Center for Modeling Immunity to Enteric Pathogens

MIB Annual Meeting
November 2-3, 2011

A contract between the
Virginia Bioinformatics Institute and the
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Program: Modeling Immunity for Biodefense

MIEP Programmatic 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

- Use the newly generated knowledge to develop more efficacious vaccines and therapeutics

VIRGINIA BIOINFORMATICS INSTITUTE AT VIRGINIA TECH

MIEP

MODELING IMMUNITY TO ENTERIC PATHOGENS

Task Name	% Complete	Finish
Modeling Immunity to Enteric Pathogens	11%	Fri 10/30/15
Administrative	0%	Fri 10/30/15
Contract Initiation	100%	Thu 9/30/10
Contract Initiation Meeting	100%	Thu 11/4/10
Fully executed contract received	100%	Thu 11/18/10
Quarterly Conference Calls	0%	Thu 8/20/15
Schedule quarterly teleconferences with COTR	100%	Fri 11/12/10
Yr1 Q1 conference call	100%	Thu 2/17/11
Yr1 Q2 conference call	100%	Thu 5/19/11
Yr1 Q3 conference call	100%	Thu 8/18/11
Quarterly Progress Reports	0%	Thu 7/30/15
Yr1 Q1 progress report	100%	Fri 1/28/11
Yr1 Q2 progress report	100%	Fri 4/29/11
Yr1 Q3 progress report	100%	Fri 7/29/11
IT Security Plan	100%	Fri 10/29/10
IT Risk Assessment	100%	Fri 10/29/10
Security Training Roster	100%	Wed 10/13/10
IMWG	100%	Mon 10/18/10
Recommendations due to CO	100%	Mon 10/18/10
Annual Site Visits	0%	Mon 6/9/14
Annual Site Visit 1	100%	Thu 6/9/11
Modeling	0%	Thu 9/17/15
ENISI Enteric Immunity Simulator	0%	Fri 9/11/15
ENISI v0.9 available (user interface to results of ENISI model runs)	100%	Wed 9/28/11
COPASI	0%	Thu 9/17/15
Training workshop on COPASI for MIEP team	100%	Mon 12/20/10
COPASI Model Updates (SBML and COPASI format)	0%	Thu 9/10/15
COPASI Yr1 Q1 model update	100%	Wed 10/20/10
COPASI Yr1 Q2 model update	100%	Tue 2/15/11
COPASI Yr1 Q3 model update	100%	Fri 6/17/11
COPASI Yr1 Q4 model update	100%	Thu 9/15/11
Immunological Experimentation	10%	Thu 9/24/15
H. pylori	21%	Fri 6/12/15
Expand collection of H. pylori strains for experimentation	100%	Wed 12/29/10
Add GFP to H. pylori strains to allow tracking of movement	100%	Wed 9/28/11
H. pylori animal models for parameter estimation and model calibration	21%	Fri 6/12/15
expand existing mouse colonies and purchase new	100%	Wed 11/24/10
initiate H. pylori challenge studies	100%	Fri 12/31/10
collect H. pylori data for model calibration and reporting	100%	Wed 3/30/11
Prepare list of H. pylori challenge experiments in mice for COPASI	100%	Tue 3/15/11
EAEC	13%	Mon 8/24/15
Approval received from NIAID to issue subcontracts	100%	Mon 1/10/11
Execute subcontract with UVA	100%	Mon 2/28/11
Expand collection of EAEC strains for experimentation	100%	Fri 12/31/10
EAEC animal models for parameter estimation and model calibration	13%	Mon 8/24/15
initiate EAEC challenge studies	100%	Fri 5/20/11
collect EAEC data for model calibration and reporting	100%	Tue 8/23/11
Prepare list of EAEC challenge experiments in mice for ENISI	100%	Tue 9/27/11
Human subjects	0%	Thu 9/24/15
Initiate recruitment	100%	Mon 9/26/11
Bioinformatics	17%	Wed 9/30/15
Database	17%	Wed 9/30/15
Complete initial database design for experimental data	100%	Fri 12/31/10
Manual submission process to ImmPort complete	100%	Thu 9/29/11
Website	0%	Thu 9/27/12
Portal v1 (layout and navigation, publications, initial CD4 model)	100%	Thu 9/30/10
Portal v1 (data, analysis, ImmPort connection)	100%	Fri 12/31/10
Portal v2 (data download & models)	100%	Thu 3/31/11
Link to IMMPORT	100%	Fri 12/31/10
Education	0%	Thu 6/5/14
Release basic COPASI training materials	100%	Fri 1/14/11
Release ENISI tutorial on website	100%	Thu 9/29/11

MIEP Year 1

- Developed four ODE-based models using COPASI

- Created an agent-based model using the newly developed ENISI 0.9

- Launched public website, improved model annotation tool (CellPublisher) and released data

- Performed immunology experimentation studies on *H. pylori* and EAEC

- Disseminated MIEP's efforts through AAI, MARCE and research collaborations
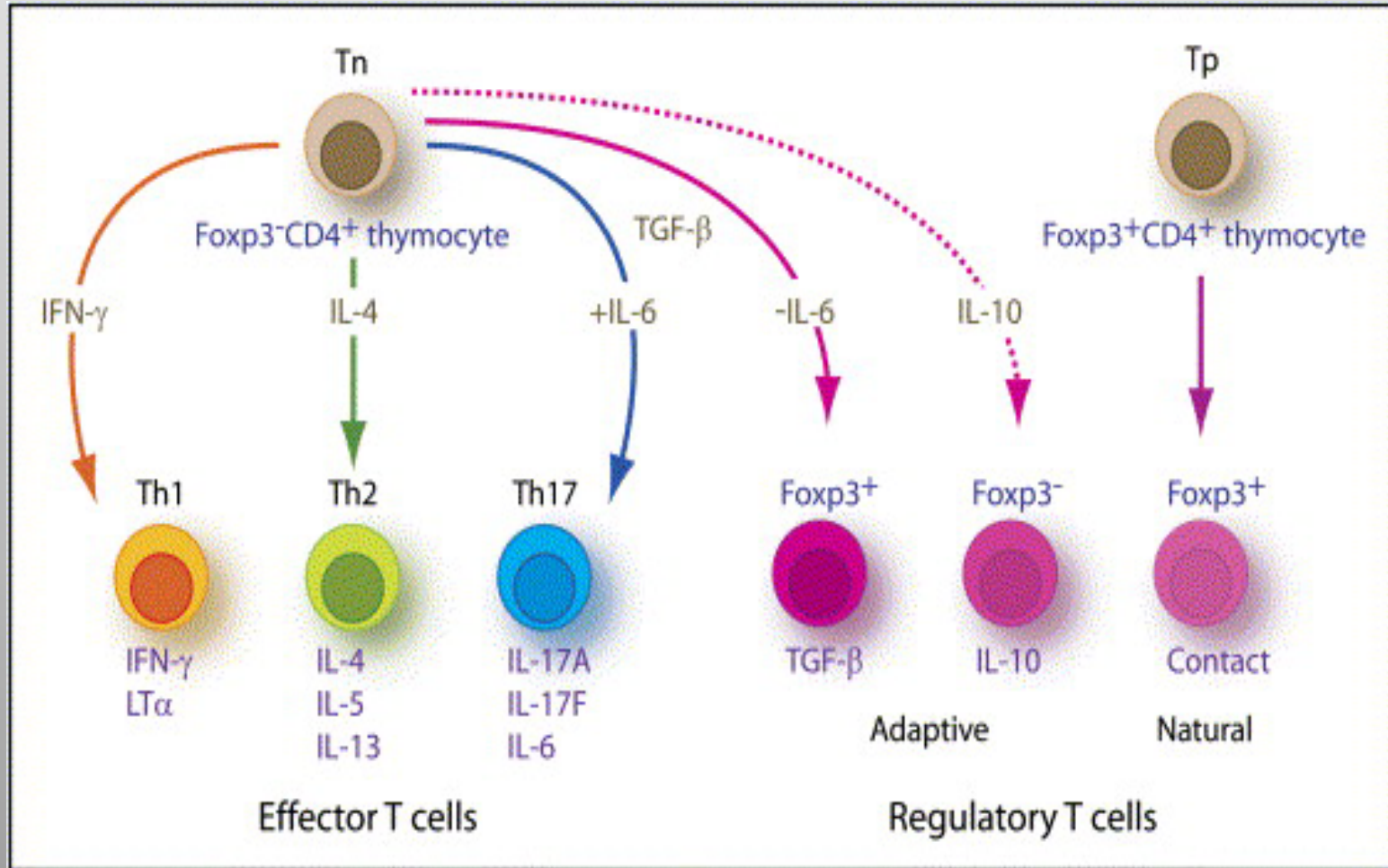
MODELING CD4+ T CELL DIFFERENTIATION

CD4+ T cell Differentiation

CD4+ T Cell Differentiation Model
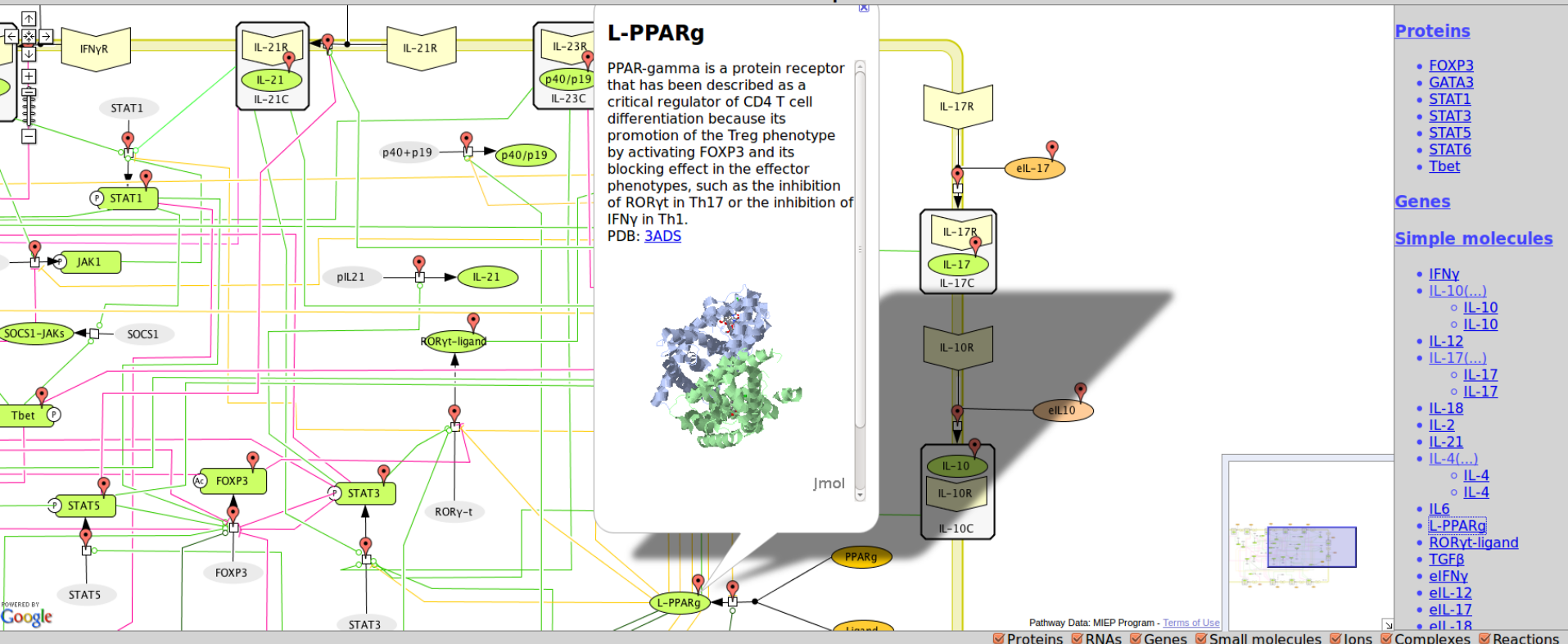
CD4+ T cell Computational Model

CD4+ T cell Computational Model
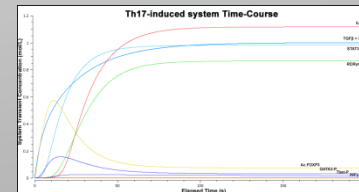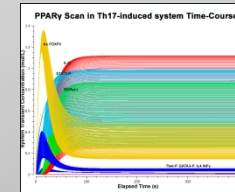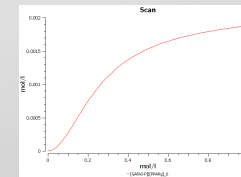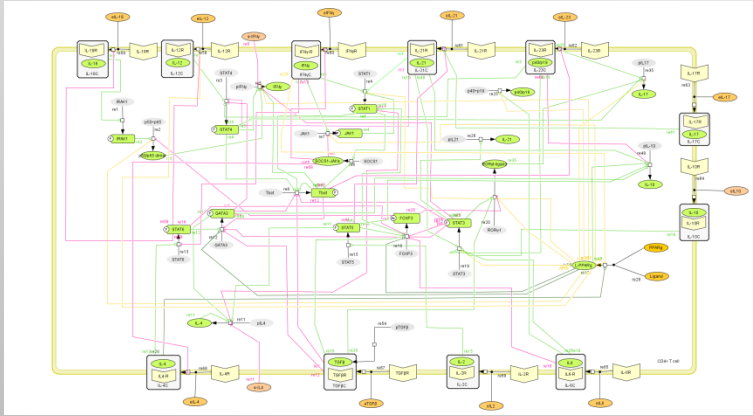
Model Calibration and Time-Course Results
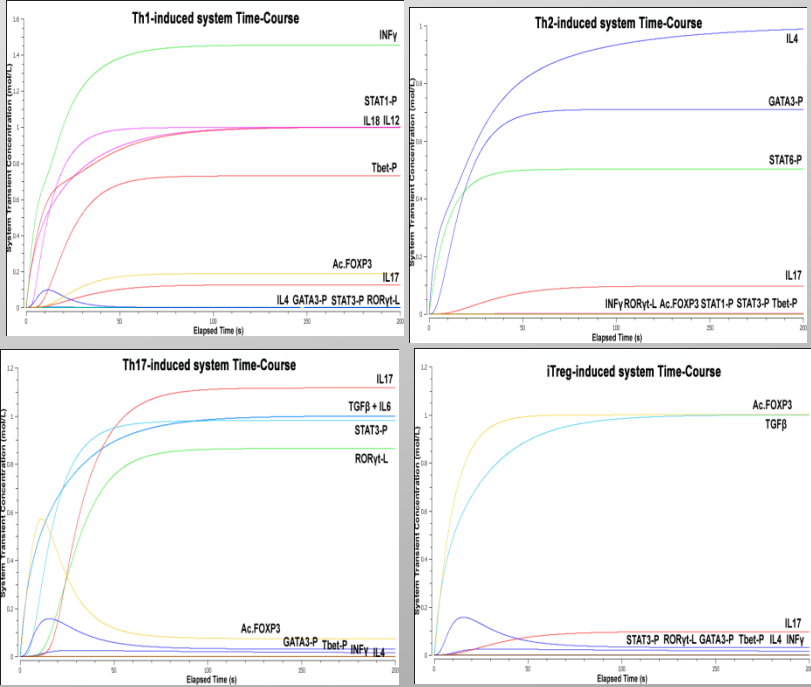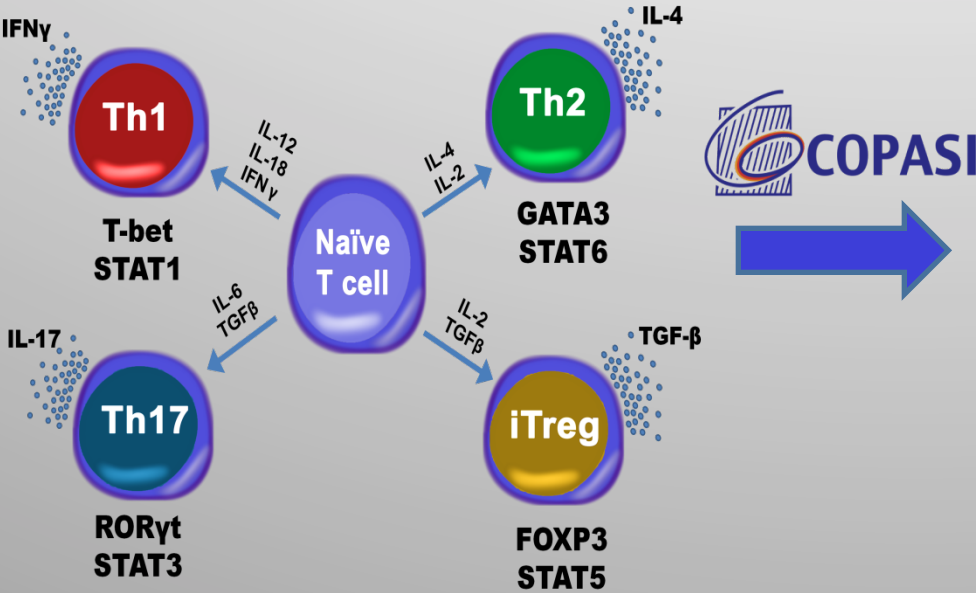
In Silico Experimentation

Time-Course experiment

- Addition of TGFβ and IL-6 to the system
- At t=300s. PPARγ was added
- The inductors were maintained
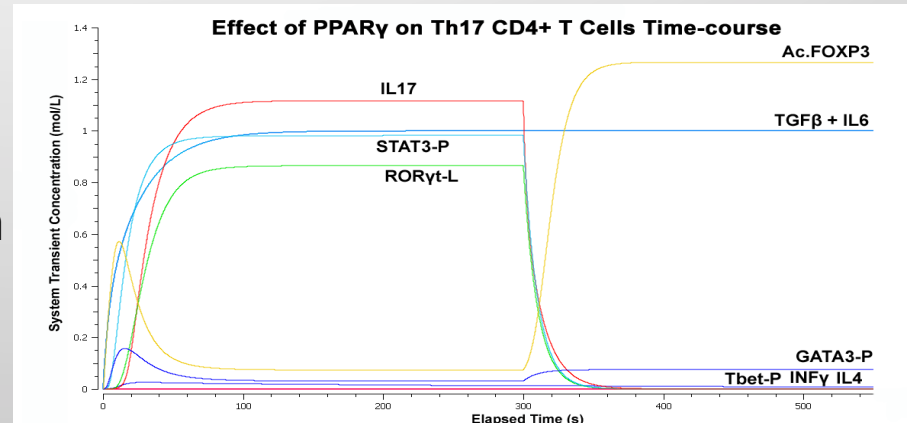
PPARγ Scan experiment

- Addition of TGFβ and IL-6 to the system
- Increasing concentrations of PPARγ
- IL-17, RORγt and FOXP3 assessed.
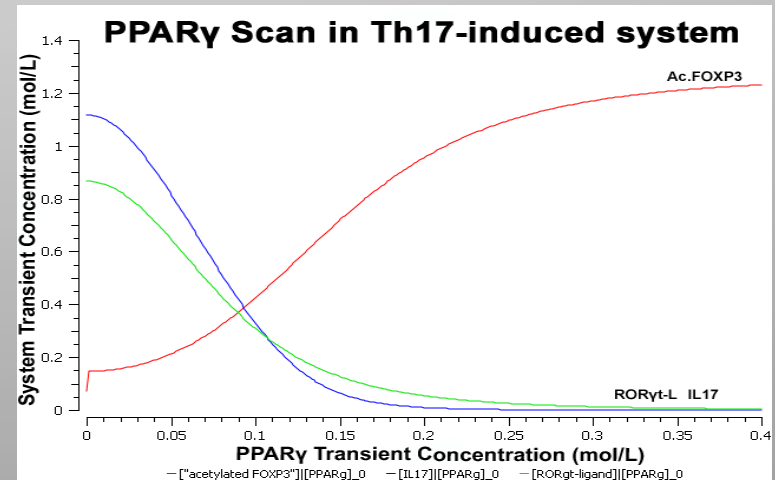
In Silico Experimentation

Time-Course and Scan combination experiment

- Addition of TGFβ and IL-6 to the system
- Increasing concentrations of PPARγ
- IL-17, RORγt, STAT-3 and FOXP3 assessed.
- Assessment over time
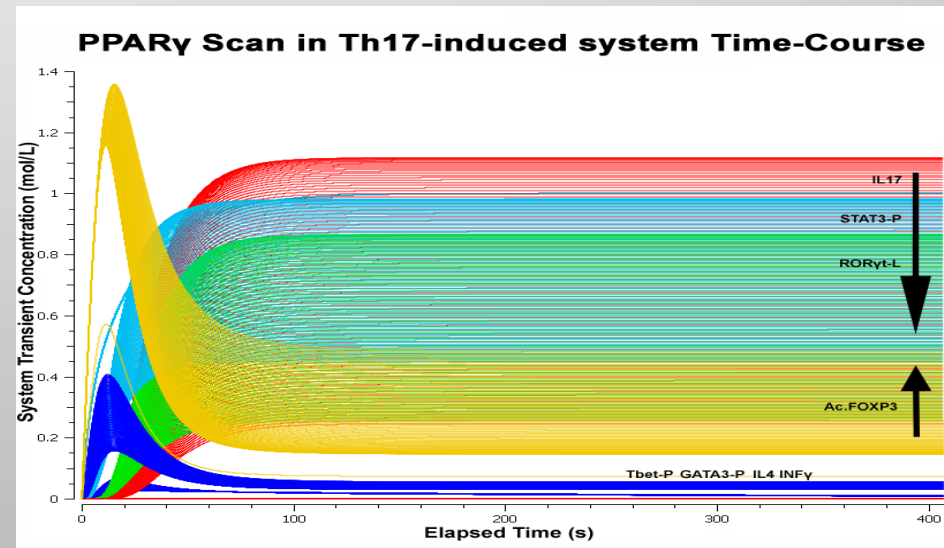
In vivo validation results
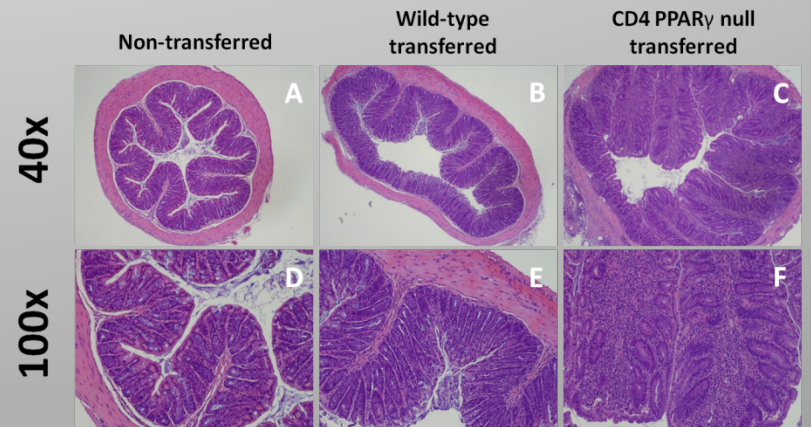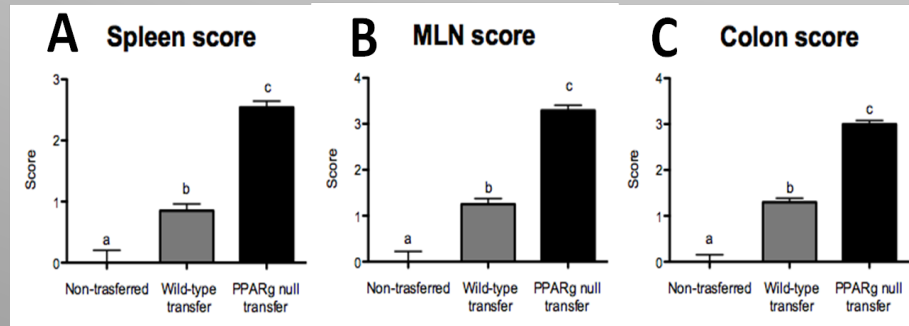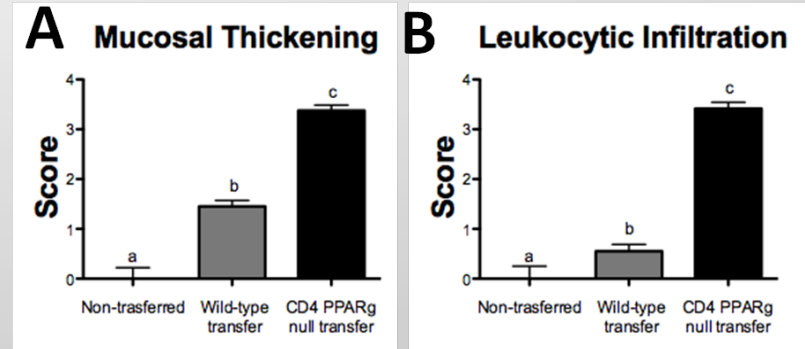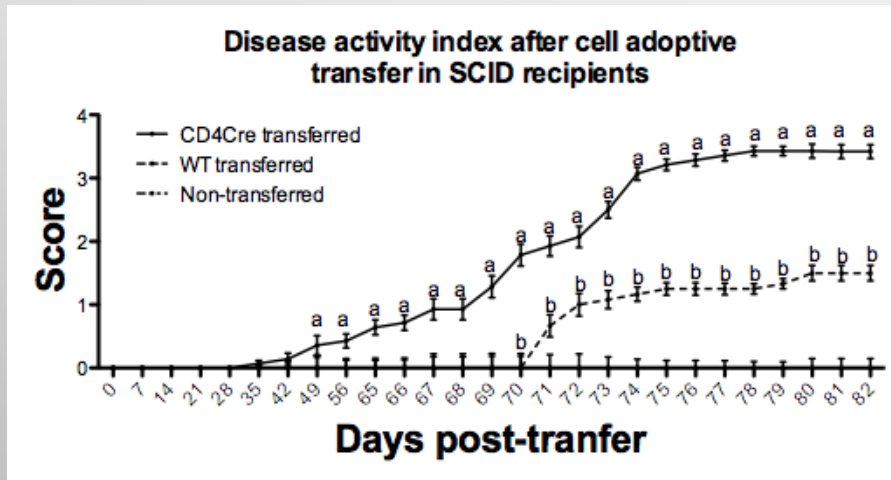
In vivo validation results
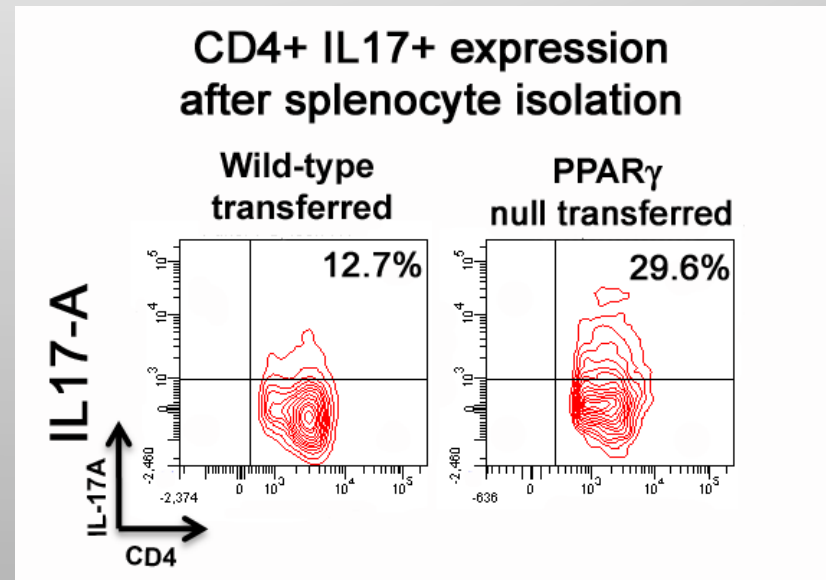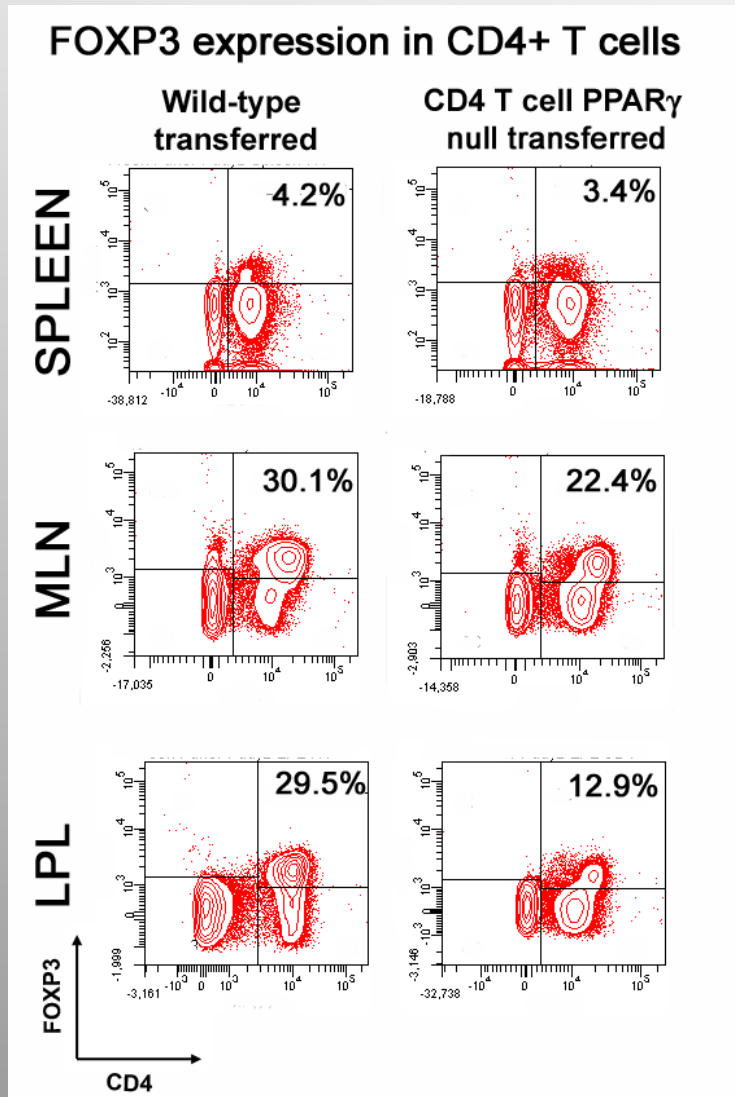
Validation Approaches
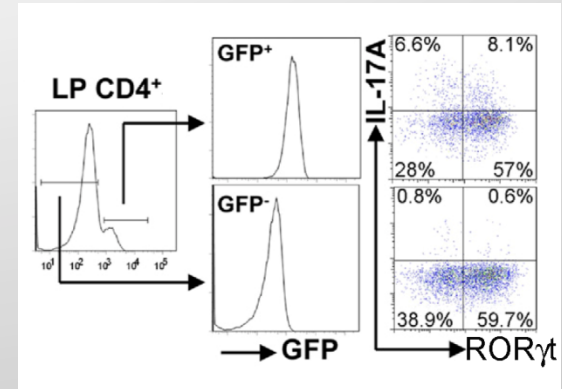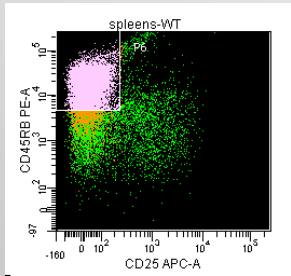
RORγt^{gfp/+} Donor Mice
CD4+ CD62L^{high} CD25-
CD45RB^{high} T cells

Adoptive Transfer to
RAG2-/- mice

Th17 cells in LP

Treatment of mice and Th17
cells with PPAR γ agonists

Assessment of phenotype
switch from Th17 to iTreg
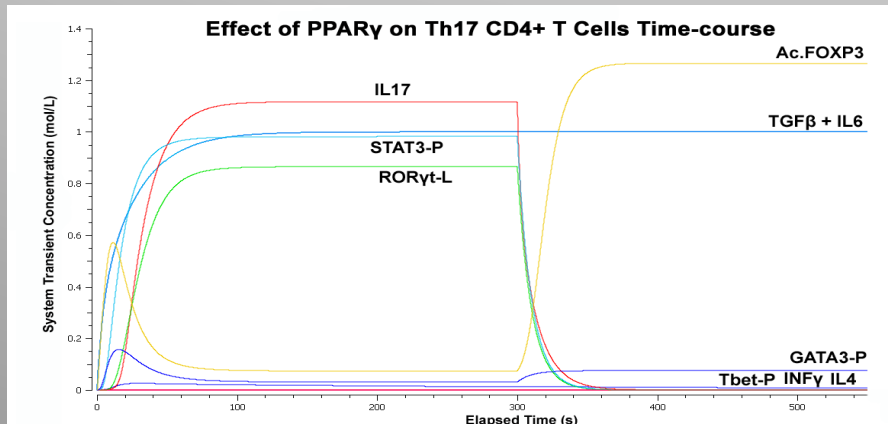
Conclusions and Future Directions

1. The model mimics the behavior of the Th1, Th2, Th17 and Treg phenotypes in a robust and reproducible way
2. The model can predict novel unforeseen CD4+ T cell behaviors
3. The model can be re-calibrated with *H. pylori,* EAEC or other pathogen data
4. In combination with molecular modeling, it will help discover novel immune therapeutics
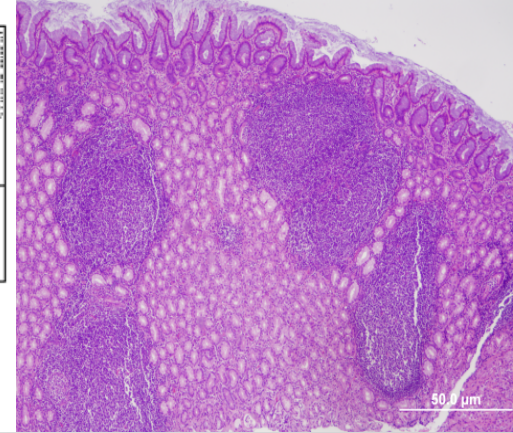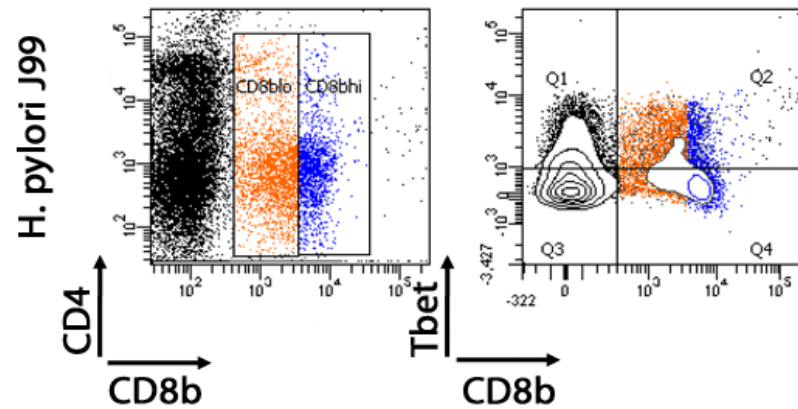
Modeling Immune Responses to *Helicobacter pylori*
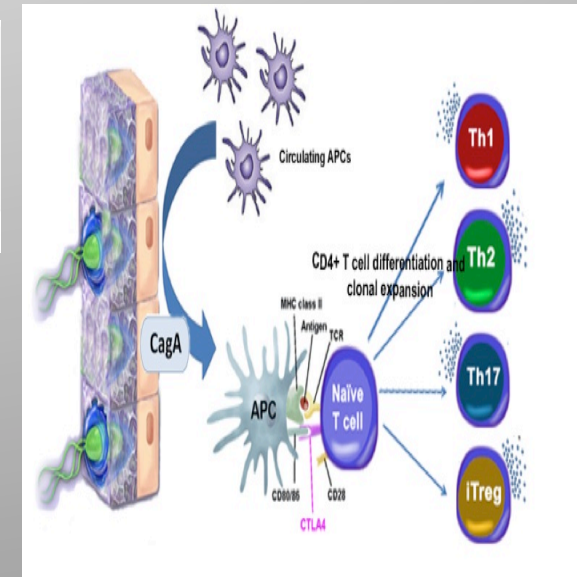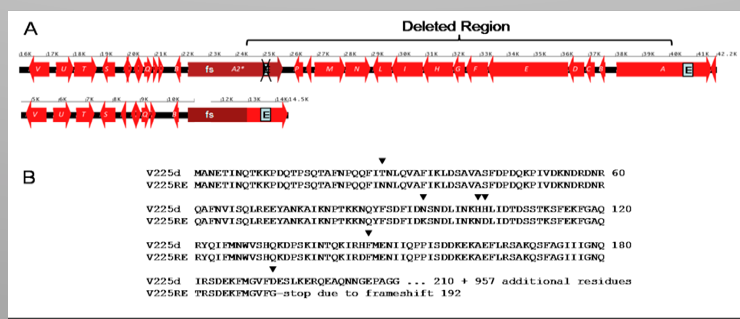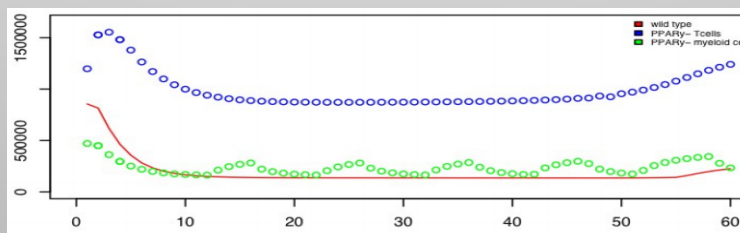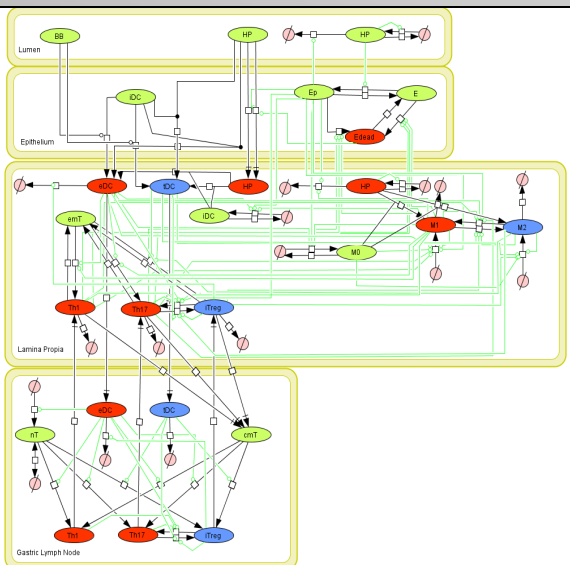
H. pylori computational model

Included as a potential immunogenic stimulant

Inflammatory

Regulatory

Can participate in both Regulatory & inflammatory

Variables
HP: *H. pylori*
Epithelial cells: E, Ep, Edead
Dendritic cells: iDC, eDC, tDC
Macrophages: M0, M1, M2
Tcells: nT, emT, cmT, Th1, Th17, iTreg
Gastric tissues
Lumen
Epithelium
Lamina propria
Gastric lymph nodes

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H. pylori Model Annotation

Model calibration: Time course study of T cell responses to *H. pylori* 26695
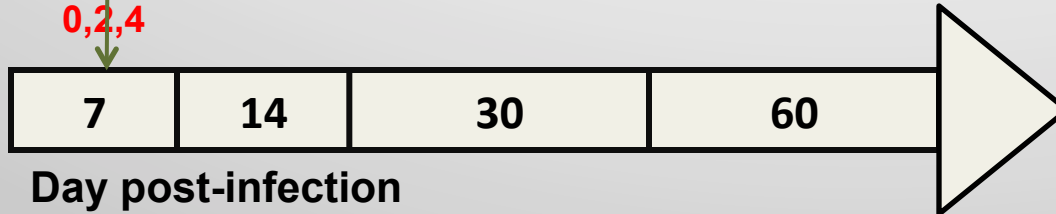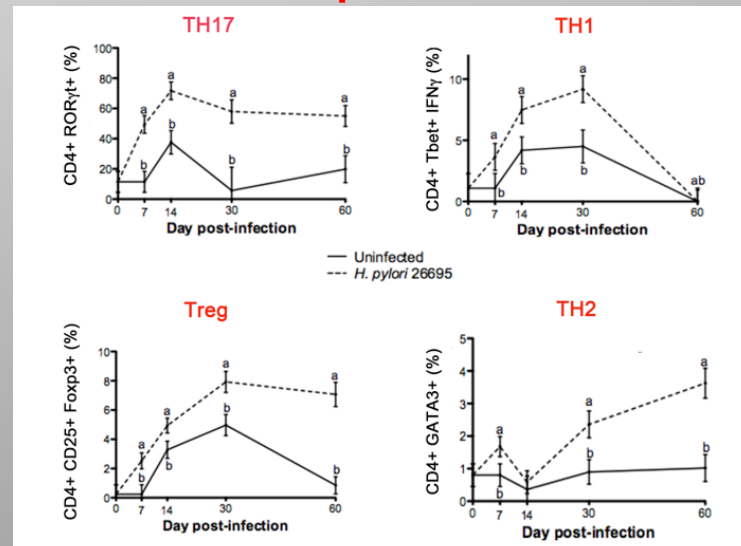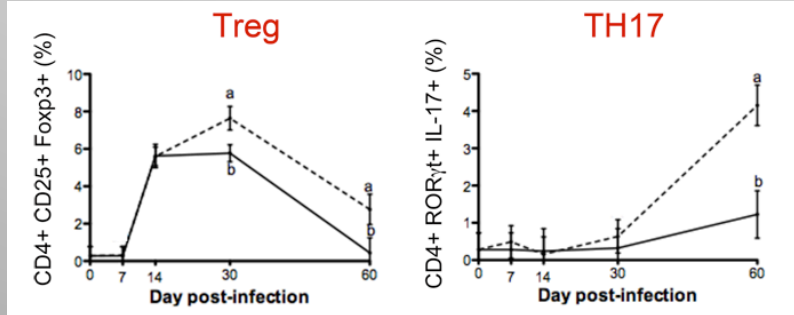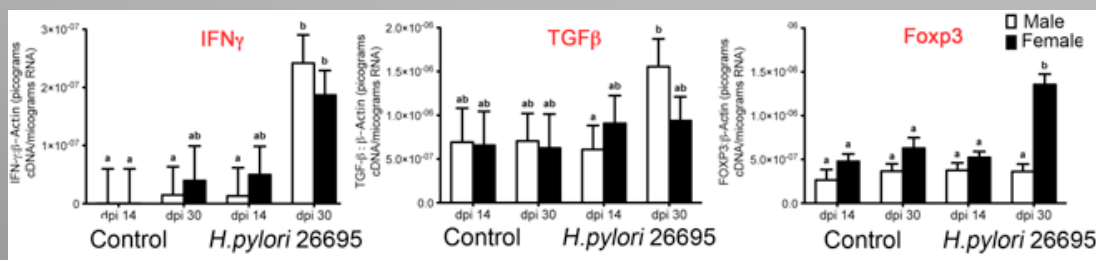
In silico Infection of PPARγ knockout mice
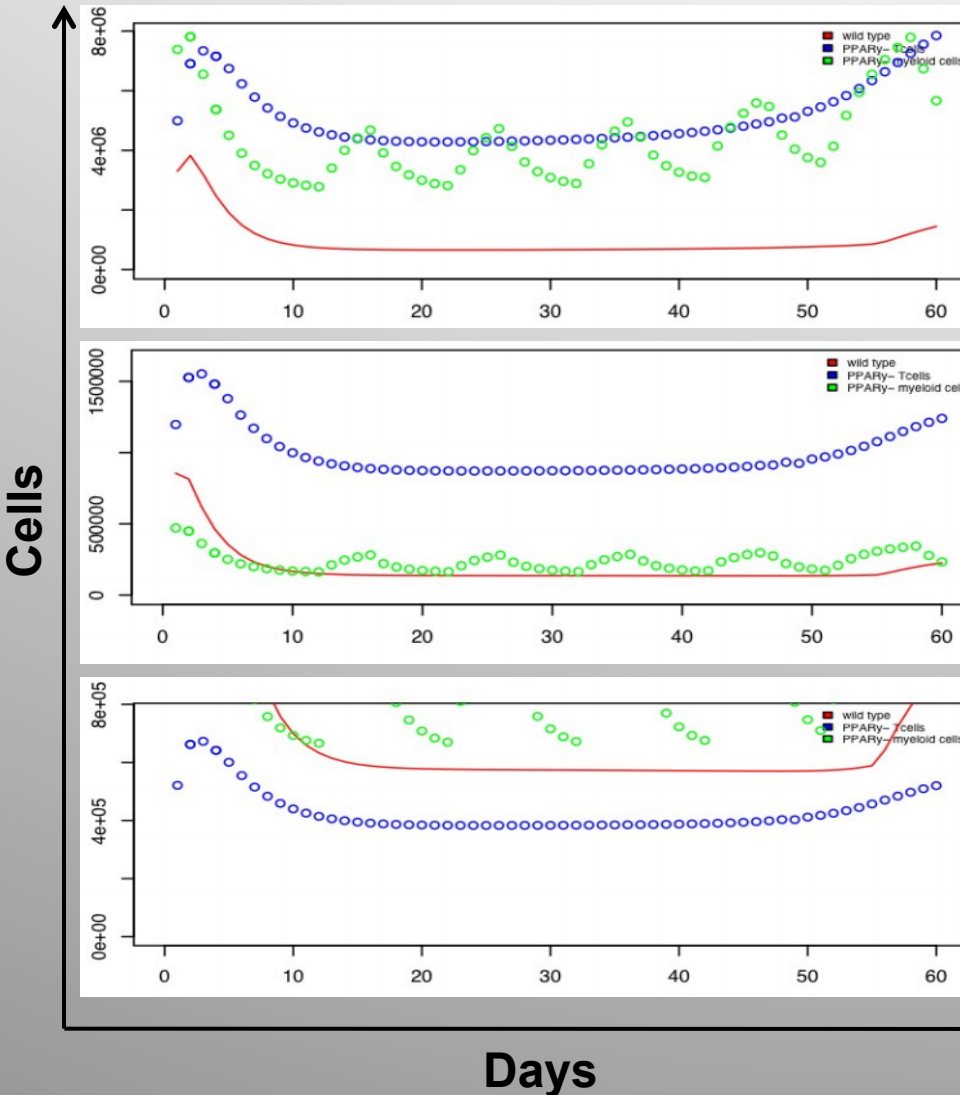
C57BL/6 Wild type
T cell-specific PPARγ null mice
Myeloid cell-specific PPARγ null mice

Th1

Th17

iTreg

Cells

Days

T cell subset dynamics in the gastric lamina propria of wild-type, T cell-specific and myeloid cell-specific PPAR γ null mice

Immune responses towards *H. pylori* in pigs

2 doses of 5x10^7 CFU
Control
H. pylori J99
H. pylori SS1

Mucosal responses

0 2   7   14   21   28   35   42   49   56

Systemic responses: PBMC
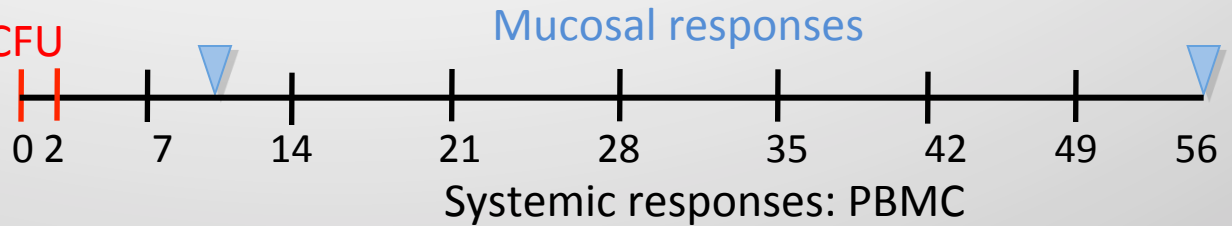
Stomach lesions dpi 57: cardiac region
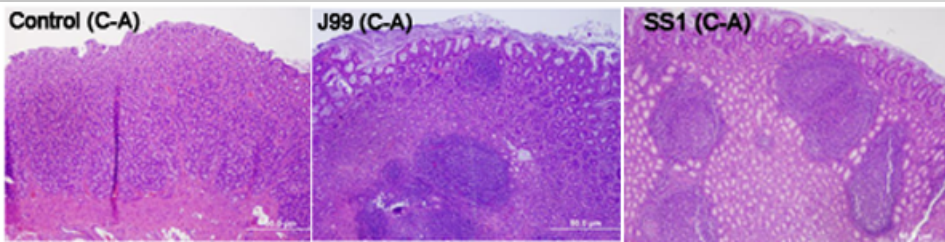
Bacterial persistence: re-isolation

Stomach region	*H.pylori* J99		*H.pylori* SS1	
	9 dpi	57 dpi	9 dpi	57 dpi
C-A	1/2	0/3	1/2	2/3
C-B	0/2	0/3	1/2	3/3
F-A	0/2	0/3	0/2	2/3
F-B	0/2	0/3	0/2	2/3
P-A	0/2	0/3	0/2	3/3
P-B	0/2	1/3	0/2	3/3
P-C	0/2	0/3	0/2	2/3
Total isolates	1	1	2	17

Systemic Th1 response
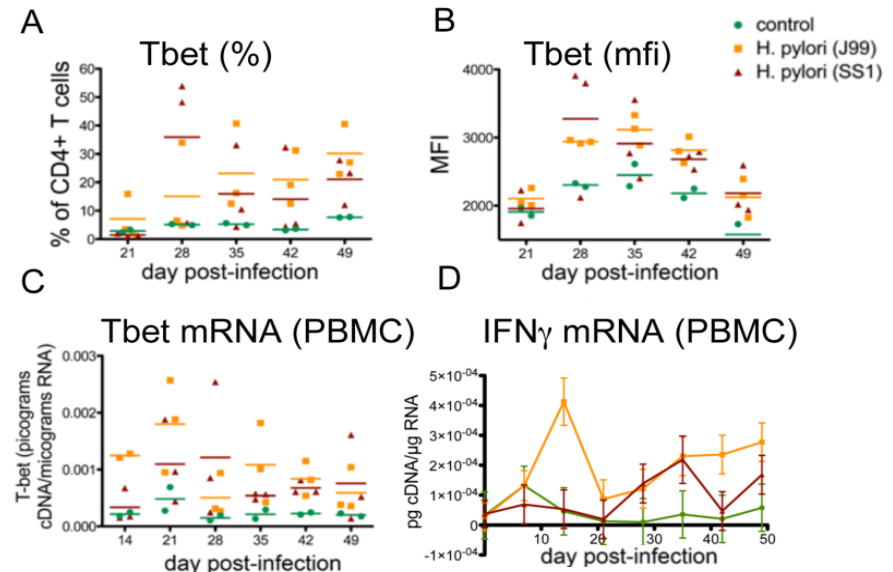
Immune responses towards *H. pylori* in pigs

2 doses of 5x10^7 CFU
Control
H. pylori J99
H. pylori SS1

Mucosal responses

0 2 7 14 21 28 35 42 49 56
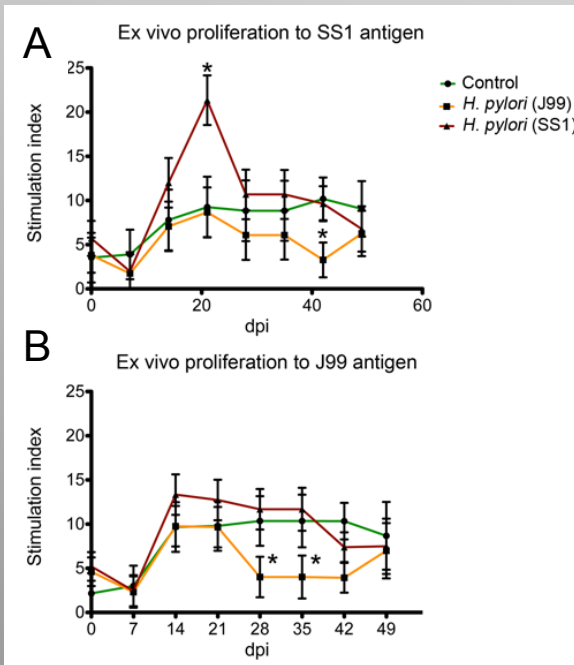
Systemic responses: PBMC
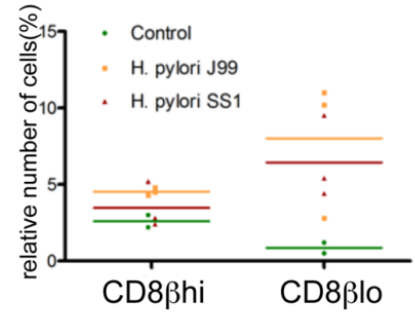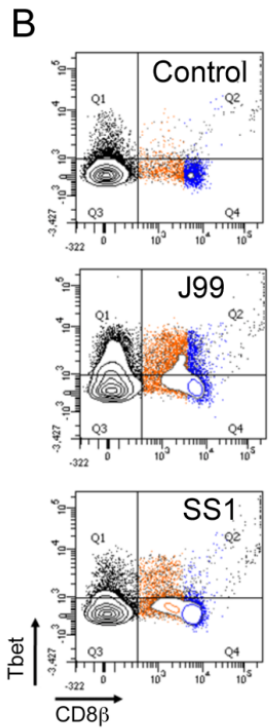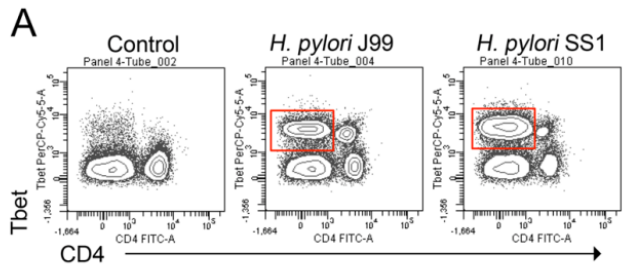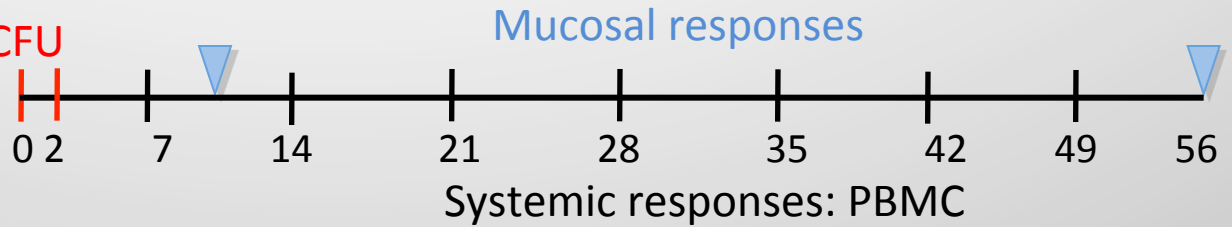
A. Increased CD4$^-$/Tbet$^+$

B. Increased CD8β^{lo}/Tbet$^+$

C. Increased NK and CD8β
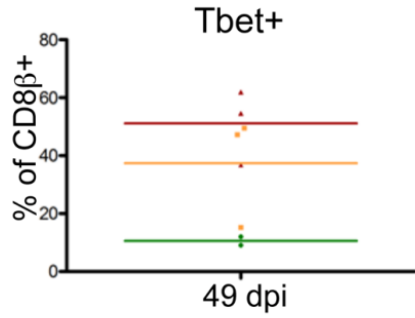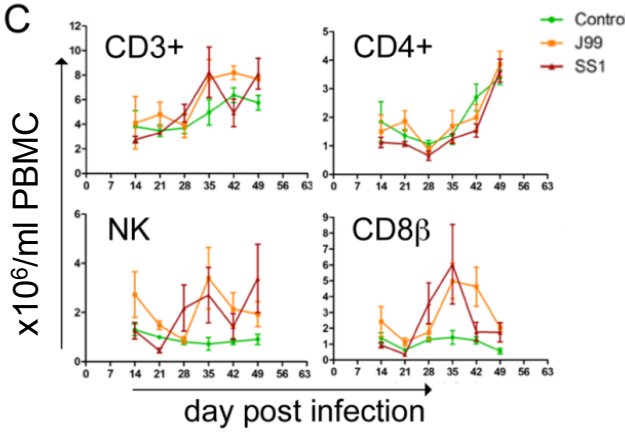
Possible Immune Evasion in *H. pylori* Infection

- Immune evasion of *H. pylori*
  - Infection of pigs with strain J99 renders PBMC unresponsive towards inactivated antigen ex vivo
  - Following immunization *in vivo,* inactivated *H. pylori* SS1 antigen induces suppression of murine splenocyte proliferation upon mitogenic stimulation *ex vivo*
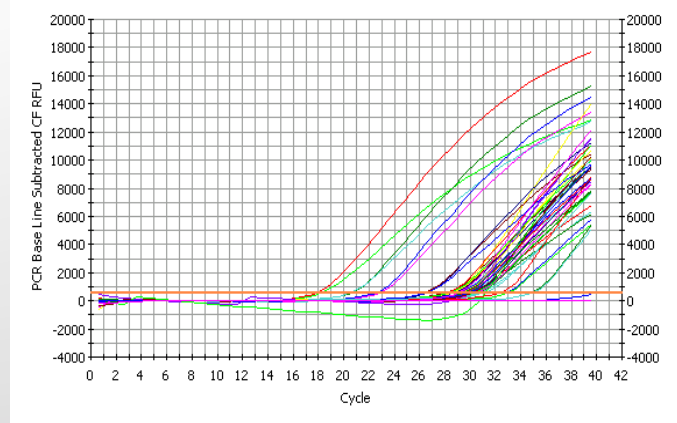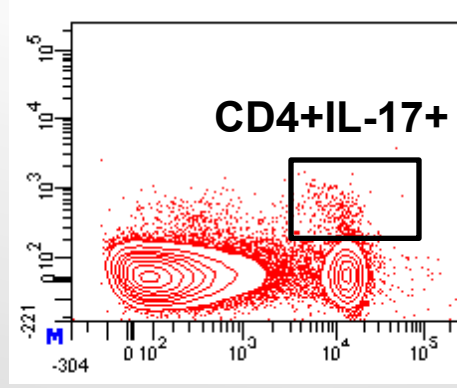   - Possible role of antigen presenting cells (APC) in *H. pylori* immune evasion
    - We will conduct studies to assess a possible induction of APC tolerance
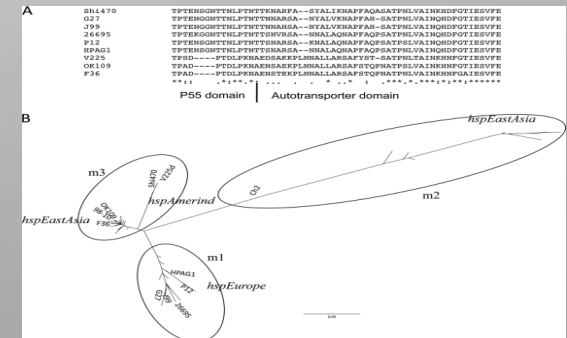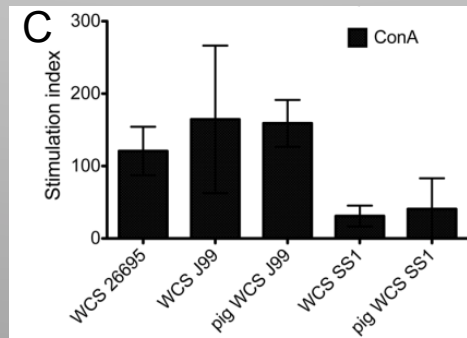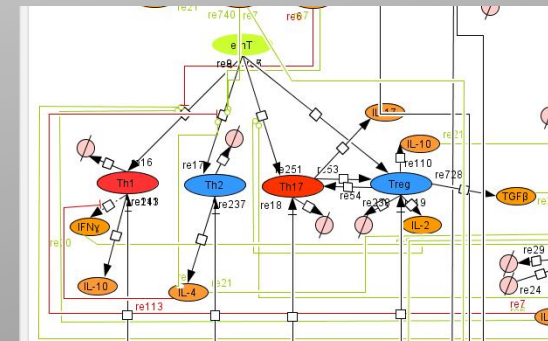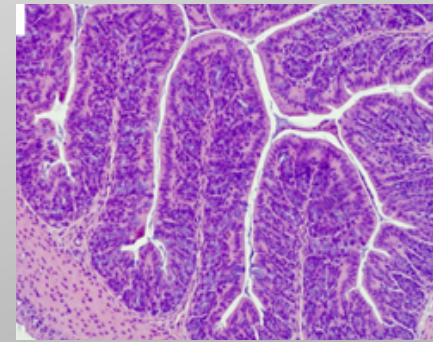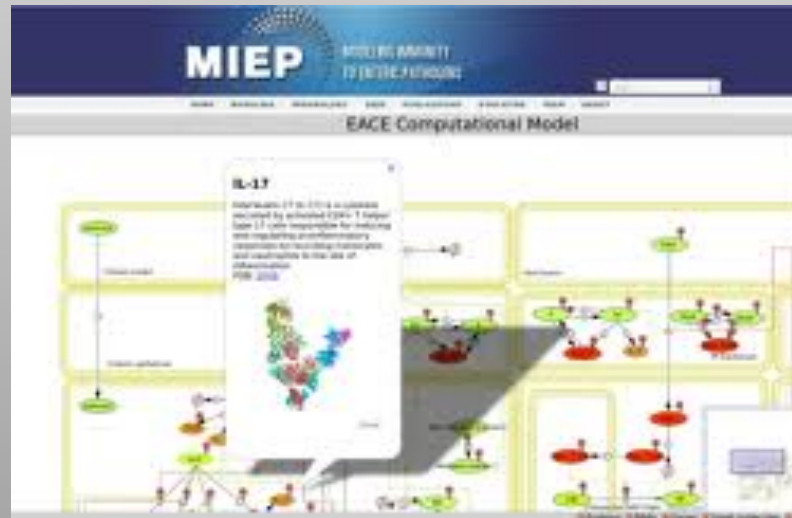
Conclusions and Outlook

- To develop a predictive mathematical model of immune responses to *H. pylori*
  - Analyze the effect of pathogenicity factors on host response
  - Consider the role of CD8+ T cells and NK cells
  - Early dominance of Th1 response followed by Th17
  - Consider effect of vehicle (brucella broth)
- Predominant Th1 and cytotoxic cell responses in pigs
  - Expansion of circulating CTL and NK cells during infection
  - Investigate the role of *H. pylori* as an intracellular pathogen
  - *H. pylori* strain specific differences regarding: persistence of bacteria, strength of Th1 response (IFN-γ levels), secondary proliferative responses *ex vivo* (J99: suppression of proliferation)

Modeling Immune Responses
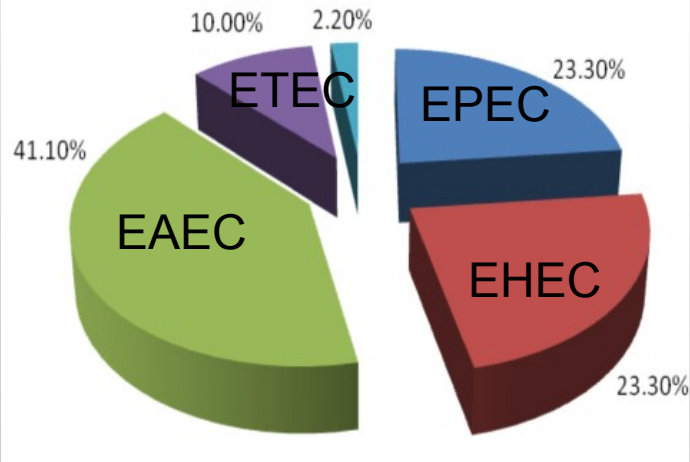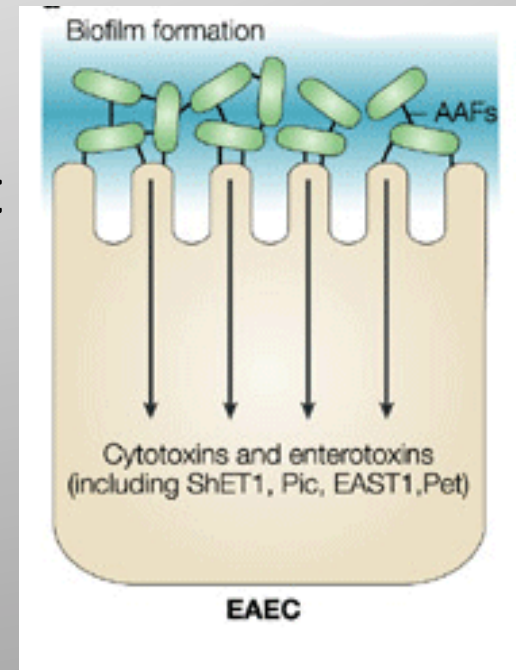# to Enteroaggregative *E. coli*

Introduction to EAEC

EAEC is a leading cause of diarrhea around the world

- Aff fimbria is the main virulence factor
- Immune response during infection is not well understood
- Malnutrition increases disease severity
- Currently no treatments are available

• The MIEP is studying immune responses to EAEC strains JM221 and 042

Enteroaggregative *E. coli* (EAEC) causes
Persistent Diarrhea (PD), Intestinal Inflammation, Growth Shortfalls and is the Leading Bacterial Enteropathogen in US

- EAEC is associated with 36% of PD; 30-74% w HIV
- EAEC-PD has elevated fecal LF, IL-8, IL-1
- EAEC-no D. also had elevated LF & Growth Shortfalls

- EAEC filtrates induce IL-8 release in Caco-2 cells.
- EAEC FIi-C (unique flagellin) cloned/sequenced is responsible for the IL-8 release.

- EAEC significantly associated with 4.5% diarrhea in Baltimore and New Haven

JM221 vs 042 EAEC strain Comparison
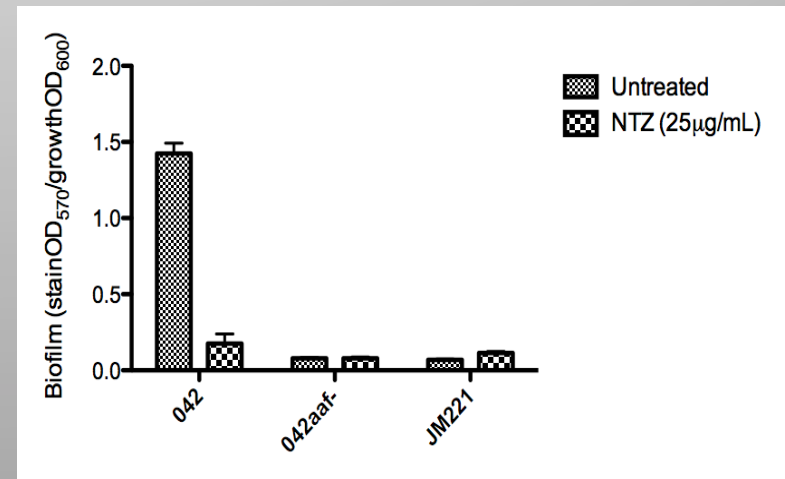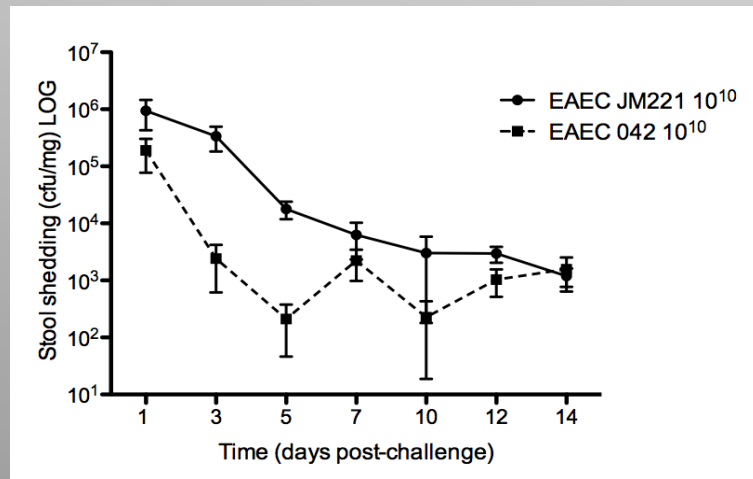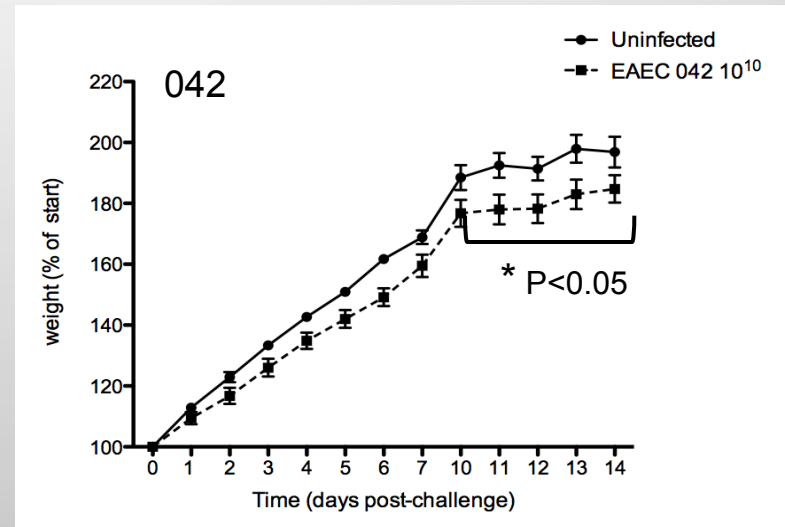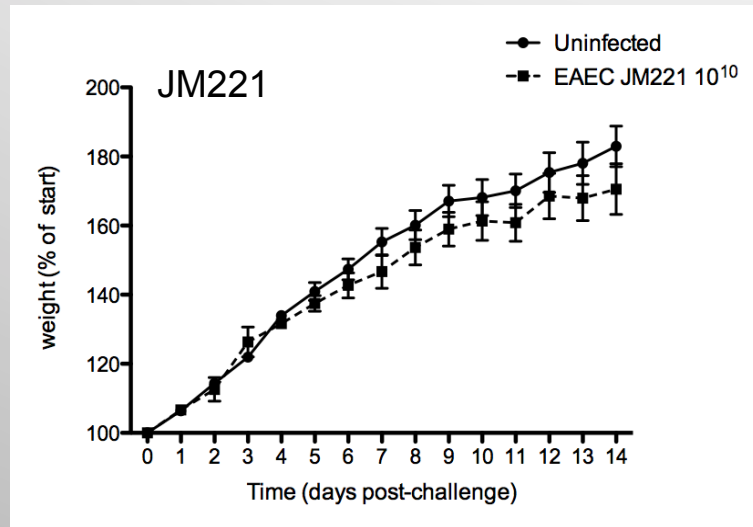
Introduction: PPARγ

- PPAR γ elicits immunoregulatory
effects during inflammatory responses
  - Suppression of Th1/Th17
  - Enhance Treg
  - Regulate macrophage phenotypes
- Current experiments that target PPARγ
  - Specific PPARγ knock out mice
  - Exogenous administration of drugs
    - Agonists (Pioglitazone)
    - Antagonists (GW9662)
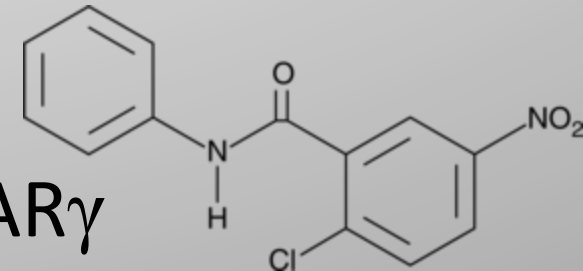
GW9662

Loss of PPARγ favors a Th17 Phenotype

• Malnutrition and the loss of PPAR γ favors IL-17 production by CD4+ T cells and EAEC clearance
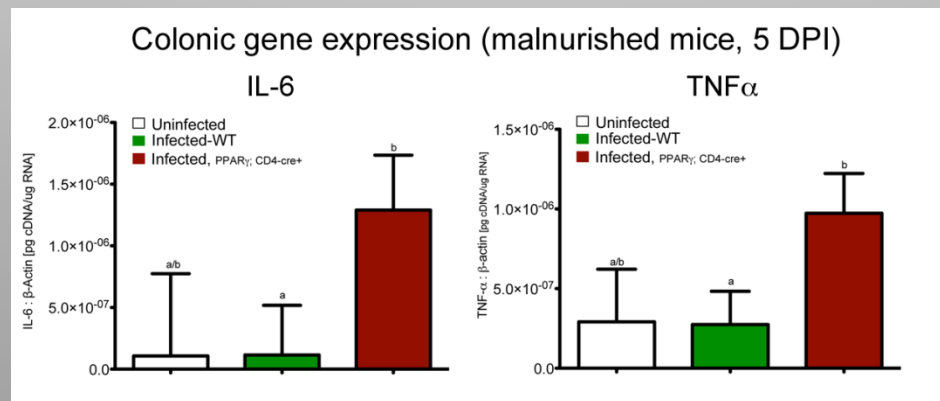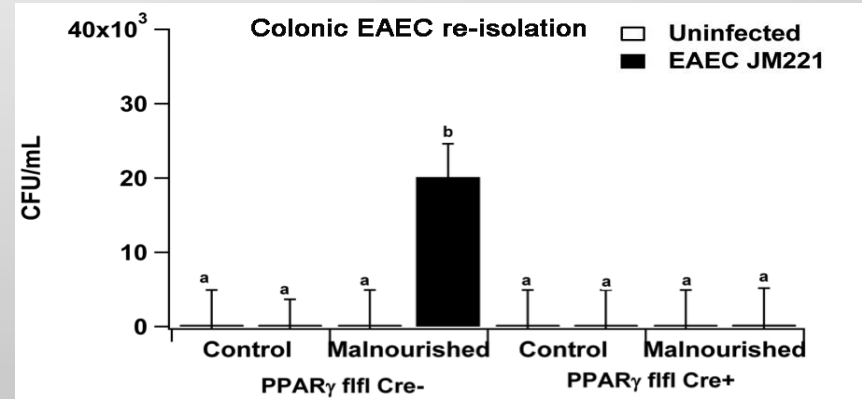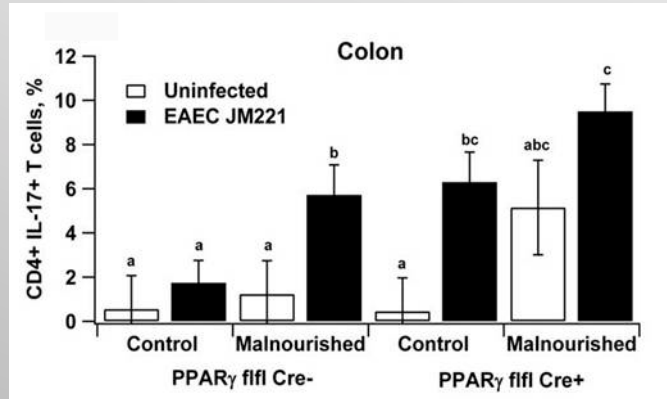
Additional Data Suggesting TH17 Differentiation
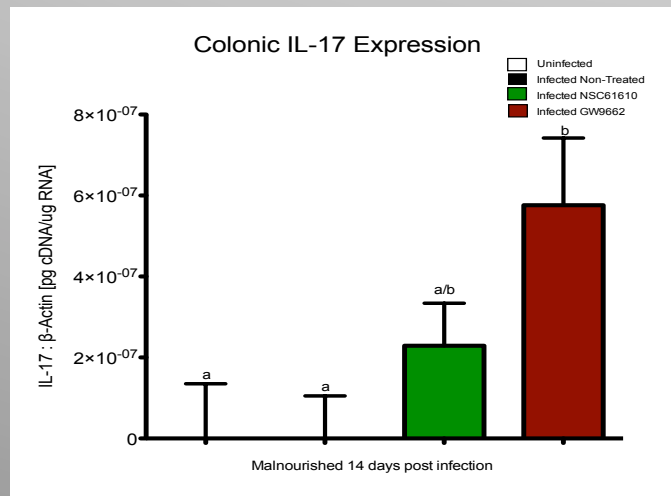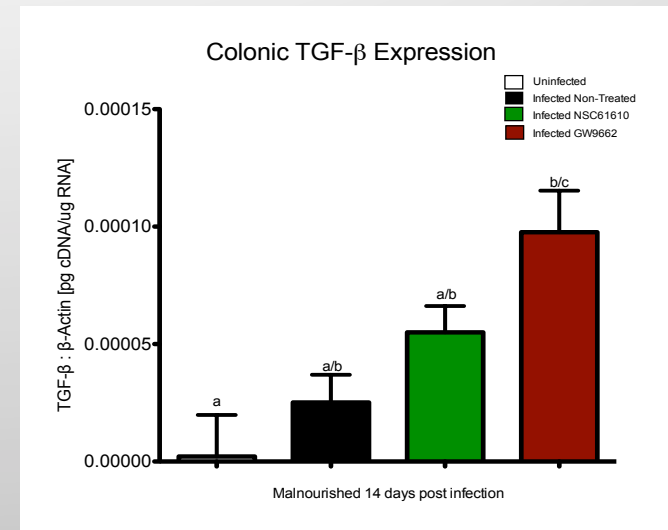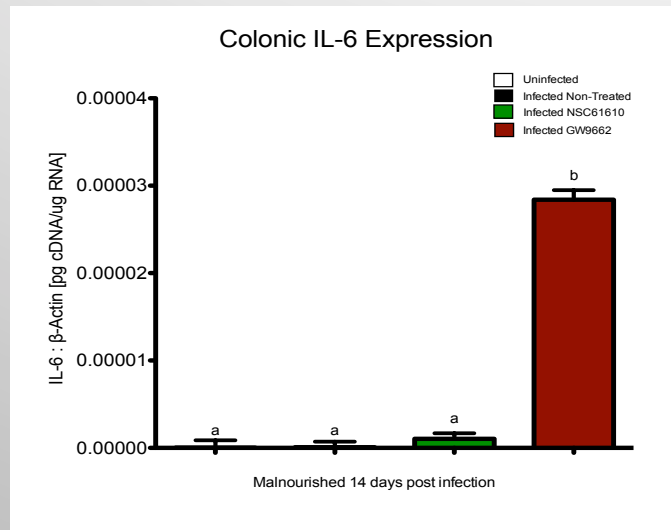
Treatment with an exogenous PPARγ antagonist results in increased colonic IL-6, TGF-β, and IL-17 expression at day 14 post-infection, suggesting that bloacking PPAR γ favors TH17 phenotype in the colonic LP of EAEC infected mice

T cell PPAR γ modulates colonic EAEC lesions
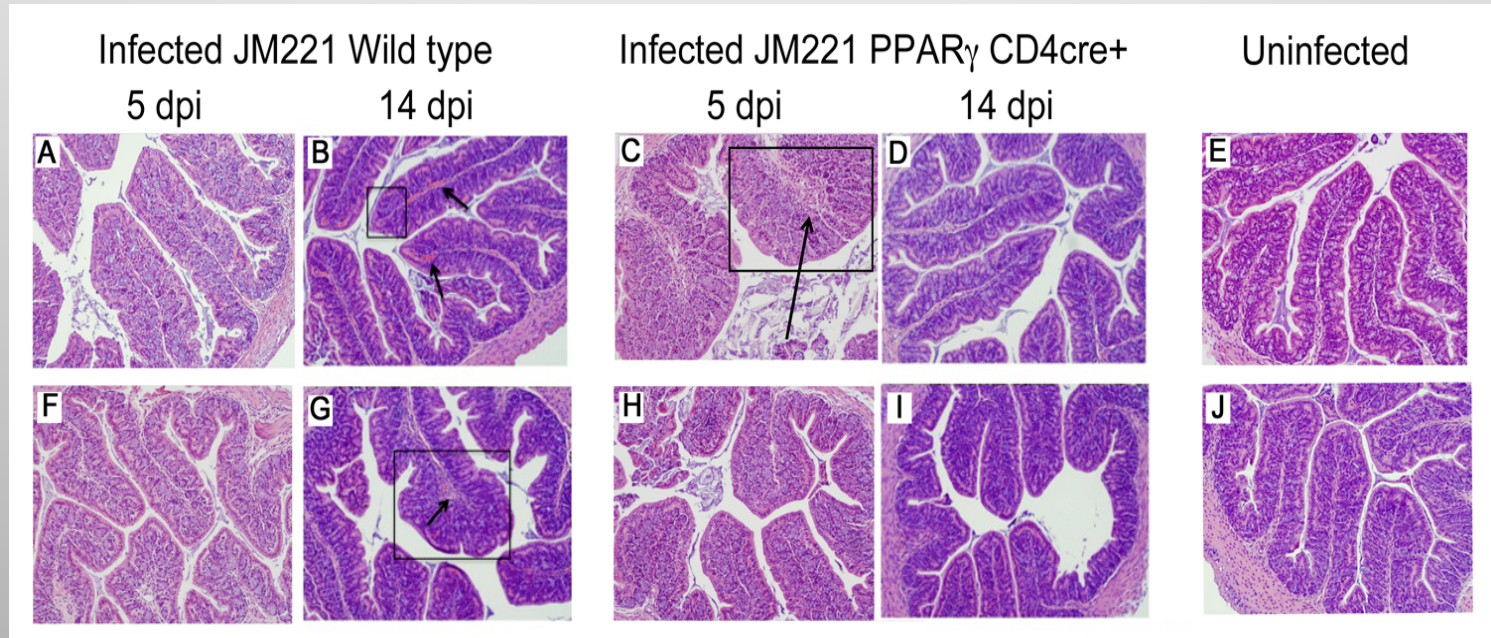
The loss of PPARγ in T cells results in greater leukocytic infiltration and inflammation at the peak of infection (day 5) and faster recovery on day 14

EAEC Model Annotation

Conclusions and Outlook

- EAEC mainly colonizes the colonic epithelium and modulates immune responses at the colonic LP

- LP Th17 responses play an important role in controlling EAEC infection

- PPAR γ represents a promising broad-based host-targeted therapeutic for EAEC infection

- Our initial modeling efforts will focus around modulating the Th17 response *in silico*

Future directions

-Time-course study designed to better understand the cellular changes throughout the course of infection
- Prepare data on EAEC challenge experiments for ENISI and COPASI model calibration
- Pig model of EAEC infection
-Antigen-specific response studies in human PBMCs

- Calibration of the COPASI and ENISI models
- Fully understand the intricate cellular changes in PPAR γ null mice using modeling
-Identify new therapeutic targets
- Investigate mechanism of action of novel broad-based therapeutics
        - LANCL2/PPAR γ agonists and antagonists
        - Gut Repair agents
        - Immunomodulators

*EN*teric *I*mmunity *SI*mulator
ENISI

ENISI: Architecture of *in silico* mucosa

Tissue Sites:
- Lumen (*H. pylori* and commensal bacteria)
- Lamina Propria(LP)
- **Lymph Node**

Phenotypes*:*
- CD4+ T helper cells
  - Resting
  - Inflammatory Th1
  - **Inflammatory Th17**
  - iTreg
- Natural T regulatory cells (nTreg)
  - Resting
  - Active
- Macrophages
  - Resting M0
  - Inflammatory M1
  - Regulatory M2
- Dendritic Cells
  - Resting iDC
  - Inflammatory eDC
  - Regulatory tDC
- Epithelial Cells
  - Normal, healthy
  - Damaged, pro-inflammatory
  - **Impaired, pro-inflammatory**
  - Dead
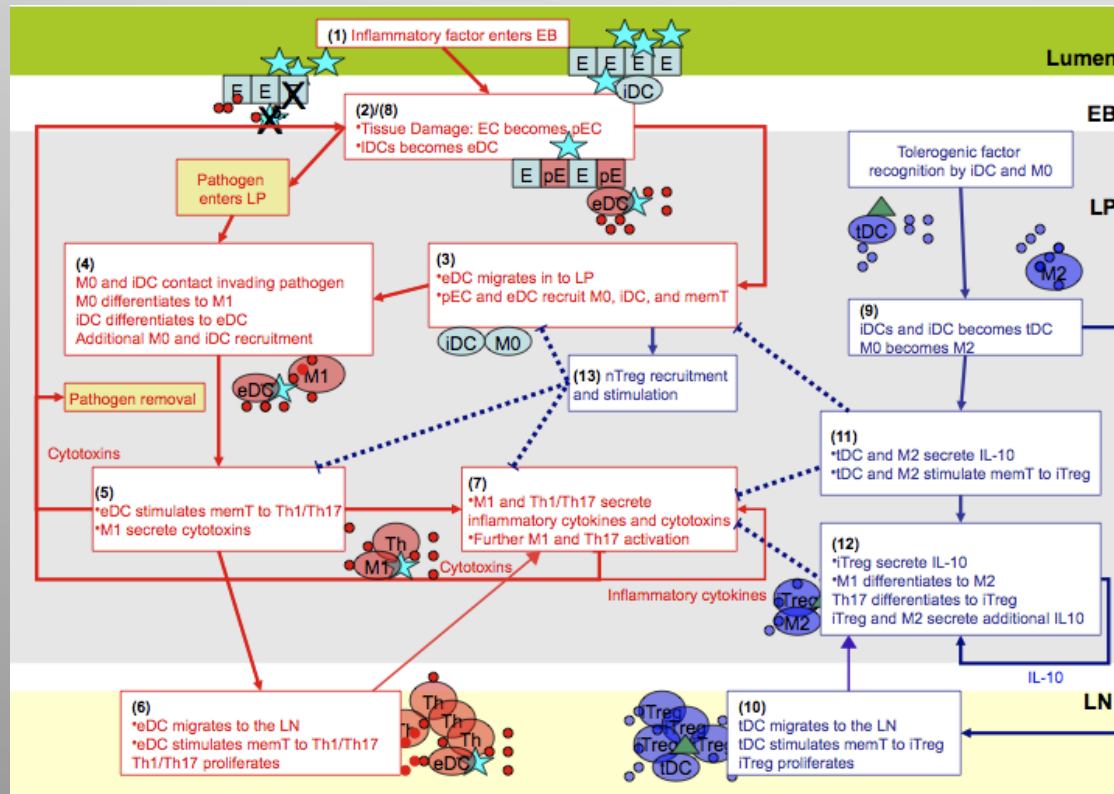
ENISI Simulations
Replicate *H. pylori* infections
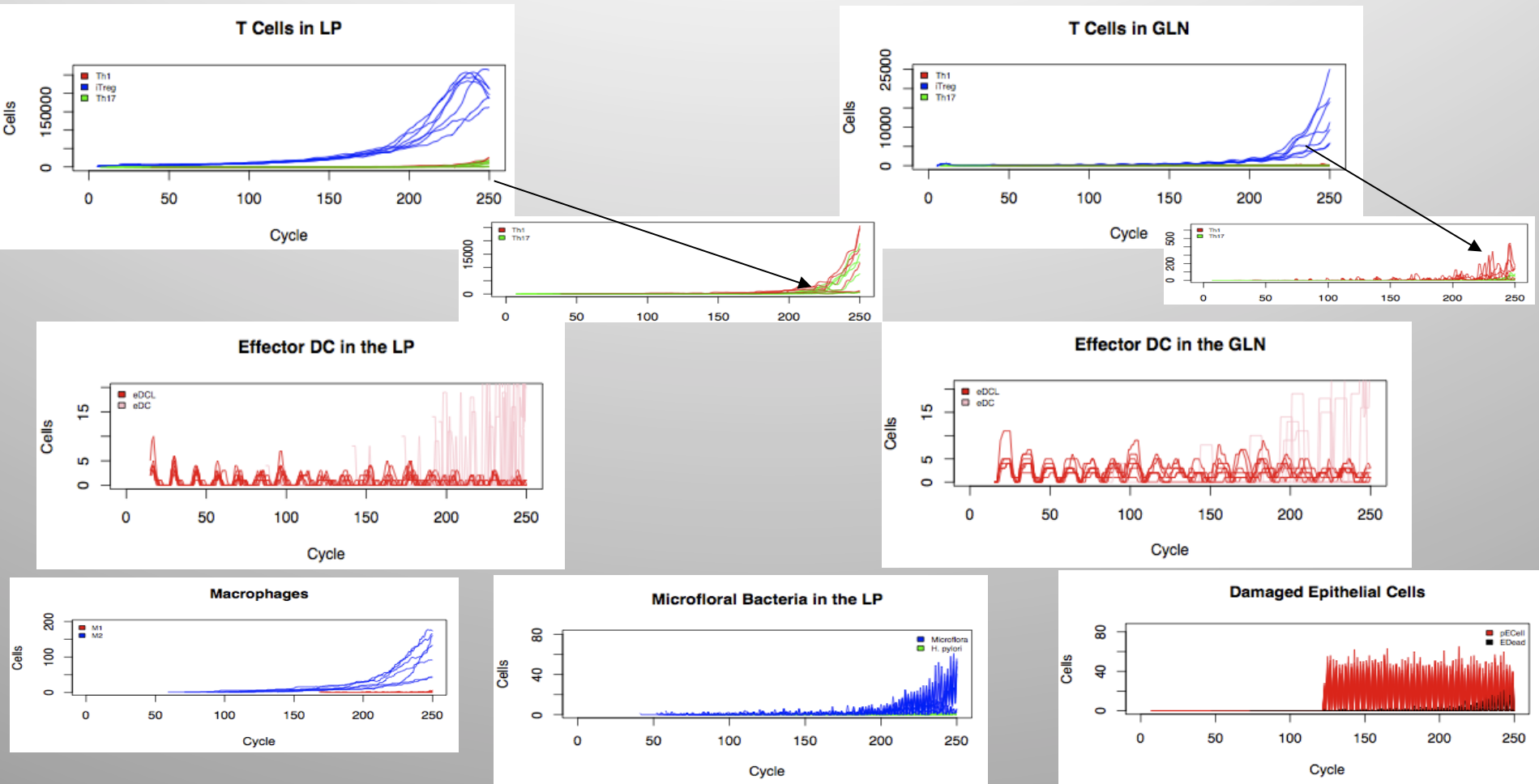
Response to *H. pylori 26695* in 7 individuals

Predicted immune response to *Helicobacter pylori* strain 26695 over 2 months showing a delayed Th1-dominant inflammatory response that results in epithelial damage.

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis

What is the cause of EC damage?
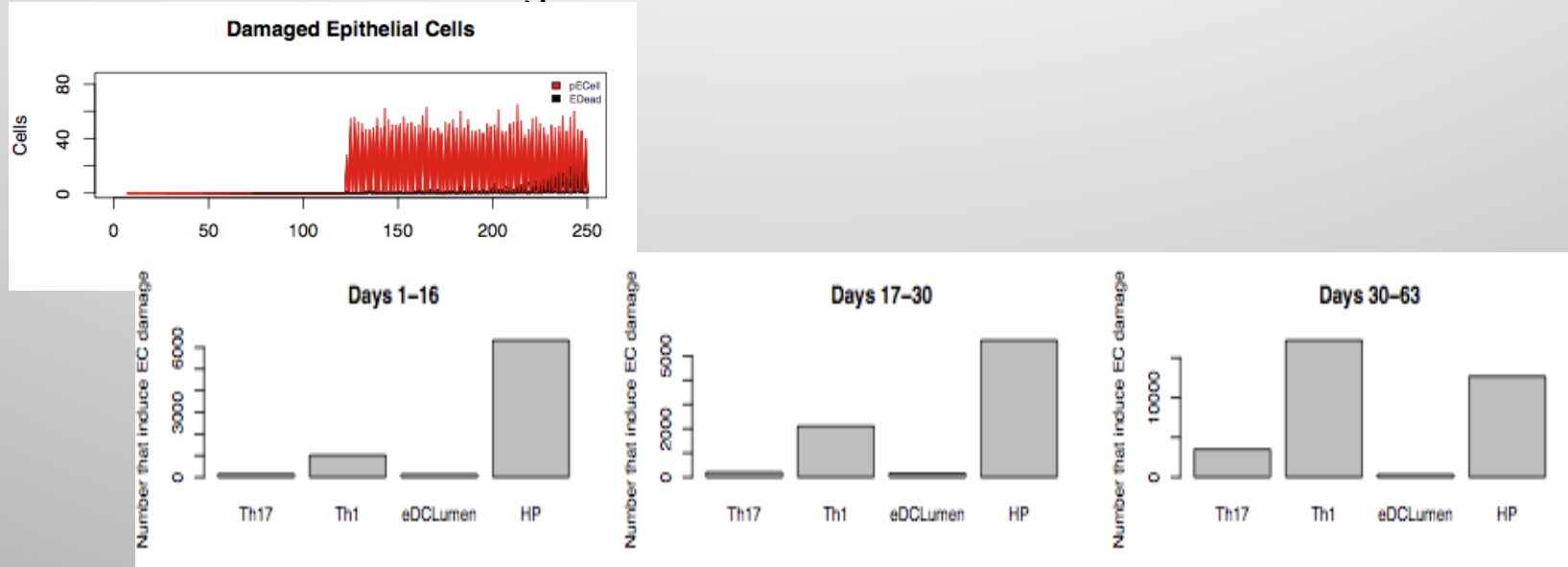
- **Histogram of number of individuals in each phenotype that induce state transition EC → pEC**

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis

What is the cause of EC damage? **Increased Th1 levels**
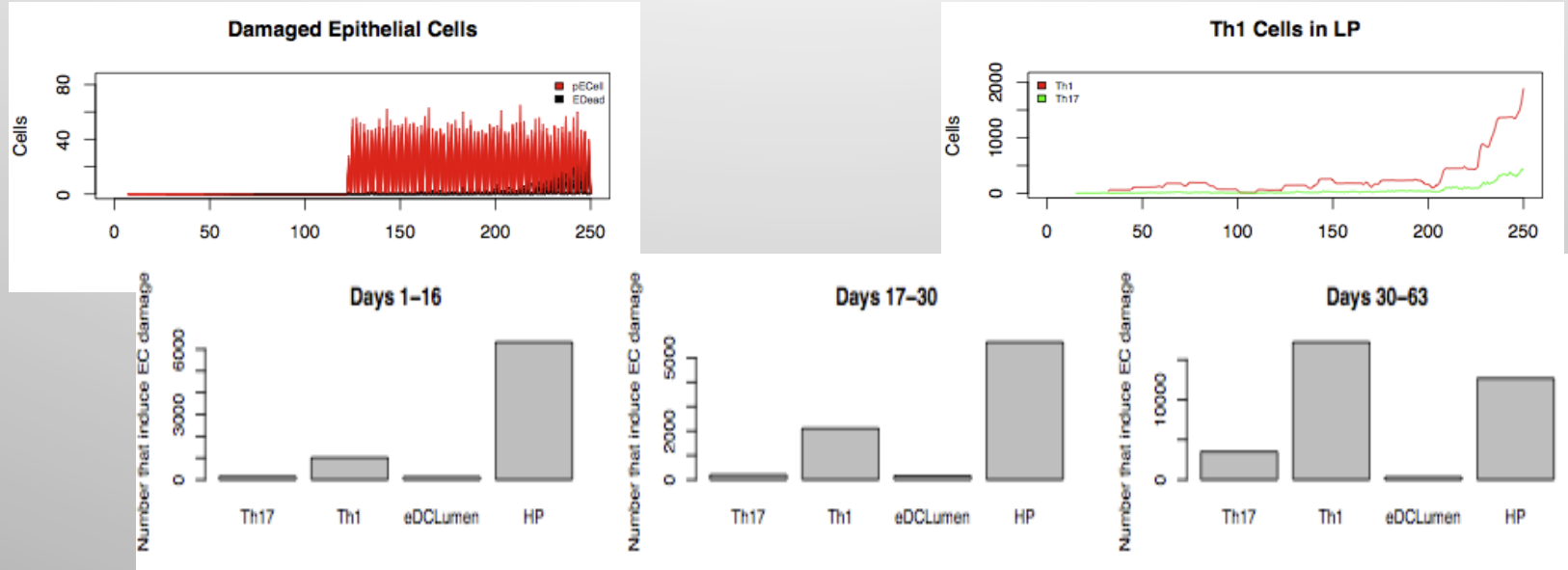
- Histogram of number of individuals in each phenotype that induce state transition EC → pEC
- Increased epithelial damage coincides with rising Th1 levels

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis

What is the cause of EC damage? **Increased Th1 levels**

What is the cause of increased Th1 levels? **T cells stimulated solely by 'sampling' eDC in the lumen**

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis

What is the cause of EC damage? **Increased Th1 levels**

What is the cause of increased Th1 levels?

The effector 'sampling' DC levels do not rise over time

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis

What is the cause of EC damage? **Increased Th1 levels**

What is the cause of increased Th1 levels?

The effector 'sampling' DC levels do not rise over time

Resting T cells in the LP increase due to recruitment

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis

What is the cause of EC damage? **Increased Th1 levels**

What is the cause of increased Th1 levels? **Effector 'sampling' dendritic cells and recruitment**

The effector 'sampling' DC levels do not rise over time

Resting T cells in the LP increase due to recruitment

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis

What is the cause of EC damage? **Increased Th1 levels**
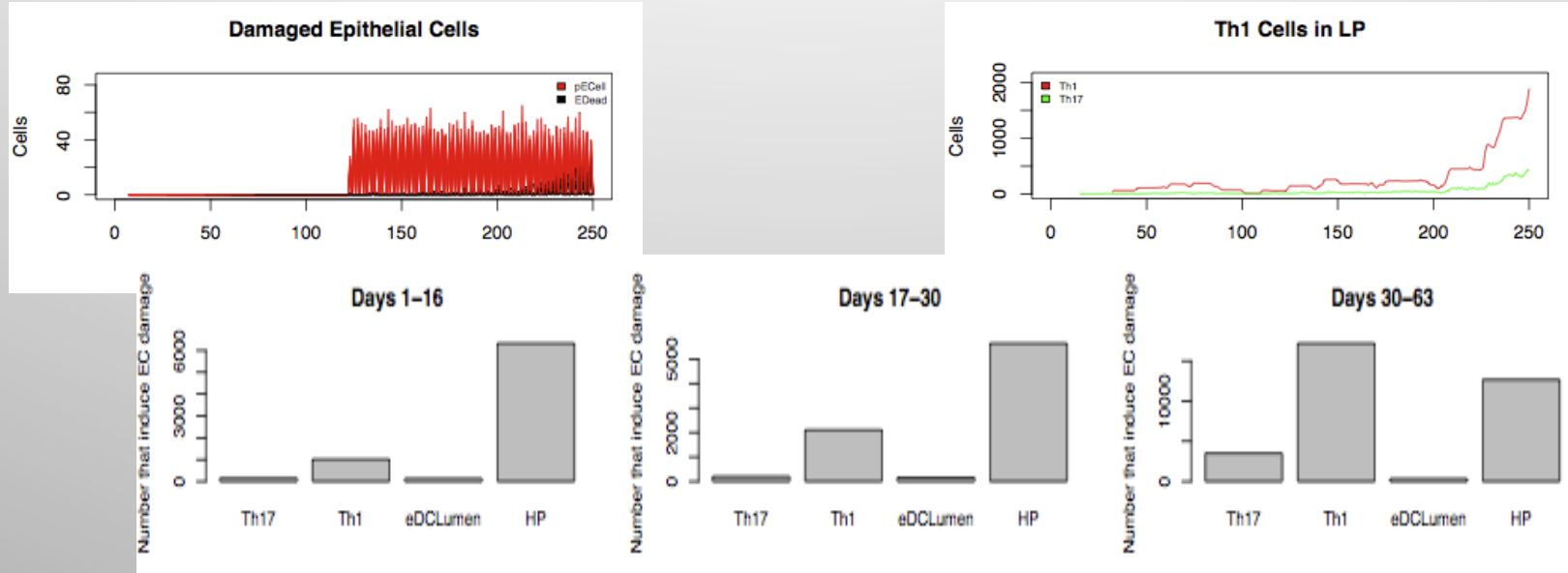
What is the cause of increased Th1 levels? **Effector 'sampling' dendritic cells and recruitment**
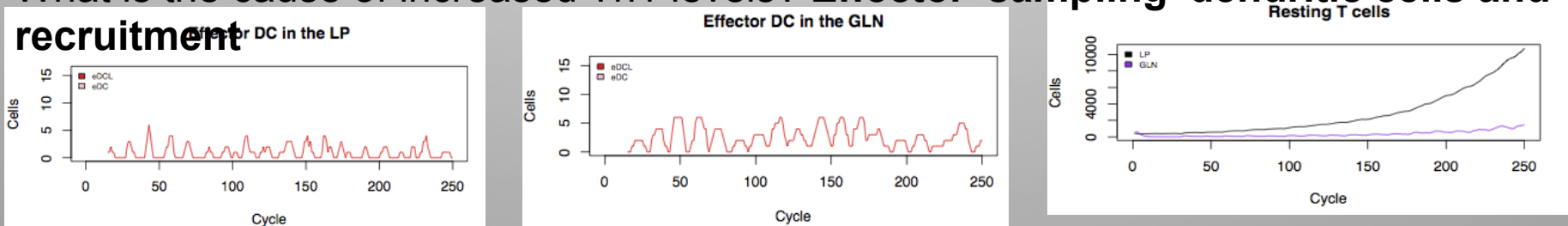
Which factors are contributing most to resting T cell recruitment?

ENISI Simulations

Mucosal cell types contributing the most to pathogenesis
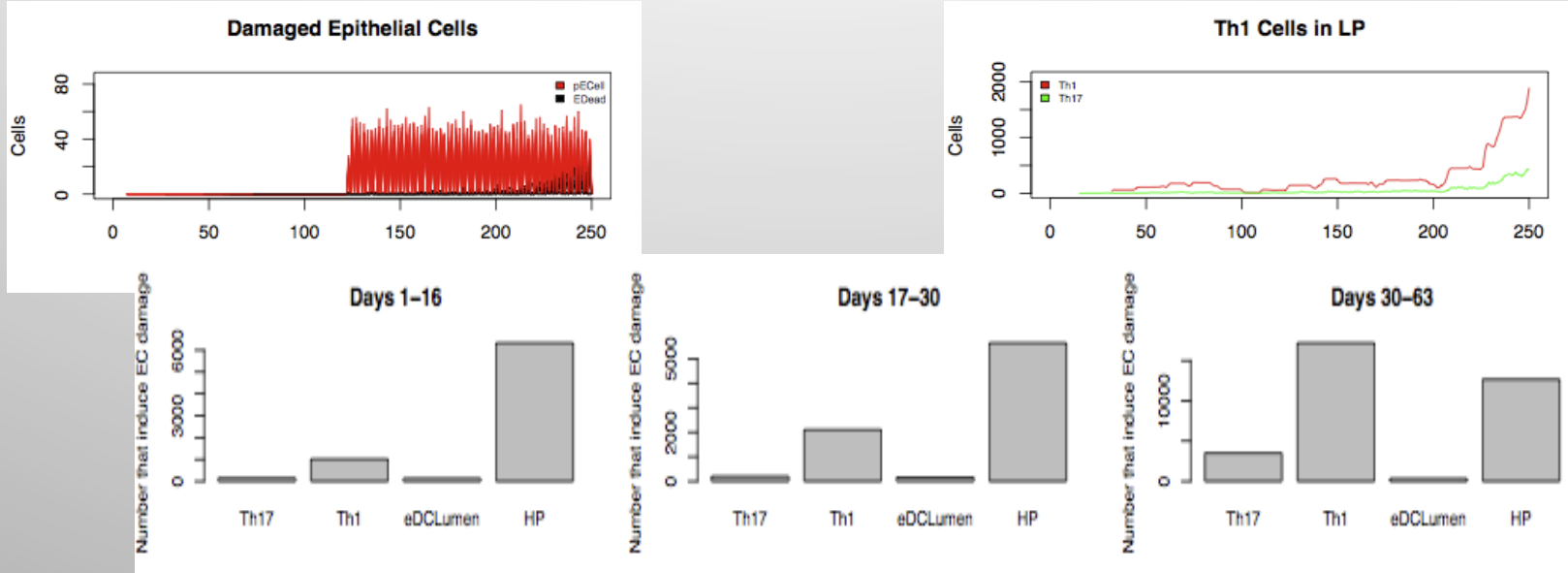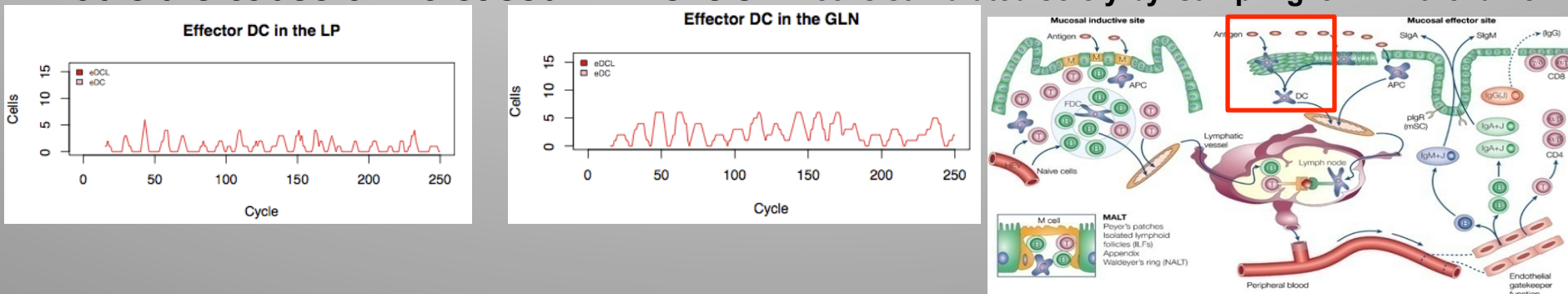
What is the cause of EC damage? **Increased Th1 levels**

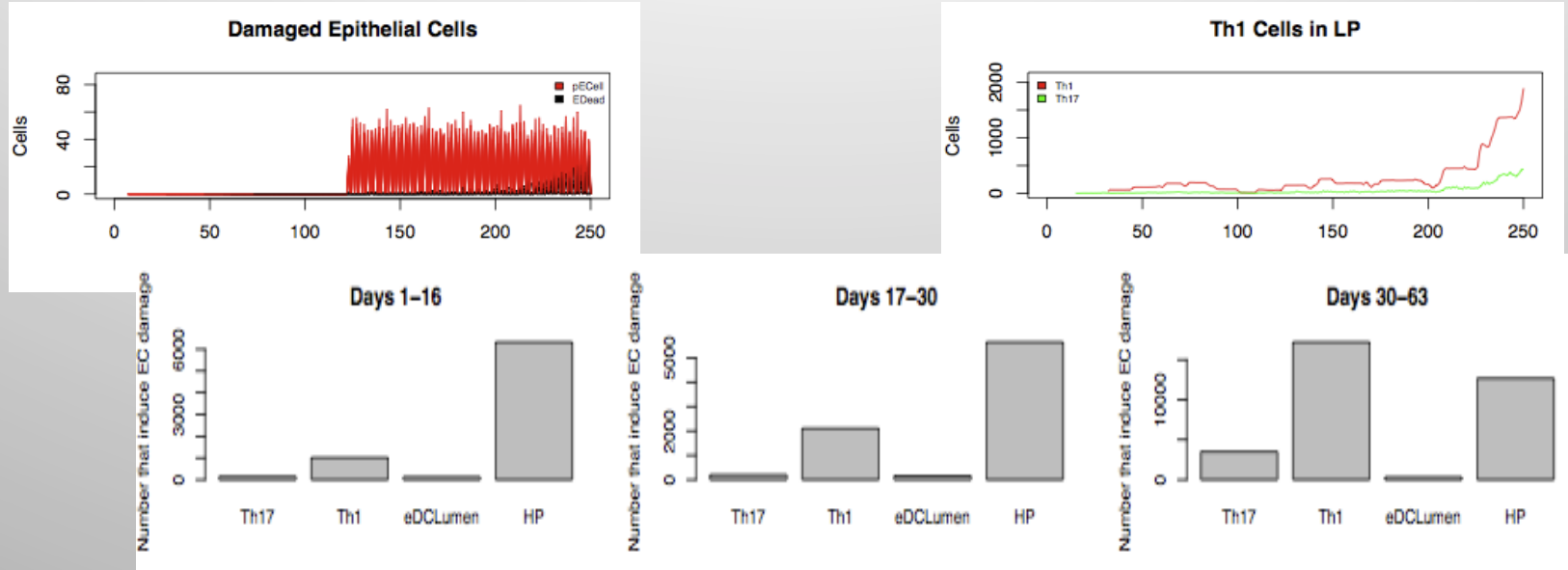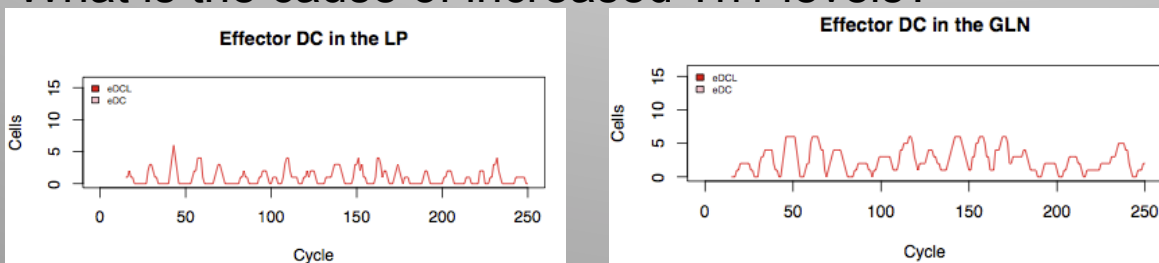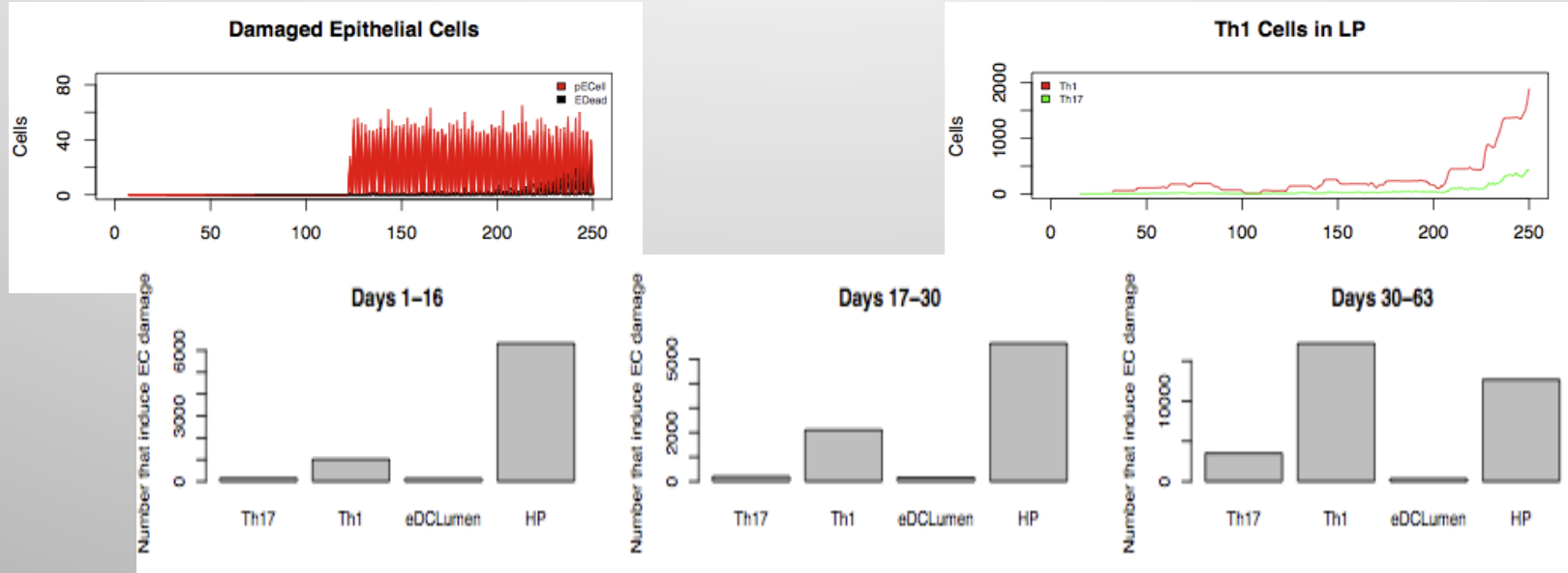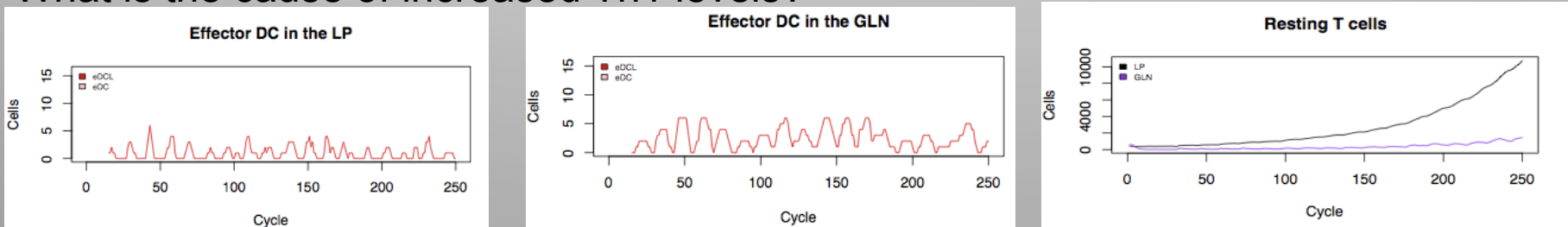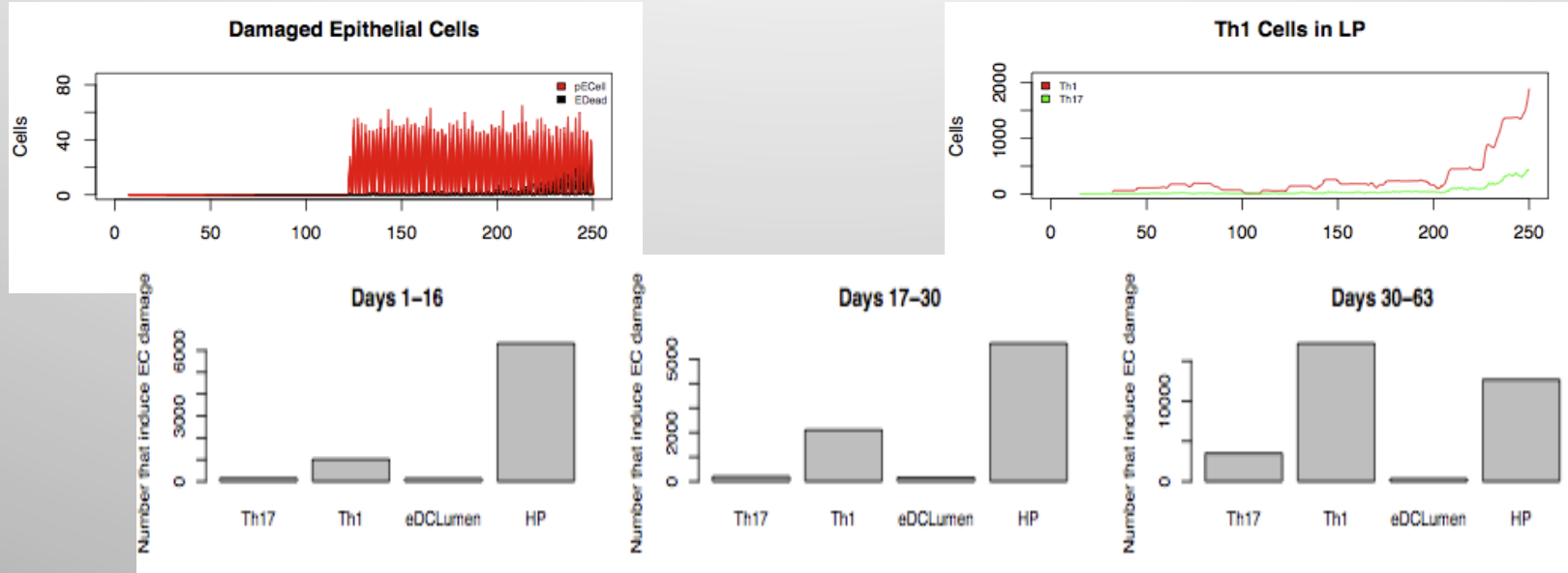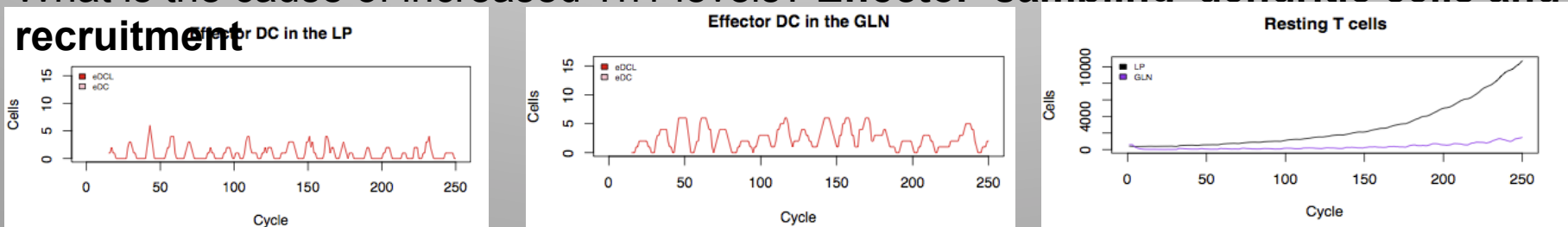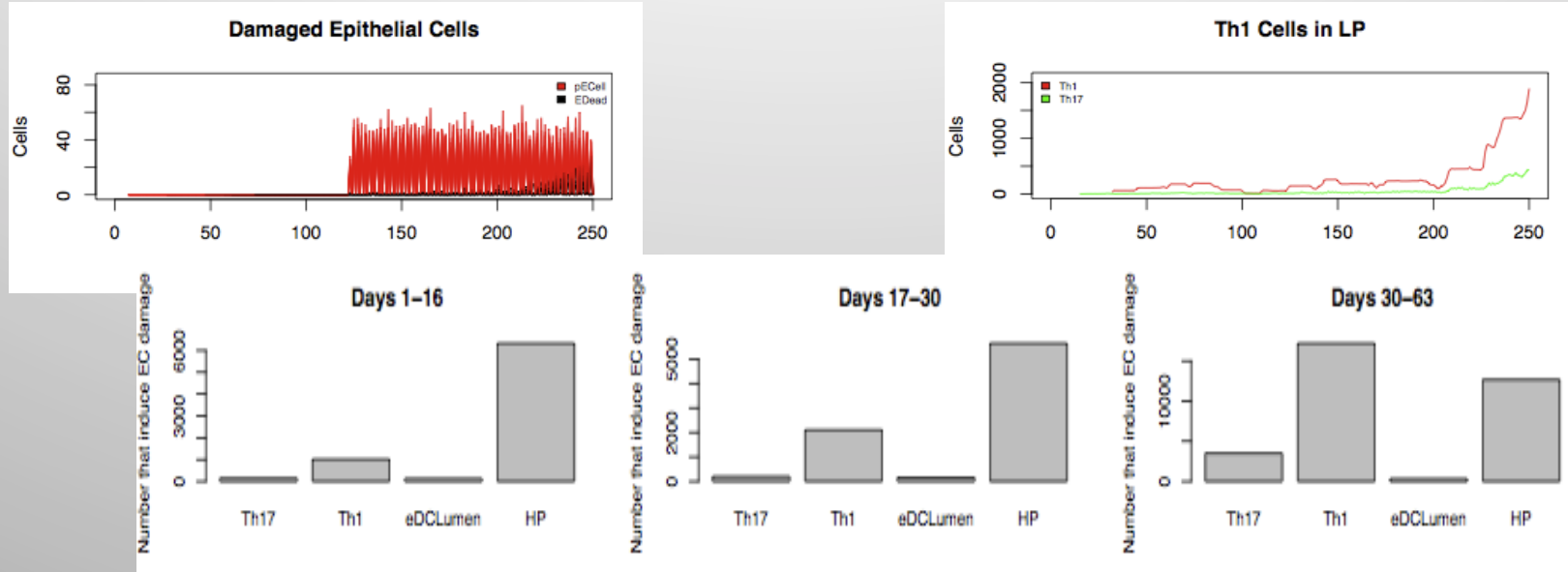What is the cause of increased Th1 levels? **Effector 'sampling' dendritic cells and recruitment**
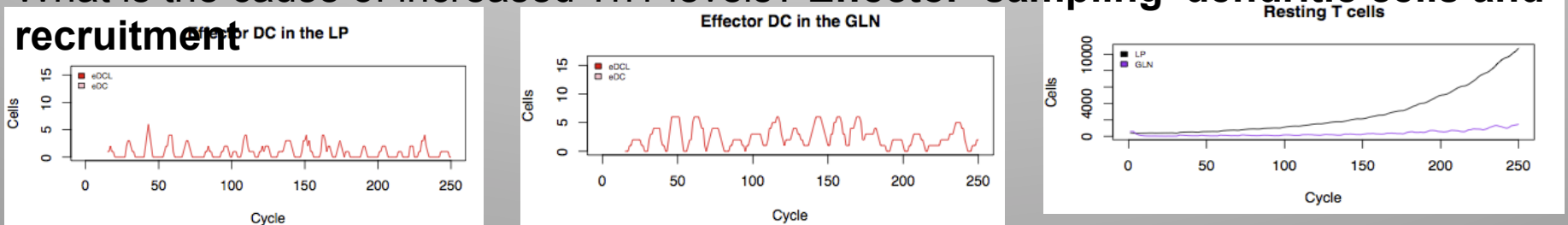
Which factors are contributing most to resting T cell recruitment?
Effector 'sampling' dendritic cells and pro-inflammatory epithelial cells

ENISI Result Viewer

ENISI Result Viewer

ENISI Directions

- A tool to
  - Generate hypothesis for cellular level interactions that give rise to tissue level observations
  - Predict immune cell dynamics and clinical outcome in different scenarios
- The current model is able to reproduce infection dynamics in a mucosal tissue sample for different pathogens
- Simulation output allow *in silico* identification of key mechanisms underlying dynamics in experimental tissue samples.
- Future directions
  - Visualization of spatial distribution of cells
  - Automated methods for calibrating the simulator to experimental data
  - Higher resolution multiscale model that includes cell differentiation models
  - Evolution allowing pathogens to "choose" an immunomodulation strategy

VBI VIRGINIA BIOINFORMATICS INSTITUTE AT VIRGINIA TECH

MIEP MODELING IMMUNITY TO ENTERIC PATHOGENS

MIEP

MODELING IMMUNITY TO ENTERIC PATHOGENS

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High Performance Computing

NEWS AND ANNOUNCEMENTS

Center for Modeling Immunity to Enteric Pathogens Releases a Revolutionary Modeling and Simulation Software: ENteric Immunity SImulator

BLACKSBURG, Va., Oct. 5th, 2011 – Researchers from the Center for Modeling Immunity to Enteric Pathogens (MIEP) at the Virginia Bioinformatics Institute have released an upgrade to the revolutionary ENteric Immunity SImulator (ENISI) software. The ENISI models immune responses to beneficial and harmful bacteria that enter the gastrointestinal tract (GI) of mice, pigs and humans. ENISI allows users to create enteric systems such as the gut-associated mucosal immune system *in silico*, providing a better glimpse of how the immune system responds to pathogens that invade the bacteria-rich environment of the gut. [More ...]

Healthy Volunteers Needed to Study Immune Responses to Intestinal Pathogens

BLACKSBURG, Va., September 28, 2011 – You may be interested in a clinical study the Center for Modeling Immunity to Enteric Pathogens (MIEP) is conducting. We Are Looking for Healthy Volunteers to Study Immune Responses to Intestinal Pathogens. Compensation is available if you qualify and are enrolled in the study. Please Contact (434) 924-9922 if you live near Charlottesville or (540) 231-7276 if you live near Blacksburg for more information. [More ...]

PRESS RELEASES

- Center for Modeling Immunity to Enteric Pathogens Releases a Revolutionary Modeling and Simulation Software: ENteric Immunity SImulator
- Center for Modeling Immunity to Enteric Pathogens Contributes Code to The Open Source Community
- Center for Modeling Immunity to Enteric Pathogens to Release New

SELECTED PUBLICATIONS

- ENteric Immunity SImulator: A tool for *in silico* study of gut immunopathologies
- Modeling the Mechanisms of Action Underlying the Plasticity of the CD4+ T cell Differentiation Process
- Abscisic acid Regulates Inflammation via Ligand-Binding Domain-Independent Activation of PPAR gamma

MIEP MISSION

The Center for Modeling Immunity to Enteric Pathogens (MIEP) is a NIAID funded program with the mission of understanding the mechanisms of action underlying immune responses to enteric pathogens.

UPCOMING EVENTS

MIEP team to present ENteric Immunity SImulator (ENISI) at IEEE International Conference on Bioinformatics and Biomedicine.

MIEP team to attend Annual Meeting of the Modeling Immunity for Biodefense Program, Bethesda, MD Nov 1-2.

RESEARCH HIGHLIGHTS

PPARγ Modulates the Plasticity between Th17 and iTreg

The MIEP team has created a network model of CD4+ T cell differentiation that reveals how the transcription factor peroxisome proliferator-activated receptor γ (PPARγ) modulates differentiation from Th17 to iTreg. [more ...]

MIEP Website Statistics: Visitors
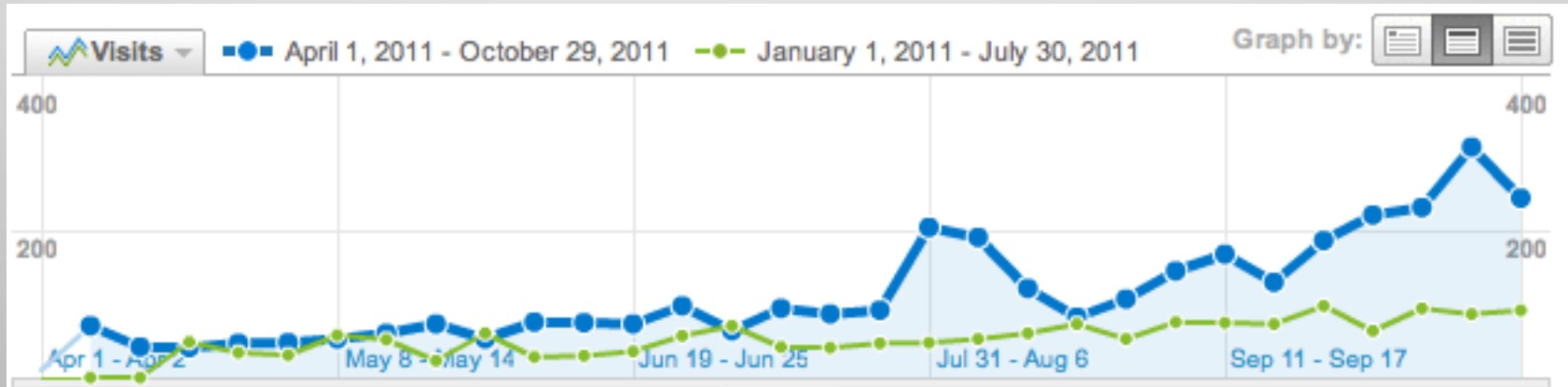
❑Comparison of number of visitors to the time period 3 month prior

MIEP Website Statistics: Page Views
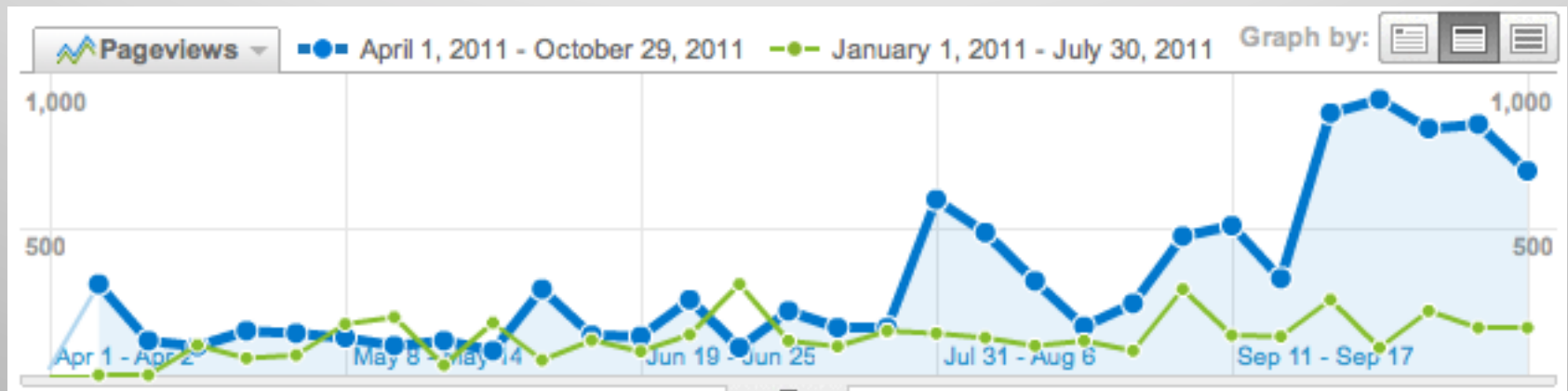
❑Comparison of number of page views to the time period 3 month prior

Enhanced CellPublisher Features

- Animated protein structure with the help of JMol

- Link to publications through Pubmed Ids

- Protein identification through Uniprot Ids

- Google map-based navigation and annotation of reactions and species

- Source code available at: http://www.modelingimmunity.org/modeling-tools/cell-publisher/

Acknowledgements

Virginia Bioinformatics Institute

Josep Bassaganya-Riera - Principal Investigator and Center Director

Raquel Hontecillas - Immunology Lead
   Barbara Kronsteiner-Dobramysl - Immunology Post-Doc
   Xia Wang - Laboratory Manager
   Xiaoying Zhang - Immunology Post-Doc
   Pinyi Lu - Immunology and Modeling GRA
   Adria Carbo - Immunology and Modeling GRA
   Kevin Muite - VT-PREP Scholar
   Mireia Pedragosa - Immunology and Modeling GRA
   Salvador Vento - Immunology Visiting Student
   Monica Viladomiu - Immunology and Modeling GRA
   Cassandra Washington - Laboratory Technician
   Caroline Moseley - Immunology and Modeling Intern
   Patrick Heizer - Immunology Intern

Jim Walke - Project Manager

Virginia Bioinformatics Institute (continued)

Madhav Marathe - Modeling Lead
   Keith Bisset - Modeling Expert
   Stephen Eubank - Modeling Expert
   Katherine Wendelsdorf - Modeling GRA
   Nikki Lewis - Modeling GRA
   Maksudul Alam - Modeling GRA

David Bevan- Education Lead

Stefan Hoops - Bioinformatics Lead
   Yongguo Mei - Research Software Engineer

University of Virginia

Richard Guerrant - Infectious Disease Expert
   James Roche - Infectious Disease Expert
   Cirle A. Warren - Infectious Disease Expert
   David Bolick - Sr. Laboratory and Research Specialist

Caprion Proteomics Inc.

Eustache Paramithiotis - Proteomics Director

Questions & Discussion

"And that's why we need a computer."