Subclinical mastitis regulation by extracellular vesicles miRNAs in dairy cows

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INRAE

UNIVERSITÀ DEGLI STUDI FIRENZE

Mastitis

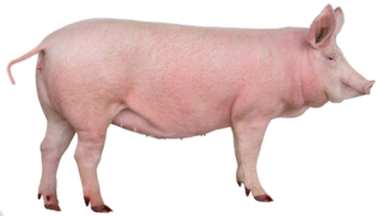
Bovine mastitis

Antimicrobials

EU Regulation
N. 6/2019

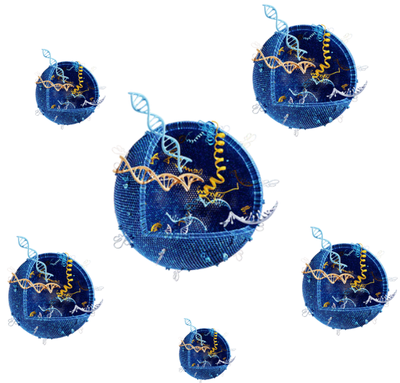
Antimicrobial prophylaxes in veterinary medicine



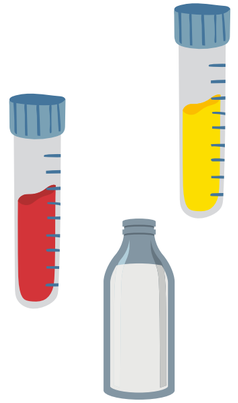




EVs and miRNAs

Bovine farming

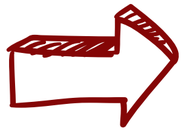


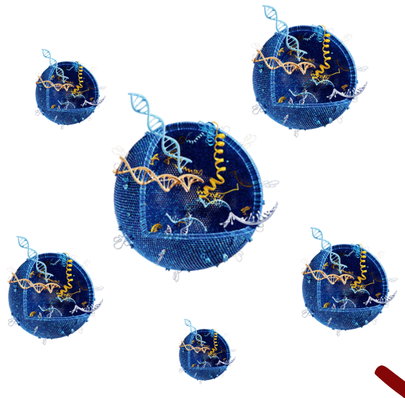
miRNAs in EVs as potential biomarkers



Biological fluids

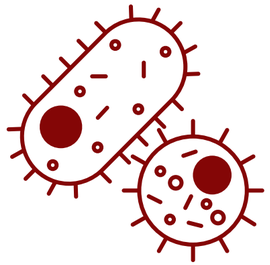
miRNAs in mastitis





miRNAs in EVs

Inflammation



Pathogens response

Immune response

Aims of the study

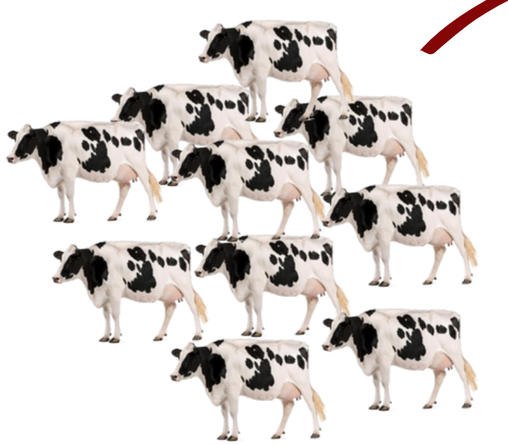
1

Evaluation of biological pathways regulated by miRNAs

2

Identification of putative early biomarkers of mastitis

Sampling



60 dairy cows in 10 farms from Piedmont

2 milk aliquots (50 ml each)

Microbiology

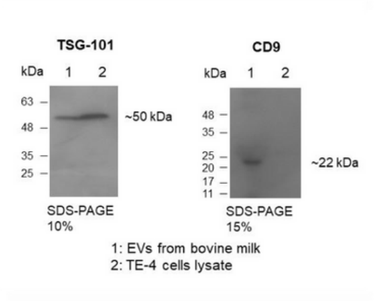
ARAP

Somatic cell count (SCC)

DSV

EVs

miRNAs workflow



Size exclusion chromatography and western blot (TSG-101 and CD9)

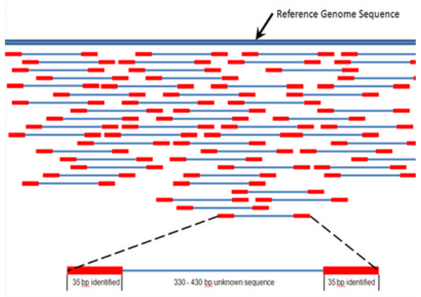
miRNAs extraction

smallRNA-seq on HiSeq (Illumina)

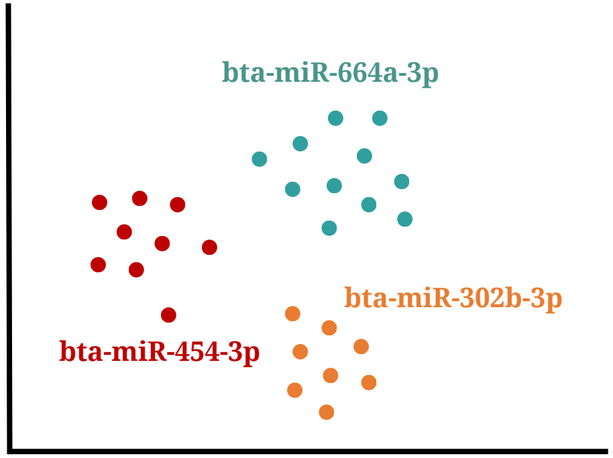
Bioinformatic analysis

miRNAs workflow

Trimming with cutadapt and trimmomatic



Reads were mapped to bovine miRNA precursors using BWAligner



Mature 3p or 5p forms were assigned and counted using docker4seq workflow

SCC < **200.000** cells/ml

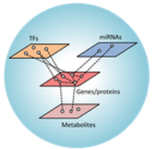
VS

SCC > **200.000** cells/ml

Differential analysis in RStudio

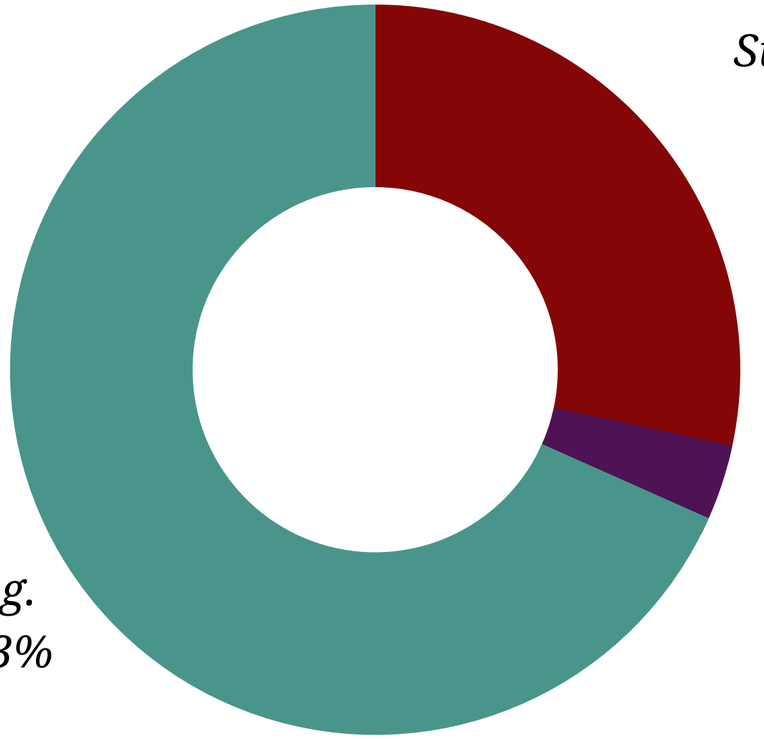
miRWalk 2.0

Cytoscape



Functional analysis

Microbiology and SCC

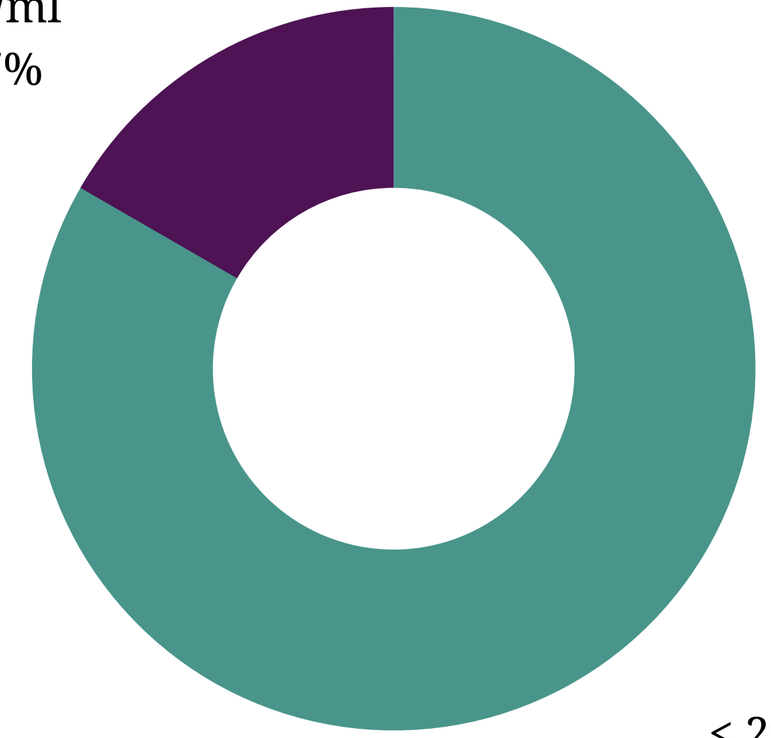


Staphylococcus spp.
28.3%

Streptococcus uberis
3.3%

Neg.
68.3%

> 200.000
cells/ml
16.7%



< 200.000
cells/ml
83.3%

(Cuccato et al., 2022)

DE miRNAs

1997

Differentially expressed miRNAs according to
SCC cut-off of 200.000 cells/ml

1267

Down-regulated miRNAs

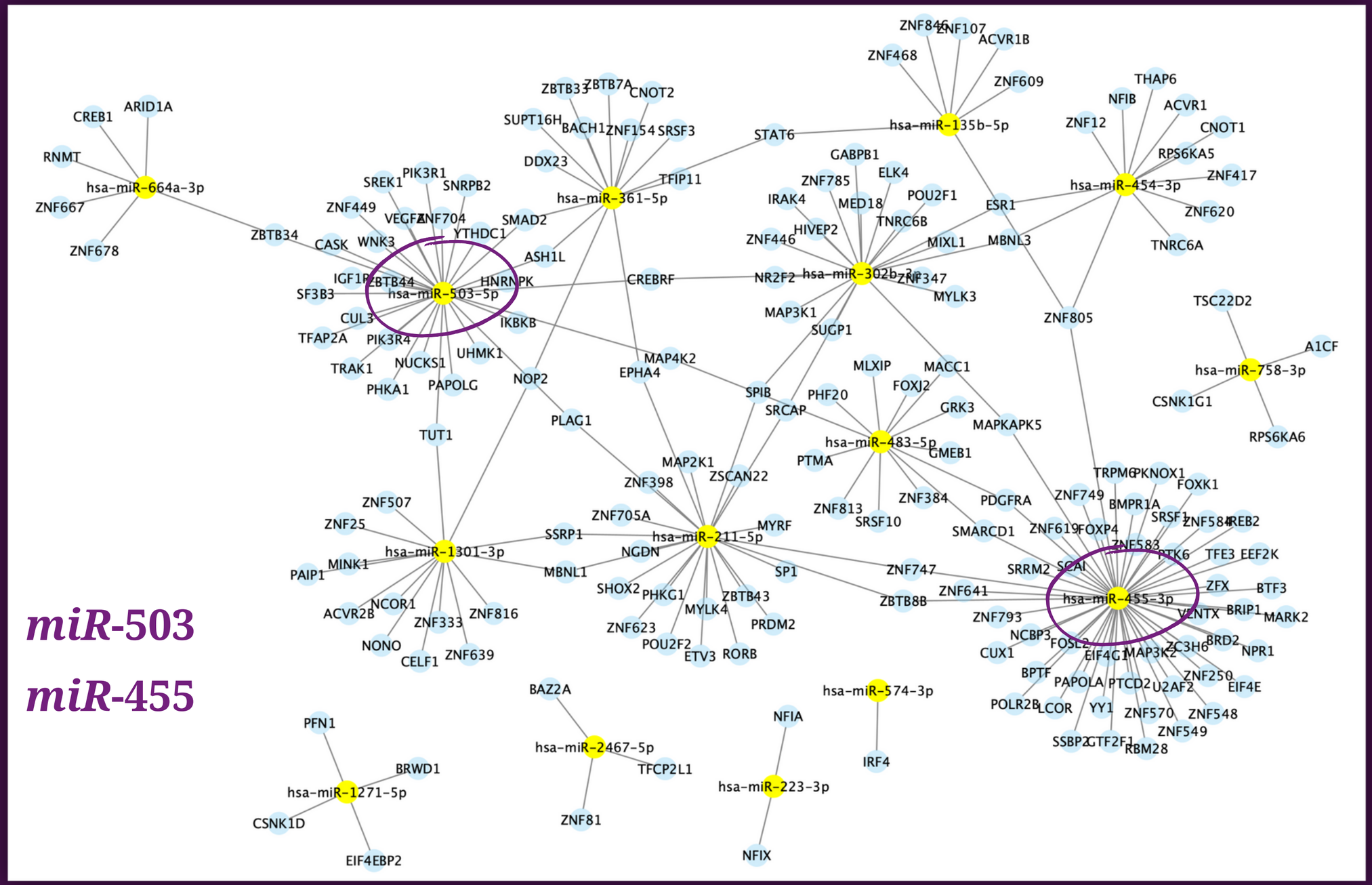
730

Up-regulated miRNAs

68

FDR < 0.05
Mostly (67) down-regulated miRNAs

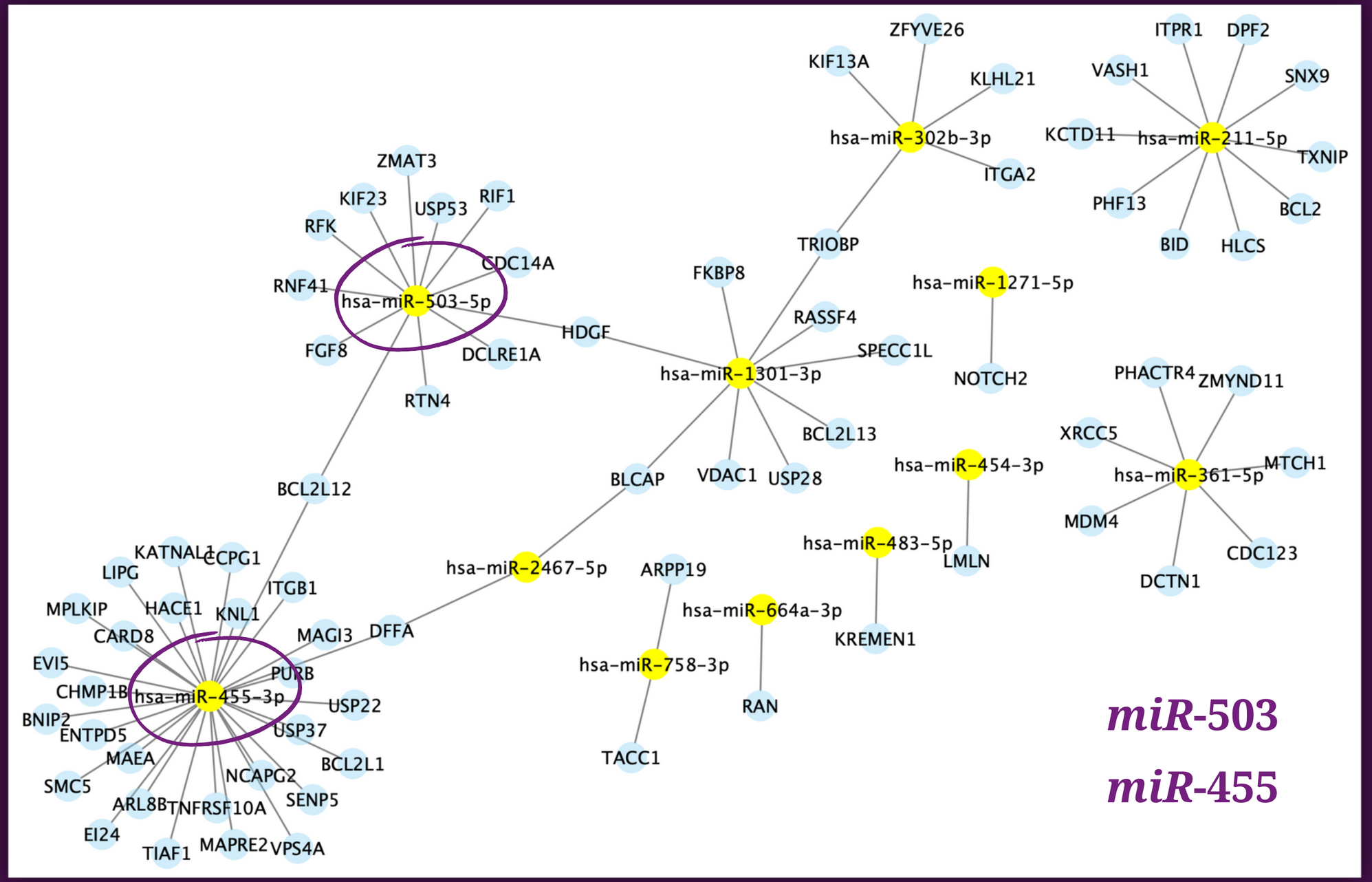
miRNA-gene interactions involved in gene expression processes



***miR*-503**

***miR*-455**

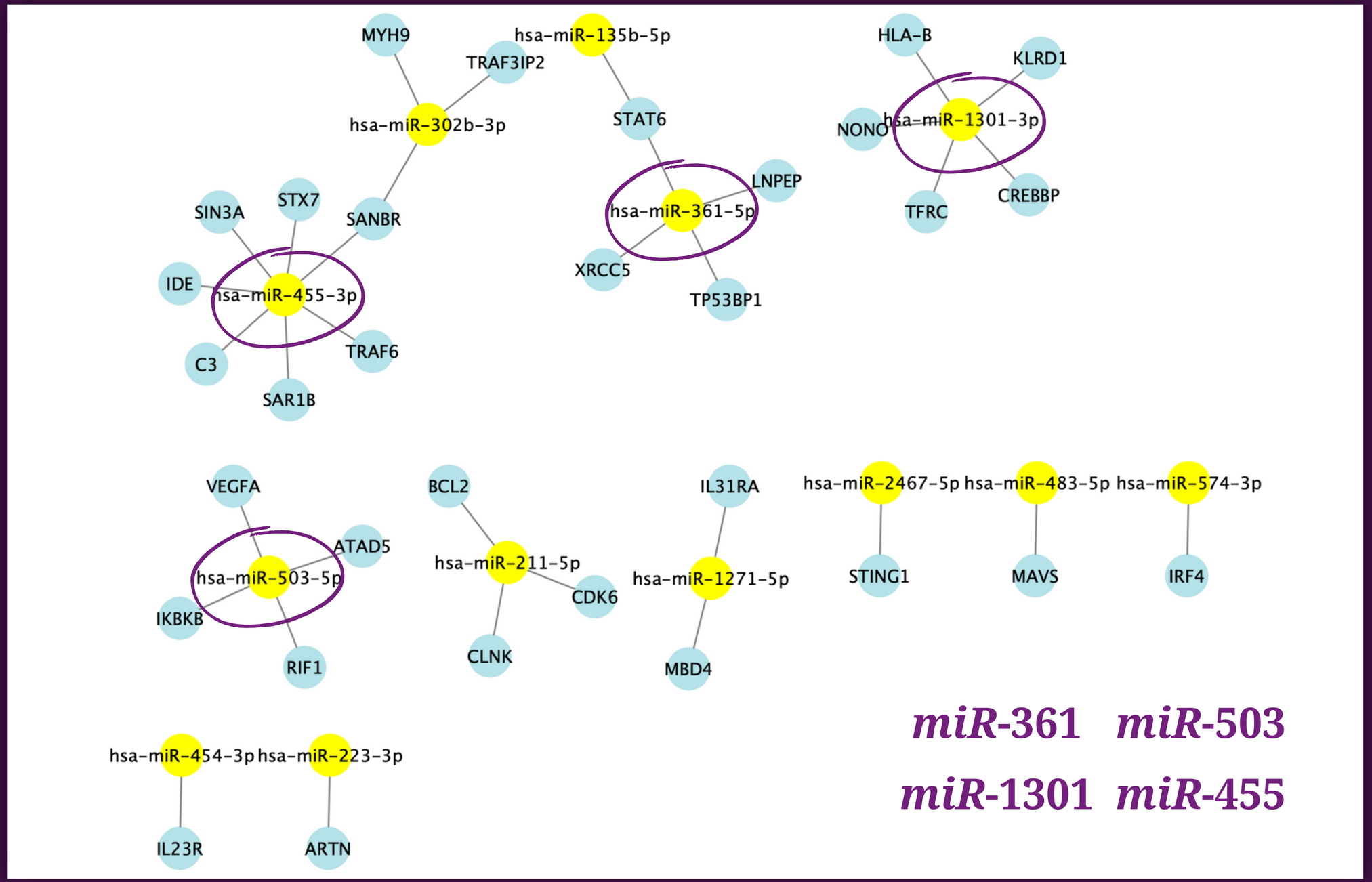
miRNA-gene interactions involved in cell life processes



***miR*-503**

***miR*-455**

miRNA-gene interactions involved in immunity processes



***miR*-361** ***miR*-503**

***miR*-1301** ***miR*-455**

The promising four

miR-361

Involved in the regulation of host-pathogen interaction (Qi et al., 2012; Ndzi et al., 2019)

In dairy farming, miR-361 is differently regulated between free-grazing and housed cows (Muroya et al., 2015)

miR-455

Similar results in dairy cows subjected to dietary restriction (Webb et al., 2020)

Activation of inflammatory pathways (Torabi et al., 2015)

The promising four

miR-503

Involved in the pathogenesis of diabetes and LPS injury in human (De Silva et al. 2018; Zapala et al., 2023)

miR-503 downregulation seems to be involved in the activation of NF-kB signalling and PPAR-gamma pathway (Zhou et al., 2013; Lee et al., 2017)

miR-1301

Upregulation in blood collected from dairy cows experimentally infected by *S. aureus* (Luoreng et al., 2018)

miR-1301 may be strictly related to the etiological agent causing the mastitis

Conclusion

This study presents an in-field scenario with naturally mastitis-affected cows, more reliable than experimental conditions

Moreover, the large dataset of this study (60 dairy cows) provides new insights in the study of bovine mastitis

Conclusion

One limitation of this research is the narrow representation of mastidogen bacteria (i.e. absence of gram negative bacteria)

In conclusion, the four main miRNAs highlighted here and their affected biological processes seem promising results in the discovery of new biomarkers for mastitis detection

Thank you for your attention

A special mention to all the people involved in this project

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