Characterisation of milk small extracellular vesicles to study adaptation to lactation in ruminants

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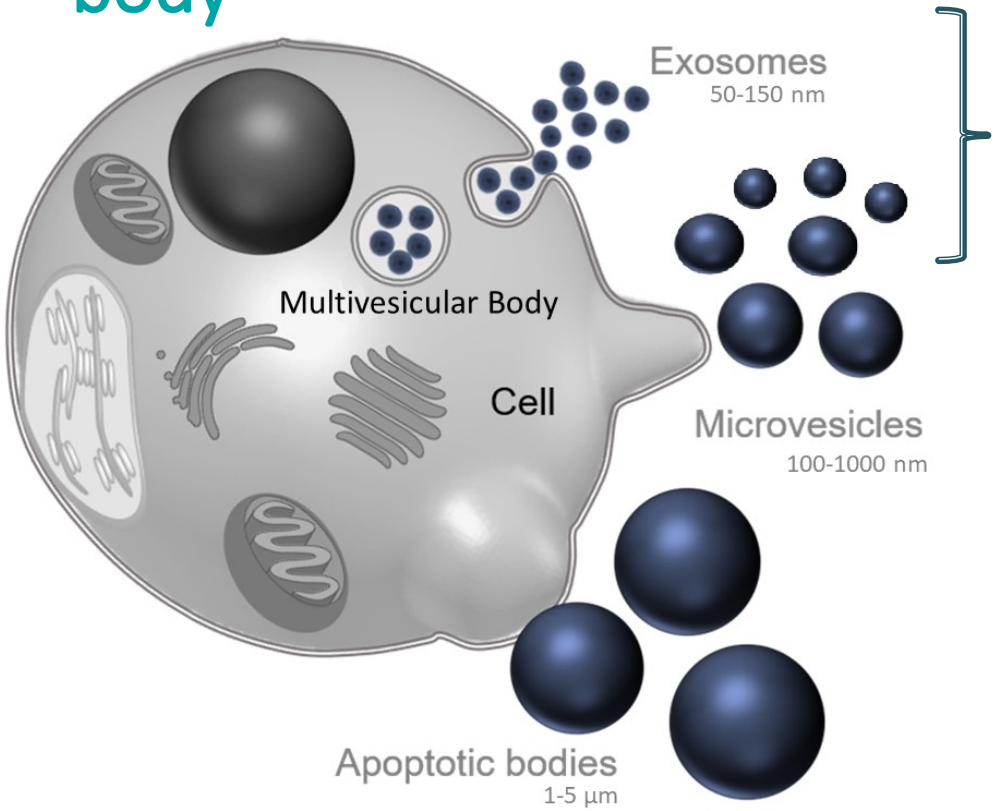
Small extracellular vesicles (EVs) are secreted by all tissues in the body



small Extracellular Vesicles

50-200 nm

adapted from everzom.com/exosomes

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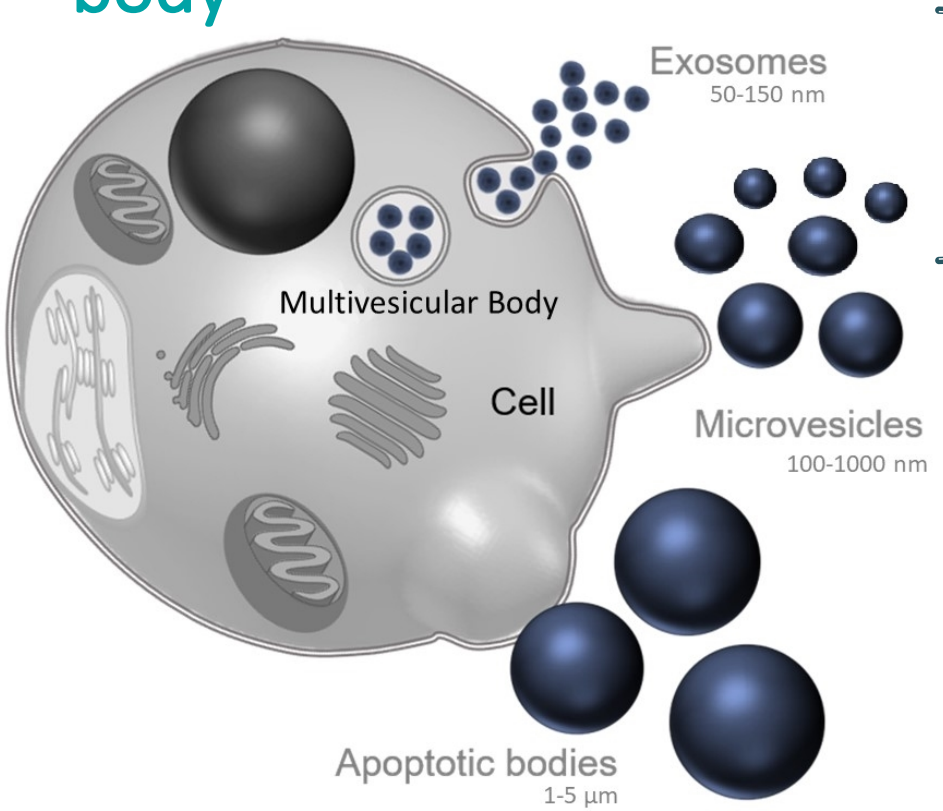

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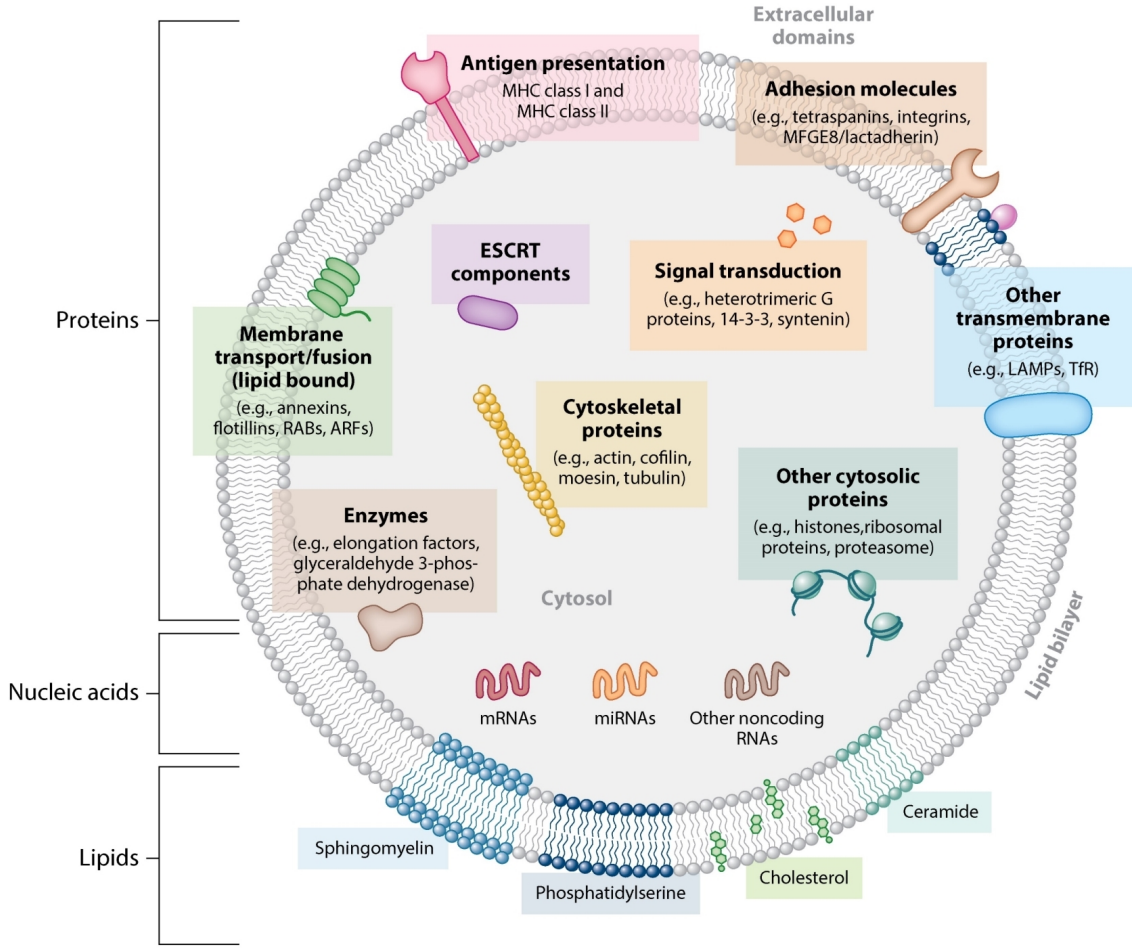
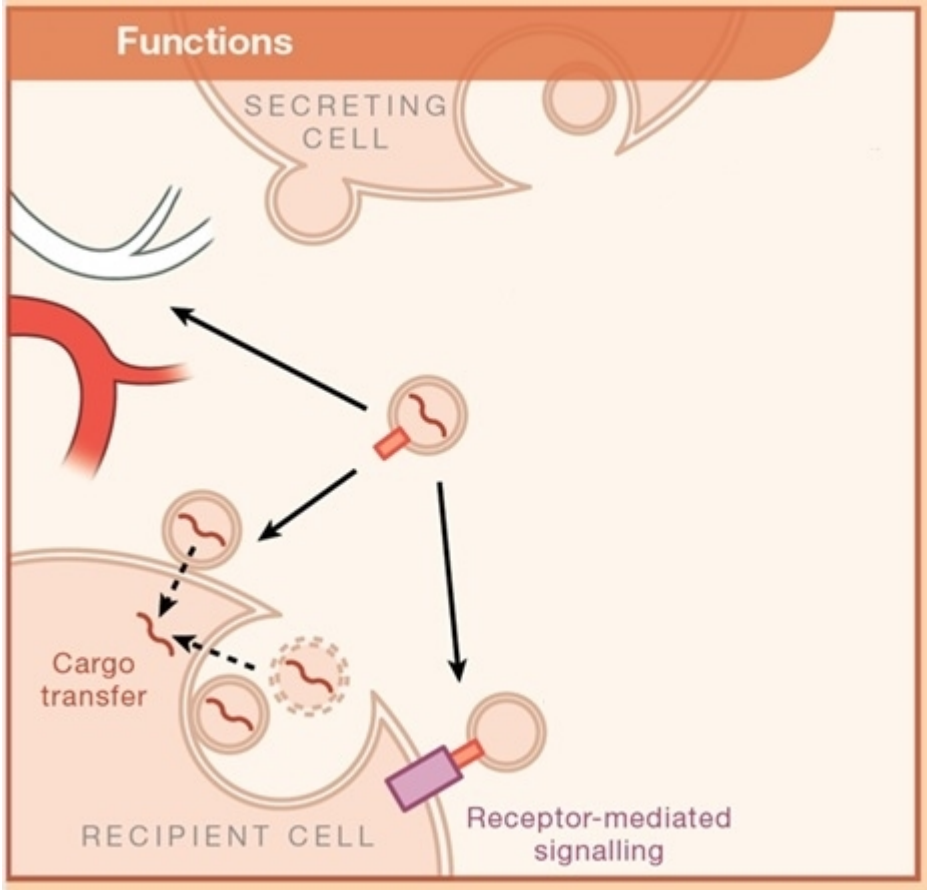


Colombo M, et al. 2014.
Annu. Rev. Cell Dev. Biol. 30:255–89

Small EVs conveyed tissular molecules from secreting cells to recipient cells



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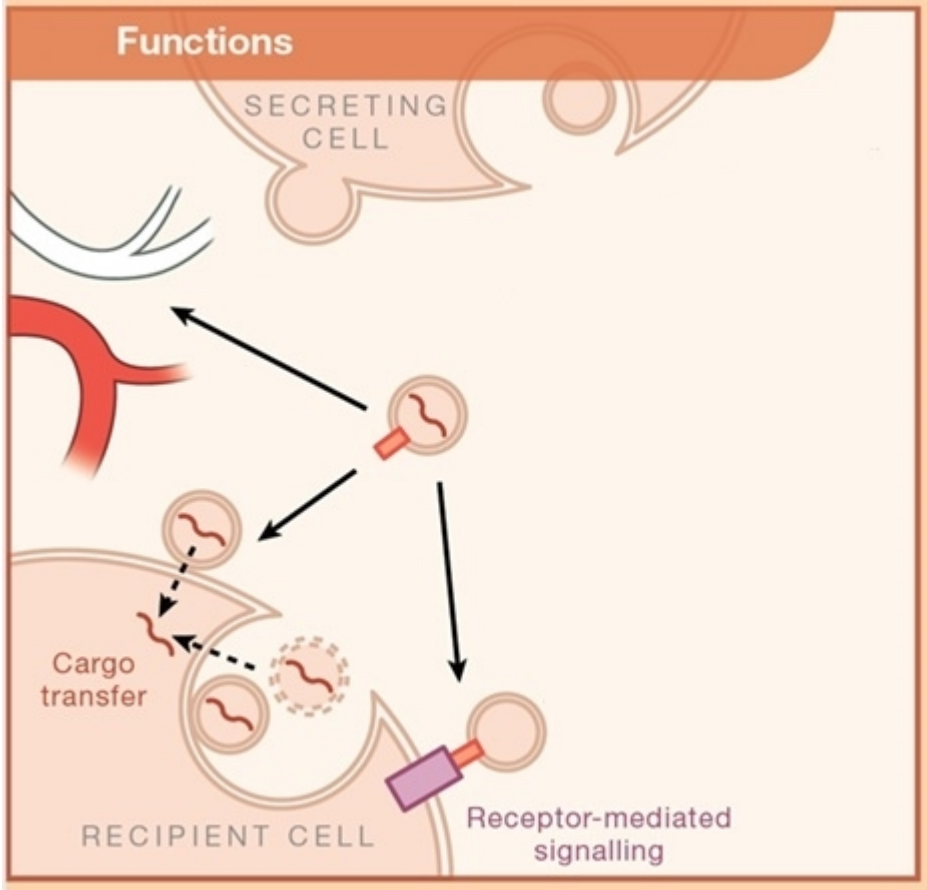

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Circulation into biological fluids (blood, milk…)

→ **Mediators of long-distance communication:** immune response, angiogenesis, signaling and transport

→ **Source of circulating biomarkers**

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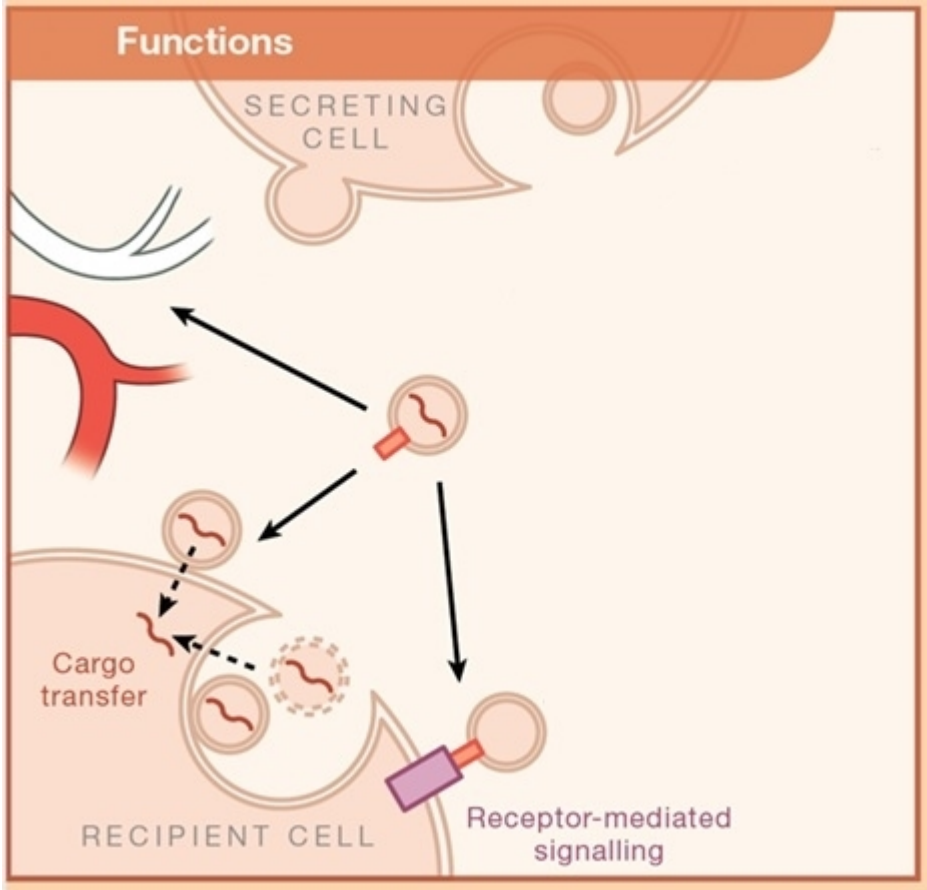

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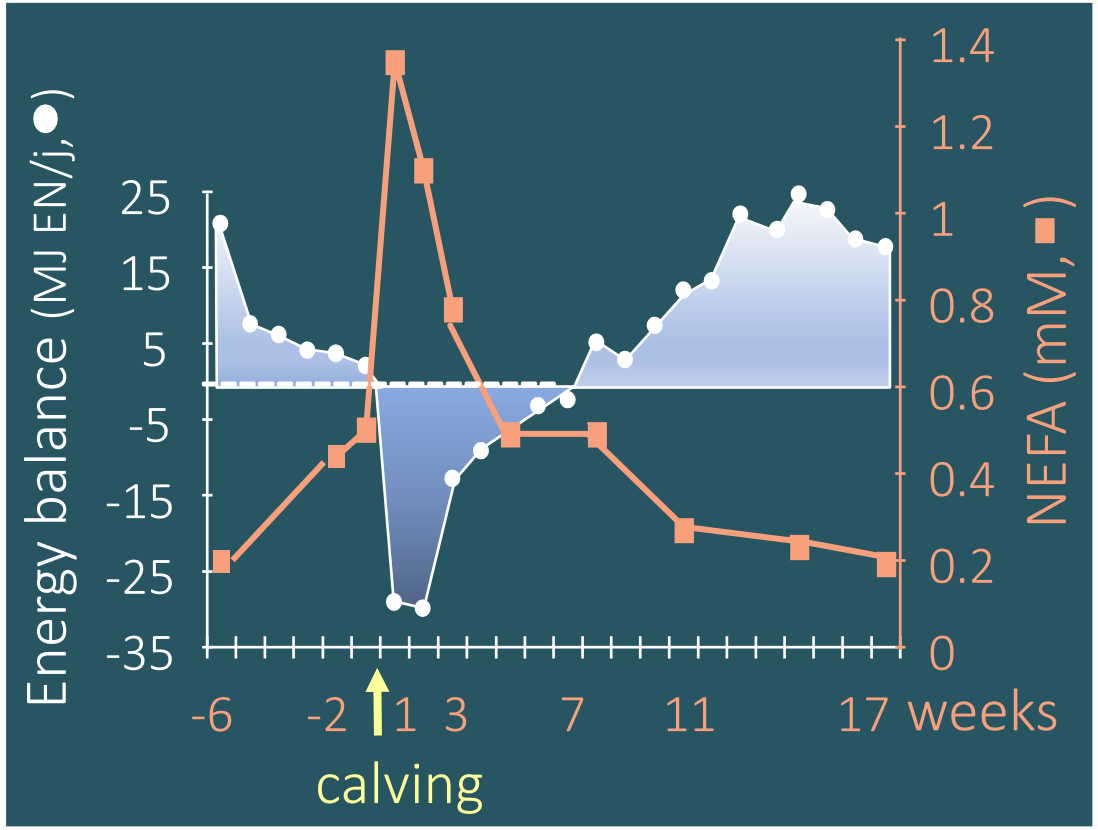
→ **Source of circulating biomarkers**

Milk small EVs: non invasive sources of biomarkers in dairy cows (mastitis, milk yield , heat-stress resistance…)
Ozdemir, 2020 ; Wang et al. 2022

Periparturient dairy cows face a strong negative energy balance by the mobilisation of body reserves

Negative energy balance (EB) during **early lactation**:

- **Mobilisation of body reserves**, mainly adipose tissue → ↗ NEFA in blood



Chilliard et al. 1984

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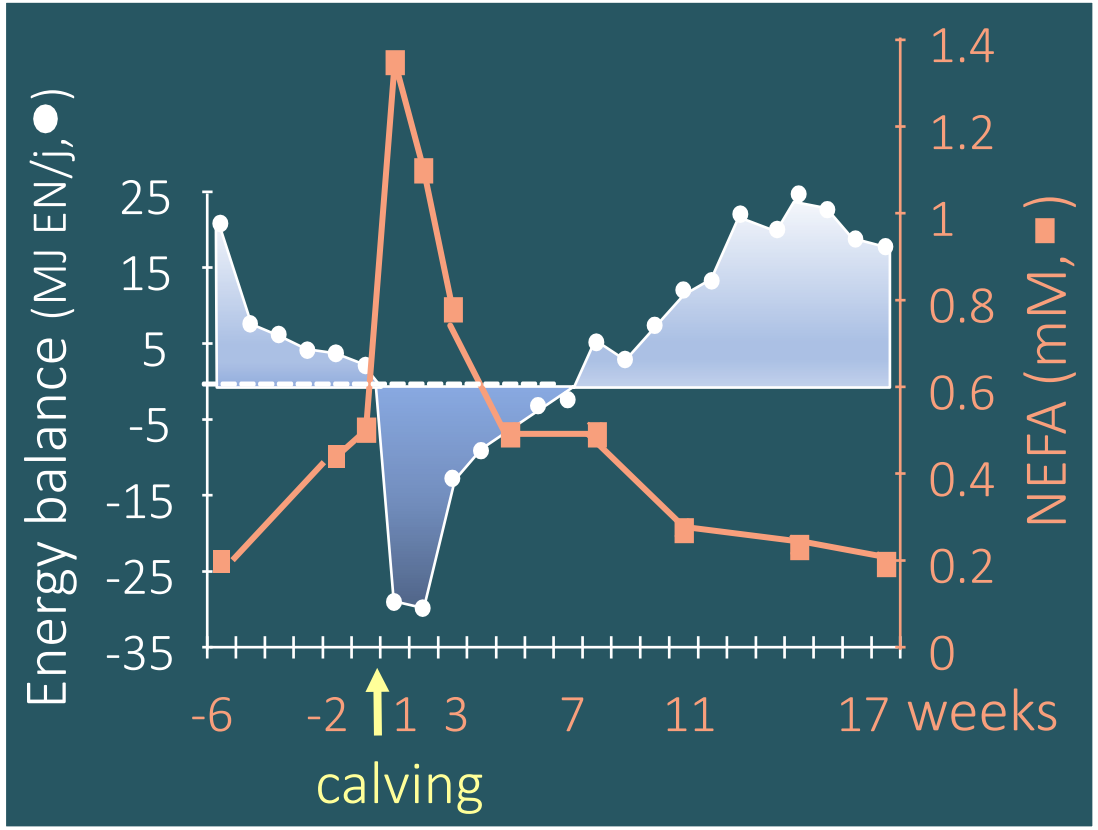

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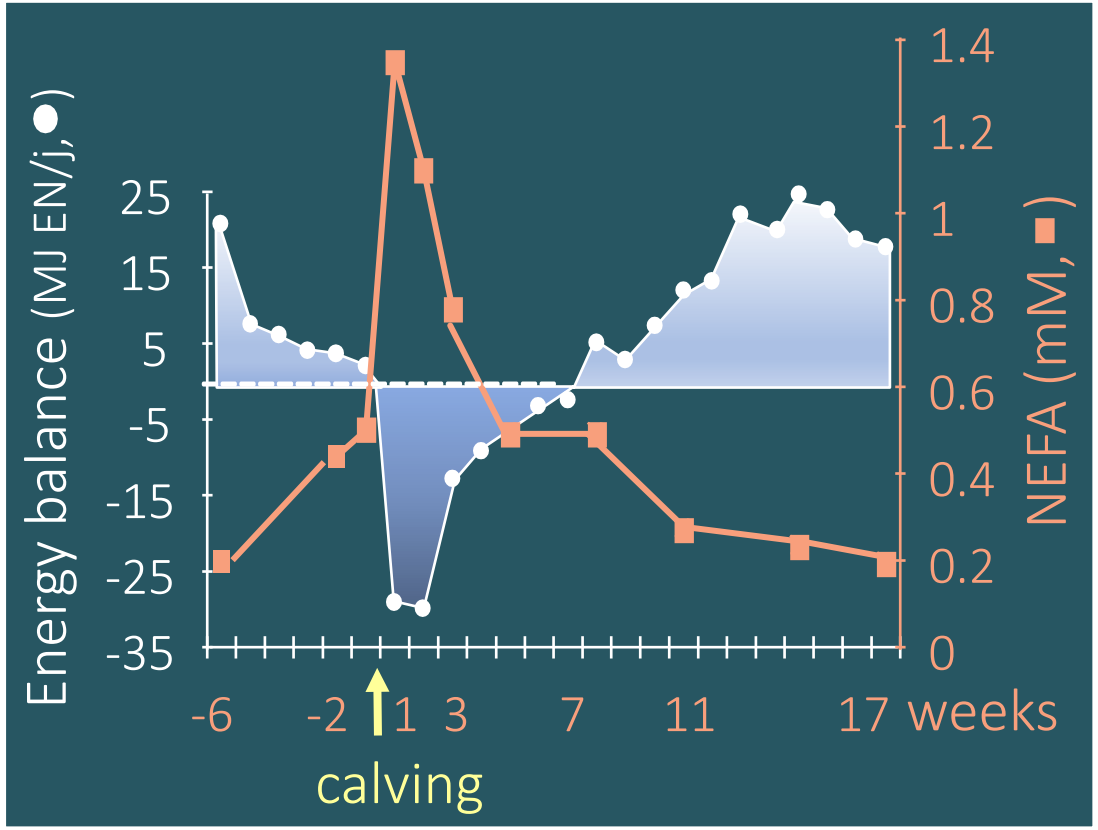
- ↗ Risk of **metabolic disorders and disease** → loss of performance and profitability



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Hypothesis

Milk small EVs carry the proteomic signatures of adaptation to negative energy balance in early lactating cows

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Objectives

- To **isolate** and **characterise** small EVs from milk of early lactating dairy cows with **different energy balances**
- To characterise small EVs **proteomic signature** associated with negative energy balance

Experimental design:

✓ **8 Holstein cows**

Parity 3 ± 1

Morning milk sampling:

- Week 2 : 14 ± 3 DIM
- Week 7 : 49 ± 3 DIM

70% forage (grass and corn silage, hay)

30% concentrate (soybean meal, corn)

Week 2 of lactation
Negative EB

Week 7 of lactation
Neutral or positive EB

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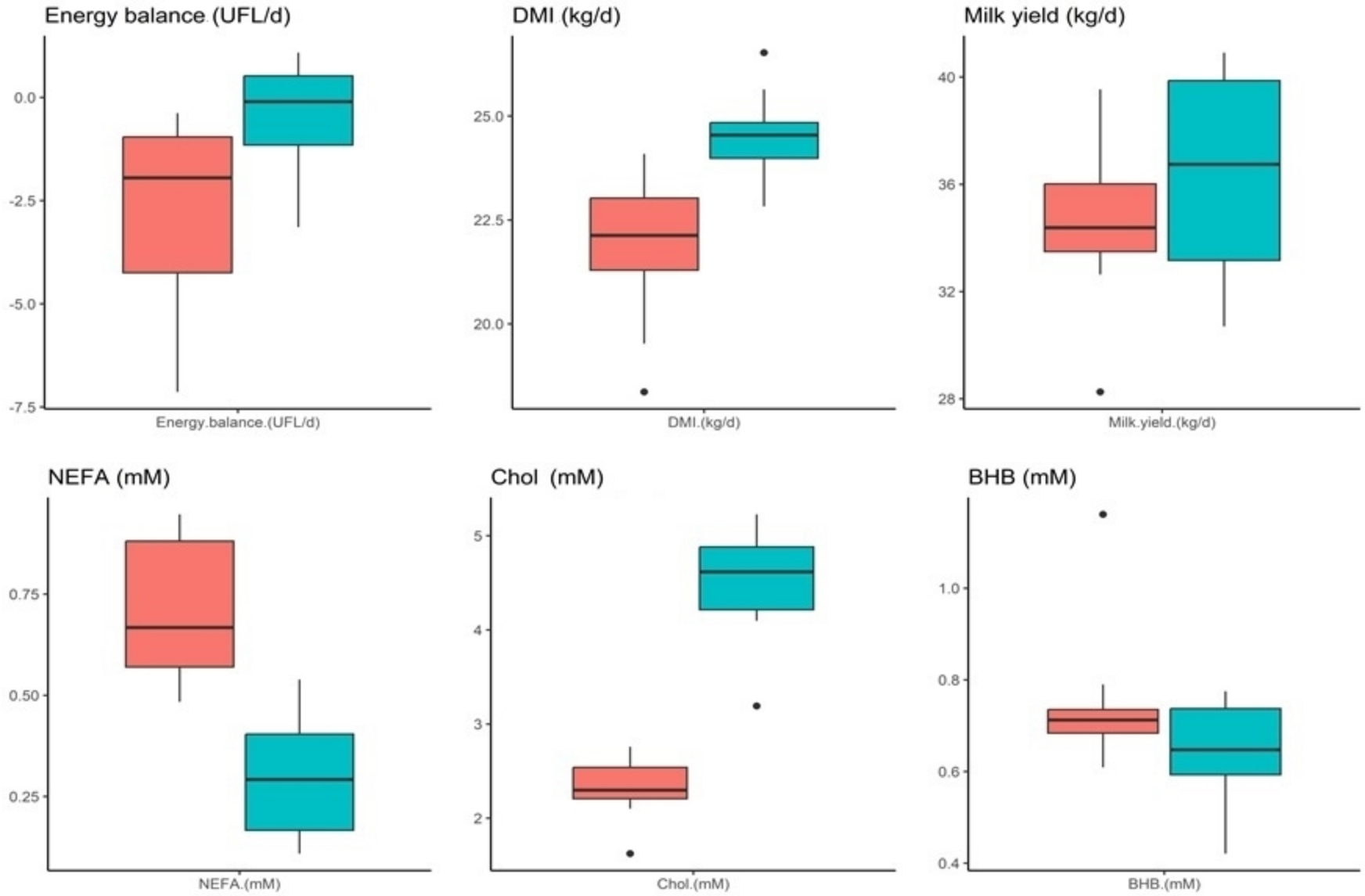
Zootechnical and metabolic indicators → Small milk EVs isolation → Small Evs Characterisation → Statistical Analysis → Proteomic Data mining

Zootechnical and metabolic indicators confirm different EB and adaptive metabolism of early lactating cows



Isolation of milk small EVs

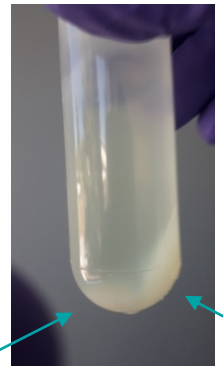
Fresh

Centrifugation

Elimination of cream and debris

Ultra Centrifugation

Pelleting and concentration of vesicles



small EVs

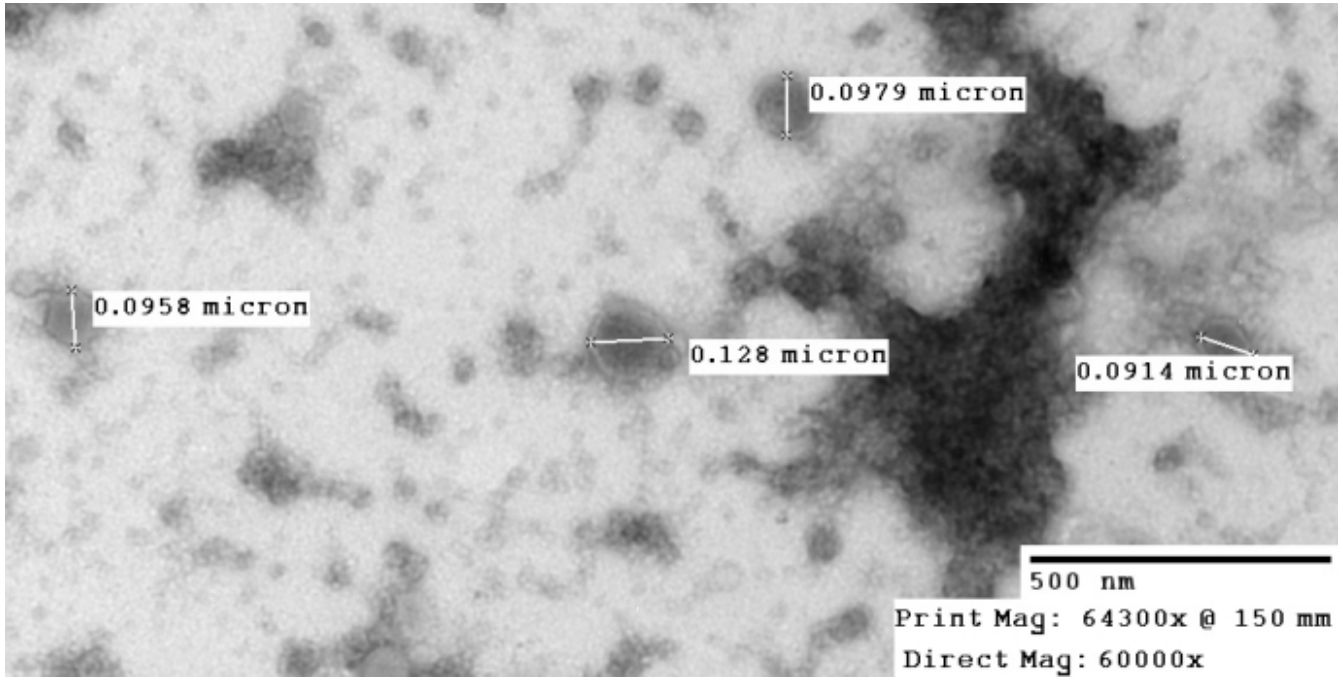
caseins

Purification

Size exclusion chromatography

Characterisation of milk small EVs

- Morphology:



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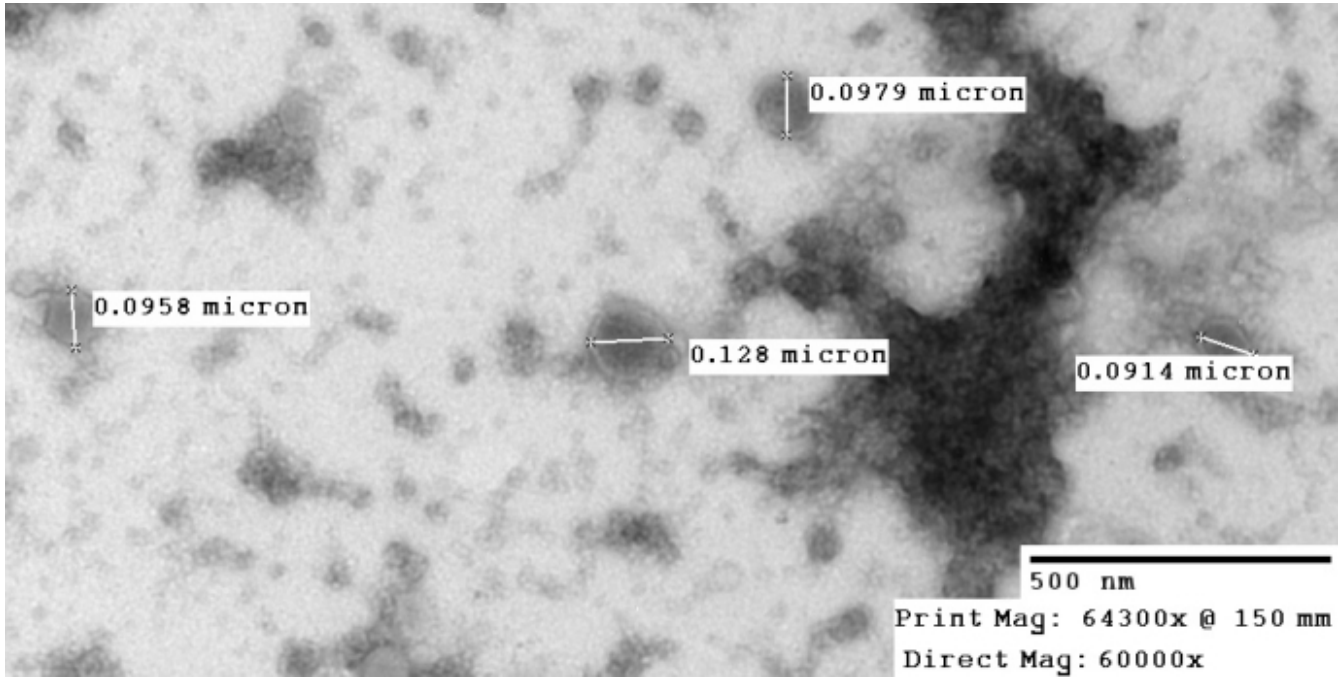
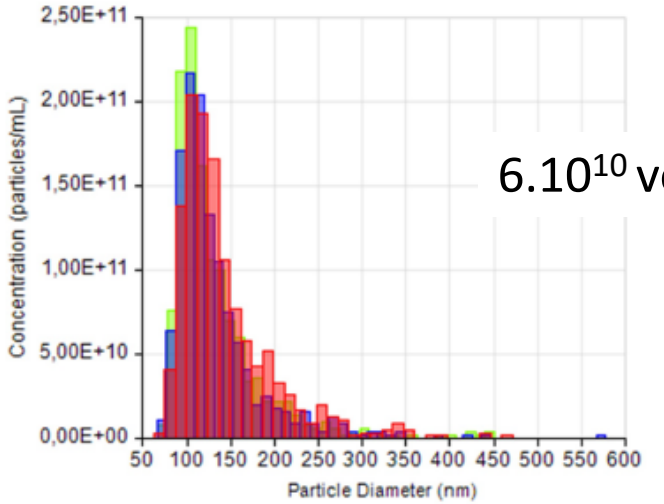


- Diameter and concentration:

2 methods:
- Nanoparticle Tracking Analysis
- Tunable resistive pulse sensing



6.10^{10} vesicles/ml milk

Characterisation of milk small EVs

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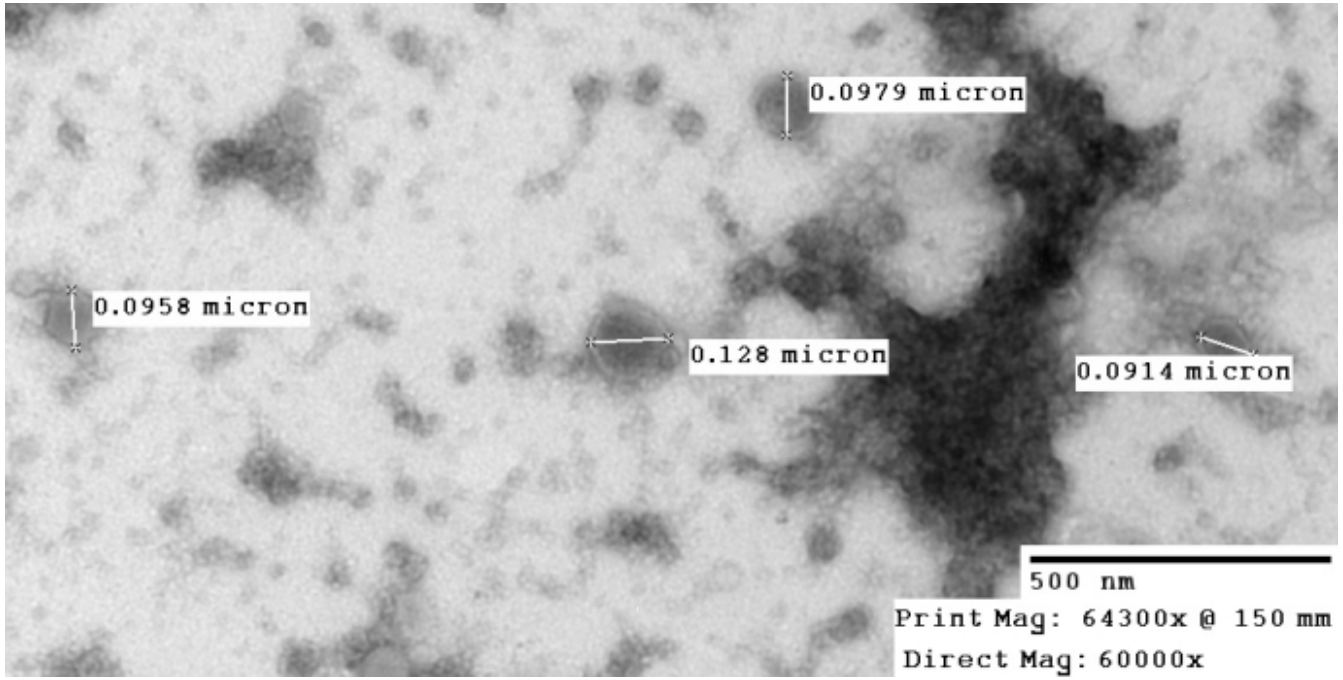

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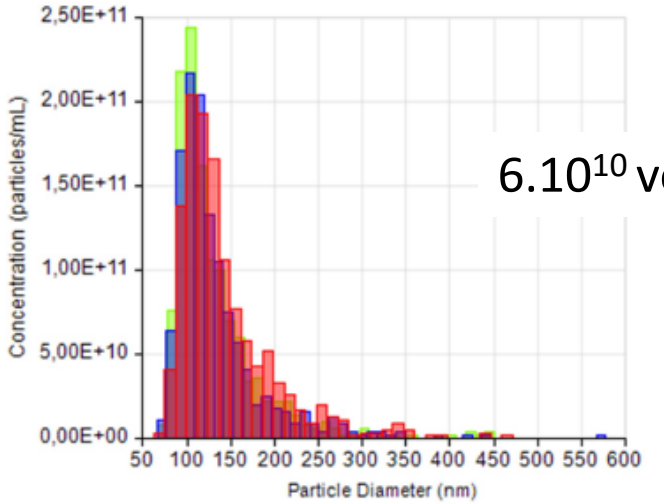

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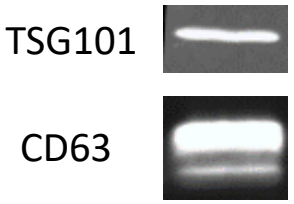
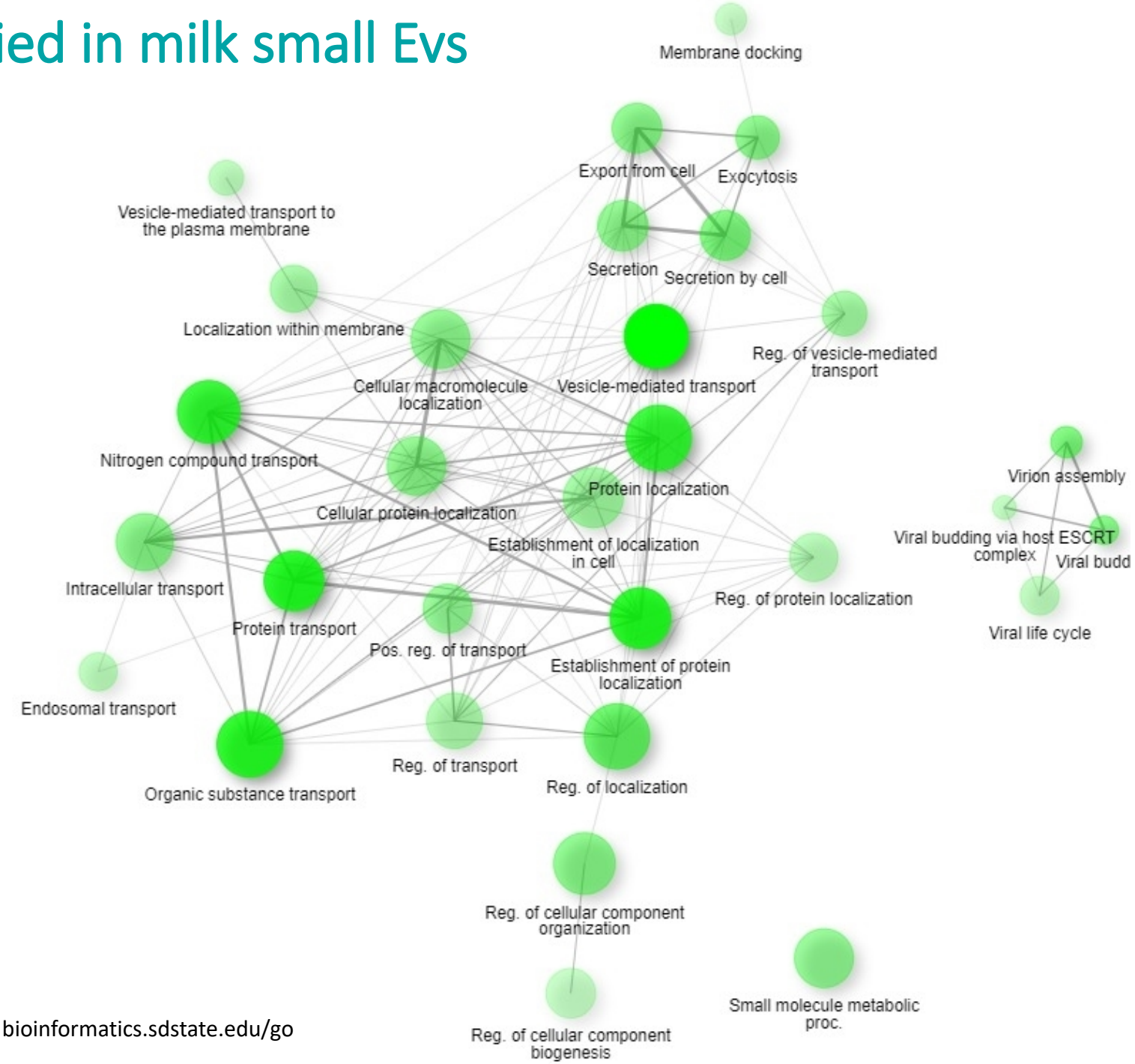
- Markers specific to small EVs:



502 proteins were identified in milk small Evs

Extraction of 600 µg of proteins
from 2.10^{10} small EVs (≈1ml of milk)

Top 30 most enriched GO Biological Processes:



ShinyGO, bioinformatics.sdstate.edu/go

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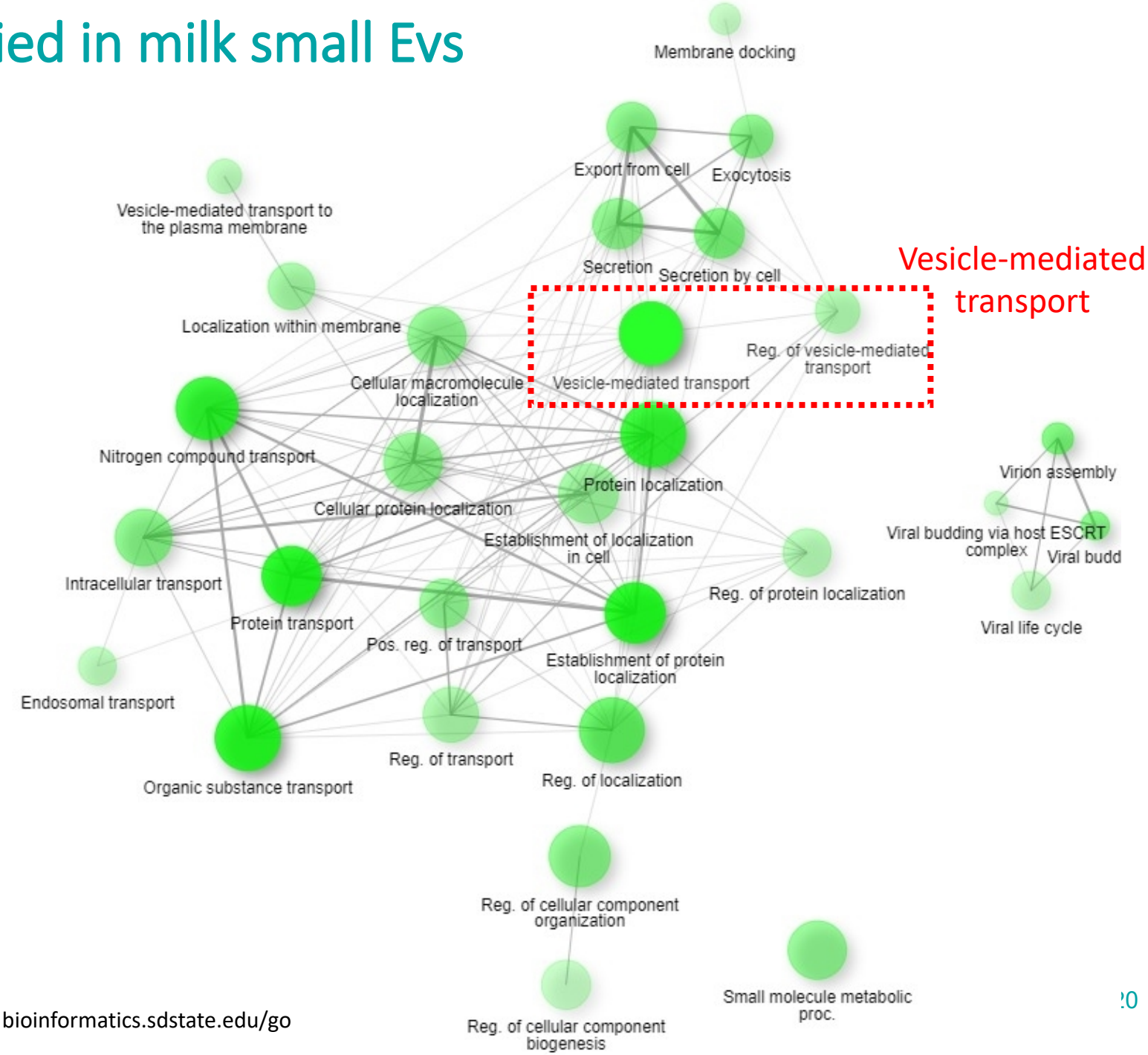

Vesicle-mediated transport

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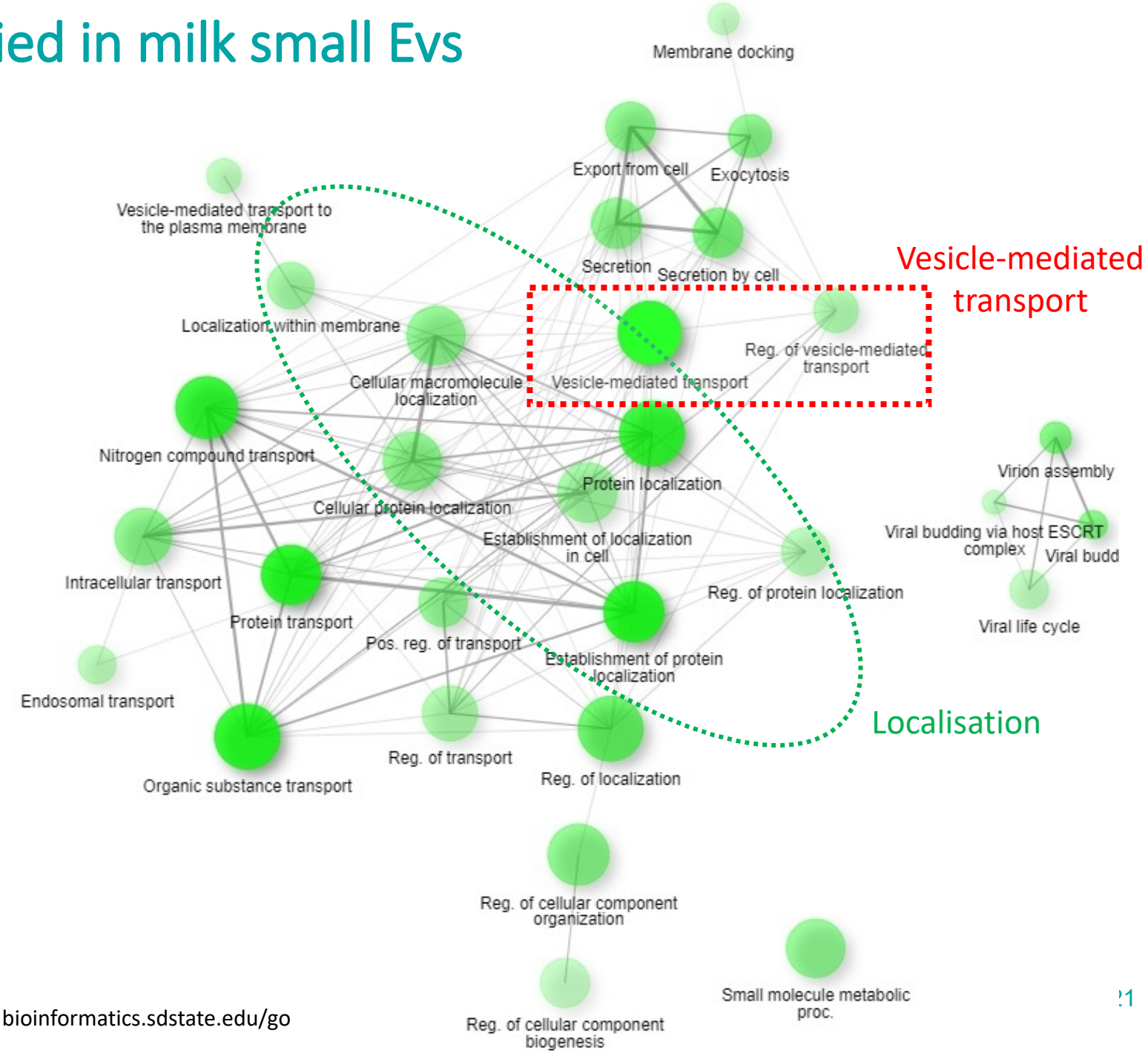

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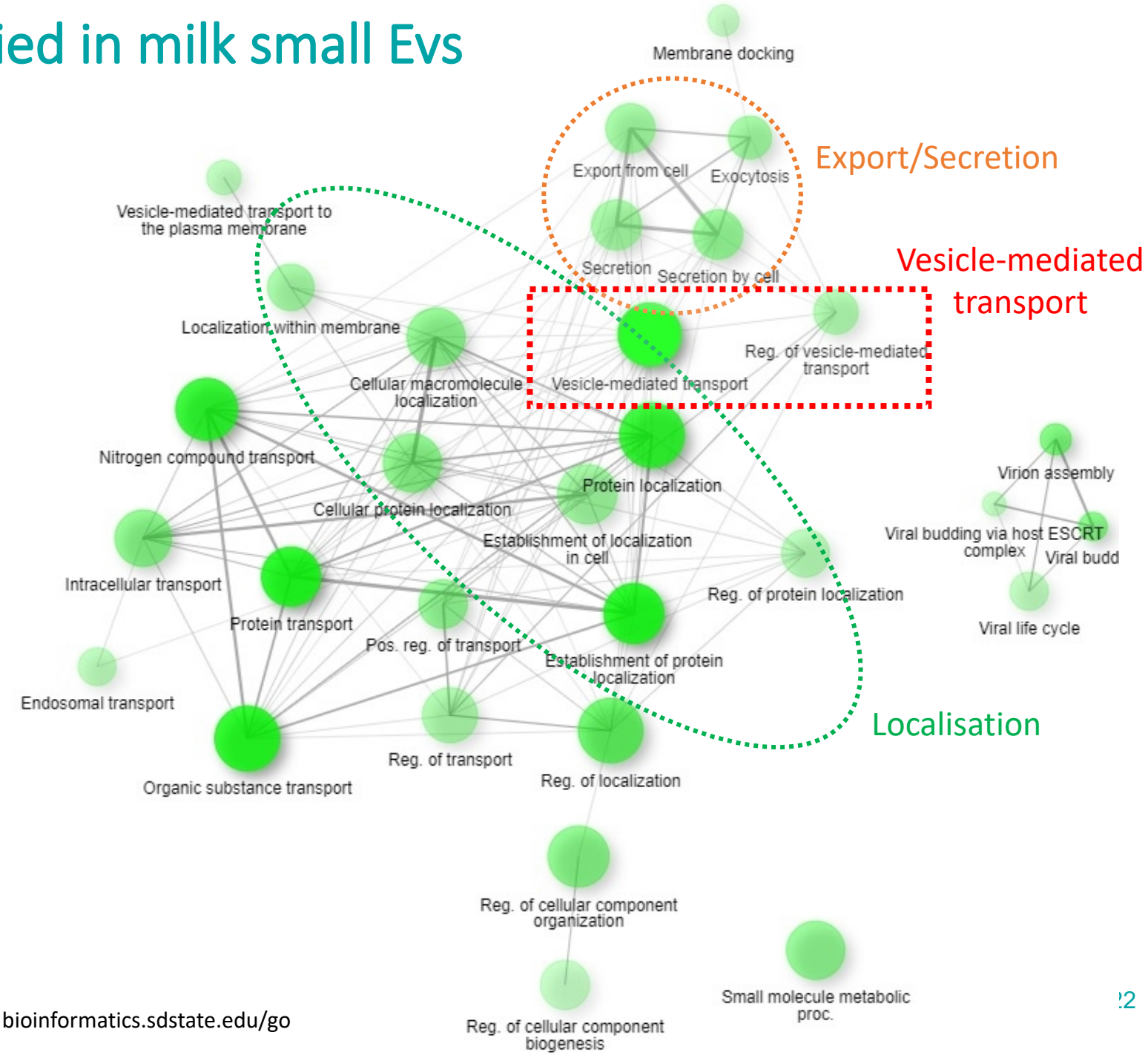

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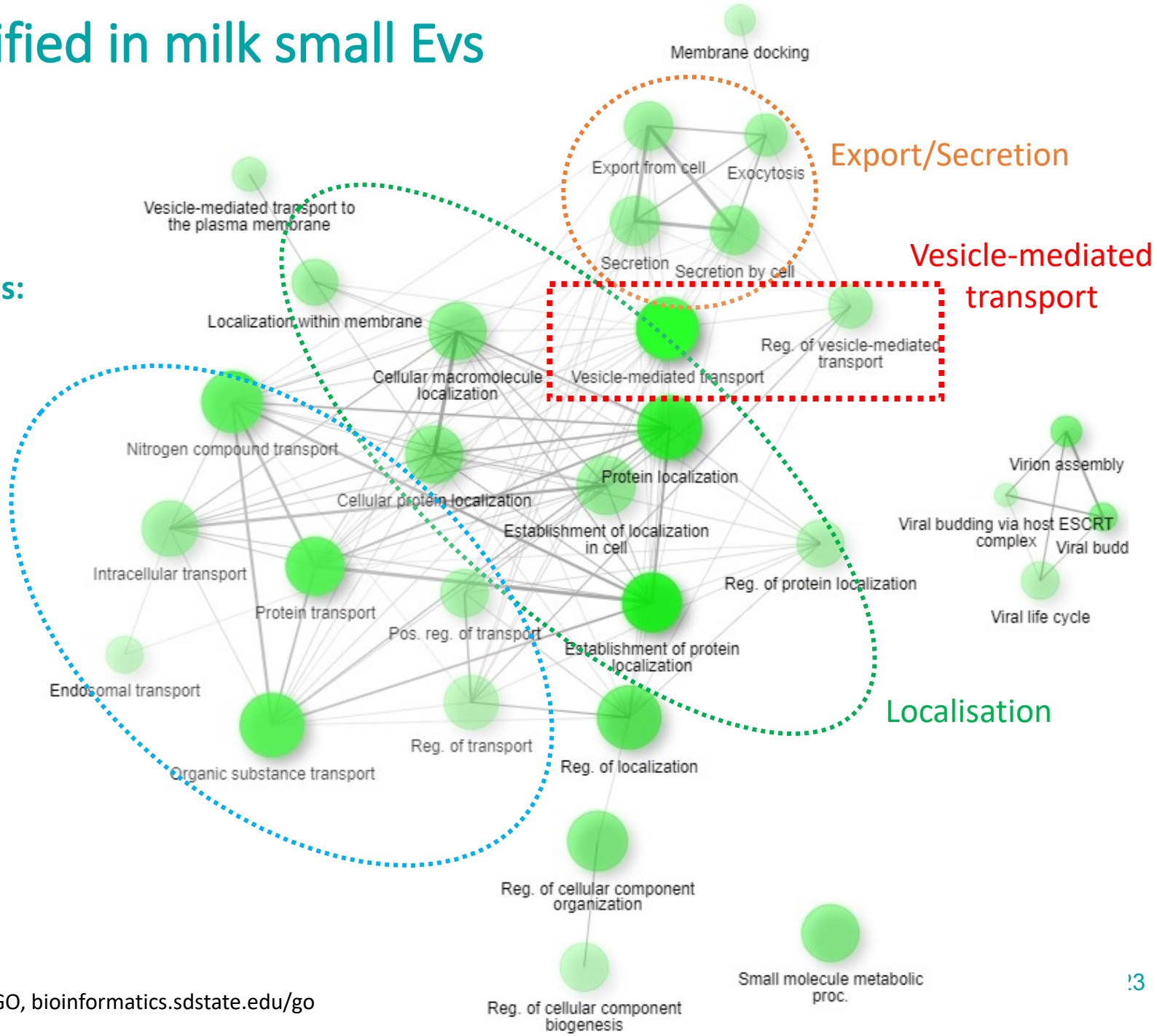

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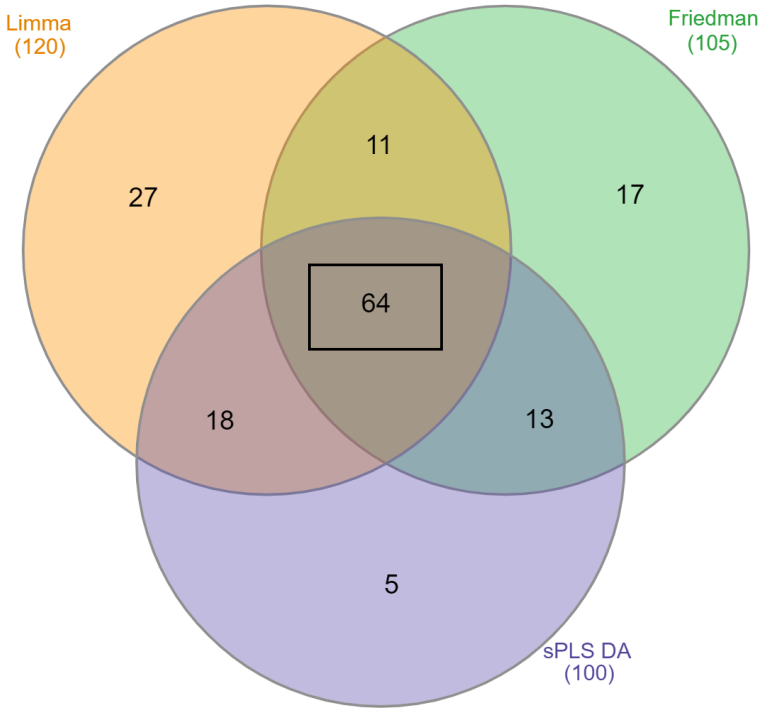
64 proteins discriminate early lactating cows divergent by the EB

Implementation of 2 univariate and 1 multivariate statistical analyses to identify differentially abundant proteins



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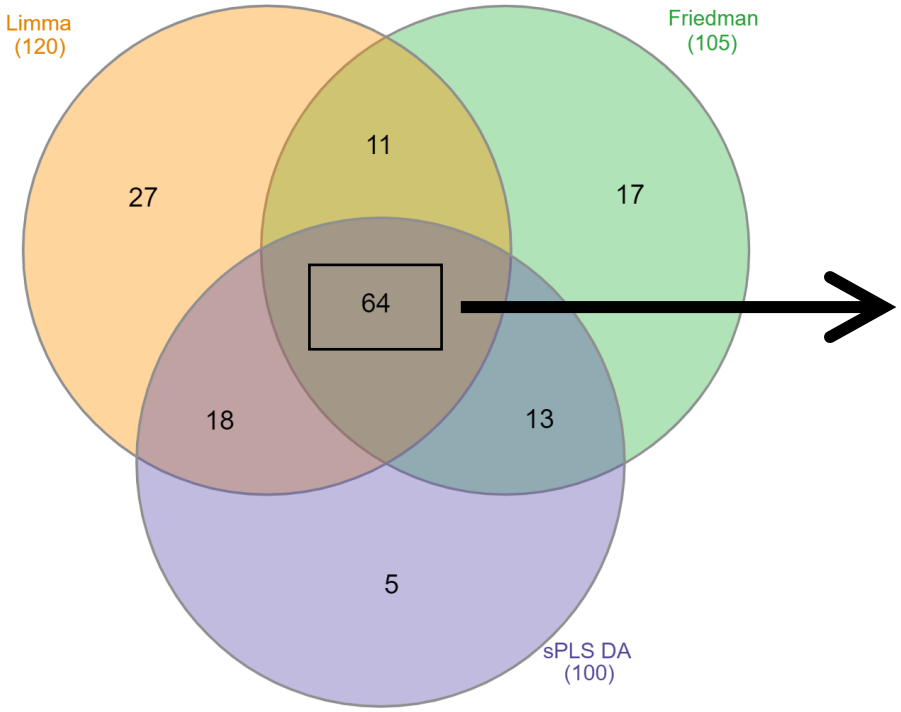
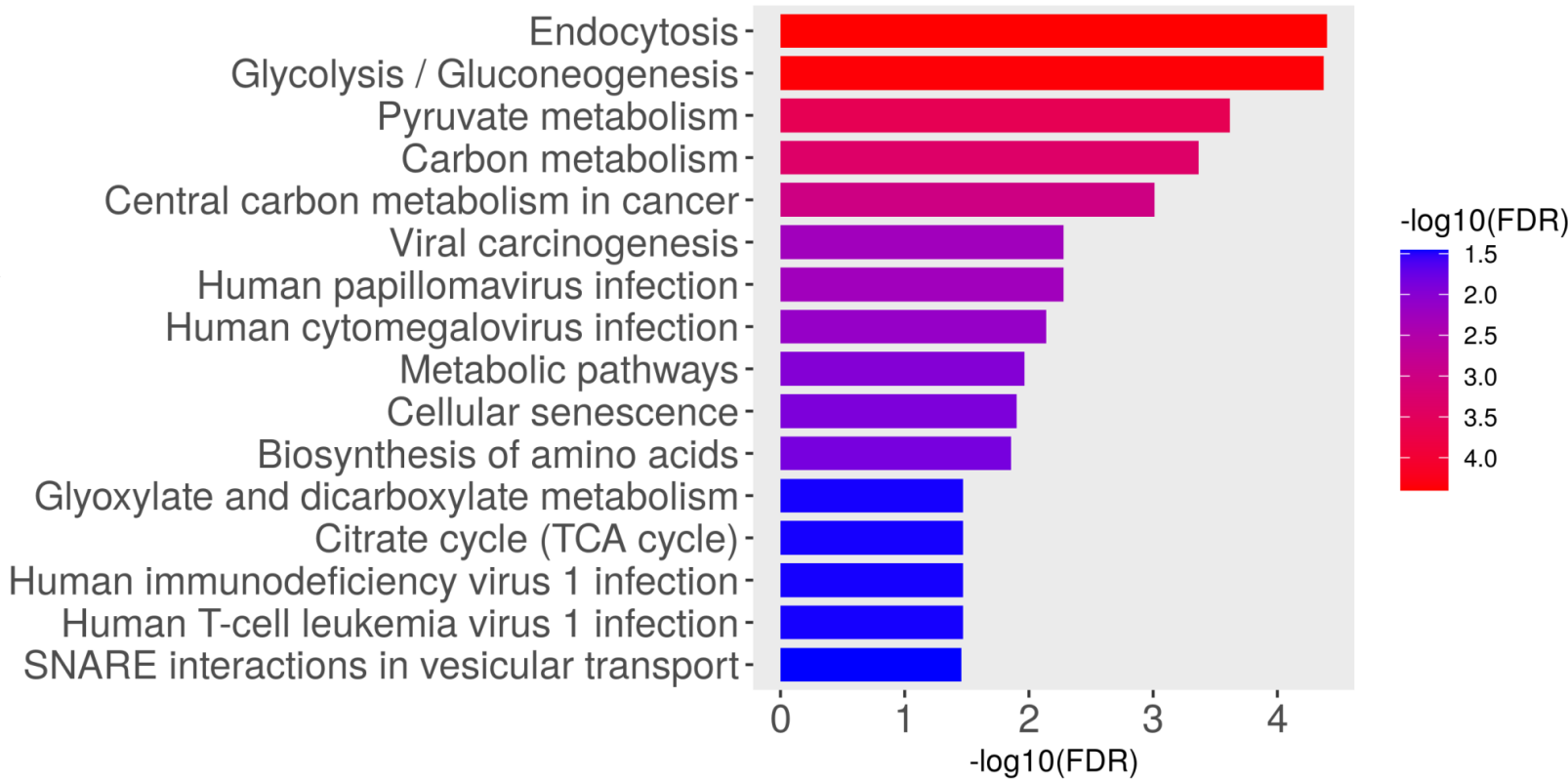


Most enriched KEGG pathways:



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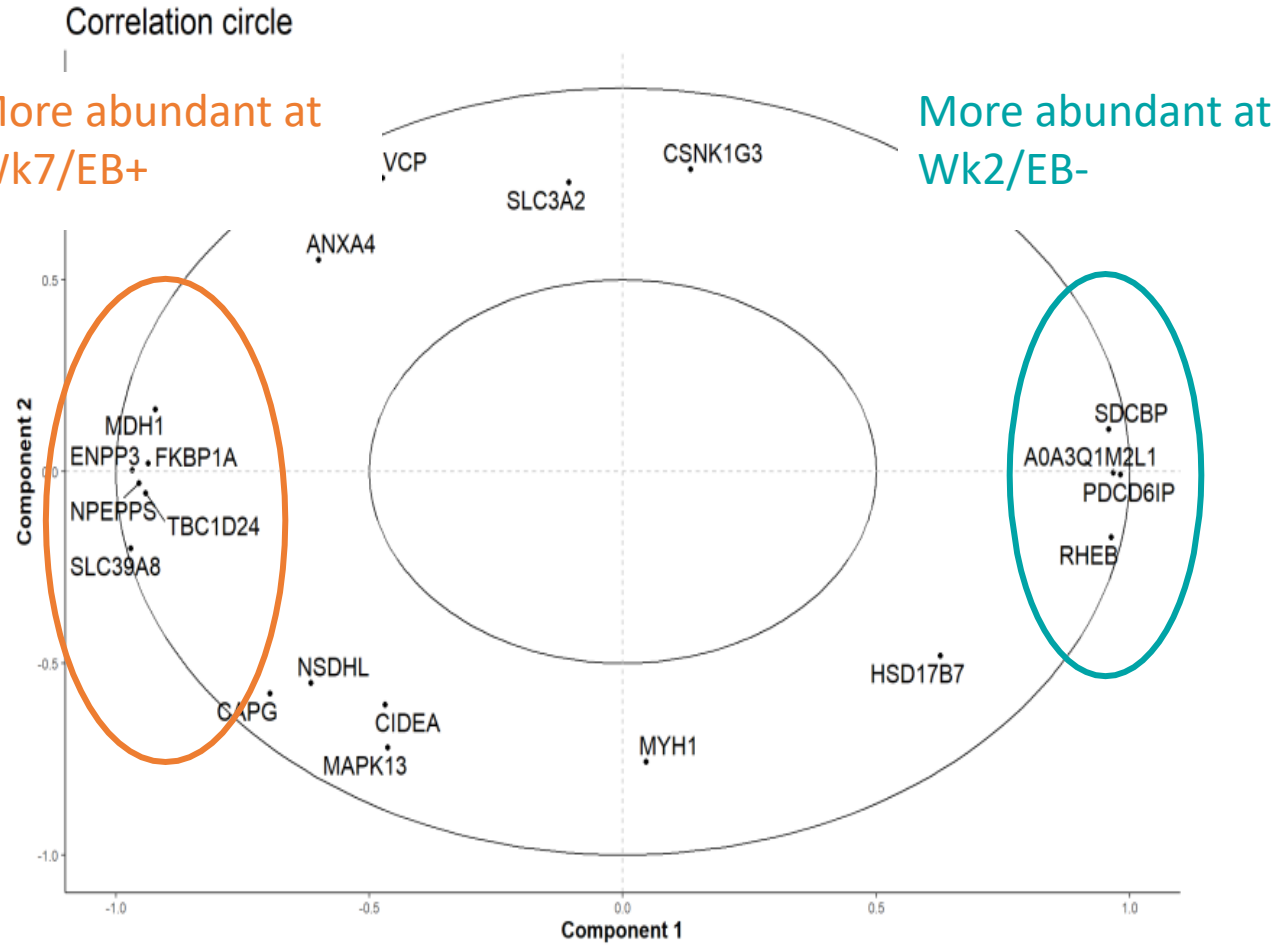
Proteomic signature of EB in early lactating dairy cows

Discriminant analysis (sparse PLSDA) on the groups week2/EB- and week7/EB+
→ Identification of the 10 most explicatives proteins



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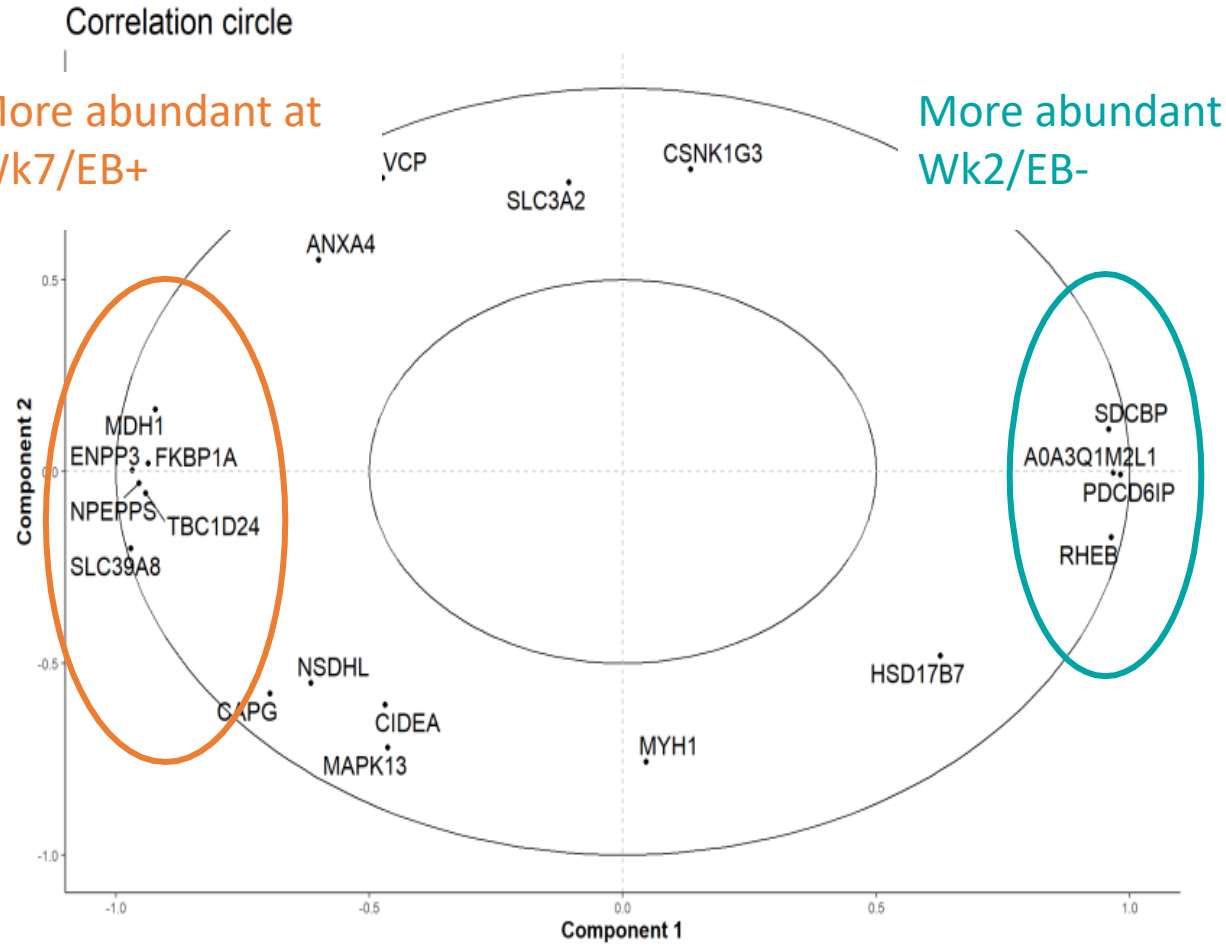
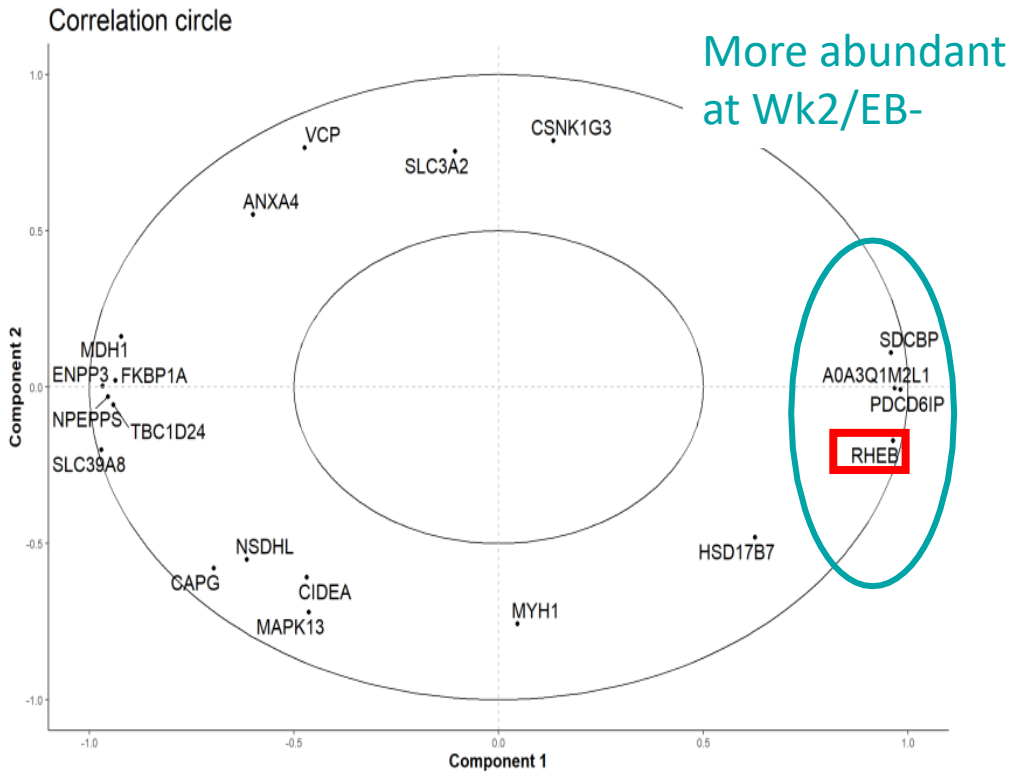
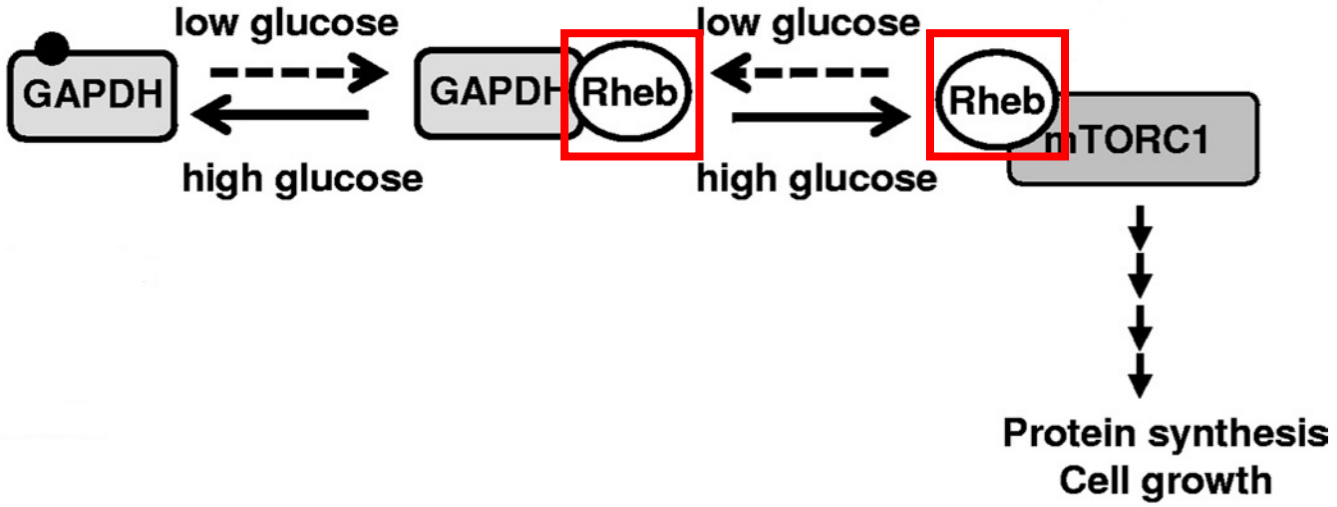


Proteins	Log2-FoldChange
SDCBP	1.9372607
A0A3Q1M2L1	3.6441336
PDCD6IP	1.9721681
RHEB	0.6323398
MDH1	-0.6554129
ENPP3	-0.9237812
FKBP1A	-0.2927596
NPEPPS	-0.6033501
TBC1D24	-0.896133
SLC39A8	-0.6215984

Physiological functions of proteins related to a negative EB



- **Rheb** activates mTORC1 signaling, strongly dependant of glycolytic flux



Lee et al. 2009

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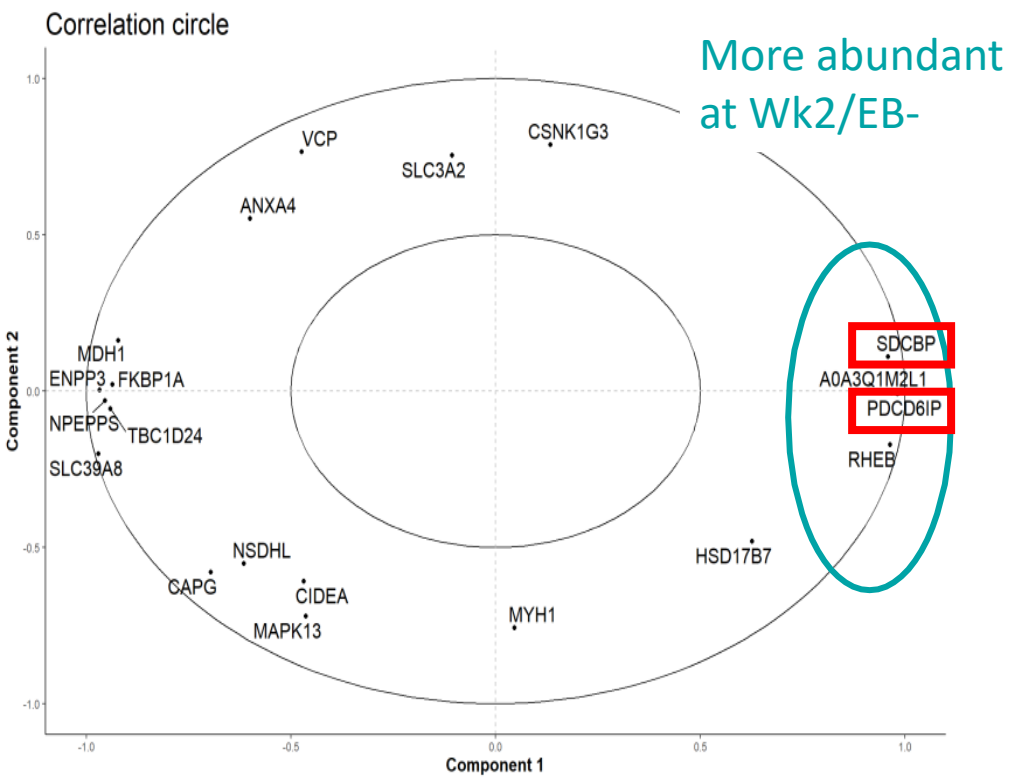
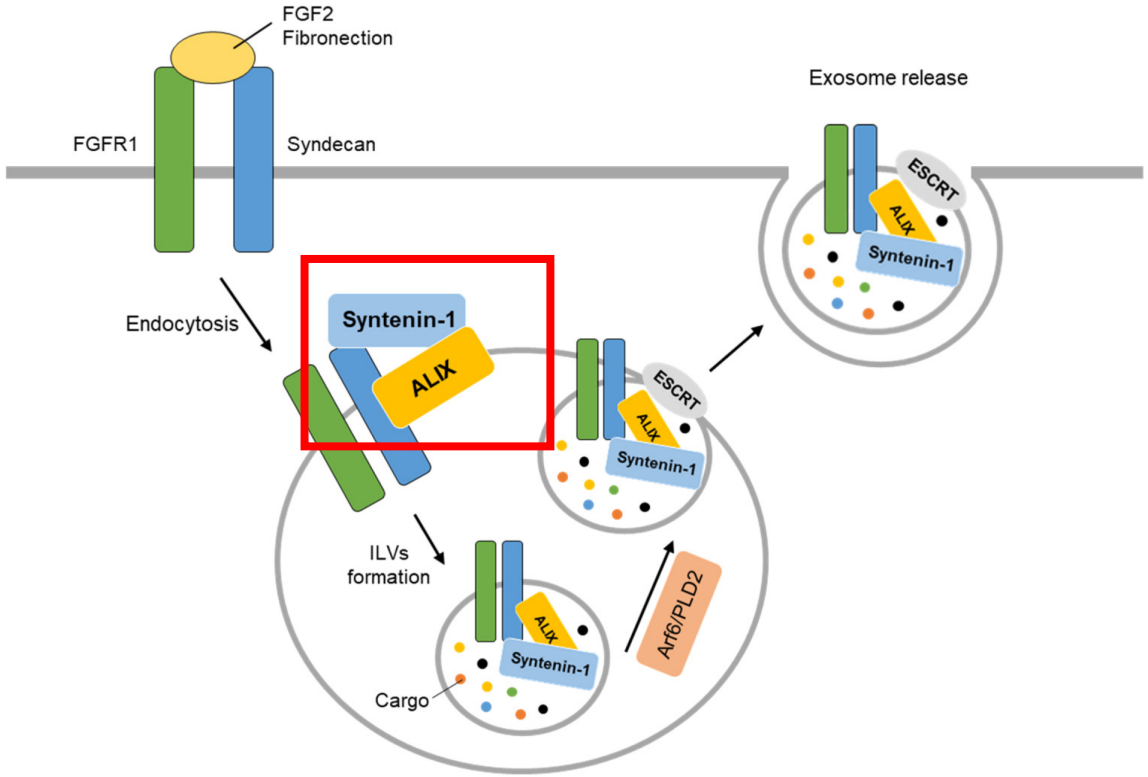


- **SDCBP = Syntenin-1** regulates the production and secretion of exosomes through interactions with **PDCD6IP = ALIX**



Baietti et al. 2012

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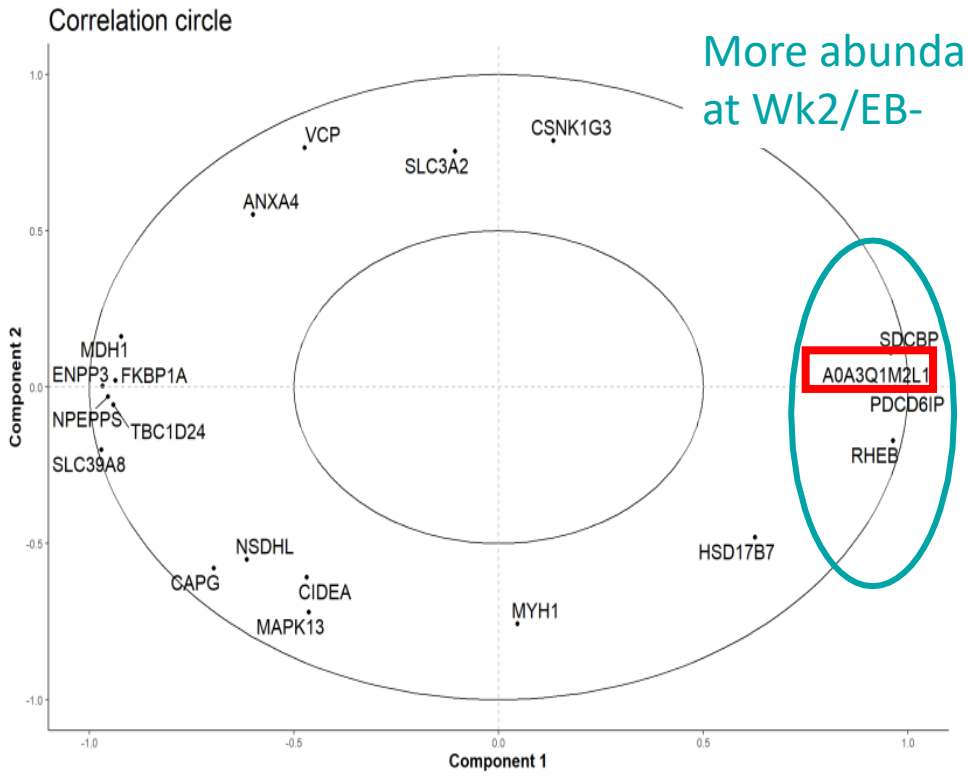
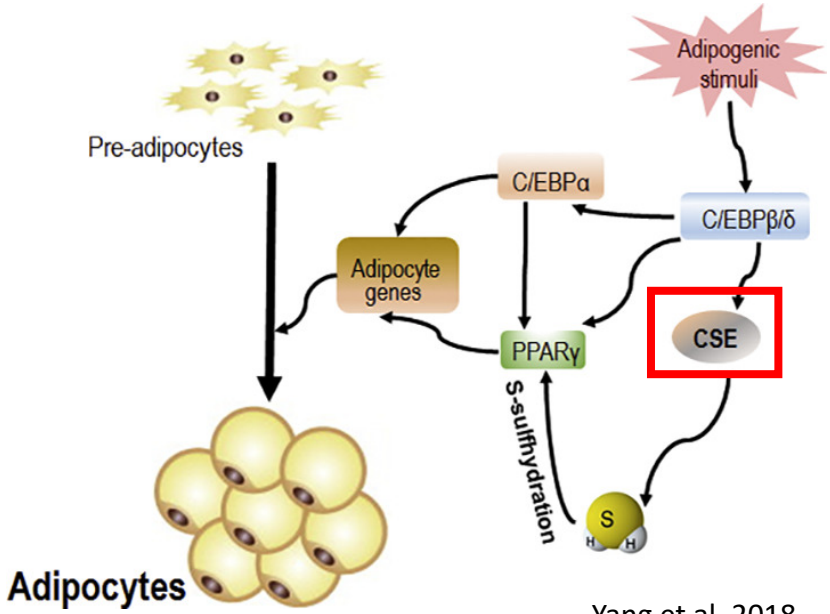
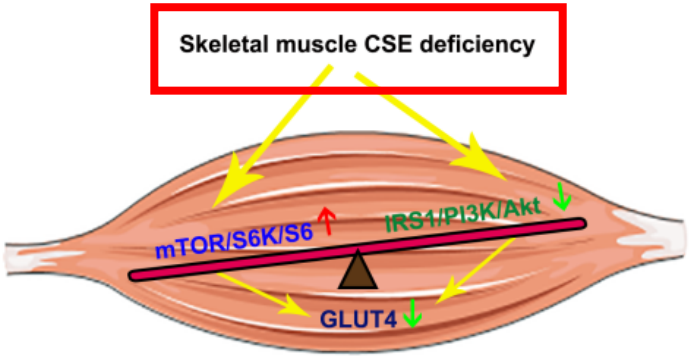


- **A0A3Q1M2L1 = CSE** is involved:
  - in glucose uptake and lipid deposition in adipose tissue (Cai et al. 2016 ; Yang et al. 2018)
  - in insulin sensitivity in muscle (Xu et al. 2022)



Yang et al. 2018



Xu et al. 2022

Conclusions and perspectives

Milk small EVs from 2 early lactating weeks **with different energy balance** were isolated and characterized:

- ✓ **502 proteins were identified in milk small EVs**, involved in vesicle-mediated transport, protein localization, export, secretion and transport.
- ✓ **64 proteins, mainly involved in metabolism and signaling, have different abundances between the 2 groups.**
- ✓ **10 proteins allow the discrimination of the 2 groups.**

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Perspectives:

Lipidomic and miRnomic analyses + Multi-omic data integration

→ To investigate pathways related to adaptation

Acknowledgments

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UNI BONN
Helga Sauerwein
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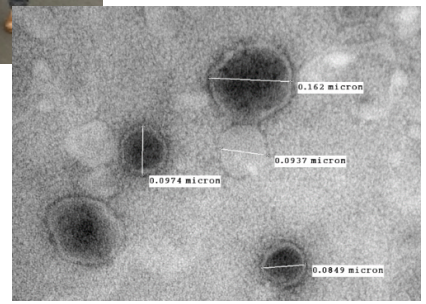
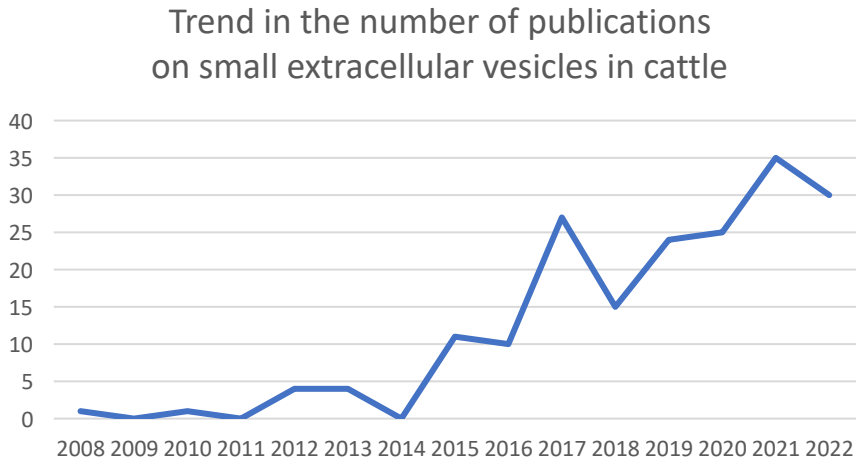


iCS Centre Imagerie Cellulaire Santé
Lorraine Novais Gameiro
Claire Szczepaniak
Christelle Blavignac

Context: small Evs in milk

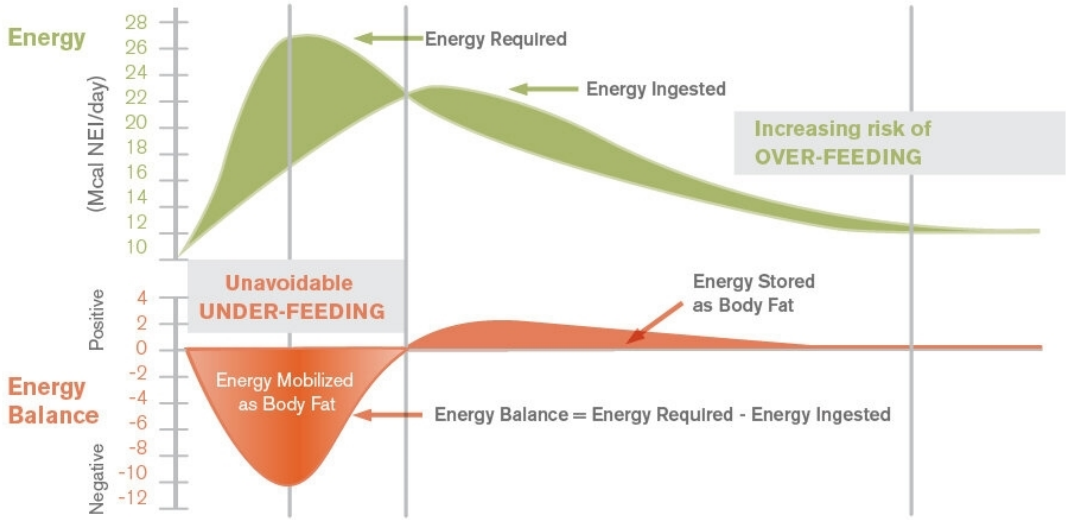


- **Recent data (mainly since 2013) indicate the presence of small EVs in bovine biological fluids (milk, uterine fluid, plasma…)**

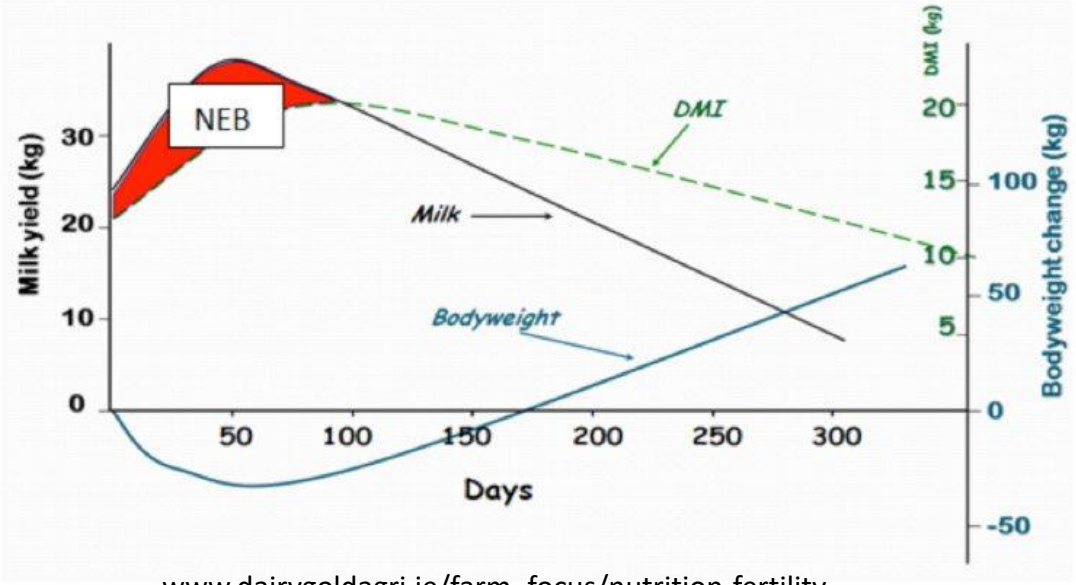
Milk small EVs:

- **Consumer health** (human/calf): development of the newborn, immune status, intestinal inflammation, bone health, stability in industrial processes (UHT, pasteurisation) ...
- **Therapeutic potential**: stable, biocompatible and available **nano-vehicles** to encapsulate therapeutic molecules
- **Non invasive sources of health biomarkers** (mastitis, enzootic bovine leukosis, tick resistance, estrus) and **efficiency of dairy cows** (milk yield (Osdemir, 2020), heat-stress resistance)
- Cargo studied: essentialy miRNAs

Context: adaptation during early lactation



www.fermented-nutrition.com



www.dairygoldagri.ie/farm_focus/nutrition-fertility

Negative energy balance (NEB) during early lactation
→ metabolic disorders, pathology, loss of performance

Period of intense adaptation involving inter-organ dialogue to prioritize metabolisms