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Extracellular vesicles from *Staphylococcus aureus* remodulate the bovine mammary epithelial cell transcriptome

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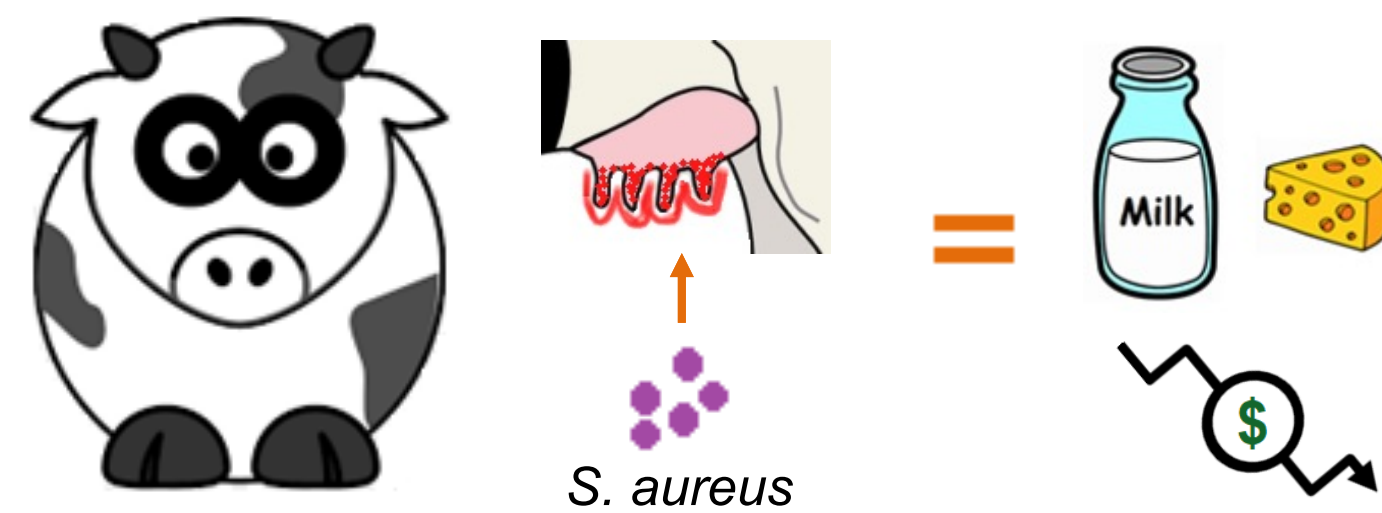
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INTRODUCTION

- Mastitis, an inflammation of the mammary gland, is a prevalent production disease in dairy herds worldwide affecting animal health and milk quality and causing economic losses.



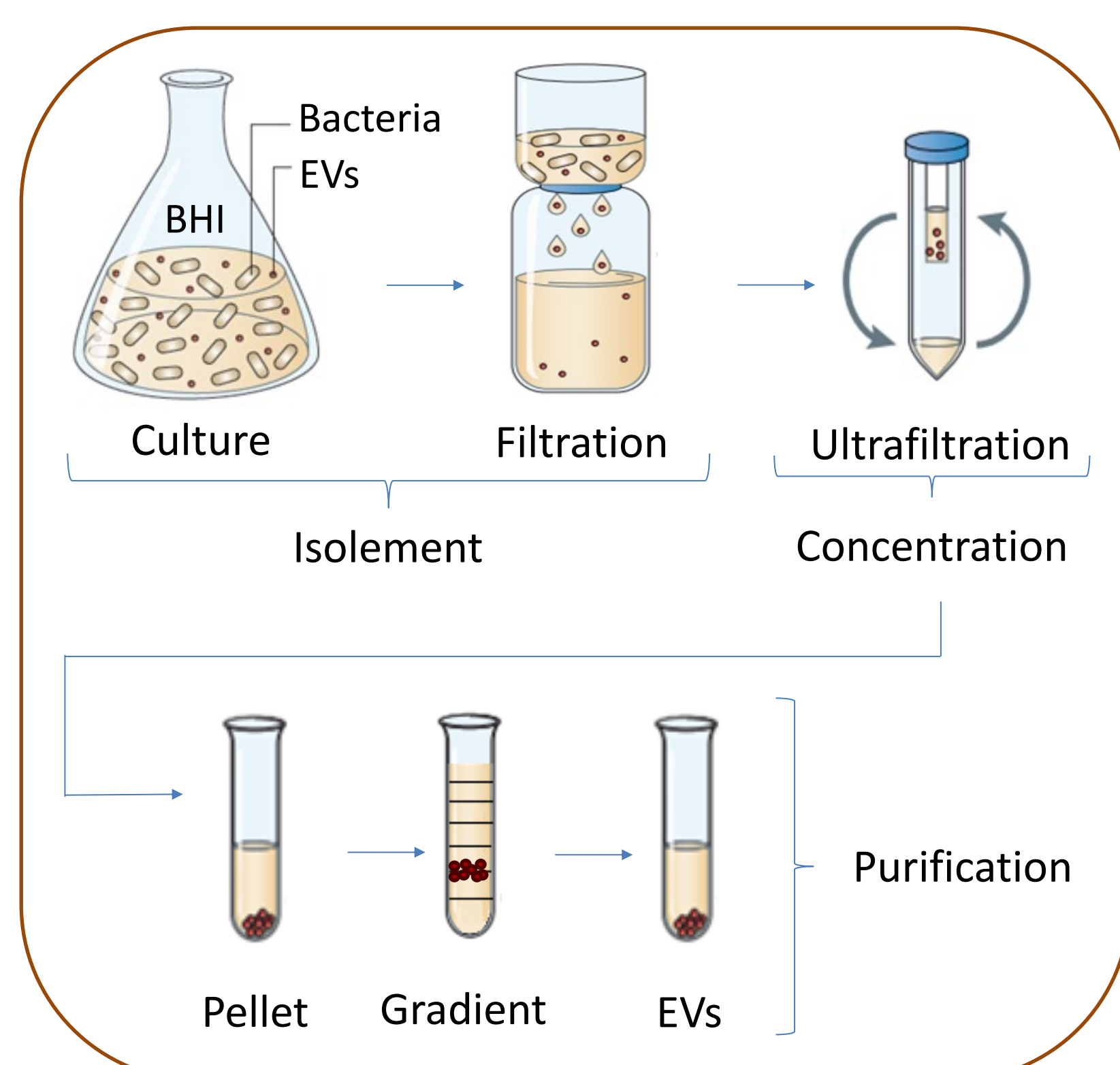
- *Staphylococcus aureus* is one of the most important etiological agent of mastitis.
- Treatment against *S. aureus*-induced mastitis is still ineffective.

- A better knowledge on host-pathogen interactions is required to develop novel preventive or curative strategies against *S. aureus* mastitis.
- Extracellular vesicles (EVs) are nano-sized particles secreted by most cells and involved in intercellular communication.
- EVs secreted by *S. aureus* N305, a bovine mastitis isolate, induce a pro-inflammatory response expression *in vitro* and promote tissue inflammation *in vivo*.

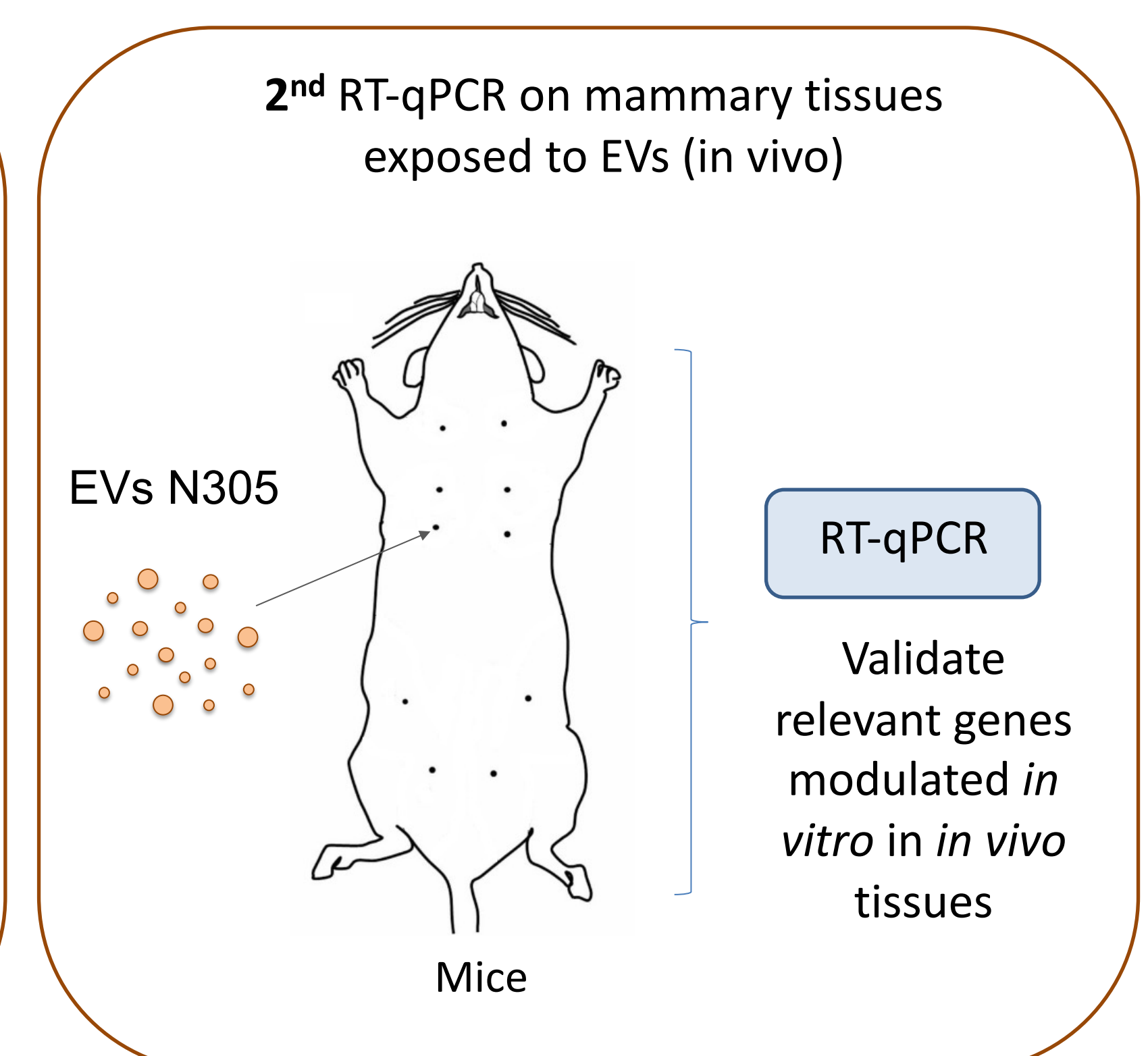
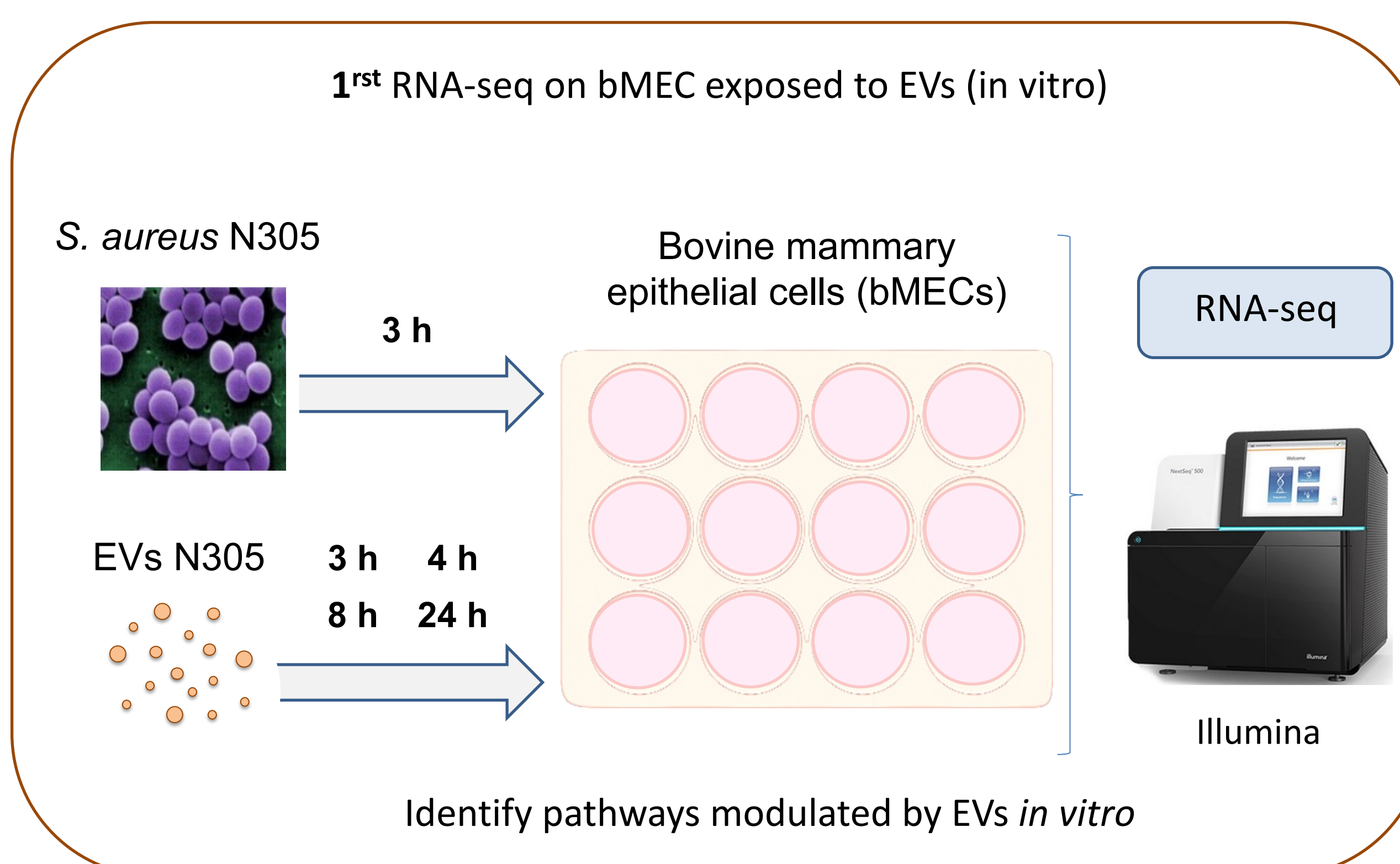
Objective: identification of genes and signaling pathways involved in EVs-host cell interactions

MATERIAL AND METHODS

EVs purification protocol



Wide identification and validation of genes and signaling pathways triggered by *S. aureus* EVs



RESULTS & DISCUSSION

Threshold: $|\log_2 FC| > 0.5$

Time	Down	Up	Total
EV 3h	3	113	116
EV 4h	10	100	110
EV 8h	13	219	232
EV 24h	85	433	518

Number of modulated genes increase with time

Each time point have specific modulated genes

57 genes are modulated at all times

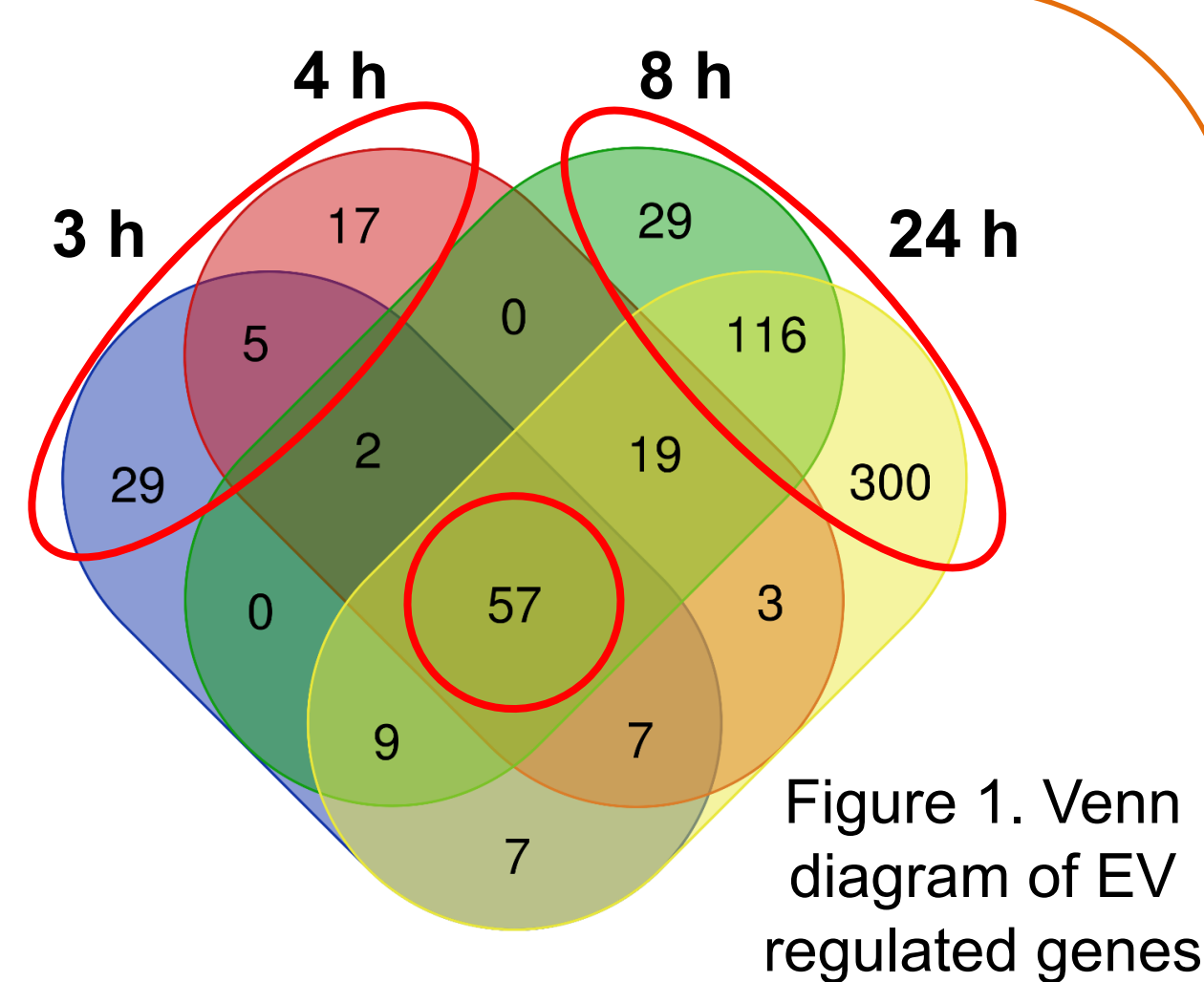


Figure 1. Venn diagram of EV regulated genes

IL-1 β	NF- κ B
IL-6	TNF- α
IL-8	CASP8

1 EVs activate a vast number of genes, regulating inflammatory, necroptotic and apoptotic pathways

Gene	$\log_2 FC$
APOL-3	4.8
CD83	4.3
ICAM-1	1.1

49 genes modulated exclusively by EVs at 3h

EVs modulate the host immune response by different routes from those of the bacteria cell

2

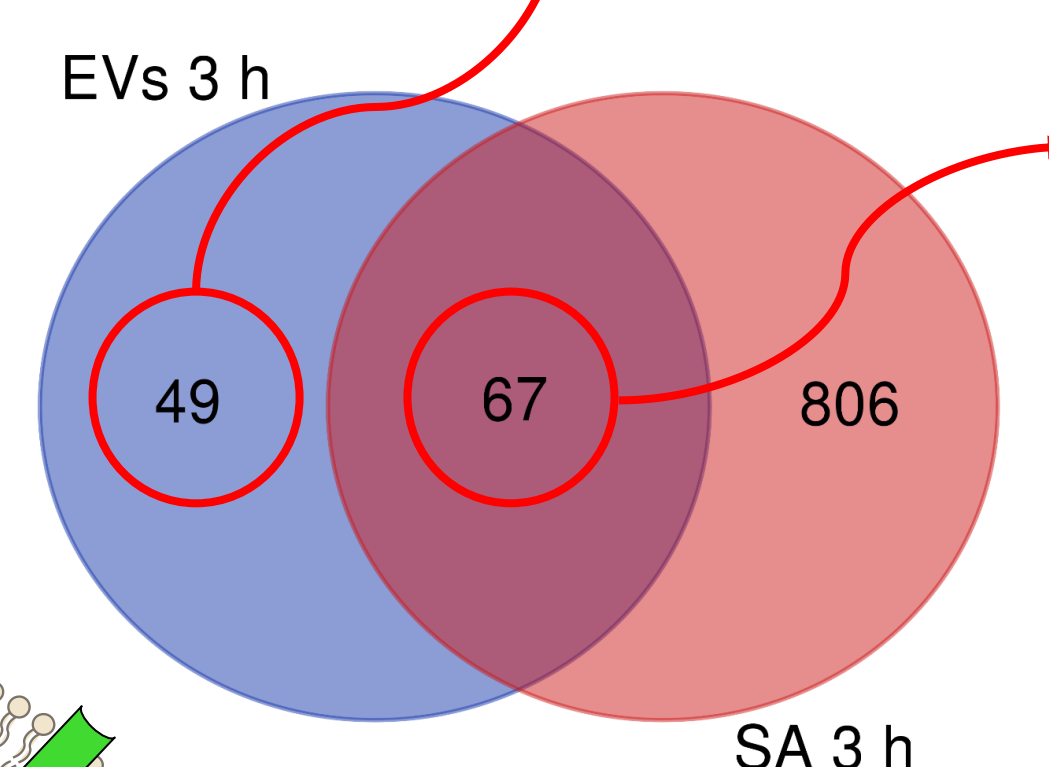


Figure 2. Venn diagram of genes regulated by EVs and *S. aureus* at 3h.

Common regulated genes include those with similar and different expression

Gene	EVs	SA
TLR10	1.1	1.4
PLAT	0.6	1.9
IL-1 β	5.6	2.8
IL-8	4.4	2.9

- PAMPs recognition
- Plasminogen activation
- Inflammatory response

EVs modulate the host response with intensities different from those of bacteria

3

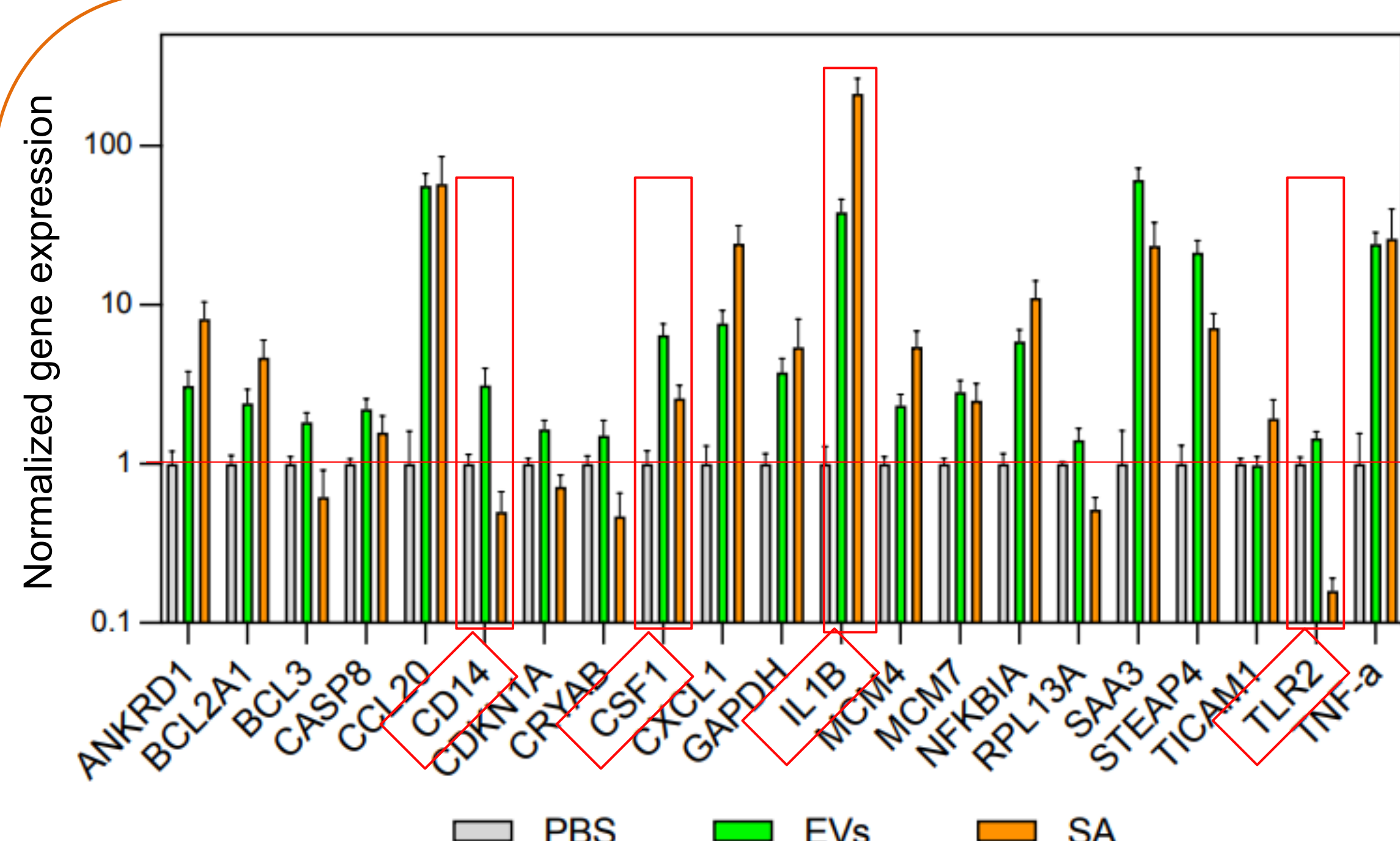


Figure 3. Twenty-one bMEC cell genes modulated by EVs *in vitro* in a bovine model were validated *in vivo* in a murine model

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EVs also exerts specific functions in a mastitis murine model

Secreted EVs modulate the host response differently from live bacteria...

Intensity
Time
Routes

... and may contribute to the control and development of *S. aureus* pathogenesis



Targets to fight infections



Drug and vaccine development