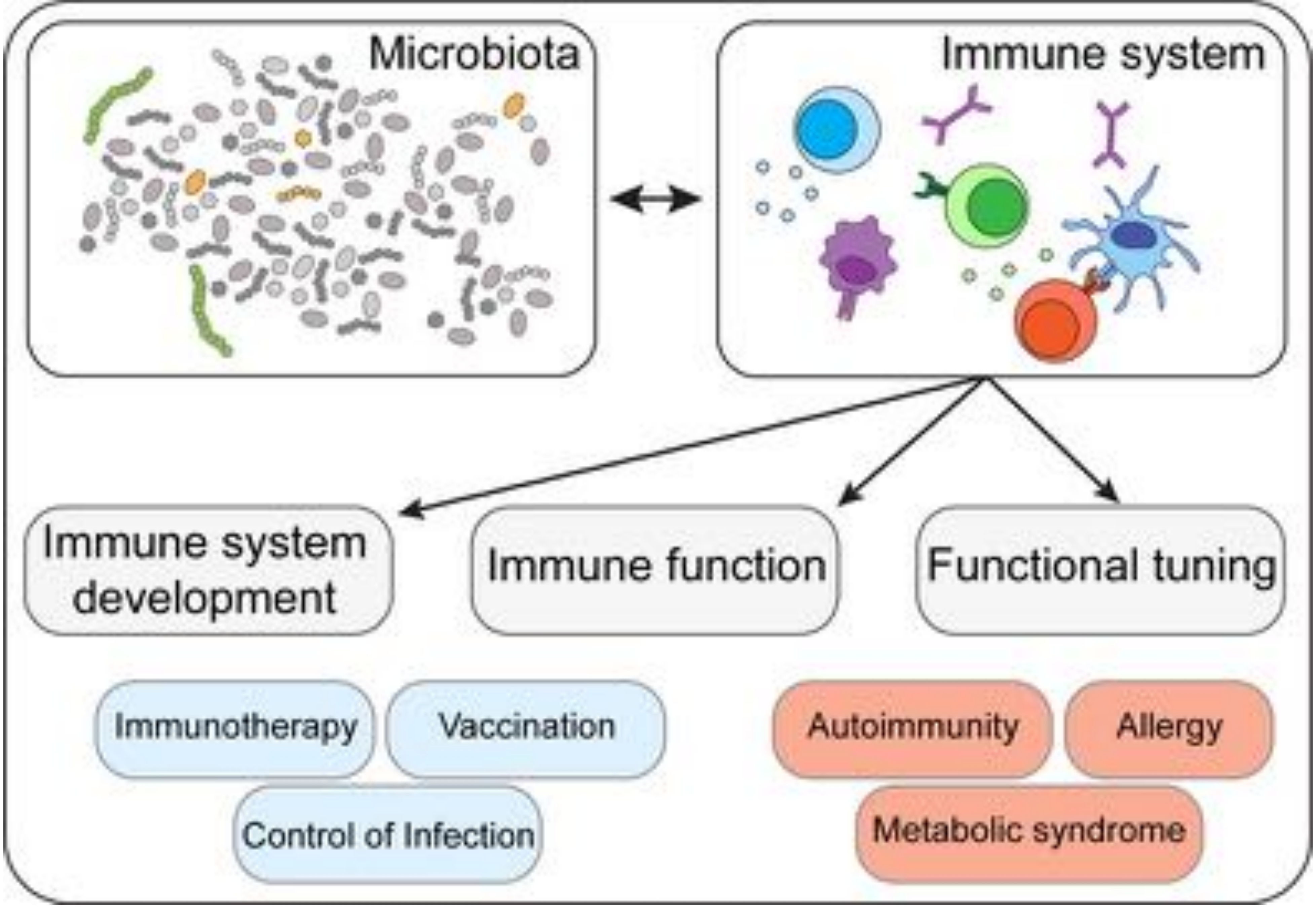
IID 209 Lecture #3: Mechanisms of host interaction, major microbial sensing pathways, and cell subsets.

LEARNING OBJECTIVES

- Describe major immune pathways responsible for microbial interactions.
- Identify molecular mechanisms responsible for common host-microbial interaction types.
- Gain a broad understanding of the host cell subsets that mediate host-microbiota interactions

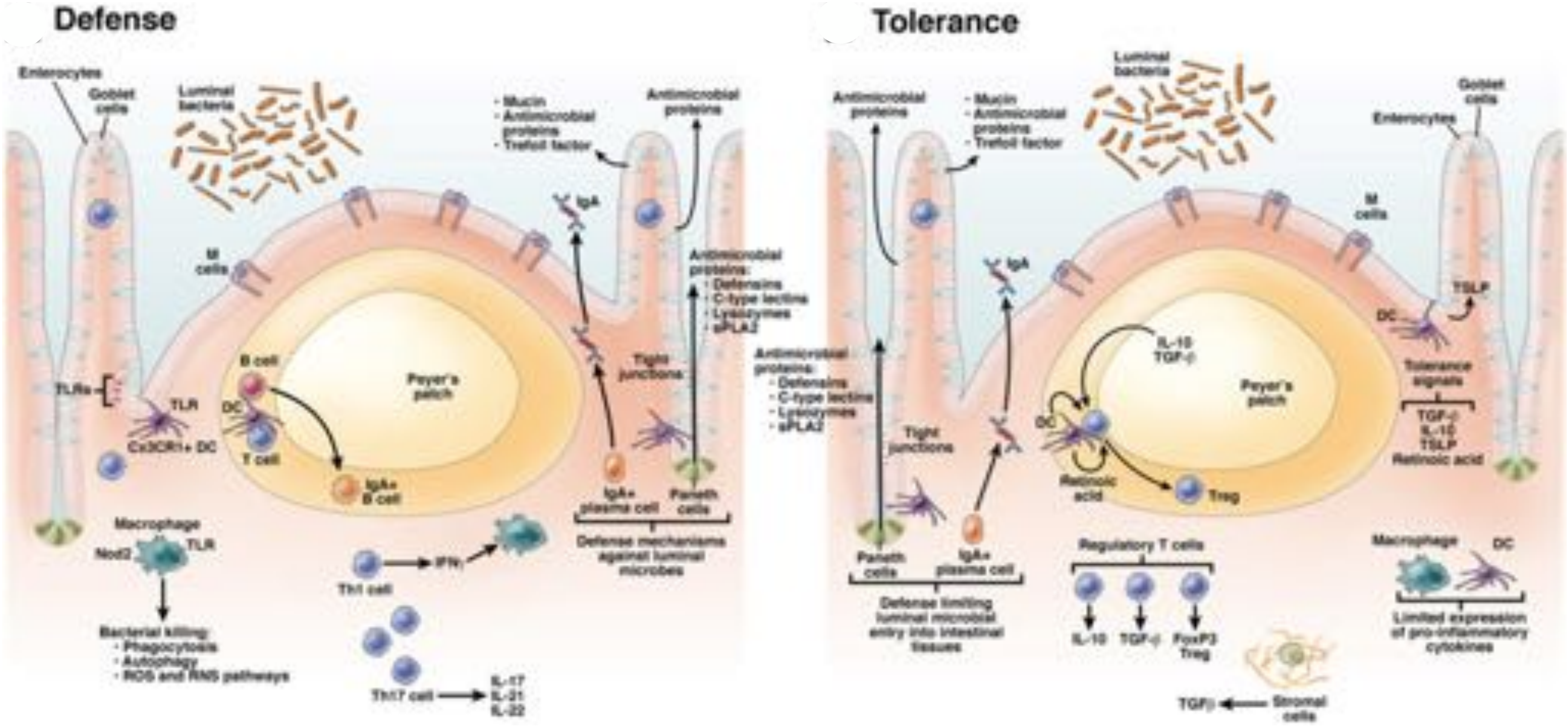
Outline

- **Introduction**
- Microbial Sensing
  - Classic Pattern Recognition Receptors (PRRs) & Microbial Associated Molecular Patterns (MAMPs)
  - Next-generation Microbial Sensors
- Host Cell Mediators of Host-Microbiota Interactions
  - Innate
  - Adaptive



Belkaid and Harrison. [Immunity. 2017 Apr 18; 46(4): 562–576.]

Think rheostat not switch in navigating the balance between immune defense and tolerance



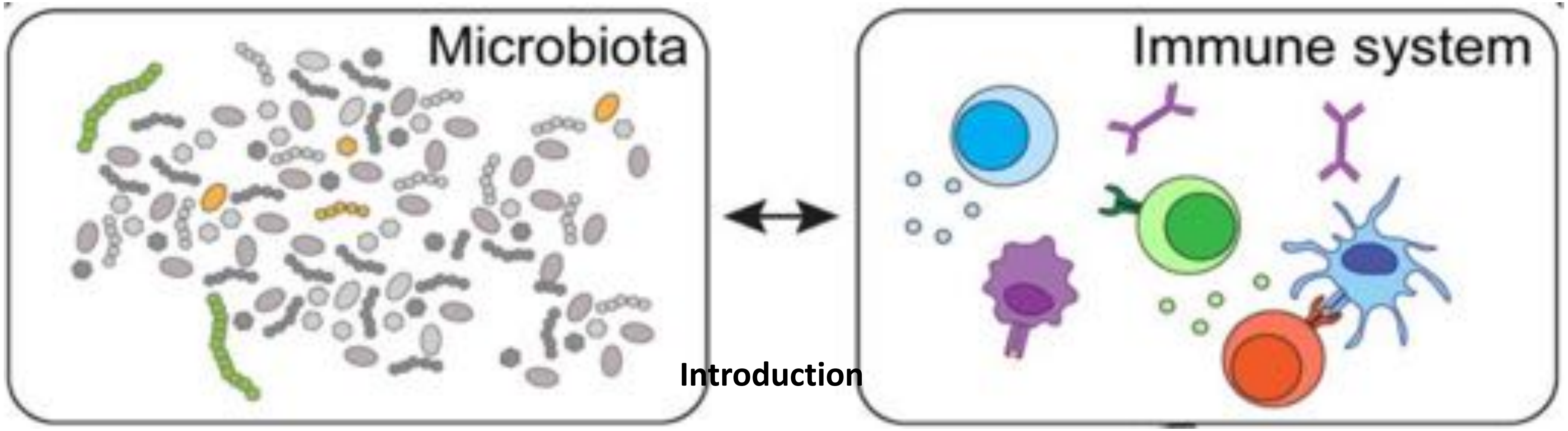
Abraham and Medzhitov. Gastroenterology. 2011 May; 140(6): 1729–1737.

The three lines of tolerance_defense of the immune system

NON-SPECIFIC DEFENCES (INNATE IMMUNITY)		SPECIFIC DEFENCES (ADAPTIVE IMMUNITY)
First line of defense	**Second line of defense**	**Third line of defense**
• Skin • Mucous membranes • Secretions of skin and mucous membranes	• Phagocytic leukocytes • Antimicrobial proteins • Inflammatory response • Fever	• Lymphocytes • Antibodies • Memory cells

http://ib.bioninja.com.au/standard-level/topic-6-human-physiology/63-defence-against-infectio/lines-of-defense.html

What helps to ensure homeostasis between host & microbiota



Introduction

LIMITING CONTACT

- Epithelial Barrier
- Mucus
- IgA
- Antimicrobial Peptides
- Immune cells that clear microbes quietly

Belkaid and Harrison. Immunity. 2017 Apr 18; 46(4): 562–576.

Outline

- Introduction
- **Microbial Sensing**
  - Classic Pattern Recognition Receptors (PRRs) & Microbial Associated Molecular Patterns (MAMPs)
  - Next-generation Microbial sensors
- Host cell mediators of mediate host-microbiota interactions
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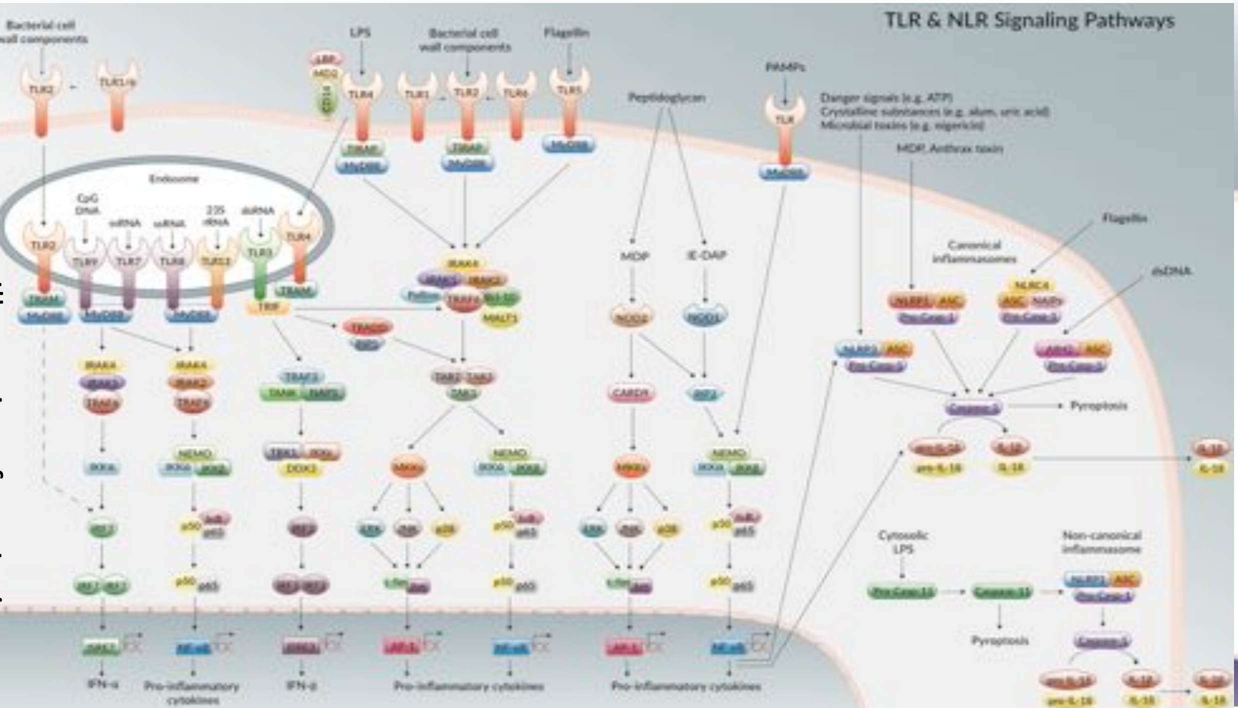
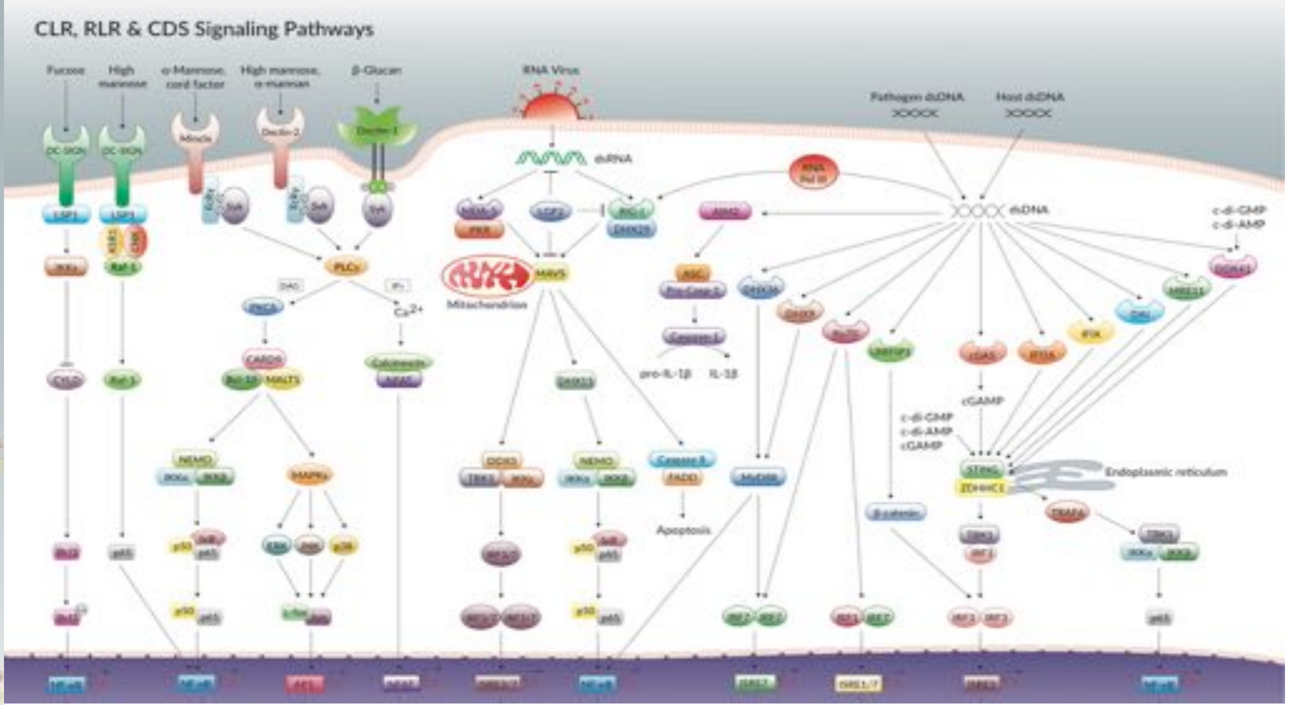
  - Adaptive

How are microbes sensed?

- Microbial-associated molecular patterns (MAMPs)
  - Sugars, lipids, proteins, DNA, RNA, metabolites
- Pattern recognition receptors (PRRs)
  - **T**oll-**l**ike **R**eceptors (TLRs), **N**ucleotide-binding Oligomerization-Domain (NOD) –**L**ike Containing **R**eceptors (NLRs), **C**-type **L**ectin **R**eceptors (CLRs), **R**etinoic-acid inducible gene-I (RIG-I) **L**ike **R**eceptors(RLR), **C**ytosolic **D**NA **S**ensors (CDs), Short-chain fatty receptors, and Aryl hydrocarbon receptors



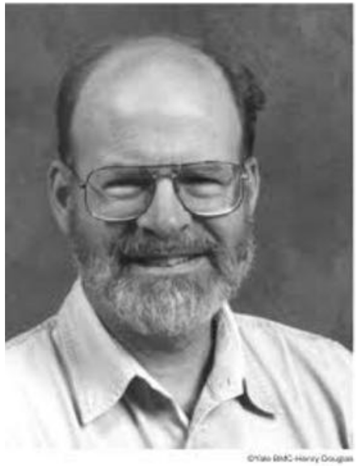


Illustrations from *Invivogen*

A brief history of how Toll-like receptors was discovered….

- 1960-1980, Two mouse strains described that were non-responsive to LPS (outer membrane component of Gram-negative bacteria that stimulates humoral and cell-mediate immune responses).
  - Resistance to infection, bone marrow chimeras,, and classic genetics lead to the discovery of the *lps locus* that non-redundantly encoded a receptor for LPS
- 1989, Charles Janeway proposes a framework for immune recognition that involves PAMPs and PRRs
- 1998, Bruce Beutler uses genetic evidence supporting TLR4 is the receptor of LPS
- 1998, Shizuo Akira uses functional and genetic data supporting TLR4 is the LPS receptors
- 2009, Crystal structure data show that lipid A, a component of LPS, binds TLR4

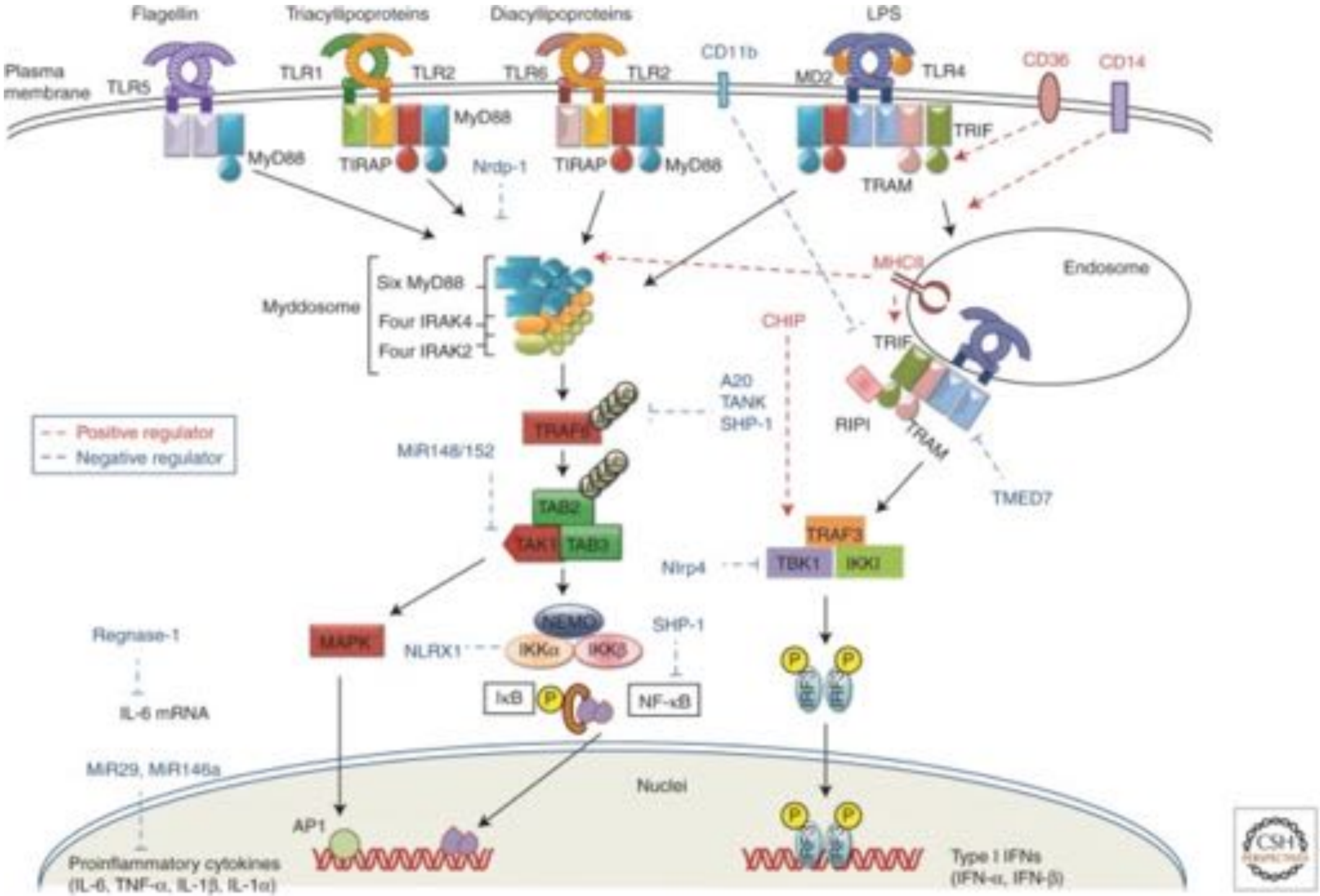
- 1985-1997, data in drosophila demonstrate that a gene involved in dorsal-ventral patterning functioned in drosophila anti-fungal immunity, Toll has high homology to the human IL-1R, and when stimulated Toll activated dorsal (NF-kB homolog)



http://www.jimmunol.org/content/197/7/2561

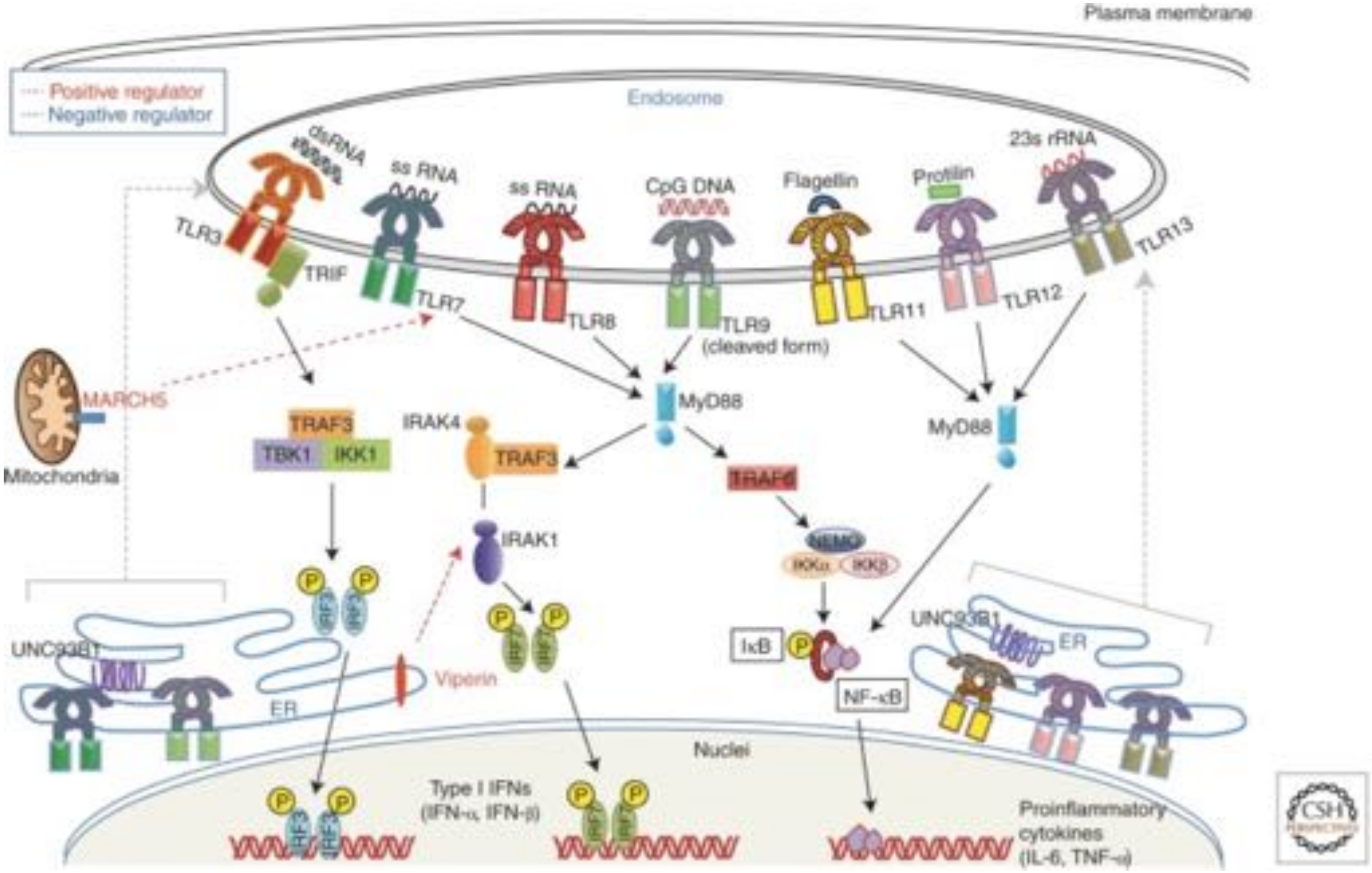
PRR	Localization	MAMP recognized
TLRs		
TLR1	Cell surface	Triacylated lipopeptides
TLR2	Cell surface	Di/triacylated lipopeptides
TLR3	Endosomes	dsRNA
TLR4	Cell surface	LPS
TLR5	Cell surface	Flagellin
TLR6	Cell surface	Diacylated lipopeptides
TLR7	Endosomes	ssRNA
TLR8	Endosomes	ssRNA
TLR9	Endosomes	CpG DNA
TLR11	Endosomes	Profilin, flagellin
TLR12	Endosomes	Profilin
TLR13	Endosomes	23s rRNA
RLRs		
RIG-I	Cytoplasm	Short dsRNA, ssRNA
MDA5	Cytoplasm	Long dsRNA
LGP2	Cytoplasm	dsRNA
DDX3	Cytoplasm	Viral RNA
Cytosolic DNA sensors		
DAI	Cytoplasm	dsDNA
RNA Pol III		AT rich dsDNA
IFI16	Nucleus and cytoplasm	dsDNA
AIM2	Cytoplasm	dsDNA
Ku70	Cytoplasm	dsDNA
MRE11		dsDNA, ISD
cGAS	Cytoplasm	dsDNA
LRRFIP1	Cytoplasm	dsDNA, dsRNA
DHX36	Cytoplasm	dsDNA
DHX9	Cytoplasm	dsDNA
DDX41	Cytoplasm	c-di-GMP, c-di-AMP
STING	Cytoplasm	c-di-GMP
HMGB	Cytoplasm	dsDNA, ssDNA
Histone H2B	Nucleus and cytoplasm	Poly (dA:dT), genomic DNA

Cell-surface TLRs



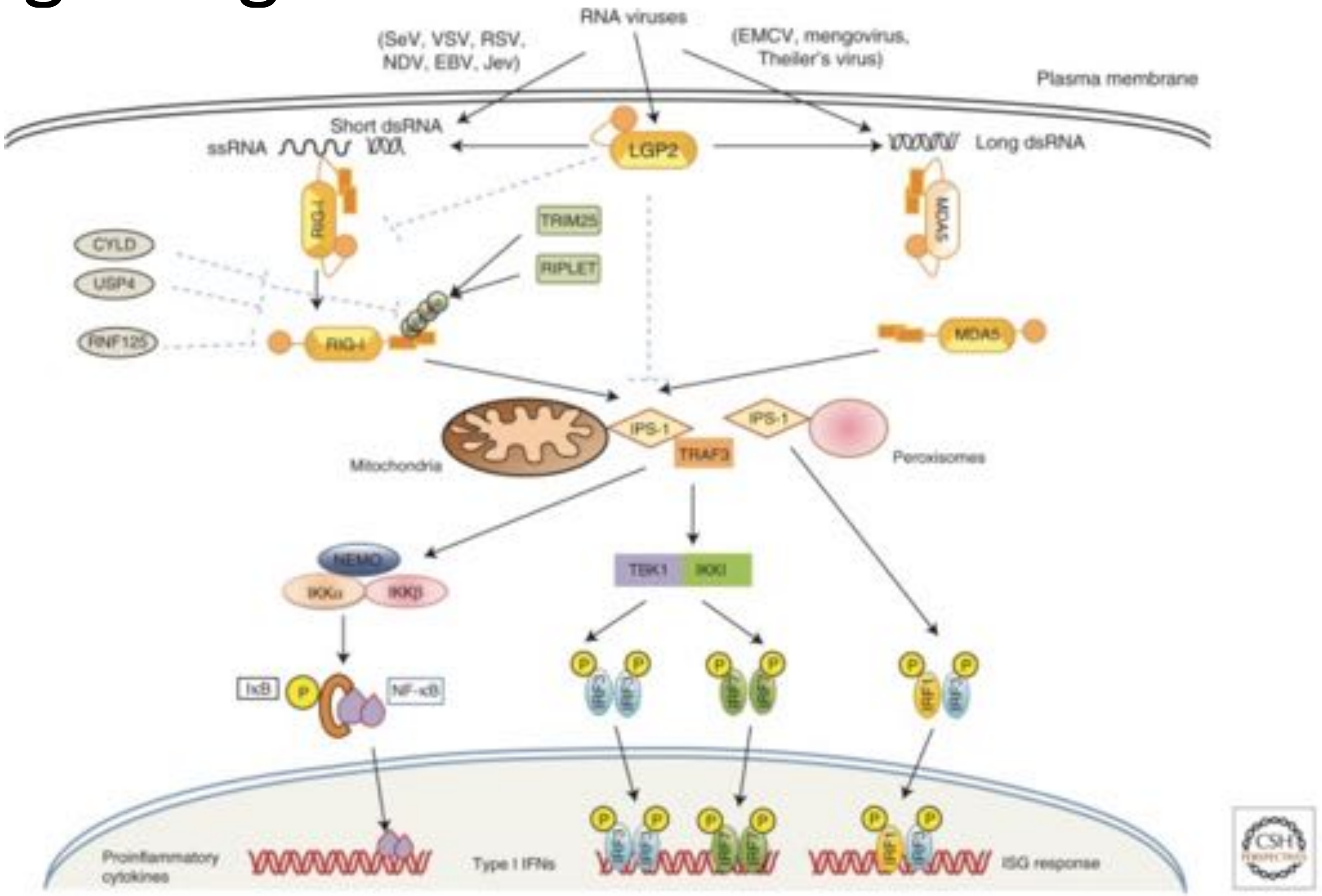
Pandey S et al. Cold Spring Harb Perspect Biol. 2015 Jan; 7(1): a016246.

Endosomal TLRs



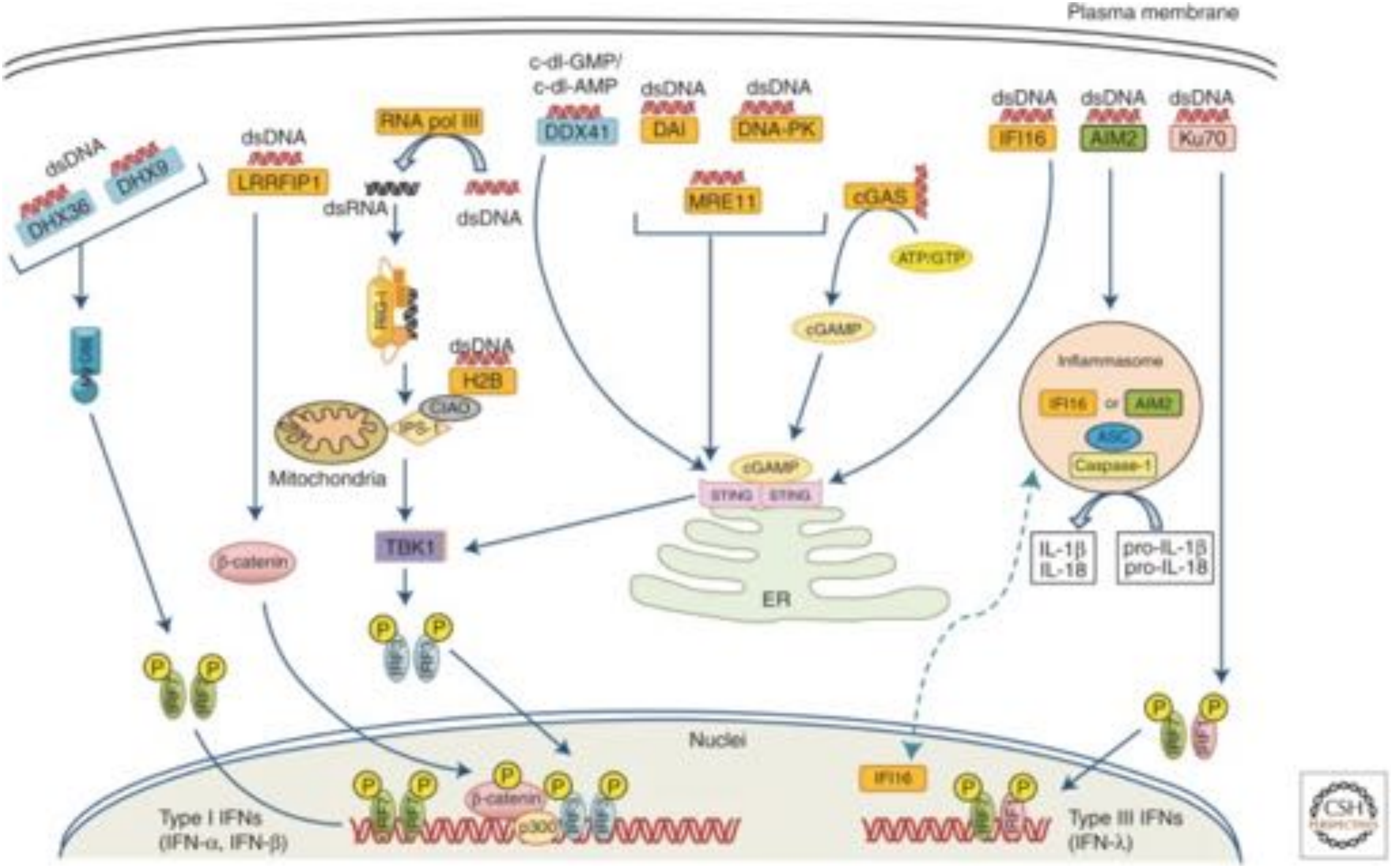
Pandey S et al. Cold Spring Harb Perspect Biol. 2015 Jan; 7(1): a016246.

RLR signaling



Pandey S et al. Cold Spring Harb Perspect Biol. 2015 Jan; 7(1): a016246.

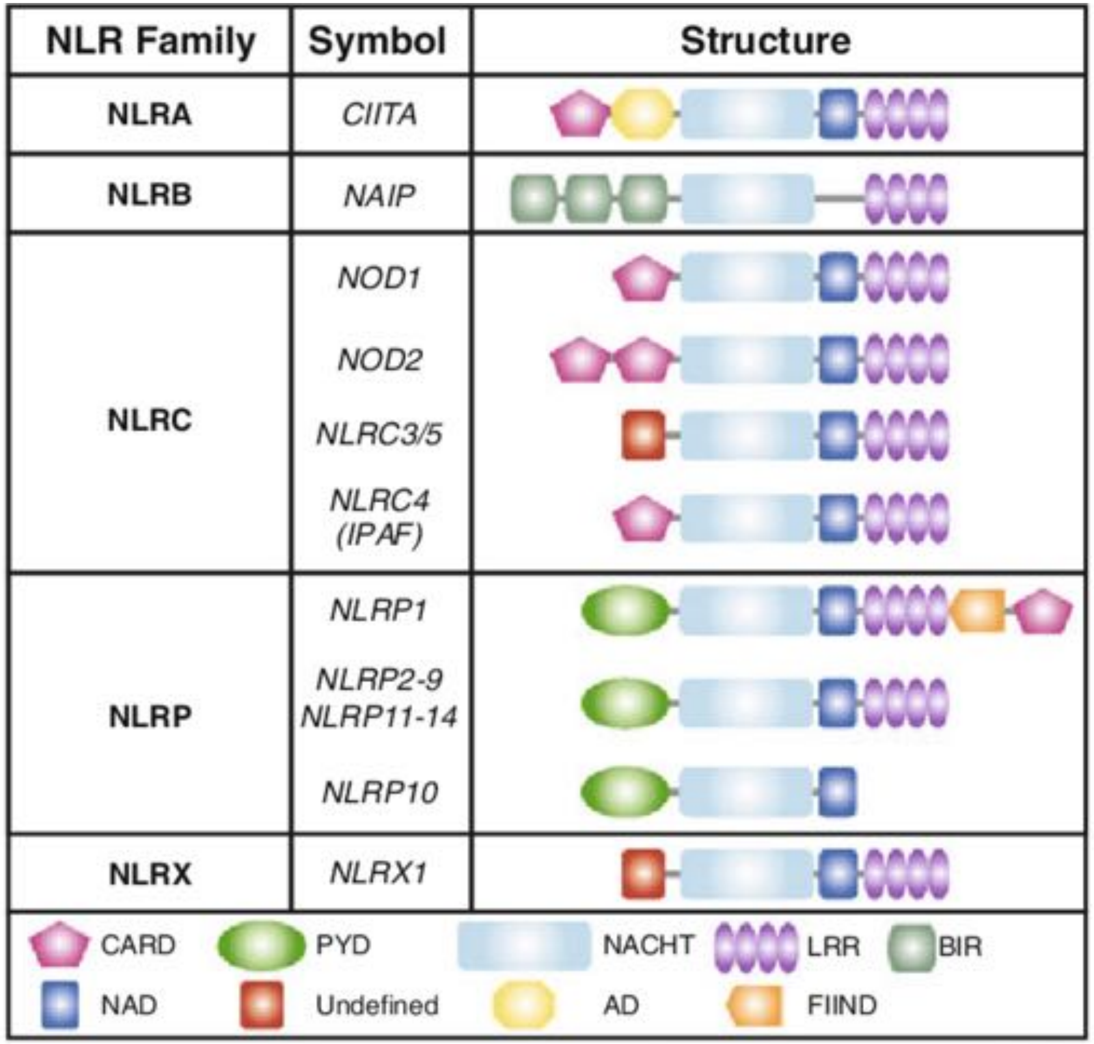
Cytosolic DNA Sensors



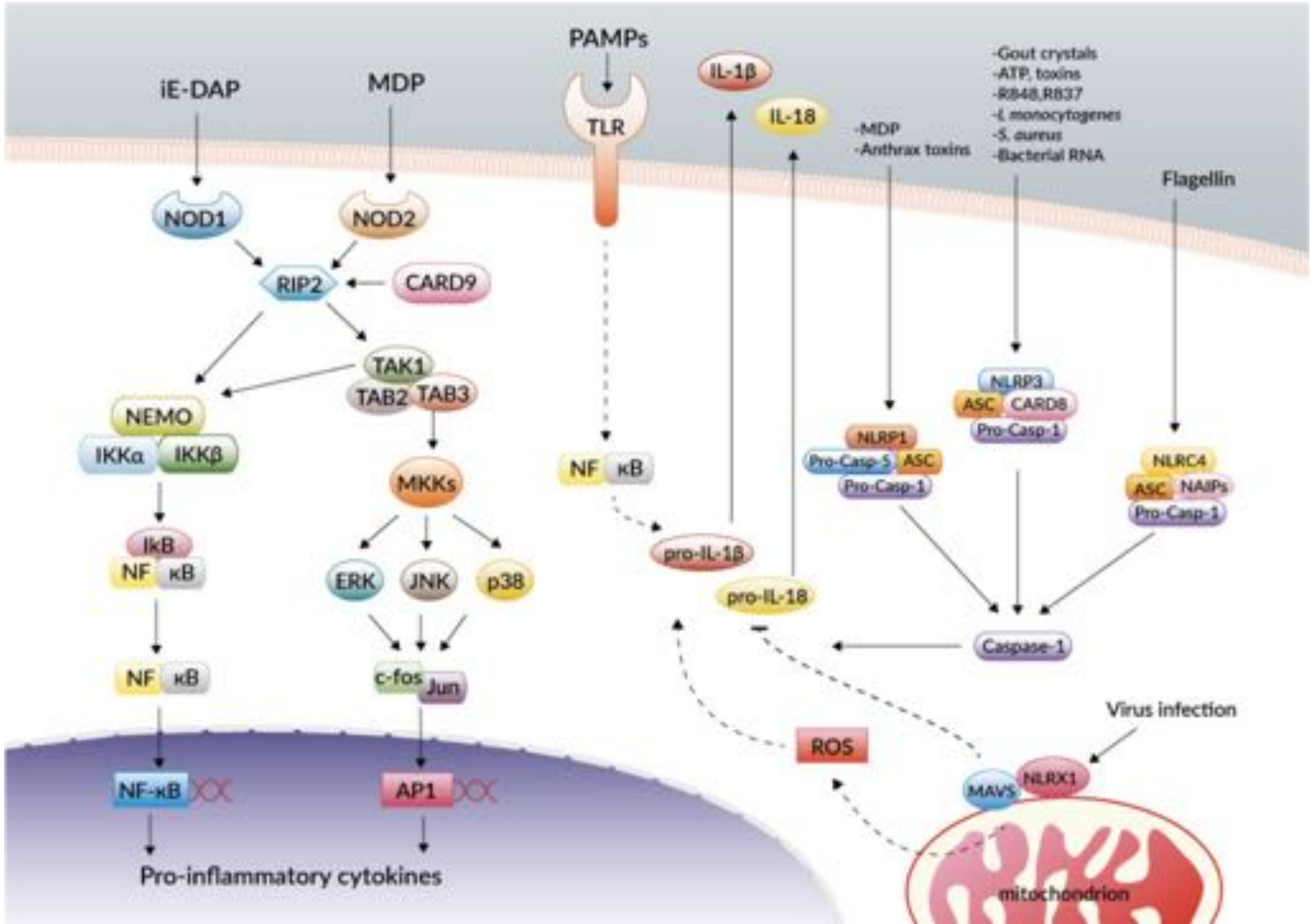
Pandey S et al. Cold Spring Harb Perspect Biol. 2015 Jan; 7(1): a016246.

NOD-like Receptors

- There are 22 NLRs identified to date
- They are divided into 5 sub-families
-     based on the N-term effector domains
- NLRs sense infection and stress through MAMPs and DAMPs
- They orchestrate inflammatory responses, autophagy or cell death
- A high incidence of mutations are found in these genes in patients with inflammatory/'auto-immune' disorders



NLR signaling overview



NOD 1 and NOD2

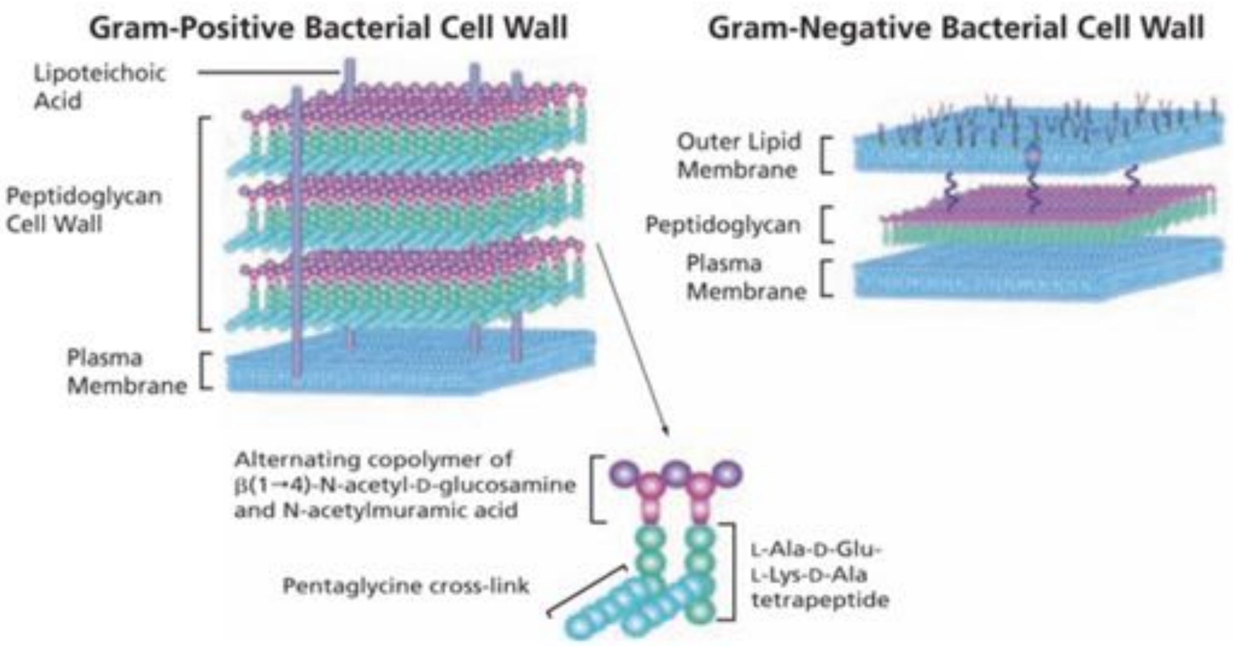
Recognize distinct motifs of **peptidoglycan** (PGN), a key part of the bacterial cell wall

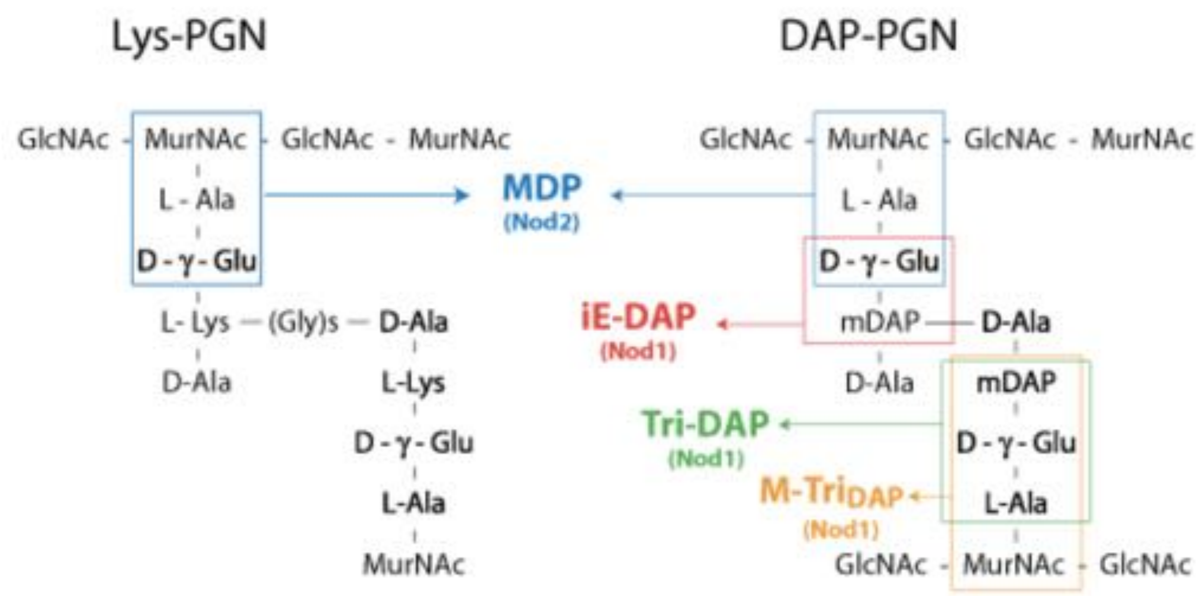
NOD1 senses senses the D-γ-glutamyl-meso-DAP dipeptide (**iE-DAP**)

- iE-DAP is found in the PGN of all Gram-negative & certain Gram-positive bacteria

NOD2 recognizes the muramyl dipeptide (**MDP**) structure found in almost all bacteria.

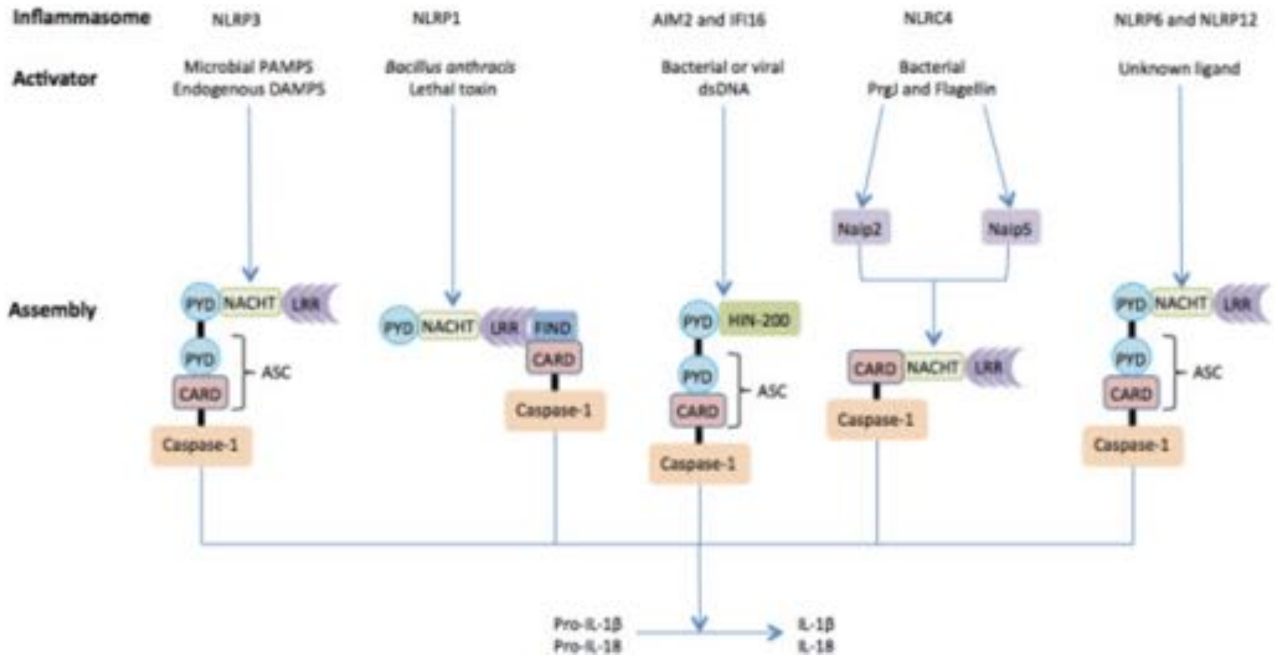
Genetic mutations in NOD2 are associated with **Crohn's disease**, a type of inflammatory bowel disease





NLRs and the Inflammasome

Some members of the NLR family plays a key role in the regulation of caspase-1 by forming a multiprotein complex called the “inflammasome”



Caspase-1 participates in the processing and subsequent release of proinflammatory cytokines, e.g. IL-1β and IL-18.

Classic PRRs completed & Now for The Next Generation of PRRs….

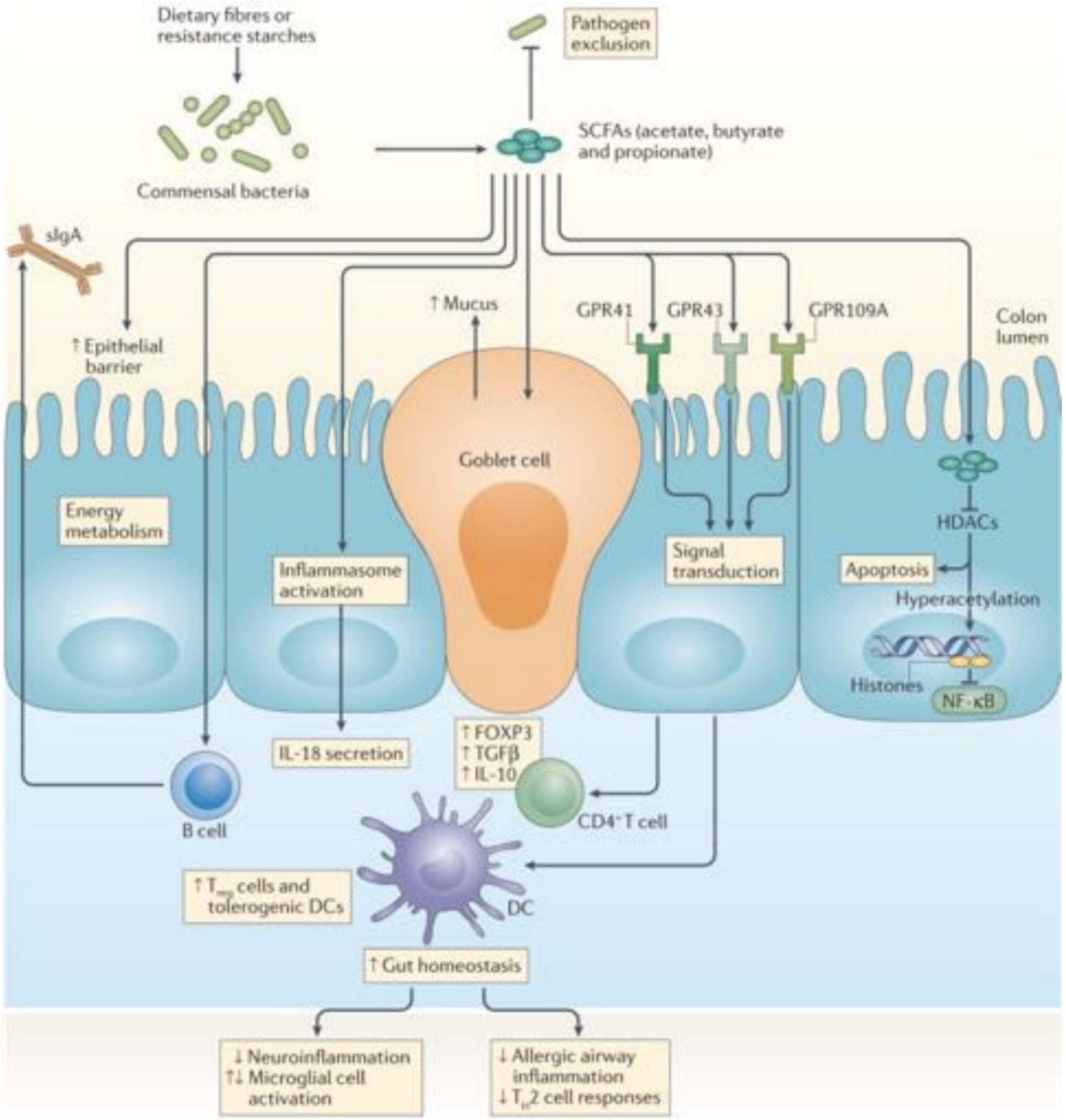
Short-chain fatty acids….

SCFA are 2, 3, 4 and carbon carboxylic acids generated from the microbial metabolism of carbohydrates and amino acids

They include acetic acid, propionic acid, and butyric acid.

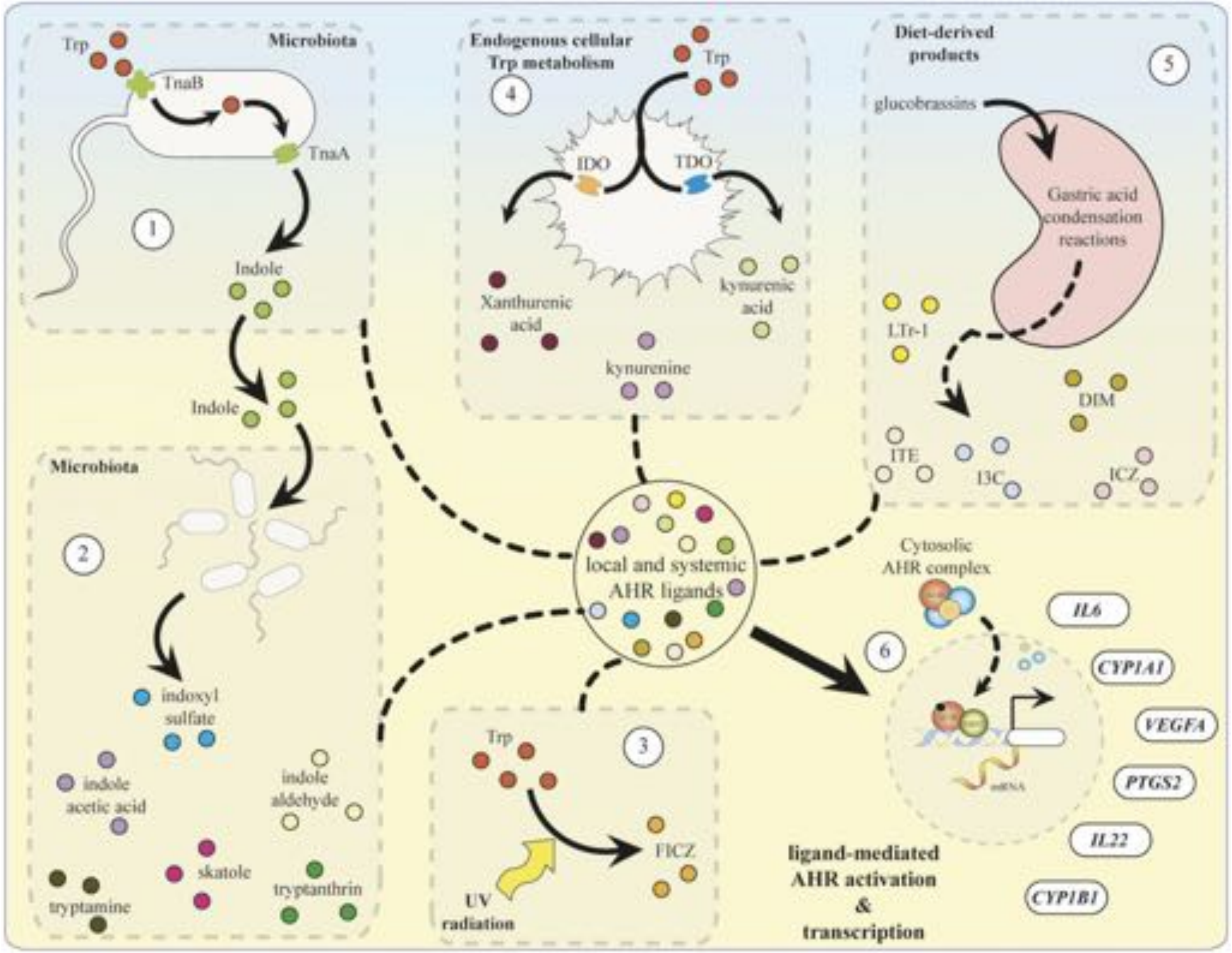
They are abundant mM levels in human (and mouse) gut

They influence the function of the microbiota and the immune system



Rooks and Garrett. Nat Rev Immunol. 2016 May 27;16(6):341-52

Microbially-modified tryptophan metabolites engage the Aryl Hydrocarbon Receptor



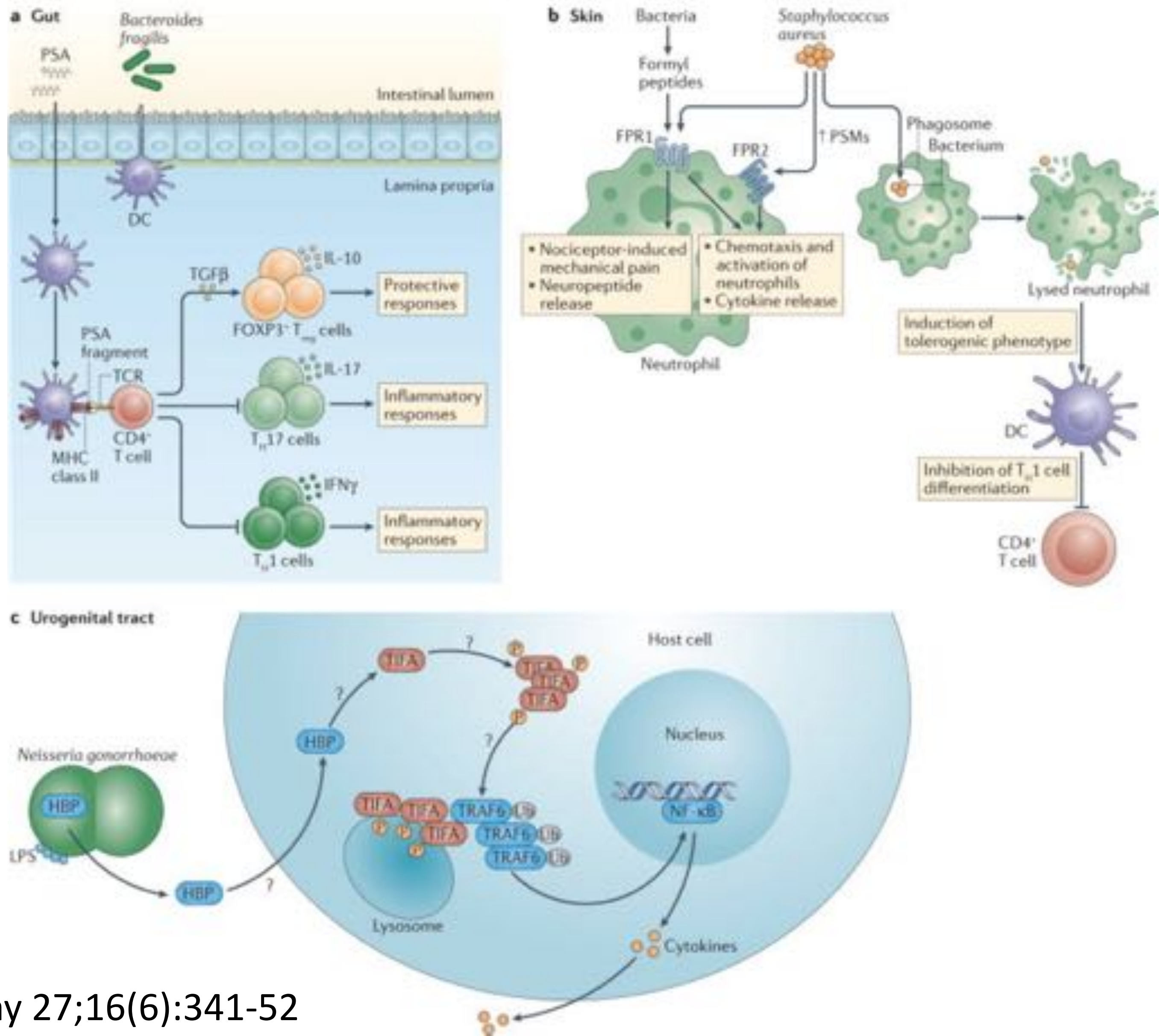
Hubbard TD et al. Drug Metab Dispos. 2015 Oct;43(10):1522-35.

And yes there are more and many to discover….

polysaccharide A (PSA)

formyl peptides (phenol-soluble modulins)

D-glycero-β-D-manno-heptose-1,7-biphosphate (HBP)



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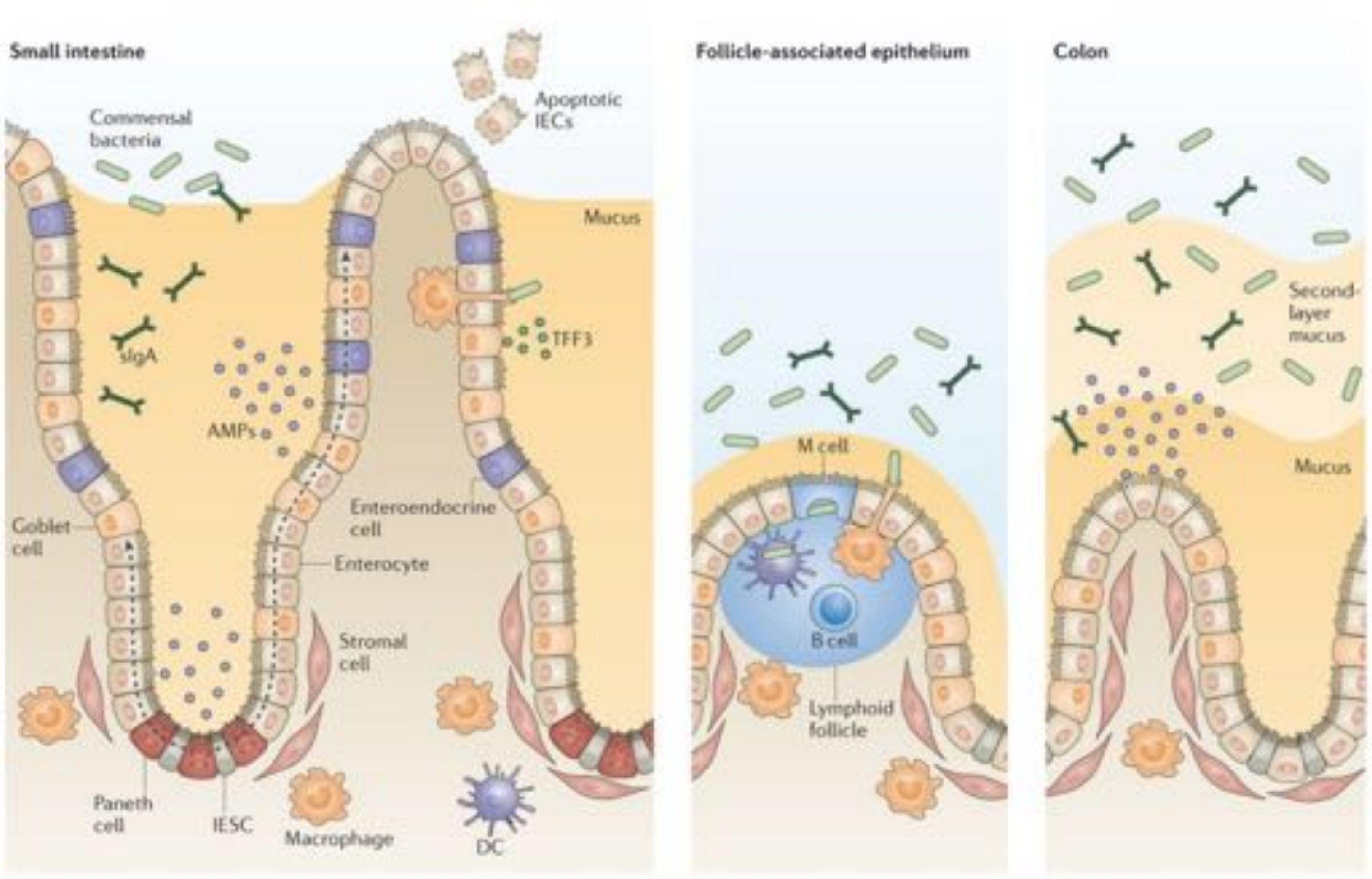
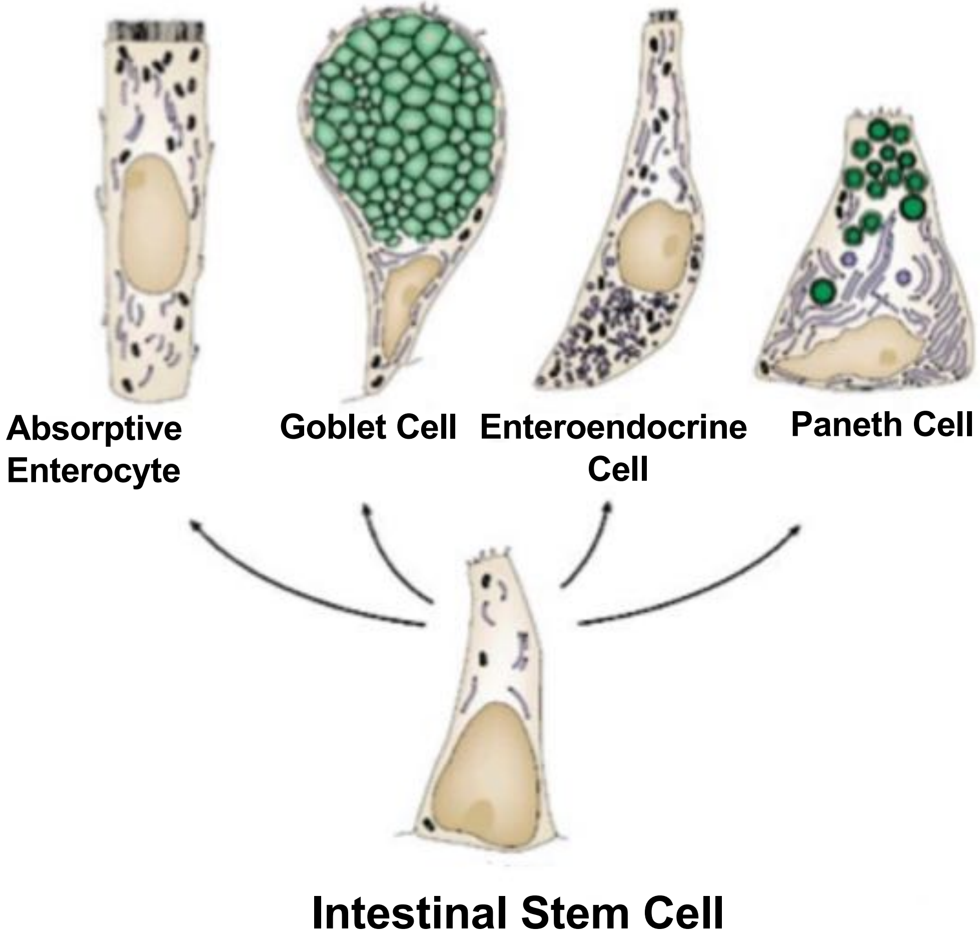
  - **Innate**
  - Adaptive

Innate Immunity

The Epithelium– a review

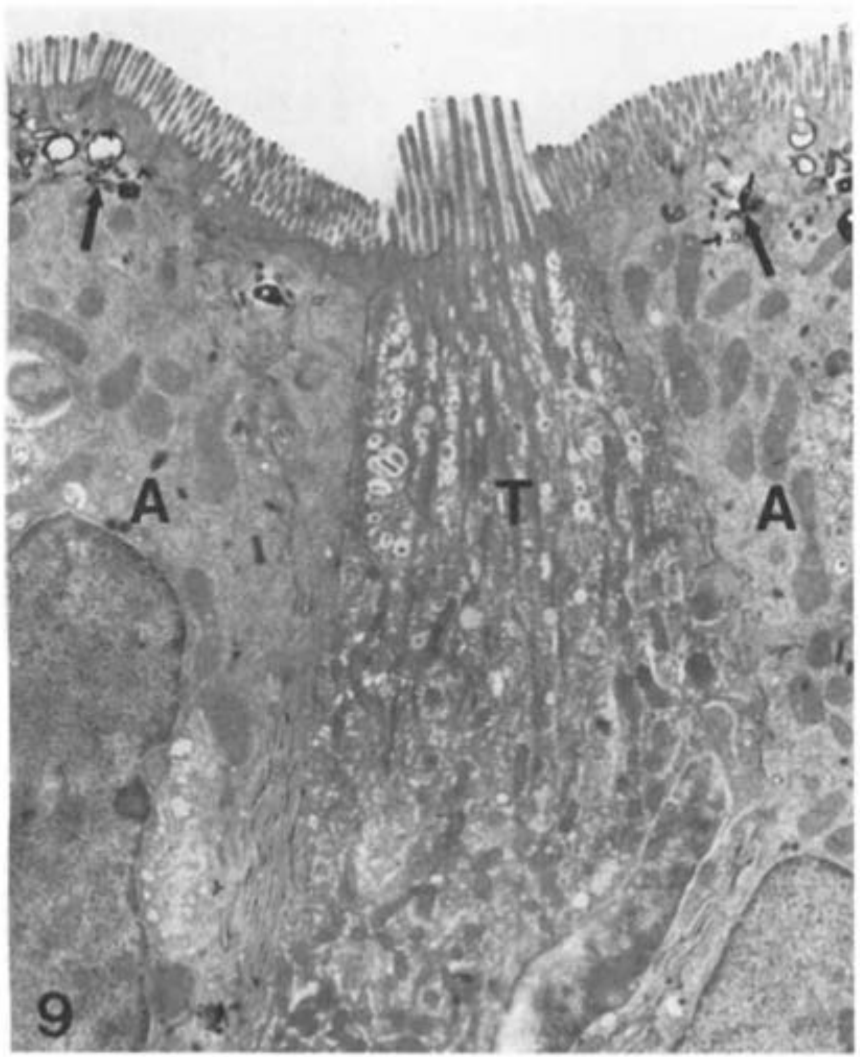


Nature Reviews | Immunology



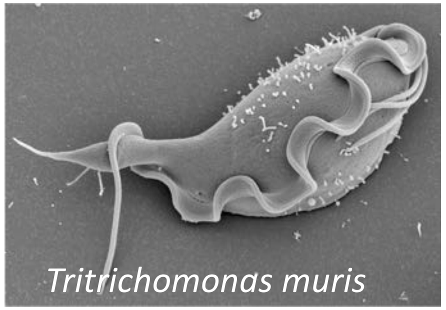
Crosnier *et al. Nature Reviews Genetics* (2006)

Tuft Cell

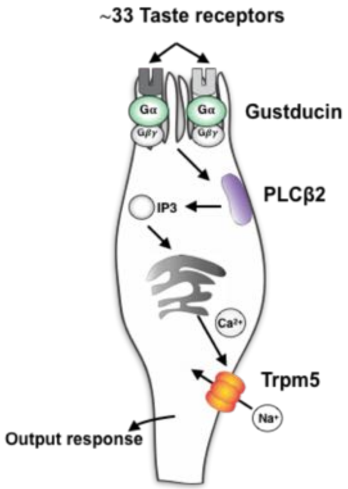


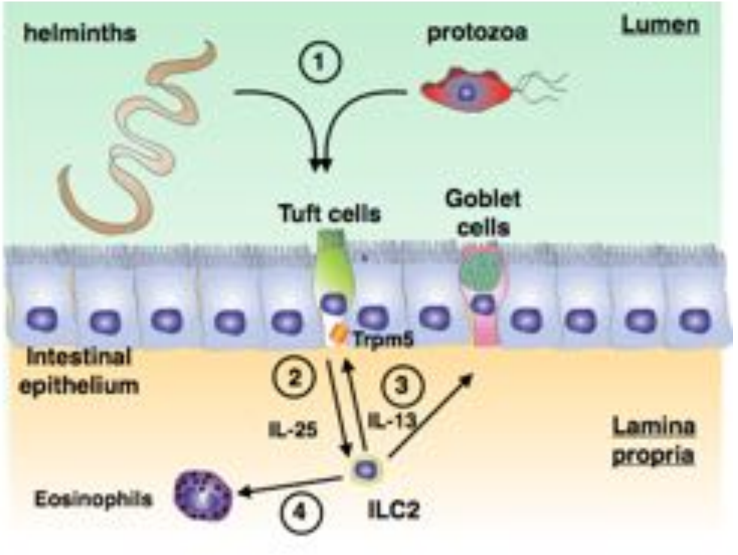
Trier *et al. Anat. Rec.* (1987)

Tuft cells, taste-chemosensory cells, orchestrate parasite immunity (defense_tolerance) in the gut



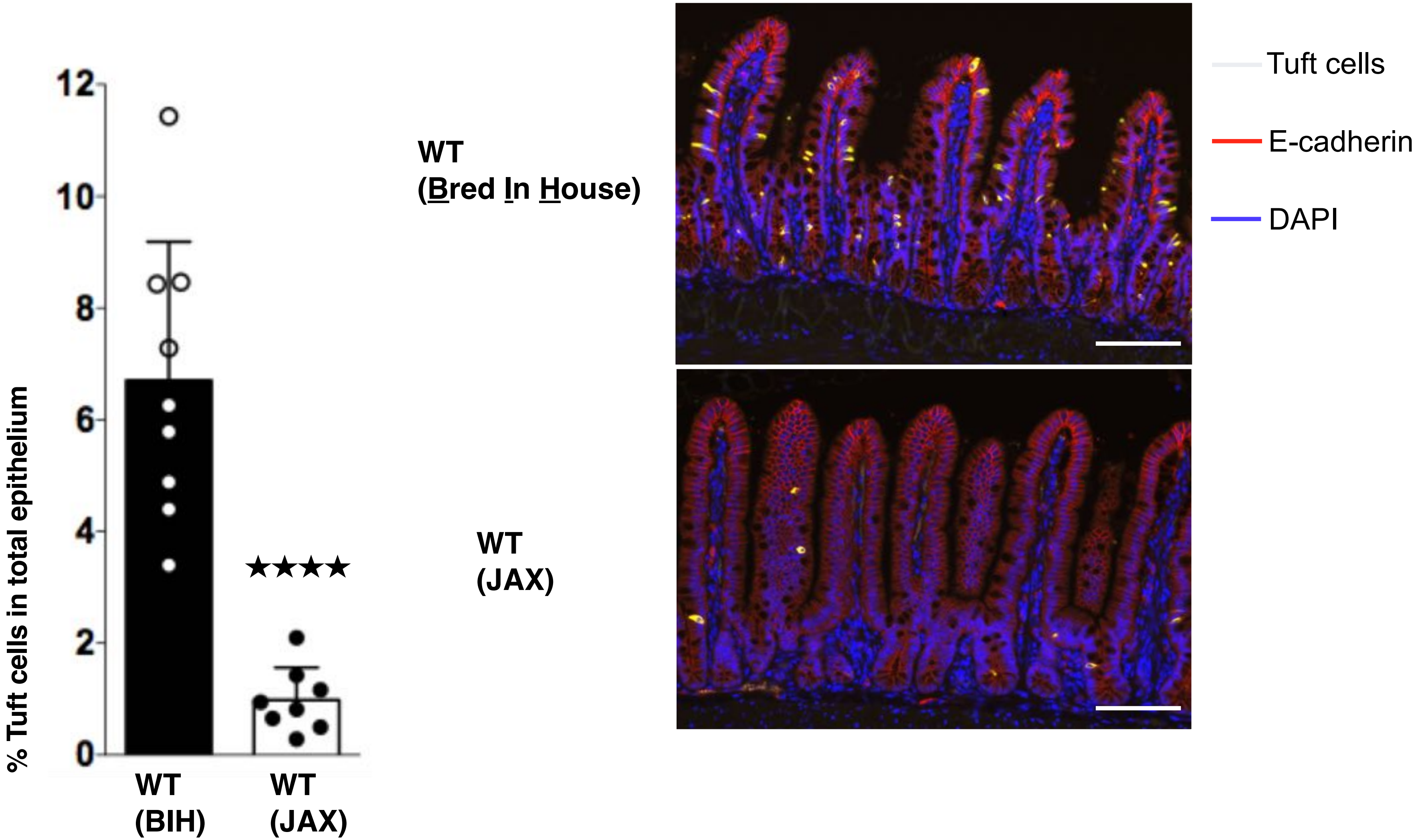
Tuft cells





- **HOW DO YOU UNCOVER WHAT KIND OF MICROBE AN 'INNATE' IMMUNE CELL CAN RECOGNIZE?**
- **HOW IT DOES IT?**
- **WHAT THE PRR IS AND WHAT THE MAMP IS?**

Michael Howitt, Ph.D.
Howitt et al. 2016. *Science.*



12
10
8
6
4
2
0
% Tuft cells in total epithelium
★★★★
WT (BIH)
WT (JAX)
WT
(Bred In House)
WT
(JAX)
Tuft cells
E-cadherin
DAPI

WT
(Bred In House)

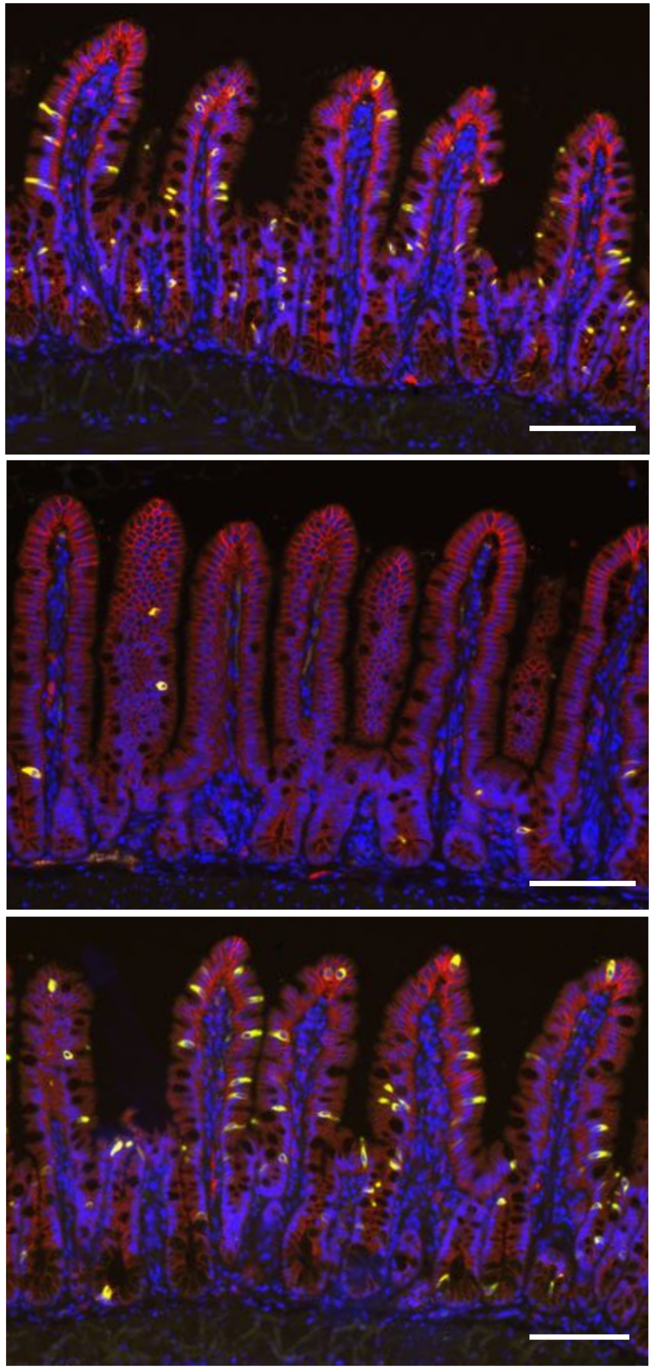
Feed the cecal
contents

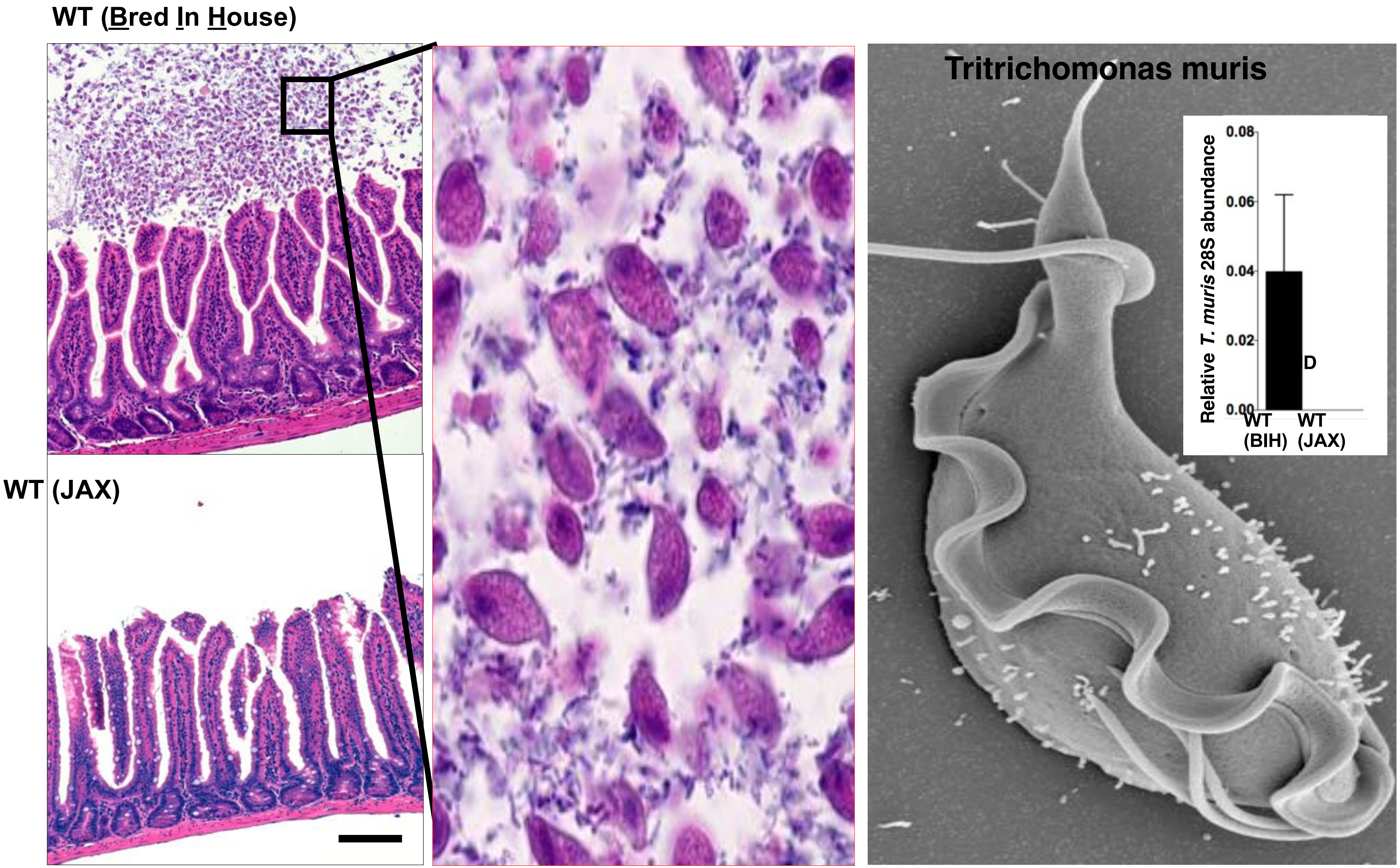
WT
(JAX)

Sacrifice mice
3 weeks later

WT (JAX)
+
cecal contents
(BIH)

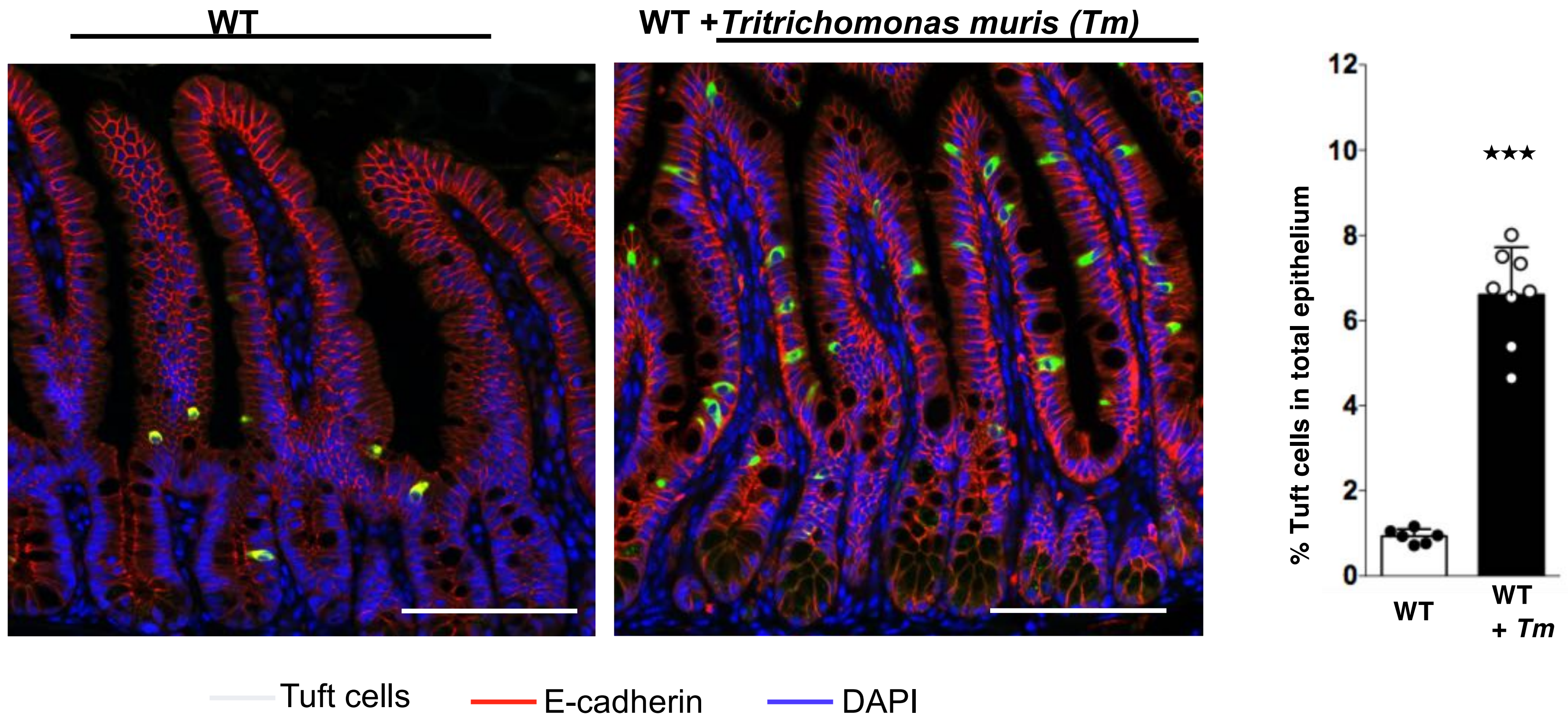
26847546





WT (Bred In House)
WT (JAX)
Tritrichomonas muris
Relative T. muris 28S abundance
0.08
0.06
0.04
0.02
0.00
ND
WT (BIH)
WT (JAX)

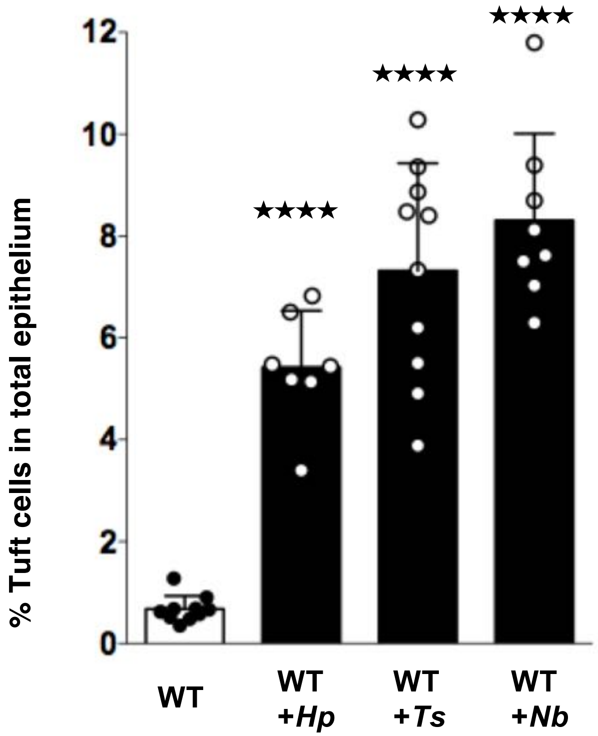
Colonize mice with *Tritrichomonas muris*



Heligmosomoides polygyrus (*Hp*)

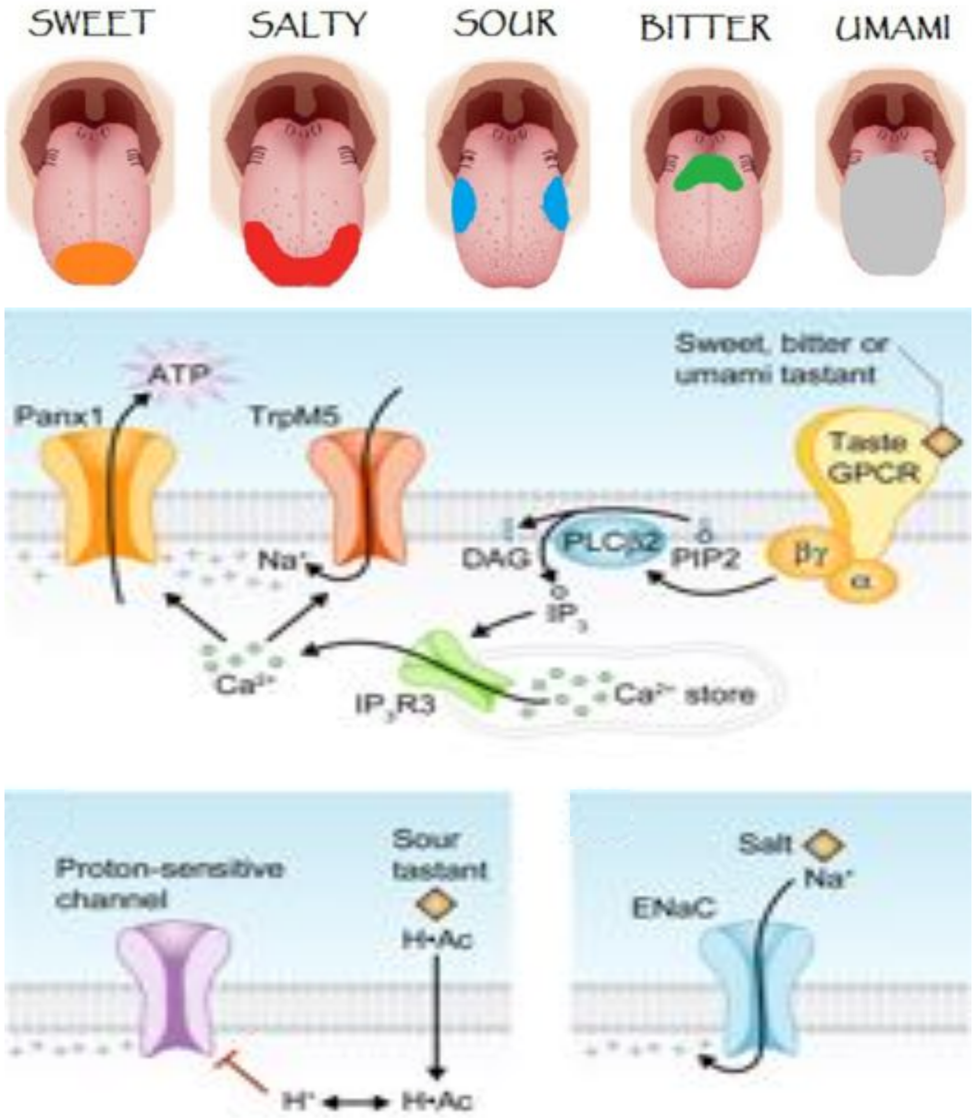
Trichinella spiralis (*Ts*)

Nippostrongylus brasiliensis (*Nb*)

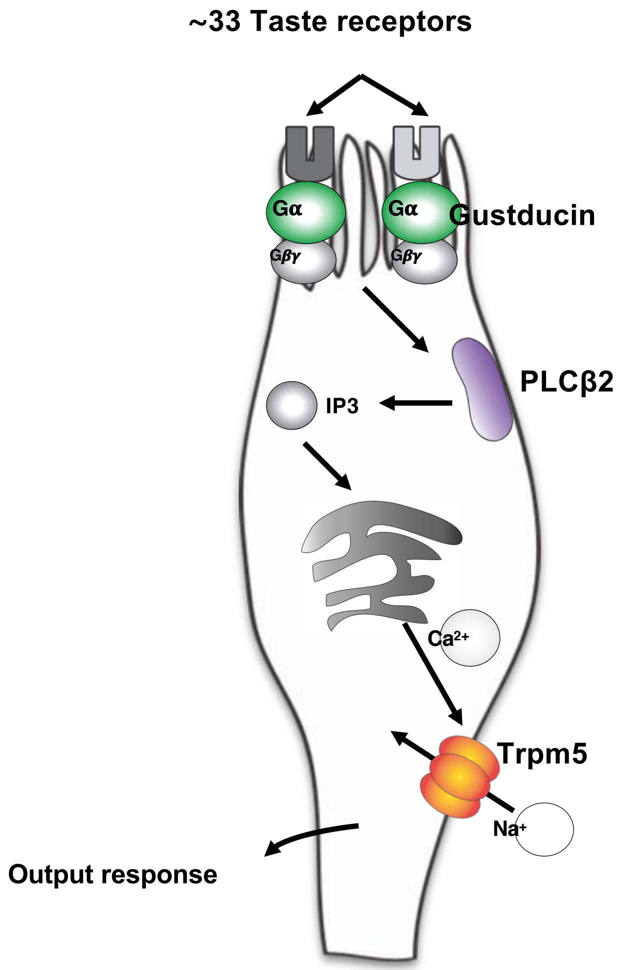


Tuft cells express taste chemosensory components

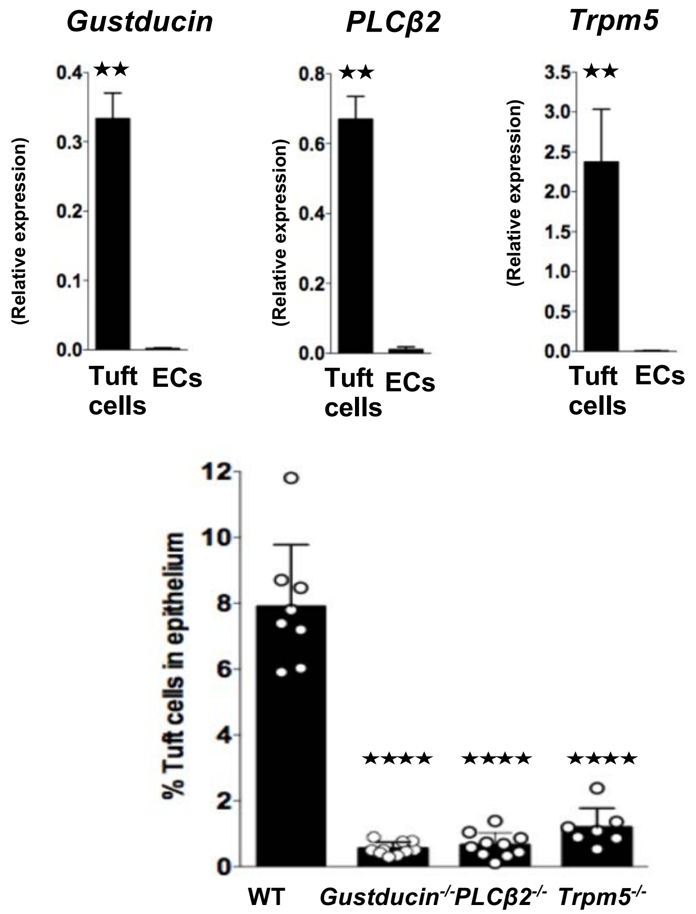
5 principal components of taste



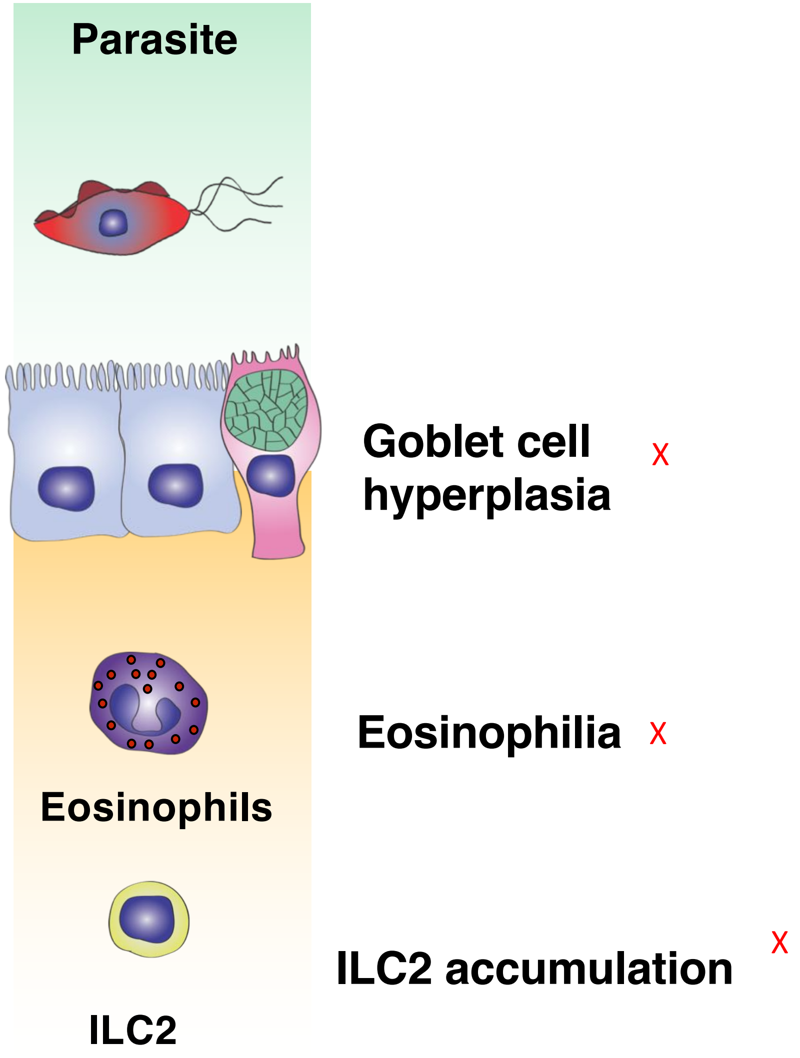
Gut tuft cells express taste chemosensory components



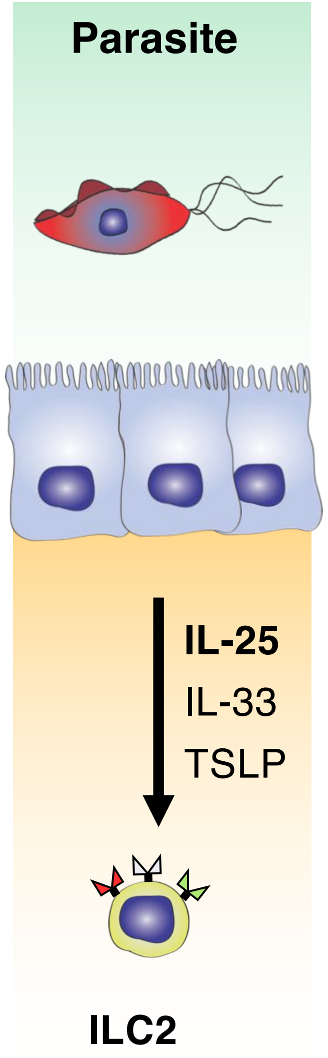
Does taste play a role in response to parasites?

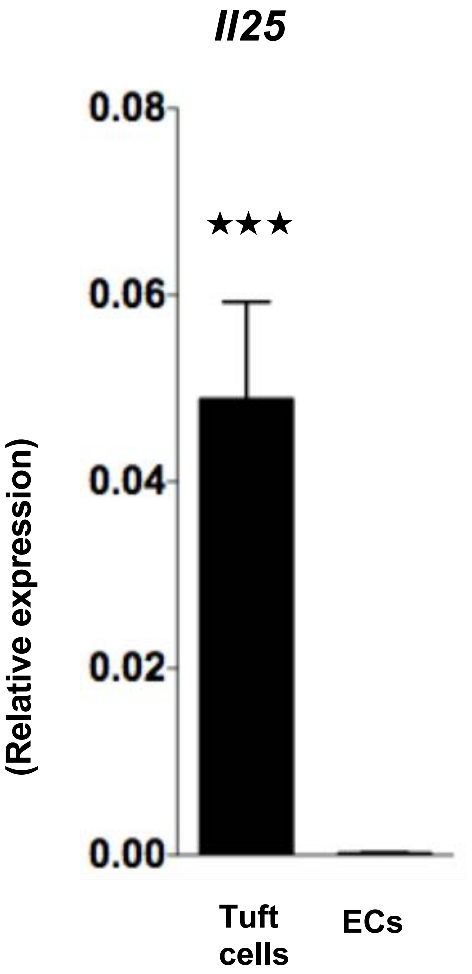


Trpm5$^{-/-}$ mice fail to initiate anti-parasitic immunity

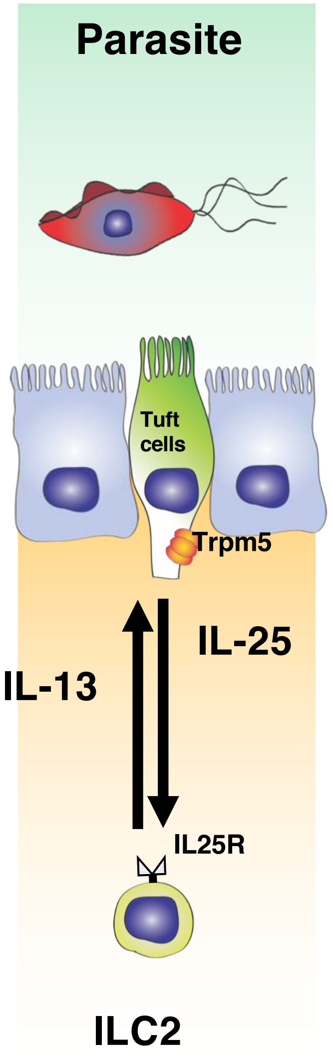


Epithelial cells induce anti-parasite immunity though release of IL-25, IL-33, and TSLP

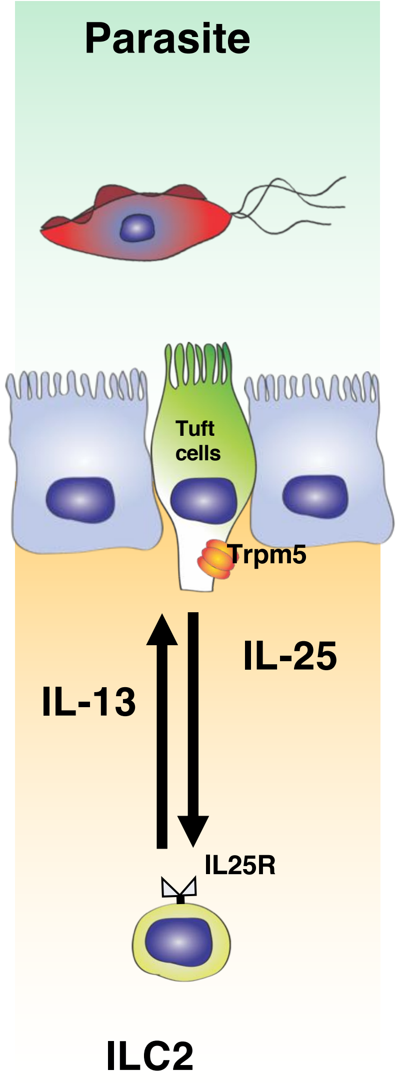


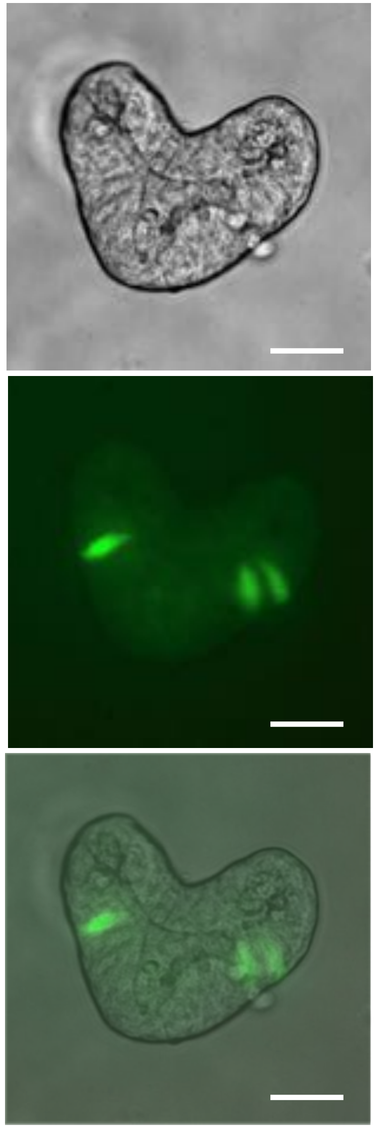


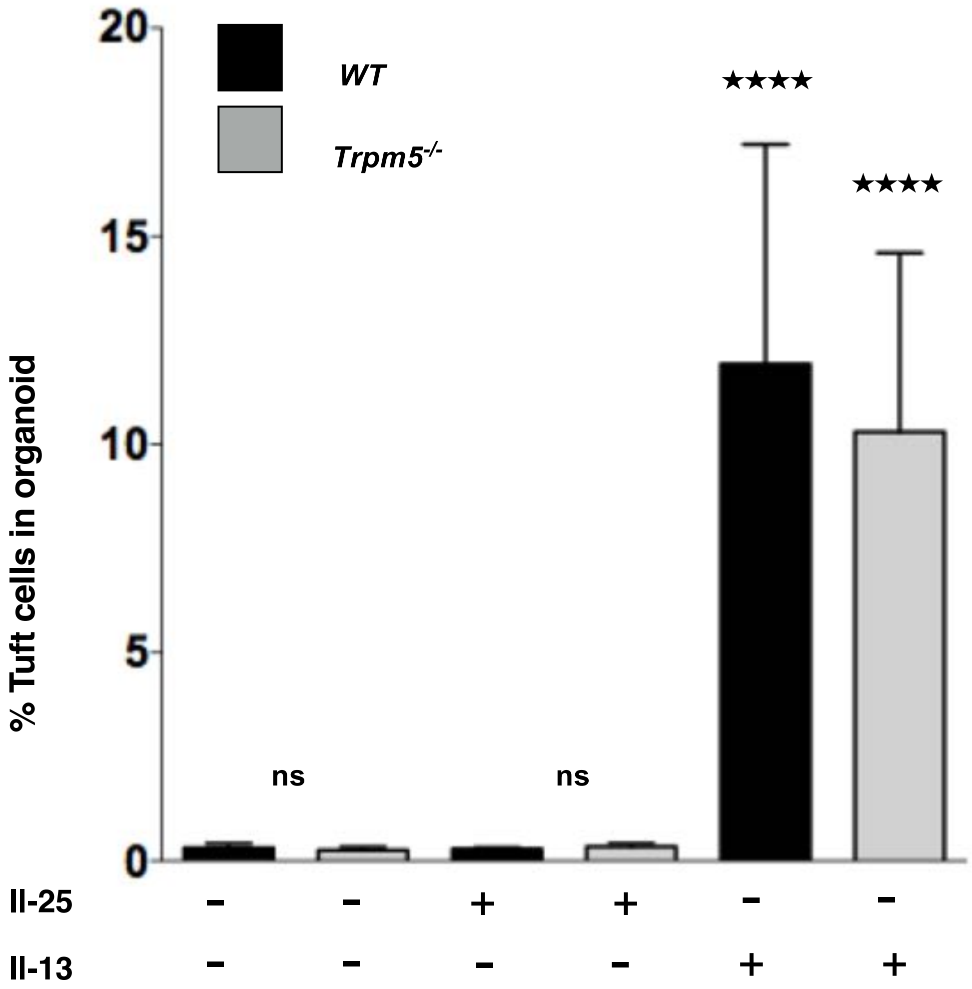
How does IL-25 increase tuft cell abundance?



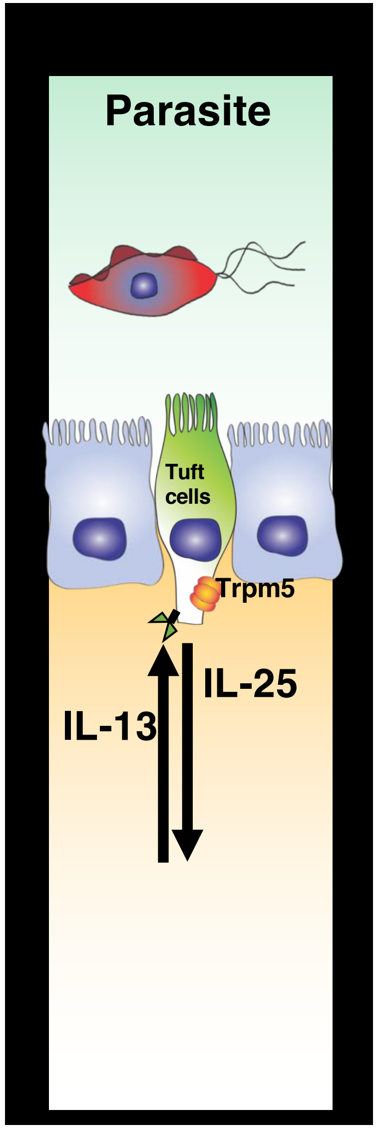
Tuft cells in primary epithelial organoid cultures

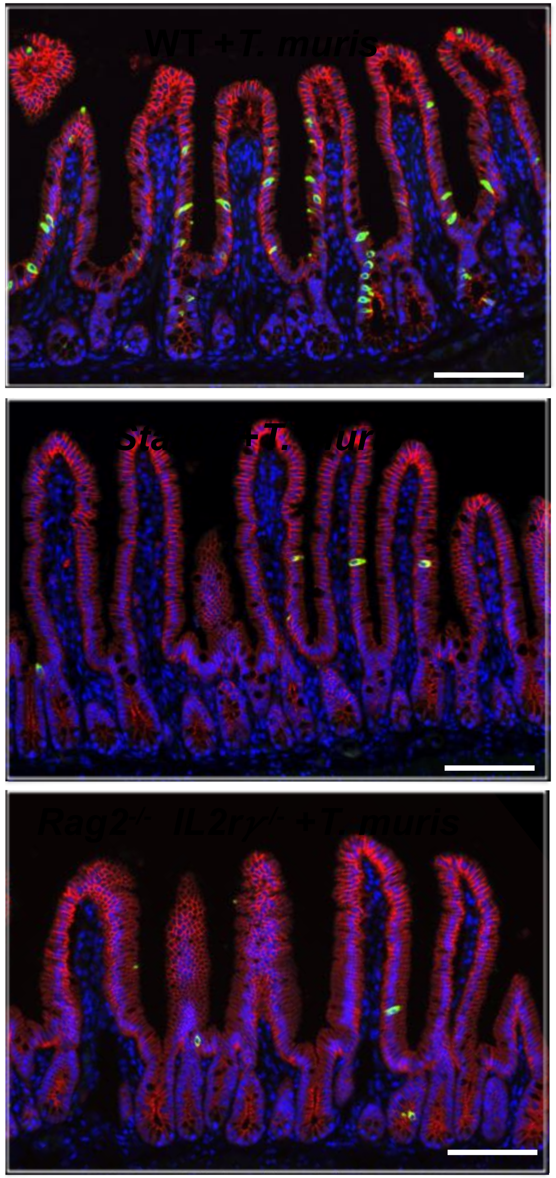


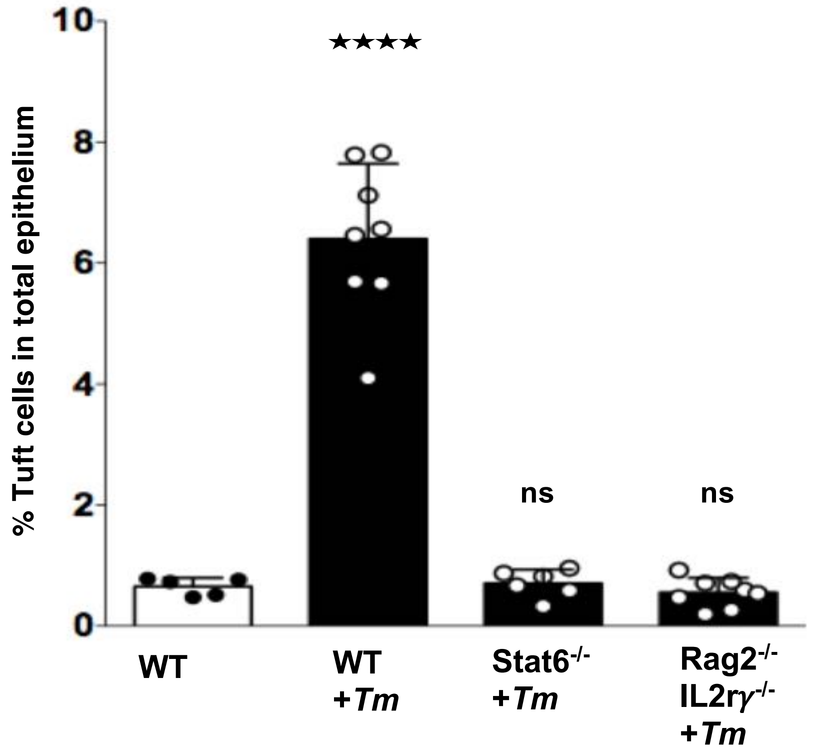




Tuft cell expansion and anti-parasitic immunity *in vivo*



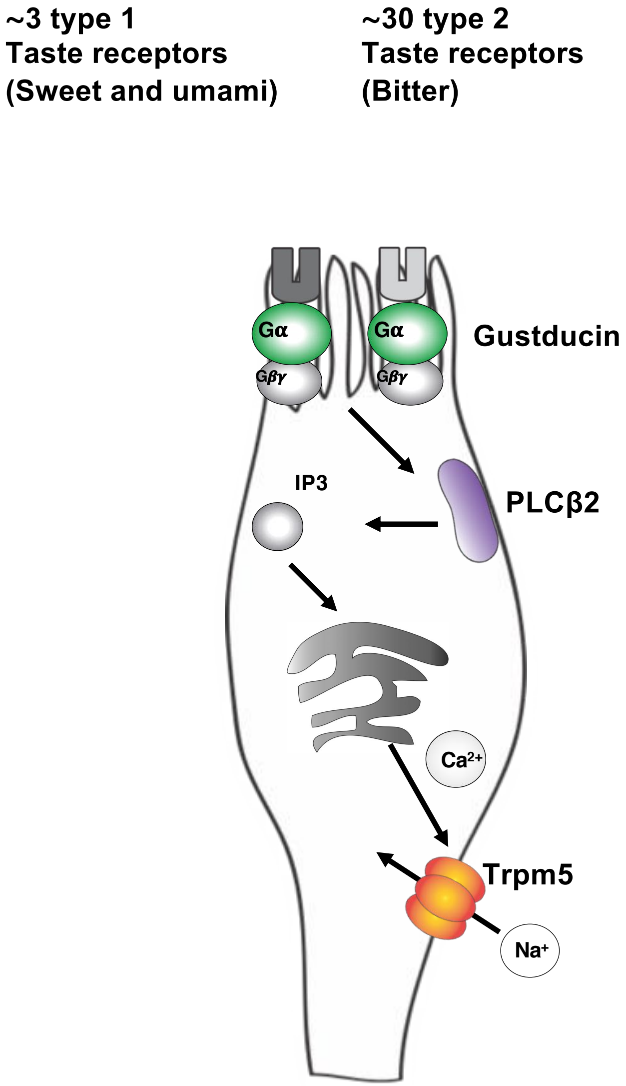


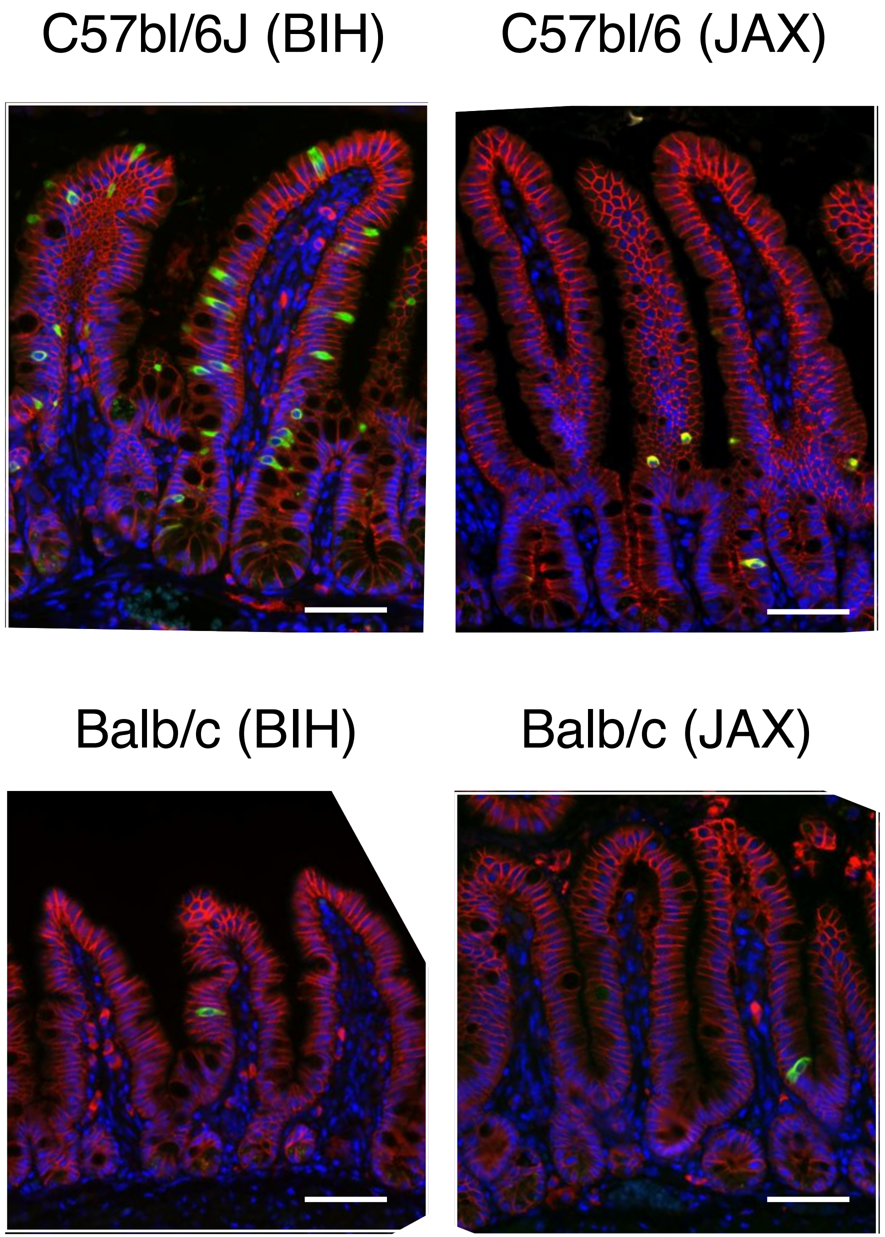


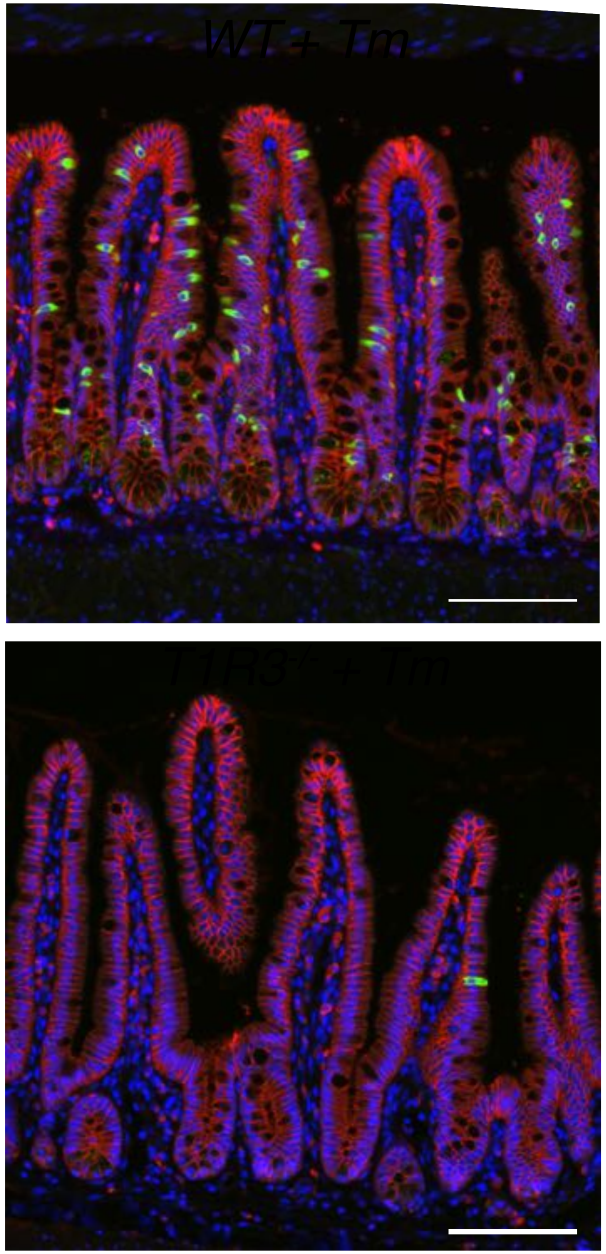
No IL13 receptor signalling

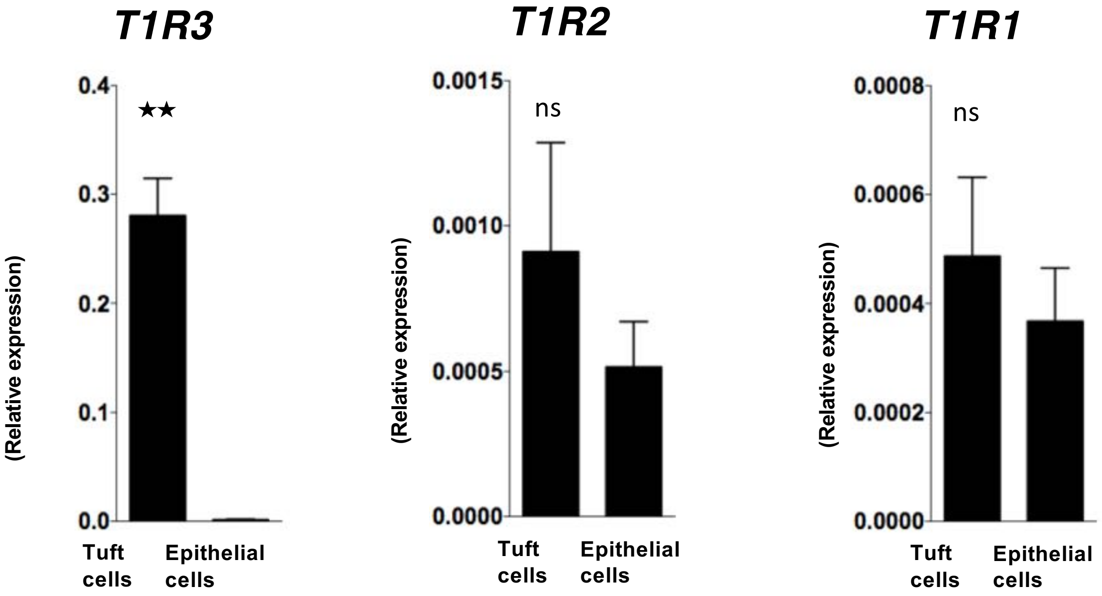
No ILCS

How do tuft cell sense parasites?

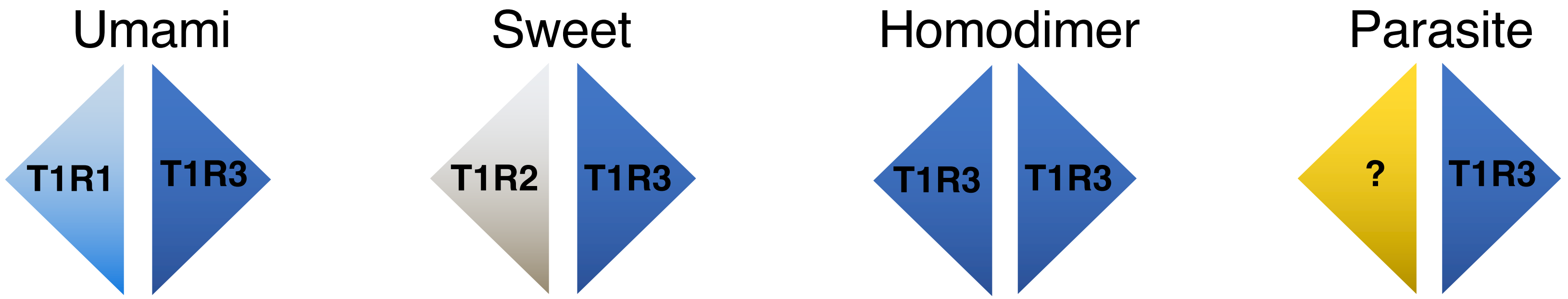





WT + Tm


T1R3
0.4
0.3
0.2
0.1
0.0
★★
(Relative expression)
Tuft cells
Epithelial cells
T1R2
0.0015
0.0010
0.0005
0.0000
ns
(Relative expression)
Tuft cells
Epithelial cells
T1R1
0.0008
0.0006
0.0004
0.0002
0.0000
ns
(Relative expression)
Tuft cells
Epithelial cells

How and what do tuft cells chemosense?
what do parasites 'taste' like



Take home points:

- There are cell types being uncovered that influence host-microbiota interactions
- Taste receptors and parasitic metabolites (succinate, sugars) are MAMPs

Outline

https://blog.thryveinside.com/the-layout-of-the-human-microbiota/

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    - Classic Pattern Recognition Receptors (PRRs) & Microbial Associated Molecular Patterns (MAMPs)
    - Next-generation Microbial sensors
- **Host cell mediators of mediate host-microbiota interactions**
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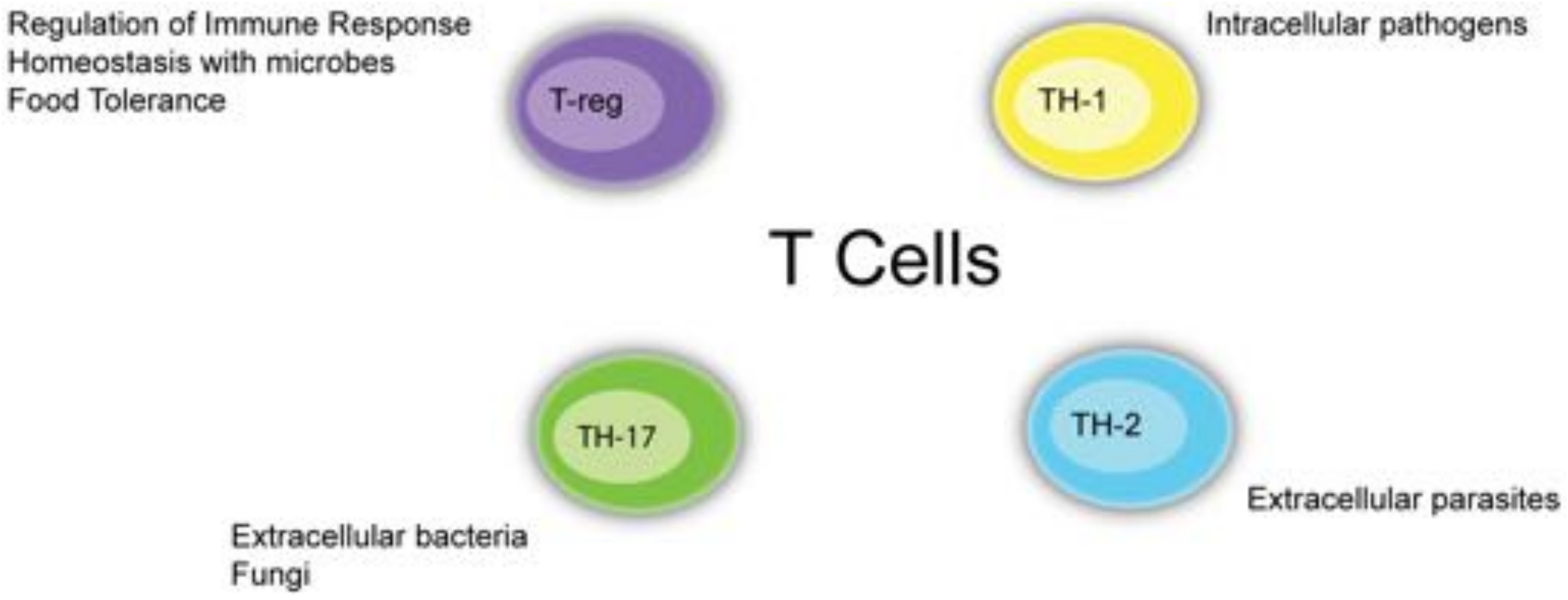
CD4+ T cell Review:

The many subsets of gut mucosal CD4+ T cells



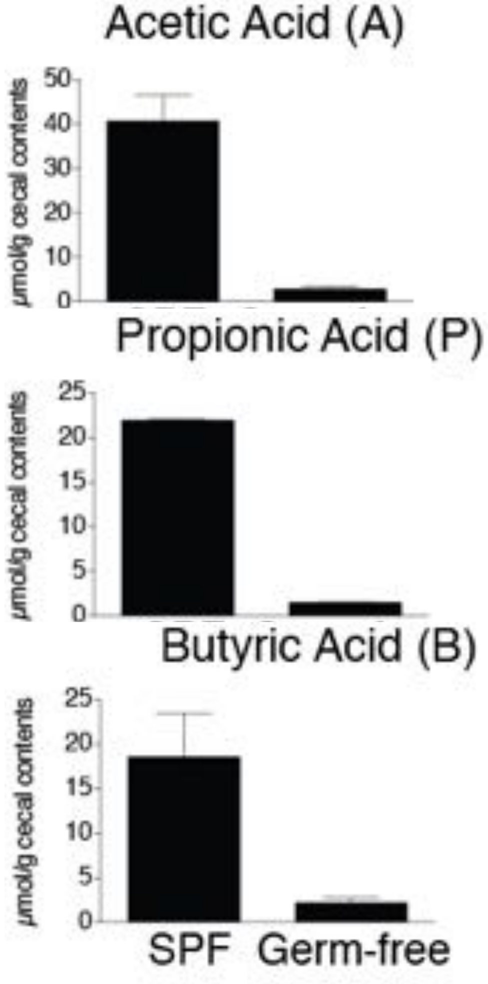
Regulatory T cells and Short-chain fatty acids

How do you uncover what MAMPs regulate what immune cells?

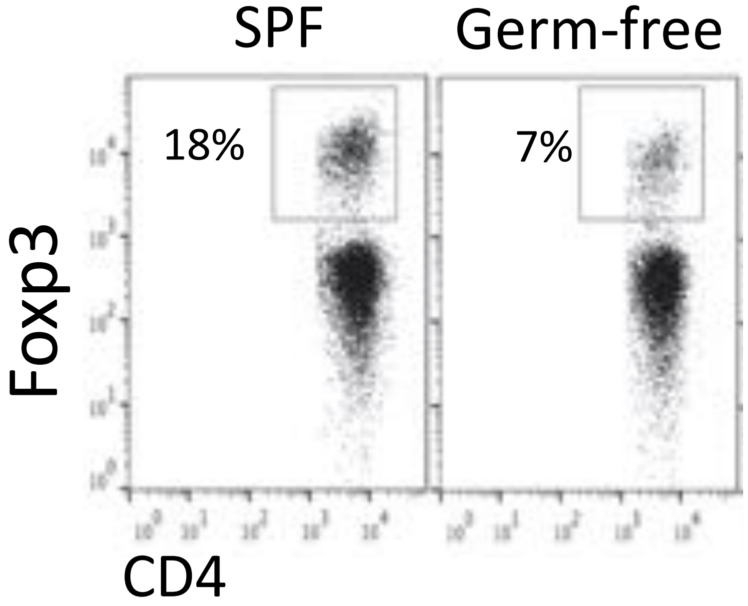
How do you determine what PRRs are responsible?

If we examine what is missing in the absence of microbes, can we learn what we need to build a healthy and resilient gut?

Short-chain fatty acids



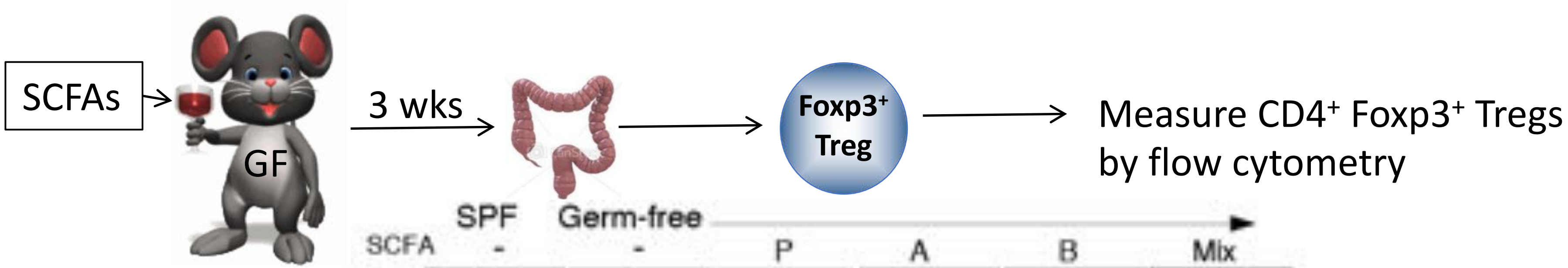
Colonic regulatory T cells

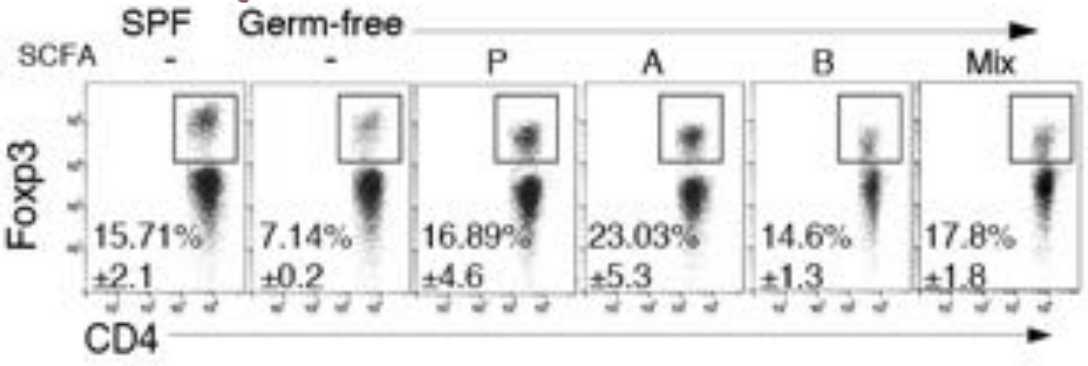


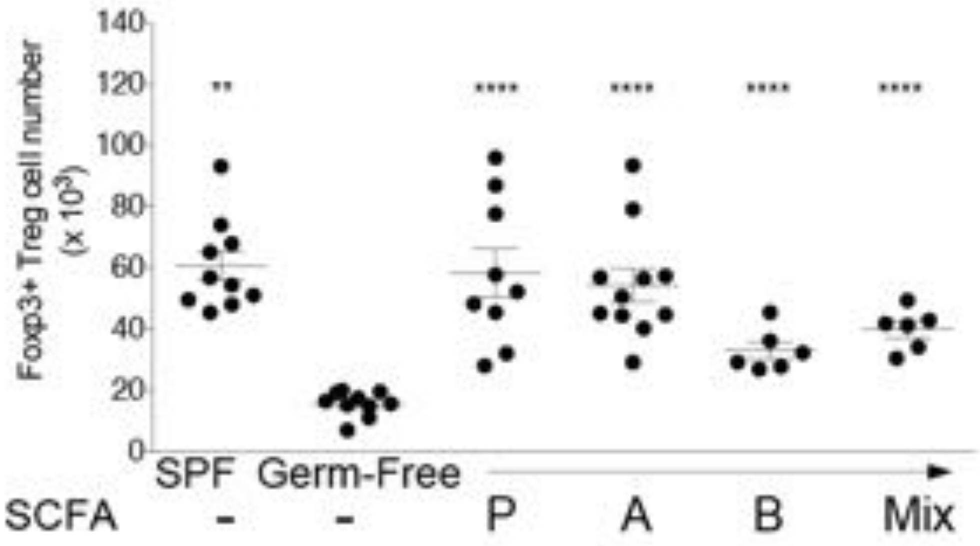
<u>Why focus on colon Tregs?</u>

- **cTregs regulate intestinal inflammation**
- **cTregs mediate intestinal immune homeostasis**
- **The microbiota affect cTreg #s and function**

Administration of SCFA to germ-free mice increases colon Tregs



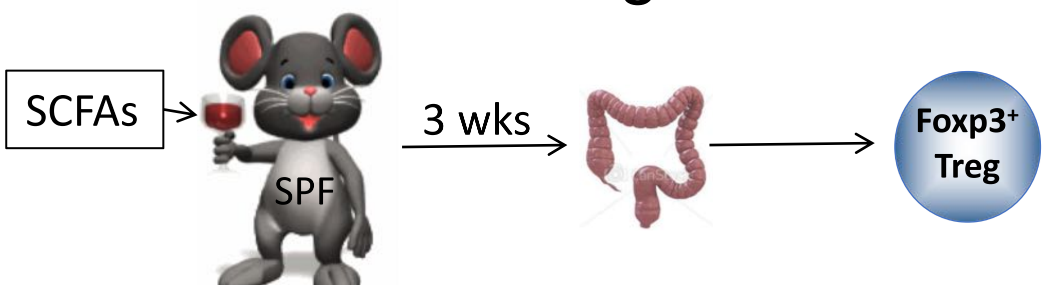




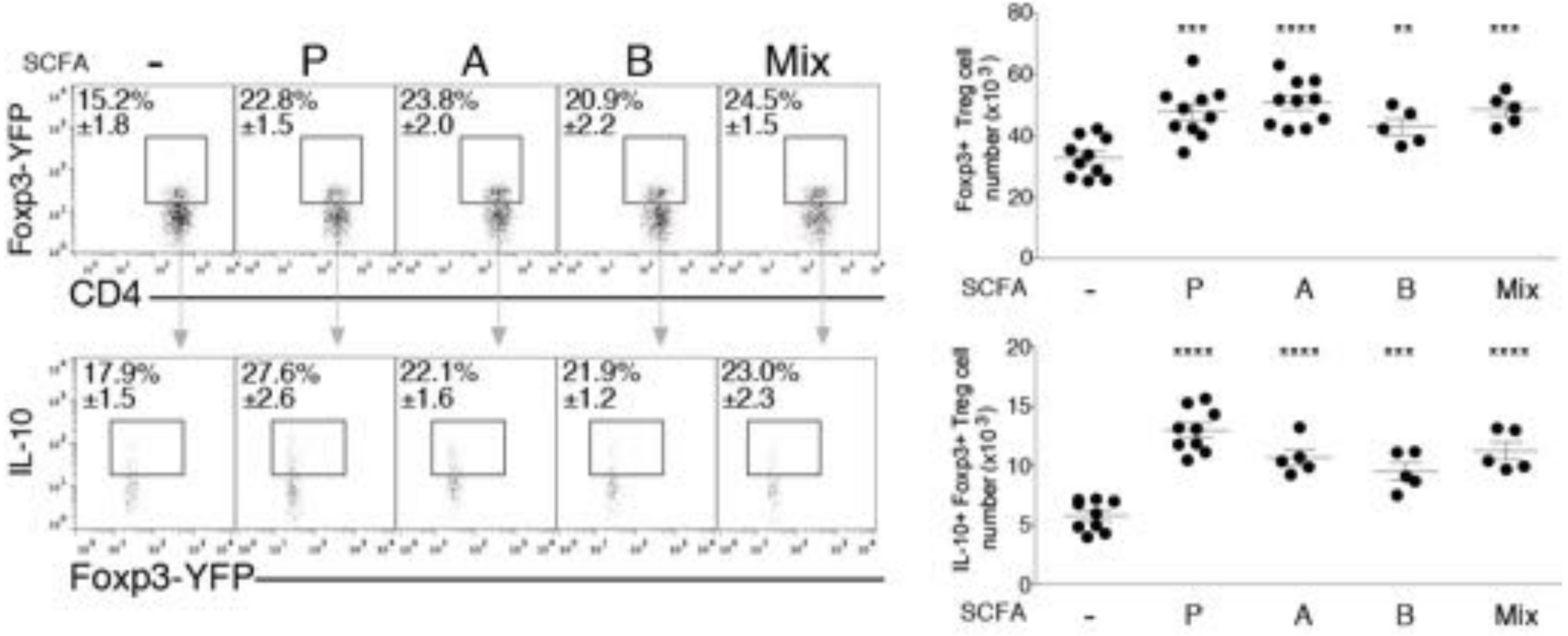
Pat Smith, Tufts PhD

Smith PM et al. 2013. *Science*

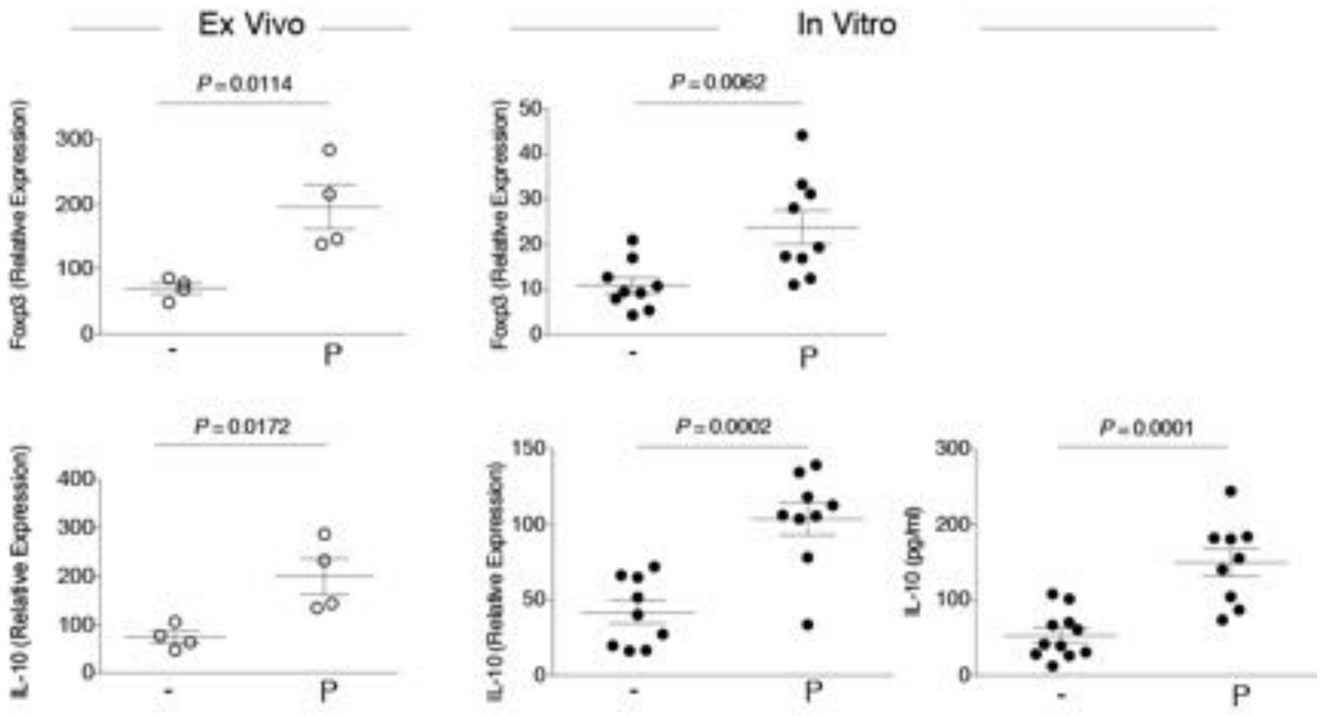
Administration of SCFA to conventional (SPF) mice increases colon Tregs



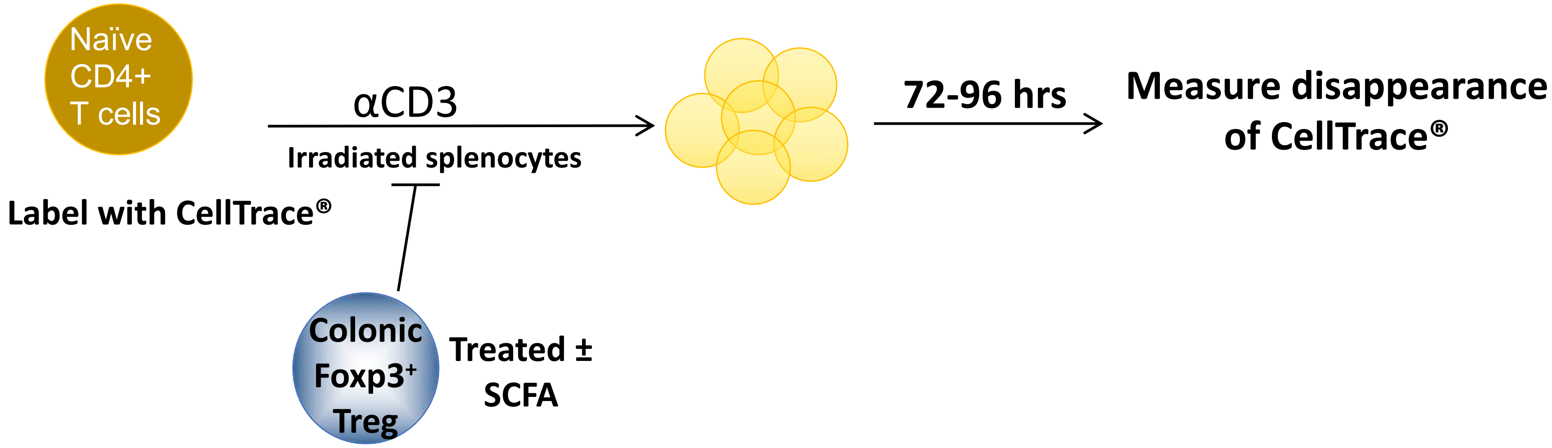
Measure CD4+ Foxp3+ Tregs by flow cytometry

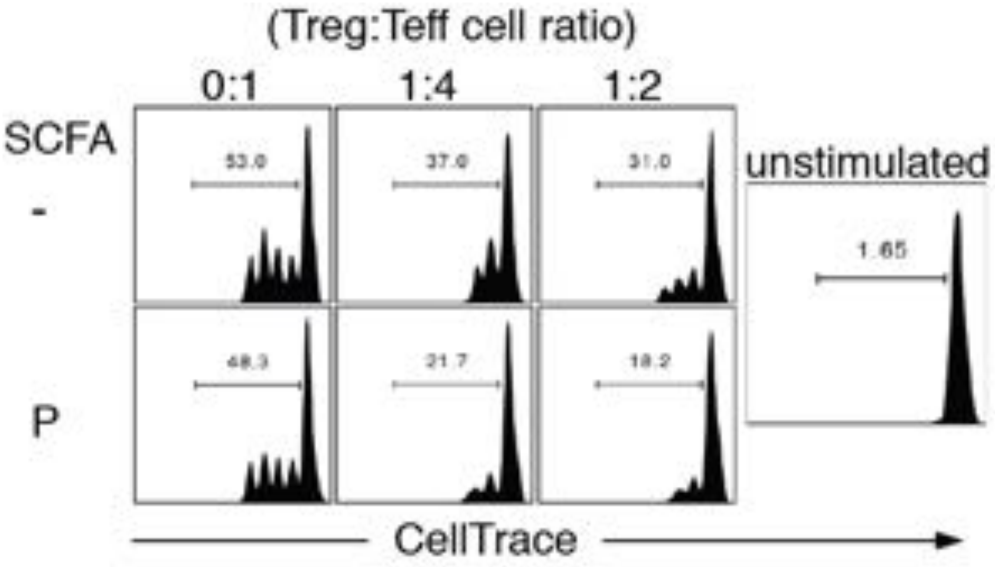


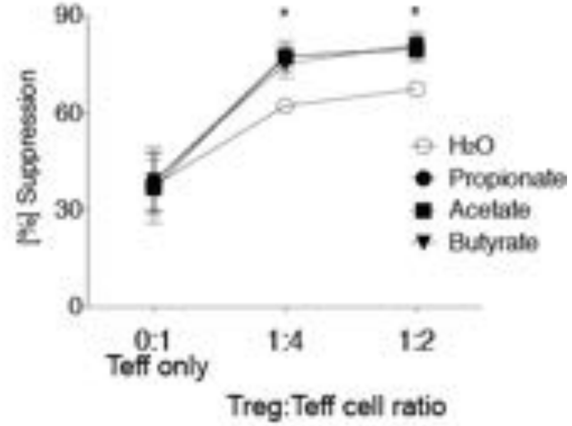
SCFA enhance Foxp3 and IL-10 expression in cTregs



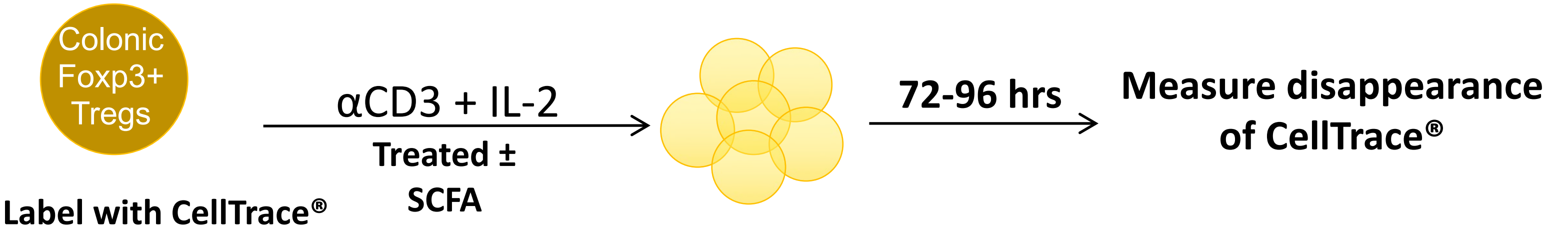
SCFA increase Treg suppressive ability

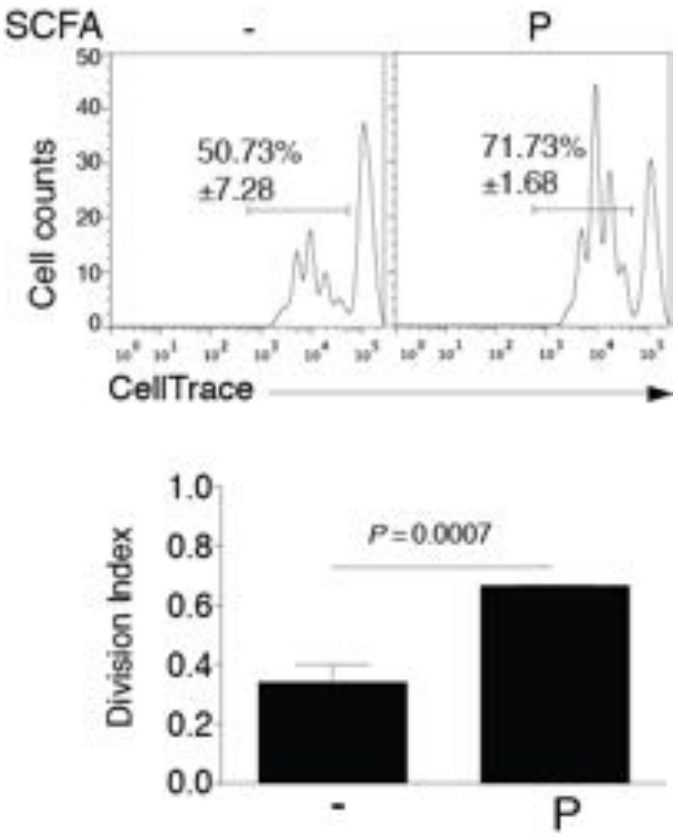






SCFA increase colon Treg proliferation





SCFA– what they don't do

Do not alter:

colon Th1, Th2, or Th17 total cell numbers

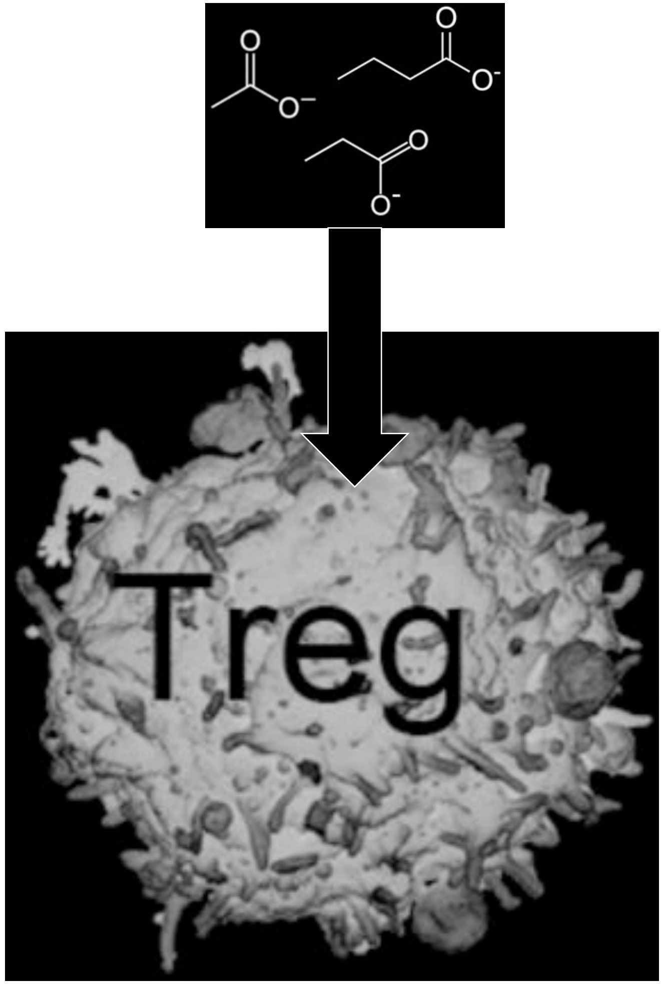
Treg Foxp3 levels from
mesenteric lymph node, small intestine, spleen,
peripheral lymph node or thymus

colon Treg TGFβ levels at the transcriptional or protein level

colon Treg CCR4 or $\alpha_4\beta_7$ expression levels

How do SCFAs **enter** and alter Tregs?

passively, channels, receptors

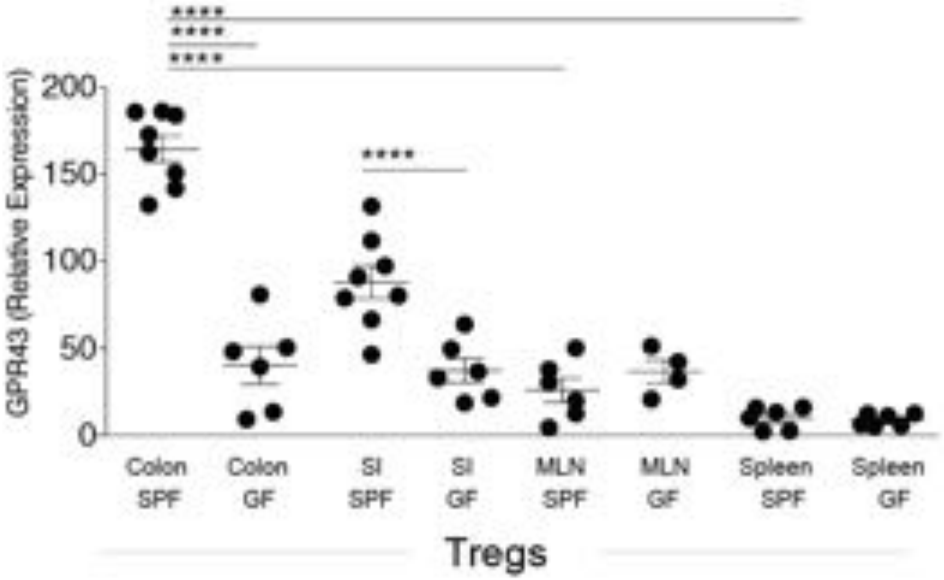


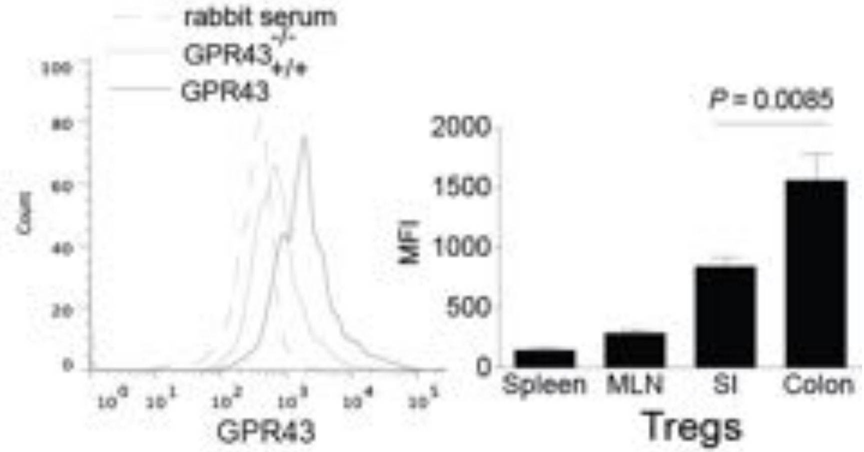
The 'short-chain fatty acid' receptors

GPR43, GPR41, GPR109a, GPR109b, and Olfr78

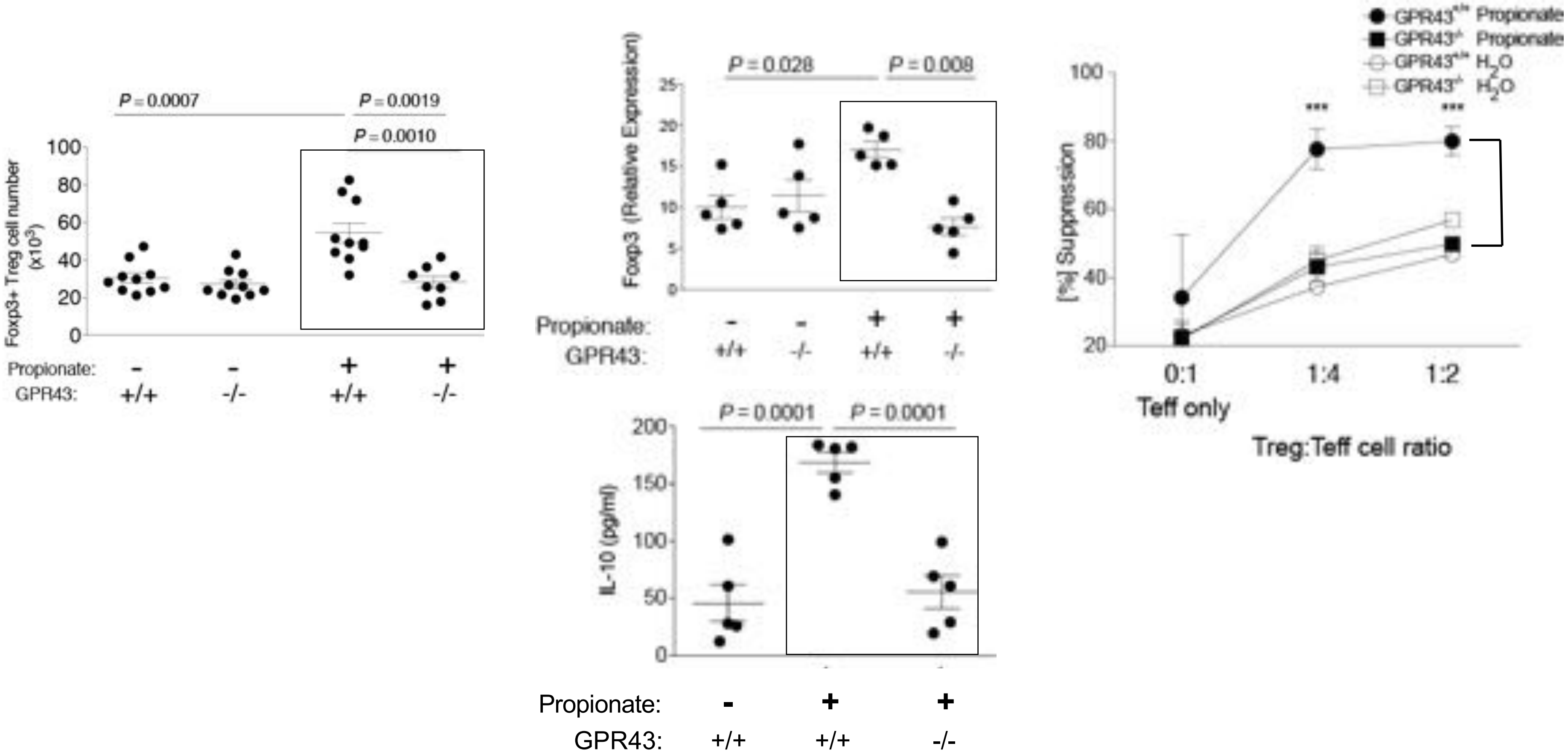
- Expressed by epithelial, fat, muscle, and immune cells
- Have distinct binding affinities for C2-C4 short-chain fatty acids
- Have pharmacologically unimpressive pEC50s
  - GPR43-- Propionate pEC50 (3.0-4.9), binding affinity: propionate > acetate = butyrate

Tissue microenvironment
(anatomic location and presence of a microbiota)
affect GPR43 levels

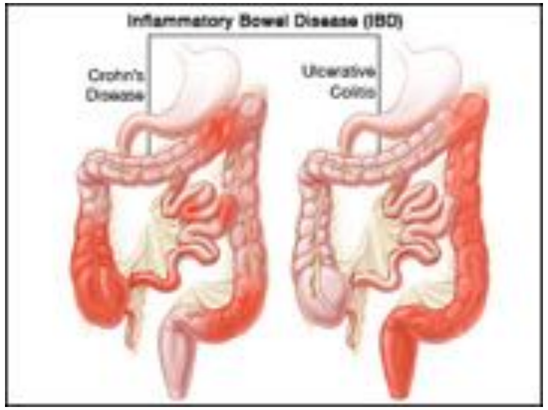




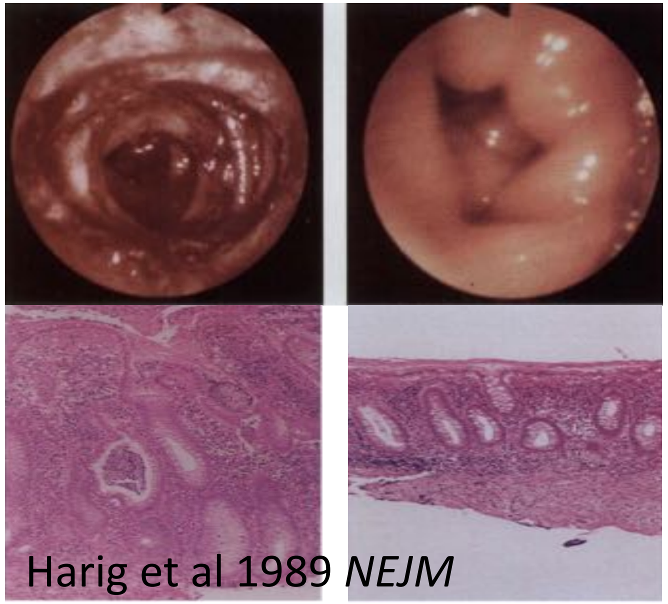
SCFA-mediated function effects on colonic Tregs are dependent upon GPR43



Are short-chain fatty acids, GPR43, and Tregs relevant for human health and disease?

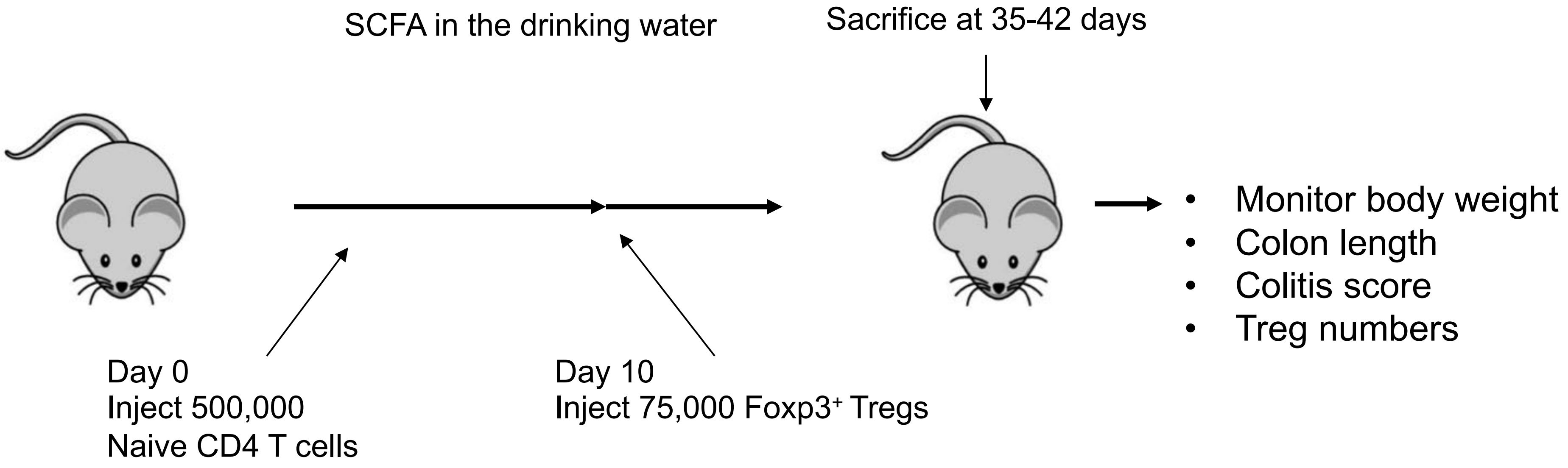


Pre-SCFA Post-SCFA



Harig et al 1989 *NEJM*

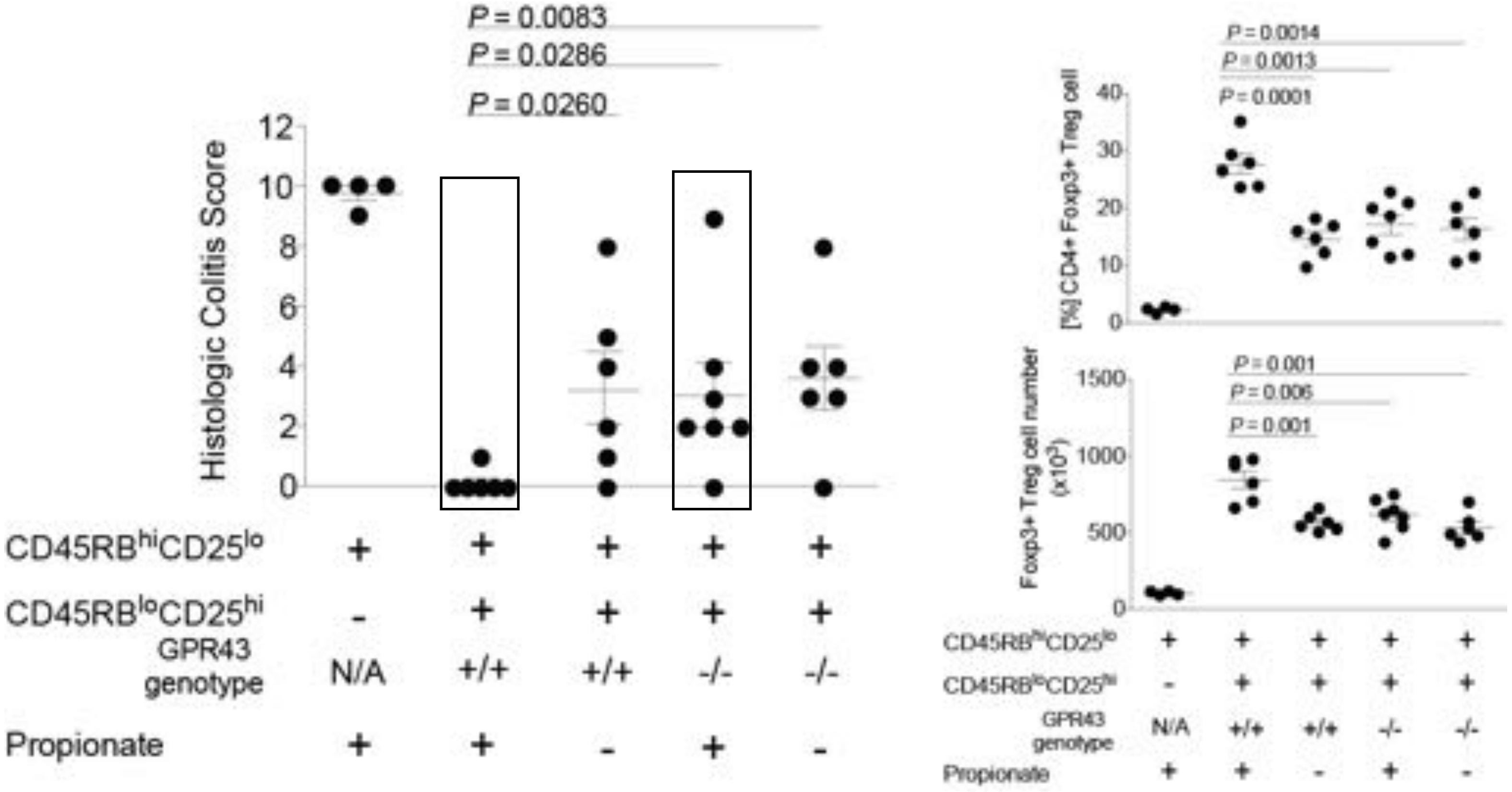
T cell transfer (Powrie) model of colitis



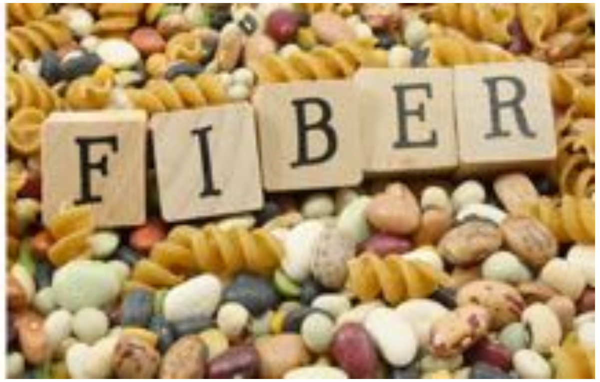
Naive T cells	+	+	+	+	+
Tregs	−	−	+	+	+
Propionate	−	+	−	−	+
Mix	−	−	−	+	−

FFAR2 (GPR43) $^{+/+}$ **vs** ***FFAR2*** $^{-/-}$

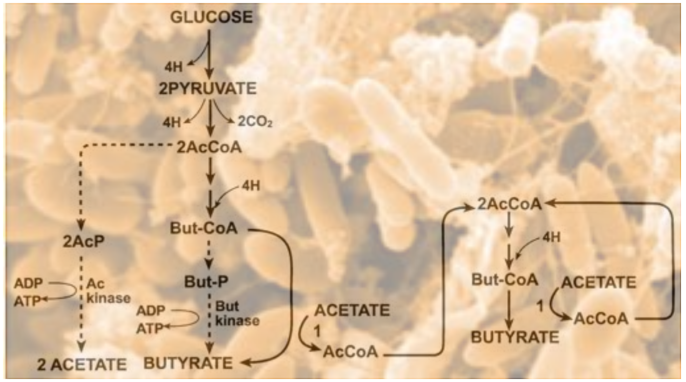
SCFA beneficial effects in the T cell transfer model are Treg intrinsic and GPR43 dependent



Are there more palatable alternatives to SCFA?

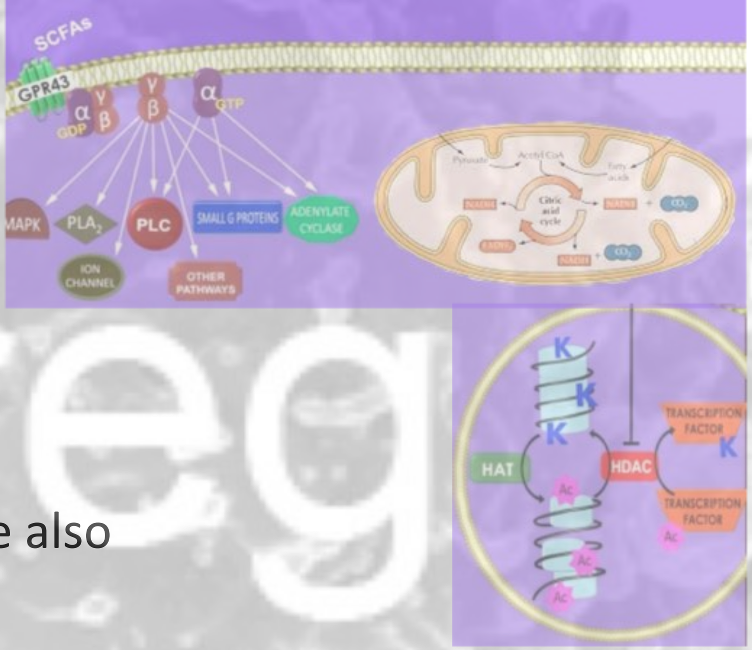


Diet



Microbiome Sculpting
Bacterial Engineering

Host receptor targeted approaches



Short-chain fatty acids

are abundant microbial metabolites that are also

- host epigenetic factors
- host energy sources
- host signaling molecules

promote colon Treg numbers and function

ameliorate colitis in a Treg intrinsic and GPR43 dependent manner in mice

GPR43 agonism

may represent a translational opportunity for IBD

Outline

- **Introduction**
- **Microbial Sensing**
  - **Classic Pattern Recognition Receptors (PRRs) & Microbial Associated Molecular Patterns (MAMPs)**
  - **Next-generation Microbial sensors**
- **Host cell mediators of mediate host-microbiota interactions**
  - **Innate**
  - **Adaptive**

Now it’s time for Immunology & Microbiota Jeopardy

https://jeopardylabs.com/play/immunology-and-the-microbiota