



# Modeling Immunity to Enteric Pathogens



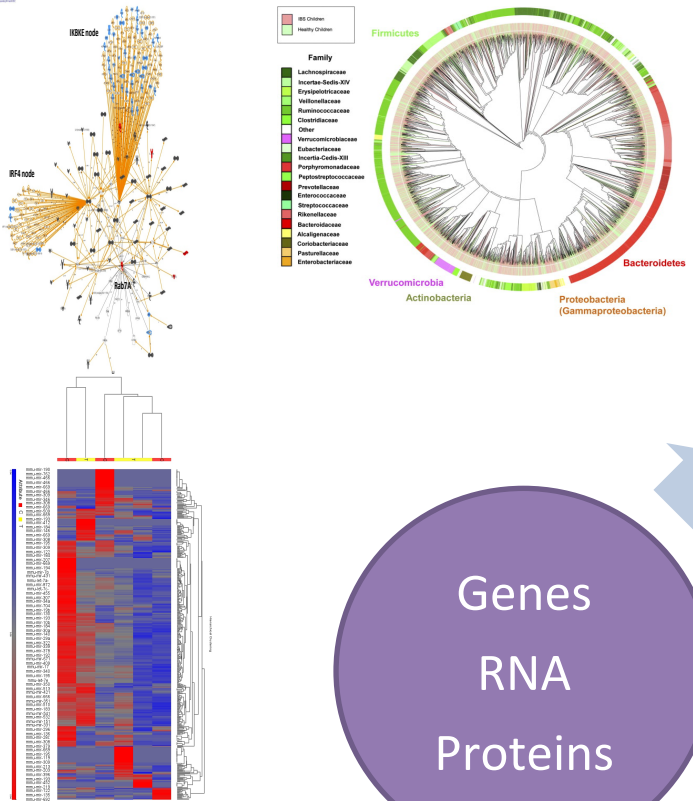
Josep Bassaganya-Riera DVM, PhD

Nutritional Immunology & Molecular Medicine Lab.

Virginia Bioinformatics Institute

Modeling Immunity for Biodefense Program





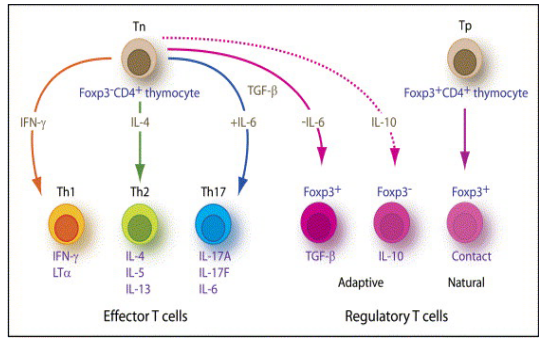
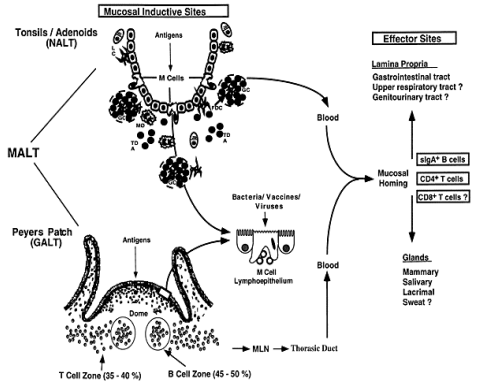
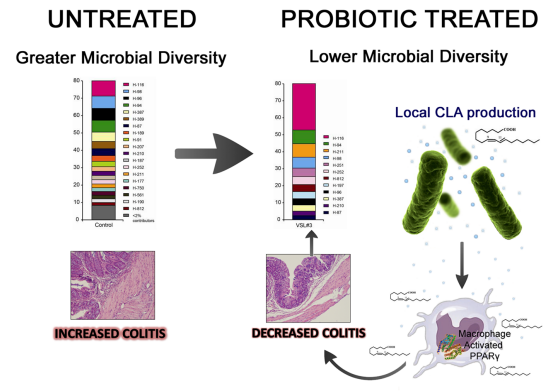
# Microbiome

# Genes RNA Proteins

# Health vs. Disease

# Diet

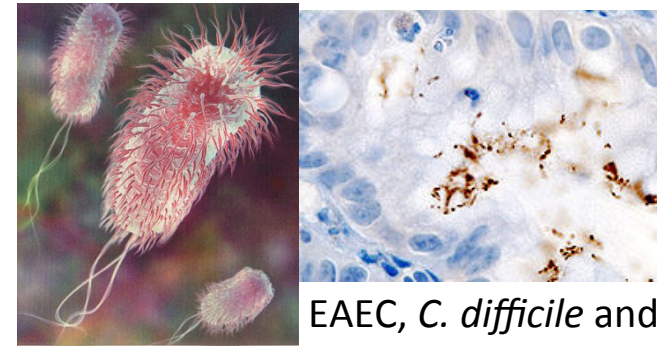
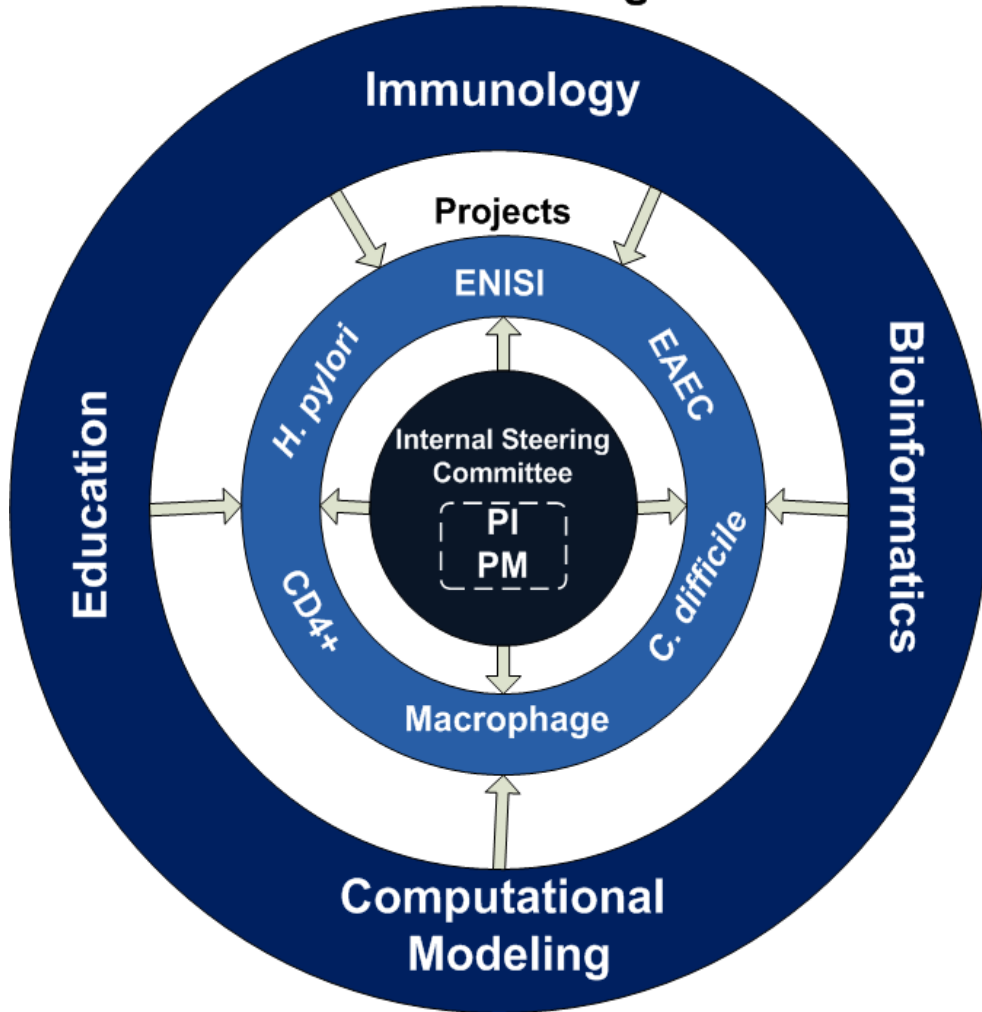
# Inflammation & Immunity



# MIEP Program Goals

- Elucidate mechanisms of action underlying immune responses to gut pathogens
- Create predictive computational/mathematical models of gut immunity
- Disseminate computational models of the gut mucosal immune system
- Develop more efficacious vaccines and immunotherapeutics

# Center for Modeling Immunity to Enteric Pathogens



EAEC, *C. difficile* and *H. pylori*

\$10.6M, 30 researchers, 3 Institutions

## Virginia Bioinformatics Institute

- Nutritional Immunology & Molecular Medicine Lab
- Network Dynamics and Simulation Science Lab

## University of Virginia

- Center for Global Health

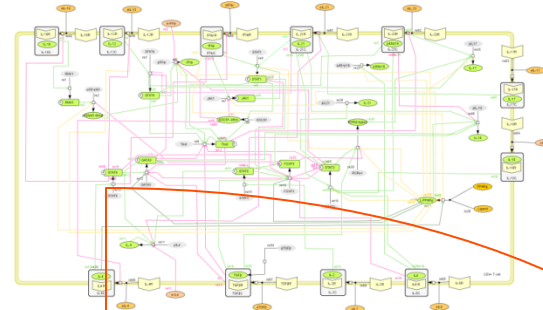
## Caprion Proteomics

[www.modelingimmunity.org](http://www.modelingimmunity.org)

# Computational Immunology



Literature & data mining



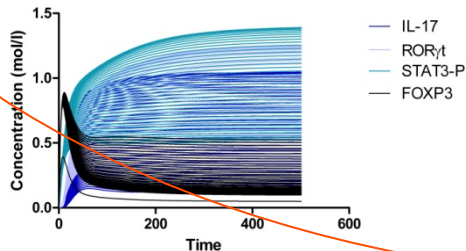
The Network Model



REFINEMENT



*In vivo* hypothesis testing



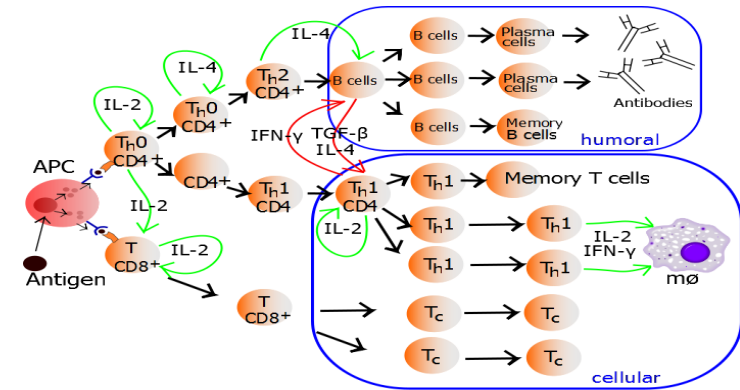
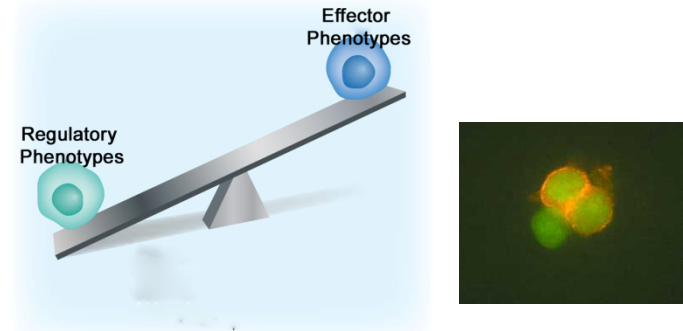
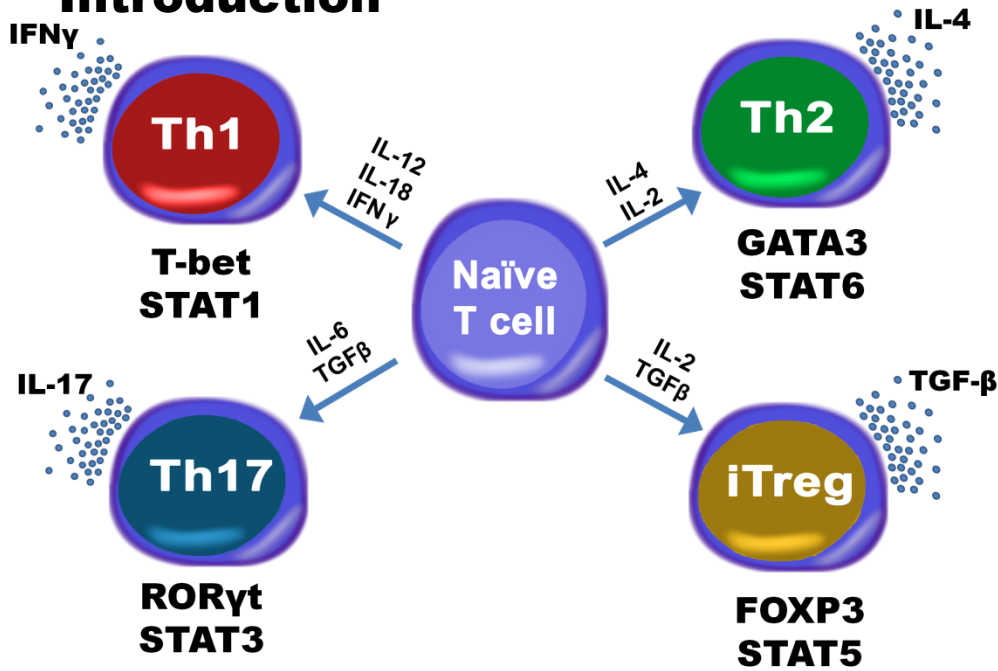
*In silico* experiments  
Hypothesis generation



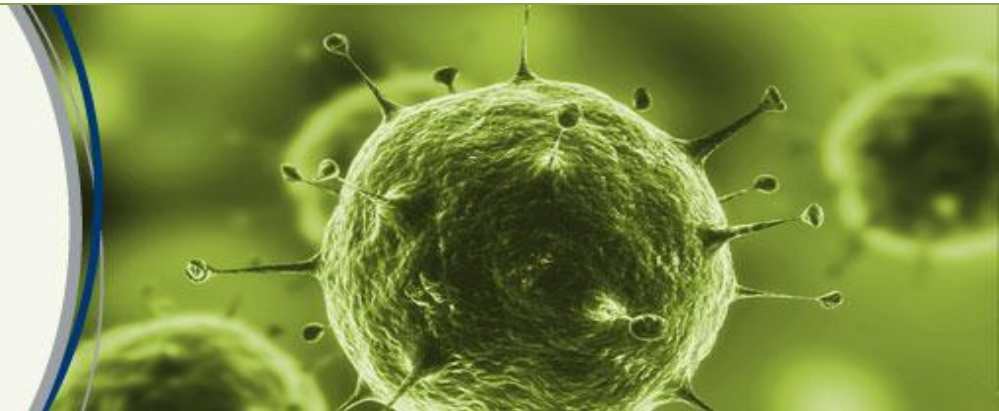
Modeling tools

# The CD4+ T cell computational model

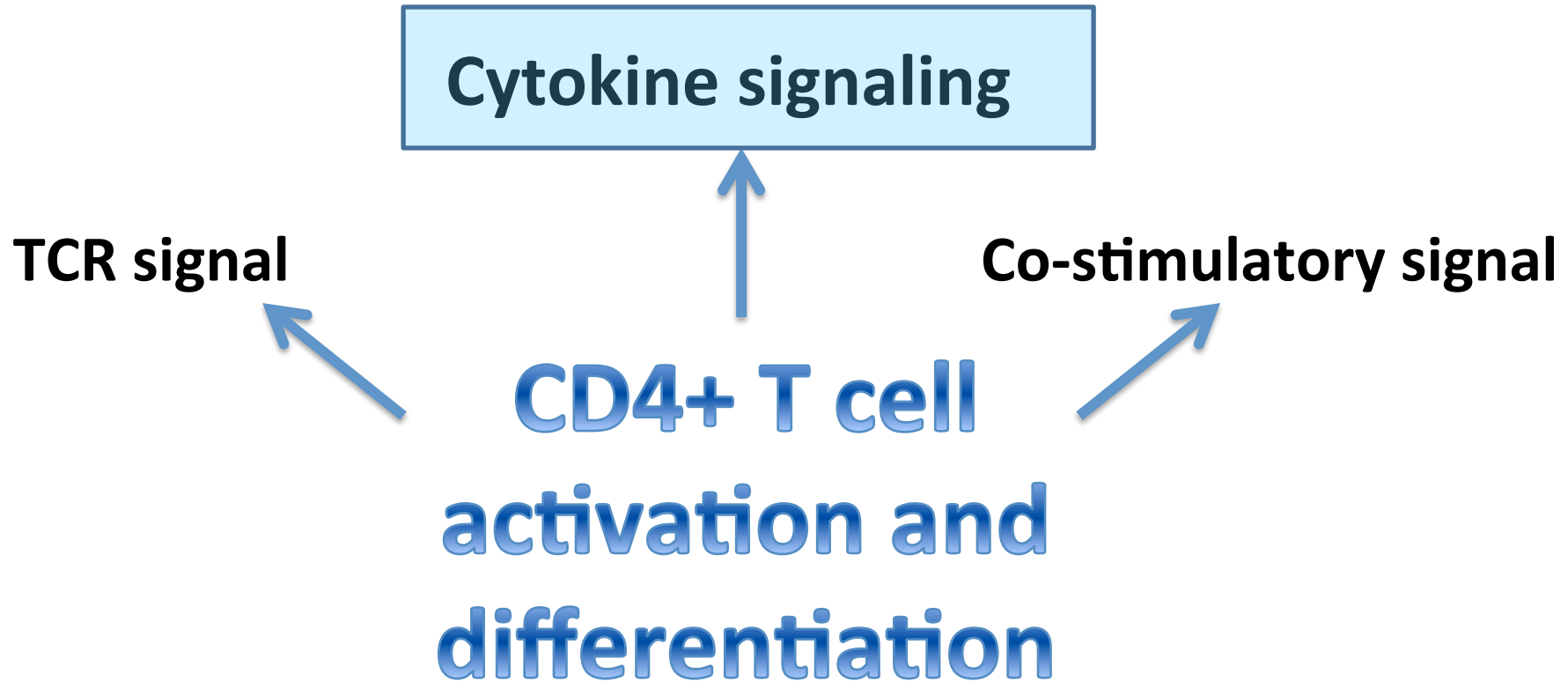
## Introduction

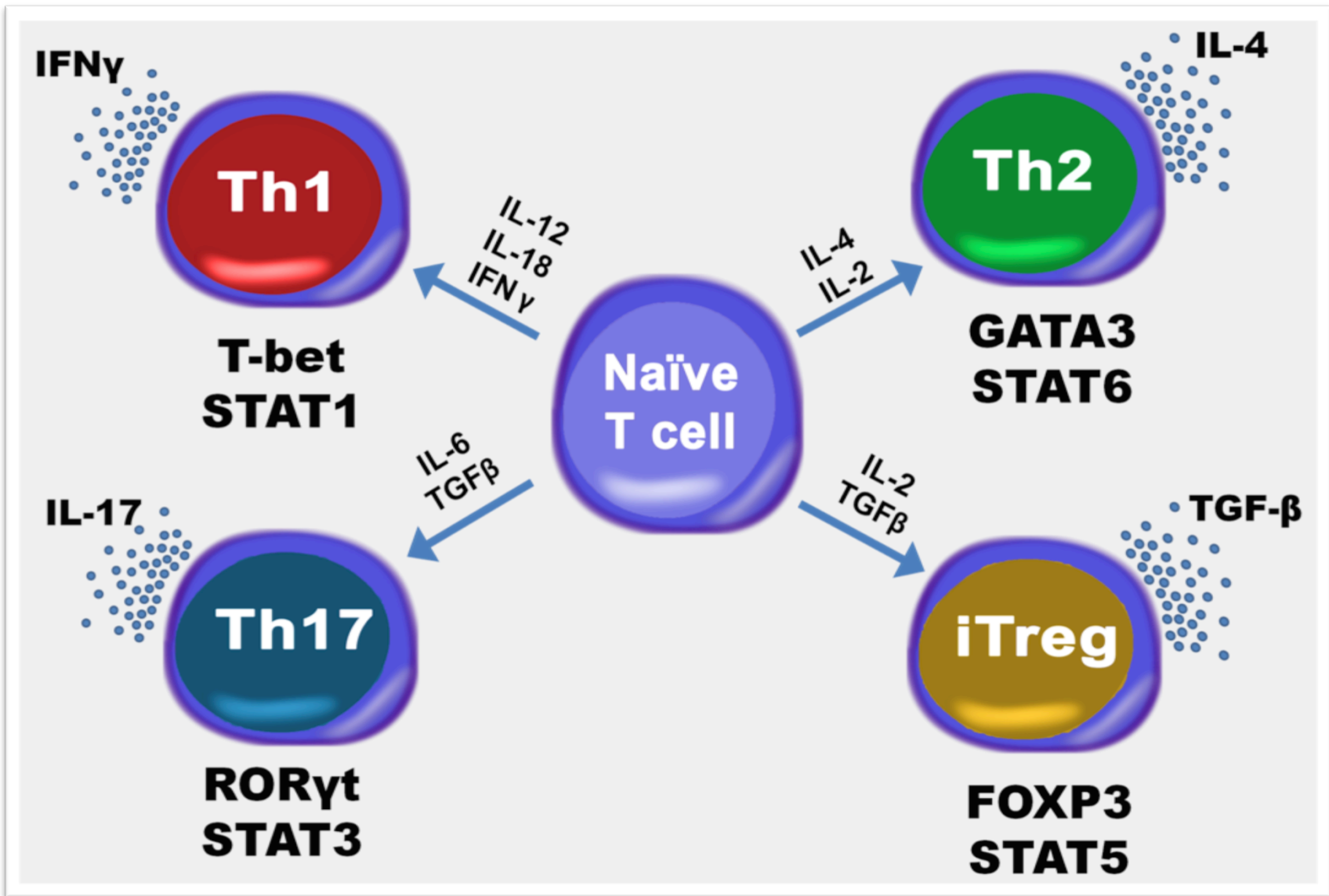


CD4+ T cells orchestrate the specific adaptive immune response by secretion of cytokines and other soluble factors in the environment

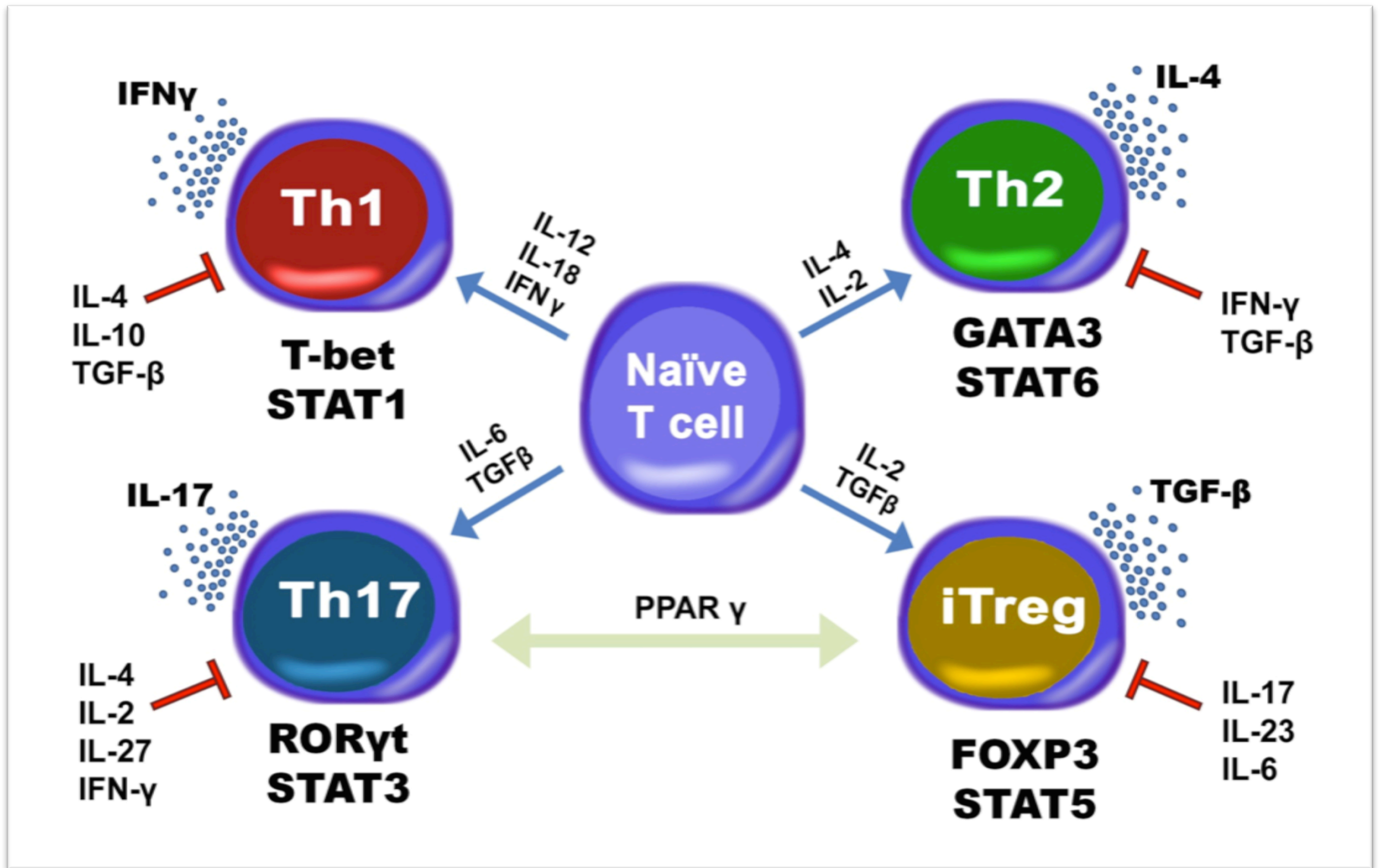


# CD4+ T CELL DIFFERENTIATION

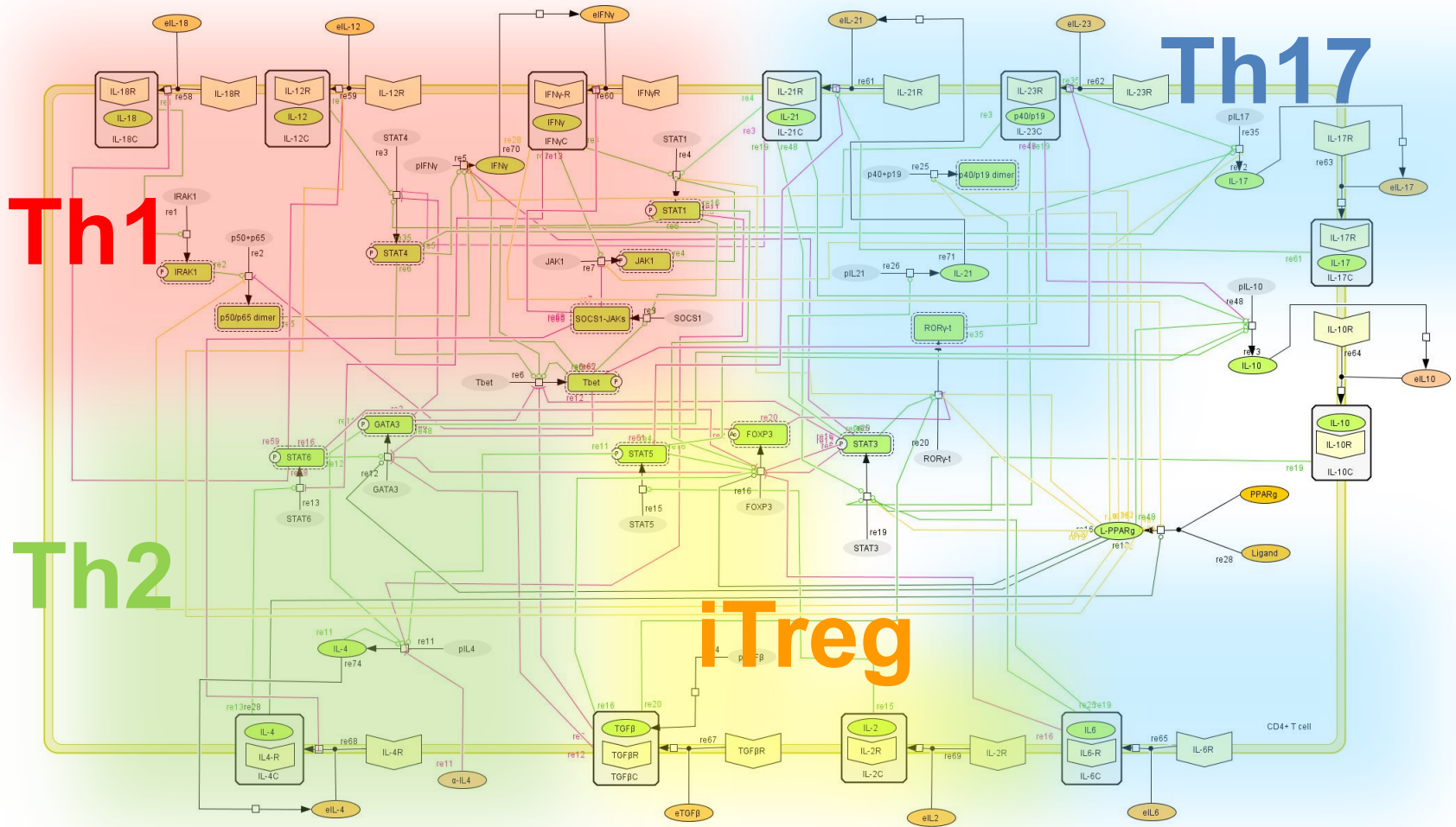






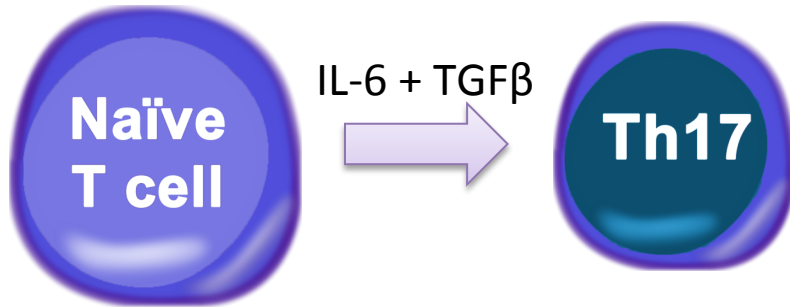


# CD4+ T CELL DIFFERENTIATION MODEL



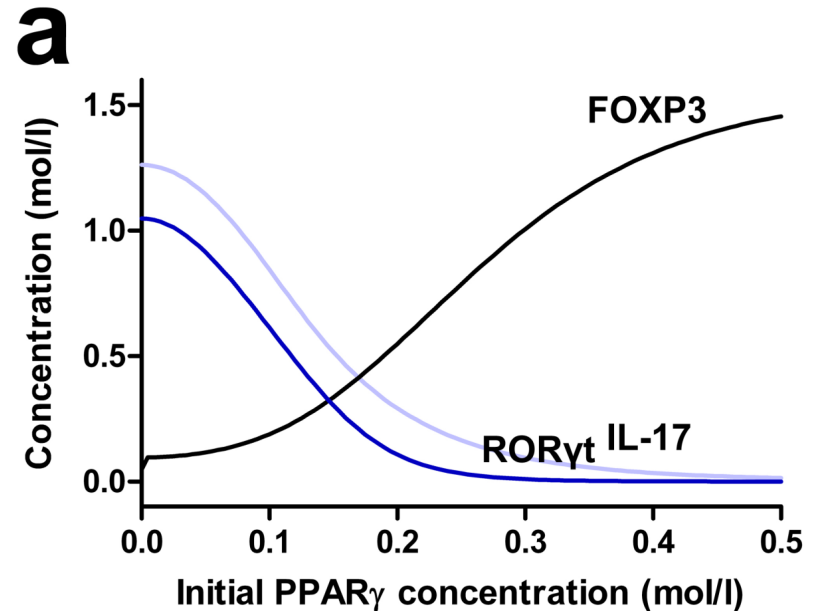
# *In silico* experimentation

GOAL: To determine whether activation of PPAR $\gamma$  influences differentiation in either a naïve or differentiated T cell



## Parameter SCAN:

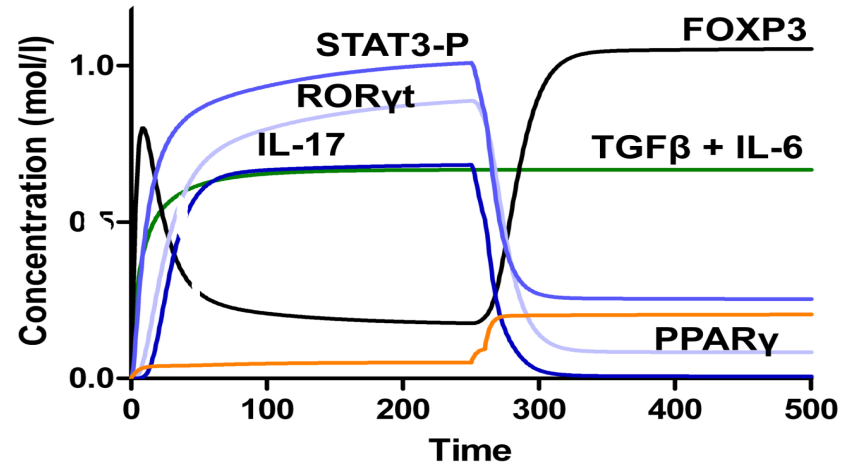
We are scanning the concentration of PPAR $\gamma$ , from 0 mol/l to 0.5 mol/l to determine the impact on the dynamics of the system



### TIME-COURSE:

We differentiate to Th17 and  
t=250h we activate  
PPAR $\gamma$  in the system.

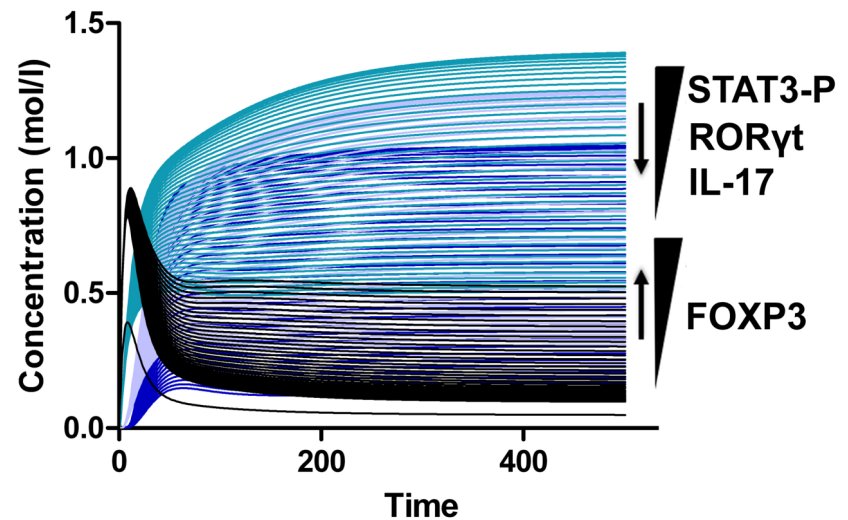
**b**



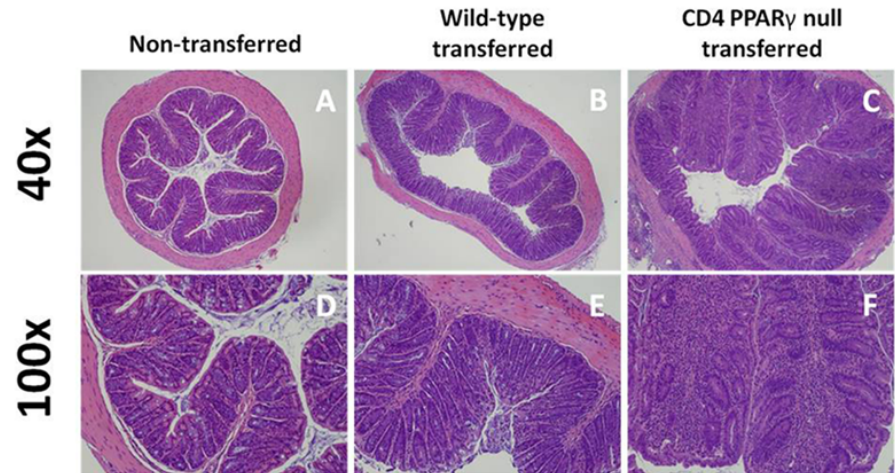
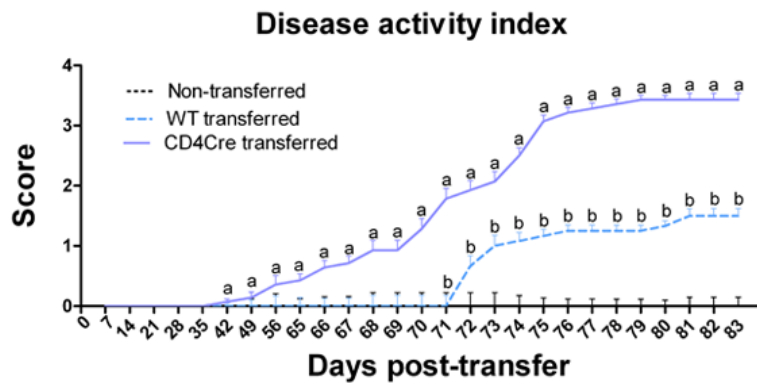
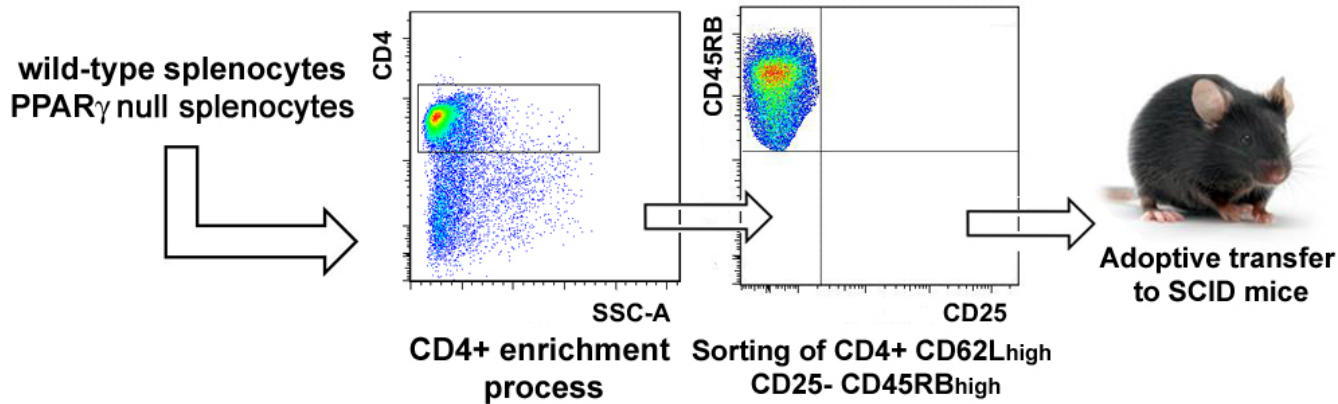
### TIME-COURSE & Parameter scan together:

We differentiate to Th17 and we  
run several time-courses  
together, each one with an  
increased concentration of PPAR $\gamma$

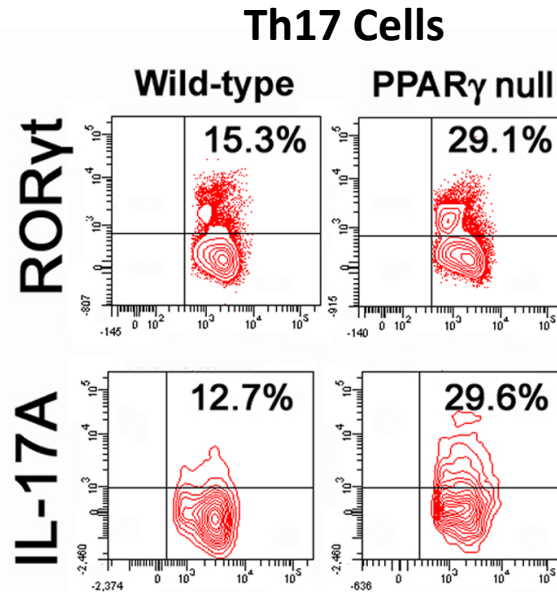
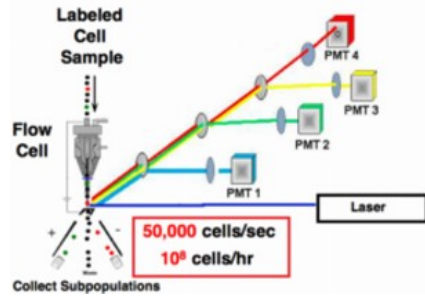
**c**



# IN VIVO EXPERIMENTATION

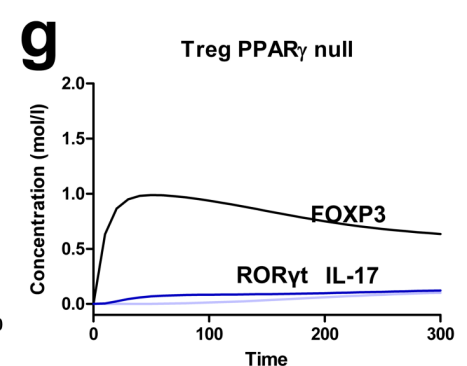
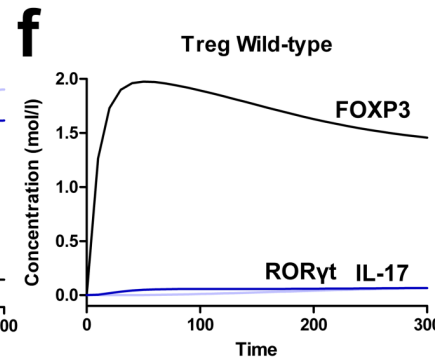
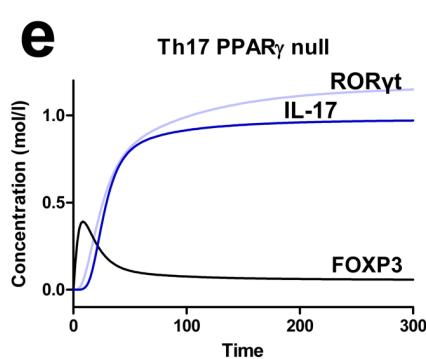
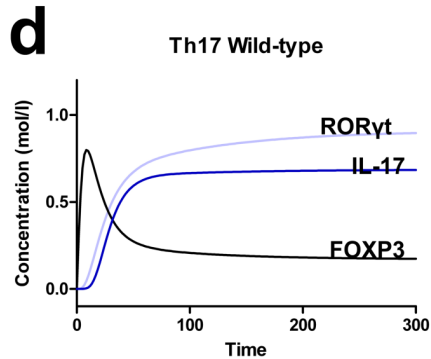
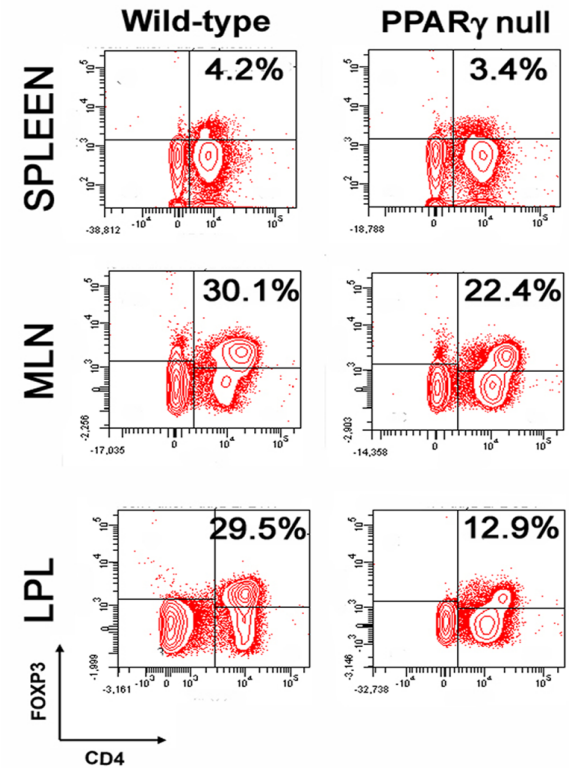


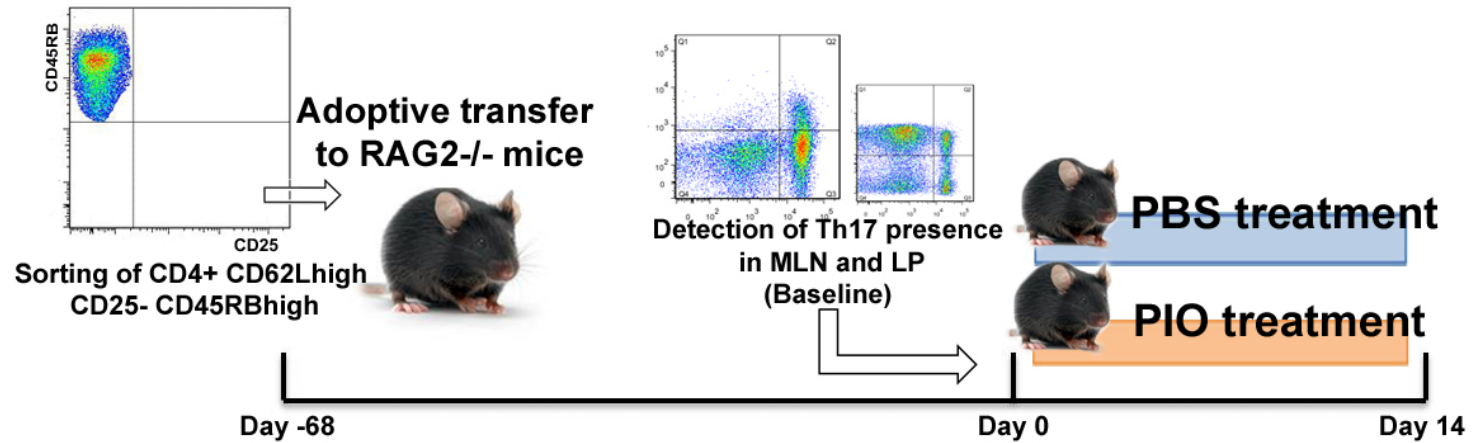
# IN VIVO EXPERIMENTATION



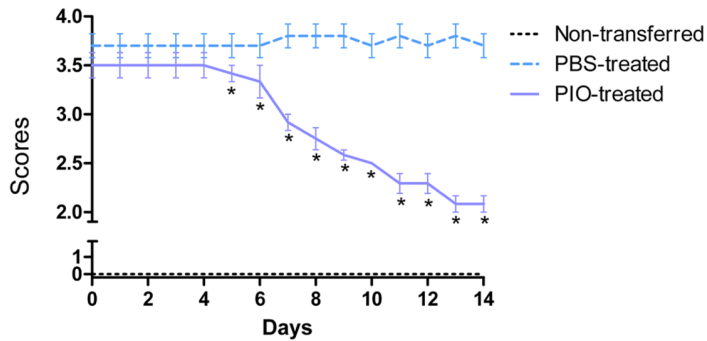
## Treg Cells

### FOXP3 expression

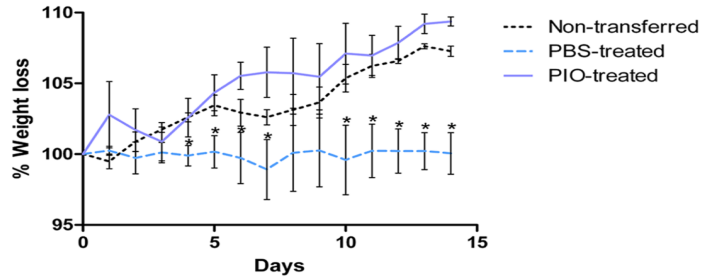




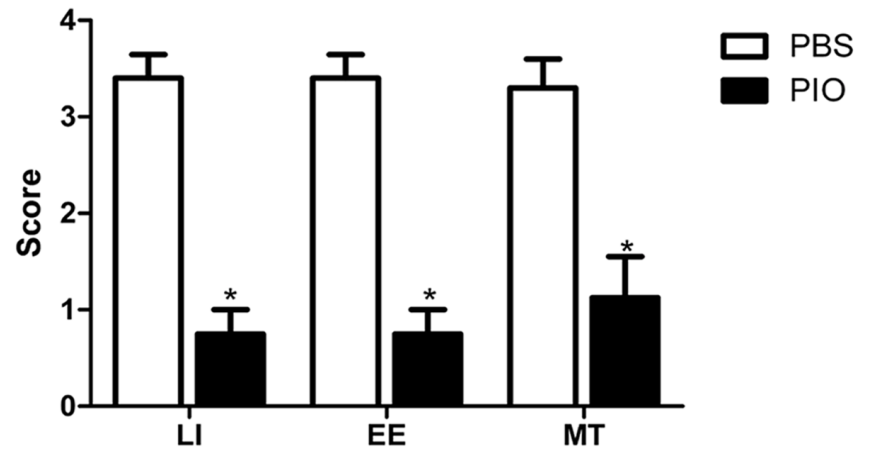
### Disease activity index



### Percentage of Weight Loss

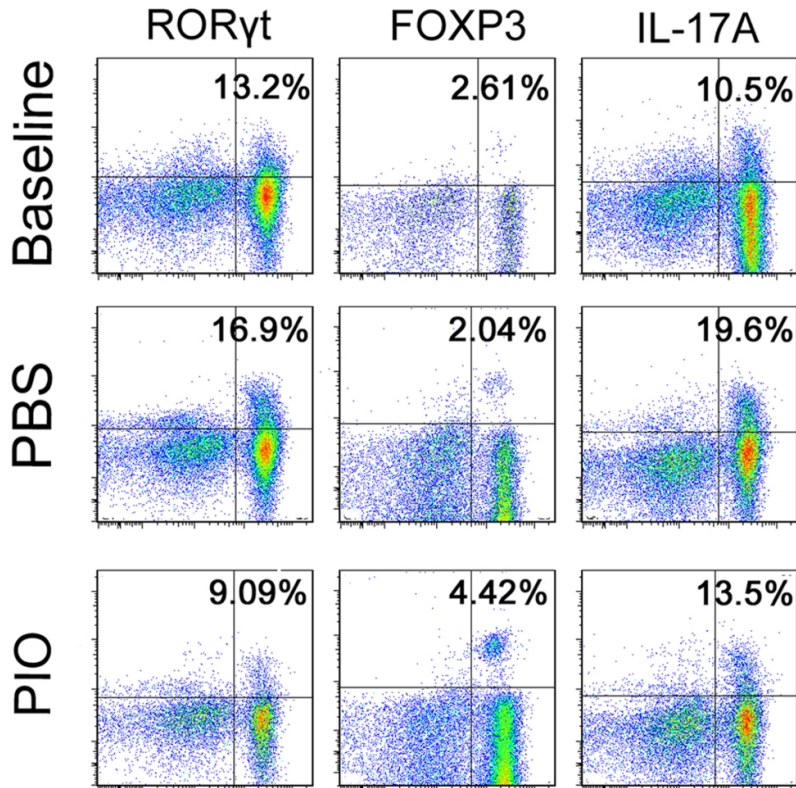


### Colonic Histopathology

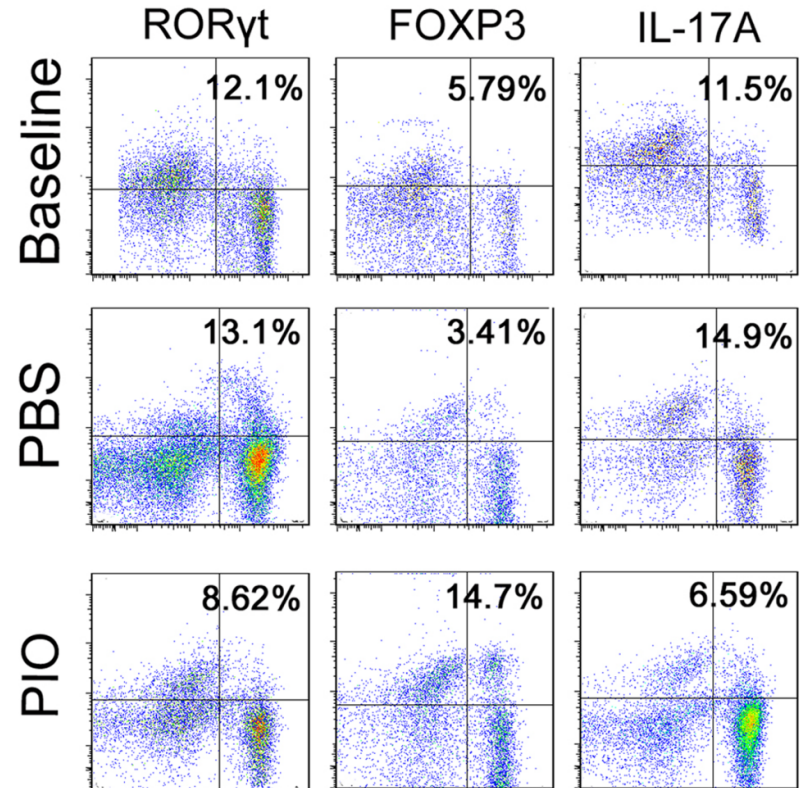


# IN VIVO EXPERIMENTATION

## Mesenteric Lymph nodes (MLN)



## Lamina Propria Lymphocytes (LPL)



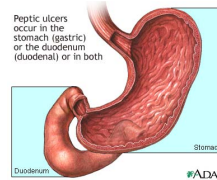


# Helicobacter pylori

Gram negative bacterium

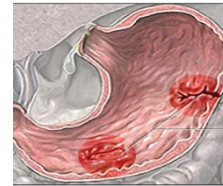


85 %



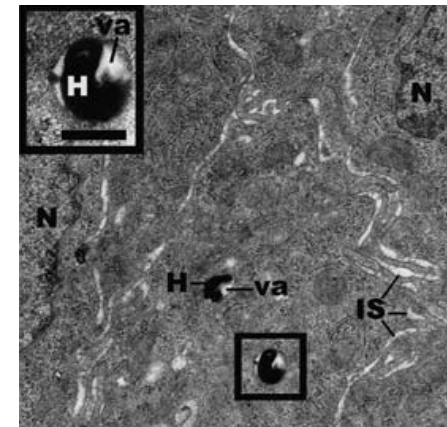
Asymptomatic infection

15 %



Peptic ulcer  
MALT lymphoma  
Adenocarcinoma

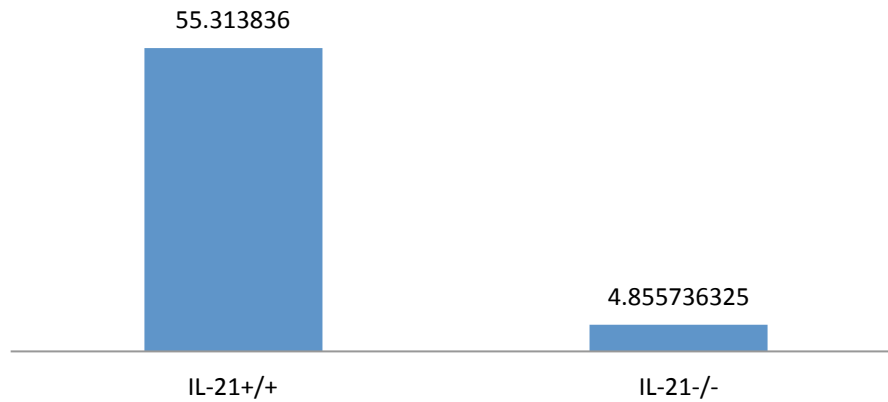
- Main pathogenicity factor: CagA (cytotoxin-associated gene A)
- Evidence that *H. pylori* is a facultative intracellular organism
- *H. pylori* was found in human gastric epithelium from infected patients
- Reported Th1 response involving IFN- $\gamma$  and IL-12



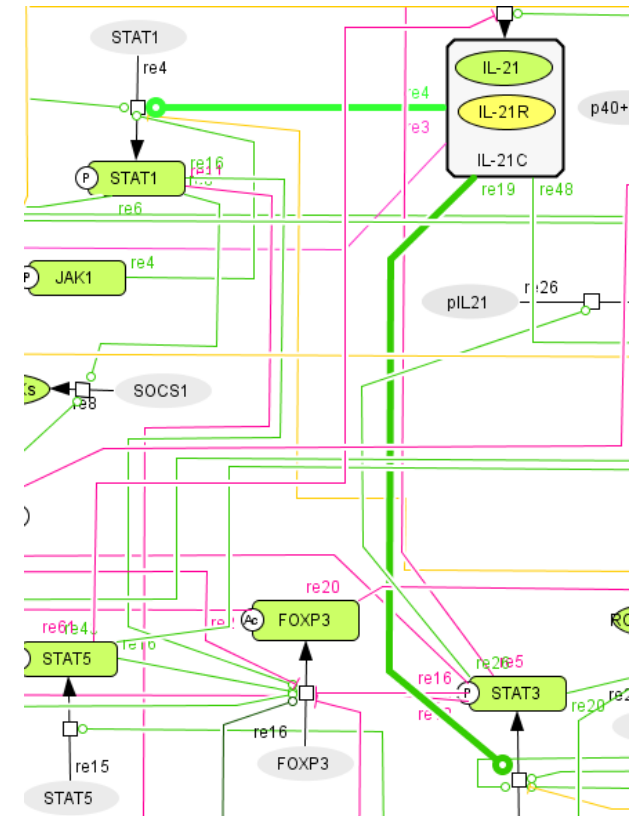
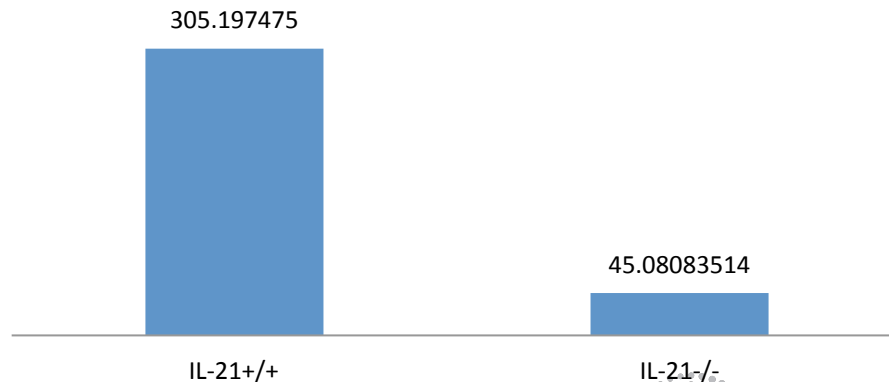
Oezbek, 2010 J Clin Gastroenterol

# ROLE OF IL-21 IN DIFFERENTIATION

RealTime data for IFN $\gamma$  production after HP infection (3 months)

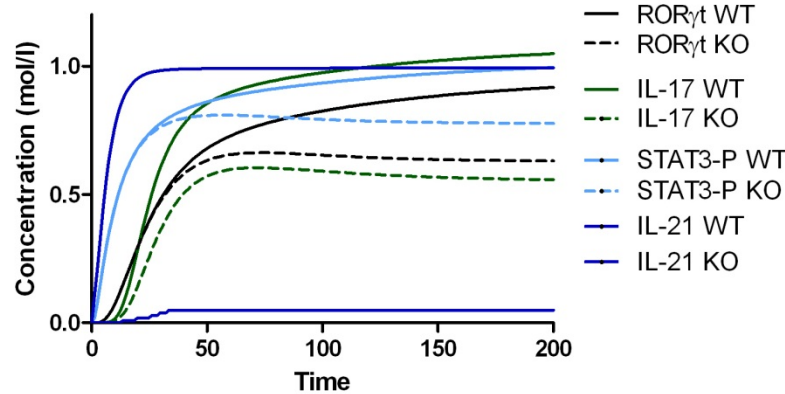


RealTime data for IL-17 production after HP infection (3 months)

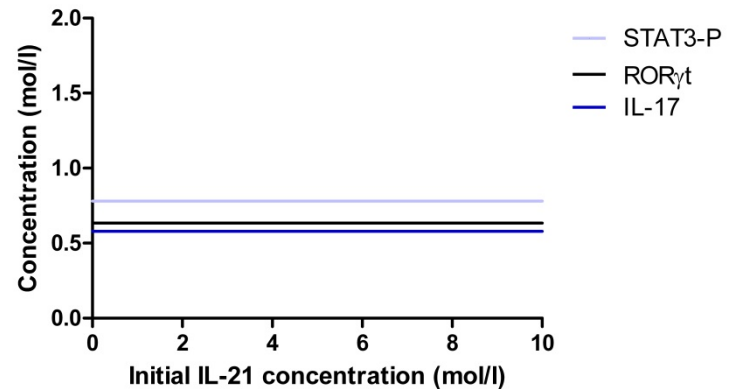
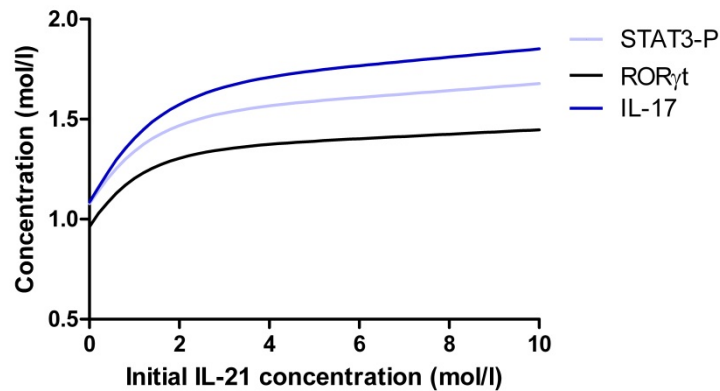
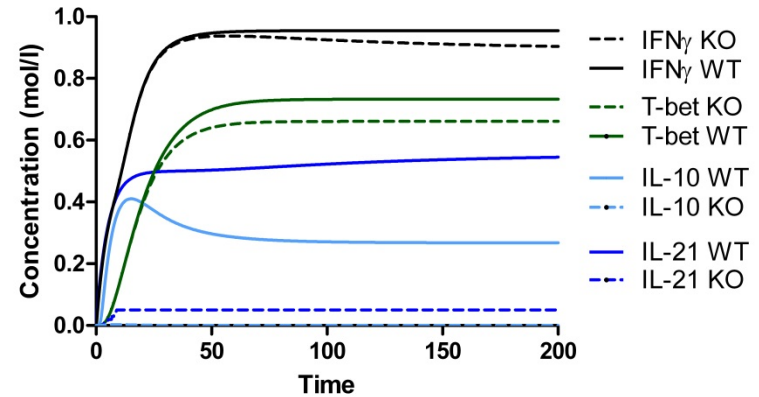


# IL-21 AND CD4+ T CELL DIFFERENTIATION

ROR $\gamma$ t, IL-17, IL-21 and STAT3 in Th17 system

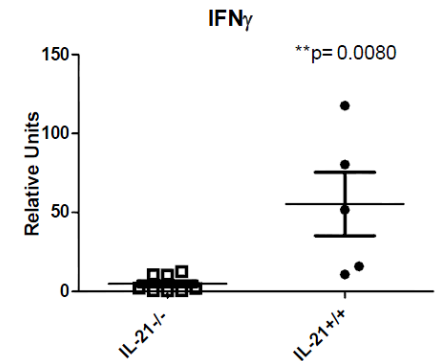
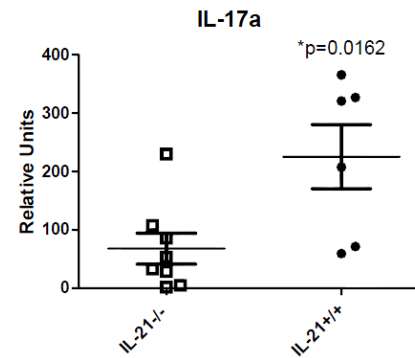
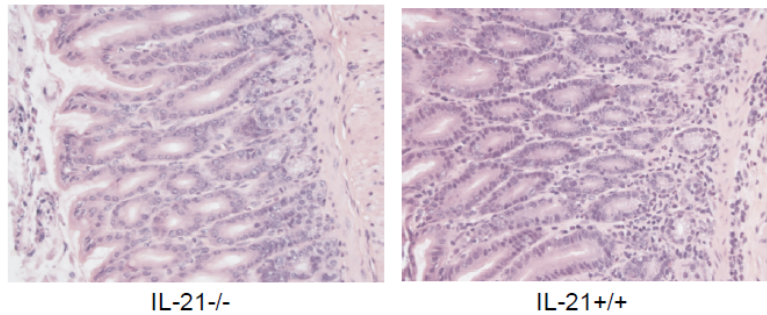


Th1 system simulation

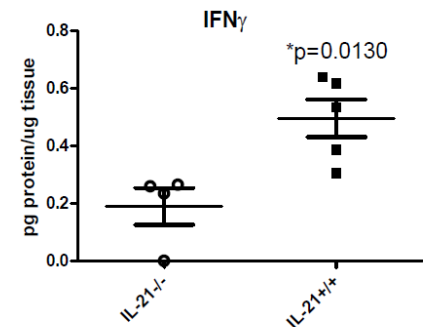
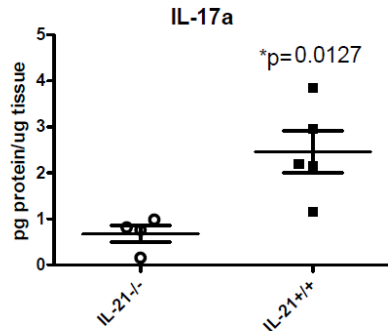
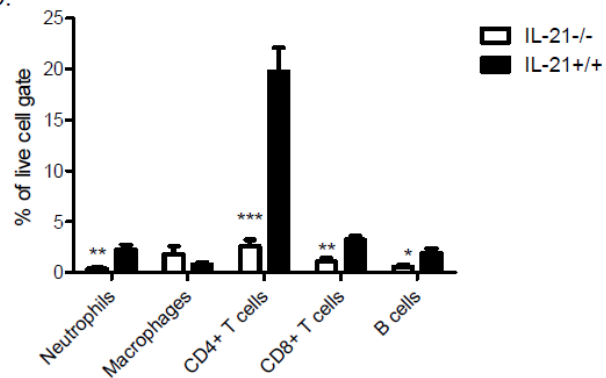


# IL-21 AND CD4+ T CELL DIFFERENTIATION

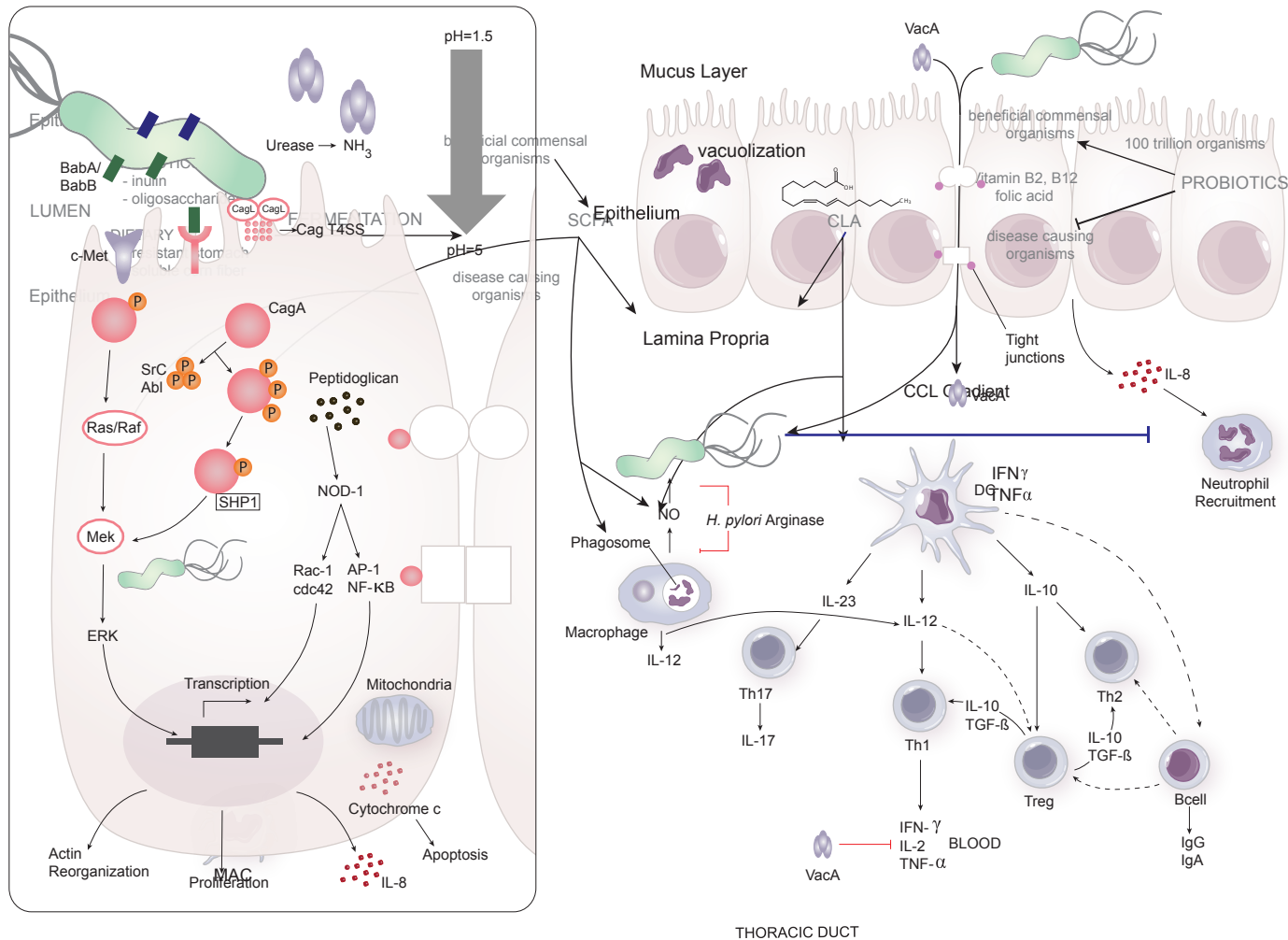
B.



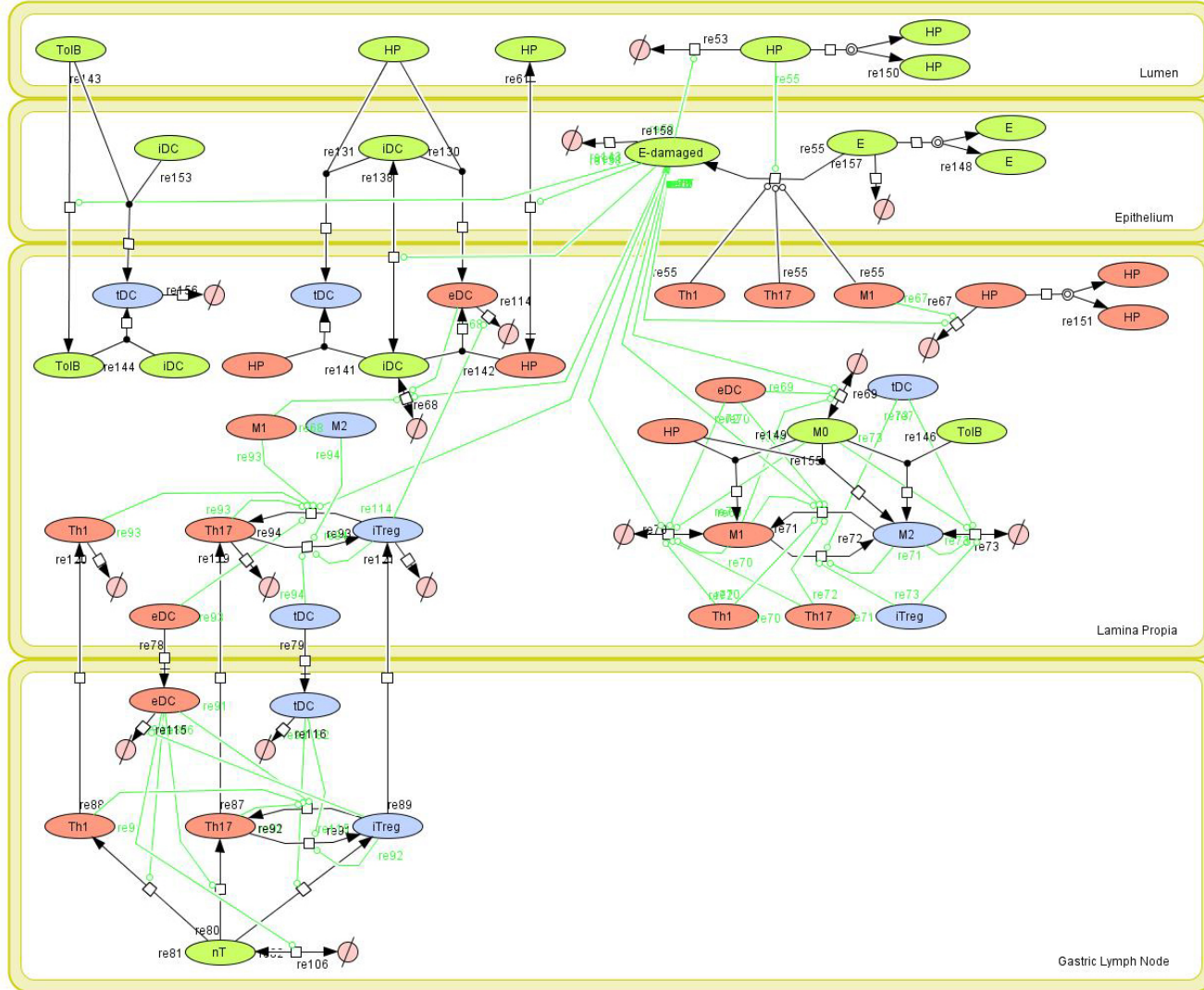
C.



# Host-*Helicobacter pylori* Interactions

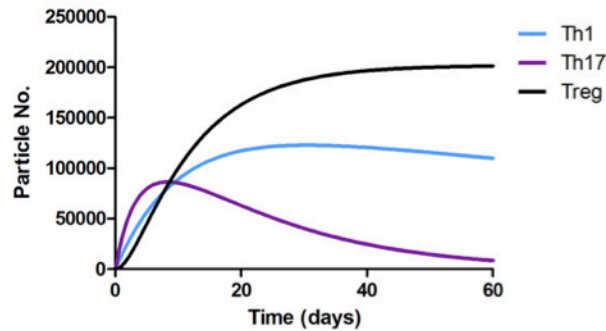


# Immune responses towards *H. pylori*



# Modeling Host Responses to *H. pylori*

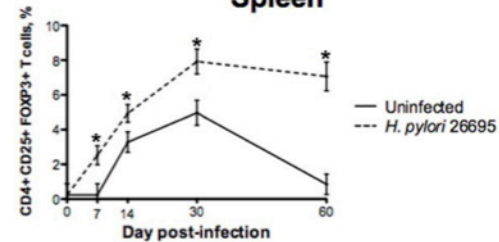
**A** CD4+ T cells in *H. pylori* infected system



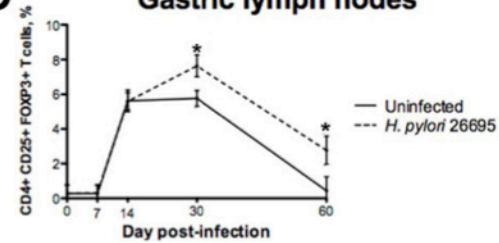
**B**

$$k_{eq} = \frac{n_{Treg} \cdot k \cdot eDC\{GLN\}}{n_{Treg} \cdot v_{max} + n_{Treg} \cdot k \cdot eDC\{GLN\}} \cdot \frac{1}{nT}$$

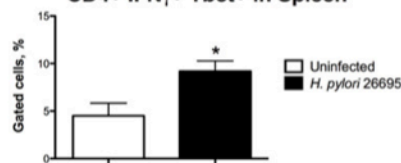
**C** Spleen



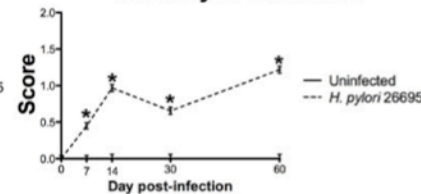
**D** Gastric lymph nodes



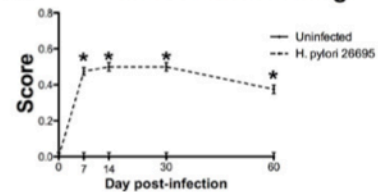
**E** CD4+ IFN $\gamma$ + Tbet+ in Spleen



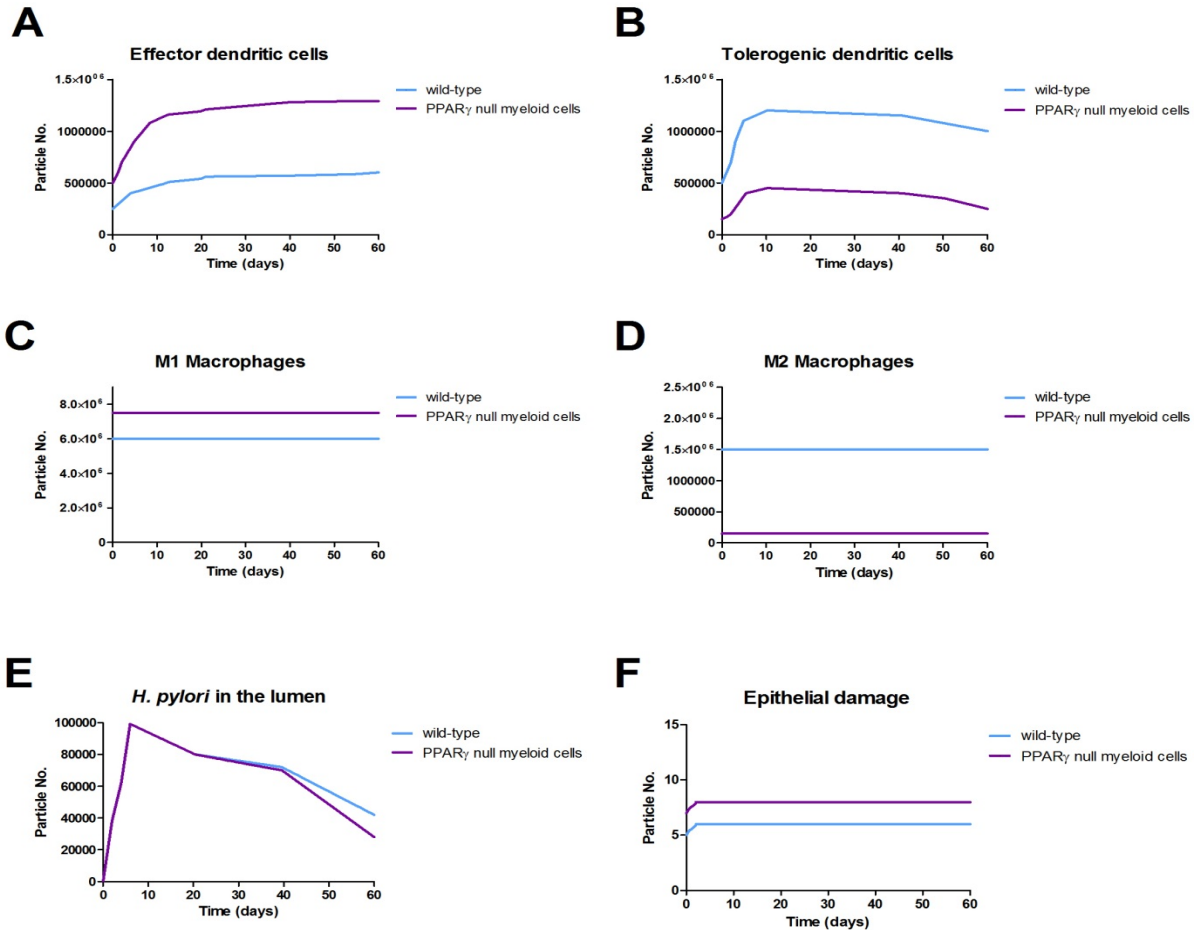
**F** Leukocytic Infiltration



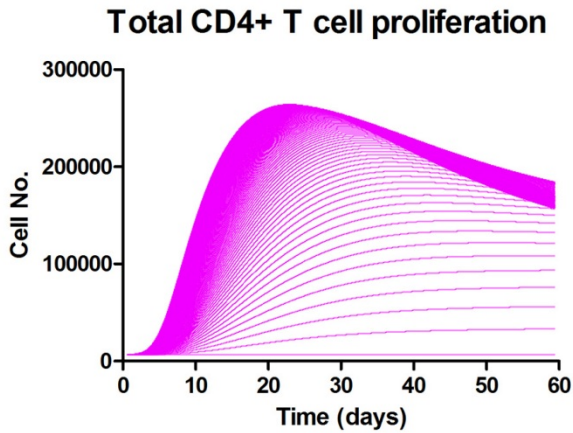
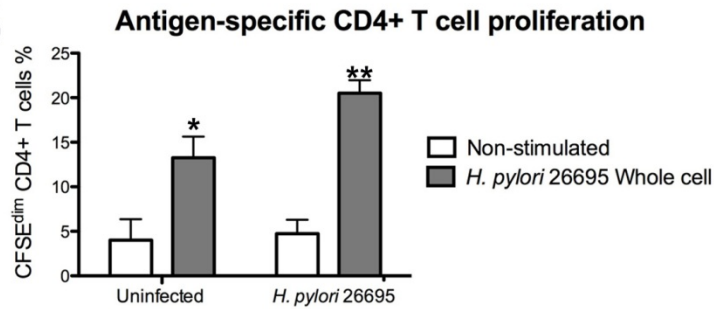
**G** Mucosal Thickening



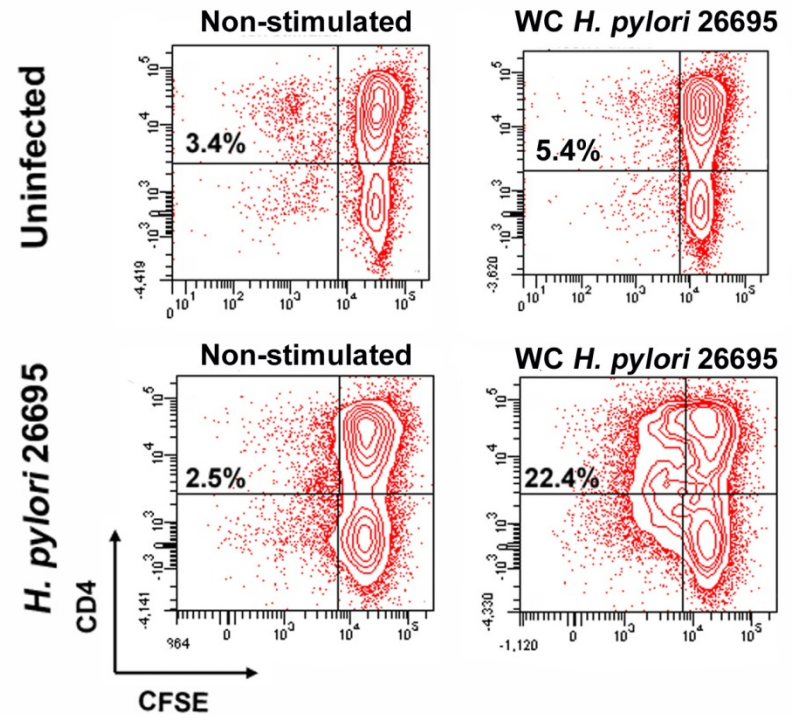
# *In silico* knockout Generation

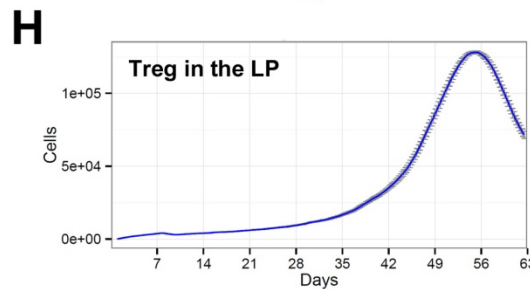
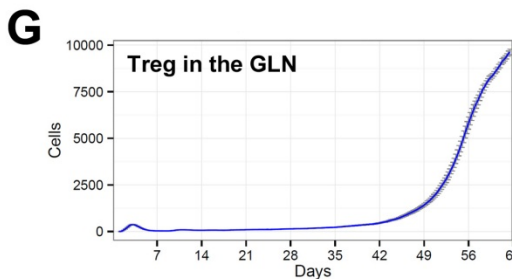
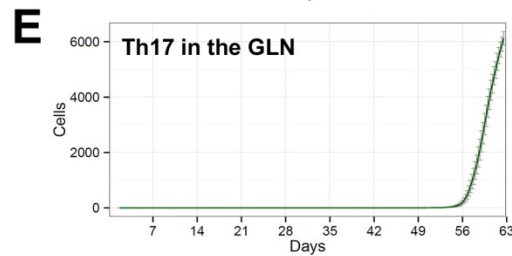
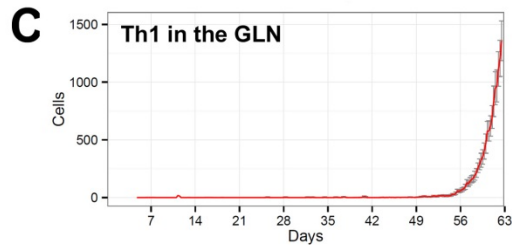
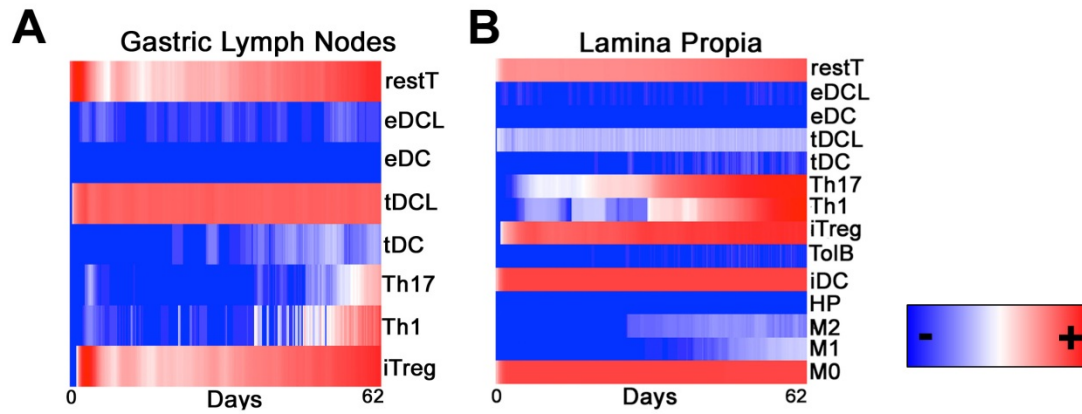




**A****B****C**

**Antigen-specific  
CD4+ T cell proliferation**

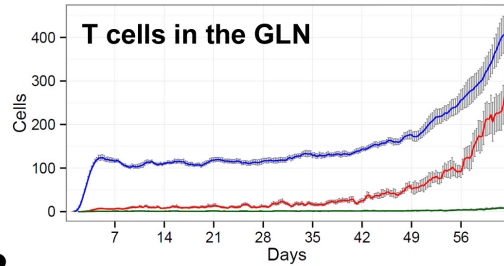
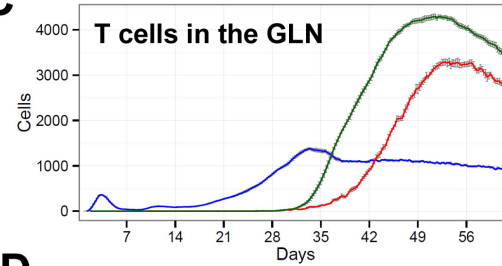
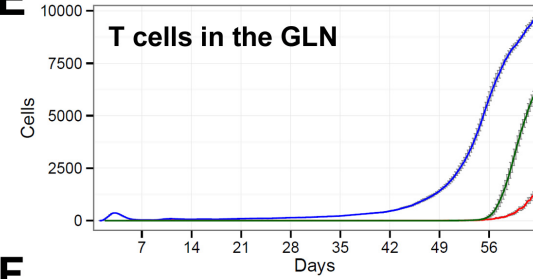
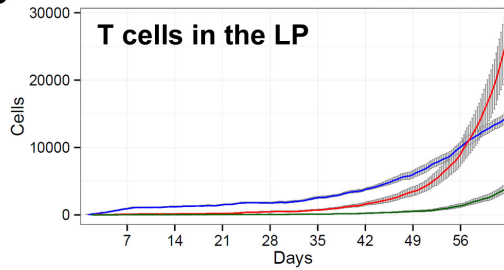
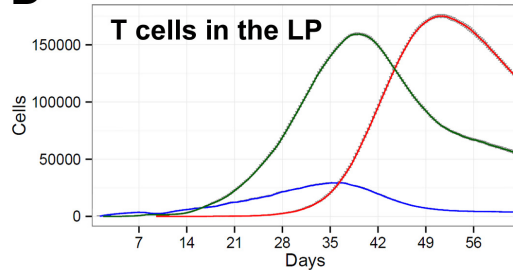
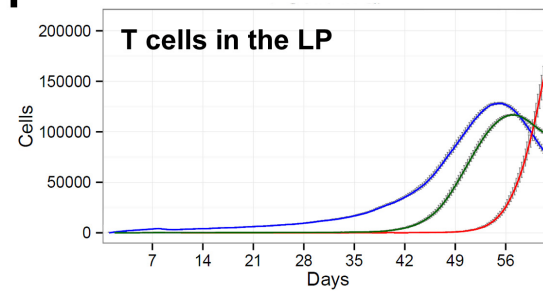




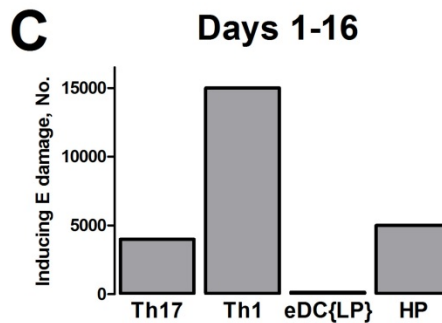
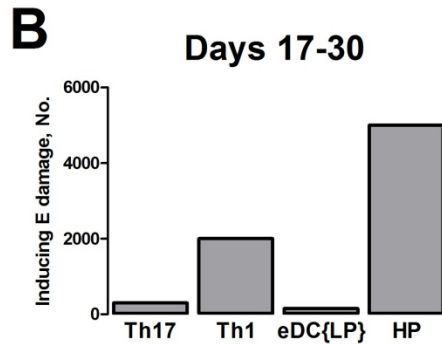
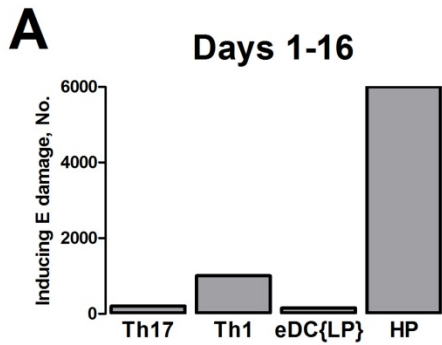
### Myeloid cell-specific PPAR $\gamma$ null

### T cell-specific PPAR $\gamma$ null

### Wild-type

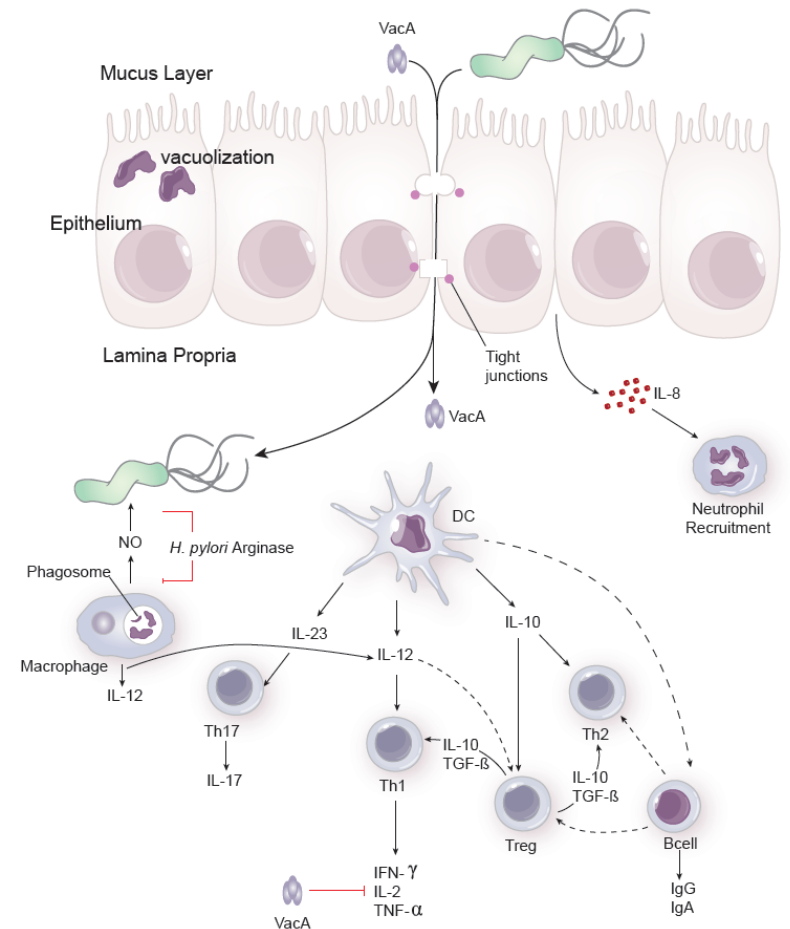
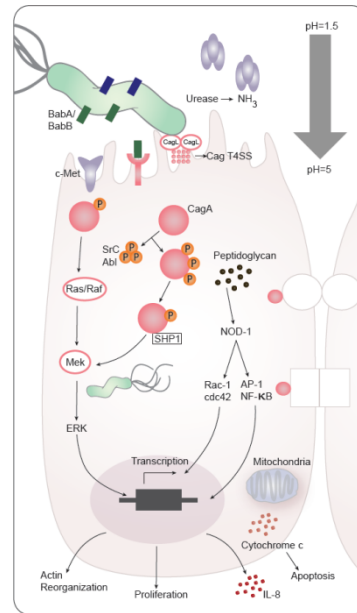
**A****C****E****B****D****F**

— Th1  
— Th17  
— Treg



### D

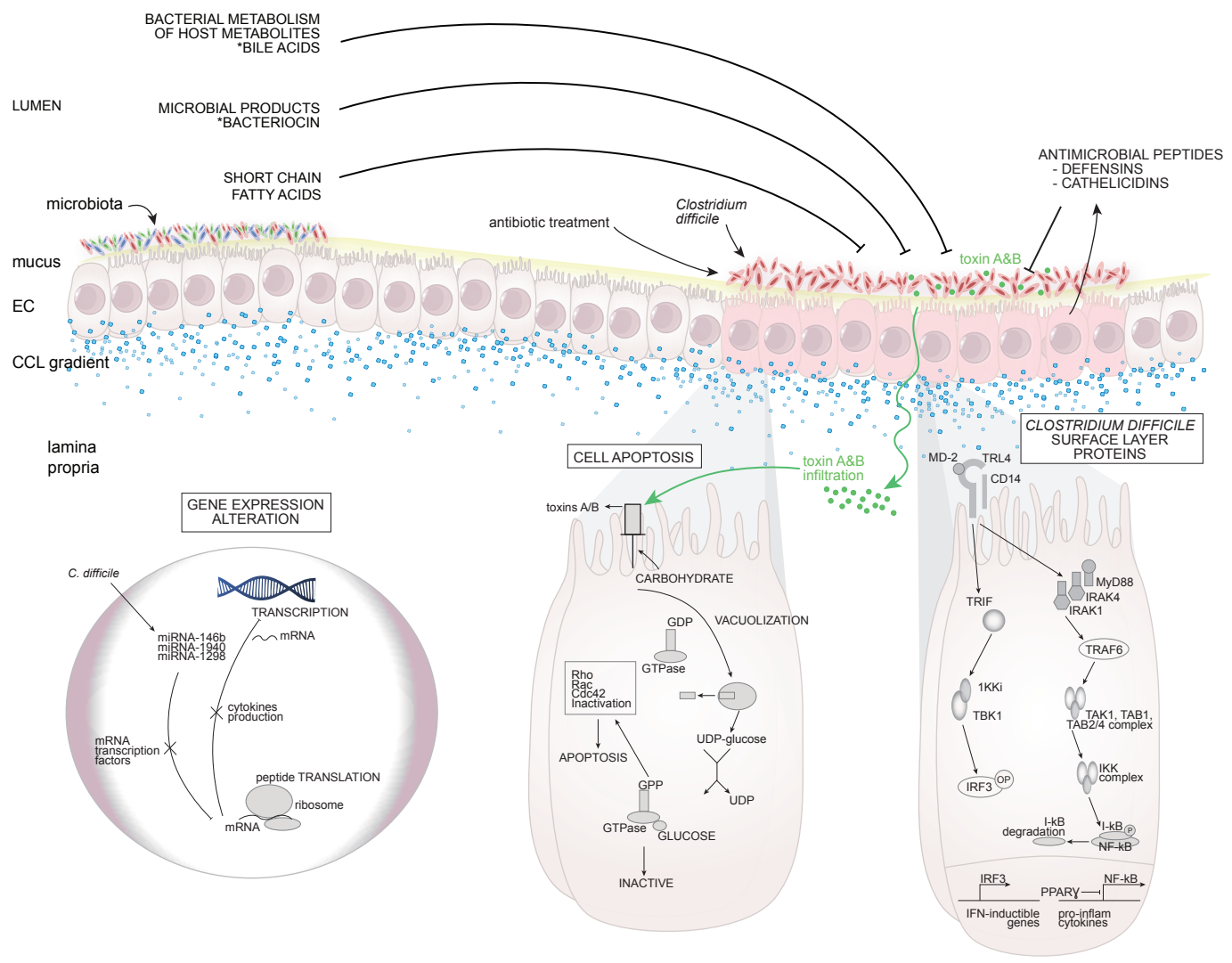
Target	Correlation
M0	-5.80E+04
E	-1.73E+02
HP{Lumen}	0.253797
HP{LP}	0.570211
nT	29802.3
eDC{GLN}	5.38E+05
tDC{GLN}	5.38E+05
tDC{LP}	7.35E+05
Th17{GLN}	1.46E+06
Th1{GLN}	3.37E+06
iTreg{GLN}	4.80E+06
M2	8.11E+06
M1	3.22E+07
Th17{LP}	4.92E+07
iTreg{LP}	7.12E+07
Th1{LP}	8.71E+07



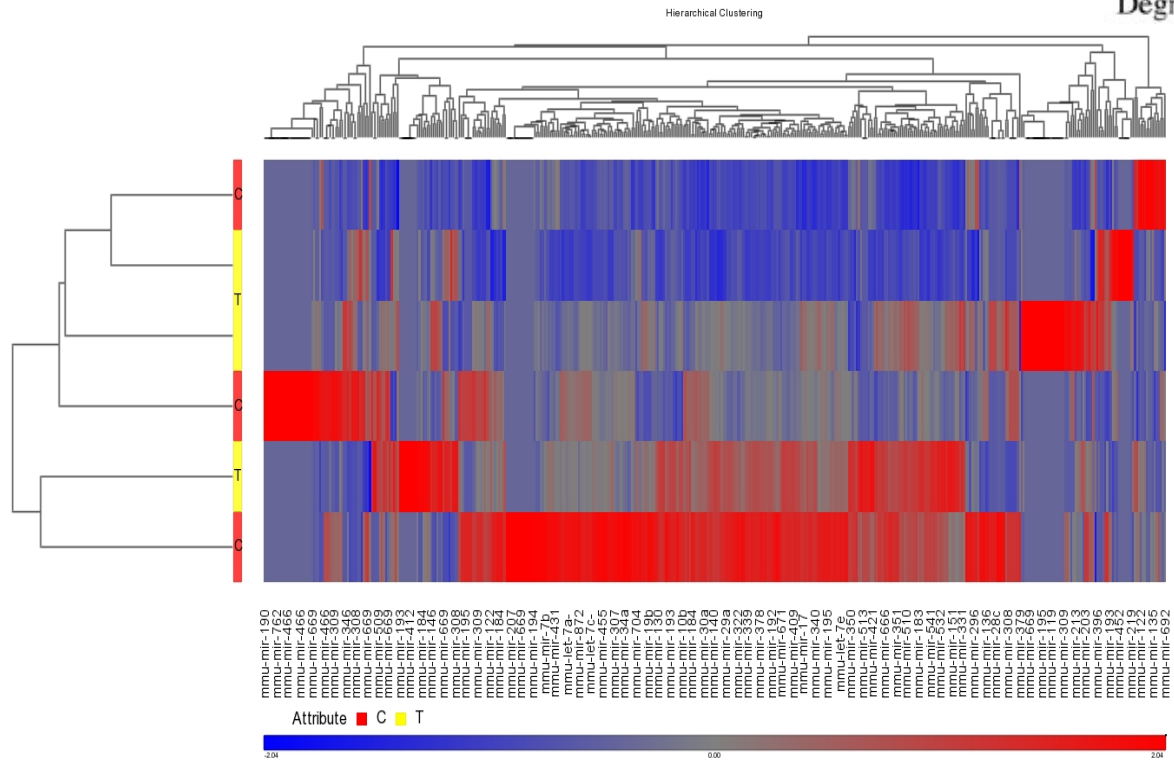
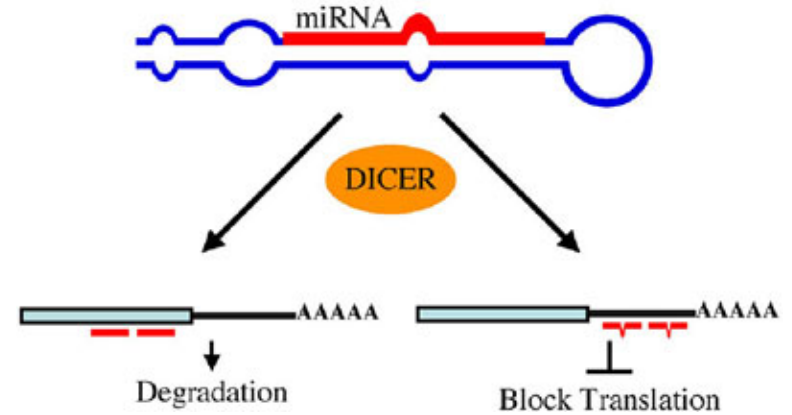
# *Clostridium difficile*

- Gram-positive anaerobic bacterium
- Nosocomial diarrhea and colitis
- Grows in the intestine of individuals with altered microflora
  - Antimicrobial therapy
  - Immunosuppressant treatment
- Emergence of several virulent strains
  - Increased incidence and severity

# Host-*Clostridium difficile* Interactions



# RNA-sequencing



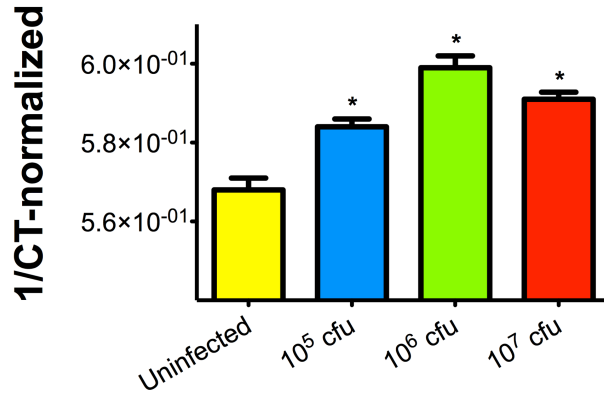
**miR-146b**

**miR-1940**

**miR-1298**

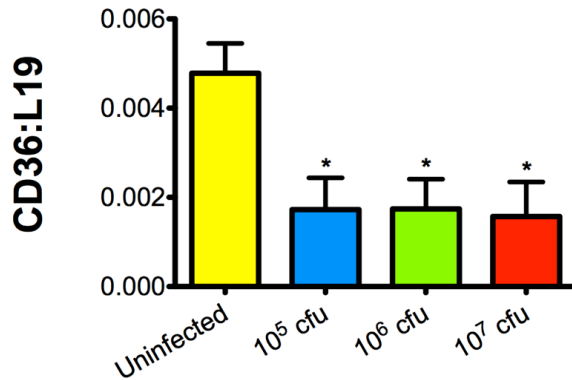
# RNA-sequencing

## miRNA-146b

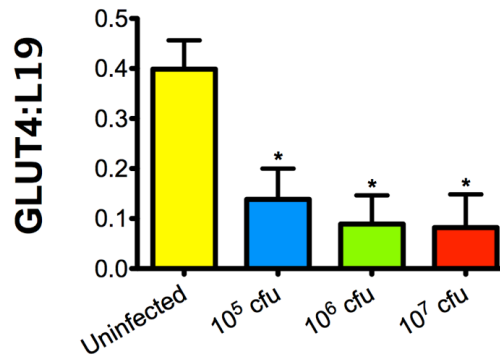


TARGET MRNA	FI	ASSOCIATED BIOLOGICAL PROCESS	MICROCOSM TARGETS P-VALUE
<b>ST (mmu-miR-146b FI = 1.91)</b>			
ALS2CL	0.59	GTPase activator activity	0.0035
AP4S1	0.57	Vesicle mediated transport	0.0028
APLP2	0.48	ECM organization	0.0097
CARD10	0.55	Apoptosis/NF-κB activation	0.0003
CFLAR	0.66	Apoptosis/NF-κB activation	0.0371
CLEC4D	0.39	Immune response	0.0016
GPR116	0.57	Cell signalling	0.0038
KLF13	0.66	Transcription regulation	0.0436
<b>NCOA4</b>	0.64	Nuclear receptor activator	0.0191
NUMB	0.62	Cell signalling	0.0089
RASIP1	0.66	Angiogenesis	0.0000

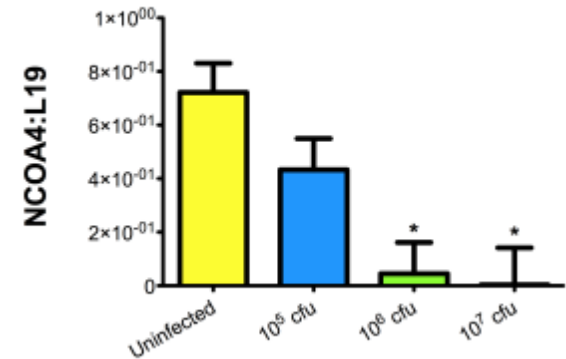
## CD36



## GLUT4

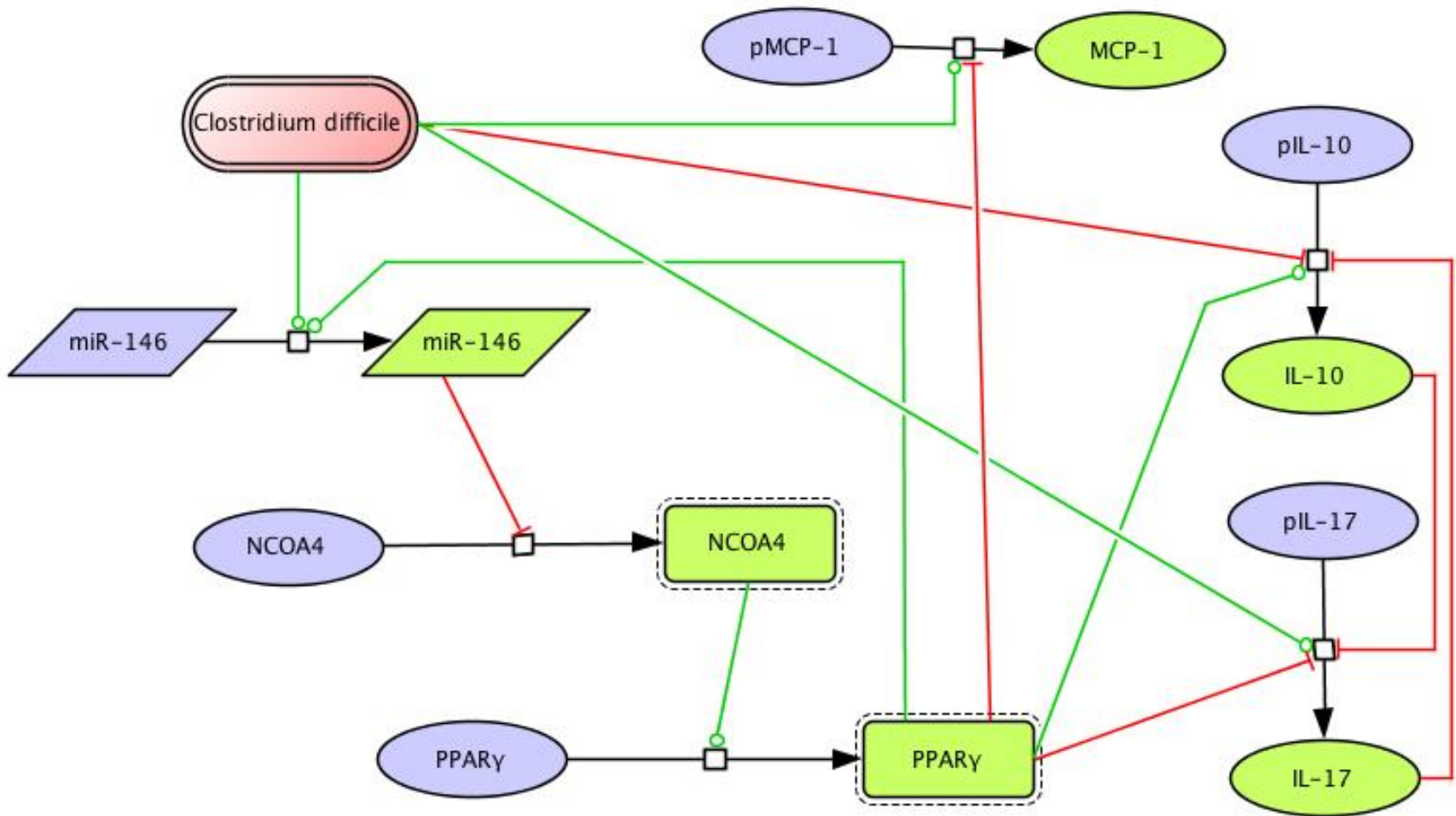


## NCOA4

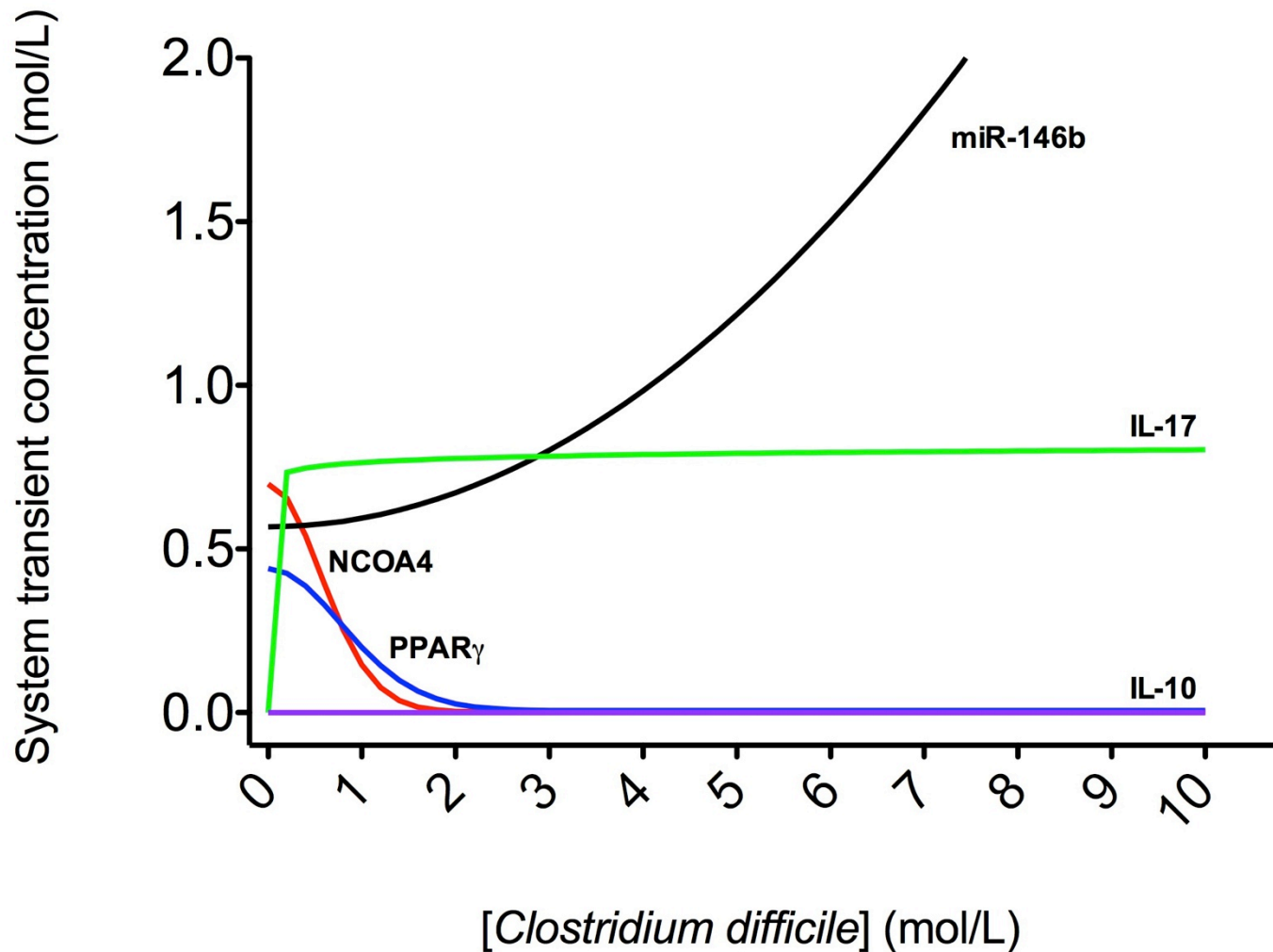




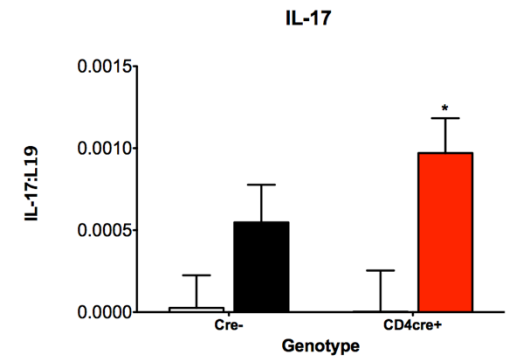
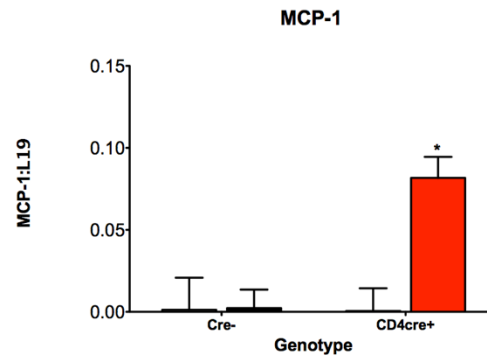
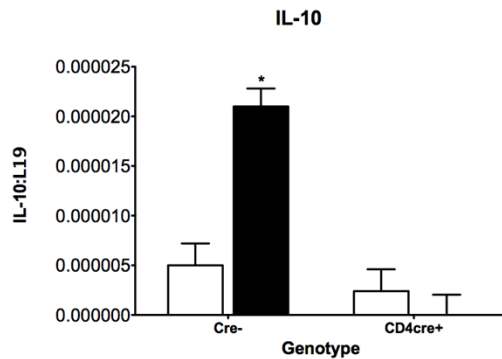
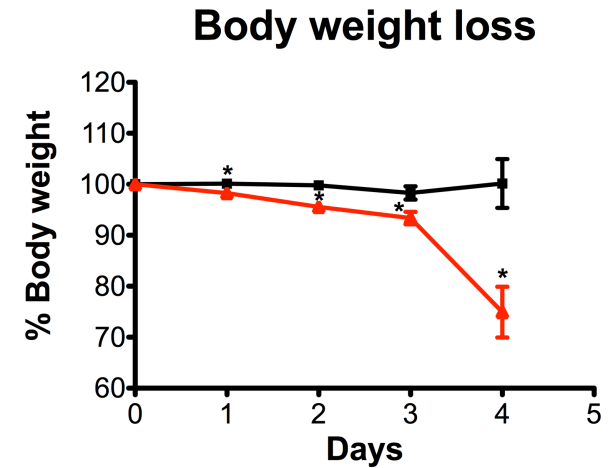
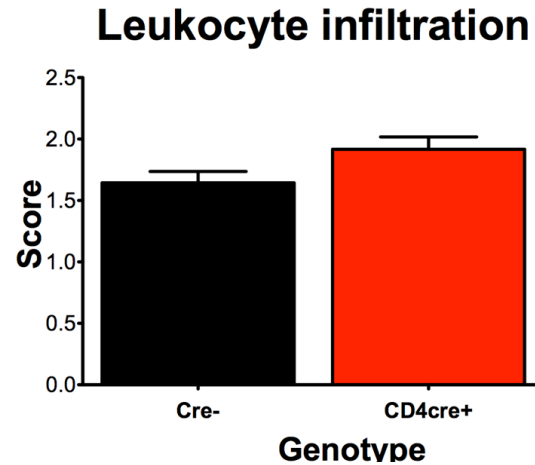
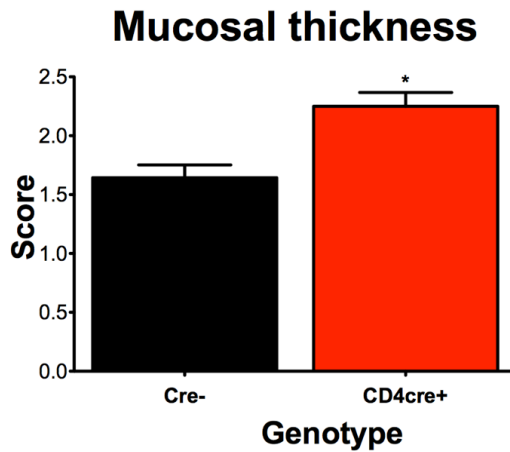
# Modeling Immune Responses to *C. difficile*



# *In silico* simulation of *C. difficile* infection



# The Loss of T cell PPAR $\gamma$ Increases CDAD

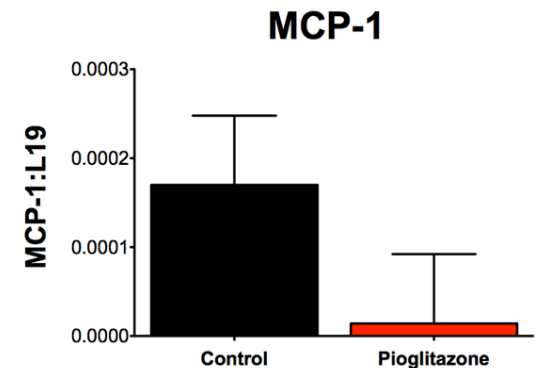
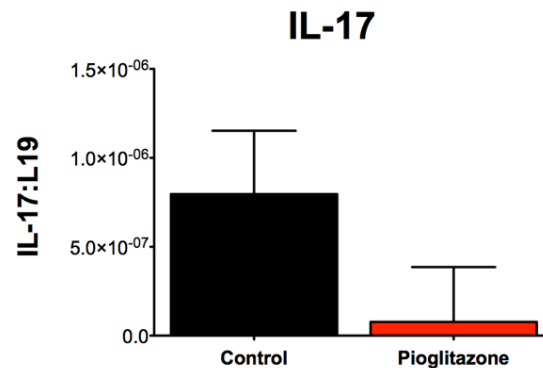
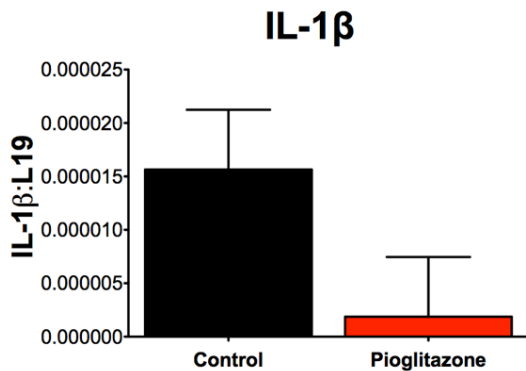
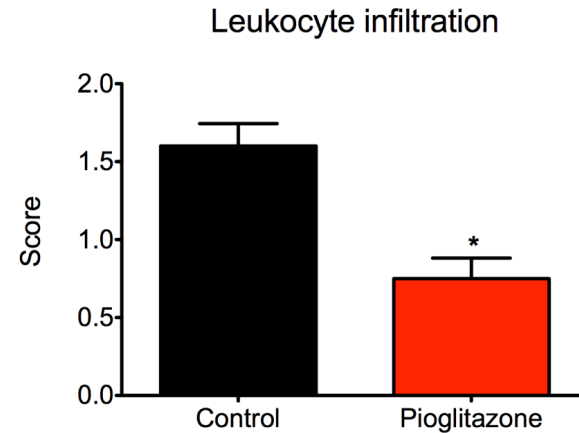
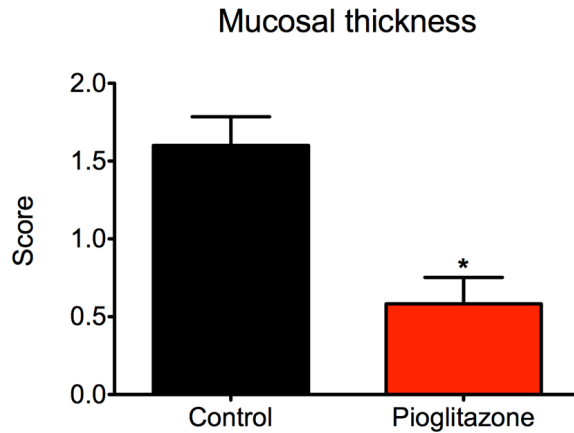


□ Uninfected

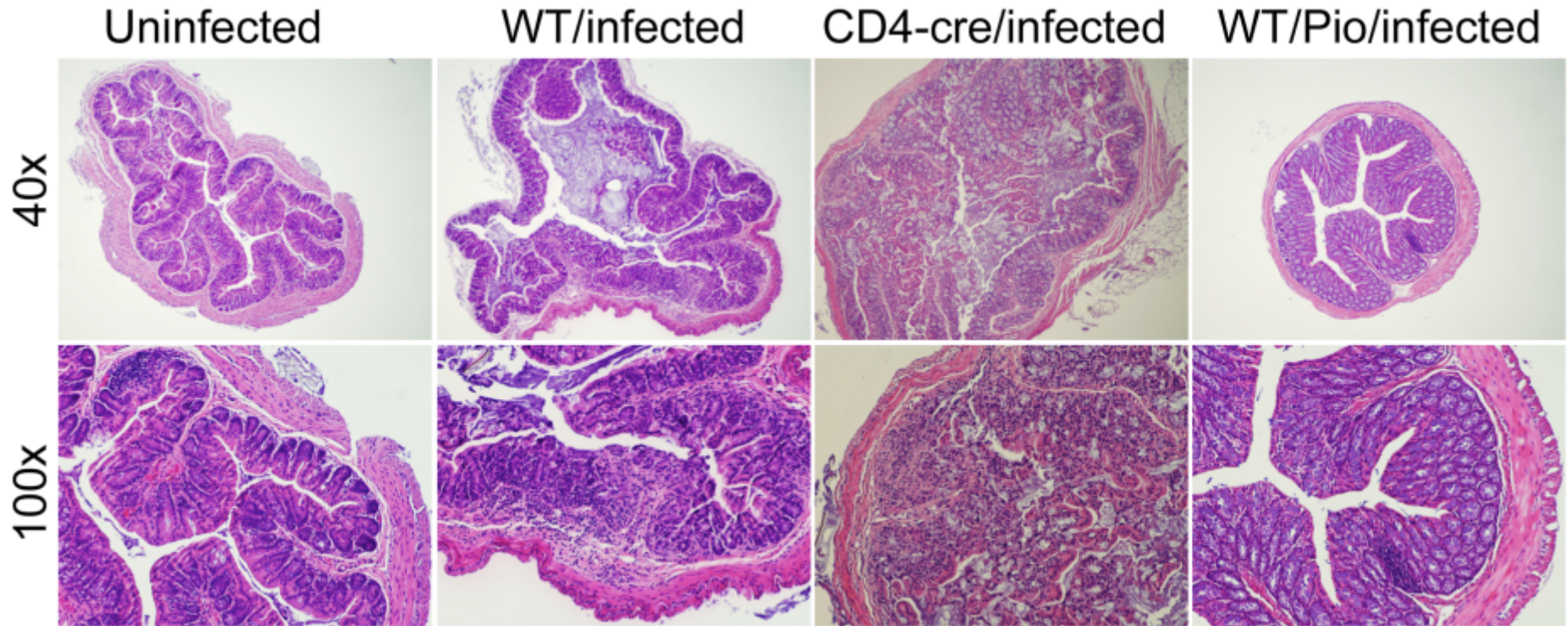
■ WT Infected

■ CD4cre+ Infected

# PPAR $\gamma$ Agonists Ameliorate CDAD

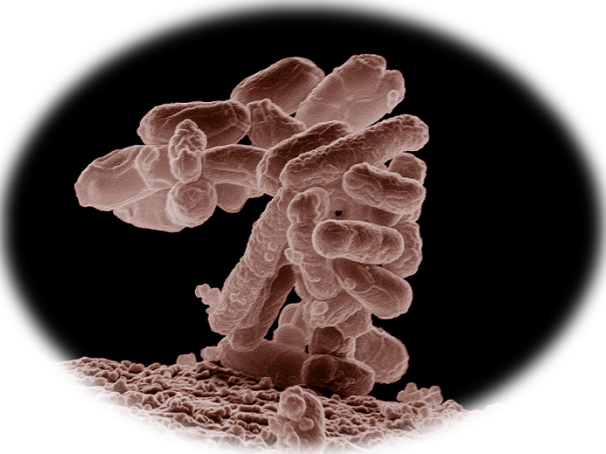


# Colonic Histology

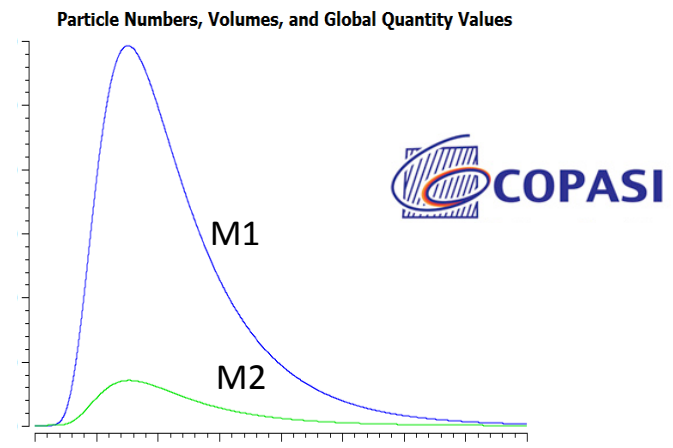


# Summary

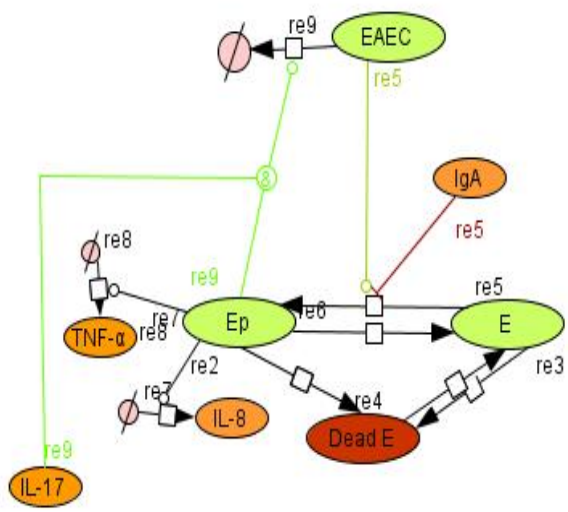
- *C. difficile* increases miRNA-146b expression, which blocks NCOA4, thus reducing PPAR  $\gamma$  activation
- The loss of PPAR  $\gamma$  in T cells worsens the disease severity associated to *C. difficile* infection by inducing a Th17 response
- Oral administration of PPAR  $\gamma$  agonists represents a novel broad-based host targeted therapeutic for CDAD



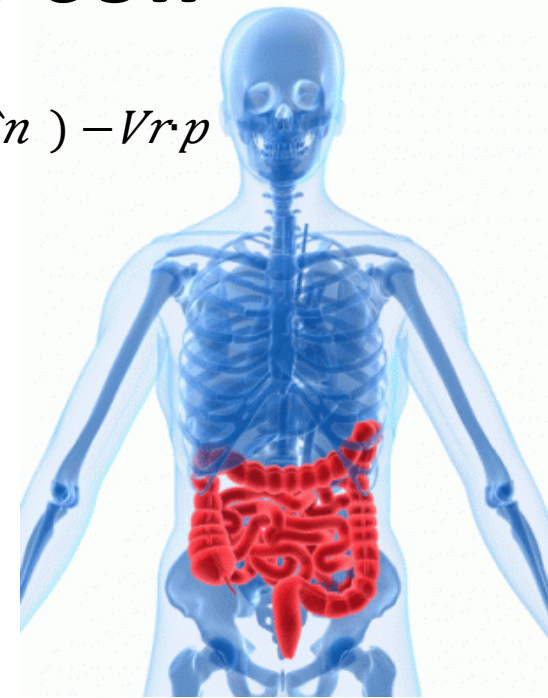
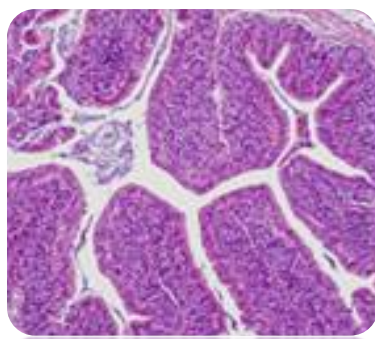
Fisher Information	EAEC Death
Epithelial cell contacts antigen	8.88E-22
IL-17 production by Th17	1.23E-24
TNF-a secretion by Ep	1.73E-20
EAEC death by neutrophil	0
IL-8 production by Ep	4.12E-26
IL-18 production by M1	1.24E-25



# Modeling Immune Responses to Enteroaggregative *E. coli*



$$[cytokine] = Vf \cdot r1 \cdot (1 + A \uparrow n / A \uparrow n + k \uparrow n) - Vr \cdot p$$

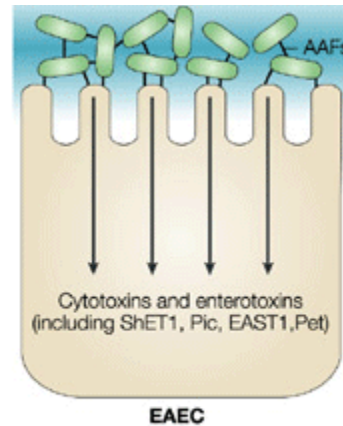
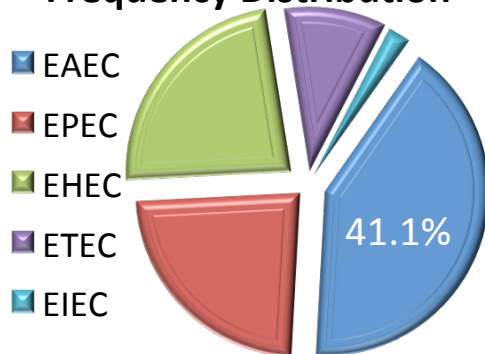


# EAEC *a leading cause of enteritis and persistent diarrhea worldwide*

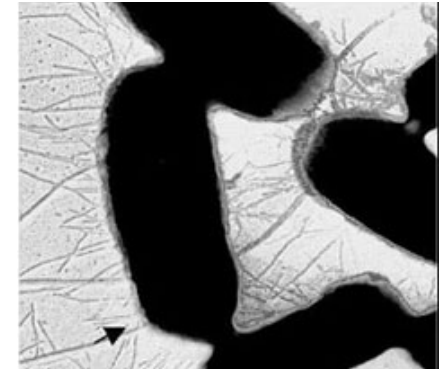
## High risk populations:

- Travelers
- HIV infected
- Malnourished children

### Diarrheagenic Isolate Frequency Distribution



AAF fimbria:  
primary virulence factor attributed to mucosal adherence

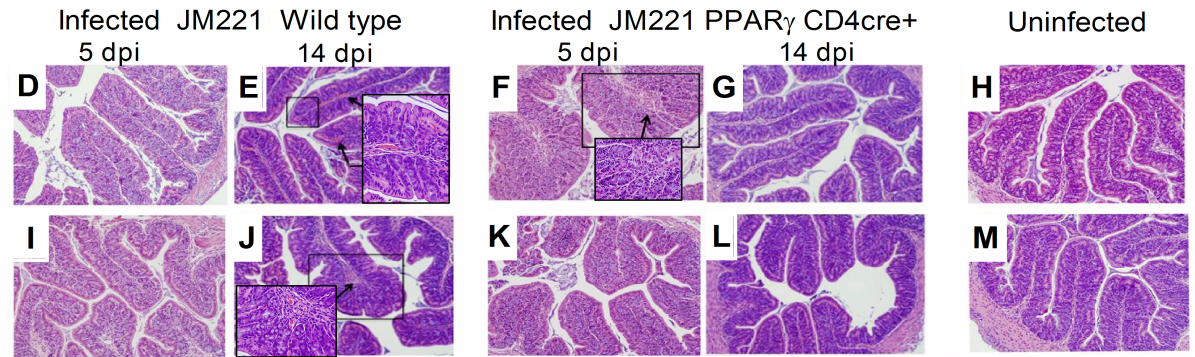
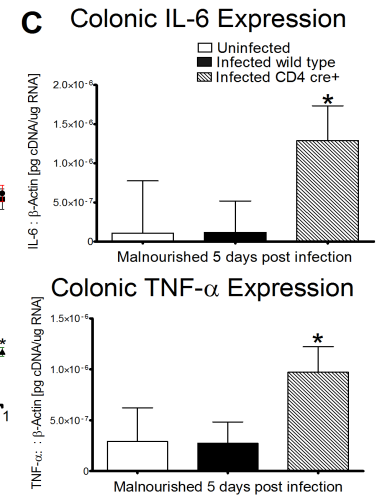
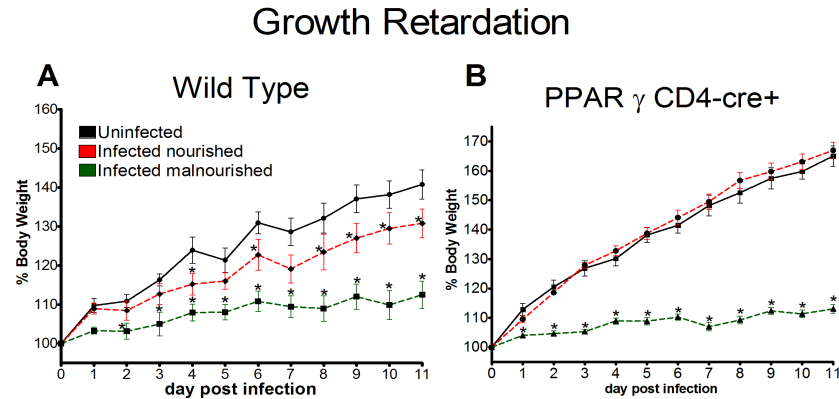
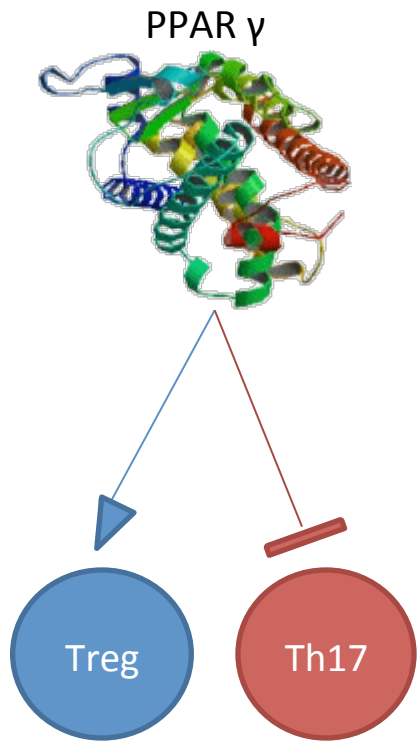


Fli-C flagellin:  
responsible for IL-8 secretion

Dispersin:  
Allows dissociation from biofilm and spread of colonization

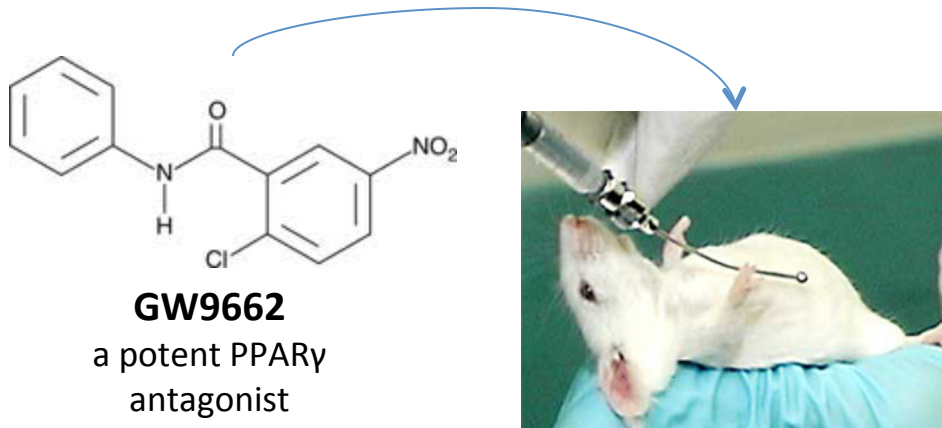


# Targeting PPAR $\gamma$ as an inflammatory mediator

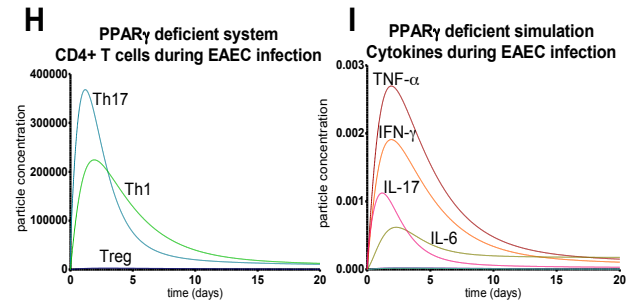
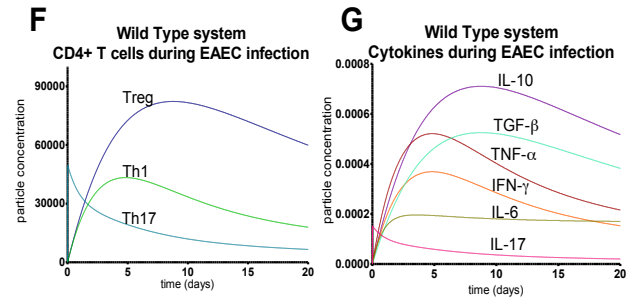
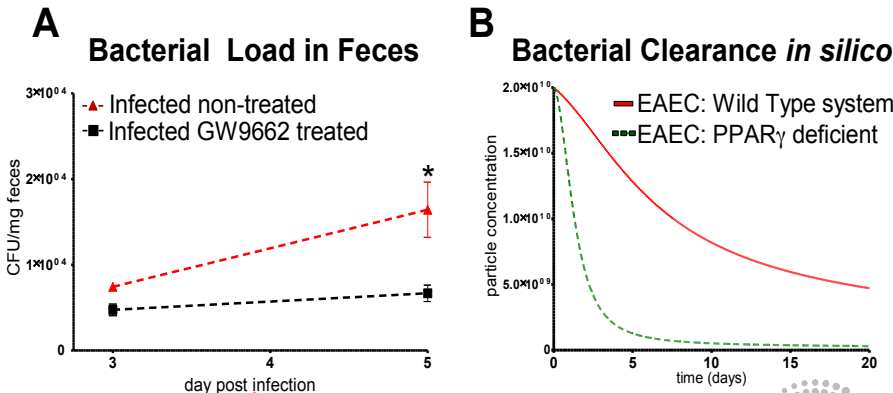
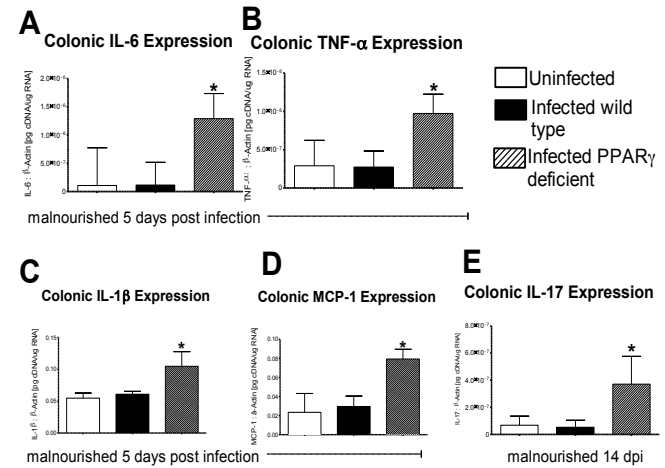


- Gene expression: Upregulation of proinflammatory markers in CD4Cre+
- Histopathology: High leukocytic infiltration early during infection in CD4Cre+ followed by amelioration of colonic inflammation by day 14

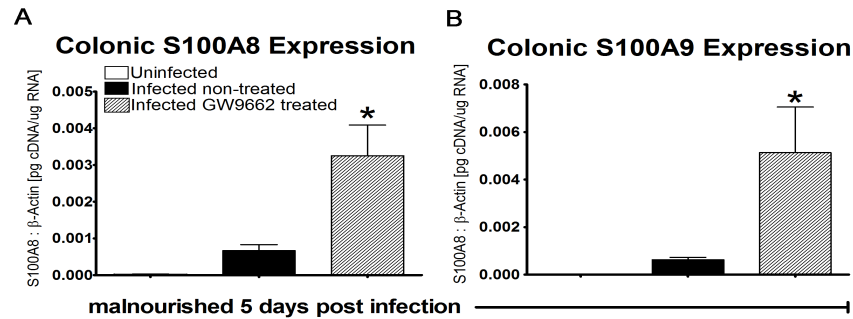
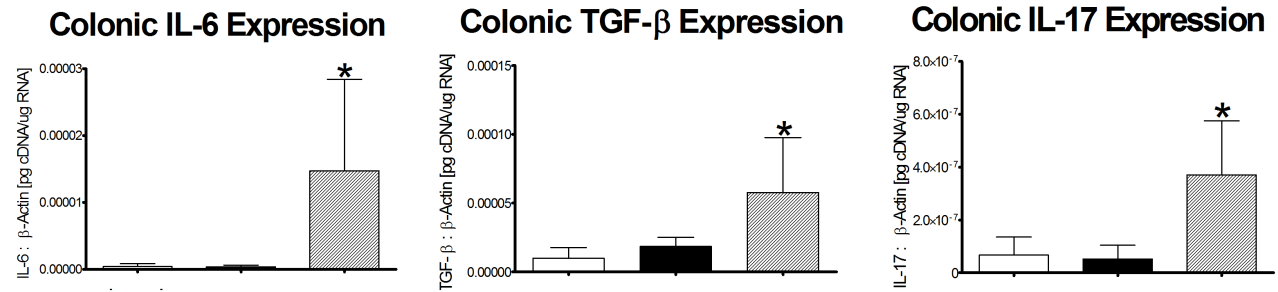
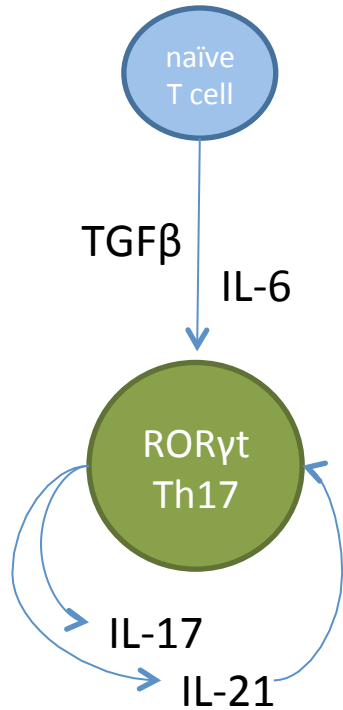
# Pharmacological blockade of PPAR $\gamma$



Administration of **GW9662** promoted the upregulation of proinflammatory cytokines that correlated to significantly *lower levels of EAEC in feces* early during infection

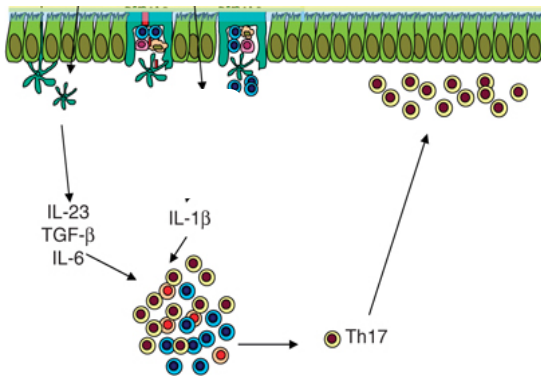


# Th17 response and antimicrobial peptides



## Pharmacological blockade of PPARγ beneficial

Late during infection GW9662 treated mice expressed cytokines responsible for potentiating Th17 differentiation in addition to significantly higher levels of anti-microbial peptides.



# MIEP Team

## Virginia Bioinformatics Institute

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**Sunny Liu** – Lab Assistant

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**Stefan Hoops** - Bioinformatics Lead

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**James Roche** - Infectious Disease Expert

**Circle A. Warren** - Infectious Disease Expert

**David Bolick** - Sr. Laboratory and Research  
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## Caprion Proteomics Inc.

**Eustache Paramithiotis** - Proteomics Director



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