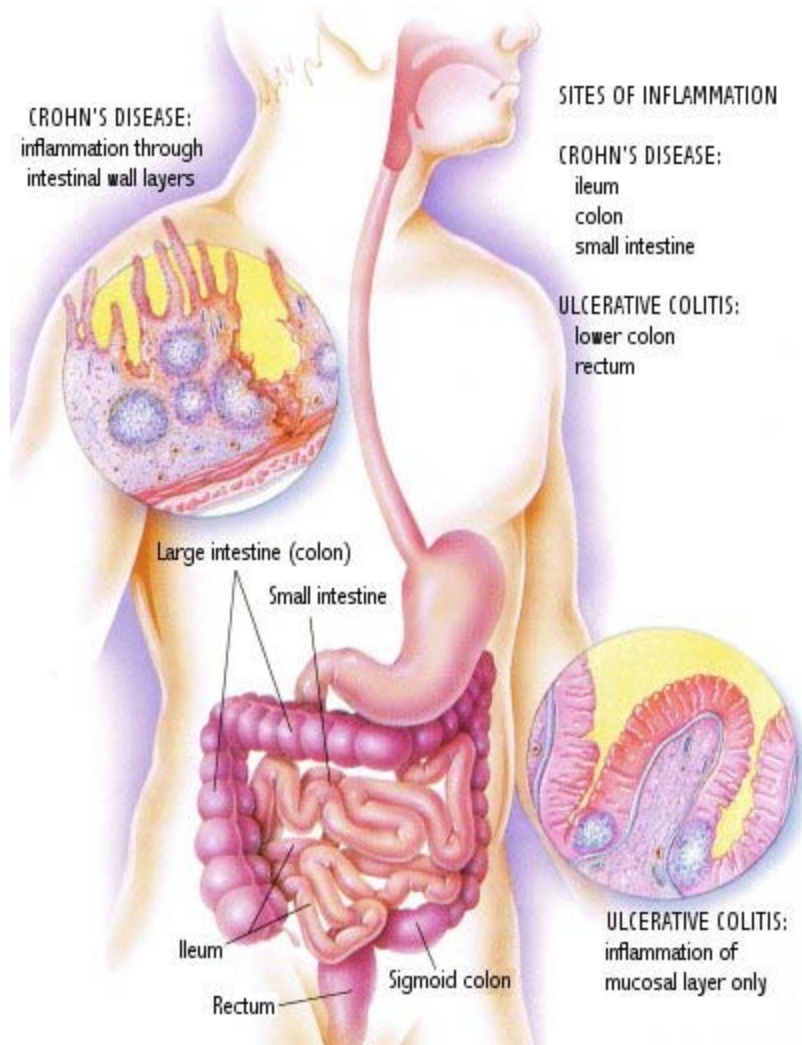




Unlocking the Evolutionary Mysteries Of Microbiome-Immune Symbiosis

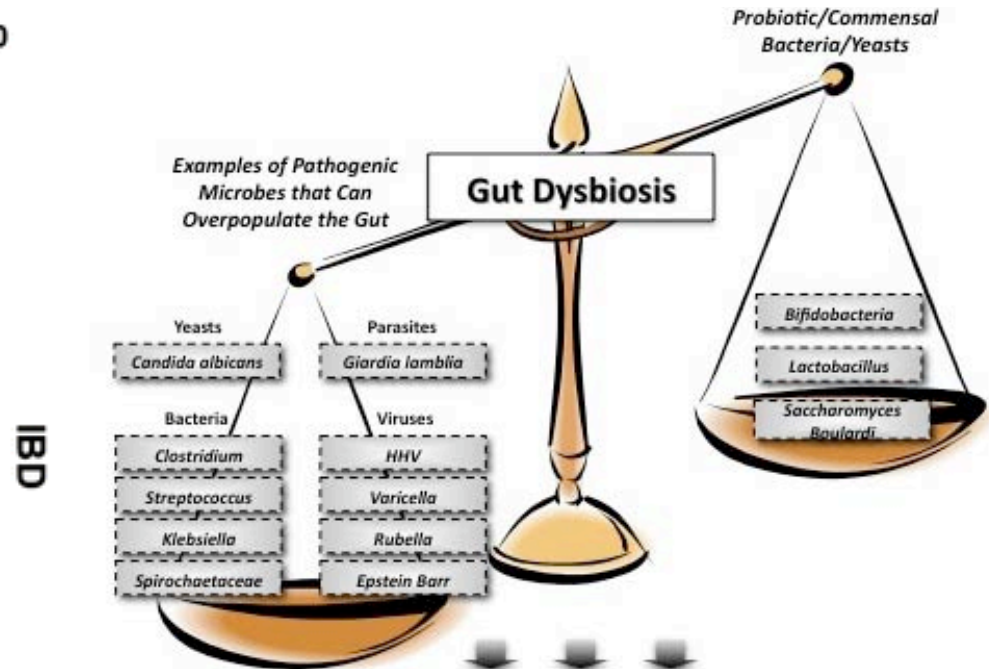
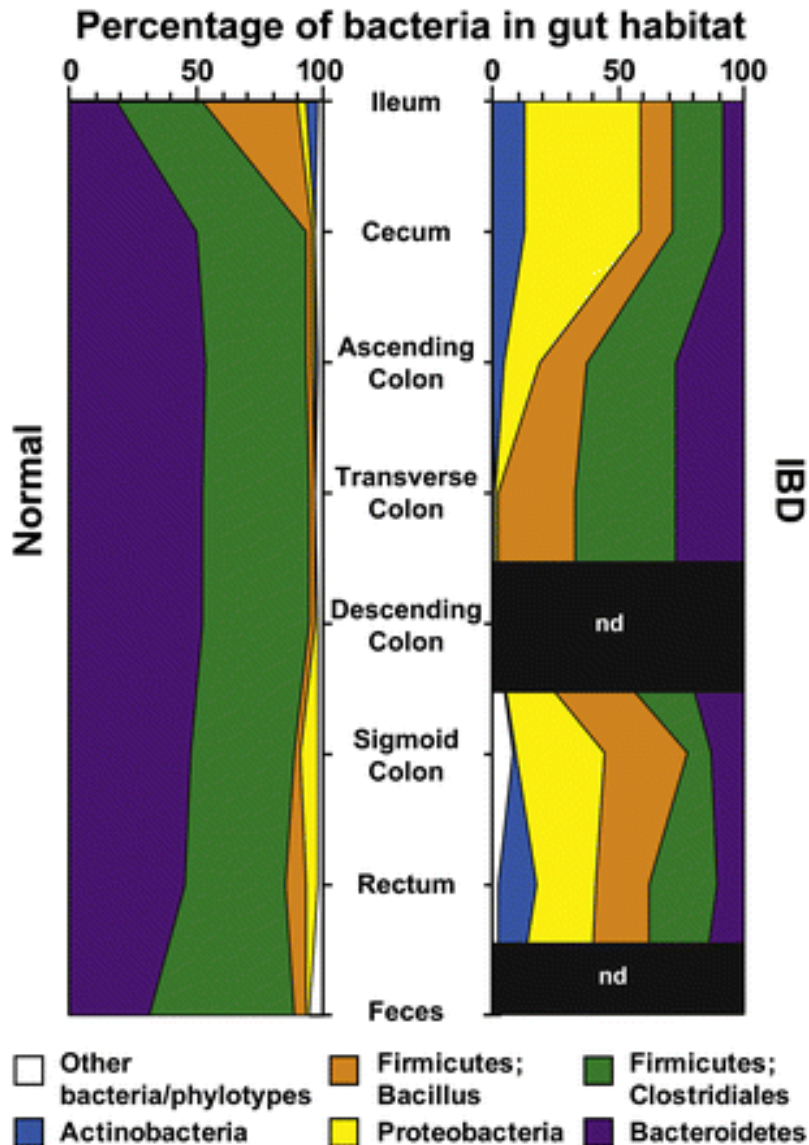
Sarkis K Mazmanian, California Institute of Technology

Bacteria play a critical role in inflammatory bowel disease (IBD)



- Antibiotics ameliorate symptoms in humans and animals
- Germ-free animals do not develop IBD in several models
- No definitive pathogens identified
- Immune reactivity to commensal organisms
- Predicted defects in immune regulation
- Alterations in the microbiota of IBD patients

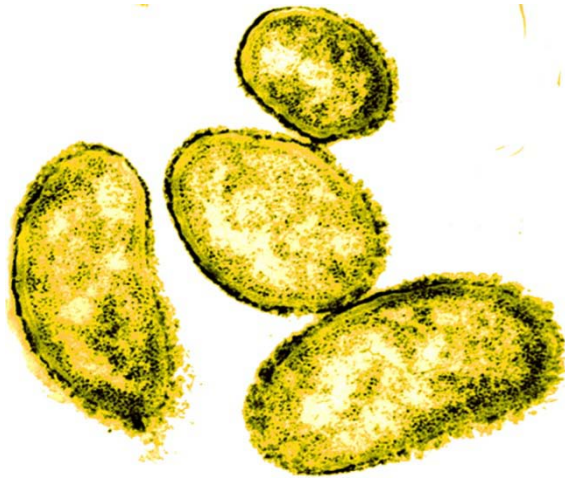
The human gastrointestinal tract is populated by both harmful and beneficial bacteria



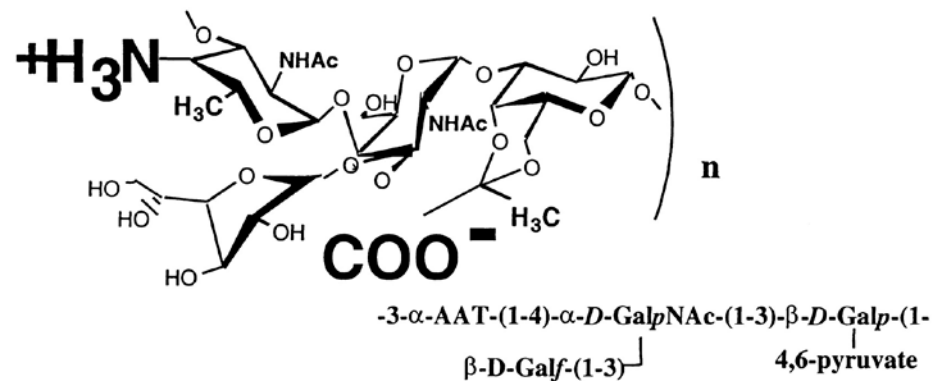
Some Consequences of Gut Dysbiosis

- Localized gut inflammation
- Systemic inflammation
- Increased oxidative stress
- Increased production of endotoxins and other biotoxins
- Altered production/synthesis of neurotransmitters
- Intestinal permeability
- Chronic infections
- Impaired detoxification/regulation of oxidative stress (e.g., sulfation)
- Impaired energy metabolism
- Impaired nutrient synthesis (e.g., vitamins, minerals and short-chain fatty acids)
- Impaired enzyme activity
- Autoimmunity

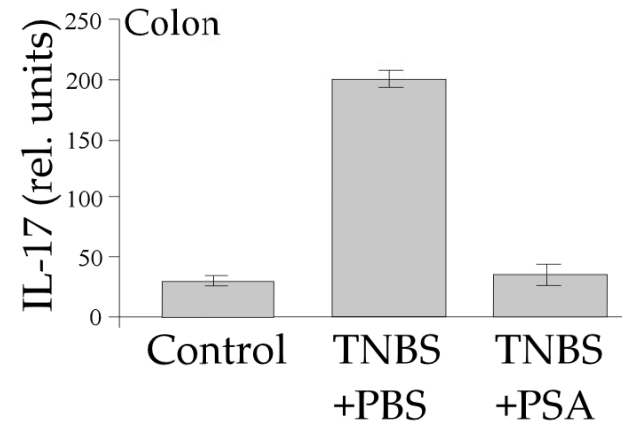
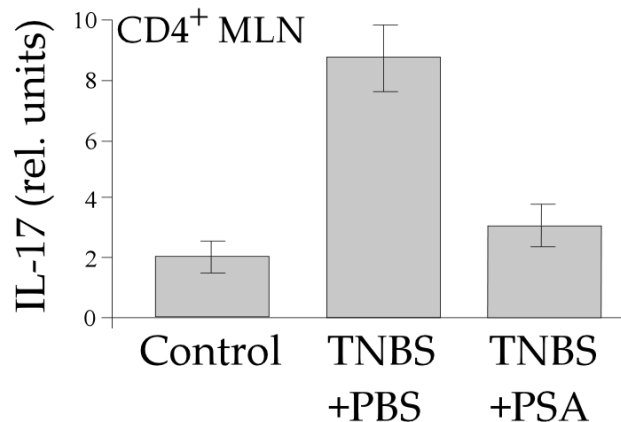
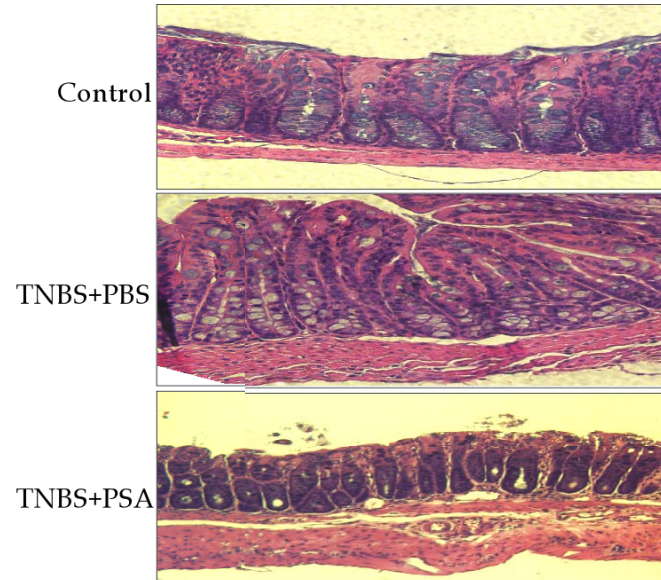
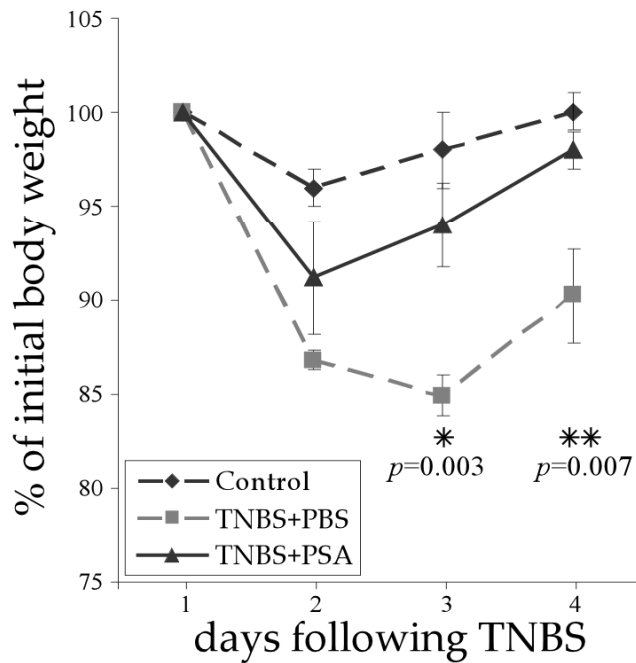
- *Bacteroides fragilis* is a gram-negative, obligate anaerobic bacteria
- *B. fragilis* is a prominent mutualist of in the human GI tract,
- *B. fragilis* synthesizes at least eight unique capsular polysaccharide complexes from distinct genomic loci
- At least two of these polysaccharides (PSA and PSB) have a novel zwitterionic structure (both a positive and negative charge within each repeating subunit)



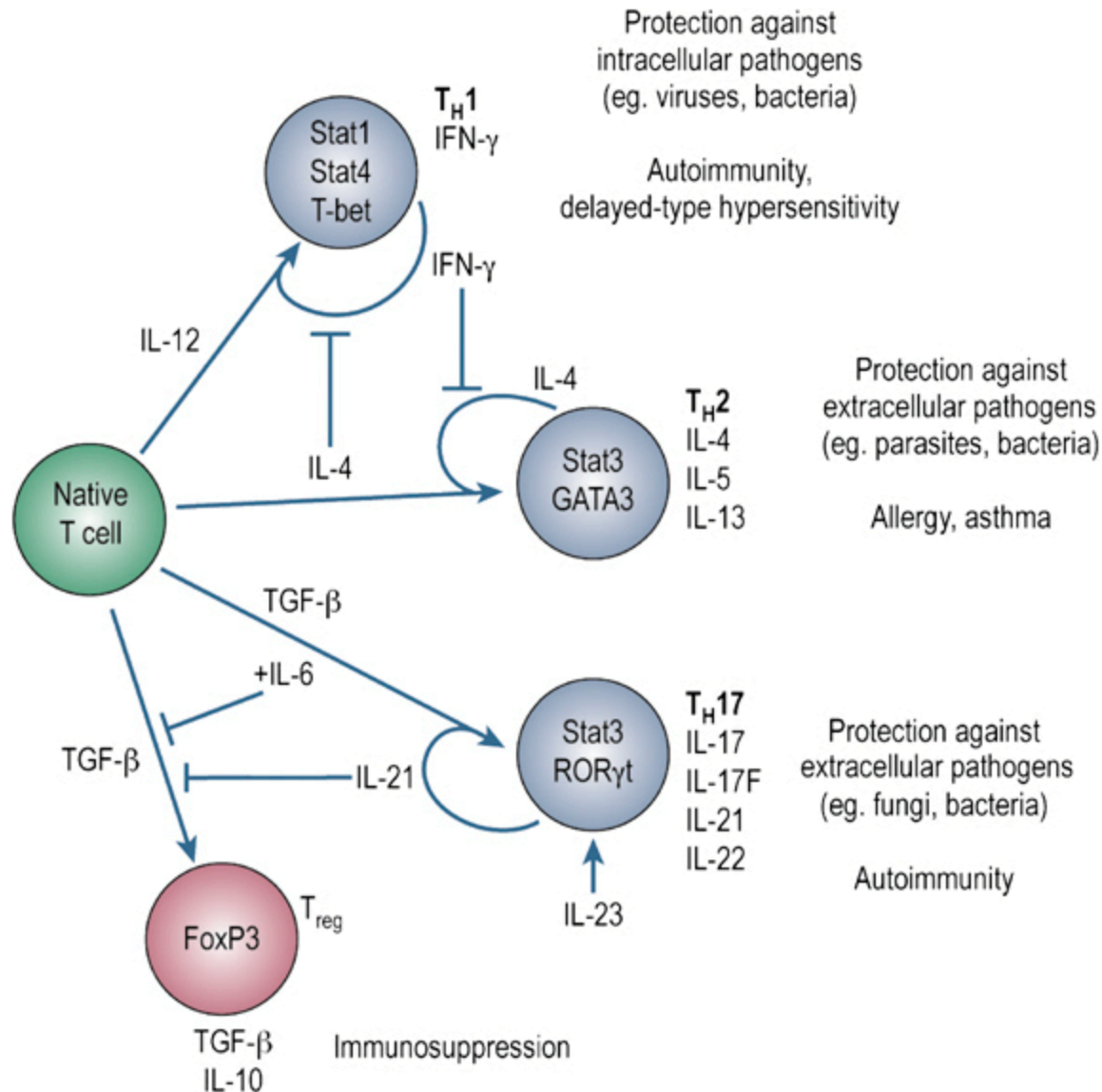
Polysaccharide A (PSA)



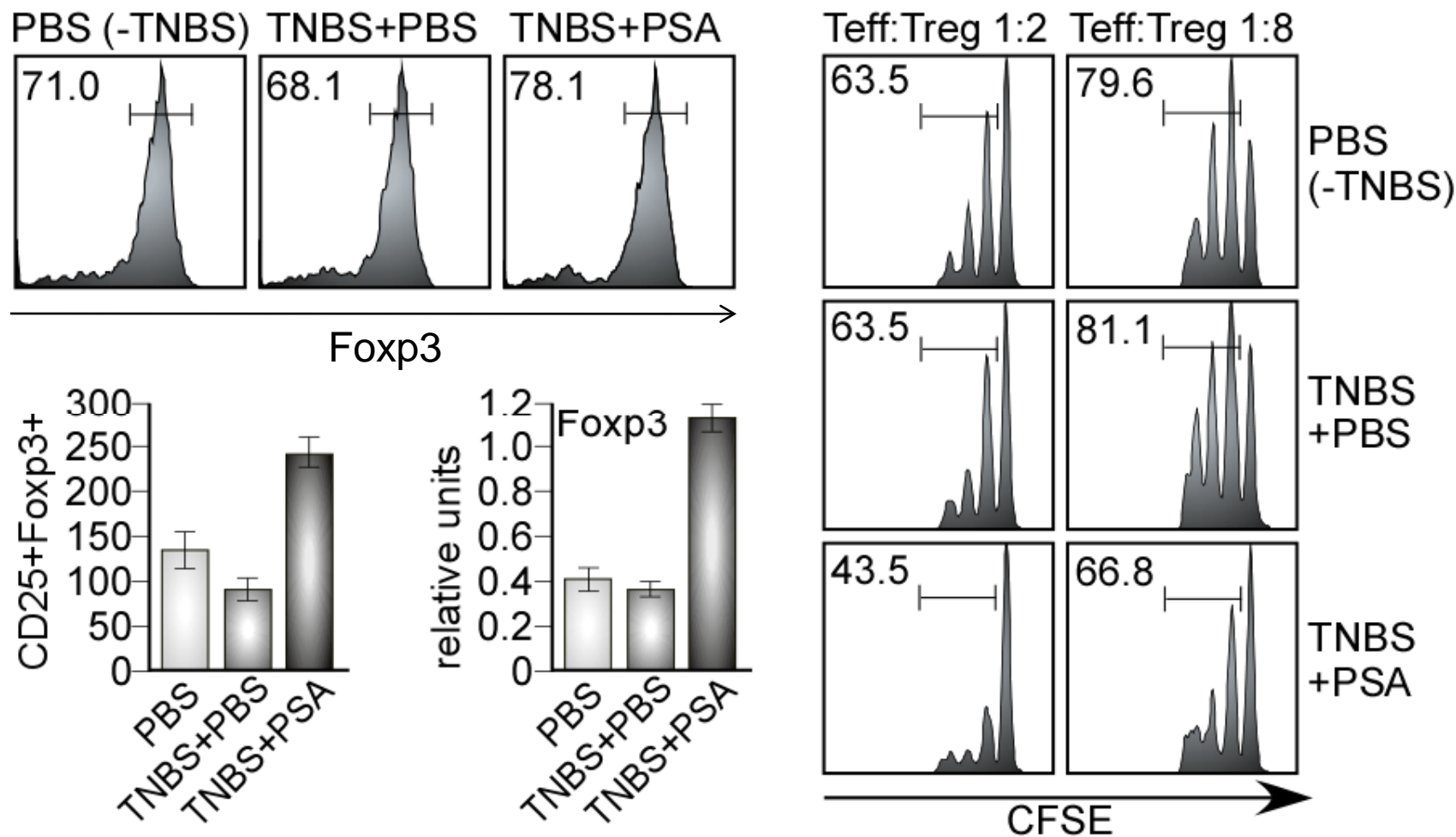
PSA protects from chemically induced intestinal inflammation



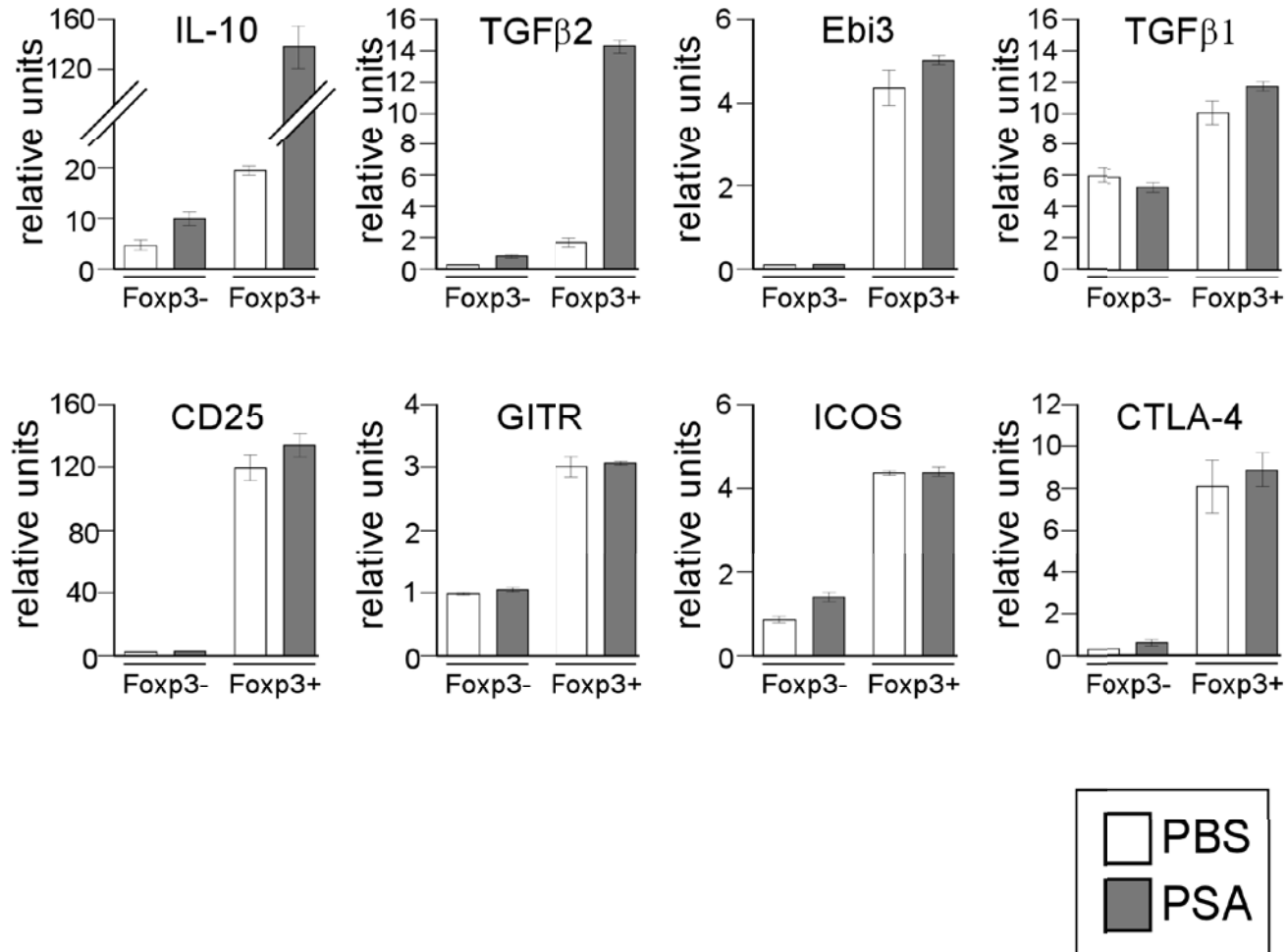
Experimental colitis is driven by inflammatory T-helper cells and prevented regulatory T cells



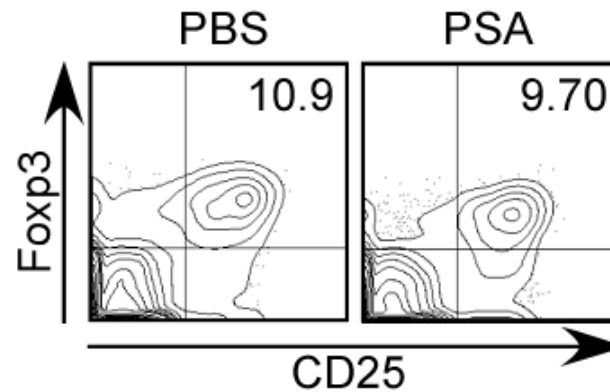
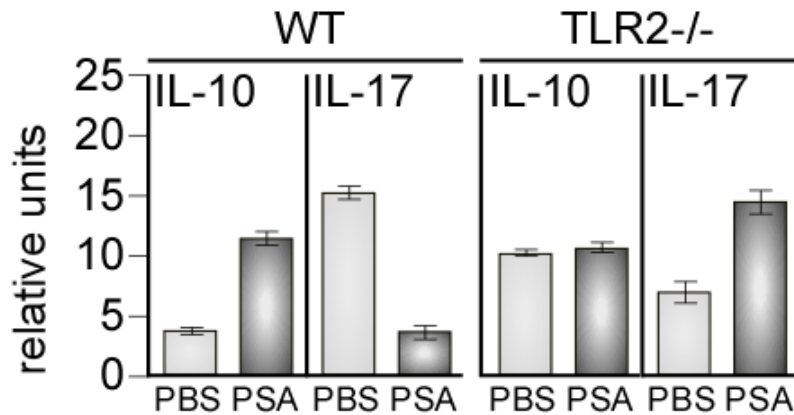
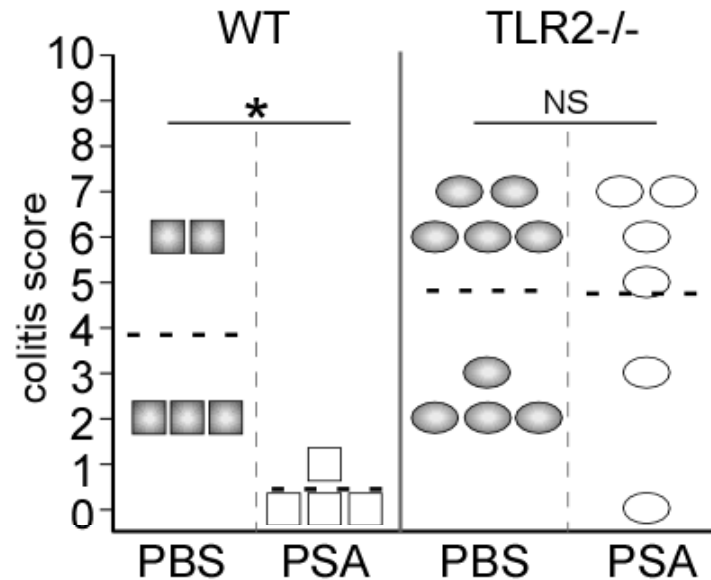
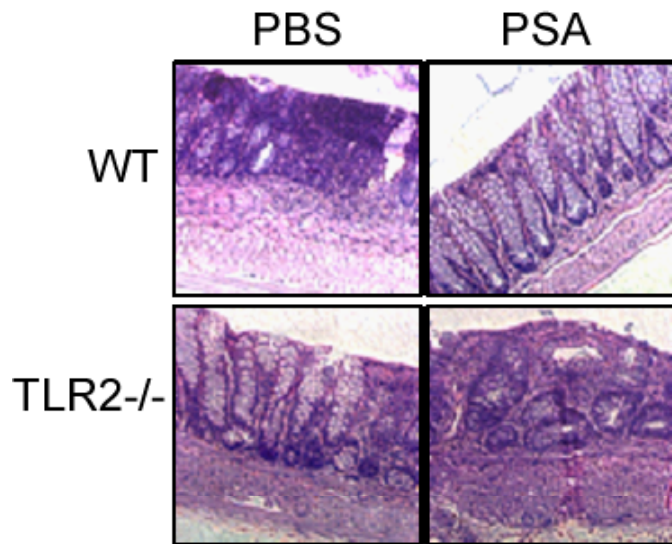
PSA mediates the expansion of functionally suppressive CD4+CD25+Foxp3+ Tregs during colitis



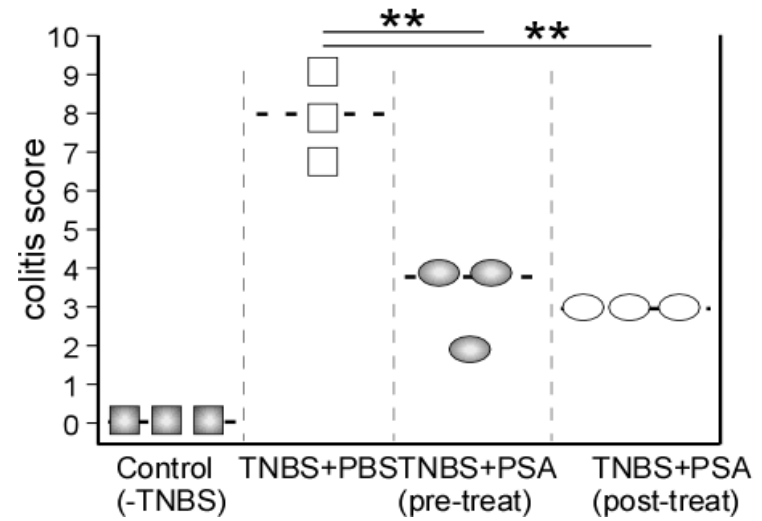
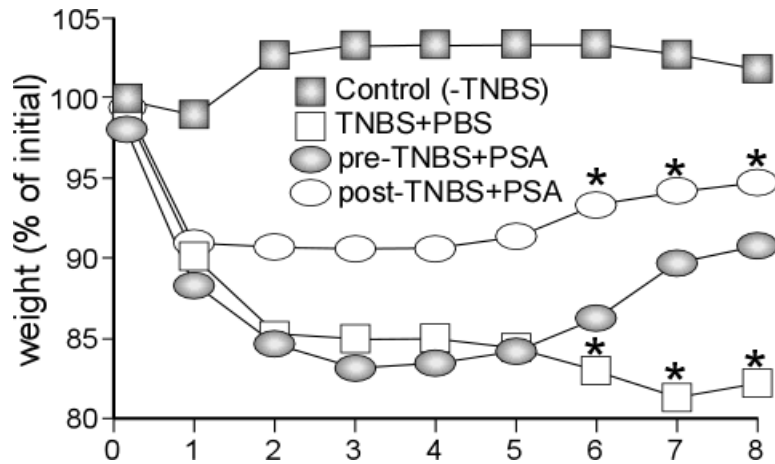
PSA directs the development of a specific gene expression profile for inducible Foxp3+ Tregs



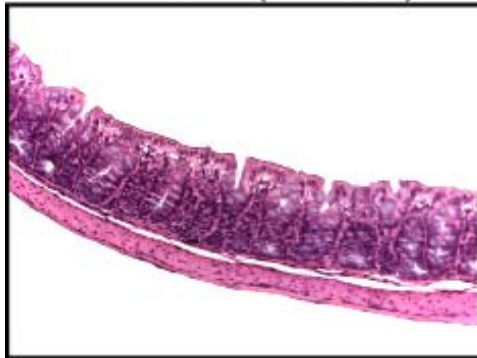
Protection from colitis by PSA requires Toll-like receptor 2 signaling



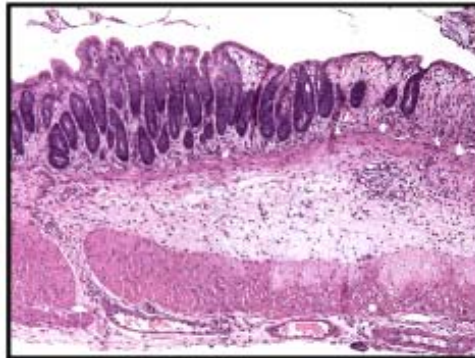
PSA cures established TNBS colitis



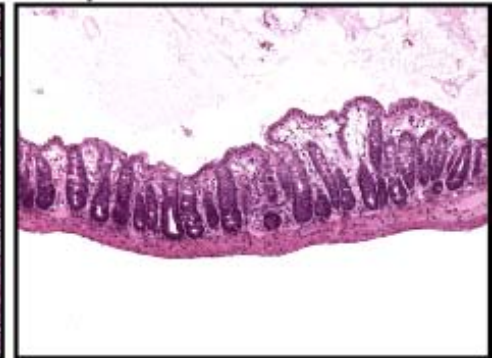
Control (-TNBS)



TNBS+PBS

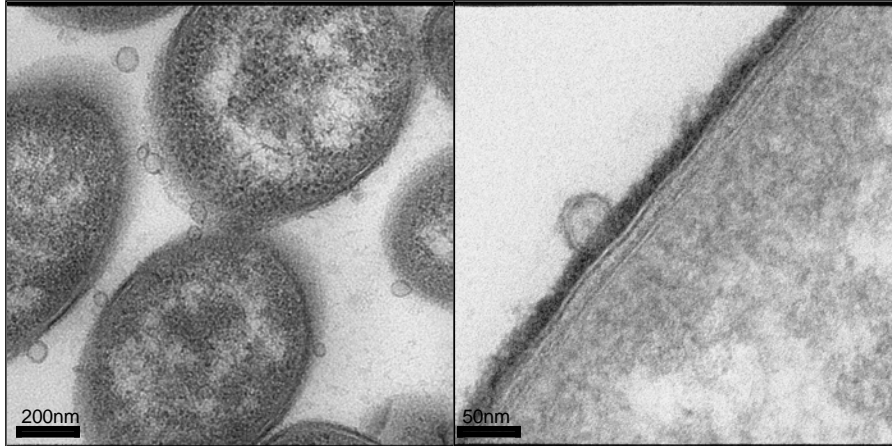


post-TNBS+PSA

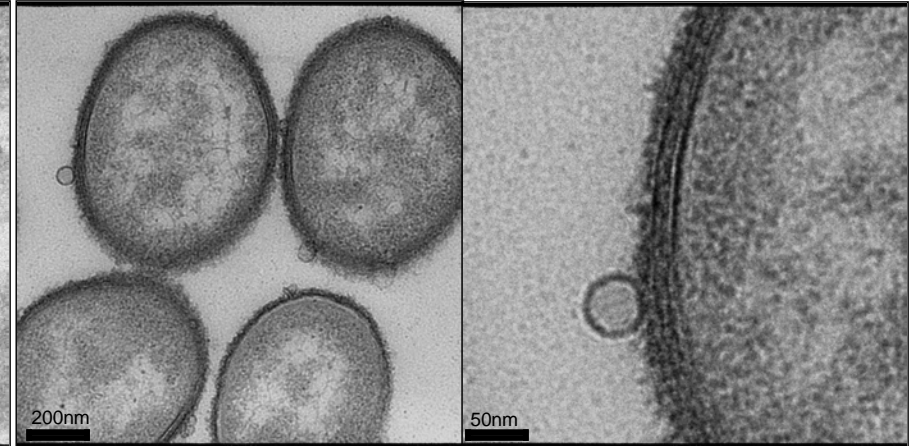


PSA is selectively packaged into outer membrane vesicles of *B. fragilis*

B. fragilis

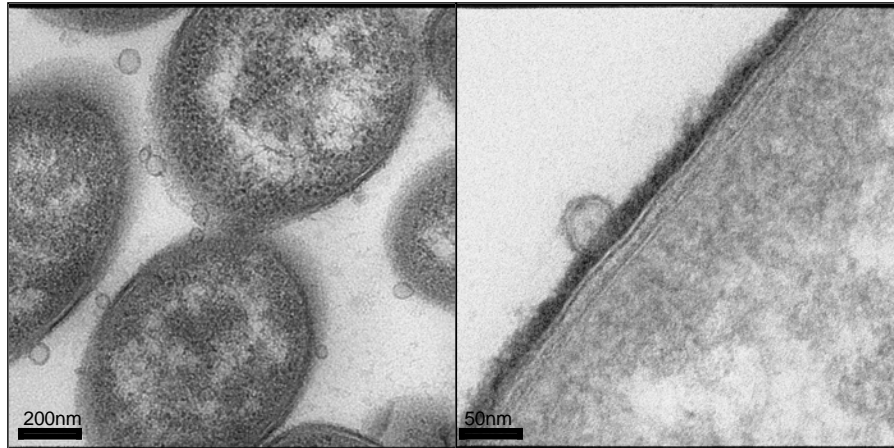


B. fragilis Δ PSA

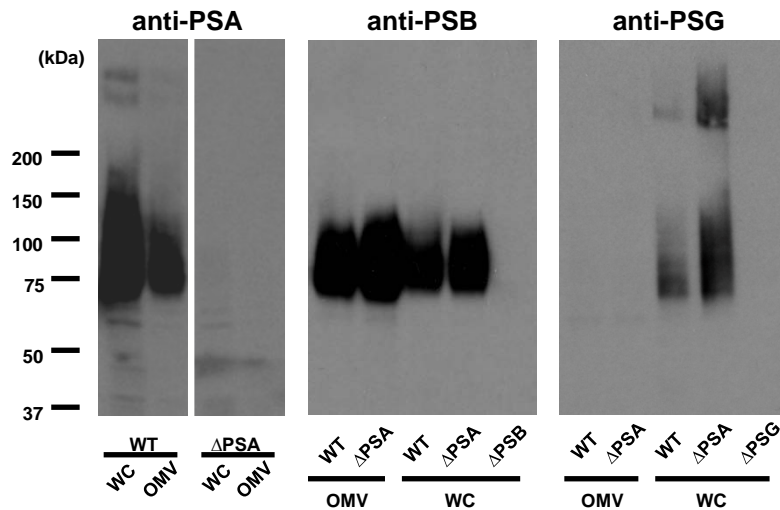


PSA is selectively packaged into outer membrane vesicles of *B. fragilis*

B. fragilis

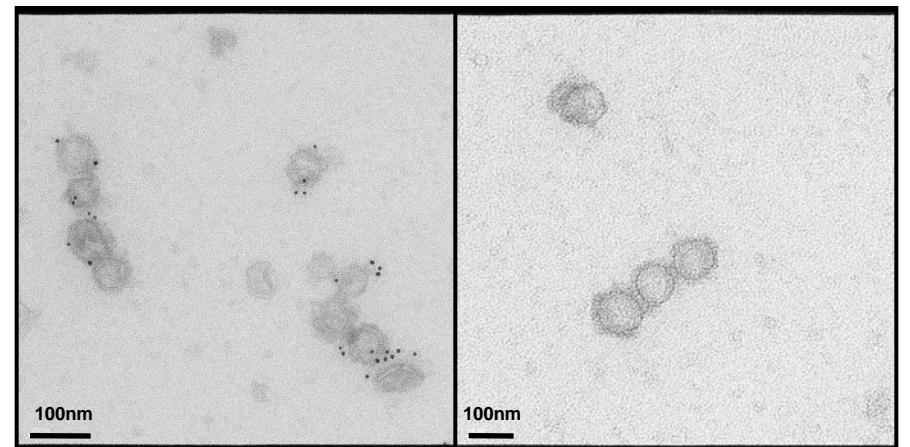


B. fragilis Δ PSA

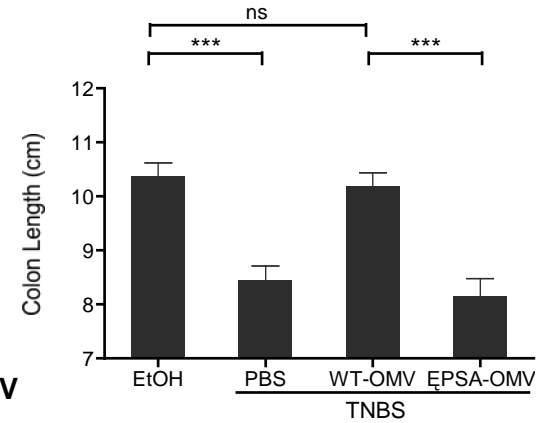
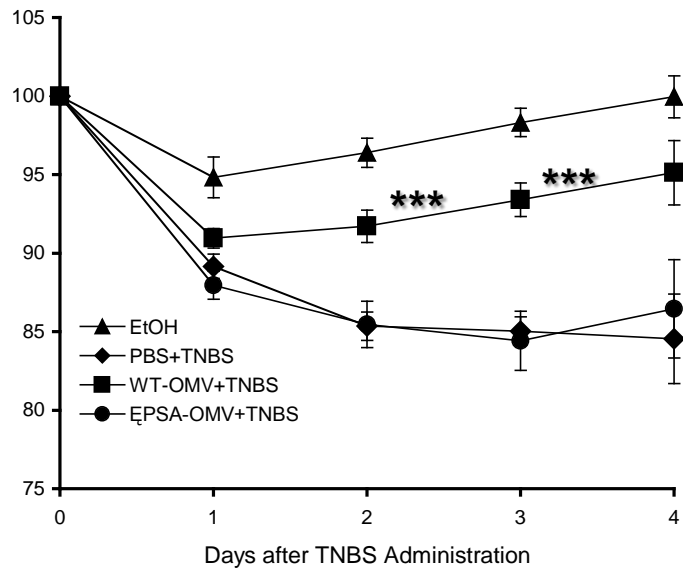


WT-OMV

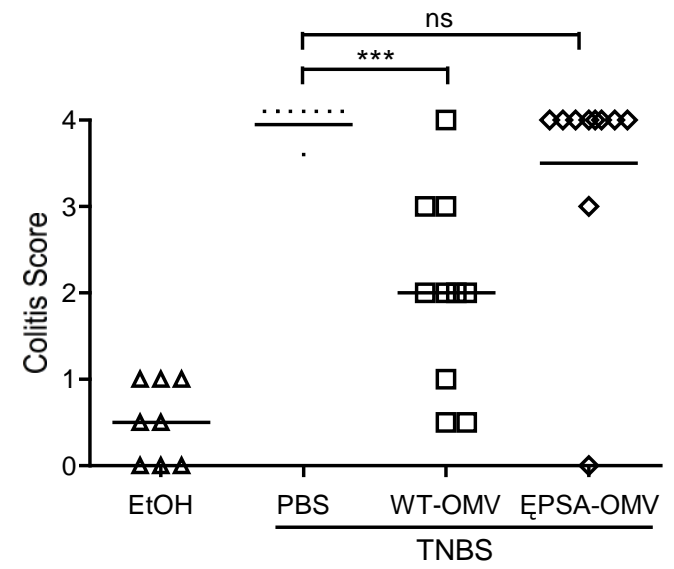
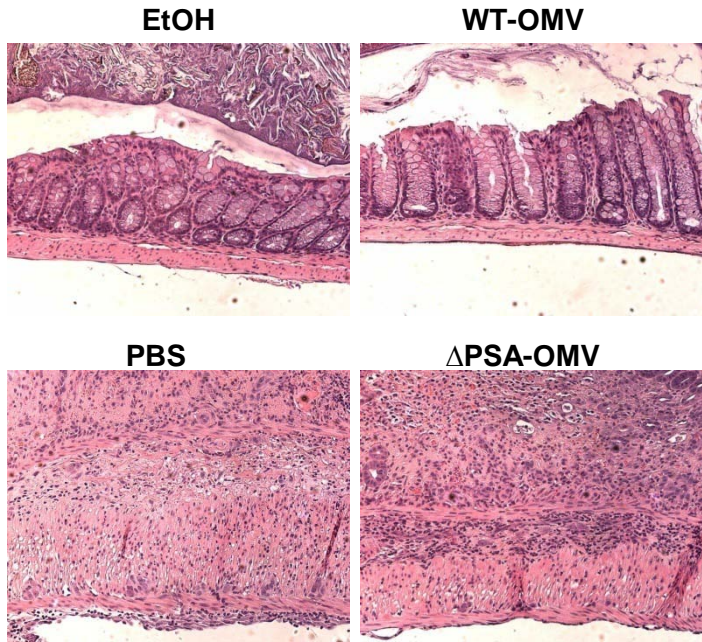
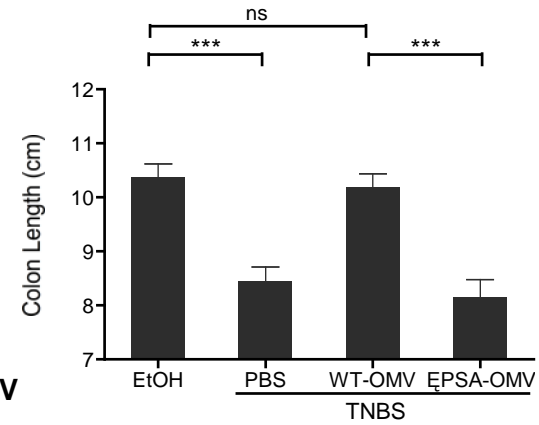
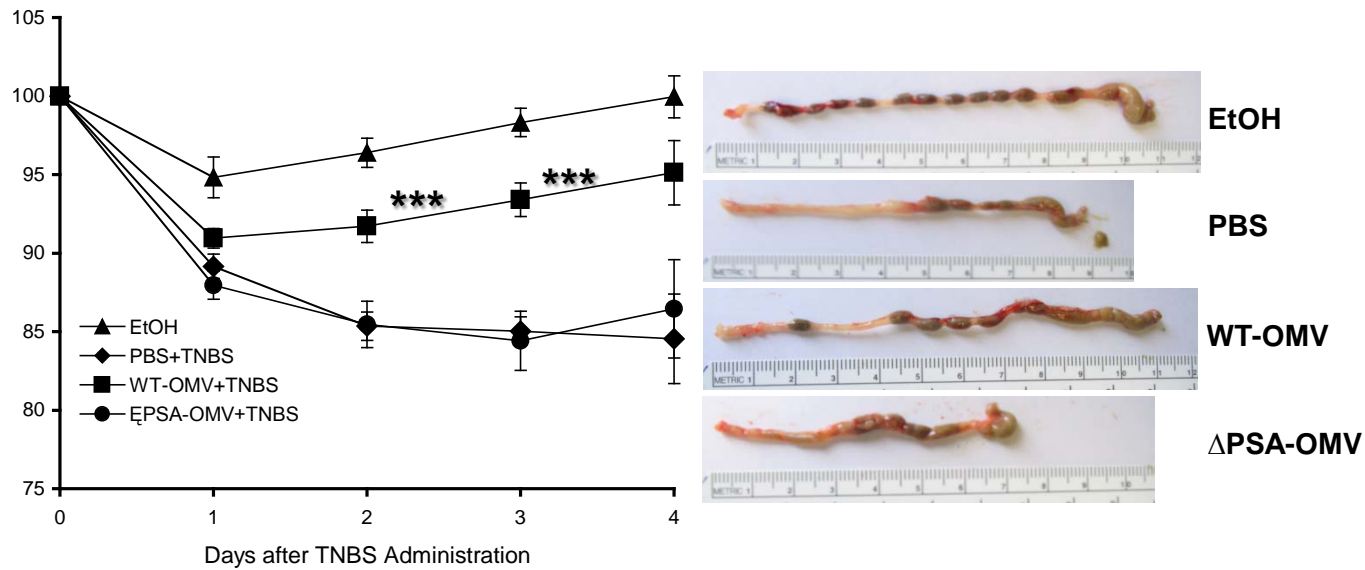
Δ PSA-OMV



OMVs containing PSA protect animals from colitis

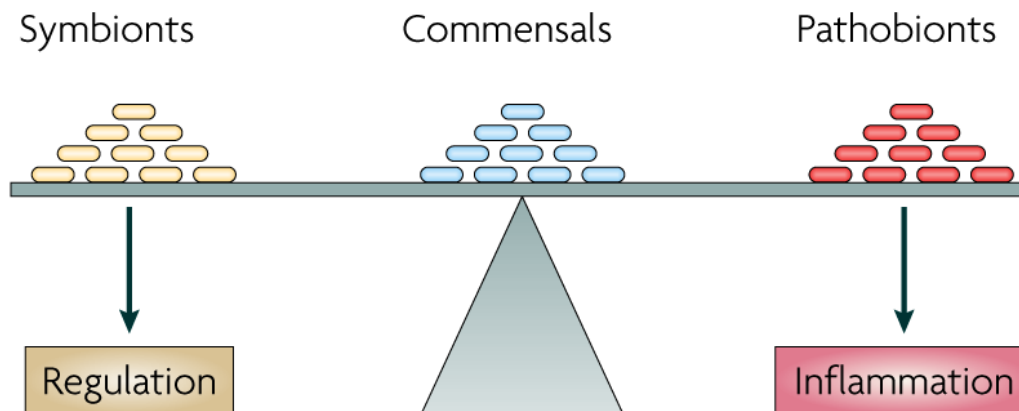


OMVs containing PSA protect animals from colitis

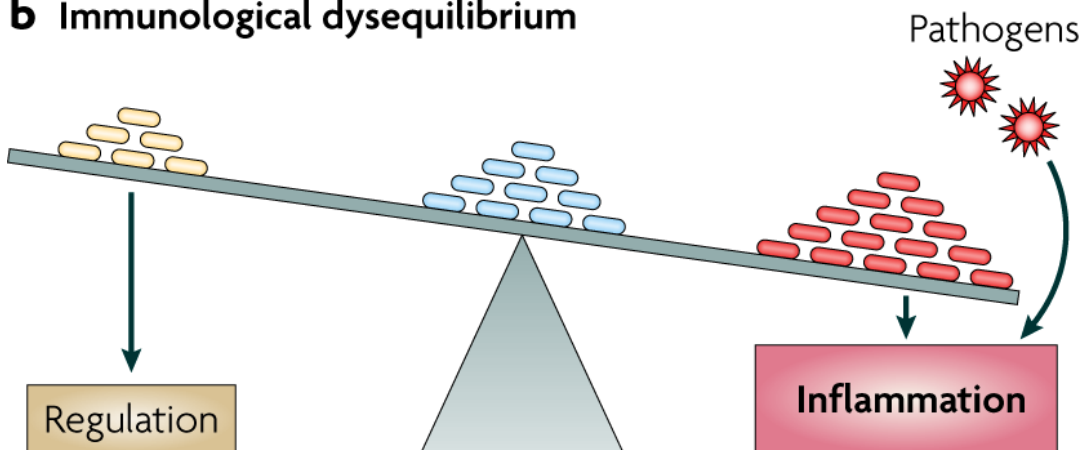


The interactions between harmful and beneficial bacteria may affect health and disease

a Immunological equilibrium

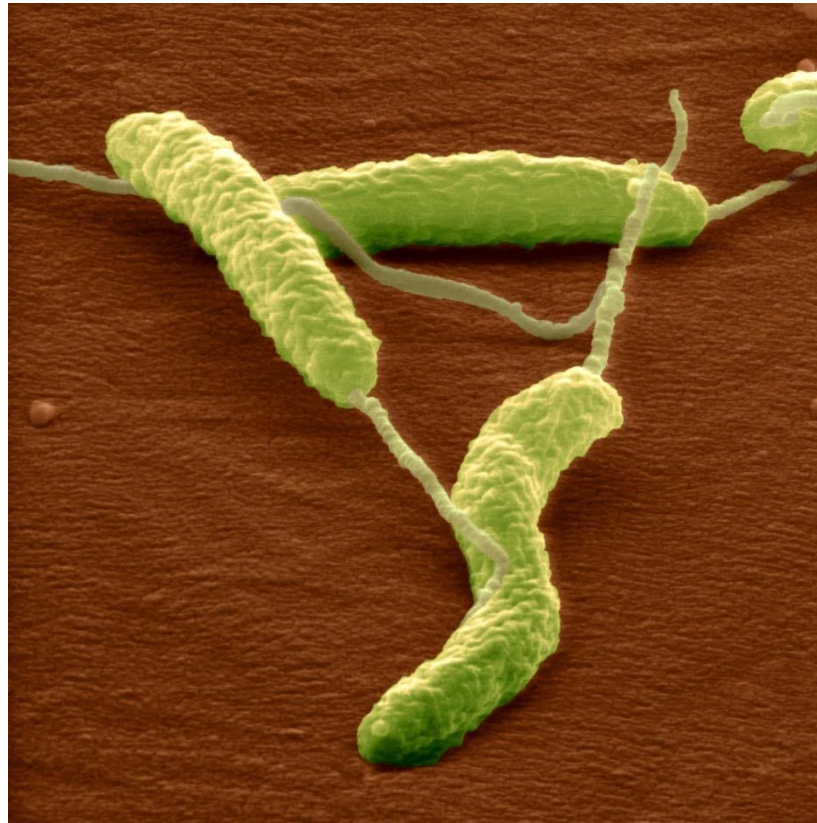


b Immunological dysequilibrium

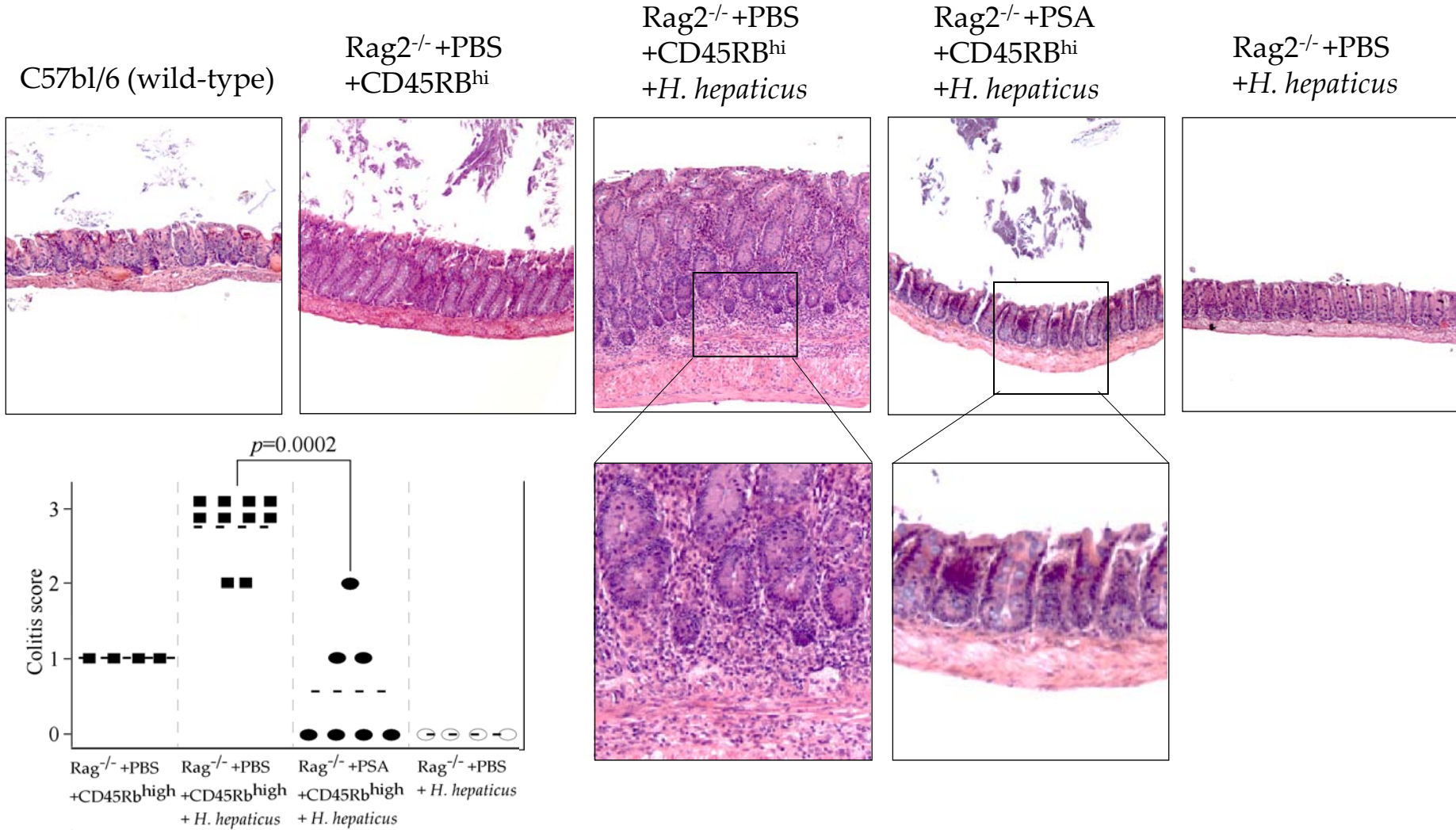


***Helicobacter hepaticus* as a model pathobiont**

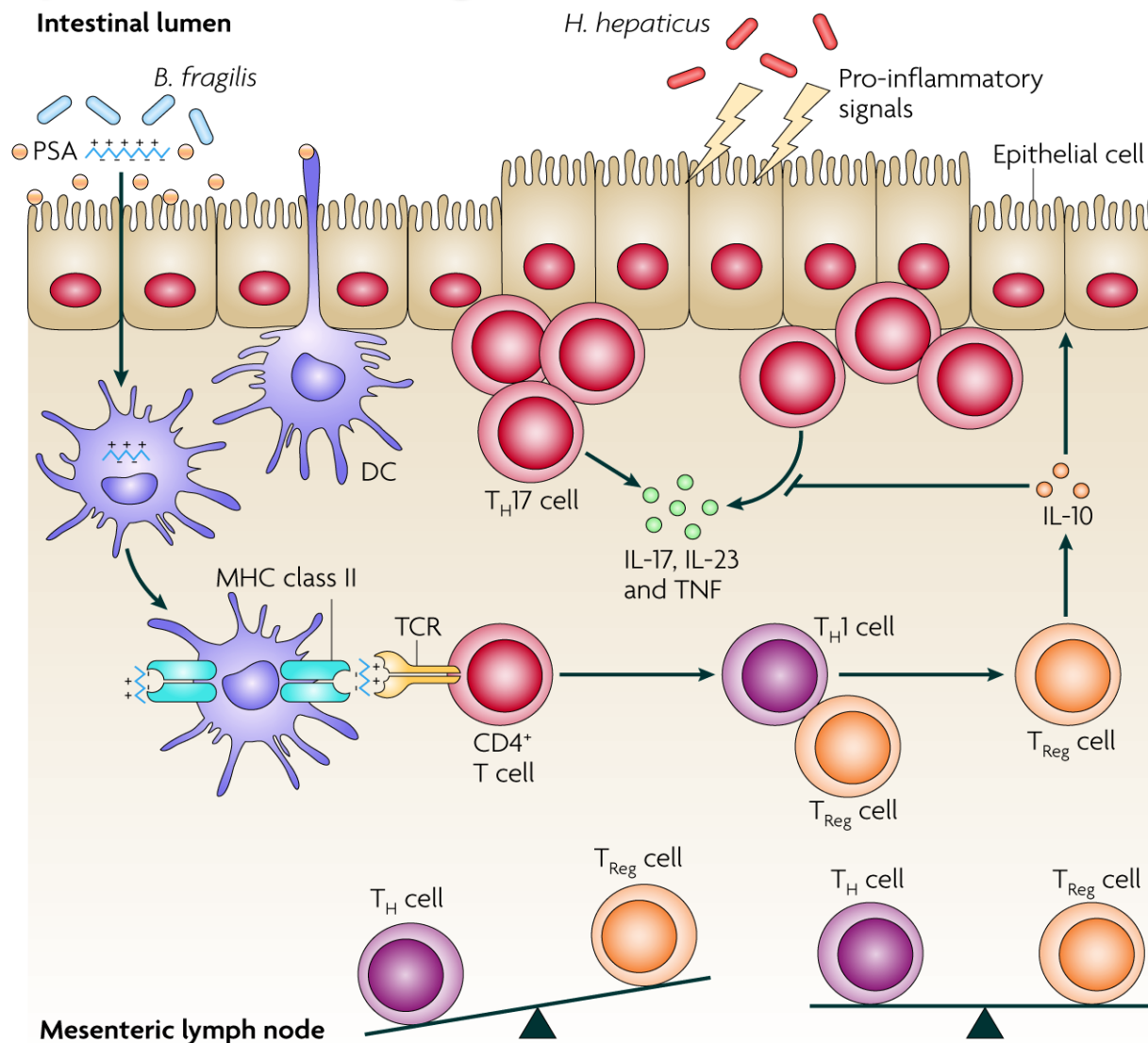
- ***Helicobacter hepaticus* is a gram-negative, microaerophilic spiral bacteria that colonizes mice**
- ***H. hepaticus* does not induce disease in wild-type animals, but upon chronic infection of immunocompromised animals causes intestinal inflammation with similarities to Crohn's disease**



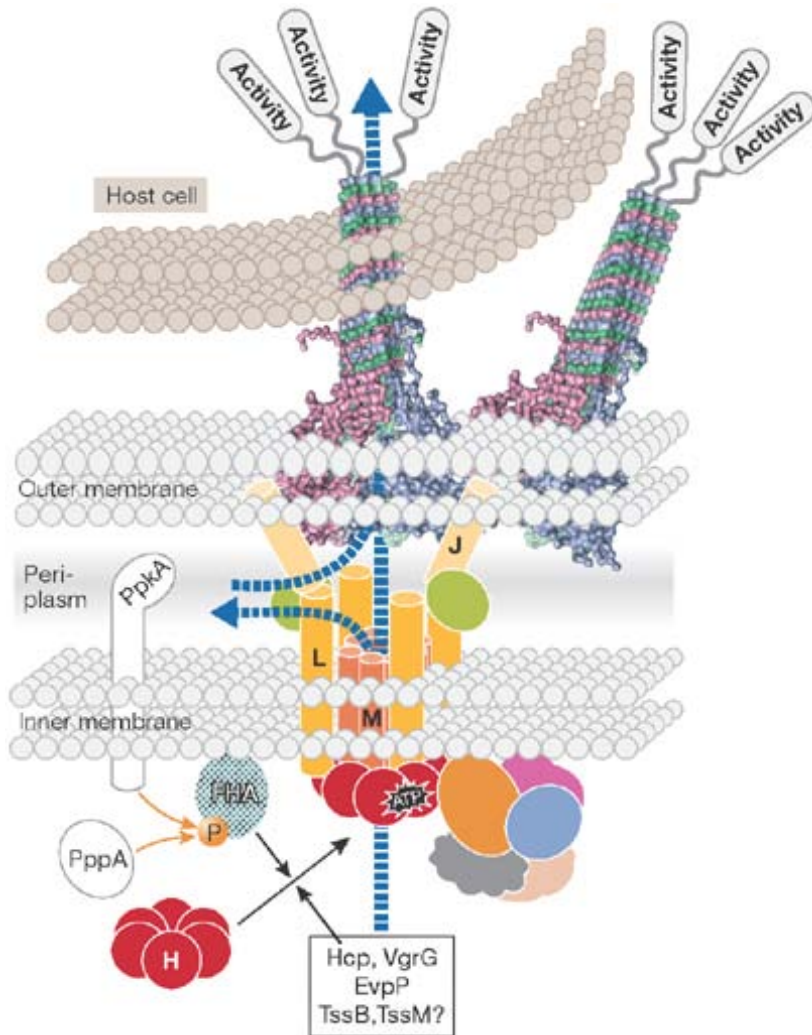
Induction of *H. hepaticus* colitis is prevented by PSA treatment



PSA of *B. fragilis* prevents pro-inflammatory immune responses in the gut to maintain homeostasis



The type VI secretion system (T6SS) of *Helicobacter hepaticus*



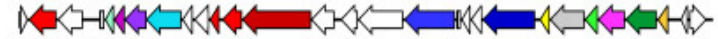
SPI-6 T6SS *S. Typhimurium* LT2



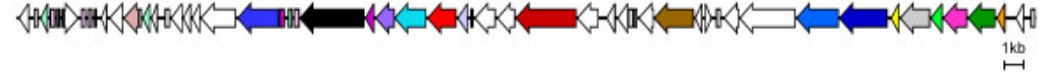
SPI-19 *S. Gallinarum* 287/91



SPI-20 *S. Illia* 62:z4,z23:- RKS2980



SPI-21 *S. Illia* 62:z4,z23:- RKS2980



T6SS core components

COG3522	COG3518/gp25-like
COG3519/VasA	COG3523/lcmF
COG3455/DotU	COG3501/"Core" VgrG
COG3516/VipA	COG0542/ClpV
COG3520	COG3515/ImpA
COG3517/VipB	COG3521/SciN
COG3157/Hcp	

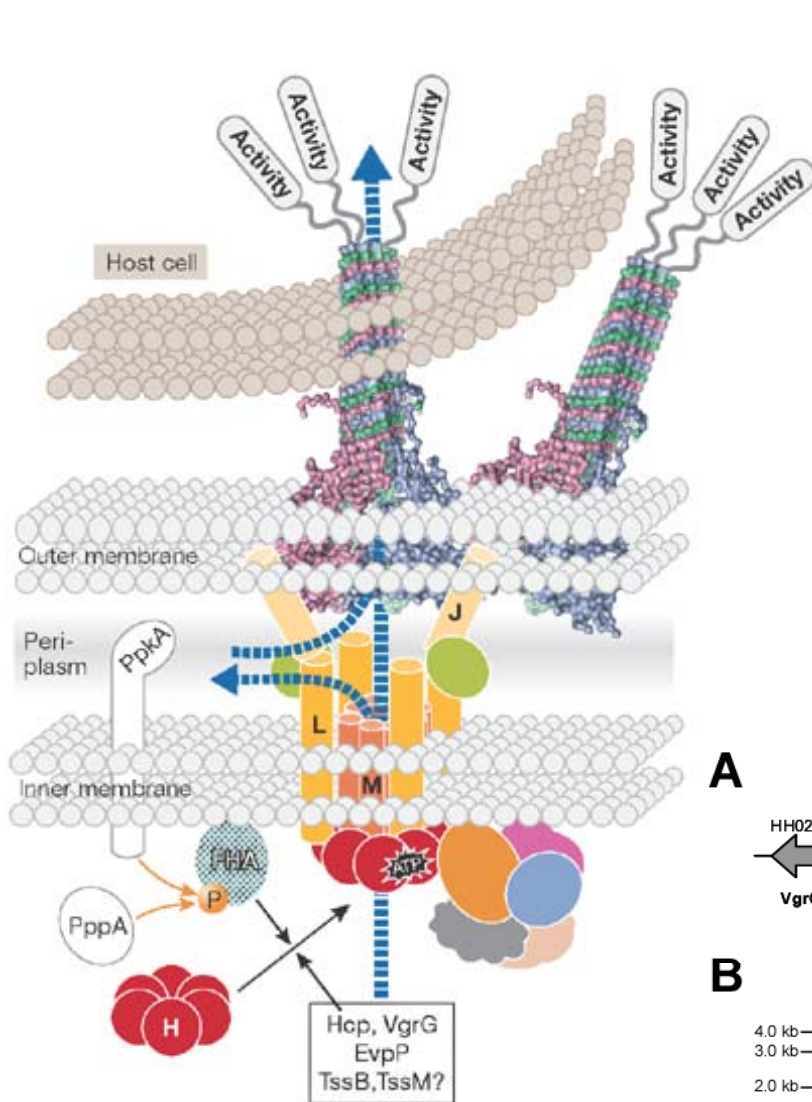
T6SS conserved accessory components

COG3456/FHA	COG2885/OmpA
COG4455/ImpE	COG3209/Rhs element
COG4104	COG4253

Additional relevant protein domains

COG5529/"Evolved" VgrG	pfam01320/colicin immunity protein
cd06577/PASTA	COG3179/putative chitinase

The type VI secretion system (T6SS) of *Helicobacter hepaticus*



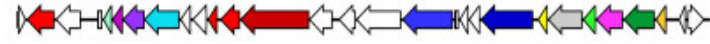
SPI-6 T6SS *S. Typhimurium* LT2



SPI-19 *S. Gallinarum* 287/91



SPI-20 *S. Illia* 62:z4,z23:- RKS2980



SPI-21 *S. Illia* 62:z4,z23:- RKS2980



T6SS core components

COG3522	COG3518/gp25-like
COG3519/VasA	COG3523/lcmF
COG3455/DotU	COG3501/*Core* VgrG
COG3516/VipA	COG0542/ClpV
COG3520	COG3517/VipB
COG3517/VipB	COG3515/ImpA
COG3157/Hcp	COG3521/ScIN

T6SS conserved accessory components

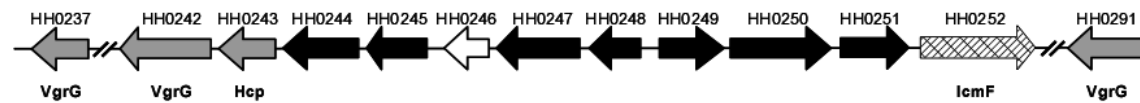
COG3456/FHA	COG2885/OmpA
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Additional relevant protein domains

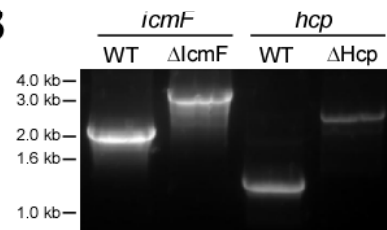
COG5529/*Evolved* VgrG	pfam01320/colicin immunity protein
cd06577/PASTA	COG3179/putative chitinase

A

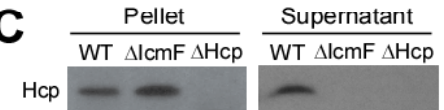
Helicobacter hepaticus



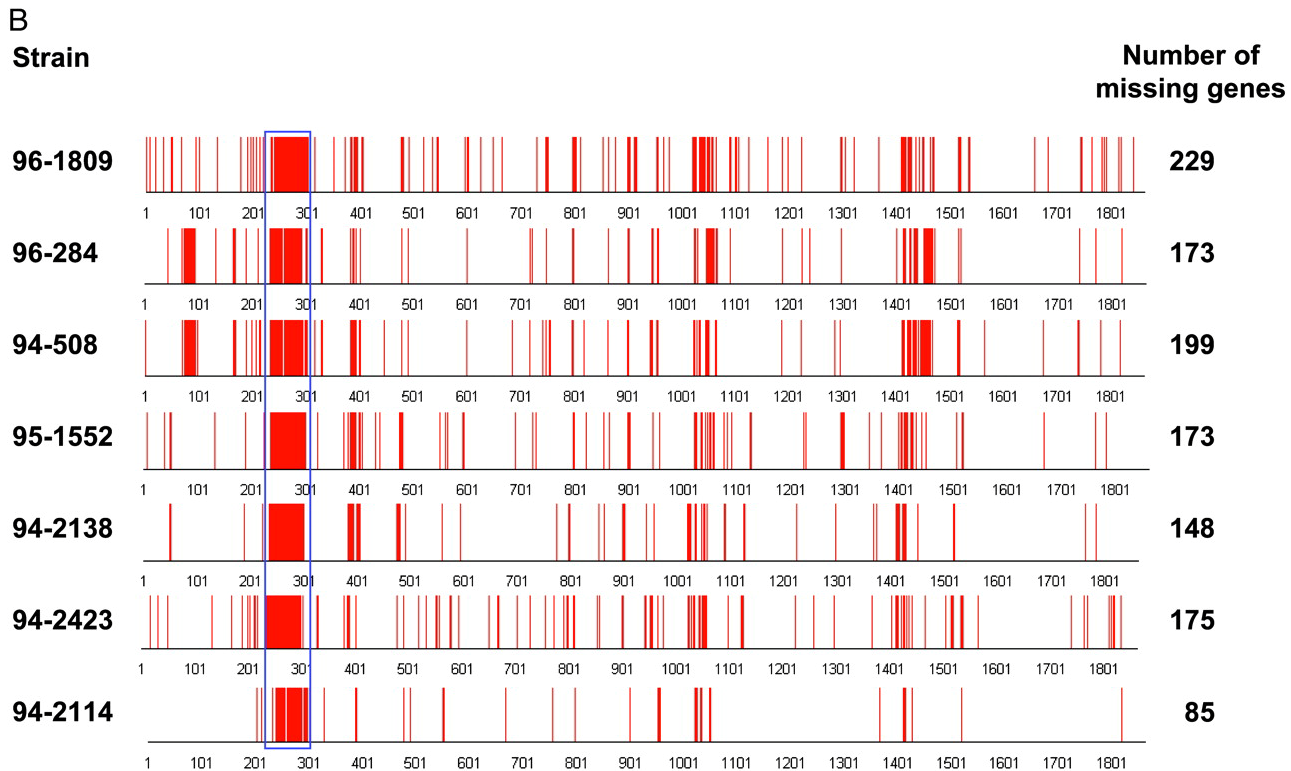
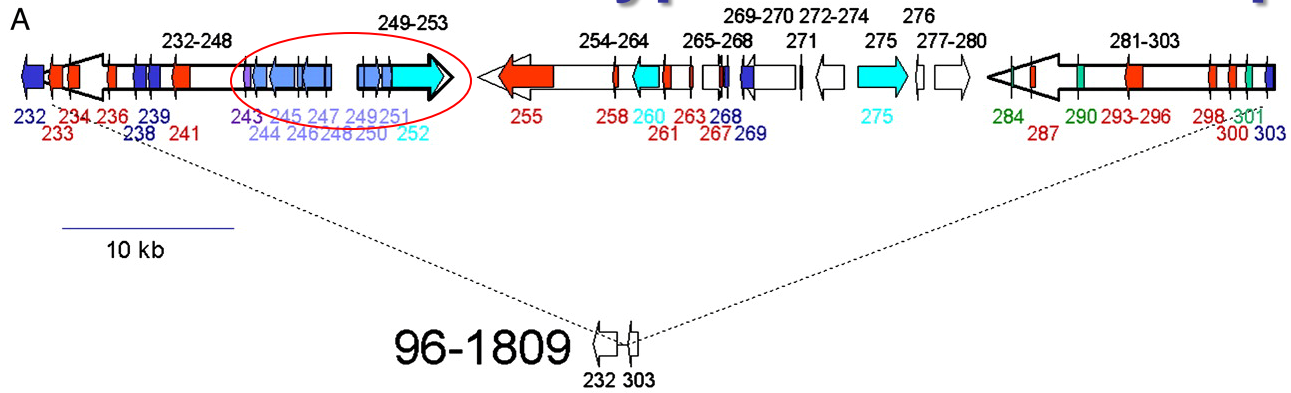
B



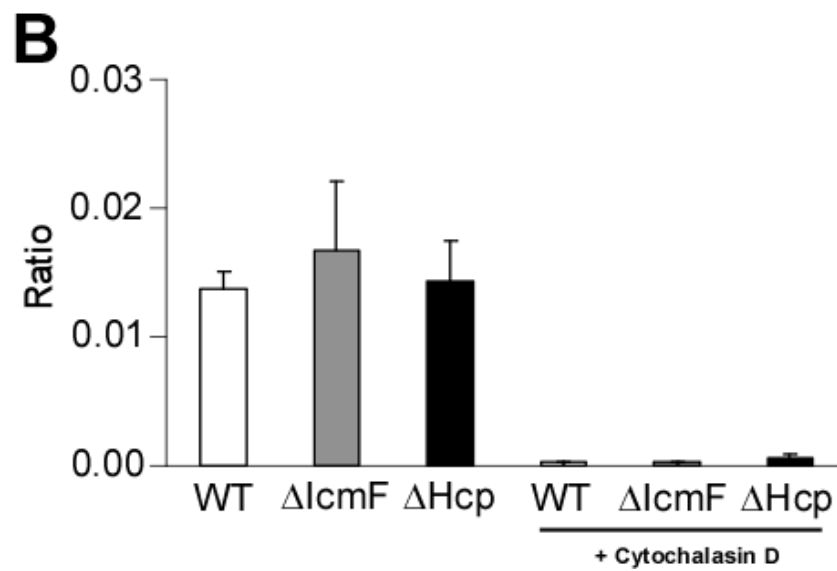
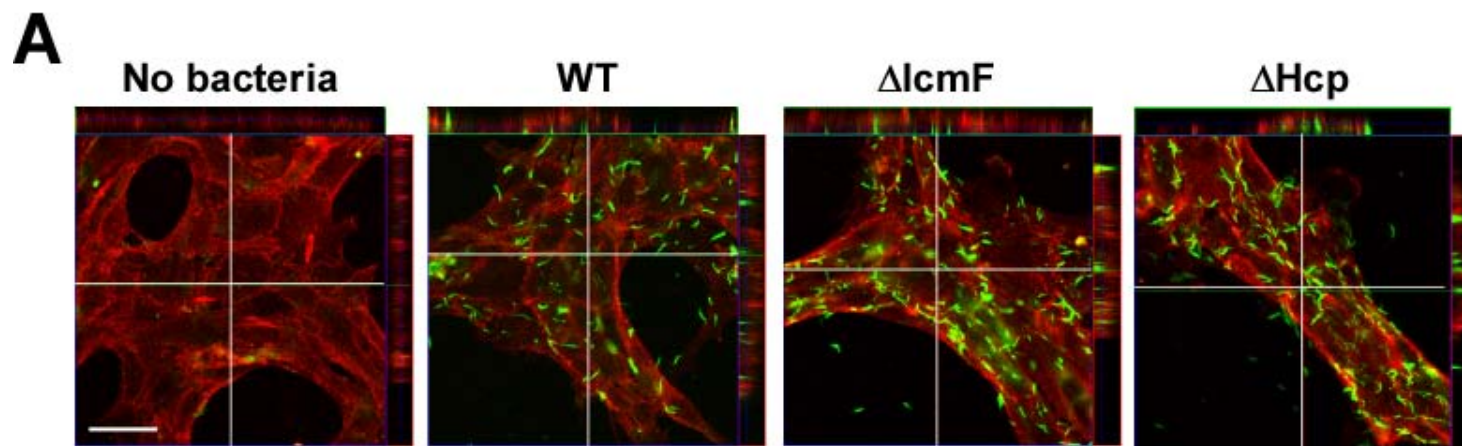
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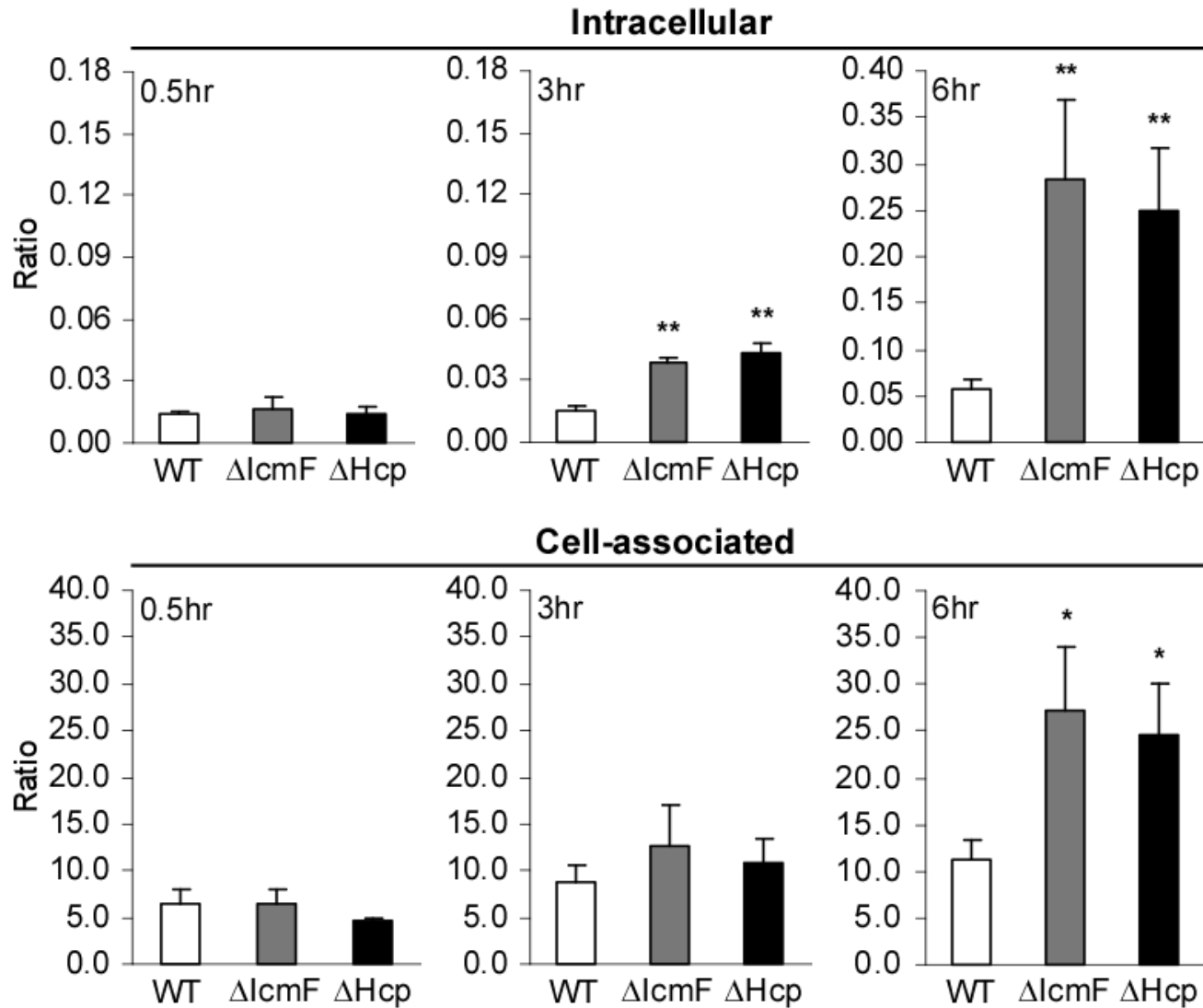
H. hepaticus encodes a type VI secretion apparatus



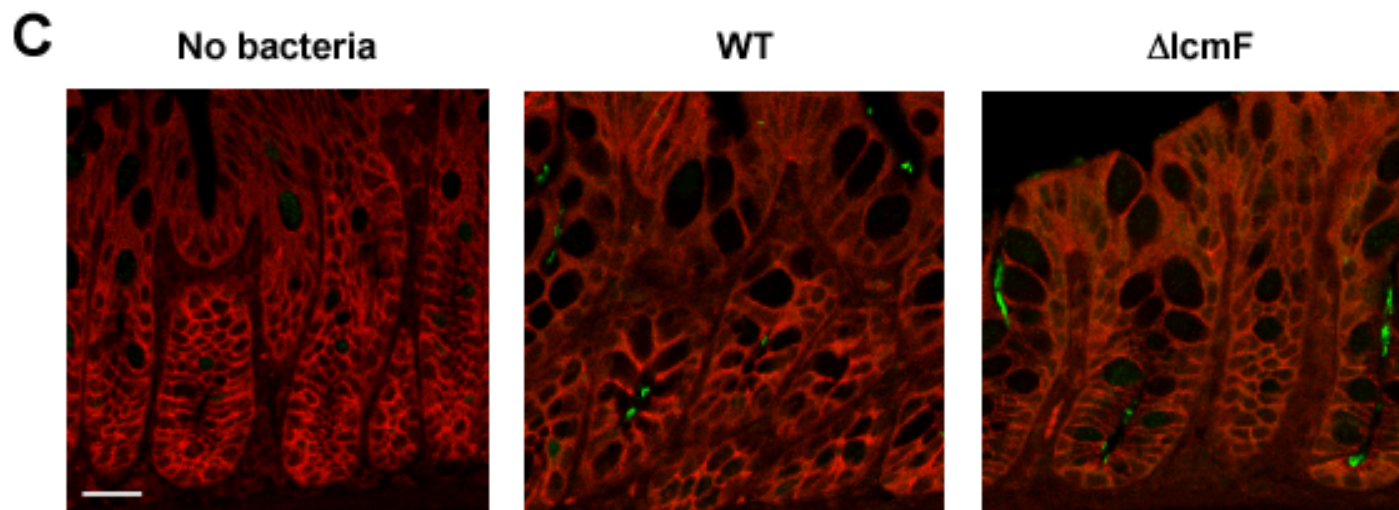
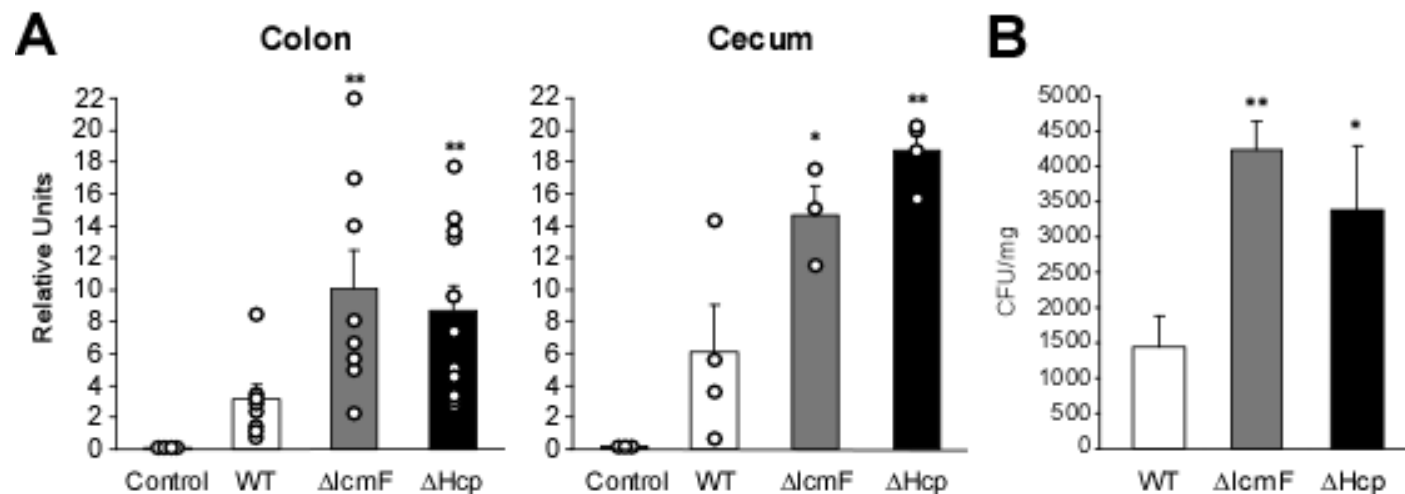
Helicobacter hepaticus enters intestinal epithelial cells



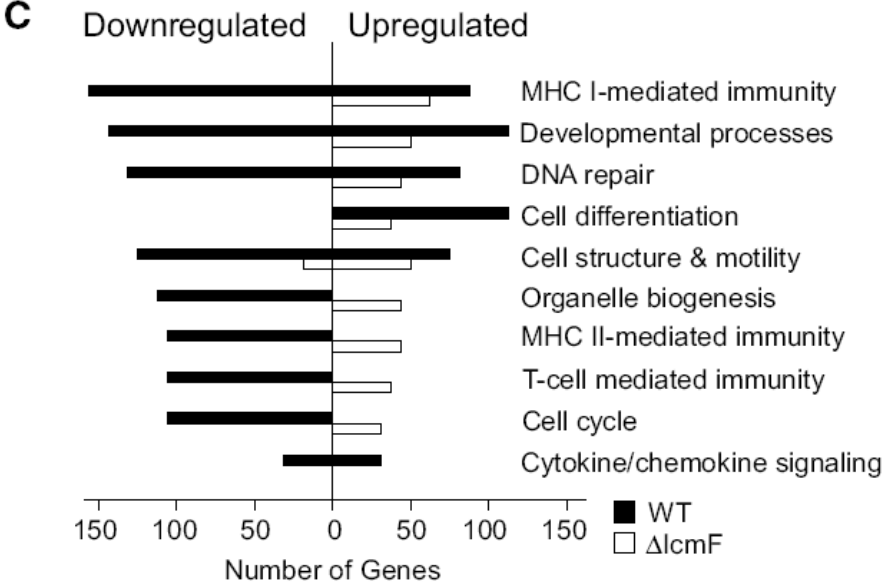
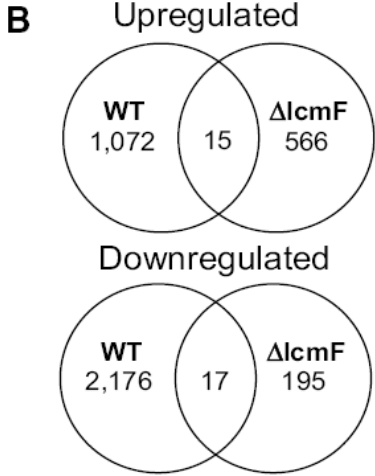
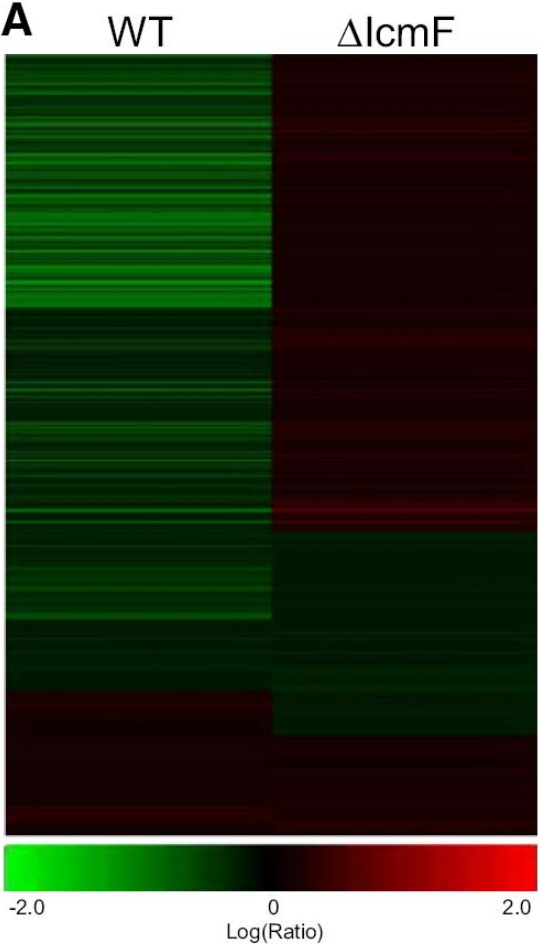
T6SS mutants of *Helicobacter hepaticus* display increased association with intestinal epithelial cells



T6SS mutants are increased in animal colonization



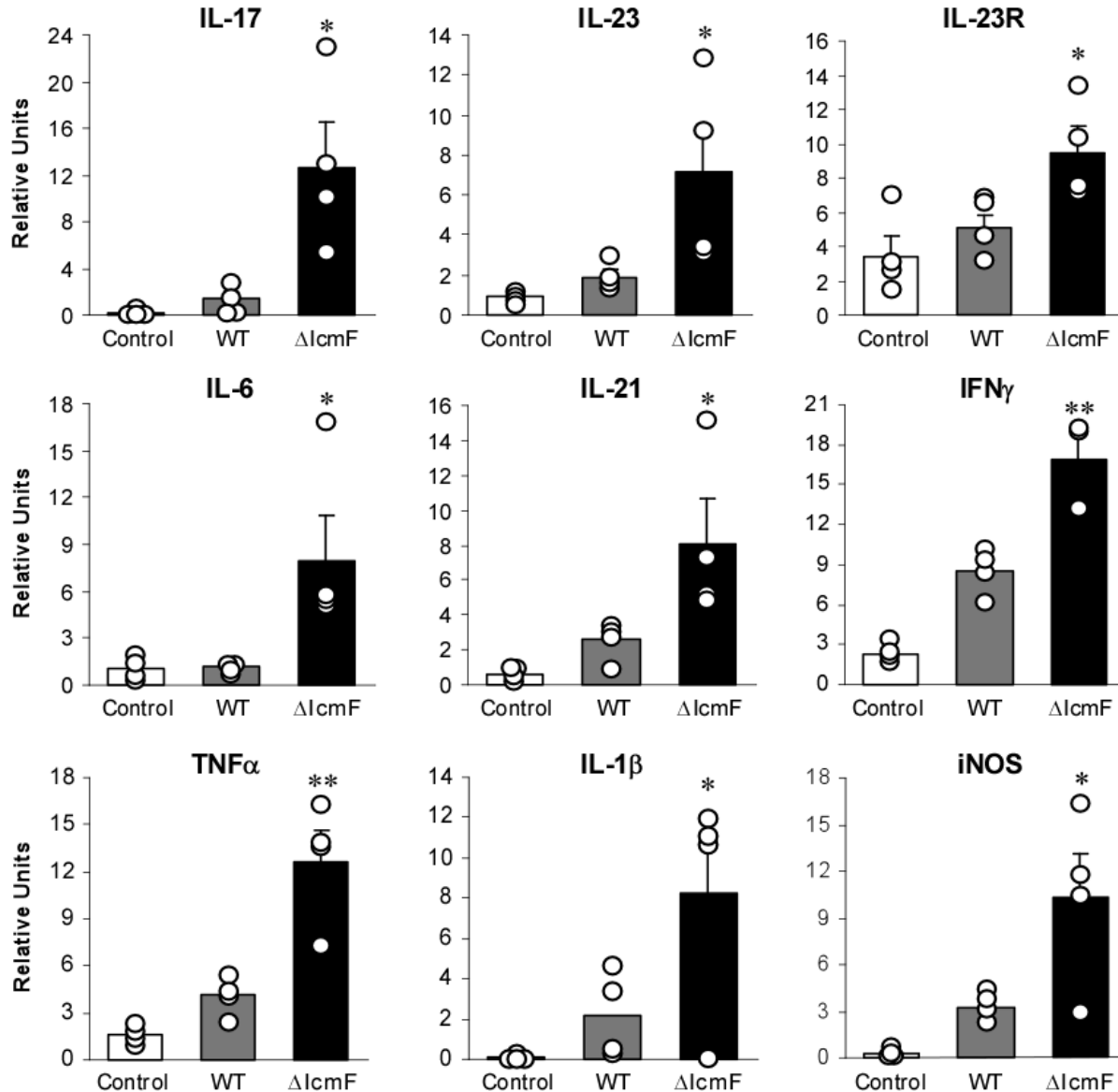
H. hepaticus employs the T6SS to suppress immune-related gene expression in epithelial cells



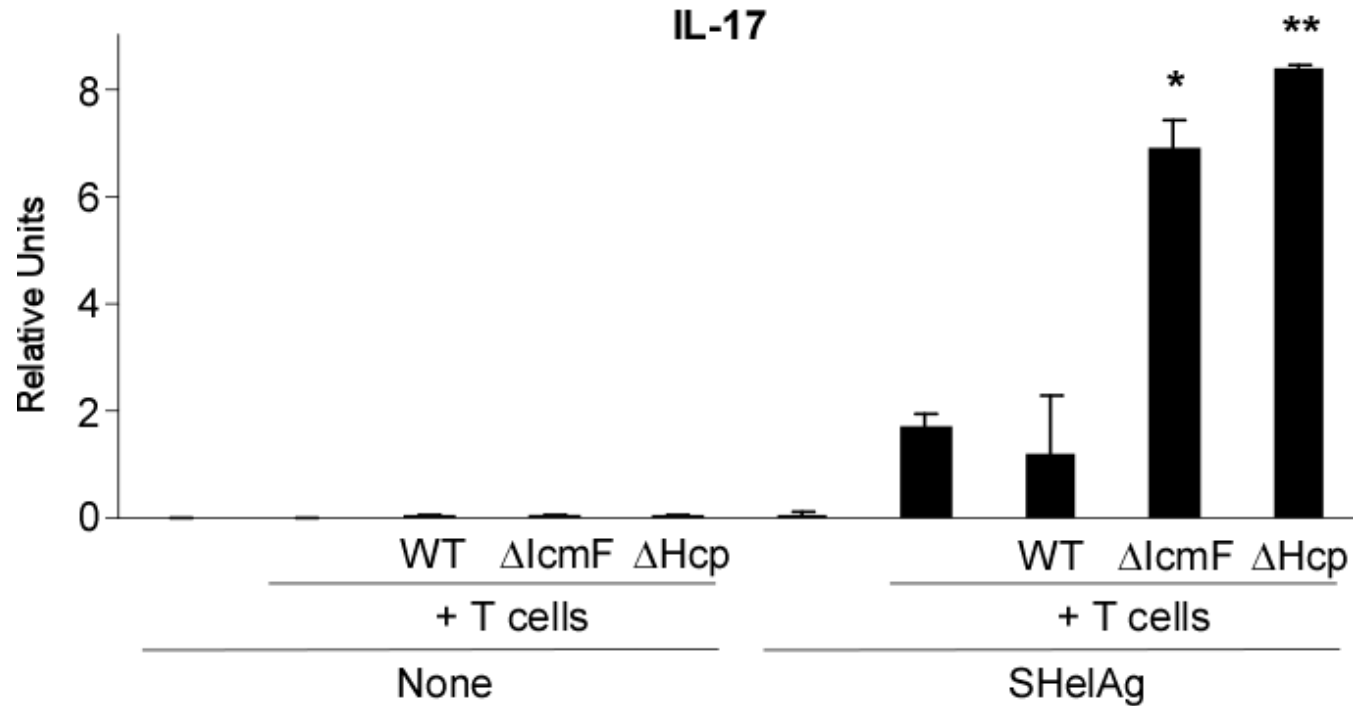
D

WT Fold Change	Gene	Gene Title
-1.52	Nfkb1	Nfkb1, p105
-2.07	Tlr4	Toll-like receptor 4
-3.23	Tlr3	Toll-like receptor 3
-4.05	IL17ra	Interleukin 17 receptor A
-4.13	Apc	Adenomatosis polyposis coli
-8.11	Ki67	Ki-67

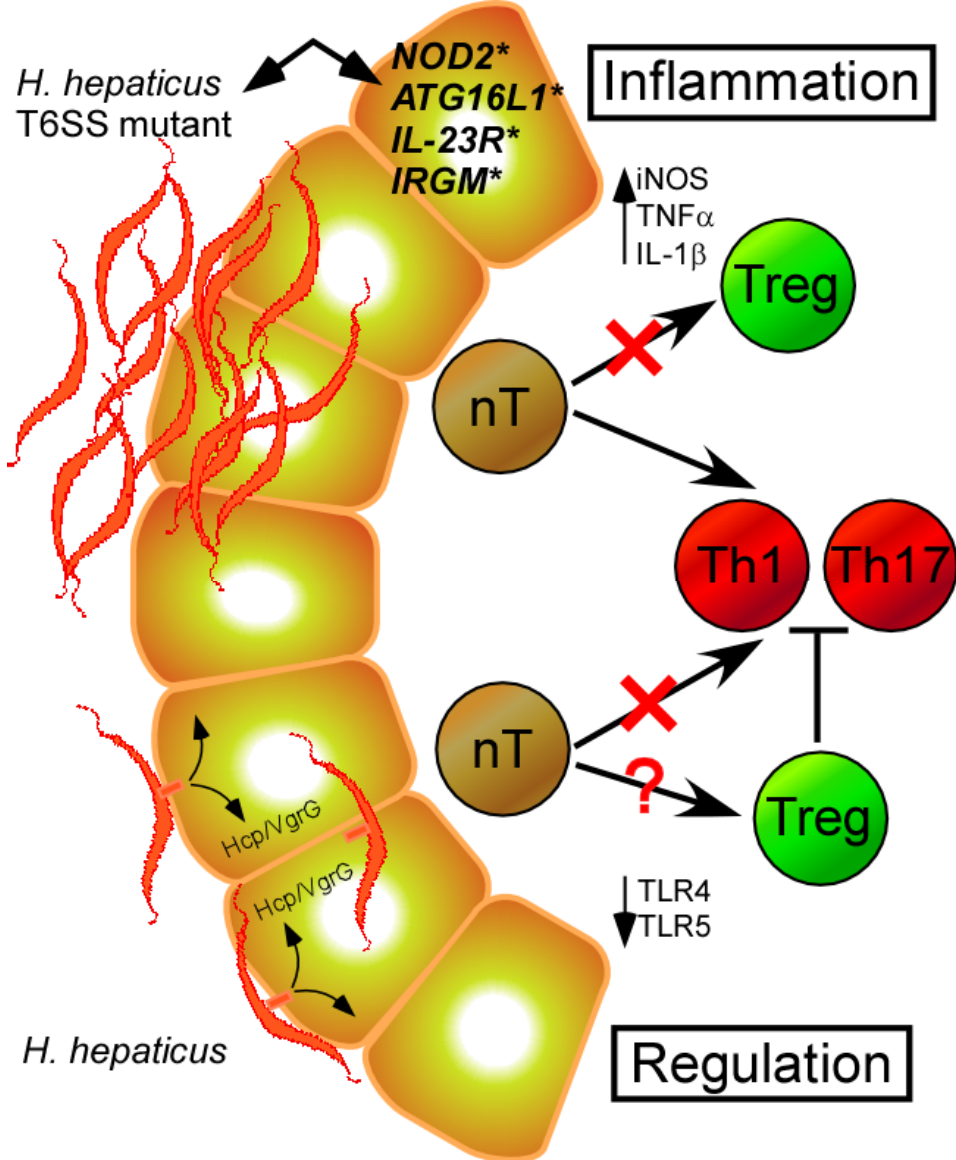
T6SS mutants of *H. hepaticus* induce increased inflammatory markers compared to wild-type bacteria



CD4+ T cells from T6SS mutants of *H. hepaticus* exhibit increased pro-inflammatory responses to intestinal epithelial cell-presented antigens

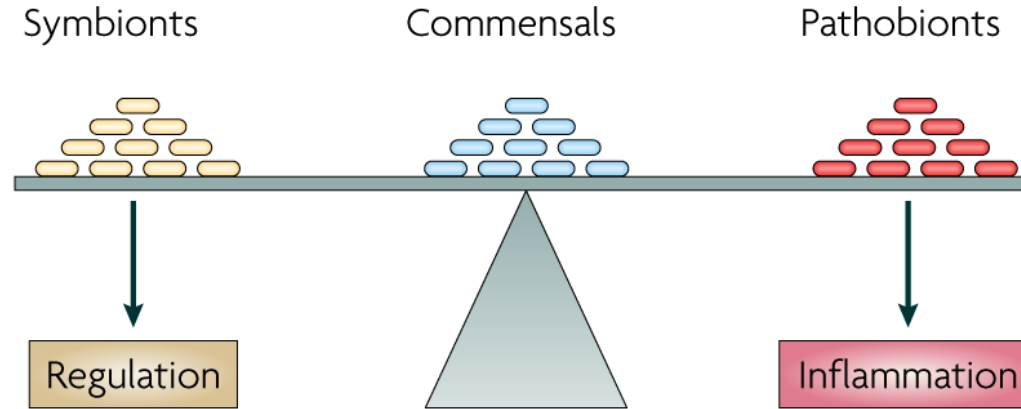


T6SS balances host colonization and intestinal inflammation

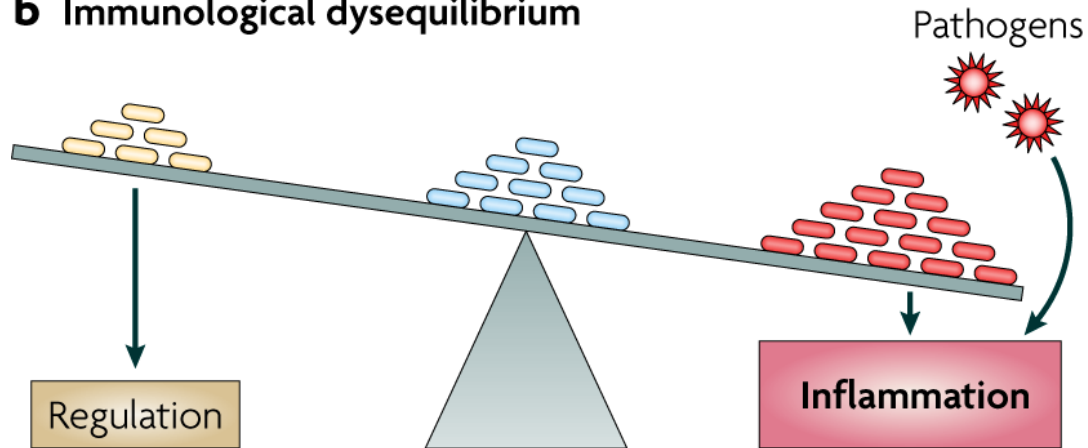


The interactions between harmful and beneficial bacteria may affect health and disease

a Immunological equilibrium

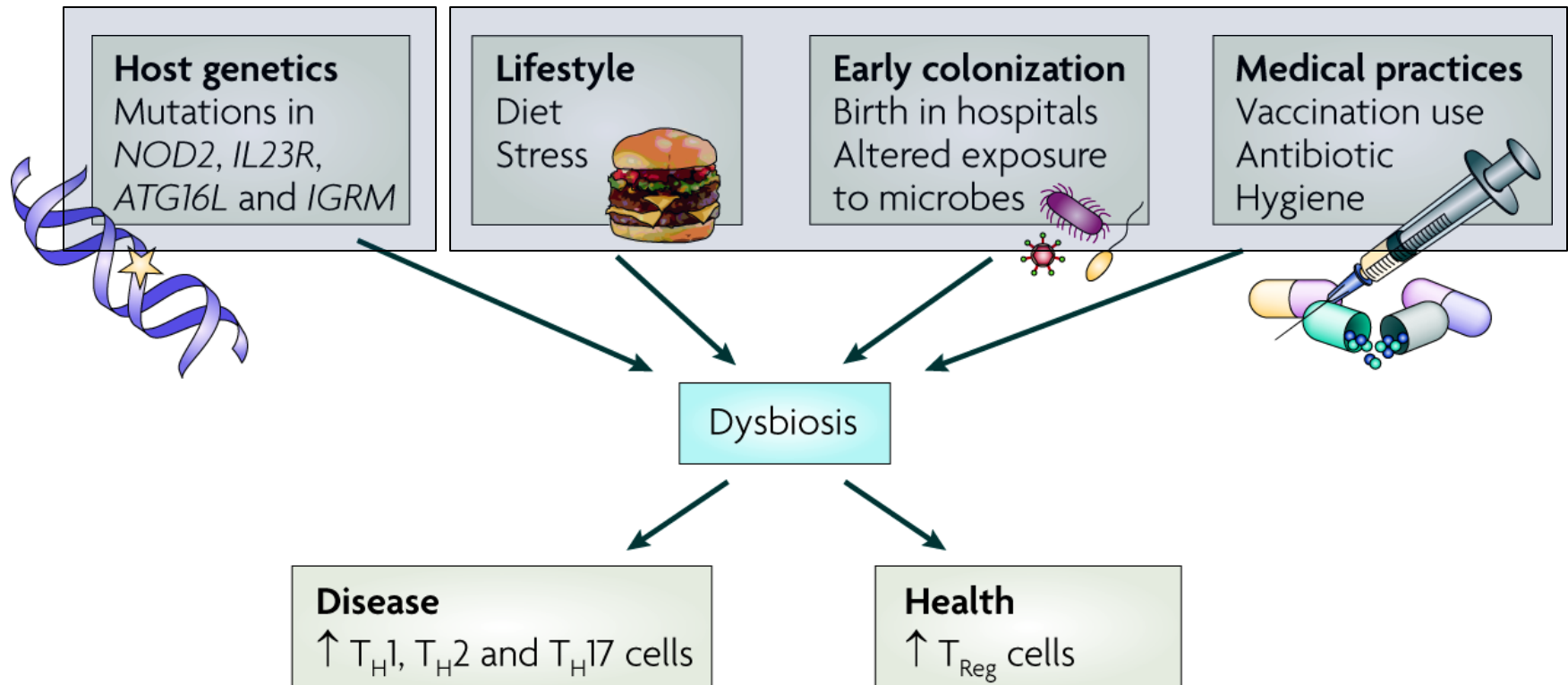


b Immunological dysequilibrium



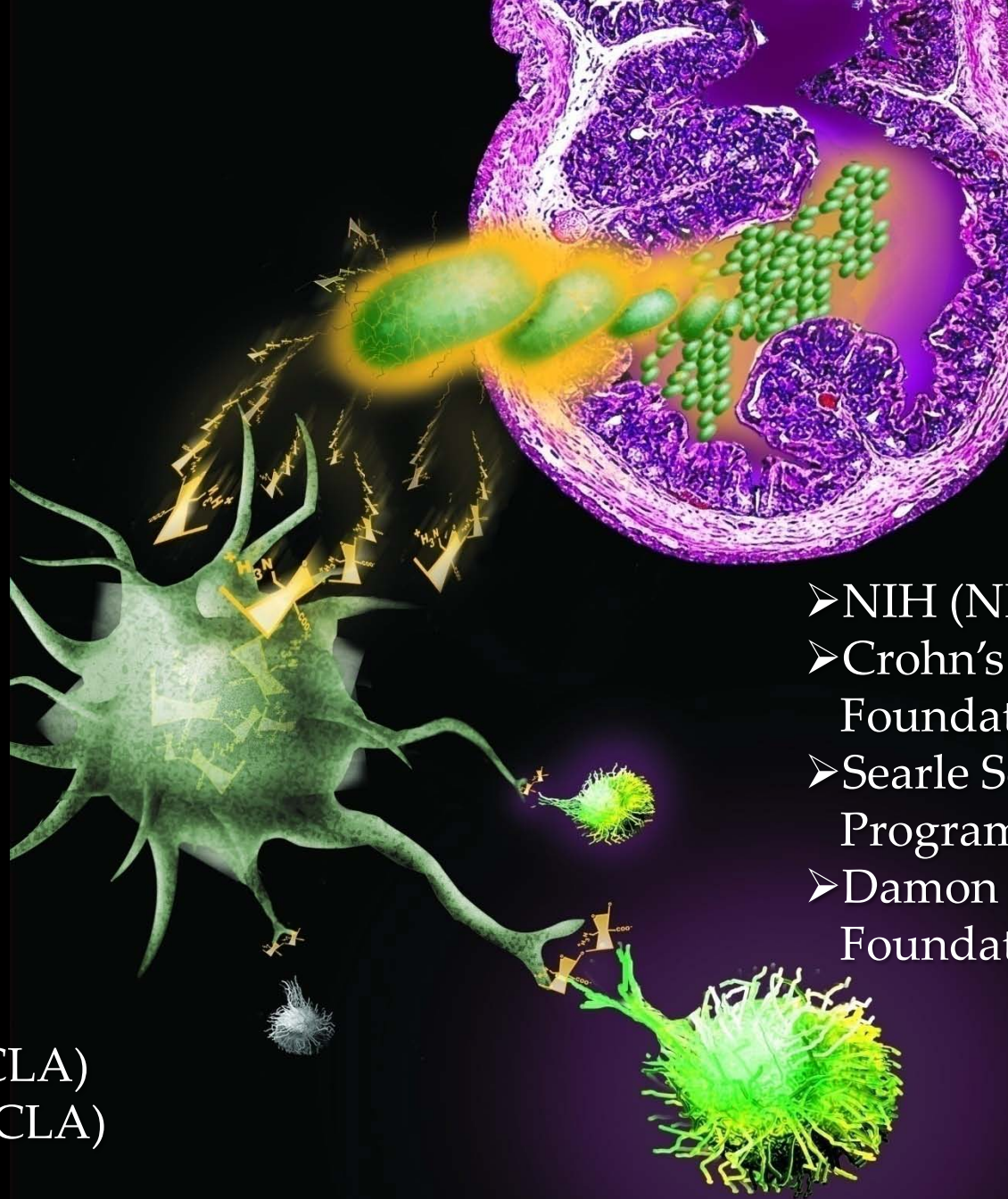
Potential causes of dysbiosis due to 'western' lifestyles its role in intestinal health and disease

Genetics and Environment



June Round
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Janet Chow
Yunkyung Lee
Sara McBride
Melanie Lee
Arya Khosravi
Vivian Yang
Gloria Tran
Taren Johnson
Rachel Galemidi
Haiqing Tang

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- Crohn's and Colitis Foundation
- Searle Scholars Program
- Damon Runyon Foundation