

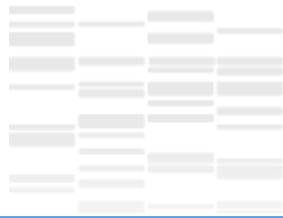


Inflammation, DNA damage and *Staphylococcus aureus* infection

Nadejda Berkova

UMR1253 STLO (Science Technologie du Lait et de l'Oeuf)
INRA Agrocampus Ouest, Rennes)





INFLAMMATION

Cause of inflammation

- Microbial infection
- Noxious substances
- Tissue stress
- Tissue injury

Physiological purpose

- Defense from microbial infection
- Expulsion of noxious substances
- Tissue repair
- Adaptation to stress

Outcome

- Tissue damage
- Autoimmune diseases
- Disease of homeostasis
- Fibrosis, metaplasie, cancer

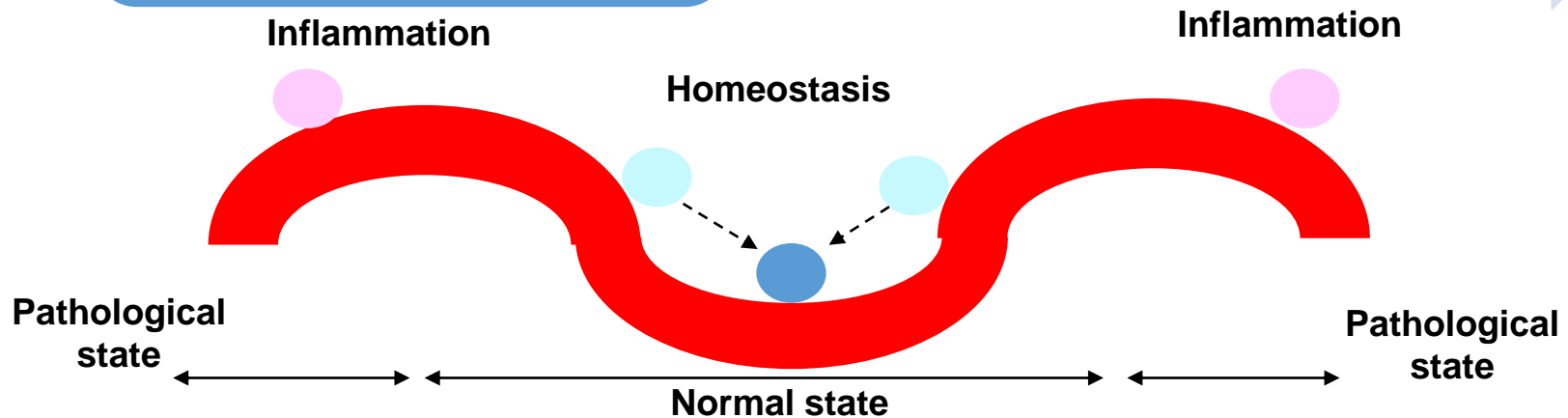
HOMEOSTATIS AND INFLAMMATION

Homeostasis

- Maintains the stability of biological system

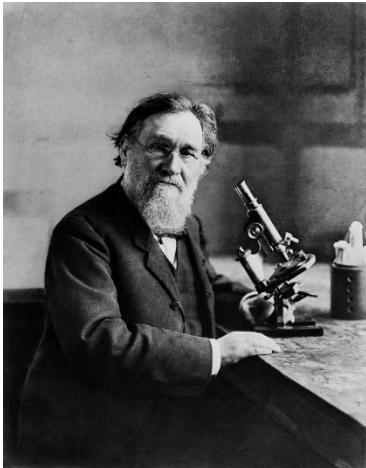
Inflammation

- Result of perturbations that exceed the homeostatic capacity of the system

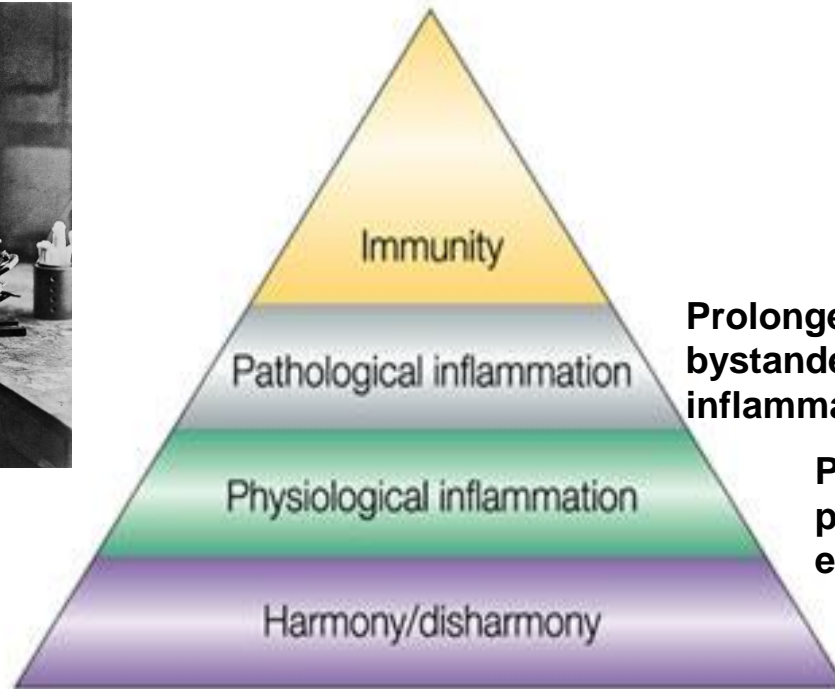


Medzhitov R. IBiology

Metchnikoff's theory of inflammation



1845-1916



Prolonged inflammation causes harm in bystander normal tissues and promotes inflammatory diseases

Physiological inflammation is a protective response mediating the elimination of injurious agents

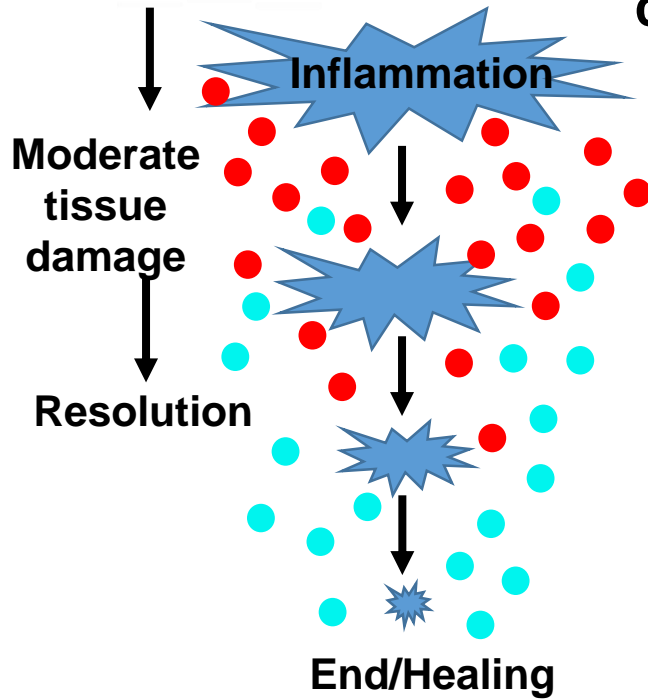
Homeostasis

Nature Reviews | Molecular Cell Biology

Alfred Tauber

Acute inflammation

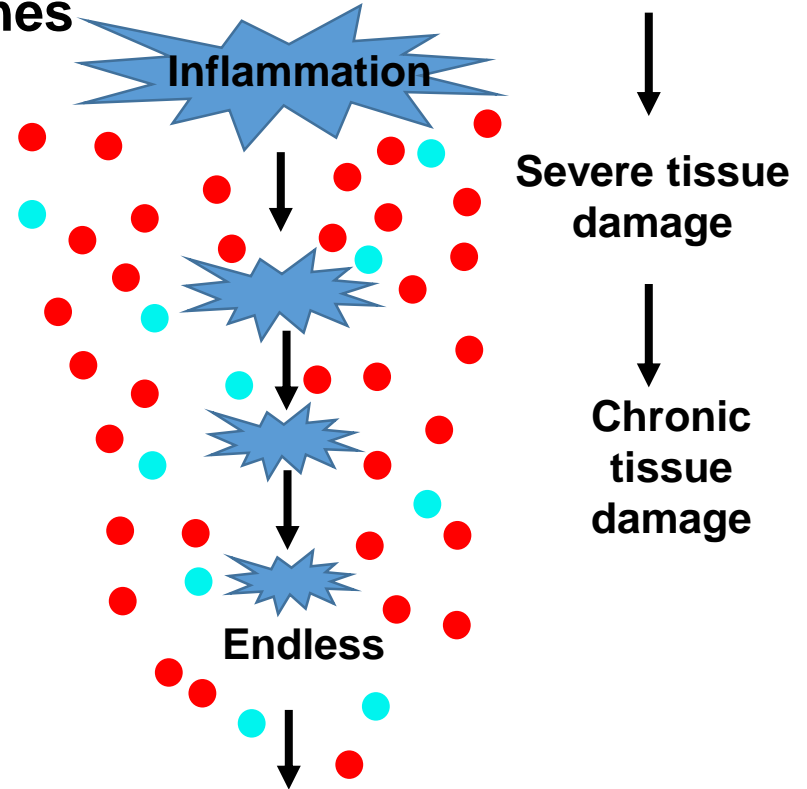
Innate immunity



Concentration of cytokines

Chronic inflammation

Adaptive immunity



- Pro-inflammatory cytokines
- Anti-inflammatory cytokines

INFECTION MEDIATED-INFLAMMATION

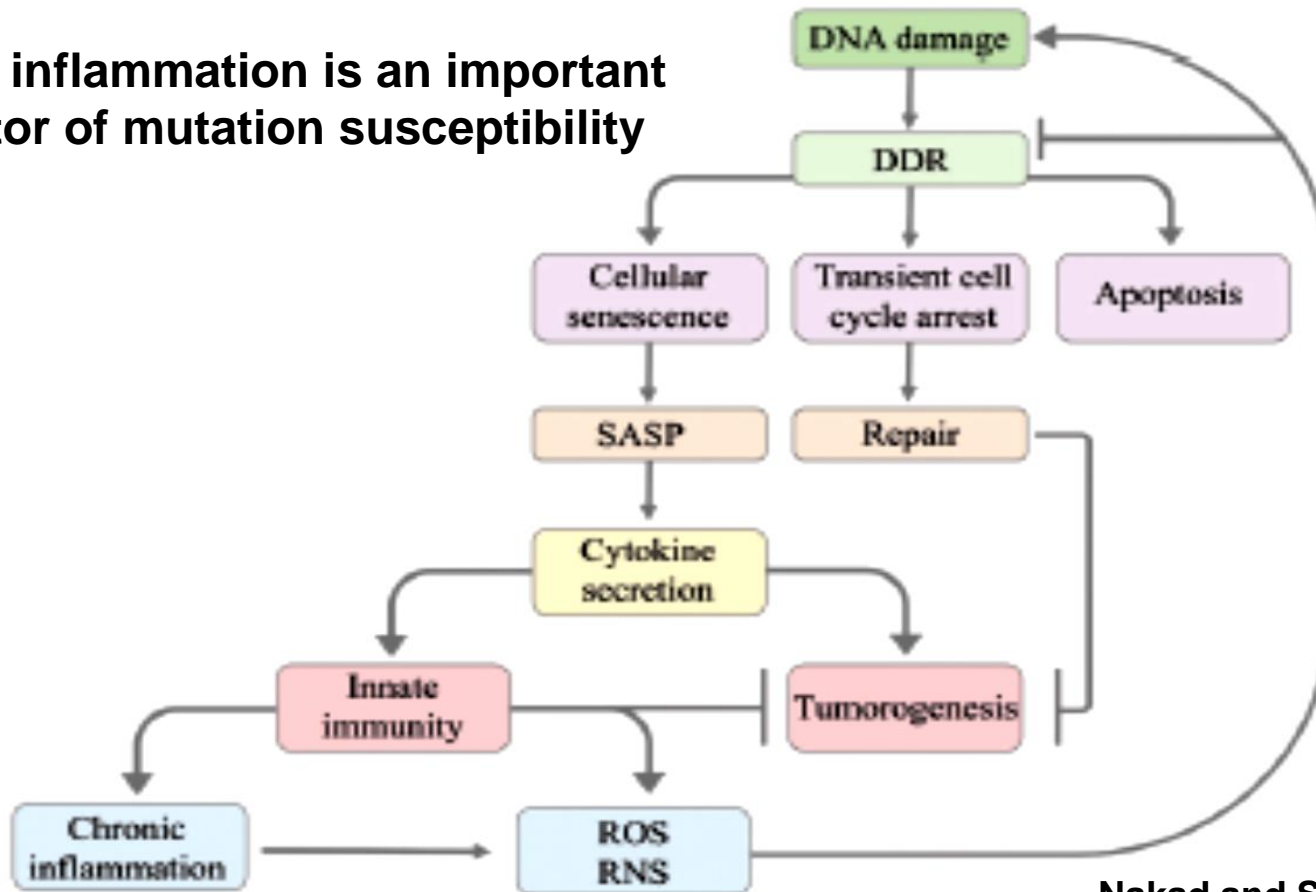
Pathogens induce various levels of inflammation in the host

Inflammation caused by infection leads to increased production of mutagenic compounds as reactive oxygen species (ROS)/reactive nitrogen species (RNS) and subsequent oxidative DNA damage

These in turn cause further inflammation and exacerbation of DNA damage

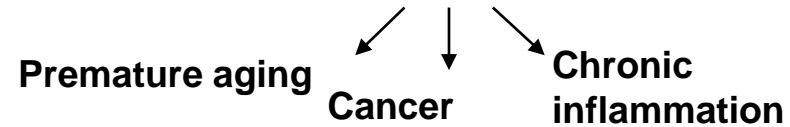
CHRONIC INFLAMMATION AS A DRIVING FORCE IN THE GENESIS OF DNA DAMAGE

Chronic inflammation is an important modulator of mutation susceptibility



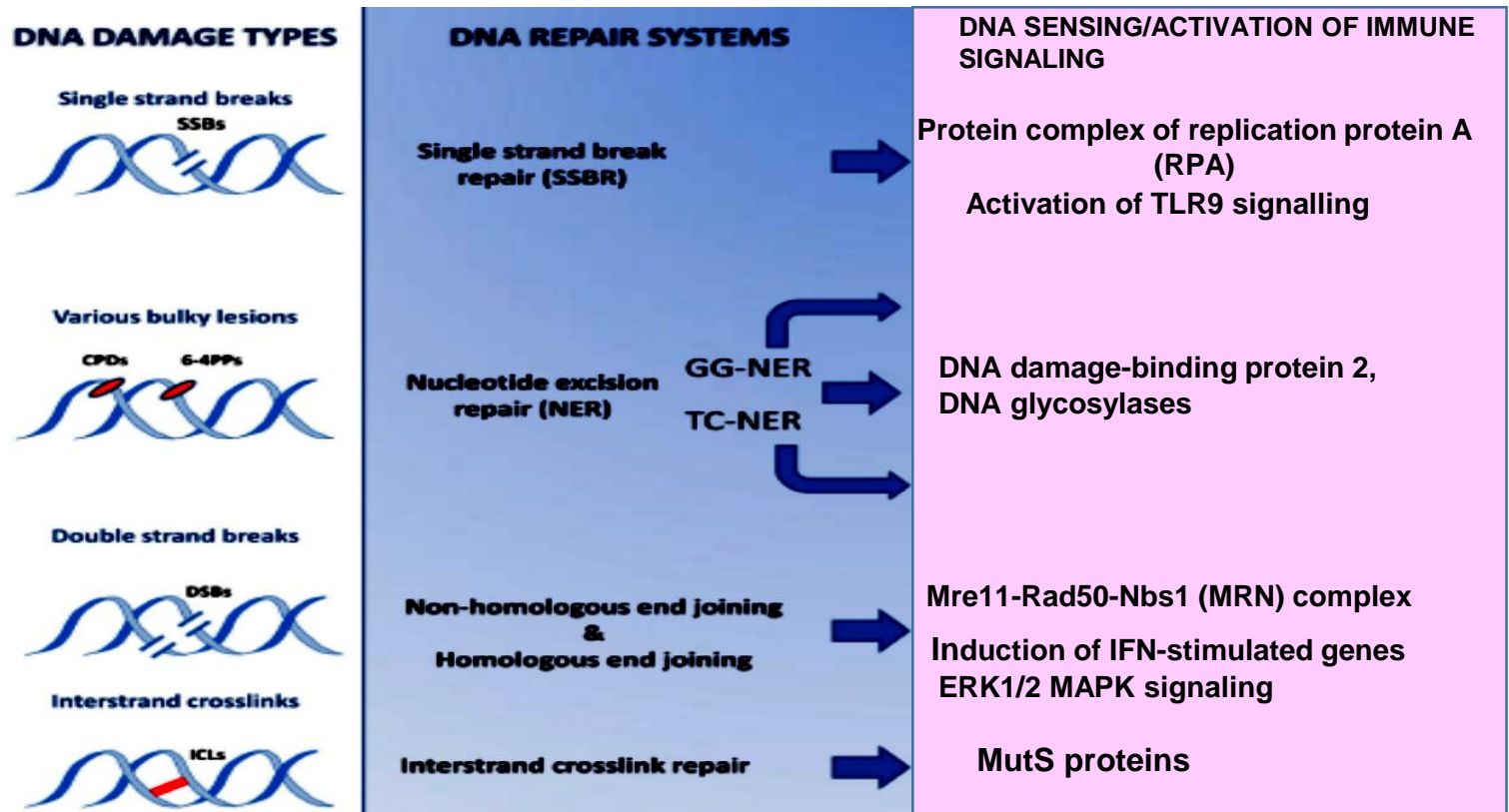
Nakad and Schumacher
Frontiers in Genetics 2016

THE IMMUNE SYSTEM RESPONDS TO DNA DAMAGE



Damaged endogenous DNA triggers inflammatory gene expression.

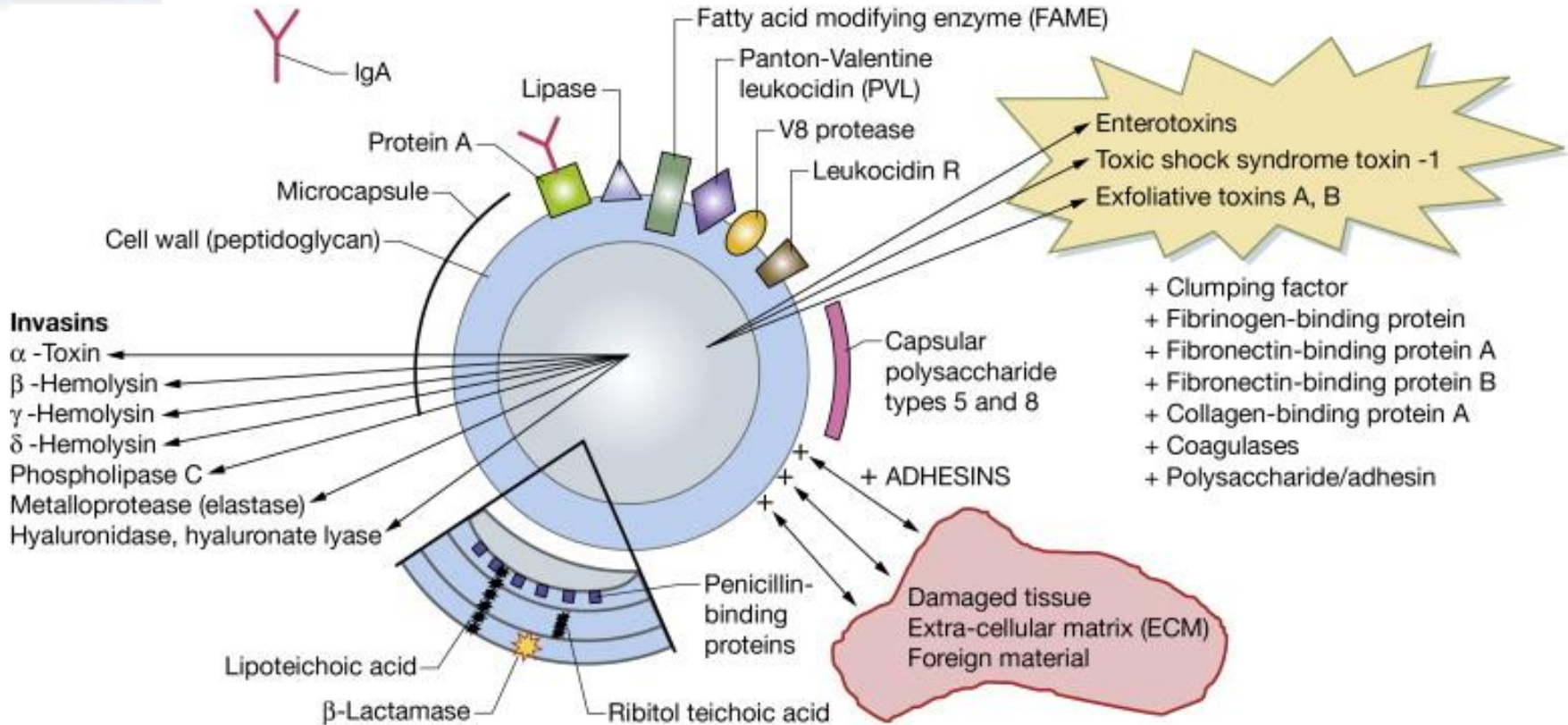
DNA damage response (DDR) orchestrates DNA damage checkpoint activation



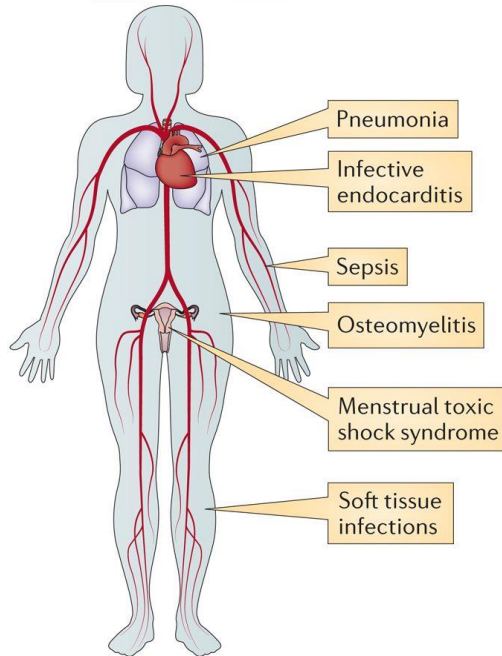


Staphylococcus aureus

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



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



Nature Reviews | Microbiology

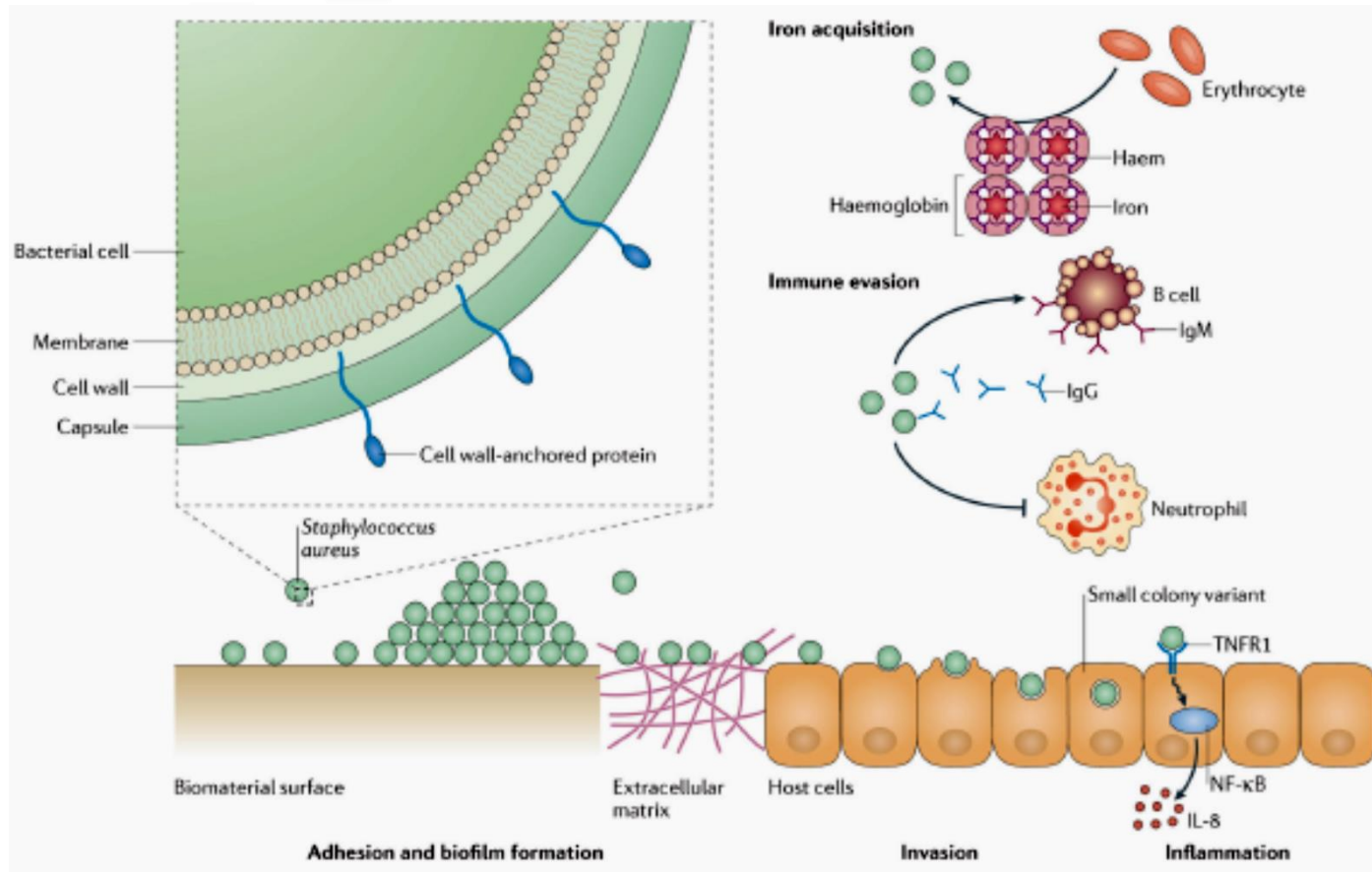
Human:

Pneumonia, osteomyelitis, meningitis, endocarditis, sepsis, chronic infections as **bone and joint infections**

Dairy cattle:

Chronic mastitis

S. aureus, FACULTATIVE INTRACELLULAR PATHOGEN



Foster T. et al, Nat Rev Microbio, 2016



Many pathogens such as *Helicobacter sp.*, *Chlamydia sp.*, *Salmonella sp.*, or *Escherichia coli* induce DNA damage in the host, demonstrating that it may lead to genomic alterations and cancer-associated transformation

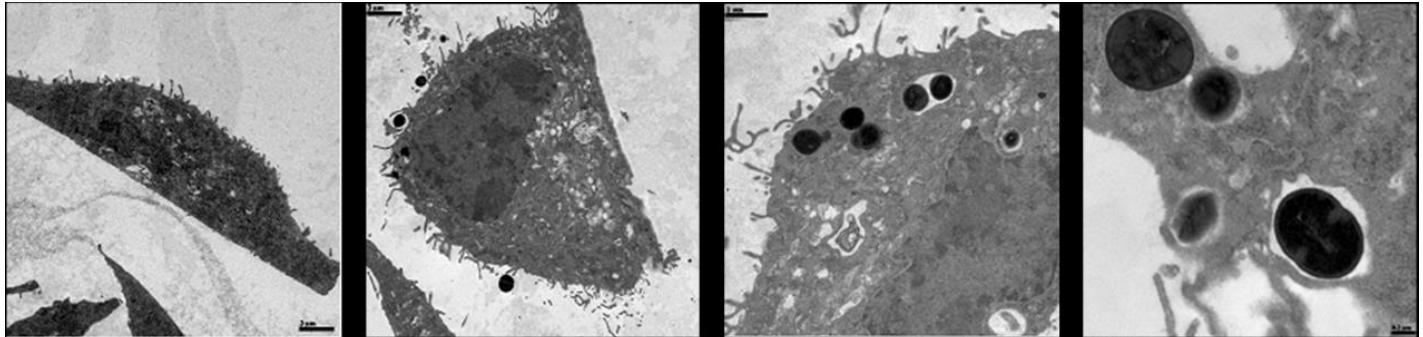
Does *Staphylococcus aureus* induce DNA damage in host cells?

S. aureus INFECTION

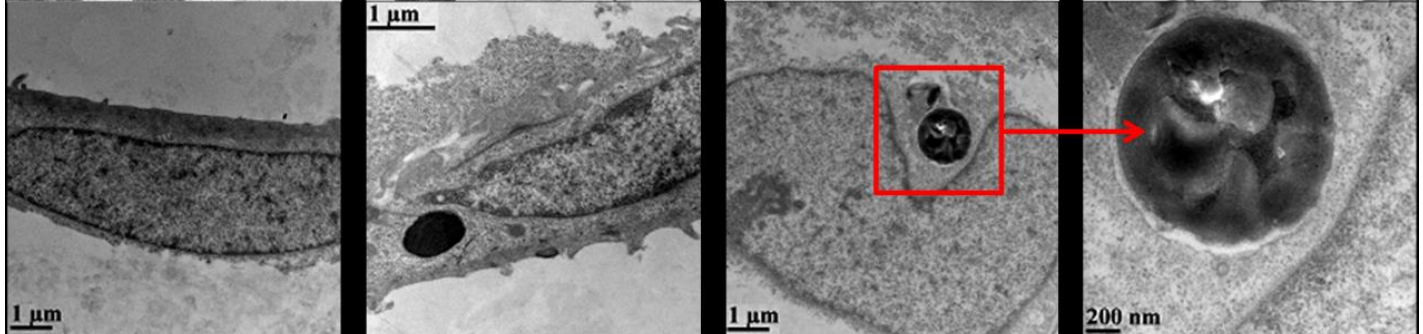
Control cells

S. aureus-infected cells

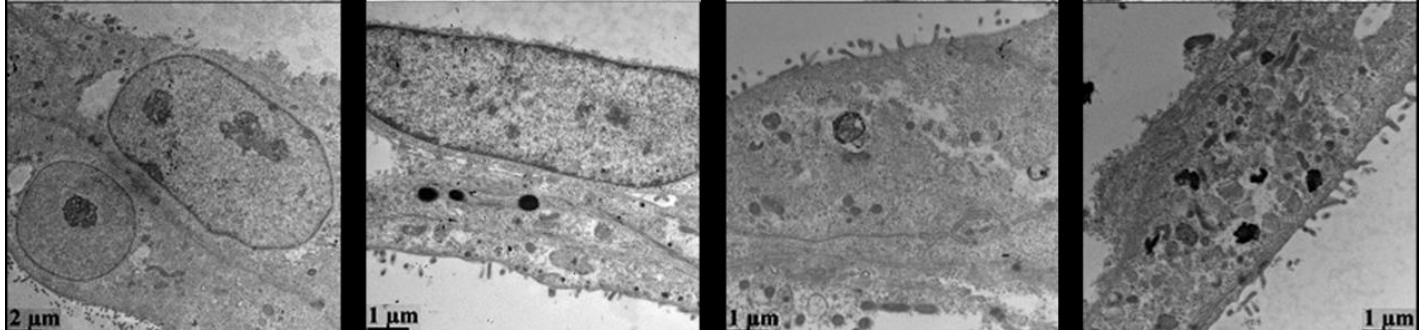
45 min



6 days

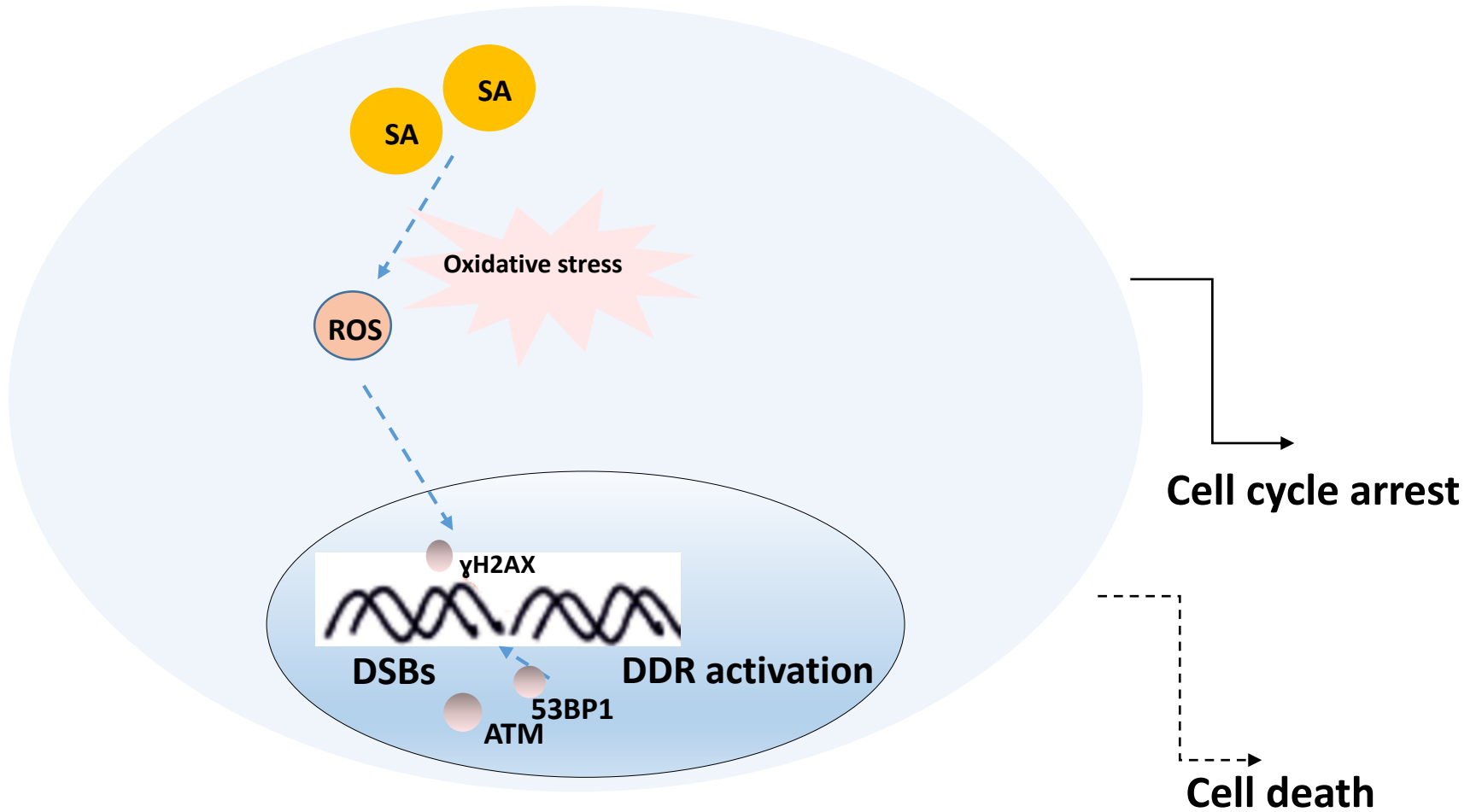


12 days



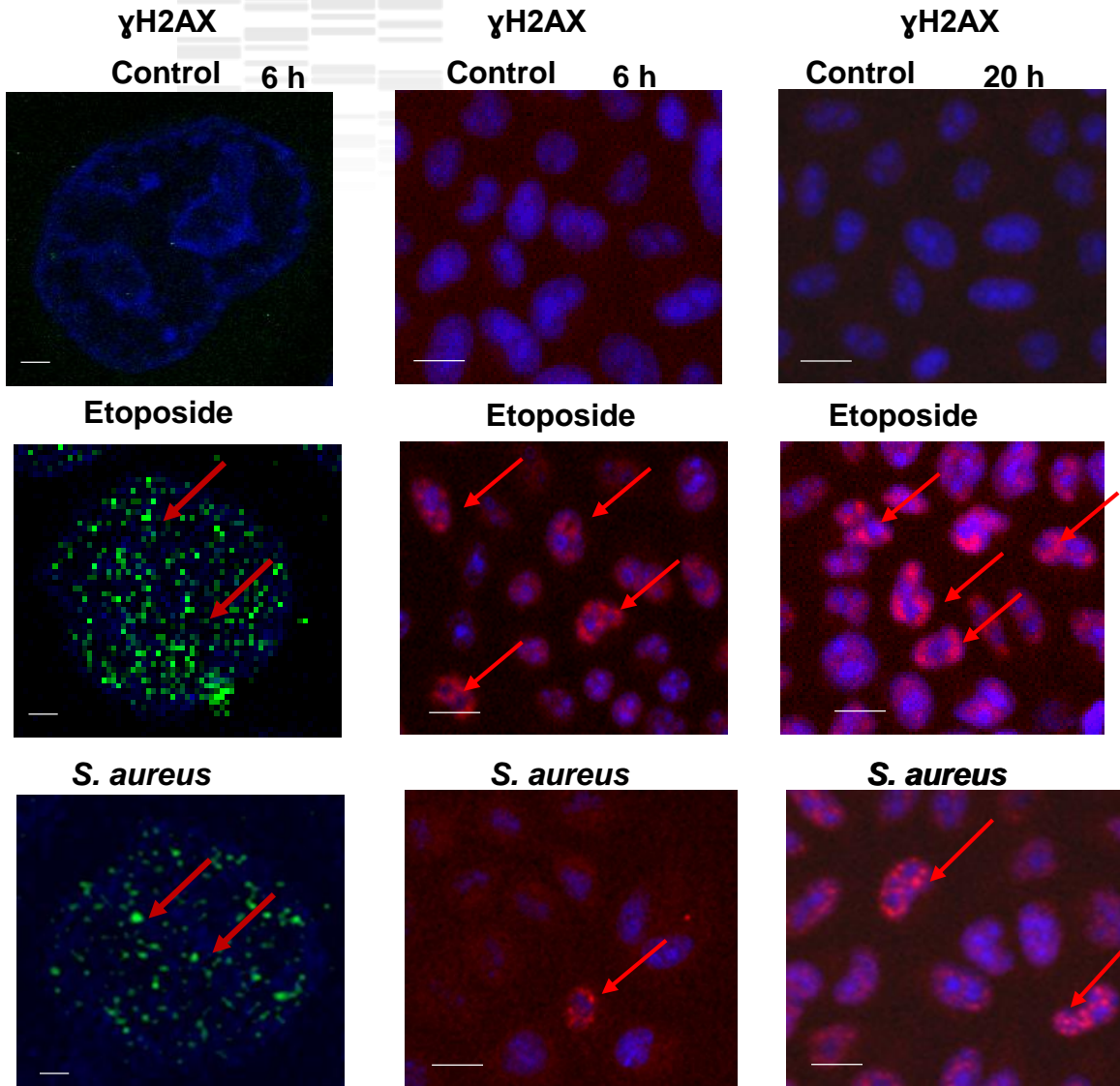
TEM

S. aureus-induced DNA damage

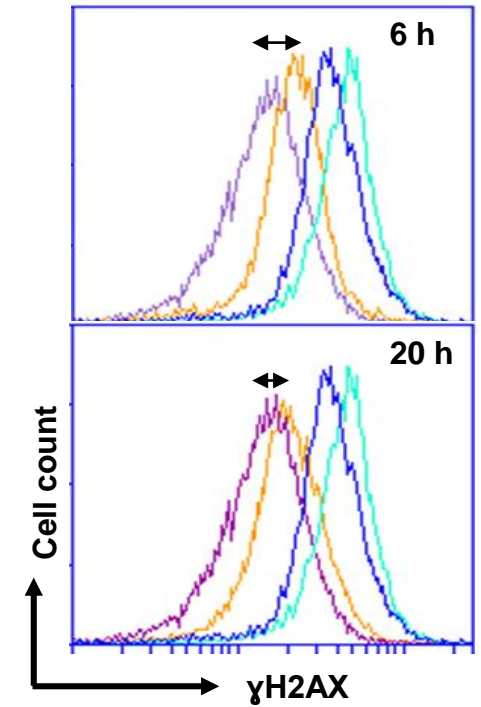


S. aureus induces DNA damage

HeLa



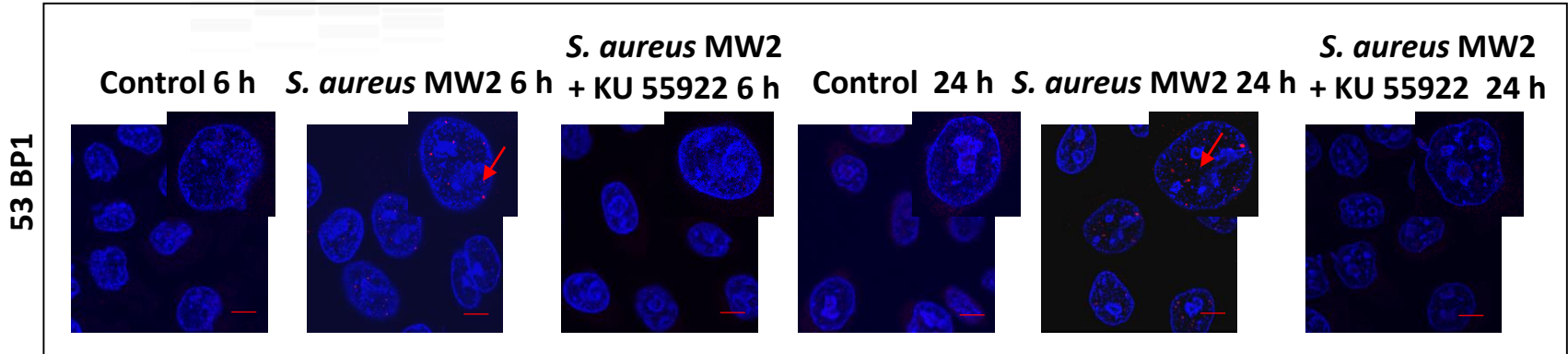
- Control
- *S. aureus* MOI 1:25
- *S. aureus* MOI 1:50
- Etoposide



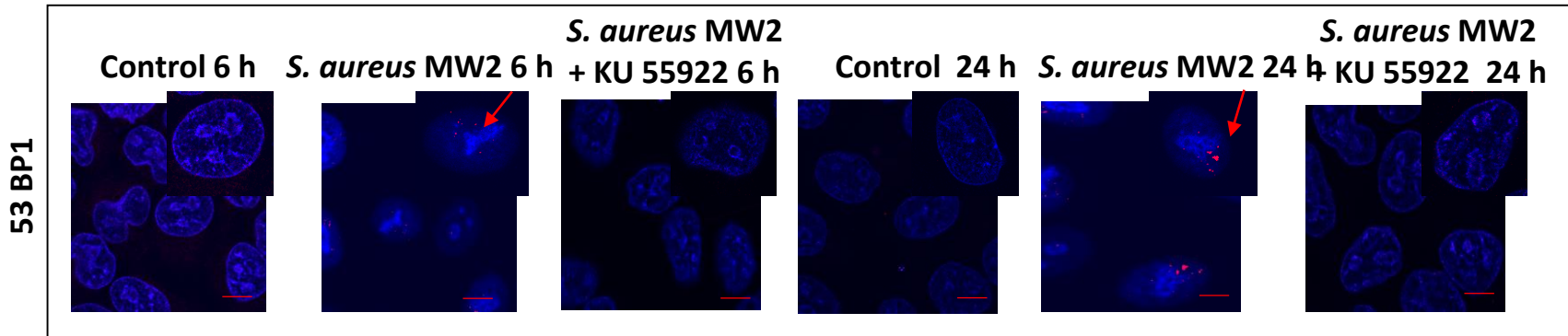
***** γ H2AX is a marker for DNA damage in the absence of apoptosis

S. aureus triggers DNA repair


HeLa



MG-63

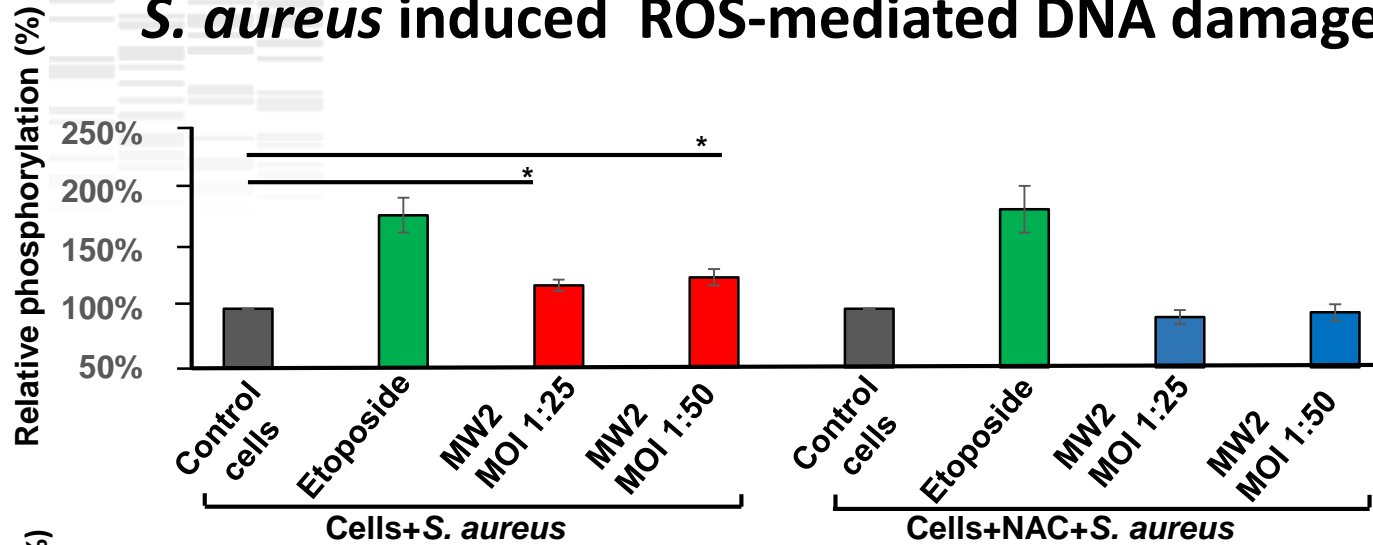


 53BP1 is the early repair protein

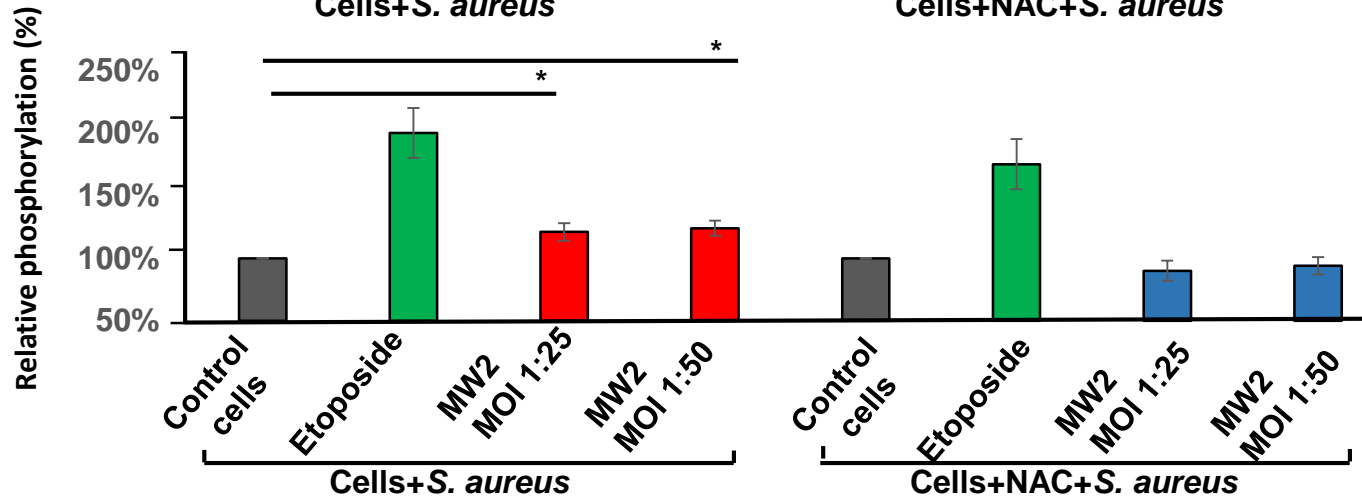
 To define whether the formation of 53BP1 foci was associated with a canonical DDR comprising the triggering of the ATM kinase-signaling pathway, HeLa and MG-63 cells were treated with the ATM inhibitor KU-55933.

S. aureus induced ROS-mediated DNA damage

6 h



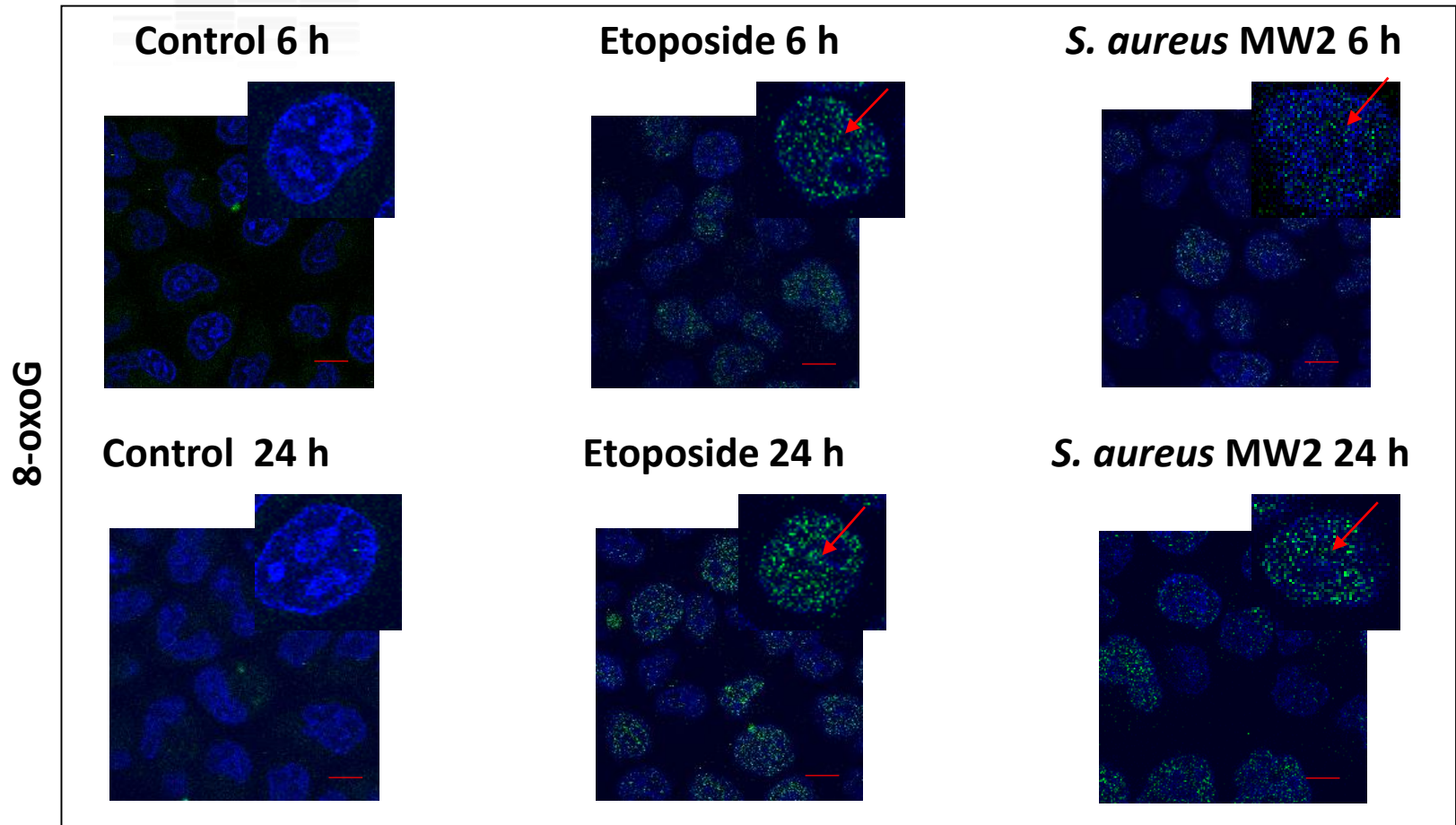
20 h



- ★ ROS can trigger DNA damage in the host cells
- ★ N-acetyl-L-cysteine (NAC) is an inhibitor of ROS

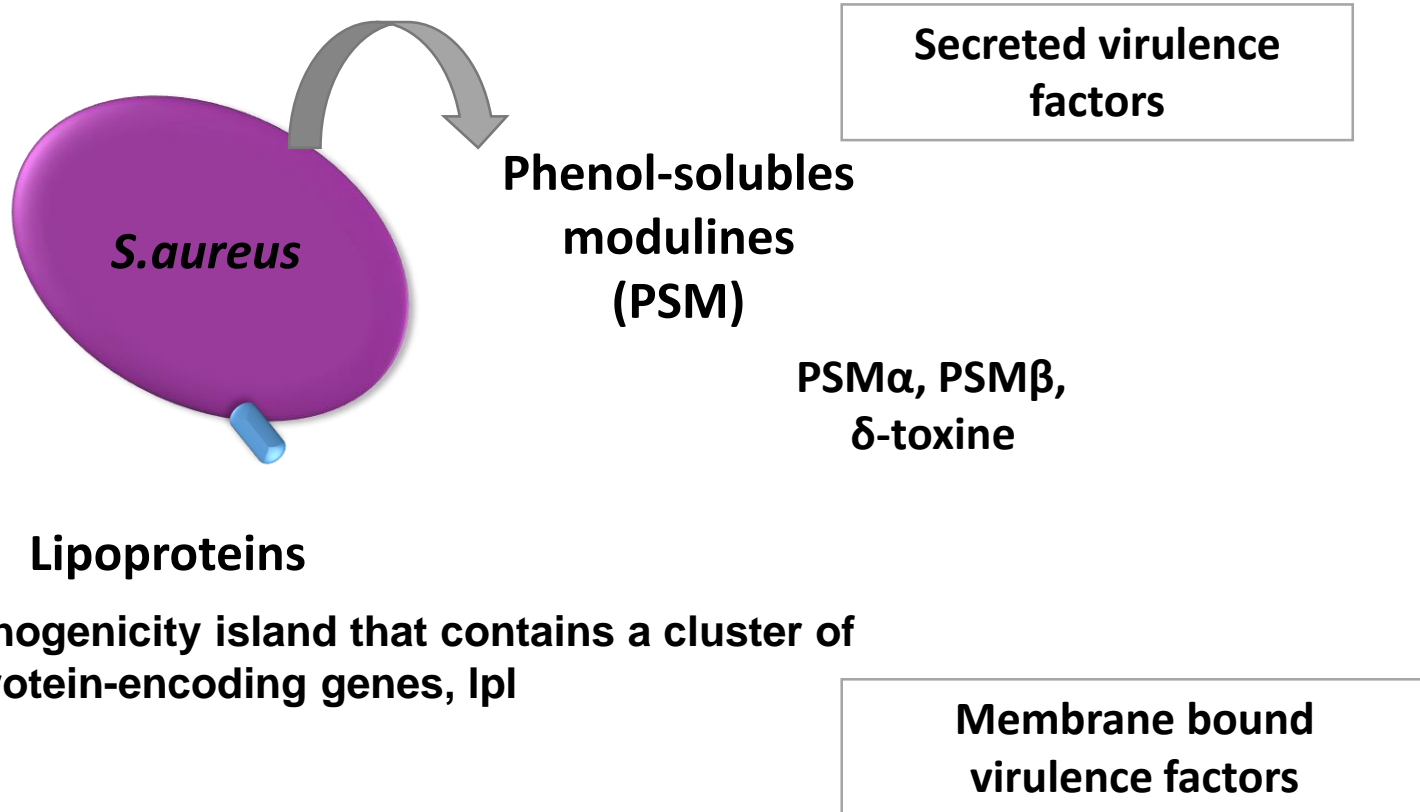
S. aureus prompts a ROS production, which induces 8-oxoG DNA lesion

HeLA



 Mutagenic lesion 8-oxoG is most often involved in oxidative DNA damage

Are *S. aureus* virulence factors PSMs and LPL involved in DNA damage?



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

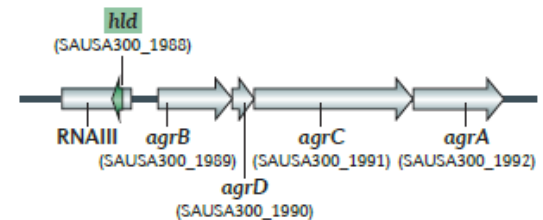
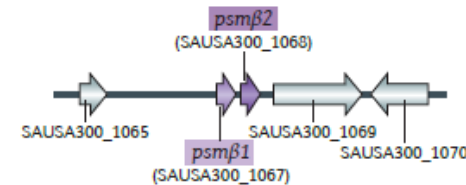
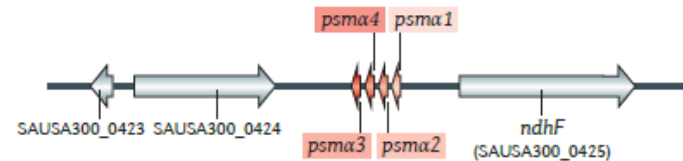
PSM α 1-PSM α 4
 δ -toxin 20-25 amino acids

PSM β 44 amino acids

PSM α 1-PSM α 4 are encoded in the *psm α* operon

PSM β 1 and PSM β 2 are encoded in the *psm β* operon

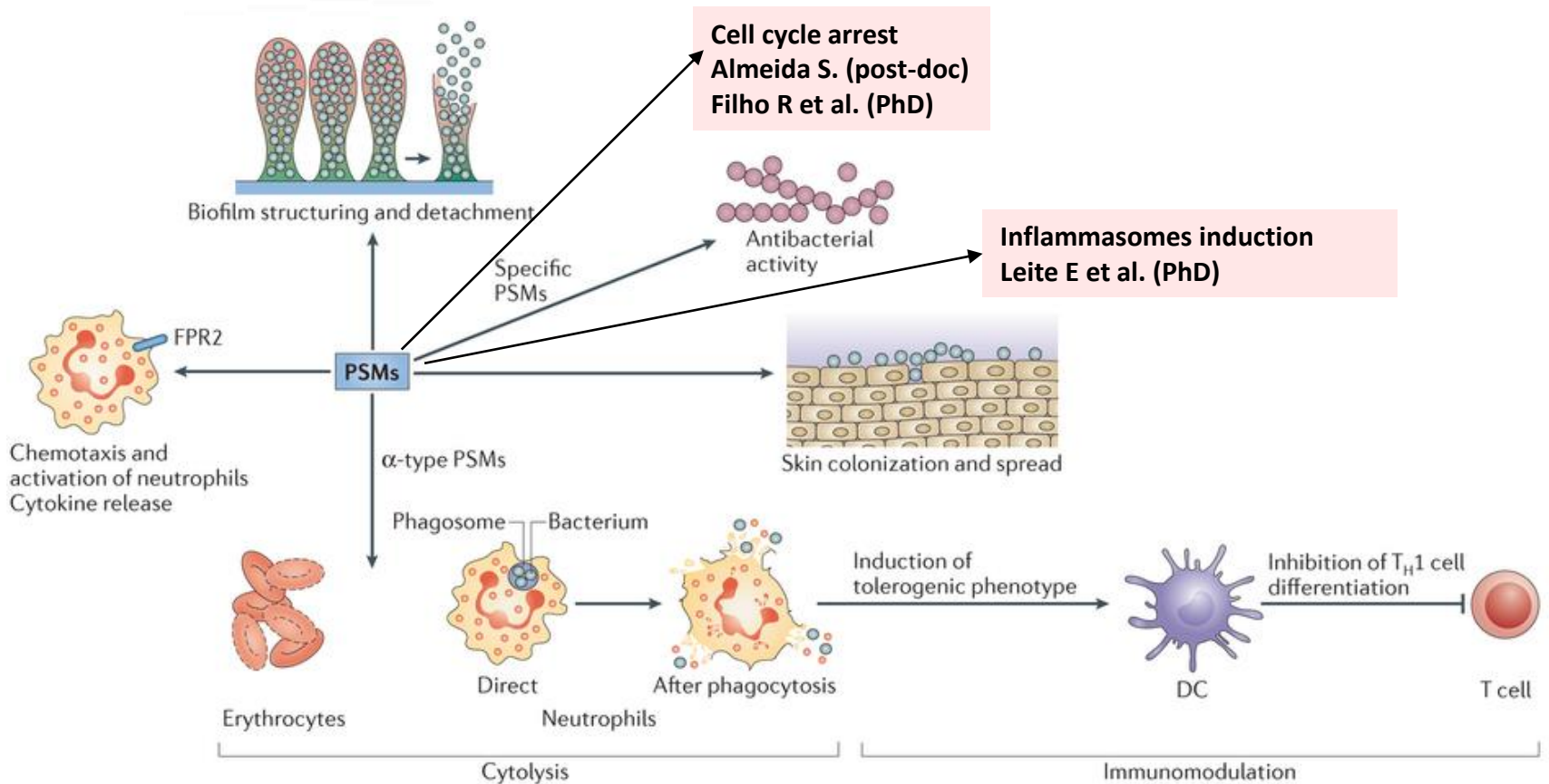
δ -toxin is encoded within the coding sequence for RNAIII, the RNA effector molecule of the accessory gene regulator (AGR) quorum-sensing system



δ -toxin	fMAADIISTIGDLVKWIIDTVNKFKKK	26 (0)
PSM α 1	fMGIIAGIIKVIKSLIEQPTGK	21 (+1)
PSM α 2	fMGIIAGIIKPIKGLIEKFTGK	21 (+2)
PSM α 3	fMEFVAKLFFKPKDLLGKFLGNN	22 (+1)
PSM α 4	fMDFTGVITSIIDLKTCIQAFG	22 (+1)
PSM β 1	fMEGLFNAIKDVTVAAINNDGAKLGTSIIVSIVENGVLLGKFLGFG	44 (-2)
PSM β 2	fMEGLAEAIANTVQAAQQHDSVKLGTSIIVDIVANGVLLGKFLGFG	44 (-1.9)

Peschel and Michael Otto, 2013

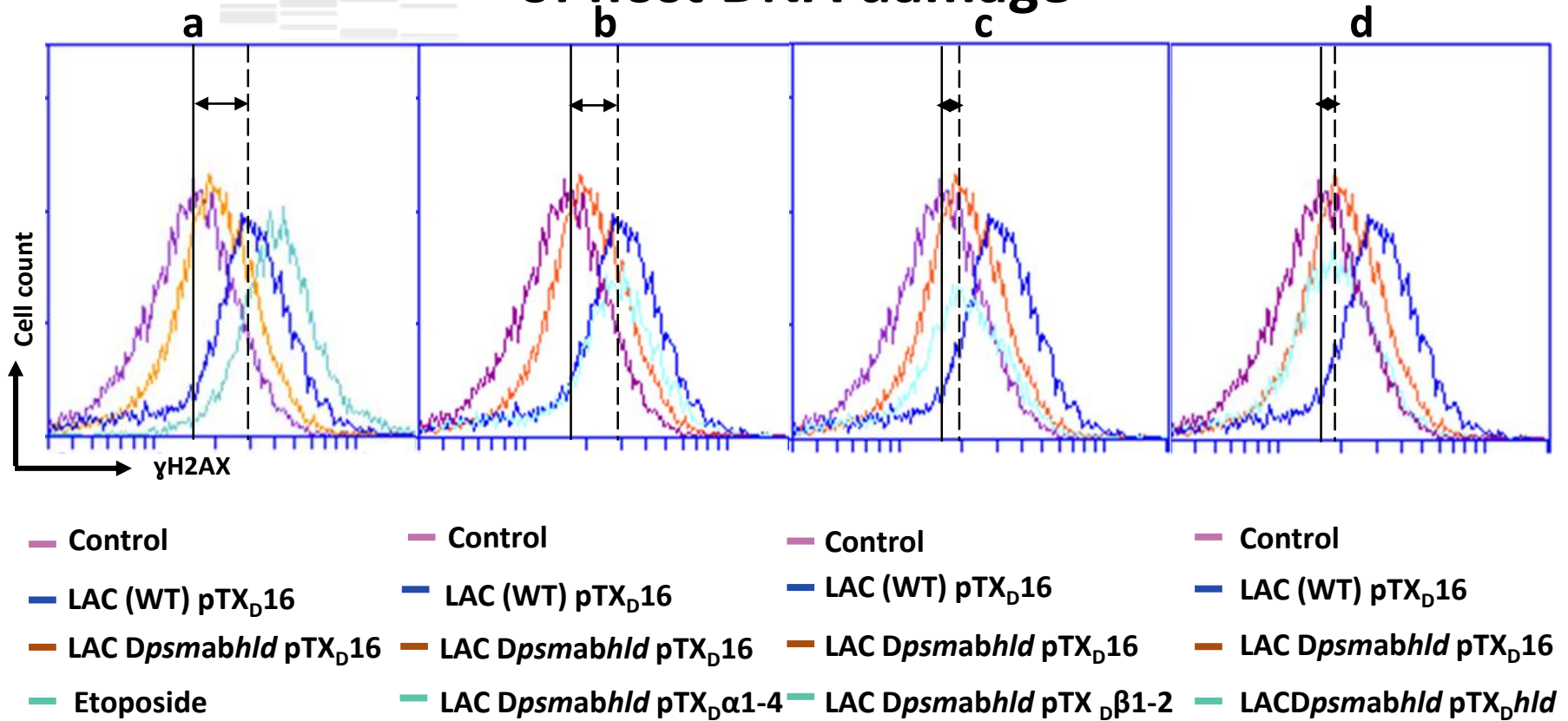
OVERVIEW OF PHENOL-SOLUBLE MODULIN ACTIVITIES



Nature Reviews | Microbiology

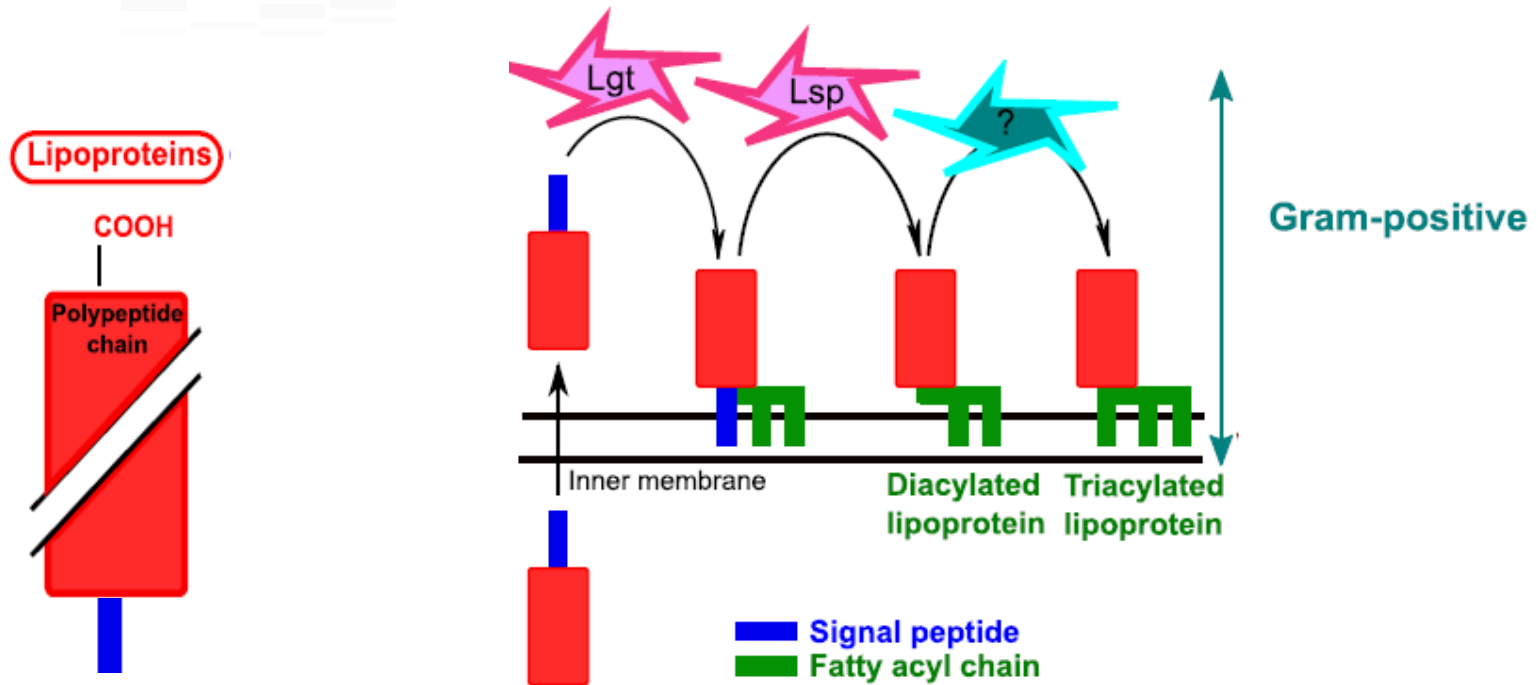
Peschel and Michael Otto, 2013

Pivotal role of *S. aureus* PSM α 1–4 toxins in the induction of host DNA damage



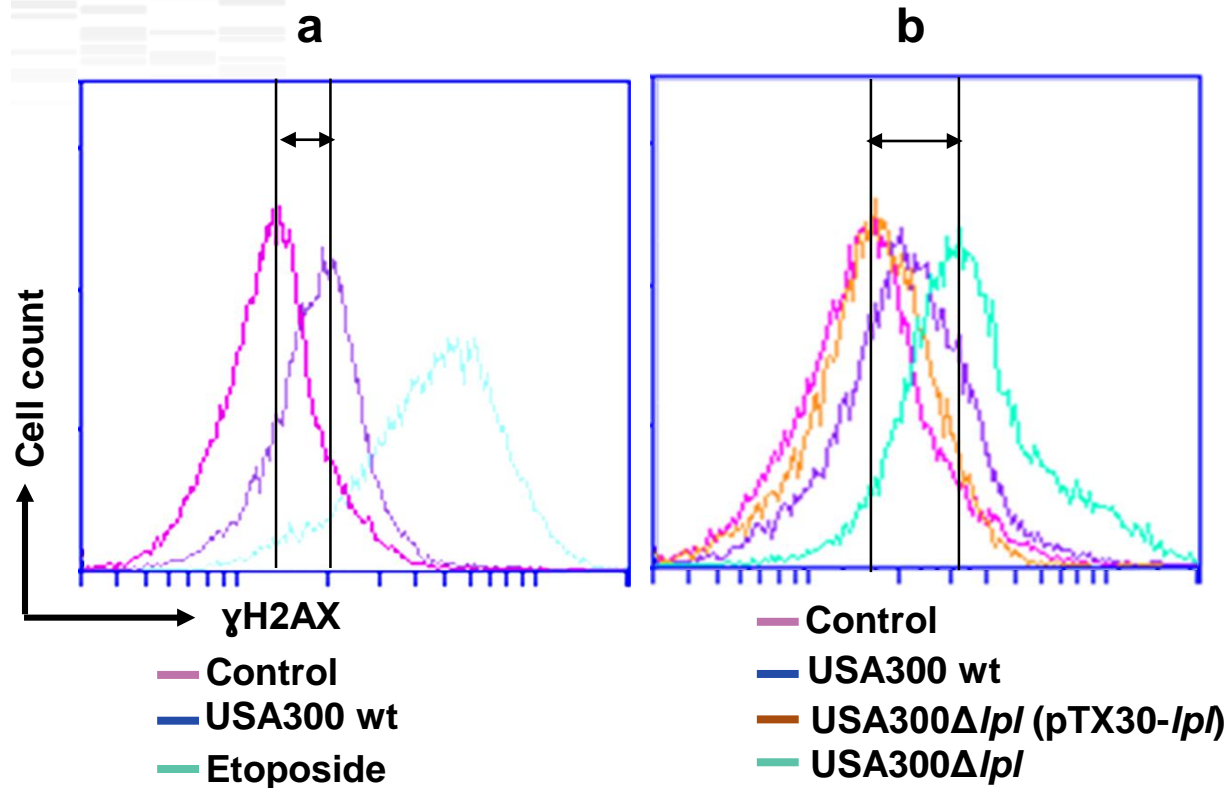
S. aureus LAC (WT) pTX Δ 16 contains plasmid pTX Δ 16 (blue line)
 PSM-deficient deletion mutant LAC Δ *psma* β *hld* pTX Δ 16 (yellow line)
 Complemented mutant LAC Δ *psma* β *hld* pTX Δ α 1-4 (blue-green line, b)
 LAC Δ *psma* β *hld* pTX Δ β (blue-green line, c) and
 LAC Δ *psma* β *hld* pTX Δ *hld* (blue-green line d)
 Constructed in the Michael Otto laboratory, NIH, USA

Bacterial Lipoproteins



After crossing the cytoplasmic membrane Lgt (prolipoprotein diacyl glyceryl transferase) transfers a diacylglyceride to the polypeptide chain and Lsp (lipoprotein signal peptidase) cleaves the signal peptide

Lpls dampen DNA damage of infected cells



Mutant USA300 Δ /*lpl* in which the entire *lpl* cluster was deleted and the complemented mutant USA300 Δ /*lpl* (pTX30-*lpl*) were constructed in the Fritz Goetz laboratory, University of Tübingen, Germany



S. aureus clinical isolats

Three couples of *S. aureus* isolates were selected from patients P1, P2, P3 who were diagnosed with initial acute (i) and recurrent (r) staphylococcal BJI

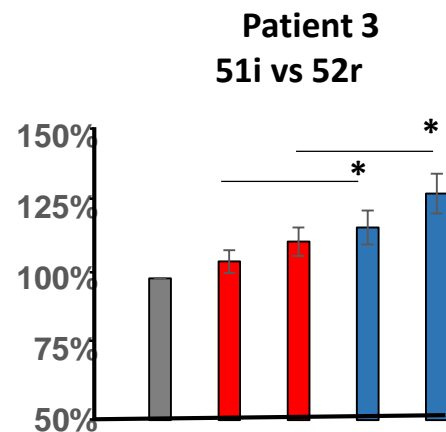
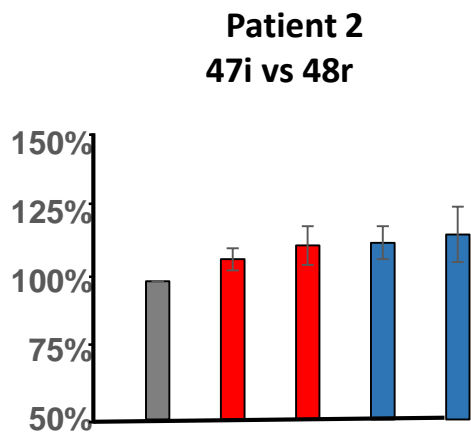
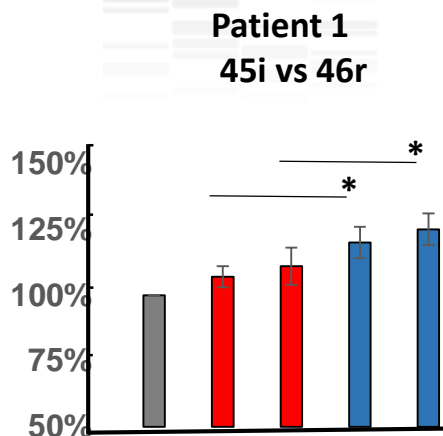
Patient P1	Patient P2	Patient P3
45i	47i	51i
46r	48r	52r

Genomic comparison did not reveal mutations in the major regulatory systems (*agr*, *sar*, *sigma* genes) or in virulence genes between initial and recurrent strains

S. aureus recurrent isolates induce stronger DNA damage than initial acute isolates

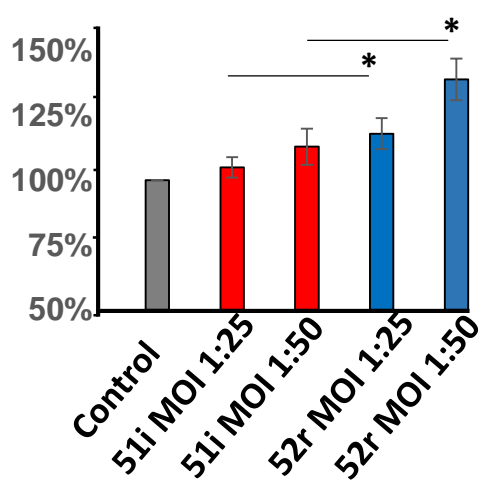
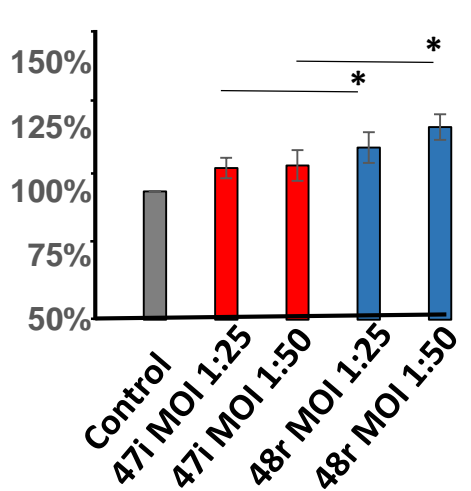
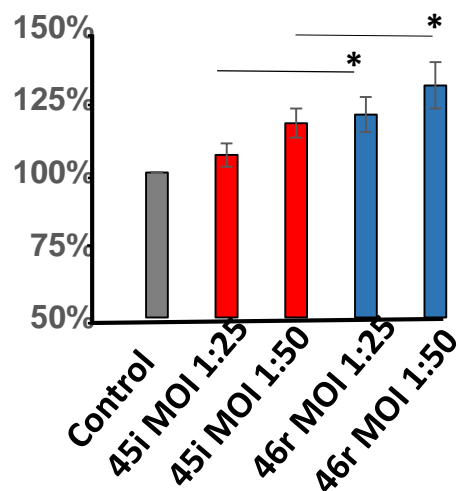
6 h

Relative phosphorylation (%)



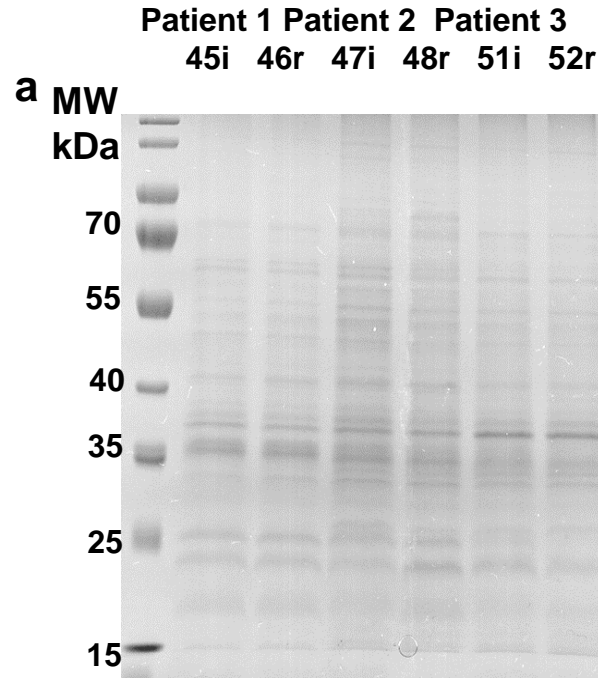
20 h

Relative phosphorylation (%)



High content screening analysis

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






P1: 45i vs 46r
P2: 47i vs 48r
P3: 51i vs 52r



OPEN

Staphylococcus aureus induces DNA damage in host cell

Martine Deplanche¹, Nassim Mouhali¹, Minh-Thu Nguyen², Chantal Cauty¹, Frédéric Ezan³, Alan Diot^{4,5}, Lesly Raulin⁶, Stephanie Dutertre⁶, Sophie Langouet³, Patrick Legembre⁷, Frederic Taieb⁸, Michael Otto ⁹, Frédéric Laurent^{4,5}, Friedrich Götz², Yves Le Loir ¹ & Nadia Berkova ¹

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

Staphylococcus aureus Lpl Lipoproteins Delay G2/M Phase Transition in HeLa Cells

Minh-Thu Nguyen¹, Martine Deplanche², Mulugeta Nega¹, Yves Le Loir², Loulou Peisl¹, Friedrich Götz^{1*} and Nadia Berkova^{2*}

December 2018 | Volume 6 | Article 201

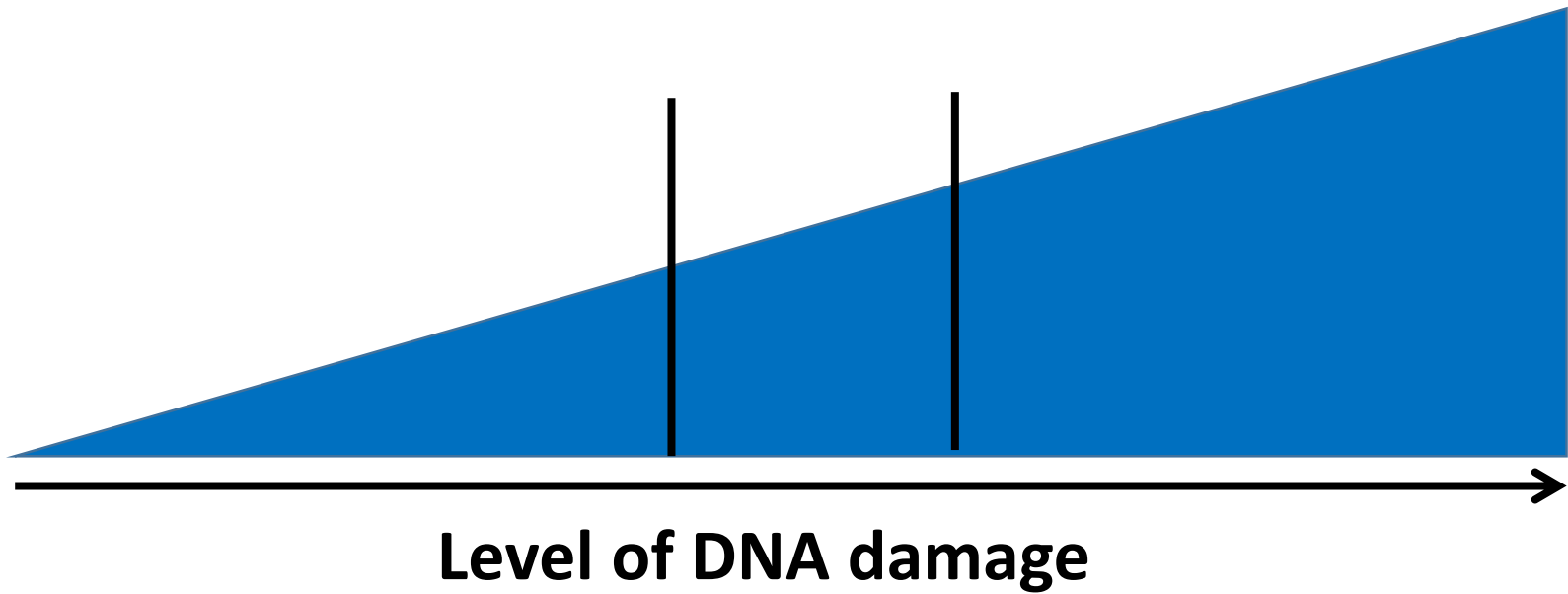


DNA DAMAGE DURING INFECTION

DNA repair

Cell cycle arrest

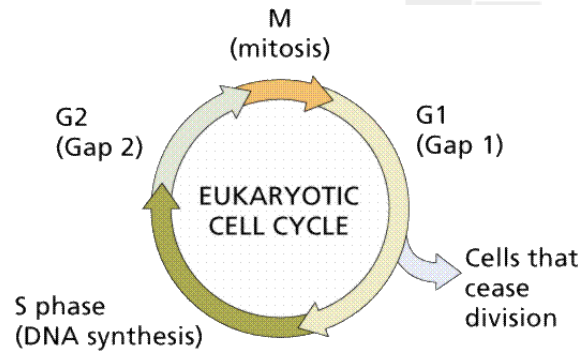
Apoptosis





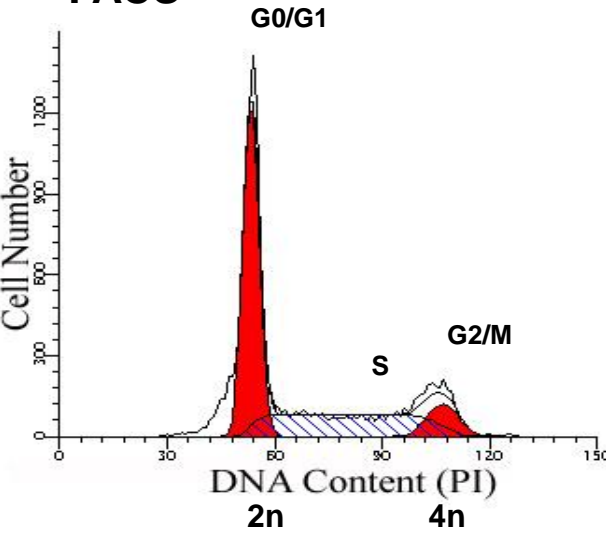
Does *Staphylococcus aureus* induce cell cycle arrest in host cells?

CELL CYCLE ANALYSIS



State	Phase	Abbreviation	Description
quiescent/senescent	Gap0	G₀	A resting phase where the cell has left the cycle and has stopped dividing.
	Gap1	G₁	Cells increase in size in Gap 1. The G₁ checkpoint control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	S	DNA replication
	Gap2	G₂	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The G₂ checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	M	Cell growth stops. Division into two daughter cells. A Mitosis checkpoint ensures that the cell is ready to complete division.

FACS

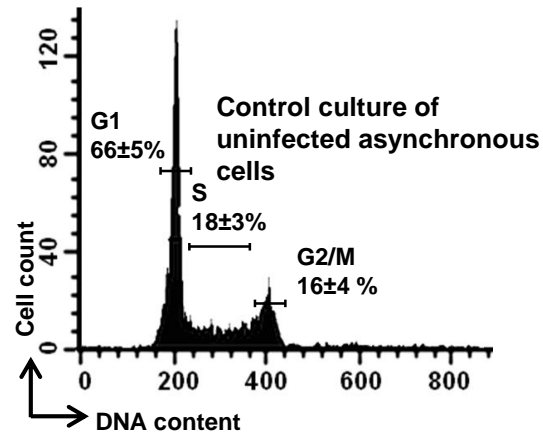


Cyclomodulins is a term for bacterial effectors that interfere with the eukaryotic cell cycle and may affect pathogen virulence.

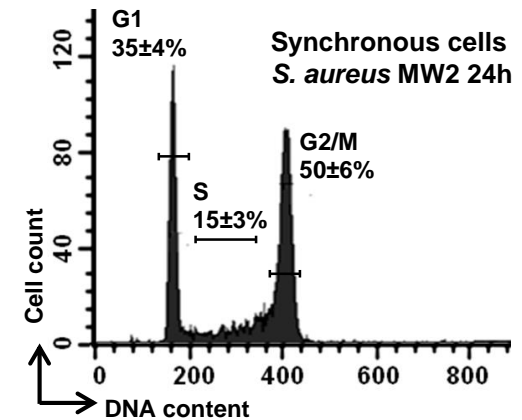
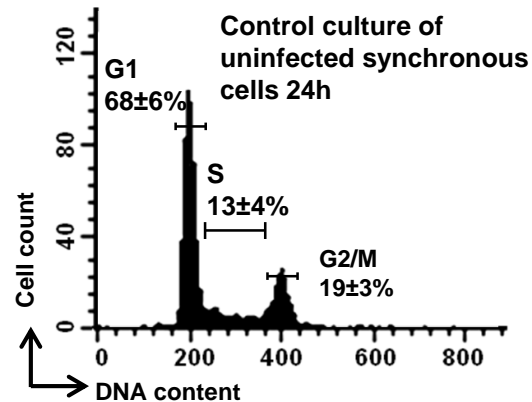
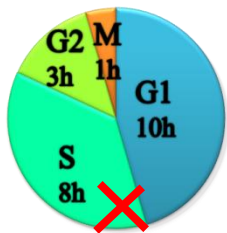
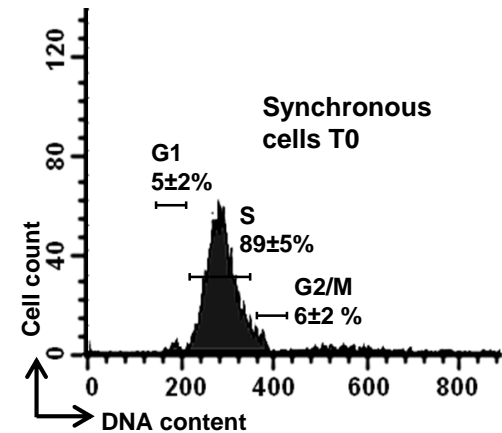
S. aureus induced a G2/M phase delay

HeLa

Asynchronous cells



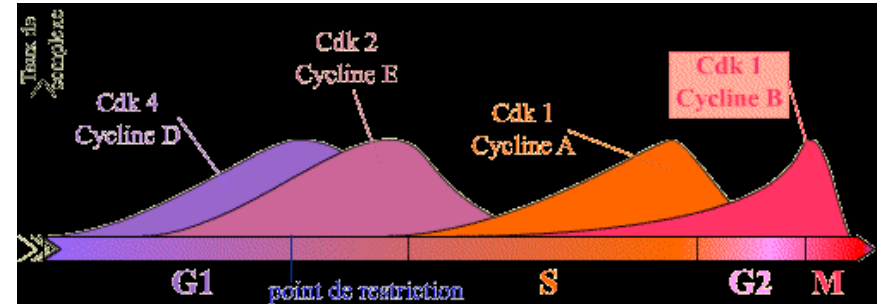
Synchronous cells



CELL CYCLE REGULATION

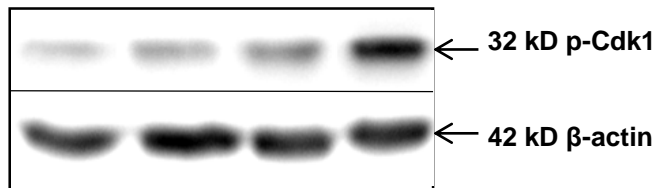
Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases.

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



S. aureus-induced accumulation of phosphorylated Cdk1

Western blot

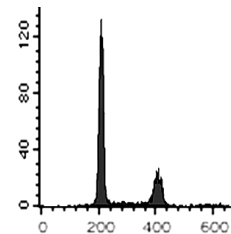


Control
20:1

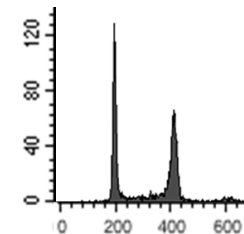
5:1 10:1

S. aureus MOI

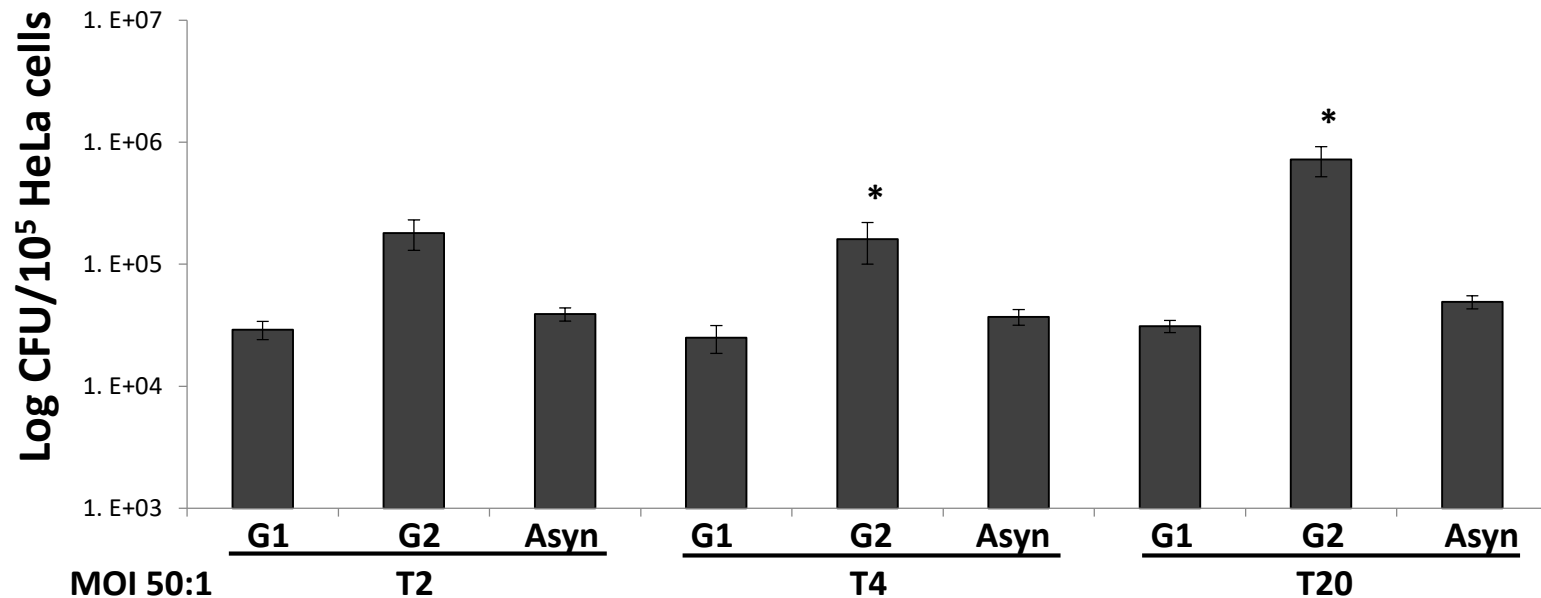
Control cells



Cells + *S. aureus*



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





OPEN ACCESS Freely available online

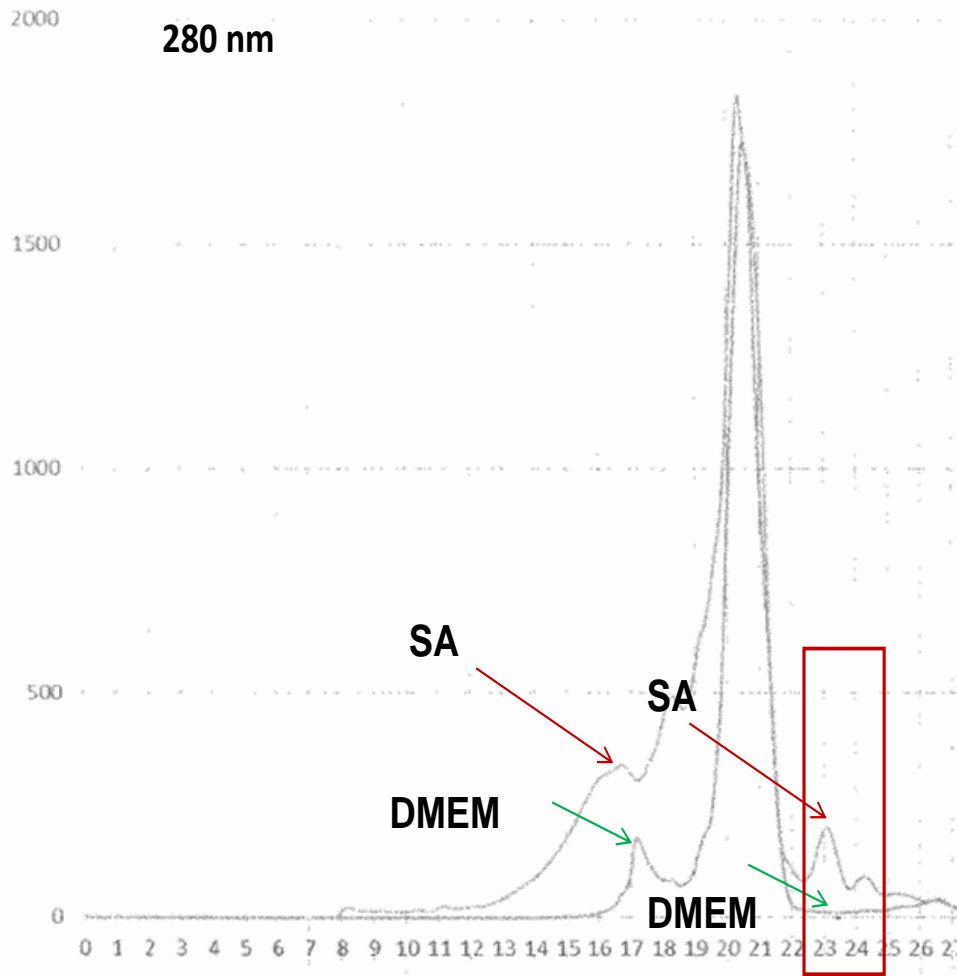
 PLOS ONE

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

Ludmila Alekseeva^{1,2,3*}, Lucie Rault^{2,3*}, Sintia Almeida^{2,3,4}, Patrick Legembre⁵, Valérie Edmond⁵, Vasco Azevedo⁴, Anderson Miyoshi⁴, Sergine Even^{2,3}, Frédéric Taieb⁶, Yannick Arlot-Bonnemains⁷, Yves Le Loir^{2,3}, Nadia Berkova^{2,3*}

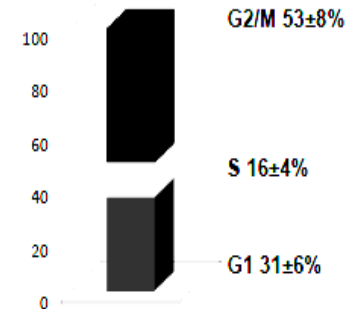
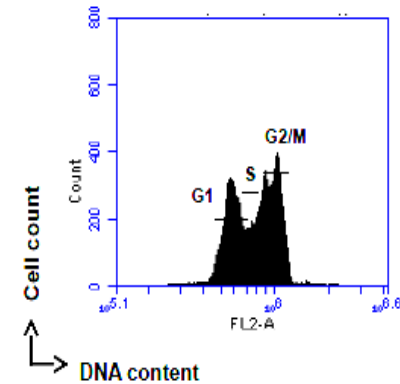
May 2013 | Volume 8 | Issue 5 | e63279

Gel-filtration chromatography of *S. aureus* supernatants

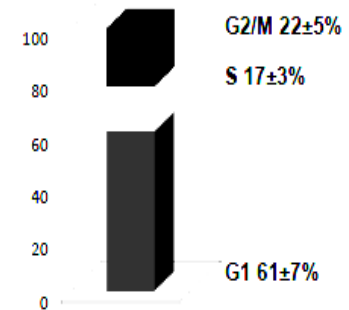
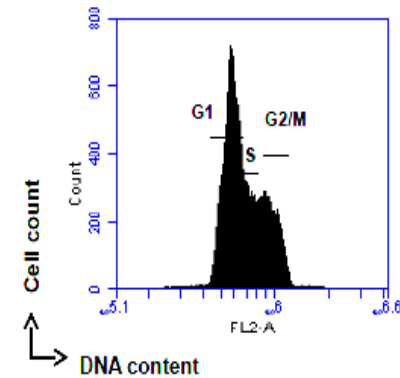


Fractions 23-25 induce G2/M transition delay

S. aureus



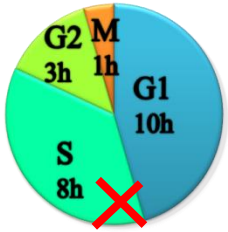
DMEM



Detection of PSM α peptide derivatives in *S. aureus* chromatography fractions

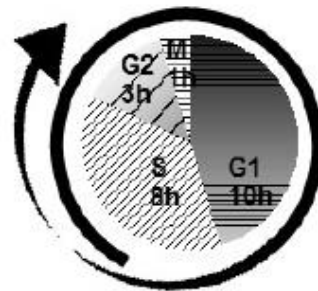
Uniprot Entry	Gene Name	Description	Peptide sequence	X!Tandem e-value	SEC Fractions identification
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	GIKVIKS	1,7E-3	23
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	IIAGIIKV	1,2E-2	23, 24
PSMA1_STAAW	psmA1	Phenol-soluble modulin alpha 1	IIKVIKS	1,8E-2	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	AKLKF	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

PSM α 1 and PSM α 2 induce the G2/M phases transition delay

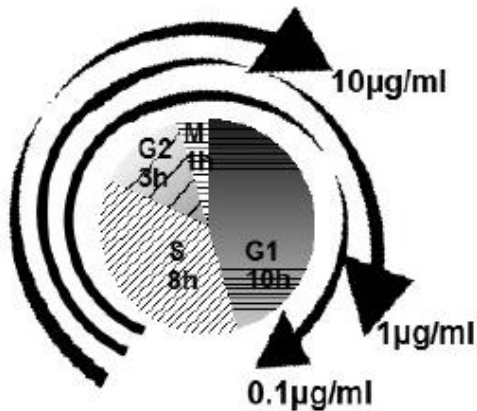


Control cell

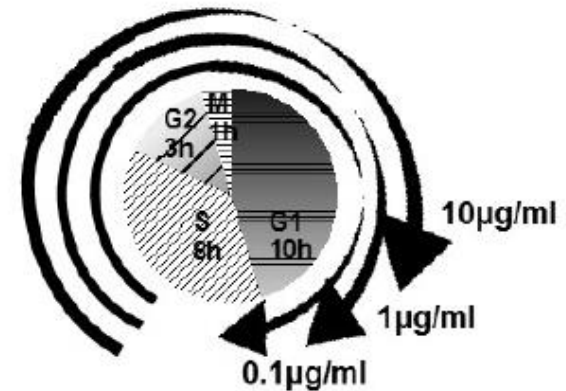
25h



PSM1

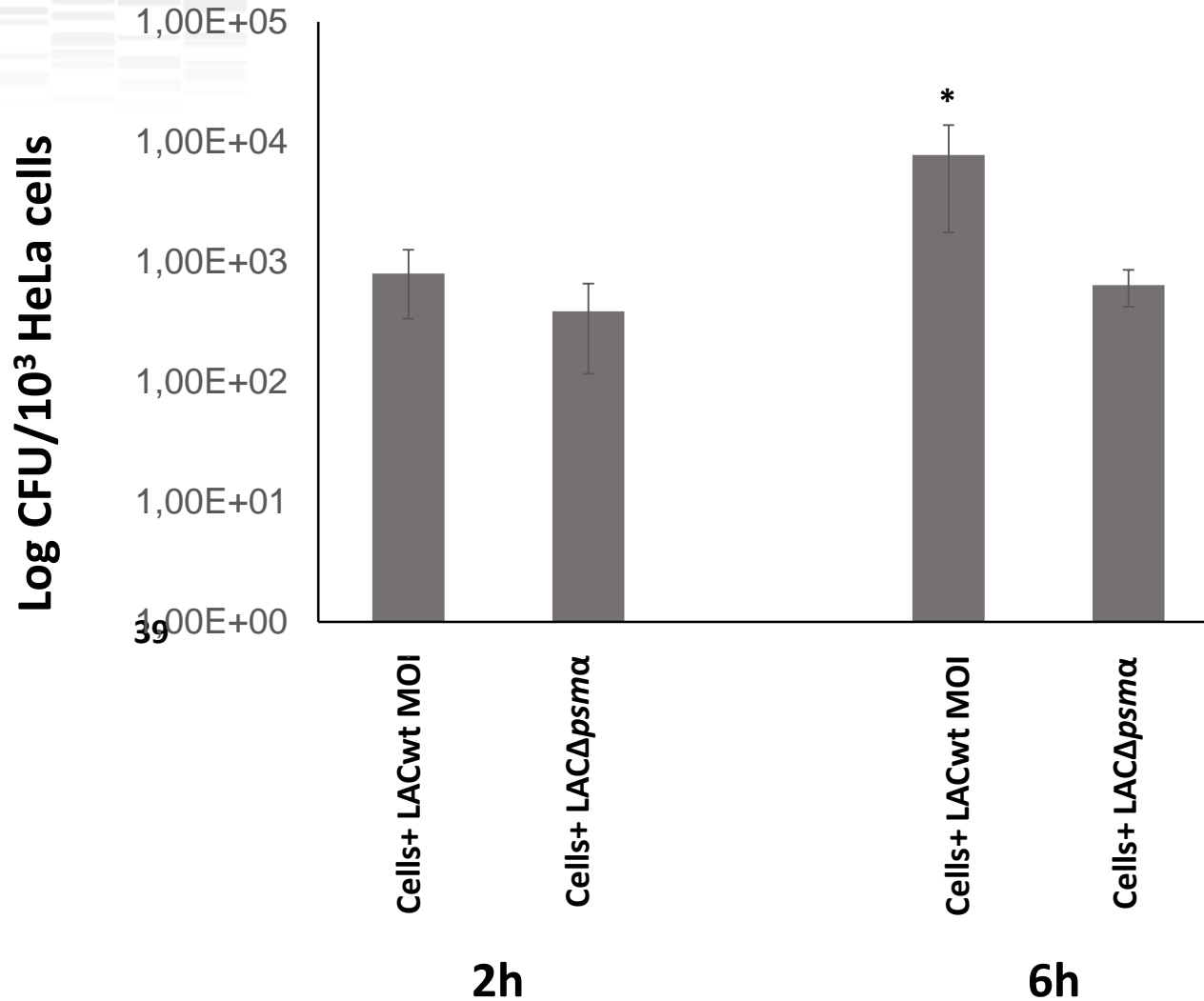


PSM3

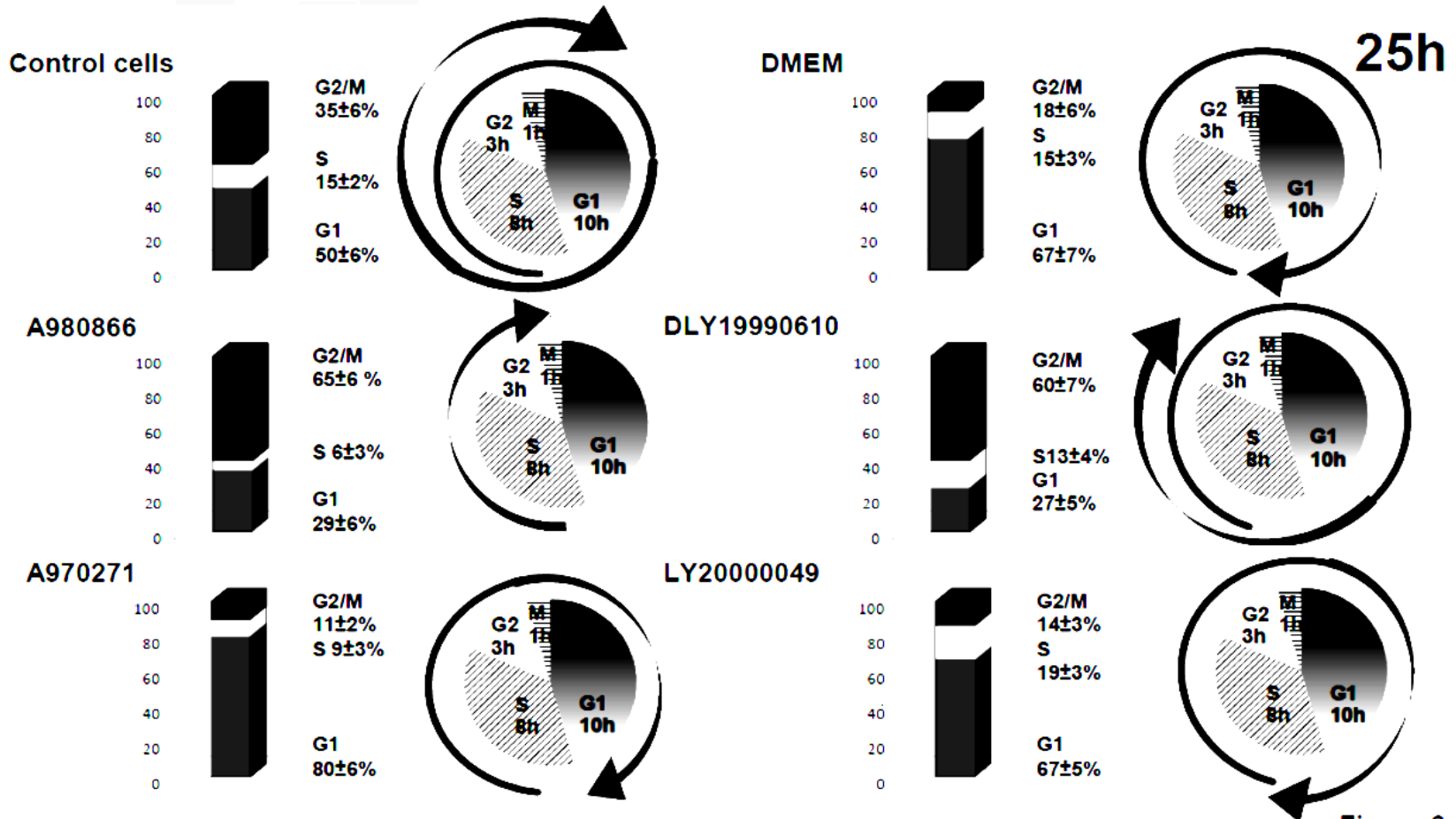


Internalized LAC wt replicates inside HeLa cells in contrast to its isogenic $LAC\Delta psm\alpha$ mutant

MOI 100:1

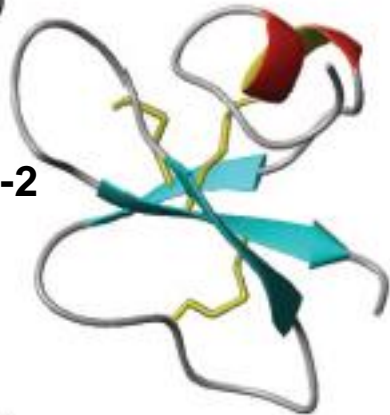


S. aureus-induced G2/M transition delay is strain-dependent



Structural classes of antimicrobial peptides.

(A)
Human β -defensin-2



(D)



Rabbit kidney α -defensin-1

Looped thanatin

(B)



(C)



B-sheeted
polyphemusin

(E)

α -helical maganin-2



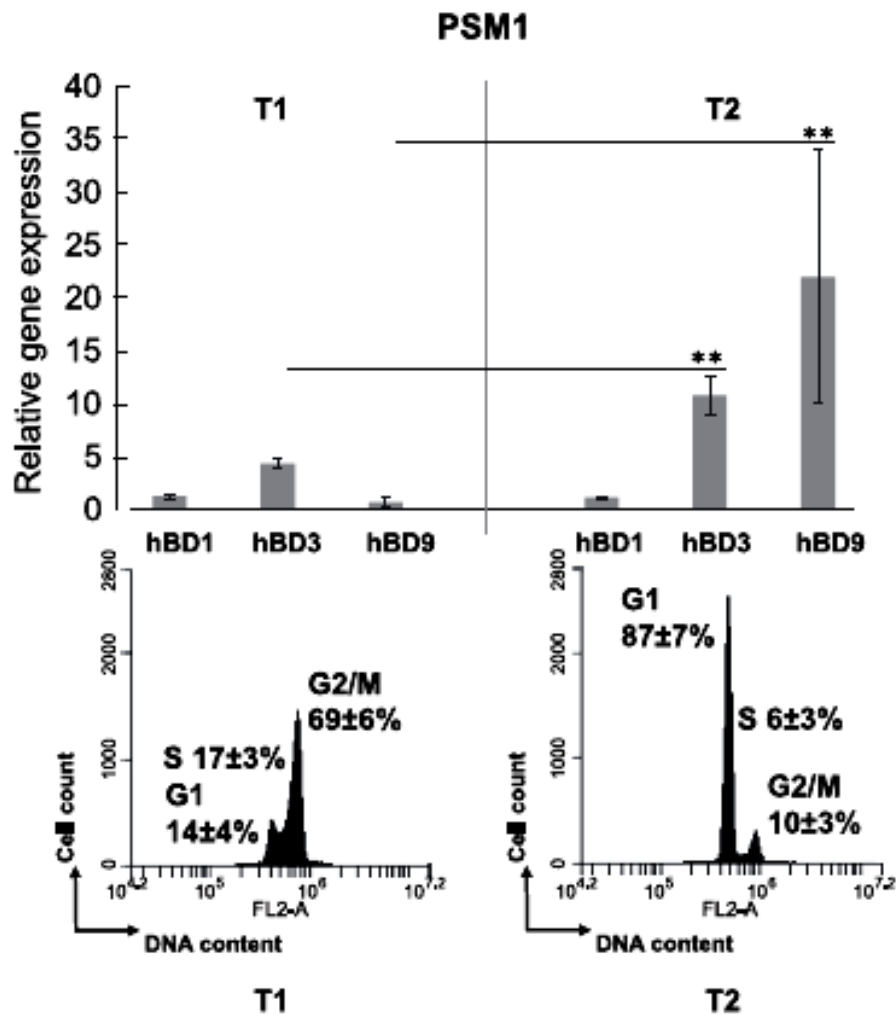
(F)



12-100 amino acids
Positively charged
Amphiphilic

-Important effector molecules of
the innate immune system
-Adaptive immune system

mRNA levels of HBD-1, -3 and -9 in PSM α 1-treated cells





The FASEB Journal • Research Communication

Phenol-soluble modulin α induces G2/M phase transition delay in eukaryotic HeLa cells

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

29, 1950–1959 (2015). www.fasebj.org



CONCLUSIONS

S. aureus triggers ROS-mediated DNA damage thus affecting the **genome integrity**

DNA damage depends on the balance between the levels of PSM α and Lpls

Host cells detect the DNA damage and transiently block cell cycle progression in the G2/M phases

PSMs α and membrane-anchored Lpls are responsible for a G2/M arrest

S. aureus-induced cell cycle alteration is associated with an increased bacterial **intracellular proliferation** as well as with the **decreased production of antibacterial peptides**

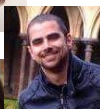
These findings open a new avenue for the development of innovative therapeutic strategies that either suppress DNA damage or boost DNA repair during *S. aureus* infection

COLLABORATIONS

Deplanche M,
Ladier E,
Mouhali N,
Jardin J,
Henry G,
Cauty C,
Le Loir Y
INRA, UMR1253,
STLO, Rennes.



Belo Horizonte
MG university, Brazil
Sintia Almeida,
Aref El Aouar Filho R,
Lima, Leite E,
Miyoshi A
Azevedo V,



NIH, Bethesda,
Maryland, USA
Michael Otto



CNRS: Arlot-Bonnemains Y,
INSERM: Legembre P,
Langouet S.



INRA, INSERM, Toulouse
Frederic Taieb (Toulouse)



Centre International de Recherche en
Infectiologie, INSERM U1111, CNRS
UMR5308, University of Lyon, France
Lina G, Laurent F, Vandenesch F



Chemyakin-Ovchinnikov
Institute of Bioorganic
Chemistry, Moscow, RF,
Alexseeva L



Hospices Civils de Lyon

Hospices Civil de Lyon
Beraud B, Steghens JP



University of Tübingen,
Germany,
Fritz Goetz





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

ABSENCE OF APOPTOSIS

