



Transcriptomic characterization of water buffalo's extracellular vesicles from colostrum and milk for their Immunomodulatory Potential

Samanta Mecocci (1) - Daniele Pietrucci (2) - Marco Milanese (3) - Stefano Capomaccio (1) – Luisa Pascucci (1) - Chiara Evangelista (3) - Loredana Basiricò (4) - Umberto Bernabucci (4) - Giovanni Chillemi (3) - Katia Cappelli (1)

Department of Veterinary Medicine, University of Perugia, Perugia, Italy (1)

Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, IBIOM, CNR, Bari, Italy (2)

Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy (3)

Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy (4)



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Aim: To characterize the Buffalo colostrum and milk miRNA EV cargo



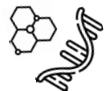
Milk is one of the most promising sources of Extracellular Vesicles (EVs)



Milk-derived EVs (mEVs) can have anti-inflammatory and immunomodulatory properties



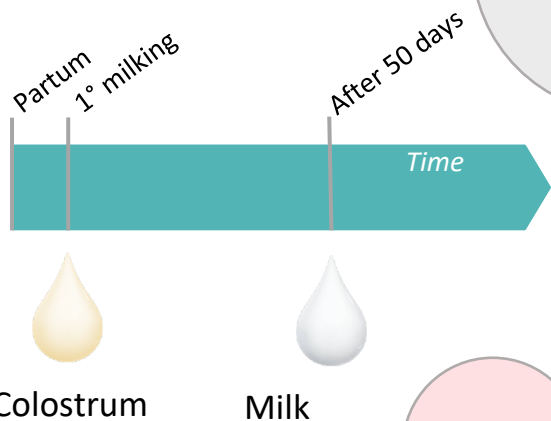
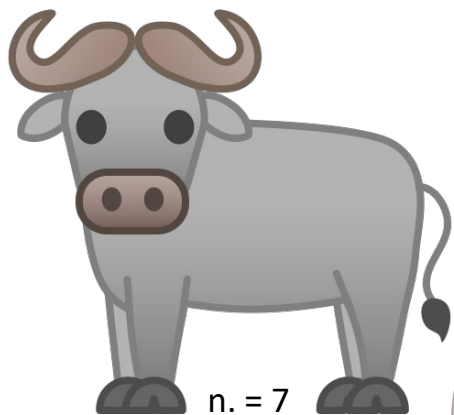
mEVs pass the difficult conditions of the gastric environment reaching the intestinal cells



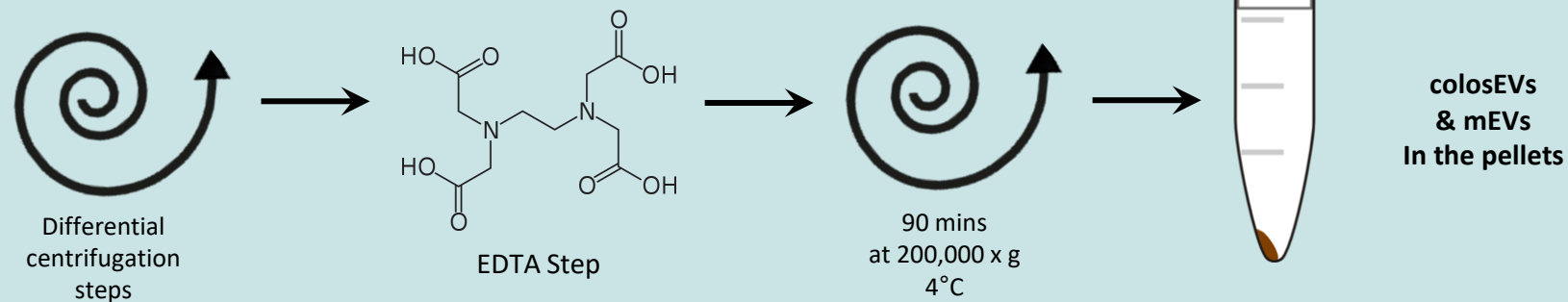
mEVs contain bioactive molecules such as proteins, lipids, RNAs, and metabolites.

Experimental design

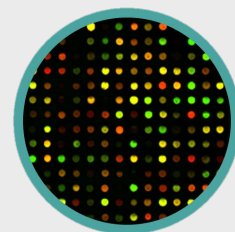
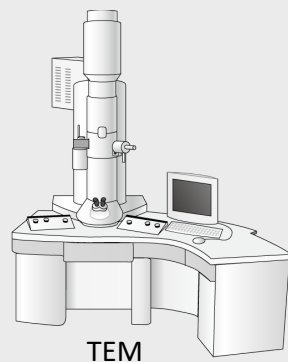
Sampling



EV isolation



EV characterization



Illumina small RNA sequencing

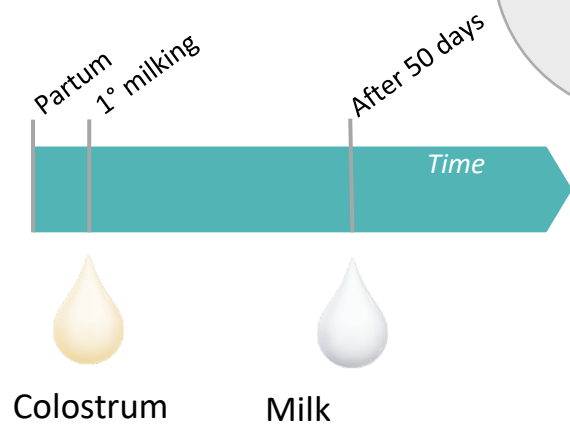
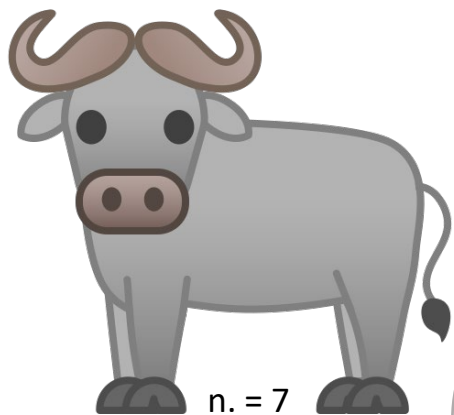


Bioinformatic analysis



Experimental design

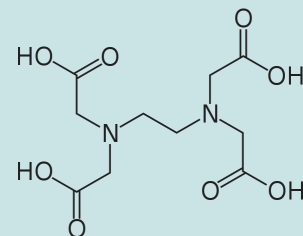
Sampling



EV isolation



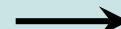
Differential centrifugation steps



EDTA Step

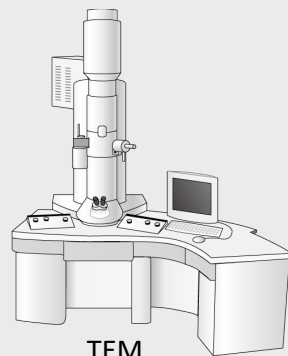


90 mins
at 200,000 x g
4°C

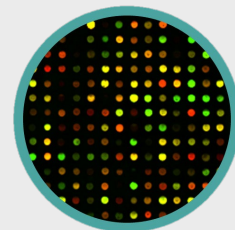


colosEVs
& mEVs
in the pellets

EV characterization



TEM



EXOVIEW

Illumina small RNA sequencing



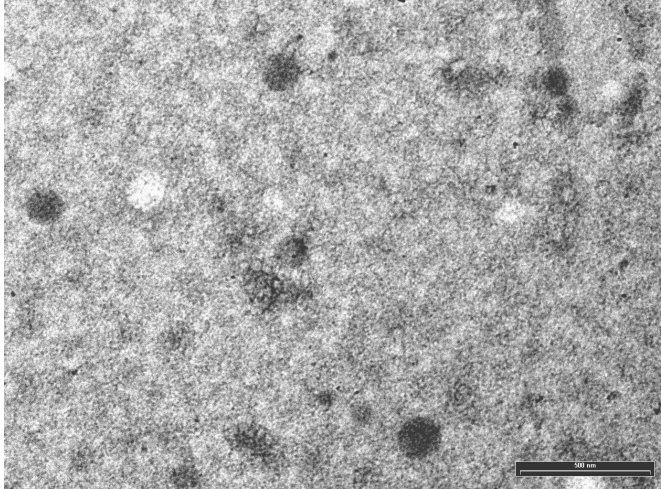
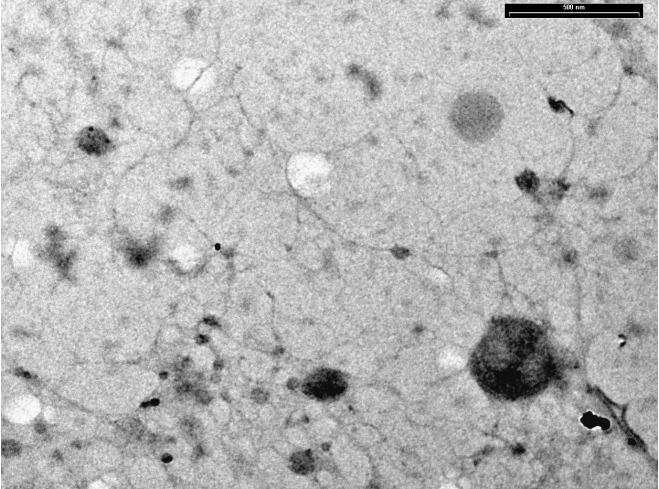
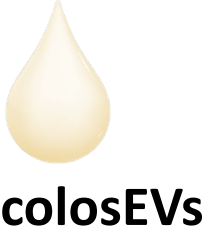
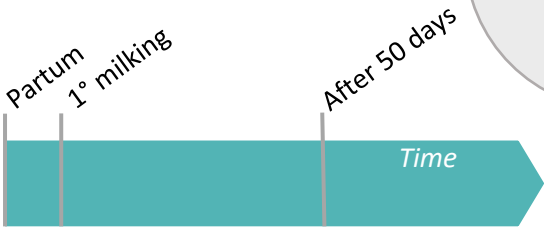
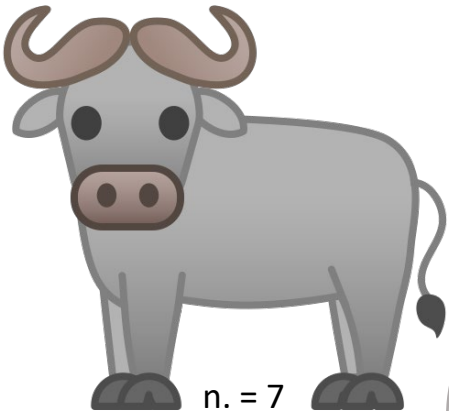
Total RNA extraction



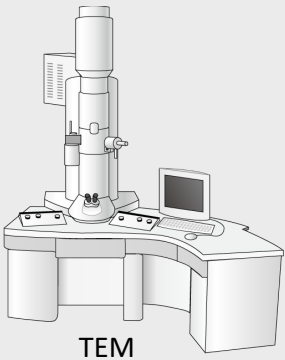
smallRNA library preparation
(150bp single-end reads)

Experimental design

Sampling



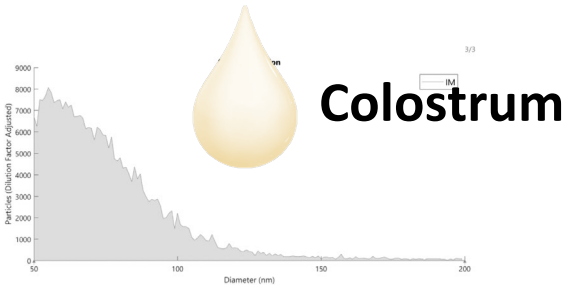
EV characterization



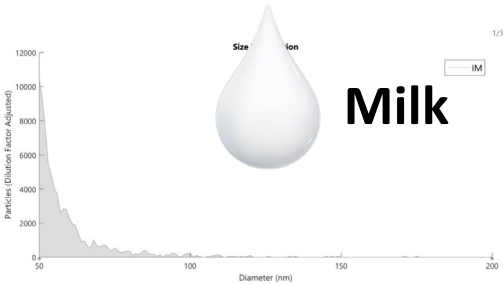
TEM



EXOVIEW



Colostrum



Milk

Particles
Avg Count
281788

Avg % Total
100.0

Diameter (nm)
Mean
76

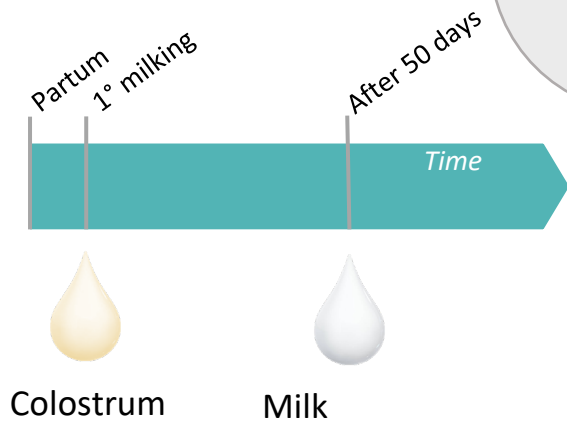
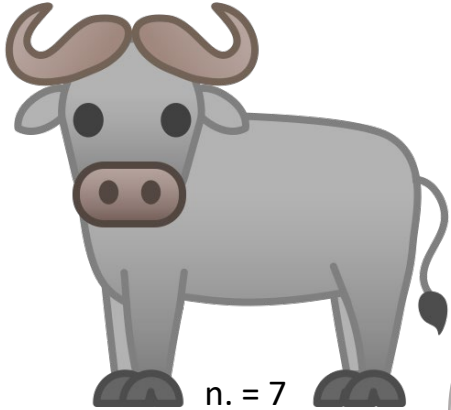
Particles
Avg Count
70232

Avg % Total
100.0

Diameter (nm)
Mean
60

SD
13

Sampling

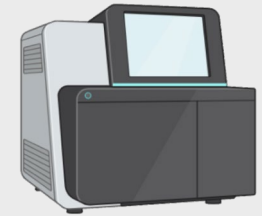


Total RNA extraction and
smallRNA library
preparation
(150bp single-end reads)

Illumina small RNA sequencing



Total RNA extraction

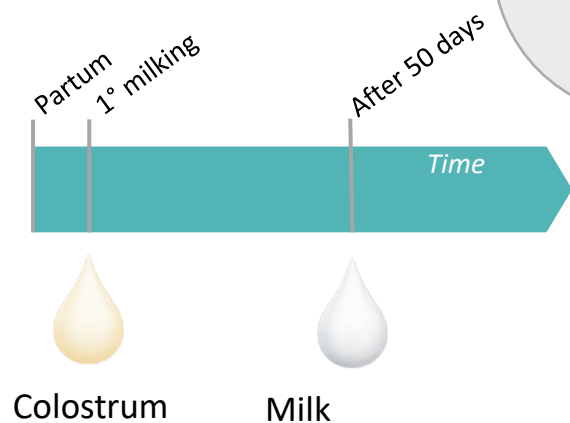
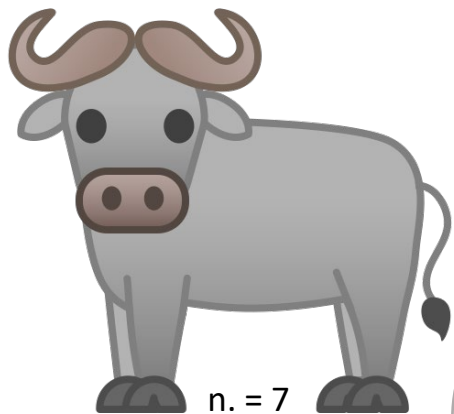


smallRNA library preparation
(150bp single-end reads)

Form the Illumina sequencing, a mean of over **33×10^6** raw
reads per sample were obtained

Experimental design

Sampling



Illumina small RNA sequencing



Total RNA extraction



smallRNA library preparation
(150bp single-end reads)

Bioinformatic analysis



Read
quality control
& trimming



2-step mapping
on miRbase
and genome



Explorative
analysis



Differential
Expression analysis
and sample-specific
miRNA identification



colosEV-specific
mEV-specific
and shared
miRNA targets



Functional analysis on
colosEV-specific
mEV-specific
and shared
miRNA targets

Two mapping steps were carried out:

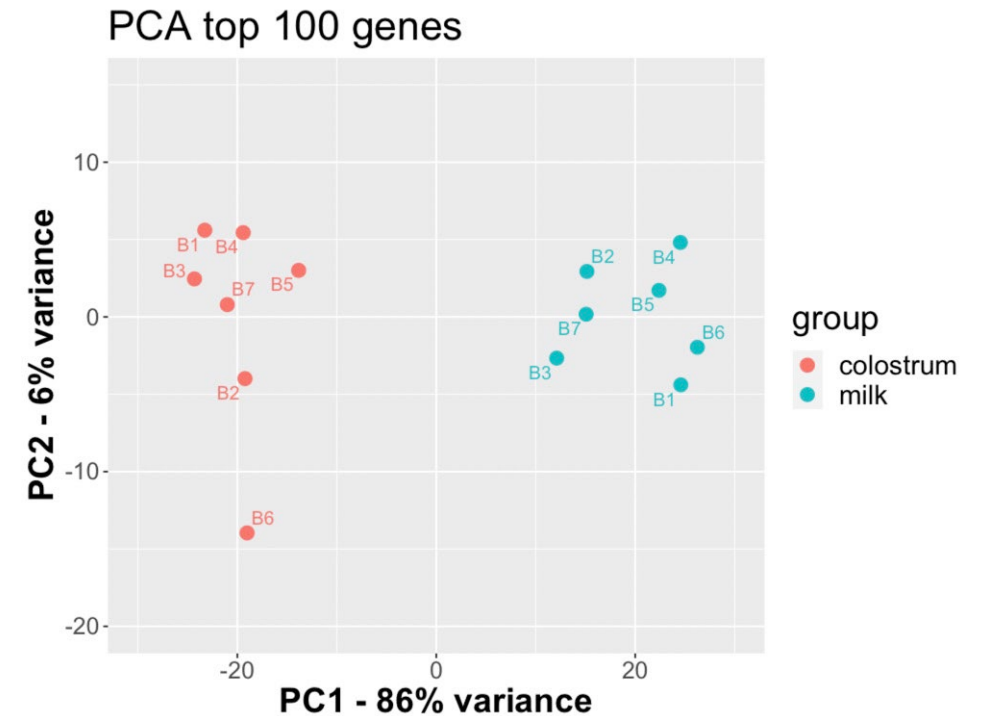
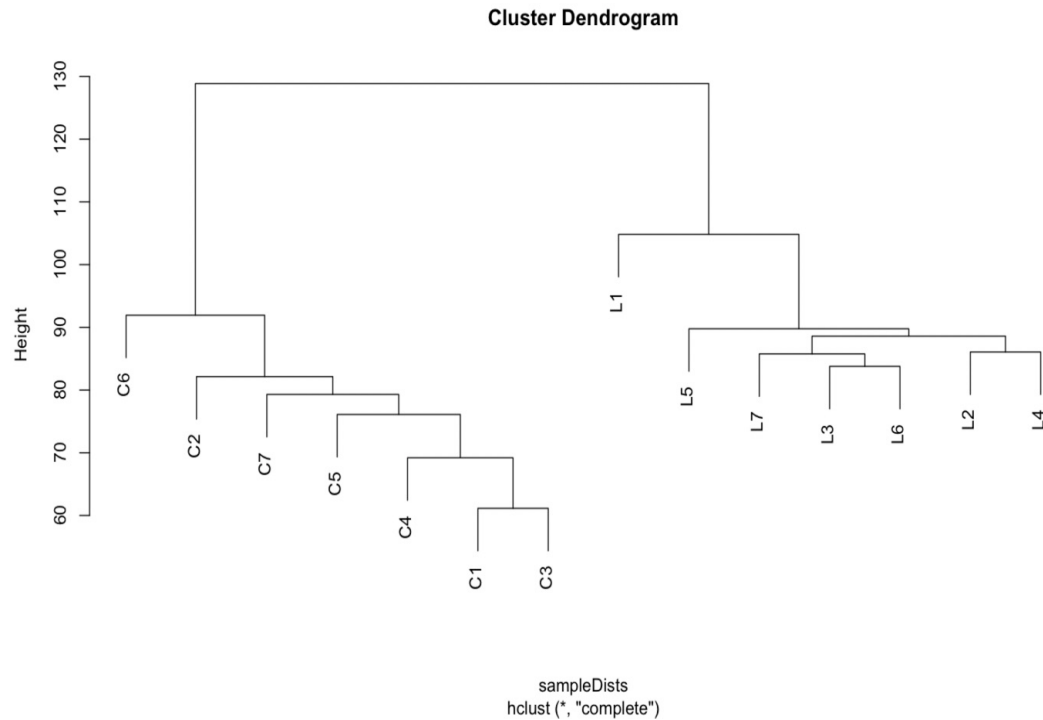
- First alignment on miRNA database (miRbase 22)
- Second alignment on the genome

In both cases ***Bos taurus*** was used instead of *Bubalus bubalis* because there is no specific annotation for **buffalo miRNAs**

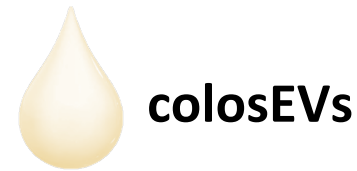
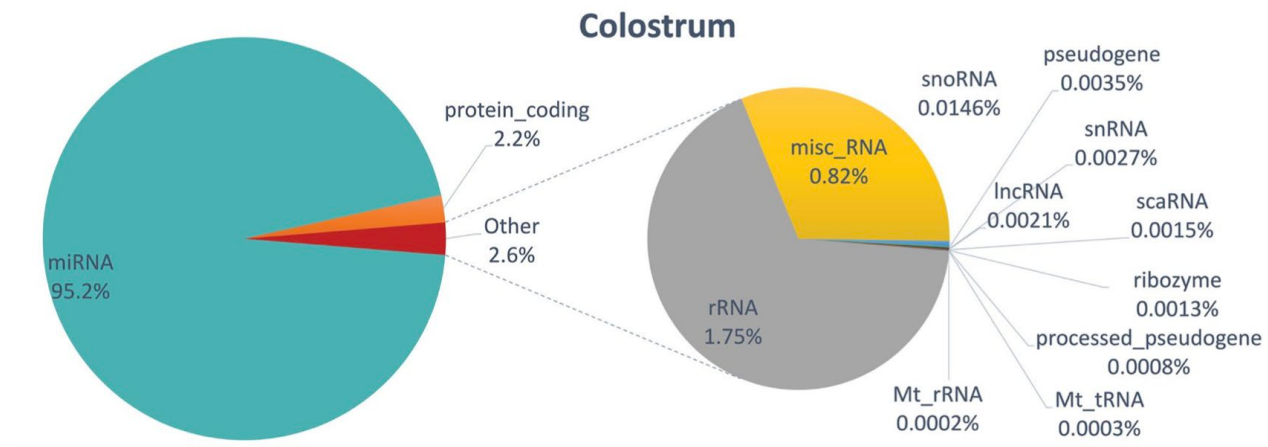
33 million of raw reads → 28 million after trimming → 33% overall alignment rate of uniquely mapped reads



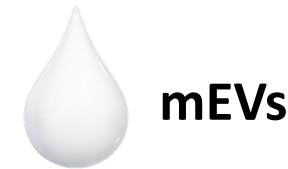
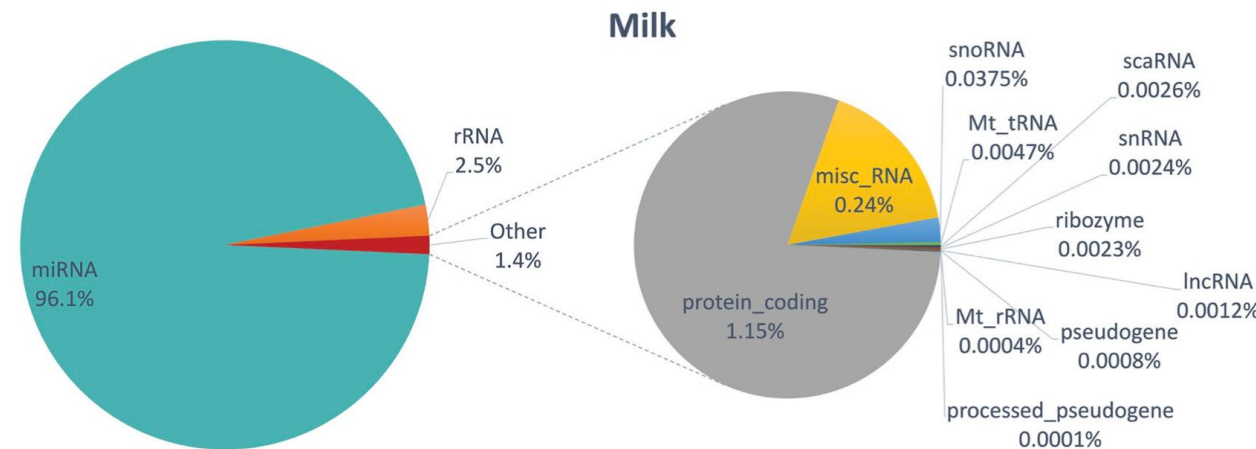
Considering genes with a mean normalized count (normDESeq2) >1 in at least 4 out of 7 samples



Type abundancies



colosEVs

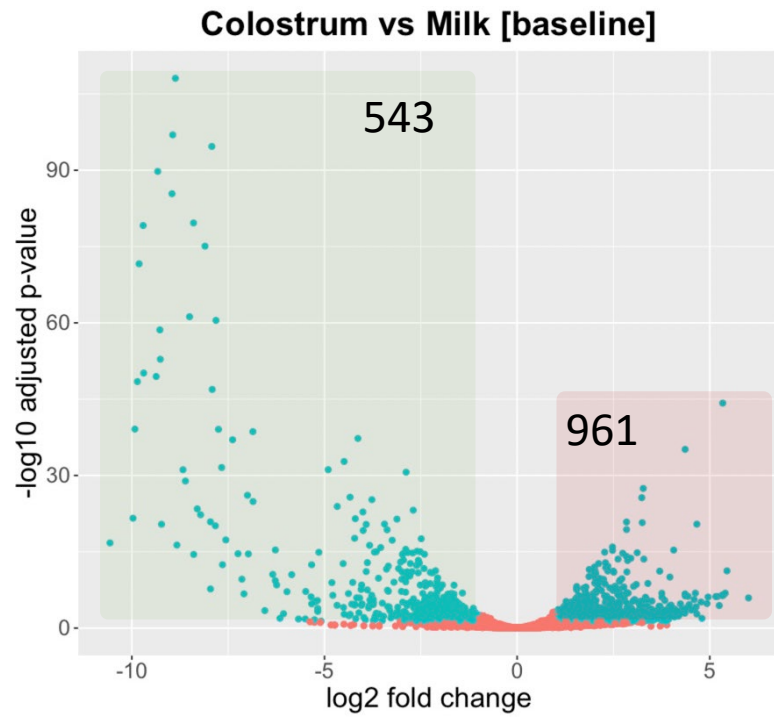


mEVs

Considering genes with a mean normalized count (normDESeq2) >1 in at least 4 out of 7 samples

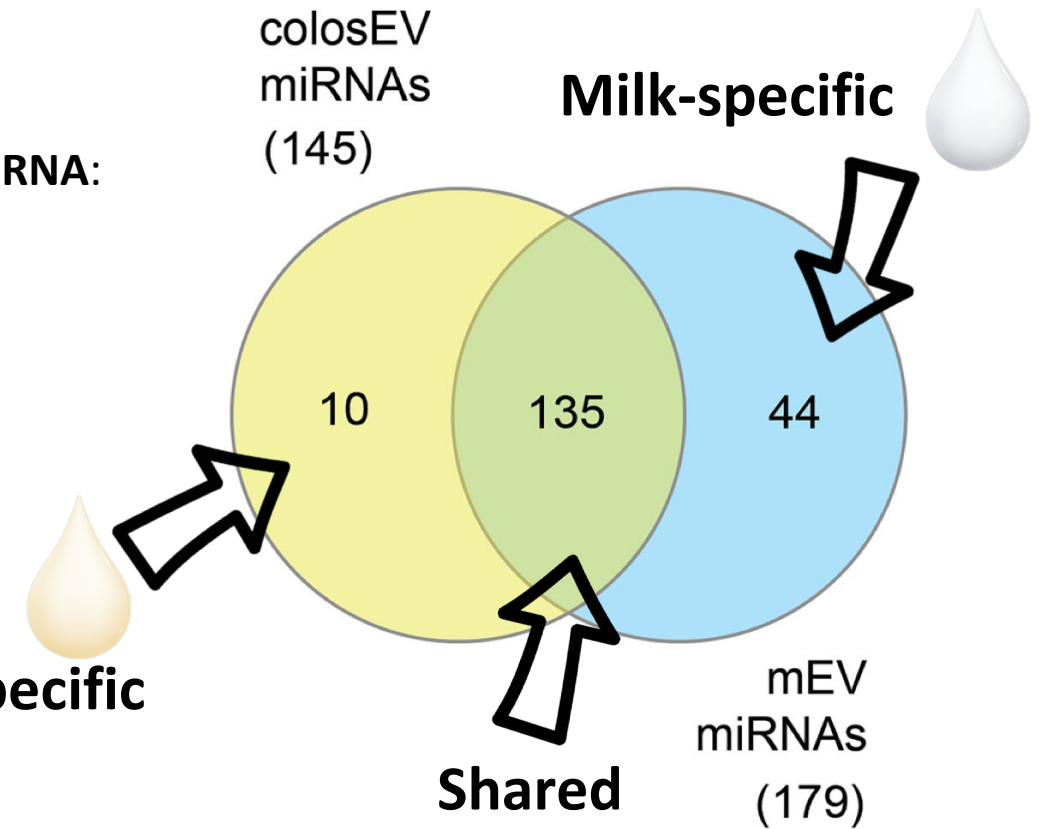


Cargo comparison



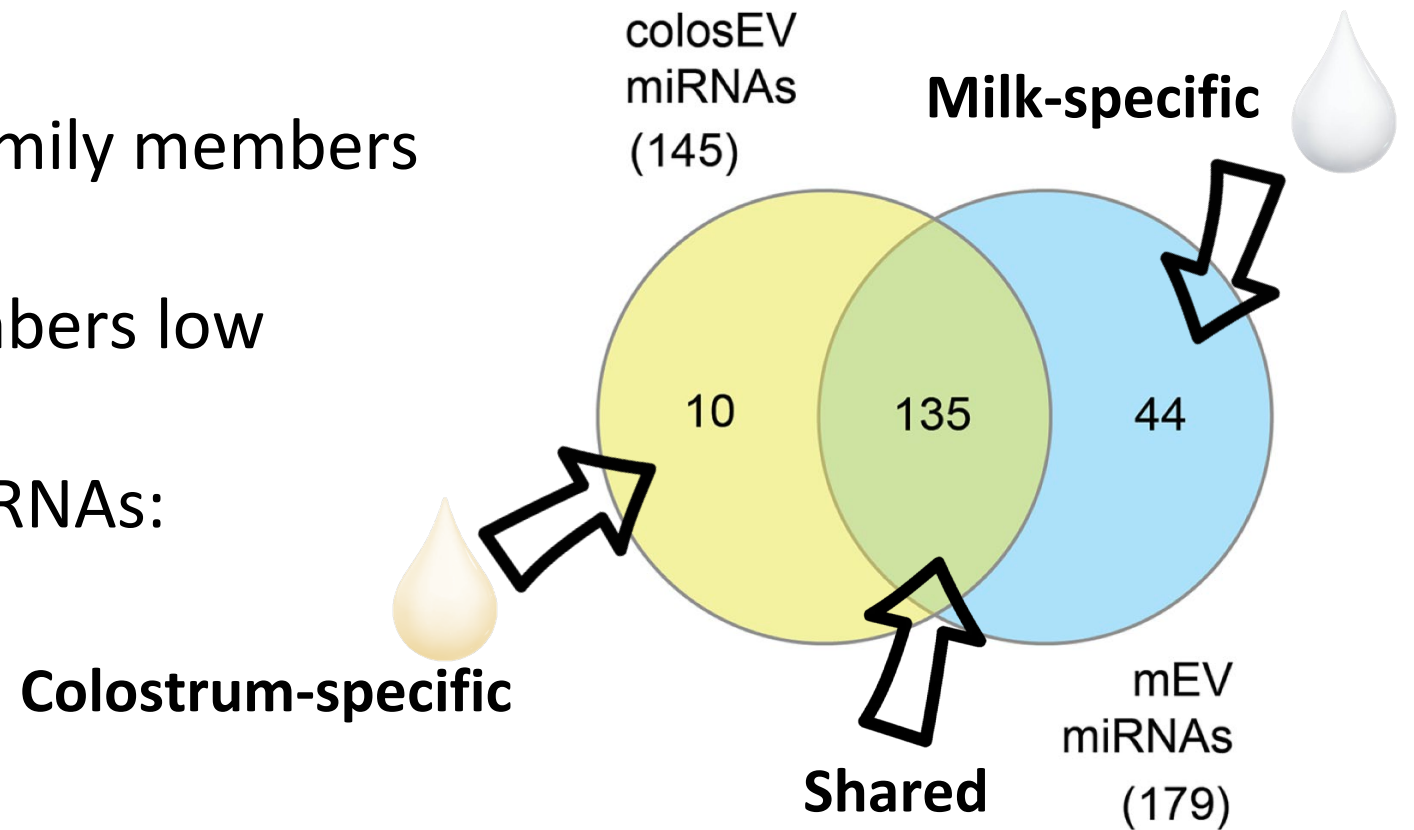
For the predominant class **miRNA**:
28 were up-regulated
184 down-regulated

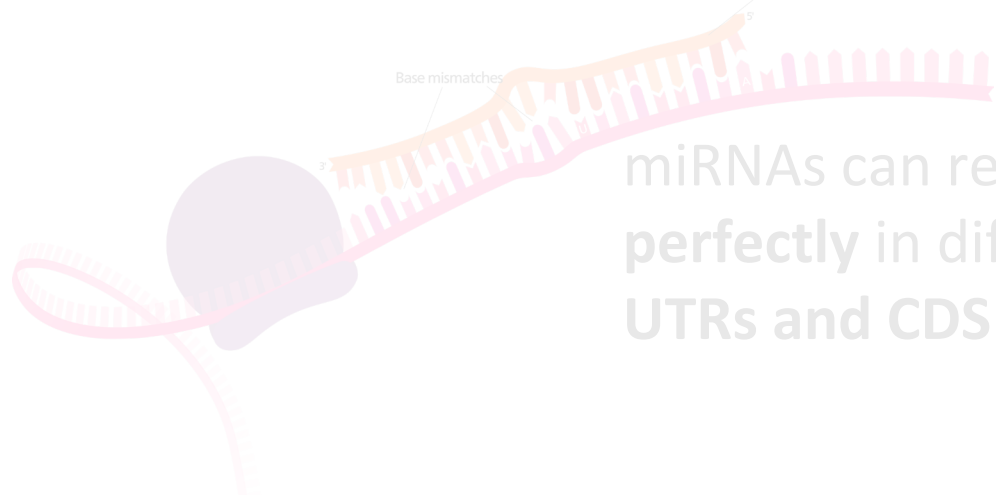
DEGs, $\log_2$ Fold Change – $\log_2FC > |1|$ and adjusted $p < 0.05$



Cargo comparison

- bta- mir-30 and bta-mir-200 family members enriched in both
- bta-mir-148 and bta-let-7 members low represented
- ColosEV- specific important miRNAs: mir-2284a, mir-301a, mir-211



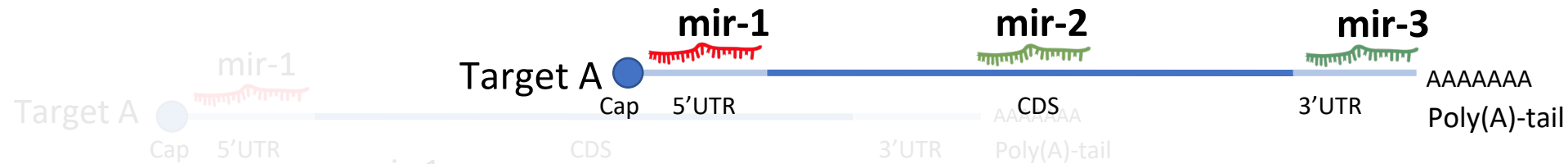


miRNAs can repress gene expression matching **non perfectly** in different mRNA compartments, UTRs and CDS



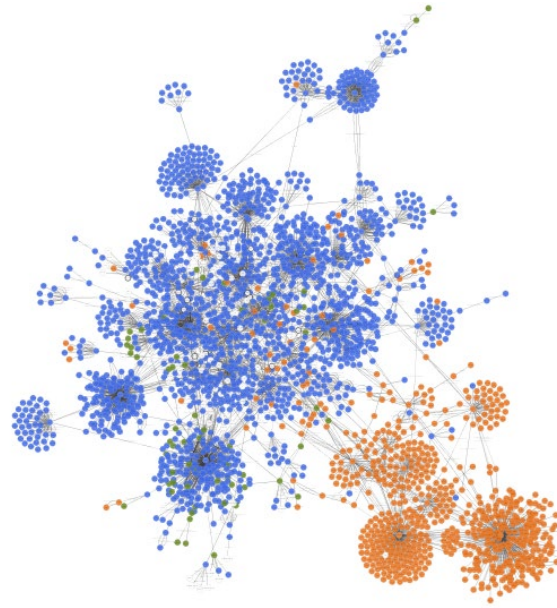
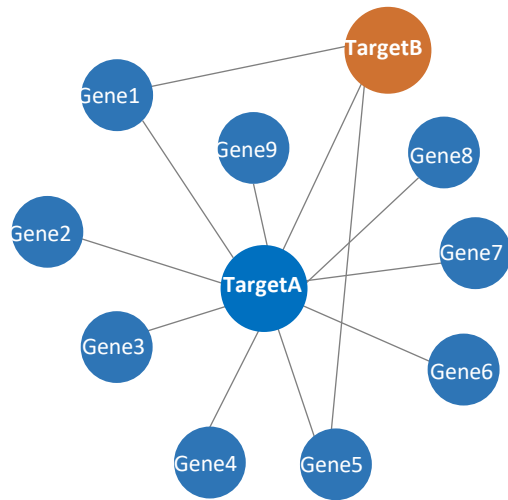
Each miRNA can bind many genes (targets)

Target selection

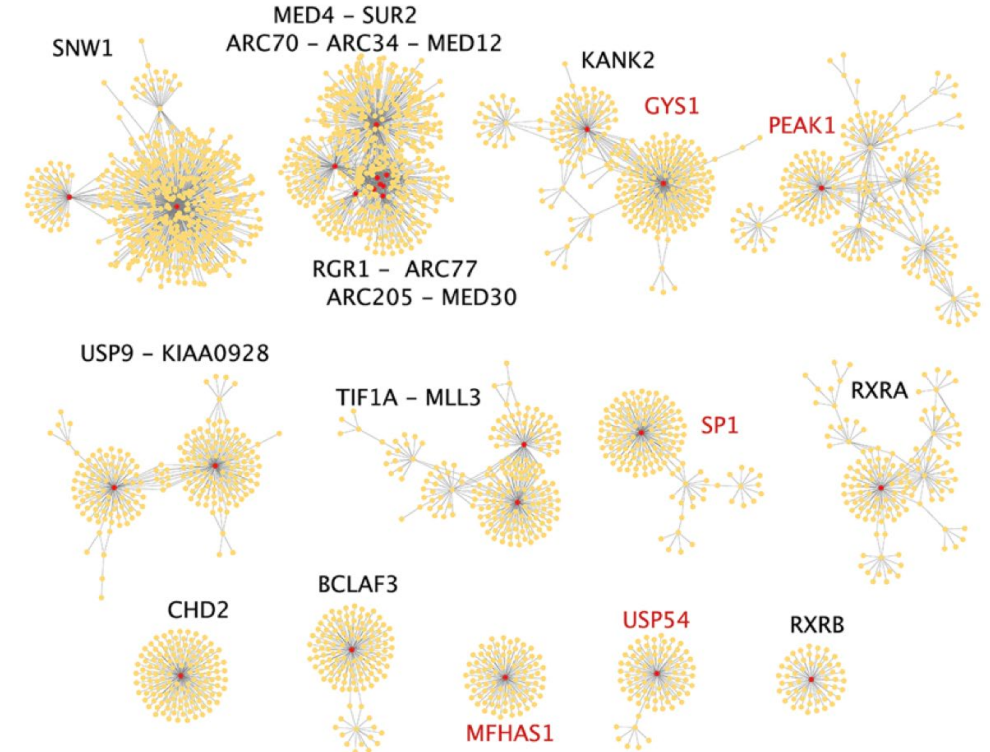


Targets were filtered basing on a threshold of miRNA hits
50% of miRNAs

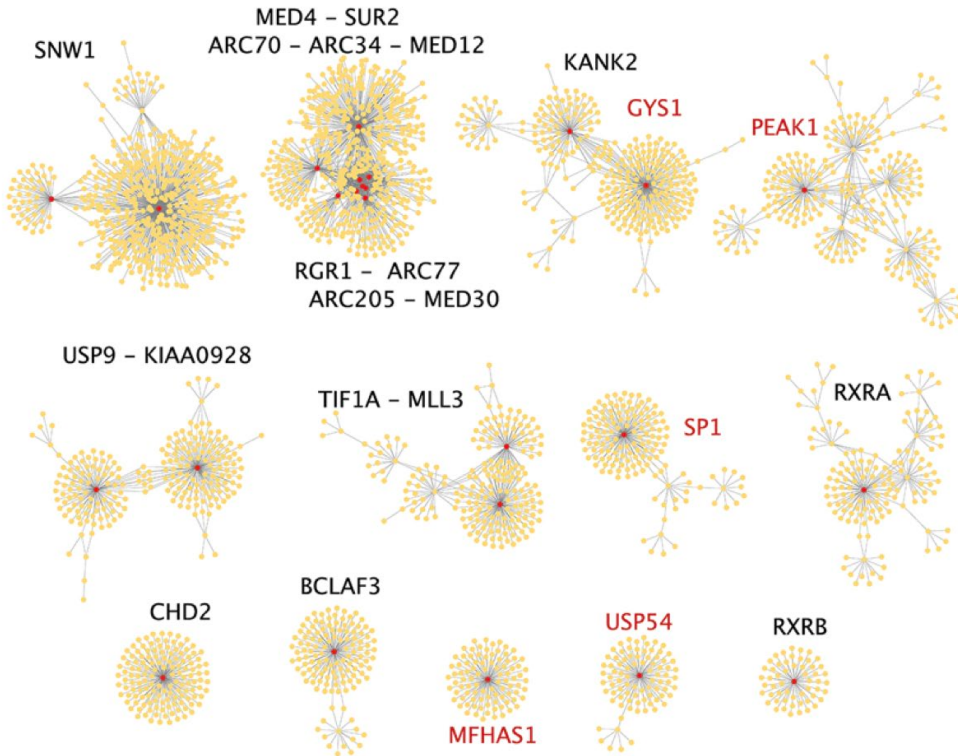
Huge complexity



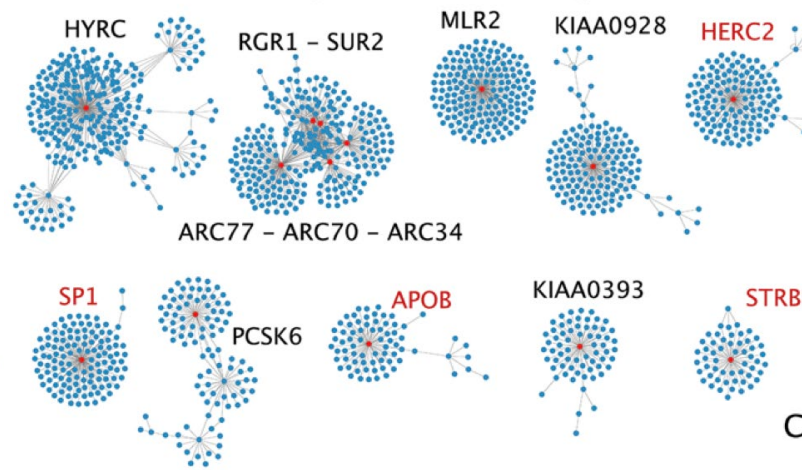
A) colosEV-specific miRNA targets



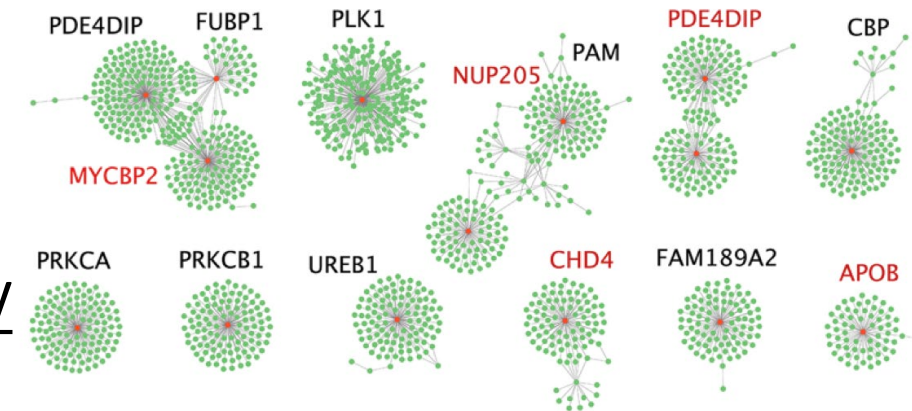
A) colosEV-specific miRNA targets



B) mEV-specific miRNA targets



C) shared most expressed miRNA targets

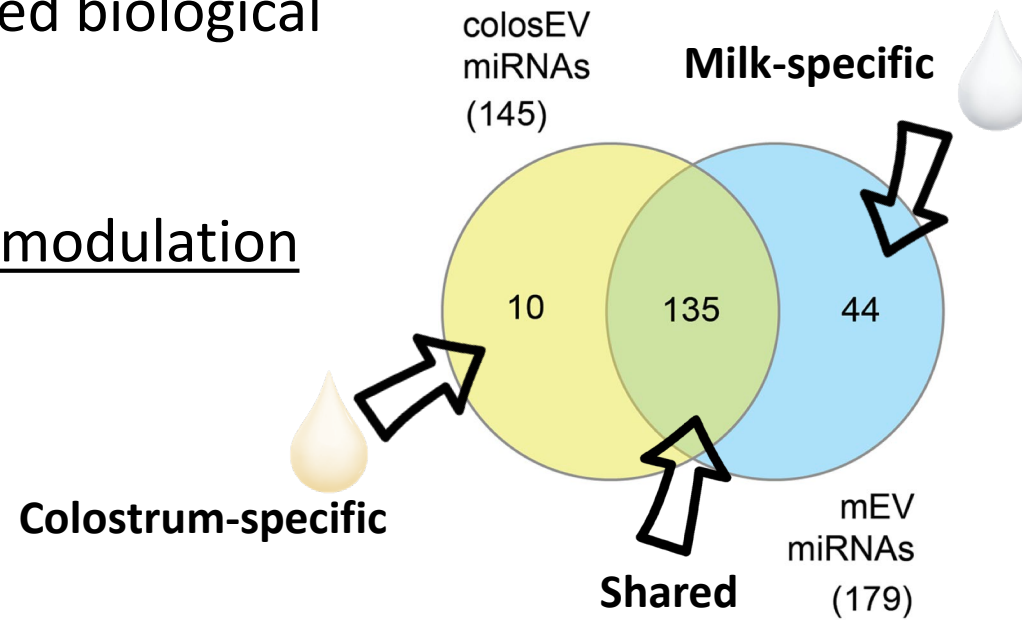


The functional analysis
on targets showed a
greater number of
interactions for colosEV
miRNAs



Enriched GO Biological Processes

Huge amount of enriched biological processes for:
Intestinal health
Immune-inflammatory modulation
epigenetic regulation



Many biological processes related to immune-inflammatory regulation

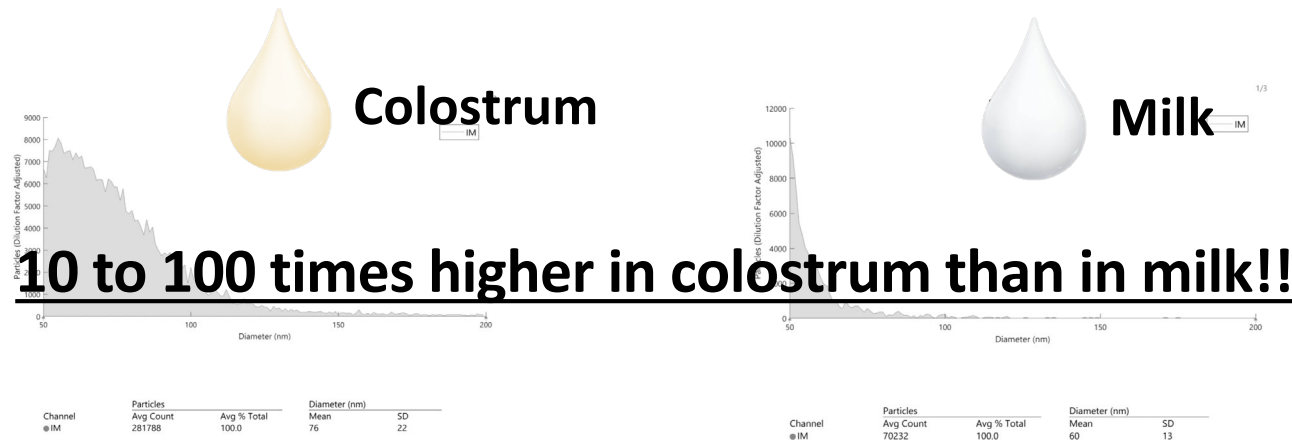
Enriched GO terms related to cell cycle, cytoskeleton organization, vesicular transport and ion transport, as well as terms related to the intestinal homestays.



- ✓ Presence of molecules, especially miRNAs, potentially capable of modifying metabolic processes of recipient cells involved in **signal transduction**, cell cycle and **immune response** as for EVs of other previously characterized species
- ✓ **Epigenetic** message from colosEVs
- ✓ the **difference is made by the quantity**



EXOVIEW



Pay attention to the **conservation of milk and colostrum** which can alter the EV structure thus literally **erasing the beneficial effects** of the cargo molecules in **receiving individuals**



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Prof. Katia Cappelli
Associate Professor AGR/17

Prof. Stefano Capomaccio
Associate Professor AGR/17

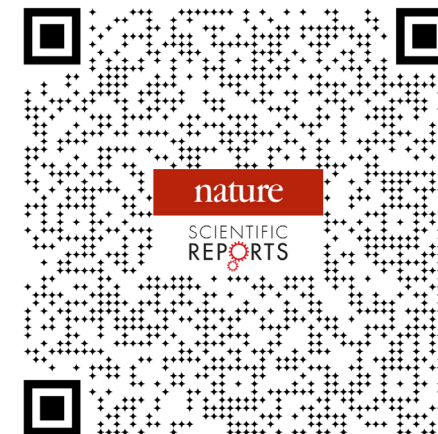
Samanta Mecocci, PhD
Postdoctoral researcher

Cristina Nocelli, PhD
Postdoctoral researcher

Prof. Luisa Pascucci
Associate Professor VET/01



Full paper!



Thank you for the attention!!



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Prof. Giovanni Chillemi
Associate Professor AGR/17



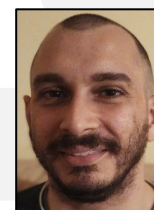
Prof. Umberto Bernabucci
Full Professor AGR/18



Prof. Loredana Basiricò
Associate Professor AGR/18



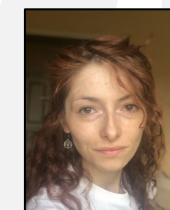
Marco Milanese, PhD
Junior researcher (RTDa)



Daniele Pietrucci, PhD
Postdoctoral researcher



Federica Gabbianelli, PhD
Technician (DNA lab)



Chiara Evangelista
PhD students