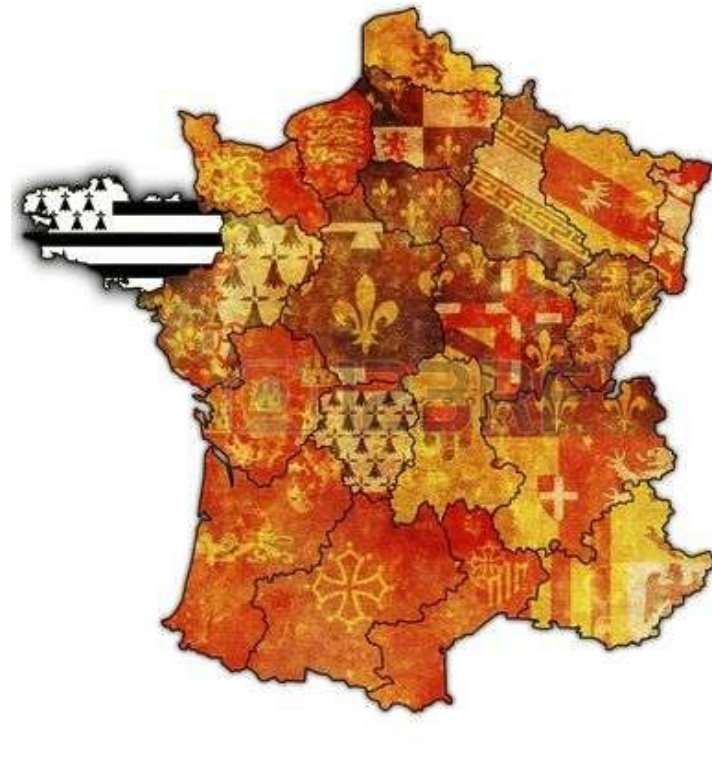




# The secret weapon that allows *Staphylococcus aureus* to hijack your cell cycle

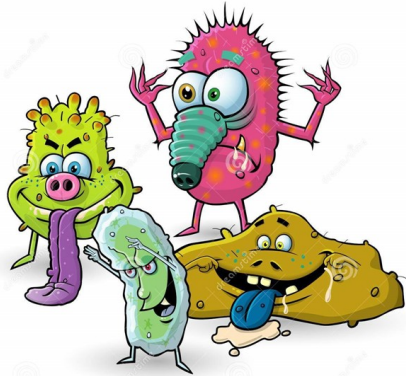
**Nadia Berkova, PhD**

UMR1253, STLO,  
Health Department, INRA, Rennes, France



# Host cells- Pathogens interactions

## How do pathogens affect host cell pathways?



**Pathogens**

**Host cells**

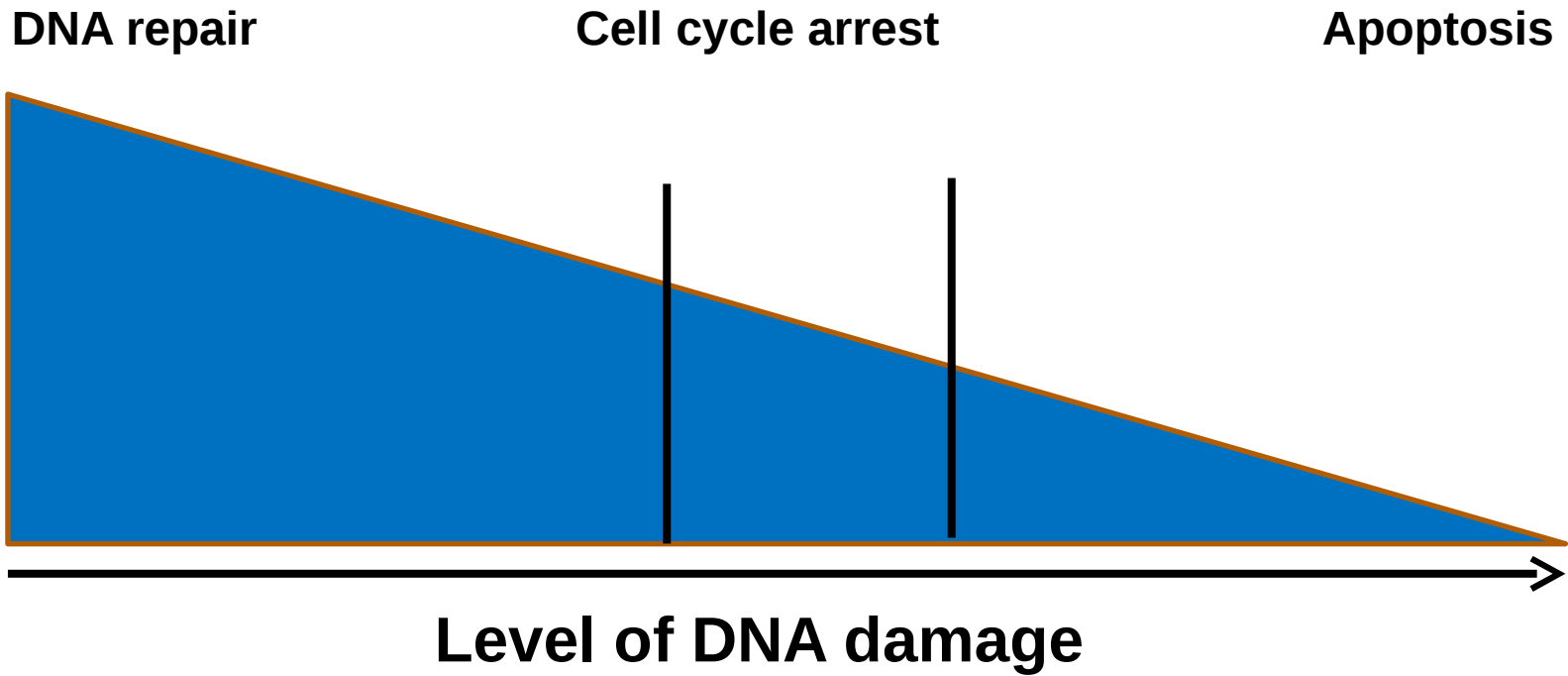
Promotion/inhibition of proliferation

Alteration of apoptosis

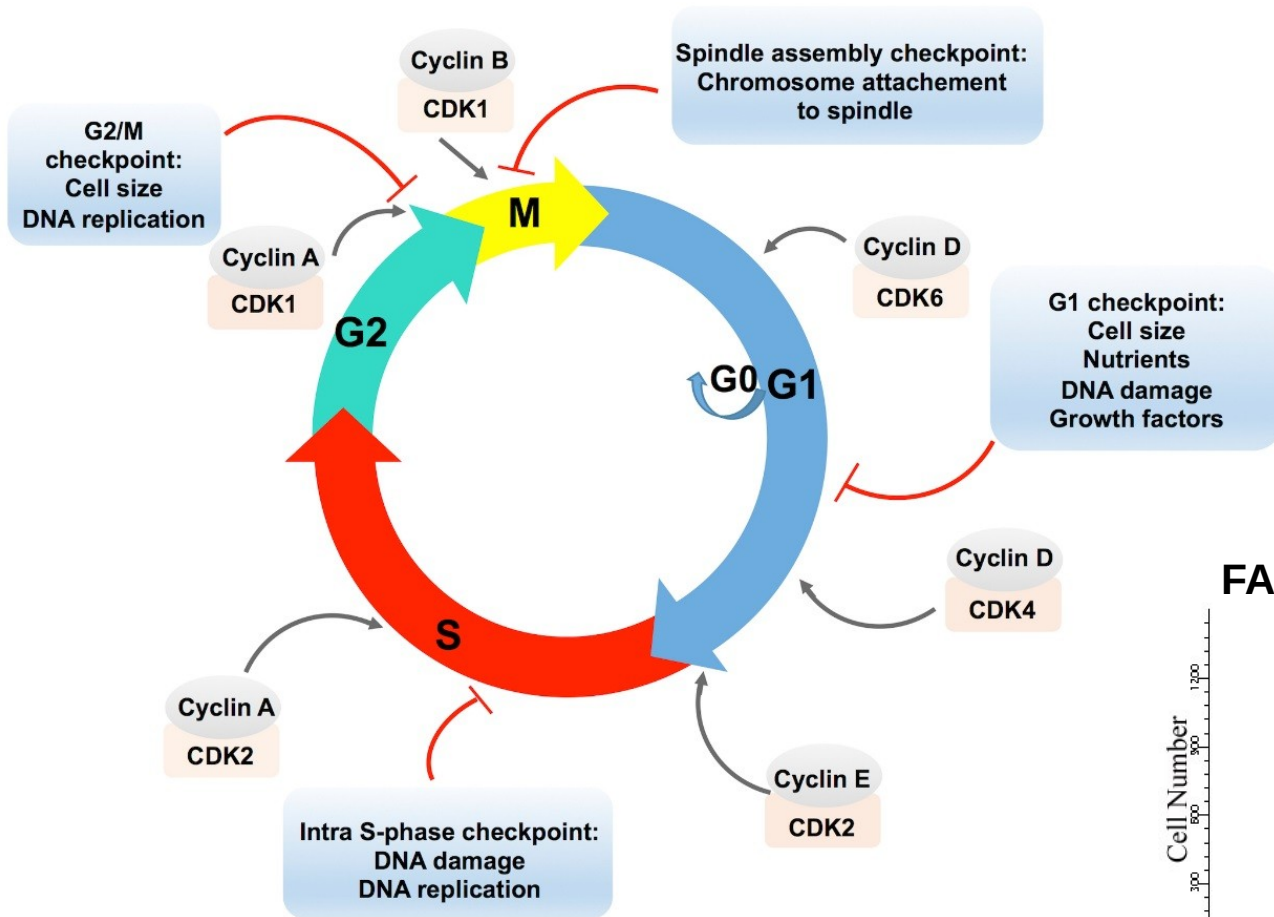
Cell cycle modulation

DNA degradation/repair

# DNA damage during infection

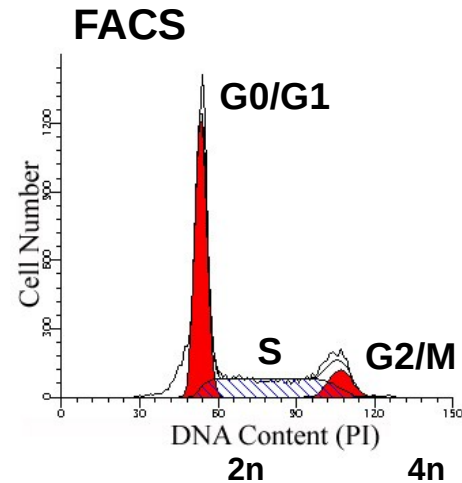


# The cell cycle of eukaryotic cells



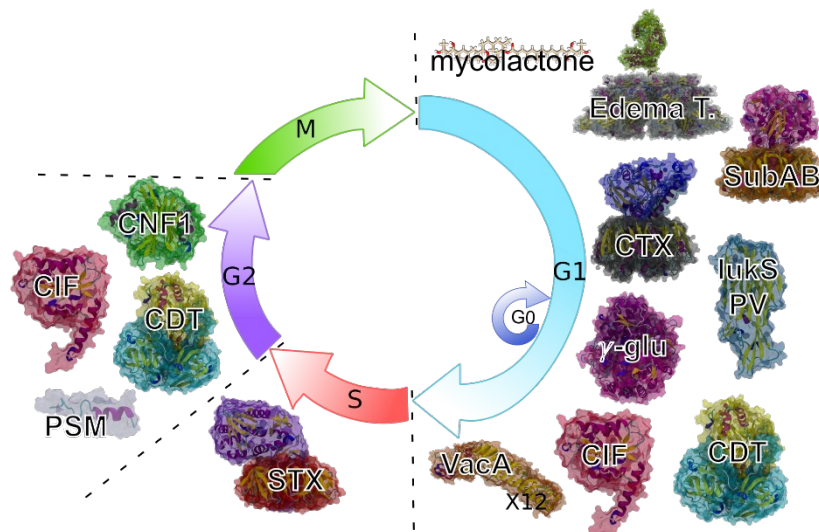
The eukaryotic cell cycle involves

- DNA replication
- Chromosomes segregation
- Cell division



# Cyclomodulins are bacterial effectors that interfere with the eukaryotic cell cycle

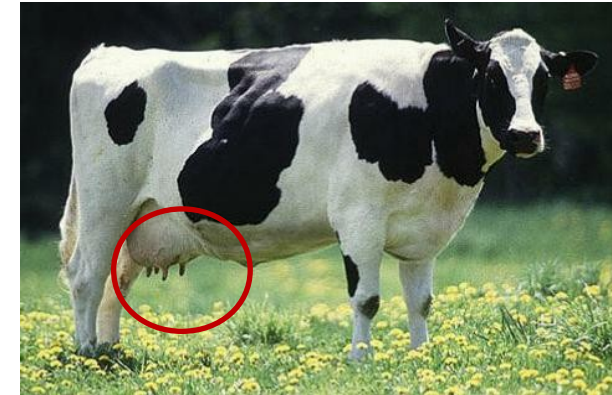
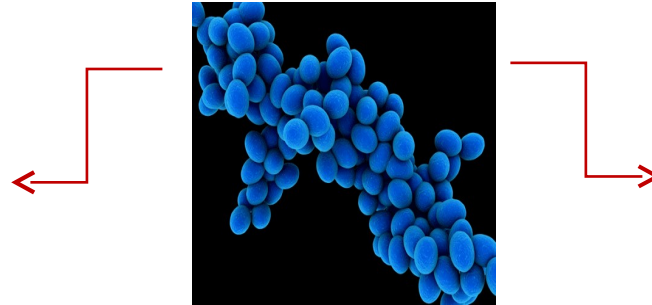
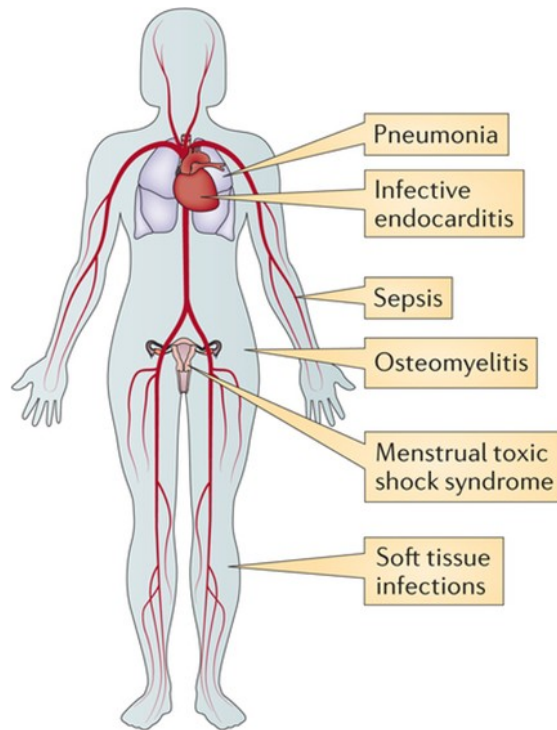
Cytotoxic Distending Toxin (CDT)	Three globular subunits	<i>E. coli</i> <i>H. hepaticus</i> <i>S. enterica serovar Typhimurium</i>	CdtB catalytic subunit CdtA and CdtC cell binding subunits	CdtB subunit: Dnase and phosphatase	G1/S G2/M
Shiga toxin (Stx) (Verotoxin)	AB5 toxin	<i>S. dysenteriae</i> <i>E. Coli (STEC)</i>	stxA enzymatic subunit StxB binding subunit	A subunit: N-glycosidase	S
Subtilase AB (SubAB)	AB5 toxin	<i>E. coli (STEC)</i>	SubA enzymatic subunit SubB binding subunit	A subunit: protease	G1/S
Anthrax toxin (Edema toxin/Lethal toxin)	Tripartite toxin	<i>B. anthracis</i>	Edema and/or Lethal factor (A enzymatic subunit) Protective Antigen (B binding subunit)	Edema factor : adenylate cyclase Lethal factor: zinc metalloprotease	G1/S



## Heterogeneous Family of Cyclomodulins: Smart Weapons That Allow Bacteria to Hijack the Eukaryotic Cell Cycle and Promote Infections

Rachid A. El-Aouar Filho<sup>1,2†</sup>, Aurélie Nicolas<sup>1†</sup>, Thiago L. De Paula Castro<sup>2</sup>, Martine Deplanche<sup>1</sup>, Vasco A. De Carvalho Azevedo<sup>2</sup>, Pierre L. Goossens<sup>3</sup>, Frédéric Taieb<sup>4</sup>, Gerard Lina<sup>5,6,7</sup>, Yves Le Loir<sup>1</sup> and Nadia Berkova<sup>1\*</sup>

# Staphylococcus aureus-induced pathologies

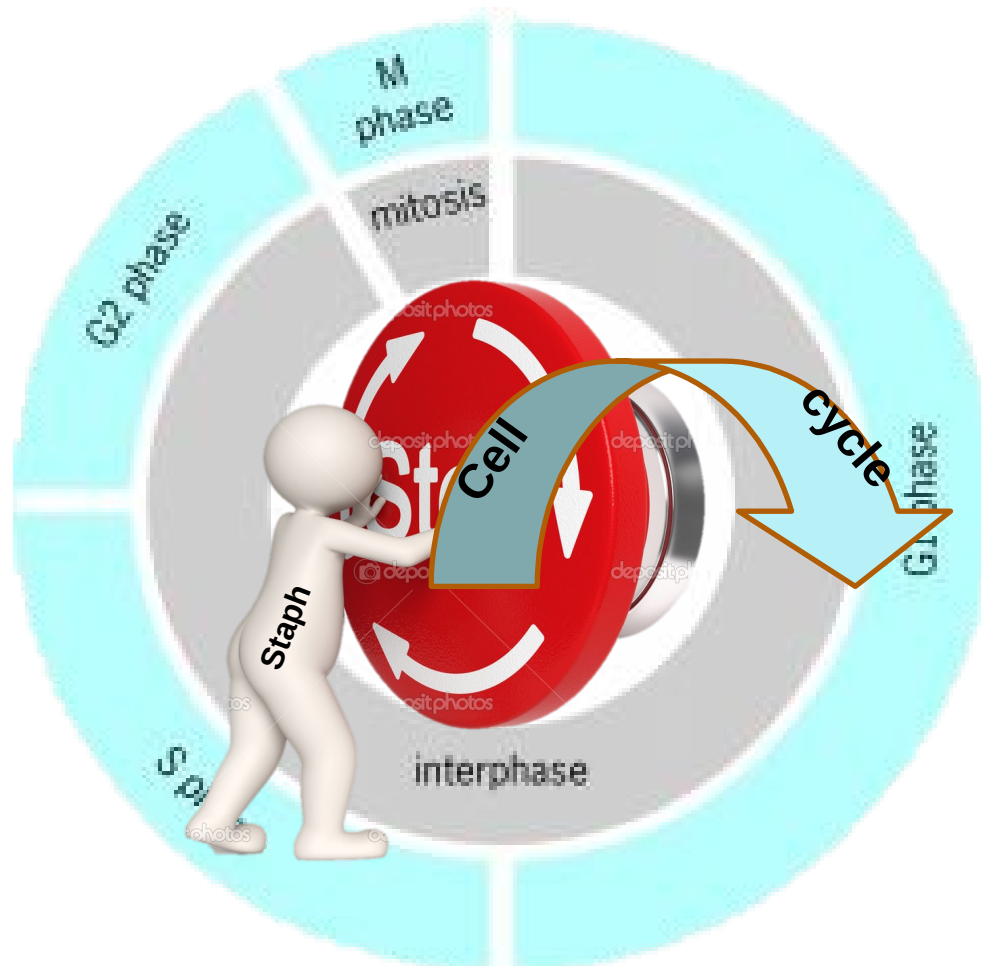


**Dairy cattle:** mastitis  
The cost:  
78 € par animal /1 year

**Human:** a wide range of clinical presentations

- **Staphylococcus aureus**
- Gram-positive bacterium
- is carried asymptotically by up to 50% of healthy people
- In Europe, methicillin-resistant strains affect 150,000 patients annually
- The extra costs=380 millions €

# Does *S. aureus* produce **cyclomodulins**? What is the biological significance of the cyclomodulin action?

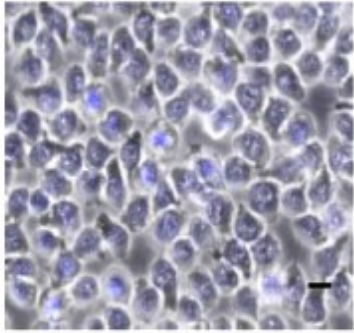


# Cytopathic effect

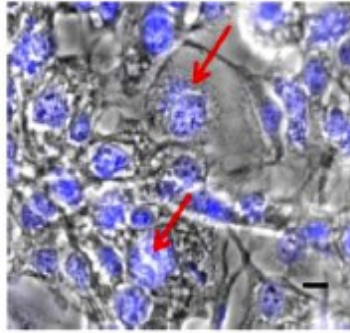
## Enlargement of the cells exposed to *S. aureus*.

72h

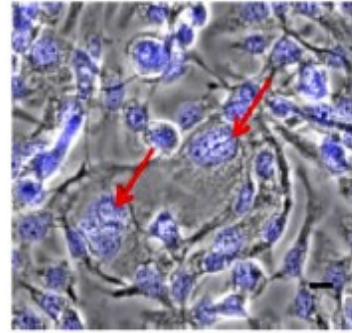
MAC-T Control



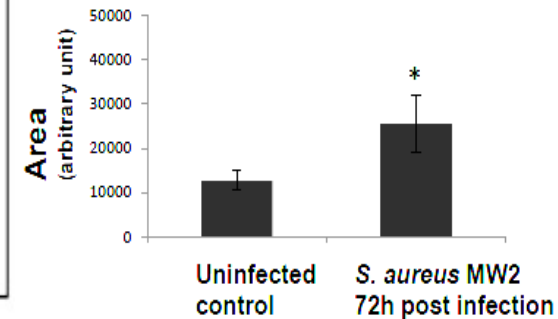
+*S. aureus* O46



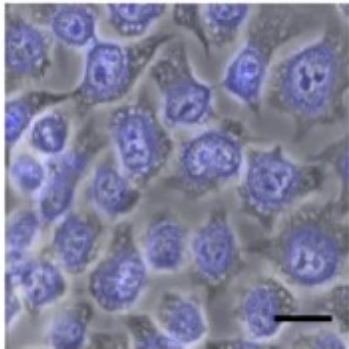
+*S. aureus* MW2



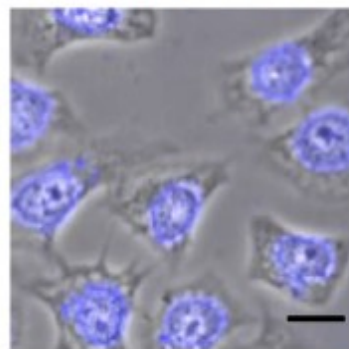
MAC-T



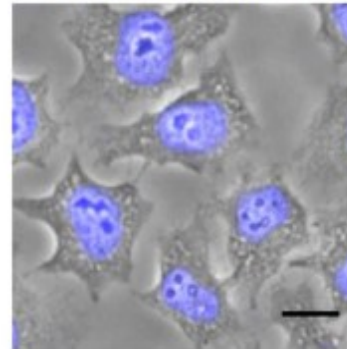
HeLa Control



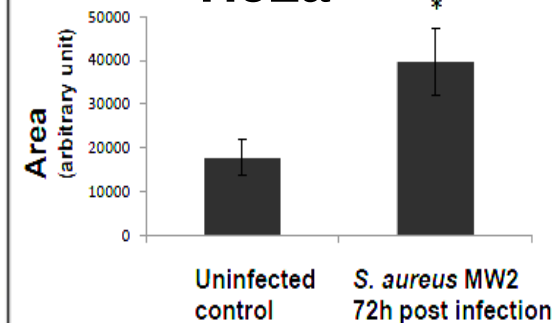
+*S. aureus* O46



+*S. aureus* MW2



HeLa

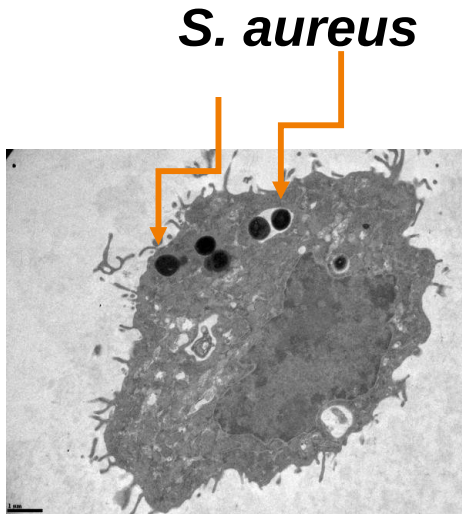


**MAC-T**, cells produced from primary bovine mammary alveolar cells

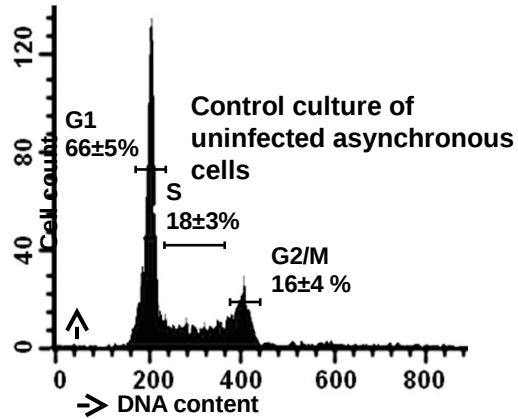
**HeLa**, human epithelial cells from a fatal cervical carcinoma

# *S. aureus* induces a G2/M phase delay

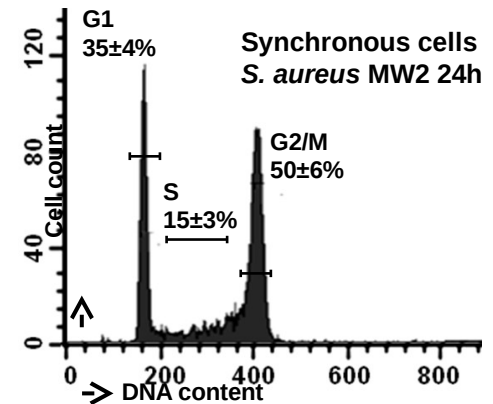
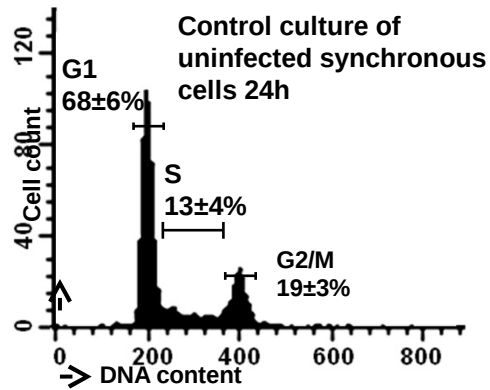
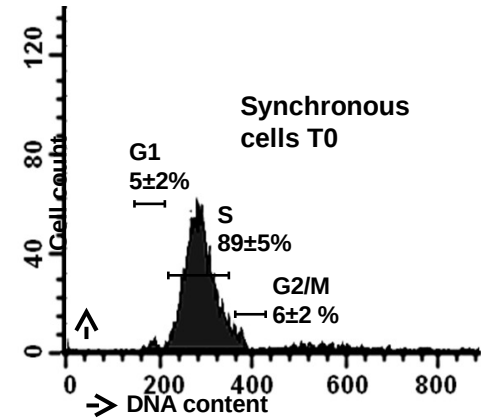
HeLa



Asynchronous cells

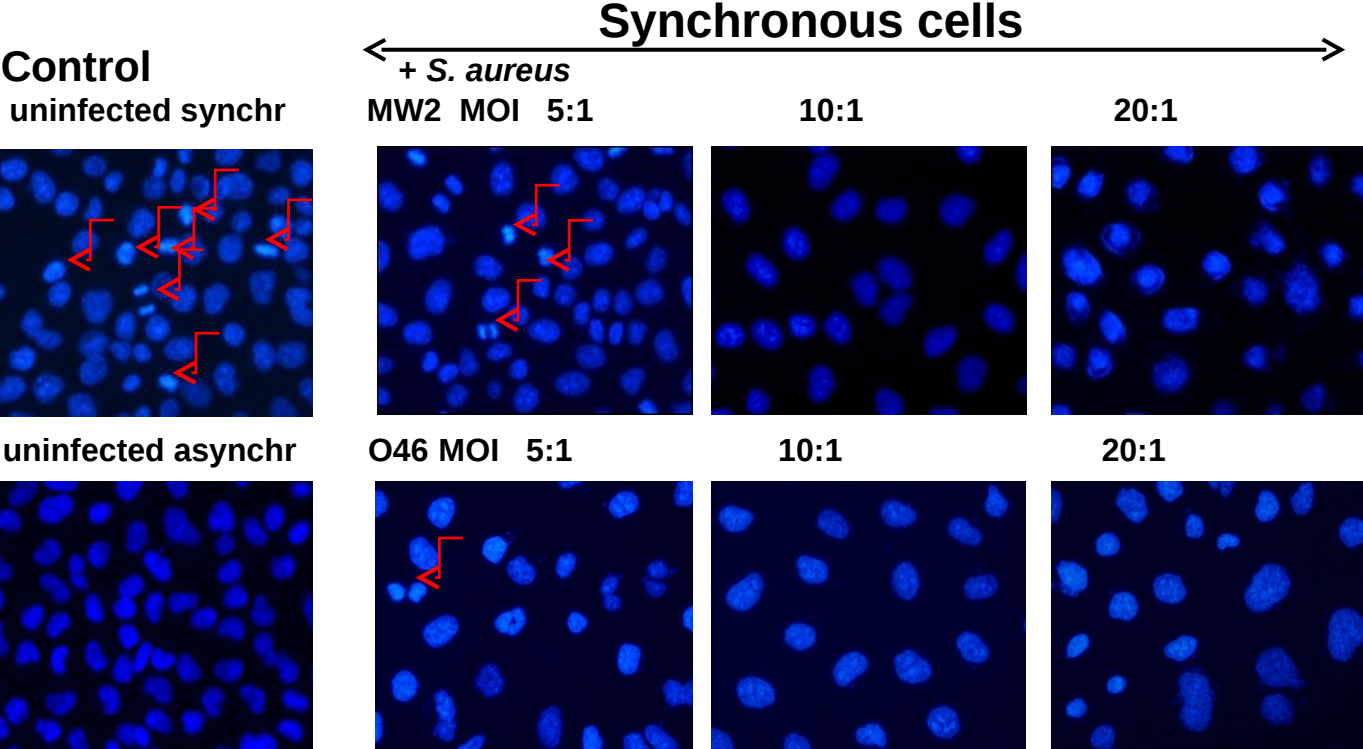


Synchronous cells



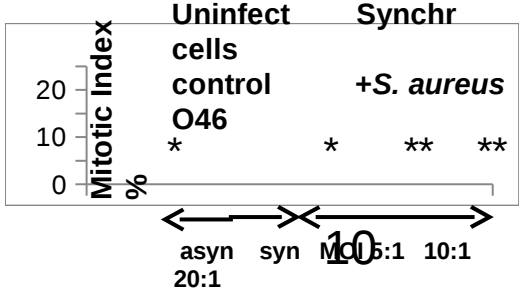
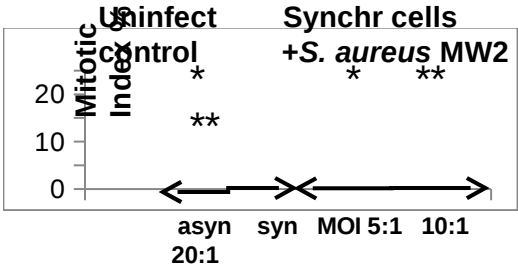
Heat-killed *S. aureus* or Latex beads don't induce a cell cycle arrest

# Drop in the mitotic index in *S. aureus*-infected cells



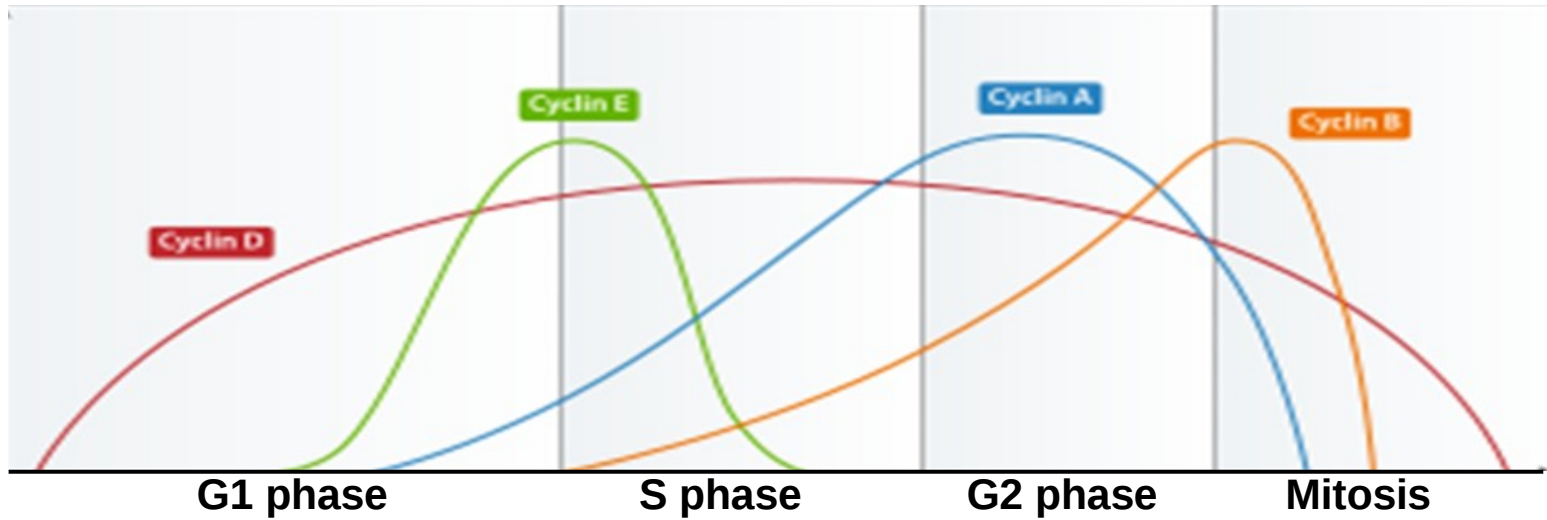
HeLa

**Number of cells in metaphase or anaphase/telophase** X 100%  
**total number of cells**

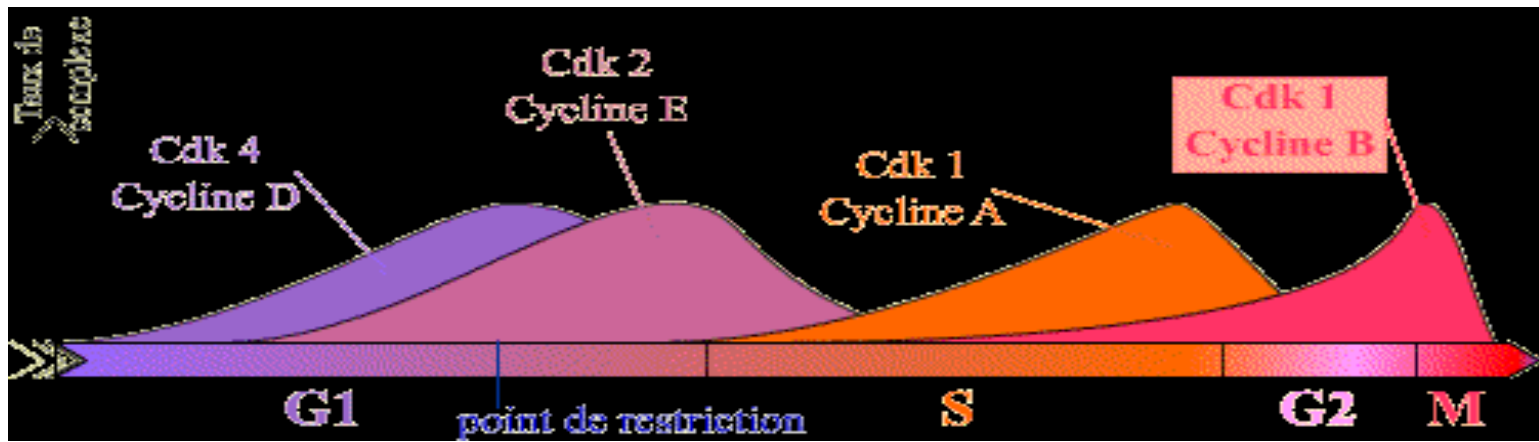


# Cell cycle regulation

**Cyclins** and **cyclin-dependent kinases** are 2 classes of regulatory molecules



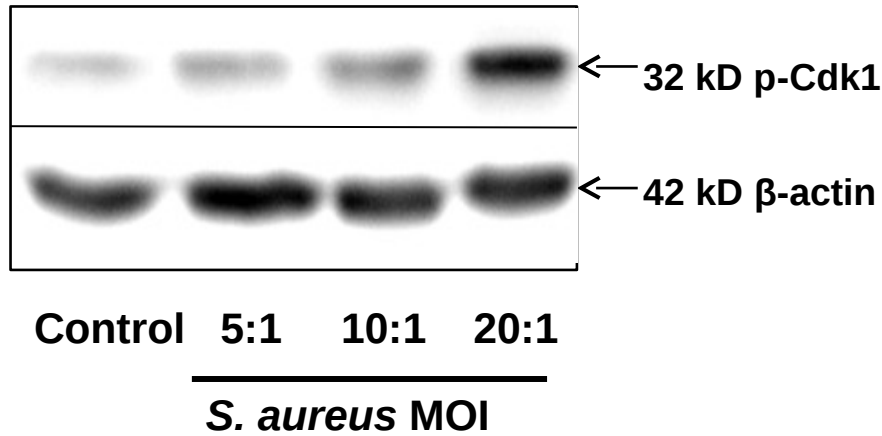
**Cyclins** control the cell cycle progression by activating **cyclin-dependent kinase**



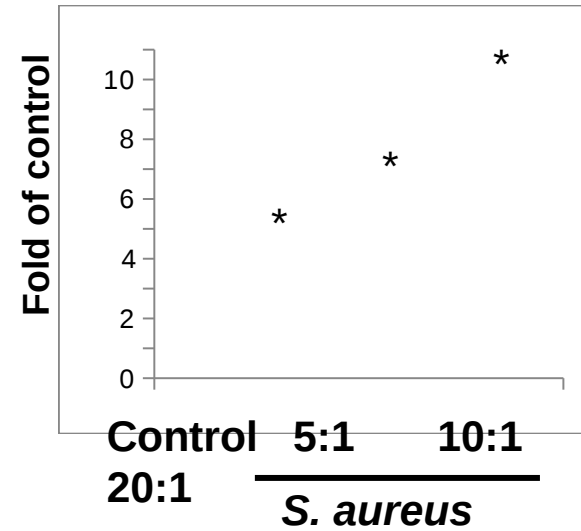
**Dephosphorylation** of Cdk1 at the late G2 phase activates Cdk1/cyclinB1 complex and triggers **mitotic** entry 11

# *S. aureus*-induced accumulation of phosphorylated Cdk1

## Western blot

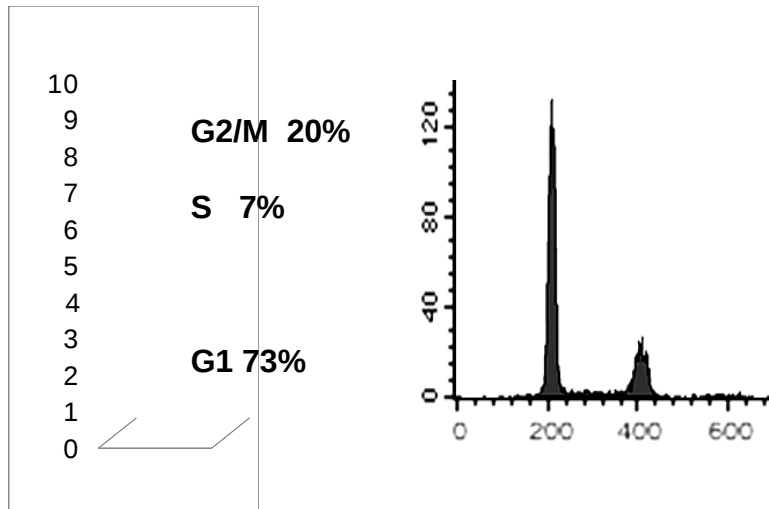


## Synchronous cells HeLa

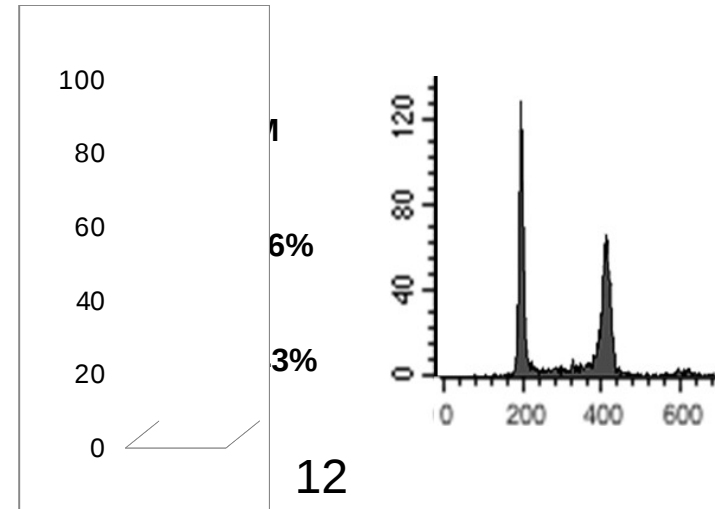


18h

Control cells



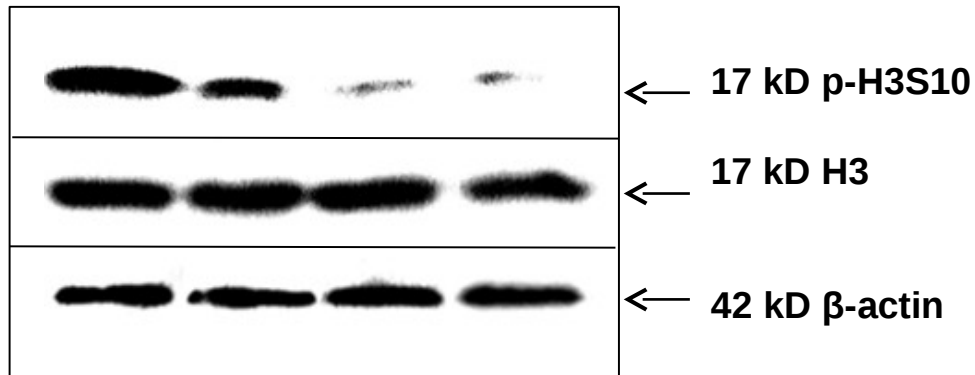
Cells + *S. aureus*





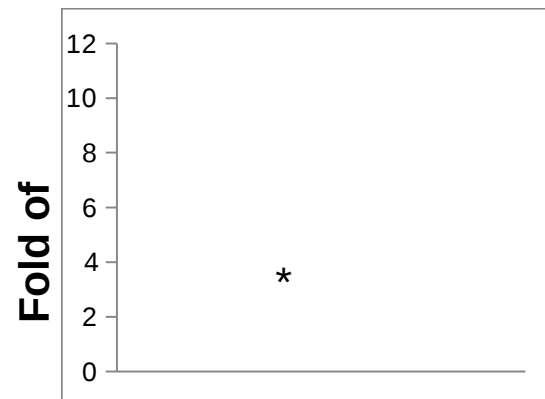
# *S. aureus*-induced dephosphorylation of histone H3 Ser10

## Western blot



Control 5:1 10:1 20:1  
*S. aureus* MOI

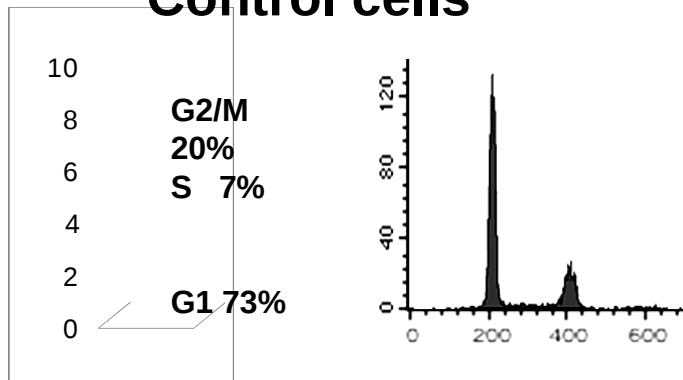
## Synchronous cells HeLa



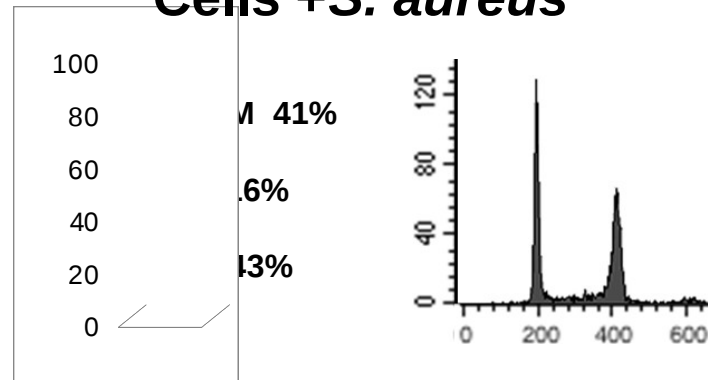
Control 5:1 10:1  
 20:1 *S. aureus* MOI

18h

## Control cells



## Cells + *S. aureus*

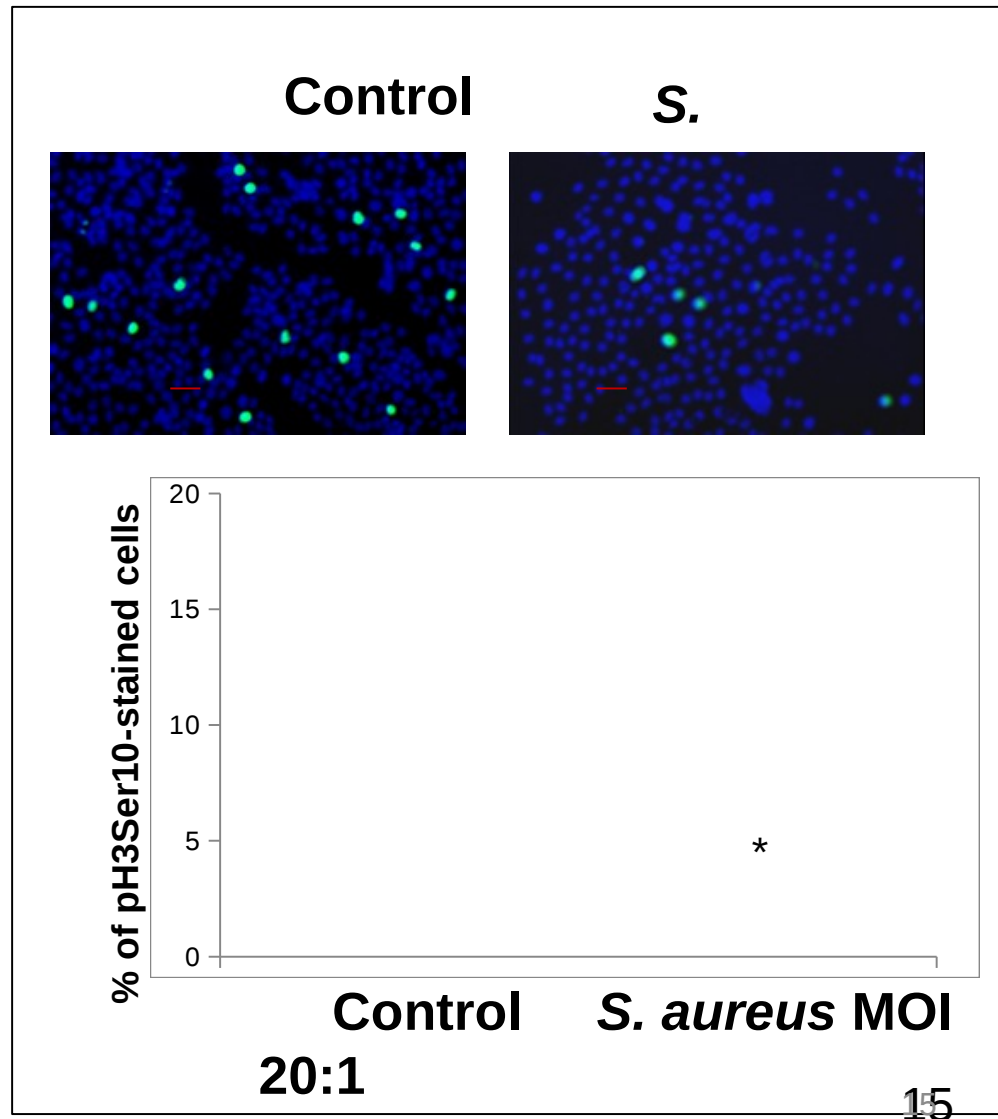


*S. aureus* induces a Ser10 dephosphorylation of histone H3

# Decrease in the number of p-Ser10 Histone H3-positive nuclei in *S. aureus*-infected cells

pH3Ser10

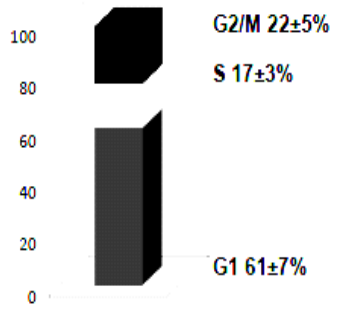
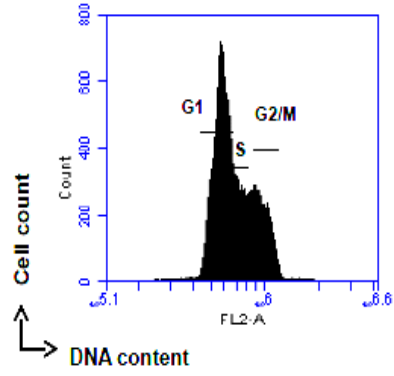
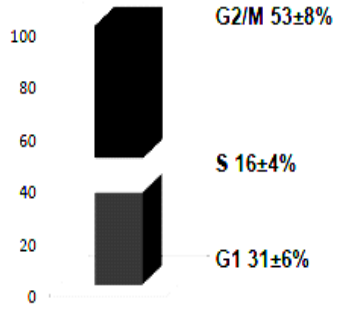
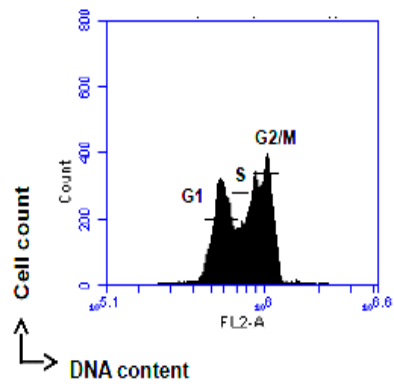
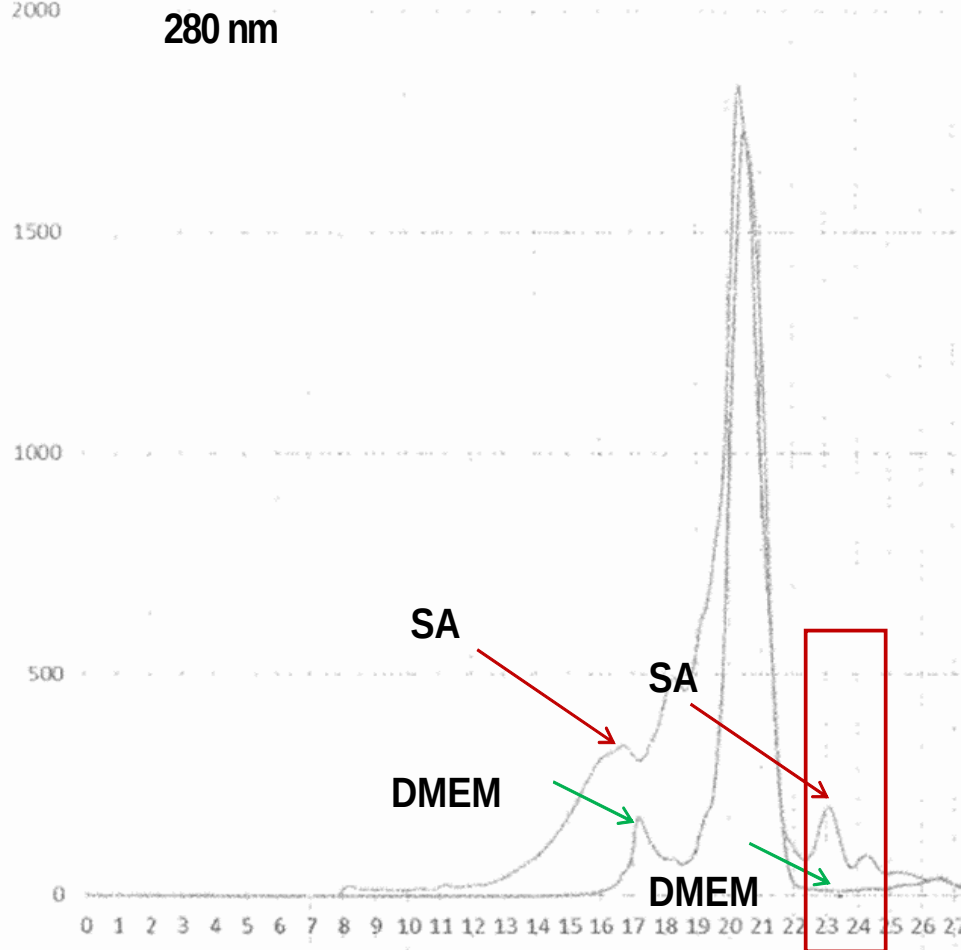
HeLa



# Gel-filtration chromatography of *S. aureus* supernatans

Bacterial cells and *S. aureus*-produced soluble factor induce the similar G2/M transition delay

25h



Fractions 23-25 induce G2/M transition delay

# Detection of PSM $\alpha$ peptide derivatives in *S. aureus* chromatography fractions.

Uniprot Entry	Gene Name	Description	Peptide sequence	XITandem e-value	SEC Fractions identification
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	GIKVIKS	1,7E-3	23
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	IIAGIIKV	1,2E-2	23, 24
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	IIKVIKS	1,8E-2	23, 24
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	LIEQFTGK	1,1E-2	23, 24, 25
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	IIAGIIKVIKS	3,2E-5	23, 25
PSMA3_STAAW	psmA3	Phenol-soluble modulin alpha 3	FKDLLGKF	8,8E-4	23, 24, 25
PSMA3_STAAW	psmA3	Phenol-soluble modulin alpha 3	AKLFKF	4,7E-2	24, 25, 26
PSMA3_STAAW	psmA3	Phenol-soluble modulin alpha 3	FFKDLLGK	3,6E-2	25
PSMA3_STAAW	psmA3	Phenol-soluble modulin alpha 3	FVAKLF	3,6E-2	25
PSMA3_STAAW	psmA3	Phenol-soluble modulin alpha 3	FVAKLFKF	4,7E-3	26
PSMA2_STAAW	psmA2	Phenol-soluble modulin alpha 2	GIKFIKG	4,6E-2	23
PSMA2_STAAW	psmA2	Phenol-soluble modulin alpha 2	IIAGIIKF	1,1E-3	23
PSMA2_STAAW	psmA2	Phenol-soluble modulin alpha 2	IIKFIKGL	5,1E-3	23
PSMA4_STAAW	psmA4	Phenol-soluble modulin alpha 4	IIDIFAK	4,2E-4	23, 24, 26
PSMA4_STAAW	psmA4	Phenol-soluble modulin alpha 4	IDIFAK	8,2E-3	24

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

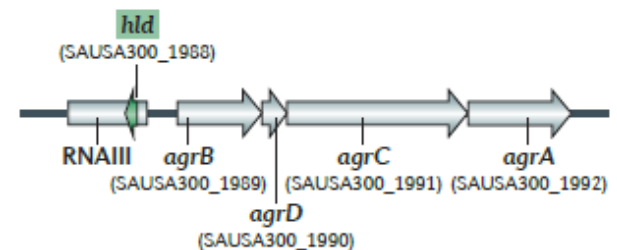
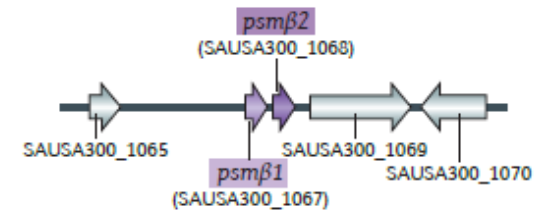
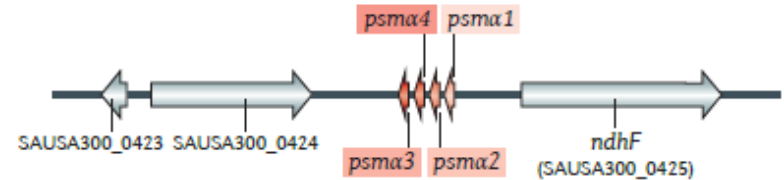
**PSM $\alpha$ 1-PSM $\alpha$ 4**  
 **$\delta$ -toxin** 20-25 amino acids

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

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

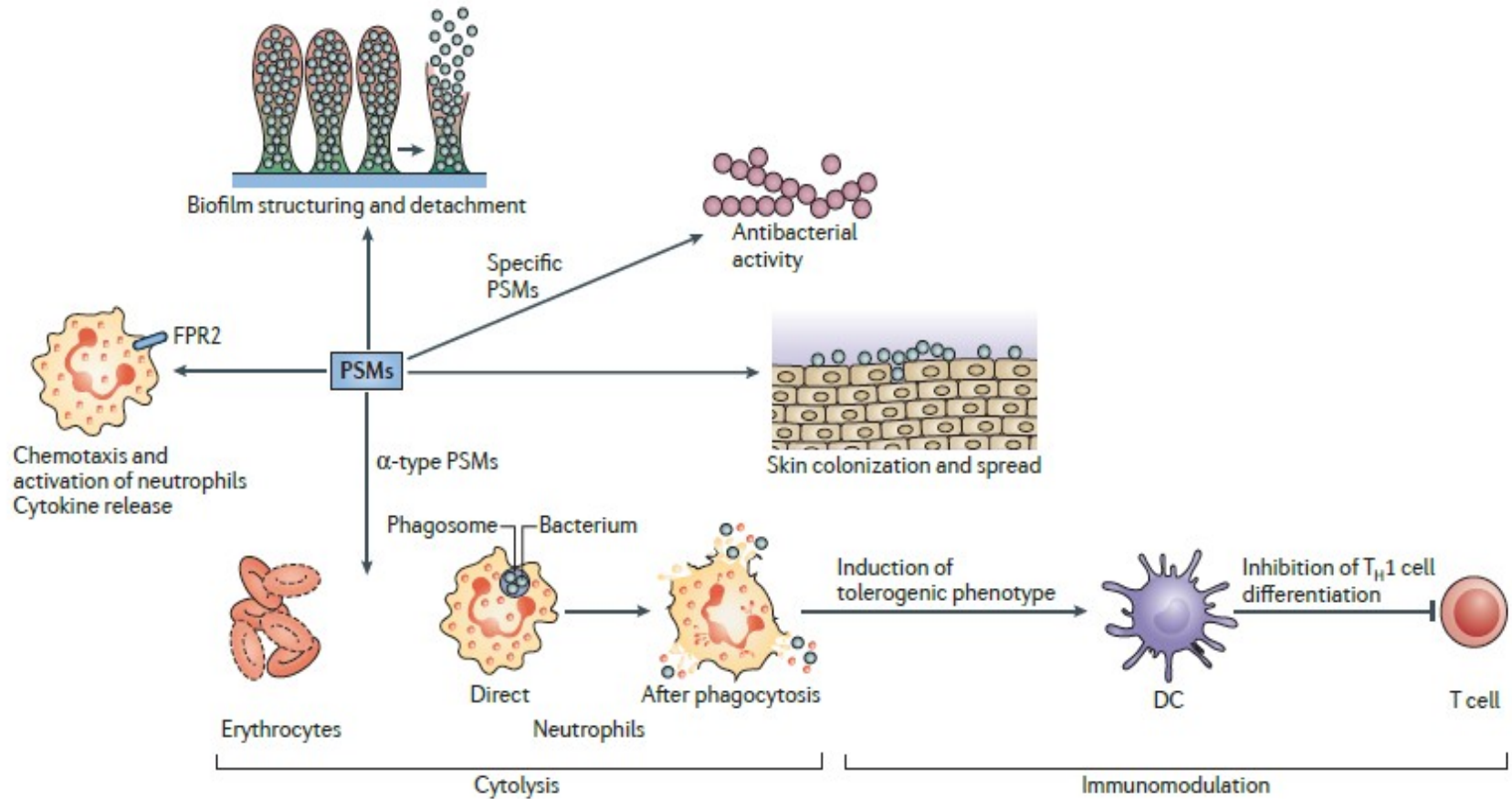
**PSM $\beta$ 1 and PSM $\beta$ 2** are encoded in the *psm $\beta$*  operon

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



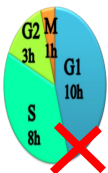
<b><math>\delta</math>-toxin</b>	fMAADIISTIGDLVVKWIIDTVNKFKKK	26 (0)
<b>PSM<math>\alpha</math>1</b>	fMGIIAGI IKVIKSLIEQFTGK	21 (+1)
<b>PSM<math>\alpha</math>2</b>	fMGIIAGI IKFIKGLIEKFTGK	21 (+2)
<b>PSM<math>\alpha</math>3</b>	fMEFVAKLFPKFFKDLLGKFLGNN	22 (+1)
<b>PSM<math>\alpha</math>4</b>	fMDFTGVITSIIDLIKTCTIQAFG	22 (+1)
<b>PSM<math>\beta</math>1</b>	fMEGLFNAIKD'TVTAAINNDGAKLGT'SIVSIVENGVLLGKLF'GF	44 (-2)
<b>PSM<math>\beta</math>2</b>	fMEGLARAIANT'VQAAQQHDSVKLGT'SIVDIVANGVLLGKLF'GF	44 (-1.9)

# Overview of phenol-soluble modulins activities



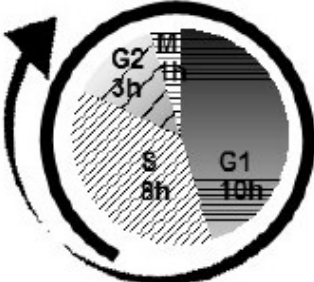
# PSM $\alpha$ 1 and PSM $\alpha$ 2 induced the G2/M phases transition delay

Temoir A980866



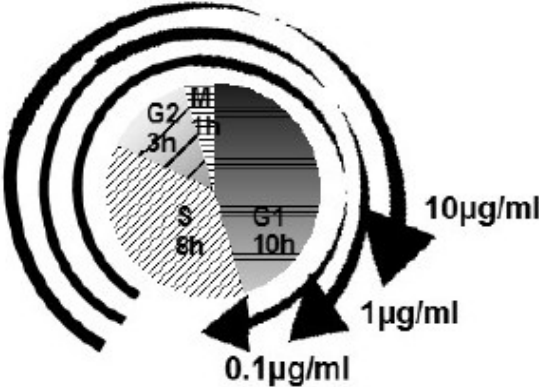
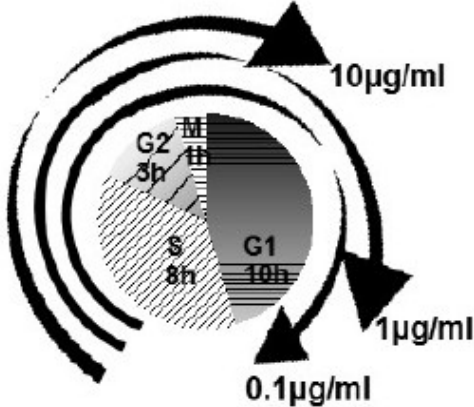
Control cell

25h



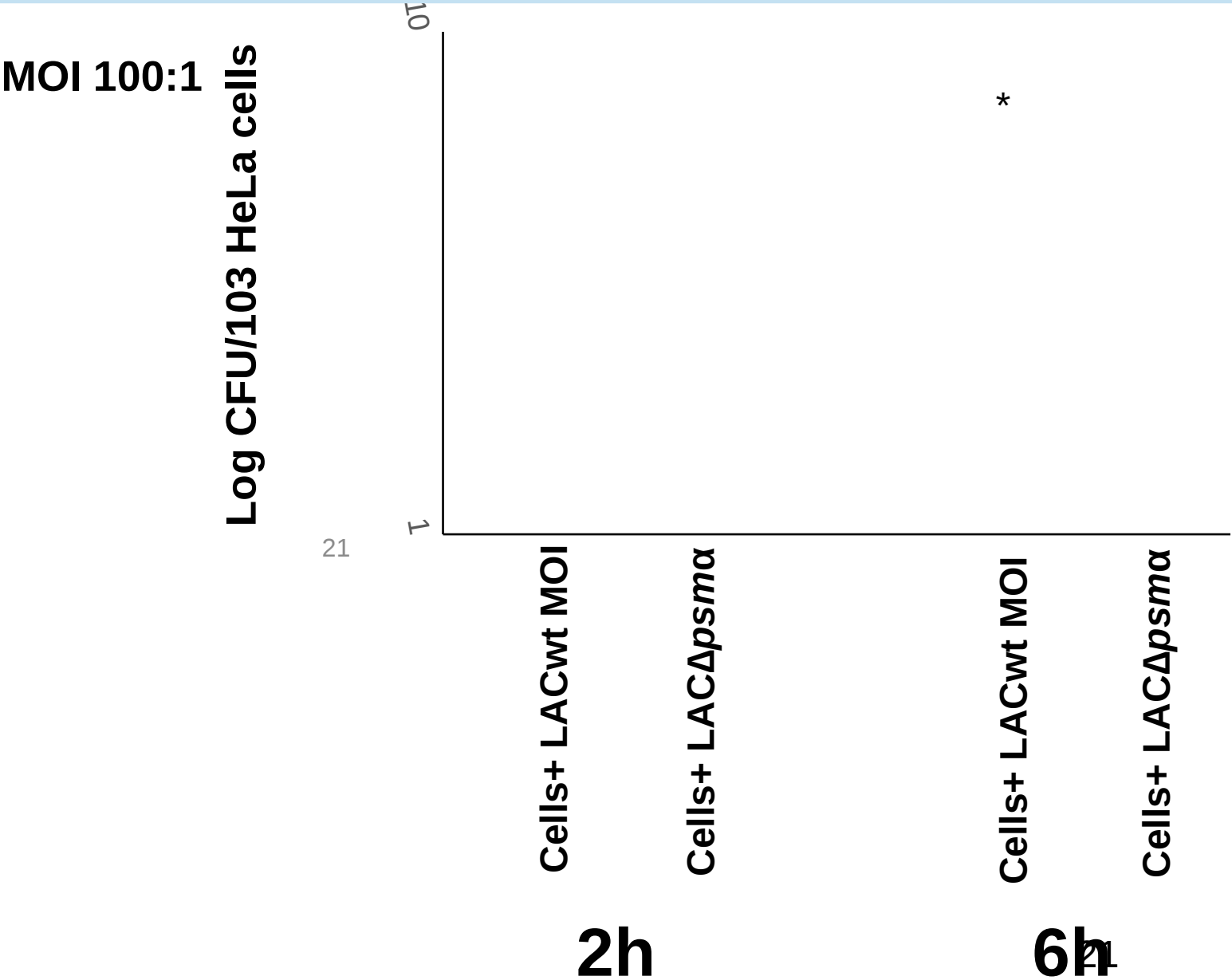
PSM1

PSM3

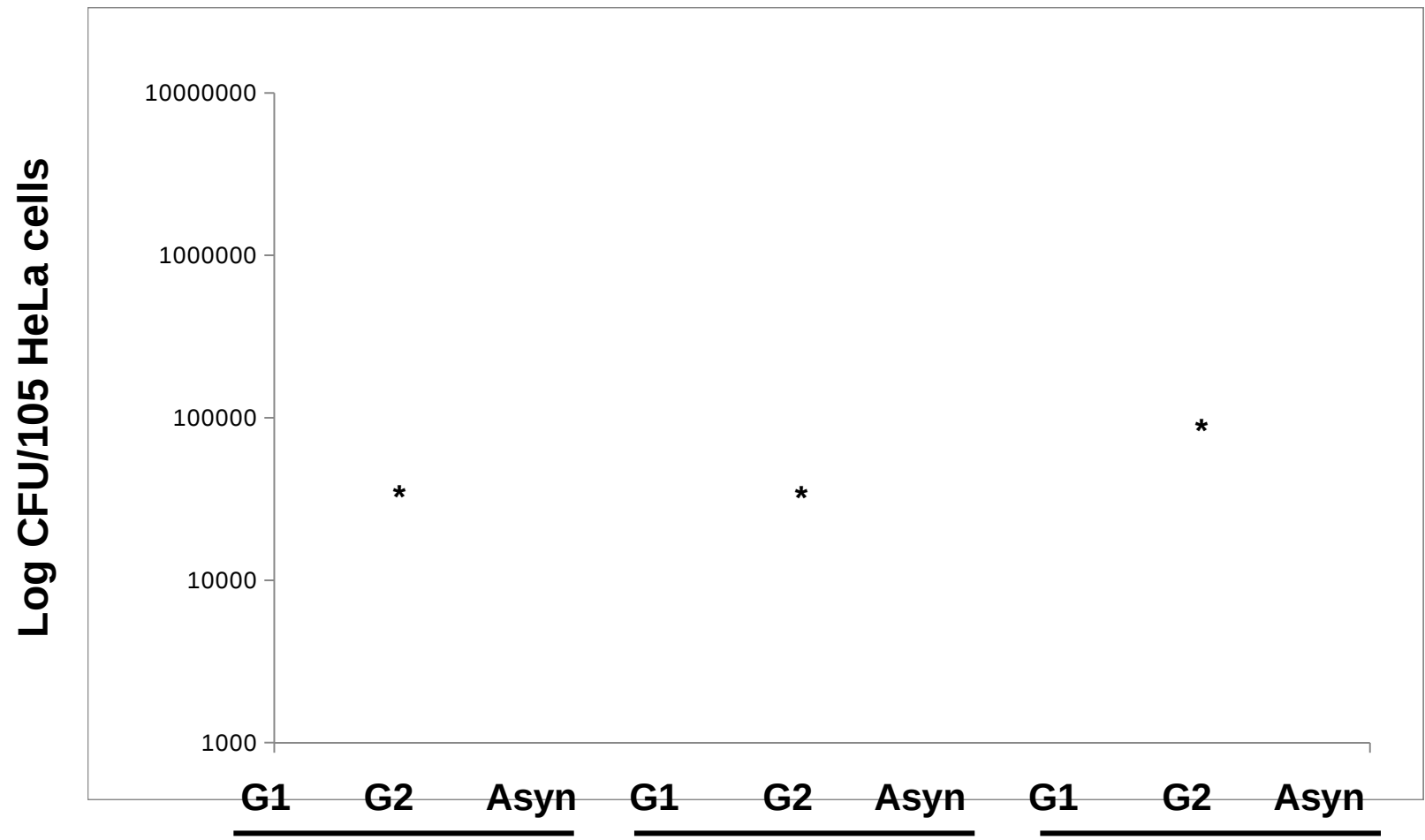


Isogenic USA300 mutant lacking the PSM $\alpha$  operon does not induce G2/M phase transition delay

# Internalized LAC wt replicated inside HeLa cells in contrast to its isogenic $LAC\Delta psm\alpha$ mutant



# The G2/M transition delay results in the increase of internalization and intracellular bacterial replication



MOI 50:1

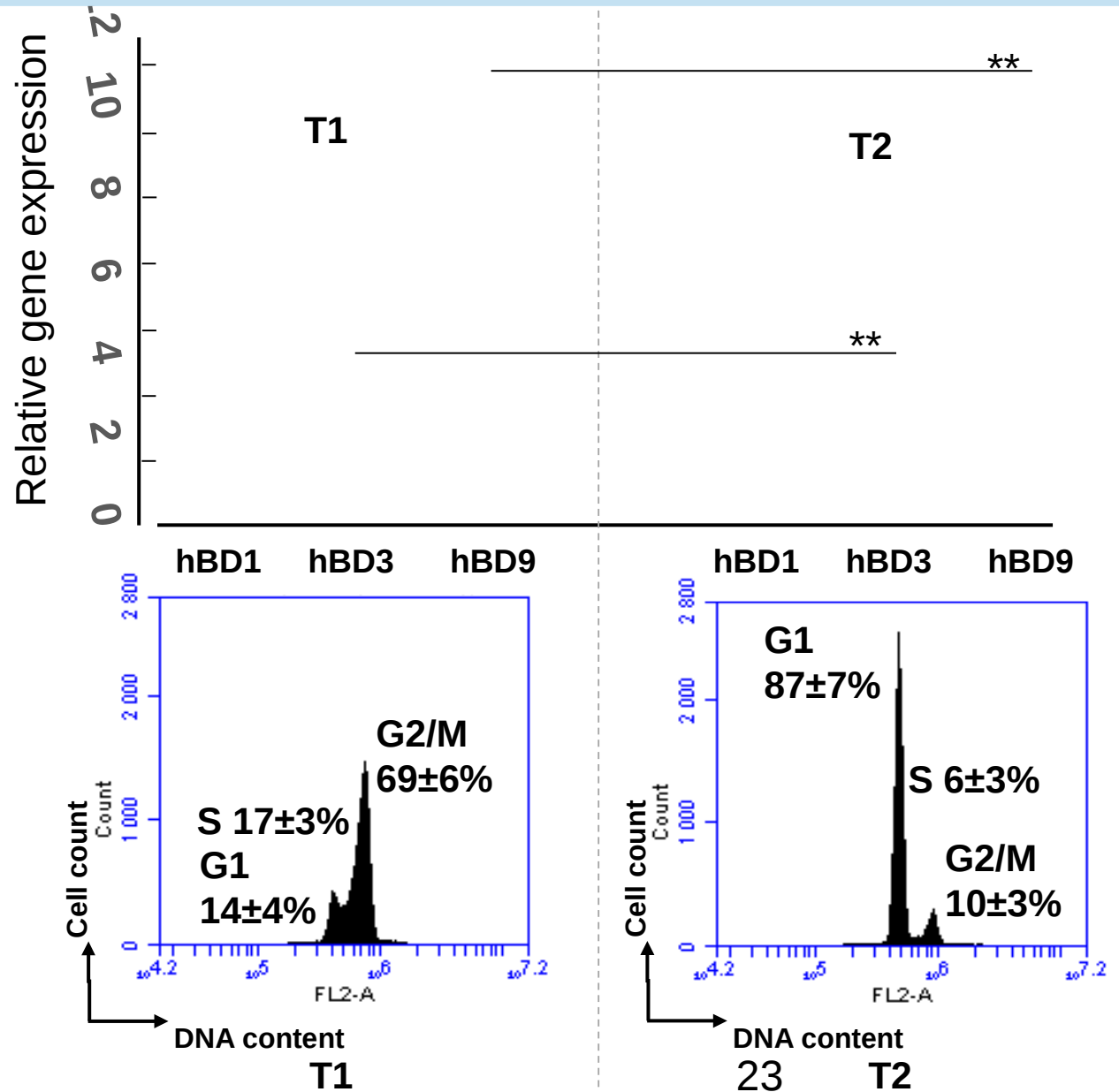
T2

T4

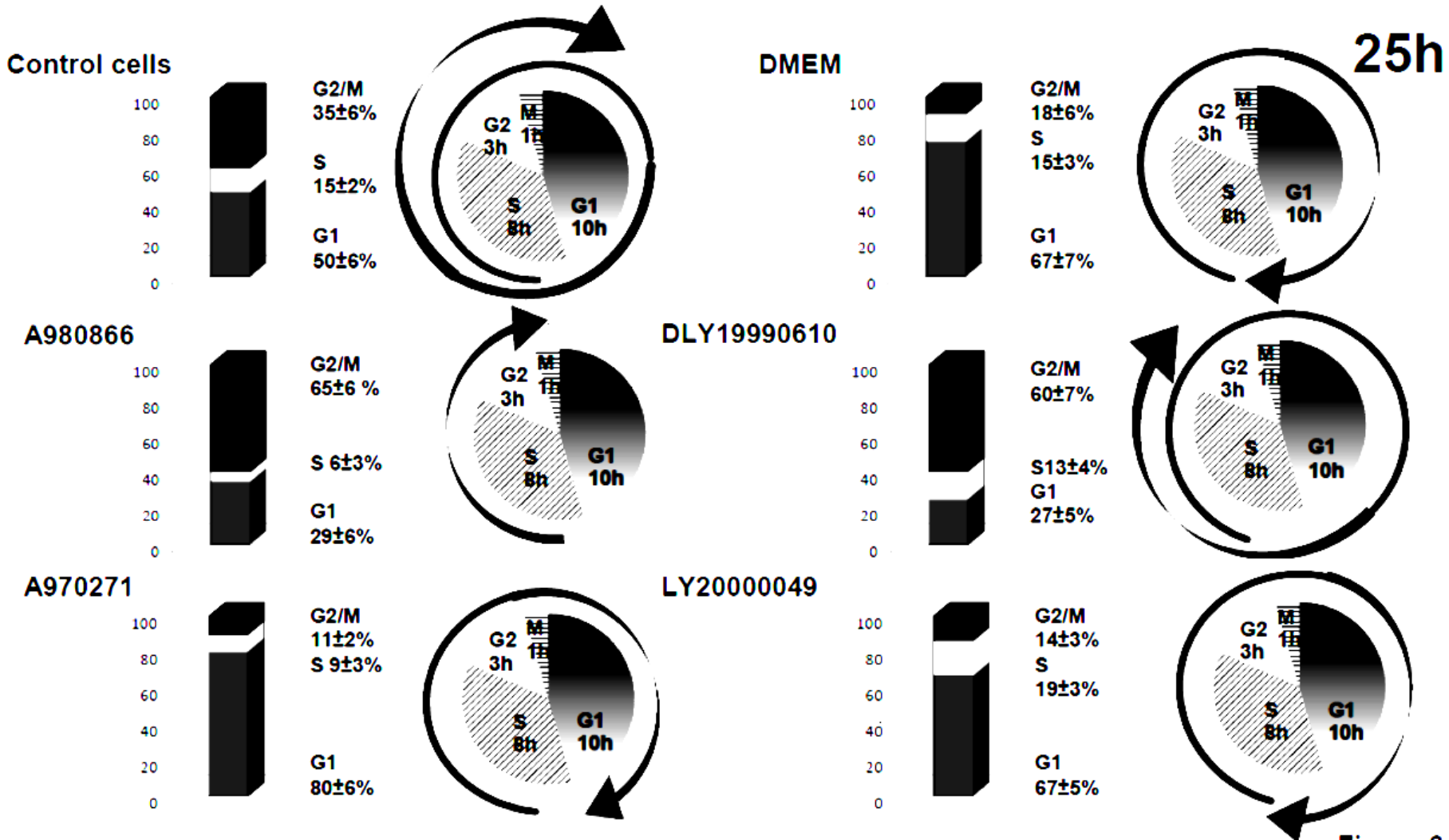
T20

Intracellular bacteria in asynchronous, G1- and G2-phase enriched HeLa cells

# Increase of mRNA levels of HBD-3 and -9 in PSM $\alpha$ 1-treated cells



# *S. aureus*-induced G2/M transition delay is strain-dependent



25h

# *Staphylococcus aureus*-Induced G2/M Phase Transition Delay in Host Epithelial Cells Increases Bacterial Infective Efficiency

Ludmila Alekseeva<sup>1,2,3,9</sup>, Lucie Rault<sup>2,3,9</sup>, Sintia Almeida<sup>2,3,4</sup>, Patrick Legembre<sup>5</sup>, Valérie Edmond<sup>5</sup>, Vasco Azevedo<sup>4</sup>, Anderson Miyoshi<sup>4</sup>, Sergine Even<sup>2,3</sup>, Frédéric Taieb<sup>6</sup>, Yannick Arlot-Bonnemains<sup>7</sup>, Yves Le Loir<sup>2,3</sup>, Nadia Berkova<sup>2,3\*</sup>

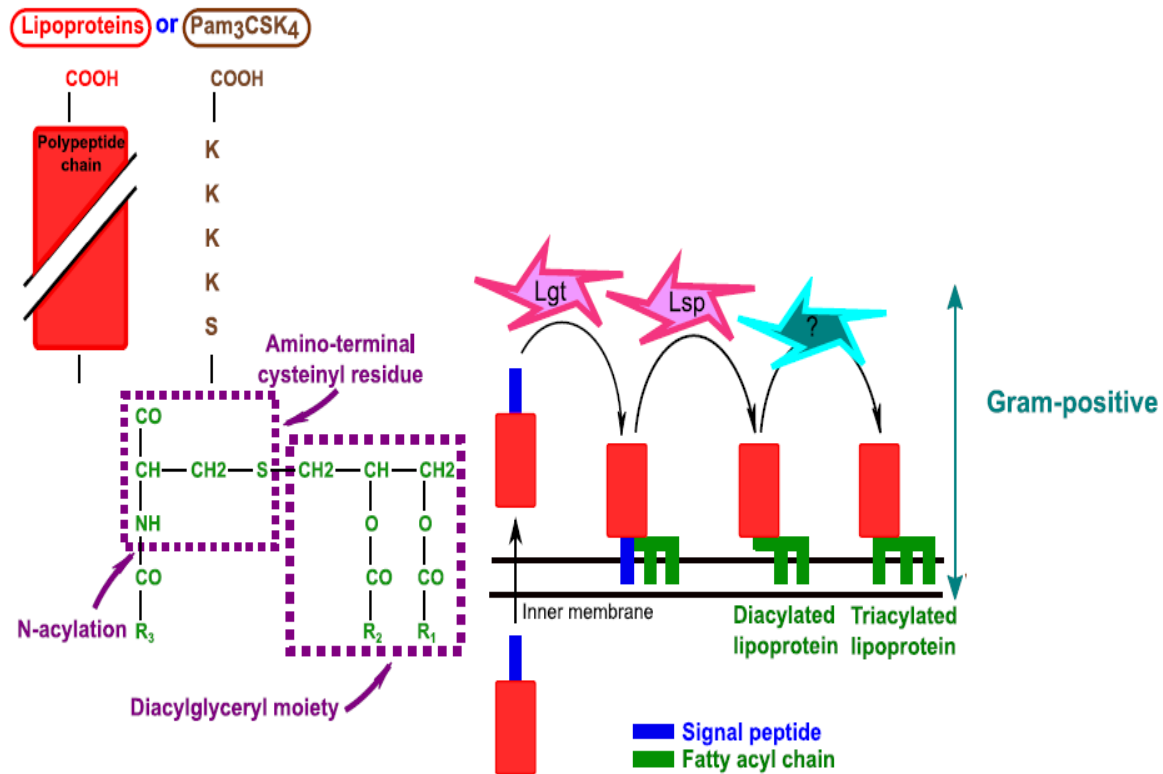
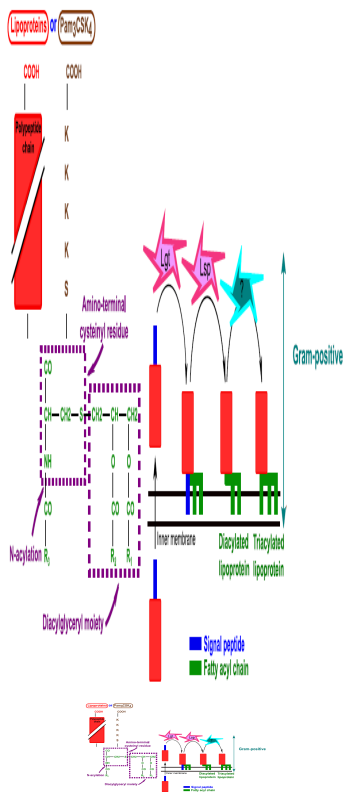
*The FASEB Journal* • Research Communication

## Phenol-soluble modulin $\alpha$ induces G2/M phase transition delay in eukaryotic HeLa cells

Martine Deplanche,<sup>\*,†,1</sup> Rachid Aref El-Aouar Filho,<sup>\*,†,‡,1</sup> Ludmila Alekseeva,<sup>\*,†,§</sup> Emilie Ladier,<sup>\*,†</sup> Julien Jardin,<sup>\*,†</sup> Gwénaële Henry,<sup>\*,†</sup> Vasco Azevedo,<sup>‡</sup> Anderson Miyoshi,<sup>‡</sup> Laetitia Beraud,<sup>¶</sup> Frederic Laurent,<sup>¶,||</sup> Gerard Lina,<sup>¶,||</sup> François Vandenesch,<sup>¶,||</sup> Jean-Paul Steghens,<sup>||</sup> Yves Le Loir,<sup>\*,†</sup> Michael Otto,<sup>#</sup> Friedrich Götz,<sup>\*\*</sup> and Nadia Berkova<sup>\*,†,2</sup>

# Impact of *S. aureus* lipoproteins on the cell cycle progression

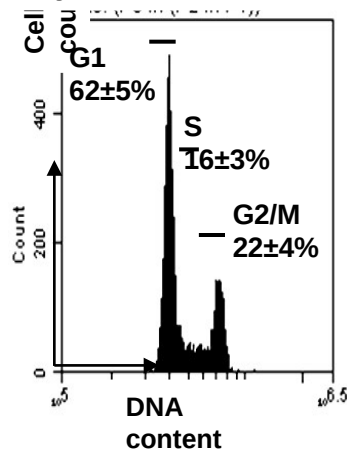
## Structure and post-translational modifications of *S. aureus* lipoproteins



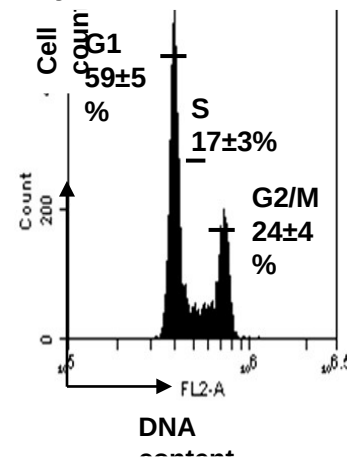
# The *lpl* gene cluster of *S. aureus* is involved in the induction of the G2/M delay

## A

Asynchronous cells



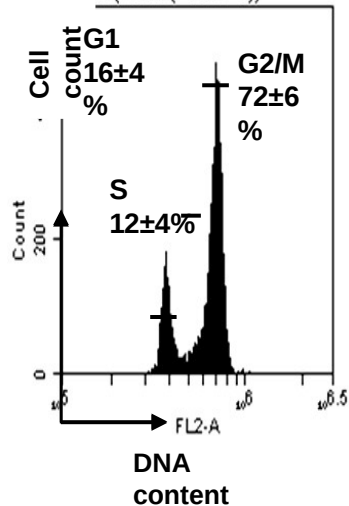
Control synchronous cells



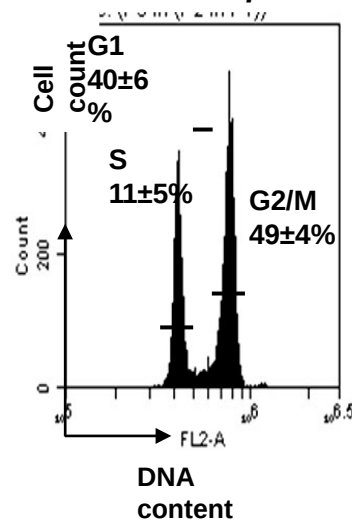
## B

USA300

(P3 in (P2 in P1))

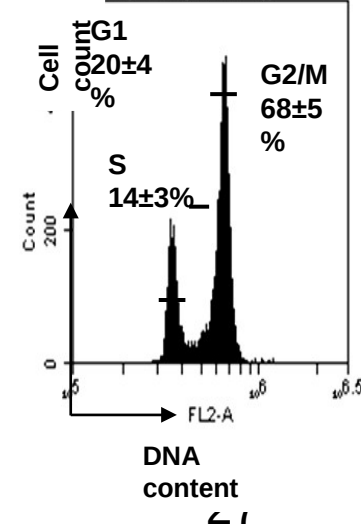


USA300Δ*lpl*

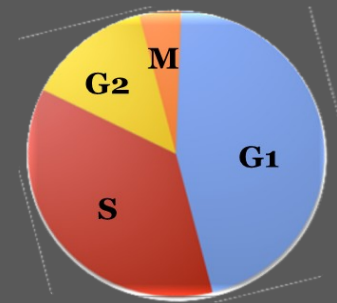
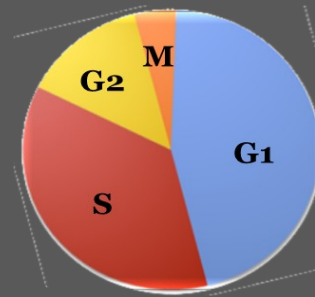
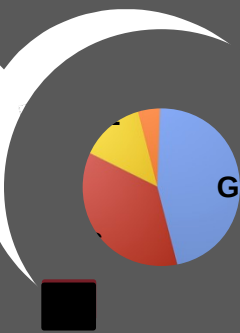


USA300Δ*lpl*  
(pTX30-*lpl*)

(P3 in (P2 in P1))

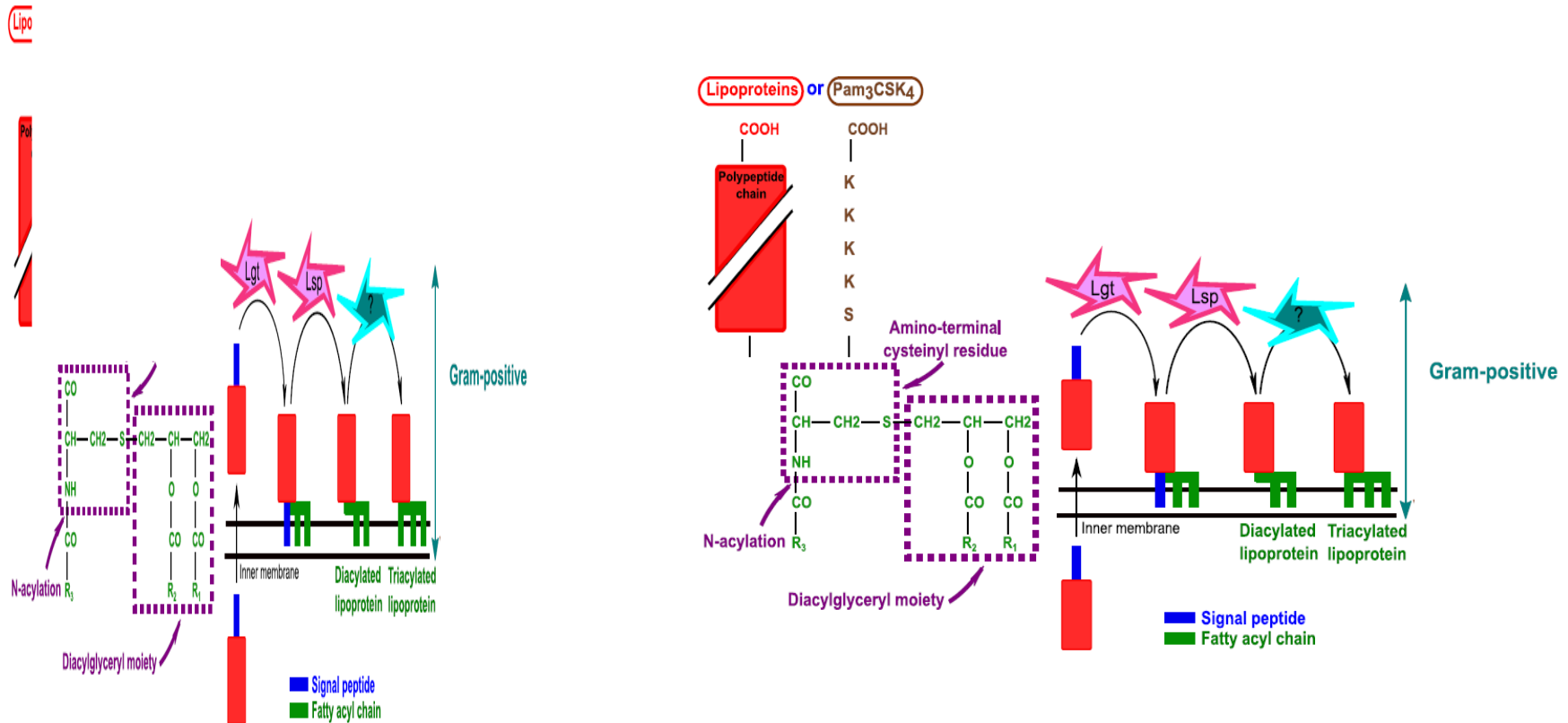


# Impact of *S. aureus* lipoproteins on the cell cycle progression

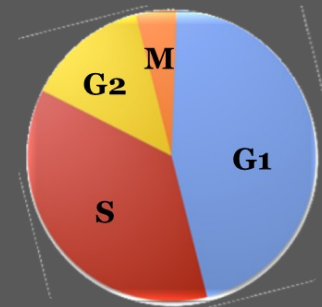
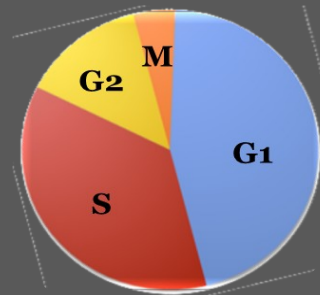
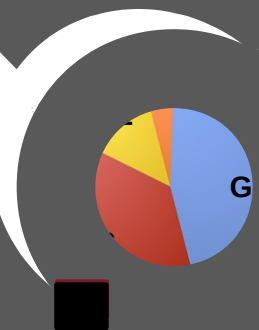


Protein portion of Ipl plays a role in cell cycle delay

# Impact of *S. aureus* lipoproteins on the cell cycle progression



Structure of Pam3 of *S. aureus* lipoprotein

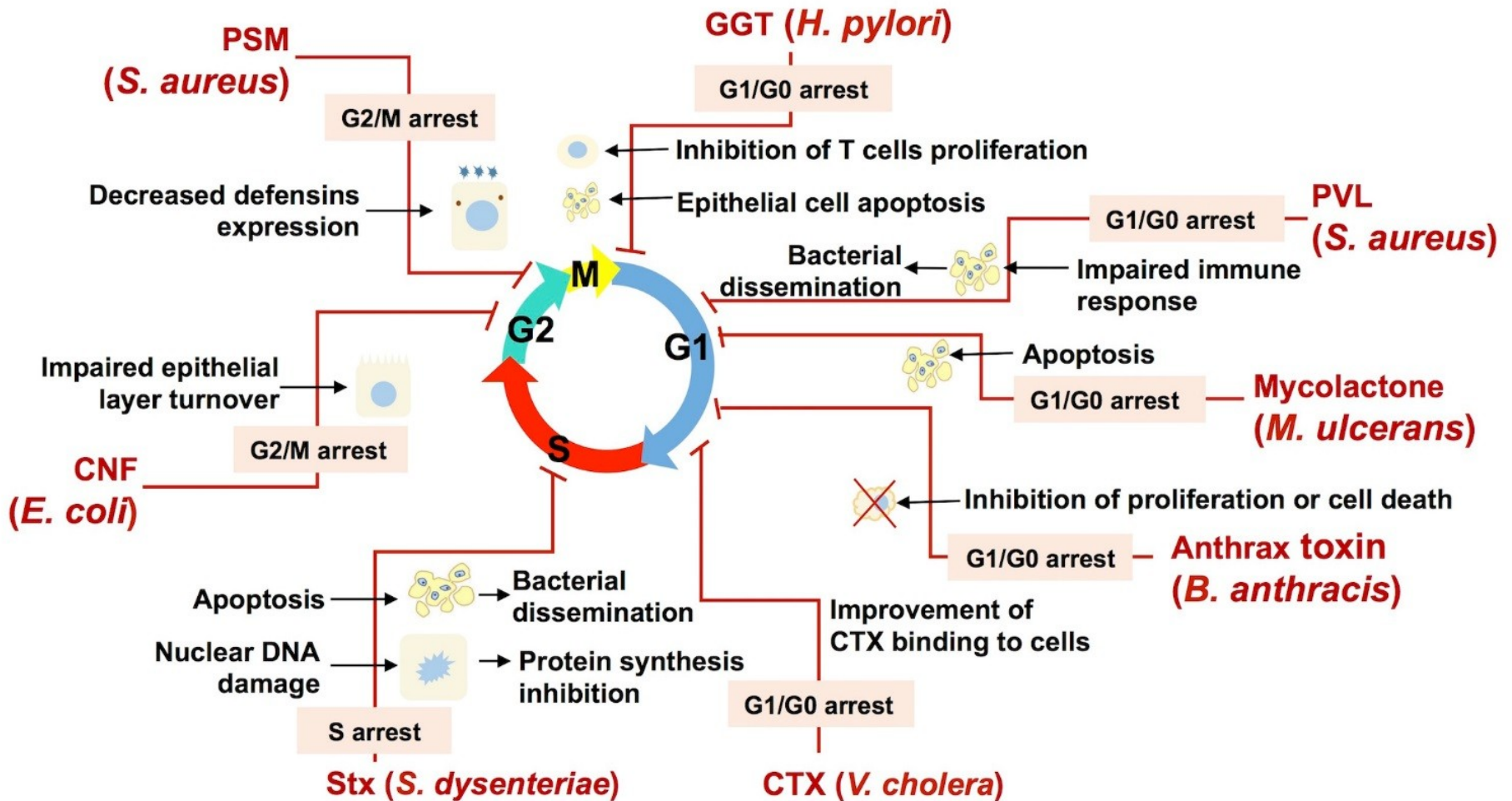


Pam2 > Pam3

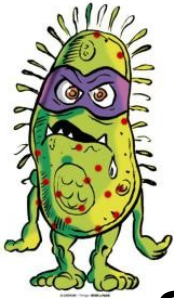
Pams are synthetic lipopeptides that mimic the acylated amino terminus of bacterial lipoproteins

**The protein part of *S. aureus* lipoproteins, a new cyclomodulin, plays a pivotal role in the G2/M transition delay**

# Bacterial cyclomodulins alter the eukaryotic cell cycle for their own benefit



# CONCLUSIONS



*S. aureus* **PSMs** and **Ipl** are **new cyclomodulins**, which

- subvert the host cell cycle progression
- increase a bacterial internalisation
- augment an intracellular bacterial proliferation
- inhibit a host protective response

**This is a newly identified mechanisms of *S. aureus* strategy for fostering an infection**

# Participants/Collaborations



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**Thank you for your attention**