight |
| RAB | RAS-related protein |
| RAL | Ras-related GTPase |
| RDX | Radixin |
| RHOA | Ras homolog family member A |
| RNA | Ribonucleic acid |

| | |
|--------------------------------|---|
| RT | Room temperature |
| SDCBP | Syntenin-1 |
| SDS | Sodium dodecyl sulfate |
| SDS-PAGE | Sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| SEC | Size exclusion chromatography |
| SEM | Scanning electron microscopy |
| SERPINA1 | Alpha-1-antitrypsin |
| SLC9A3R1 | Exchange regulatory cofactor NHE-RF protein |
| SM | Sphingomyelin |
| SNAP23 | Synaptosomal-associated protein 23 |
| SNARE | Soluble NSF attachment protein receptor |
| SPON-2 | Spondin-2 |
| SPR | Surface plasmon resonance |
| STAM1 | Signal transducing adapter molecule 1 |
| Syx1A | Syntaxin 1A |
| TG | Triacylglycerol |
| TEAB | Triethylammonium bicarbonate |
| TEM | Transmission electron microscopy |
| TGF-α | Protransforming growth factor alpha |
| TINAGL1 | Tubulointerstitial nephritis antigen-like |
| TMT | Tandem mass tag |
| TSG101 | Tumor susceptibility gene 101 protein |
| UC | Ultracentrifugation |
| VAMP7 | Vesicle-associated membrane protein 7 |
| VPS4B | Vacuolar protein sorting-associated protein 4B |
| VSN | Variance Stabilizing Normalization |
| WB | Western blotting |
| YKT6 | Synaptobrevin homolog YKT6 |
| YWHAZ | 14-3-3 protein zeta/delta |
| ω-3 | Omega-3 fatty acids |
| ω-6 | Omega-6 fatty acids |

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1 INTRODUCTION

Extracellular vesicles (EV) are membrane-bound nano-sized vesicles released from cells in both physiological and pathological conditions (POL et al., 2012; YUANA et al., 2013). First described in 1967 as “platelet dust” (WOLF, 1967), EV have been increasingly studied for their role in mediating intercellular communication in short and longer-range signaling events and for their ability to transfer various proteins, lipids, DNA, RNA, and metabolites between different cell types (KALRA et al., 2016).

EVs can be classified into three types based on their biogenesis and size: exosomes, ectosomes or shedding microvesicles (MV), and apoptotic bodies (KALRA et al., 2016). Exosomes were first described as vehicles for eliminating specific proteins and for remodeling the plasma membrane for reticulocyte maturation, and were initially proposed to represent cellular waste (JOHNSTONE et al., 1987). Since that, the term “exosomes” has been loosely used to describe many EV (GOULD and RAPOSO, 2013), but the most actual accepted definition describes exosomes as vesicles released to the extracellular environment after fusion of late endosomes/multivesicular bodies (MVB) with the plasma membrane (HESSVIK and LLORENTE, 2018; EDGAR, 2016).

Exosome biogenesis starts on the endocytic pathway: through the inward budding of the plasma membrane, early endosomes mature into late endosomes or MVB - characterized by the presence of vesicles in their lumen (ILV) formed by the invagination of the endosomal membrane (KOWAL et al., 2014). The MVB can either traffic to lysosomes and be subjected to proteasome degradation (i.e., ‘degradative MVB’) or can continue to further steps for releasing exosomes by the transport and fusion of MVB with the plasma membrane (i.e., ‘exocytic MVB’), being the ILV released into extracellular space referred to as ‘exosomes’ (MATHIVANAN et al., 2010). A schematic representation of biogenesis and release of exosomes is shown in Figure 1, indicating several molecules that have been implicated in these processes. The exact process that regulates the fate of MVB in both pathways is still unknown, but it appears that cholesterol levels play a significant role: cholesterol-rich MVB are directed for exosomal release, and cholesterol-poor MVB are directed to lysosomes (MOBIUS et al., 2002).

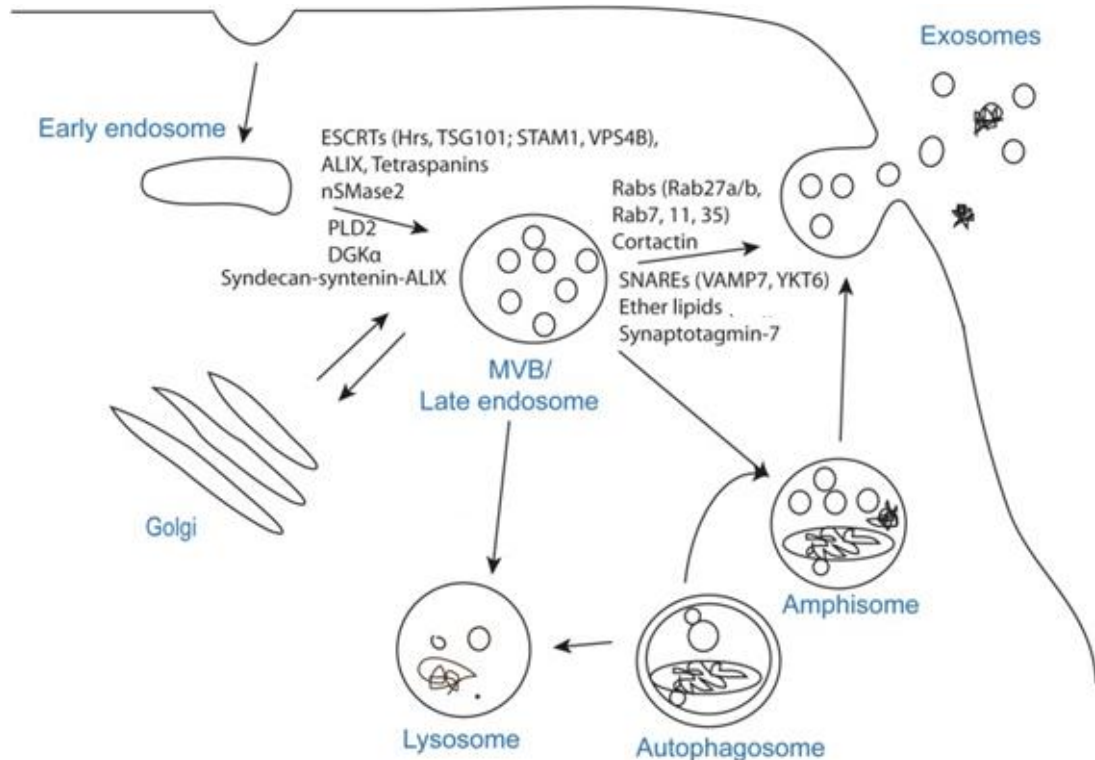


Figure 1. Exosome biogenesis with main molecules shown to affect its formation, transport of MVB to the plasma membrane, and fusion of MVB with the plasma membrane, releasing the exosomes. Abbreviations: ALIX, ALG-2 interacting protein X; DGK, diacylglycerol kinase; ESCRT, endosome sorting complex required for transport; Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate; nSMase2, neutral sphingomyelinase 2; SNAREs, SNAP receptors; RAB, RAS-related protein; STAM1, signal transducing adapter molecule 1; TSG101, tumor susceptibility gene 101; VPS4B, vacuolar protein sorting 4 homolog B; YKT6, synaptobrevin homolog. Adapted from HESSVIK and LLORENTE (2018).

The protein content of exosomes has been recently analyzed from different cell types from cell cultures and different body fluids, by mass spectrometry, Western blotting, and fluorescence-activated cell sorting (YANG et al., 2017; THÉRY et al., 2002). Since exosomes originate from endosomes, proteins involved in MVB formation (e.g., Alix and TSG101), membrane transport and fusion (e.g., annexins, flotillins, GTPases), adhesion (e.g., integrins), tetraspanins (e.g., CD9, CD63, CD81, CD82), antigen presentation (MHC

class molecules), heat shock proteins (HSP70, HSP90) and lipid-related proteins are often identified in exosomes irrespective of the cell type of origin (KALRA et al., 2016).

Lipids are essential components of exosomal membranes, and exosomes are also known to be enriched in lipids such as cholesterol, sphingomyelins, glycosphingolipids, and phosphatidylserines (SKOTLAND et al., 2017a). Exosomes have been reported to carry RNA, including mRNA, miRNA, and some non-coding RNA, and have polysaccharide and glycan signatures on their outer surface (KALRA et al., 2016).

However, it is important to understand that whatever exosomes contain, it is sorted into them during their formation (as ILV). EDGAR (2016) claimed that any cargo on the ILV/exosome membrane must first be on the limiting membrane of the endosome, which means it must first come from the cytosol. The cargo may thus be concentrated in exosomes, but it will also be found elsewhere and there will never be an exclusive marker for exosomes. Hence, it is becoming clear that despite some molecules such as Alix, TSG101, CD63, and CD9 have been previously claimed as exosomal markers (MATHIVANAN et al., 2010), they are perceived to be enriched in exosomes, but are not specific markers (LÖTVÄL et al., 2014).

Milk is a biofluid with complex composition, important as the primary source of nutrition for infants and as a vector involved in the transfer of bioactive compounds and cells (JENSEN 1995; INOUE and TSUKAHARA, 2021). Although the major functional aspects of milk are known, the specific roles of each component and its working pathways have yet to be revealed in detail. The EV cargo is suggested to have an essential role in the development of the infant immune system (ZHOU et al., 2012).

While the technology for manufacturing milk replacers advances, mother's milk is still considered the best source of nutrition for infants across different species. In humans, breastfeeding decreases the development of allergic disorders (VERHASSELT et al., 2008) and is associated with a dose-response reduction of the risk for pneumonia, necrotizing enterocolitis, and inflammatory diseases in infants (EIDELMAN and SCHANLER, 2012). Compared with breastfeeding, formula feeding is associated with altered body composition in infancy (GALE et al., 2012), different effects on immunocompetence (TARRANT et al., 2010), and different gut microbiota (POULSEN et al., 2017). In pigs, the genetic selection for increased litter size exceeding the number of teats impelled farmers to use milk replacers

for enabling weight gain and improving survival chances (VOS et al., 2014), although it is known that the piglets' gut development (FAN et al., 2014) and digestive functions (THYMANN et al., 2006) are impaired when compared to suckling sows' milk. The need for revising large litter size as a breeding goal is now increasingly recognized, however, both animal and human neonates need to be adequately nourished when the supply of their mother's milk is lacking or too low. Therefore, bridging the gap between mother's milk and milk replacers is important for human or animal health and also has significant economical perspectives.

Milk contains abundant quantities of EV that may originate from multiple cellular sources, which are bioavailable and able to deliver their cargo across species boundaries (BAIER et al., 2014; KUSUMA et al., 2016). It has been described that bovine milk exosomes can enter intestinal cells (WOLF et al., 2015; KUSUMA et al., 2016) and circulating immune cells (IZUMI et al., 2014), and accumulate in the liver (MUNAGALA et al., 2017). Milk EV are filled with immune modulatory features (ADMYRE et al., 2007) and are suggested to have an essential role in the development of the infant's immune system (ZHOU et al., 2012), but also assist in various functions such as inducing cell proliferation (MARTIN et al., 2018), supporting the oral (ZONNEVELD et al., 2021) and gut epithelial barrier (MAGHRABY et al., 2021). Insofar, the in-depth analysis of milk exosomes in different lactation periods may provide information to understand the transfer of immunity from mother to child, along with insights for being used as novel biocomponents in milk replacers. As milk EV are bioavailable and can be taken up by various cell types, including in inter-individual or even cross-species (ZEMPLINI, 2017), milk has been pointed out to be of particular interest as a scalable source of exosomes for drug loading and delivery (MUNAGALA et al., 2016). Moreover, the study of milk EV is also of importance given the use of milk and dairy products as food (ZEMPLINI, 2017). The physiology and associated metabolic processes in pigs are alike in humans, making it a good animal model for translational research, including proteomics analysis (BASSOLS et al., 2014).

Finally, nanoparticle-delivery systems are a relatively new and rapidly developing technology, which allow the delivery of unstable molecules, enhance the efficacy of therapeutic agents, and may allow site-specific, target-oriented delivery agents (PATRA et al., 2018; JABIR et al., 2012). Despite many advantages, factors have hindered the clinical use of drug-delivery nanoparticles, such as the high cost, poor stability, non-specific binding,

toxicity issues, and large-scale production (LIM et al., 2013). Milk exosomes could potentially overcome some of the limitations of synthetic liposomes, due to their superior half-life circulation and bioavailability, avoidance of degradation, and cross-species tolerance (FENG et al., 2021; DEL POZO-ACEBO et al., 2021). Therefore, understanding the structural, functional, and stability characteristics of milk exosomes is of particular interest.

The ratio of dietary omega-6 (ω -6) and omega-3 (ω -3) polyunsaturated fatty acids (PUFA) is known to affect health: a high ratio (15:1 and greater) is considered to promote chronic diseases such as inflammatory and cardiovascular ones, whereas a lower ω -6: ω -3 ratio counteracts such diseases and exerts anti-inflammatory effects (SIMOPOULOS, 2010; CANDELA et al., 2011). The ω -6: ω -3 ratio in the diet is relevant both in human and livestock nutrition to support health and provide healthier foods. In pig production, the use of diets enriched in ω -3 PUFA has been tested in sows and was shown to be associated with anti-inflammatory and antioxidative properties (EASTWOOD et al., 2014; TANGHE et al., 2015). Moreover, recent works suggest that the metabolism of the sows and their piglet's health might also be modulated (YANG et al., 2019; MCAFEE et al., 2019). Besides these findings, it is still not clear how the effects of ω -3 and ω -6 PUFA in the diets fed during gestation and lactation may be transmitted from the mother to the offspring. The dietary supplementation of ω -3 PUFA is known to induce changes in the milk fatty acid composition in several mammals, including pigs (YIN et al., 2017; NGUYEN et al., 2020), cows (MOALLEM, 2018; PALMQUIST, 2009), and humans (MAZURIER et al., 2017; BÜYÜKUSLU et al., 2018). The use of ω -3 PUFA enriched diets has also been associated with an overall reduction in milk fat percentage of dairy cows (MOALLEM, 2018; MUSTAFA et al., 2003; DONOVAN et al., 2000), although no changes in milk protein (MOALLEM, 2018; MUSTAFA et al., 2003; AKRAIM et al., 2007) or milk lactose (DONOVAN et al., 2000; MOALLEM et al., 2013; GONTHIER et al., 2005) percentages have been found, and reports on the milk EV fraction were unavailable until now. While the diet is an important external factor in many molecular processes (YAN, 2015; NASIR et al., 2020), its impact on exosome composition has hardly been investigated so far. Thus, this study aims to bridge this gap by providing knowledge on how the lipid and protein composition of porcine milk exosomes may be affected by different ratios of PUFA in the diet.

Milk exosomes have gained increasing interest in research, and major findings have revealed their prospective use as drug vehicles, carriers of potential biomarkers, and provide information on physiological and pathological functions. Exosomal metabolites are potentially related to growth performance and immune response, opening a perspective on animal production and of development of dietary exosomal supplements (“exosome mimetics”). Therefore, knowledge on milk exosome characterization is relevant for basic research, but also has applied aspects in animal nutrition and health and may provide new perspectives for feeding additives, including milk replacers.

The experiment of this thesis will use two groups of sows, allocated to diets with different ratios of ω -6: ω -3 PUFA, and compare the composition of sow’s milk exosomes on different lactating stages from both groups by proteomics and lipidomics analyses. The dietary ω -6: ω -3 ratio during gestation and lactation of sows is a critical factor affecting performance in sows and their litters. The main aims of this thesis are to identify how the protein and lipid composition of milk exosomes are modified during the transition from the colostrum until mature milk and to identify how different ratios of ω -6: ω -3 PUFA affect the composition of milk exosomes.

2 LITERATURE REVIEW

2.1 Introduction to extracellular vesicles

2.1.1 *The history and the problem of extracellular vesicles nomenclature*

Extracellular vesicles (EV) are nano-sized membrane vesicles bound by a lipid membrane that are released by all cell types into the extracellular space (RAPOSO and STOORVOGEL, 2013; MATHIVANAN et al., 2021). While EV research continues to advance, the nomenclature of EV is continuously debated (WITWER and THÉRY, 2019, THÉRY et al., 2018) and various subtypes have been proposed over time (KANG et al., 2021). The EV are classically divided into three main subtypes: microvesicles, exosomes, and apoptotic bodies. These terms, even today, may mean different things to different investigators, and whilst it must be pointed out that to this date there is no consensus on the EV nomenclature (BAZZAN et al., 2021), generally, it is accepted that these three main types can be defined, based primarily on the EV biogenesis and release pathways (DOYLE and WANG., 2019).

The first report of the possible existence of EV-like entities was initially proposed by CHARGAFF and WEST (1946), that postulated that a "variety of minute breakdown products of the blood corpuscles" were involved in the clotting properties of thromboplastic agents. Years later, WOLF (1967) described "platelet-dust" as lipid-rich particulate material originating from platelets, visualized by electron microscopy, and considered the first description of EV. The term "extracellular vesicles", however, appeared first only in 1971 when AARONSON et al. reported the production of intra and extracellular membrane-bound structures in the eukaryotic algae *Ochromonas danica*. AARONSON et al. (1971) also hinted at the EV biogenesis by noticing that these EV structures arose from distinct cell organelles, distinguishing them from virus-like particles or cellular debris.

It was in blood research that "exosomes" were primarily reported, associated with small vesicles with transferrin receptors secreted by blood reticulocytes into the extracellular space (HARDRING et al., 1983; PAN and JOHNSTONE, 1983). Rose Johnstone is officially considered to discover and coin the name exosome (borrowing from the ancient Greek "*exo*" = outside, and "*soma*" = body) when she proposed this term referring to these EV arising from reticulocytes (JOHNSTONE, 2009). Although the term had appeared a few

years before by TRAMS et al. (1981) when referring to “vesicles exfoliated from the plasma membrane” which “may serve a physiologic function”, it was JOHNSTONE et al. (1987) who were able to describe the formation and the secretion pathway of said vesicles in reticulocytes. The studies that followed up were able to first elucidate the biogenesis of exosomes by the formation of intraluminal vesicles, and the release of their cargo after fusion with the membrane, thus providing the basis for differentiating exosomes from microvesicles (PAN et al., 1985; JOHNSTONE et al., 1989).

From these findings on, EV research continuously progressed, and the term “exosome” has quickly surpassed in popularity among researchers (Figure 2). However, the term “exosomes” has spread among EV studies not only as means to describe EVs originating from multivesicular bodies, but indiscriminately to refer to any type of nano or micro vesicles, or as an alternative to “extracellular vesicles”. An empiric approach has also been used to define exosomes, and many authors have started to use it to describe vesicles that sediment over differential centrifugation at 100,000 x g (GOULD and RAPOSO, 2013, THÉRY et al., 2006). This has created challenges in the interpretation and reproducibility of results inside the EV field.

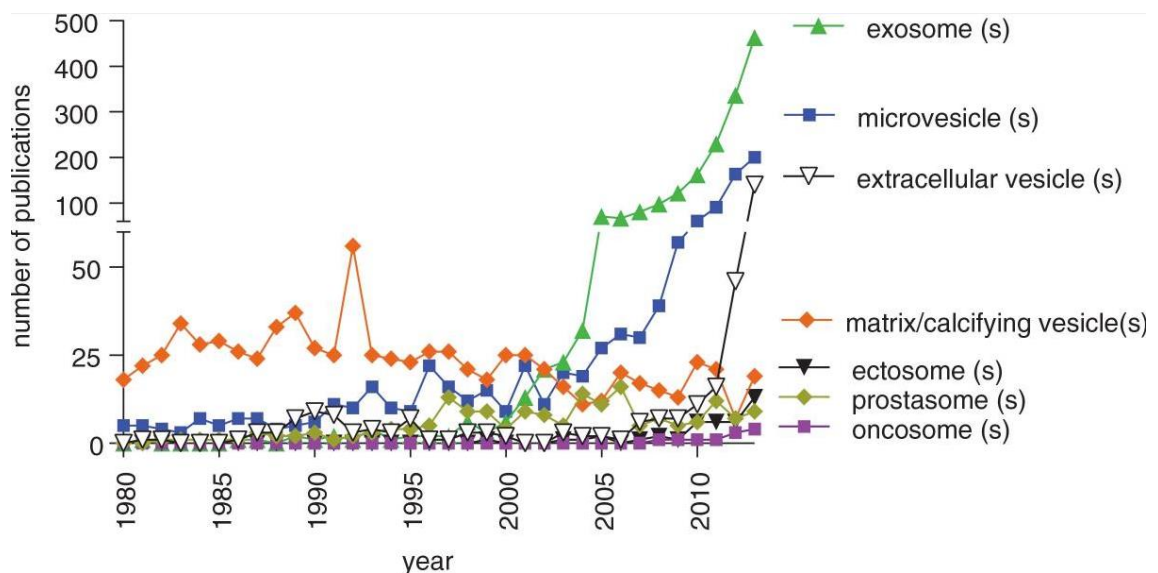


Figure 2. Comparative evolution of the use of different terms for extracellular vesicles and their subtypes by the cumulative number of publications in the literature from 1980 to 2010. Reproduced from LÖTVALL et al. (2014).

To circumvent this problem, scientists from the International Society for Extracellular Vesicles (ISEV) have proposed a guideline, based on state-of-the-art practices, which researchers should follow to help them characterize EV in their studies (LÖTVALL et al., 2014). The minimal experimental requirements for definition of extracellular vesicles (MISEV) general practice includes: (1) selection of isolation methodology on the downstream application and scientific question, and rigorous report of all details of the method to ensure reproducibility; (2) assessment of "exosome-enriched" proteins, typically transmembrane proteins and cytosolic membrane-binding proteins, in at least a semi-quantitative manner; (3) the comparison of their protein isolates by searches within databases, such as EVpedia; (4) the characterization of the EV isolation, using two different technologies – preferably size distribution measurements for a large number of vesicles, and single vesicle visualization by microscopy techniques. The study here presented therefore followed these recommendations and understands exosomes as the classically postulated EV subtype with endosomal origin via multivesicular bodies.

The MISEV guidelines were mainly accepted within the EV community. Although several scientists reportedly still prefer the term "exosome" as a generic replacement to EV (WITWER and THÉRY, 2018), a follow-up survey by ISEV showed that 71% of recently publishing authors indicated that they followed and/or cited the MISEV2014 in their work (WITWER et al., 2017). A consensus, however, was still not achieved, and EV research continues to enthusiastically expand. Recent discoveries and the continuation of the nomenclature discussion have led to a revision in the MISEV in 2018 (THÉRY et al., 2018). ISEV has endorsed that "extracellular vesicle" remains the general term for particles released from cells limited by a lipid bilayer and without a functional nucleus, and exosomes the endosomal-origin EV subtype. Since there are no specific markers that could validate the particular EV biogenesis pathway, it has arisen the recommendation to consider operational terms for EV subtypes. Therefore, categories should be created by authors, using for example their size (e.g., "small EV" for vesicles under 200 nm, including consequently exosomes) or their biochemical composition (e.g., containing CD63+ EV or Annexin+ EV). With the advantage of being more accurate, the free categorizing proposed on the last MISEV may lack comparability between studies. As so, the debate continues, and updates on the MISEV are already planned (WITWER et al., 2021). In conclusion, as the EV field has evolved significantly since its first description in 1946 (CHARGAFF and WEST, 1946), the

advances should certainly come together with further standardization efforts and procedures to enhance precision and avoid misinterpretation of results.

2.1.2 Extracellular vesicle biogenesis

The classical distinction of EV subtypes recognizes three subtypes of EV: microvesicles, apoptotic bodies, and exosomes, which are defined based on their biological pathways (Figure 3) (YÁÑEZ-MÓ et al., 2015; TENG and FUSSENEGGER, 2020). These EV subtypes have distinct biogenesis, release pathways, size, content, and functions (DOYLE and WANG, 2019).

Microvesicles (MV) (100 nm to 1 μ m in diameter) are formed by direct outward budding of the plasma membrane of healthy cells (ZABOROWSKI et al., 2015). The MV formation involves vertical trafficking of molecular cargo to the cellular membrane, use of contractile machinery, and redistribution of membrane lipids, allowing the scission of the plasma membrane. Although the specific route of formation is not yet completely elucidated, it is known that this process depends on dynamic reactions between cholesterol-rich microdomains, and the interaction of actin and myosin together with a subsequent ATP-dependent contraction for MV fission and release (TRICARICO et al., 2017).

Apoptotic bodies (AB) are the largest subtype of EV, with sizes that can range from 50 nm up to 5 μ m in diameter, with most AB measuring approximately 1 to 5 μ m in diameter (BORGES et al., 2013). Unlike other types of EV, which are generated in both physiological and pathological conditions, AB are released by the blebbing of the plasma membrane in dying cells (BATTISTELLI and FALCIERI, 2020). The AB appear as a natural process of apoptosis, where extensive plasma membrane blebbing occurs followed by karyorrhexis and separation of cell fragments (ELMORE, 2007). Thus, the composition and cargo are also clearly distinct from other EV, containing chromatin remnants, cytosol portions, degraded proteins and DNA fragments, and even intact organelles (BORGES et al., 2013; BATTISTELLI and FALCIERI, 2020).

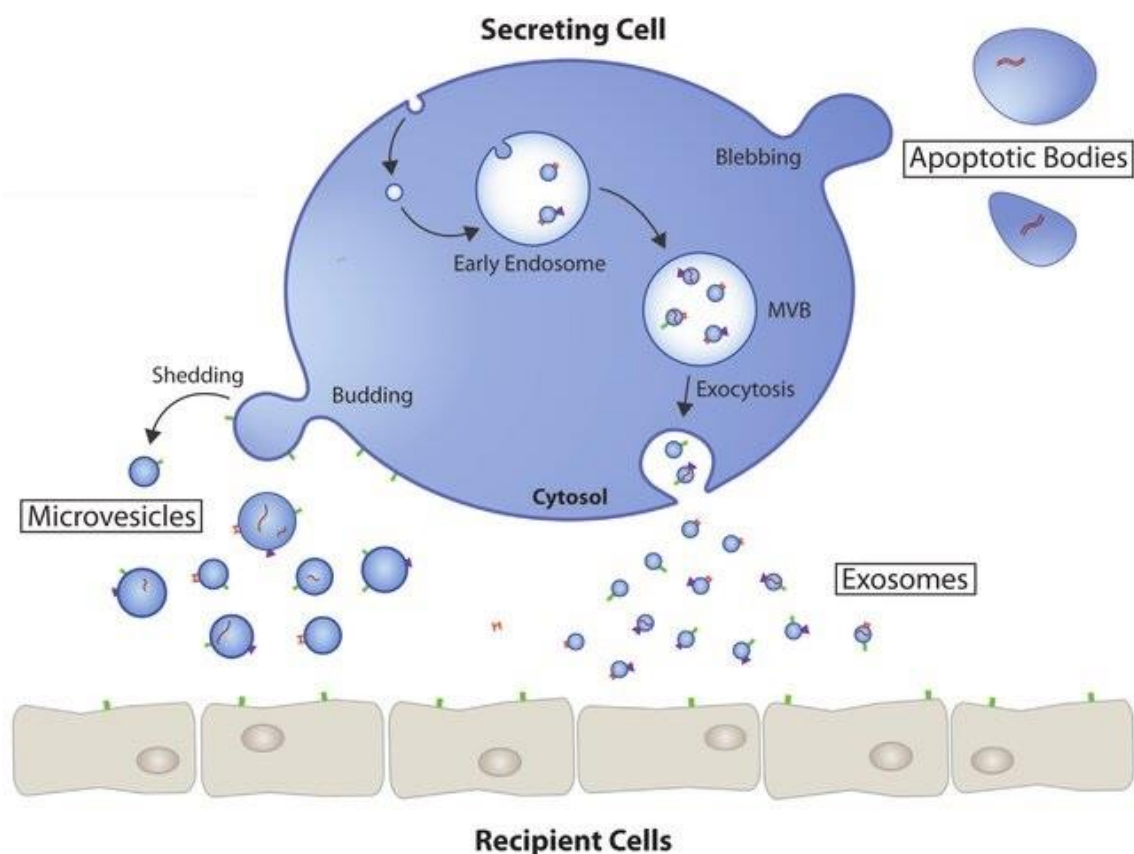


Figure 3. Schematic representation of the different biogenesis of extracellular vesicles. Exosomes are formed as intraluminal vesicles by budding into early endosomes, which by fusing with the plasma membrane can release their cargo into the extracellular milieu. Microvesicles are formed as a simple outward budding and fission of the plasma membrane mediated by phospholipid redistribution and cytoskeletal protein contraction. Finally, apoptotic bodies, are formed during programmed cell death and represent the largest EV subtype. Abbreviations: MVB, multivesicular bodies. Adapted from GUSTAFSON et al., 2017.

Exosome biogenesis follows a more complex route than the other EV subtypes, which has been extensively reviewed (HARDING et al., 2013; GURUNG et al., 2021; ZHANG et al., 2019; HESSVIK and LLORENTE, 2017). Exosomes are typically 30 to 150 nm in diameter, and their formation consists of regulated steps for their formation, transport, and release. Early endosomes are formed via the invagination of the plasma membrane, which follows a maturation process and the subsequent invagination of the endosomal membrane into the lumen to form intraluminal vesicles (ILV), creating the so-called multivesicular bodies (MVB). The MVB are eventually either sent to the lysosome to be degraded along

with all of its components or fused with the cell's plasma membrane to release its content, including exosomes, into the extracellular space (Figure 4).

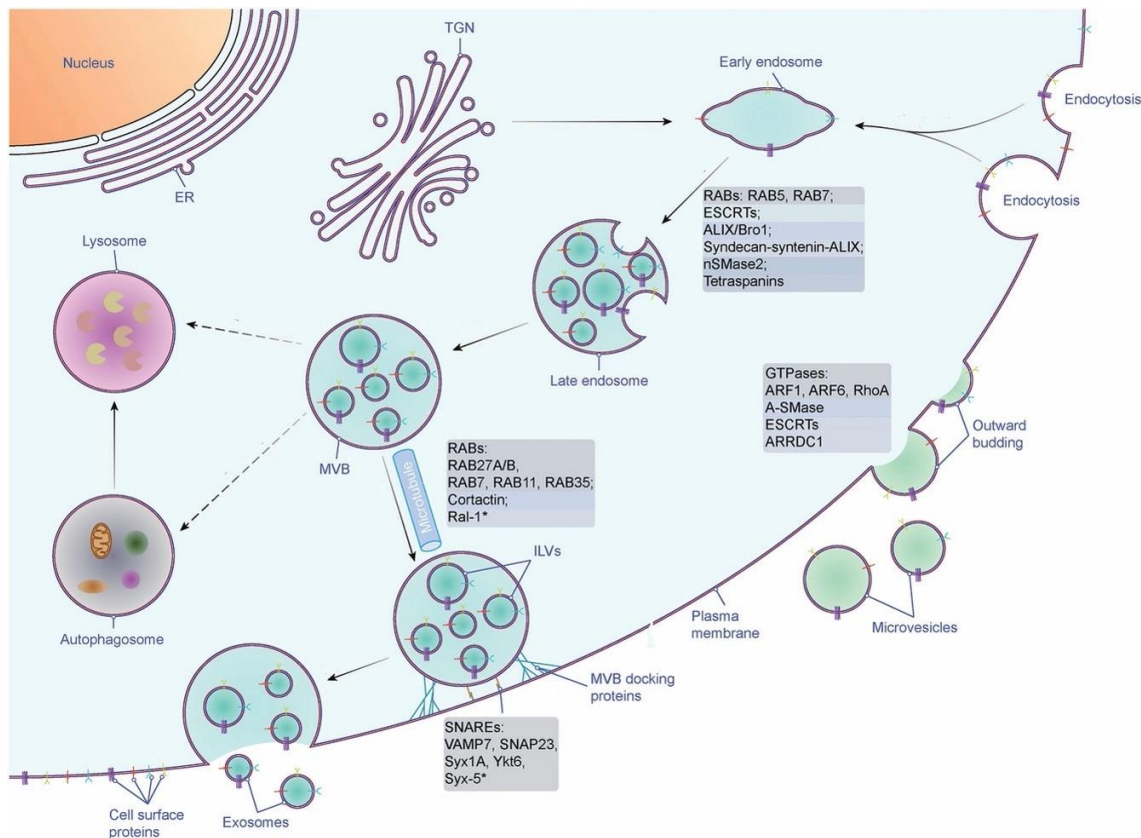


Figure 4. Biogenesis and secretion of exosomes. Early endosomes originate from the inward budding of the plasma membrane and mature into late endosomes, generating MVB filled with ILV. The MVB can proceed to either be fused with the plasma membrane, resulting in the release of the exosomes or fuse with lysosomes/autophagosomes, resulting in MVB degradation. Several molecules are involved in exosome biogenesis, trafficking, and fusion of MVB, which are highlighted in the Figure. The outward budding of the plasma membrane, originating another subtype of EV known as microvesicles, is also schematized to differentiate from exosome biogenesis. Abbreviations: EV, extracellular vesicle; ESCRT, endosome sorting complex required for transport; MVB, multivesicular body; ILV, intraluminal vesicle; RAB, RAS-related protein; ALIX, ALG-2 interacting protein X; nSMase2, neutral sphingomyelinase 2; Ral-1, RAL (Ras-related GTPase) homolog; SNARE, soluble NSF attachment protein receptor; VAMP7, vesicle-associated membrane protein 7; SNAP23, synaptosomal-associated protein 23; Syx1A, syntaxin 1A; ARF, ADP ribosylation factor; RhoA, Ras homolog family member A; A-SMase, acid sphingomyelinase; ARRDC1, arrestin domain containing protein 1. *, homologs in *C. elegans*. Adapted from TENG and FUSSENEGGER, 2020.

The endosomal sorting complex required for transport (ESCRT) function is essential for the creation of ILVs. The ESCRTs is a detailed machinery of four proteins (nominated from 0 to III) that collaborate to ensure MVB formation, vesicle budding, and sorting of exosomal cargo (HENNE et al., 2011; HURLEY 2015). The process is initiated by phosphoinositides in endosomal membranes, which recruit the ESCRT-0 complex to early endosomes, via its hepatocyte growth factor-regulated tyrosine kinase substrate (HRS). The HRS interacts with the tumor susceptibility gene 101 (TSG101) subunit of ESCRT-I, and subsequently with the ESCRT-II complex, resulting in the invagination of the endosomal membrane. The ESCRT-III assembly is activated, leading to sequential rounds of ILV scission into the lumen, originating the MVB. Non-canonical ESCRT-dependent pathways are also described to play a role in exosome biogenesis, via the syndecan-syntenin-Alix pathway, which can happen independently of ubiquitin and ESCRT-0, but still requires ESCRT-III for the scission step (BAIETTI et al., 2012). The ALG-2 interacting protein X (Alix) is a typical exosome protein, which has also been associated with several ESCRT proteins, such as TSG101 and charged multivesicular body protein 4a (CHMP4A) (VILLARROYA-BELTRI et al., 2014). The biogenesis via ESCRT proteins can be directly used to understand its composition, and therefore proteins from this machinery and its accessory proteins (namely Alix, TSG101, and heat shock proteins) are expected to be found in exosomes of all types of cells, and commonly referred as "exosome marker proteins" (DOYLE and WANG, 2019).

2.2 Introduction to milk exosomes

2.2.1 Composition of porcine colostrum and milk

Colostrum and milk provide adequate nutrition during early life, along with bioactive components such as hormones, cytokines, immunoglobulins, and other immune-modulatory factors (STELWAGEN et al., 2009; BALLARD and MORROW, 2013). The structure of the placenta in swine prevents the transfer of immunoglobulins in utero; neonates are born agammaglobulinemic - leaving colostrum and milk the sole source of maternal antibodies (BORGHESI et al., 2014; WAGSTROM et al., 2000). As so, timely colostrum intake is decisive for the piglets to gain sufficient nutrients and passive immunoglobulins. It is reported the ingestion of 200 g of colostrum per piglet during the first 24 h after birth can

reduce mortality to 10 %, compared to over 60 % of mortality rate when ingesting less than 100 g (QUESNEL et al., 2012). The early consumption of colostrum can also induce long-lasting effects on the piglets' growth (DEVILLERS et al., 2011) and their intestinal epithelium (MEI et al., 2006).

Alike other mammals, protein, fat, and lactose are the three predominant components in sow milk, although milk function is also significantly mediated by biocomponents that come in small concentrations, such as milk EV, oligosaccharides, vitamins, leukocytes, and hormones (HURLEY et al., 2015). Sow milk has a comparatively higher milk fat concentration than the median mammal level, but not milk protein or lactose (Table 1). The composition of sow milk also markedly changes during lactation: a higher protein concentration is found in the colostrum, which gradually decreases over time, while lactose concentration increases as milk production matures (Figure 5) (KLOBASA et al., 1987; ZHANG et al., 2018). The lactation period in farmed pigs is usually limited to 21 – 28 days when the piglets are removed and moved to fattening units.

2.2.2 Milk exosomes and their function

Milk is a nutrient-rich biofluid with a complex composition, including an abundant quantity of EVs as reported for different species (VASWANI et al., 2021; REINHARDT et al., 2012; CHEN et al., 2017; van HERWIJNEN et al., 2016; QUAN et al., 2020). The studies of milk exosomes in humans and different species of domestic animals and their major findings are summarized in Table 2.

Milk exosomes act on the development of the infant's intestinal tract (MAGHRABY et al., 2021; GOOD et al., 2005) and immune system (PETERS et al., 2015; ADMYRE et al., 2007; SAMUEL et al., 2017), while new functions are being discovered such as inducing cell proliferation (CHEN et al., 2016) and enhancing the epithelial barrier (HE et al., 2021; ZONNEVELD et al., 2021). The cargo encapsulated by the exosomal membrane is protected from enzymatic and non-enzymatic degradation (LIAO et al., 2017) and can remain bioactive even after pasteurization (PIETERS et al., 2015). This is reportedly mainly by the preservation of activity of milk exosomal miRNA under simulated conditions (FENG et al., 2021), such as the ability to survive gastric and pancreatic digestion (LIAO et al., 2017).

Commercial milk has also been shown to contain stable EV, including exosomes, which were able to be taken up by murine macrophages and facilitate T-cell differentiation (PIETERS et al., 2015).

Table 1. Milk composition of fat, protein, lactose, ash, and total solids in domestic animals, by percentage (%) of the total composition.

| Species | Fat | Protein | Lactose | Ash | Total solids |
|------------|------|---------|---------|------|--------------|
| Buffalo | 10.4 | 5.9 | 4.3 | 0.8 | 21.5 |
| Camel | 4.9 | 3.7 | 5.1 | 0.7 | 14.4 |
| Cat | 10.9 | 11.1 | 3.4 | --- | 25.4 |
| Cow | 4.5 | 3.6 | 5 | 0.7 | 15 |
| Deer | 19.7 | 10.4 | 2.6 | 1.4 | 34.1 |
| Dog | 8.3 | 9.5 | 3.7 | 1.2 | 20.7 |
| Goat | 3.5 | 3.1 | 4.6 | 0.79 | 12 |
| Guinea Pig | 3.9 | 8.1 | 3 | 0.82 | 15.8 |
| Horse | 1.6 | 2.7 | 6.1 | 0.51 | 11 |
| Human | 4.5 | 1.1 | 6.8 | 0.2 | 12.6 |
| Pig | 8.2 | 5.8 | 4.8 | 0.63 | 19.9 |
| Rabbit | 12.2 | 10.4 | 1.8 | 2 | 26.4 |
| Rat | 14.8 | 11.3 | 2.9 | 1.5 | 31.7 |

¹Data adapted from Jensen (1995) and Zhang et al. (2018)

Exosome content can be taken up by diverse cell types, including cross-species (BAIER et al., 2015; KUSUMA et al., 2016), which has signaled the importance of studies on milk EVs given their biological function and the use of milk and dairy products as food (ZEMPLINI 2016). First studies suggest that bovine milk exosomes can enter various immune and epithelial human cells (KUSUMA et al., 2016; IZUMI et al., 2015), and were able to maintain cellular metabolic activity without exerting cytotoxic effects (ROSS et al., 2020). However, it has been recently hypothesized that the chronic ingestion of bovine milk exosomes in humans could be associated with the development of chronic diseases such as obesity, type2-2 diabetes mellitus, osteoporosis, and common cancers (MELNIK and

SCHMITZ 2019) – although no direct evidence so far has been discovered to back up these claims. Still, the ubiquitous presence of exosomes in milk justifies enough cause for concern, and future studies should disclose if milk exosomes can be associated with derogatory other than the previously described protective effects (WANG et al., 2021).

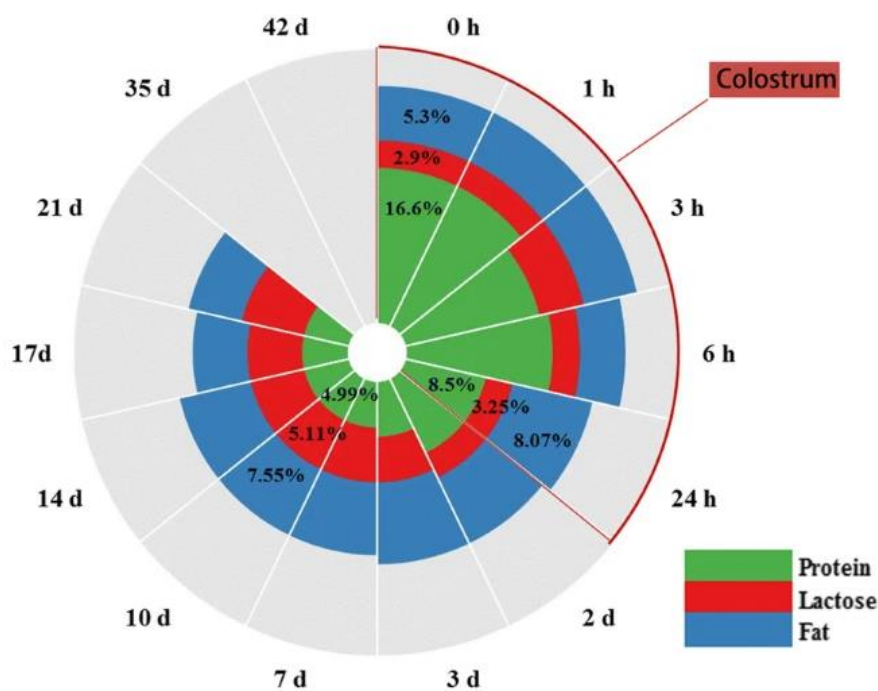


Figure 5. Composition of sow milk throughout lactation. Adapted from Zhang et al. (2018)

Milk EVs have also been targeted as a potential natural source of lipid-based-nanocarriers for drug or additives delivery systems (ZHONG et al., 2021; MUNANGALA et al., 2017; ADRIANO et al., 2021). Many drug molecules and biological agents are unstable in *in vivo* environments, and distinct nanoparticulate delivery systems have been developed to circumvent this issue. Liposomes and polymeric nanoparticles are the most common, although with limitations: Liposomes are highly biocompatible but less stable systemically, while polymeric nanoparticles exhibit superior stability but relatively less

biocompatible material (YALLAPU et al., 2021; GANJU et al., 2014). Milk exosomes have been appointed as a possible solution, evidenced due to (1) their superior circulation capacity and bioavailability (negative zeta potential), (2) their smaller size with deep tissue penetration capacity, (3) similarity with cellular membranes, and (4) avoidance of degradation and quick elimination (ADRIANO et al., 2021). Recent studies report promising approaches using milk exosomes to deliver doxorubicin (ZHANG et al., 2021) and paclitaxel (AGRAWAL et al., 2017) with promising stability and sustained release, and remarkably lower systemic and immunologic toxicities as compared to free oral delivery.

Table 2. Summary of literature on milk-derived exosomes from domestic animals and human breast milk.

| Species of origin of milk exosomes | Isolation and characterization strategies | Key findings | Reference |
|---|--|---|------------------------|
| Bovine | DG/UC, NTA, TEM, WB | Characterization of miRNA profiling of bovine milk exosomes by deep sequencing in healthy and mastitic cows revealed potential miRNA biomarkers for early detection of mastitis. | CAI et al., 2018 |
| Bovine | PS | Bovine milk exosomes in early pregnant cows showed differentially expressed miRNA than the normal group, revealing the potential role of miRNA as biomarkers of early pregnancy in dairy cattle. | MARKKADAN et al., 2018 |
| Bovine | DG/UC, NTA, TEM, WB | Proteomic profiling of bovine milk exosomes revealed 94 proteins – 86 unique in milk exosomes and 8 commons with exosomes from bovine plasma. | KOH et al., 2017 |
| Bovine | DG/UC, NTA, TEM, WB | High throughput proteomic analysis of bovine milk exosomes revealed more than 4980 proteins uniquely to colostrum, indicating a fundamental role of these proteins in modulating the gastrointestinal tract and immune system of the infant | SAMUEL et al., 2017 |
| Bovine | DG/UC, WB | The proteomics approach revealed several differentially expressed exosome proteins between human and bovine milk exosomes. Pathway analysis revealed that identified proteins were mostly involved in response to stimulus. | YANG et al., 2017 |
| Bovine | DG/UC, TEM, WB | Characterization of bovine milk exosome proteome, identifying 2107 proteins, which were mostly shared between exosomes and milk fat globules. | REINHARDT et al., 2012 |

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|--------|-------------------------------|---|------------------------|
| Bovine | DG/UC, WB | Characterization of proteins upregulated during <i>S. aureus</i> infection and their relative abundance in exosomes, milk fat globules, and whey milk fractions | REINHARDT et al., 2013 |
| Bovine | UC | Most mRNA and miRNA present in bovine milk were highly expressed in exosomes more than in other forms. Human macrophages could uptake bovine whey-derived exosomes | IZUMI et al., 2015 |
| Bovine | PS, DLS, SEM | Bovine milk exosomes contain small interfering RNA that resists the digestive process and uptake by cells <i>in vitro</i> , suggesting it would be able to cross the intestinal barrier and transfer their content to the blood circulation | SHANDILYA et al., 2017 |
| Bovine | DG/UC, AFM, DLS, NTA, SEM, WB | Bovine milk exosomes showed the potential to serve as biocompatible, safe, and cost-effective drug carriers for hydrophilic and lipophilic agents, and could eliminate off-target side effects | MUNAGALA et al., 2015 |
| Bovine | UC, TEM, WB | Intestinal uptake of microRNA encapsulated in bovine milk exosomes is likely mediated by endocytosis in humans and rats, and protein/protein recognition is vital for the intestinal uptake of exosomes. | WOLF et al., 2015 |
| Bovine | UC, NTA, TEM, WB | Exosomes from commercial dairy milk are bioavailable and accumulate in the liver and the spleen of mice. Resident macrophages in these tissues are responsible for clearing foreign exosomes administrated to mice. | MANCA et al., 2018 |
| Bovine | UC, AFM, NTA, SEM | Bovine milk exosomes are an alternative for drug delivery of anthocyanidins efficient <i>in vitro</i> and in mice. | MUNAGALA et al., 2017 |
| Bovine | UC, SEC, NTA, TEM, WB | Time efficient and less labor-intensive method for isolating and purifying bovine milk exosomes incorporating SEC to UC. | VASWANI et al., 2017 |

| | | | |
|--------|------------------|---|-----------------------|
| Bovine | DG/UC, SEC, PS | Isoelectric precipitation can be used to remove caseins and reduce operation time to isolate exosomes from raw bovine milk. | YAMAUCHI et al., 2018 |
| Bovine | UC, NTA, TEM, WB | Oral administration of milk exosomes increased the expression levels of genes related to intestinal immunity and the integrity of the mucus layer in mice. | TONG et al., 2020 |
| Bovine | UC, TEM, WB | Milk exosomes were conjugated to doxorubicin by a pH-cleavable bond to build a new milk-exosome-based drug delivery system. | ZHANG et al., 2021 |
| Bovine | UC | Milk exosomes enhanced the expression of CD69 on natural killer cells. | KOMINE-AIZAWA et al. |
| Bovine | UC, DLS, TEM, WB | A total of 3,475 novel and 6 annotated long coding RNA were identified in milk exosomes, which were resistant to <i>in vitro</i> digestion simulating the intestinal tract | FENG et al., 2021 |
| Bovine | UC, DLS, TEM, WB | Absorption of milk exosomes on the gastrointestinal tract occurs via neonatal Fc receptor and is suggested to occur as intact particles that can be modified with ligands in target tissues | BETKER et al., 2018 |
| Bovine | UC, DLS, WB | Milk exosomes affect the proliferation of macrophages and exert protective effects against chemotherapeutic drug-induced cytotoxicity | MATIC et al., 2020 |
| Bovine | UC, WB | Bovine milk exosomes were uptake by human intestinal epithelial cells, were able to maintain cellular metabolic activity, and were not cytotoxic. | ROSS et al., 2020 |
| Bovine | UC, DLS, TEM, WB | Epicatechin gallate was successfully delivered by milk exosomes in neuroblastoma cells and exerted enhanced neuroprotective effects. | LUO et al., 2021 |
| Bovine | UC, DLS, SEM, WB | Milk exosomes could attenuate purine nucleotide catabolism and improve energy status in intestinal cells and provide protection against oxidative stress | WANG et al., 2021 |

| | | | |
|-------------------------|---------------------|--|-----------------------------|
| Bovine | UC, SEC, NTA, WB | Milk exosomes were suitable vehicles for extracellular RNA via the absorption of miR-148a-3p in hepatic and intestinal cell lines | DEL POZO-ACEBO et al., 2021 |
| Bovine | UC, SEC, TEM, WB | Milk exosomes can be taken up by intestinal epithelial cells and mediated functional intracellular delivery of small interfering RNA. | WARREN et al., 2013 |
| Bovine | UC/DG, NTA, TEM, WB | Treatment with milk exosomes improved intestinal permeability, intestinal architecture, and cellular proliferation | MAGHRABY et al., 2021 |
| Bovine | PS, IB, NTA, TEM | Processed commercial milk contains stable exosomes, which were able to be taken up by murine macrophages and express immunoregulatory cargo | PIETERS et al., 2015 |
| Bovine | SEC, NTA, TEM, WB | Milk exosomes can be successfully in a three-step-based size exclusion chromatography | BLANS et al., 2016 |
| Bovine and yak | UC, DLS, TEM, WB | Proteomes of cow and yak milk exosomes differ. Milk exosomes from both species were able to activate the PI3K/AKT/C3 signaling pathway, alleviating LPS-induced intestinal inflammation and promoting cell survival. | GAO et al., 2021 |
| Bovine and goat | PS, WB | Beneficial miRNA were reported in human, bovine, and goat milk exosomes and also in the fat layer. | GOLAN-GERSTL et al., 2017 |
| Bovine, goat, and human | UC | Comparative analysis reveals that abundant miRNAs expressed in human milk are similarly conserved across species, with highlights to immune-related miRNAs | YUN et al., 2020 |
| Bovine and human | UC, SEC, TEM, WB | A total of 239 and 227 proteins were identified in human and bovine milk exosomes, respectively, of which 186 proteins were unique to bovine exosomes. | VASWANI et al., 2021 |
| Bovine | UC, TEM, WB | Human vascular endothelial cells can transport exosomes from commercial milk via endocytosis | KUSUMA et al., 2016 |

| | | | |
|-----------|--------------------|--|----------------------------|
| Dromedary | UC, TEM, WB | Different lactation periods had a different abundance of dromedary milk exosome proteins by electrophoresis and a stable expression of casein family proteins | YASSIN et al., 2016 |
| Dog | UC, DLS, TEM, WB | MS/MS proteomic analysis of colostrum exosomes identified 826 proteins. Colostrum exosomes increased the proliferation of mesenchymal stem cells | VILLATORO et al., 2020 |
| Horse | UC, FPLC, TEM | MS/MS MALDI proteomic analysis revealed a small number of major proteins in horse milk exosomes | SEDYKH et al., 2017 |
| Human | DG/UC, IB, TEM, WB | Milk exosomes inhibited anti-CD3-induced IL-2 and IFN- γ production <i>in vitro</i> and increased the number of Foxp3+CD4+CD25+ T regulatory cell | ADMYRE et al., 2007 |
| Human | PS | Exosomal miRNA miR-148a and miR-30b were negatively associated with overweight and obese newborns, and miR-30b was positively associated with infant weight, percent body fat, and fat mass at 1 month. | SHAH et al., 2021 |
| Human | UC, NTA, TEM, WB | Term and preterm exosomes in human milk ameliorated necrotizing enterocolitis severity <i>in vivo</i> and <i>in vitro</i> . A total of 395 distinct lipids were identified in milk exosomes, and 10 lipids were remarkably distinct between preterm and term groups. | CHEN et al., 2011 |
| Human | PS, NTA, TEM, WB | Milk exosomes exert beneficial effects in preventing necrotizing enterocolitis and reducing inflammation on the intestinal epithelium. | HE et al., 2012 |
| Human | PS, DLS, TEM | Milk exosomes induced proliferation and epithelial mesenchymal transformation in normal cells and not in tumor cells. A miRNA-148a target gene was downregulated in normal cells after being challenged with milk exosomes. | REIF et al., 2019 |
| Human | DG/UC, WB | LC-MS/MS proteomic analysis identified 1963 proteins in milk exosomes, of which 633 differ from the milk proteome database, revealing a functional proteome distinct from other milk components. | VAN HERWIJNEN et al., 2016 |

| | | | |
|----------------|------------------|---|---------------------------------|
| Human | PS | Milk exosome miRNA can survive simulated gastric and pancreatic digestion. | LIAO et al., 2017 |
| Pig | PS, AFM | Milk exosomes carry miRNA related to various immune and pathological responses and their expression profiles show a lactation specific pattern | GU et al., 2012 |
| Pig | UC, TEM | 491 miRNAs in porcine milk exosomes have been revealed by deep sequencing. Top miRNA target genes enriched in transcription, immunity, and metabolism processes. | CHEN et al., 2014 |
| Pig | UC | Porcine milk exosomes can facilitate intestinal cell proliferation and development of the intestinal tract in mice | CHEN et al., 2016 |
| Pig | DG/UC, TEM, WB | RNA-sequencing and LC-ESI-MS/MS proteomics analysis revealed 16,304 mRNAs and 66 proteins | CHEN et al., 2017 |
| Pig | UC, DLS, TEM, WB | Milk exosomes increased intestinal secretory immunoglobulin A levels and the expression levels of polymeric immunoglobulin receptors in mice and piglets. | ZENG et al., 2020a |
| Pig | UC, DLS, TEM, WB | RNA-sequencing reveals the presence of 2,466 novel and 809 long coding RNAs. Functional analysis reveals pathways involved in cellular macromolecule metabolic, RNA metabolic, and immune processes | ZENG et al., 2020 |
| Pig | UC, TEM | Milk exosomes attenuated deoxynivalenol induced damage on mice body weight and intestinal epithelium growth | XIE et al., 2020 |
| Pig and bovine | UC | Oral administration of porcine and bovine milk exosomes alters the level of selected miRNA in piglet serum | LIN et al., 2020 |
| Pig | - | Bioinformatics analysis of data from miRNA composition in human and porcine milk exosomes showed vesicles carry plant miRNA | LUKASIK and ZIELENKIEWICZ, 2014 |

| | | | |
|-------|--------------|---|-------------------|
| Pig | UC, TEM | miRNA and genes in porcine milk exosomes showed differential expression in response to diet with Ginseng polysaccharides supplementation | SUN et al., 2018 |
| Sheep | PS, TEM, DLS | Immune-related exosomal miRNA occupied 98% of total miRNA expression. miR-26a, miR-191, let-7f, let-7b and miR-10b were highly expressed. | QUAN et al., 2020 |

Abbreviations: AFM, Atomic force microscopy; AKT, Protein kinase B; C3, Complement C3; CD25, Interleukin-2 receptor alpha chain; CD4, T-cell surface glycoprotein; CD69, Early activation antigen CD69; DG/UC, Density gradient ultracentrifugation; DLS, Dynamic light scattering; Foxp3, Forkhead-Box-Protein; FPLC, Fast protein liquid chromatography; IB, Immuno-magnetic beads; IFN- γ , Interferon- γ ; IL-2, Interleukin-2; LC-ESI-MS/MS, Liquid chromatography electrospray ionization tandem mass spectrometry; LC-MS/MS, Liquid chromatography coupled with mass spectrometry; MALDI, Matrix assisted laser desorption/ionization; MS/MS, Tandem mass spectrometry; NTA, Nanoparticle tracking analysis; UC, Ultracentrifugation; PI3K, Phosphatidylinositol 3-kinase; PS, Centrifugation with precipitation solution (commercial kit); SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; WB, Western blotting; SEC, Size exclusion chromatography.

2.2.3 Porcine milk exosomes

Only a few studies on milk exosomes have focused on sow milk (Table 2). Many milk exosome components and their subsequent function seem to be parallel between species, specifically for miRNA (YUN et al., 2020, FENG et al., 2021). Yet, particularities and species-specific constituents are also reported. Vaswani et al. (2021) determined the proteome of bovine and human milk exosomes, and less than 25 % of identified proteins were shared between both species. Studies on porcine milk exosomes have been mostly limited to the miRNA, mRNA, and long coding RNA cargo (GU et al., 2021; CHEN et al., 2014; ZENG et al., 2020; LIN et al., 2020; SUN et al., 2018; LUKASIK and ZIELENKIEWICZ, 2014). These suggest that porcine milk exosomes transfer encapsulated RNA materials related to the development of immunity and gastrointestinal health of newborns, and their expression profiles show a lactation-specific pattern although the underlying regulatory mechanisms remain unknown.

The proteome of porcine milk exosomes has been investigated by LC-ESI-MS/MS analysis (CHEN et al., 2017). A total of 639 (571 known, 66 candidate, and 2 putative proteins) proteins were identified. A major part of the proteins included in the cytoplasm and cytoplasmic part, but specific membrane-bounded vesicle lumen, granule lumen, vesicle, lytic vacuole, and reticulum lumen proteins were also identified, and functional analysis highlighted multiple biological processes. Proteins involved in DNA or RNA synthesis, and related to intracellular trafficking, secretion, and vesicular transport were particularly abundant, and conserved proteins involved in cell cycle control and division were also identified. However, no information on the lactation stage of the milk samples is described in this study.

2.3 Isolation and characterization of milk exosomes

2.3.1 Techniques for isolation and purification of milk exosomes

Milk is a complex body fluid consisting of proteins, carbohydrates, lipids, cells, and other biologically important components, especially immunological components that affect the development of the infant's immune system. Isolation of EV is particularly complicated from milk by the high lipid content, released as milk fat globules (MFGs) (WITWER et al.,

2013). These MFGs are surrounded by a complex phospholipid trilayer and their disintegration can result in the formation of EV-like structures which can contaminate and be co-isolated with other EV populations in milk (BLANS et al., 2017).

A checklist of information should be collected specifically for the research project, considering that a large number of factors influence the composition of milk, such as stage of lactation, parity, the volume of milk production, infant feeding, maternal diet, energy status, maternal health, illness, and stress (WITWER et al., 2013). The storage conditions of milk have also been shown to be a crucial factor for the final exosome concentration and integrity. Storage of breast milk in a refrigerator or freezer may induce cell death and cause contamination to the composition of milk EVs (ZONNEVELD et al., 2014). Thus, although it is recommended to prepare fresh milk samples right after collection, removing milk cells before storage (defatting and removal of cells), this approach is not feasible for most studies in the animal field, where samples are collected directly on the farm and stored for further processing. Lee et al. (2016) evaluated the effects of short-term storage on milk exosome recovery and revealed that while all representative exosome markers were detected at 4°C, 37°C, and room temperature (RT), samples incubated at RT showed a loss of HSP70. Therefore, the choice of the method of isolation should be intended to act on clearing these possible contaminations, and samples should be directly refrigerated or frozen upon collection.

The long-term storage condition is also known to affect exosome yield and recovery (LEE et al., 2016; ZHOU et al., 2006). Exosome-associated protein levels remained intact in samples stored at -20 and -70 °C for 10 days, while storage at 4 °C and at RT resulted in complete loss of the CD63 protein and reduction in RNA levels. Repetitive freeze-thawing cycles also affect exosomal membranes and could change their properties and are therefore recommended to be avoided as much as possible (CHENG et al., 2018). Storage on acidic pH is suggested to reduce the degradation of exosome-associated proteins in urine (BAN et al., 2015), but reports also show a decrease in exosome concentration when stored at pH 4 (CHENG et al., 2018). While the study of pH influence on milk exosomes warrants further research, pH 7.4 PBS continues to be the preferred approach for storing milk exosomes (FENG et al., 2021).

Ultracentrifugation-based techniques are most commonly used in exosome isolation (ZAROVNI et al., 2015). The most widely used method is differential centrifugation (CHIA et al., 2017), which usually consists of a series of centrifugation cycles of different centrifugal forces and duration to isolate exosomes based on their density and size differences from other components in a sample. The limitations of this method include its length (4–5 h), its requirement for high-speed ultracentrifuge, and its rather low purity of exosomes (MOMEM-HERAVI et al., 2013). As so, further steps may be included to increase the purity of ultracentrifugation isolates.

Exosomes have also been isolated from bovine milk samples using differential centrifugation coupled with ultracentrifugation, with further purification using density gradient in sucrose (REINHARDT et al., 2013) and iodixanol (SAMUEL et al., 2017). It has been reported that differential centrifugation followed by top-down density gradient ultracentrifugation allows efficient density separation of EV from breast milk, while a bottom-up approach for density-based purification of EVs from milk was indicated to be not efficient (ZONNEVELD et al., 2014).

Strategies to isolate milk exosomes from bovine milk using ultracentrifugation and further enrichment of exosomes using size exclusion chromatography (SEC) proved to be an efficient method for isolation of exosomes with increased yield and reduced contamination (VASWANI et al., 2017). The combination of ultracentrifugation and SEC techniques is known to improve exosome enrichment, purity, and integrity for subsequent use, and has arisen as the preferred method for milk exosome isolation in the latest years (DEL POZO-ACEBO et al., 2021). The SEC has also been used alone to isolate bovine milk EVs, performed at milk samples after being skimmed (centrifugation at 3,400 x g for 35 min) and further centrifugation at 10,000 to 20,000 x g to sediment both main casein components and apoptotic bodies (BLANS et al., 2017). Yamada et al. (2012) have evaluated a commercially available precipitation solution called ExoQuick (System Biosciences, USA) for exosome isolation from bovine milk. This method was considered not efficient if used alone, but successful if paired with ultracentrifugation. However, ultracentrifugation paired with density gradient centrifugation was considered to have higher purity and be more suitable for protein analyses.

Isolation of exosomes from porcine milk has been reported using ultracentrifugation (GU et al., 2012; CHEN et al., 2014; 2016), ultracentrifugation with sucrose gradient and filtration steps (CHEN et al., 2017), and ultracentrifugation with the use of commercial precipitation kits (GU et al., 2012). The isolation of milk exosomes from sow milk can be additionally challenging, due to the scarce volume of samples when compared to other mammals, such as dairy cows. For OMIC studies, this can be especially limiting, as the multiple numbers of steps may lead to substantial sample loss, and a considerable pure isolate is needed for optimal results (KASSEM et al., 2021).

Finally, it is factual that every step when processing the samples will affect the sample composition to some extent. In milk EV research, the different sources and isolation methods can make the comparison between studies difficult, and detailed reports of the isolating techniques should be included and differences in the processing should be put into consideration for the reader.

2.3.2 Techniques for characterization and quantification of milk exosomes

The ISEV states as a minimal requirement to provide a general composition of each EV preparation, at least in a first publication, reporting the amount of several proteins (3 or more) that should be expected in EV isolates and may include also the levels of proteins that should not be expected to be enriched in EV (LÖTVALL et al., 2014).

At least one protein of each category should be quantified (at least by a semi-quantitative method) in the EV preparations: (1) transmembrane or lipid-bound extracellular proteins, present or enriched in EV/exosomes (e.g. CD9, CD63, integrins); (2) cytosolic proteins, present or enriched in EV/exosomes (e.g. TSG101, annexins, syntenin); (3) intracellular proteins, absent or under-represented in EV/exosomes (e.g. HSP90B1, CANX, GM130). Lötvall et al. (2014) also stated that analysis should be performed in a semi-quantitative manner, and analytic approaches can include Western blots (WB), high-resolution flow cytometry, or global proteomic analysis by mass spectrometry techniques.

Researchers are also encouraged to compare their results with existing EV and exosome databases. ExoCarta (exocarta.org), Vesiclepedia (microvesicles.org/), and EVpedia (evpedia.info) are databases of studies of extracellular vesicles based on

community annotation of results (KIM et al., 2013; KALRA et a., 2012; KEERTHIKUMAR et al., 2016). These online databases of comprehensive vesicle-specific components contain information on mRNAs, miRNAs, proteins, and lipids identified in EV. Proteins that are more often identified in exosomal studies based on the number of occurrences of these molecules in ExoCarta and whether they have been identified in milk exosome studies are listed in Table 3.

Table 3. List of the 20 most commonly reported exosomal proteins according to ExoCarta databases and whether they have been reported in milk.

| Gene name | Protein name | Identified in milk* |
|-----------|--|---------------------|
| CD9 | CD9 antigen | Yes |
| HSPA8 | Heat shock cognate 70 kDa protein | Yes |
| PDCD6IP | Programmed cell death 6-interacting protein | No |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase | Yes |
| ACTB | Actin, cytoplasmic 1 | No |
| ANXA2 | Annexin A2 | Yes |
| CD63 | CD63 antigen | Yes |
| SDCBP | Syntenin-1 | Yes |
| ENO1 | Alpha-enolase | Yes |
| HSP90AA1 | Heat shock protein HSP 90-alpha | Yes |
| TSG101 | Tumor susceptibility gene 101 protein | Yes |
| PKM | Pyruvate kinase PKM | Yes |
| LDHA | L-lactate dehydrogenase A chain | No |
| EEF1A1 | eukaryotic translation elongation factor 1 alpha 1 | Yes |
| YWHAZ | 14-3-3 protein zeta/delta | No |
| PGK1 | Phosphoglycerate kinase 1 | Yes |
| EEF2 | Elongation factor 2 | Yes |
| ALDOA | Fructose-bisphosphate aldolase A | Yes |
| HSP90AB1 | Heat shock protein HSP 90-beta | Yes |
| ANXA5 | Annexin A5 | No |

*If the respective protein has been previously identified in milk studies which are uploaded to the database.

Benmoussa et al. (2018) have identified 41 proteins specific to a pellet over 100,000 x g centrifugation in bovine milk, and compared them to a 35,000 x g centrifugation pellet, making these proteins possibly specific milk exosomal markers. TSG101, SDCBP, CD9, CD63, CD81, Complement proteins C2, C6, and C7, and ITGAV have been pinpointed as

being the most markedly enriched in the 100,000 x *g* EV subsets, although they are recommended to be used in combination.

Electron microscopy (EM) techniques are valuable for assessments of morphology, size, and the presence of markers (by immuno-EM). EM techniques are well established in EV research and can be used to prove direct evidence for the presence of vesicular structure (WITWER et al., 2013). Atomic force microscopy (AFM) has also been applied to the study of exosomes. The sub-nanometer resolution is a suitable technique for assessing the EV morphology. It is recommended that EM or AFM images show a wide field encompassing multiple vesicles and close-up images of single vesicles (LÖTVALL et al., 2014).

Transmission electron microscopy (TEM) is the most common method used to assess milk exosomes (BENMOUSSA et al., 2017; VASWANI et al., 2017; MANCA et al., 2018; SAMUEL et al., 2018; BLANS et al., 2017), but scanning electron microscopy (SEM) (MUNAGALA et al., 2016) and atomic force microscopy (MUNAGALA et al., 2016) have also been employed. In porcine milk exosomes, previous studies reported the use of atomic force microscopy (GU et al., 2012), transmission electron microscopy (CHEN et al., 2016), and transmission electron microscopy in addition to the identification of enriched tetraspanins (CD63 and CD9) (CHEN et al., 2017).

Quantification techniques are based primarily on the physical properties of the particles, such as the size, mass, and density of membrane proteins presented on their surface. The main techniques used for the quantification of exosomes are immunoaffinity capture (IAC), nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and surface plasmon resonance (SPR) (CHIA et al., 2017). The NTA is the most popular technique and has been used in bovine milk exosomes (MANCA et al., 2018; SAMUEL et al., 2017; VASWANI et al., 2017; BLANS et al., 2017). Munagala et al. (2017) used a combined approach of NTA and DLS to evaluate the size distribution of isolated bovine milk exosomes. It is recommended that the values acquired with distribution measurement techniques should be compared with microscopy techniques (e.g., TEM, AFM) since they do not distinguish membrane vesicles from co-isolated non-membranous particles of the same size (LÖTVALL et al., 2014).

2.4 Current knowledge of milk exosome composition

2.4.1 Current knowledge on the proteome of milk exosomes

Vesicle content has been extensively investigated by MS-based proteomic analysis, playing a particularly important role in our understanding of the composition of extracellular vesicles from various cell types and body fluids (CHOI et al., 2012). The proteome of milk exosomes can provide important information on different mechanisms and pathways of physiological functions and diseases, particularly the development of the immune system in the neonate, and facilitate biomarker discovery.

Proteomic investigations have been performed in exosomes from milk from different mammals (Table 2). A comparison of proteins identified in studies with bovine milk exosomes, with data comprised in Exocarta, Vesiclepedia, and EVpedia, revealed a significant number of concurrent proteins (Figure 6). Unique proteins have also been pinpointed in each study, showing a different composition in milk exosomes related to the individuality of animals, different treatments, lactation period, and health status.

The major milk fat globule membrane proteins (xanthine oxidase, butyrophilin, lactadherin/MGF8, and adipophilin/perilipin-2) are reported to be the most abundant proteins found in bovine milk exosomes (REINHARDT et al., 2012), but were not found in porcine milk exosomes (CHEN et al., 2017). Despite that, proteins of ECM-receptor interaction, focal adhesion, regulation of actin cytoskeleton, and leukocyte transendothelial migration pathways appear to be enriched in both bovine and porcine exosomes (CHEN et al., 2017).

Samuel et al. (2017) identified different proteins in colostrum and mature milk bovine samples. Colostrum was enriched with proteins related to inflammatory, acute-phase, and innate immune response, while mature milk was enriched in proteins related to protein transport. Thus, colostrum exosomes have a primordial role in cellular growth and innate immune response and modulate the gastrointestinal system and hematopoietic development of the offspring. Results also showed that many proteins (e.g. L-amino-acid oxidase, 2, agrin, and glypican 4) are highly abundant in 24 h colostrum exosomes and are gradually depleted until mature milk is produced. Only one study has investigated the proteome of milk exosomes, albeit without describing the lactation stage of the samples. Proteins found related

majorly to immune system functions and vesicle trafficking, as previously described in section 2.2.3.

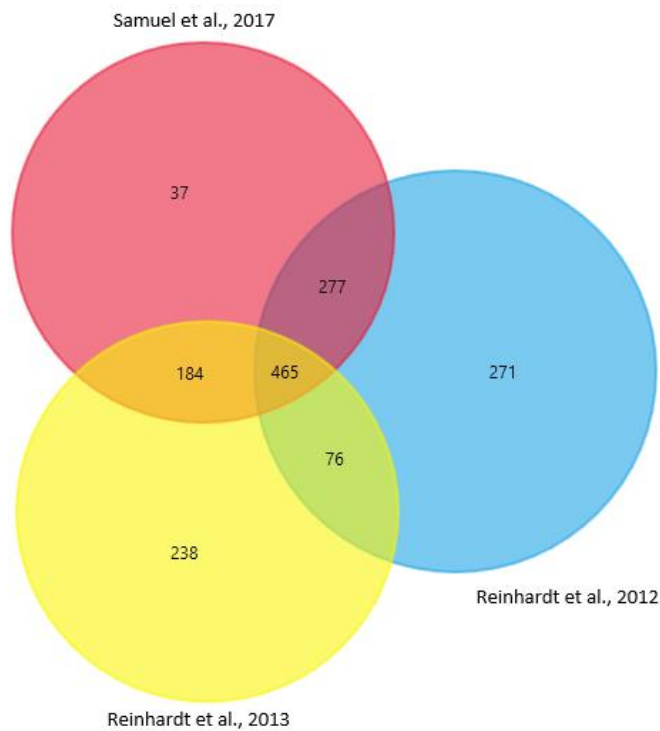


Figure 6. Venn diagram of unique proteins identified in different bovine milk exosome studies with databases available at Vesiclepedia. Source: Analysis made by the author using FunRich software v.3.1.3.

Exosomes from both bovine milk and serum appear to have common proteins, but their composition is mostly unique to each body fluid (KOH et al., 2017). The biological process and molecular function targeted by these proteins using Panther and gene ontology algorithms revealed similar results, although milk exosomes contained proteins with a function in the reproduction and development processes. Koh et al. (2017) also highlighted the need for expanded detailed exosome analysis of proteins together with lipids and miRNA profiles.

2.4.2 *Current knowledge on the lipidome of milk exosomes*

Lipidomics has gained special interest as a novel field in EV research, but only a handful of lipids have been identified, and the roles of lipids in vesicle biogenesis and their biological functions are yet to be elucidated (CHOI et al., 2013). Thin layer chromatography, gas liquid chromatography, and mass spectrometry have been commonly employed for exosome lipidomic analysis (SKOTLAND et al., 2017a). Lipid contents have been analyzed from exosomes of different cell types (HARASZTI et al., 2016) including reticulocytes (CARAYON et al., 2011), prostate cells (LLORENTE et al., 2013), cortical collecting duct cells (DANG et al., 2017), ovarian surface epithelial cells (CHEN et al., 2020), and urine (SKOTLAND et al., 2017b; DEL BOCCIO et al., 2012).

Phospholipid profiles from milk serum, fluff (phospholipid concentrate obtained by ultracentrifugation of skim milk), and fat globules in bovine and human milk have been analyzed by high-performance thin layer chromatography (BLANS et al., 2017). Sphingomyelins were reported to be the main component of all isolates, and milk EVs appeared to be enriched in sphingomyelins and phosphatidylserines at the expense of phosphatidylcholines in comparison to milk fat globules. Only one study has investigated the composition of milk exosomes using untargeted lipidomics, in human milk (CHEN et al., 2021). A total of 395 lipids were identified, and the lipidome of exosomes present in term and preterm milk were predominantly shared (only 10 distinct lipid elements). In total, 15 lipid sub-classes, including phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylethanolamine (PE), were identified in both groups. There is no previous report of lipidomics studies in porcine milk exosomes.

2.5 Effects of different ratios of dietary omega-6:omega-3 polyunsaturated fatty acids

Omega-6 (ω -6) and omega-3 (ω -3) fatty acids are polyunsaturated fatty acids (PUFA), by definition containing more than one cis double bond. Although humans and other mammals can synthesize saturated fatty acids and some monounsaturated fatty acids from carbon groups in carbohydrates and proteins, they lack the delta (Δ) 12 and Δ 15 desaturase enzymes necessary to insert a cis double bond at the ω -6 or the ω -3 position of fatty acid

(TRUMBO et al., 2002). Consequently, ω -6 and ω -3 are essential nutrients and represent a crucial component of the diet of humans and animals.

Linoleic acid (LA) is the parent fatty acid (FA) of the ω -6 series, while α -linolenic acid (ALA) is the parent FA of the ω -3 series, and both FA are part of a longer chain of longer derivatives inside the body, which can also be obtained from different food sources (Figure 7) (HIGDON et al., 2019).

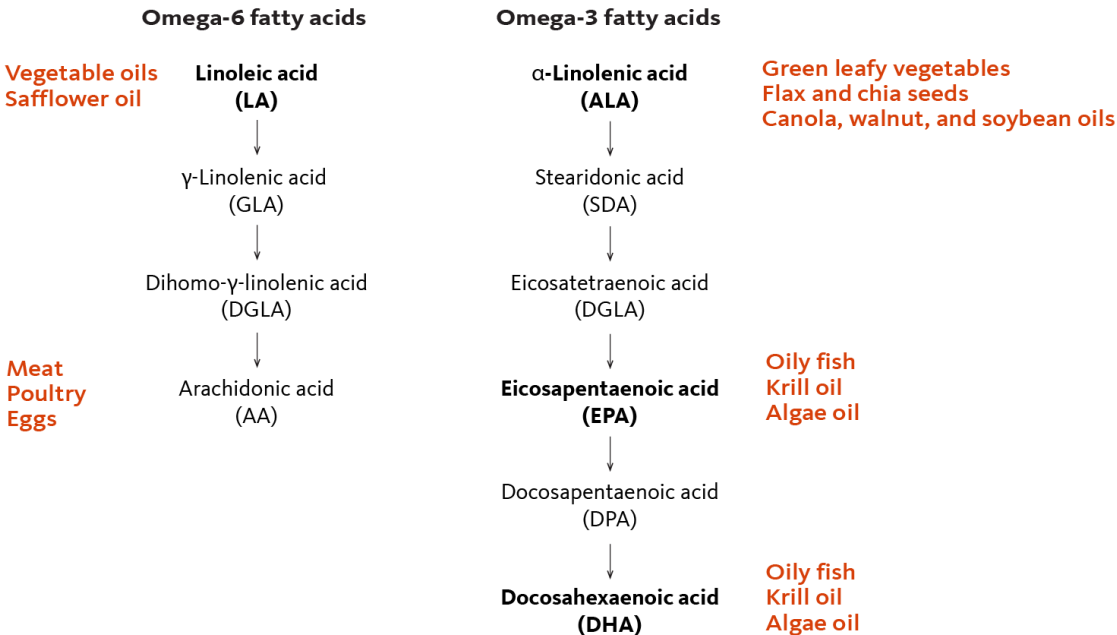


Figure 7. Omega-6 and omega-3 and their respective longer chain derivative compounds. Dietary sources of the different long-chain polyunsaturated fatty acids are listed in orange. Adapted from Higdon et al., 2019.

PUFA are involved in a wide variety of physiological functions such as fat deposition, muscle development, and glycolipid metabolism and often have not inter-convertible and opposing functions (MOERNO-ALIAGRA et al., 2010; VAIDYA and CHEEMA, 2014; TACHTSIS et al., 2018). During the synthesis of their longer chain derivatives, the 18-carbon ω -3, and ω -6 share a common enzymatic pathway and thus compete for it (PALMQUIST, 2009). As a result, the balance between ω -6: ω -3 plays an important role to maximize the beneficial effects of adding ω -3 into the diet. The optimal ratio of ω -6: ω -3

supports the body's health and development and could inhibit immune stimulation to increase the efficiency in using energy and nutrients for optimum performance and homeostatic pathways (CALDER, 2013; DUAN et al., 2013). In contrast, the pathogenesis of many diseases such as cardiovascular disease, cancer, and inflammatory and autoimmune diseases is promoted by a high ω -6: ω -3 ratio (SIMOPOULOS, 2006).

The ω -6: ω -3 ratio in the diet is relevant both in human and livestock nutrition to support health and provide healthier foods. In pig production, there is a growing interest in lowering the ω -6: ω -3 by using divergent sources of ω -3 PUFA (e.g., fish oil, linseed oil) to promote the health and performance of sows and piglets (GREEF et al., 2016; YIN et al., 2017). Present commercial diets can provoke an imbalance in the ω -6: ω -3 due to the increased amount of ω -6 from corn and other grains (KOUBA and MOURROT, 2011; SIMOPOULOS, 1991). To induce the beneficial effects on health and performance of sows and piglets, sources containing high amounts of ω -3, such as fish oil and linseed oil, are used as a supplement in the maternal diet to lower the ratio of ω 6: ω 3 PUFA often below 6:1 (YAO et al., 2012; DE GREEFF et al., 2016; YIN et al., 2017; MCAFEE et al., 2019; YANG et al., 2019).

Pregnancy is the most sensitive phase in the mammal life cycle, with complex physiological changes such as weight gain, and fetal and placental development (CORRALES et al., 2021). Particularly in gestating sows, the nutrient requirements change according to the reproduction phases and massive metabolic alterations can occur in many tissues, including increased oxidation levels and anti-oxidant strategies are recommended to avoid high oxidative damage during late gestation and lactation (KIM et al., 2013). In that sense, the enrichment of ω -3 PUFA in the diet can stimulate the antioxidative capacity in sow plasma and increase glutathione peroxidase activity, an enzyme protecting cells against oxidative damage in piglet liver, and decreased lipid peroxidation in piglet plasma (TANGHE et al., 2015). Nguyen et al. (2021) have investigated the effects of low ratios of dietary ω -6: ω -3 on the plasma proteome of sows at different time points throughout gestation and lactation. Unique proteins were found to be differentially abundant between the treatments in different periods, and functional analysis revealed that major proteins were involved in the regulation of plasma lipoproteins, stimulus- and defense-response proteins. Besides these findings, it is still not clear how the effects of ω -3 and ω -6 PUFA in the diets fed during gestation and lactation may be transmitted from the mother to the offspring, and

EV-mediated intercellular communication via milk exosomes and placental exosomes could potentially play a role (JIN and MENON, 2017).

Moreover, early studies have already shown that the addition of fat reaching from 7.5% to 15 % to the sow's diet before farrowing and (or) during lactation results in a higher fat content of the colostrum and milk (PETTIGREW, 1981). Besides, the fatty acid composition of the milk can be significantly influenced by the sow diet or rather by the fat source. Among a variety of fat components such as rapeseed oil, fish oil, coconut oil, palm oil, or sunflower oil, the availability of PUFA could be increased to the greatest extent by the addition of fish oil (LAURIDSEN and DANIELSEN, 2004). It has also been reported that IgG synthesis in piglets positively correlates with the amount of absorbed maternal IgG (ROOKE and BLAND, 2002), which reinforces the importance of the IgG intake from colostrum and milk. However, a clear pattern between the change of immunoglobulin concentrations in piglet plasma and the ratio of ω -6: ω -3 could not be reported. On the other hand, the cytokines IL-1 β and TNF- α were decreased, suggesting that piglet inflammation was prevented at a ω 6: ω 3 ratio of 9:1 (YAO et al. 2012).

The dietary supplementation of ω -3 PUFA is known to induce changes in the milk fatty acid composition in several mammals, including pigs (YIN et al., 2017; NGUYEN et al., 2020). In the colostrum, a low ω -6: ω -3 ratio reduced concentrations of γ -linolenic acid and docosahexaenoic acid, while in mature milk, a low ω 6: ω 3 ratio increased concentrations of total ω -3 FAs, specifically of ALA, eicosatrienoic acid, and eicosapentaenoic acid (NGUYEN et al., 2020). Further effects of the dietary enrichment of ω -3 PUFA have also been observed in bovine milk, by the reduction in milk fat percentage, but no alterations to the milk protein or milk lactose concentrations were found (MOALLEM et al., 2018; MUSTAFA et al., 2003). No studies have included the milk EV fraction, and thus the effects of ω -3 supplementation on milk exosomes are unknown.

3 AIMS AND HYPOTHESES

The main aims of this thesis are: (1) to identify how the protein and lipid composition of porcine milk exosomes are modified during the transition from the colostrum until the weaning stage; and (2) to assess how different ratios of ω -6: ω -3 PUFA in the diet of gestating and lactating sows affect the protein and lipid composition of porcine milk exosomes.

The current knowledge on the molecular characterization of exosomes indicated that their proteome comprehends both a unique protein signature and dynamic protein changes, which can be modulated by variations on the physiological state of the cell, adapting to external and local stimuli. Recent evidence has also underlined the pivotal role of milk exosomes on the infant's adequate growth and cellular development. Therefore, the hypothesis of this study is that sow colostrum exosomes and milk exosomes have distinct specific characteristics and contains cargo that will play a role modulating the piglet's immune system and growth.

The ratio of dietary ω -6 and ω -3 PUFA is known to affect health: a high ratio is considered to promote chronic diseases such as inflammatory and cardiovascular ones, whereas a lower ω -6: ω -3 ratio counteracts such diseases and exerts anti-inflammatory effects. The enrichment of ω 3 PUFA is a strategy used in pig production, although it is still not clear how the effects of ω 3 and ω 6 PUFA in the diets fed during gestation and lactation may be transmitted from the mother to the offspring, and EVs are suggested to play a role in that matter. The overall effect of the diet on the milk exosome composition is also largely unexplored, although lowering the dietary ω -6: ω -3 ratio has been associated with variations on the blood proteome and other metabolic changes. Therefore, the second hypothesis of this study is that different maternal feeds, by different ratios of ω -6 and ω -3 PUFA on the sow's diet, may affect the protein and lipid composition of porcine milk exosomes.

4 MATERIAL AND METHODS

4.1 Experimental design and sample collection

4.1.1 Animals and housing

The animal trial was conducted on a commercial swine farm (Arioli and Sangalli Agricultural Company S. S.; Genzone, Italy), and was approved by the Ethical Committee of the University of Milan (OPBA 67/2018) and the Italian Ministry of Health (authorization n. 168/2019 PR). Sows were artificially inseminated with pooled semen (Topdelta boar) and kept in groups from one week after artificial insemination until one week before farrowing. All sows in this study were at second parity, with the insemination for second parity performed at 13th months of age and the average age at parturition being around 17th months of age. All sows had similar body weight (202.57 ± 7.16 kg, mean \pm SEM) and body condition score (2.36 ± 0.12 , mean \pm SEM) at the beginning of the trial. Details on body weight and body condition of sows from this study during gestation and at the end of lactation are provided in Table 4. On day 108, gestating sows were moved to individual farrowing crates and stayed there until weaning. Within 24 h of birth, ear notching and tagging, iron injection, needle teeth clipping, and tail docking were performed.

Table 4. Sow's body weight (BW) and body condition score (BCS) at different points of the experimental trial. Data are presented as mean \pm standard deviation. The BW and BCS were not statistically different between the feeding groups (p-value = 0.36 and 0.9, for BW and BCS respectively).

| Timepoint | Body Weight (kg, mean \pm SD) | BCS (mean \pm SD) |
|-------------------|---|---------------------------------------|
| Gestation Day 28 | 210 \pm 25 | 2.44 \pm 0,46 |
| Gestation Day 79 | 242 \pm 19 | 2.55 \pm 0,53 |
| Gestation Day 108 | 251 \pm 19 | 2.88 \pm 0,22 |
| Lactation Day 26 | 217 \pm 32 | 2.12 \pm 0,64 |

4.1.2 Experimental diets

Sixteen multiparous sows with similar body weight and body condition scores were randomly allocated to one of two dietary treatments: the control group (Group S - GS) received a standard diet with a ω -6: ω -3 ratio of 13:1 from day 28 of gestation until farrowing, and of 10:1 during lactation. The treatment group (Group L - GL) was fed a diet with a low ω -6: ω -3 ratio (4:1 from day 28 of gestation until the end of lactation, i.e. 24 days after parturition). The ω 6 and ω 3 fatty acids for this study were derived from soybean oil (GS) and linseed oil (GL), respectively (Mazzoleni s.pa., Bergamo, Italy). The ω -6 and ω -3 PUFAs content (per total fatty acids) of soybean oil was 54.3% and 8.5%, (ω -6: ω -3 = 6.26) of linseed oil, was 16.2% and 52.9% (ω -6: ω -3 = 0.31). All diets were calculated to be isonitrogenous and isoenergetic and to meet or exceed the estimated nutrient requirements for sows during gestation and lactation (National Research Council, 2012). The complete information on the composition of the basal diets of the sows is provided in Table 5 and the adjustments to the final ratios of ω -6: ω -3 PUFAs are described in Table 6.

Experimental diets (fed as a liquid feed by mixing with water) were supplied from the day (d) 28 of gestation until the end of lactation. Soybean oil and linseed oil were added to the barley meal at a rate of 10% to create a mixture before the daily feeding of the sows. The gestation diet was provided at 2.4 kg/d with 15 g/d of soybean or linseed oil from d 28 to d 79 and 2.9 kg/d with 18 g/d of soybean or linseed oil from d 80 to the end of gestation. Sows were fed per pen (8 sows/pen). The lactation diet was fed at 1 kg/d on the farrowing day (d 0) and then gradually increased to a maximum of 7.5 kg/d at weaning. During lactation, soybean oil and linseed oil were added daily to the individually basal diet administered according to the lactation feeding plan. Feed was provided twice a day and sows had unlimited access to freshwater. Feed was offered (increased daily) based on the sows' feed consumed during the previous day.

4.1.3 Sample collection

To ensure the wellbeing of the animals, the milk samples were non-invasively obtained during the naturally occurring milk-let down reflex when the piglets were suckled. Colostrum and milk samples were collected after an overnight fast (12 h), at the first 24 hours post-partum (day 0 - colostrum), day 7, and day 14 post-partum. Samples were divided

into two aliquots and frozen immediately after collection and kept frozen at $-80\text{ }^{\circ}\text{C}$ until exosome isolation.

Table 5. Composition of basal sow diets used on the experimental trial from this study.

| Item <i>Ingredients (g/kg as fed basis)</i> | Gestation | Lactation |
|---|------------------|------------------|
| Corn | 284.60 | 249.10 |
| Barley | 224.20 | 216.70 |
| Wheat bran | 208.00 | 115.80 |
| Distillers grains | 125.00 | 40.00 |
| Biscuit | 50.20 | 52.10 |
| Rice | 35.00 | 35.00 |
| Commercial concentrate * | 25.00 | 250.00 |
| Soybean oil | 12.90 | 14.00 |
| Fish meal | - | 14.50 |
| Mineral-vitamin premix ** | 20.00 | 11.70 |
| HCl-Lysine | 11.20 | 15.00 |
| <i>Composition (% DM)</i> | | |
| Crude protein | 15.85 | 19.92 |
| Crude fat | 4.55 | 4.93 |
| Crude fiber | 5.69 | 5.66 |
| Ash | 5.68 | 4.46 |
| Ca | 1.70 | 1.21 |
| P | 0.56 | 0.57 |
| Ca/P | 3.04 | 2.12 |
| Lysine | 1.04 | 1.34 |
| Methionine | 0.18 | 0.22 |
| Met +Cis | 0.37 | 0.50 |

* Providing (as fed basis): 32.36% crude protein, 6.80% crude fat, 6.77% crude fiber, 0.80% Na, 2.43% lysine, 0.56% methionine. ** Providing (per kg of complete diet): vitamin A, 10,000 IU; vitamin D3, 2000 IU; vitamin E, 48 IU; vitamin K3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; biotin, 0.2 mg; d-pantothenic, 17 mg; folic acid, 2 mg; choline, 166 mg; vitamin B6, 2 mg; and vitamin B12, 28 mg. Fe (as FeSO_4), 90 mg; Cu (as CuSO_4), 15 mg; Zn (as ZnSO_4), 50 mg; Mn (as MnO_2), 54 mg; I (as KI), 0.99 mg; and Se (as Na_2SeO_3), 0.25 mg.

Table 6. Fatty Acid composition (g/100 g total fatty acids) of the control ratio (CR) and low ratio (LR) diets, adjusted for different ratios of omega-3 (ω -3) and omega-6 (ω -6) polyunsaturated fatty acids.

| Item | Gestation CR | Gestation LR | Lactation CR | Lactation LR |
|---------------------------|--------------|--------------|--------------|--------------|
| 10:00 | 0.26 | 0.22 | - | - |
| 12:00 | 0.35 | 0.30 | 1.80 | 1.56 |
| 14:00 | 0.25 | 0.21 | - | - |
| 16:00 | 20.23 | 18.08 | 17.58 | 16.06 |
| 16:1 ω 7 | 0.50 | 0.42 | - | - |
| 18:00 | 3.06 | 3.30 | 4.36 | 4.40 |
| 18:1 ω 9 cis | 19.88 | 19.80 | 24.51 | 23.84 |
| 18:1 ω 7 | 1.05 | 1.03 | - | 0.12 |
| 18:2 ω -6 cis 9,12 | 49.79 | 44.73 | 46.92 | 42.90 |
| 18:3 ω -3 | 3.72 | 11.12 | 4.83 | 11.12 |
| 20:00 | 0.28 | 0.24 | - | - |
| 20:1 ω -9 | 0.41 | 0.35 | - | - |
| 22:00 | 0.23 | 0.20 | - | - |
| ω -6 | 49.79 | 44.73 | 46.92 | 42.90 |
| ω -3 | 3.72 | 11.12 | 4.83 | 11.12 |
| ω -6: ω -3 | 13.40 | 4.02 | 9.71 | 3.88 |

4.2 Isolation and characterization of milk exosomes

4.2.1 Isolation of exosomes from colostrum and milk

Milk exosomes were isolated separately in two different batches, from two aliquots derived from each sample, one for proteomics analysis and one for lipidomics analysis, respectively. Milk exosomes were isolated using ultracentrifugation coupled with size exclusion chromatography to enrich milk-derived exosomes from extracellular vesicles as described by Vaswani et al. (2009) with modifications. Samples with an initial volume of 2 mL were centrifuged at 4,000 x g for 30 min at 4°C. Avoiding the fat layer, approximately 1.5 mL of skimmed milk were removed and centrifuged at 12,000 x g for 30 min at 4 °C and the supernatant was then centrifuged at 100,000 x g for 1 h at 4°C. The pellet that was formed on top of the firm casein pellet was removed and resuspended in PBS. After a further

centrifugation step of 150,000 x g for 2 h at 4°C, the exosome pellet was collected again removed from the firm casein pellet and resuspended in PBS to 500 µL. This suspension was then loaded on a qEVoriginal 35 nm size exclusion chromatography column (Izon Science, Oxford, UK), following the manufacturer's instructions. After the void volume, 4 fractions of each 500 µL were collected. Fractions 2 and 3 contained higher concentrations of milk exosomes and were used in the following analysis.

4.2.2 Nanoparticle Tracking Analysis (NTA)

The concentration and size distribution of particles in the fractions were measured with NanoSight NS300 (NTA 3.1, Malvern Panalytical, Malvern, UK). Fractions were diluted to 1:25-1:1,000-fold in PBS to keep the number of particles in the field between 50 to 200/frame. Three videos of 30-sec duration were captured with camera gain set at 5, camera level set at 12, and detection threshold set at 5.

The statistical analysis of the NTA data (exosomal size and concentration) was performed using SAS (version 9.4, SAS Institute Inc.). All data were tested for normality of distribution (Shapiro-Wilk statistic) using the UNIVARIATE procedure of SAS, and where appropriate, they were transformed using a log₁₀ transformation. A repeated-measures model was fitted to the data using the PROC MIXED procedure of SAS. The first-order autoregressive covariance structure was the best fit for these data as determined by the lowest Bayesian information criterion. The model consisted of treatment, time (day), and the interaction of treatment and time as fixed effects, and animal as a random effect. Data are reported as least squares mean calculated using the post hoc procedure (Tukey-Kramer), with $p < 0.05$ considered statistically significant.

4.2.3 Transmission Electron Microscopy (TEM)

For electron microscopy analysis, 50 µL of purified exosomes were pipetted on Parafilm® (Bemis, Neenah, WI, USA) and immediately adsorbed to an Athene old 400 mesh copper grid (45 µm square, Agar Scientific, Essex, UK) coated with 1% Pioloform® (Agar Scientific) in chloroform (w/v), and then incubated for 5 min at RT. The grid was carefully washed twice with distilled water and negatively stained with 50 µL of 2% uranyl acetate

(w/v) (SERVA, Rosenheim, Germany). The samples were then viewed using the Zeiss EM 109 (Carl Zeiss QEC GmbH, Köln, Germany) transmission electron microscope.

4.2.4 Verification of exosomal marker by Western Blotting

Proteins from all fractions were dissolved in a reducing sample buffer, boiled, and loaded on 12% SDS-PAGE. The fractionated proteins were transferred to a polyvinylidene difluoride membrane with the use of the Trans-Blot Turbo transfer unit for 30 min, at 1 A and 25 V (Bio-Rad Laboratories, Munich, Germany). The membranes were blocked with Tris-buffered saline containing 0.05% Tween 20 (TBST) and Rotiblock (Carl Roth, Karlsruhe, Germany) for 60 min at RT. Blots were incubated with mouse monoclonal anti-TSG101 IgG2a- κ (sc-7964, Santa Cruz Biotechnology, Dallas, TX, USA) overnight at 4°C, washed three times with TBS-T, and incubated for 120 min at RT with secondary mouse IgG κ light chain binding protein (m-IgG κ BP) conjugated to horseradish peroxidase (sc-516102, Santa Cruz Biotechnology). After washing, the immune complex was detected with Amersham™ ECL Select™ Western Blotting Detection Reagent chemiluminescence detection system (RPN2235, Cytiva, Freiburg, Germany). Imaging was performed with a VersaDoc MP4000 imaging system (Bio-Rad, Hercules, CA, USA).

4.2.5 Total protein concentration

Total protein concentration on exosome fractions was estimated using Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), immediately after isolation.

4.3 Proteomic analysis

4.3.1 In-gel digestion

Exosome fractions were concentrated to a final volume of 20 μ L using a 10 kDa cut-off column (11,000 x g for 30 min at 4 °C) and the proteins were separated in a 12% SDS-PAGE at 90 V for 15 min. Proteins were fixed with 25% isopropanol/10% acetic acid for 15 min and washed three times for 10 min each with ultrapure water. The gel was stained with PageBlue™ Protein Staining Solution (24620, Thermo Fisher Scientific, Waltham, USA)

for 1 h at RT, rinsed, and incubated with ultrapure water. A large single band was excised from the gel, cut into 1 mm³ cubes, and transferred to a microcentrifuge tube. Bands were shrunk and destained with acetonitrile (ACN) and incubated with 10 mM DTT/50 mM triethylammonium bicarbonate (TEAB) for 30 min at 56 °C to reduce the proteins. Bands were again shrunk with ACN and incubated for 20 min at RT in the dark with 55 mM iodoacetamide in 50 mM TEAB for alkylation of cysteines, washed with 50 mM TEAB for 15 min, and incubated with 20 µg/mL of trypsin (Trypsin Gold, Promega, Madison, WI, USA) for 1 h at RT. The addition of trypsin solution was repeated, and the incubation continued for another 15 min followed by the addition of TEAB/10% ACN buffer to keep gel pieces wet during enzymatic cleavage. Bands were incubated overnight at 37 °C for maximum peptide recovery, and peptides were extracted using 1:2 5% formic acid/ACN for 15 min at 37 °C in a Thermomixer shaker (HLC Biotech, Bovenden, Germany, Model: MHR11) at 250 rpm. The extracted peptides were dried at 45 °C (SpeedVac™, Thermo Fisher Scientific, Waltham, USA) and stored at -80°C until LC-MS/MS analysis.

4.3.2 Peptide labeling and LC-MS/MS

The peptides were dissolved in 15 µL 100 mM TEAB and labeled with isobaric tandem mass tags using the TMTpro 16plex reagents (Thermo Fisher Scientific, Darmstadt, Germany) according to the manufacturer's recommendations. Animals were assigned randomly to two pools with 15 labels each, detailed assignments are shown in Figure 8. Pooled peptides were acidified with formic acid (pH 2.5) and desalted on Oasis HLB cartridges (30 mg sorbent, Waters GmbH, Eschborn, Germany). Eluates containing 70% ACN, 0.1% formic acid were dried and stored at - 20°C.

Peptide separation was performed on a Dionex Ultimate 3000 RSLC nano HPLC system (Dionex GmbH, Idstein, Germany). The autosampler was operated in µL-pickup mode. Peptides were dissolved in 10 µL 0.1% FA (solvent A). One µL was injected onto a C18 analytical column (self-packed 300 mm length, 75 µm inner diameter, ReproSil-Pur 120 C18-AQ, 1.9 µm, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). Peptides were separated during a linear gradient from 7% to 35% solvent B (90% ACN, 0.1% FA) at 300 nL/min. The nanoHPLC was coupled online to an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

Gradient length was 180 min. Peptide ions between 350 and 1400 m/z were scanned in the Orbitrap detector every 3 sec with a resolution of 120,000 (maximum fill time 50 ms, AGC target 400,000). Polysiloxane (445.12002 Da) was used for internal calibration (typical mass error ≤ 1.5 ppm). In a top-speed method, peptides were subjected to higher energy CID (HCD) (0.7 Da isolation, threshold intensity 20,000, normalized energy 30%) and fragments were analyzed in the Orbitrap with target 80,000 and maximum fill time 86 ms, 50,000 resolution. Fragmented peptide ions were excluded from repeat analysis for 30 s.

4.3.3 Data processing and statistical analysis

Raw data processing and database search were performed with the Proteome Discoverer software 2.4.0.305 (Thermo Fisher Scientific). Peptide identification was done with an in-house Mascot server version 2.6.1 (Matrix Science Ltd, London, UK). MS data were searched against sequences from the Uniprot pig reference proteome (2020/04, 49865 sequences) and contaminants database (cRAP) (MELLACHERUVU et al., 2013). Precursor Ion m/z tolerance was 10 ppm, fragment ion tolerance 0.02 Da. Tryptic peptides with up to two missed cleavages were searched. Propionamide on cysteines and TMTpro on N-termini and lysines were set as static modifications. Oxidation was allowed as a dynamic modification of methionine. Mascot results were evaluated by the Percolator algorithm version 3.02.1 as implemented in Proteome Discoverer. Spectra with identifications below 1% q-value were sent to the second round of database search with semi-tryptic enzyme specificity (one missed cleavage allowed). Protein N-terminal acetylation, methionine oxidation, and TMT were then set as dynamic modifications. Actual FDR values were typically $\leq 0.6\%$ (peptide spectrum matches) and $\leq 1.1\%$ (peptides). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD025515 and 10.6019/PXD025515.

| Label | Pool 1 | Pool 2 |
|-------|--------------------|--------------------|
| 126 | Day 0 Animal Y20 | Day 0 Animal K69 |
| 127N | Day 0 Animal Y145 | Day 0 Animal K25 |
| 127C | Day 0 Animal Y32 | Day 0 Animal Z127 |
| 128N | Day 0 Animal D95 | Day 7 Animal D80 |
| 128C | Day 14 Animal D80 | Day 0 Animal D80 |
| 129N | Day 14 Animal K25 | Day 7 Animal K25 |
| 129C | Day 7 Animal Y32 | Day 7 Animal Y145 |
| 130N | Day 0 Animal Y132 | Day 14 Animal D95 |
| 130C | Day 7 Animal D95 | Day 7 Animal Y20 |
| 131N | Day 14 Animal Y145 | Day 14 Animal Y132 |
| 131C | Day 7 Animal Y132 | Day 7 Animal K69 |
| 132N | Day 7 Animal K120 | Day 14 Animal K69 |
| 132C | Day 14 Animal Y32 | Day 0 Animal K120 |
| 133N | Day 14 Animal K120 | Day 7 Animal Z127 |
| 133C | Day 14 Animal Y20 | Day 14 Animal Z127 |

Figure 8. Assignment of tandem mass tags (TMT) labels for multiplex liquid chromatography mass spectrometry quantification analysis between milk exosome samples. Tags were randomly assigned to two pools with 15 labels each.

The statistical analyses of the peptide-spectrum match (PSM) level data were performed in R environment (R version 3.6) (R Core Team, 2018) using an in-house developed workflow. Non-unique peptides and single-hit proteins were filtered out before the statistical analysis. Only PSMs with co-isolation <50% were used. From all available fractions, only those with the least number of missing values per PSM and across all TMT channels were selected. Wherever this filter returns more than one fraction per PSM, the one with the highest average intensity across all TMT channels was selected. The PSM-level data were variance-stabilized and transformed using the VSN package (HUBER et al., 2002) and then aggregated to protein-level intensities using Tukey's median polish procedure. The statistical analysis was carried out using the R package Limma (Ritchie et al., 2015). As samples were taken from the same animals repeatedly over time, animals were

considered as a random effect in the statistical model and passed to the `duplicateCorrelation` function in Limma package as the blocking parameter. In addition, the peptide pool was modeled as a fixed effect. For each statistical contrast, the resulting P-values were adjusted for multiple testing and the false discovery rates (FDR) were calculated by the Benjamini-Hochberg method. An adjusted p-value of $p < 0.05$ was considered statistically significant and used to determine the differentially abundant proteins between the two contrasts. The Volcano plots, heatmaps, and PCA plots were generated using `ggplot2` (Wickham, 2016), `ComplexHeatmap` (GU et al., 2016), and `FactoMineR` (LE et al., 2008) packages, respectively.

4.3.4 Gene ontology and protein pathway analysis

Protein accession numbers were converted into Gene ID using the UniProt database conversion tool. Proteins from which the Gene ID was not available for *Sus scrofa* and/or proteins protein names defined as “Uncharacterized protein” in the UniProt *Sus scrofa* database were replaced using the UniProt BLAST tool with the best match on *Homo sapiens* orthologue annotated genes and/or names when providing a match hit of at least 70% identity. The protein-protein interaction (PPI) network, Gene Ontology (GO) analysis containing Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis were performed in STRING software version 11.0 (SZKLARCZYK et al., 2019), and complemented in Cytoscape v3.8.2 using ClueGo plugin v2.5.7 (BINDEA et al., 2009) with the following parameters: GO_BiologicalProcess in *Homo sapiens*, evidence codes used=All_without_IEA, GO tree interval level from 3 to 5, Kappa score threshold=0.4, correction method=Bonferroni step down. Generated GO terms were submitted to a refinement step by Revigo (revigo.irb.hr) to remove redundant terms and assign a term as leading GO.

4.4 Lipidomics analysis

4.4.1 Lipid extraction

Lipid extraction of milk exosome samples was prepared as previously described (CECILIANI et al., 2021) with minor alterations. Briefly, two aliquots (100 μ L) from each

sample were added with internal standards and extracted according to the Folch method (FOLCH et al., 1957). The organic residue was reconstituted with 200 μ L of 2-propanol: acetonitrile (90:10, vol/vol), 0.1% formic acid, and 10 mM ammonium acetate. Aliquots of 20 μ L were then diluted 1:10 with mobile phase B for lipidomic analysis in positive mode. Aliquots of 5 μ L were analyzed in negative ion mode for free fatty acid analysis. Aliquots of 50 μ L, after acid saponification, were prepared for total fatty acid quantification in negative mode. Each sample was extracted in duplicate, and 2 runs were performed for each extraction.

4.4.2 Lipidomics analysis

Samples were analyzed at the UNITECH platform OMICS (Università degli Studi di Milano, Italy) as follows: 2 and 5 μ L of sample for the positive and negative ion modes, respectively, were separated by liquid chromatography with a Kinetex EVO C18 column (2.1 \times 100 mm, 1.7 μ m; Phenomenex) at 45°C, connected to an ExionLC AD system (ABSciex) maintained at 15°C. Separated metabolites were then ionized through an electrospray ionization source and analyzed in a TripleTOF 6600 (quadrupole time-of-flight, QTOF, ABSciex) mass spectrometer. Mobile phases were (A) water with 0.1% formic acid and 10 mM ammonium acetate/acetonitrile (60:40), and (B) 2-propanol with 0.1% formic acid and 10 mM ammonium acetate/acetonitrile (90:10). The following elution gradient was used: 0 min 55% B; 2 min 55% B; 12 min 3% B; 17 min 3% B; 17.10 min 55% B; 20 min 55% B. The flow rate was 0.4 mL/min.

4.4.3 Data processing

Data processing was carried out using the untargeted data processing program MSDIAL (v. 3.98; <http://prime.psc.riken.jp/compms/msdial/main.html>) with LipidBlast database v. 2019 (<https://fiehnlab.ucdavis.edu/projects/LipidBlast>). This database contains 143,342 tandem mass spectra relating to 110,833 analytes belonging to 32 lipid classes. Data are expressed as ratio of analyte to internal standard area (1-phenoxy-2-propanol) and total protein content.

4.4.4 Lipid nomenclature

Lipid classification in categories, main class, and subclass was carried out according to the LIPID MAPS database (LIEBSICH et al., 2020). Lipids are abbreviated as follows: fatty acids – acylcarnitines (CAR), free fatty acids (FA), n-acyl ethanolamines (NAE), n-acyl glycines (NAGly), n-acyl glyceryl serines (NAGlySer), n-acyl ornithines (NAOrn), oxidized fatty acids (OxFA); glycerolipids – acyl diacylglyceryl glucuronides (ADGGA), diacylglycerols (DG), diacylglyceryl glucuronides (DGGA), diacylglyceryl trimethylhomoserine/diacylglyceryl hydroxymethyl-n,n,n-trimethyl- β -alanines (DGTS), diacylglyceryl-3-o-carboxyhydroxymethylcholines (DGCC), digalactosyldiacylglycerols (DGDG), lysodiacylglyceryl trimethylhomoserine/lysodiacylglyceryl hydroxymethyl-n,n,n-trimethyl- β -alanines (LDGTS), monoacylglycerols (MG), triacylglycerols (TG); glycerophospholipids – bismonoacylglycerophosphates (BMP), cardiolipins (CL), dimethyl-phosphatidylethanolamines (DMPE), hemibismonoacylglycerophosphates (HBMP), lysophosphatidylcholines (LPC), lysophosphatidic acids (LPA), lysophosphatidylethanolamine (LPE), n-acyl-lysophosphatidylserines (LNAPS), n-monomethyl phosphatidylethanolamines (MMPE), phosphatidic acids (PA), phosphatidylcholines (PC), phosphatidylethanol (PetOH), phosphatidylethanolamines (PE), phosphatidylglycerols (PG), phosphatidylinositols (PI), phosphatidylmethanol (PMeOH), phosphatidylserines (PS); prenol lipids - coenzyme Q (CoQ10); sphingolipids – acylhexosylceramides (AHexCer), ceramide 1-phosphates (CerP), ceramide phosphoethanolamines (PE-Cer), ceramide phosphoinositol (PI-Cer), ceramides (Cer), hexosylceramides (HexCer), sphingomyelins (SM), sulfatides (SHexCer), sulfonolipid (SL); and sterol lipids - cholesteryl ester (CE), sterol esters (SE), sterols (ST). Alkyl ether and plasmalogen linkages are denoted by O- and P- respectively. The side-chain structures are denoted as carbon chain length:number of double bonds and are provided for each chain where they could be determined, or as a total number of all carbons and double bonds where individual chains could not be determined.

4.4.5 Univariate and Multivariable Statistical Analysis

MS data were checked for integrity, and variables containing more than 20% missing values (i.e., values lower than the limit of detection) were not considered for the statistical analysis using the MetaboAnalyst 5.0 webtool (PANG et al., 2021). When present, missing

values were imputed by Bayesian principal component analysis by MetaboAnalyst 5.0. The data were then transformed by log₁₀-transformation and Pareto scaled to correct for heteroscedasticity, reduce the skewness of the data, and reduce mask effects (CECILIANI et al., 2021). Volcano plots were created to provide an overview of the lipid profiles in two diets (GS vs. GL) as well as different sampling times (day 0 vs. 7; day 0 vs 14; day 7 vs.14) animals using the log-transformed data. Volcano plots are used to relate fold change (FC) to statistical significance. Significance was set using t-test FDR adjusted p-value threshold at 0.05 and fold change threshold at 2. The heatmaps were clustered by Euclidean distance and Ward's minimum variance method (ward.D).

5 RESULTS

5.1 Characterization of the porcine milk exosome preparation

Porcine milk and colostrum exosomes, isolated in two different batches designated respectively for proteomics and lipidomics analysis, were characterized by NTA, TEM, and Western Blotting. Results from the two batches were consistent and presented both overall and described separately in detail to substantiate each exosome isolation, as recommended by ISEV (LÖTVALL et al., 2014; THÉRY et al., 2018).

5.1.1 Porcine milk exosome size and concentration

The ANOVA mixed-model using the NTA measurements revealed that the treatments and the interaction between time and treatment yielded no differences in EV size, while the different lactation stages (colostrum vs milk) did. Overall results are presented in Table 7 and Figure 9.

Table 7. Descriptive summary of nanoparticle tracking analysis results on size (diameter in nm) and concentration (particles/mL) of milk exosomes from Group L (GL) and Group S (GS) on different lactation stages.

| Group | Concentration (particles / mL) | | Size (nm) | |
|----------------|-----------------------------------|------------------------|--------------|-------|
| | GL | GS | GL | GS |
| Mean | 3.3 x 10 ¹¹ | 2.6 x 10 ¹¹ | 156.5 | 157.1 |
| Std. Deviation | 2.8 x 10 ¹¹ | 1.7 x 10 ¹¹ | 17 | 18.9 |

| Day | 0 | 7 | 14 | 0 | 7 | 14 |
|----------------|------------------------|------------------------|----------------------|-------|------|-------|
| Mean | 4.4 x 10 ¹¹ | 2.3 x 10 ¹¹ | 2 x 10 ¹¹ | 145.3 | 163 | 162.4 |
| Std. Deviation | 3.2 x 10 ¹¹ | 1.3 x 10 ¹¹ | 9 x 10 ¹¹ | 20.7 | 13.9 | 12.2 |

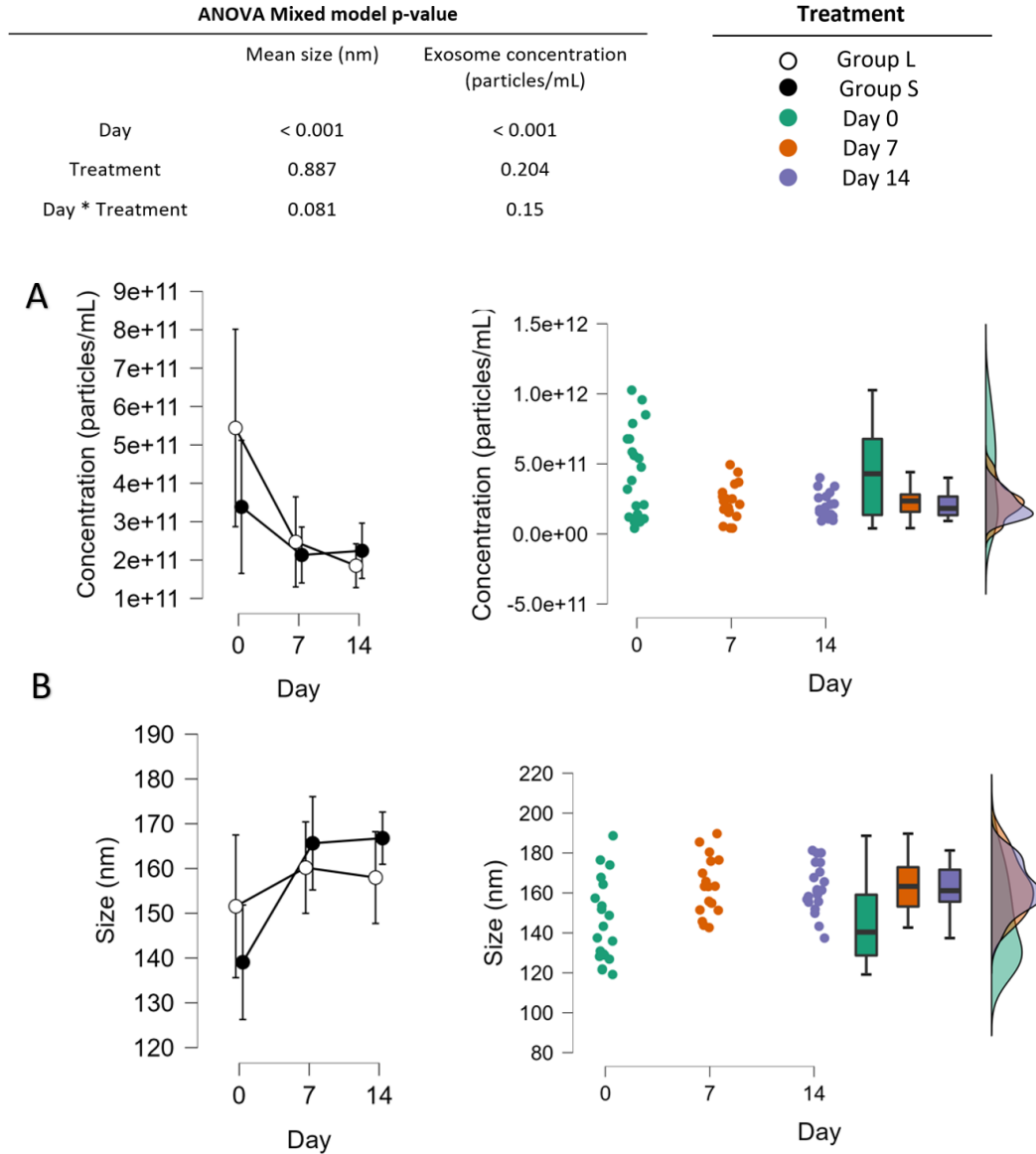


Figure 9. ANOVA mixed model comparison using the data from nanoparticle tracking analysis in response to **(A)** mean size (nm) and **(B)** exosome concentration (particles/ mL) concerning the diets, time, and interaction of diet and time, and raincloud plots with respective data distribution. Data are presented as means \pm SEM.

The average exosome diameter (nm) did not differ between the two groups (GL = 151 ± 18.5 ; GS = 154 ± 19.3 ; means \pm SD, on the isolation for proteomics analysis; and GL = 162 ± 14.7 ; GS = 156 ± 20.6 , on the isolation for lipidomics analysis). The exosome population was smaller ($p < 0.05$) in colostrum (139 ± 19 , proteomics isolation; 147 ± 21.3 , lipidomics isolation) than in mature milk at day 7 (163 ± 15 , proteomics isolation; $165 \pm$

14.2 lipidomics isolation) and 14 (160 ± 10, proteomics isolation; 166 ± 11, lipidomics isolation).

The treatment, time, and their interaction also did not influence the number of EV obtained from 2 mL starting material and the average concentration was similar in both groups, and throughout lactation, with a mean concentration of 2.4×10^{11} particles/mL on proteomics isolation and 3.5×10^{11} particles/mL on lipidomics isolation.

The exosome concentration had a positive correlation with the protein concentration (Pearson's $R^2 = 0.802$, p -value < 0.05) of the samples, corroborating with a feasible pure isolation free of contamination from other milk biocomponents.

5.1.2 Porcine milk exosome morphology

The examination by TEM showed round and cup-like concave spheres with morphology compatible with exomes. Representative images displayed both in wide and in close-up views are provided in Figure 10.

5.1.3 Verification of exosomal markers

The exosomal marker protein TSG101 was identified in colostrum and milk exosomes of both groups by Western Blotting (Figure 11). In addition, LC-MS/MS results were mapped against exosome protein databases and discovered that colostrum and milk porcine exosomes from this study contained most of those proteins that were commonly demonstrated in exosome proteome experiments, and which are also referred to as “exosome markers”: From the 25 most common exosome proteins in Exocarta (http://exocarta.org/exosome_markers_new) (KEERTHIKUMAR et al., 2015), 17 were also identified in this study, including all top 10 exosome markers according to this database (Table 8).

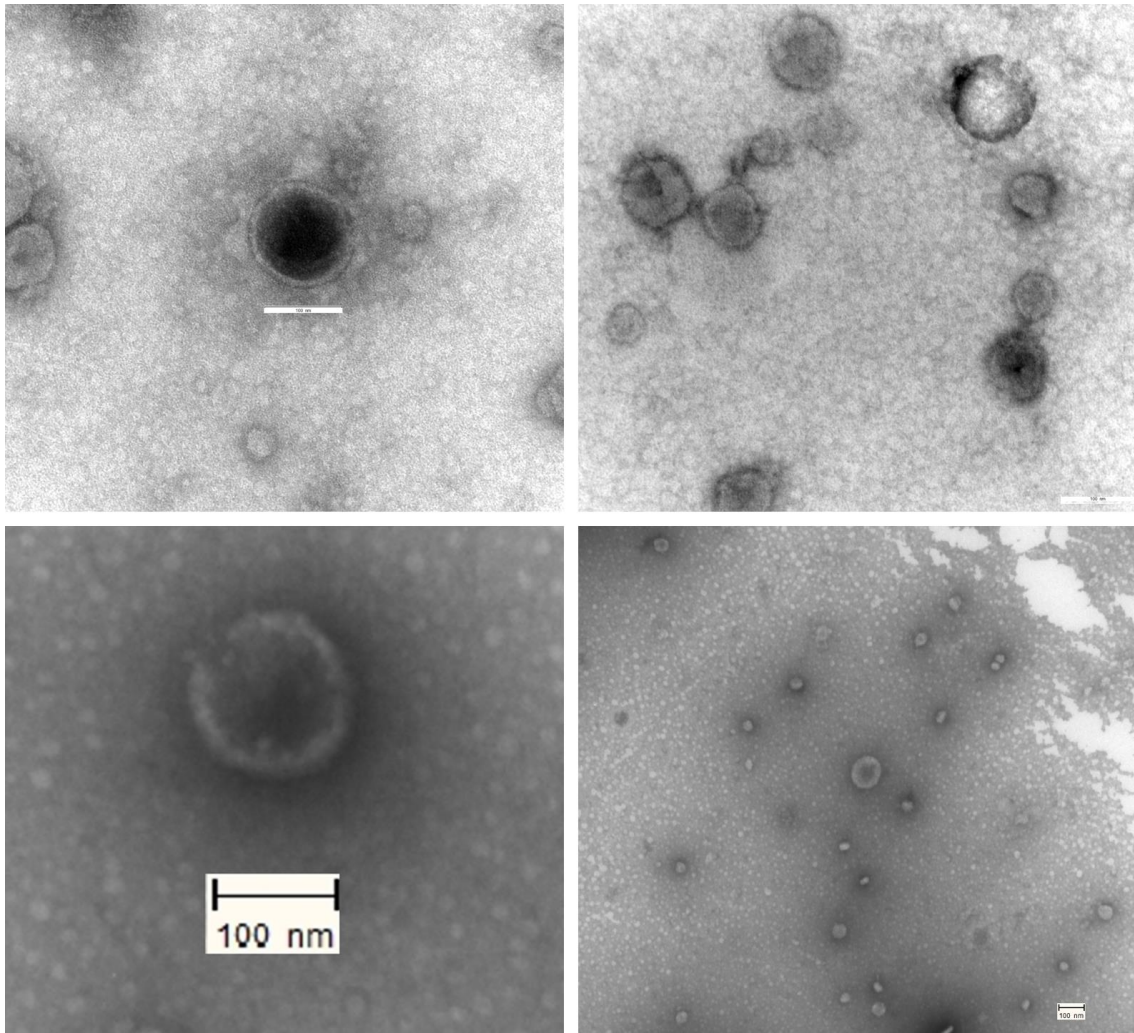


Figure 10. Representative electron micrograph of porcine milk exosomes in a close-up of a single vesicle (pictures on the right side) and wide view (pictures on the left side). Micrographs represent samples from both batches of isolation forwarded to lipidomics (upper pictures) and proteomics (lower pictures). The bar scale is equal to 100 nm in all micrographs.

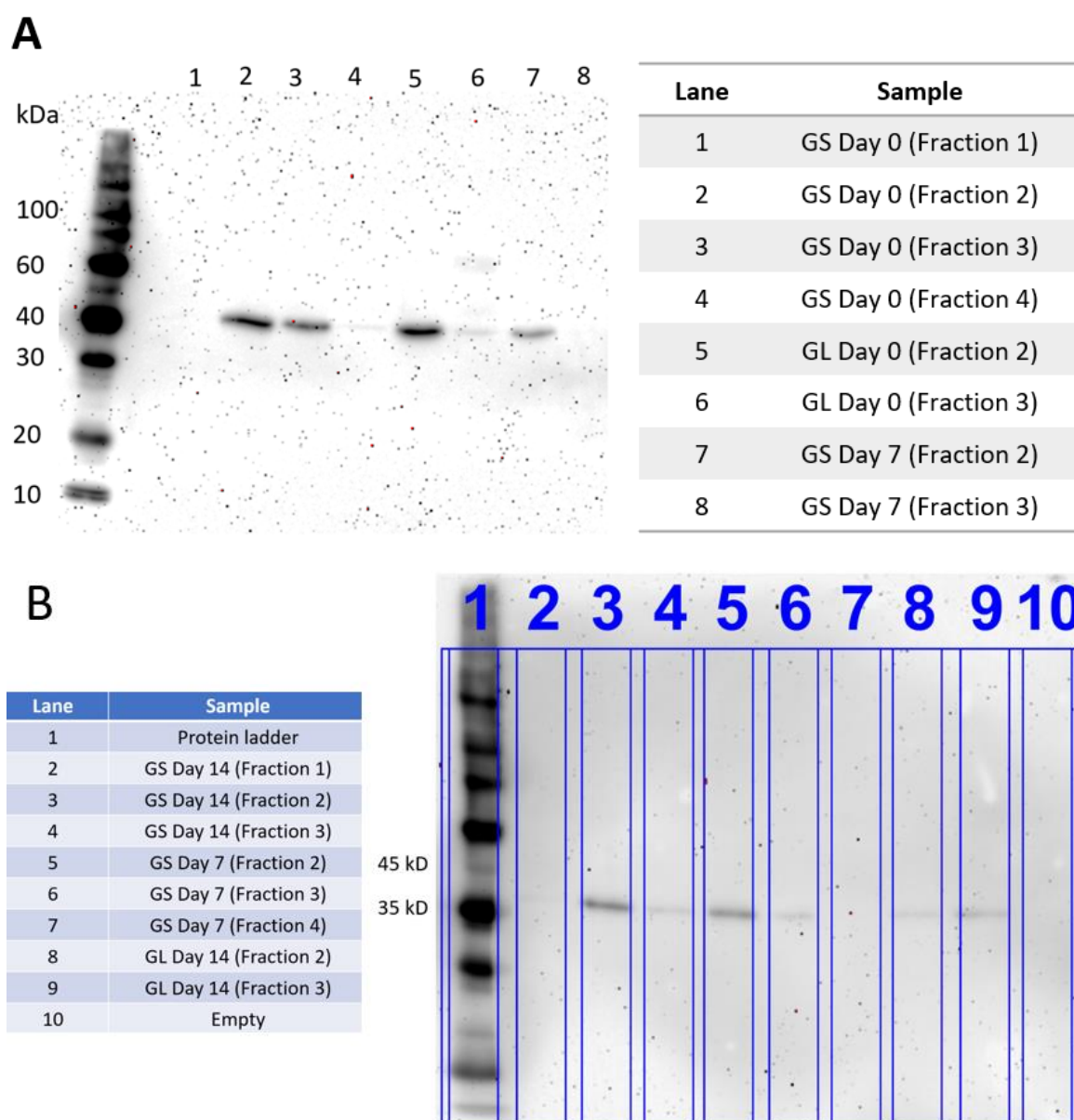


Figure 11. Western blotting of exosome marker TSG 101 in samples from both treatment groups after ultracentrifugation coupled with size exclusion chromatography (SEC), identified in milk exosomes isolation forwarded to (A) lipidomics and (B) proteomics analysis. Fractions 2 and 3 from SEC contained exosomes and were pooled together for proteomics analysis.

Table 8. Comparison between proteins identified by liquid chromatography–mass spectrometry (LC-MS/MS) and top exosome markers ranked in the Exocarta database (Keerthikumar et al., 2015).

| Rank in Exocarta database * | Gene Symbol | Number of studies identifying this marker in the Exocarta database | Identified by LC-MS/MS in this study |
|-----------------------------|-------------|--|--------------------------------------|
| 1 | CD9 | 98 | Yes |
| 2 | HSPA8 | 97 | Yes |
| 3 | PDCD6IP | 96 | Yes |
| 4 | GAPDH | 95 | Yes |
| 5 | ACTB | 93 | Yes |
| 6 | ANXA2 | 83 | Yes |
| 7 | CD63 | 82 | Yes |
| 8 | SDCBP | 78 | Yes |
| 9 | ENO1 | 78 | Yes |
| 10 | HSP90AA1 | 77 | Yes |
| 11 | TSG101 | 76 | Yes |
| 12 | PKM | 72 | No |
| 13 | LDHA | 72 | No |
| 14 | EEF1A1 | 71 | Yes |
| 15 | YWHAZ | 69 | No |
| 16 | PGK1 | 69 | Yes |
| 17 | EEF2 | 69 | Yes |
| 18 | ALDOA | 69 | Yes |
| 19 | HSP90AB1 | 67 | No |
| 20 | ANXA5 | 67 | Yes |
| 21 | FASN | 66 | Yes |
| 22 | YWHAE | 65 | No |
| 23 | CLTC | 64 | No |
| 24 | CD81 | 64 | No |
| 25 | ALB | 64 | Yes |

5.2 Proteomics analysis

Following in-gel digestion and TMT-labelling, peptides were analyzed by LC-MS/MS, revealing 5072 features, and mapping 3989 peptides which represented 637 proteins. PSM-level data variance stabilization and transformation are shown in Appendix

1. After exclusion criteria were applied ($FDR \leq 0.5\%$ and at least two unique peptides, removing redundant peptide-spectral-matches, single-shot proteins, and peptides with high isolation interference), 319 proteins were present at each time point were maintained for statistical analysis. Appendix 2 contains all proteins identified in each time point and the statistically significant proteins in each contrast.

5.2.1 Proteomics analysis from colostrum exosomes vs milk exosomes

Exosomes from colostrum presented 162 differentially abundant proteins (DAP; 82 increased and 80 decreased) when compared to exosomes from milk at day 7 p.p., and 170 DAP (81 increased and 89 decreased) from day 14. Comparing milk exosomes from day 7 versus day 14 yielded no DAP. The Volcano plots of \log_2 fold changes (x-axis) and their associated $-\log_{10}$ transformed p-values (y-axis) of all identified proteins in the different conditions are given in Figure 12A, together with the parallel analysis of effect-size of the top 20 genes per contrast (Figure 12B). Most DAP were found in both milk exosomes from day 7 p.p. versus colostrum exosomes and milk exosomes at day 14 p.p. versus colostrum exosomes (Figure 13) and were also common between the top upregulated and downregulated proteins in the comparisons (Tables 9 and 10). A distinct proteome profile from colostrum exosomes and milk exosomes was also noted in the principal component analysis of the top 5% proteins with the highest variance (Figure 14) and on the hierarchically clustered heatmaps (Figure 15).

5.2.2 Proteomics analysis from Group S vs Group L

In colostrum, there were no proteins with significantly different abundance between the two treatments. The abundances of GB1/RHD3-type G domain-containing protein and ATPase H(+)-transporting lysosomal accessory protein 2 were marginally greater in GS than GL (Table 9). In milk EV at 7 d, spondin-2 (SPON2) and 78 kDa glucose-regulated protein (HSPA5) were more abundant in GS than GL, while the Chromosome 8 C4orf19 homolog (C4orf19) was less abundant in group GS than GL at d 14. Proteins above the 10% FDR threshold in each contrast are displayed in Table 10. The Volcano plots of \log_2 fold changes

(x-axis) and their associated $-\log_{10}$ transformed p -values (y-axis) of all identified proteins in the different conditions are given in Figure 16.

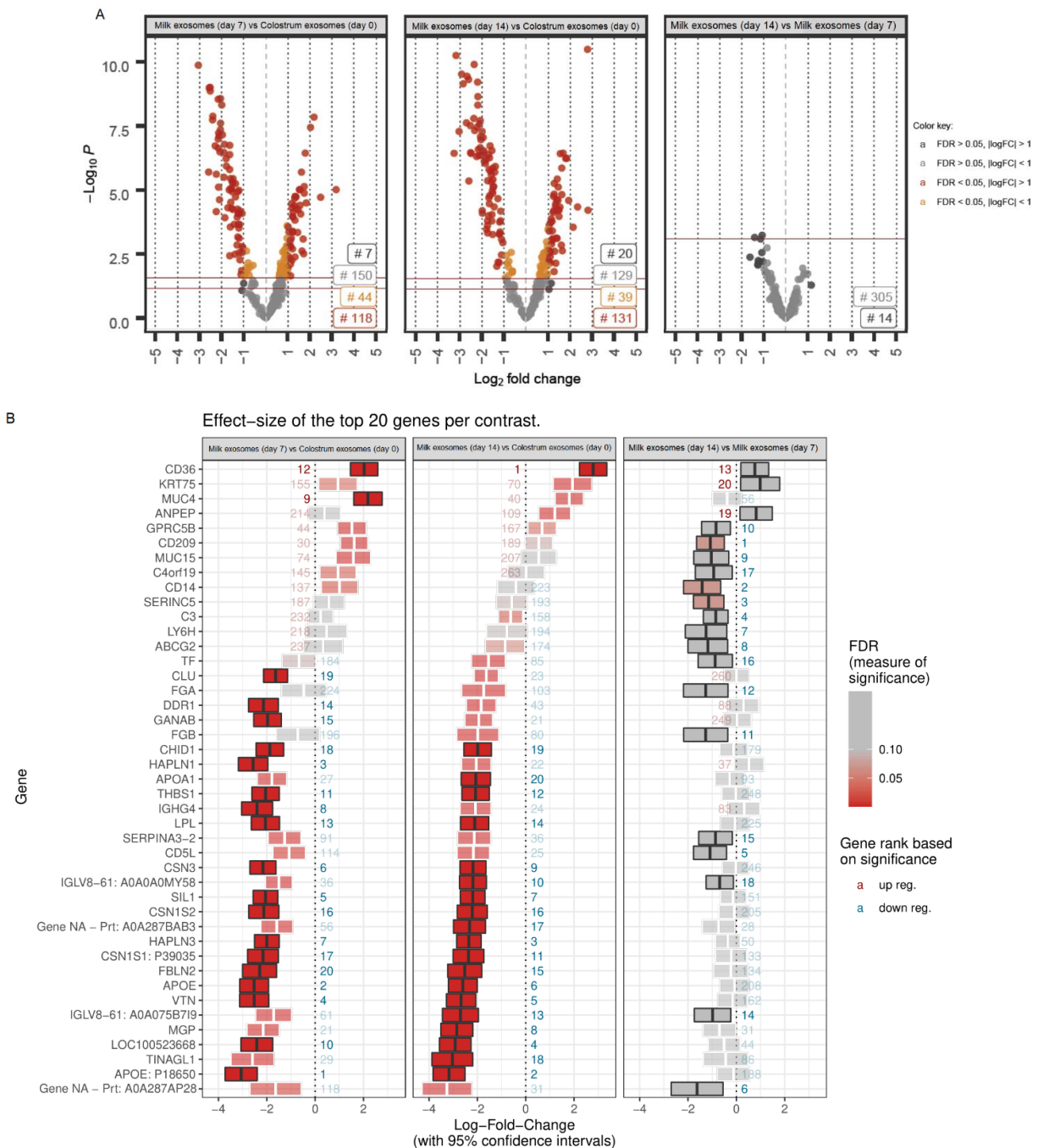


Figure 12. (A) Volcano plots of \log_2 fold changes (x-axis) and their associated $-\log_{10}$ transformed p -values (y-axis) of all identified proteins in the different time points. The horizontal lines in red represent the 5% and 10% FDR threshold on the first contrast, and the 10% FDR threshold on the third contrast. (B) Parallel analysis of effect size of the top 20 genes per contrast. Red boxes correspond to differentially expressed genes in a given comparison and shallow-grey boxes correspond to genes that were not among the top 20 genes in a given comparison but are among the top hits in the other comparisons.

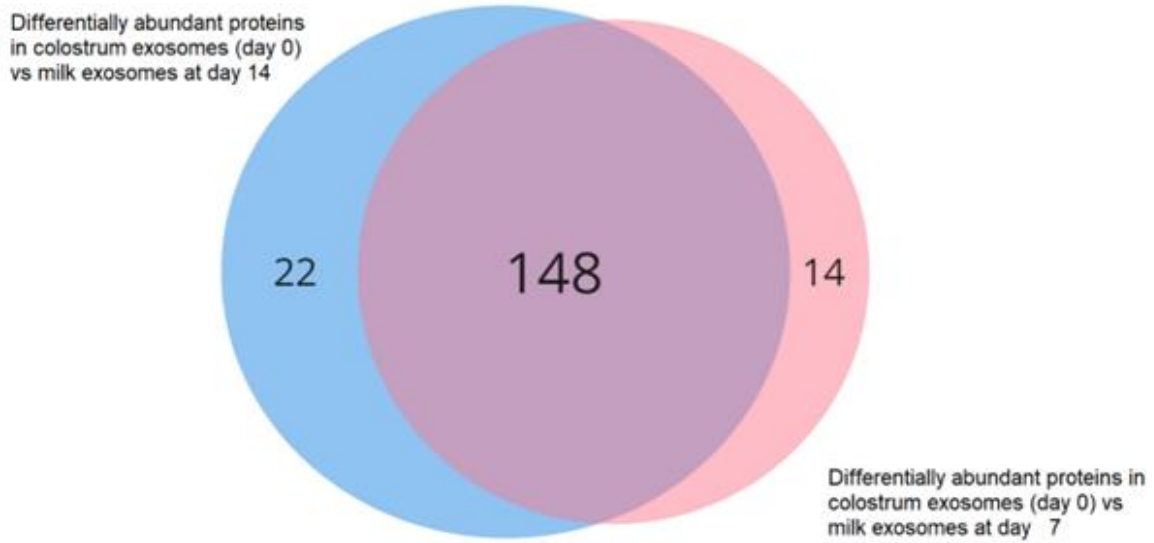


Figure 13. Venn diagram showing the comparison of DAP identified in colostrum exosomes and milk exosomes on day 7, and day 14. From a total of 184 unique DAP between colostrum exosomes and milk exosomes on day 7 and day 14, 148 proteins were significantly different ($p < 1.0e-16$) from colostrum on both timepoints.

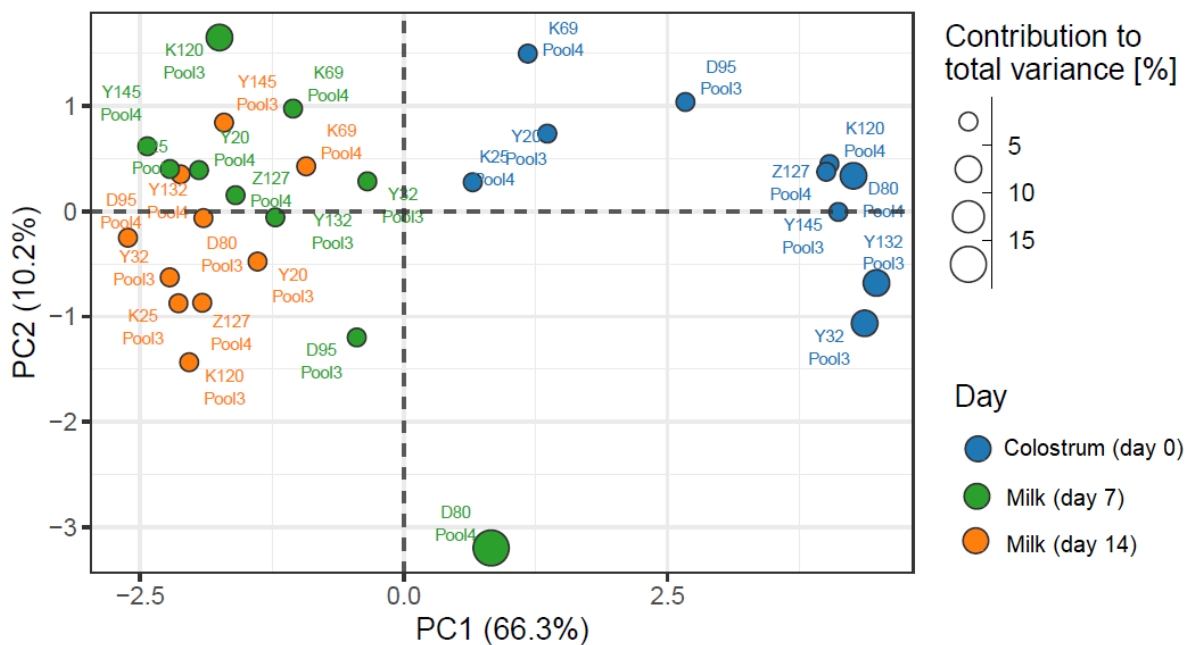


Figure 14. Principal component analysis of the top 5% proteins with the highest variance.

Table 9. Top 10 upregulated and downregulated DAP in milk exosomes at day 7 post-partum as compared to colostrum exosomes. Protein names indicated with * were defined as “Uncharacterized protein” in the UniProt *Sus scrofa* database and thus were replaced with the best match on *Homo sapiens* database, using UniProt BLAST tool.

^a Accession number from UniProt protein database for *Sus scrofa*.

| Protein name | Accession number ^a | log ₂ FC | Adjusted p-value | Number of peptides |
|---|-------------------------------|---------------------|------------------|--------------------|
| Lipocln_cytosolic_FA-bd_dom domain-containing protein | I3LTW5;O02772 | 3.19 | 5.79E-05 | 2 |
| Radixin / Moesin-B / Ezrin | P26044;F1SB42 | 2.48 | 9.62E-05 | 2 |
| Mucin-4 | A0A287B5M2 | 2.18 | 4.22E-07 | 23 |
| Glycoprotein IIIb | Q3HUX1 | 2.03 | 7.9E-07 | 24 |
| Mucin 1, cell surface associated | A0A5G2QGF8;F1RGR9 | 1.83 | 0.000148 | 9 |
| Tetraspanin | F1S1M4 | 1.79 | 3.99E-06 | 4 |
| Na(+)/H(+) exchange regulatory cofactor NHE-RF | A0A5G2R543 | 1.74 | 7.15E-05 | 16 |
| Fatty acid synthase | I3LCW1 | 1.74 | 5.09E-05 | 4 |
| Ras homolog family member A | I3LVS7 | 1.69 | 0.002287 | 5 |
| Mucin 1. cell surface associated | F1RGR9 | 1.64 | 0.005719 | 3 |
| Apolipoprotein E | P18650 | -3.06 | 3.54E-08 | 8 |
| Tubulointerstitial nephritis antigen like 1 | F1SVA2 | -2.59 | 1.77E-05 | 3 |
| Hyaluronan and proteoglycan link protein 1 | P10859 | -2.57 | 9.02E-08 | 2 |
| Apolipoprotein E | F1RM45;P18650 | -2.52 | 9.02E-08 | 28 |
| Vitronectin | P48819 | -2.52 | 9.02E-08 | 7 |
| GB1/RHD3-type G domain-containing protein | A0A5G2R4L0 | -2.41 | 4.83E-07 | 3 |
| Immunoglobulin heavy constant gamma 4 * | A0A5G2QXT5 | -2.40 | 4.22E-07 | 5 |
| Fibulin-2 | A0A287B5Q1 | -2.30 | 2.5E-06 | 10 |
| Alpha-S1-casein | A0A5S6G633 | -2.25 | 0.000267 | 3 |
| Immunoglobulin heavy constant gamma 2 * | A0A287ATT2 | -2.24 | 2.06E-05 | 3 |

Table 10. Top 10 upregulated and downregulated DAP in milk exosomes at day 14 post partum as compared to colostrum exosomes. Protein names indicated with * were defined as “Uncharacterized protein” in the UniProt *Sus scrofa* database and thus were replaced with the best match on *Homo sapiens* database, using UniProt BLAST tool.

^a Accession number from UniProt protein database for *Sus scrofa*.

| Protein name | Accession number ^a | log ₂ FC | Adjusted p-value | Number of peptides |
|--|--|---------------------|------------------|--------------------|
| Glycoprotein IIIb | Q3HUX1 | 2.79 | 7.41E-09 | 24 |
| Acyl-CoA synthetase long chain family member 3 | A0A287B8R8 | 1.68 | 3.04E-06 | 41 |
| Ras-related protein Rab-18 | I3LC07 | 1.55 | 3.04E-06 | 11 |
| Sphingomyelin phosphodiesterase acid like 3B | A0A287BJW1;F1STN0 | 1.85 | 3.87E-06 | 10 |
| Mucin-4 | A0A287B5M2 | 1.80 | 3.87E-06 | 23 |
| Fatty acid-binding protein 3 | O02772 | 1.62 | 8E-06 | 22 |
| Ras-related protein Rab-1A | F2Z5U4 | 1.41 | 1.93E-05 | 9 |
| Myoferlin | A0A5G2Q8T2 | 1.28 | 2.61E-05 | 30 |
| Ras-related protein Rab-1A/ Ras-related protein Rab-1B | F2Z5U4;Q06AU7 | 1.46 | 3.46E-05 | 6 |
| G protein-coupled receptor class C group 5 member C | A0A287ASV2 | 1.27 | 6.19E-05 | 5 |
| Ig-like domain-containing protein | A0A287AP28; A0A286ZIM1;A0A287BAB3 | -3.26 | 3.04E-06 | 3 |
| Apolipoprotein E | P18650 | -3.17 | 7.41E-09 | 8 |
| Tubulointerstitial nephritis antigen like 1 | F1SVA2 | -3.04 | 7.24E-07 | 3 |
| GB1/RHD3-type G domain-containing protein | A0A5G2R4L0 | -2.92 | 1.93E-08 | 3 |
| Matrix Gla protein | A0A5G2QKI1 | -2.85 | 2.36E-08 | 10 |
| Ig-like domain-containing protein | A0A075B7I9; A0A075B7H9; A0A075B7J0; A0A075B7I5 | -2.70 | 4.85E-07 | 4 |
| Vitronectin | P48819 | -2.67 | 1.93E-08 | 7 |
| Alpha-S1-casein | A0A5S6G633 | -2.59 | 2.18E-05 | 3 |
| Fibulin-2 | A0A287B5Q1 | -2.52 | 5.39E-07 | 10 |
| Immunoglobulin heavy constant gamma 2 * | A0A287ATT2 | -2.52 | 3.04E-06 | 3 |

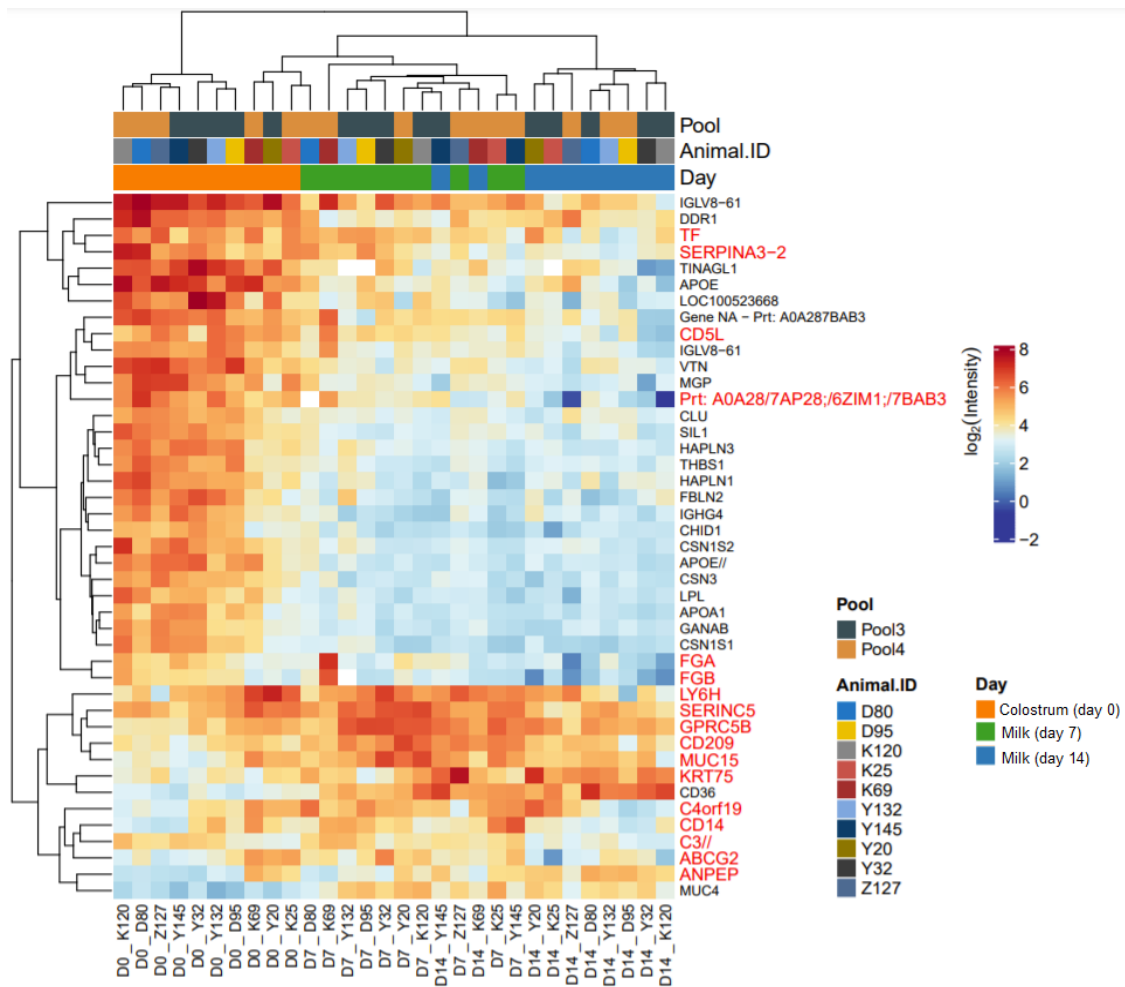


Figure 15. Hierarchically-clustered heatmap of the top 20 genes with the smallest P-values in each of the three contrasts. The subset contains 43 genes, whereby 18 genes are among the top 20 in more than one contrast (overlapping genes). Genes/Proteins indicated in red were not significantly regulated (FDR > 0.05) and were entirely from the milk exosomes on day 7 versus milk exosomes on day 14 contrast which yielded no DAP.

Table 11. Milk-derived exosome proteins above the 10% FDR threshold in the comparison between GS and GL at each time point. Significantly different proteins are accentuated in bold. Protein names indicated with * were not available for porcine and were blasted for the best match in *Homo sapiens*.

| Group S vs Group L at day 0 post-partum | | | | |
|--|-------------------|---------------|------------------|--------------------|
| Protein name | Accession number | logFC | Adjusted p-value | Number of peptides |
| ATPase H(+)-transporting lysosomal accessory protein 2 | A0A287AJK1 | 1.509 | 0.092 | 5 |
| GB1/RHD3-type G domain-containing protein | A0A5G2R4L0 | 1.691 | 0.092 | 3 |
| Group S vs Group L on day 7 post-partum | | | | |
| 78 kDa glucose-regulated protein (HSPA5) | A0A287BIL8 | 1.821 | 0.022 | 4 |
| Spondin-2 | A0A481AED0 | 2.638 | 0.022 | 4 |
| Equilibrative nucleoside transporter 1 isoform 2 | A0A287B8K5 | -1.948 | 0.102 | 6 |
| Tumor susceptibility gene 101 protein* | A0A5G2QN35 | -2.117 | 0.102 | 4 |
| Group S vs Group L at day 14 post-partum | | | | |
| Chromosome 8 C4orf19 homolog | F1S4L8 | -2.373 | 0.015 | 2 |

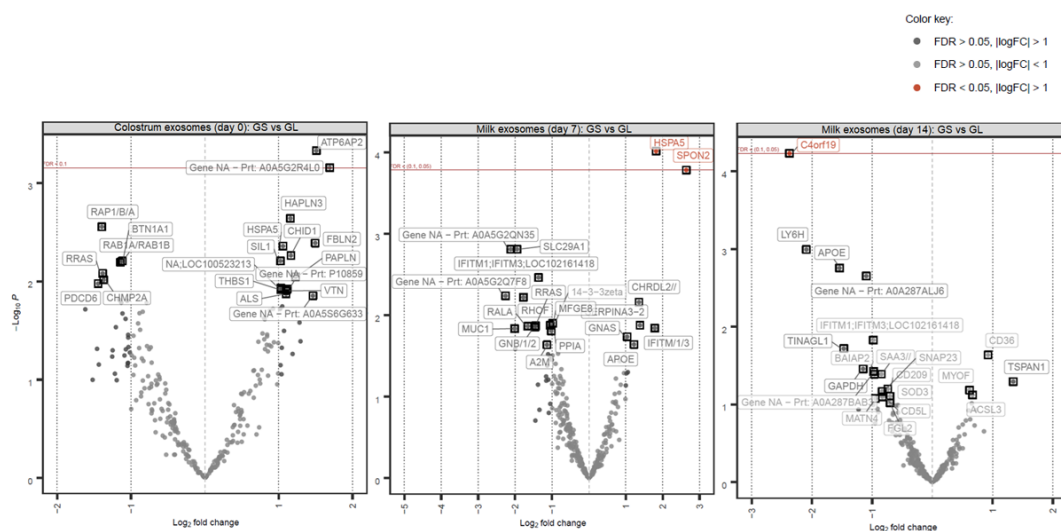


Figure 16. Volcano plots of log₂ fold changes (x-axis) and their associated -log₁₀ transformed p-values (y-axis) of all identified proteins in the different time points on the comparison between GS and GL. The horizontal lines in red represent the 5% FDR threshold (adjusted p-value < 0.05) on the second and third contrast.

5.3 GO terms and pathway enrichment analysis

The comprehensive protein-protein interaction (PPI) network of all 319 proteins retained in this study was highly significant (552 edges, $p < 1.0e-16$) and distinctly connected, with 1931 terms and only 11 secluded from any pathway (Figure 17). The most significantly overrepresented GO terms on cellular components were vesicle (GO.0031982), cytoplasmic vesicle (GO.0031410), secretory granule (GO.0030141), endomembrane system (GO.0012505), and secretory vesicle (GO.0099503). The most significant GO terms on biological processes were vesicle-mediated transport (GO.0016192), transport (GO.0006810), establishment of localization (GO.0051234), exocytosis (GO.0006887), and regulated exocytosis (GO.0045055). The most significant GO terms on biological function were GTPase activity (GO.0003924), carbohydrate derivative binding (GO.0097367), protein binding (GO.0005515), anion binding (GO.0043168), and GTP binding (GO.00055259).

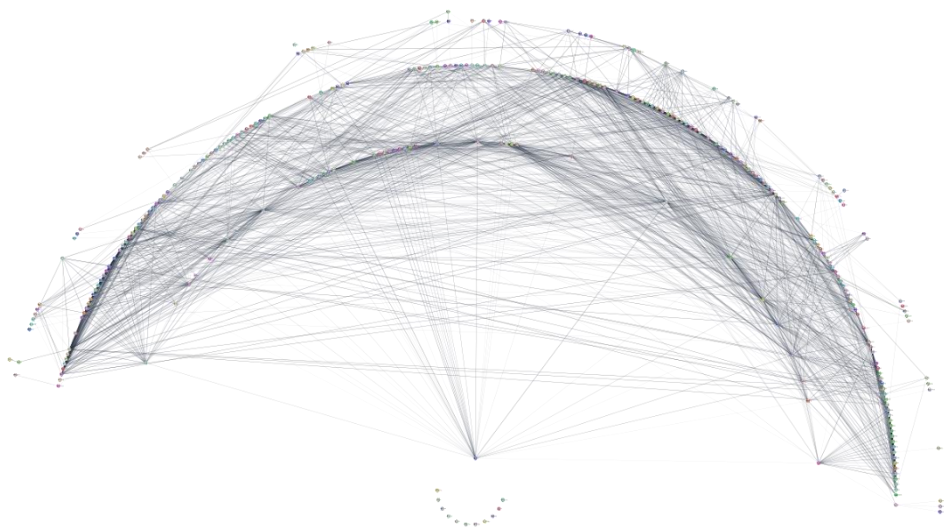


Figure 17. Comprehensive protein-protein interaction (PPI) network of all 319 proteins retained in this study was highly significant (552 edges, $p < 1.0e-16$) and distinctly connected, with 1931 terms and only 11 secluded from any pathway.

5.3.1 GO term and pathway enrichment analysis from colostrum exosomes vs milk exosomes

The PPI network in the DAP between colostrum and milk on day 7 was overall highly significant (495 edges, $p < 1.0e-16$), for both increased (244 edges, $p < 1.0e-16$) and decreased proteins (125 edges, $p < 1.0e-16$). The PPI network in the DAP between colostrum and milk on day 14 was overall highly significant (552 edges, $p < 1.0e-16$) for both increased (240 edges, $p < 1.0e-16$) and decreased proteins (186 edges, $p < 1.0e-16$). The list of final GO terms corresponding to the DAP between colostrum exosomes and milk exosomes is presented with their respective number of genes inside each term and their associated p-value (expressed as $-\log_{10}$) in Appendix 3. The top 20 leading GO terms are illustrated in Figure 18 (A) for the comparison between milk exosomes at day 7 vs colostrum exosomes, and in Figure 18 (B) for the comparison between milk exosomes at day 14 vs colostrum exosomes.

5.3.2 GO term and pathway enrichment analysis from Group S vs Group L

The comprehensive protein-protein interaction (PPI) network of the lead proteins in the comparison of GS and GL was significantly interactive at all time points. The GO terms found for comparisons at d 0 and 14 p.p. did not contain any significantly abundant proteins (p -value < 0.5) and thus they were not further considered.

The PPI network of the lead milk EV proteins between GS vs GL at d 7 p. p. (PPI enrichment p -value = $3.35e-06$) with 36 nodes and 153 predicted protein associations (edges) is given in Figure 19. The leading GO terms for the GS vs GL analysis at d 7 are displayed as histograms for GO terms related to biological processes (Figure 20A), molecular function (Figure 20B), and cellular components (Figure 20C). The GO terms related to biological processes as illustrated by Cytoscape with ClueGO application are shown in Figure 21.

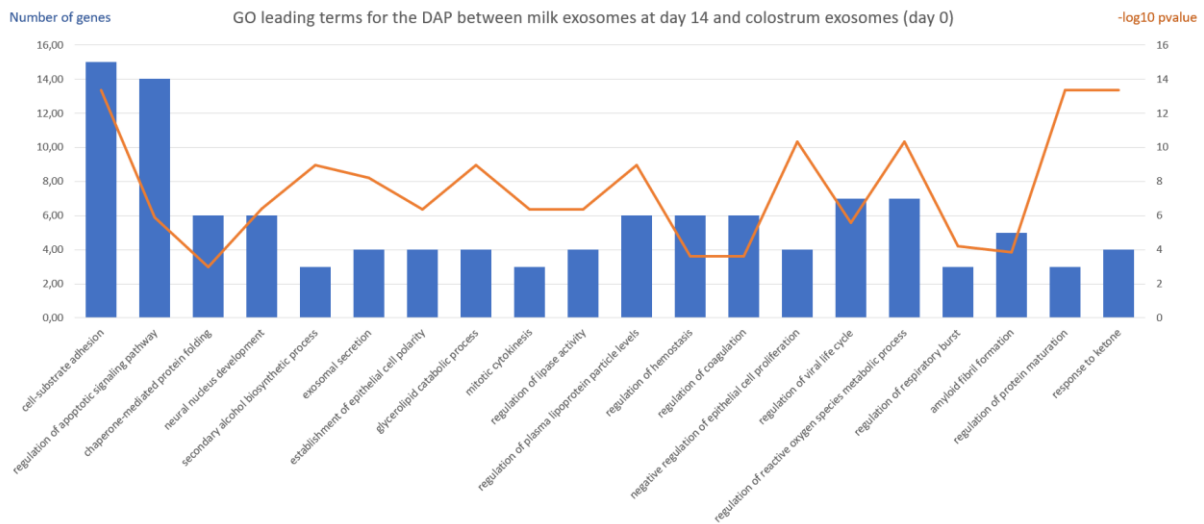
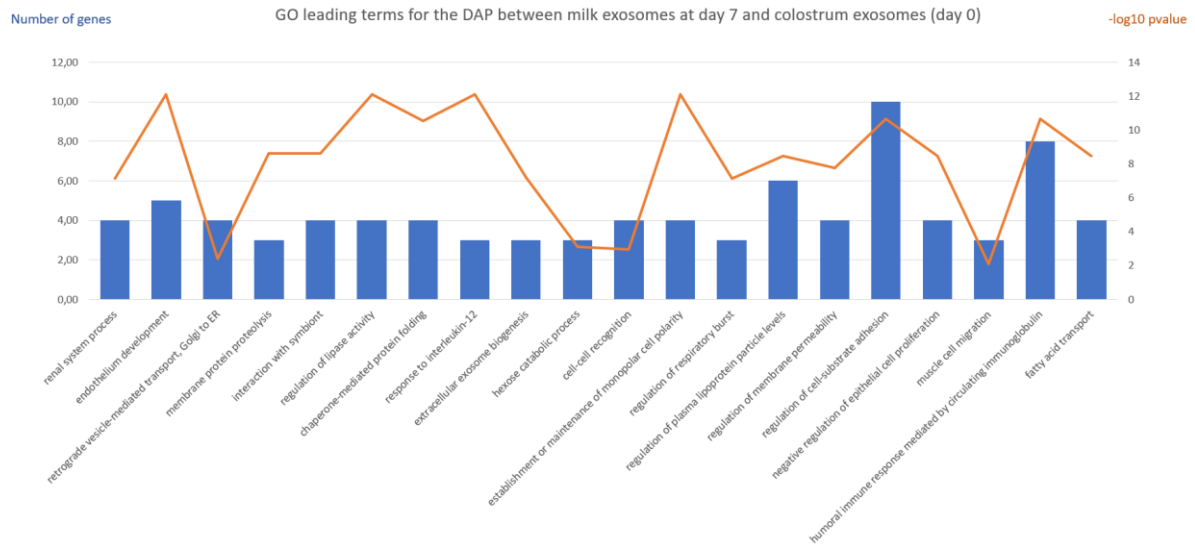


Figure 18. A. The top 20 significantly overrepresented GO leader terms with number of genes associated in the comparison between GS and GL (left y-axis) and their $-\log p$ value (right y-axis) for the DAP between milk exosomes at day 7 and colostrum exosomes (day 0), performed in ClueGO and cured by REVIGO. B. Top 20 significant GO leader terms with the number of genes associated in the comparison between GS and GL (left y-axis) and their $-\log p$ value (right y-axis) for the DAP between milk exosomes at day 14 and colostrum exosomes (day 0), performed in ClueGO and cured by REVIGO

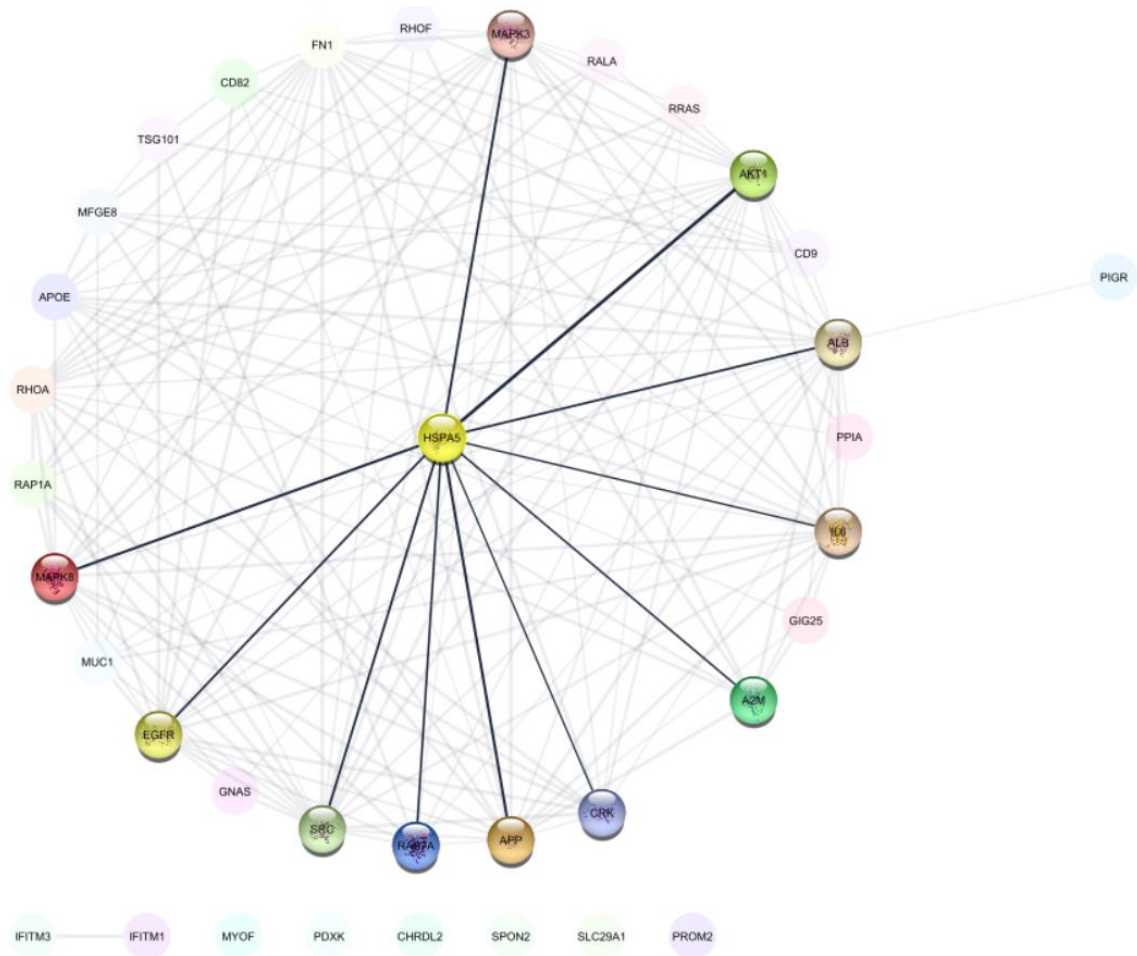


Figure 19. Protein-protein interaction network of the leader milk EV proteins in the comparison between GS and GL at day 7 p.p. (PPI enrichment p value = 3.35e-06), with 36 nodes and 153 predicted protein associations (edges), enriched in STRING and displayed in Cytoscape. HSPA5 was significantly more abundant in milk EVs in group S than group L in LC-MS/MS and it is part of the top-level interactions of the PPI network; highlighted nodes represent direct interaction with HSPA5.



Figure 20. Leader Gene Ontology terms with the number of genes (left y-axis) (converted from proteins) and their $-\log p$ -value (right y-axis) for the comparison between milk exosomes from Group S vs Group L at day 7 p.p., related to biological process (A), molecular function (B) and cellular component (C), performed in Cytoscape using the STRING enrichment database. Only GO terms containing significantly expressed proteins (p -value < 0.05) were retained.

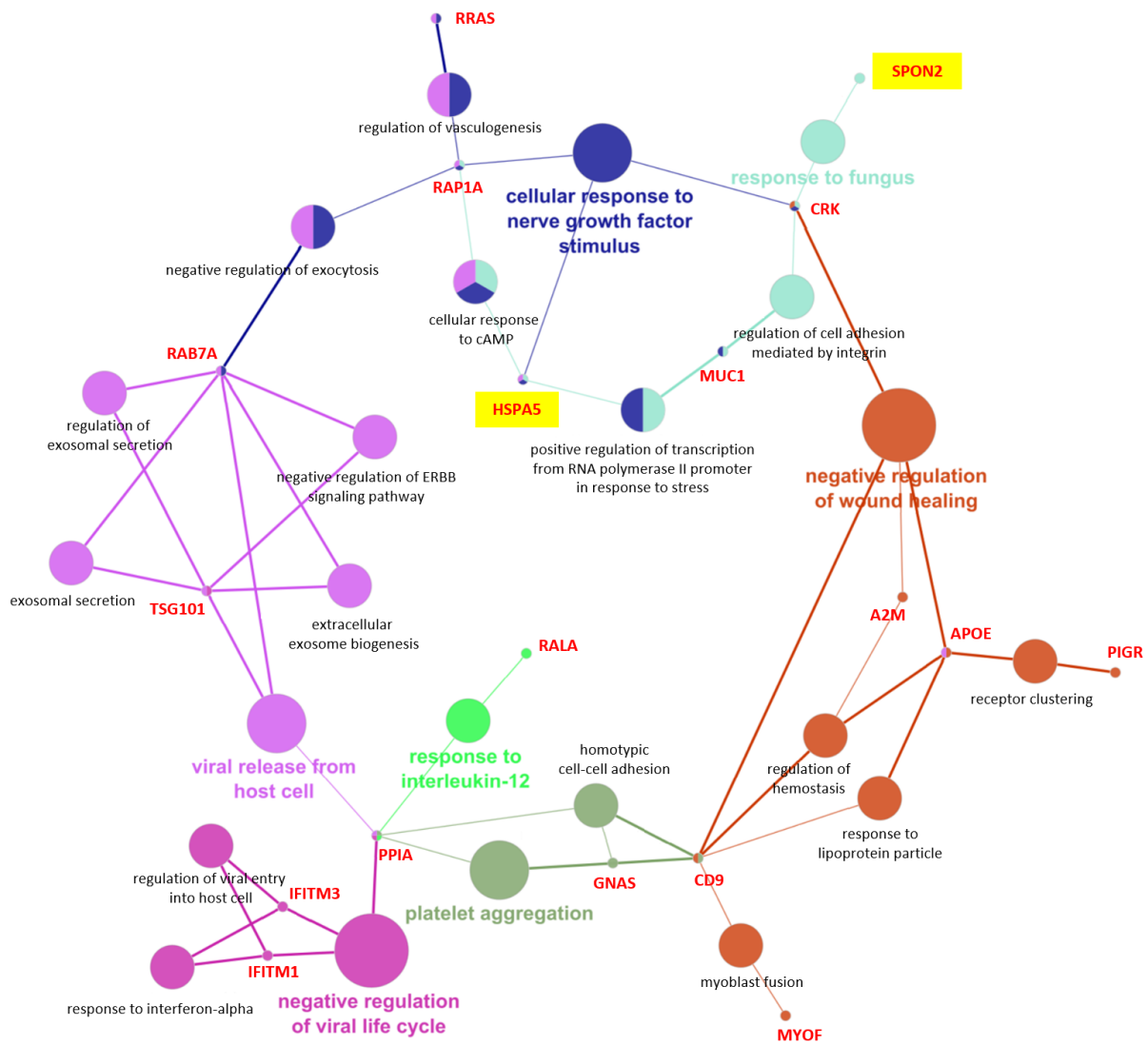


Figure 21. Comprehensive interactive network using the generated gene ontology terms (converted from proteins) for Biological Process by the leader milk EV proteins in the comparison between Group S and Group L at day 7, displayed in Cytoscape using the ClueGO application. Gene names are annotated in red, with significantly different genes highlighted in yellow.

5.4 Lipidomics analysis

The liquid chromatography–quadrupole time-of-flight mass spectrometry lipidomics analysis was able to identify a total of 947 lipids in milk and colostrum exosome samples. The lipid elements identified covered six categories and were divided into 25 main classes (Table 12, Figure 22) and 47 subclasses.

Table 12. Classification of all 947 lipid elements identified in milk and colostrum exosome samples by liquid chromatography–quadrupole time-of-flight mass spectrometry lipidomics analysis.

| Lipid category | Number of lipids | Main class | Number of lipids |
|-----------------------|-------------------------|---------------------------------------|-------------------------|
| Fatty Acids | 105 | Fatty acids and Conjugates | 50 |
| | | Fatty amides | 46 |
| | | Fatty esters | 7 |
| | | Oxygenated hydrocarbons | 2 |
| Glycerolipids | 391 | Diradylglycerols | 185 |
| | | Glycosyldiradylglycerols | 3 |
| | | Monoradylglycerols | 21 |
| | | Other Glycerolipids | 10 |
| | | Triradylglycerols | 172 |
| Glycerophospholipids | 216 | Glycerophosphates | 6 |
| | | Glycerophosphocholines | 73 |
| | | Glycerophosphoethanolamines | 71 |
| | | Glycerophosphoglycerols | 17 |
| | | Glycerophosphoglycerophosphoglycerols | 8 |
| | | Glycerophosphoinositols | 24 |
| | | Glycerophosphoserines | 15 |
| | | Other Glycerophospholipids | 2 |
| Prenol Lipids | 1 | Quinones and hydroquinone | 1 |
| Sphingolipids | 190 | Acidic glycosphingolipids | 10 |
| | | Ceramides | 69 |
| | | Neutral glycosphingolipids | 19 |
| | | Phosphosphingolipids | 78 |
| | | Sphingoid bases | 14 |
| Sterol Lipids | 44 | Secosteroids | 1 |
| | | Sterols | 43 |

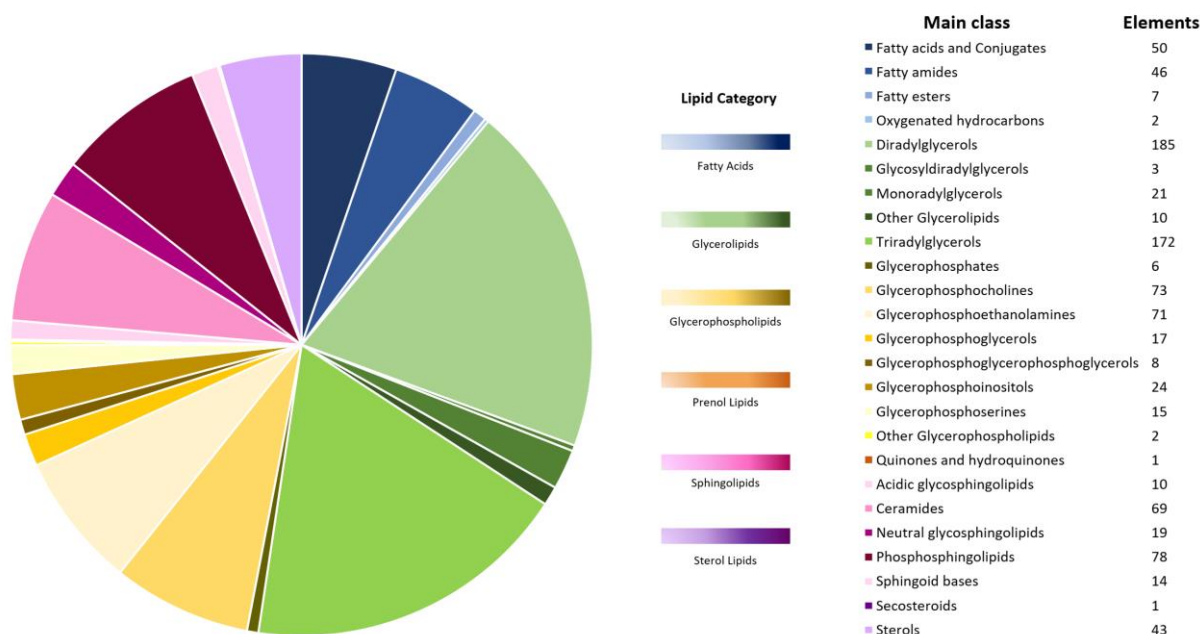


Figure 22. Summary of the total of 947 lipid elements identified in milk and colostrum exosomes by liquid chromatography–quadrupole time-of-flight mass spectrometry lipidomics analysis, color-coded by lipid category and stratified in lipid main classes.

The most abundant lipid classes included: (1) diradylglycerols, a glyceride consisting of two fatty acid chains covalently bound to a glycerol molecule through ester linkages; (2) triradylglycerols, formed by linking fatty acids with an ester linkage to three alcohol groups in glycerol; and (3) phosphosphingolipids, consisting of sphingolipids, mainly sphingomyelins, which include a phosphoryl group. The complete list of analyzed lipids within their respective lipid species classes, subclasses, and the number of lipids annotated from the untargeted lipidomic analysis is summarized in Appendix 4.

The principal component analysis indicates a clear separation between the time of sampling (day 0 vs. 7 and 14) and no separation between treatments (GS and GL) based on the lipid composition of exosomes from colostrum and milk (Figure 23A-B). The hierarchical clustering heat map of the top 50 statistically significant differences at days 0, 7, and 14 when sows were fed diets with different ratios of ω -6 to ω -3 fatty acids show a clear distinct lipidomic profile between colostrum and milk exosomes (Figure 24).

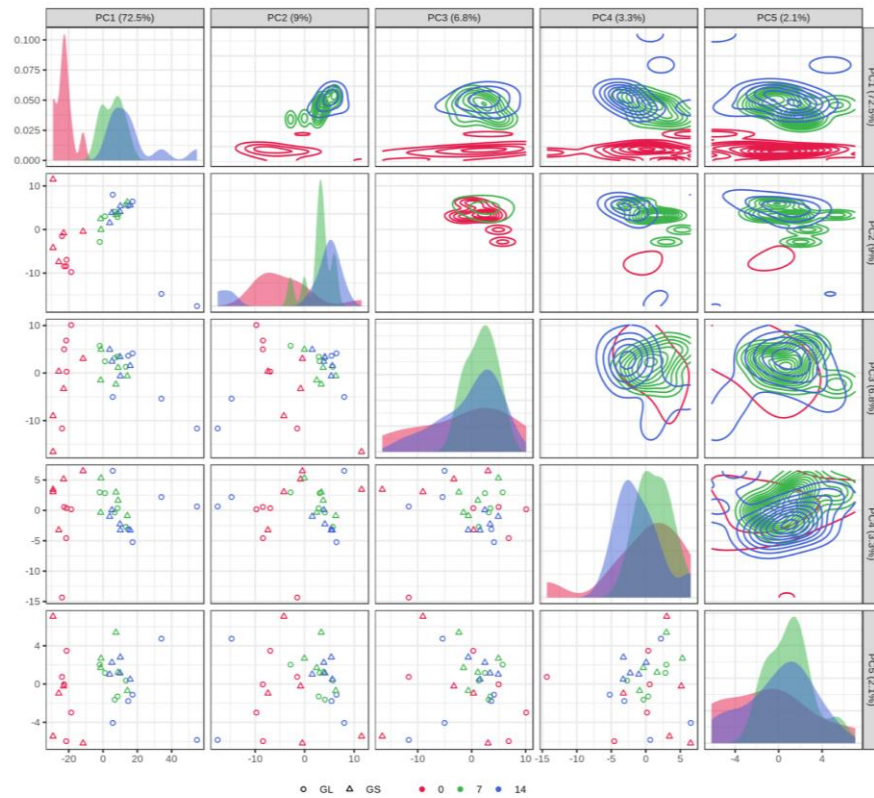
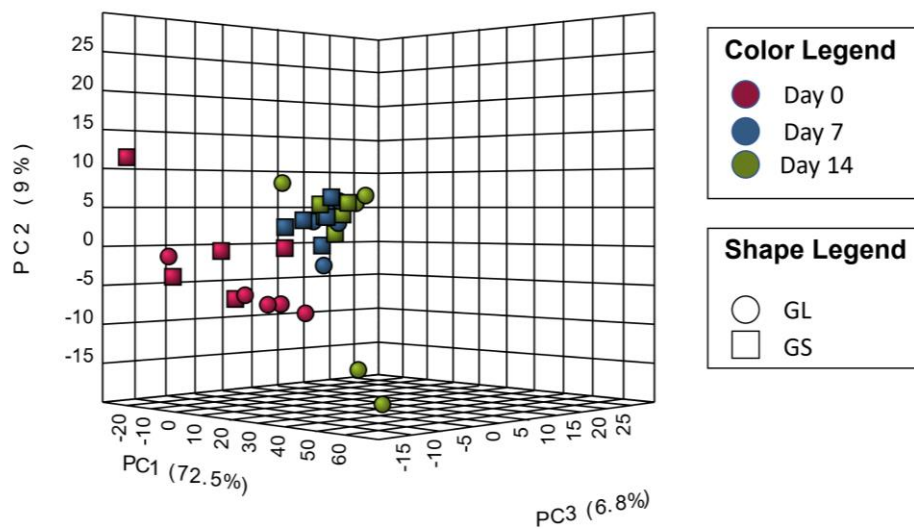
A**B**

Figure 23. Principal component analysis (PCA) score plot of lipidomic profiling of exosomes from colostrum and milk shows a distinct lipidomic profile between colostrum (day 0) and mature milk (day 7 and 14) exosomes, without clear separation between feeding diets with different ratios of omega-6 to omega-3 fatty acids (GS and GL groups).

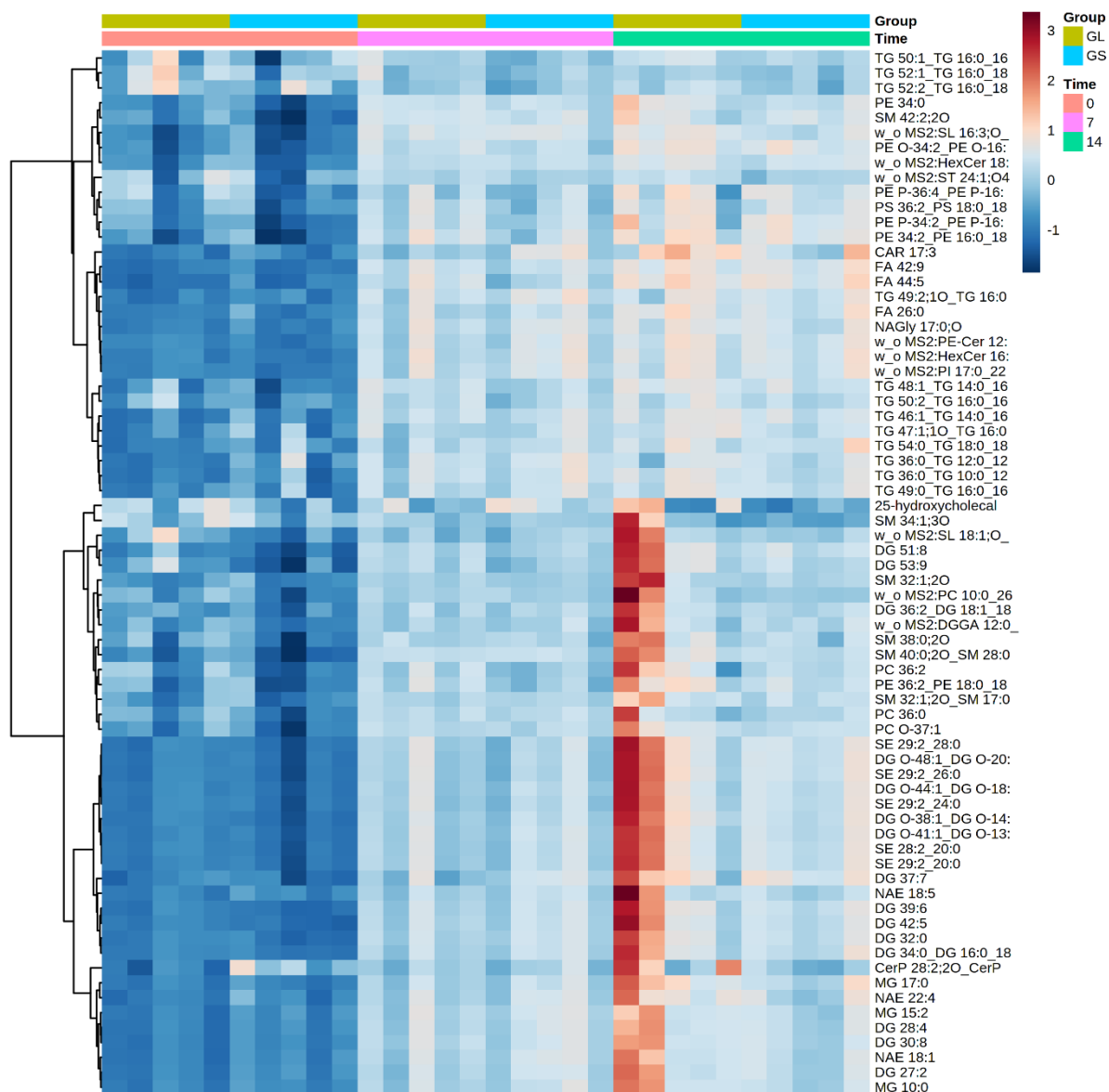


Figure 24. Hierarchically-clustered heatmap of the top 50 statistically significant lipids with smallest P-values in each grouped by day and time in milk exosomes from sows fed diets with different ratios of ω -6 to ω -3 PUFA on days 0, 7, and 14. The colors in the heat map reflect the different profiles abundance of the lipids in milk exosomes and colostrum exosomes (mean combined and divided by the range of each variable).

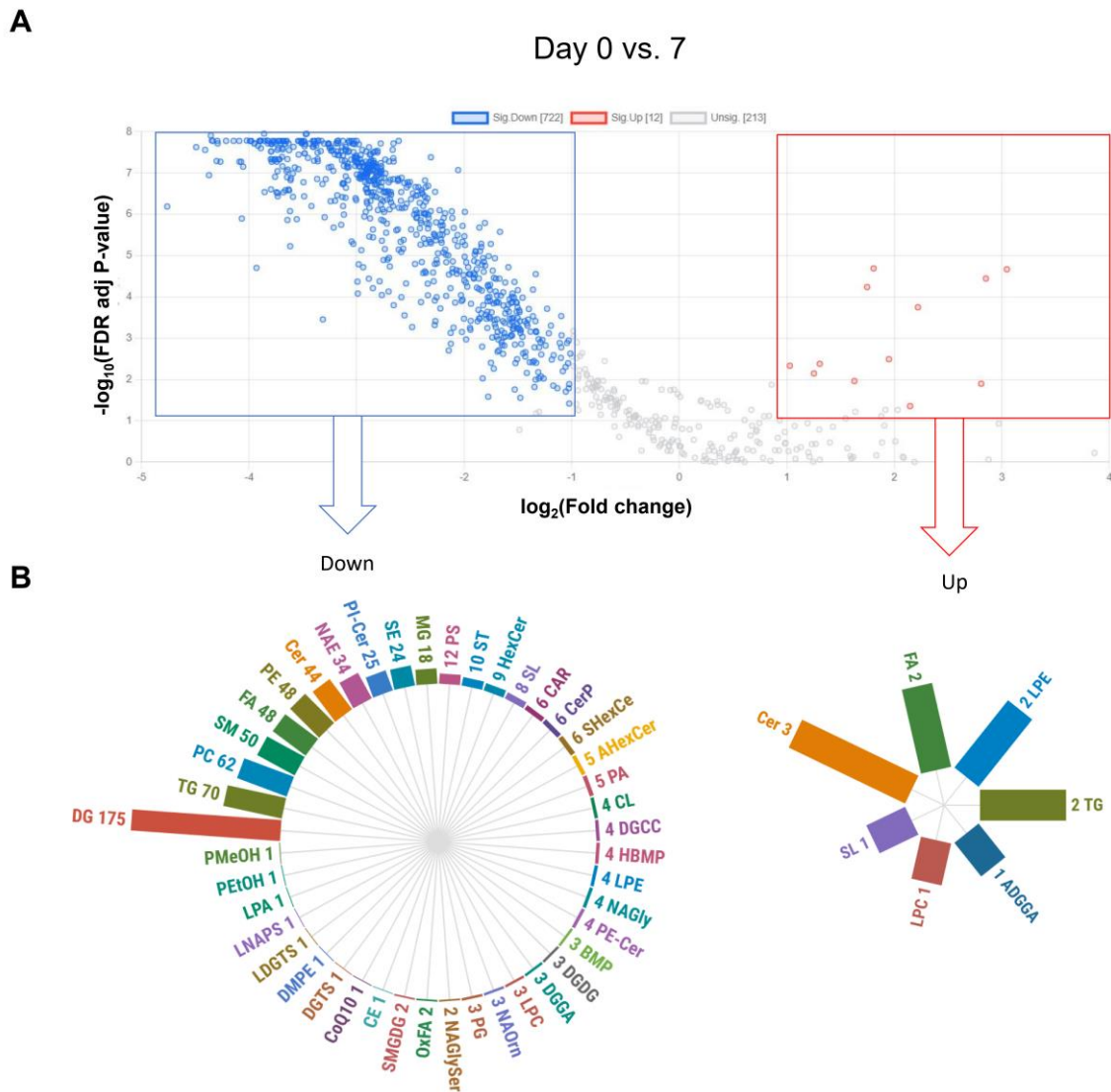


Figure 25. (A) Volcano plot of \log_2 fold changes (x-axis) and their associated $-\log_{10}$ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between colostrum exosomes (day 0) and milk exosomes at day 7. Significantly different lipids (t-test FDR adjusted p-value < 0.05, fold change > 2) are highlighted indicating up (red) and down (blue) regulated differentially expressed lipids. (B) The circular bar plots represent the number of lipid elements in each subclass that were up- or down-regulated on exosomes from colostrum compared to milk at day 7.

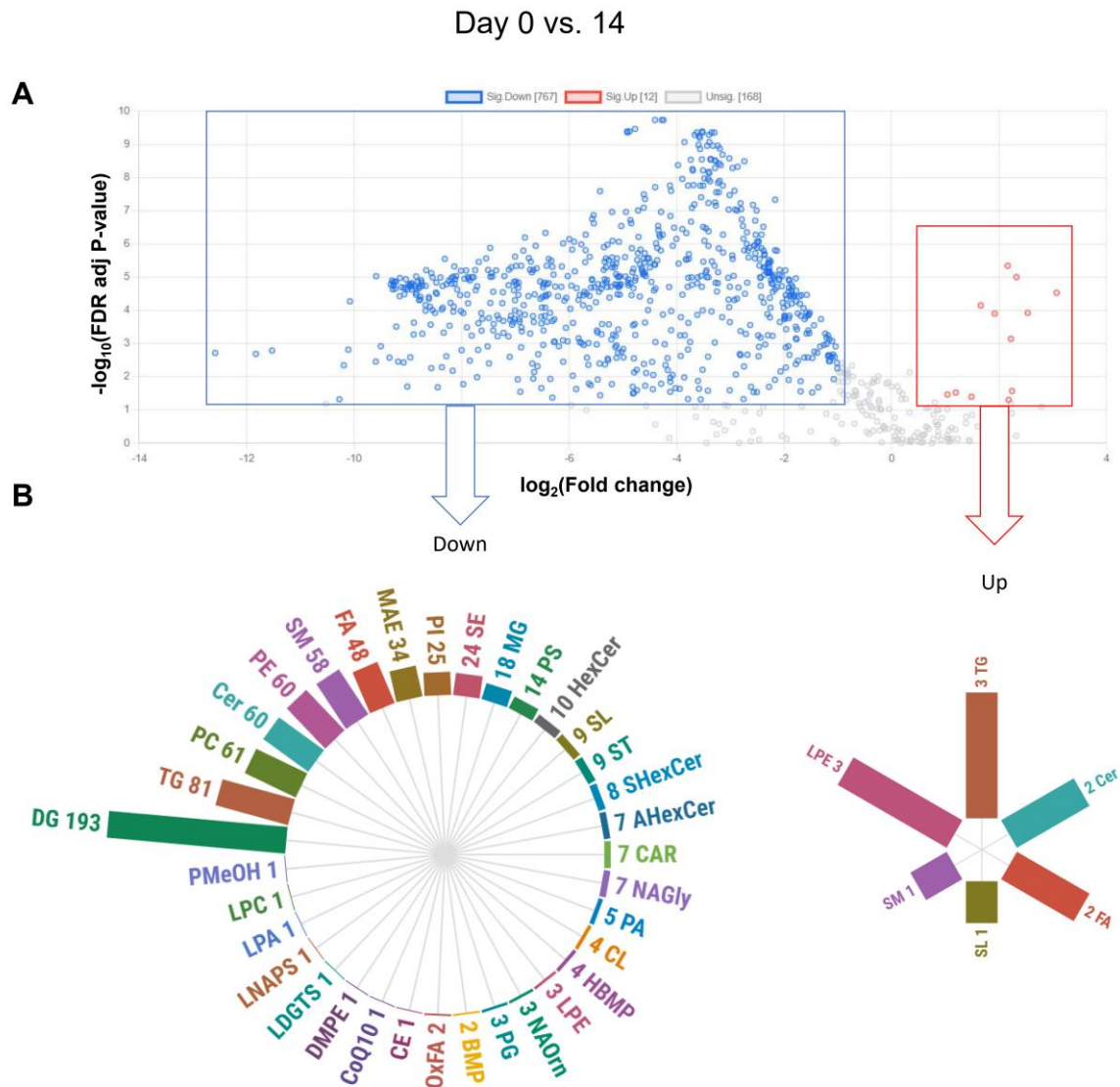


Figure 26. (A) Volcano plot of \log_2 fold changes (x-axis) and their associated $-\log_{10}$ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between colostrum exosomes (day 0) and milk exosomes at day 14. Significantly different lipids (t-test FDR adjusted p-value < 0.05 , fold change > 2) are highlighted indicating up (red) and down (blue) regulated differentially expressed lipids. (B) The circular bar plots represent the number of lipid elements in each subclass that were up- or down-regulated on exosomes from colostrum compared to milk at day 14.

A total of 734 significantly different expressed lipid elements between day 0 and day 7 (722 down- and 12 up-regulated) in the lipid composition of exosomes from colostrum and milk (Appendix 5a). Compared with exosomes from milk at day 7, exosomes of colostrum had lower DG (n = 176), TG (n = 70), PC (n = 62), SM (n = 50), FA (n = 48), and PE (n = 48) (Figure 25). On the contrast between day 0 and day 14, we observed 779 significantly different lipids (767 down- and 12 up-regulated) (Appendix 5b). Compared with exosomes from milk at day 14, exosomes of colostrum had lower DG (n = 193), TG (n = 81), PC (n = 61), Cer (n = 61), PE (n = 60), SM (n = 58), and FA (n = 48) (Figure 26). We observed no significant differences in the lipid composition of milk exosomes between days 7 and 14 (Figure 27). Moreover, most of the up-regulated and down-regulated lipid elements in comparison between colostrum exosomes versus milk exosomes from day 7 were shared with the comparison between colostrum exosomes versus milk exosomes from day 14 (Figure 28), evidencing distinct lipid signatures between colostrum and mature milk exosomes.

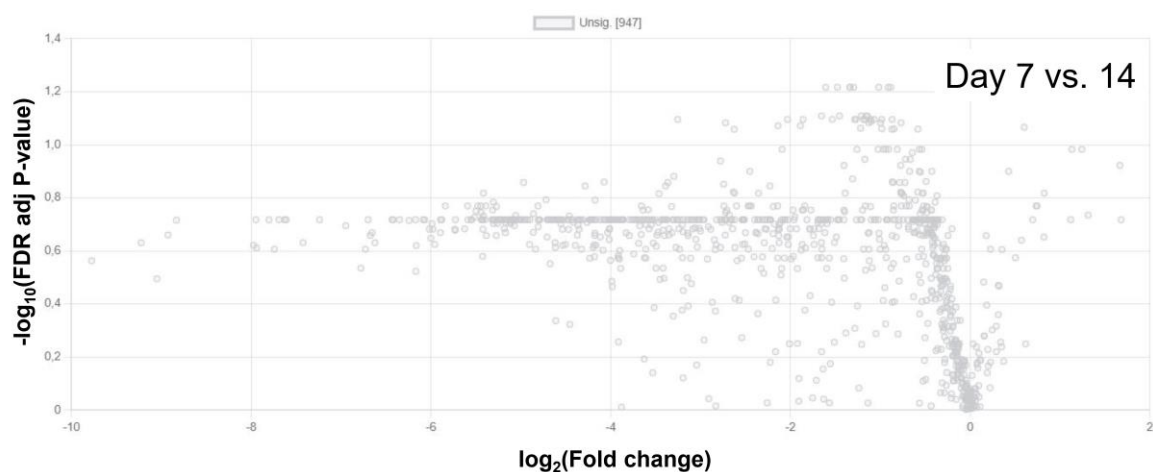


Figure 27. Volcano plot of log₂ fold changes (x-axis) and their associated -log₁₀ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between milk exosomes at day 7 and milk exosomes at day 14. No significantly different lipids were found (t-test FDR adjusted p-value threshold at 0.05, fold change threshold at 2).

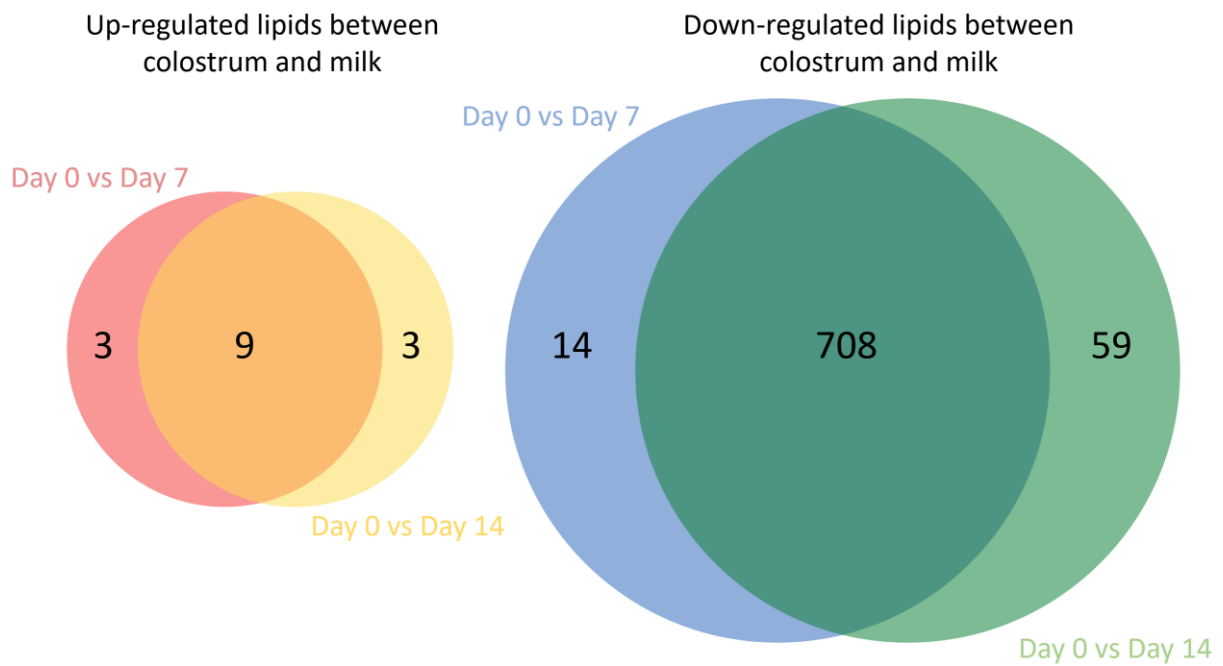


Figure 28. Venn diagram representing the number of differentially expressed lipids elements found in comparison between colostrum exosomes (day 0) and milk exosomes at day 7 and 14, featuring that most of lipid elements were shared between comparisons.

In addition, we examined differences in lipid composition between the treatments with different feeding ratios of ω -6 to ω -3 fatty acids. No differently expressed lipid element was identified in milk exosomes between treatments at day 0 and 7 (Figure 29A-B), and only one lipid (PA 17:0_28:6) was down-regulated in the comparison between milk exosomes from GS and GL at day 14 (Figure 29C).

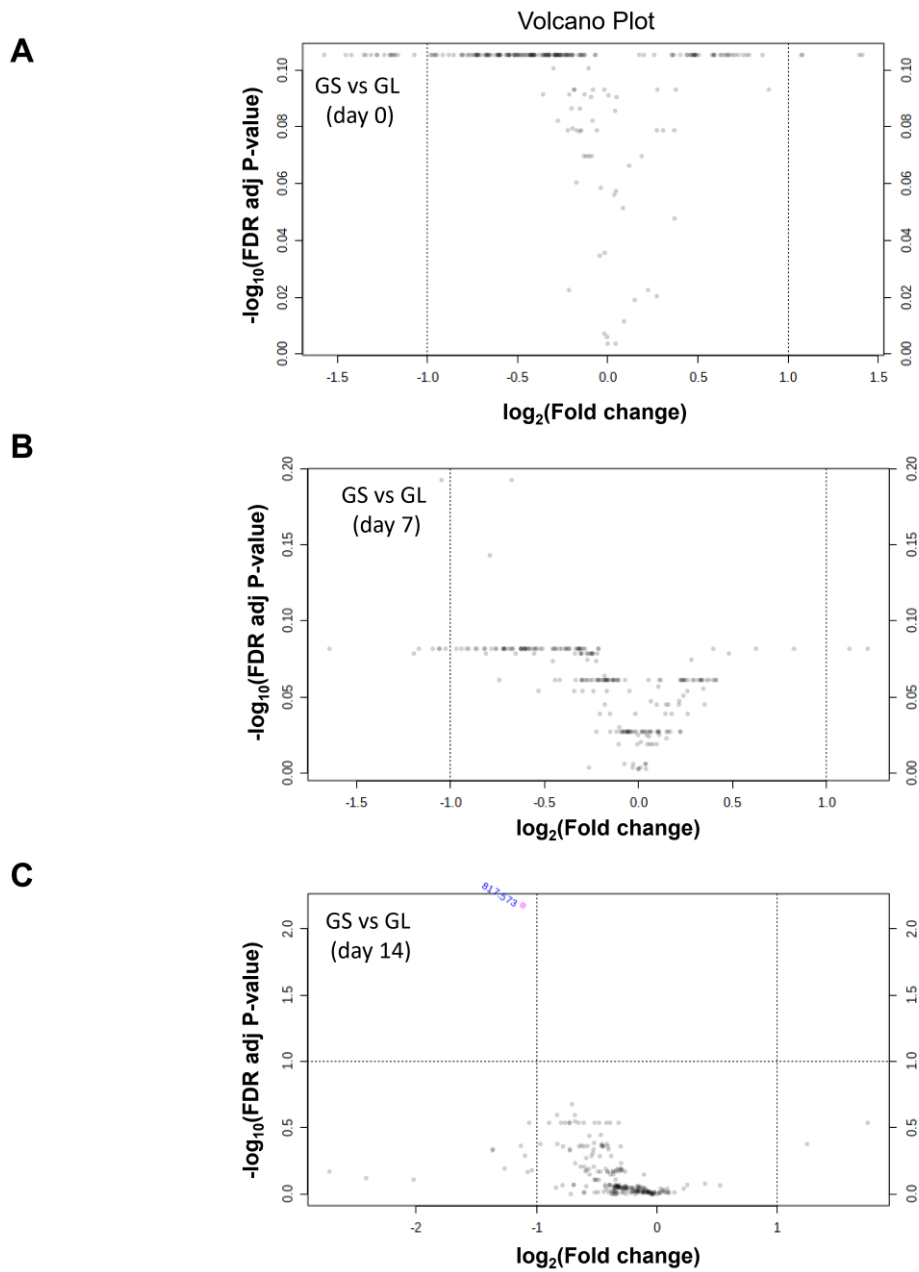


Figure 29. Volcano plot of \log_2 fold changes (x-axis) and their associated $-\log_{10}$ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between milk exosomes from GS and GL sows in different stages of lactation - at (A) day 0, (B) day 7, and (C) day 14. No significantly different lipid was identified at timepoints (A) and (B), and one lipid was down-regulated in the comparison between GS and GL on day 14 (C).

6 DISCUSSION

The molecular characterization of exosomes indicates that while there are basic protein signatures in the exosomal composition, their unique proteome is dynamic and continuously modulated by subtle changes in the abundance of different proteins and post-translational modifications (QUIROZ-BAEZ et al., 2020). Exosome cargo loading does not occur arbitrarily but favors the sorting of specific compounds during the EV biogenesis and may not always mirror the protein composition of the parental cells (ANAND et al, 2019). The exosome proteome changes and adapts to external and local stimuli (MALLIA et al., 2020), and the same cell types can produce exosomes of different cargo and composition in different stages. This effect can be clearly observed in the results of this study, by the distinct proteome and lipidomic profiles identified in colostrum and mature milk exosomes. Similar effects of different lactation stages on milk exosome proteome have been previously reported in bovines (SAMUEL et al., 2017), albeit not yet studied in porcine, nor on the exosomal lipid composition of any species. Similarly, while the diet is an important external factor in many molecular processes (YAN, 2015; NASIR et al., 2020), its impact on exosome composition has hardly been investigated so far.

Whilst a variety of methods can be used to isolate milk exosomes, each methodology has its advantages and disadvantages. There is no “one size fits all” for EV isolation, and the isolation and purification technique should be designed specifically for each study (LÖTVALL et al., 2014; THÉRY et al., 2018). Yet, an absolute pure preparation of exosomes is to this day not obtainable, especially when isolating EVs from complex body fluids, such as milk. Milk-EVs are known to contain a unique functional proteome distinct from other milk components, although a significant number of these proteins are shared with the milk proteome (van HERWIJNEN et al., 2016). As exosome isolation protocols advance over time and more and more studies have been published, we have started to grasp what the milk exosome proteome looks like – however, it is still to some extent limited to understanding which proteins are definitely shared with milk and represent factual constituents of the exosomal proteome, and which proteins are co-isolated as contaminants from the exosome isolation. For instance, several forms of casein have invariably been identified in concurrent milk-EVs proteomics studies (REINHARDT, 2013; CHEN et al.,

2017; VASWANI et al., 2021; SAMUEL et al., 2017; YANG et al., 2017), and also found among the LC-MS/MS results from this study.

The porcine milk exosome proteome has been previously reported (CHEN et al., 2017), although their distinct characterization in different stages of lactation, including colostrum, despite its importance was yet unexplored. Over 160 DAP were identified between colostrum and mature milk at days 7 and 14, revealing that porcine colostrum exosomes contain unique features that distinguish them from milk exosomes. Proteins related to the regulation of hemostasis were upregulated in colostrum as compared to milk exosomes from both days 7 and 14. From the top 10 DAP in colostrum exosomes in comparison to milk exosomes on days 7 and 14, GO terms were associated with negative regulation of coagulation (GO:0050819) and negative regulation of hemostasis (GO:1900047), represented by proteins vitronectin, apolipoprotein E and thrombospondin-1.

In humans, it is known that the neonatal hemostatic system is characterized by hypocoagulation, reflected in prolonged activated partial thromboplastin time, which may be a process of adaptation for extrauterine life (ANDREW et al., 1987). The functions of EV in coagulation and vascular homeostasis have been thoroughly investigated (OGGERO et al., 2019; BERCKMANS et al., 2019), ever since the first description of EV in 1967 as “platelet dust” (WOLF, 1967). Colostrum EV are also suggested to play a role in modulating the innate immune system of the infant (SAMUEL et al., 2017). Vitronectin, which participates in the regulation of coagulation, fibrinolysis, and the complement cascade (PREISSNER, 1989) was among the top upregulated proteins in colostrum exosomes in comparison to milk exosomes. Tubulointerstitial nephritis antigen like 1 (TINAGL1), a matricellular protein that promotes cell adhesion and spreading (TAJIRI et al., 2010), has also appeared as a leader protein upregulated in colostrum exosomes when compared to milk exosomes. TINAGL1 was pointed out as a regulator of angiogenesis (MARY et al., 2017), although its physiological roles are not yet clearly understood. These results are consistent with a previous study that has found TINAGL1 to be enriched in exosomes from bovine colostrum when compared to milk exosomes of mature milk (SAMUEL et al., 2017), endorsing the need for future studies to unveil its role in neonate health and development.

From the top 10 of the DAP that were upregulated in exosomes from milk (day 7) as compared to colostrum exosomes, proteins ezrin (EZR), fatty acid synthase (FASN), and radixin (RDX) were related to the establishment of the endothelial barrier (GO:0061028)

and endothelial cell development (GO:0001885). EZR and RDX, together with Na(+)/H(+) exchange regulatory cofactor NHE-RF protein (SLC9A3R1) were also related to microvillus assembly (GO:0030033); and EZR, SLC9A3R1 together with Ras homolog family member A (RHOA) were related to the establishment or maintenance of apical/basal cell (GO:0035088). This suggests that the transition from colostrum exosomes to milk exosomes is likely associated with cellular development. Zonneveld et al. (2020) performed a gap closure assay with gingival epithelial cells in the presence of milk EV and suggested that milk EV increases the re-epithelialization rate of epithelial cells, comparable to positive control TGF- α , while EV-depleted milk did not. The first interaction of milk components with the infant's mucosa occurs in the oral cavity, and the authors pointed out that milk EV may play a role in maintaining its integrity. Moreover, vascular endothelial cells can transport milk exosomes by endocytosis (KUSUMA et al., 2016), and since the identity of the glycoproteins facilitating their endocytosis is still unknown, the exosome proteome presented here and in past studies may help to identify targets for that function.

Additionally, ERM proteins (ezrin, radixin, and moesin), known to constitute mechanisms of membrane microfilament attachment, were upregulated in milk exosomes at both day 7 and day 14 as compared to colostrum exosomes. The ERM proteins can be also found in microvilli on endothelial cells and are present at high levels in the intestine on the absorptive epithelium (SAUVANET et al., 2015), where they play an essential role in configuring the developing intestine (SAOTOME et al., 2004). This corroborates a previous study in which milk exosomes were demonstrated to facilitate intestinal cell proliferation and intestinal tract development both *in vitro* and *in vivo* (CHEN et al., 2016). The results presented here may indicate different functions of milk EV in different stages of lactation. Weaning in piglets is a stressful process, accompanied by marked changes in gastrointestinal physiology, including changes in villous height and functional changes in the enterocytes (HEO et al., 2013). The EV is likely involved not only in early nutrition but may also be a part of preparing the infant for post-weaning nutrition, albeit more studies are needed to clarify that role.

Another interesting aspect was that the transition from colostrum to milk was paralleled by changes in the exosomal proteome associated with cellular lipid intake. From the top 10 upregulated DAP between milk and colostrum exosomes at day 14, three proteins - Glycoprotein IIIb, Fatty acid-binding protein (FABP3), and Acyl-CoA synthetase long chain family member 3 - were related to the long-chain fatty acid import into the cell

(GO:0044539). Exosomes act as a biological vehicle and can directly transport lipids such as cholesterol, fatty acids, and eicosanoids from parent cells to recipient cells (GARCIA et al., 2019; RECORD et al., 2015), and have been related to all steps of lipid metabolism, including lipid synthesis, transportation, and degradation (WANG et al., 2020). It is known that exosome-mediated intracellular lipid exchange differs from lipoprotein-mediated lipid transport, and exosomes carry a set of enzymes related to lipid metabolism (ESSER et al., 2010; SUBRA et al., 2011). In this study, not only proteins related to lipid metabolism were found in all time points, but proteins such as FABP3, fatty acid synthase, sphingomyelin phosphodiesterase acid like 3B, lipoprotein lipase, apolipoprotein E, and apolipoprotein A1 were differentially expressed between colostrum and milk at 7 and/or 14 days. In pigs, milk composition changes from 5.3% of fat in colostrum to 13.1% at 48 to 72 h after farrowing and decreases afterward to 6.5% at the end of lactation (CSAPÓ et al., 1996), which may indicate that the changes in milk exosome composition during this time could work in parallel with the remaining biocomponents, supporting fat metabolism in the newborn. It is worth mentioning that the intrinsic exosome biogenesis process involves as well various lipids, which by their own precursors (intraluminal vesicles) are generated from the membrane of late endosomes in a process which requires the Endosomal Sorting Complex Required for Transport (ESCRT), which includes TSG101 and Alix proteins, and the constitutive heat-shock protein HSC70 (SAHU et al., 2011; ZHANG et al., 2019) – proteins which were also identified by LC-MS/MS in the milk exosomes of this study.

Moreover, reports about changes in the exosome composition induced by diet are currently limited to the lipid and the miRNA cargo: Kumar et al. (2021) found that feeding a high-fat diet altered the lipid composition of exosomes, and further administration of exosomes isolated from the feces of obese mice led to insulin resistance in lean mice. In addition, modulation of microRNA present in milk exosomes was reported upon dietary supplementation of ginseng polysaccharides in sows (SUN et al., 2019) and by replacing alfalfa hay with whole cottonseed in the diet of dairy cows (QUAN et al., 2020). To the best of our knowledge, our study is the first to address changes induced by the diet in the milk exosome proteins.

PUFA-enriched diets are linked to decreased DNA methylation (KARIMI et al., 2017) and can induce changes in many metabolic pathways, including epigenetic changes in gene expression (KITAJKA et al., 2022; TREMBLAY et al., 2017; SZOSTAK et al., 2016). The ω -3 and ω -6 PUFA form bioactive mediators which act on different receptors and proteins

in the body, while previous proteomic profiles were obtained in mice in response to increased supply of ω -3 PUFA mainly focused on liver tissue and identified proteins involved in regulating lipid, carbohydrate, one-carbon, citric acid cycle and protein metabolism (AHMED et al., 2014). The increase of dietary ω -3 has also been demonstrated to induce changes in the proteome of high-density lipoprotein (HDL) particles (Burillo et al., 2012). Apolipoprotein A-I, clusterin, fibrinogen B, and serum paraoxonase were the main proteins differentially upregulated in HDL after ω -3 supplementation in humans (Burillo et al., 2012), similarly to results obtained in cows (VESHKINI et al.; 2022). Although these proteins were all also identified in the milk EV of this study, their abundance was not significantly different between our diet groups. In pigs, differences in the plasma proteome between the feeding groups of this study were previously established (NGUYEN et al., 2022) and concerned haptoglobin (HP), alpha-1-antitrypsin (SERPINA1), and serum amyloid P-component (APCS), although these changes were not translated to the milk EV proteome profile.

The 78 kDa glucose-regulated protein (HSPA5) was found at greater abundance in EV from GS than in GL on both d 0 ($p < 0.1$) and d 7 ($p < 0.05$) of lactation. In the PPI network, HSPA5 was observed playing a central role in the top-level interactions at day 7 p. p. Heat shock proteins (HSP), such as HSPA5, are ubiquitous polypeptides that act as molecular chaperones, participating in several basic cellular processes including protein folding (HARTL et al., 2022). The HSPA5 is essential for the normal function of the endoplasmic reticulum (ER) and is widely used as a marker of ER stress (NI et al; 2011). It is also part of the plasma membrane where it acts as a signal-transducing receptor, involved in tumor survival, proliferation, and resistance (DORES-SILVA et al., 2020); it has been regularly reported in EV when related to stress response (LIN et al., 2020; CHETTIMADA et al., 2018; SUNDAR et al., 2019; KIM et al., 2019) and accessory to the regulation of EV biogenesis in the ILV formation (MALLIA et al., 2020). Therefore, the upregulation of HSPA5 in milk EV from GS could have outcomes in a series of pathways, as displayed by the leader GO terms in Fig. 4 and the central relationship to the leader proteins in this study (Fig. 5). The effects of ω -3 in ER stress have also been explored in the protective effects of ER response by upregulation of HSP (OKADA et al., 2018; MCGUINNESS et al., 2006; OLIVERA-PEREZ et al., 2017), and our results suggest that the EV secretory pathway may be involved.

Spondin-2 (SPON-2) is part of a family of extracellular matrix proteins that acts on pattern recognition for initiating innate immune responses (HIGASHIJIMA et al., 1997). It

has been related to the proliferation of various cancer cells (HIGASHIJIMA et al., 1997; LUCARELLI et al., 2013), and upregulation of their respective tumor-derived exosomes have also been reported (DOGRA et al., 2020). The protein C4orf19 maps on human chromosome 19 and encodes for a protein of 141 amino acid residues, highly conserved among vertebrates, although the knowledge of its biological and cellular functions is still very limited (MIGNANI et al., 2020). It has a putative role in lipid homeostasis, evidenced in experimental observations by high expression levels of C19orf12 in adipose tissue, particularly during adipocyte differentiation (MIGNANI et al., 2020). The C19orf12-coregulated gene interactions are also predominant for genes involved in fatty acid metabolism (MIGNANI et al., 2020). Still, the roles of exosome-mediated communication events on the specific function of these proteins in milk exosomes remain to be explored, together with possible interactions they may have with other milk components.

The ω -6 and ω -3 PUFA, and the lipids formed by them, are also components of the cellular membrane, and therefore important to maintaining the structure and functional integrity of cells and exosomes (SINCLAIR et al., 2003; KARIMI et al., 2017). The length and degree of unsaturation of the phospholipid fatty acids and the relative content of membrane phospholipids are determinants for cellular and exosome functionality (CASARES et al., 2019; STUBBS and SMITH; 1984). In our study, the GO analysis highlighted terms relevant to vesicle mediated transport (GO:0016192) and transport (GO:0006810), which could be influenced by changes in PUFA composition. For instance, changes in dietary PUFA have been related to influencing the lipid composition of the cellular membrane of red blood cells (DE-LUCCHI et al., 1988), although detailed studies would be necessary to explore these effects on the exosome membrane.

Lipids comprise a main structural role in the exosomal membrane, while are also essential to exosome formation, release, targeting, and uptake (DONOSO-QUEZADA et al., 2021 SKOTLAND et al., 2017). While EVs have received a quite extensive characterization of both protein and nucleic acid (mRNA and microRNA) content, their lipid components have been largely overlooked, including in milk exosomes (SKOTLAND et al., 2020). Sphingomyelin (SM), phosphatidylcholine (PC), ceramides (Cer), and cholesterol (Chol) are lipid classes commonly found in cell membranes and therefore in exosomes (CHOI et al., 2012), although their relative abundance and enrichment of other lipid classes may depend on the producer cell type (HARASZTI et al., 2016) and physiological state of the cell (BRZOZOWSKI et al., 2018; CARAYON et al., 2016).

The use of exosomes as drug-delivery agents is highly sought, and while many attempts are undergoing, understanding their lipid composition and organization is a crucial point for their use as "natural liposomes" (IGNATIUS et al., 2000). Still, there are several limitations for lipidomics studies involving EV, mainly due to the current state-of-the-art inability to produce entirely pure EV isolations, and the knowledge on the lipid composition of exosomes from body fluids has been in part empirical, based on knowledge acquired in lipid organization in membranes and their subsequent physicochemical properties (SUBRA et al., 2007).

There are particular challenges in isolating lipids from milk-derived exosomes, due to the invariable co-isolation of milk lipids and MFG. The MFG contain a great number of triacylglycerols (TG) in their core, potentially interfering with the interpretation of lipidomics results of milk exosomes (ONG et al., 2021). Several studies in exosomes from various cell types have also reported high levels of cholesterol esters (CE) and TG, likely due to the co-isolation of lipid droplets or lipoproteins (SKOTLAND et al., 2019). The CE and TG are part of the structure of the hydrophobic core of lipid droplets and lipoproteins (MEER et al., 2008), and if considerably large amounts of these lipids are obtained in exosome preparations, they were likely co-isolated. Lipid droplets are also commonly co-isolated with EV when cells have been ruptured, which often occurs during the freezing and storage of biological samples. Interpretation of results on lipid exosome studies should be made with caution, emphasizing lipids that fit the bilayer membrane structure formed by plasma membrane or endosomal membranes (SKOTLAND et al., 2017).

The TG were one of the main subclasses identified in this study, ranked in second place with 172 lipid elements and representing 18% of the total number of lipid elements. TG were also one of the predominant subclasses of lipid elements differentially abundant in the comparison between milk exosomes and colostrum exosomes. Studies have spotted TG as a quantitatively minor intrinsic membrane component and playing a specific role in cellular stimulation and metastatic processes (LERIQUE et al., 1994). It is plausible that some of the identified TG represent compounds from the exosome membrane, since it is expected that many of these lipids derive likely from MFG and lipid droplets, inferences extended to exosome composition should be made with caution.

Only three studies, respectively on bovine, dromedary, and human milk, have also specifically examined the lipid composition of milk exosomes (BLANS et al. 2016, YASSIN

et al. 2016), and only one (CHEN et al., 2021) used an untargeted approach. TG was the most prominent lipid subclass found by Chen et al., (2021), comprising 22% of the total lipid elements identified using sing an LC-MS/MS metabolomics strategy. Blans et al. (2016) used SEC to isolate fractions of both milk exosomes and MFG and noticed a higher TG-to-cholesterol ratio in MFG fractions when compared to exosome fractions. The results from Yassin et al. (2016), however, were limited to the phospholipid fraction. The MFG have a notably distinct cargo, size (4 μm), and biophysical properties (tri-layered membrane) than exosomes, and are mostly removed in exosome isolation strategies, albeit it cannot be entirely excluded that the lipids from their membrane reminiscent are co-isolated and therefore will appear in lipidomic studies.

The SM, PC, Chol, and Cer represent the main lipid components of the exosome membrane (MATHIVANAN et al., 2010) and have been identified in our study in high numbers. SM was the third class with the higher number of lipid elements herein identified (69), with PC and Cer ranked in fourth and fifth place, with 67 and 61 lipid elements respectively. These classes were also part of the top significant differentially abundant lipids between exosomes from colostrum and mature milk at both 7 and 14. These results indicate that porcine exosomes and milk exosomes have a distinct profile, and the exosomal membrane composition is altered during lactation phases.

SM is a major class of phospholipids, which has been identified in the membrane of exosomes of all cell types (CHOI et al., 2013). Exosome membrane is also found to be enriched in SM when compared with their parent cells (SUBRA et al., 2007; VALLEJO et al., 2012). Yet, the exosome lipid content and specific lipid enrichment are dependent on the originating cell. SM was found to be more enriched in B-lymphocytes and dendritic cells than in oligodendroglial precursor cells (SKOTLAND et al., 2017). Mammary gland tissue is particularly rich in SM in both lactating and non-lactating stages (PRABHAKAR et al., 1980), and thus expected to be enriched in milk exosomes, although literature lacks data for comparison with exosomes in distinct body fluids. The SM provide exosomes with stability and structural rigidity (DONOSO-QUEZADA et al., 2021), but are also associated with exosome function. It has been reported that the angiogenic activity of tumor-derived EV is mediated mainly by SM both *in vitro* and *in vivo* (KIM et al., 2012). The underlying effects of the SM downregulation found in colostrum exosomes remain for further investigation.

Cer is one of the essential lipids found in ILVs and on the MVE membrane, as they are involved in the inward budding of endosomes to form multivesicular bodies (HEINRICHS, 2008; SKYRABIN et al., 2019). In consequence, the inner leaflet of the exosome membrane is enriched in Cer (MARSH et al., 2008). A high number of Cer elements were downregulated in colostrum exosomes when compared with milk exosomes (53 Cer on day 7, and 60 on day 14). The structure of the lipid bilayer directly influences its rigidity, and with so exosome size. Colostrum exosomes were significantly smaller than milk exosomes, as verified in two different isolations batches, and the modulation of SM, PC, and Cer lipid components may be causing this effect.

Chol is one of the major structural components of the plasma membrane of cells, and consequently exosomes (MAXFIELD and MEER, 2010). Although it has been identified in all exosome samples from this study, its abundance did not differ between lactation stages. A significant number of other sterol components (39) were identified in both milk and colostrum exosomes. Sterols are considered membrane reinforcers, as they sustain the domain structure of the cellular membrane (RIBEIRO et al., 2007). A total of 7 differentially abundant sterols were identified to be downregulated in colostrum exosomes and milk exosomes at both day 7 and 14, namely stigmasterols (ST 29:2;O;Hex;FA 20:1, ST 29:1;O;Hex;FA 15:2, and ST 29:1;O;Hex;FA 13:0) and steroid conjugates (ST 24:1;O4_19:2;1O, ST 24:2;O4_2:0, ST 24:1;O4;G_16:2;1O, ST 24:1;O3;G_28). Stigmasterols are major constituents of the sterol profiles of plant species (SCHALLER et al., 2003), and although cholesterol is a predominant sterol in the animal membrane, plant sterols (phytosterols) can also be found (HAC-WYDRO et al., 2007). Phytosterols are absorbed from the diet and are incorporated into cellular membranes but since their absorption via the intestinal tract is very poor, their content in mammal membranes is relatively low (IKEDA et al., 2001, HALLING and SLOTTE, 2004, SUDHOP et al., 2005, RATNAYAKE et al., 2000).

Sterols together with sphingolipids are also crucial for the formation of lipid rafts, that play an important role in signal transduction, cytoskeleton reorganization, and cellular sorting (DUFOURC, 2008). Particularly in exosomes, lipid rafts are involved both in cargo loading using an ESCRT-independent mechanism, and the shift of protein and molecules facilitating their secretion (LI et al., 2018; GASSART et al., 2003), thus directly involved in exosome function in intercellular communication. A major number of lipid compounds

downregulated on colostrum exosomes were involved in lipid raft composition, which may have thus a direct effect on the exosome biogenesis and trafficking.

The combination of lipidomic and proteomic analysis can also provide valuable information on exosome studies (SKOTLAND et al., 2019). The joint interpretation of the results can help corroborate the successful isolation of the exosome preparation, and also provide an extended picture of the complex metabolic interactions behind exosome-mediated communication. Diverse proteins and lipids related to exosome biogenesis, cargo sorting, and secretion were found in parallel in both studies. ESCRT complex and accessory proteins comprised a substantial portion of the proteins identified in milk exosomes. Interestingly, these are enriched in lipid rafts (BUKRINSKY et al., 2020), and a major part of the lipids identified were involved in the lipid raft's structure and function.

Finally, it is important to notice that different storage and processing steps can influence the physical properties and overall composition of the EV isolated (MIGNANI et al., 2020; ZONNEVELD et al., 2014; WIJENAYAKE et al., 2021; KLEINJAN et al., 2021). Specific aspects such as the use of fresh or frozen milk and the use of distinct isolation procedures should be taken into consideration when comparing studies in milk exosomes. The experimental design herein used strategies for the isolation of exosomes derived from frozen milk and opted for the combined use of ultracentrifugation coupled with SEC, reportedly to recover a consistent yield and significantly pure exosome isolations in complex body fluids, including milk (ALAMELDIN et al., 2021; KOH et al., 2018).

7 CONCLUSIONS

This study endorses the importance of exosomes as active biocomponents of milk and provides knowledge for future studies exploring their role in the regulation of immunity and growth of the newborn. The identified functional proteome and protein-protein interaction networks identified in our study help to elucidate the role of milk exosomes in different lactation periods. This study explored the proteome of exosomes from porcine colostrum and milk and showed that proteins with functions in the development of immune response, regulation of cellular processes, and cellular development were differentially expressed between the time points.

The proteome of colostrum exosomes presented a unique profile distinct from the proteome of exosomes from mature milk. The functional analysis highlighted pathways related to the regulation of homeostasis to be upregulated in colostrum exosomes, and pathways such as endothelial cell development and lipid metabolism to be upregulated in mature milk exosomes. As a result, further studies are encouraged to unveil the distinct physiological functions of milk exosomes in different lactating stages.

Functional analysis of the proteins in milk exosomes revealed proteins that play a key role in health during the early stage of life and development, corroborating to the suggestion that exosome-mediated communication is a pivotal part of the infant's immune system and cellular development. These results are of relevance for the basic understanding of their impact on the infant's development but also for bringing forward the manufacturing of milk replacers.

This study also explored the effects of different ratios of ω -6: ω -3 fatty acids in the maternal diet on the sow's milk exosome proteome, revealing variations in the milk exosome proteome between the diet groups. Protein abundance changes included the key exosome protein HSPA5, involved in processes of exosome biogenesis, cargo sorting and secretion. Spondin-2, also downregulated in the low ω -6: ω -3 ratio diets on milk exosomes at day 7, is an extracellular matrix protein that acts on pattern recognition for initiating innate immune responses. The results reported here provide the first evidence that the milk exosome proteome can be modulated by the diet. These results are relevant not only for fundamental research on the understanding of the sources of exosome components but also have applied

aspects in animal and human nutrition and health and may provide new perspectives for feeding additives.

The lipidomic characterization provided knowledge on the structural, function, and stability characteristics of milk exosomes. Main lipid components of the exosome membrane, such as SM, PC, Chol, and Cer were identified in our results, and were downregulated in colostrum exosomes when compared to milk exosomes. Lipid elements related to lipid rafts were also among the major lipids identified on this study, which are involved in numerous processes of exosome biogenesis and secretion, including for a cargo sorting mechanism not depending on ESCRT machinery. This study helps to bridge the gap on the limited knowledge of lipid characterization of milk exosomes, and the underlying effects of lipid modulation on exosome structure and function remain unexplored. The complete lipidome characterization of milk exosomes on different lactation stages provided here is also of major importance for innovative bionanotechnological approaches, as milk exosomes are suggested as a potential natural drug delivery system.

Finally, while methodological improvements for the isolation of milk exosomes are still to take place in EV research, UC coupled with SEC proved to be an effective and feasible strategy for the isolation and purification of milk exosomes. The development of strategies for the depletion of MFG and casein micelles is desired, as they could potentially enhance the purity of milk exosome isolation, and therefore reduce confounding effects inherent to milk EV studies and improve the understanding and interpretation of results.

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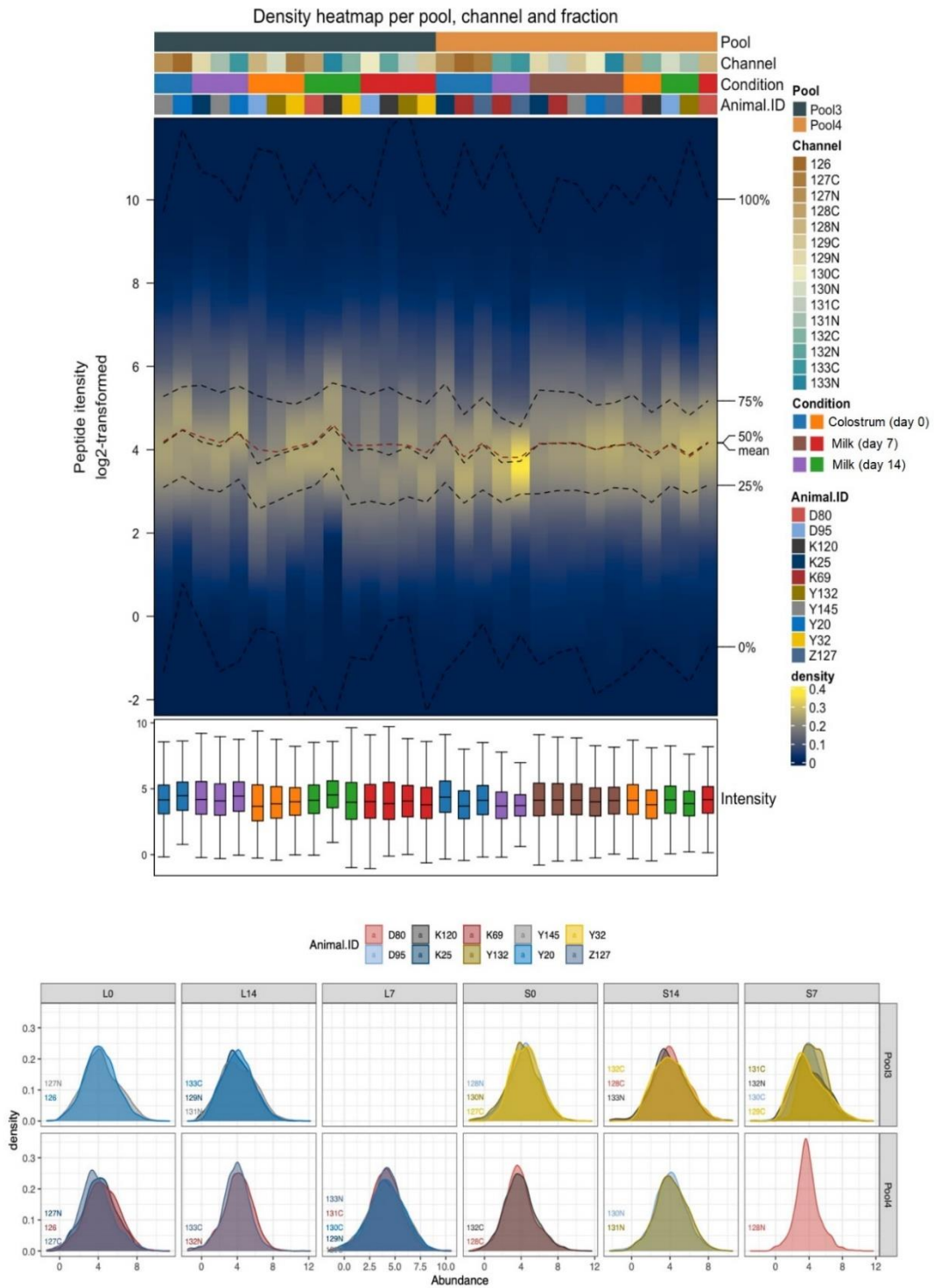
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9 APPENDICES

Appendix 1



Appendix 1. The PSM-level data were variance-stabilized and transformed using the VSN package in R environment and then aggregated to protein-level intensities using Tukey's median polish procedure.

Appendix 2

Table A2-1. List of all proteins identified by LC-MS/MS maintained for statistical analysis for analysis on comparison between colostrum exosomes (day 0) and milk exosomes at day 7, ranked by significance.

| Rank | Gene | Protein | logFC | P Value | Adjusted P Value | Average expression | Peptide count |
|------|-------------------------------------|-------------------|----------|----------|------------------|--------------------|---------------|
| 1 | APOE | P18650 | -3.05884 | 1.34E-10 | 3.54E-08 | 4.580658 | 8 |
| 3 | Gene NA - Prt: P10859 | P10859 | -2.56844 | 1.03E-09 | 9.02E-08 | 3.716168 | 2 |
| 2 | APOE// | F1RM45;P 18650 | -2.52347 | 9.79E-10 | 9.02E-08 | 3.62444 | 28 |
| 4 | VTN | P48819 | -2.51944 | 1.37E-09 | 9.02E-08 | 4.276618 | 7 |
| 6 | CSN3 | P11841 | -2.16003 | 2.87E-09 | 1.26E-07 | 3.436882 | 27 |
| 5 | SIL1 | F1RGI9 | -2.0533 | 2.69E-09 | 1.26E-07 | 3.792533 | 8 |
| 7 | HAPLN3 | A0A5G2R 305 | -1.99782 | 4.8E-09 | 1.81E-07 | 3.833653 | 20 |
| 8 | Gene NA - Prt: A0A5G2QXT 5 | A0A5G2Q XT5 | -2.40265 | 1.32E-08 | 4.22E-07 | 3.449775 | 5 |
| 9 | MUC4 | A0A287B 5M2 | 2.184258 | 1.44E-08 | 4.22E-07 | 3.497925 | 23 |
| 10 | Gene NA - Prt: A0A5G2R4L0 | A0A5G2R 4L0 | -2.4133 | 1.83E-08 | 4.83E-07 | 4.125335 | 3 |
| 11 | THBS1 | F1SS26 | -2.05115 | 2.47E-08 | 5.94E-07 | 3.711806 | 41 |
| 12 | CD36 | Q3HUX1 | 2.026453 | 3.59E-08 | 7.9E-07 | 4.671275 | 24 |
| 13 | LPL | A0A287A TV6 | -2.0572 | 4.44E-08 | 9.03E-07 | 3.338335 | 15 |
| 14 | DDR1 | K7GSX5 | -2.13766 | 5.93E-08 | 1.12E-06 | 4.792415 | 2 |
| 15 | GANAB | P79403 | -1.96872 | 7.01E-08 | 1.23E-06 | 3.357891 | 24 |
| 16 | CSN1S2 | P39036 | -2.1243 | 7.79E-08 | 1.29E-06 | 3.715798 | 39 |
| 17 | CSN1S1 | P39035 | -2.15744 | 1.02E-07 | 1.58E-06 | 3.239364 | 31 |
| 18 | CHID1 | F1RZ01 | -1.87334 | 1.65E-07 | 2.42E-06 | 3.185273 | 11 |
| 20 | FBLN2 | A0A287B 5Q1 | -2.29675 | 1.9E-07 | 2.5E-06 | 3.6693 | 10 |
| 19 | CLU | A0A5S8K LN1 | -1.63593 | 1.81E-07 | 2.5E-06 | 3.847397 | 27 |
| 21 | MGP | A0A5G2Q KI1 | -2.1489 | 2.33E-07 | 2.93E-06 | 4.000976 | 10 |
| 22 | PGLYRP1 | A0A286ZI 97 | -2.07871 | 3.26E-07 | 3.91E-06 | 3.716147 | 14 |
| 23 | CP | A0A5G2Q W05 | -1.90183 | 3.42E-07 | 3.92E-06 | 3.659874 | 11 |
| 24 | TSPAN6 | F1S1M4 | 1.789332 | 3.63E-07 | 3.99E-06 | 4.167684 | 4 |
| 25 | HSPA5 | A0A287BI L8 | -1.63728 | 4.16E-07 | 4.4E-06 | 2.279501 | 4 |
| 26 | ATP6AP2 | A0A287AJ K1 | -1.79991 | 5.98E-07 | 6.07E-06 | 3.929251 | 5 |
| 27 | APOA1 | K7GM40 | -1.78832 | 1.16E-06 | 1.14E-05 | 3.357979 | 30 |
| 28 | PPIB | A0A286Z KG9 | -1.63939 | 1.58E-06 | 1.49E-05 | 3.286902 | 3 |
| 29 | TINAGL1 | F1SVA2 | -2.5922 | 2E-06 | 1.77E-05 | 4.590614 | 3 |

| | | | | | | | |
|----|-------------------------------------|-------------------------------|----------|----------|----------|----------|----|
| 30 | CD209 | B3FVQ2 | 1.615881 | 2.01E-06 | 1.77E-05 | 4.766564 | 2 |
| 31 | Gene NA - Prt: A0A287ATT2 | A0A287A TT2 | -2.24384 | 2.42E-06 | 2.06E-05 | 3.373407 | 3 |
| 32 | B4GALT1 | A0A287B G16 | -1.89939 | 3.18E-06 | 2.62E-05 | 3.320653 | 20 |
| 33 | TTR | A0A5G2Q IE9 | -1.59931 | 3.59E-06 | 2.8E-05 | 3.478735 | 3 |
| 34 | SERPINC1 | A0A5G2Q UE0 | -1.48287 | 3.61E-06 | 2.8E-05 | 3.234506 | 9 |
| 35 | ITIH2 | A0A5G2Q EV5 | -1.52173 | 4.74E-06 | 3.58E-05 | 3.574539 | 7 |
| 36 | Gene NA - Prt: A0A0A0MY5 8 | A0A0A0M Y58 | -1.49819 | 5.37E-06 | 3.94E-05 | 4.034749 | 19 |
| 37 | SERPINF1 | A0A287A YJ8 | -1.3751 | 5.66E-06 | 4.04E-05 | 3.303514 | 10 |
| 38 | FASN | I3LCW1 | 1.741019 | 7.33E-06 | 5.09E-05 | 3.770177 | 4 |
| 39 | C1QA | Q69DL0 | -2.14218 | 7.74E-06 | 5.24E-05 | 4.078393 | 2 |
| 40 | CHRDL2// | A0A5G2Q XF8;A0A5 G2RH74 | -1.5885 | 8.73E-06 | 5.76E-05 | 3.581001 | 8 |
| 45 | FUCA1 | I3LNS9 | -1.65992 | 9.87E-06 | 5.79E-05 | 3.774814 | 2 |
| 41 | ERAP1 | K7GLU6 | -1.55396 | 9.06E-06 | 5.79E-05 | 2.754582 | 6 |
| 44 | GPRC5B | A0A480W 2U9 | 1.523077 | 9.74E-06 | 5.79E-05 | 5.240895 | 5 |
| 42 | SLC34A2 | F1S5A6 | 1.544571 | 9.64E-06 | 5.79E-05 | 4.131015 | 66 |
| 43 | NA;FABP3 | I3LTW5;O 02772 | 3.186529 | 9.68E-06 | 5.79E-05 | 6.001107 | 2 |
| 46 | GSN | A0A287A 6P1 | -1.21755 | 1.03E-05 | 5.94E-05 | 3.579003 | 19 |
| 48 | CSN2// | P39037;A0 A2C9F376 | -1.52755 | 1.17E-05 | 6.43E-05 | 2.792655 | 68 |
| 47 | ALB | A0A287B AY9 | -1.26931 | 1.16E-05 | 6.43E-05 | 3.830738 | 18 |
| 49 | SLC9A3R1 | A0A5G2R 543 | 1.742217 | 1.33E-05 | 7.15E-05 | 4.747528 | 16 |
| 50 | RAB3D | A0A287B LN4 | 1.327575 | 1.76E-05 | 9.29E-05 | 3.960266 | 4 |
| 52 | LTF | A0A5G2Q UQ4 | -1.37492 | 1.87E-05 | 9.49E-05 | 3.41711 | 26 |
| 51 | TTYH2 | A0A287A AJ2 | 1.371667 | 1.84E-05 | 9.49E-05 | 5.144515 | 8 |
| 53 | RDX;EZR | P26044;F1 SB42 | 2.484253 | 1.93E-05 | 9.62E-05 | 4.44116 | 2 |
| 54 | ACSL3 | A0A287B 8R8 | 1.308643 | 2.12E-05 | 0.000103 | 4.195918 | 41 |
| 55 | FMOD | F1S6B5 | -2.09937 | 2.4E-05 | 0.000115 | 2.122226 | 5 |
| 56 | Gene NA - Prt: A0A287BAB3 | A0A287B AB3 | -1.58745 | 3.01E-05 | 0.000142 | 4.473881 | 4 |
| 57 | MUC1// | A0A5G2Q GF8;F1RG R9 | 1.834236 | 3.19E-05 | 0.000148 | 4.559646 | 9 |
| 58 | RAB18 | I3LC07 | 1.171828 | 3.37E-05 | 0.000153 | 4.32985 | 11 |
| 59 | BAIAP2// | A0A287B RR9;F1RZ B0 | 1.550944 | 3.93E-05 | 0.000176 | 4.191112 | 16 |

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|----|--|---|----------|----------|----------|----------|----|
| 60 | FABP3 | O02772 | 1.297847 | 4.07E-05 | 0.000179 | 4.383504 | 22 |
| 61 | Gene NA - Prt: A0A075B7I9; A0A075B7H9 ;A0A075B7J0 ;A0A075B7I5 | A0A075B 7I9;A0A07 5B7H9;A0 A075B7J0; A0A075B 7I5 | -1.71783 | 4.96E-05 | 0.000214 | 5.663481 | 4 |
| 62 | RAB1A | F2Z5U4 | 1.186049 | 5.02E-05 | 0.000214 | 4.315186 | 9 |
| 63 | Gene NA - Prt: A0A075B7I6 | A0A075B 7I6 | -1.54786 | 5.17E-05 | 0.000217 | 3.55741 | 2 |
| 64 | SMPDL3B// | A0A287BJ W1;F1ST N0 | 1.380332 | 5.27E-05 | 0.000218 | 3.57818 | 10 |
| 65 | NA;LOC1005 23213 | A0A0A0M Y58;F1RL 06 | -1.28115 | 5.61E-05 | 0.000228 | 3.985018 | 6 |
| 66 | PAPLN | F1S3J7 | -1.49688 | 5.74E-05 | 0.00023 | 3.598821 | 4 |
| 67 | RDX;MSN;E ZR | P26044;A0 A287AK1 9;F1SB42 | 1.469399 | 5.96E-05 | 0.000235 | 4.397996 | 10 |
| 68 | ATP6AP1 | A0A5G2R D35 | -1.17875 | 6.52E-05 | 0.000253 | 3.264964 | 4 |
| 69 | Gene NA - Prt: A0A5S6G633 | A0A5S6G 633 | -2.24612 | 6.97E-05 | 0.000267 | 2.838299 | 3 |
| 70 | ARF/3/4 | A0A287B 8I6;A0A5 G2R3T3 | 1.233139 | 7.46E-05 | 0.000281 | 3.238186 | 4 |
| 73 | NA;CSN1S1 | A0A5S6G 633;P3903 5 | -1.25937 | 8.41E-05 | 0.000304 | 3.32349 | 13 |
| 72 | Gene NA - Prt: A0A287ALC1 | A0A287A LC1 | -1.2067 | 8.4E-05 | 0.000304 | 3.088551 | 68 |
| 71 | Gene NA - Prt: A0A287ALJ6 | A0A287A LJ6 | -1.07694 | 8.22E-05 | 0.000304 | 4.016404 | 4 |
| 74 | MUC15 | F1SFZ5 | 1.58103 | 8.66E-05 | 0.000309 | 4.71967 | 9 |
| 75 | RAB1A/RAB 1B | F2Z5U4;Q 06AU7 | 1.206563 | 0.000109 | 0.000383 | 3.36718 | 6 |
| 76 | XDH | F1S3Y7 | 1.229709 | 0.000113 | 0.000391 | 4.814699 | 66 |
| 77 | MSTN | O18831 | -1.29607 | 0.000115 | 0.000395 | 2.954647 | 10 |
| 78 | SERPIND1 | F1RKY2 | -1.85453 | 0.00012 | 0.000408 | 3.397077 | 7 |
| 79 | FASN | I3LC73 | 1.322274 | 0.000151 | 0.000504 | 3.94284 | 17 |
| 81 | JCHAIN | A0A287B QC8 | -1.4338 | 0.00022 | 0.000716 | 4.707291 | 2 |
| 80 | PAEP | A0A5S6H Q91 | -1.25135 | 0.000219 | 0.000716 | 3.980301 | 12 |
| 82 | Gene NA - Prt: A0A287A4Y3 | A0A287A 4Y3 | -1.18388 | 0.000223 | 0.000716 | 4.354942 | 13 |
| 83 | MYOF | A0A5G2Q 8T2 | 0.965459 | 0.000249 | 0.000793 | 3.989779 | 30 |
| 84 | SPON2 | A0A481A ED0 | -1.60841 | 0.000278 | 0.000868 | 3.364732 | 4 |
| 85 | GPRC5C | A0A287A SV2 | 1.015776 | 0.00028 | 0.000868 | 4.592204 | 5 |

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|-----|--|---------------------------------|----------|----------|----------|----------|-----|
| 86 | APOE | F1RM45 | -1.22529 | 0.000305 | 0.000938 | 3.395127 | 2 |
| 87 | SLC6A14 | K7GNX7 | 1.217545 | 0.000408 | 0.001238 | 4.229918 | 13 |
| 88 | ENO1 | I3LK59 | 1.048299 | 0.000435 | 0.001304 | 4.114588 | 7 |
| 89 | CIDEA | A0A5G2R 9X9 | 1.22115 | 0.000443 | 0.001315 | 4.788696 | 3 |
| 90 | RAB11A | A0A5G2Q E74 | 1.443548 | 0.000454 | 0.001332 | 4.928738 | 6 |
| 91 | SERPINA3-2 | A0A287B 9R5 | -1.26669 | 0.000686 | 0.00199 | 4.440938 | 5 |
| 93 | Gene NA - Prt: A0A287B626 | A0A287B 626 | -1.20554 | 0.000768 | 0.002161 | 3.970228 | 29 |
| 94 | CKB | A0A5G2R 6X7 | 0.960056 | 0.00077 | 0.002161 | 4.131502 | 7 |
| 92 | PLIN3 | K4P7U5 | 1.1538 | 0.000759 | 0.002161 | 4.318163 | 12 |
| 95 | RHOA | I3LVS7 | 1.692316 | 0.000823 | 0.002287 | 5.26511 | 5 |
| 96 | TLR2 | Q59HI8 | 1.104358 | 0.000947 | 0.002605 | 4.359128 | 26 |
| 97 | C9 | A0A287B H90 | -1.30456 | 0.001406 | 0.003827 | 3.484615 | 3 |
| 98 | CDC42 | Q007T2 | 1.022553 | 0.001474 | 0.00397 | 1.875831 | 2 |
| 100 | PGK1 | F1RPH0 | 0.93556 | 0.001521 | 0.004006 | 4.45063 | 4 |
| 101 | BTN1A1 | A0A5K1U 1P1 | 0.972775 | 0.001533 | 0.004006 | 4.268103 | 61 |
| 99 | RAC2 | A0A5G2R 6S0 | 1.111124 | 0.001511 | 0.004006 | 4.062857 | 2 |
| 102 | PLOD1 | F1RF77 | -1.07263 | 0.001615 | 0.004179 | 3.585215 | 3 |
| 103 | BAIAP2 | A0A287B RR9 | 1.119257 | 0.001632 | 0.004183 | 5.518362 | 2 |
| 104 | KRAS | I3LCQ9 | 1.045186 | 0.001649 | 0.004186 | 3.835119 | 3 |
| 105 | SOD3 | I3LUD1 | -1.03019 | 0.001843 | 0.004634 | 3.206342 | 2 |
| 107 | MFGE8// | A0A5G2R KJ7;A0A5 G2QXS8 | 0.887413 | 0.002007 | 0.004953 | 4.588095 | 105 |
| 106 | SLC29A1 | A0A287B 8K5 | 1.207747 | 0.001989 | 0.004953 | 4.707259 | 6 |
| 108 | PPIA | P62936 | 0.845717 | 0.002201 | 0.005381 | 4.659886 | 14 |
| 109 | ANXA6 | A0A5G2R BI3 | 0.91366 | 0.002336 | 0.005657 | 4.400298 | 6 |
| 110 | Gene NA - Prt: A0A287A7S8 | A0A287A 7S8 | -0.87628 | 0.002403 | 0.005719 | 3.275506 | 2 |
| 111 | MUC1 | F1RGR9 | 1.638827 | 0.002405 | 0.005719 | 3.142075 | 3 |
| 112 | ATP2B2 | A0A286Z SF2 | 0.76404 | 0.002657 | 0.006229 | 4.769486 | 7 |
| 113 | SRC | K7GM45 | 0.839798 | 0.002666 | 0.006229 | 4.3238 | 6 |
| 115 | COL4A2 | A0A5G2R J53 | -1.20742 | 0.0028 | 0.006427 | 3.017329 | 3 |
| 114 | CD5L | F1RN76 | -1.07683 | 0.00278 | 0.006427 | 4.184892 | 9 |
| 116 | Gene NA - Prt: I3LTW5 | I3LTW5 | 1.713666 | 0.003099 | 0.007054 | 3.116072 | 2 |
| 117 | Gene NA - Prt: A0A5G2R9S9 ;A0A287ALJ6 | A0A5G2R 9S9;A0A2 87ALJ6 | -1.39581 | 0.003241 | 0.007314 | 3.153838 | 2 |
| 118 | Gene NA - Prt: A0A287AP28; | A0A287A P28;A0A2 86ZIM1;A | -1.63365 | 0.003696 | 0.008269 | 3.773167 | 3 |

| | A0A286ZIM1 ;A0A287BAB 3 | 0A287BA B3 | | | | | |
|-----|--|--|----------|----------|----------|----------|----|
| 119 | MSN | A0A287A K19 | 1.026988 | 0.003869 | 0.008583 | 4.993424 | 19 |
| 120 | RDX | P26044 | 0.883987 | 0.004037 | 0.00888 | 3.883106 | 5 |
| 121 | MVB12A | A0A287A P77 | 1.321117 | 0.004128 | 0.009007 | 4.637288 | 4 |
| 122 | RAB2A/RAB 2B | F1RT87;F 1S8J6 | 0.981515 | 0.004422 | 0.009568 | 3.736955 | 3 |
| 123 | ARF3 | A0A287B 8I6 | 1.0729 | 0.004724 | 0.010139 | 4.867116 | 4 |
| 124 | IFITM1;IFIT M3;LOC1021 61418 | A0A5G2Q WL4;A0A 287B7R8; F1RGC5 | 0.832938 | 0.004842 | 0.010309 | 3.46739 | 4 |
| 125 | MATN4 | F1SDQ7 | -1.17435 | 0.005314 | 0.011224 | 2.441729 | 2 |
| 126 | 14-3- 3zeta;YWHA H;YWHAQ;C RK;YWHAB | A0A480P LY3;F2Z4 Y1;A0A5 G2QM17; A0A5G2Q SR7;A0A2 87BPX7 | 1.175426 | 0.005671 | 0.011882 | 5.657113 | 2 |
| 127 | Gene NA - Prt: A0A075B7H9 | A0A075B 7H9 | -0.80249 | 0.006983 | 0.014515 | 3.212066 | 4 |
| 128 | CD82 | A0A5G2Q KD8 | 0.658262 | 0.007147 | 0.014696 | 4.252814 | 8 |
| 129 | KRT1 | F1SGG3 | 0.864733 | 0.007181 | 0.014696 | 5.116696 | 9 |
| 130 | EZR | F1SB42 | 1.119353 | 0.00777 | 0.015778 | 3.956502 | 22 |
| 131 | STX3 | F1RI88 | 0.987971 | 0.008453 | 0.017035 | 3.589462 | 9 |
| 132 | AEBP1 | A0A286Z ZM9 | -0.57936 | 0.008719 | 0.017438 | 3.833016 | 4 |
| 133 | LOC10030236 8 | A0A287B 5W2 | 0.680148 | 0.009078 | 0.018019 | 4.415311 | 34 |
| 134 | RAB35 | A0A287B FE6 | 0.768843 | 0.009574 | 0.018862 | 4.809324 | 5 |
| 135 | PDCD6IP | A0A287A 5B4 | 0.937747 | 0.009907 | 0.019374 | 4.405182 | 30 |
| 136 | ITIH4 | A0A286Z N24 | -0.71567 | 0.010322 | 0.020037 | 3.40089 | 4 |
| 137 | CD14 | A2SW51 | 1.01012 | 0.010828 | 0.020866 | 3.953437 | 3 |
| 138 | PIGR// | A0A287B 644;Q2924 4 | -0.89284 | 0.01092 | 0.02089 | 3.790705 | 5 |
| 139 | CPM | I3LTE1 | 0.886343 | 0.011023 | 0.020936 | 4.389205 | 28 |
| 140 | PROM2 | F1SU61 | 0.656004 | 0.011954 | 0.022541 | 3.750727 | 2 |
| 141 | ARHGAP18 | A0A287B HC4 | 0.702267 | 0.013452 | 0.025186 | 4.917479 | 4 |
| 142 | Gene NA - Prt: A0A5G2R9S9 | A0A5G2R 9S9 | -1.09679 | 0.014055 | 0.025858 | 3.738216 | 2 |
| 143 | TM4SF1 | A0A5G2Q C13 | -0.83057 | 0.014077 | 0.025858 | 3.680237 | 2 |
| 144 | PRDX5 | A0A5G2R 9D6 | 0.659936 | 0.014105 | 0.025858 | 3.401712 | 6 |
| 145 | C4orf19 | F1S4L8 | 0.948851 | 0.015086 | 0.027468 | 4.353937 | 2 |

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|-----|---------------------------------|-------------------------------|----------|----------|----------|----------|----|
| 146 | RHOF | Q06AT7 | 0.980713 | 0.015743 | 0.028467 | 5.064607 | 2 |
| 147 | PPIC | A0A5G2R B35 | -0.90975 | 0.017663 | 0.031467 | 2.948777 | 4 |
| 149 | GDI2 | A0A5G2Q SK4 | 0.640722 | 0.01776 | 0.031467 | 3.627553 | 15 |
| 148 | RAB7A | A0A286Z P69 | 0.695541 | 0.017744 | 0.031467 | 4.306068 | 9 |
| 150 | MDH1 | A0A5G2Q FC2 | 0.635617 | 0.018048 | 0.031765 | 4.533037 | 4 |
| 151 | ST13 | F1SRB9 | 0.805281 | 0.019229 | 0.033618 | 4.607381 | 3 |
| 152 | UBC | A0A5G2Q DJ2 | 0.627925 | 0.01952 | 0.033904 | 4.733632 | 12 |
| 153 | GPRC5A | I3LN87 | -1.12708 | 0.019748 | 0.034075 | 2.411634 | 2 |
| 154 | ALS | Q1KS52 | -0.75255 | 0.021216 | 0.036371 | 1.561597 | 4 |
| 155 | KRT75 | F1SGI7 | 0.946073 | 0.021667 | 0.036903 | 4.699503 | 2 |
| 156 | KRT10 | I3LDS3 | 0.647603 | 0.02234 | 0.037621 | 4.41125 | 5 |
| 157 | GALM | A0A5G2Q Z46 | 0.655284 | 0.022453 | 0.037621 | 3.897957 | 4 |
| 158 | PARK7 | A0A5G2Q IK1 | 1.127233 | 0.022515 | 0.037621 | 5.159512 | 2 |
| 159 | DAG1 | A0A287B BR1 | -0.98116 | 0.023326 | 0.03873 | 2.410085 | 2 |
| 160 | MFGE8 | A0A5G2R KJ7 | 0.599344 | 0.024807 | 0.040932 | 5.037166 | 11 |
| 161 | KRT1/4/0 | A0A287A EL2;I3LD S3 | 0.900781 | 0.025025 | 0.041035 | 5.18532 | 3 |
| 162 | STXBP2 | A0A5G2Q A44 | 0.729406 | 0.026892 | 0.043824 | 4.857438 | 11 |
| 163 | EEF1A1 | A0A288C G57 | 0.704532 | 0.030952 | 0.050131 | 5.317222 | 10 |
| 164 | KRT5 | A0A287A ZL3 | 0.728007 | 0.03257 | 0.052429 | 3.506331 | 3 |
| 166 | APOA4 | A0A287B M29 | -0.58592 | 0.033323 | 0.052996 | 3.986276 | 7 |
| 165 | SNAP23 | A0A287B 2U6 | 0.707816 | 0.033308 | 0.052996 | 4.028723 | 5 |
| 167 | HSPA/1B/6 | Q6S4N2;Q 04967 | 0.619092 | 0.035608 | 0.056291 | 5.257768 | 3 |
| 168 | CD63 | F1SPK8 | 0.688495 | 0.042119 | 0.066187 | 4.220068 | 3 |
| 169 | RRAS | F1RHW4 | 0.741922 | 0.042825 | 0.066898 | 4.486447 | 3 |
| 171 | Gene NA - Prt: A0A5G2Q7F8 | A0A5G2Q 7F8 | -1.0033 | 0.044259 | 0.068149 | 2.486988 | 2 |
| 172 | FLOT1 | Q767L6 | -0.62912 | 0.0444 | 0.068149 | 3.905262 | 6 |
| 170 | VWA2 | F1S5H3 | -0.50493 | 0.044139 | 0.068149 | 3.350119 | 7 |
| 173 | SAA3// | A0A480K NV0;A0A 5G2R2X7 | 0.594472 | 0.046534 | 0.071011 | 4.899096 | 7 |
| 174 | HSPA8 | F1S9Q3 | 0.529534 | 0.047349 | 0.071468 | 3.712596 | 22 |
| 175 | GNAS | A5GFT9 | 0.548258 | 0.047374 | 0.071468 | 4.08505 | 2 |
| 176 | SELENBP1 | F1ST01 | 0.51584 | 0.048281 | 0.072422 | 3.456086 | 2 |
| 177 | HEXA | F1SI88 | -0.48961 | 0.048669 | 0.072591 | 2.413714 | 4 |
| 179 | RAB2A | F1RT87 | 0.459988 | 0.052726 | 0.077764 | 4.826399 | 4 |
| 178 | RAB14 | Q52NJ6 | 0.729694 | 0.052718 | 0.077764 | 4.846186 | 2 |

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|-----|---------------------------------|---|----------|----------|----------|----------|----|
| 180 | TMBIM1 | A0A5G2R 7N2 | 0.766063 | 0.053391 | 0.078306 | 5.668077 | 2 |
| 181 | RAC1 | A0A287B 4G4 | 0.677151 | 0.056982 | 0.083112 | 4.270526 | 3 |
| 182 | SDCBP | A0A286Z KH9 | 0.53583 | 0.057867 | 0.08394 | 3.258615 | 11 |
| 184 | TF | A0A5G2R 5N9 | -0.66656 | 0.06144 | 0.088154 | 4.641461 | 2 |
| 183 | TTYH3 | F1RI78 | 0.548322 | 0.061162 | 0.088154 | 4.253275 | 3 |
| 185 | PDXK | O46560 | 0.636445 | 0.067545 | 0.096388 | 3.521722 | 3 |
| 186 | CDK2 | A0A286Z S27 | 0.528704 | 0.071347 | 0.101266 | 3.908768 | 4 |
| 187 | SERINC5 | F1RF26 | 0.568497 | 0.072462 | 0.102299 | 5.354037 | 2 |
| 188 | ACLY | F1SON2 | 0.471032 | 0.075551 | 0.106093 | 2.983594 | 4 |
| 189 | CLDN3 | A0A5G2Q JG3 | 0.657004 | 0.076535 | 0.106906 | 4.344975 | 3 |
| 190 | DNAJC3 | I3LBK9 | -1.08076 | 0.083611 | 0.116176 | 3.527757 | 2 |
| 191 | RAC/2/1 | A0A5G2R 6S0;A0A2 87B4G4 | 0.681555 | 0.085511 | 0.118193 | 5.47119 | 2 |
| 192 | VPS37B | F1REY0 | 0.481797 | 0.087599 | 0.120449 | 3.943706 | 7 |
| 193 | PON1 | I3L5K0 | -0.90364 | 0.103953 | 0.142115 | 2.249963 | 4 |
| 194 | RAB5C | A0A287B N36 | 0.544742 | 0.104433 | 0.142115 | 2.520809 | 4 |
| 195 | CD9 | Q8WMQ3 | 0.907597 | 0.105237 | 0.142474 | 5.56161 | 4 |
| 196 | FGB | A0A5G2R HA4 | -0.71452 | 0.116385 | 0.156764 | 2.991589 | 7 |
| 197 | C3 | F1SBS4 | 0.436642 | 0.123992 | 0.166162 | 3.724701 | 4 |
| 198 | EEF2 | I3LII3 | 0.514595 | 0.125706 | 0.167608 | 2.515056 | 2 |
| 199 | SAA3 | A0A480K NV0 | -0.65475 | 0.12915 | 0.171335 | 5.54745 | 2 |
| 201 | PFN1 | A0A5G2R 9A9 | 0.456534 | 0.136242 | 0.178945 | 3.252424 | 4 |
| 200 | ACT/A1/B/G1 | A0A5S6G 831;Q6QA Q1;I3LVD 5 | 0.517173 | 0.136196 | 0.178945 | 4.611775 | 18 |
| 202 | GPI | P08059 | 0.383493 | 0.13754 | 0.179755 | 4.692694 | 4 |
| 203 | RALA | I3LAJ6 | 0.600719 | 0.146074 | 0.189968 | 4.085954 | 5 |
| 204 | VTA1 | F1S6Z8 | -0.59522 | 0.147884 | 0.19138 | 3.461418 | 5 |
| 205 | Gene NA - Prt: A0A075B7J0 | A0A075B 7J0 | -0.65449 | 0.15291 | 0.196919 | 2.635656 | 2 |
| 206 | TSPAN1 | F1S3V5 | 0.709392 | 0.160755 | 0.206016 | 4.832822 | 2 |
| 207 | PIGR | A0A287B 644 | -0.41692 | 0.16249 | 0.207233 | 3.942603 | 21 |
| 208 | CD109 | A0A5G2Q PT1 | -0.58239 | 0.163454 | 0.207461 | 3.34873 | 2 |
| 209 | VAMP3 | A0A5G2R 1F8 | 0.325182 | 0.172824 | 0.218304 | 2.71867 | 2 |
| 210 | LALBA | P18137 | 0.71311 | 0.182052 | 0.228866 | 4.058392 | 2 |
| 211 | HSPA/8/1B/6/ 1L | F1S9Q3;Q 6S4N2;Q0 4967;A5A 8V7 | 0.506597 | 0.185274 | 0.231812 | 4.239478 | 3 |

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| 212 | VPS4/A/B | I3LL27;A0 A287BA1 2 | -0.58048 | 0.207552 | 0.258462 | 3.75846 | 4 |
| 213 | CHMP2A | I3LSS2 | -0.44117 | 0.224114 | 0.277776 | 4.856903 | 8 |
| 214 | ANPEP | K7GMF9 | 0.38722 | 0.240604 | 0.29682 | 3.724721 | 37 |
| 215 | RAB10 | F2Z5F2 | 0.548762 | 0.247333 | 0.303702 | 5.116465 | 3 |
| 216 | ACT/B/G1 | Q6QAQ1;I 3LVD5 | 0.320812 | 0.249445 | 0.303817 | 3.531901 | 9 |
| 217 | ANXA11 | F1S2E2 | 0.353845 | 0.249728 | 0.303817 | 4.544183 | 9 |
| 218 | LY6H | A0A286Z QV1 | 0.47347 | 0.264379 | 0.320166 | 5.135652 | 2 |
| 219 | ARHGDI A | A0A287A QC2 | 0.289857 | 0.286778 | 0.345705 | 4.198994 | 8 |
| 224 | FGA | F1RX36 | -0.47319 | 0.297037 | 0.350079 | 3.361582 | 9 |
| 221 | IST1 | A0A287A 426 | -0.41877 | 0.295651 | 0.350079 | 4.034732 | 8 |
| 223 | CNP | A0A287A 9L8 | 0.296639 | 0.296957 | 0.350079 | 4.197055 | 4 |
| 220 | ACTB | Q6QAQ1 | 0.355729 | 0.295004 | 0.350079 | 3.635767 | 2 |
| 222 | PDCD6 | A0A287A KU6 | 0.436159 | 0.296417 | 0.350079 | 3.468693 | 4 |
| 225 | GNB/1/2 | A0A287A 7Q3;F2Z4 Z8 | 0.36053 | 0.320789 | 0.375805 | 4.30146 | 5 |
| 226 | IFITM/1/3 | A0A5G2Q WL4;A0A 287B7R8 | 0.440999 | 0.322589 | 0.375805 | 4.615226 | 4 |
| 227 | CHMP5 | F1SE87 | 0.567977 | 0.323135 | 0.375805 | 4.236682 | 2 |
| 228 | ARRDC1 | A0A286ZJ R9 | 0.246068 | 0.327908 | 0.379253 | 3.880001 | 4 |
| 229 | CLIC1 | A0A287B 2P1 | 0.338684 | 0.328973 | 0.379253 | 4.235843 | 9 |
| 230 | Gene NA - Prt: A0A5G2QN3 5 | A0A5G2Q N35 | 0.36409 | 0.356269 | 0.408935 | 3.774462 | 4 |
| 232 | C3// | F1SBS4;P 01025 | 0.228073 | 0.363336 | 0.413451 | 3.897809 | 18 |
| 231 | ANXA2 | P19620 | 0.250061 | 0.36314 | 0.413451 | 4.321955 | 12 |
| 233 | GNA13 | A0A287B C83 | -0.1966 | 0.383955 | 0.435039 | 3.59591 | 5 |
| 234 | VPS4B | A0A287B A12 | -0.33771 | 0.399504 | 0.450722 | 4.101608 | 8 |
| 236 | 14-3-3zeta | A0A480P LY3 | 0.196922 | 0.409297 | 0.457858 | 3.095345 | 6 |
| 235 | try | C6L245 | 0.278095 | 0.408403 | 0.457858 | 3.758574 | 3 |
| 237 | ABCG2 | Q8MIB3 | 0.328094 | 0.414802 | 0.462058 | 3.772382 | 12 |
| 238 | VAT1 | I3L9V2 | 0.210155 | 0.428257 | 0.475041 | 2.022158 | 7 |
| 239 | C4A | A0A5G2Q CL1 | 0.194068 | 0.470999 | 0.520266 | 3.140668 | 6 |
| 241 | CFL1 | P10668 | 0.247084 | 0.499054 | 0.546682 | 4.136279 | 6 |
| 240 | PGAM1 | A0A287AJ Q2 | 0.261435 | 0.497482 | 0.546682 | 2.992217 | 2 |
| 242 | RAB5A | Q06AU6 | 0.256079 | 0.511286 | 0.557766 | 2.725639 | 3 |
| 243 | PZP | A0A287B DU7 | -0.16932 | 0.521545 | 0.566616 | 2.407524 | 5 |
| 244 | GAPDH | P00355 | 0.230859 | 0.544144 | 0.588746 | 3.678831 | 4 |

| | | | | | | | |
|-----|-------------------------------------|--|----------|----------|----------|----------|----|
| 245 | RAP1B/A | A0A286Z VJ2;F2Z5 K3 | 0.167147 | 0.590438 | 0.636227 | 4.35698 | 9 |
| 246 | ACSL6 | A0A5G2Q 912 | 0.197649 | 0.627029 | 0.672909 | 3.649641 | 3 |
| 247 | FGG | F1RX35 | 0.124802 | 0.654233 | 0.699261 | 4.145301 | 8 |
| 248 | RAL/A/B | I3LAJ6;I3 LV17 | -0.2914 | 0.676877 | 0.720547 | 3.104039 | 4 |
| 249 | CHMP4B | F1S4Z2 | -0.13443 | 0.70163 | 0.743897 | 4.979629 | 2 |
| 250 | LOC10073916 3 | A0A287B Q81 | 0.113397 | 0.714954 | 0.754992 | 3.889458 | 4 |
| 251 | ADFP | D0G0B6 | -0.08051 | 0.735925 | 0.772909 | 4.142014 | 29 |
| 252 | EPS8 | I3LP99 | -0.07818 | 0.737777 | 0.772909 | 3.694047 | 9 |
| 253 | CRK | A0A5G2Q SR7 | 0.105652 | 0.743008 | 0.775312 | 4.007048 | 4 |
| 254 | MRAS | A0A5G2Q JB5 | 0.06939 | 0.762215 | 0.792223 | 3.061752 | 3 |
| 255 | EPS8L2 | A0A286Z VR9 | 0.073404 | 0.794483 | 0.822523 | 3.676723 | 6 |
| 257 | TACSTD1 | Q75QW1 | -0.1118 | 0.84806 | 0.871159 | 4.620366 | 3 |
| 256 | CHMP1A | I3LU89 | -0.07755 | 0.847903 | 0.871159 | 4.261307 | 4 |
| 258 | BROX | A0A287A AM4 | -0.057 | 0.857751 | 0.877699 | 3.516861 | 8 |
| 259 | FGL2 | F6Q194 | -0.03733 | 0.895327 | 0.912611 | 3.24415 | 3 |
| 260 | VPS4A | I3LL27 | -0.03933 | 0.902852 | 0.916742 | 3.815098 | 4 |
| 261 | A2M | A0A5G2Q 8I9 | -0.03327 | 0.915692 | 0.926218 | 2.536434 | 9 |
| 262 | ACTG1 | I3LVD5 | 0.047854 | 0.930211 | 0.937312 | 3.067914 | 2 |
| 263 | CEACAM1 | I3L9E5 | 0.026414 | 0.941503 | 0.945083 | 4.408131 | 7 |
| 264 | RAP1A | F2Z5K3 | -0.01195 | 0.968232 | 0.968232 | 3.470279 | 3 |
| 265 | PDCD6// | A0A5G2Q CX9;A0A 287AKU6 | -1.67925 | | | 3.582214 | 2 |
| 266 | FLOT2 | A0A480T GV2 | -1.03469 | | | 4.538231 | 4 |
| 267 | SLC3A2 | A0A5G2R CQ0 | -0.95781 | | | 3.103297 | 3 |
| 268 | Gene NA - Prt: A0A286ZIM1 | A0A286ZI M1 | -0.82395 | | | 3.68489 | 2 |
| 269 | HSPA1B | Q6S4N2 | -0.69848 | | | 4.982824 | 2 |
| 270 | RAB5A;CDK 2;RAB5C | Q06AU6; A0A286Z S27;A0A2 87BN36 | -0.54691 | | | 4.325362 | 2 |
| 271 | RALB | I3LV17 | -0.48541 | | | 4.330499 | 2 |
| 272 | SAR1A | A0A5S6G 7K0 | -0.32045 | | | 3.514933 | 2 |
| 273 | SEMA7A// | I3LC80;I3 LQF4 | -0.31968 | | | 3.204831 | 7 |
| 274 | Gene NA - Prt: A0A5G2QXC 1 | A0A5G2Q XC1 | -0.2875 | | | 4.693061 | 2 |
| 275 | BSG | A0A286Z S77 | -0.16315 | | | 4.53726 | 2 |
| 276 | COL18A1 | I3LH70 | -0.10653 | | | 4.222196 | 4 |

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|-----|---------|-------------------------------|----------|----------|---|
| 277 | RAB27B | A0A286Z TH5 | -0.02048 | 3.929521 | 4 |
| 278 | SOD1 | P04178 | -0.01406 | 4.882674 | 3 |
| 279 | TMEM19 | A0A287A 4W6 | 0.065255 | 3.568603 | 2 |
| 280 | PTGFRN | F1SAY0 | 0.082311 | 3.232651 | 2 |
| 281 | PTPRJ | A0A287A WI4 | 0.084169 | 3.254381 | 2 |
| 282 | PEBP1 | F1RKG8 | 0.092333 | 4.257097 | 4 |
| 283 | YKT6 | F1SSG1 | 0.153408 | 4.286261 | 3 |
| 284 | SLA-2 | A0A287A QG3 | 0.171871 | 4.682111 | 2 |
| 285 | LDHB | A0A5G2Q HL7 | 0.226899 | 4.244291 | 2 |
| 286 | LBP | A0A287B 028 | 0.240003 | 3.437749 | 4 |
| 287 | SCAMP2 | A0A5G2R 660 | 0.269796 | 3.410068 | 2 |
| 288 | CDC42// | Q007T2;F 2Z5W2 | 0.293609 | 3.629216 | 4 |
| 289 | ACTA1 | A0A5S6G 831 | 0.350123 | 2.871411 | 3 |
| 290 | NIT2 | A0A5G2R GN4 | 0.38321 | 4.490943 | 2 |
| 291 | NAPA | F1RLB5 | 0.399539 | 4.338879 | 3 |
| 292 | VPS28 | K7GM88 | 0.405172 | 3.844972 | 4 |
| 293 | SLA-2// | A0A287A EH7;A0A2 87AQG3 | 0.406926 | 3.42832 | 3 |
| 294 | SLC44A2 | A0A5G2Q TD4 | 0.415279 | 4.523337 | 6 |
| 295 | ANXA4 | A0A287A YJ2 | 0.559065 | 3.656262 | 2 |
| 296 | PRSS8 | A0A5G2Q SK0 | 0.569814 | 3.307351 | 3 |
| 297 | SCARB1 | Q8SQC1 | 0.570655 | 3.984991 | 3 |
| 298 | EHD4 | I3LDA5 | 0.584663 | 3.530621 | 3 |
| 299 | NCSTN | A0A286Z K33 | 0.609611 | 4.984004 | 3 |
| 300 | PGD | A0A5G2Q R34 | 0.656442 | 4.105394 | 2 |
| 301 | CYSTM1 | A0A287A UM3 | 0.672297 | 4.740443 | 2 |
| 302 | TXN | P82460 | 0.70453 | 2.914253 | 3 |
| 303 | AK1 | A0A286Z Q79 | 0.732717 | 5.301423 | 2 |
| 304 | NME2 | Q2EN76 | 0.740228 | 4.570478 | 4 |
| 305 | ANXA5 | F2Z5C1 | 0.790246 | 3.397106 | 2 |
| 306 | HSD17B7 | A0A5G2Q GH2 | 0.795076 | 3.386419 | 2 |
| 307 | VPS25 | A0A287A AM6 | 0.933669 | 4.546764 | 4 |
| 308 | IDI1 | I3LDY2 | 1.275815 | 2.701298 | 2 |
| 309 | CAB39 | F1SMX5 | 1.408023 | 3.965521 | 3 |
| 310 | SRM | I3LPB8 | 2.028283 | 4.429186 | 3 |
| 317 | GNB2 | F2Z4Z8 | | 2.838204 | 2 |

| | | | | | | | |
|------------|----------|--------|--|--|--|----------|---|
| 318 | OLFML3 | H6UWK6 | | | | 3.489077 | 2 |
| 319 | SERPINA1 | P50447 | | | | 2.66335 | 2 |

Table A2-2. List of all proteins identified by LC-MS/MS maintained for statistical analysis for analysis on comparison between colostrum exosomes (day 0) and milk exosomes at day 14, ranked by significance.

| Rank | Gene | Protein | logFC | P Value | Adjusted P Value | Average expression | Peptide count |
|-------------|--|---|--------------|----------------|-------------------------|---------------------------|----------------------|
| 1 | CD36 | Q3HUX1 | 2.787974 | 3.24E-11 | 7.41E-09 | 4.671275 | 24 |
| 2 | APOE | P18650 | -3.17454 | 5.62E-11 | 7.41E-09 | 4.580658 | 8 |
| 3 | HAPLN3 | A0A5G2R305 | -2.35313 | 1.26E-10 | 1.11E-08 | 3.833653 | 20 |
| 4 | Gene NA - Prt: A0A5G2R4L0 | A0A5G2R4L0 | -2.91948 | 2.99E-10 | 1.93E-08 | 4.125335 | 3 |
| 5 | VTN | P48819 | -2.67255 | 3.66E-10 | 1.93E-08 | 4.276618 | 7 |
| 6 | APOE// | F1RM45;P18650 | -2.60378 | 4.86E-10 | 2.14E-08 | 3.62444 | 28 |
| 7 | SIL1 | F1RGI9 | -2.1956 | 6.15E-10 | 2.32E-08 | 3.792533 | 8 |
| 8 | MGP | A0A5G2QK11 | -2.85145 | 7.15E-10 | 2.36E-08 | 4.000976 | 10 |
| 9 | CSN3 | P11841 | -2.18434 | 2.25E-09 | 6.6E-08 | 3.436882 | 27 |
| 10 | Gene NA - Prt: A0A0A0MY58 | A0A0A0MY58 | -2.18735 | 4.84E-09 | 1.28E-07 | 4.034749 | 19 |
| 11 | CSN1S1 | P39035 | -2.36836 | 1.61E-08 | 3.87E-07 | 3.239364 | 31 |
| 12 | THBS1 | F1SS26 | -2.07583 | 1.94E-08 | 4.27E-07 | 3.711806 | 41 |
| 13 | Gene NA - Prt: A0A075B7I9;A0A075B7H9;A0A075B7J0;A0A075B7I5 | A0A075B7I9;A0A075B7H9;A0A075B7J0;A0A075B7I5 | -2.70424 | 2.39E-08 | 4.85E-07 | 5.663481 | 4 |
| 14 | LPL | A0A287ATV6 | -2.11343 | 2.6E-08 | 4.9E-07 | 3.338335 | 15 |
| 15 | FBLN2 | A0A287B5Q1 | -2.52406 | 3.07E-08 | 5.39E-07 | 3.6693 | 10 |
| 16 | CSN1S2 | P39036 | -2.21227 | 3.52E-08 | 5.8E-07 | 3.715798 | 39 |
| 17 | Gene NA - Prt: A0A287BAB3 | A0A287BAB3 | -2.32708 | 4.79E-08 | 7.24E-07 | 4.473881 | 4 |
| 18 | TINAGL1 | F1SVA2 | -3.03646 | 5.16E-08 | 7.24E-07 | 4.590614 | 3 |
| 19 | CHID1 | F1RZ01 | -1.98956 | 5.21E-08 | 7.24E-07 | 3.185273 | 11 |
| 20 | APOA1 | K7GM40 | -2.06713 | 8.33E-08 | 1.1E-06 | 3.357979 | 30 |
| 21 | GANAB | P79403 | -1.94385 | 8.96E-08 | 1.13E-06 | 3.357891 | 24 |
| 22 | Gene NA - Prt: P10859 | P10859 | -2.0488 | 1.09E-07 | 1.31E-06 | 3.716168 | 2 |

| | | | | | | | |
|----|---|--|----------|----------|----------|----------|----|
| 23 | CLU | A0A5S8K LN1 | -1.63238 | 1.89E-07 | 2.16E-06 | 3.847397 | 27 |
| 24 | Gene NA - Prt: A0A5G2Q XT5 | A0A5G2Q XT5 | -2.07965 | 2.23E-07 | 2.45E-06 | 3.449775 | 5 |
| 25 | CD5L | F1RN76 | -2.16757 | 2.64E-07 | 2.79E-06 | 4.184892 | 9 |
| 26 | Gene NA - Prt: A0A287A LJ6 | A0A287A LJ6 | -1.54779 | 2.89E-07 | 2.88E-06 | 4.016404 | 4 |
| 27 | CP | A0A5G2Q W05 | -1.91726 | 2.95E-07 | 2.88E-06 | 3.659874 | 11 |
| 28 | Gene NA - Prt: A0A287A TT2 | A0A287A TT2 | -2.51536 | 3.48E-07 | 3.04E-06 | 3.373407 | 3 |
| 29 | CHRD2// | A0A5G2Q XF8;A0A5 G2RH74 | -1.92443 | 3.55E-07 | 3.04E-06 | 3.581001 | 8 |
| 30 | ACSL3 | A0A287B 8R8 | 1.680737 | 3.64E-07 | 3.04E-06 | 4.195918 | 41 |
| 31 | Gene NA - Prt: A0A287A P28;A0A2 86ZIM1;A 0A287BA B3 | A0A287A P28;A0A2 86ZIM1;A 0A287BA B3 | -3.26085 | 3.69E-07 | 3.04E-06 | 3.773167 | 3 |
| 32 | C1QA | Q69DL0 | -2.49001 | 3.76E-07 | 3.04E-06 | 4.078393 | 2 |
| 33 | RAB18 | I3LC07 | 1.551818 | 3.8E-07 | 3.04E-06 | 4.32985 | 11 |
| 34 | Gene NA - Prt: A0A287A LC1 | A0A287A LC1 | -1.70228 | 4.17E-07 | 3.24E-06 | 3.088551 | 68 |
| 35 | PGLYRP1 | A0A286ZI 97 | -2.04348 | 4.44E-07 | 3.34E-06 | 3.716147 | 14 |
| 36 | SERPINA 3-2 | A0A287B 9R5 | -2.13211 | 4.55E-07 | 3.34E-06 | 4.440938 | 5 |
| 37 | JCHAIN | A0A287B QC8 | -2.2442 | 4.93E-07 | 3.52E-06 | 4.707291 | 2 |
| 38 | SERPINC 1 | A0A5G2Q UE0 | -1.65629 | 5.55E-07 | 3.86E-06 | 3.234506 | 9 |
| 39 | SMPDL3B // | A0A287BJ W1;F1ST N0 | 1.845312 | 5.78E-07 | 3.87E-06 | 3.57818 | 10 |
| 40 | MUC4 | A0A287B 5M2 | 1.799223 | 5.87E-07 | 3.87E-06 | 3.497925 | 23 |
| 41 | ATP6AP2 | A0A287AJ K1 | -1.7957 | 6.11E-07 | 3.93E-06 | 3.929251 | 5 |
| 42 | Gene NA - Prt: A0A075B 7I6 | A0A075B 7I6 | -1.89857 | 7.55E-07 | 4.74E-06 | 3.55741 | 2 |
| 43 | DDR1 | K7GSX5 | -1.84211 | 9.2E-07 | 5.65E-06 | 4.792415 | 2 |
| 44 | GSN | A0A287A 6P1 | -1.39196 | 1.2E-06 | 7.2E-06 | 3.579003 | 19 |
| 45 | SERPINF1 | A0A287A YJ8 | -1.49888 | 1.39E-06 | 8E-06 | 3.303514 | 10 |
| 46 | FABP3 | O02772 | 1.617887 | 1.39E-06 | 8E-06 | 4.383504 | 22 |

| | | | | | | | |
|----|-------------------------------------|----------------------------------|----------|----------|----------|----------|----|
| 47 | Gene NA - Prt: A0A287A 4Y3 | A0A287A 4Y3 | -1.67471 | 1.58E-06 | 8.9E-06 | 4.354942 | 13 |
| 48 | ITIH2 | A0A5G2Q EV5 | -1.61108 | 1.87E-06 | 1.03E-05 | 3.574539 | 7 |
| 49 | ALB | A0A287B AY9 | -1.41839 | 2.01E-06 | 1.08E-05 | 3.830738 | 18 |
| 50 | NA;LOC1 00523213 | A0A0A0M Y58;F1RL 06 | -1.57585 | 2.59E-06 | 1.37E-05 | 3.985018 | 6 |
| 51 | RAB1A | F2Z5U4 | 1.414846 | 3.73E-06 | 1.93E-05 | 4.315186 | 9 |
| 52 | PPIB | A0A286Z KG9 | -1.50136 | 3.91E-06 | 1.98E-05 | 3.286902 | 3 |
| 53 | PAPLN | F1S3J7 | -1.74303 | 4.35E-06 | 2.16E-05 | 3.598821 | 4 |
| 54 | Gene NA - Prt: A0A5S6G 633 | A0A5S6G 633 | -2.59191 | 4.46E-06 | 2.18E-05 | 2.838299 | 3 |
| 55 | TTR | A0A5G2Q IE9 | -1.56841 | 4.9E-06 | 2.35E-05 | 3.478735 | 3 |
| 56 | MYOF | A0A5G2Q 8T2 | 1.27693 | 5.54E-06 | 2.61E-05 | 3.989779 | 30 |
| 57 | LTF | A0A5G2Q UQ4 | -1.46424 | 7.29E-06 | 3.38E-05 | 3.41711 | 26 |
| 58 | RAB1A/R AB1B | F2Z5U4;Q 06AU7 | 1.458813 | 7.68E-06 | 3.46E-05 | 3.36718 | 6 |
| 59 | NA;CSN1 S1 | A0A5S6G 633;P3903 5 | -1.49164 | 7.73E-06 | 3.46E-05 | 3.32349 | 13 |
| 60 | APOE | F1RM45 | -1.53219 | 1.08E-05 | 4.76E-05 | 3.395127 | 2 |
| 61 | CSN2// | P39037;A0 A2C9F376 | -1.51269 | 1.35E-05 | 5.86E-05 | 2.792655 | 68 |
| 62 | GPRC5C | A0A287A SV2 | 1.273678 | 1.45E-05 | 6.19E-05 | 4.592204 | 5 |
| 63 | RDX;MSN ;EZR | P26044;A0 A287AK1 9;F1SB42 | 1.620933 | 1.51E-05 | 6.34E-05 | 4.397996 | 10 |
| 64 | Gene NA - Prt: A0A287B 626 | A0A287B 626 | -1.64572 | 1.64E-05 | 6.78E-05 | 3.970228 | 29 |
| 65 | LOC10030 2368 | A0A287B 5W2 | 1.238396 | 1.76E-05 | 7.16E-05 | 4.415311 | 34 |
| 66 | B4GALT1 | A0A287B G16 | -1.68319 | 2.02E-05 | 8.09E-05 | 3.320653 | 20 |
| 67 | ATP6AP1 | A0A5G2R D35 | -1.27672 | 2.18E-05 | 8.59E-05 | 3.264964 | 4 |
| 68 | TSPAN6 | F1S1M4 | 1.382491 | 2.36E-05 | 9.15E-05 | 4.167684 | 4 |
| 69 | SLC34A2 | F1S5A6 | 1.447573 | 2.49E-05 | 9.55E-05 | 4.131015 | 66 |
| 70 | KRT75 | F1SGI7 | 1.931457 | 2.57E-05 | 9.68E-05 | 4.699503 | 2 |
| 71 | C9 | A0A287B H90 | -1.66305 | 3.44E-05 | 0.000128 | 3.484615 | 3 |
| 72 | XDH | F1S3Y7 | 1.344054 | 3.49E-05 | 0.000128 | 4.814699 | 66 |
| 73 | BTN1A1 | A0A5K1U 1P1 | 1.332199 | 4.28E-05 | 0.000155 | 4.268103 | 61 |
| 74 | FUCA1 | I3LNS9 | -1.44575 | 4.57E-05 | 0.000161 | 3.774814 | 2 |
| 75 | RDX;EZR | P26044;F1 SB42 | 2.334737 | 4.6E-05 | 0.000161 | 4.44116 | 2 |

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|-----|--|-------------------------------|----------|----------|----------|----------|----|
| 76 | FASN | I3LCW1 | 1.532428 | 4.64E-05 | 0.000161 | 3.770177 | 4 |
| 77 | BAIAP2// | A0A287B RR9;F1RZ B0 | 1.501731 | 6.07E-05 | 0.000208 | 4.191112 | 16 |
| 78 | NA;FABP 3 | I3LTW5;O 02772 | 2.808464 | 6.19E-05 | 0.000209 | 6.001107 | 2 |
| 79 | CIDEA | A0A5G2R 9X9 | 1.436374 | 6.3E-05 | 0.000211 | 4.788696 | 3 |
| 80 | FGB | A0A5G2R HA4 | -1.98563 | 6.39E-05 | 0.000211 | 2.991589 | 7 |
| 81 | TTYH2 | A0A287A AJ2 | 1.251632 | 6.53E-05 | 0.000213 | 5.144515 | 8 |
| 82 | HSPA5 | A0A287BI L8 | -1.14988 | 6.72E-05 | 0.000216 | 2.279501 | 4 |
| 83 | PGK1 | F1RPH0 | 1.226537 | 7.56E-05 | 0.00024 | 4.45063 | 4 |
| 84 | FMOD | F1S6B5 | -1.97912 | 8.08E-05 | 0.000254 | 2.122226 | 5 |
| 85 | TF | A0A5G2R 5N9 | -1.54338 | 9.1E-05 | 0.000283 | 4.641461 | 2 |
| 86 | MATN4 | F1SDQ7 | -1.69833 | 0.0001 | 0.000308 | 2.441729 | 2 |
| 87 | SLC9A3R 1 | A0A5G2R 543 | 1.495403 | 0.000108 | 0.000326 | 4.747528 | 16 |
| 88 | ARF/3/4 | A0A287B 8I6;A0A5 G2R3T3 | 1.139308 | 0.000134 | 0.000403 | 3.238186 | 4 |
| 89 | Gene NA - Prt: A0A075B 7H9 | A0A075B 7H9 | -1.15795 | 0.000159 | 0.00047 | 3.212066 | 4 |
| 90 | Gene NA - Prt: A0A5G2R 9S9;A0A2 87ALJ6 | A0A5G2R 9S9;A0A2 87ALJ6 | -1.74858 | 0.00016 | 0.00047 | 3.153838 | 2 |
| 91 | COL4A2 | A0A5G2R J53 | -1.55004 | 0.000163 | 0.000472 | 3.017329 | 3 |
| 92 | ANXA6 | A0A5G2R BI3 | 1.178973 | 0.000167 | 0.000479 | 4.400298 | 6 |
| 93 | SOD3 | I3LUD1 | -1.19672 | 0.00018 | 0.00051 | 3.206342 | 2 |
| 94 | RAB3D | A0A287B LN4 | 1.110317 | 0.000187 | 0.000525 | 3.960266 | 4 |
| 95 | PPIA | P62936 | 1.054179 | 0.000233 | 0.000646 | 4.659886 | 14 |
| 96 | SERPIND 1 | F1RKY2 | -1.74799 | 0.000245 | 0.000675 | 3.397077 | 7 |
| 97 | Gene NA - Prt: I3LTW5 | I3LTW5 | 2.132129 | 0.000285 | 0.000774 | 3.116072 | 2 |
| 98 | GPRC5A | I3LN87 | -1.78933 | 0.000343 | 0.000919 | 2.411634 | 2 |
| 99 | PROM2 | F1SU61 | 0.991174 | 0.000345 | 0.000919 | 3.750727 | 2 |
| 100 | KRT1 | F1SGG3 | 1.193279 | 0.000395 | 0.001042 | 5.116696 | 9 |
| 101 | CDC42 | Q007T2 | 1.053134 | 0.000404 | 0.001056 | 1.875831 | 2 |
| 102 | PAEP | A0A5S6H Q91 | -1.17835 | 0.000432 | 0.001119 | 3.980301 | 12 |
| 103 | FGA | F1RX36 | -1.74174 | 0.000476 | 0.001219 | 3.361582 | 9 |
| 104 | TM4SF1 | A0A5G2Q C13 | -1.20096 | 0.00051 | 0.001294 | 3.680237 | 2 |
| 105 | DAG1 | A0A287B BR1 | -1.21693 | 0.000533 | 0.001341 | 2.410085 | 2 |

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|-----|-------------------------------------|---------------------------|----------|----------|----------|----------|----|
| 106 | SPON2 | A0A481A ED0 | -1.51264 | 0.000547 | 0.001363 | 3.364732 | 4 |
| 107 | RAB11A | A0A5G2Q E74 | 1.376893 | 0.000748 | 0.001844 | 4.928738 | 6 |
| 108 | MUC1// | A0A5G2Q GF8;F1RG R9 | 1.406629 | 0.000772 | 0.001888 | 4.559646 | 9 |
| 109 | ANPEP | K7GMF9 | 1.203276 | 0.000794 | 0.001923 | 3.724721 | 37 |
| 110 | PLOD1 | F1RF77 | -1.11114 | 0.000844 | 0.002025 | 3.585215 | 3 |
| 111 | ST13 | F1SRB9 | 1.20013 | 0.000879 | 0.00209 | 4.607381 | 3 |
| 112 | RAC2 | A0A5G2R 6S0 | 1.163842 | 0.000968 | 0.002281 | 4.062857 | 2 |
| 113 | CKB | A0A5G2R 6X7 | 0.93028 | 0.001054 | 0.002463 | 4.131502 | 7 |
| 114 | MSTN | O18831 | -1.05346 | 0.00114 | 0.002639 | 2.954647 | 10 |
| 115 | CPM | I3LTE1 | 1.161156 | 0.001278 | 0.002934 | 4.389205 | 28 |
| 116 | RHOA | I3LVS7 | 1.600098 | 0.001421 | 0.003234 | 5.26511 | 5 |
| 117 | RAB35 | A0A287B FE6 | 0.968397 | 0.001575 | 0.003553 | 4.809324 | 5 |
| 118 | STX3 | F1RI88 | 0.996383 | 0.002305 | 0.005156 | 3.589462 | 9 |
| 119 | MUC1 | F1RGR9 | 1.642195 | 0.002362 | 0.005182 | 3.142075 | 3 |
| 120 | FASN | I3LC73 | 1.014165 | 0.002375 | 0.005182 | 3.94284 | 17 |
| 121 | KRAS | I3LCQ9 | 1.003446 | 0.002375 | 0.005182 | 3.835119 | 3 |
| 122 | try | C6L245 | 1.095114 | 0.002433 | 0.005265 | 3.758574 | 3 |
| 123 | EZR | F1SB42 | 1.295345 | 0.002474 | 0.00531 | 3.956502 | 22 |
| 124 | KRT10 | I3LDS3 | 0.882225 | 0.002594 | 0.005522 | 4.41125 | 5 |
| 125 | AEBP1 | A0A286Z ZM9 | -0.67377 | 0.002732 | 0.005771 | 3.833016 | 4 |
| 126 | MSN | A0A287A K19 | 1.066692 | 0.002829 | 0.005912 | 4.993424 | 19 |
| 127 | TLR2 | Q59HI8 | 0.979083 | 0.00286 | 0.005912 | 4.359128 | 26 |
| 128 | GPI | P08059 | 0.814504 | 0.002866 | 0.005912 | 4.692694 | 4 |
| 129 | PRDX5 | A0A5G2R 9D6 | 0.793333 | 0.002945 | 0.006027 | 3.401712 | 6 |
| 130 | KRT5 | A0A287A ZL3 | 1.016064 | 0.002989 | 0.006069 | 3.506331 | 3 |
| 131 | HSPA/1B/ 6 | Q6S4N2;Q 04967 | 0.875539 | 0.003294 | 0.006639 | 5.257768 | 3 |
| 132 | PPIC | A0A5G2R B35 | -1.11432 | 0.003437 | 0.006874 | 2.948777 | 4 |
| 133 | ERAP1 | K7GLU6 | -0.89184 | 0.00359 | 0.007127 | 2.754582 | 6 |
| 134 | PDXK | O46560 | 1.090229 | 0.003968 | 0.007817 | 3.521722 | 3 |
| 135 | SELENBP 1 | F1ST01 | 0.749338 | 0.004023 | 0.007868 | 3.456086 | 2 |
| 136 | CD82 | A0A5G2Q KD8 | 0.706357 | 0.004197 | 0.008146 | 4.252814 | 8 |
| 137 | PDCD6IP | A0A287A 5B4 | 1.016596 | 0.005578 | 0.010749 | 4.405182 | 30 |
| 138 | UBC | A0A5G2Q DJ2 | 0.756449 | 0.005741 | 0.010982 | 4.733632 | 12 |
| 139 | Gene NA - Prt: A0A287A 7S8 | A0A287A 7S8 | -0.77892 | 0.006085 | 0.011557 | 3.275506 | 2 |
| 140 | RRAS | F1RHW4 | 1.027646 | 0.006377 | 0.012026 | 4.486447 | 3 |

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| 141 | DNAJC3 | I3LBK9 | -1.2679 | 0.006537 | 0.01224 | 3.527757 | 2 |
| 142 | SAA3 | A0A480K NV0 | -1.21728 | 0.006826 | 0.012671 | 5.54745 | 2 |
| 143 | RHOF | Q06AT7 | 1.111703 | 0.006863 | 0.012671 | 5.064607 | 2 |
| 144 | PARK7 | A0A5G2Q IK1 | 1.349727 | 0.007231 | 0.013257 | 5.159512 | 2 |
| 145 | FLOT1 | Q767L6 | -0.86194 | 0.007314 | 0.013316 | 3.905262 | 6 |
| 146 | RAB7A | A0A286Z P69 | 0.792978 | 0.007608 | 0.013756 | 4.306068 | 9 |
| 147 | ARF3 | A0A287B 8I6 | 0.980421 | 0.009113 | 0.016367 | 4.867116 | 4 |
| 148 | ATP2B2 | A0A286Z SF2 | 0.649128 | 0.009257 | 0.016485 | 4.769486 | 7 |
| 149 | GALM | A0A5G2Q Z46 | 0.756429 | 0.009304 | 0.016485 | 3.897957 | 4 |
| 150 | Gene NA - Prt: A0A5G2Q 7F8 | A0A5G2Q 7F8 | -1.32455 | 0.009492 | 0.016706 | 2.486988 | 2 |
| 151 | PIGR | A0A287B 644 | -0.7977 | 0.010154 | 0.017753 | 3.942603 | 21 |
| 152 | RAB2A/R AB2B | F1RT87;F 1S8J6 | 0.836642 | 0.011173 | 0.019406 | 3.736955 | 3 |
| 153 | MFGE8 | A0A5G2R KJ7 | 0.684264 | 0.011314 | 0.019522 | 5.037166 | 11 |
| 154 | GDI2 | A0A5G2Q SK4 | 0.682434 | 0.012058 | 0.020671 | 3.627553 | 15 |
| 155 | HEXA | F1SI88 | -0.61733 | 0.014573 | 0.024822 | 2.413714 | 4 |
| 156 | VWA2 | F1S5H3 | -0.62055 | 0.014891 | 0.0252 | 3.350119 | 7 |
| 157 | TSPAN1 | F1S3V5 | 1.271756 | 0.015365 | 0.025837 | 4.832822 | 2 |
| 158 | C3// | F1SBS4;P 01025 | -0.62601 | 0.016605 | 0.027745 | 3.897809 | 18 |
| 159 | MFGE8// | A0A5G2R KJ7;A0A5 G2QXS8 A0A480P LY3;F2Z4 | 0.664273 | 0.016896 | 0.028054 | 4.588095 | 105 |
| 160 | 14-3- 3zeta;YW HAH;YW HAQ;CRK ;YWHAB | Y1;A0A5 G2QM17; A0A5G2Q SR7;A0A2 87BPX7 | 0.979989 | 0.018858 | 0.031116 | 5.657113 | 2 |
| 161 | MDH1 | A0A5G2Q FC2 | 0.606665 | 0.019589 | 0.03212 | 4.533037 | 4 |
| 162 | RDX | P26044 | 0.692097 | 0.021007 | 0.034234 | 3.883106 | 5 |
| 163 | PIGR// | A0A287B 644;Q2924 4 | -0.79823 | 0.021531 | 0.034873 | 3.790705 | 5 |
| 164 | Gene NA - Prt: A0A5G2Q N35 | A0A5G2Q N35 | 0.932155 | 0.022708 | 0.036554 | 3.774462 | 4 |
| 165 | KRT1/4/0 | A0A287A EL2;I3LD S3 | 0.913294 | 0.023227 | 0.037163 | 5.18532 | 3 |
| 166 | ARHGAP 18 | A0A287B HC4 | 0.63425 | 0.024701 | 0.039284 | 4.917479 | 4 |
| 167 | GPRC5B | A0A480W 2U9 | 0.679624 | 0.025105 | 0.039686 | 5.240895 | 5 |

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|-----|--|--|----------|----------|----------|----------|----|
| 168 | IFITM1;IF ITM3;LO C1021614 18 | A0A5G2Q WL4;A0A 287B7R8; F1RGC5 | 0.638931 | 0.026623 | 0.041836 | 3.46739 | 4 |
| 169 | Gene NA - Prt: A0A075B 7J0 | A0A075B 7J0 | -0.94126 | 0.026907 | 0.042033 | 2.635656 | 2 |
| 170 | ENO1 | I3LK59 | 0.612814 | 0.028254 | 0.043876 | 4.114588 | 7 |
| 171 | RALA | I3LAJ6 | 0.899265 | 0.032977 | 0.050912 | 4.085954 | 5 |
| 172 | CD109 | A0A5G2Q PT1 | -0.87241 | 0.033406 | 0.051274 | 3.34873 | 2 |
| 173 | SDCBP | A0A286Z KH9 | 0.595325 | 0.0363 | 0.055395 | 3.258615 | 11 |
| 174 | ABCG2 | Q8MIB3 | -0.85362 | 0.039418 | 0.059768 | 3.772382 | 12 |
| 175 | RAB2A | F1RT87 | 0.490576 | 0.039619 | 0.059768 | 4.826399 | 4 |
| 176 | EEF1A1 | A0A288C G57 | 0.665873 | 0.040636 | 0.060954 | 5.317222 | 10 |
| 177 | CD9 | Q8WMQ3 | 1.142423 | 0.04391 | 0.065493 | 5.56161 | 4 |
| 178 | VTA1 | F1S6Z8 | -0.83663 | 0.045295 | 0.06718 | 3.461418 | 5 |
| 179 | SRC | K7GM45 | 0.534227 | 0.046145 | 0.068058 | 4.3238 | 6 |
| 180 | MVB12A | A0A287A P77 | 0.882353 | 0.047407 | 0.06953 | 4.637288 | 4 |
| 181 | SAA3// | A0A480K NV0;A0A 5G2R2X7 | 0.591138 | 0.047699 | 0.069572 | 4.899096 | 7 |
| 182 | ALS | Q1KS52 | -0.61632 | 0.048474 | 0.070314 | 1.561597 | 4 |
| 183 | SLC6A14 | K7GNX7 | 0.63026 | 0.048926 | 0.070582 | 4.229918 | 13 |
| 184 | CLIC1 | A0A287B 2P1 | 0.691502 | 0.051546 | 0.073646 | 4.235843 | 9 |
| 185 | APOA4 | A0A287B M29 | -0.53258 | 0.051608 | 0.073646 | 3.986276 | 7 |
| 186 | HSPA8 | F1S9Q3 | 0.517331 | 0.052357 | 0.074313 | 3.712596 | 22 |
| 187 | ARRDC1 | A0A286ZJ R9 | 0.489955 | 0.056722 | 0.080078 | 3.880001 | 4 |
| 188 | Gene NA - Prt: A0A5G2R 9S9 | A0A5G2R 9S9 | -0.77875 | 0.060096 | 0.08439 | 3.738216 | 2 |
| 189 | CD209 | B3FVQ2 | 0.540163 | 0.060622 | 0.084423 | 4.766564 | 2 |
| 190 | VAMP3 | A0A5G2R 1F8 | 0.44183 | 0.060759 | 0.084423 | 2.71867 | 2 |
| 191 | FGG | F1RX35 | -0.52766 | 0.06513 | 0.090023 | 4.145301 | 8 |
| 192 | GNB1/2 | A0A287A 7Q3;F2Z4 Z8 | 0.681641 | 0.065727 | 0.090375 | 4.30146 | 5 |
| 193 | SERINC5 | F1RF26 | -0.57897 | 0.067614 | 0.092487 | 5.354037 | 2 |
| 194 | LY6H | A0A286Z QV1 | -0.78056 | 0.070385 | 0.095782 | 5.135652 | 2 |
| 195 | PFN1 | A0A5G2R 9A9 | 0.538192 | 0.072088 | 0.097596 | 3.252424 | 4 |
| 196 | TACSTD1 | Q75QW1 | 1.038773 | 0.075865 | 0.102185 | 4.620366 | 3 |
| 197 | ACLY | F1SON2 | 0.468321 | 0.086003 | 0.114861 | 2.983594 | 4 |
| 198 | VAT1 | I3L9V2 | 0.449533 | 0.086146 | 0.114861 | 2.022158 | 7 |
| 199 | SLC29A1 | A0A287B 8K5 | 0.632019 | 0.087064 | 0.115502 | 4.707259 | 6 |

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| 200 | TMBIM1 | A0A5G2R7N2 | 0.657117 | 0.095796 | 0.126451 | 5.668077 | 2 |
| 201 | STXBP2 | A0A5G2QA44 | 0.537629 | 0.096803 | 0.127144 | 4.857438 | 11 |
| 202 | ACT/A1/B/G1 | A0A5S6G831;Q6QAQ1;I3LVD5 | 0.573716 | 0.099694 | 0.130294 | 4.611775 | 18 |
| 203 | ACTB | Q6QAQ1 | 0.543499 | 0.113751 | 0.147932 | 3.635767 | 2 |
| 204 | CHMP4B | F1S4Z2 | -0.54918 | 0.115173 | 0.149048 | 4.979629 | 2 |
| 205 | VPS4/A/B | I3LL27;A0A287BA12 | -0.71497 | 0.124202 | 0.159948 | 3.75846 | 4 |
| 206 | CD63 | F1SPK8 | 0.492295 | 0.127383 | 0.163249 | 4.220068 | 3 |
| 207 | MUC15 | F1SFZ5 | 0.540082 | 0.133473 | 0.169587 | 4.71967 | 9 |
| 208 | PLIN3 | K4P7U5 | 0.475927 | 0.133614 | 0.169587 | 4.318163 | 12 |
| 209 | CDK2 | A0A286ZS27 | 0.4326 | 0.136697 | 0.172669 | 3.908768 | 4 |
| 210 | RAB14 | Q52NJ6 | 0.550711 | 0.138587 | 0.173917 | 4.846186 | 2 |
| 211 | VPS37B | F1REY0 | 0.414788 | 0.139002 | 0.173917 | 3.943706 | 7 |
| 212 | IFITM/1/3 | A0A5G2QWL4;A0A287B7R8 | 0.646385 | 0.150739 | 0.187713 | 4.615226 | 4 |
| 213 | VPS4B | A0A287BA12 | -0.57213 | 0.157858 | 0.195655 | 4.101608 | 8 |
| 214 | 14-3-3zeta | A0A480PLY3 | 0.331055 | 0.1696 | 0.208864 | 3.095345 | 6 |
| 215 | PZP | A0A287BDU7 | -0.36682 | 0.170098 | 0.208864 | 2.407524 | 5 |
| 216 | PON1 | I3L5K0 | -0.73345 | 0.183737 | 0.224568 | 2.249963 | 4 |
| 217 | HSPA/8/1B/6/1L | F1S9Q3;Q6S4N2;Q04967;A5A8V7 | 0.49768 | 0.192912 | 0.234695 | 4.239478 | 3 |
| 218 | FGL2 | F6Q194 | -0.35961 | 0.202802 | 0.245595 | 3.24415 | 3 |
| 219 | IST1 | A0A287A426 | -0.50098 | 0.212617 | 0.256306 | 4.034732 | 8 |
| 220 | ACT/B/G1 | Q6QAQ1;I3LVD5 | 0.338912 | 0.2243 | 0.269159 | 3.531901 | 9 |
| 221 | RAB5A | Q06AU6 | 0.355557 | 0.23744 | 0.282436 | 2.725639 | 3 |
| 222 | BAIAP2 | A0A287BRR9 | 0.390657 | 0.237503 | 0.282436 | 5.518362 | 2 |
| 223 | CD14 | A2SW51 | -0.40736 | 0.282662 | 0.334631 | 3.953437 | 3 |
| 224 | ITIH4 | A0A286ZN24 | -0.2708 | 0.29331 | 0.345687 | 3.40089 | 4 |
| 225 | CFL1 | P10668 | 0.374499 | 0.307879 | 0.361245 | 4.136279 | 6 |
| 226 | TTYH3 | F1RI78 | 0.282189 | 0.310453 | 0.362653 | 4.253275 | 3 |
| 227 | ACSL6 | A0A5G2Q912 | 0.411101 | 0.315233 | 0.366615 | 3.649641 | 3 |
| 228 | ANXA11 | F1S2E2 | 0.298541 | 0.33 | 0.382105 | 4.544183 | 9 |
| 229 | RAB5C | A0A287BN36 | 0.270885 | 0.372354 | 0.429264 | 2.520809 | 4 |
| 230 | ANXA2 | P19620 | 0.221027 | 0.420825 | 0.483034 | 4.321955 | 12 |
| 231 | RAC/2/1 | A0A5G2R6S0;A0A287B4G4 | 0.300704 | 0.440278 | 0.502798 | 5.47119 | 2 |

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|-----|------------------|---------------------------|----------|----------|----------|----------|----|
| 232 | RAC1 | A0A287B 4G4 | 0.266834 | 0.441853 | 0.502798 | 4.270526 | 3 |
| 233 | ARHGDI A | A0A287A QC2 | 0.205567 | 0.447901 | 0.507493 | 4.198994 | 8 |
| 234 | EPS8 | I3LP99 | -0.16508 | 0.480983 | 0.54177 | 3.694047 | 9 |
| 235 | CLDN3 | A0A5G2Q JG3 | 0.255039 | 0.482257 | 0.54177 | 4.344975 | 3 |
| 236 | C3 | F1SBS4 | -0.19344 | 0.488864 | 0.546272 | 3.724701 | 4 |
| 237 | C4A | A0A5G2Q CL1 | 0.185605 | 0.490403 | 0.546272 | 3.140668 | 6 |
| 238 | CRK | A0A5G2Q SR7 | 0.218436 | 0.500422 | 0.55509 | 4.007048 | 4 |
| 239 | GNAS | A5GFT9 | 0.163538 | 0.542437 | 0.599177 | 4.08505 | 2 |
| 240 | EEF2 | I3LII3 | 0.199972 | 0.556066 | 0.611672 | 2.515056 | 2 |
| 241 | CHMP2A | I3LSS2 | -0.2103 | 0.5586 | 0.61191 | 4.856903 | 8 |
| 242 | GNA13 | A0A287B C83 | -0.12974 | 0.564231 | 0.615525 | 3.59591 | 5 |
| 243 | GAPDH | P00355 | 0.209409 | 0.581982 | 0.632276 | 3.678831 | 4 |
| 244 | CEACAM 1 | I3L9E5 | -0.19155 | 0.595451 | 0.644258 | 4.408131 | 7 |
| 245 | PGAM1 | A0A287AJ Q2 | -0.19012 | 0.604974 | 0.65189 | 2.992217 | 2 |
| 246 | EPS8L2 | A0A286Z VR9 | -0.12867 | 0.631436 | 0.677639 | 3.676723 | 6 |
| 247 | RAP1A | F2Z5K3 | -0.13105 | 0.662644 | 0.706351 | 3.470279 | 3 |
| 248 | PDCD6 | A0A287A KU6 | 0.171198 | 0.663542 | 0.706351 | 3.468693 | 4 |
| 249 | CHMP5 | F1SE87 | 0.226292 | 0.682243 | 0.723341 | 4.236682 | 2 |
| 250 | MRAS | A0A5G2Q JB5 | 0.089784 | 0.686107 | 0.724529 | 3.061752 | 3 |
| 251 | RAP1/B/A | A0A286Z VJ2;F2Z5 K3 | 0.119871 | 0.699187 | 0.7354 | 4.35698 | 9 |
| 252 | CNP | A0A287A 9L8 | 0.107811 | 0.702464 | 0.735915 | 4.197055 | 4 |
| 253 | RAB10 | F2Z5F2 | 0.162696 | 0.7204 | 0.751722 | 5.116465 | 3 |
| 254 | VPS4A | I3LL27 | -0.10563 | 0.743237 | 0.772498 | 3.815098 | 4 |
| 255 | LOC10073 9163 | A0A287B Q81 | -0.07458 | 0.795557 | 0.823636 | 3.889458 | 4 |
| 256 | CHMP1A | I3LU89 | -0.09048 | 0.823495 | 0.849229 | 4.261307 | 4 |
| 257 | ADFP | D0G0B6 | 0.049973 | 0.834098 | 0.856817 | 4.142014 | 29 |
| 258 | RAL/A/B | I3LAJ6;I3 LV17 | 0.102452 | 0.879553 | 0.897575 | 3.104039 | 4 |
| 259 | SNAP23 | A0A287B 2U6 | -0.04814 | 0.880575 | 0.897575 | 4.028723 | 5 |
| 260 | ACTG1 | I3LVD5 | -0.0477 | 0.925074 | 0.937761 | 3.067914 | 2 |
| 261 | BROX | A0A287A AM4 | 0.029086 | 0.927105 | 0.937761 | 3.516861 | 8 |
| 262 | LALBA | P18137 | -0.02123 | 0.967774 | 0.975161 | 4.058392 | 2 |
| 263 | C4orf19 | F1S4L8 | 0.011717 | 0.974857 | 0.977245 | 4.353937 | 2 |
| 264 | A2M | A0A5G2Q 8I9 | 0.00899 | 0.977245 | 0.977245 | 2.536434 | 9 |
| 265 | CHRDL2 | A0A5G2R H74 | -2.91355 | | | 3.439313 | 2 |
| 266 | OLFML3 | H6UWK6 | -2.37574 | | | 3.489077 | 2 |

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| 267 | TGFBR3 | A0A287B PI7 | -2.35933 | 3.625966 | 2 |
| 268 | CHI3L1 | A0A287B KG2 | -2.09004 | 3.343129 | 2 |
| 269 | FCN2 | A0A5K1U ZA2 | -1.57296 | 4.034965 | 2 |
| 270 | FLOT2 | A0A480T GV2 | -1.34148 | 4.538231 | 4 |
| 271 | PDCD6// | A0A5G2Q CX9;A0A 287AKU6 | -1.18419 | 3.582214 | 2 |
| 272 | Gene NA - Prt: A0A286ZI M1 | A0A286ZI M1 | -0.92992 | 3.68489 | 2 |
| 273 | COL18A1 | I3LH70 | -0.78059 | 4.222196 | 4 |
| 274 | SERPINA 1 | P50447 | -0.50064 | 2.66335 | 2 |
| 275 | TMEM19 | A0A287A 4W6 | -0.41074 | 3.568603 | 2 |
| 276 | LDHB | A0A5G2Q HL7 | -0.38671 | 4.244291 | 2 |
| 277 | LBP | A0A287B 028 | -0.38113 | 3.437749 | 4 |
| 278 | RAB5A;C DK2;RAB 5C | Q06AU6; A0A286Z S27;A0A2 87BN36 | -0.21396 | 4.325362 | 2 |
| 279 | PTGFRN | F1SAY0 | -0.20516 | 3.232651 | 2 |
| 280 | SLC3A2 | A0A5G2R CQ0 | -0.11464 | 3.103297 | 3 |
| 281 | Gene NA - Prt: A0A5G2Q XC1 | A0A5G2Q XC1 | -0.06124 | 4.693061 | 2 |
| 282 | PRSS8 | A0A5G2Q SK0 | -0.02916 | 3.307351 | 3 |
| 283 | SLA-2 | A0A287A QG3 | -0.02578 | 4.682111 | 2 |
| 284 | CSN2 | A0A2C9F 376 | 0.028999 | 1.685854 | 3 |
| 285 | VPS28 | K7GM88 | 0.106097 | 3.844972 | 4 |
| 286 | PTPRJ | A0A287A WI4 | 0.115492 | 3.254381 | 2 |
| 287 | RALB | I3LV17 | 0.243288 | 4.330499 | 2 |
| 288 | NAPA | F1RLB5 | 0.246132 | 4.338879 | 3 |
| 289 | SCARB1 | Q8SQC1 | 0.267341 | 3.984991 | 3 |
| 290 | PGD | A0A5G2Q R34 | 0.28659 | 4.105394 | 2 |
| 291 | ACTA1 | A0A5S6G 831 | 0.336615 | 2.871411 | 3 |
| 292 | SEMA7A// | I3LC80;I3 LQF4 | 0.357643 | 3.204831 | 7 |
| 293 | NCSTN | A0A286Z K33 | 0.391173 | 4.984004 | 3 |
| 294 | GNB2 | F2Z4Z8 | 0.401695 | 2.838204 | 2 |
| 295 | SCAMP2 | A0A5G2R 660 | 0.473635 | 3.410068 | 2 |

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|-----|---------|-------------------------------|----------|--|----------|---|
| 296 | HSD17B7 | A0A5G2Q GH2 | 0.490597 | | 3.386419 | 2 |
| 297 | TXN | P82460 | 0.559431 | | 2.914253 | 3 |
| 298 | SAR1A | A0A5S6G 7K0 | 0.559527 | | 3.514933 | 2 |
| 299 | SOD1 | P04178 | 0.570862 | | 4.882674 | 3 |
| 300 | ANXA5 | F2Z5C1 | 0.627685 | | 3.397106 | 2 |
| 301 | SLA-2// | A0A287A EH7;A0A2 87AQG3 | 0.661826 | | 3.42832 | 3 |
| 302 | NIT2 | A0A5G2R GN4 | 0.703802 | | 4.490943 | 2 |
| 303 | RAB27B | A0A286Z TH5 | 0.743441 | | 3.929521 | 4 |
| 304 | PEBP1 | F1RKG8 | 0.747405 | | 4.257097 | 4 |
| 305 | BSG | A0A286Z S77 | 0.869228 | | 4.53726 | 2 |
| 306 | ANXA4 | A0A287A YJ2 | 0.876266 | | 3.656262 | 2 |
| 307 | EHD4 | I3LDA5 | 0.922947 | | 3.530621 | 3 |
| 308 | IDI1 | I3LDY2 | 0.947231 | | 2.701298 | 2 |
| 309 | YKT6 | F1SSG1 | 1.024874 | | 4.286261 | 3 |
| 310 | HSPA1B | Q6S4N2 | 1.033207 | | 4.982824 | 2 |
| 311 | CYSTM1 | A0A287A UM3 | 1.072653 | | 4.740443 | 2 |
| 312 | CAB39 | F1SMX5 | 1.280263 | | 3.965521 | 3 |
| 313 | SLC44A2 | A0A5G2Q TD4 | 1.315642 | | 4.523337 | 6 |
| 314 | NME2 | Q2EN76 | 1.332111 | | 4.570478 | 4 |
| 315 | CDC42// | Q007T2;F 2Z5W2 | 1.419767 | | 3.629216 | 4 |
| 316 | VPS25 | A0A287A AM6 | 1.432011 | | 4.546764 | 4 |
| 317 | AK1 | A0A286Z Q79 | 1.446713 | | 5.301423 | 2 |
| 318 | KRT14 | A0A287A EL2 | 1.629547 | | 4.917632 | 2 |
| 319 | SRM | I3LPB8 | 1.882885 | | 4.429186 | 3 |

Table A2-3. List of all proteins identified by LC-MS/MS maintained for statistical analysis for analysis on comparison between milk exosomes at day 7 and milk exosomes at day 14, ranked by significance.

| Rank | Gene | Protein | logFC | P Value | Adjusted P Value | Average expression | Peptide count |
|------|------------------------------|---------------------------------|----------|----------|------------------|--------------------|---------------|
| 1 | CD209 | B3FVQ2 | -1.07572 | 0.000585 | 0.072106 | 4.766564 | 2 |
| 2 | CD14 | A2SW51 | -1.41748 | 0.000714 | 0.072106 | 3.953437 | 3 |
| 3 | SERINC5 | F1RF26 | -1.14747 | 0.000819 | 0.072106 | 5.354037 | 2 |
| 4 | C3// | F1SBS4;P 01025 | -0.85408 | 0.001812 | 0.119611 | 3.897809 | 18 |
| 5 | CD5L | F1RN76 | -1.09074 | 0.002775 | 0.1465 | 4.184892 | 9 |
| 6 | Gene NA - Prt: A0A287A | A0A287A P28;A0A2 86ZIM1;A | -1.62721 | 0.004186 | 0.183708 | 3.773167 | 3 |

P28;A0A2
86ZIM1;A
0A287BA
B3

0A287BA
B3

| | | | | | | | |
|----|--|---|----------|----------|----------|----------|----|
| 7 | LY6H | A0A286Z QV1 | -1.25403 | 0.005661 | 0.183708 | 5.135652 | 2 |
| 8 | ABCG2 | Q8MIB3 | -1.18171 | 0.006154 | 0.183708 | 3.772382 | 12 |
| 9 | MUC15 | F1SFZ5 | -1.04095 | 0.006263 | 0.183708 | 4.71967 | 9 |
| 10 | GPRC5B | A0A480W 2U9 | -0.84345 | 0.00707 | 0.186635 | 5.240895 | 5 |
| 11 | FGB | A0A5G2R HA4 | -1.27111 | 0.008025 | 0.188018 | 2.991589 | 7 |
| 12 | FGA | F1RX36 | -1.26855 | 0.008546 | 0.188018 | 3.361582 | 9 |
| 13 | CD36 | Q3HUX1 | 0.76152 | 0.011284 | 0.226258 | 4.671275 | 24 |
| 14 | Gene NA - Prt: A0A075B 7I9;A0A07 5B7H9;A0 A075B7J0; A0A075B 7I5 | A0A075B 7I9;A0A07 5B7H9;A0 A075B7J0; A0A075B 7I5 | -0.98641 | 0.011999 | 0.226258 | 5.663481 | 4 |
| 15 | SERPINA 3-2 | A0A287B 9R5 | -0.86541 | 0.016147 | 0.243795 | 4.440938 | 5 |
| 16 | TF | A0A5G2R 5N9 | -0.87682 | 0.017065 | 0.243795 | 4.641461 | 2 |
| 17 | C4orf19 | F1S4L8 | -0.93713 | 0.017527 | 0.243795 | 4.353937 | 2 |
| 18 | Gene NA - Prt: A0A0A0M Y58 | A0A0A0M Y58 | -0.68916 | 0.018332 | 0.243795 | 4.034749 | 19 |
| 19 | ANPEP | K7GMF9 | 0.816056 | 0.018346 | 0.243795 | 3.724721 | 37 |
| 20 | KRT75 | F1SGI7 | 0.985384 | 0.018469 | 0.243795 | 4.699503 | 2 |
| 21 | try | C6L245 | 0.817019 | 0.021012 | 0.264151 | 3.758574 | 3 |
| 22 | SNAP23 | A0A287B 2U6 | -0.75595 | 0.025364 | 0.30073 | 4.028723 | 5 |
| 23 | FGG | F1RX35 | -0.65246 | 0.0262 | 0.30073 | 4.145301 | 8 |
| 24 | ERAP1 | K7GLU6 | 0.662127 | 0.029828 | 0.30159 | 2.754582 | 6 |
| 25 | JCHAIN | A0A287B QC8 | -0.8104 | 0.030111 | 0.30159 | 4.707291 | 2 |
| 26 | LOC10030 2368 | A0A287B 5W2 | 0.558248 | 0.031272 | 0.30159 | 4.415311 | 34 |
| 27 | C3 | F1SBS4 | -0.63008 | 0.031472 | 0.30159 | 3.724701 | 4 |
| 28 | Gene NA - Prt: A0A287B AB3 | A0A287B AB3 | -0.73963 | 0.031987 | 0.30159 | 4.473881 | 4 |
| 29 | BAIAP2 | A0A287B RR9 | -0.7286 | 0.033981 | 0.309347 | 5.518362 | 2 |
| 30 | PLIN3 | K4P7U5 | -0.67787 | 0.038074 | 0.335047 | 4.318163 | 12 |
| 31 | MGP | A0A5G2Q K11 | -0.70255 | 0.041561 | 0.353937 | 4.000976 | 10 |
| 32 | TACSTD1 | Q75QW1 | 1.150569 | 0.05167 | 0.426279 | 4.620366 | 3 |
| 33 | Gene NA - Prt: A0A287A LJ6 | A0A287A LJ6 | -0.47086 | 0.059593 | 0.476747 | 4.016404 | 4 |

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|----|-------------------------------------|---------------------------|----------|----------|----------|----------|----|
| 34 | SLC6A14 | K7GNX7 | -0.58729 | 0.068686 | 0.5192 | 4.229918 | 13 |
| 35 | HSPA5 | A0A287BI L8 | 0.487404 | 0.068833 | 0.5192 | 2.279501 | 4 |
| 36 | Gene NA - Prt: A0A287A LC1 | A0A287A LC1 | -0.49557 | 0.076316 | 0.559653 | 3.088551 | 68 |
| 37 | Gene NA - Prt: P10859 | P10859 | 0.519637 | 0.09589 | 0.675687 | 3.716168 | 2 |
| 38 | Gene NA - Prt: A0A287A 4Y3 | A0A287A 4Y3 | -0.49083 | 0.097258 | 0.675687 | 4.354942 | 13 |
| 39 | GPI | P08059 | 0.431011 | 0.100704 | 0.681692 | 4.692694 | 4 |
| 40 | ITIH4 | A0A286Z N24 | 0.444867 | 0.103406 | 0.682479 | 3.40089 | 4 |
| 41 | ENO1 | I3LK59 | -0.43548 | 0.116467 | 0.749932 | 4.114588 | 7 |
| 42 | SLC29A1 | A0A287B 8K5 | -0.57573 | 0.122099 | 0.767481 | 4.707259 | 6 |
| 43 | SMPDL3B // | A0A287BJ W1;F1ST N0 | 0.46498 | 0.129283 | 0.769757 | 3.57818 | 10 |
| 44 | Gene NA - Prt: A0A5G2R 4L0 | A0A5G2R 4L0 | -0.50619 | 0.130095 | 0.769757 | 4.125335 | 3 |
| 45 | RAB18 | I3LC07 | 0.379991 | 0.131209 | 0.769757 | 4.32985 | 11 |
| 46 | TSPAN6 | F1S1M4 | -0.40684 | 0.158971 | 0.879809 | 4.167684 | 4 |
| 47 | Gene NA - Prt: A0A5G2Q N35 | A0A5G2Q N35 | 0.568066 | 0.159099 | 0.879809 | 3.774462 | 4 |
| 48 | GNAS | A5GFT9 | -0.38472 | 0.162485 | 0.879809 | 4.08505 | 2 |
| 49 | GPRC5A | I3LN87 | -0.66225 | 0.163423 | 0.879809 | 2.411634 | 2 |
| 50 | HAPLN3 | A0A5G2R 305 | -0.35531 | 0.169609 | 0.879809 | 3.833653 | 20 |
| 51 | ACSL3 | A0A287B 8R8 | 0.372095 | 0.169963 | 0.879809 | 4.195918 | 41 |
| 52 | PROM2 | F1SU61 | 0.33517 | 0.18162 | 0.913404 | 3.750727 | 2 |
| 53 | LALBA | P18137 | -0.73434 | 0.186589 | 0.913404 | 4.058392 | 2 |
| 54 | Gene NA - Prt: A0A287B 626 | A0A287B 626 | -0.44019 | 0.188411 | 0.913404 | 3.970228 | 29 |
| 55 | SAA3 | A0A480K NV0 | -0.56253 | 0.195635 | 0.913404 | 5.54745 | 2 |
| 56 | MUC4 | A0A287B 5M2 | -0.38504 | 0.195711 | 0.913404 | 3.497925 | 23 |
| 57 | MYOF | A0A5G2Q 8T2 | 0.311471 | 0.19725 | 0.913404 | 3.989779 | 30 |
| 58 | MATN4 | F1SDQ7 | -0.52398 | 0.200672 | 0.913404 | 2.441729 | 2 |
| 59 | PIGR | A0A287B 644 | -0.38078 | 0.206452 | 0.923785 | 3.942603 | 21 |
| 60 | BTN1A1 | A0A5K1U 1P1 | 0.359423 | 0.214349 | 0.929227 | 4.268103 | 61 |
| 61 | Gene NA - Prt: | A0A075B 7H9 | -0.35546 | 0.215045 | 0.929227 | 3.212066 | 4 |

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|----|---------------------------------|-----------------------|----------|----------|----------|----------|----|
| | A0A075B7H9 | | | | | | |
| 62 | PDXK | O46560 | 0.453784 | 0.218227 | 0.929227 | 3.521722 | 3 |
| 63 | CHMP4B | F1S4Z2 | -0.41475 | 0.233778 | 0.970847 | 4.979629 | 2 |
| 64 | ST13 | F1SRB9 | 0.394849 | 0.240861 | 0.970847 | 4.607381 | 3 |
| 65 | RAC1 | A0A287B4G4 | -0.41032 | 0.245816 | 0.970847 | 4.270526 | 3 |
| 66 | PGAM1 | A0A287AJQ2 | -0.45156 | 0.249405 | 0.970847 | 2.992217 | 2 |
| 67 | SRC | K7GM45 | -0.30557 | 0.249621 | 0.970847 | 4.3238 | 6 |
| 68 | TSPAN1 | F1S3V5 | 0.562364 | 0.253643 | 0.970847 | 4.832822 | 2 |
| 69 | FABP3 | O02772 | 0.320039 | 0.253744 | 0.970847 | 4.383504 | 22 |
| 70 | TM4SF1 | A0A5G2QC13 | -0.37039 | 0.260045 | 0.980743 | 3.680237 | 2 |
| 71 | MUC1// | A0A5G2QGF8;F1RGR9 | -0.42761 | 0.271718 | 0.986904 | 4.559646 | 9 |
| 72 | CHRD2// | A0A5G2QXF8;A0A5G2RH74 | -0.33593 | 0.275776 | 0.986904 | 3.581001 | 8 |
| 73 | CLDN3 | A0A5G2QJG3 | -0.40196 | 0.276842 | 0.986904 | 4.344975 | 3 |
| 74 | KRT1 | F1SGG3 | 0.328546 | 0.288564 | 0.986904 | 5.116696 | 9 |
| 75 | FGL2 | F6Q194 | -0.32228 | 0.290746 | 0.986904 | 3.24415 | 3 |
| 76 | PGK1 | F1RPH0 | 0.290977 | 0.29379 | 0.986904 | 4.45063 | 4 |
| 77 | Gene NA - Prt: A0A075B7I6 | A0A075B7I6 | -0.35071 | 0.297088 | 0.986904 | 3.55741 | 2 |
| 78 | NA;LOC100523213 | A0A0A0MY58;F1RL06 | -0.29471 | 0.297639 | 0.986904 | 3.985018 | 6 |
| 79 | MVB12A | A0A287AP77 | -0.43876 | 0.30084 | 0.986904 | 4.637288 | 4 |
| 80 | GPRC5C | A0A287ASV2 | 0.257901 | 0.312479 | 0.986904 | 4.592204 | 5 |
| 81 | CLIC1 | A0A287B2P1 | 0.352818 | 0.315511 | 0.986904 | 4.235843 | 9 |
| 82 | RAC/2/1 | A0A5G2R6S0;A0A287B4G4 | -0.38085 | 0.318499 | 0.986904 | 5.47119 | 2 |
| 83 | Gene NA - Prt: A0A5G2QXT5 | A0A5G2QXT5 | 0.323004 | 0.319117 | 0.986904 | 3.449775 | 5 |
| 84 | APOE | F1RM45 | -0.30689 | 0.321017 | 0.986904 | 3.395127 | 2 |
| 85 | FASN | I3LC73 | -0.30811 | 0.328478 | 0.986904 | 3.94284 | 17 |
| 86 | TINAGL1 | F1SVA2 | -0.44426 | 0.332998 | 0.986904 | 4.590614 | 3 |
| 87 | ARRDC1 | A0A286ZJR9 | 0.243887 | 0.338151 | 0.986904 | 3.880001 | 4 |
| 88 | DDR1 | K7GSX5 | 0.295549 | 0.341369 | 0.986904 | 4.792415 | 2 |
| 89 | C9 | A0A287BH90 | -0.35849 | 0.34607 | 0.986904 | 3.484615 | 3 |
| 90 | ANXA6 | A0A5G2RBI3 | 0.265313 | 0.349068 | 0.986904 | 4.400298 | 6 |
| 91 | TTYH3 | F1RI78 | -0.26613 | 0.358168 | 0.986904 | 4.253275 | 3 |

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|-----|--|-------------------------------|----------|----------|----------|----------|-----|
| 92 | HSPA1B/ 6 | Q6S4N2;Q 04967 | 0.256447 | 0.360055 | 0.986904 | 5.257768 | 3 |
| 93 | APOA1 | K7GM40 | -0.27881 | 0.361472 | 0.986904 | 3.357979 | 30 |
| 94 | SELENBP 1 | F1ST01 | 0.233498 | 0.362005 | 0.986904 | 3.456086 | 2 |
| 95 | RAB1A/R AB1B | F2Z5U4;Q 06AU7 | 0.252249 | 0.367404 | 0.986904 | 3.36718 | 6 |
| 96 | COL4A2 | A0A5G2R J53 | -0.34262 | 0.368405 | 0.986904 | 3.017329 | 3 |
| 97 | VAT1 | I3L9V2 | 0.239378 | 0.373637 | 0.986904 | 2.022158 | 7 |
| 98 | RAB1A | F2Z5U4 | 0.228798 | 0.377337 | 0.986904 | 4.315186 | 9 |
| 99 | EEF2 | I3LII3 | -0.31462 | 0.377798 | 0.986904 | 2.515056 | 2 |
| 100 | GNB1/2 | A0A287A 7Q3;F2Z4 Z8 | 0.321111 | 0.381693 | 0.986904 | 4.30146 | 5 |
| 101 | KRT5 | A0A287A ZL3 | 0.288057 | 0.388253 | 0.986904 | 3.506331 | 3 |
| 102 | C1QA | Q69DL0 | -0.34783 | 0.394515 | 0.986904 | 4.078393 | 2 |
| 103 | KRT10 | I3LDS3 | 0.234622 | 0.396237 | 0.986904 | 4.41125 | 5 |
| 104 | MFGE8// | A0A5G2R KJ7;A0A5 G2QXS8 | -0.22314 | 0.4087 | 0.986904 | 4.588095 | 105 |
| 105 | CPM | I3LTE1 | 0.274812 | 0.414106 | 0.986904 | 4.389205 | 28 |
| 106 | NA;CSN1 S1 | A0A5S6G 633;P3903 5 | -0.23227 | 0.416586 | 0.986904 | 3.32349 | 13 |
| 107 | RAB5C | A0A287B N36 | -0.27386 | 0.418944 | 0.986904 | 2.520809 | 4 |
| 108 | RAB10 | F2Z5F2 | -0.38607 | 0.418944 | 0.986904 | 5.116465 | 3 |
| 109 | RAB3D | A0A287B LN4 | -0.21726 | 0.419016 | 0.986904 | 3.960266 | 4 |
| 110 | MSTN | O18831 | 0.24261 | 0.421179 | 0.986904 | 2.954647 | 10 |
| 111 | PPIA | P62936 | 0.208462 | 0.422681 | 0.986904 | 4.659886 | 14 |
| 112 | RRAS | F1RHW4 | 0.285725 | 0.427968 | 0.986904 | 4.486447 | 3 |
| 113 | Gene NA - Prt: I3LTW5 | I3LTW5 | 0.418464 | 0.428771 | 0.986904 | 3.116072 | 2 |
| 114 | Gene NA - Prt: A0A5G2R 9S9;A0A2 87ALJ6 | A0A5G2R 9S9;A0A2 87ALJ6 | -0.35277 | 0.429424 | 0.986904 | 3.153838 | 2 |
| 115 | PAPLN | F1S3J7 | -0.24615 | 0.445984 | 0.986904 | 3.598821 | 4 |
| 116 | FLOT1 | Q767L6 | -0.23283 | 0.449641 | 0.986904 | 3.905262 | 6 |
| 117 | PZP | A0A287B DU7 | -0.19749 | 0.460864 | 0.986904 | 2.407524 | 5 |
| 118 | GSN | A0A287A 6P1 | -0.17442 | 0.46299 | 0.986904 | 3.579003 | 19 |
| 119 | RAB35 | A0A287B FE6 | 0.199554 | 0.469017 | 0.986904 | 4.809324 | 5 |
| 120 | RALA | I3LAJ6 | 0.298547 | 0.469856 | 0.986904 | 4.085954 | 5 |
| 121 | Gene NA - Prt: A0A5G2R 9S9 | A0A5G2R 9S9 | 0.318045 | 0.470163 | 0.986904 | 3.738216 | 2 |
| 122 | SLC9A3R 1 | A0A5G2R 543 | -0.24681 | 0.475251 | 0.986904 | 4.747528 | 16 |

| | | | | | | | |
|-----|--|--|----------|----------|----------|----------|----|
| 123 | IFITM1;IF ITM3;LO C1021614 18 | A0A5G2Q WL4;A0A 287B7R8; F1RGC5 | -0.19401 | 0.49043 | 0.986904 | 3.46739 | 4 |
| 124 | EPS8L2 | A0A286Z VR9 | -0.20208 | 0.491362 | 0.986904 | 3.676723 | 6 |
| 125 | CD109 | A0A5G2Q PT1 | -0.29003 | 0.498039 | 0.986904 | 3.34873 | 2 |
| 126 | CIDEA | A0A5G2R 9X9 | 0.215224 | 0.499105 | 0.986904 | 4.788696 | 3 |
| 127 | NA;FABP 3 | I3LTW5;O 02772 | -0.37807 | 0.499374 | 0.986904 | 6.001107 | 2 |
| 128 | Gene NA - Prt: A0A287A TT2 | A0A287A TT2 | -0.27152 | 0.50241 | 0.986904 | 3.373407 | 3 |
| 129 | FUCA1 | I3LNS9 | 0.214172 | 0.504067 | 0.986904 | 3.774814 | 2 |
| 130 | Gene NA - Prt: A0A5S6G 633 | A0A5S6G 633 | -0.34578 | 0.508873 | 0.986904 | 2.838299 | 3 |
| 131 | CNP | A0A287A 9L8 | -0.18883 | 0.50982 | 0.986904 | 4.197055 | 4 |
| 132 | RDX | P26044 | -0.19189 | 0.510538 | 0.986904 | 3.883106 | 5 |
| 133 | CSN1S1 | P39035 | -0.21092 | 0.511384 | 0.986904 | 3.239364 | 31 |
| 134 | FBLN2 | A0A287B 5Q1 | -0.22731 | 0.519991 | 0.986904 | 3.6693 | 10 |
| 135 | SERPINC 1 | A0A5G2Q UE0 | -0.17341 | 0.52111 | 0.986904 | 3.234506 | 9 |
| 136 | Gene NA - Prt: A0A5G2Q 7F8 | A0A5G2Q 7F8 | -0.32125 | 0.52303 | 0.986904 | 2.486988 | 2 |
| 137 | CHMP2A | I3LSS2 | 0.230866 | 0.52624 | 0.986904 | 4.856903 | 8 |
| 138 | B4GALT1 | A0A287B G16 | 0.216202 | 0.528991 | 0.986904 | 3.320653 | 20 |
| 139 | FASN | I3LCW1 | -0.20859 | 0.529619 | 0.986904 | 3.770177 | 4 |
| 140 | Gene NA - Prt: A0A075B 7J0 | A0A075B 7J0 | -0.28677 | 0.536241 | 0.986904 | 2.635656 | 2 |
| 141 | PDCD6 | A0A287A KU6 | -0.26496 | 0.538537 | 0.986904 | 3.468693 | 4 |
| 142 | ALB | A0A287B AY9 | -0.14908 | 0.549883 | 0.986904 | 3.830738 | 18 |
| 143 | LOC10073 9163 | A0A287B Q81 | -0.18798 | 0.550449 | 0.986904 | 3.889458 | 4 |
| 144 | STXBP2 | A0A5G2Q A44 | -0.19178 | 0.550784 | 0.986904 | 4.857438 | 11 |
| 145 | CEACAM 1 | I3L9E5 | -0.21796 | 0.551017 | 0.986904 | 4.408131 | 7 |
| 146 | CD63 | F1SPK8 | -0.1962 | 0.554791 | 0.986904 | 4.220068 | 3 |
| 147 | CHMP5 | F1SE87 | -0.34168 | 0.555196 | 0.986904 | 4.236682 | 2 |
| 148 | VTA1 | F1S6Z8 | -0.24141 | 0.556589 | 0.986904 | 3.461418 | 5 |
| 149 | VPS4B | A0A287B A12 | -0.23441 | 0.562426 | 0.986904 | 4.101608 | 8 |
| 150 | DAG1 | A0A287B BR1 | -0.23576 | 0.57036 | 0.986904 | 2.410085 | 2 |

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| 151 | SIL1 | FIRGI9 | -0.1423 | 0.577473 | 0.986904 | 3.792533 | 8 |
| 152 | 14-3-3zeta | A0A480P LY3 | 0.134133 | 0.577748 | 0.986904 | 3.095345 | 6 |
| 153 | RAL/A/B | I3LAJ6;I3 LV17 | 0.393847 | 0.578562 | 0.986904 | 3.104039 | 4 |
| 154 | PPIC | A0A5G2R B35 | -0.20457 | 0.581366 | 0.986904 | 2.948777 | 4 |
| 155 | ACTB | Q6QAQ1 | 0.18777 | 0.582732 | 0.986904 | 3.635767 | 2 |
| 156 | SOD3 | I3LUD1 | -0.16652 | 0.588277 | 0.986904 | 3.206342 | 2 |
| 157 | ADFP | D0G0B6 | 0.130486 | 0.589919 | 0.986904 | 4.142014 | 29 |
| 158 | HEXA | F1SI88 | -0.12772 | 0.600832 | 0.986904 | 2.413714 | 4 |
| 159 | ACSL6 | A0A5G2Q 912 | 0.213451 | 0.604425 | 0.986904 | 3.649641 | 3 |
| 160 | PRDX5 | A0A5G2R 9D6 | 0.133397 | 0.606754 | 0.986904 | 3.401712 | 6 |
| 161 | VAMP3 | A0A5G2R 1F8 | 0.116648 | 0.613428 | 0.986904 | 2.71867 | 2 |
| 162 | VTN | P48819 | -0.15311 | 0.614153 | 0.986904 | 4.276618 | 7 |
| 163 | UBC | A0A5G2Q DJ2 | 0.128525 | 0.622112 | 0.986904 | 4.733632 | 12 |
| 164 | PPIB | A0A286Z KG9 | 0.138031 | 0.624301 | 0.986904 | 3.286902 | 3 |
| 165 | 14-3-3zeta;YWHAH;YWHAB;YWHAB | A0A480P LY3;F2Z4 Y1;A0A5 G2QM17; A0A5G2Q SR7;A0A2 87BPX7 | -0.19544 | 0.628997 | 0.986904 | 5.657113 | 2 |
| 166 | RAB14 | Q52NJ6 | -0.17898 | 0.629068 | 0.986904 | 4.846186 | 2 |
| 167 | SERPINF1 | A0A287A YJ8 | -0.12378 | 0.630657 | 0.986904 | 3.303514 | 10 |
| 168 | ATP2B2 | A0A286Z SF2 | -0.11491 | 0.630977 | 0.986904 | 4.769486 | 7 |
| 169 | VWA2 | F1S5H3 | -0.11562 | 0.638547 | 0.986904 | 3.350119 | 7 |
| 170 | RDX;MSN;EZR | P26044;A0 A287AK1 9;F1SB42 | 0.151534 | 0.639712 | 0.986904 | 4.397996 | 10 |
| 171 | PARK7 | A0A5G2Q IK1 | 0.222495 | 0.64304 | 0.986904 | 5.159512 | 2 |
| 172 | IFITM1/3 | A0A5G2Q WL4;A0A 287B7R8 | 0.205385 | 0.647115 | 0.986904 | 4.615226 | 4 |
| 173 | AEBP1 | A0A286Z ZM9 | -0.09442 | 0.655454 | 0.986904 | 3.833016 | 4 |
| 174 | RAB2A/RAB2B | F1RT87;F 1S8J6 | -0.14487 | 0.656624 | 0.986904 | 3.736955 | 3 |
| 175 | EZR | F1SB42 | 0.175992 | 0.661619 | 0.986904 | 3.956502 | 22 |
| 176 | TTYH2 | A0A287A AJ2 | -0.12003 | 0.66545 | 0.986904 | 5.144515 | 8 |
| 177 | ALS | Q1KS52 | 0.136222 | 0.666995 | 0.986904 | 1.561597 | 4 |
| 178 | CD9 | Q8WMQ3 | 0.234826 | 0.672807 | 0.986904 | 5.56161 | 4 |
| 179 | CHID1 | F1RZ01 | -0.11622 | 0.683931 | 0.986904 | 3.185273 | 11 |
| 180 | TLR2 | Q59HI8 | -0.12528 | 0.68522 | 0.986904 | 4.359128 | 26 |
| 181 | XDH | F1S3Y7 | 0.114345 | 0.687751 | 0.986904 | 4.814699 | 66 |
| 182 | RAP1A | F2Z5K3 | -0.11911 | 0.695318 | 0.986904 | 3.470279 | 3 |

| | | | | | | | |
|-----|-------------------------------------|-------------------------------|----------|----------|----------|----------|----|
| 183 | ATP6AP1 | A0A5G2R D35 | -0.09797 | 0.707734 | 0.986904 | 3.264964 | 4 |
| 184 | EPS8 | I3LP99 | -0.0869 | 0.713314 | 0.986904 | 3.694047 | 9 |
| 185 | GALM | A0A5G2Q Z46 | 0.101145 | 0.716716 | 0.986904 | 3.897957 | 4 |
| 186 | CRK | A0A5G2Q SR7 | 0.112784 | 0.720891 | 0.986904 | 4.007048 | 4 |
| 187 | Gene NA - Prt: A0A287A 7S8 | A0A287A 7S8 | 0.097369 | 0.725166 | 0.986904 | 3.275506 | 2 |
| 188 | APOE | P18650 | -0.1157 | 0.727767 | 0.986904 | 4.580658 | 8 |
| 189 | CFL1 | P10668 | 0.127415 | 0.729969 | 0.986904 | 4.136279 | 6 |
| 190 | RAB7A | A0A286Z P69 | 0.097438 | 0.731387 | 0.986904 | 4.306068 | 9 |
| 191 | ARF/3/4 | A0A287B 8I6;A0A5 G2R3T3 | -0.09383 | 0.732853 | 0.986904 | 3.238186 | 4 |
| 192 | RHOF | Q06AT7 | 0.130991 | 0.738406 | 0.986904 | 5.064607 | 2 |
| 193 | CDK2 | A0A286Z S27 | -0.0961 | 0.739769 | 0.986904 | 3.908768 | 4 |
| 194 | MFGE8 | A0A5G2R KJ7 | 0.084919 | 0.7436 | 0.986904 | 5.037166 | 11 |
| 195 | SLC34A2 | F1S5A6 | -0.097 | 0.745641 | 0.986904 | 4.131015 | 66 |
| 196 | LTF | A0A5G2Q UQ4 | -0.08931 | 0.748292 | 0.986904 | 3.41711 | 26 |
| 197 | ITIH2 | A0A5G2Q EV5 | -0.08935 | 0.750844 | 0.986904 | 3.574539 | 7 |
| 198 | PON1 | I3L5K0 | 0.170191 | 0.757472 | 0.986904 | 2.249963 | 4 |
| 199 | ARHGDI A | A0A287A QC2 | -0.08429 | 0.757704 | 0.986904 | 4.198994 | 8 |
| 200 | DNAJC3 | I3LBK9 | -0.18714 | 0.760735 | 0.986904 | 3.527757 | 2 |
| 201 | VPS4/A/B | I3LL27;A0 A287BA1 2 | -0.13449 | 0.762713 | 0.986904 | 3.75846 | 4 |
| 202 | RDX;EZR | P26044;F1 SB42 | -0.14952 | 0.766715 | 0.986904 | 4.44116 | 2 |
| 203 | GNA13 | A0A287B C83 | 0.066855 | 0.768783 | 0.986904 | 3.59591 | 5 |
| 204 | TMBIM1 | A0A5G2R 7N2 | -0.10895 | 0.772328 | 0.986904 | 5.668077 | 2 |
| 205 | CSN1S2 | P39036 | -0.08798 | 0.777238 | 0.986904 | 3.715798 | 39 |
| 206 | PIGR// | A0A287B 644;Q2924 4 | 0.094616 | 0.778801 | 0.986904 | 3.790705 | 5 |
| 207 | FMOD | F1S6B5 | 0.120246 | 0.782244 | 0.986904 | 2.122226 | 5 |
| 208 | APOE// | F1RM45;P 18650 | -0.08031 | 0.788384 | 0.986904 | 3.62444 | 28 |
| 209 | PFN1 | A0A5G2R 9A9 | 0.081658 | 0.788725 | 0.986904 | 3.252424 | 4 |
| 210 | BROX | A0A287A AM4 | 0.086082 | 0.789293 | 0.986904 | 3.516861 | 8 |
| 211 | ARHGAP 18 | A0A287B HC4 | -0.06802 | 0.796874 | 0.986904 | 4.917479 | 4 |
| 212 | ARF3 | A0A287B 8I6 | -0.09248 | 0.797272 | 0.986904 | 4.867116 | 4 |
| 213 | SERPIND 1 | F1RKY2 | 0.106542 | 0.804783 | 0.986904 | 3.397077 | 7 |

| | | | | | | | |
|-----|-----------------|--------------------------------------|----------|----------|----------|----------|----|
| 214 | RAB5A | Q06AU6 | 0.099478 | 0.805621 | 0.986904 | 2.725639 | 3 |
| 215 | VPS37B | F1REY0 | -0.06701 | 0.810187 | 0.986904 | 3.943706 | 7 |
| 216 | PAEP | A0A5S6H Q91 | 0.073 | 0.811188 | 0.986904 | 3.980301 | 12 |
| 217 | SPON2 | A0A481A ED0 | 0.095761 | 0.811232 | 0.986904 | 3.364732 | 4 |
| 218 | PDCD6IP | A0A287A 5B4 | 0.078849 | 0.821041 | 0.986904 | 4.405182 | 30 |
| 219 | SDCBP | A0A286Z KH9 | 0.059495 | 0.830442 | 0.986904 | 3.258615 | 11 |
| 220 | CD82 | A0A5G2Q KD8 | 0.048096 | 0.836755 | 0.986904 | 4.252814 | 8 |
| 221 | IST1 | A0A287A 426 | -0.08221 | 0.837966 | 0.986904 | 4.034732 | 8 |
| 222 | VPS4A | I3LL27 | -0.0663 | 0.839029 | 0.986904 | 3.815098 | 4 |
| 223 | APOA4 | A0A287B M29 | 0.053348 | 0.842599 | 0.986904 | 3.986276 | 7 |
| 224 | RHOA | I3LVS7 | -0.09222 | 0.843299 | 0.986904 | 5.26511 | 5 |
| 225 | LPL | A0A287A TV6 | -0.05623 | 0.847554 | 0.986904 | 3.338335 | 15 |
| 226 | ANXA11 | F1S2E2 | -0.0553 | 0.857518 | 0.986904 | 4.544183 | 9 |
| 227 | ACTG1 | I3LVD5 | -0.09556 | 0.859058 | 0.986904 | 3.067914 | 2 |
| 228 | RAB11A | A0A5G2Q E74 | -0.06665 | 0.859279 | 0.986904 | 4.928738 | 6 |
| 229 | ACT/A1/B /G1 | A0A5S6G 831;Q6QA Q1;I3LVD 5 | 0.056543 | 0.869891 | 0.986904 | 4.611775 | 18 |
| 230 | RAC2 | A0A5G2R 6S0 | 0.052717 | 0.87155 | 0.986904 | 4.062857 | 2 |
| 231 | GDI2 | A0A5G2Q SK4 | 0.041712 | 0.873177 | 0.986904 | 3.627553 | 15 |
| 232 | RAP1/B/A | A0A286Z VJ2;F2Z5 K3 | -0.04728 | 0.880254 | 0.986904 | 4.35698 | 9 |
| 233 | BAIAP2// | A0A287B RR9;F1RZ B0 | -0.04921 | 0.881734 | 0.986904 | 4.191112 | 16 |
| 234 | A2M | A0A5G2Q 8I9 | 0.042256 | 0.890815 | 0.986904 | 2.536434 | 9 |
| 235 | KRAS | I3LCQ9 | -0.04174 | 0.892754 | 0.986904 | 3.835119 | 3 |
| 236 | RAB2A | F1RT87 | 0.030589 | 0.895632 | 0.986904 | 4.826399 | 4 |
| 237 | PLOD1 | F1RF77 | -0.0385 | 0.903057 | 0.986904 | 3.585215 | 3 |
| 238 | EEF1A1 | A0A288C G57 | -0.03866 | 0.903309 | 0.986904 | 5.317222 | 10 |
| 239 | MSN | A0A287A K19 | 0.039705 | 0.905895 | 0.986904 | 4.993424 | 19 |
| 240 | CKB | A0A5G2R 6X7 | -0.02978 | 0.909807 | 0.986904 | 4.131502 | 7 |
| 241 | MDH1 | A0A5G2Q FC2 | -0.02895 | 0.911179 | 0.986904 | 4.533037 | 4 |
| 242 | PGLYRP1 | A0A286ZI 97 | 0.035225 | 0.914538 | 0.986904 | 3.716147 | 14 |
| 243 | TTR | A0A5G2Q IE9 | 0.030907 | 0.915245 | 0.986904 | 3.478735 | 3 |
| 244 | ANXA2 | P19620 | -0.02903 | 0.916406 | 0.986904 | 4.321955 | 12 |
| 245 | CDC42 | Q007T2 | 0.030581 | 0.918237 | 0.986904 | 1.875831 | 2 |
| 246 | CSN3 | P11841 | -0.02431 | 0.927956 | 0.986904 | 3.436882 | 27 |

| | | | | | | | |
|-----|--------------------|---|----------|----------|----------|----------|----|
| 247 | MRAS | A0A5G2Q JB5 | 0.020393 | 0.929987 | 0.986904 | 3.061752 | 3 |
| 248 | THBS1 | F1SS26 | -0.02468 | 0.930535 | 0.986904 | 3.711806 | 41 |
| 249 | GANAB | P79403 | 0.024865 | 0.93083 | 0.986904 | 3.357891 | 24 |
| 250 | ACT/B/G1 | Q6QAQ1;I 3LVD5 | 0.0181 | 0.948284 | 0.994693 | 3.531901 | 9 |
| 251 | GAPDH | P00355 | -0.02145 | 0.955487 | 0.994693 | 3.678831 | 4 |
| 252 | CP | A0A5G2Q W05 | -0.01543 | 0.959131 | 0.994693 | 3.659874 | 11 |
| 253 | CSN2// | P39037;A0 A2C9F376 | 0.014858 | 0.960407 | 0.994693 | 2.792655 | 68 |
| 254 | HSPA8 | F1S9Q3 | -0.0122 | 0.962822 | 0.994693 | 3.712596 | 22 |
| 255 | CHMP1A | I3LU89 | -0.01293 | 0.973957 | 0.994693 | 4.261307 | 4 |
| 256 | KRT1/4/0 | A0A287A EL2;I3LD S3 | 0.012513 | 0.974436 | 0.994693 | 5.18532 | 3 |
| 257 | C4A | A0A5G2Q CL1 | -0.00846 | 0.975131 | 0.994693 | 3.140668 | 6 |
| 258 | STX3 | F1RI88 | 0.008412 | 0.980674 | 0.994693 | 3.589462 | 9 |
| 259 | HSPA/8/1 B/6/1L | F1S9Q3;Q 6S4N2;Q0 4967;A5A 8V7 | -0.00892 | 0.981365 | 0.994693 | 4.239478 | 3 |
| 260 | CLU | A0A5S8K LN1 | 0.003552 | 0.988672 | 0.994693 | 3.847397 | 27 |
| 261 | ATP6AP2 | A0A287AJ K1 | 0.004209 | 0.988702 | 0.994693 | 3.929251 | 5 |
| 262 | SAA3// | A0A480K NV0;A0A 5G2R2X7 | -0.00333 | 0.990911 | 0.994693 | 4.899096 | 7 |
| 263 | ACLY | F1S0N2 | -0.00271 | 0.992185 | 0.994693 | 2.983594 | 4 |
| 264 | MUC1 | F1RGR9 | 0.003367 | 0.994693 | 0.994693 | 3.142075 | 3 |
| 265 | COL18A1 | I3LH70 | -0.67405 | | | 4.222196 | 4 |
| 266 | LBP | A0A287B 028 | -0.62114 | | | 3.437749 | 4 |
| 267 | LDHB | A0A5G2Q HL7 | -0.61361 | | | 4.244291 | 2 |
| 268 | PRSS8 | A0A5G2Q SK0 | -0.59897 | | | 3.307351 | 3 |
| 269 | TMEM19 | A0A287A 4W6 | -0.476 | | | 3.568603 | 2 |
| 270 | PGD | A0A5G2Q R34 | -0.36985 | | | 4.105394 | 2 |
| 271 | IDI1 | I3LDY2 | -0.32858 | | | 2.701298 | 2 |
| 272 | FLOT2 | A0A480T GV2 | -0.30679 | | | 4.538231 | 4 |
| 273 | HSD17B7 | A0A5G2Q GH2 | -0.30448 | | | 3.386419 | 2 |
| 274 | SCARB1 | Q8SQC1 | -0.30331 | | | 3.984991 | 3 |
| 275 | VPS28 | K7GM88 | -0.29908 | | | 3.844972 | 4 |
| 276 | PTGFRN | F1SAY0 | -0.28747 | | | 3.232651 | 2 |
| 277 | NCSTN | A0A286Z K33 | -0.21844 | | | 4.984004 | 3 |
| 278 | SLA-2 | A0A287A QG3 | -0.19766 | | | 4.682111 | 2 |
| 279 | ANXA5 | F2Z5C1 | -0.16256 | | | 3.397106 | 2 |
| 280 | NAPA | F1RLB5 | -0.15341 | | | 4.338879 | 3 |

| | | | | | |
|-----|-------------------------------------|--|----------|----------|---|
| 281 | SRM | I3LPB8 | -0.1454 | 4.429186 | 3 |
| 282 | TXN | P82460 | -0.1451 | 2.914253 | 3 |
| 283 | CAB39 | F1SMX5 | -0.12776 | 3.965521 | 3 |
| 284 | Gene NA - Prt: A0A286ZI M1 | A0A286ZI M1 | -0.10597 | 3.68489 | 2 |
| 285 | ACTA1 | A0A5S6G 831 | -0.01351 | 2.871411 | 3 |
| 286 | PTPRJ | A0A287A WI4 | 0.031323 | 3.254381 | 2 |
| 287 | SCAMP2 | A0A5G2R 660 | 0.203839 | 3.410068 | 2 |
| 288 | Gene NA - Prt: A0A5G2Q XC1 | A0A5G2Q XC1 | 0.226257 | 4.693061 | 2 |
| 289 | SLA-2// | A0A287A EH7;A0A2 87AQG3 | 0.2549 | 3.42832 | 3 |
| 290 | ANXA4 | A0A287A YJ2 | 0.3172 | 3.656262 | 2 |
| 291 | NIT2 | A0A5G2R GN4 | 0.320592 | 4.490943 | 2 |
| 292 | RAB5A;C DK2;RAB 5C | Q06AU6; A0A286Z S27;A0A2 87BN36 | 0.332946 | 4.325362 | 2 |
| 293 | EHD4 | I3LDA5 | 0.338284 | 3.530621 | 3 |
| 294 | CYSTM1 | A0A287A UM3 | 0.400356 | 4.740443 | 2 |
| 295 | PDCD6// | A0A5G2Q CX9;A0A 287AKU6 | 0.495063 | 3.582214 | 2 |
| 296 | VPS25 | A0A287A AM6 | 0.498342 | 4.546764 | 4 |
| 297 | SOD1 | P04178 | 0.584922 | 4.882674 | 3 |
| 298 | NME2 | Q2EN76 | 0.591883 | 4.570478 | 4 |
| 299 | PEBP1 | F1RKG8 | 0.655071 | 4.257097 | 4 |
| 300 | SEMA7A// | I3LC80;I3 LQF4 | 0.677322 | 3.204831 | 7 |
| 301 | AK1 | A0A286Z Q79 | 0.713996 | 5.301423 | 2 |
| 302 | RALB | I3LV17 | 0.728694 | 4.330499 | 2 |
| 303 | RAB27B | A0A286Z TH5 | 0.763921 | 3.929521 | 4 |
| 304 | SLC3A2 | A0A5G2R CQ0 | 0.843177 | 3.103297 | 3 |
| 305 | YKT6 | F1SSG1 | 0.871466 | 4.286261 | 3 |
| 306 | SAR1A | A0A5S6G 7K0 | 0.879974 | 3.514933 | 2 |
| 307 | SLC44A2 | A0A5G2Q TD4 | 0.900363 | 4.523337 | 6 |
| 308 | BSG | A0A286Z S77 | 1.032375 | 4.53726 | 2 |
| 309 | CDC42// | Q007T2;F 2Z5W2 | 1.126158 | 3.629216 | 4 |
| 310 | HSPA1B | Q6S4N2 | 1.731683 | 4.982824 | 2 |

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|------------|----------|----------------|----------|---|
| 311 | KRT14 | A0A287AEL 2 | 4.917632 | 2 |
| 312 | CHI3L1 | A0A287BK G2 | 3.343129 | 2 |
| 313 | TGFBR3 | A0A287BPI 7 | 3.625966 | 2 |
| 314 | CSN2 | A0A2C9F37 6 | 1.685854 | 3 |
| 315 | CHRD2 | A0A5G2RH 74 | 3.439313 | 2 |
| 316 | FCN2 | A0A5K1UZ A2 | 4.034965 | 2 |
| 317 | GNB2 | F2Z4Z8 | 2.838204 | 2 |
| 318 | OLFML3 | H6UWK6 | 3.489077 | 2 |
| 319 | SERPINA1 | P50447 | 2.66335 | 2 |

Appendix 3

Table A3-1. List of significant gene ontology terms associated with differential abundant proteins on the comparison between colostrum exosomes (day 0) and milk exosomes at day 7.

| GOD | GOTerm | Term PValue | Group PValue | % Genes | Nr. Genes |
|------------|---|-------------|--------------|---------|-----------|
| GO:1903053 | regulation of extracellular matrix organization | 0.00 | 0.02 | 8.11 | 3.00 |
| GO:0051438 | regulation of ubiquitin-protein transferase activity | 0.01 | 0.02 | 5.36 | 3.00 |
| GO:0007009 | plasma membrane organization | 0.01 | 0.03 | 4.35 | 4.00 |
| GO:0009988 | cell-cell recognition | 0.00 | 0.01 | 7.14 | 4.00 |
| GO:0014812 | muscle cell migration | 0.01 | 0.02 | 6.00 | 3.00 |
| GO:0034389 | lipid droplet organization | 0.00 | 0.01 | 14.29 | 3.00 |
| GO:0051898 | negative regulation of protein kinase B signaling | 0.00 | 0.00 | 10.26 | 4.00 |
| GO:0060627 | regulation of vesicle-mediated transport | 0.00 | 0.00 | 4.23 | 18.00 |
| GO:0006890 | retrograde vesicle-mediated transport. Golgi to endoplasmic reticulum | 0.01 | 0.02 | 4.21 | 4.00 |
| GO:1905037 | autophagosome organization | 0.01 | 0.02 | 4.40 | 4.00 |
| GO:0007264 | small GTPase mediated signal transduction | 0.00 | 0.00 | 4.54 | 22.00 |
| GO:0007265 | Ras protein signal transduction | 0.00 | 0.00 | 5.07 | 18.00 |
| GO:0045861 | negative regulation of proteolysis | 0.00 | 0.00 | 4.35 | 13.00 |
| GO:0061134 | peptidase regulator activity | 0.00 | 0.00 | 4.19 | 8.00 |
| GO:0048278 | vesicle docking | 0.01 | 0.02 | 5.77 | 3.00 |
| GO:0140029 | exocytic process | 0.01 | 0.02 | 5.26 | 3.00 |
| GO:0051702 | interaction with symbiont | 0.01 | 0.00 | 4.12 | 4.00 |
| GO:0031640 | killing of cells of other organism | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0019730 | antimicrobial humoral response | 0.00 | 0.00 | 4.41 | 6.00 |
| GO:0002218 | activation of innate immune response | 0.00 | 0.01 | 4.03 | 6.00 |
| GO:0002758 | innate immune response-activating signal transduction | 0.00 | 0.01 | 4.92 | 6.00 |
| GO:0002220 | innate immune response activating cell surface receptor signaling pathway | 0.00 | 0.01 | 4.96 | 6.00 |
| GO:0031333 | negative regulation of protein-containing complex assembly | 0.00 | 0.00 | 4.67 | 5.00 |
| GO:1902745 | positive regulation of lamellipodium organization | 0.00 | 0.00 | 13.33 | 4.00 |
| GO:0010592 | positive regulation of lamellipodium assembly | 0.00 | 0.00 | 13.64 | 3.00 |
| GO:0071622 | regulation of granulocyte chemotaxis | 0.01 | 0.00 | 6.52 | 3.00 |
| GO:0043149 | stress fiber assembly | 0.01 | 0.00 | 4.40 | 4.00 |
| GO:1903580 | positive regulation of ATP metabolic process | 0.00 | 0.01 | 7.50 | 3.00 |
| GO:0046365 | monosaccharide catabolic process | 0.02 | 0.01 | 4.23 | 3.00 |
| GO:0019320 | hexose catabolic process | 0.01 | 0.01 | 4.92 | 3.00 |
| GO:2001242 | regulation of intrinsic apoptotic signaling pathway | 0.00 | 0.01 | 4.44 | 6.00 |

| | | | | | |
|------------|--|------|------|-------|-------|
| GO:2001243 | negative regulation of intrinsic apoptotic signaling pathway | 0.00 | 0.01 | 5.62 | 5.00 |
| GO:1901654 | response to ketone | 0.00 | 0.00 | 7.04 | 5.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:0008625 | extrinsic apoptotic signaling pathway via death domain receptors | 0.02 | 0.00 | 4.00 | 3.00 |
| GO:2001235 | positive regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.01 | 0.00 | 4.60 | 4.00 |
| GO:0051701 | interaction with host | 0.00 | 0.00 | 4.52 | 7.00 |
| GO:0044766 | multi-organism transport | 0.00 | 0.00 | 6.06 | 4.00 |
| GO:0019058 | viral life cycle | 0.00 | 0.00 | 4.98 | 14.00 |
| GO:0019068 | virion assembly | 0.00 | 0.00 | 15.91 | 7.00 |
| GO:0019079 | viral genome replication | 0.00 | 0.00 | 5.79 | 7.00 |
| GO:0045069 | regulation of viral genome replication | 0.00 | 0.00 | 5.26 | 5.00 |
| GO:0051851 | modulation by host of symbiont process | 0.02 | 0.00 | 4.41 | 3.00 |
| GO:0044788 | modulation by host of viral process | 0.00 | 0.00 | 9.09 | 3.00 |
| GO:0060348 | bone development | 0.00 | 0.00 | 5.17 | 6.00 |
| GO:0150116 | regulation of cell-substrate junction organization | 0.02 | 0.00 | 4.69 | 3.00 |
| GO:0060349 | bone morphogenesis | 0.01 | 0.00 | 5.08 | 3.00 |
| GO:0090109 | regulation of cell-substrate junction assembly | 0.01 | 0.00 | 5.00 | 3.00 |
| GO:0061515 | myeloid cell development | 0.00 | 0.00 | 9.38 | 3.00 |
| GO:1990748 | cellular detoxification | 0.00 | 0.00 | 4.59 | 5.00 |
| GO:0042743 | hydrogen peroxide metabolic process | 0.01 | 0.00 | 6.00 | 3.00 |
| GO:0098869 | cellular oxidant detoxification | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:0034369 | plasma lipoprotein particle remodeling | 0.00 | 0.00 | 12.90 | 4.00 |
| GO:0034367 | protein-containing complex remodeling | 0.00 | 0.00 | 12.50 | 4.00 |
| GO:0016209 | antioxidant activity | 0.00 | 0.00 | 5.56 | 5.00 |
| GO:0034375 | high-density lipoprotein particle remodeling | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0010822 | positive regulation of mitochondrion organization | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0003014 | renal system process | 0.01 | 0.00 | 4.08 | 4.00 |
| GO:0031640 | killing of cells of other organism | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0060263 | regulation of respiratory burst | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0001894 | tissue homeostasis | 0.00 | 0.00 | 5.32 | 10.00 |
| GO:0019730 | antimicrobial humoral response | 0.00 | 0.00 | 4.41 | 6.00 |
| GO:0006910 | phagocytosis. recognition | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:0001895 | retina homeostasis | 0.00 | 0.00 | 9.59 | 7.00 |
| GO:0019731 | antibacterial humoral response | 0.00 | 0.00 | 6.78 | 4.00 |
| GO:0071706 | tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.44 | 6.00 |
| GO:0060348 | bone development | 0.00 | 0.00 | 5.17 | 6.00 |
| GO:0062207 | regulation of pattern recognition receptor signaling pathway | 0.00 | 0.00 | 5.49 | 5.00 |
| GO:0032640 | tumor necrosis factor production | 0.00 | 0.00 | 4.62 | 6.00 |

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|------------|---|------|------|-------|-------|
| GO:1901889 | negative regulation of cell junction assembly | 0.00 | 0.00 | 10.00 | 3.00 |
| GO:1903555 | regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.58 | 6.00 |
| GO:0002755 | MyD88-dependent toll-like receptor signaling pathway | 0.00 | 0.00 | 8.82 | 3.00 |
| GO:0034121 | regulation of toll-like receptor signaling pathway | 0.00 | 0.00 | 6.06 | 4.00 |
| GO:0031663 | lipopolysaccharide-mediated signaling pathway | 0.01 | 0.00 | 6.25 | 3.00 |
| GO:0032680 | regulation of tumor necrosis factor production | 0.00 | 0.00 | 4.69 | 6.00 |
| GO:0061515 | myeloid cell development | 0.00 | 0.00 | 9.38 | 3.00 |
| GO:0044766 | multi-organism transport | 0.00 | 0.00 | 6.06 | 4.00 |
| GO:0019058 | viral life cycle | 0.00 | 0.00 | 4.98 | 14.00 |
| GO:0140112 | extracellular vesicle biogenesis | 0.00 | 0.00 | 13.64 | 3.00 |
| GO:0046794 | transport of virus | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:0097734 | extracellular exosome biogenesis | 0.00 | 0.00 | 15.00 | 3.00 |
| GO:0007032 | endosome organization | 0.00 | 0.00 | 5.33 | 4.00 |
| GO:0075733 | intracellular transport of virus | 0.01 | 0.00 | 5.08 | 3.00 |
| GO:1990182 | exosomal secretion | 0.00 | 0.00 | 15.79 | 3.00 |
| GO:0045921 | positive regulation of exocytosis | 0.00 | 0.00 | 5.63 | 4.00 |
| GO:1901184 | regulation of ERBB signaling pathway | 0.01 | 0.00 | 4.40 | 4.00 |
| GO:1901185 | negative regulation of ERBB signaling pathway | 0.01 | 0.00 | 5.88 | 3.00 |
| GO:0042058 | regulation of epidermal growth factor receptor signaling pathway | 0.01 | 0.00 | 4.71 | 4.00 |
| GO:0042059 | negative regulation of epidermal growth factor receptor signaling pathway | 0.01 | 0.00 | 6.52 | 3.00 |
| GO:0034381 | plasma lipoprotein particle clearance | 0.01 | 0.00 | 5.45 | 3.00 |
| GO:0071402 | cellular response to lipoprotein particle stimulus | 0.00 | 0.00 | 12.00 | 3.00 |
| GO:0019915 | lipid storage | 0.00 | 0.00 | 7.25 | 5.00 |
| GO:0055094 | response to lipoprotein particle | 0.00 | 0.00 | 12.50 | 3.00 |
| GO:0010232 | vascular transport | 0.01 | 0.00 | 4.44 | 4.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:0008361 | regulation of cell size | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0045807 | positive regulation of endocytosis | 0.00 | 0.00 | 4.88 | 4.00 |
| GO:1903426 | regulation of reactive oxygen species biosynthetic process | 0.02 | 0.00 | 4.17 | 3.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0008637 | apoptotic mitochondrial changes | 0.00 | 0.00 | 4.39 | 5.00 |
| GO:0150104 | transport across blood-brain barrier | 0.01 | 0.00 | 4.44 | 4.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0015908 | fatty acid transport | 0.00 | 0.00 | 5.41 | 4.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:2000377 | regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 4.79 | 7.00 |
| GO:0050680 | negative regulation of epithelial cell proliferation | 0.01 | 0.00 | 4.49 | 4.00 |

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|------------|---|------|------|-------|-------|
| GO:1903426 | regulation of reactive oxygen species biosynthetic process | 0.02 | 0.00 | 4.17 | 3.00 |
| GO:1903573 | negative regulation of response to endoplasmic reticulum stress | 0.01 | 0.00 | 6.52 | 3.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:0140354 | lipid import into cell | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0001937 | negative regulation of endothelial cell proliferation | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0002576 | platelet degranulation | 0.00 | 0.00 | 5.80 | 8.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0008625 | extrinsic apoptotic signaling pathway via death domain receptors | 0.02 | 0.00 | 4.00 | 3.00 |
| GO:1903557 | positive regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.94 | 4.00 |
| GO:0015908 | fatty acid transport | 0.00 | 0.00 | 5.41 | 4.00 |
| GO:0032760 | positive regulation of tumor necrosis factor production | 0.00 | 0.00 | 5.06 | 4.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.01 | 0.00 | 4.60 | 4.00 |
| GO:0031589 | cell-substrate adhesion | 0.00 | 0.00 | 4.12 | 12.00 |
| GO:0006909 | phagocytosis | 0.00 | 0.00 | 4.64 | 17.00 |
| GO:0006910 | phagocytosis. recognition | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:0002920 | regulation of humoral immune response | 0.00 | 0.00 | 6.67 | 9.00 |
| GO:0010810 | regulation of cell-substrate adhesion | 0.00 | 0.00 | 6.02 | 10.00 |
| GO:0002433 | immune response-regulating cell surface receptor signaling pathway involved in phagocytosis | 0.00 | 0.00 | 5.96 | 9.00 |
| GO:0006956 | complement activation | 0.00 | 0.00 | 5.94 | 12.00 |
| GO:0010324 | membrane invagination | 0.00 | 0.00 | 6.49 | 10.00 |
| GO:0002455 | humoral immune response mediated by circulating immunoglobulin | 0.00 | 0.00 | 5.33 | 8.00 |
| GO:0019731 | antibacterial humoral response | 0.00 | 0.00 | 6.78 | 4.00 |
| GO:0006911 | phagocytosis. engulfment | 0.00 | 0.00 | 6.52 | 9.00 |
| GO:0006958 | complement activation. classical pathway | 0.00 | 0.00 | 5.63 | 8.00 |
| GO:0030449 | regulation of complement activation | 0.00 | 0.00 | 7.20 | 9.00 |
| GO:0002431 | Fc receptor mediated stimulatory signaling pathway | 0.00 | 0.00 | 5.77 | 9.00 |
| GO:0038096 | Fc-gamma receptor signaling pathway involved in phagocytosis | 0.00 | 0.00 | 5.96 | 9.00 |
| GO:0051702 | interaction with symbiont | 0.01 | 0.00 | 4.12 | 4.00 |
| GO:0031640 | killing of cells of other organism | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0051701 | interaction with host | 0.00 | 0.00 | 4.52 | 7.00 |
| GO:0051817 | modulation of process of other organism involved in symbiotic interaction | 0.00 | 0.00 | 4.95 | 5.00 |
| GO:0051851 | modulation by host of symbiont process | 0.02 | 0.00 | 4.41 | 3.00 |
| GO:0019058 | viral life cycle | 0.00 | 0.00 | 4.98 | 14.00 |
| GO:0052126 | movement in host environment | 0.00 | 0.00 | 4.39 | 5.00 |
| GO:0019079 | viral genome replication | 0.00 | 0.00 | 5.79 | 7.00 |

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|------------|--|------|------|-------|------|
| GO:0044788 | modulation by host of viral process | 0.00 | 0.00 | 9.09 | 3.00 |
| GO:0044409 | entry into host | 0.01 | 0.00 | 4.65 | 4.00 |
| GO:0060348 | bone development | 0.00 | 0.00 | 5.17 | 6.00 |
| GO:0033619 | membrane protein proteolysis | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0045069 | regulation of viral genome replication | 0.00 | 0.00 | 5.26 | 5.00 |
| GO:0045071 | negative regulation of viral genome replication | 0.01 | 0.00 | 4.92 | 3.00 |
| GO:0046718 | viral entry into host cell | 0.02 | 0.00 | 4.29 | 3.00 |
| GO:1905952 | regulation of lipid localization | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:0010883 | regulation of lipid storage | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:0010884 | positive regulation of lipid storage | 0.00 | 0.00 | 13.04 | 3.00 |
| GO:0030730 | sequestering of triglyceride | 0.00 | 0.00 | 15.79 | 3.00 |
| GO:0071706 | tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.44 | 6.00 |
| GO:0010889 | regulation of sequestering of triglyceride | 0.00 | 0.00 | 18.75 | 3.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:0010890 | positive regulation of sequestering of triglyceride | 0.00 | 0.00 | 27.27 | 3.00 |
| GO:0032640 | tumor necrosis factor production | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:1901889 | negative regulation of cell junction assembly | 0.00 | 0.00 | 10.00 | 3.00 |
| GO:1903555 | regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.58 | 6.00 |
| GO:1903557 | positive regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.94 | 4.00 |
| GO:0031663 | lipopolysaccharide-mediated signaling pathway | 0.01 | 0.00 | 6.25 | 3.00 |
| GO:0021782 | glial cell development | 0.01 | 0.00 | 4.92 | 3.00 |
| GO:0032680 | regulation of tumor necrosis factor production | 0.00 | 0.00 | 4.69 | 6.00 |
| GO:0061515 | myeloid cell development | 0.00 | 0.00 | 9.38 | 3.00 |
| GO:0032760 | positive regulation of tumor necrosis factor production | 0.00 | 0.00 | 5.06 | 4.00 |
| GO:0072604 | interleukin-6 secretion | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:2000778 | positive regulation of interleukin-6 secretion | 0.00 | 0.00 | 8.82 | 3.00 |
| GO:0061077 | chaperone-mediated protein folding | 0.00 | 0.00 | 6.67 | 4.00 |
| GO:0098927 | vesicle-mediated transport between endosomal compartments | 0.00 | 0.00 | 11.63 | 5.00 |
| GO:1901654 | response to ketone | 0.00 | 0.00 | 7.04 | 5.00 |
| GO:1903580 | positive regulation of ATP metabolic process | 0.00 | 0.00 | 7.50 | 3.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:2000377 | regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 4.79 | 7.00 |
| GO:0045022 | early endosome to late endosome transport | 0.00 | 0.00 | 12.20 | 5.00 |
| GO:0045807 | positive regulation of endocytosis | 0.00 | 0.00 | 4.88 | 4.00 |
| GO:1901655 | cellular response to ketone | 0.00 | 0.00 | 7.14 | 3.00 |
| GO:1903426 | regulation of reactive oxygen species biosynthetic process | 0.02 | 0.00 | 4.17 | 3.00 |

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|------------|---|------|------|-------|------|
| GO:1903573 | negative regulation of response to endoplasmic reticulum stress | 0.01 | 0.00 | 6.52 | 3.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:0031333 | negative regulation of protein-containing complex assembly | 0.00 | 0.00 | 4.67 | 5.00 |
| GO:1903649 | regulation of cytoplasmic transport | 0.00 | 0.00 | 14.81 | 4.00 |
| GO:2000641 | regulation of early endosome to late endosome transport | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:1902745 | positive regulation of lamellipodium organization | 0.00 | 0.00 | 13.33 | 4.00 |
| GO:2000249 | regulation of actin cytoskeleton reorganization | 0.00 | 0.00 | 7.50 | 3.00 |
| GO:2001242 | regulation of intrinsic apoptotic signaling pathway | 0.00 | 0.00 | 4.44 | 6.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.01 | 0.00 | 4.60 | 4.00 |
| GO:2001243 | negative regulation of intrinsic apoptotic signaling pathway | 0.00 | 0.00 | 5.62 | 5.00 |
| GO:0043149 | stress fiber assembly | 0.01 | 0.00 | 4.40 | 4.00 |
| GO:0048857 | neural nucleus development | 0.00 | 0.00 | 10.34 | 6.00 |
| GO:0090559 | regulation of membrane permeability | 0.00 | 0.00 | 5.33 | 4.00 |
| GO:1905710 | positive regulation of membrane permeability | 0.01 | 0.00 | 4.92 | 3.00 |
| GO:0042743 | hydrogen peroxide metabolic process | 0.01 | 0.00 | 6.00 | 3.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:0008637 | apoptotic mitochondrial changes | 0.00 | 0.00 | 4.39 | 5.00 |
| GO:0046902 | regulation of mitochondrial membrane permeability | 0.02 | 0.00 | 4.62 | 3.00 |
| GO:1905477 | positive regulation of protein localization to membrane | 0.00 | 0.00 | 4.46 | 5.00 |
| GO:0030901 | midbrain development | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0008625 | extrinsic apoptotic signaling pathway via death domain receptors | 0.02 | 0.00 | 4.00 | 3.00 |
| GO:0010822 | positive regulation of mitochondrion organization | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0051205 | protein insertion into membrane | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:0090151 | establishment of protein localization to mitochondrial membrane | 0.01 | 0.00 | 6.25 | 3.00 |
| GO:1903749 | positive regulation of establishment of protein localization to mitochondrion | 0.00 | 0.00 | 6.45 | 4.00 |
| GO:2001235 | positive regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:0035794 | positive regulation of mitochondrial membrane permeability | 0.01 | 0.00 | 5.08 | 3.00 |
| GO:1902108 | regulation of mitochondrial membrane permeability involved in apoptotic process | 0.01 | 0.00 | 5.00 | 3.00 |
| GO:1903747 | regulation of establishment of protein localization to mitochondrion | 0.00 | 0.00 | 5.41 | 4.00 |
| GO:0021762 | substantia nigra development | 0.00 | 0.00 | 11.32 | 6.00 |
| GO:1902686 | mitochondrial outer membrane permeabilization involved in programmed cell death | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0051204 | protein insertion into mitochondrial membrane | 0.01 | 0.00 | 6.52 | 3.00 |

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|------------|--|------|------|-------|-------|
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0097345 | mitochondrial outer membrane permeabilization | 0.01 | 0.00 | 5.66 | 3.00 |
| GO:1902110 | positive regulation of mitochondrial membrane permeability involved in apoptotic process | 0.01 | 0.00 | 5.36 | 3.00 |
| GO:1901028 | regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway | 0.01 | 0.00 | 6.82 | 3.00 |
| GO:1901030 | positive regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway | 0.00 | 0.00 | 8.57 | 3.00 |
| GO:0001844 | protein insertion into mitochondrial membrane involved in apoptotic signaling pathway | 0.00 | 0.00 | 9.68 | 3.00 |
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |
| GO:1900739 | regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway | 0.00 | 0.00 | 11.11 | 3.00 |
| GO:1900740 | positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway | 0.00 | 0.00 | 11.11 | 3.00 |
| GO:0031589 | cell-substrate adhesion | 0.00 | 0.00 | 4.12 | 12.00 |
| GO:1901654 | response to ketone | 0.00 | 0.00 | 7.04 | 5.00 |
| GO:0010810 | regulation of cell-substrate adhesion | 0.00 | 0.00 | 6.02 | 10.00 |
| GO:0071706 | tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.44 | 6.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:0010812 | negative regulation of cell-substrate adhesion | 0.00 | 0.00 | 7.02 | 4.00 |
| GO:0032570 | response to progesterone | 0.00 | 0.00 | 15.00 | 3.00 |
| GO:0060348 | bone development | 0.00 | 0.00 | 5.17 | 6.00 |
| GO:0150116 | regulation of cell-substrate junction organization | 0.02 | 0.00 | 4.69 | 3.00 |
| GO:1901655 | cellular response to ketone | 0.00 | 0.00 | 7.14 | 3.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0010633 | negative regulation of epithelial cell migration | 0.02 | 0.00 | 4.35 | 3.00 |
| GO:0031333 | negative regulation of protein-containing complex assembly | 0.00 | 0.00 | 4.67 | 5.00 |
| GO:0032640 | tumor necrosis factor production | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:0140354 | lipid import into cell | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:1901889 | negative regulation of cell junction assembly | 0.00 | 0.00 | 10.00 | 3.00 |
| GO:1903555 | regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.58 | 6.00 |
| GO:1903649 | regulation of cytoplasmic transport | 0.00 | 0.00 | 14.81 | 4.00 |
| GO:2000641 | regulation of early endosome to late endosome transport | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:0001953 | negative regulation of cell-matrix adhesion | 0.00 | 0.00 | 8.33 | 3.00 |

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|------------|--|------|------|-------|-------|
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0008625 | extrinsic apoptotic signaling pathway via death domain receptors | 0.02 | 0.00 | 4.00 | 3.00 |
| GO:0090109 | regulation of cell-substrate junction assembly | 0.01 | 0.00 | 5.00 | 3.00 |
| GO:1901343 | negative regulation of vasculature development | 0.00 | 0.00 | 5.00 | 6.00 |
| GO:1903557 | positive regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.94 | 4.00 |
| GO:0010596 | negative regulation of endothelial cell migration | 0.01 | 0.00 | 5.77 | 3.00 |
| GO:0032680 | regulation of tumor necrosis factor production | 0.00 | 0.00 | 4.69 | 6.00 |
| GO:0061515 | myeloid cell development | 0.00 | 0.00 | 9.38 | 3.00 |
| GO:0015908 | fatty acid transport | 0.00 | 0.00 | 5.41 | 4.00 |
| GO:0032760 | positive regulation of tumor necrosis factor production | 0.00 | 0.00 | 5.06 | 4.00 |
| GO:2000181 | negative regulation of blood vessel morphogenesis | 0.00 | 0.00 | 5.56 | 6.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.01 | 0.00 | 4.60 | 4.00 |
| GO:2001243 | negative regulation of intrinsic apoptotic signaling pathway | 0.00 | 0.00 | 5.62 | 5.00 |
| GO:0043537 | negative regulation of blood vessel endothelial cell migration | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0016525 | negative regulation of angiogenesis | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0043149 | stress fiber assembly | 0.01 | 0.00 | 4.40 | 4.00 |
| GO:0022600 | digestive system process | 0.02 | 0.00 | 4.48 | 3.00 |
| GO:0031589 | cell-substrate adhesion | 0.00 | 0.00 | 4.12 | 12.00 |
| GO:0034381 | plasma lipoprotein particle clearance | 0.01 | 0.00 | 5.45 | 3.00 |
| GO:0048857 | neural nucleus development | 0.00 | 0.00 | 10.34 | 6.00 |
| GO:0061245 | establishment or maintenance of bipolar cell polarity | 0.00 | 0.00 | 13.51 | 5.00 |
| GO:0061339 | establishment or maintenance of monopolar cell polarity | 0.00 | 0.00 | 26.67 | 4.00 |
| GO:0006909 | phagocytosis | 0.00 | 0.00 | 4.64 | 17.00 |
| GO:0035088 | establishment or maintenance of apical/basal cell polarity | 0.00 | 0.00 | 13.51 | 5.00 |
| GO:0061162 | establishment of monopolar cell polarity | 0.00 | 0.00 | 28.57 | 4.00 |
| GO:0090162 | establishment of epithelial cell polarity | 0.00 | 0.00 | 22.22 | 4.00 |
| GO:0098927 | vesicle-mediated transport between endosomal compartments | 0.00 | 0.00 | 11.63 | 5.00 |
| GO:1901654 | response to ketone | 0.00 | 0.00 | 7.04 | 5.00 |
| GO:0010232 | vascular transport | 0.01 | 0.00 | 4.44 | 4.00 |
| GO:0010810 | regulation of cell-substrate adhesion | 0.00 | 0.00 | 6.02 | 10.00 |
| GO:0008360 | regulation of cell shape | 0.00 | 0.00 | 8.13 | 10.00 |
| GO:0000281 | mitotic cytokinesis | 0.02 | 0.00 | 4.17 | 3.00 |
| GO:0003158 | endothelium development | 0.00 | 0.00 | 5.10 | 5.00 |
| GO:0008361 | regulation of cell size | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0010324 | membrane invagination | 0.00 | 0.00 | 6.49 | 10.00 |
| GO:0010811 | positive regulation of cell-substrate adhesion | 0.00 | 0.00 | 5.75 | 5.00 |

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|------------|---|------|------|-------|------|
| GO:0010812 | negative regulation of cell-substrate adhesion | 0.00 | 0.00 | 7.02 | 4.00 |
| GO:0034446 | substrate adhesion-dependent cell spreading | 0.01 | 0.00 | 4.21 | 4.00 |
| GO:0035089 | establishment of apical/basal cell polarity | 0.00 | 0.00 | 33.33 | 4.00 |
| GO:0045022 | early endosome to late endosome transport | 0.00 | 0.00 | 12.20 | 5.00 |
| GO:0045197 | establishment or maintenance of epithelial cell apical/basal polarity | 0.00 | 0.00 | 15.15 | 5.00 |
| GO:0045807 | positive regulation of endocytosis | 0.00 | 0.00 | 4.88 | 4.00 |
| GO:0060191 | regulation of lipase activity | 0.01 | 0.00 | 4.08 | 4.00 |
| GO:0070671 | response to interleukin-12 | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0150116 | regulation of cell-substrate junction organization | 0.02 | 0.00 | 4.69 | 3.00 |
| GO:1901655 | cellular response to ketone | 0.00 | 0.00 | 7.14 | 3.00 |
| GO:0031122 | cytoplasmic microtubule organization | 0.01 | 0.00 | 5.00 | 3.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0010633 | negative regulation of epithelial cell migration | 0.02 | 0.00 | 4.35 | 3.00 |
| GO:0031333 | negative regulation of protein-containing complex assembly | 0.00 | 0.00 | 4.67 | 5.00 |
| GO:0031532 | actin cytoskeleton reorganization | 0.00 | 0.00 | 4.72 | 5.00 |
| GO:0045446 | endothelial cell differentiation | 0.01 | 0.00 | 4.76 | 4.00 |
| GO:0150104 | transport across blood-brain barrier | 0.01 | 0.00 | 4.44 | 4.00 |
| GO:1903649 | regulation of cytoplasmic transport | 0.00 | 0.00 | 14.81 | 4.00 |
| GO:1903651 | positive regulation of cytoplasmic transport | 0.00 | 0.00 | 21.43 | 3.00 |
| GO:2000641 | regulation of early endosome to late endosome transport | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:0030901 | midbrain development | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0001885 | endothelial cell development | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:0001953 | negative regulation of cell-matrix adhesion | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0030859 | polarized epithelial cell differentiation | 0.00 | 0.00 | 28.57 | 4.00 |
| GO:0035722 | interleukin-12-mediated signaling pathway | 0.01 | 0.00 | 5.36 | 3.00 |
| GO:0090109 | regulation of cell-substrate junction assembly | 0.01 | 0.00 | 5.00 | 3.00 |
| GO:1900024 | regulation of substrate adhesion-dependent cell spreading | 0.01 | 0.00 | 5.77 | 3.00 |
| GO:1905666 | regulation of protein localization to endosome | 0.00 | 0.00 | 30.00 | 3.00 |
| GO:1905668 | positive regulation of protein localization to endosome | 0.00 | 0.00 | 33.33 | 3.00 |
| GO:0061572 | actin filament bundle organization | 0.00 | 0.00 | 4.96 | 7.00 |
| GO:0010596 | negative regulation of endothelial cell migration | 0.01 | 0.00 | 5.77 | 3.00 |
| GO:0045198 | establishment of epithelial cell apical/basal polarity | 0.00 | 0.00 | 44.44 | 4.00 |
| GO:1900026 | positive regulation of substrate adhesion-dependent cell spreading | 0.00 | 0.00 | 7.89 | 3.00 |
| GO:1902745 | positive regulation of lamellipodium organization | 0.00 | 0.00 | 13.33 | 4.00 |
| GO:2000249 | regulation of actin cytoskeleton reorganization | 0.00 | 0.00 | 7.50 | 3.00 |

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|------------|--|------|------|-------|------|
| GO:2000643 | positive regulation of early endosome to late endosome transport | 0.00 | 0.00 | 37.50 | 3.00 |
| GO:0051017 | actin filament bundle assembly | 0.00 | 0.00 | 5.07 | 7.00 |
| GO:0021762 | substantia nigra development | 0.00 | 0.00 | 11.32 | 6.00 |
| GO:0032231 | regulation of actin filament bundle assembly | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0050701 | interleukin-1 secretion | 0.01 | 0.00 | 4.92 | 3.00 |
| GO:1902966 | positive regulation of protein localization to early endosome | 0.00 | 0.00 | 33.33 | 3.00 |
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0043537 | negative regulation of blood vessel endothelial cell migration | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0043149 | stress fiber assembly | 0.01 | 0.00 | 4.40 | 4.00 |
| GO:0050702 | interleukin-1 beta secretion | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |
| GO:0097006 | regulation of plasma lipoprotein particle levels | 0.00 | 0.00 | 6.90 | 6.00 |
| GO:0022600 | digestive system process | 0.02 | 0.00 | 4.48 | 3.00 |
| GO:0034381 | plasma lipoprotein particle clearance | 0.01 | 0.00 | 5.45 | 3.00 |
| GO:0071402 | cellular response to lipoprotein particle stimulus | 0.00 | 0.00 | 12.00 | 3.00 |
| GO:0019915 | lipid storage | 0.00 | 0.00 | 7.25 | 5.00 |
| GO:0050818 | regulation of coagulation | 0.00 | 0.00 | 4.94 | 4.00 |
| GO:1900046 | regulation of hemostasis | 0.00 | 0.00 | 5.13 | 4.00 |
| GO:1905952 | regulation of lipid localization | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:1905954 | positive regulation of lipid localization | 0.00 | 0.00 | 6.49 | 5.00 |
| GO:1990748 | cellular detoxification | 0.00 | 0.00 | 4.59 | 5.00 |
| GO:0010883 | regulation of lipid storage | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:0050819 | negative regulation of coagulation | 0.00 | 0.00 | 7.69 | 4.00 |
| GO:0055094 | response to lipoprotein particle | 0.00 | 0.00 | 12.50 | 3.00 |
| GO:1900047 | negative regulation of hemostasis | 0.00 | 0.00 | 8.16 | 4.00 |
| GO:0010232 | vascular transport | 0.01 | 0.00 | 4.44 | 4.00 |
| GO:0010884 | positive regulation of lipid storage | 0.00 | 0.00 | 13.04 | 3.00 |
| GO:0030730 | sequestering of triglyceride | 0.00 | 0.00 | 15.79 | 3.00 |
| GO:0071706 | tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.44 | 6.00 |
| GO:0098869 | cellular oxidant detoxification | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:1903035 | negative regulation of response to wounding | 0.00 | 0.00 | 6.10 | 5.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:0034369 | plasma lipoprotein particle remodeling | 0.00 | 0.00 | 12.90 | 4.00 |
| GO:0034377 | plasma lipoprotein particle assembly | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0008361 | regulation of cell size | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0010811 | positive regulation of cell-substrate adhesion | 0.00 | 0.00 | 5.75 | 5.00 |
| GO:0010812 | negative regulation of cell-substrate adhesion | 0.00 | 0.00 | 7.02 | 4.00 |
| GO:0010889 | regulation of sequestering of triglyceride | 0.00 | 0.00 | 18.75 | 3.00 |
| GO:0030193 | regulation of blood coagulation | 0.00 | 0.00 | 5.13 | 4.00 |

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|------------|---|------|------|-------|------|
| GO:0034367 | protein-containing complex remodeling | 0.00 | 0.00 | 12.50 | 4.00 |
| GO:0034446 | substrate adhesion-dependent cell spreading | 0.01 | 0.00 | 4.21 | 4.00 |
| GO:0045807 | positive regulation of endocytosis | 0.00 | 0.00 | 4.88 | 4.00 |
| GO:0050680 | negative regulation of epithelial cell proliferation | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0060191 | regulation of lipase activity | 0.01 | 0.00 | 4.08 | 4.00 |
| GO:0071825 | protein-lipid complex subunit organization | 0.00 | 0.00 | 9.80 | 5.00 |
| GO:0150116 | regulation of cell-substrate junction organization | 0.02 | 0.00 | 4.69 | 3.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0010633 | negative regulation of epithelial cell migration | 0.02 | 0.00 | 4.35 | 3.00 |
| GO:0010890 | positive regulation of sequestering of triglyceride | 0.00 | 0.00 | 27.27 | 3.00 |
| GO:0016209 | antioxidant activity | 0.00 | 0.00 | 5.56 | 5.00 |
| GO:0030195 | negative regulation of blood coagulation | 0.00 | 0.00 | 8.16 | 4.00 |
| GO:0031333 | negative regulation of protein-containing complex assembly | 0.00 | 0.00 | 4.67 | 5.00 |
| GO:0031532 | actin cytoskeleton reorganization | 0.00 | 0.00 | 4.72 | 5.00 |
| GO:0032640 | tumor necrosis factor production | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:0044070 | regulation of anion transport | 0.02 | 0.00 | 4.69 | 3.00 |
| GO:0046503 | glycerolipid catabolic process | 0.02 | 0.00 | 4.29 | 3.00 |
| GO:0046889 | positive regulation of lipid biosynthetic process | 0.02 | 0.00 | 4.29 | 3.00 |
| GO:0061045 | negative regulation of wound healing | 0.00 | 0.00 | 7.14 | 5.00 |
| GO:0140354 | lipid import into cell | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0150104 | transport across blood-brain barrier | 0.01 | 0.00 | 4.44 | 4.00 |
| GO:1902653 | secondary alcohol biosynthetic process | 0.02 | 0.00 | 4.11 | 3.00 |
| GO:1903555 | regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.58 | 6.00 |
| GO:0034370 | triglyceride-rich lipoprotein particle remodeling | 0.00 | 0.00 | 20.00 | 3.00 |
| GO:0034375 | high-density lipoprotein particle remodeling | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0001937 | negative regulation of endothelial cell proliferation | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0090109 | regulation of cell-substrate junction assembly | 0.01 | 0.00 | 5.00 | 3.00 |
| GO:1901343 | negative regulation of vasculature development | 0.00 | 0.00 | 5.00 | 6.00 |
| GO:0006656 | phosphatidylcholine biosynthetic process | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0010596 | negative regulation of endothelial cell migration | 0.01 | 0.00 | 5.77 | 3.00 |
| GO:0032680 | regulation of tumor necrosis factor production | 0.00 | 0.00 | 4.69 | 6.00 |
| GO:2000249 | regulation of actin cytoskeleton reorganization | 0.00 | 0.00 | 7.50 | 3.00 |
| GO:0034371 | chylomicron remodeling | 0.00 | 0.00 | 27.27 | 3.00 |

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|------------|--|------|------|-------|------|
| GO:0034372 | very-low-density lipoprotein particle remodeling | 0.00 | 0.00 | 23.08 | 3.00 |
| GO:0015908 | fatty acid transport | 0.00 | 0.00 | 5.41 | 4.00 |
| GO:0032231 | regulation of actin filament bundle assembly | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0050701 | interleukin-1 secretion | 0.01 | 0.00 | 4.92 | 3.00 |
| GO:0050710 | negative regulation of cytokine secretion | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:2000181 | negative regulation of blood vessel morphogenesis | 0.00 | 0.00 | 5.56 | 6.00 |
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0043537 | negative regulation of blood vessel endothelial cell migration | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0060996 | dendritic spine development | 0.02 | 0.00 | 4.23 | 3.00 |
| GO:0016525 | negative regulation of angiogenesis | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0043149 | stress fiber assembly | 0.01 | 0.00 | 4.40 | 4.00 |
| GO:0050702 | interleukin-1 beta secretion | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |

Table A3-2. List of significant gene ontology terms associated with differential abundant proteins on the comparison between colostrum exosomes (day 0) and milk exosomes at day 7.

| GOID | GOTerm | Term PValue | Group PValue | % Associated Genes | Nr. Genes |
|------------|---|-------------|--------------|--------------------|-----------|
| GO:0006887 | exocytosis | 0.00 | 0.00 | 4.72 | 41.00 |
| GO:0045055 | regulated exocytosis | 0.00 | 0.00 | 4.62 | 36.00 |
| GO:0043312 | neutrophil degranulation | 0.00 | 0.00 | 5.23 | 28.00 |
| GO:0002443 | leukocyte mediated immunity | 0.00 | 0.00 | 4.01 | 35.00 |
| GO:0002283 | neutrophil activation involved in immune response | 0.00 | 0.00 | 5.20 | 28.00 |
| GO:0002446 | neutrophil mediated immunity | 0.00 | 0.00 | 5.14 | 28.00 |
| GO:0042119 | neutrophil activation | 0.00 | 0.00 | 5.12 | 28.00 |
| GO:0036230 | granulocyte activation | 0.00 | 0.00 | 5.06 | 28.00 |
| GO:0002274 | myeloid leukocyte activation | 0.00 | 0.00 | 4.61 | 30.00 |
| GO:0043299 | leukocyte degranulation | 0.00 | 0.00 | 4.95 | 28.00 |
| GO:0002275 | myeloid cell activation involved in immune response | 0.00 | 0.00 | 4.90 | 28.00 |
| GO:0002444 | myeloid leukocyte mediated immunity | 0.00 | 0.00 | 4.85 | 28.00 |
| GO:0060627 | regulation of vesicle-mediated transport | 0.00 | 0.00 | 5.40 | 23.00 |
| GO:0060627 | regulation of vesicle-mediated transport | 0.00 | 0.00 | 5.40 | 23.00 |
| GO:0060627 | regulation of vesicle-mediated transport | 0.00 | 0.00 | 5.40 | 23.00 |
| GO:0007265 | Ras protein signal transduction | 0.00 | 0.00 | 5.63 | 20.00 |
| GO:0007264 | small GTPase mediated signal transduction | 0.00 | 0.00 | 4.74 | 23.00 |
| GO:0006897 | endocytosis | 0.00 | 0.00 | 4.04 | 23.00 |
| GO:0006959 | humoral immune response | 0.00 | 0.00 | 4.65 | 18.00 |
| GO:0006959 | humoral immune response | 0.00 | 0.00 | 4.65 | 18.00 |

| | | | | | |
|------------|--|------|------|-------|-------|
| GO:0010324 | membrane invagination | 0.00 | 0.00 | 7.79 | 12.00 |
| GO:0006909 | phagocytosis | 0.00 | 0.00 | 4.64 | 17.00 |
| GO:0010810 | regulation of cell-substrate adhesion | 0.00 | 0.00 | 7.23 | 12.00 |
| GO:0010810 | regulation of cell-substrate adhesion | 0.00 | 0.00 | 7.23 | 12.00 |
| GO:0010810 | regulation of cell-substrate adhesion | 0.00 | 0.00 | 7.23 | 12.00 |
| GO:0006911 | phagocytosis. engulfment | 0.00 | 0.00 | 7.97 | 11.00 |
| GO:0006911 | phagocytosis. engulfment | 0.00 | 0.00 | 7.97 | 11.00 |
| GO:0031589 | cell-substrate adhesion | 0.00 | 0.00 | 5.15 | 15.00 |
| GO:0031589 | cell-substrate adhesion | 0.00 | 0.00 | 5.15 | 15.00 |
| GO:0019068 | virion assembly | 0.00 | 0.00 | 15.91 | 7.00 |
| GO:0008360 | regulation of cell shape | 0.00 | 0.00 | 8.13 | 10.00 |
| GO:0008360 | regulation of cell shape | 0.00 | 0.00 | 8.13 | 10.00 |
| GO:0019058 | viral life cycle | 0.00 | 0.00 | 4.98 | 14.00 |
| GO:0045921 | positive regulation of exocytosis | 0.00 | 0.00 | 11.27 | 8.00 |
| GO:0045921 | positive regulation of exocytosis | 0.00 | 0.00 | 11.27 | 8.00 |
| GO:0045921 | positive regulation of exocytosis | 0.00 | 0.00 | 11.27 | 8.00 |
| GO:0006956 | complement activation | 0.00 | 0.00 | 5.94 | 12.00 |
| GO:0051047 | positive regulation of secretion | 0.00 | 0.00 | 4.53 | 15.00 |
| GO:0051047 | positive regulation of secretion | 0.00 | 0.00 | 4.53 | 15.00 |
| GO:0001895 | retina homeostasis | 0.00 | 0.00 | 10.96 | 8.00 |
| GO:0001895 | retina homeostasis | 0.00 | 0.00 | 10.96 | 8.00 |
| GO:0022604 | regulation of cell morphogenesis | 0.00 | 0.00 | 4.16 | 16.00 |
| GO:0022604 | regulation of cell morphogenesis | 0.00 | 0.00 | 4.16 | 16.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 26.32 | 5.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 26.32 | 5.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 26.32 | 5.00 |
| GO:1990000 | amyloid fibril formation | 0.00 | 0.00 | 26.32 | 5.00 |
| GO:0045198 | establishment of epithelial cell apical/basal polarity | 0.00 | 0.00 | 44.44 | 4.00 |
| GO:1903532 | positive regulation of secretion by cell | 0.00 | 0.00 | 4.40 | 14.00 |
| GO:1903532 | positive regulation of secretion by cell | 0.00 | 0.00 | 4.40 | 14.00 |
| GO:0001894 | tissue homeostasis | 0.00 | 0.00 | 5.85 | 11.00 |
| GO:0010811 | positive regulation of cell-substrate adhesion | 0.00 | 0.00 | 9.20 | 8.00 |
| GO:0010811 | positive regulation of cell-substrate adhesion | 0.00 | 0.00 | 9.20 | 8.00 |
| GO:2001233 | regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.32 | 14.00 |
| GO:2001233 | regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.32 | 14.00 |
| GO:2001233 | regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.32 | 14.00 |
| GO:2001233 | regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.32 | 14.00 |
| GO:0031647 | regulation of protein stability | 0.00 | 0.00 | 4.59 | 13.00 |
| GO:0030449 | regulation of complement activation | 0.00 | 0.00 | 7.20 | 9.00 |
| GO:0035089 | establishment of apical/basal cell polarity | 0.00 | 0.00 | 33.33 | 4.00 |
| GO:0061045 | negative regulation of wound healing | 0.00 | 0.00 | 10.00 | 7.00 |
| GO:0002920 | regulation of humoral immune response | 0.00 | 0.00 | 6.67 | 9.00 |
| GO:0032102 | negative regulation of response to external stimulus | 0.00 | 0.00 | 4.25 | 13.00 |
| GO:0032102 | negative regulation of response to external stimulus | 0.00 | 0.00 | 4.25 | 13.00 |

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|------------|---|------|------|-------|-------|
| GO:0006898 | receptor-mediated endocytosis | 0.00 | 0.00 | 4.22 | 13.00 |
| GO:0006898 | receptor-mediated endocytosis | 0.00 | 0.00 | 4.22 | 13.00 |
| GO:0006898 | receptor-mediated endocytosis | 0.00 | 0.00 | 4.22 | 13.00 |
| GO:0002576 | platelet degranulation | 0.00 | 0.00 | 6.52 | 9.00 |
| GO:0002576 | platelet degranulation | 0.00 | 0.00 | 6.52 | 9.00 |
| GO:0002576 | platelet degranulation | 0.00 | 0.00 | 6.52 | 9.00 |
| GO:0002576 | platelet degranulation | 0.00 | 0.00 | 6.52 | 9.00 |
| GO:1900047 | negative regulation of hemostasis | 0.00 | 0.00 | 12.24 | 6.00 |
| GO:0030195 | negative regulation of blood coagulation | 0.00 | 0.00 | 12.24 | 6.00 |
| GO:1900047 | negative regulation of hemostasis | 0.00 | 0.00 | 12.24 | 6.00 |
| GO:0030195 | negative regulation of blood coagulation | 0.00 | 0.00 | 12.24 | 6.00 |
| GO:1900047 | negative regulation of hemostasis | 0.00 | 0.00 | 12.24 | 6.00 |
| GO:0030195 | negative regulation of blood coagulation | 0.00 | 0.00 | 12.24 | 6.00 |
| GO:0061162 | establishment of monopolar cell polarity | 0.00 | 0.00 | 28.57 | 4.00 |
| GO:0030859 | polarized epithelial cell differentiation | 0.00 | 0.00 | 28.57 | 4.00 |
| GO:0002221 | pattern recognition receptor signaling pathway | 0.00 | 0.00 | 5.38 | 10.00 |
| GO:0002221 | pattern recognition receptor signaling pathway | 0.00 | 0.00 | 5.38 | 10.00 |
| GO:0050819 | negative regulation of coagulation | 0.00 | 0.00 | 11.54 | 6.00 |
| GO:0050819 | negative regulation of coagulation | 0.00 | 0.00 | 11.54 | 6.00 |
| GO:0050819 | negative regulation of coagulation | 0.00 | 0.00 | 11.54 | 6.00 |
| GO:0021762 | substantia nigra development | 0.00 | 0.00 | 11.32 | 6.00 |
| GO:0021762 | substantia nigra development | 0.00 | 0.00 | 11.32 | 6.00 |
| GO:0016485 | protein processing | 0.00 | 0.00 | 5.26 | 10.00 |
| GO:0061339 | establishment or maintenance of monopolar cell polarity | 0.00 | 0.00 | 26.67 | 4.00 |
| GO:0045807 | positive regulation of endocytosis | 0.00 | 0.00 | 8.54 | 7.00 |
| GO:0045807 | positive regulation of endocytosis | 0.00 | 0.00 | 8.54 | 7.00 |
| GO:0045807 | positive regulation of endocytosis | 0.00 | 0.00 | 8.54 | 7.00 |
| GO:1903035 | negative regulation of response to wounding | 0.00 | 0.00 | 8.54 | 7.00 |
| GO:0050821 | protein stabilization | 0.00 | 0.00 | 5.18 | 10.00 |
| GO:0050821 | protein stabilization | 0.00 | 0.00 | 5.18 | 10.00 |
| GO:0007160 | cell-matrix adhesion | 0.00 | 0.00 | 5.13 | 10.00 |
| GO:0007160 | cell-matrix adhesion | 0.00 | 0.00 | 5.13 | 10.00 |
| GO:0045197 | establishment or maintenance of epithelial cell apical/basal polarity | 0.00 | 0.00 | 15.15 | 5.00 |
| GO:0042742 | defense response to bacterium | 0.00 | 0.00 | 4.10 | 12.00 |
| GO:0042742 | defense response to bacterium | 0.00 | 0.00 | 4.10 | 12.00 |
| GO:0048857 | neural nucleus development | 0.00 | 0.00 | 10.34 | 6.00 |
| GO:0048857 | neural nucleus development | 0.00 | 0.00 | 10.34 | 6.00 |
| GO:0019731 | antibacterial humoral response | 0.00 | 0.00 | 10.17 | 6.00 |
| GO:0019731 | antibacterial humoral response | 0.00 | 0.00 | 10.17 | 6.00 |
| GO:0019731 | antibacterial humoral response | 0.00 | 0.00 | 10.17 | 6.00 |
| GO:0019731 | antibacterial humoral response | 0.00 | 0.00 | 10.17 | 6.00 |
| GO:0061077 | chaperone-mediated protein folding | 0.00 | 0.00 | 10.00 | 6.00 |
| GO:0062207 | regulation of pattern recognition receptor signaling pathway | 0.00 | 0.00 | 7.69 | 7.00 |
| GO:0140354 | lipid import into cell | 0.00 | 0.00 | 22.22 | 4.00 |

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|------------|--|------|------|-------|-------|
| GO:0140354 | lipid import into cell | 0.00 | 0.00 | 22.22 | 4.00 |
| GO:0090162 | establishment of epithelial cell polarity | 0.00 | 0.00 | 22.22 | 4.00 |
| GO:0140354 | lipid import into cell | 0.00 | 0.00 | 22.22 | 4.00 |
| GO:0061245 | establishment or maintenance of bipolar cell polarity | 0.00 | 0.00 | 13.51 | 5.00 |
| GO:0035088 | establishment or maintenance of apical/basal cell polarity | 0.00 | 0.00 | 13.51 | 5.00 |
| GO:1900026 | positive regulation of substrate adhesion-dependent cell spreading | 0.00 | 0.00 | 13.16 | 5.00 |
| GO:1900026 | positive regulation of substrate adhesion-dependent cell spreading | 0.00 | 0.00 | 13.16 | 5.00 |
| GO:1990182 | exosomal secretion | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:1990182 | exosomal secretion | 0.00 | 0.00 | 21.05 | 4.00 |
| GO:0097734 | extracellular exosome biogenesis | 0.00 | 0.00 | 20.00 | 4.00 |
| GO:0097734 | extracellular exosome biogenesis | 0.00 | 0.00 | 20.00 | 4.00 |
| GO:0019730 | antimicrobial humoral response | 0.00 | 0.00 | 5.88 | 8.00 |
| GO:0019730 | antimicrobial humoral response | 0.00 | 0.00 | 5.88 | 8.00 |
| GO:2000643 | positive regulation of early endosome to late endosome transport | 0.00 | 0.00 | 37.50 | 3.00 |
| GO:0002224 | toll-like receptor signaling pathway | 0.00 | 0.00 | 5.63 | 8.00 |
| GO:0002224 | toll-like receptor signaling pathway | 0.00 | 0.00 | 5.63 | 8.00 |
| GO:0140112 | extracellular vesicle biogenesis | 0.00 | 0.00 | 18.18 | 4.00 |
| GO:0140112 | extracellular vesicle biogenesis | 0.00 | 0.00 | 18.18 | 4.00 |
| GO:0043903 | regulation of interspecies interactions between organisms | 0.00 | 0.00 | 4.33 | 10.00 |
| GO:0043903 | regulation of interspecies interactions between organisms | 0.00 | 0.00 | 4.33 | 10.00 |
| GO:1905668 | positive regulation of protein localization to endosome | 0.00 | 0.00 | 33.33 | 3.00 |
| GO:1902966 | positive regulation of protein localization to early endosome | 0.00 | 0.00 | 33.33 | 3.00 |
| GO:2001234 | negative regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.64 | 9.00 |
| GO:2001234 | negative regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.64 | 9.00 |
| GO:2001234 | negative regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.64 | 9.00 |
| GO:2001234 | negative regulation of apoptotic signaling pathway | 0.00 | 0.00 | 4.64 | 9.00 |
| GO:1905954 | positive regulation of lipid localization | 0.00 | 0.00 | 7.79 | 6.00 |
| GO:1905954 | positive regulation of lipid localization | 0.00 | 0.00 | 7.79 | 6.00 |
| GO:0017157 | regulation of exocytosis | 0.00 | 0.00 | 5.26 | 8.00 |
| GO:0017157 | regulation of exocytosis | 0.00 | 0.00 | 5.26 | 8.00 |
| GO:0017157 | regulation of exocytosis | 0.00 | 0.00 | 5.26 | 8.00 |
| GO:1900046 | regulation of hemostasis | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0030193 | regulation of blood coagulation | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0030901 | midbrain development | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0030901 | midbrain development | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:1900046 | regulation of hemostasis | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0030193 | regulation of blood coagulation | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:1900046 | regulation of hemostasis | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0030193 | regulation of blood coagulation | 0.00 | 0.00 | 7.69 | 6.00 |
| GO:0034114 | regulation of heterotypic cell-cell adhesion | 0.00 | 0.00 | 15.38 | 4.00 |

| | | | | | |
|------------|--|------|------|-------|------|
| GO:0034114 | regulation of heterotypic cell-cell adhesion | 0.00 | 0.00 | 15.38 | 4.00 |
| GO:0034114 | regulation of heterotypic cell-cell adhesion | 0.00 | 0.00 | 15.38 | 4.00 |
| GO:0060348 | bone development | 0.00 | 0.00 | 6.03 | 7.00 |
| GO:1905666 | regulation of protein localization to endosome | 0.00 | 0.00 | 30.00 | 3.00 |
| GO:0050818 | regulation of coagulation | 0.00 | 0.00 | 7.41 | 6.00 |
| GO:0050818 | regulation of coagulation | 0.00 | 0.00 | 7.41 | 6.00 |
| GO:0050818 | regulation of coagulation | 0.00 | 0.00 | 7.41 | 6.00 |
| GO:0071825 | protein-lipid complex subunit organization | 0.00 | 0.00 | 9.80 | 5.00 |
| GO:0051085 | chaperone cofactor-dependent protein refolding | 0.00 | 0.00 | 14.81 | 4.00 |
| GO:0042730 | fibrinolysis | 0.00 | 0.00 | 14.81 | 4.00 |
| GO:0042730 | fibrinolysis | 0.00 | 0.00 | 14.81 | 4.00 |
| GO:0042730 | fibrinolysis | 0.00 | 0.00 | 14.81 | 4.00 |
| GO:1900024 | regulation of substrate adhesion-dependent cell spreading | 0.00 | 0.00 | 9.62 | 5.00 |
| GO:1900024 | regulation of substrate adhesion-dependent cell spreading | 0.00 | 0.00 | 9.62 | 5.00 |
| GO:1902115 | regulation of organelle assembly | 0.00 | 0.00 | 4.91 | 8.00 |
| GO:0034371 | chylomicron remodeling | 0.00 | 0.00 | 27.27 | 3.00 |
| GO:0030100 | regulation of endocytosis | 0.00 | 0.00 | 4.82 | 8.00 |
| GO:0030100 | regulation of endocytosis | 0.00 | 0.00 | 4.82 | 8.00 |
| GO:0030100 | regulation of endocytosis | 0.00 | 0.00 | 4.82 | 8.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 6.90 | 6.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 6.90 | 6.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 6.90 | 6.00 |
| GO:2001237 | negative regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 6.90 | 6.00 |
| GO:0097006 | regulation of plasma lipoprotein particle levels | 0.00 | 0.00 | 6.90 | 6.00 |
| GO:0050792 | regulation of viral process | 0.00 | 0.00 | 4.15 | 9.00 |
| GO:0050792 | regulation of viral process | 0.00 | 0.00 | 4.15 | 9.00 |
| GO:0034369 | plasma lipoprotein particle remodeling | 0.00 | 0.00 | 12.90 | 4.00 |
| GO:0034369 | plasma lipoprotein particle remodeling | 0.00 | 0.00 | 12.90 | 4.00 |
| GO:2001235 | positive regulation of apoptotic signaling pathway | 0.00 | 0.00 | 5.38 | 7.00 |
| GO:2001235 | positive regulation of apoptotic signaling pathway | 0.00 | 0.00 | 5.38 | 7.00 |
| GO:1905952 | regulation of lipid localization | 0.00 | 0.00 | 5.38 | 7.00 |
| GO:1905952 | regulation of lipid localization | 0.00 | 0.00 | 5.38 | 7.00 |
| GO:0007009 | plasma membrane organization | 0.00 | 0.00 | 6.52 | 6.00 |
| GO:0051084 | 'de novo' posttranslational protein folding | 0.00 | 0.00 | 12.50 | 4.00 |
| GO:0034367 | protein-containing complex remodeling | 0.00 | 0.00 | 12.50 | 4.00 |
| GO:0034367 | protein-containing complex remodeling | 0.00 | 0.00 | 12.50 | 4.00 |
| GO:0034372 | very-low-density lipoprotein particle remodeling | 0.00 | 0.00 | 23.08 | 3.00 |
| GO:0034446 | substrate adhesion-dependent cell spreading | 0.00 | 0.00 | 6.32 | 6.00 |
| GO:0034446 | substrate adhesion-dependent cell spreading | 0.00 | 0.00 | 6.32 | 6.00 |

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|------------|--|------|------|-------|------|
| GO:0061041 | regulation of wound healing | 0.00 | 0.00 | 5.11 | 7.00 |
| GO:0002062 | chondrocyte differentiation | 0.03 | 0.00 | 4.00 | 3.00 |
| GO:2001267 | regulation of cysteine-type endopeptidase activity involved in apoptotic signaling pathway | 0.00 | 0.00 | 21.43 | 3.00 |
| GO:1903651 | positive regulation of cytoplasmic transport | 0.00 | 0.00 | 21.43 | 3.00 |
| GO:2001267 | regulation of cysteine-type endopeptidase activity involved in apoptotic signaling pathway | 0.00 | 0.00 | 21.43 | 3.00 |
| GO:1902905 | positive regulation of supramolecular fiber organization | 0.00 | 0.00 | 4.28 | 8.00 |
| GO:1902905 | positive regulation of supramolecular fiber organization | 0.00 | 0.00 | 4.28 | 8.00 |
| GO:1902905 | positive regulation of supramolecular fiber organization | 0.00 | 0.00 | 4.28 | 8.00 |
| GO:1902905 | positive regulation of supramolecular fiber organization | 0.00 | 0.00 | 4.28 | 8.00 |
| GO:0006958 | complement activation, classical pathway | 0.00 | 0.00 | 4.93 | 7.00 |
| GO:0006958 | complement activation, classical pathway | 0.00 | 0.00 | 4.93 | 7.00 |
| GO:0006458 | 'de novo' protein folding | 0.00 | 0.00 | 11.11 | 4.00 |
| GO:0034116 | positive regulation of heterotypic cell-cell adhesion | 0.00 | 0.00 | 20.00 | 3.00 |
| GO:1903543 | positive regulation of exosomal secretion | 0.00 | 0.00 | 20.00 | 3.00 |
| GO:0034116 | positive regulation of heterotypic cell-cell adhesion | 0.00 | 0.00 | 20.00 | 3.00 |
| GO:1903543 | positive regulation of exosomal secretion | 0.00 | 0.00 | 20.00 | 3.00 |
| GO:0034116 | positive regulation of heterotypic cell-cell adhesion | 0.00 | 0.00 | 20.00 | 3.00 |
| GO:0034370 | triglyceride-rich lipoprotein particle remodeling | 0.00 | 0.00 | 20.00 | 3.00 |
| GO:0034121 | regulation of toll-like receptor signaling pathway | 0.00 | 0.00 | 7.58 | 5.00 |
| GO:2000377 | regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 4.79 | 7.00 |
| GO:1903541 | regulation of exosomal secretion | 0.00 | 0.00 | 18.75 | 3.00 |
| GO:0046597 | negative regulation of viral entry into host cell | 0.00 | 0.00 | 18.75 | 3.00 |
| GO:1903541 | regulation of exosomal secretion | 0.00 | 0.00 | 18.75 | 3.00 |
| GO:0031532 | actin cytoskeleton reorganization | 0.00 | 0.00 | 5.66 | 6.00 |
| GO:0031532 | actin cytoskeleton reorganization | 0.00 | 0.00 | 5.66 | 6.00 |
| GO:0031532 | actin cytoskeleton reorganization | 0.00 | 0.00 | 5.66 | 6.00 |
| GO:0031532 | actin cytoskeleton reorganization | 0.00 | 0.00 | 5.66 | 6.00 |
| GO:0019915 | lipid storage | 0.00 | 0.00 | 7.25 | 5.00 |
| GO:0002455 | humoral immune response mediated by circulating immunoglobulin | 0.00 | 0.00 | 4.67 | 7.00 |
| GO:0002455 | humoral immune response mediated by circulating immunoglobulin | 0.00 | 0.00 | 4.67 | 7.00 |
| GO:0051898 | negative regulation of protein kinase B signaling | 0.00 | 0.00 | 10.26 | 4.00 |
| GO:0062208 | positive regulation of pattern recognition receptor signaling pathway | 0.00 | 0.00 | 10.26 | 4.00 |
| GO:0062208 | positive regulation of pattern recognition receptor signaling pathway | 0.00 | 0.00 | 10.26 | 4.00 |
| GO:2000181 | negative regulation of blood vessel morphogenesis | 0.00 | 0.00 | 5.56 | 6.00 |

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|------------|---|------|------|-------|------|
| GO:0010770 | positive regulation of cell morphogenesis involved in differentiation | 0.00 | 0.00 | 5.56 | 6.00 |
| GO:0010770 | positive regulation of cell morphogenesis involved in differentiation | 0.00 | 0.00 | 5.56 | 6.00 |
| GO:2000181 | negative regulation of blood vessel morphogenesis | 0.00 | 0.00 | 5.56 | 6.00 |
| GO:0002433 | immune response-regulating cell surface receptor signaling pathway involved in phagocytosis | 0.00 | 0.00 | 4.64 | 7.00 |
| GO:0038096 | Fc-gamma receptor signaling pathway involved in phagocytosis | 0.00 | 0.00 | 4.64 | 7.00 |
| GO:0051092 | positive regulation of NF-kappaB transcription factor activity | 0.00 | 0.00 | 4.64 | 7.00 |
| GO:1903900 | regulation of viral life cycle | 0.00 | 0.00 | 4.58 | 7.00 |
| GO:0045022 | early endosome to late endosome transport | 0.00 | 0.00 | 9.76 | 4.00 |
| GO:0051701 | interaction with host | 0.00 | 0.00 | 4.52 | 7.00 |
| GO:1903034 | regulation of response to wounding | 0.00 | 0.00 | 4.52 | 7.00 |
| GO:0002431 | Fc receptor mediated stimulatory signaling pathway | 0.00 | 0.00 | 4.49 | 7.00 |
| GO:1902653 | secondary alcohol biosynthetic process | 0.03 | 0.00 | 4.11 | 3.00 |
| GO:0060263 | regulation of respiratory burst | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0060263 | regulation of respiratory burst | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0034375 | high-density lipoprotein particle remodeling | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0034375 | high-density lipoprotein particle remodeling | 0.00 | 0.00 | 16.67 | 3.00 |
| GO:0015908 | fatty acid transport | 0.00 | 0.00 | 6.76 | 5.00 |
| GO:0008625 | extrinsic apoptotic signaling pathway via death domain receptors | 0.00 | 0.00 | 6.67 | 5.00 |
| GO:0008625 | extrinsic apoptotic signaling pathway via death domain receptors | 0.00 | 0.00 | 6.67 | 5.00 |
| GO:0008625 | extrinsic apoptotic signaling pathway via death domain receptors | 0.00 | 0.00 | 6.67 | 5.00 |
| GO:0070268 | cornification | 0.00 | 0.00 | 5.26 | 6.00 |
| GO:0052126 | movement in host environment | 0.00 | 0.00 | 5.26 | 6.00 |
| GO:0098927 | vesicle-mediated transport between endosomal compartments | 0.00 | 0.00 | 9.30 | 4.00 |
| GO:2000641 | regulation of early endosome to late endosome transport | 0.00 | 0.00 | 15.79 | 3.00 |
| GO:0042026 | protein refolding | 0.00 | 0.00 | 15.00 | 3.00 |
| GO:0003413 | chondrocyte differentiation involved in endochondral bone morphogenesis | 0.00 | 0.00 | 15.00 | 3.00 |
| GO:1901343 | negative regulation of vasculature development | 0.00 | 0.00 | 5.00 | 6.00 |
| GO:1901343 | negative regulation of vasculature development | 0.00 | 0.00 | 5.00 | 6.00 |
| GO:0019079 | viral genome replication | 0.00 | 0.00 | 4.96 | 6.00 |
| GO:0019079 | viral genome replication | 0.00 | 0.00 | 4.96 | 6.00 |
| GO:0019079 | viral genome replication | 0.00 | 0.00 | 4.96 | 6.00 |
| GO:0042059 | negative regulation of epidermal growth factor receptor signaling pathway | 0.00 | 0.00 | 8.70 | 4.00 |
| GO:1903573 | negative regulation of response to endoplasmic reticulum stress | 0.00 | 0.00 | 8.70 | 4.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |

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|------------|--|------|------|-------|------|
| GO:2000379 | positive regulation of reactive oxygen species metabolic process | 0.00 | 0.00 | 6.25 | 5.00 |
| GO:1903426 | regulation of reactive oxygen species biosynthetic process | 0.03 | 0.00 | 4.17 | 3.00 |
| GO:1903426 | regulation of reactive oxygen species biosynthetic process | 0.03 | 0.00 | 4.17 | 3.00 |
| GO:0000281 | mitotic cytokinesis | 0.03 | 0.00 | 4.17 | 3.00 |
| GO:0034389 | lipid droplet organization | 0.00 | 0.00 | 14.29 | 3.00 |
| GO:0044183 | protein folding chaperone | 0.00 | 0.00 | 14.29 | 3.00 |
| GO:0048260 | positive regulation of receptor-mediated endocytosis | 0.00 | 0.00 | 8.16 | 4.00 |
| GO:0010592 | positive regulation of lamellipodium assembly | 0.00 | 0.00 | 13.64 | 3.00 |
| GO:0097199 | cysteine-type endopeptidase activity involved in apoptotic signaling pathway | 0.00 | 0.00 | 13.64 | 3.00 |
| GO:0097199 | cysteine-type endopeptidase activity involved in apoptotic signaling pathway | 0.00 | 0.00 | 13.64 | 3.00 |
| GO:0032680 | regulation of tumor necrosis factor production | 0.00 | 0.00 | 4.69 | 6.00 |
| GO:0032680 | regulation of tumor necrosis factor production | 0.00 | 0.00 | 4.69 | 6.00 |
| GO:2001236 | regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 4.65 | 6.00 |
| GO:2001236 | regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 4.65 | 6.00 |
| GO:2001236 | regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 4.65 | 6.00 |
| GO:2001236 | regulation of extrinsic apoptotic signaling pathway | 0.00 | 0.00 | 4.65 | 6.00 |
| GO:1901185 | negative regulation of ERBB signaling pathway | 0.00 | 0.00 | 7.84 | 4.00 |
| GO:0003417 | growth plate cartilage development | 0.00 | 0.00 | 13.04 | 3.00 |
| GO:0010884 | positive regulation of lipid storage | 0.00 | 0.00 | 13.04 | 3.00 |
| GO:0010884 | positive regulation of lipid storage | 0.00 | 0.00 | 13.04 | 3.00 |
| GO:0032640 | tumor necrosis factor production | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:0032640 | tumor necrosis factor production | 0.00 | 0.00 | 4.62 | 6.00 |
| GO:1903555 | regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.58 | 6.00 |
| GO:1903555 | regulation of tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.58 | 6.00 |
| GO:0048259 | regulation of receptor-mediated endocytosis | 0.00 | 0.00 | 5.75 | 5.00 |
| GO:0048259 | regulation of receptor-mediated endocytosis | 0.00 | 0.00 | 5.75 | 5.00 |
| GO:0060996 | dendritic spine development | 0.02 | 0.02 | 4.23 | 3.00 |
| GO:1903364 | positive regulation of cellular protein catabolic process | 0.00 | 0.00 | 4.55 | 6.00 |
| GO:0055094 | response to lipoprotein particle | 0.00 | 0.00 | 12.50 | 3.00 |
| GO:0055094 | response to lipoprotein particle | 0.00 | 0.00 | 12.50 | 3.00 |
| GO:0016209 | antioxidant activity | 0.00 | 0.00 | 5.56 | 5.00 |
| GO:0016209 | antioxidant activity | 0.00 | 0.00 | 5.56 | 5.00 |
| GO:0071706 | tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.44 | 6.00 |
| GO:0071706 | tumor necrosis factor superfamily cytokine production | 0.00 | 0.00 | 4.44 | 6.00 |
| GO:0010171 | body morphogenesis | 0.00 | 0.00 | 12.00 | 3.00 |

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|------------|--|------|------|-------|------|
| GO:0071402 | cellular response to lipoprotein particle stimulus | 0.00 | 0.00 | 12.00 | 3.00 |
| GO:0060351 | cartilage development involved in endochondral bone morphogenesis | 0.00 | 0.00 | 12.00 | 3.00 |
| GO:0071402 | cellular response to lipoprotein particle stimulus | 0.00 | 0.00 | 12.00 | 3.00 |
| GO:0010633 | negative regulation of epithelial cell migration | 0.02 | 0.00 | 4.35 | 3.00 |
| GO:0010633 | negative regulation of epithelial cell migration | 0.02 | 0.00 | 4.35 | 3.00 |
| GO:0010633 | negative regulation of epithelial cell migration | 0.02 | 0.00 | 4.35 | 3.00 |
| GO:0010633 | negative regulation of epithelial cell migration | 0.02 | 0.00 | 4.35 | 3.00 |
| GO:0051017 | actin filament bundle assembly | 0.00 | 0.00 | 4.35 | 6.00 |
| GO:0051017 | actin filament bundle assembly | 0.00 | 0.00 | 4.35 | 6.00 |
| GO:0051017 | actin filament bundle assembly | 0.00 | 0.00 | 4.35 | 6.00 |
| GO:0051017 | actin filament bundle assembly | 0.00 | 0.00 | 4.35 | 6.00 |
| GO:0051017 | actin filament bundle assembly | 0.00 | 0.00 | 4.35 | 6.00 |
| GO:0003416 | endochondral bone growth | 0.00 | 0.00 | 11.54 | 3.00 |
| GO:0034123 | positive regulation of toll-like receptor signaling pathway | 0.00 | 0.00 | 11.54 | 3.00 |
| GO:0045069 | regulation of viral genome replication | 0.00 | 0.00 | 5.26 | 5.00 |
| GO:0045069 | regulation of viral genome replication | 0.00 | 0.00 | 5.26 | 5.00 |
| GO:0045069 | regulation of viral genome replication | 0.00 | 0.00 | 5.26 | 5.00 |
| GO:0061572 | actin filament bundle organization | 0.00 | 0.00 | 4.26 | 6.00 |
| GO:0061572 | actin filament bundle organization | 0.00 | 0.00 | 4.26 | 6.00 |
| GO:0061572 | actin filament bundle organization | 0.00 | 0.00 | 4.26 | 6.00 |
| GO:0061572 | actin filament bundle organization | 0.00 | 0.00 | 4.26 | 6.00 |
| GO:0061572 | actin filament bundle organization | 0.00 | 0.00 | 4.26 | 6.00 |
| GO:1900739 | regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway | 0.00 | 0.00 | 11.11 | 3.00 |
| GO:1900740 | positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway | 0.00 | 0.00 | 11.11 | 3.00 |
| GO:1903649 | regulation of cytoplasmic transport | 0.00 | 0.00 | 11.11 | 3.00 |
| GO:0051851 | modulation by host of symbiont process | 0.02 | 0.00 | 4.41 | 3.00 |
| GO:0051851 | modulation by host of symbiont process | 0.02 | 0.00 | 4.41 | 3.00 |
| GO:0006910 | phagocytosis, recognition | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:0006910 | phagocytosis, recognition | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:0098869 | cellular oxidant detoxification | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:0098869 | cellular oxidant detoxification | 0.00 | 0.00 | 5.15 | 5.00 |
| GO:0003014 | renal system process | 0.00 | 0.00 | 5.10 | 5.00 |
| GO:0003158 | endothelium development | 0.00 | 0.00 | 5.10 | 5.00 |
| GO:0003158 | endothelium development | 0.00 | 0.00 | 5.10 | 5.00 |
| GO:0034113 | heterotypic cell-cell adhesion | 0.00 | 0.00 | 6.78 | 4.00 |
| GO:0060349 | bone morphogenesis | 0.00 | 0.00 | 6.78 | 4.00 |
| GO:0034113 | heterotypic cell-cell adhesion | 0.00 | 0.00 | 6.78 | 4.00 |
| GO:0034113 | heterotypic cell-cell adhesion | 0.00 | 0.00 | 6.78 | 4.00 |
| GO:0045778 | positive regulation of ossification | 0.02 | 0.00 | 4.48 | 3.00 |
| GO:0045778 | positive regulation of ossification | 0.02 | 0.00 | 4.48 | 3.00 |

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|------------|---|------|------|-------|------|
| GO:0045778 | positive regulation of ossification | 0.02 | 0.00 | 4.48 | 3.00 |
| GO:0022600 | digestive system process | 0.02 | 0.00 | 4.48 | 3.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0098868 | bone growth | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0034377 | plasma lipoprotein particle assembly | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0007263 | nitric oxide mediated signal transduction | 0.00 | 0.00 | 10.34 | 3.00 |
| GO:0019320 | hexose catabolic process | 0.00 | 0.00 | 6.56 | 4.00 |
| GO:1903749 | positive regulation of establishment of protein localization to mitochondrion | 0.00 | 0.00 | 6.45 | 4.00 |
| GO:0000302 | response to reactive oxygen species | 0.00 | 0.00 | 4.00 | 6.00 |
| GO:0044766 | multi-organism transport | 0.02 | 0.00 | 4.55 | 3.00 |
| GO:1902745 | positive regulation of lamellipodium organization | 0.00 | 0.00 | 10.00 | 3.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0016525 | negative regulation of angiogenesis | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0001952 | regulation of cell-matrix adhesion | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0016525 | negative regulation of angiogenesis | 0.00 | 0.00 | 4.76 | 5.00 |
| GO:0030010 | establishment of cell polarity | 0.00 | 0.00 | 4.72 | 5.00 |
| GO:0030010 | establishment of cell polarity | 0.00 | 0.00 | 4.72 | 5.00 |
| GO:0001844 | protein insertion into mitochondrial membrane involved in apoptotic signaling pathway | 0.00 | 0.00 | 9.68 | 3.00 |
| GO:0044070 | regulation of anion transport | 0.00 | 0.00 | 6.25 | 4.00 |
| GO:0031333 | negative regulation of protein-containing complex assembly | 0.00 | 0.00 | 4.67 | 5.00 |
| GO:0031333 | negative regulation of protein-containing complex assembly | 0.00 | 0.00 | 4.67 | 5.00 |
| GO:0032092 | positive regulation of protein binding | 0.02 | 0.00 | 4.62 | 3.00 |
| GO:0032092 | positive regulation of protein binding | 0.02 | 0.00 | 4.62 | 3.00 |
| GO:0046902 | regulation of mitochondrial membrane permeability | 0.02 | 0.00 | 4.62 | 3.00 |
| GO:0032092 | positive regulation of protein binding | 0.02 | 0.00 | 4.62 | 3.00 |
| GO:1990748 | cellular detoxification | 0.00 | 0.00 | 4.59 | 5.00 |
| GO:1990748 | cellular detoxification | 0.00 | 0.00 | 4.59 | 5.00 |
| GO:0061515 | myeloid cell development | 0.00 | 0.00 | 9.38 | 3.00 |
| GO:0061515 | myeloid cell development | 0.00 | 0.00 | 9.38 | 3.00 |
| GO:0048705 | skeletal system morphogenesis | 0.00 | 0.00 | 4.55 | 5.00 |
| GO:0006026 | aminoglycan catabolic process | 0.02 | 0.01 | 4.69 | 3.00 |
| GO:1903902 | positive regulation of viral life cycle | 0.02 | 0.00 | 4.69 | 3.00 |
| GO:0044788 | modulation by host of viral process | 0.00 | 0.00 | 9.09 | 3.00 |
| GO:0044788 | modulation by host of viral process | 0.00 | 0.00 | 9.09 | 3.00 |
| GO:1903793 | positive regulation of anion transport | 0.00 | 0.00 | 9.09 | 3.00 |
| GO:0048524 | positive regulation of viral process | 0.00 | 0.00 | 4.46 | 5.00 |
| GO:1905477 | positive regulation of protein localization to membrane | 0.00 | 0.00 | 4.46 | 5.00 |
| GO:0048524 | positive regulation of viral process | 0.00 | 0.00 | 4.46 | 5.00 |

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|------------|--|------|------|------|------|
| GO:0046718 | viral entry into host cell | 0.00 | 0.00 | 5.71 | 4.00 |
| GO:0046503 | glycerolipid catabolic process | 0.00 | 0.00 | 5.71 | 4.00 |
| GO:0046889 | positive regulation of lipid biosynthetic process | 0.00 | 0.00 | 5.71 | 4.00 |
| GO:0042339 | keratan sulfate metabolic process | 0.00 | 0.01 | 8.82 | 3.00 |
| GO:0002755 | MyD88-dependent toll-like receptor signaling pathway | 0.00 | 0.00 | 8.82 | 3.00 |
| GO:2000778 | positive regulation of interleukin-6 secretion | 0.00 | 0.00 | 8.82 | 3.00 |
| GO:0071695 | anatomical structure maturation | 0.00 | 0.00 | 4.42 | 5.00 |
| GO:0050710 | negative regulation of cytokine secretion | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:0051205 | protein insertion into membrane | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:0032507 | maintenance of protein location in cell | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:0050710 | negative regulation of cytokine secretion | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:0050710 | negative regulation of cytokine secretion | 0.02 | 0.00 | 4.76 | 3.00 |
| GO:1901030 | positive regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway | 0.00 | 0.00 | 8.57 | 3.00 |
| GO:0046596 | regulation of viral entry into host cell | 0.00 | 0.00 | 8.57 | 3.00 |
| GO:0008637 | apoptotic mitochondrial changes | 0.00 | 0.00 | 4.39 | 5.00 |
| GO:0006638 | neutral lipid metabolic process | 0.00 | 0.00 | 4.39 | 5.00 |
| GO:0006639 | acylglycerol metabolic process | 0.00 | 0.00 | 4.39 | 5.00 |
| GO:0046365 | monosaccharide catabolic process | 0.00 | 0.00 | 5.63 | 4.00 |
| GO:1901654 | response to ketone | 0.00 | 0.00 | 5.63 | 4.00 |
| GO:1901654 | response to ketone | 0.00 | 0.00 | 5.63 | 4.00 |
| GO:1904019 | epithelial cell apoptotic process | 0.00 | 0.00 | 5.56 | 4.00 |
| GO:1904019 | epithelial cell apoptotic process | 0.00 | 0.00 | 5.56 | 4.00 |
| GO:1904019 | epithelial cell apoptotic process | 0.00 | 0.00 | 5.56 | 4.00 |
| GO:0070301 | cellular response to hydrogen peroxide | 0.02 | 0.00 | 4.84 | 3.00 |
| GO:0070301 | cellular response to hydrogen peroxide | 0.02 | 0.00 | 4.84 | 3.00 |
| GO:0060350 | endochondral bone morphogenesis | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0043537 | negative regulation of blood vessel endothelial cell migration | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0043537 | negative regulation of blood vessel endothelial cell migration | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0043537 | negative regulation of blood vessel endothelial cell migration | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0043537 | negative regulation of blood vessel endothelial cell migration | 0.00 | 0.00 | 8.33 | 3.00 |
| GO:0034614 | cellular response to reactive oxygen species | 0.00 | 0.00 | 4.17 | 5.00 |
| GO:0007032 | endosome organization | 0.00 | 0.00 | 5.33 | 4.00 |
| GO:0090559 | regulation of membrane permeability | 0.00 | 0.00 | 5.33 | 4.00 |
| GO:0007032 | endosome organization | 0.00 | 0.00 | 5.33 | 4.00 |
| GO:1903053 | regulation of extracellular matrix organization | 0.00 | 0.00 | 8.11 | 3.00 |
| GO:1903747 | regulation of establishment of protein localization to mitochondrion | 0.00 | 0.00 | 5.41 | 4.00 |
| GO:0010822 | positive regulation of mitochondrion organization | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0010822 | positive regulation of mitochondrion organization | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0008361 | regulation of cell size | 0.00 | 0.00 | 4.20 | 5.00 |

| | | | | | |
|------------|---|------|------|------|------|
| GO:0008361 | regulation of cell size | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0008361 | regulation of cell size | 0.00 | 0.00 | 4.20 | 5.00 |
| GO:0090307 | mitotic spindle assembly | 0.02 | 0.00 | 4.92 | 3.00 |
| GO:0045071 | negative regulation of viral genome replication | 0.02 | 0.00 | 4.92 | 3.00 |
| GO:1905710 | positive regulation of membrane permeability | 0.02 | 0.00 | 4.92 | 3.00 |
| GO:0021782 | glial cell development | 0.02 | 0.00 | 4.92 | 3.00 |
| GO:0045071 | negative regulation of viral genome replication | 0.02 | 0.00 | 4.92 | 3.00 |
| GO:0050701 | interleukin-1 secretion | 0.02 | 0.00 | 4.92 | 3.00 |
| GO:0035890 | exit from host | 0.00 | 0.00 | 7.89 | 3.00 |
| GO:0019076 | viral release from host cell | 0.00 | 0.00 | 7.89 | 3.00 |
| GO:0031122 | cytoplasmic microtubule organization | 0.02 | 0.00 | 5.00 | 3.00 |
| GO:0031122 | cytoplasmic microtubule organization | 0.02 | 0.00 | 5.00 | 3.00 |
| GO:1902108 | regulation of mitochondrial membrane permeability involved in apoptotic process | 0.02 | 0.00 | 5.00 | 3.00 |
| GO:0002753 | cytoplasmic pattern recognition receptor signaling pathway | 0.02 | 0.00 | 5.00 | 3.00 |
| GO:0031122 | cytoplasmic microtubule organization | 0.02 | 0.00 | 5.00 | 3.00 |
| GO:0035794 | positive regulation of mitochondrial membrane permeability | 0.02 | 0.00 | 5.08 | 3.00 |
| GO:0061621 | canonical glycolysis | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0006735 | NADH regeneration | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0061615 | glycolytic process through fructose-6-phosphate | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0001937 | negative regulation of endothelial cell proliferation | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0001937 | negative regulation of endothelial cell proliferation | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0001937 | negative regulation of endothelial cell proliferation | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0006656 | phosphatidylcholine biosynthetic process | 0.00 | 0.00 | 7.69 | 3.00 |
| GO:0033619 | membrane protein proteolysis | 0.01 | 0.01 | 5.17 | 3.00 |
| GO:0031640 | killing of cells of other organism | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:1902686 | mitochondrial outer membrane permeabilization involved in programmed cell death | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0070671 | response to interleukin-12 | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0070671 | response to interleukin-12 | 0.01 | 0.00 | 5.17 | 3.00 |
| GO:0032760 | positive regulation of tumor necrosis factor production | 0.01 | 0.00 | 5.06 | 4.00 |
| GO:0032760 | positive regulation of tumor necrosis factor production | 0.01 | 0.00 | 5.06 | 4.00 |
| GO:0032757 | positive regulation of interleukin-8 production | 0.01 | 0.00 | 5.26 | 3.00 |
| GO:0010812 | negative regulation of cell-substrate adhesion | 0.01 | 0.00 | 5.26 | 3.00 |
| GO:0032757 | positive regulation of interleukin-8 production | 0.01 | 0.00 | 5.26 | 3.00 |
| GO:0010812 | negative regulation of cell-substrate adhesion | 0.01 | 0.00 | 5.26 | 3.00 |
| GO:0032757 | positive regulation of interleukin-8 production | 0.01 | 0.00 | 5.26 | 3.00 |
| GO:0010812 | negative regulation of cell-substrate adhesion | 0.01 | 0.00 | 5.26 | 3.00 |

| | | | | | |
|------------|--|------|------|------|------|
| GO:0009988 | cell-cell recognition | 0.01 | 0.01 | 5.36 | 3.00 |
| GO:0051438 | regulation of ubiquitin-protein transferase activity | 0.01 | 0.00 | 5.36 | 3.00 |
| GO:1902110 | positive regulation of mitochondrial membrane permeability involved in apoptotic process | 0.01 | 0.00 | 5.36 | 3.00 |
| GO:0035722 | interleukin-12-mediated signaling pathway | 0.01 | 0.00 | 5.36 | 3.00 |
| GO:0035722 | interleukin-12-mediated signaling pathway | 0.01 | 0.00 | 5.36 | 3.00 |
| GO:0097345 | mitochondrial outer membrane permeabilization | 0.01 | 0.00 | 5.66 | 3.00 |
| GO:1903317 | regulation of protein maturation | 0.01 | 0.00 | 5.66 | 3.00 |
| GO:0034381 | plasma lipoprotein particle clearance | 0.01 | 0.00 | 5.45 | 3.00 |
| GO:0034381 | plasma lipoprotein particle clearance | 0.01 | 0.00 | 5.45 | 3.00 |
| GO:0032370 | positive regulation of lipid transport | 0.01 | 0.00 | 5.45 | 3.00 |
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0032233 | positive regulation of actin filament bundle assembly | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:0050702 | interleukin-1 beta secretion | 0.01 | 0.00 | 5.56 | 3.00 |
| GO:1903580 | positive regulation of ATP metabolic process | 0.01 | 0.00 | 7.50 | 3.00 |
| GO:2000249 | regulation of actin cytoskeleton reorganization | 0.01 | 0.00 | 7.50 | 3.00 |
| GO:1903580 | positive regulation of ATP metabolic process | 0.01 | 0.00 | 7.50 | 3.00 |
| GO:2000249 | regulation of actin cytoskeleton reorganization | 0.01 | 0.00 | 7.50 | 3.00 |
| GO:2000249 | regulation of actin cytoskeleton reorganization | 0.01 | 0.00 | 7.50 | 3.00 |
| GO:0060191 | regulation of lipase activity | 0.01 | 0.00 | 4.08 | 4.00 |
| GO:0060191 | regulation of lipase activity | 0.01 | 0.00 | 4.08 | 4.00 |
| GO:1903557 | positive regulation of tumor necrosis factor superfamily cytokine production | 0.01 | 0.00 | 4.94 | 4.00 |
| GO:1903557 | positive regulation of tumor necrosis factor superfamily cytokine production | 0.01 | 0.00 | 4.94 | 4.00 |
| GO:0052372 | modulation by symbiont of entry into host | 0.01 | 0.00 | 7.14 | 3.00 |
| GO:0043277 | apoptotic cell clearance | 0.01 | 0.00 | 7.32 | 3.00 |
| GO:0043277 | apoptotic cell clearance | 0.01 | 0.00 | 7.32 | 3.00 |
| GO:0006890 | retrograde vesicle-mediated transport. Golgi to endoplasmic reticulum | 0.01 | 0.01 | 4.21 | 4.00 |
| GO:0150115 | cell-substrate junction organization | 0.01 | 0.00 | 4.21 | 4.00 |
| GO:0045185 | maintenance of protein location | 0.01 | 0.00 | 4.21 | 4.00 |
| GO:0045185 | maintenance of protein location | 0.01 | 0.00 | 4.21 | 4.00 |
| GO:0150115 | cell-substrate junction organization | 0.01 | 0.00 | 4.21 | 4.00 |
| GO:0010596 | negative regulation of endothelial cell migration | 0.01 | 0.00 | 5.77 | 3.00 |
| GO:0010596 | negative regulation of endothelial cell migration | 0.01 | 0.00 | 5.77 | 3.00 |
| GO:0010596 | negative regulation of endothelial cell migration | 0.01 | 0.00 | 5.77 | 3.00 |

| | | | | | |
|------------|---|------|------|------|------|
| GO:0010596 | negative regulation of endothelial cell migration | 0.01 | 0.00 | 5.77 | 3.00 |
| GO:0051668 | localization within membrane | 0.01 | 0.00 | 4.88 | 4.00 |
| GO:1905897 | regulation of response to endoplasmic reticulum stress | 0.01 | 0.00 | 4.88 | 4.00 |
| GO:1901028 | regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway | 0.01 | 0.00 | 6.82 | 3.00 |
| GO:0046461 | neutral lipid catabolic process | 0.01 | 0.00 | 6.82 | 3.00 |
| GO:0014812 | muscle cell migration | 0.01 | 0.01 | 6.00 | 3.00 |
| GO:0042743 | hydrogen peroxide metabolic process | 0.01 | 0.00 | 6.00 | 3.00 |
| GO:0042743 | hydrogen peroxide metabolic process | 0.01 | 0.00 | 6.00 | 3.00 |
| GO:1903901 | negative regulation of viral life cycle | 0.01 | 0.00 | 4.76 | 4.00 |
| GO:1903901 | negative regulation of viral life cycle | 0.01 | 0.00 | 4.76 | 4.00 |
| GO:0045446 | endothelial cell differentiation | 0.01 | 0.00 | 4.76 | 4.00 |
| GO:0019319 | hexose biosynthetic process | 0.01 | 0.00 | 4.65 | 4.00 |
| GO:0044409 | entry into host | 0.01 | 0.00 | 4.65 | 4.00 |
| GO:0045669 | positive regulation of osteoblast differentiation | 0.01 | 0.00 | 6.98 | 3.00 |
| GO:0045669 | positive regulation of osteoblast differentiation | 0.01 | 0.00 | 6.98 | 3.00 |
| GO:0045669 | positive regulation of osteoblast differentiation | 0.01 | 0.00 | 6.98 | 3.00 |
| GO:0051702 | interaction with symbiont | 0.01 | 0.00 | 4.12 | 4.00 |
| GO:0051702 | interaction with symbiont | 0.01 | 0.00 | 4.12 | 4.00 |
| GO:0042058 | regulation of epidermal growth factor receptor signaling pathway | 0.01 | 0.00 | 4.71 | 4.00 |
| GO:0046364 | monosaccharide biosynthetic process | 0.01 | 0.00 | 4.30 | 4.00 |
| GO:0032368 | regulation of lipid transport | 0.01 | 0.00 | 4.26 | 4.00 |
| GO:0090151 | establishment of protein localization to mitochondrial membrane | 0.01 | 0.00 | 6.25 | 3.00 |
| GO:0031663 | lipopolysaccharide-mediated signaling pathway | 0.01 | 0.00 | 6.25 | 3.00 |
| GO:2001243 | negative regulation of intrinsic apoptotic signaling pathway | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:2001243 | negative regulation of intrinsic apoptotic signaling pathway | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0032231 | regulation of actin filament bundle assembly | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0050680 | negative regulation of epithelial cell proliferation | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:2001243 | negative regulation of intrinsic apoptotic signaling pathway | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0032231 | regulation of actin filament bundle assembly | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0032231 | regulation of actin filament bundle assembly | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0050680 | negative regulation of epithelial cell proliferation | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0032231 | regulation of actin filament bundle assembly | 0.01 | 0.00 | 4.49 | 4.00 |
| GO:0043113 | receptor clustering | 0.01 | 0.00 | 6.38 | 3.00 |
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |

| | | | | | |
|------------|---|------|------|------|------|
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |
| GO:0051496 | positive regulation of stress fiber assembly | 0.01 | 0.00 | 6.38 | 3.00 |
| GO:0071622 | regulation of granulocyte chemotaxis | 0.01 | 0.00 | 6.52 | 3.00 |
| GO:0051204 | protein insertion into mitochondrial membrane | 0.01 | 0.00 | 6.52 | 3.00 |
| GO:1905037 | autophagosome organization | 0.01 | 0.01 | 4.40 | 4.00 |
| GO:1901184 | regulation of ERBB signaling pathway | 0.01 | 0.00 | 4.40 | 4.00 |
| GO:0010883 | regulation of lipid storage | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:0072604 | interleukin-6 secretion | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:0001885 | endothelial cell development | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:0010883 | regulation of lipid storage | 0.01 | 0.00 | 6.12 | 3.00 |
| GO:0032637 | interleukin-8 production | 0.01 | 0.00 | 4.55 | 4.00 |
| GO:0032637 | interleukin-8 production | 0.01 | 0.00 | 4.55 | 4.00 |

Appendix 4.

| Main class | Number of elements | Lipid category | Number of elements |
|---------------------------------------|--------------------|----------------------|--------------------|
| Fatty acids and Conjugates | 50 | Fatty Acids | 105 |
| Fatty amides | 46 | | |
| Fatty esters | 7 | | |
| Oxygenated hydrocarbons | 2 | | |
| Diradylglycerols | 185 | Glycerolipids | 391 |
| Glycosyldiradylglycerols | 3 | | |
| Monoradylglycerols | 21 | | |
| Other Glycerolipids | 10 | | |
| Triradylglycerols | 172 | | |
| Glycerophosphates | 6 | Glycerophospholipids | 216 |
| Glycerophosphocholines | 73 | | |
| Glycerophosphoethanolamines | 71 | | |
| Glycerophosphoglycerols | 17 | | |
| Glycerophosphoglycerophosphoglycerols | 8 | | |
| Glycerophosphoinositols | 24 | | |
| Glycerophosphoserines | 15 | | |
| Other Glycerophospholipids | 2 | | |
| Quinones and hydroquinones | 1 | Prenol Lipids | 1 |
| Acidic glycosphingolipids | 10 | Sphingolipids | 190 |
| Ceramides | 69 | | |
| Neutral glycosphingolipids | 19 | | |
| Phosphosphingolipids | 78 | | |
| Sphingoid bases | 14 | | |
| Secosteroids | 1 | Sterol Lipids | 44 |
| Sterols | 43 | | |

Appendix 4. Summarization of the complete list of analyzed lipids within their respective lipid species classes, subclasses and number of lipids annotated from the untargeted lipidomic analysis.

Appendix 5

Appendix Table 5-1. List of all significantly up- and down-regulated lipids elements in comparison between porcine colostrum exosomes (day 0) and milk exosomes at day 7. Significance was set using t-test FDR adjusted p-value threshold at 0.05 and fold change threshold at 2 ($|\log_2 FC| > 1$).

| Day 0 vs 7 | | | | |
|-------------|---|-----------------------|----------------------|---------|
| Lipid class | Lipid name | log ₂ (FC) | FDR Adjusted P value | Up/Down |
| ADGGA | ADGGA (O-28:0)17:2_22:6 | 1.253 | 0.007 | UP |
| AHexCer | AHexCer 59:9;3O | -3.162 | 0.000 | Down |
| AHexCer | AHexCer 40:4;3O | -2.493 | 0.000 | Down |
| AHexCer | AHexCer 39:4;3O_AHexCer (O-14:1)25:3;3O | -2.029 | 0.000 | Down |
| AHexCer | AHexCer 60:8;3O | -1.653 | 0.001 | Down |
| AHexCer | AHexCer 72:9;3O | -1.451 | 0.002 | Down |
| BMP | BMP 17:1_17:1 | -3.217 | 0.000 | Down |
| BMP | BMP 15:0_8:0 | -2.698 | 0.000 | Down |
| BMP | BMP 8:0_28:2 | -2.083 | 0.000 | Down |
| CAR | CAR 17:3 | -3.046 | 0.000 | Down |
| CAR | CAR 13:0 | -2.731 | 0.000 | Down |
| CAR | CAR 7:0 | -2.701 | 0.000 | Down |
| CAR | CAR 5:0 | -2.655 | 0.000 | Down |
| CAR | CAR 19:3 | -2.167 | 0.000 | Down |
| CAR | CAR 16:0 | -1.693 | 0.000 | Down |
| CE | CE 18:1 | -2.549 | 0.000 | Down |
| Cer | Cer 34:1;2O_Cer 18:1;2O_16:0. | 1.028 | 0.005 | UP |
| Cer | Cer 12:0;2O_19:0;(2OH) | 1.807 | 0.000 | UP |
| Cer | Cer 12:1;2O_24:3;(2OH) | 3.046 | 0.000 | UP |
| Cer | Cer 13:2;2O_40:3 | -4.407 | 0.000 | Down |
| Cer | Cer 65:5;4O | -3.724 | 0.000 | Down |
| Cer | Cer 38:0;2O_Cer 18:0;2O_20:0 | -3.406 | 0.000 | Down |
| Cer | Cer 40:7;4O | -3.382 | 0.000 | Down |
| Cer | Cer 12:0;2O_23:0;O | -3.238 | 0.000 | Down |
| Cer | Cer 42:0;4O | -3.126 | 0.000 | Down |
| Cer | Cer 33:6;4O | -3.101 | 0.000 | Down |
| Cer | Cer 50:10;4O | -3.024 | 0.000 | Down |
| Cer | Cer 40:0;4O_Cer 30:0;3O_10:0;(2OH) | -3.009 | 0.000 | Down |
| Cer | Cer 34:3;4O_Cer 19:2;3O_15:1;(2OH) | -2.952 | 0.000 | Down |
| Cer | Cer 24:3;3O_Cer 16:3;2O_8:0;O | -2.946 | 0.000 | Down |
| Cer | Cer 38:3;5O_Cer 21:2;3O_17:1;(2OH) | -2.909 | 0.000 | Down |
| Cer | Cer 35:5;5O_Cer 19:2;3O_16:3;(2OH) | -2.904 | 0.000 | Down |
| Cer | Cer 36:4;4O | -2.896 | 0.000 | Down |
| Cer | Cer 36:1;3O_Cer 19:0;2O_17:1;O | -2.842 | 0.000 | Down |
| Cer | Cer 36:2;3O_Cer 19:0;2O_17:2;O | -2.830 | 0.000 | Down |
| Cer | Cer 38:5;4O | -2.786 | 0.000 | Down |
| Cer | Cer 33:5;4O | -2.754 | 0.000 | Down |

| | | | | |
|-------|--------------------------------|--------|-------|------|
| Cer | Cer 34:0;3O_Cer 18:0;2O_16:0;O | -2.749 | 0.000 | Down |
| Cer | Cer 34:1;3O_Cer 19:0;2O_15:1;O | -2.733 | 0.000 | Down |
| Cer | Cer 35:5;4O | -2.707 | 0.000 | Down |
| Cer | Cer 28:3;4O. | -2.690 | 0.000 | Down |
| Cer | Cer 28:3;4O | -2.630 | 0.000 | Down |
| Cer | Cer 35:6;2O_Cer 17:3;2O_18:3 | -2.588 | 0.000 | Down |
| Cer | Cer 94:3;4O | -2.443 | 0.000 | Down |
| Cer | Cer 60:11;4O | -2.288 | 0.000 | Down |
| Cer | Cer 58:11;4O | -2.282 | 0.000 | Down |
| Cer | Cer 12:2;2O_24:1 | -2.274 | 0.000 | Down |
| Cer | Cer 12:1;3O_31:0;(2OH) | -2.272 | 0.000 | Down |
| Cer | Cer 12:2;2O_39:10;2O | -2.255 | 0.000 | Down |
| Cer | Cer 12:1;2O_30:0 | -2.201 | 0.000 | Down |
| Cer | Cer 42:1;2O_Cer 18:1;2O_24:0. | -2.061 | 0.000 | Down |
| Cer | Cer 92:5;4O | -2.013 | 0.000 | Down |
| Cer | Cer 42:1;2O_Cer 18:1;2O_24:0 | -1.991 | 0.000 | Down |
| Cer | Cer 31:4;2O_Cer 12:2;2O_19:2 | -1.799 | 0.000 | Down |
| Cer | Cer 58:5;4O | -1.688 | 0.001 | Down |
| Cer | Cer 94:5;4O | -1.652 | 0.001 | Down |
| Cer | Cer 61:13;4O | -1.627 | 0.001 | Down |
| Cer | Cer 12:2;2O_42:10;2O | -1.617 | 0.001 | Down |
| Cer | Cer 16:3;2O_30:3 | -1.551 | 0.000 | Down |
| Cer | Cer 12:2;2O_30:0 | -1.359 | 0.000 | Down |
| Cer | Cer 42:2;2O_Cer 18:1;2O_24:1 | -1.334 | 0.000 | Down |
| Cer | Cer 12:1;2O_28:0 | -1.192 | 0.000 | Down |
| Cer | Cer 42:1;3O_Cer 18:1;2O_24:0;O | -1.097 | 0.000 | Down |
| CerP | CerP 24:2;2O_28:3 | -3.030 | 0.000 | Down |
| CerP | CerP 29:2;2O_CerP 16:1;2O_13:1 | -2.605 | 0.000 | Down |
| CerP | CerP 30:3;2O_CerP 12:0;2O_18:3 | -2.356 | 0.000 | Down |
| CerP | CerP 34:2;2O_CerP 19:1;2O_15:1 | -1.854 | 0.000 | Down |
| CerP | CerP 16:1;2O_28:3 | -1.473 | 0.001 | Down |
| CerP | CerP 14:1;2O_28:3 | -1.082 | 0.006 | Down |
| CL | CL 14:1_22:6_26:0_28:0 | -3.012 | 0.000 | Down |
| CL | CL 12:0_22:6_28:0_28:0 | -2.901 | 0.000 | Down |
| CL | CL 72:6_CL 18:0_18:0_16:1_20:5 | -2.126 | 0.000 | Down |
| CL | CL 15:0_22:5_28:0_28:0 | -1.626 | 0.002 | Down |
| CoQ10 | CoQ10 | -2.013 | 0.000 | Down |
| DG | DG 47:6 | -4.354 | 0.000 | Down |
| DG | DG 42:0 | -4.345 | 0.000 | Down |
| DG | DG 86:5 | -4.335 | 0.000 | Down |
| DG | DG 43:2 | -4.300 | 0.000 | Down |
| DG | DG 50:6 | -4.293 | 0.000 | Down |
| DG | DG 41:0 | -4.291 | 0.000 | Down |
| DG | DG 43:0 | -4.213 | 0.000 | Down |
| DG | DG 51:4 | -4.143 | 0.000 | Down |
| DG | DG 28:0 | -4.090 | 0.000 | Down |
| DG | DG O-45:6_DG O-17:0_28:6 | -4.072 | 0.000 | Down |
| DG | DG 39:1 | -4.020 | 0.000 | Down |
| DG | DG 44:0 | -3.991 | 0.000 | Down |

| | | | | |
|----|--------------------------|--------|-------|------|
| DG | DG 42:5 | -3.980 | 0.000 | Down |
| DG | DG 40:0 | -3.979 | 0.000 | Down |
| DG | DG 45:0 | -3.950 | 0.000 | Down |
| DG | DG 52:6 | -3.950 | 0.000 | Down |
| DG | DG 46:0 | -3.915 | 0.000 | Down |
| DG | DG 48:6 | -3.903 | 0.000 | Down |
| DG | DG 49:6 | -3.899 | 0.000 | Down |
| DG | DG 49:7 | -3.892 | 0.000 | Down |
| DG | DG O-42:6_DG O-18:0_24:6 | -3.890 | 0.000 | Down |
| DG | DG 45:6 | -3.889 | 0.000 | Down |
| DG | DG 46:6 | -3.853 | 0.000 | Down |
| DG | DG 30:0 | -3.823 | 0.000 | Down |
| DG | DG 39:6 | -3.822 | 0.000 | Down |
| DG | DG 51:0 | -3.797 | 0.000 | Down |
| DG | DG 49:8 | -3.796 | 0.000 | Down |
| DG | DG 47:0 | -3.793 | 0.000 | Down |
| DG | DG 50:0 | -3.789 | 0.000 | Down |
| DG | DG 43:6 | -3.789 | 0.000 | Down |
| DG | DG 49:0 | -3.783 | 0.000 | Down |
| DG | DG 38:6 | -3.781 | 0.000 | Down |
| DG | DG 39:0 | -3.770 | 0.000 | Down |
| DG | DG 45:7 | -3.765 | 0.000 | Down |
| DG | DG O-55:1_DG O-27:0_28:1 | -3.758 | 0.000 | Down |
| DG | DG O-38:1_DG O-14:0_24:1 | -3.756 | 0.000 | Down |
| DG | DG 40:5 | -3.752 | 0.000 | Down |
| DG | DG O-41:1_DG O-13:0_28:1 | -3.742 | 0.000 | Down |
| DG | DG O-48:1_DG O-20:0_28:1 | -3.734 | 0.000 | Down |
| DG | DG 35:6 | -3.728 | 0.000 | Down |
| DG | DG 35:0 | -3.725 | 0.000 | Down |
| DG | DG O-39:1_DG O-13:0_26:1 | -3.716 | 0.000 | Down |
| DG | DG 43:7 | -3.710 | 0.000 | Down |
| DG | DG 40:2 | -3.689 | 0.000 | Down |
| DG | DG 41:7 | -3.680 | 0.000 | Down |
| DG | DG 39:7 | -3.673 | 0.000 | Down |
| DG | DG 41:6 | -3.654 | 0.000 | Down |
| DG | DG O-44:1_DG O-18:0_26:1 | -3.645 | 0.000 | Down |
| DG | DG O-40:1_DG O-16:0_24:1 | -3.641 | 0.000 | Down |
| DG | DG 36:0 | -3.631 | 0.000 | Down |
| DG | DG 27:0 | -3.627 | 0.000 | Down |
| DG | DG 37:7 | -3.617 | 0.000 | Down |
| DG | DG 51:6 | -3.609 | 0.000 | Down |
| DG | DG 34:0 | -3.544 | 0.000 | Down |
| DG | DG 36:0_DG 18:0_18:0 | -3.531 | 0.000 | Down |
| DG | DG 40:6 | -3.514 | 0.000 | Down |
| DG | DG 45:8 | -3.513 | 0.000 | Down |
| DG | DG 43:8 | -3.508 | 0.000 | Down |
| DG | DG 38:2 | -3.502 | 0.000 | Down |
| DG | DG 44:6_DG 16:0_28:6 | -3.493 | 0.000 | Down |
| DG | DG O-35:0_DG O-19:0_16:0 | -3.483 | 0.000 | Down |

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|----|--------------------------|--------|-------|------|
| DG | DG 52:7 | -3.483 | 0.000 | Down |
| DG | DG 42:6_DG 16:0_26:6 | -3.478 | 0.000 | Down |
| DG | DG O-30:1_DG O-16:0_14:1 | -3.451 | 0.000 | Down |
| DG | DG 32:0 | -3.437 | 0.000 | Down |
| DG | DG 31:0 | -3.405 | 0.000 | Down |
| DG | DG 27:4 | -3.400 | 0.000 | Down |
| DG | DG 36:6 | -3.397 | 0.000 | Down |
| DG | DG O-36:1_DG O-17:0_19:1 | -3.395 | 0.000 | Down |
| DG | DG 33:1 | -3.387 | 0.000 | Down |
| DG | DG 34:2_DG 16:0_18:2 | -3.373 | 0.000 | Down |
| DG | DG O-33:1_DG O-17:0_16:1 | -3.360 | 0.000 | Down |
| DG | DG 48:12 | -3.327 | 0.000 | Down |
| DG | DG 52:11 | -3.301 | 0.000 | Down |
| DG | DG 34:0_DG 16:0_18:0 | -3.265 | 0.000 | Down |
| DG | DG 27:5 | -3.259 | 0.000 | Down |
| DG | DG 52:14 | -3.259 | 0.000 | Down |
| DG | DG 42:10 | -3.235 | 0.000 | Down |
| DG | DG 32:0_DG 16:0_16:0 | -3.190 | 0.000 | Down |
| DG | DG 40:9 | -3.176 | 0.000 | Down |
| DG | DG 41:6_DG 15:0_26:6 | -3.136 | 0.000 | Down |
| DG | DG 31:4 | -3.132 | 0.000 | Down |
| DG | DG 51:7 | -3.125 | 0.000 | Down |
| DG | DG 46:3 | -3.108 | 0.000 | Down |
| DG | DG 30:5 | -3.099 | 0.000 | Down |
| DG | DG 38:5 | -3.099 | 0.000 | Down |
| DG | DG 38:8 | -3.088 | 0.000 | Down |
| DG | DG 30:7 | -3.085 | 0.000 | Down |
| DG | DG 16:0 | -3.078 | 0.000 | Down |
| DG | DG 45:11 | -3.073 | 0.000 | Down |
| DG | DG 35:2 | -3.067 | 0.000 | Down |
| DG | DG 42:11 | -3.050 | 0.000 | Down |
| DG | DG 30:8 | -3.028 | 0.000 | Down |
| DG | DG 32:6 | -3.017 | 0.000 | Down |
| DG | DG 41:11 | -3.010 | 0.000 | Down |
| DG | DG 28:2 | -3.004 | 0.000 | Down |
| DG | DG 49:2 | -3.003 | 0.000 | Down |
| DG | DG 27:3 | -3.000 | 0.000 | Down |
| DG | DG 38:7 | -2.997 | 0.000 | Down |
| DG | DG 39:10 | -2.996 | 0.000 | Down |
| DG | DG 19:0 | -2.995 | 0.000 | Down |
| DG | DG 30:4 | -2.995 | 0.000 | Down |
| DG | DG 24:1 | -2.965 | 0.000 | Down |
| DG | DG 34:5 | -2.958 | 0.000 | Down |
| DG | DG 24:2 | -2.953 | 0.000 | Down |
| DG | DG 46:11 | -2.946 | 0.000 | Down |
| DG | DG 36:5 | -2.937 | 0.000 | Down |
| DG | DG 32:2 | -2.934 | 0.000 | Down |
| DG | DG 33:7 | -2.931 | 0.000 | Down |
| DG | DG 37:4 | -2.919 | 0.000 | Down |

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|----|----------------------|--------|-------|------|
| DG | DG 34:3 | -2.915 | 0.000 | Down |
| DG | DG 26:0 | -2.905 | 0.000 | Down |
| DG | DG 32:7 | -2.905 | 0.000 | Down |
| DG | DG 44:6 | -2.904 | 0.000 | Down |
| DG | DG 28:4 | -2.904 | 0.000 | Down |
| DG | DG 29:3 | -2.896 | 0.000 | Down |
| DG | DG 29:4 | -2.895 | 0.000 | Down |
| DG | DG 36:4 | -2.893 | 0.000 | Down |
| DG | DG 32:8 | -2.889 | 0.000 | Down |
| DG | DG 26:2 | -2.889 | 0.000 | Down |
| DG | DG 30:6 | -2.872 | 0.000 | Down |
| DG | DG 29:5 | -2.869 | 0.000 | Down |
| DG | DG 20:0 | -2.862 | 0.000 | Down |
| DG | DG 31:7 | -2.862 | 0.000 | Down |
| DG | DG 32:3 | -2.858 | 0.000 | Down |
| DG | DG 25:2 | -2.856 | 0.000 | Down |
| DG | DG 30:3 | -2.848 | 0.000 | Down |
| DG | DG 26:5 | -2.848 | 0.000 | Down |
| DG | DG 29:0 | -2.845 | 0.000 | Down |
| DG | DG 31:8 | -2.845 | 0.000 | Down |
| DG | DG 23:0 | -2.845 | 0.000 | Down |
| DG | DG 31:5 | -2.845 | 0.000 | Down |
| DG | DG 32:1 | -2.842 | 0.000 | Down |
| DG | DG 25:0 | -2.832 | 0.000 | Down |
| DG | DG 25:1 | -2.830 | 0.000 | Down |
| DG | DG 28:3 | -2.829 | 0.000 | Down |
| DG | DG 23:4 | -2.819 | 0.000 | Down |
| DG | DG 39:9 | -2.817 | 0.000 | Down |
| DG | DG 23:1 | -2.815 | 0.000 | Down |
| DG | DG 28:5 | -2.814 | 0.000 | Down |
| DG | DG 40:8 | -2.812 | 0.000 | Down |
| DG | DG 39:8 | -2.795 | 0.000 | Down |
| DG | DG 23:2 | -2.785 | 0.000 | Down |
| DG | DG 31:6 | -2.775 | 0.000 | Down |
| DG | DG 24:0 | -2.772 | 0.000 | Down |
| DG | DG 36:1_DG 18:0_18:1 | -2.771 | 0.000 | Down |
| DG | DG 34:4 | -2.770 | 0.000 | Down |
| DG | DG 27:2 | -2.765 | 0.000 | Down |
| DG | DG 34:2 | -2.744 | 0.000 | Down |
| DG | DG 22:0 | -2.681 | 0.000 | Down |
| DG | DG 29:2 | -2.678 | 0.000 | Down |
| DG | DG 18:0 | -2.657 | 0.000 | Down |
| DG | DG 24:3 | -2.616 | 0.000 | Down |
| DG | DG 51:8 | -2.607 | 0.000 | Down |
| DG | DG 22:1 | -2.566 | 0.000 | Down |
| DG | DG 30:2 | -2.464 | 0.000 | Down |
| DG | DG 46:8 | -2.451 | 0.000 | Down |
| DG | DG 36:1 | -2.361 | 0.000 | Down |
| DG | DG 44:7 | -2.252 | 0.000 | Down |

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|------|--------------------------|--------|-------|------|
| DG | DG 34:1_DG 16:0_18:1 | -2.243 | 0.000 | Down |
| DG | DG 36:2 | -2.223 | 0.000 | Down |
| DG | DG 34:1 | -2.107 | 0.000 | Down |
| DG | DG 51:13 | -2.067 | 0.000 | Down |
| DG | DG 52:10 | -1.945 | 0.001 | Down |
| DG | DG 48:13 | -1.935 | 0.000 | Down |
| DG | DG 36:2_DG 18:1_18:1 | -1.899 | 0.000 | Down |
| DG | DG 52:9 | -1.860 | 0.000 | Down |
| DG | DG 28:1 | -1.840 | 0.000 | Down |
| DG | DG 36:3_DG 18:1_18:2 | -1.783 | 0.000 | Down |
| DG | DG 41:8 | -1.578 | 0.000 | Down |
| DG | DG 50:1 | -1.574 | 0.000 | Down |
| DG | DG 38:4 | -1.542 | 0.001 | Down |
| DG | DG 50:9 | -1.363 | 0.004 | Down |
| DG | DG 53:9 | -1.185 | 0.003 | Down |
| DG | DG 49:1 | -1.028 | 0.016 | Down |
| DGCC | DGCC 36:2_DGCC 18:1_18:1 | -3.236 | 0.000 | Down |
| DGCC | DGCC 16:0_19:5 | -1.752 | 0.000 | Down |
| DGCC | DGCC 15:2_18:5 | -1.695 | 0.000 | Down |
| DGCC | DGCC 15:0_22:6 | -1.583 | 0.000 | Down |
| DGDG | DGDG 8:0_20:2 | -1.997 | 0.000 | Down |
| DGDG | DGDG O-8:0_17:0 | -1.714 | 0.000 | Down |
| DGDG | DGDG 17:1_22:6 | -1.549 | 0.000 | Down |
| DGGA | DGGA 10:0_22:1 | -2.713 | 0.000 | Down |
| DGGA | DGGA 22:0_22:6 | -1.995 | 0.000 | Down |
| DGGA | DGGA 12:0_22:1 | -1.659 | 0.000 | Down |
| DGTS | DGTS 16:0_17:3 | -3.016 | 0.000 | Down |
| DMPE | DMPE 17:0_22:5 | -1.816 | 0.000 | Down |
| FA | FA 20:4;30 | -3.930 | 0.000 | Down |
| FA | FA 16:0;30 | -3.859 | 0.000 | Down |
| FA | FA 16:1;30 | -3.689 | 0.000 | Down |
| FA | FA 44:5 | -3.651 | 0.000 | Down |
| FA | FA 40:5 | -3.592 | 0.000 | Down |
| FA | FA 42:5 | -3.586 | 0.000 | Down |
| FA | FA 16:1 | -3.493 | 0.000 | Down |
| FA | FA 15:4 | -3.486 | 0.000 | Down |
| FA | FA 42:9 | -3.472 | 0.000 | Down |
| FA | FA 18:3;40 | -3.355 | 0.000 | Down |
| FA | FA 16:2;30 | -3.313 | 0.000 | Down |
| FA | FA 22:0;40 | -3.180 | 0.000 | Down |
| FA | FA 19:4;10 | -3.073 | 0.000 | Down |
| FA | FA 17:4;20 | -2.955 | 0.000 | Down |
| FA | FA 38:5 | -2.914 | 0.000 | Down |
| FA | FA 19:1;20 | -2.864 | 0.000 | Down |
| FA | FA 20:0;40 | -2.806 | 0.000 | Down |
| FA | FA 20:3;40 | -2.804 | 0.000 | Down |
| FA | FA 22:6 | -2.775 | 0.000 | Down |
| FA | FA 25:0 | -2.715 | 0.000 | Down |
| FA | FA 22:5;40 | -2.664 | 0.000 | Down |

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|--------|--------------------------------------|--------|-------|------|
| FA | FA 14:0 | -2.610 | 0.000 | Down |
| FA | FA 28:7 | -2.608 | 0.000 | Down |
| FA | FA 22:6;40 | -2.581 | 0.000 | Down |
| FA | FA 29:0 | -2.557 | 0.000 | Down |
| FA | FA 32:0 | -2.534 | 0.000 | Down |
| FA | FA 30:0 | -2.525 | 0.000 | Down |
| FA | FA 26:0 | -2.516 | 0.000 | Down |
| FA | FA 24:0 | -2.504 | 0.000 | Down |
| FA | FA 31:0 | -2.493 | 0.000 | Down |
| FA | FA 27:0 | -2.449 | 0.000 | Down |
| FA | FA 18:1 | -2.440 | 0.000 | Down |
| FA | FA 20:0 | -2.413 | 0.000 | Down |
| FA | FA 23:0 | -2.412 | 0.000 | Down |
| FA | FA 16:0 | -2.408 | 0.000 | Down |
| FA | FA 28:1;20 | -2.399 | 0.000 | Down |
| FA | FA 28:0 | -2.364 | 0.000 | Down |
| FA | FA 34:0 | -2.362 | 0.000 | Down |
| FA | FA 18:0 | -2.346 | 0.000 | Down |
| FA | FA 22:0 | -2.341 | 0.000 | Down |
| FA | FA 17:0 | -2.325 | 0.000 | Down |
| FA | FA 26:1;10 | -2.280 | 0.000 | Down |
| FA | FA 36:5 | -2.217 | 0.000 | Down |
| FA | FA 33:0 | -2.169 | 0.000 | Down |
| FA | FA 18:1;0 | -2.084 | 0.000 | Down |
| FA | FA 15:0 | -1.822 | 0.000 | Down |
| FA | FA 18:1;20 | -1.734 | 0.000 | Down |
| FA | FA 21:0 | -1.724 | 0.005 | Down |
| FA | FA 42:10 | 1.747 | 0.000 | UP |
| FA | FA 44:10 | 2.220 | 0.000 | UP |
| HBMP | HBMP 22:2_12:0_12:0 | -3.143 | 0.000 | Down |
| HBMP | HBMP 20:1_12:0_12:0 | -2.698 | 0.000 | Down |
| HBMP | HBMP 22:3_12:0_12:0 | -2.210 | 0.000 | Down |
| HBMP | HBMP 13:1_12:0_13:1 | -1.094 | 0.001 | Down |
| HexCer | HexCer 18:1;20_18:5 | -3.642 | 0.000 | Down |
| HexCer | HexCer 16:1;30_17:0;(2OH) | -3.555 | 0.000 | Down |
| HexCer | HexCer 16:0;20_24:1 | -3.203 | 0.000 | Down |
| HexCer | HexCer 16:1;30_26:7;(2OH) | -2.961 | 0.000 | Down |
| HexCer | HexCer 18:0;20_18:5 | -2.109 | 0.000 | Down |
| HexCer | HexCer 16:0;20_30:4;0 | -1.874 | 0.000 | Down |
| HexCer | HexCer 34:0;20 | -1.557 | 0.000 | Down |
| HexCer | HexCer 34:1;30_HexCer 18:1;20_16:0;0 | -1.502 | 0.000 | Down |
| HexCer | HexCer 20:2;20_20:5 | -1.335 | 0.010 | Down |
| LDGTS | LDGTS 15:0 | -2.660 | 0.000 | Down |
| LNAPS | LNAPS 14:0_N-28:0 | -2.309 | 0.000 | Down |
| LPA | LPA 28:2 | -3.049 | 0.000 | Down |
| LPC | LPC 28:7 | -2.267 | 0.000 | Down |
| LPC | LPC 38:6 | -1.310 | 0.007 | Down |
| LPC | LPC 38:5 | -1.301 | 0.002 | Down |
| LPC | LPC 18:0 | 1.306 | 0.004 | UP |

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|-----|------------------|--------|-------|------|
| LPE | LPE O-17:1 | -2.884 | 0.000 | Down |
| LPE | LPE 18:1. | -2.055 | 0.000 | Down |
| LPE | LPE 16:0 | -1.784 | 0.001 | Down |
| LPE | LPE 18:1 | -1.672 | 0.000 | Down |
| LPE | LPE O-16:1. | 1.950 | 0.003 | UP |
| LPE | LPE O-18:1 | 2.851 | 0.000 | UP |
| MG | MG 22:5 | -3.705 | 0.000 | Down |
| MG | MG 12:0 | -3.486 | 0.000 | Down |
| MG | MG 17:0 | -2.962 | 0.000 | Down |
| MG | MG 16:0 | -2.914 | 0.000 | Down |
| MG | MG 18:0 | -2.914 | 0.000 | Down |
| MG | MG 15:4 | -2.880 | 0.000 | Down |
| MG | MG 18:3 | -2.844 | 0.000 | Down |
| MG | MG 15:0 | -2.843 | 0.000 | Down |
| MG | MG 17:4 | -2.838 | 0.000 | Down |
| MG | MG 15:3 | -2.806 | 0.000 | Down |
| MG | MG 15:2 | -2.800 | 0.000 | Down |
| MG | MG 19:5 | -2.775 | 0.000 | Down |
| MG | MG 10:0 | -2.731 | 0.000 | Down |
| MG | MG 16:3 | -2.729 | 0.000 | Down |
| MG | MG 9:0 | -2.689 | 0.000 | Down |
| MG | MG 21:1 | -2.579 | 0.000 | Down |
| MG | MG 13:0 | -2.563 | 0.000 | Down |
| MG | MGDG O-16:4_22:6 | -2.267 | 0.000 | Down |
| NAE | NAE 16:1 | -3.394 | 0.000 | Down |
| NAE | NAE 26:5 | -3.287 | 0.000 | Down |
| NAE | NAE 19:5 | -3.237 | 0.000 | Down |
| NAE | NAE 26:6 | -3.179 | 0.000 | Down |
| NAE | NAE 17:4 | -3.126 | 0.000 | Down |
| NAE | NAE 24:5 | -3.117 | 0.000 | Down |
| NAE | NAE 14:1 | -3.103 | 0.000 | Down |
| NAE | NAE 18:4 | -3.102 | 0.000 | Down |
| NAE | NAE 14:0 | -3.100 | 0.000 | Down |
| NAE | NAE 20:3 | -3.087 | 0.000 | Down |
| NAE | NAE 15:1 | -3.018 | 0.000 | Down |
| NAE | NAE 18:3 | -2.995 | 0.000 | Down |
| NAE | NAE 22:5 | -2.981 | 0.000 | Down |
| NAE | NAE 20:5 | -2.980 | 0.000 | Down |
| NAE | NAE 18:5 | -2.936 | 0.000 | Down |
| NAE | NAE 18:1 | -2.931 | 0.000 | Down |
| NAE | NAE 18:2 | -2.929 | 0.000 | Down |
| NAE | NAE 16:0 | -2.908 | 0.000 | Down |
| NAE | NAE 15:4 | -2.901 | 0.000 | Down |
| NAE | NAE 20:4 | -2.900 | 0.000 | Down |
| NAE | NAE 20:2 | -2.900 | 0.000 | Down |
| NAE | NAE 16:4 | -2.899 | 0.000 | Down |
| NAE | NAE 13:1 | -2.888 | 0.000 | Down |
| NAE | NAE 21:4 | -2.881 | 0.000 | Down |
| NAE | NAE 16:3 | -2.877 | 0.000 | Down |

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|----------|----------------------------|--------|-------|------|
| NAE | NAE 19:4 | -2.873 | 0.000 | Down |
| NAE | NAE 6:0 | -2.837 | 0.000 | Down |
| NAE | NAE 15:0 | -2.814 | 0.000 | Down |
| NAE | NAE 22:4 | -2.770 | 0.000 | Down |
| NAE | NAE 22:3 | -2.767 | 0.000 | Down |
| NAE | NAE 15:3 | -2.746 | 0.000 | Down |
| NAE | NAE 20:1 | -2.693 | 0.000 | Down |
| NAE | NAE 7:0 | -2.685 | 0.000 | Down |
| NAE | NAE 16:2 | -2.255 | 0.000 | Down |
| NAGly | NAGly 22:6_21:5 | -3.689 | 0.000 | Down |
| NAGly | NAGly 17:0;O | -2.884 | 0.000 | Down |
| NAGly | NAGly 13:1;O | -2.878 | 0.000 | Down |
| NAGly | NAGly 21:1_9:0 | -2.540 | 0.000 | Down |
| NAGlySer | NAGly 30:0_NAGly 20:0_10:0 | -2.971 | 0.000 | Down |
| NAGlySer | NAGlySer 22:6_20:1 | -1.992 | 0.000 | Down |
| NAOrn | NAOrn 13:0;O | -2.830 | 0.000 | Down |
| NAOrn | NAOrn 14:1;O | -2.770 | 0.000 | Down |
| NAOrn | NAGlySer 22:6_21:4 | -1.507 | 0.003 | Down |
| OxFA | OxFA 18:0;(2OH) | -2.866 | 0.000 | Down |
| OxFA | NAOrn 22:2_20:0 | -1.715 | 0.000 | Down |
| PA | PA 23:0_28:7 | -1.817 | 0.000 | Down |
| PA | OxFA 18:2;(2OH) | -1.511 | 0.000 | Down |
| PA | PA 17:0_28:6 | -1.330 | 0.004 | Down |
| PA | PA 15:0_28:7 | -1.171 | 0.014 | Down |
| PA | PA 21:0_28:6 | -1.055 | 0.020 | Down |
| PC | PC O-39:0 | -4.053 | 0.000 | Down |
| PC | PC 30:1 | -3.735 | 0.000 | Down |
| PC | PC O-30:0 | -3.540 | 0.000 | Down |
| PC | PC O-32:1 | -3.362 | 0.000 | Down |
| PC | PC O-12:0_22:5;4O | -3.251 | 0.000 | Down |
| PC | PC 32:2_PC 16:1_16:1 | -3.241 | 0.000 | Down |
| PC | PC 32:1_PC 16:0_16:1 | -3.213 | 0.000 | Down |
| PC | PC 30:0 | -3.200 | 0.000 | Down |
| PC | PC 15:0_18:1(d7) | -3.127 | 0.000 | Down |
| PC | PC O-35:7 | -3.117 | 0.000 | Down |
| PC | PC 28:0_PC 12:0_16:0 | -3.079 | 0.000 | Down |
| PC | PC O-32:0 | -2.978 | 0.000 | Down |
| PC | PC O-10:0_22:3;4O | -2.885 | 0.000 | Down |
| PC | PC 32:1 | -2.756 | 0.000 | Down |
| PC | PC 34:3_PC 16:1_18:2 | -2.702 | 0.000 | Down |
| PC | PC O-36:7 | -2.657 | 0.000 | Down |
| PC | PC 33:1 | -2.388 | 0.000 | Down |
| PC | PC O-39:3 | -2.364 | 0.000 | Down |
| PC | PC 32:0. | -2.309 | 0.000 | Down |
| PC | PC O-34:0 | -2.269 | 0.000 | Down |
| PC | PC 34:2 | -2.221 | 0.000 | Down |
| PC | PC O-34:1 | -2.198 | 0.000 | Down |
| PC | PC O-37:1 | -2.192 | 0.000 | Down |
| PC | PC O-37:8 | -2.177 | 0.000 | Down |

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|----|--------------------------|--------|-------|------|
| PC | PC 36:4_PC 18:2_18:2 | -2.125 | 0.001 | Down |
| PC | PC 34:3 | -2.098 | 0.001 | Down |
| PC | PC O-14:1_24:0;10 | -2.097 | 0.000 | Down |
| PC | PC 34:2_PC 16:0_18:2 | -1.981 | 0.001 | Down |
| PC | PC 34:1_PC 16:0_18:1 | -1.944 | 0.000 | Down |
| PC | PC O-14:0_22:5;30 | -1.912 | 0.000 | Down |
| PC | PC 33:0 | -1.900 | 0.000 | Down |
| PC | PC 32:0_PC 16:0_16:0 | -1.891 | 0.000 | Down |
| PC | PC O-18:0_18:1;10 | -1.883 | 0.000 | Down |
| PC | PC O-38:7 | -1.858 | 0.000 | Down |
| PC | PC O-36:4 | -1.770 | 0.000 | Down |
| PC | PC 36:0 | -1.758 | 0.000 | Down |
| PC | PC 38:6 | -1.719 | 0.000 | Down |
| PC | PC 36:3 | -1.682 | 0.001 | Down |
| PC | PC 32:0 | -1.636 | 0.000 | Down |
| PC | PC 34:0_PC 16:0_18:0 | -1.617 | 0.000 | Down |
| PC | PC 36:1 | -1.604 | 0.000 | Down |
| PC | PC 34:1 | -1.591 | 0.000 | Down |
| PC | PC O-12:0_22:3;20 | -1.560 | 0.001 | Down |
| PC | PC 10:0_26:1 | -1.546 | 0.001 | Down |
| PC | PC 40:5 | -1.522 | 0.004 | Down |
| PC | PC 35:1 | -1.501 | 0.000 | Down |
| PC | PC O-39:10 | -1.487 | 0.000 | Down |
| PC | PC 37:6. | -1.475 | 0.001 | Down |
| PC | PC 35:2 | -1.457 | 0.001 | Down |
| PC | PC O-39:8 | -1.456 | 0.000 | Down |
| PC | PC 39:2 | -1.431 | 0.003 | Down |
| PC | PC O-30:7 | -1.398 | 0.000 | Down |
| PC | PC O-37:9 | -1.332 | 0.001 | Down |
| PC | PC O-39:7 | -1.328 | 0.001 | Down |
| PC | PC 37:6 | -1.304 | 0.009 | Down |
| PC | PC 38:5 | -1.285 | 0.008 | Down |
| PC | PC 36:1_PC 18:0_18:1 | -1.282 | 0.002 | Down |
| PC | PC 36:2 | -1.260 | 0.009 | Down |
| PC | PC 36:3_PC 18:1_18:2 | -1.133 | 0.016 | Down |
| PC | PC 39:8 | -1.057 | 0.005 | Down |
| PC | PC 40:6 | -1.043 | 0.001 | Down |
| PC | PC 36:2_PC 18:0_18:2 | -1.023 | 0.038 | Down |
| PE | PE 32:1_PE 16:0_16:1 | -4.760 | 0.000 | Down |
| PE | PE 34:3 | -4.371 | 0.000 | Down |
| PE | PE O-32:2_PE O-16:1_16:1 | -4.067 | 0.000 | Down |
| PE | PE 32:1 | -3.831 | 0.000 | Down |
| PE | PE O-26:6_14:0 | -3.618 | 0.000 | Down |
| PE | PE P-32:1_PE P-16:0_16:1 | -3.463 | 0.000 | Down |
| PE | PE P-32:0_PE P-16:0_16:0 | -3.334 | 0.000 | Down |
| PE | PE O-32:1_PE O-16:1_16:0 | -3.317 | 0.000 | Down |
| PE | PE 32:0_PE 16:0_16:0 | -3.297 | 0.000 | Down |
| PE | PE 34:2_PE 16:1_18:1 | -3.118 | 0.000 | Down |
| PE | PE 34:2_PE 16:0_18:2 | -2.991 | 0.000 | Down |

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|--------|----------------------------|--------|-------|------|
| PE | PE 34:1_PE 16:0_18:1 | -2.927 | 0.000 | Down |
| PE | PE P-34:1_PE P-16:0_18:1 | -2.915 | 0.000 | Down |
| PE | PE 34:1_PE 16:0_18:1. | -2.874 | 0.000 | Down |
| PE | PE 34:2 | -2.827 | 0.000 | Down |
| PE | PE O-34:2_PE O-16:1_18:1 | -2.801 | 0.000 | Down |
| PE | PE P-36:2_PE P-18:1_18:1 | -2.764 | 0.000 | Down |
| PE | PE O-18:3_22:5;4O | -2.749 | 0.000 | Down |
| PE | PE 34:2. | -2.718 | 0.000 | Down |
| PE | PE 36:3_PE 18:1_18:2. | -2.655 | 0.000 | Down |
| PE | PE 34:0 | -2.585 | 0.000 | Down |
| PE | PE 36:3 | -2.585 | 0.000 | Down |
| PE | PE 36:3_PE 18:1_18:2 | -2.583 | 0.000 | Down |
| PE | PE P-34:2_PE P-16:0_18:2 | -2.338 | 0.000 | Down |
| PE | PE P-36:3_PE P-18:1_18:2 | -2.336 | 0.000 | Down |
| PE | PE O-36:3_PE O-18:2_18:1 | -2.273 | 0.000 | Down |
| PE | PE 36:3_PE 18:0_18:3 | -2.143 | 0.002 | Down |
| PE | PE 34:0_PE 16:0_18:0 | -2.141 | 0.000 | Down |
| PE | PE O-34:3_PE O-16:1_18:2 | -2.123 | 0.001 | Down |
| PE | PE 36:2 | -2.109 | 0.000 | Down |
| PE | PE 36:2;O_PE 18:0_18:2;O | -1.998 | 0.000 | Down |
| PE | PE O-24:5_16:0 | -1.894 | 0.000 | Down |
| PE | PE 36:2_PE 18:1_18:1. | -1.875 | 0.000 | Down |
| PE | PE P-38:5_PE P-18:1_20:4 | -1.832 | 0.002 | Down |
| PE | PE O-19:0_28:6 | -1.827 | 0.009 | Down |
| PE | PE O-22:4_22:6;3O | -1.824 | 0.000 | Down |
| PE | PE O-36:2_PE O-18:1_18:1 | -1.758 | 0.000 | Down |
| PE | PE 38:5 | -1.756 | 0.000 | Down |
| PE | PE 40:5 | -1.698 | 0.001 | Down |
| PE | PE 36:2_PE 18:0_18:2 | -1.676 | 0.002 | Down |
| PE | PE 36:1_PE 18:0_18:1 | -1.639 | 0.000 | Down |
| PE | PE 36:2_PE 18:1_18:1 | -1.638 | 0.002 | Down |
| PE | PE 36:1_PE 18:0_18:1. | -1.506 | 0.001 | Down |
| PE | PE O-38:6_PE O-18:2_20:4 | -1.477 | 0.028 | Down |
| PE | PE O-26:5_16:0 | -1.437 | 0.000 | Down |
| PE | PE 20:4_22:5 | -1.390 | 0.004 | Down |
| PE | PE 38:3 | -1.345 | 0.002 | Down |
| PE | PE O-37:4 | -1.289 | 0.001 | Down |
| PE-Cer | PE-Cer 13:1;2O_30:1 | -3.359 | 0.000 | Down |
| PE-Cer | PE-Cer 12:1;2O_16:1 | -2.786 | 0.000 | Down |
| PE-Cer | PE-Cer 12:1;2O_32:0 | -1.781 | 0.000 | Down |
| PE-Cer | PE-Cer 17:1;2O_36:8;O | -1.701 | 0.000 | Down |
| PEtOH | PEtOH 26:2_PEtOH 13:1_13:1 | -2.658 | 0.000 | Down |
| PG | PG 28:0_8:0 | -2.988 | 0.000 | Down |
| PG | PG O-15:0_28:0 | -2.901 | 0.000 | Down |
| PG | PG 18:2_18:1;1O | -1.349 | 0.004 | Down |
| PI-Cer | PI-Cer 39:4;3O | -3.259 | 0.000 | Down |
| PI-Cer | PI-Cer 13:2;2O_22:6;O | -3.216 | 0.000 | Down |
| PI-Cer | PI O-11:0_28:6 | -3.170 | 0.000 | Down |
| PI-Cer | PI 36:0 | -3.038 | 0.000 | Down |

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| PI-Cer | PI 17:0_22:3;2O | -2.999 | 0.000 | Down |
| PI-Cer | PI-Cer 13:1;2O_32:7;O | -2.967 | 0.000 | Down |
| PI-Cer | PI-Cer 39:1;3O | -2.964 | 0.000 | Down |
| PI-Cer | PI 34:1. | -2.938 | 0.000 | Down |
| PI-Cer | PI 34:1 | -2.880 | 0.000 | Down |
| PI-Cer | PI 34:0 | -2.848 | 0.000 | Down |
| PI-Cer | PI 24:0_18:2;3O | -2.367 | 0.000 | Down |
| PI-Cer | PI 18:0_28:6 | -2.159 | 0.000 | Down |
| PI-Cer | PI 18:0_28:5 | -2.114 | 0.000 | Down |
| PI-Cer | PI 36:1 | -2.060 | 0.000 | Down |
| PI-Cer | PI-Cer 36:2;3O | -2.020 | 0.001 | Down |
| PI-Cer | PI 36:2. | -2.001 | 0.000 | Down |
| PI-Cer | PI 24:0_22:6;4O | -1.979 | 0.000 | Down |
| PI-Cer | PI 26:0_17:0;2O | -1.840 | 0.000 | Down |
| PI-Cer | PI 16:0_28:5 | -1.804 | 0.000 | Down |
| PI-Cer | PI O-13:1_26:7 | -1.772 | 0.000 | Down |
| PI-Cer | PI 24:0_18:1;4O | -1.708 | 0.000 | Down |
| PI-Cer | PI 20:5_24:0;1O | -1.707 | 0.001 | Down |
| PI-Cer | PI 36:1_PI 18:0_18:1 | -1.623 | 0.000 | Down |
| PI-Cer | PI 16:0_22:6;4O | -1.588 | 0.000 | Down |
| PI-Cer | PI 36:2 | -1.482 | 0.001 | Down |
| PMeOH | PMeOH 28:7_28:7 | -2.785 | 0.000 | Down |
| PS | PS 34:0 | -3.176 | 0.000 | Down |
| PS | PS 22:5_22:6;4O | -2.977 | 0.000 | Down |
| PS | PS 8:0_28:1 | -2.615 | 0.000 | Down |
| PS | PS 44:2 | -2.311 | 0.000 | Down |
| PS | PS 36:2 | -1.867 | 0.000 | Down |
| PS | PS 36:2_PS 18:0_18:2 | -1.792 | 0.000 | Down |
| PS | PS 36:2. | -1.570 | 0.001 | Down |
| PS | PS 36:1_PS 18:0_18:1 | -1.515 | 0.000 | Down |
| PS | PS 36:2_PS 18:0_18:2. | -1.429 | 0.002 | Down |
| PS | PS 36:3 | -1.416 | 0.007 | Down |
| PS | PS 22:6_22:6 | -1.345 | 0.015 | Down |
| PS | PS 38:2 | -1.242 | 0.002 | Down |
| SE | SE 28:2_16:0 | -4.088 | 0.000 | Down |
| SE | SE 29:2_28:0 | -3.796 | 0.000 | Down |
| SE | SE 29:2_38:5 | -3.795 | 0.000 | Down |
| SE | SE 28:2_35:0 | -3.765 | 0.000 | Down |
| SE | SE 28:2_28:0 | -3.744 | 0.000 | Down |
| SE | SE 29:2_20:0 | -3.735 | 0.000 | Down |
| SE | SE 29:2_26:0 | -3.733 | 0.000 | Down |
| SE | SE 28:2_34:5 | -3.693 | 0.000 | Down |
| SE | SE 28:2_42:5 | -3.687 | 0.000 | Down |
| SE | SE 28:2_30:0 | -3.679 | 0.000 | Down |
| SE | SE 29:2_34:5 | -3.664 | 0.000 | Down |
| SE | SE 29:2_30:0 | -3.661 | 0.000 | Down |
| SE | SE 28:2_13:1 | -3.649 | 0.000 | Down |
| SE | SE 28:2_20:0 | -3.638 | 0.000 | Down |
| SE | SE 29:2_23:0 | -3.632 | 0.000 | Down |

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| SE | SE 28:2_32:0 | -3.626 | 0.000 | Down |
| SE | SE 29:2_22:0 | -3.613 | 0.000 | Down |
| SE | SE 29:2_24:0 | -3.590 | 0.000 | Down |
| SE | SE 27:2_16:0 | -3.574 | 0.000 | Down |
| SE | SE 28:2_10:0 | -3.538 | 0.000 | Down |
| SE | SE 28:2_36:5 | -3.481 | 0.000 | Down |
| SE | SE 28:4_32:6 | -2.374 | 0.000 | Down |
| SE | SE 28:2_19:4 | -2.228 | 0.000 | Down |
| SE | SE 28:2_19:5 | -1.667 | 0.000 | Down |
| SHexCe | SHexCer 39:0;2O | -3.175 | 0.000 | Down |
| SHexCe | SHexCer 32:0;3O | -2.736 | 0.000 | Down |
| SHexCe | SHexCer 38:3;3O | -1.841 | 0.000 | Down |
| SHexCe | SHexCer 43:3;3O | -1.835 | 0.000 | Down |
| SHexCe | SHexCer 35:0;3O | -1.689 | 0.002 | Down |
| SHexCe | SHexCer 12:1;2O_26:1 | -1.417 | 0.000 | Down |
| SL | SL 16:3;O_36:1;O | -3.366 | 0.000 | Down |
| SL | SL 12:1;O_34:6 | -3.362 | 0.000 | Down |
| SL | SL 12:2;O_34:0;O | -3.263 | 0.000 | Down |
| SL | SL 13:2;O_36:5;O | -2.826 | 0.000 | Down |
| SL | SL 13:2;O_32:2;O | -2.724 | 0.000 | Down |
| SL | SHexCer 45:4;3O | -1.954 | 0.000 | Down |
| SL | SL 16:3;O_30:8 | -1.529 | 0.017 | Down |
| SL | SL 22:1;O_36:9 | -1.212 | 0.001 | Down |
| SL | SL 13:1;O_34:5 | 2.147 | 0.044 | UP |
| SM | SM 42:0;2O | -4.491 | 0.000 | Down |
| SM | SM 41:0;2O | -4.265 | 0.000 | Down |
| SM | SM 42:1;2O | -3.855 | 0.000 | Down |
| SM | SM 40:0;2O_SM 28:0;2O_12:0 | -3.419 | 0.000 | Down |
| SM | SM 21:0;3O | -3.134 | 0.000 | Down |
| SM | SM 41:1;2O_SM 18:1;2O_23:0 | -3.131 | 0.000 | Down |
| SM | SM 42:1;3O | -2.981 | 0.000 | Down |
| SM | SM 42:2;2O | -2.847 | 0.000 | Down |
| SM | SM 42:2;2O_SM 18:1;2O_24:1 | -2.847 | 0.000 | Down |
| SM | SM 32:0;2O_SM 23:0;2O_9:0 | -2.773 | 0.000 | Down |
| SM | SM 32:0;2O | -2.697 | 0.000 | Down |
| SM | SM 39:1;3O | -2.677 | 0.000 | Down |
| SM | SM 42:1;2O_SM 18:1;2O_24:0 | -2.615 | 0.000 | Down |
| SM | SM 12:0;2O_27:0 | -2.590 | 0.000 | Down |
| SM | SM 15:3;2O_30:3 | -2.483 | 0.001 | Down |
| SM | SM 39:0;2O | -2.456 | 0.000 | Down |
| SM | SM 42:1;2O | -2.404 | 0.000 | Down |
| SM | SM 42:2;3O | -2.401 | 0.000 | Down |
| SM | SM 40:1;2O_SM 18:1;2O_22:0 | -2.381 | 0.000 | Down |
| SM | SM 40:1;2O. | -2.355 | 0.000 | Down |
| SM | SM 44:2;2O_SM 12:1;2O_32:1 | -2.334 | 0.000 | Down |
| SM | SM 41:4;3O | -2.272 | 0.000 | Down |
| SM | SM 28:5;2O(FA 22:6) | -2.200 | 0.000 | Down |
| SM | SM 42:3;2O_SM 18:1;2O_24:2 | -2.023 | 0.000 | Down |
| SM | SM 44:2;2O | -2.013 | 0.000 | Down |

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| SM | SM 44:3;2O | -2.004 | 0.000 | Down |
| SM | SM 13:1;2O_28:1 | -1.969 | 0.000 | Down |
| SM | SM 42:4;2O | -1.938 | 0.000 | Down |
| SM | SM 35:0;2O | -1.907 | 0.000 | Down |
| SM | SM 44:1;2O | -1.852 | 0.000 | Down |
| SM | SM 40:2;2O. | -1.834 | 0.000 | Down |
| SM | SM 32:1;2O | -1.807 | 0.000 | Down |
| SM | SM 42:3;3O. | -1.750 | 0.004 | Down |
| SM | SM 42:3;3O | -1.738 | 0.001 | Down |
| SM | SM 41:0;3O | -1.723 | 0.000 | Down |
| SM | SM 12:1;2O_29:0 | -1.694 | 0.000 | Down |
| SM | SM 13:1;2O_28:2 | -1.647 | 0.001 | Down |
| SM | SM 38:0;2O | -1.633 | 0.001 | Down |
| SM | SM 12:1;2O_28:3 | -1.618 | 0.000 | Down |
| SM | SM 12:0;2O_25:0 | -1.554 | 0.001 | Down |
| SM | SM 25:3;2O(FA 20:5) | -1.538 | 0.001 | Down |
| SM | SM 32:1;2O_SM 17:0;2O_15:1 | -1.532 | 0.000 | Down |
| SM | SM 40:1;2O | -1.527 | 0.001 | Down |
| SM | SM 39:0;3O | -1.491 | 0.001 | Down |
| SM | SM 34:0;2O | -1.469 | 0.001 | Down |
| SM | SM 40:2;2O | -1.404 | 0.000 | Down |
| SM | SM 30:3;2O(FA 22:6) | -1.306 | 0.001 | Down |
| SM | SM 12:1;2O_26:3 | -1.271 | 0.001 | Down |
| SM | SM 34:1;2O | -1.228 | 0.000 | Down |
| SM | SM 36:0;2O_SM 28:0;2O_8:0 | -1.023 | 0.013 | Down |
| SMGDG | SMGDG O-21:4_28:7 | -2.260 | 0.000 | Down |
| SMGDG | SMGDG O-17:0_28:5 | -1.511 | 0.000 | Down |
| ST | ST 29:2;O;Hex;FA 20:1 | -3.726 | 0.000 | Down |
| ST | ST 24:1;O4_19:2;1O | -3.072 | 0.000 | Down |
| ST | ST 24:2;O4_2:0 | -2.773 | 0.000 | Down |
| ST | SMGDG O-9:0_26:1 | -2.764 | 0.000 | Down |
| ST | ST 24:1;O4;G_16:2;1O | -2.554 | 0.000 | Down |
| ST | ST 29:1;O;Hex;FA 15:2 | -1.941 | 0.001 | Down |
| ST | ST 24:1;O3;G_28:6 | -1.776 | 0.026 | Down |
| ST | ST 29:1;O;Hex;FA 13:0 | -1.542 | 0.000 | Down |
| ST | ST 24:1;O4;T_21:1 | -1.485 | 0.000 | Down |
| ST | ST 24:1;O3_23:0 | -1.097 | 0.002 | Down |
| TG | TG 49:2;1O_TG 16:0_16:0_17:2;1O | -3.144 | 0.000 | Down |
| TG | TG 49:2_TG 16:0_16:1_17:1 | -3.029 | 0.000 | Down |
| TG | TG 49:2_TG 15:0_16:1_18:1 | -2.743 | 0.000 | Down |
| TG | TG 44:0_TG 14:0_14:0_16:0. | -2.533 | 0.000 | Down |
| TG | TG O-41:0_TG O-11:0_14:0_16:0 | -2.476 | 0.000 | Down |
| TG | TG 46:1_TG 14:0_14:0_18:1 | -2.452 | 0.000 | Down |
| TG | TG 38:1_TG 10:0_10:0_18:1 | -2.443 | 0.000 | Down |
| TG | TG 44:1_TG 10:0_16:0_18:1 | -2.439 | 0.000 | Down |
| TG | TG 43:0_TG 13:0_14:0_16:0 | -2.434 | 0.000 | Down |
| TG | TG 46:1_TG 14:0_16:0_16:1 | -2.425 | 0.000 | Down |
| TG | TG 36:0_TG 10:0_12:0_14:0 | -2.405 | 0.000 | Down |
| TG | TG 36:0_TG 10:0_12:0_14:0. | -2.401 | 0.000 | Down |

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| TG | TG 47:0_TG 15:0_16:0_16:0. | -2.394 | 0.000 | Down |
| TG | TG 46:0_TG 14:0_16:0_16:0 | -2.392 | 0.000 | Down |
| TG | TG O-52:1_TG O-19:1_16:0_17:0 | -2.380 | 0.000 | Down |
| TG | TG 44:0_TG 14:0_14:0_16:0 | -2.374 | 0.000 | Down |
| TG | TG 56:0;1O_TG 22:0_22:0_12:0;1O | -2.358 | 0.000 | Down |
| TG | TG 45:1_TG 14:0_15:0_16:1 | -2.357 | 0.000 | Down |
| TG | TG 42:0_TG 12:0_14:0_16:0 | -2.335 | 0.000 | Down |
| TG | TG 38:1;1O_TG 8:0_16:0_14:1;1O | -2.334 | 0.000 | Down |
| TG | TG 47:1_TG 15:0_16:0_16:1 | -2.330 | 0.000 | Down |
| TG | TG 40:0_TG 12:0_12:0_16:0 | -2.326 | 0.000 | Down |
| TG | TG 45:0_TG 15:0_15:0_15:0 | -2.317 | 0.000 | Down |
| TG | TG 40:0_TG 10:0_14:0_16:0 | -2.262 | 0.000 | Down |
| TG | TG 46:0_TG 14:0_16:0_16:0. | -2.215 | 0.000 | Down |
| TG | TG 42:0_TG 12:0_14:0_16:0. | -2.208 | 0.000 | Down |
| TG | TG 52:1;3O_TG 17:0_17:0_18:1;3O | -2.189 | 0.000 | Down |
| TG | TG 43:0_TG 12:0_15:0_16:0 | -2.177 | 0.000 | Down |
| TG | TG 49:0_TG 15:0_17:0_17:0 | -2.165 | 0.000 | Down |
| TG | TG 38:0_TG 8:0_14:0_16:0 | -2.147 | 0.000 | Down |
| TG | TG 48:0_TG 16:0_16:0_16:0. | -2.105 | 0.000 | Down |
| TG | TG 38:0_TG 12:0_12:0_14:0 | -2.071 | 0.000 | Down |
| TG | TG 38:1_TG 10:0_14:0_14:1 | -2.043 | 0.000 | Down |
| TG | TG 40:1_TG 8:0_16:0_16:1 | -1.983 | 0.000 | Down |
| TG | TG 48:2_TG 16:0_16:1_16:1 | -1.961 | 0.000 | Down |
| TG | TG O-54:1_TG O-18:0_18:0_18:1 | -1.953 | 0.000 | Down |
| TG | TG 49:0_TG 16:0_16:0_17:0 | -1.911 | 0.000 | Down |
| TG | TG 42:2_TG 10:0_14:1_18:1 | -1.910 | 0.000 | Down |
| TG | TG 38:1_TG 10:0_10:0_18:1. | -1.881 | 0.000 | Down |
| TG | TG 56:0_TG 15:0_16:0_25:0 | -1.862 | 0.000 | Down |
| TG | TG O-57:1_TG O-19:0_16:1_22:0 | -1.860 | 0.000 | Down |
| TG | TG 49:0_TG 16:0_16:0_17:0. | -1.780 | 0.000 | Down |
| TG | TG 48:4_TG 14:0_16:1_18:3 | -1.780 | 0.000 | Down |
| TG | TG 48:1_TG 14:0_16:0_18:1 | -1.776 | 0.000 | Down |
| TG | TG 36:0_TG 12:0_12:0_12:0 | -1.764 | 0.000 | Down |
| TG | TG 47:0_TG 15:0_16:0_16:0 | -1.761 | 0.000 | Down |
| TG | TG 42:1_TG 10:0_14:0_18:1 | -1.745 | 0.000 | Down |
| TG | TG 40:2_TG 12:0_14:1_14:1 | -1.726 | 0.000 | Down |
| TG | TG 54:0_TG 18:0_18:0_18:0 | -1.704 | 0.000 | Down |
| TG | TG 58:0_TG 16:0_16:0_26:0 | -1.679 | 0.000 | Down |
| TG | TG O-55:1_TG O-19:1_18:0_18:0 | -1.634 | 0.000 | Down |
| TG | TG 48:3_TG 14:0_16:1_18:2. | -1.575 | 0.000 | Down |
| TG | TG 55:0_TG 15:0_16:0_24:0 | -1.562 | 0.000 | Down |
| TG | TG 55:2_TG 18:0_18:1_19:1 | -1.555 | 0.000 | Down |
| TG | TG 48:0_TG 16:0_16:0_16:0 | -1.554 | 0.000 | Down |
| TG | TG 49:1_TG 16:0_16:0_17:1 | -1.537 | 0.000 | Down |
| TG | TG 51:0_TG 17:0_17:0_17:0 | -1.509 | 0.000 | Down |
| TG | TG 8:0_9:0_22:1 | -1.503 | 0.000 | Down |
| TG | TG 47:1;1O_TG 16:0_16:0_15:1;1O | -1.481 | 0.000 | Down |
| TG | TG 51:0_TG 16:0_17:0_18:0 | -1.410 | 0.000 | Down |
| TG | TG 38:0_TG 8:0_12:0_18:0 | -1.382 | 0.001 | Down |

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|-----------|---------------------------------|--------|-------|------|
| TG | TG 54:0_TG 18:0_18:0_18:0. | -1.359 | 0.000 | Down |
| TG | TG 53:0_TG 14:0_16:0_23:0 | -1.336 | 0.000 | Down |
| TG | TG 48:2_TG 14:0_16:1_18:1 | -1.331 | 0.001 | Down |
| TG | TG 50:0_TG 16:0_16:0_18:0. | -1.168 | 0.003 | Down |
| TG | TG 50:2_TG 16:0_16:1_18:1. | -1.111 | 0.001 | Down |
| TG | TG 56:1_TG 16:0_22:0_18:1 | -1.100 | 0.003 | Down |
| TG | TG 48:3_TG 14:0_16:1_18:2 | -1.033 | 0.004 | Down |
| TG | TG 34:0_TG 8:0_12:0_14:0 | -1.028 | 0.001 | Down |
| TG | TG O-49:1_TG O-17:1_14:0_18:0 | -1.012 | 0.002 | Down |
| TG | TG 54:3;1O_TG 18:1_18:1_18:1;1O | 1.629 | 0.011 | UP |
| TG | TG 54:4_TG 18:1_18:1_18:2 | 2.808 | 0.013 | UP |

Appendix Table 5-2. List of all significantly up- and down-regulated lipids elements in comparison between porcine colostrum exosomes (day 0) and milk exosomes at day 7. Significance was set using T-test FDR adjusted p-value threshold at 0.05 and fold change threshold at 2 ($|\log_2 FC| > 1$).

| Day 0 vs 14 | | | | |
|-------------|------------------------------------|-----------------------|----------------------|---------|
| Lipid class | Lipid name | log ₂ (FC) | FDR Adjusted P value | UP/Down |
| SL | SL 12:1;O_34:6 | -12.586 | 0.002 | Down |
| Cer | Cer 35:5;5O_Cer 19:2;3O_16:3;(2OH) | -11.829 | 0.002 | Down |
| SM | SM 32:0;2O | -11.528 | 0.002 | Down |
| SM | SM 34:1;2O | -10.275 | 0.048 | Down |
| MAE | NAE 16:2 | -10.194 | 0.005 | Down |
| PC | PC 32:0. | -10.109 | 0.002 | Down |
| DG | DG 35:6 | -10.078 | 0.000 | Down |
| Cer | Cer 94:5;4O | -9.599 | 0.003 | Down |
| DG | DG 42:0 | -9.590 | 0.000 | Down |
| MG | MGDG O-16:4_22:6 | -9.505 | 0.001 | Down |
| AHexCer | AHexCer 60:8;3O | -9.343 | 0.003 | Down |
| DG | DG 49:6 | -9.321 | 0.000 | Down |
| DG | DG 43:0 | -9.297 | 0.000 | Down |
| DG | DG 42:5 | -9.281 | 0.000 | Down |
| DG | DG O-55:1_DG O-27:0_28:1 | -9.273 | 0.000 | Down |
| DG | DG 45:0 | -9.273 | 0.000 | Down |
| SE | SE 28:2_30:0 | -9.271 | 0.000 | Down |
| SE | SE 29:2_28:0 | -9.239 | 0.000 | Down |
| DG | DG 86:5 | -9.237 | 0.000 | Down |
| SE | SE 28:2_28:0 | -9.222 | 0.000 | Down |
| Cer | Cer 12:0;2O_23:0;O | -9.217 | 0.000 | Down |
| SM | SM 42:1;2O | -9.183 | 0.003 | Down |
| DG | DG 35:0 | -9.153 | 0.000 | Down |
| SE | SE 29:2_26:0 | -9.147 | 0.000 | Down |
| SE | SE 28:2_32:0 | -9.123 | 0.000 | Down |
| AHexCer | AHexCer 72:9;3O | -9.079 | 0.004 | Down |
| SE | SE 29:2_30:0 | -9.077 | 0.000 | Down |
| DG | DG 36:0 | -9.067 | 0.000 | Down |
| DG | DG 50:0 | -9.041 | 0.000 | Down |
| DG | DG O-48:1_DG O-20:0_28:1 | -9.037 | 0.000 | Down |
| SM | SM 12:1;2O_26:3 | -9.012 | 0.020 | Down |
| DG | DG 39:0 | -9.006 | 0.000 | Down |
| DG | DG 51:0 | -8.987 | 0.000 | Down |
| DG | DG 47:0 | -8.965 | 0.000 | Down |
| SM | SM 44:2;2O_SM 12:1;2O_32:1 | -8.956 | 0.003 | Down |
| DG | DG 46:0 | -8.946 | 0.000 | Down |
| SE | SE 29:2_23:0 | -8.945 | 0.000 | Down |
| DG | DG 40:0 | -8.928 | 0.000 | Down |
| SE | SE 28:2_34:5 | -8.923 | 0.000 | Down |
| MAE | NAE 18:5 | -8.893 | 0.001 | Down |
| DG | DG 19:0 | -8.889 | 0.000 | Down |

| | | | | |
|---------|--------------------------------|--------|-------|------|
| SE | SE 29:2_24:0 | -8.862 | 0.000 | Down |
| SE | SE 29:2_38:5 | -8.852 | 0.000 | Down |
| SE | SE 28:2_42:5 | -8.851 | 0.000 | Down |
| DG | DG 41:0 | -8.846 | 0.000 | Down |
| SE | SE 28:2_35:0 | -8.842 | 0.000 | Down |
| DG | DG 39:1 | -8.816 | 0.000 | Down |
| DG | DG 44:6_DG 16:0_28:6 | -8.793 | 0.000 | Down |
| DG | DG O-44:1_DG O-18:0_26:1 | -8.793 | 0.000 | Down |
| SE | SE 28:2_16:0 | -8.786 | 0.000 | Down |
| DG | DG 43:8 | -8.769 | 0.000 | Down |
| DG | DG 41:6 | -8.768 | 0.000 | Down |
| DG | DG 52:6 | -8.756 | 0.000 | Down |
| DG | DG 49:0 | -8.747 | 0.000 | Down |
| ST | ST 29:2;O;Hex;FA 20:1 | -8.734 | 0.000 | Down |
| MG | MG 13:0 | -8.733 | 0.001 | Down |
| DG | DG 46:11 | -8.673 | 0.000 | Down |
| DG | DG 51:7 | -8.672 | 0.000 | Down |
| SE | SE 29:2_22:0 | -8.669 | 0.000 | Down |
| DG | DG 50:6 | -8.661 | 0.000 | Down |
| SE | SE 28:2_10:0 | -8.611 | 0.000 | Down |
| DG | DG O-36:1_DG O-17:0_19:1 | -8.610 | 0.000 | Down |
| DG | DG 49:2 | -8.590 | 0.000 | Down |
| MG | MG 18:3 | -8.577 | 0.000 | Down |
| DG | DG O-38:1_DG O-14:0_24:1 | -8.565 | 0.000 | Down |
| SM | SM 12:1;2O_28:3 | -8.565 | 0.005 | Down |
| DG | DGCC 36:2_DGCC 18:1_18:1 | -8.543 | 0.000 | Down |
| DG | DG 44:0 | -8.541 | 0.000 | Down |
| DG | DG 30:5 | -8.496 | 0.000 | Down |
| DG | DG O-30:1_DG O-16:0_14:1 | -8.481 | 0.000 | Down |
| SM | SM 42:0;2O | -8.472 | 0.000 | Down |
| DG | DG O-33:1_DG O-17:0_16:1 | -8.463 | 0.000 | Down |
| SM | SM 36:0;2O_SM 28:0;2O_8:0 | -8.445 | 0.021 | Down |
| DG | DG 39:6 | -8.396 | 0.000 | Down |
| DG | DG 27:5 | -8.371 | 0.000 | Down |
| CAR | CAR 16:0 | -8.368 | 0.005 | Down |
| MG | MG 22:5 | -8.353 | 0.000 | Down |
| SM | SM 41:1;2O_SM 18:1;2O_23:0 | -8.350 | 0.000 | Down |
| Cer | Cer 94:3;4O | -8.335 | 0.000 | Down |
| DG | DG 47:6 | -8.313 | 0.000 | Down |
| PS | PS 36:2 | -8.286 | 0.002 | Down |
| SE | SE 27:2_16:0 | -8.281 | 0.000 | Down |
| DG | DG 45:7 | -8.239 | 0.000 | Down |
| Cer | Cer 36:1;3O_Cer 19:0;2O_17:1;O | -8.226 | 0.000 | Down |
| DG | DG 27:4 | -8.207 | 0.000 | Down |
| SE | SE 29:2_20:0 | -8.195 | 0.000 | Down |
| DG | DG 43:7 | -8.183 | 0.000 | Down |
| AHexCer | AHexCer 54:6;3O | -8.167 | 0.007 | Down |
| DG | DG 46:6 | -8.159 | 0.000 | Down |
| DG | DG 28:2 | -8.154 | 0.000 | Down |

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|---------|------------------------------------|--------|-------|------|
| DG | DG 41:6_DG 15:0_26:6 | -8.148 | 0.000 | Down |
| SM | SM 41:0;20 | -8.137 | 0.000 | Down |
| DG | DG 45:8 | -8.129 | 0.000 | Down |
| DG | DG 38:6 | -8.120 | 0.000 | Down |
| DG | DG 48:6 | -8.105 | 0.000 | Down |
| DG | DG 40:6 | -8.084 | 0.000 | Down |
| MAE | NAE 14:1 | -8.028 | 0.000 | Down |
| MAE | NAE 15:4 | -8.015 | 0.000 | Down |
| DG | DG 49:8 | -8.013 | 0.000 | Down |
| Cer | Cer 92:5;40 | -8.001 | 0.002 | Down |
| DG | DG 20:0 | -7.970 | 0.000 | Down |
| PS | PS 38:2 | -7.970 | 0.017 | Down |
| DG | DG O-41:1_DG O-13:0_28:1 | -7.947 | 0.000 | Down |
| DG | DG 49:7 | -7.916 | 0.000 | Down |
| MG | MG 16:3 | -7.901 | 0.000 | Down |
| DG | DG 52:7 | -7.898 | 0.000 | Down |
| DG | DG O-39:1_DG O-13:0_26:1 | -7.883 | 0.000 | Down |
| AHexCer | AHexCer 40:4;30 | -7.881 | 0.000 | Down |
| SE | SE 28:2_20:0 | -7.873 | 0.000 | Down |
| PS | PS 36:3 | -7.848 | 0.003 | Down |
| DG | DG O-40:1_DG O-16:0_24:1 | -7.846 | 0.000 | Down |
| SE | SE 28:4_32:6 | -7.812 | 0.001 | Down |
| Cer | Cer 65:5;40 | -7.808 | 0.000 | Down |
| SE | SE 29:2_34:5 | -7.779 | 0.000 | Down |
| DG | DG 45:6 | -7.749 | 0.000 | Down |
| DG | DG 42:6_DG 16:0_26:6 | -7.740 | 0.000 | Down |
| DG | DG 41:7 | -7.712 | 0.000 | Down |
| PC | PC 10:0_26:1 | -7.712 | 0.010 | Down |
| Cer | Cer 61:13;40 | -7.694 | 0.002 | Down |
| DG | DG 25:1 | -7.690 | 0.000 | Down |
| DG | DG 51:6 | -7.681 | 0.000 | Down |
| MAE | NAE 18:4 | -7.636 | 0.000 | Down |
| DG | DG 39:8 | -7.630 | 0.000 | Down |
| Cer | Cer 38:3;50_Cer 21:2;30_17:1;(2OH) | -7.615 | 0.000 | Down |
| DG | DG 51:4 | -7.545 | 0.000 | Down |
| PC | PC 34:3 | -7.518 | 0.001 | Down |
| Cer | Cer 13:2;20_40:3 | -7.509 | 0.000 | Down |
| MAE | NAE 14:0 | -7.493 | 0.000 | Down |
| DG | DG 51:13 | -7.492 | 0.004 | Down |
| PE | PE 32:1 | -7.469 | 0.000 | Down |
| DG | DG 51:8 | -7.466 | 0.000 | Down |
| DG | DG 43:6 | -7.465 | 0.000 | Down |
| DG | DG 36:0_DG 18:0_18:0 | -7.447 | 0.000 | Down |
| SE | SE 28:2_36:5 | -7.430 | 0.000 | Down |
| DG | DG 50:1 | -7.413 | 0.000 | Down |
| DG | DG 36:6 | -7.412 | 0.000 | Down |
| Cer | Cer 38:5;40 | -7.405 | 0.000 | Down |
| SE | SE 28:2_13:1 | -7.358 | 0.000 | Down |
| CE | CE 18:1 | -7.338 | 0.000 | Down |

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|--------|--------------------------------------|--------|-------|------|
| DG | DG 18:0 | -7.331 | 0.001 | Down |
| DG | DG 36:1_DG 18:0_18:1 | -7.307 | 0.000 | Down |
| PC | PC O-36:7 | -7.243 | 0.000 | Down |
| DG | DG 31:0 | -7.232 | 0.000 | Down |
| MAE | NAE 16:4 | -7.224 | 0.000 | Down |
| Cer | CerP 29:2;2O_CerP 16:1;2O_13:1 | -7.220 | 0.000 | Down |
| SM | SM 42:1;2O | -7.205 | 0.000 | Down |
| Cer | Cer 58:5;4O | -7.130 | 0.001 | Down |
| MG | MG 15:0 | -7.127 | 0.000 | Down |
| DG | DG 34:0 | -7.122 | 0.000 | Down |
| SM | SM 40:2;2O. | -7.106 | 0.003 | Down |
| DG | DG 22:1 | -7.096 | 0.000 | Down |
| DG | DG 37:4 | -7.083 | 0.000 | Down |
| DG | DG 39:7 | -7.077 | 0.000 | Down |
| Cer | Cer 36:2;3O_Cer 19:0;2O_17:2;O | -7.061 | 0.000 | Down |
| DG | DG 25:2 | -7.043 | 0.000 | Down |
| MAE | NAE 26:5 | -7.029 | 0.000 | Down |
| DG | DG 42:10 | -7.026 | 0.000 | Down |
| MG | MG 21:1 | -7.015 | 0.000 | Down |
| Cer | Cer 40:0;4O_Cer 30:0;3O_10:0;(2OH) | -7.008 | 0.000 | Down |
| DG | DG 25:0 | -7.007 | 0.000 | Down |
| DG | DG 37:7 | -7.005 | 0.000 | Down |
| DG | DG 34:0_DG 16:0_18:0 | -6.994 | 0.000 | Down |
| DG | DG O-35:0_DG O-19:0_16:0 | -6.986 | 0.000 | Down |
| DG | DG 34:2_DG 16:0_18:2 | -6.980 | 0.000 | Down |
| CAR | CAR 18:1 | -6.957 | 0.012 | Down |
| DG | DG 16:0 | -6.946 | 0.000 | Down |
| PI | PI 34:1 | -6.944 | 0.000 | Down |
| MAE | NAE 22:3 | -6.940 | 0.000 | Down |
| Cer | Cer 34:1;3O_Cer 19:0;2O_15:1;O | -6.927 | 0.000 | Down |
| DG | DG 34:3 | -6.926 | 0.000 | Down |
| DG | DG 36:5 | -6.924 | 0.000 | Down |
| PC | PC 38:5. | -6.909 | 0.028 | Down |
| SM | SM 42:1;3O | -6.894 | 0.001 | Down |
| DG | DG 27:0 | -6.892 | 0.000 | Down |
| Cer | Cer 12:2;2O_24:1 | -6.889 | 0.000 | Down |
| DG | DG 36:2 | -6.882 | 0.000 | Down |
| DG | DG O-45:6_DG O-17:0_28:6 | -6.852 | 0.000 | Down |
| MAE | NAE 19:5 | -6.846 | 0.000 | Down |
| DG | DG 43:2 | -6.824 | 0.000 | Down |
| Cer | CerP 34:2;2O_CerP 19:1;2O_15:1 | -6.823 | 0.000 | Down |
| MAE | NAE 18:3 | -6.758 | 0.000 | Down |
| DG | DG 40:2 | -6.751 | 0.000 | Down |
| ST | ST 24:1;O4_19:2;1O | -6.729 | 0.000 | Down |
| DG | DG 38:5 | -6.725 | 0.000 | Down |
| DG | DG 40:5 | -6.713 | 0.000 | Down |
| DG | DG 32:0 | -6.692 | 0.000 | Down |
| HexCer | HexCer 34:1;3O_HexCer 18:1;2O_16:0;O | -6.685 | 0.004 | Down |
| Cer | Cer 12:2;2O_39:10;2O | -6.673 | 0.000 | Down |

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|-----|------------------------------|--------|-------|------|
| ST | ST 24:1;O4;T_26:2 | -6.668 | 0.011 | Down |
| PC | PC O-39:0 | -6.668 | 0.000 | Down |
| DG | DG 34:2 | -6.658 | 0.000 | Down |
| DG | DG 55:9 | -6.652 | 0.007 | Down |
| DG | DGTS 16:0_17:3 | -6.642 | 0.000 | Down |
| DG | DG O-42:6_DG O-18:0_24:6 | -6.623 | 0.000 | Down |
| DG | DG 38:2 | -6.603 | 0.000 | Down |
| DG | DG 53:10 | -6.589 | 0.005 | Down |
| DG | DG 41:8 | -6.575 | 0.002 | Down |
| DG | DG 52:11 | -6.541 | 0.000 | Down |
| PC | PC O-35:7 | -6.534 | 0.000 | Down |
| Cer | Cer 60:11;40 | -6.519 | 0.000 | Down |
| CAR | CAR 19:3 | -6.517 | 0.000 | Down |
| Cer | Cer 81:9;40 | -6.516 | 0.022 | Down |
| Cer | Cer 36:4;40 | -6.506 | 0.000 | Down |
| MAE | NAE 26:6 | -6.497 | 0.000 | Down |
| PE | PE 34:2 | -6.485 | 0.000 | Down |
| SM | SM 32:1;2O | -6.483 | 0.001 | Down |
| SM | SM 40:0;2O_SM 28:0;2O_12:0 | -6.479 | 0.000 | Down |
| DG | DG 36:1 | -6.476 | 0.000 | Down |
| MAE | NAE 21:4 | -6.463 | 0.000 | Down |
| DG | DG 46:8 | -6.456 | 0.000 | Down |
| SM | SM 39:0;2O | -6.445 | 0.000 | Down |
| PC | PC 30:1 | -6.441 | 0.000 | Down |
| DG | DG 24:1 | -6.433 | 0.000 | Down |
| DG | DG 32:3 | -6.430 | 0.000 | Down |
| DG | DG 33:1 | -6.418 | 0.000 | Down |
| DG | DG 46:3 | -6.416 | 0.000 | Down |
| DG | DGCC 15:2_18:5 | -6.414 | 0.000 | Down |
| DG | DG 29:0 | -6.382 | 0.000 | Down |
| Cer | Cer 38:0;2O_Cer 18:0;2O_20:0 | -6.380 | 0.000 | Down |
| MAE | NAE 20:3 | -6.379 | 0.000 | Down |
| DG | DG 26:0 | -6.355 | 0.000 | Down |
| DG | DG 52:13 | -6.349 | 0.012 | Down |
| DG | DG 30:0 | -6.331 | 0.000 | Down |
| Cer | CerP 16:2;2O_28:3 | -6.312 | 0.042 | Down |
| PS | PS 34:0 | -6.311 | 0.000 | Down |
| Cer | Cer 58:11;40 | -6.310 | 0.000 | Down |
| DG | DG 32:0_DG 16:0_16:0 | -6.290 | 0.000 | Down |
| DG | DG 34:1 | -6.281 | 0.001 | Down |
| Cer | Cer 35:6;2O_Cer 17:3;2O_18:3 | -6.276 | 0.000 | Down |
| Cer | Cer 31:4;2O_Cer 12:2;2O_19:2 | -6.270 | 0.001 | Down |
| MAE | NAE 24:5 | -6.258 | 0.000 | Down |
| DG | DG 23:4 | -6.207 | 0.000 | Down |
| DG | DG 28:1 | -6.191 | 0.001 | Down |
| MG | MG 19:5 | -6.145 | 0.000 | Down |
| ST | ST 24:2;O4_2:0 | -6.137 | 0.000 | Down |
| SE | SE 28:2_19:4 | -6.116 | 0.001 | Down |
| DG | DG 28:0 | -6.112 | 0.000 | Down |

| | | | | |
|----------------|------------------------------------|--------|-------|------|
| SL | SL 22:1;O_36:9 | -6.111 | DDG | Down |
| MAE | NAE 20:5 | -6.089 | 0.000 | Down |
| DG | DG 29:5 | -6.061 | 0.000 | Down |
| MAE | NAE 15:1 | -6.054 | 0.000 | Down |
| CoQ10 | CoQ10 | -6.030 | 0.001 | Down |
| CAR | CAR 13:0 | -6.027 | 0.000 | Down |
| PC | PC O-32:1 | -5.997 | 0.000 | Down |
| NAGly | NAGly 21:1_9:0 | -5.978 | 0.000 | Down |
| DG | DG 32:2 | -5.958 | 0.000 | Down |
| Cer | Cer 28:3;4O. | -5.943 | 0.000 | Down |
| SM | SM 42:2;3O | -5.932 | 0.009 | Down |
| SM | SM 44:3;2O | -5.929 | 0.000 | Down |
| DG | DG 31:4 | -5.909 | 0.000 | Down |
| DG | DG 45:11 | -5.894 | 0.000 | Down |
| SHexCer | SHexCer 12:1;2O_26:1 | -5.870 | 0.023 | Down |
| Cer | Cer 50:10;4O | -5.861 | 0.000 | Down |
| DG | DG 40:8 | -5.826 | 0.000 | Down |
| Cer | Cer 12:1;2O_30:0 | -5.824 | 0.001 | Down |
| Cer | Cer 42:1;2O_Cer 18:1;2O_24:0. | -5.804 | 0.001 | Down |
| PC | PC 33:1 | -5.803 | 0.000 | Down |
| DG | DG 40:9 | -5.776 | 0.000 | Down |
| DG | DG 53:9 | -5.766 | 0.001 | Down |
| CAR | CAR 5:0 | -5.740 | 0.000 | Down |
| DG | DG 24:2 | -5.730 | 0.000 | Down |
| SE | SE 28:2_19:5 | -5.728 | 0.001 | Down |
| DG | DG 44:7 | -5.713 | 0.000 | Down |
| MG | MG 17:0 | -5.701 | 0.000 | Down |
| Cer | Cer 34:3;4O_Cer 19:2;3O_15:1;(2OH) | -5.694 | 0.000 | Down |
| PC | PC 32:2_PC 16:1_16:1 | -5.692 | 0.000 | Down |
| Cer | CerP 30:3;2O_CerP 12:0;2O_18:3 | -5.678 | 0.000 | Down |
| Cer | Cer 42:2;2O_Cer 18:1;2O_24:1 | -5.668 | 0.003 | Down |
| HexCer | HexCer 34:0;2O | -5.642 | 0.004 | Down |
| DG | DGGA 12:0_22:1 | -5.640 | 0.001 | Down |
| DG | DG 57:9 | -5.636 | 0.016 | Down |
| DG | DGGA 10:0_22:1 | -5.625 | 0.000 | Down |
| DG | DG 36:4 | -5.618 | 0.000 | Down |
| PC | PC 28:0_PC 12:0_16:0 | -5.617 | 0.000 | Down |
| Cer | Cer 89:6;4O | -5.598 | 0.018 | Down |
| Cer | Cer 42:0;4O | -5.593 | 0.000 | Down |
| DG | DG 23:1 | -5.575 | 0.000 | Down |
| MAE | NAE 16:0 | -5.572 | 0.000 | Down |
| PE | PE 32:1_PE 16:0_16:1 | -5.571 | 0.000 | Down |
| PE | PE O-32:2_PE O-16:1_16:1 | -5.544 | 0.000 | Down |
| NAGly | NAGly 22:6_21:5 | -5.542 | 0.000 | Down |
| MAE | NAE 13:1 | -5.531 | 0.000 | Down |
| PS | PS 8:0_28:1 | -5.502 | 0.000 | Down |
| Cer | Cer 42:1;2O_Cer 18:1;2O_24:0 | -5.487 | 0.001 | Down |
| DG | DG 48:13 | -5.481 | 0.000 | Down |
| SM | SM 42:4;2O | -5.472 | 0.000 | Down |

| | | | | |
|----------------|---|--------|-------|------|
| Cer | Cer 38:1;2O_Cer 18:1;2O_20:0 | -5.468 | 0.012 | Down |
| MAE | NAE 15:0 | -5.466 | 0.000 | Down |
| MAE | NAE 20:4 | -5.445 | 0.000 | Down |
| PS | PS 36:1_PS 18:0_18:1 | -5.434 | 0.004 | Down |
| PE | PE 34:3 | -5.424 | 0.000 | Down |
| AHexCer | AHexCer 72:4;3O | -5.421 | 0.036 | Down |
| MG | MG 9:0 | -5.404 | 0.000 | Down |
| PS | PS 36:2_PS 18:0_18:2 | -5.399 | 0.001 | Down |
| DG | DG 34:1_DG 16:0_18:1 | -5.386 | 0.000 | Down |
| DG | DG 35:2 | -5.378 | 0.000 | Down |
| DG | DG 34:5 | -5.356 | 0.000 | Down |
| Cer | Cer 28:3;4O | -5.351 | 0.000 | Down |
| MAE | NAE 22:5 | -5.340 | 0.000 | Down |
| PE | PE 34:2. | -5.340 | 0.000 | Down |
| AHexCer | AHexCer 39:4;3O_AHexCer (O-14:1)25:3;3O | -5.333 | 0.004 | Down |
| MAE | NAE 16:1 | -5.316 | 0.000 | Down |
| DG | DG 23:0 | -5.315 | 0.000 | Down |
| MAE | NAE 20:1 | -5.314 | 0.000 | Down |
| DG | DG 23:2 | -5.296 | 0.000 | Down |
| DG | DG 32:7 | -5.289 | 0.000 | Down |
| LDGTS | LDGTS 15:0 | -5.286 | 0.000 | Down |
| SM | SM 30:3;2O(FA 22:6) | -5.282 | 0.003 | Down |
| Cer | Cer 42:1;3O_Cer 18:1;2O_24:0;O | -5.274 | 0.003 | Down |
| Cer | Cer 40:7;4O | -5.262 | 0.000 | Down |
| PI | PI 36:1 | -5.261 | 0.000 | Down |
| DG | DG 38:8 | -5.249 | 0.000 | Down |
| DG | DG 33:7 | -5.222 | 0.000 | Down |
| SM | SM 44:2;2O | -5.215 | 0.001 | Down |
| MG | MGDG O-14:1_20:4 | -5.205 | 0.017 | Down |
| FA | FA 16:2;3O | -5.202 | 0.000 | Down |
| Cer | Cer 33:6;4O | -5.201 | 0.000 | Down |
| Cer | Cer 24:3;3O_Cer 16:3;2O_8:0;O | -5.200 | 0.000 | Down |
| PI | PI-Cer 39:1;3O | -5.191 | 0.000 | Down |
| Cer | Cer 34:0;3O_Cer 18:0;2O_16:0;O | -5.186 | 0.000 | Down |
| PS | PS 44:2 | -5.179 | 0.001 | Down |
| BMP | BMP 15:0_8:0 | -5.152 | 0.000 | Down |
| DG | DG 30:2 | -5.143 | 0.000 | Down |
| PC | PC 35:2 | -5.142 | 0.001 | Down |
| PC | PC 33:0 | -5.135 | 0.001 | Down |
| DG | DG 26:5 | -5.128 | 0.000 | Down |
| DG | DG 42:11 | -5.126 | 0.000 | Down |
| DG | DGDG O-8:0_17:0 | -5.120 | 0.003 | Down |
| PC | PC 38:6 | -5.120 | 0.001 | Down |
| PC | PC 32:1 | -5.110 | 0.000 | Down |
| MAE | NAE 15:3 | -5.110 | 0.000 | Down |
| DG | DG 38:7 | -5.101 | 0.000 | Down |
| MAE | NAE 17:4 | -5.094 | 0.000 | Down |
| DG | DG 26:2 | -5.092 | 0.000 | Down |

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| DG | DG 38:4 | -5.090 | 0.002 | Down |
| DG | DG 36:2_DG 18:1_18:1 | -5.088 | 0.000 | Down |
| PI | PI-Cer 39:4;3O | -5.074 | 0.000 | Down |
| DG | DG 29:3 | -5.074 | 0.000 | Down |
| NAGly | NAGly 30:0_NAGly 20:0_10:0 | -5.068 | 0.000 | Down |
| SM | SM 39:1;3O | -5.060 | 0.000 | Down |
| DG | DG 32:6 | -5.058 | 0.000 | Down |
| DG | DG 27:2 | -5.050 | 0.000 | Down |
| DG | DG 30:3 | -5.041 | 0.000 | Down |
| AHexCer | AHexCer 59:9;3O | -5.029 | 0.000 | Down |
| PC | PC 35:1 | -5.026 | 0.002 | Down |
| Cer | Cer 12:2;2O_30:0 | -4.993 | 0.001 | Down |
| SM | SM 44:1;2O | -4.989 | 0.003 | Down |
| DG | DG 34:4 | -4.960 | 0.000 | Down |
| DG | DG 32:1 | -4.951 | 0.000 | Down |
| PC | PC O-30:0 | -4.944 | 0.000 | Down |
| FA | FA 44:5 | -4.921 | 0.000 | Down |
| PE | PE P-32:1_PE P-16:0_16:1 | -4.917 | 0.000 | Down |
| DG | DG 22:0 | -4.907 | 0.000 | Down |
| DG | DG 31:6 | -4.898 | 0.000 | Down |
| PC | PC O-37:8 | -4.897 | 0.000 | Down |
| FA | FA 40:5 | -4.876 | 0.000 | Down |
| FA | FA 20:4;3O | -4.859 | 0.000 | Down |
| MG | MG 10:0 | -4.858 | 0.000 | Down |
| Cer | Cer 35:5;4O | -4.844 | 0.000 | Down |
| SM | SM 42:2;2O_SM 18:1;2O_24:1 | -4.839 | 0.000 | Down |
| BMP | BMP 17:1_17:1 | -4.834 | 0.000 | Down |
| DG | DG 32:8 | -4.821 | 0.000 | Down |
| Cer | Cer 76:7;4O | -4.816 | 0.020 | Down |
| ST | ST 24:1;O4;T_20:3;1O | -4.812 | 0.032 | Down |
| DG | DG 36:3_DG 18:1_18:2 | -4.810 | 0.000 | Down |
| DG | DG 29:2 | -4.803 | 0.000 | Down |
| DG | DG 24:0 | -4.803 | 0.000 | Down |
| SL | SL 21:1;O_36:9;O | -4.796 | 0.031 | Down |
| PC | PC 40:5 | -4.795 | 0.003 | Down |
| Cer | Cer 40:1;2O_Cer 18:1;2O_22:0. | -4.789 | 0.007 | Down |
| PC | PC O-36:4 | -4.780 | 0.000 | Down |
| FA | FA 42:5 | -4.773 | 0.000 | Down |
| MAE | NAE 22:4 | -4.772 | 0.000 | Down |
| SM | SM 28:5;2O(FA 22:6) | -4.769 | 0.001 | Down |
| DG | DG 29:4 | -4.764 | 0.000 | Down |
| Cer | CerP 14:1;2O_28:3 | -4.742 | 0.006 | Down |
| DG | DG 48:9 | -4.737 | 0.025 | Down |
| CAR | CAR 17:3 | -4.733 | 0.000 | Down |
| NAOrn | NAOrn 14:1;O | -4.721 | 0.000 | Down |
| MAE | NAE 7:0 | -4.717 | 0.000 | Down |
| DG | DG 24:3 | -4.708 | 0.000 | Down |
| SM | SM 42:1;2O_SM 18:1;2O_24:0 | -4.684 | 0.000 | Down |
| DG | DG 30:8 | -4.684 | 0.000 | Down |

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| CAR | CAR 7:0 | -4.671 | 0.000 | Down |
| DG | DG 30:7 | -4.651 | 0.000 | Down |
| DG | DG 39:10 | -4.644 | 0.000 | Down |
| DG | DG 31:8 | -4.626 | 0.000 | Down |
| MAE | NAE 18:1 | -4.624 | 0.000 | Down |
| NAOrn | NAOrn 13:0;O | -4.615 | 0.000 | Down |
| Cer | Cer 33:5;4O | -4.613 | 0.000 | Down |
| FA | FA 17:4;2O | -4.609 | 0.000 | Down |
| Cer | Cer 12:1;2O_28:0 | -4.608 | 0.004 | Down |
| DG | DG 41:11 | -4.606 | 0.000 | Down |
| DG | DG 31:7 | -4.599 | 0.000 | Down |
| DG | DG 27:3 | -4.593 | 0.000 | Down |
| DG | DG 31:5 | -4.589 | 0.000 | Down |
| SM | SM 35:0;2O | -4.589 | 0.000 | Down |
| MG | MG 12:0 | -4.587 | 0.000 | Down |
| SL | SL 18:1;O_36:4;O | -4.584 | 0.019 | Down |
| MG | MG 17:4 | -4.574 | 0.000 | Down |
| SM | SM 38:2;2O | -4.567 | 0.008 | Down |
| SM | SM 32:0;2O_SM 23:0;2O_9:0 | -4.544 | 0.000 | Down |
| PC | PC 34:2 | -4.531 | 0.000 | Down |
| NAGly | NAGly 13:1;O | -4.517 | 0.000 | Down |
| DG | DG 28:5 | -4.497 | 0.000 | Down |
| SM | SM 41:4;3O | -4.491 | 0.000 | Down |
| DG | DG 28:3 | -4.485 | 0.000 | Down |
| DG | DG 39:9 | -4.469 | 0.000 | Down |
| PC | PC 36:2 | -4.455 | 0.004 | Down |
| PC | PC O-30:7 | -4.441 | 0.024 | Down |
| SM | SM 34:0;3O | -4.435 | 0.009 | Down |
| PC | PC O-39:3 | -4.422 | 0.000 | Down |
| DG | DG 28:4 | -4.414 | 0.000 | Down |
| DG | DG 30:4 | -4.401 | 0.000 | Down |
| FA | FA 16:1;3O | -4.401 | 0.000 | Down |
| FA | FA 19:1;2O | -4.399 | 0.000 | Down |
| SM | SM 38:0;2O | -4.394 | 0.001 | Down |
| PC | PC 34:3_PC 16:1_18:2 | -4.393 | 0.000 | Down |
| PC | PC O-32:0 | -4.383 | 0.000 | Down |
| DG | DG 30:6 | -4.362 | 0.000 | Down |
| MAE | NAE 20:2 | -4.361 | 0.000 | Down |
| PE | PE O-26:6_14:0 | -4.345 | 0.000 | Down |
| HexCer | HexCer 16:1;3O_17:0;(2OH) | -4.339 | 0.000 | Down |
| PC | PC 32:1_PC 16:0_16:1 | -4.320 | 0.000 | Down |
| HexCer | HexCer 18:1;2O_18:5 | -4.308 | 0.000 | Down |
| MAE | NAE 6:0 | -4.294 | 0.000 | Down |
| PE | PE 34:2_PE 16:1_18:1 | -4.290 | 0.000 | Down |
| PC | PC O-39:7 | -4.288 | 0.016 | Down |
| FA | FA 16:0;3O | -4.275 | 0.000 | Down |
| SM | SM 21:0;3O | -4.259 | 0.000 | Down |
| SM | SM 42:3;2O_SM 18:1;2O_24:2 | -4.256 | 0.000 | Down |
| FA | FA 42:9 | -4.245 | 0.000 | Down |

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| PC | PC 36:1 | -4.222 | 0.001 | Down |
| Cer | Cer 40:1;2O_Cer 18:1;2O_22:0 | -4.208 | 0.004 | Down |
| DG | DG 52:10 | -4.169 | 0.003 | Down |
| DG | DG 49:1 | -4.165 | 0.006 | Down |
| DG | DGDG 8:0_20:2 | -4.139 | 0.000 | Down |
| PE | PE P-34:1_PE P-16:0_18:1 | -4.124 | 0.000 | Down |
| PC | PC 38:5 | -4.119 | 0.011 | Down |
| Cer | CerP 28:2;2O_CerP 13:1;2O_15:1 | -4.119 | 0.028 | Down |
| PC | PC O-38:7 | -4.117 | 0.014 | Down |
| DG | DG 52:14 | -4.114 | 0.000 | Down |
| FA | FA 16:1 | -4.104 | 0.000 | Down |
| DG | DGCC 15:0_22:6 | -4.101 | 0.000 | Down |
| DG | DGCC 16:0_19:5 | -4.100 | 0.000 | Down |
| PC | PC 36:2_PC 18:0_18:2 | -4.074 | 0.009 | Down |
| PE | PE P-36:2_PE P-18:1_18:1 | -4.072 | 0.000 | Down |
| PC | PC 30:0 | -4.047 | 0.000 | Down |
| LPC | LPC 28:7 | -4.020 | 0.022 | Down |
| MG | MG 15:4 | -4.005 | 0.000 | Down |
| FA | FA 15:4 | -3.975 | 0.000 | Down |
| MG | MG 15:2 | -3.964 | 0.000 | Down |
| SM | SM 38:1;2O | -3.943 | 0.023 | Down |
| SM | SM 40:2;2O | -3.933 | 0.001 | Down |
| PC | PC 36:0 | -3.923 | 0.002 | Down |
| SM | SM 40:1;2O_SM 18:1;2O_22:0 | -3.921 | 0.000 | Down |
| DG | DG 48:12 | -3.894 | 0.000 | Down |
| SHexCer | SHexCer 32:0;3O | -3.893 | 0.000 | Down |
| PE | PE P-36:3_PE P-18:1_18:2 | -3.891 | 0.000 | Down |
| Cer | Cer 38:1;2O_Cer 18:1;2O_20:0. | -3.860 | 0.010 | Down |
| FA | FA 38:5 | -3.853 | 0.000 | Down |
| NAOrn | NAOrn 22:2_20:0 | -3.849 | 0.001 | Down |
| PE | PE 34:2_PE 16:0_18:2 | -3.826 | 0.000 | Down |
| PE | PE 32:0_PE 16:0_16:0 | -3.819 | 0.000 | Down |
| MAE | NAE 16:3 | -3.818 | 0.000 | Down |
| PC | PC O-37:1 | -3.793 | 0.000 | Down |
| DG | DG 57:11 | -3.788 | 0.047 | Down |
| PE | PE O-37:4 | -3.788 | 0.002 | Down |
| PE | PE P-32:0_PE P-16:0_16:0 | -3.788 | 0.000 | Down |
| PC | PC O-34:1 | -3.783 | 0.000 | Down |
| PC | PC O-34:0 | -3.781 | 0.000 | Down |
| MG | MG 15:3 | -3.774 | 0.000 | Down |
| MAE | NAE 18:2 | -3.771 | 0.000 | Down |
| PI | PI 36:2 | -3.749 | 0.001 | Down |
| LPE | LPE O-17:1 | -3.737 | 0.000 | Down |
| CL | CL 72:6_CL 18:0_18:0_16:1_20:5 | -3.733 | 0.000 | Down |
| PE | PE 36:3_PE 18:1_18:2. | -3.722 | 0.000 | Down |
| PE | PE O-32:1_PE O-16:1_16:0 | -3.716 | 0.000 | Down |
| PE | PE 36:2 | -3.713 | 0.000 | Down |
| FA | FA 16:0 | -3.704 | 0.000 | Down |
| FA | FA 34:0 | -3.698 | 0.000 | Down |

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| FA | FA 18:0 | -3.686 | 0.000 | Down |
| PE | PE O-34:2_PE O-16:1_18:1 | -3.670 | 0.000 | Down |
| SL | SL 12:2;O_34:0;O | -3.664 | 0.000 | Down |
| PC | PC 15:0_18:1(d7) | -3.625 | 0.000 | Down |
| PG | PG 28:0_8:0 | -3.616 | 0.000 | Down |
| PC | PC 34:2_PC 16:0_18:2 | -3.615 | 0.000 | Down |
| PE | PE 36:3_PE 18:1_18:2 | -3.606 | 0.000 | Down |
| SM | SM 37:1;2O | -3.602 | 0.013 | Down |
| PS | PS 22:5_22:6;4O | -3.592 | 0.000 | Down |
| DG | DG 48:11 | -3.584 | 0.023 | Down |
| PI | PI O-11:0_28:6 | -3.582 | 0.000 | Down |
| HexCer | HexCer 16:0;2O_24:1 | -3.568 | 0.000 | Down |
| FA | FA 22:6;4O | -3.564 | 0.000 | Down |
| MAE | NAE 19:4 | -3.559 | 0.000 | Down |
| SHexCer | SHexCer 16:3;2O_22:3 | -3.552 | 0.048 | Down |
| PE | PE P-34:2_PE P-16:0_18:2 | -3.544 | 0.000 | Down |
| FA | FA 17:0 | -3.544 | 0.000 | Down |
| FA | FA 32:0 | -3.531 | 0.000 | Down |
| PC | PC 36:4_PC 18:2_18:2 | -3.530 | 0.000 | Down |
| FA | FA 18:3;4O | -3.524 | 0.000 | Down |
| SM | SM 34:2;2O_SM 19:0;2O_15:2 | -3.518 | 0.006 | Down |
| PE | PE 36:2_PE 18:0_18:2 | -3.516 | 0.000 | Down |
| PE | PE-Cer 13:1;2O_30:1 | -3.515 | 0.000 | Down |
| FA | FA 30:0 | -3.509 | 0.000 | Down |
| FA | FA 25:0 | -3.507 | 0.000 | Down |
| SL | SL 16:3;O_36:1;O | -3.505 | 0.000 | Down |
| PE | PE 36:3 | -3.503 | 0.000 | Down |
| FA | FA 22:0 | -3.485 | 0.000 | Down |
| HBMP | HBMP 22:2_12:0_12:0 | -3.483 | 0.000 | Down |
| PE | PE 34:1_PE 16:0_18:1. | -3.456 | 0.000 | Down |
| PI | PI 34:1. | -3.455 | 0.000 | Down |
| SM | SMGDG O-9:0_26:1 | -3.444 | 0.000 | Down |
| PE | PE O-36:3_PE O-18:2_18:1 | -3.440 | 0.000 | Down |
| PI | PI-Cer 13:1;2O_32:7;O | -3.432 | 0.000 | Down |
| Cer | Cer 12:1;2O_26:0 | -3.418 | 0.026 | Down |
| DG | DG 52:9 | -3.415 | 0.002 | Down |
| FA | FA 29:0 | -3.414 | 0.000 | Down |
| ST | ST 24:1;O4;G_16:2;1O | -3.409 | 0.000 | Down |
| Cer | CerP 24:2;2O_28:3 | -3.409 | 0.000 | Down |
| FA | FA 26:1;1O | -3.409 | 0.000 | Down |
| PE | PE 34:1_PE 16:0_18:1 | -3.406 | 0.000 | Down |
| FA | FA 31:0 | -3.402 | 0.000 | Down |
| FA | FA 19:4;1O | -3.397 | 0.000 | Down |
| FA | FA 20:3;4O | -3.395 | 0.000 | Down |
| HexCer | HexCer 16:1;3O_26:7;(2OH) | -3.393 | 0.000 | Down |
| FA | FA 20:0 | -3.378 | 0.000 | Down |
| CL | CL 14:1_22:6_26:0_28:0 | -3.369 | 0.000 | Down |
| FA | FA 14:0 | -3.365 | 0.000 | Down |
| PC | PC 36:3 | -3.351 | 0.000 | Down |

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| LPA | LPA 28:2 | -3.348 | 0.000 | Down |
| PE | PE O-18:3_22:5;4O | -3.344 | 0.000 | Down |
| PI | PI 17:0_22:3;2O | -3.325 | 0.000 | Down |
| Cer | Cer 12:1;3O_31:0;(2OH) | -3.297 | 0.000 | Down |
| PI | PI 36:0 | -3.297 | 0.000 | Down |
| PC | PC O-39:8 | -3.290 | 0.001 | Down |
| FA | FA 26:0 | -3.286 | 0.000 | Down |
| OxFA | OxFA 18:0;(2OH) | -3.286 | 0.000 | Down |
| FA | FA 36:5 | -3.285 | 0.000 | Down |
| FA | FA 24:0 | -3.276 | 0.000 | Down |
| PC | PC O-10:0_22:3;4O | -3.275 | 0.000 | Down |
| FA | FA 33:0 | -3.270 | 0.000 | Down |
| FA | FA 23:0 | -3.261 | 0.000 | Down |
| PI | PI 34:0 | -3.249 | 0.000 | Down |
| PE | PE-Cer 12:1;2O_16:1 | -3.234 | 0.000 | Down |
| PE | PE O-34:3_PE O-16:1_18:2 | -3.233 | 0.000 | Down |
| FA | FA 28:0 | -3.229 | 0.000 | Down |
| SM | SM 42:2;2O | -3.228 | 0.000 | Down |
| SL | SL 13:2;O_32:2;O | -3.216 | 0.000 | Down |
| FA | FA 27:0 | -3.214 | 0.000 | Down |
| SL | SL 13:2;O_36:5;O | -3.210 | 0.000 | Down |
| TG | TG 49:2;1O_TG 16:0_16:0_17:2;1O | -3.202 | 0.000 | Down |
| SHexCer | SHexCer 39:0;2O | -3.196 | 0.000 | Down |
| PC | PC O-12:0_22:5;4O | -3.178 | 0.000 | Down |
| PI | PI-Cer 36:2;3O | -3.154 | 0.000 | Down |
| NAGly | NAGly 17:0;O | -3.145 | 0.000 | Down |
| FA | FA 28:1;2O | -3.139 | 0.000 | Down |
| PG | PG O-15:0_28:0 | -3.136 | 0.000 | Down |
| PE | PE 34:0 | -3.135 | 0.000 | Down |
| PE | PE 36:2_PE 18:1_18:1. | -3.041 | 0.000 | Down |
| PC | PC 32:0 | -3.033 | 0.000 | Down |
| CL | CL 12:0_22:6_28:0_28:0 | -3.031 | 0.000 | Down |
| PE | PE 38:5 | -3.021 | 0.000 | Down |
| TG | TG 49:2_TG 16:0_16:1_17:1 | -3.019 | 0.000 | Down |
| PE | PEtOH 26:2_PEtOH 13:1_13:1 | -3.014 | 0.000 | Down |
| SM | SM 33:1;2O | -3.012 | 0.026 | Down |
| PE | PE 36:3_PE 18:0_18:3 | -3.009 | 0.000 | Down |
| PMeOH | PMeOH 28:7_28:7 | -3.009 | 0.000 | Down |
| PC | PC 34:1_PC 16:0_18:1 | -2.962 | 0.000 | Down |
| FA | FA 22:6 | -2.959 | 0.000 | Down |
| SM | SM 12:0;2O_27:0 | -2.957 | 0.000 | Down |
| HBMP | HBMP 20:1_12:0_12:0 | -2.938 | 0.000 | Down |
| PI | PI 36:2. | -2.924 | 0.000 | Down |
| TG | TG 46:0_TG 14:0_16:0_16:0 | -2.922 | 0.000 | Down |
| FA | FA 18:1 | -2.911 | 0.000 | Down |
| PS | PS 36:2 | -2.897 | 0.009 | Down |
| PC | PC O-39:10 | -2.877 | 0.000 | Down |
| PI | PI-Cer 13:2;2O_22:6;O | -2.875 | 0.000 | Down |
| FA | FA 22:5;4O | -2.866 | 0.000 | Down |

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|----------------|---------------------------------|--------|-------|------|
| PC | PC 34:1 | -2.851 | 0.000 | Down |
| Cer | Cer 12:2;2O_42:10;2O | -2.825 | 0.002 | Down |
| PC | PC O-37:9 | -2.810 | 0.000 | Down |
| SM | SM 40:1;2O | -2.810 | 0.002 | Down |
| TG | TG 46:1_TG 14:0_16:0_16:1 | -2.809 | 0.000 | Down |
| PI | PI 24:0_18:2;3O | -2.799 | 0.000 | Down |
| TG | TG 46:1_TG 14:0_14:0_18:1 | -2.787 | 0.000 | Down |
| TG | TG 44:0_TG 14:0_14:0_16:0 | -2.766 | 0.000 | Down |
| PC | PC 40:6 | -2.756 | 0.047 | Down |
| TG | TG 44:1_TG 10:0_16:0_18:1 | -2.755 | 0.000 | Down |
| DG | DG 50:9 | -2.746 | 0.005 | Down |
| TG | TG 43:0_TG 12:0_15:0_16:0 | -2.742 | 0.000 | Down |
| FA | FA 28:7 | -2.739 | 0.000 | Down |
| SM | SM 32:1;2O_SM 17:0;2O_15:1 | -2.730 | 0.000 | Down |
| PE | PE P-38:5_PE P-18:1_20:4 | -2.712 | 0.001 | Down |
| TG | TG 49:2_TG 15:0_16:1_18:1 | -2.695 | 0.000 | Down |
| TG | TG 44:0_TG 14:0_14:0_16:0. | -2.691 | 0.000 | Down |
| ST | ST 29:1;O;Hex;FA 15:2 | -2.686 | 0.000 | Down |
| PC | PC 36:1_PC 18:0_18:1 | -2.682 | 0.002 | Down |
| TG | TG 48:0_TG 16:0_16:0_16:0. | -2.671 | 0.000 | Down |
| LNAPS | LNAPS 14:0_N-28:0 | -2.660 | 0.000 | Down |
| TG | TG 47:1_TG 15:0_16:0_16:1 | -2.645 | 0.000 | Down |
| PE | PE 40:5 | -2.632 | 0.000 | Down |
| SM | SM 38:1;2O_SM 18:1;2O_20:0 | -2.617 | 0.020 | Down |
| TG | TG 56:0;1O_TG 22:0_22:0_12:0;1O | -2.578 | 0.000 | Down |
| PC | PC 39:2 | -2.568 | 0.029 | Down |
| TG | TG O-41:0_TG O-11:0_14:0_16:0 | -2.559 | 0.000 | Down |
| TG | TG 36:0_TG 10:0_12:0_14:0. | -2.552 | 0.000 | Down |
| PE | PE O-22:4_22:6;3O | -2.544 | 0.000 | Down |
| TG | TG 43:0_TG 13:0_14:0_16:0 | -2.538 | 0.000 | Down |
| TG | TG 48:2_TG 16:0_16:1_16:1 | -2.536 | 0.000 | Down |
| PI | PI 18:0_28:6 | -2.530 | 0.000 | Down |
| TG | TG O-52:1_TG O-19:1_16:0_17:0 | -2.511 | 0.000 | Down |
| PE | PE 38:4_PE 18:0_20:4. | -2.507 | 0.029 | Down |
| HBMP | HBMP 22:3_12:0_12:0 | -2.506 | 0.000 | Down |
| TG | TG 46:0_TG 14:0_16:0_16:0. | -2.504 | 0.000 | Down |
| SM | SM 34:0;2O | -2.483 | 0.001 | Down |
| HexCer | HexCer 18:0;2O_18:5 | -2.479 | 0.000 | Down |
| PE | PE O-38:6_PE O-18:2_20:4 | -2.477 | 0.002 | Down |
| TG | TG 40:0_TG 12:0_12:0_16:0 | -2.474 | 0.000 | Down |
| SL | SL 16:3;O_30:8 | -2.469 | 0.001 | Down |
| TG | TG 49:0_TG 15:0_17:0_17:0 | -2.460 | 0.000 | Down |
| PC | PC 32:0_PC 16:0_16:0 | -2.456 | 0.000 | Down |
| TG | TG 47:0_TG 15:0_16:0_16:0. | -2.446 | 0.000 | Down |
| TG | TG 48:0_TG 16:0_16:0_16:0 | -2.439 | 0.000 | Down |
| FA | FA 22:0;4O | -2.436 | 0.001 | Down |
| PE | PE O-36:2_PE O-18:1_18:1 | -2.436 | 0.000 | Down |
| PE | PE 34:0_PE 16:0_18:0 | -2.424 | 0.000 | Down |
| SHexCer | SHexCer 38:3;3O | -2.422 | 0.000 | Down |

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|----------------|---------------------------------|--------|-------|------|
| NAGly | NAGlySer 22:6_20:1 | -2.419 | 0.000 | Down |
| TG | TG 52:1;3O_TG 17:0_17:0_18:1;3O | -2.387 | 0.000 | Down |
| TG | TG 36:0_TG 10:0_12:0_14:0 | -2.383 | 0.000 | Down |
| OxFA | OxFA 18:2;(2OH) | -2.382 | 0.000 | Down |
| SM | SM 40:1;2O. | -2.380 | 0.000 | Down |
| TG | TG O-54:1_TG O-18:0_18:0_18:1 | -2.369 | 0.000 | Down |
| SM | SM 13:1;2O_28:1 | -2.367 | 0.000 | Down |
| TG | TG 45:1_TG 14:0_15:0_16:1 | -2.361 | 0.000 | Down |
| TG | TG 45:0_TG 15:0_15:0_15:0 | -2.354 | 0.000 | Down |
| PC | PC 36:3_PC 18:1_18:2 | -2.351 | 0.001 | Down |
| PC | PC O-14:0_22:5;3O | -2.317 | 0.000 | Down |
| TG | TG 58:0_TG 16:0_16:0_26:0 | -2.316 | 0.000 | Down |
| SHexCer | SHexCer 35:0;3O | -2.312 | 0.000 | Down |
| TG | TG 56:0_TG 15:0_16:0_25:0 | -2.308 | 0.000 | Down |
| PE | PE 38:3 | -2.302 | 0.001 | Down |
| TG | TG 48:1_TG 14:0_16:0_18:1 | -2.299 | 0.000 | Down |
| PE | PE O-24:5_16:0 | -2.285 | 0.000 | Down |
| TG | TG 54:0_TG 18:0_18:0_18:0 | -2.283 | 0.000 | Down |
| TG | TG 42:0_TG 12:0_14:0_16:0. | -2.283 | 0.000 | Down |
| SM | SM 36:0;2O_SM 24:0;2O_12:0 | -2.280 | 0.020 | Down |
| FA | FA 18:1;O | -2.280 | 0.000 | Down |
| TG | TG 40:0_TG 10:0_14:0_16:0 | -2.280 | 0.000 | Down |
| TG | TG 49:0_TG 16:0_16:0_17:0 | -2.275 | 0.000 | Down |
| TG | TG 42:0_TG 12:0_14:0_16:0 | -2.274 | 0.000 | Down |
| TG | TG 38:0_TG 8:0_14:0_16:0 | -2.273 | 0.000 | Down |
| TG | TG O-57:1_TG O-19:0_16:1_22:0 | -2.269 | 0.000 | Down |
| PS | PS 36:2. | -2.266 | 0.000 | Down |
| PE | PE 36:2_PE 18:1_18:1 | -2.264 | 0.000 | Down |
| PC | PC 34:0_PC 16:0_18:0 | -2.254 | 0.000 | Down |
| SM | SMGDG O-21:4_28:7 | -2.249 | 0.000 | Down |
| TG | TG 38:1_TG 10:0_10:0_18:1 | -2.247 | 0.000 | Down |
| TG | TG 42:2_TG 10:0_14:1_18:1 | -2.234 | 0.000 | Down |
| NAGly | NAGlySer 22:6_21:4 | -2.215 | 0.001 | Down |
| SM | SM 13:1;2O_28:2 | -2.211 | 0.000 | Down |
| TG | TG 38:0_TG 12:0_12:0_14:0 | -2.210 | 0.000 | Down |
| TG | TG 38:1_TG 10:0_14:0_14:1 | -2.202 | 0.000 | Down |
| PC | PC O-18:0_18:1;1O | -2.196 | 0.000 | Down |
| PE | PE-Cer 17:1;2O_36:8;O | -2.174 | 0.000 | Down |
| DG | DG 44:6 | -2.169 | 0.012 | Down |
| PI | PI 18:0_28:5 | -2.169 | 0.000 | Down |
| TG | TG 48:4_TG 14:0_16:1_18:3 | -2.168 | 0.000 | Down |
| LPE | LPE 18:1. | -2.166 | 0.000 | Down |
| TG | TG 38:1;1O_TG 8:0_16:0_14:1;1O | -2.132 | 0.000 | Down |
| ST | ST 24:1;O4;T_21:1 | -2.128 | 0.000 | Down |
| SHexCer | SHexCer 45:4;3O | -2.098 | 0.000 | Down |
| TG | TG 47:0_TG 15:0_16:0_16:0 | -2.086 | 0.000 | Down |
| PC | PC O-14:1_24:0;1O | -2.083 | 0.000 | Down |
| DMPE | DMPE 17:0_22:5 | -2.080 | 0.000 | Down |
| SM | SM 12:0;2O_25:0 | -2.078 | 0.000 | Down |

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|----------------|---------------------------------|--------|-------|------|
| PE | PE P-38:4_PE P-18:0_20:4 | -2.066 | 0.007 | Down |
| TG | TG 55:0_TG 15:0_16:0_24:0 | -2.042 | 0.000 | Down |
| DG | DGDG 17:1_22:6 | -2.038 | 0.000 | Down |
| TG | TG 42:1_TG 10:0_14:0_18:1 | -2.026 | 0.000 | Down |
| TG | TG 40:1_TG 8:0_16:0_16:1 | -2.007 | 0.000 | Down |
| PI | PI 36:1_PI 18:0_18:1 | -1.994 | 0.000 | Down |
| PS | PS 36:2_PS 18:0_18:2. | -1.992 | 0.000 | Down |
| PI | PI 20:5_24:0;1O | -1.989 | 0.000 | Down |
| FA | FA 20:0;4O | -1.982 | 0.000 | Down |
| TG | TG 55:2_TG 18:0_18:1_19:1 | -1.977 | 0.000 | Down |
| SM | SM 25:3;2O(FA 20:5) | -1.969 | 0.000 | Down |
| PA | PA 15:0_28:7 | -1.967 | 0.001 | Down |
| PE | PE O-36:5_PE O-16:1_20:4 | -1.967 | 0.005 | Down |
| PE | PE 36:1_PE 18:0_18:1 | -1.961 | 0.000 | Down |
| TG | TG 49:0_TG 16:0_16:0_17:0. | -1.949 | 0.000 | Down |
| TG | TG 38:1_TG 10:0_10:0_18:1. | -1.939 | 0.000 | Down |
| PS | PS 22:6_22:6 | -1.920 | 0.001 | Down |
| FA | FA 18:1;2O | -1.917 | 0.000 | Down |
| PC | PC O-12:0_17:2;2O | -1.916 | 0.012 | Down |
| TG | TG 40:2_TG 12:0_14:1_14:1 | -1.914 | 0.000 | Down |
| TG | TG O-55:1_TG O-19:1_18:0_18:0 | -1.913 | 0.000 | Down |
| TG | TG 47:1;1O_TG 16:0_16:0_15:1;1O | -1.910 | 0.000 | Down |
| PI | PI O-13:1_26:7 | -1.905 | 0.000 | Down |
| Cer | CerP 16:1;2O_28:3 | -1.902 | 0.000 | Down |
| PE | PE 36:2;O_PE 18:0_18:2;O | -1.900 | 0.002 | Down |
| TG | TG 48:2_TG 14:0_16:1_18:1 | -1.887 | 0.000 | Down |
| PA | PA 23:0_28:7 | -1.864 | 0.000 | Down |
| PI | PI 16:0_28:5 | -1.863 | 0.000 | Down |
| PE | PE O-26:5_16:0 | -1.858 | 0.000 | Down |
| TG | TG 53:0_TG 14:0_16:0_23:0 | -1.855 | 0.000 | Down |
| DG | DGGA 22:0_22:6 | -1.845 | 0.000 | Down |
| SHexCer | SHexCer 43:3;3O | -1.835 | 0.000 | Down |
| PI | PI 24:0_22:6;4O | -1.822 | 0.000 | Down |
| TG | TG 51:0_TG 16:0_17:0_18:0 | -1.808 | 0.000 | Down |
| MG | MG 18:0 | -1.782 | 0.004 | Down |
| PE | PE-Cer 12:1;2O_32:0 | -1.782 | 0.000 | Down |
| PI | PI 26:0_17:0;2O | -1.782 | 0.000 | Down |
| PG | PG 18:2_18:1;1O | -1.780 | 0.001 | Down |
| TG | TG 51:0_TG 17:0_17:0_17:0 | -1.777 | 0.000 | Down |
| PI | PI 24:0_18:1;4O | -1.774 | 0.000 | Down |
| HexCer | HexCer 20:2;2O_20:5 | -1.767 | 0.001 | Down |
| SM | SM 41:0;3O | -1.762 | 0.000 | Down |
| ST | ST 29:1;O;Hex;FA 13:0 | -1.744 | 0.000 | Down |
| PE | PE P-36:4_PE P-16:0_20:4 | -1.743 | 0.003 | Down |
| FA | FA 15:0 | -1.712 | 0.000 | Down |
| TG | TG 50:0_TG 16:0_16:0_18:0. | -1.705 | 0.000 | Down |
| PE | PE 38:5_PE 18:1_20:4 | -1.693 | 0.027 | Down |
| CL | CL 15:0_22:5_28:0_28:0 | -1.693 | 0.014 | Down |
| TG | TG 36:0_TG 12:0_12:0_12:0 | -1.691 | 0.000 | Down |

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|--------|---------------------------------|--------|-------|------|
| TG | TG 49:1_TG 16:0_16:0_17:1 | -1.679 | 0.000 | Down |
| Cer | Cer 16:3;2O_30:3 | -1.638 | 0.000 | Down |
| PE | PE O-26:5_18:2 | -1.625 | 0.003 | Down |
| SM | SM 12:1;2O_29:0 | -1.619 | 0.000 | Down |
| LPE | LPE 18:1 | -1.592 | 0.000 | Down |
| SM | SMGDG O-17:0_28:5 | -1.586 | 0.000 | Down |
| TG | TG 50:0_TG 16:0_16:0_18:0 | -1.583 | 0.000 | Down |
| PI | PI 16:0_22:6;4O | -1.578 | 0.000 | Down |
| PA | PA 17:0_28:6 | -1.559 | 0.003 | Down |
| PE | PE 36:1_PE 18:0_18:1. | -1.550 | 0.000 | Down |
| TG | TG 54:0_TG 18:0_18:0_18:0. | -1.546 | 0.000 | Down |
| TG | TG 56:1_TG 16:0_22:0_18:1 | -1.541 | 0.000 | Down |
| TG | TG 38:0_TG 8:0_12:0_18:0 | -1.533 | 0.000 | Down |
| TG | TG 8:0_9:0_22:1 | -1.525 | 0.000 | Down |
| PE | PE O-19:0_28:6 | -1.476 | 0.030 | Down |
| PC | PC O-12:0_22:3;2O | -1.474 | 0.003 | Down |
| HexCer | HexCer 16:0;2O_30:4;O | -1.445 | 0.000 | Down |
| PE | PE 20:4_22:5 | -1.436 | 0.002 | Down |
| SM | SM 39:0;3O | -1.383 | 0.002 | Down |
| PA | PA 21:0_28:6 | -1.365 | 0.021 | Down |
| TG | TG 48:3_TG 14:0_16:1_18:2. | -1.329 | 0.001 | Down |
| TG | TG 50:2_TG 16:0_16:1_18:1. | -1.314 | 0.000 | Down |
| PS | PS 36:1_PS 18:0_18:1. | -1.256 | 0.001 | Down |
| TG | TG O-49:1_TG O-17:1_14:0_18:0 | -1.234 | 0.001 | Down |
| TG | TG 49:3;1O_TG 16:0_16:0_17:3;1O | -1.210 | 0.001 | Down |
| TG | TG 34:0_TG 8:0_12:0_14:0 | -1.207 | 0.001 | Down |
| HBMP | HBMP 13:1_12:0_13:1 | -1.184 | 0.001 | Down |
| TG | TG 52:0_TG 16:0_18:0_18:0 | -1.182 | 0.002 | Down |
| HexCer | HexCer 16:0;2O_24:6;O | -1.173 | 0.002 | Down |
| PE | PE O-13:0_28:7 | -1.164 | 0.003 | Down |
| TG | TG O-50:1_TG O-16:0_16:0_18:1 | -1.157 | 0.001 | Down |
| TG | TG 48:3_TG 12:0_18:1_18:2 | -1.155 | 0.002 | Down |
| TG | TG 50:5_TG 18:1_16:2_16:2 | -1.145 | 0.003 | Down |
| TG | TG 48:3_TG 14:0_16:1_18:2 | -1.115 | 0.003 | Down |
| FA | FA 21:0 | -1.106 | 0.001 | Down |
| TG | TG 55:3_TG 18:1_18:1_19:1 | -1.090 | 0.002 | Down |
| TG | TG 43:3;1O_TG 10:0_18:1_15:2;1O | -1.075 | 0.012 | Down |
| TG | TG 52:0_TG 16:0_18:0_18:0. | -1.059 | 0.002 | Down |
| TG | TG 50:1_TG 16:0_16:0_18:1. | -1.042 | 0.001 | Down |
| PA | PA 17:0_28:7 | -1.017 | 0.004 | Down |
| TG | TG 43:1;1O_TG 10:0_16:0_17:1;1O | -1.009 | 0.006 | Down |
| SM | SM 12:1;2O_20:0 | 1.040 | 0.034 | Up |
| TG | TG 54:3;1O_TG 18:1_18:1_18:1;1O | 1.195 | 0.030 | Up |
| TG | TG O-53:6_TG O-15:4_19:1_19:1 | 1.486 | 0.040 | Up |
| FA | FA 42:10 | 1.660 | 0.000 | Up |
| LPE | LPE 18:0. | 1.920 | 0.000 | Up |
| Cer | Cer 12:0;2O_19:0;(2OH) | 2.162 | 0.000 | Up |
| SL | SL 13:1;O_34:5 | 2.179 | 0.050 | Up |
| LPE | LPE O-18:1 | 2.221 | 0.001 | Up |

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|------------|---------------------------|-------|-------|----|
| TG | TG 54:4_TG 18:1_18:1_18:2 | 2.244 | 0.027 | Up |
| LPE | LPE O-16:1. | 2.324 | 0.000 | Up |
| FA | FA 44:10 | 2.534 | 0.000 | Up |
| Cer | Cer 12:1;2O_24:3;(2OH) | 3.071 | 0.000 | Up |

10 BIOGRAPHY OF THE AUTHOR WITH BIBLIOGRAPHY OF PUBLISHED WORK

Rafaela Furioso Ferreira is a Veterinarian (D. V. M.), graduated with honours at the Federal University of Paraná (Curitiba, Brazil), and an Environmental, Health and Safety Technician certified by the Federal Technological University of Paraná (Curitiba, Brazil). She has worked as a research intern at the VetMedZg Laboratory of Proteomics (Zagreb, Croatia) and at the Laboratory of Veterinary Clinical Pathology HV-UFPR (Curitiba, Brazil). She is currently an Early Stage Researcher of the European Joint Doctorate in Molecular Animal Nutrition (MANNA), under the supervision of Prof. Vladimir Mrljak at the University of Zagreb, and Prof. Helga Sauerwein at the University of Bonn, and industrial advice from Dr. Mike Salter (AB Agri, UK). She has experience in Veterinary Medicine, Veterinary Clinical Pathology, Clinical Proteomics, Exosomes, Extracellular Vesicles, Veterinary Parasitology, and Molecular Biology. Her research interests include the development of novelty diagnosis tools for veterinary medicine and the use of innovative approaches such as OMICs tools for animal health and animal production.

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