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## Staphylococcus aureus induces DNA damage in host cell

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INRAE

➤ ***Staphylococcus aureus* induces DNA damage  
in host cell**

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**Déclaration de conflit d'intérêt**

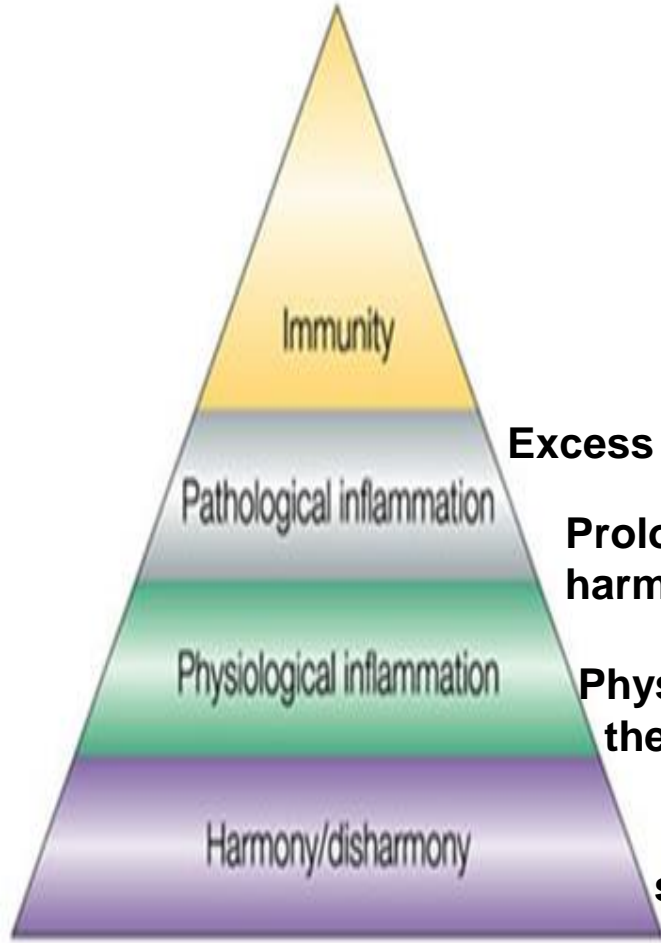
Pour cette présentation,  
je déclare n'avoir aucun conflit d'intérêt.



# ➤ HOMEOSTASIS AND INFLAMMATION

Chronic inflammation as a driving force in the genesis of DNA damage

Metchnikoff's theory of inflammation

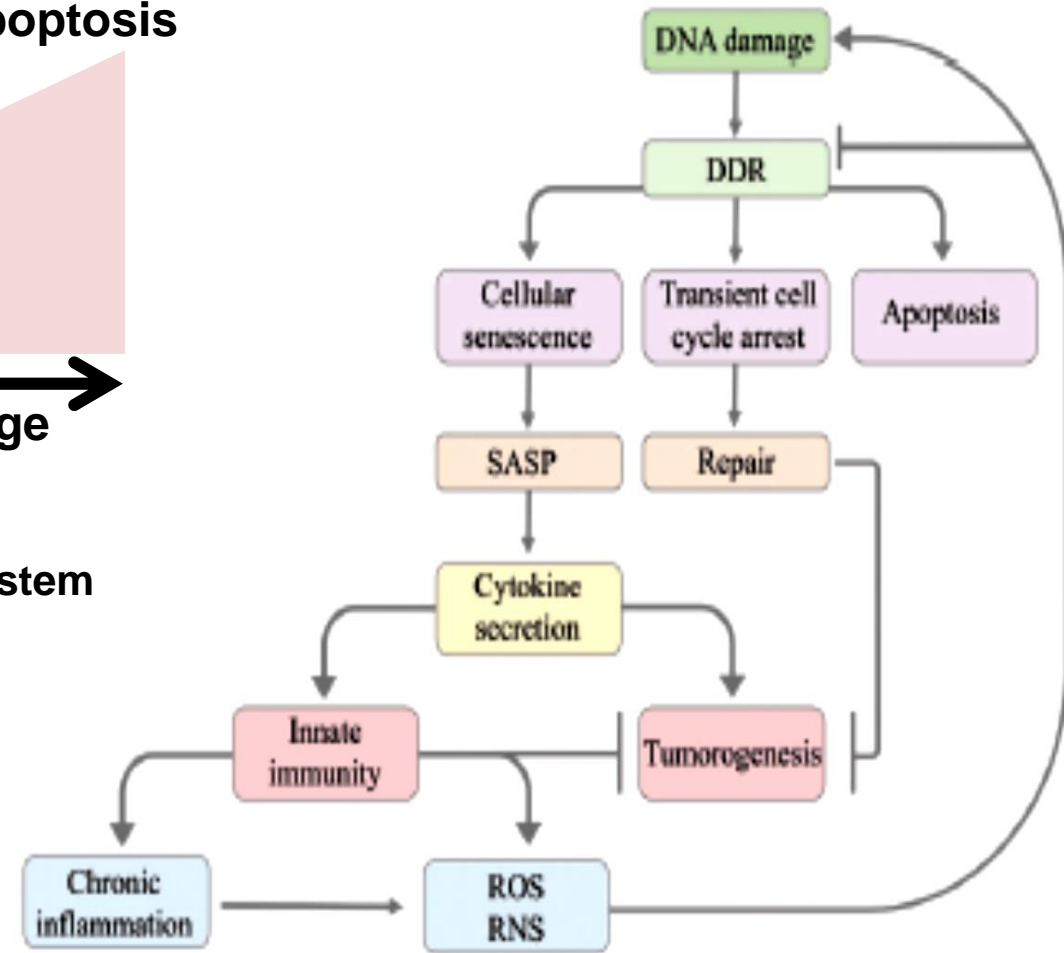
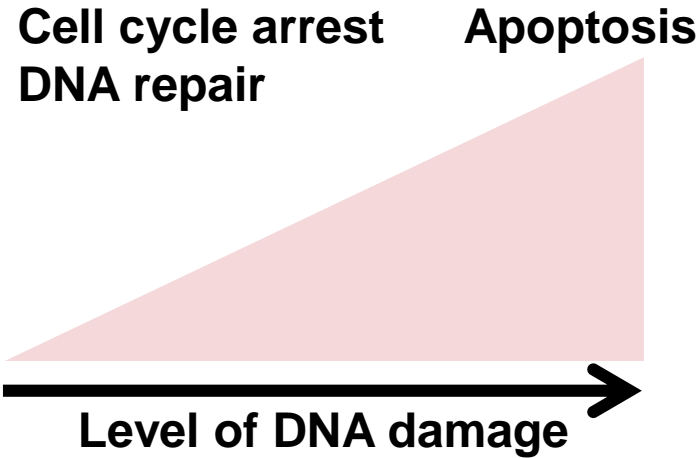


Excess of the homeostatic capacity of the system

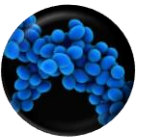
Prolonged inflammation causes harm in bystander tissues

Physiological inflammation mediates the elimination of injurious agents

Homeostasis maintains the stability of biological system

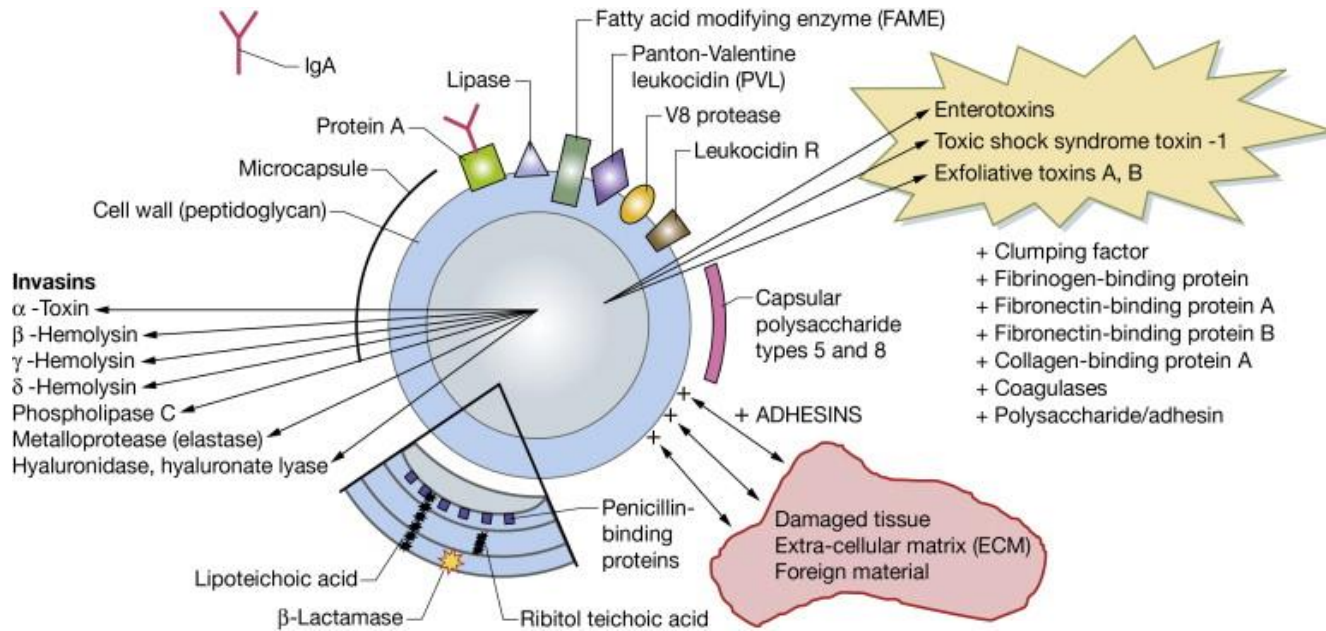


Pathogens induce various levels of inflammation



# ➤ *Staphylococcus aureus* IS RESPONSIBLE FOR A WIDE RANGE OF INFECTIONS IN HUMAN AND ANIMALS

## Gram-positive bacterium



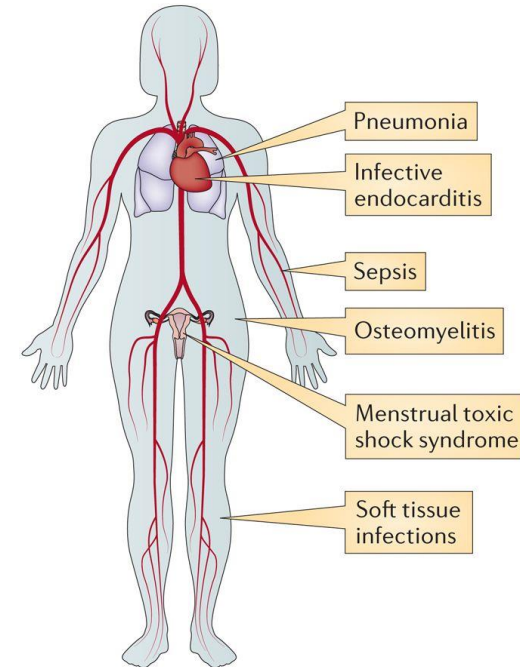
***S. aureus*-induced diseases represent serious clinical problems, especially during chronic infections**

## Human

### Mild skin infections



### Life-threatening infections

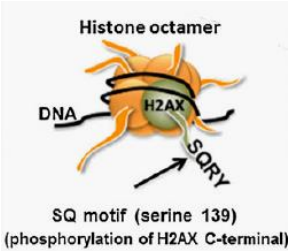


## Dairy\_cattle:

### Chronic mastitis

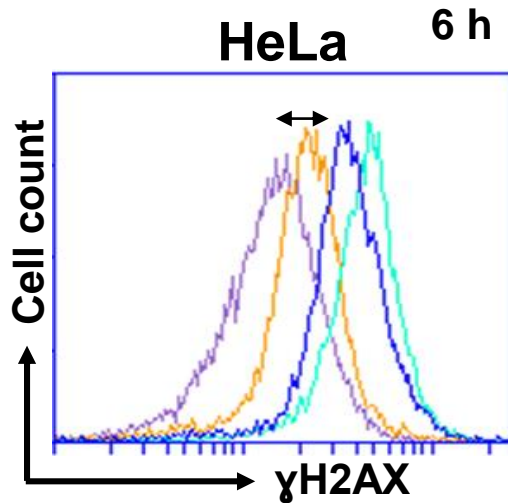


# ➤ *S. aureus* INDUCES DNA DAMAGE IN HOST CELLS



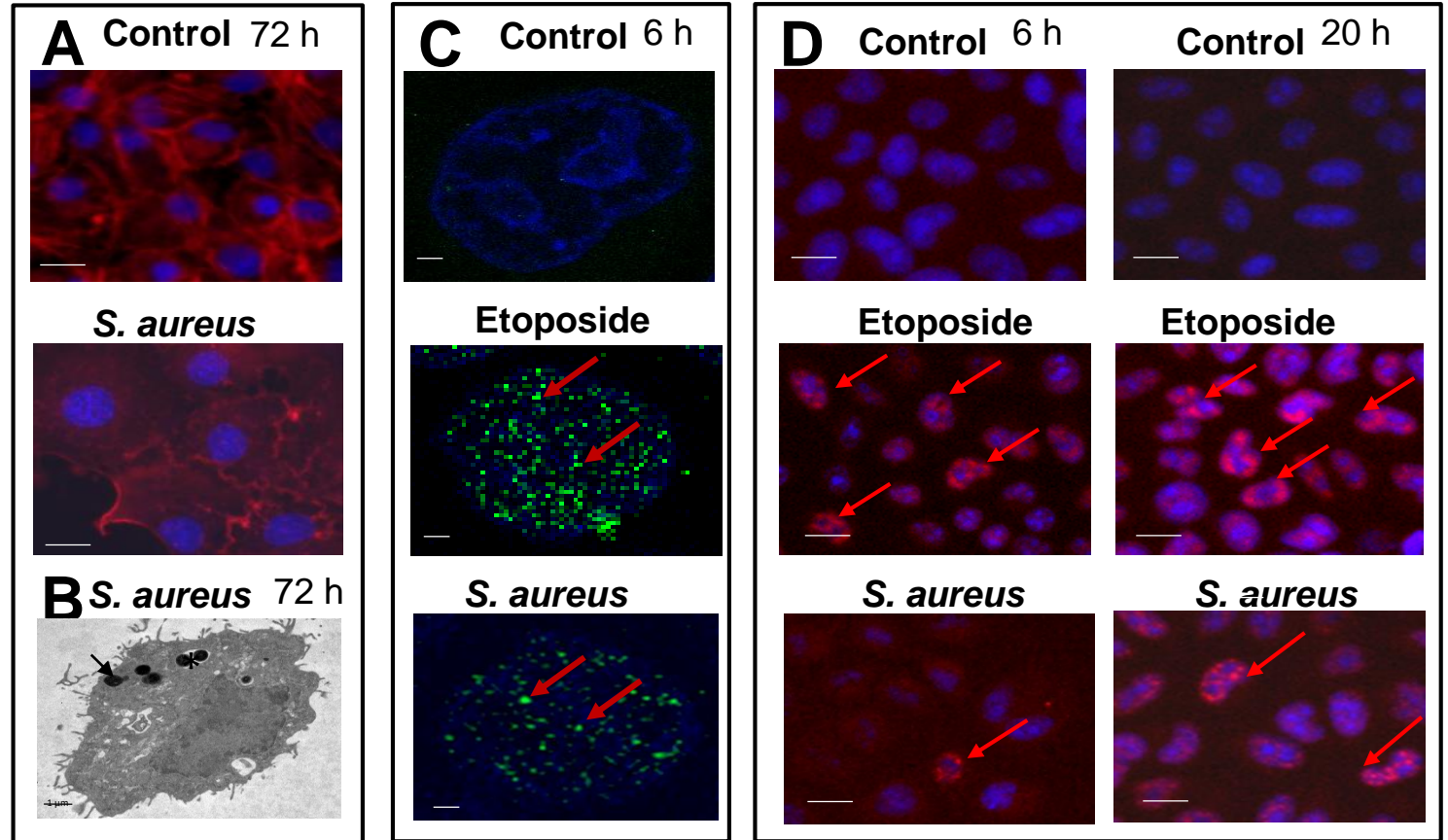
Phosphorylation of H2AX at Ser 139 ( $\gamma$ -H2AX) is the most sensitive marker for the examination of the DNA damage

Ibuki et al., 2015



- Control
- *S. aureus* MOI 1:25
- *S. aureus* MOI 1:50
- Etoposide

$\gamma$ H2AX was quantified by flow cytometry  
Double arrow shows the shift between control cells and *S. aureus*-infected cells

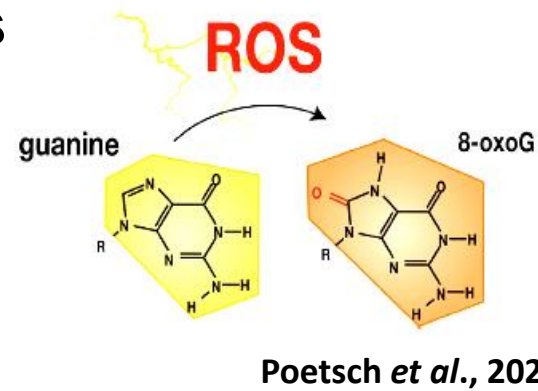
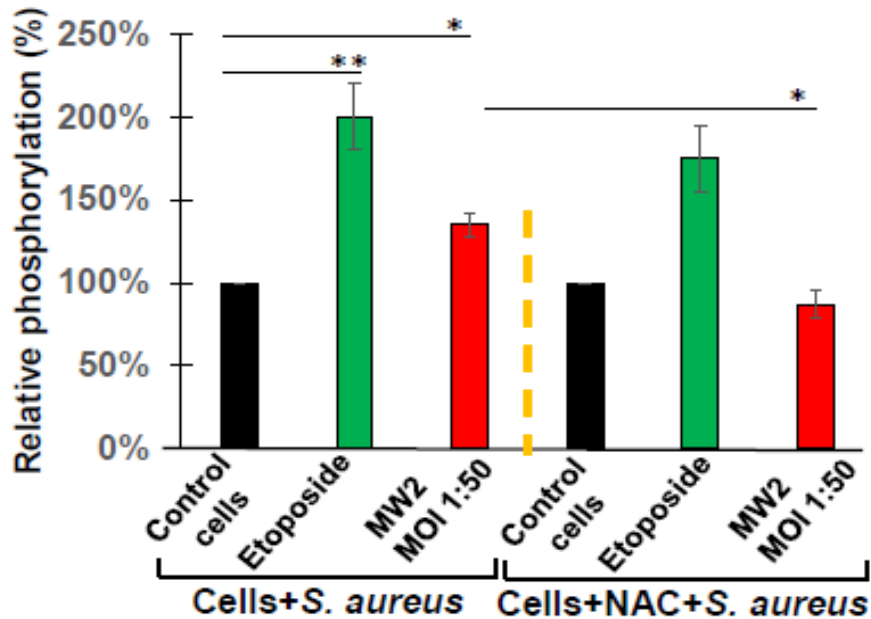


- (A) HeLa cells were infected with *S. aureus*. TRITC-conjugated phalloidin labels F-actin  
(B) Transmission electron micrograph. Bacteria appear to be free within the cytoplasm or in vacuoles  
(C) Infected cells were stained for  $\gamma$ H2AX. Etoposide-treated cells were used as a positive control  
(D) High Content Screening analysis. Cells were stained for  $\gamma$ H2AX

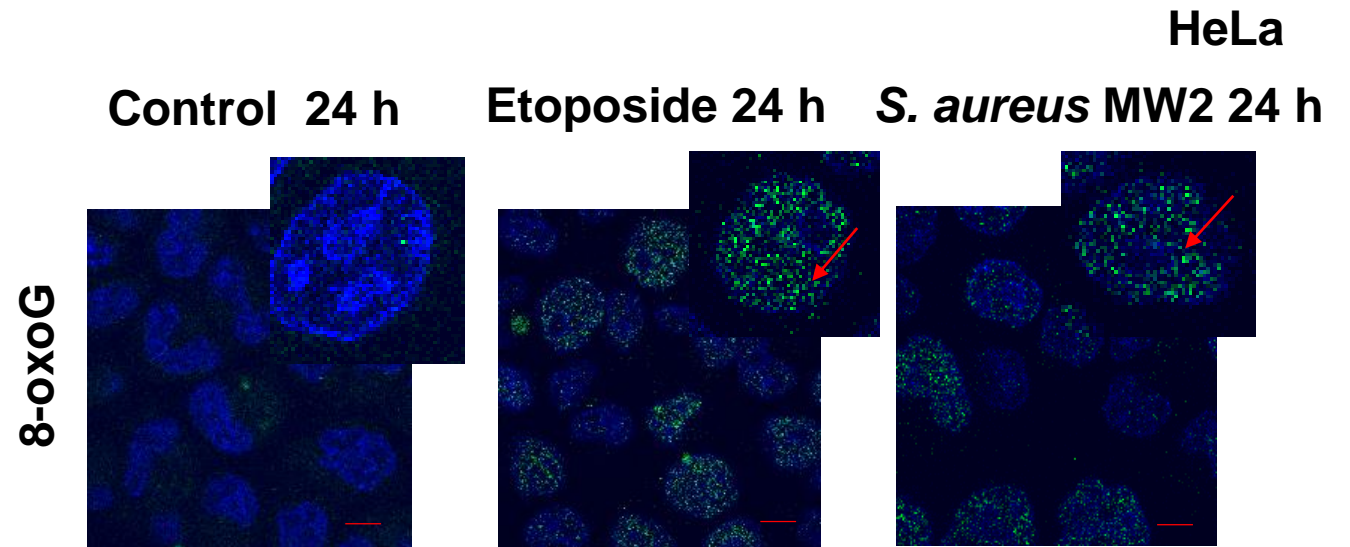
# ➤ *S. aureus* INDUCES 8-OXOG DNA LESIONS IN THE HOST CELLS

**a** *S. aureus* infection induces reactive oxygen species (ROS)

ROS inhibitor reduces a *S. aureus*-induced H2AX phosphorylation

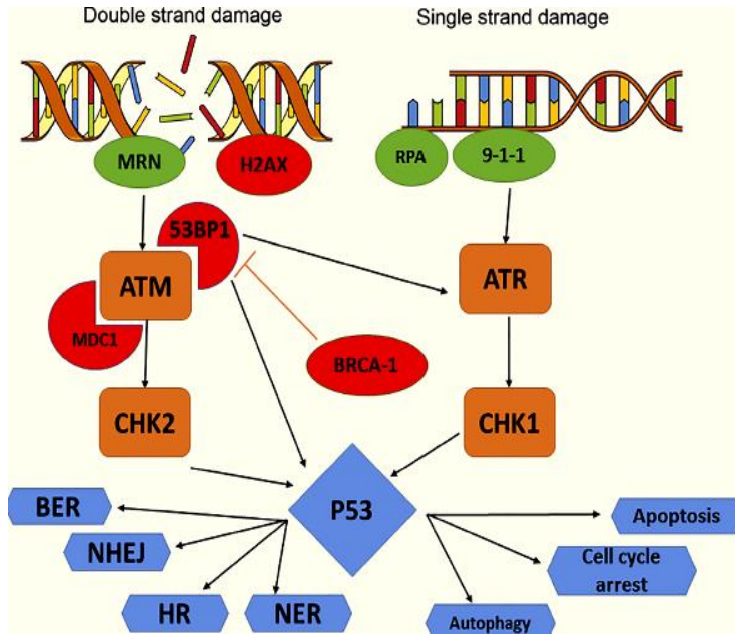


**b** ROS primarily damage guanine among the DNA bases. Guanine's oxidized product 7,8-dihydro-8-oxoguanine (8-oxoG) is the most predominant DNA oxidative lesion in the genome



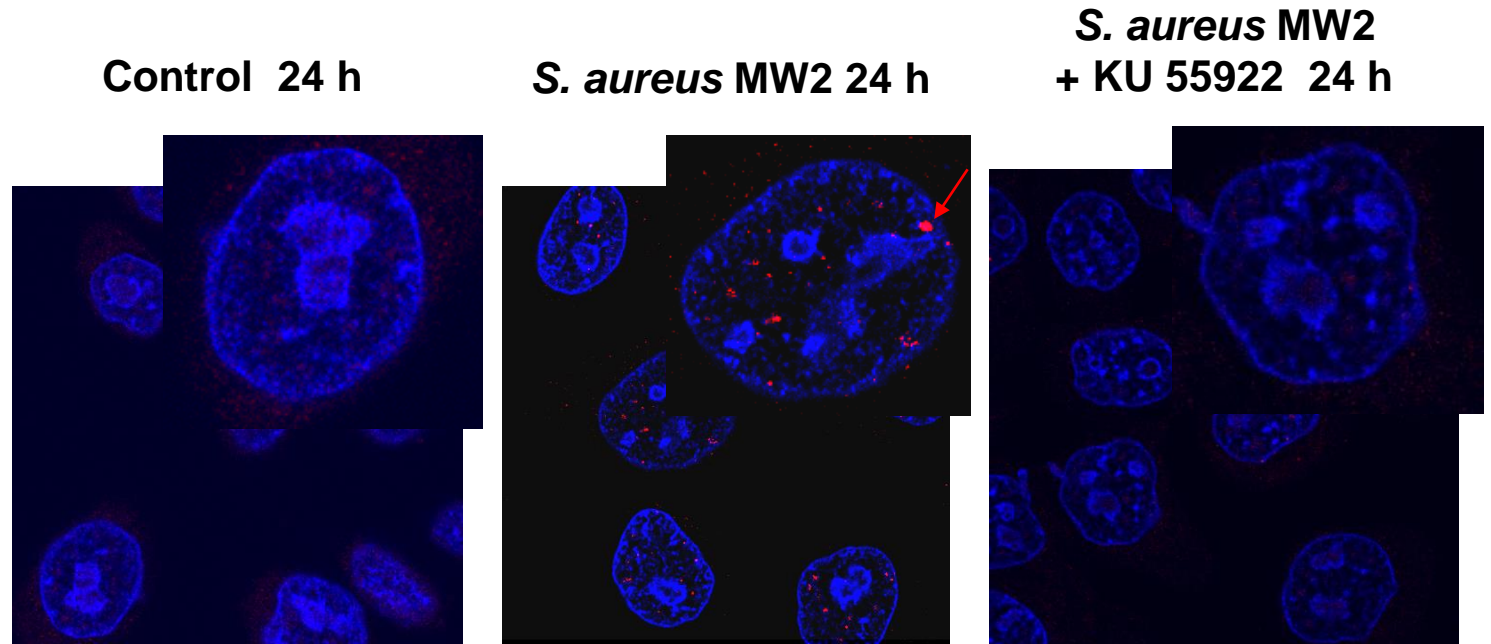
Cells were exposed to *S. aureus* for 24 h and were immunostained with anti 8-oxoG DNA lesion antibody

# ➤ The damaged DNA triggers a DDR involving the recruitment of the early repair factor 53BP1



Mirza-Aghazadeh-Attari et al., 2019

53BP1



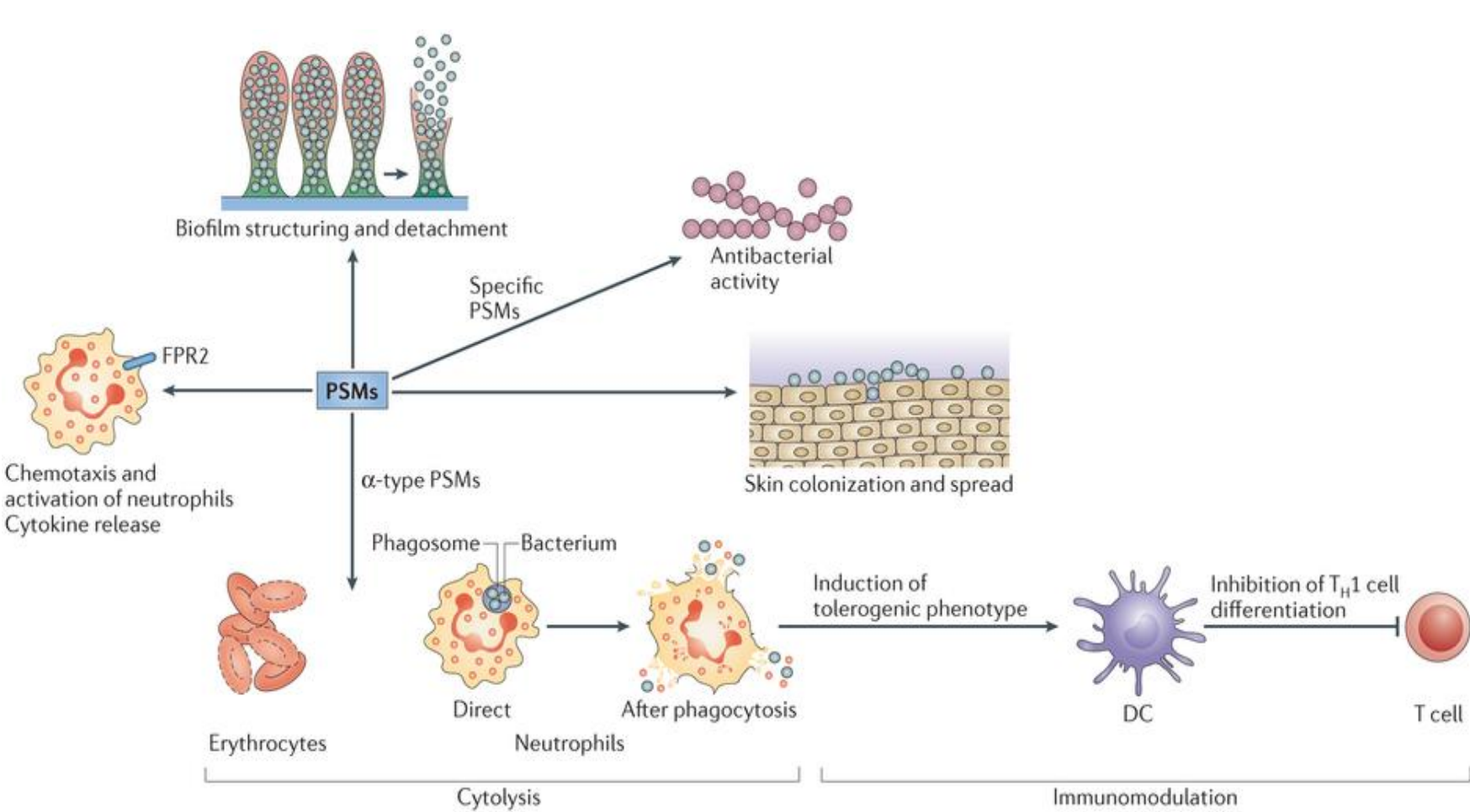
53BP1 accumulates on the chromatin surrounding DSB due to the signaling that is initiated by the ATM-mediated phosphorylation of the histone H2AX

The nuclear staining of 53BP1.

53BP1 promotes the end-joining of distal DNA ends

An addition of the ATM inhibitor **KU-55933** resulted in a strong decrease of the proportion of positive 53BP1-stained infected cells.

# ➤ PHENOL-SOLUBLE MODULINS PEPTIDES (PSMs) DEFINE THE VIRULENCE POTENTIAL OF *S. aureus*



**PSM $\alpha$ 1-PSM $\alpha$ 4  
 $\delta$ -toxin** 20-25 amino acids

**PSM $\beta$**  44 amino acids

**PSM $\alpha$ 1-PSM $\alpha$ 4** are encoded in the *psm $\alpha$*  operon

**PSM $\beta$ 1** and **PSM $\beta$ 2** are encoded in the *psm $\beta$*  operon

**$\delta$ -toxin** is encoded within the coding sequence for RNAIII, the RNA effector molecule of the accessory gene regulator (AGR) quorum-sensing system

Nature Reviews | Microbiology

Peschel and Michael Otto, 2013

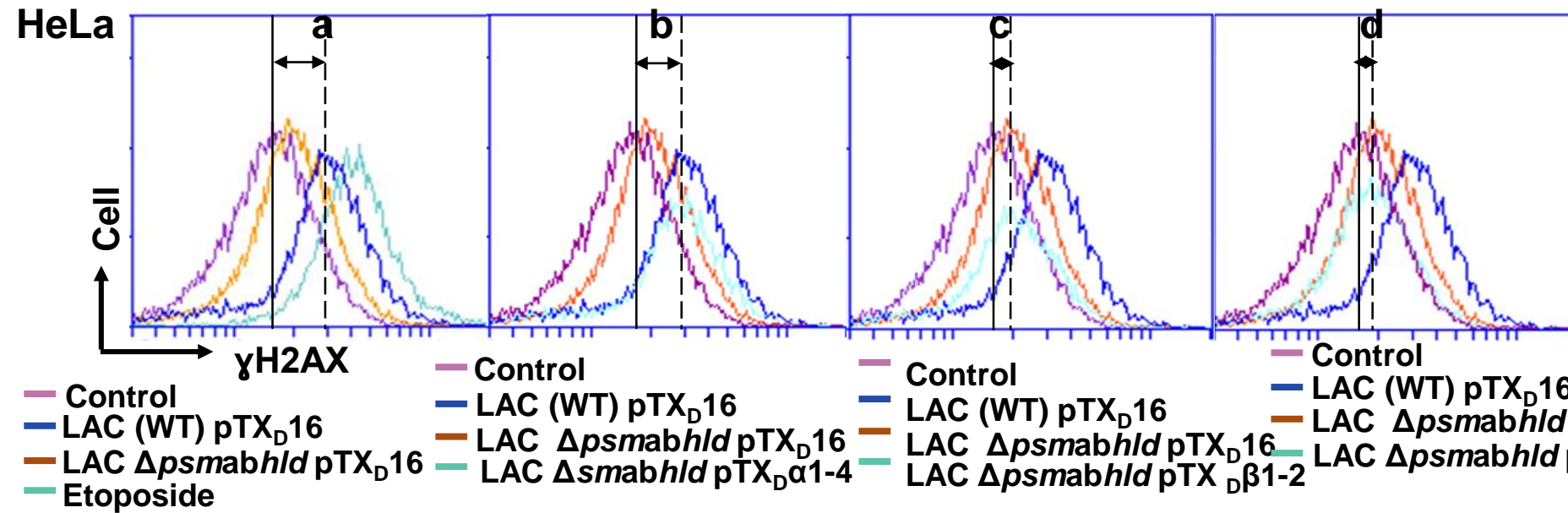


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# ➤ *S. aureus* PHENOL-SOLUBLES MODULINES PSM $\alpha$ 1-4 INDUCE, WHILE LPLs DAMPEN HOST DNA DAMAGE

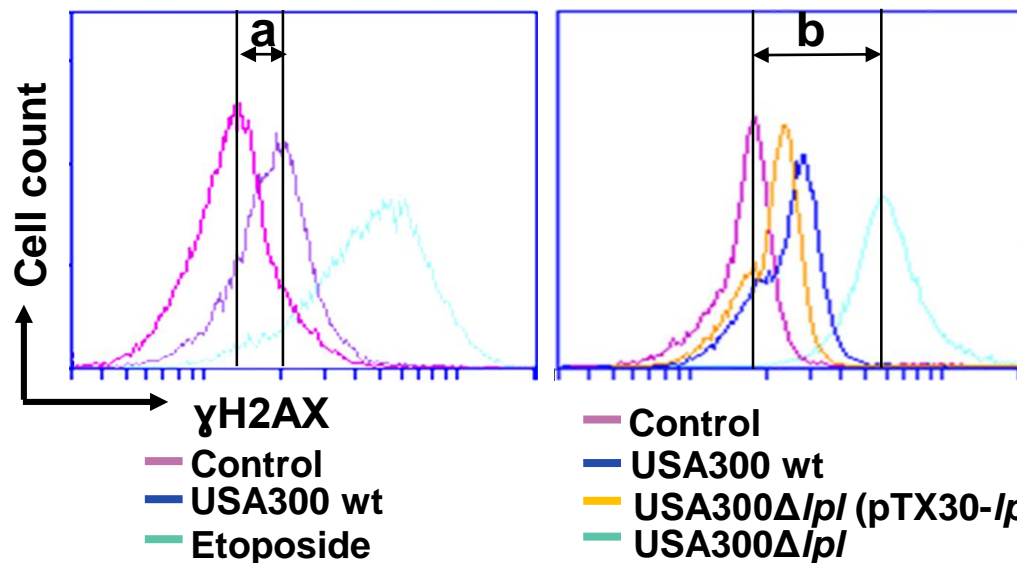


LAC (WT) pTX $\Delta$ 16 contains plasmid pTX $\Delta$ 16 (blue line)  
 LAC  $\Delta$ psm $\alpha$  $\beta$ hld pTX $\Delta$ 16-PSM deletion mutant (yellow line)  
 Complemented mutants:  
 LAC $\Delta$ psm $\alpha$  $\beta$ hld pTX $\Delta$  $\alpha$ 1-4 (blue-green line, b)  
 LAC $\Delta$ psm $\alpha$  $\beta$ hld pTX $\Delta$  $\beta$  (blue-green line, c)  
 LAC $\Delta$ psm $\alpha$  $\beta$ hld pTX $\Delta$ hld (blue-green line d)

Constructed in the Michael Otto laboratory, NIH, USA

## Lipoproteins

A pathogenicity island that contains a cluster of lipoprotein-encoding genes, *lpl*



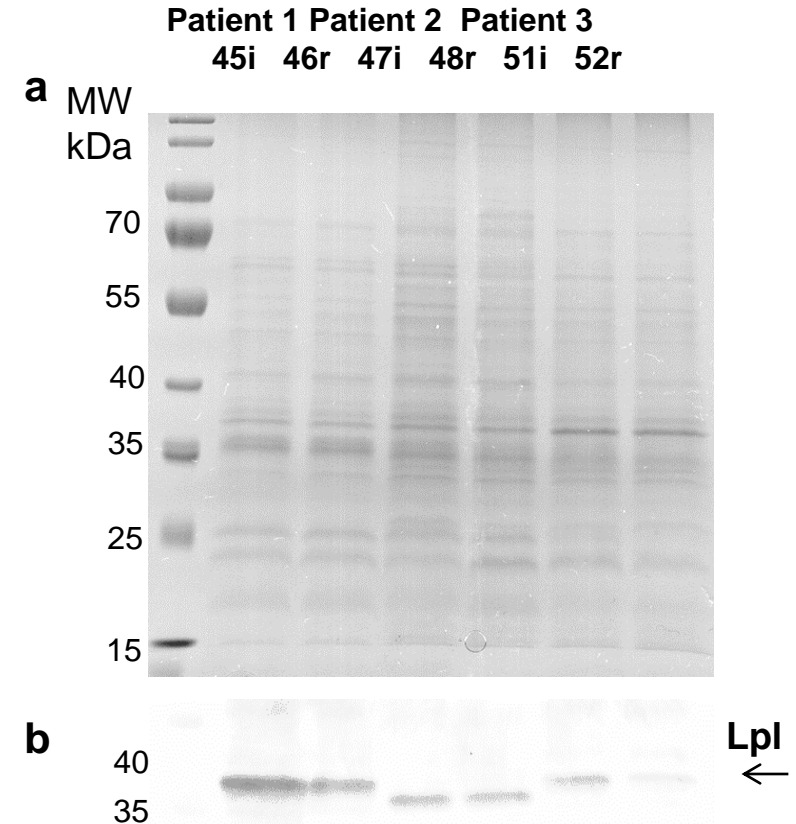
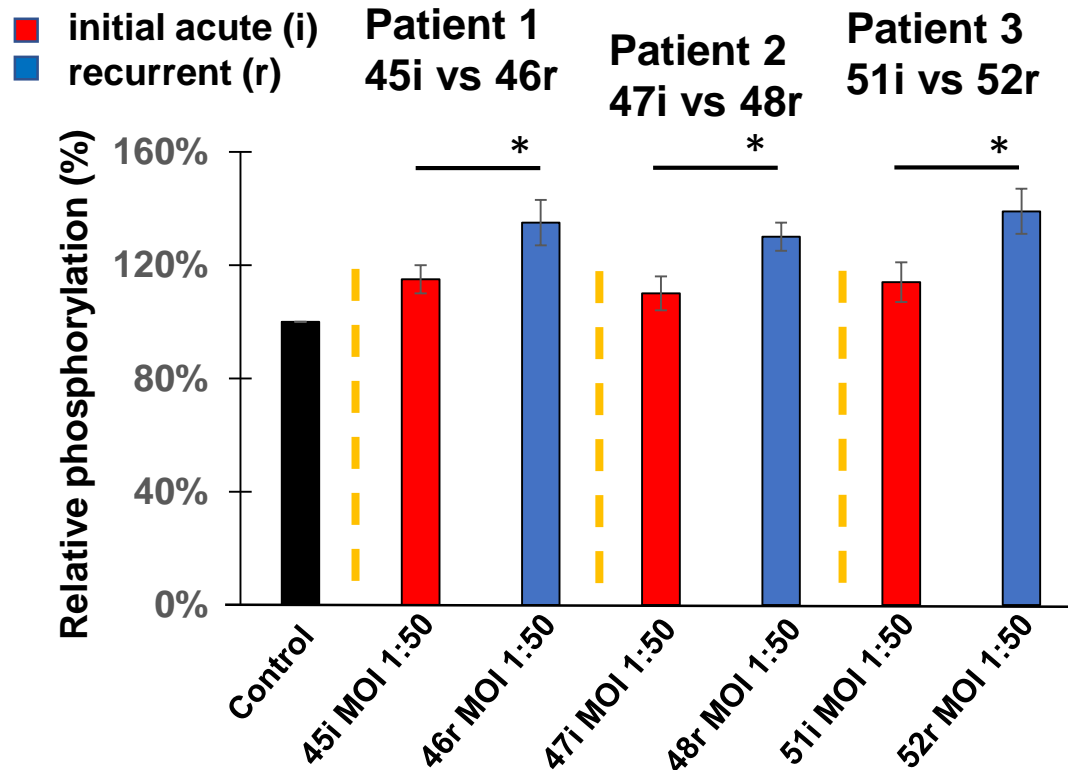
USA300 wt (a, b, blue) ↑  
 USA300 $\Delta$ lpl, the deletion mutant (b, blue-green) ↓  
 USA300 $\Delta$ lpl (pTX30-*lpl*) the complemented mutant (b, yellow)

Constructed in the Fritz Götz laboratory, University of Tübingen, Germany

# > *S. aureus* RECURRENT ISOLATES INDUCE STRONGER DNA DAMAGE THAN INITIAL ACUTE ISOLATES

HeLa

*S. aureus* recurrent isolates express a lower amount of Lpls than initial acute isolates

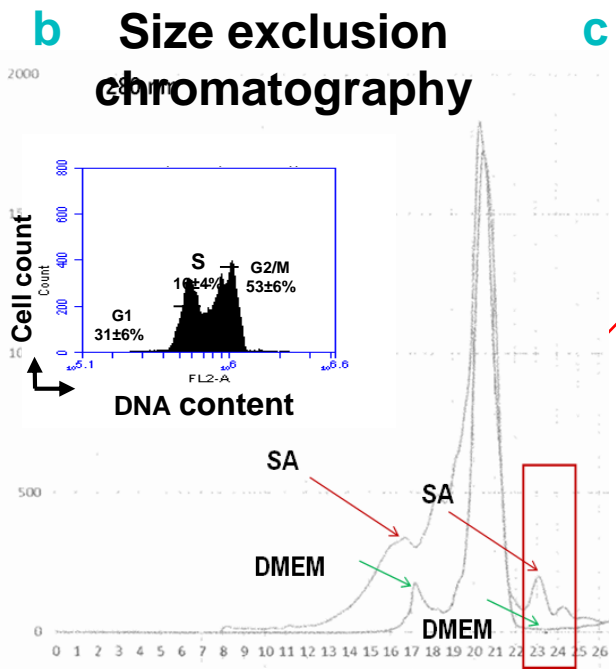
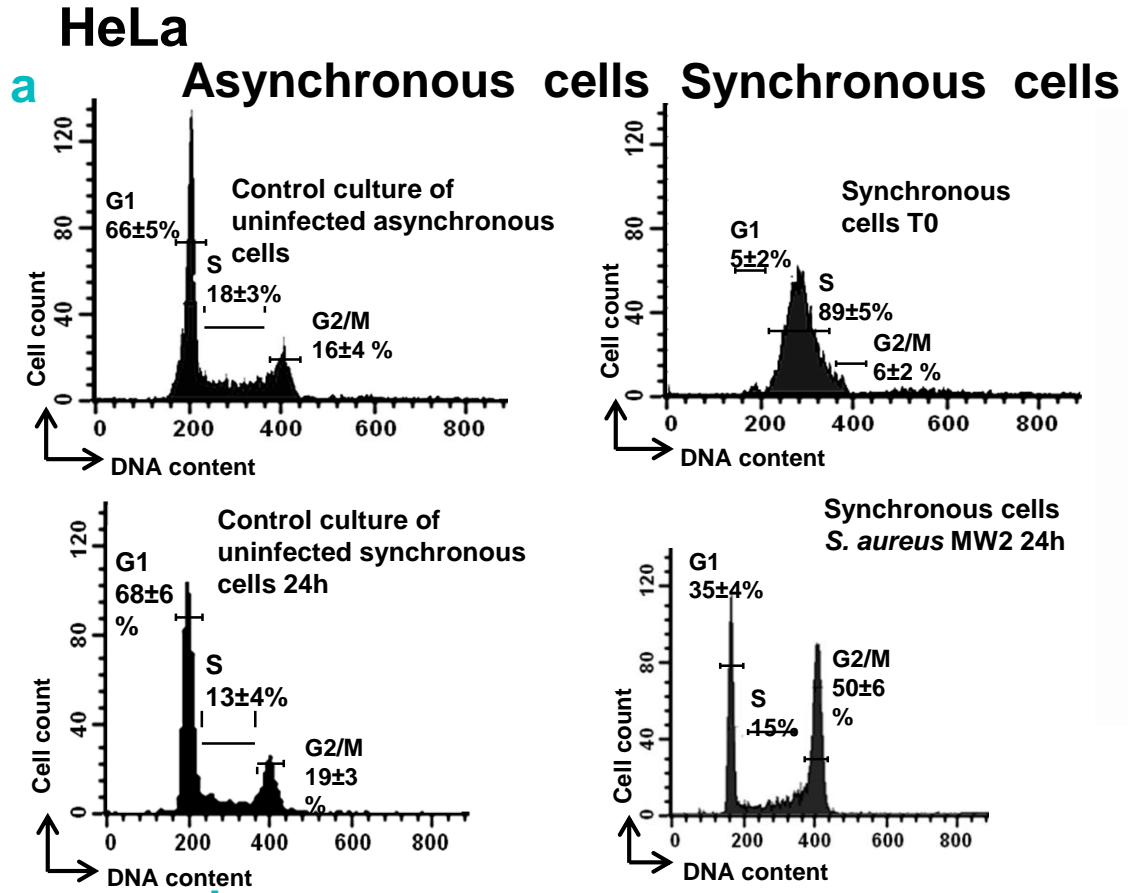


Recurrent *S. aureus* isolates induced a stronger DNA damage than initial ones and this capacity could be linked at least partially to the low level of Lpls production

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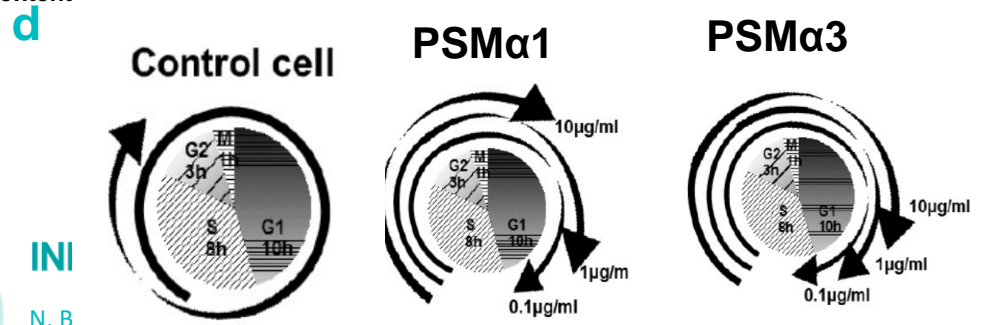
N. Berkova  
SFM 2022

# > *S. aureus* INDUCED A G2/M PHASE TRANSITION DELAY



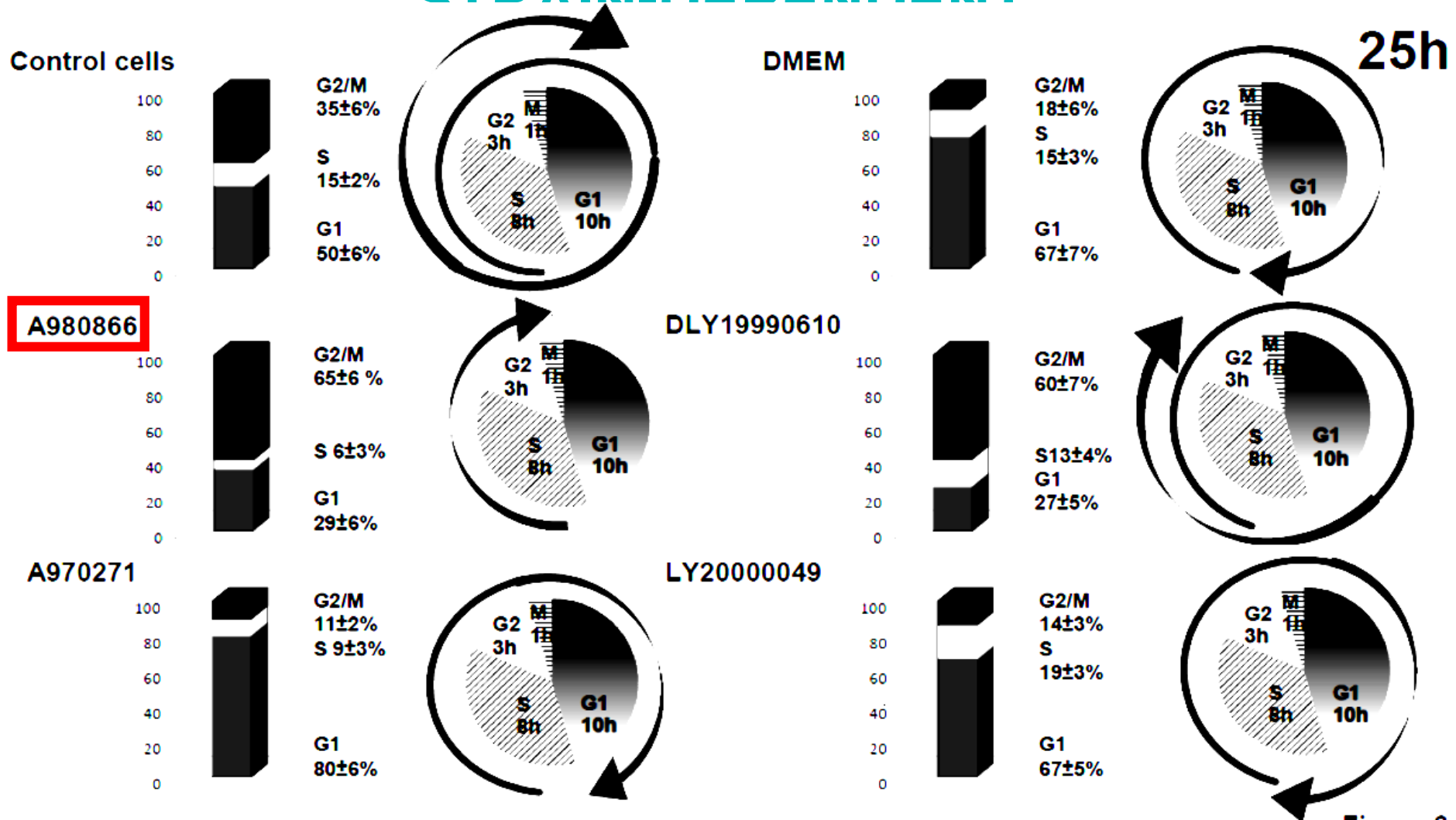
**c** Tandem mass spectrometry

PSM peptide derivatives: the majority were PSMα1 and PSMα3. PSMα2 and PSMα4 derivatives were also identified



Synthetic PSMα1 and PSMα3 induce a G2/M phase transition delay

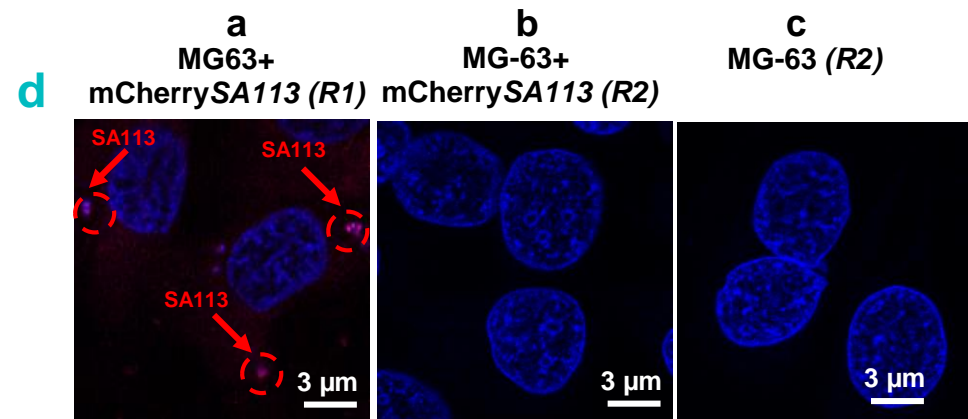
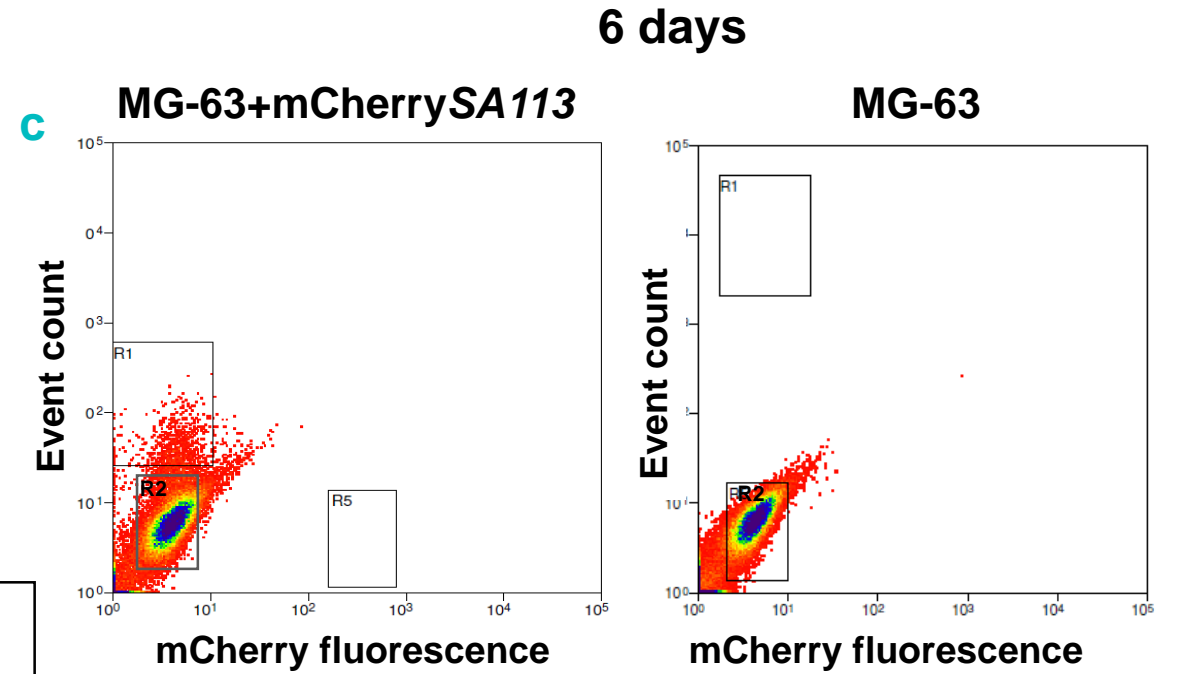
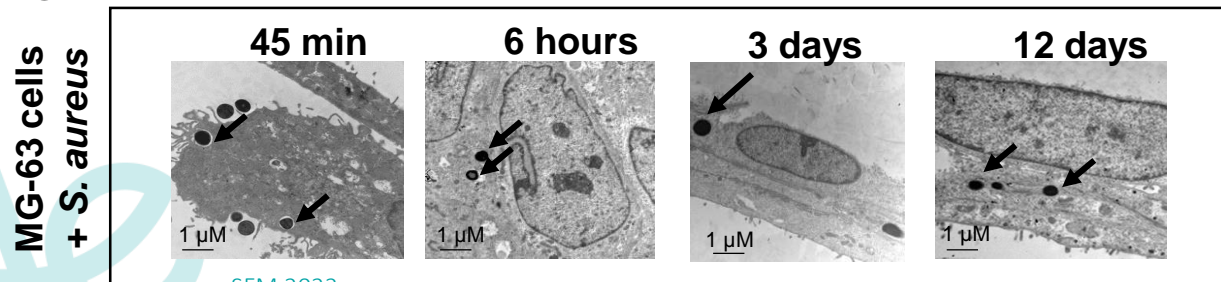
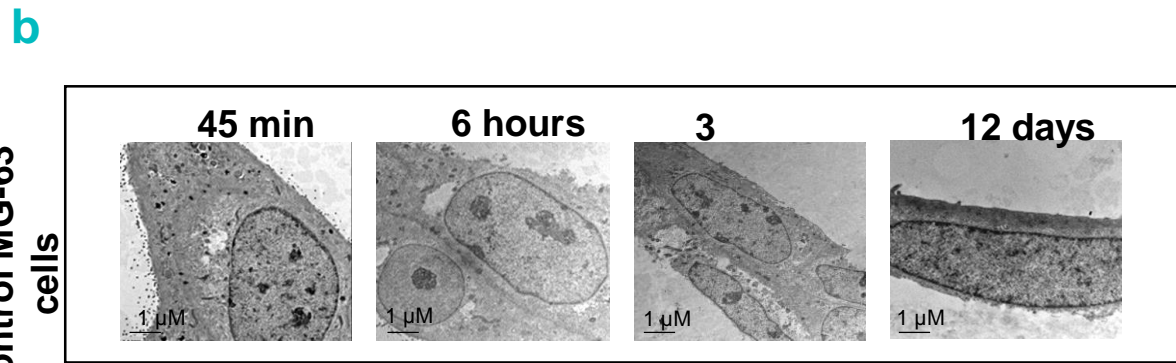
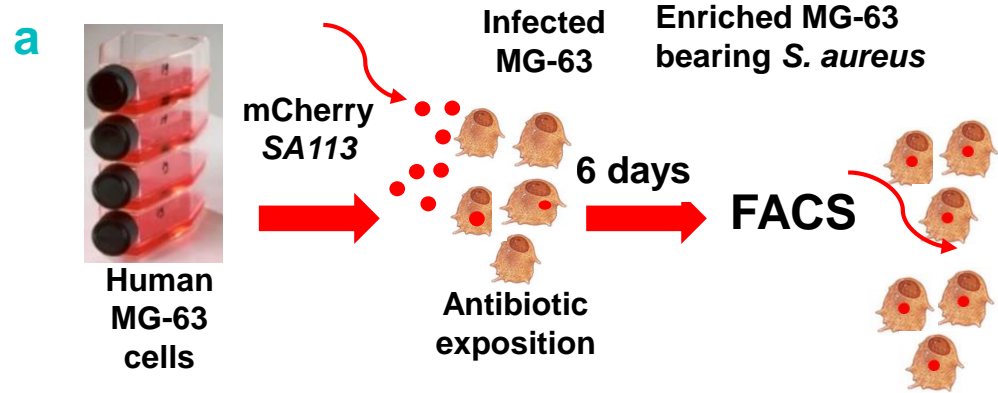
# > *S. aureus*-INDUCED G2/M TRANSITION DELAY IS STDBAINDEPENDENT



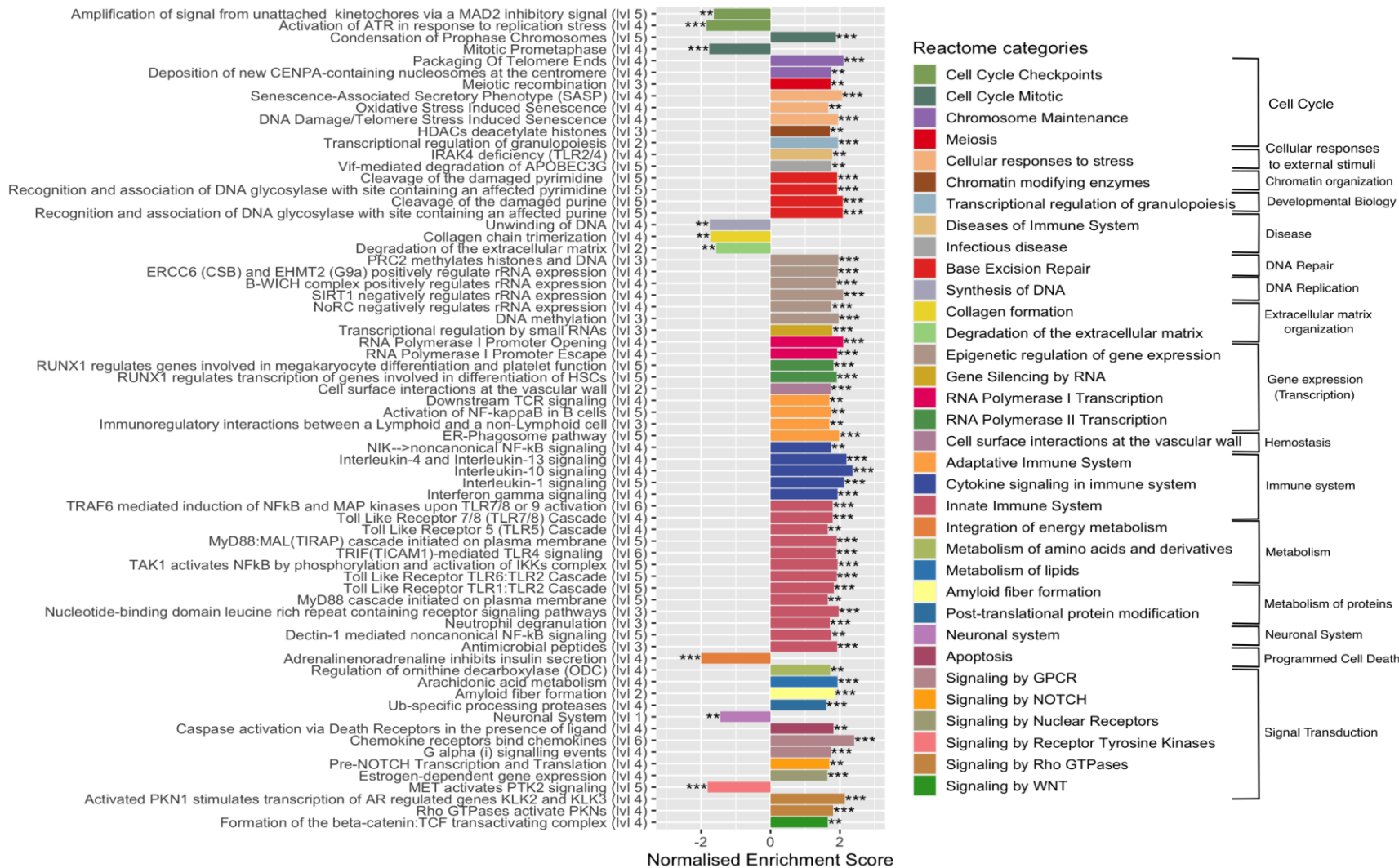
The results of reversed-phase HPLC coupled to tandem mass spectroscopy showed that PSMA1 was only detectable in A980866 supernatant that delay G2/M transition



# ➤ DEVELOPMENT OF AN INFECTION MODEL TO ISOLATE SOLELY CELLS CONTAINING INTERNALIZED *S. aureus*



# 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

RNA sequencing of hosting intracellular *S. aureus* cells or non-infected cells  
Gene-Set Enrichment Analysis using Reactome Database

# ➤ TRANSCRIPTIONAL REPROGRAMMING OF GENES INVOLVED IN EPIGENETIC REGULATION

Genes expression depends on the interaction of transcription factors with epigenetic modulators (“epifactors”), which regulate DNA accessibility by controlling the structure of chromatin

## Epifactor database

117 DEGs encoding epifactors

**92 downregulated**

25 upregulated

## GO-BP enrichment analysis

Chromatin-Repressive Complexes:

BAHD1, NurD, Polycomb PRC1

mSin3A, CoREST complexes

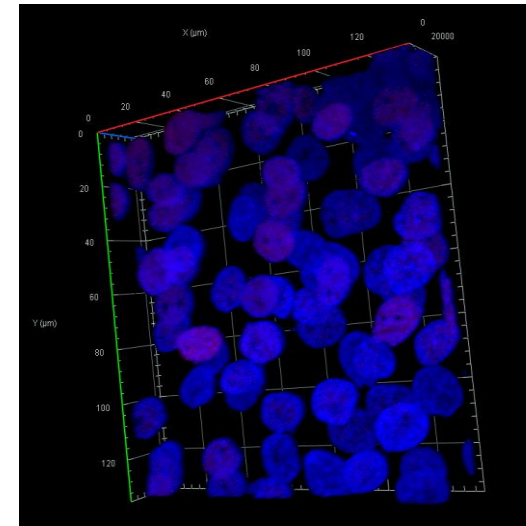
Downregulated DEGs “epifactors”:

histone deacetylases,

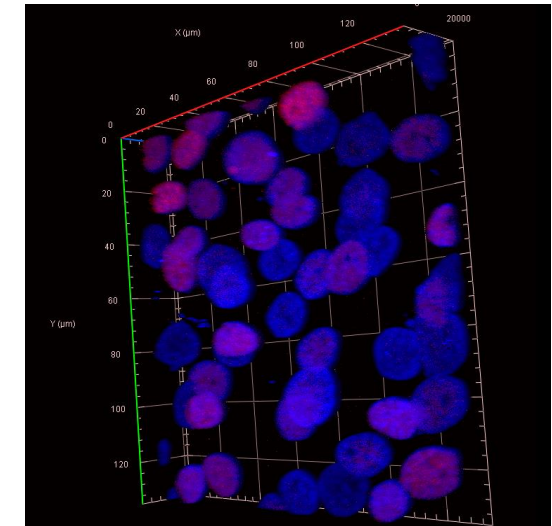
methyltransferase

Histone 3 acetylation lysine 27  
in *S. aureus*-treated cells

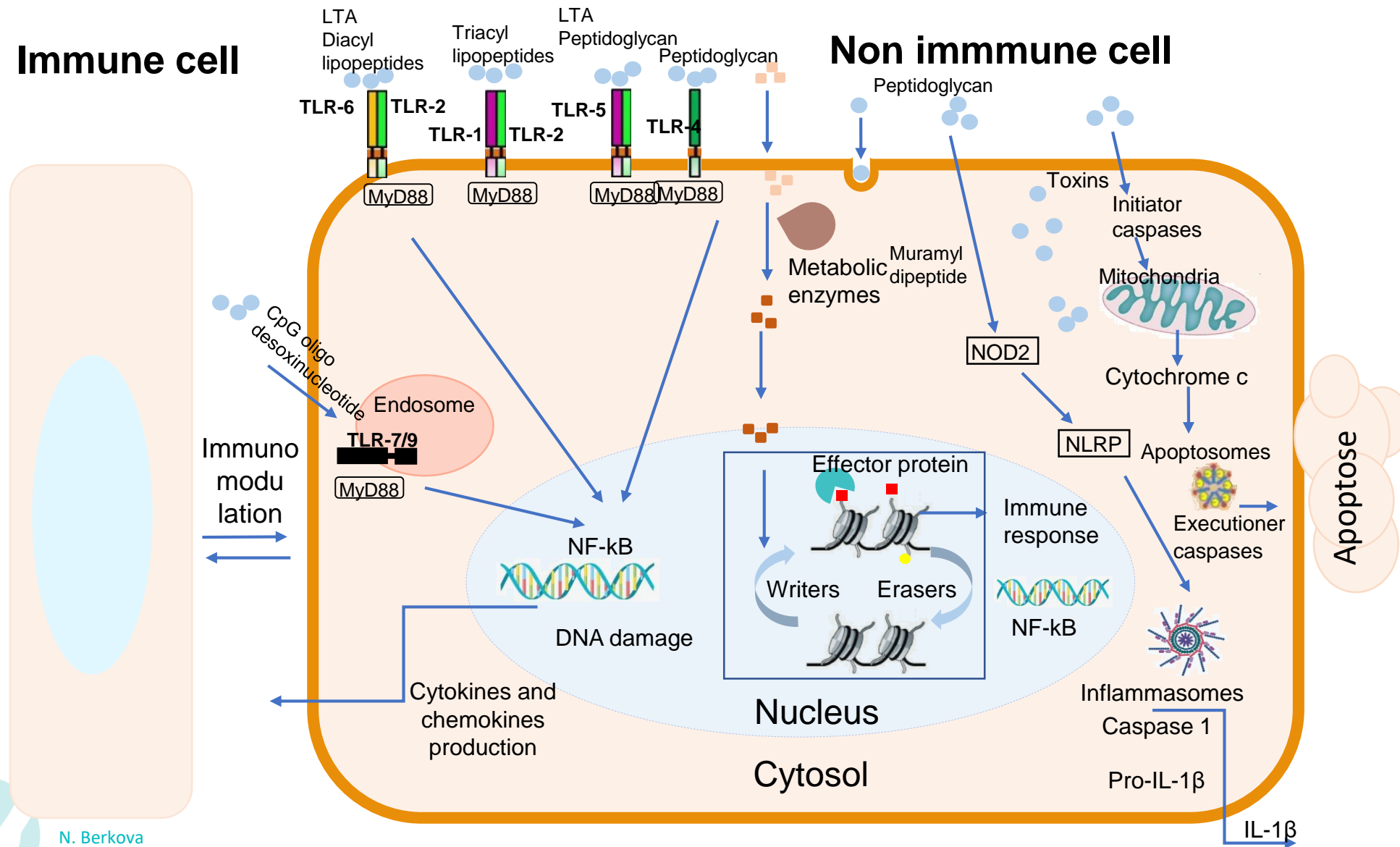
Control



Cells+*S. aureus*



# ➤ Model of the immune, metabolic and epigenetic dysregulated signatures induced by long-term *S. aureus* infection





# THANK YOU FOR YOUR ATTENTION

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