Crohn's and Colitis Canada
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Microbial tryptophan metabolism activates AhR and reduces colitis in humanized and gnotobiotic mice

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Diet and microbes in IBD management

- IBD treatment remains limited by high costs, loss of drug efficacy, and adverse effects.

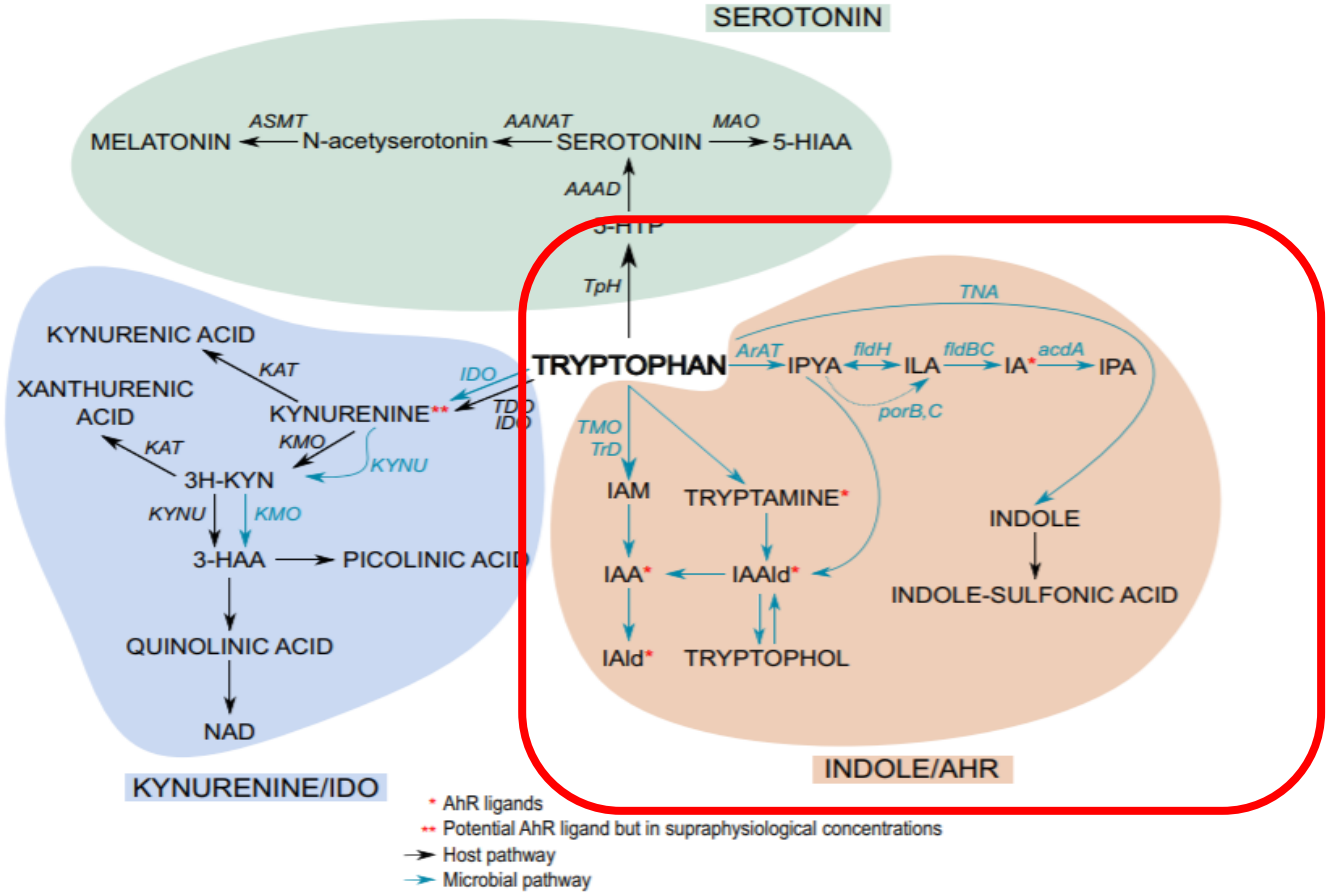
- Diet is an emerging area of clinical interest as a complement to pharmacological treatments, but how dietary components interact with the microbiota to influence disease is not clear.

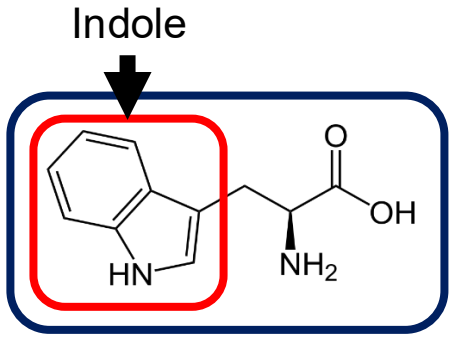
- Gut microbes transform dietary components into bioactive metabolites that modulate gut immunity – including **tryptophan-derived aryl hydrocarbon receptor (AhR) ligands**

Fitzpatrick JA et al. *Nature Reviews Gastroenterology and Heptatology* (2022).

Gut microbe tryptophan metabolism produces AhR ligands

- Tryptophan is an essential amino acid found in protein-rich foods and is metabolized by gut microbes to produce AhR ligands





Tryptophan

Tryptamine – *Clostridium sporogenes, Ruminococcus gnavus*

Indoleacetic acid – *Bifidobacterium longum*

Indolepropionic acid – *Peptostreptococcus stomatis, C. sporogenes*

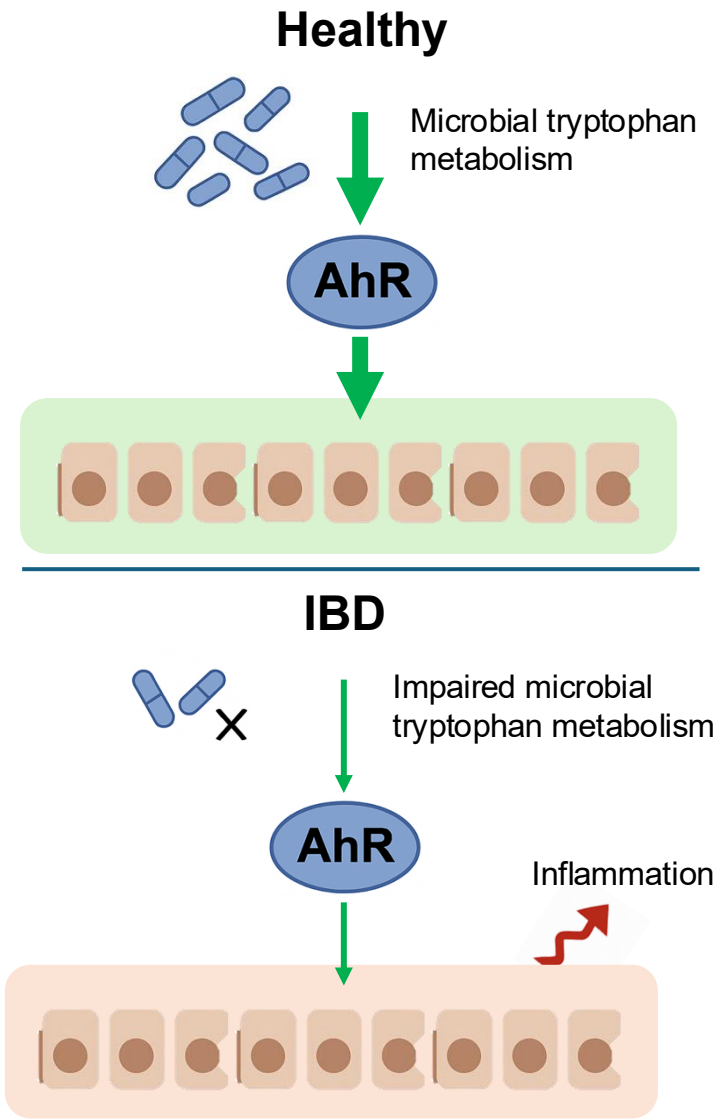
Indolealdehyde - *Lactobacillus reuteri, C. sporogenes*

Agus, Planchais & Sokol. *Cell Host Microbe*. (2018).
Roager & Licht. *Nature Communications*. (2018)

Dodd D et al. *Nature* (2017).
Strickland LH. *Biochem J* (1934).

AhR senses microbial tryptophan metabolites to maintain gut immune balance—but is impaired in IBD

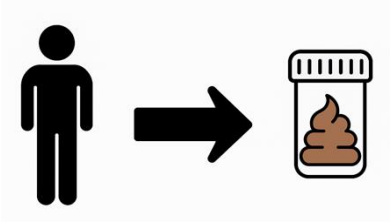
- Ligand activated transcription factor in intestinal epithelial and immune cells
- Activated by microbial tryptophan metabolites → stimulates IL-22 production, **promoting mucosal healing and barrier function**
- **In IBD**:
  - Lower expression of AhR in intestinal tissue
  - Decreased fecal AhR ligands
  - Reduced microbial genes for tryptophan metabolism



Lamas B et al. *Nature Medicine*. (2016). Monteleone I et al. *Gastroenterology* (2011). Franzosa EA et al. *Nature Microbiology* (2019).

What is the role of the microbiota in AhR activation in IBD?

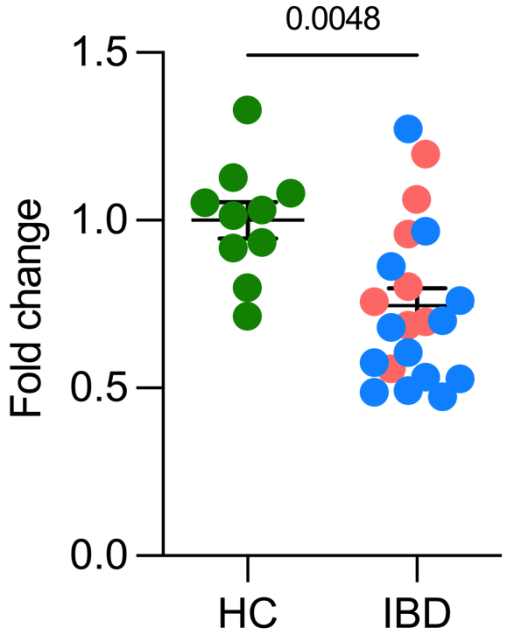
Tryptophan metabolism is reduced in IBD



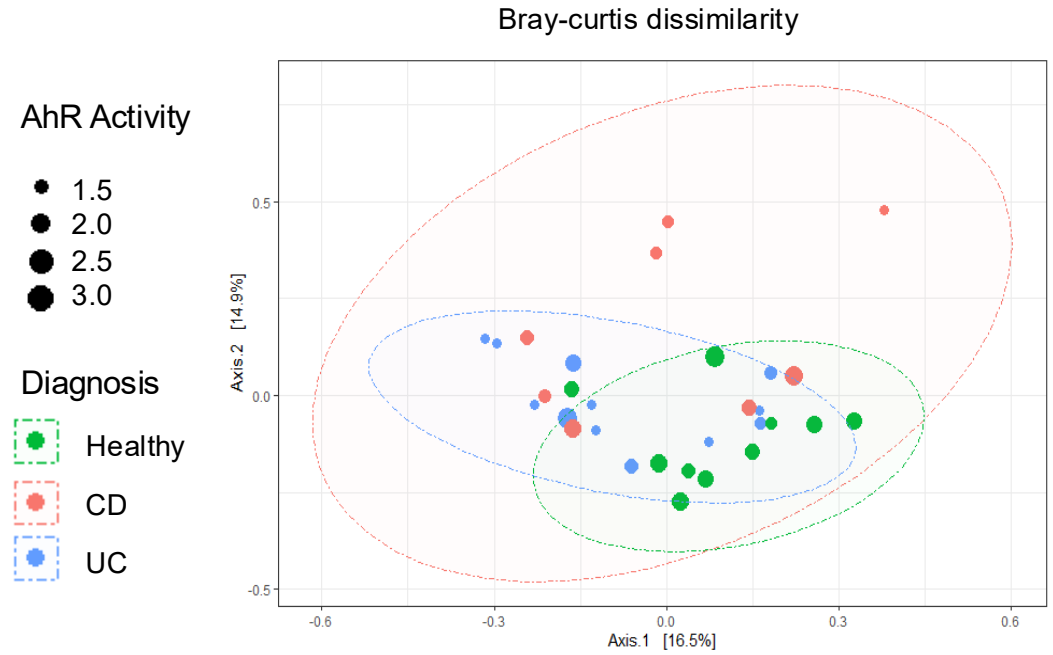
Diagnosis

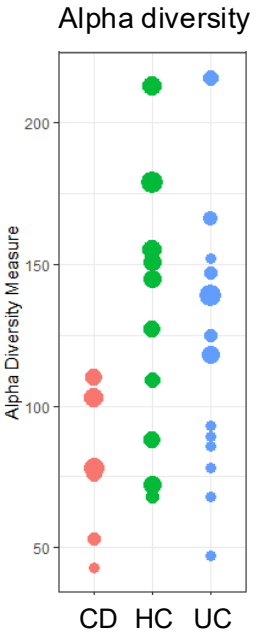
- Healthy n=10
- CD n= 8
- UC n=13

AhR activation (feces)

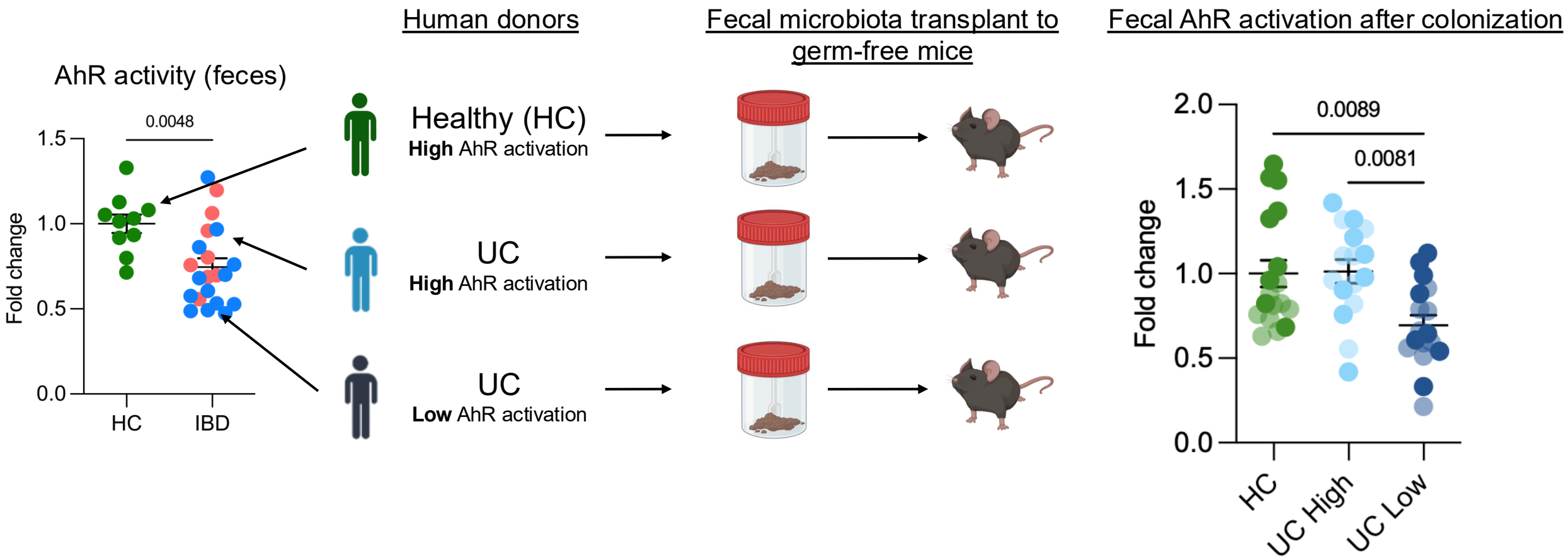


Human microbiota





Human AhR activation is microbially-driven



AhR activation phenotype transferred from human → mouse

What is the association between AhR activation and colitis severity in vivo?

Mouse AhR activation is microbially-driven and associated with colitis severity

Conventional (SPF) microbiota

Minimal Microbiota (10-12 strains)

AhR characterization →

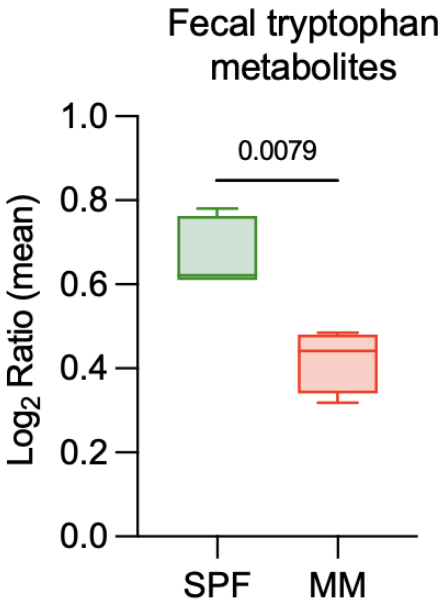
High production of AhR agonists

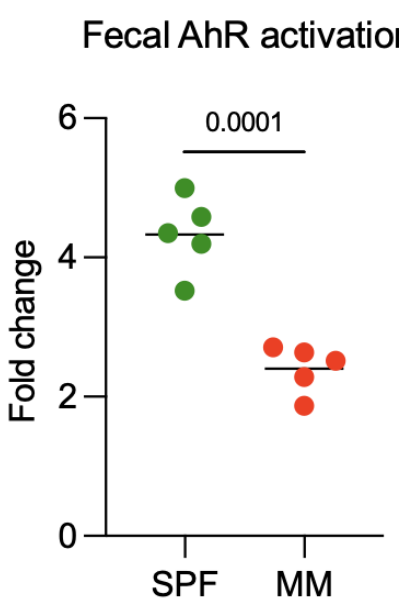
Impaired production AhR agonists

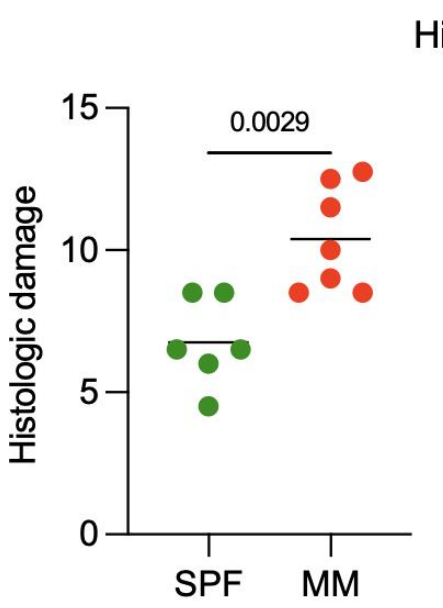
DSS colitis (1 week) →

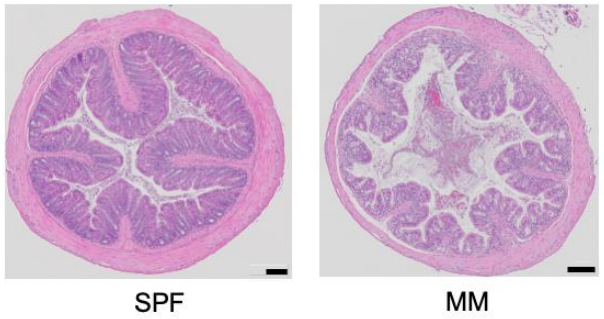
Mild colitis

Severe colitis









Can this pathway be modulated with diet to reduce colitis severity?

Dietary tryptophan is efficiently metabolized by microbiota and activates AhR

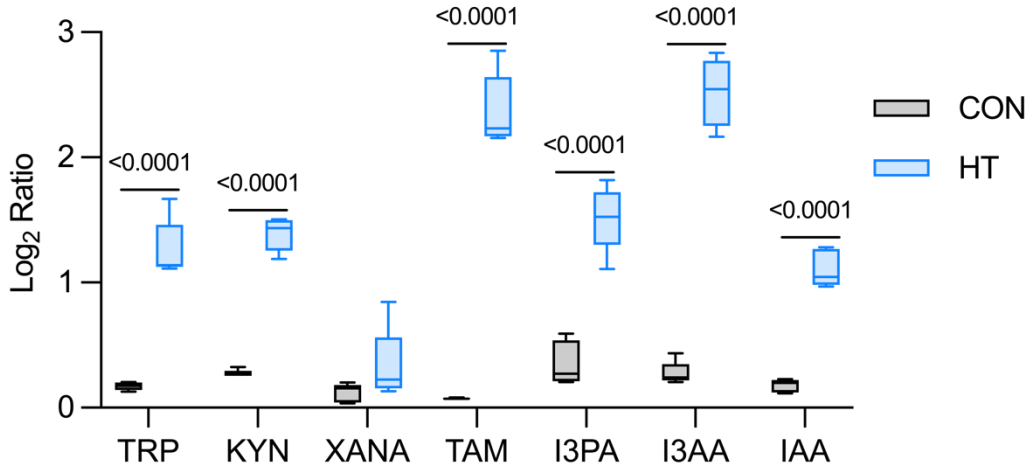
Conventional microbiota mice

Protocol

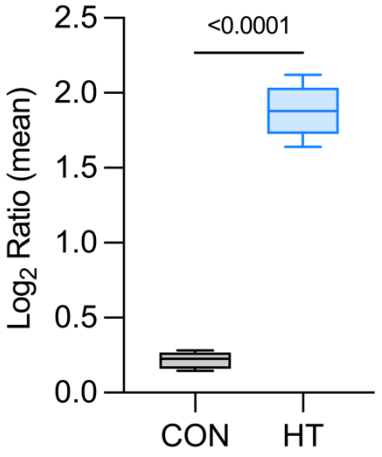
3 weeks

Control (CON) or **High (HT)** tryptophan diet

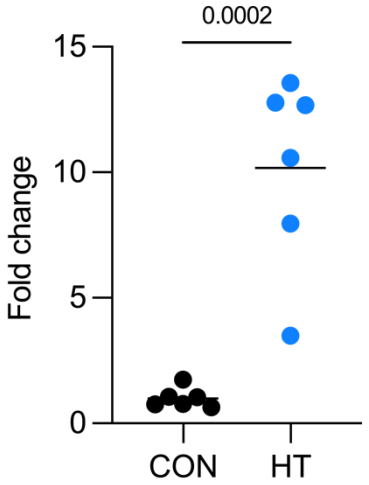
Tryptophan metabolites



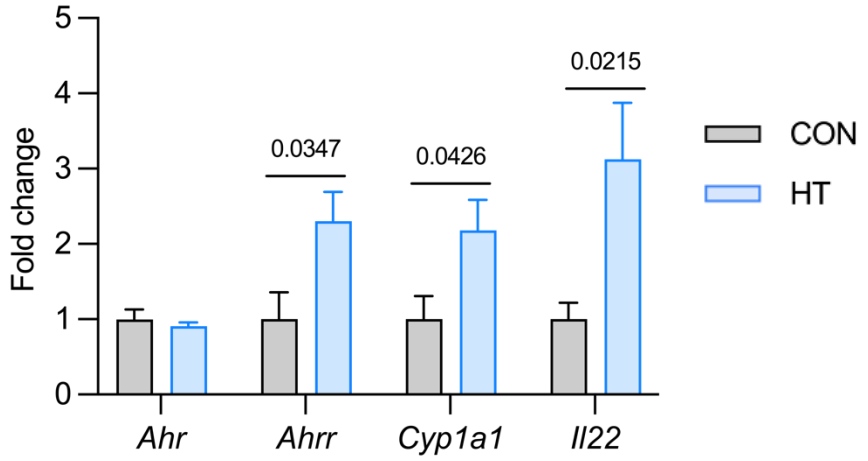
Microbial tryptophan metabolites



AhR activity (feces)



Gene expression



Dietary tryptophan reduces colitis severity through AhR

Colitis Protocol

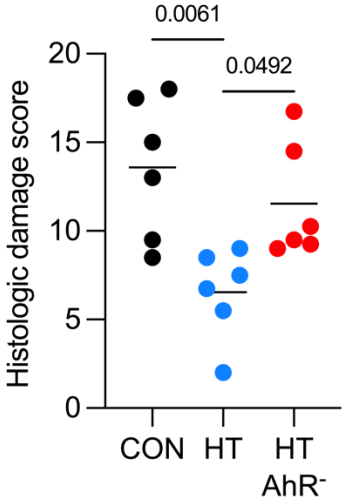
Conventional microbiota mice

AhR$^-$ (CH223191; 1x/day)

3 weeks	DSS (1wk)

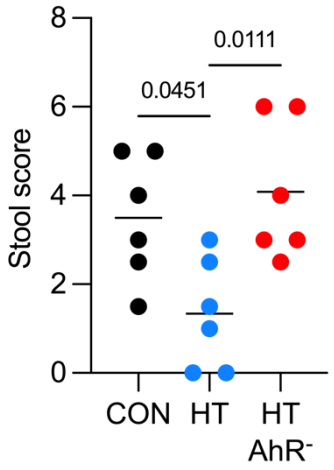
CON or **HT** diet

Histologic damage

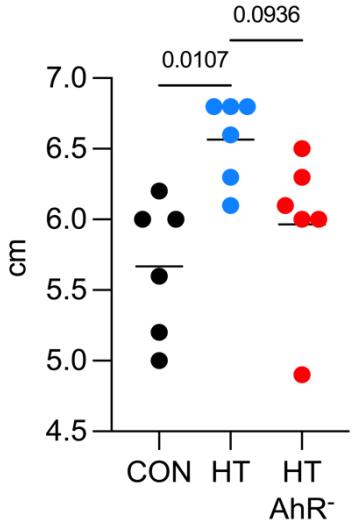


CON HT HT AhR$^-$

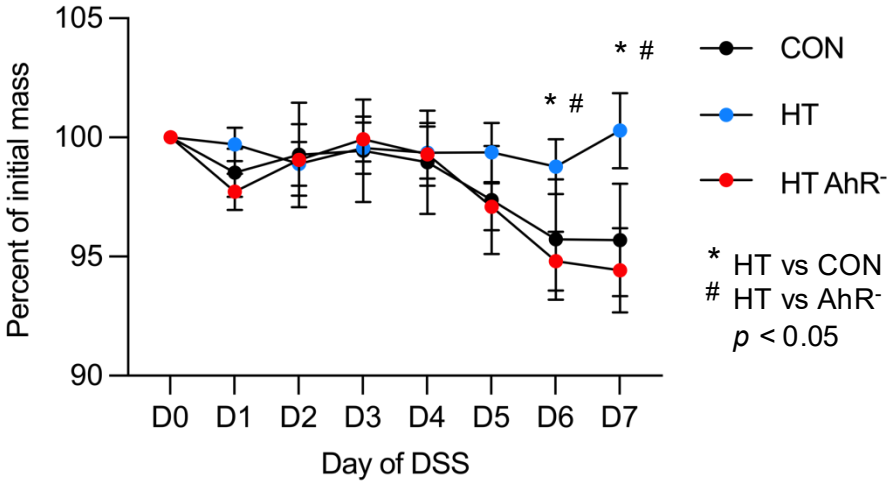
Stool score



Colon length

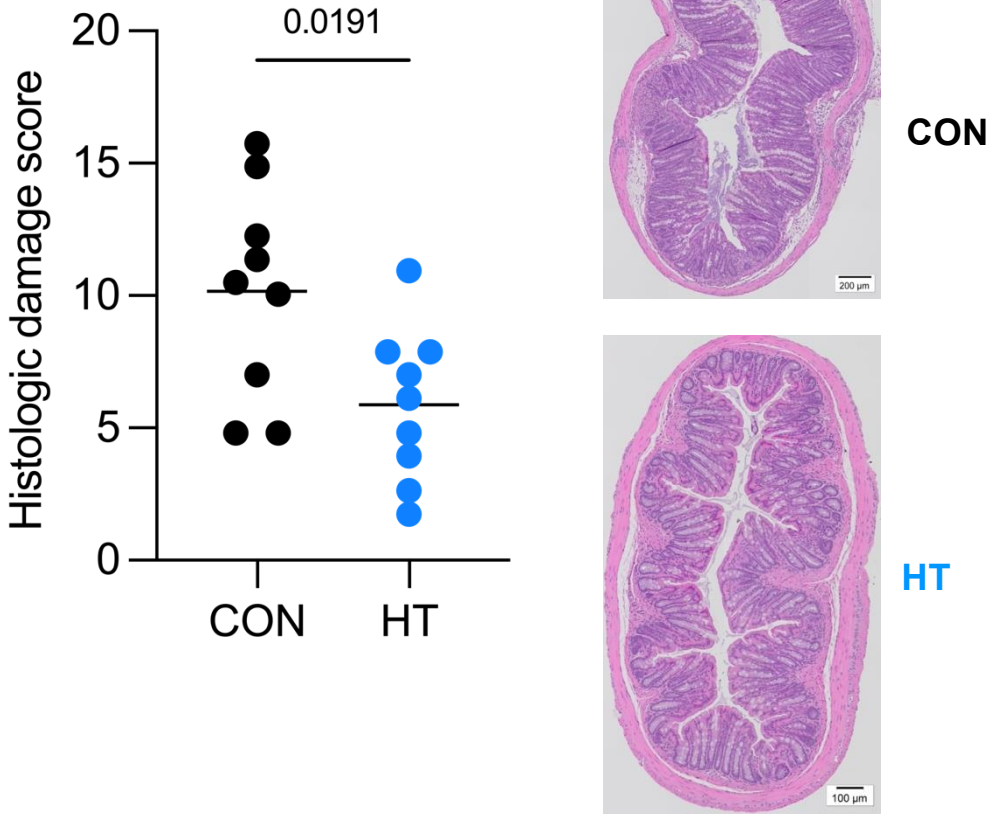


Weight loss

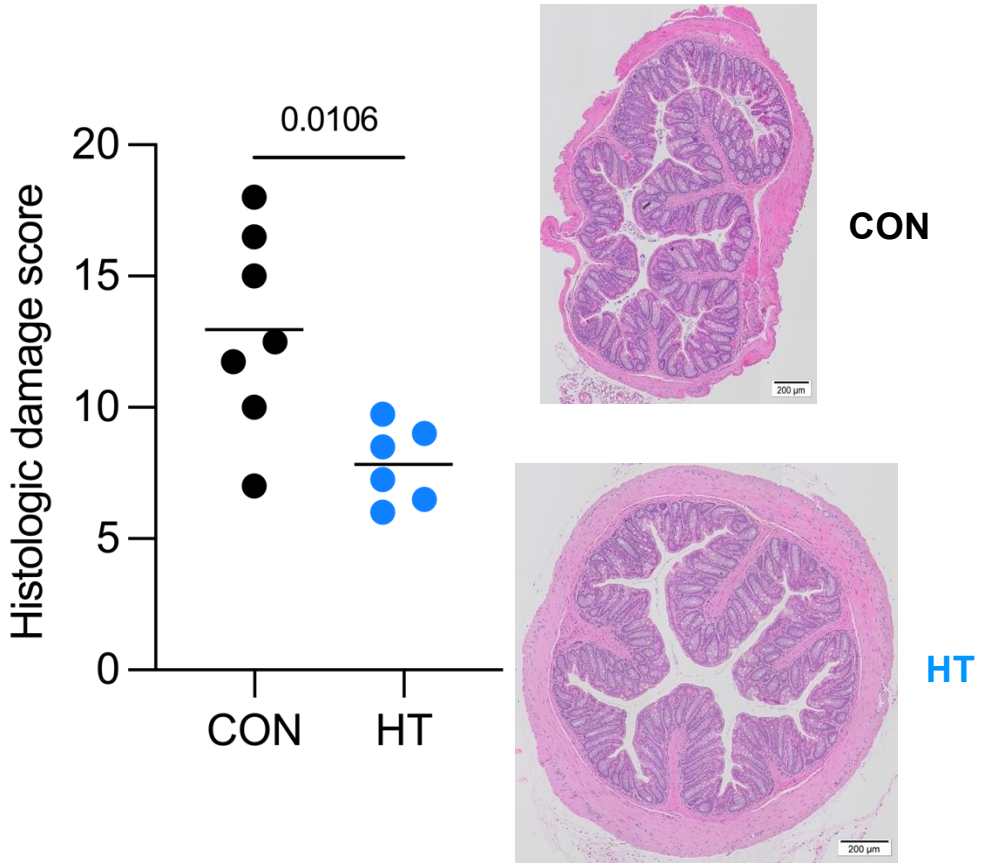


Dietary tryptophan reduces colitis severity through AhR

TNBS (2%)
Conventional microbiota



IL-10-deficient
Conventional microbiota



What about in the case of impaired tryptophan metabolism?

Dietary tryptophan and *C. sporogenes* boost tryptophan metabolism in mice with impaired metabolism

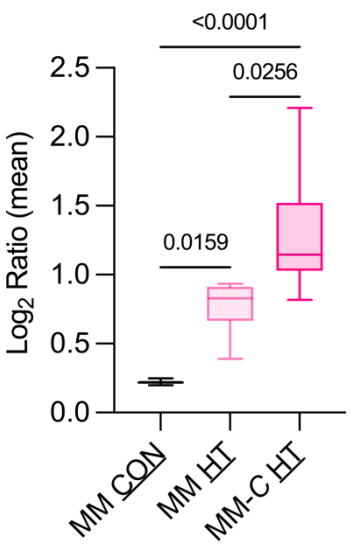
Protocol

Minimal microbiota

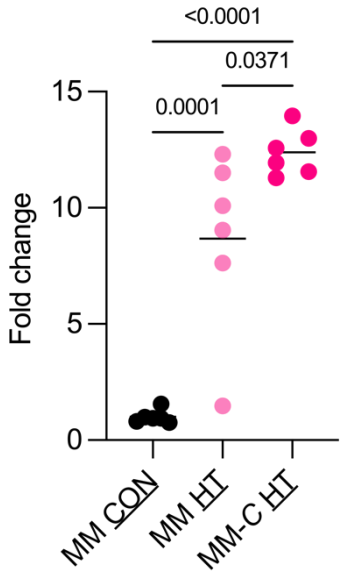
3 weeks

CON diet
HT diet
HT diet + *Clostridium sporogenes*

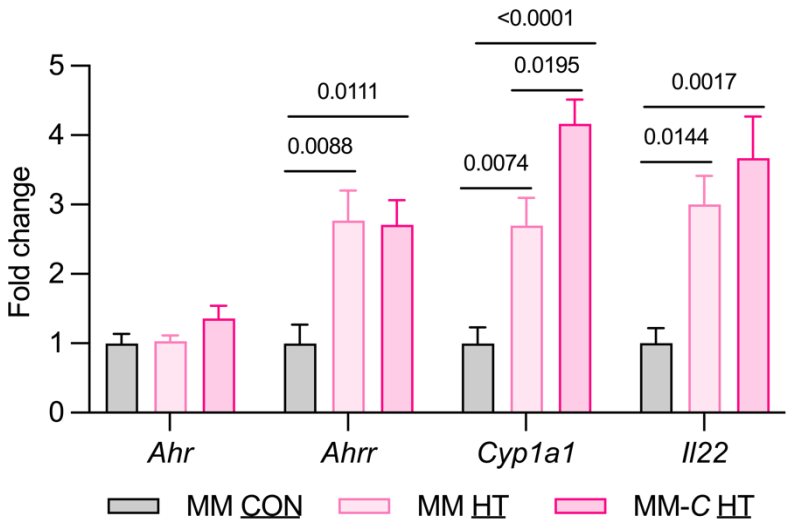
Microbial tryptophan metabolites



AhR activity (feces)



Gene expression



C. sporogenes enhances the protective effects of tryptophan during colitis

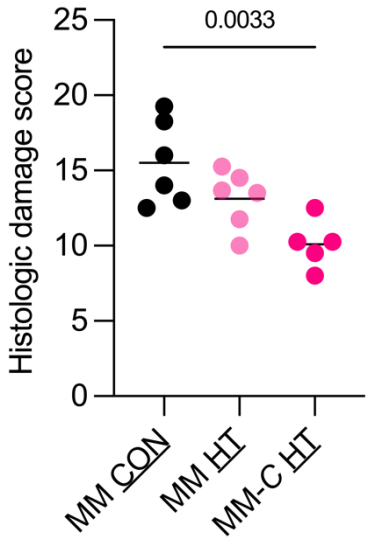
Protocol

Minimal microbiota

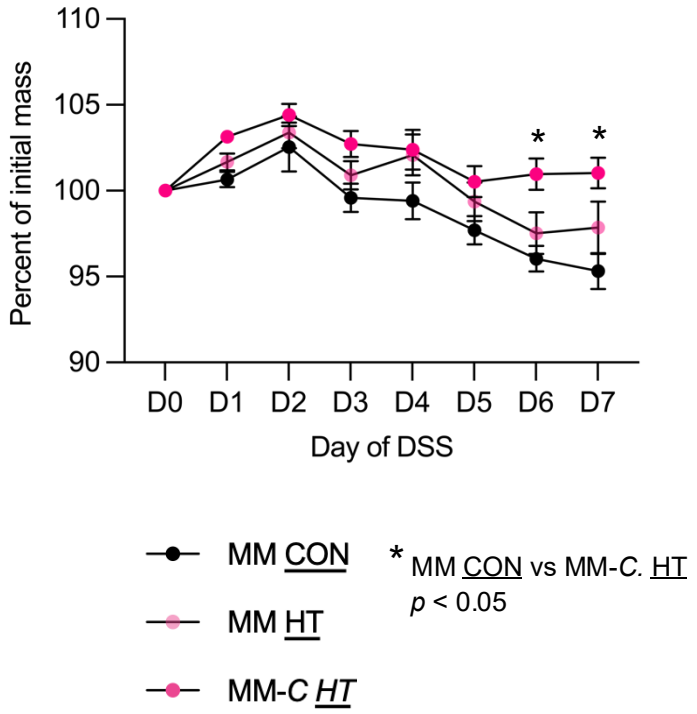
3 weeks | DSS colitis

CON diet
HT diet
HT diet + *Clostridium sporogenes*

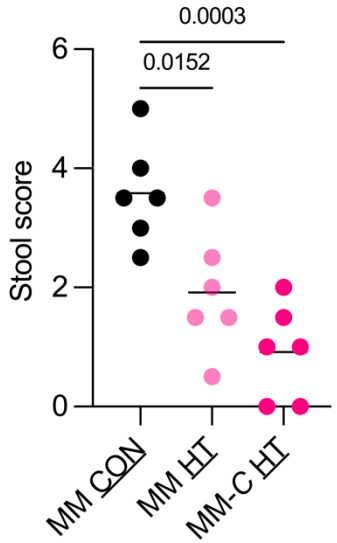
Histologic damage



Weight loss



Stool score



Tryptophan diet reduces colitis severity in UC-humanized mice

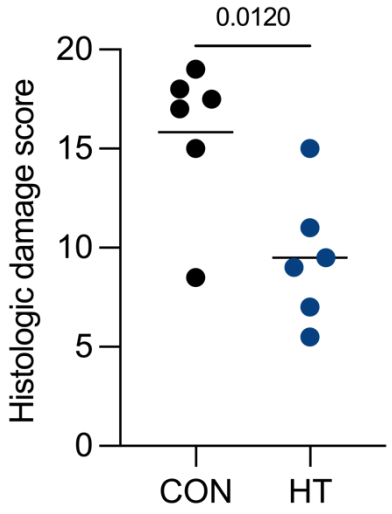
UC – Low AhR humanized mice

Colitis Protocol

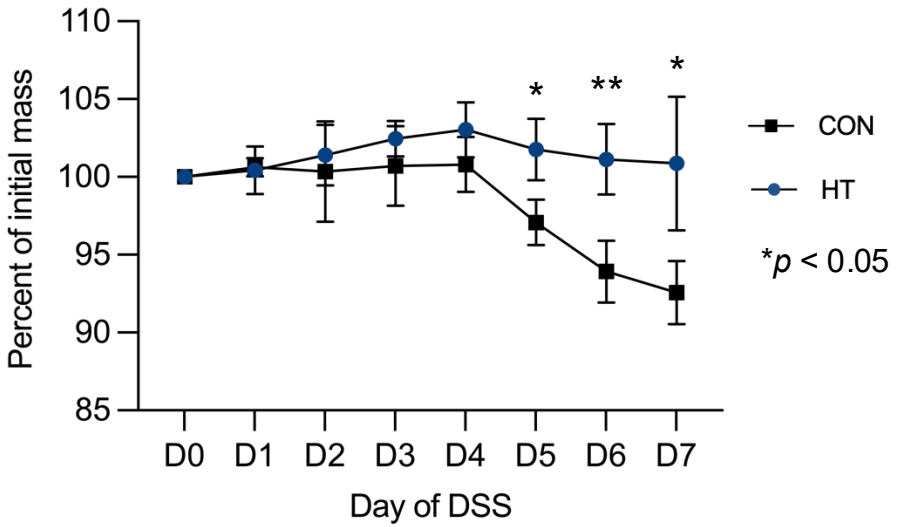
3 weeks	DSS

HT or **CON** diet

Histologic damage



Weight loss



CON

HT

Summary & Conclusions

- Microbial tryptophan metabolism is impaired in IBD and associated with worsened colitis severity in mouse models.
- Microbial tryptophan metabolism drives AhR activation and mucosal healing.
- Diet and microbial interventions can restore AhR function and reduce inflammation in mouse models.

Modulating microbial tryptophan metabolism through diet may represent a strategy to support mucosal healing and complement IBD therapies.

Thank you!

Dr. Alberto Caminero

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McMaster Genomics Facility
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TMIC The Metabolomics Innovation Centre

Crohn's and Colitis Canada
Crohn et Colite Canada

CIHR IRSC

Farncombe Family Digestive Health Research Institute

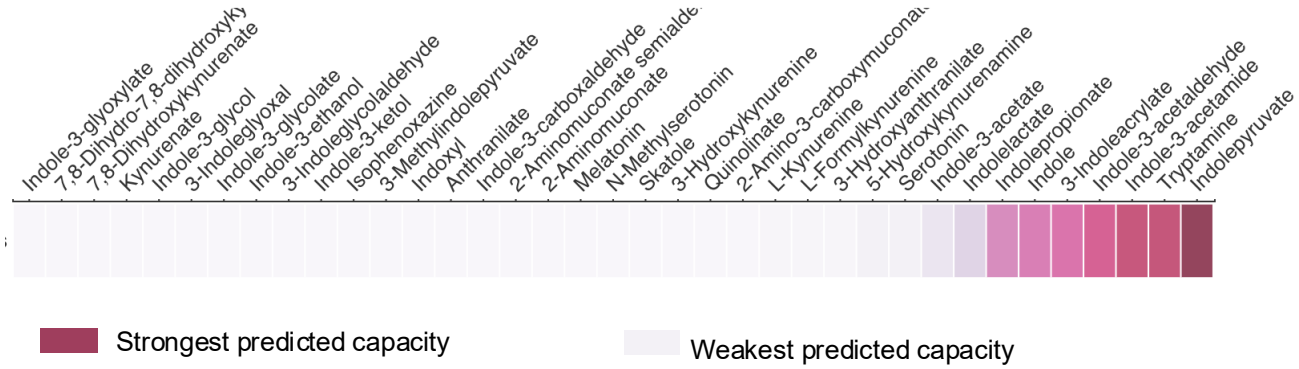
Farncombe Institute NUTRITION INITIATIVE

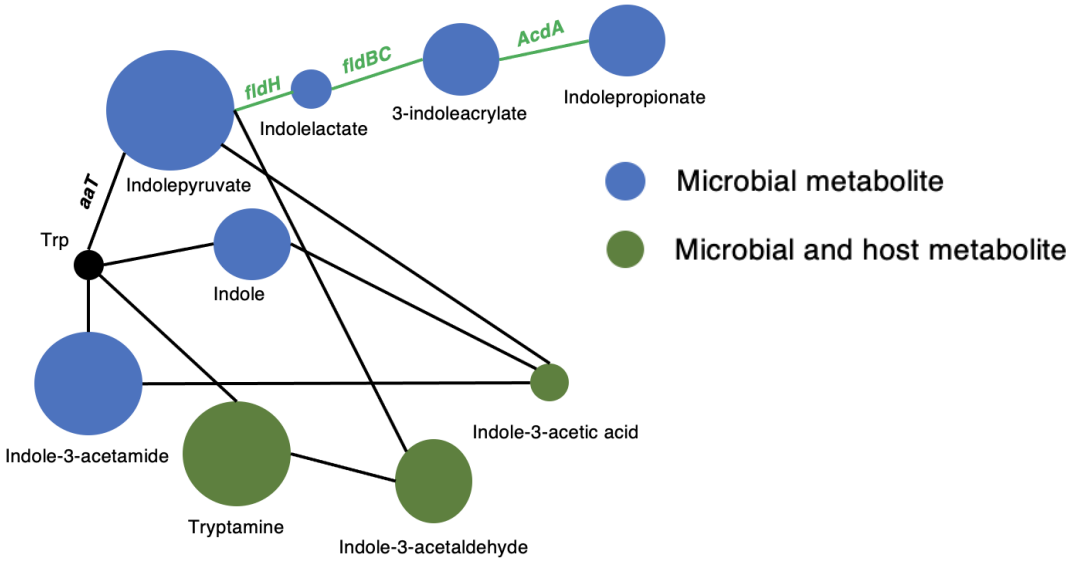
McMaster GENOMICS FACILITY

McMaster University

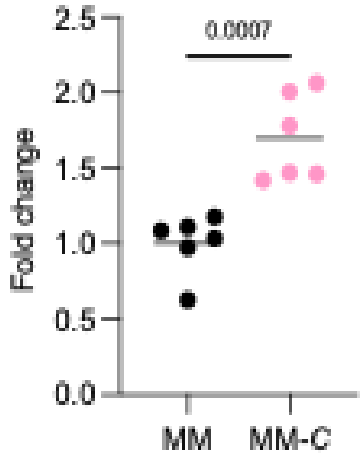
C. sporogenes isolate harbors tryptophan metabolism genes

C. sporogenes predicted tryptophan metabolism network





AhR activation (feces) post-colonization and pre-tryptophan diet

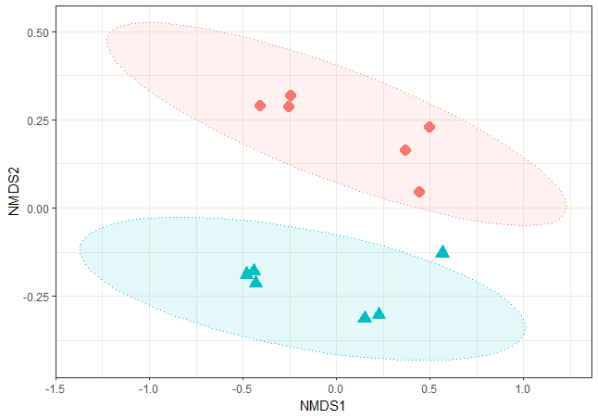


<u>Enzyme genes confirmed by whole-genome sequencing</u>

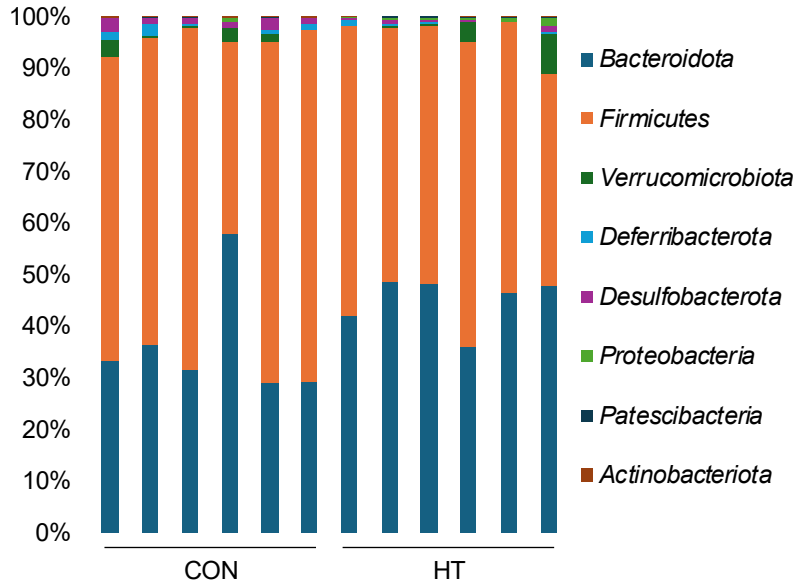
fldH: indolelactate dehydrogenase
fldBC: indolelactate dehydratase
AcdA: acylCoA dehydrogenase
TDC: tryptophan decarboxylase
aaT: aromatic amino transferase

Microbiota is altered by HT diet consumption

A Bray-curtis dissimilarity



B Phylum composition



C Differentially abundant genera