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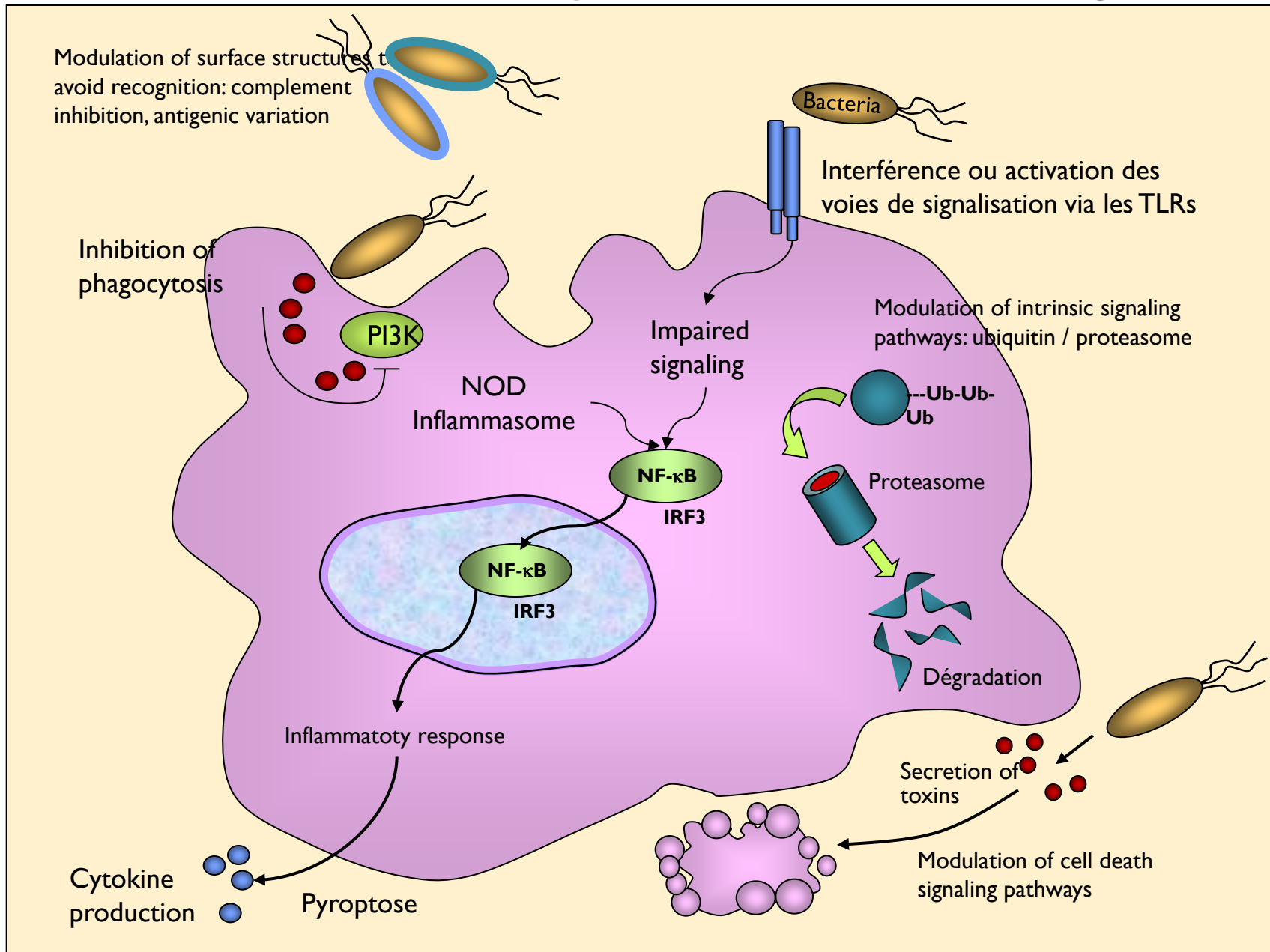
# Host-pathogen interaction: innate immunity

Pr Imad KANSAU

Equipe Bactéries Pathogènes et Santé



# Main mechanisms of manipulation of the immune system



# Innate immunity and recognition of non-self

## In bacteria:

### PAMPs :

- Lipopolysaccharide (LPS)
- Peptidoglycane
- Lipopeptide
- Lipoarabinomannanes
- Flagellines
- ADN C+G

**PAMPs:** Pathogen Associated Molecular Patterns

**DAMPs:** damage-associated molecular patterns

**HAMPs:** homeostasis-altering molecular processes

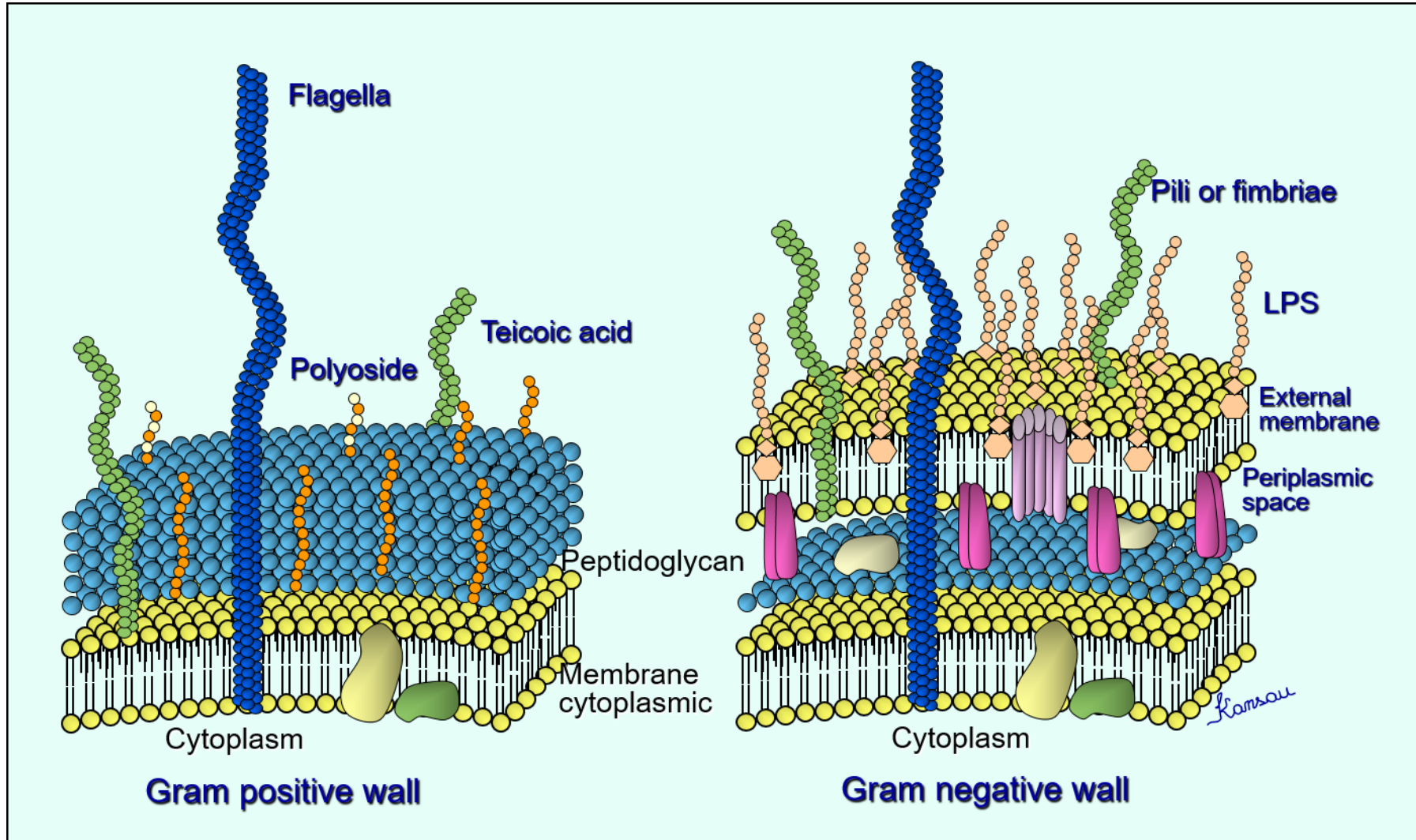
## In the host:

### PRR : *Pattern Recognition Receptors*

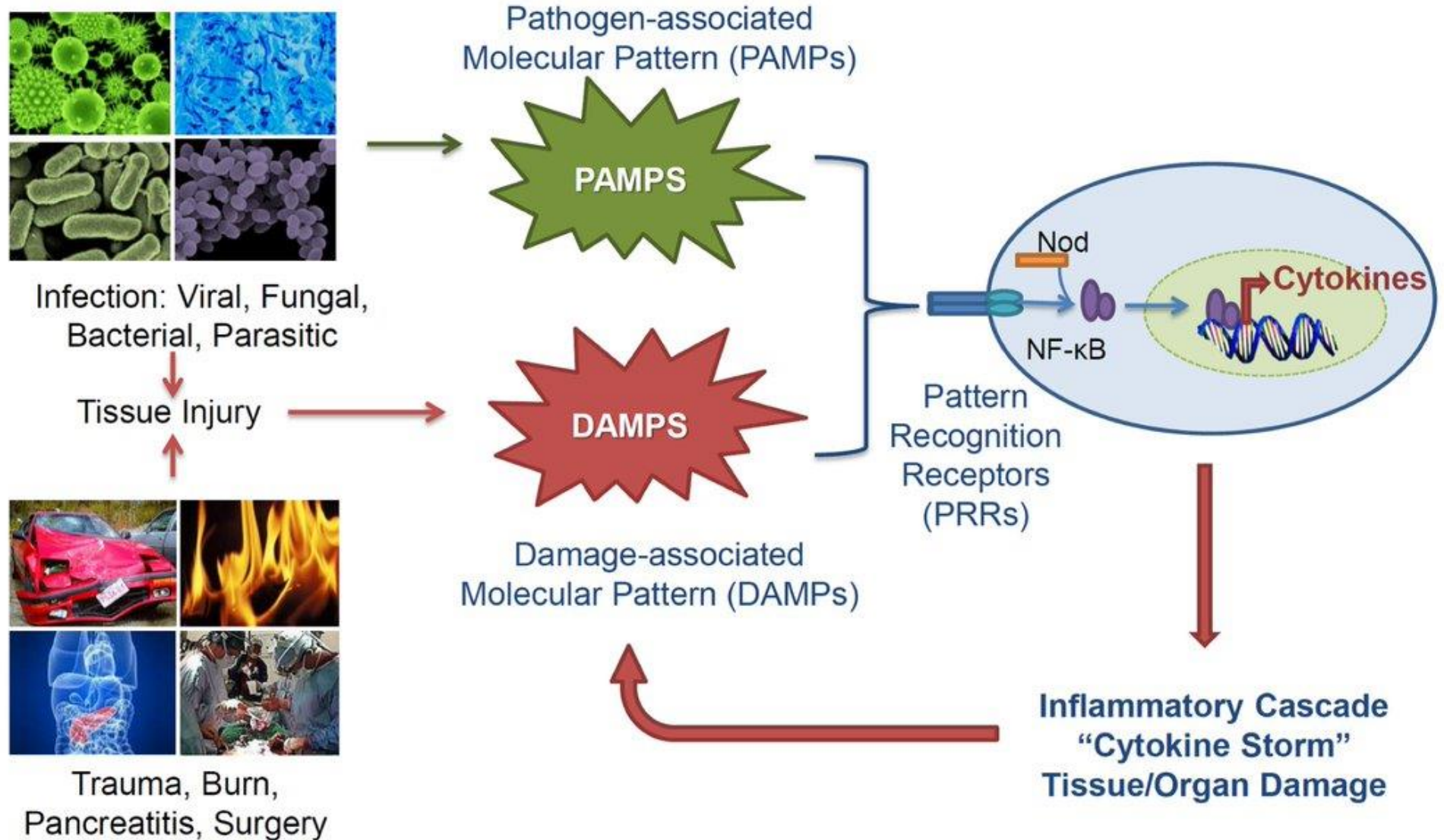
Récepteurs non opsonisants qui reconnaissent les PAMPs :

- Scavenger Receptors (SR)
- C-type lectin receptors (CLRs)
- Toll like receptors (TLR)
- Nucleotide-binding domain and leucine-rich repeat receptors (NLR) or NOD-like receptors
- retinoic acid-inducible gene (RIG)-I-like receptors (RLRs)
- DNA sensors

# Pathogen-associated molecular patterns (PAMP) on the bacterial surface



# Inflammatory Mechanisms

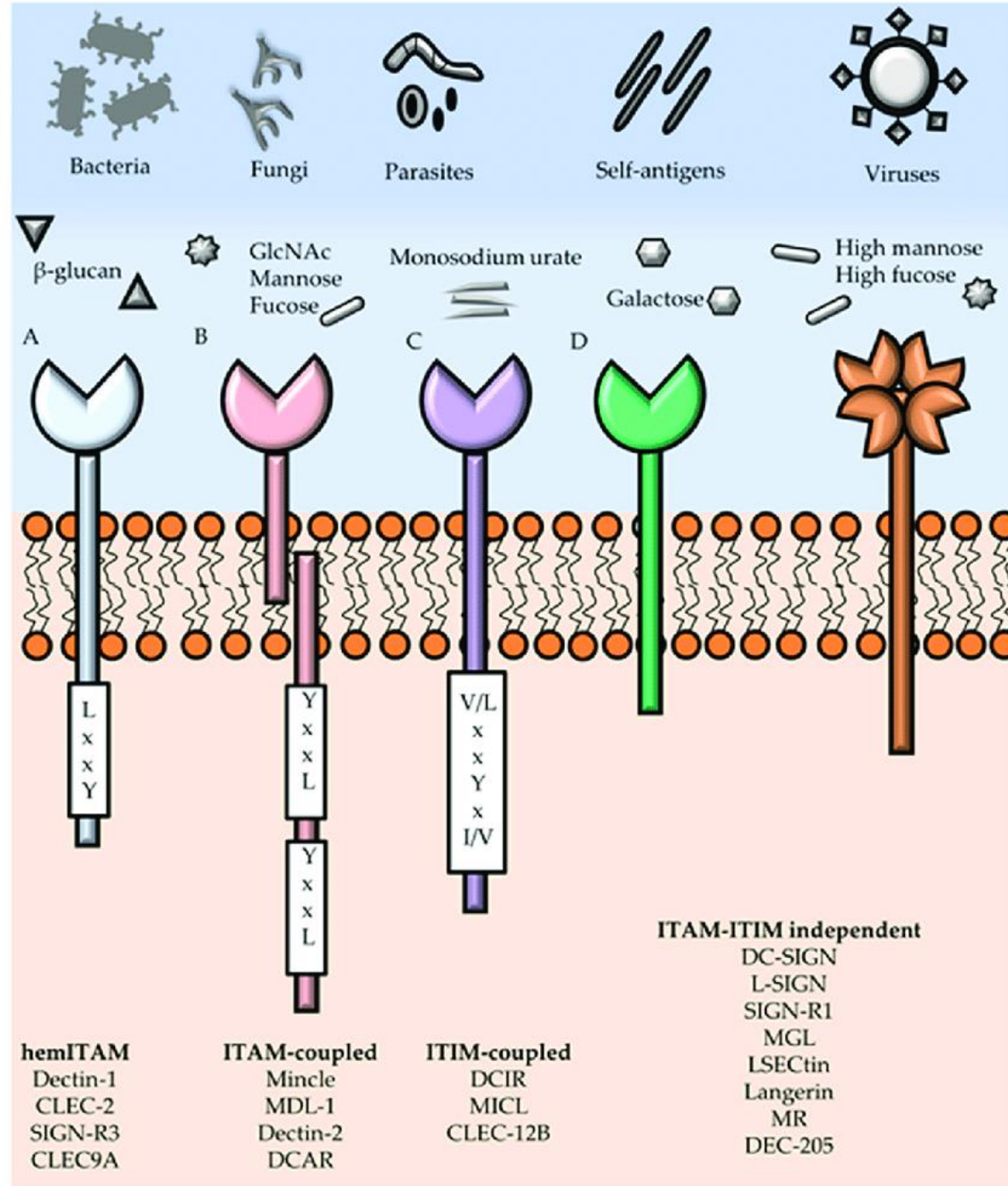


# CLRs: Myeloid C-type lectin receptors

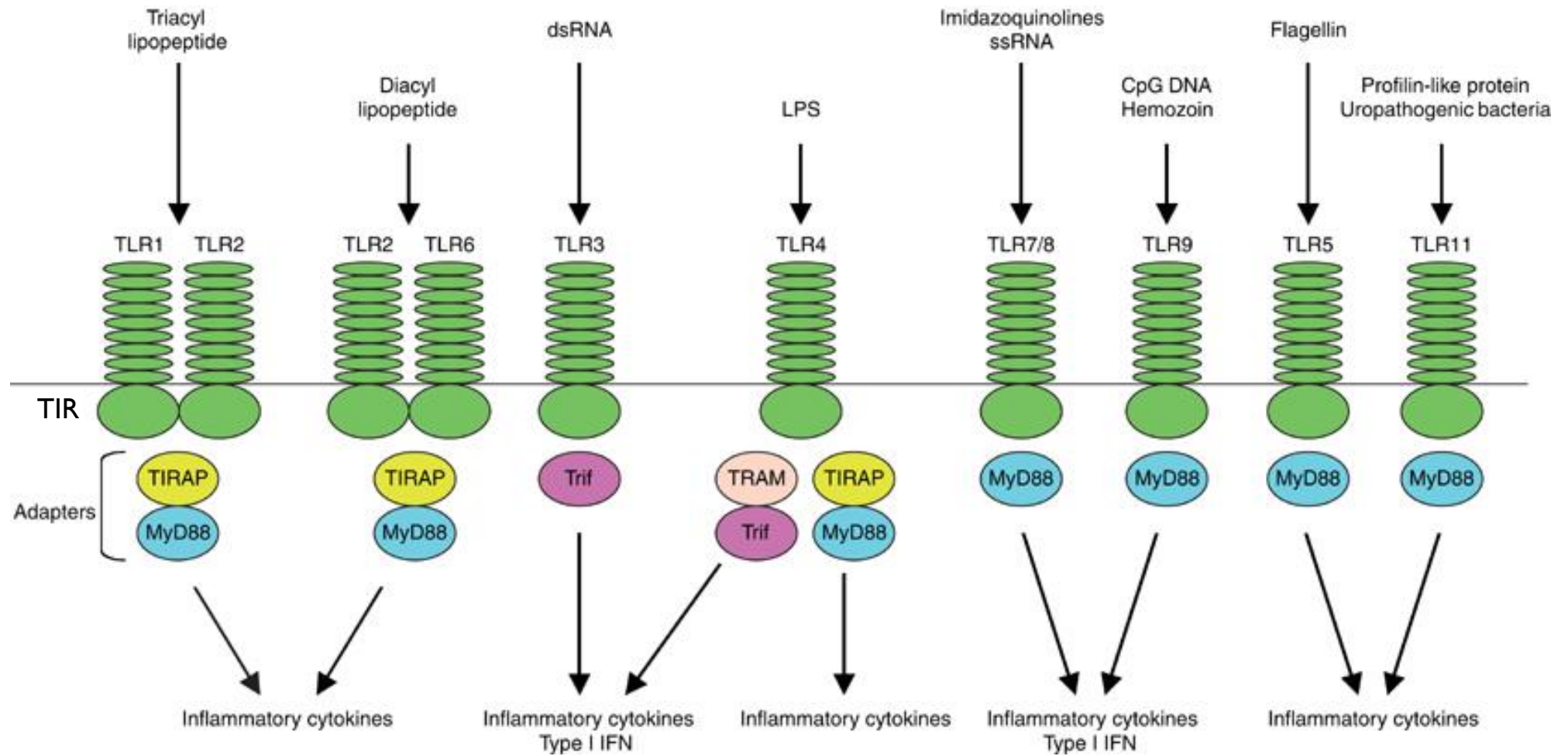
Expressed by APCs

ITAM: immunoreceptor tyrosine-based activating motif

ITIM: immunoreceptor tyrosine-based inhibitory motif



# Toll-like receptors (TLR) and host response



# Agonists of mouse and human TLRs

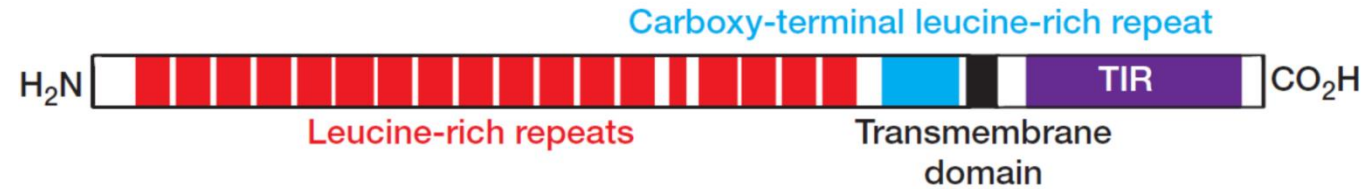
TLR	Ligand
1/2	Triacyl lipopeptides
2/6	Diacyl lipopeptides
3	dsRNA
4	Lipopolysaccharide
5	Flagellin
7	ssRNA
8	ssRNA in humans; unclear in mice
9	CpG DNA, malarial hemozoin
10 <sup>a</sup>	Unknown
11 <sup>b</sup>	Uropathogenic bacteria, <i>Toxoplasma gondii</i> profilin-like protein
12 <sup>b</sup>	Unknown
13 <sup>b</sup>	Unknown

<sup>a</sup>Expressed only in humans.

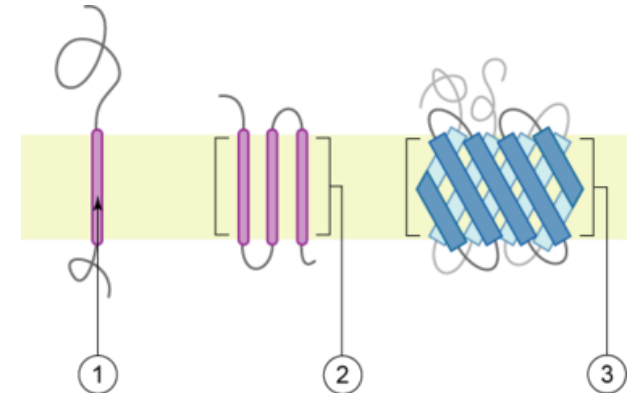
<sup>b</sup>Expressed only in mice.



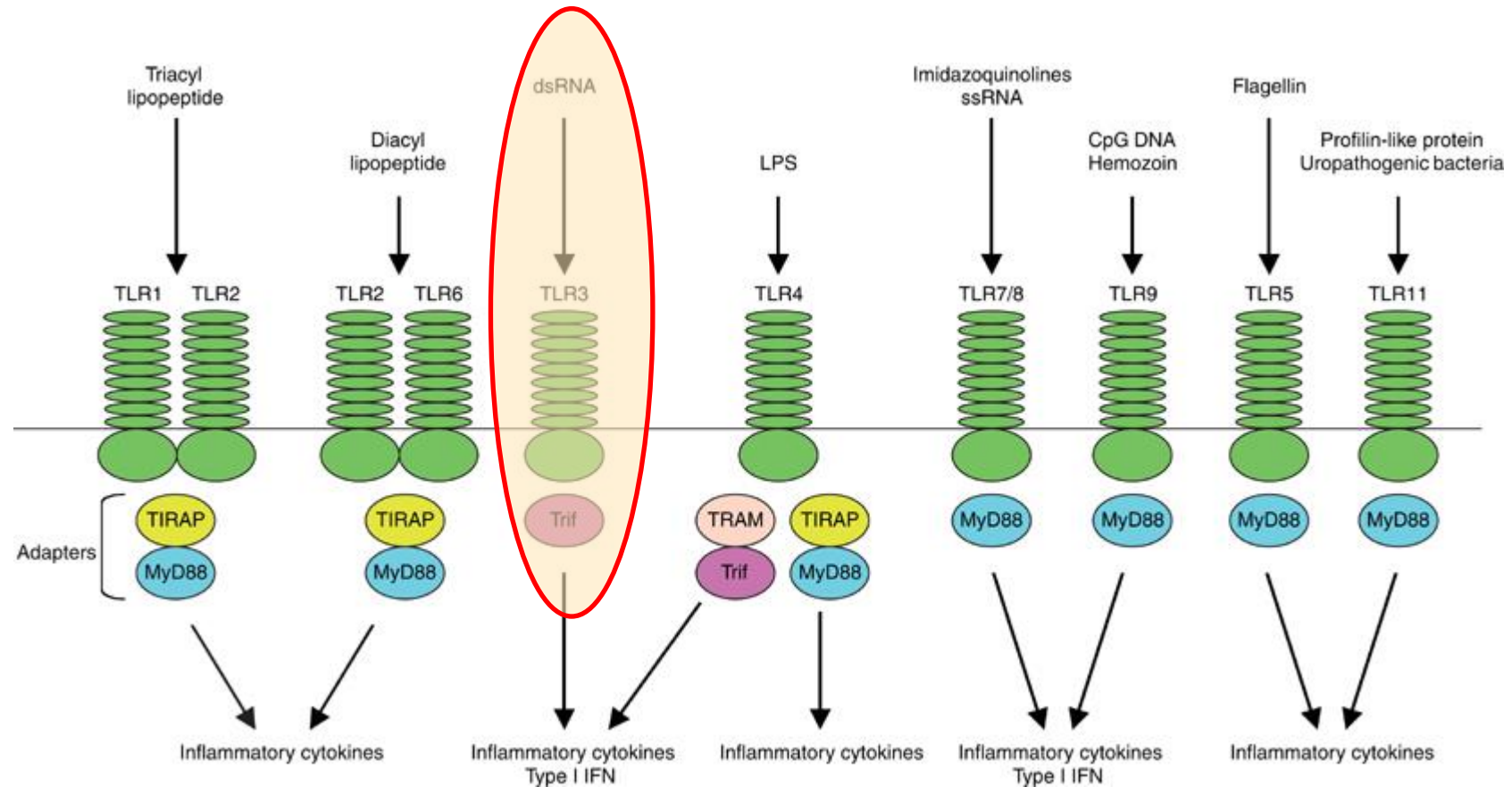
# TLRs



- ✓ Type I transmembrane proteins
- ✓ LRR : *leucine-riche repeats*
- ✓ Recognize extracellular bacterial and viral PAMPs: TLR1, 2, 4, 5, 6 et 11
- ✓ Recognize bacterial and viral PAMPs in endolysosomes: TLR3, 7, 8, 9 et 10
- ✓ Translation of cytoplasmic signal via a domain TIR : *Toll/interleukin 1 receptor*



# MyD88-dependent activation of TAK1 and IKK

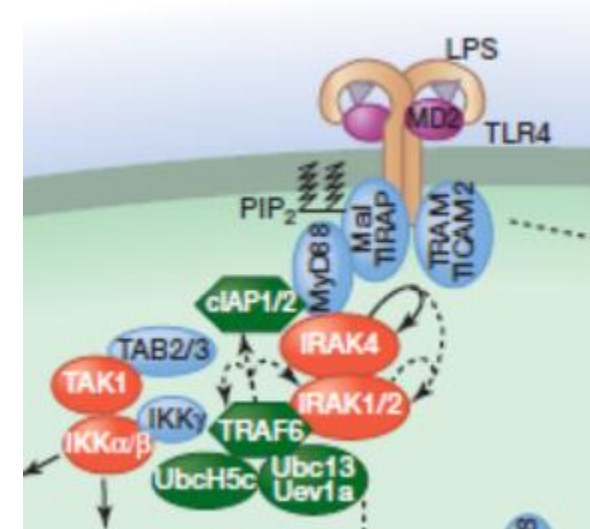


# MyD88-dependent activation of TAK1 and IKK

MyD88 : *Myeloid differentiation primary response gene 88*

## TLR4

- ✓ MyD88 : a DD domain (death d) and TIR
- ✓ Interacts with IRAK4 (serin/threonin kinase)
- ✓ IRAK4 autophosphorylation and link to DD of IRAK1 and 2
- ✓ Nucleation with ubiquitine ligases E3: TRAF6, cIAP1 and cIAP2
- ✓ ...and with ubiquitine-conjugase E2: Ubc13
- ✓ TRAF6 and Ubc13 catalyze the formation of polyubiquitin chains on TRAF6, IRAK1 and cIAP1/2
- ✓ Recruitment of TAB2 / 3 adapters complexed with TAK1 kinase
- ✓ Recrutement of IKK $\alpha$  (NEMO): regulatory subunit of the I $\kappa$ B kinase complex (IKK)

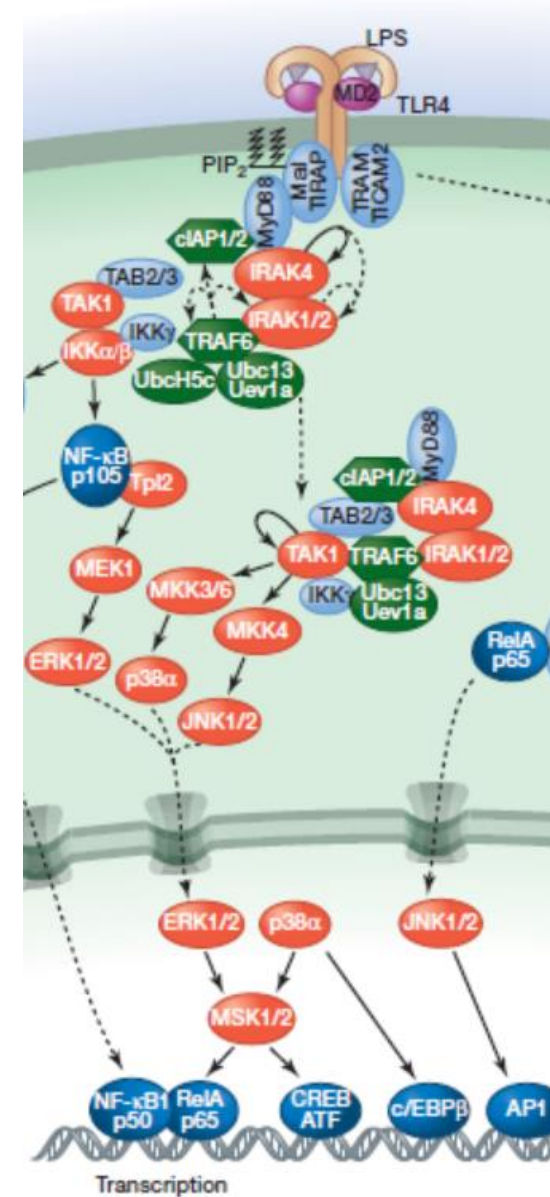


Blue: adaptor  
Red: kinase  
Green: ubiquitin enzyme

# MyD88-dependent activation of TAK1 and IKK

## ...TLR4

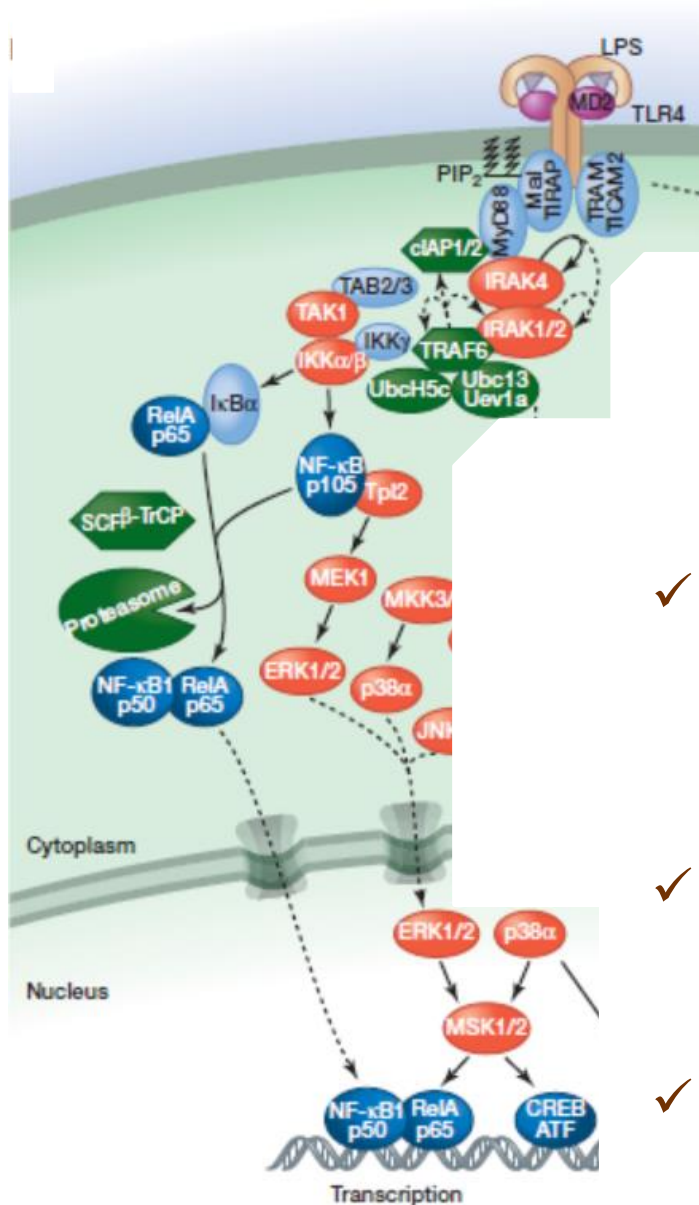
- ✓ Translocation of the MyD88-TRAF6-Ubc13-cIAP-TAK1-IKK $\gamma$  complex in the cytosol
- ✓ TAK1 kinase autophosphorylation
- ✓ Activation of JNK et p38 MAPK
- ✓ p38 activates CREB et c/EBP $\beta$  transcription factors:
  - chemokines (Cxcl1, Cxcl2), cytokines (IL10, IL12 $\beta$ , IL1a, and IL1b), ECM remodeling (Mmp13) and adhesion (Vcam1) regulators
- ✓ JNK activates the transcription factor AP1:
  - TNF and other pro-inflammatory mediators



Blue: adaptor  
Red: kinase  
Green: ubiquitin enzyme  
Blue: transcription factor

# MyD88-dependent activation of TAK1 and IKK

...on the side of NF- $\kappa$ B



✓ IKK $\beta$  (kinase) substrates:

p105 precursor of p50 NF- $\kappa$ B

I $\alpha$ B

✓ E3 ubiquitine ligase SCF<sup>b-TrCP</sup> and proteasome degradation

✓ p105 degradation: Tpl2-MEK1-ERK activation, *Ptgs2* gene transcription (by CREB/ATF) and COX2 synthesis

PG : pain, fever...

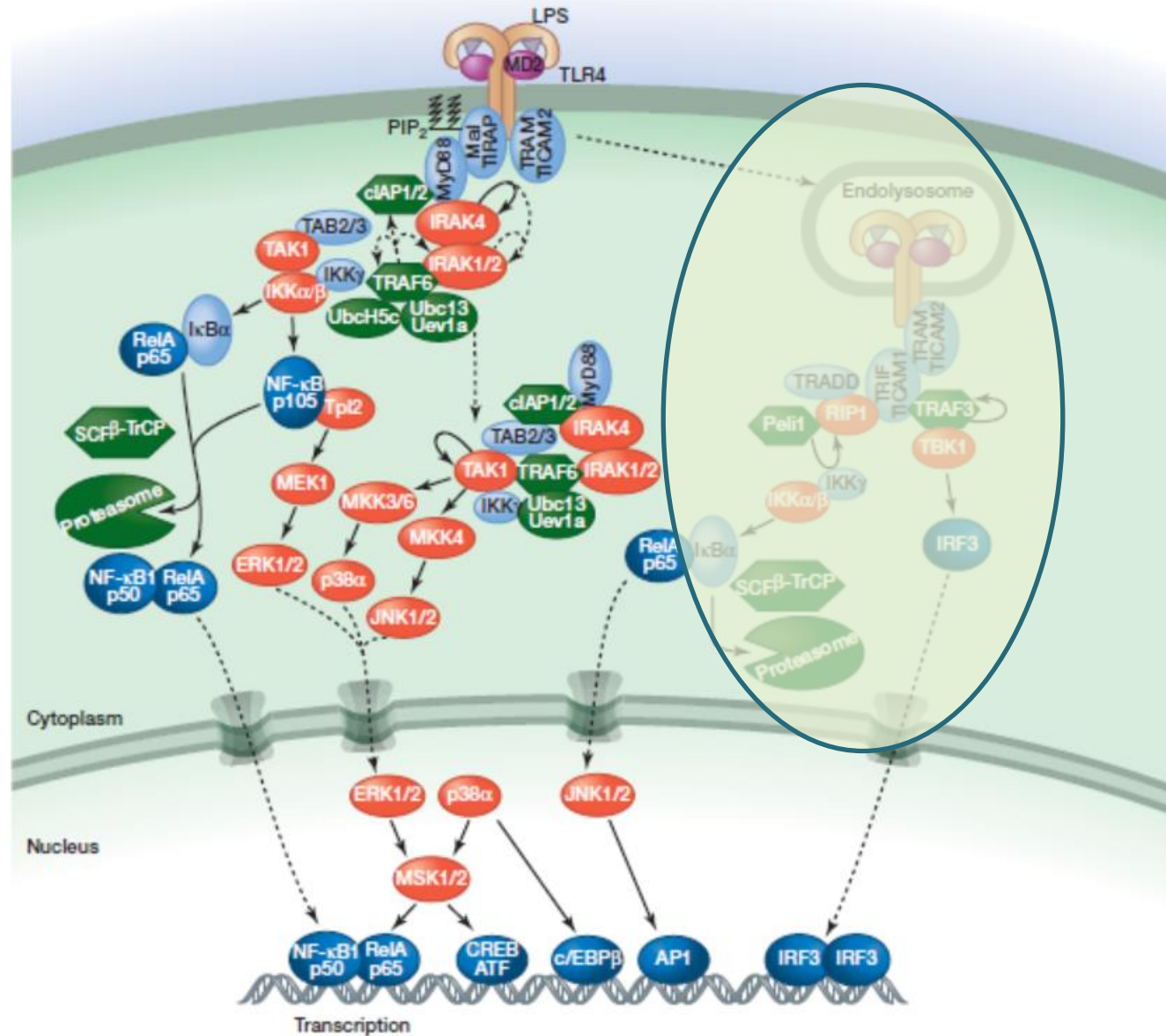
✓ I $\kappa$ B degradation: translocation to the nucleus of the NF- $\kappa$ B complex consisting of RelA (p65) and NF- $\kappa$ B1 (p50)

✓ Expression of pro-inflammatory genes ...

## Protéines inducées par NF-κB

Protein	Function
RAGE	PRR belonging to the immunoglobulin superfamily that recognizes multiple ligands including HMGB1
cIAP2	Ubiquitin ligase that regulates NF-κB activation
Caspase-11	Aspartate-specific cysteine protease implicated in inflammation
MCP1	Chemokine for monocyte recruitment
MIP1a	Chemokine for leukocyte recruitment
RANTES	Chemokine for monocyte and T-cell recruitment
CD200	Binds CD200R1 and inhibits macrophage activation
Complement factor B	Serine protease in the alternative complement activation pathway
c-FLIP	Inhibitor of death receptor-induced apoptosis
GM-CSF	Growth factor that promotes differentiation and activation of DCs, macrophages, and neutrophils
KC	Chemokine for neutrophil recruitment
MIP2	Chemokine for neutrophil recruitment
Tissue factor	Coagulation factor
ICAM1	Cell adhesion molecule that interacts with β2 integrins
IFNβ	Suppressor of virus replication
IL1β	Cytokine that amplifies the inflammatory response
IL6	Pleiotropic cytokine that stimulates fever, production of hepatocyte acute phase proteins, and lymphocyte differentiation
IL12 p40	Component of heterodimeric IL12 and IL23, which modulate NK cell and lymphocyte effector functions
MMP9	Metalloproteinase that degrades extracellular matrix
IκBa	Inhibitor of NF-κB signaling
IκBβ	NF-κB transcriptional coactivator
iNOS	Enzyme that makes anti-microbial nitric oxide
E-selectin	Cell adhesion molecule
P-selectin	Cell adhesion molecule
MnSOD	Enzyme that converts superoxide to hydrogen peroxide
TNF	Cytokine that amplifies the inflammatory response
A20	Inhibitor of NF-κB signaling by TLRs and TNF-R1
VCAM1	Cell adhesion molecule that interacts with β1 integrin

# MyD88-dependent activation of TAK1 and IKK



# Functions of genes over-expressed by TLR4

## Genes

*Ccl2, Ccl3, Ccl4, Ccl5, Cxcl1, Cxcl2, Cxcl5, Cxcl10, Ccr12*

*Icam1, Vcam1*

*Bcl2a1, Cflar*

*Mmp13*

*Edn1*

*Hdc, Nos2, Ptges, Ptgs2*

*Il1a, Il1b, Il6, Il18, Tnf*

*Ifnb*

*Birc2, Birc3, Casp4, Mefv, Nfkbiz*

*Bcl3, Dusp1, Nfkbia, Socs3, Tnfaip3, Zc3h12a*

*Fpr1, Nlrp3*

*Ch25h, Icosl, Il10, Il12a, Il12b, Il15, Tnfsf9*

## Output

Leukocyte recruitment

Cell adhesion

Cell survival

Remodeling of extracellular matrix

Vascular effects

Synthesis of inflammatory mediators

Inflammatory cytokines

Antiviral response

Intracellular signaling (positive)

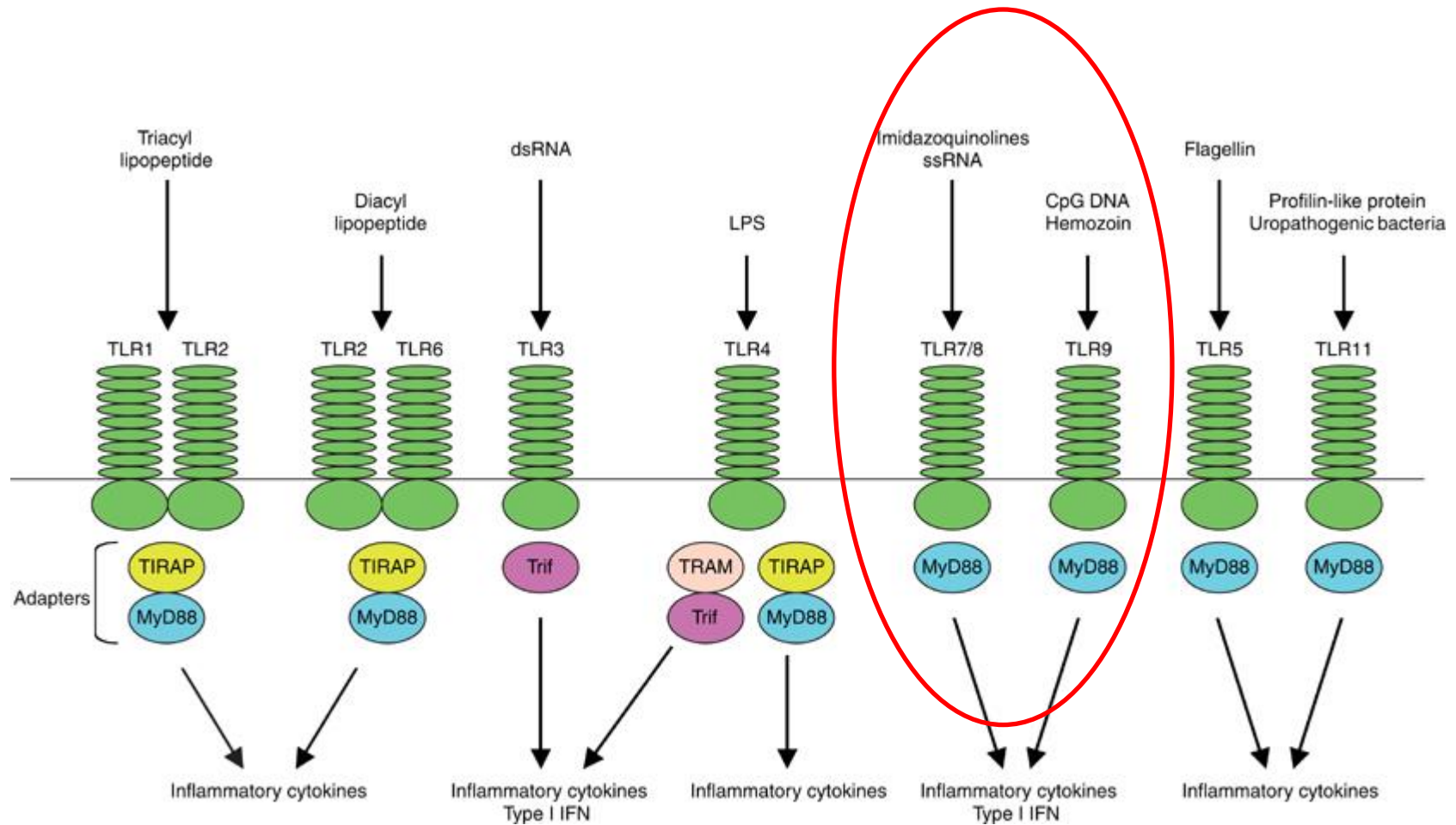
Intracellular signaling (negative)

PRRs

Regulators of adaptive immune response

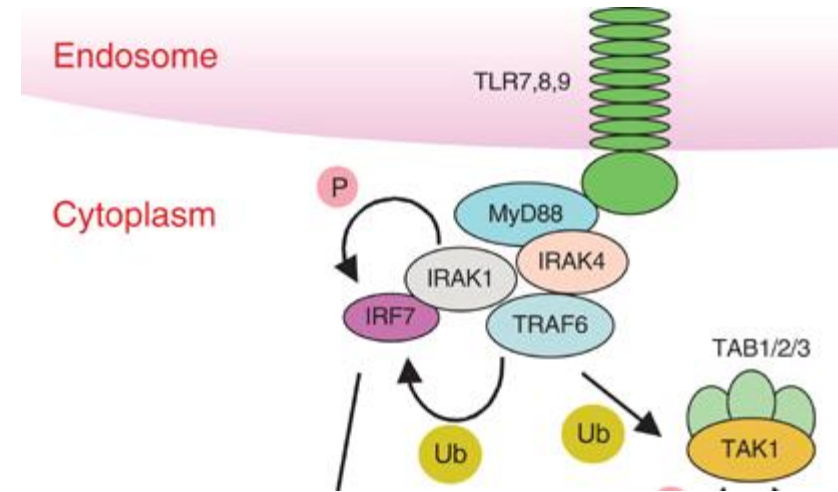


# TLR7 and 9: MyD88-dependent induction of type I IFN

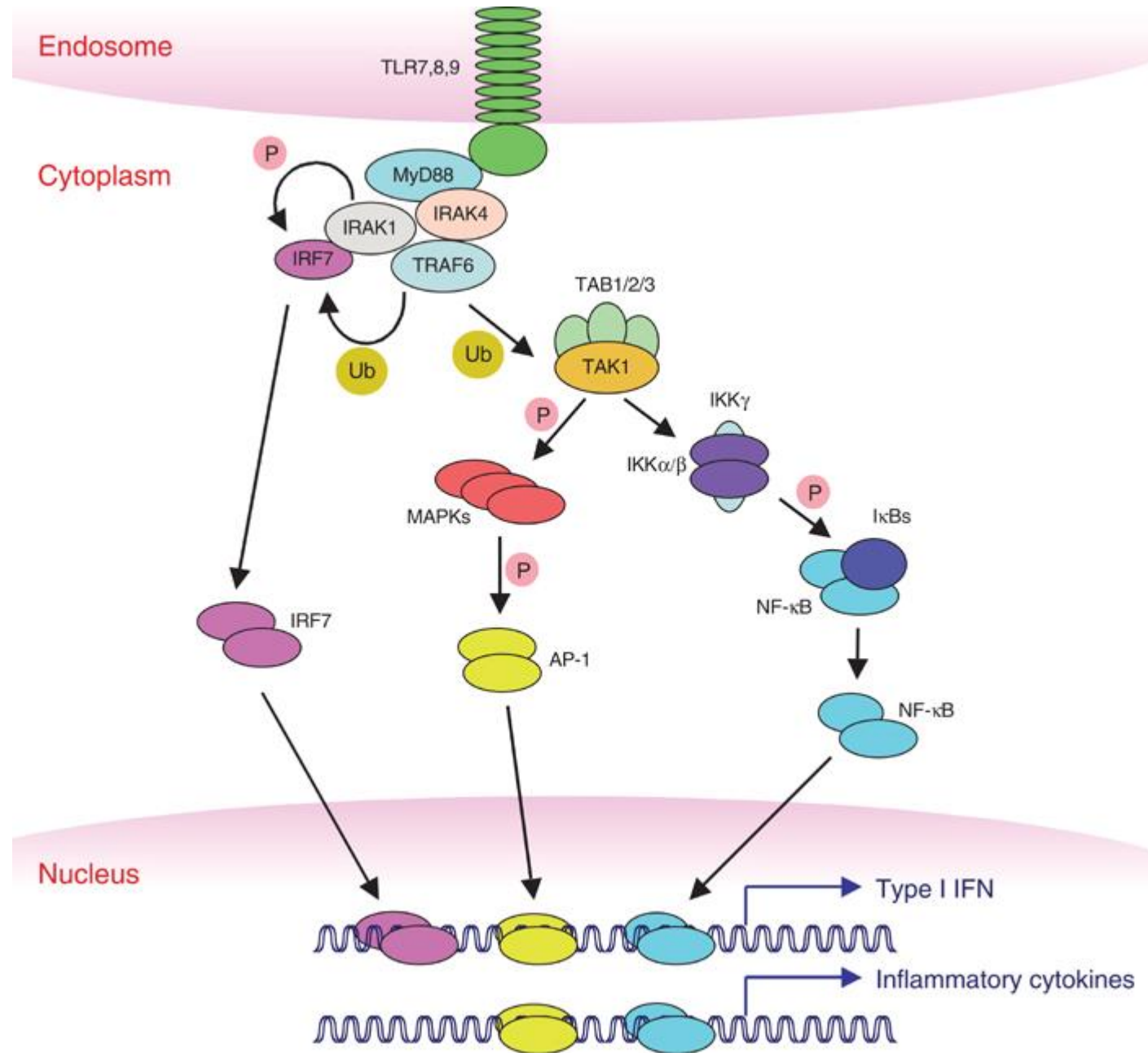


# TLR7 and 9: MyD88-dependent induction of type I IFN

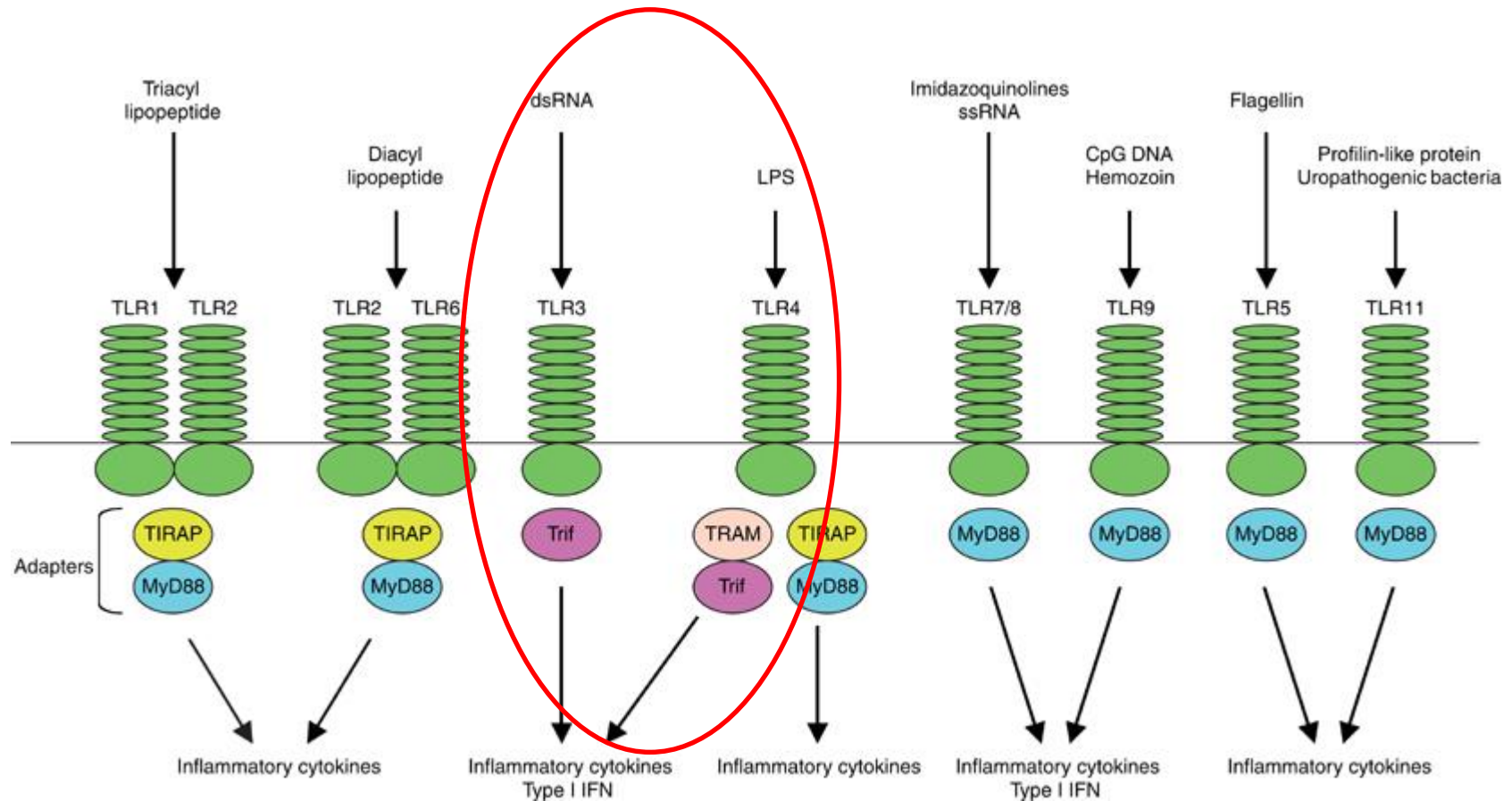
- ✓ From the endosome of plasmacytoid dendritic cells
- ✓ Anti-viral response: IFN $\alpha$  and IFN $\beta$



- ✓ Commitment of the MyD88-IRAK4-IRAK1-TRAF6 complex
- ✓ Dimerization of IRF7 and translocation in the nucleus



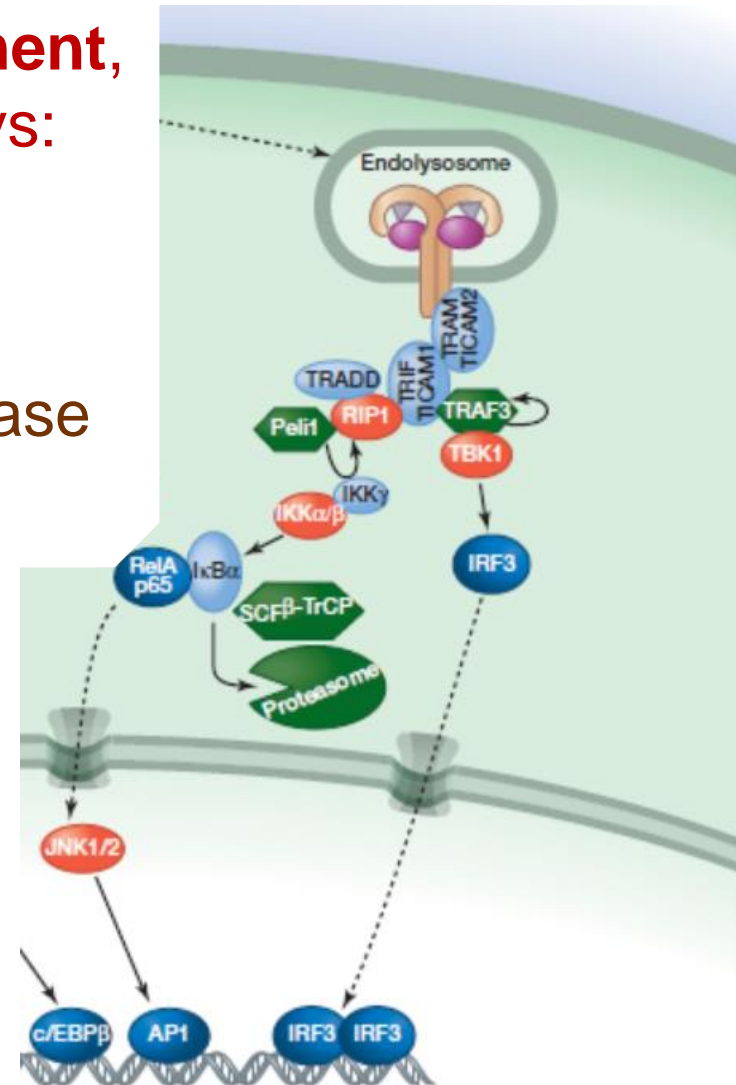
# TLR3 and 4: TRIF (TICAM1) dependent signaling



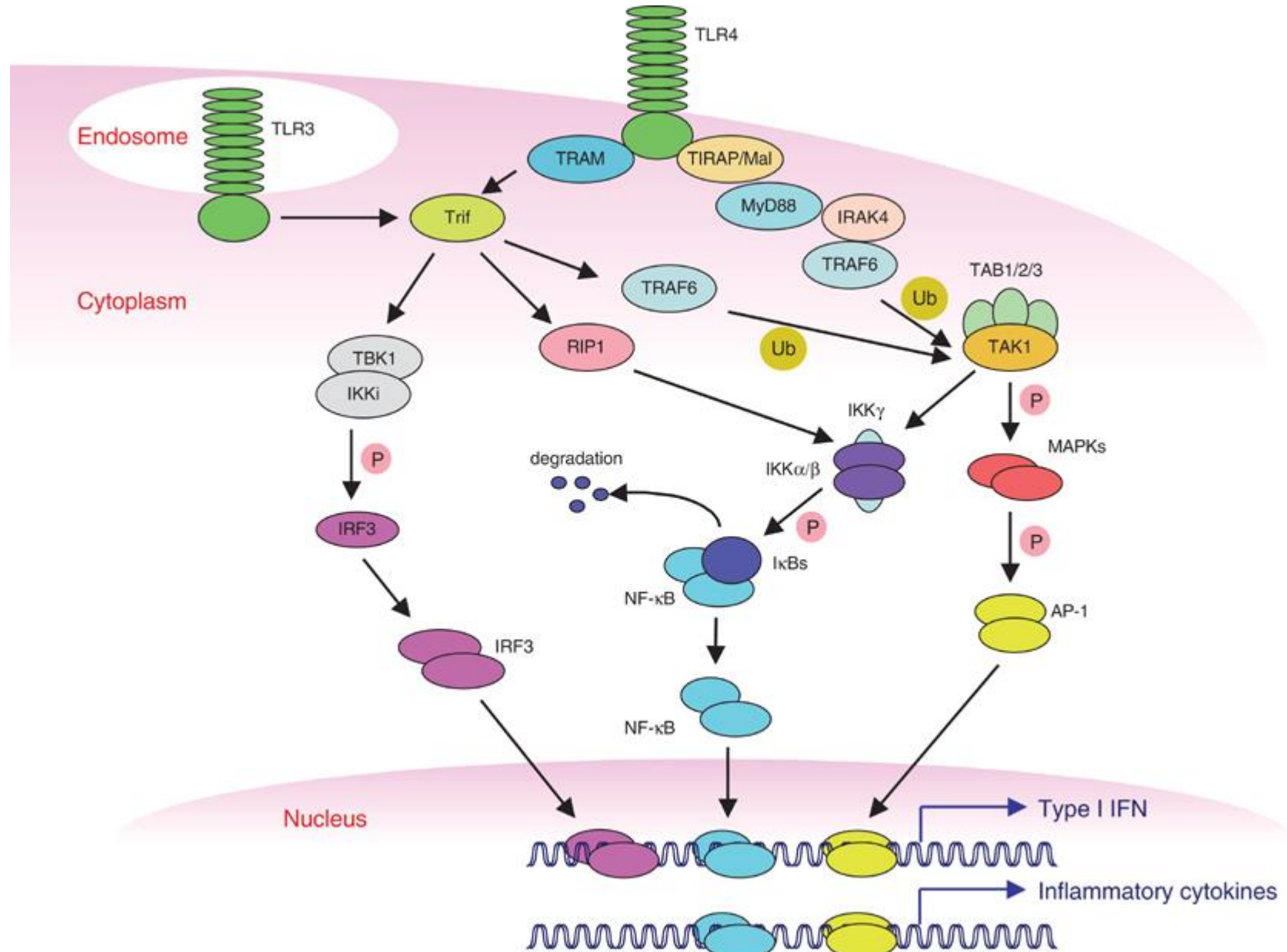
# TLR3 and 4: TRIF (TICAM1) dependent signaling

From the **endosomal compartment**,  
(anti-viral dsRNA) two pathways:

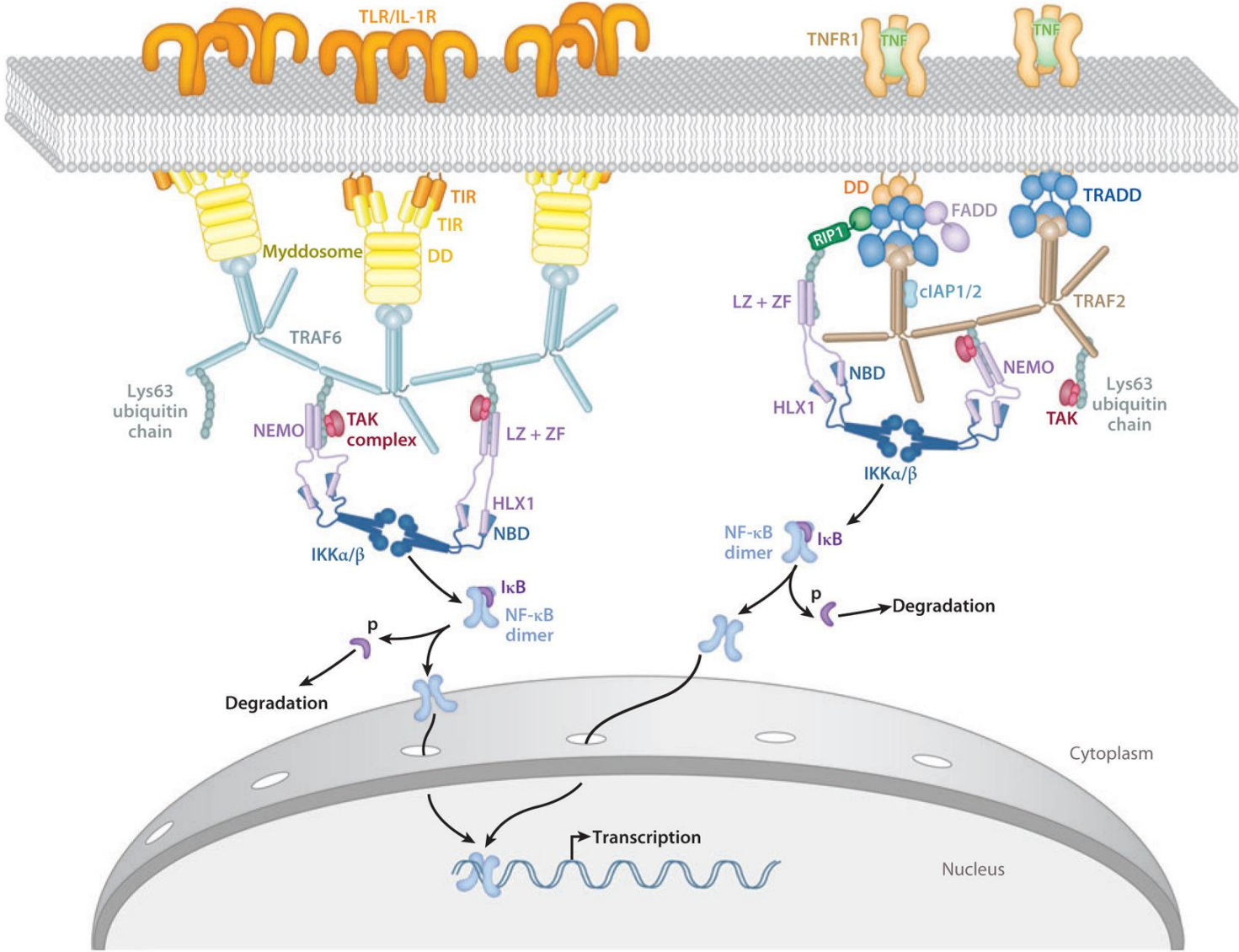
- ✓ Via TRIF (TICAM1) and TRAM (TICAM2) adapters
- ✓ Interaction of TRIF with RIP1 kinase and E3 ubiquitin ligase Peli I: activation of NF- $\kappa$ B
- ✓ Interaction of TRIF with TBK1 kinase and TRAF3 ubiquitin ligase: activation of the IRF3 transcription factor



# TLR3 and 4: TRIF (TICAM1) dependent signaling



# Simplified view of TLR / IL-1R TNFR signaling pathways



# The NLR family and host response

- **NODs: nucleotide oligomerization domain receptors**
- **NLRP / NALPs: NACHT, LRR and pyrin domains containing proteases**
- **NLRC4 / IPAF: NLR family CARD domain-containing protein 4 / IL-1 $\beta$ -converting enzyme (ICE)-protease activating factor**
- **NAIPs: Neuronal apoptosis inhibitor factors**
- **CIITA: MHC class II transactivator**



# The NLR family and host response

According to the phylogenetic relationships, NLRs can be divided into 3 subfamilies:

- **NODs:** NOD1, NOD2, NOD3 (NLRC3), NOD4 (NLRC5), NOD5 (NLRX1), CIITA
- **NLRPs (NALPs):** NLRP1, NLRP2, NLRP3, NLRP4, NLRP5, NLRP6, NLRP7, NLRP8, NLRP9, NLRP10, NLRP11, NLRP12, NLRP13, NLRP14
- **IAPF:** IAPF (NLRC4), NAIP

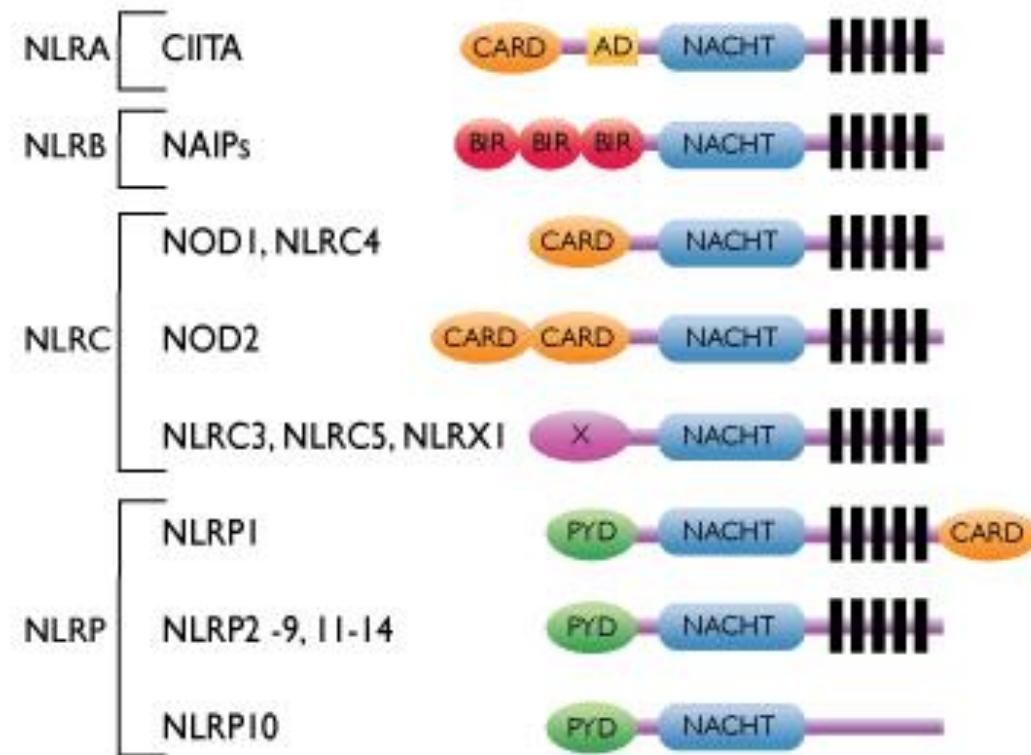
# The NLR family and host response

**23 NLRs encoded in the human genome (34 in mouse).**

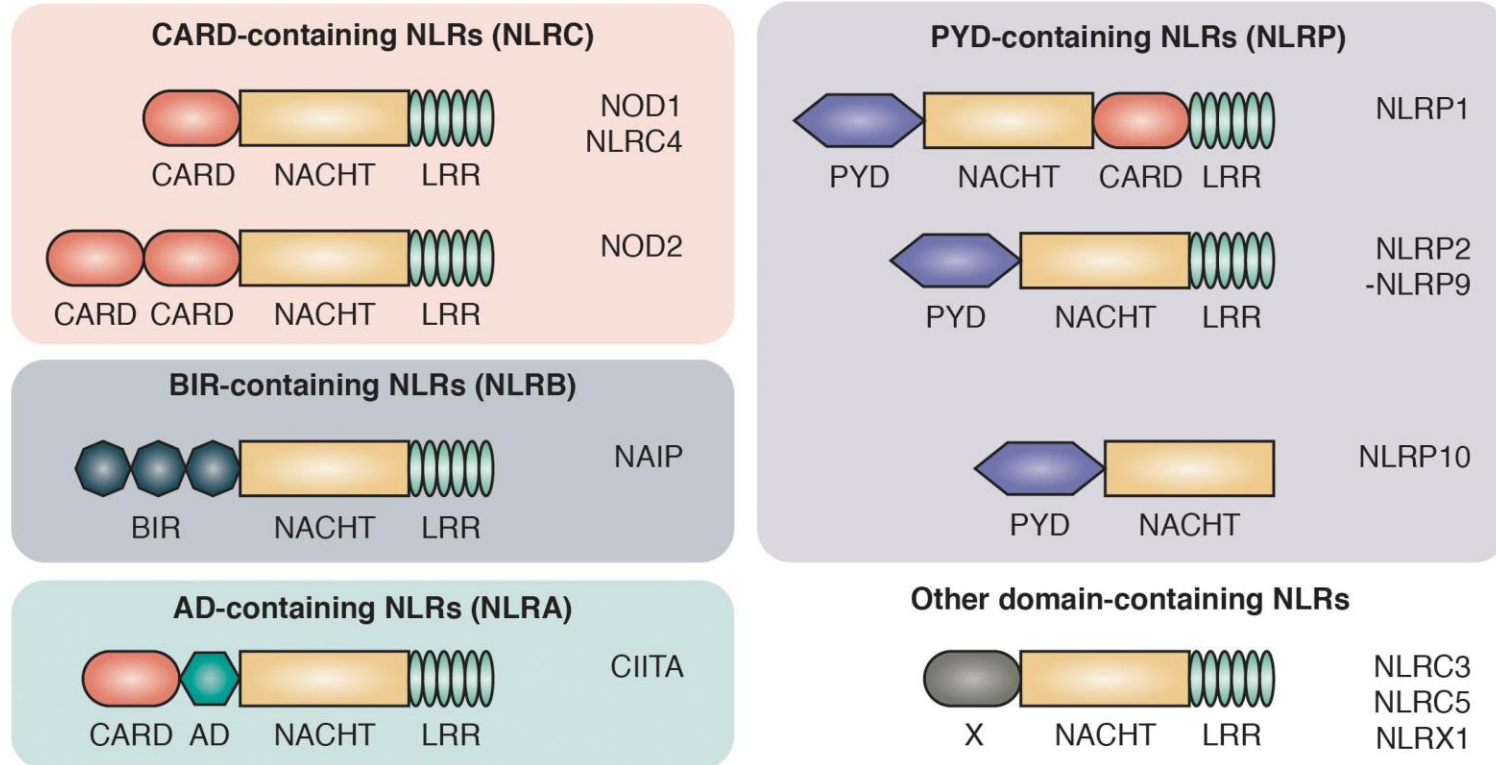
**NLRs are structured by three distinct domains:**

1. The ligand-sensing LRRs (leucine-rich repeat) domain
2. The NACHT or nucleotide binding domain (NBD), which is responsible for the capacity of NLRs to oligomerize
3. The effector pyrin domain (**PYD**), or The caspase recruitment domain family (**CARD**), or The baculovirus inhibitor of apoptosis repeat (**BIR**) domains

## NLR Subfamilies

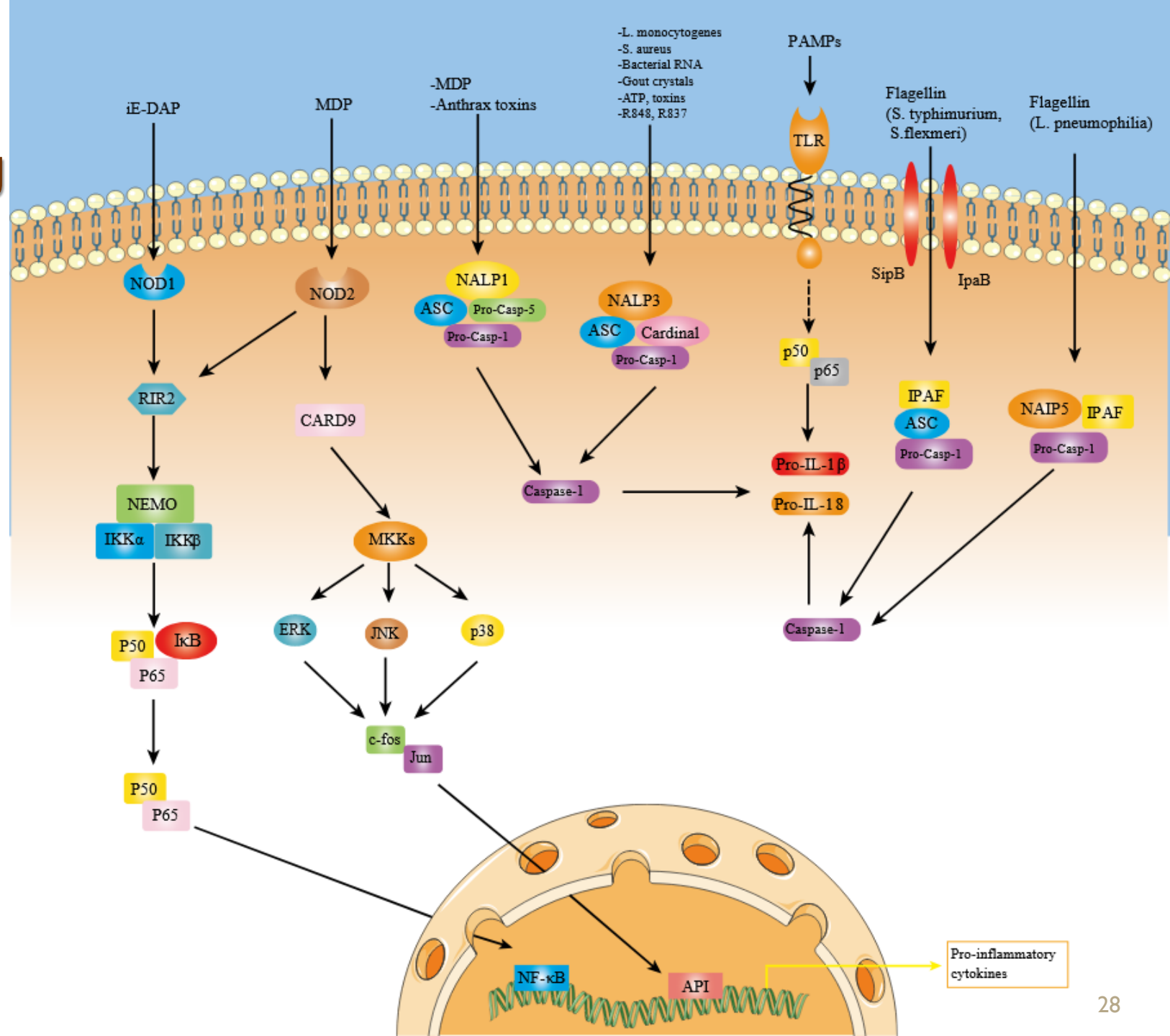


# The NLR family and host response

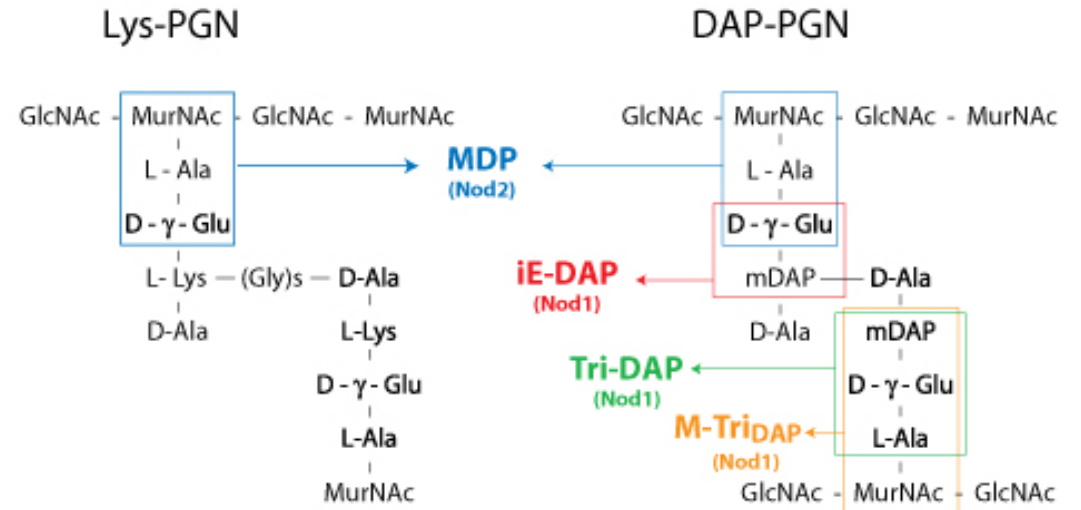
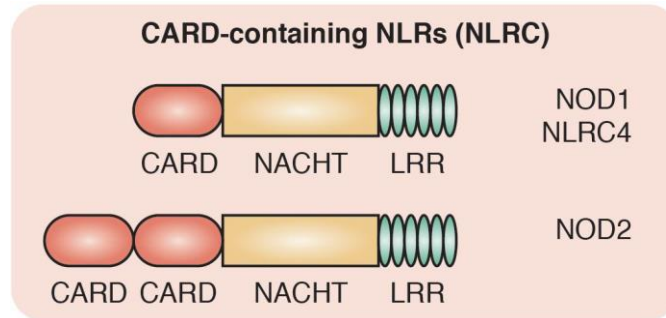


# All NLRs signaling pathways

- Transcription factor's signaling pathways
- Inflammasome



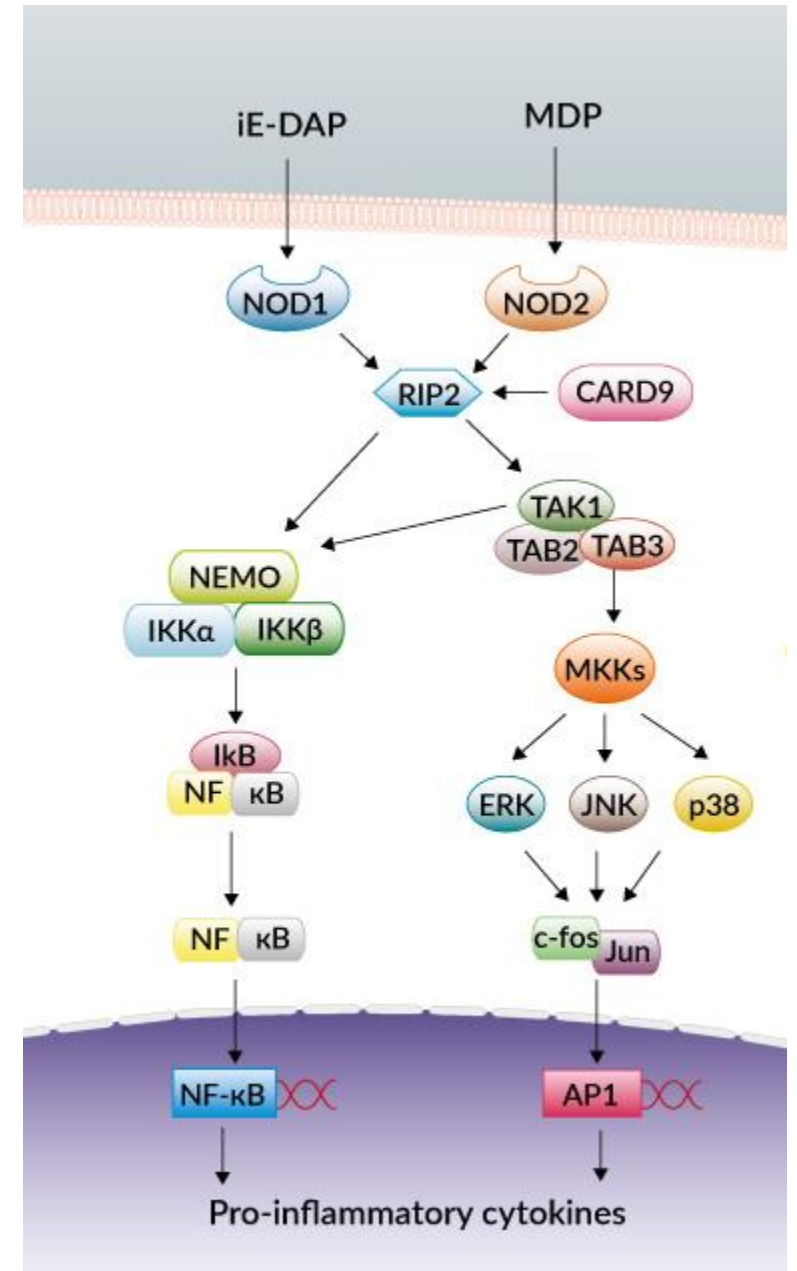
# The NOD nucleotide-binding oligomerization domain proteins



- Sense conserved fragments from the cell wall of many types of bacteria
- **NOD1** senses the D-γ-glutamyl-meso-DAP dipeptide (iE-DAP), found in PGN of all Gram-negative and certain Gram-positive bacteria
- **NOD2** recognizes the muramyl dipeptide (MDP) found in almost all bacteria
- In the **host cytosol** (microbial surveillance of pathogen invasion)
- Activate intracellular signaling pathways that drive proinflammatory and antimicrobial responses

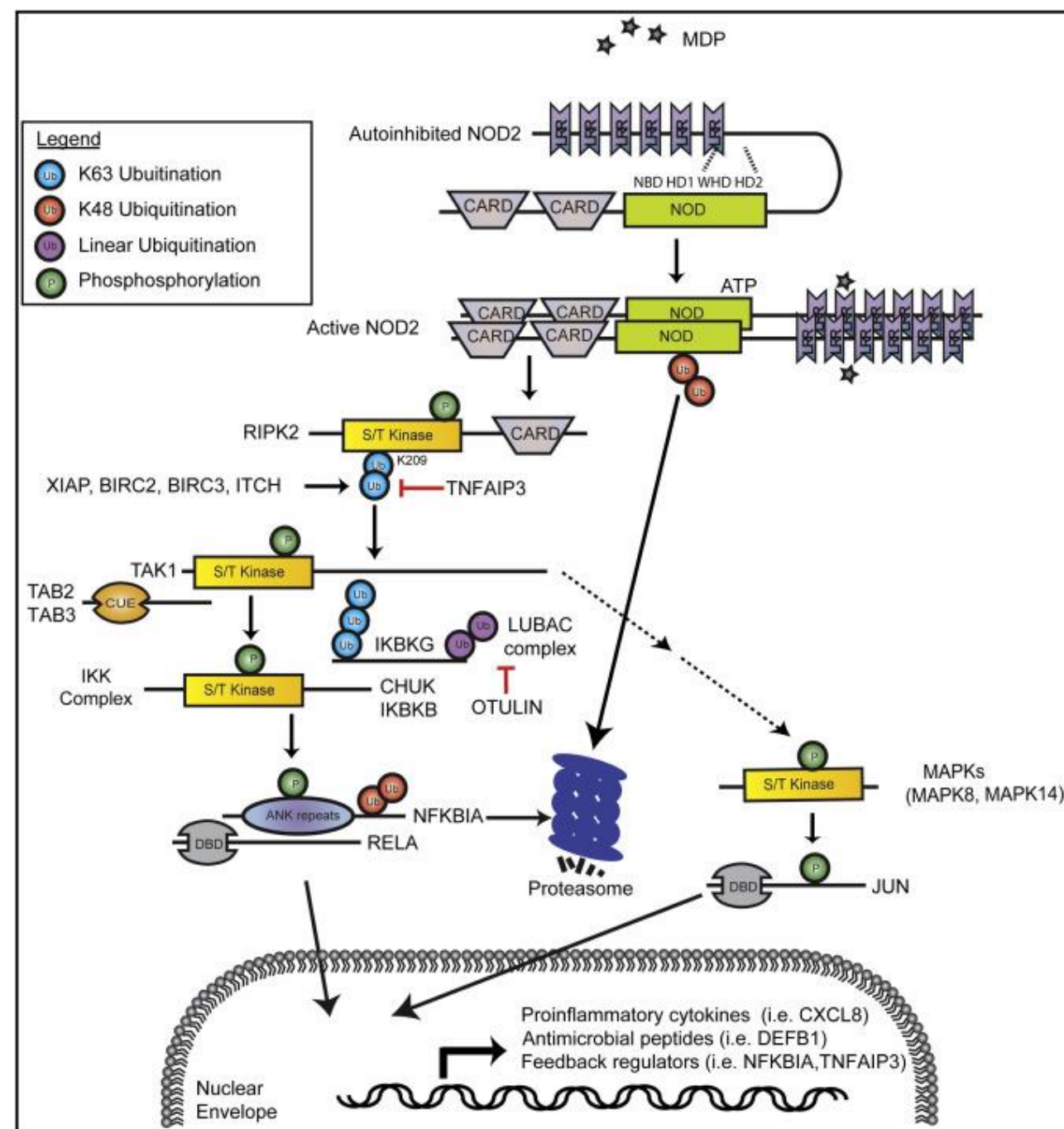
# The NOD (nucleotide-binding oligomerization domain) proteins

- Ligand bound NOD1 and NOD2 oligomerize and signal via the serine/threonine **RIP2** (RICK,CARDIAK) kinase through CARD-CARD homophilic interactions. Activated RIP2 mediates ubiquitination of **NEMO/IKK $\gamma$**  leading to the activation of **NF- $\kappa$ B** and the production of inflammatory cytokines.
- Furthermore, poly-ubiquitinated RIP2 recruits **TAK1**, which leads to **IKK** complex activation and the activation of **MAPKs**
- Signaling by NOD2 has been shown to involve the adapter protein CARD9, to mediate p38 and JNK signaling through RIP2 .
- Genetic mutations in NOD2 are associated with Crohn's disease, a chronic inflammatory bowel disease

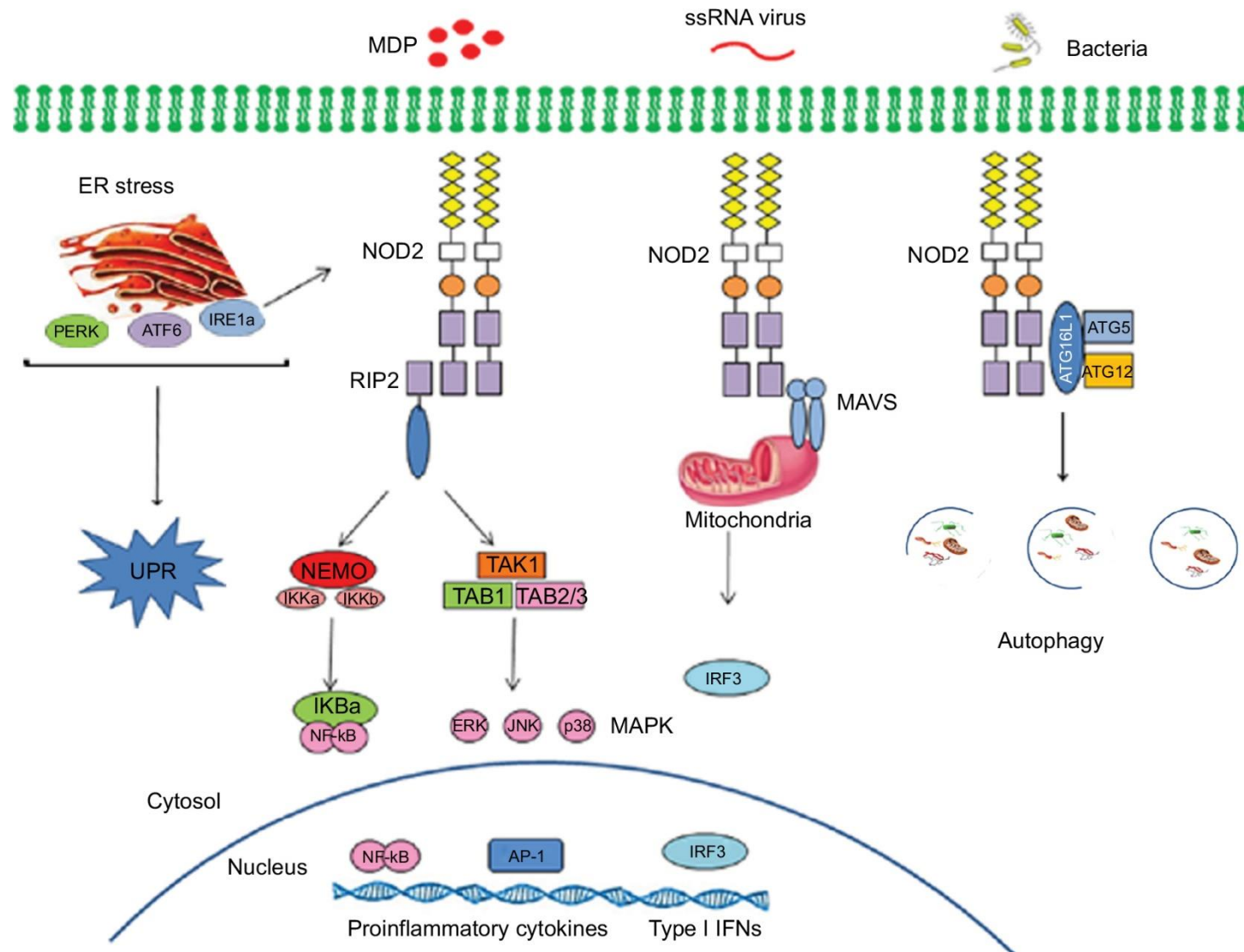


# The NOD (nucleotide-binding oligomerization domain) proteins

- Ligand recognition relieves intramolecular autoinhibitory interactions, leading to NOD oligomerization.
- Recruitment of the downstream serine/threonine kinase (S/T kinase) **RIPK2** occurs through CARD-CARD interactions.
- Positive or negative regulation by posttranslational modifications (phosphorylation and pUb events).
- Multiple regulatory effectors: LUBAC (linear ubiquitin chain assembly complex), OTULIN (deubiquitinase)

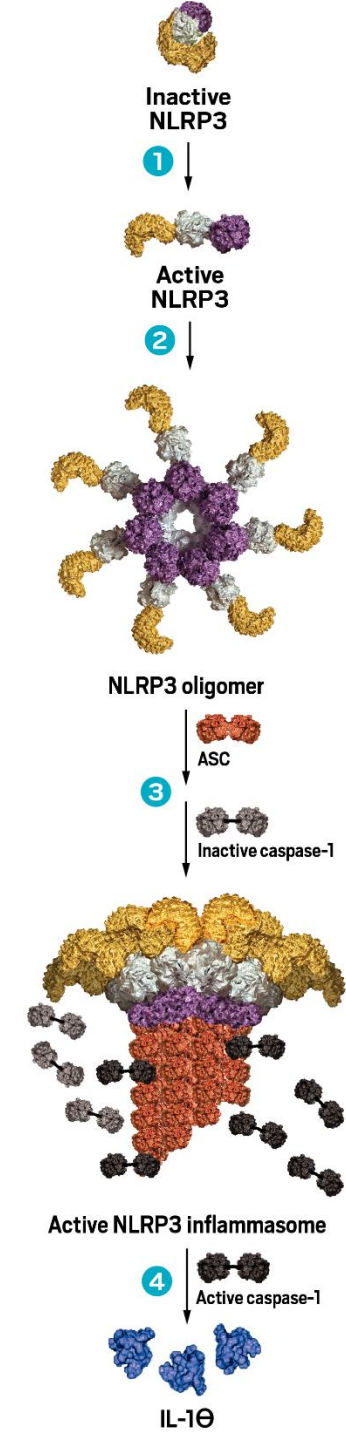
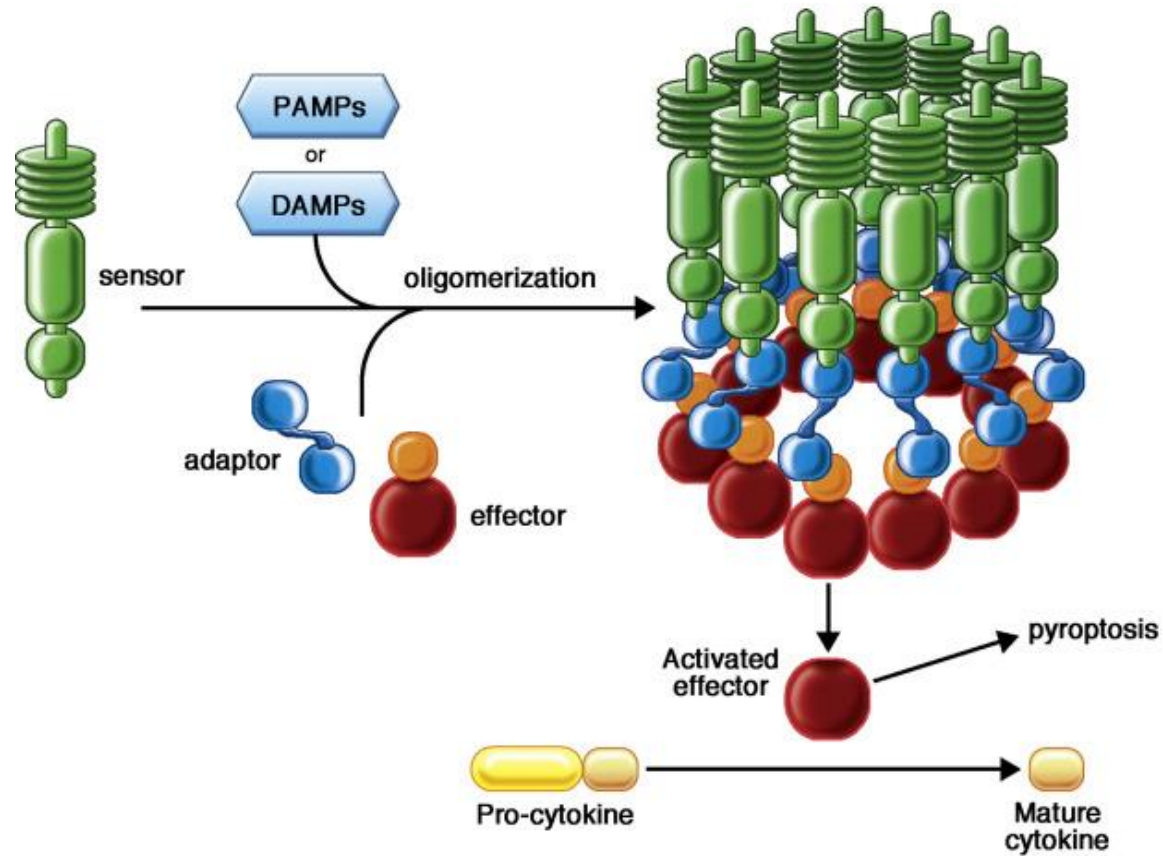


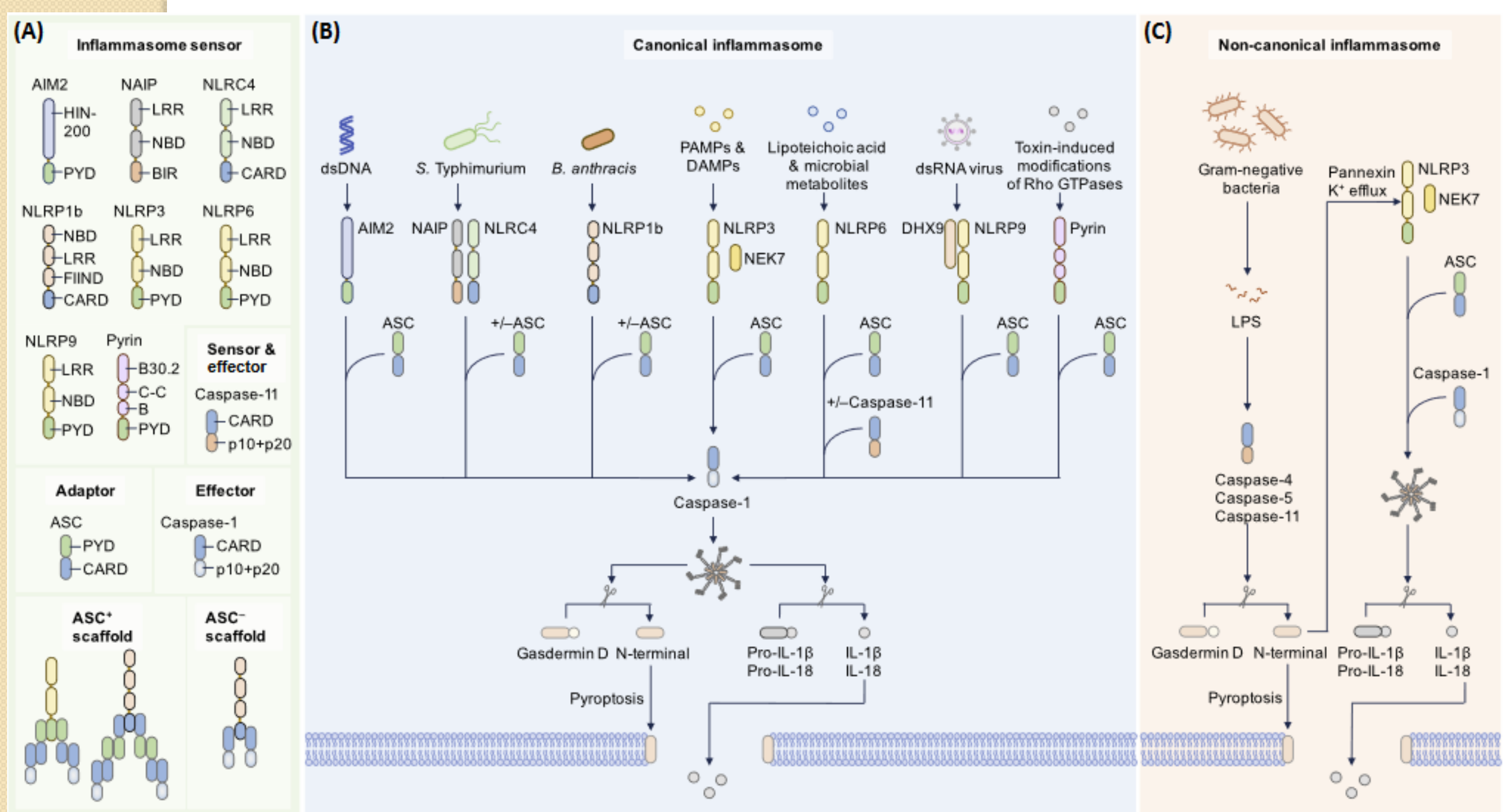
# The NOD (nucleotide-binding oligomerization domain) proteins





# The NLR family and the inflammasome





# Inflammasome :

## In professional innate immune cells

## In epithelial cells

**NLR**, nucleotide-binding domain and leucine-rich repeat receptors

**AIM2r**, absent in melanoma 2

**NAIP**, NOD-like receptor family apoptosis inhibitory protein

**NLRC4 (IPAF)**, NLR family CARD domain-containing protein 4

**NLRP / NALP**, NACHT, LRR, FIIND, CARD domain and PYD domains-containing protein

**NACHT**, nucleoside-triphosphatase (NTPase) domain found in apoptosis proteins

**CARD**, caspase-activation and recruitment domain

**NEK7**, NIMA-related kinase 7

**PYD**, pyrin domain

**LRR**, leucine-rich-repeat domain

**NBD**, nucleotide-binding domain

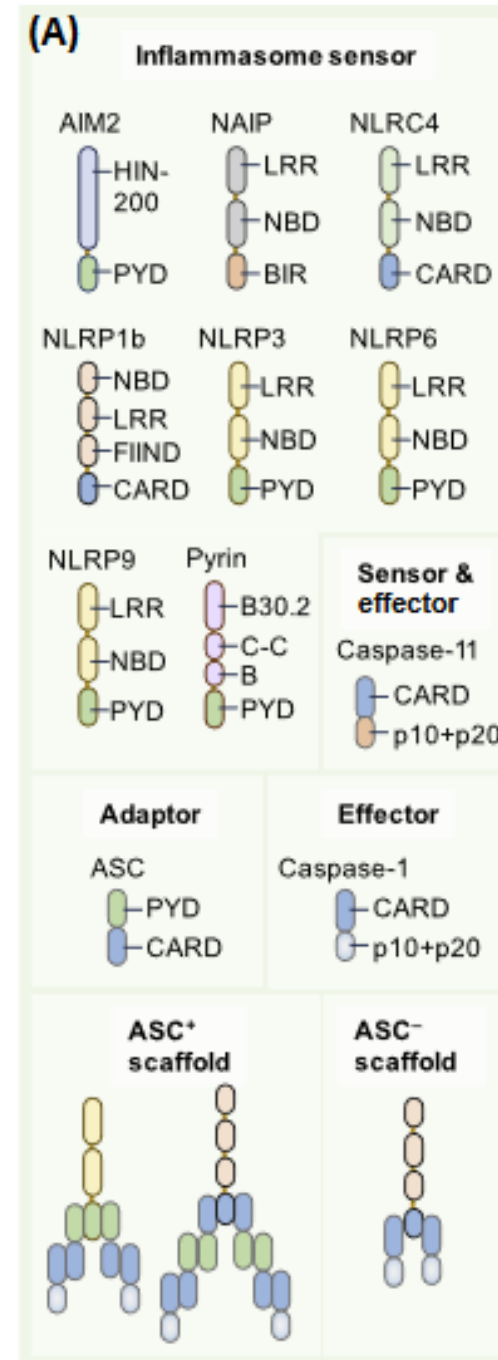
**CC**, coiled-coil domain

**FIIND**, function-to-find domain

**BIR**, baculovirus inhibitor of apoptosis repeat

**ASC**, apoptosis-associated speck-like protein containing a CARD

**DHX9**, DEAH-box helicase 9



**AIM2r**, absent in melanoma 2

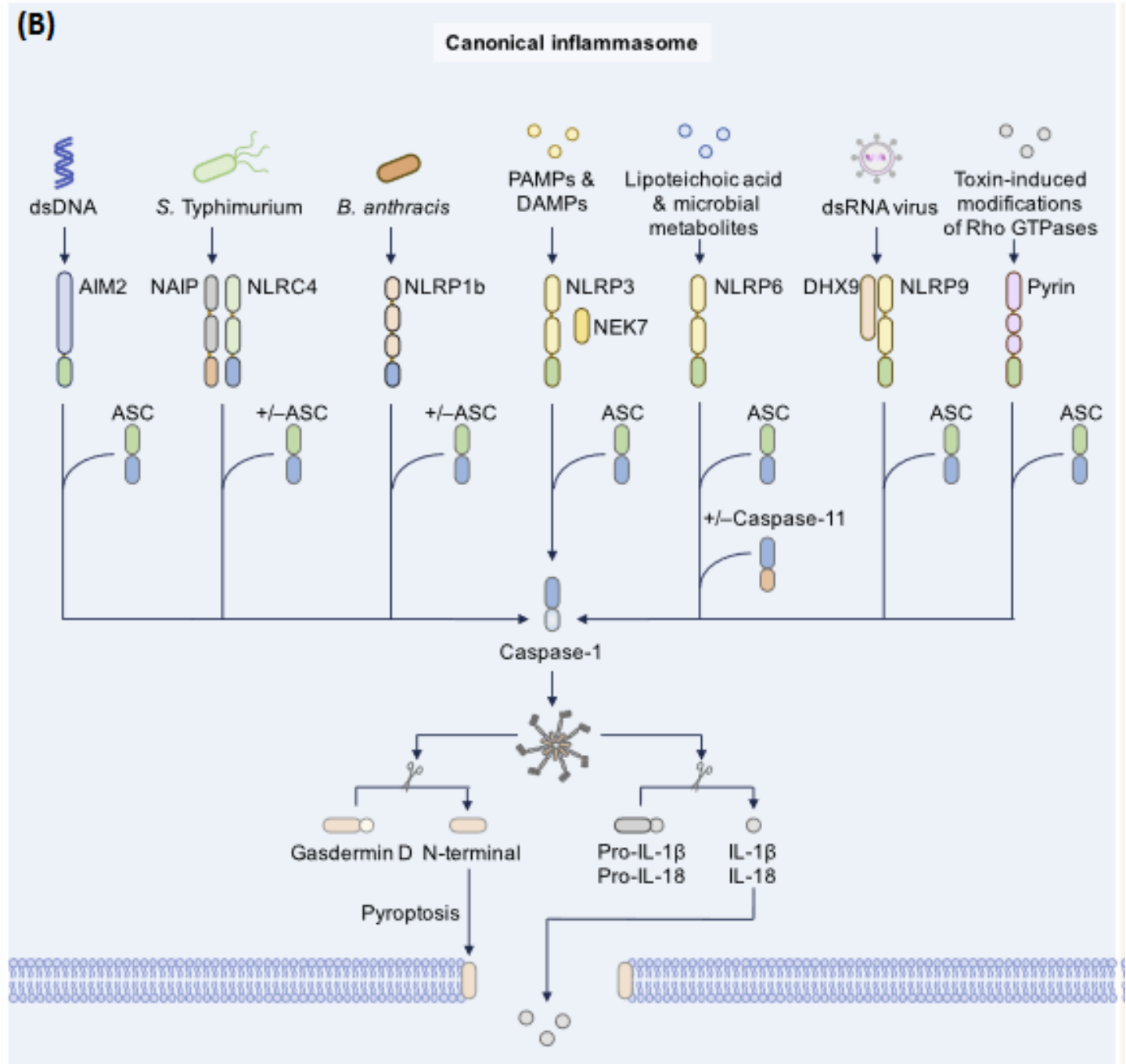
**NAIP**, NOD-like receptor family apoptosis inhibitory protein

**NLRC4**, NLR family CARD domain-containing protein 4

**NLRP**, NACHT, LRR, FIIND, CARD domain and PYD domains-containing protein

**NACHT**, nucleoside-triphosphatase (NTPase) domain found in apoptosis proteins

**ASC**, apoptosis-associated speck-like protein containing a CARD



# NLRP1b Inflammasome

**NLRP**, NACHT, LRR, FIIND, CARD domain and PYD domains-containing protein

**NACHT**, nucleoside-triphosphatase (NTPase) domain found in apoptosis proteins

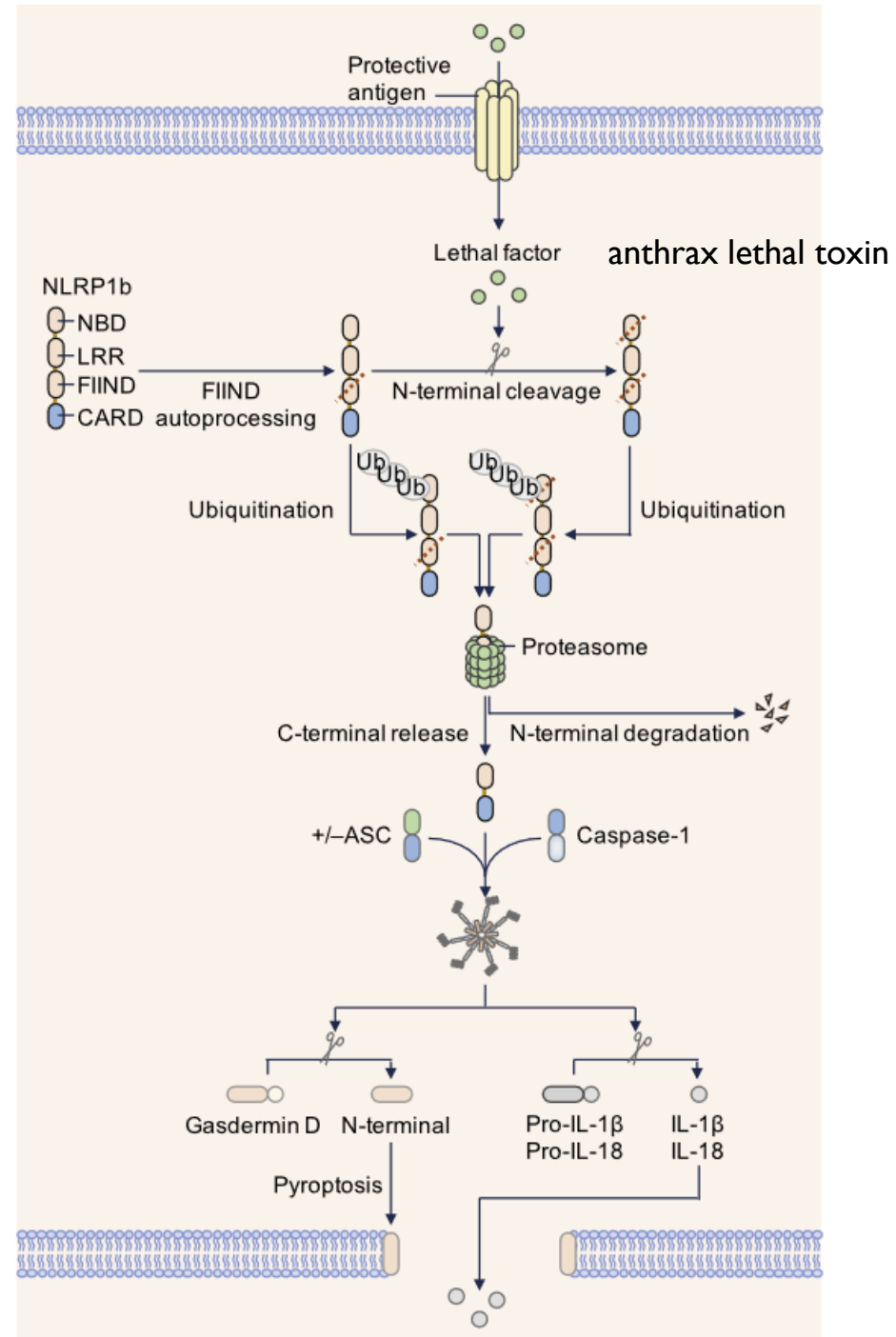
**NBD**, nucleotide-binding domain

**LRR**, leucine-rich-repeat domain

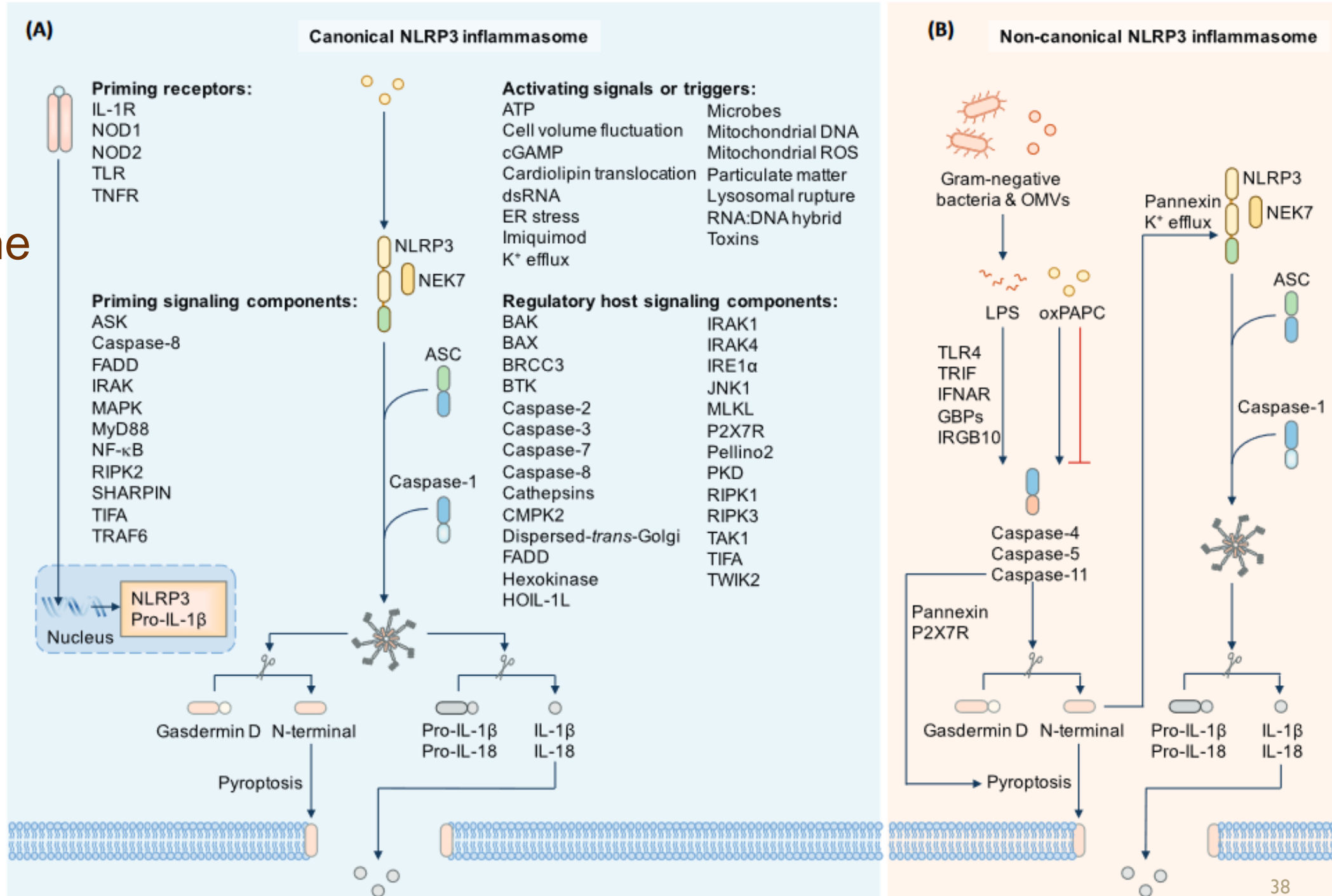
**FIIND**, function-to-find domain

**CARD**, caspase-activation and recruitment domain

**ASC**, apoptosis-associated speck-like protein containing a CARD

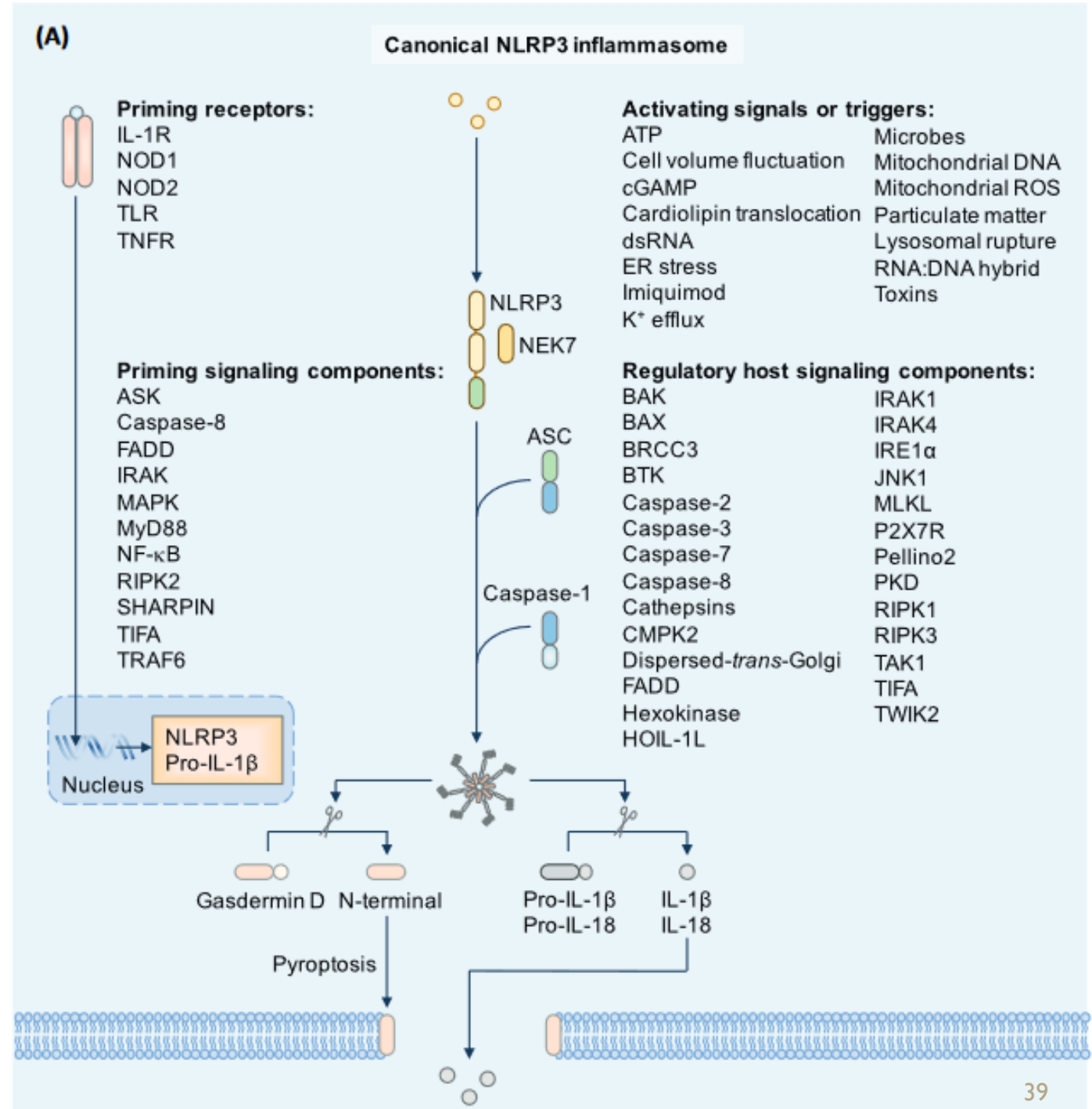


# NLRP3 Inflammasome



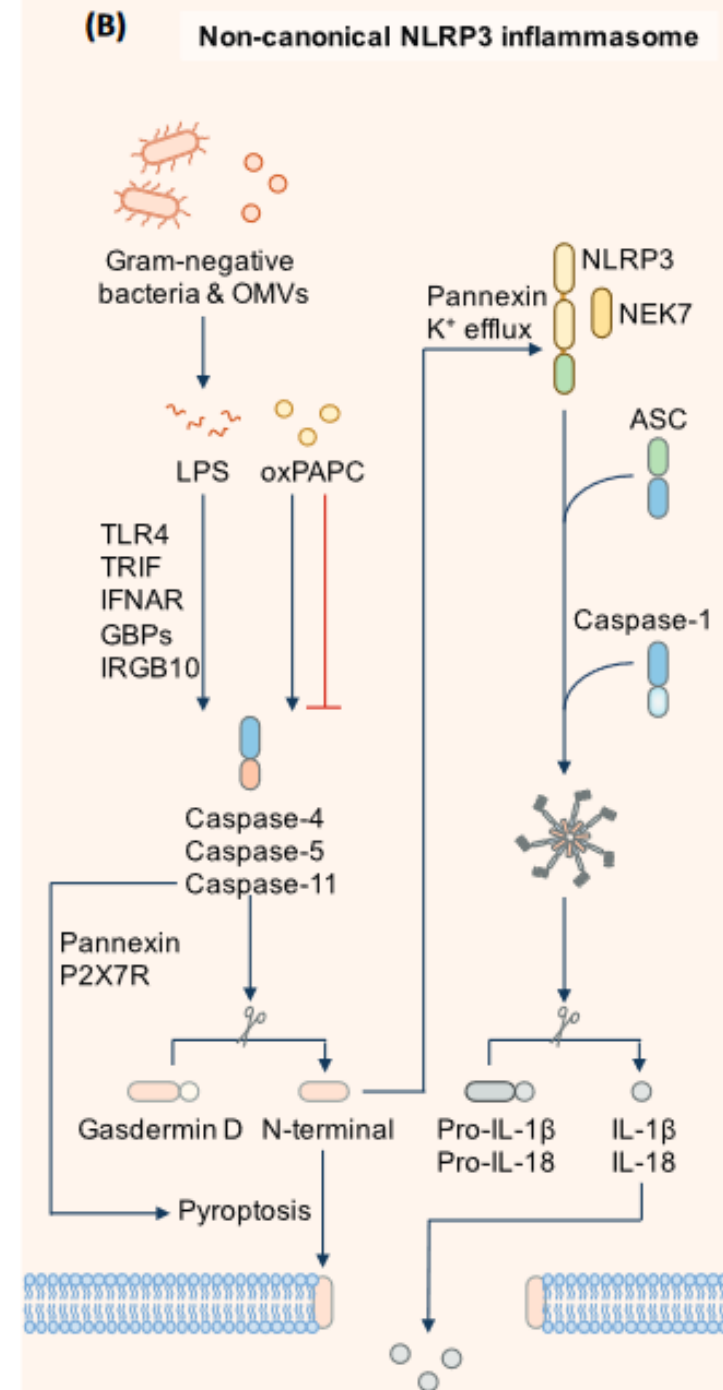
# Canonical NLRP3 Inflammasome

NIMA-related kinase 7 (NEK7)



# Non-cannonical NLRP3 Inflammasome

**oxPAPC**, oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphorylcholine





# NAIP–NLRC4 inflammasome

**NAIP**, NOD-like receptor family apoptosis inhibitory protein

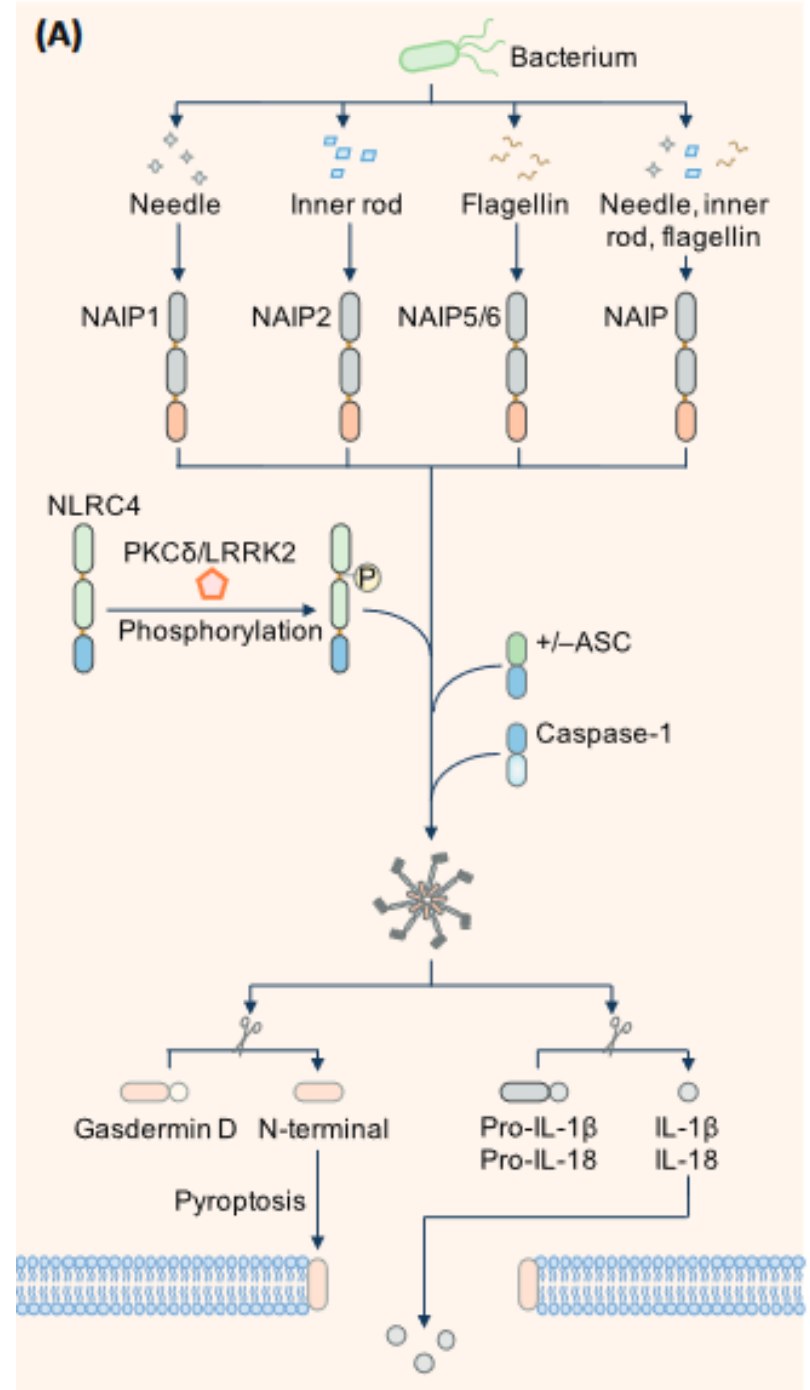
**NLRC4 (IPAF)**, NLR family CARD domain-containing protein 4

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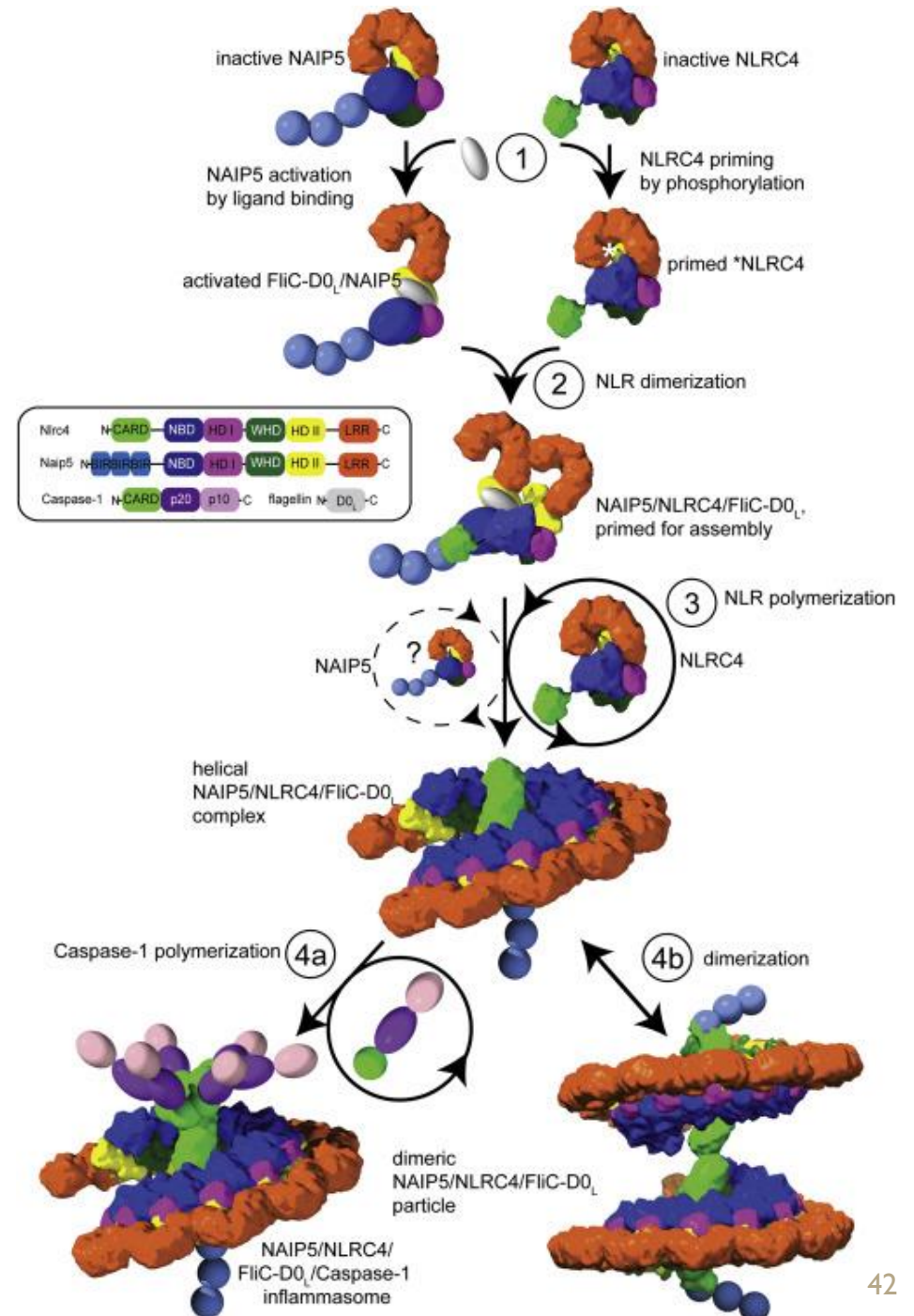


# NAIP–NLRC4 inflammasome

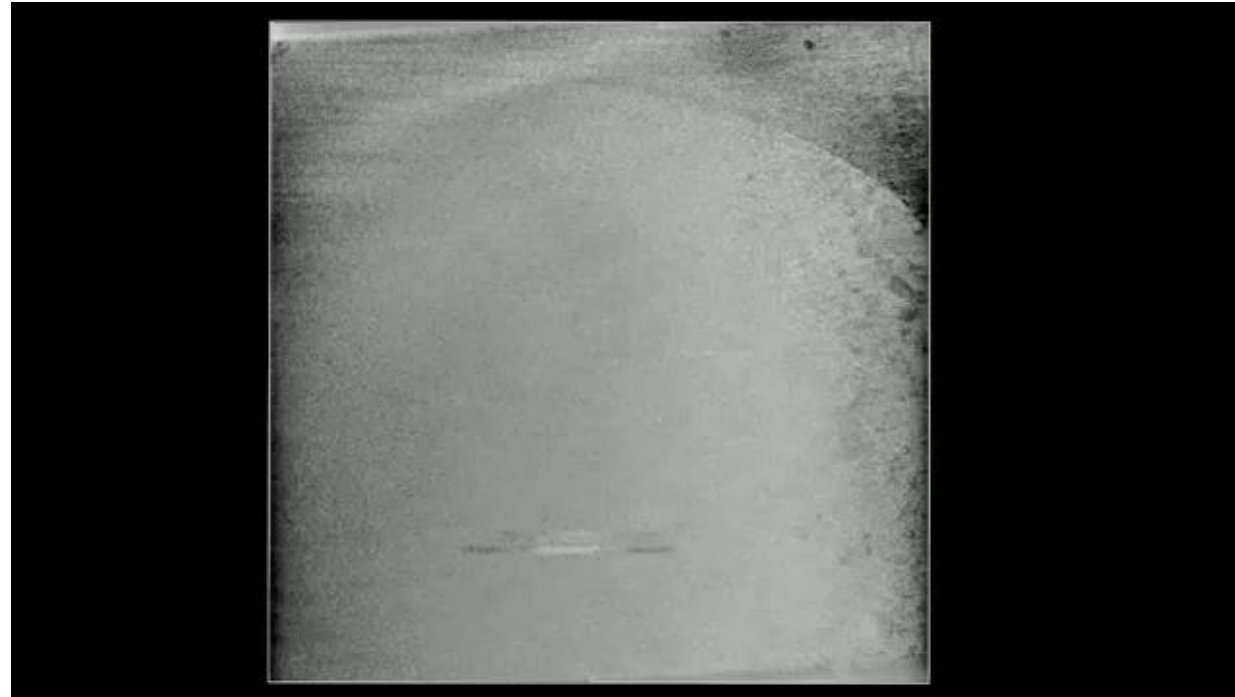
## Cryoelectron Tomography of the NAIP5/NLRC4 Inflammasome.

### Stepwise NAIP5/NLRC4 Inflammasome Activation Model:

1. Flagellin primes NLRC4 by inducing phosphorylation, and binds and activates NAIP5.
2. Activated NAIP5 recruits and activates primed NLRC4 by inducing similar conformational rearrangements to form the NAIP5/NLRC4/FliC-D0L complex.
3. This complex forms a nucleation site for helical NLRC4 polymerization. Activated NAIP5 might also be incorporated at low frequency.
4. a, The CARD-exposing side forms a nucleation complex for caspase-1 multimerization.
5. b, Alternatively, the CARD-exposing side of the helical complex allows dimerization



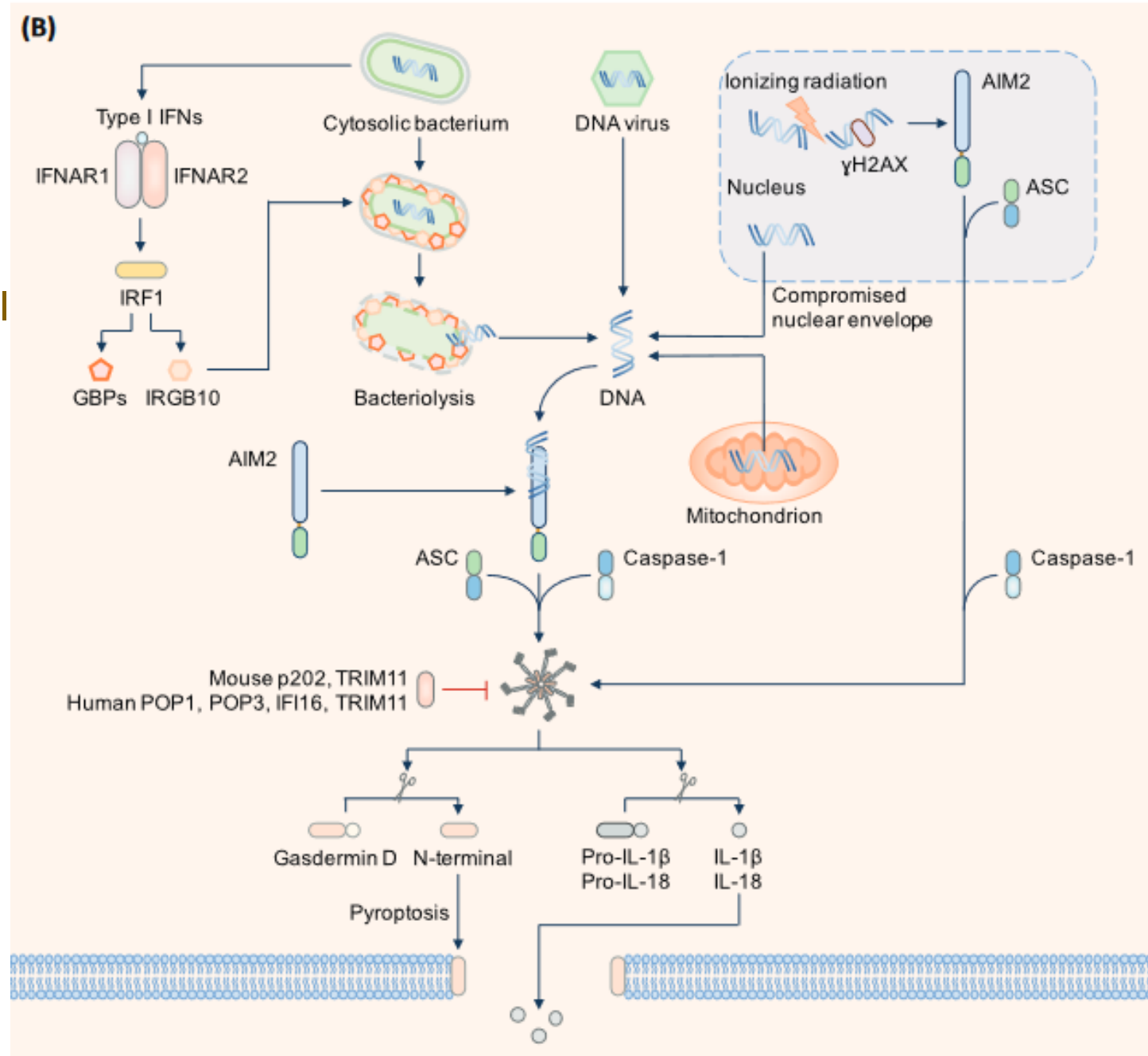
## NAIP5-PKG/NLRC4/FliC



Cryoelectron Tomography of the NAIP5/NLRC4 Inflammasome.

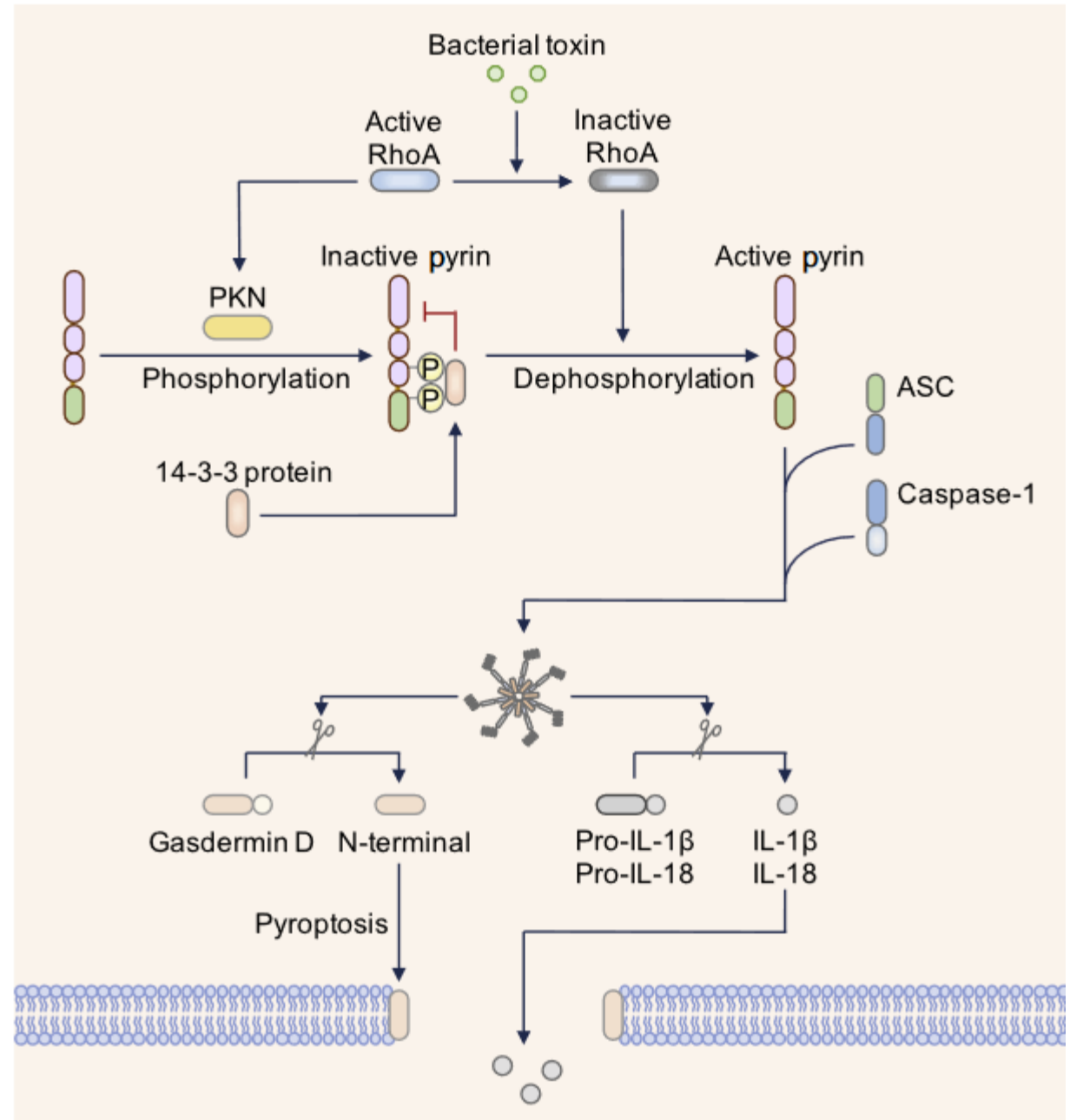
## AIM2 inflammasome

- activated in response to microbial or self-DNA.
- type I IFNs drives the expression of GBPs and IRGB10 and target bacterial and vacuolar membranes for destruction (releasing bacterial DNA) => AIM2 activation.
- DNA viruses can activate AIM2 independently of type I IFN.
- AIM2 detects radiation-induced host DNA damaged in the nucleus. AIM2 also recognizes host DNA that has leaked into the cytoplasm following damage to the nucleus or mitochondria.
- Upon binding to DNA, AIM2 recruits ASC and caspase-1 to form an active inflammasome complex.

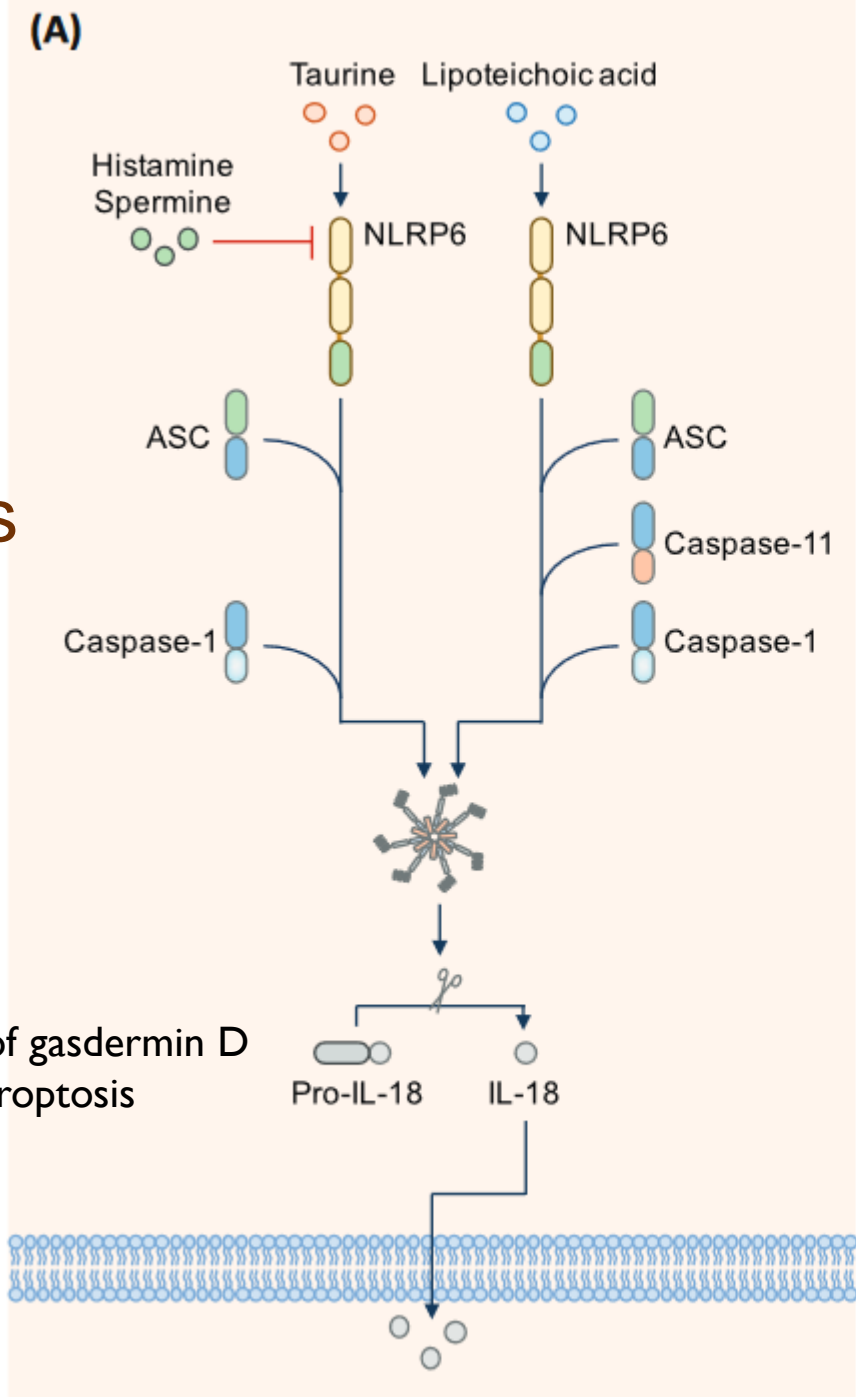


# Pyrin Inflammasome

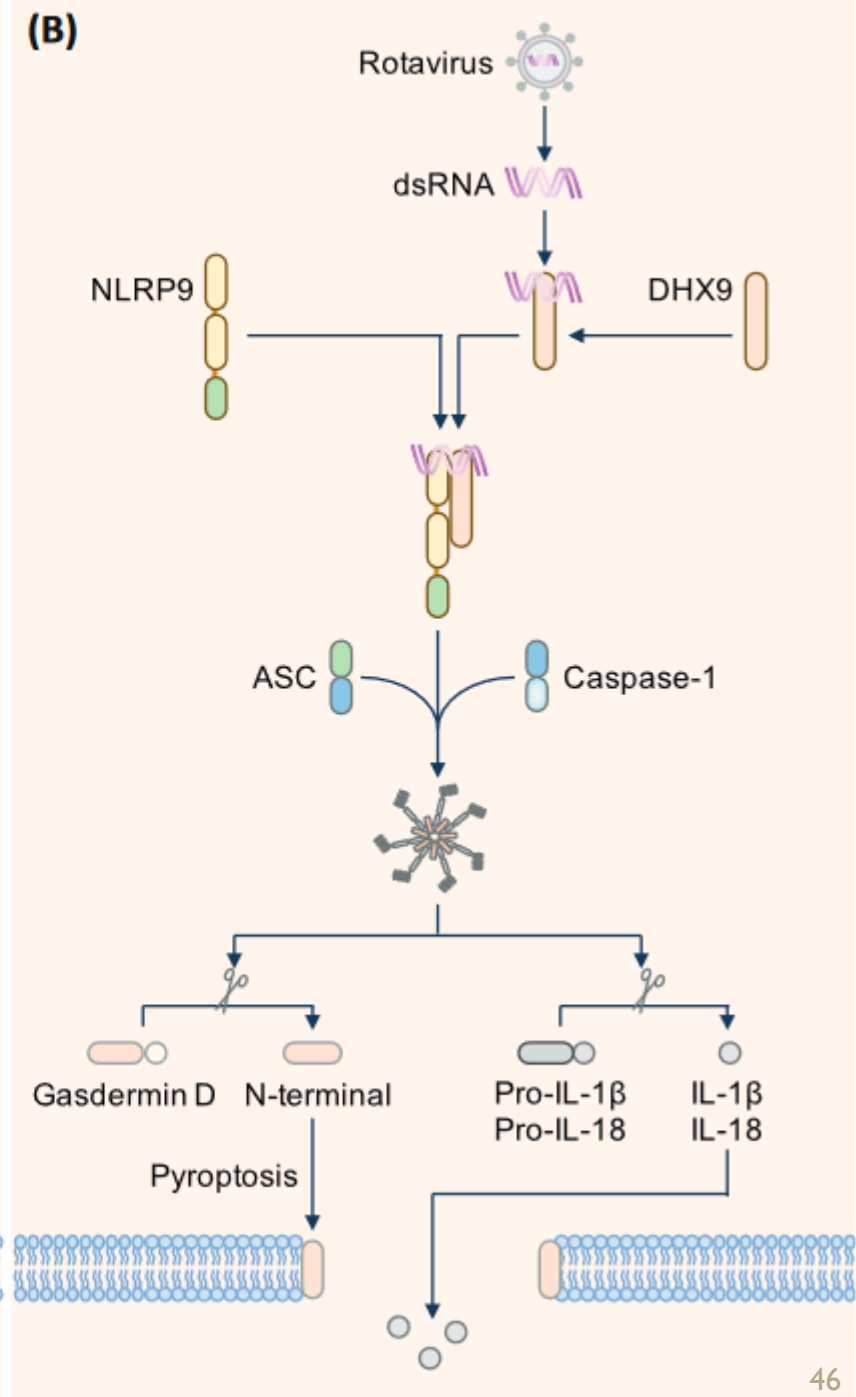
- phosphorylated by the Ras homolog family member A (RhoA) effector kinases, protein kinase N1/2 (PKN1/2), and is bound to 14-3-3 proteins that keep pyrin in an inactive state.
- Bacterial toxins such as *Clostridium difficile* TcdA and TcdB can inhibit RhoA activity and subsequent PKN1/2 phosphorylation, thereby leading to pyrin dephosphorylation and 14-3-3 protein disassociation.
- Unhindered pyrin initiates the recruitment of ASC and caspase-1, forming an active inflammasome complex.



# NLRP6 and NLRP9b Inflammasomes



independently of gasdermin D cleavage and pyroptosis



**DHX9**, DEAH-box helicase 9

# RIG-I antiviral signaling

**MAVS:** mitochondrial antiviral signaling protein (IPS-I, VISA or Cardif)

**TRAF3:** TNF receptor-associated factor 3

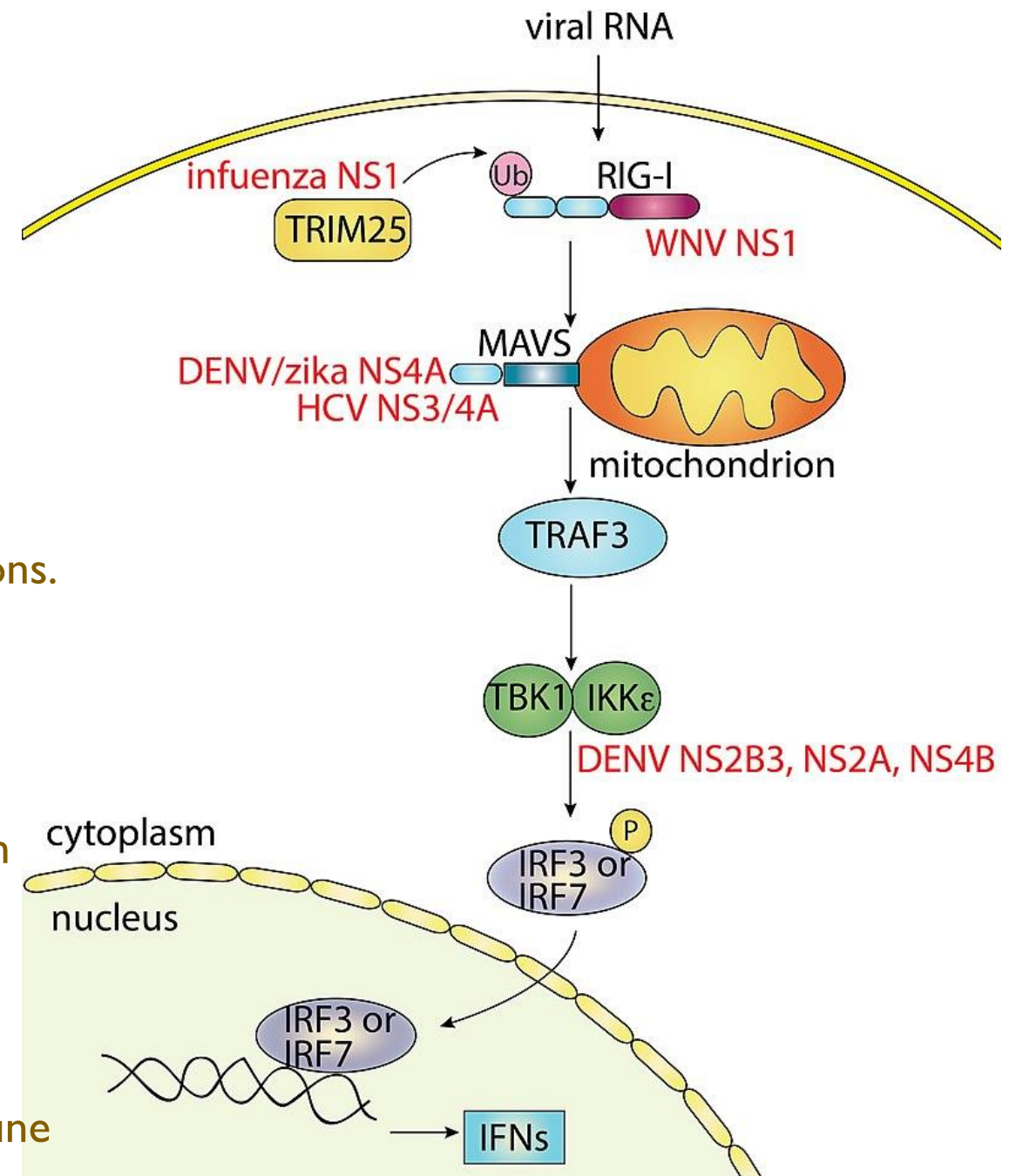
**IKK $\epsilon$ /TBK1:** I-kappa-B kinase-epsilon/TANK-binding kinase I

**IRF:** interferon regulatory factor (transcription factors)

**IFN:** type I (IFN $\alpha$  and IFN $\beta$ ) and type III interferons.

The type I IFNs bind type I IFN receptors on the surface of cells to activate JAK-STAT (Janus kinase/signal transducers and activators of transcription) signaling. This leads to the induction of hundreds of interferon stimulated genes (ISGs) that amplify the IFN response:

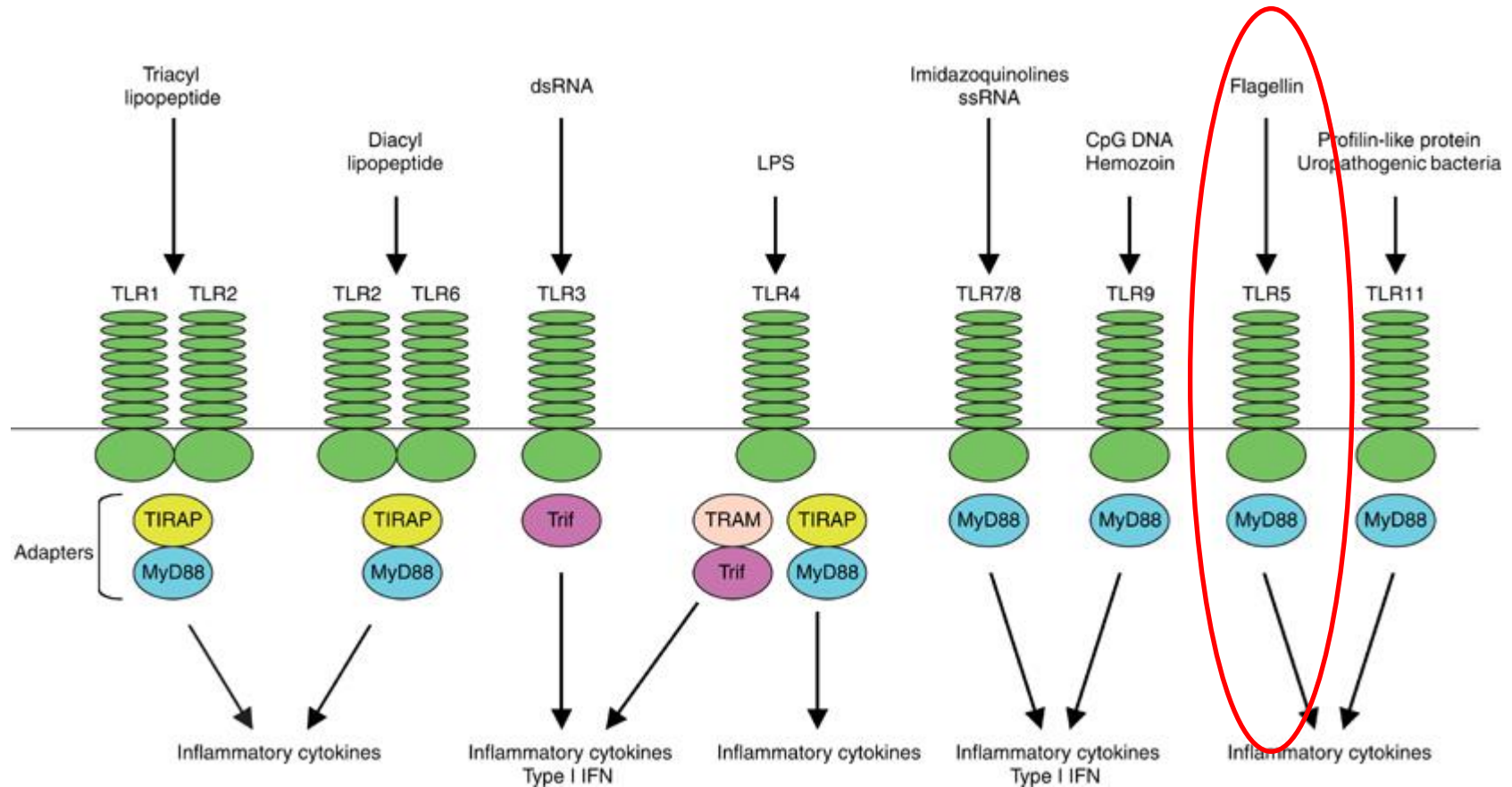
- death of infected cells
- protection of surrounding cells
- activation of the antigen-specific antiviral immune response



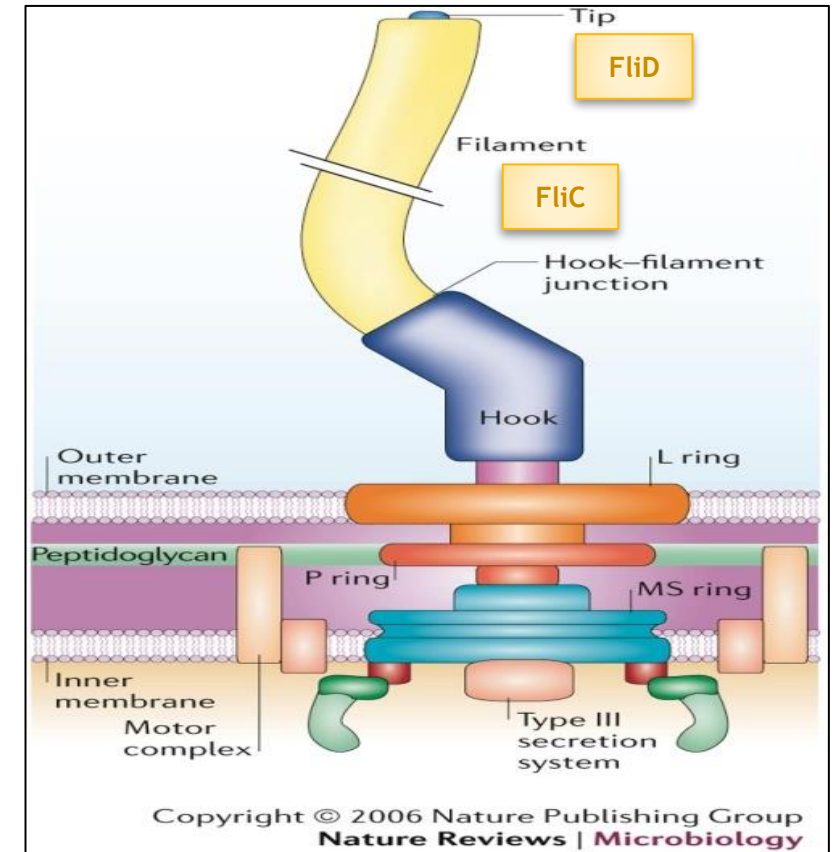
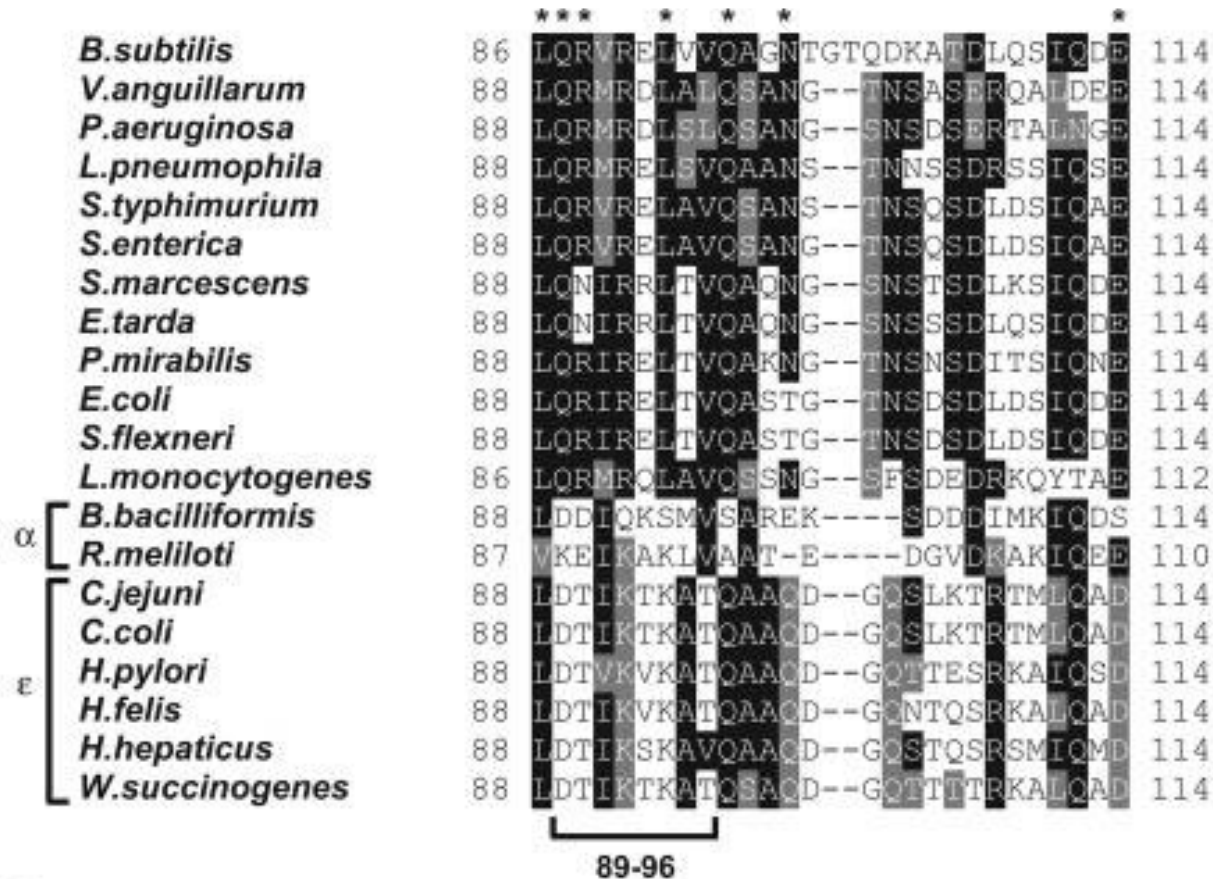
# Experimental study: TL5 and bacterial flagellins



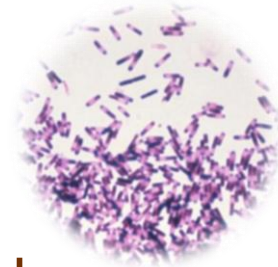
# TLR5 and bacterial flagellins



# TLR5 and bacterial flagellins



# *Clostridioides (Clostridium) difficile*

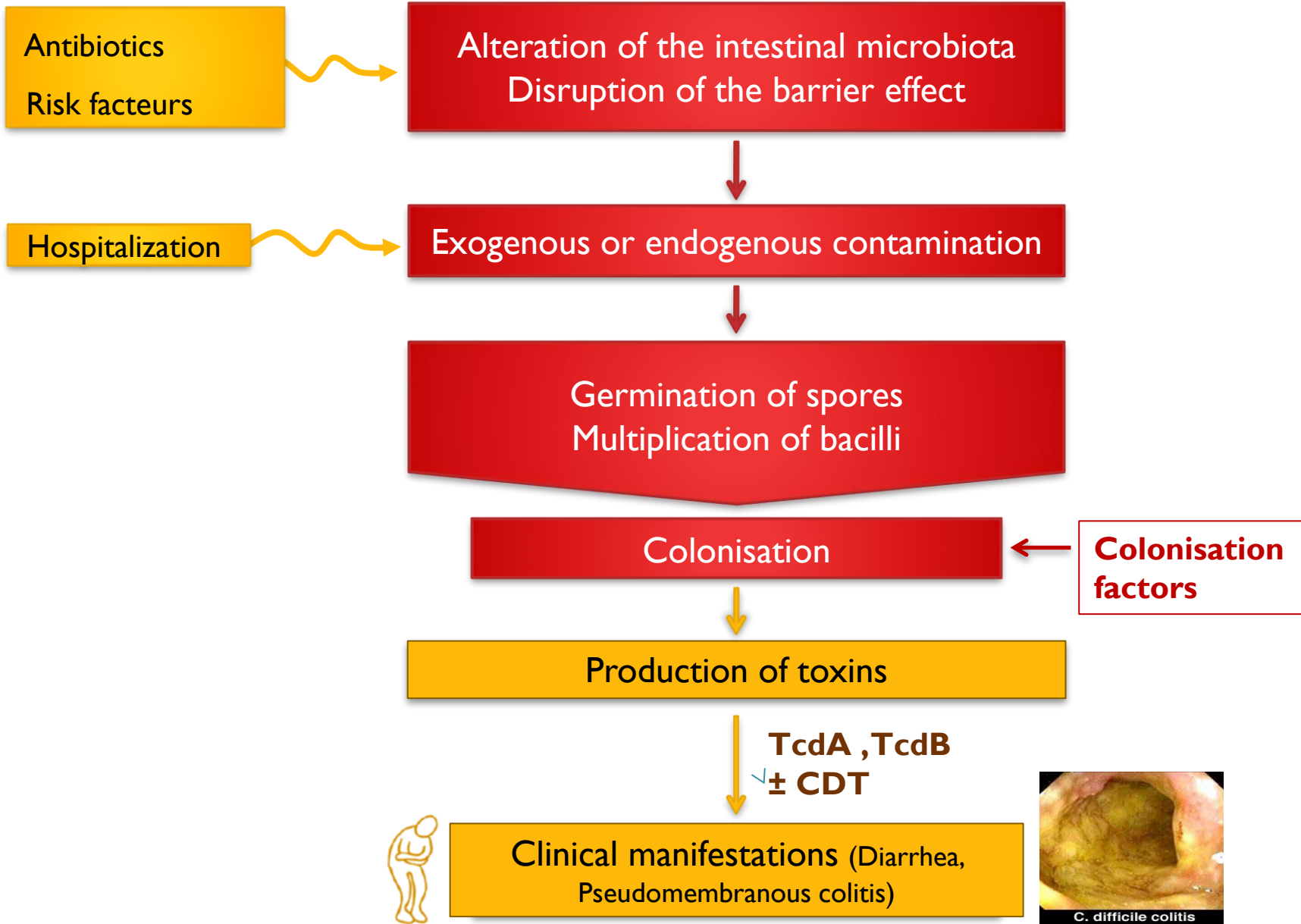


Gram + bacillus, strict anaerobic, sporulated and motile

- ✓ Responsible for 10 to 25% of post-antibiotic diarrhea.
- ✓ Responsible for 95% of cases of pseudomembranous colitis.
- ✓ Leading cause of nosocomial infectious diarrhea in adults.
- ✓ Risk of frequent recurrence (20% 1st episode, 60% beyond).
- ✓ High risk of mortality (up to 50%) .



# Pathogenicity of *C. difficile*



# Virulence factors

✓ Toxins TcdA et TcdB

✓ Colonization factors:

- Adhesin-acting proteins:

- S layer *(Calabi et al., 2007)*

- Cwp66 *(Waligora et al., 2001)*

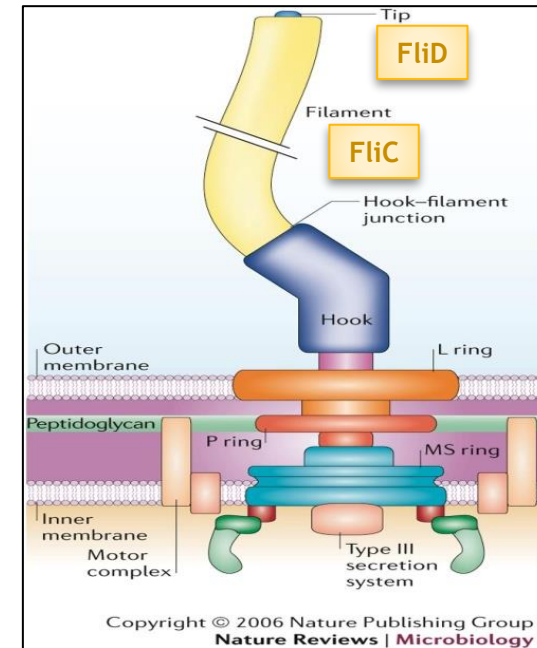
- Fbp68 *(Hennequin et al., 2003)*

- GroEL *(Hennequin et al., 2001)*

- Proteolytic enzymes (Cwp84). *(Janoir et al., 2007)*

✓ Flagella: FliC, FliD

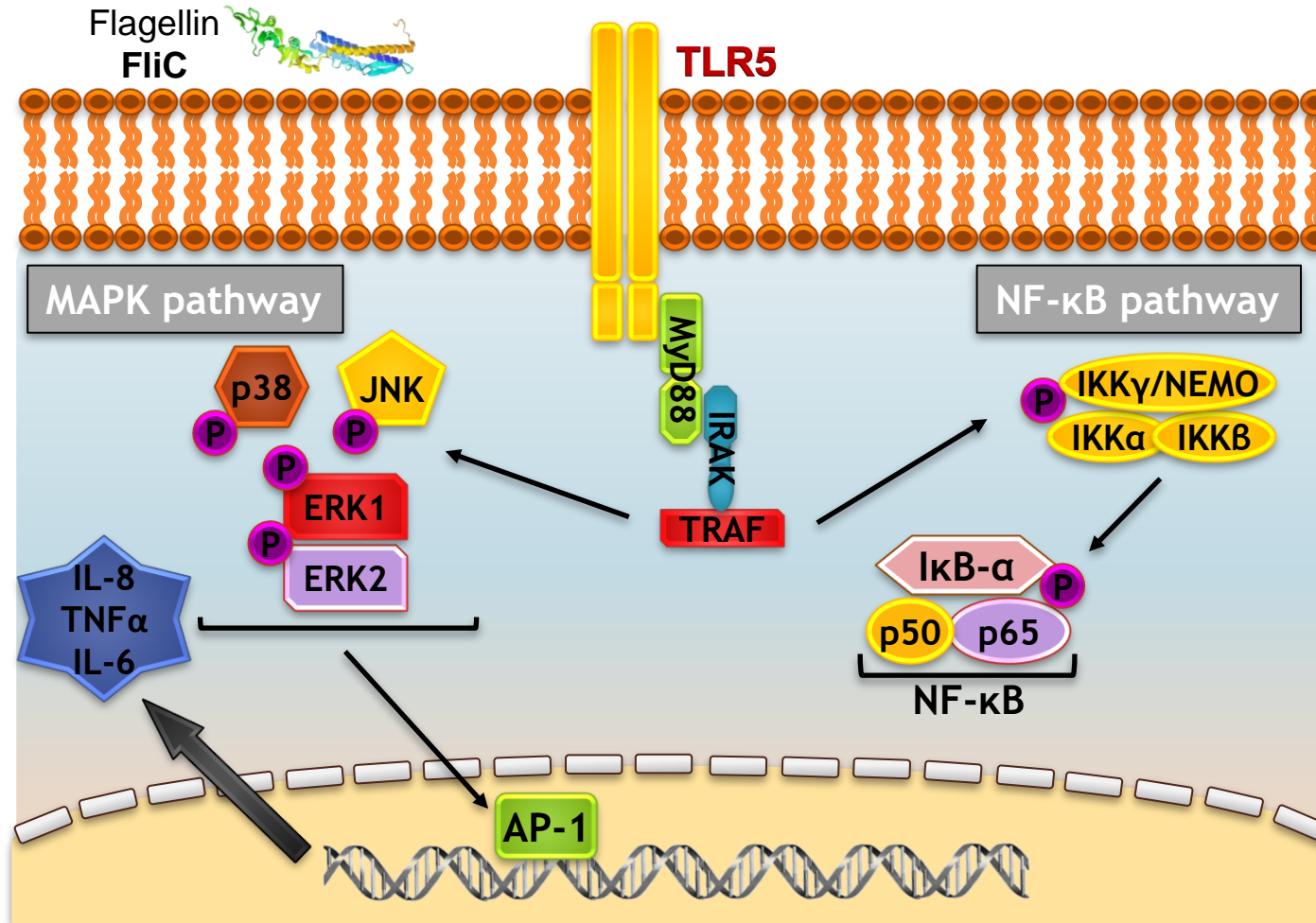
*(Cunningham et al., 2004)*



# Flagellar proteins

- ✓ Mobility. *(Tasteyre et al., 2001)*
- ✓ Adhesion and colonization of mucous membranes.  
*(Lillehoj et al., 2002)*
- ✓ Auto-agglutination and formation of biofilms.  
*(Blair et al., 2008)*
- ✓ Cellular invasion.  
*(Grant et al., 1993)*
- ✓ Regulation of the secretion of non-flagellar proteins involved in virulence.  
*(Anderson et al., 2010; Barketi et al. 2014)*
- ✓ Immune response. *(Cunningham et al., 2004)*

# Role of flagella in inducing inflammatory response



Hayashi, 2001  
Zeng et al., 2006

## Role flagella of *C. difficile* in the amplification of the intestinal inflammatory response during infection

To characterize in vitro and in vivo the signaling pathways involved in the interaction between *C. difficile* flagella and the epithelial cell via the innate immune response receptor TLR5

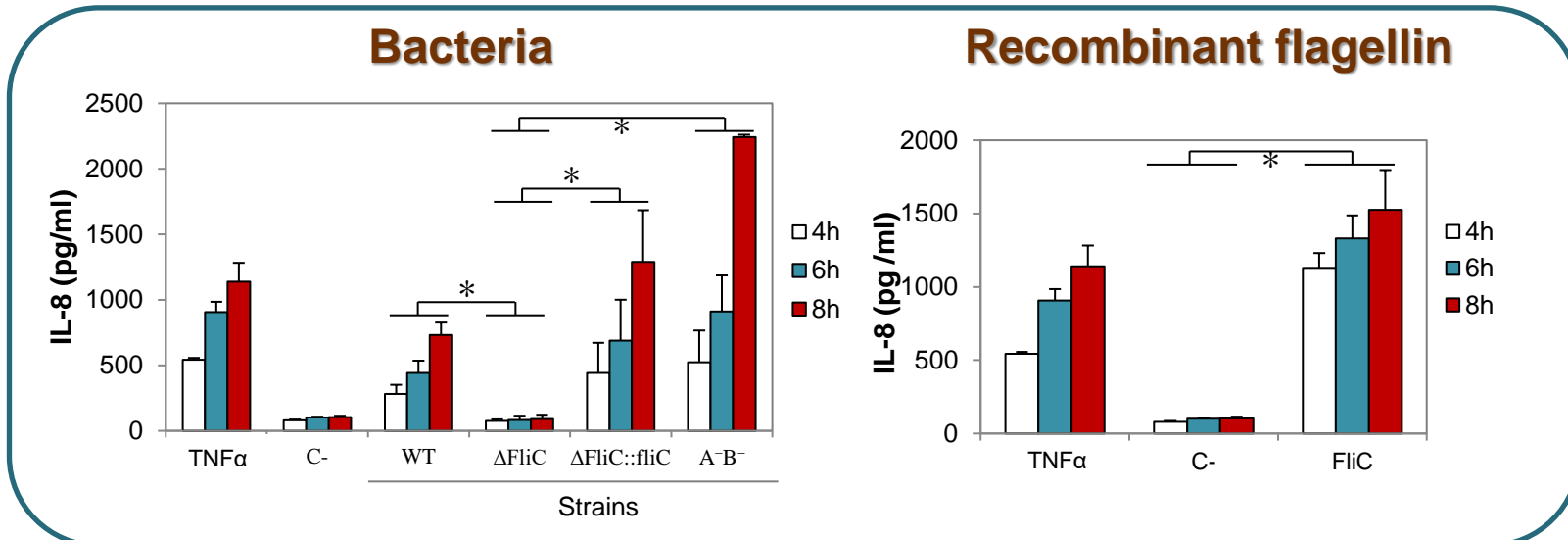
		***	*	*	*					*																										
<i>C. difficile</i> 630 (YP_001086707.1)	86	L	Q	R	M	R	I	L	S	L	Q	S	A	N	E	I	N	N	T	E	E	R	E	K	I	A	D	E	L	T	Q	L	K	D	E	120
<i>C. difficile</i> R20291 (YP_003216748.1)	86	L	Q	R	M	R	I	L	S	V	Q	S	S	N	E	T	N	T	A	E	E	R	Q	K	I	A	D	E	L	L	Q	L	K	D	E	120
<i>S. typhimurium</i> LT2 (NC_003197.1)	88	L	Q	R	V	R	E	L	A	V	Q	S	A	N	S	T	N	S	Q	D	L	D	S	S	I	Q	A	E	I	T	Q	R	L	N	E	122
<i>H. pylori</i> B8 (NC_014256.1)	88	L	D	T	I	K	T	K	A	V	Q	A	A	Q	D	G	Q	T	L	E	S	R	R	A	L	Q	S	D	I	Q	R	L	E	E	122	



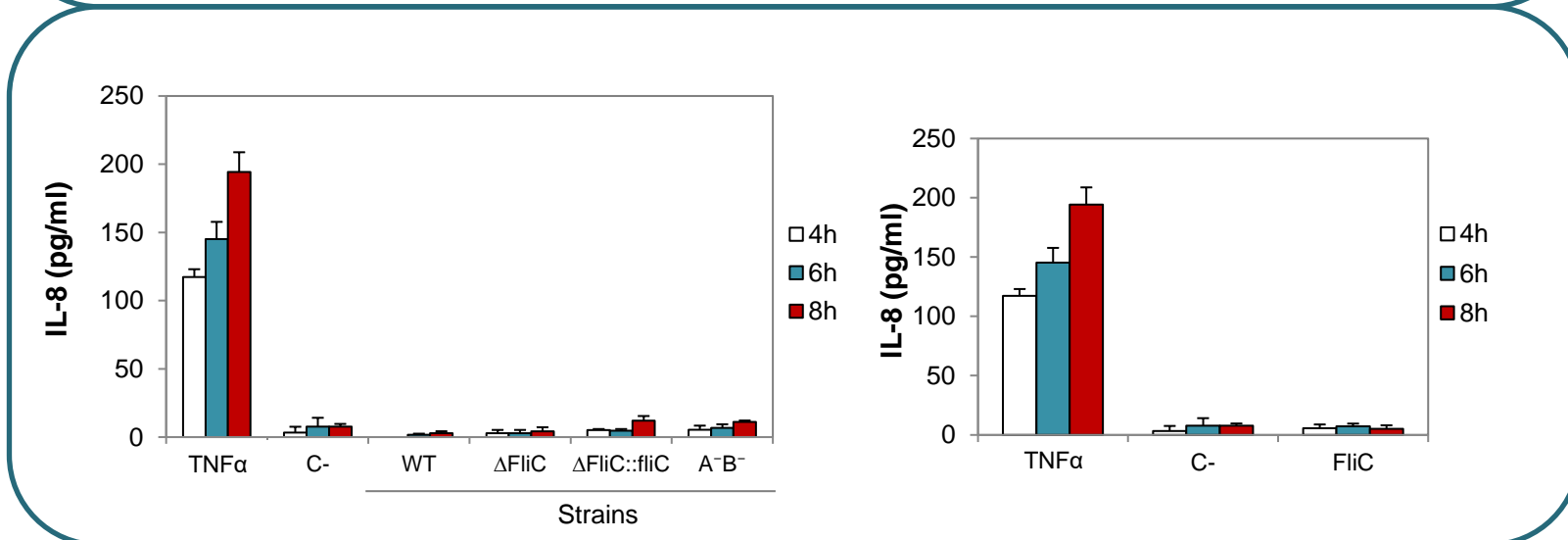
# Induction of IL-8 by *C. difficile* flagellin via TLR5

## MDCK-TLR5 epithelial cell model

MDCK-TLR5

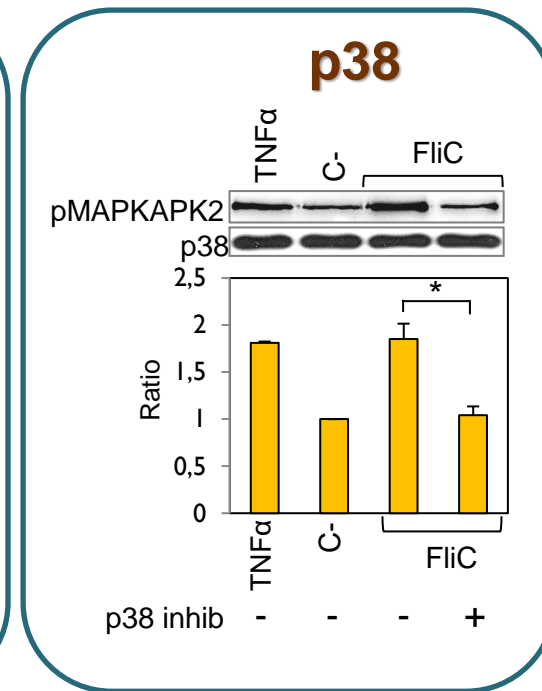
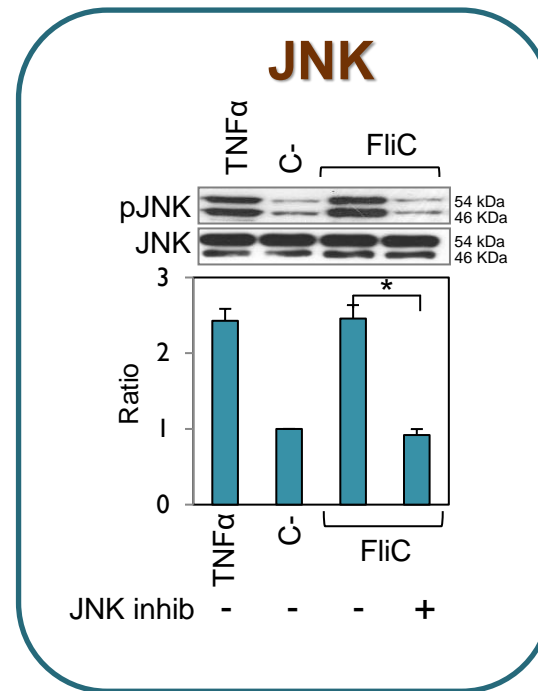
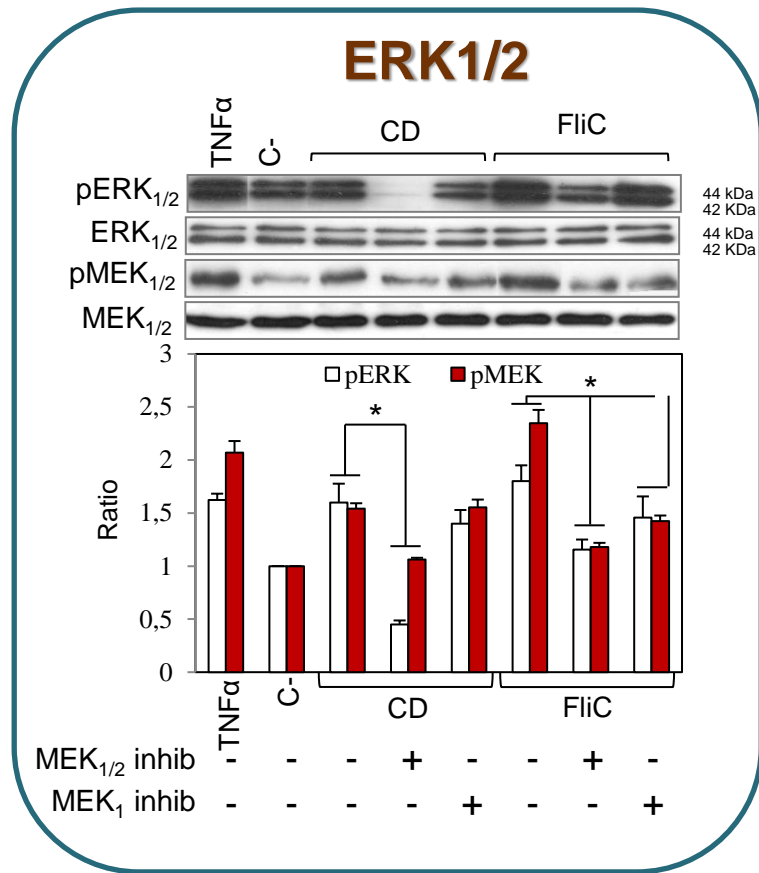


MDCK contrôle



# Activation of MAPKs by *C. difficile* flagellin via TLR5

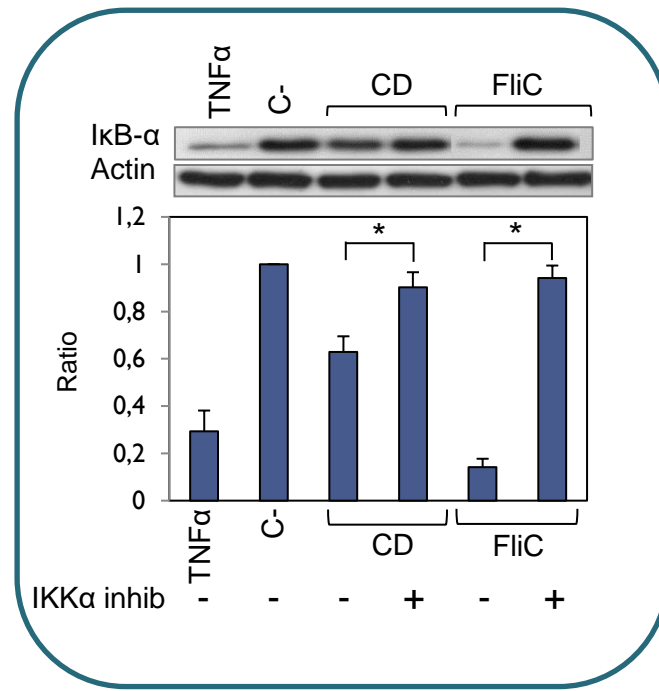
## MDCK-TLR5 epithelial cell model



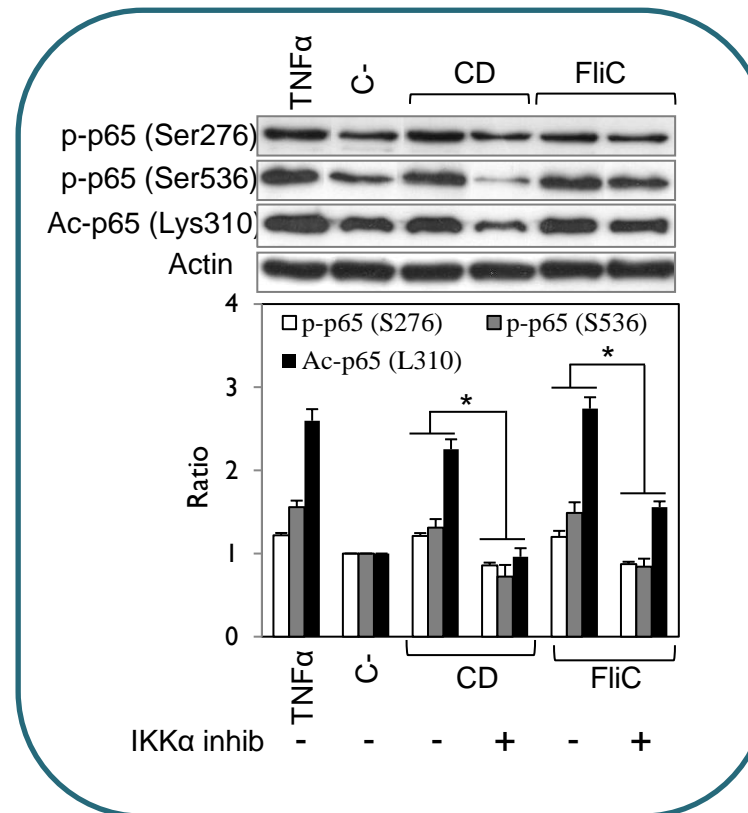
# Activation of NF- $\kappa$ B by *C. difficile* flagellin via TLR5

MDCK-TLR5 epithelial cell model

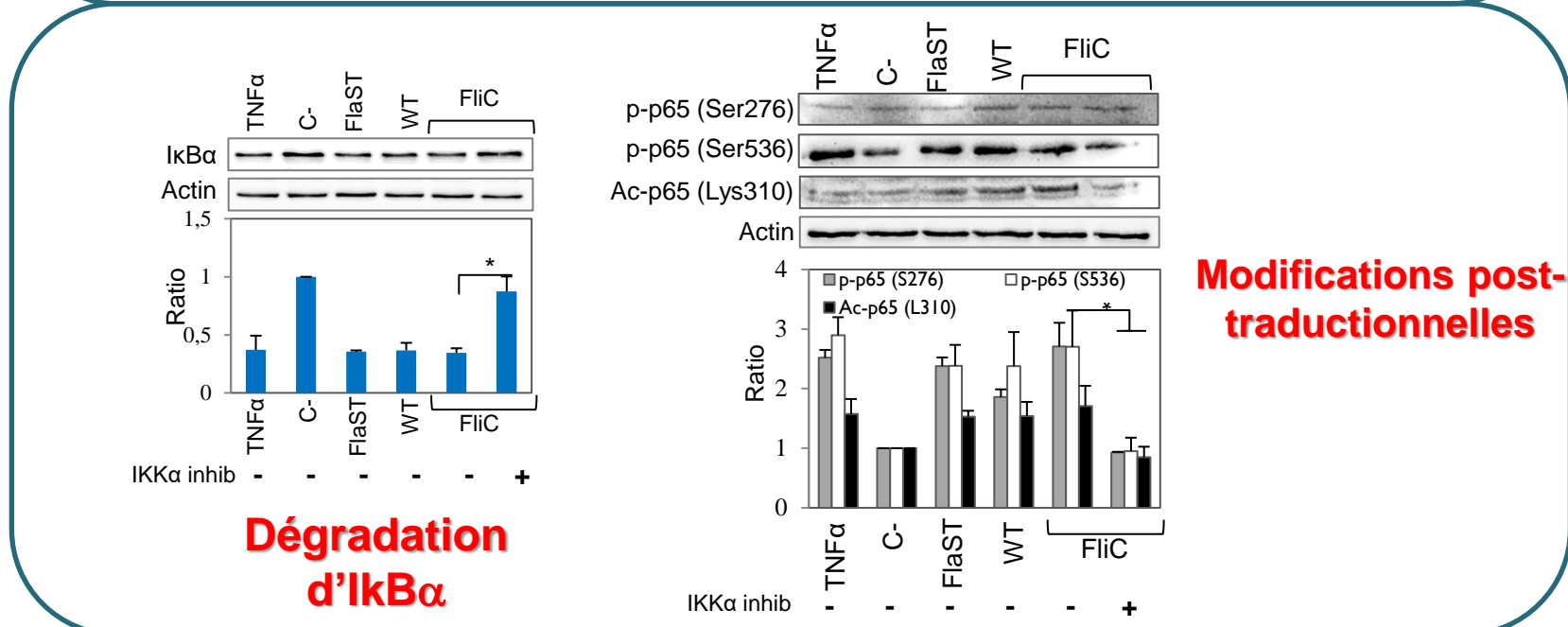
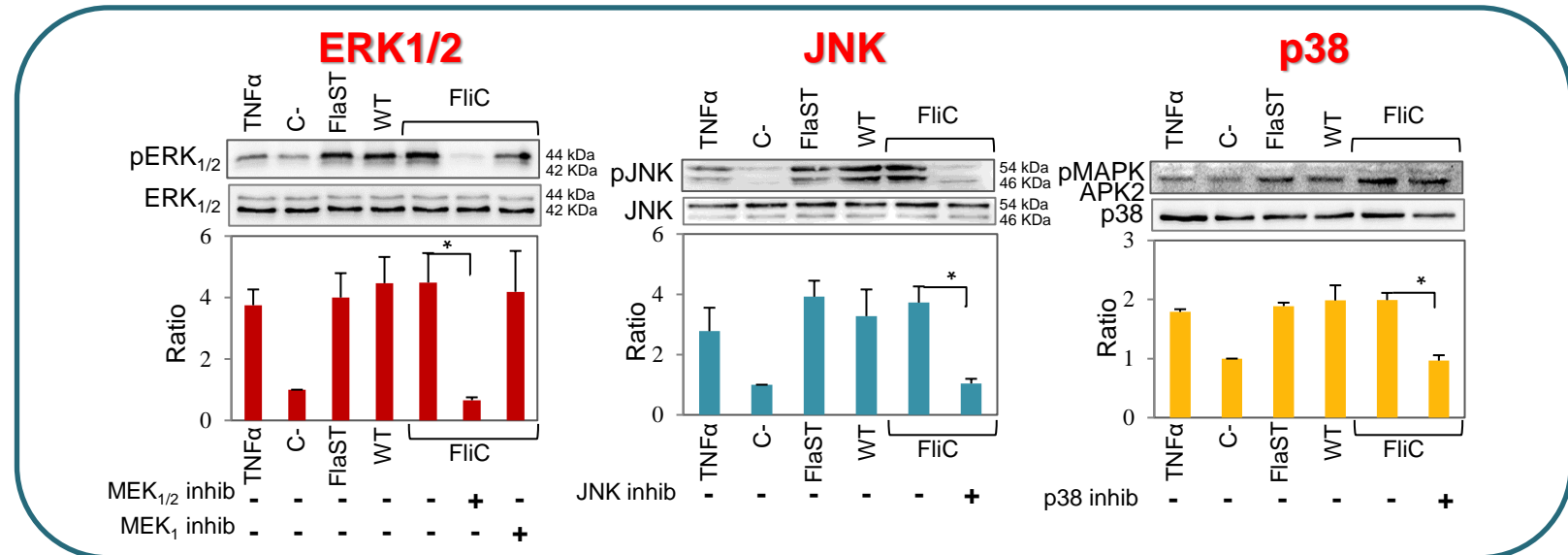
## I $\kappa$ B $\alpha$ degradation



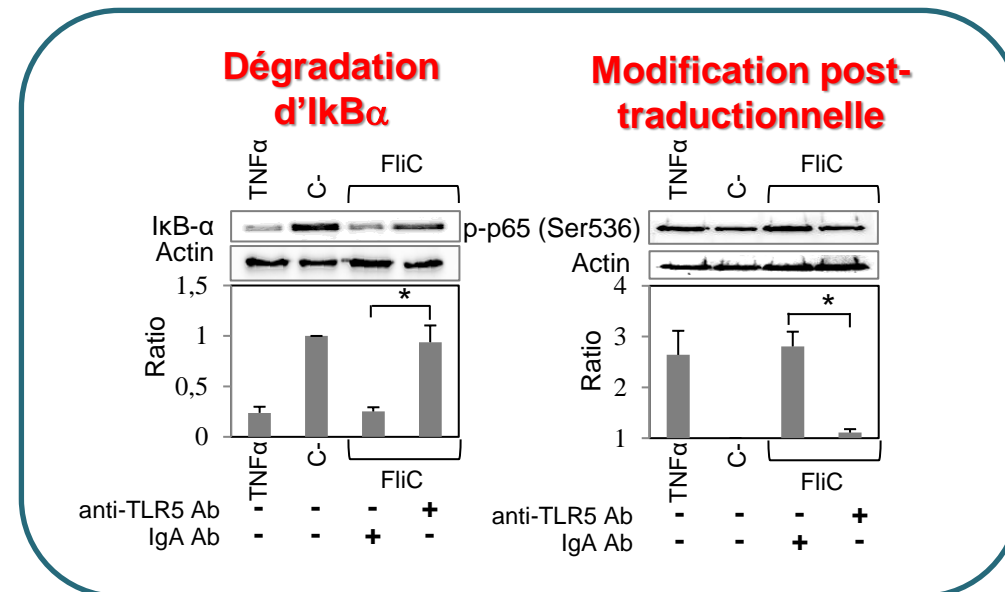
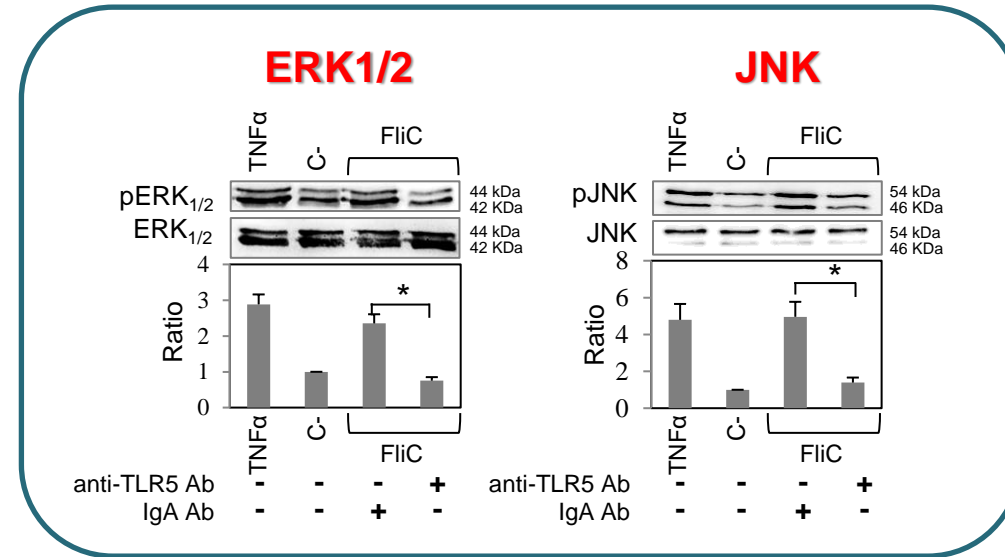
## Post-translational modifications



# Activation of MAPKs and NF- $\kappa$ B by *C. difficile* flagellin via TLR5: Caco-2 epithelial cell model

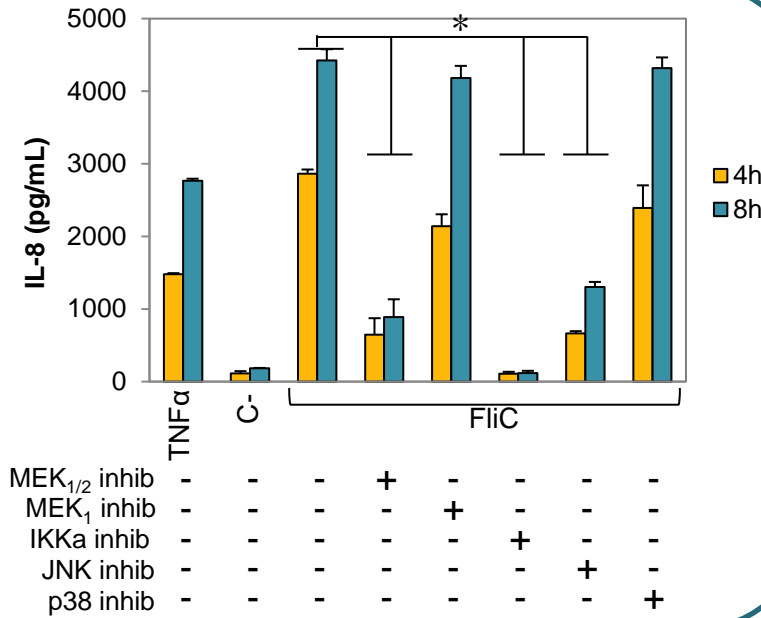


# Activation of MAPKs and NF- $\kappa$ B by *C. difficile* flagellin via TLR5: Caco-2 epithelial cell model



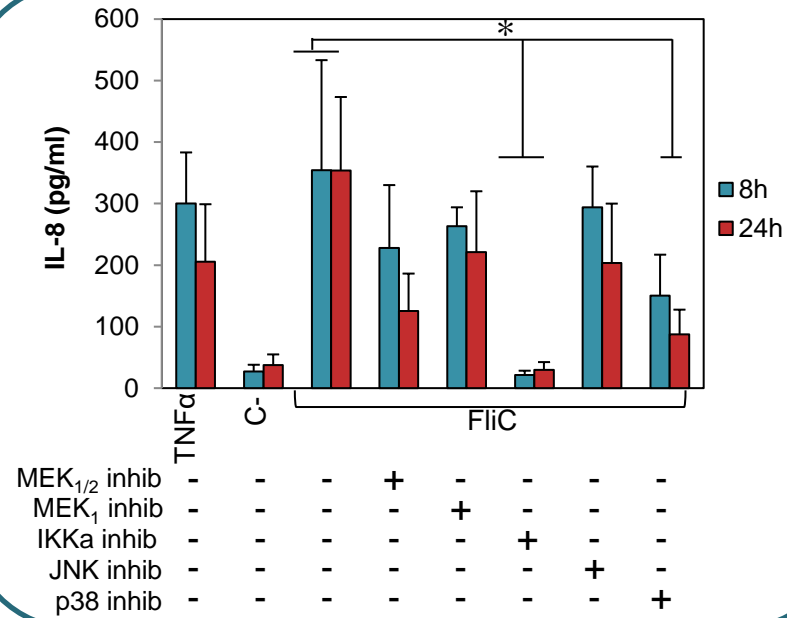
# Predominantly NF- $\kappa$ B activation by *C. difficile* flagellin via TLR5: chemical inhibition

**MDCK-TLR5**



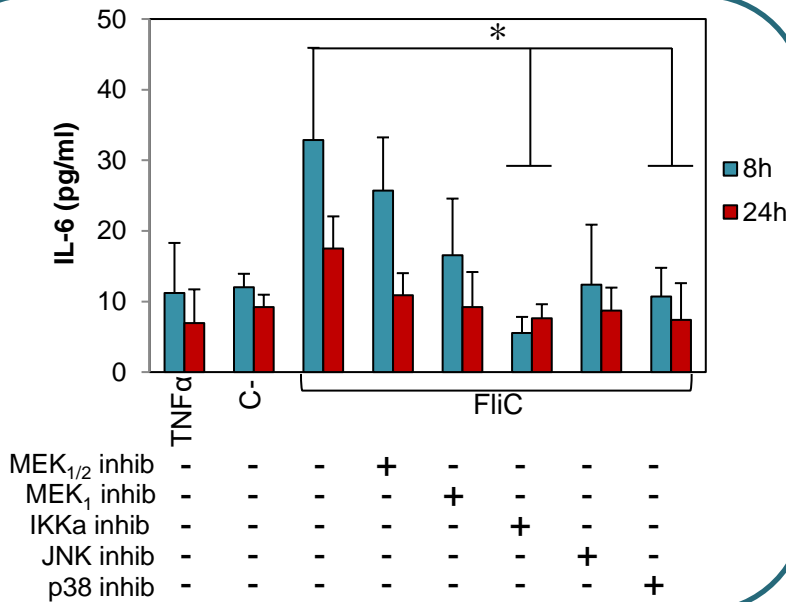
MEK<sub>1/2</sub> inhib  
MEK<sub>1</sub> inhib  
IKK $\alpha$  inhib  
JNK inhib  
p38 inhib

**Caco-2**



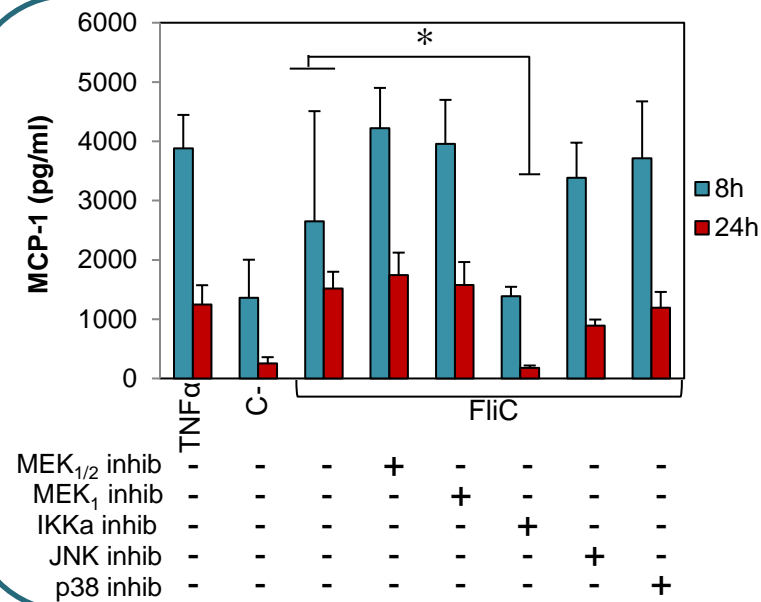
MEK<sub>1/2</sub> inhib  
MEK<sub>1</sub> inhib  
IKK $\alpha$  inhib  
JNK inhib  
p38 inhib

**Caco-2**



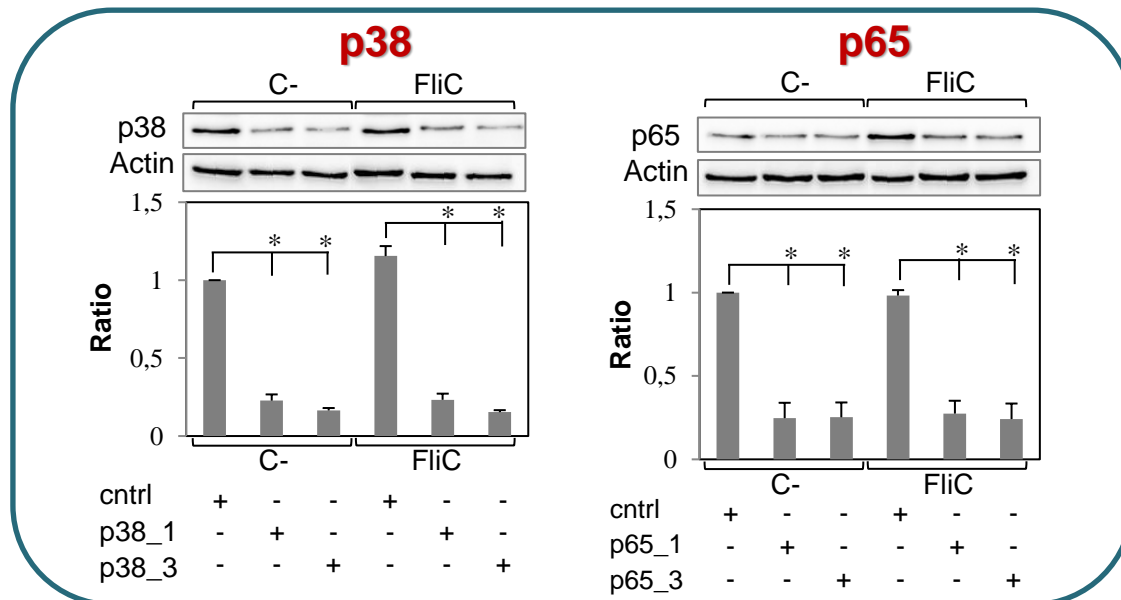
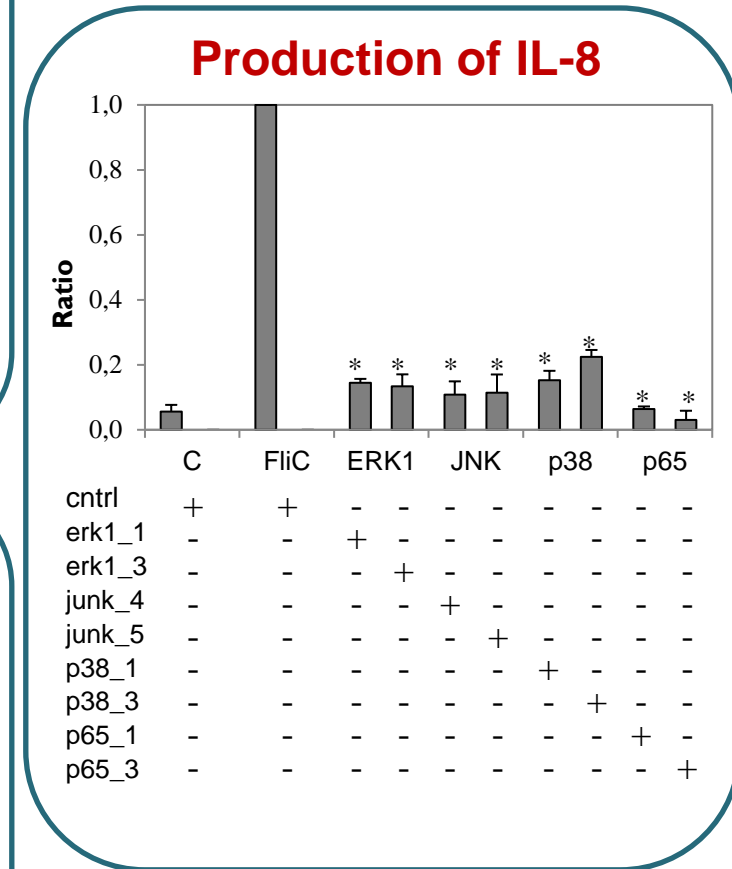
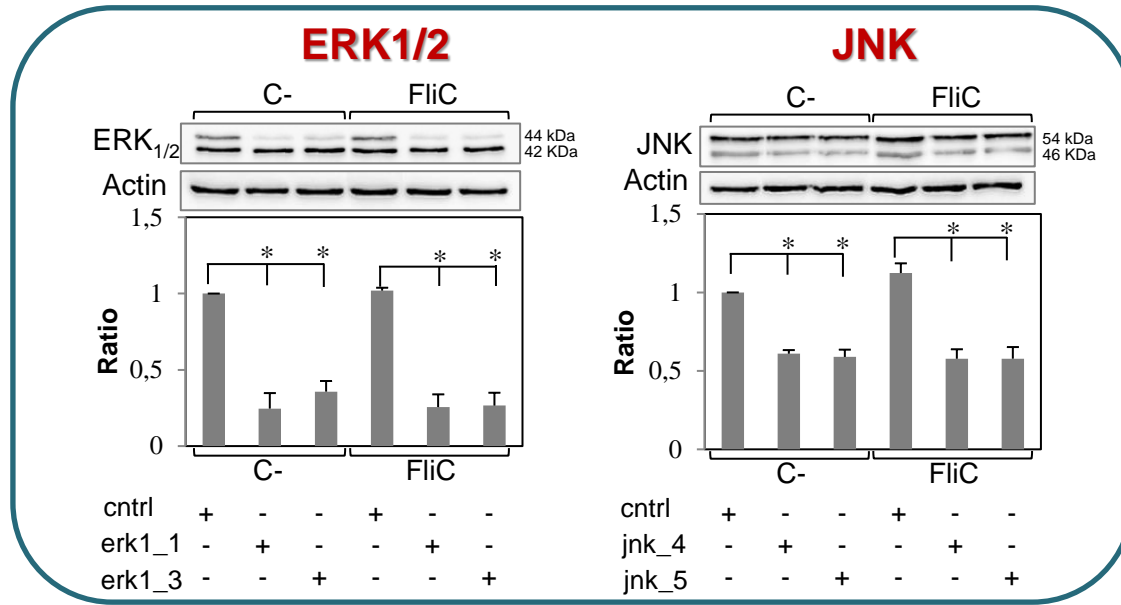
MEK<sub>1/2</sub> inhib  
MEK<sub>1</sub> inhib  
IKK $\alpha$  inhib  
JNK inhib  
p38 inhib

**Caco-2**

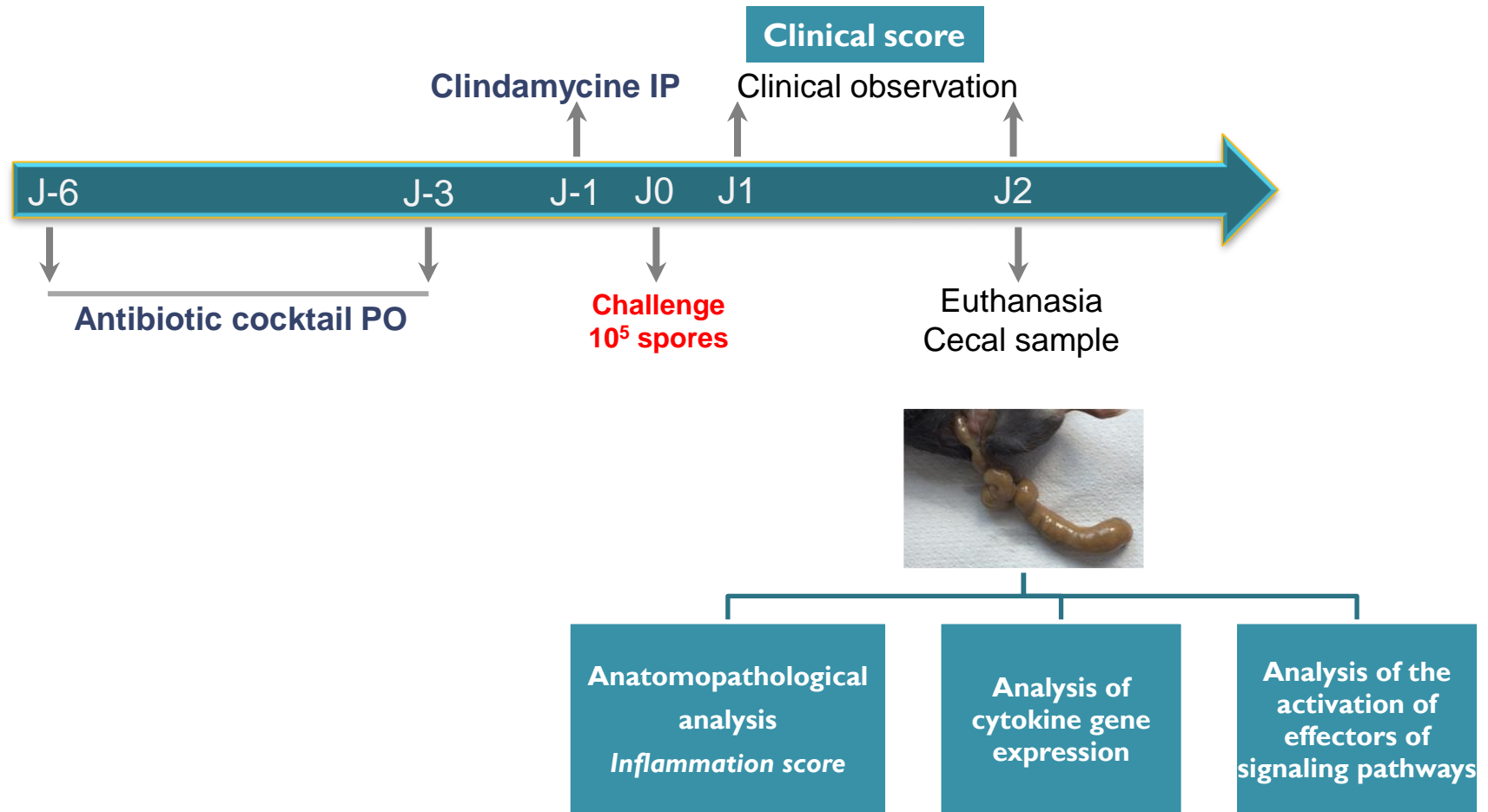


MEK<sub>1/2</sub> inhib  
MEK<sub>1</sub> inhib  
IKK $\alpha$  inhib  
JNK inhib  
p38 inhib

# Predominantly NF- $\kappa$ B activation by *C. difficile* flagellin via TLR5: RNA interference (siRNA)







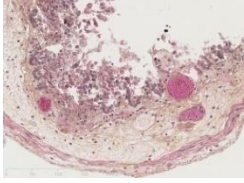


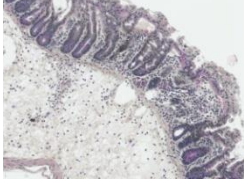


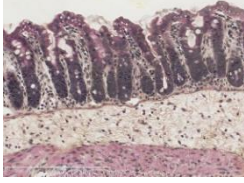


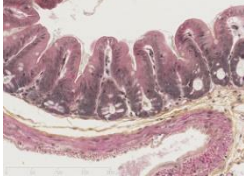


# In vivo study (mouse model)





# Anatomopathological analysis of caecums

C57BL/6 mice	<i>C. difficile</i> 027 (10 <sup>5</sup> spores)	Clinical score				Anatomopathological analysis	
		Loose stools 	Reduced activity 	Spiky coat 	Death 	Macro	Micro
 n = 6	Wild type strain	6	6	6	3		
 n = 6	Unflagellated mutant ( $\Delta$ FliC)	2	2	2	-		
 n = 6	Non toxinogenic, flagellated mutant (A <sup>-</sup> B <sup>-</sup> )	-	-	-	-		
 n = 6	Uninfected control	-	-	-	-		

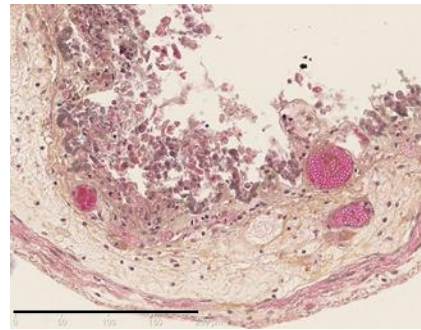
The  $\Delta$ FliC et A-B- mutants induce less inflammation than the wild-type 027 strain

# Intestinal inflammation induced by the wild strain of *C. difficile*

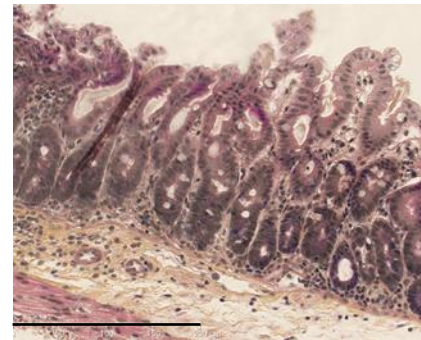
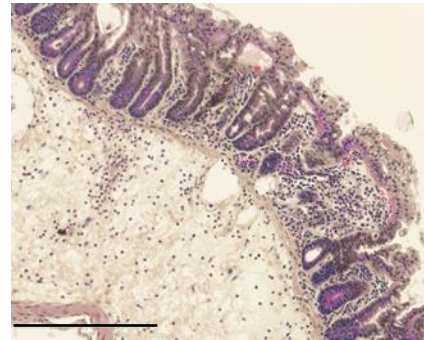
**Negative control**



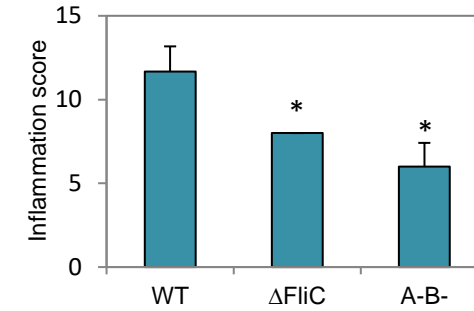
**Wild type strain**



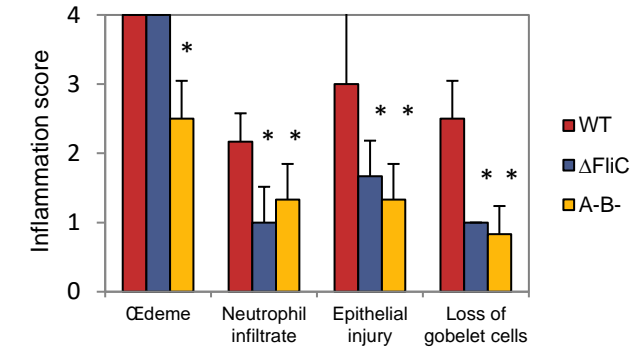
**Unflagellated mutant Non toxinogenic mutant**



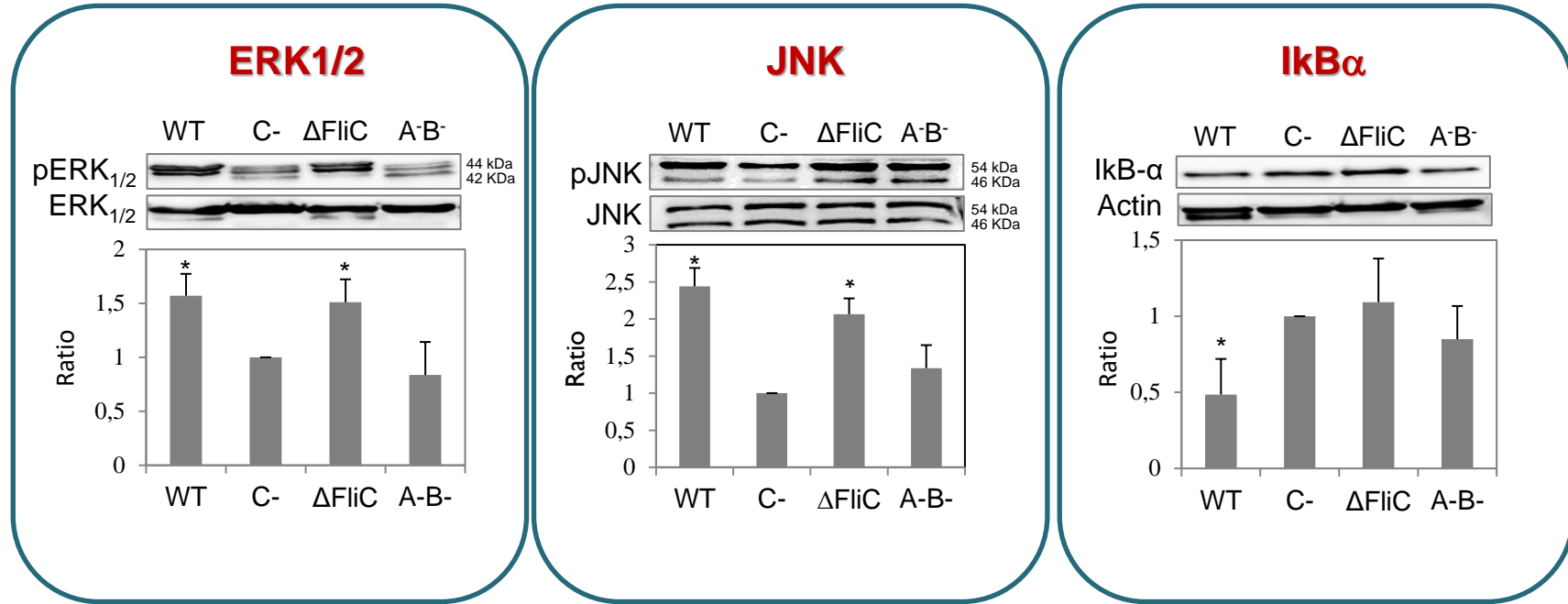
**Total scores**



**Partial scores**



# Activation of MAPKs and NF- $\kappa$ B by the wild strain of *C. difficile*



# Pathogenesis and inflammatory response

- Adhesion / colonization
- Effect of toxins
- Flagellin is recognized by TLR5 at the basolateral level of epithelial cells
- Production of pro-inflammatory cytokines and induction of an immune response

