

Connecting Data to Models



Josep Bassaganya-Riera, DVM, PhD

[Nutritional Immunology & Molecular Medicine Lab](#)
[Center for Modeling Immunity to Enteric Pathogens](#)

Virginia Tech, Blacksburg, Virginia



MODELING IMMUNITY
TO ENTERIC PATHOGENS
Modeling Mucosal Immunity
Summer School & Symposium

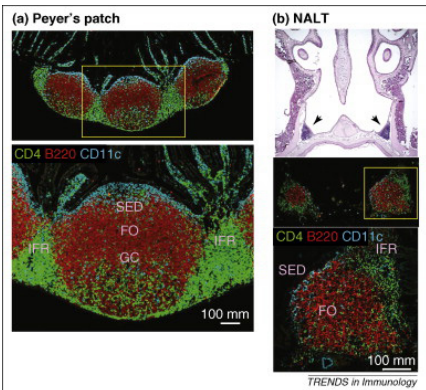
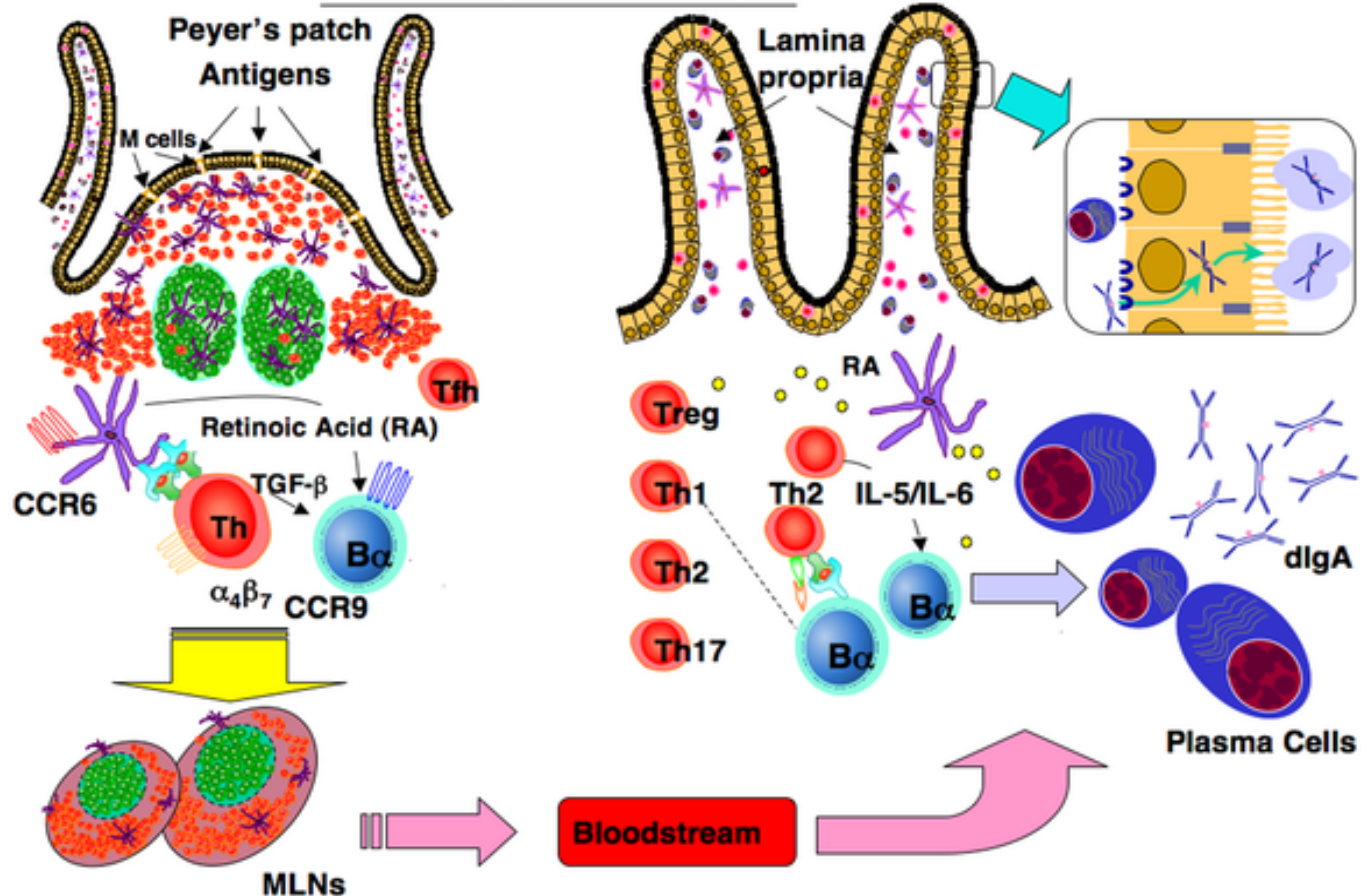


Mucosal Immune System

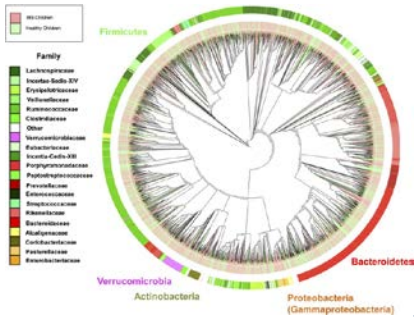


A Mucosal Communication System

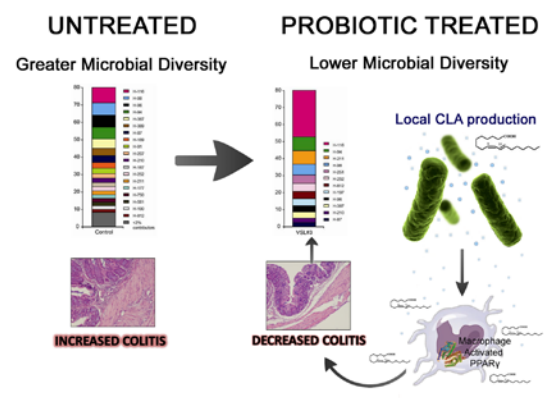
Inductive Versus Effector Sites



McGhee JR, Fujihashi K (2012) Inside the Mucosal Immune System. PLoS Biol 10(9): e1001397. doi:10.1371/journal.pbio.1001397



Microbiome

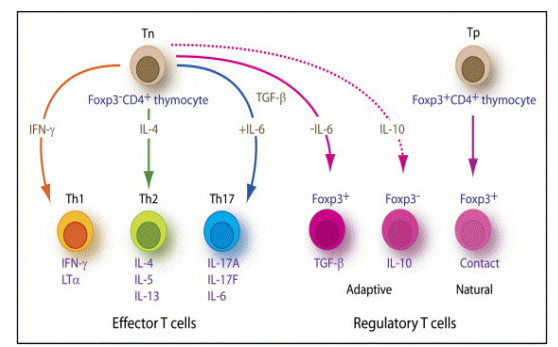
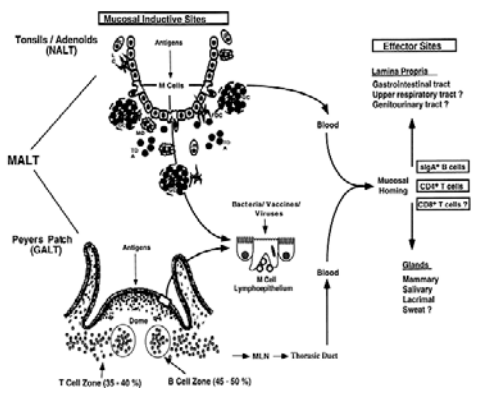
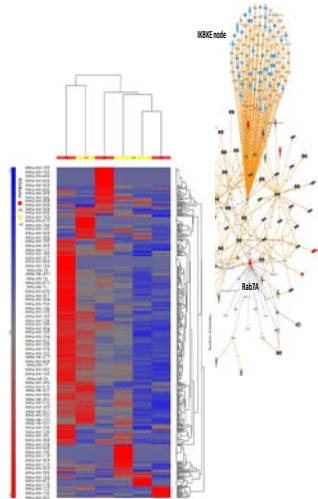


**Genes
RNA
Proteins**

**Health vs.
Disease**

Diet

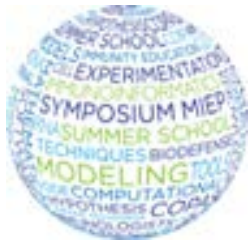
**Inflammation
& Immunity**





MMI Goals

- Introduce immunologists to the latest methods and tools for using computational modeling
- Present MIEP and MIB work to a wider audience
- Disseminate computational models of the gut mucosal immune system



What you have learned?

- Mucosal immune responses (CD4+ T cells and epithelial cells)
 - Inductive and effector sites
- Types of computational models of the MIS and tools
- How to build network models from data and theory
- Mining immunological datasets using Cytobank or IPA, signaling-regulatory network modules
- Using CellDesigner, COPASI and ENISI for modeling
 - Calibration, sensitivity analysis, parameter estimation, simulation, model-driven hypothesis generation & experimental validation

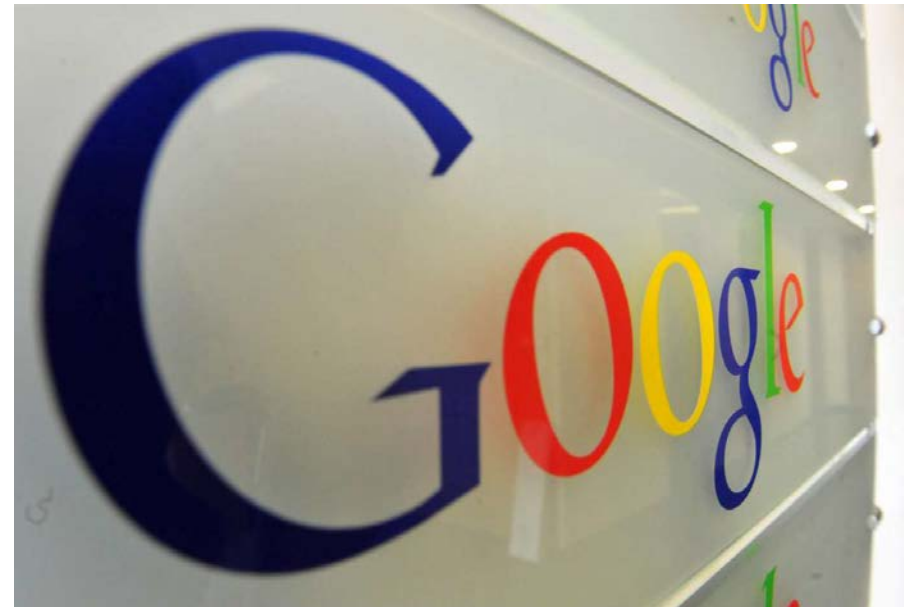


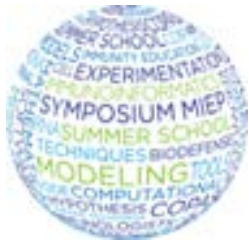
MIEP Modeling

- Build models that are portable and comply with standards (i.e., SBML)
- Models of the immune system are applicable to infectious and autoimmune diseases
- Models can be recycled for new uses following re-calibration with new datasets
- Combine theoretical and data-driven approaches to make models predictive
- Integrate diverse datasets and explore conflicting results



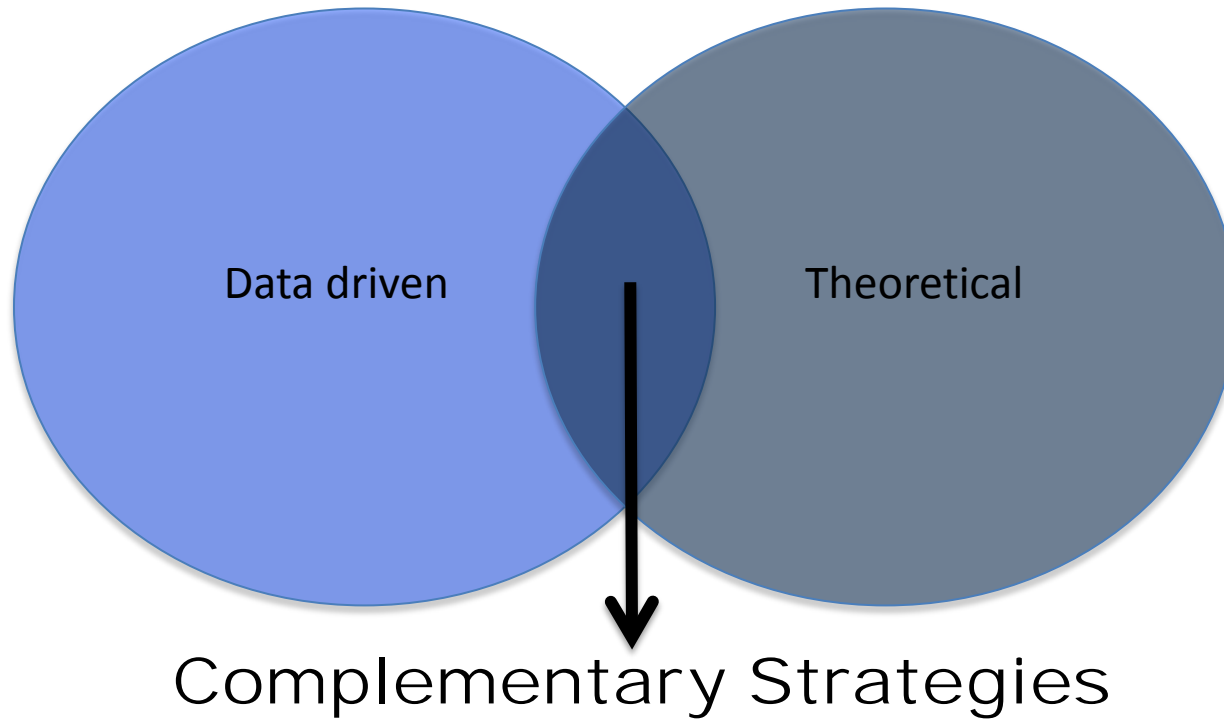
Common Themes





Data-driven vs. theoretical

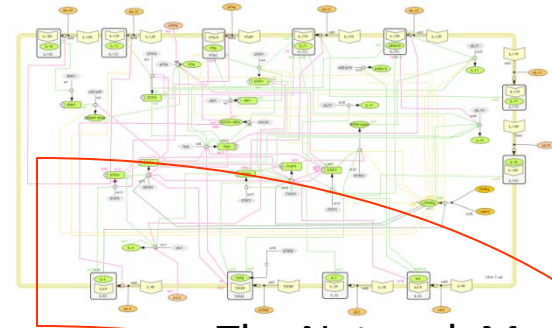
WHAT IS BEST?



Computational Immunology



Literature & data mining



The Network Model



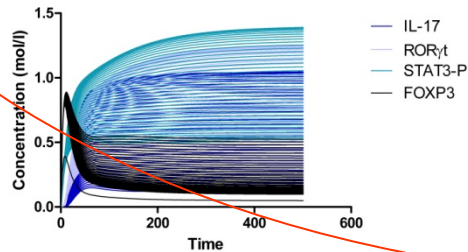
ENteric Immunity Simulator

Modeling tools

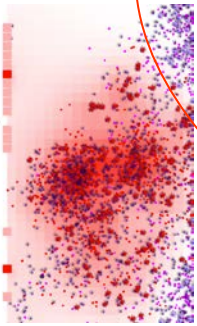


REFINEMENT

In vivo hypothesis testing



In silico experiments
Hypothesis generation



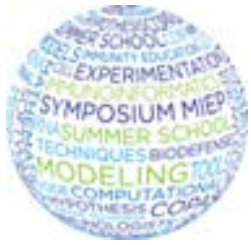


Helicobacter pylori

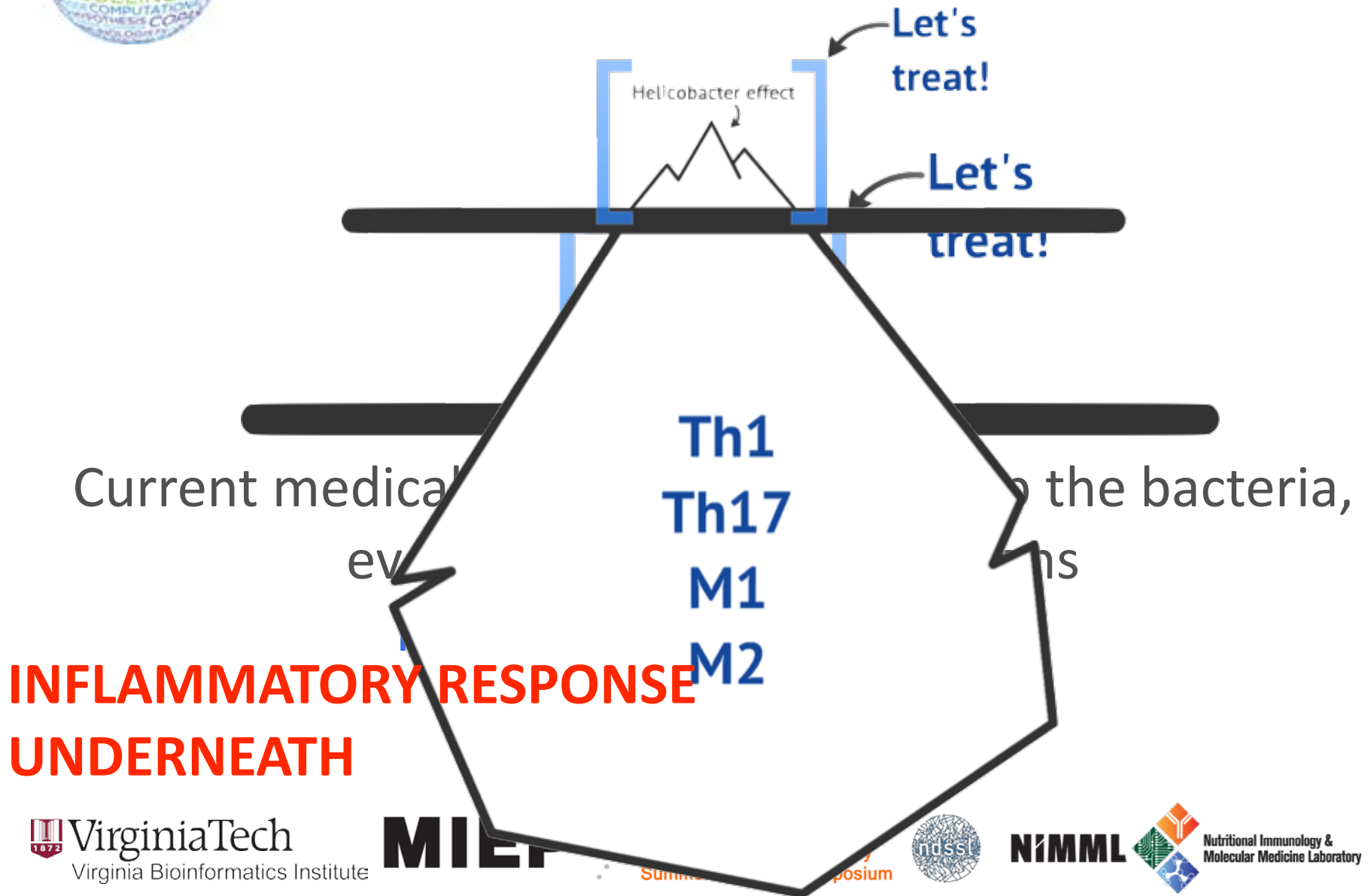
- *H. pylori* was classified as a type I carcinogen by the WHO... Should it be eradicated?
- *H. pylori* should be included in the list of most endangered species (M. Blaser)...and preserved as a beneficial commensal
- Inverse correlation between *H. pylori* prevalence and rate of overweight/obesity (Lender, 2014)

***Helicobacter pylori* Colonization Ameliorates Glucose Homeostasis in Mice through a PPAR γ -Dependent Mechanism**

Josep Bassaganya-Riera^{1,4*}, Maria Gloria Dominguez-Bello², Barbara Kronsteiner¹, Adria Carbo¹, Pinyi Lu¹, Monica Viladomiu¹, Mireia Pedragosa¹, Xiaoying Zhang¹, Bruno W. Sobral^{1,2}, Shrinivasrao P. Mane¹, Saroj K. Mohapatra¹, William T. Horne¹, Amir J. Guri¹, Michael Groeschl³, Gabriela Lopez-Velasco¹, Raquel Hontecillas¹



Host Responses to *H. pylori*





Host Responses to *H. pylori*

We have given evidence supporting the following:

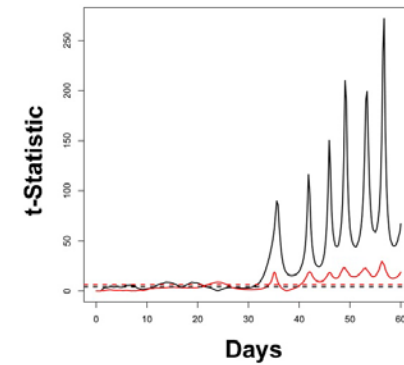
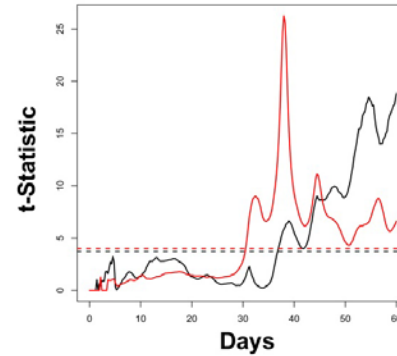
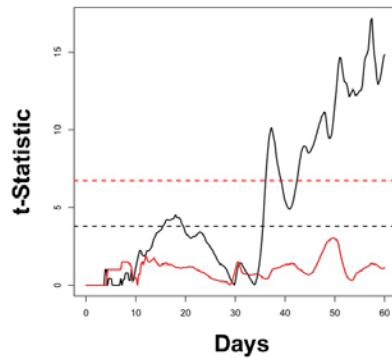
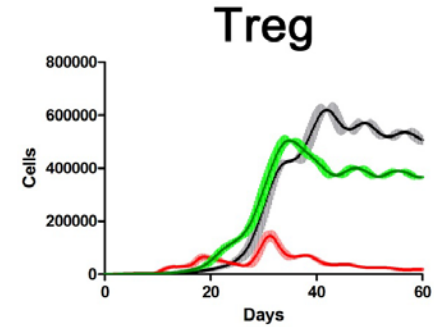
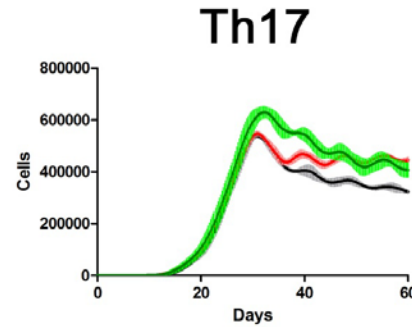
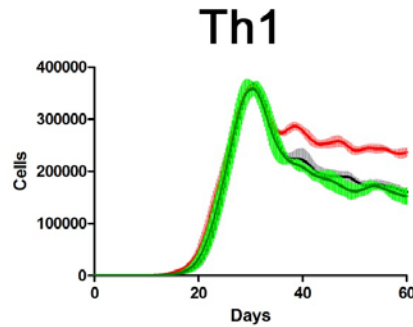
- CD4+ T cells are key mediators during *H. pylori* infection
- Cytokines and transcription factors activated in CD4+ T cells are crucial to modulate myeloid cell function
- We need to target the immune system and not the bacterium itself if we want to reduce inflammatory processes during chronic infections

HOST-TARGETED THERAPEUTIC APPROACHES



ENISI LP Simulation Results

— Wild-type
 — T cell-specific PPAR γ null
 — Myeloid-specific PPAR γ null



— Wild-type vs. T cell-specific PPAR γ null
 — Wild-type vs. Myeloid-specific PPAR γ null



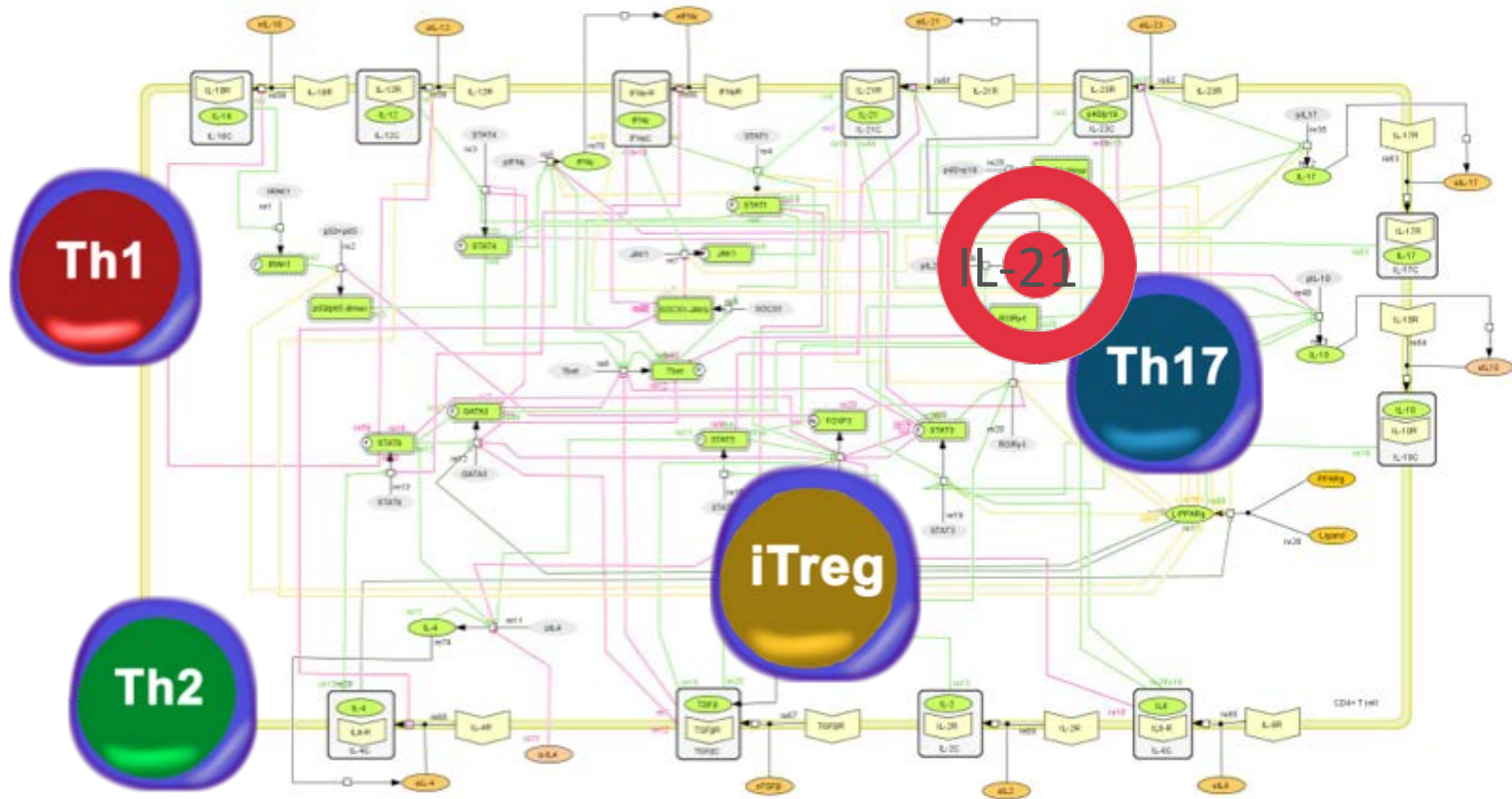
CD4+ T cell differentiation

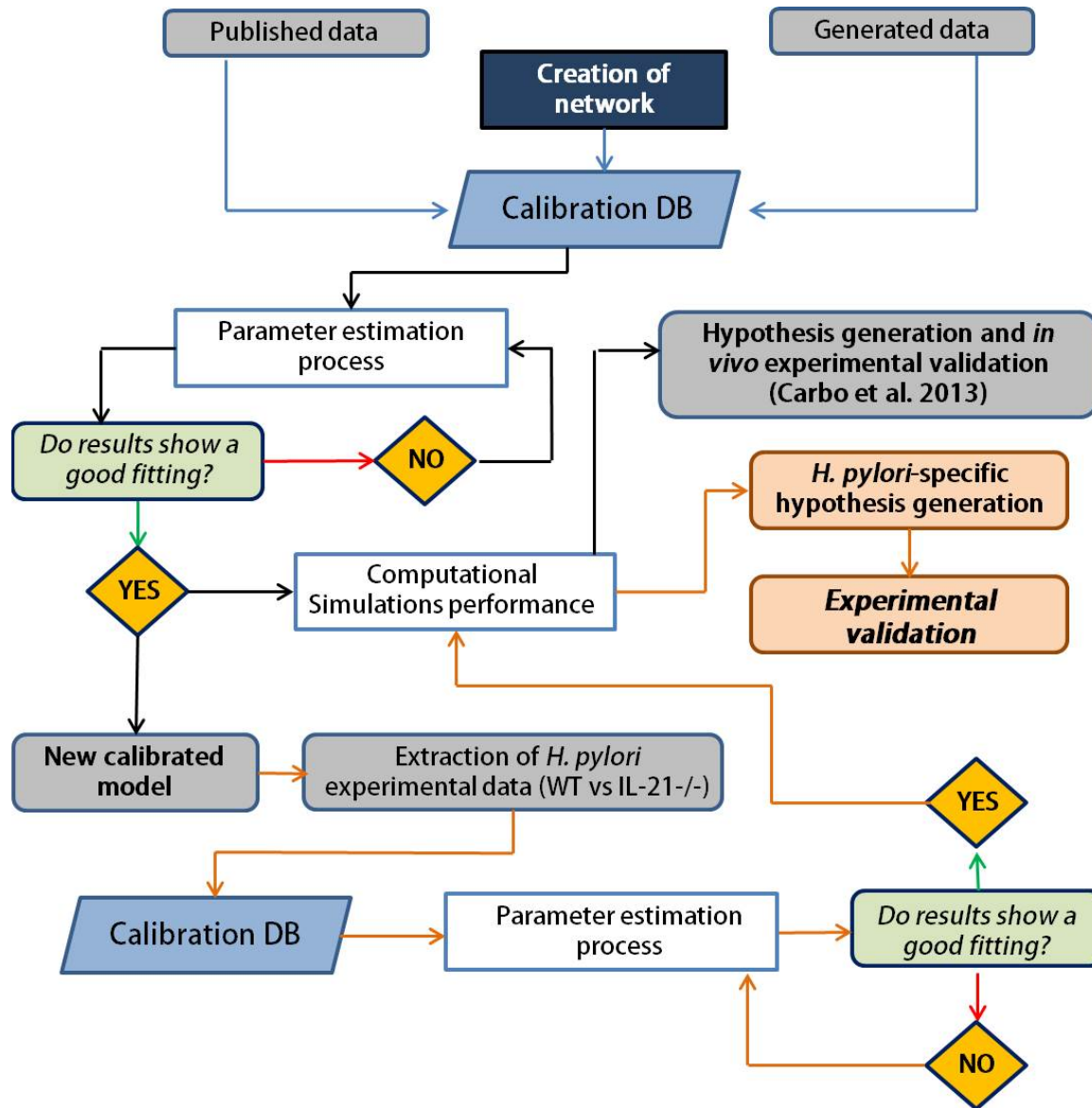
Interleukin-21

- i. IL-21 is mostly produced by activated CD4+ T cells (especially Th17) fTh and NKT cells
- ii. IL-21 helps in the maintenance of Th17 and impairs Treg homeostasis by IL-2 inhibition
- iii. IL-21 is increased with *H. pylori* infection and correlates with levels of gastritis in the mouse model



CD4+ T cell differentiation

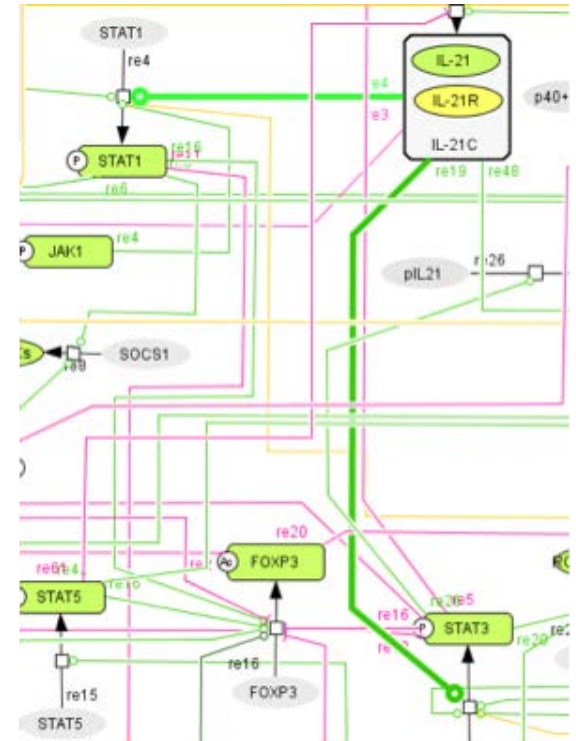
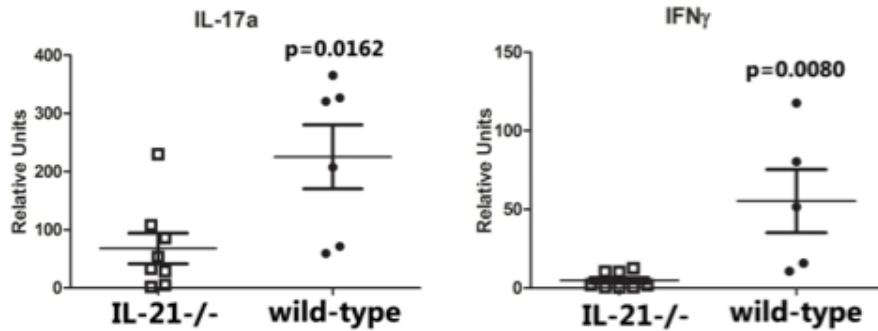






CD4+ T cell differentiation

Stomach RT-PCR data



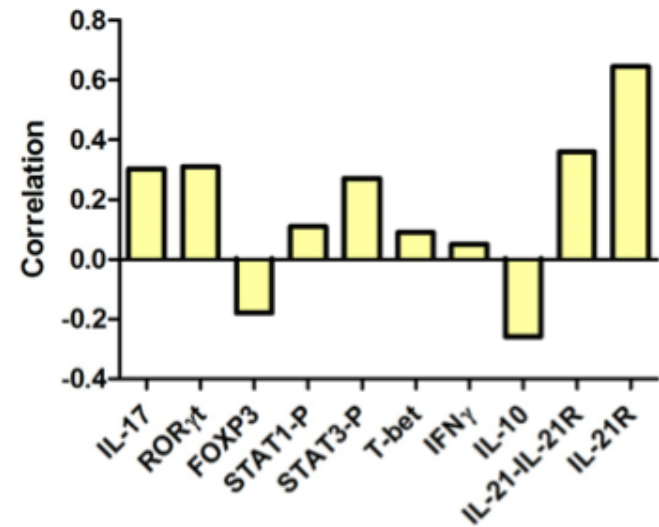
Re-calibration of the CD4+ T cell model with experimental data coming from *H. pylori* infections



CD4+ T cell differentiation

Sensitivity Analysis

How sensitive are different molecules to the change in concentration of IL-21 following *H. pylori* infection?

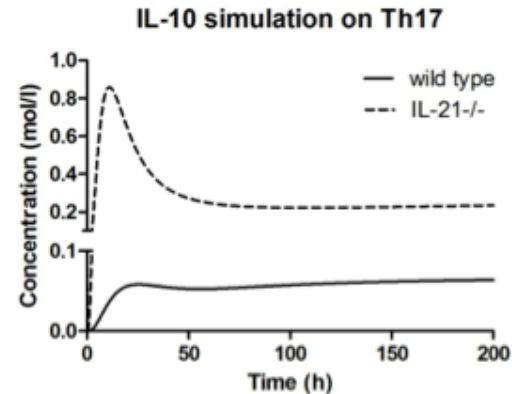
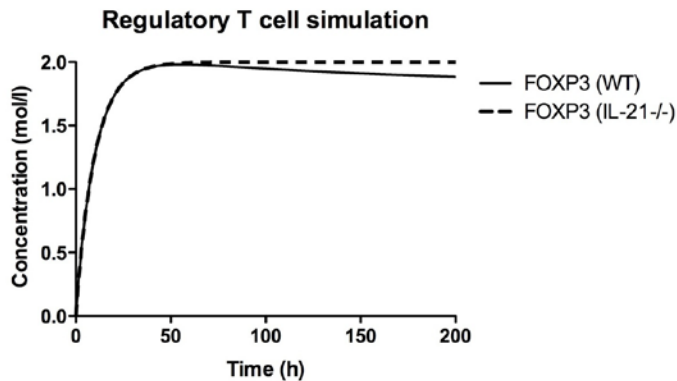


IL-21 activation is positively correlated with Th1- and Th17-related molecules and negatively correlated to both FOXP3 and IL-10



CD4+ T cell differentiation

In silico experimentation

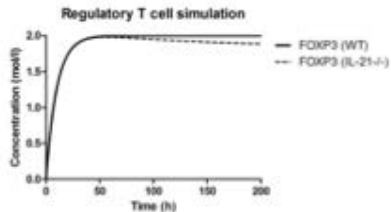
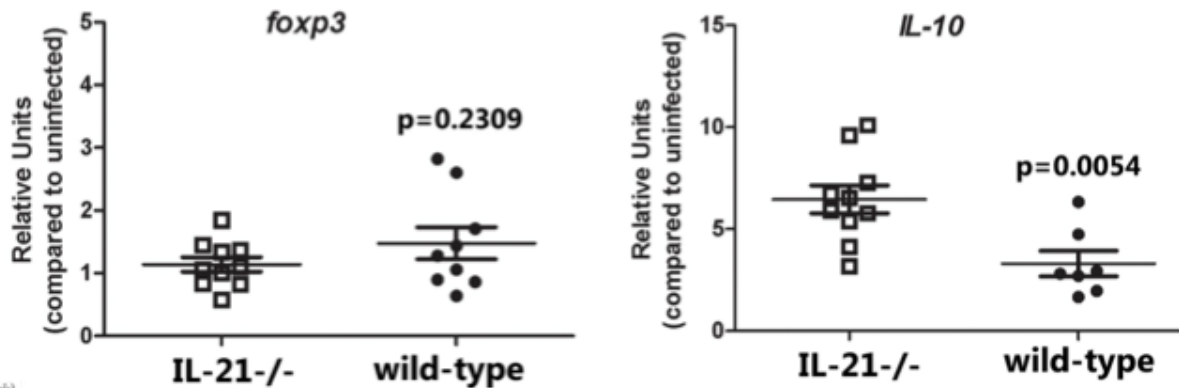
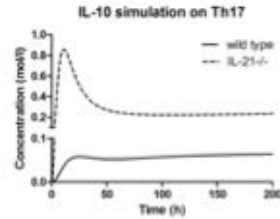


IL-21 does not modulate FOXP3 expression during *H. pylori* infection. However, IL-21 has a significant impact on the IL-10 response by Th17 cells



CD4+ T cell differentiation

In vivo validation

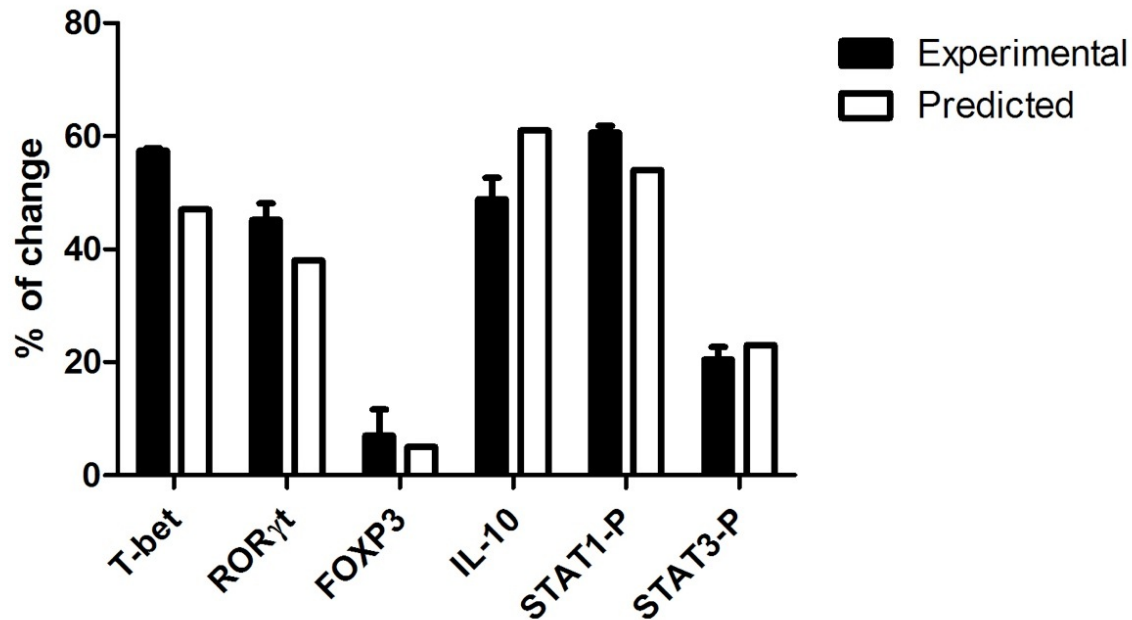


As predicted, IL-10 expression was significantly higher in *H. pylori*-infected IL-21-/- mice and IL-21 does not modulate FOXP3 expression in CD4+ T cells from infected mice



CD4+ T cell differentiation

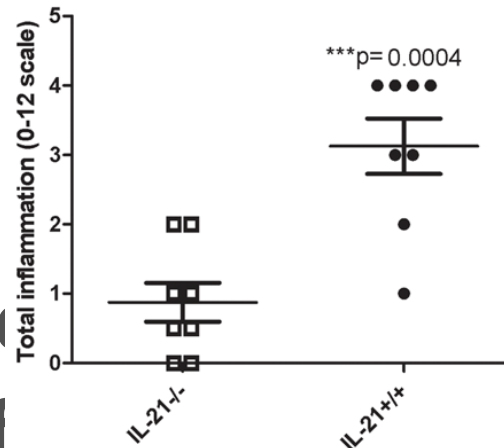
Percentage change side-by-side comparison



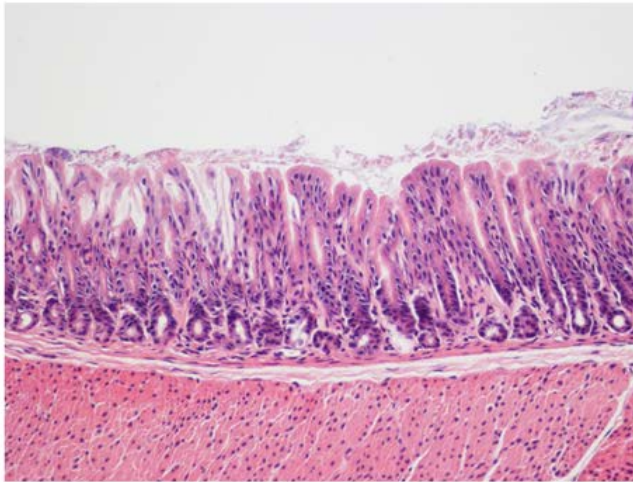


CD4+ T cell differentiation

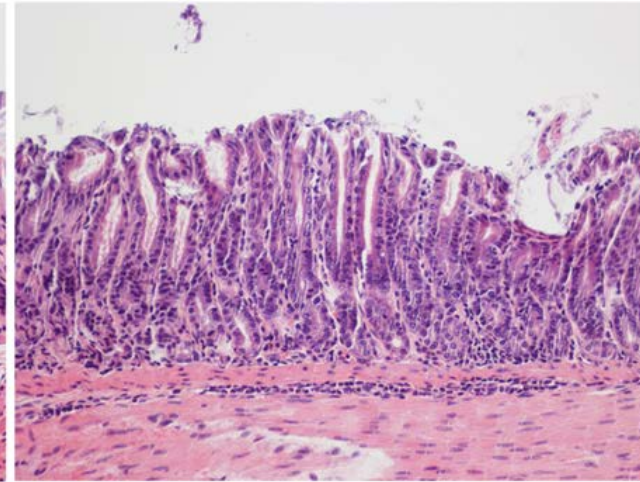
Can we find a better approach to reduce the inflammation?



targeted approach response triggered **YES**



IL-21^{-/-}



IL-21^{+/+}



IL-21-based Therapeutics

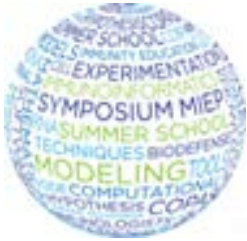
IL-21 inhibitor: PF-05230900

Trade Name: ATR-107

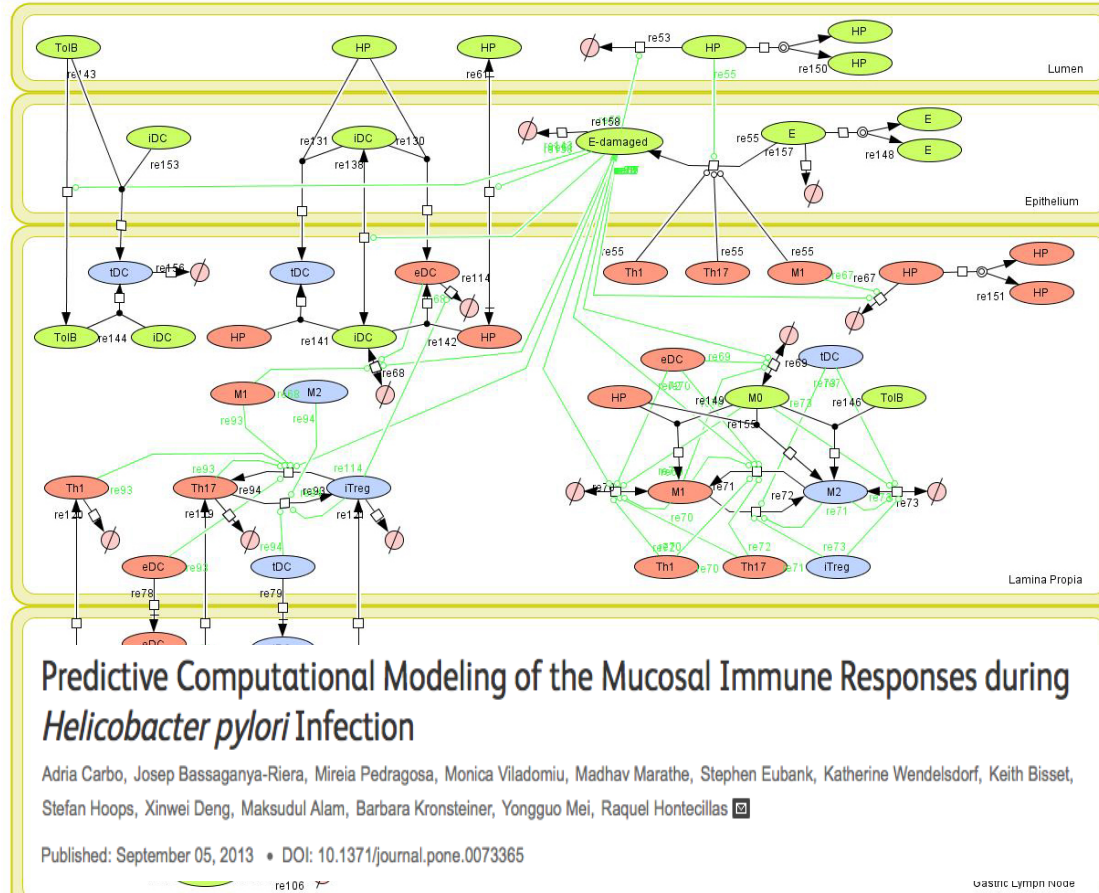
Company: Pfizer

Biological Target: IL-21 in IBD

Mechanism: binds to IL-21 and blocks processes leading to inflammatory activity



Immune response to *H. pylori*

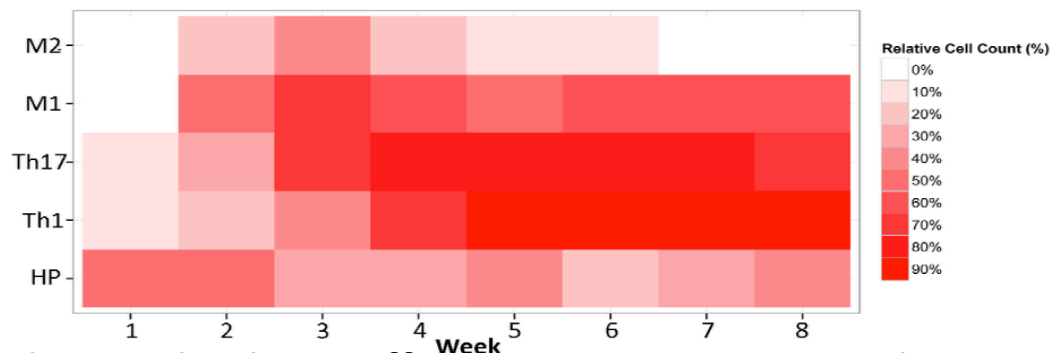


<http://www.modelingimmunity.org/models/copasi-helicobacter-pylori-computational-model-archive/>

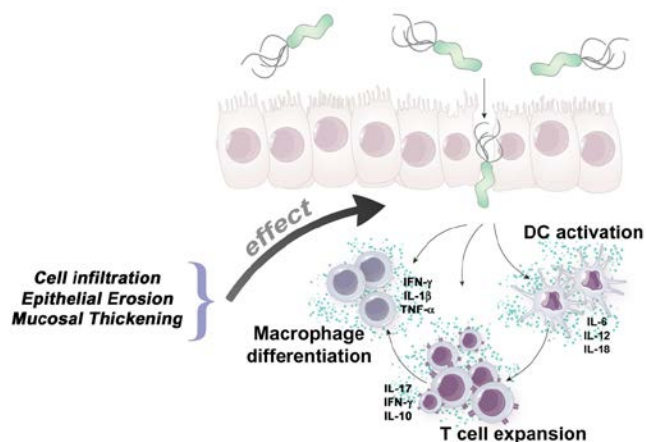


Previous Model predictions

Main cause of epithelial damage



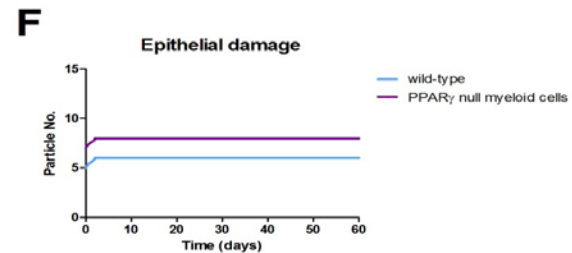
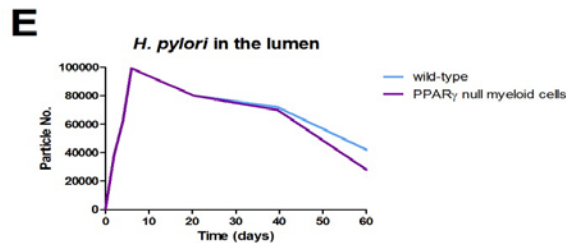
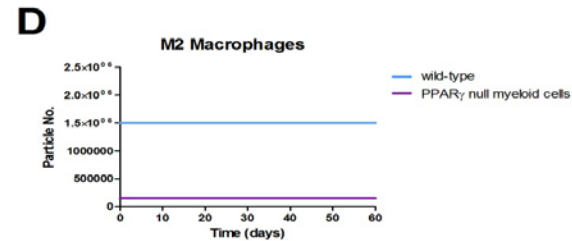
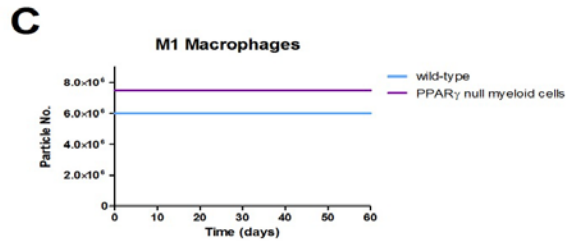
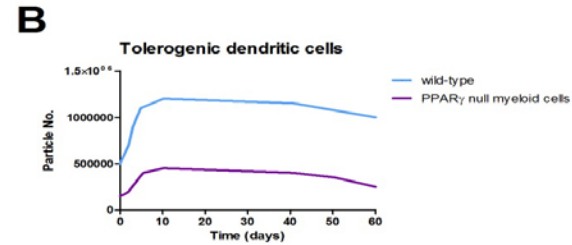
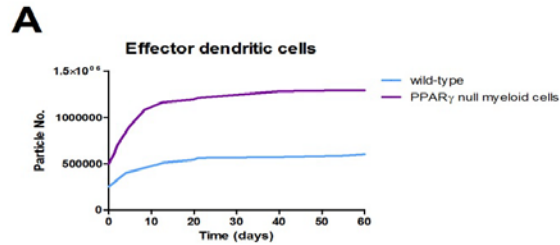
Th1 and Th17 effector responses contribute to gastritis in the chronic phase of infection.



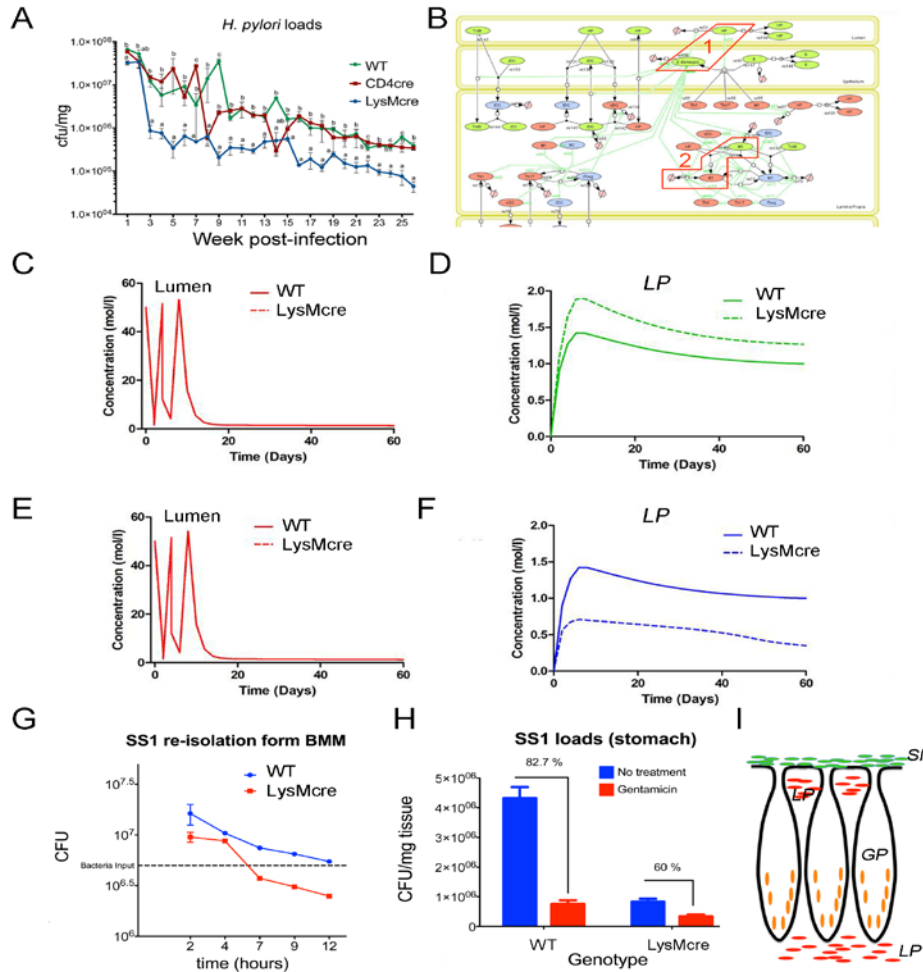
Target	Correlation
M0	-5.80E+04
E	-1.73E+02
HP{Lumen}	0.253797
HP{LP}	0.570211
nT	29802.3
eDC{GLN}	5.38E+05
tDC{GLN}	5.38E+05
tDC{LP}	7.35E+05
Th17{GLN}	1.46E+06
Th1{GLN}	3.37E+06
iTreg{GLN}	4.80E+06
M2	8.11E+06
M1	3.22E+07
Th17{LP}	4.92E+07
iTreg{LP}	7.12E+07
Th1{LP}	8.71E+07



Simulation of PPAR γ deletion



Epithelial vs Myeloid Cell



Epithelial antimicrobial response

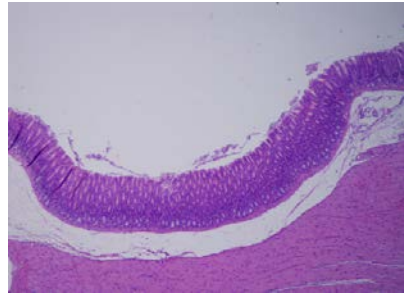
M1 macrophage differentiation



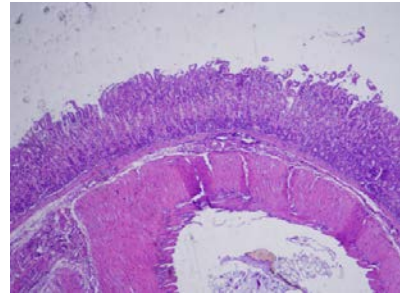
H. pylori Loads and Lesions

STOMACH
WPI 16

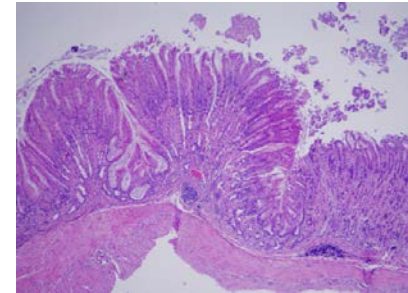
Uninfected



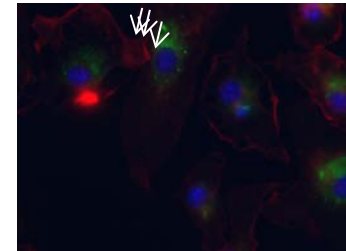
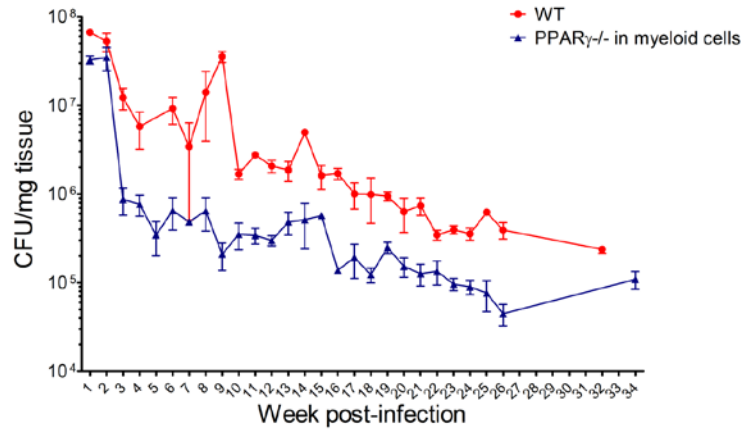
Wild Type



Myeloid cell
PPAR γ -deficient



Bacterial re-isolation





HUMAN & ANIMAL STUDIES

Publicly available data (GEO)

In-house generated NGS data

ANALYSIS with GALAXY pipeline

Sequencing RESULTS (gene reads)

Read Averages, Read Trimming, and Calculations of FCs and Log2

Data TREATMENT

IPA® Integration of data into Ingenuity Pathway Analysis

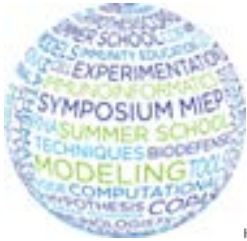
Core analysis
Identification of Canonical Pathways
Differences in expression
Network inference

Extraction of data and construction of SBML-compliant network

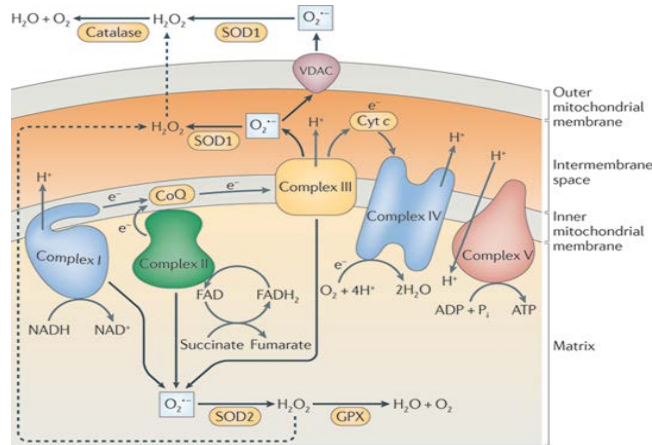
Importation into COPASI and ENISI for Model Calibration, Simulation, and Analysis

GENERATION of NEW HYPOTHESES

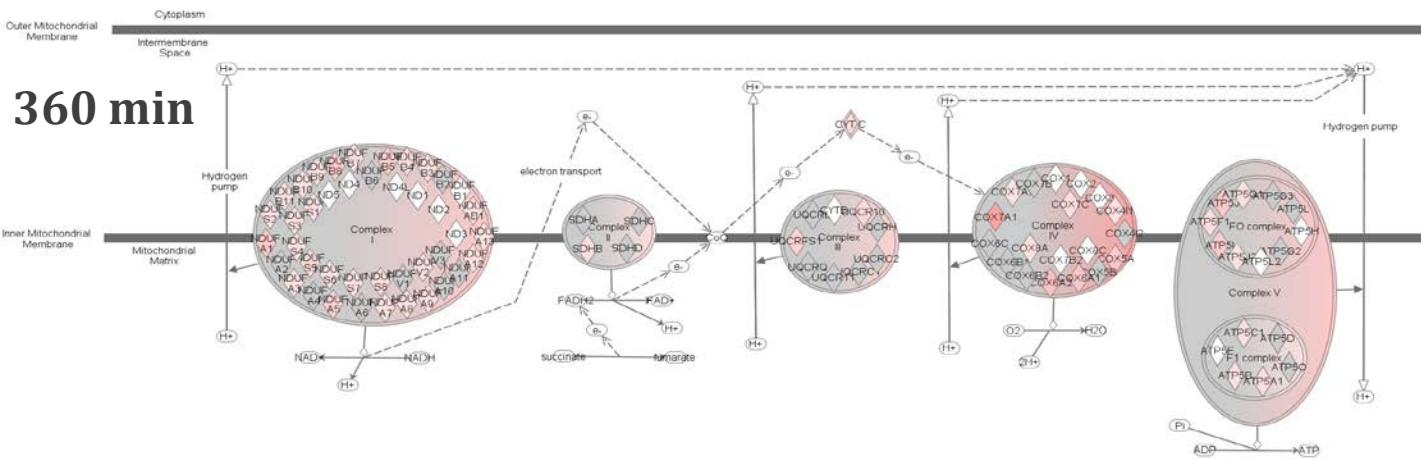
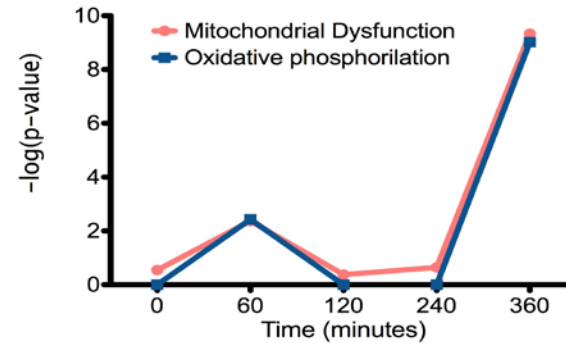




Response to *H. pylori*

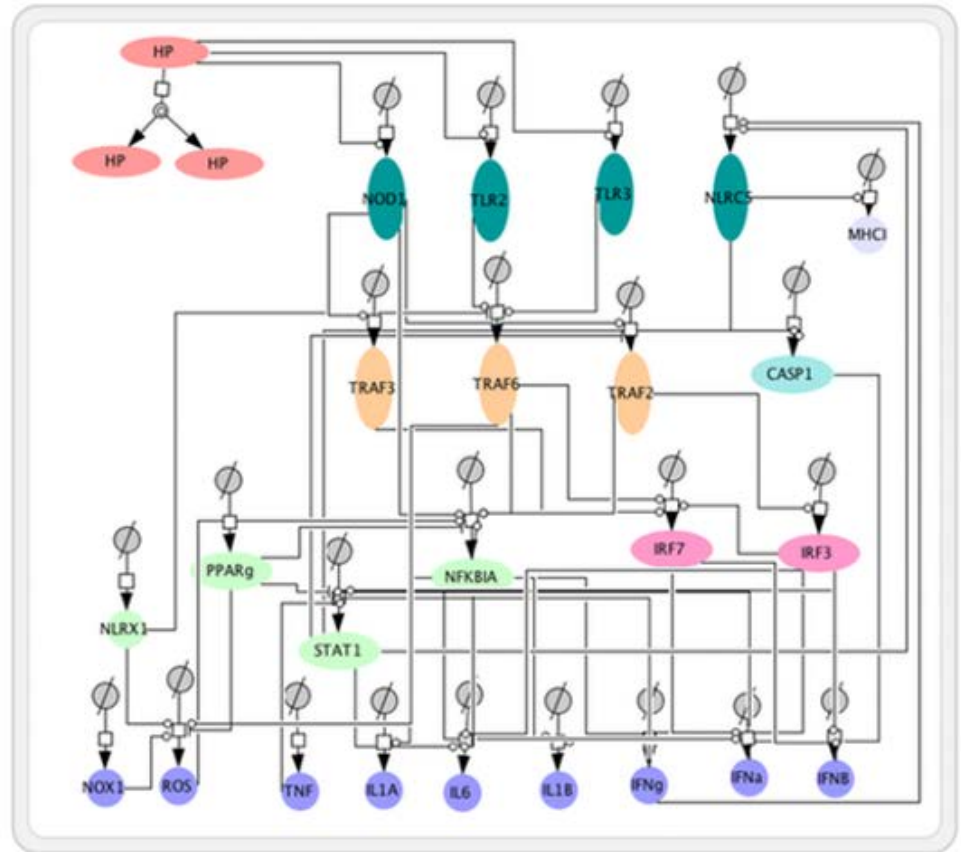
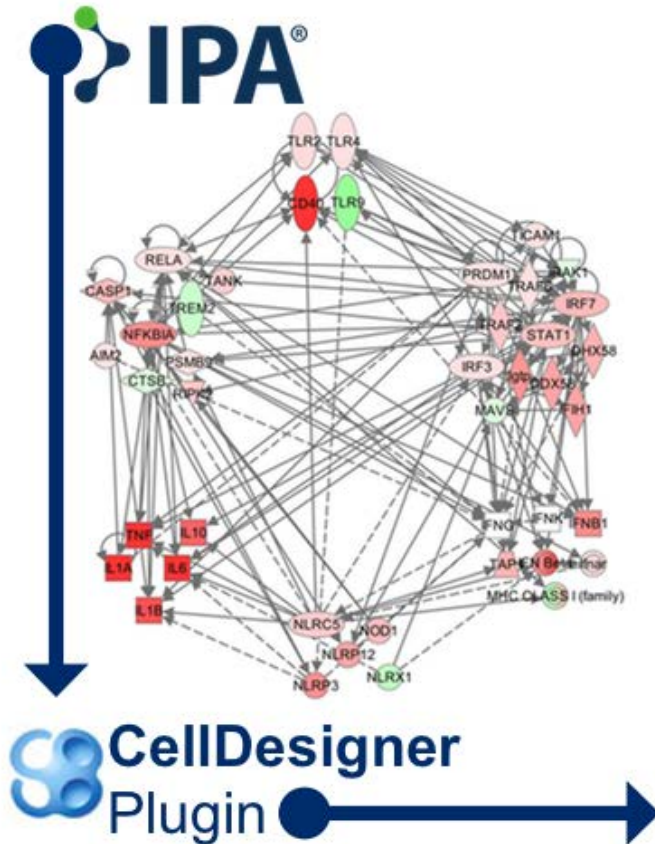


Upregulated pathways in LysMcre mice





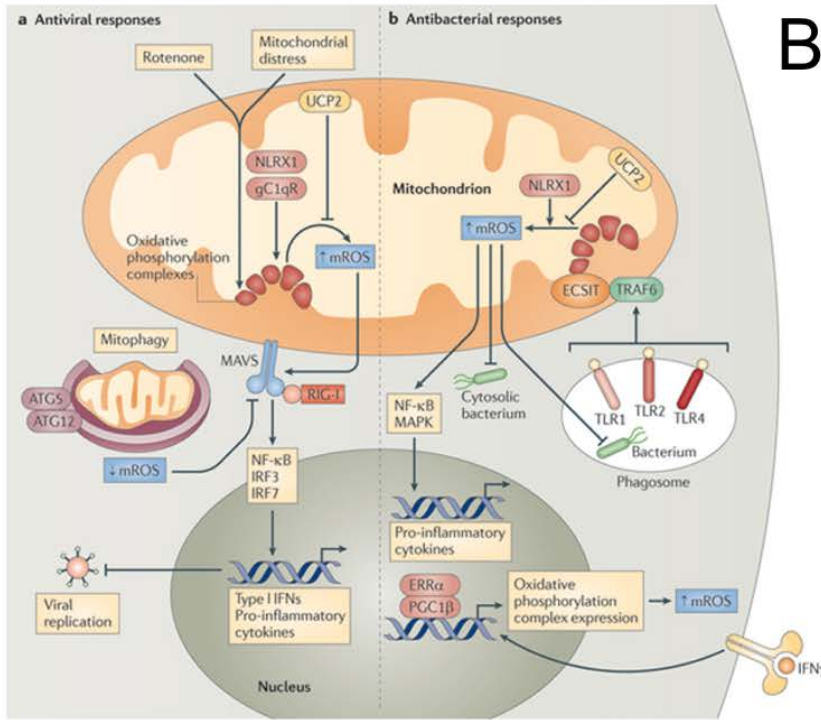
Modeling Innate Responses to *H. pylori*





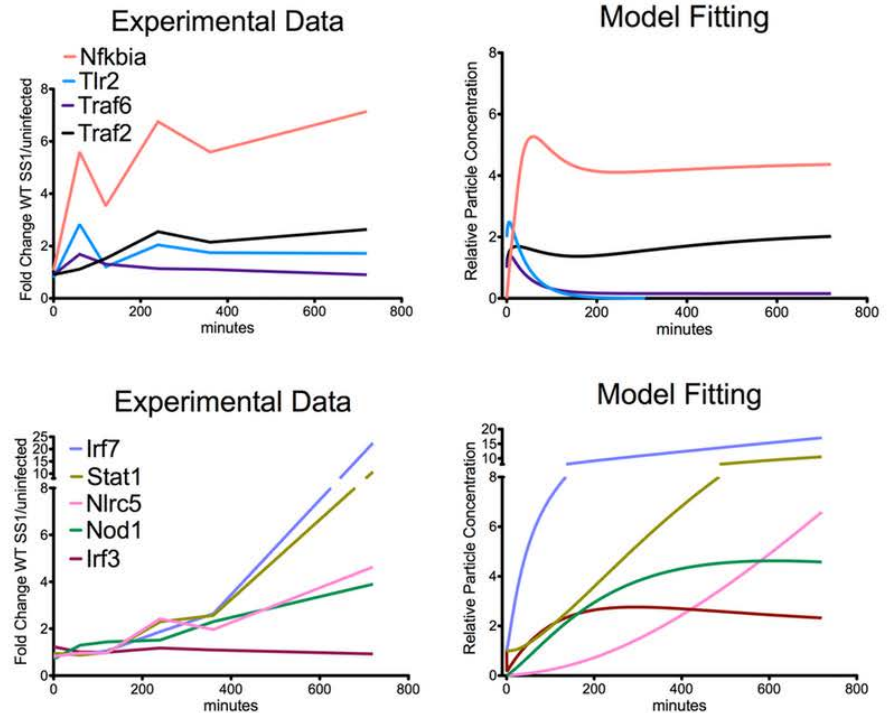
Modeling Innate Responses to *H. pylori*

A



Nature Reviews | Immunology

B





NLRX1 Sensitivity Analysis

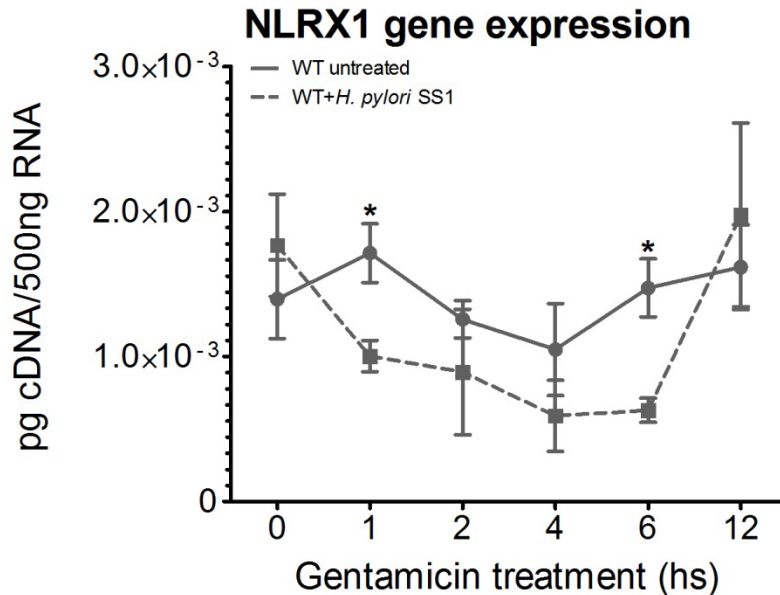
TRAF6	3.96E+16
IRF7	5.84E+16
PPARg	8.55E+16
HP	1.11E+17
NOD1	1.16E+17
TLR2	1.44E+17
IRF7	2.02E+17
STAT1	2.02E+17
TNF	3.20E+17
IRF3	3.51E+17
TRAF2	4.29E+17
MHCI	7.14E+18
IFNb	3.52E+21

- Local sensitivity analysis portrays relationship between NLRX1 and viral signaling cascades during intracellular *H. pylori* infection
- NLRX1 and IFN signaling demonstrate intimate link within our model; could translate biologically
- Sensitivities suggest there may be a role for NLRX1 in MHC class I signaling as well

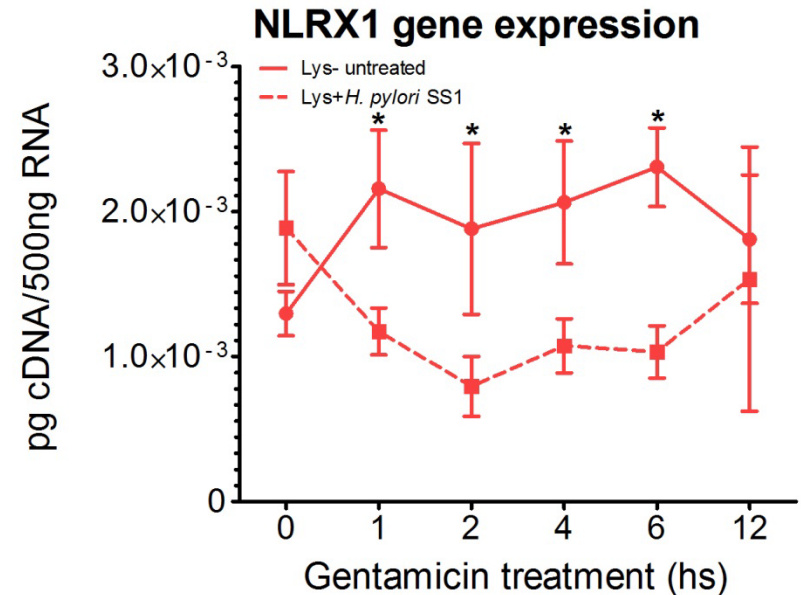


NLRX1 Expression Validation in Macrophages

Wild type



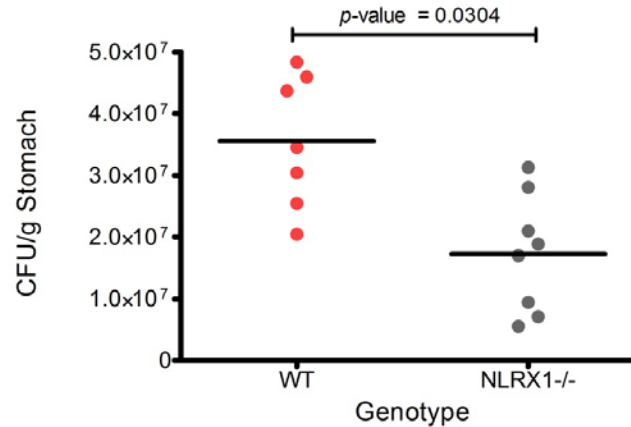
PPAR γ -deficient



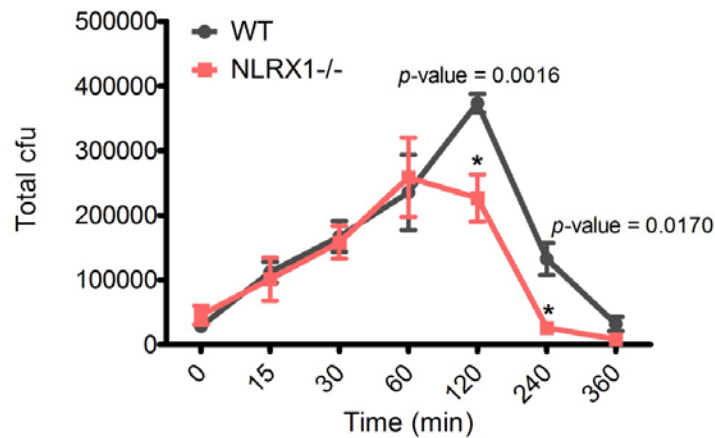


Validation in NLRX1 ko

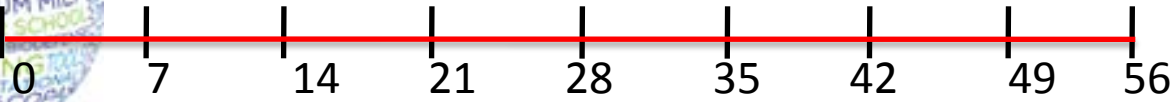
Reisolation 3 weeks post-infection



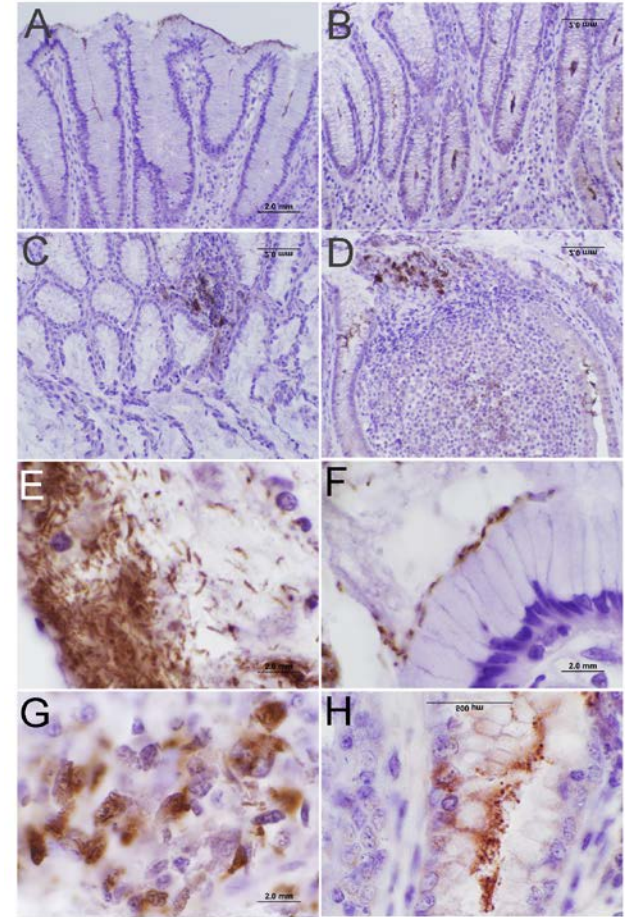
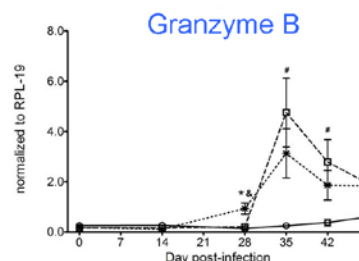
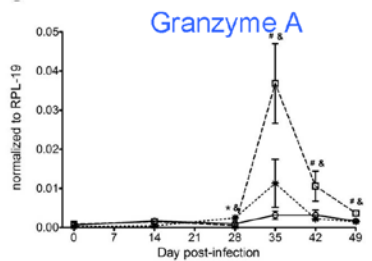
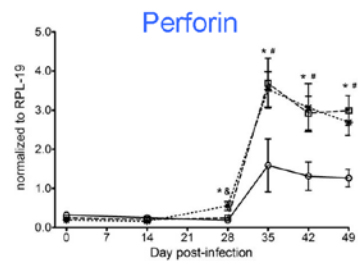
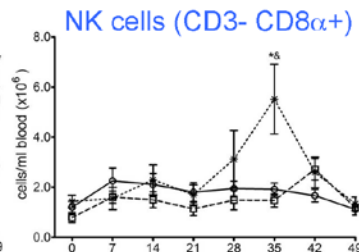
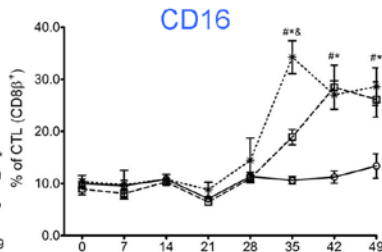
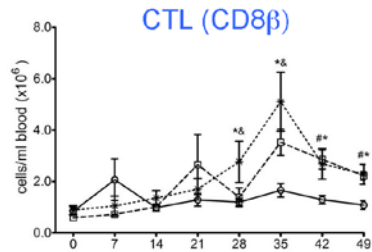
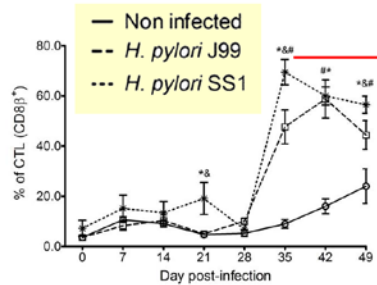
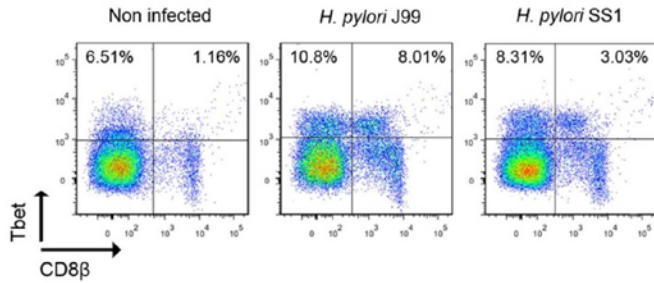
BMDM

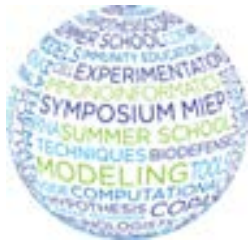


CD8+ T cell responses



Control/ *H. pylori* J99/SS1





Next Steps

- Run local and global sensitivity analyses by using COPASI
 - Sensitivities across scales to link molecular changes with tissue-level lesion formation
 - Sensitivities of the model to changes in NLRP3, NLRC5, NOD1
- Generation of *in silico* KOs
 - Calibration, sensitivity analysis, parameter estimation, simulation, model-driven hypothesis generation, stochastic simulations of sensitive nodes
 - Integrate this gene expression model with tissue level



MIEP Team

Virginia Bioinformatics Institute

Josep Bassaganya-Riera - Principal Investigator and Center Director

Jim Walke – Project Manager

Raquel Hontecillas - Immunology Lead

Barbara Kronsteiner-Dobramysl – Immunology Researcher

Xiaoying Zhang – Immunology

Pinyi Lu - Bioinformatics and Modeling

Adria Carbo - Immunology and Modeling

Kristin Eden- Immunology and Modeling

Monica Viladomiu – Immunology

Irving C. Allen - Immunology

Ken Oestreich - Immunology

Casandra Philipson – Immunology and Modeling

Eric Schiff, Patrick Heizer, Nathan Palmer, Mark Langowski, Chase Hetzel, Emily Fung – Interns

David Bevan- Education Lead



Funding: Supported by NIAID Contract No. HHSN272201000056C



MODELING IMMUNITY
TO ENTERIC PATHOGENS
Modeling Mucosal Immunity
Summer School & Symposium



Virginia Bioinformatics Institute (continued)

Madhav Marathe - Modeling Lead

Keith Bisset - Modeling Expert

Stephen Eubank - Modeling Expert

Tricity Andrew- Modeling GRA

Maksudul Alam - Modeling GRA

Stefan Hoops/Yongguo Mei - Bioinformatics Leads

Pinyi Lu – Bioinformatics GRA

Pawel Michalak – Genomics Tools

Nathan Liles - Bioinformatician

Xinwei Deng – Statistical Analysis

University of Virginia

Richard Guerrant - Infectious Disease Expert

Circle A. Warren - Infectious Disease Expert

David Bolick - Sr. Laboratory and Research Specialist



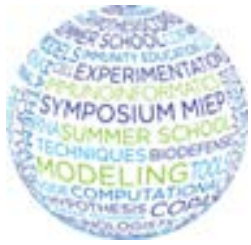
MMI Acknowledgements

- **Adria Carbo**
- **Kimberly Borkowski**
- **David Bevan**
- **Jim Walke**
- **Kathy O'hara**
- **Rachel Robinson**
- **Traci Roberts**
- **Tiffany Trent**
- **Kristopher Monger**
- **Ivan Morozov**
- **Josh Dunbar**



Entereroaggregative E. coli





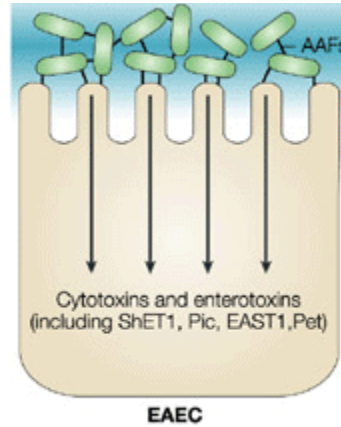
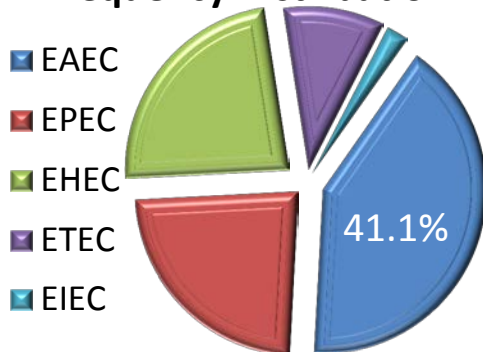
EAEC

a leading cause of enteritis & persistent diarrhea worldwide

High risk populations:

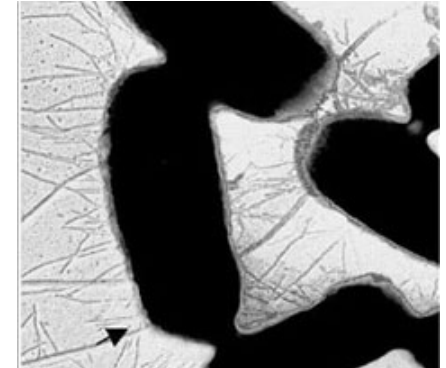
- Travelers
- HIV infected
- Malnourished children

Diarrheagenic Isolate Frequency Distribution



AAF fimbria:

primary virulence factor attributed to mucosal adherence



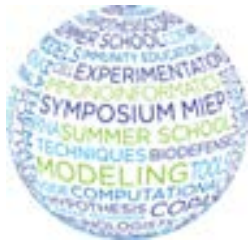
Fli-C flagellin:

responsible for IL-8 secretion

Dispersin:

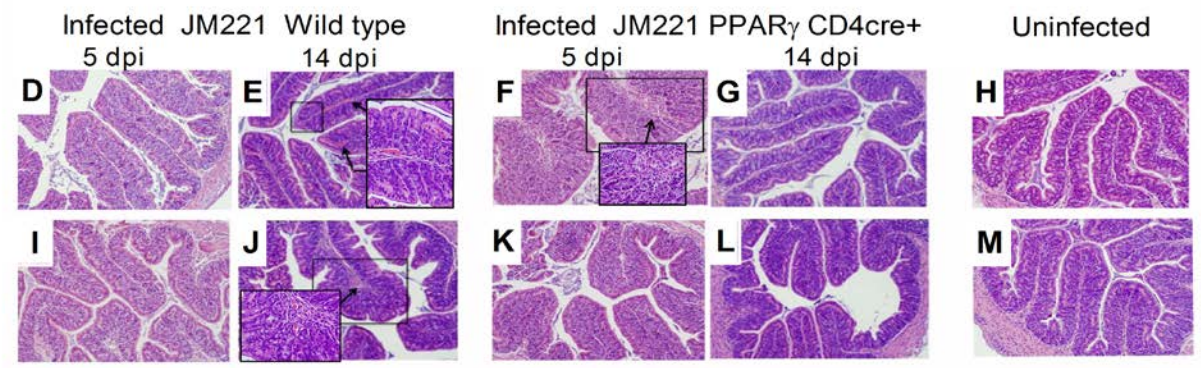
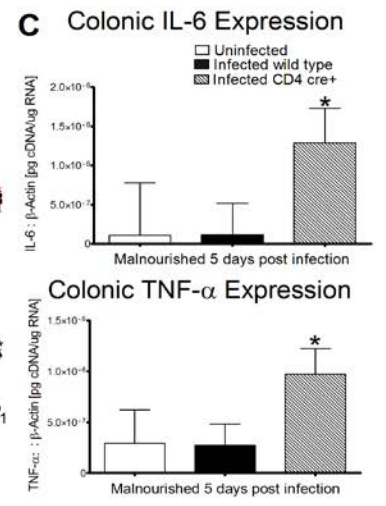
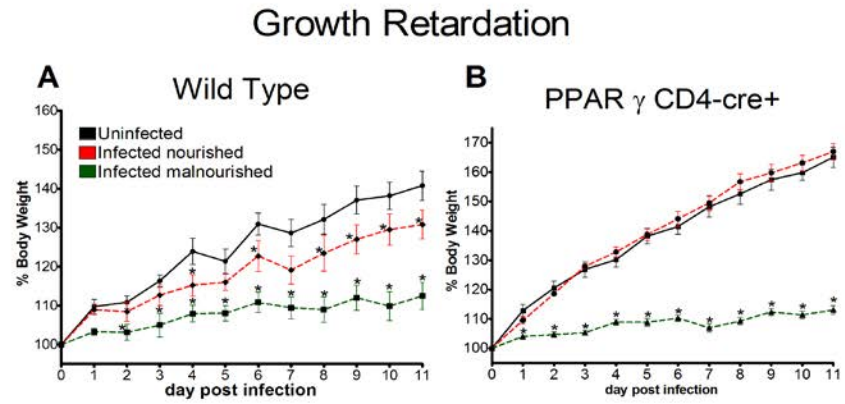
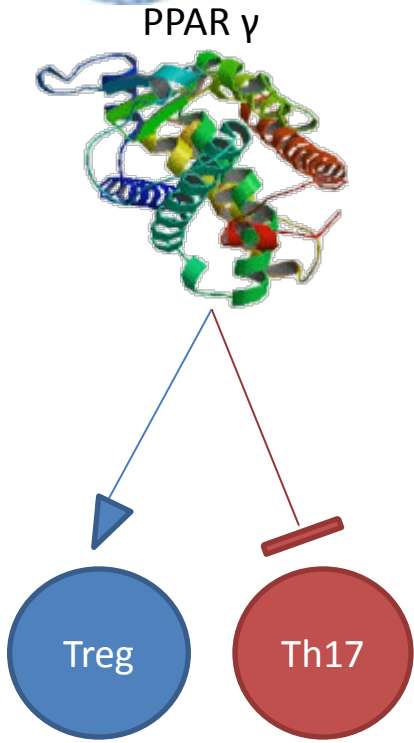
Allows dissociation from biofilm and spread of colonization

EAEC

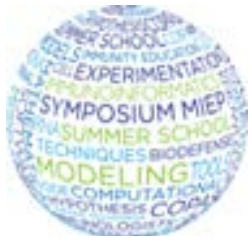


- Our *in vivo* murine model data suggested a beneficial role for Th17 cells and IL17A
- We used computational modeling to predict the effects of enhancing effector T cell populations during EAEC infection

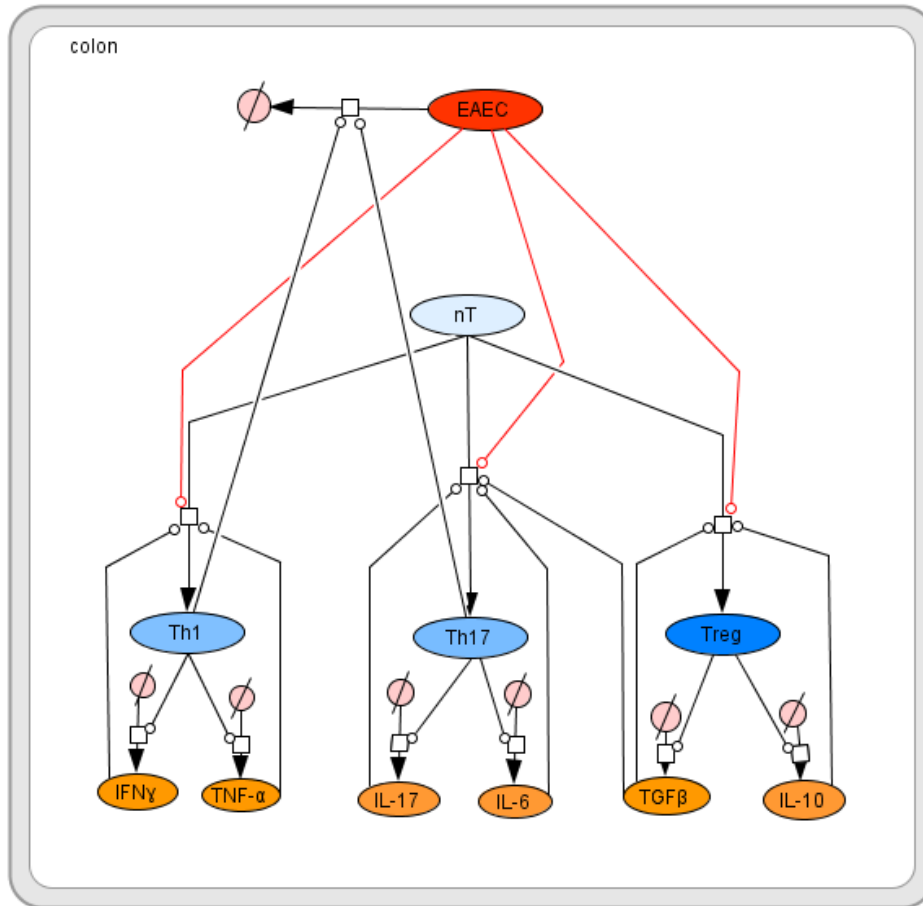
Targeting PPAR γ as an inflammatory mediator



- 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



EAEC T cell Model



Ordinary differential equations from EAEC T cell differentiation model

$$\frac{d([Treg])}{dt} = -V_{colon} \cdot (K1_{(re17)} \cdot [Treg]) + ([emT] \cdot (K1_{(re21)} \cdot [TGF\beta] + K2_{(re21)} \cdot [IL10])) + V_{colon} \cdot ([nt] \cdot (K1_{(re7)})) \cdot [EAEC]$$

$$\frac{d([Th17])}{dt} = -V_{colon} \cdot (K1_{(re16)} \cdot [Th17]) + ([emT] \cdot (K1_{(re9)} \cdot [IL6] + K2_{(re9)} \cdot [TGF\beta] + K3_{(re9)} \cdot [IL17])) + V_{colon} \cdot ([nt] \cdot K1_{(re7)}) \cdot [EAEC]$$

$$\frac{d([Th1])}{dt} = -V_{colon} \cdot (K1_{(re16)} \cdot [Th1]) + ([emT] \cdot (K1_{(re20)} \cdot [IFN\gamma] + K2_{(re20)} \cdot [TNF\alpha])) + V_{colon} \cdot ([nt] \cdot K1_{(re8)}) \cdot [EAEC]$$

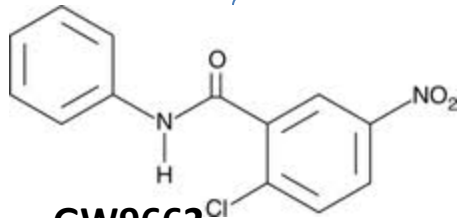
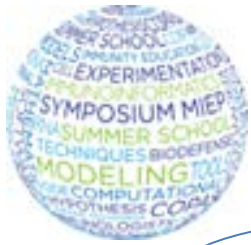
$$\frac{d([EAEC])}{dt} = -V_{colon} \cdot ([EAEC] \cdot K1_{(re22)} \cdot [Th1] + K2_{(re22)} \cdot [Th17])$$

Parameter estimation → Calibration

Bacterial Load in Feces		T cell populations using Flow Cytometry			
time	EAEC quantification	time	IL17 producing Th17	IFNγ producing Th1	Regulatory T cells
3	7123.13	14	90888.75	145422	327199.5
3	8110.87	14	92340	295488	203148
3	7029.98	14	65667.6	98816.64	86464.56
3	9648.13	14	38165.85	45002.25	64881.945
3	6342.8	14	103774.65	42936.39	45900
3	7262.77	14	65667.6	34765.2	38628
3	5831.49	14	56359.8	61065.36	31311
3	8028.2	14	73266.32143	103356.5486	113933.2864



Pharmacological blockade

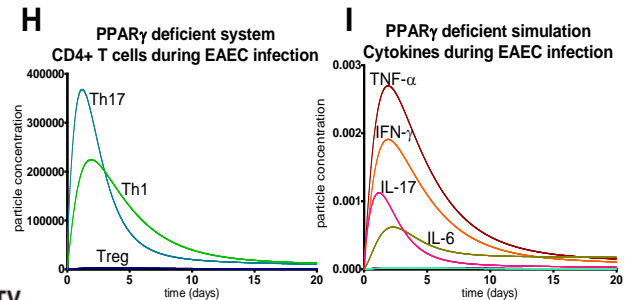
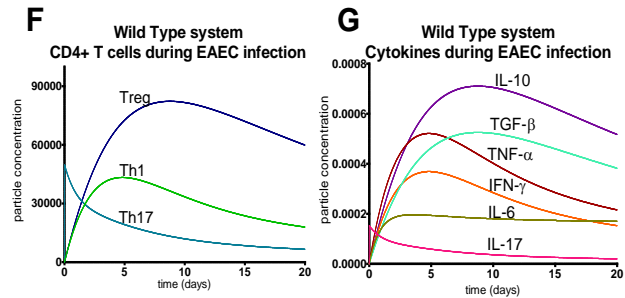
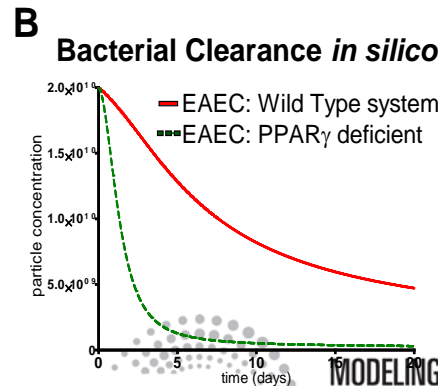
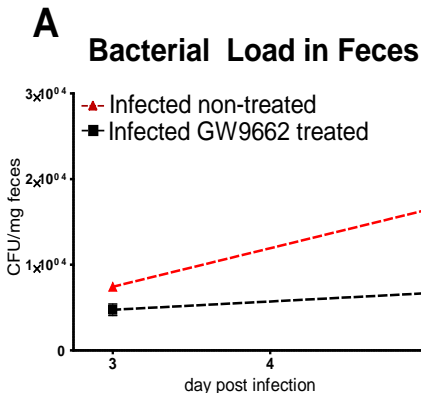
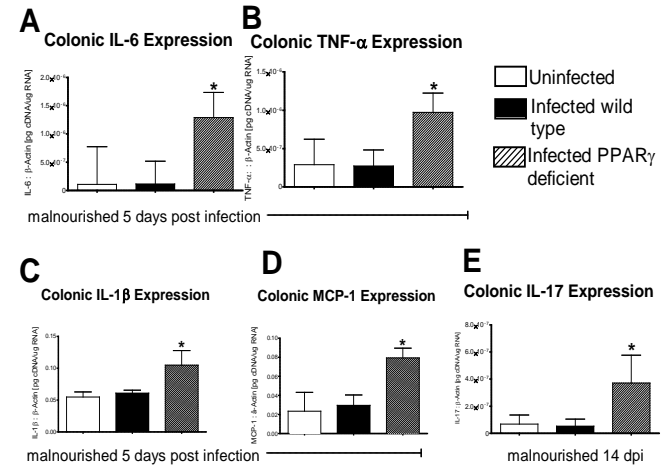


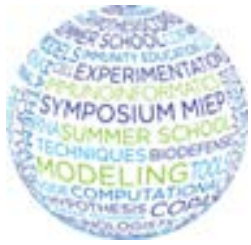
GW9662

a potent PPAR γ antagonist

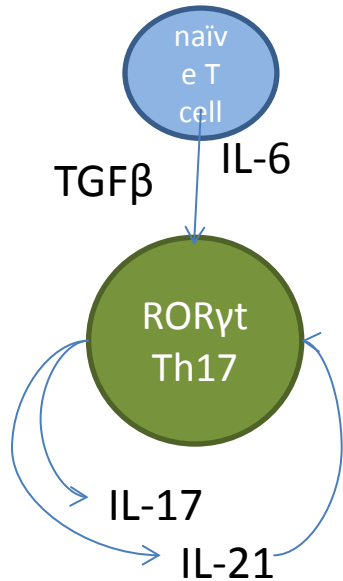


Administration of **GW9662** promoted the upregulation of proinflammatory cytokines that correlated to significantly *lower levels of EAEC in feces* early during infection

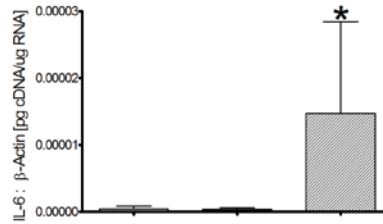




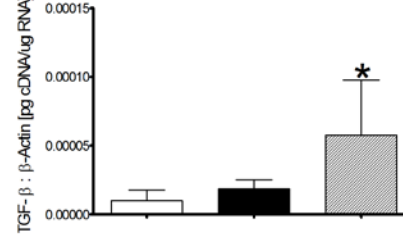
Antimicrobial Peptides



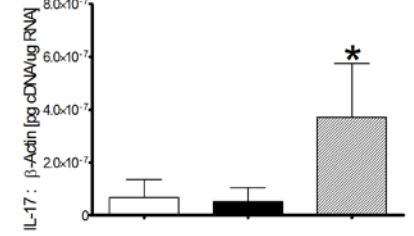
Colonic IL-6 Expression



Colonic TGF-β Expression

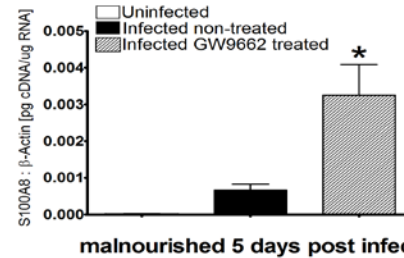


Colonic IL-17 Expression



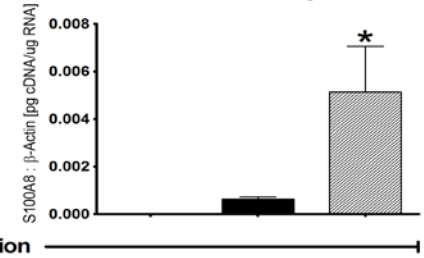
A

Colonic S100A8 Expression



B

Colonic S100A9 Expression



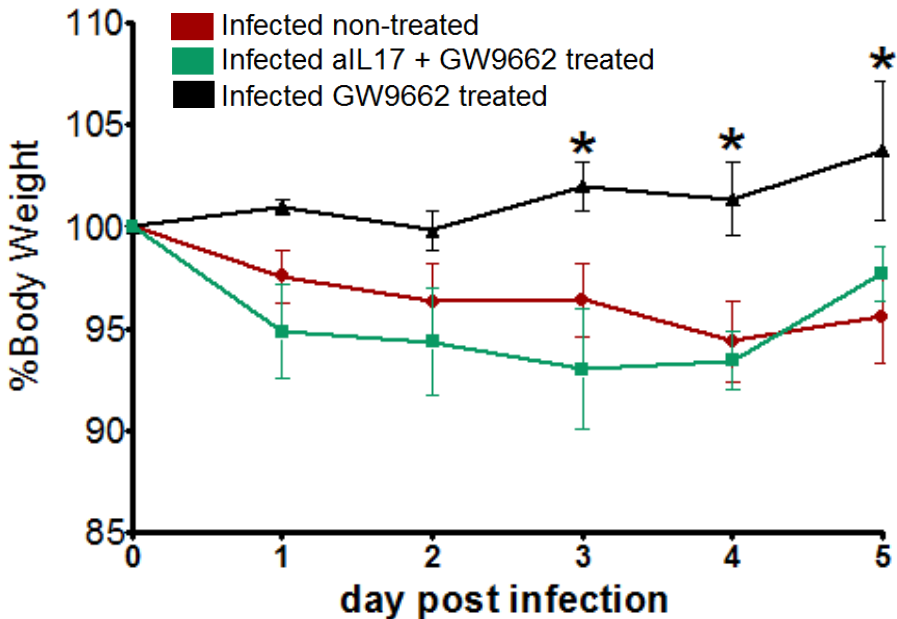
Pharmacological blockade of PPARγ beneficial

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

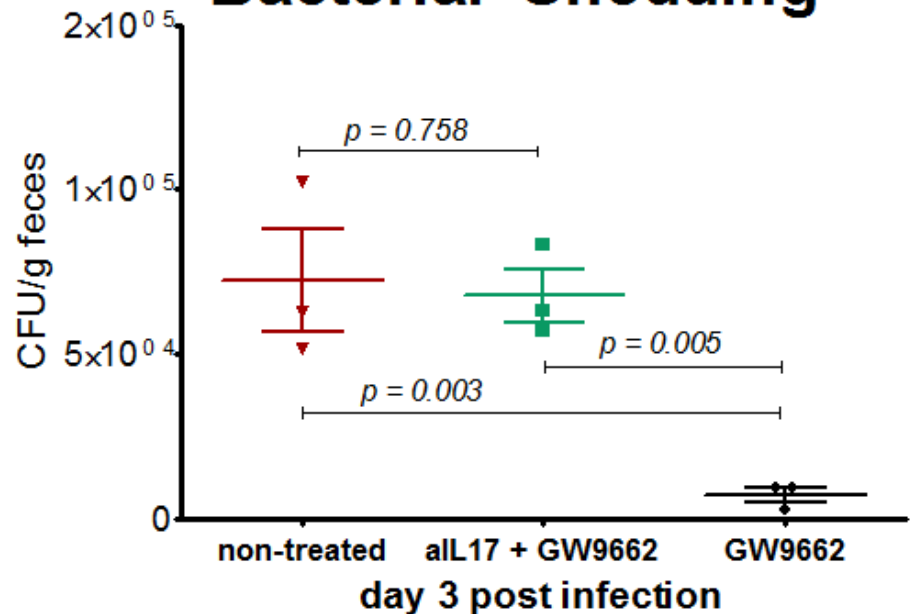
IL-17A Neutralization abrogates benefits of PPAR γ Blockade



Growth Retardation



Bacterial Shedding



Anti-IL-17A neutralizing antibody abrogates the beneficial effects of GW9662 in ameliorating disease based on weight loss and bacterial shedding