

A scanning electron micrograph (SEM) showing a dense population of diverse, rod-shaped bacteria. The bacteria are rendered in various colors (blue, green, yellow, orange, red, pink, purple) against a dark background, illustrating the complexity and diversity of the gut microbiome.

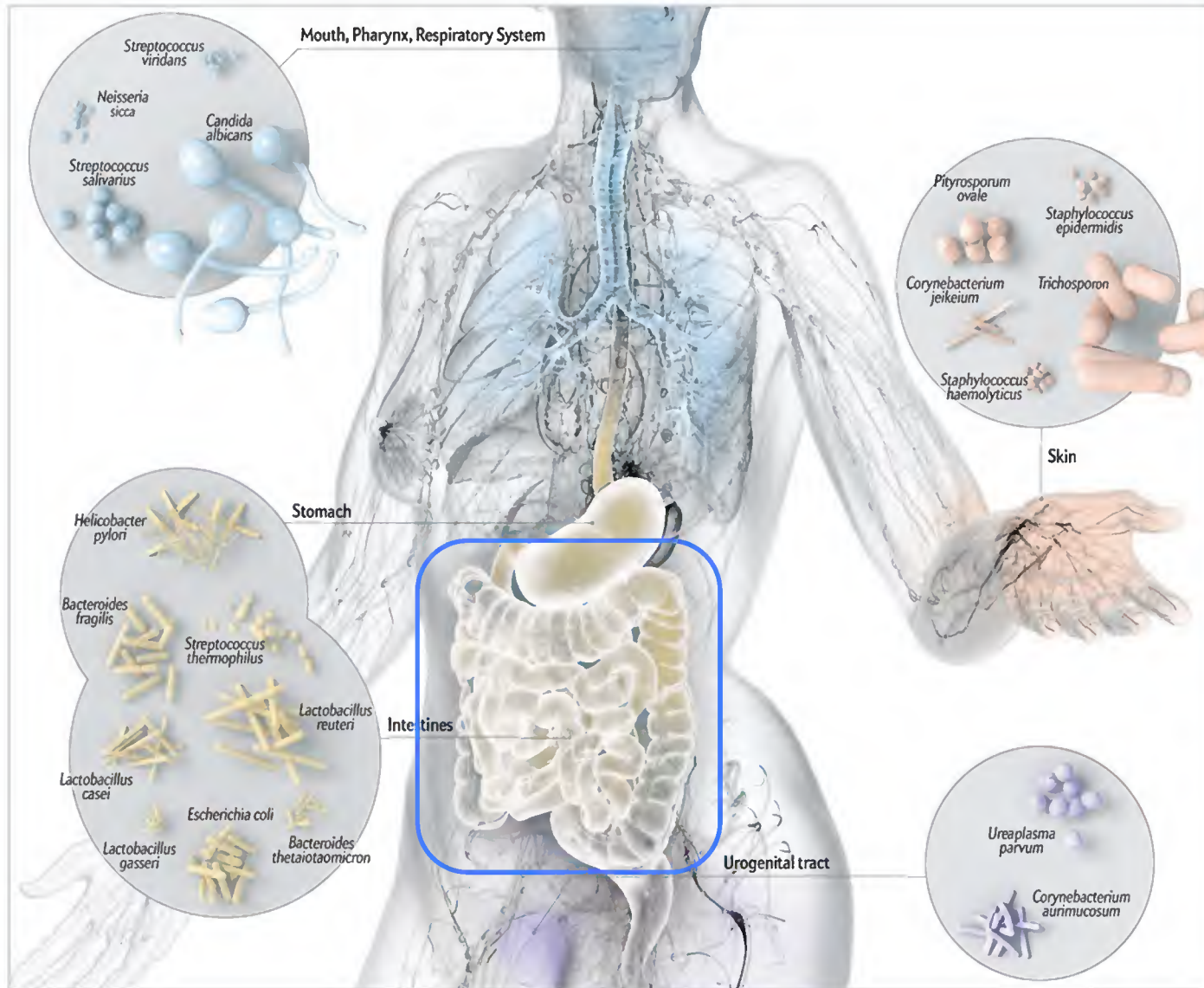
# L'intestino come secondo cervello

Chiara Pozzi

Humanitas Clinical and Research Center

# The microbiota

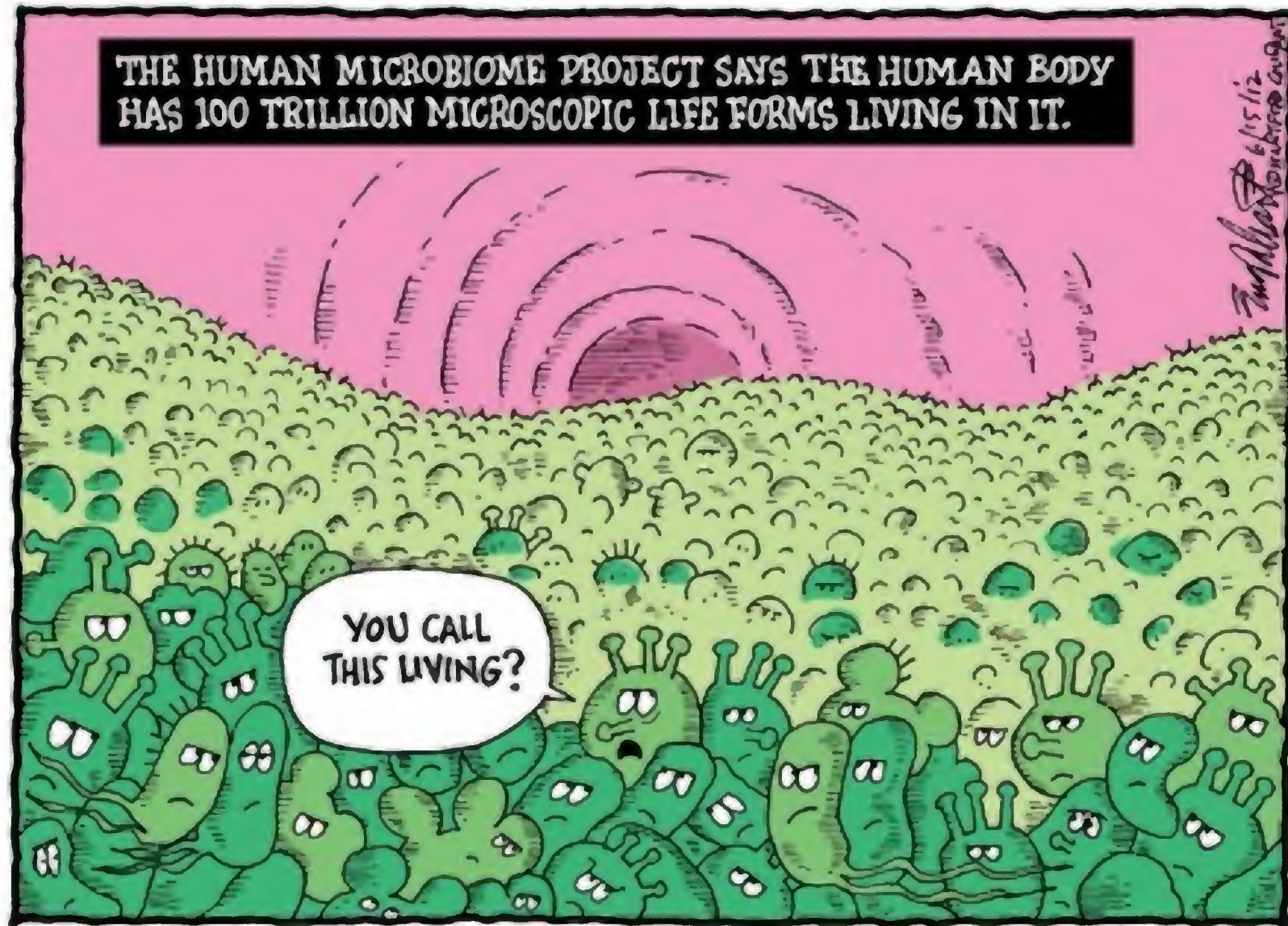
“The entire microbial community living in symbiosis with our body”



- skin,
- mammary glands,
- placenta,
- seminal fluid,
- uterus,
- ovarian follicles,
- lung,
- saliva,
- oral mucosa,
- conjunctiva,
- gastrointestinal tract

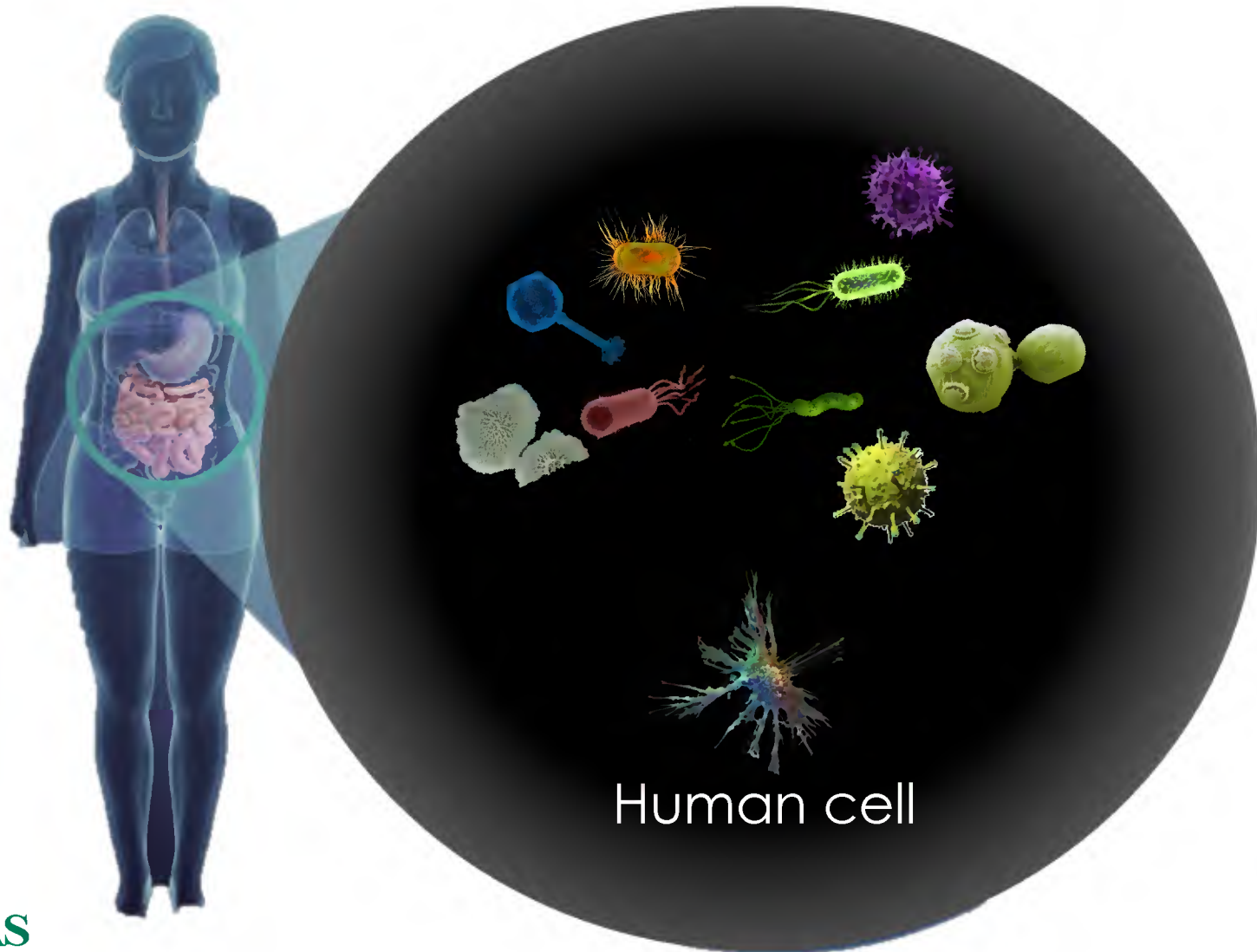


# The microbiota

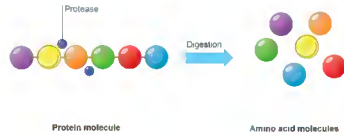


# The intestinal microbiota

- ✓  $10^{14}$  bacteria (vs.  $10^{13}$  mammalian cells): 10 times more microbial cells than mammalian cells
- ✓  $10^6$  microbial genes (100 times more than us)
- ✓ Resident microbiota consisting of  $10^{10}$  –  $10^{12}$  organisms per gram of luminal content.



# The intestinal microbiota

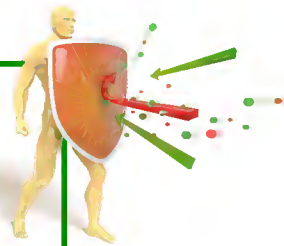


Food  
breakdown

Vitamin  
metabolism



Immune  
system  
maturation



**Intestinal  
microbiota**

Brain  
development



Defense  
against  
pathogens

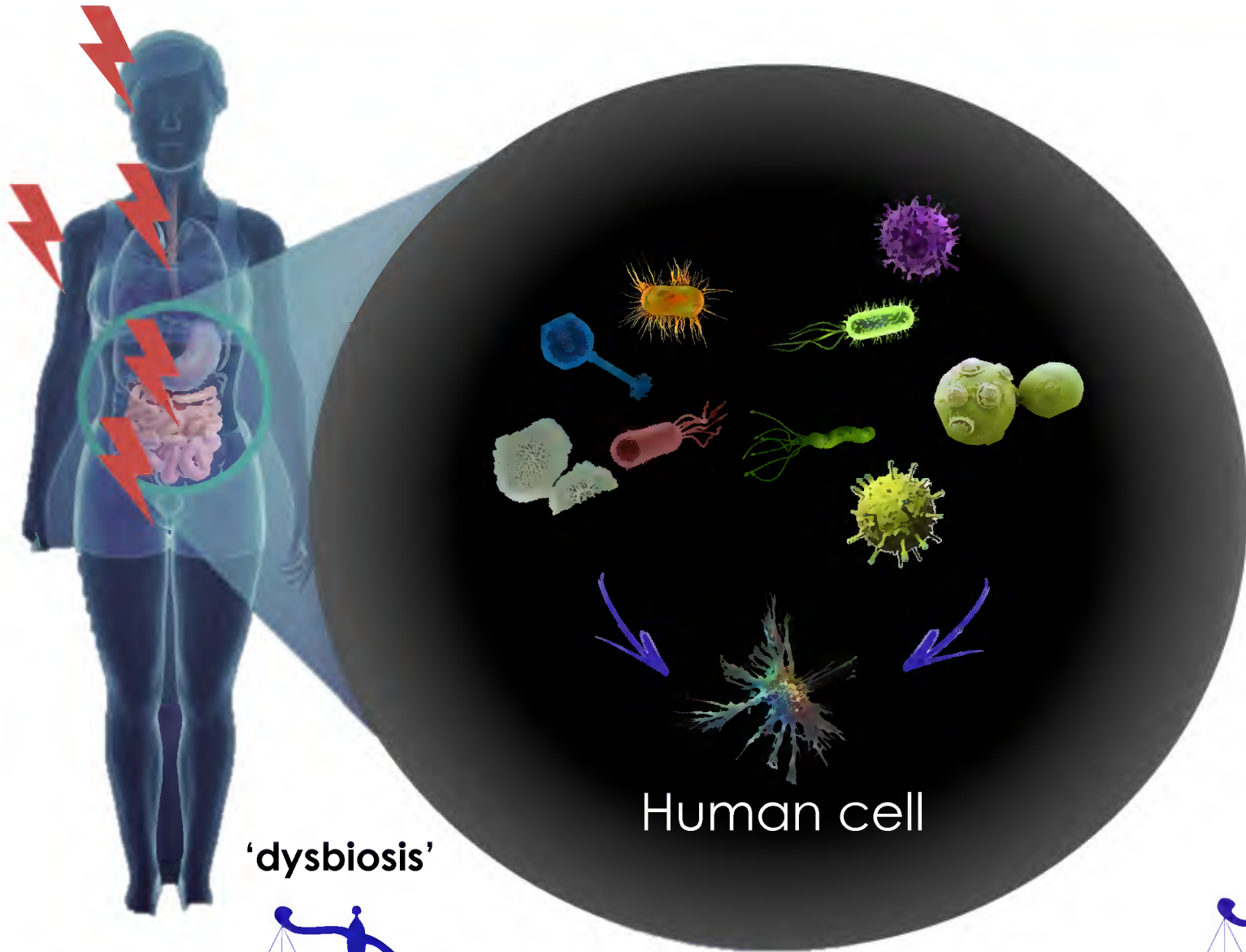
Xenobiotic  
metabolism



*Eberl, Mucosal Immunology. 2010*



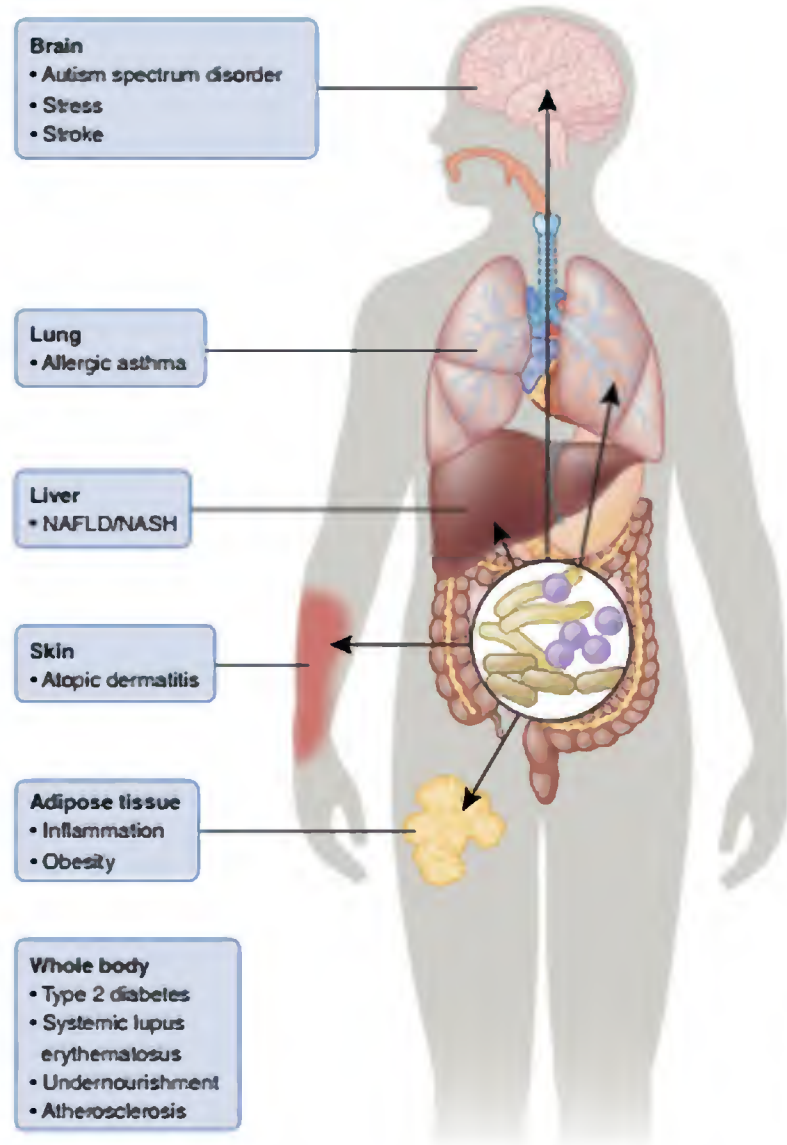
# Dysbiosis



'dysbiosis'

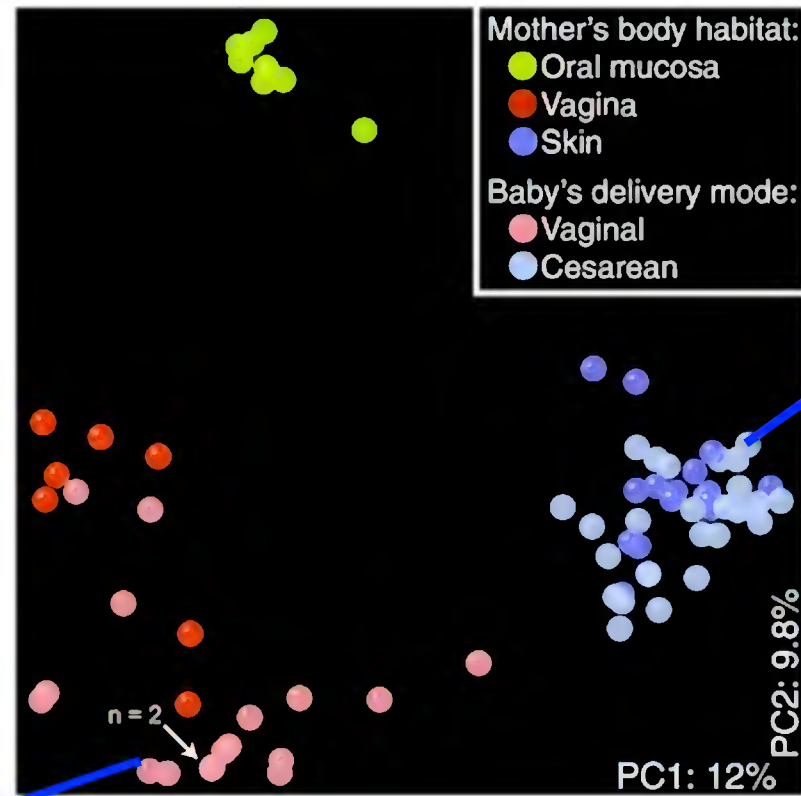


# The intestinal microbiota is associated with various diseases in humans



Alterations in composition, diversity and metabolites derived from the intestinal microbiota are associated with diseases affecting different organs of the human body.

# Microbiota is acquired at birth and during lactation



**C-section:** typical skin taxa (Staphylococcus spp.)

## Natural delivery

(Lactobacillus, Prevotella, Atopobium, or Sneathia spp.)



# Microbiota is acquired at birth

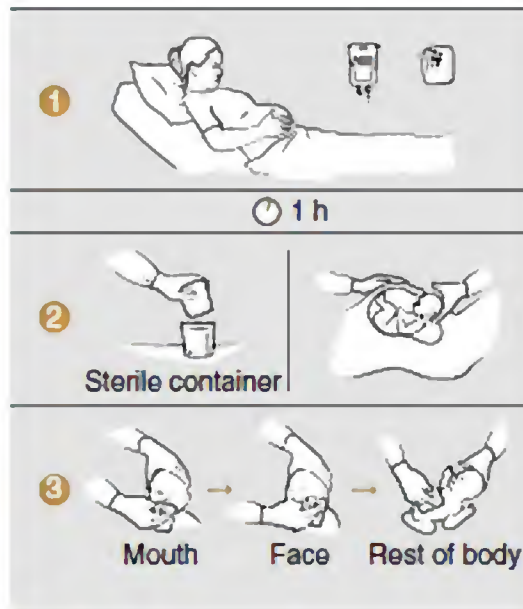
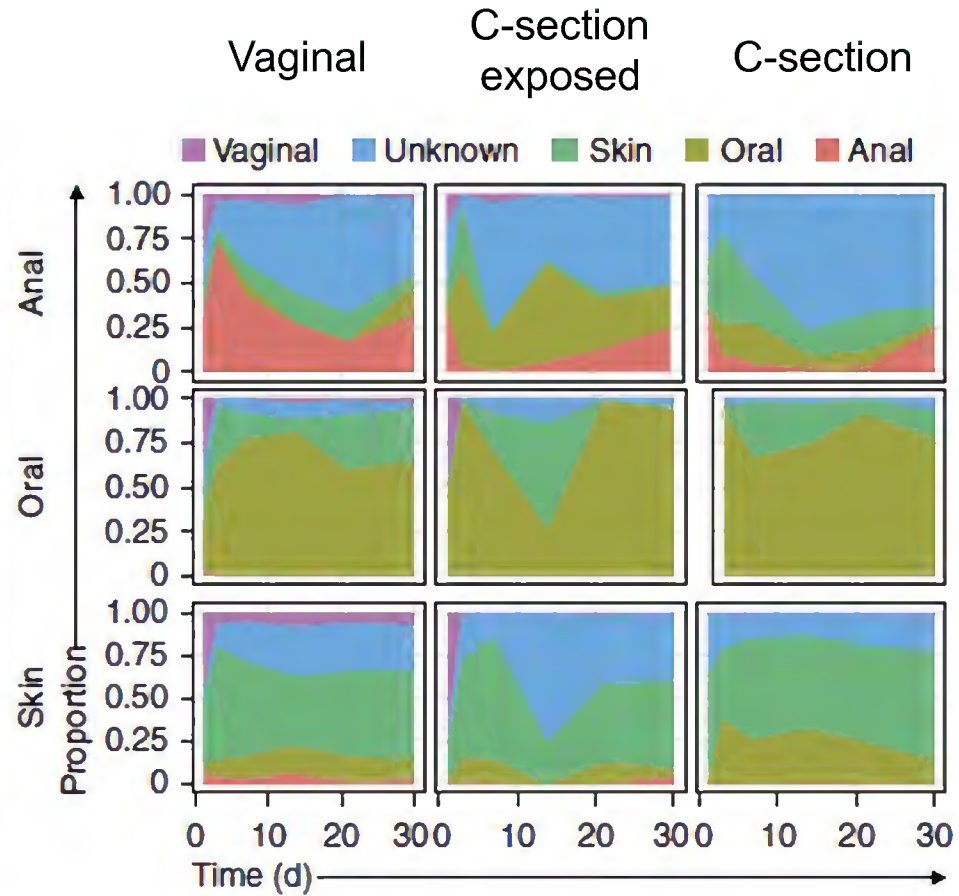
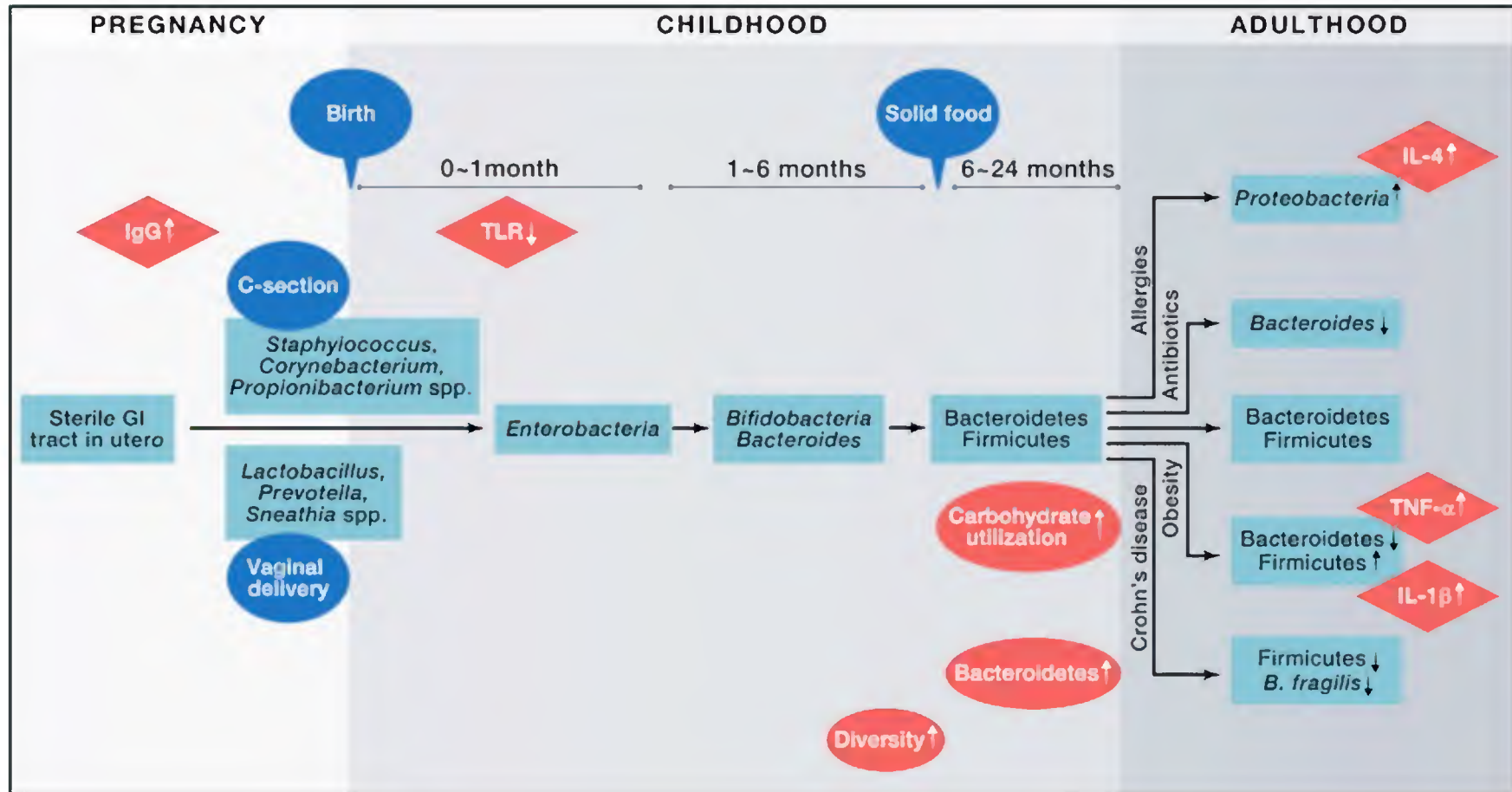


Image: M.J. Schoen



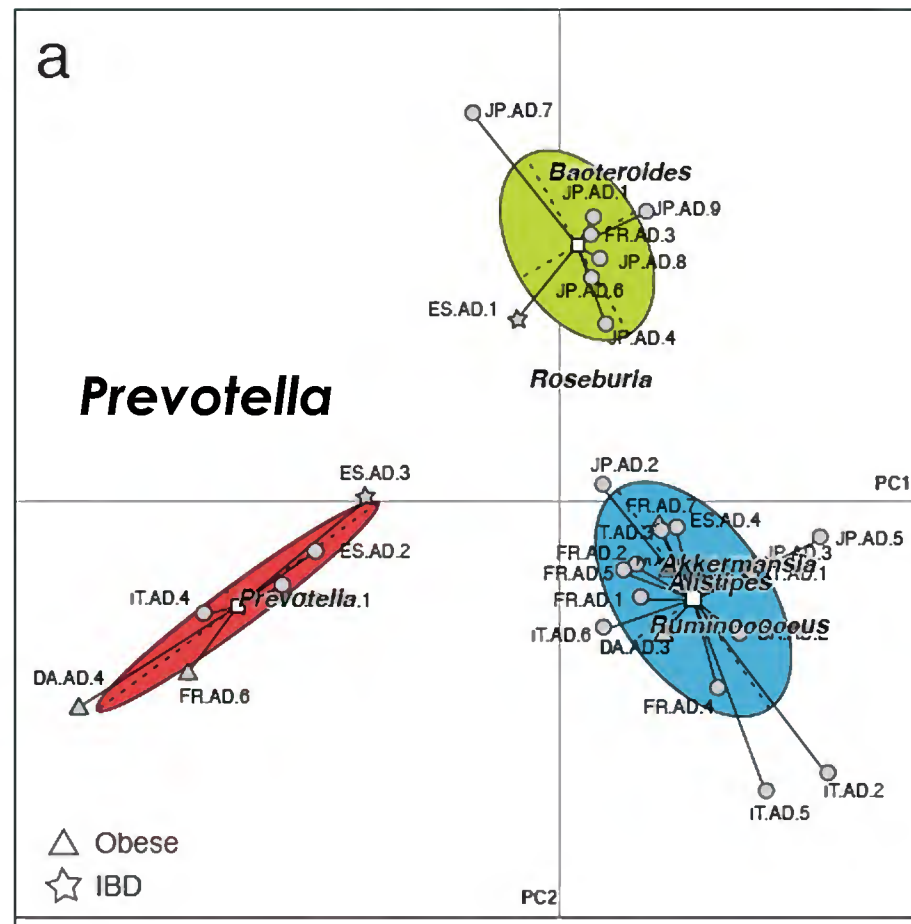
# Establishment of the microbiota



Clemente et al. Cell 148, (2012), Pages 1258–1270

# Three different enterotypes characterize the human population

## Bacteroides

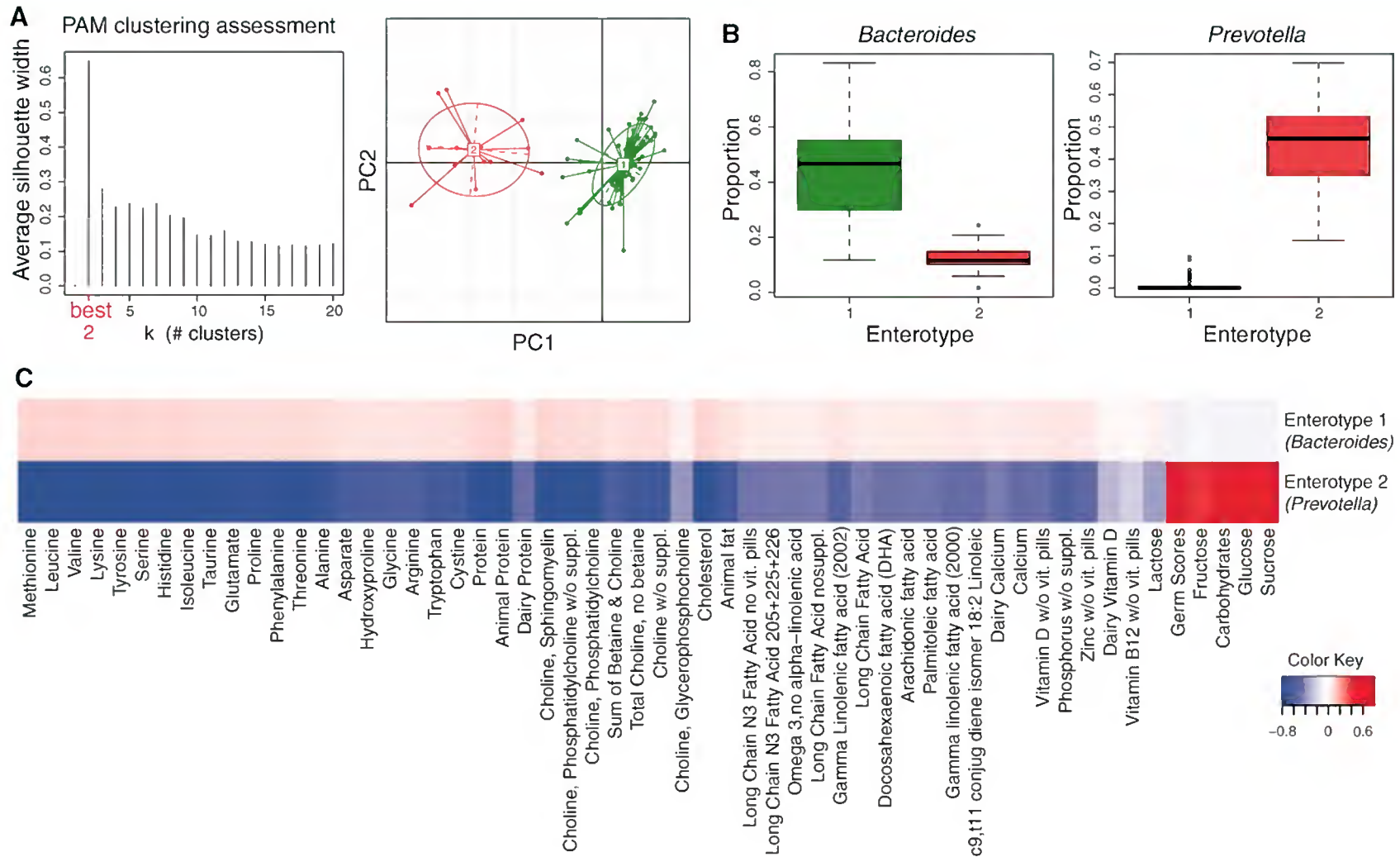


39 individuals including  
Europeans, American  
Japanese

Enterotypes of the human gut microbiome. Arumugam M. et al. Nature April 2011 (Metahit consortium)

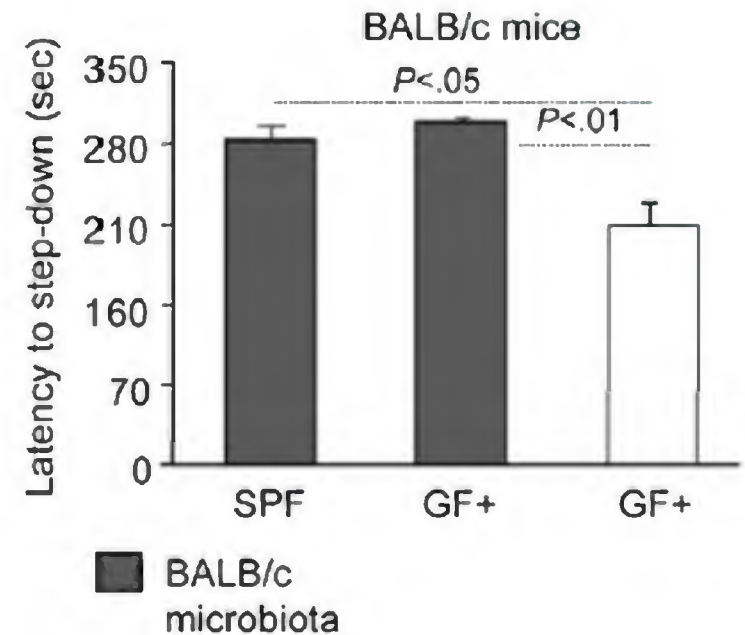
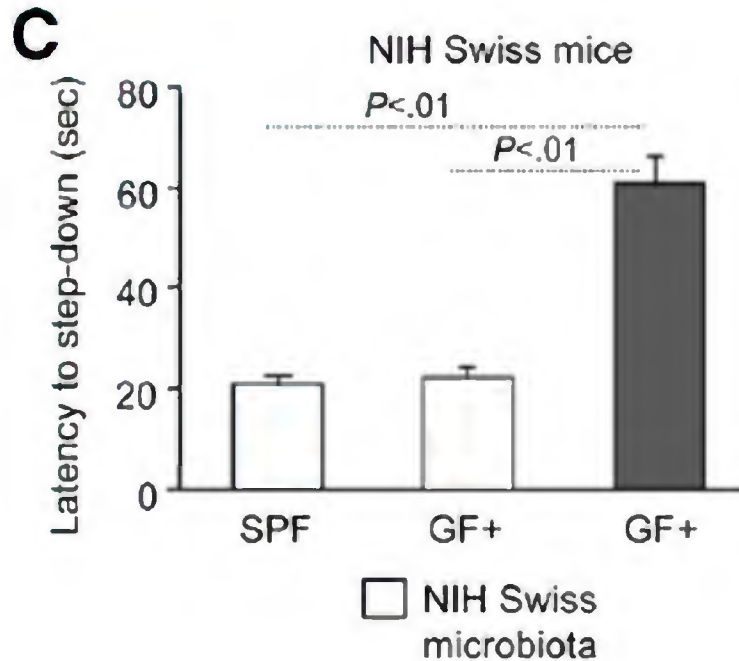
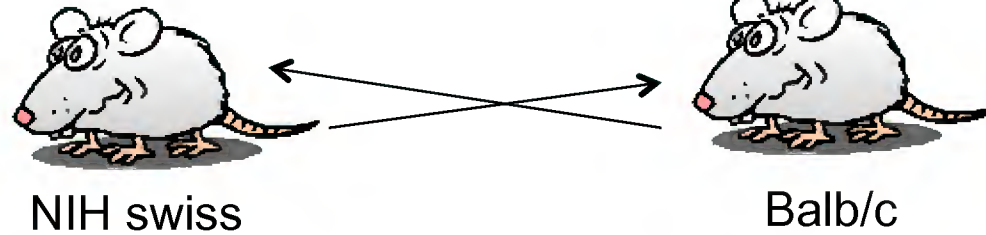
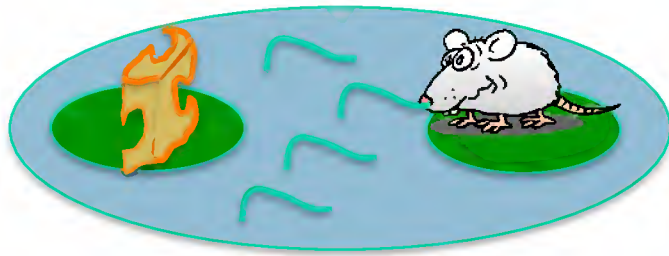


# Diet can affect the microbiota



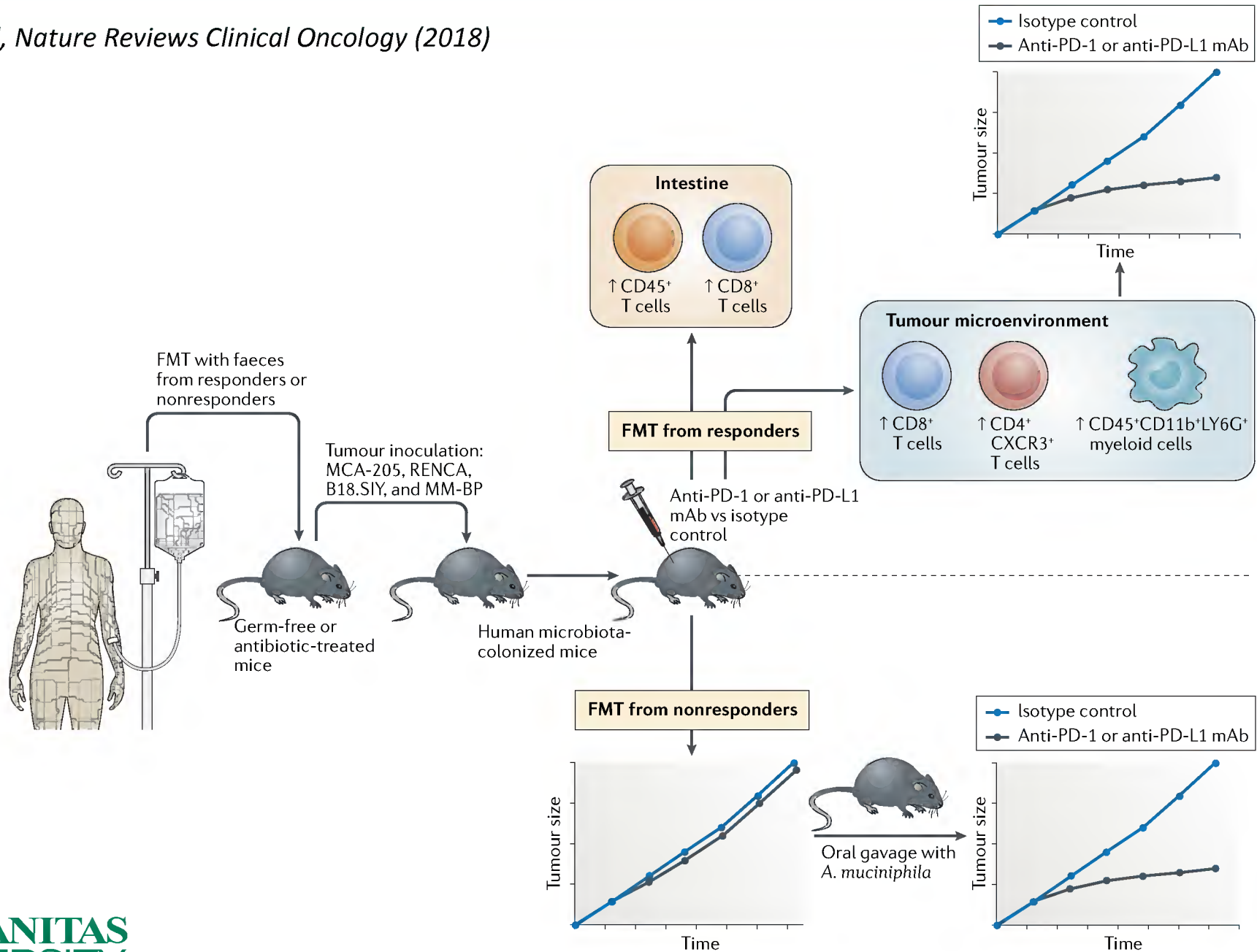
Wu et al *Science* 7 October 2011:

# The microbiota controls behaviour



# Correlations between the clinical efficacy of immune-checkpoint blockade and microbiota composition

Routy et al, Nature Reviews Clinical Oncology (2018)

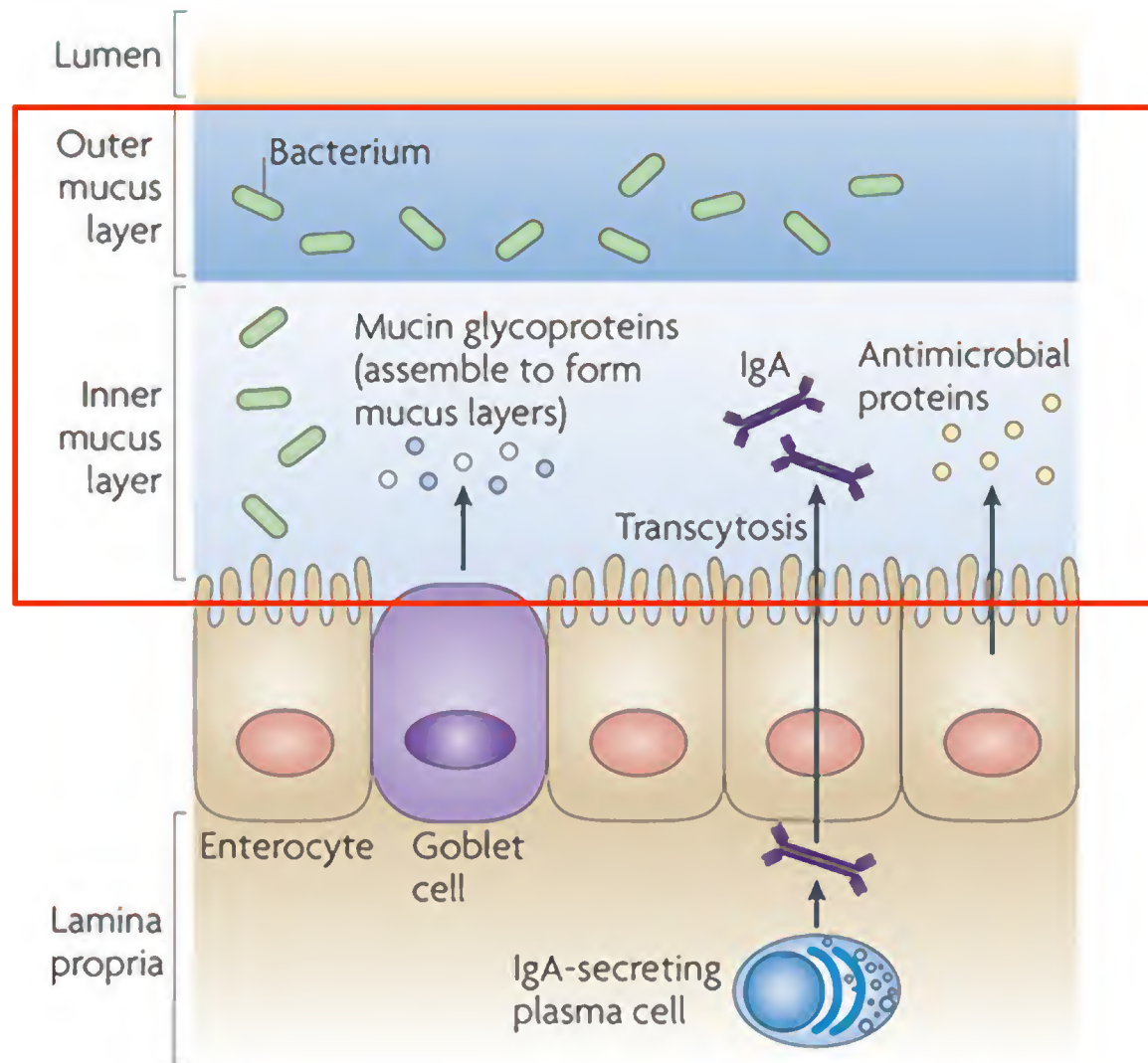




# The host-microbiota interface

Multiple components:

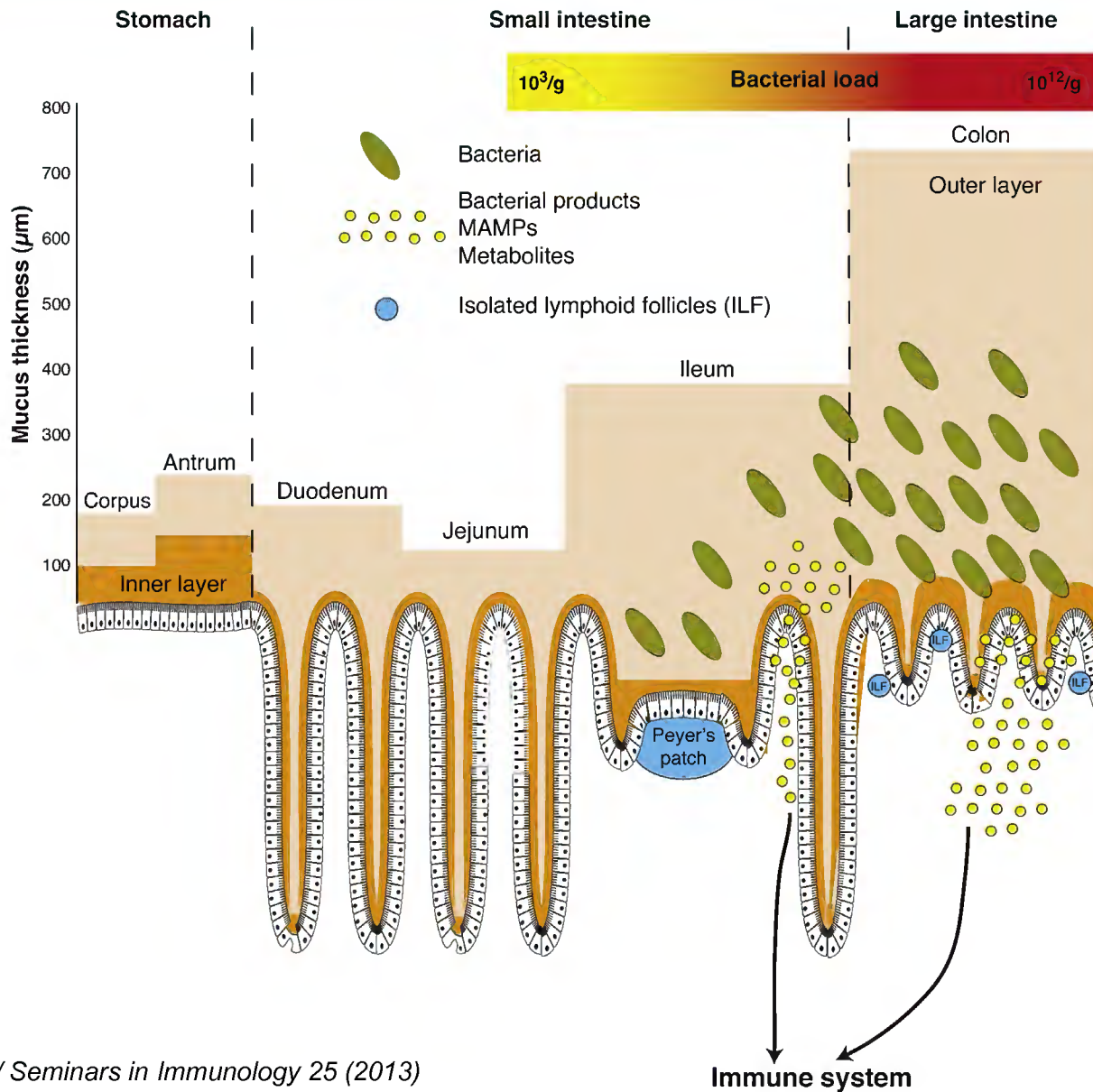
- ✓ **Mucus** (goblet cells).
- ✓ Epithelial cells;
- ✓ Antimicrobial peptides;
- ✓ Secretory IgA;
- ✓ Immune cells.



Johansson et al., PNAS, 2008

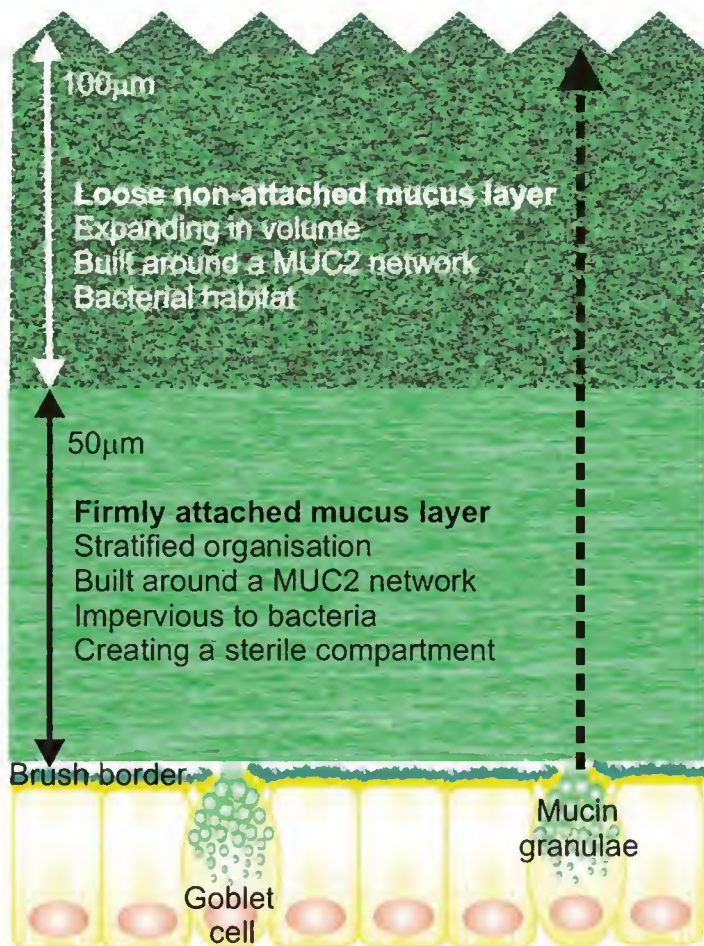
Hooper et al., Nat. Rev. Immunol. 2010

# The intestinal microbiota is separated from the epithelium by the mucus

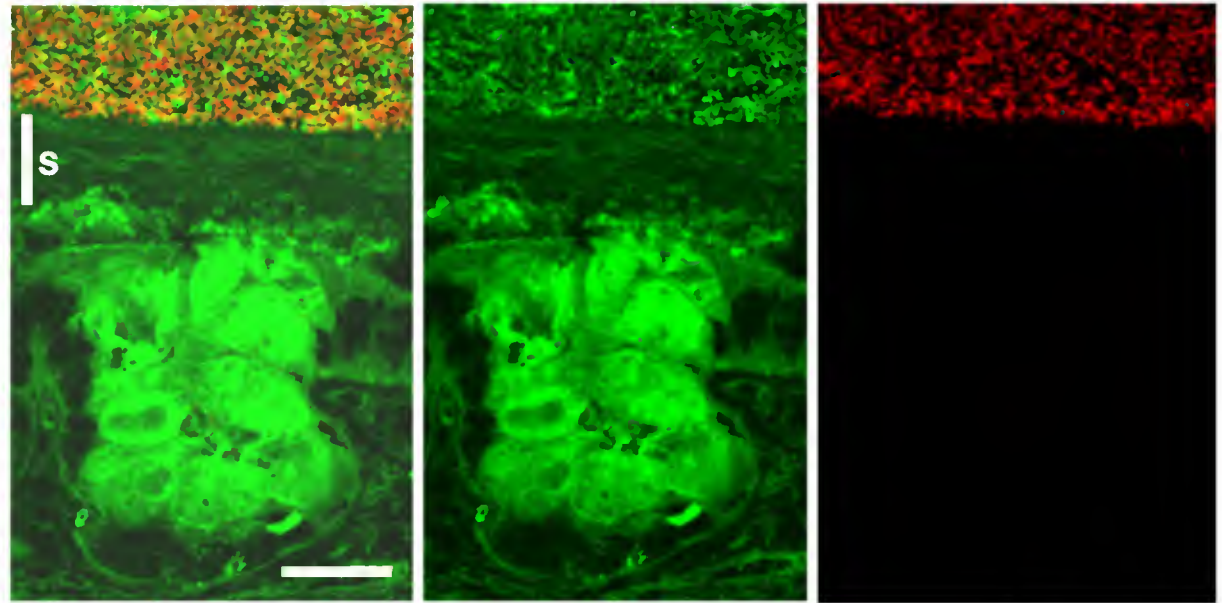


A.J. Macpherson, K.D. McCoy / *Seminars in Immunology* 25 (2013)

# The mucus layer



Johansson et al., *Gut Microbes*, 2010



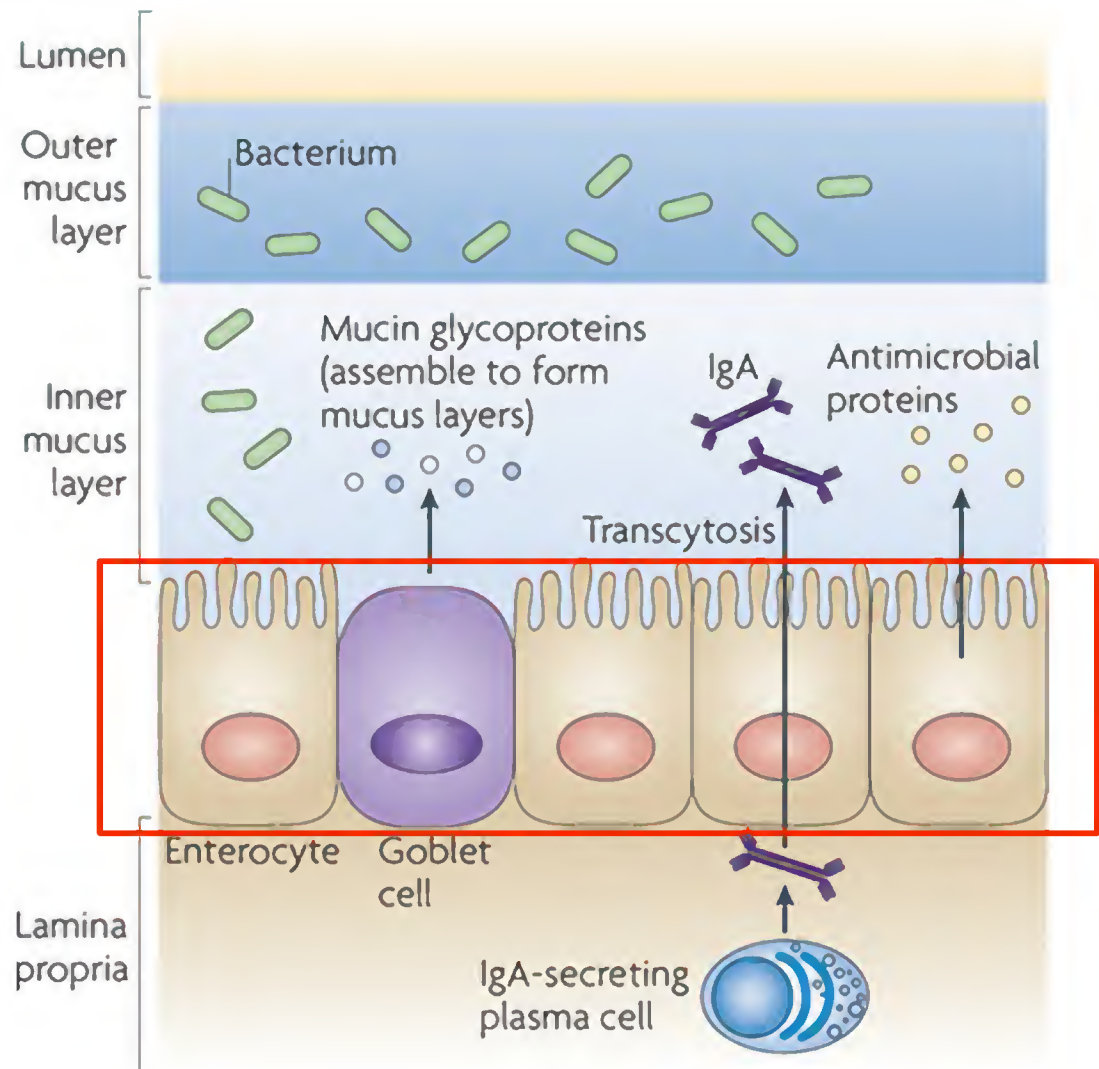
**MUC2**  
**16S**



# The host-microbiota interface

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- ✓ Mucus (goblet cells).
- ✓ **Epithelial cells;**
- ✓ Antimicrobial peptides;
- ✓ Secretory IgA;
- ✓ Immune cells.



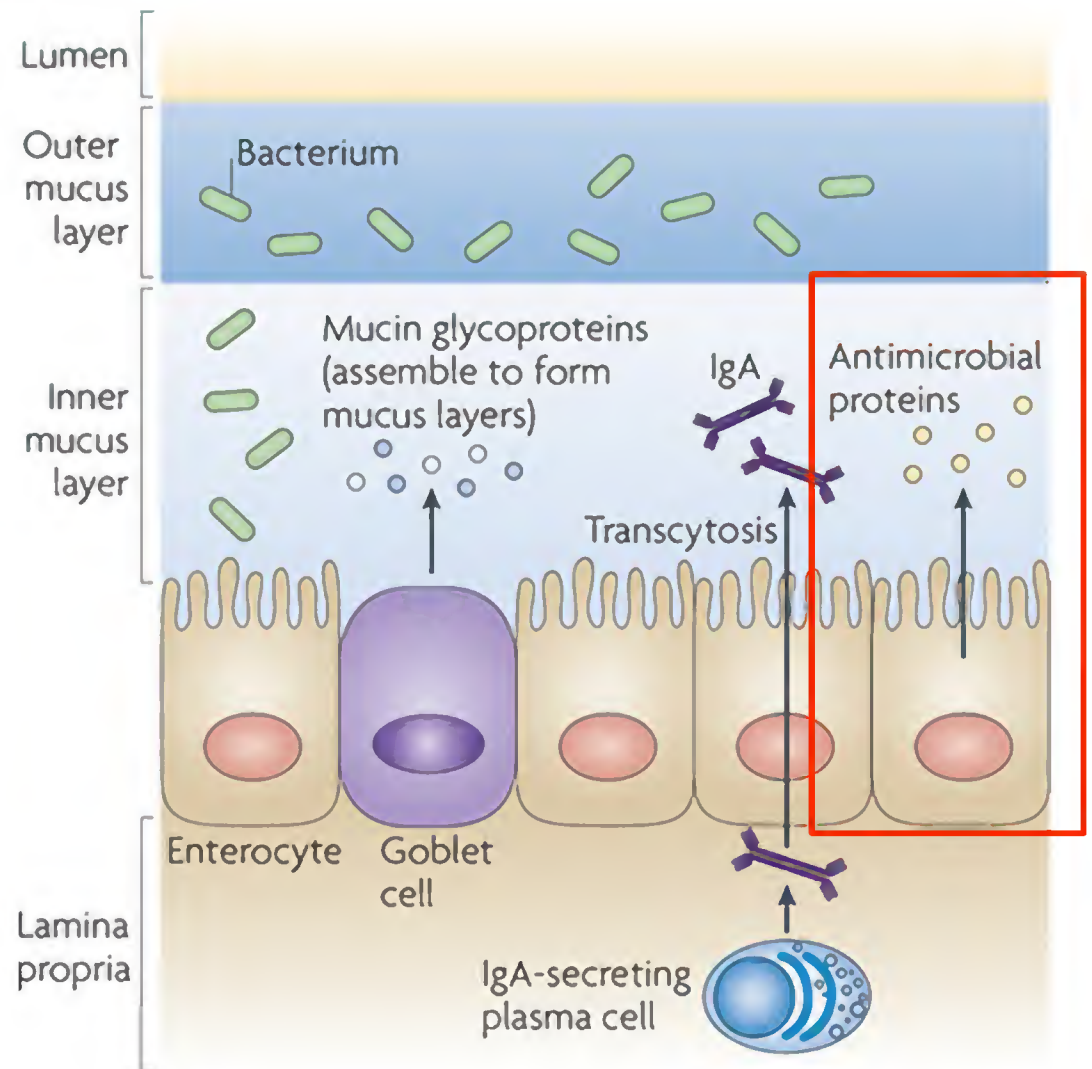
Johansson et al., PNAS, 2008

Hooper et al., Nat. Rev. Immunol. 2010

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Multiple components:

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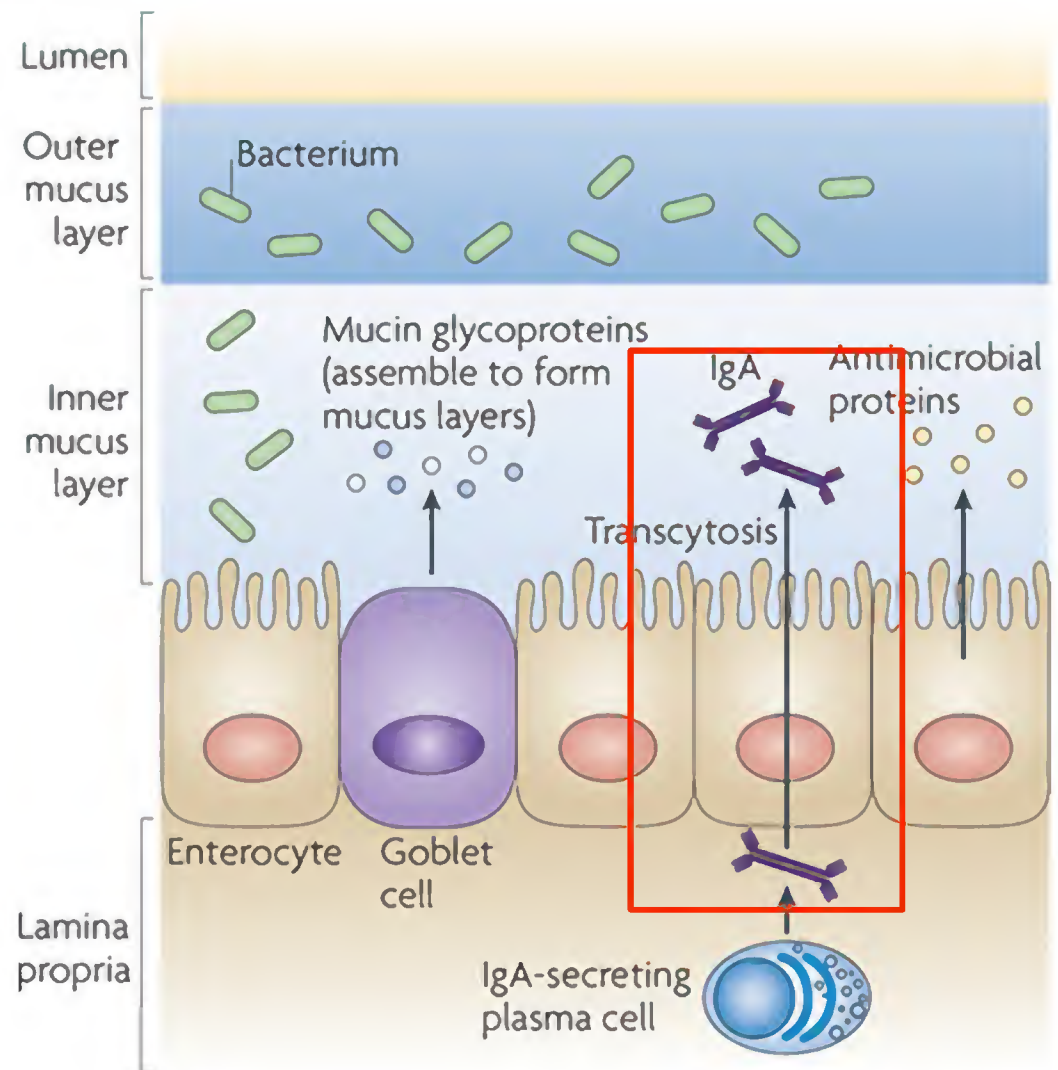
Johansson et al., PNAS, 2008

Hooper et al., Nat. Rev. Immunol. 2010

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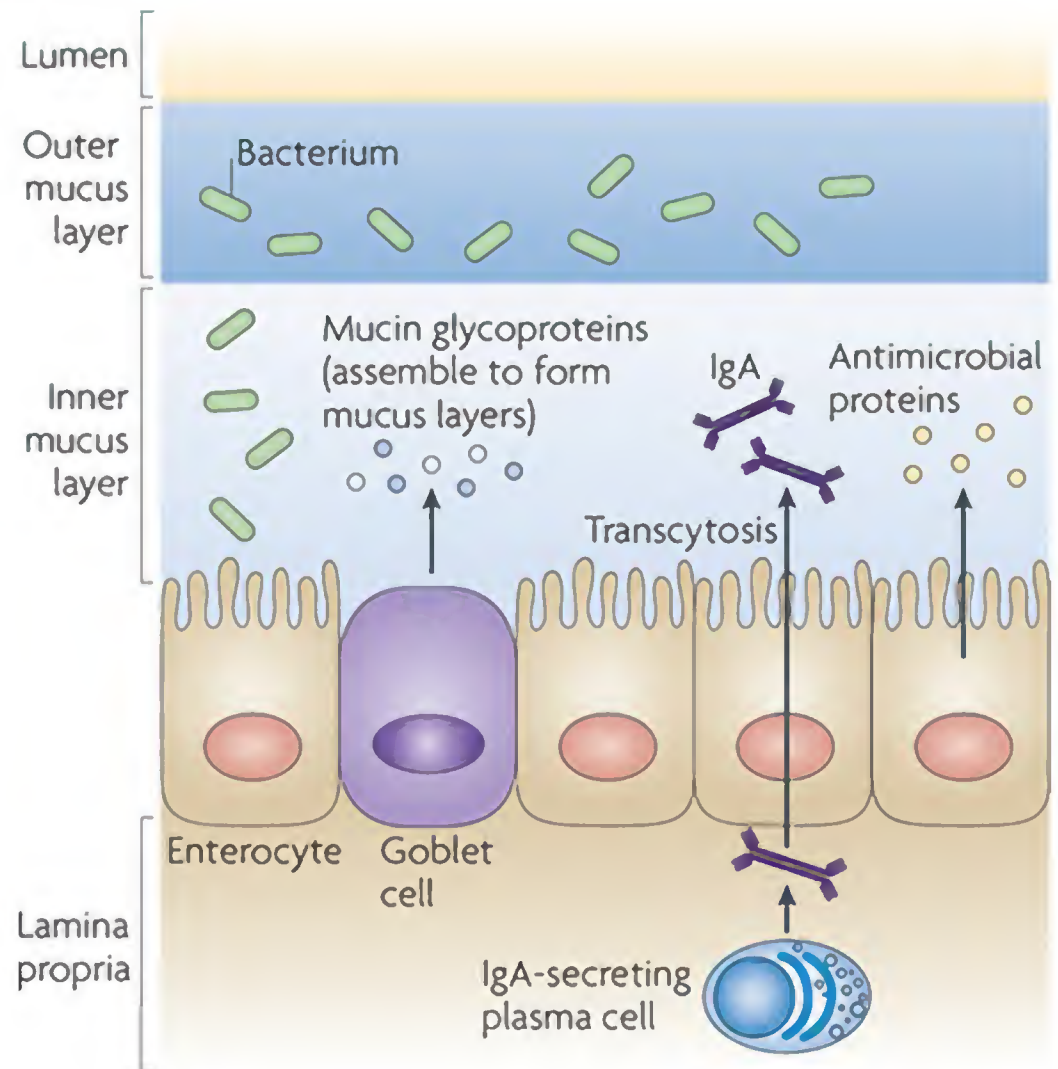
Johansson et al., PNAS, 2008

Hooper et al., Nat. Rev. Immunol. 2010

# The host-microbiota interface

## Multiple components:

- ✓ Mucus (goblet cells).
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- ✓ Antimicrobial peptides;
- ✓ Secretory IgA;
- ✓ **Immune cells.**

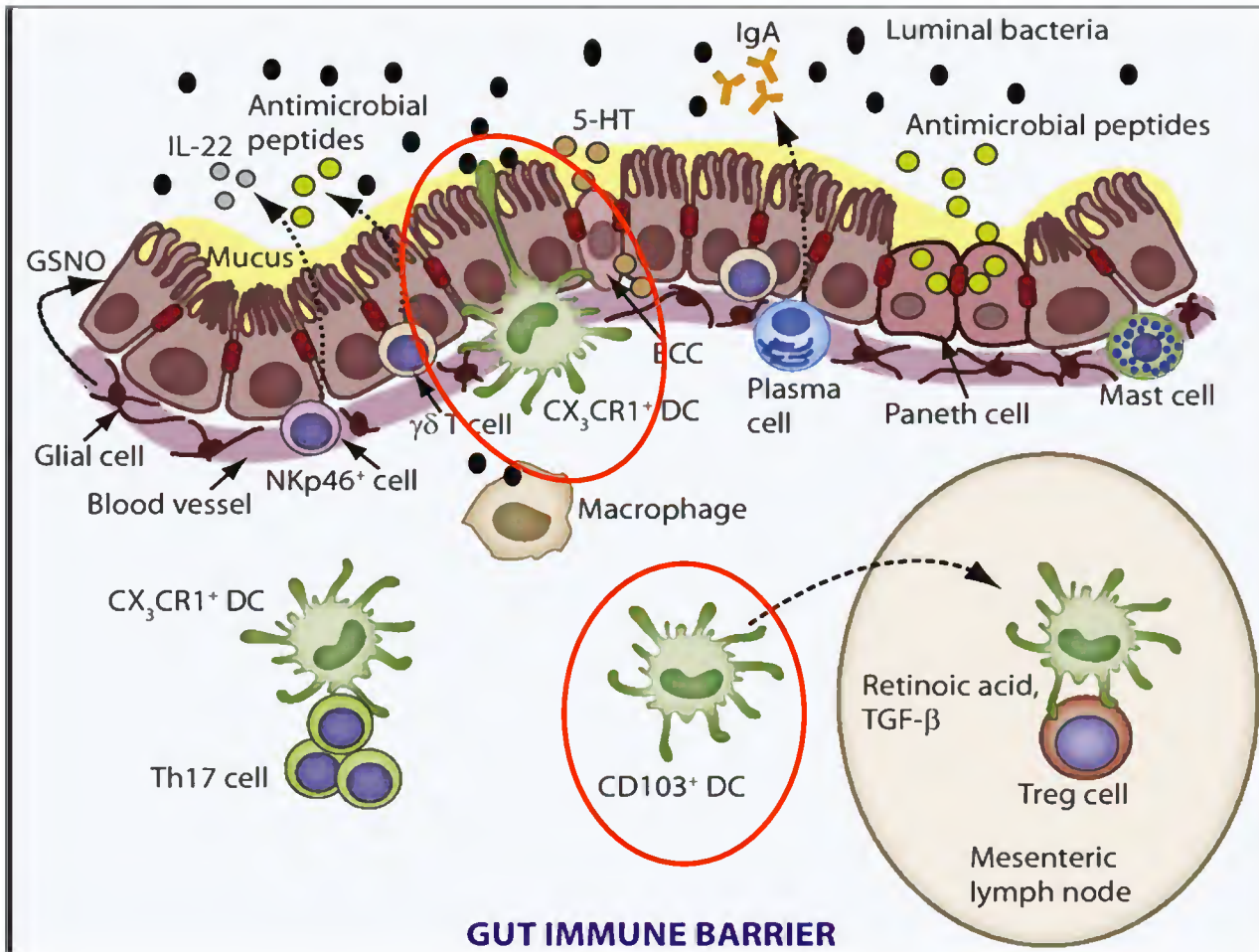


Johansson et al., PNAS, 2008

Hooper et al., Nat. Rev. Immunol. 2010



# Immune cells

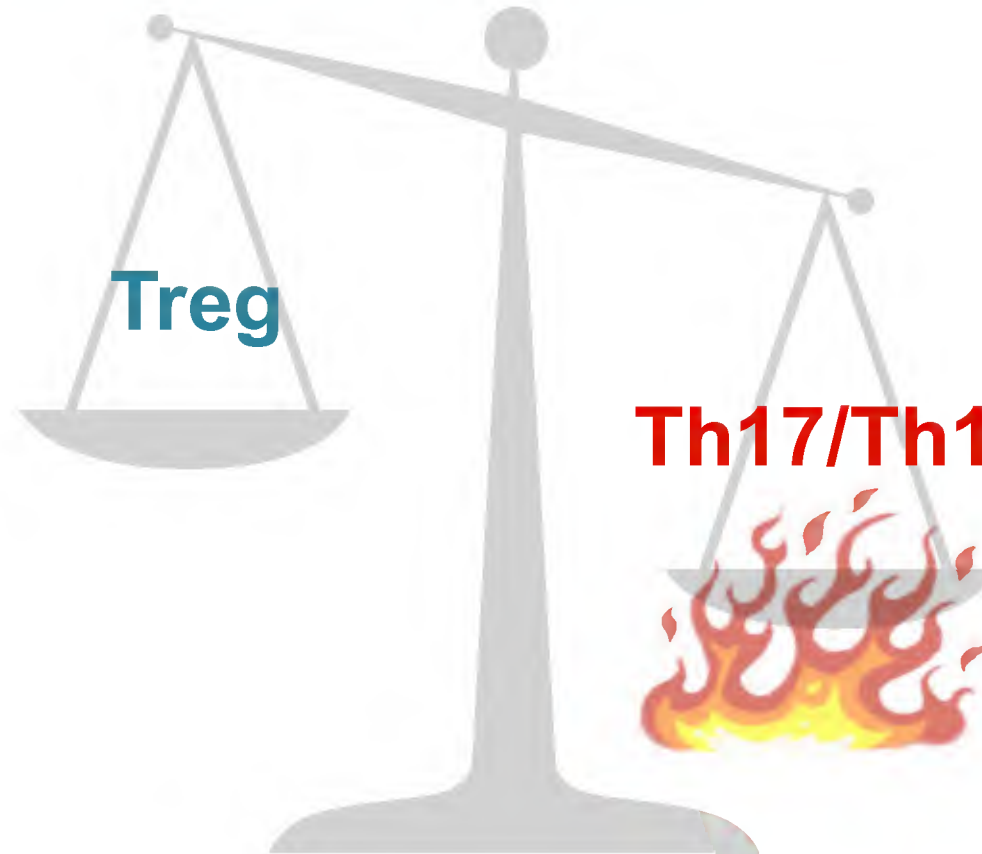


**Two major subsets of APCs in the gut:**

$\text{CD11c}^+ \text{CX3CR1}^{\text{high}} \text{F4/80}^+$  macrophages:  
intra-epithelial  $\rightarrow$  Th17, Treg restimulation

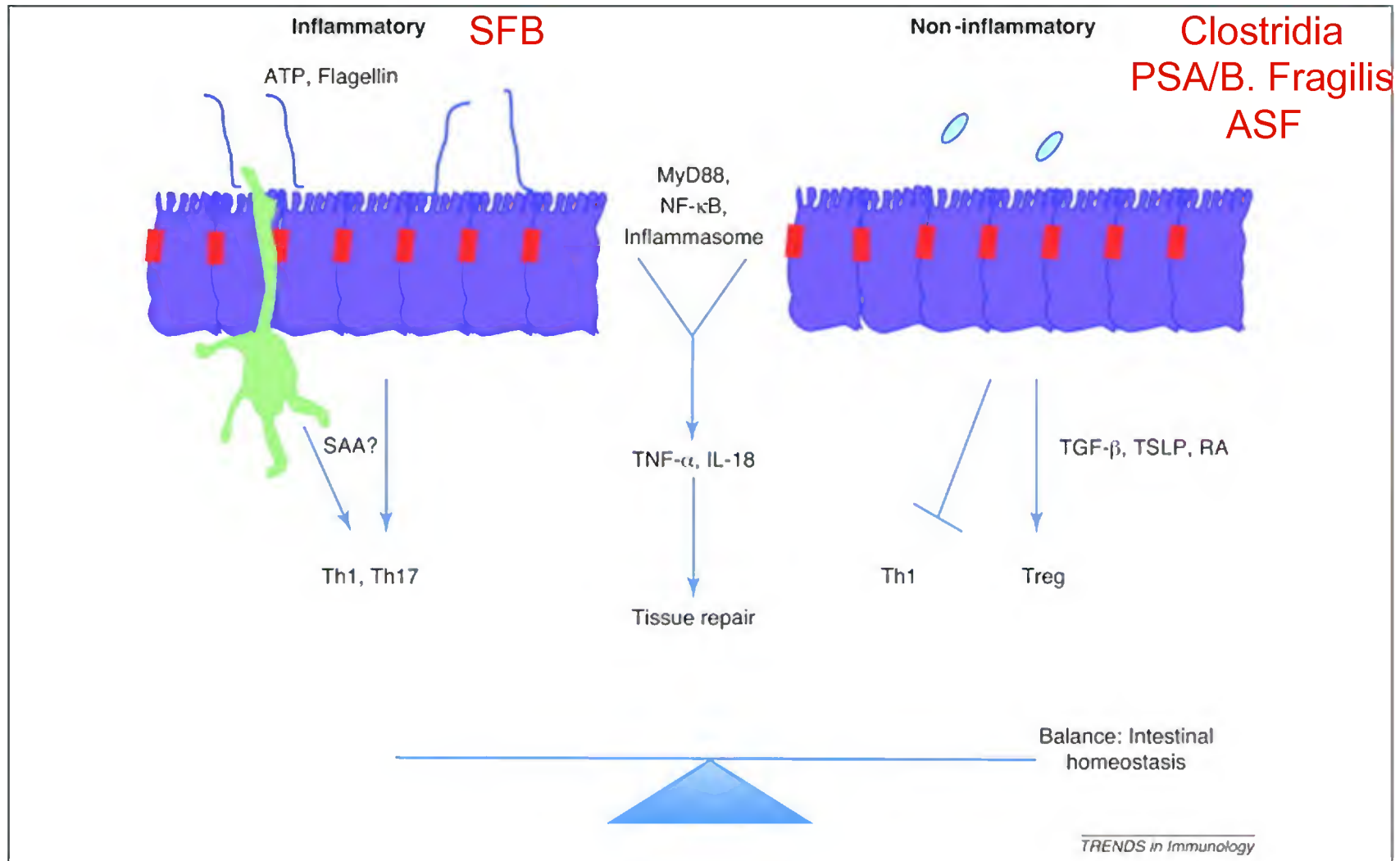
$\text{CD103}^+$  DCs: tolerogenic

# Unbalanced immune response



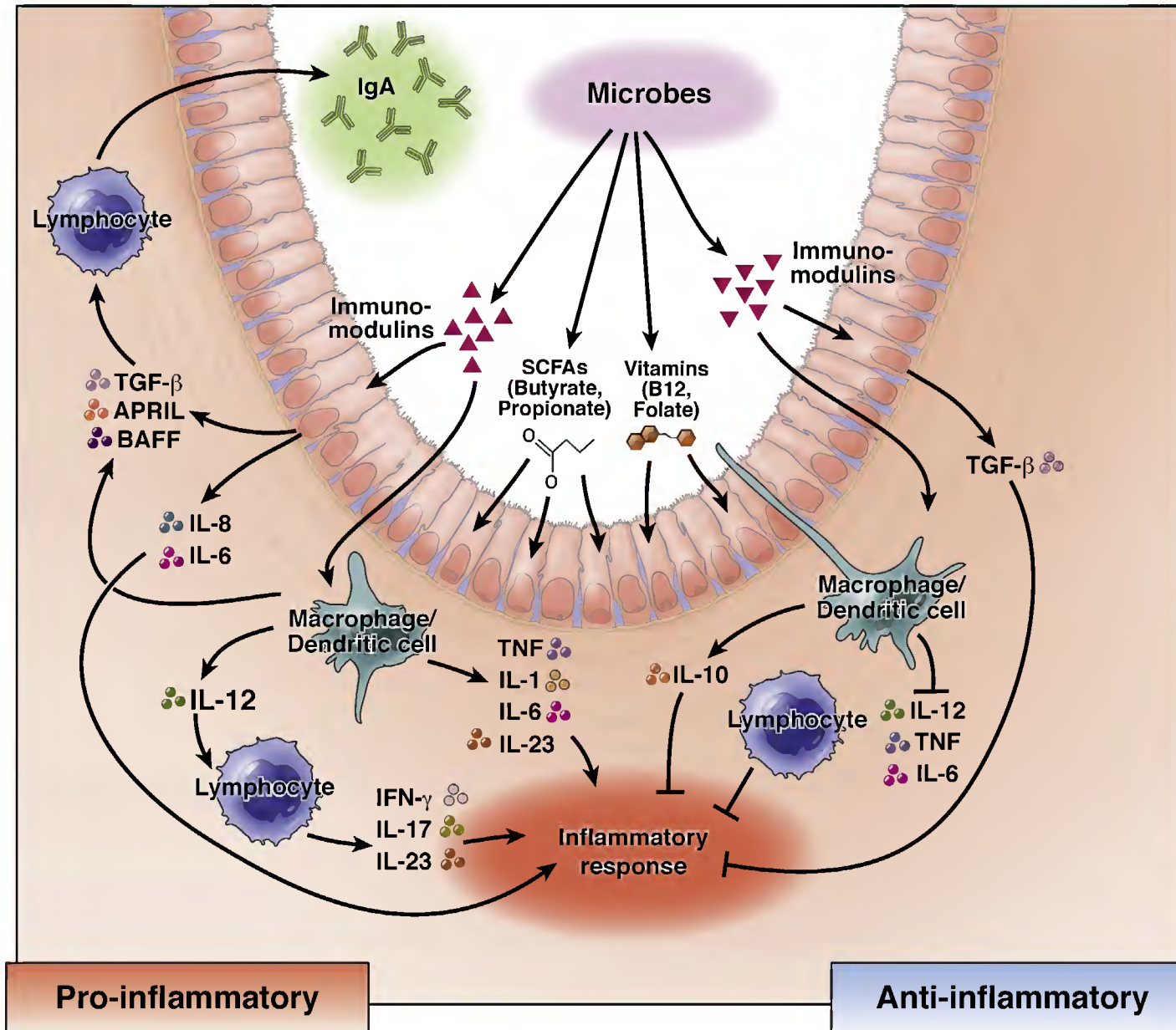
Inflammation as a result of an unbalanced immune response

# Inflammatory/tolerogenic bacteria control intestinal immune homeostasis



Rescigno Trends Immunol 2011, 32: 256–264

# Most of the homeostatic activities are dependent on metabolites (Postbiotics)



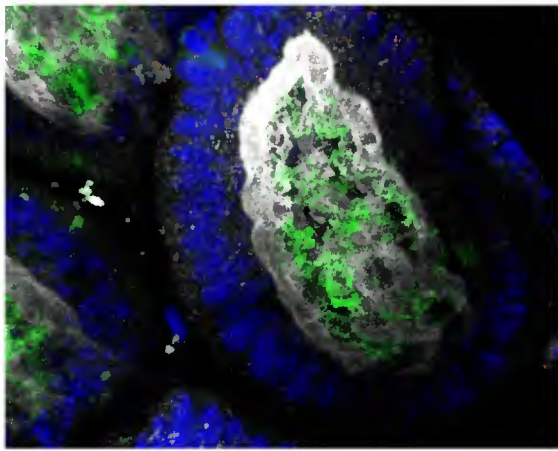


# The microbiota is ignored systemically

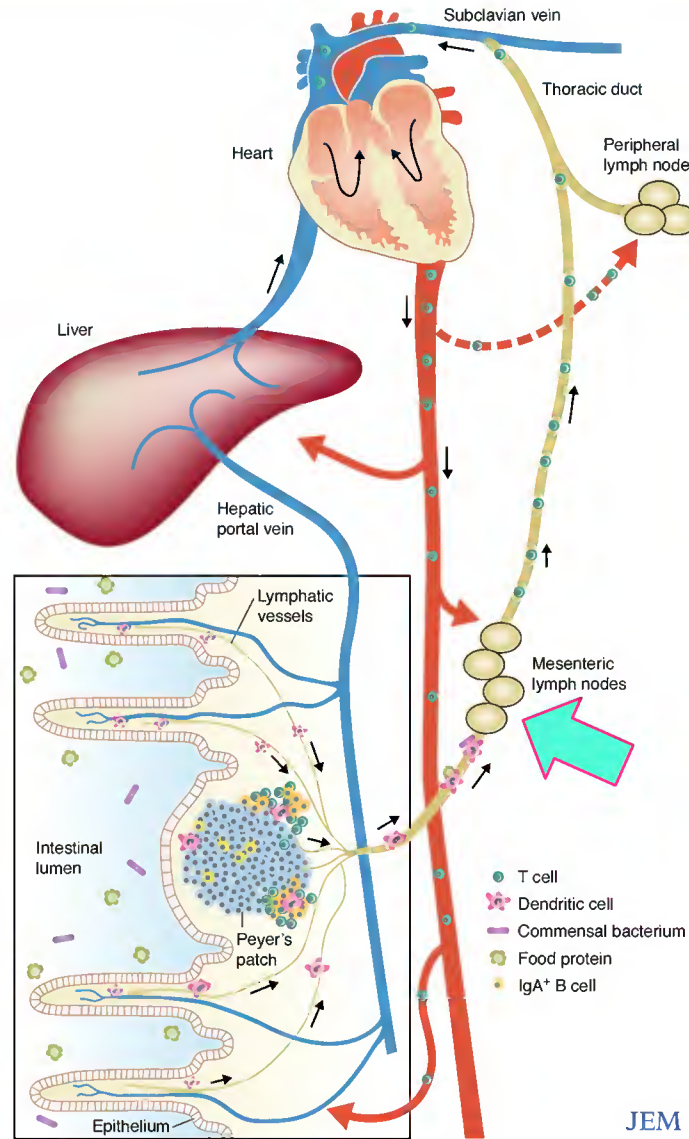
Soluble Antigens can disseminate systemically via lymph and blood.



**ORAL TOLERANCE**  
to food proteins



Blue: Dapi; White: CD31; Red: Lyve-1



MLNs have a role as a "firewall" against bacteria that have penetrated epithelial defenses or sampled by intestinal DCs.



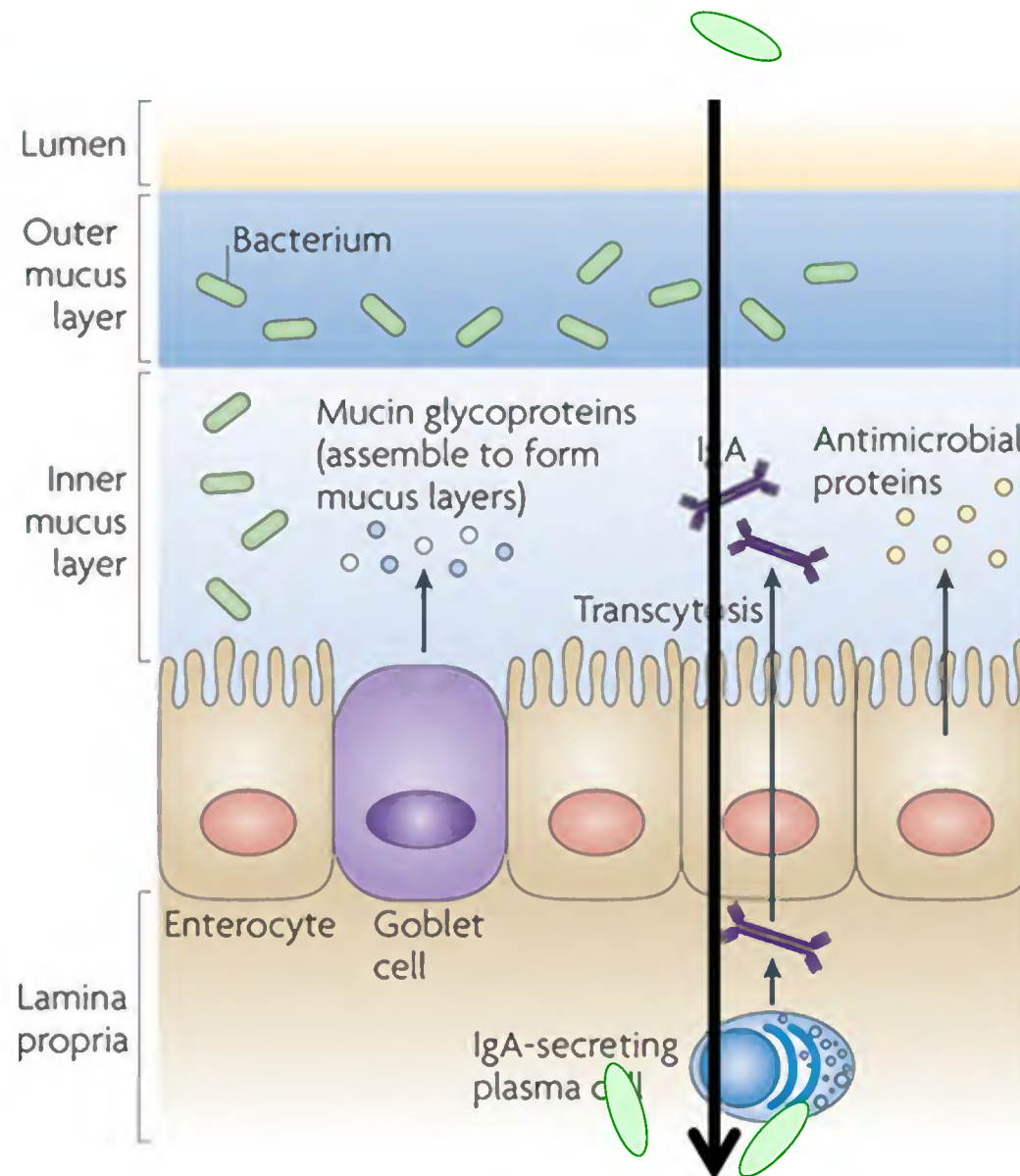
**SYSTEMIC IMMUNE IGNORANCE** to the microbiota

**Some pathogens spread systemically, some other do not**

JEM

From Macpherson A J , Smith K J Exp Med 2006

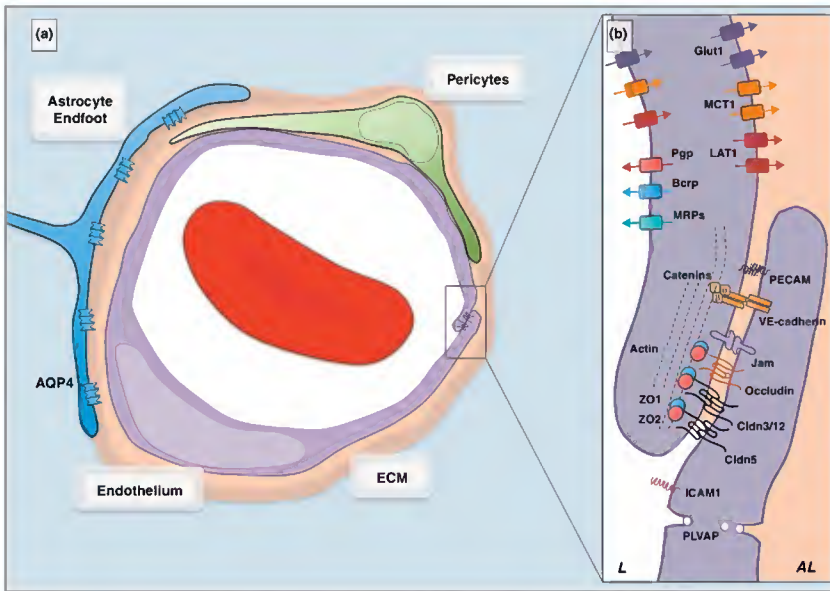
# What controls the systemic dissemination of bacteria?



# Hypothesis

Existence of a **gut vascular barrier** that, similarly to the **blood brain barrier**, is selective in the passage of molecules.

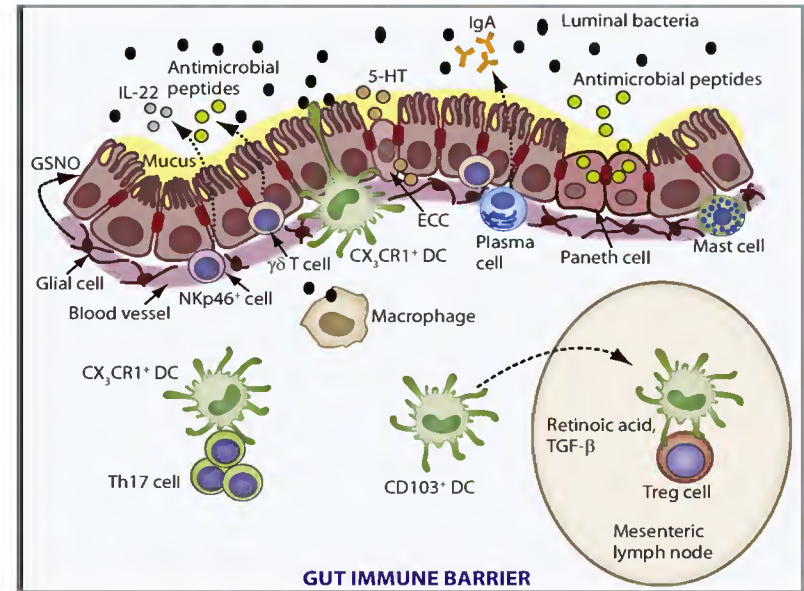
# Blood Brain Barrier VS Gut Vascular Barrier



From Siegenthaler *et al.* Current Opinion in Neurobiology 2013

**NeuroVascular Unit:** ENDOTHELIAL CELLS  
PERICYTES  
ASTROCYTES  
NEURONS

**ENDOTHELIAL CELLS CHARACTERISTICS:**  
ADHERENS JUNCTIONS  
TIGHT JUNCTIONS  
POLARIZED EXPRESSION OF TRANSPORTERS



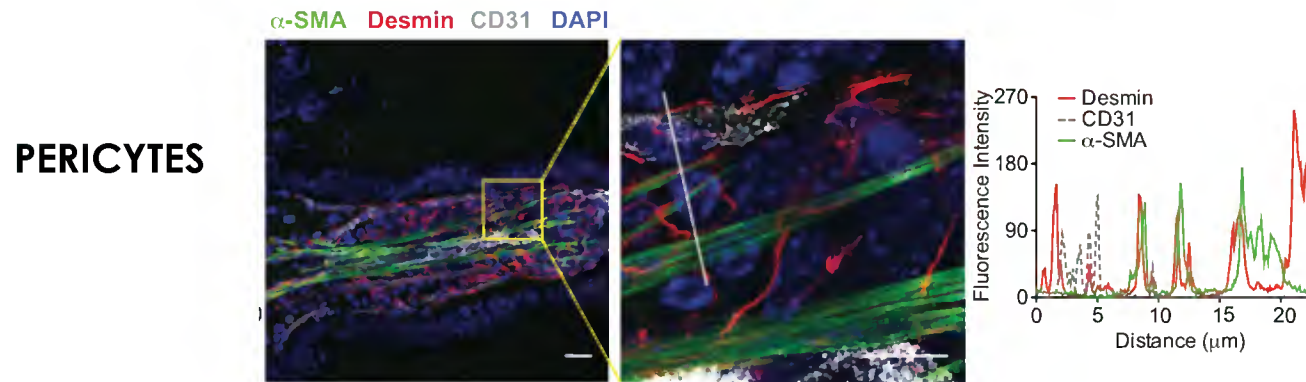
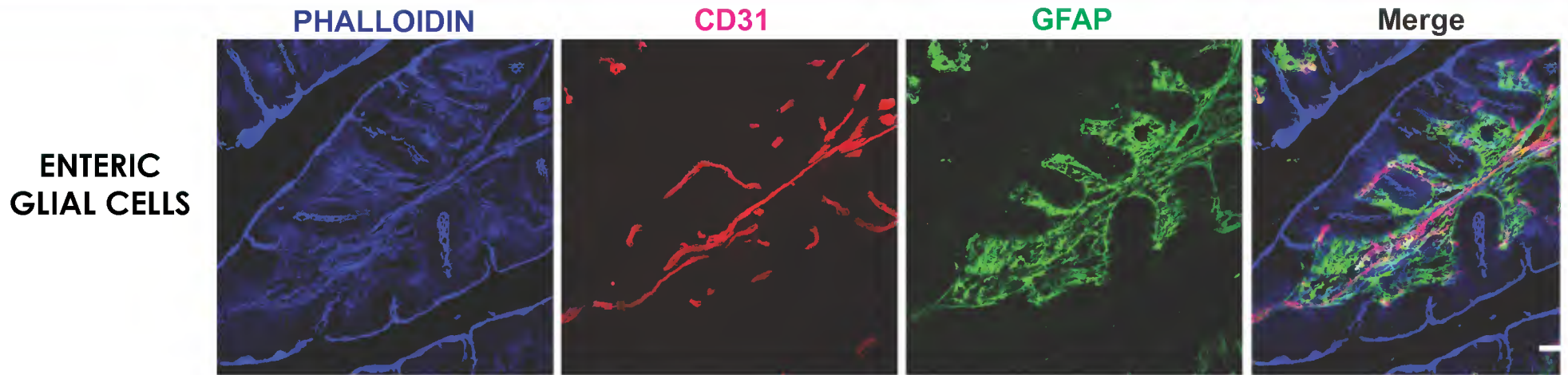
Adapted from Daneman R. and Rescigno Immunity 2009

**“Gut Vascular Unit”:** ENDOTHELIAL CELLS  
And ?

**ENDOTHELIAL CELLS CHARACTERISTICS ?**



# Gut Vascular Unit

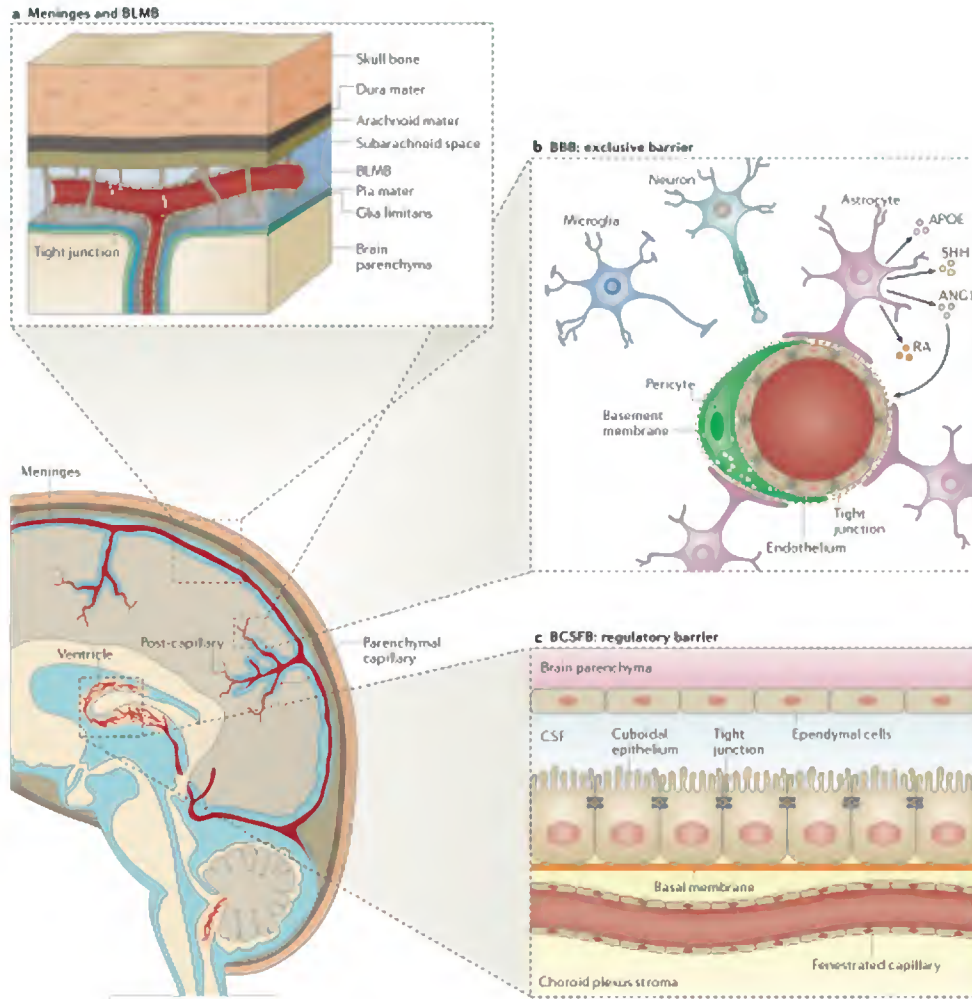


**EGCs** preserve **epithelial barrier integrity**

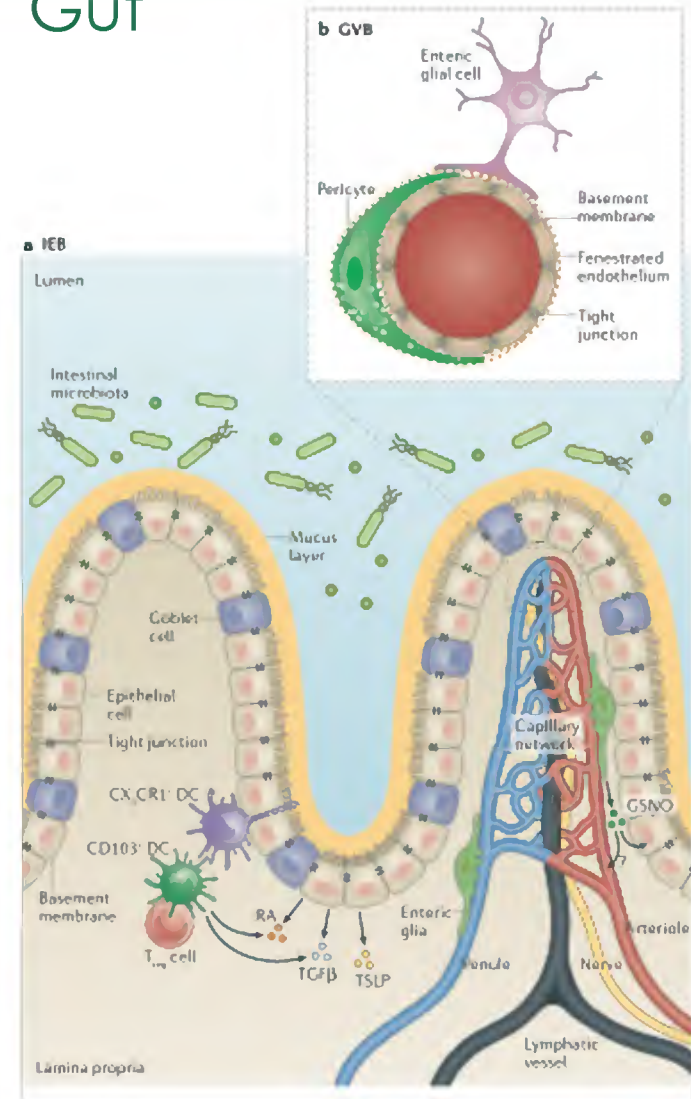
**Pericytes** are associated to intestinal vessel but they role remains to be assessed.

# The Gut Vascular Barrier resembles the Blood brain barrier

## Brain



## Gut

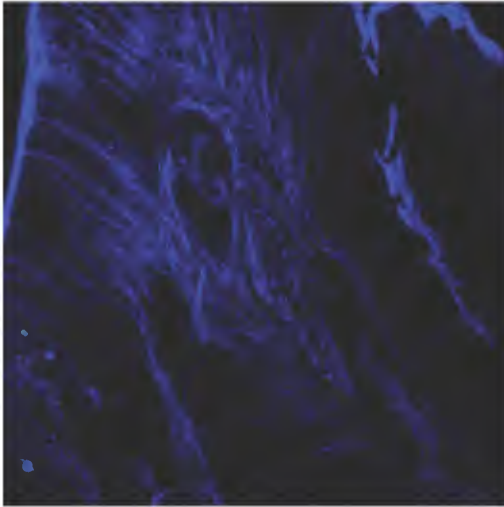


*Spadoni et al. Science 2015*  
*Spadoni et al Nature Rev Immunol. 2017*

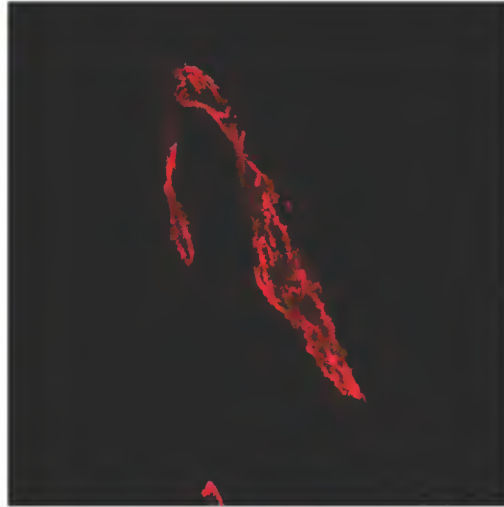
# Intestinal endothelium characterization (I)

## Adherens Junctions

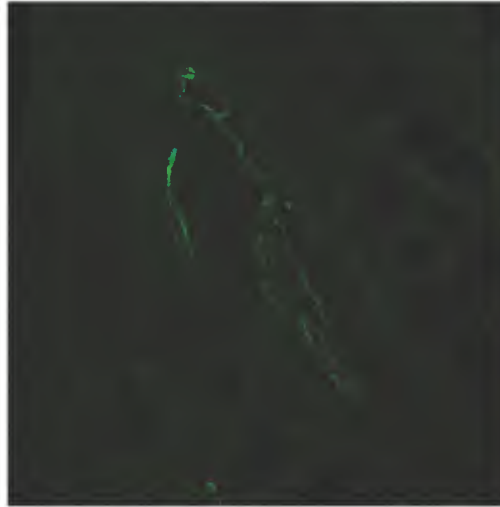
PHALLOIDIN



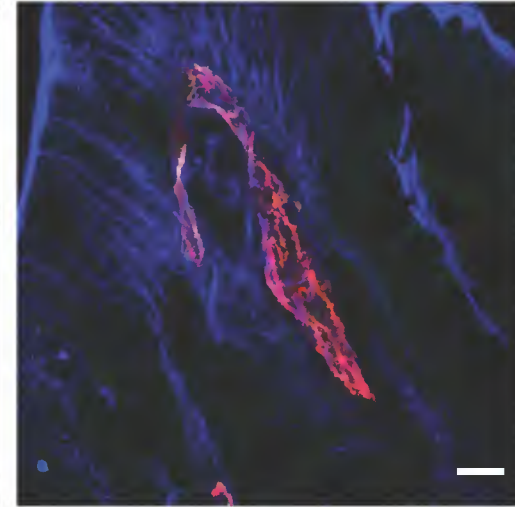
CD31



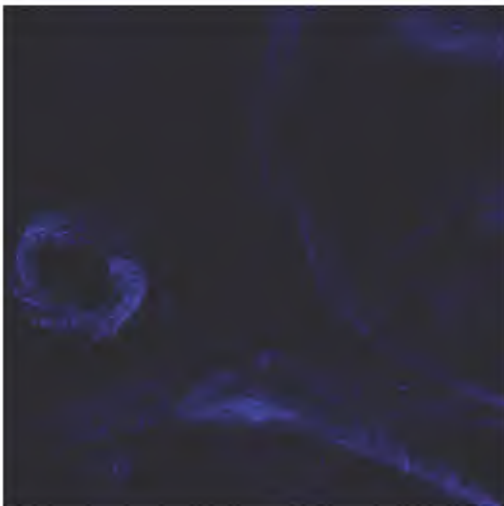
VE-Cadherin



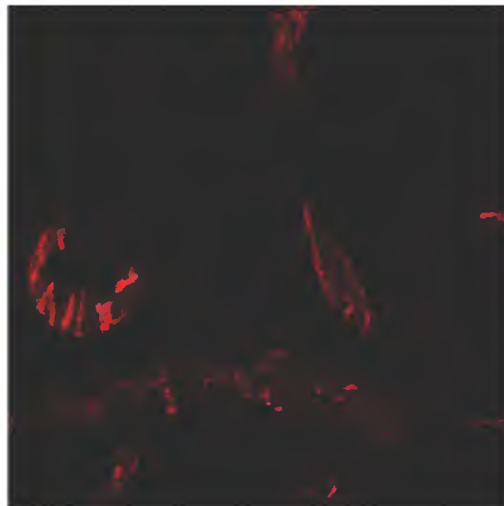
Merge



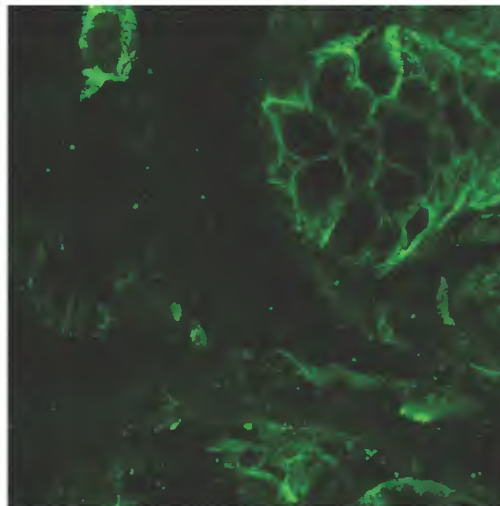
PHALLOIDIN



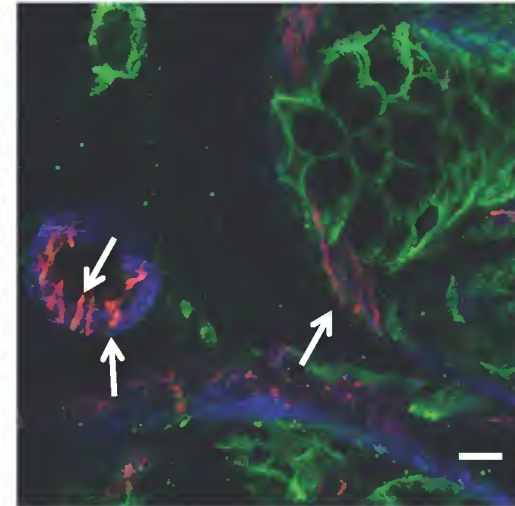
CD31



$\beta$ -catenin



Merge

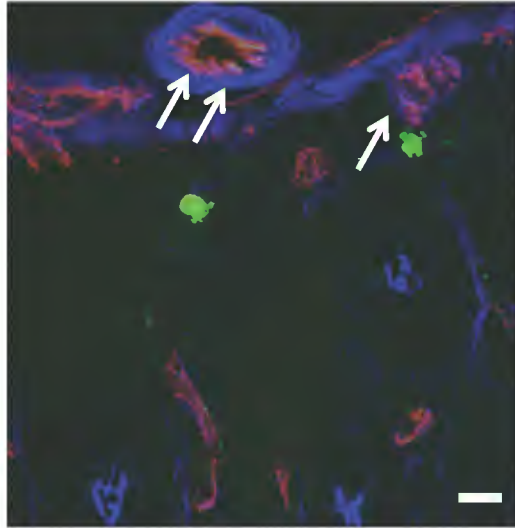




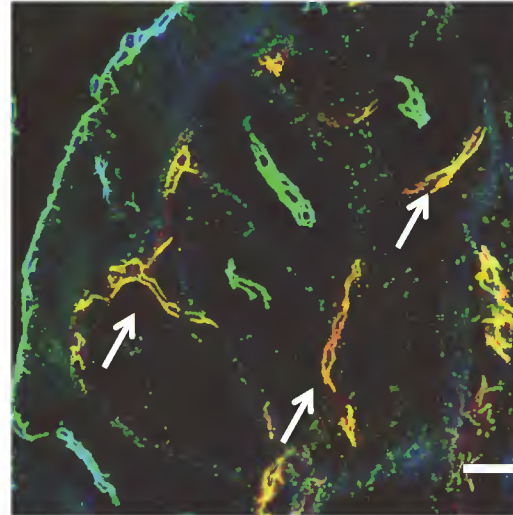
# Intestinal endothelium characterization (II)

## Tight Junctions

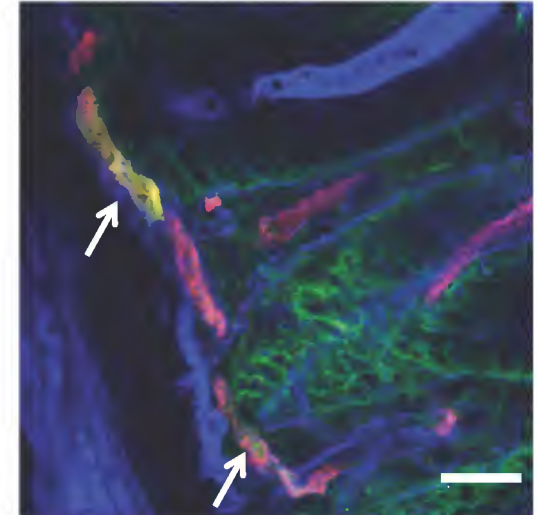
Occludin



ZO-1

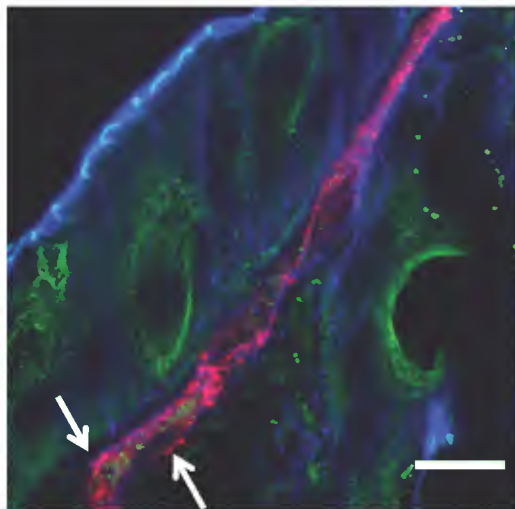


JAM-A

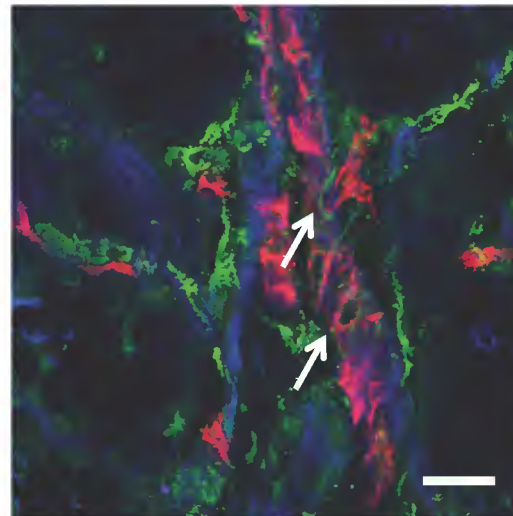


PHALLOIDIN

Cingulin



CLDN12



CD31

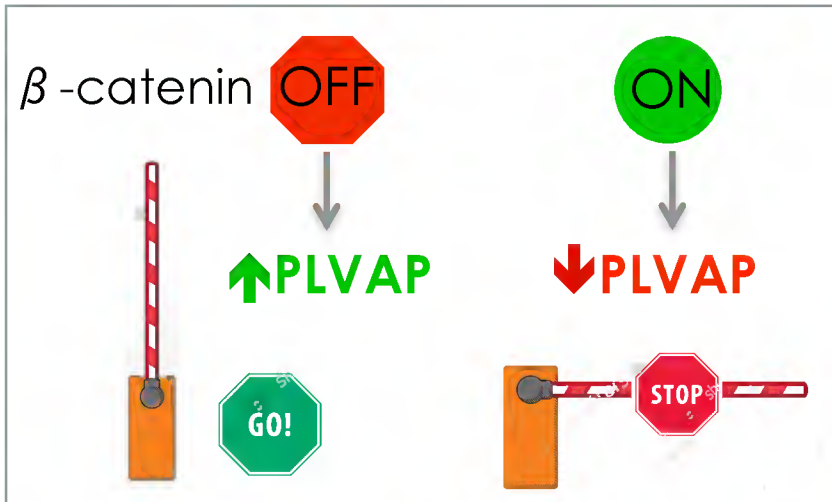


# Is this a functional GVB?

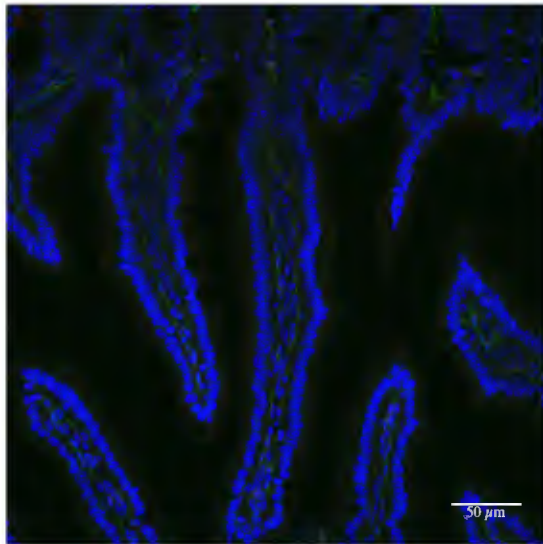
Analysis of markers of effective blood barrier

# Identification of a barrier marker in the intestinal endothelium

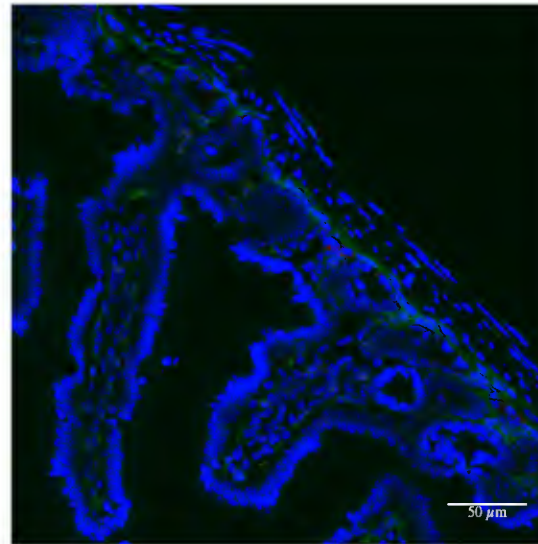
## PLVAP (Plasmalemma Vescicle Associated Protein)



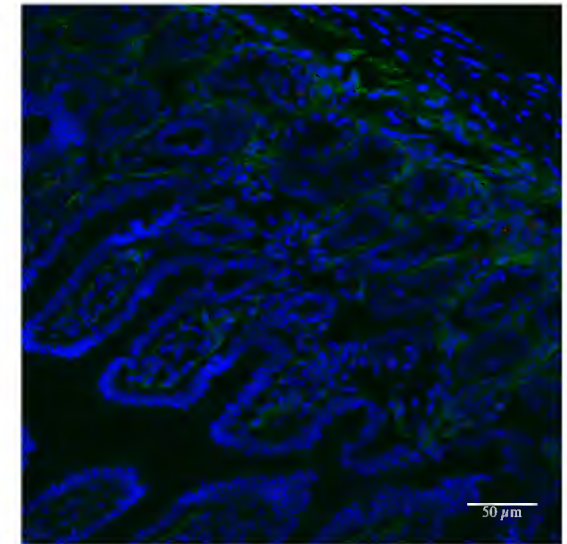
Duodenum



Jejunum



Ileum



CD34

PLVAP

DAPI

- It is associated with the caveolae of fenestrated microvascular endothelial cells (in the lung, liver, kidney);  
Herrnberger L et al. Histochem Cell Biol 2012; Stan RV et al. Dev Cell 2012

- **Maturation of the BBB:** activation of the Wnt/ $\beta$ -catenin signaling pathway  $\rightarrow$  down-regulation of PLVAP  
Liebner S et al. JCB 2008

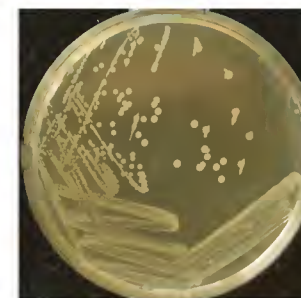
# PLVAP expression after *S. typhimurium* infection

$10^9$  *Salmonella*  
 $\Delta$ AroA



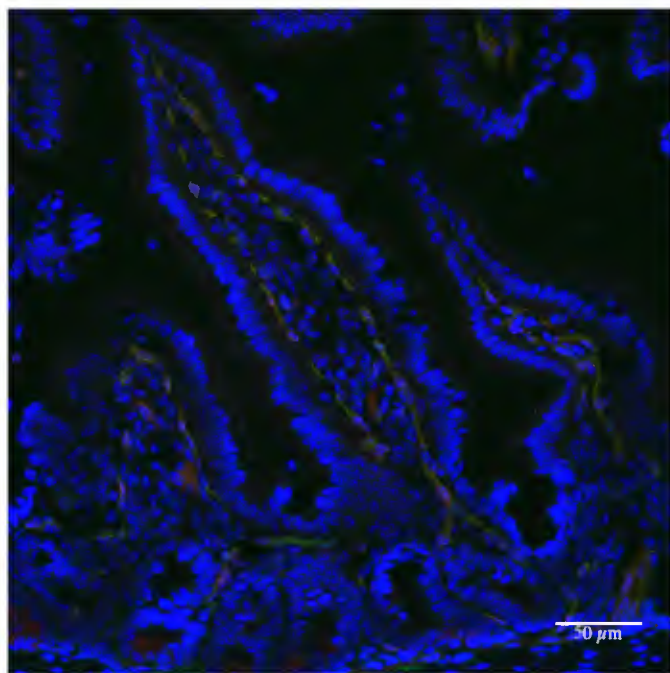
2h/6h/24h

Organ collection,  
plating and IF on  
intestine cryosections

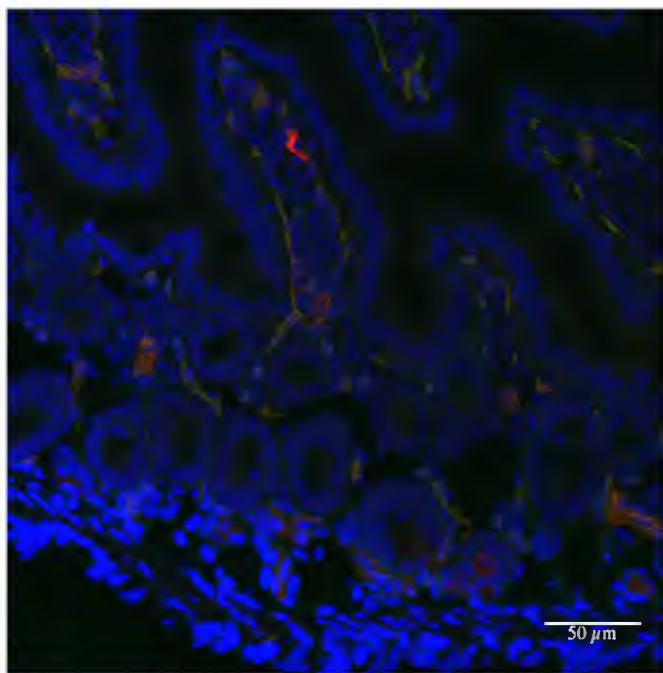


DUODENUM

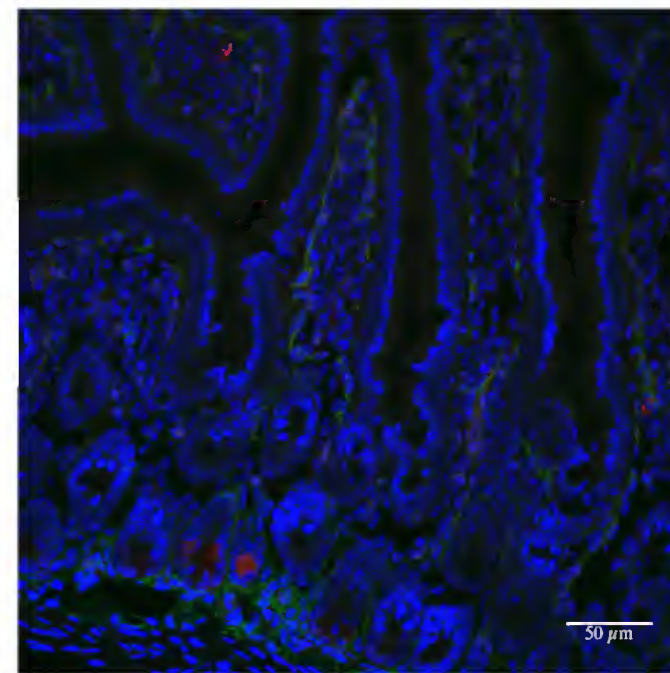
2h



6h



24h



CD34 PLVAP DAPI



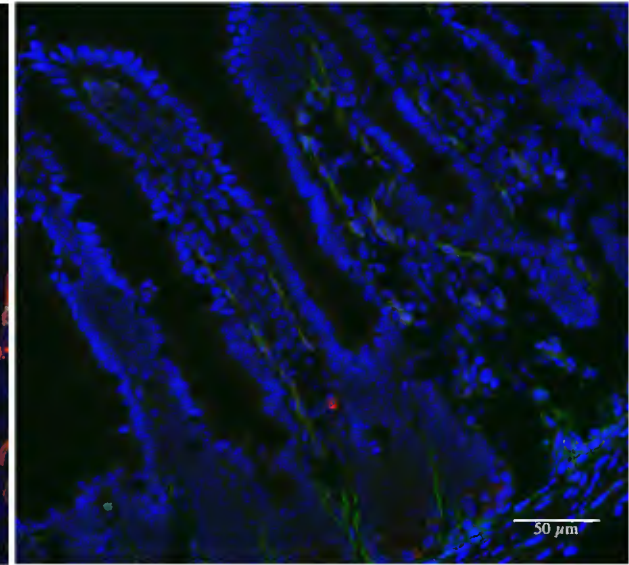
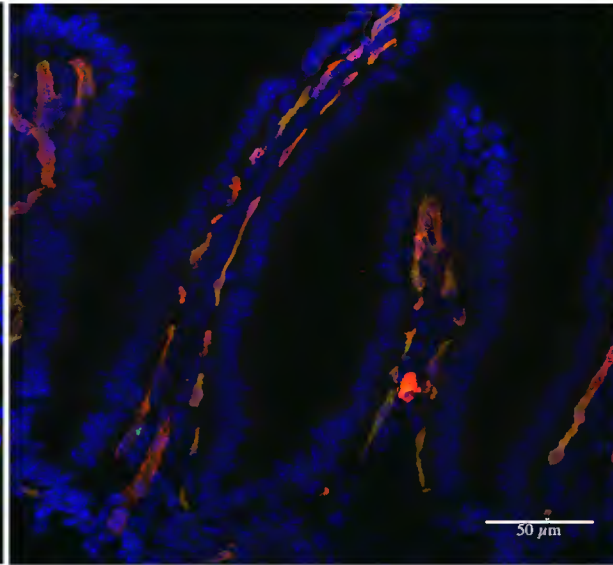
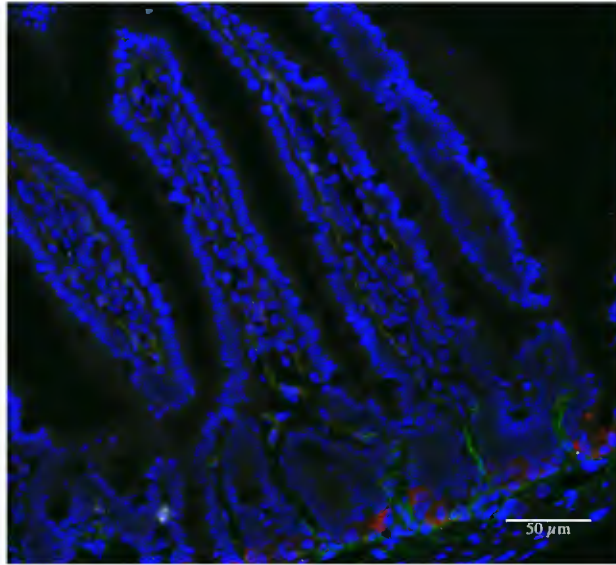
# PLVAP expression after *S. typhimurium* infection

2h

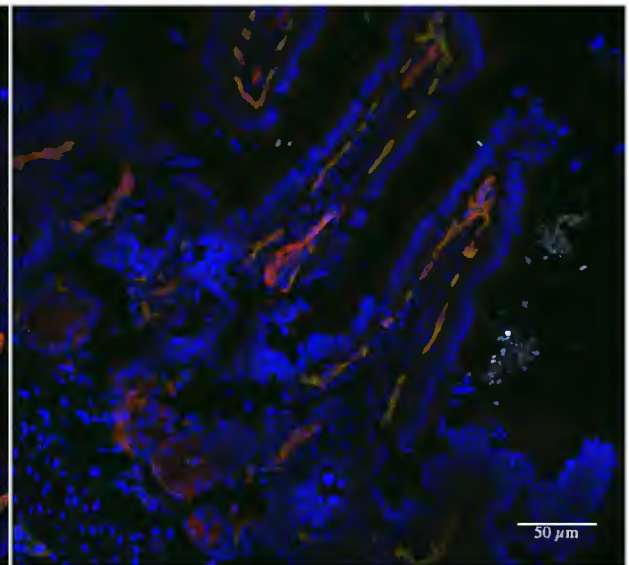
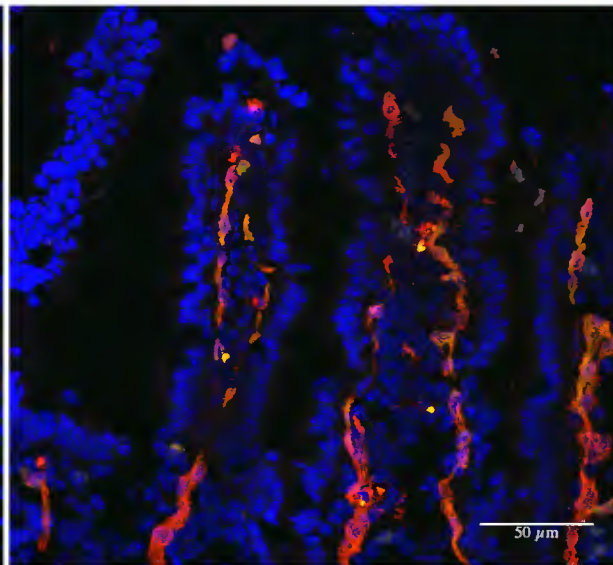
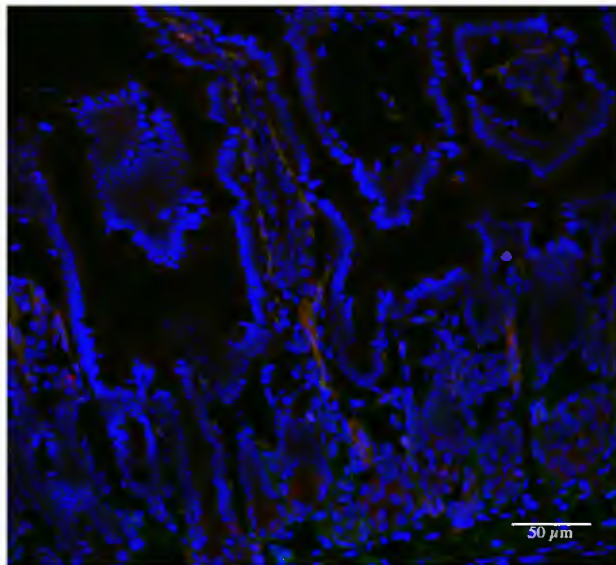
6h

24h

JEJUNUM



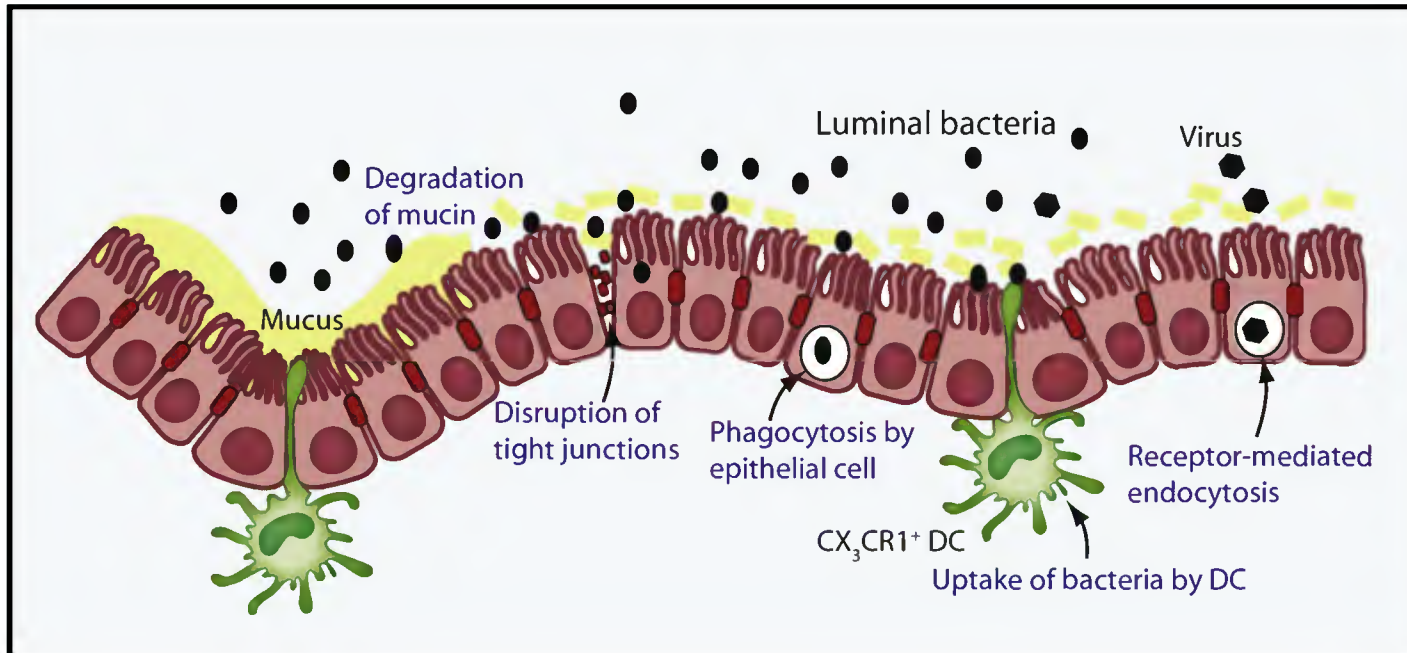
ILEUM



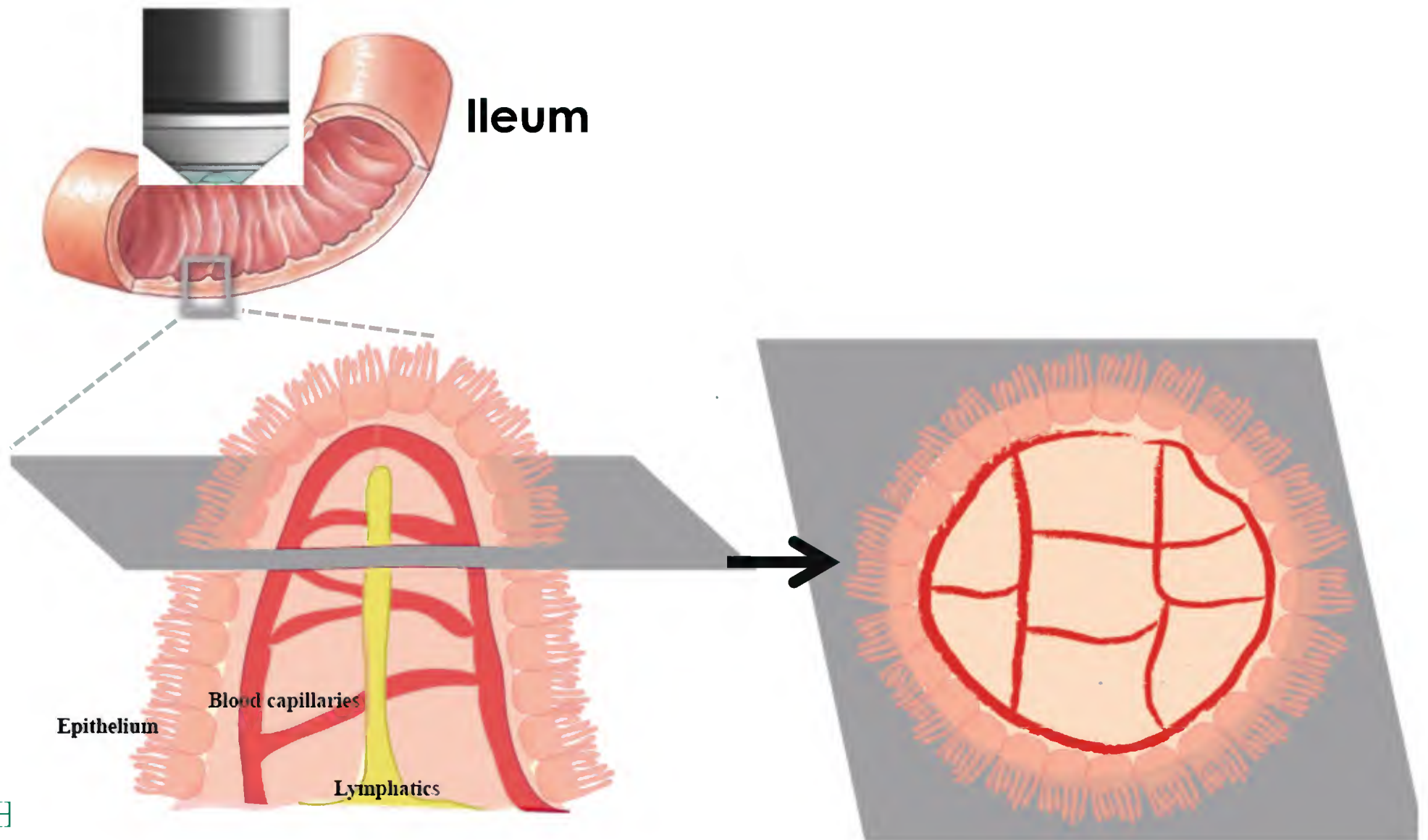




# Is the disruption of the barrier due to endothelial or epithelial dysfunction?

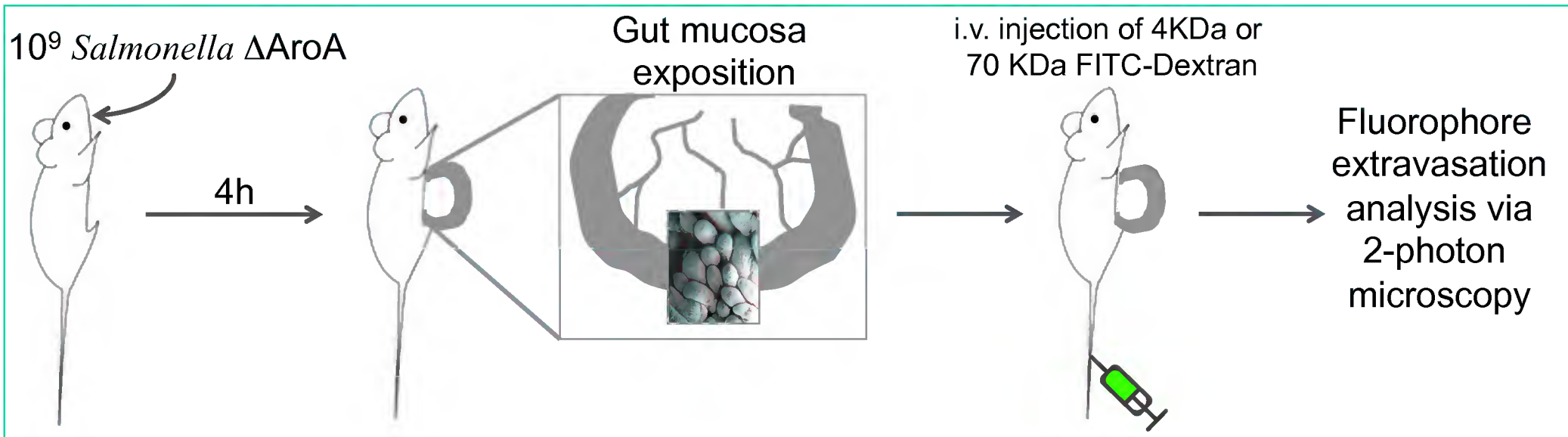


# *In vivo* ileum permeability



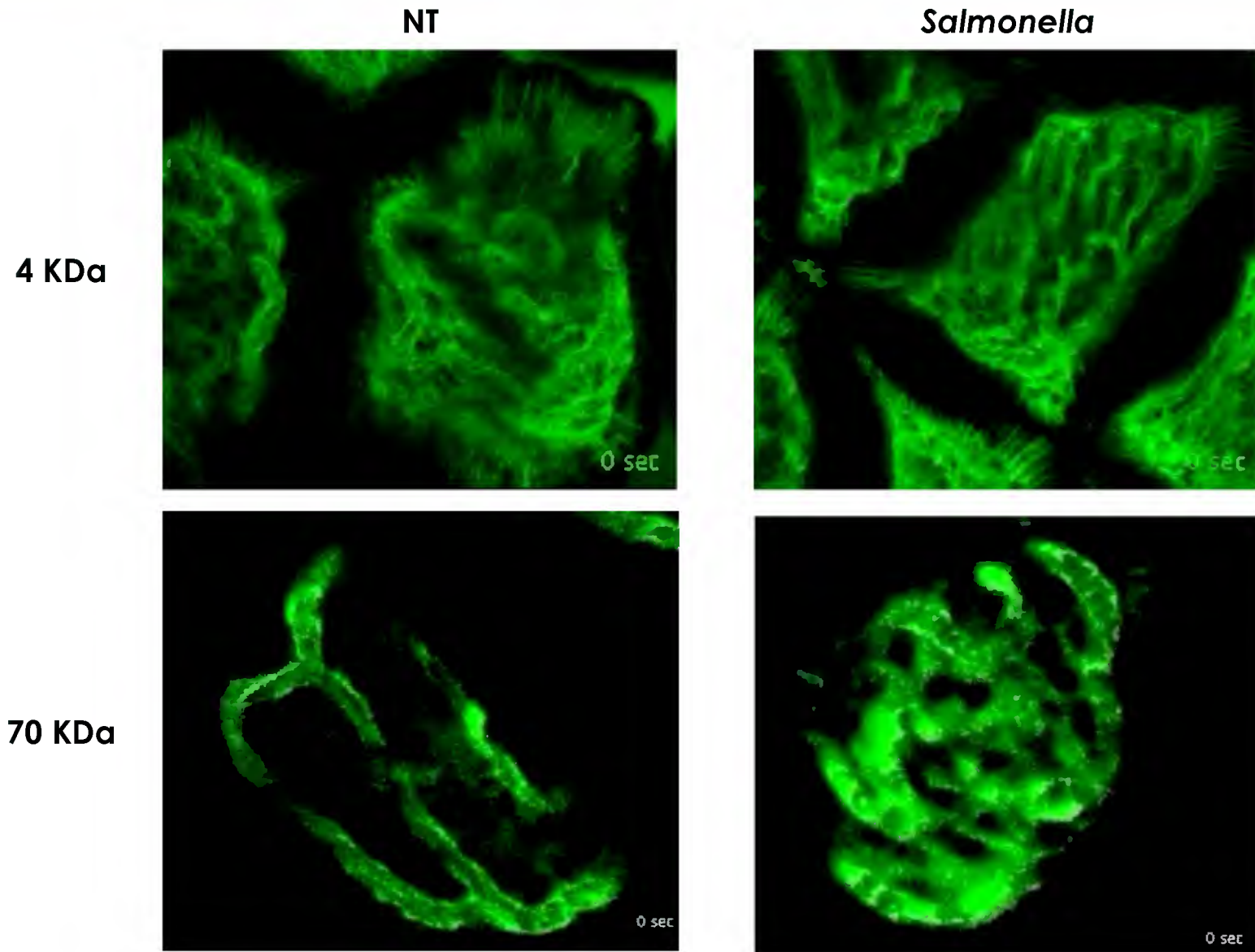
# A vascular barrier is present below the epithelial barrier

Inside → out



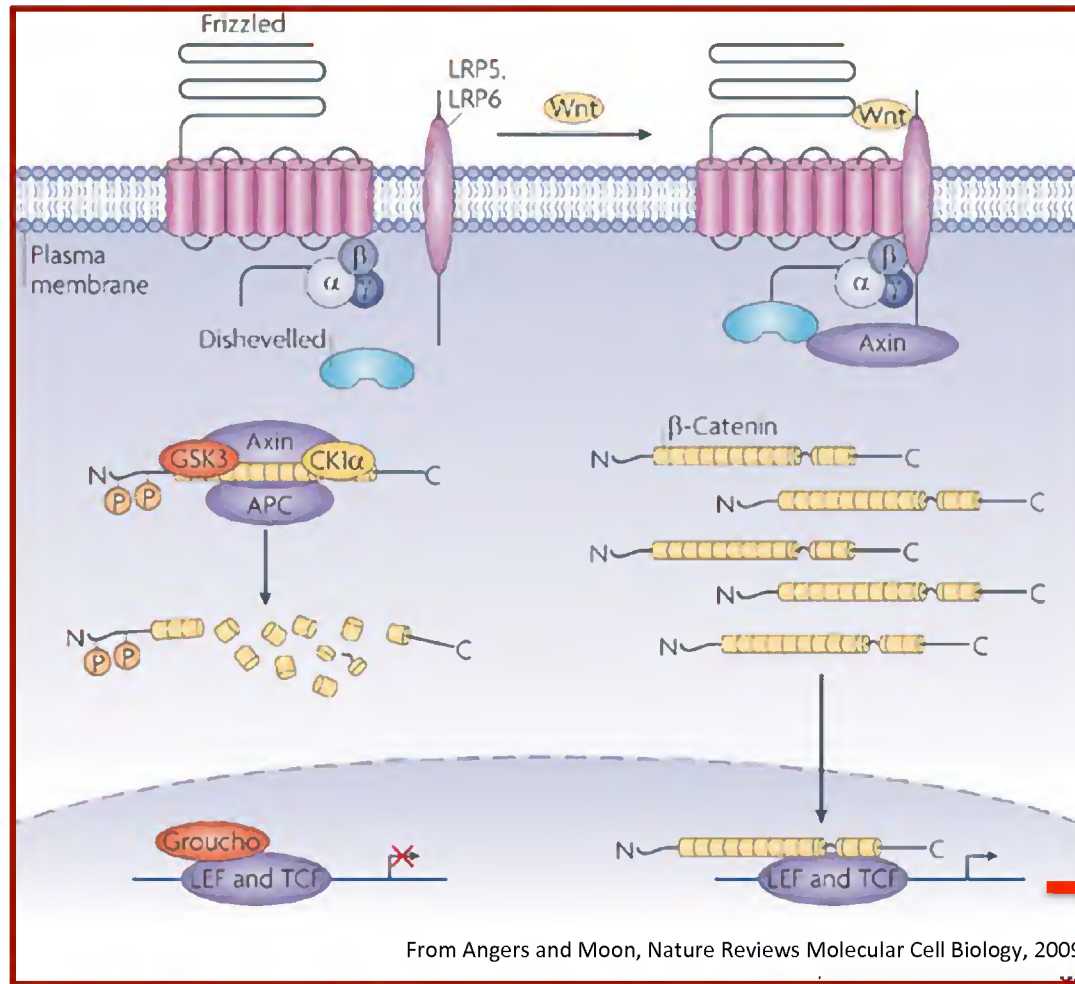


# Intestinal blood vessel permeability is modified after infection

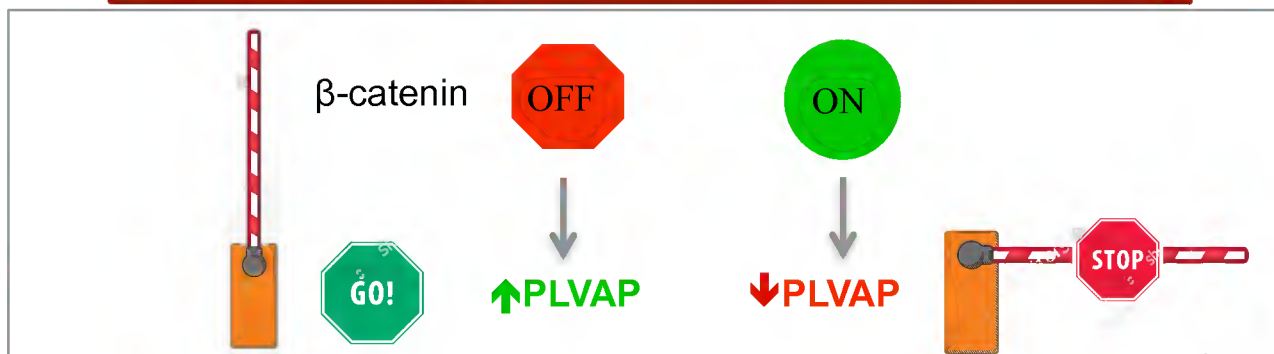


**What is the mechanism employed by  
*Salmonella*?**

# Canonical WNT/ $\beta$ -CATENIN signaling pathway



**Axin 2**



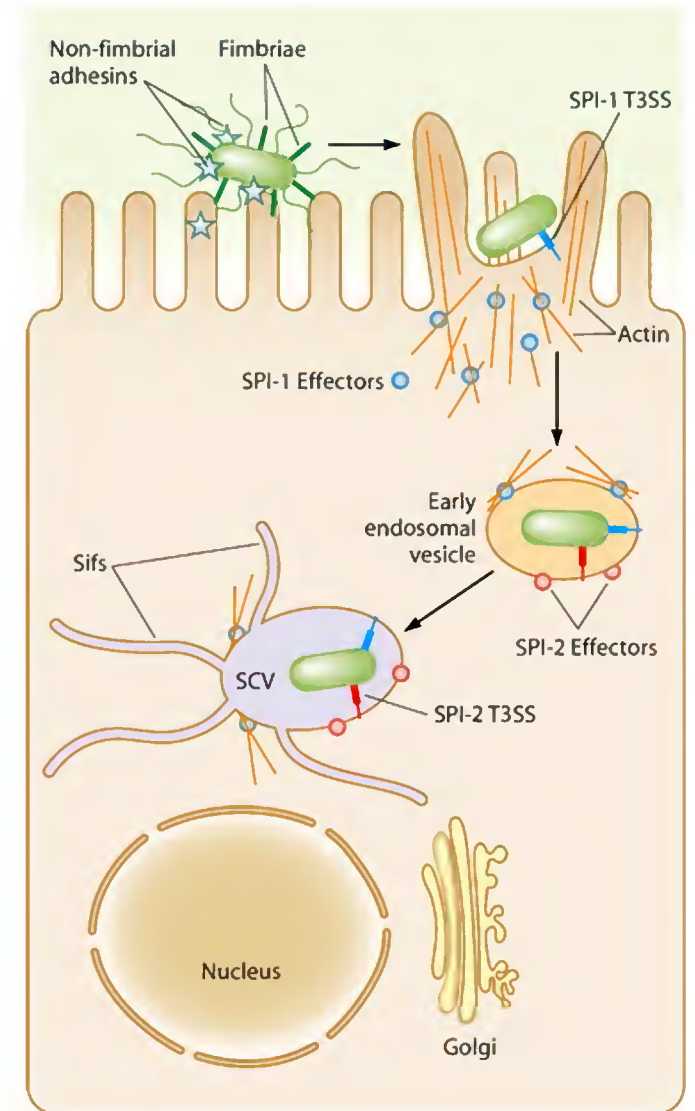
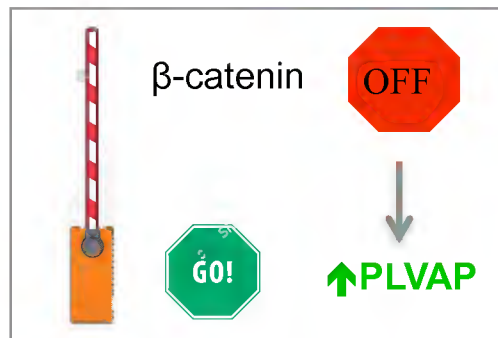
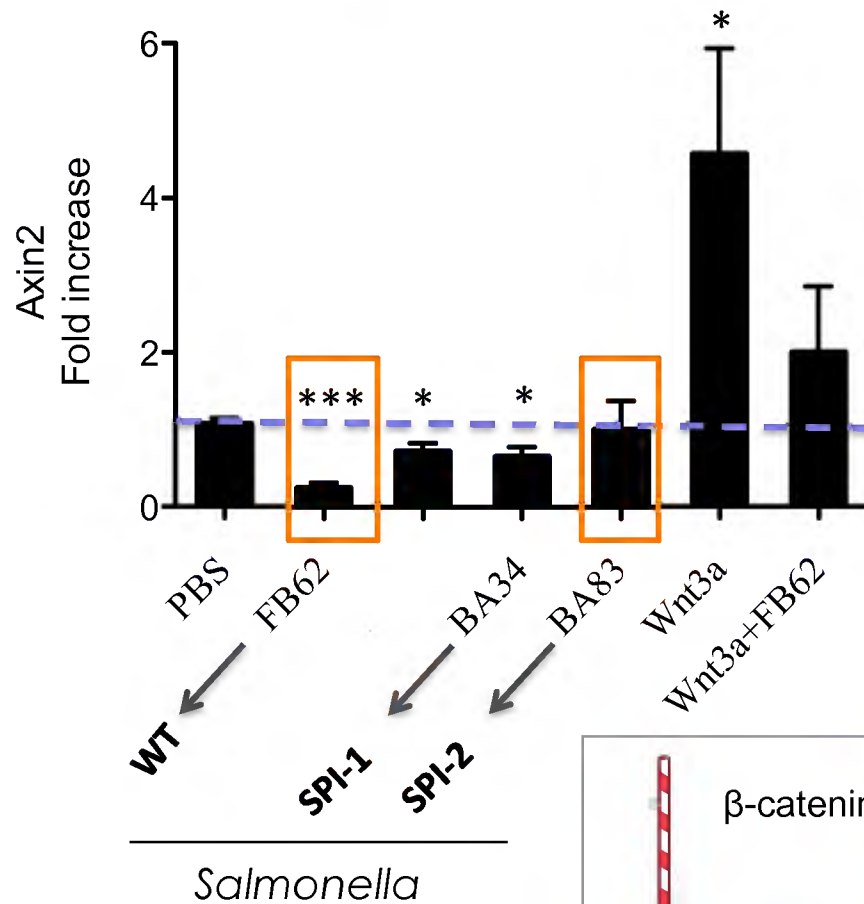
# *S. typhimurium* regulates WNT/ $\beta$ -CATENIN signaling pathway

Primary Lung Endothelial Cells

4h

Infection with *Salmonella*

Stimulation with Wnt3a (positive control)

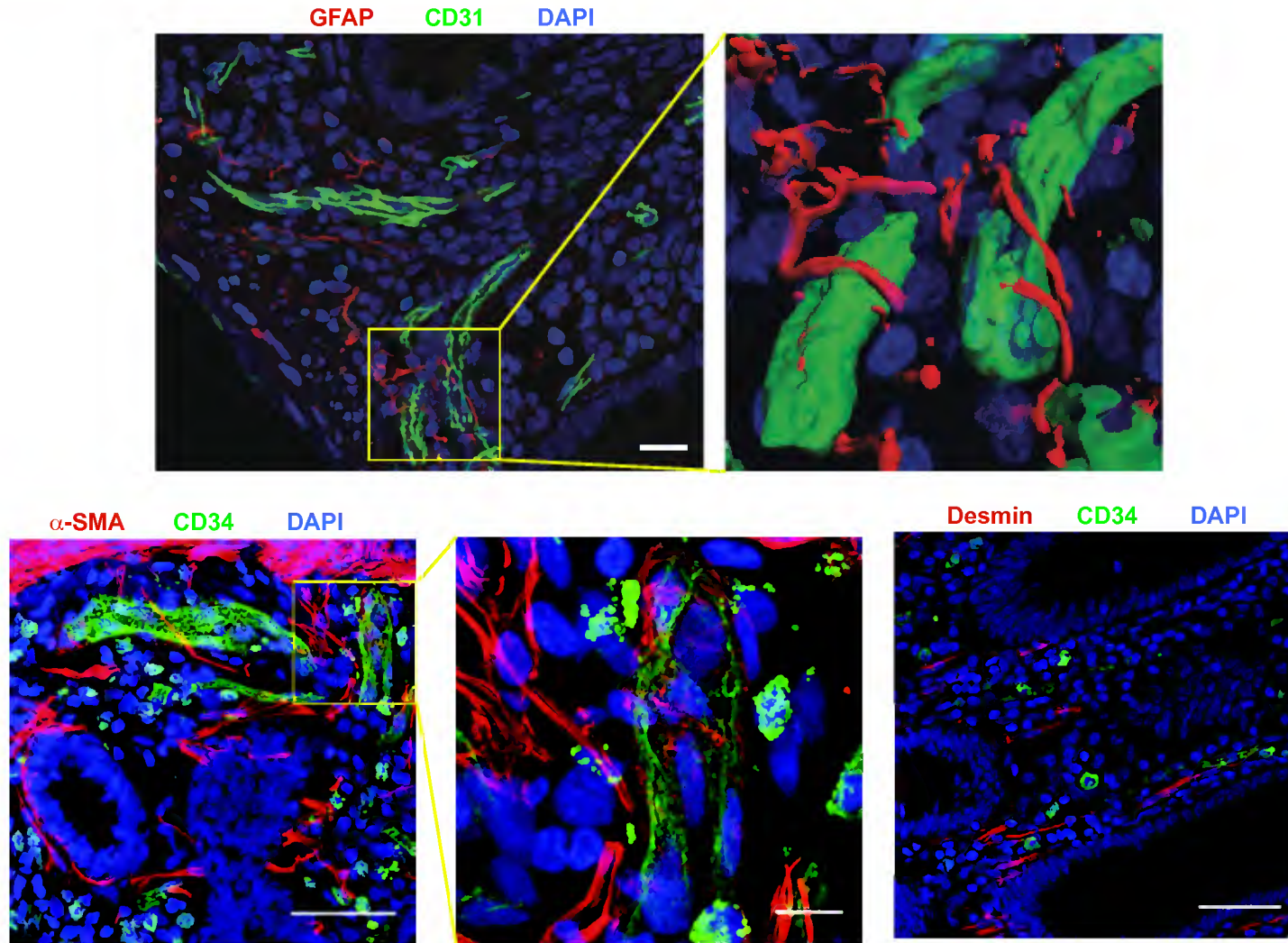


From Gilbreath JJ, Microbiology and molecular biology reviews 2011

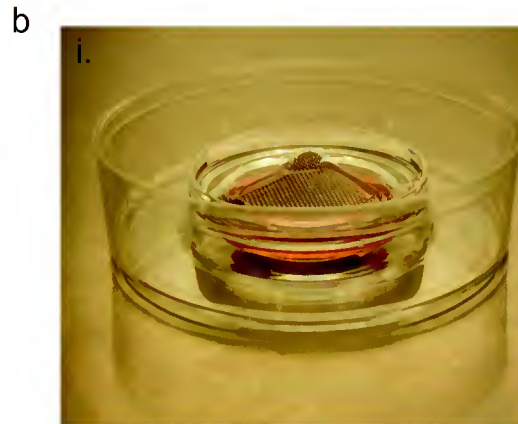
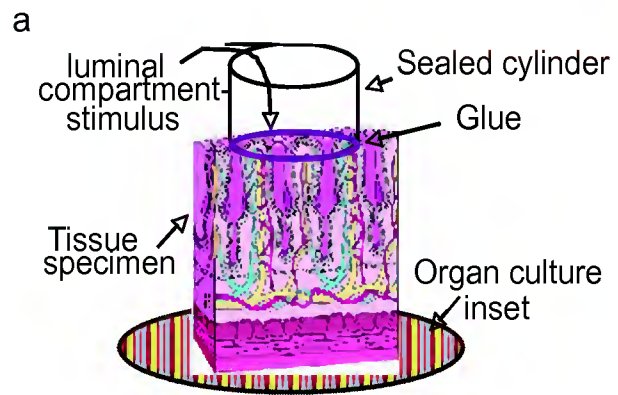
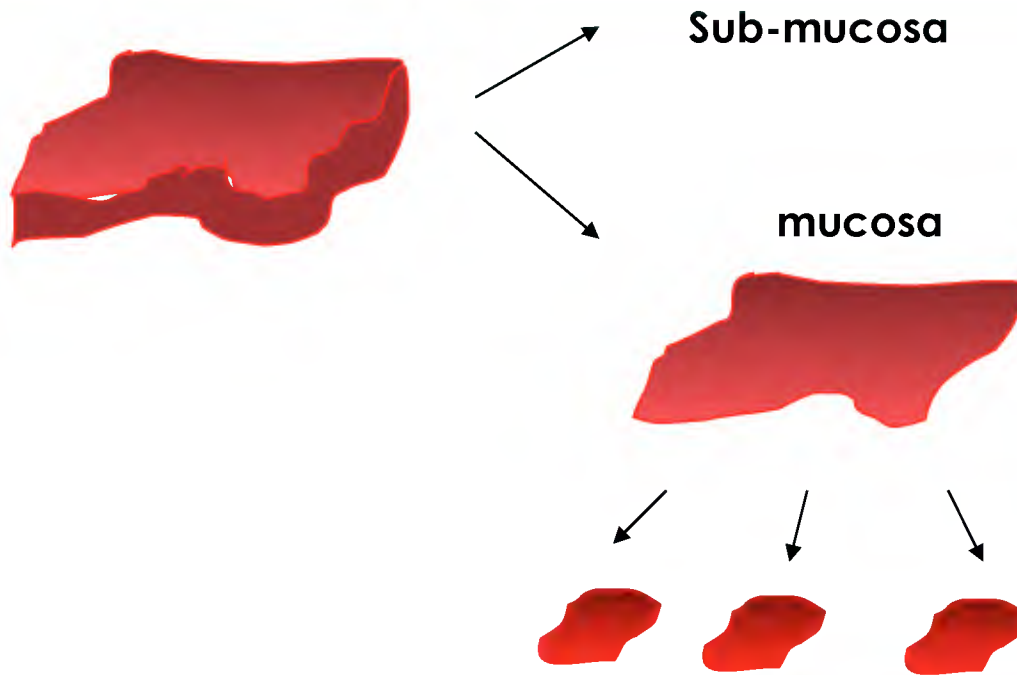




# The human gut has a gut vascular unit



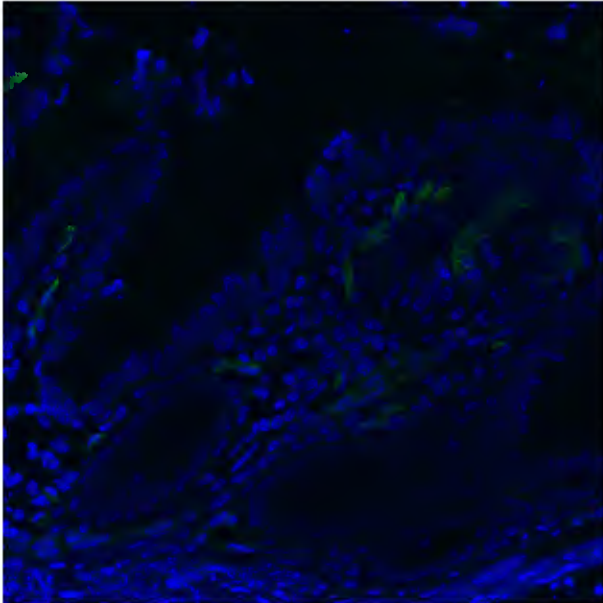
# Is this barrier functional?



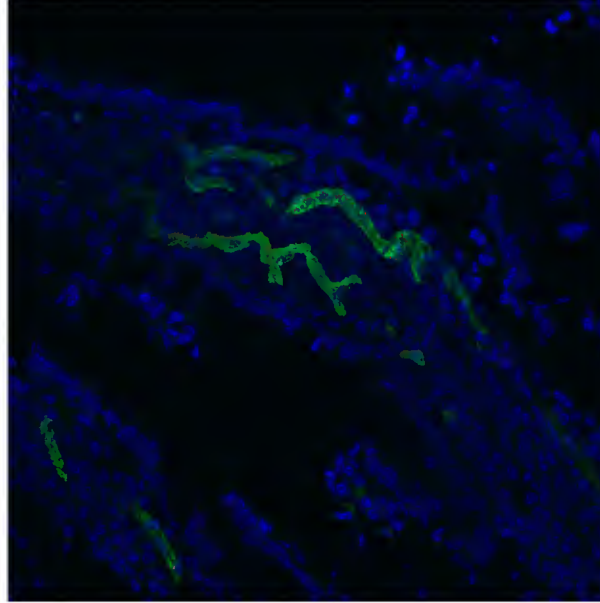


# Is this barrier functional?

Untreated



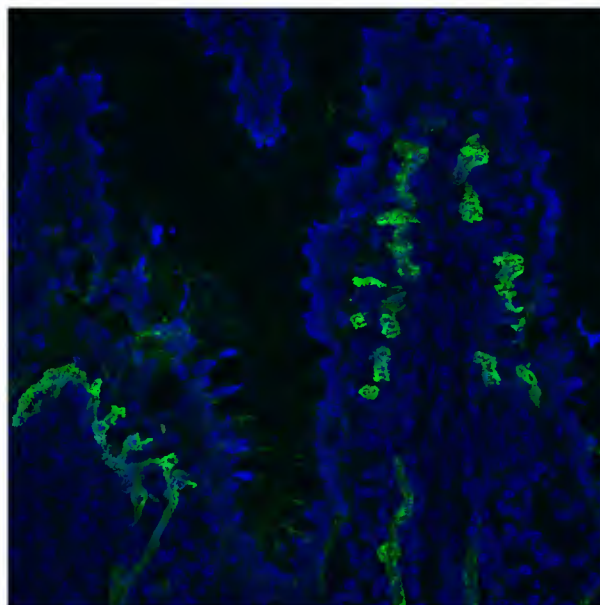
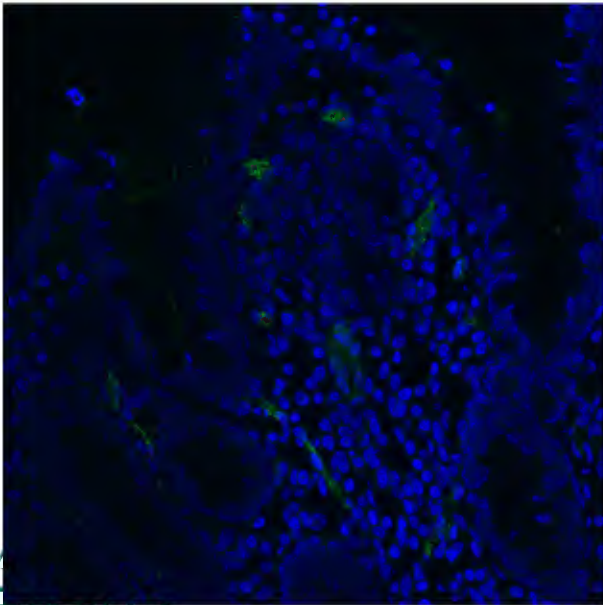
*Salmonella* 10<sup>5</sup>



*Salmonella*



Ileum samples



PLVAP

DAPI



# Implications for human disease

## Celiac disease (CD) patients under gluten-free diet

- High levels of serum ALT/AST
- Low/normal levels of serum ALT/AST

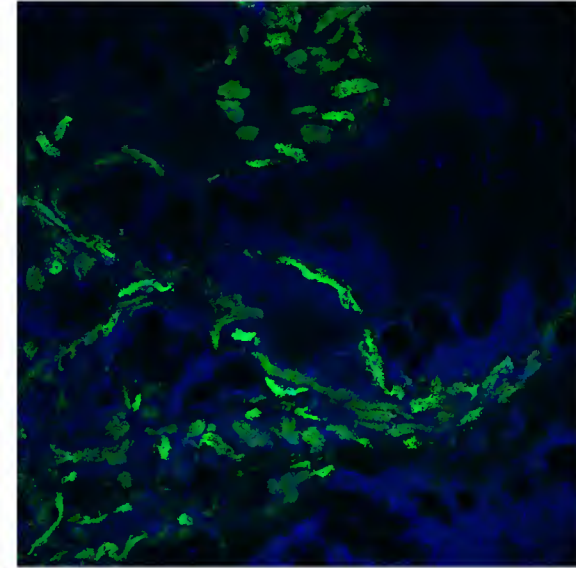
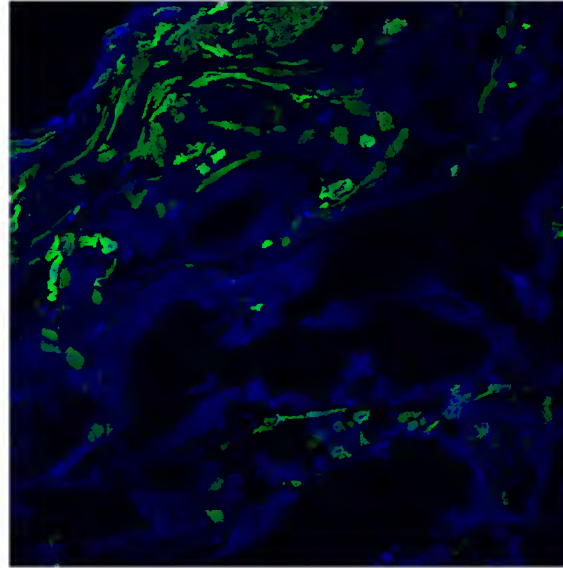
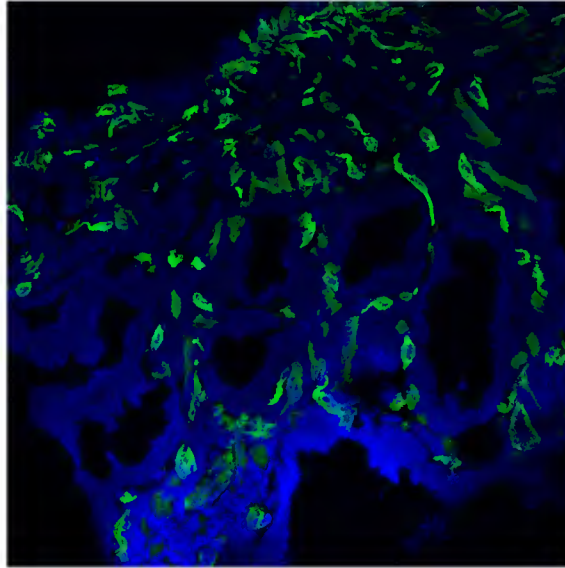
# Implications for human disease

Celiac disease patients with:

**HIGH  
LEVELS  
ALT/AST**



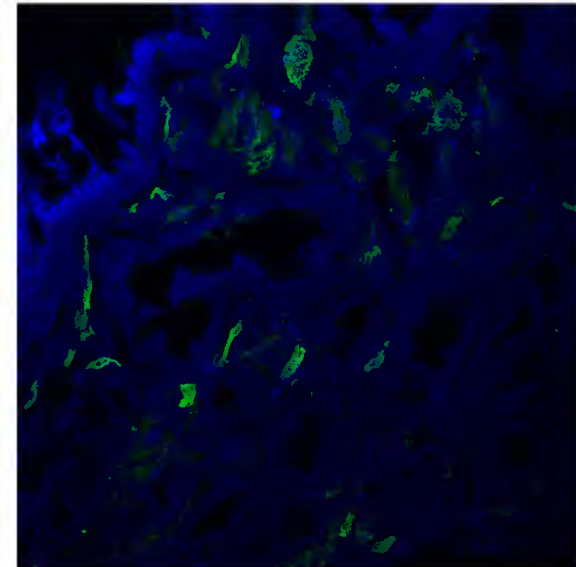
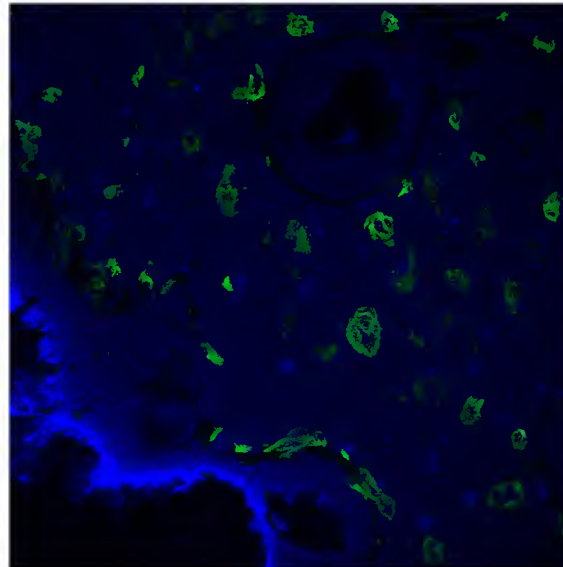
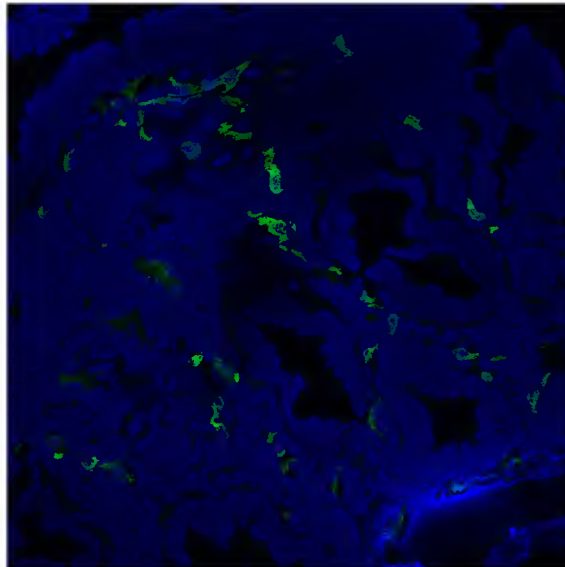
**HIGH PLVAP  
EXPRESSION**



**NORMAL  
LEVELS  
ALT/AST**

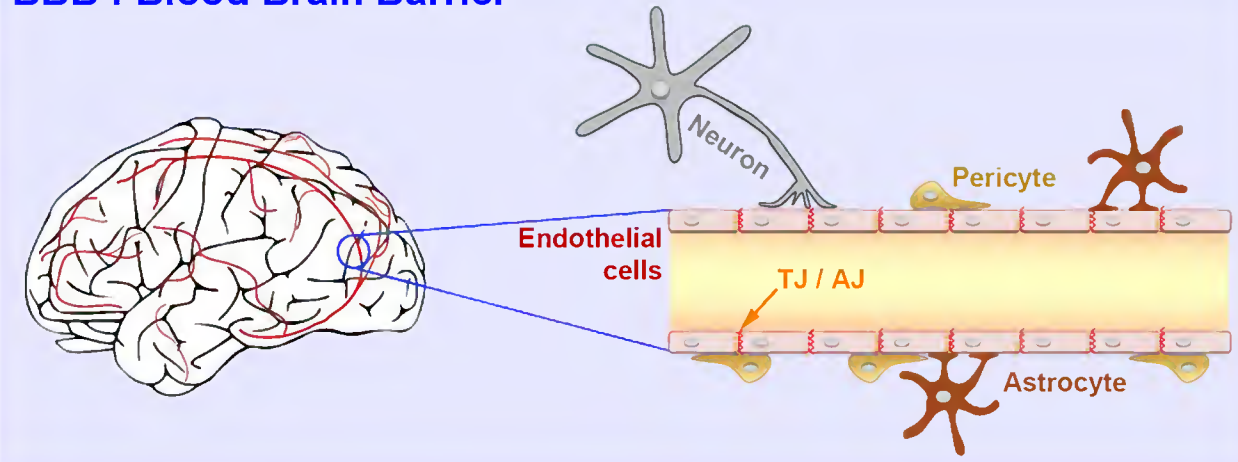


**LOW PLVAP  
EXPRESSION**



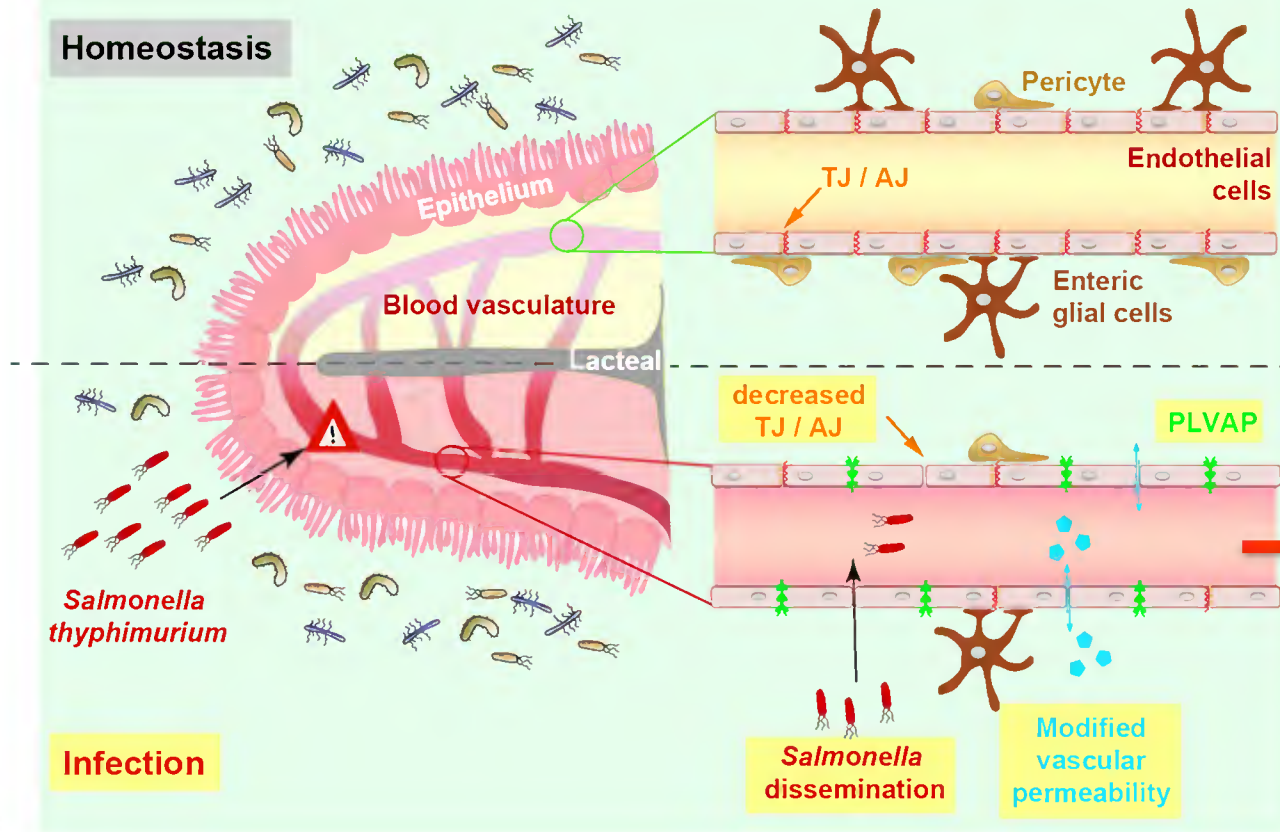
# The Gut Vascular Barrier resembles the Blood brain barrier

## BBB : Blood Brain Barrier

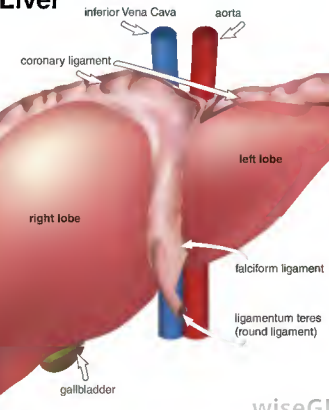


## GVB : Gut vascular Barrier

Homeostasis



## Human Liver



wiseGEEK

Spadoni et al. Science.  
2015 Nov 13;350(6262):830-4



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