



# Identification of host-microbial interaction networks that mediate intestinal epithelial barrier maturation

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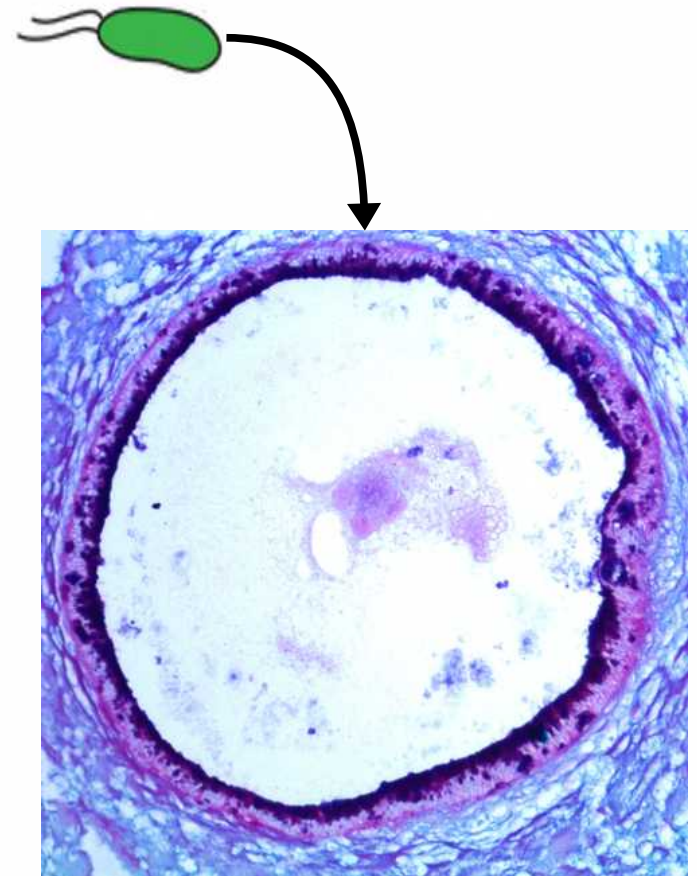
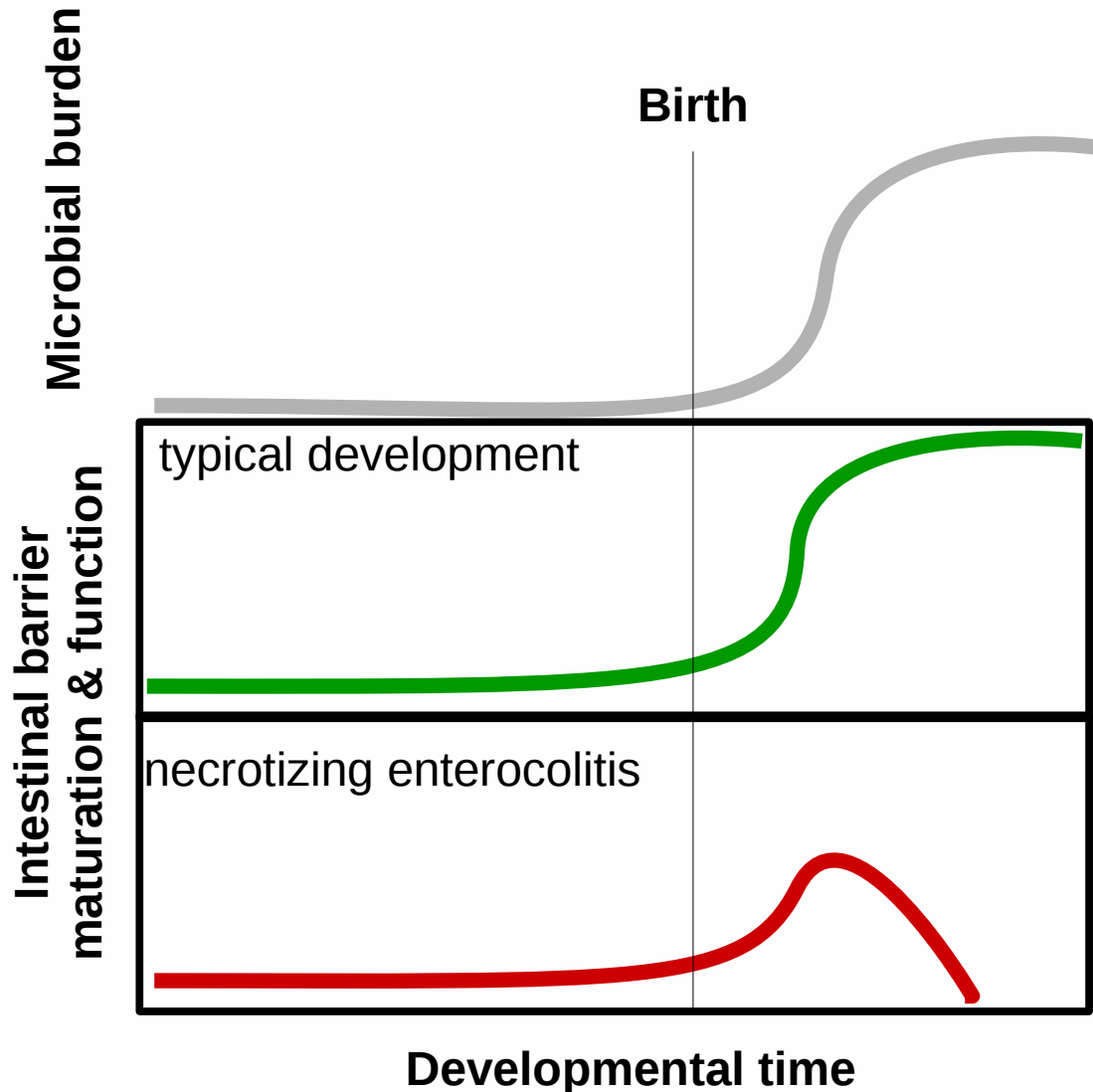
 hilldr

 @DRHill\_PhD

# *How is host-microbe mutualism in the gut established and maintained?*

*Multicellular life did not take over the globe by combat, but by networking*

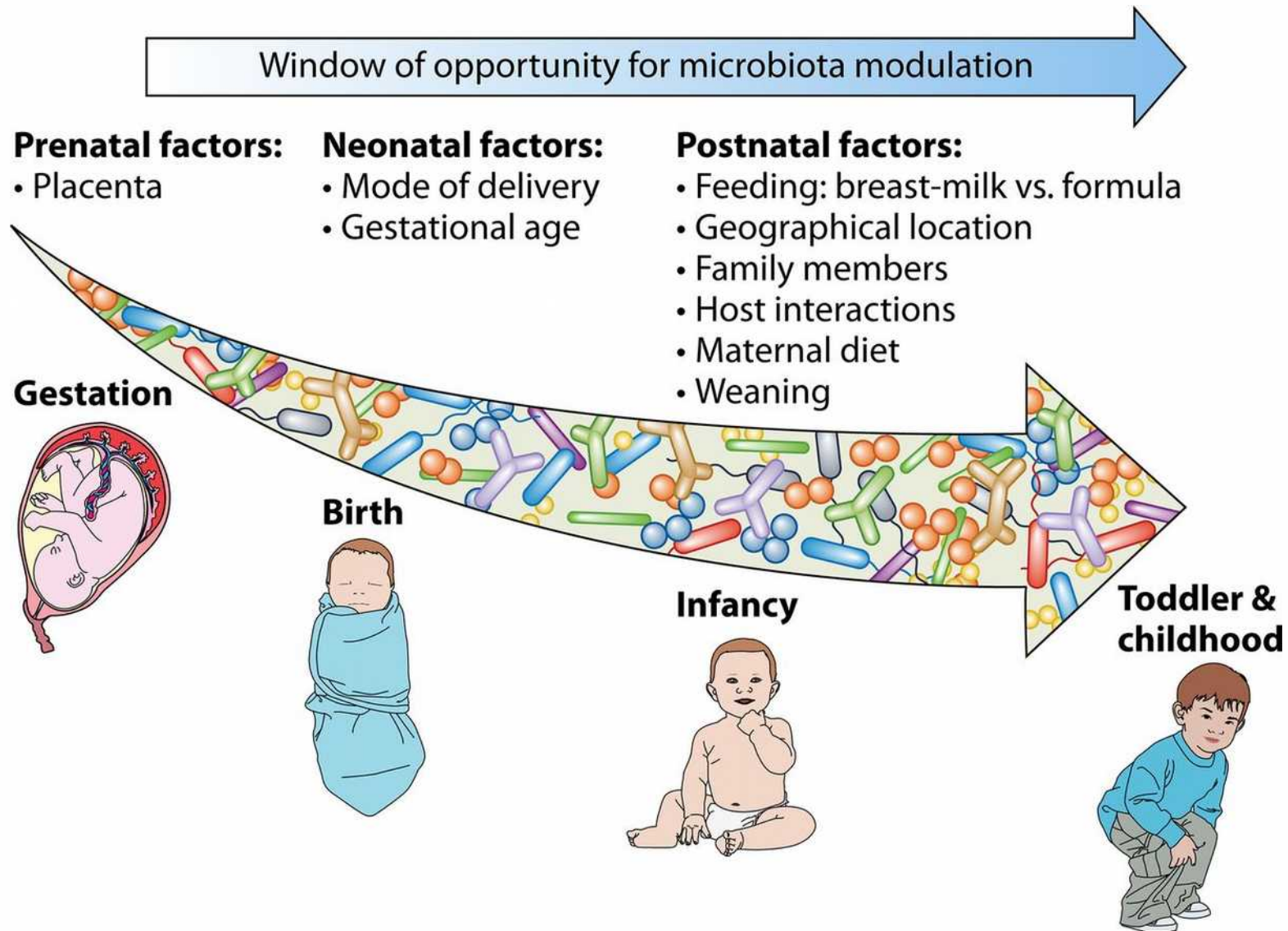
- Dr. Lynn Margulis



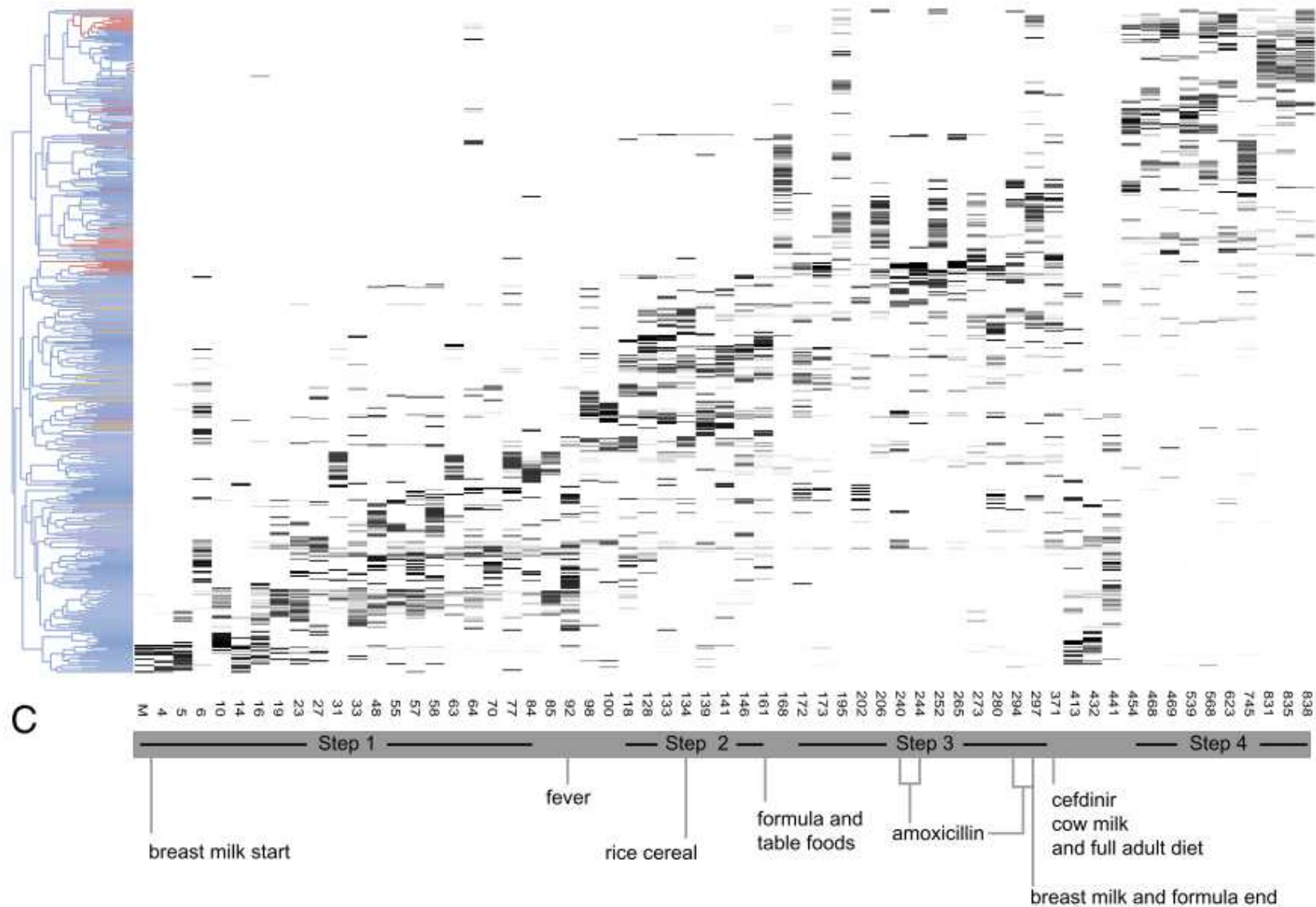
- ***Tissue maturation***
- ***Innate defense***
- ***Barrier resilience***



# Window of opportunity for microbiota modulation from gestation to childhood.



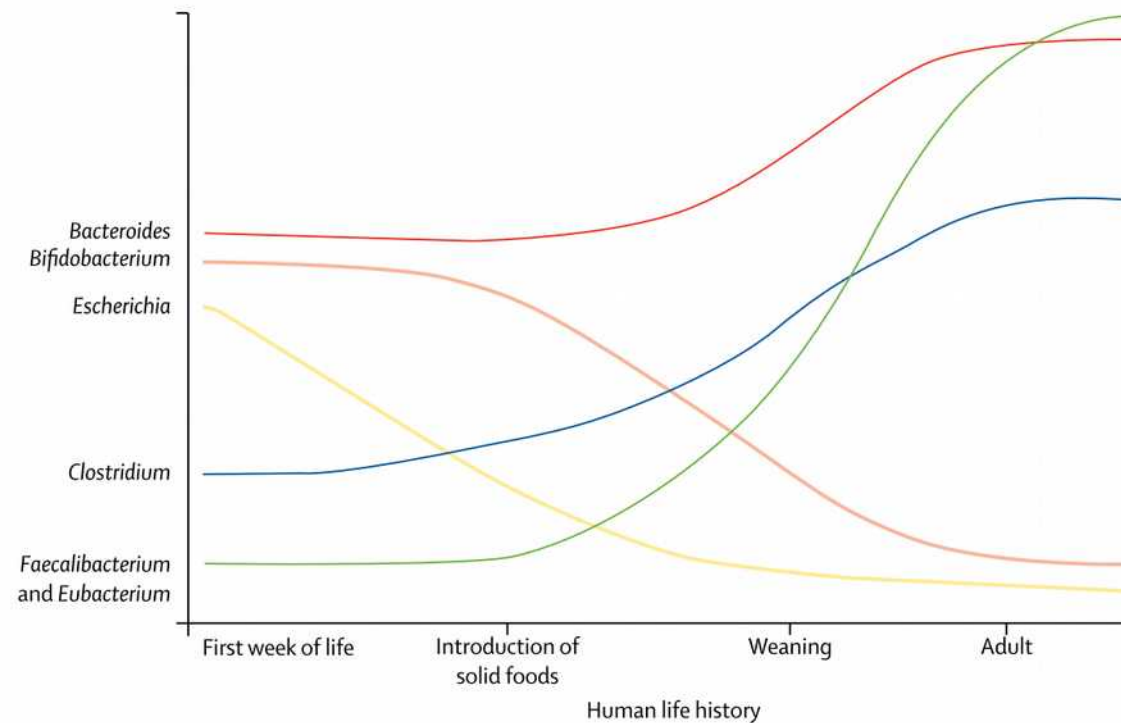
# Development of an intestinal microbial community



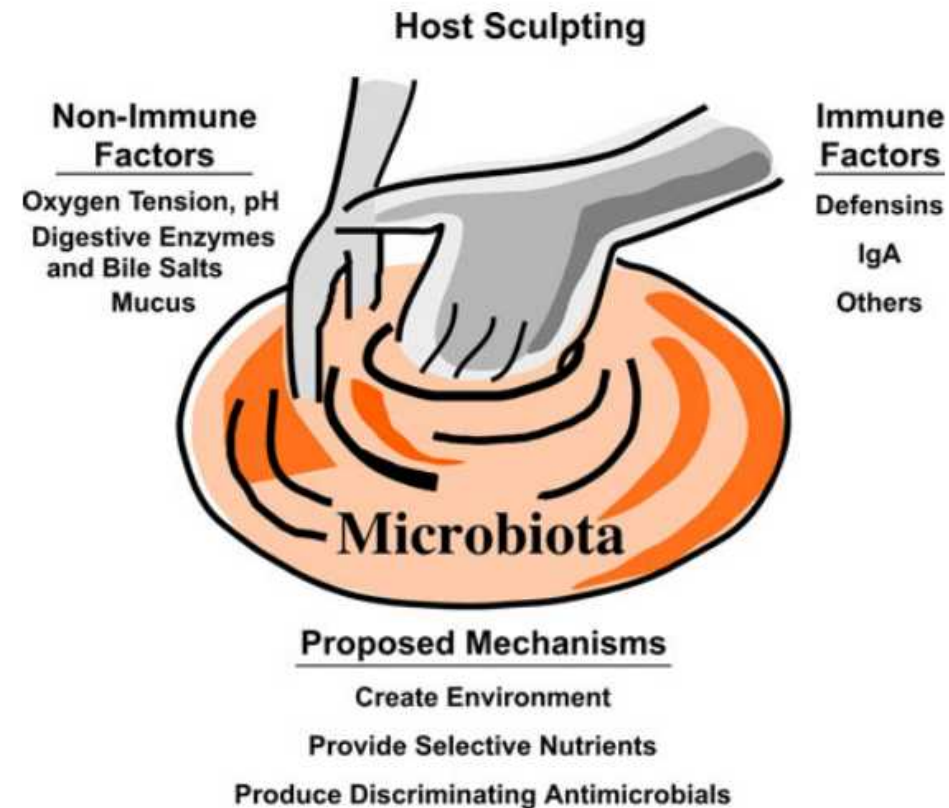
Koenig *et al. Proc Natl Acad Sci.* 2011; 108 Suppl 1:4578-85

# Bacterial succession and host sculpting shape the developing intestine and its microbiota

## Bacterial succession

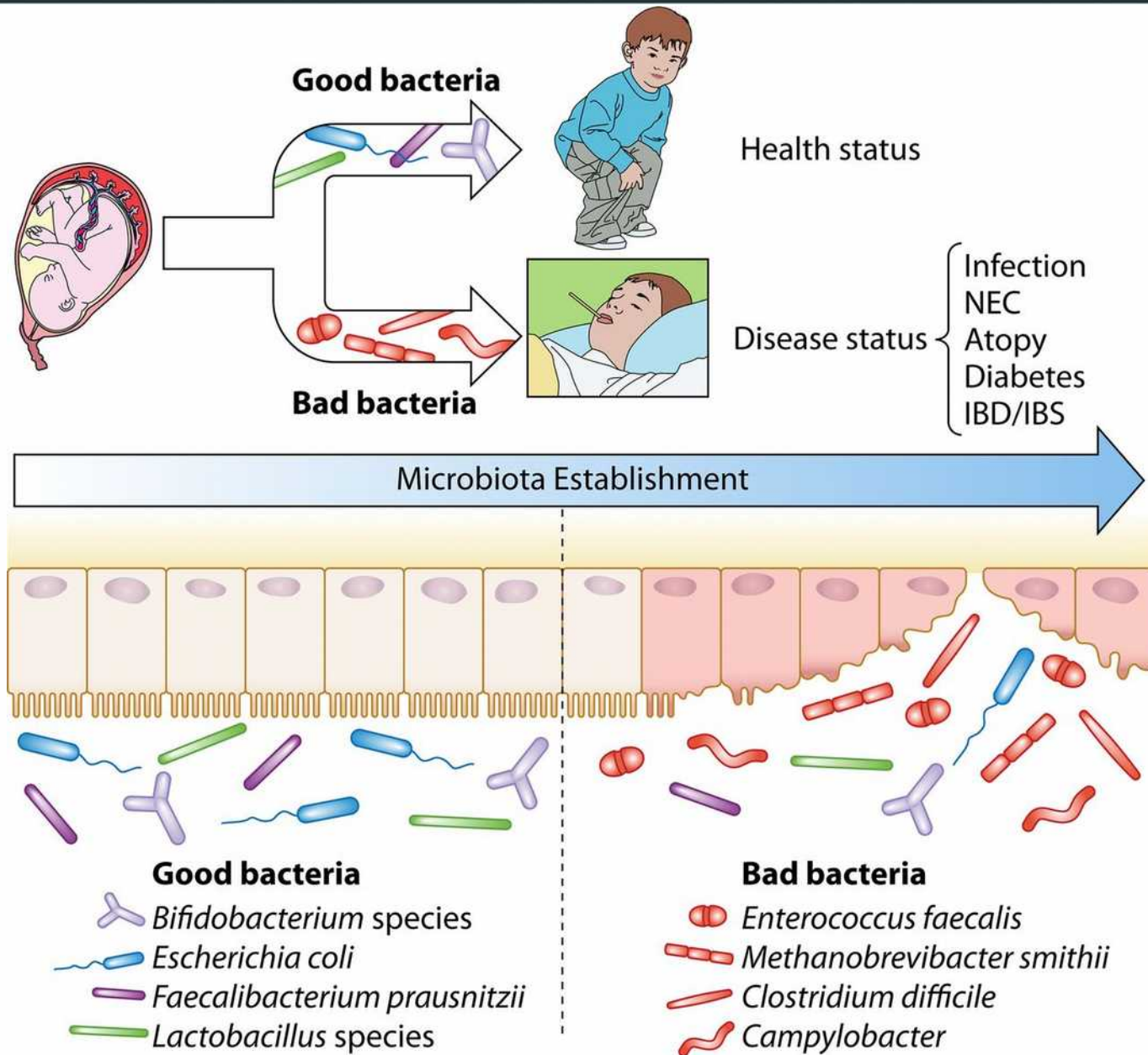


Rook et al 2017 *The Lancet*



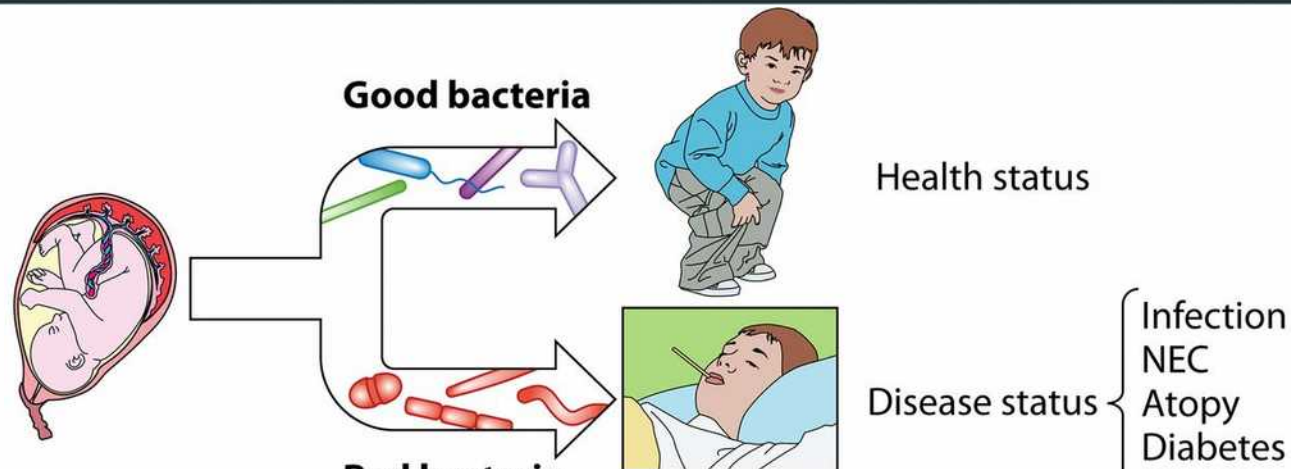
Bevins and Salzman 2011 *CMLS*

# Infant health status and microbiota establishment

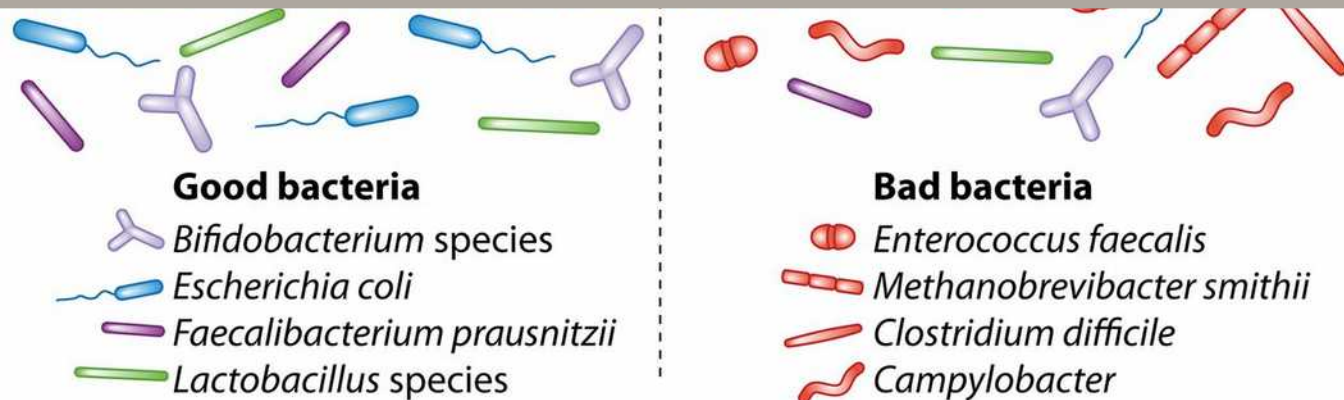




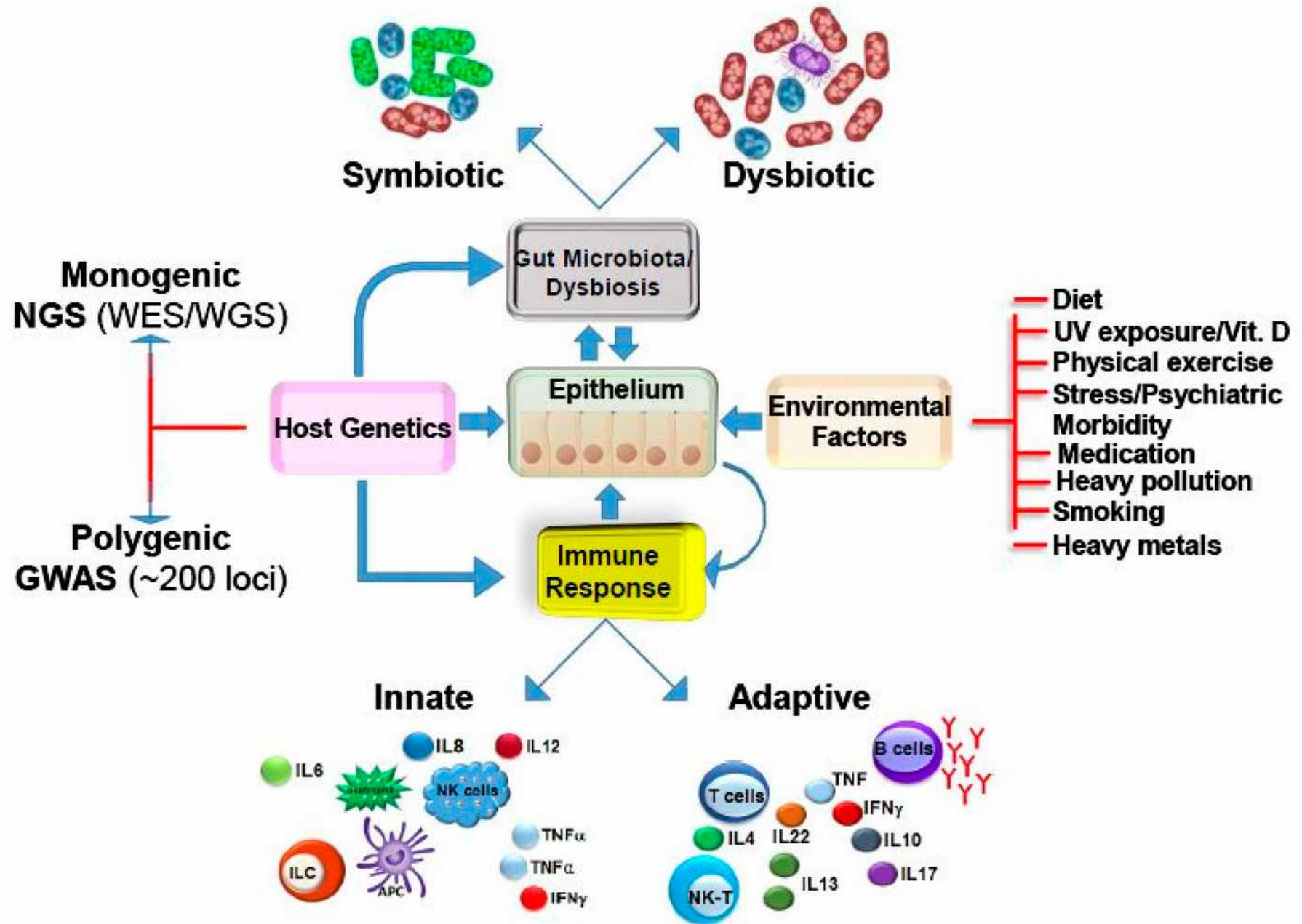
# Infant health status and microbiota establishment



How do differences in microbial colonization produce different health and developmental outcomes?

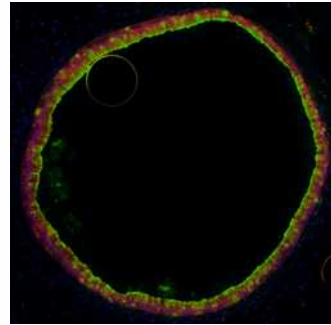
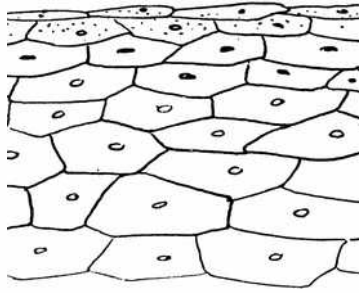


# Bacterial interactions with epithelium are central to understanding the mechanistic causes of microbiota-associated diseases

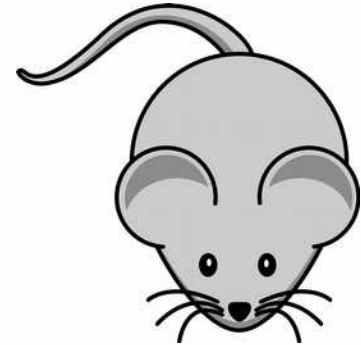
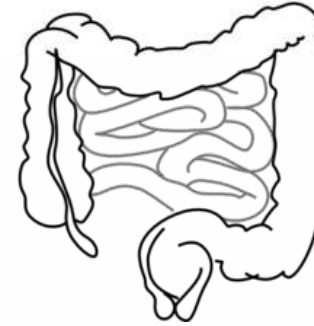




# Comparing intestinal model systems



increasing complexity



cell lines

polarized  
monolayers

organoids

tissue  
explants

animal  
models

*single cell type*

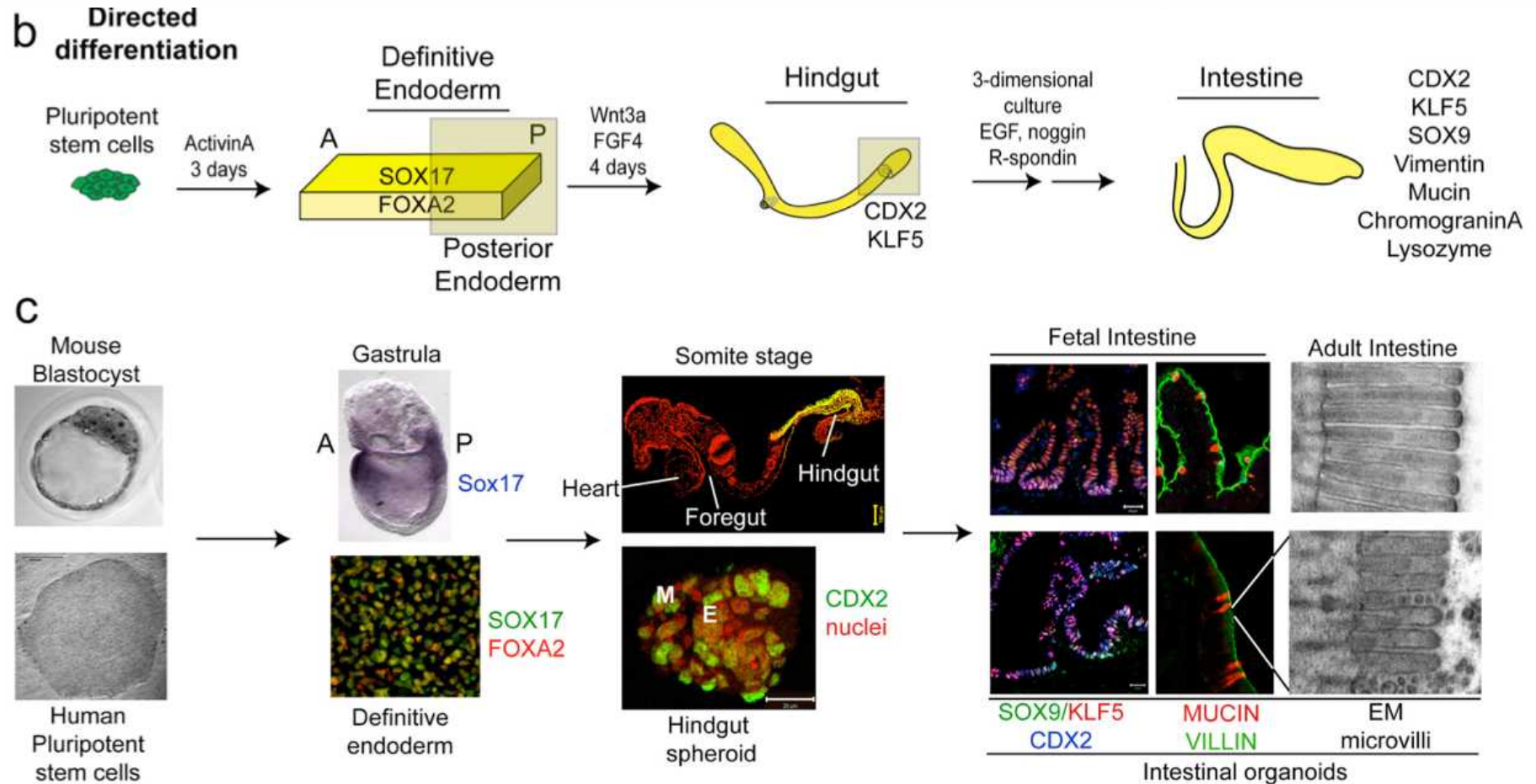
*multiple cell types  
(epithelium, goblet cells, Paneth cells, enteroendocrine cells)*

*no circulating immune cells*

*indigenous microbiota*

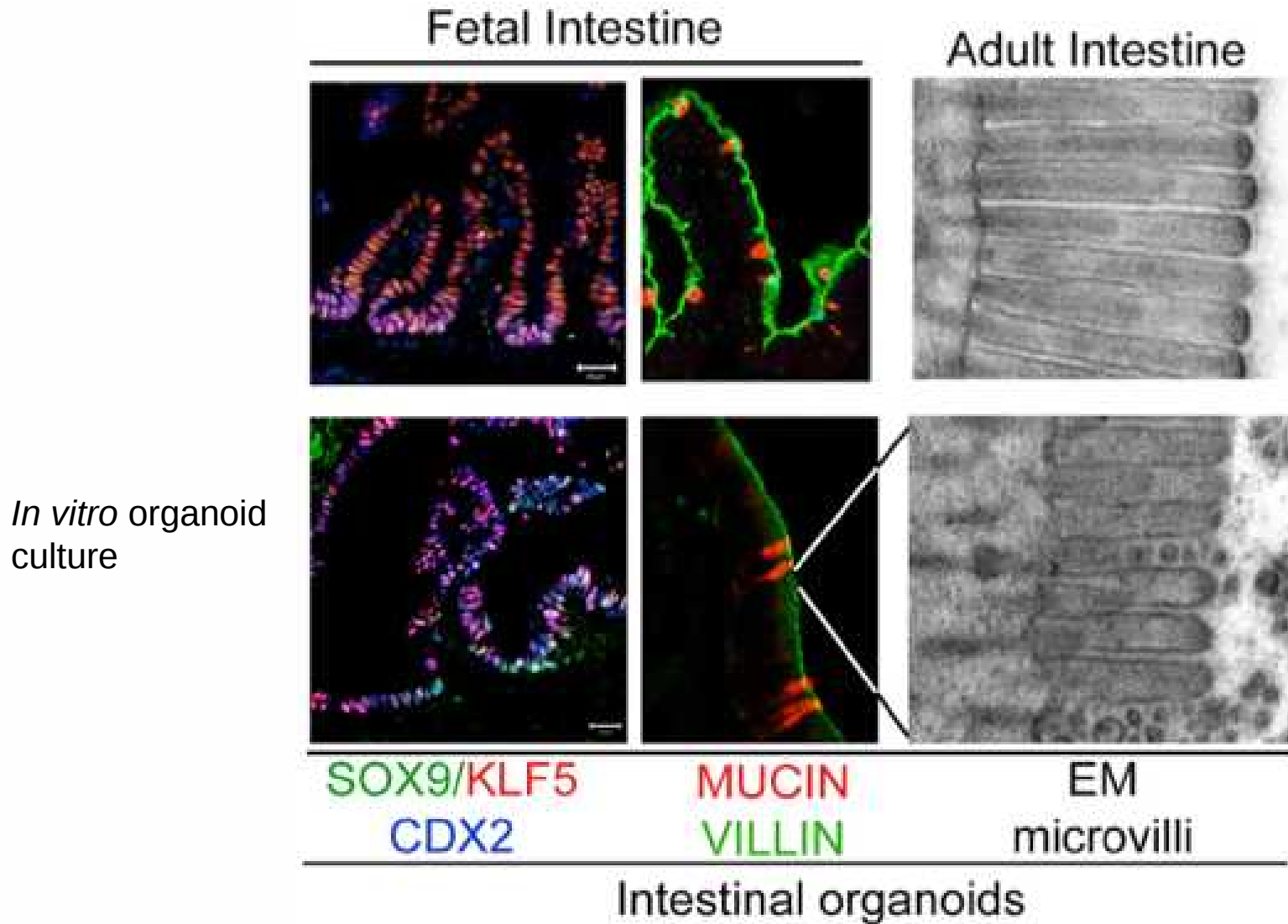
Young VB. Old and new models for studying host-microbe interactions in health and disease: *C. difficile* as an example. *Am J Physiol Gastrointest Liver Physiol*. 2017

# Directed differentiation of pluripotent stem cells into intestinal tissue



Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V. V, Wells, S.I., Zorn, A.M., et al. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* 470, 105–109.

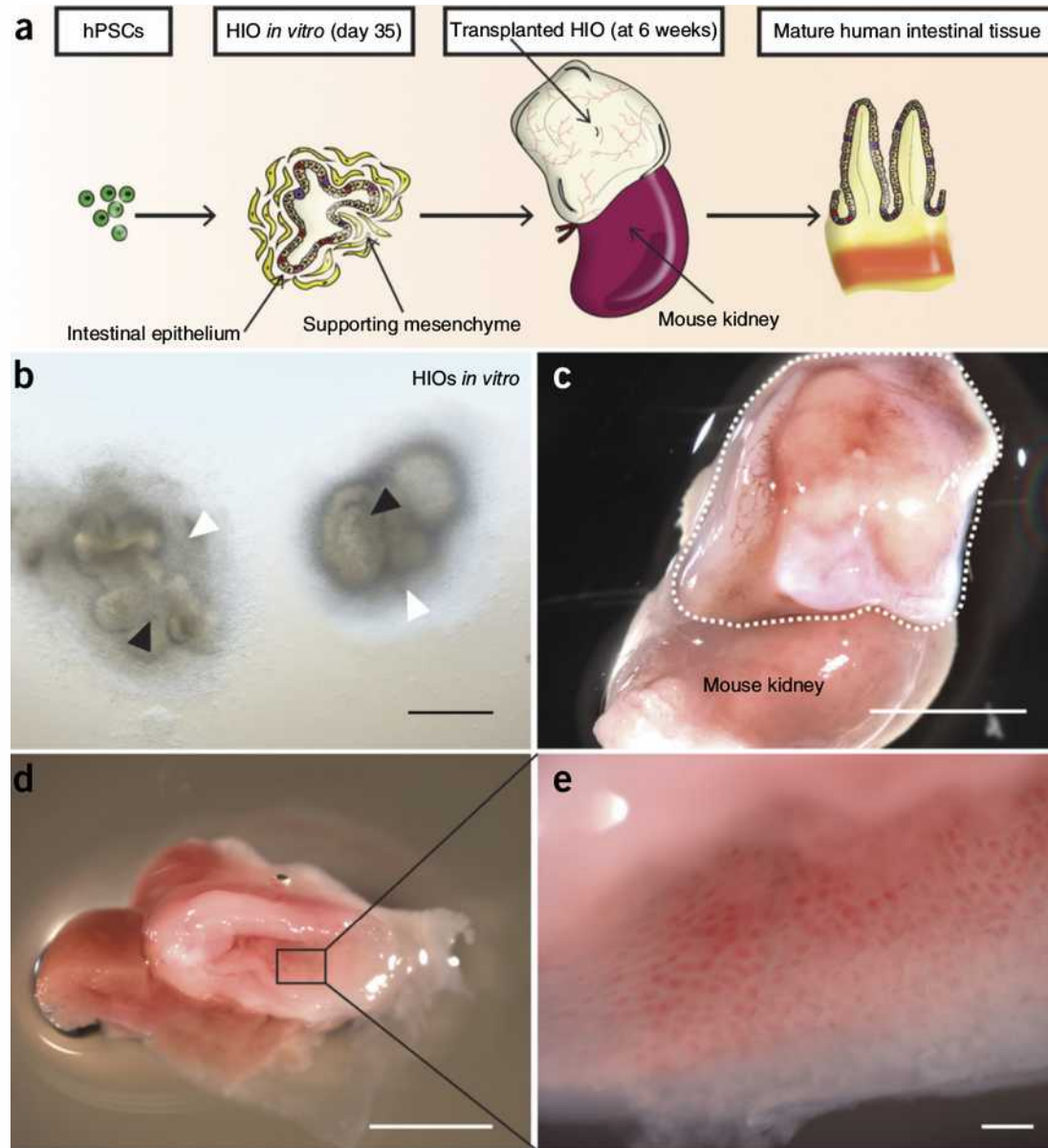
# Directed differentiation of pluripotent stem cells into intestinal tissue



Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V. V, Wells, S.I., Zorn, A.M., et al. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* 470, 105–109.

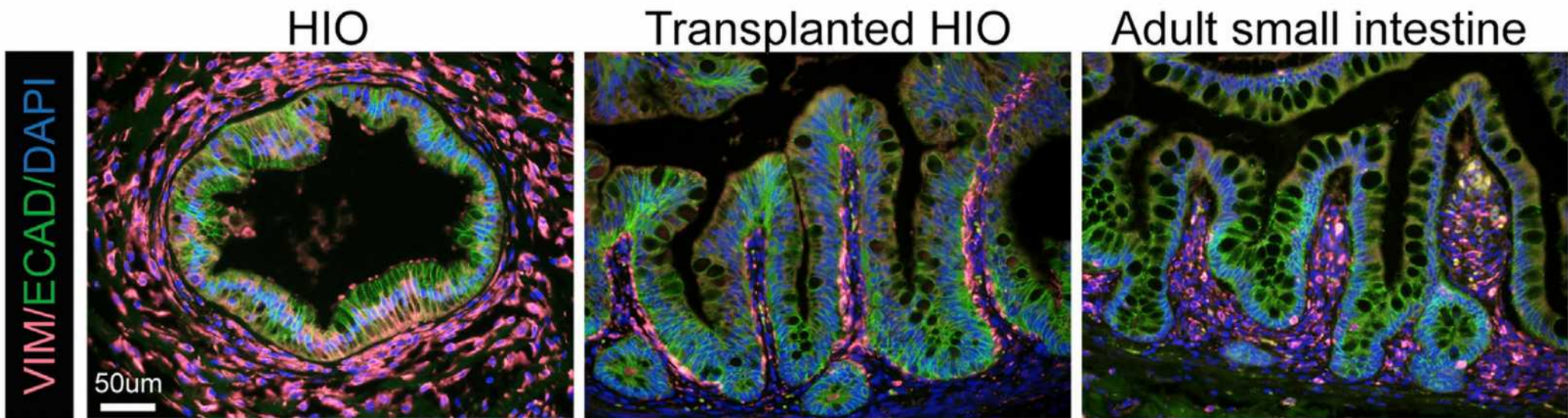


# Stem-cell derived organoids are capable of maturation into adult-like structures upon transplantation



Watson et al. (2014). An in vivo model of human small intestine using pluripotent stem cells. *Nat. Med.* 20, 1310–1314.

# Stem-cell derived organoids are capable of maturation into adult-like structures upon transplantation



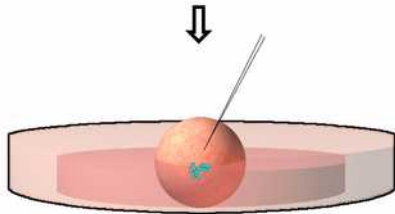
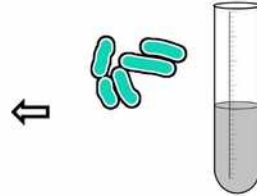
Finkbeiner, S.R., Hill, D.R., Altheim, C.H., Dedhia, P.H., Taylor, M.J., Tsai, Y.H., Chin, A.M., Mahe, M.M., Watson, C.L., Freeman, J.J., et al. (2015). *Stem Cell Reports* 4, 1140–1155.

# Methodology for colonizing HIOs with live bacteria

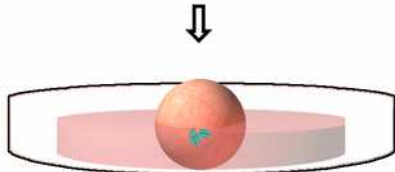
**DAY -30 to -1:** Prepare HIOs according to McCracken *et al* (2011) and plate 1 HIO per well (24-well plate) in 50  $\mu$ l Matrigel and 500  $\mu$ l ENR media



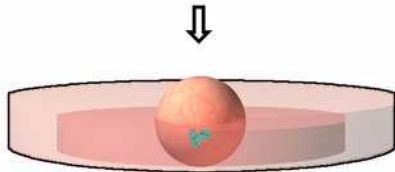
**DAY -1:** Culture *E. coli* from glycerol stock in LB broth overnight at 37° C



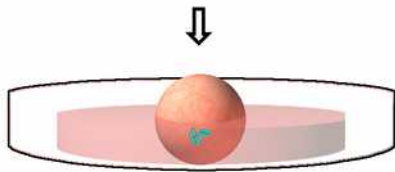
**DAY 0:** Microinject approximately 1  $\mu$ L of the overnight *E. coli* culture (diluted in PBS) into the HIO lumen



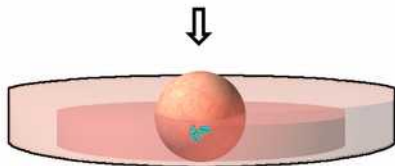
**After microinjection:** Remove ENR media and wash 1X with sterile PBS.



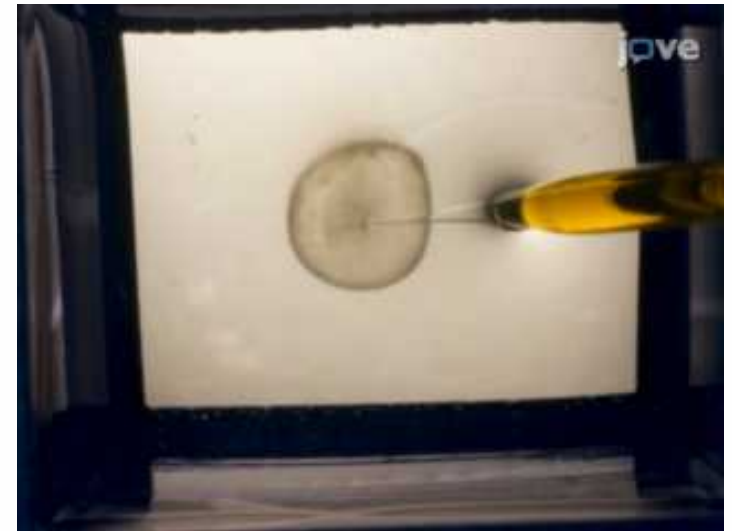
**0-1 h post-microinjection:** Add ENR media containing Pen/Strep mix and incubate at 37 °C for 1 hour



**1 h post-microinjection:** Remove ENR containing antibiotics and wash 1X with sterile PBS.



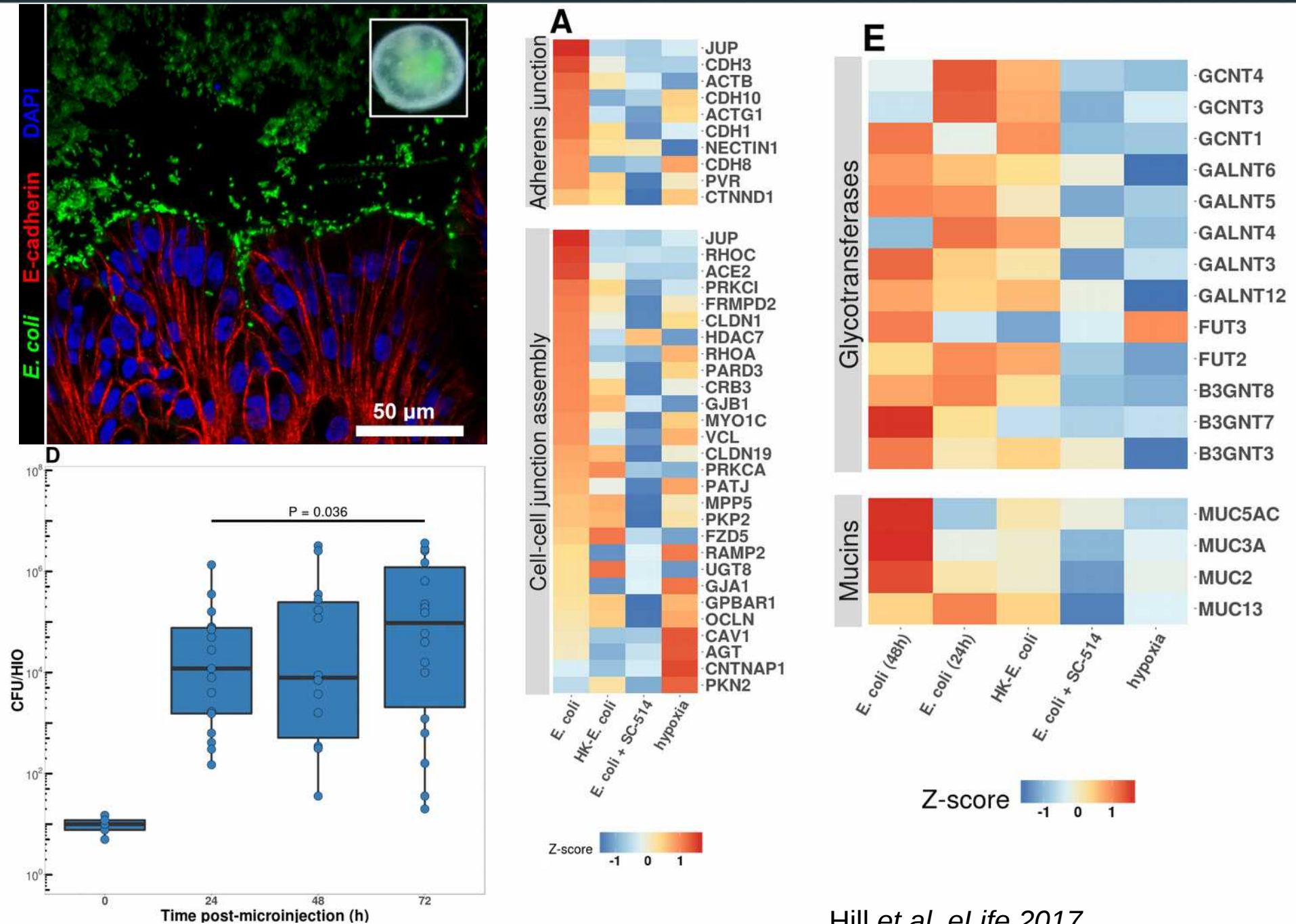
Replace fresh ENR without antibiotics and culture at 37 °C and 5% CO<sub>2</sub>



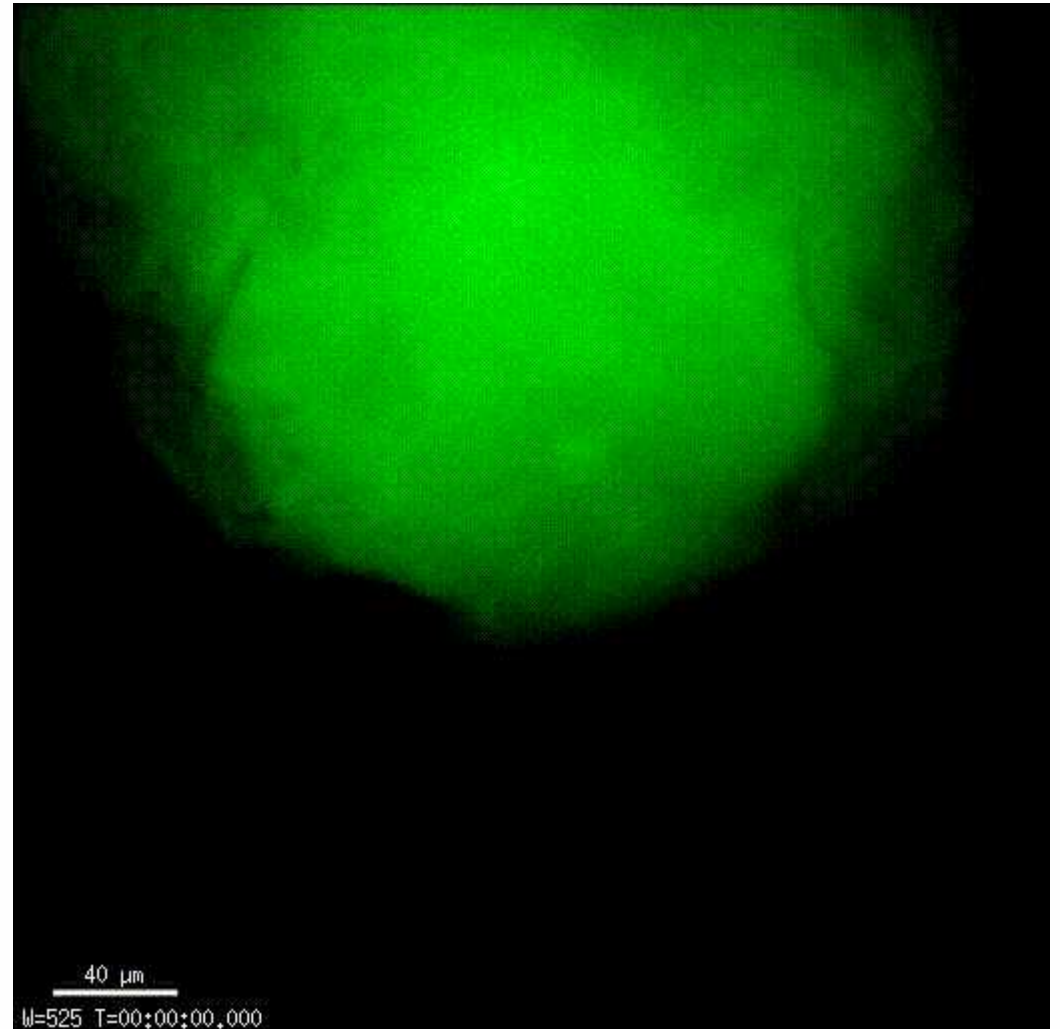
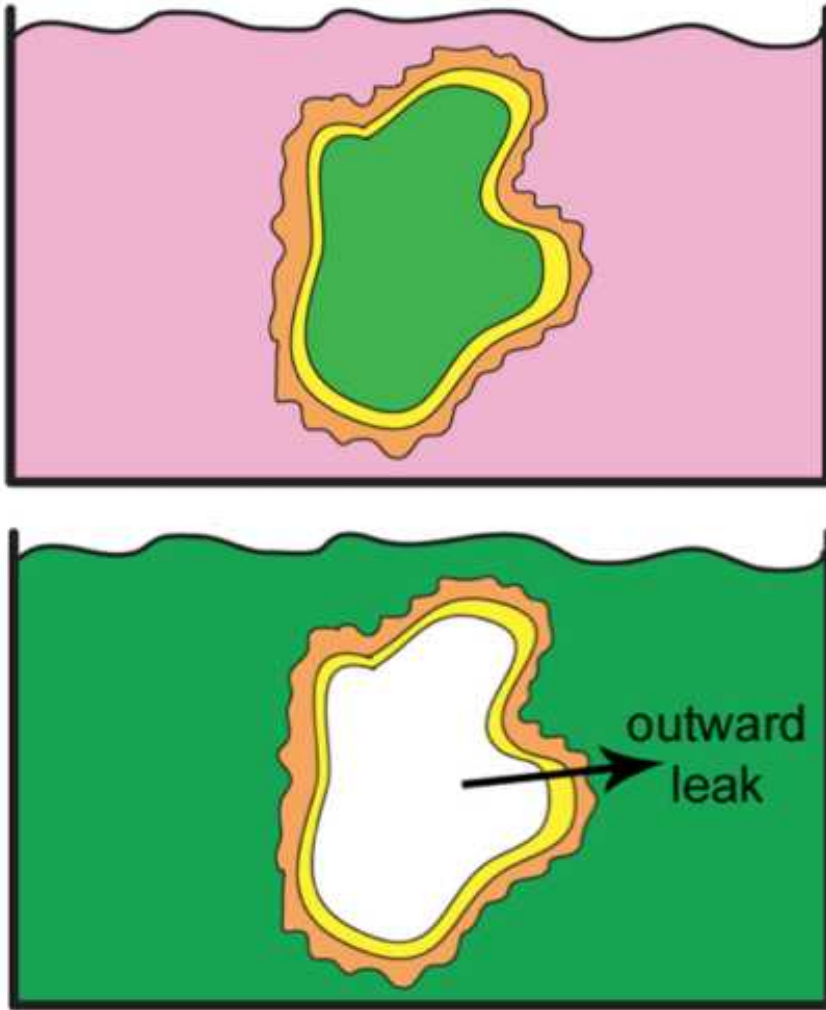
Hill *et al.* (2017). *eLife*  
Hill *et al.* (2017). *JoVE*



# Stable bacterial colonization promotes expression of gene sets critical for epithelial barrier function

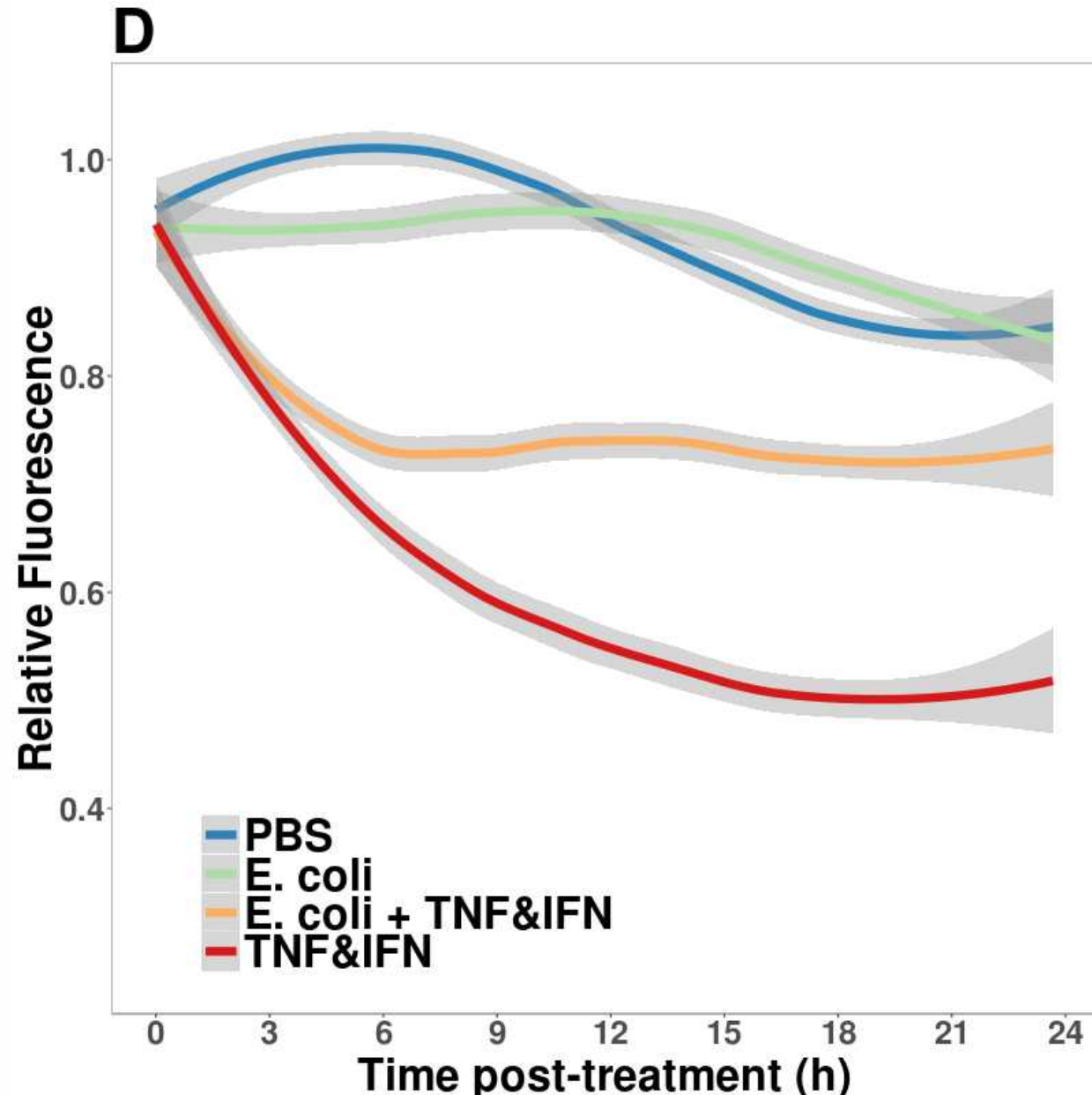


# Measuring epithelial barrier permeability in HIOs



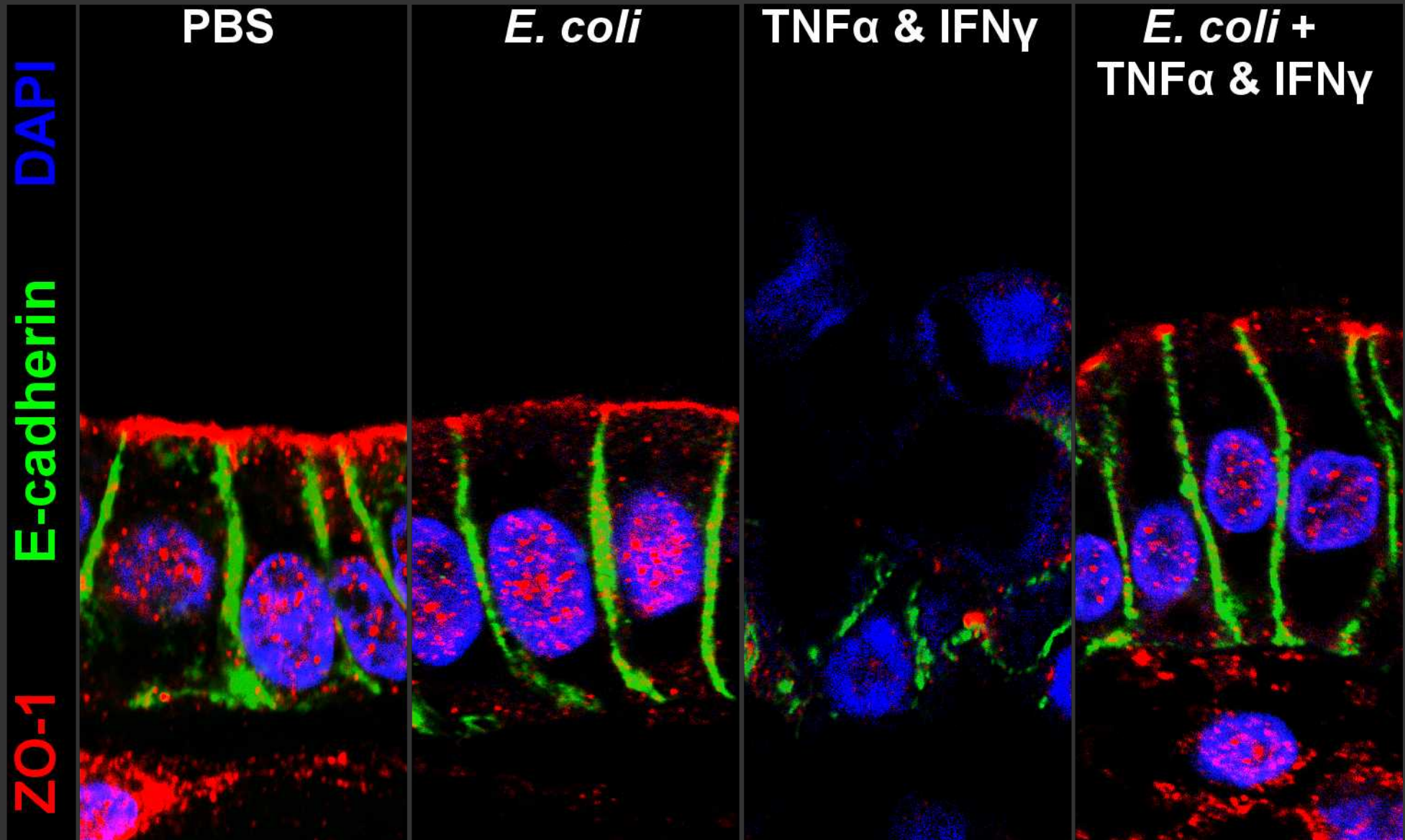
# *E. coli* colonization enhances epithelial barrier resilience

- TNF $\alpha$  and IFN $\gamma$  released during intestinal inflammation increases epithelial barrier permeability
- Control HIOs or HIO pre-colonized with *E. coli* treated with TNF $\alpha$  and IFN $\gamma$  for 24 hrs
- **Epithelial barrier integrity is maintained during cytokine challenge in colonized HIOs**



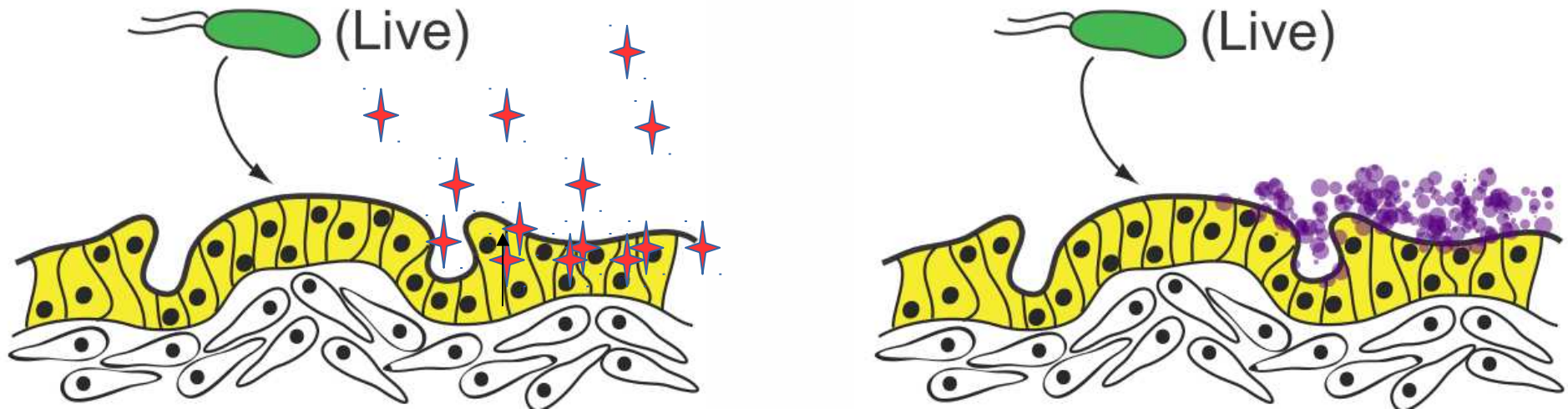


# *E. coli* colonization enhances epithelial barrier resilience



# Broad Implications

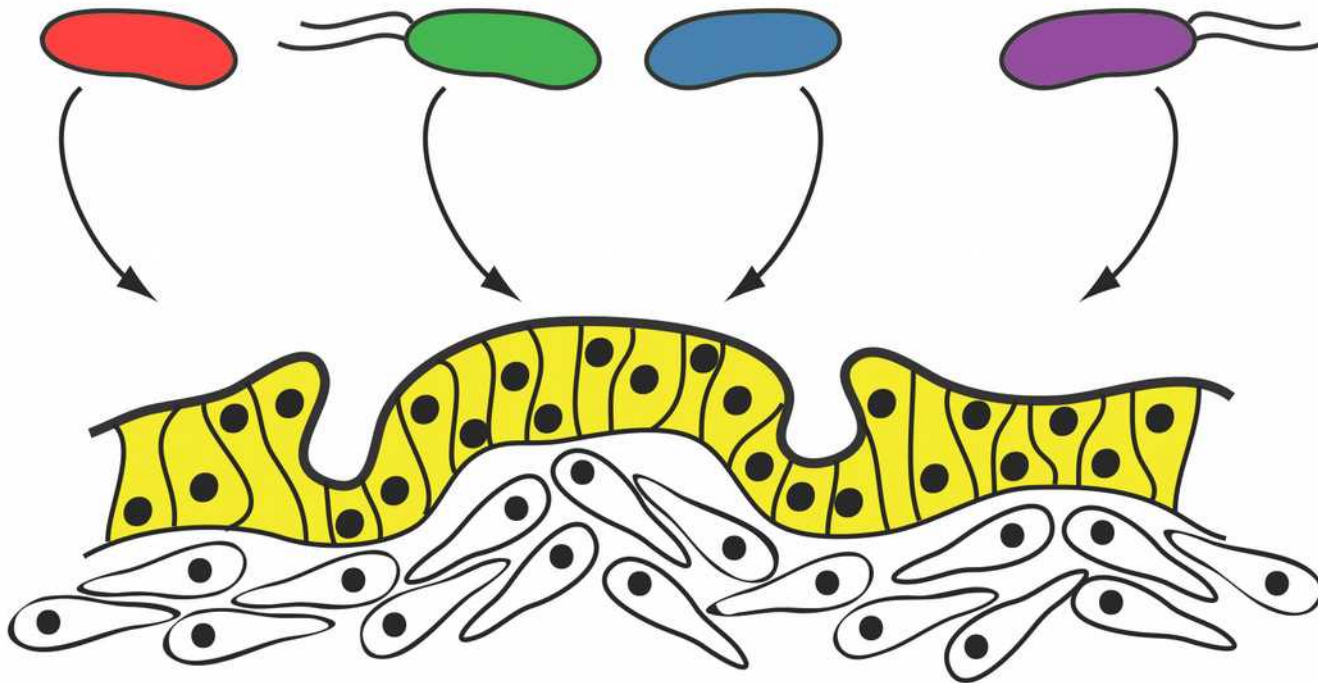
- Immature epithelium and underlying mesenchyme is sufficient to maintain symbiosis with bacteria
  - Achieved through enhanced epithelial barrier defense
  - No immune cells required



Hill DR, Huang S, Nagy MS, Yadagiri VK, Fields C, Mukherjee D, Bons B, Dedhia PH, Chin AM, Tsai YH, Thodla S, Schmidt TM, Walk S, Young VB, Spence JR. Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium. *Elife*. 2017 Nov 7;6. pii: e29132. doi:10.7554/eLife.29132.

## Key questions

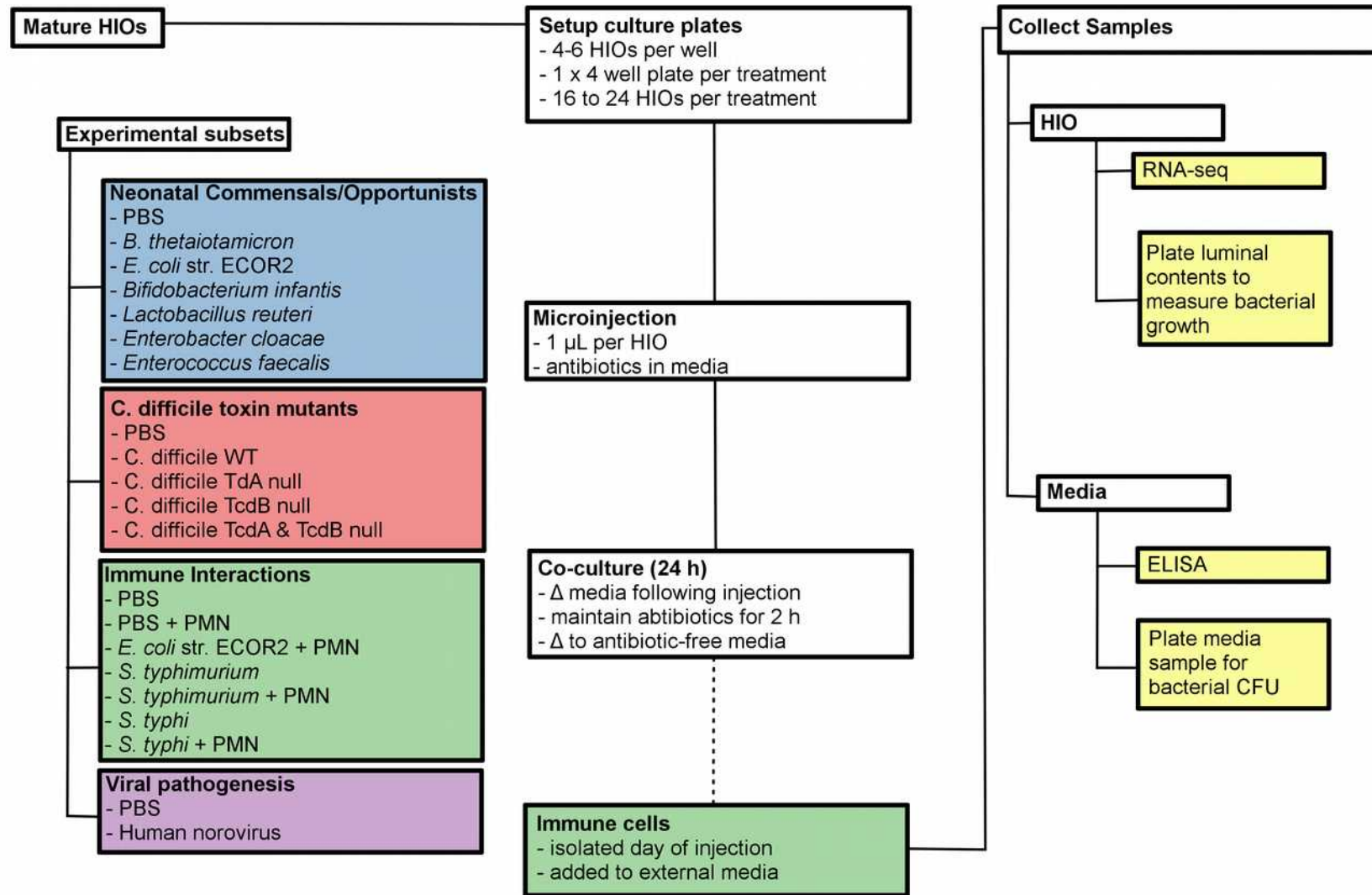
***Is the epithelial response to primary microbial colonization species-specific?***



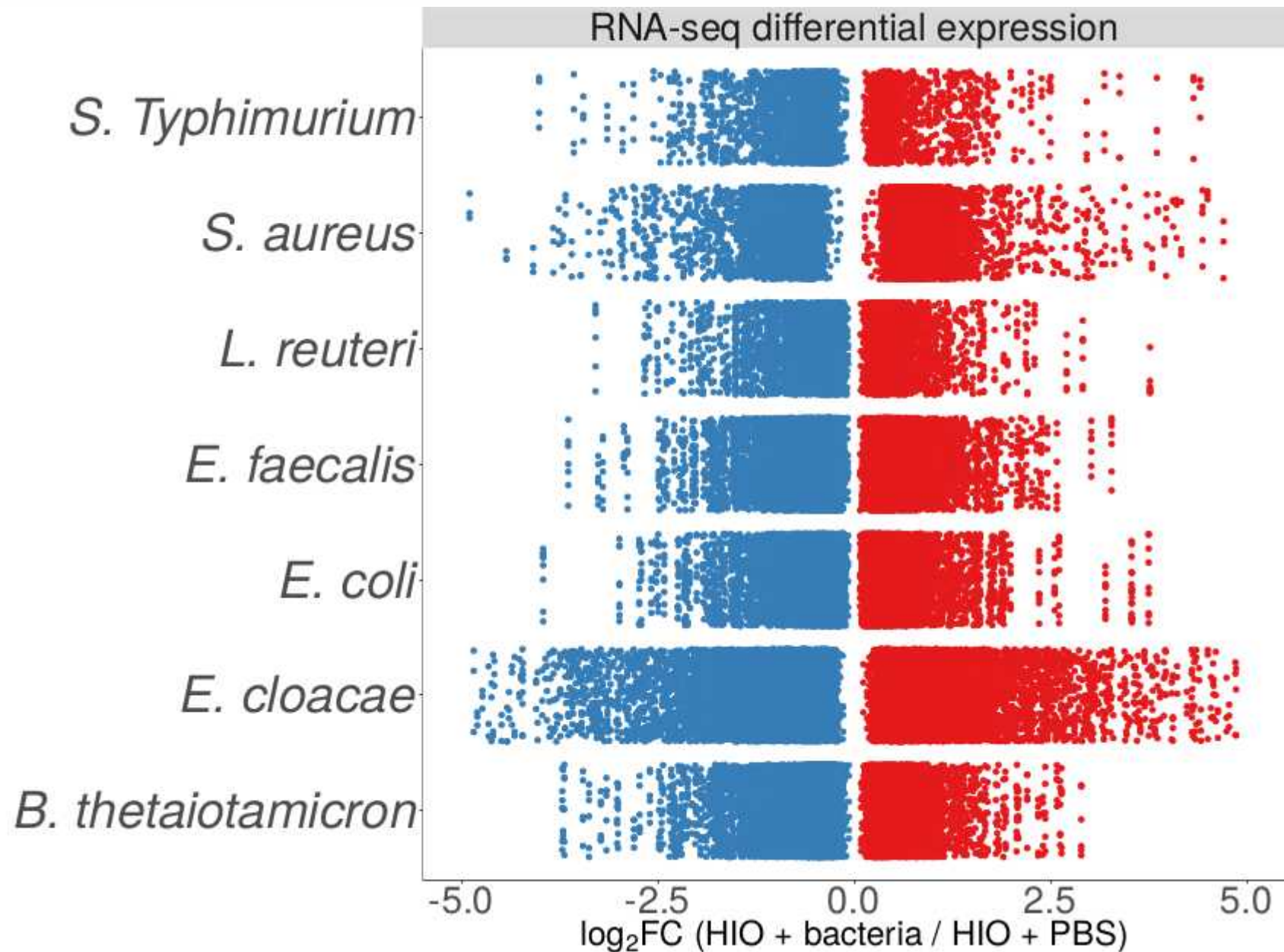


# Designing a screen of representative microbial colonists

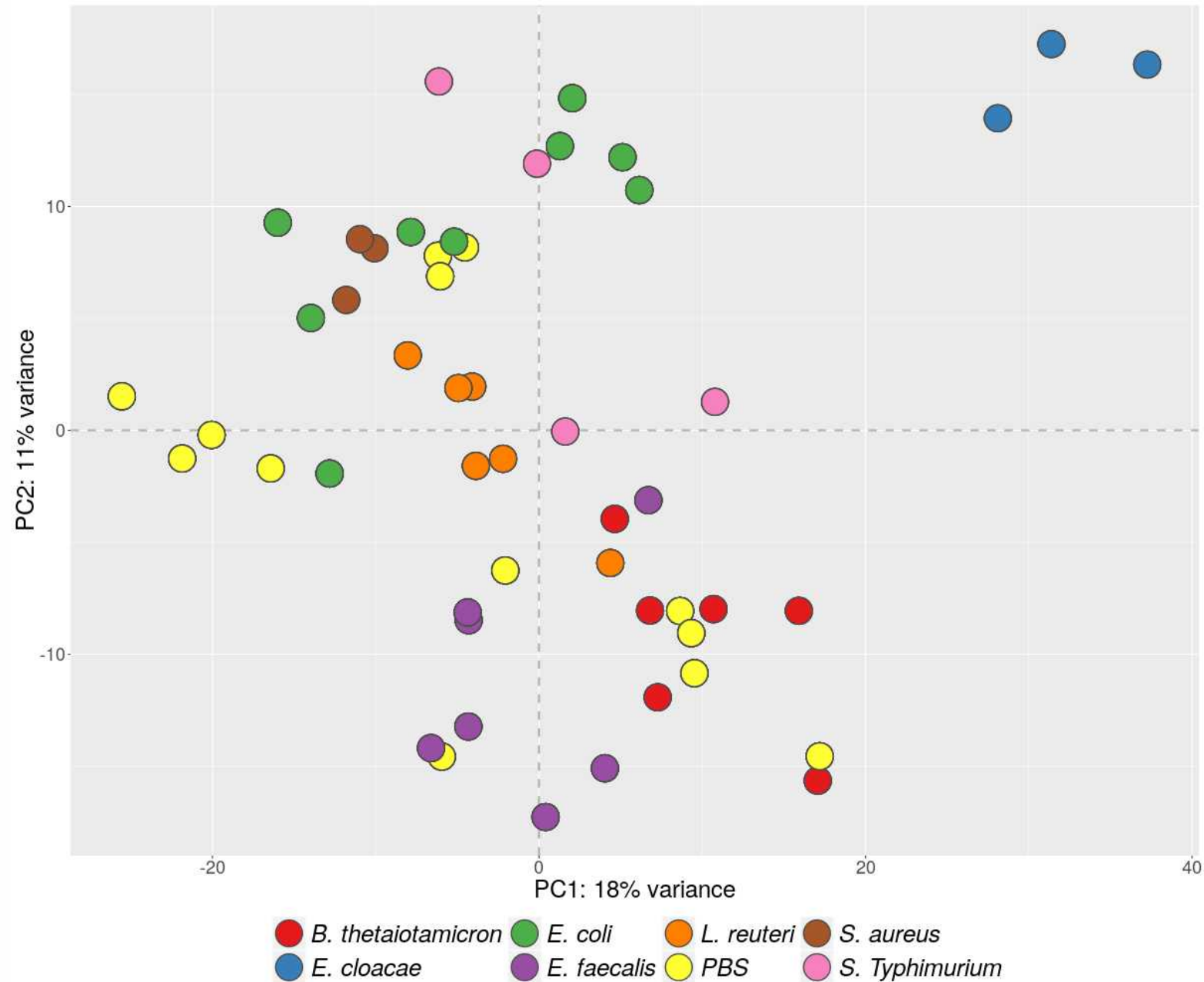
## Novel Alternative Model Systems of Enteric Disease (NAMSED)



# Robust host transcriptional response to bacterial colonization

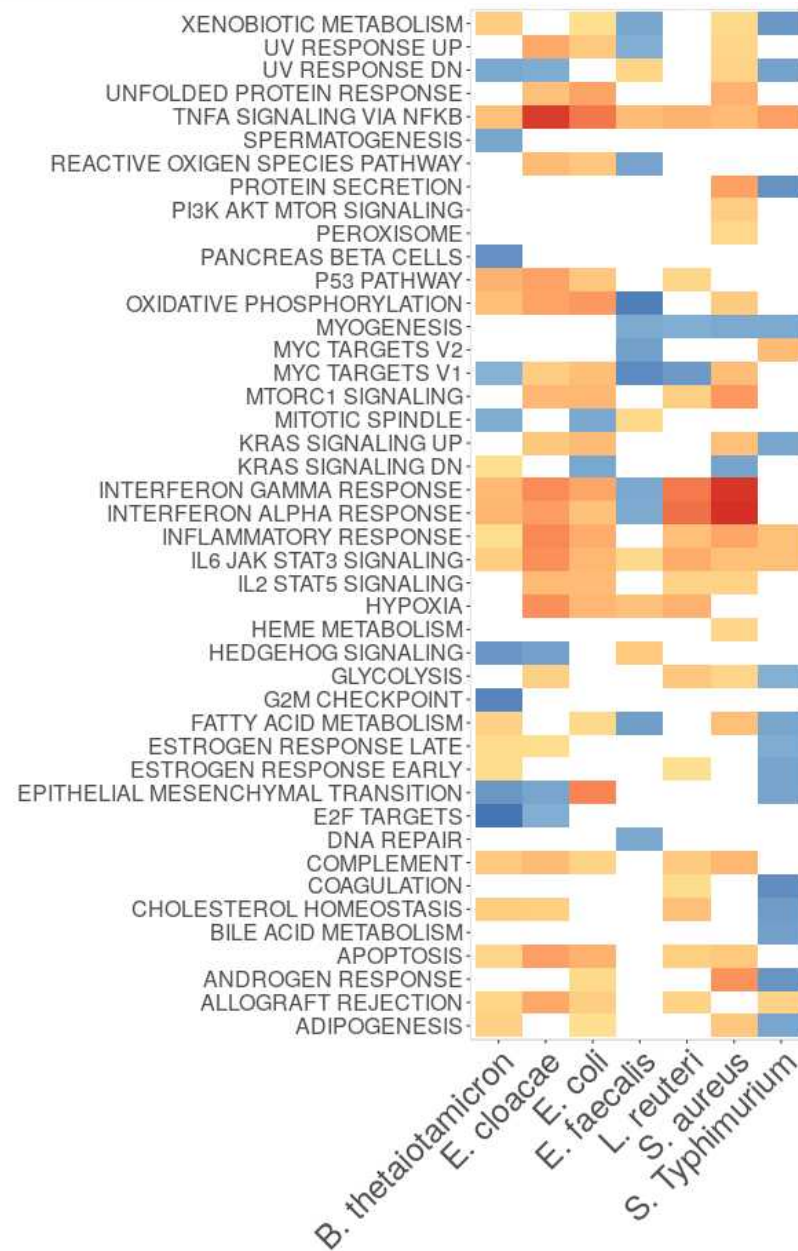


# Identification of strain specific host response gene sets



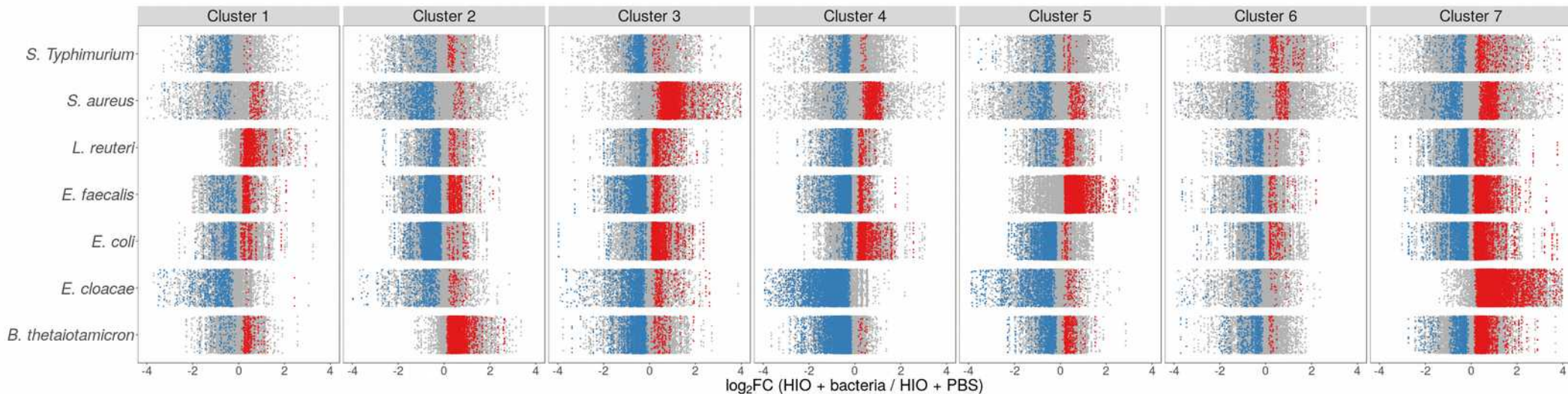


# The host transcriptional response to bacterial colonization is species-specific



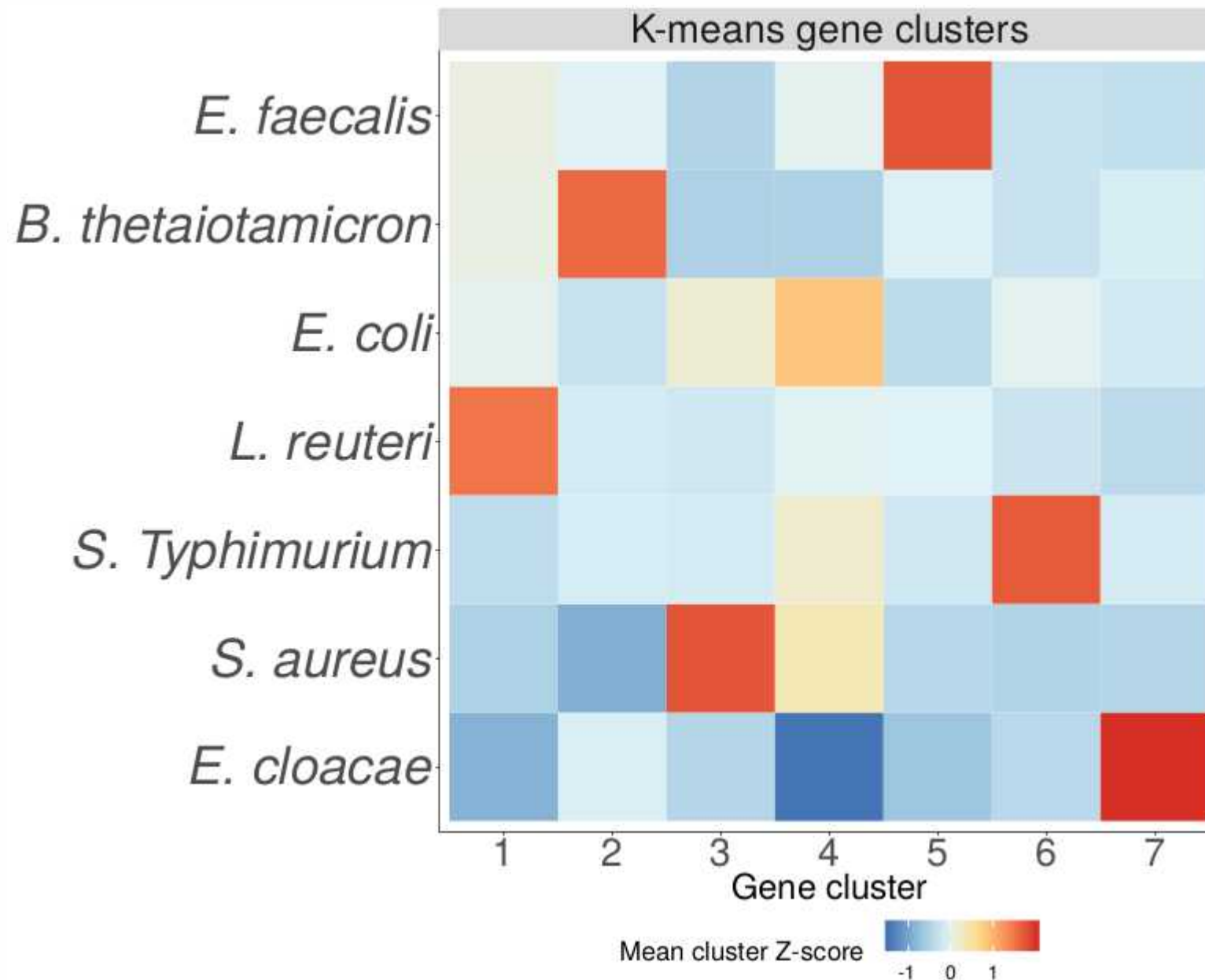
NES  
-2 0 2

# The host transcriptional response to bacterial colonization is species-specific



- **K-means algorithm** finds patterns in a large data set without prior knowledge
- Each gene is assigned to one of  $K$  groups based on expression across all colonization conditions
- $K$  is defined *a priori* as part of the hypothesis (e.g. *There are 7 distinct transcriptional responses to the 7 bacterial colonization conditions*)
- Each *cluster* is a list of genes that have a shared pattern across conditions

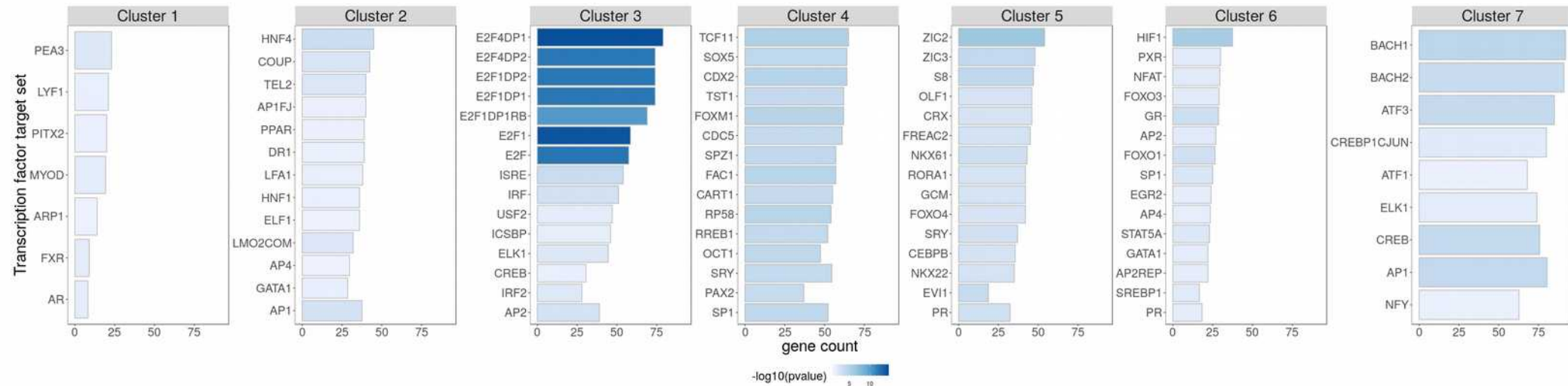
# The host transcriptional response to bacterial colonization is species-specific





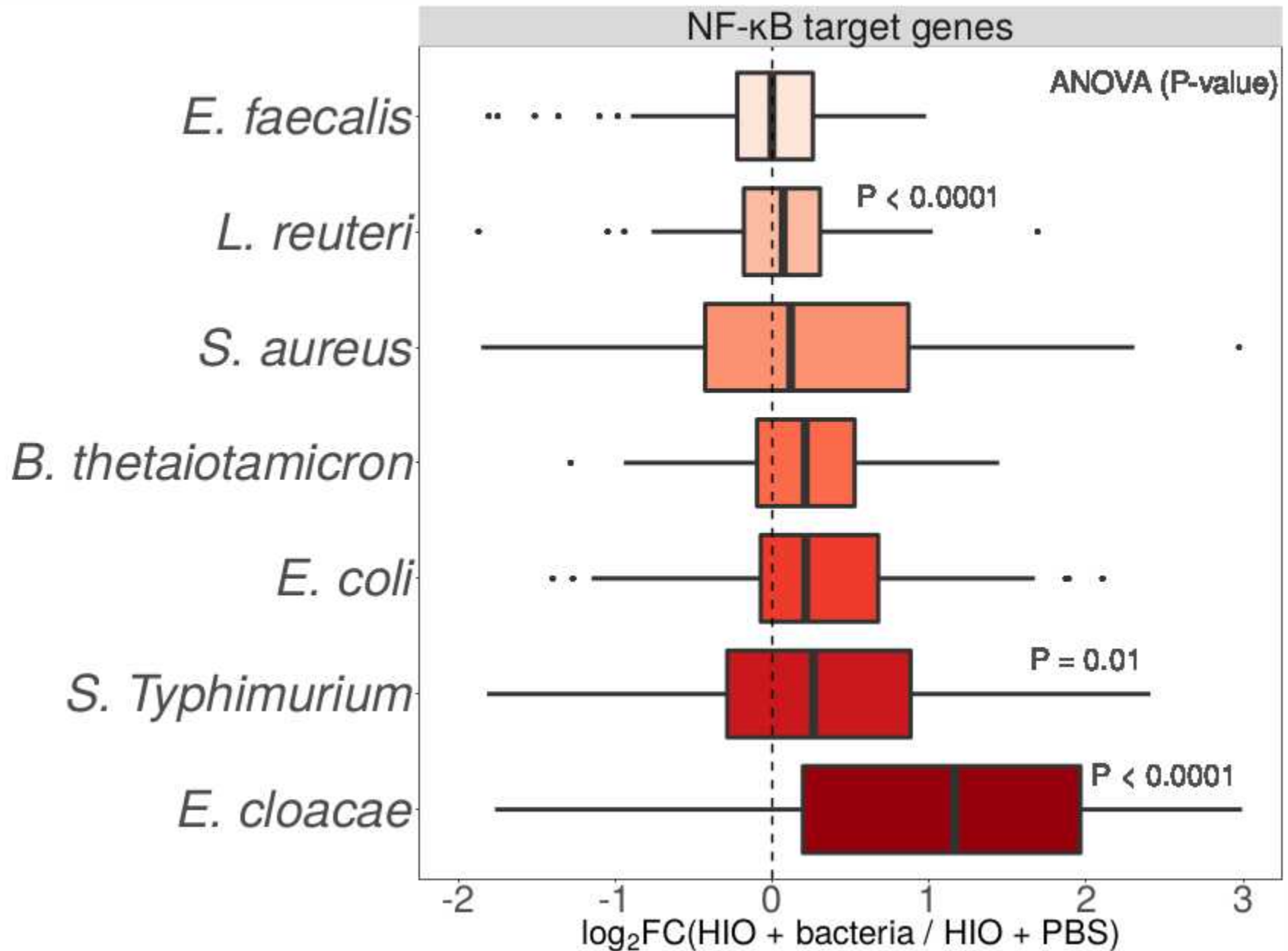
# Evidence of specific transcriptional control over genes in each cluster

## Transcription factor target set over-abundance test



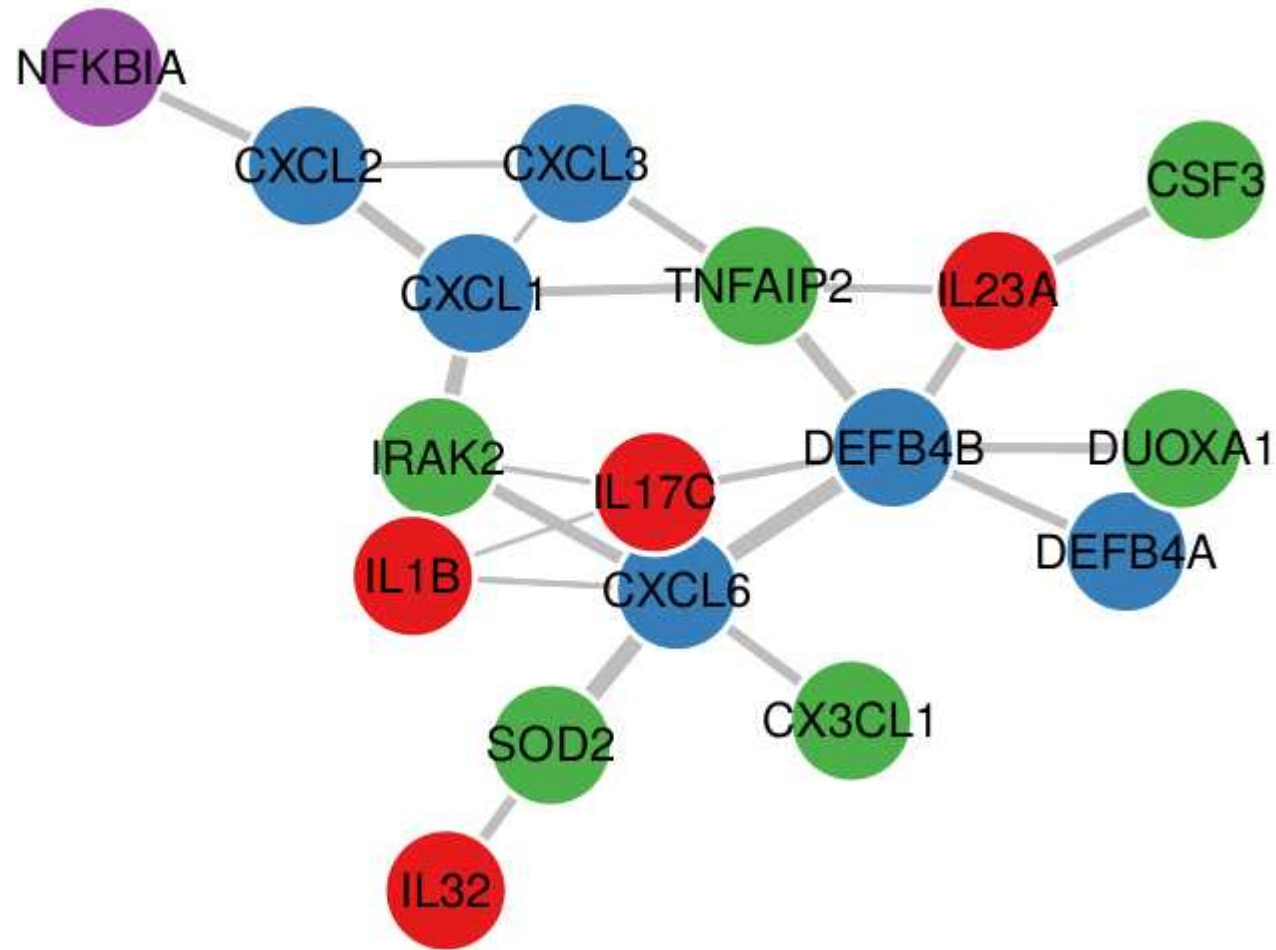
**Over-abundance test:** more genes in a list from a given gene set (TFT) than expected by chance.

# Expression of downstream NF- $\kappa$ B targets varies by bacterial strain



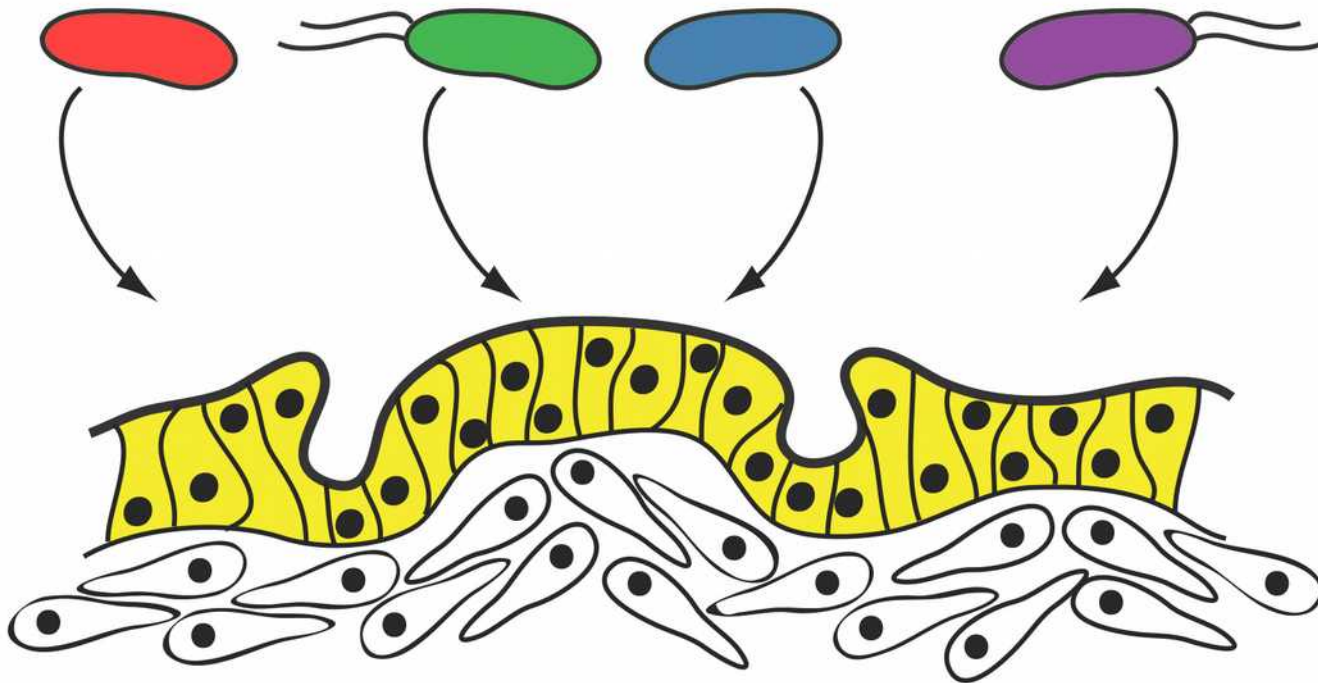
# Network analysis reveals novel correlations between gene expression events

Inferred Gene Regulatory Network

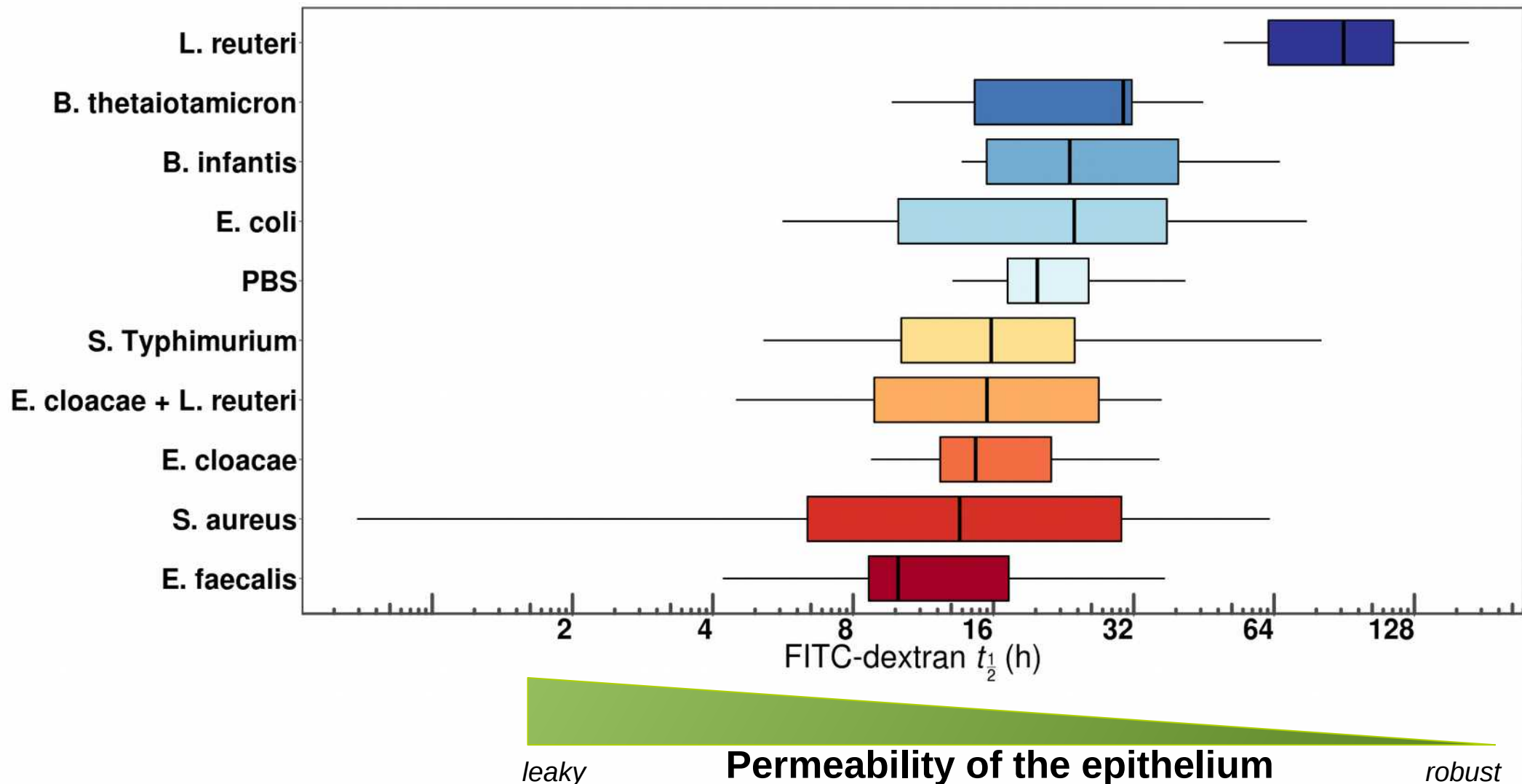




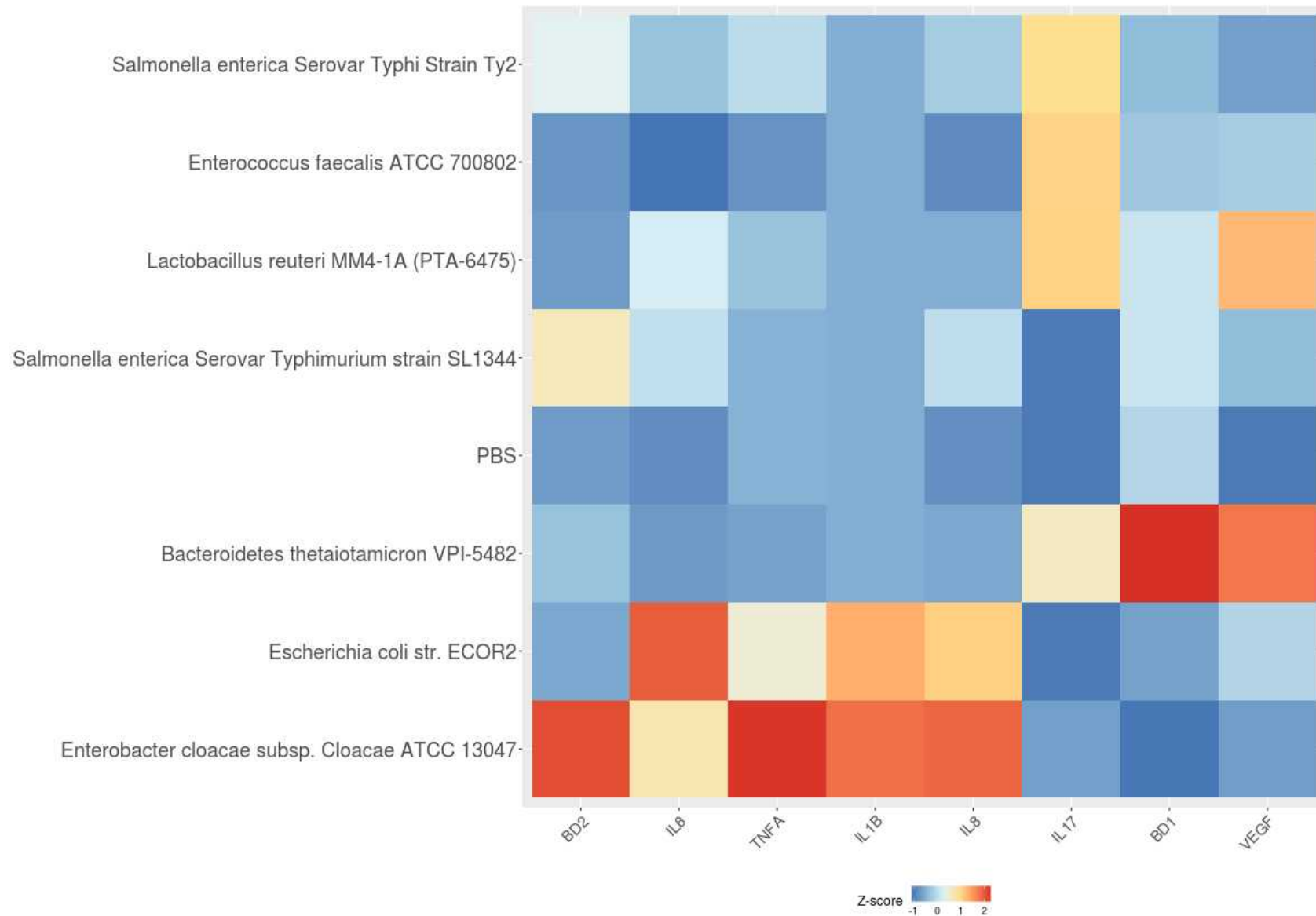
**What are the functional consequences of variation in epithelial transcriptional response?**



# Effect of bacteria on epithelial barrier function is highly strain-dependent



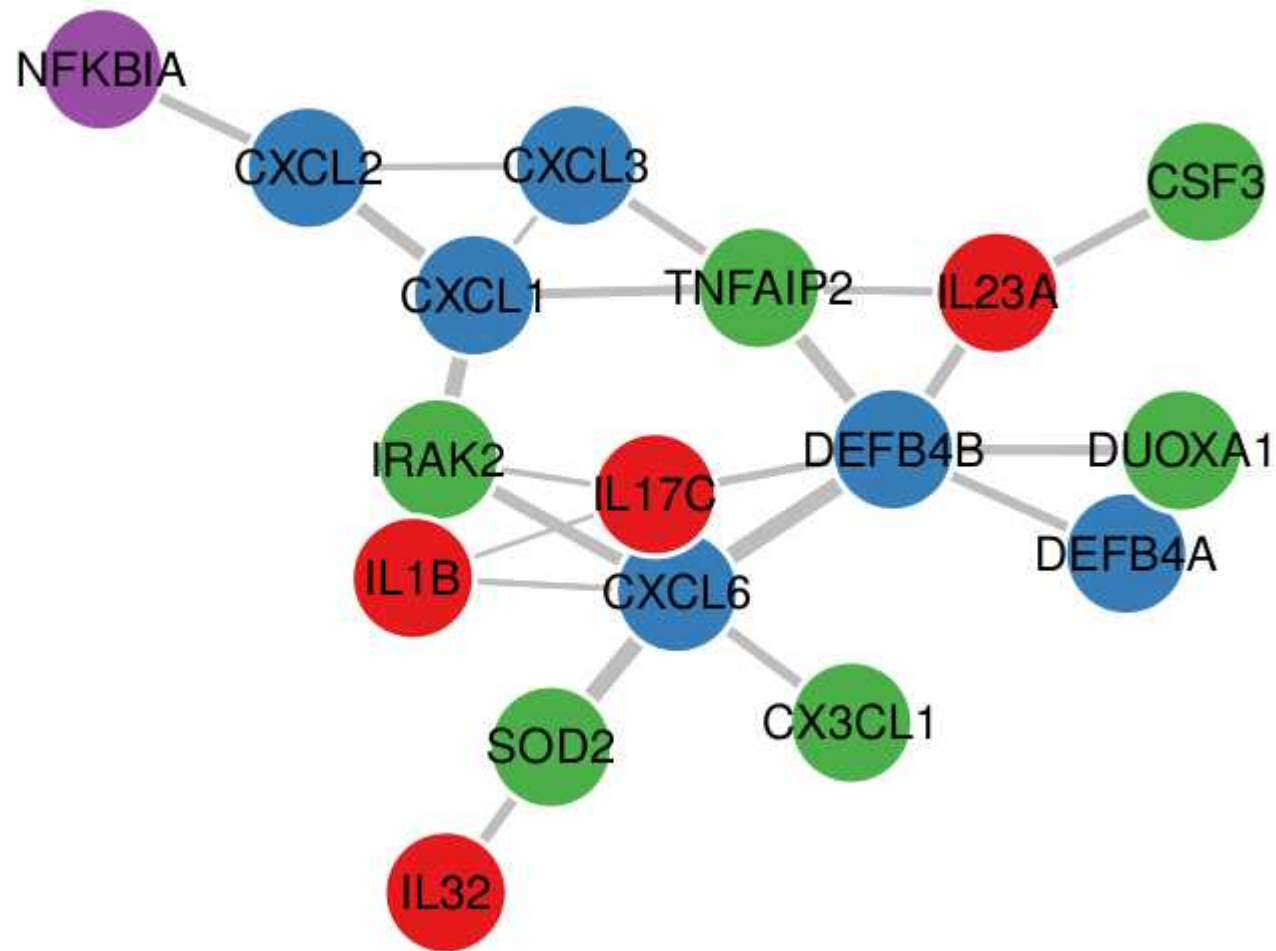
# Secreted cytokine response to bacterial colonists varies widely



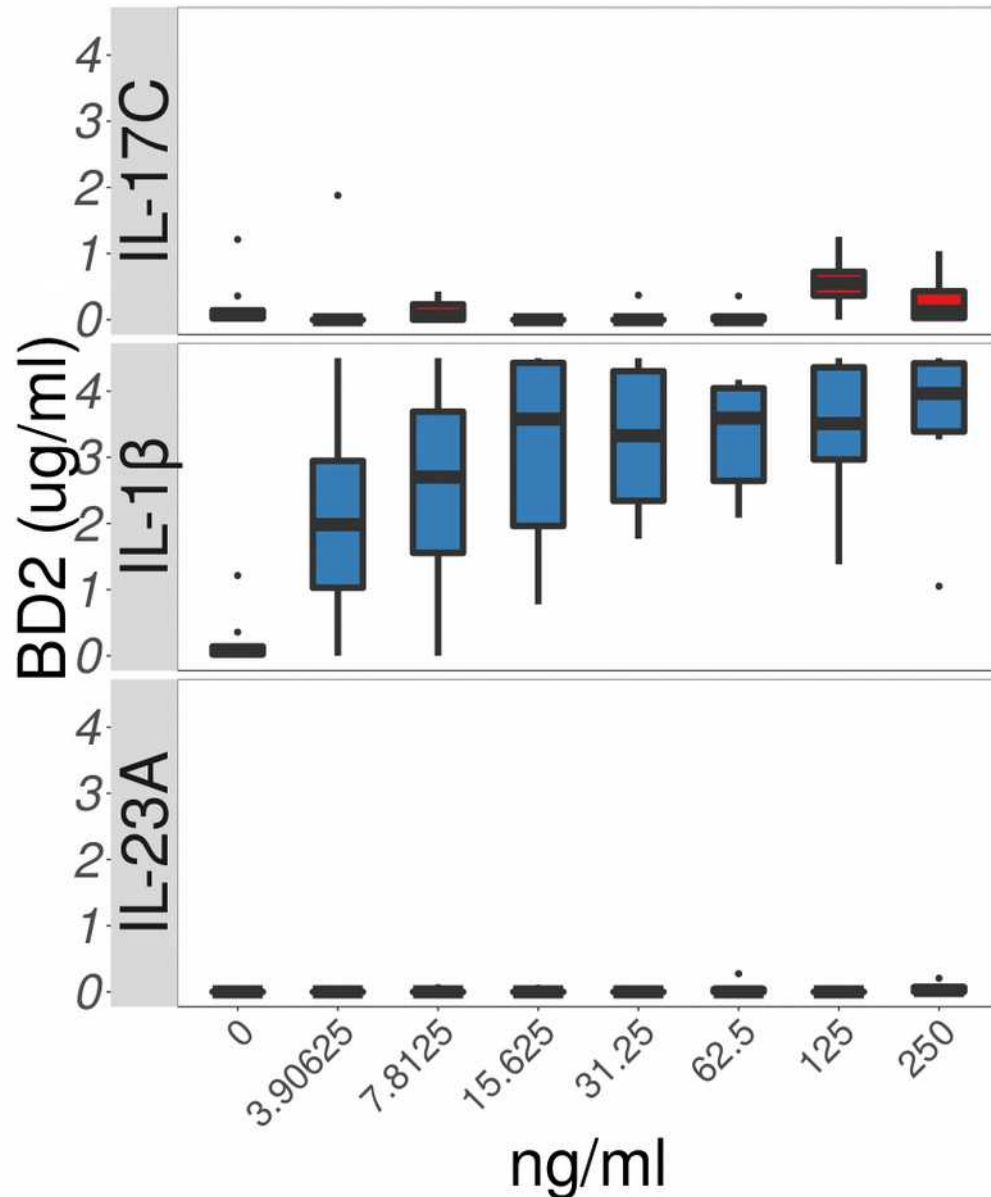


# Network analysis reveals novel correlations between gene expression events

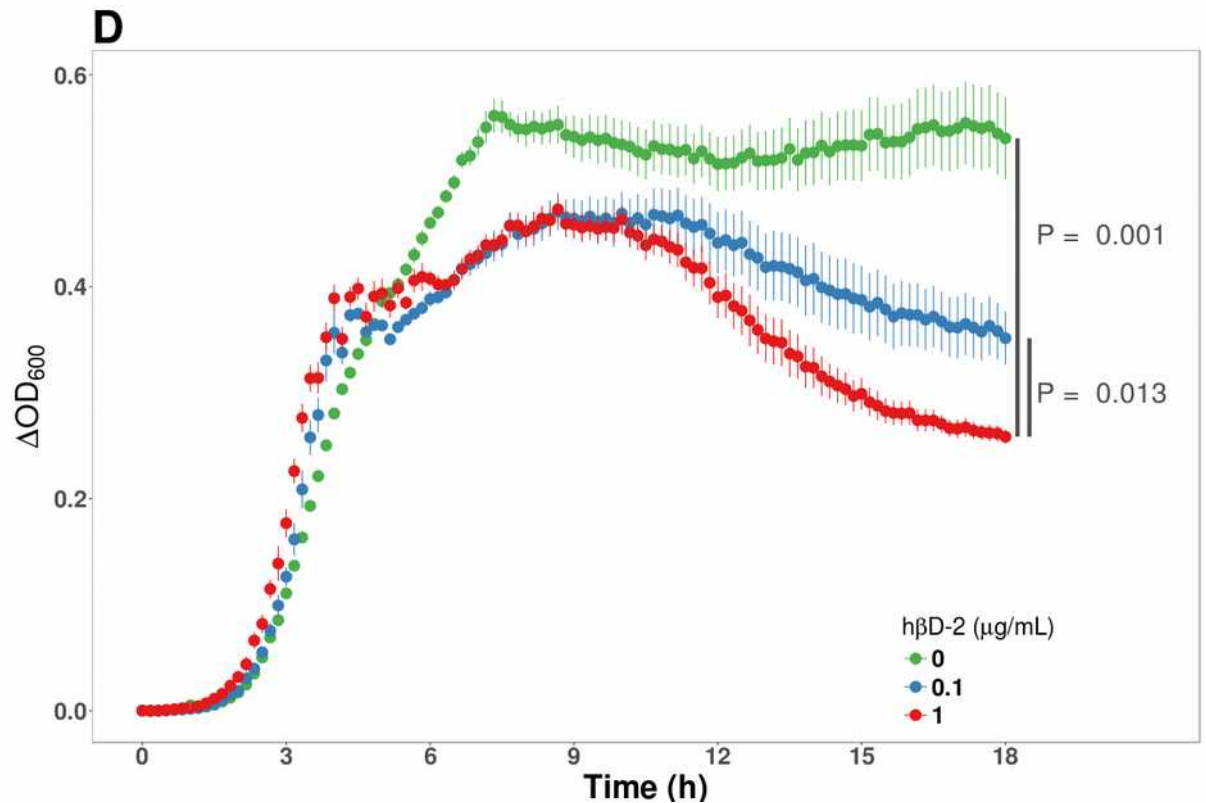
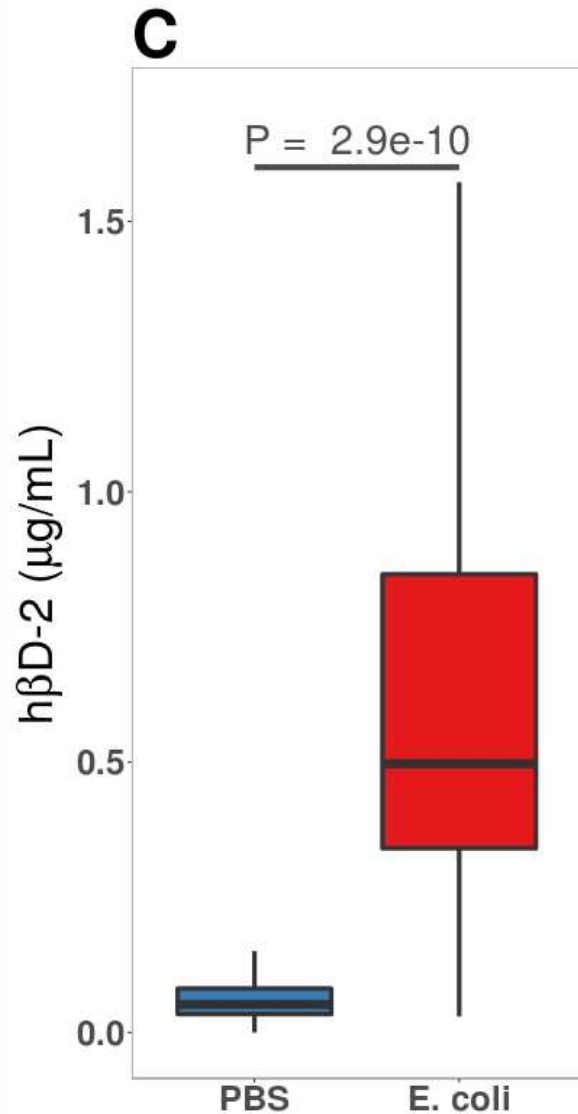
Inferred Gene Regulatory Network



# Epithelial cytokines control AMP secretion

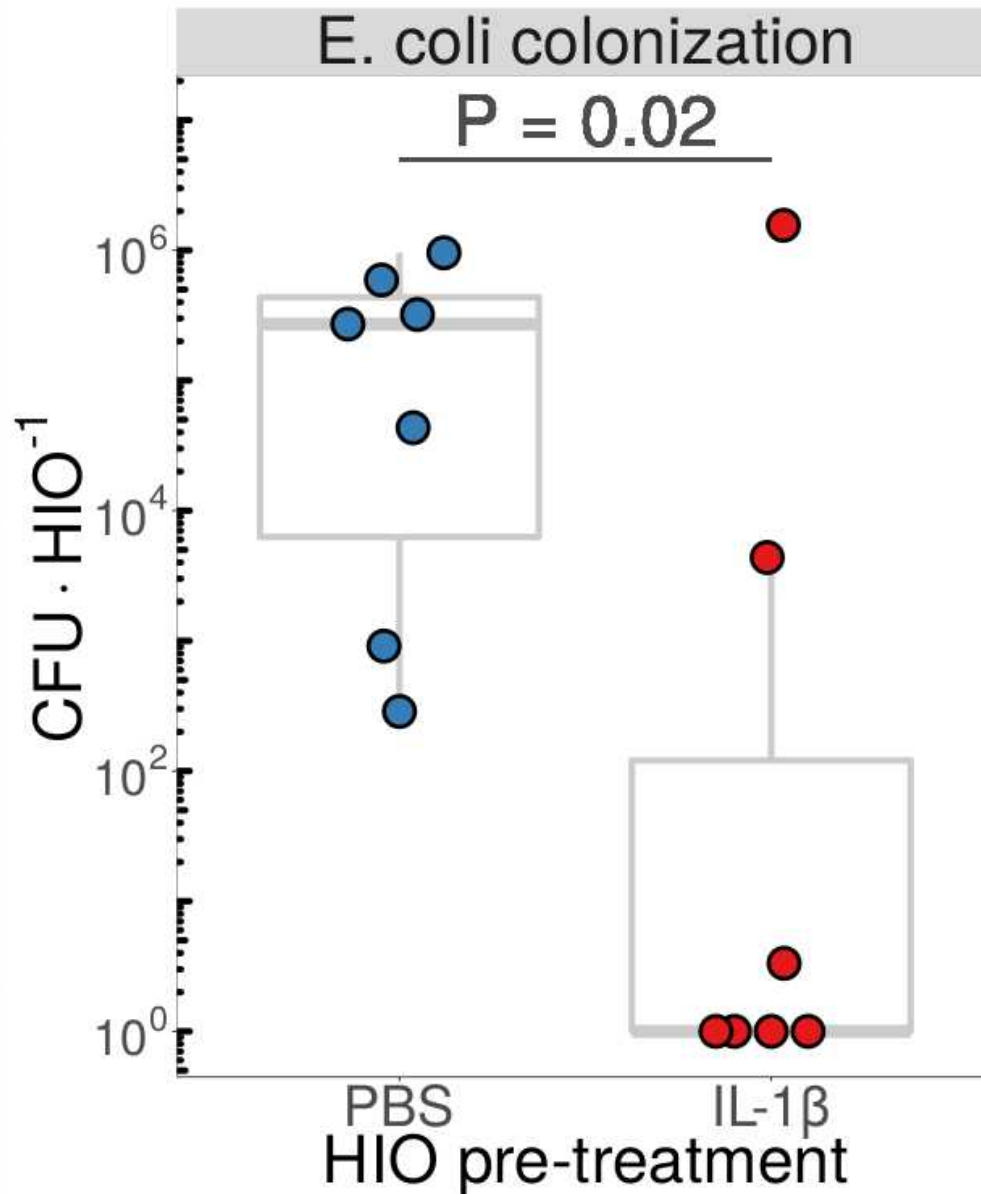


# BD-2 inhibits *E. coli* growth *in vitro*

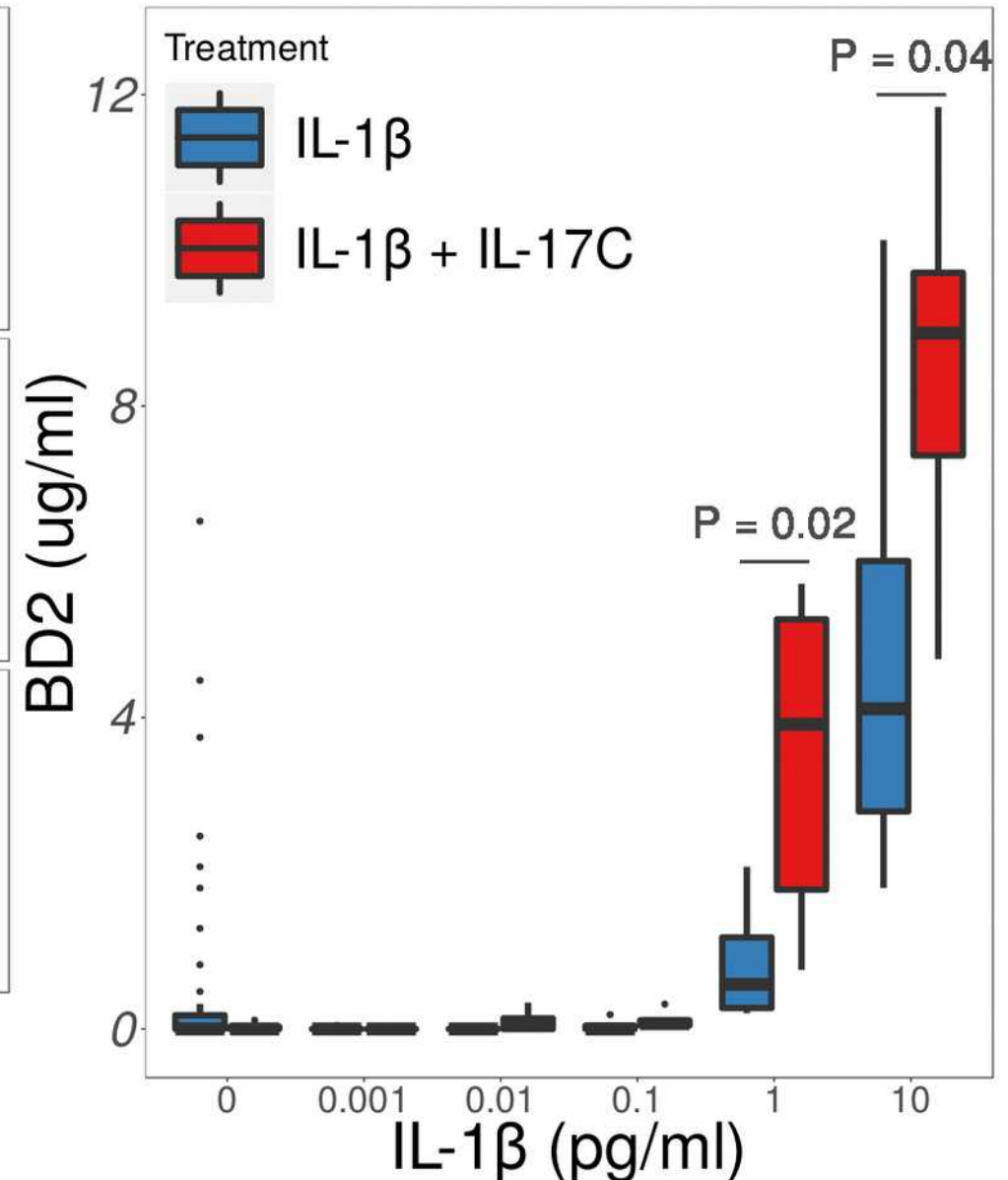
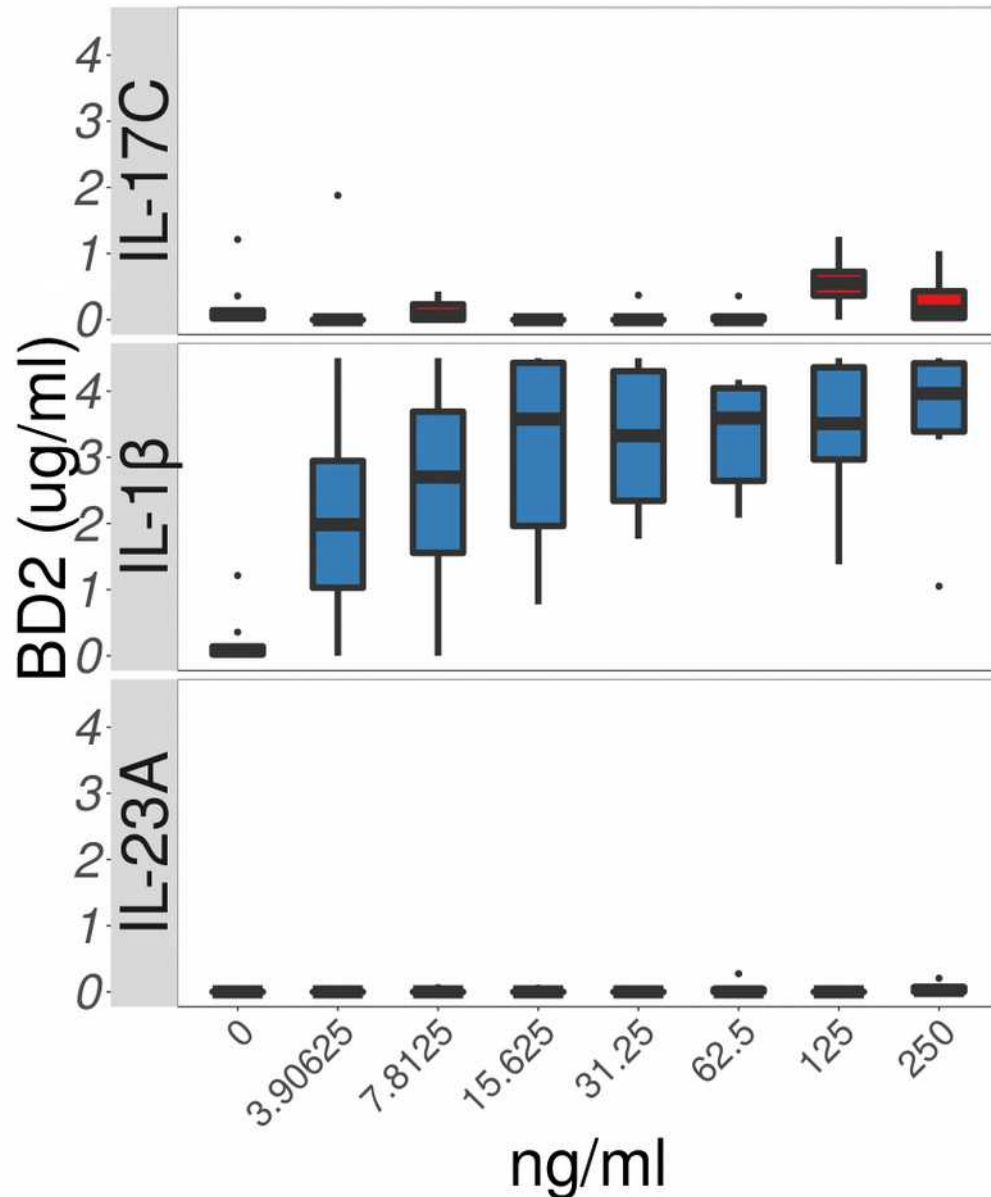




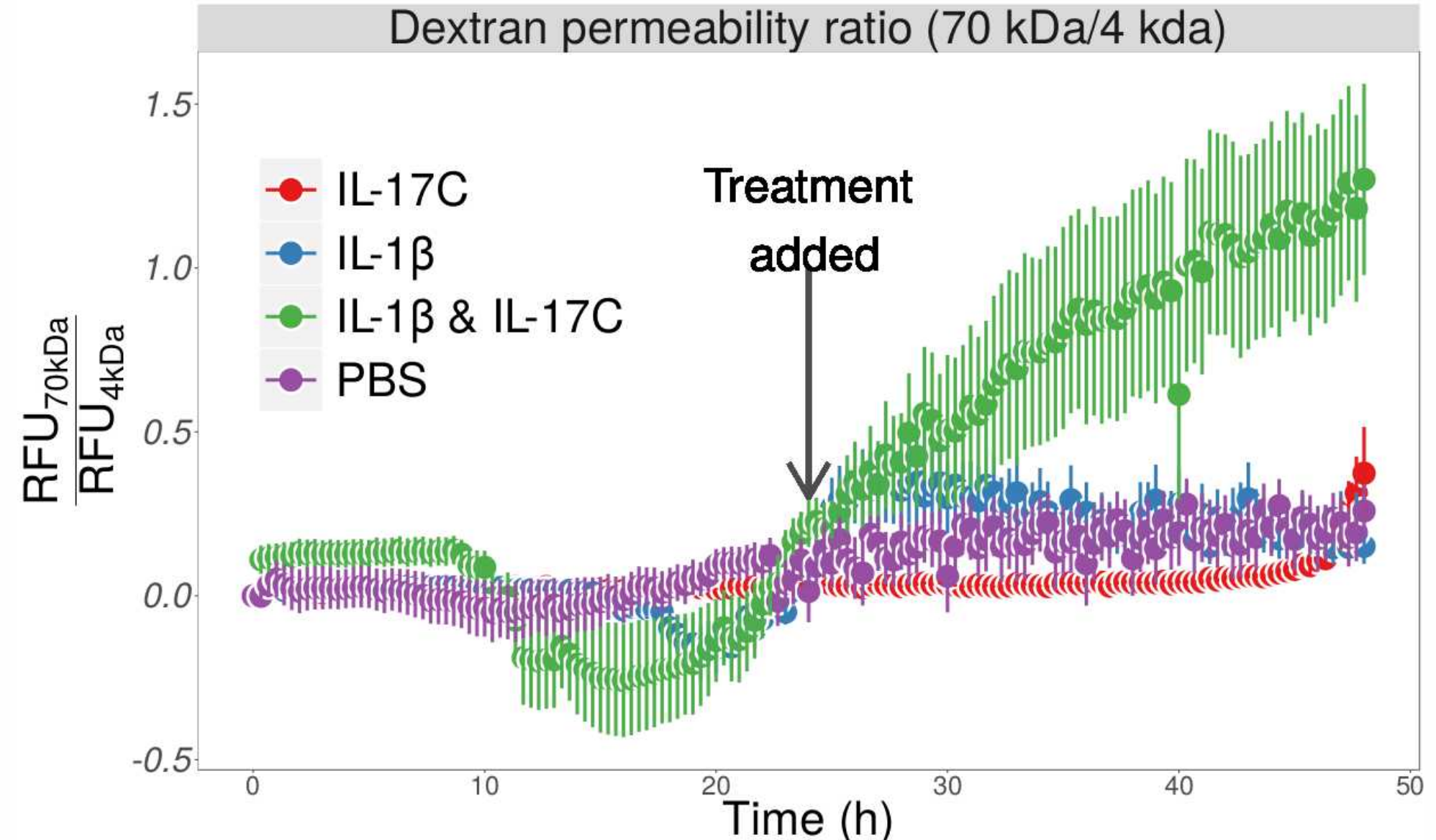
# Pre-treatment with IL-1 $\beta$ suppresses microbial growth



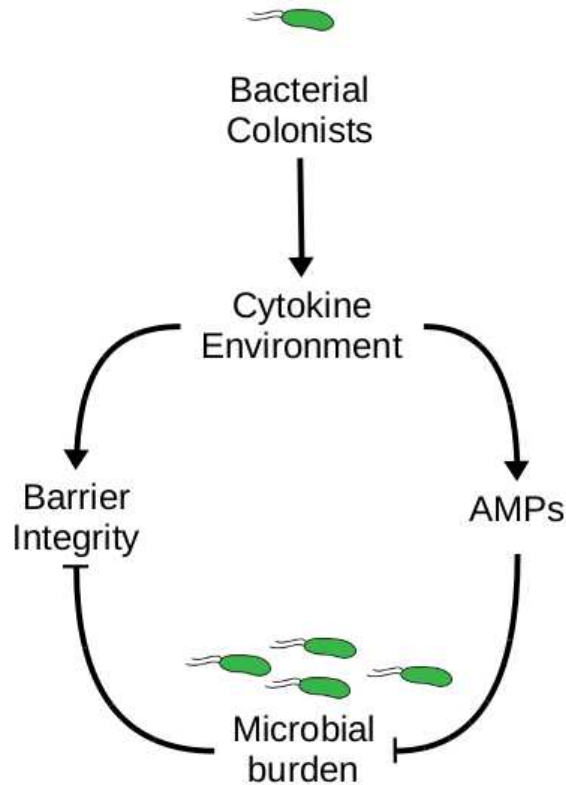
# Epithelial cytokines control AMP secretion



# Cytokine environment alters epithelial permeability

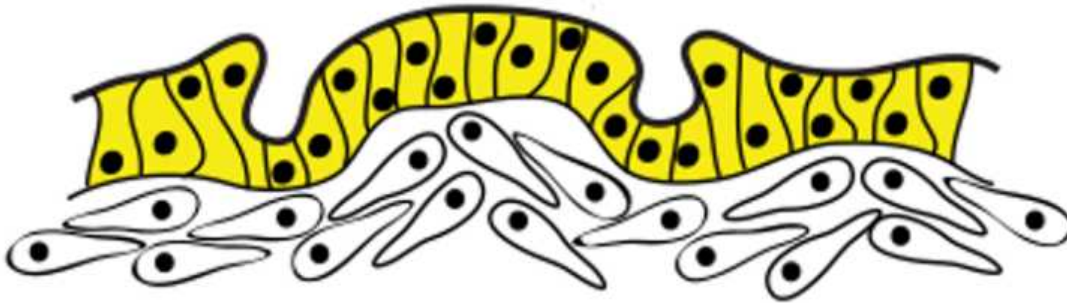


# *A working hypothesis on bacterial-epithelial interactions during gut colonization*



## *Many questions remain*

- How do bacteria elicit distinct epithelial responses?
- How do we distinguish the initial response to bacteria from the response to cytokines induced by bacteria?
- Does the epithelial response to initial colonists shape the environment in ways that affect subsequent colonists?
- Does the epithelial response reflect microbial community composition?



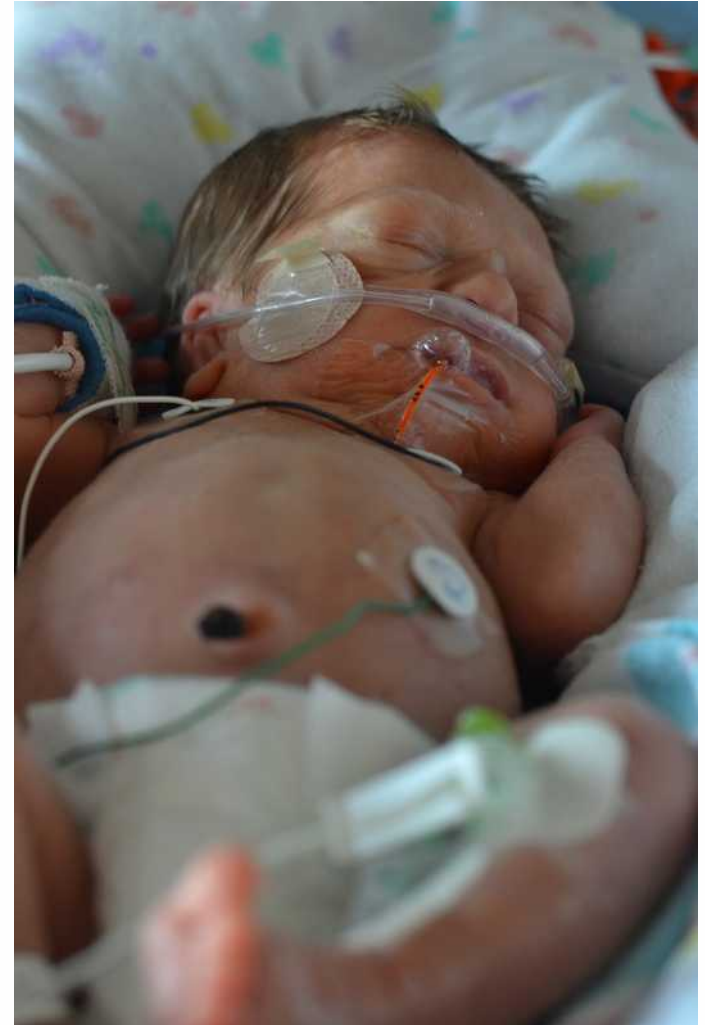


# **Extending the HIO platform for application to clinical sample sets**



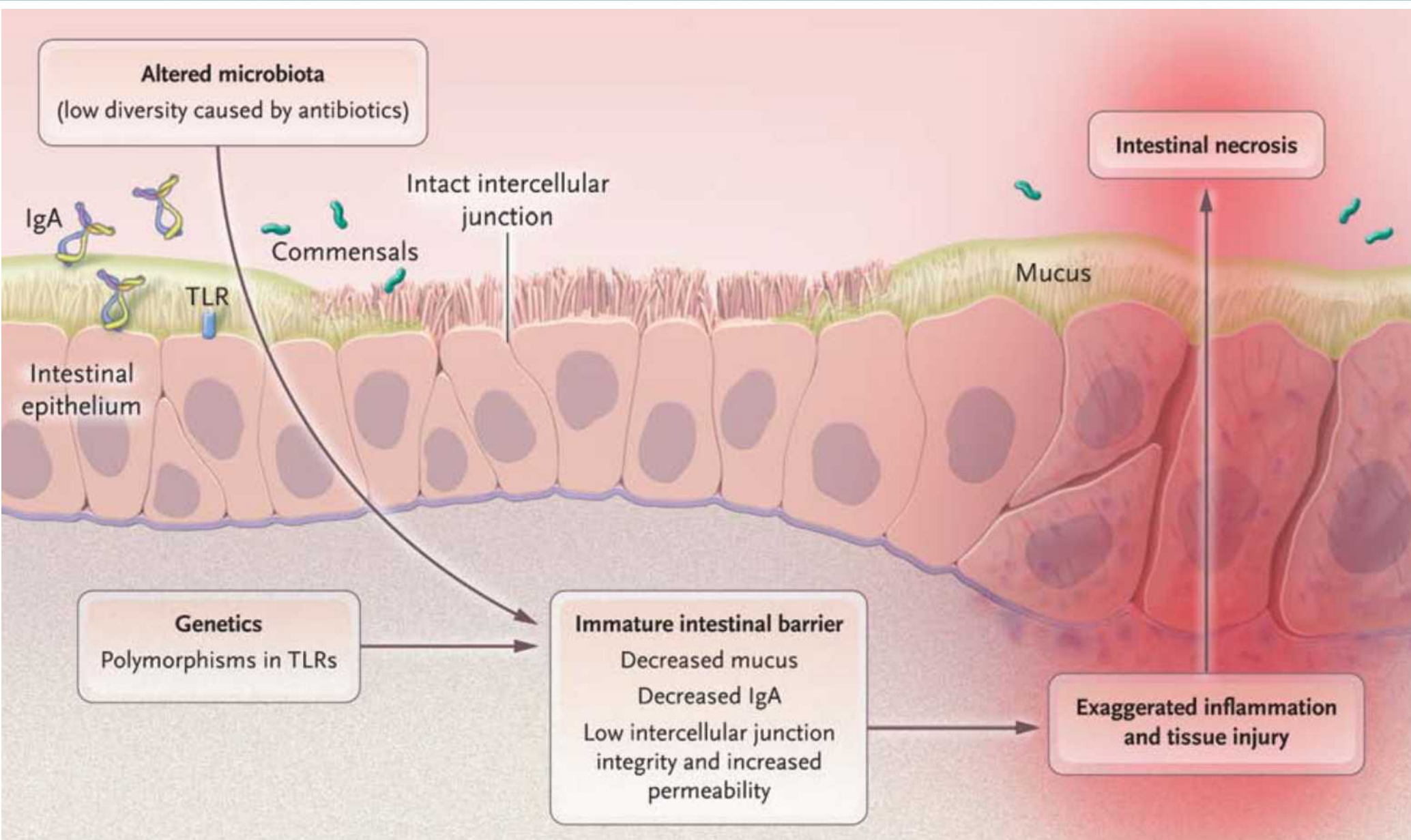
# Necrotizing enterocolitis is a critical health challenge

- Severe intestinal inflammation and necrosis
- Affects up to 0.5% of US newborns
- 7-fold elevated risk among premature and low birth weight infants
- In-hospital mortality of up to 30%
- Lifelong complications and disability among survivors



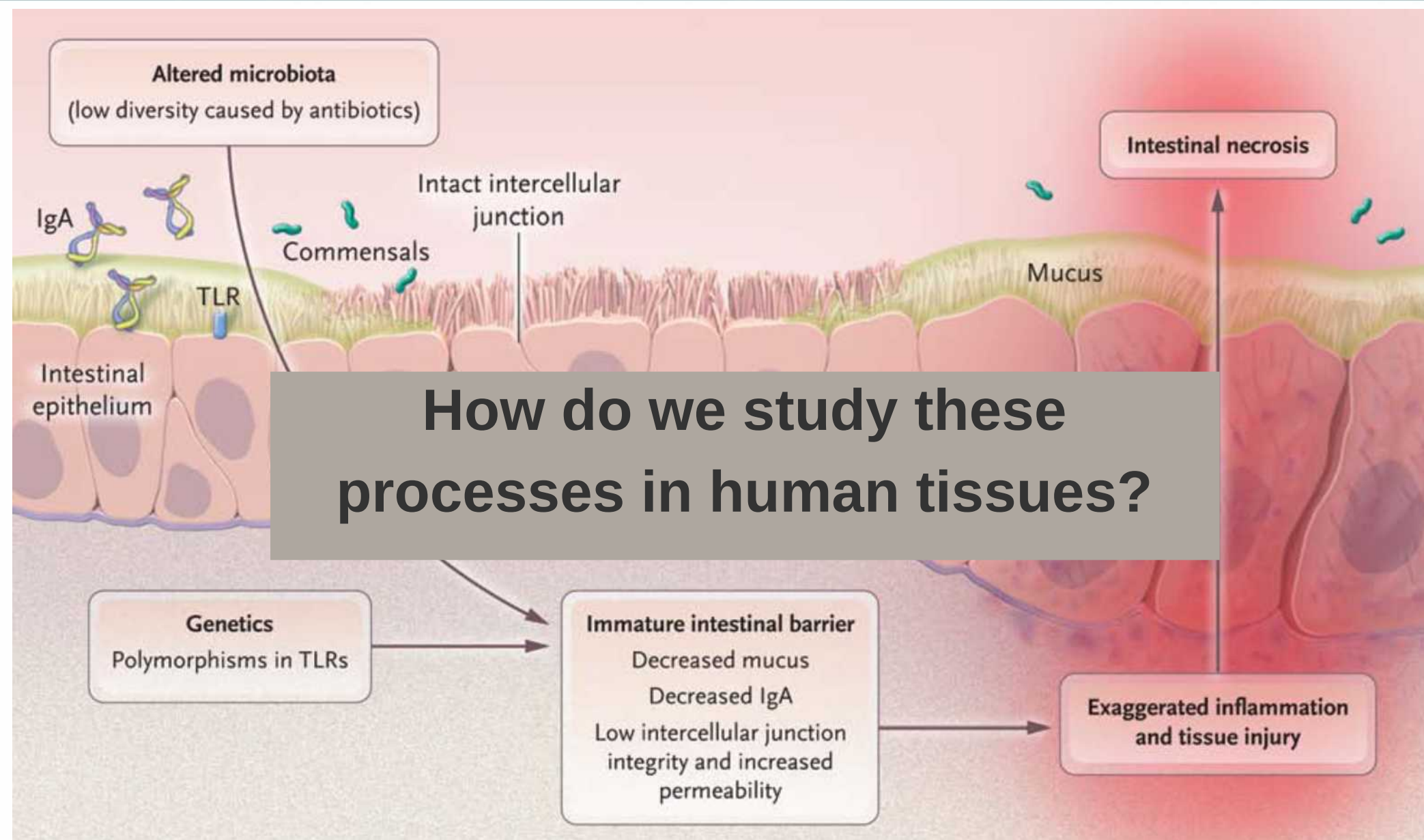
***There has been no improvement in NEC incidence or mortality in over 40 years***

# NEC results from intestinal immaturity and aberrant microbial colonization



Neu, J. & Walker, W. A. Necrotizing enterocolitis. *N. Engl. J. Med.* 364, 255–64 (2011).

# NEC results from intestinal immaturity and aberrant microbial colonization



Neu, J. & Walker, W. A. Necrotizing enterocolitis. *N. Engl. J. Med.* 364, 255–64 (2011).



# Metagenomic Sequencing with Strain-Level Resolution Implicates Uropathogenic *E. coli* in Necrotizing Enterocolitis and Mortality in Preterm Infants

Doyle V. Ward,<sup>1,4,\*</sup> Matthias Scholz,<sup>2,4</sup> Moreno Zolfo,<sup>2</sup> Diana H. Taft,<sup>3</sup> Kurt R. Schibler,<sup>3</sup> Adrian Tett,<sup>2</sup> Nicola Segata,<sup>2,5</sup> and Ardythe L. Morrow<sup>3,5</sup>

<sup>1</sup>Center for Microbiome Research, University of Massachusetts Medical School, Worcester, MA 01655, USA

<sup>2</sup>Centre for Integrative Biology, University of Trento, Trento, TN 38123, Italy

<sup>3</sup>Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

<sup>4</sup>Co-first author

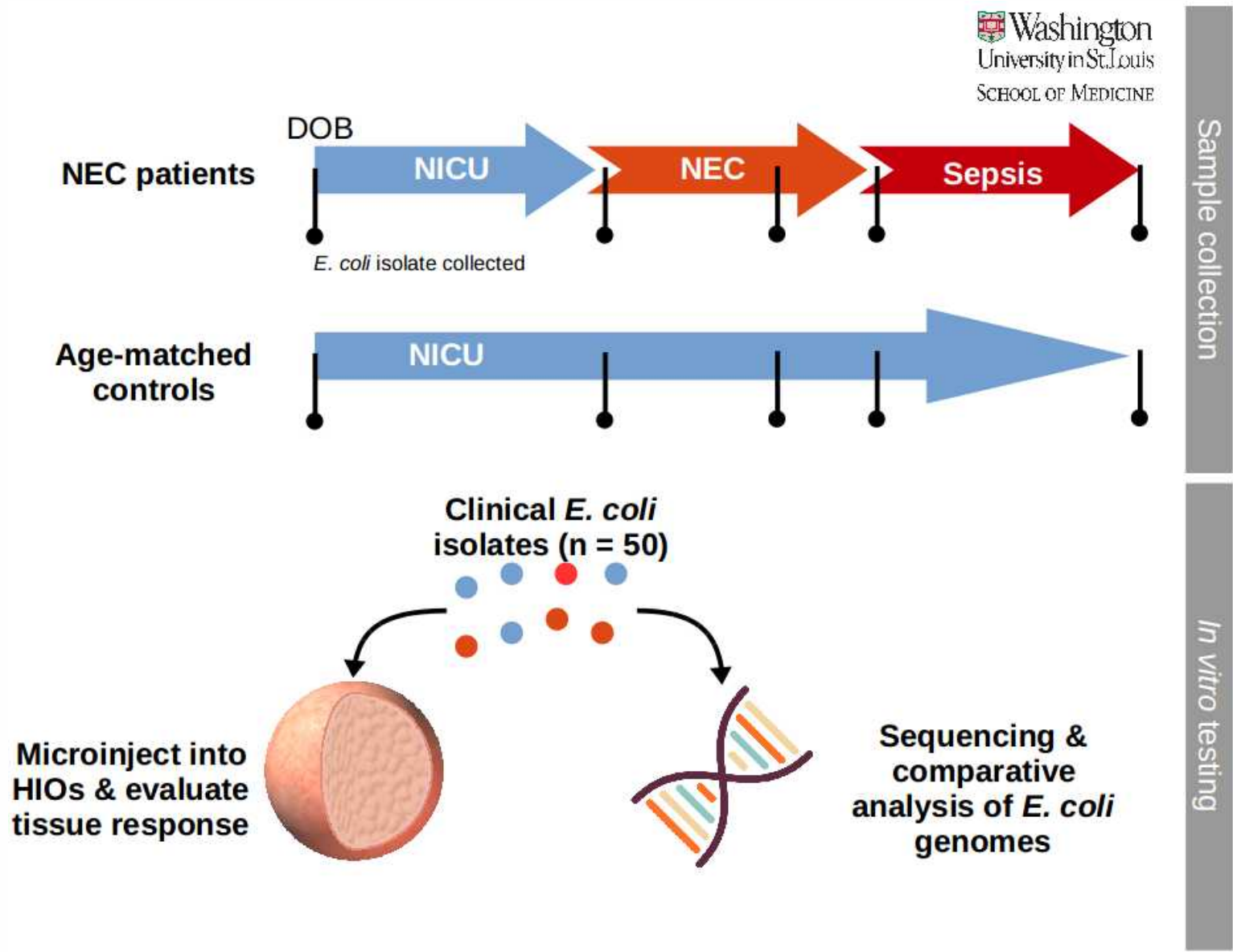
<sup>5</sup>Co-senior author

\*Correspondence: [doyle.ward@umassmed.edu](mailto:doyle.ward@umassmed.edu)

<http://dx.doi.org/10.1016/j.celrep.2016.03.015>

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# Extending the HIO platform for application to clinical sample sets



**GOAL: Identify and characterize clinical *E. coli* strains with physiologically relevant effects on the immature intestinal epithelium**

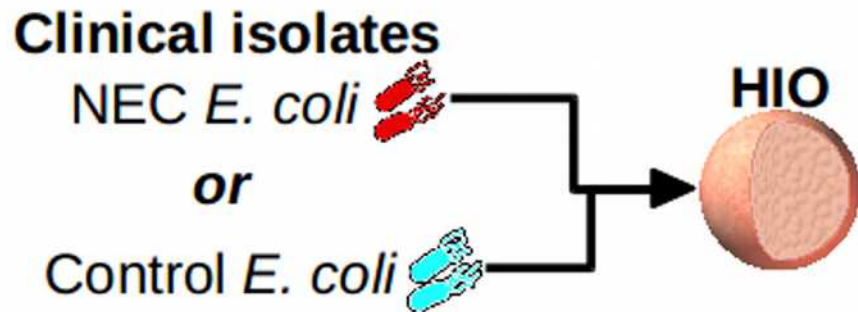
# Description of clinical isolates

Table 1: Clinical *E. coli* isolates cohort

Clinical status	Unique Isolates <i>n</i>	DOL* acquisition mean (range)	DOL NEC onset mean (range)
Non-NEC	19	33.7 (8-59)	-
NEC	28	26.4 (2-58)	31.2 (17-61)
<i>post</i> -NEC	5	33.3 (20-58)	31.6 (17-56)
<i>pre</i> -NEC	23	24.9 (2-56)	31.1 (17-61)
<b>Total</b>	47	29.3 (2-59)	31.2 (17-61)

\* Day of Life or days post-partum

# Elucidating the mechanistic connections between tissue response, *E. coli* genetics, and clinical outcomes

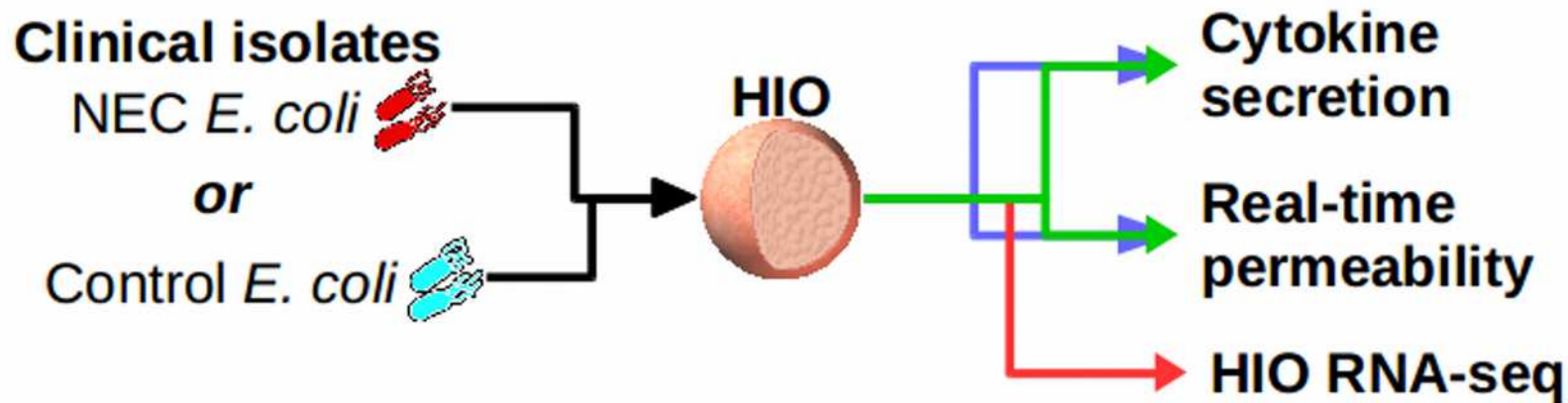


## GOALS:

- Evaluate effect of clinical *E. coli* isolates on HIO
- Identify *E. coli* genetic factors that predict the HIO response



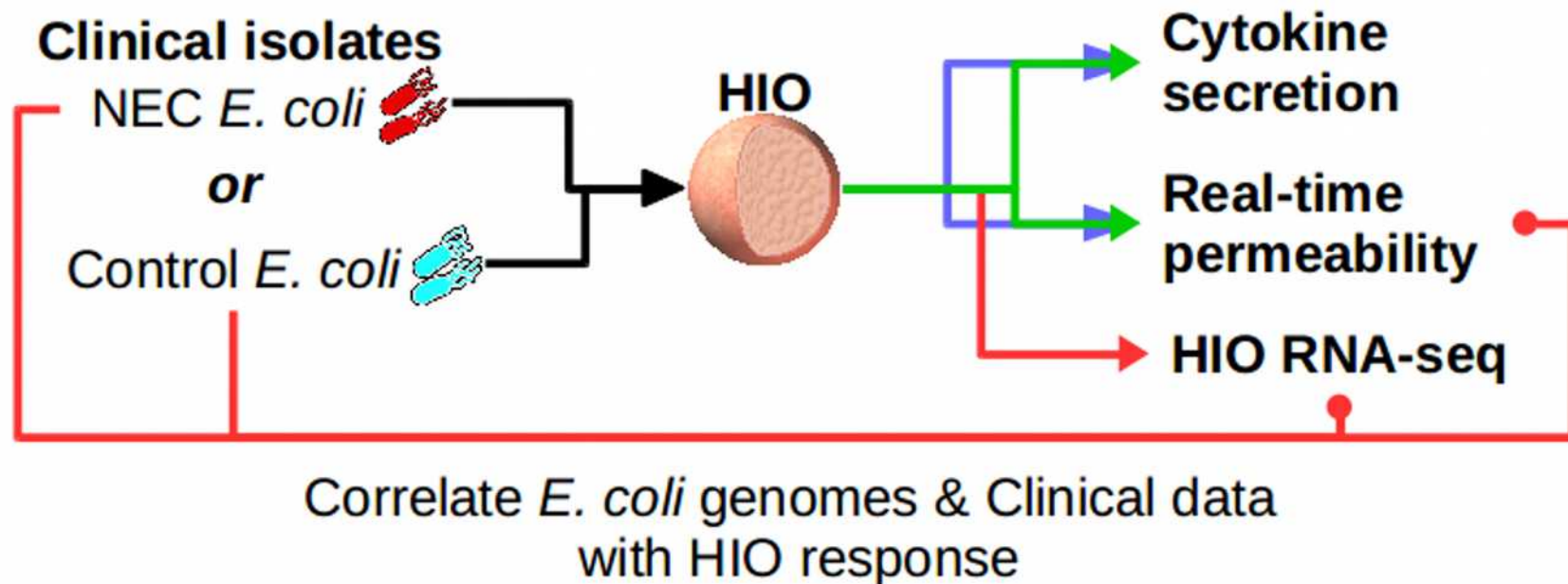
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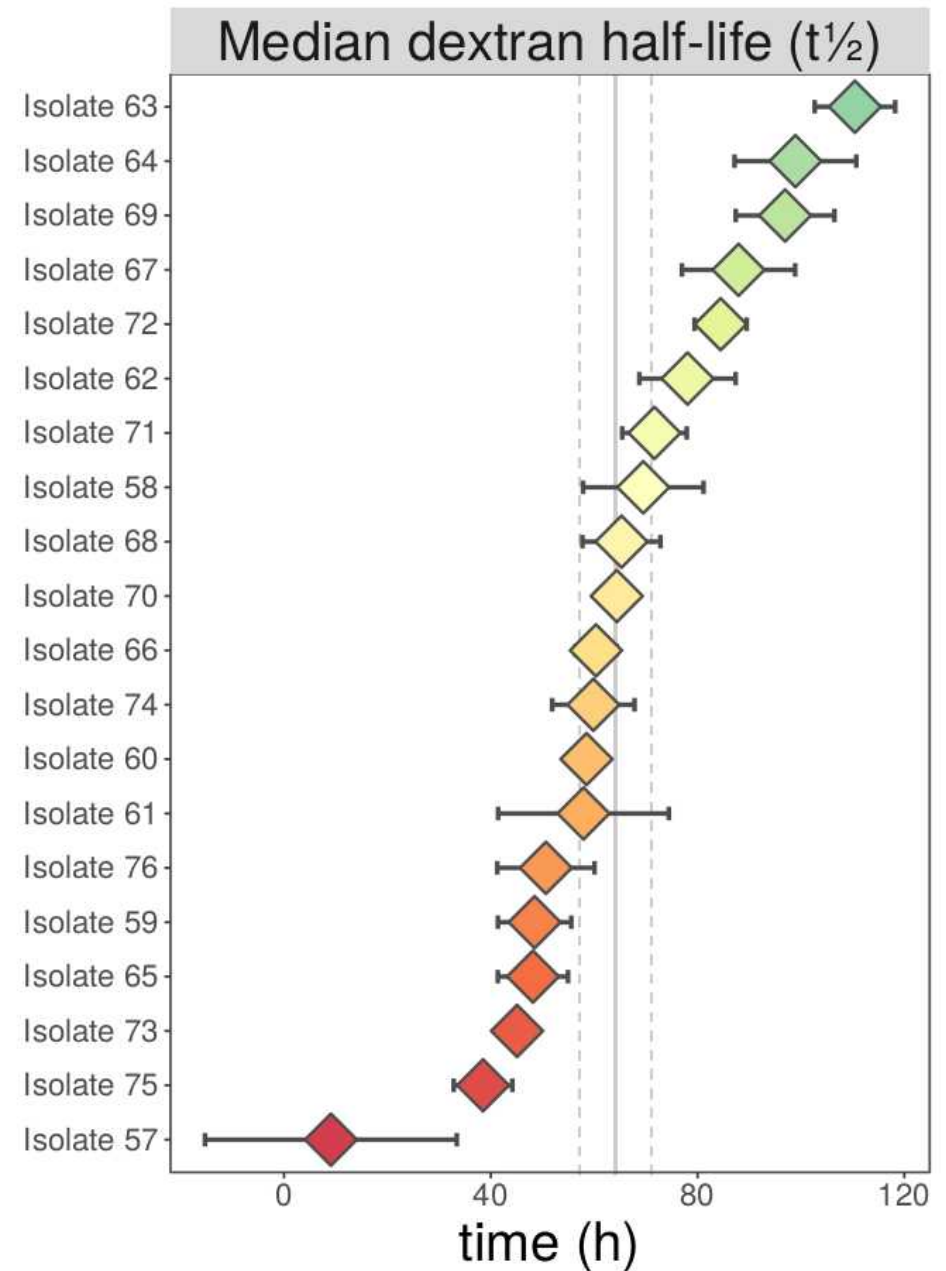
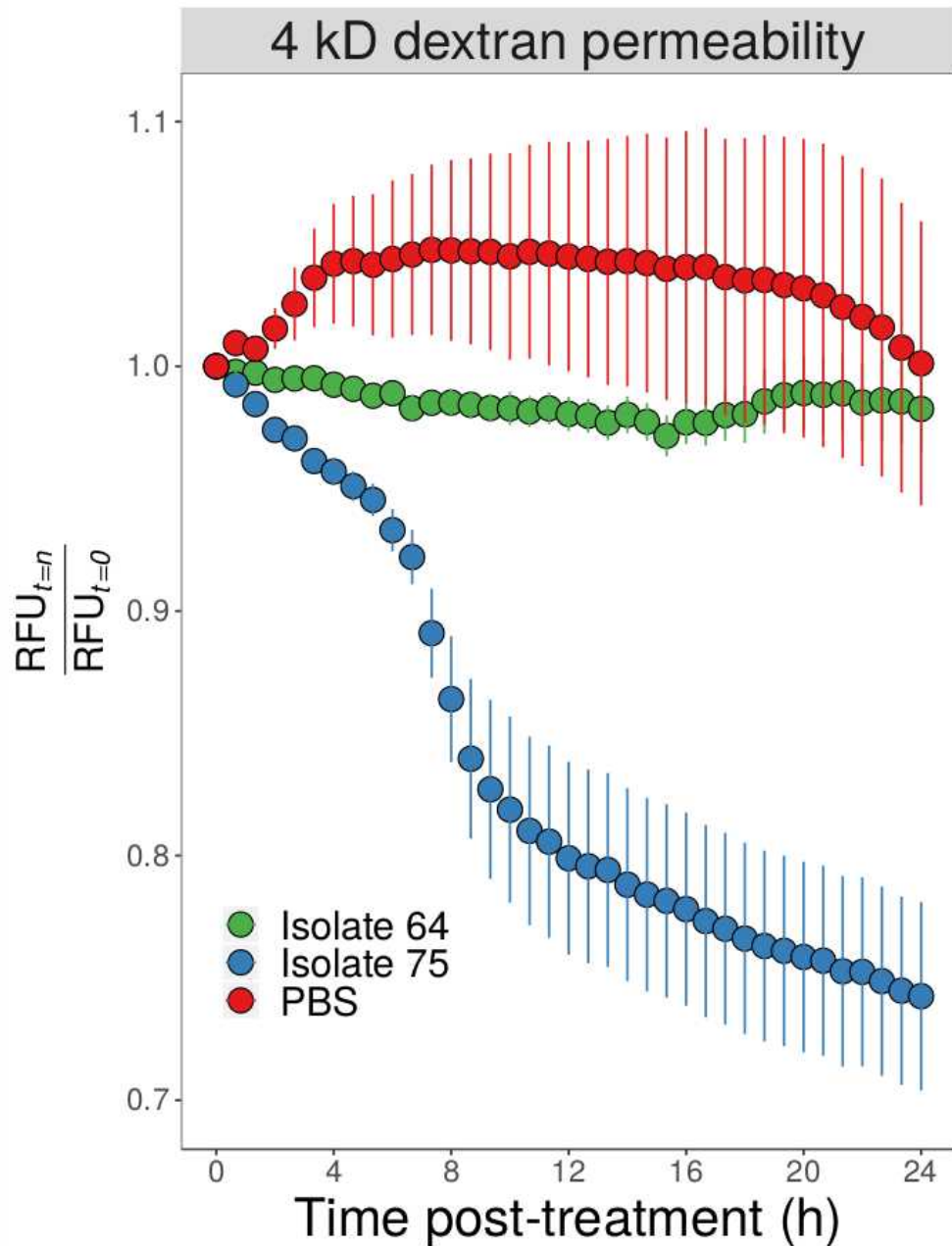
# Elucidating the mechanistic connections between tissue response, *E. coli* genetics, and clinical outcomes



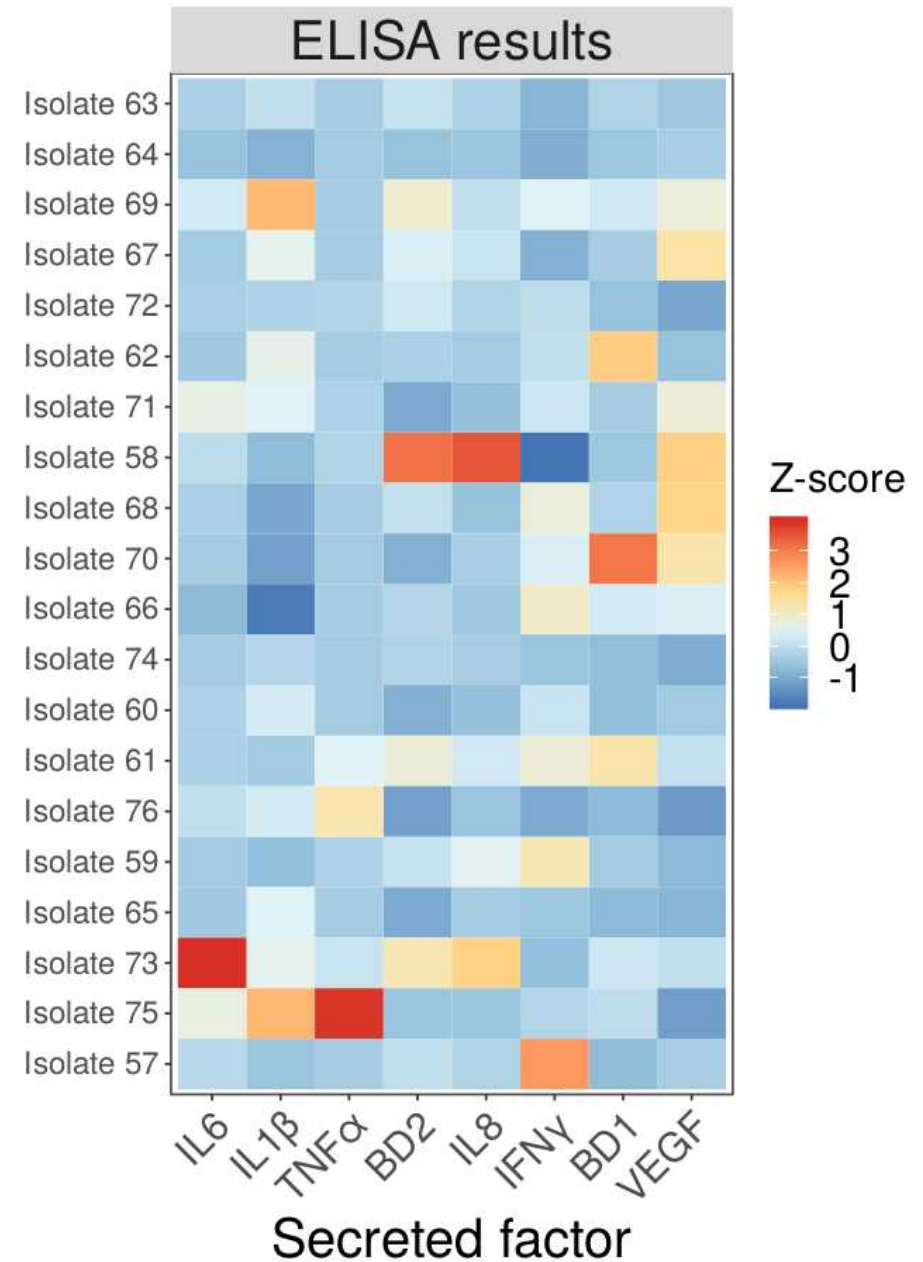
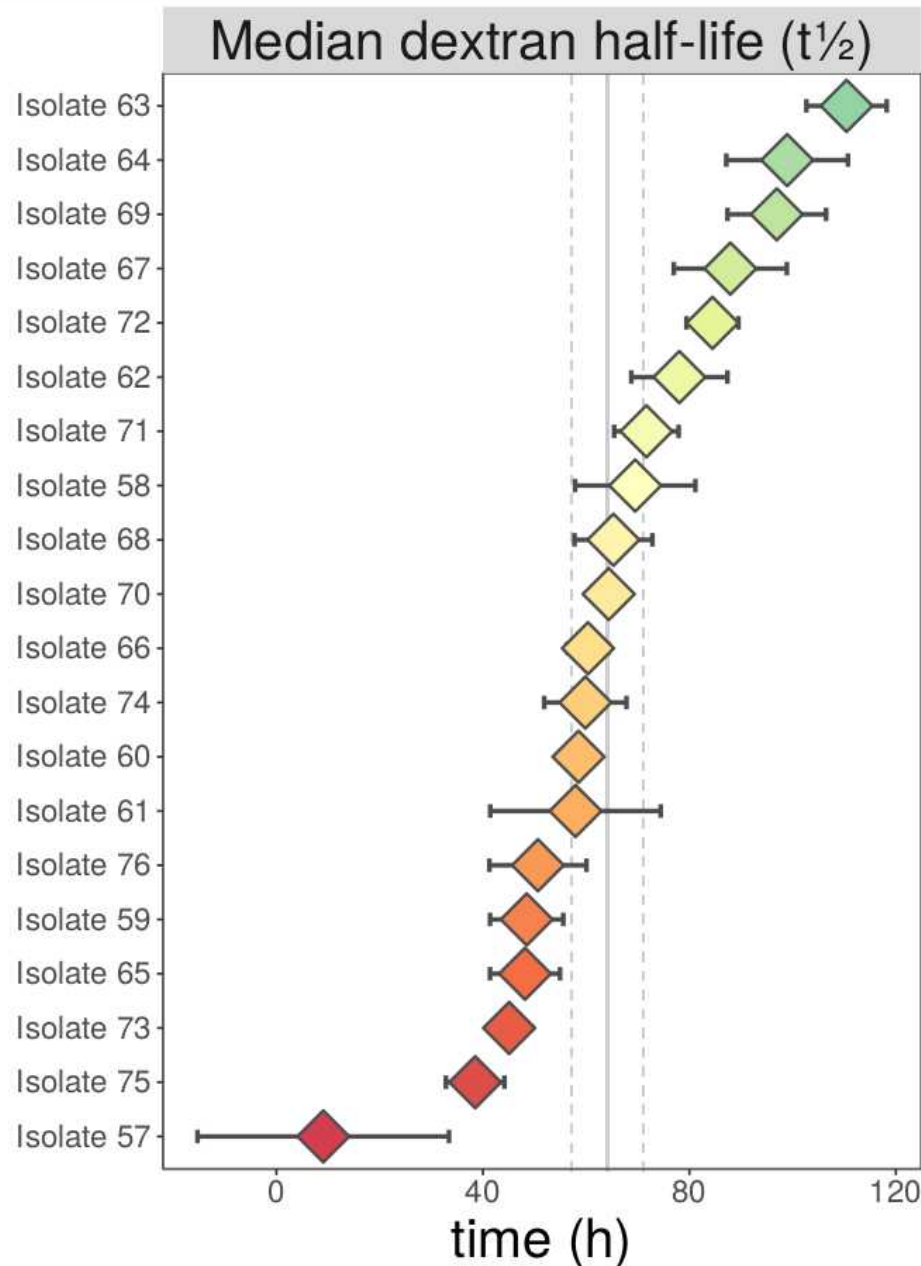
## GOALS:

- Evaluate effect of clinical *E. coli* isolates on HIO
- Identify *E. coli* genetic factors that predict the HIO response

# Screening distinct *E. coli* isolates for effects on epithelial barrier permeability in HIOs

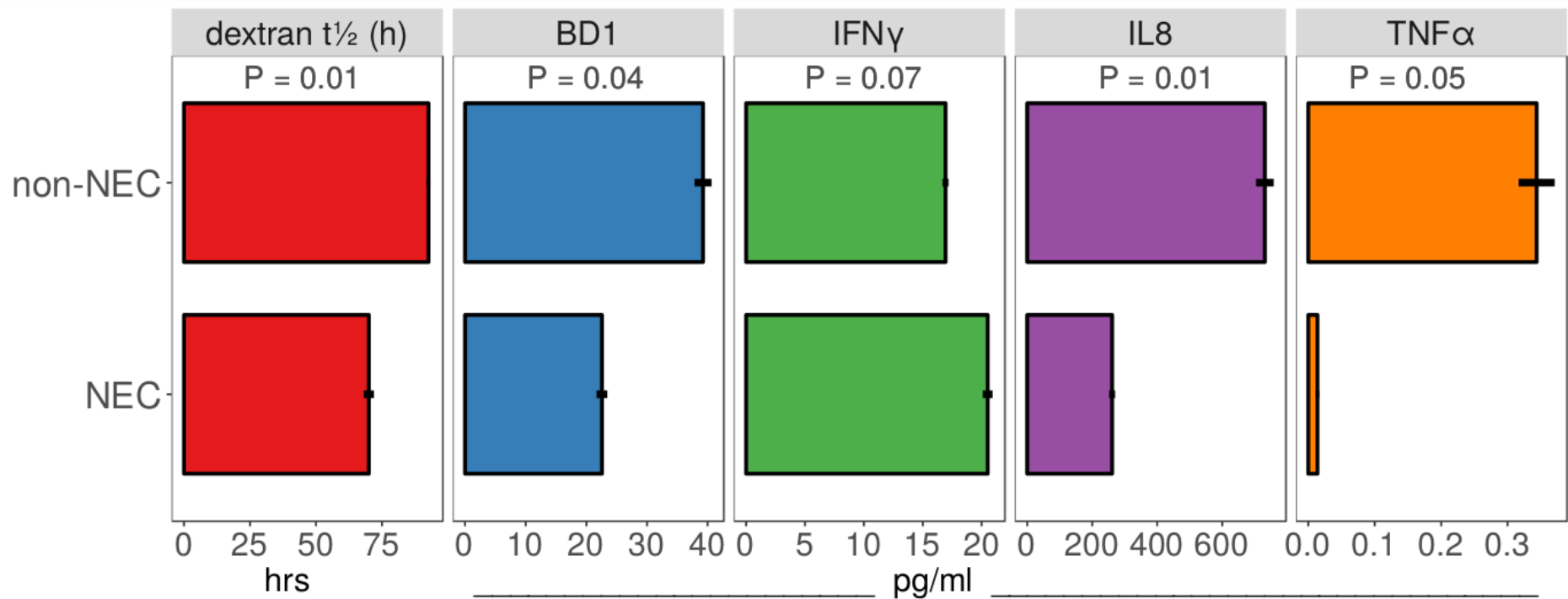


# Clinical *E. coli* isolates elicit distinct patterns of cytokine secretion

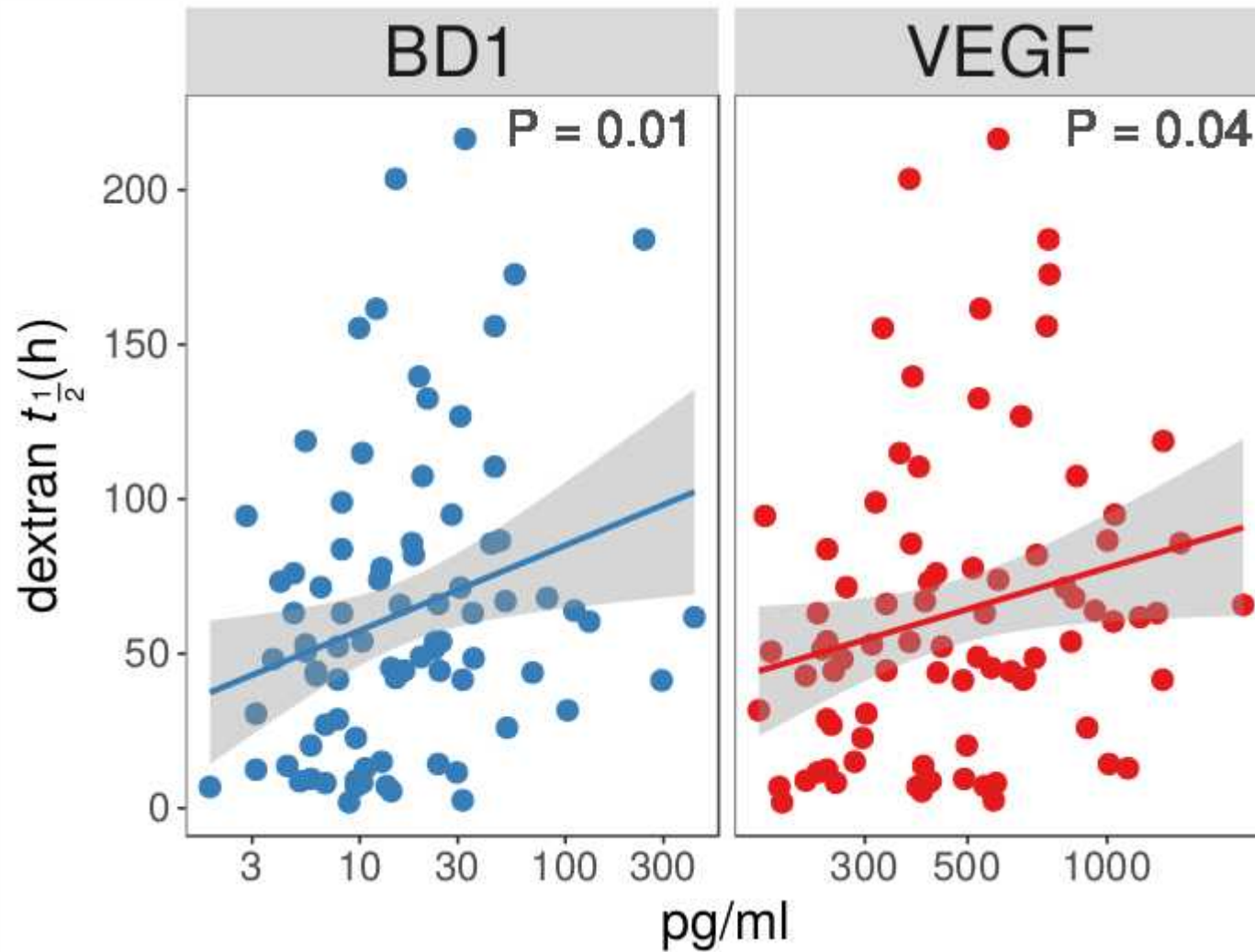




# Correlating clinical status with HIO response



# Correlating epithelial barrier permeability with epithelial secretions



# A Web of Correlated Data

***...and the tools to test those correlations directly***



## ***Ongoing work***

- Sequence, assemble, and annotate genomes for all clinical isolates (Dr. Roberto Cieza)
- Correlate genomic features with HIO response and identify genes associated with barrier function and cytokine secretion
- Test role of specific bacterial and host genes *in vitro* (Molecular Koch's postulates)
  - Can we identify bacterial genes associated with epithelial barrier integrity?
  - Are these bacterial genes associated with NEC risk in clinical populations?

# HIOs are a flexible and modular model system

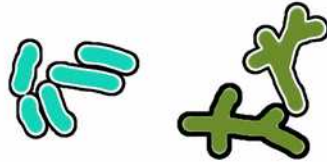
## Microorganism(s)

**Genetics:** Reference strains  
Clinical isolates  
Genetically engineered

**Products:** Metabolites  
PAMPs  
toxins

### Microbial communities

#### Symbionts



#### Pathogens

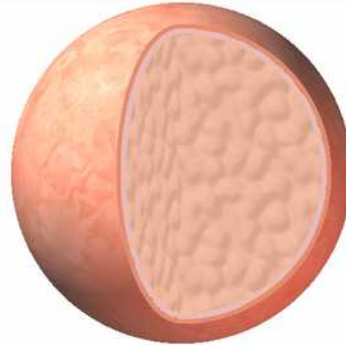


## Organoid

**Source:** Tissue  
iPSCs  
ESCs

**Genetics:** Standardized cell lines  
Patient genotype  
Genetically engineered

**Structure:** 3D spheroid  
2D monolayer



## ECM

Matrix composition  
Biologic or synthetic origin



## Media

Growth factors  
Cytokines  
Microbial products  
pharmacological agents  
pH, hypoxia







## **Young Laboratory**

Vince Young  
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