CHARACTERIZATION OF PORCINE MILK EXOSOMES FROM DIFFERENT STAGES OF LACTATION

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RAFAELA FURIOSO FERREIRA

KARAKTERIZACIJA EGZOSOMA MLIJEKA KRMAČA U RAZLIČITIM RAZDOBLJIMA LAKTACIJE

MEĐUNARODNI DVOJNI DOKTORAT

Mentori:
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Characterization of porcine milk exosomes from different stages of lactation

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By

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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 Funktion 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 mittlels 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 Milchexosomen 947 Lipide aus sechzehn Subklassen identifiziert. Im Vergleich zwischen Kolostrum- und Milchexosomen wurden an Tag 7 post partum 734 unterschiedlicher Menge vorliegende Lipide (*differentially abundant lipids;* DAL) identifiziert und 779 DAL an Tag 14. Die unterscheidlichen Futterrationen wirkten sich auf das Proteom der Milchexosomen aus, nicht aber auf ihr Lipidom. Die Ergebnisse zeigen ein unterschiedliches Proteom- und Lipidom-Profil in Milchexosomen von Sauen in verschiedenen Laktationsstadien, was Auswirkungen auf ihre funktionelle Biologie hat. Zudem sind die Resultate für mögliche Ansätze zur

Verwendung von Milchexosomen 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 prasenja, 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 prasenja). 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 ± 18,5; GS = 154 ± 19,3; (± SD u nm), za izolaciju korištenu u proteomskoj analizi; i GL = 162 ± 14,7 GS = 156 ± 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 ± 21,3 u izolaciji korištenoj u analizi lipida) od mlijeka (162 ± 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 ≤ 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 endotelnih stanica 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 diradilgliceroli, triradilgliceroli 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 istražit 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	Endoplasmin
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	Linoneic 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).

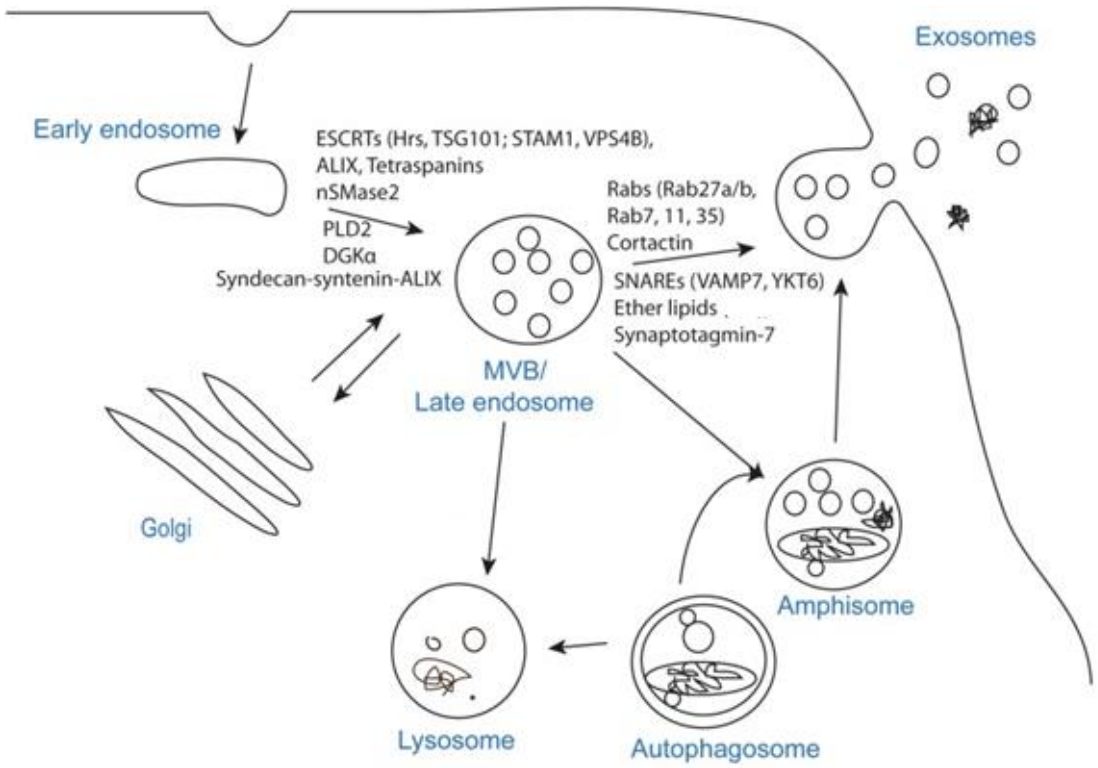


Figure 1. Exosome biogenesis with main molecules shown to affect its formation, transport of MVB to the plasma membrane, and fusion of MVB with the plasma membrane, releasing the exosomes. Abbreviations: ALIX, ALG-2 interacting protein X; DGK, diacylglycerol kinase; ESCRT, endosome sorting complex required 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 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 (LÖTVAL 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 (ZEMPLENI, 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 (ZEMPLENI, 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 colostral 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*. AARSONSON 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.

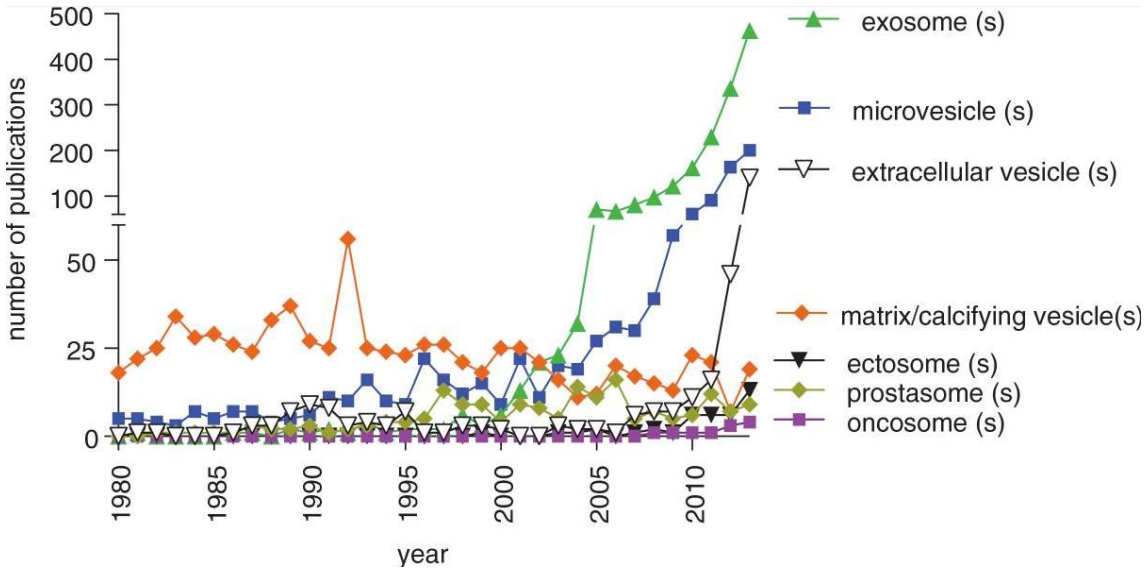


Figure 2. Comparative evolution of the use of different terms for extracellular vesicles and their subtypes by the cumulative number of publications in the literature from 1980 to 2010. Reproduced from LÖTVALL et al. (2014).

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 μm 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 μm in diameter, with most AB measuring approximately 1 to 5 μm 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).

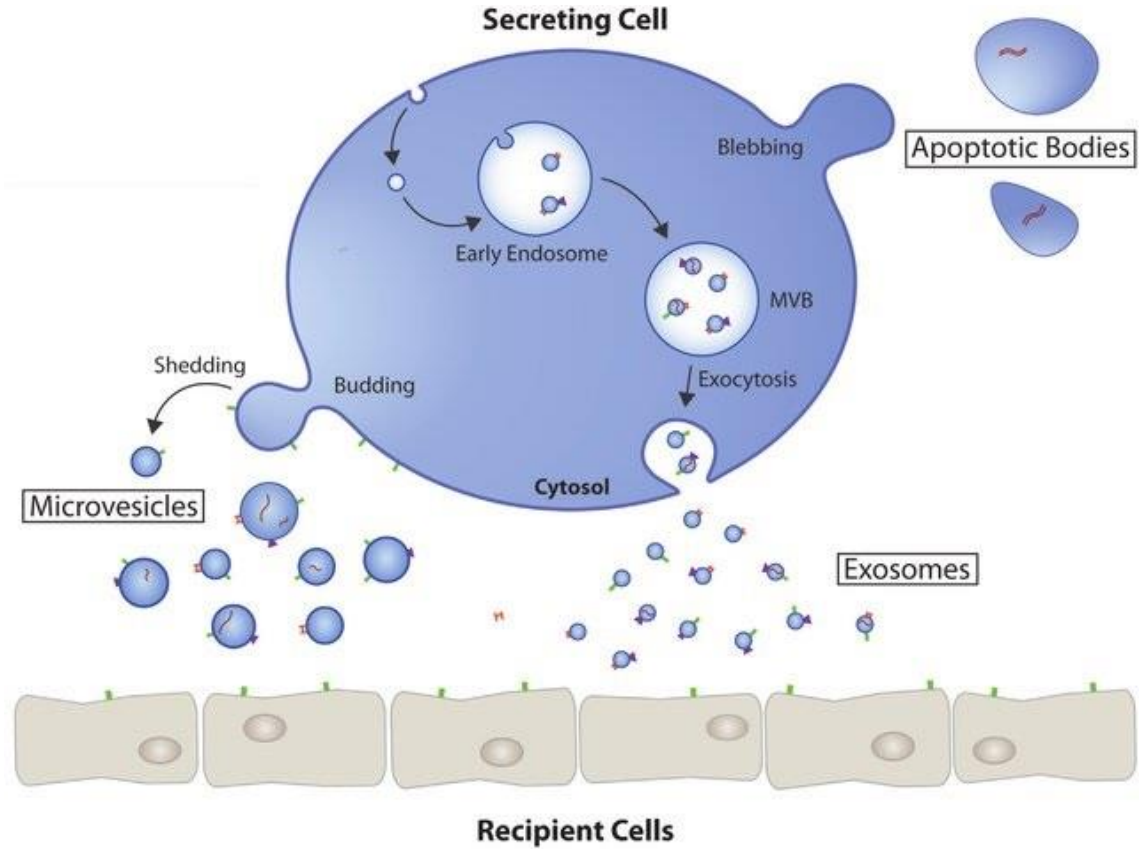


Figure 3. Schematic representation of the different biogenesis of extracellular vesicles. Exosomes are formed as intraluminal vesicles by budding into early endosomes, which by fusing with the plasma membrane can release their cargo into the extracellular milieu. Microvesicles are formed as a simple 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).

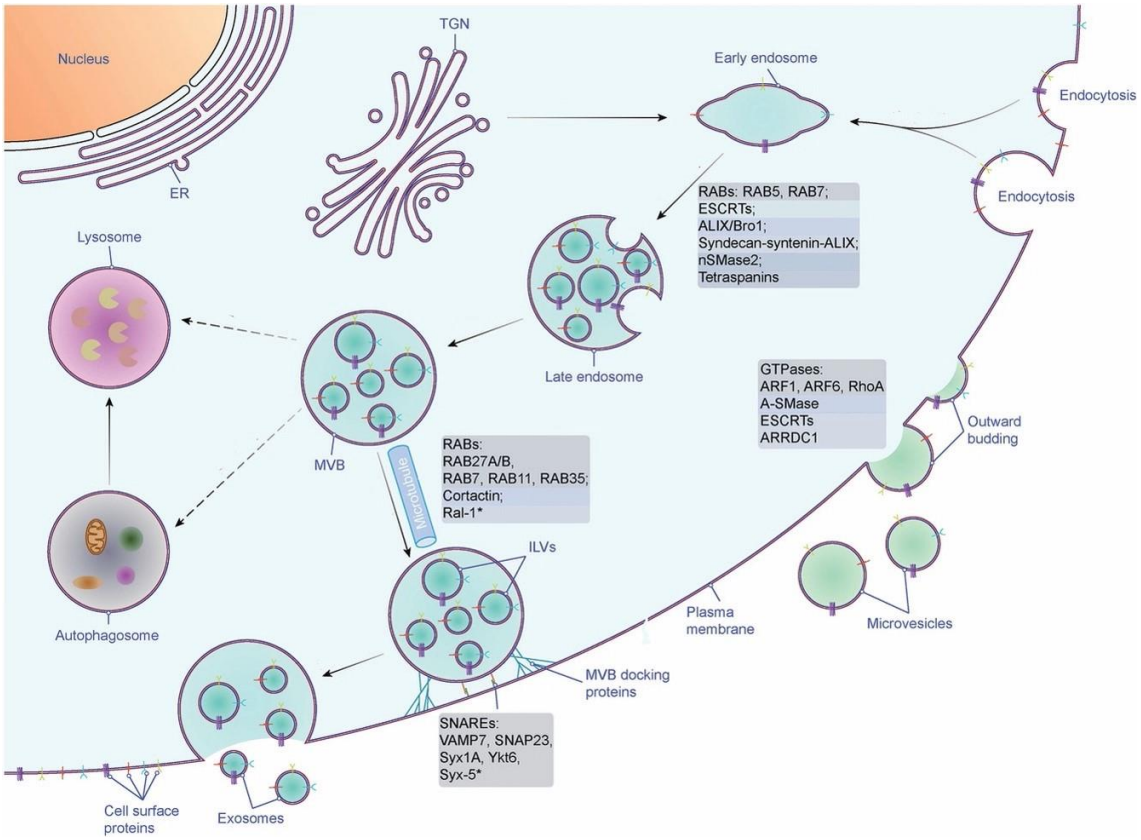


Figure 4. Biogenesis and secretion of exosomes. Early endosomes originate from the inward budding of the plasma membrane and mature into late endosomes, generating MVB filled with ILV. The MVB can proceed to either be fused with the plasma membrane, resulting in the release of the exosomes or fuse with 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; RohA, 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.

Specious	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

[1]Data 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 signalized the importance of studies on milk EVs given their biological function and the use of milk and dairy products as food (ZEMPLENI 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).

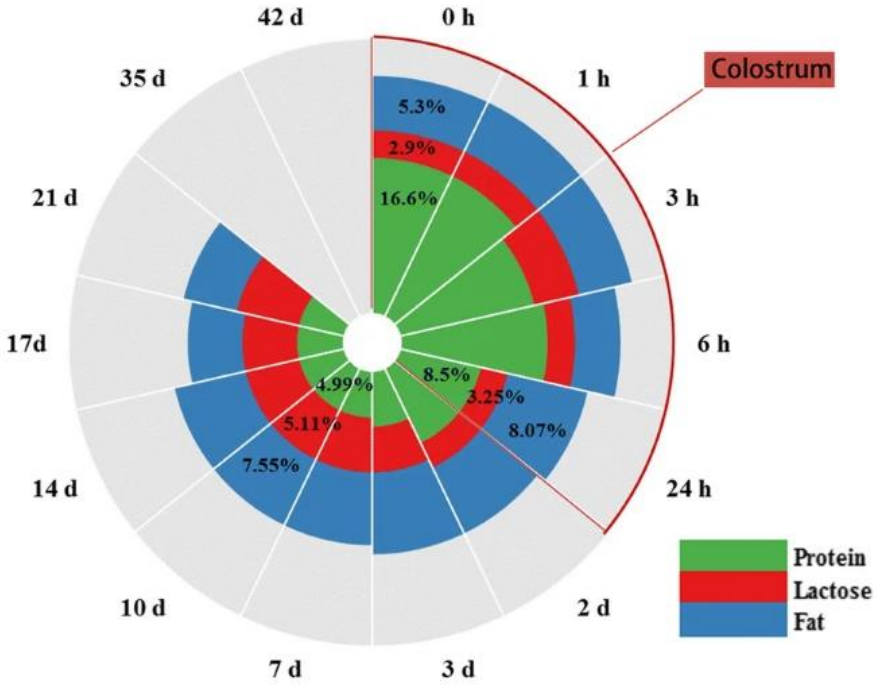


Figure 5. Composition of sow milk throughout lactation. Adapted from Zhang et al. (2018)

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

Bovine	DG/UC, WB	Characterization of proteins upregulated during *S. aureus* 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 *in vitro*, 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 *in vitro* 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 *in vitro* 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 *in vitro* 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 *in vivo* and *in vitro*. 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 antigenCD69; 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 subsequential 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 technics 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 (SEC) (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.

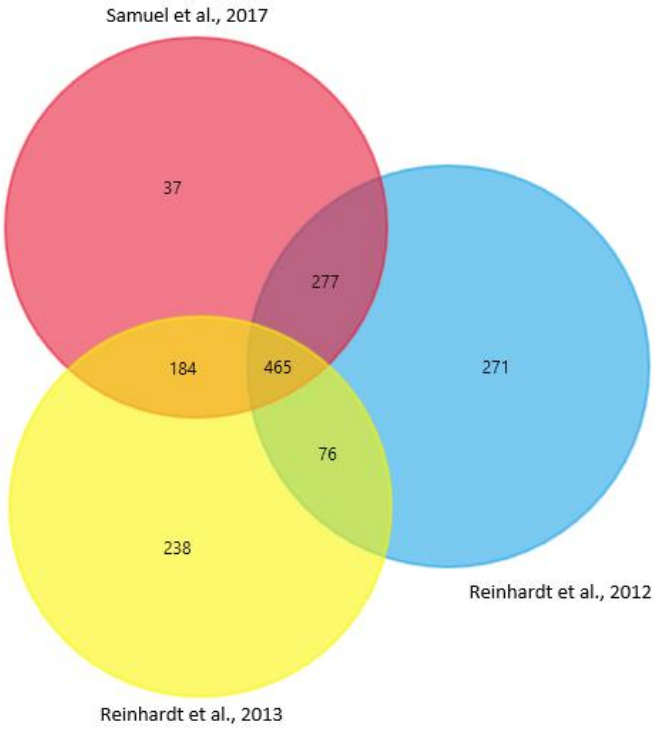


Figure 6. Venn diagram of unique proteins identified in different bovine milk exosome studies with databases available at Vesiclepedia. Source: Analysis made by the author using FunRich software v.3.1.3.

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 Δ15 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 derivates inside the body, which can also be obtained from different food sources (Figure 7) (HIGDON et al., 2019).

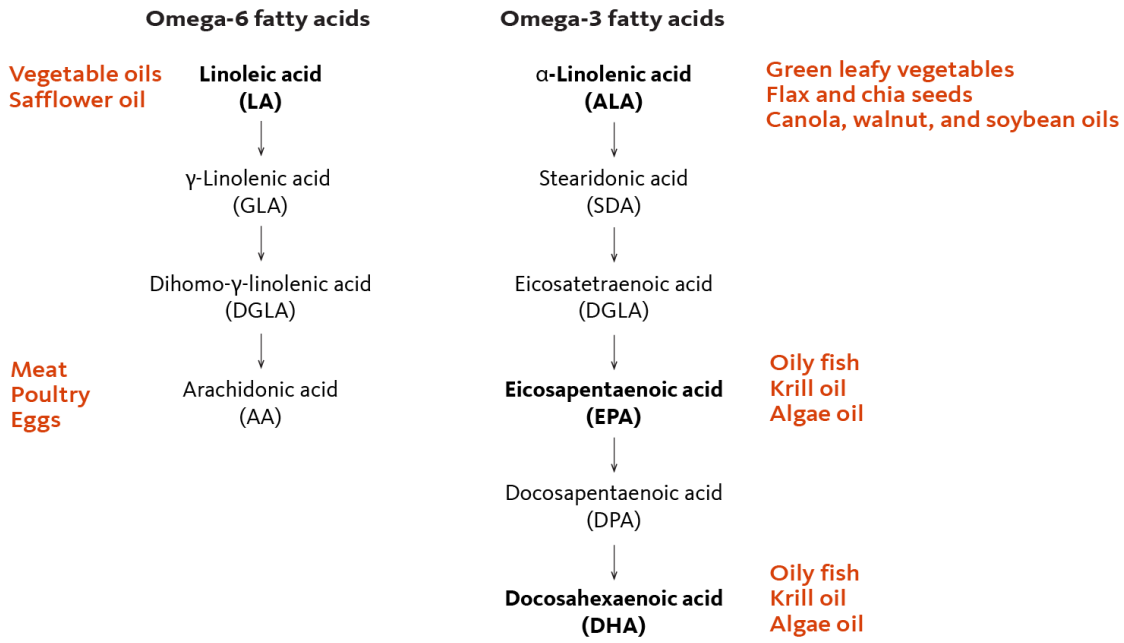


Figure 7. Omega-6 and omega-3 and their respective longer chain derivative compounds. Dietary sources of the different long-chain polyunsaturated fatty acids are listed in orange. Adapted from Higdon et al., 2019.

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 MOUROT, 2011; SIMOPOULOS, 1991). To induce the beneficial effects on heath 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 ω6:ω3 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-1β and TNF-α were decreased, suggesting that piglet inflammation was prevented at a ω6:ω3 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 ω6:ω3 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 ω3 PUFA is a strategy used in pig production, although it is still not clear how the effects of ω3 and ω6 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 ± SEM) and body condition score (2.36 ± 0.12, mean ± 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 ± 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 ± SD)	BCS (mean ± SD)
Gestation Day 28	210 ± 25	2.44 ± 0,46
Gestation Day 79	242 ± 19	2.55 ± 0,53
Gestation Day 108	251 ± 19	2.88 ± 0,22
Lactation Day 26	217 ± 32	2.12 ± 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 ω6 and ω3 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 °C until exosome isolation.

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

Item *Ingredients (g/kg as fed basis)*	**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
Composition (% DM)		
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 ω7	0.50	0.42	-	-
18:00	3.06	3.30	4.36	4.40
18:1 ω9 cis	19.88	19.80	24.51	23.84
18:1 ω7	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% Piolofom® (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 IgGκ light chain binding protein (m-IgGκ 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 (Termo 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 µL 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 mm^3 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 ≤ 0.6% (peptide spectrum matches) and ≤ 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 mutiplex 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 µL) 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 µL of 2-propanol: acetonitrile (90:10, vol/vol), 0.1% formic acid, and 10 mM ammonium acetate. Aliquots of 20 µL were then diluted 1:10 with mobile phase B for lipidomic analysis in positive mode. Aliquots of 5 µL were analyzed in negative ion mode for free fatty acid analysis. Aliquots of 50 µL, 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 µL of sample for the positive and negative ion modes, respectively, were separated by liquid chromatography with a Kinetex EVO C18 column (2.1 × 100 mm, 1.7 µm; 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 glycyl 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), lysophophatidylcholines (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.

	Concentration (particles / mL)		**Size (nm)**	
Group	**GL**	**GS**	**GL**	**GS**
Mean	3.3×10^{11}	2.6×10^{11}	156.5	157.1
Std. Deviation	2.8×10^{11}	1.7×10^{11}	17	18.9

Day	**0**	**7**	**14**	**0**	**7**	**14**
Mean	4.4×10^{11}	2.3×10^{11}	$2 \times 10^{11}1$	145.3	163	162.4
Std. Deviation	3.2×10^{11}	1.3×10^{11}	9×10^{11}	20.7	13.9	12.2

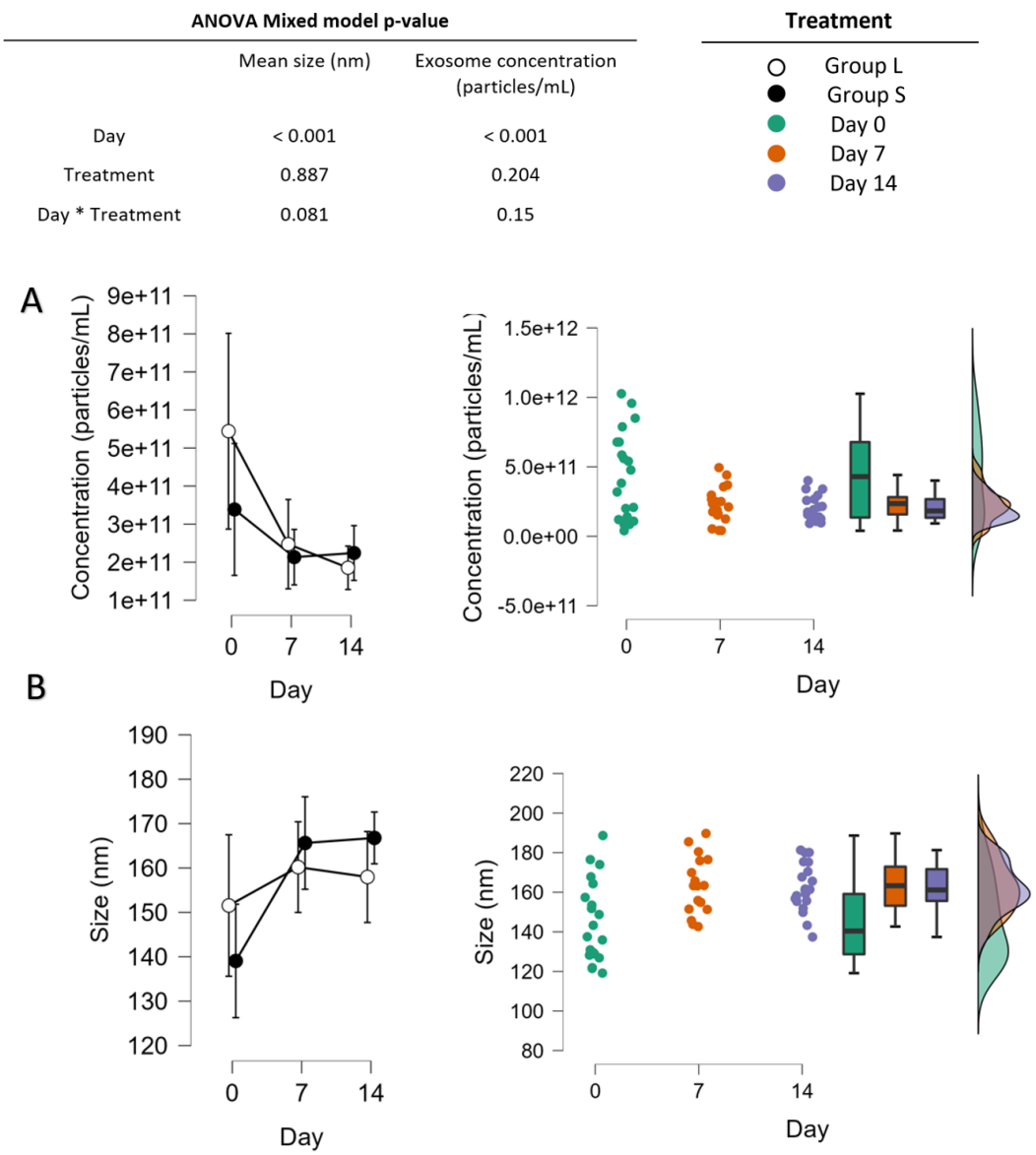


Figure 9. ANOVA mixed model comparison using the data from nanoparticle tracking analysis in response to **(A)** mean size (nm) and **(B)** exosome concentration (particles/ mL) concerning the diets, time, and interaction of diet and time, and raincloud plots with respective data distribution. Data are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

The average exosome diameter (nm) did not differ between the two groups (GL = 151 ± 18.5; GS = 154 ± 19.3; means ± 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 ±

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 x 10^{11} particles/mL on proteomics isolation and 3.5 x 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).

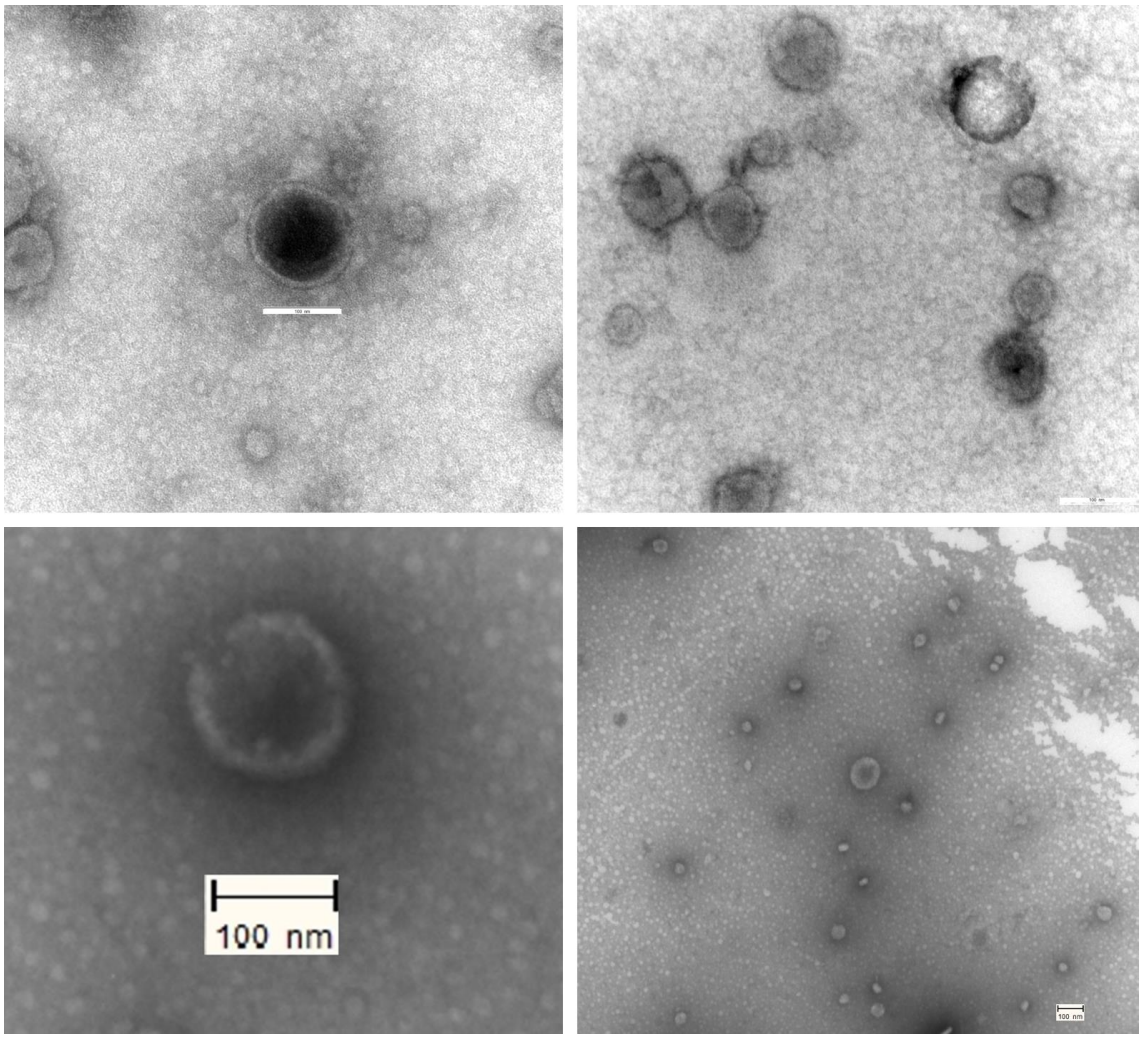
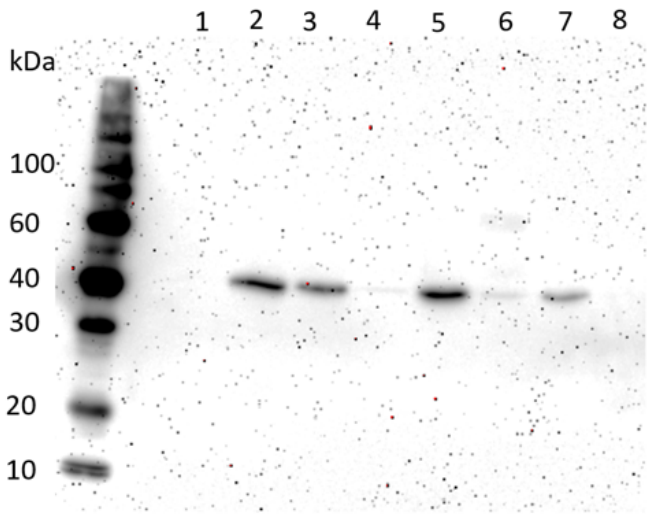


Figure 10. Representative electron micrograph of porcine milk exosomes in a close-up of a single vesicle (pictures on the right side) and wide view (pictures on the left side). Micrographs represent samples from both batches of isolation forwarded to lipidomics (upper pictures) and proteomics (lower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

A



Lane	Sample
1	GS Day 0 (Fraction 1)
2	GS Day 0 (Fraction 2)
3	GS Day 0 (Fraction 3)
4	GS Day 0 (Fraction 4)
5	GL Day 0 (Fraction 2)
6	GL Day 0 (Fraction 3)
7	GS Day 7 (Fraction 2)
8	GS Day 7 (Fraction 3)

B

Lane	Sample
1	Protein ladder
2	GS Day 14 (Fraction 1)
3	GS Day 14 (Fraction 2)
4	GS Day 14 (Fraction 3)
5	GS Day 7 (Fraction 2)
6	GS Day 7 (Fraction 3)
7	GS Day 7 (Fraction 4)
8	GL Day 14 (Fraction 2)
9	GL Day 14 (Fraction 3)
10	Empty

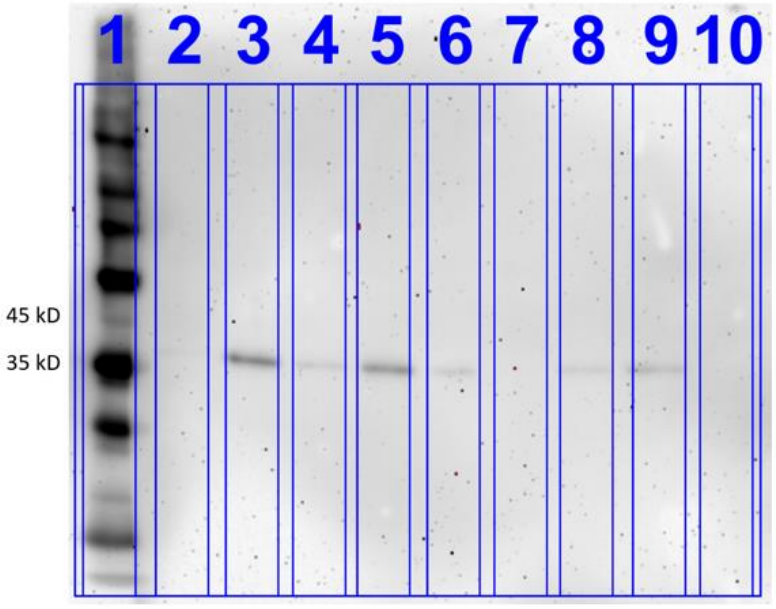


Figure 11. Western blotting of exosome marker TSG 101 in samples from both treatment groups after ultracentrifugation coupled with size exclusion chromatography (SEC), identified in milk exosomes isolation forwarded to **(A)** lipidomics and **(B)** proteomics analysis. Fractions 2 and 3 from SEC contained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

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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 ≤ 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 log2 fold changes (x-axis) and their associated -log10 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 log2 fold changes

(x-axis) and their associated -log10 transformed *p*-values (y-axis) of all identified proteins in the different conditions are given in Figure 16.

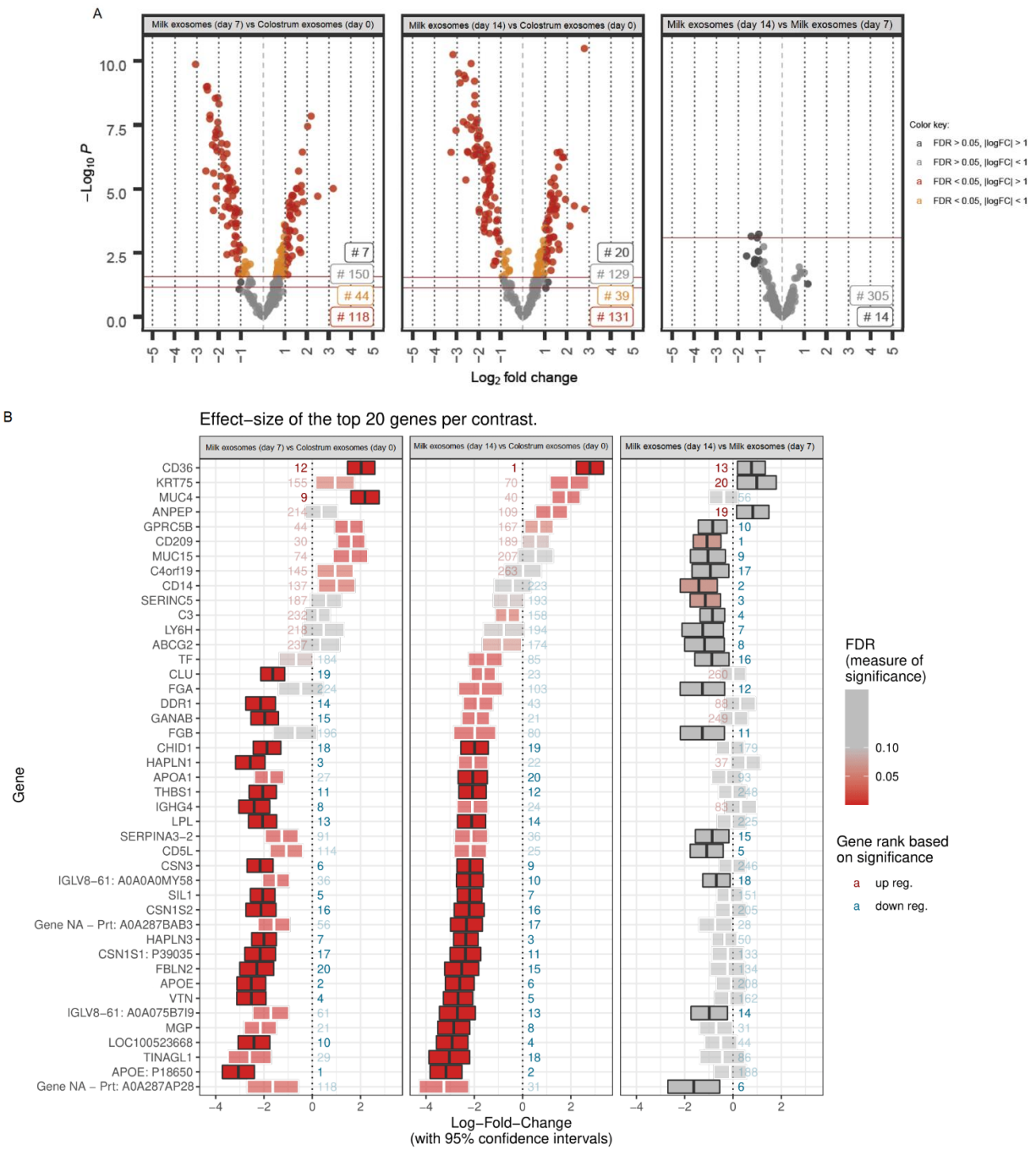

Figure 12. (A) Volcano plots of log2 fold changes (x-axis) and their associated -log10 transformed p-values (y-axis) of all identified proteins in the different time points. The horizontal lines in red represent the 5% and 10% FDR threshold on the first contrast, and the 10% FDR threshold on the third 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.

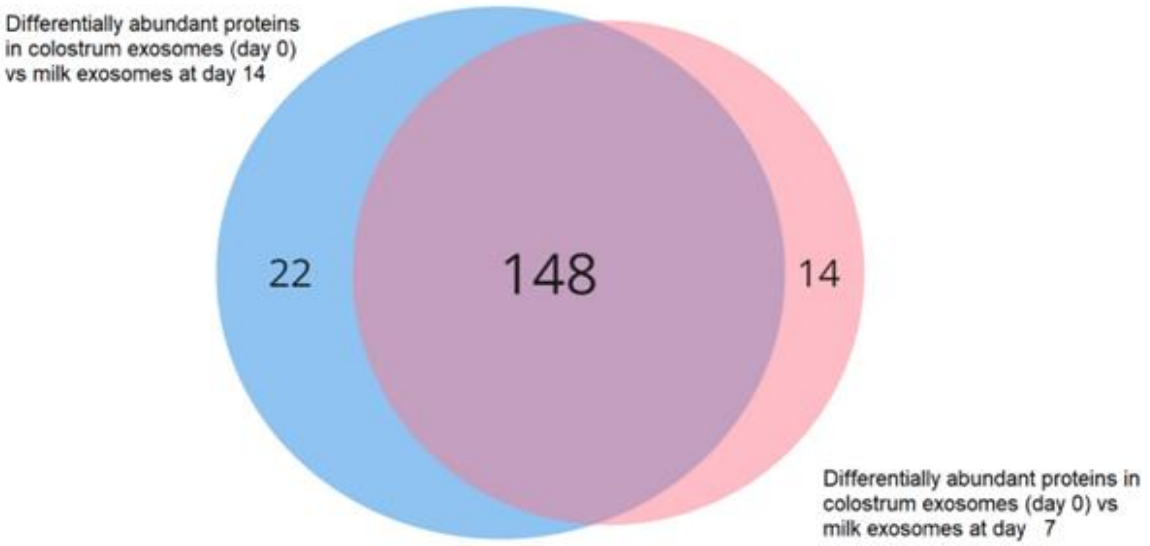

Figure 13. Venn diagram showing the comparison of DAP identified in colostrum exosomes and milk exosomes on day 7, and day 14. From a total of 184 unique DAP between colostrum exosomes and milk exosomes on day 7 and day 14, 148 proteins were significantly different ($p < 1.0e-16$) from colostrum on both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

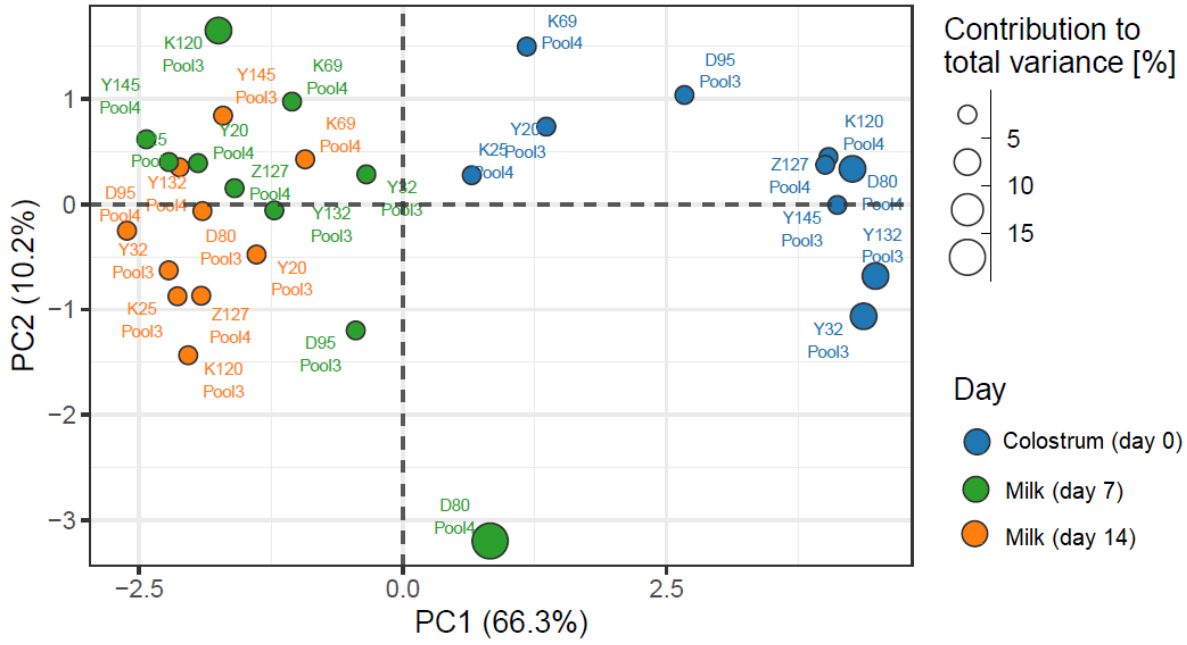

Figure 14. Principal component analysis of the top 5% proteins with the highest variance.

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_2FC	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_2FC	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

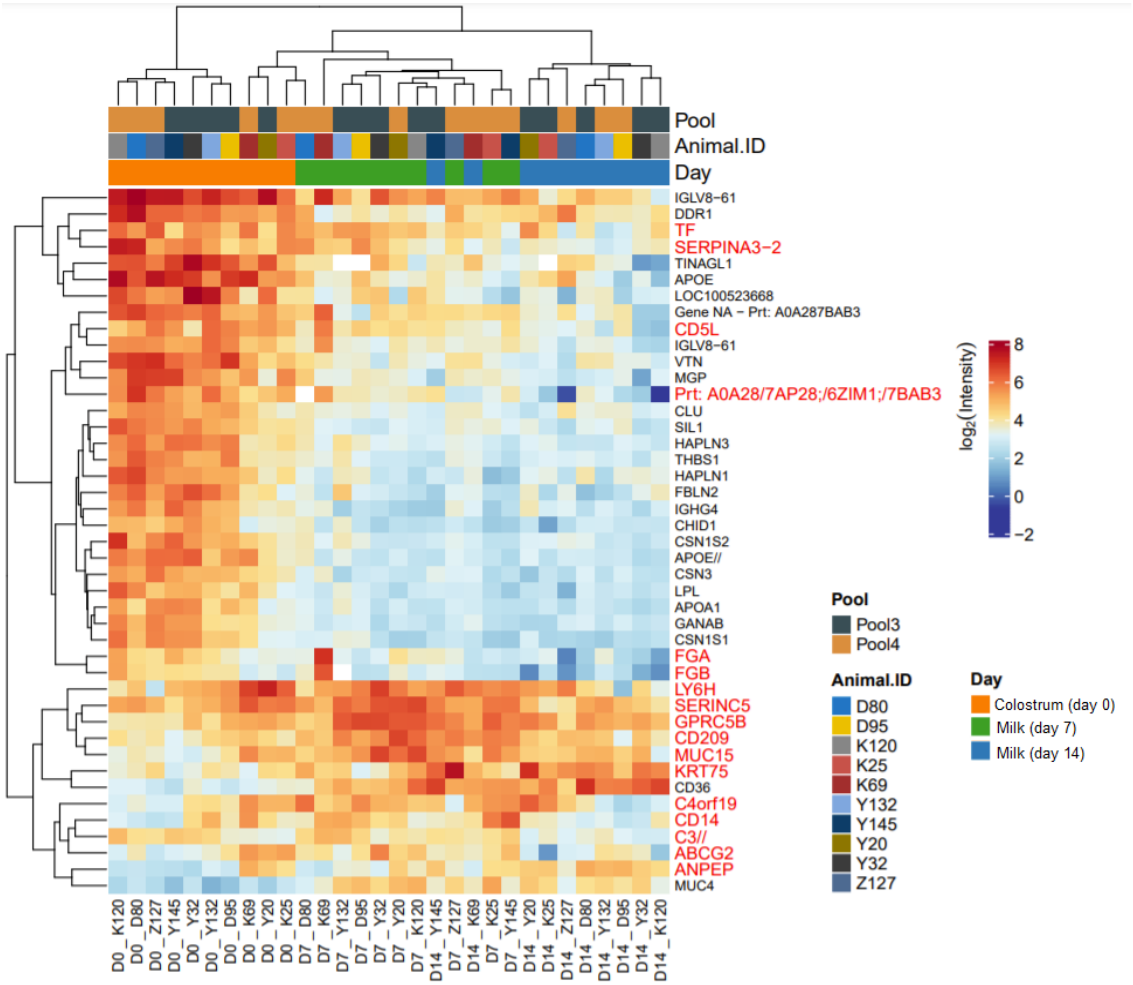

Figure 15. Hierarchically-clustered heatmap of the top 20 genes with the smallest P-values in each of the three contrasts. The subset contains 43 genes, whereby 18 genes are among the top 20 in more than one contrast (overlapping genes). Genes/Proteins indicated in red were not significantly regulated (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*.

Protein name	Accession number	logFC	Adjusted p-value	Number of peptides
Group S vs Group L at day 0 post-partum				
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**

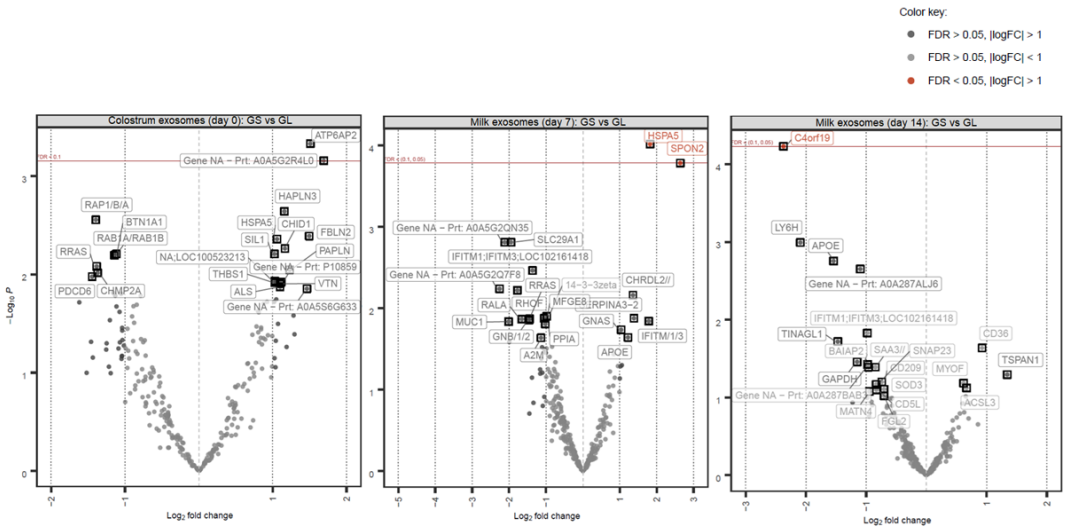

Figure 16. Volcano plots of log2 fold changes (x-axis) and their associated -log10 transformed p-values (y-axis) of all identified proteins in the different time points on the comparison between GS and GL. The horizontal lines in red represent the 5% FDR threshold (adjusted p-value < 0.05) on the second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

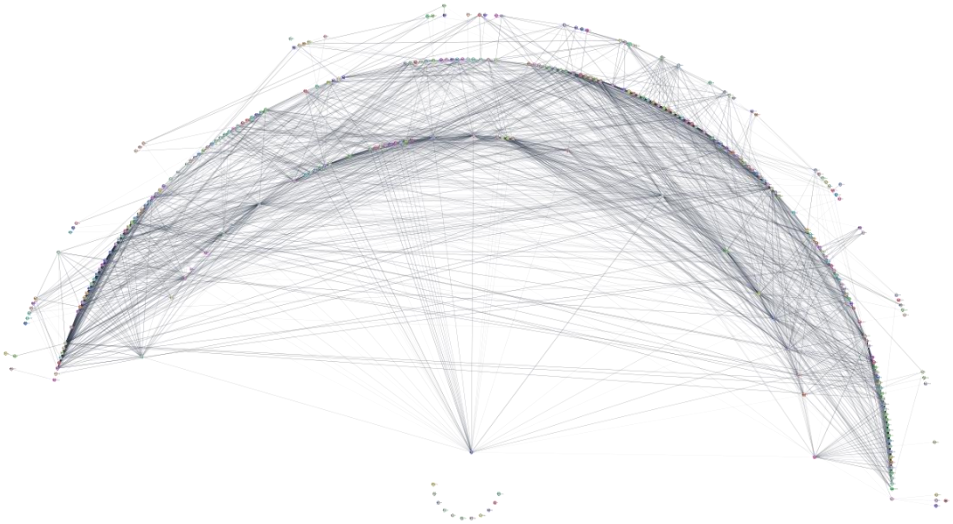

Figure 17. Comprehensive protein-protein interaction (PPI) network of all 319 proteins retained in this study was highly significant (552 edges, p < 1.0e-16) and distinctly connected, with 1931 terms and only 11 secluded from any pathway.

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 -log10) 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.

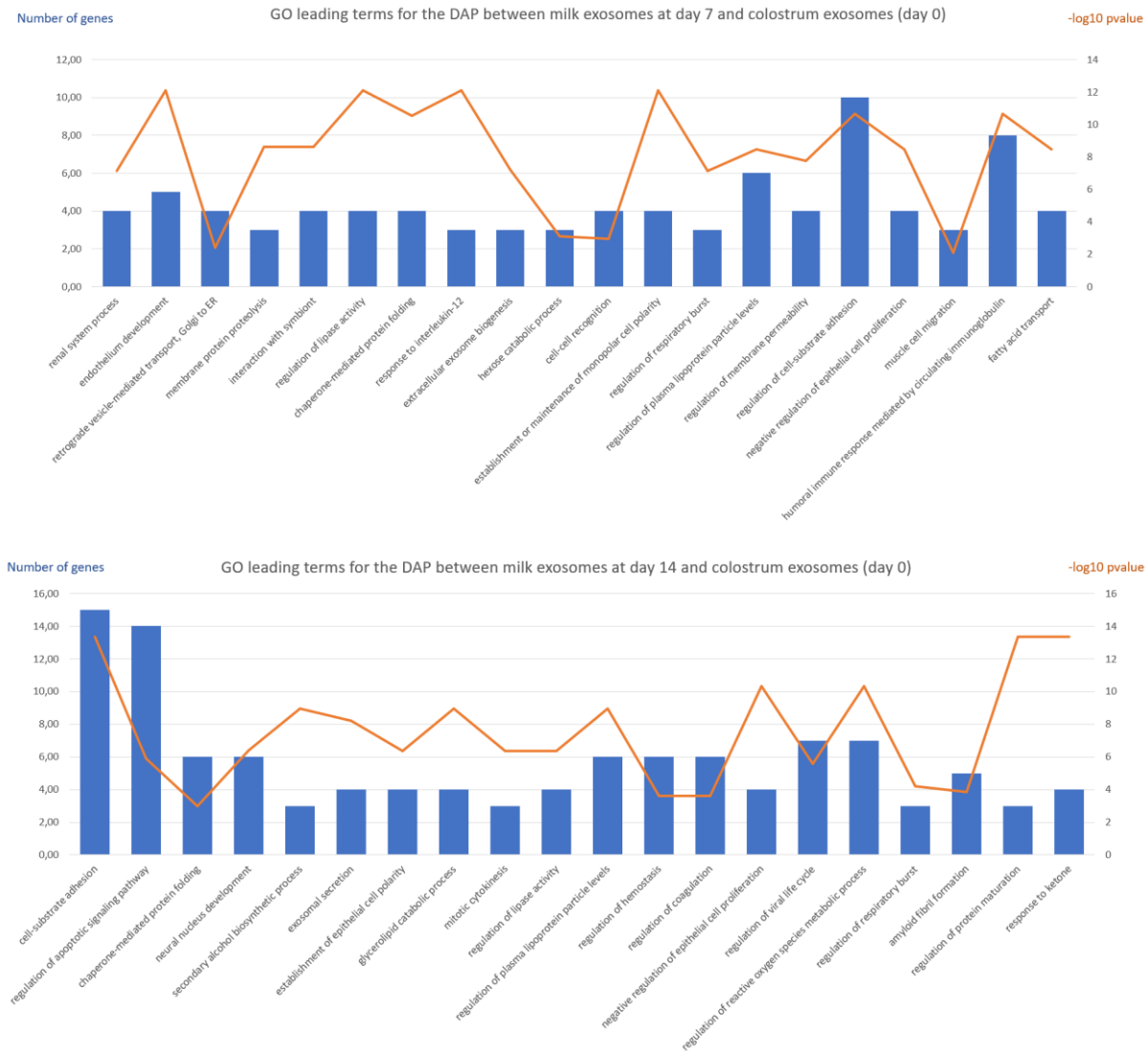

Figure 18. A. The top 20 significantly overrepresented GO leader terms with number of genes associated in the comparison between GS and GL (left y-axis) and their –log p value (right y-axis) for the DAP between milk exosomes at day 7 and colostrum exosomes (day 0), performed in ClueGO and cured by REVIGO.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

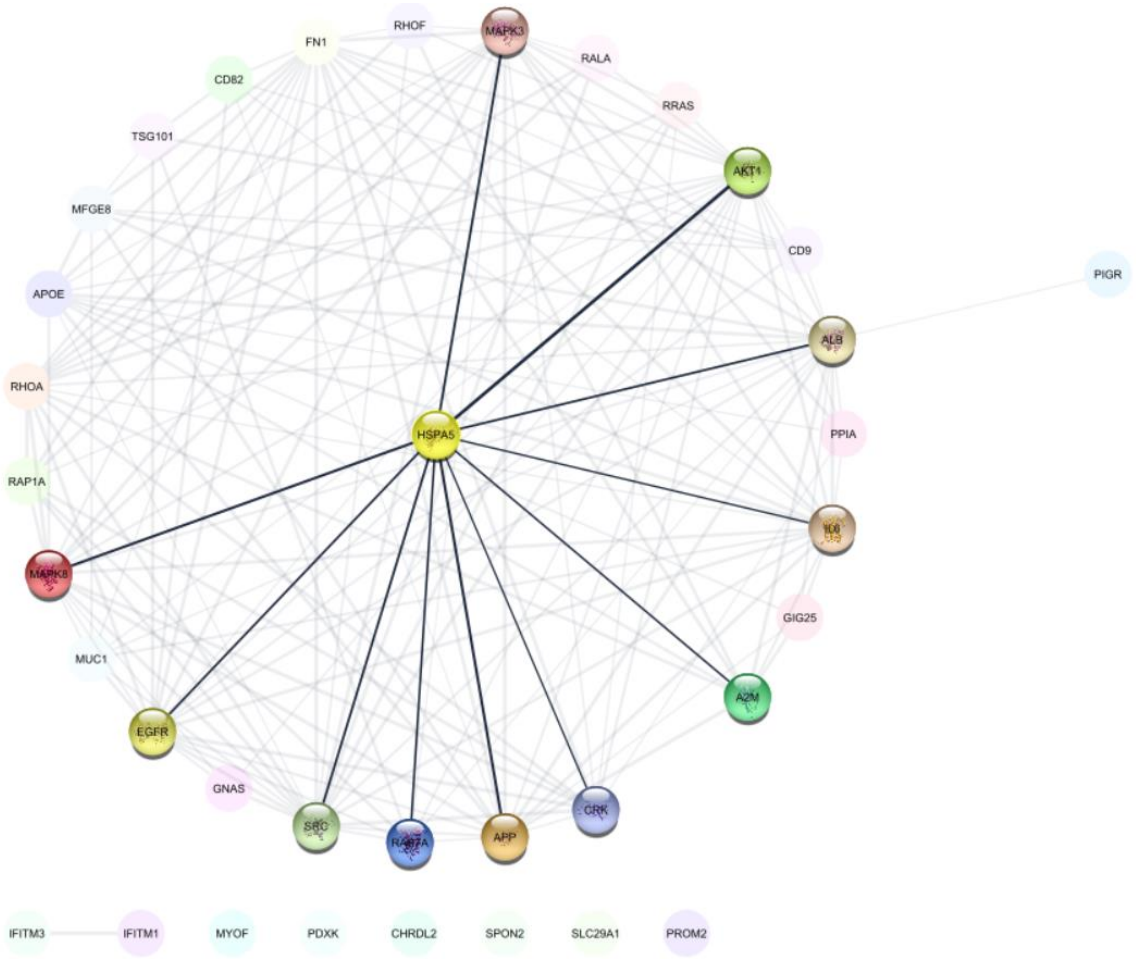

Figure 19. Protein-protein interaction network of the leader milk EV proteins in the comparison between GS and GL at day 7 p.p. (PPI enrichment p value = 3.35e-06), with 36 nodes and 153 predicted protein associations (edges), enriched in STRING and displayed in Cytoscape. HSPA5 was significantly more 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.

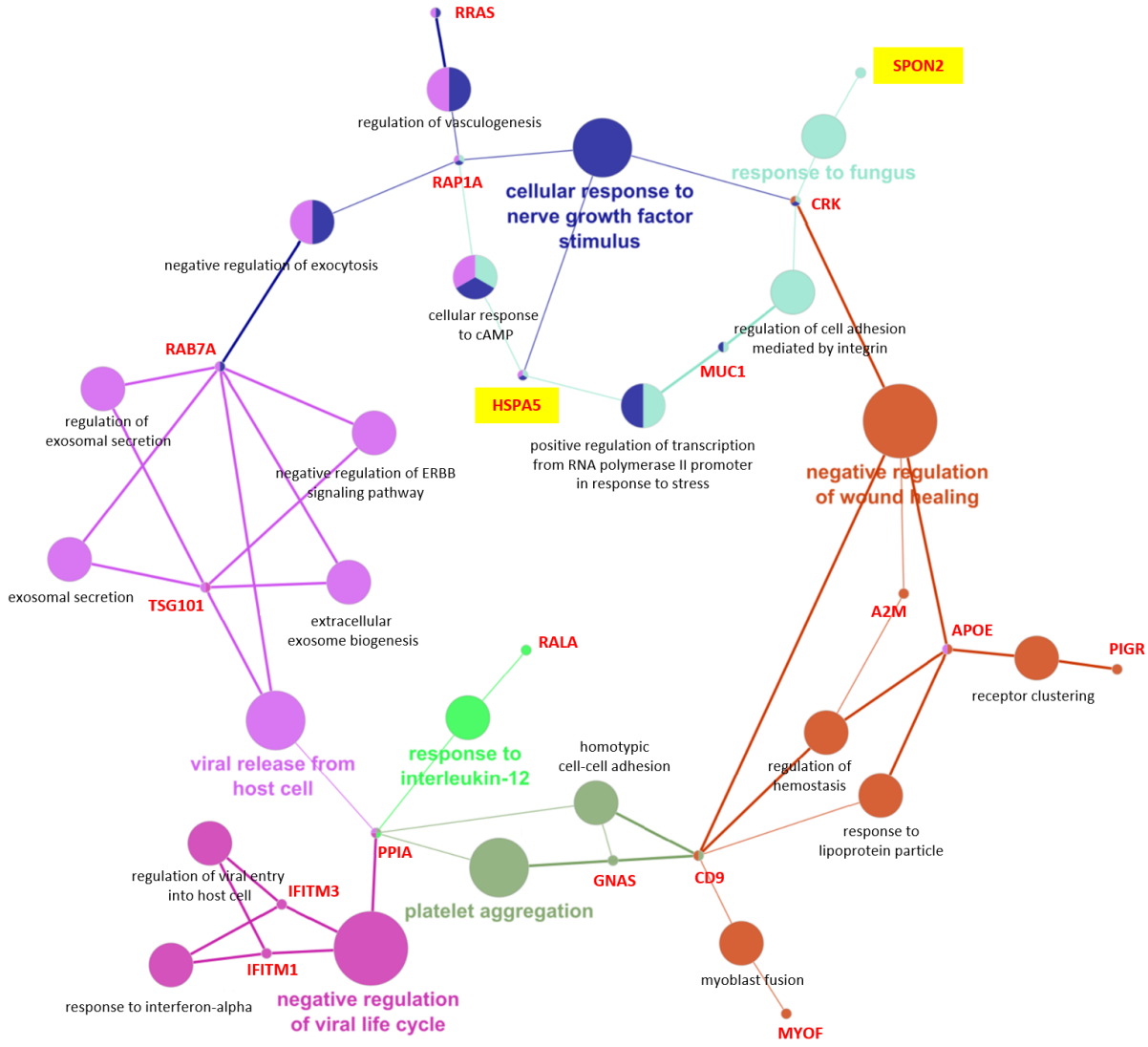

Figure 21. Comprehensive interactive network using the generated gene ontology terms (converted from proteins) for Biological Process by the leader milk EV proteins in the comparison between Group S and Group L at day 7, displayed in Cytoscape using the ClueGO application. Gene names are annotated in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

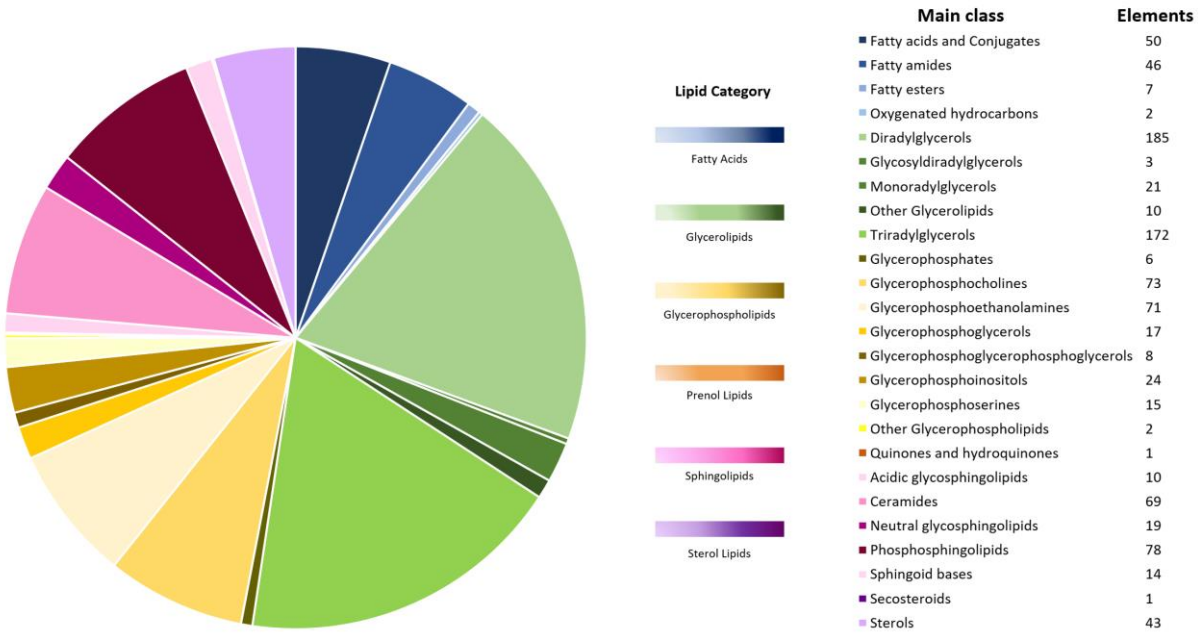

Figure 22. Summary of the total of 947 lipid elements identified in milk and colostrum exosomes by liquid chromatography–quadrupole time-of-flight mass spectrometry lipidomics analysis, color-coded by lipid category and stratified in lipid main classes.

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).

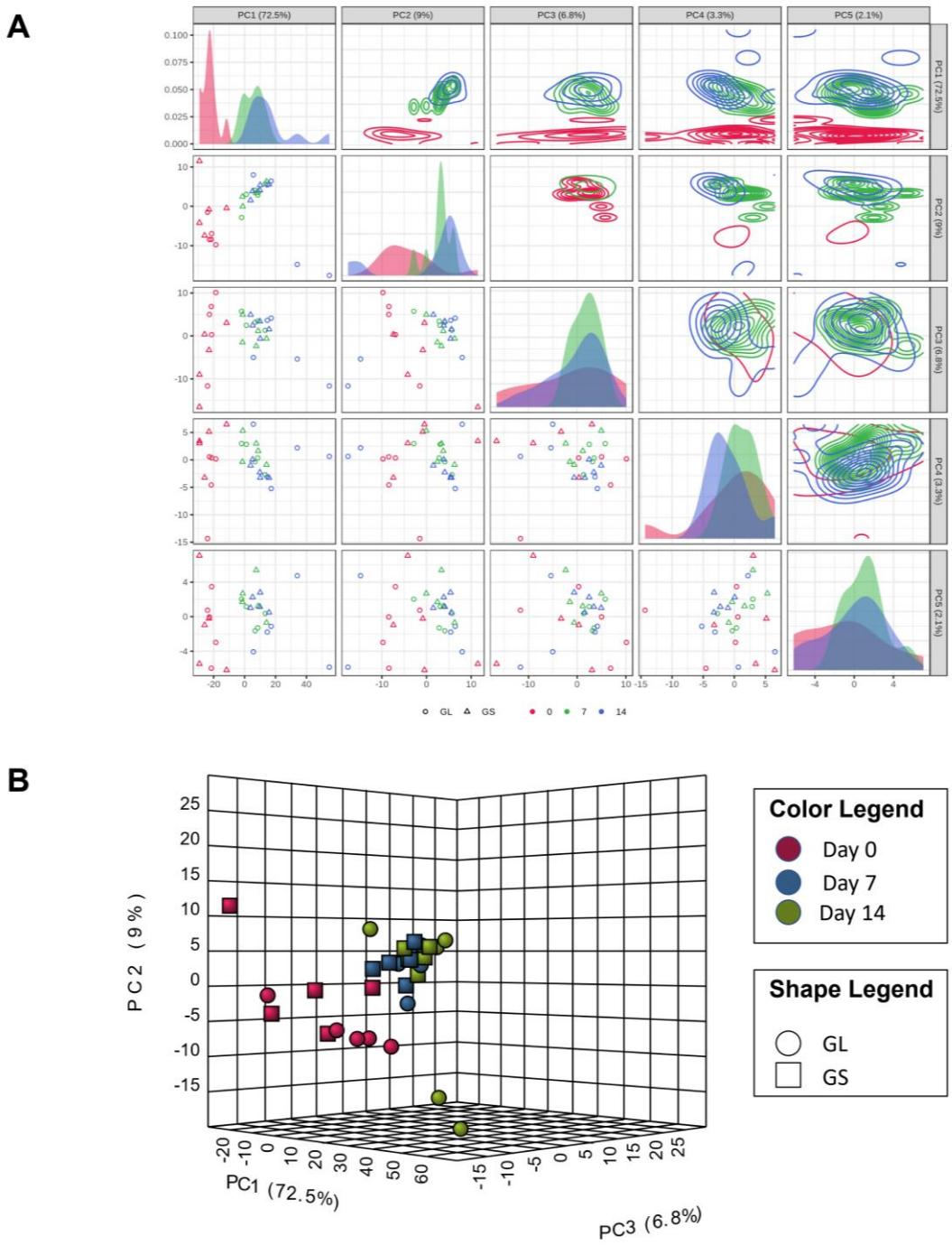

Figure 23. Principal component analysis (PCA) score plot of lipidomic profiling of exosomes from colostrum and milk shows a distinct lipidomic profile between colostrum (day 0) and mature milk (day 7 and 14) exosomes, without clear separation between feeding diets with different ratios of omega-6 to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

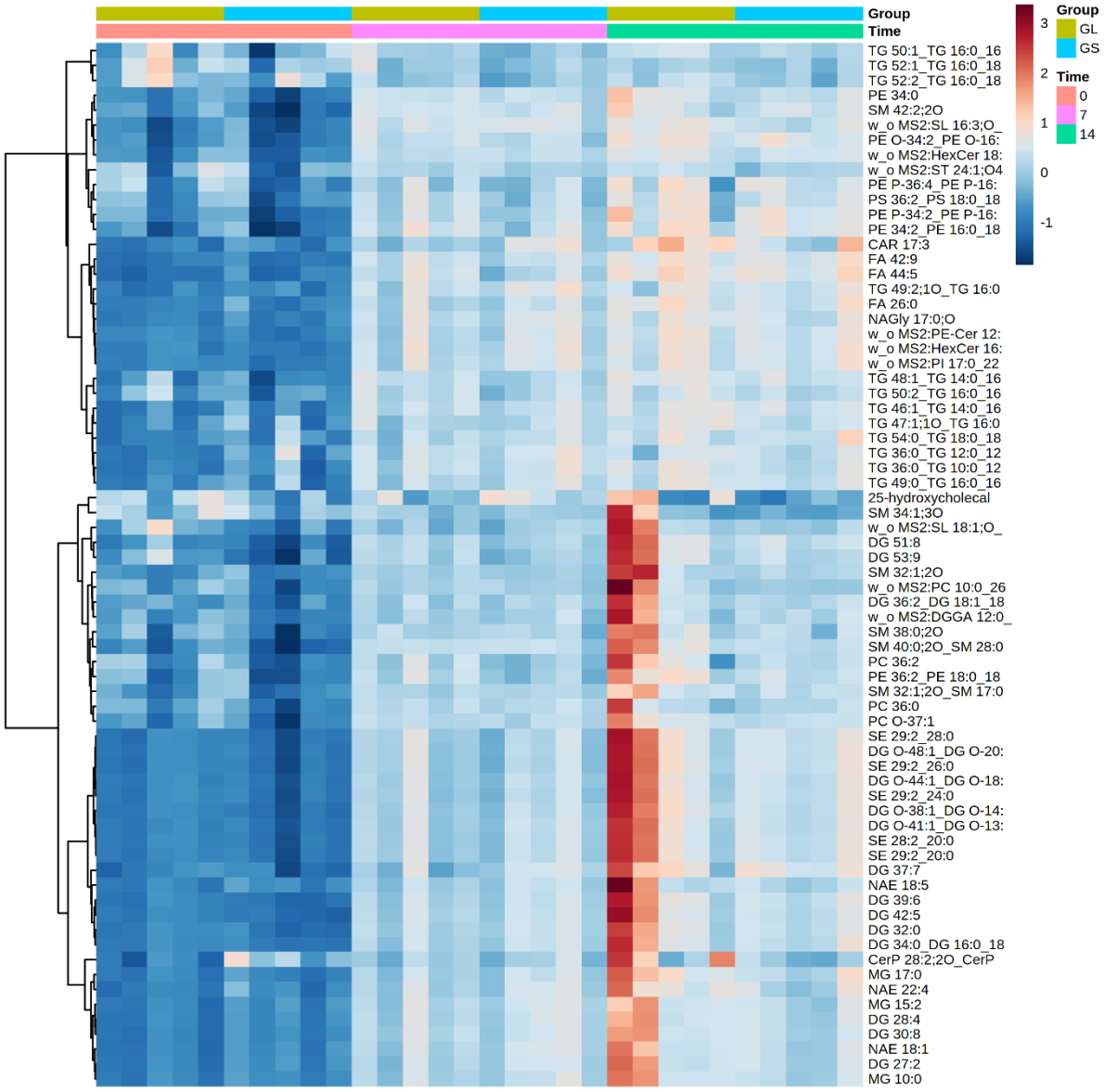

Figure 24. Hierarchically-clustered heatmap of the top 50 statistically significant lipids with smallest P-values in each grouped by day and time in milk exosomes from sows fed diets with different ratios of ω-6 to ω-3 PUFA on days 0, 7, and 14. The colors in the heat map reflect the different profiles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

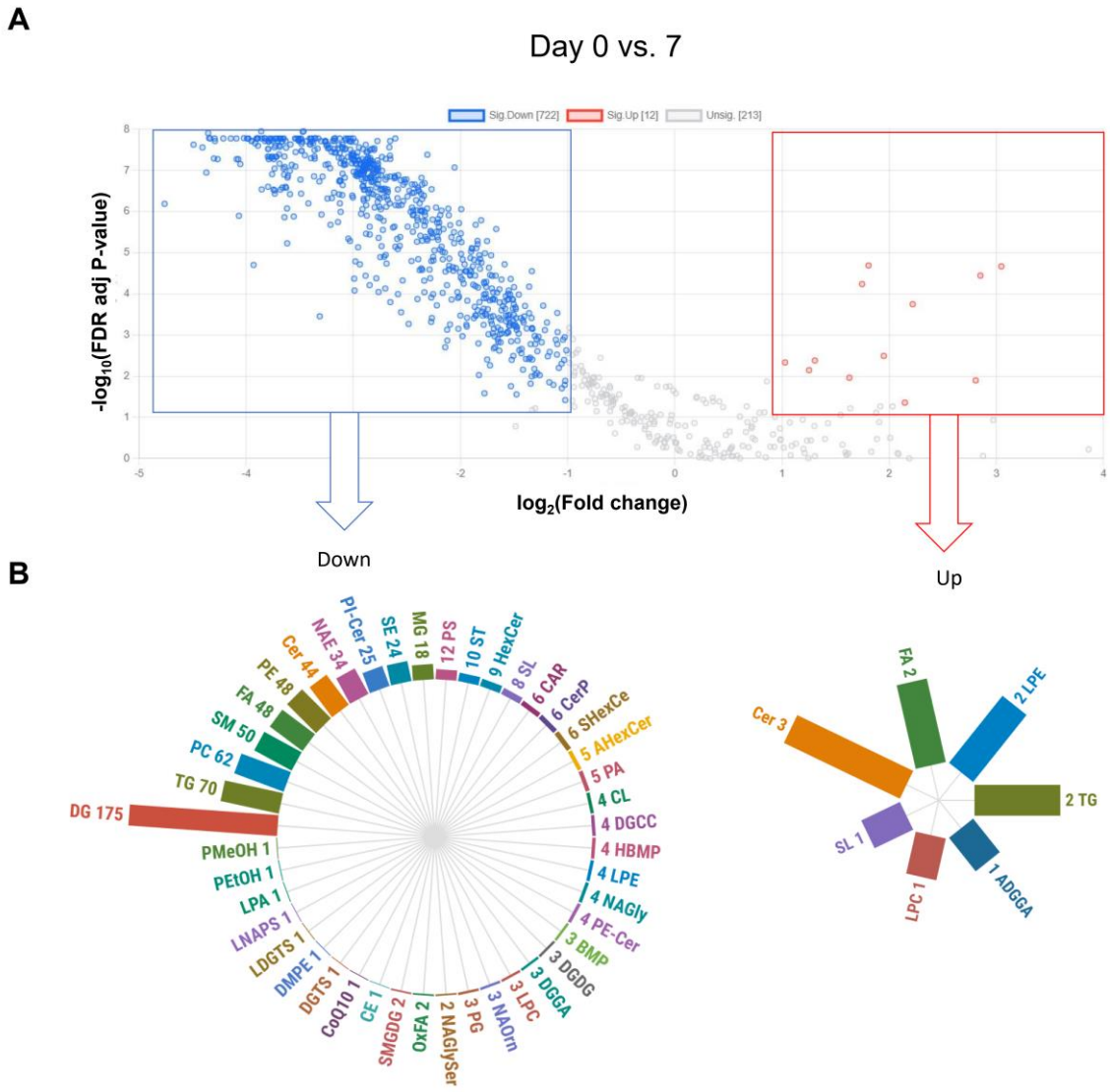

Figure 25. (A) Volcano plot of $\log_2$ fold changes (x-axis) and their associated $-\log_{10}$ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between colostrum exosomes (day 0) and milk exosomes at day 7. Significantly different lipids (t-test FDR adjusted p-value < 0.05, 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.

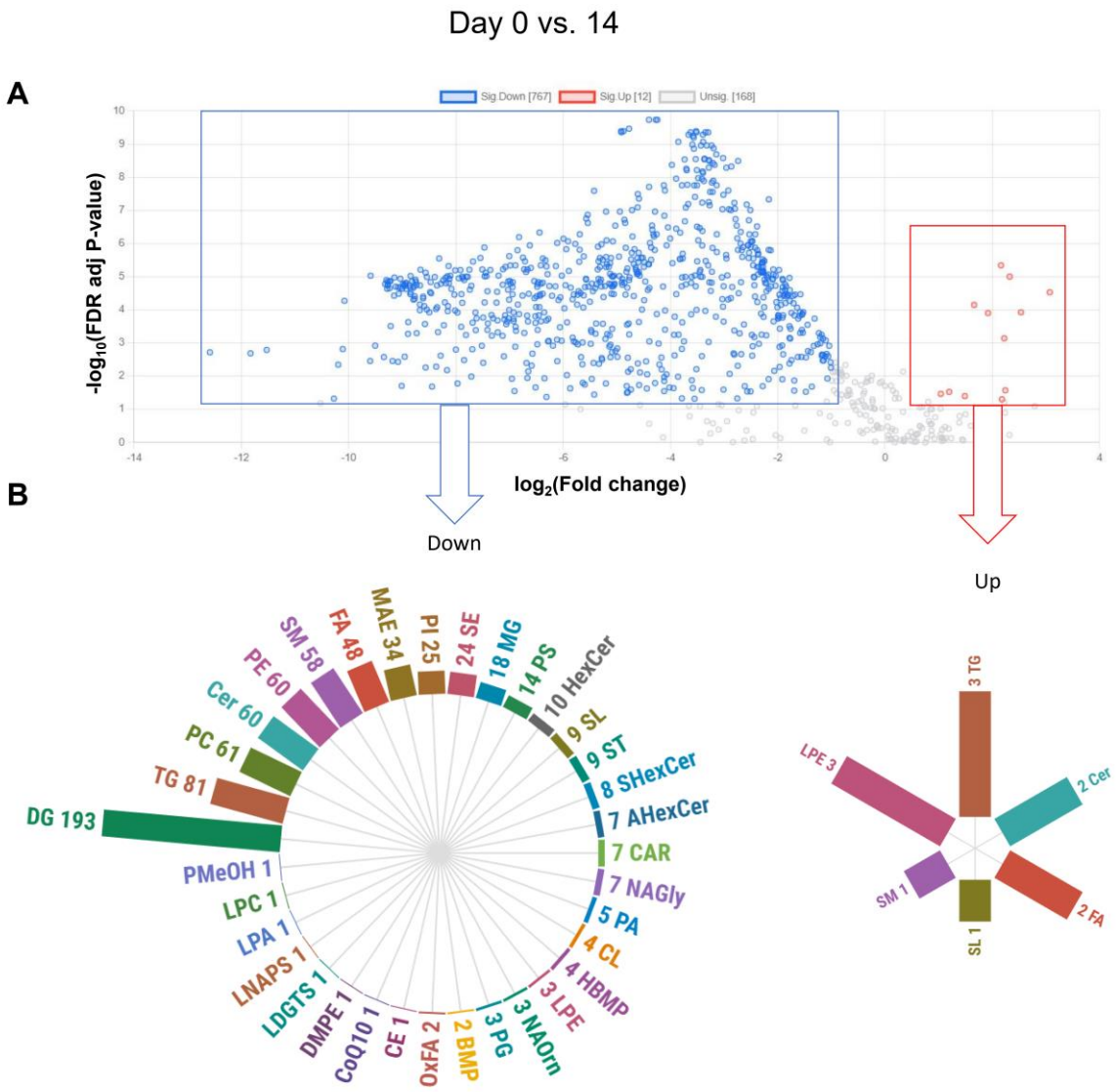

Figure 26. (A) Volcano plot of log_2 fold changes (x-axis) and their associated $-log_{10}$ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between colostrum exosomes (day 0) and milk exosomes at day 14. Significantly different lipids (t-test FDR adjusted p-value < 0.05, fold 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.

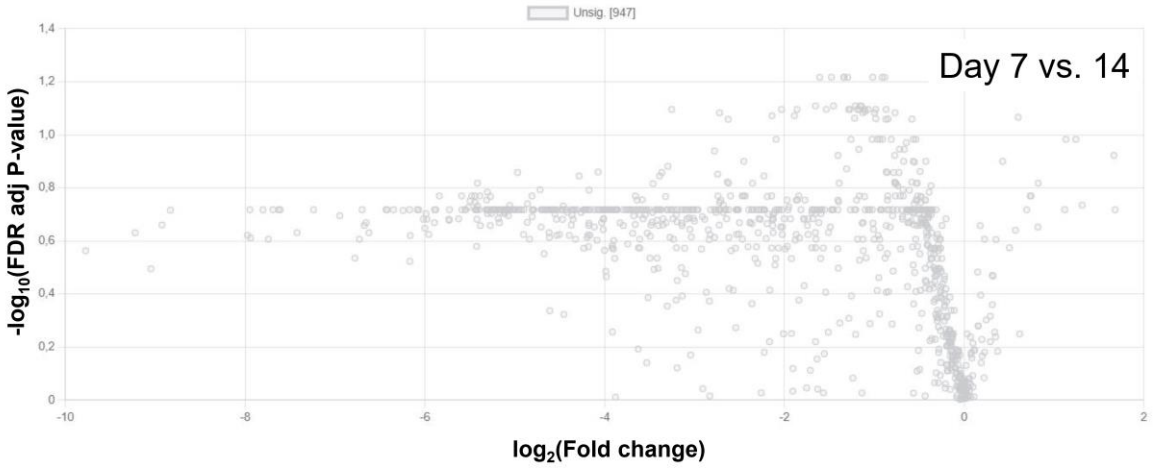

Figure 27. Volcano plot of $\log_2$ fold changes (x-axis) and their associated $-\log_{10}$ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between milk exosomes at day 7 and milk exosomes at day 14. No significantly different lipids were found (t-test FDR adjusted p-value threshold at 0.05, fold change threshold at 2).

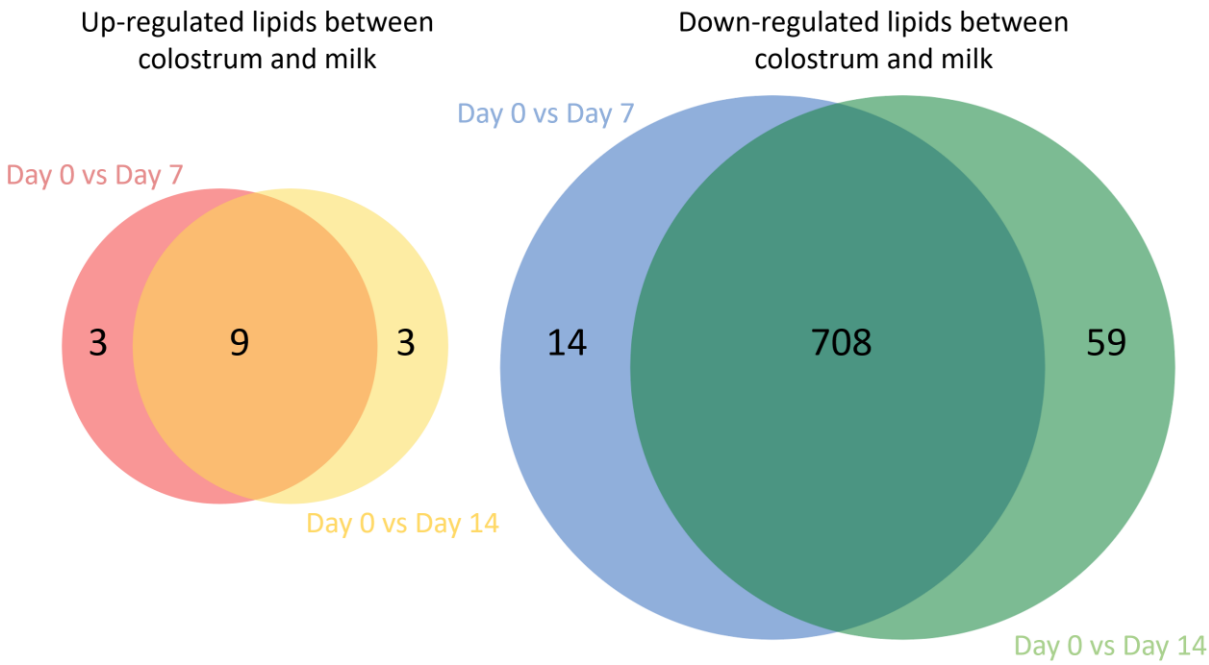

Figure 28. Venn diagram representing the number of differentially expressed lipids elements found in comparison between colostrum exosomes (day 0) and milk exosomes at day 7 and 14, featuring that most of lipid elements were shared between comparisons.

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).

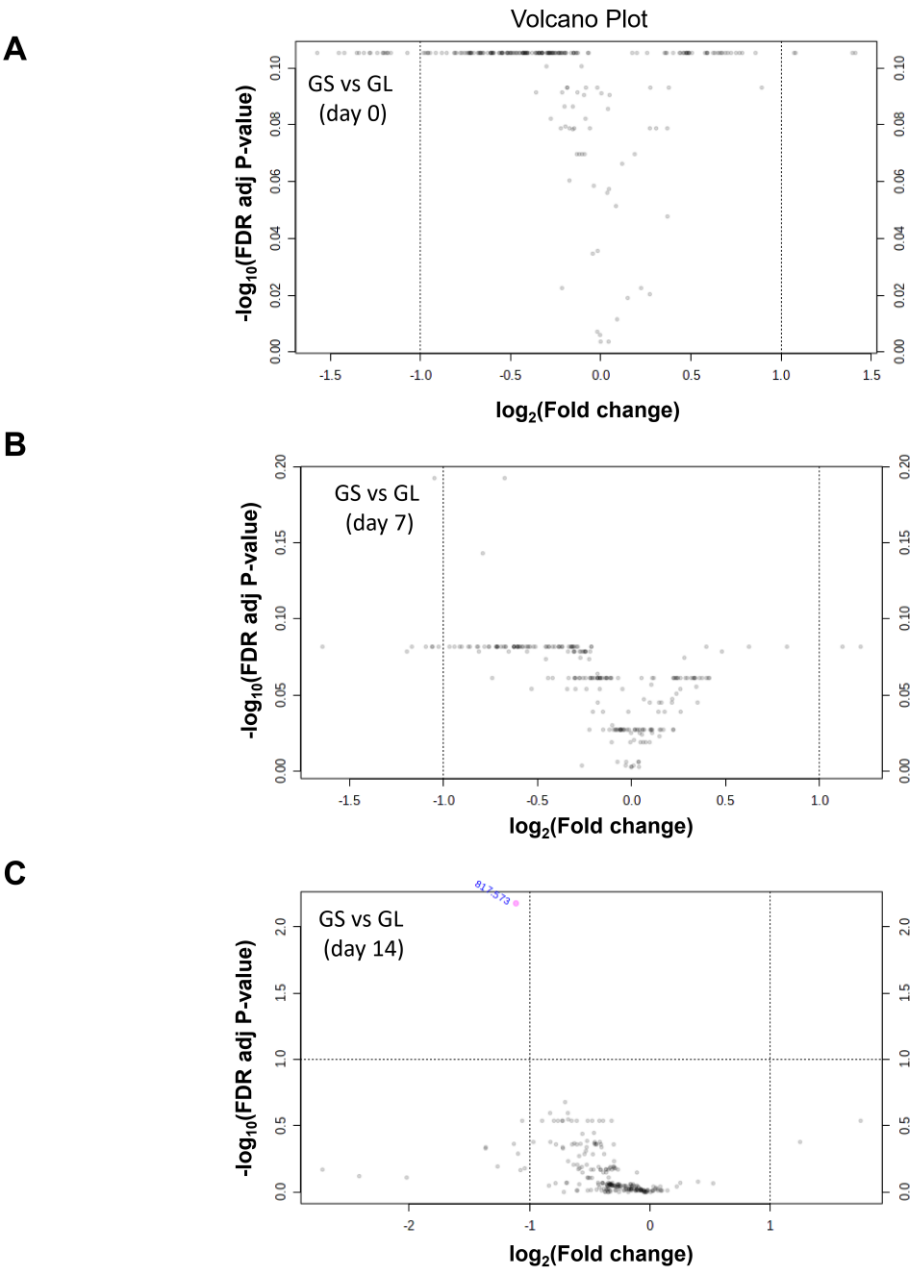

Figure 29. Volcano plot of $\log_2$ fold changes (x-axis) and their associated $-\log_{10}$ FDR adjusted p-values (y-axis) of all identified lipids in the comparison between milk exosomes from GS and GL sows in different stages of lactation - at **(A)** day 0, **(B)** day 7, and **(C)** day 14. No significantly 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 colostral 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 chrematistics 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 where also among the major lipids identified on this study, which are involved in numeral 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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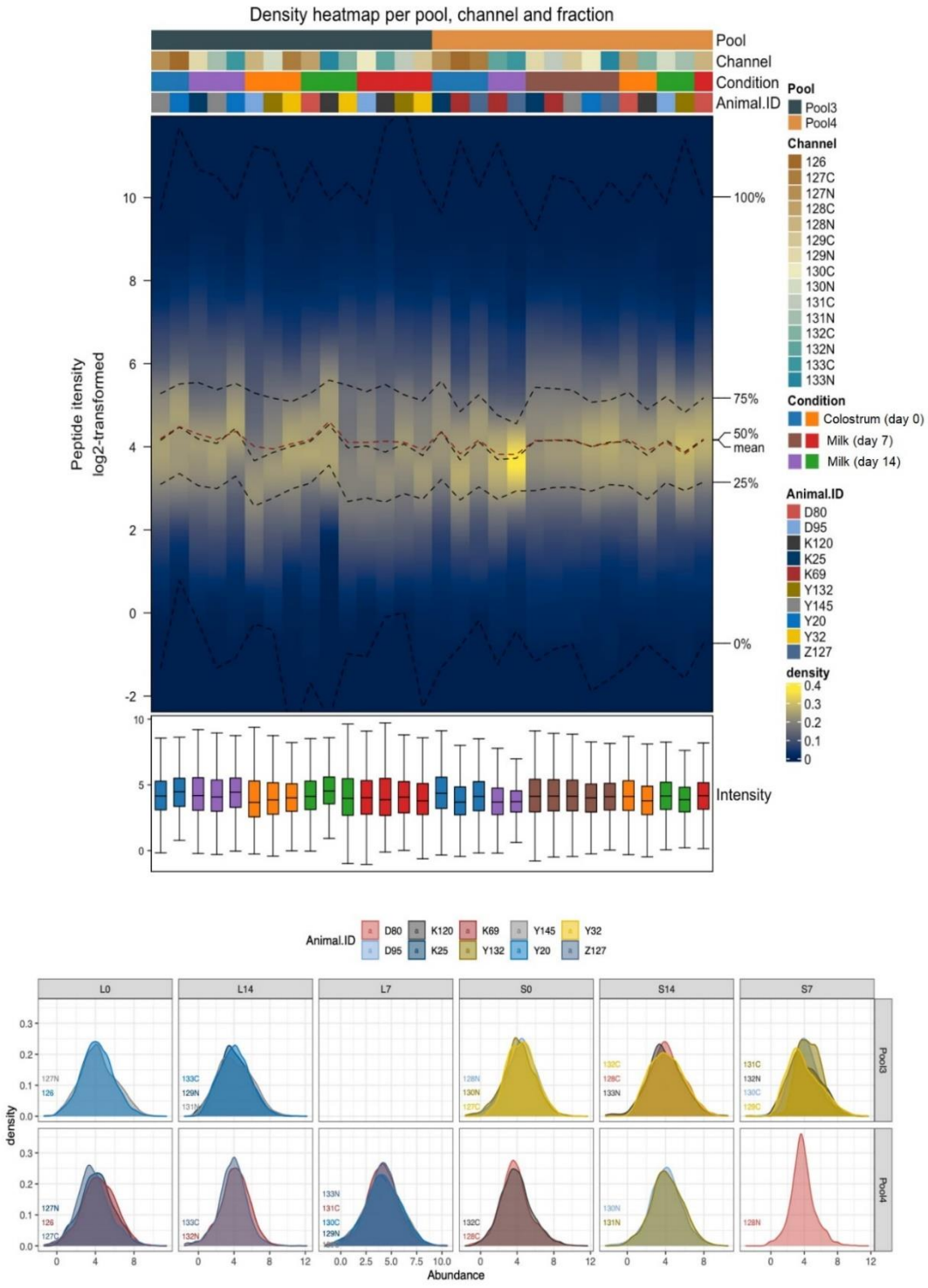
9 APPENDICES

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;P18650	-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	A0A5G2R305	-1.99782	4.8E-09	1.81E-07	3.833653	20
8	Gene NA - Prt: A0A5G2QXT5	A0A5G2QXT5	-2.40265	1.32E-08	4.22E-07	3.449775	5
9	MUC4	A0A287B5M2	2.184258	1.44E-08	4.22E-07	3.497925	23
10	Gene NA - Prt: A0A5G2R4L0	A0A5G2R4L0	-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	A0A287ATV6	-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	A0A287B5Q1	-2.29675	1.9E-07	2.5E-06	3.6693	10
19	CLU	A0A5S8KLN1	-1.63593	1.81E-07	2.5E-06	3.847397	27
21	MGP	A0A5G2QKI1	-2.1489	2.33E-07	2.93E-06	4.000976	10
22	PGLYRP1	A0A286ZI97	-2.07871	3.26E-07	3.91E-06	3.716147	14
23	CP	A0A5G2QW05	-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	A0A287BIL8	-1.63728	4.16E-07	4.4E-06	2.279501	4
26	ATP6AP2	A0A287AJK1	-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	A0A286ZKG9	-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	A0A287ATT2	-2.24384	2.42E-06	2.06E-05	3.373407	3
32	B4GALT1	A0A287BG16	-1.89939	3.18E-06	2.62E-05	3.320653	20
33	TTR	A0A5G2QIE9	-1.59931	3.59E-06	2.8E-05	3.478735	3
34	SERPINC1	A0A5G2QUE0	-1.48287	3.61E-06	2.8E-05	3.234506	9
35	ITIH2	A0A5G2QEV5	-1.52173	4.74E-06	3.58E-05	3.574539	7
36	Gene NA - Prt: A0A0A0MY58	A0A0A0MY58	-1.49819	5.37E-06	3.94E-05	4.034749	19
37	SERPINF1	A0A287AYJ8	-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//	A0A5G2QXF8;A0A5G2RH74	-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	A0A480W2U9	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;O02772	3.186529	9.68E-06	5.79E-05	6.001107	2
46	GSN	A0A287A6P1	-1.21755	1.03E-05	5.94E-05	3.579003	19
48	CSN2//	P39037;A0A2C9F376	-1.52755	1.17E-05	6.43E-05	2.792655	68
47	ALB	A0A287BAY9	-1.26931	1.16E-05	6.43E-05	3.830738	18
49	SLC9A3R1	A0A5G2R543	1.742217	1.33E-05	7.15E-05	4.747528	16
50	RAB3D	A0A287BLN4	1.327575	1.76E-05	9.29E-05	3.960266	4
52	LTF	A0A5G2QUQ4	-1.37492	1.87E-05	9.49E-05	3.41711	26
51	TTYH2	A0A287AAJ2	1.371667	1.84E-05	9.49E-05	5.144515	8
53	RDX;EZR	P26044;F1SB42	2.484253	1.93E-05	9.62E-05	4.44116	2
54	ACSL3	A0A287B8R8	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	A0A287BAB3	-1.58745	3.01E-05	0.000142	4.473881	4
57	MUC1//	A0A5G2QGF8;F1RGR9	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//	A0A287BRR9;F1RZB0	1.550944	3.93E-05	0.000176	4.191112	16

60	FABP3	O02772	1.297847	4.07E-05	0.000179	4.383504	22
61	Gene NA - Prt: A0A075B7I9;A0A075B7H9;A0A075B7J0;A0A075B7I5	A0A075B7I9;A0A075B7H9;A0A075B7J0;A0A075B7I5	-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	A0A075B7I6	-1.54786	5.17E-05	0.000217	3.55741	2
64	SMPDL3B//	A0A287BJW1;F1STN0	1.380332	5.27E-05	0.000218	3.57818	10
65	NA;LOC100523213	A0A0A0MY58;F1RL06	-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;EZR	P26044;A0A287AK19;F1SB42	1.469399	5.96E-05	0.000235	4.397996	10
68	ATP6AP1	A0A5G2RD35	-1.17875	6.52E-05	0.000253	3.264964	4
69	Gene NA - Prt: A0A5S6G633	A0A5S6G633	-2.24612	6.97E-05	0.000267	2.838299	3
70	ARF/3/4	A0A287B8I6;A0A5G2R3T3	1.233139	7.46E-05	0.000281	3.238186	4
73	NA;CSN1S1	A0A5S6G633;P390335	-1.25937	8.41E-05	0.000304	3.32349	13
72	Gene NA - Prt: A0A287ALC1	A0A287ALC1	-1.2067	8.4E-05	0.000304	3.088551	68
71	Gene NA - Prt: A0A287ALJ6	A0A287ALJ6	-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/RAB1B	F2Z5U4;Q06AU7	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	A0A287BQC8	-1.4338	0.00022	0.000716	4.707291	2
80	PAEP	A0A5S6HQ91	-1.25135	0.000219	0.000716	3.980301	12
82	Gene NA - Prt: A0A287A4Y3	A0A287A4Y3	-1.18388	0.000223	0.000716	4.354942	13
83	MYOF	A0A5G2Q8T2	0.965459	0.000249	0.000793	3.989779	30
84	SPON2	A0A481AED0	-1.60841	0.000278	0.000868	3.364732	4
85	GPRC5C	A0A287ASV2	1.015776	0.00028	0.000868	4.592204	5

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	A0A5G2R9X9	1.22115	0.000443	0.001315	4.788696	3
90	RAB11A	A0A5G2QE74	1.443548	0.000454	0.001332	4.928738	6
91	SERPINA3-2	A0A287B9R5	-1.26669	0.000686	0.00199	4.440938	5
93	Gene NA - Prt: A0A287B626	A0A287B626	-1.20554	0.000768	0.002161	3.970228	29
94	CKB	A0A5G2R6X7	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	A0A287BH90	-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	A0A5K1U1P1	0.972775	0.001533	0.004006	4.268103	61
99	RAC2	A0A5G2R6S0	1.111124	0.001511	0.004006	4.062857	2
102	PLOD1	F1RF77	-1.07263	0.001615	0.004179	3.585215	3
103	BAIAP2	A0A287BRR9	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//	A0A5G2RKJ7;A0A5G2QXS8	0.887413	0.002007	0.004953	4.588095	105
106	SLC29A1	A0A287B8K5	1.207747	0.001989	0.004953	4.707259	6
108	PPIA	P62936	0.845717	0.002201	0.005381	4.659886	14
109	ANXA6	A0A5G2RBI3	0.91366	0.002336	0.005657	4.400298	6
110	Gene NA - Prt: A0A287A7S8	A0A287A7S8	-0.87628	0.002403	0.005719	3.275506	2
111	MUC1	F1RGR9	1.638827	0.002405	0.005719	3.142075	3
112	ATP2B2	A0A286ZSF2	0.76404	0.002657	0.006229	4.769486	7
113	SRC	K7GM45	0.839798	0.002666	0.006229	4.3238	6
115	COL4A2	A0A5G2RJ53	-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	A0A5G2R9S9;A0A287ALJ6	-1.39581	0.003241	0.007314	3.153838	2
118	Gene NA - Prt: A0A287AP28;	A0A287AP28;A0A286ZIM1;A	-1.63365	0.003696	0.008269	3.773167	3

	A0A286ZIM1;A0A287BAB3	0A287BAB3					
119	MSN	A0A287AK19	1.026988	0.003869	0.008583	4.993424	19
120	RDX	P26044	0.883987	0.004037	0.00888	3.883106	5
121	MVB12A	A0A287AP77	1.321117	0.004128	0.009007	4.637288	4
122	RAB2A/RAB2B	F1RT87;F1S8J6	0.981515	0.004422	0.009568	3.736955	3
123	ARF3	A0A287B8I6	1.0729	0.004724	0.010139	4.867116	4
124	IFITM1;IFITM3;LOC102161418	A0A5G2QWL4;A0A287B7R8;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;YWHAH;YWHAQ;CRK;YWHAB	A0A480PLY3;F2Z4Y1;A0A5G2QM17;A0A5G2QSR7;A0A287BPX7	1.175426	0.005671	0.011882	5.657113	2
127	Gene NA - Prt: A0A075B7H9	A0A075B7H9	-0.80249	0.006983	0.014515	3.212066	4
128	CD82	A0A5G2QKD8	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	A0A286ZZM9	-0.57936	0.008719	0.017438	3.833016	4
133	LOC100302368	A0A287B5W2	0.680148	0.009078	0.018019	4.415311	34
134	RAB35	A0A287BFE6	0.768843	0.009574	0.018862	4.809324	5
135	PDCD6IP	A0A287A5B4	0.937747	0.009907	0.019374	4.405182	30
136	ITIH4	A0A286ZN24	-0.71567	0.010322	0.020037	3.40089	4
137	CD14	A2SW51	1.01012	0.010828	0.020866	3.953437	3
138	PIGR//	A0A287B644;Q29244	-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	A0A287BHC4	0.702267	0.013452	0.025186	4.917479	4
142	Gene NA - Prt: A0A5G2R9S9	A0A5G2R9S9	-1.09679	0.014055	0.025858	3.738216	2
143	TM4SF1	A0A5G2QC13	-0.83057	0.014077	0.025858	3.680237	2
144	PRDX5	A0A5G2R9D6	0.659936	0.014105	0.025858	3.401712	6
145	C4orf19	F1S4L8	0.948851	0.015086	0.027468	4.353937	2

146	RHOF	Q06AT7	0.980713	0.015743	0.028467	5.064607	2
147	PPIC	A0A5G2RB35	-0.90975	0.017663	0.031467	2.948777	4
149	GDI2	A0A5G2QSK4	0.640722	0.01776	0.031467	3.627553	15
148	RAB7A	A0A286ZP69	0.695541	0.017744	0.031467	4.306068	9
150	MDH1	A0A5G2QFC2	0.635617	0.018048	0.031765	4.533037	4
151	ST13	F1SRB9	0.805281	0.019229	0.033618	4.607381	3
152	UBC	A0A5G2QDJ2	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	A0A5G2QZ46	0.655284	0.022453	0.037621	3.897957	4
158	PARK7	A0A5G2QIK1	1.127233	0.022515	0.037621	5.159512	2
159	DAG1	A0A287BBR1	-0.98116	0.023326	0.03873	2.410085	2
160	MFGE8	A0A5G2RKJ7	0.599344	0.024807	0.040932	5.037166	11
161	KRT1/4/0	A0A287AEL2;I3LDS3	0.900781	0.025025	0.041035	5.18532	3
162	STXBP2	A0A5G2QA44	0.729406	0.026892	0.043824	4.857438	11
163	EEF1A1	A0A288CG57	0.704532	0.030952	0.050131	5.317222	10
164	KRT5	A0A287AZL3	0.728007	0.03257	0.052429	3.506331	3
166	APOA4	A0A287BM29	-0.58592	0.033323	0.052996	3.986276	7
165	SNAP23	A0A287B2U6	0.707816	0.033308	0.052996	4.028723	5
167	HSPA/1B/6	Q6S4N2;Q04967	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	A0A5G2Q7F8	-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//	A0A480KNV0;A0A5G2R2X7	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

180	TMBIM1	A0A5G2R7N2	0.766063	0.053391	0.078306	5.668077	2
181	RAC1	A0A287B4G4	0.677151	0.056982	0.083112	4.270526	3
182	SDCBP	A0A286ZKH9	0.53583	0.057867	0.08394	3.258615	11
184	TF	A0A5G2R5N9	-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	A0A286ZS27	0.528704	0.071347	0.101266	3.908768	4
187	SERINC5	F1RF26	0.568497	0.072462	0.102299	5.354037	2
188	ACLY	F1S0N2	0.471032	0.075551	0.106093	2.983594	4
189	CLDN3	A0A5G2QJG3	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	A0A5G2R6S0;A0A287B4G4	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	A0A287BN36	0.544742	0.104433	0.142115	2.520809	4
195	CD9	Q8WMQ3	0.907597	0.105237	0.142474	5.56161	4
196	FGB	A0A5G2RHA4	-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	A0A480KNV0	-0.65475	0.12915	0.171335	5.54745	2
201	PFN1	A0A5G2R9A9	0.456534	0.136242	0.178945	3.252424	4
200	ACT/A1/B/G1	A0A5S6G831;Q6QAQ1;I3LVD5	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	A0A075B7J0	-0.65449	0.15291	0.196919	2.635656	2
206	TSPAN1	F1S3V5	0.709392	0.160755	0.206016	4.832822	2
207	PIGR	A0A287B644	-0.41692	0.16249	0.207233	3.942603	21
208	CD109	A0A5G2QPT1	-0.58239	0.163454	0.207461	3.34873	2
209	VAMP3	A0A5G2R1F8	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;Q6S4N2;Q04967;A5A8V7	0.506597	0.185274	0.231812	4.239478	3

212	VPS4/A/B	I3LL27;A0A287BA12	-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;I3LVD5	0.320812	0.249445	0.303817	3.531901	9
217	ANXA11	F1S2E2	0.353845	0.249728	0.303817	4.544183	9
218	LY6H	A0A286ZQV1	0.47347	0.264379	0.320166	5.135652	2
219	ARHGDIA	A0A287AQC2	0.289857	0.286778	0.345705	4.198994	8
224	FGA	F1RX36	-0.47319	0.297037	0.350079	3.361582	9
221	IST1	A0A287A426	-0.41877	0.295651	0.350079	4.034732	8
223	CNP	A0A287A9L8	0.296639	0.296957	0.350079	4.197055	4
220	ACTB	Q6QAQ1	0.355729	0.295004	0.350079	3.635767	2
222	PDCD6	A0A287AKU6	0.436159	0.296417	0.350079	3.468693	4
225	GNB/1/2	A0A287A7Q3;F2Z4Z8	0.36053	0.320789	0.375805	4.30146	5
226	IFITM/1/3	A0A5G2QWL4;A0A287B7R8	0.440999	0.322589	0.375805	4.615226	4
227	CHMP5	F1SE87	0.567977	0.323135	0.375805	4.236682	2
228	ARRDC1	A0A286ZJR9	0.246068	0.327908	0.379253	3.880001	4
229	CLIC1	A0A287B2P1	0.338684	0.328973	0.379253	4.235843	9
230	Gene NA - Prt: A0A5G2QN35	A0A5G2QN35	0.36409	0.356269	0.408935	3.774462	4
232	C3//	F1SBS4;P01025	0.228073	0.363336	0.413451	3.897809	18
231	ANXA2	P19620	0.250061	0.36314	0.413451	4.321955	12
233	GNA13	A0A287BC83	-0.1966	0.383955	0.435039	3.59591	5
234	VPS4B	A0A287BA12	-0.33771	0.399504	0.450722	4.101608	8
236	14-3-3zeta	A0A480PLY3	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	A0A5G2QCL1	0.194068	0.470999	0.520266	3.140668	6
241	CFL1	P10668	0.247084	0.499054	0.546682	4.136279	6
240	PGAM1	A0A287AJQ2	0.261435	0.497482	0.546682	2.992217	2
242	RAB5A	Q06AU6	0.256079	0.511286	0.557766	2.725639	3
243	PZP	A0A287BDU7	-0.16932	0.521545	0.566616	2.407524	5
244	GAPDH	P00355	0.230859	0.544144	0.588746	3.678831	4

245	RAP1/B/A	A0A286ZVJ2;F2Z5K3	0.167147	0.590438	0.636227	4.35698	9
246	ACSL6	A0A5G2Q912	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;I3LV17	-0.2914	0.676877	0.720547	3.104039	4
249	CHMP4B	F1S4Z2	-0.13443	0.70163	0.743897	4.979629	2
250	LOC100739163	A0A287BQ81	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	A0A5G2QSR7	0.105652	0.743008	0.775312	4.007048	4
254	MRAS	A0A5G2QJB5	0.06939	0.762215	0.792223	3.061752	3
255	EPS8L2	A0A286ZVR9	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	A0A287AAM4	-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	A0A5G2Q8I9	-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//	A0A5G2QCX9;A0A287AKU6	-1.67925			3.582214	2
266	FLOT2	A0A480TGV2	-1.03469			4.538231	4
267	SLC3A2	A0A5G2RCQ0	-0.95781			3.103297	3
268	Gene NA - Prt: A0A286ZIM1	A0A286ZIM1	-0.82395			3.68489	2
269	HSPA1B	Q6S4N2	-0.69848			4.982824	2
270	RAB5A;CDK2;RAB5C	Q06AU6;A0A286ZS27;A0A287BN36	-0.54691			4.325362	2
271	RALB	I3LV17	-0.48541			4.330499	2
272	SAR1A	A0A5S6G7K0	-0.32045			3.514933	2
273	SEMA7A//	I3LC80;I3LQF4	-0.31968			3.204831	7
274	Gene NA - Prt: A0A5G2QXC1	A0A5G2QXC1	-0.2875			4.693061	2
275	BSG	A0A286ZS77	-0.16315			4.53726	2
276	COL18A1	I3LH70	-0.10653			4.222196	4

277	RAB27B	A0A286ZTH5	-0.02048	3.929521	4
278	SOD1	P04178	-0.01406	4.882674	3
279	TMEM19	A0A287A4W6	0.065255	3.568603	2
280	PTGFRN	F1SAY0	0.082311	3.232651	2
281	PTPRJ	A0A287AWI4	0.084169	3.254381	2
282	PEBP1	F1RKG8	0.092333	4.257097	4
283	YKT6	F1SSG1	0.153408	4.286261	3
284	SLA-2	A0A287AQG3	0.171871	4.682111	2
285	LDHB	A0A5G2QHL7	0.226899	4.244291	2
286	LBP	A0A287B028	0.240003	3.437749	4
287	SCAMP2	A0A5G2R660	0.269796	3.410068	2
288	CDC42//	Q007T2;F2Z5W2	0.293609	3.629216	4
289	ACTA1	A0A5S6G831	0.350123	2.871411	3
290	NIT2	A0A5G2RGN4	0.38321	4.490943	2
291	NAPA	F1RLB5	0.399539	4.338879	3
292	VPS28	K7GM88	0.405172	3.844972	4
293	SLA-2//	A0A287AEH7;A0A287AQG3	0.406926	3.42832	3
294	SLC44A2	A0A5G2QTD4	0.415279	4.523337	6
295	ANXA4	A0A287AYJ2	0.559065	3.656262	2
296	PRSS8	A0A5G2QSK0	0.569814	3.307351	3
297	SCARB1	Q8SQC1	0.570655	3.984991	3
298	EHD4	I3LDA5	0.584663	3.530621	3
299	NCSTN	A0A286ZK33	0.609611	4.984004	3
300	PGD	A0A5G2QR34	0.656442	4.105394	2
301	CYSTM1	A0A287AUM3	0.672297	4.740443	2
302	TXN	P82460	0.70453	2.914253	3
303	AK1	A0A286ZQ79	0.732717	5.301423	2
304	NME2	Q2EN76	0.740228	4.570478	4
305	ANXA5	F2Z5C1	0.790246	3.397106	2
306	HSD17B7	A0A5G2QGH2	0.795076	3.386419	2
307	VPS25	A0A287AAM6	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	A0A5G2QKI1	-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	A0A5S8KLN1	-1.63238	1.89E-07	2.16E-06	3.847397	27
24	Gene NA - Prt: A0A5G2QXT5	A0A5G2QXT5	-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: A0A287ALJ6	A0A287ALJ6	-1.54779	2.89E-07	2.88E-06	4.016404	4
27	CP	A0A5G2QW05	-1.91726	2.95E-07	2.88E-06	3.659874	11
28	Gene NA - Prt: A0A287ATT2	A0A287ATT2	-2.51536	3.48E-07	3.04E-06	3.373407	3
29	CHRDL2//	A0A5G2QXF8;A0A5G2RH74	-1.92443	3.55E-07	3.04E-06	3.581001	8
30	ACSL3	A0A287B8R8	1.680737	3.64E-07	3.04E-06	4.195918	41
31	Gene NA - Prt: A0A287AP28;A0A286ZIM1;A0A287BAB3	A0A287AP28;A0A286ZIM1;A0A287BAB3	-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: A0A287ALC1	A0A287ALC1	-1.70228	4.17E-07	3.24E-06	3.088551	68
35	PGLYRP1	A0A286ZI97	-2.04348	4.44E-07	3.34E-06	3.716147	14
36	SERPINA3-2	A0A287B9R5	-2.13211	4.55E-07	3.34E-06	4.440938	5
37	JCHAIN	A0A287BQC8	-2.2442	4.93E-07	3.52E-06	4.707291	2
38	SERPINC1	A0A5G2QUE0	-1.65629	5.55E-07	3.86E-06	3.234506	9
39	SMPDL3B//	A0A287BJW1;F1STN0	1.845312	5.78E-07	3.87E-06	3.57818	10
40	MUC4	A0A287B5M2	1.799223	5.87E-07	3.87E-06	3.497925	23
41	ATP6AP2	A0A287AJK1	-1.7957	6.11E-07	3.93E-06	3.929251	5
42	Gene NA - Prt: A0A075B7I6	A0A075B7I6	-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	A0A287A6P1	-1.39196	1.2E-06	7.2E-06	3.579003	19
45	SERPINF1	A0A287AYJ8	-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: A0A287A4Y3	A0A287A4Y3	-1.67471	1.58E-06	8.9E-06	4.354942	13
48	ITIH2	A0A5G2QEV5	-1.61108	1.87E-06	1.03E-05	3.574539	7
49	ALB	A0A287BAY9	-1.41839	2.01E-06	1.08E-05	3.830738	18
50	NA;LOC100523213	A0A0A0MY58;F1RL06	-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	A0A286ZKG9	-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: A0A5S6G633	A0A5S6G633	-2.59191	4.46E-06	2.18E-05	2.838299	3
55	TTR	A0A5G2QIE9	-1.56841	4.9E-06	2.35E-05	3.478735	3
56	MYOF	A0A5G2Q8T2	1.27693	5.54E-06	2.61E-05	3.989779	30
57	LTF	A0A5G2QUQ4	-1.46424	7.29E-06	3.38E-05	3.41711	26
58	RAB1A/RAB1B	F2Z5U4;Q06AU7	1.458813	7.68E-06	3.46E-05	3.36718	6
59	NA;CSN1S1	A0A5S6G633;P39035	-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;A0A2C9F376	-1.51269	1.35E-05	5.86E-05	2.792655	68
62	GPRC5C	A0A287ASV2	1.273678	1.45E-05	6.19E-05	4.592204	5
63	RDX;MSN;EZR	P26044;A0A287AK19;F1SB42	1.620933	1.51E-05	6.34E-05	4.397996	10
64	Gene NA - Prt: A0A287B626	A0A287B626	-1.64572	1.64E-05	6.78E-05	3.970228	29
65	LOC100302368	A0A287B5W2	1.238396	1.76E-05	7.16E-05	4.415311	34
66	B4GALT1	A0A287BG16	-1.68319	2.02E-05	8.09E-05	3.320653	20
67	ATP6AP1	A0A5G2RD35	-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	A0A287BH90	-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	A0A5K1U1P1	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;F1SB42	2.334737	4.6E-05	0.000161	4.44116	2

76	FASN	I3LCW1	1.532428	4.64E-05	0.000161	3.770177	4
77	BAIAP2//	A0A287BRR9;F1RZB0	1.501731	6.07E-05	0.000208	4.191112	16
78	NA;FABP3	I3LTW5;O02772	2.808464	6.19E-05	0.000209	6.001107	2
79	CIDEA	A0A5G2R9X9	1.436374	6.3E-05	0.000211	4.788696	3
80	FGB	A0A5G2RHA4	-1.98563	6.39E-05	0.000211	2.991589	7
81	TTYH2	A0A287AAJ2	1.251632	6.53E-05	0.000213	5.144515	8
82	HSPA5	A0A287BIL8	-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	A0A5G2R5N9	-1.54338	9.1E-05	0.000283	4.641461	2
86	MATN4	F1SDQ7	-1.69833	0.0001	0.000308	2.441729	2
87	SLC9A3R1	A0A5G2R543	1.495403	0.000108	0.000326	4.747528	16
88	ARF/3/4	A0A287B8I6;A0A5G2R3T3	1.139308	0.000134	0.000403	3.238186	4
89	Gene NA - Prt: A0A075B7H9	A0A075B7H9	-1.15795	0.000159	0.00047	3.212066	4
90	Gene NA - Prt: A0A5G2R9S9;A0A287ALJ6	A0A5G2R9S9;A0A287ALJ6	-1.74858	0.00016	0.00047	3.153838	2
91	COL4A2	A0A5G2RJ53	-1.55004	0.000163	0.000472	3.017329	3
92	ANXA6	A0A5G2RBI3	1.178973	0.000167	0.000479	4.400298	6
93	SOD3	I3LUD1	-1.19672	0.00018	0.00051	3.206342	2
94	RAB3D	A0A287BLN4	1.110317	0.000187	0.000525	3.960266	4
95	PPIA	P62936	1.054179	0.000233	0.000646	4.659886	14
96	SERPIND1	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	A0A5S6HQ91	-1.17835	0.000432	0.001119	3.980301	12
103	FGA	F1RX36	-1.74174	0.000476	0.001219	3.361582	9
104	TM4SF1	A0A5G2QC13	-1.20096	0.00051	0.001294	3.680237	2
105	DAG1	A0A287BBR1	-1.21693	0.000533	0.001341	2.410085	2

106	SPON2	A0A481AED0	-1.51264	0.000547	0.001363	3.364732	4
107	RAB11A	A0A5G2QE74	1.376893	0.000748	0.001844	4.928738	6
108	MUC1//	A0A5G2QGF8;F1RGR9	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	A0A5G2R6S0	1.163842	0.000968	0.002281	4.062857	2
113	CKB	A0A5G2R6X7	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	A0A287BFE6	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	A0A286ZZM9	-0.67377	0.002732	0.005771	3.833016	4
126	MSN	A0A287AK19	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	A0A5G2R9D6	0.793333	0.002945	0.006027	3.401712	6
130	KRT5	A0A287AZL3	1.016064	0.002989	0.006069	3.506331	3
131	HSPA/1B/6	Q6S4N2;Q04967	0.875539	0.003294	0.006639	5.257768	3
132	PPIC	A0A5G2RB35	-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	SELENBP1	F1ST01	0.749338	0.004023	0.007868	3.456086	2
136	CD82	A0A5G2QKD8	0.706357	0.004197	0.008146	4.252814	8
137	PDCD6IP	A0A287A5B4	1.016596	0.005578	0.010749	4.405182	30
138	UBC	A0A5G2QDJ2	0.756449	0.005741	0.010982	4.733632	12
139	Gene NA - Prt: A0A287A7S8	A0A287A7S8	-0.77892	0.006085	0.011557	3.275506	2
140	RRAS	F1RHW4	1.027646	0.006377	0.012026	4.486447	3

141	DNAJC3	I3LBK9	-1.2679	0.006537	0.01224	3.527757	2
142	SAA3	A0A480KNV0	-1.21728	0.006826	0.012671	5.54745	2
143	RHOF	Q06AT7	1.111703	0.006863	0.012671	5.064607	2
144	PARK7	A0A5G2QIK1	1.349727	0.007231	0.013257	5.159512	2
145	FLOT1	Q767L6	-0.86194	0.007314	0.013316	3.905262	6
146	RAB7A	A0A286ZP69	0.792978	0.007608	0.013756	4.306068	9
147	ARF3	A0A287B8I6	0.980421	0.009113	0.016367	4.867116	4
148	ATP2B2	A0A286ZSF2	0.649128	0.009257	0.016485	4.769486	7
149	GALM	A0A5G2QZ46	0.756429	0.009304	0.016485	3.897957	4
150	Gene NA - Prt: A0A5G2Q7F8	A0A5G2Q7F8	-1.32455	0.009492	0.016706	2.486988	2
151	PIGR	A0A287B644	-0.7977	0.010154	0.017753	3.942603	21
152	RAB2A/RAB2B	F1RT87;F1S8J6	0.836642	0.011173	0.019406	3.736955	3
153	MFGE8	A0A5G2RKJ7	0.684264	0.011314	0.019522	5.037166	11
154	GDI2	A0A5G2QSK4	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;P01025	-0.62601	0.016605	0.027745	3.897809	18
159	MFGE8//	A0A5G2RKJ7;A0A5G2QXS8	0.664273	0.016896	0.028054	4.588095	105
160	14-3-3zeta;YWHAH;YWHAQ;CRK;YWHAB	A0A480PLY3;F2Z4Y1;A0A5G2QM17;A0A5G2QSR7;A0A287BPX7	0.979989	0.018858	0.031116	5.657113	2
161	MDH1	A0A5G2QFC2	0.606665	0.019589	0.03212	4.533037	4
162	RDX	P26044	0.692097	0.021007	0.034234	3.883106	5
163	PIGR//	A0A287B644;Q29244	-0.79823	0.021531	0.034873	3.790705	5
164	Gene NA - Prt: A0A5G2QN35	A0A5G2QN35	0.932155	0.022708	0.036554	3.774462	4
165	KRT1/4/0	A0A287AEL2;I3LDS3	0.913294	0.023227	0.037163	5.18532	3
166	ARHGAP18	A0A287BHC4	0.63425	0.024701	0.039284	4.917479	4
167	GPRC5B	A0A480W2U9	0.679624	0.025105	0.039686	5.240895	5

168	IFITM1;IFITM3;LOC102161418	A0A5G2QWL4;A0A287B7R8;F1RGC5	0.638931	0.026623	0.041836	3.46739	4
169	Gene NA - Prt: A0A075B7J0	A0A075B7J0	-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	A0A5G2QPT1	-0.87241	0.033406	0.051274	3.34873	2
173	SDCBP	A0A286ZKH9	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	A0A288CG57	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	A0A287AP77	0.882353	0.047407	0.06953	4.637288	4
181	SAA3//	A0A480KNV0;A0A5G2R2X7	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	A0A287B2P1	0.691502	0.051546	0.073646	4.235843	9
185	APOA4	A0A287BM29	-0.53258	0.051608	0.073646	3.986276	7
186	HSPA8	F1S9Q3	0.517331	0.052357	0.074313	3.712596	22
187	ARRDC1	A0A286ZJR9	0.489955	0.056722	0.080078	3.880001	4
188	Gene NA - Prt: A0A5G2R9S9	A0A5G2R9S9	-0.77875	0.060096	0.08439	3.738216	2
189	CD209	B3FVQ2	0.540163	0.060622	0.084423	4.766564	2
190	VAMP3	A0A5G2R1F8	0.44183	0.060759	0.084423	2.71867	2
191	FGG	F1RX35	-0.52766	0.06513	0.090023	4.145301	8
192	GNB/1/2	A0A287A7Q3;F2Z4Z8	0.681641	0.065727	0.090375	4.30146	5
193	SERINC5	F1RF26	-0.57897	0.067614	0.092487	5.354037	2
194	LY6H	A0A286ZQV1	-0.78056	0.070385	0.095782	5.135652	2
195	PFN1	A0A5G2R9A9	0.538192	0.072088	0.097596	3.252424	4
196	TACSTD1	Q75QW1	1.038773	0.075865	0.102185	4.620366	3
197	ACLY	F1S0N2	0.468321	0.086003	0.114861	2.983594	4
198	VAT1	I3L9V2	0.449533	0.086146	0.114861	2.022158	7
199	SLC29A1	A0A287B8K5	0.632019	0.087064	0.115502	4.707259	6

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

232	RAC1	A0A287B4G4	0.266834	0.441853	0.502798	4.270526	3
233	ARHGDIA	A0A287AQC2	0.205567	0.447901	0.507493	4.198994	8
234	EPS8	I3LP99	-0.16508	0.480983	0.54177	3.694047	9
235	CLDN3	A0A5G2QJG3	0.255039	0.482257	0.54177	4.344975	3
236	C3	F1SBS4	-0.19344	0.488864	0.546272	3.724701	4
237	C4A	A0A5G2QCL1	0.185605	0.490403	0.546272	3.140668	6
238	CRK	A0A5G2QSR7	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	A0A287BC83	-0.12974	0.564231	0.615525	3.59591	5
243	GAPDH	P00355	0.209409	0.581982	0.632276	3.678831	4
244	CEACAM1	I3L9E5	-0.19155	0.595451	0.644258	4.408131	7
245	PGAM1	A0A287AJQ2	-0.19012	0.604974	0.65189	2.992217	2
246	EPS8L2	A0A286ZVR9	-0.12867	0.631436	0.677639	3.676723	6
247	RAP1A	F2Z5K3	-0.13105	0.662644	0.706351	3.470279	3
248	PDCD6	A0A287AKU6	0.171198	0.663542	0.706351	3.468693	4
249	CHMP5	F1SE87	0.226292	0.682243	0.723341	4.236682	2
250	MRAS	A0A5G2QJB5	0.089784	0.686107	0.724529	3.061752	3
251	RAP1/B/A	A0A286ZVJ2;F2Z5K3	0.119871	0.699187	0.7354	4.35698	9
252	CNP	A0A287A9L8	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	LOC100739163	A0A287BQ81	-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;I3LV17	0.102452	0.879553	0.897575	3.104039	4
259	SNAP23	A0A287B2U6	-0.04814	0.880575	0.897575	4.028723	5
260	ACTG1	I3LVD5	-0.0477	0.925074	0.937761	3.067914	2
261	BROX	A0A287AAM4	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	A0A5G2Q8I9	0.00899	0.977245	0.977245	2.536434	9
265	CHRDL2	A0A5G2RH74	-2.91355			3.439313	2
266	OLFML3	H6UWK6	-2.37574			3.489077	2

267	TGFBR3	A0A287BPI7	-2.35933	3.625966	2
268	CHI3L1	A0A287BKG2	-2.09004	3.343129	2
269	FCN2	A0A5K1UZA2	-1.57296	4.034965	2
270	FLOT2	A0A480TGV2	-1.34148	4.538231	4
271	PDCD6//	A0A5G2QCX9;A0A287AKU6	-1.18419	3.582214	2
272	Gene NA - Prt: A0A286ZIM1	A0A286ZIM1	-0.92992	3.68489	2
273	COL18A1	I3LH70	-0.78059	4.222196	4
274	SERPINA1	P50447	-0.50064	2.66335	2
275	TMEM19	A0A287A4W6	-0.41074	3.568603	2
276	LDHB	A0A5G2QHL7	-0.38671	4.244291	2
277	LBP	A0A287B028	-0.38113	3.437749	4
278	RAB5A;CDK2;RAB5C	Q06AU6;A0A286ZS27;A0A287BN36	-0.21396	4.325362	2
279	PTGFRN	F1SAY0	-0.20516	3.232651	2
280	SLC3A2	A0A5G2RCQ0	-0.11464	3.103297	3
281	Gene NA - Prt: A0A5G2QXC1	A0A5G2QXC1	-0.06124	4.693061	2
282	PRSS8	A0A5G2QSK0	-0.02916	3.307351	3
283	SLA-2	A0A287AQG3	-0.02578	4.682111	2
284	CSN2	A0A2C9F376	0.028999	1.685854	3
285	VPS28	K7GM88	0.106097	3.844972	4
286	PTPRJ	A0A287AWI4	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	A0A5G2QR34	0.28659	4.105394	2
291	ACTA1	A0A5S6G831	0.336615	2.871411	3
292	SEMA7A//	I3LC80;I3LQF4	0.357643	3.204831	7
293	NCSTN	A0A286ZK33	0.391173	4.984004	3
294	GNB2	F2Z4Z8	0.401695	2.838204	2
295	SCAMP2	A0A5G2R660	0.473635	3.410068	2

296	HSD17B7	A0A5G2QGH2	0.490597		3.386419	2
297	TXN	P82460	0.559431		2.914253	3
298	SAR1A	A0A5S6G7K0	0.559527		3.514933	2
299	SOD1	P04178	0.570862		4.882674	3
300	ANXA5	F2Z5C1	0.627685		3.397106	2
301	SLA-2//	A0A287AEH7;A0A287AQG3	0.661826		3.42832	3
302	NIT2	A0A5G2RGN4	0.703802		4.490943	2
303	RAB27B	A0A286ZTH5	0.743441		3.929521	4
304	PEBP1	F1RKG8	0.747405		4.257097	4
305	BSG	A0A286ZS77	0.869228		4.53726	2
306	ANXA4	A0A287AYJ2	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	A0A287AUM3	1.072653		4.740443	2
312	CAB39	F1SMX5	1.280263		3.965521	3
313	SLC44A2	A0A5G2QTD4	1.315642		4.523337	6
314	NME2	Q2EN76	1.332111		4.570478	4
315	CDC42//	Q007T2;F2Z5W2	1.419767		3.629216	4
316	VPS25	A0A287AAM6	1.432011		4.546764	4
317	AK1	A0A286ZQ79	1.446713		5.301423	2
318	KRT14	A0A287AEL2	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;P01025	-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	A0A287AP28;A0A286ZIM1;A	-1.62721	0.004186	0.183708	3.773167	3

	P28;A0A286ZIM1;A0A287BAB3	0A287BAB3					
7	LY6H	A0A286ZQV1	-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	A0A480W2U9	-0.84345	0.00707	0.186635	5.240895	5
11	FGB	A0A5G2RHA4	-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: A0A075B7I9;A0A075B7H9;A0A075B7J0;A0A075B7I5	A0A075B7I9;A0A075B7H9;A0A075B7J0;A0A075B7I5	-0.98641	0.011999	0.226258	5.663481	4
15	SERPINA3-2	A0A287B9R5	-0.86541	0.016147	0.243795	4.440938	5
16	TF	A0A5G2R5N9	-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: A0A0A0MY58	A0A0A0MY58	-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	A0A287B2U6	-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	A0A287BQC8	-0.8104	0.030111	0.30159	4.707291	2
26	LOC100302368	A0A287B5W2	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: A0A287BAB3	A0A287BAB3	-0.73963	0.031987	0.30159	4.473881	4
29	BAIAP2	A0A287BRR9	-0.7286	0.033981	0.309347	5.518362	2
30	PLIN3	K4P7U5	-0.67787	0.038074	0.335047	4.318163	12
31	MGP	A0A5G2QKI1	-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: A0A287ALJ6	A0A287ALJ6	-0.47086	0.059593	0.476747	4.016404	4

34	SLC6A14	K7GNX7	-0.58729	0.068686	0.5192	4.229918	13
35	HSPA5	A0A287BIL8	0.487404	0.068833	0.5192	2.279501	4
36	Gene NA - Prt: A0A287ALC1	A0A287ALC1	-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: A0A287A4Y3	A0A287A4Y3	-0.49083	0.097258	0.675687	4.354942	13
39	GPI	P08059	0.431011	0.100704	0.681692	4.692694	4
40	ITIH4	A0A286ZN24	0.444867	0.103406	0.682479	3.40089	4
41	ENO1	I3LK59	-0.43548	0.116467	0.749932	4.114588	7
42	SLC29A1	A0A287B8K5	-0.57573	0.122099	0.767481	4.707259	6
43	SMPDL3B //	A0A287BJW1;F1STN0	0.46498	0.129283	0.769757	3.57818	10
44	Gene NA - Prt: A0A5G2R4L0	A0A5G2R4L0	-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: A0A5G2QN35	A0A5G2QN35	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	A0A5G2R305	-0.35531	0.169609	0.879809	3.833653	20
51	ACSL3	A0A287B8R8	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: A0A287B626	A0A287B626	-0.44019	0.188411	0.913404	3.970228	29
55	SAA3	A0A480KNV0	-0.56253	0.195635	0.913404	5.54745	2
56	MUC4	A0A287B5M2	-0.38504	0.195711	0.913404	3.497925	23
57	MYOF	A0A5G2Q8T2	0.311471	0.19725	0.913404	3.989779	30
58	MATN4	F1SDQ7	-0.52398	0.200672	0.913404	2.441729	2
59	PIGR	A0A287B644	-0.38078	0.206452	0.923785	3.942603	21
60	BTN1A1	A0A5K1U1P1	0.359423	0.214349	0.929227	4.268103	61
61	Gene NA - Prt:	A0A075B7H9	-0.35546	0.215045	0.929227	3.212066	4

	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	CHRDL2//	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

92	HSPA/1B/6	Q6S4N2;Q04967	0.256447	0.360055	0.986904	5.257768	3
93	APOA1	K7GM40	-0.27881	0.361472	0.986904	3.357979	30
94	SELENBP1	F1ST01	0.233498	0.362005	0.986904	3.456086	2
95	RAB1A/RAB1B	F2Z5U4;Q06AU7	0.252249	0.367404	0.986904	3.36718	6
96	COL4A2	A0A5G2RJ53	-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	GNB/1/2	A0A287A7Q3;F2Z4Z8	0.321111	0.381693	0.986904	4.30146	5
101	KRT5	A0A287AZL3	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//	A0A5G2RKJ7;A0A5G2QXS8	-0.22314	0.4087	0.986904	4.588095	105
105	CPM	I3LTE1	0.274812	0.414106	0.986904	4.389205	28
106	NA;CSN1S1	A0A5S6G633;P39035	-0.23227	0.416586	0.986904	3.32349	13
107	RAB5C	A0A287BN36	-0.27386	0.418944	0.986904	2.520809	4
108	RAB10	F2Z5F2	-0.38607	0.418944	0.986904	5.116465	3
109	RAB3D	A0A287BLN4	-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: A0A5G2R9S9;A0A287ALJ6	A0A5G2R9S9;A0A287ALJ6	-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	A0A287BDU7	-0.19749	0.460864	0.986904	2.407524	5
118	GSN	A0A287A6P1	-0.17442	0.46299	0.986904	3.579003	19
119	RAB35	A0A287BFE6	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: A0A5G2R9S9	A0A5G2R9S9	0.318045	0.470163	0.986904	3.738216	2
122	SLC9A3R1	A0A5G2R543	-0.24681	0.475251	0.986904	4.747528	16

123	IFITM1;IFITM3;LOC102161418	A0A5G2QWL4;A0A287B7R8;F1RGC5	-0.19401	0.49043	0.986904	3.46739	4
124	EPS8L2	A0A286ZVR9	-0.20208	0.491362	0.986904	3.676723	6
125	CD109	A0A5G2QPT1	-0.29003	0.498039	0.986904	3.34873	2
126	CIDEA	A0A5G2R9X9	0.215224	0.499105	0.986904	4.788696	3
127	NA;FABP3	I3LTW5;O02772	-0.37807	0.499374	0.986904	6.001107	2
128	Gene NA - Prt: A0A287ATT2	A0A287ATT2	-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: A0A5S6G633	A0A5S6G633	-0.34578	0.508873	0.986904	2.838299	3
131	CNP	A0A287A9L8	-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	A0A287B5Q1	-0.22731	0.519991	0.986904	3.6693	10
135	SERPINC1	A0A5G2QUE0	-0.17341	0.52111	0.986904	3.234506	9
136	Gene NA - Prt: A0A5G2Q7F8	A0A5G2Q7F8	-0.32125	0.52303	0.986904	2.486988	2
137	CHMP2A	I3LSS2	0.230866	0.52624	0.986904	4.856903	8
138	B4GALT1	A0A287BG16	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: A0A075B7J0	A0A075B7J0	-0.28677	0.536241	0.986904	2.635656	2
141	PDCD6	A0A287AKU6	-0.26496	0.538537	0.986904	3.468693	4
142	ALB	A0A287BAY9	-0.14908	0.549883	0.986904	3.830738	18
143	LOC100739163	A0A287BQ81	-0.18798	0.550449	0.986904	3.889458	4
144	STXBP2	A0A5G2QA44	-0.19178	0.550784	0.986904	4.857438	11
145	CEACAM1	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	A0A287BA12	-0.23441	0.562426	0.986904	4.101608	8
150	DAG1	A0A287BBR1	-0.23576	0.57036	0.986904	2.410085	2

151	SIL1	F1RGI9	-0.1423	0.577473	0.986904	3.792533	8
152	14-3-3zeta	A0A480PLY3	0.134133	0.577748	0.986904	3.095345	6
153	RAL/A/B	I3LAJ6;I3LV17	0.393847	0.578562	0.986904	3.104039	4
154	PPIC	A0A5G2RB35	-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	A0A5G2Q912	0.213451	0.604425	0.986904	3.649641	3
160	PRDX5	A0A5G2R9D6	0.133397	0.606754	0.986904	3.401712	6
161	VAMP3	A0A5G2R1F8	0.116648	0.613428	0.986904	2.71867	2
162	VTN	P48819	-0.15311	0.614153	0.986904	4.276618	7
163	UBC	A0A5G2QDJ2	0.128525	0.622112	0.986904	4.733632	12
164	PPIB	A0A286ZKG9	0.138031	0.624301	0.986904	3.286902	3
165	14-3-3zeta;YWHAH;YWHAQ;CRK;YWHAB	A0A480PLY3;F2Z4Y1;A0A5G2QM17;A0A5G2QSR7;A0A287BPX7	-0.19544	0.628997	0.986904	5.657113	2
166	RAB14	Q52NJ6	-0.17898	0.629068	0.986904	4.846186	2
167	SERPINF1	A0A287AYJ8	-0.12378	0.630657	0.986904	3.303514	10
168	ATP2B2	A0A286ZSF2	-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;A0A287AK19;F1SB42	0.151534	0.639712	0.986904	4.397996	10
171	PARK7	A0A5G2QIK1	0.222495	0.64304	0.986904	5.159512	2
172	IFITM/1/3	A0A5G2QWL4;A0A287B7R8	0.205385	0.647115	0.986904	4.615226	4
173	AEBP1	A0A286ZZM9	-0.09442	0.655454	0.986904	3.833016	4
174	RAB2A/RAB2B	F1RT87;F1S8J6	-0.14487	0.656624	0.986904	3.736955	3
175	EZR	F1SB42	0.175992	0.661619	0.986904	3.956502	22
176	TTYH2	A0A287AAJ2	-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	A0A5G2RD35	-0.09797	0.707734	0.986904	3.264964	4
184	EPS8	I3LP99	-0.0869	0.713314	0.986904	3.694047	9
185	GALM	A0A5G2QZ46	0.101145	0.716716	0.986904	3.897957	4
186	CRK	A0A5G2QSR7	0.112784	0.720891	0.986904	4.007048	4
187	Gene NA - Prt: A0A287A7S8	A0A287A7S8	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	A0A286ZP69	0.097438	0.731387	0.986904	4.306068	9
191	ARF/3/4	A0A287B8I6;A0A5G2R3T3	-0.09383	0.732853	0.986904	3.238186	4
192	RHOF	Q06AT7	0.130991	0.738406	0.986904	5.064607	2
193	CDK2	A0A286ZS27	-0.0961	0.739769	0.986904	3.908768	4
194	MFGE8	A0A5G2RKJ7	0.084919	0.7436	0.986904	5.037166	11
195	SLC34A2	F1S5A6	-0.097	0.745641	0.986904	4.131015	66
196	LTF	A0A5G2QUQ4	-0.08931	0.748292	0.986904	3.41711	26
197	ITIH2	A0A5G2QEV5	-0.08935	0.750844	0.986904	3.574539	7
198	PON1	I3L5K0	0.170191	0.757472	0.986904	2.249963	4
199	ARHGDIA	A0A287AQC2	-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;A0A287BA12	-0.13449	0.762713	0.986904	3.75846	4
202	RDX;EZR	P26044;F1SB42	-0.14952	0.766715	0.986904	4.44116	2
203	GNA13	A0A287BC83	0.066855	0.768783	0.986904	3.59591	5
204	TMBIM1	A0A5G2R7N2	-0.10895	0.772328	0.986904	5.668077	2
205	CSN1S2	P39036	-0.08798	0.777238	0.986904	3.715798	39
206	PIGR//	A0A287B644;Q29244	0.094616	0.778801	0.986904	3.790705	5
207	FMOD	F1S6B5	0.120246	0.782244	0.986904	2.122226	5
208	APOE//	F1RM45;P18650	-0.08031	0.788384	0.986904	3.62444	28
209	PFN1	A0A5G2R9A9	0.081658	0.788725	0.986904	3.252424	4
210	BROX	A0A287AAM4	0.086082	0.789293	0.986904	3.516861	8
211	ARHGAP18	A0A287BHC4	-0.06802	0.796874	0.986904	4.917479	4
212	ARF3	A0A287B8I6	-0.09248	0.797272	0.986904	4.867116	4
213	SERPIND1	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	A0A5S6HQ91	0.073	0.811188	0.986904	3.980301	12
217	SPON2	A0A481AED0	0.095761	0.811232	0.986904	3.364732	4
218	PDCD6IP	A0A287A5B4	0.078849	0.821041	0.986904	4.405182	30
219	SDCBP	A0A286ZKH9	0.059495	0.830442	0.986904	3.258615	11
220	CD82	A0A5G2QKD8	0.048096	0.836755	0.986904	4.252814	8
221	IST1	A0A287A426	-0.08221	0.837966	0.986904	4.034732	8
222	VPS4A	I3LL27	-0.0663	0.839029	0.986904	3.815098	4
223	APOA4	A0A287BM29	0.053348	0.842599	0.986904	3.986276	7
224	RHOA	I3LVS7	-0.09222	0.843299	0.986904	5.26511	5
225	LPL	A0A287ATV6	-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	A0A5G2QE74	-0.06665	0.859279	0.986904	4.928738	6
229	ACT/A1/B/G1	A0A5S6G831;Q6QAQ1;I3LVD5	0.056543	0.869891	0.986904	4.611775	18
230	RAC2	A0A5G2R6S0	0.052717	0.87155	0.986904	4.062857	2
231	GDI2	A0A5G2QSK4	0.041712	0.873177	0.986904	3.627553	15
232	RAP1/B/A	A0A286ZVJ2;F2Z5K3	-0.04728	0.880254	0.986904	4.35698	9
233	BAIAP2//	A0A287BRR9;F1RZB0	-0.04921	0.881734	0.986904	4.191112	16
234	A2M	A0A5G2Q8I9	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	A0A288CG57	-0.03866	0.903309	0.986904	5.317222	10
239	MSN	A0A287AK19	0.039705	0.905895	0.986904	4.993424	19
240	CKB	A0A5G2R6X7	-0.02978	0.909807	0.986904	4.131502	7
241	MDH1	A0A5G2QFC2	-0.02895	0.911179	0.986904	4.533037	4
242	PGLYRP1	A0A286ZI97	0.035225	0.914538	0.986904	3.716147	14
243	TTR	A0A5G2QIE9	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	A0A5G2QJB5	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;I3LVD5	0.0181	0.948284	0.994693	3.531901	9
251	GAPDH	P00355	-0.02145	0.955487	0.994693	3.678831	4
252	CP	A0A5G2QW05	-0.01543	0.959131	0.994693	3.659874	11
253	CSN2//	P39037;A0A2C9F376	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	A0A287AEL2;I3LDS3	0.012513	0.974436	0.994693	5.18532	3
257	C4A	A0A5G2QCL1	-0.00846	0.975131	0.994693	3.140668	6
258	STX3	F1RI88	0.008412	0.980674	0.994693	3.589462	9
259	HSPA/8/1B/6/1L	F1S9Q3;Q6S4N2;Q04967;A5A8V7	-0.00892	0.981365	0.994693	4.239478	3
260	CLU	A0A5S8KLN1	0.003552	0.988672	0.994693	3.847397	27
261	ATP6AP2	A0A287AJK1	0.004209	0.988702	0.994693	3.929251	5
262	SAA3//	A0A480KNV0;A0A5G2R2X7	-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	A0A287B028	-0.62114			3.437749	4
267	LDHB	A0A5G2QHL7	-0.61361			4.244291	2
268	PRSS8	A0A5G2QSK0	-0.59897			3.307351	3
269	TMEM19	A0A287A4W6	-0.476			3.568603	2
270	PGD	A0A5G2QR34	-0.36985			4.105394	2
271	IDI1	I3LDY2	-0.32858			2.701298	2
272	FLOT2	A0A480TGV2	-0.30679			4.538231	4
273	HSD17B7	A0A5G2QGH2	-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	A0A286ZK33	-0.21844			4.984004	3
278	SLA-2	A0A287AQG3	-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: A0A286ZIM1	A0A286ZIM1	-0.10597	3.68489	2
285	ACTA1	A0A5S6G831	-0.01351	2.871411	3
286	PTPRJ	A0A287AWI4	0.031323	3.254381	2
287	SCAMP2	A0A5G2R660	0.203839	3.410068	2
288	Gene NA - Prt: A0A5G2QXC1	A0A5G2QXC1	0.226257	4.693061	2
289	SLA-2//	A0A287AEH7;A0A287AQG3	0.2549	3.42832	3
290	ANXA4	A0A287AYJ2	0.3172	3.656262	2
291	NIT2	A0A5G2RGN4	0.320592	4.490943	2
292	RAB5A;CDK2;RAB5C	Q06AU6;A0A286ZS27;A0A287BN36	0.332946	4.325362	2
293	EHD4	I3LDA5	0.338284	3.530621	3
294	CYSTM1	A0A287AUM3	0.400356	4.740443	2
295	PDCD6//	A0A5G2QCX9;A0A287AKU6	0.495063	3.582214	2
296	VPS25	A0A287AAM6	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;I3LQF4	0.677322	3.204831	7
301	AK1	A0A286ZQ79	0.713996	5.301423	2
302	RALB	I3LV17	0.728694	4.330499	2
303	RAB27B	A0A286ZTH5	0.763921	3.929521	4
304	SLC3A2	A0A5G2RCQ0	0.843177	3.103297	3
305	YKT6	F1SSG1	0.871466	4.286261	3
306	SAR1A	A0A5S6G7K0	0.879974	3.514933	2
307	SLC44A2	A0A5G2QTD4	0.900363	4.523337	6
308	BSG	A0A286ZS77	1.032375	4.53726	2
309	CDC42//	Q007T2;F2Z5W2	1.126158	3.629216	4
310	HSPA1B	Q6S4N2	1.731683	4.982824	2

311	KRT14	A0A287AEL2	4.917632	2
312	CHI3L1	A0A287BKG2	3.343129	2
313	TGFBR3	A0A287BPI7	3.625966	2
314	CSN2	A0A2C9F376	1.685854	3
315	CHRDL2	A0A5G2RH74	3.439313	2
316	FCN2	A0A5K1UZA2	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.

GOID	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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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 ($\left| \log_2 FC > 1 \right|$).

Day 0 vs 7

Lipid class	Lipid name	log2(FC)	FDR Ajusted 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

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

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

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;3O	-3.930	0.000	Down
FA	FA 16:0;3O	-3.859	0.000	Down
FA	FA 16:1;3O	-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;4O	-3.355	0.000	Down
FA	FA 16:2;3O	-3.313	0.000	Down
FA	FA 22:0;4O	-3.180	0.000	Down
FA	FA 19:4;1O	-3.073	0.000	Down
FA	FA 17:4;2O	-2.955	0.000	Down
FA	FA 38:5	-2.914	0.000	Down
FA	FA 19:1;2O	-2.864	0.000	Down
FA	FA 20:0;4O	-2.806	0.000	Down
FA	FA 20:3;4O	-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;4O	-2.664	0.000	Down

FA	FA 14:0	-2.610	0.000	Down
FA	FA 28:7	-2.608	0.000	Down
FA	FA 22:6;4O	-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;2O	-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;1O	-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;O	-2.084	0.000	Down
FA	FA 15:0	-1.822	0.000	Down
FA	FA 18:1;2O	-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;2O_18:5	-3.642	0.000	Down
HexCer	HexCer 16:1;3O_17:0;(2OH)	-3.555	0.000	Down
HexCer	HexCer 16:0;2O_24:1	-3.203	0.000	Down
HexCer	HexCer 16:1;3O_26:7;(2OH)	-2.961	0.000	Down
HexCer	HexCer 18:0;2O_18:5	-2.109	0.000	Down
HexCer	HexCer 16:0;2O_30:4;O	-1.874	0.000	Down
HexCer	HexCer 34:0;2O	-1.557	0.000	Down
HexCer	HexCer 34:1;3O_HexCer 18:1;2O_16:0;O	-1.502	0.000	Down
HexCer	HexCer 20:2;2O_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

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

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

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;1O	-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;3O	-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;1O	-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;2O	-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

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

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

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

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

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

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 ($\left|\log_2 FC > 1\right|$).

Day 0 vs 14				
Lipid class	**Lipid name**	**log2(FC)**	**FDR Ajusted 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

DG	DG 41:6_DG 15:0_26:6	-8.148	0.000	Down
SM	SM 41:0;2O	-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;4O	-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;3O	-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;4O	-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;4O	-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;5O_Cer 21:2;3O_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;2O_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;4O	-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

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

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;4O	-6.519	0.000	Down
CAR	CAR 19:3	-6.517	0.000	Down
Cer	Cer 81:9;4O	-6.516	0.022	Down
Cer	Cer 36:4;4O	-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;4O	-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

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

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

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

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

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

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

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

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

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

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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