# A novel positive feedback loop sets a dose-dependent threshold for T cell differentiation

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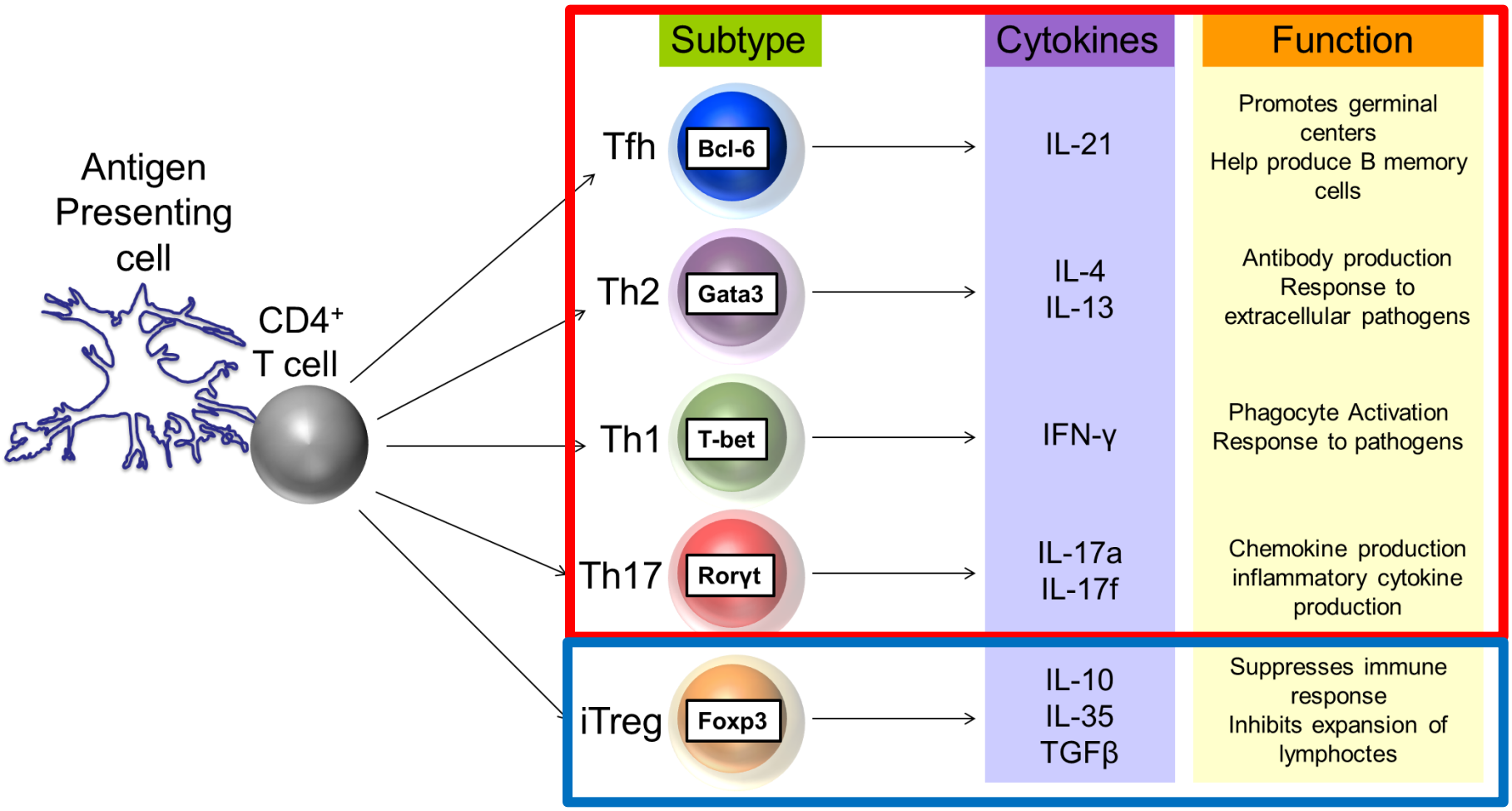
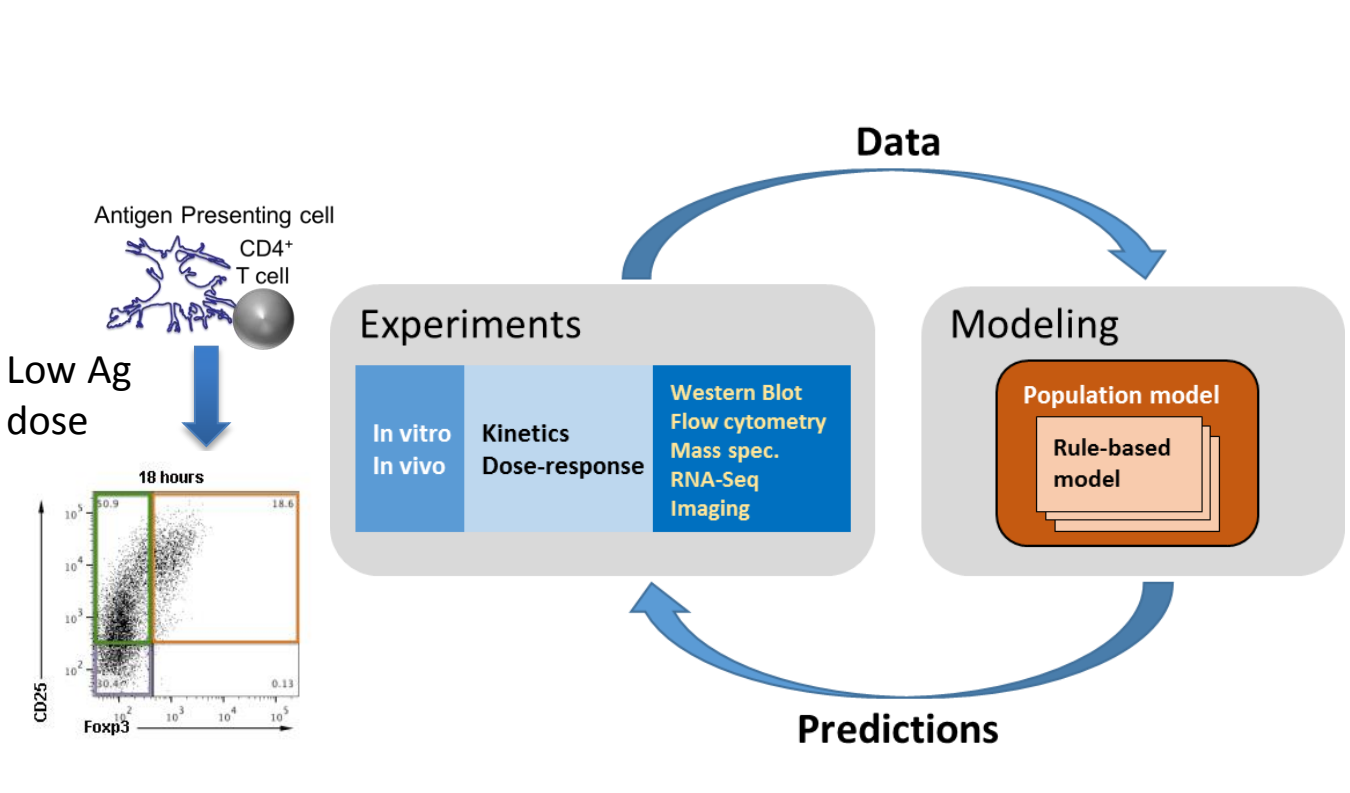
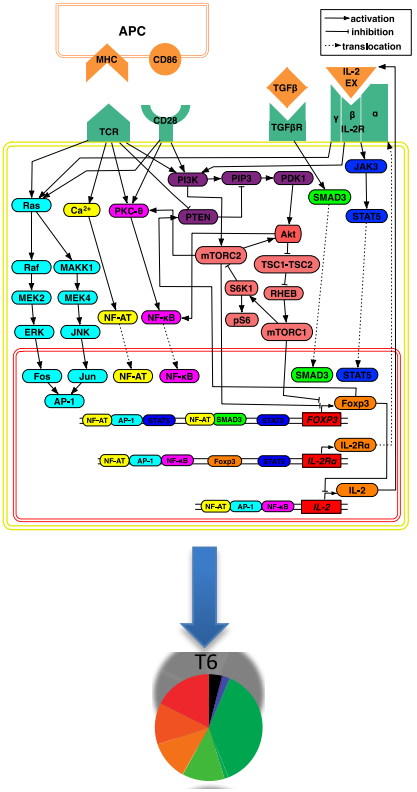
# Helper T cell fate determination



# Systems Biology Approach to T Cell Differentiation

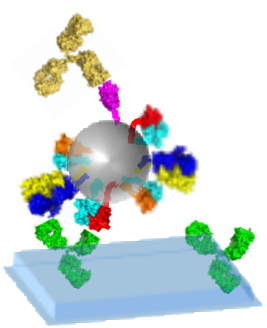


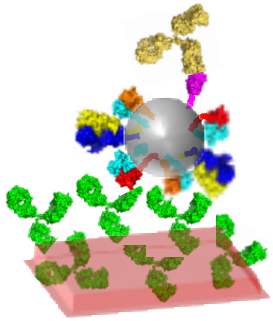




**Morel Lab**

**Faeder Lab**

# Antigen dose controls level of Treg induction



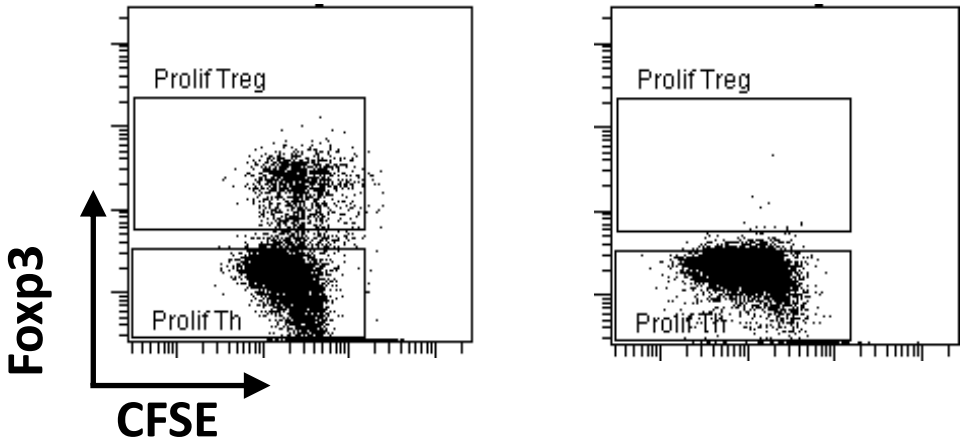


soluble aCD28

plate-bound aCD3

(0.25 µg/ml aCD3) Low Ag dose

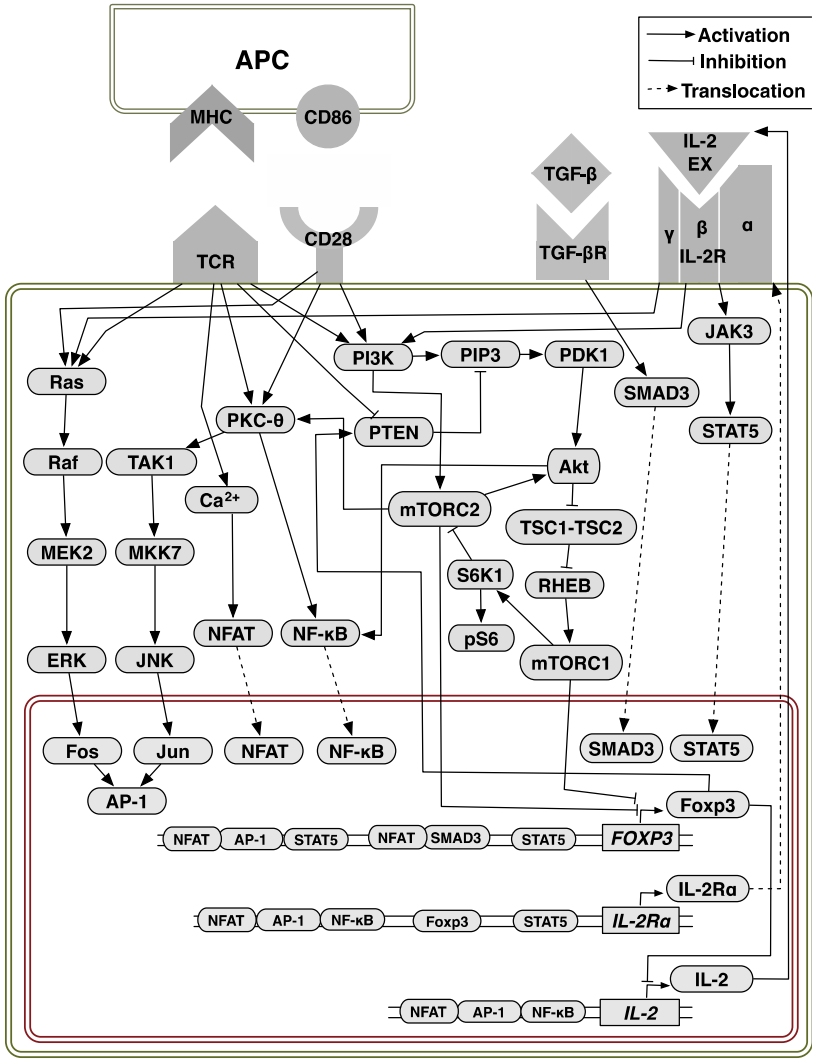
High Ag dose (1 µg/ml aCD3)

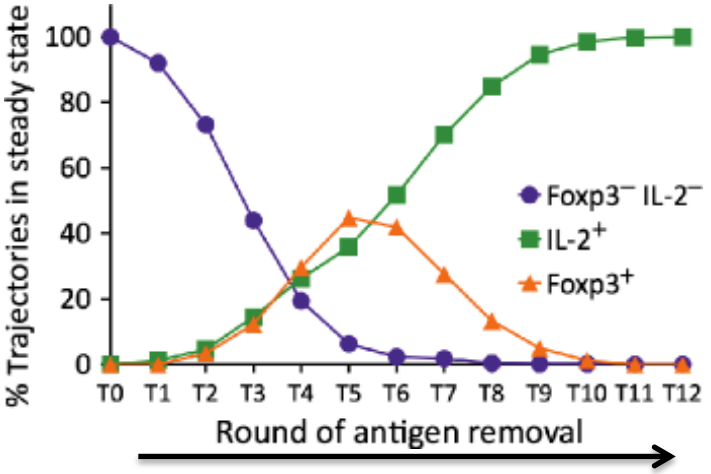



No cytokines are added

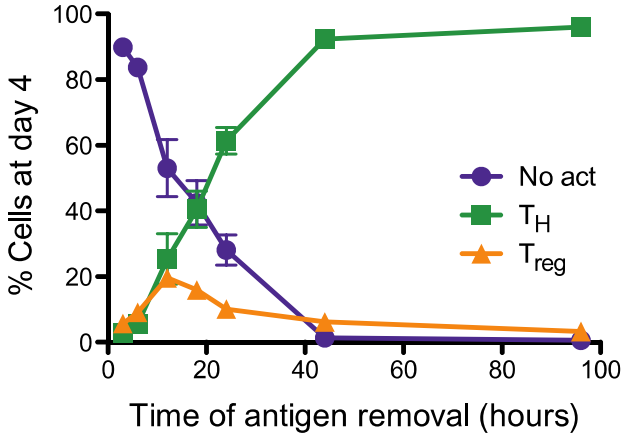
Turner *et al.*, *J. Immunol.* (2009)

# Logical model of T cell differentiation



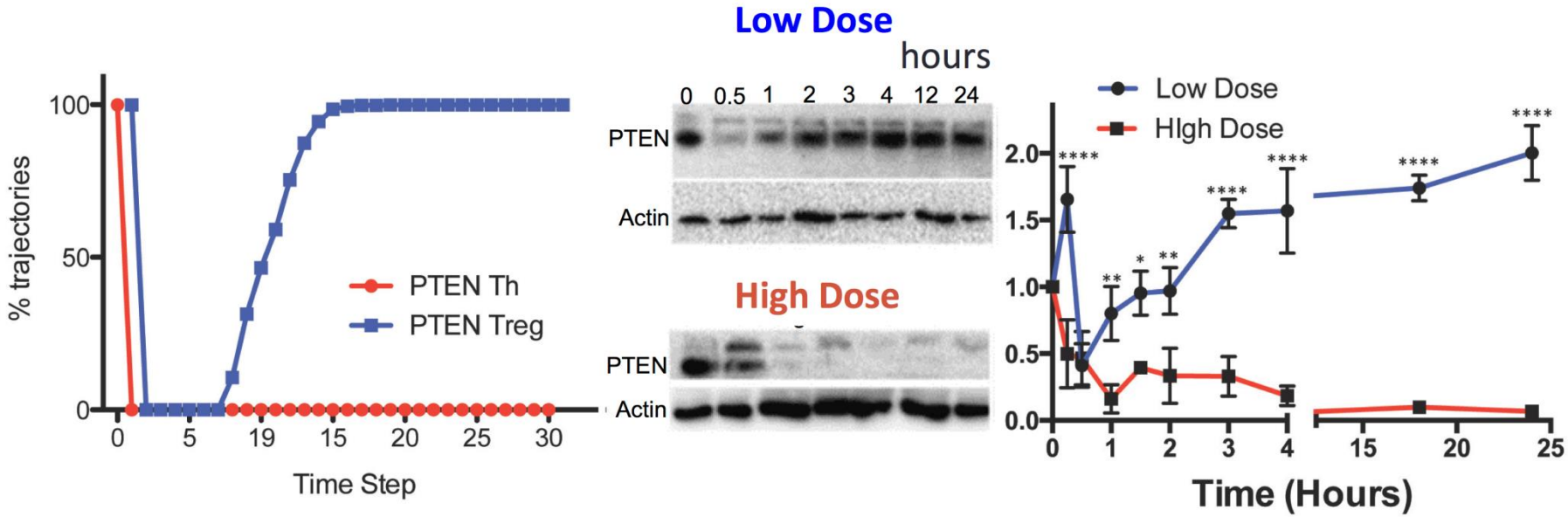

TCR signal duration




Miskov-Zivanov et al. *Science Signaling* (2013)

# Model prediction: differential kinetics of PTEN

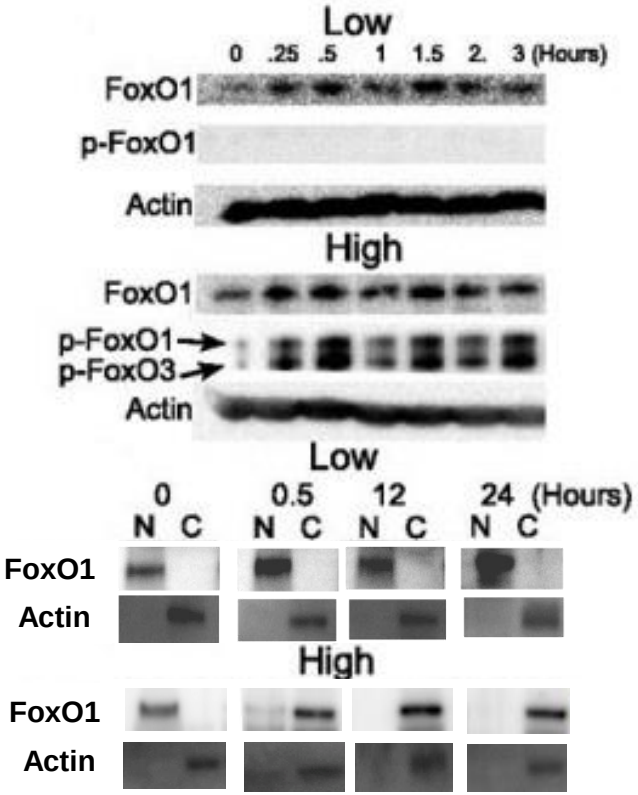
**Experiments: Dr. Bill Hawse**

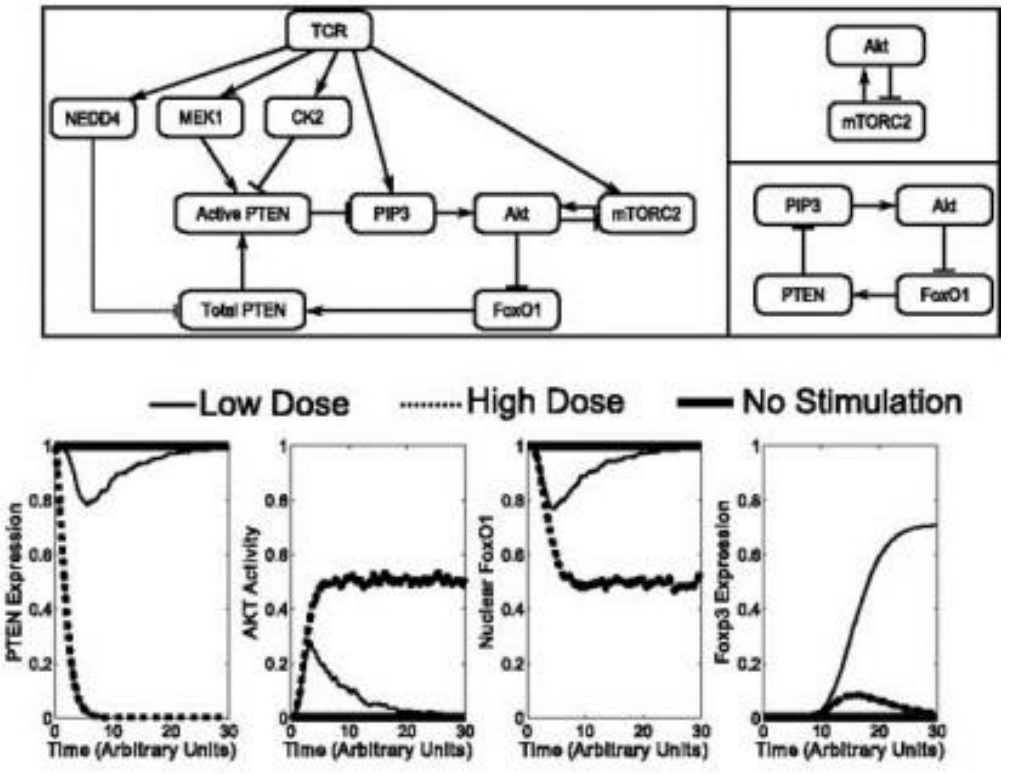



Miskov-Zivanov et al. *Science Signaling* (2013)

Hawse et al., *J. Immunol.* Cutting Edge (2015)

# FoxO1 plays a critical role in PTEN regulation

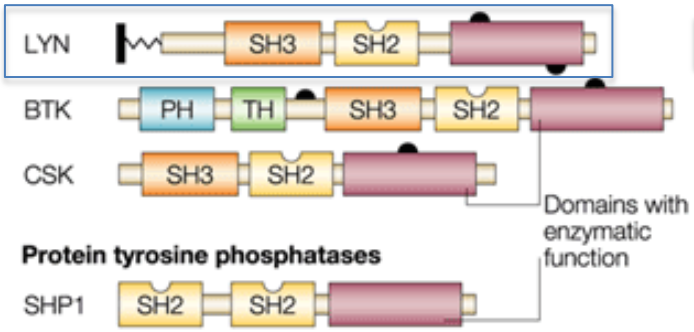



Hawse et al., *J. Immunol.* Cutting Edge (2015)

# Rule-based Modeling (RBM)

Molecules are modeled as *structured objects*

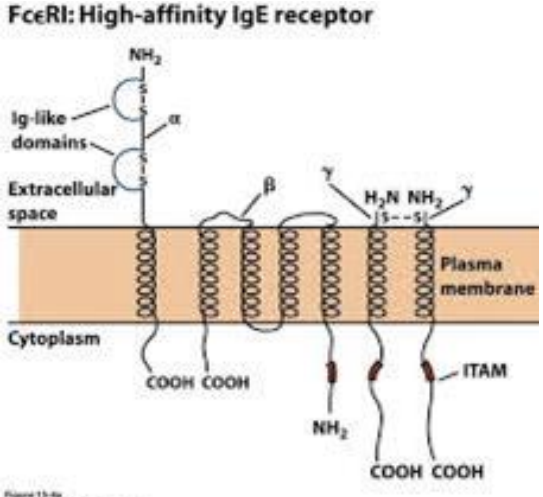



LYN(SH3,SH2,kin,Y397~0~P,Y508~0~P)

Molecule

Components

States

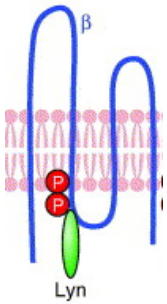



FceRI(a_Ig,b_Y218~0~P,g_ITAM~0~P)

www.bionetgen.org

# Rule-based Modeling (RBM)

## Rules define the interactions of molecules




"Lyn SH2 domain binds to phosphorylated Tyr 218 on the β subunit of FcεRI "

**Reactants** **Products**

LYN(SH2) + Rec(bY_218~P) <-> LYN(SH2!1).FceRI(bY_218~P!1) kpL, kmL

**bond**

**Center** – elements modified by the action of the rule

**Context** – elements required for reaction to occur but not modified

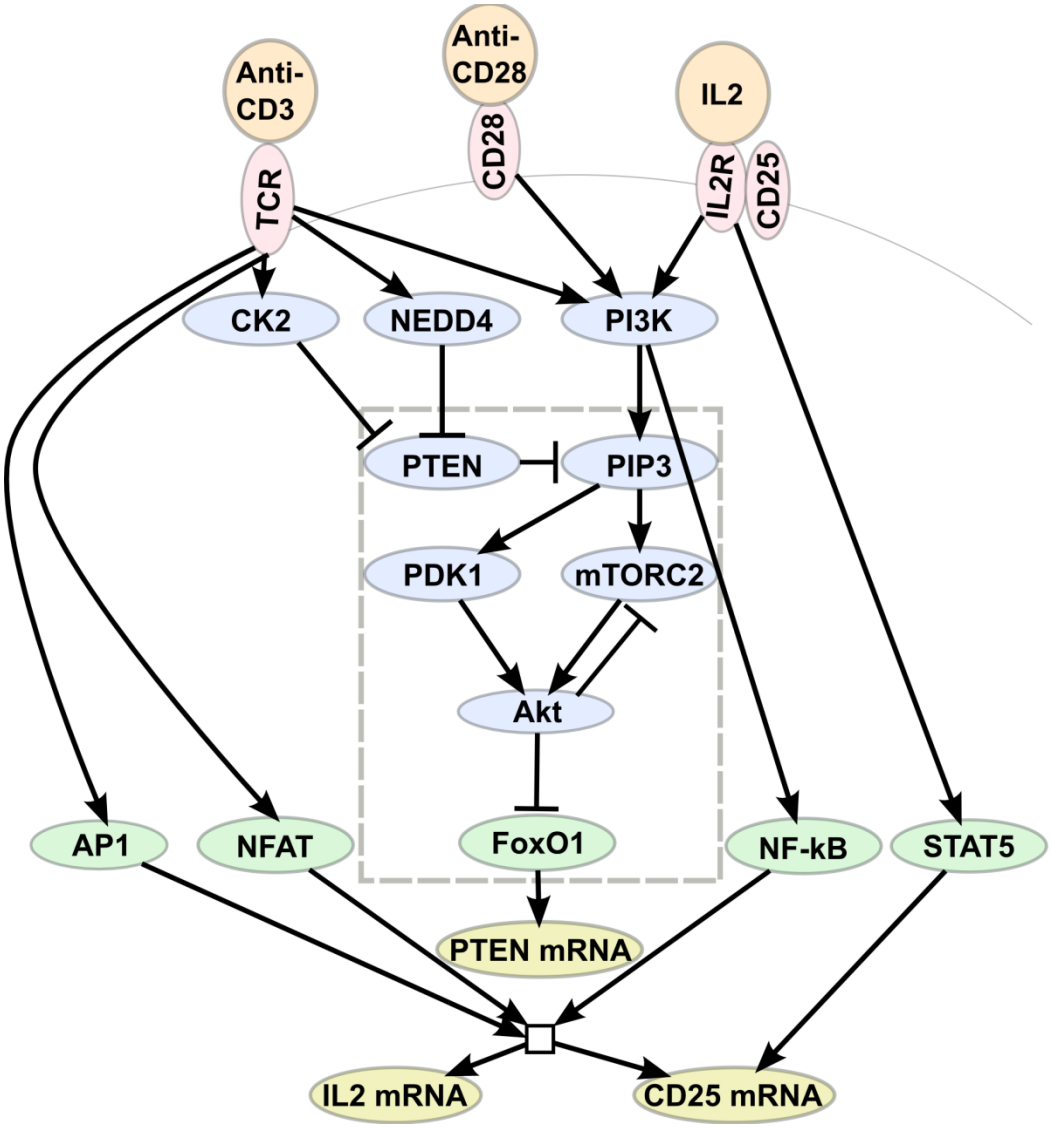
"Don't write don't care" – elements not mentioned may be in any state

➔ *One rule can generate reactions involving many different species*

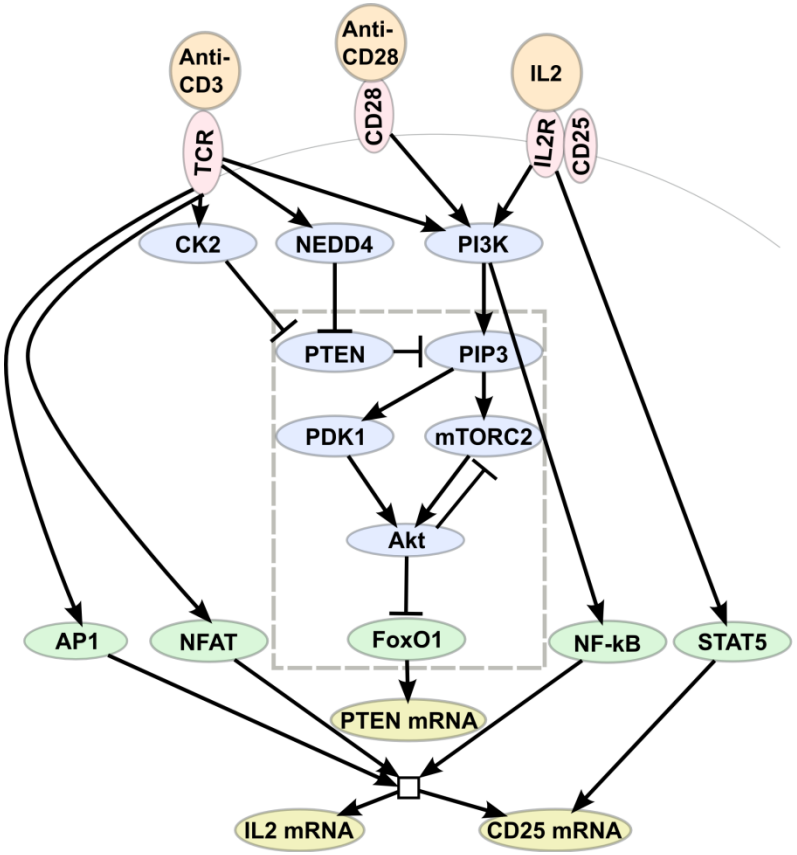
Reaction rate determined by **Mass Action kinetics**

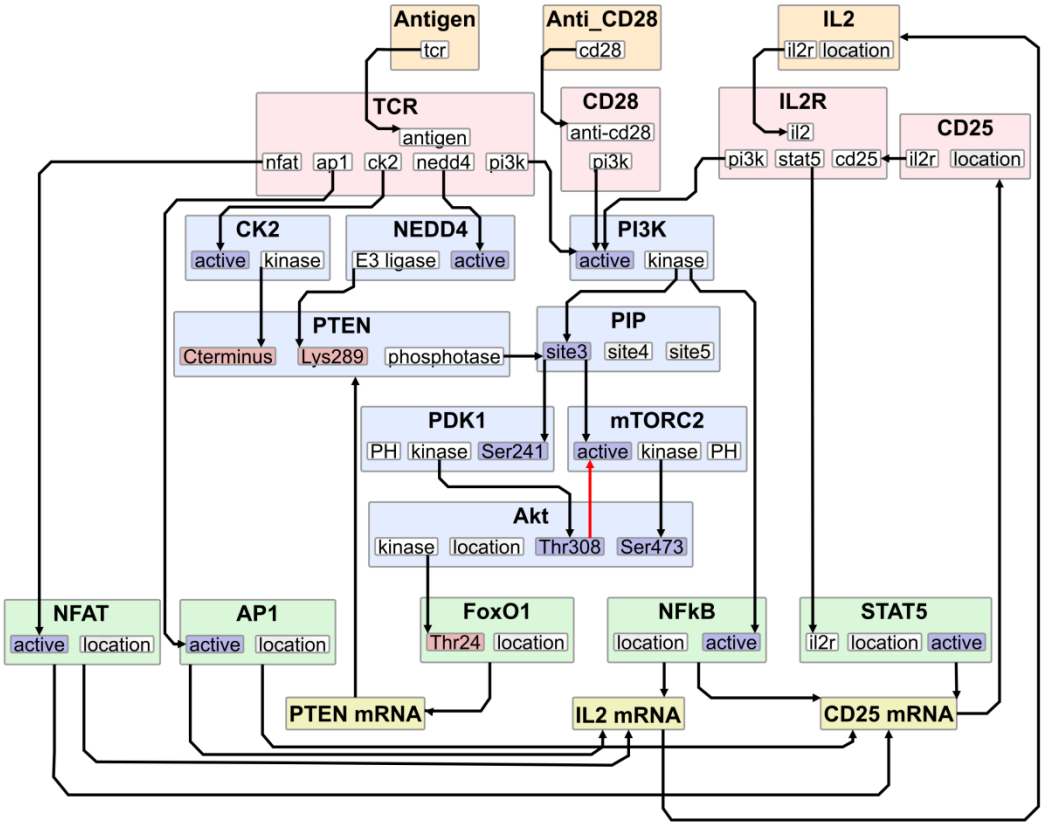
rate forward = kpL*[Lyn(SH2)]*[Rec(bY_218~P)]

# Rule based modeling of TCR signaling



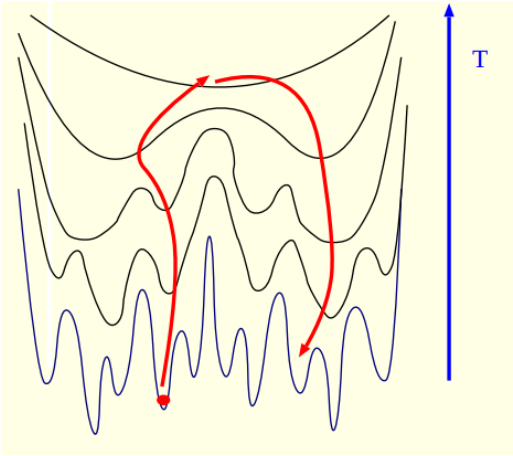
# Rule based modeling of TCR signaling

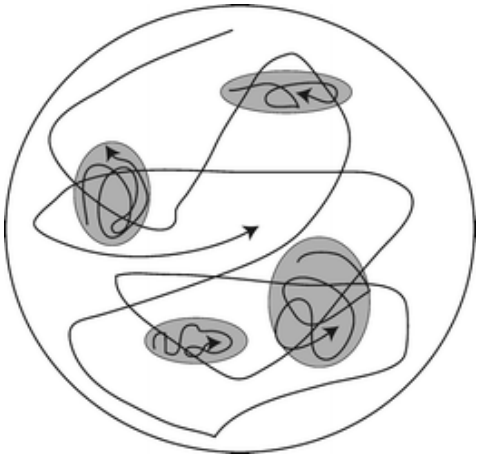


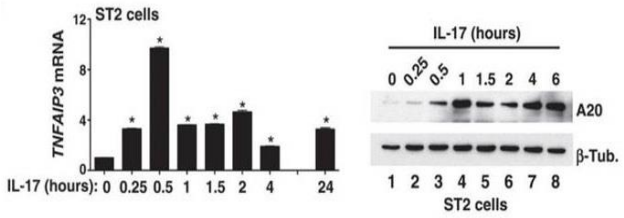
# Parameter Estimation: Parallel Tempering

- Replica exchange Bayesian MCMC
- Allows for efficient sampling of multi-dimensional parameter space
- Run parallel MCMC chains at different temperatures
- Higher temperatures smooth the energy landscape, allowing for global sampling
- Chains switch temperatures when new local minima are found
- Prior distributions constrain parameter values

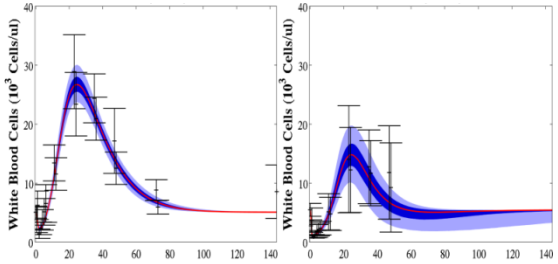


# Ptempest




Experimental Data

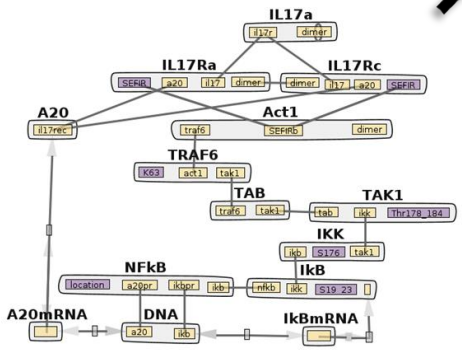


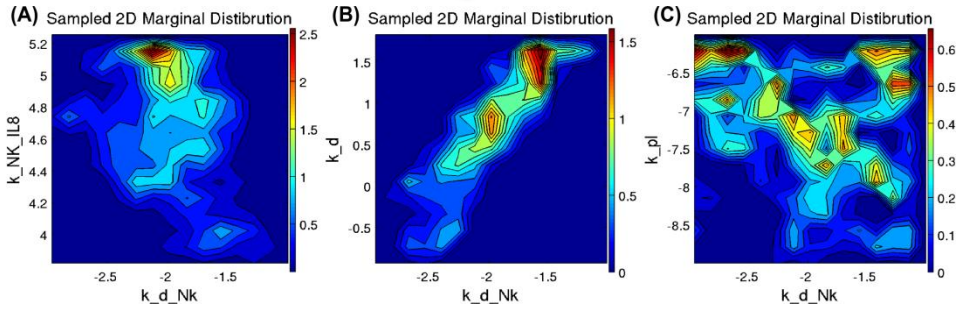

**ptempest**

Parallel tempering for Bayesian parameter estimation of dynamical systems

Best fit parameter ensembles

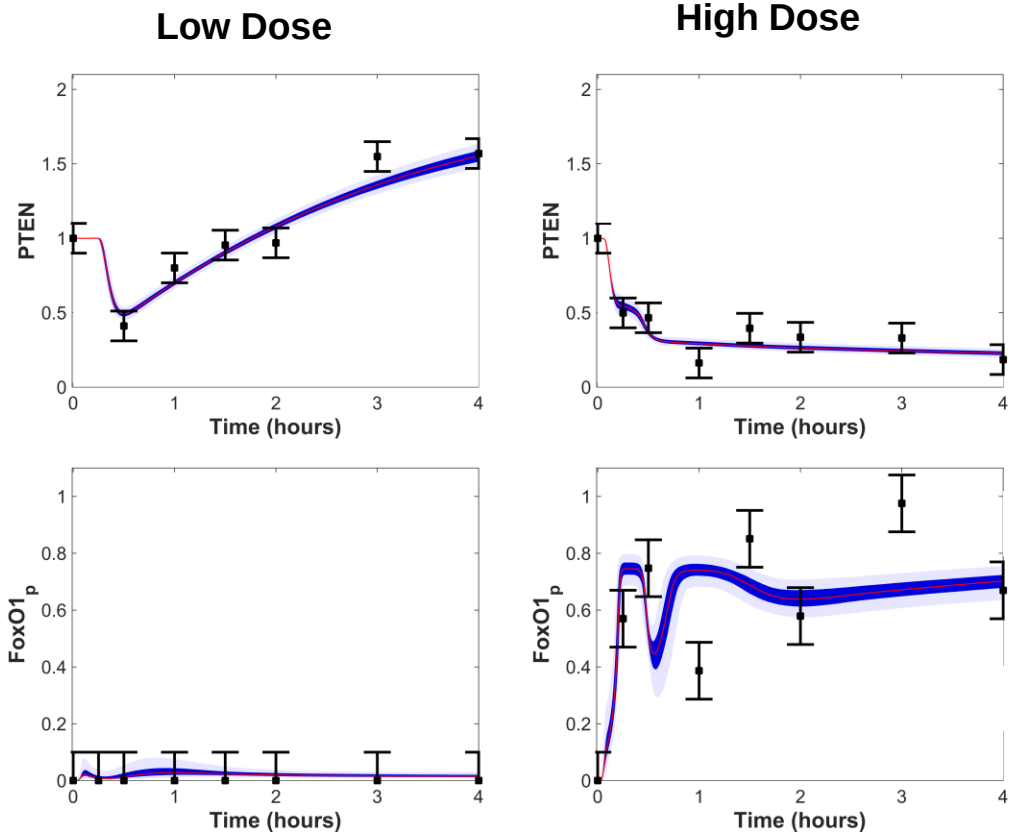
BioNetGen Model

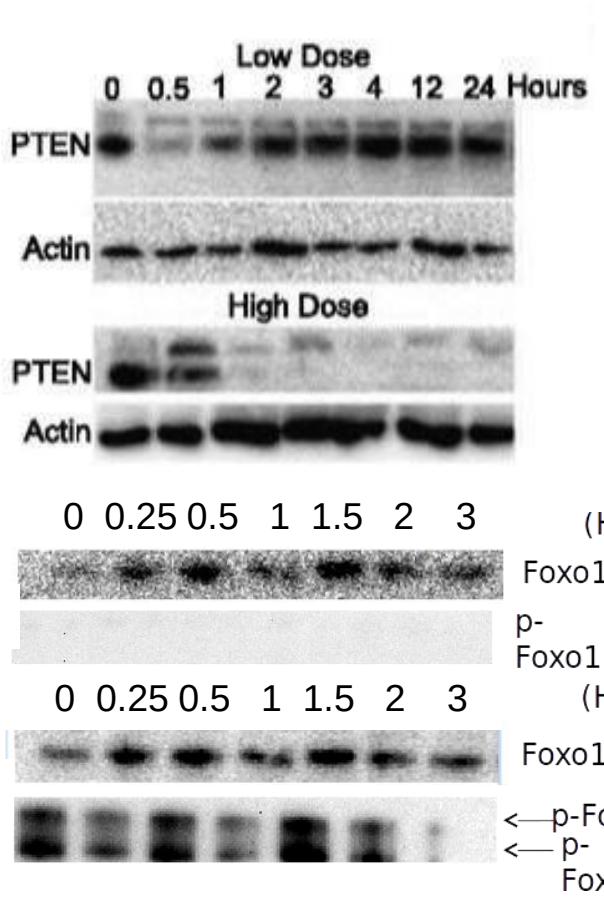



https://github.com/RuleWorld/ptempest

# Simulations capture key differences between high and low dose stimulation



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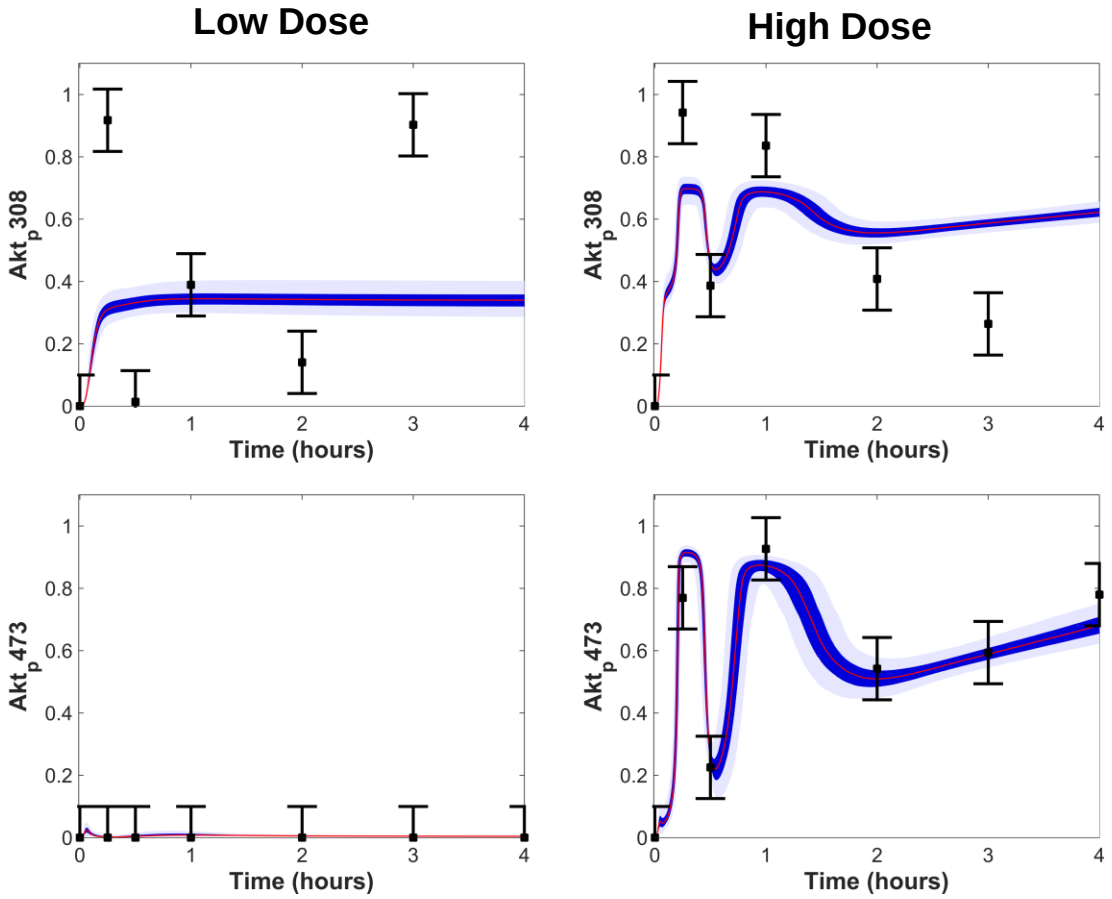
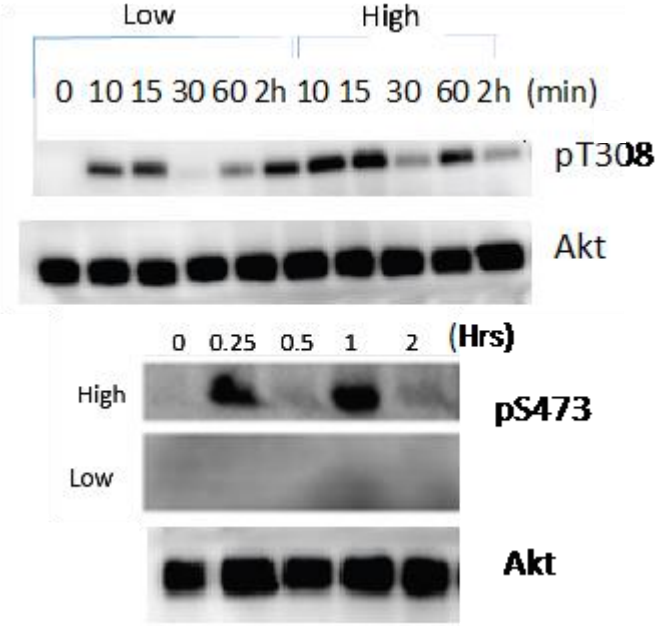
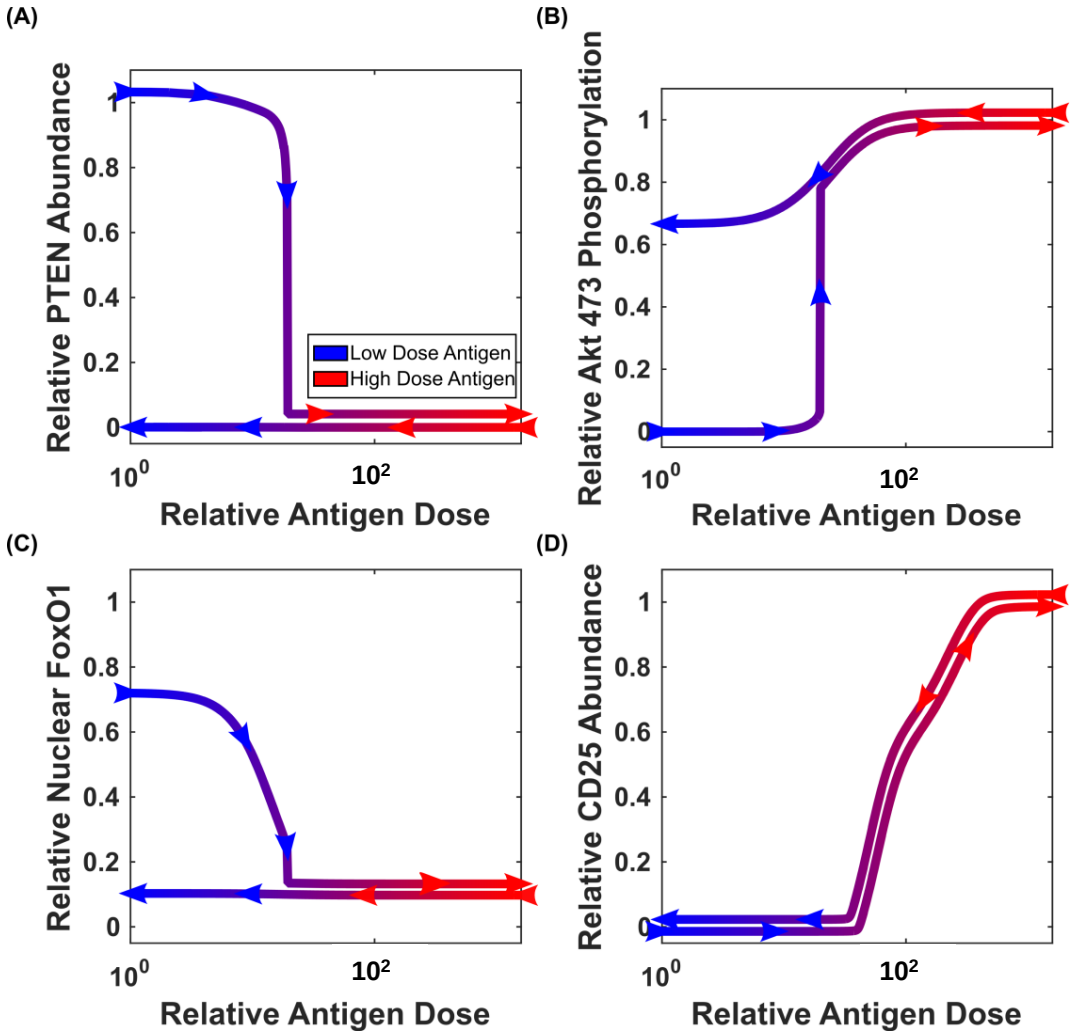
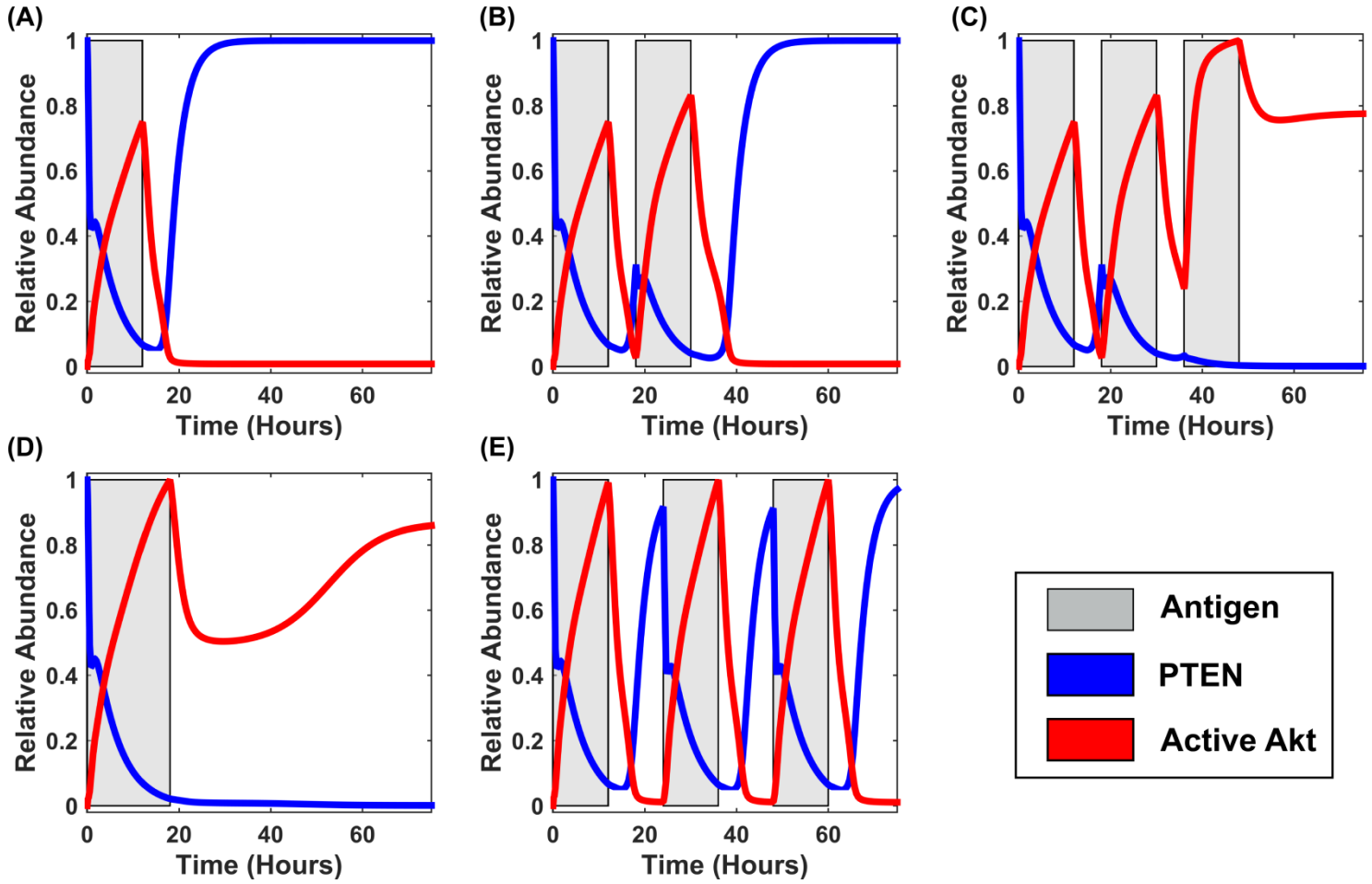


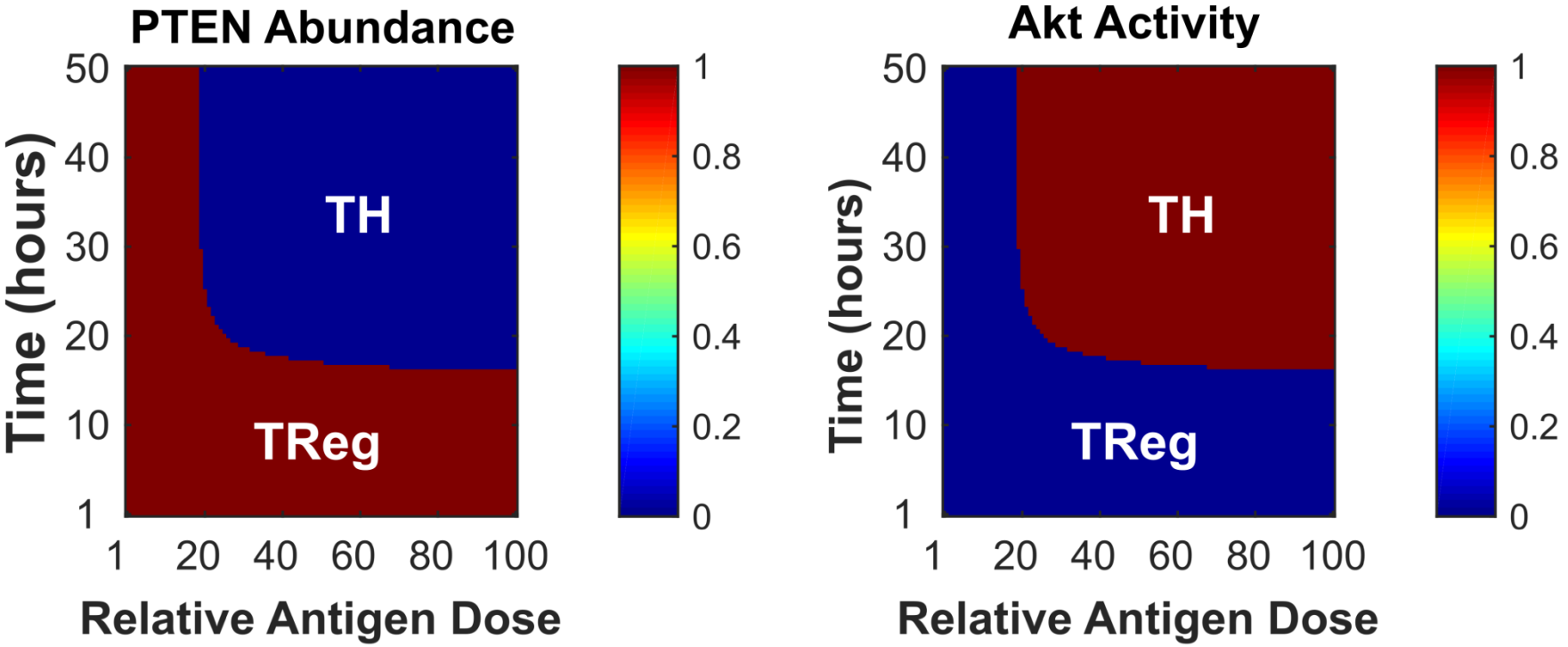
# Sharp thresholds on antigen dose govern PTEN, Akt, and FoxO1 activity



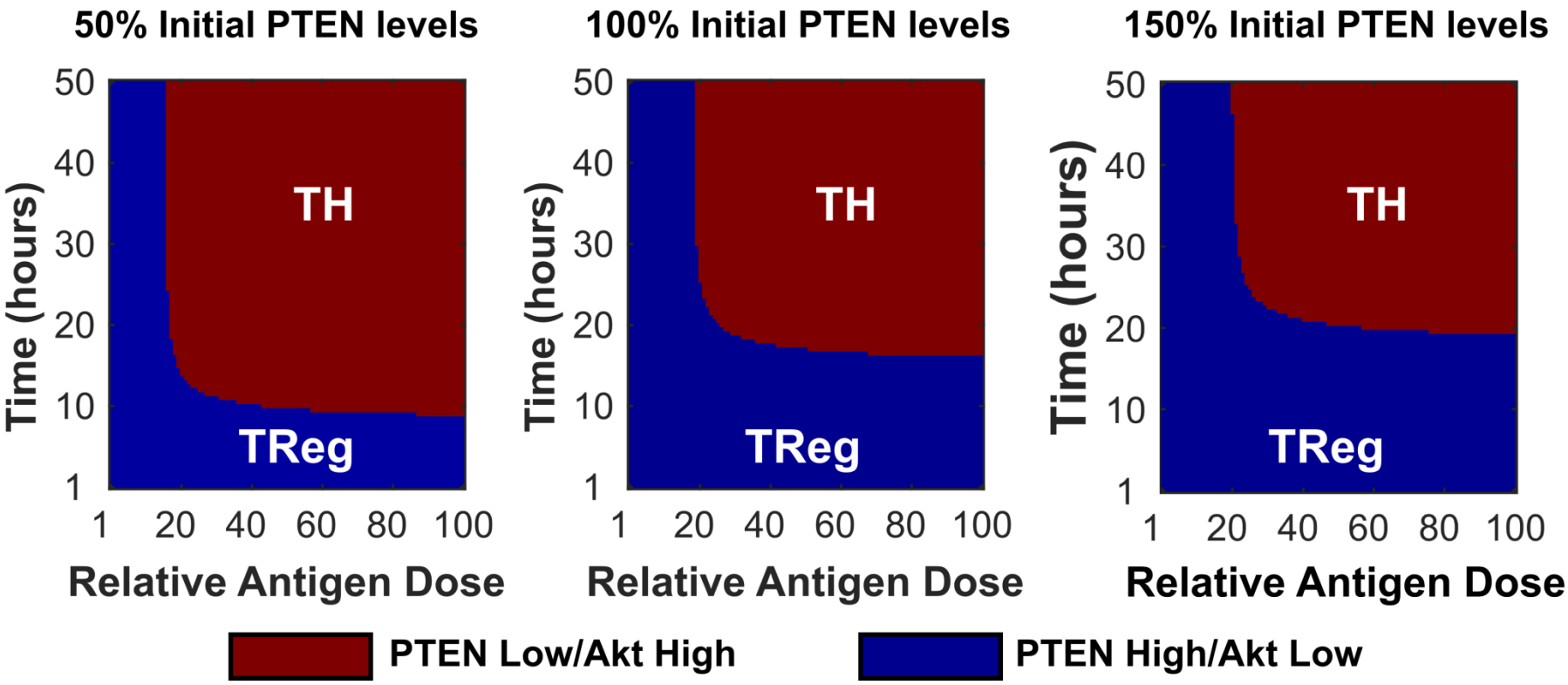
# A minimum duration of stimulation is required to remove PTEN



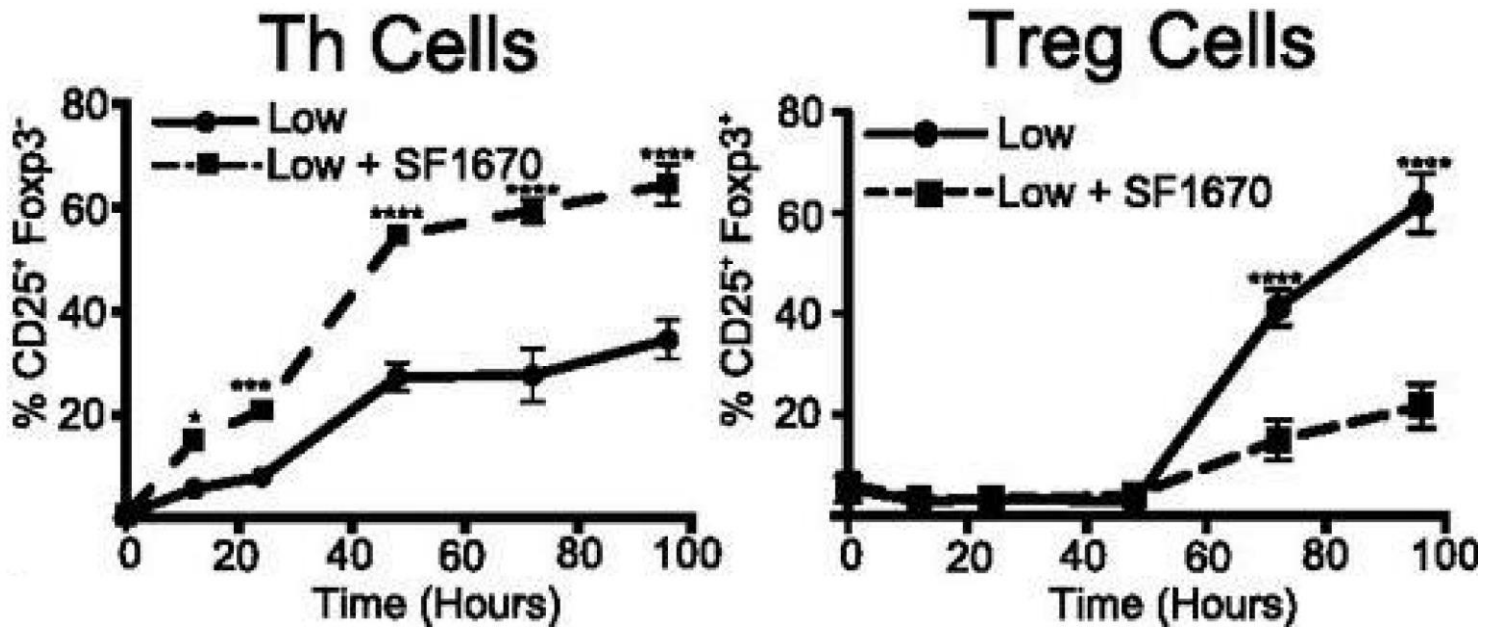
# Dose and time thresholds define stimulation necessary to reduce PTEN and activate Akt



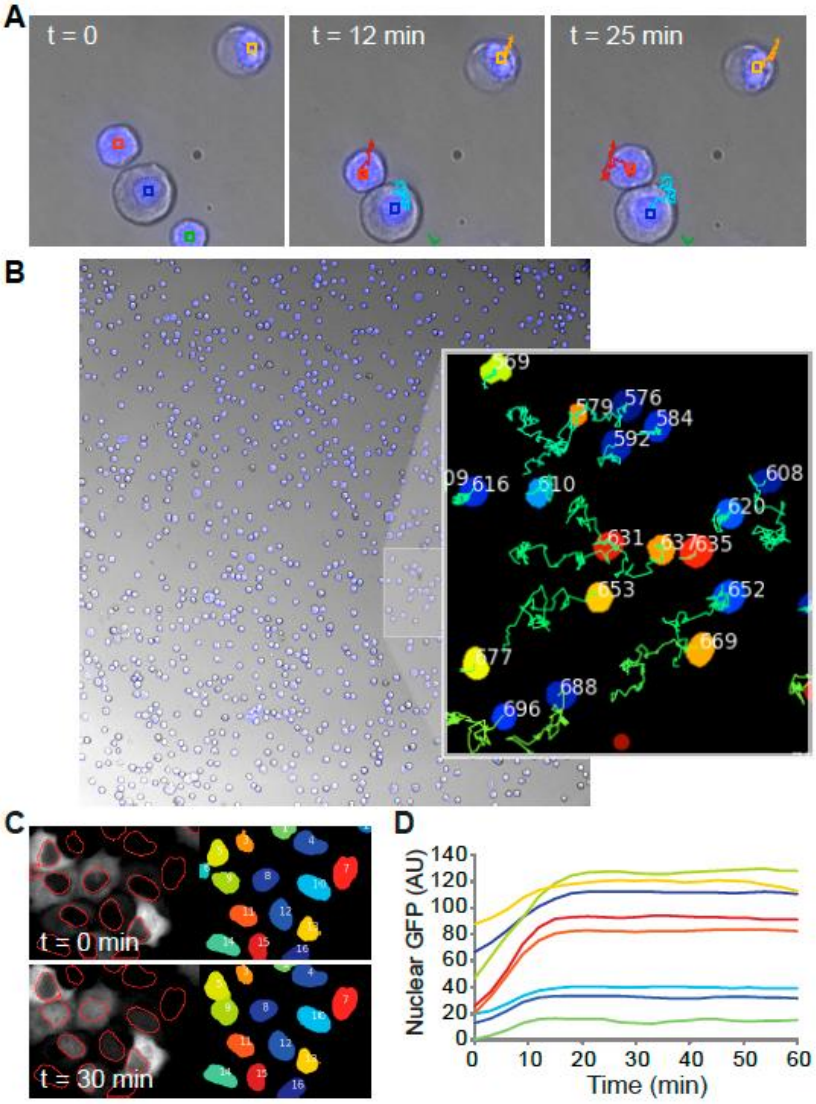
# Varying initial concentrations of PTEN shifts the threshold



# PTEN inhibitor SF1670 changes T cell populations

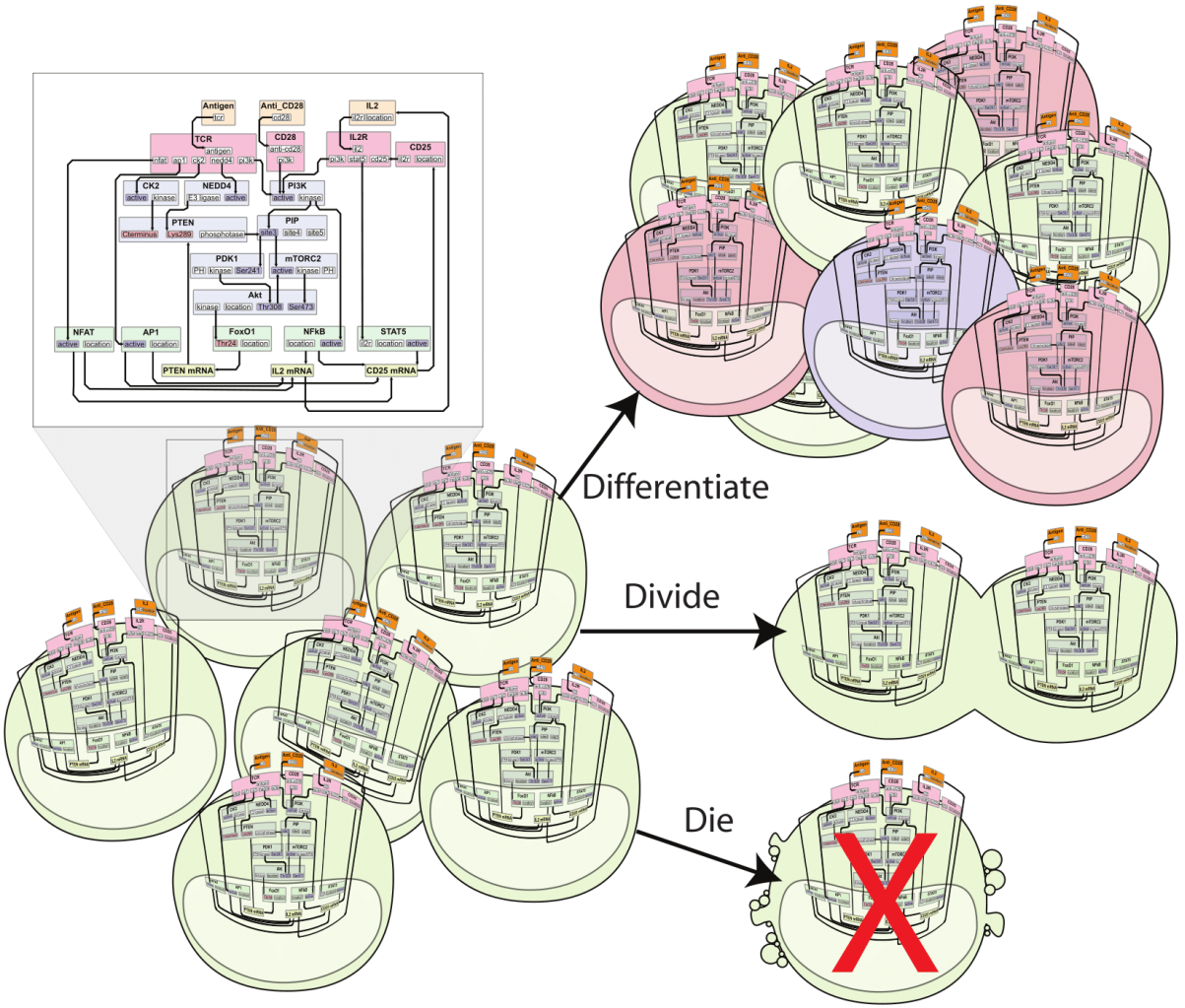


# Live cell imaging can be used to validate model predictions




Courtesy of Dr. Robin Lee

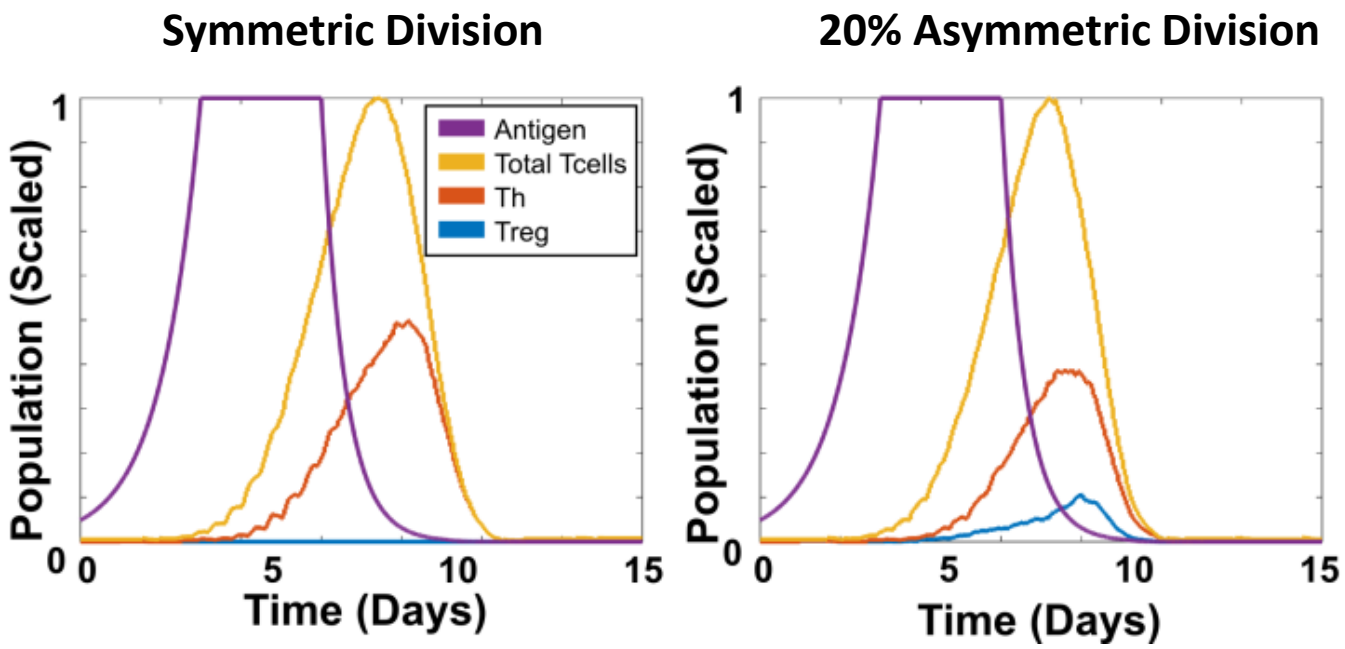
# Encoding a RBM as a population model



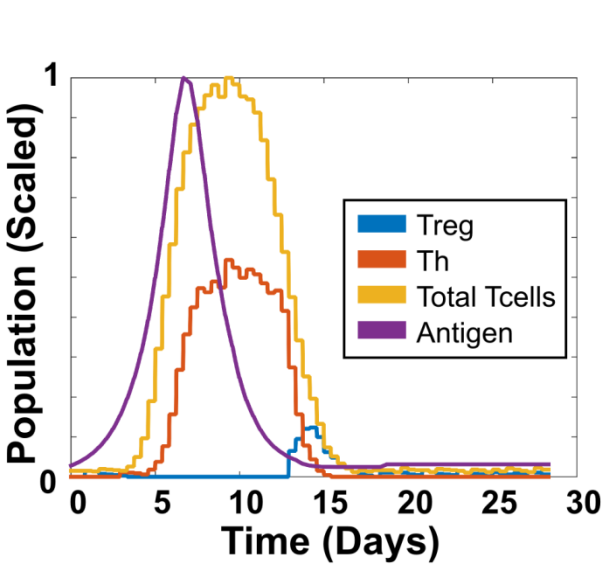
# Populations model details

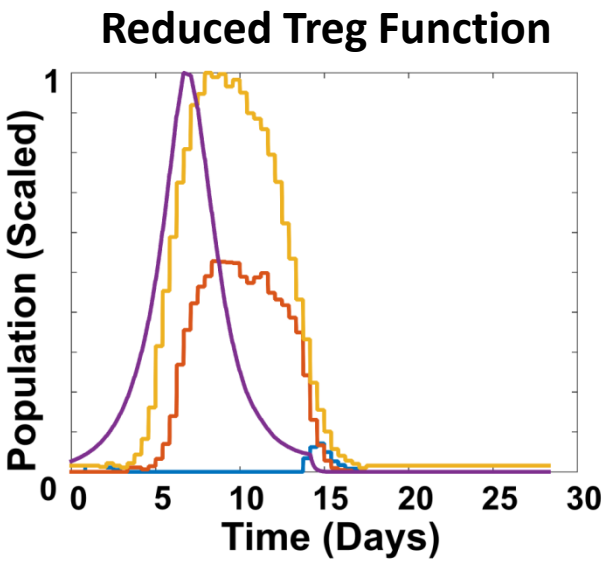
- T cells modeled as RBMs simulated independently
- Identical network architecture, heterogeneous initial conditions
- Communicate via shared IL-2 pool
- Simulated in 30 minute blocks
- Differentiate based on thresholds on total Akt activity
- Divide based on IL-2 signaling
- Die based on individual cell lifetime

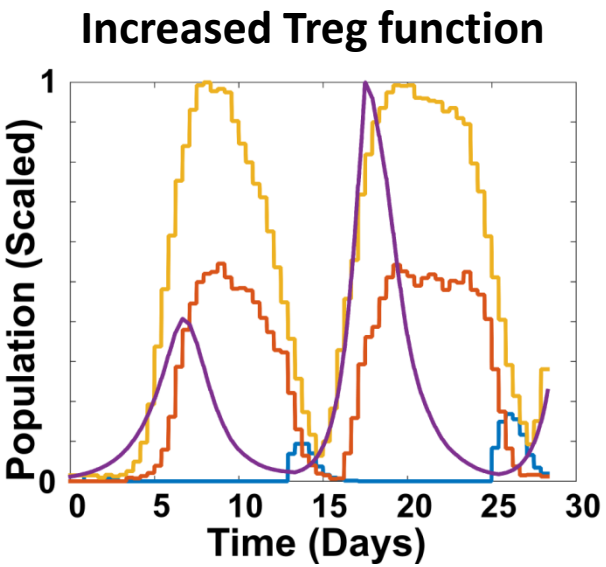
# Asymmetric T cell division allows for the emergence of a Treg population



# Tregs alter the trajectory of disease clearance



# Key extensions of the population model

- Fine tune asymmetric cell division
- More detailed differentiation
  - FOXP3, T-bet
- Include additional cell types
  - DCs, macrophages, CD8+ T cells, memory T cells
- Environment: Cytokines, spatial effects

# Conclusions

- A novel Akt-FoxO1-PTEN positive feedback loop gives rise to bistability in PTEN/Akt activity
- Thresholds in both the strength and duration of TCR activation control commitment to two steady states
- Perturbing the system to shift this threshold could tune T cell population sizes and alter immune responses

# Acknowledgements


- Faeder Lab
  - Dr. Justin Hogg
  - Dr. Natasa Miskov-Zivanov
- Morel Lab
  - Dr. Bill Hawse
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- NIH T32 AI089443