Unveiling the Secrets of Long-Term Staphylococcus aureus Infections: Insights into Inflammation, Metabolism, and Epigenetic Changes from Non-Immune Cells Transcriptomes

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Unveiling the Secrets of Long-Term *Staphylococcus aureus* Infections: Insights into Inflammation, Metabolism, and Epigenetic Changes from Non-Immune Cells Transcriptomes

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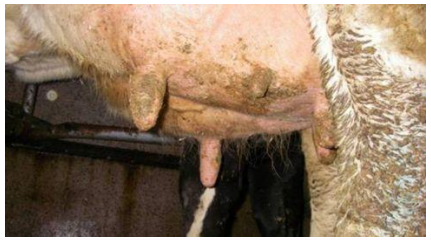
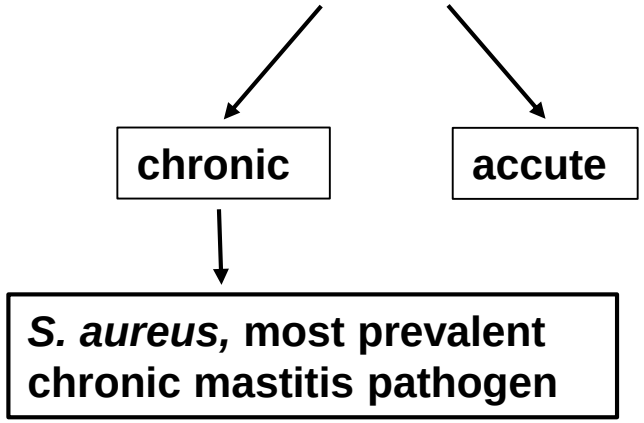
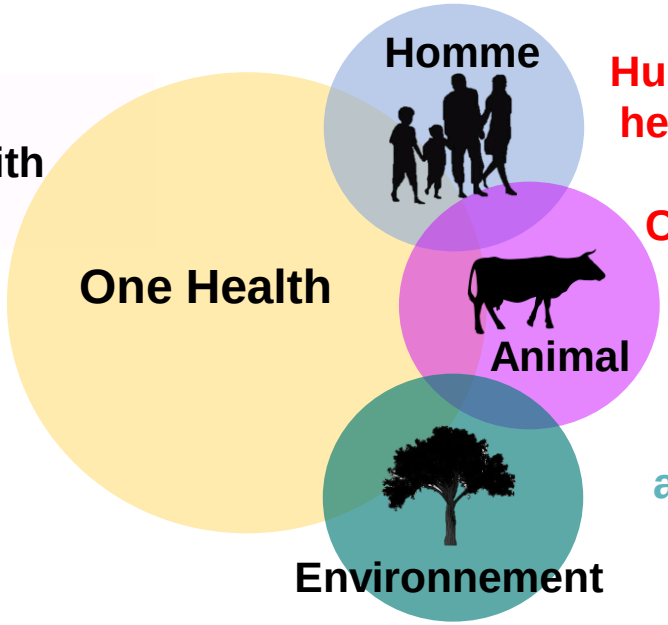
https://www6.rennes.inrae.fr/stlo

Persistent *S. aureus* infection is an economic/animal welfare problem and a serious public health burden

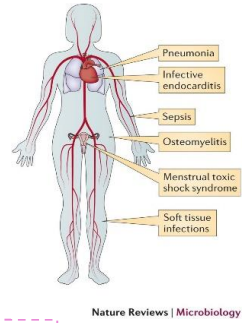


Mastitis:
inflammation (often associated with infection) of the udder





Life-threatening infections



Osteomyelitis

Cross contamination

Mastitis

Animal health/wellbeing

Emergence of antibiotic resistant bacteria

Urgent Need → **Host's defense against recurrent *S. aureus* infection**

HYPOTHESIS

Chronic infections requires intracellular asymptomatic carriage of *S. aureus*

The compelling reasons to study non-immune cells in host-pathogen dynamics

Site-Specific Defense:
Non-immune cells with an extended lifespan are located in tissues prone to infections

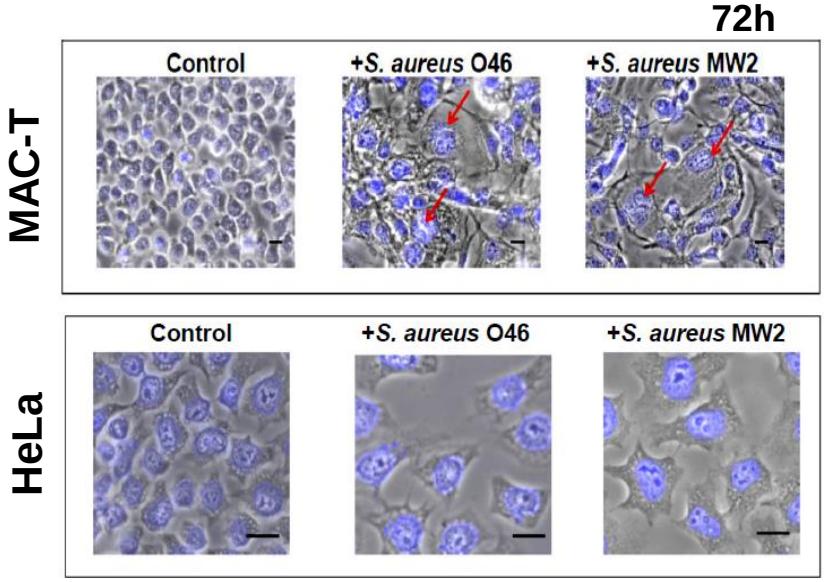
Chronic Infections:
Tissue-residents non-immune cells, contribute to infection persistence by internalizing pathogens

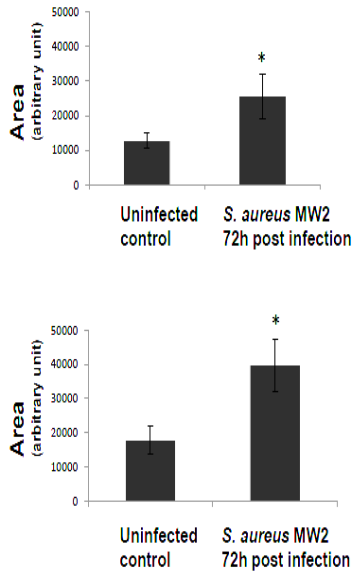
Cellular Crosstalk:
Immune cells & non-immune cells communication shapes a coordinated defense response

How non-immune cells defend against *S. aureus* Invasion?

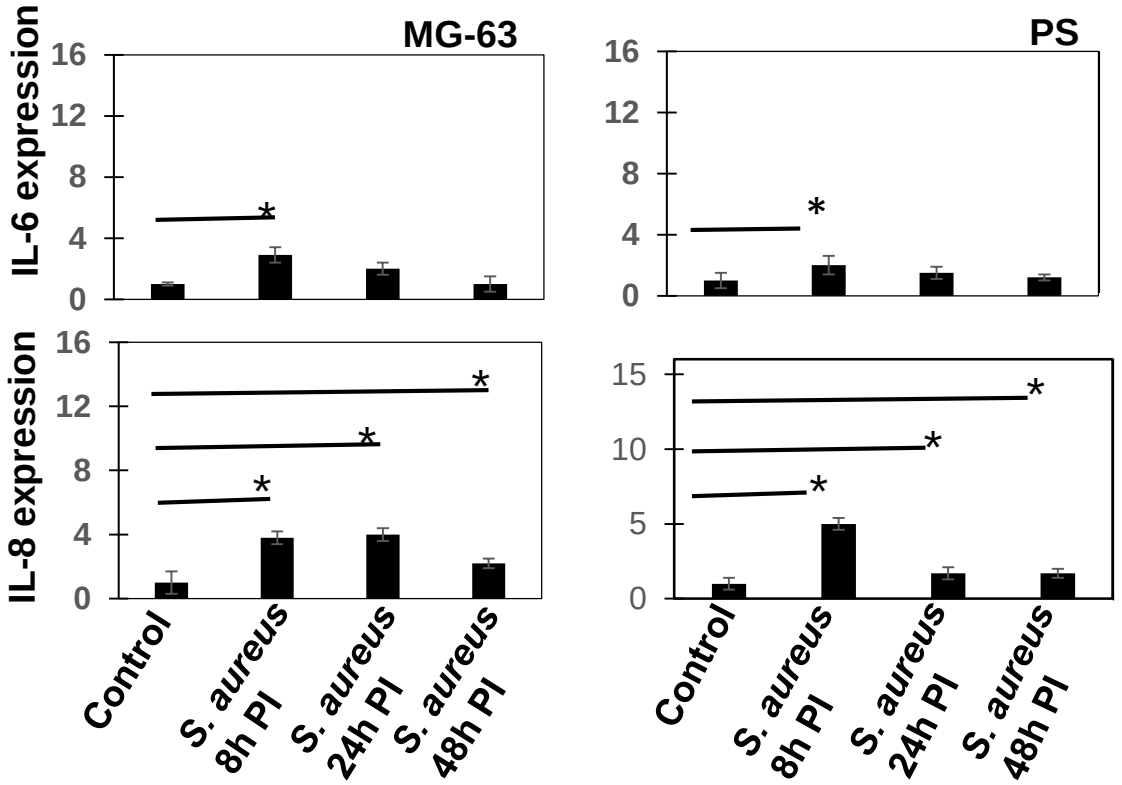
Similarities in the response of non-immune cells of various origins during *S. aureus* infection

Cytopathic effect:
Enlargement of the cells exposed to *S. aureus*





Expression of IL-6 and IL-8 in human MG-63 and bovine PS cells exposed to *S. aureus*



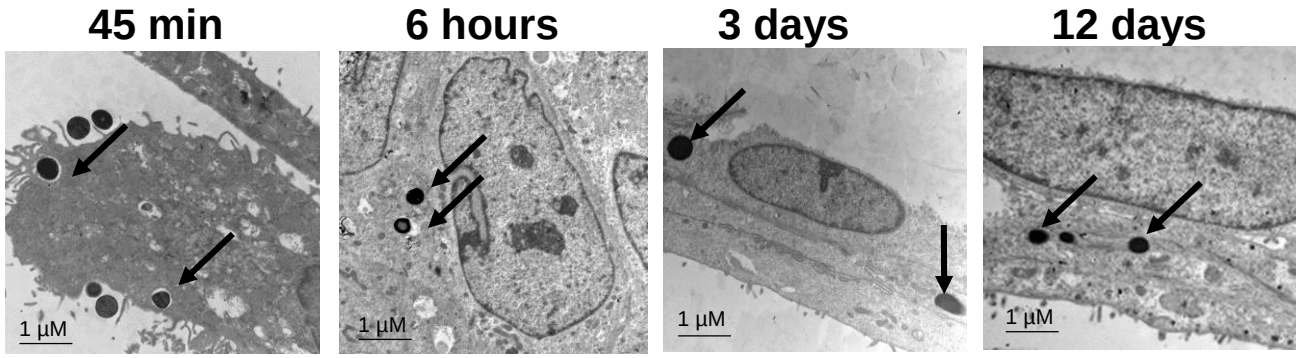
MAC-T, bovine mammary epithelial cells
PS, bovine mammary epithelial cells isolated from the secretory parenchyma
HeLa, human epithelial cells from a cervical carcinoma
MG-63, human osteoblast-like cells

Alekseeva *et al*., PlosOne, 2013
Deplanche *et al*., Infect. Immun, 2016

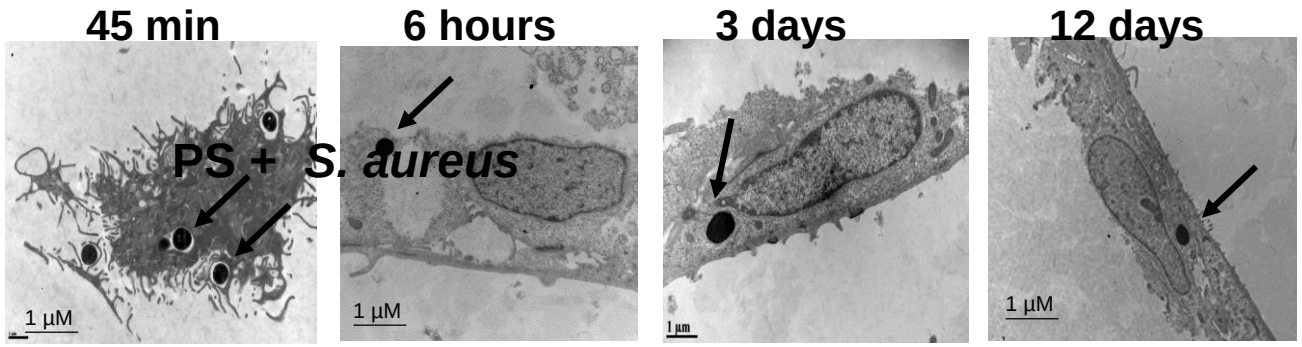
Long-term *S. aureus* infection of human and bovine non-immune cells

Transmission electron microscopy analysis of *S. aureus*-infected cells

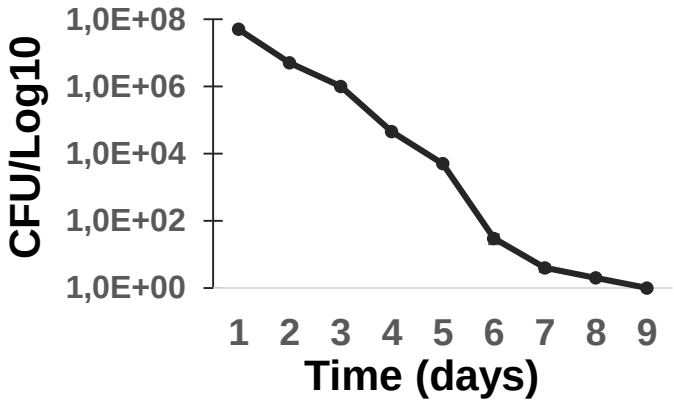
MG-63 + *S. aureus*



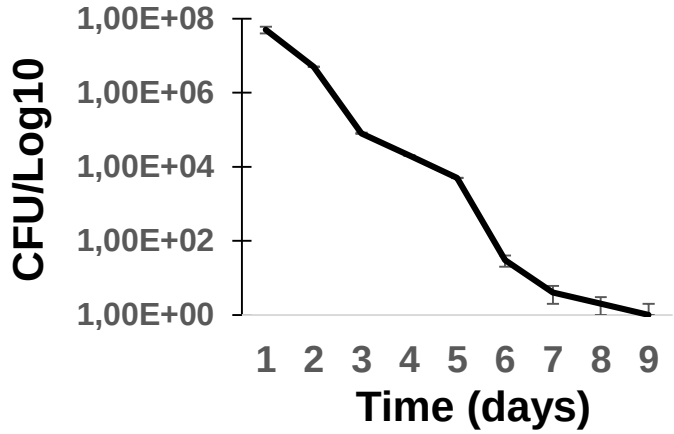
PS + *S. aureus*



Human MG-63 cells+mCherry*SA113*



Bovin PS cells +mCherry*SA113*



Most internalized bacteria are surrounded by phagosomal/lysosomal membranes, some bacteria are scattered freely in the cytosol

Lima Leite *et al.* Cell. Microbiol., 2020

Development of a model for long-term *S. aureus* infection

Human osteoblasts MG-63
Bovine mammry epithelial PS cells

Infection

mCherry
S. aureus

Infected eukaryotic cells

6 days

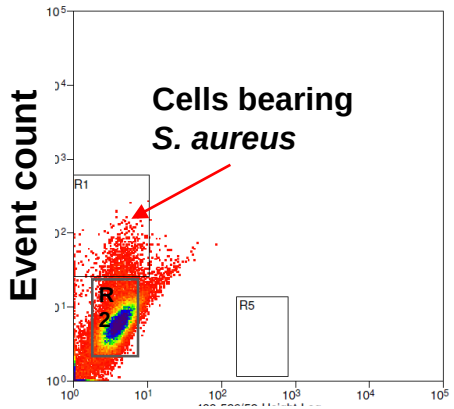
Antibiotic treatment

FACS

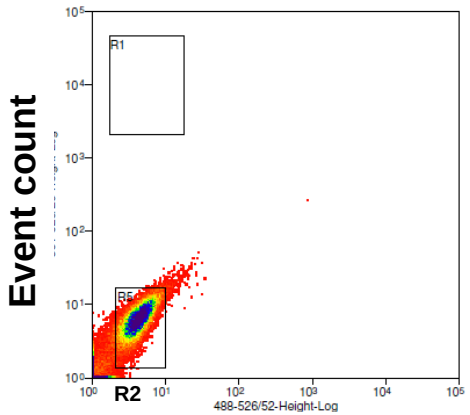
Enriched eukaryotic cells bearing *S. aureus*

5% of MG-63 and 1% of PC cells bearing internalized *S. aureus*

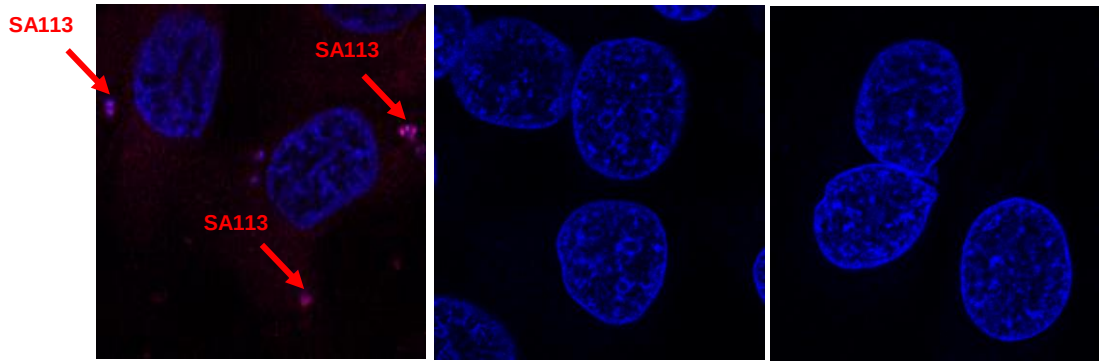
MG-63+ mCherry*SA113*



MG-63

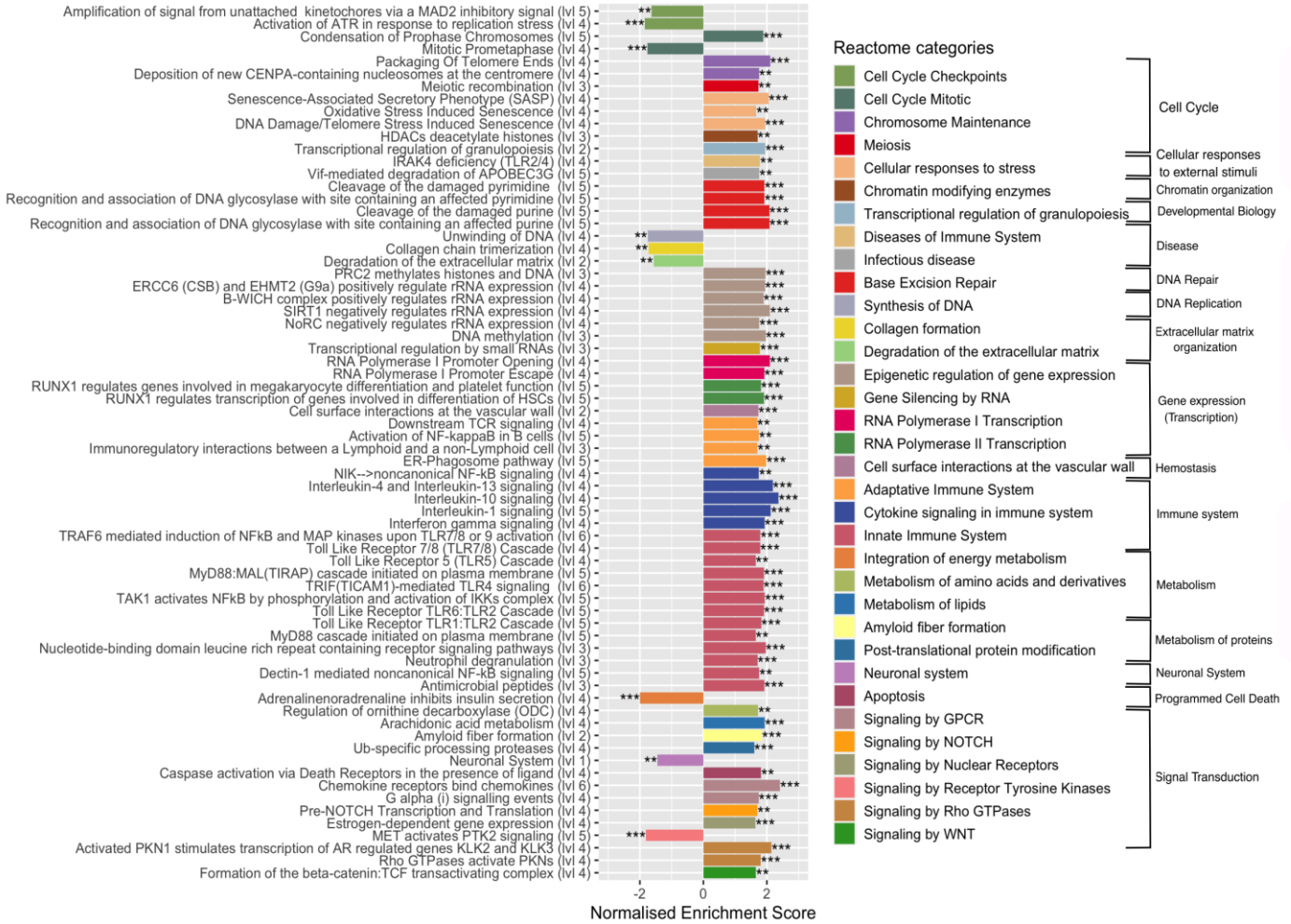


MG-63+*SA113* (R1) MG-63+*SA113* (R2) MG-63+(R2)



Isolating only S. aureus-bearing cells from mixed populations helps avoid bystander effects of uninfected cells and enables the identification of signals specific to intracellular infection

Enriched Reactome pathways



Immune system genes are among the top highly induced DEGs

Transcriptional reprogramming of genes associated to the cell cycle progression, DNA damage and repair

Transcriptional reprogramming of genes involved in metabolism

Transcriptional reprogramming of genes involved in epigenetic regulation

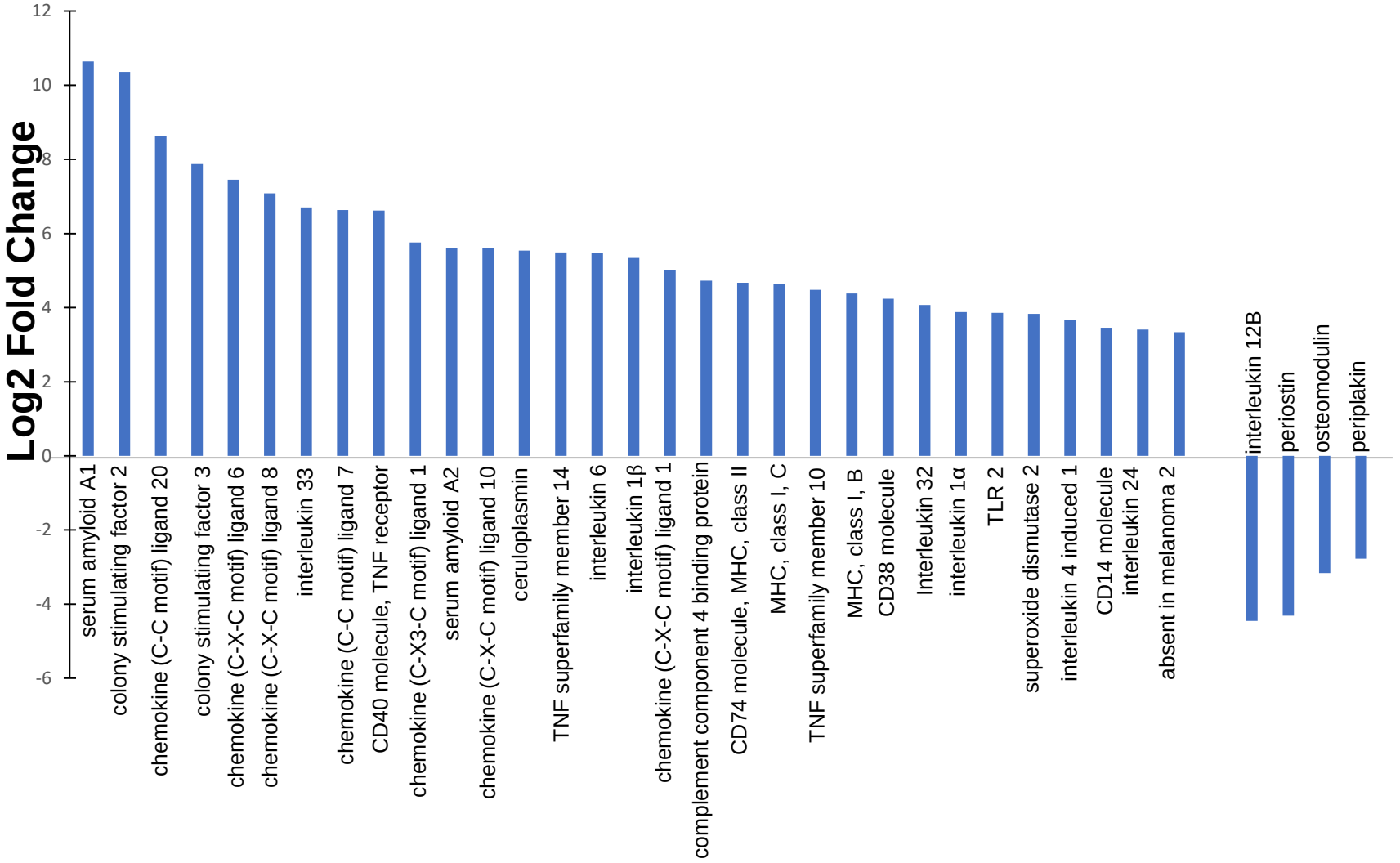
RNAseq

2,850 differential expressed genes (DEGs) with a threshold log2 FC -0.3 > log2FC > 0.3
1,514 of DEGs were upregulated, 1,336 were downregulate

Gene-Set Enrichment Analysis using Reactome Database
70 Reactome enriched pathways: 61 upregulated and 9 downregulated pathways

Nicolas et *al.*,
Front. Cell. Infect. Microbiol, 2022

Immune system and signal transduction genes are among the top highly induced DEGs

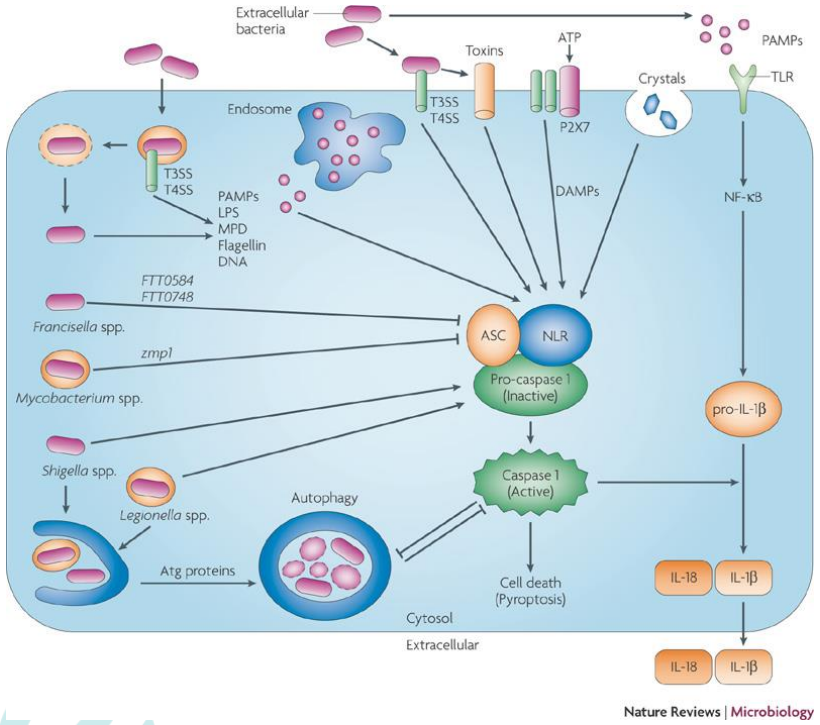


22 upregulated pathways were associated with the immune system

INFLAMMASOMES activation as a defense mechanism against infection and injury

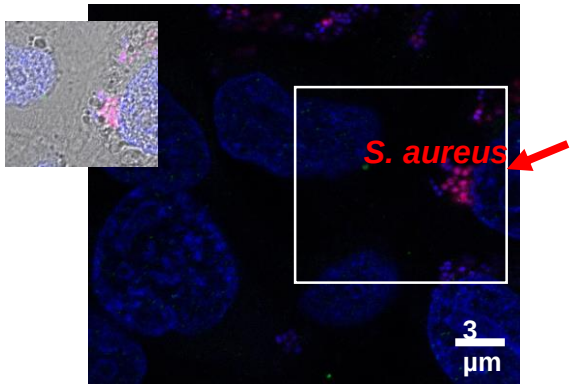
Inflammasomes, which assemble in response to danger signals or pathogens, are composed of a sensor (NLR), an adaptor (ASC), and procaspase-1

Inflammasomes activate Caspase-1, which proteolytically matures pro-IL-1β and pro-IL-18

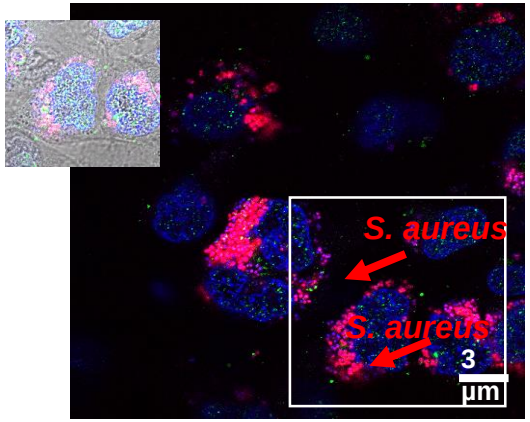


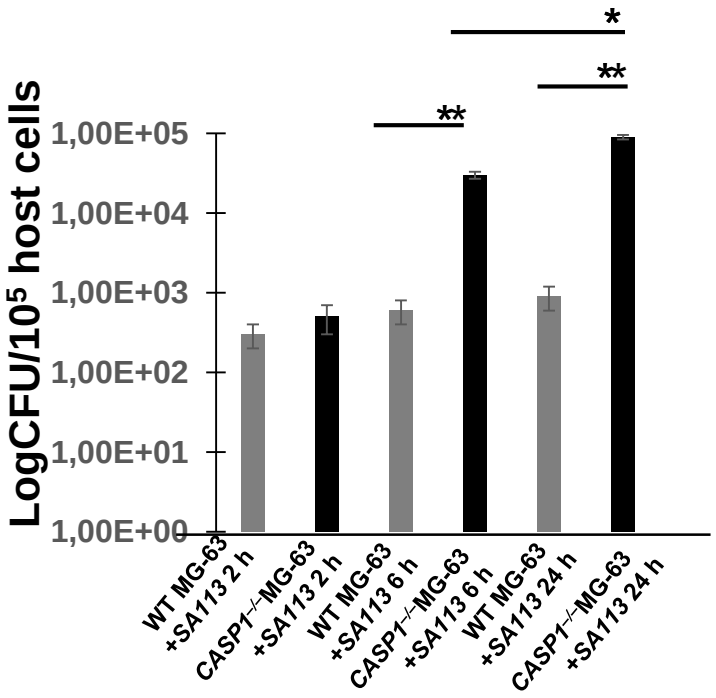
Generation of *CASP1*$^{-/-}$ MG-63 cells using the CRISPR-Cas9 gene editing system

WT MG-63 +*SA113*



CASP1*$^{-/-}$MG-63 +*SA113





Internalization of *S. aureus* (2h) was not impaired in CASP1$^{-/-}$MG-63 cells

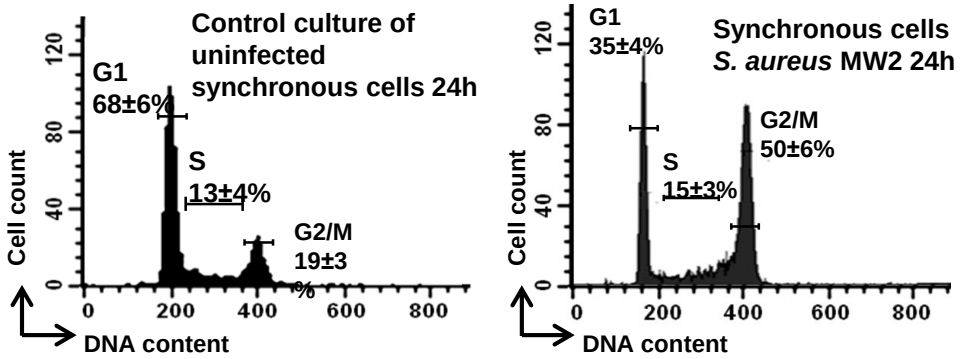
Higher number of intracellular S. aureus cells was observed in CASP1$^{-/-}$ MG-63 cells compared to WT MG-63 cells

Caspase-1 is required for the killing of internalized *S. aureus*

Lima Leite *et al.* Cell. Microbiol., 2020

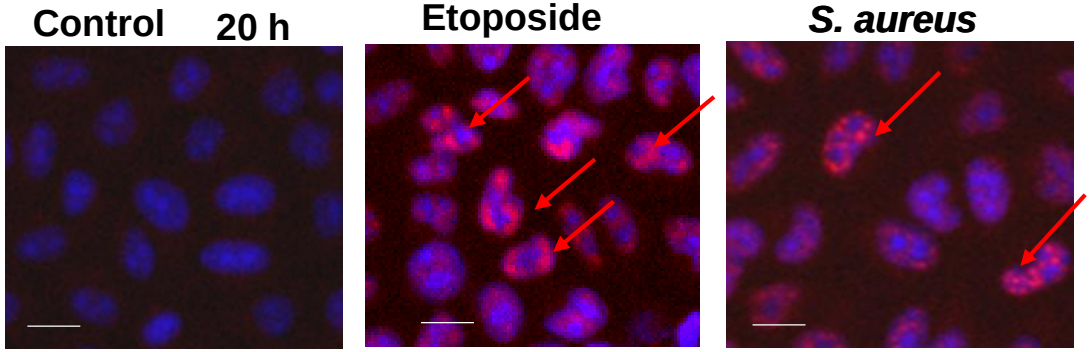
Transcriptional reprogramming of genes associated to the cell cycle progression, DNA damage and repair

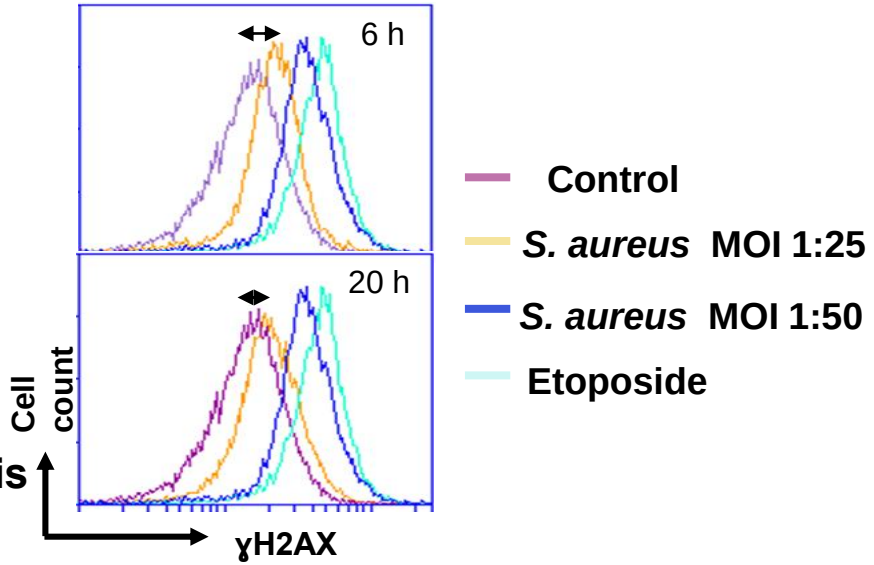
S. aureus induces a G2/M phase delay



Reactome GSEA highlighted 7 enriched pathways that belong to cell cycle progression

S. aureus induces DNA damage





γH2AX is a marker for DNA damage in the absence of apoptosis

Alekseeva et *al., Plos. One* 2013 Deplanche et *al.,* FASEB J., 2015 Deplanche et *al., Scient., Reports* 2019

S. aureus infection triggers transcriptional reprogramming of genes involved in metabolism

UniProt ID	Gene name	Gene description	FC	Log2FC	Adj.p-value	
		Upregulated genes				
P14555	pla2g2a	phospholipase A2 group IIA	75.85	6.25	3.52E-03	Lipid metabolism
P28845	hsd11b1	hydroxysteroid (11-beta) dehydrogenase 1	53.22	5.73	3.69E-04	Hormone metabolism
134339	saa2	serum amyloid A 2	48.93	5.61	1.58E-04	
P00167	cyb5a	cytochrome b5 type A	24.61	4.62	7.45E-05	
P04179	sod2	superoxide dismutase 2. mitochondrial	14.24	3.83	3.17E-23	Metabolic reprogramming
Q8TDS4	hcar2	hydroxycarboxylic acid receptor 2	13.22	3.72	5.34E-07	Regulates lipolysis
C9JRZ8	akr1b15	aldo-keto reductase family 1	10.51	3.39	1.21E-02	
A1L3X0	elovl7	ELOVL fatty acid elongase 7	7.70	2.94	1.68E-06	Synthesis of fatty acids
O95992	ch25h	cholesterol 25-hydroxylase	6.26	2.65	3.36E-03	Converts cholesterol to oxysterol
P43490	nampt	nicotinamide phosphoribosyltransferase	5.51	2.46	6.99E-56	
Q9H2J7	slc6a15	solute carrier family 6 member 15	4.56	2.19	3.76E-09	
Q99541	plin2	Perilipin2	4.46	2.16	2.42E-23	Lipid droplet binding protein
Q9NXB9	elovl2	ELOVL fatty acid elongase 2	3.56	1.83	2.06E-19	Synthesis of fatty acids
Q9Y5L2	hilpda	hypoxia inducible lipid droplet-associated	3.16	1.66	5.29E-13	
		glycolysis genes				
		Upregulated genes				
Q9BYZ2	ldhal6b	lactate dehydrogenase A-like 6B	6.52	2.71	2.74E-02	Conversion of pyruvate into lactic acid
Q6PCE3	pgm2l1	phosphoglucomutase 2-like 1	4.37	2.13	9.55E-19	Interconversion of glucose
P06733	eno1	Enolase. phosphopyruvate hydratase	1.79	0.84	1.22E-02	
P00338	ldha	lactate dehydrogenase A	1.67	0.74	5.45E-02	
P04075	aldoa	aldolase. fructose-bisphosphate A	1.66	0.73	8.12E-03	
		Downregulated genes				
P08237	pfkm	Phosphofructokinase	0.69	- 0.54	2.29E-02	
O43175	phgdh	phosphoglycerate dehydrogenase	0.61	- 0.72	4.48E-02	

The second group of the top highly regulated genes and enriched pathways belongs to the metabolism category

Nicolas et *al.*,
Front. Cell. Infect. Microbiol, 2022

Epigenetic modifications

Epigenetics is the study of heritable changes in gene expression caused by mechanisms other than changes in the primary DNA sequence.

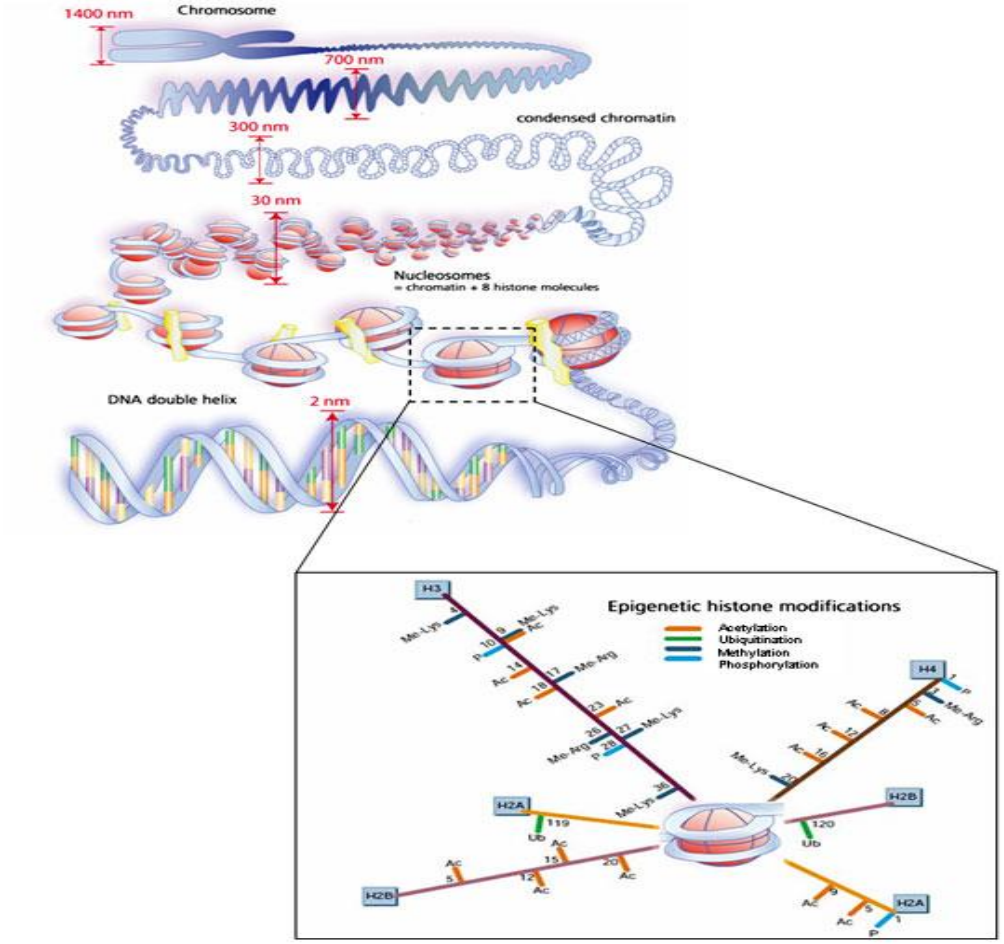
Gene expression relies on the interaction between transcription factors and 'epifactors' that control DNA accessibility

Epigenetic modifications

DNA methylation,

Histone modifications

Nucleosome positioning.



Transcriptional reprogramming of genes involved in epigenetic regulation

Helene Bierne,
Micalis, INRAE

Epifactor database
720 epifactors:
writers, erasers, readers, chromatin-remodeling enzymes, scaffold proteins, cofactors

117 DEGs encoding epifactors
92 downregulated
25 upregulated

An important number of epifactor genes were downregulated by infection: 7% of all of the downregulated DEGs

GO-BP (Gene Ontology of Biological Processes) enrichment analysis of the DAVID software

30 genes encoded epifactors with a negative effect on transcription

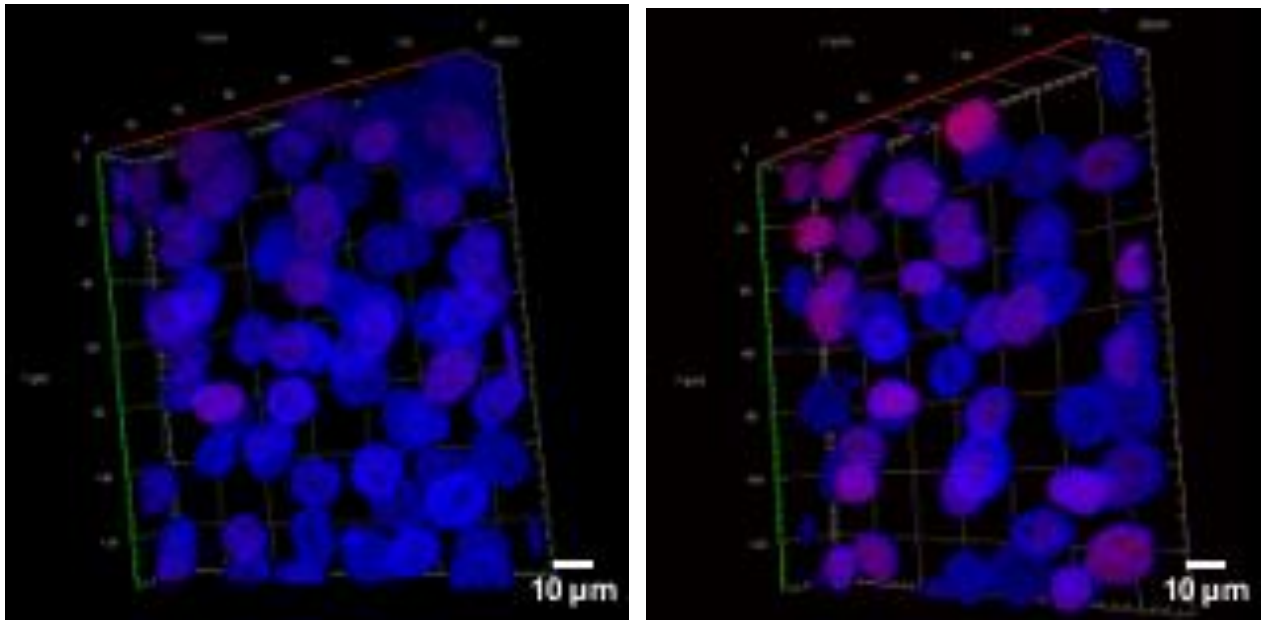
Chromatin-repressive complexes:
BAHD1, NurD, Polycomb PRC1, mSin3A and CoRES

Histone deacetylases, components of the DNA methylation and demethylation pathways

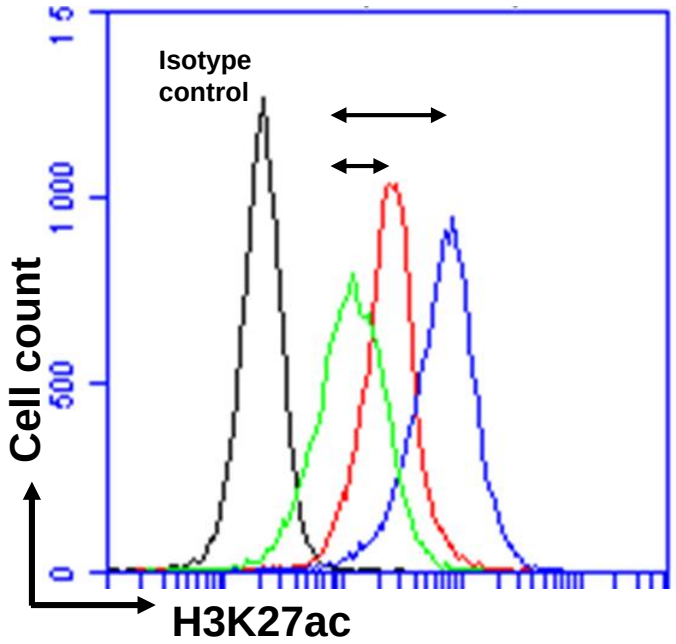
Acetylation of Histone 3 at lysine 27 in *S. aureus*-infected cells

A **Confocal microscopy**

MG-63 **MG-63+*S. aureus***



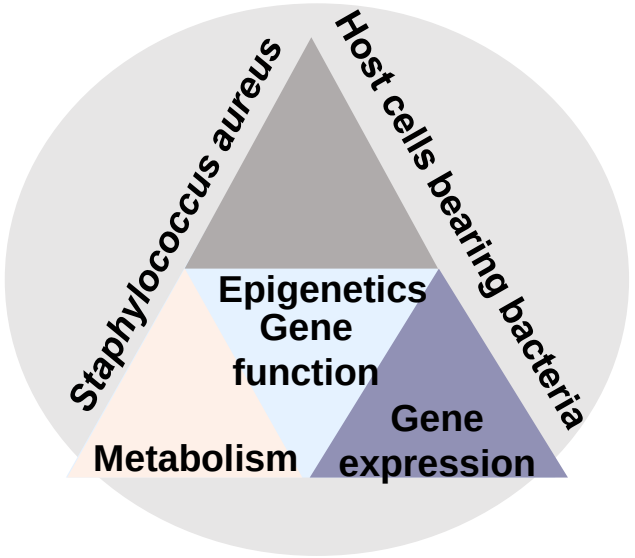
B **Flow cytometry**



- Isotype control
- MG-63
- MG-63+*S. aureus*
- MG-63 +TSA

Chaumond et *al.*, *Front Immunol*, 2023

Transcriptional, Epigenetic and Metabolic Signatures



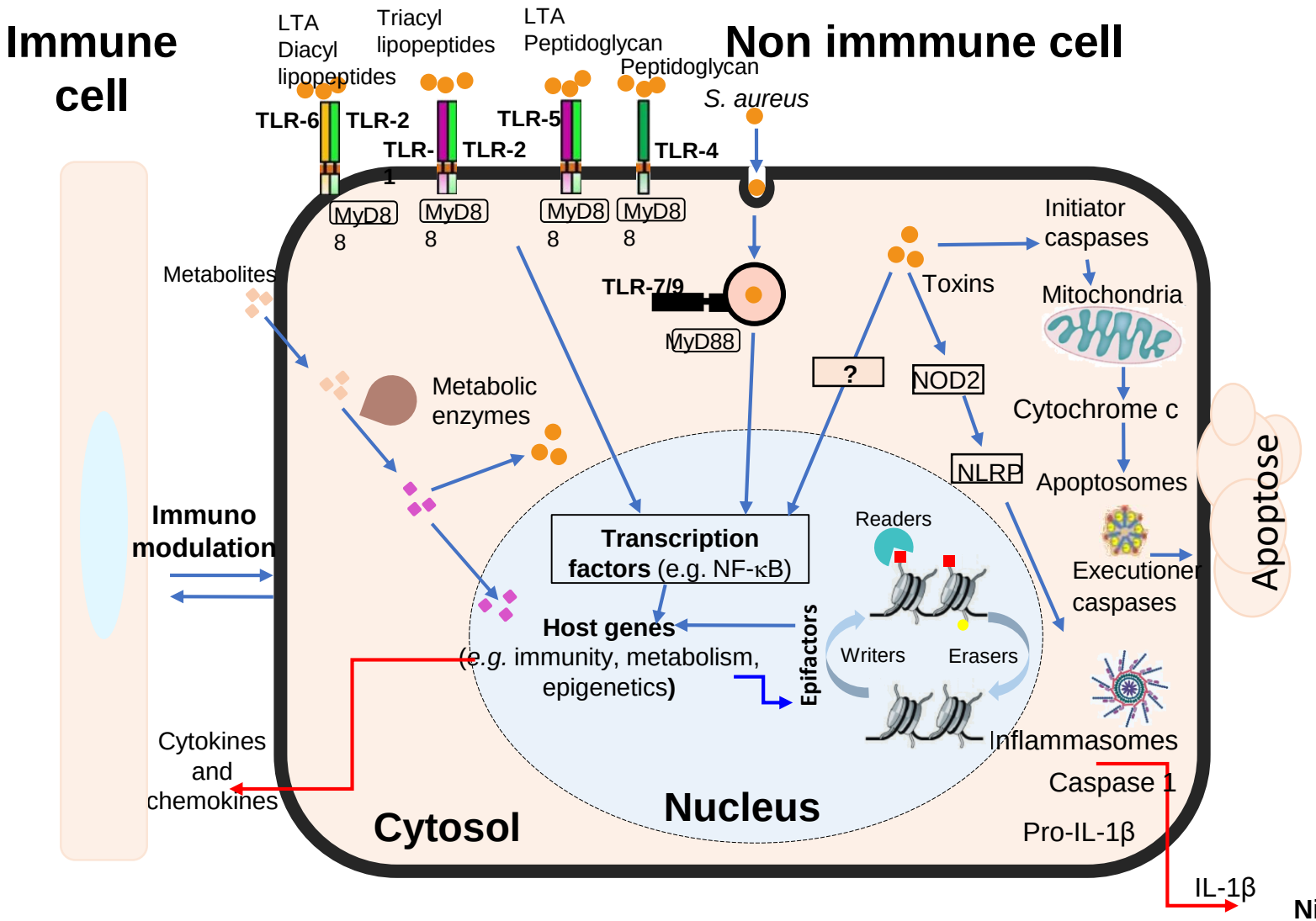
Specific categories and pathways

Epigenetic modifications, Chromatin organization, Senescence pathways, Neuronal system, Integration of energy metabolism, Metabolism of lipids

Cell cycle, DNA repair, DNA damage, DNA replication, Genes expression, Glycolysis, Metabolism of amino acids and derivatives

Innate and adoptative immune systems, Cytokines signaling, Cell death, Cell motility, Signal transduction, Extracellular matrix organization, Post-translational protein modification, Cell surface interactions

Model of the immune, metabolic and epigenetic dysregulated signatures during *S. aureus* infection

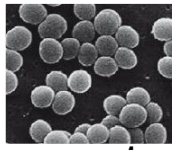


Nicolas et *al.*,
Front. Cell. Infect. Microbiol, 2022

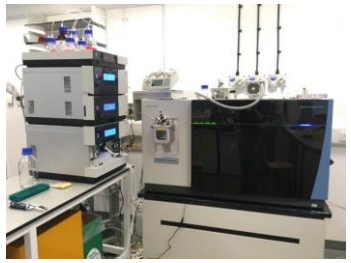
Impact of *S aureus* supernatants on the serum metabolome

HYPOTHESIS → ***S. aureus* reprograms the host's metabolism, thereby determining its susceptibility to persistent infection**

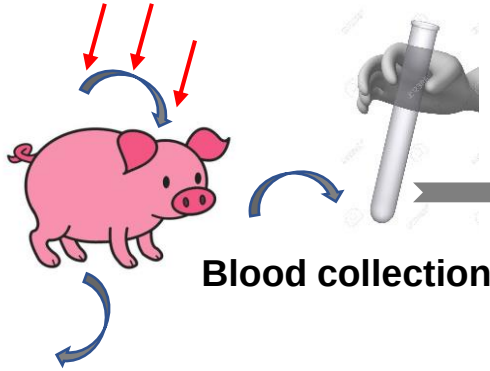
S. aureus



Supernatant



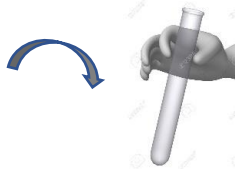
Mass spectrometry analysis → **Bioinformatic analysis**



Blood collection

Metabolomic analyses → **Bioinformatic analysis**

Microbiota analysis



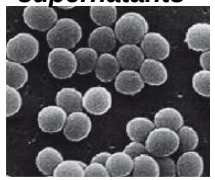
Blood collection → **Confirmation**
Markers
Metabolic pathways

Experimental approaches

a *S. aureus* supernatants preparation

STLO, Rennes:
Peron S
Jan G
Jardin J

S. aureus supernatants



Supernatants concentration → Mass spectrometry analysis → 231 groups of *S. aureus* proteins (2182 peptides) were identified

b Mini-pig model of a long-term *S. aureus* infection

NuMeCan Institute, Rennes:
Luron I.
Blat S.

S. aureus → Haptglobin, Body temperature, Weight → Blood collection

S. aureus → Haptglobin, Body temperature, Weight → Blood collection

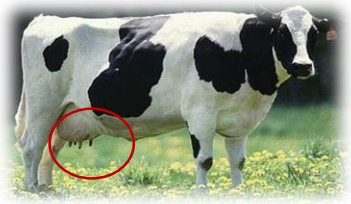
S. aureus → Haptglobin, Body temperature, Weight

→ Mass spectrometry analysis (serum)
→ Serum metabolome
→ Microbiota analysis

c Validation of the results in the context of *S. aureus* cow mastitis

Sherbrooke University, Canada
Malouin F

Agriculture and Agri-Food Canada, Sherbrooke Research Centre
Ster C



Blood collection → Confirmation, Markers, Metabolic pathways

done | in progress | not done

CONCLUSION

Besides structural functions, non-immune cells contribute to the defense response during infection

Transcriptome architecture of non-immune cells bearing internalized *S. aureus* reveals strong inflammatory responses and signatures of metabolic and epigenetic dysregulation

Non-immune cells induce an immune response against *S. aureus* through inflammasomes activation and processing of IL-1β that restrict intracellular replication of *S. aureus*

The deregulation of epigenetic and DNA repair pathways suggests that *S. aureus* infection has a long-term impact on the genome and epigenome of host cells, which may exert patho-physiological dysfunctions

Our results provide an atlas of deregulated host genes and biological pathways and identify potential candidates for prophylactic and therapeutic approaches during chronic *S. aureus* infection

COLLABORATIONS

THANK YOU FOR YOUR ATTENTION

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