Bacteria engineering for diagnostics and therapeutics

TJC 20220712

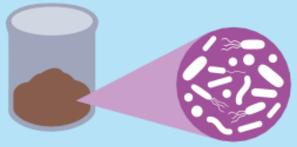
Hui Zhang

Applications of engineered bacteria

- Bacteria engineered to deliver therapies that degrade in the stomach or bloodstream.
- Achieve effective treatment with reduced systemic drug exposure.
- Record transient signals, such as reactive inflammatory metabolites, for noninvasive testing.

Microbiome-based therapeutics

Faecal microbiota transplantation



- Transfer of faeces or complex communities derived by in vitro culture or purification of spores
- Demonstrated efficacy for treatment of recurrent *Clostridioides difficile* infections

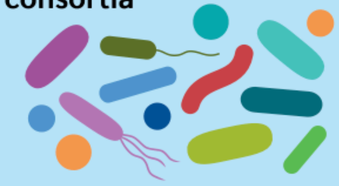
- Advantages: transfer of intact community, proven efficacy in clinic
- Challenges: screening of donor samples, scalability, potential variability in efficacy depending on donor

Diet and prebiotics

- Supplementation of microbiota-targeted substrates, such as specific dietary fibres to promote a desired compositional changes in the microbiota, or production of a desired metabolite

- Advantages: relatively easy to prepare, safety
- Challenges: predicting outcomes of supplementation across different microbiota compositions, length of impact following supplementation, targted species or activities must be present

Symbiotic microbial consortia



- Transfer of a group of isolates, selected or designed to promote specific microbiota functions

- Advantages: known composition of consortia, individual isolates and potentially self-sustaining community can be screened for safety
- Challenges: isolate selection, replicating phenotypes emerging from complex bacterial interactions, growing desired isolates in culture

Engineered symbiotic bacteria

- Transfer of bacteria that colonize the targeted site and are engineered to have a desired function or deliver a desired product or metabolite

- Advantages: potential for producing desired metabolites or compounds in the correct location using a platform strain background that could be engineered for multiple purposes
- Challenges: limited ability to manipulate many species of the microbiota, have to demonstrate safety of modifications

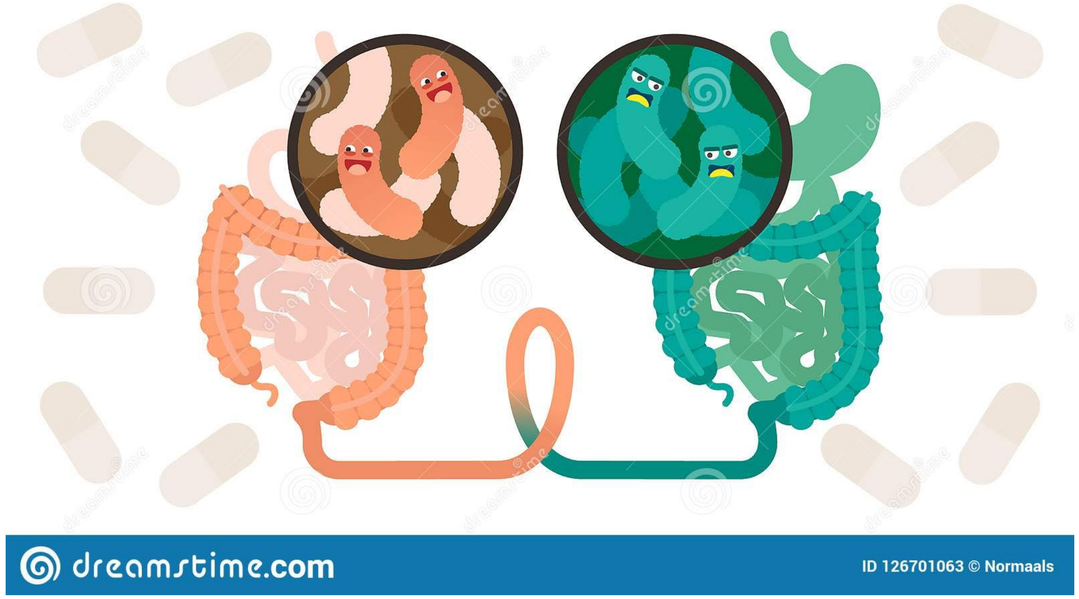
Microbiota-derived proteins and metabolites

- Direct supplemention with beneficial proteins or metabolites

- Advantages: relatively easy to prepare, assess safety, likely to follow conventional pharmaceutical development pathways
- Challenges: determining and delivering adequate concentrations to desired site

Faecal microbiota transplantation

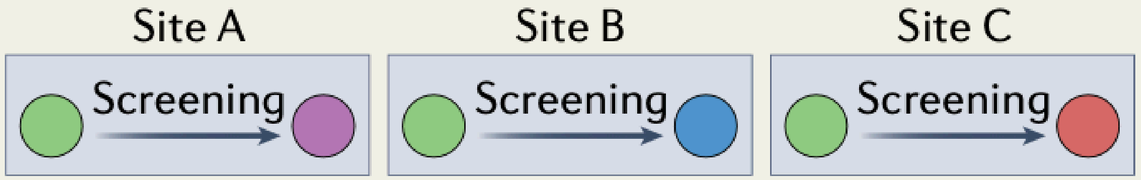
- Clostridioides difficile infection
- Inflammatory bowel disease
- Constipation
- Neurological diseases:
  - Parkinson’s disease
  - Multiple sclerosis



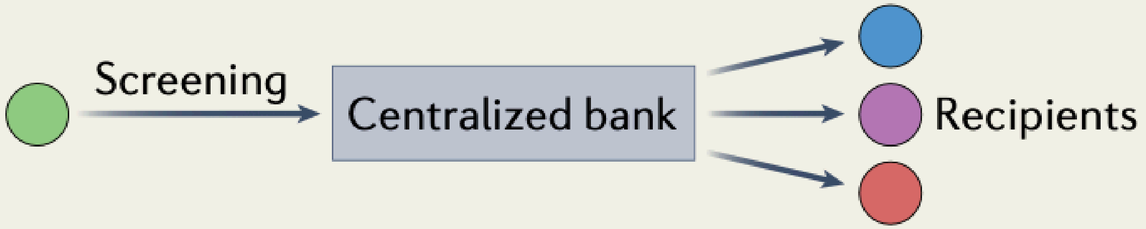
a Source

Heterologous

Patient-directed donation

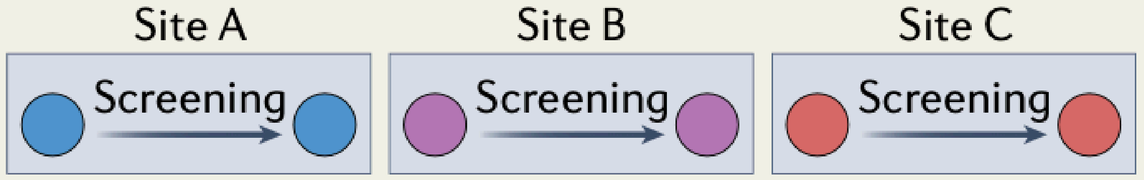


Stool bank or universal donor



Autologous

Provided by the recipient while the microbiota is in a healthy state and frozen for later administration



b Administration

Upper gastrointestinal tract

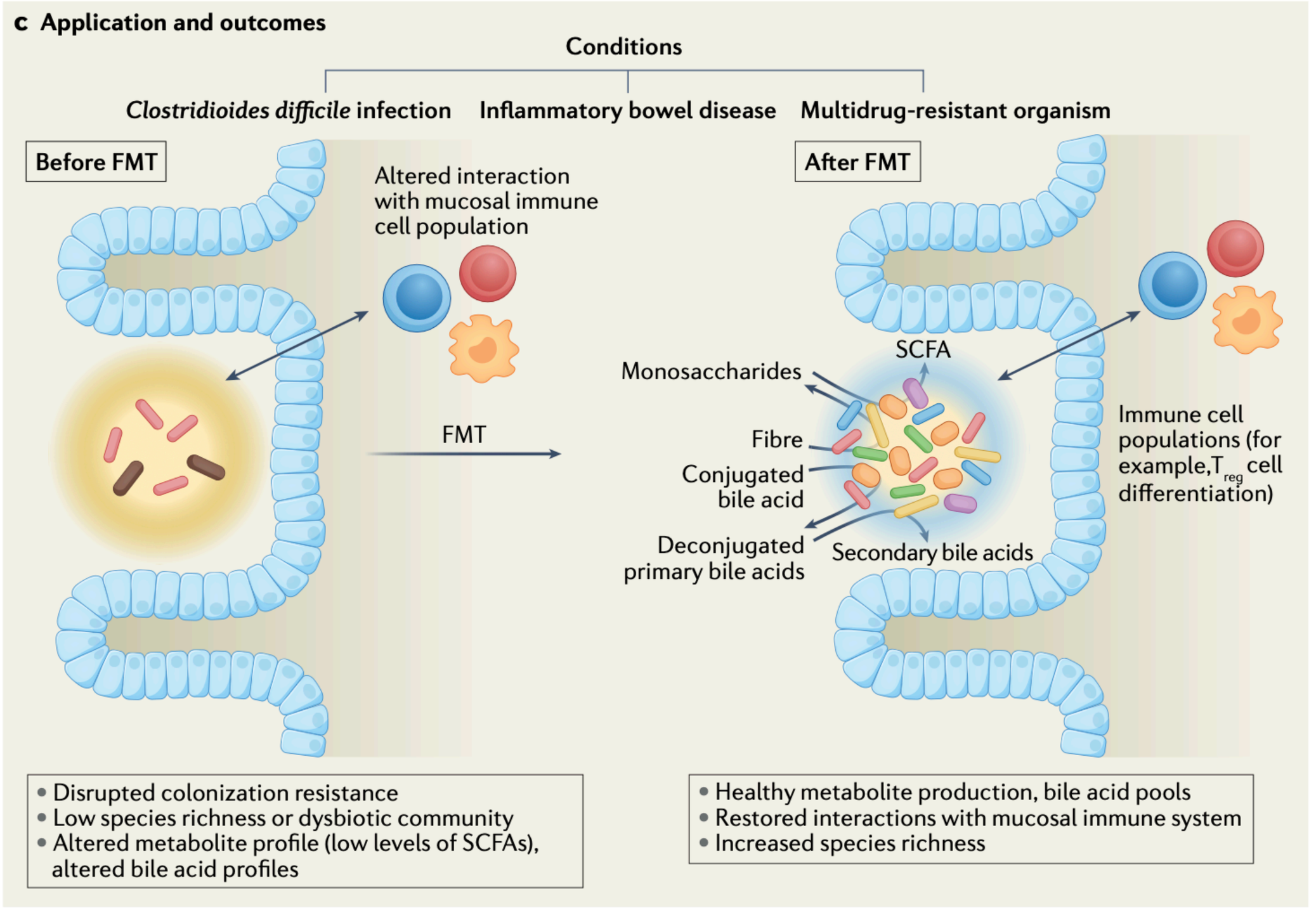
Nasoenteric tubes **Capsule delivery**

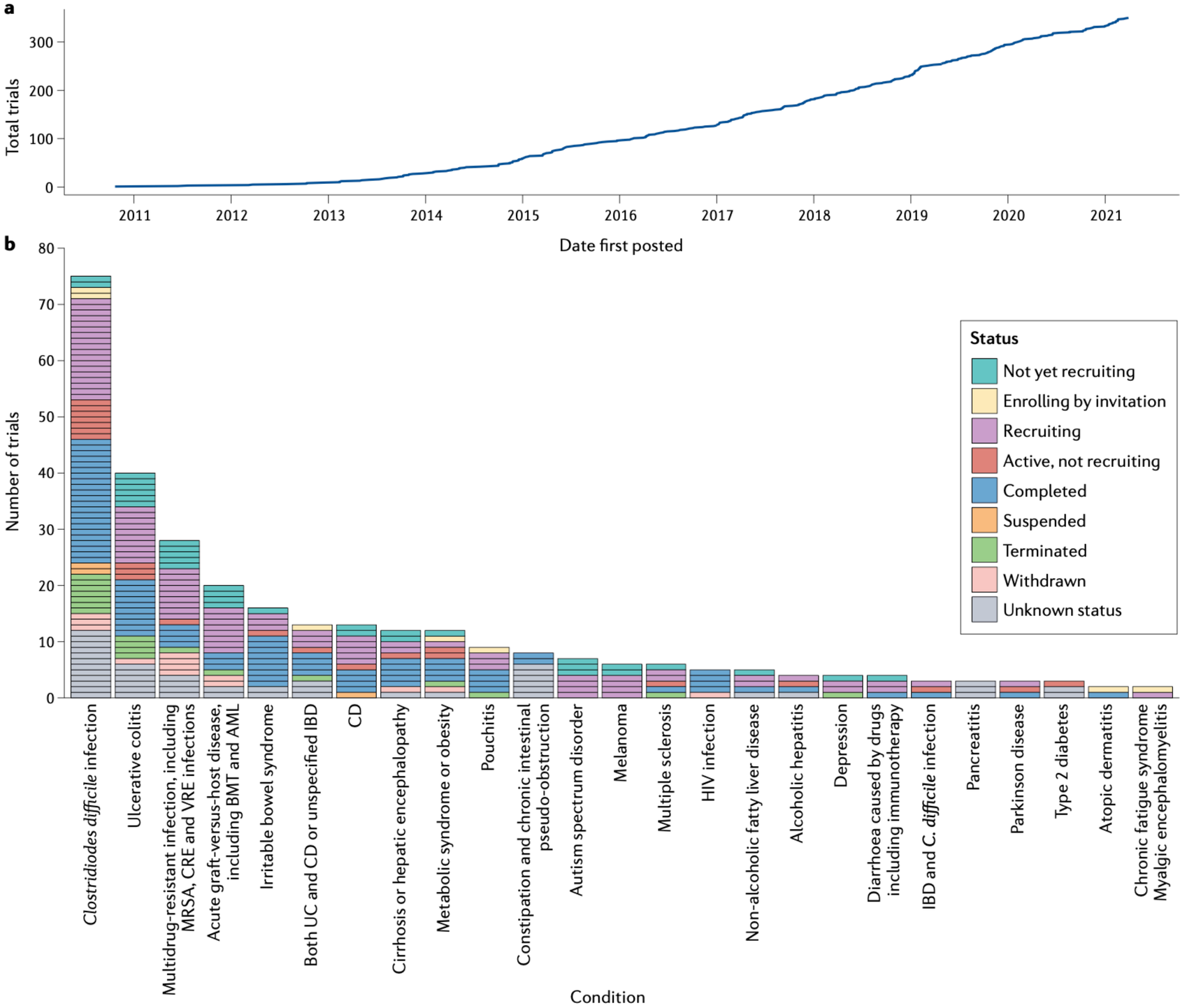
- Lower volumes, concentrated material
- Exposure of small intestinal immune cells to transferred microorganisms

Lower gastrointestinal tract

Retention enema **Sigmoidoscopy** **Colonoscopy**

- Potential for direct delivery to impacted sites
- Larger volumes possible


c Application and outcomes
Conditions
Clostridioides difficile infection
Inflammatory bowel disease
Multidrug-resistant organism
Before FMT
Altered interaction with mucosal immune cell population
FMT
After FMT
SCFA
Monosaccharides
Fibre
Conjugated bile acid
Deconjugated primary bile acids
Secondary bile acids
Immune cell populations (for example, T_{reg} cell differentiation)
• Disrupted colonization resistance
• Low species richness or dysbiotic community
• Altered metabolite profile (low levels of SCFAs), altered bile acid profiles
• Healthy metabolite production, bile acid pools
• Restored interactions with mucosal immune system
• Increased species richness


a
Total trials
300
200
100
0
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
Date first posted
b
Number of trials
80
70
60
50
40
30
20
10
0
Status
Not yet recruiting
Enrolling by invitation
Recruiting
Active, not recruiting
Completed
Suspended
Terminated
Withdrawn
Unknown status
Clostridiodes difficile infection
Ulcerative colitis
Multidrug-resistant infection, including MRSA, CRE and VRE infections
Acute graft-versus-host disease, including BMT and AML
Irritable bowel syndrome
Both UC and CD or unspecified IBD
CD
Cirrhosis or hepatic encephalopathy
Metabolic syndrome or obesity
Pouchitis
Constipation and chronic intestinal pseudo-obstruction
Autism spectrum disorder
Melanoma
Multiple sclerosis
HIV infection
Non-alcoholic fatty liver disease
Alcoholic hepatitis
Depression
Diarrhoea caused by drugs including immunotherapy
IBD and C. difficile infection
Pancreatitis
Parkinson disease
Type 2 diabetes
Atopic dermatitis
Chronic fatigue syndrome Myalgic encephalomyelitis
Condition

Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation

David T Riglar[1,2], Tobias W Giessen[1,2], Michael Baym[1,3], S Jordan Kerns[1,2,6], Matthew J Niederhuber[1,2], Roderick T Bronson[4], Jonathan W Kotula[1,2,6], Georg K Gerber[5], Jeffrey C Way[2] & Pamela A Silver[1,2]

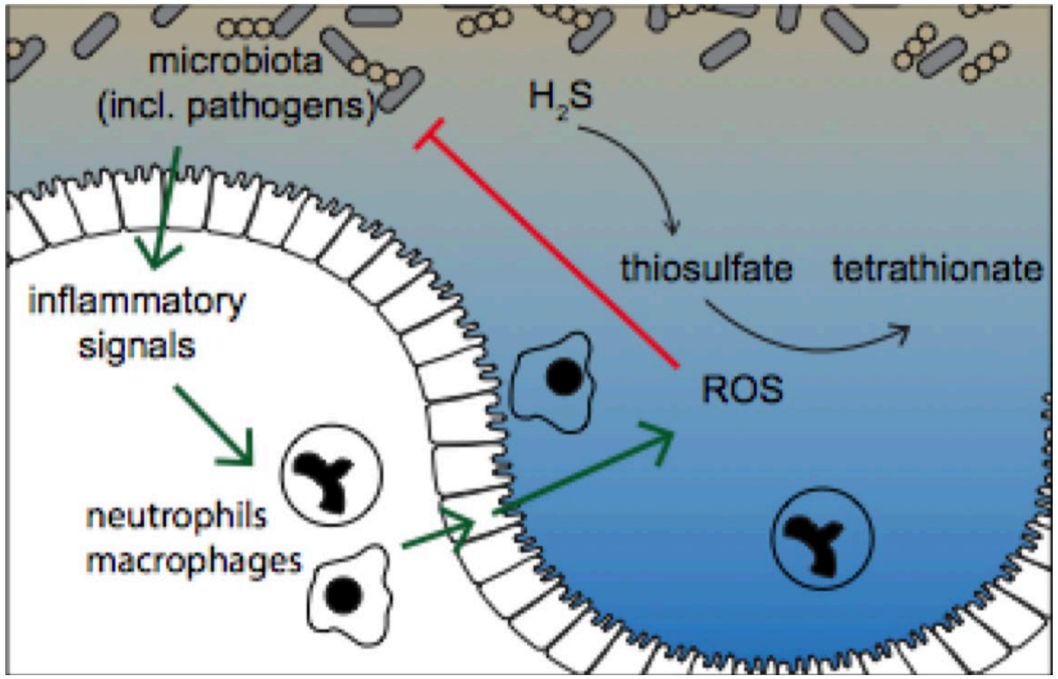
Advantages

- The gut environment is largely inaccessible.
- Products of digestion or those released by the host or microbiome can be modified by the microbiome, or absorbed by the host, before excretion.
- Live, engineered bacteria can be used as non-invasive diagnostics to detect transient (or highly localized) molecules in the gut, or as therapeutics.

Concerns about engineered bacteria

- Susceptibility of synthetic genetic circuits to mutation: loss of engineered function, lack of growth of the recombinant strain in a host and environment-dependent manner.
- Unpredictable function during extended gut colonization: cleared from the body within ~1 month.
- Stable, engineered bacterial strains that maintain their function for 6 months in the mouse gut.

Aim: to detect tetrathionate, an inflammatory marker



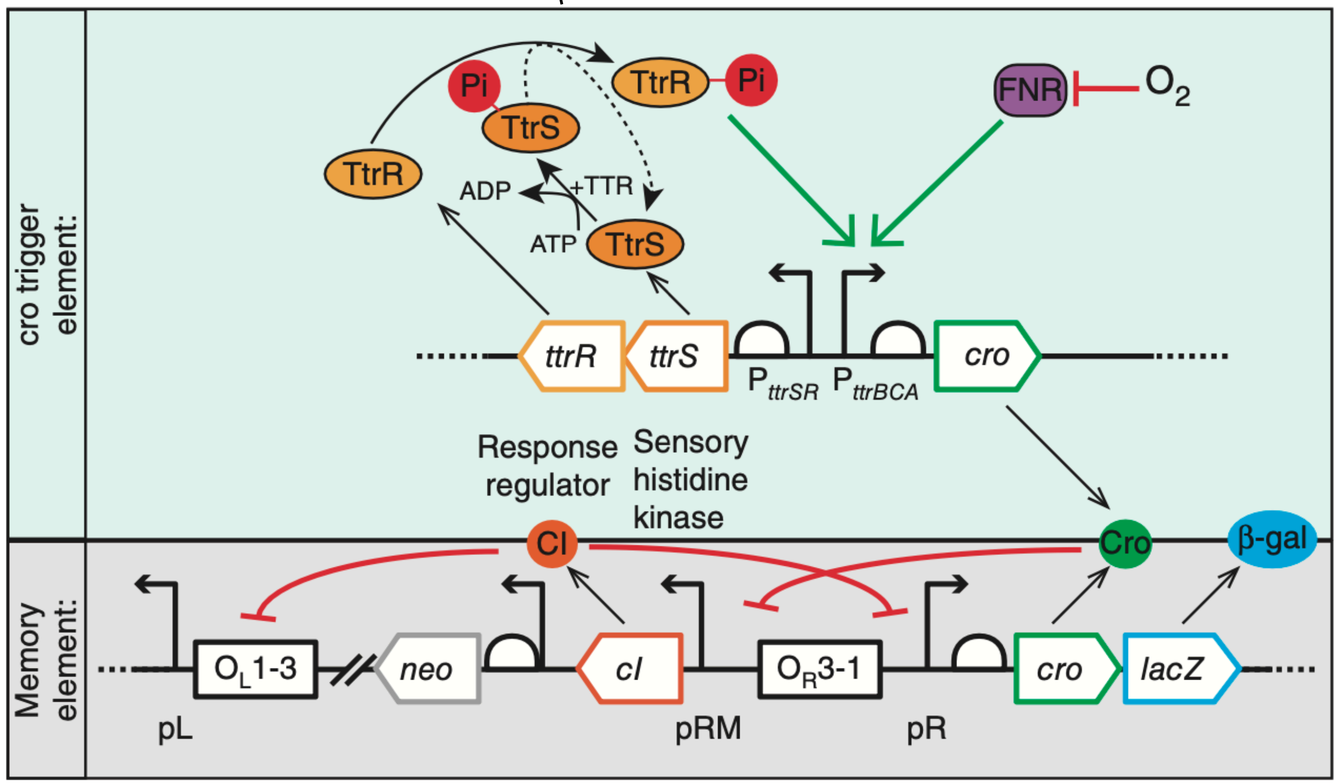
- transient product of reactive oxygen species (ROS), which are produced during inflammation, can be used as a terminal electron acceptor for anaerobic respiration, providing a growth advantage to these pathogens during inflammation.
- Induced by S. typhimuriumand Yersinia enterocolitica.
- Tetrathionate is detected in these bacteria by the TtrR/TtrS two-component system and can be used as a terminal electron acceptor for anaerobic respiration, providing a growth advantage to these pathogens during inflammation.
- A range of other bacteria, including pathogens, may also be able to grow preferentially using tetrathionate.

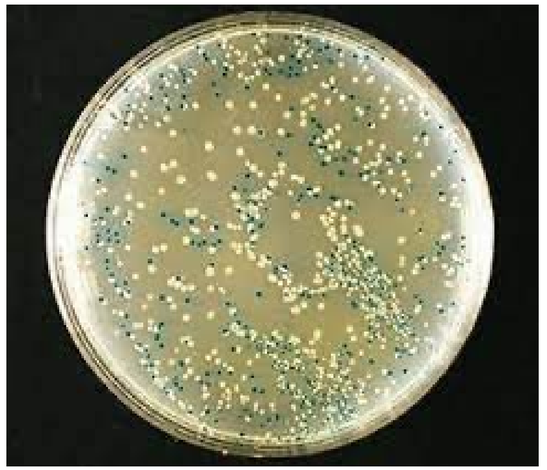
study the ability of the strain to colonise and function in the murine gut over an extended time period.

commensal mouse *E. coli* strain NGF-1 engineered to detect tetrathionate

from S. typhimurium

reduce burden and allow long- term circuit retention without selection.



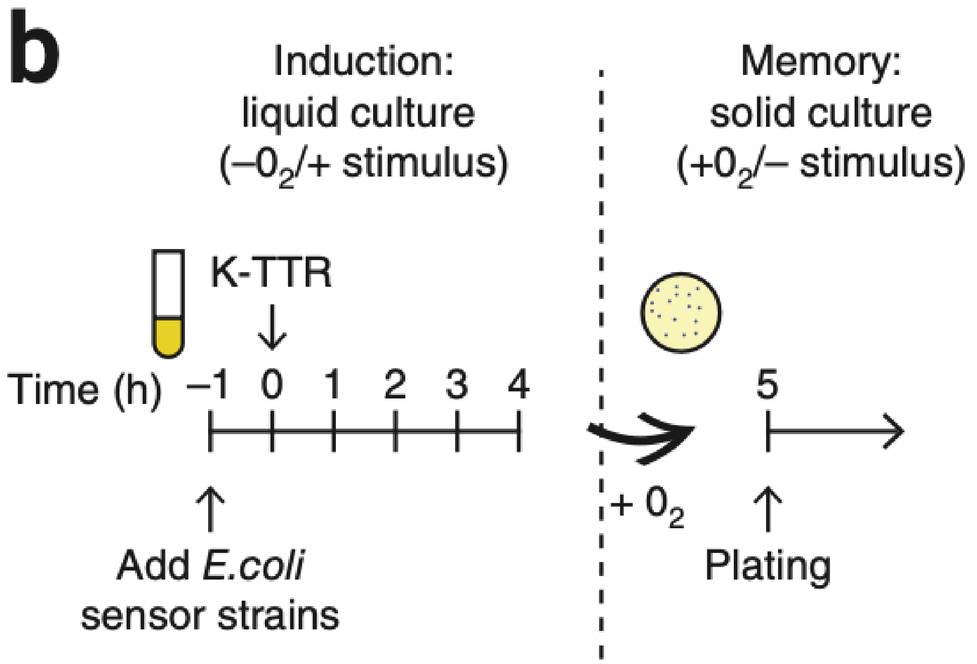


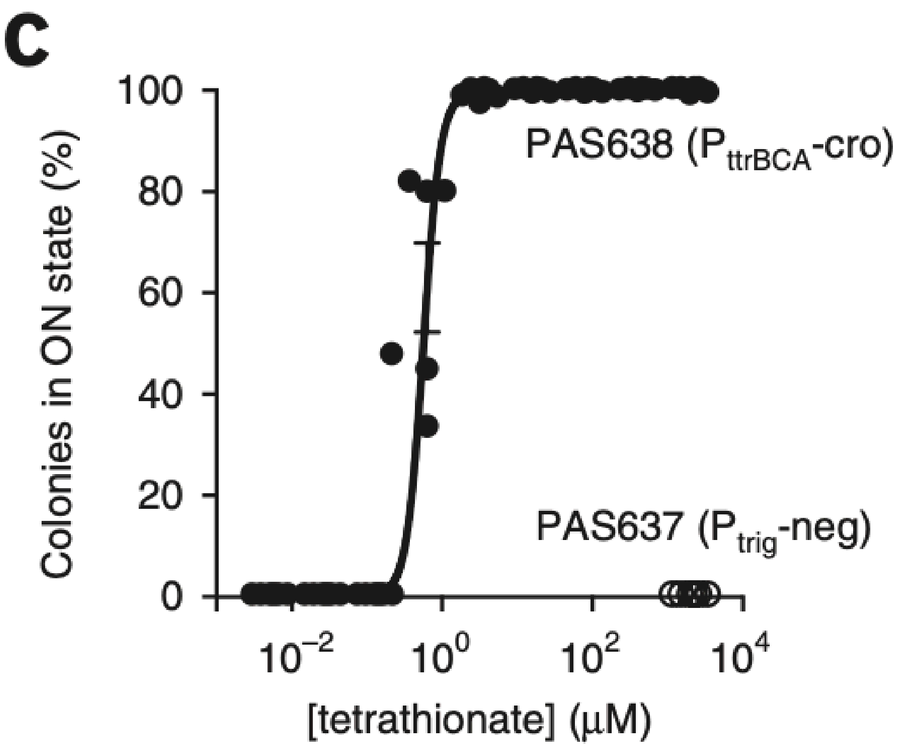
Off : CI^+/Cro and β-gal^-

On : Cro and β-gal^+/CI^-

E. coli strain PAS638

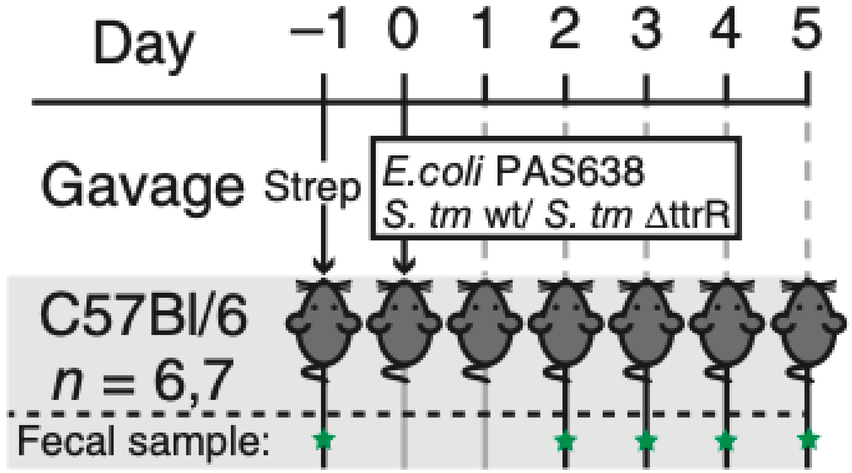
In vitro test





PAS638 could record tetrathionate exposure

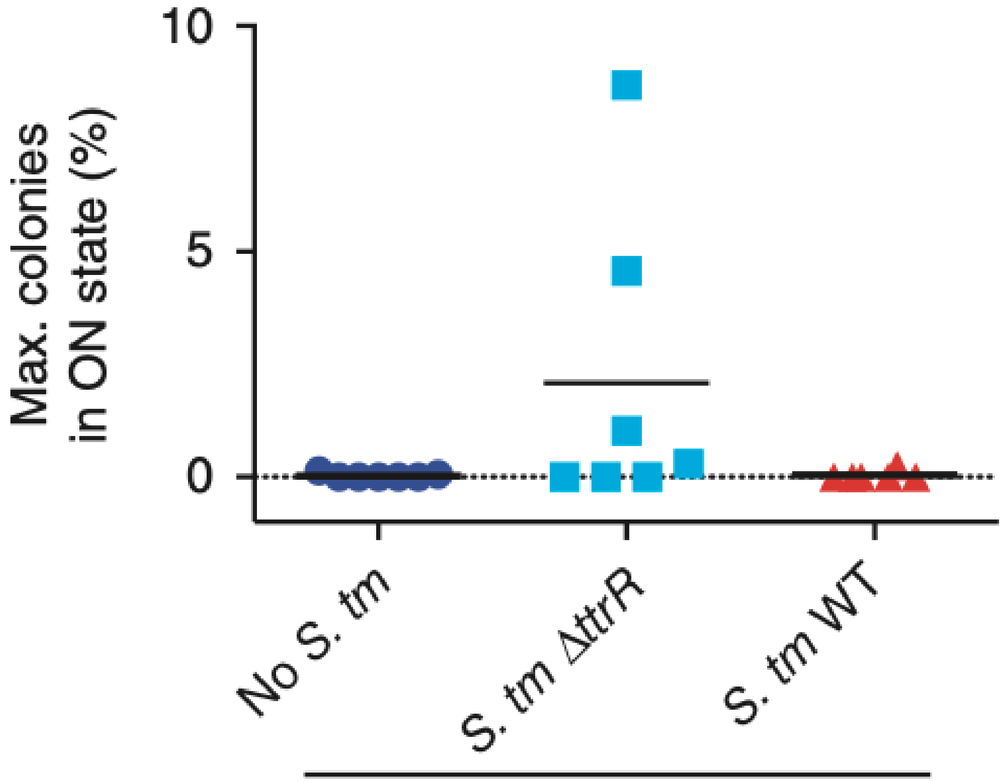
In vivo test in a murine *S. typhimurium*-induced colitis model



streptomycin treatment: to reduce colonization resistance for *S. typhimurium;*

oral gavage:

fecal samples were analyzed days 2–5 after administration

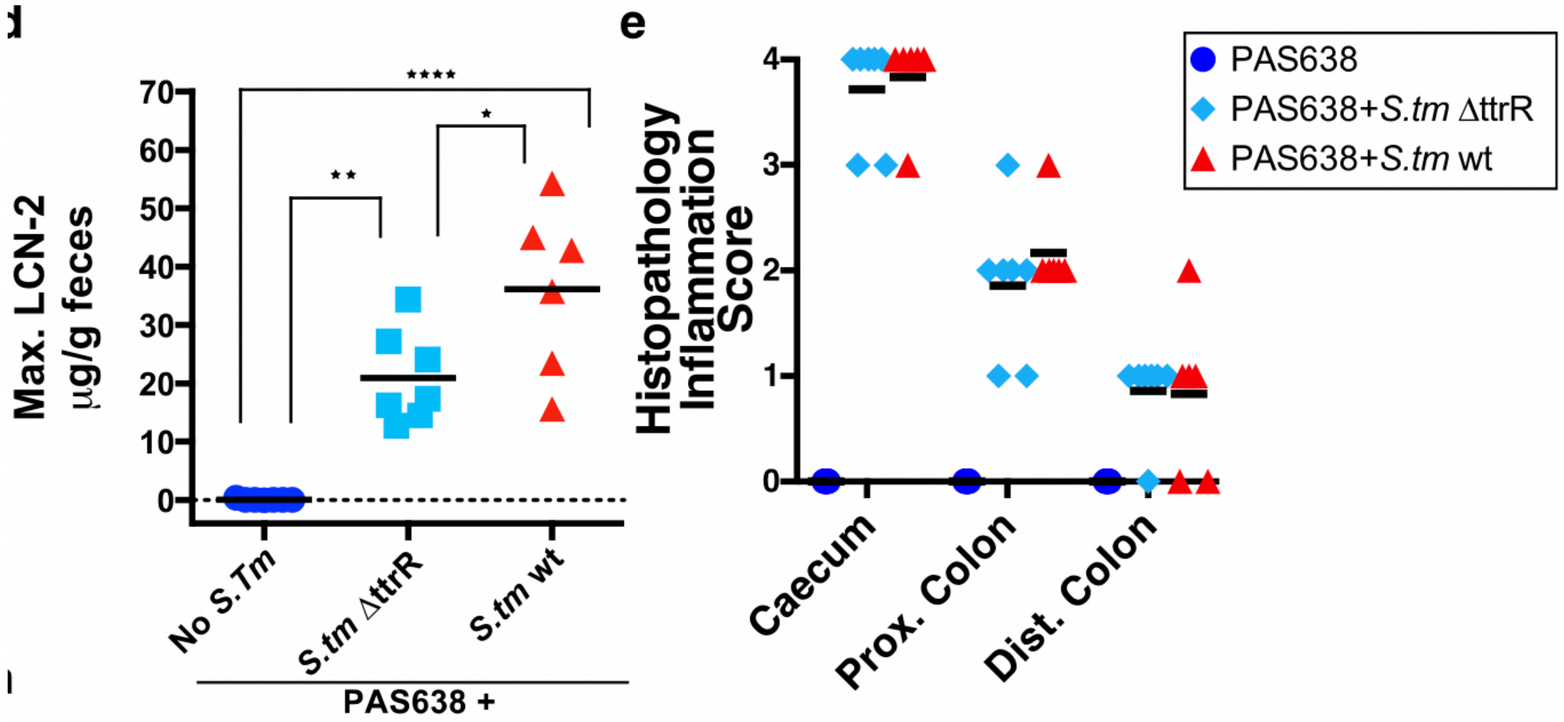


PAS638 with *S. typhimurium*

PAS638 with a *S. typhimurium* □*ttrR*

□*ttrR* variant is unable to express tetrathionate reductase

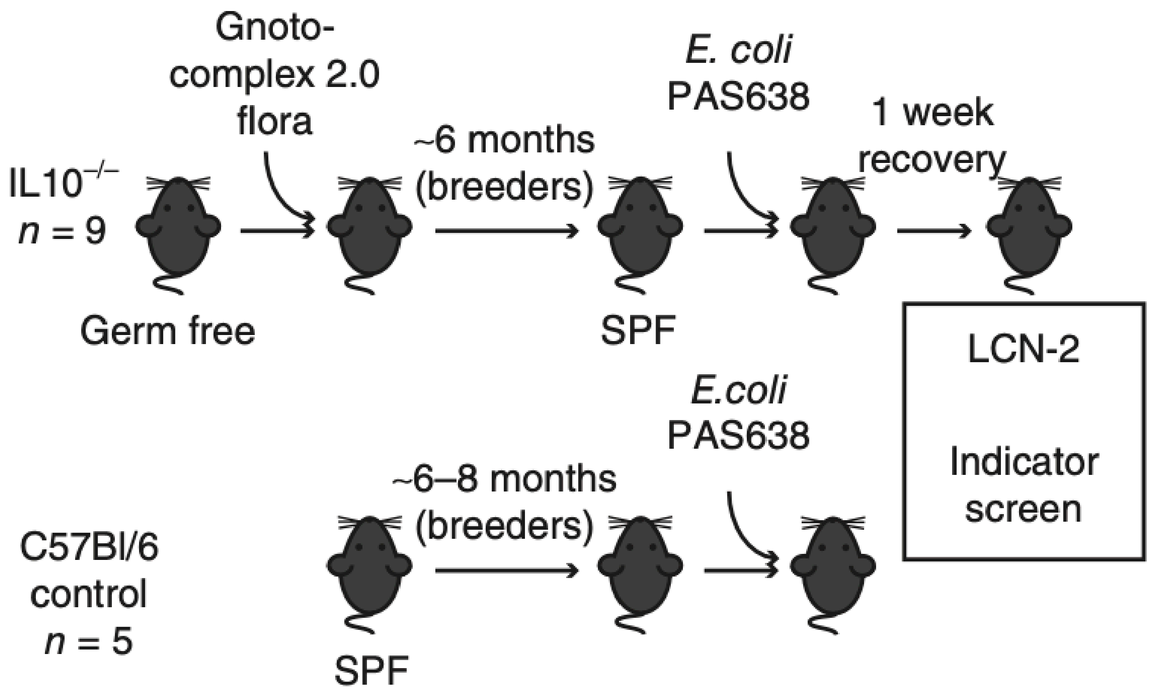
quantification of the lipocalin-2 (LCN-2) for inflammation detection

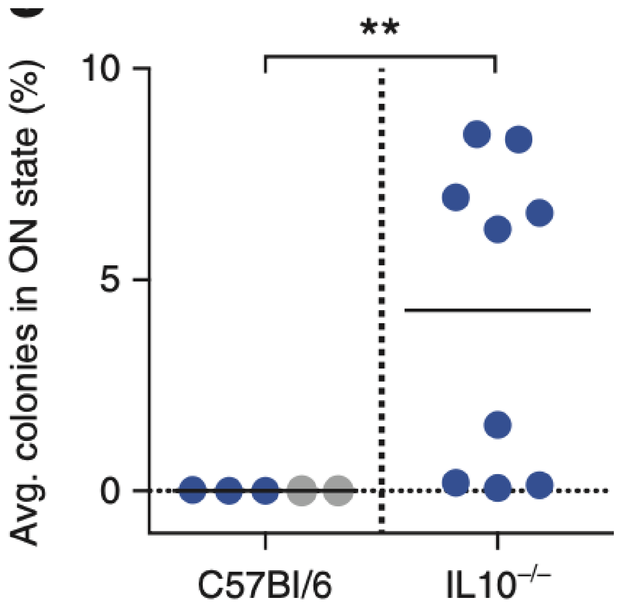


engineered memory strain specifically senses tetrathionate;

tetrathionate sensing corresponds to a more acute inflammatory response *in vivo*.

To test whether PAS638 could detect subclinical inflammation

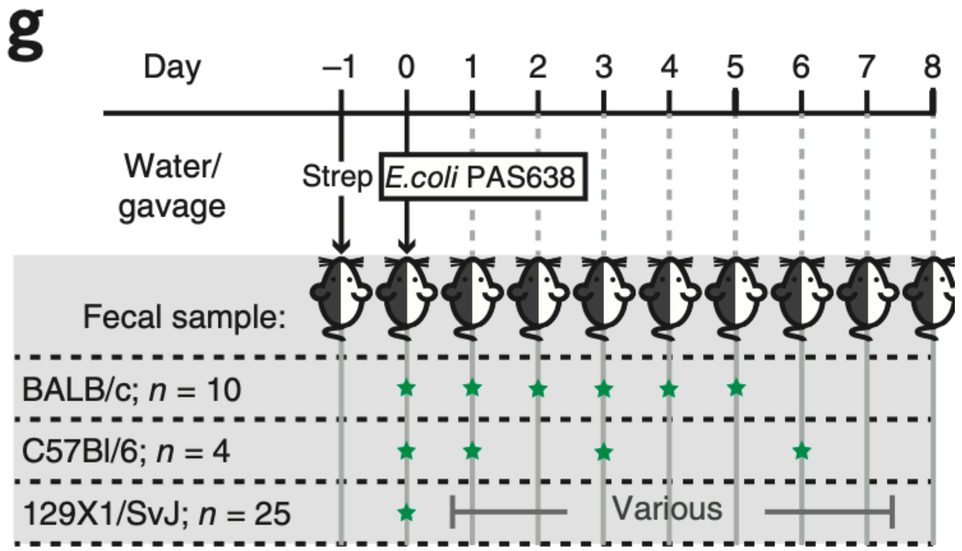


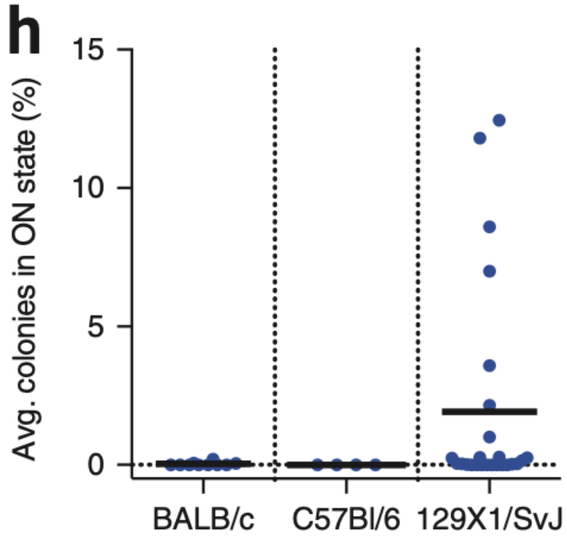


Interleukin-10-deficient mice (IL10$^{-/-}$): features of human IBD, do not have acute colitis because they have been raised in gnotobiotic and barrie

PAS638 can detect elevated tetrathionate in a physiologically relevant subclinical inflar

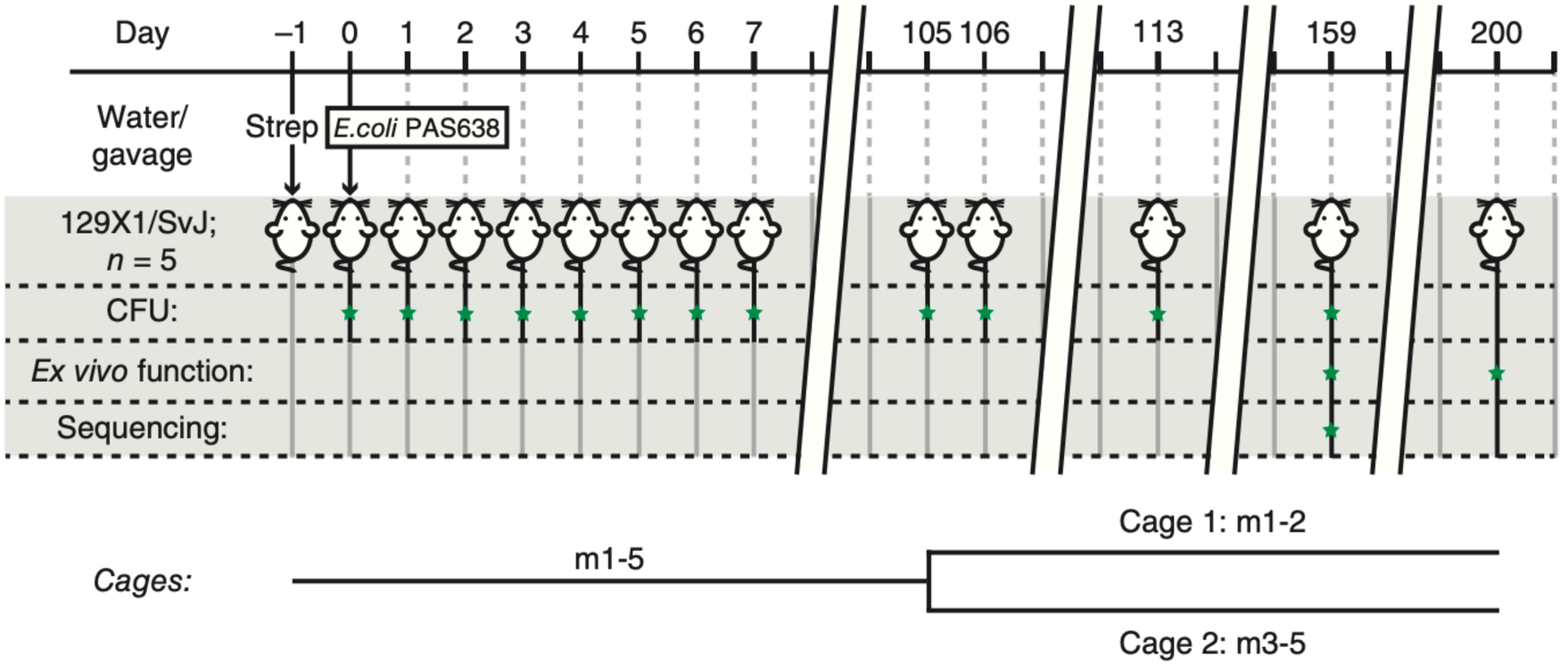
evaluate PAS638 in different mouse backgrounds





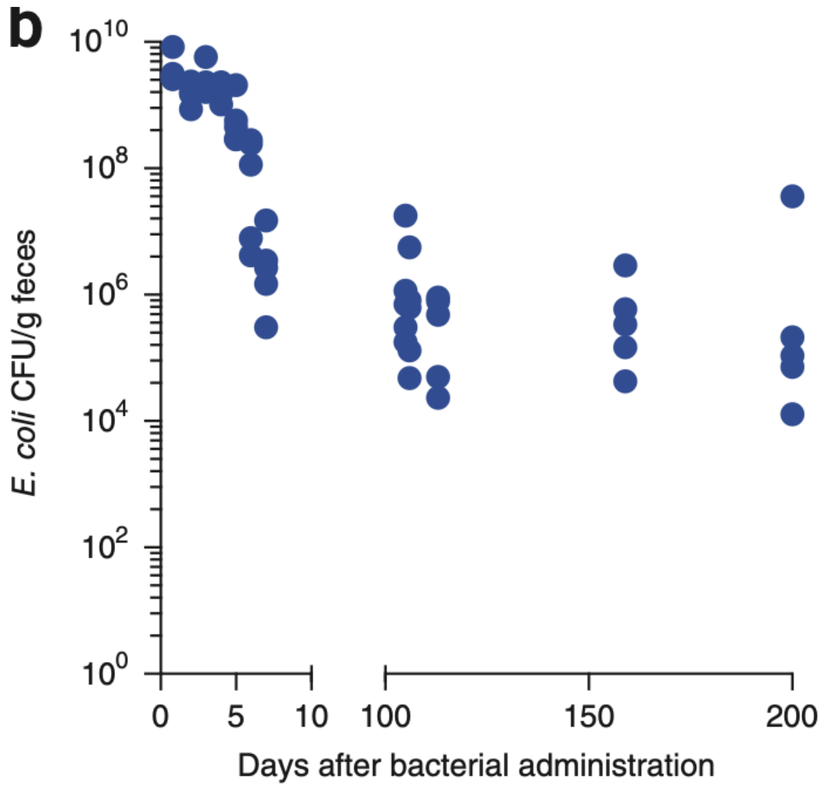
129X1/SvJ strain has documented defects in macrophage recruitment to sites of inflam

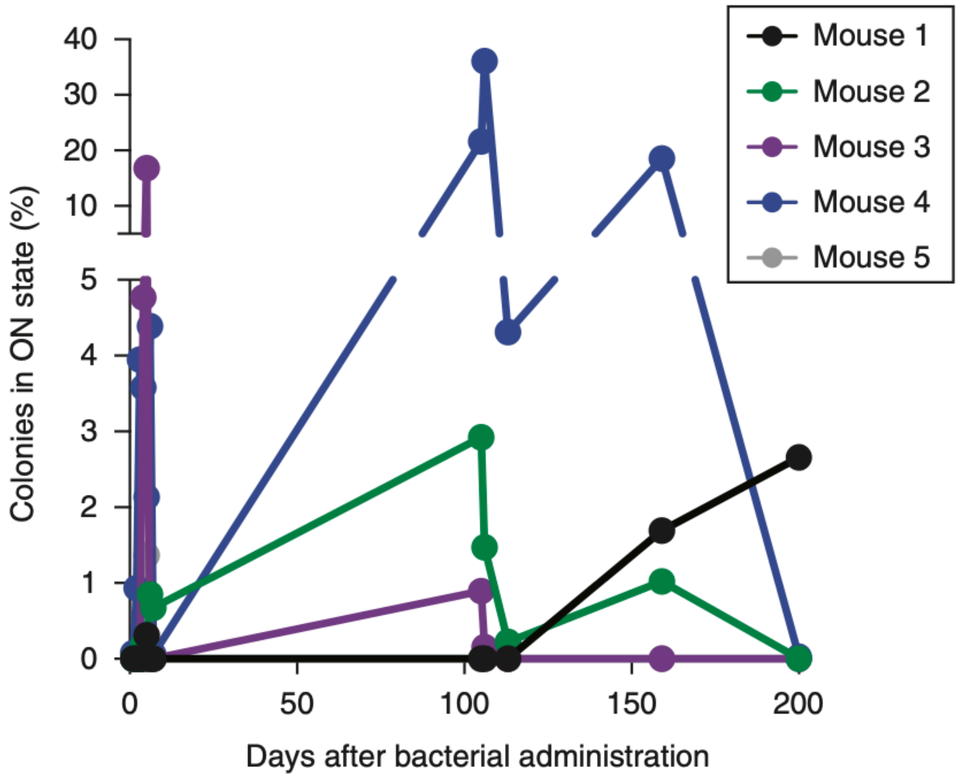
long-term monitoring of tetrathionate



200 days, >1600 bacteria generations, intermittent faecal testing

PAS638 remained colonized at detectable levels without continued antibiotic selection





colonies in the memory-on state were detected at each time point

Whole genome sequencing confirmed circuit stability

Mouse	*Ex vivo* function				Sequence analysis (d159)		
	Percentage 'off' colonies retaining ability to turn 'on'		Percentage 'on' colonies retaining ability to turn 'off'		Chromosome Incl trigger and memory elements	Plasmid 1 *Colicin*-like	Plasmid 2 *Citrobacter rodentium*-like
	d159	d200	d159	d200			
1	100% (6/6)	100% (68/68)	100% (5/5)	100% (19/19)	100%	11%	100%
2	100% (6/6)	100% (250/250)	100% (6/6)	N/A	100%	8%	100%
3	99% (112/113)	100% (243/243)	N/A	N/A	100%	100%	100%
4	100% (179/179)	100% (136/136)	100% (6/6)	100% (1/1)	100%	100%	100%
5	100% (6/6)	100% (109/109)	N/A	N/A	100%	100%	100%

No mutations or rearrangements were detected on the chromosome, including the synthetic gene elements

colonies that were in the memory-off state in fecal samples at day 159 and day 200 retained the ability to respond to tetrathionate *ex vivo;*

colonies that were in the memory-on state in fecal samples retained an ability to turn off memory under repeated streaking on agar plates.

Summary

synthetic bacterial devices can colonize the complex host mammalian gut and be used to monitor and analyze the course of a disease over an extended timeframe.

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Article

Bacterially Derived Tryptamine Increases Mucus Release by Activating a Host Receptor in a Mouse Model of Inflammatory Bowel Disease

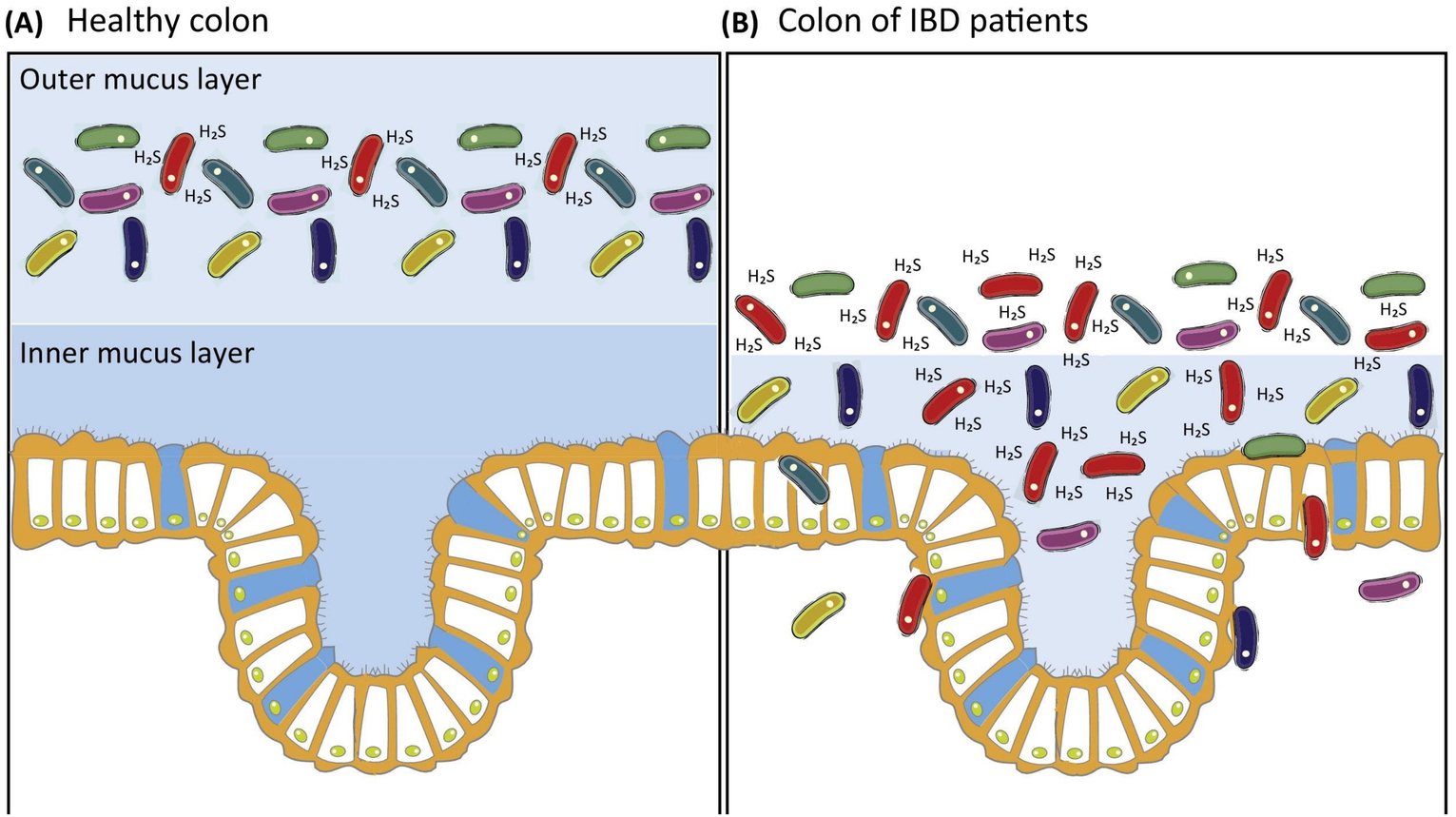
mucins from epithelial goblet cells

luminal contents (polysaccharides)

bioactive metabolites (neuropeptides, short chain fatty acids)

neurotransmitters (e.g. 5-HT)

immune factors (inflammatory cytokines)

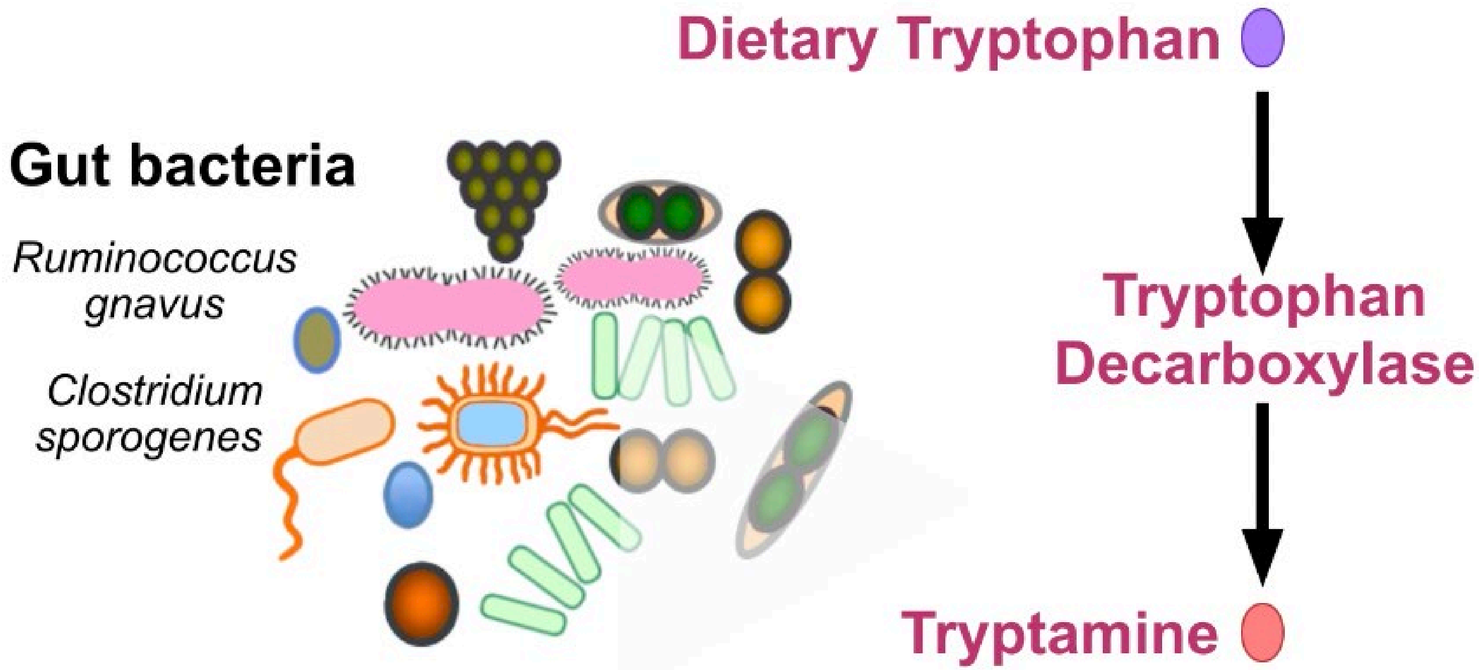


genetic factors

gut microbiota

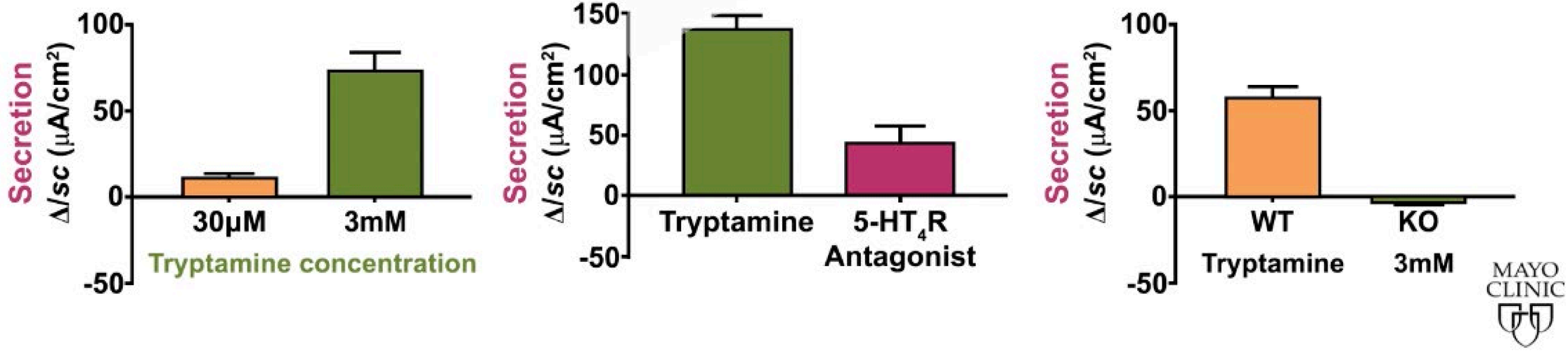
increased permeability

luminal microbes and their products causing innate immune activation and exacerbation of inflammatory processes



Pathway generally only exists in bacteria

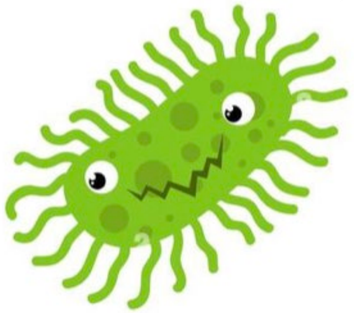
Tryptamine increases colonic secretion and tissue preparations



Biological effects of tryptamine are mediated through the 5-HT4 receptor, a G-protein-cou

The secretory effect of tryptamine is dependent on 5-HT4R activation and is blocked by 5

gene encoding tryptophan decarboxylase



R. gnavus

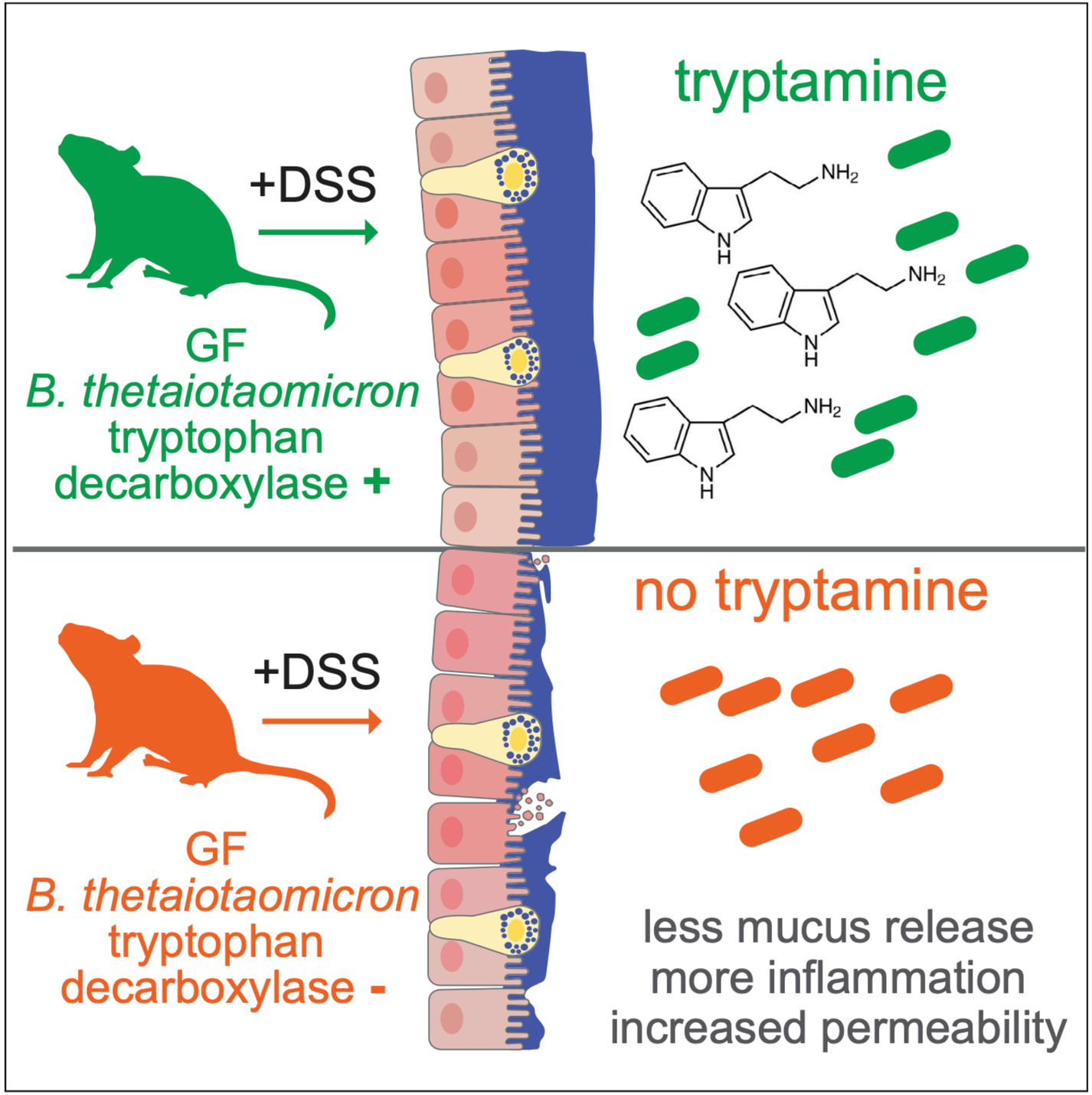
B. thetaiotaomicron

genetically tractable

effectively colonizes the gut

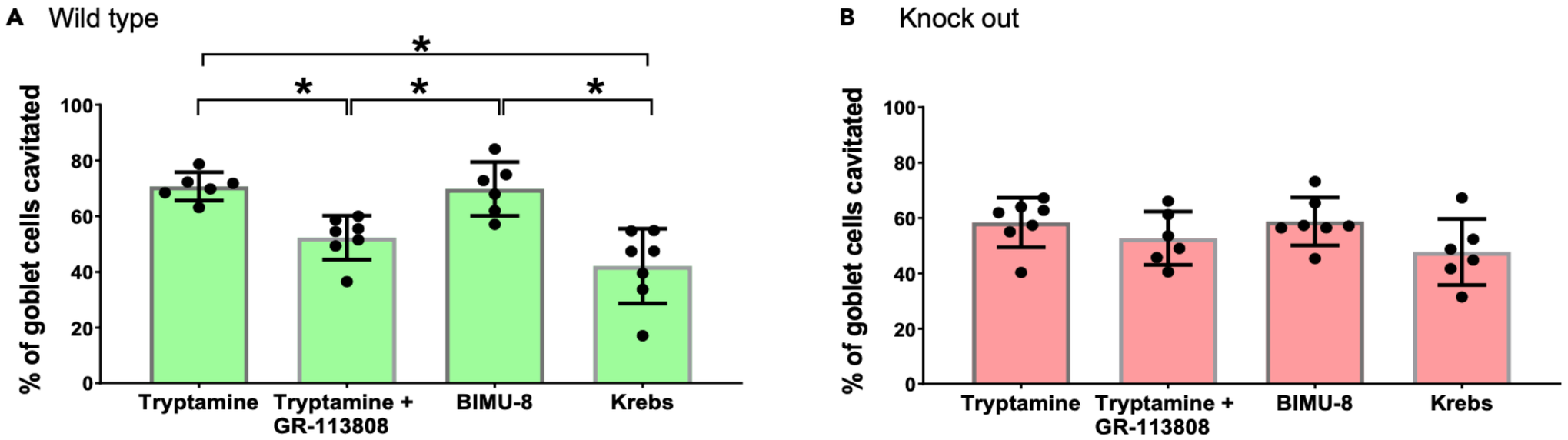
potentially can be used as a biotherapeutic

colonize germ free mice with either *B. thetaiotaomicron* Trp D+ or vector-only control *B. thetaiotaomicron* Trp D− and supplemented the drinkin



DSS: dextran sodium sulfate

Tryptamine Evokes Mucus Release Ex Vivo, Which Is Blocked by 5-HT4R Antagonist and Absent in 5-HT4R KO Mice



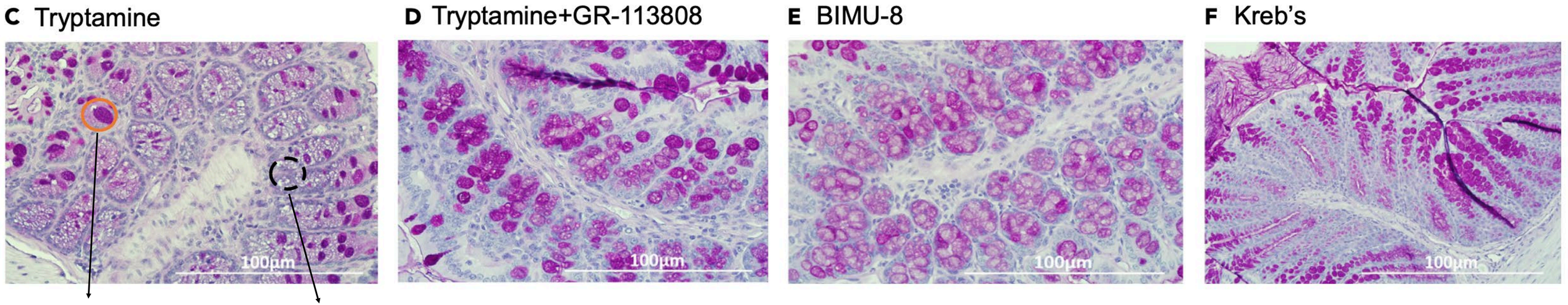
tryptamine(3mM): 5-HT4R-specific agonist, physiological concentration

GR-13808 (30 nM): 5-HT4R-antagonist GR-13808

BIMU-8 (10 mM) : a known pharmacologic 5-HT4R-agonist

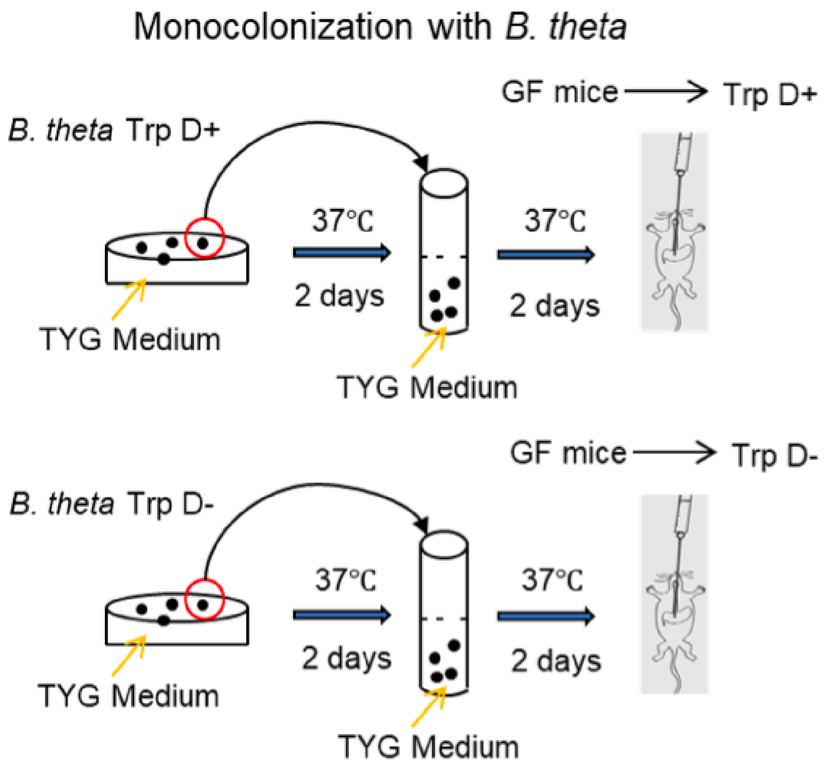
Krebs: control

Goblet cell cavitation following treatment with BIMU-8 was comparable to



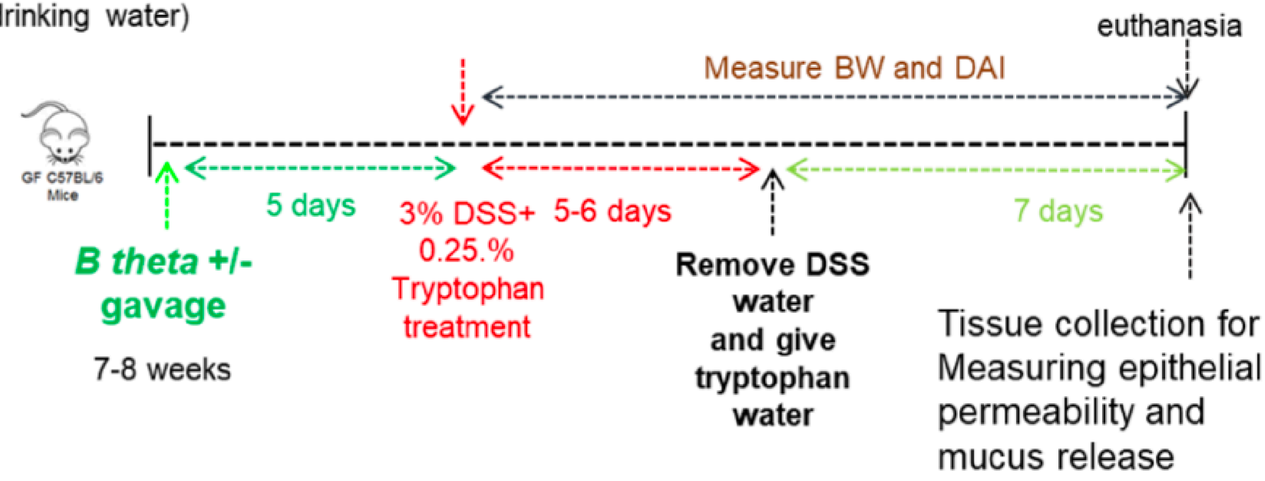
tryptamine causes goblet cell cavitation and stimulates mucus release in the mouse prox

in vivo tryptamine production by B. theta Trp D+ strain increased mucus release and pr

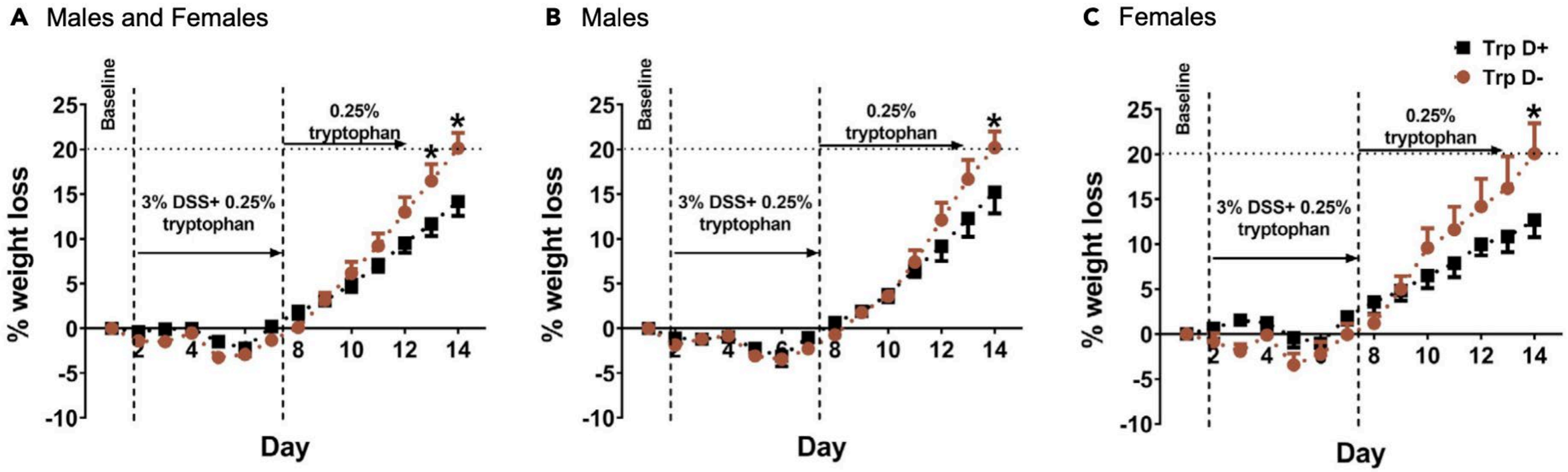


Timeline

Mice have free access to food and medicated water (water containing 0.25% tryptophan supplemented in drinking water)



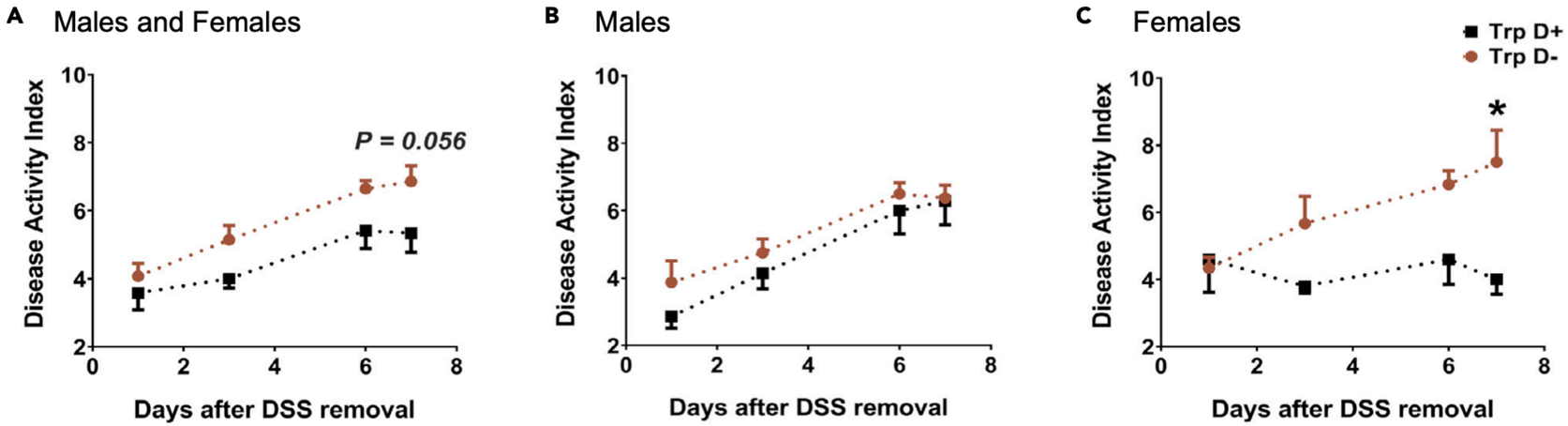
Bacterially Derived Tryptamine Attenuates Weight Loss Following DSS A



B. thetaiotaomicron Trp D+ monocolonized mice showed significantly lower percentage weight loss compared to Trp D- monocolonized mice, an

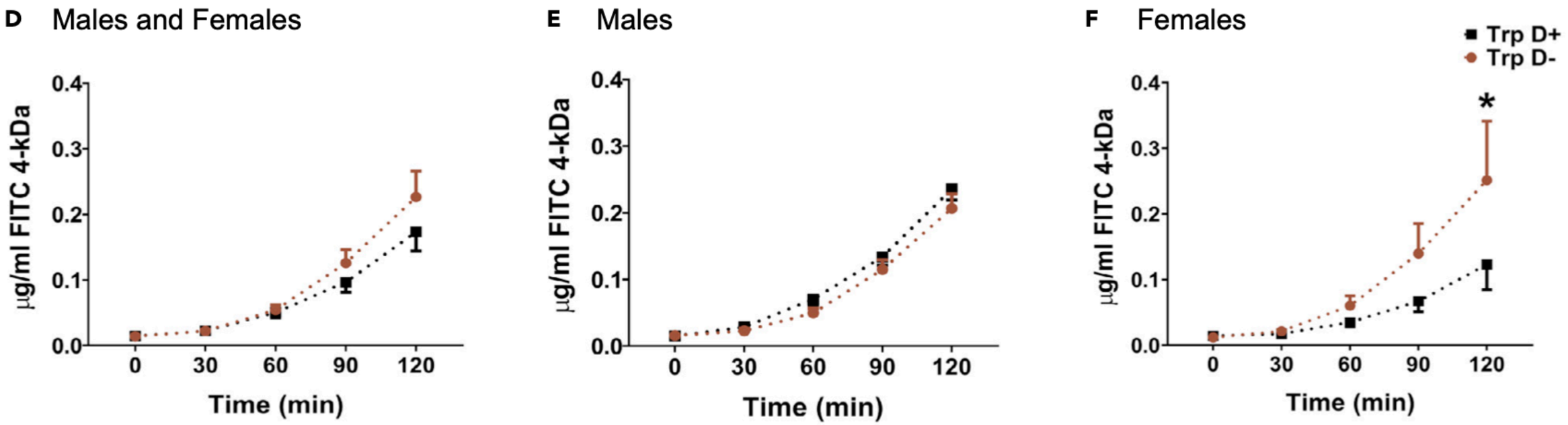
Bacterially Derived Tryptamine Reduces DAI in Female Mice

Score	Weight loss	Stool Consistency	Bleeding
0	None	Normal	Normal
1	1-5%		
2	5-10%	Loose stool	Hemoccult +
3	10-20%		Visible blood in stool only
4	>20%	Diarrhea	Gross bleeding (visible)

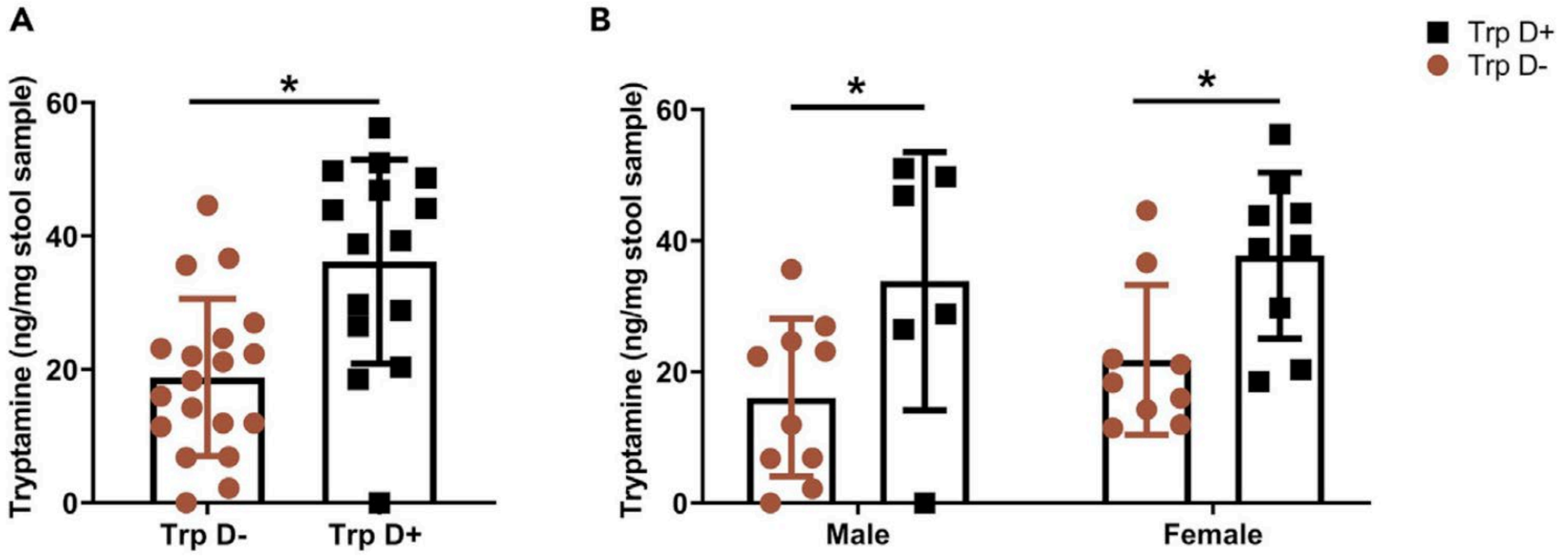


DAI: Disease Activity Index, severity index used to assess severity of colitis in DSS-treated mice

Bacterially Derived Tryptamine Reduces DSS-Induced Epithelial Barrier D

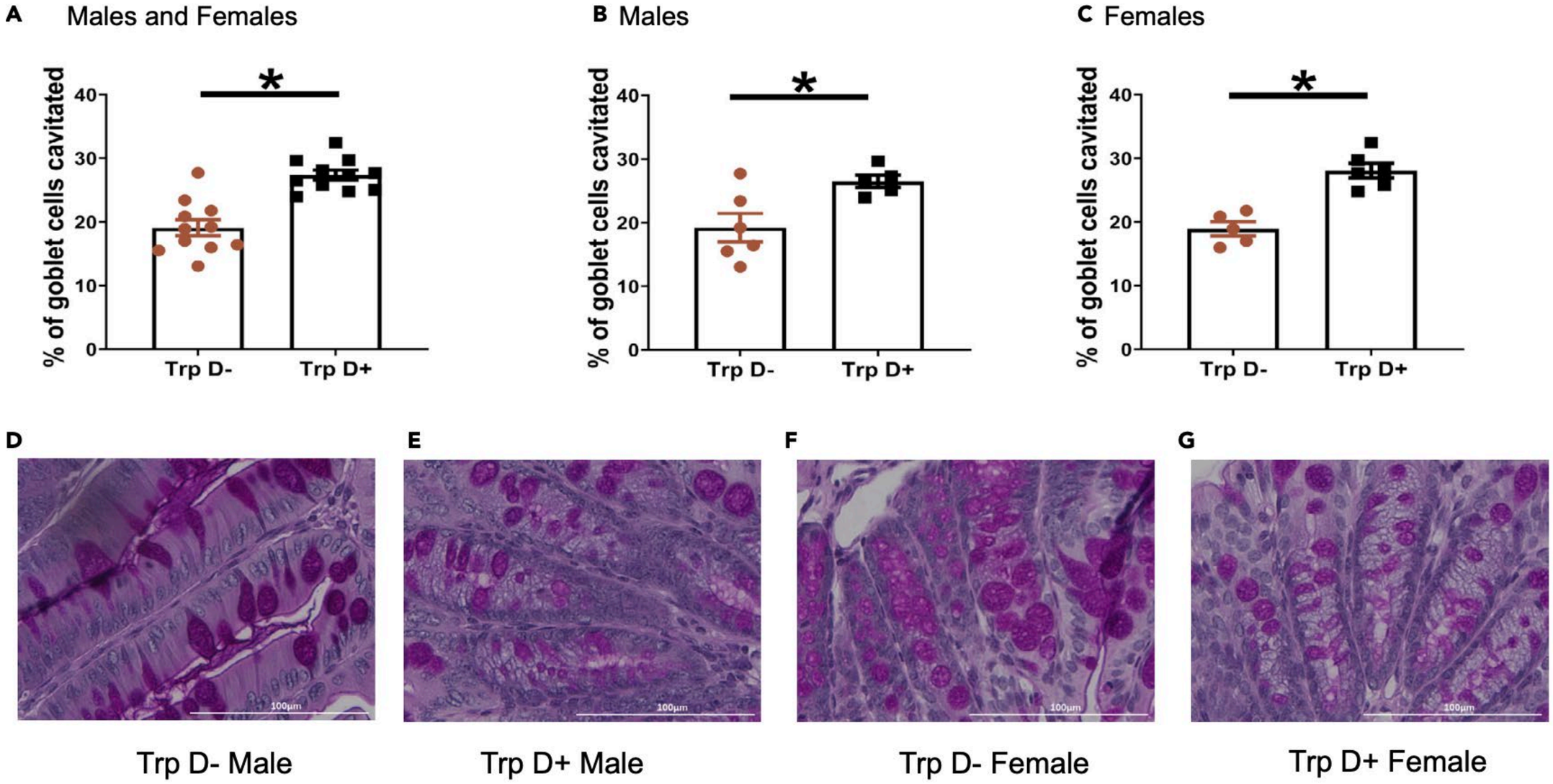


change in 4-kDa FITC flux across proximal colon tissue over two hours in both male and female DSS-treated Trp D+ and Trp D monocolonized m



sex differences observed in the DAI and colonic permeability between Trp D+ and Trp D- r

Bacterially Derived Tryptamine Increases In Vivo Mucus Release Following DSS Administration



summary

- Precise control of tryptamine production in the gut by engineering commensal bacteria can help reinforce the mucus barrier and improve overall colitis burden in response to noxious stimuli in mice.
- This study is an example of how communication between gut bacteria and the host can be exploited for development of novel therapeutics.

Thanks for listening!