Interazioni Tra Immunità Innata e Microbiota Intestinale Nelle MICI

Loris R. Lopetuso

Digestive Disease Center
Fondazione Policlinico Universitario A. Gemelli IRCCS Roma
DMSI – Università «G. D'Annunzio» Chieti-Pescara

Gemelli
Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

CEMAD
Centro Malattie Apparato Digerente
Digestive Disease Center

WORLD'S BEST HOSPITALS 2021
GASTROENTEROLOGY
Newsweek
statista
FONDAZIONE POLICLINICO UNIVERSITARIO A. GEMELLI IRCCS
3rd PLACE

Starting from the end: Microbiota and IBD

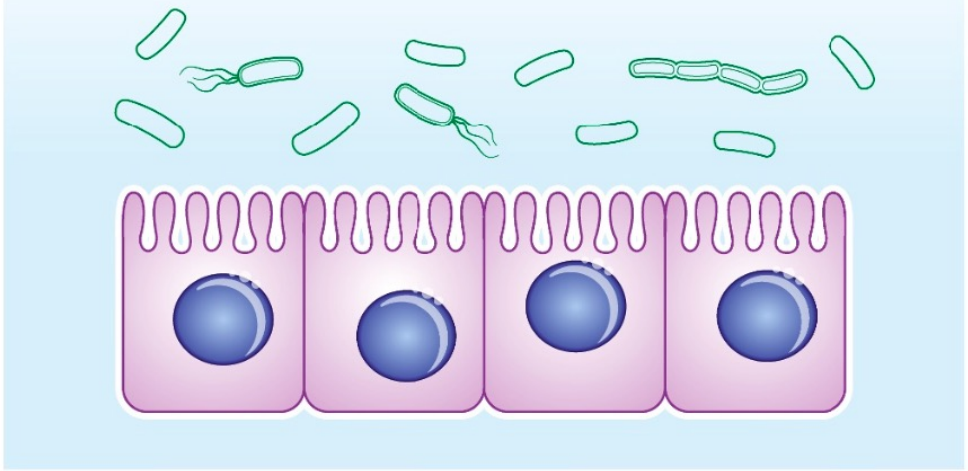
Gut microbiota composition is altered in IBD vs controls

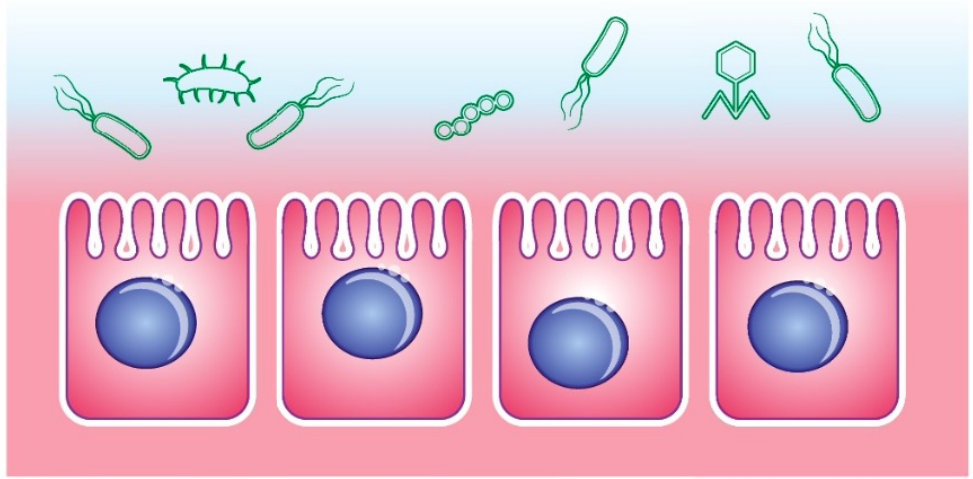
Gut microbiota composition is altered in active vs non-active IBD

Gut microbiota can influence the development of IBD

Microbial alterations in IBD

Healthy		Inflammatory bowel disease	
Firmicutes	*Bacteroidetes*	Bacterial diversity↓	*Proteobacteria*
Roseburia	*Faecalibacterium prausnitzii*	*Enterobacteriaceae*	Adherent-invasive *Escherichia coli*
Faecalibacterium	*Rosenburia hominis*	*Fusobacterium*	*Shigella species*
Dorea	*Rosenburia intestinalis*	*Enterococcus*	*Ruminococcus gnavus*
Blautia	*Akkermansia muciniphila*	*Megasphaera*	*Ruminococcus torques*
Christensenellaceae	*Saccharomyces cerevisiae*	*Campylobacter*	*Fusobacterium nucleatum*
Collinsella		*Gammproteobacteria*	*Bacteriodes fragilis*
Ruminococcus		*Deltaproteobacteria*	*Klebsiella pneumoniae*
		Candida albicans	*Caudovirales* bacteriophages
		Malassezia restricta	

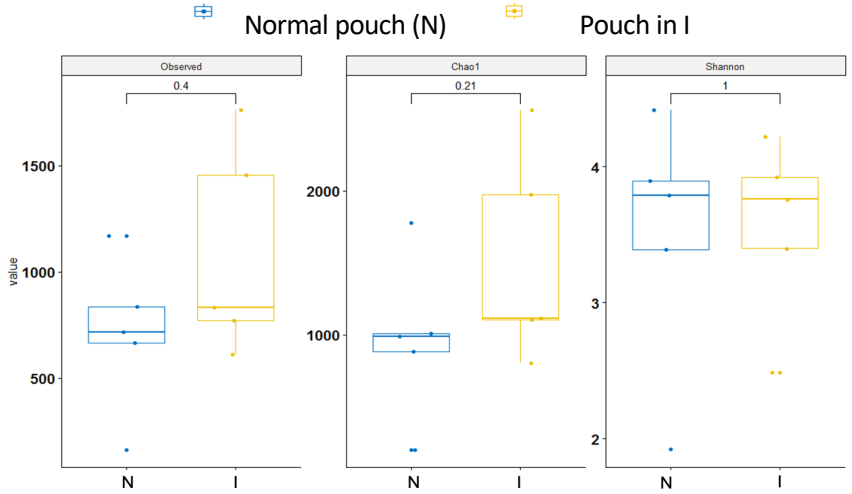




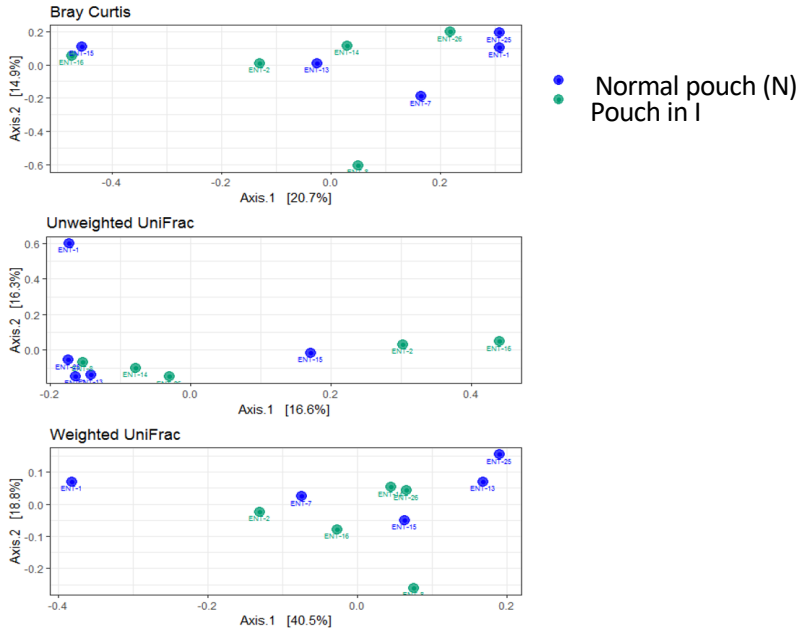
Bacterial phyla	Bacterial genus	Bacterial species	Fungi	Virus

Zheng et al, UEGJ 2022

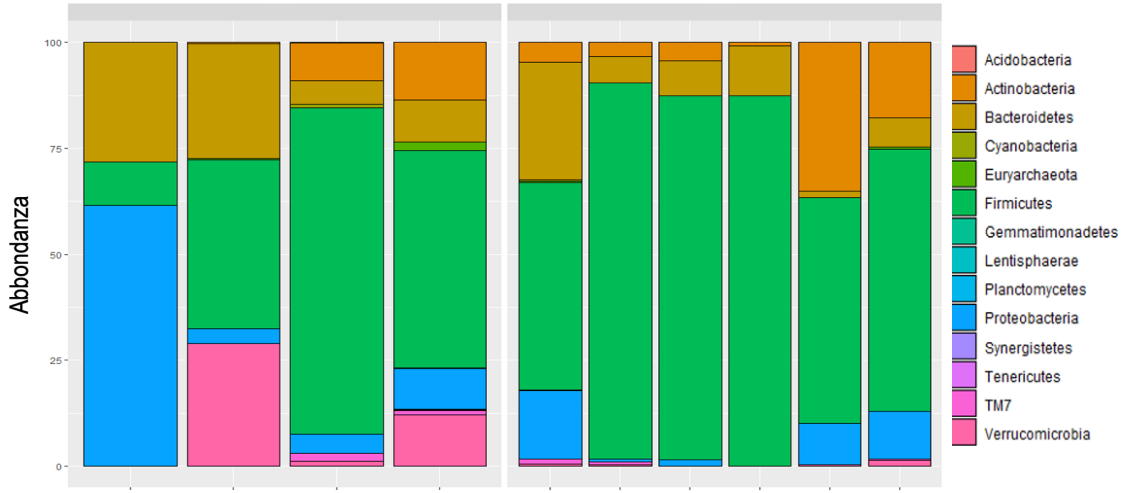
Microbial alterations in special conditions: pouchitis

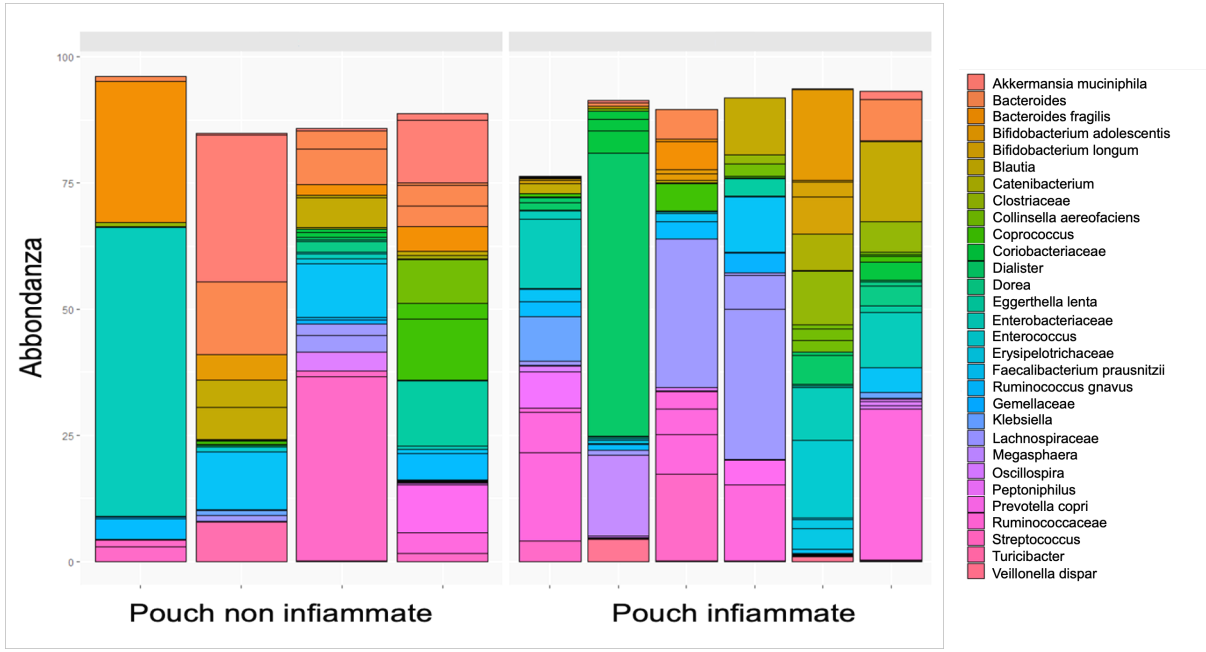


Alpha diversity



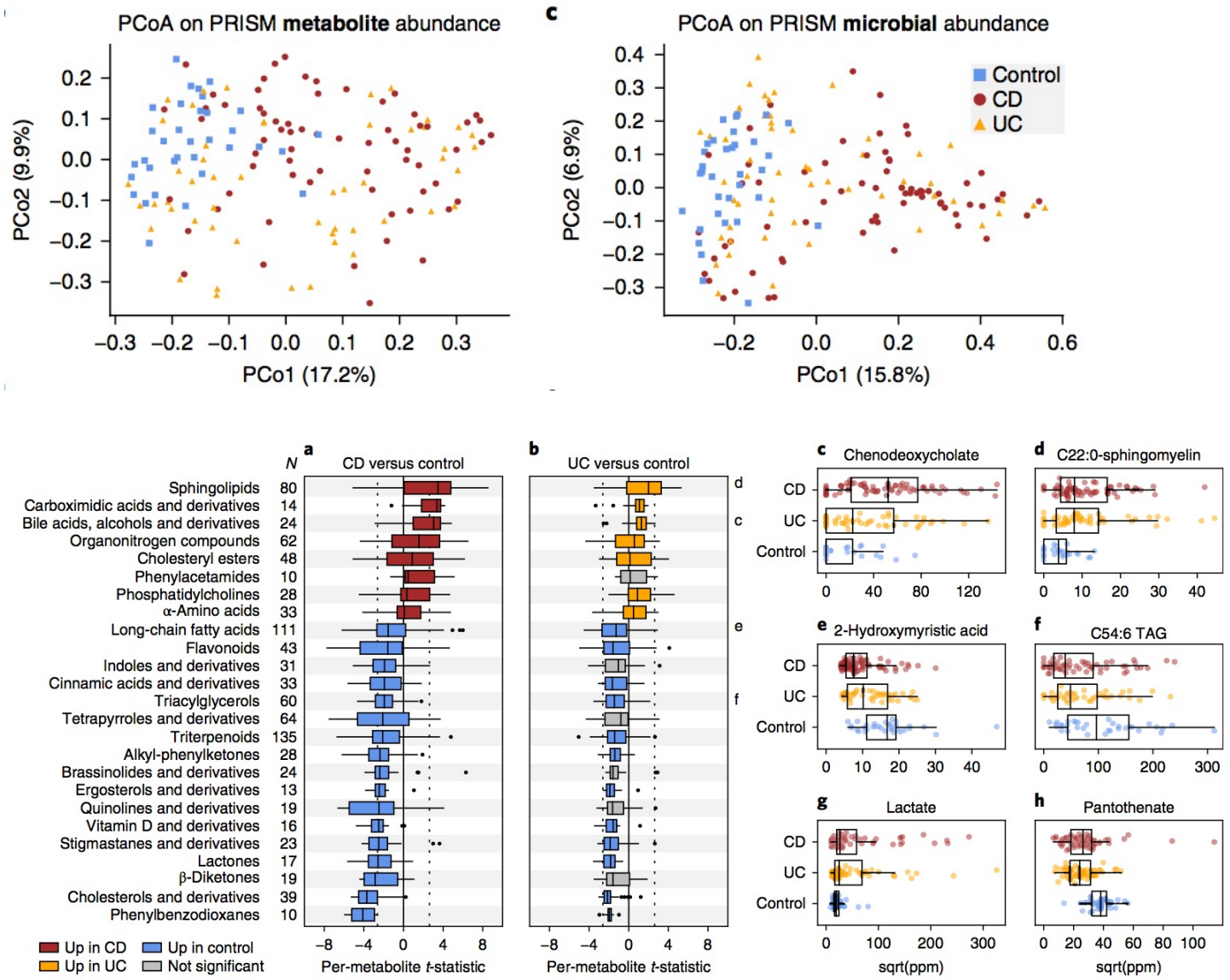
Beta diversity

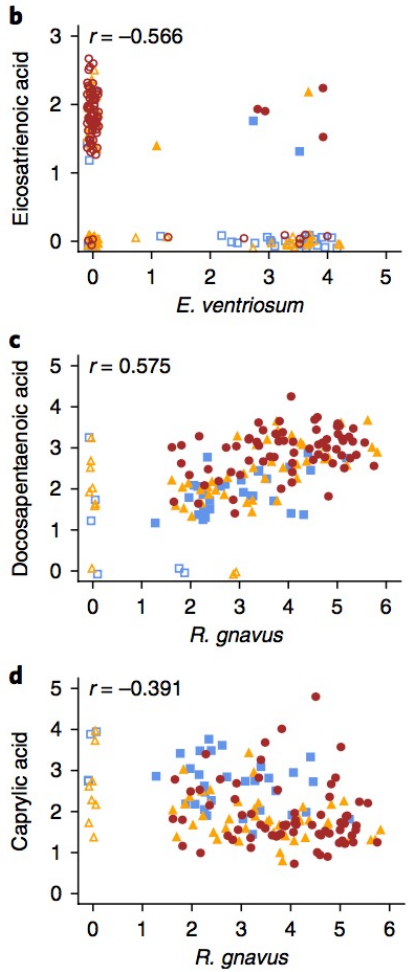




Lopetuso et al, unpublished data

Metabolomic alterations in IBD

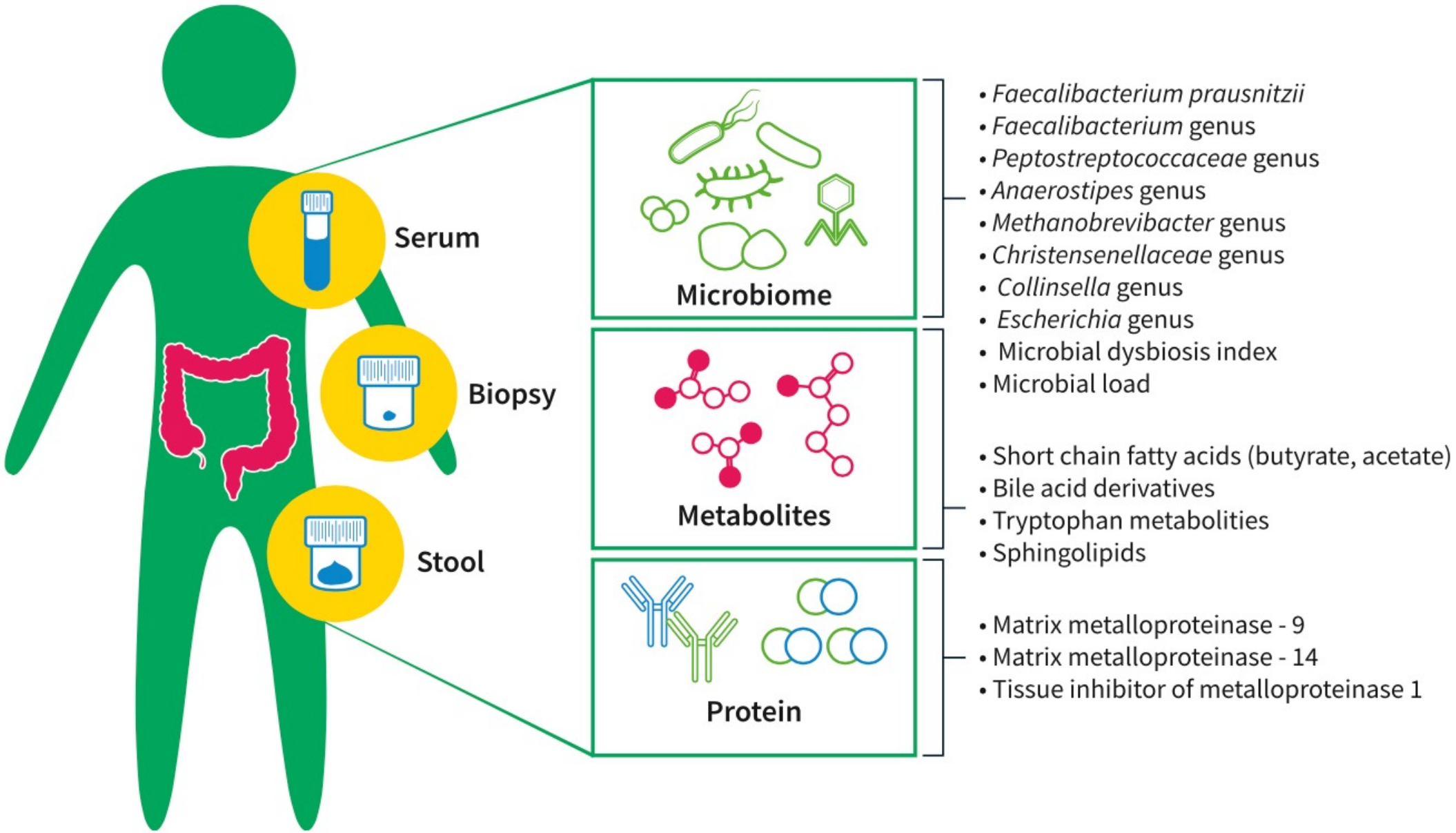




Short-chain fatty acids
Medium-chain fatty acids
Tryptophan
Bile acids
Sphingolipids

Franzosa et al, Nature 2019

Metaproteomic alterations in IBD



Zheng et al, UEGJ 2022

Starting from the end: Microbiota and IBD

Gut microbiota composition is altered in IBD vs controls

Gut microbiota composition is altered in active vs non-active IBD

Gut microbiota can influence the development of IBD

Association  **Causal relationship**

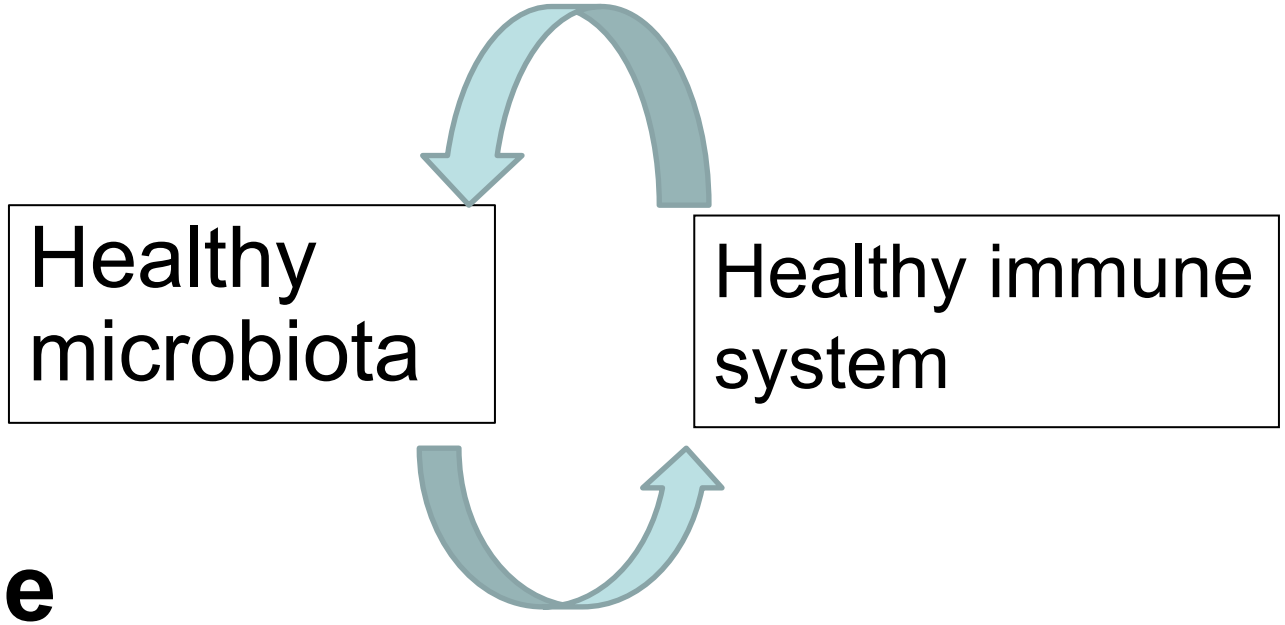
Microbiota and immune system relationship

Innate immune response

- Epithelial cells
- Myeloid cells
- Innate lymphoid cells

Adaptive immune response

- IgA
- TH17 cells
- Treg cells



Microbiota is important for the correct development of both innate and adaptive immune response

Microbiota educates immune system to react to pathogens but also to tolerance

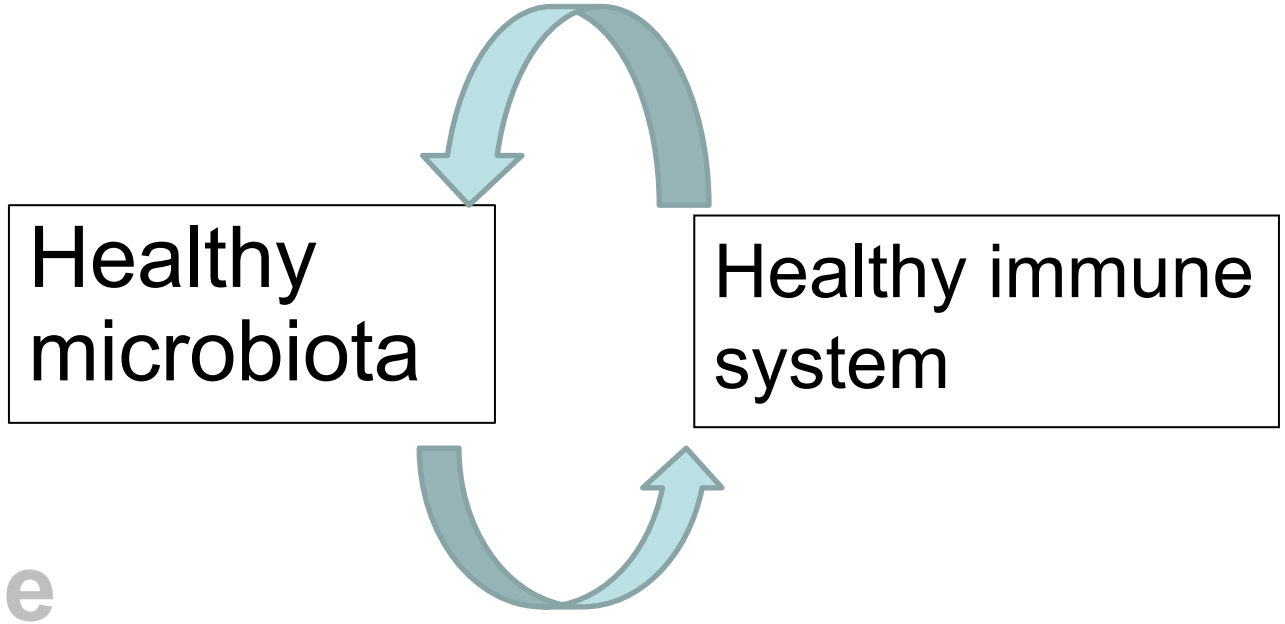
Microbiota and immune system relationship

Innate immune response

- Epithelial cells
- Myeloid cells
- Innate lymphoid cells

Adaptive immune response

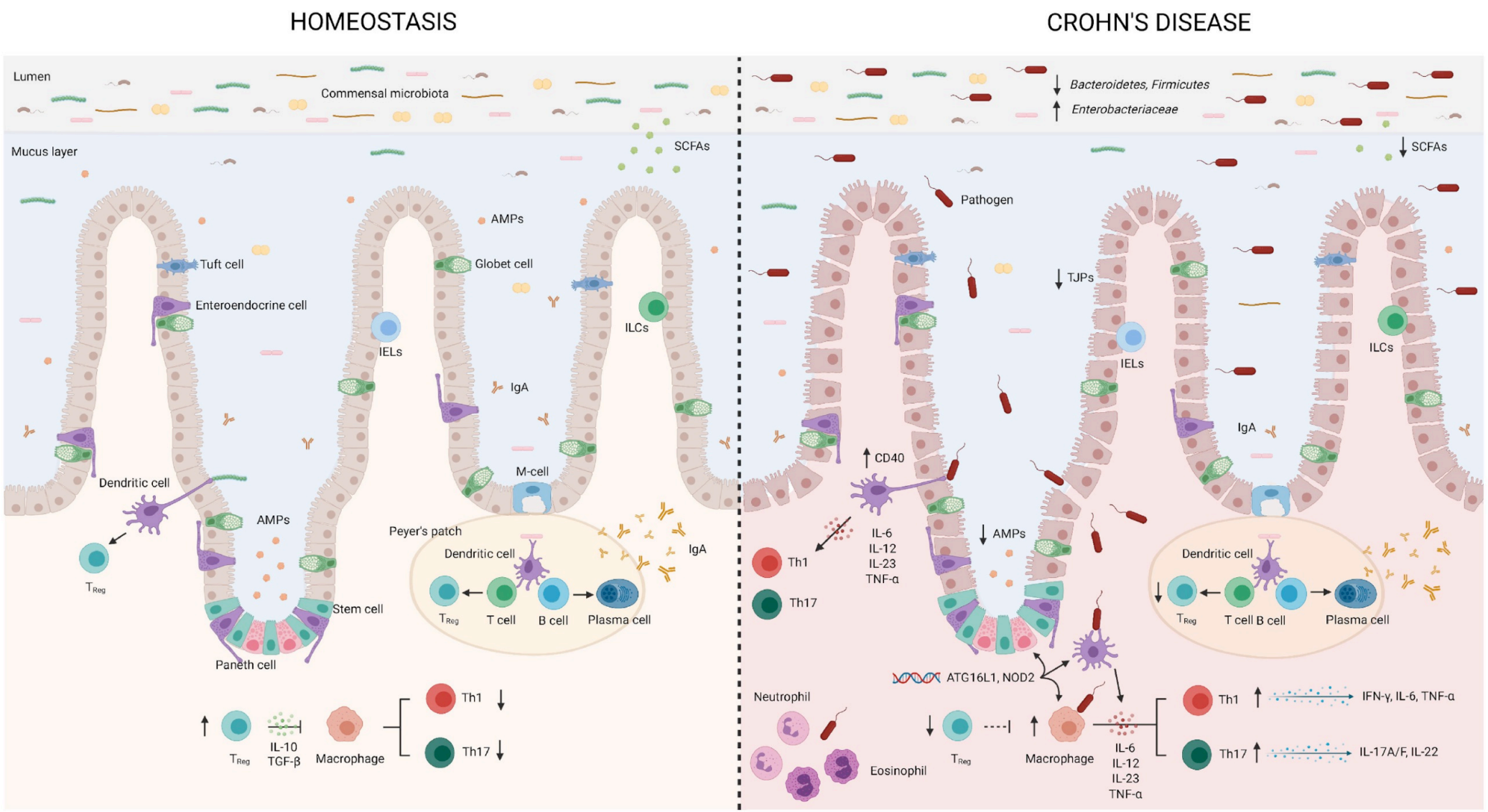
- IgA
- TH17 cells
- Treg cells



Microbiota is important for the correct development of both innate and adaptive immune response

Microbiota educates immune system to react to pathogens but also to tolerance

Bacterial translocation in IBD



Linares R, et al. Front Cell Dev Biol. 2021

Microbiota and epithelial cells

Table 1 Main immunological functions of IEC

Main cytokines produced by IEC
Proinflammatory cytokines: TNF-α and IL-15 (in IBD patients) [2, 33]
Proinflammatory cytokines IL-1β and TNF-α causing an increase of MCP-1 production in vitro by IECs (chemokine playing role in intestinal inflammation in IBD) [2]
Anti-inflammatory cytokines IL-4, IL-10, IL-13 downregulating the production of MCP-1 in vitro by IECs and monocytic lysosomal enzyme release [39]
TGF-β causing suppression inflammation in neonatal gut [55]
Main receptors
TLRs (Toll-like receptors), which recognize microbe-associated molecular patterns and activate inflammatory mechanisms. TLRs have different expression in various parts of the intestine [2, 33]
MHC-I and MHC-II molecules responsible for antigen presentation to lymphocytes [33]
Other functions
The production of mucin proteins, i.e., TSLP[a] which decreases the production of proinflammatory cytokines: IL-12 and IL-25 by DCs with simultaneously increasing production of IL-10 [33]
Influence the production of antibodies (sIgA) which prevent the adherence of antigens to gut mucosa [33]
Secretion of C3 complement component [40]
Production of serotonin [51]
Inhibition of PLT adhesion to and aggregation by fibrinogen [22]

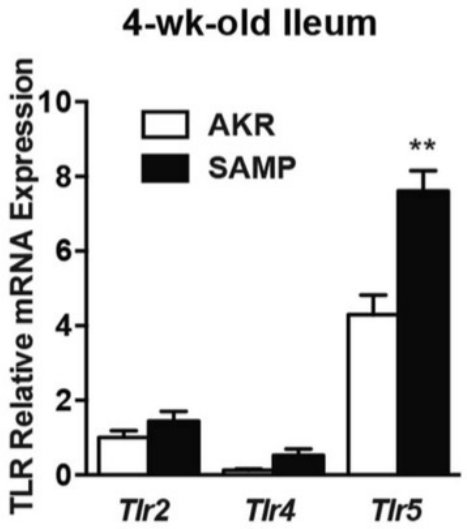
Górski et al, Cellular and Mol Sciences 2017

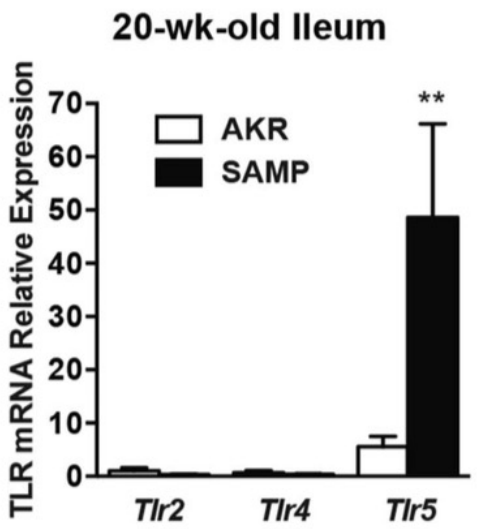
Epithelial-specific TLR5 Activation Mediates Barrier Dysfunction in Experimental Ileitis

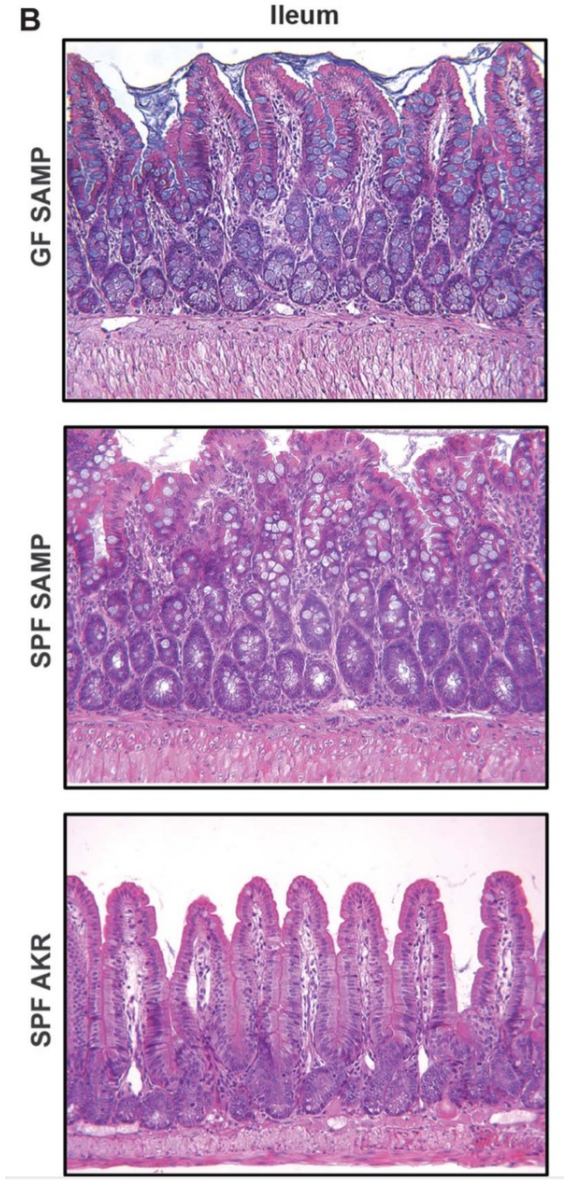
Ileal Tlr5 and serum antiflagellin IgG antibodies are increased in SAMP before the onset of inflammation and during established disease.

These trends are abrogated in the absence of colonizing commensal bacteria.

Ileal epithelial TLR5 in preinflamed SAMP is increased compared with age-matched AKR and germ-free SAMP.

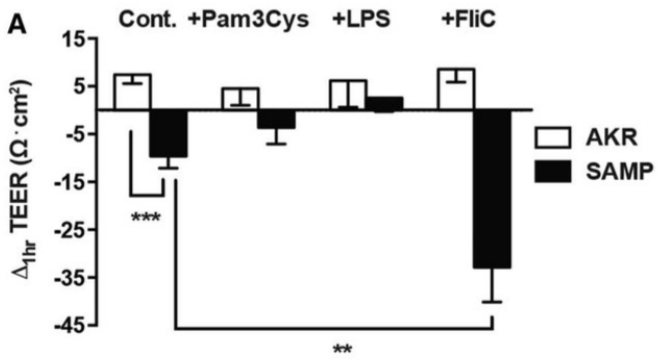


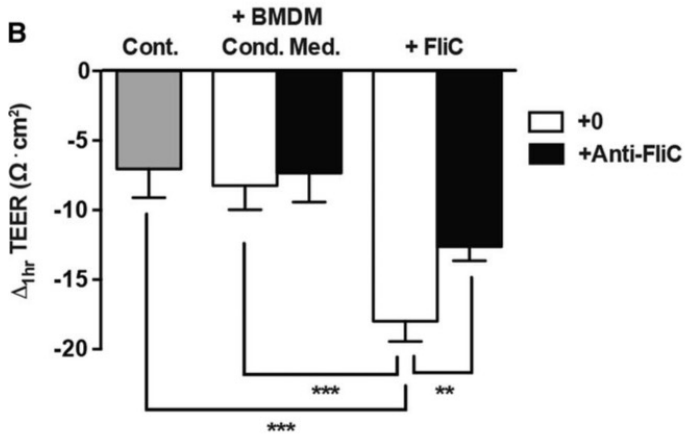


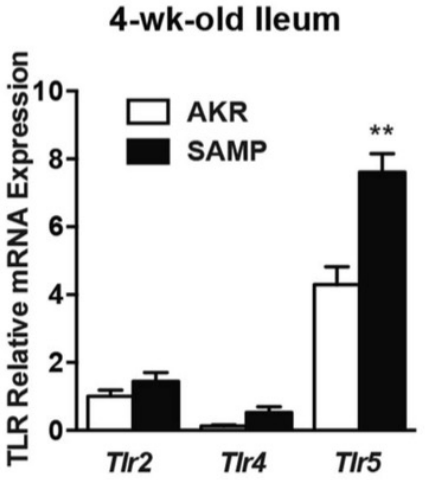


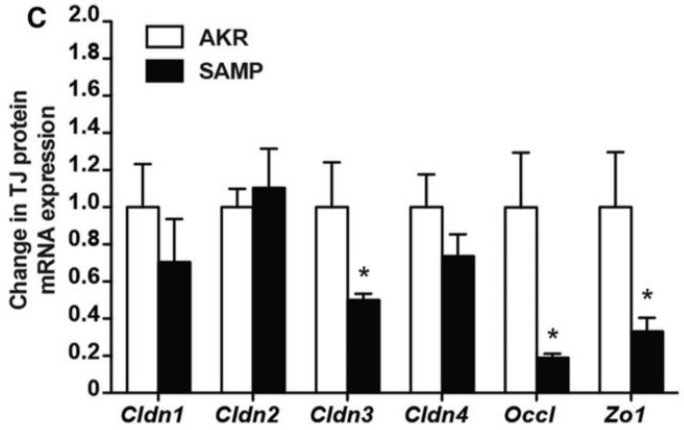
Lopetuso et al, IBD 2017

Epithelial-specific TLR5 Activation Mediates Barrier Dysfunction in Experimental Ileitis

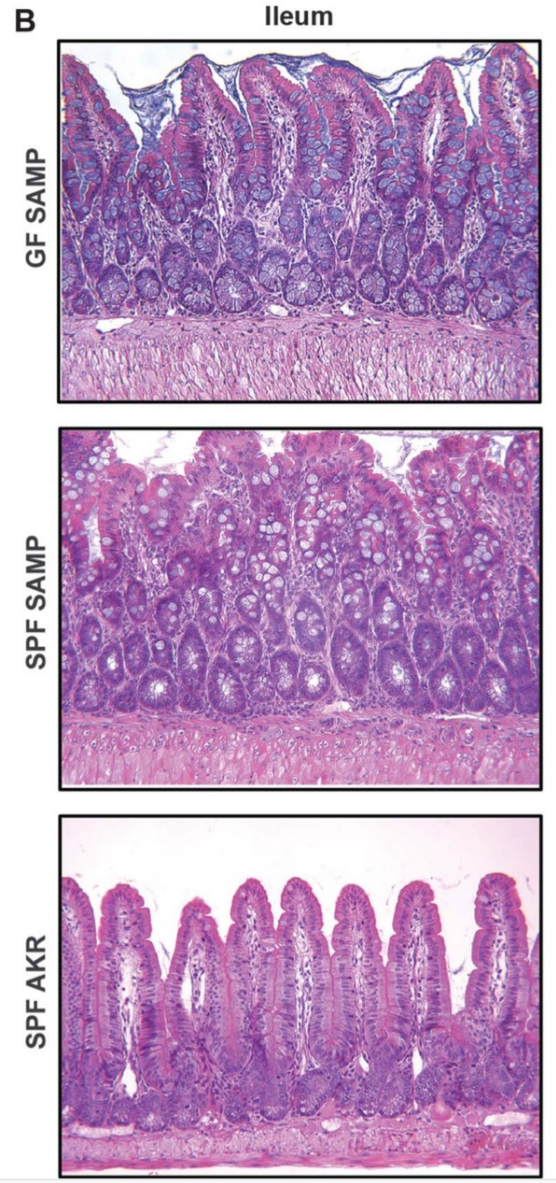








TLR5-specific activation of SAMP ileal tissues decreases epithelial barrier resistance, indicative of increased permeability, and is accompanied by altered expression of the tight junction proteins, claudin-3, occludin, and zonula occludens-1.

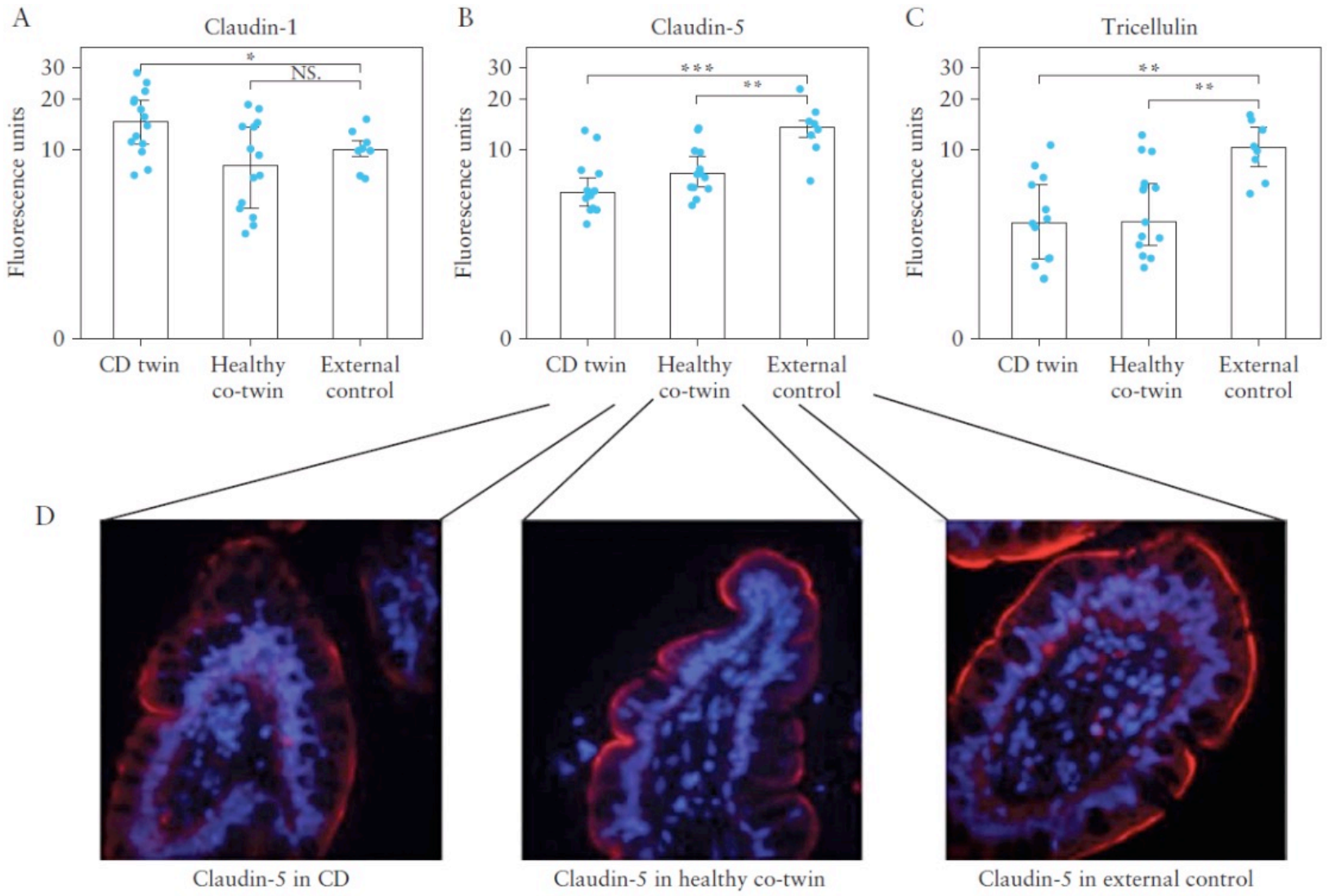


Aberrant, elevated TLR5 expression is present in the ileal epithelium of SAMP mice and is augmented in the presence of the gut microbiome.

TLR5 activation in response to bacterial flagellin results in a deficiency to maintain appropriate epithelial barrier integrity.

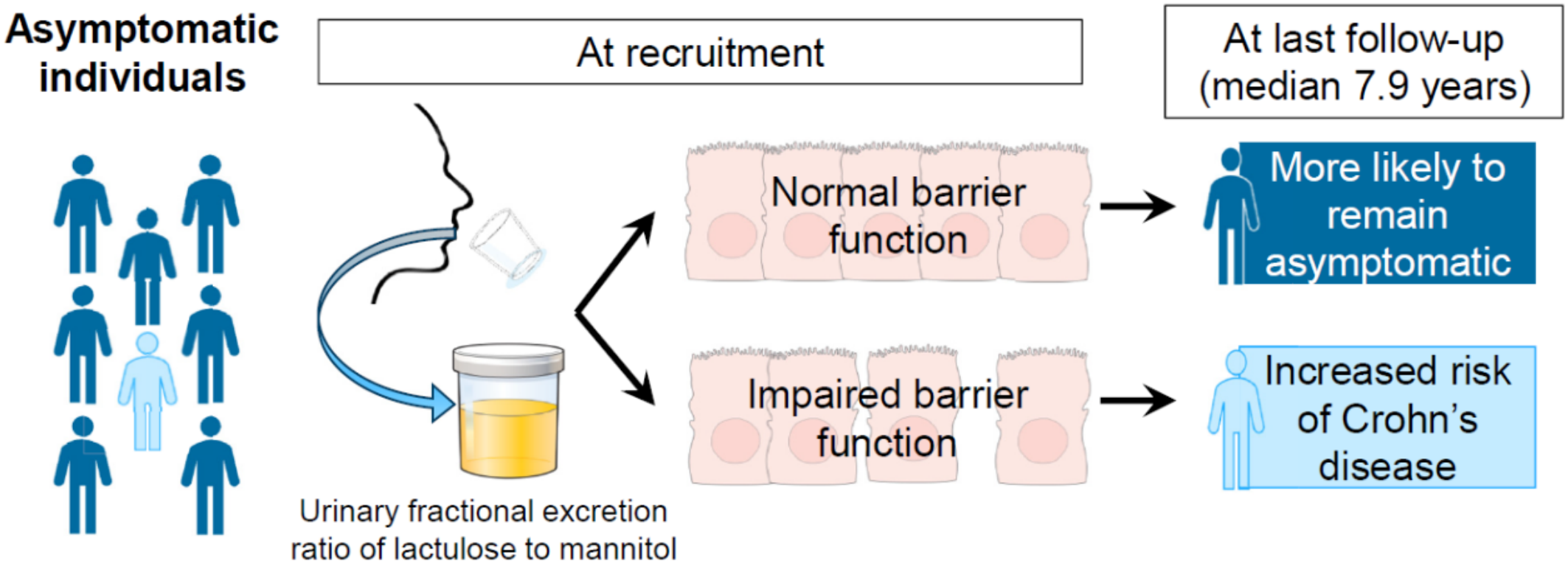
Lopetuso et al, IBD 2017

Barrier dysfunction is a primary defect in IBD: studies on twins



Keita ÅV, et al. J Crohns Colitis. 2018

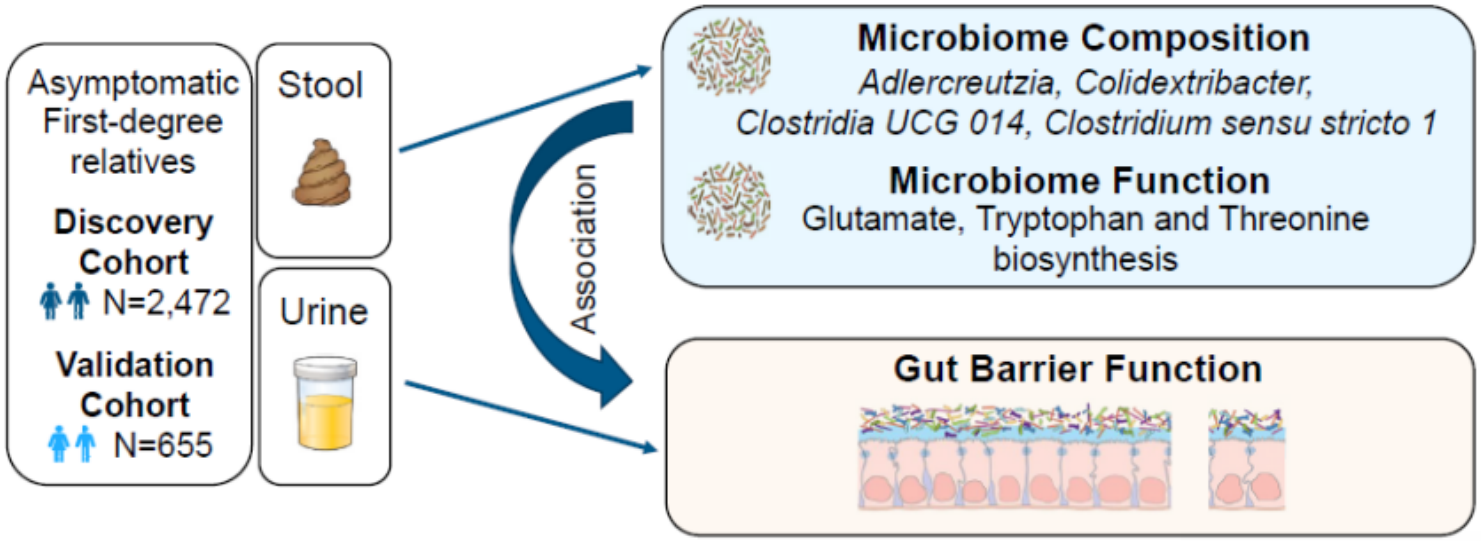
Increased Intestinal Permeability Is Associated With Later Development of IBD in relatives

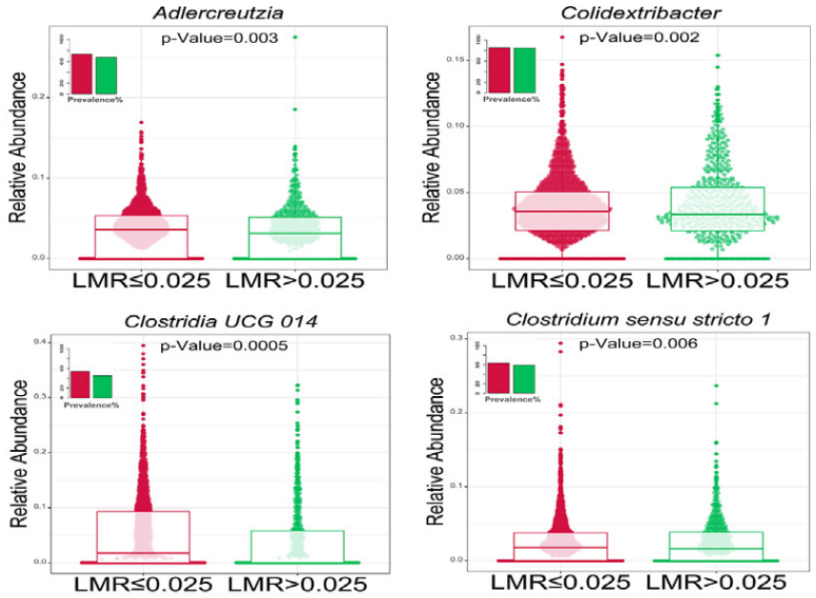


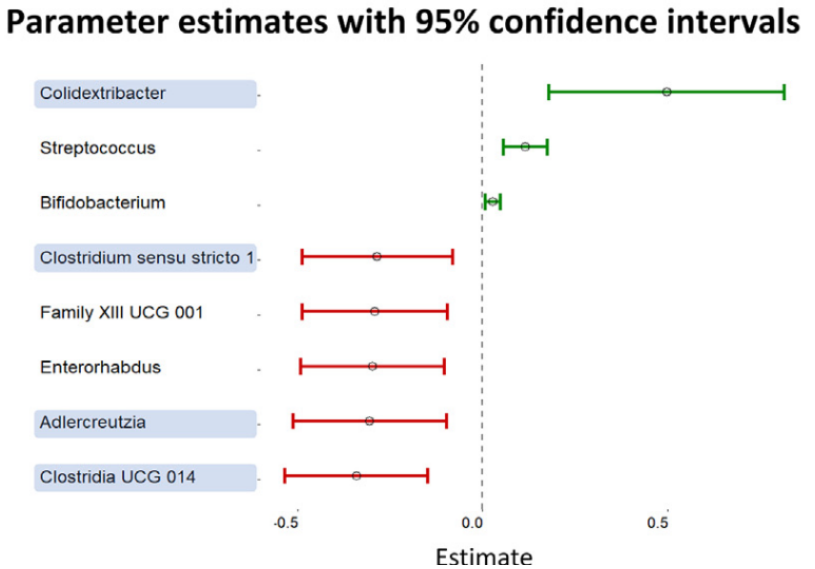
HR 3.03

Turpin W, et al. Gastroenterology. 2020

Altered Gut Microbiome Composition and Function Are Associated With Gut Barrier Dysfunction in Healthy Relatives of Patients With CD



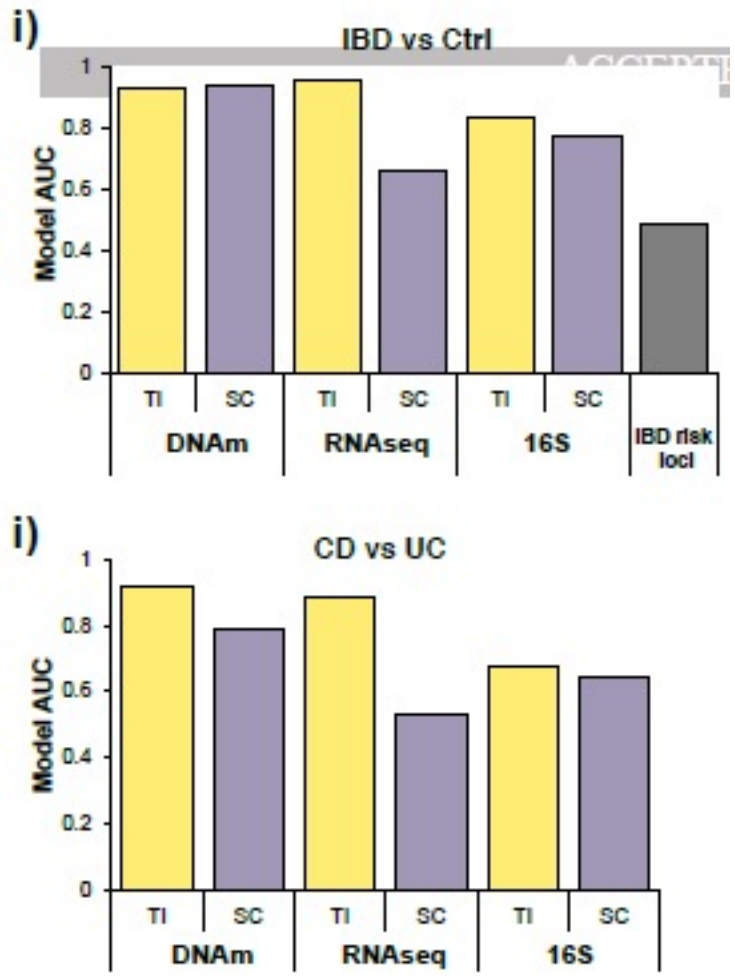




Leibovitzh H, et al. Gastroenterology. 2022

Gut barrier epigenetic dysfunction in IBD

Intestinal epithelial cells (IEC) from mucosal biopsies of children newly diagnosed with IBD



Gut segment-specific differences in DNA methylation and transcription profiles of IECs

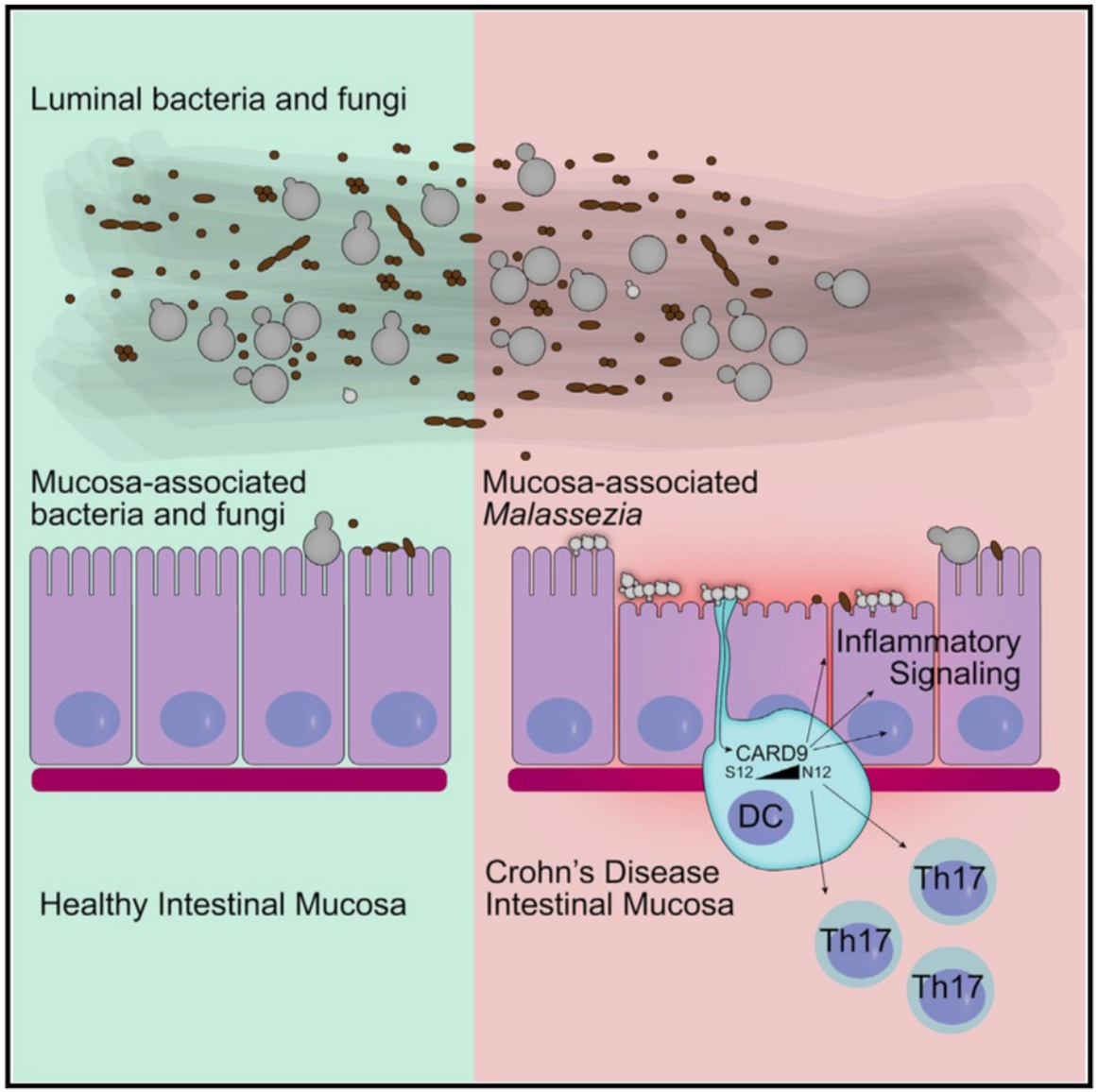
Changes in gut microbiota between IBD and control groups were not as large and were difficult to assess due large amounts of intra-individual variation.

Only IECs from patients with CD had changes in DNA methylation and transcription patterns in terminal ileum epithelium, compared with controls.

Colon epithelium from patients with CD and from patients with UC had distinct changes in DNA methylation and transcription patterns, compared with controls.

Howell et al. Gastroenterology 2017

Malassezia Is Associated with Crohn's Disease and Exacerbates Colitis in Mouse Models

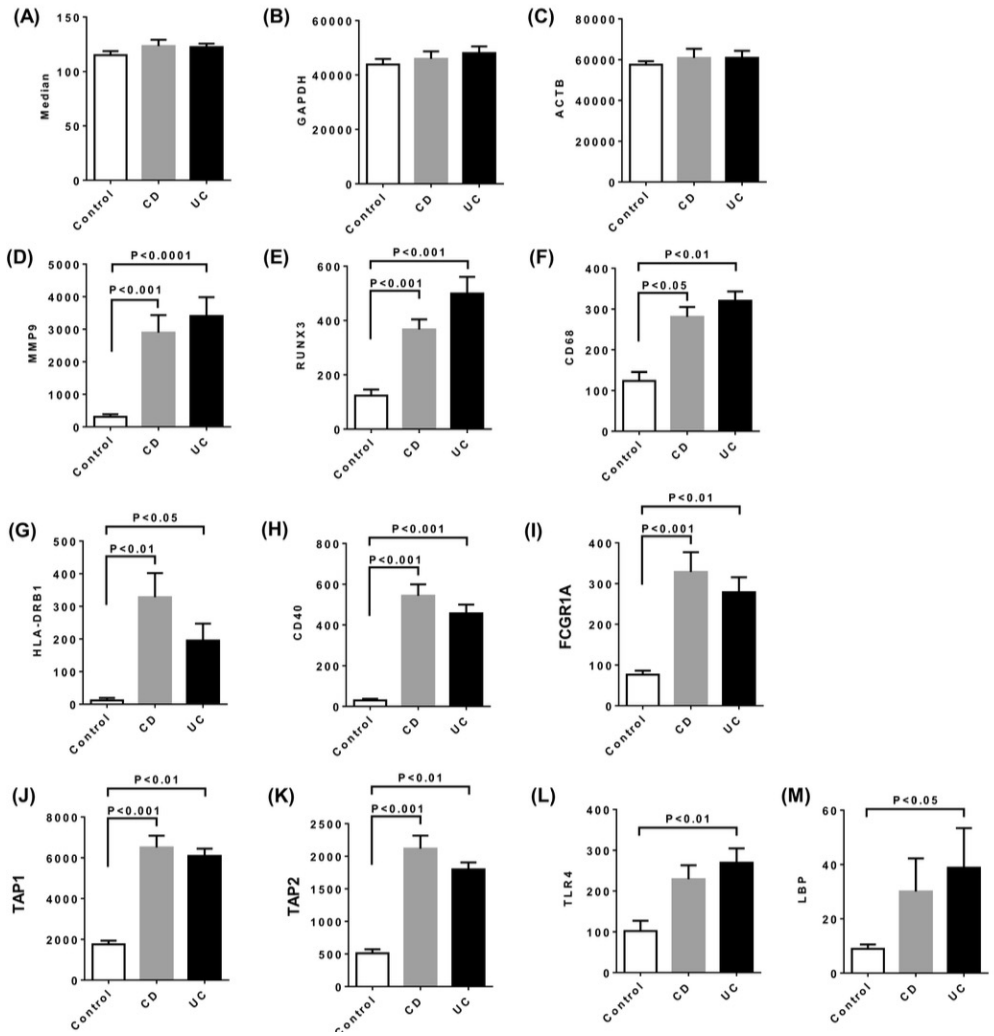


- ✓ M. restricta is associated with the colonic mucosa in CD patients
- ✓ M. restricta is found in CD patients with a disease-linked polymorphism in CARD9
- ✓ M. restricta exacerbates colitis in WT and gnotobiotic mice
- ✓ Malassezia-exacerbated colitis in mice requires signaling via CARD9

Limon et al. Cell H&M 2019

Gut barrier dysfunction in IBD: transcriptomic data

Viral infection induced autoimmunity may represent a pathomechanism for IBD, especially CD



- ✓ Differentially expressed genes in the colonic mucosa of CD and UC correlated with response to microbial antigens
- ✓ Virus infection and autoimmune pathways upregulated in CD but not in UC when compared with controls
- ✓ Some expressed genes elevated in both CD and UC, with CD exhibiting more pronounced elevations
- ✓ Gene expression levels in viral infection pathways correlated with those of autoimmune pathways
- ✓ Pattern recognition–mediated innate immune pathways (TLR4 and TLR2) were significantly elevated in UC but not in CD

Different intervention targets for CD and UC, which may lead to more effective treatments for IBD

Yang et al. IBD 2019

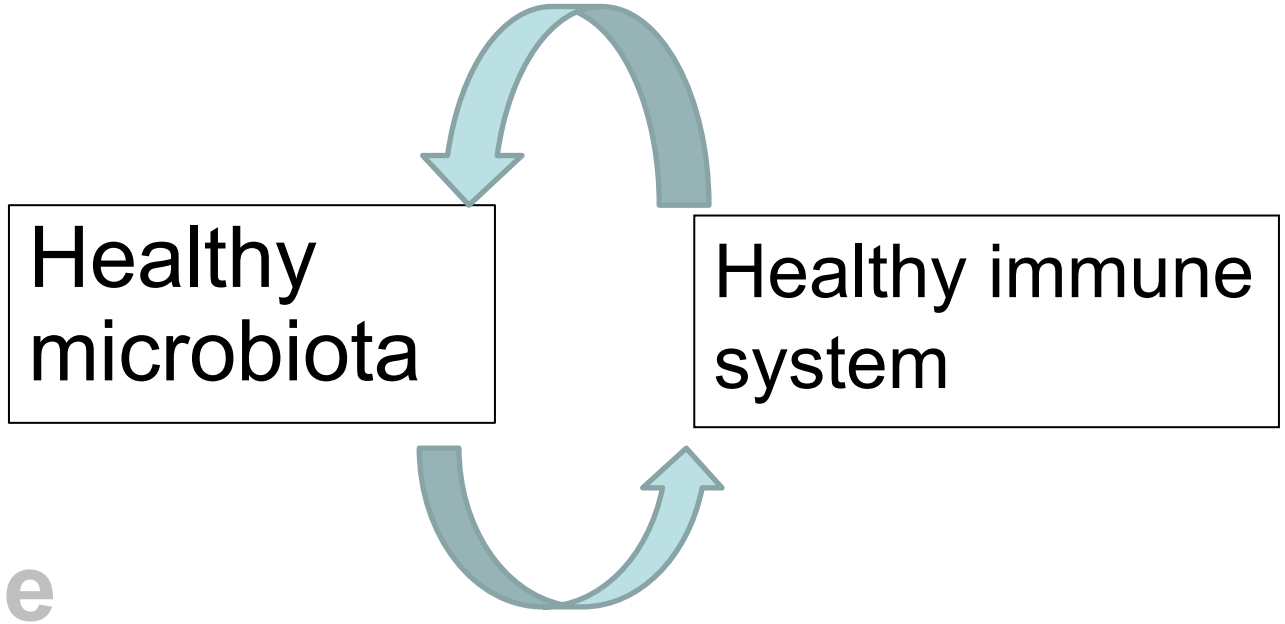
Microbiota and immune system relationship

Innate immune response

- Epithelial cells
- Myeloid cells
- Innate lymphoid cells

Adaptive immune response

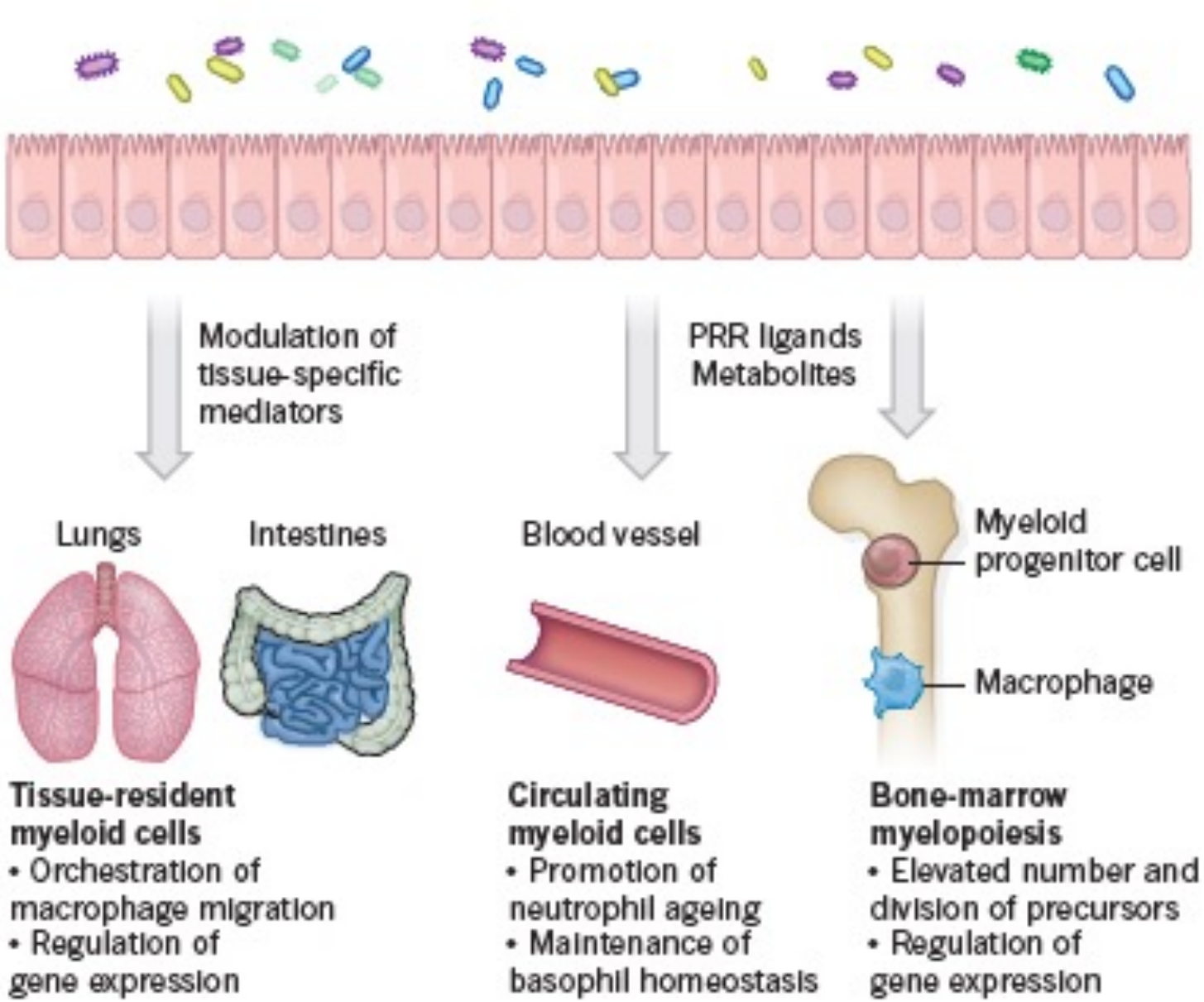
- IgA
- TH17 cells
- Treg cells



Microbiota is important for the correct development of both innate and adaptive immune response

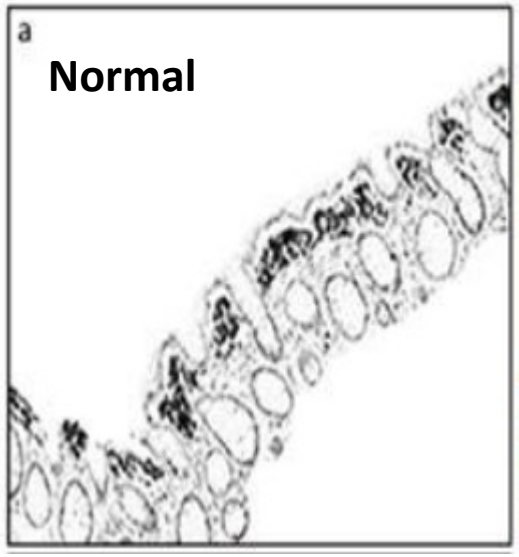
Microbiota educates immune system to react to pathogens but also to tolerance

Microbiota and myeloid cells

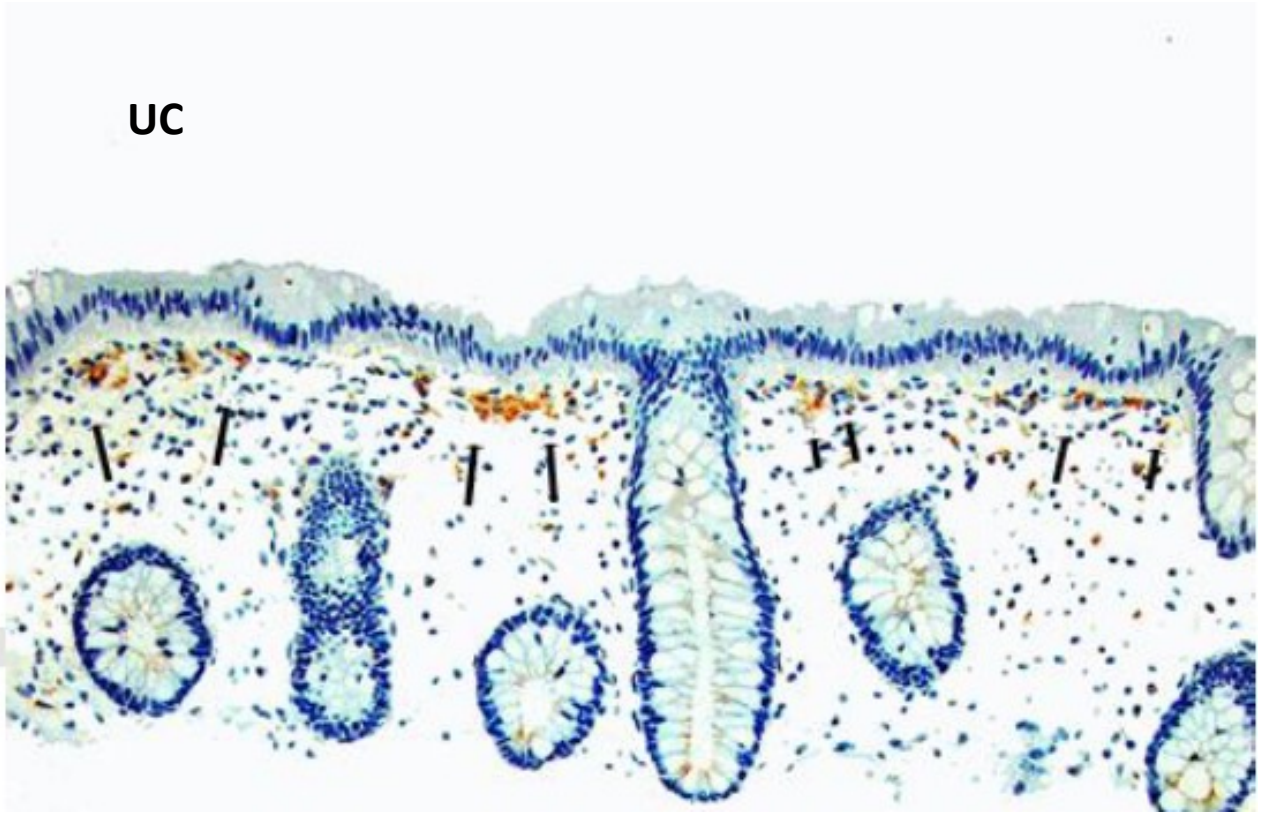


Thaiss et al, Nature 2016

Abrogation of the subepithelial macrophage barrier against the gut microbiota in IBD



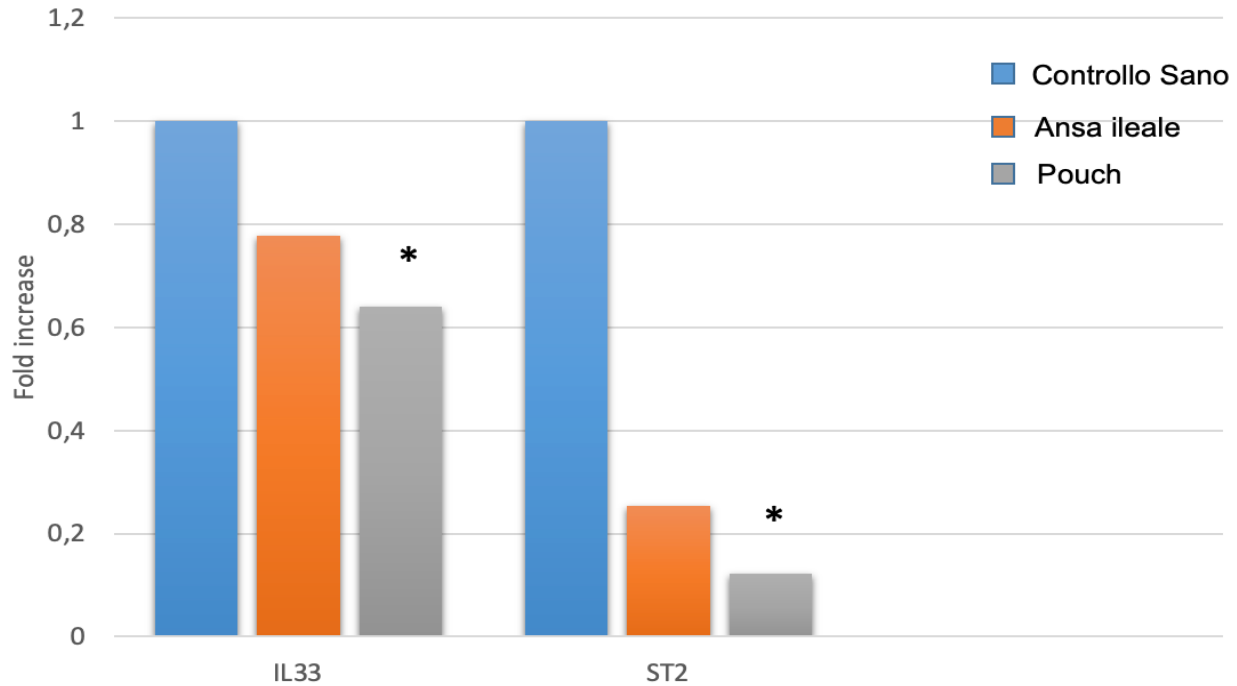
Continuous band of CD68+ macrophages underneath the luminal epithelium



fragmented CD68+ band of macrophages

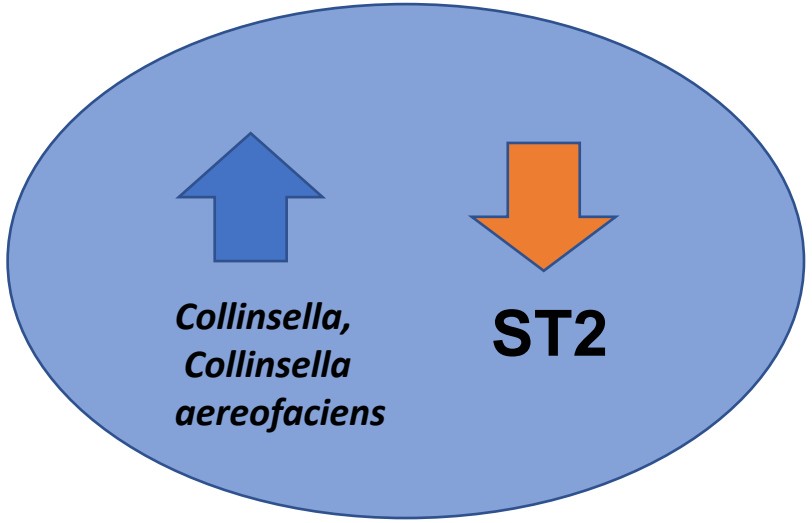
Rubio et al. Histopathology. 2017

Microbial/innate immune alterations in special conditions: pouchitis



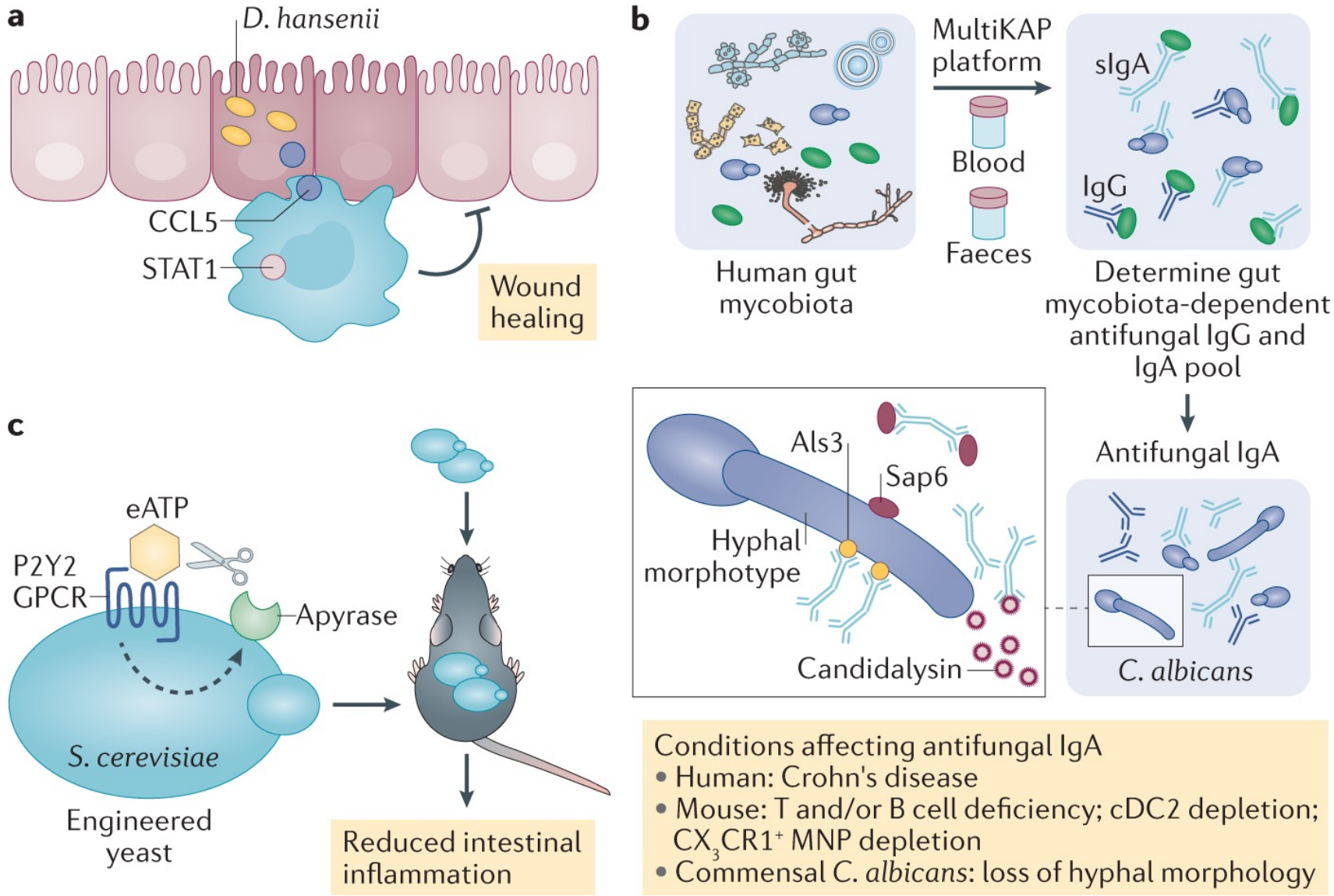
Pz. con infiammazione pouch

*P< 0.05



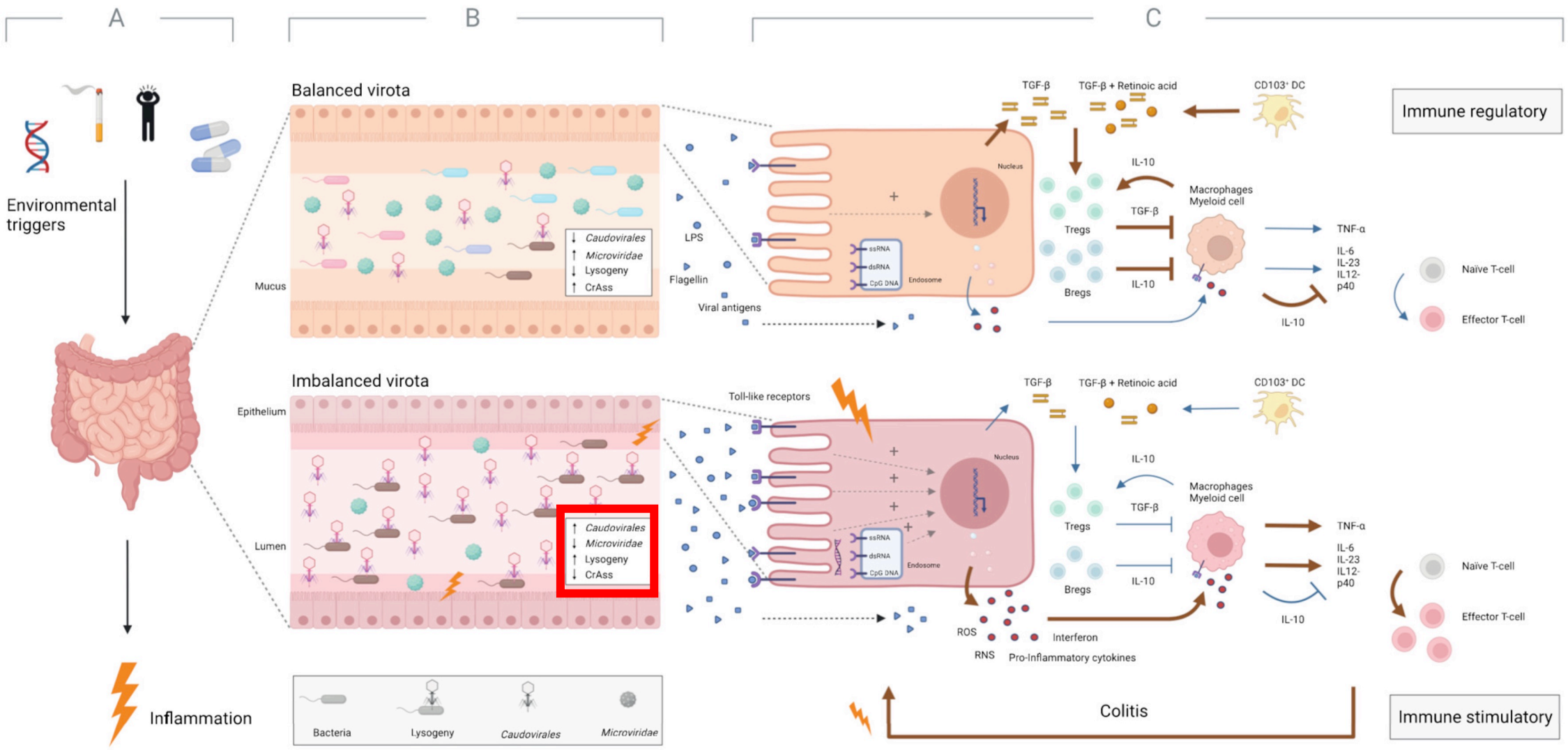
Lopetuso et al, unpublished data

Interactions between gut mycobiota and immune system in IBD



Iliev ID. Nat Rev Gastroenterol Hepatol. 2022

IBD patients possess an imbalanced intestinal Virome and a dysregulated immune system



Jansen D, et al. Viruses. 2023

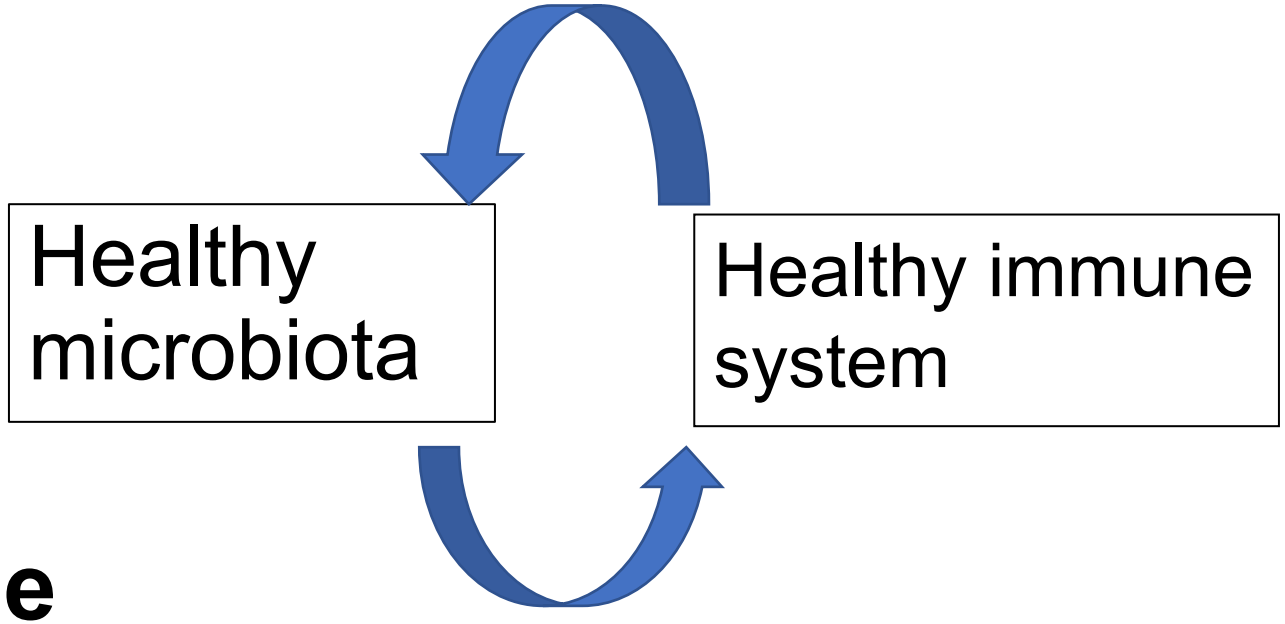
Microbiota and immune system relationship

Innate immune response

- Epithelial cells
- Myeloid cells
- Innate lymphoid cells

Adaptive immune response

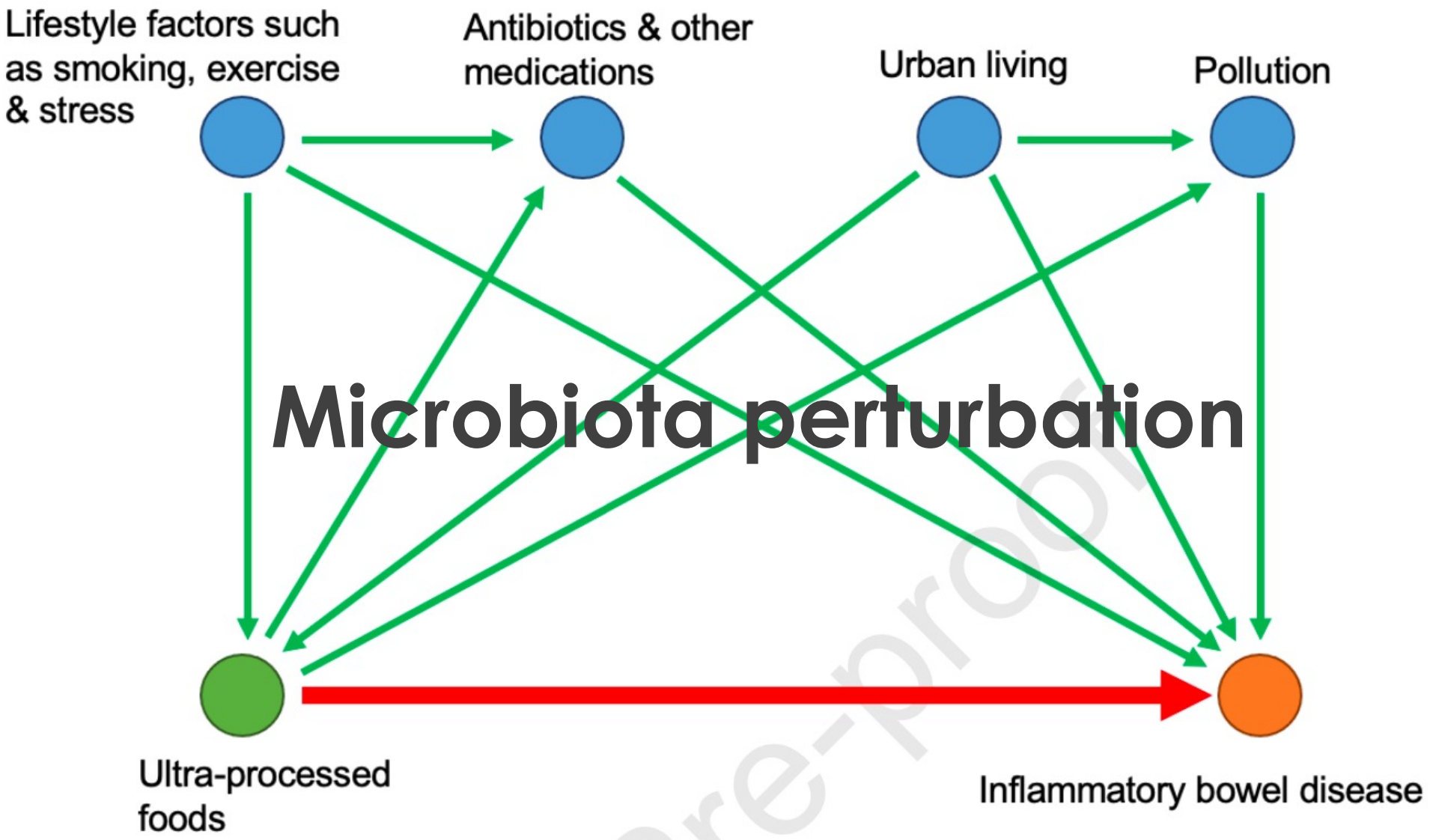
- IgA
- TH17 cells
- Treg cells



Microbiota is important for the correct development of both innate and adaptive immune response

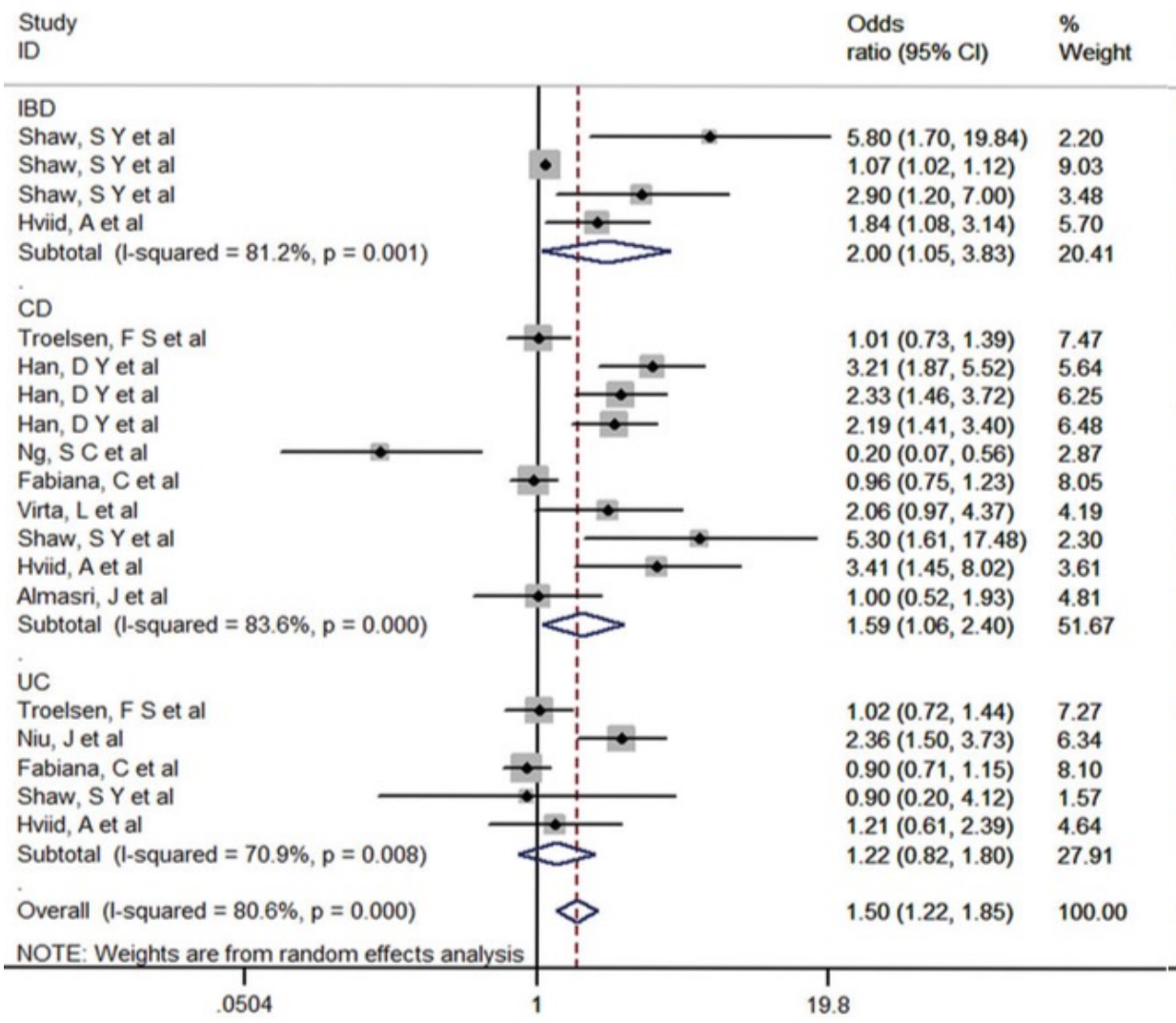
Microbiota educates immune system to react to pathogens but also to tolerance

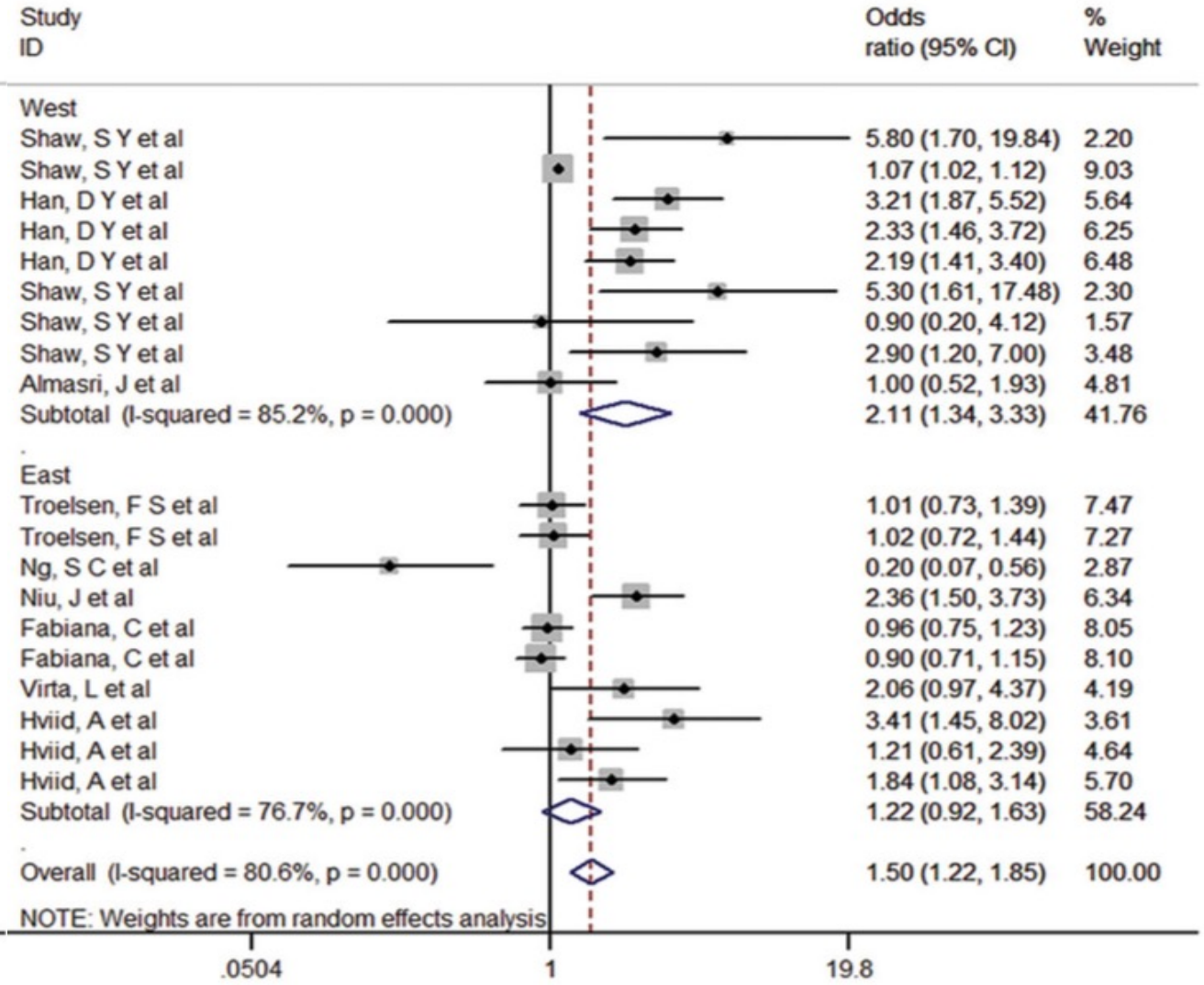
Environmental exposures and the risk of IBD



Adapted from Allin et al, J. Gastroenterol 2021

Antibiotics and environmental exposures in the early life: chronic intestinal inflammation

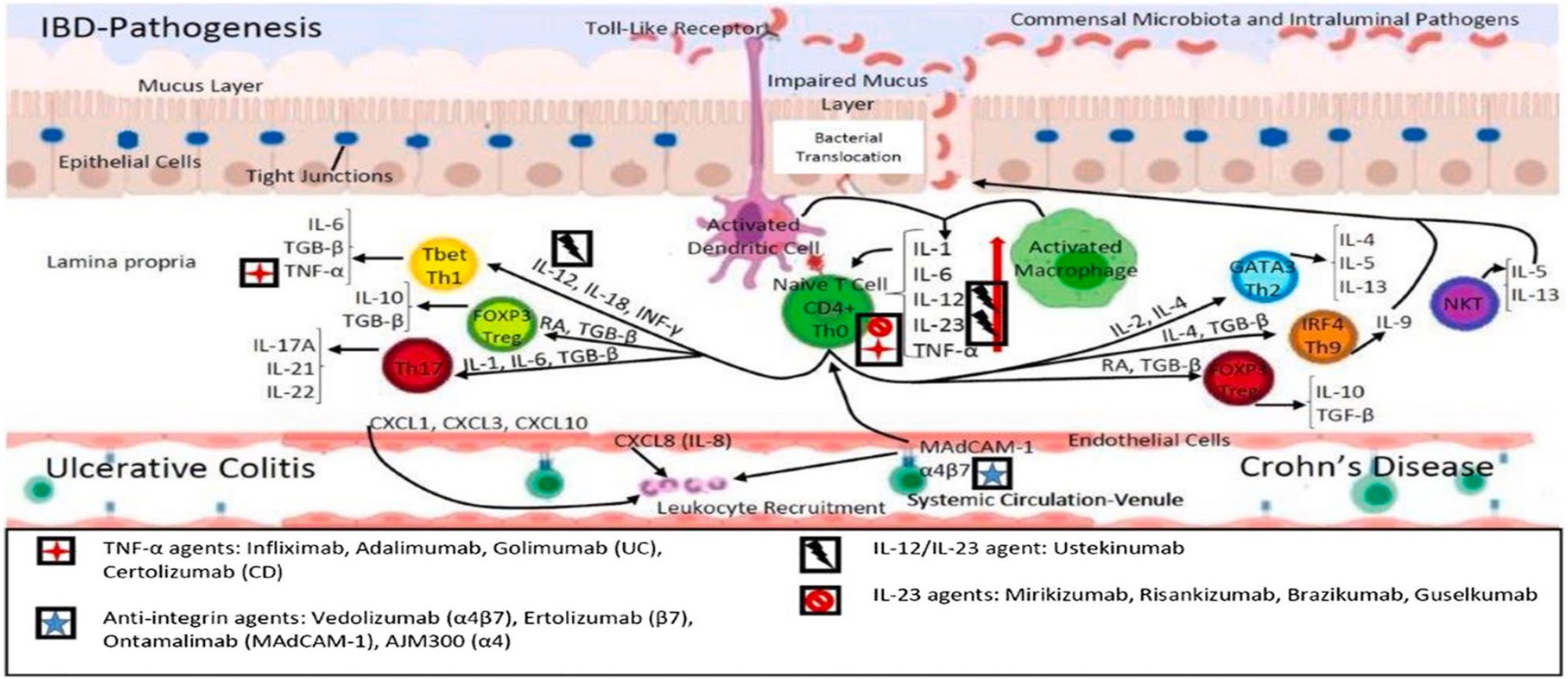




Lee et al 2020
Zhang et al 2022
Zou et al 2020

WHICH APPLICATIONS IN IBD?

TOWARDS A PERSONALIZED IBD THERAPY

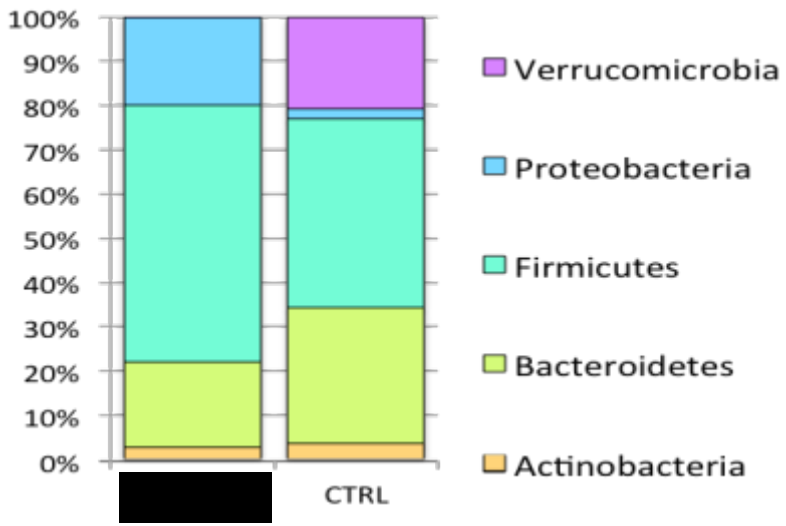


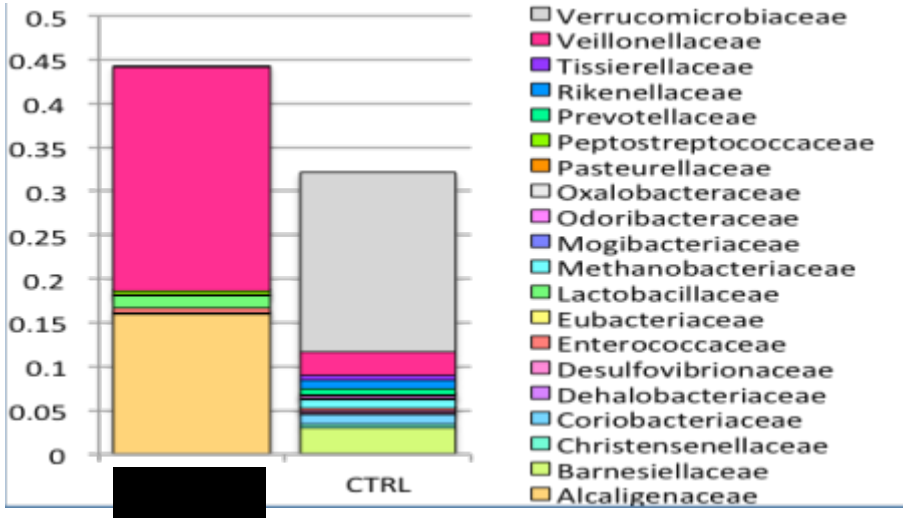
Optimize treatments → **Minimize side-effects and costs**

Moving toward a Microbiota signature for IBD patients

Nadpara et al – Dig Dis Sci 2020

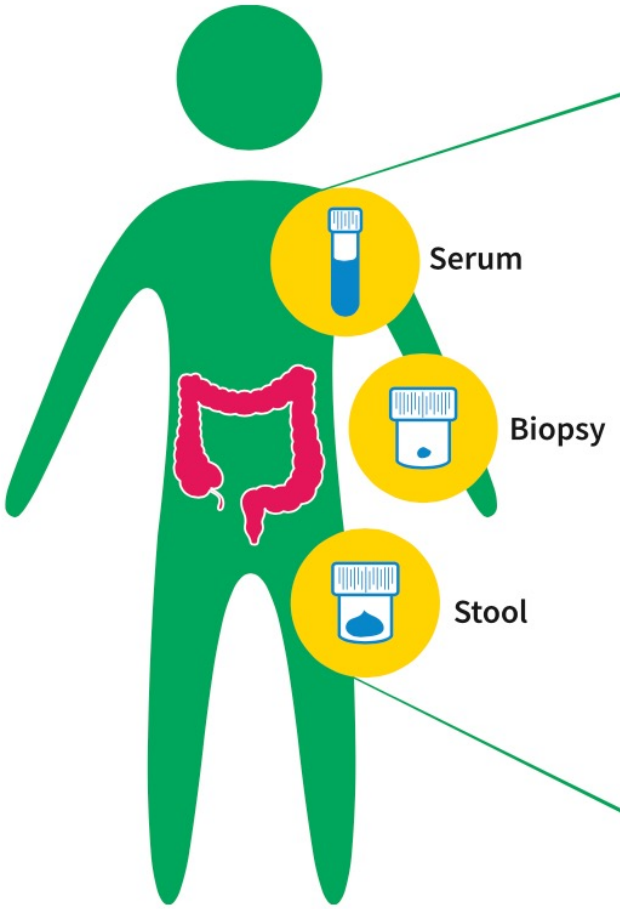
MICROBIOTA SIGNATURE:
the inflammatory microbiome of a patient with an active UC disease





Unità tassonomica	Unità tassonomica	[redacted]	CTRL	ANDAMENTO[2]
Actinobacteria	*Bifidobacterium*	0.03550	0.00621	+
Actinobacteria	*Bifidobacterium adolescentis*	0.02567	0.00027	+
Actinobacteria	*Bifidobacterium longum*	0.01599	0.00205	+
Actinobacteria	*Collinsella*	0.00092	0.00008	+
Euryarchaeota	*Methanobrevibacter*	0.00000	0.00912	-
Firmicutes	*Clostridium hiranonis*	0.00006	0.00000	+
Firmicutes	*Dorea formicigenerans*	0.00011	0.00000	+
Firmicutes	*Oscillospira*	0.00134	0.00753	-
Firmicutes	*Ruminococcus bromii*	0.00347	0.00005	+
Firmicutes	*Streptococcus*	0.00097	0.01670	-
Proteobacteria	*Acinetobacter*	0.00006	0.00001	+
Verrucomicrobia	*Akkermansia muciniphila*	0.00039	0.22569	-

MICROBIOME-ASSOCIATED BIOMARKERS IN IBD MANAGEMENT



Diagnosis
- Diagnosis
- Disease classification
- Disease activity assessment

Prognosis
- Disease recurrence prediction
- Therapeutic response

Fecal and mucosal microbiome

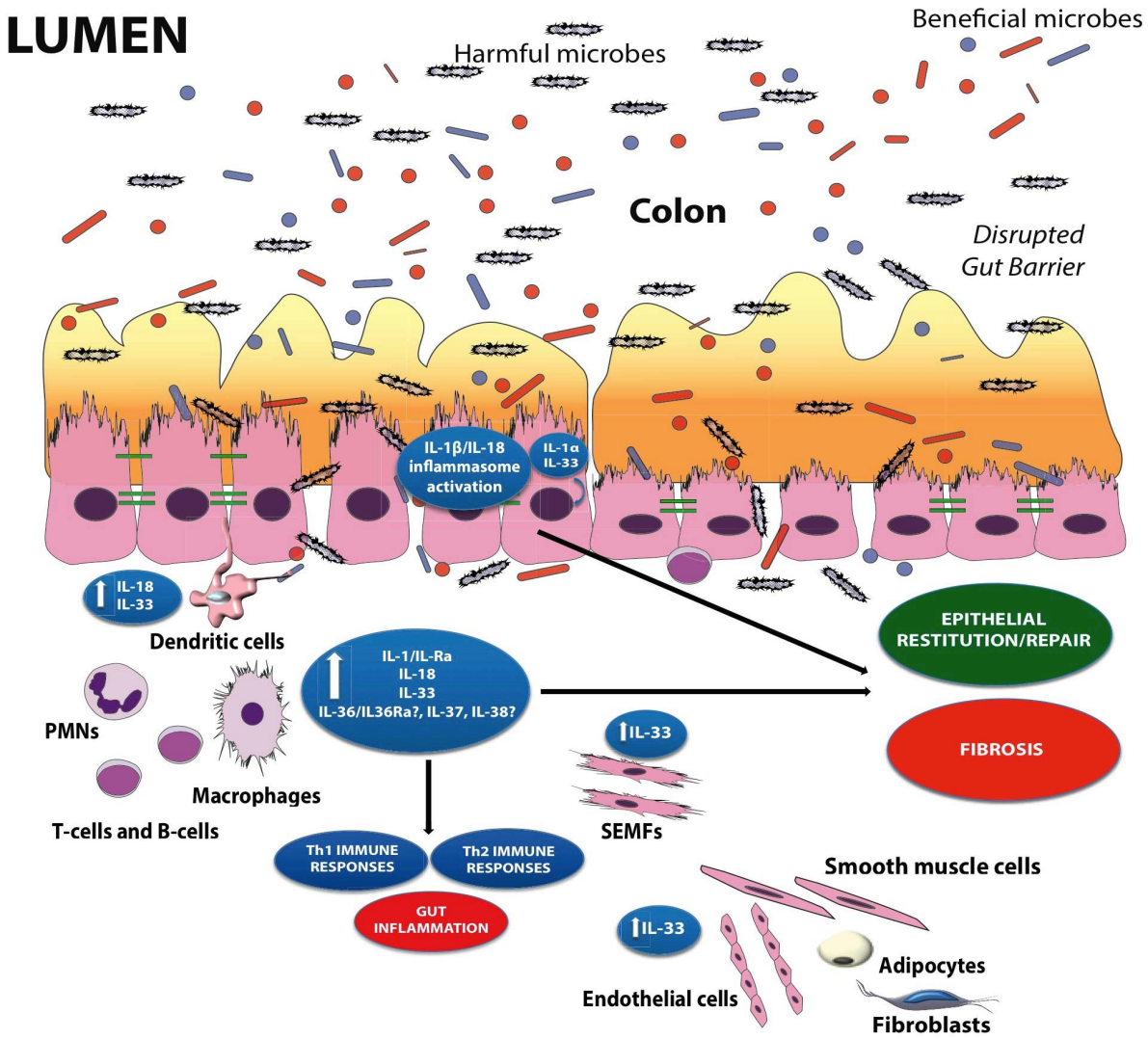
Diagnosis
Classification
Disease activity
Disease course
Recurrence after surgery
Responses to therapeutics

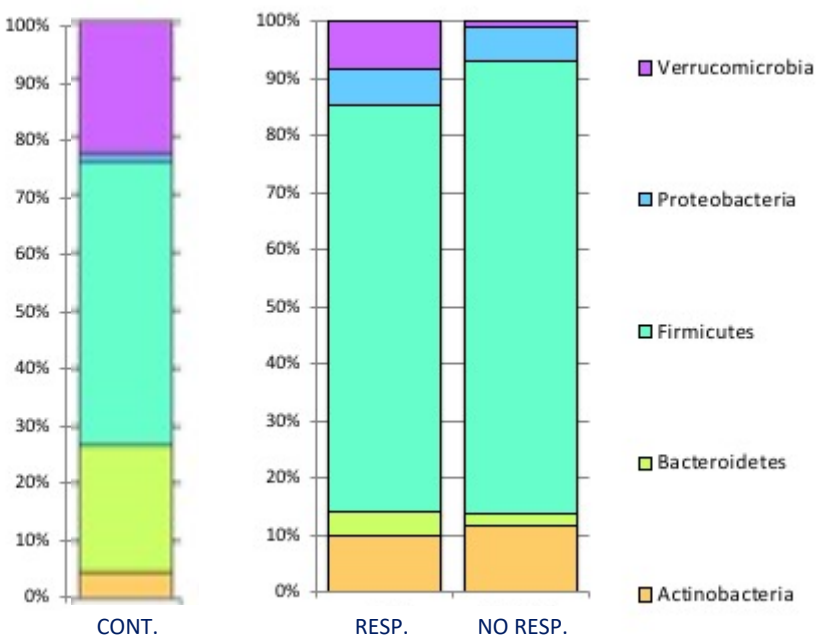
Bacteria derived metabolites,
serum and fecal microbe-associated proteins

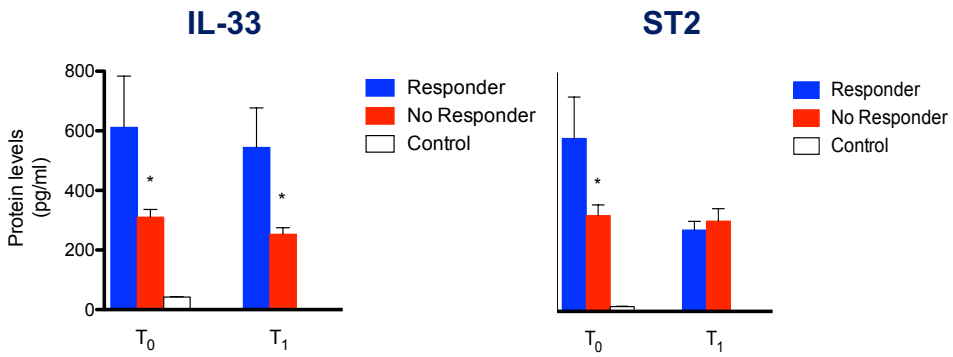
IBD determination and classification

Caenepeel et al. - APT 2020
Zheng et al, UEGJ 2022

Microbiota-IL-33/ST2 profiling can predict mucosal response to anti-TNF in UC







Lopetuso et al, PNAS 2018
Lopetuso et al, unpublished data

Starting from the end: Microbiota and IBD

Gut microbiota composition is altered in IBD vs controls

Gut microbiota composition is altered in active vs non-active IBD

Gut microbiota can influence the development of IBD

IBD changes when targeting microbiota

Therapeutic microbiota modulation

Diet & nutritional support

- Caloric amount, minerals, vitamins
- Diet composition (fibers/high glicemic index/saturated fatty acids…)

Removal of predisposing conditions

- Treat diabetes, endocrine, other motility disorders..
- Surgery or prokinetics when indicated

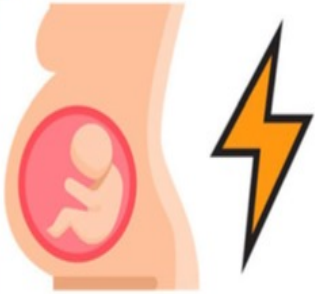
Therapeutic interventions

- Antibiotics
- Prebiotics, probiotics, postbiotics, symbiotics
- Fecal Microbiota Transplantation

Designing strategies for reconfiguring homeostasis

Under construction: More work needed in these areas:

Preventions

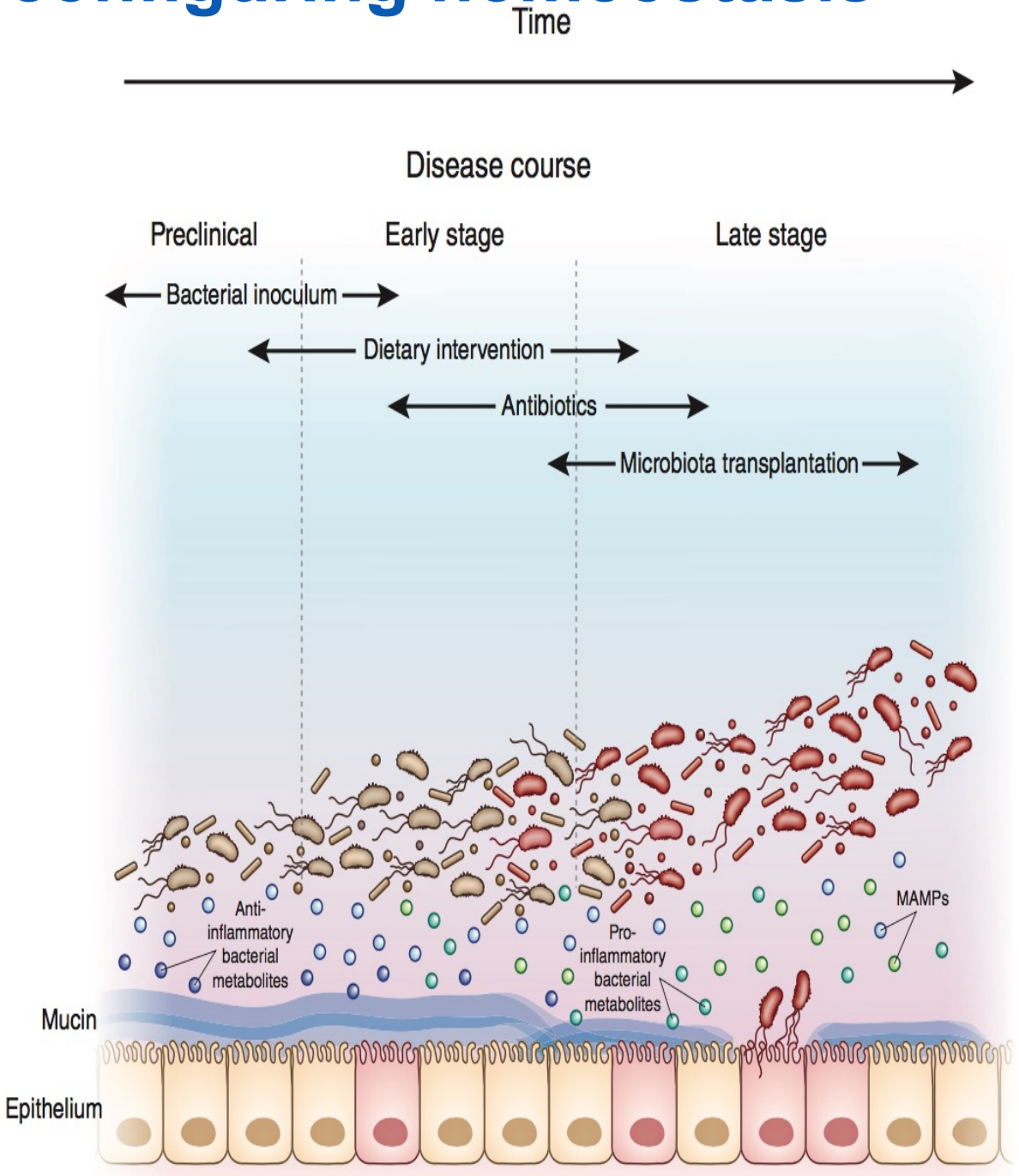


Effects of pre-conception and prenatal stress on the developing microbiome

How do living conditions, e.g. geography, density, and poverty influence the developing microbiome in ways that influence cognitive and affective health?

Interventions

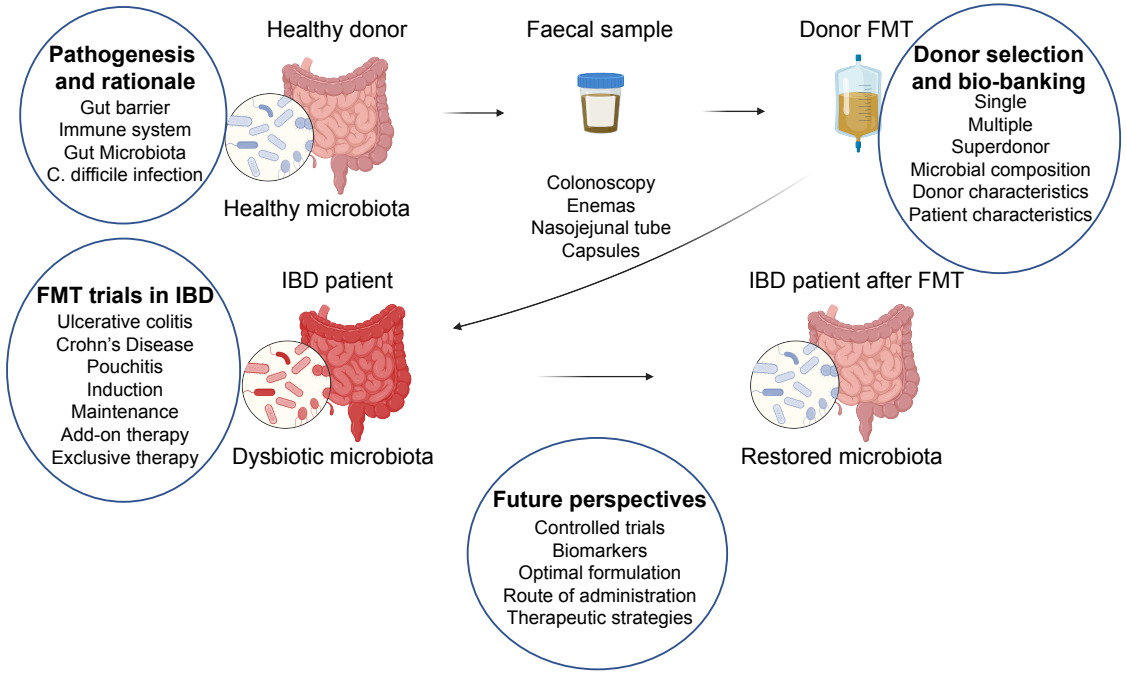
Testing for nutritional and probiotic/ prebiotic/antibiotic interventions for neurocognitive development, mental health, and early life stress



Vogel et al 2020
Tamburini et al 2016

The first international ROME consensus conference on gut microbiota and faecal microbiota transplantation (FMT) in inflammatory bowel disease (IBD).

Loris Riccardo Lopetuso[1,18,19#], Sara Deleu[2#], Lihi Godny[3], Valentina Petito[1], Pierluigi Puca[1], Federica Facciotti[4], Harry Sokol[5], Gianluca Ianiro[6], Luca Masucci[7], Maria T. Abreu[8], Iris Dotan[3], Samuel Costello[9], Ailsa Hart[10], Tariq Iqbal[11], Sudarshan Paramsothy[12], Maurizio Sanguinetti[7], Silvio Danese[13], Herbert Tilg[14], Fabio Cominelli[15], Theresa T. Pizarro[16], Alessandro Armuzzi[17], Giovanni Cammarota[6], Antonio Gasbarrini[6], Severine Vermeire[2*], Franco Scaldaferri[1*]



Pathogenesis and Rationale	
A1	The precise etiology of IBD is currently unknown; however, its pathogenesis is multifactorial, influenced by genetic susceptibility, host mucosal immune responses and the environment, including diet and the gut microbiome.
A2	Alterations in composition, relative abundance, diversity and function of gut microbiota (*i.e.*, dysbiosis) are implicated in the initiation and progression of IBD.
A3	The gut microbiome of CD and UC patients is particularly deficient in *Faecalibacterium prausnitzii*, which is recognized for its potential anti-inflammatory properties.
A4	The risk of *Clostridioides difficile* infection in IBD patients is higher than in the general population.
Donor Selection and Biobanking	
B1	Suitable donors for experimental FMT in IBD should submit to blood, as well as stool, testing in agreement with national and international guidelines, currently available for the treatment of *C. difficile* infection by FMT, and in general, for clinical practice.
B2	Stool donation should be voluntary, and donors should be notified as to the potential risks and/or benefits of donating. Moreover, written informed consent must be provided by each patient.
B3	Donors can also be managed through stool banks for experimental use, in agreement with national and international guidelines and regulations available for *C. difficile* infection and, in general, for clinical practice.
B4	Donor faeces should preferably be collected on site at a stool bank or at the site where the experimental procedure is performed, following national and international guidelines and regulations.
B5	Each donor can be enrolled to contribute different preparations of FMT, in agreement with the experimental protocol.
B6	A registry of donor information should be maintained and stored, in agreement with national and international guidelines and regulations.
B7	Patients should not have direct access to stool banks for the treatment of IBD. Provision of FMT samples should always be under the guidance of a treating healthcare provider, in agreement with national and international guidelines and regulations.
B8	Clear traceability should be available regarding the complete process of FMT, from faeces collection to FMT sample administration. Therefore, aliquots of each FMT sample should be retained for testing in case of unexpected adverse events.
B9	A common agreement regarding the technical aspects of donor FMT preparation will help to provide procedure standardization and optimization worldwide, facilitating interpretation of results.
B10	Research is needed to define donor characteristics associated with better clinical response rate and overall outcomes of FMT as a therapeutic option for IBD.
FMT Trials in IBD	
C1	Previously performed RCTs are, in general, small and methodologically heterogeneous; thus, definitive conclusions cannot be drawn at the present time.
C2	FMT is recommended as a treatment option for both mild and severe recurrent or refractory CDI in IBD patients.
C3	FMT may be effective in the induction of remission for mild to moderate UC.
C4	Currently, there is insufficient evidence to recommend FMT as a treatment for UC in routine clinical practice and its use should generally be limited to the research setting.
C5	RCTs suggest that UC patients who achieve remission following FMT generally do not have sustained remission beyond one-year post FMT treatment.
C6	Repeated infusions and donor-recipient engraftment are likely important for the therapeutic success of FMT in UC.
C7	An increase in the diversity of gut microbiome composition after FMT is likely a marker of response in UC.
C8	The available data indicate that FMT is low risk for the induction of remission in mild to moderate UC.
C9	RCTs have not demonstrated significant differences between FMT and control arms, in terms of disease worsening or symptoms attributable minor or serious adverse events.
C10	After FMT in UC, common adverse events are transient minor gastrointestinal symptoms, such as bloating, diarrhea and flatulence.
C11	There is insufficient evidence to recommend FMT as a treatment for CD in clinical practice. To date, its use should be limited to the research setting.
C12	There is insufficient evidence to recommend FMT as a treatment for pouchitis in clinical practice. To date, its use should be limited to the research setting.
C13	There is insufficient data on the safety of FMT in CD and pouchitis.
C14	Further research is needed to determine the efficacy and safety of FMT in Crohn's disease and pouchitis.
Future perspectives	
D1	Future research is needed to identify the optimal characteristics of both FMT donors and recipients in IBD.
D2	Controlled FMT trials are warranted in order to optimize efficacy in defined phenotypes of IBD.
D3	There is a need to identify biomarkers that predict response to FMT in IBD.
D4	Future research is required to determine the optimal formulation and route of administration for FMT-based therapy in IBD.
D5	Studies are needed to accumulate evidence-based information regarding the use of complementary strategies to improve FMT efficacy.
D6	Studies are required to assess the role of FMT as a stand-alone treatment for IBD or in combination with currently available treatment modalities.

Gut journal, *in press*