Gut Microbiome: Toxicant Perturbation and Stability

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Risk e-Learning Webinar Series
The Interplay Between Environmental Exposures and Infectious Agents
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Environmental Microbial and Mammalian Biomolecular
Responses to AhR Ligands

MICHIGAN STATE
UNIVERSITY

Outline

A. Gut Microbiome

- Background and Objective
- Treg/Th17 system, TCDD, and SFB
- Hypothesis and Experimental Details
- Results

B. Key Challenges Ahead

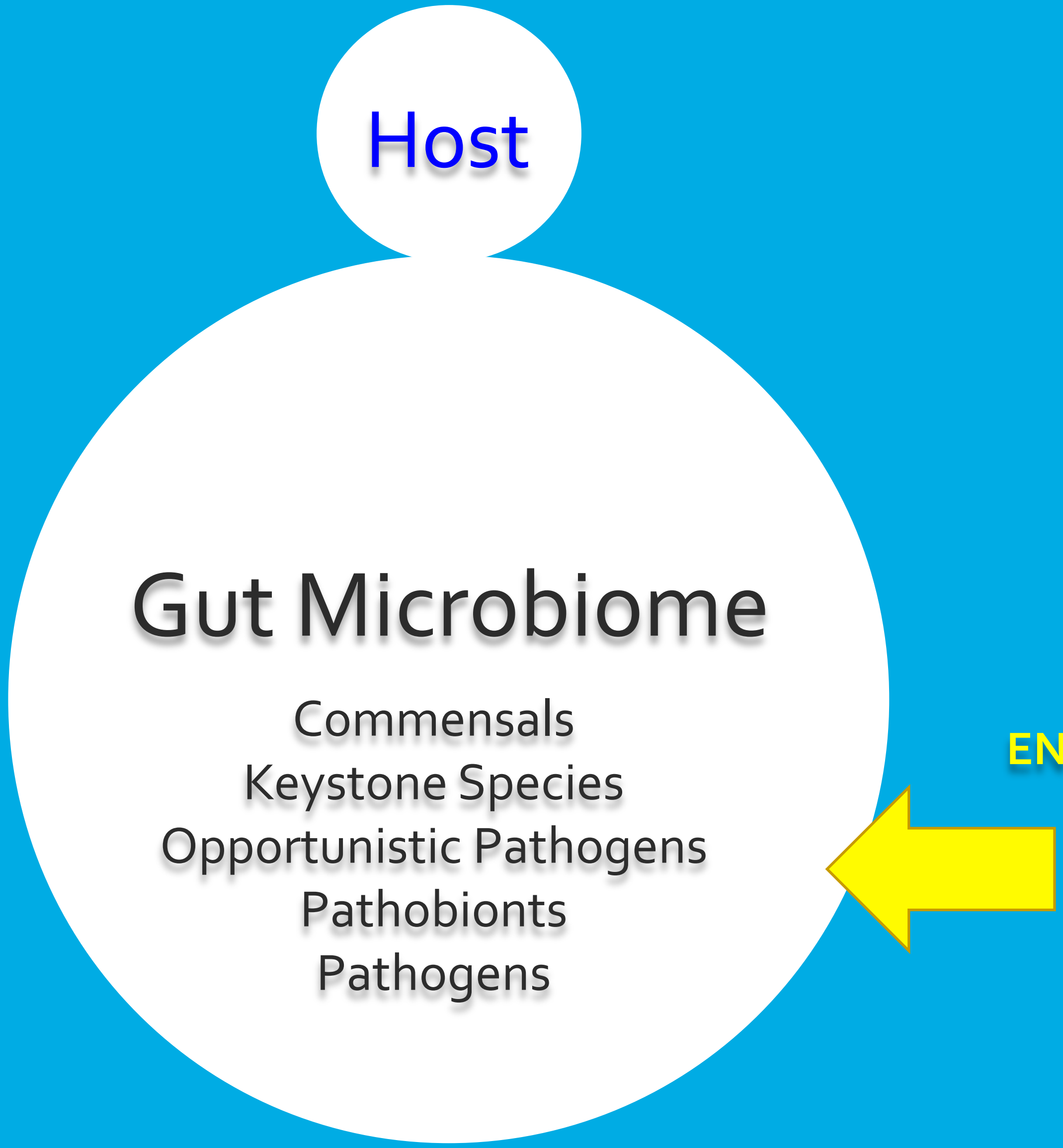
- Communication channels
- Predictive capabilities
- Markers/Gut chips
- Interventions

C. Summary

Gut Microbiome: Diseases & Interventions

DISEASES

Allergies
Asthma
Anxiety
Autism
Autoimmune
Cardiovascular
Crohn’s
Depression
IBD
Mood Disorder
Migraine
Multiple Sclerosis
NAFLD
Obesity
Parkinson's
Spinal Cord Injury
Stroke
Type 2 Diabetes



Host

Gut Microbiome

Commensals
Keystone Species
Opportunistic Pathogens
Pathobionts
Pathogens

ORGANS

Brain
Mouth
Heart
Kidney
Liver
Immune System
Colon
Tissue
Muscle

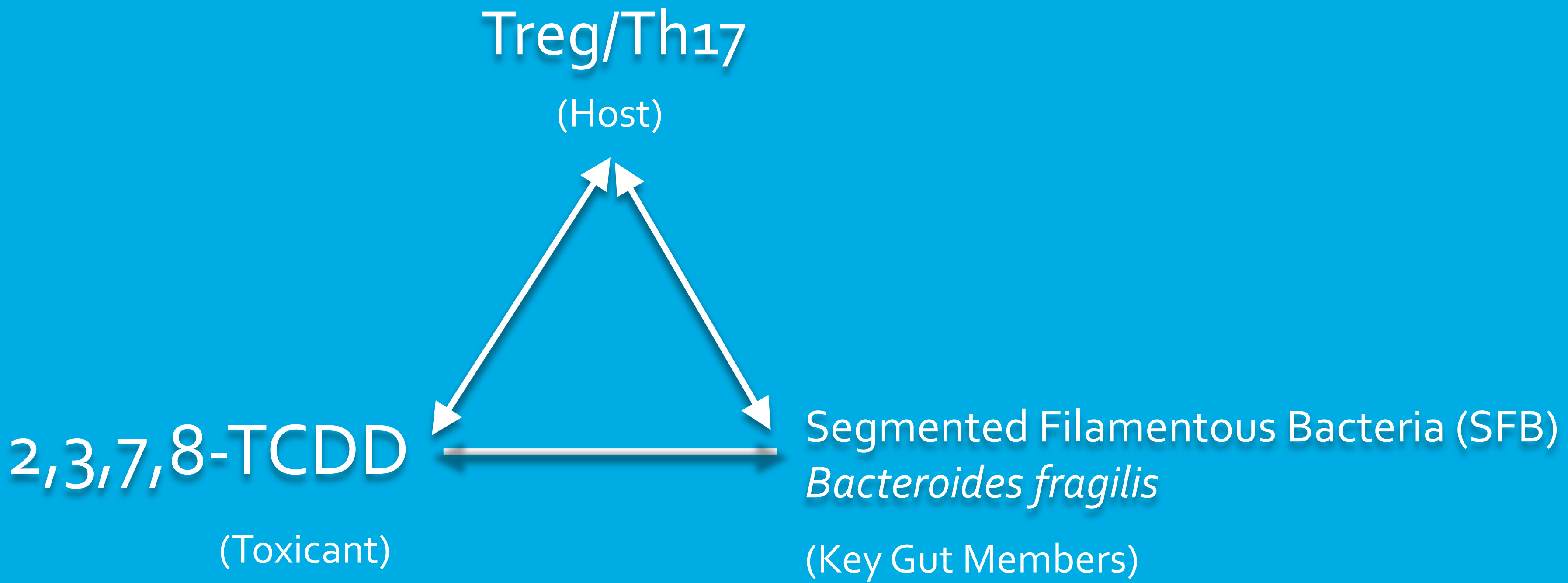
ENVRIONMENTAL EXPOSURE

Toxicants

INTERVENTIONS

Fecal Transplant
Psychobiotics
Pre- and Probiotics
Antibiotics
Food Habits
CRISPR
Phage

Objective: Characterize the effect of specific gut microbiome members on Treg/Th17 System with and without TCDD



Treg/Th17

(Host)

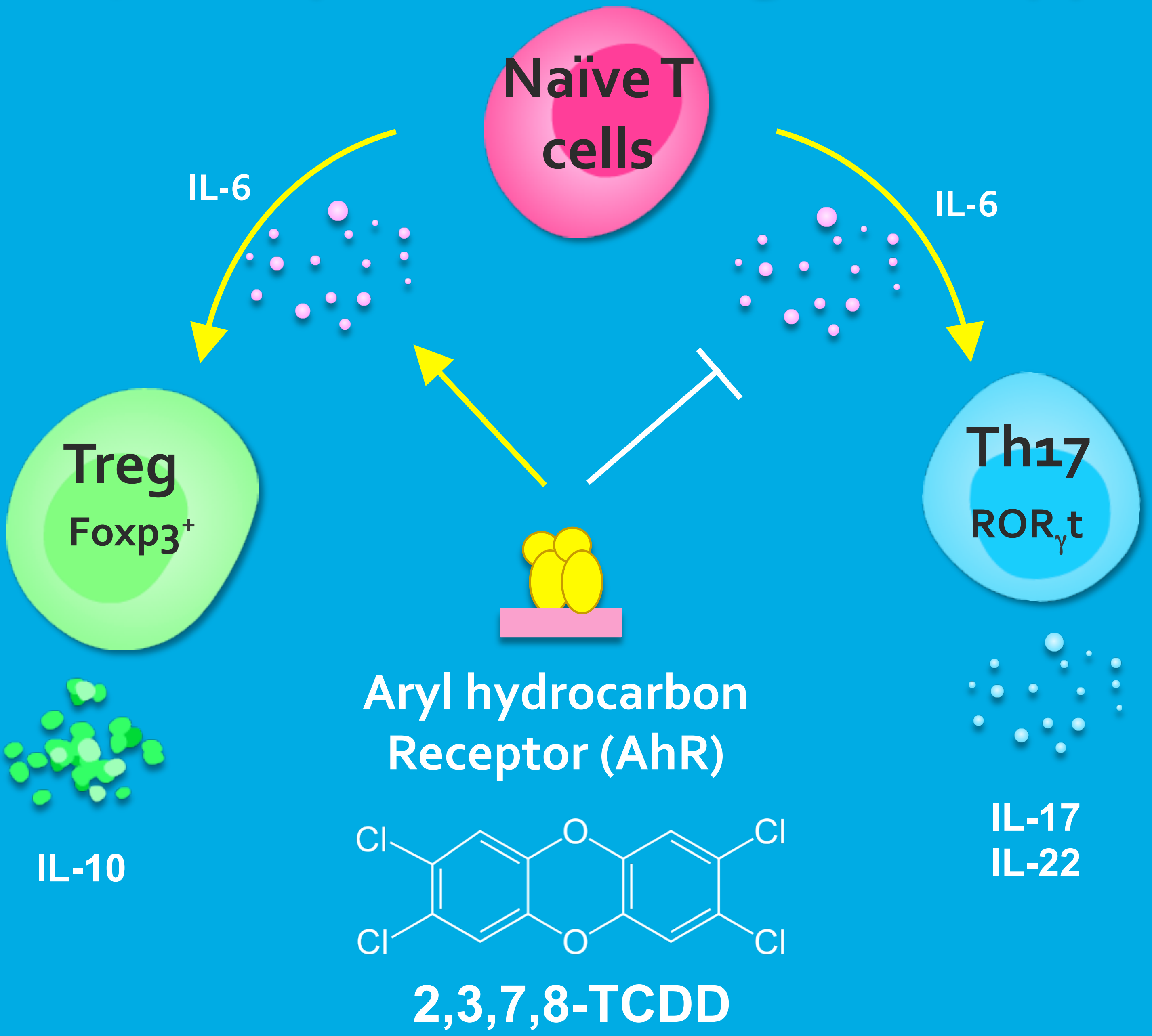
2,3,7,8-TCDD

(Toxicant)

Segmented Filamentous Bacteria (SFB)
Bacteroides fragilis

(Key Gut Members)

With TCDD, AhR promotes Treg and suppresses Th17



Naïve T cells

IL-6

IL-6

Treg
modulates and
abrogates
autoimmune
disease

Treg
Foxp3$^+$

Th17
ROR$_\gamma$t

Th17
aids in antimicrobial
response;
also causes
inflammation &
autoimmune disease

IL-10

Aryl hydrocarbon Receptor (AhR)

IL-17
IL-22

2,3,7,8-TCDD

Why Segmented Filamentous Bacteria (SFB)?

- Obligate symbiont
- Extensive auxotroph
- No genes for amino acids, vitamins/cofactors, nucleic acids
- Host-specific

Candidatus **Savagella** | *Environmental Microbiology* **14** (6): 1462-2920 | 2012

SFB cultivation is now possible using TC7 cell lines (BioTechniques, 59 (2):94–98, 2015

Ivanov et al., *Cell*: 2009

SFB in humans?

2015

Dig Dis Sci. 60(10): 2953-62

SFB in patients by qPCR. Less in IBD constipated, and more in IBD diarrhea.

2013

Yin et al., ISME Journal

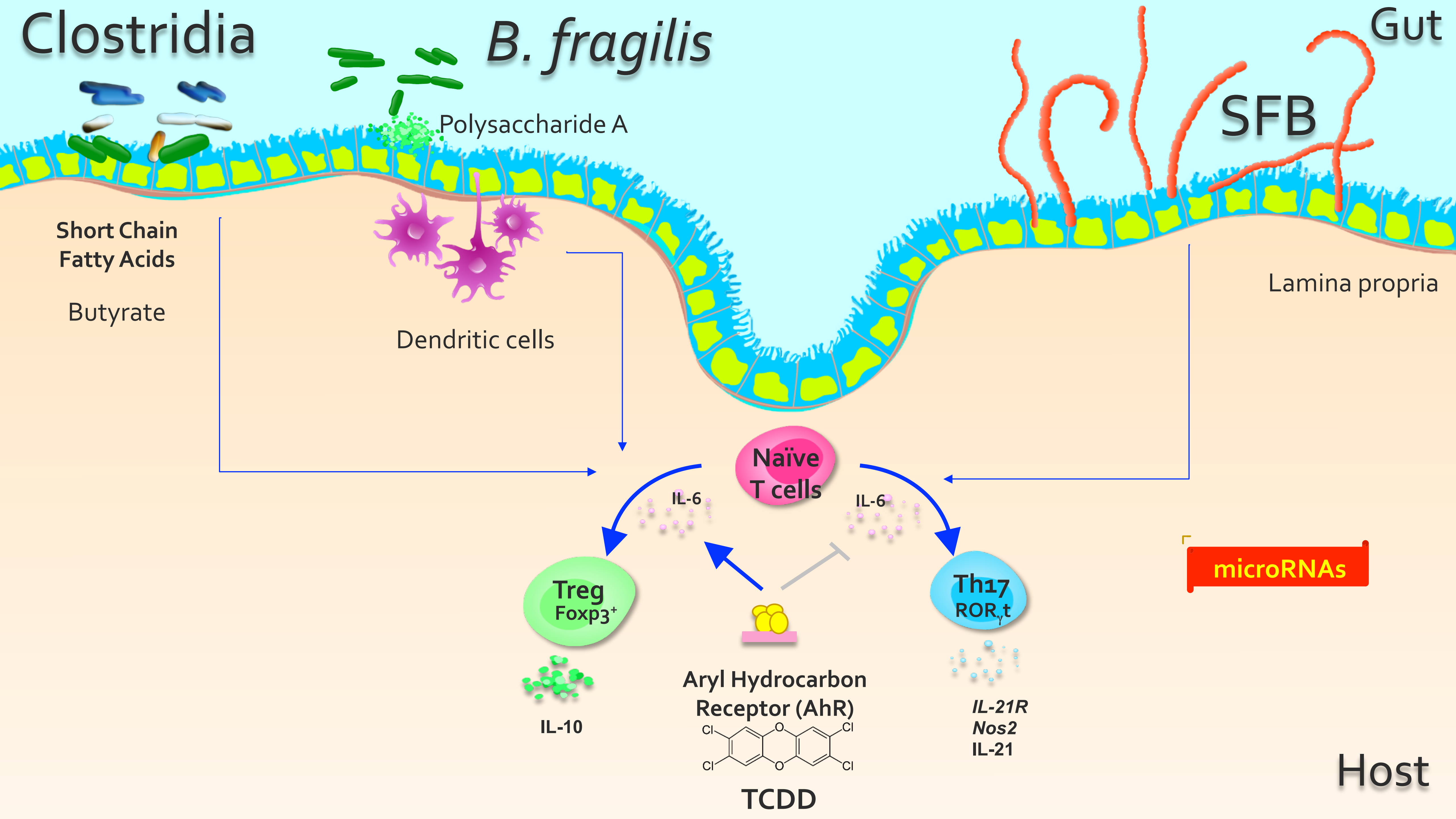
251 humans: majority colonized between ages 2 to 3

2013

Hans Jonsson

2009

Snel et al.



Clostridia

B. fragilis

Gut

Polysaccharide A

SFB

Short Chain Fatty Acids

Butyrate

Dendritic cells

Lamina propria

Naïve T cells

IL-6

IL-6

Treg
Foxp3$^+$

Th17
ROR$_\gamma$t

microRNAs

IL-10

Aryl Hydrocarbon Receptor (AhR)

TCDD

IL-21R
Nos2
IL-21

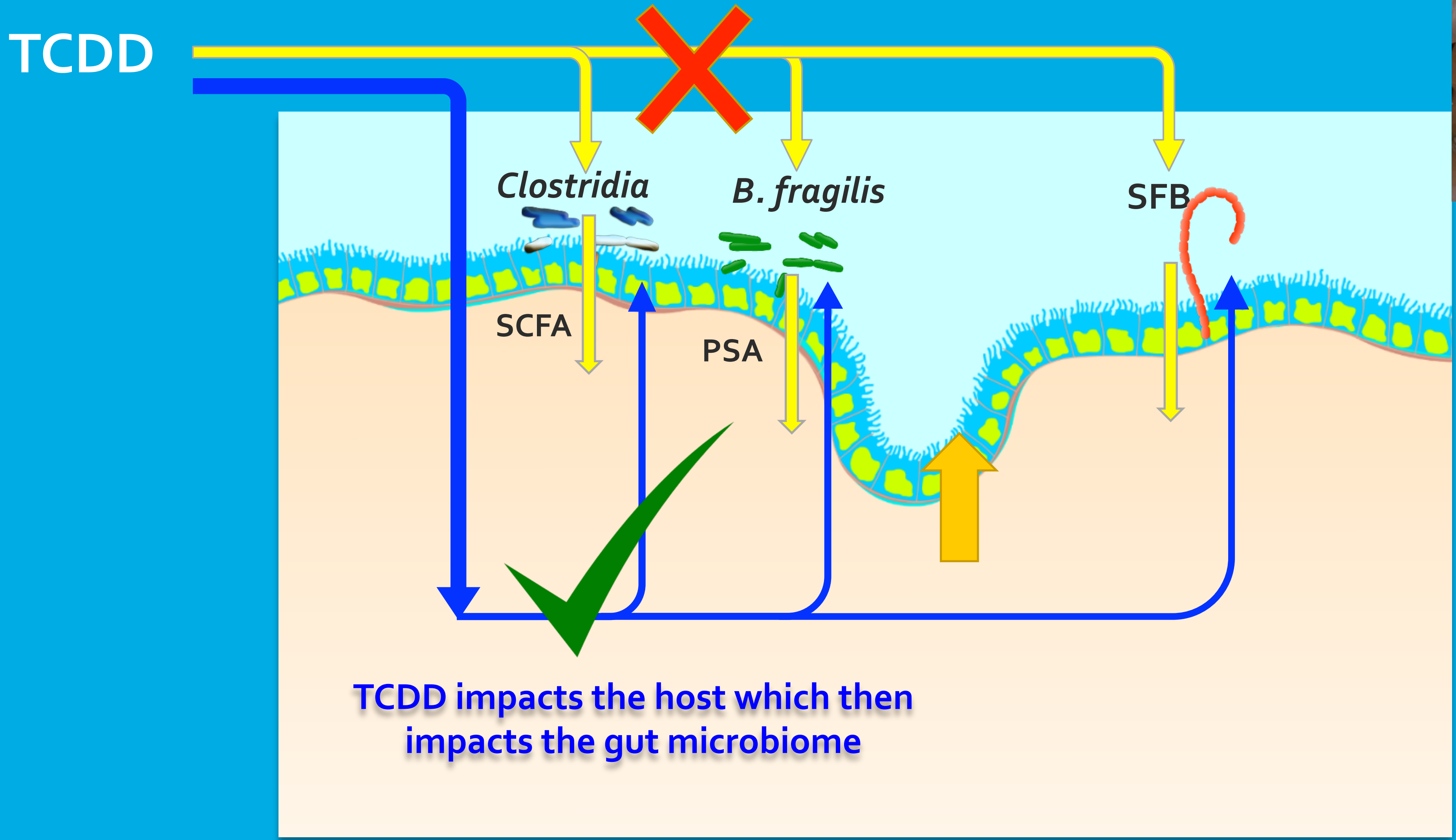
Host

Hypothesis

TCDD exposure disrupts the Treg /Th17 system and specific gut microbial members are capable of preventing this disruption.

Two Possibilities!

TCDD impacts the gut microbiome which then impacts host



TCDD impacts the host which then impacts the gut microbiome

Experimental Details

C57BL6

Traditional

TCDD: 0.01 to 30 µg/kg every 4 d
30 d study
120 d study (90 d + 30 d recovery)
8 per group

Cage separation

Tim Zacharewski’s Lab

Gnotobiotic

TCDD: 30 µg/kg every 4 d
56 d study
4 per group

- GF
- SFB
- *B. fragilis*
- *B. fragilis* + SFB

UM Germ-Free Facility

Key measurements

- mRNA expression of ileal immunology genes (nCounter: **547** Immunology gene targets)
- T-cells in blood/spleen (Flow cytometry)
- microRNA expression in ileum (nCounter: **600** mouse microRNAs)

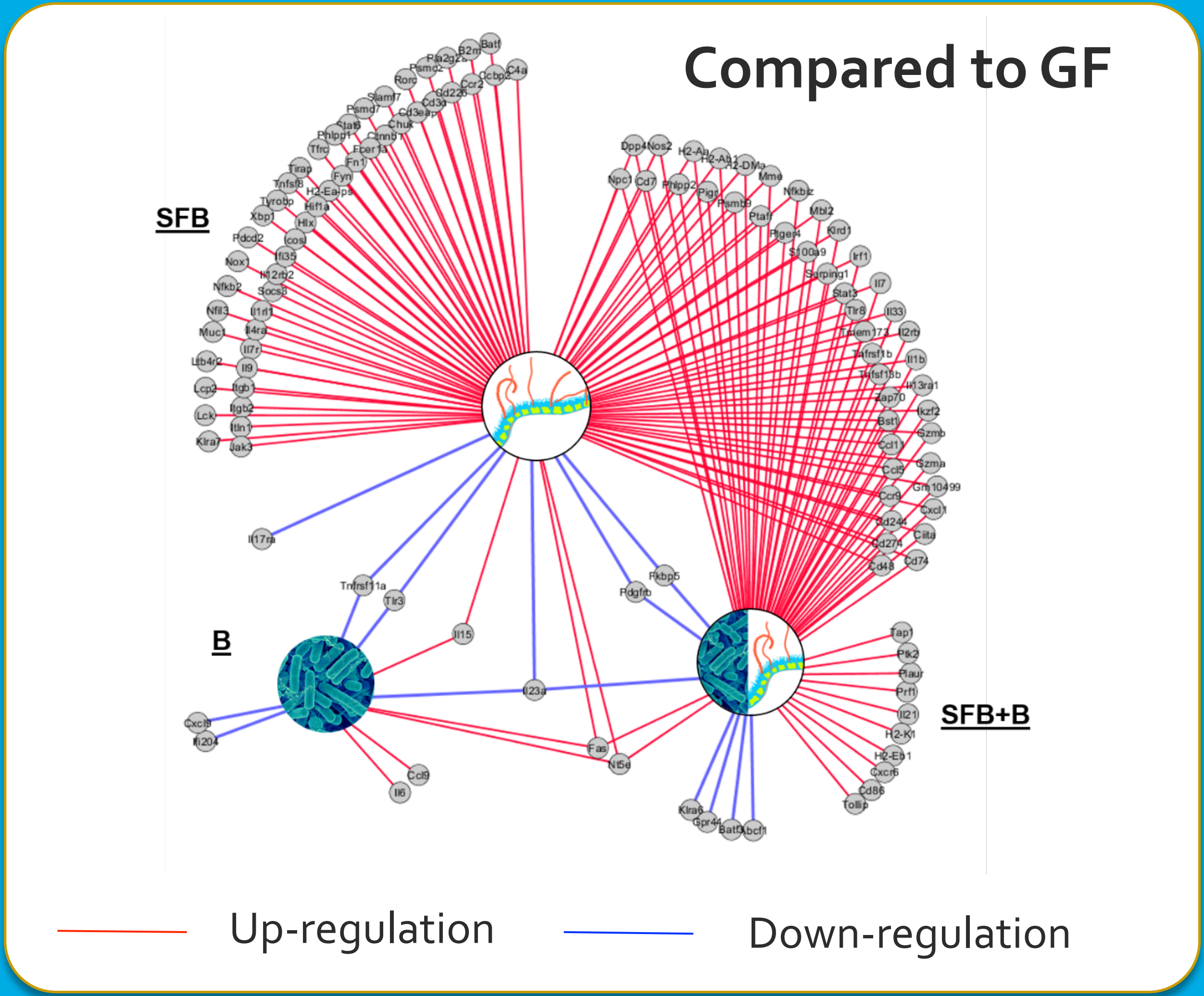
Fecal pellets, ilium, cecum, blood

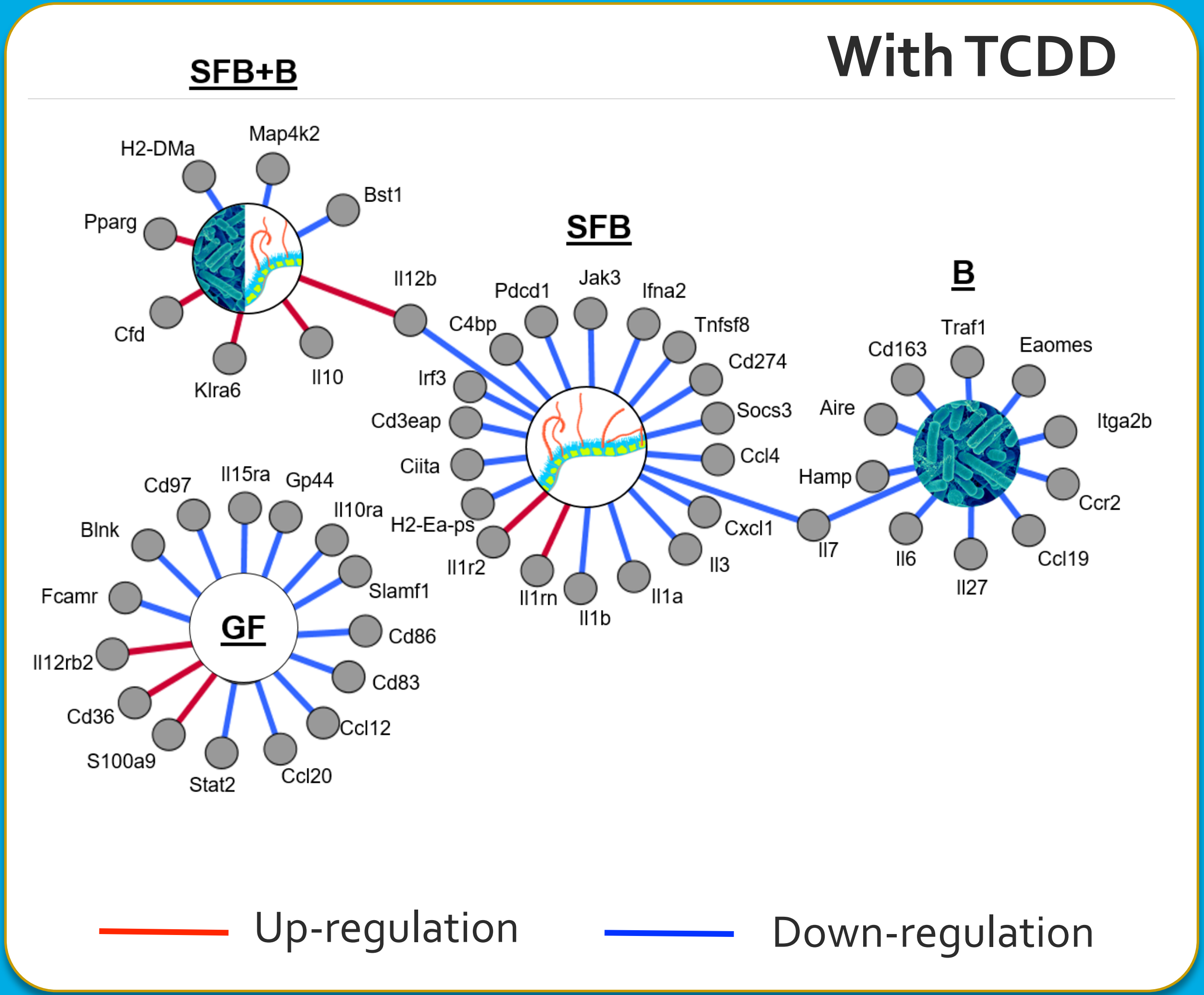
- High Throughput (Wafergen) or qPCR

Fecal pellets, cecum

Gnotobiotic C57BL6: Gene Expression

Ileum



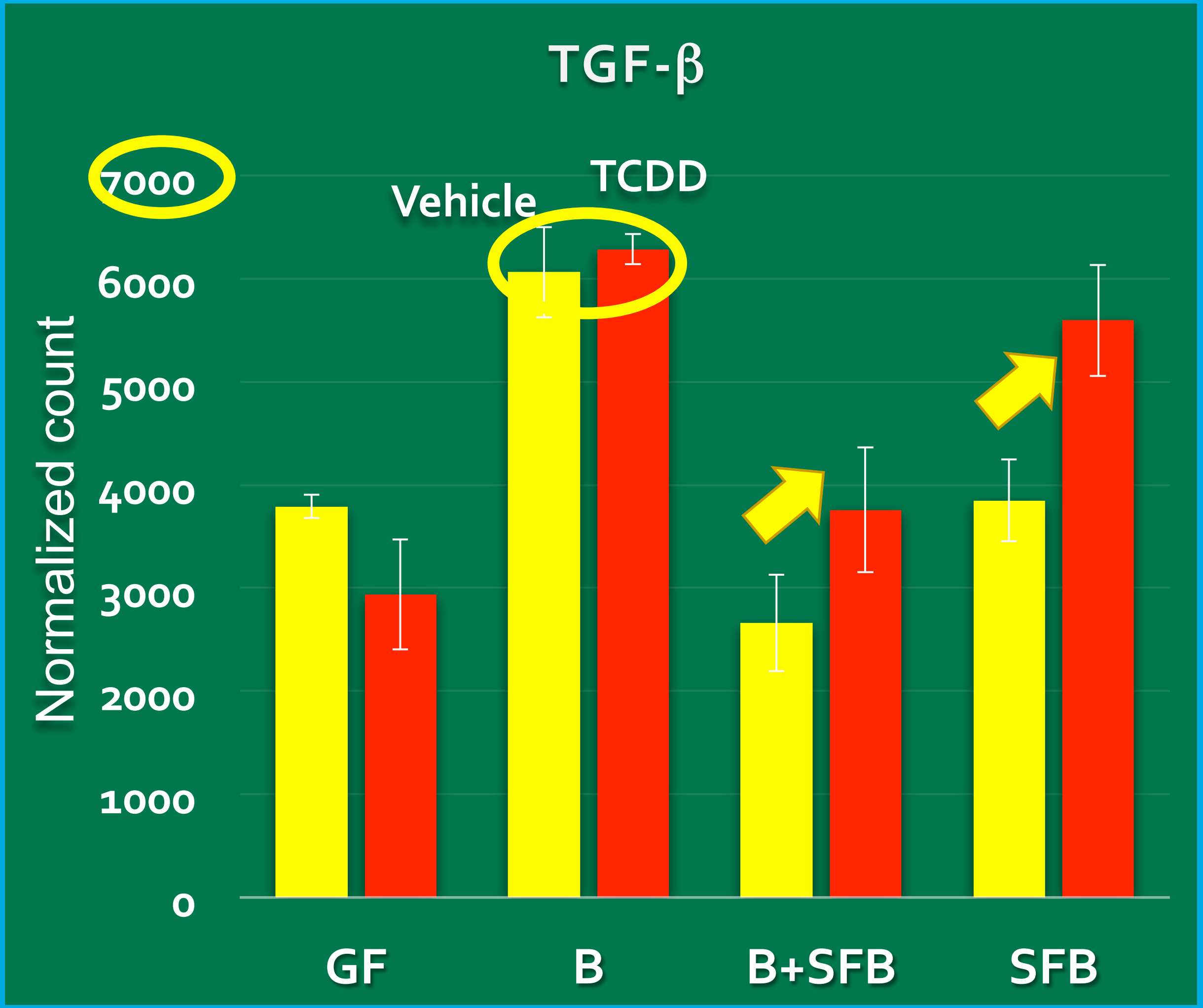


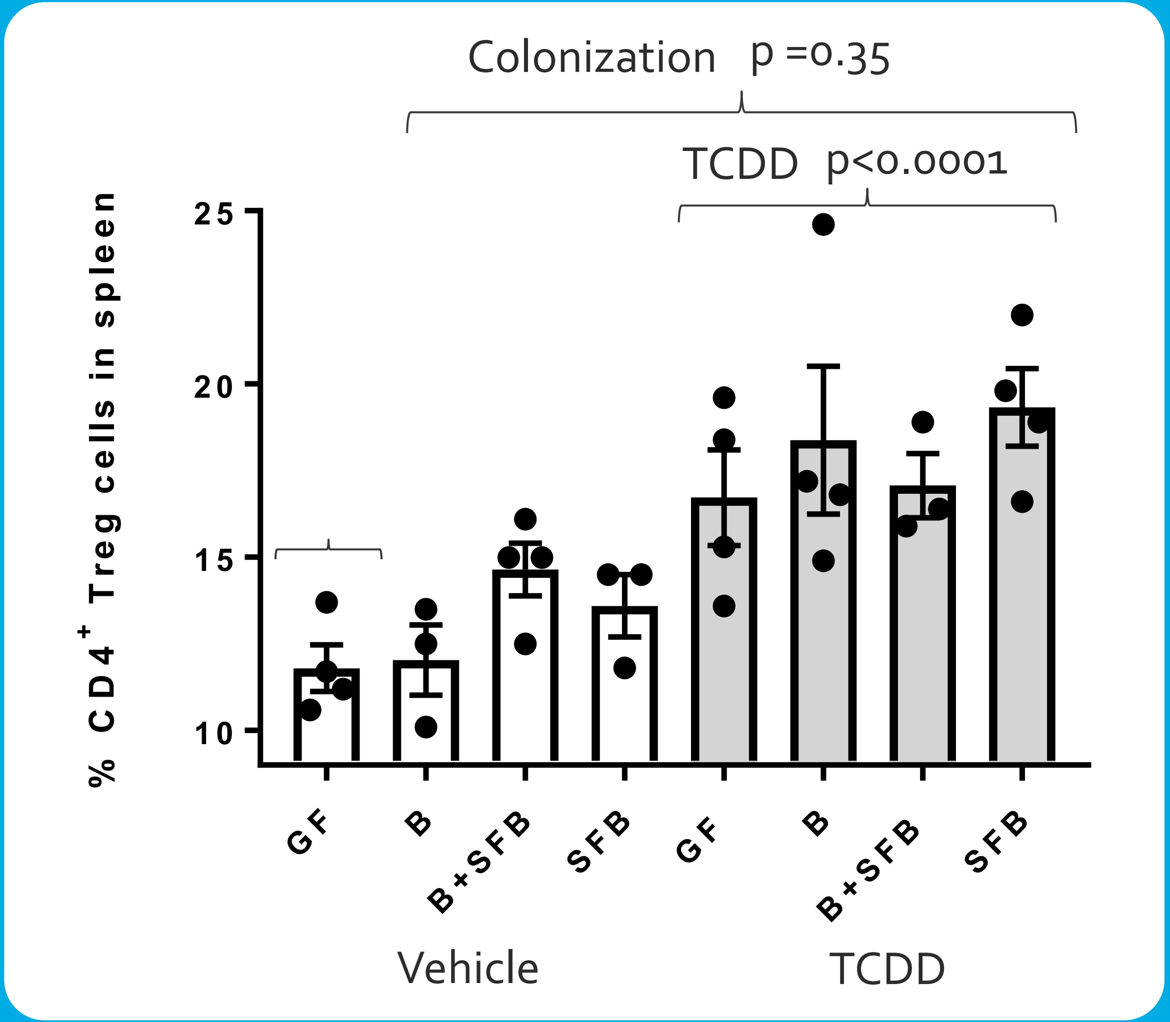
Compared to GF, SFB has more Up-regulated genes.

With TCDD, SFB has more Down-regulated genes.

Gnotobiotic C57BL6: Treg

Spleen

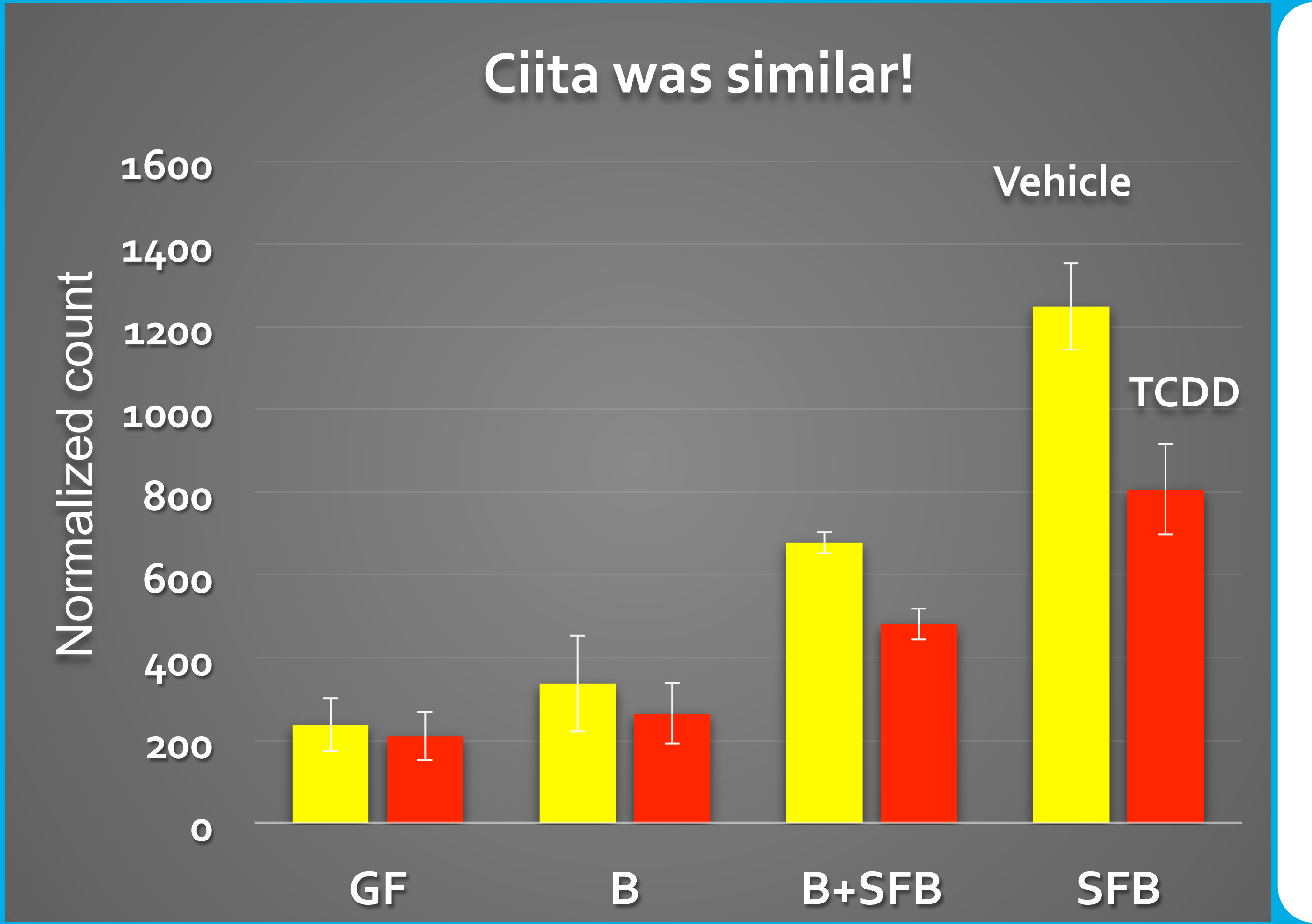


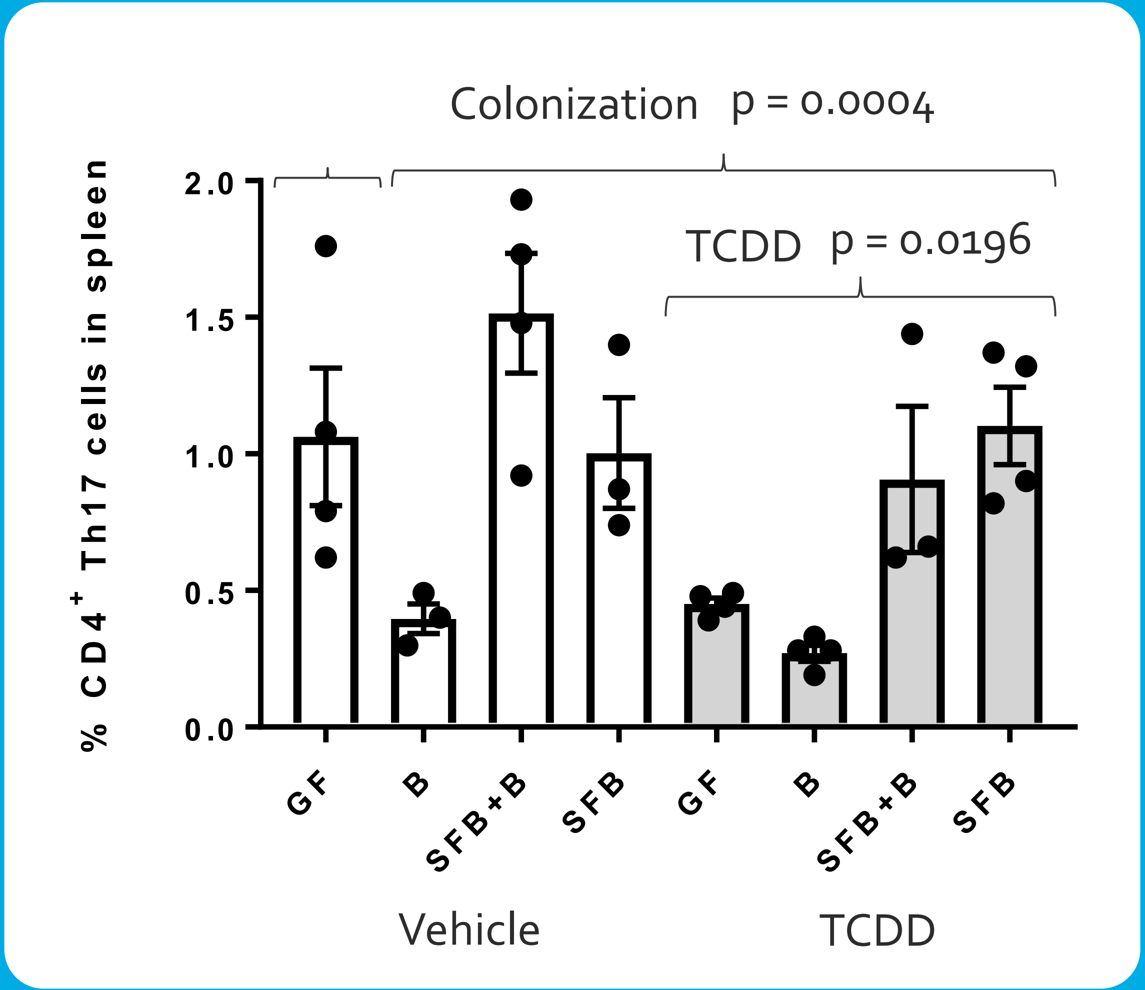


Parametric two-way ANOVA

Gnotobiotic C57BL6: Th17

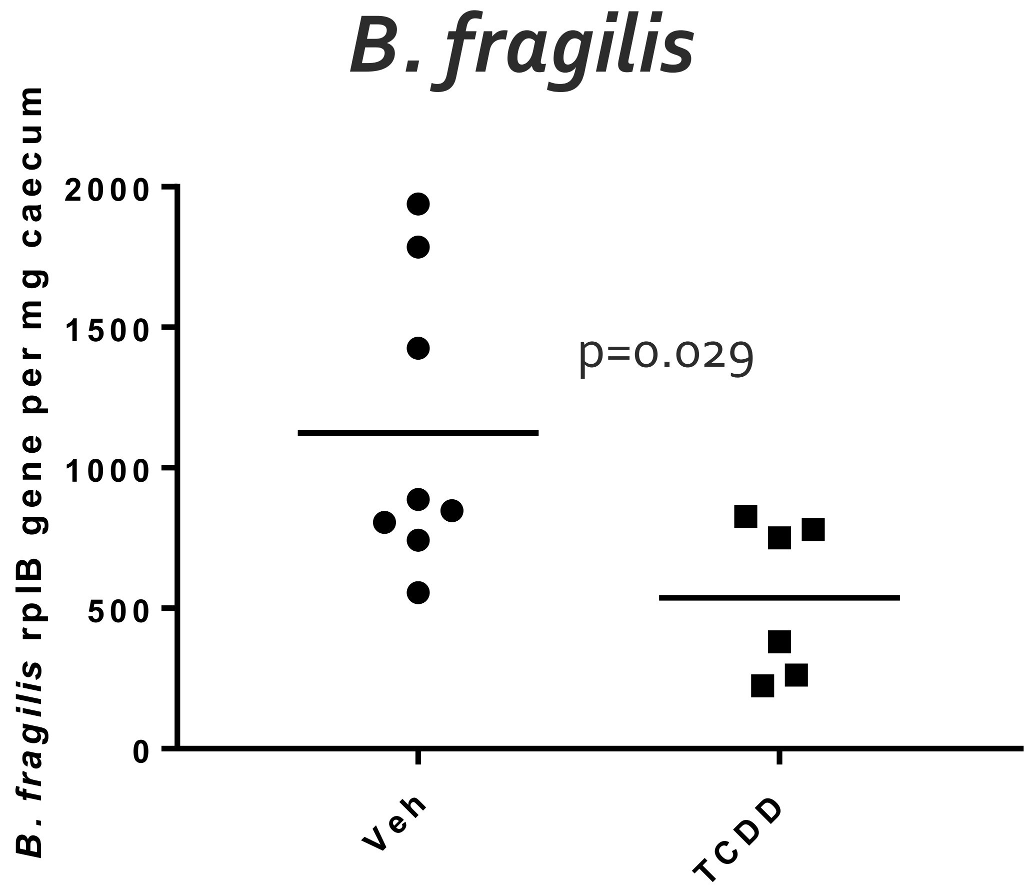
Spleen



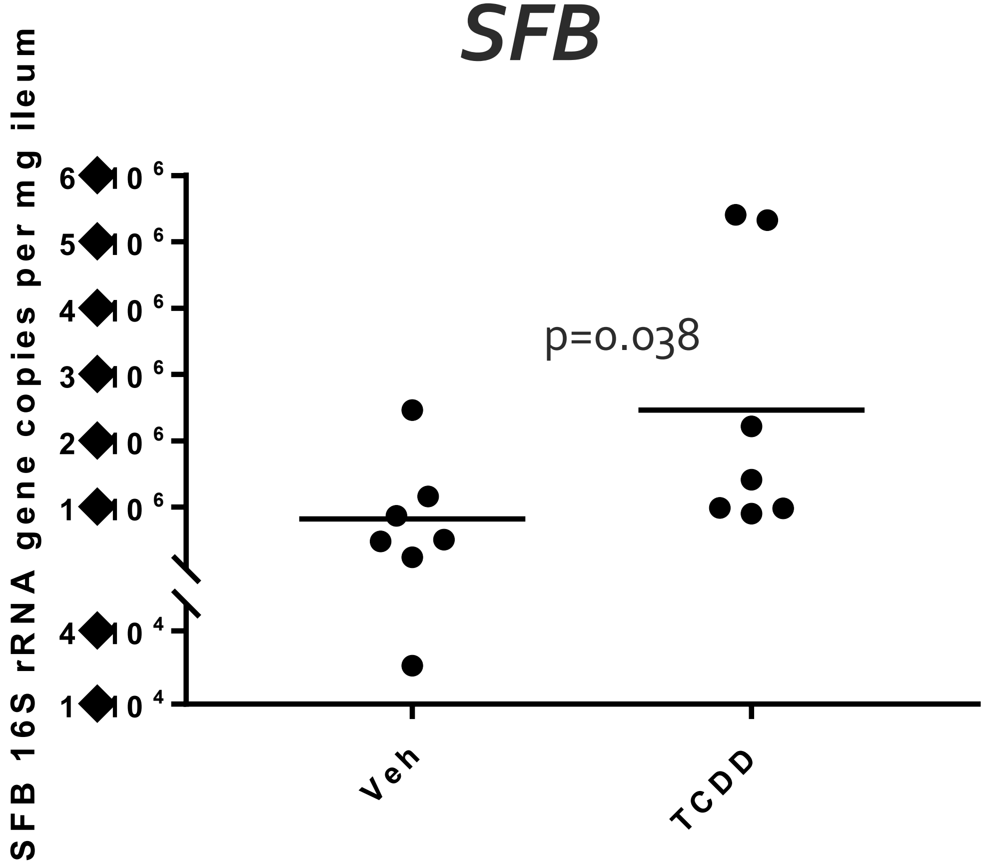


Parametric two-way ANOVA

Gnotobiotic C57BL6: *B. fragilis and* SFB

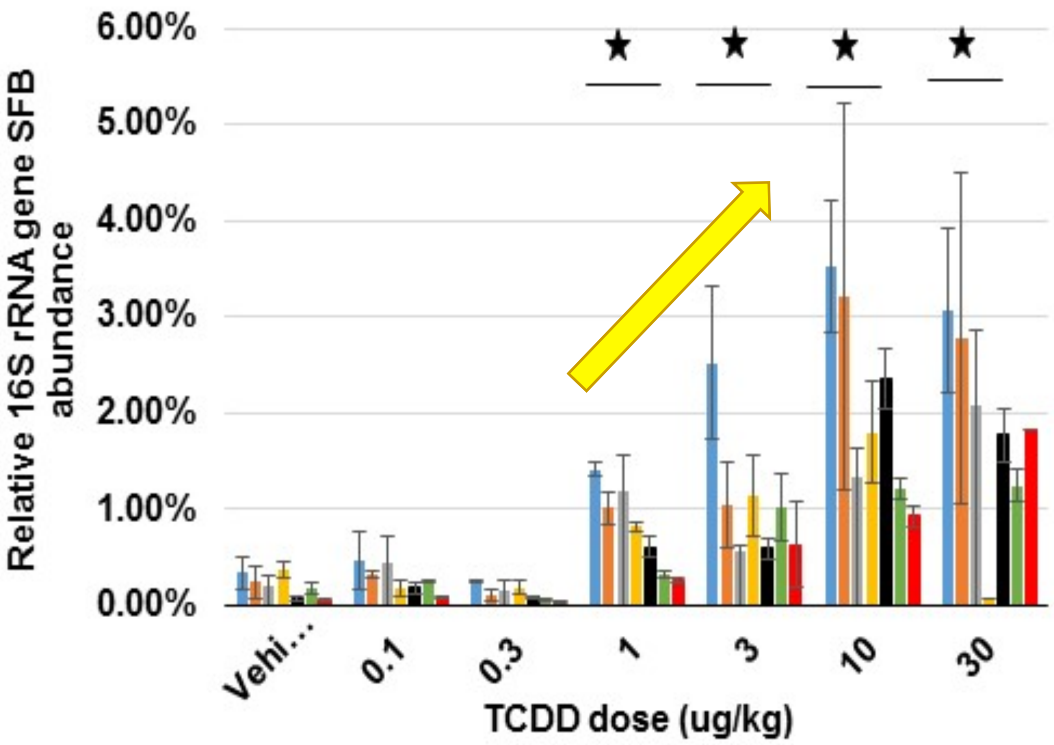


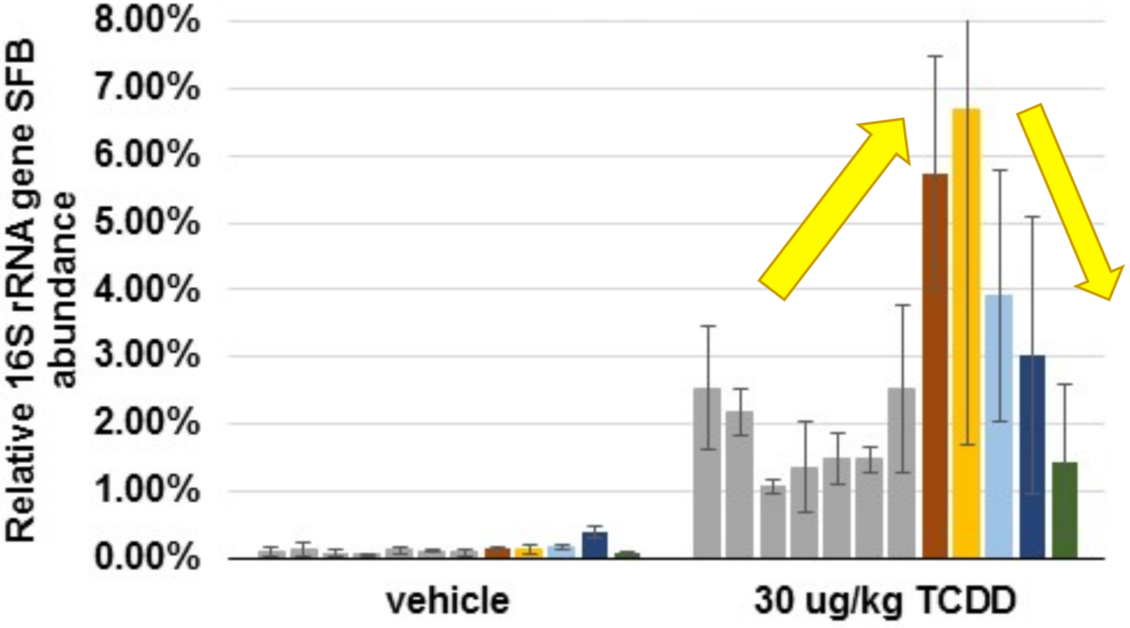
B. fragilis



SFB

Traditional C57BL6: Dose Response & Recovery



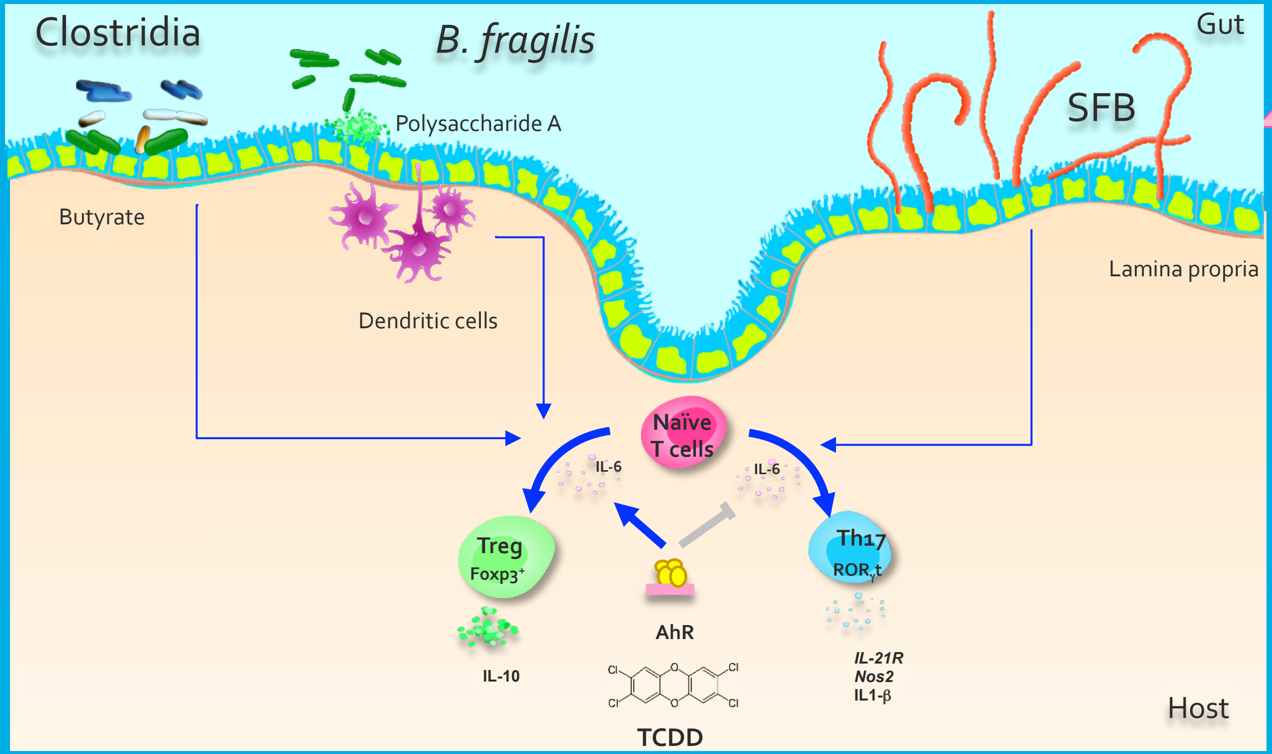


Day 38 90 120

Overall Interaction of TCDD, *B. fragilis*, and SFB

Decrease in *B. fragilis* & Clostridia

(Expected change in *B. fragilis* & Clostridia was a DECREASE)

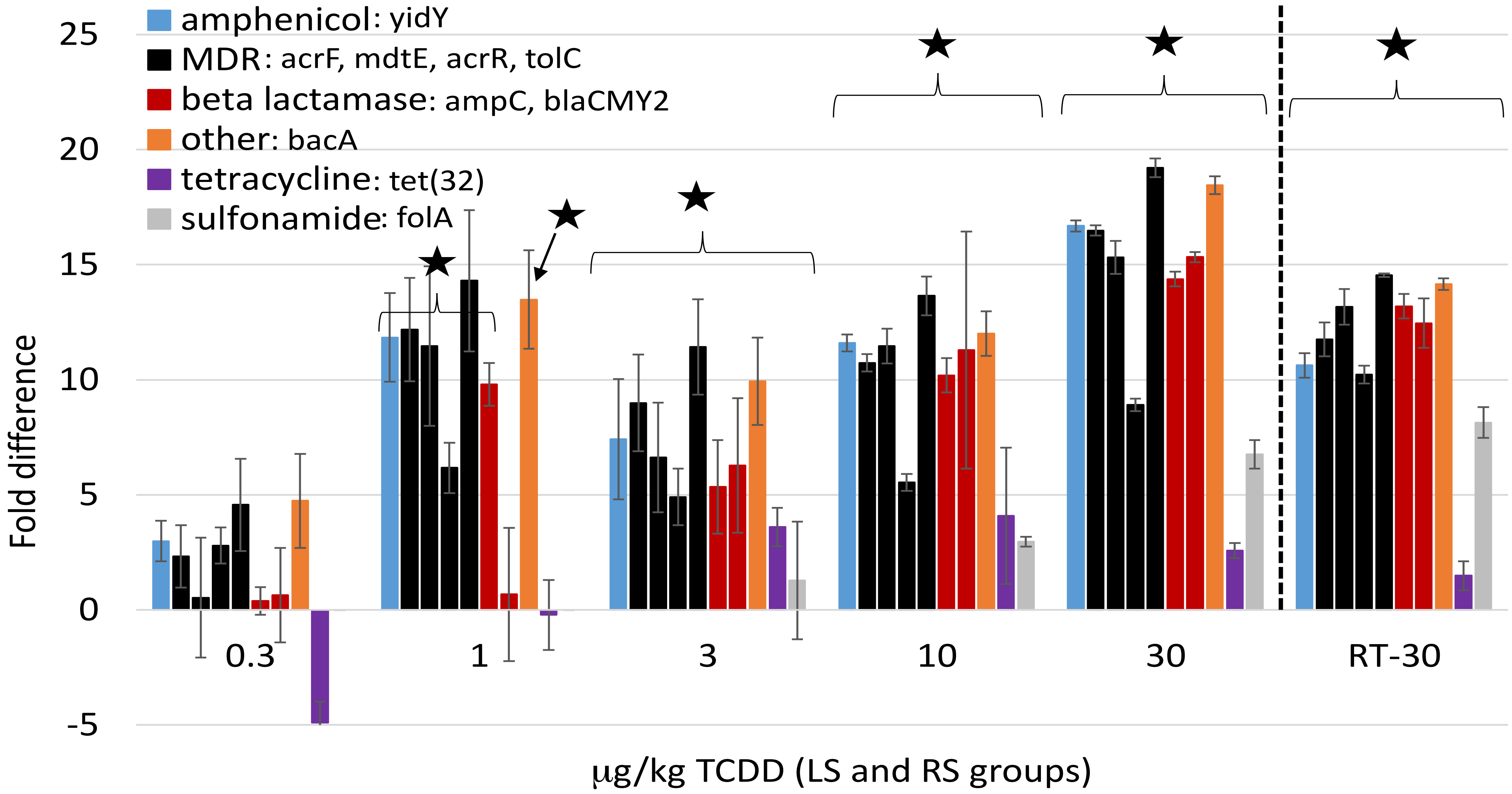


Increase in SFB

(Expected change in SFB was an INCREASE)

TCDD

Traditional C57BL6: Increase in Antimicrobial Resistance Genes



B. Key Challenges Ahead

Identity

Activity

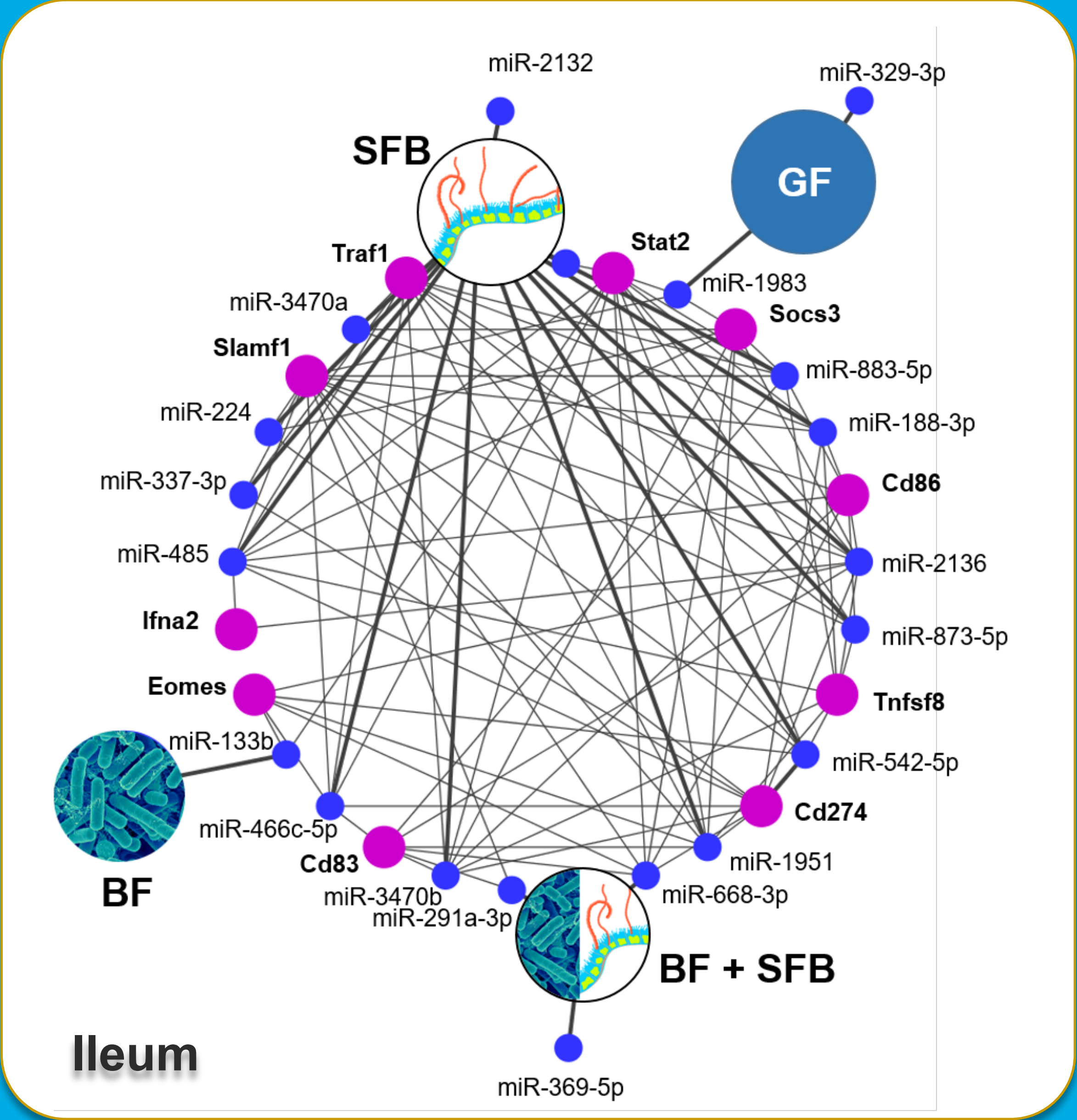
Prediction

Diagnosis

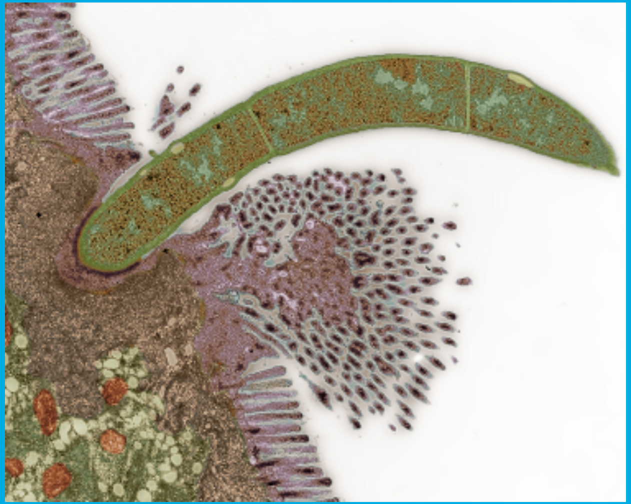
Intervention

1. Who is there?
2. Who is doing what and how?
3. Can we predict gut behavior? Quantitatively?
4. How do we know when something is wrong?
5. How to stop or encourage key members?

2. Who is doing what and how?



microRNA expression in SFB-associated mice is much greater than Germ-free or *B. fragilis*-associated groups!



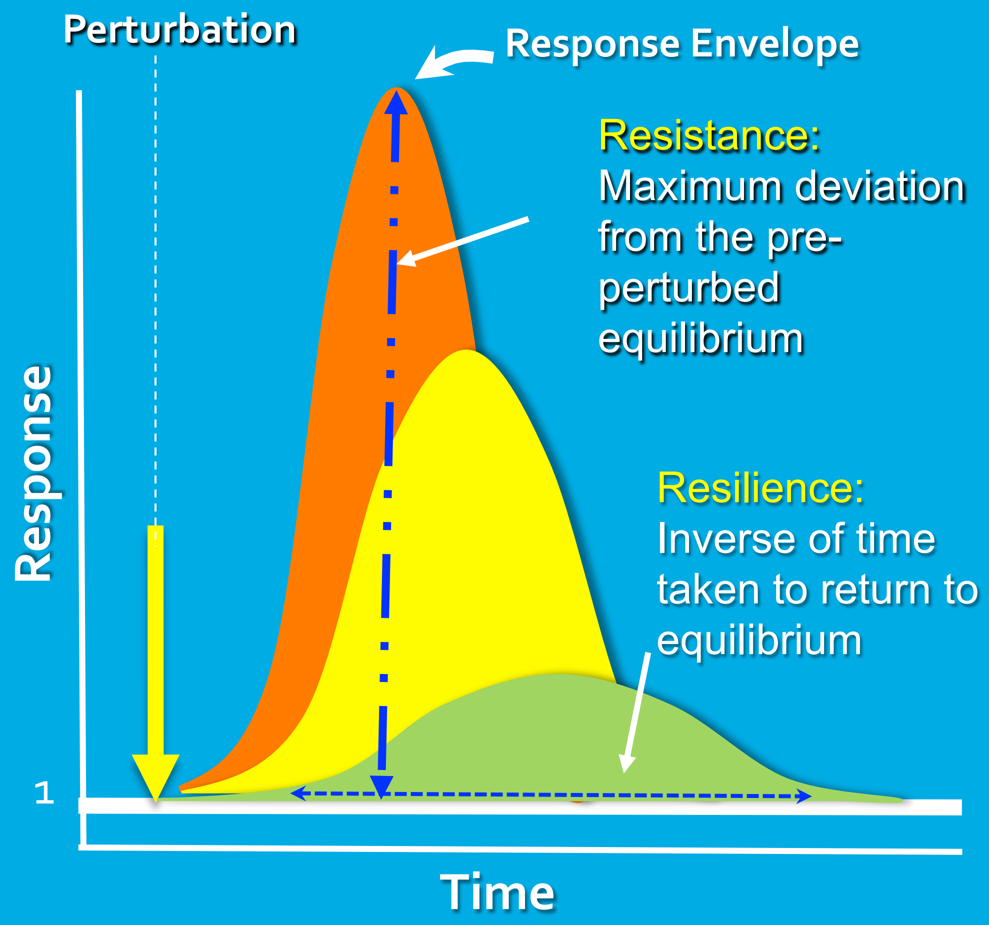
Ivanov et al., *Cell*: 2009

Spatial resolution

More sensitive to work with 1-10 µl blood
At all molecular levels

MicroRNAs may alter the gut microbiota through fecal microRNAs, affecting growth and other cellular processes (*Liu et al., 2016*).

3. Can we predict the gut behavior – quantitatively?



Resistance: Maximum deviation from the pre-perturbed equilibrium

Resilience: Inverse of time taken to return to equilibrium

Hashsham et al., Fernandez et al., AEM, 2000

MDSINE

Time series
Biomass
Perturbation

⇩

Generalized Lota Volterra Model

⇩

Trajectories
Interactions
Stability
Keystone-ness

Bucci et al. *Genome Biology* 17:121, 2016

Gut Disruption Index

VRE vs. ***E. faecium***

Halpin et al, Am. J. Infection Control 44 (2016) 830-6

Disease-specific

Deterministic
Probabilistic

4a. How do we know when something is wrong?

Markers

Functions, Guilds

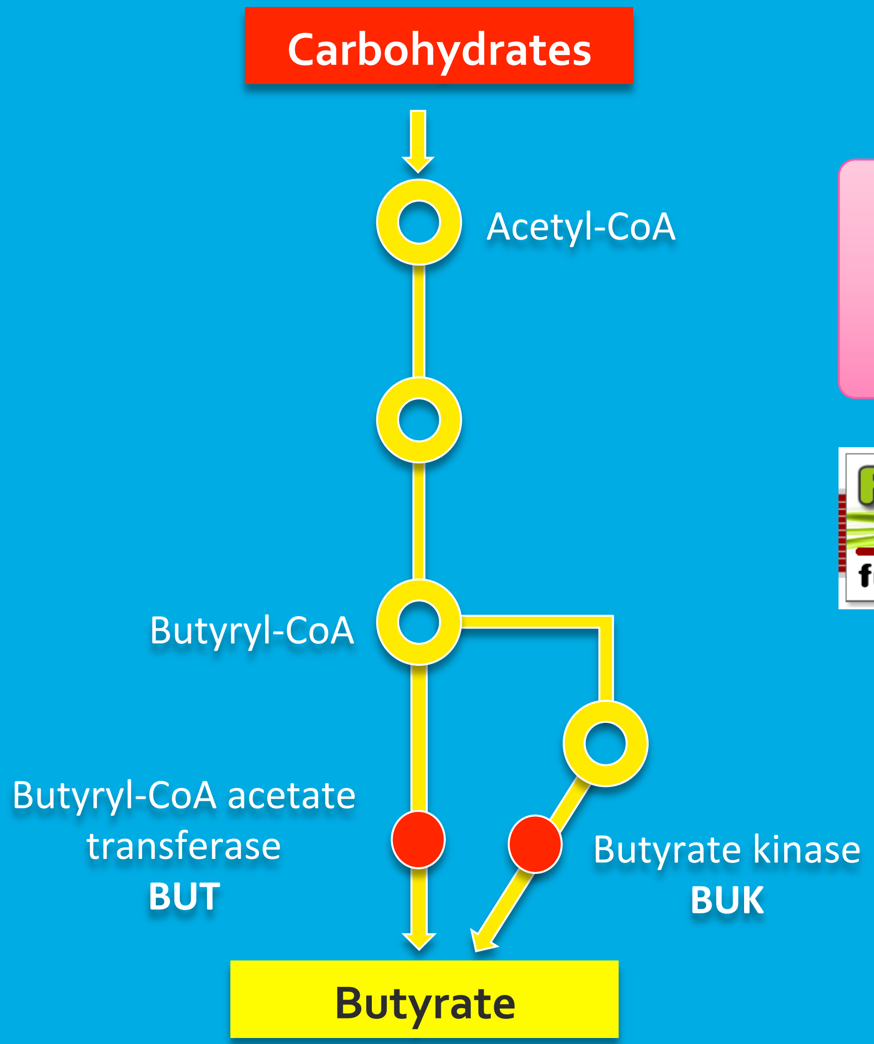
Butyrate producers

Roseburia intestinalis

Faecalibacterium prausnitzii

….

Vital et al., mBio, 2014



Carbohydrates

Acetyl-CoA

Butyryl-CoA

Butyryl-CoA acetate transferase
BUT

Butyrate kinase
BUK

Butyrate

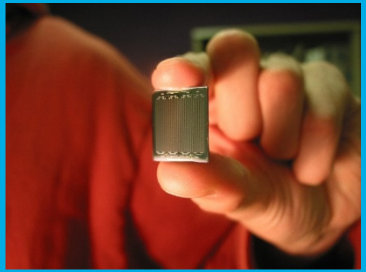
Functional Gene Diversity
Primer Coverage

FunGene
functional gene pipeline & repository

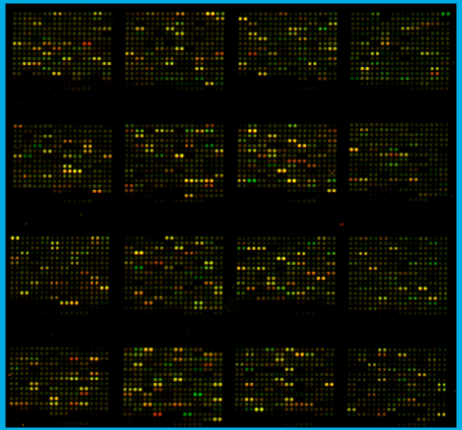
Fish et al., *Front. Microbiol.* 4: 291 2013

4b. How do we know when something is wrong?

Gut Chips



Amplification-based qPCR or Low-density Chips



Hybridization-based Arrays

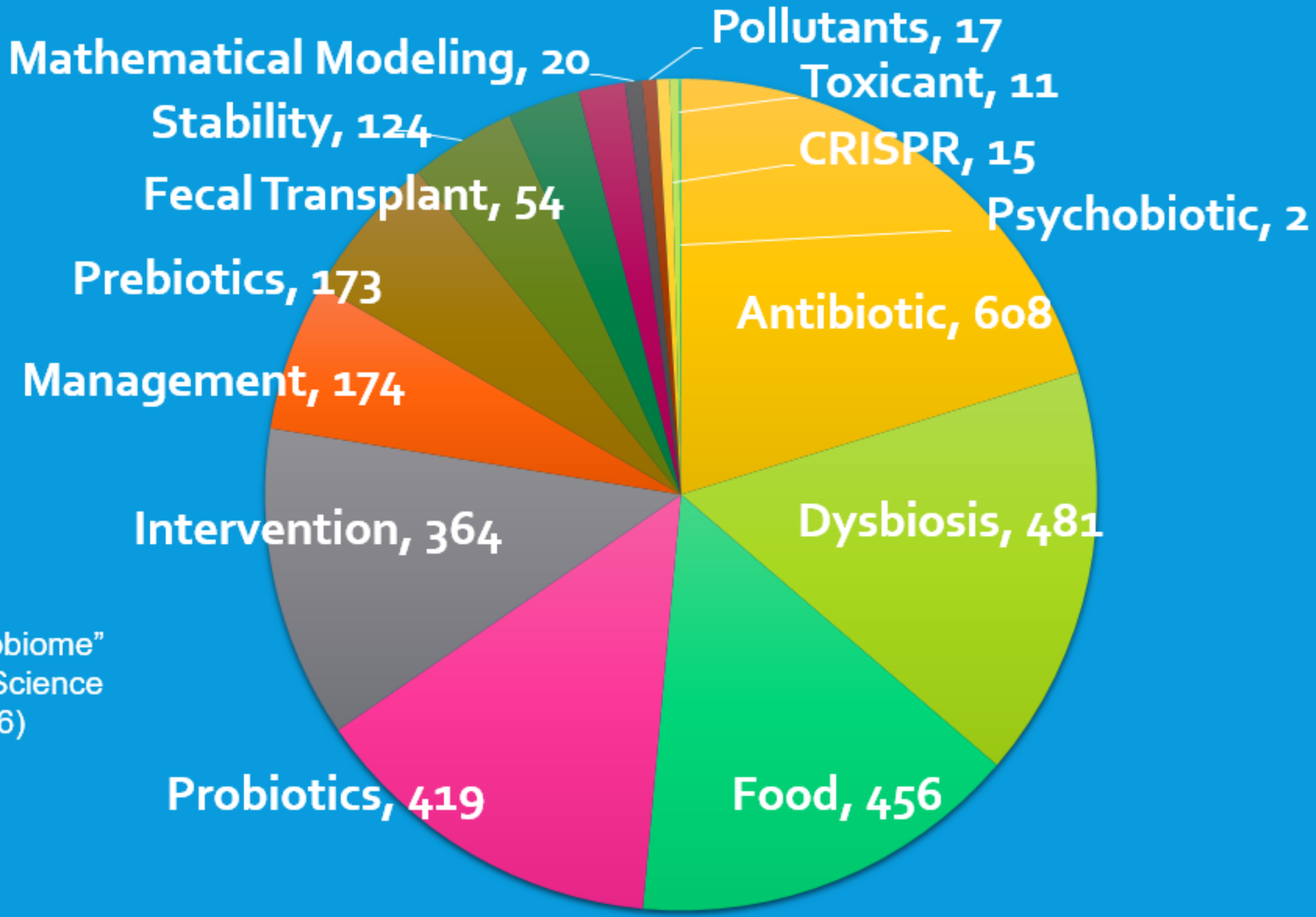
High Throughput Sequencing

Fluidigm (24 primer sets)
GULDA (Gut Low Density Array): 31 targets

HuMiChip (500 functional genes, 180,000 probes)
HuGChip (66 families, 4000 probes)
IBS/IBD Chip (300 bacteria, 54 probes)

Numerous but most focused on 16S rRNA gene based

5. How to stop, encourage, or manage them?



Total of 4600 “Gut Microbiome” Publications in Web of Science (As of Oct 26, 2016)

Summary

1. TCDD and SFB/*B. fragilis* interact through AhR in a predictable manner in terms of immune cell response. Such interactions may establish the basis for intervention.

2. Measuring smaller effects of toxicants on gut microbiome members through the host may be difficult.

3. Gut member activity, mode of communication with the host, quantitative predictive models, and markers of healthy/sick gut microbiome are some of the key challenges ahead in gut microbiome research.

Gut Microbiome

Play Store

App Store

Feedback:
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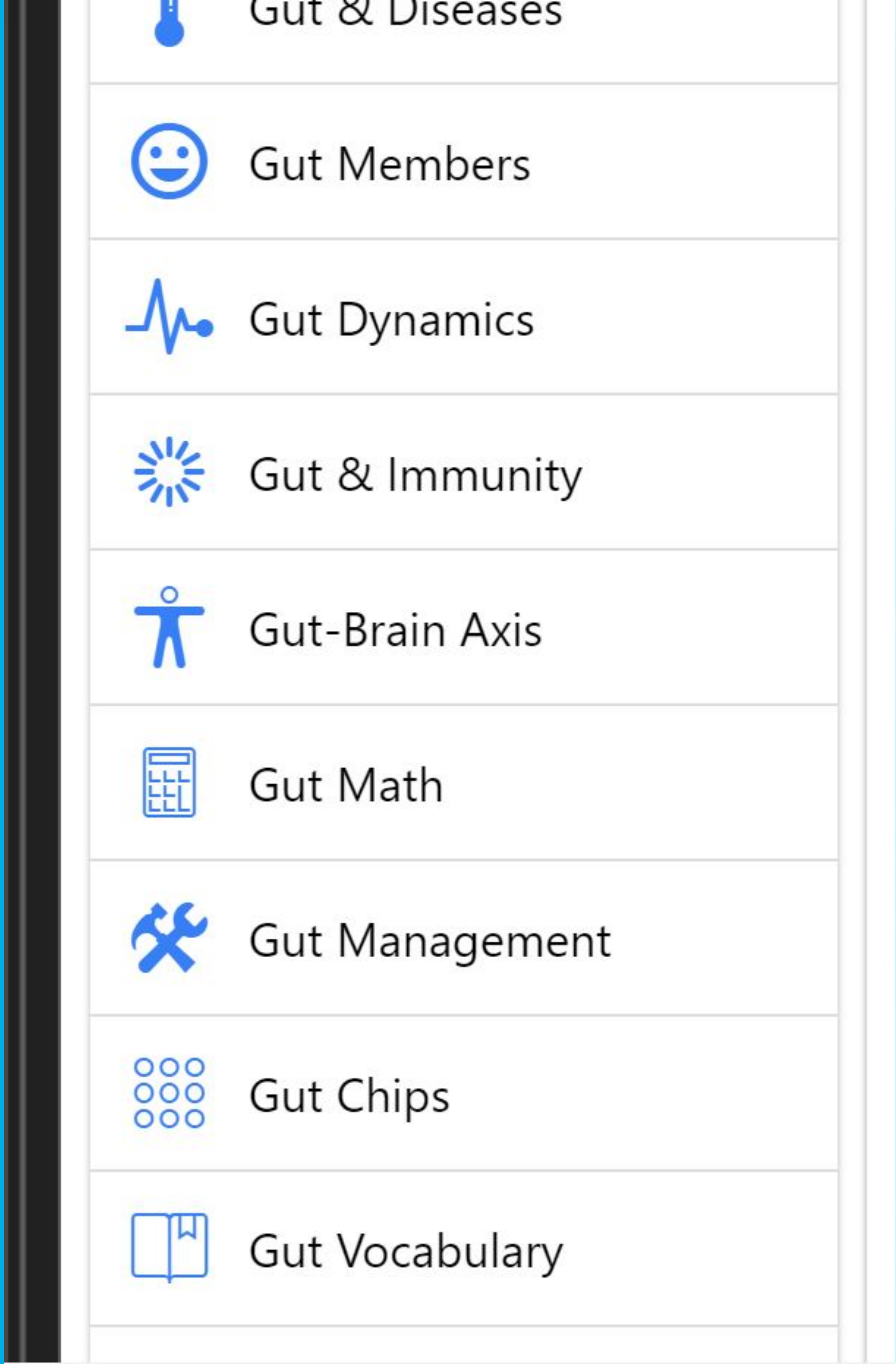

- Gut & Diseases
- Gut Members
- Gut Dynamics
- Gut & Immunity
- Gut-Brain Axis
- Gut Math
- Gut Management
- Gut Chips
- Gut Vocabulary

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SFB source: Candidatus Arthromitus SFB-mouse-Japan was provided by Dr. Tomomi Kuwahara under MTA.