Inflammasome activation and an involvement of Caspase-1 in a bacterial clearance during S. aureus infection.

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Inflammasome activation and an involvement of Caspase-1 in a bacterial clearance during *S. aureus* infection

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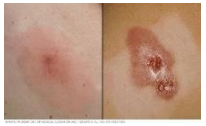
Staphylococcus aureus is responsible for a wide range of infections in human and animals

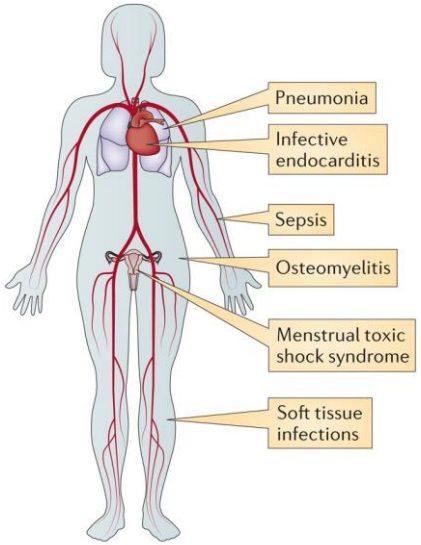
Gram-positive bacterium that is carried by up to 50% of healthy people

Human:

Mild skin infections



Life-threatening infections



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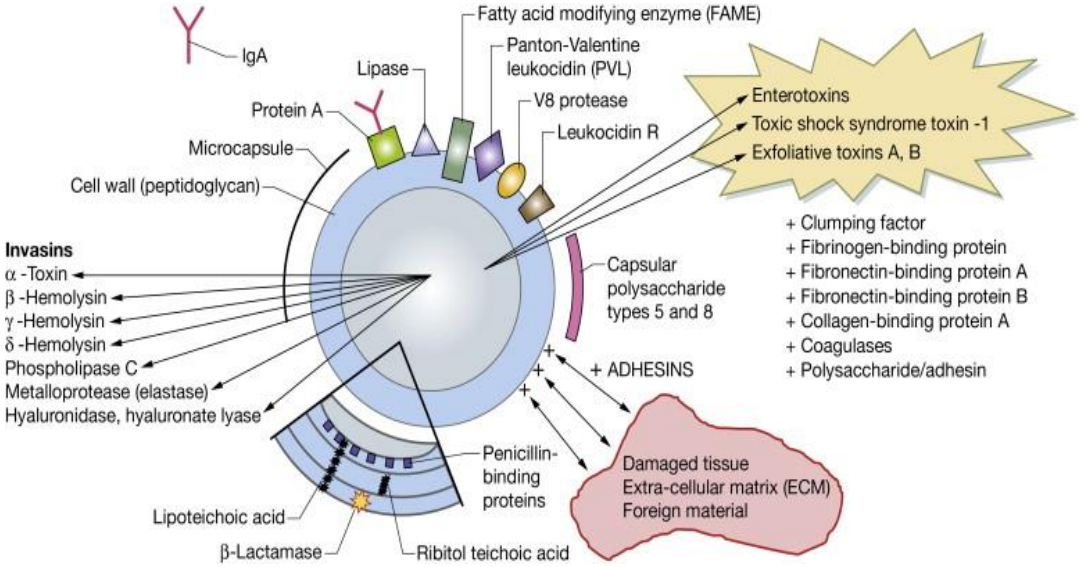
Dairy_cattle:

Chronic mastitis



***S. aureus* infections induce serious clinical problems. Despite antimicrobial therapy, there is a high percentage of relapse, leading to the chronicity**

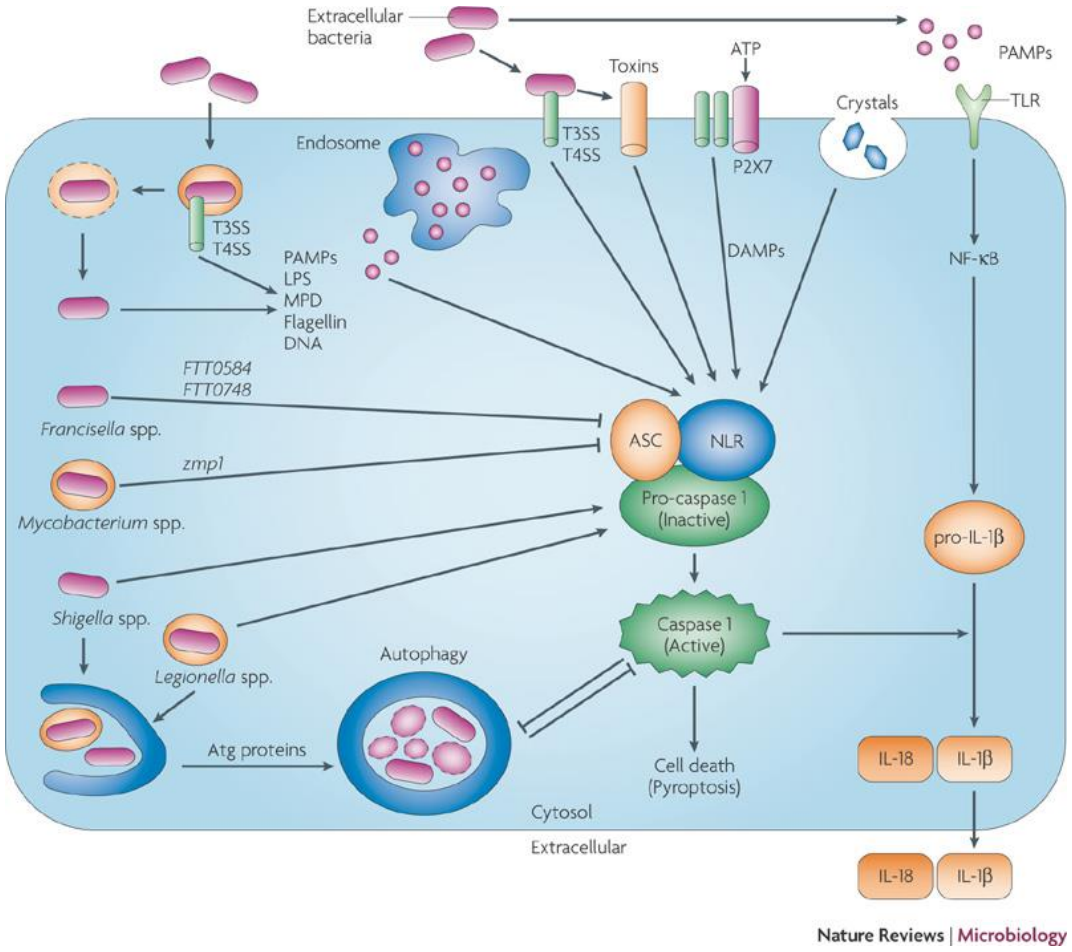
INFLAMMATION
as a defense mechanism against infection and injury

Cause of inflammation	Physiological purpose	Outcomes
• **Microbial infection** • Noxious substances • Tissue stress • Tissue injury	• **Defense from microbial infection** • Expulsion of noxious substances • Tissue repaire • Adaptation to stress	• DNA damage • Tissue damage • Autoimmune diseases • Disease of homeostasis • Fibrosis, metaplasie, cancer

Innate immune system instructs adaptive immune responses

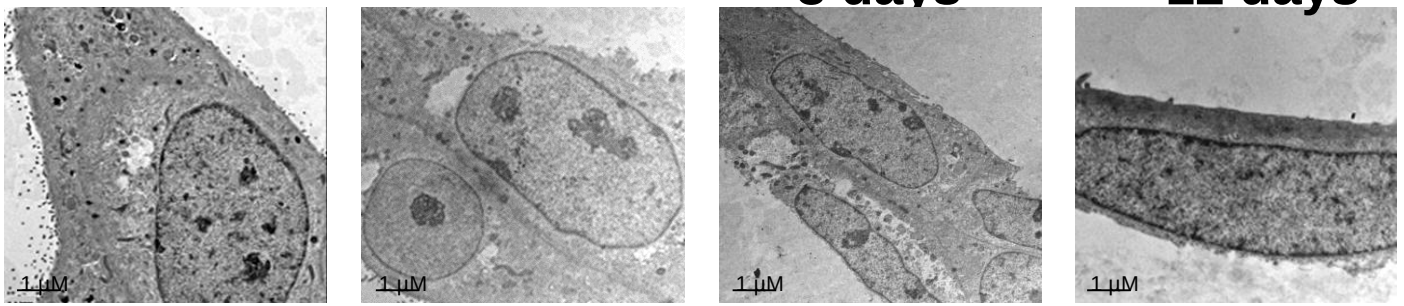
Persistent inflammation induces the activation of inflammasomes that are central players of innate immunity to pathogens

Inflammasomes are multi-protein signaling complexes that are composed of a sensor (NLR), an adaptor (ASC), and a zymogen procaspase-1

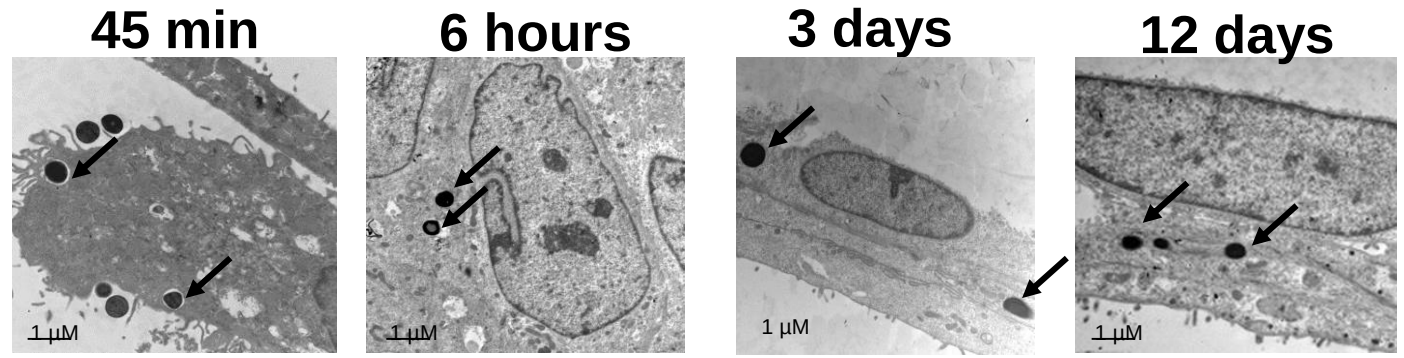


Staphylococcus aureus, a facultative intracellular pathogen

Control MG-63 cells



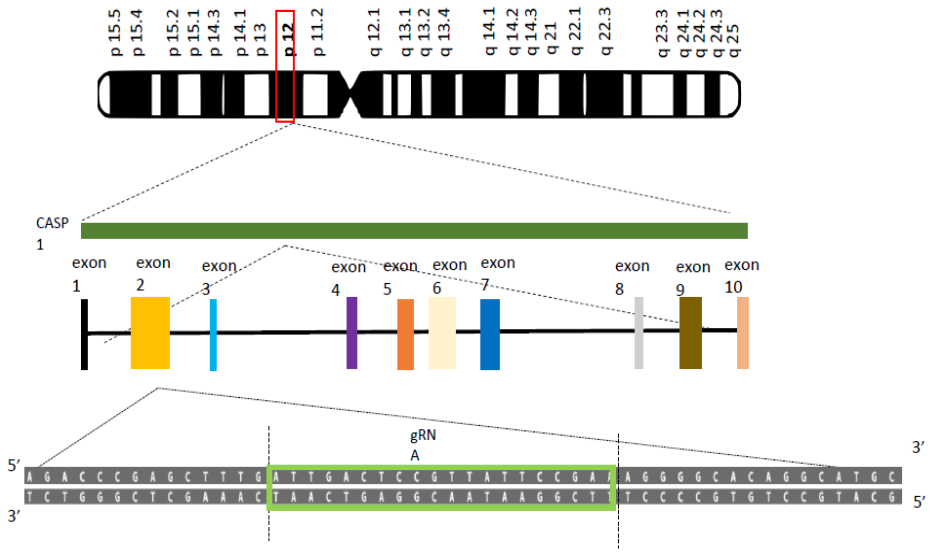
MG-63 cells + *S. aureus*



A long-term *S. aureus* infected cell culture as a model of chronic infection

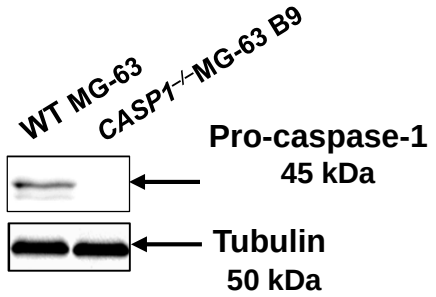
Generation of *CASP1*$^{-/-}$ *MG*-63 cells using the CRISPR-Cas9 gene editing system

Human Chromosome 11 map depicting the position of Caspase-1

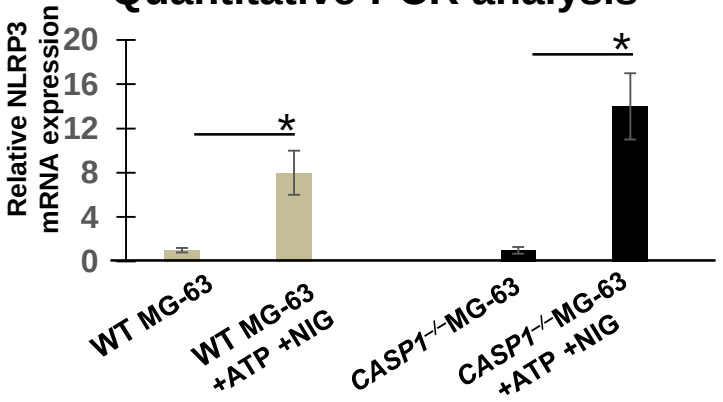


***DNA target**, GRCh38.p12 (GCF_000001405.38) Chr 11 (NC_000011.10):105,024,544 - 105,036,108

Western blot analysis



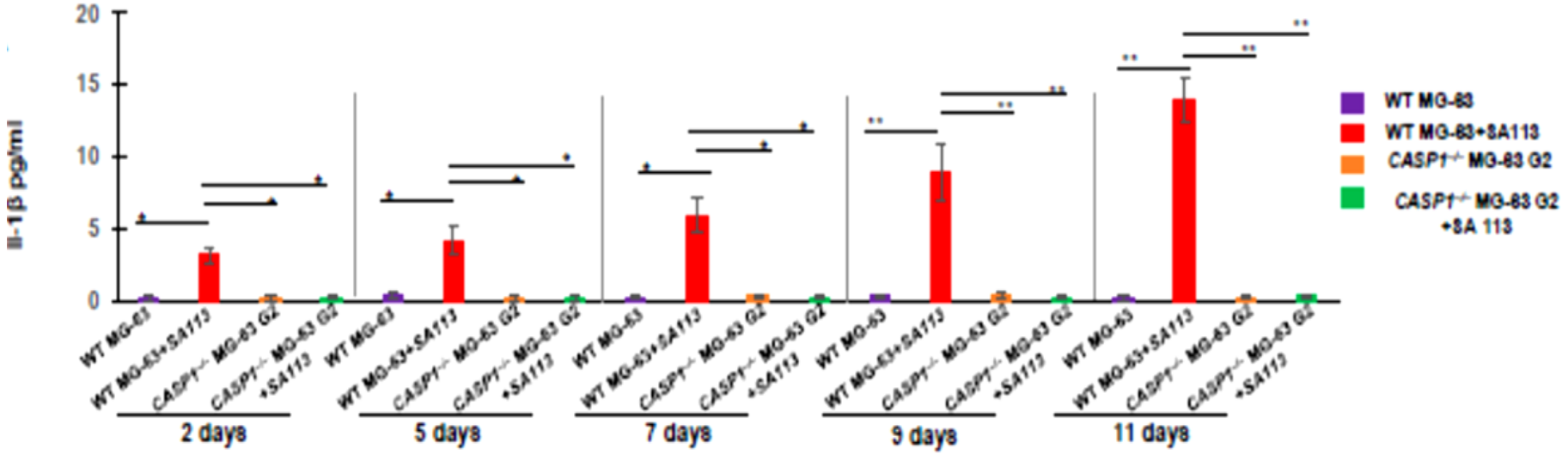
Quantitative PCR analysis



CRISPR/Cas9 edits genes by precisely cutting DNA and then letting natural DNA repair processes to take over

Depletion of Caspase-1 does not impair upstream events of inflammasome formation

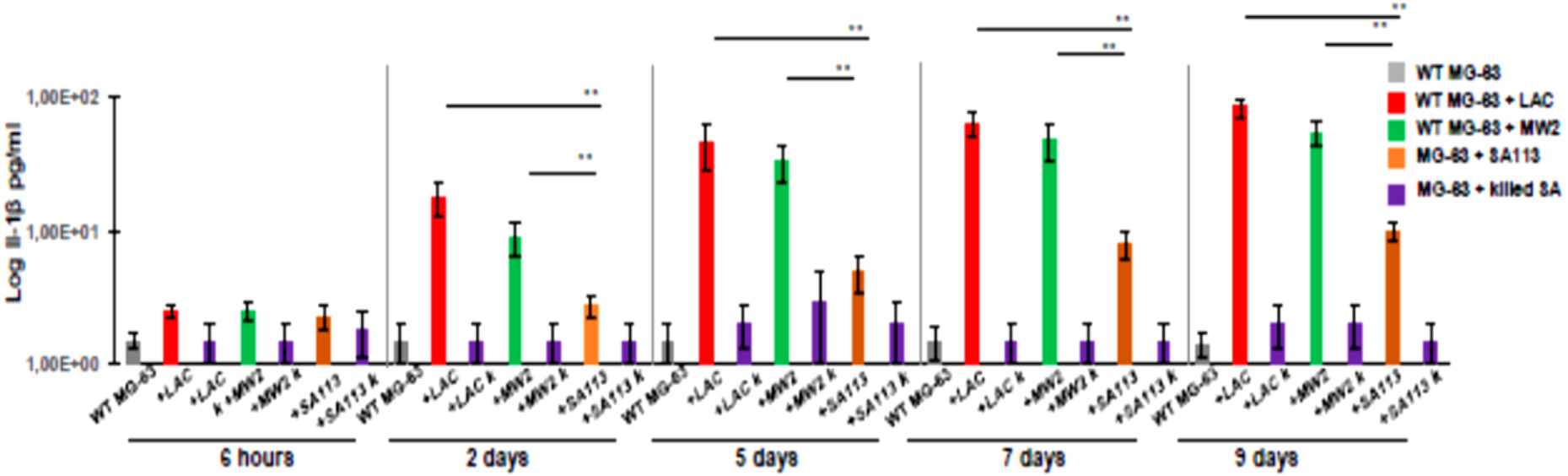
S. aureus-induced release of IL-1β by infected WT MG-63 cells and CASP1$^{-/-}$MG-63 clone



WT MG-63 cells and CASP1$^{-/-}$MG-63 clones express NLRP3 and form ASC specks
Only WT MG-63 cells produce IL-1β following the exposure *S. aureus*

Monitoring of IL-1β produced by both cell lines are used for the analysis of inflammasomes activation

S. aureus strain-dependent release of IL-1β by infected MG-63 cells

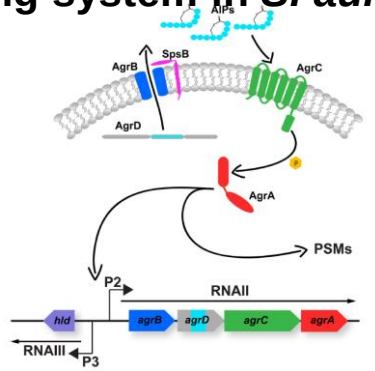


Agr-defective SA113 strain induce a low levels of IL-1β

Strains MW2 and LAC (pTXΔ16), which harbor a functional Agr system induce a high level of IL-1β

Possible involvement of the Agr

The accessory gene regulator (Agr), the gene cluster that encodes the peptide quorum-sensing system in *S. aureus*



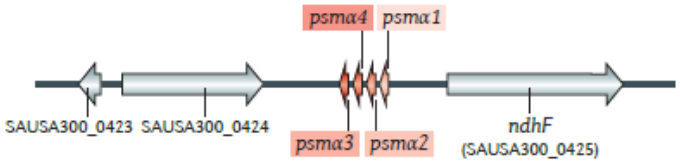
Phenol-soluble modulins peptides (PSMs) define the virulence potential of *S. aureus*

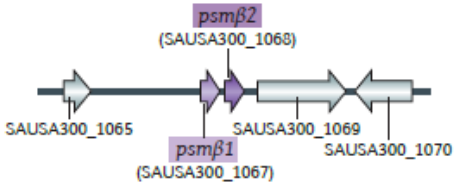
PSMα1-PSMα4
δ-toxin

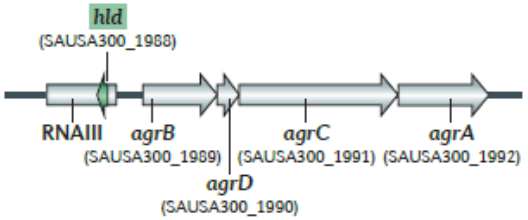
20-25 amino acids

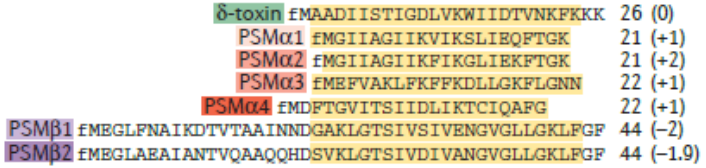
PSMβ

44 amino acids

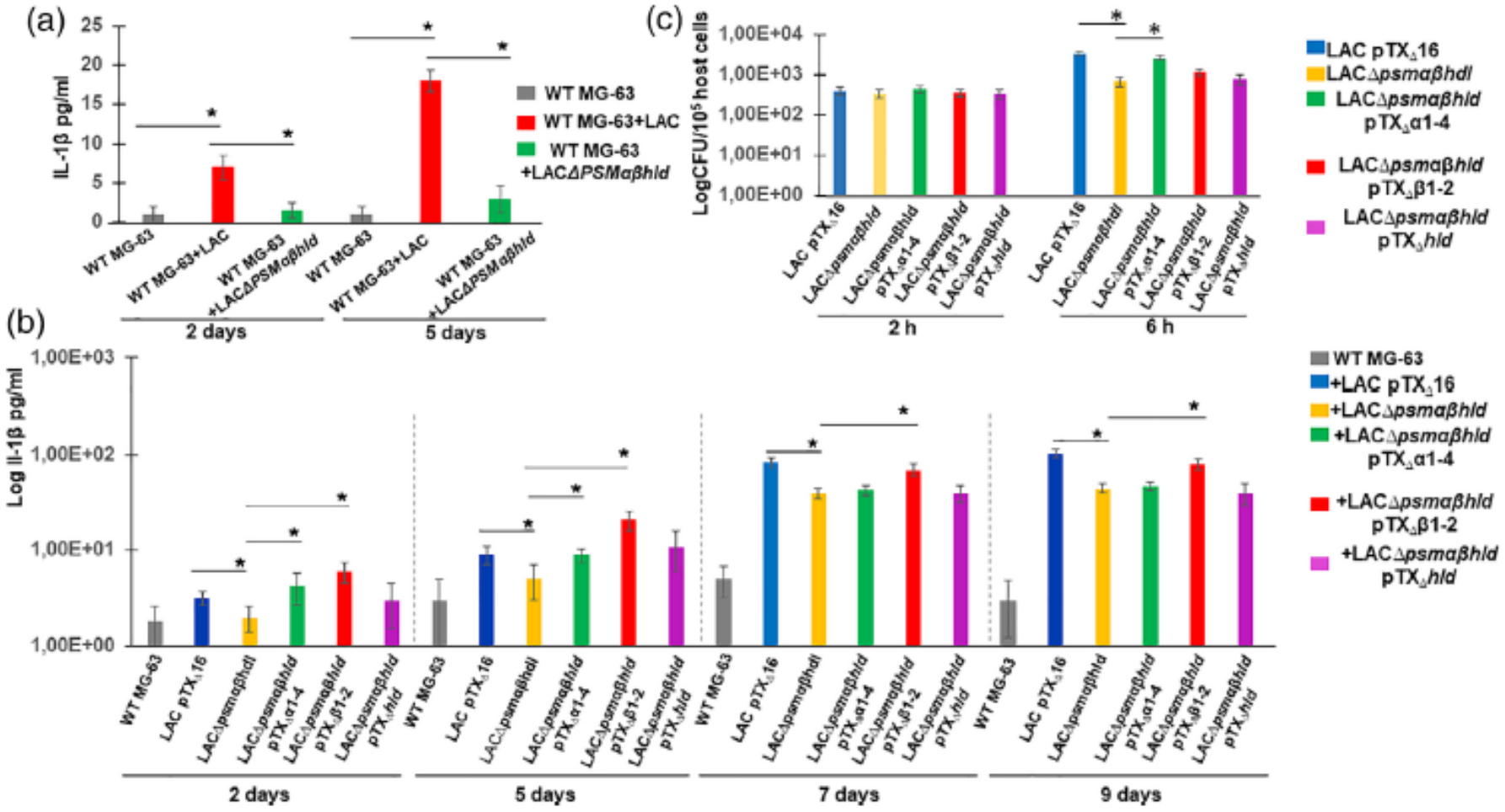




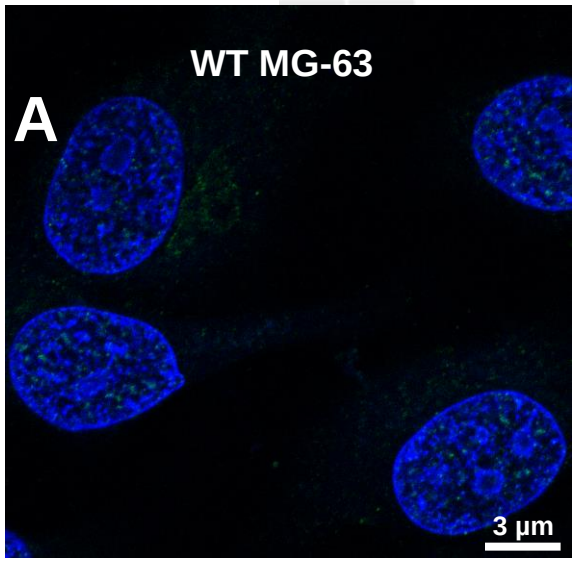


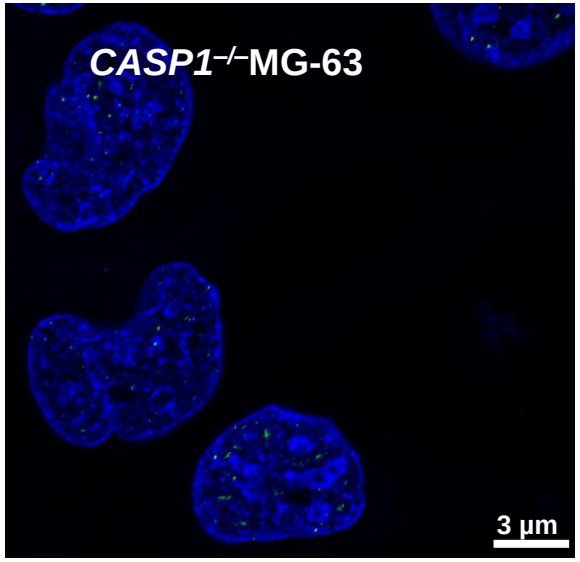


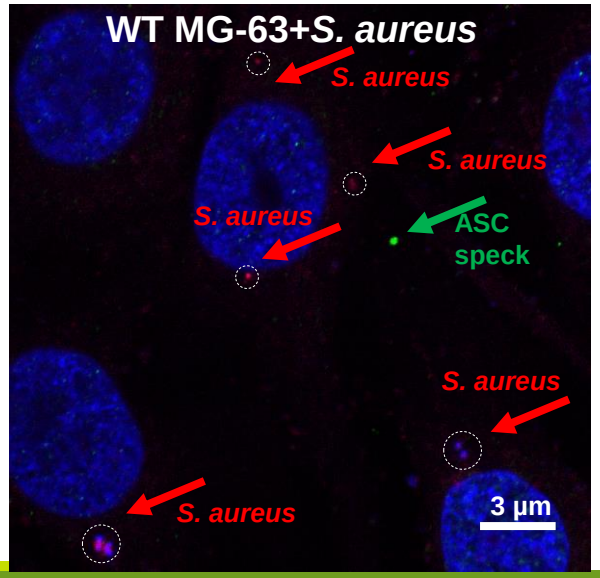
S. aureus PSM toxins are involved in stimulation of IL-1β release by infected MG-63 cells

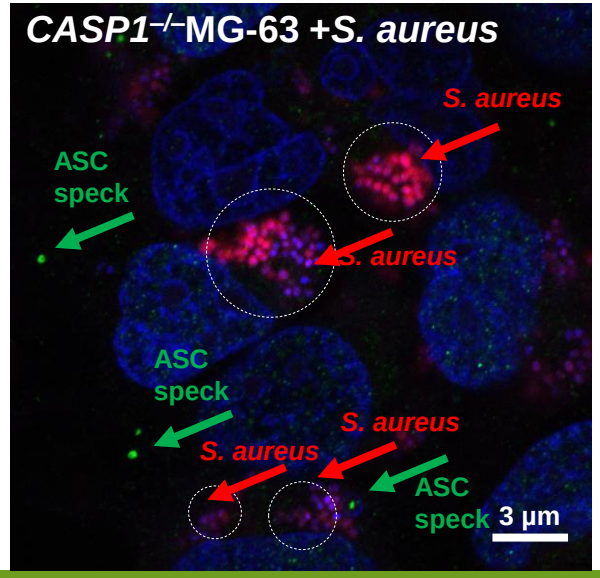


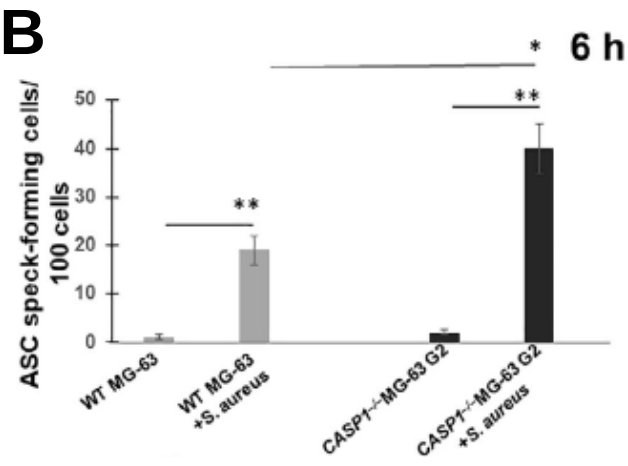
Higher number of ASC specks in *S. aureus* infected CASP1$^{-/-}$ MG-63 cells compared to WT MG-63 cells

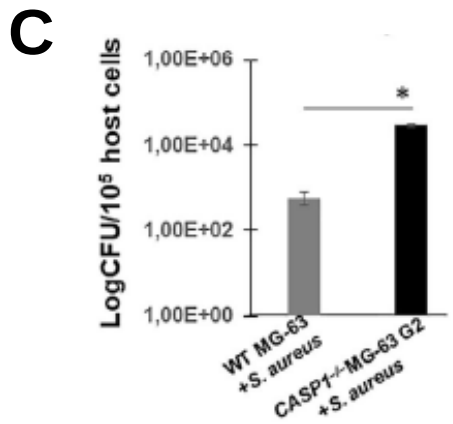




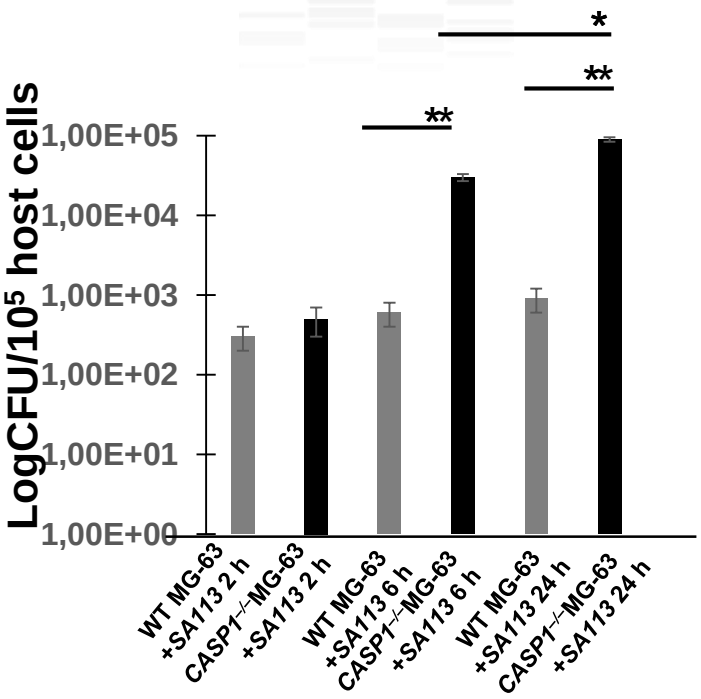


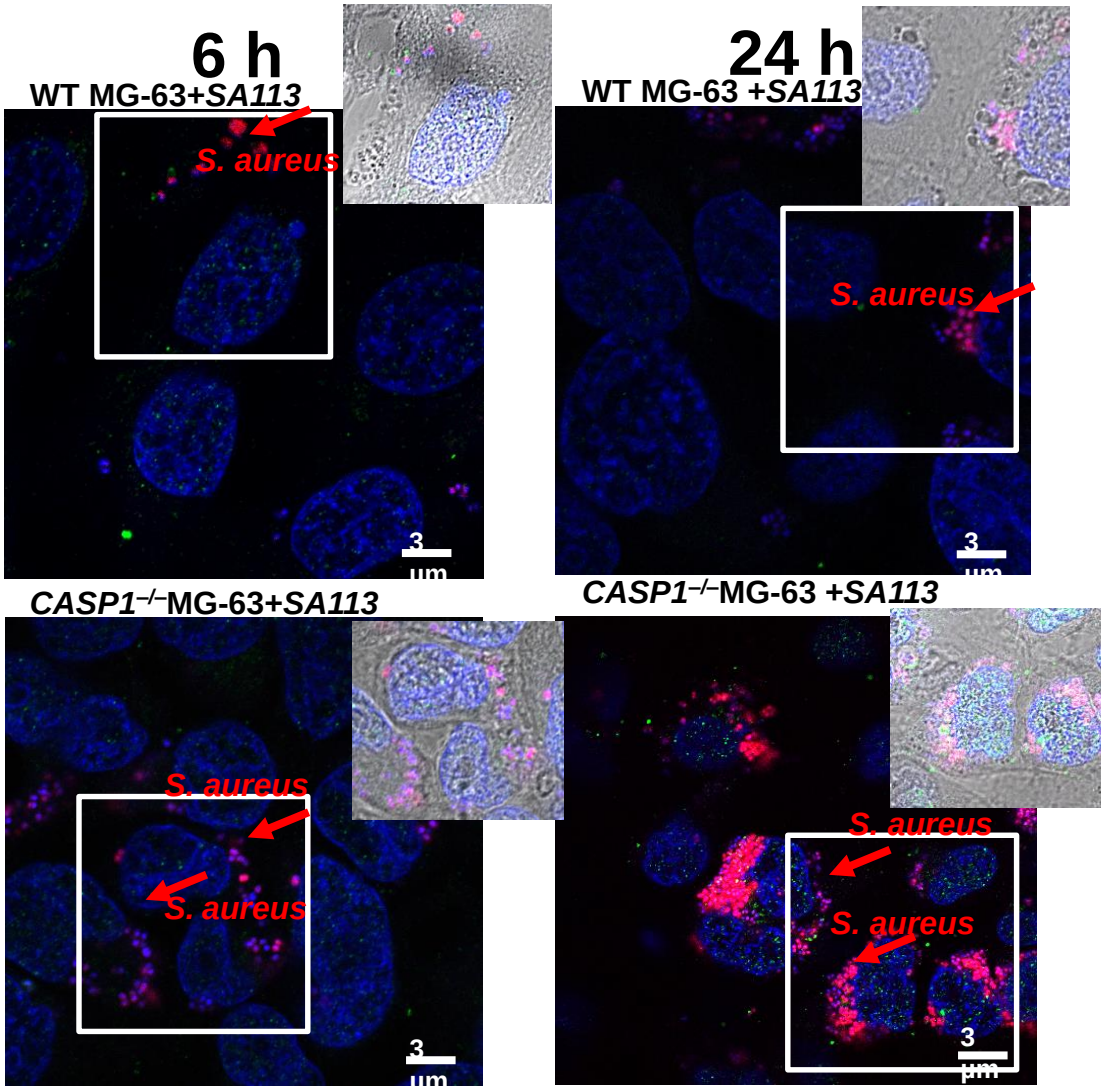






S. aureus clearance by osteoblast-like MG-63 cells depends on caspase-1

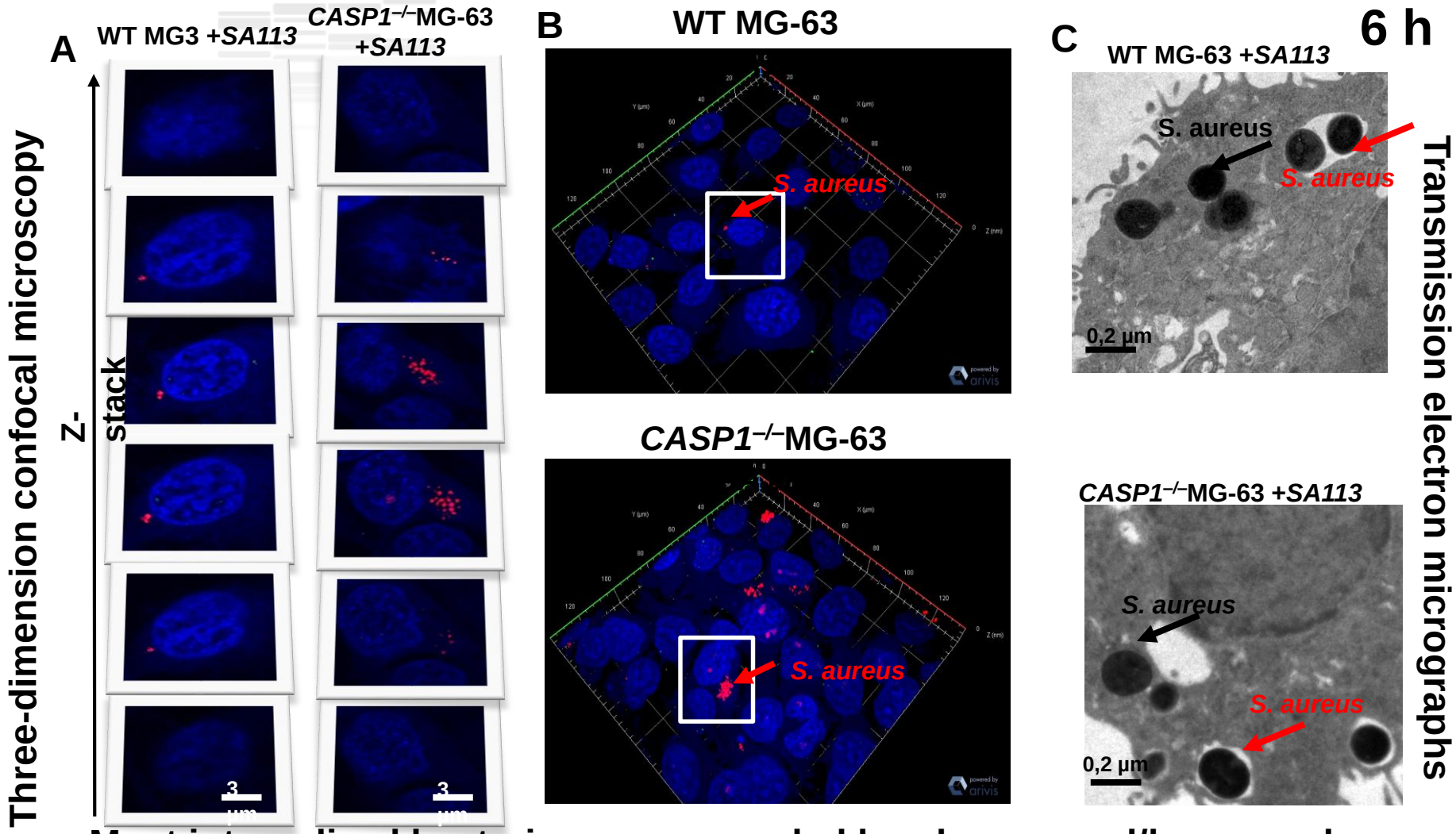




Internalization of *S. aureus* (2h) was not impaired in CASP1$^{-/-}$MG-63 cells

Higher number of intracellular *S. aureus* was observed in CASP1$^{-/-}$ MG-63 cells compared to WT MG-63 cells 6h and 24h post-infection

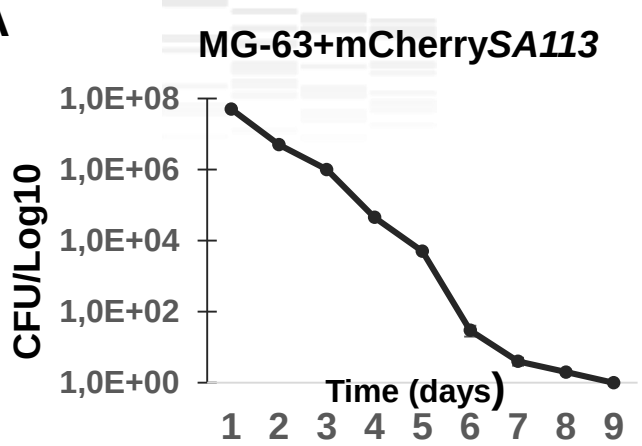
Cytoplasmic localization of internalized *S. aureus*



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

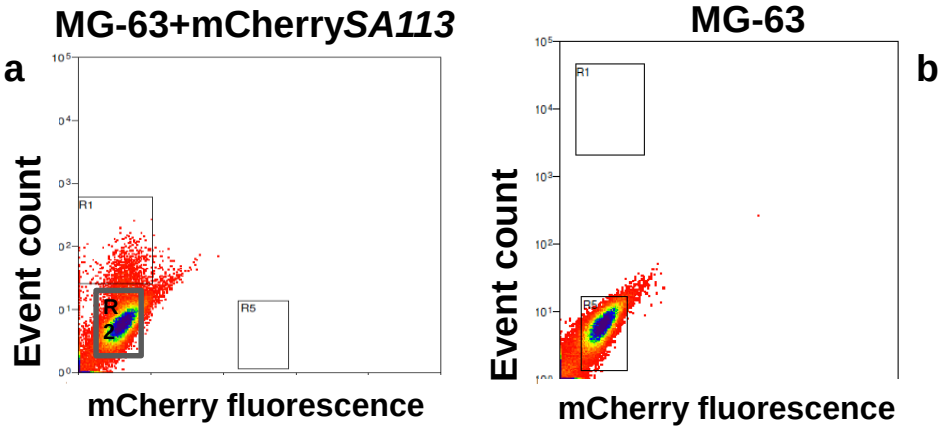
Development of an infection model to isolate solely cells containing internalized *S. aureus*

A



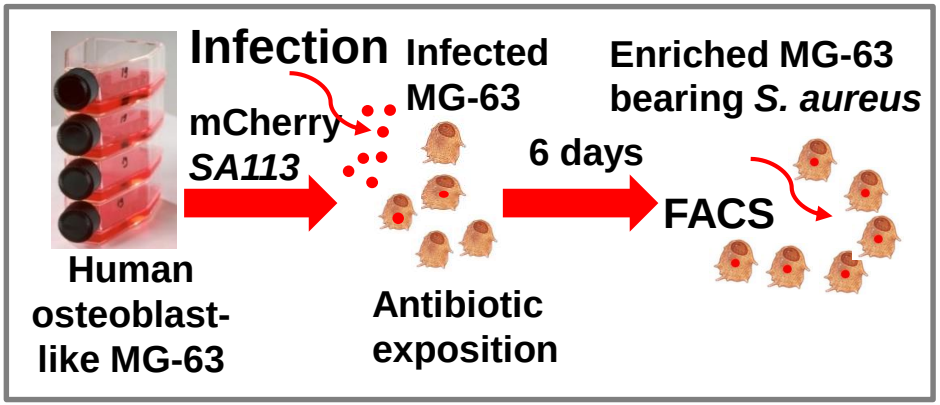
Survival curve of mCherrySA113 bacteria internalized into MG-63 cells

C



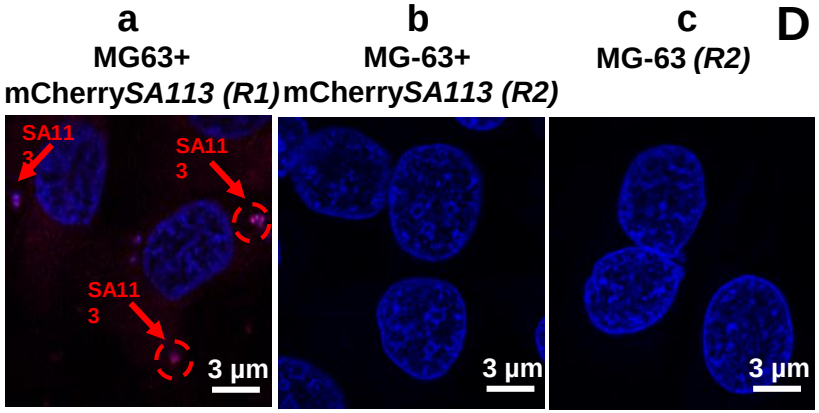
S. aureus infected cells (a) or uninfected control cells (b) were sorted using FACS

B



Scheme of the selection of mCherrySA113-containing MG-63 cells

D



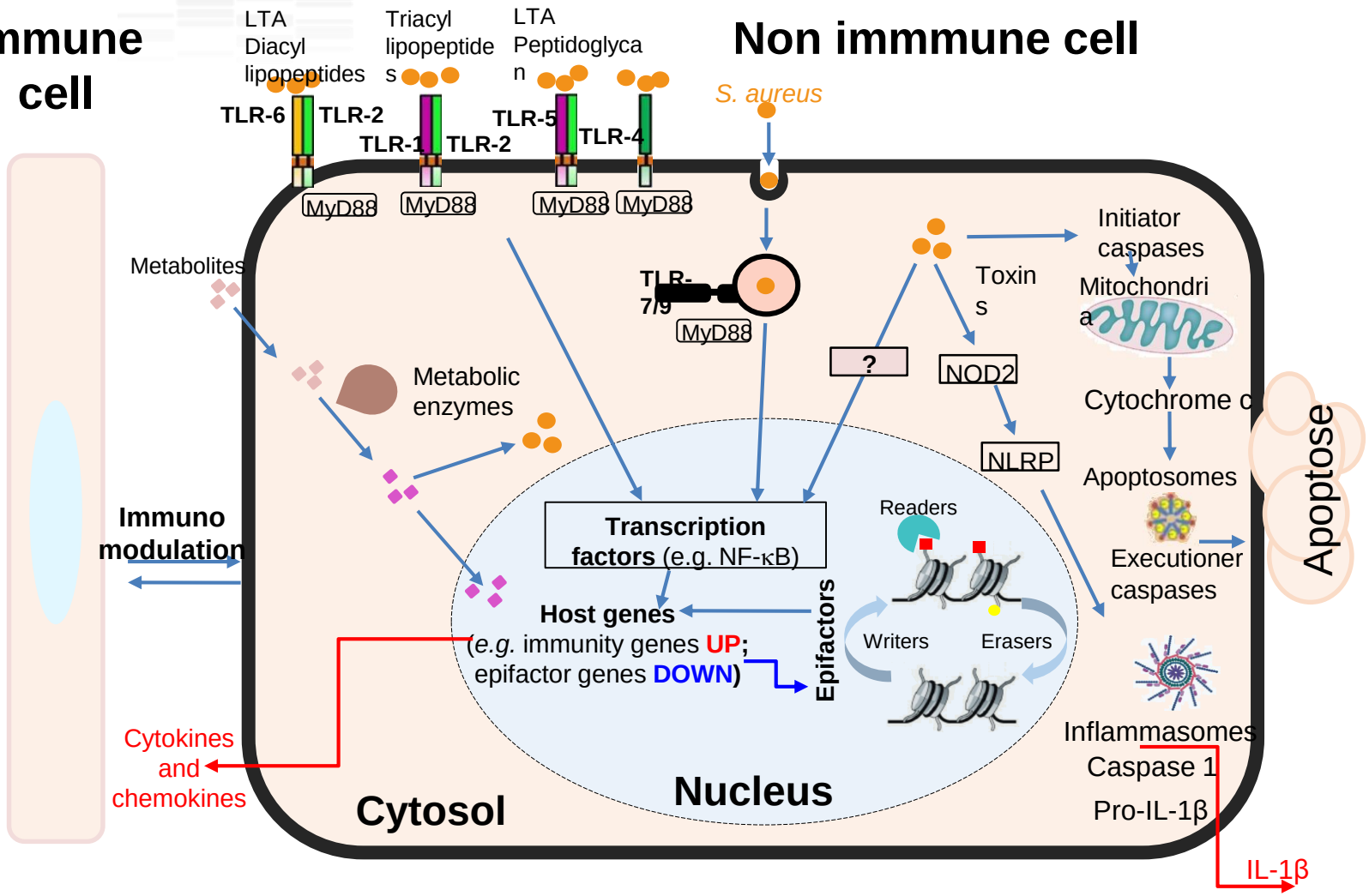
Fluorescence microscopy

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

Enriched Reactome pathways in infected cells.

Enriched Reactome pathways in infected cells. Gene-set enrichment analysis was performed in *S. aureus*-bearing cells compared to uninfected control cells by ReactomePA software.

Schematic model of the immune, metabolic and epigenetic dysregulated signatures induced *S. aureus* infection



CONCLUSIONS

Non-professional phagocytes induce an immune response against *S. aureus* through inflammasomes activation and processing of IL-1β

The outcome of the infection depends on the balance between the host immune response and the action of main *S. aureus* virulence factors, such as PSMs, whose production differ among the *S. aureus* strains

The active caspase-1 prevents exacerbated intracellular replication of *S. aureus* in non-professional phagocyte

Our findings suggest that pathogens, which inhibit caspase-1 activation, do so not only to govern the generation of inflammatory cytokines but also to permit bacterial replication

Our results provide an atlas of deregulated host genes and biological pathways and identify novel markers and potential candidates for prophylactic and therapeutic approaches

Involvement of caspase-1 in inflammasomes activation and bacterial clearance in *S. aureus*-infected osteoblast-like MG-63 cells

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Cellular Microbiology. 2020;22:e13204.

SCIENTIFIC REPORTS

Transcriptome Architecture of Osteoblastic Cells Infected With *Staphylococcus aureus* Reveals Strong Inflammatory Responses and Signatures of Metabolic and Epigenetic Dysregulation

Aurélie Nicolas[1], Martine Deplanche[1], Pierre-Henri Commere[2], Alan Diot[3,4], Clemence Genthon[5], Wanderson Marques da Silva[1,6], Vasco Azevedo[6], Pierre Germon[7], Hélène Jamme[8,9], Eric Guédon[1], Yves Le Loir[1], Fréderic Laurent[3,4], Hélène Bierne[10] and Nadia Berkova[1]*

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: xx xx 2022
doi: 10.3389/fcimb.2022.854242

OPEN

Staphylococcus aureus induces DNA damage in host cell

Martine Deplanche[1], Nassim Mouhali[1], Minh-Thu Nguyen[2], Chantal Cauty[1], Frédéric Ezan[3], Alan Diot[4,5], Lesly Raulin[6], Stephanie Dutertre[6], Sophie Langouet[3], Patrick Legembre[7], Frederic Taieb[8], Michael Otto[9], Frédéric Laurent[4,5], Friedrich Götz[2], Yves Le Loir[1] & Nadia Berkova[1]

SCIENTIFIC REPORTS | (2019) 9:7694 | https://doi.org/10.1038/s41598-019-44213-3

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INSERM

Micalis 10 ans

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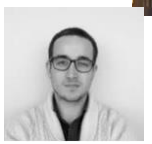

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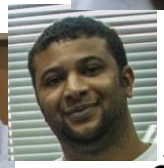

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спасибо
谢谢
GRACIAS
THANK YOU
ありがとうございました
MERCI
DANKE
धन्यवाद
شُكراً
OBRIGADO


Merci !