



Centre Hospitalier Régional  
Universitaire de Lille



**Polymorphisms in the Mannose Binding Lectin gene  
are associated with the defect of the mannose binding  
lectin functional activity in Crohn's disease patients**

Laura Choteau; Francis Vasseur; Frederic Lepretre; Martin Figeac; Corine Gower-Rousseau; Laurent Dubuquoy; Daniel Poulain; Jean-Frederic Colombel; Boualem Sendid; **Samir Jawhara**

**Fungal-Associated Invasive and Inflammatory Diseases**

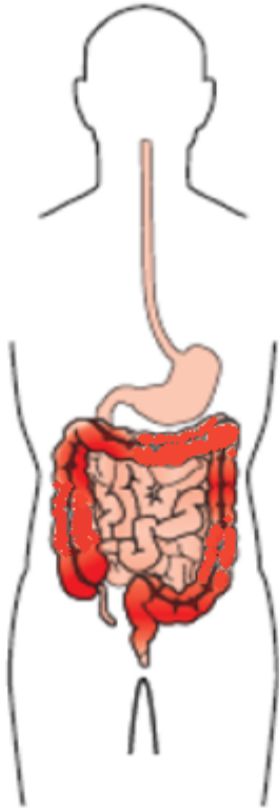
**LIRIC-INSERM U995-Equipe2**

**Lille, France**

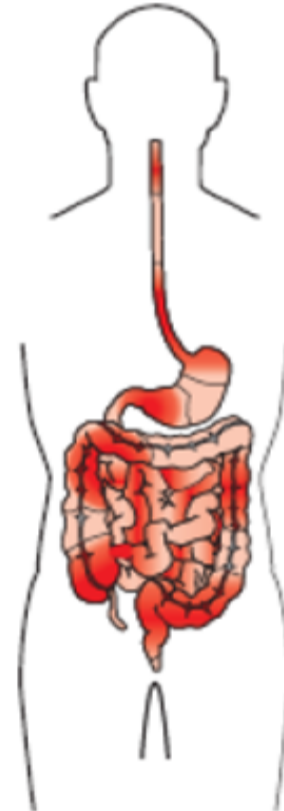


# Inflammatory Bowel Disease

**Ulcerative colitis**



**Crohn's disease**



Baumgart et Sandborn (2012) Lancet.



# IBD: multifactorial disorders

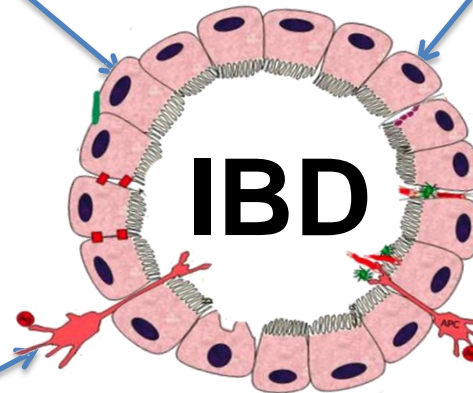
## Etiologic Hypotheses

### Persistent colonization/infection:

Mycobacteria  
Helicobacter sp.  
Listeria  
Toxigenic *E. coli*  
*C. albicans*

### Genetics:

NOD2,  
STAT1, 2 or 4  
IL12B  
IL23R  
IL10  
MBL2



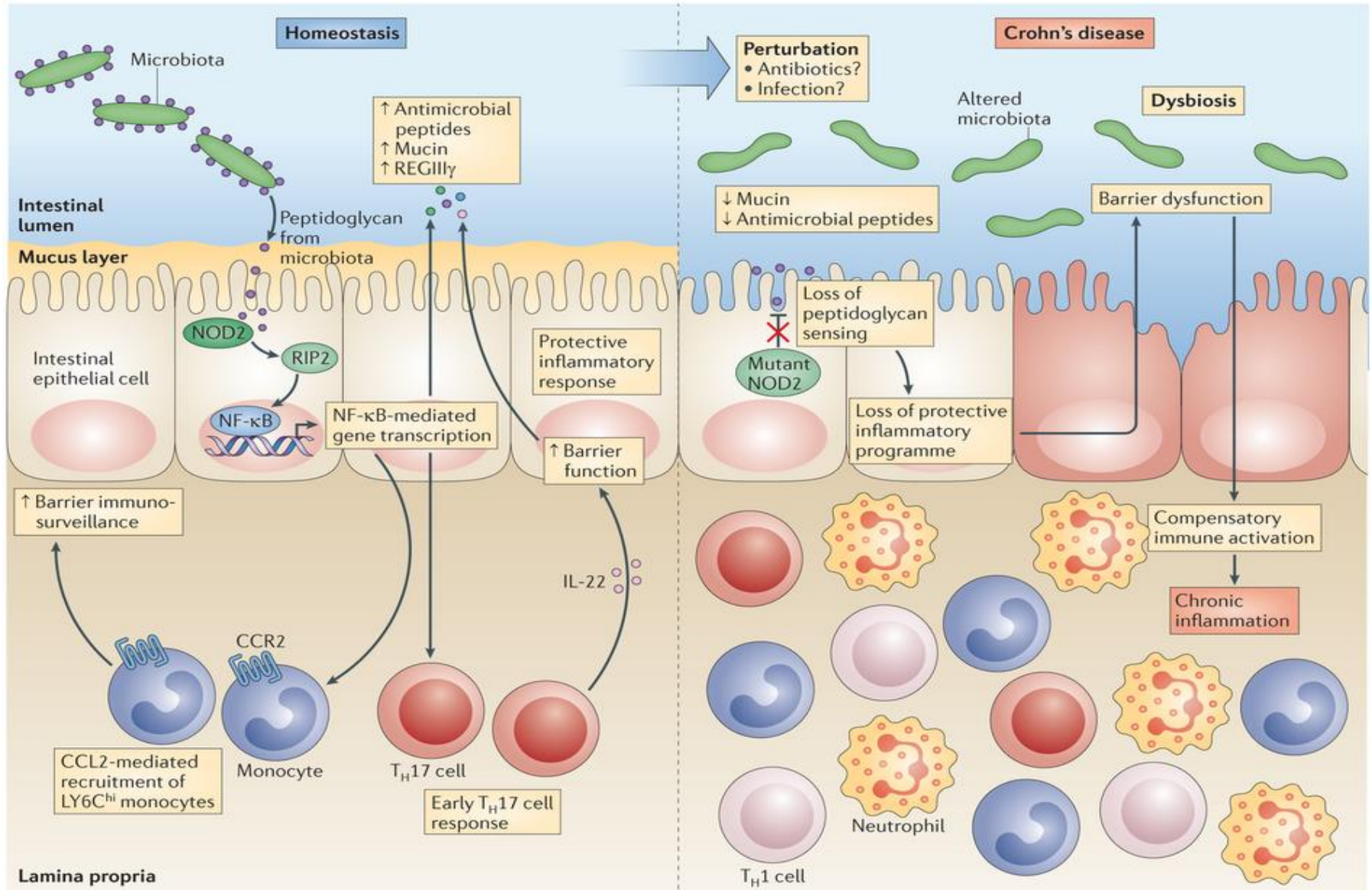
### Dysbiosis:

↓ Protective bacteria  
↑ Aggressive commensals

### Dysregulated immune response and defective mucosal integrity:

Loss of tolerance  
Aggressive cellular activation  
Altered mucus/Increased permeability

# NOD2 plays a key role in intestinal homeostasis

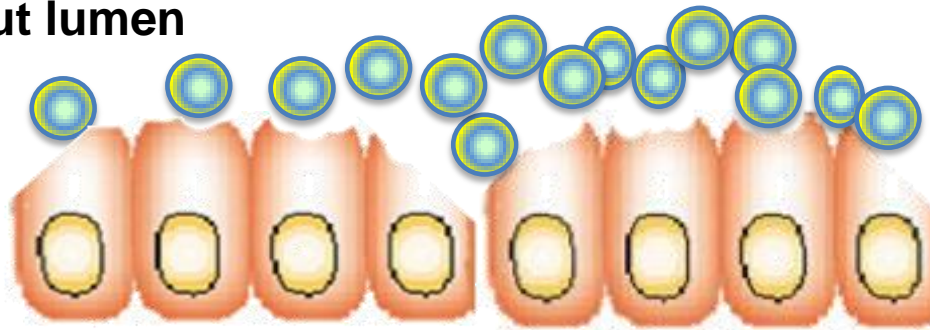




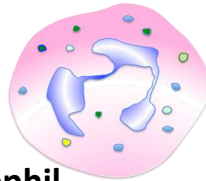
# Link between inflammatory bowel disease and *Candida albicans*

*Candida albicans*  $\longleftrightarrow$  ? **IBD** [ Anti-*Saccharomyces cerevisiae* antibodies (ASCA)  
Colonized with *C. albicans*

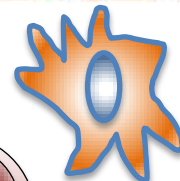
Gut lumen



Neutrophil

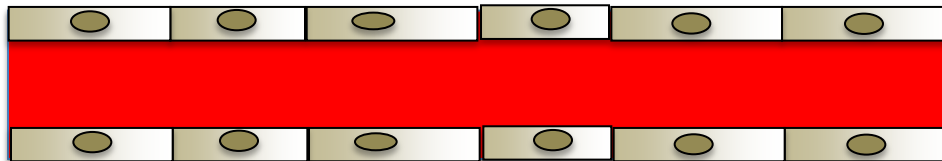
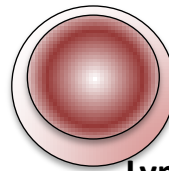


Macrophage



Bloodstream

Lymphocyte T

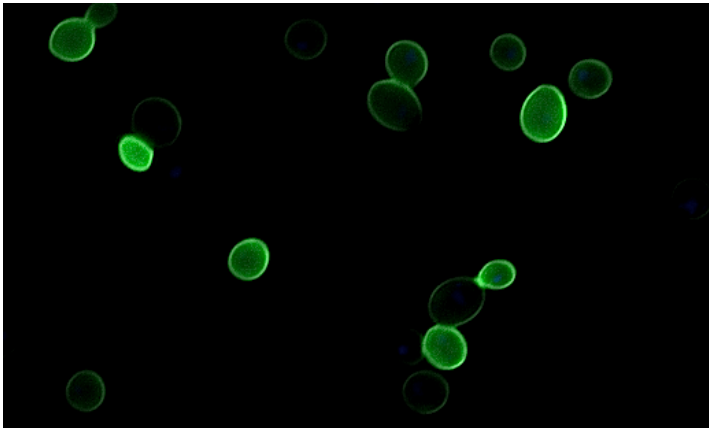


Sendid et al. (1998) Am J Gastroenterol  
Standaert-Vitse et al. (2009) Am J Gastroenterol  
Poulain et al. (2009) Digest. Dis.



# *Candida albicans*

- ❖ Opportunistic pathogen yeast causes the candidiasis
- ❖ ranked the 4<sup>th</sup> most frequent nosocomial infection
- ❖ 30–70% mortality rates for patients with systemic candidiasis
- ❖ commensal yeast resides in digestive tract and vagina
- ❖ polymorphic fungus



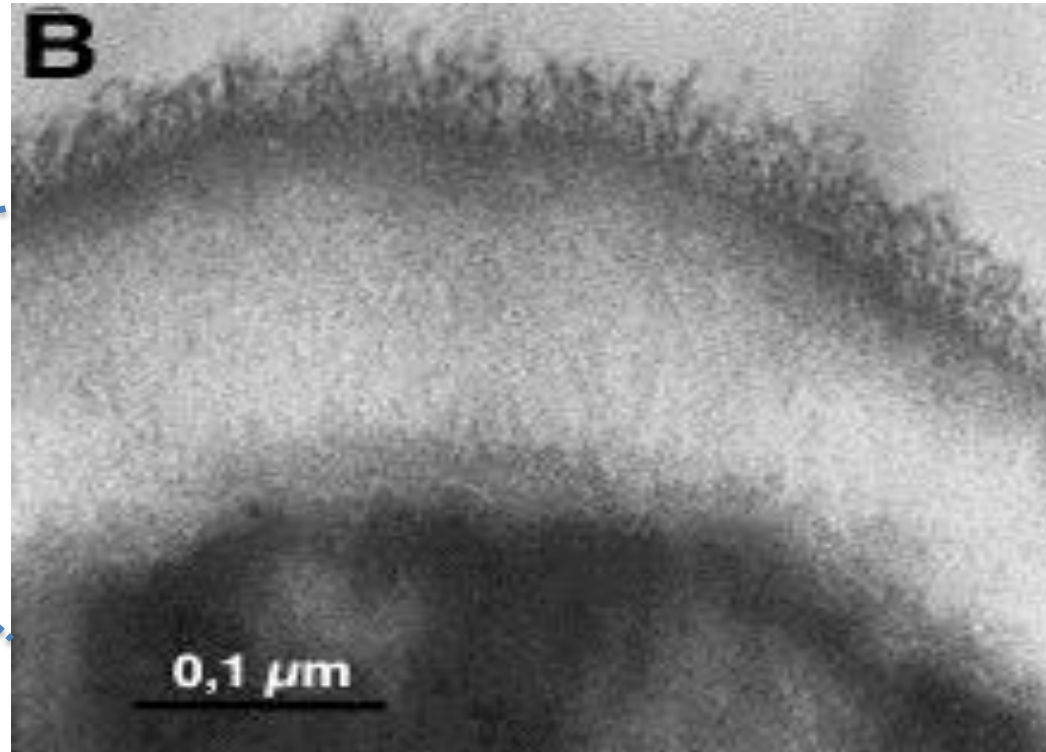
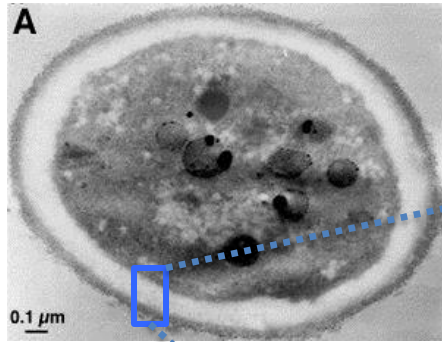
**Blastospores**



**filamentous form**

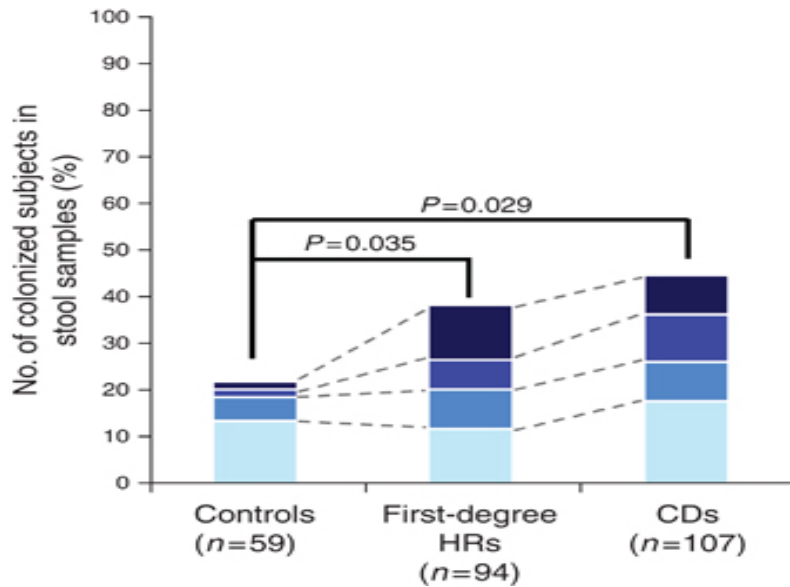


# Cell wall structure of *C. albicans*

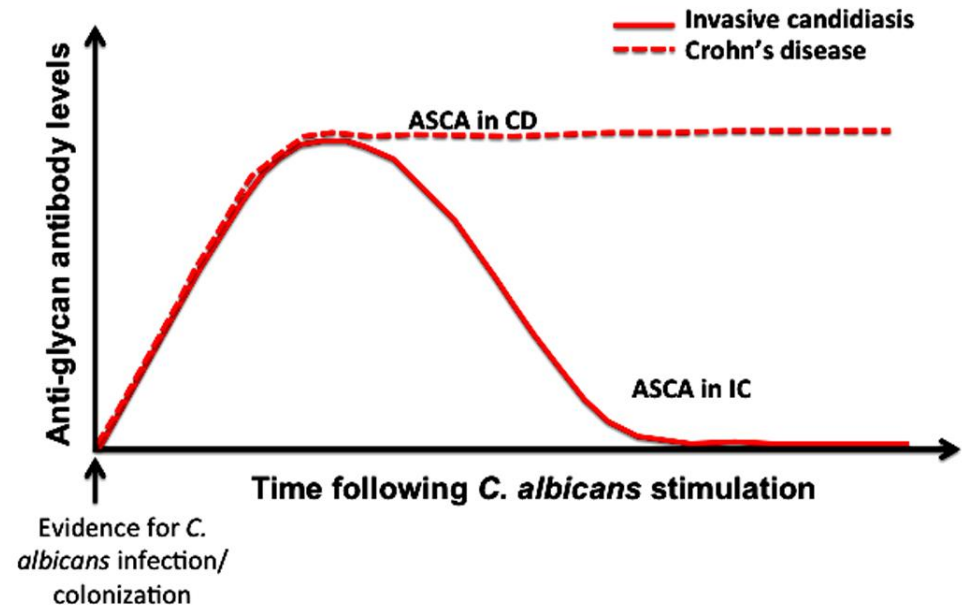


**Mannans (PPM)**  
**Glycolipids (PLM)**  
**Mannoproteins**  
**β-glucans**  
**Chitin**

## C. albicans colonization and ASCA in familial Crohn's disease



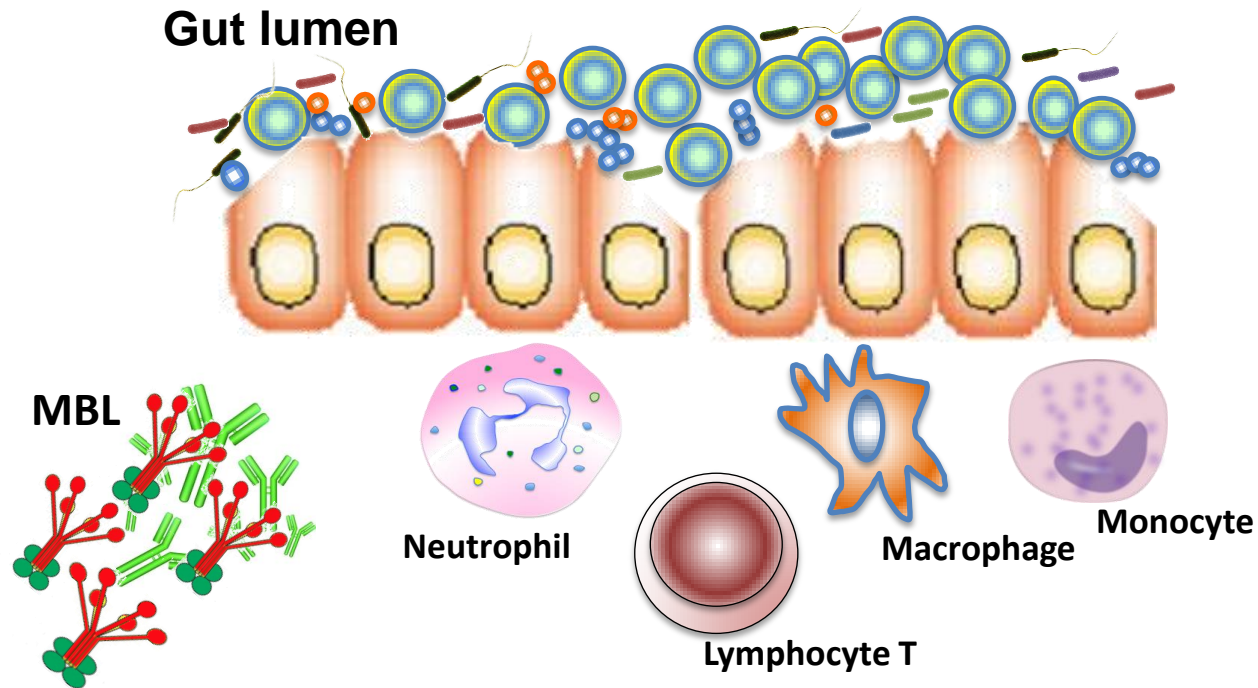
**C. albicans** was isolated frequently from stool samples from CD patients



Regardless of ASCA levels in IC, ASCA status is stable during the time course of CD



# Role of MBL in Crohn's diseases



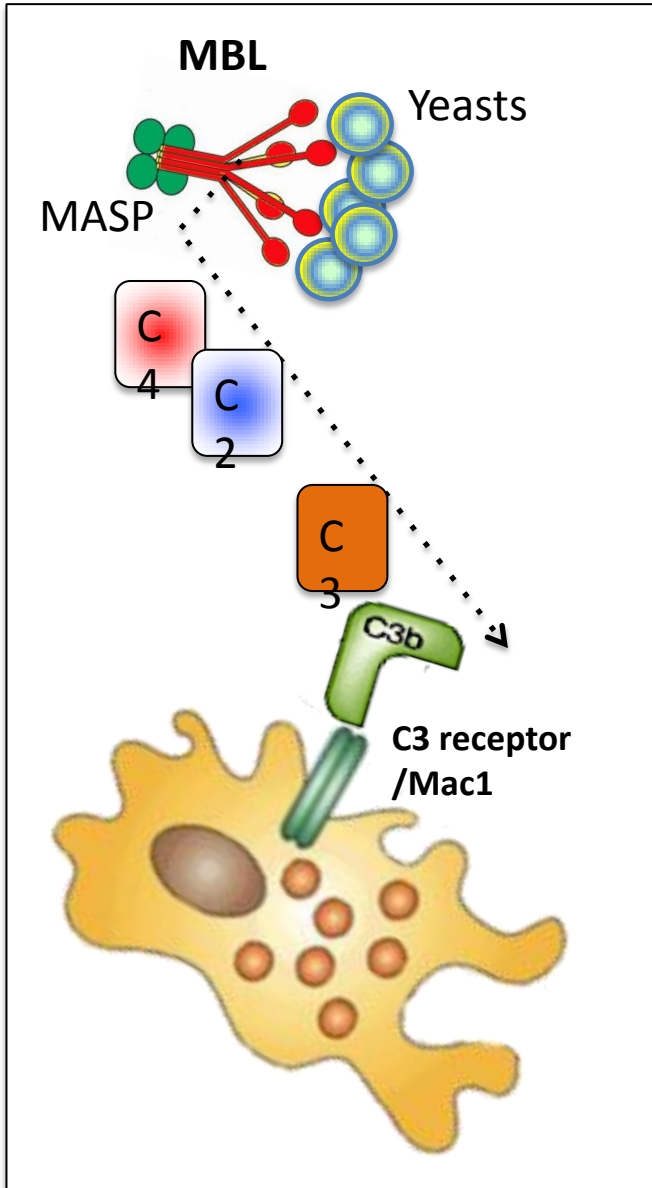
**MBL levels are inversely correlated with ASCA levels in CD patients**

**MBL deficiency was found to be related to CD in a pediatric cohort**

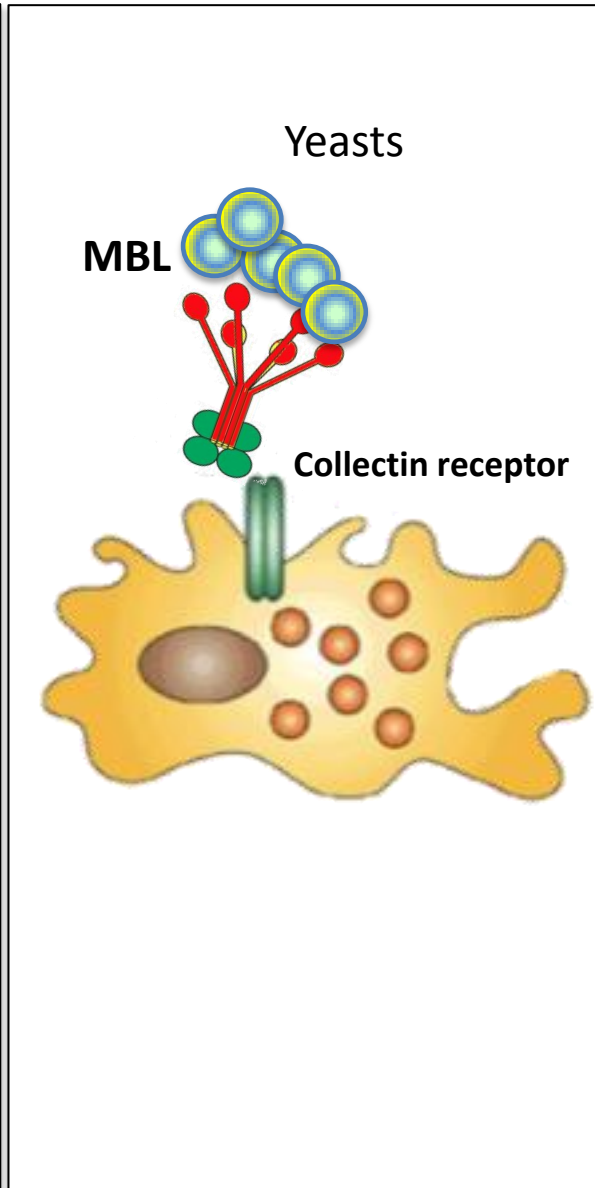


# Mannose Binding Lectin

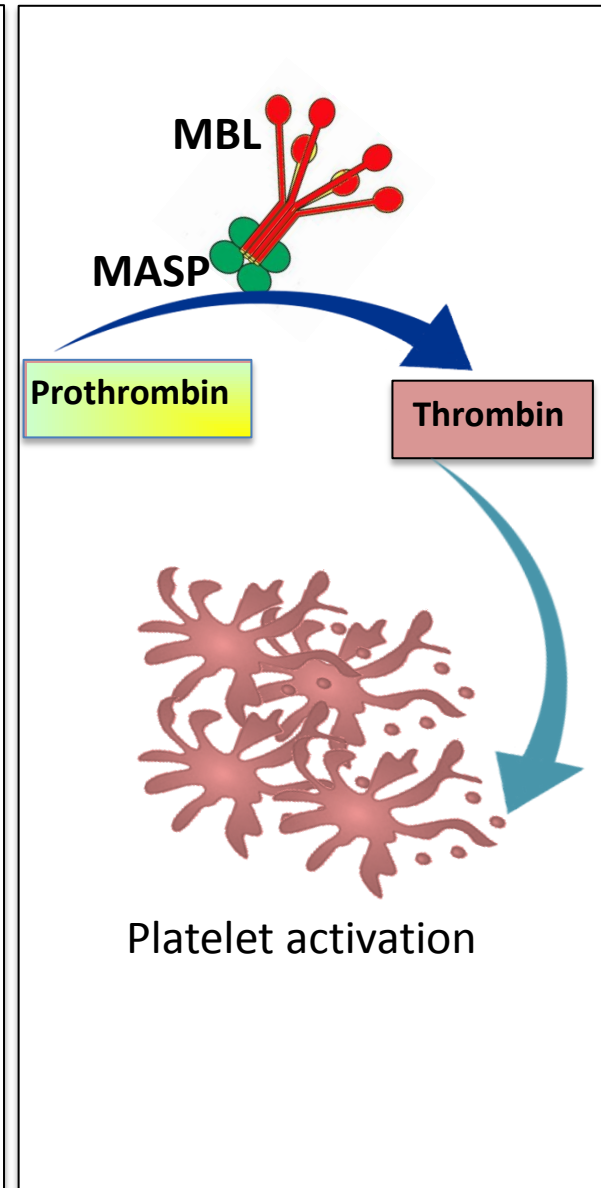
## Activation of complement lectin pathways



## Opsonization of microorganisms

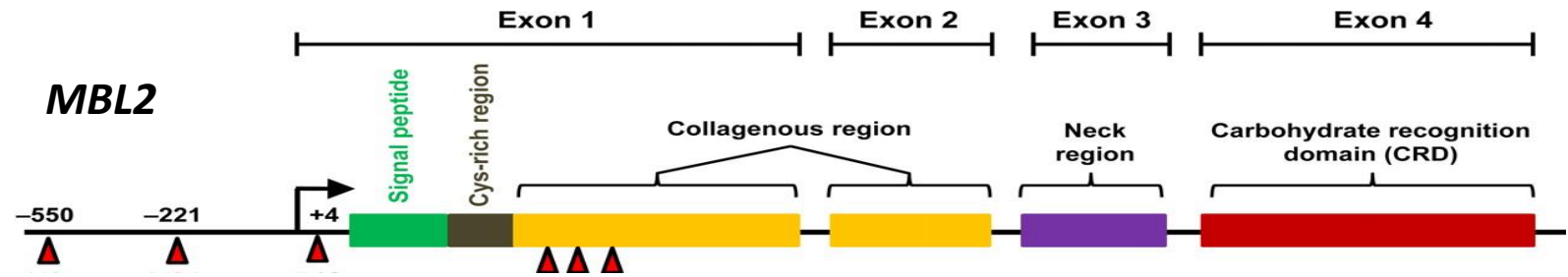


## Coagulation cascades





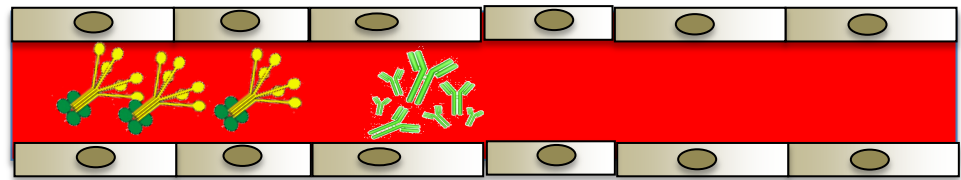
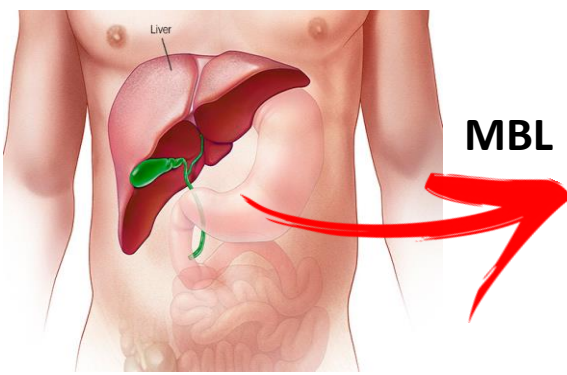
# The question is whether MBL is also produced and released locally in the gut?



**MBL?**  
**Gut lumen**



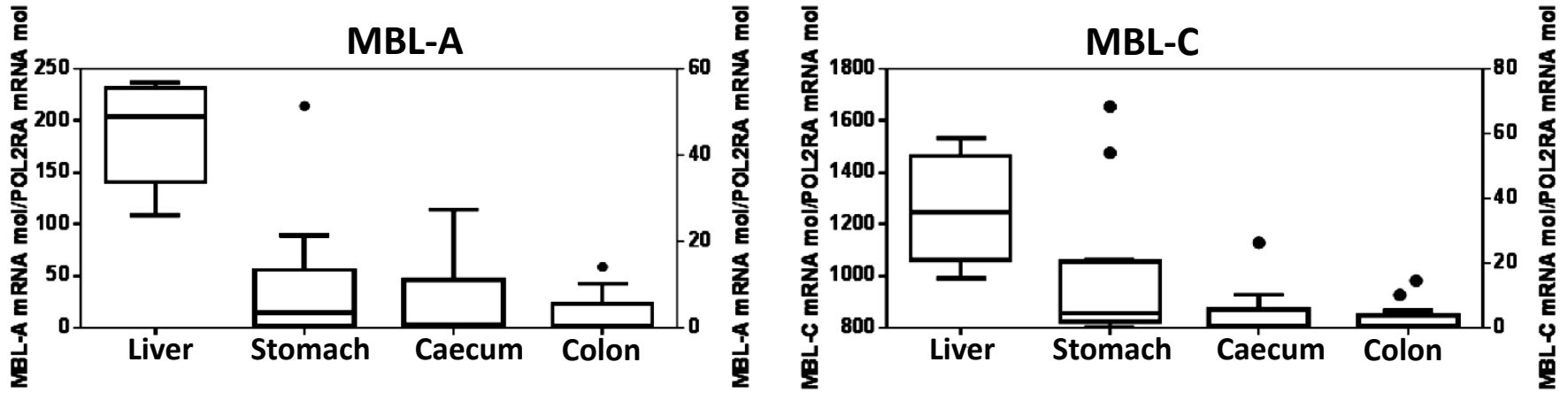
**Bloodstream**



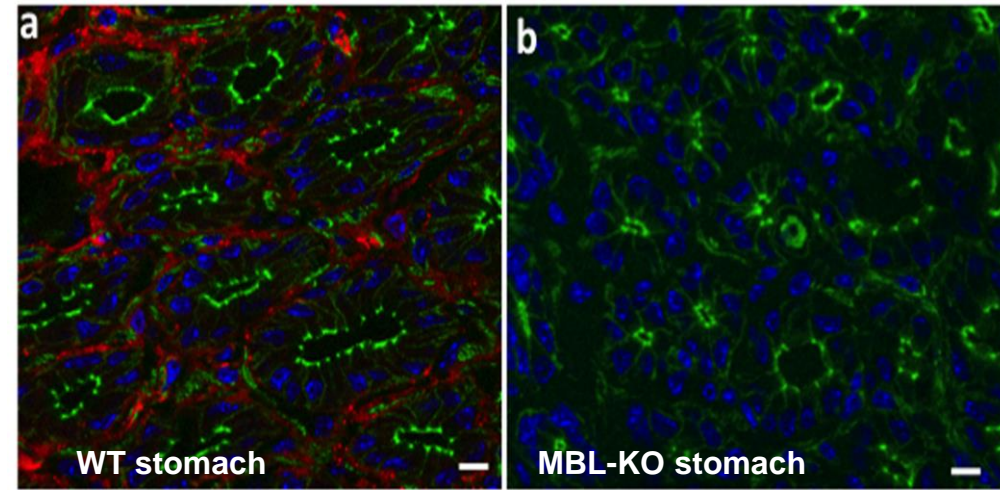
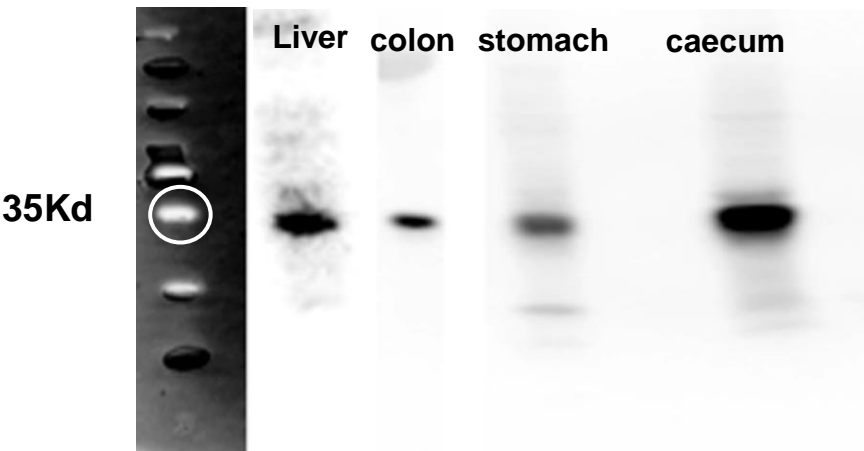


# Detection of MBL-A and MBL-C mRNA and proteins in the gastrointestinal tract of wild-type mice

## Detection of MBL-A and MBL-C mRNA in the gastrointestinal tract of mice



## Expression of MBL proteins in the gastrointestinal tract of mice

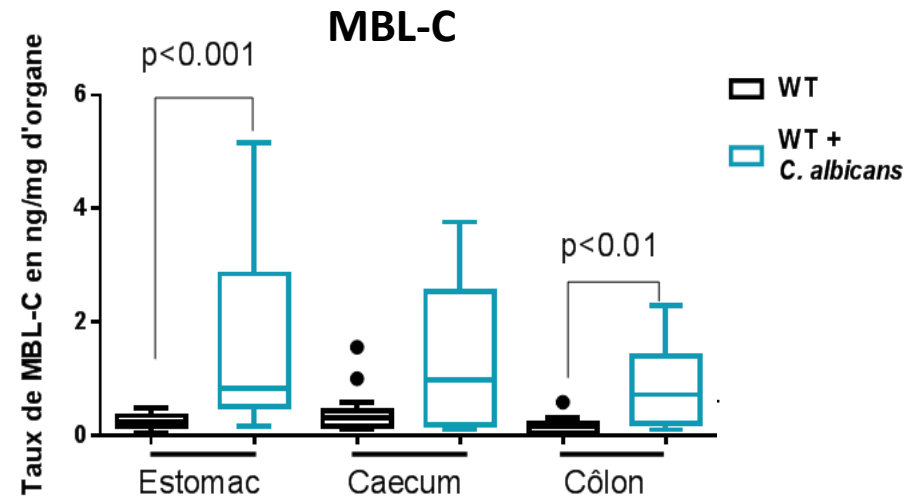
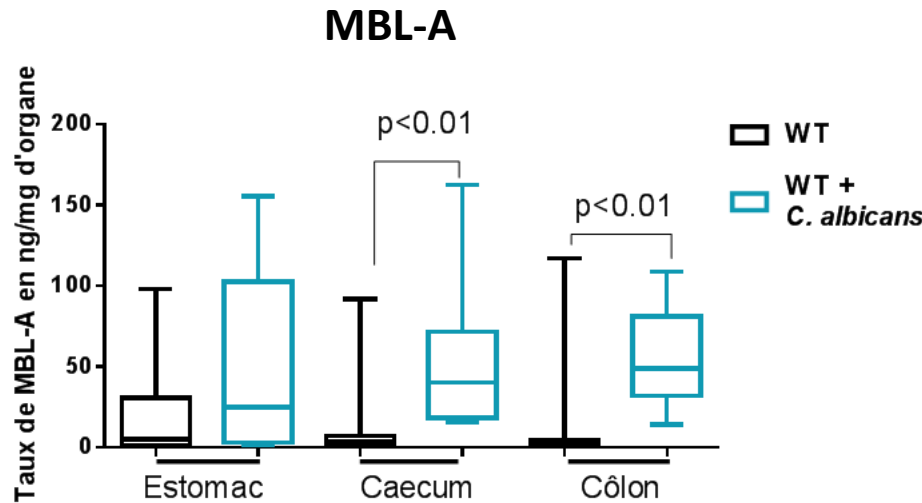




# *C. albicans* colonization increases the expression of MBL-A and MBL-C in the gastrointestinal tract of mice



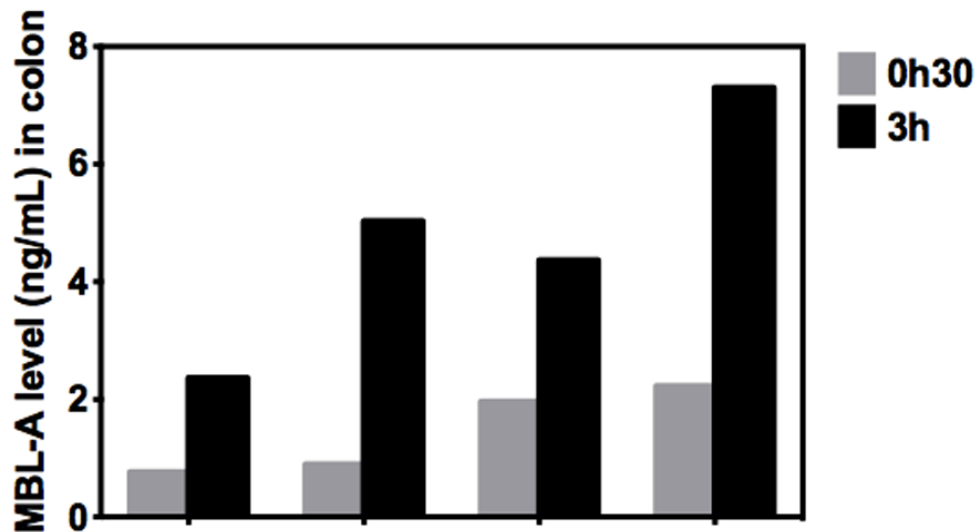
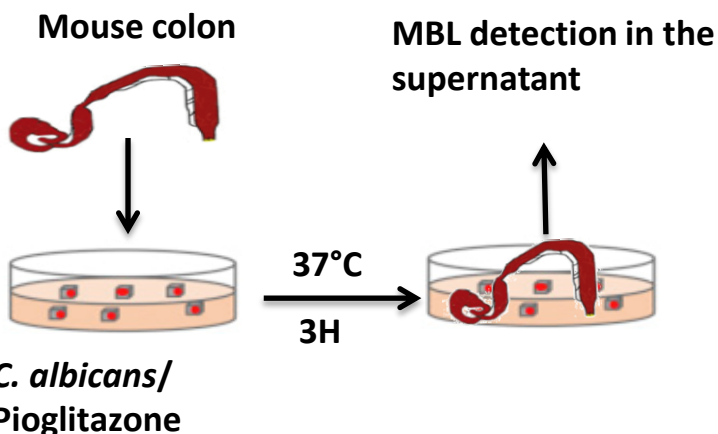
Expression of MBL proteins in the gastrointestinal tract of mice colonized or not with *C. albicans*





# Mouse intestinal explant culture produced MBL after *C. albicans* sensing alone or with pioglitazone treatment

The level of MBL-A from colons of wild-type mice increased in the supernatant at 3hrs after *C. albicans* sensing combined or not with pioglitazone.



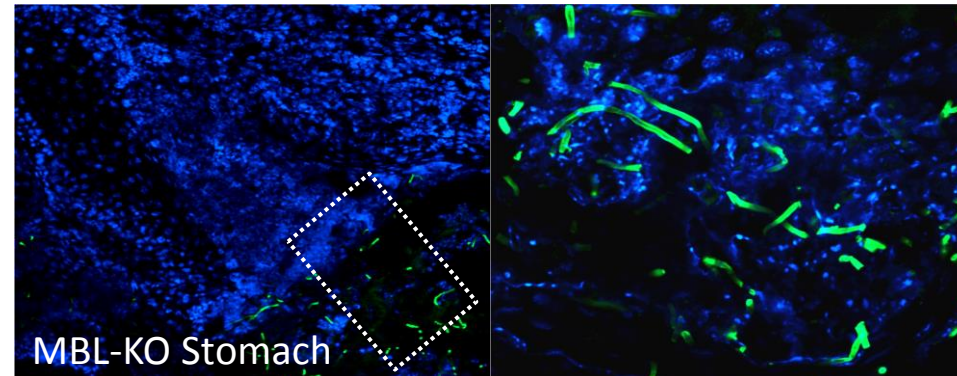
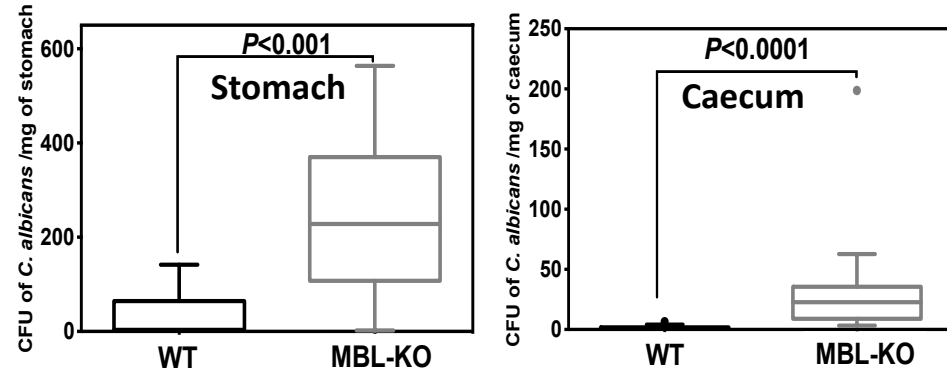
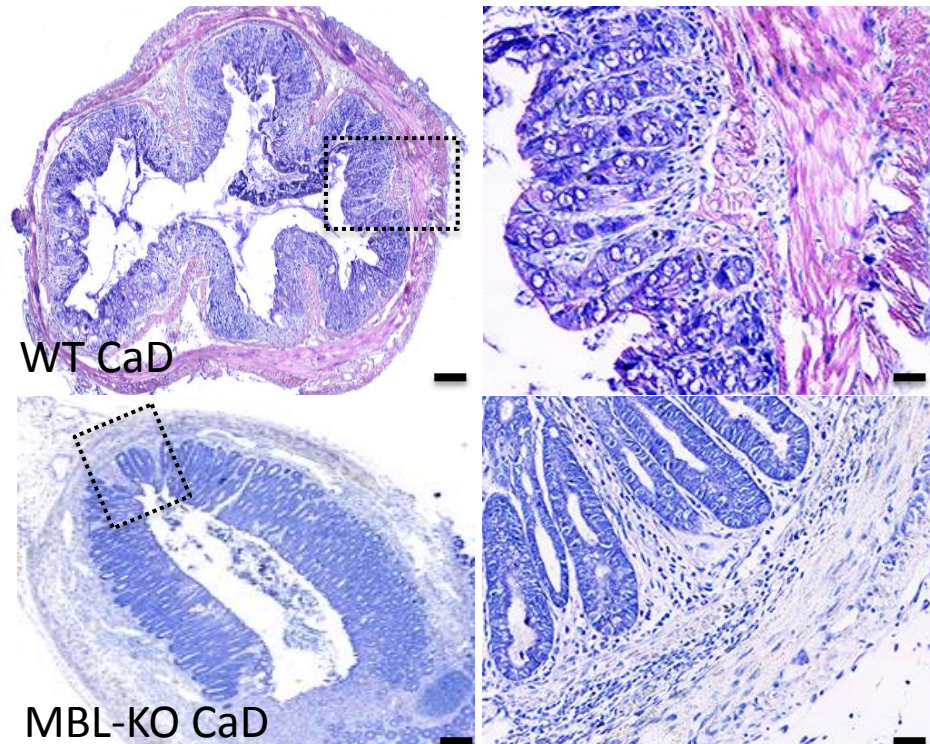
PBS	+	-	-	-
<i>C. albicans</i>	-	+	-	+
Pioglitazone	-	-	+	+

# MBL deficiency exacerbates intestinal inflammation and *C. albicans* colonization



## Histological analysis of colon sections

## MBL deficiency promoted *C. albicans* colonization in mice





The aims of the study were to assess in 70 CD patients and 30 age- and sex-matched healthy control subjects the relationship between :

**Clinical CD phenotypes**

**MBL serum concentrations**

**MBL functional activity**

***MBL2*, and *NOD2* polymorphisms**

**ASCA levels**





# Clinical characteristics of the Crohn's disease patients

Crohn's disease patients (n=70) Mean age of onset (years) 23  
Female/male 42/27

## Montreal classification

### •Age at diagnosis

A1: 16-years	17 (24.6%)
A2: 16-40-years	48 (69.6%)
A3: >40-years	3 (4.3%)

### •Behavior

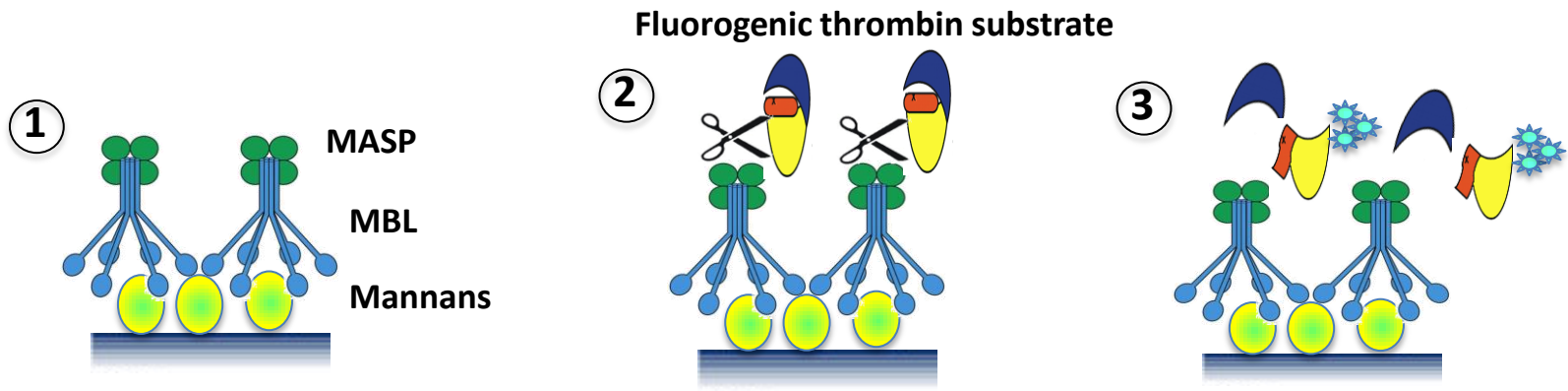
B1: Non-stricturing/non-penetrating	37 (53.6%)
B2: Stricturing	16 (23.2%)
B3: Penetrating	14 (20.3%)

### •Location

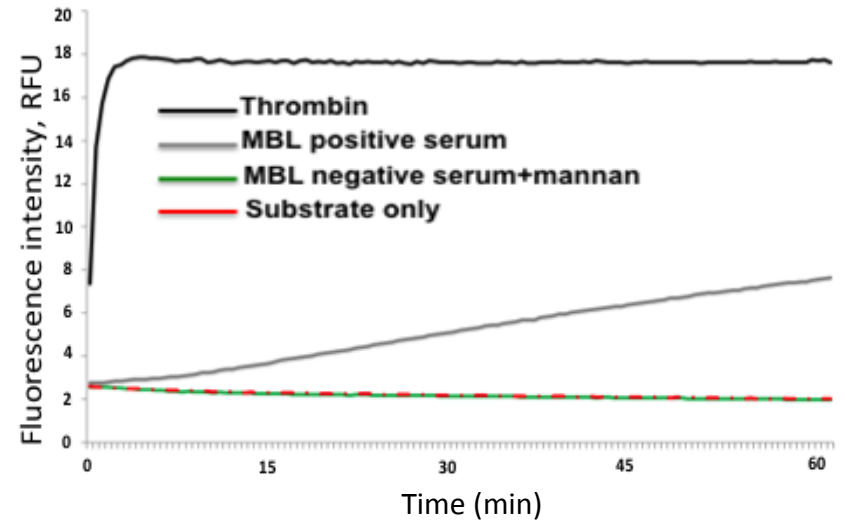
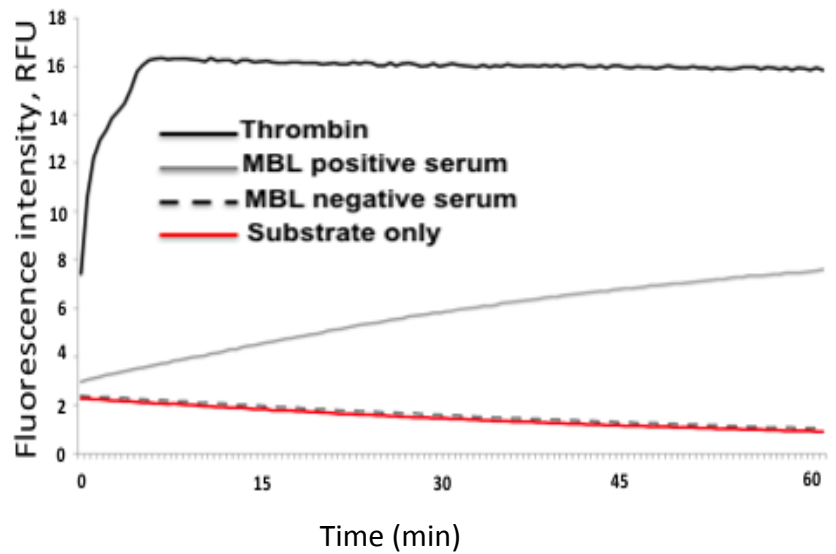
L1: Terminal ileum	15 (21.7%)
L2: Colon	14 (20.3%)
L3: Ileocolon	34 (49.3%)



# Development of assay for MBL-MASP functional activity

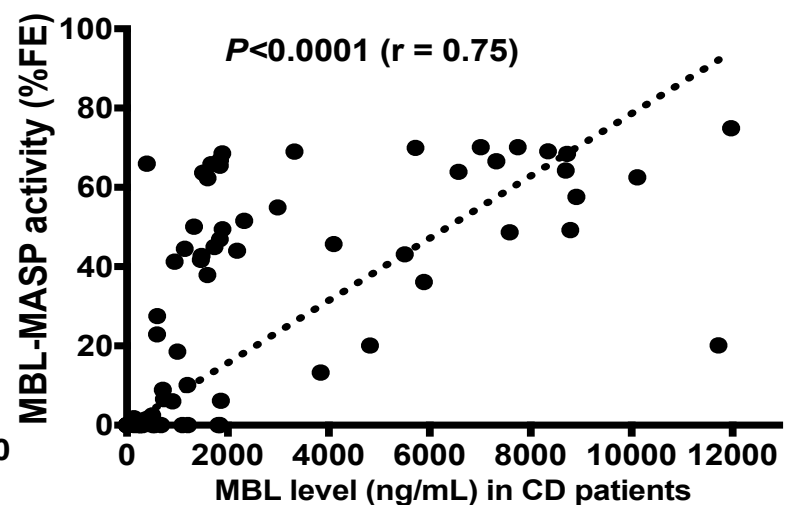
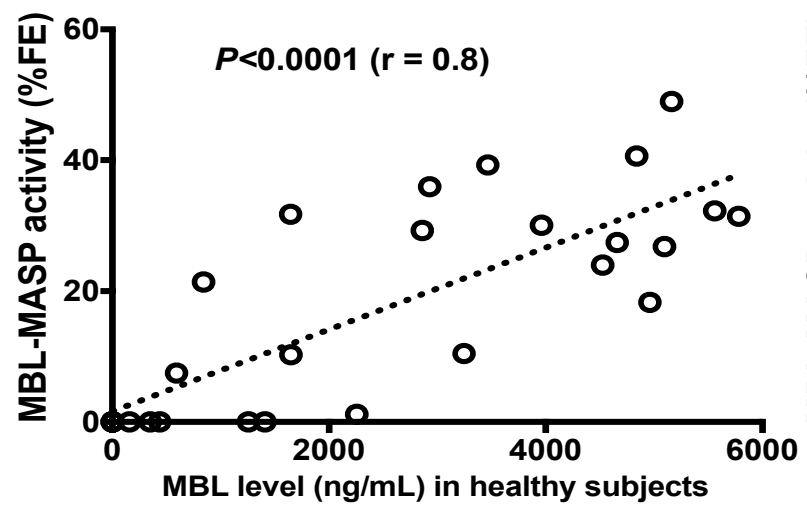
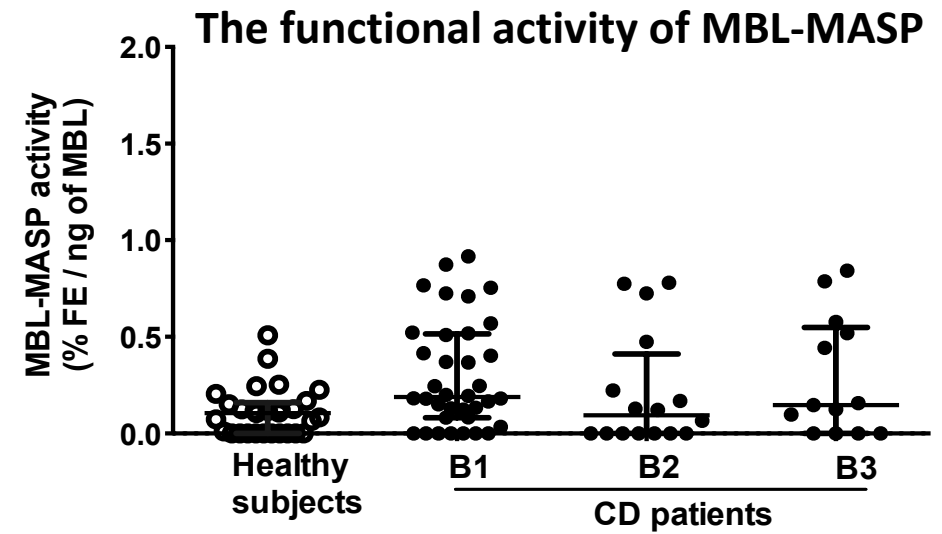
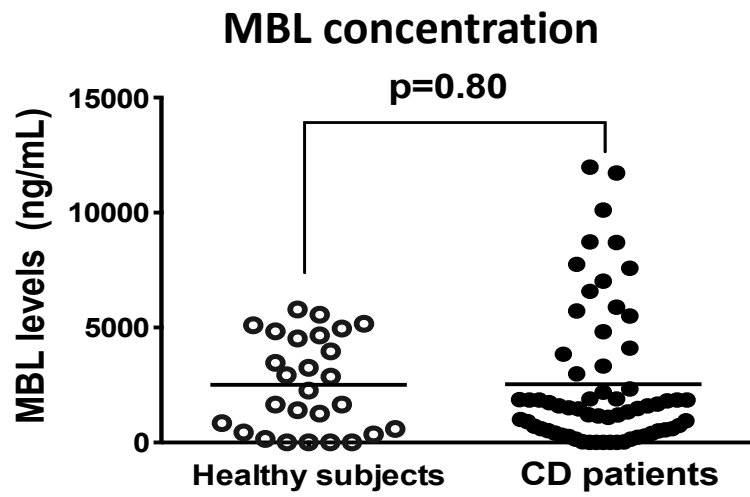


## Cleavage of the fluorogenic thrombin substrate by the MBL-MASP complex



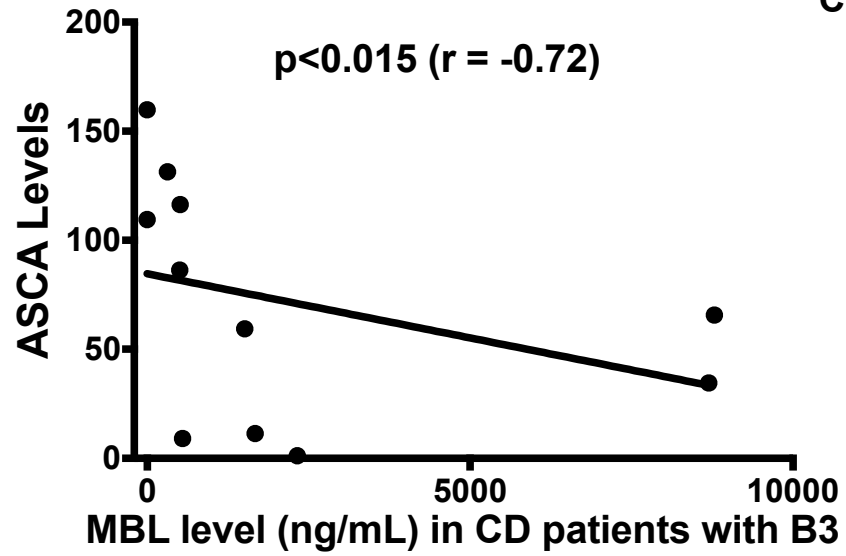
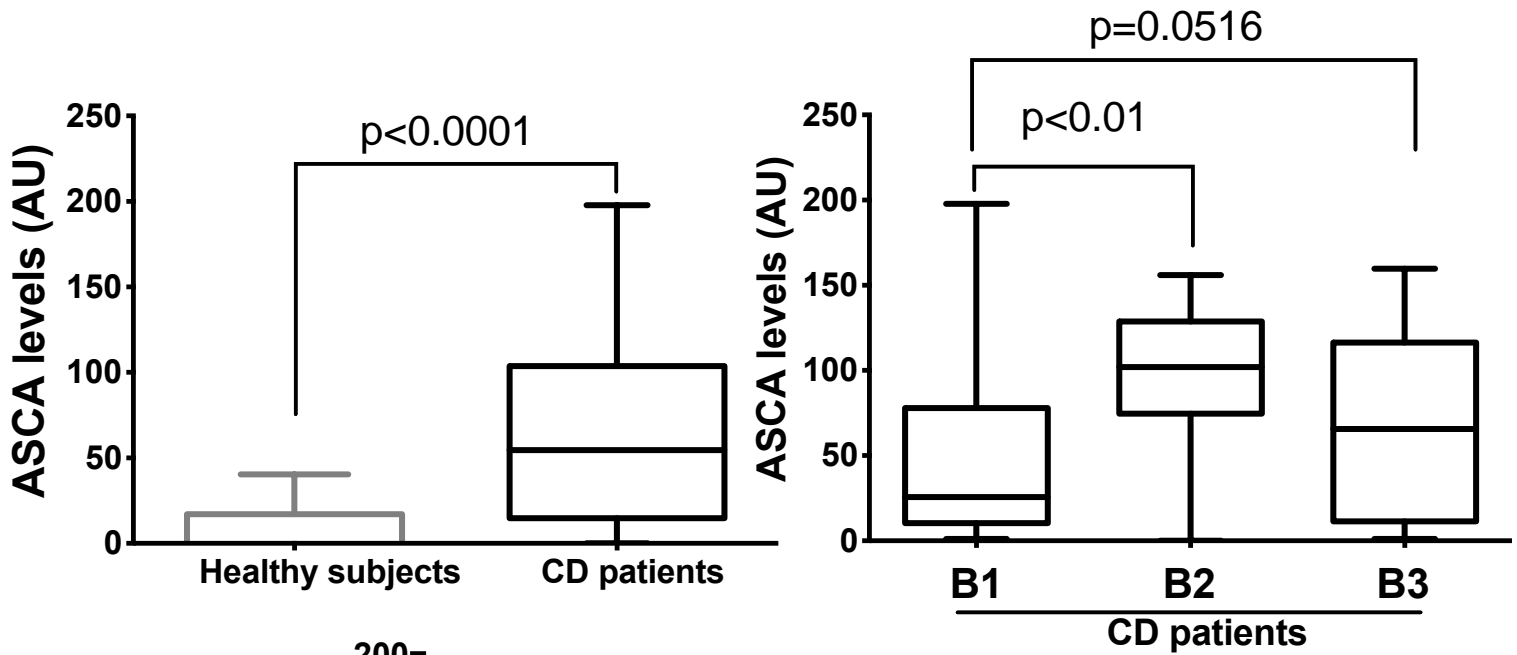


# Polymorphisms in the MBL gene are associated with the defect of the MBL activity in Crohn's disease patients





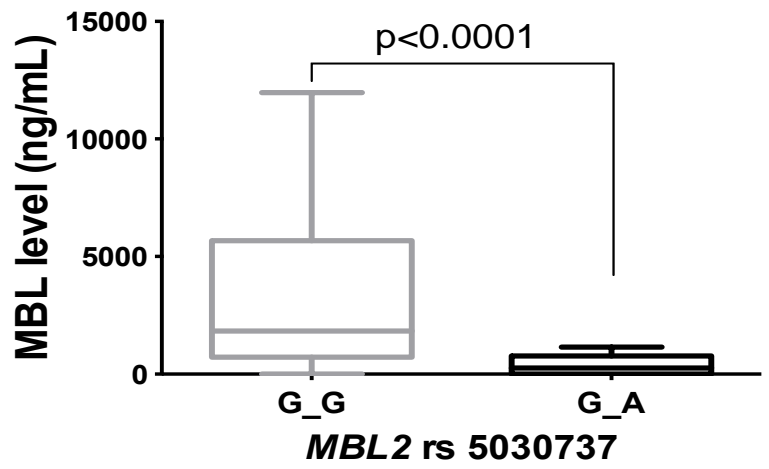
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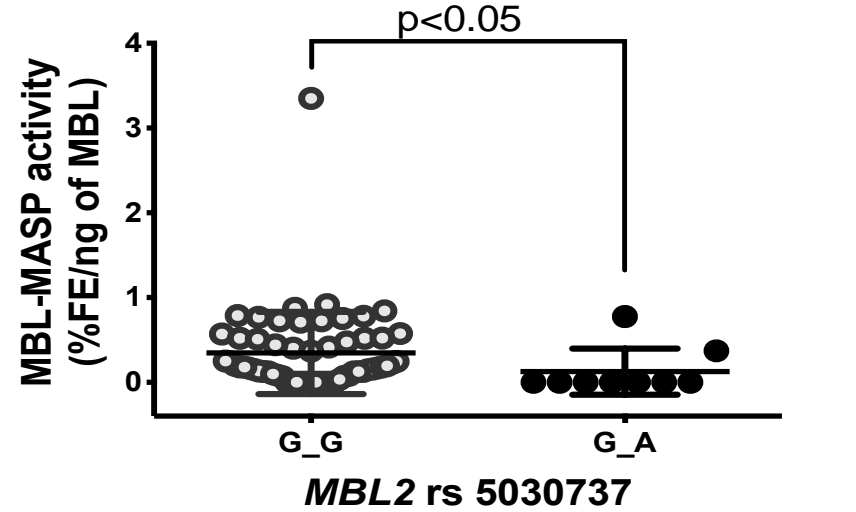


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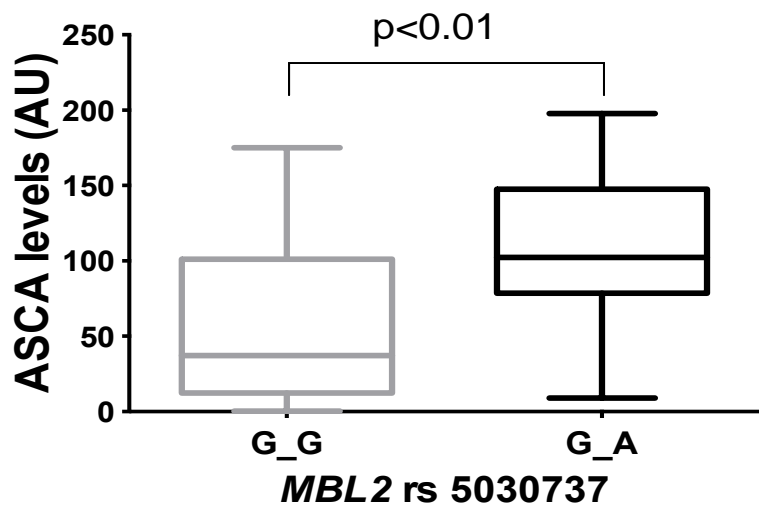
### MBL level vs. *MBL2* genotyping



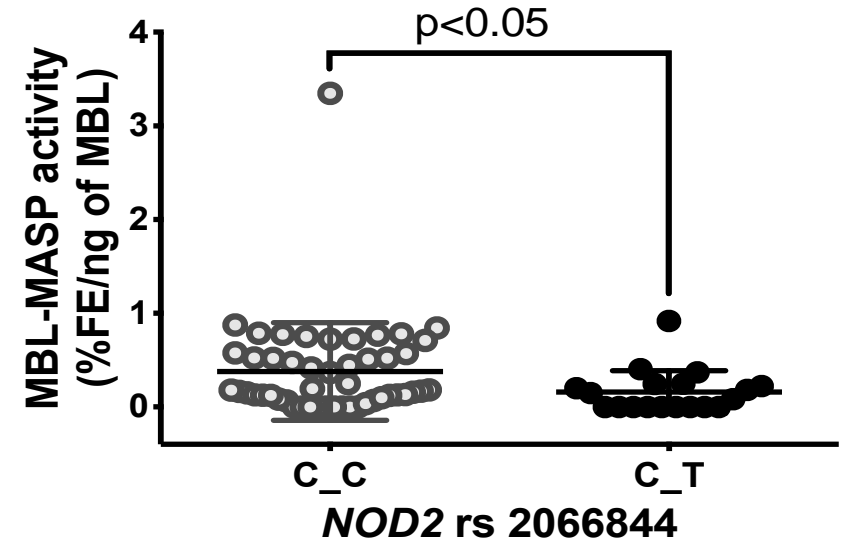
### MBL-MASP activity vs. *MBL2* genotyping



### ASCA level vs. *MBL2* genotyping

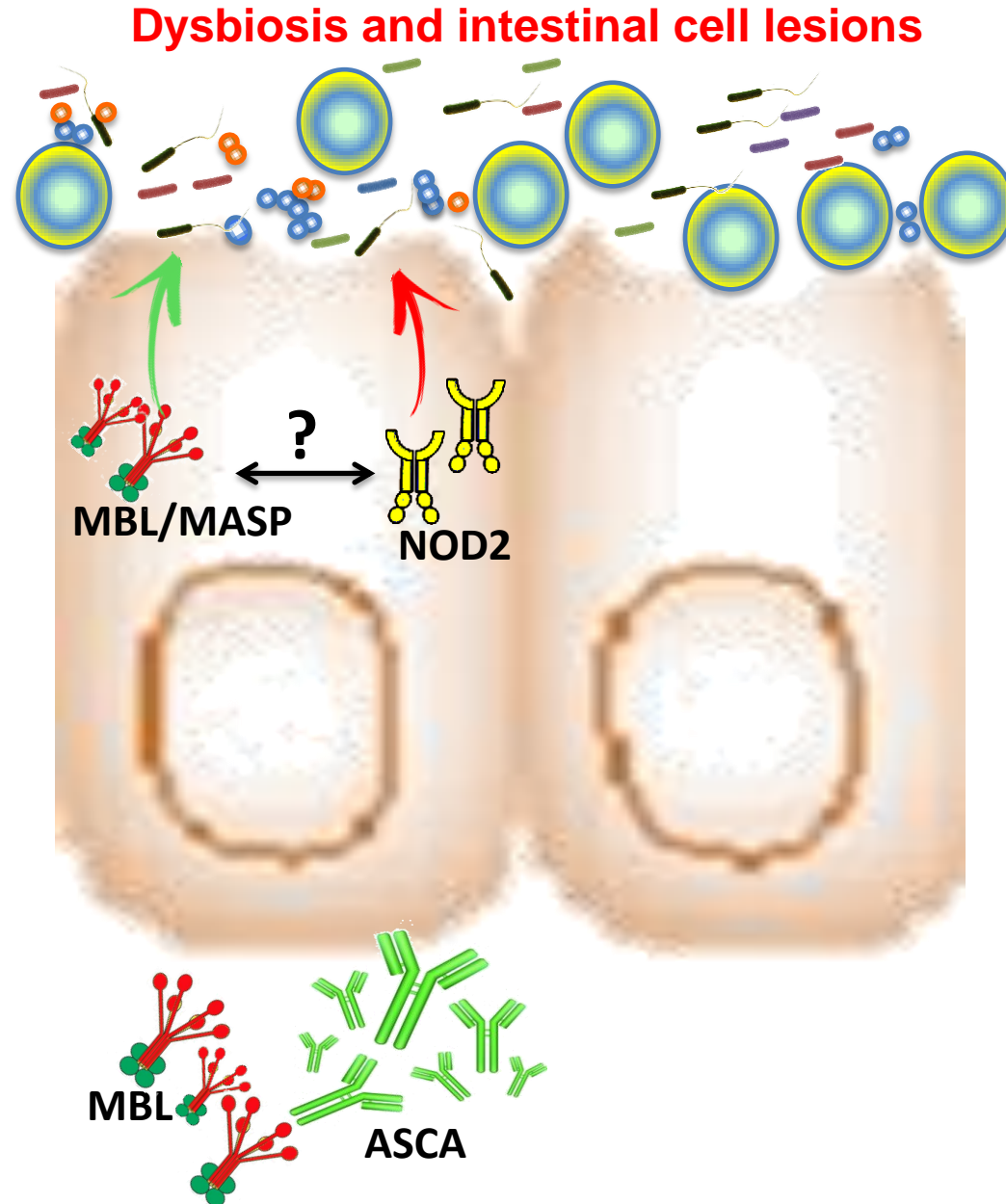


### MBL-MASP activity vs. *NOD2* genotyping



# Role of *MBL2* and *NOD2* in Crohn's diseases

- The *MBL2* variant rs5030737 was associated with a low level of MBL in CD patients.
- The *MBL2* variant rs5030737 was associated with an impaired MBL-MASP functional activity in CD patients.
- The *MBL2* variant rs5030737 was associated with a higher level of ASCA.
- The *NOD2* variant rs2066844 was significantly correlated with the impairment in MBL-MASP functional activity.





# Conclusion

**Our findings provide evidence that CD patients with severe clinical phenotypes have an impairment of MBL-MASP functional activity, and that this defect is associated with *MBL2* and *NOD2* variants.**

## Perspectives

In the clinical study:

- Further analysis of the cells and molecular pathways that regulate innate immune responses in the intestine is ongoing (*NOD2* and *MBL2*)
- Modification of the gut microbiota and their impact on MBL levels



**Dubuquoy's team  
(Inserm U995/Team1)**

**Guerardel 's team  
(CNRS, UMR 8576)**

**Plateforme d'Interaction Moléculaire  
(IMPRT-IFR114)**

**Bayry's team  
(Inserm U1138)**

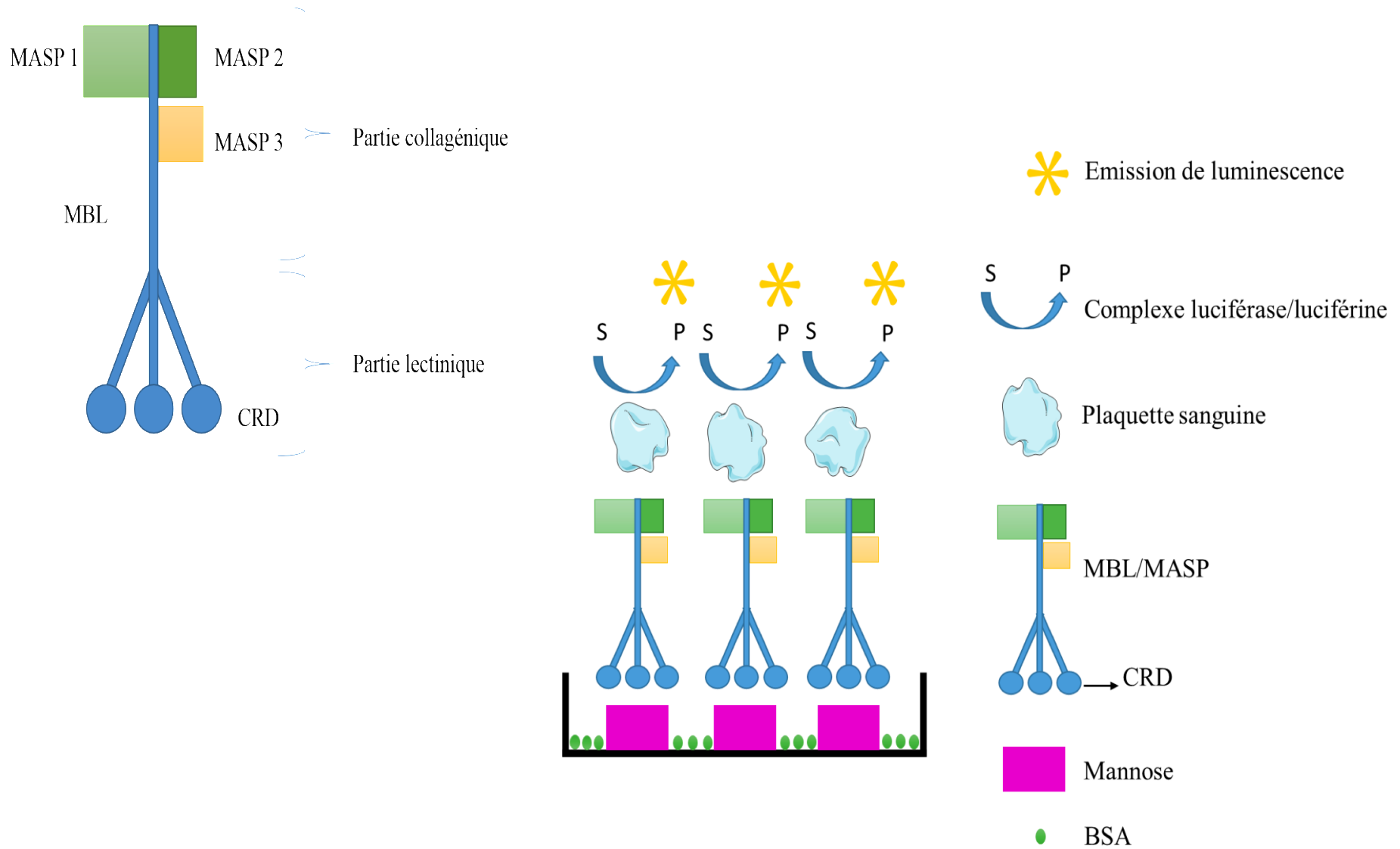
**Kuchler's team  
(Medical University of Vienna)**

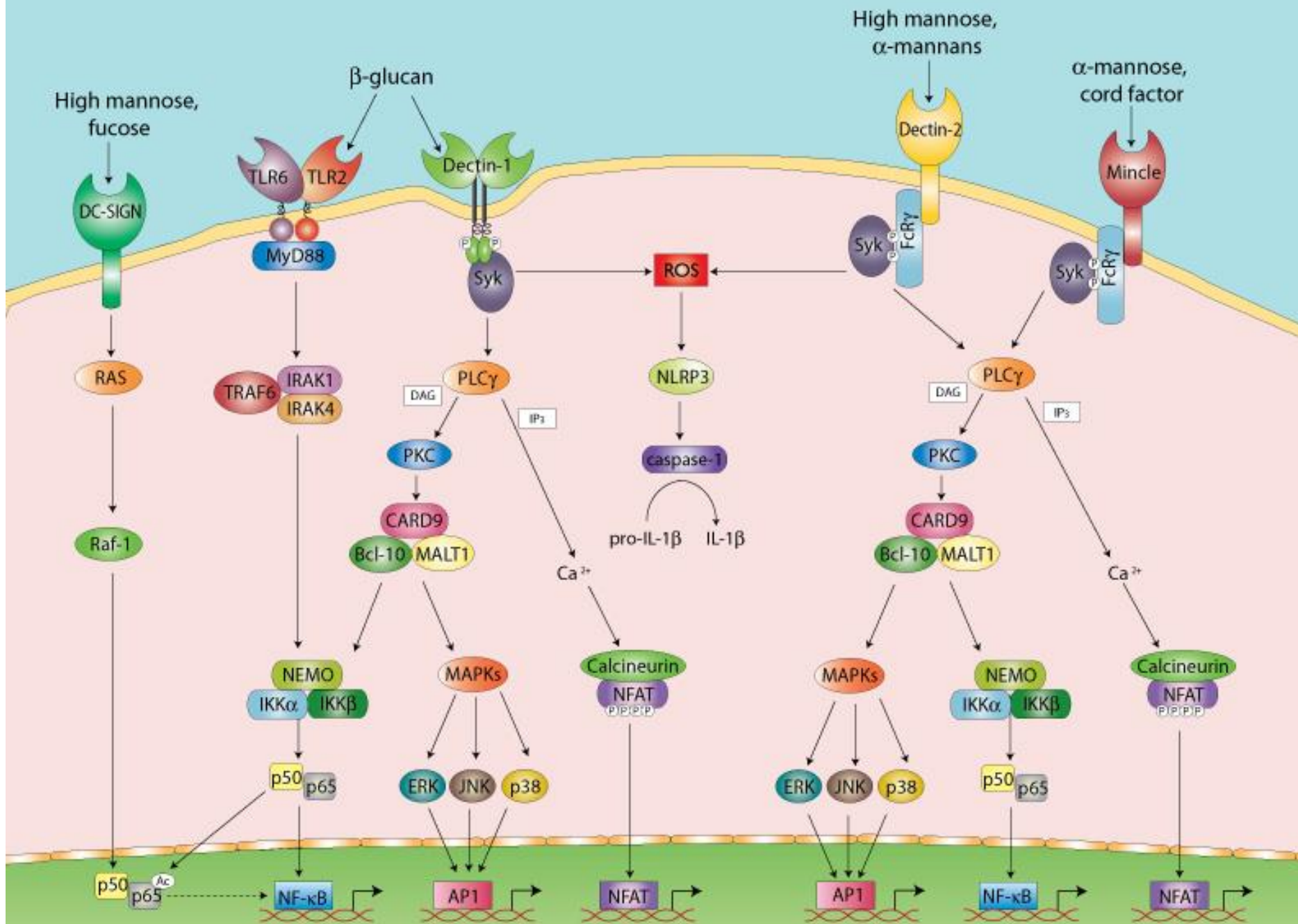
**Plow's team  
(Cleveland Clinic, Cleveland )**

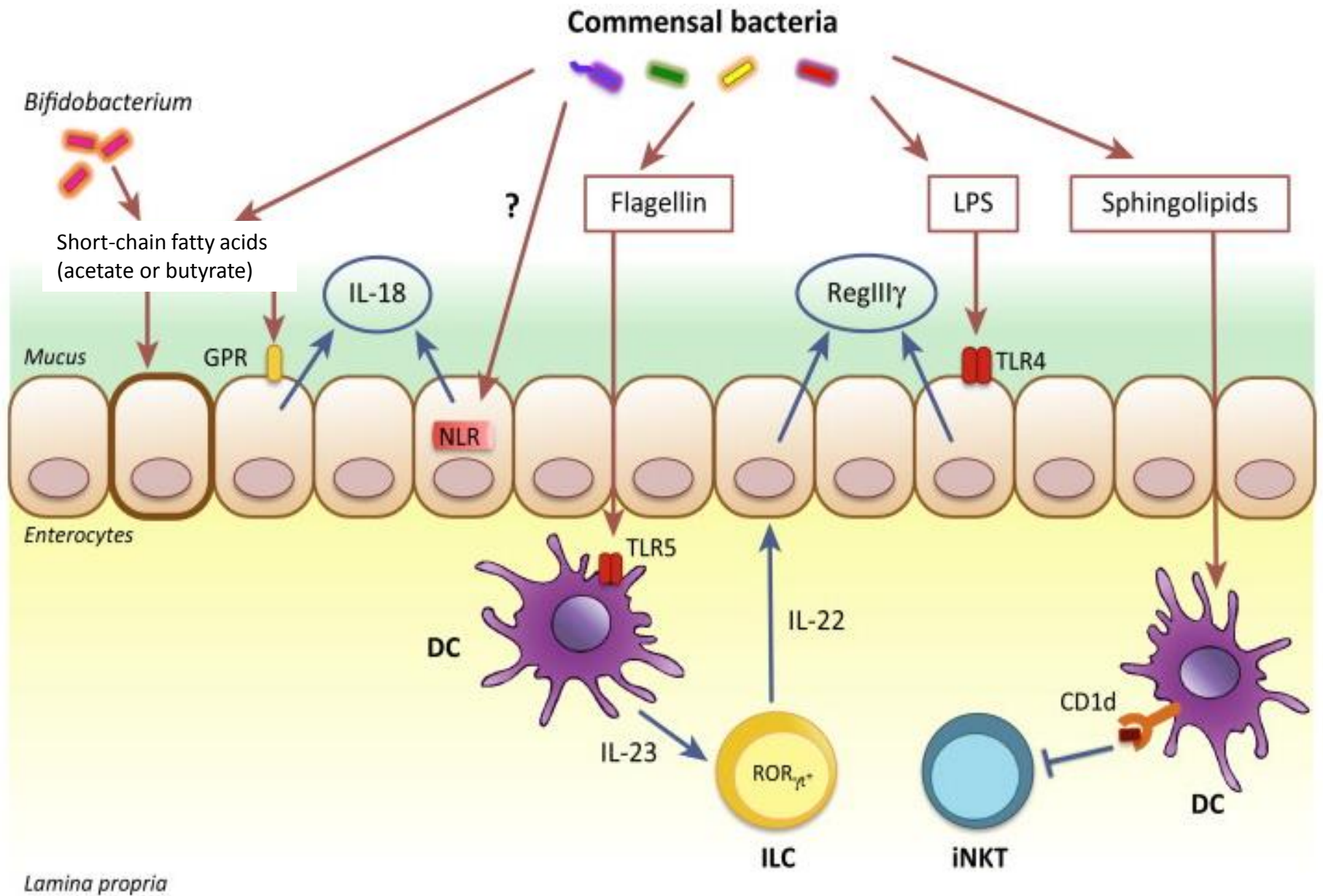
**Roger's team  
(University of Lausanne, Switzerland)**

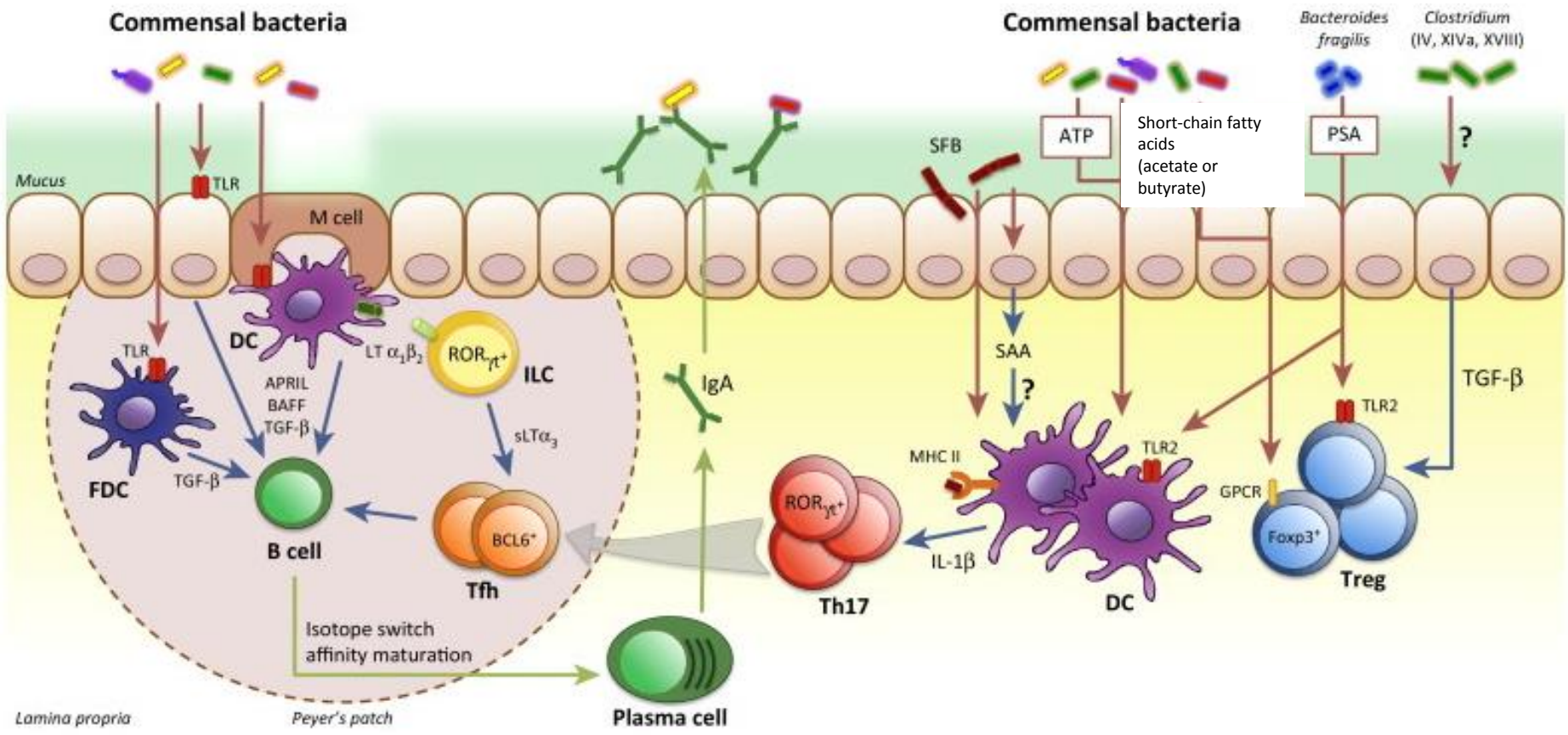


# Principe de mesure de l'activation plaquettaire par le complexe MBL-MASP









**Commensal bacteria**

**Commensal bacteria**

*Bacteroides fragilis*

*Clostridium* (IV, XIVa, XVIII)

Mucus

Short-chain fatty acids (acetate or butyrate)

Lamina propria

Peyer's patch

Plasma cell

Th17

DC

Treg

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Tfh

ILC

FDC

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