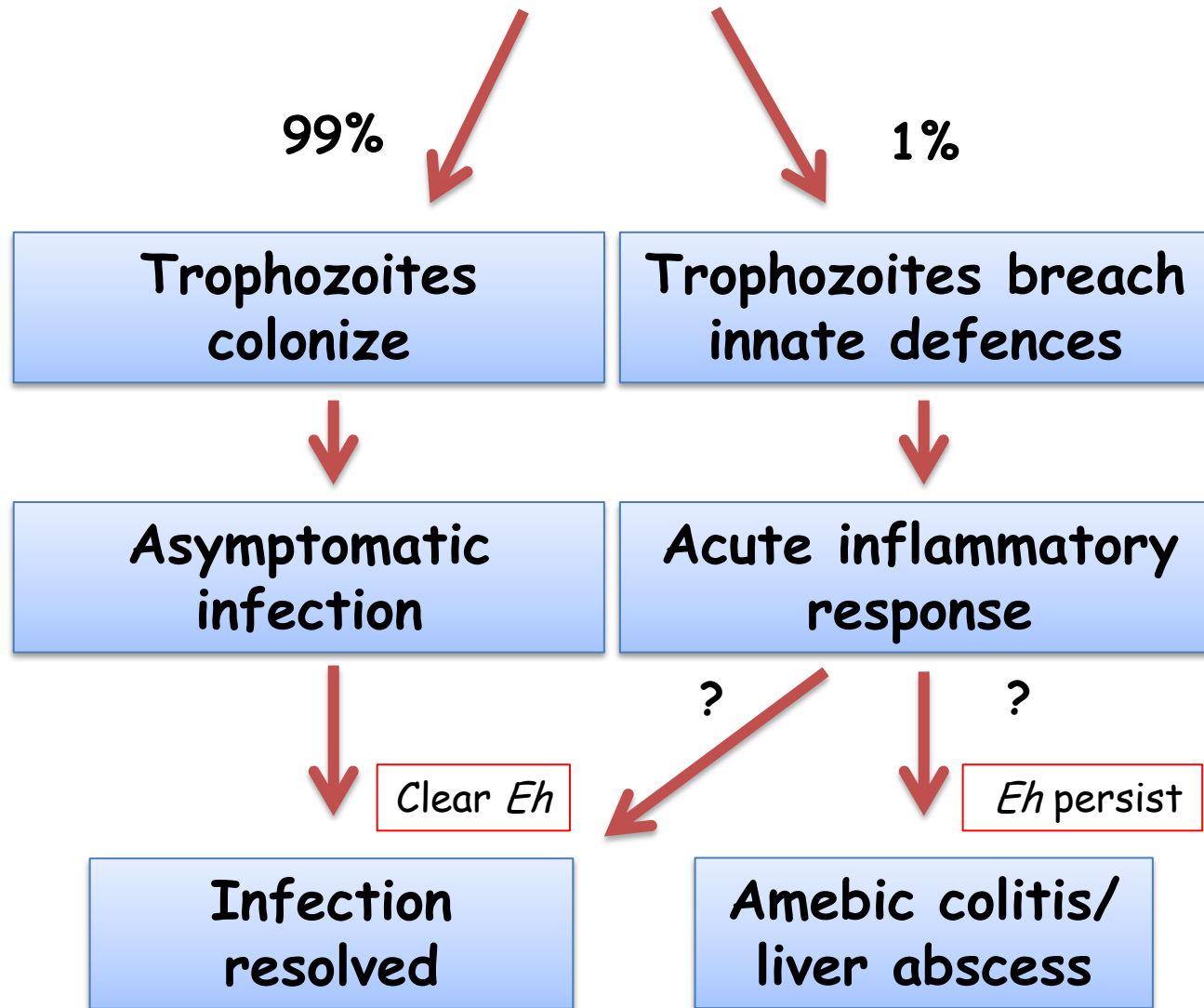


Is *Entamoeba histolytica* contact with host cells the key to disease pathogenesis?

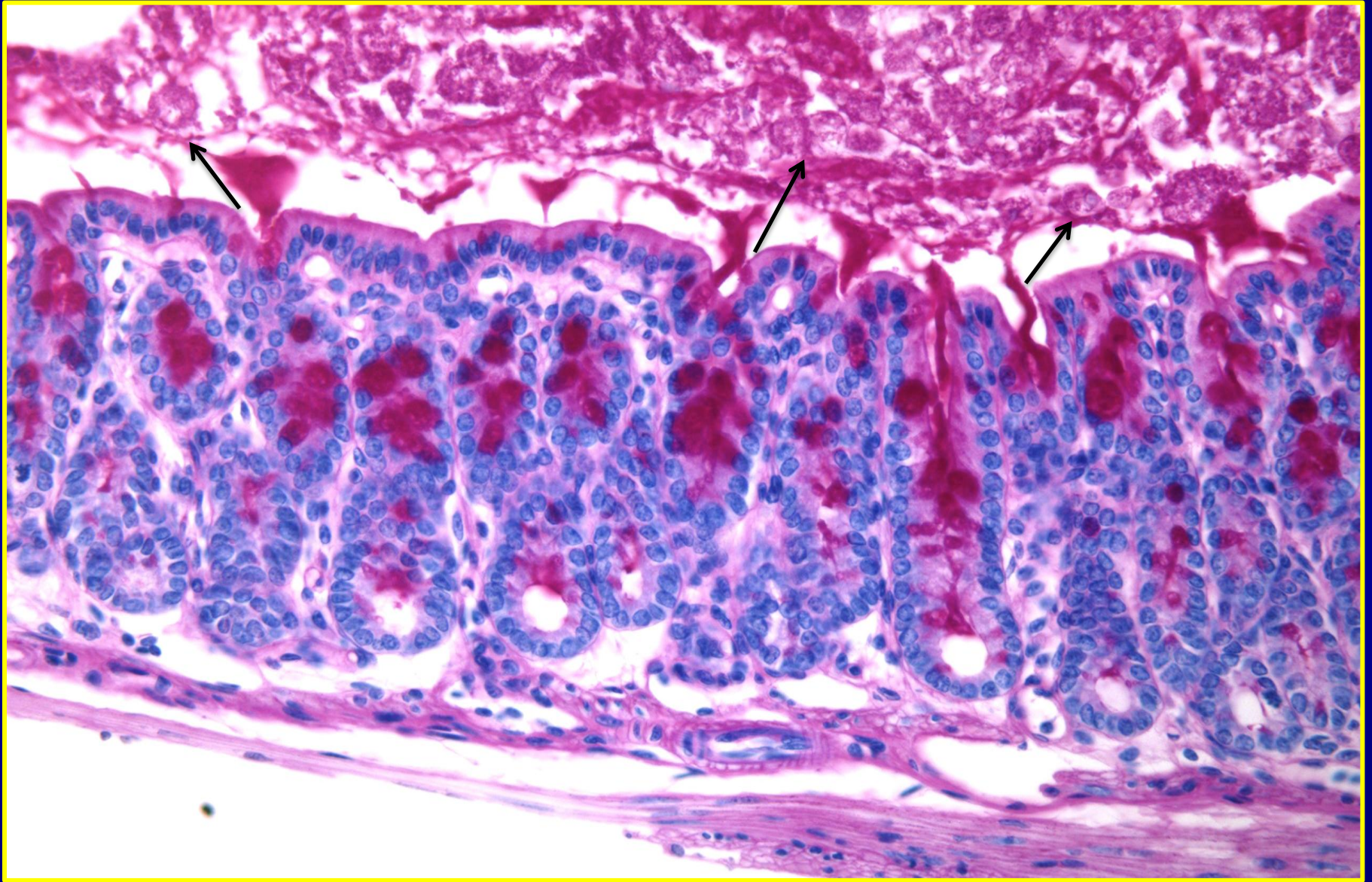
Outcome of *E. histolytica* (*Eh*) infection in the gut



Eh colonizes in/on the outer mucus layer



Mucus hypersecretion in response to *Eh*

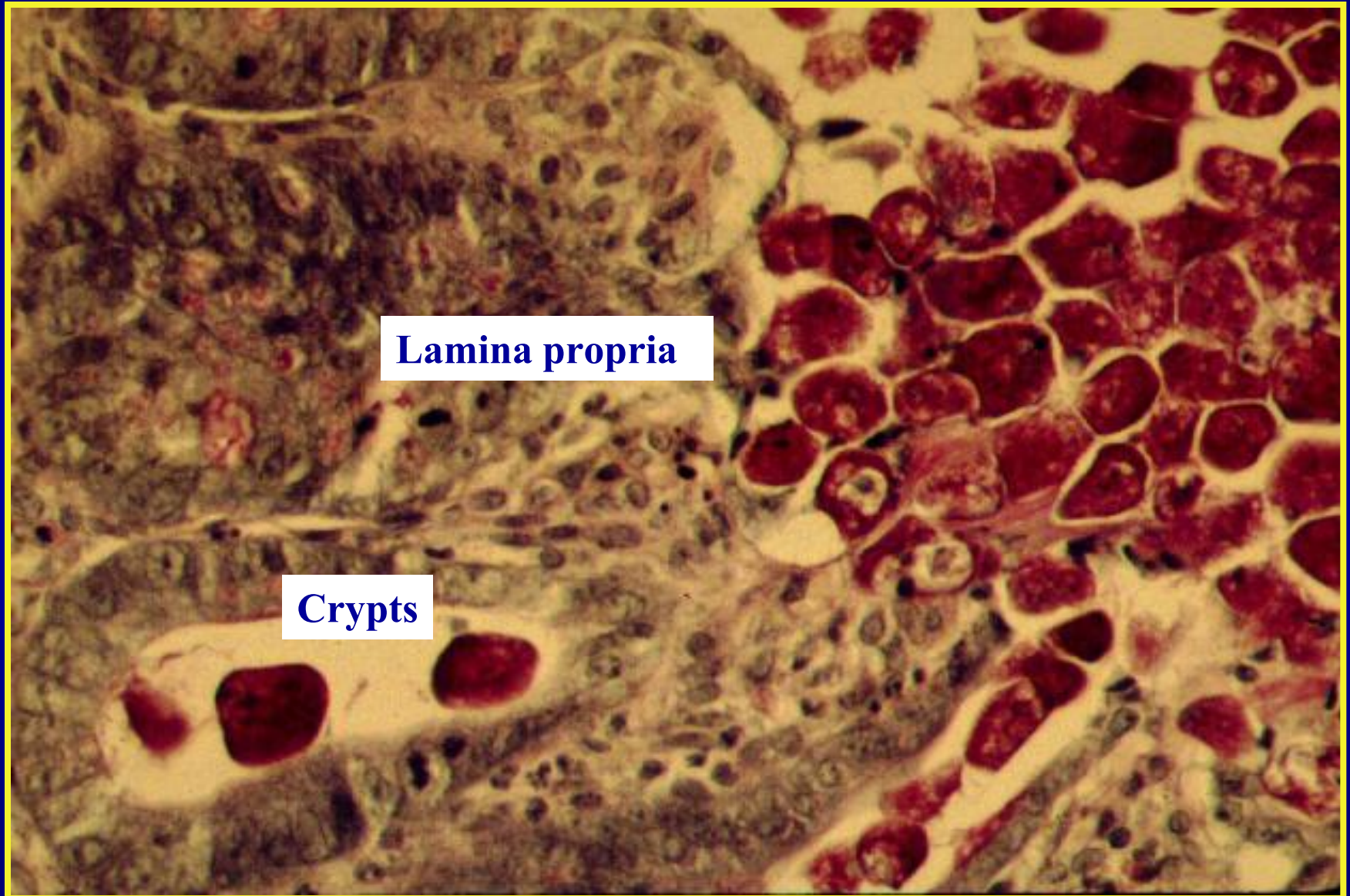


Depletion of the mucus layer and *Eh* adherence to epithelial cells

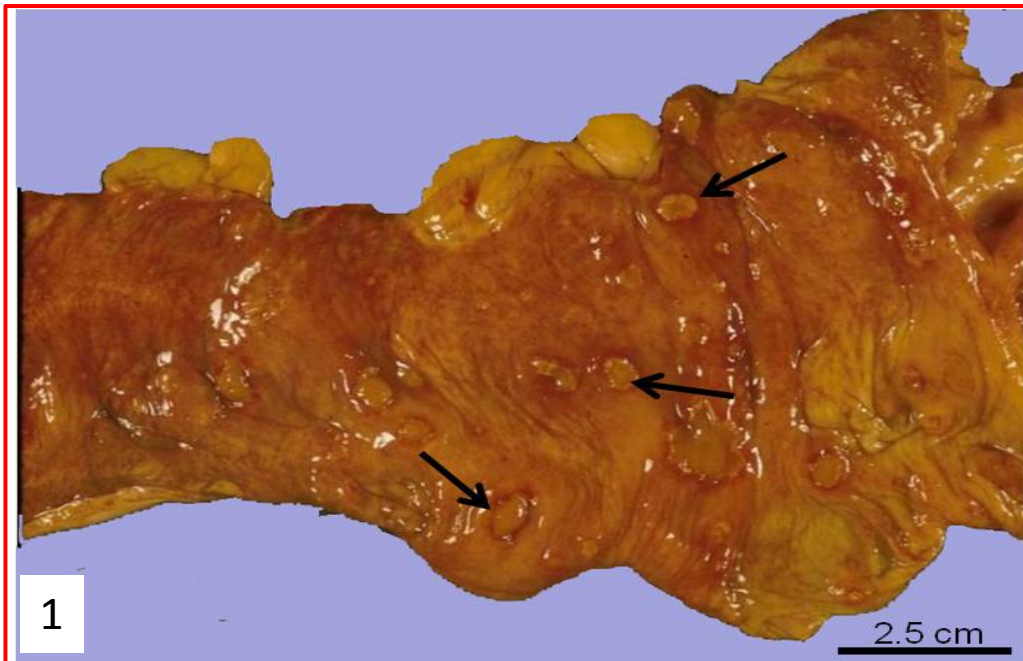


Cover Photo *J. Innate Immunity* (2009)

Eh invasion of the lamina propria and crypts



Invasive Amebiasis

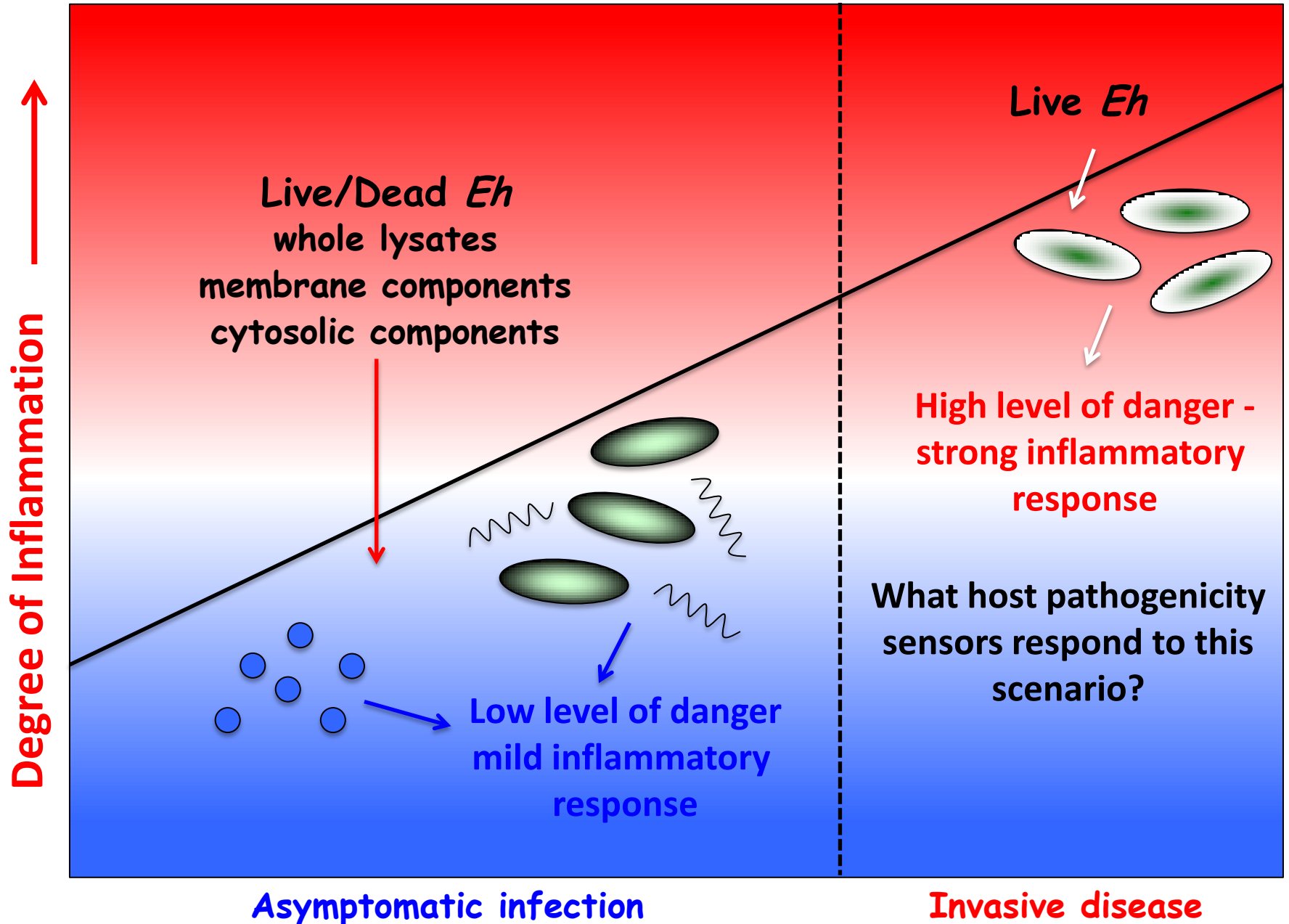


Amebic ulcerative colitis



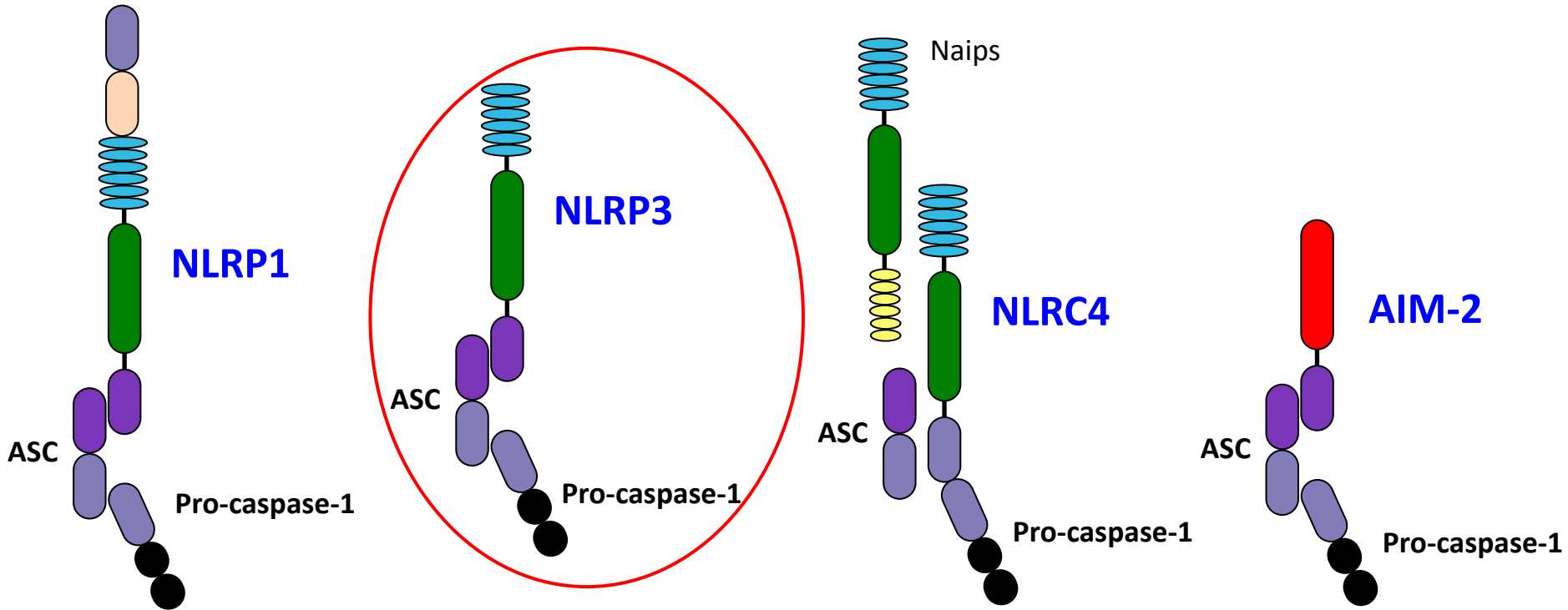
Amebic liver abscess

NLRP3 inflammasome monitor the pathogenicity of *Eh*



Inflammasomes - Caspase-1 Activating Platforms

Intracellular multi-protein complexes that link specific pro-inflammatory stimuli to caspase-1 activation



Bacillus anthracis
lethal toxin

Toxins (nigericin, maitotoxin)

Endogenous danger molecules
(ATP, uric acid crystals)

Bacteria, Fungi, Viruses

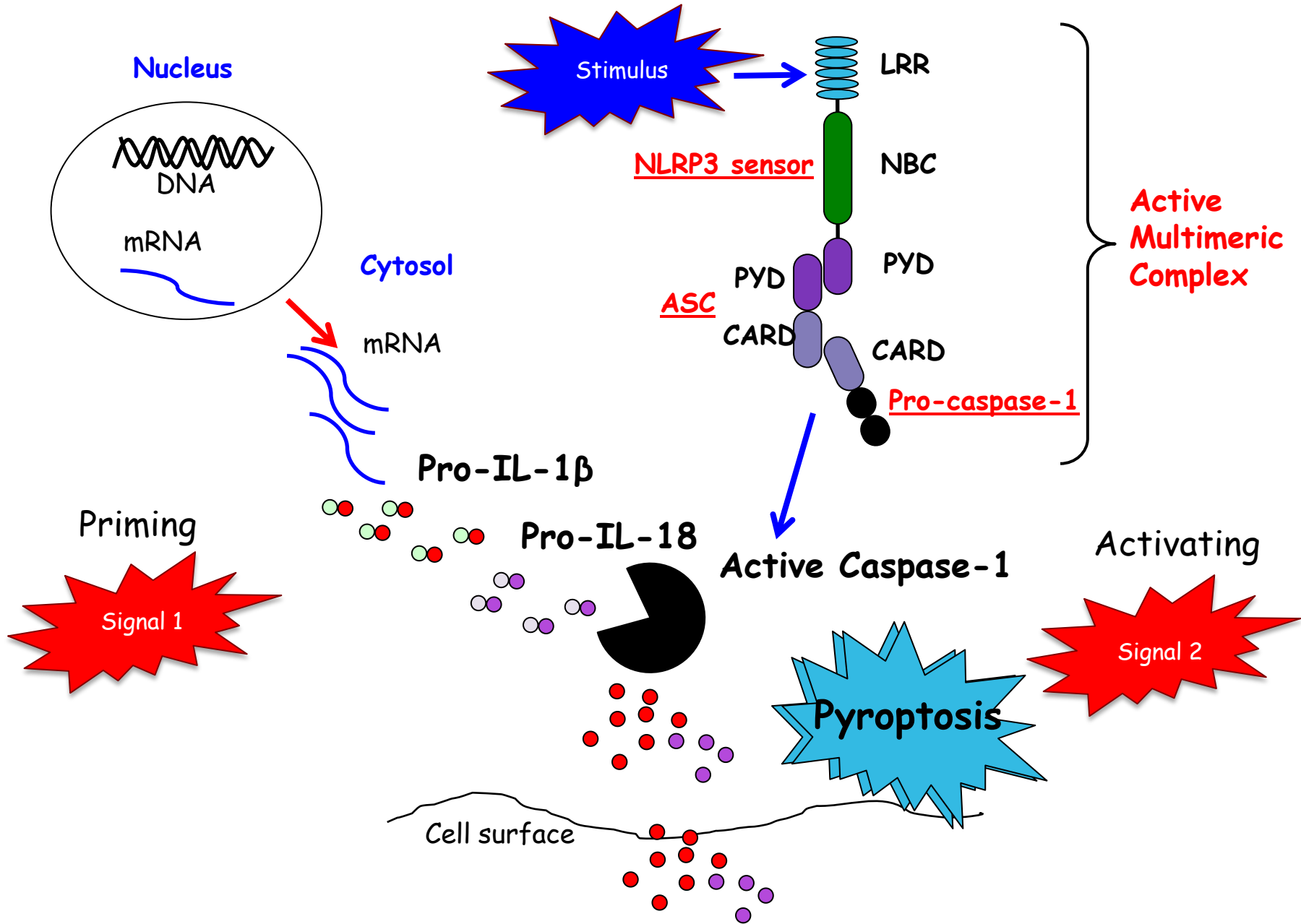
Environmental particulates (silica,
asbestos)

Cytosolic flagellin

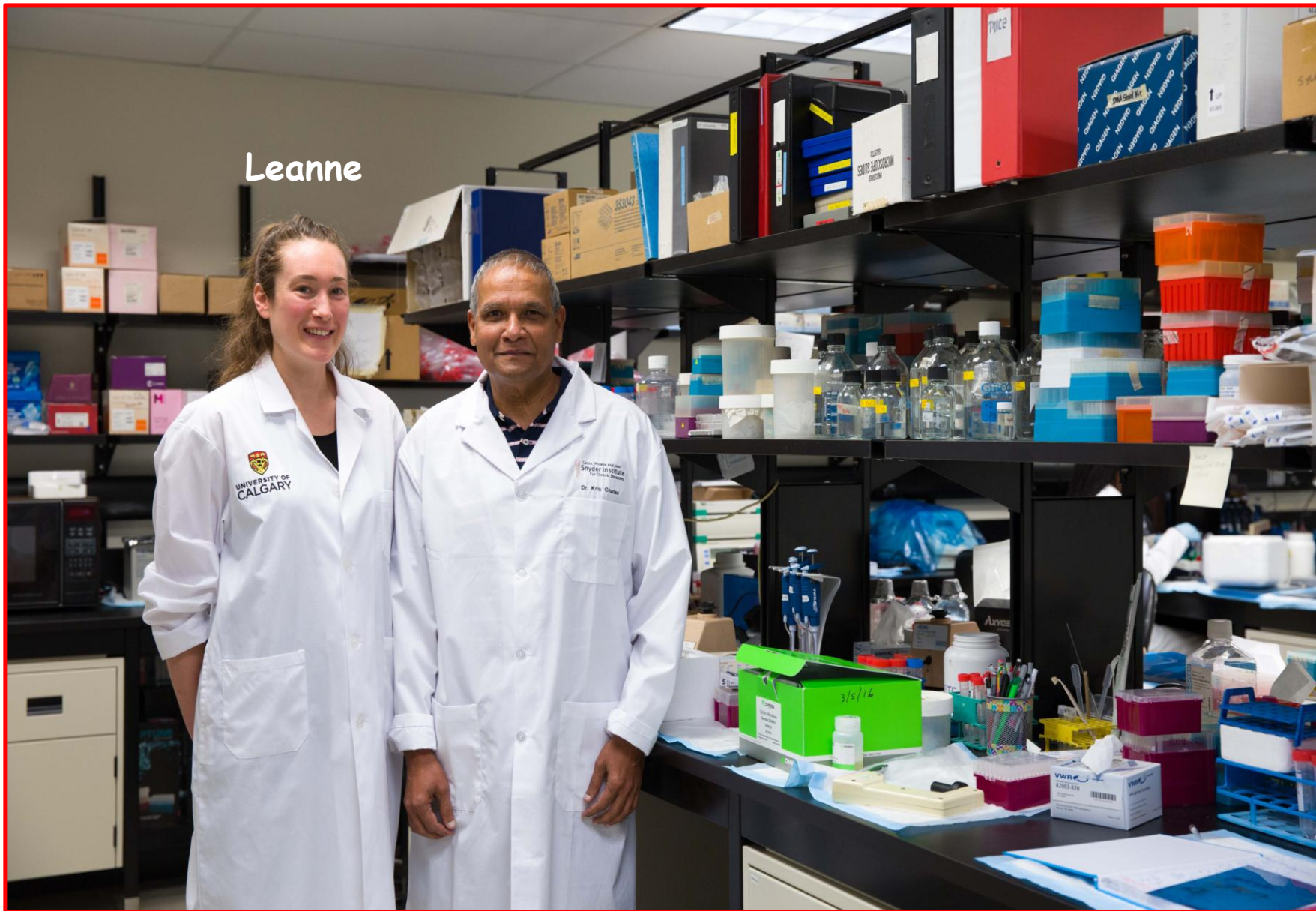
Type III and IV
secretion systems

Cytosolic dsDNA

Inflammasomes - Caspase-1 Activating Platforms



Leanne



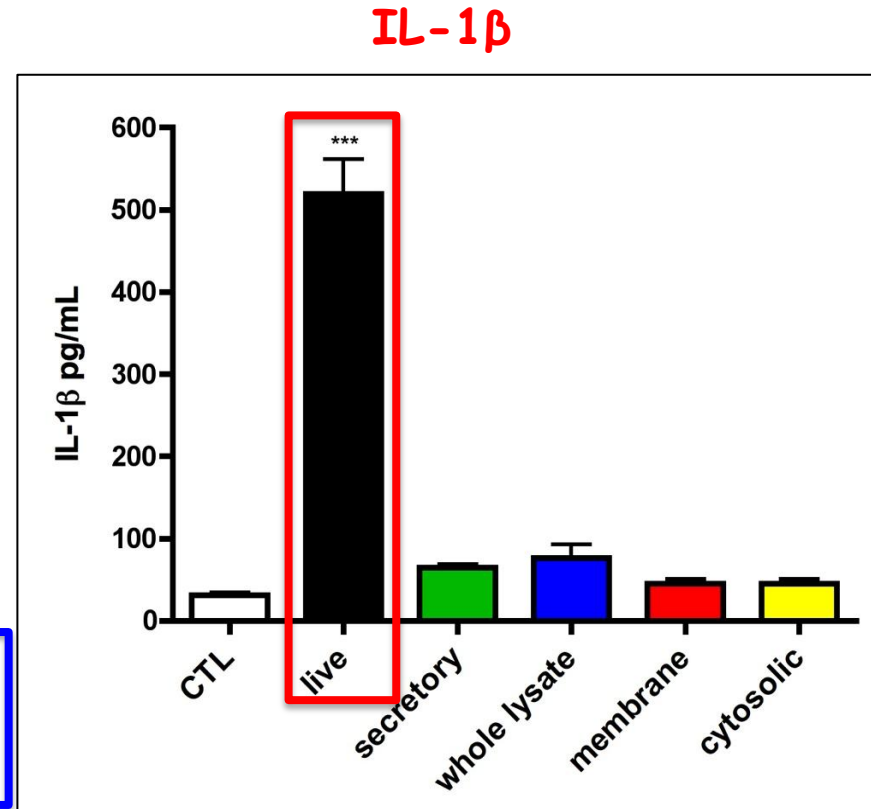
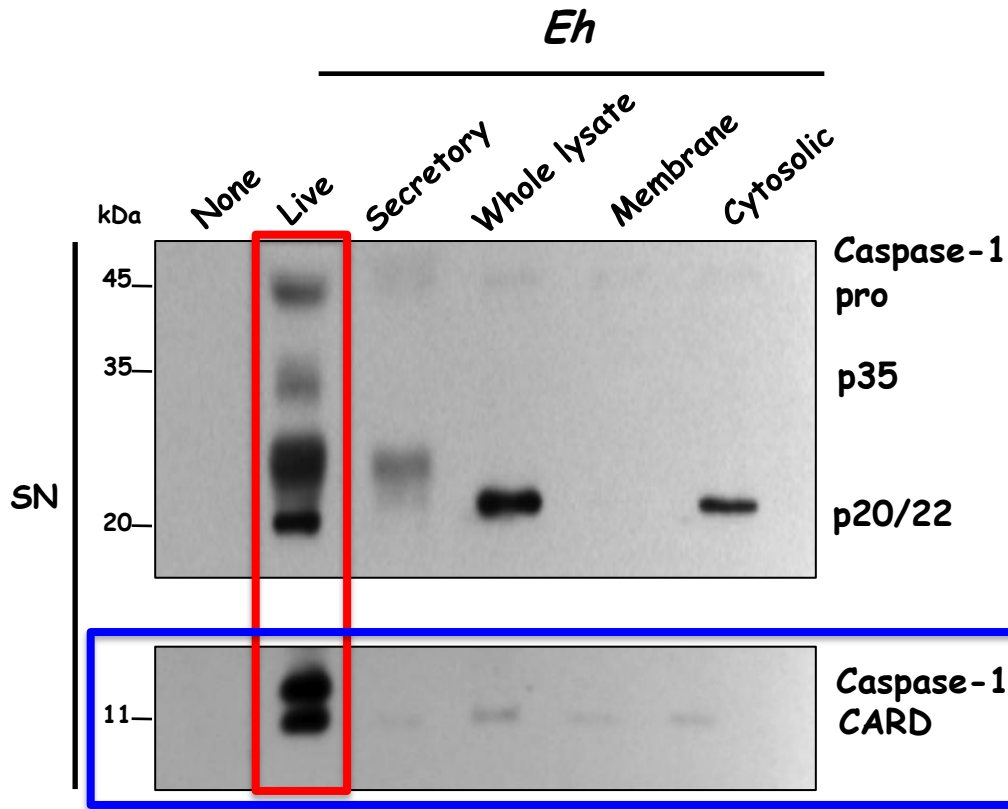


Steve

France

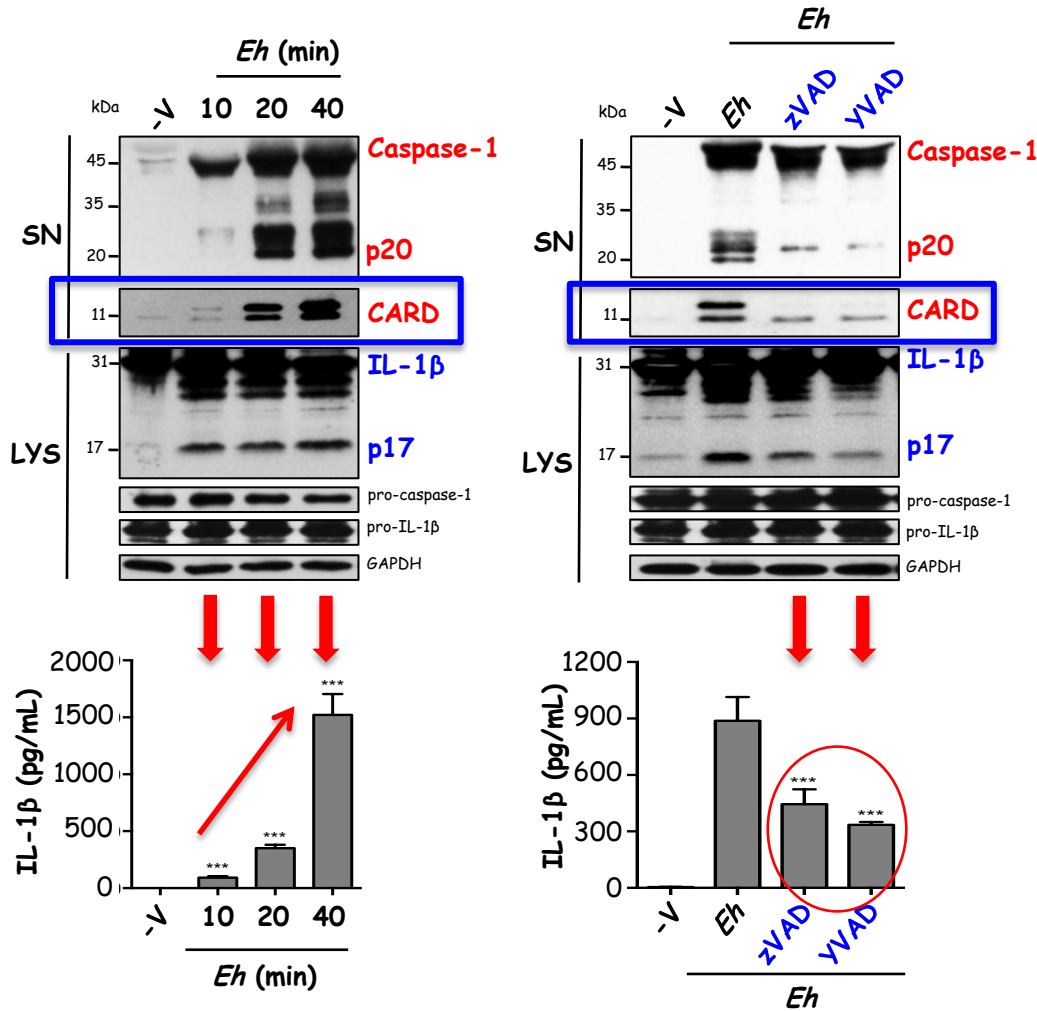
Jeanie

Only live *Eh* activates the inflammasome



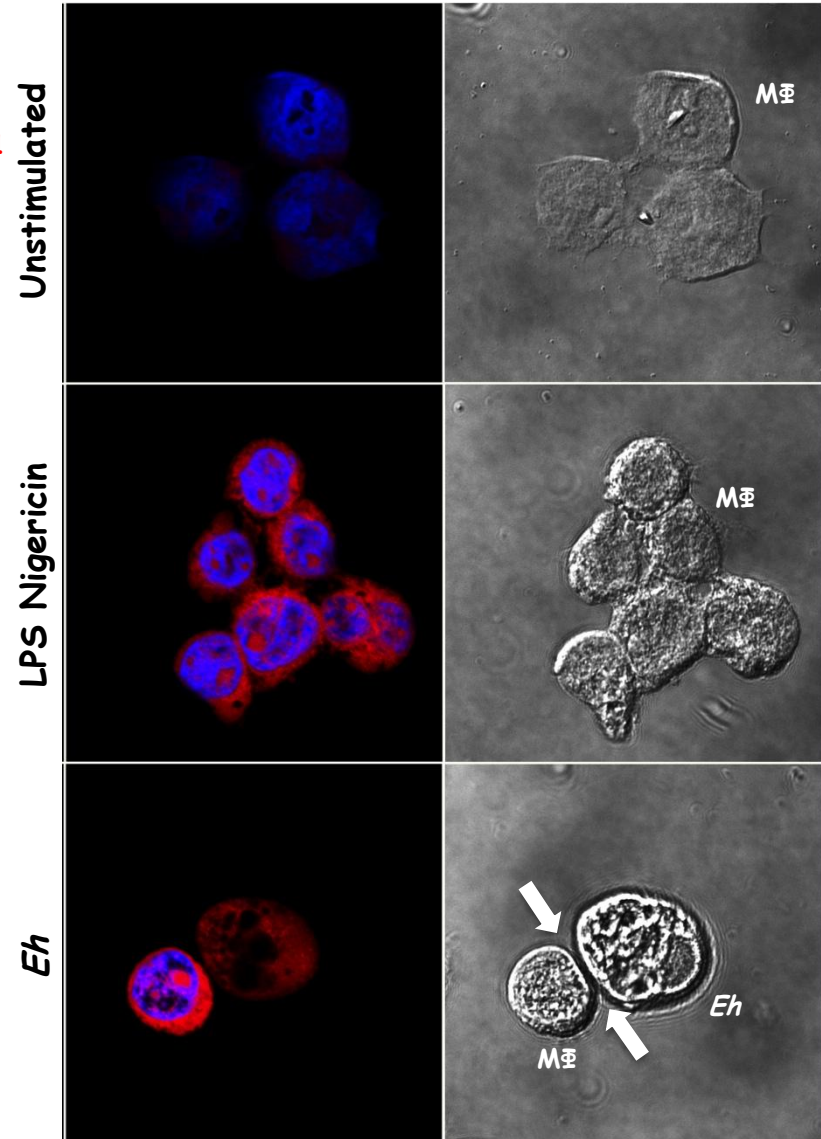
Mortimer, Moreau, Cornick and Chadee (2013) *Mucosal Immunology* 7:829

Eh activates the NLRP3 inflammasome by direct contact



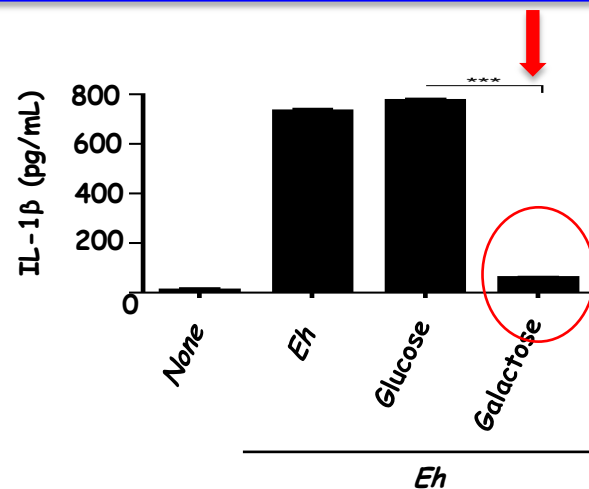
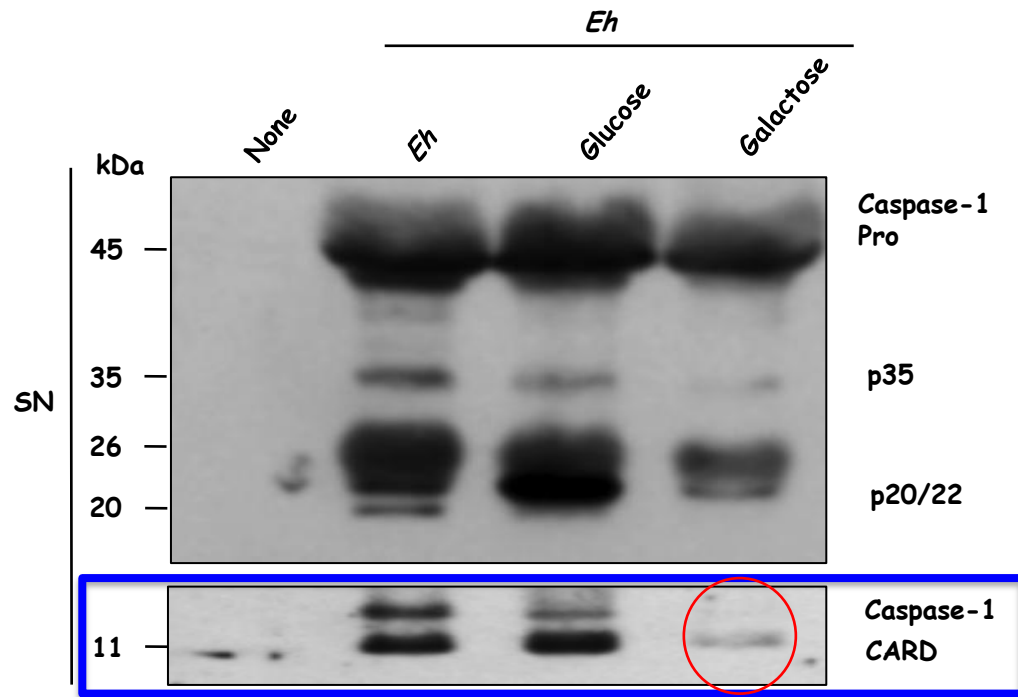
zVAD - pan-caspase inhibitor
 yVAD - caspase-1 specific inhibitor

Active caspase-1 FLICA Mammalian nuclei

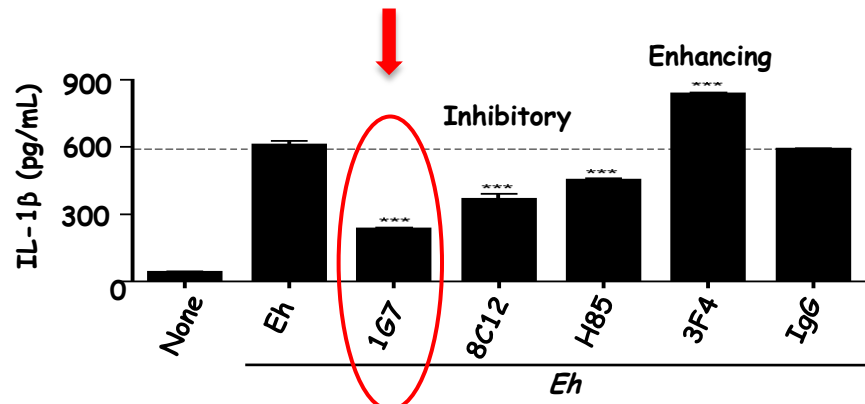
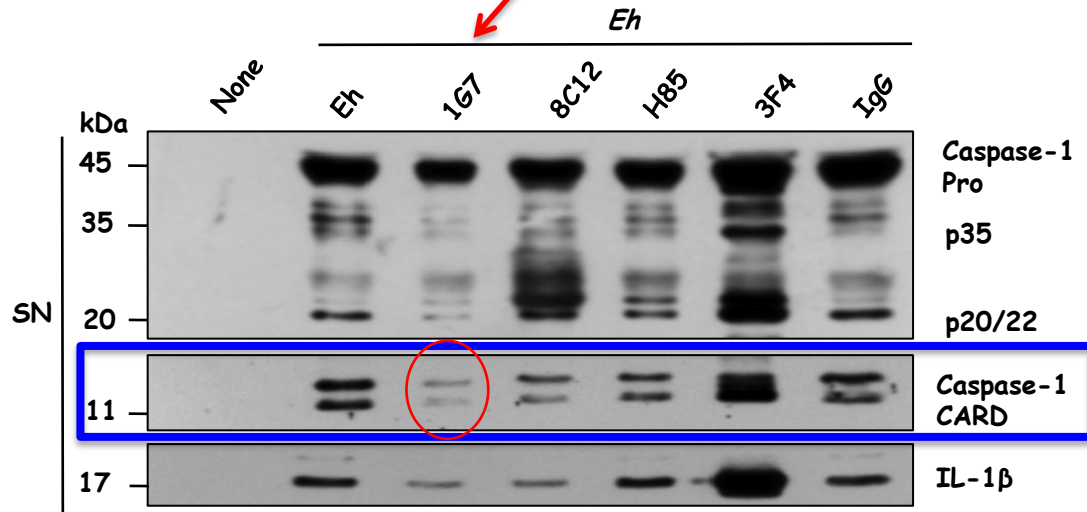
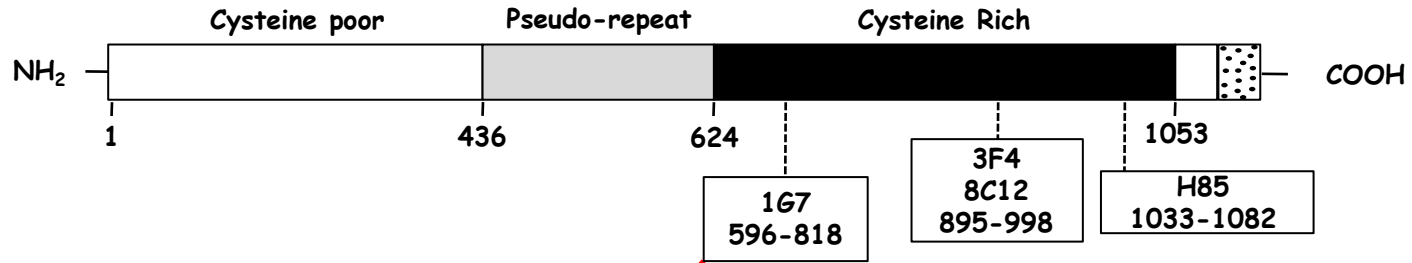


What component on the surface of *Eh* activates the inflammasome?

Surface-bound *Eh* Gal-lectin is required to activate the inflammasome



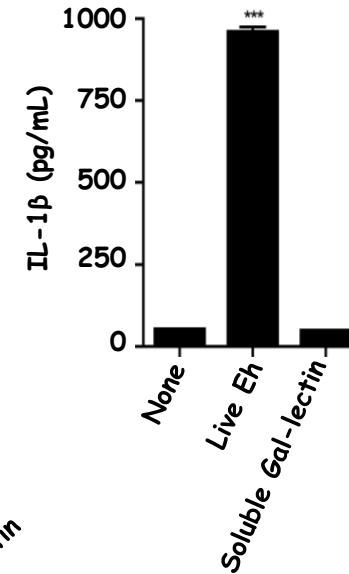
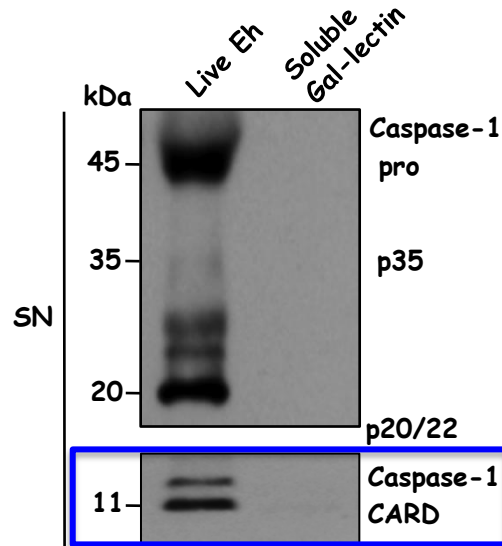
Gal-lectin MAbs bind to and inhibit *Eh*-induced inflammasome activation



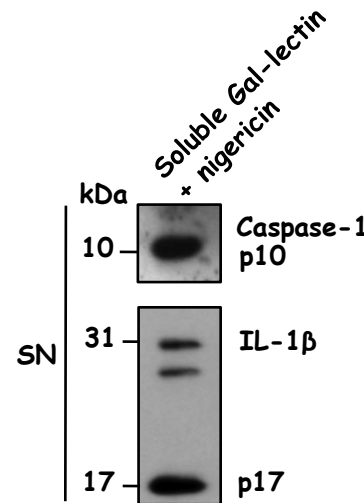
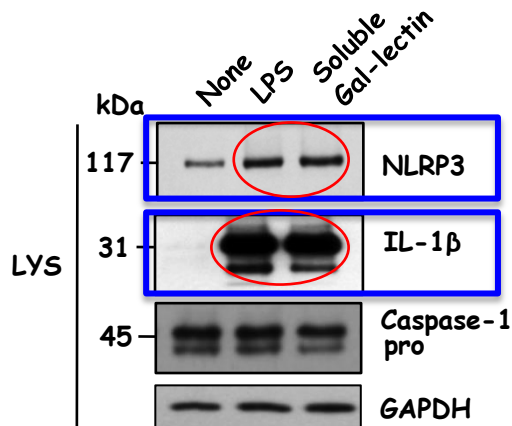
How does *Eh* Gal-lectin activate the
inflammasome?

Soluble *Eh* Gal-lectin primes but does not activate the inflammasome

Human macrophage



BMM

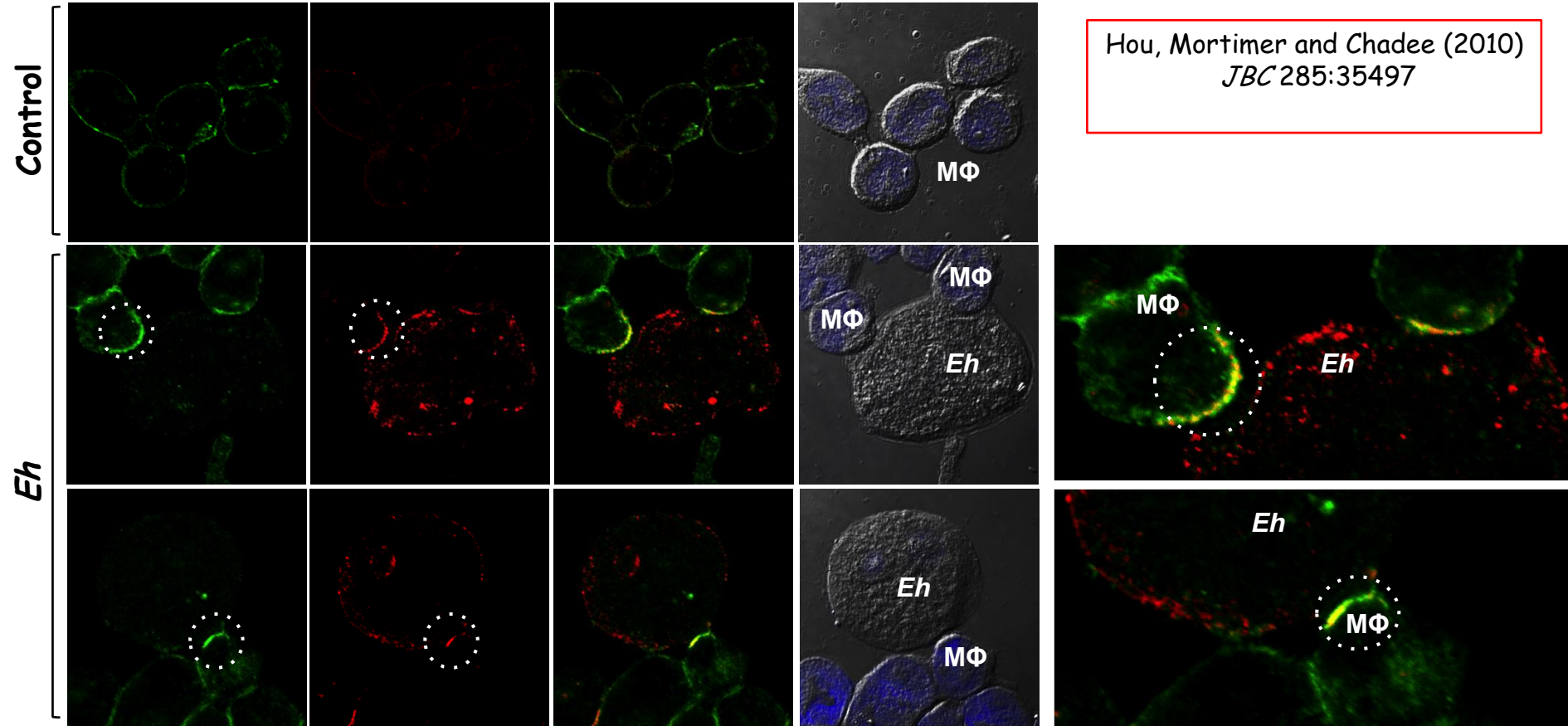


The Gal-lectin up-regulates (primes) IL-1 β and NLRP3 similar to LPS

If the Gal-lectin primes the
inflammasome - what other surface *Eh*
molecule activates it?

What host surface receptor is at the contact site?

$\alpha_5\beta_1$ integrin phospho-paxillin merge mammalian nuclei

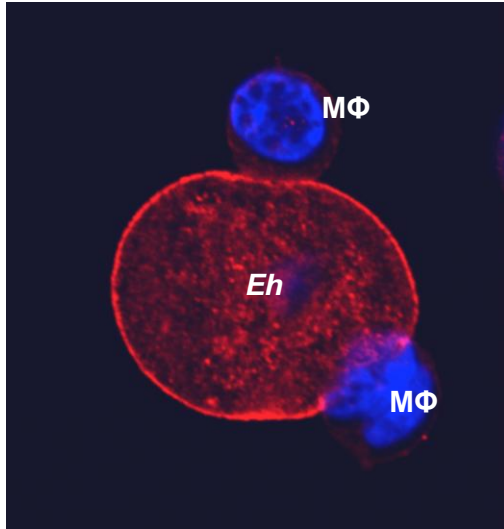


Hou, Mortimer and Chadee (2010)
JBC 285:35497

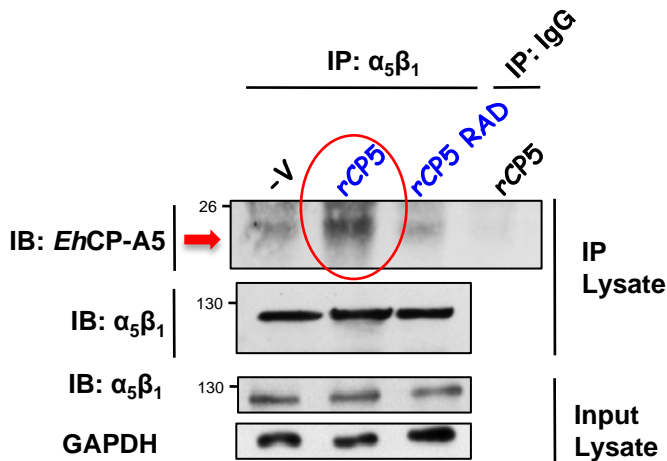
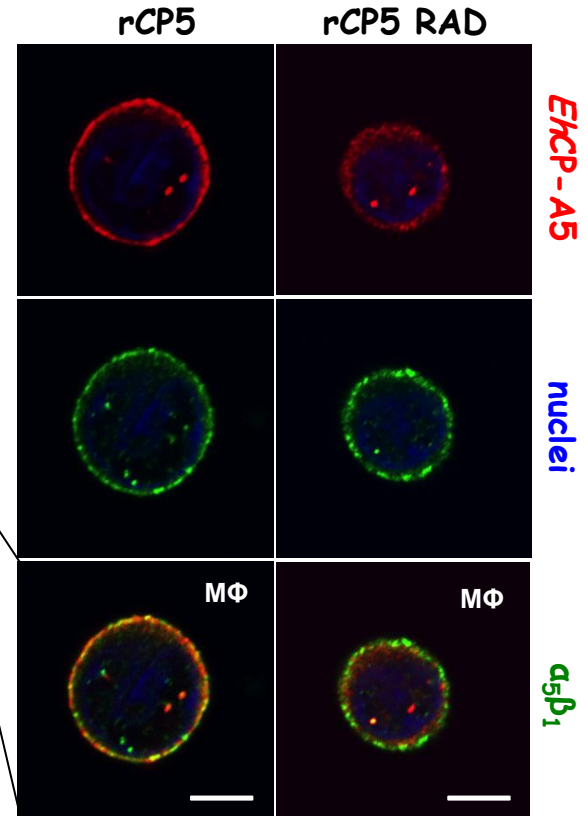
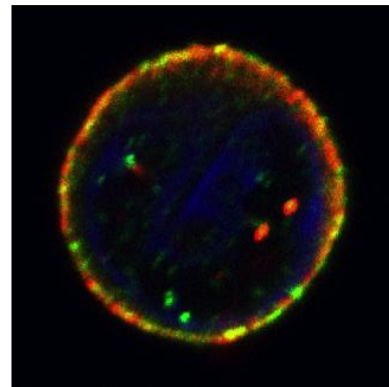
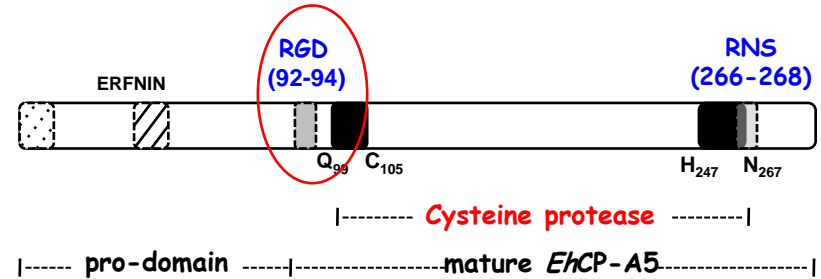
$\alpha_5\beta_1$ integrin and phosphorylated-paxillin are recruited to sites of contact where there is active integrin signaling

Surface-bound *EhCP-A5* interacts with $\alpha_5\beta_1$ integrin

EhCP-A5 mammalian nuclei

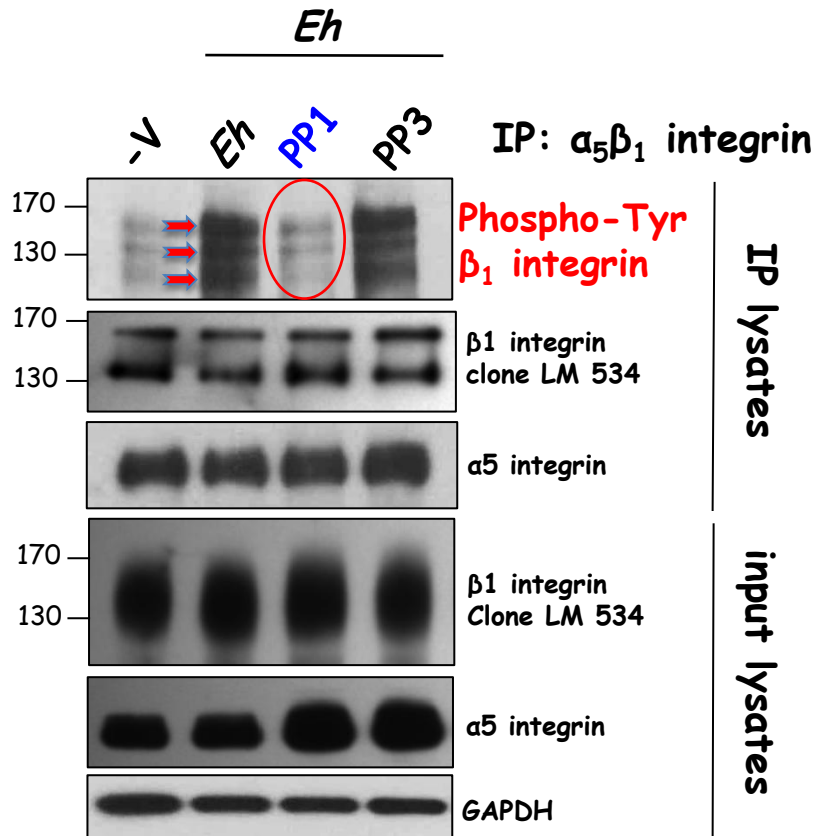


Structural Organization of *EhCP-A5*

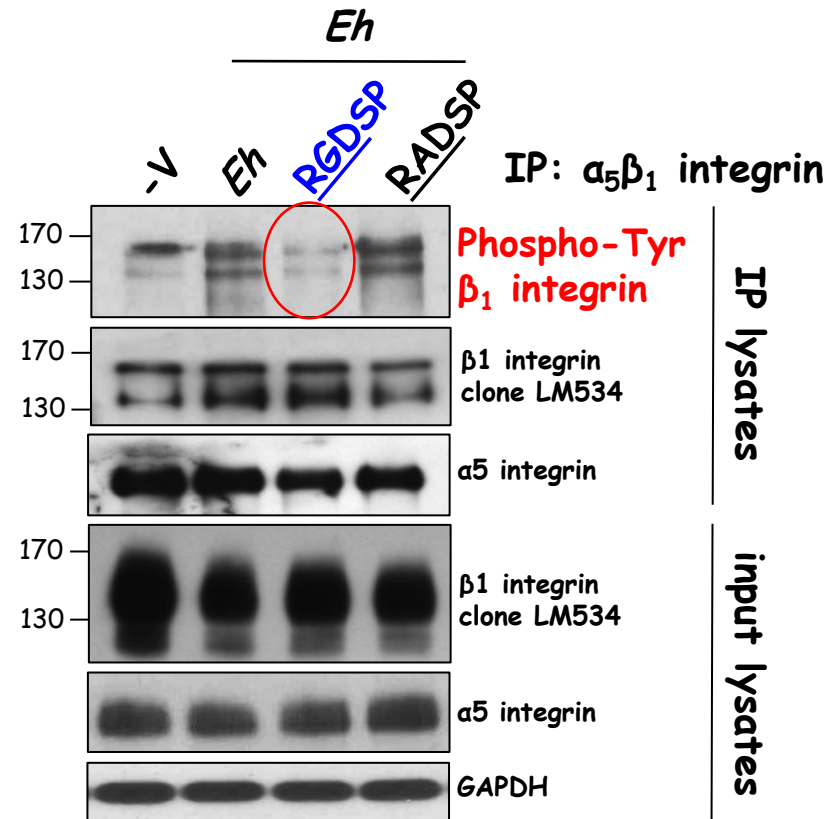


Mortimer, Moreau, Cornick and Chadee (2015) *PLoS Pathogens*.
doi: 10.1371

EhCP-A5 RGD- $\alpha_5\beta_1$ integrin signaling upon contact

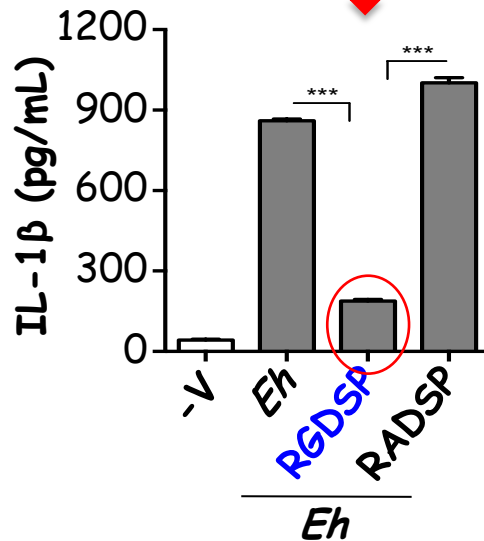
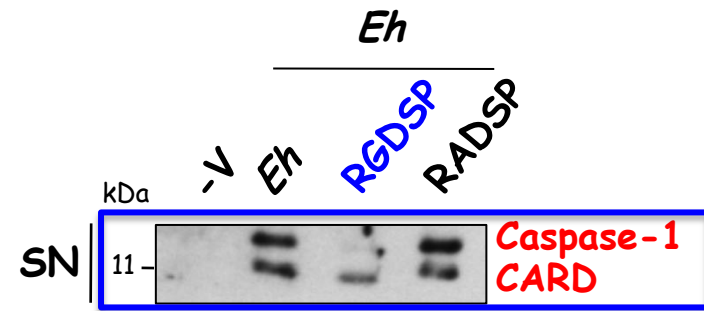


SFK phosphorylation (160/140/120kDa)
inhibited by SFK inhibitor PP1 but not
inactive analog PP3

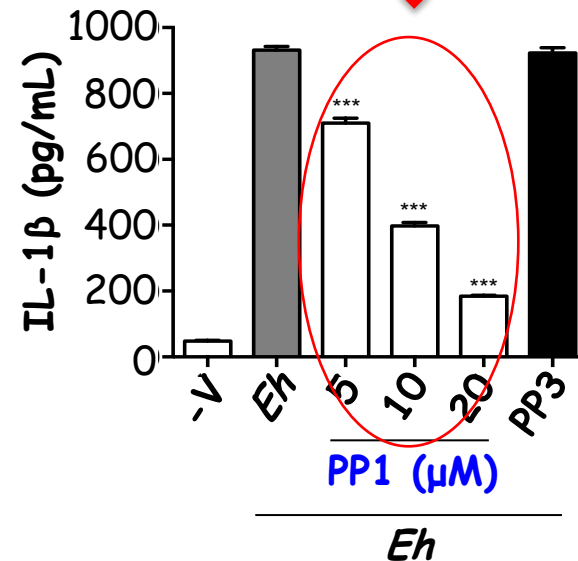
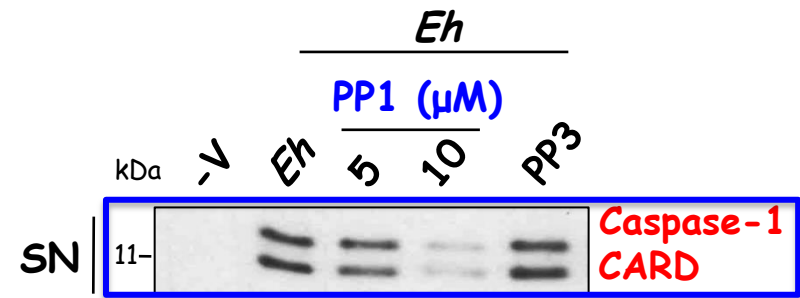


Specificity for *EhCP-A5* RGD binding
with RGDSP-inhibitory peptide but
not RADSP control

Is $\alpha_5\beta_1$ integrin activation required for *Eh* to activate the NLRP3 inflammasome?

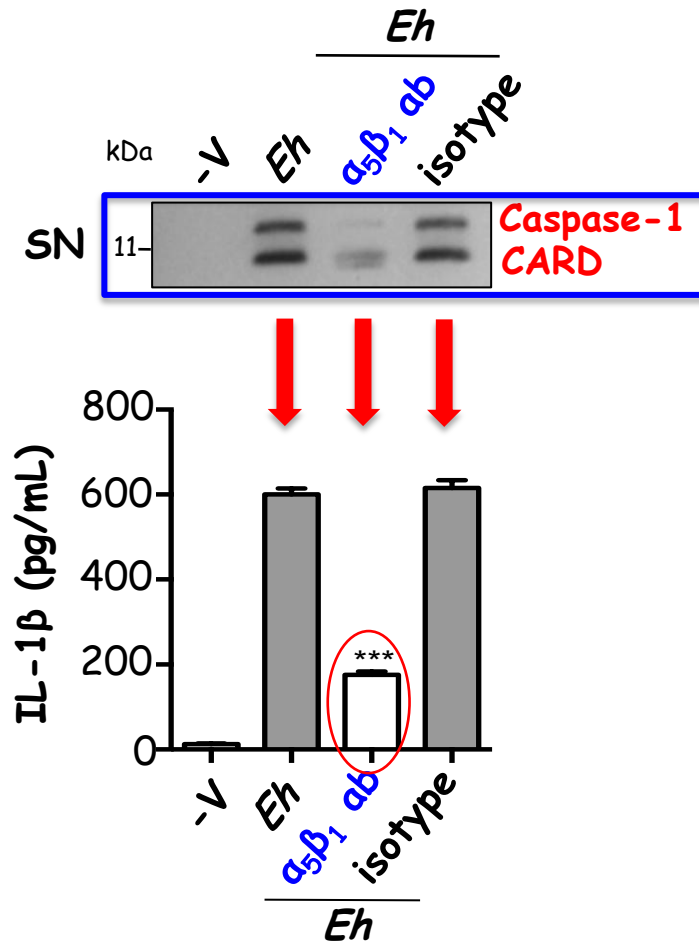


RGDSP = inhibitory peptide



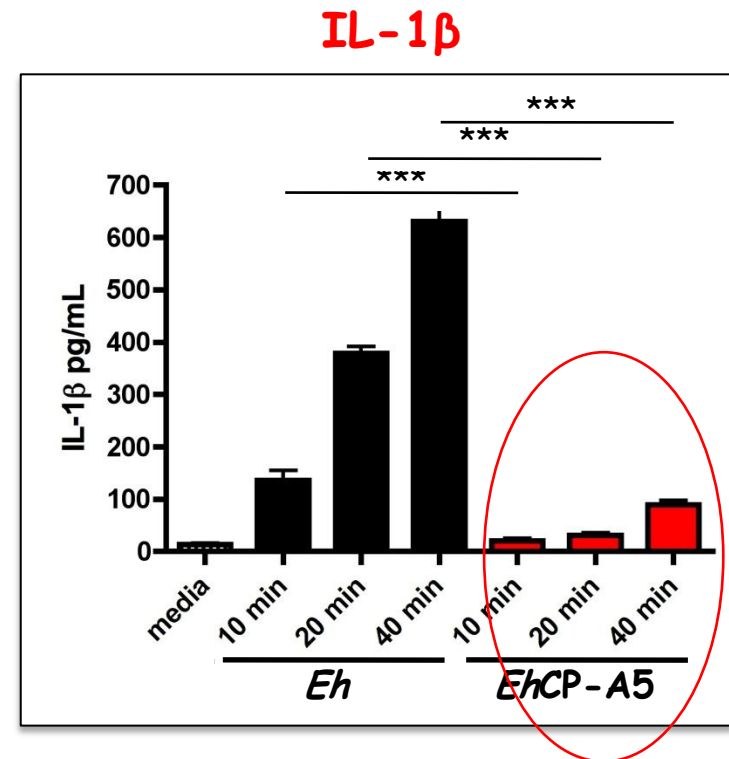
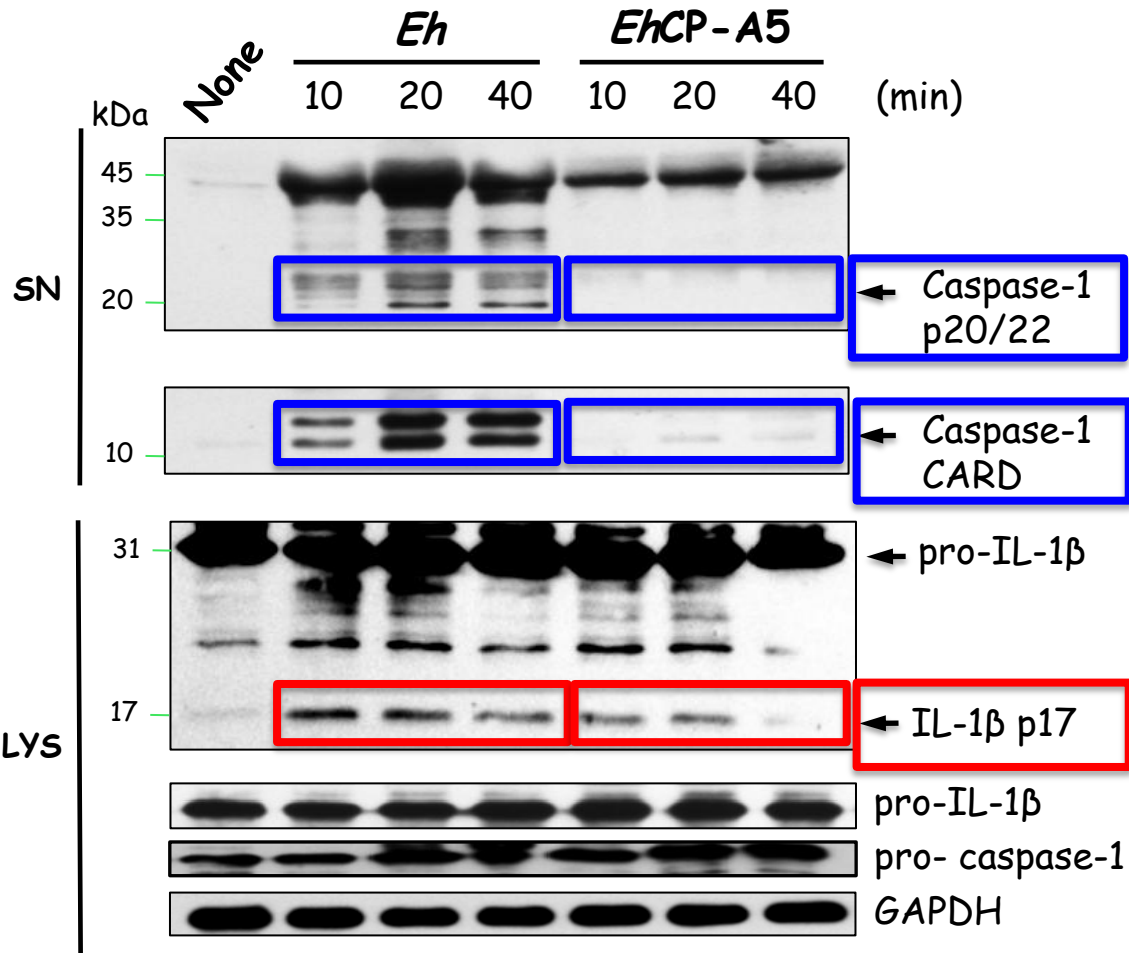
PPI = SFK inhibitor

Is activation of $\alpha_5\beta_1$ integrin required for *Eh* to activate the NLRP3 inflammasome?



Function blocking Abs to α_5 and β_1 subunits and $\alpha_5\beta_1$ abrogated caspase-1 and IL-1 β secretion

Inflammasome activation requires *EhCP-A5*

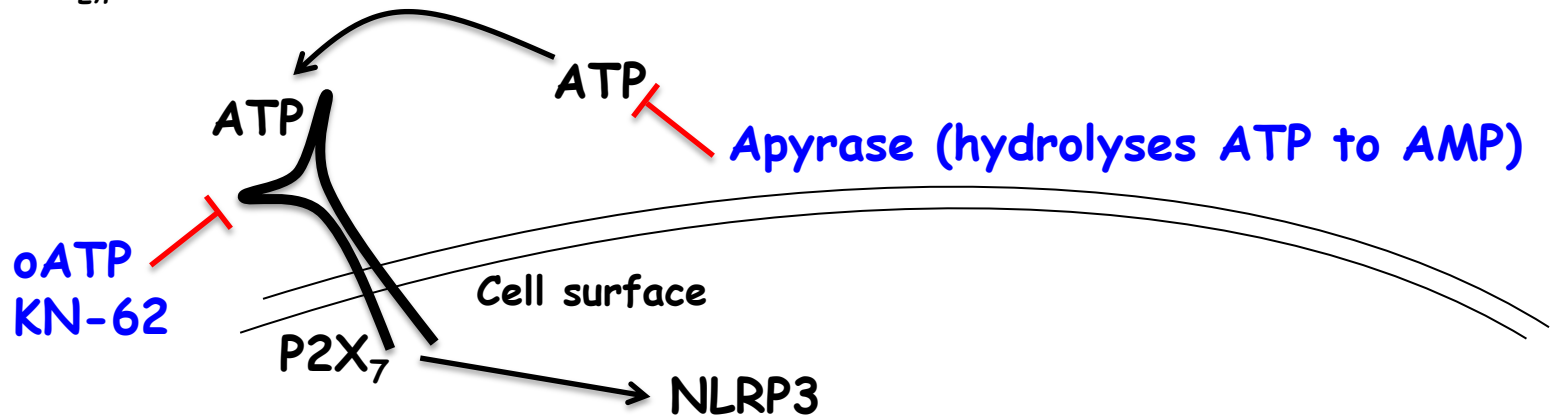
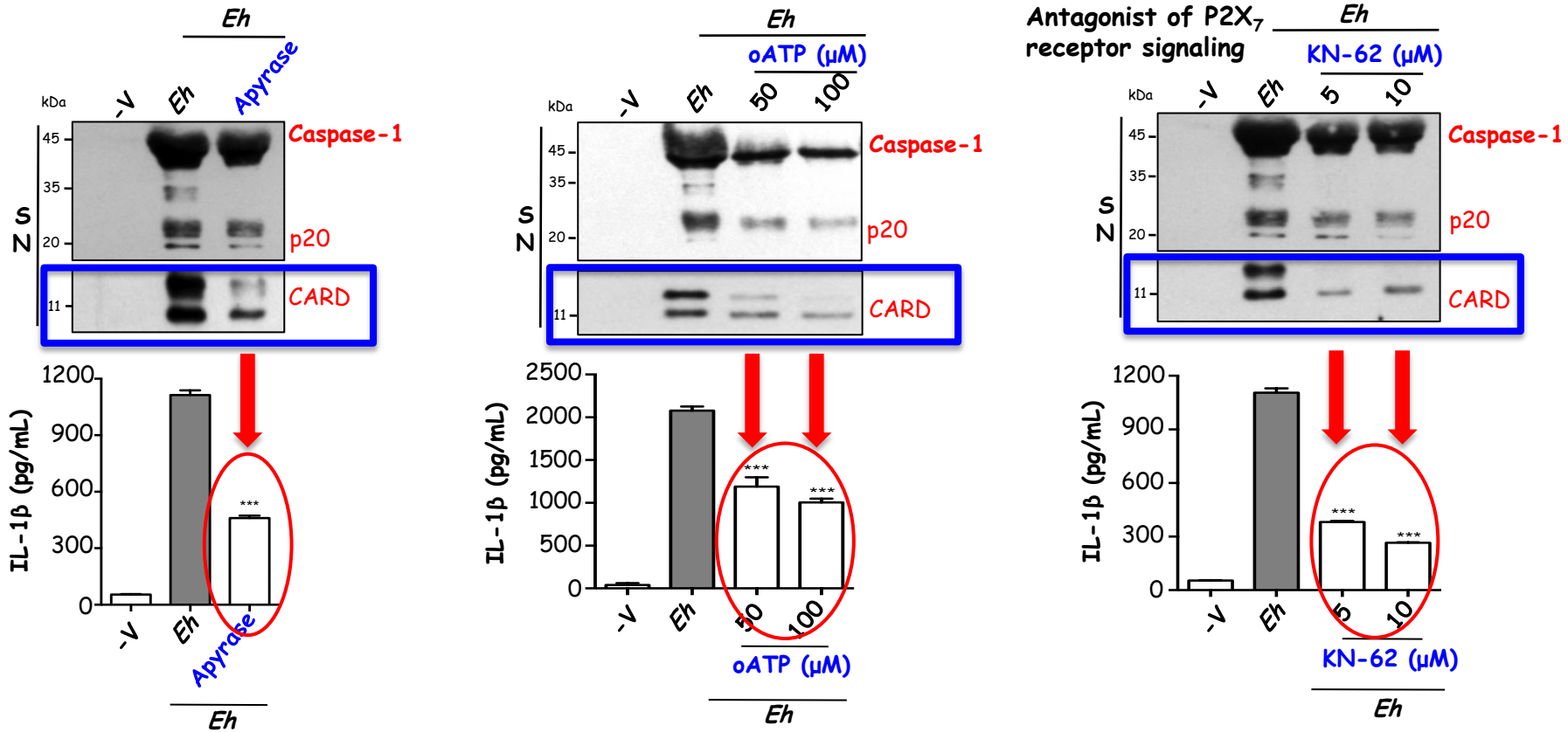


How does *EhCP-A5- $\alpha_5\beta_1$* integrin signaling regulate NLRP3 activation?

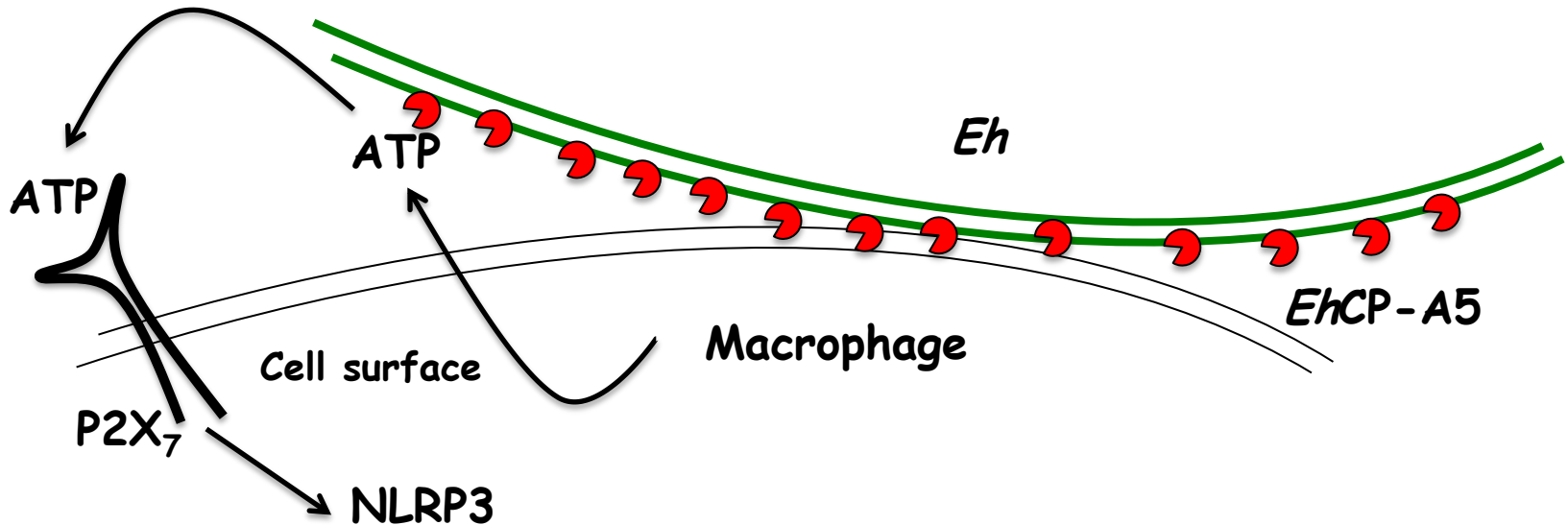
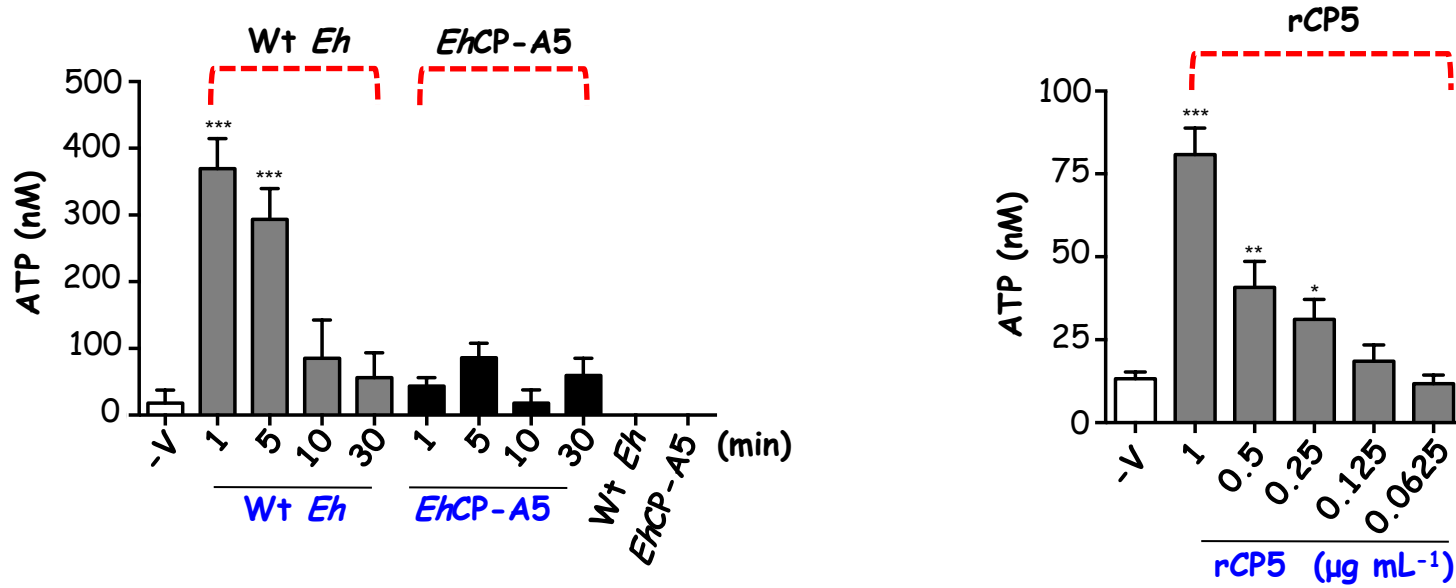
The kinetics of NLRP3 activation is very rapid
(can detect within 10 minutes)

This is similar to the rate of NLRP3 activation by ATP
(high extracellular ATP is an endogenous activator of
NLRP3)

Is ATP required for *Eh* to activate the NLRP3 inflammasome?

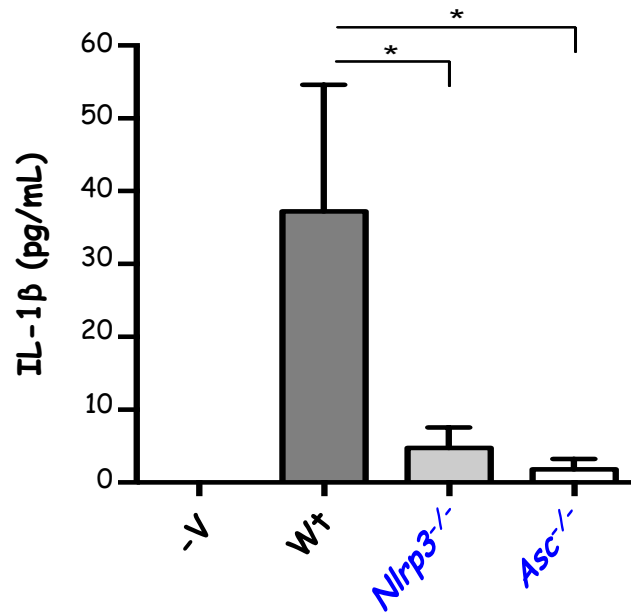


Does *EhCP-A5* trigger ATP release from macrophages?

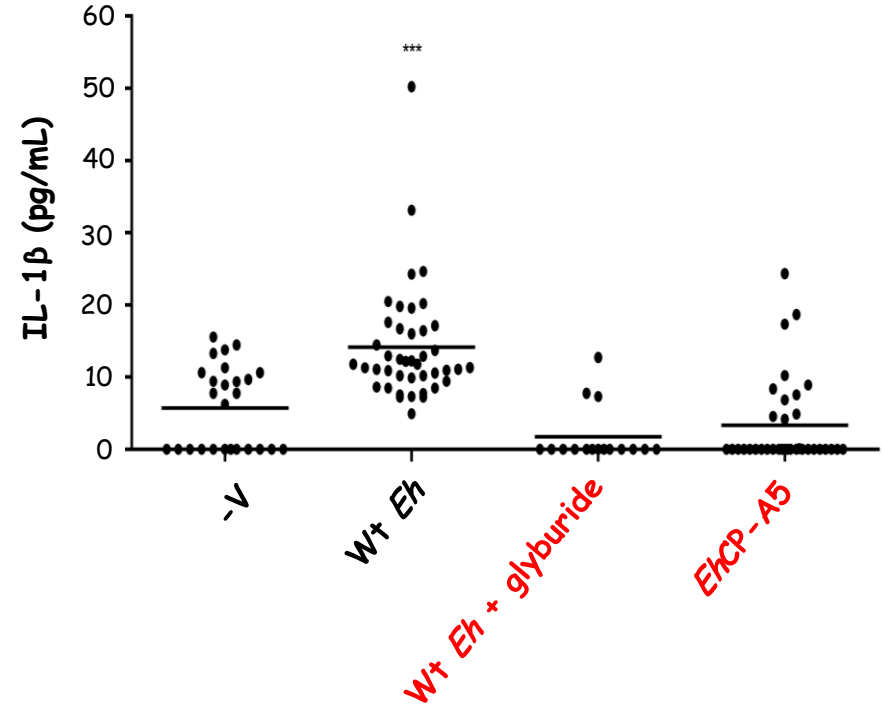


Is NLRP3 inflammasome required for *Eh*-induced pro-inflammatory responses in the colon?

Mouse colonic loops inoculated with *Eh*



Healthy human colon biopsy exposed to *Eh*



Glyburide - NLRP3 inhibitor

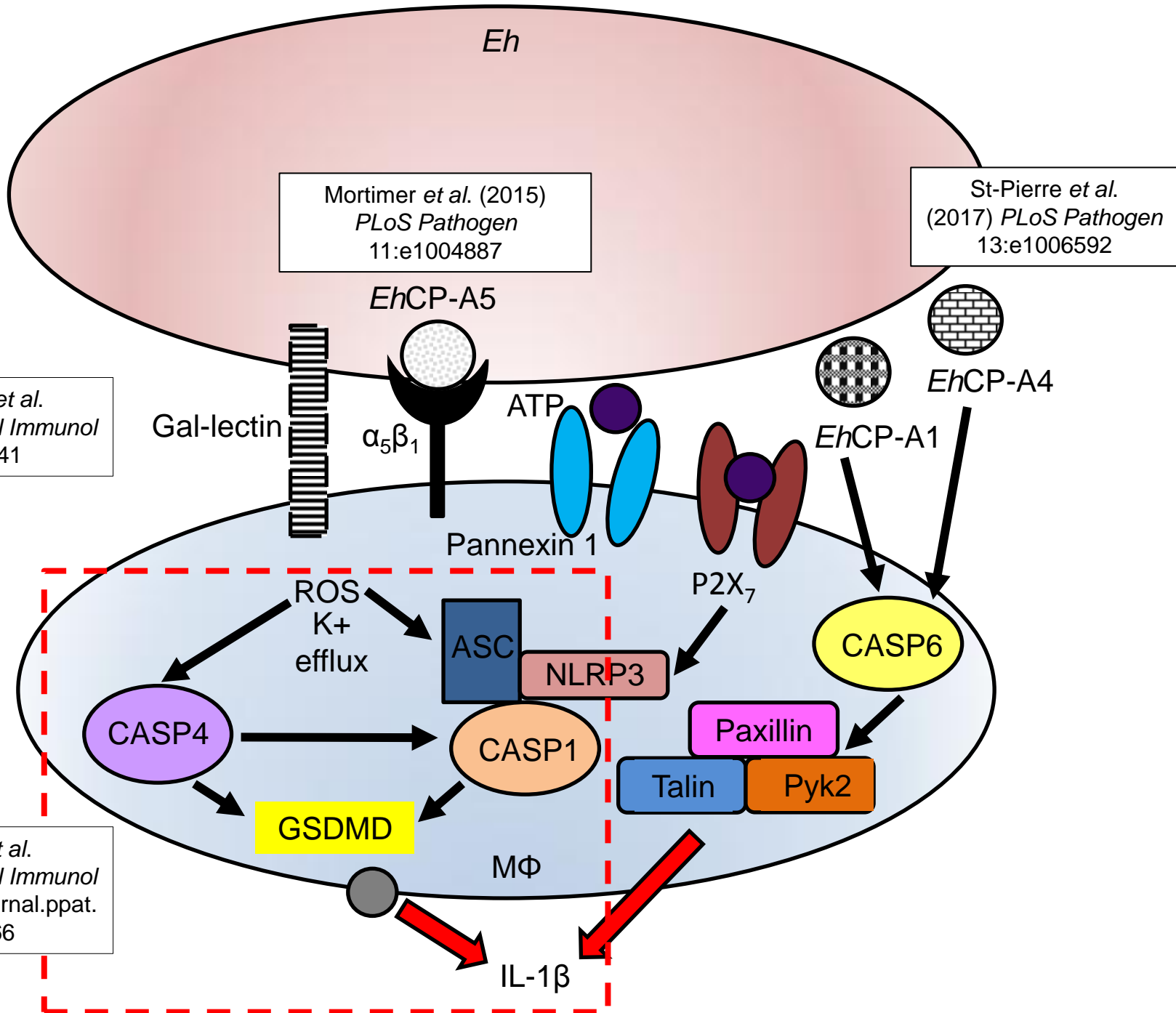
Take home messages

Eh primes the NLRP3 inflammasome upon formation of an immune synapse mediated by the Gal-lectin

Gal-lectin binding facilitates *Eh*CP-A5 ligation and activation of $\alpha_5\beta_1$ integrin and NLRP3 inflammasome activation



NLRP3 inflammasome functions as a pathogenicity sensor for detecting invading *Eh*



Acknowledgements

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Dr. Joëlle St-Pierre

Dr. Leanne Mortimer

Dr. Adelaide Tawiah

Cells/Mice

Dr. Yan Shi (Casp-1/11 KO mice),

Dr. Yates (HEK-IL-1 β cells, COS-7)

Dr. Dan Muruve (NLRP3 and ASC KO mice, NLRP3 CRISPR KO THP-1)

Dr. Bruce Vallance (Casp-11 KO mice)

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CIHR

NSERC

CCC

CFI

Trainee Scholarships



11.10.2013

***Eh* virulent molecules**

Gal-lectin

Gal-lectin + *Eh*CP-A5

PGE₂

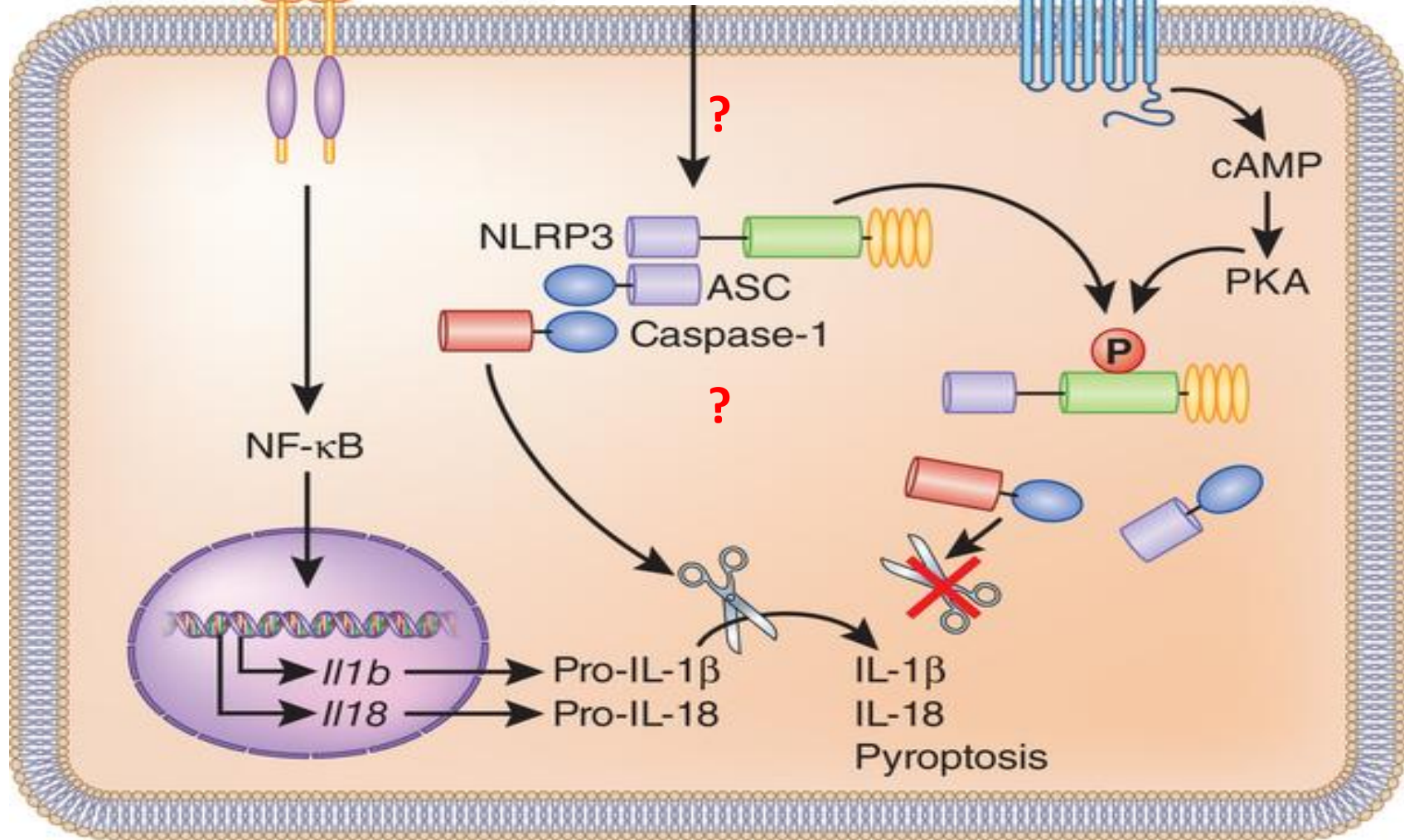
Mortimer . . Chadee (2016)
Nature Immunology 10:1

Host receptors

TLR4

$\alpha 5\beta 1$ integrin

EP4

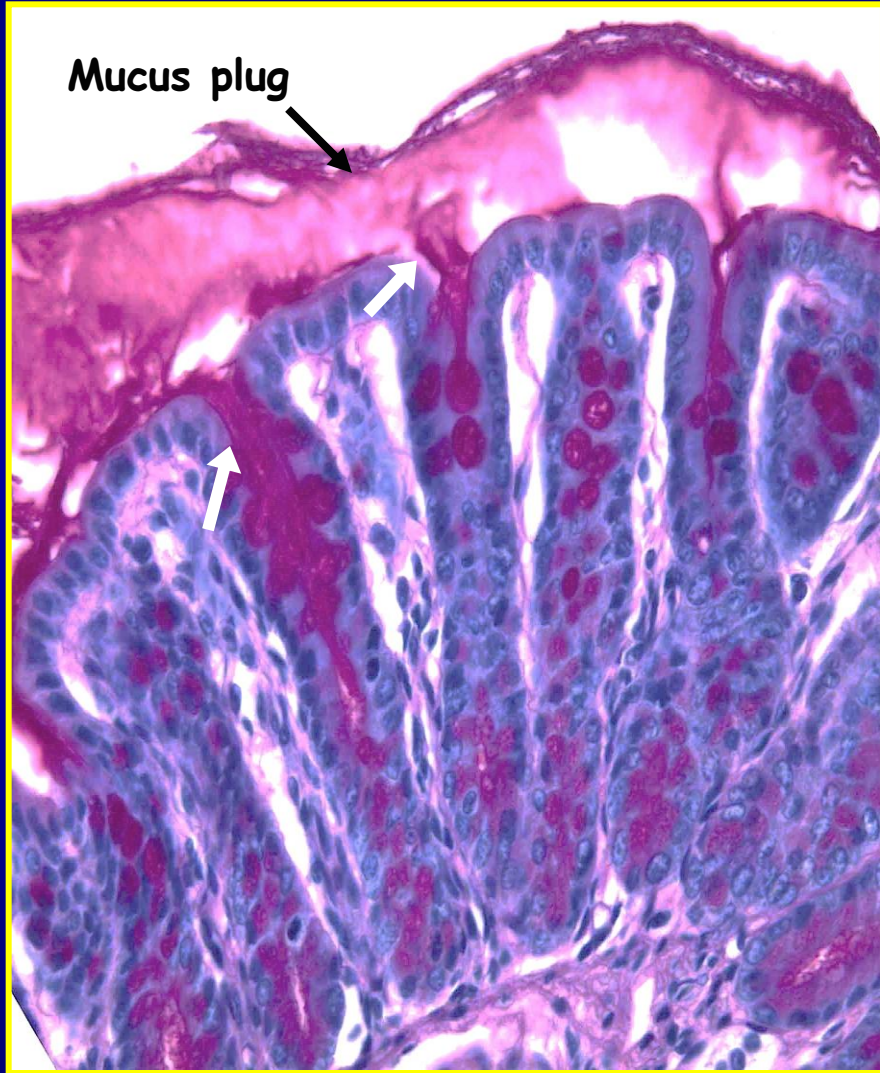


Parasite-induced inflammasome activation in disease pathogenesis

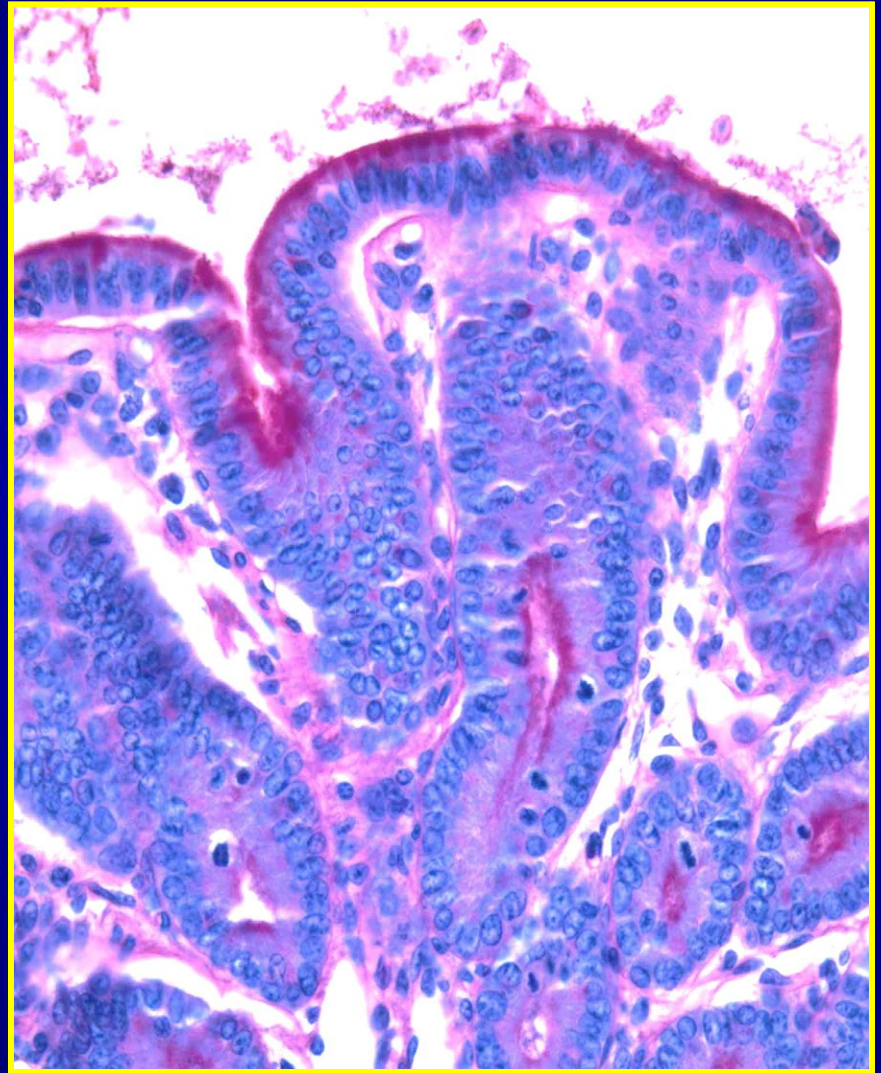
Pathogen driven NLRP3 inflammasome activation is a sensor for initiating gut inflammation

What are the predominant responses in the gut in the absence of *Eh*-epithelial cell contact?

No *Eh*-epithelial cell damage in mucus-deficient animals

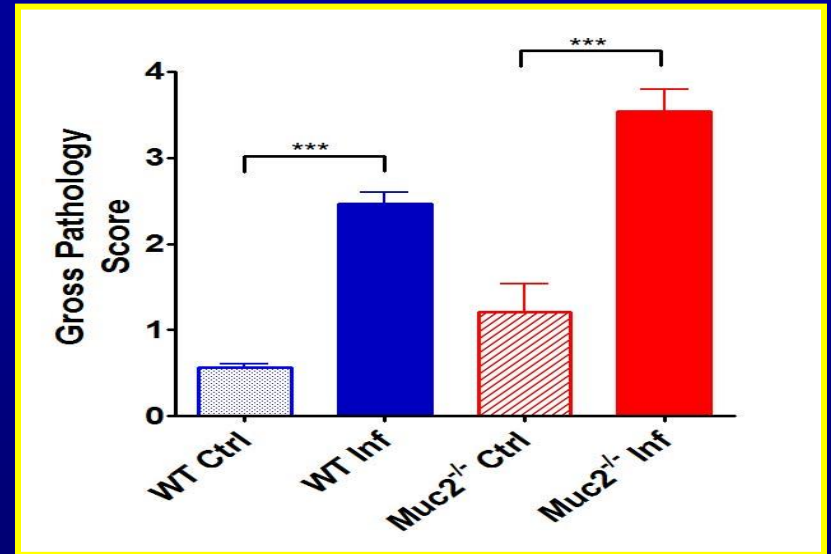
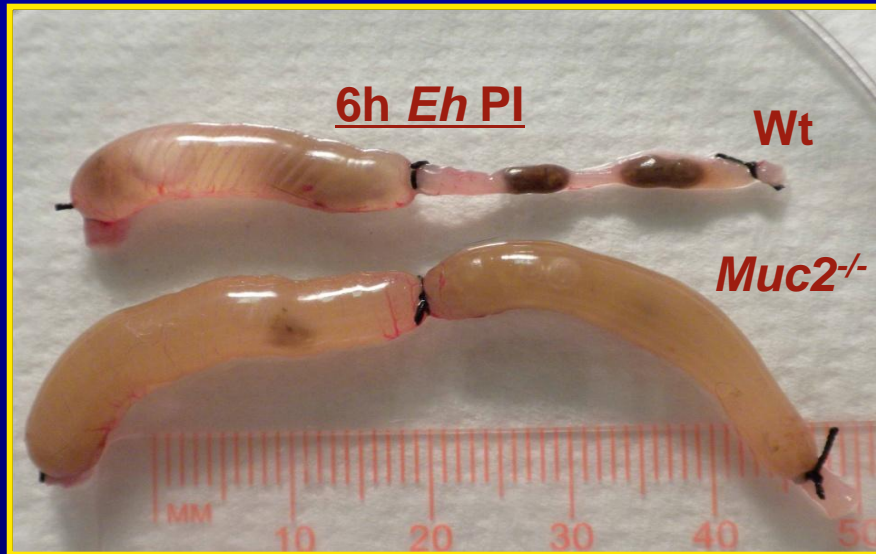
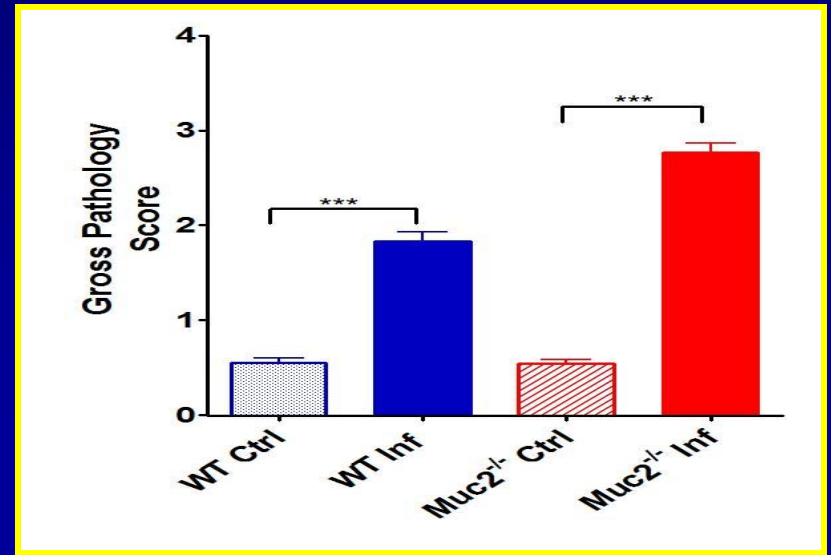
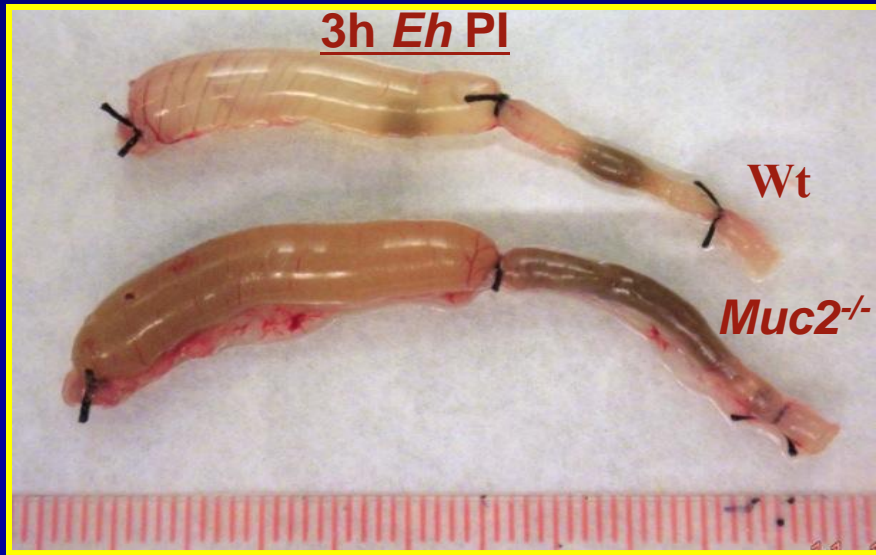


Wt 3h PI



Muc2^{-/-} 3h PI

The secretory response consist of water, mucus, serum albumin and pro-inflammatory cytokines



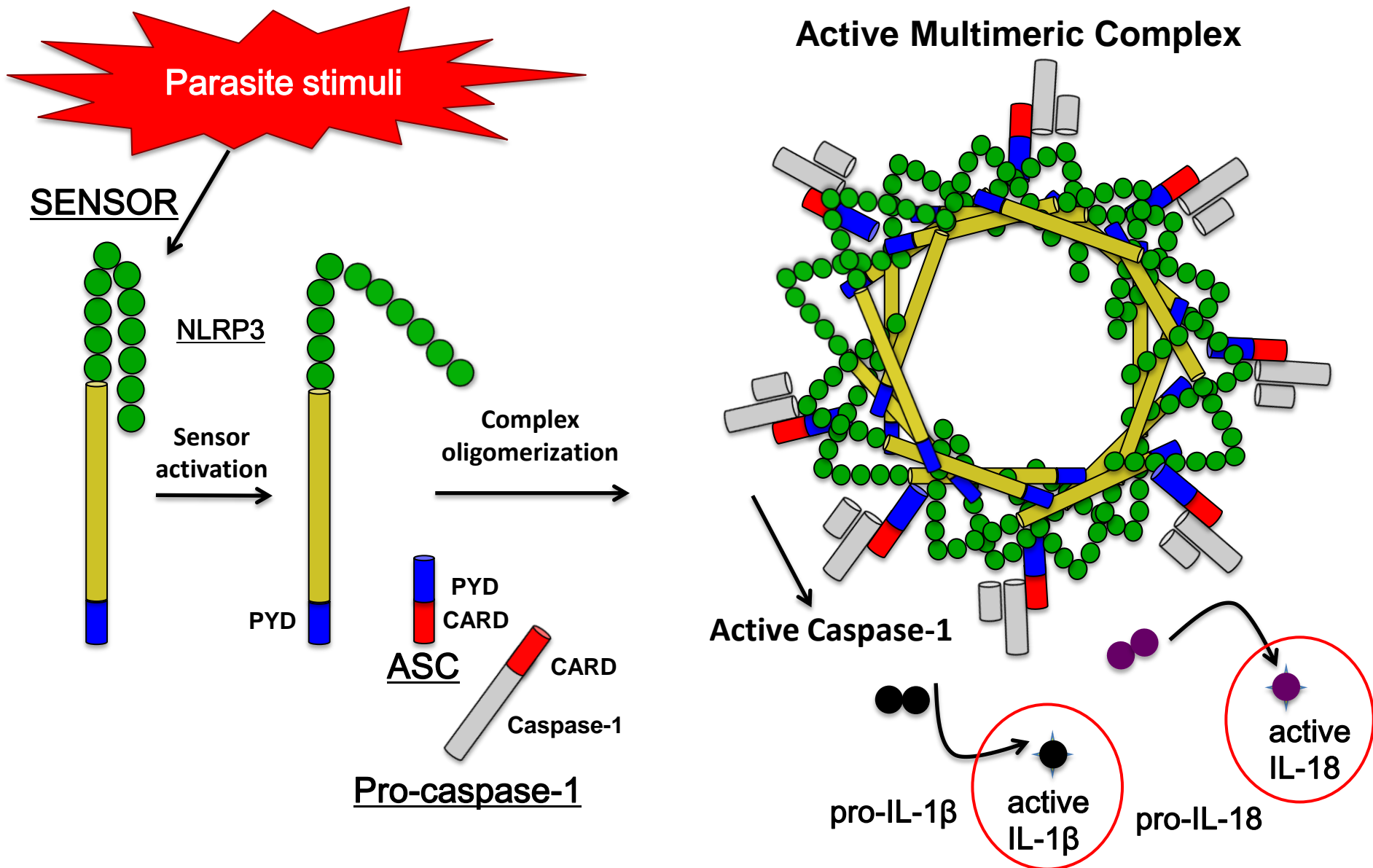


Sharmin

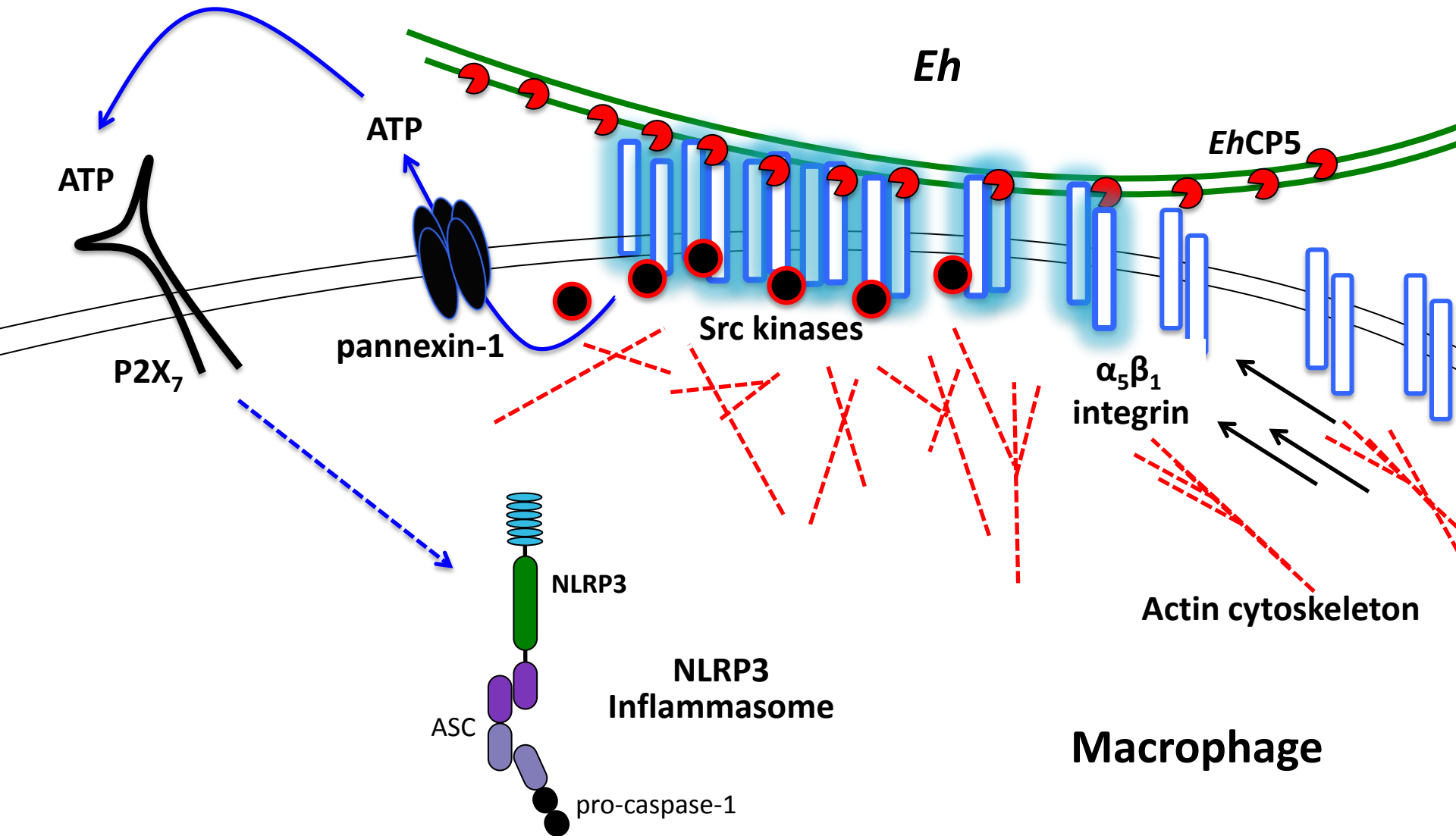
Jeanie

Aralia

Inflammasomes control the activation of caspase-1



Model of NLRP3 inflammasome activation by *EhCP5*





***Eh* virulent molecules**

Gal-lectin

Gal-lectin + *Eh*CP5

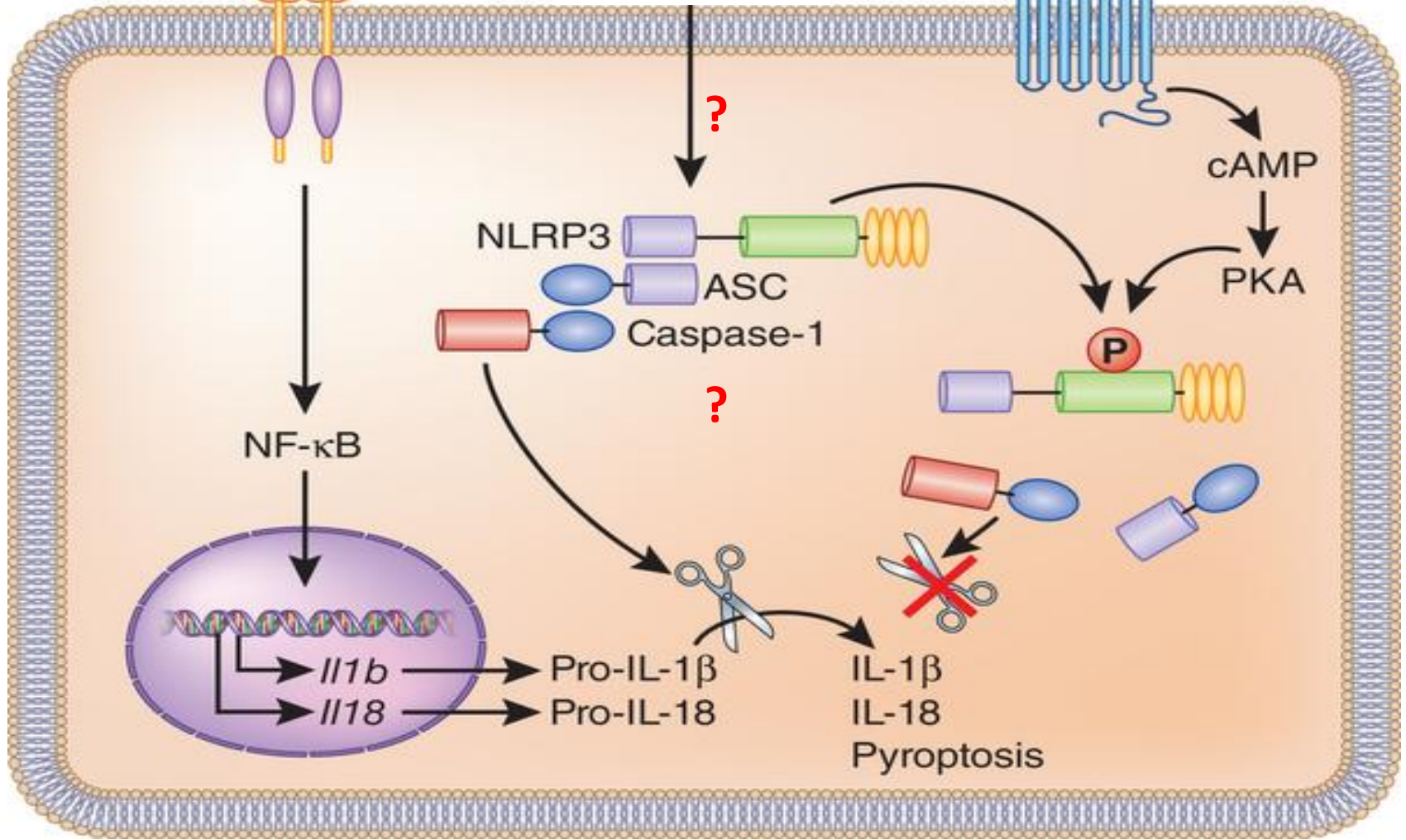
PGE₂

Host receptors

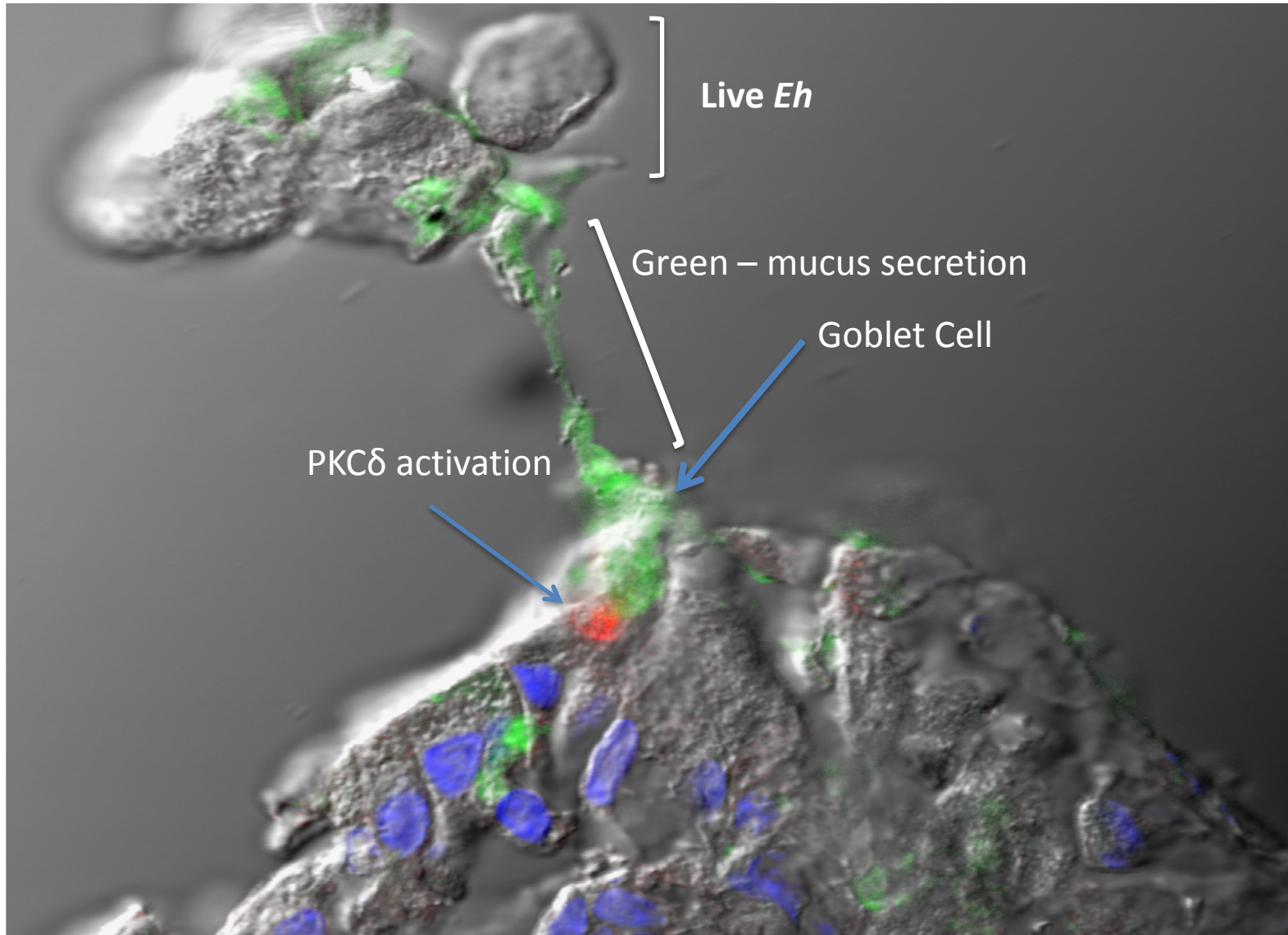
TLR4

$\alpha 5\beta 1$ integrin

EP4



Live cell imaging showing PKC activation and mucus secretion



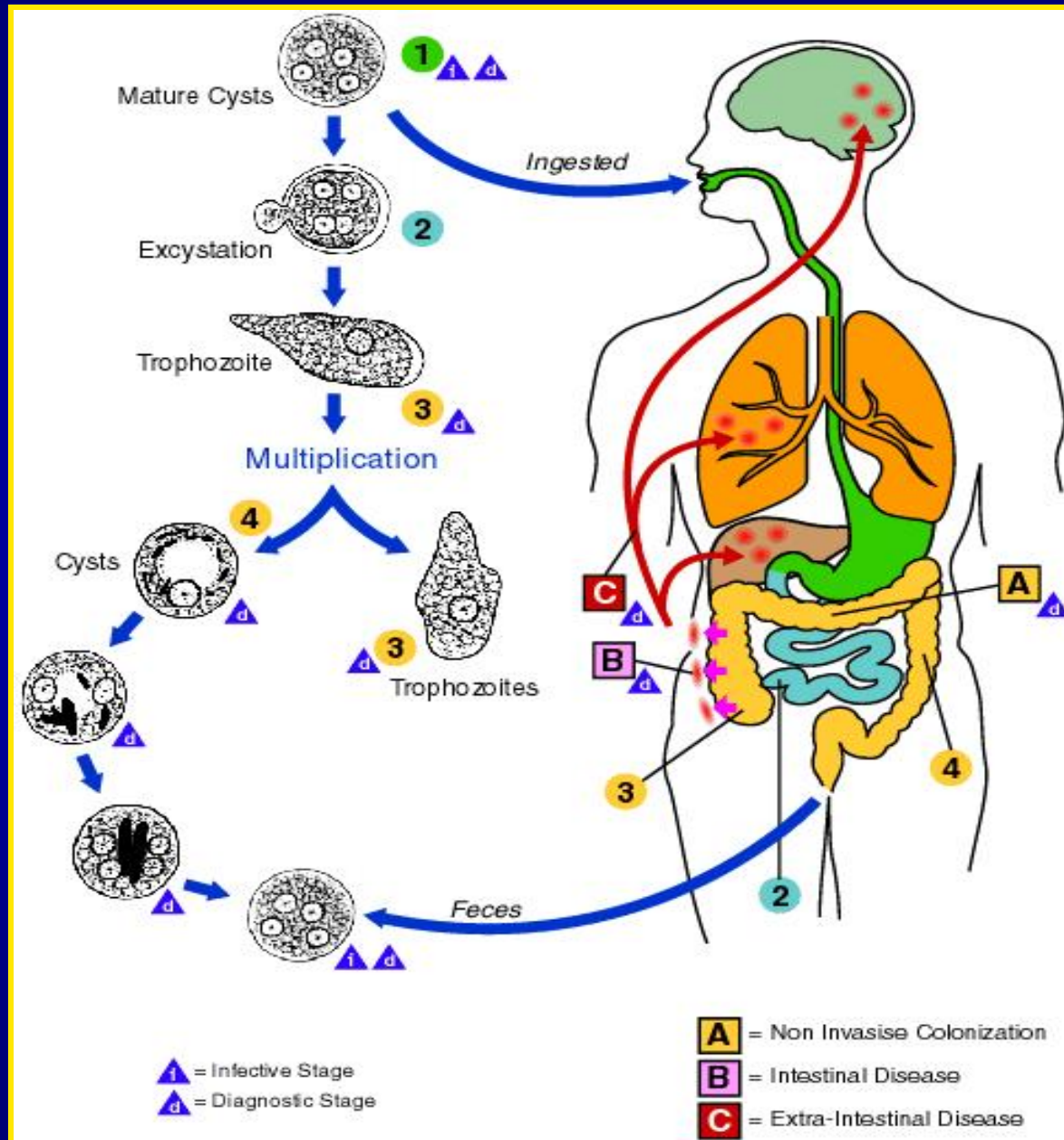
Steve

Leanne

France



Life cycle of *Entamoeba histolytica*



Global Prevalence and Incidence of Amebiasis

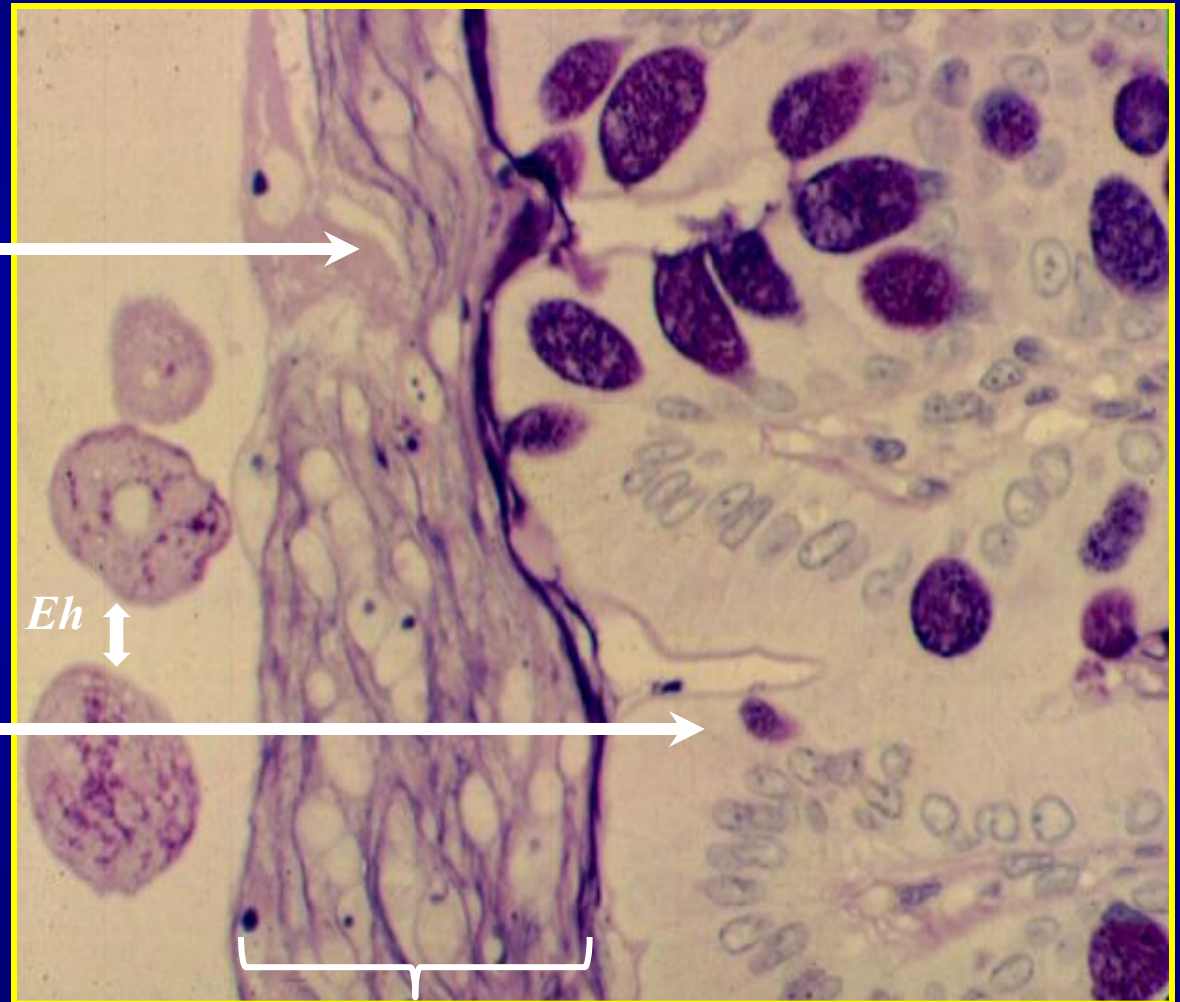
- About 10% of the world's population is infected with *Eh*
- 1% get invasive disease (amebic colitis and/or liver abscess) causing @10⁵ deaths/year
- Second leading cause of death by a protozoan parasite

Pathogenesis of *Entamoeba histolytica* colitis: a global health problem in developing nations

Unresolved issues in the biology of *Eh*

Does the host sense/respond to colonized *Eh* in the gut?

Why does *Eh* elicit a rapid pro-inflammatory response upon contact/invasion?



MUC2 Mucus Barrier

