Logical Modeling Peripheral T Cell Differentiation

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  - **Penelope Morel**
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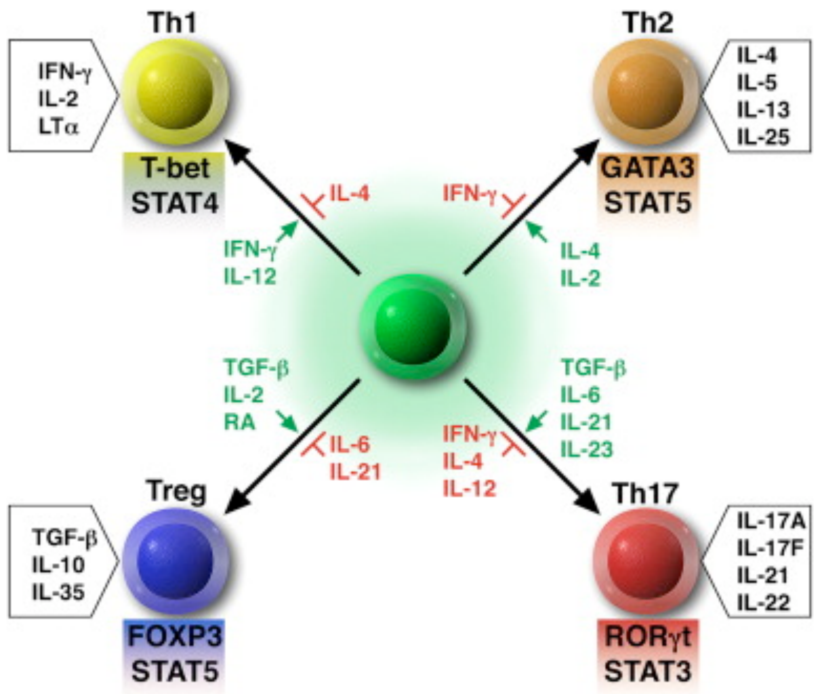
Peripheral T cell differentiation

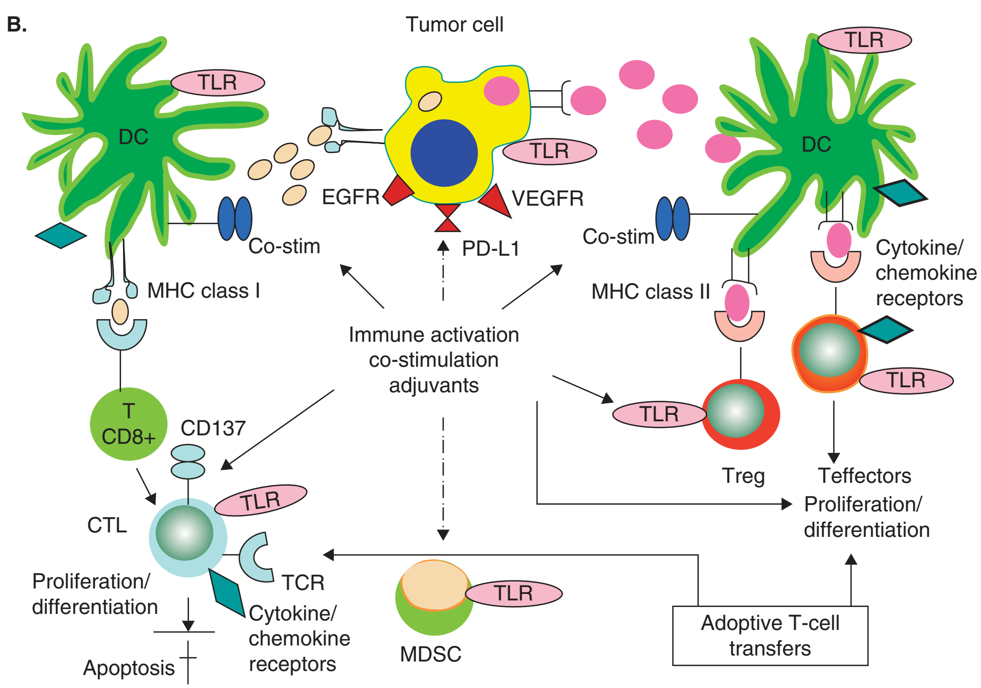
- T cell subpopulation ratios are critical for numerous immune and auto-immune pathologies



Source:
Ochs et al.,
J Allergy Clin
Immunol, 2009.

Peripheral T cell differentiation

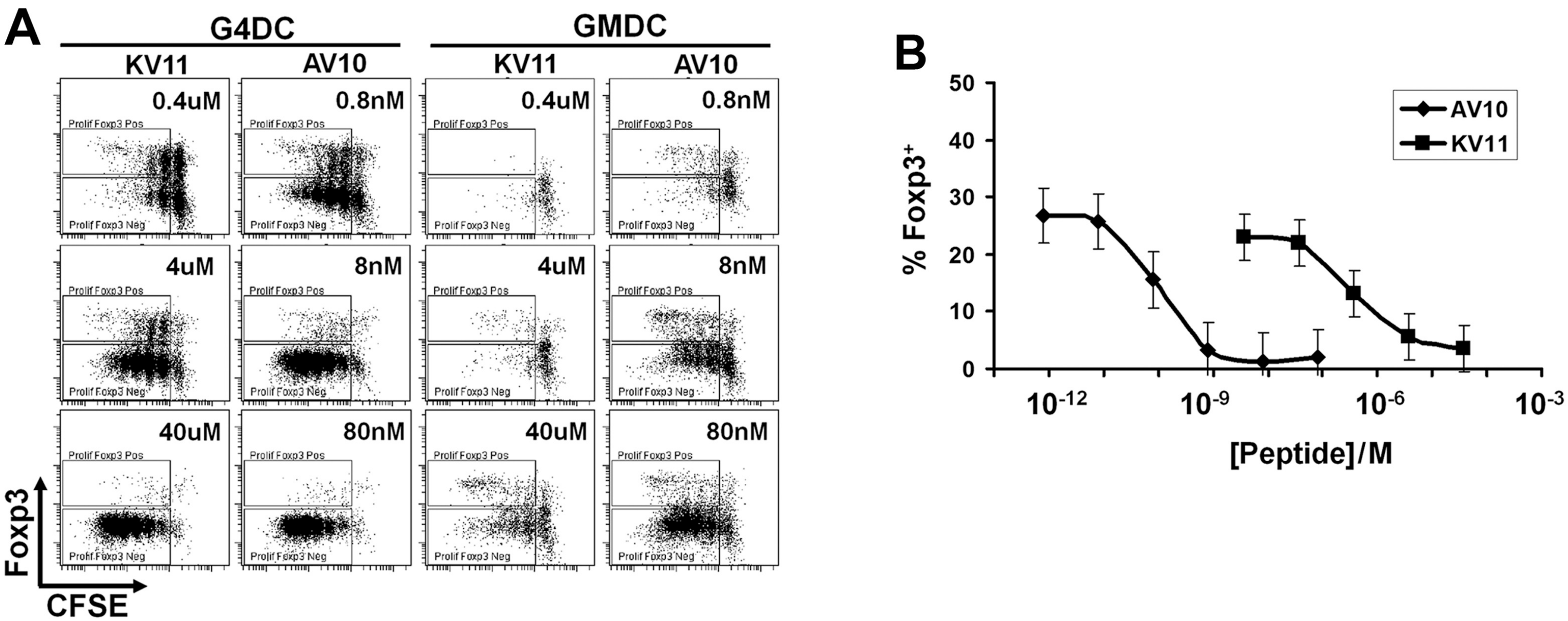
- T cell subpopulation ratios are critical for numerous immune and auto-immune pathologies
- Key target for immunomodulation therapy in cancer*



* Whiteside, T.L. "Inhibiting the Inhibitors...", Expert Opin. Biol. Ther. (2010), **10**, 1019.

Dominant Role of Antigen Dose in CD4$^+$Foxp3$^+$ Regulatory T Cell Induction and Expansion[1]

Michael S. Turner, Lawrence P. Kane, and Penelope A. Morel[2]



Naïve T cells stimulated with low Ag doses produce a high percentage of regulatory cells, which falls off as dose is increased.

Dominant Role of Antigen Dose in CD4+Foxp3+ Regulatory T Cell Induction and Expansion[1]

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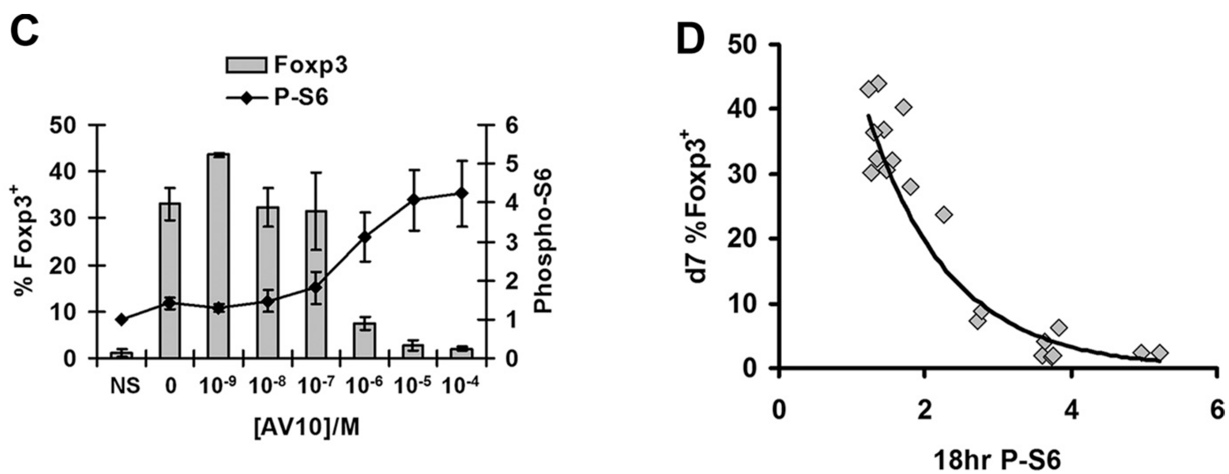


Inverse correlation between Foxp3+ Treg expansion and TCR signaling via Akt/mTOR/pS6.

Key Findings

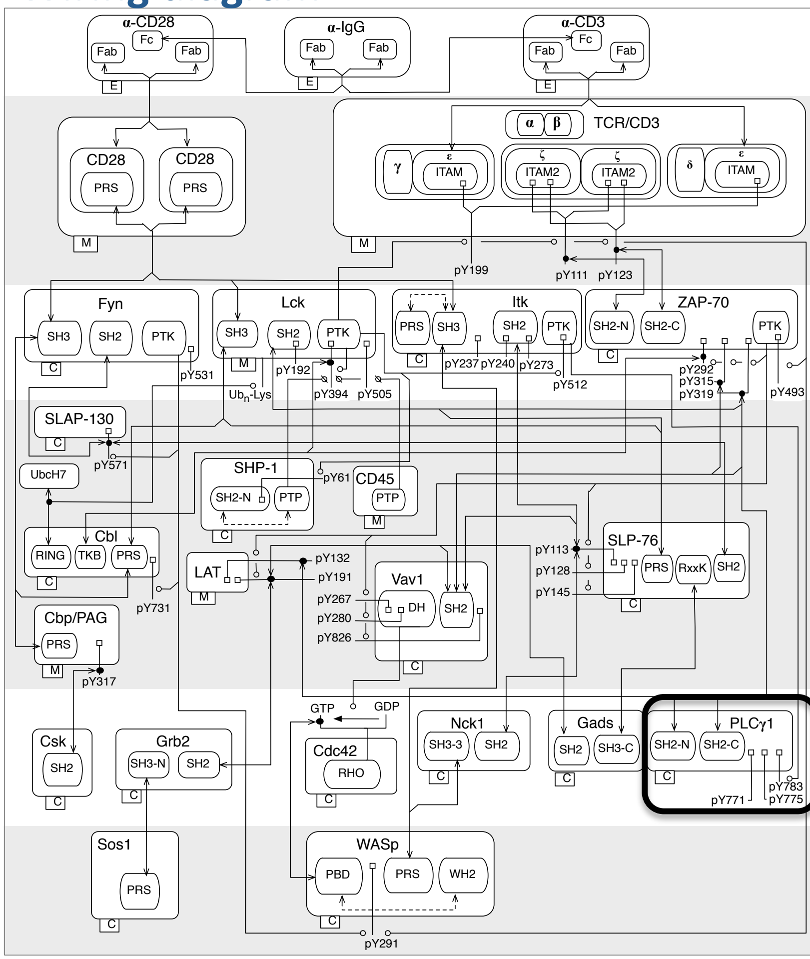
- Treg induction is determined by Ag dose
- Mechanism is T cell intrinsic
  - Observed with both iDC and mDC
  - Observed with plate-bound anti-CD3/CD28
- Inverse correlation between mTOR activation at 18h and Foxp3+ Treg at 7 days
- No exogenous TGF-β

Modeling Goals

- Determine whether known mechanisms are *sufficient* to explain experimental observations.
- Suggest *additional experiments* to identify missing mechanisms and clarifying areas of *uncertainty*.
- Identify other *early markers* of the response.
- Incorporate signals through other receptors ➔*predictive model.*

Rule-Based Modeling of Signal Transduction

Wiring diagram



Object-oriented model of protein

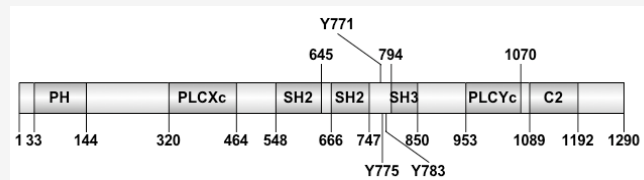
21. **PLCγ1**

Gene names: PLCG1, PLC1

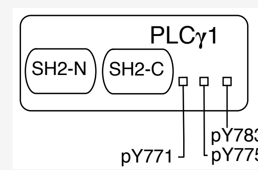
Uniprot accession number: P19174

Molecule type definiton: `PLCG1(SH2_N,SH2_C,Y771~u~p,Y775~u~p,Y783~u~p)`

Domain structure:



In the map of molecular interactions, PLCγ1 is represented with the following graph:

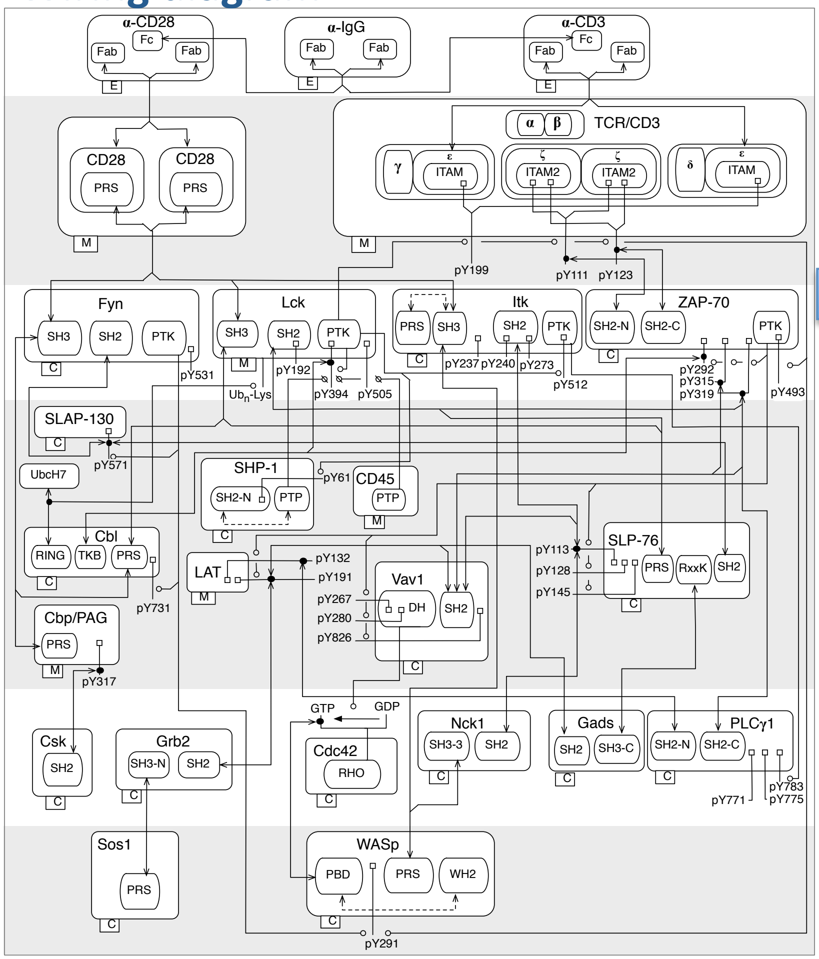


Phospholipase Cγ1 is an enzyme essential for T cell activation (*127*). It cleaves phosphatidylinositol 4,5-bisphosphate, generating the second messengers diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) (*128*). IP_3 binds to receptors on the endoplasmic reticulum, leading to release of Ca^{2+} (*129*). Itk phosphorylates PLCγ1 on Y783, which is important for activation (*51,130,131*). PLCγ1 binds to phosphorylated LAT (*111*). The

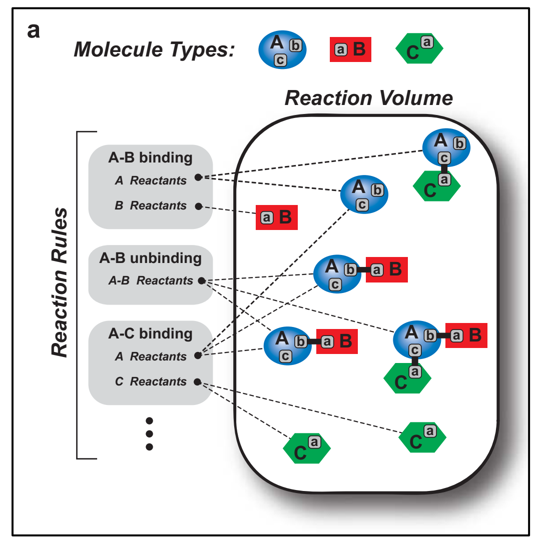
Hu, Chylek, and Hlavacek, in preparation.

Rule-Based Modeling of Signal Transduction

Wiring diagram



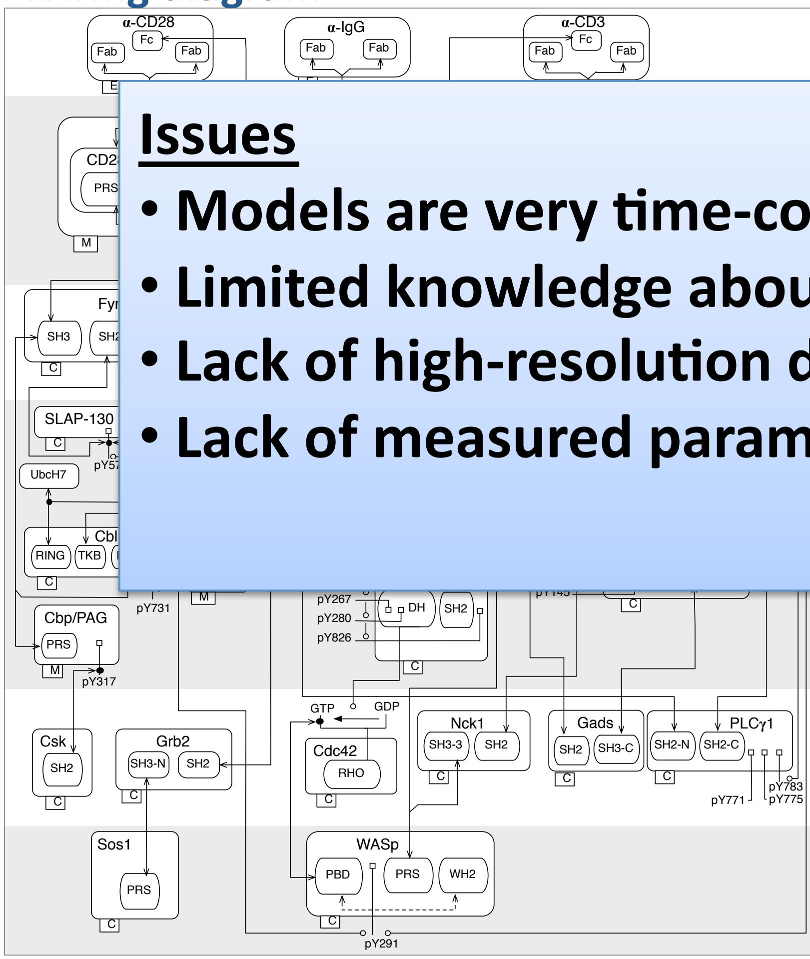
BioNetGen / NFsim



Hu, Chylek, and Hlavacek, in preparation.

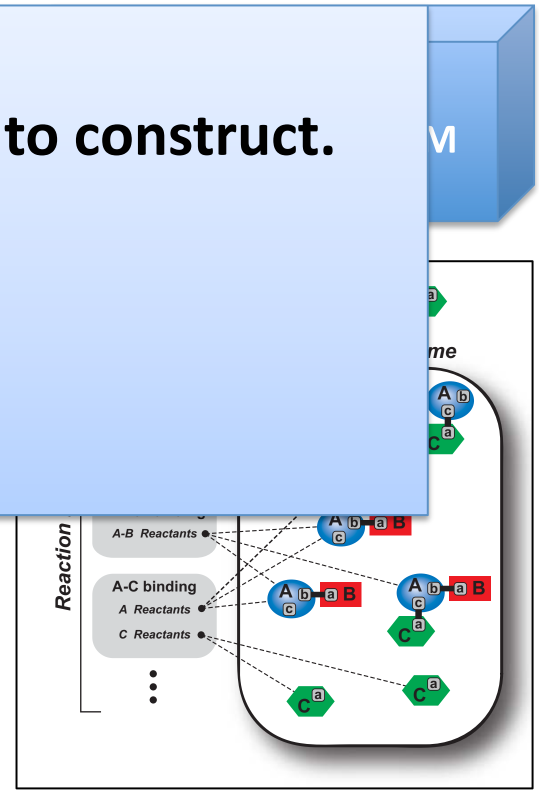
Rule-Based Modeling of Signal Transduction

Wiring diagram



Issues

- Models are very time-consuming to construct.
- Limited knowledge about wiring.
- Lack of high-resolution data.
- Lack of measured parameters.



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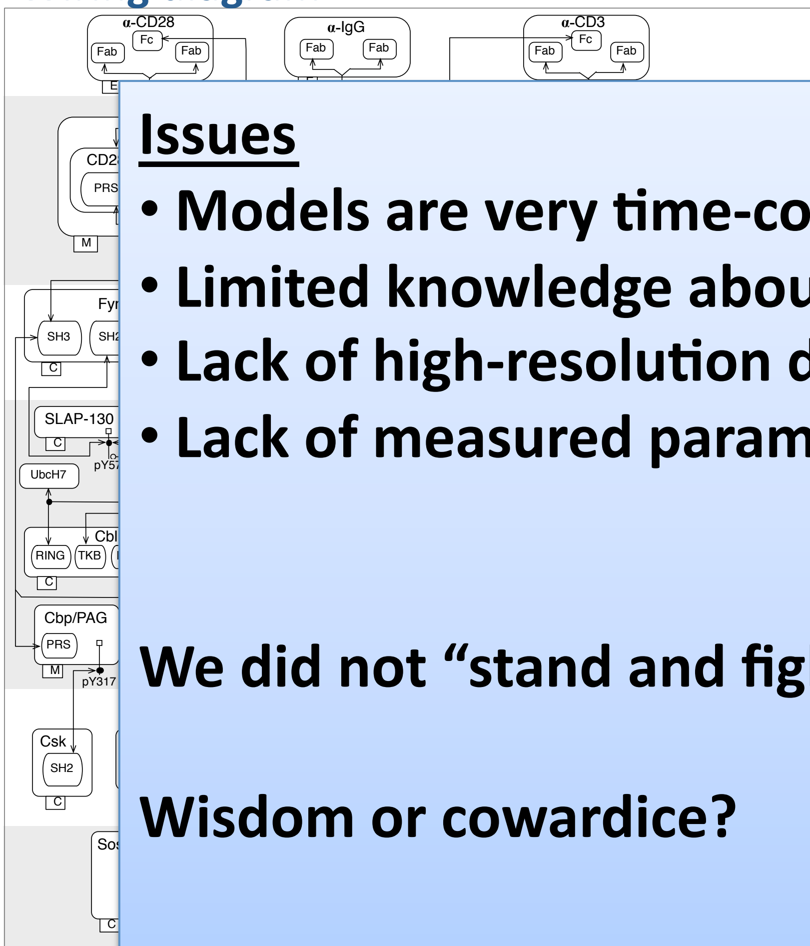
Rule-Based Modeling of Signal Transduction

Wiring diagram

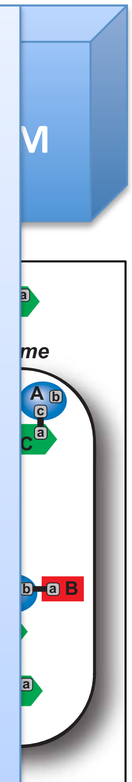


Issues

- **Models are very time-consuming to construct.**
- **Limited knowledge about wiring.**
- **Lack of high-resolution data.**
- **Lack of measured parameters.**

We did not "stand and fight" this time.

Wisdom or cowardice?



Hu, Chylek, and Hlavacek, in preparation.

A Simpler Approach
Boolean Networks

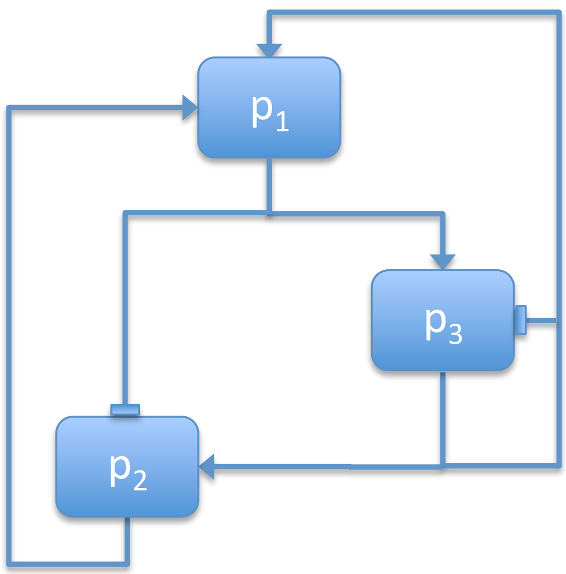
- The **state of an element** in the signaling network can be described by a **Boolean variable**, expressing that it is:
  - Active or present (on or ‘1’)
  - Inactive or absent (off or ‘0’)
- **Boolean functions**:
  - Represent interactions between elements
  - The state of an element is calculated from states of other elements
- The resulting network is a **Boolean network**
- Long history of applications to biology.

Logical Modeling Approach

- Generalization of Boolean – variables may have more than 2 values.
- Systematic study of the **dynamics** of large systems:
  - Depends largely on the interconnection structure
- *Does not require numerical parameters.*
- Discrete networks provide information about:
  - Multi-stationarity
  - Stability
  - Oscillatory behavior
- Highly relevant for obtaining **qualitative** measures
  - Perturbations
  - Environment
  - Alternative wiring of the network

Boolean Network Modeling Example

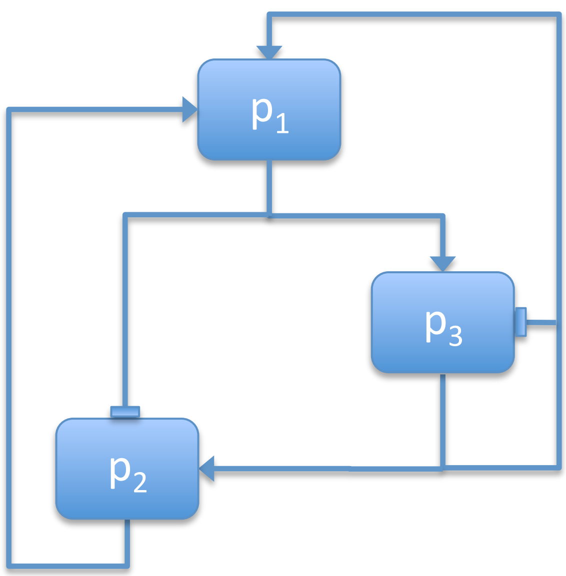
Biological network



Proteins: p_1, p_2, p_3

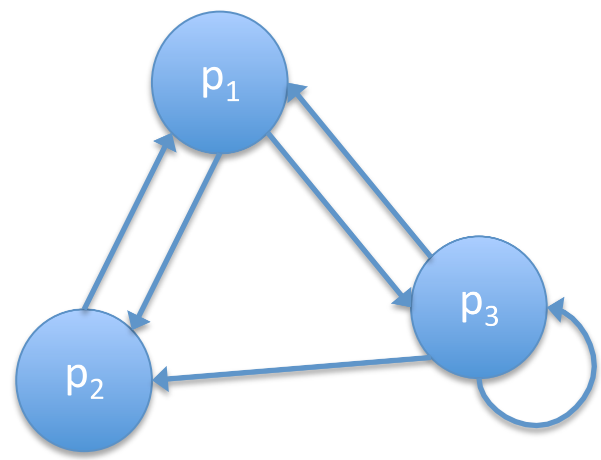
Boolean Network Modeling Example

Biological network



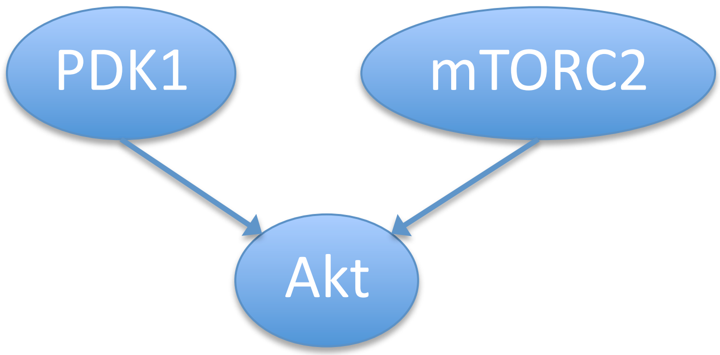
Proteins: p_1, p_2, p_3

Boolean network

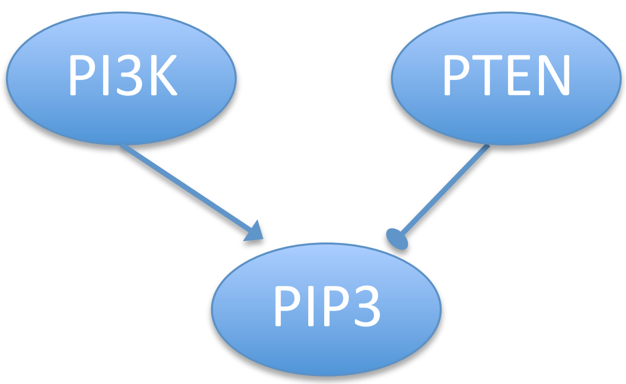


$p_1^* = p_2 \text{ OR } p_3$
$p_2^* = \text{NOT } p_1 \text{ AND } p_3$
$p_3^* = p_1 \text{ AND NOT } p_3$

Biochemical Examples



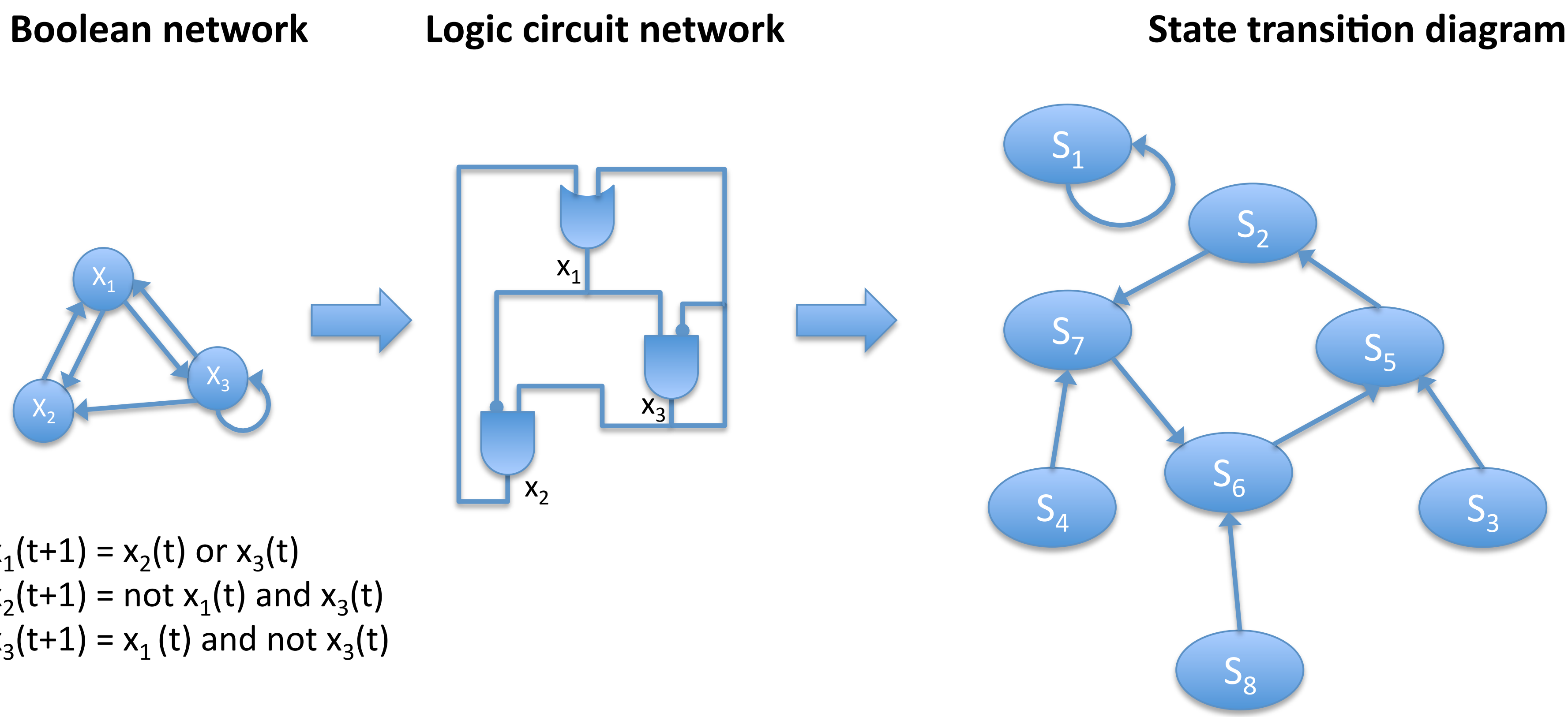
Akt’ = PDK1 AND mTORC2



PIP3’ = PI3K AND NOT PTEN

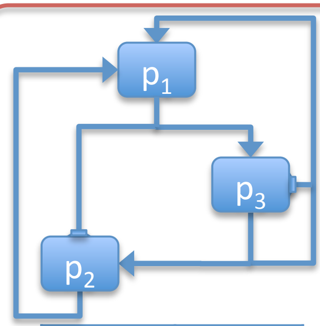
Note that PTEN overrides PI3K here.

Boolean Models Are Logic Circuits

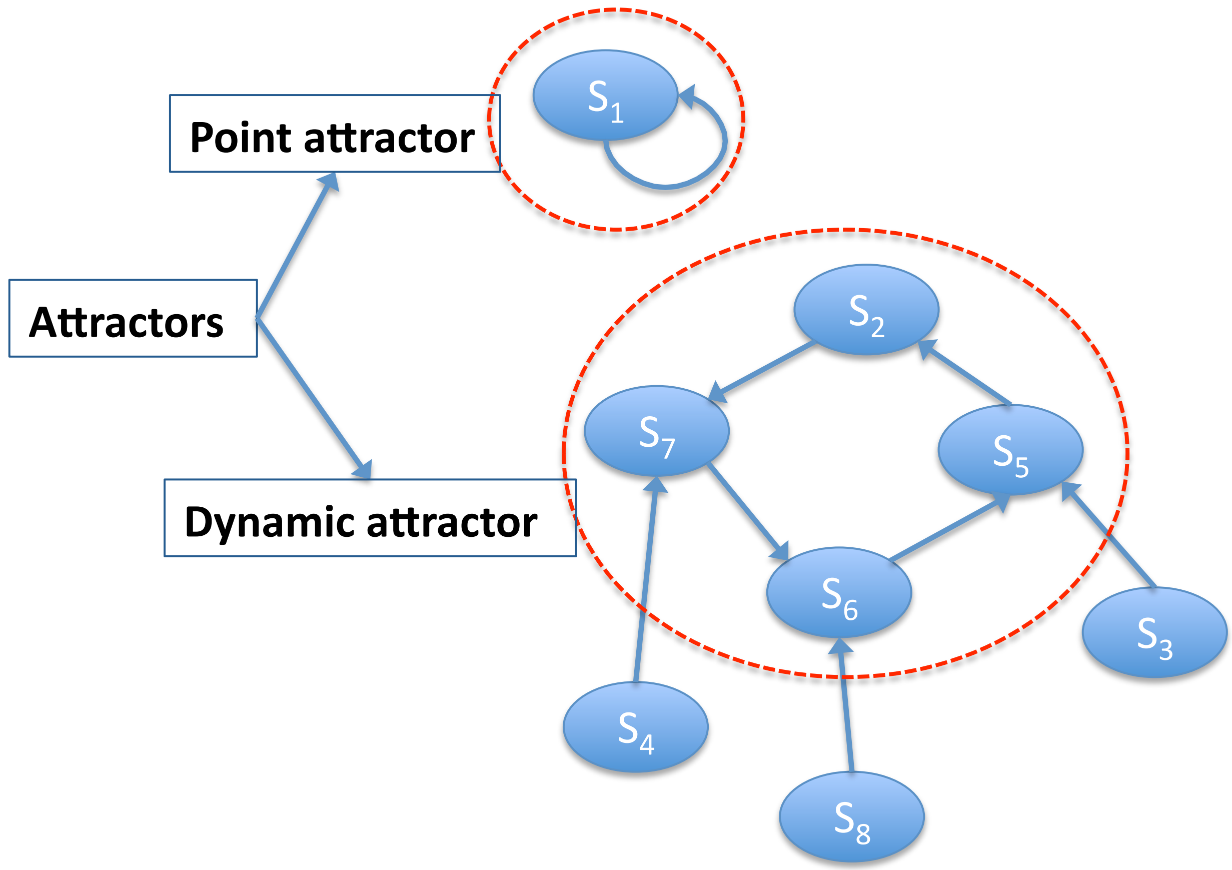


$x_1(t+1) = x_2(t)$ or $x_3(t)$
$x_2(t+1) =$ not $x_1(t)$ and $x_3(t)$
$x_3(t+1) = x_1(t)$ and not $x_3(t)$

Dynamics of a Boolean Model

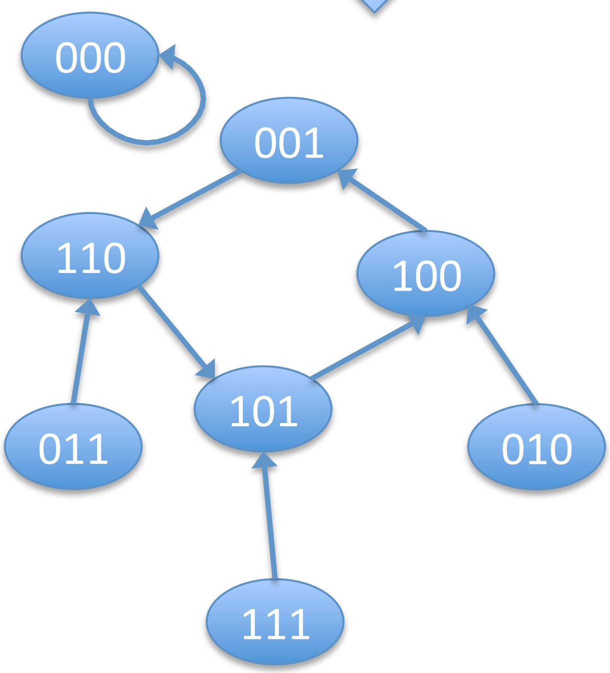


state	$x_1x_2x_3$
s_1	000
s_2	001
s_3	010
s_4	011
s_5	100
s_6	101
s_7	110
s_8	111



Different Methods for Simulating Network Dynamics

Synchronous updates



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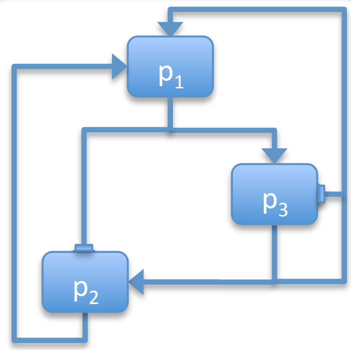

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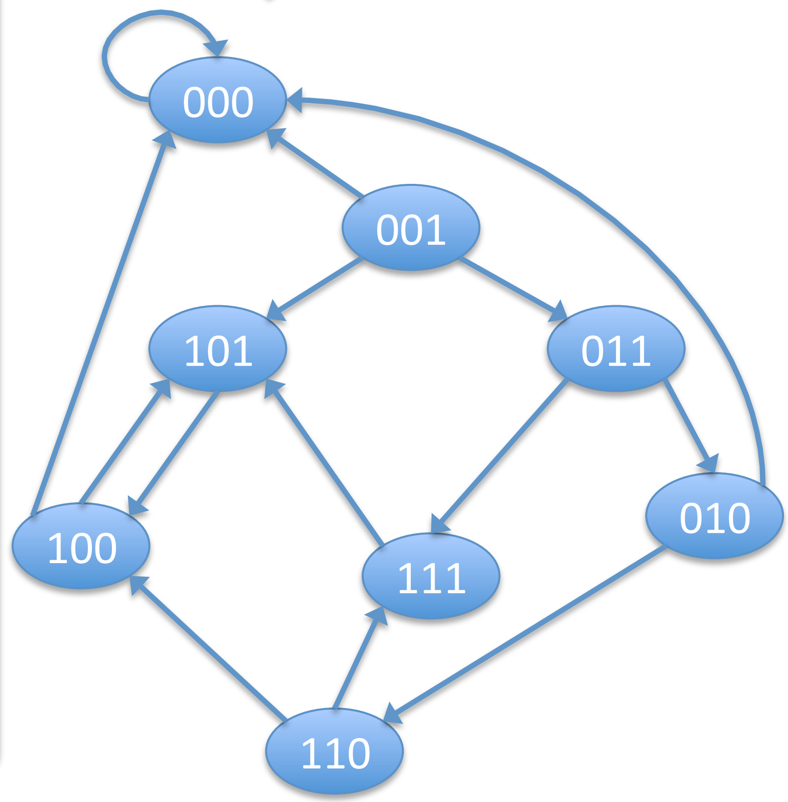
Asynchronous updates



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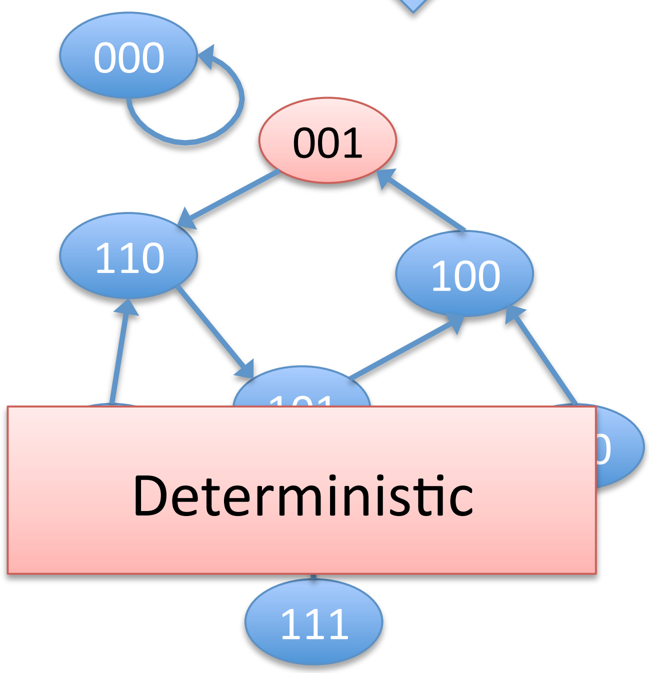

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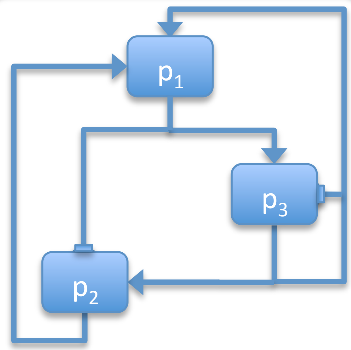

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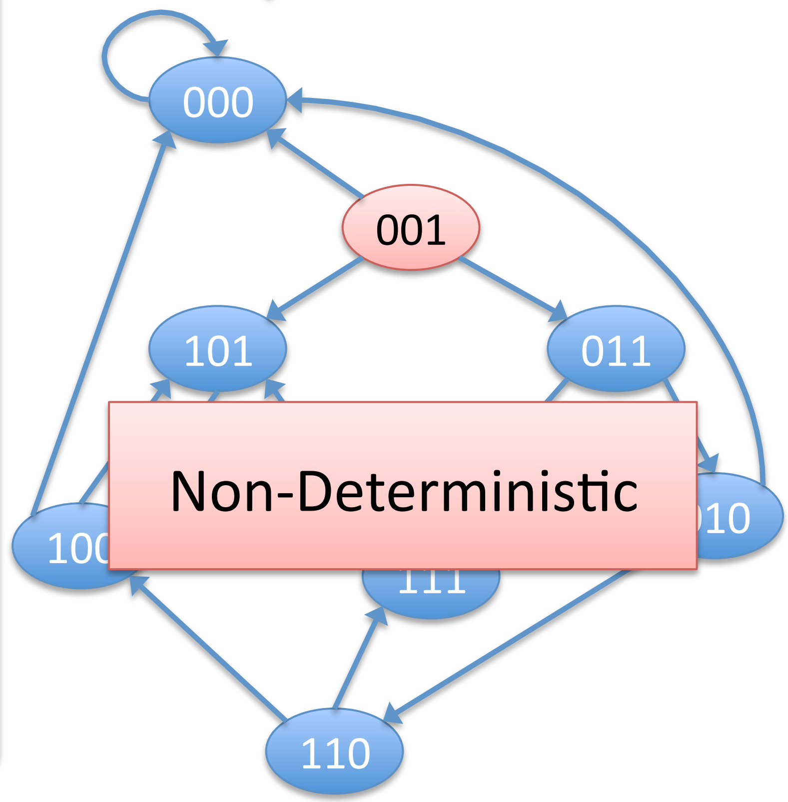
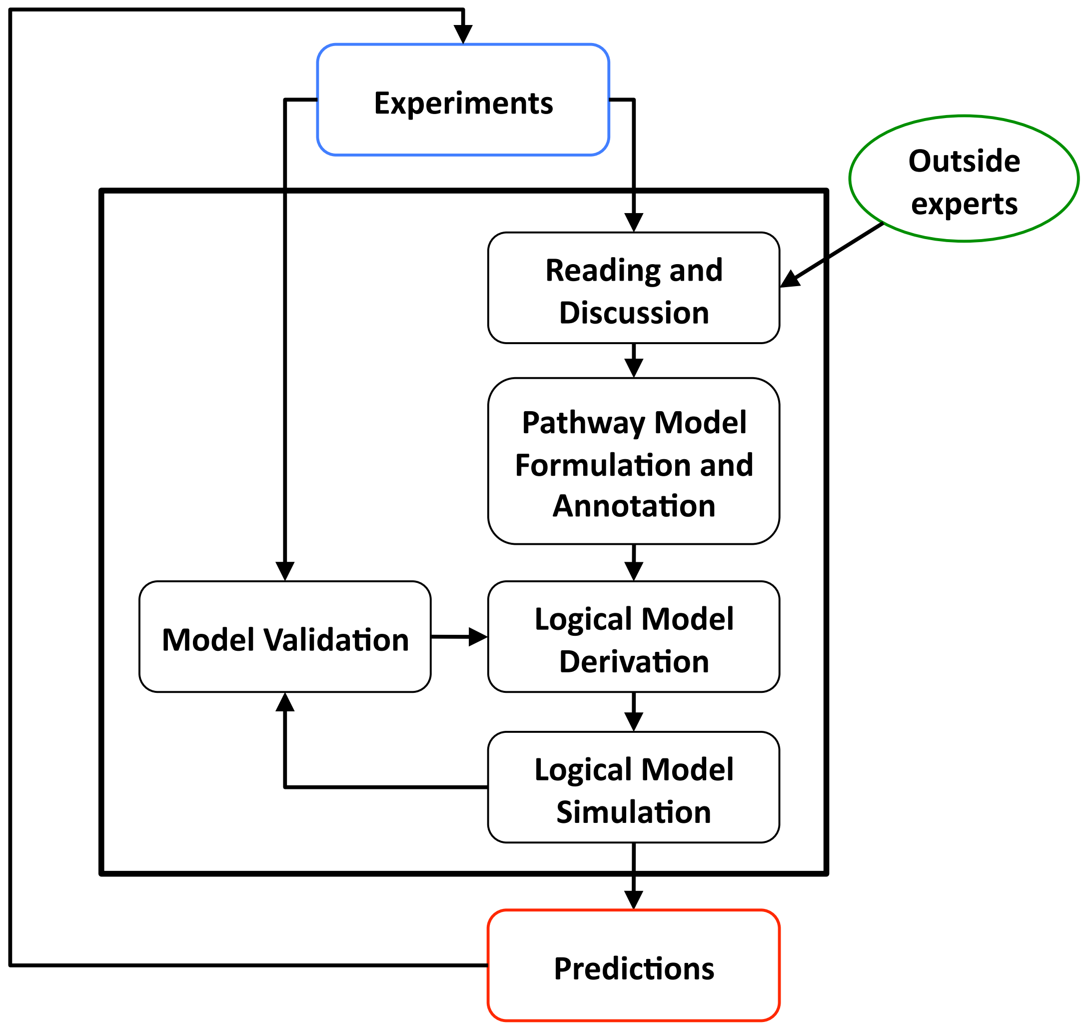
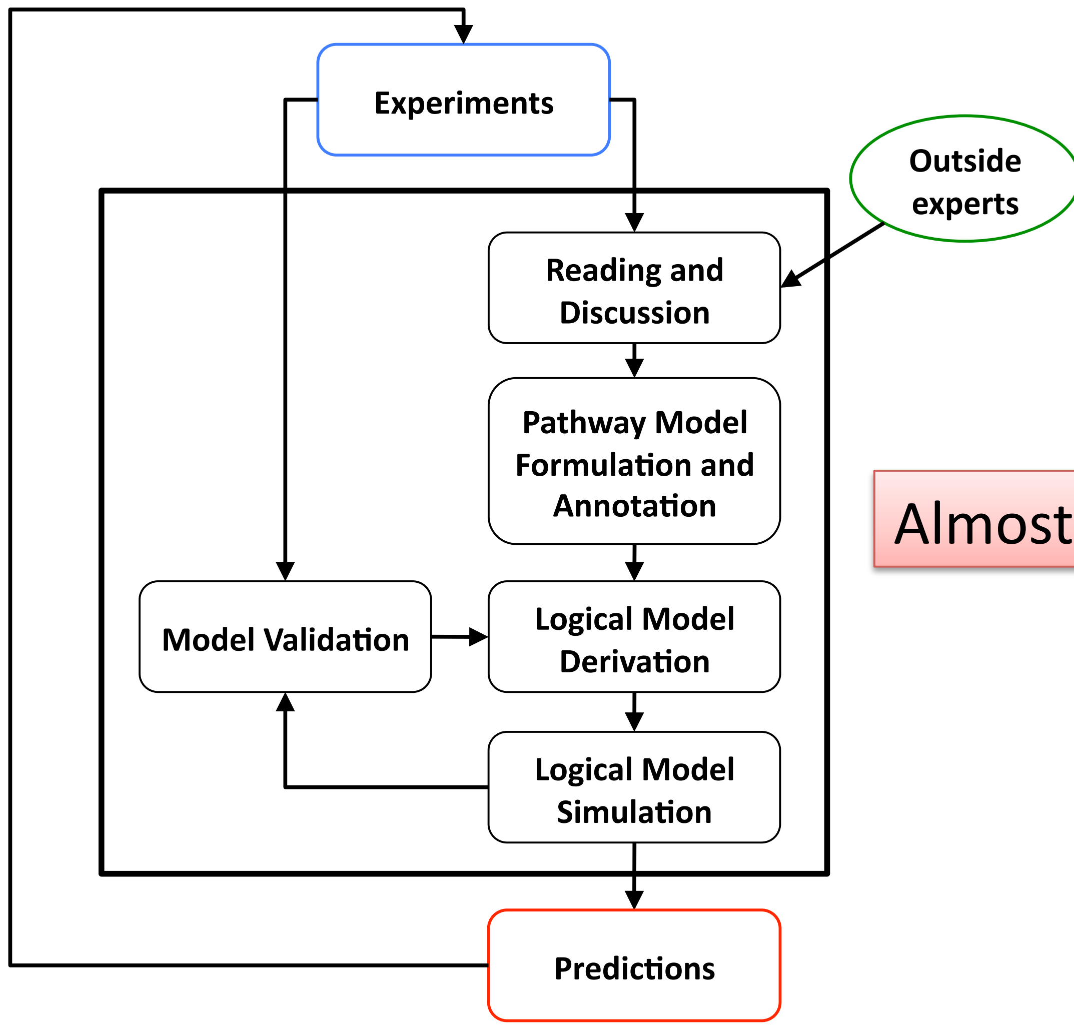


Model Construction Process

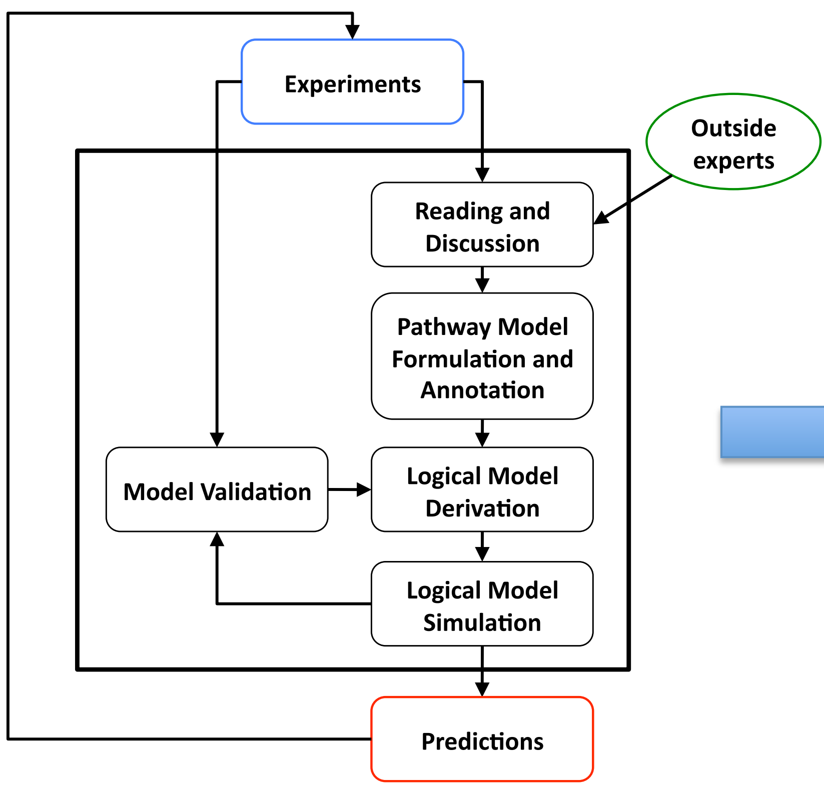


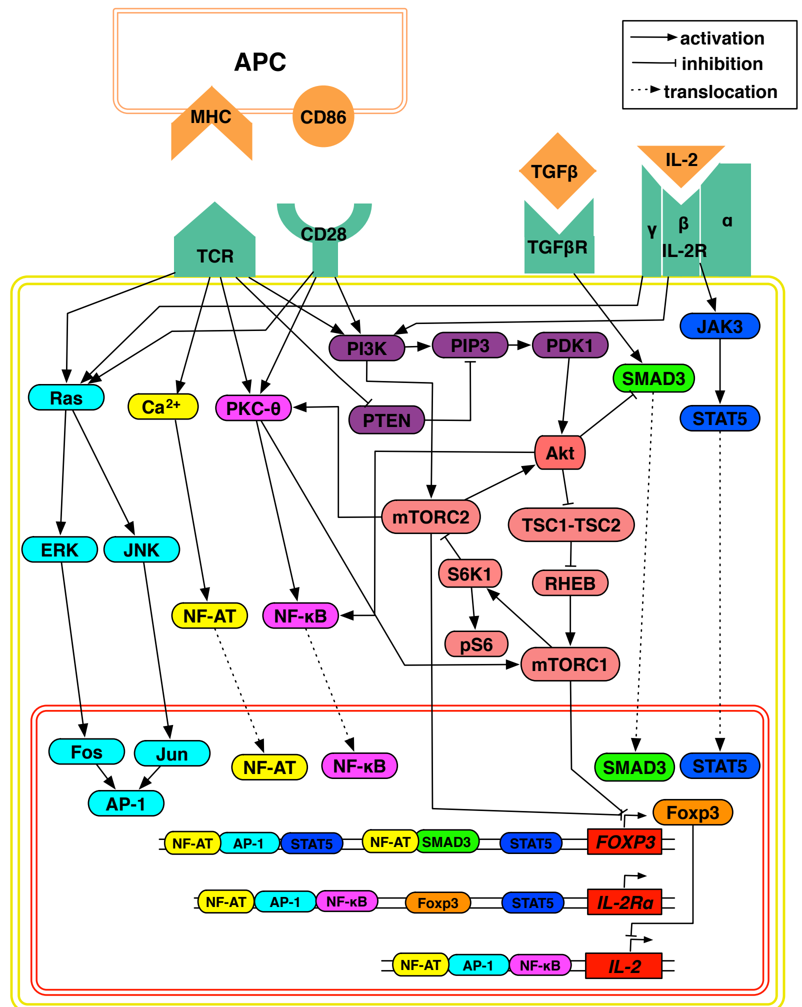
Model Construction Process



Almost 1 year!

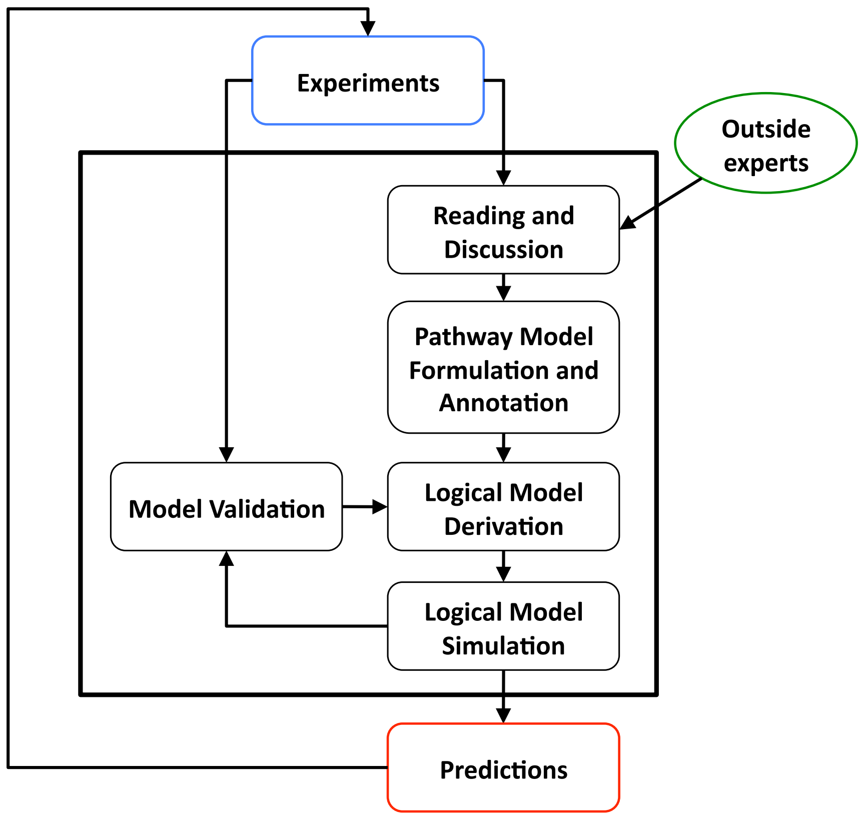
The Model





~25 variables / 50 edges

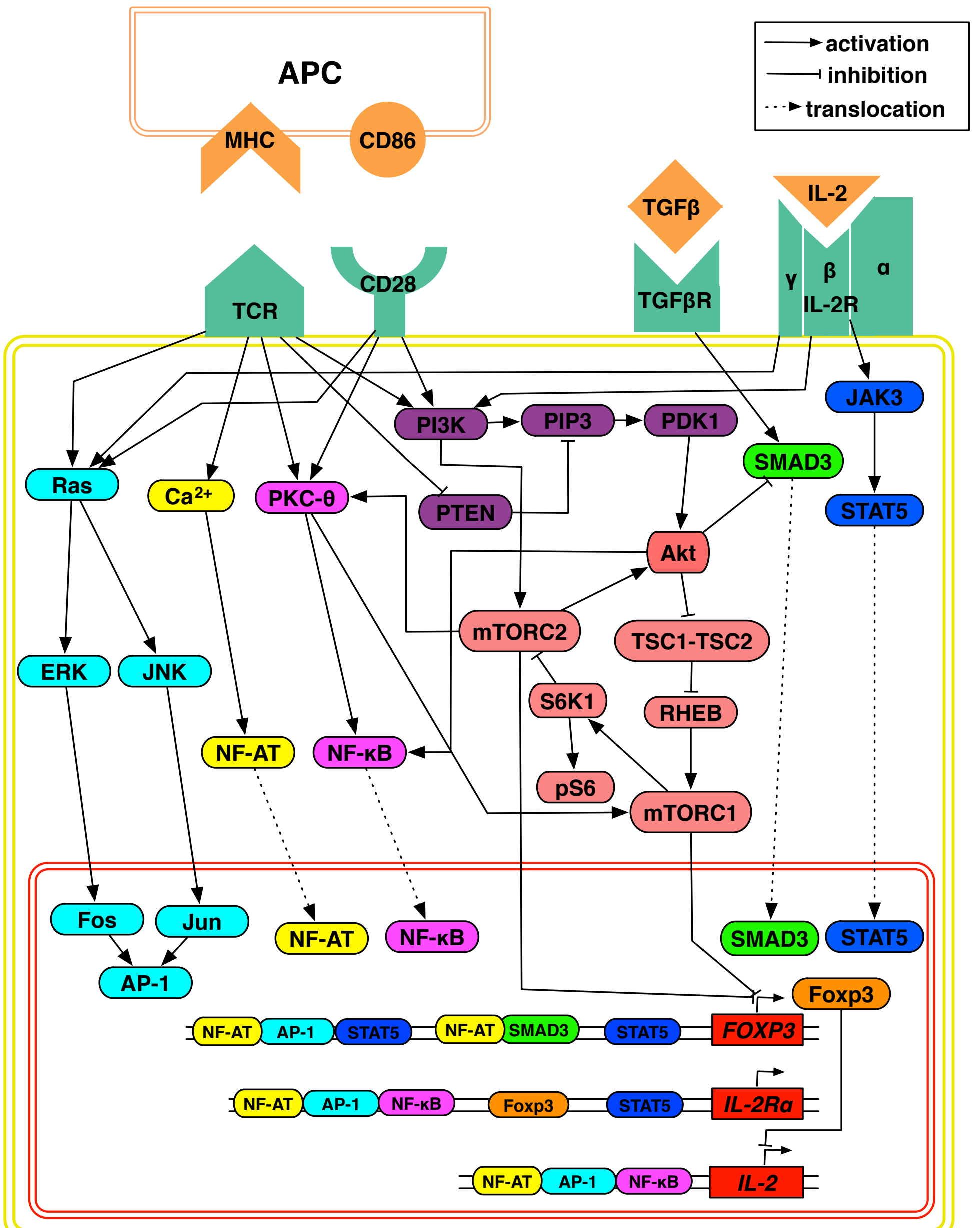
The Model



Model rules

TCR_HIGH* = TCR_HIGH
TCR* = TCR_LOW or TCR_HIGH
RAS* = (TCR and CD28) or (RAS and IL2_EX and IL2R)
ERK* = RAS
FOS* = ERK
JNK* = RAS
JUN* = JNK
AP1* = FOS and JUN
CA* = TCR
PKCTHETA* = TCR_HIGH or (TCR_LOW and CD28 and MTORC2)
NFKAPPAB* = PKCTHETA or AKT
NFAT* = CA
IL2* = (AP1 and NFAT and NFKAPPAB) and not FOXP3
IL2R* = CD25 and CD122 and CD132
PI3K* = (TCR and CD28) or (PI3K and IL2_EX and IL2R)
PIP3* = PI3K and not PTEN
PDK1* = PIP3
AKT* = PDK1 and MTORC2
TSC* = not AKT
RHEB* = not TSC
MTORC1* = RHEB and PKCTHETA
MTORC2* = PI3K and not S6K1
MTOR* = MTORC1 and MTORC2
S6K1* = MTORC1
PS6* = S6K1
SMAD3* = TGFBETA and not (AKT and MTORC1)
STAT5* = IL2R and IL2_EX
FOXP3* = (not MTOR and STAT5) or (NFAT and SMAD3)
CD25* = FOXP3 or (AP1 and NFAT and NFKAPPAB) or STAT5
PTEN* = not TCR_HIGH
IL2_EX* = IL2 or IL2_EX

~25 variables / 50 edges



Receptors:

T cell receptor (TCR)

Co-stimulation through CD28

IL-2 receptor (IL-2R)

TGFβ receptor (TGFβR)

Transcription factors:

AP-1, NFAT, NFκB, SMAD3, STAT5

Genes:

IL-2, CD25, Foxp3

Other important elements:

PTEN, PI3K, PIP3, PDK1,

Akt, mTORC1, mTORC2,

TSC1-TSC2, Rheb, S6K1, pS6

Influence sets

Element	Influence set
PI3K	TCR, CD28, IL-2, IL-2R
Akt	PDK1, mTORC2
mTORC1	Rheb, PKC-θ
mTORC2	PI3K, S6K1
Foxp3	NFAT, AP-1, STAT5, Smad3
IL-2	NFAT, AP-1, NFκB, Foxp3
CD25	NFAT, AP-1, NFκB, STAT5, Foxp3
STAT5	IL-2, IL-2R
NFκB	PKC-θ, Akt
Smad3	TGFβ, Akt, mTORC1
PIP3	PI3K, PTEN
Ras	TCR, CD28, IL-2, IL-2R

Element	Influence set
AP-1	Fos, Jun
ERK	Ras
JNK	Ras
Fos	ERK
Jun	JNK
NFAT	Ca
Ca	TCR
PDK1	PIP3
TSC1-TSC2	Akt
Rheb	TSC1-TSC2
S6K1	mTORC1
pS6	S6K1

Influence sets

Element	Influence set
PI3K	TCR, CD28, IL-2, IL-2R
Akt	PDK1, mTORC2
mTORC1	Rheb, PKC-θ
mTORC2	PI3K, S6K1
Foxp3	NFAT, AP-1, STAT5, Smad3
IL-2	NFAT, AP-1, NFκB, Foxp3
CD25	NFAT, AP-1, NFκB, STAT5, Foxp3
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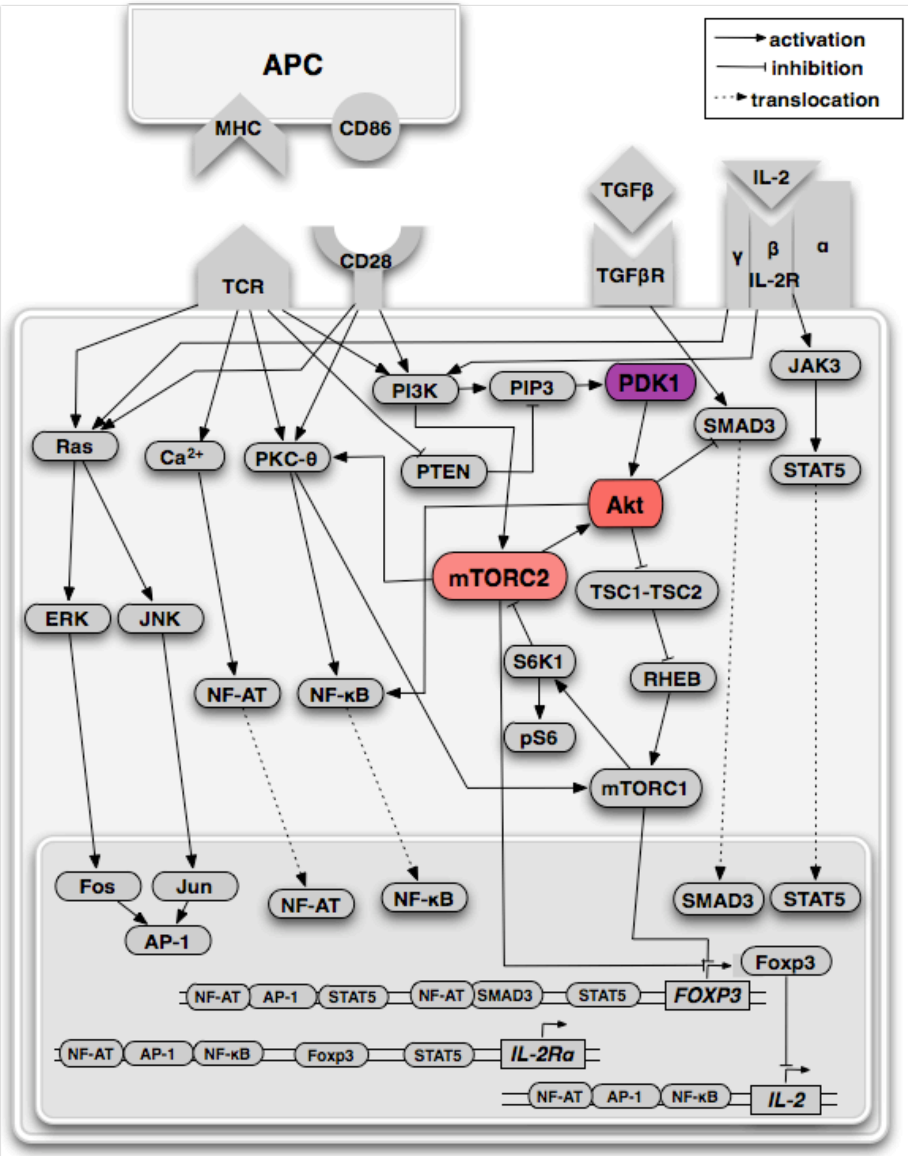
Smad3	TGFβ, Akt, mTORC1
PIP3	PI3K, PTEN
Ras	TCR, CD28, IL-2, IL-2R

Element	Influence set
AP-1	Fos, Jun
ERK	Ras
JNK	Ras
Fos	ERK
Jun	JNK
NFAT	Ca
Ca	TCR
PDK1	PIP3
TSC1-TSC2	Akt
Rheb	TSC1-TSC2
S6K1	mTORC1
pS6	S6K1

Logical modeling approach



Akt’ = PDK1 and mTORC2

Influence sets

Element	Influence set
PI3K	TCR, CD28, IL-2, IL-2R
Akt	PDK1, mTORC2
mTORC1	Rheb, PKC-θ
mTORC2	PI3K, S6K1
Foxp3	NFAT, AP-1, STAT5, Smad3
IL-2	NFAT, AP-1, NFκB, Foxp3
CD25	NFAT, AP-1, NFκB, STAT5, Foxp3
STAT5	IL-2, IL-2R
NFκB	PKC-θ, Akt
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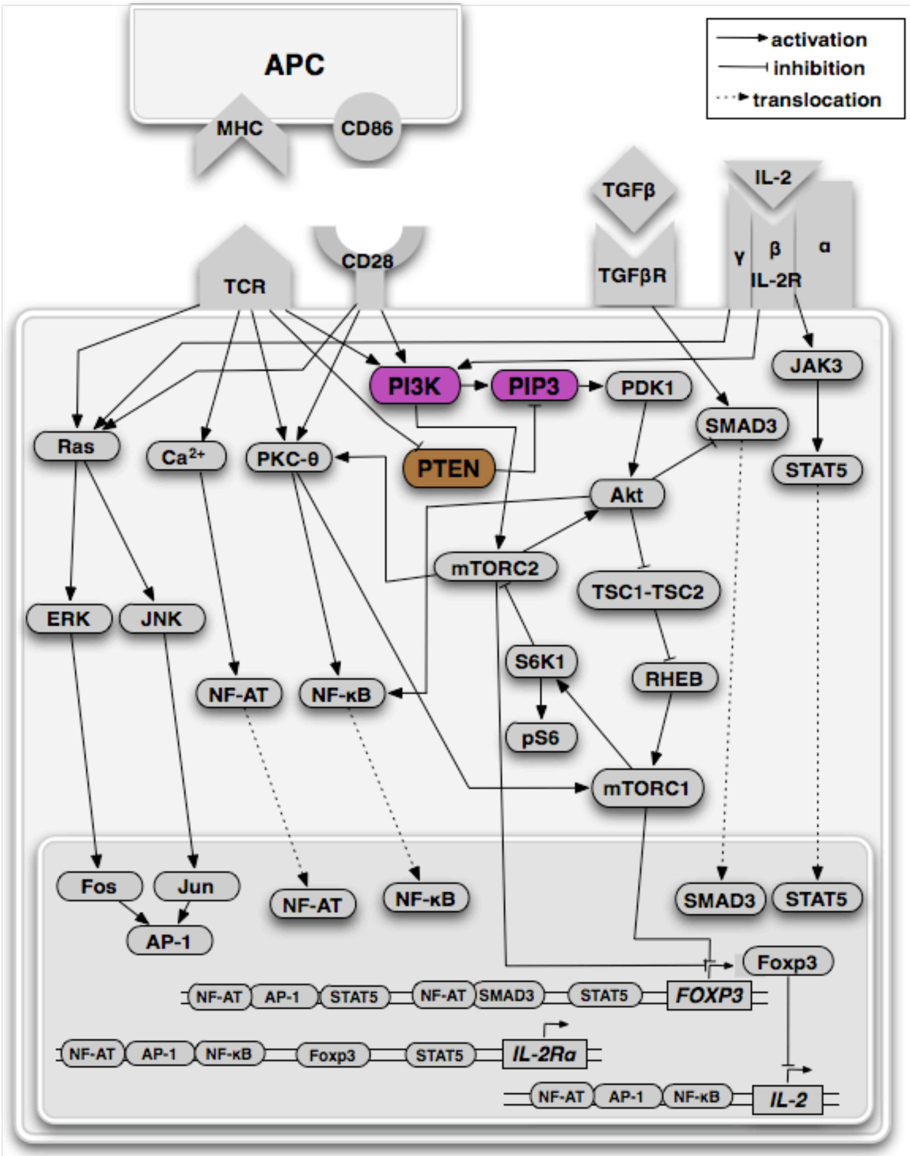
Element	Influence set
AP-1	Fos, Jun
ERK	Ras
JNK	Ras
Fos	ERK
Jun	JNK
NFAT	Ca
Ca	TCR
PDK1	PIP3
TSC1-TSC2	Akt
Rheb	TSC1-TSC2
S6K1	mTORC1
pS6	S6K1

Logical modeling approach

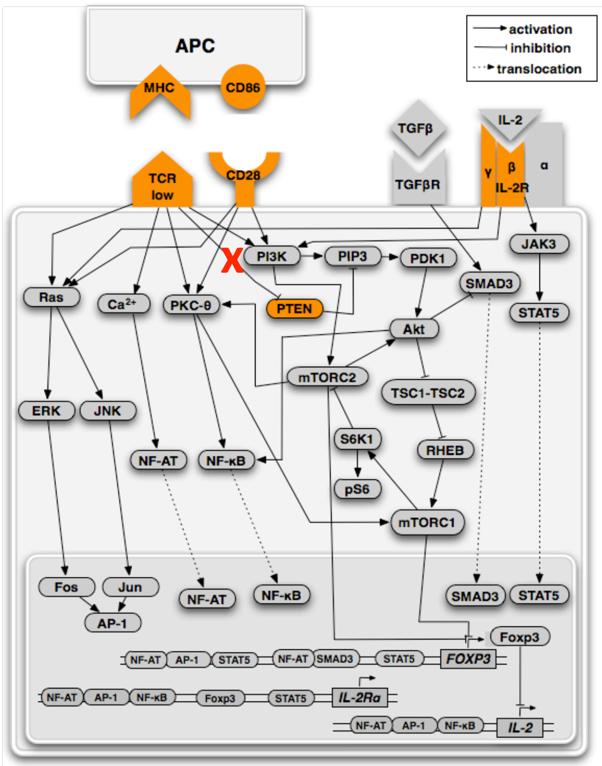


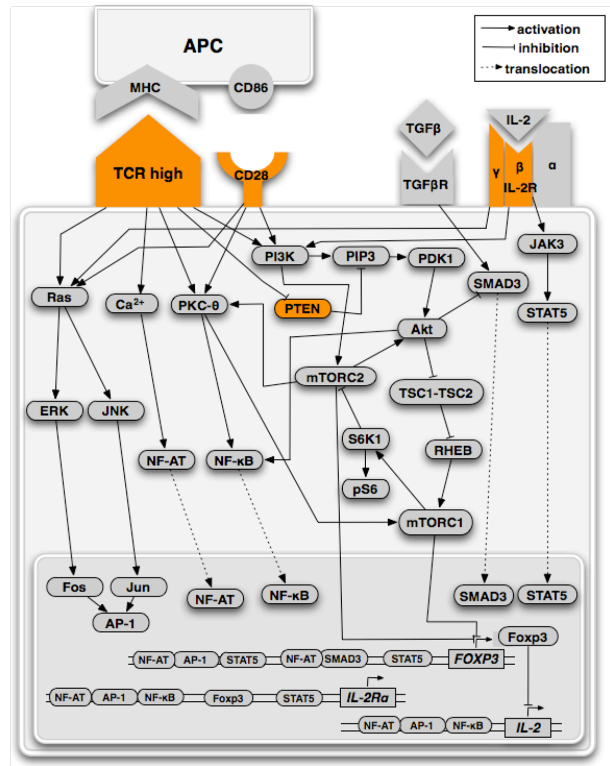
PIP3’ = PI3K and not PTEN

Logical modeling decisions

- Number of levels for element values
  - TCR variable represents level of antigen stim.
    - No antigen (TCR_LOW = 0, TCR_HIGH = 0)
    - Low antigen dose (TCR_LOW = 1, TCR_HIGH = 0)
    - High antigen dose (TCR_LOW = 0, TCR_HIGH = 1)

TCR_LOW vs. TCR_HIGH



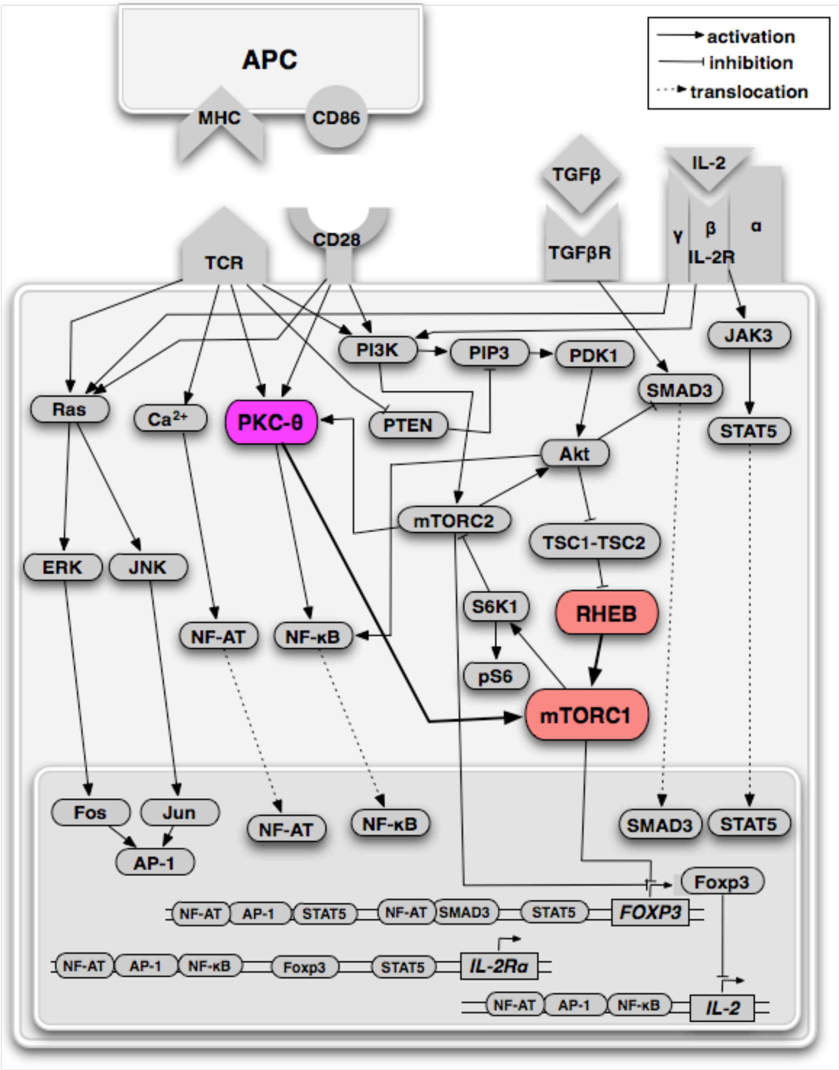


TCR_LOW not strong enough to overcome inhibition by PTEN.

Logical modeling decisions

- Choice between OR and AND:
  - Example:

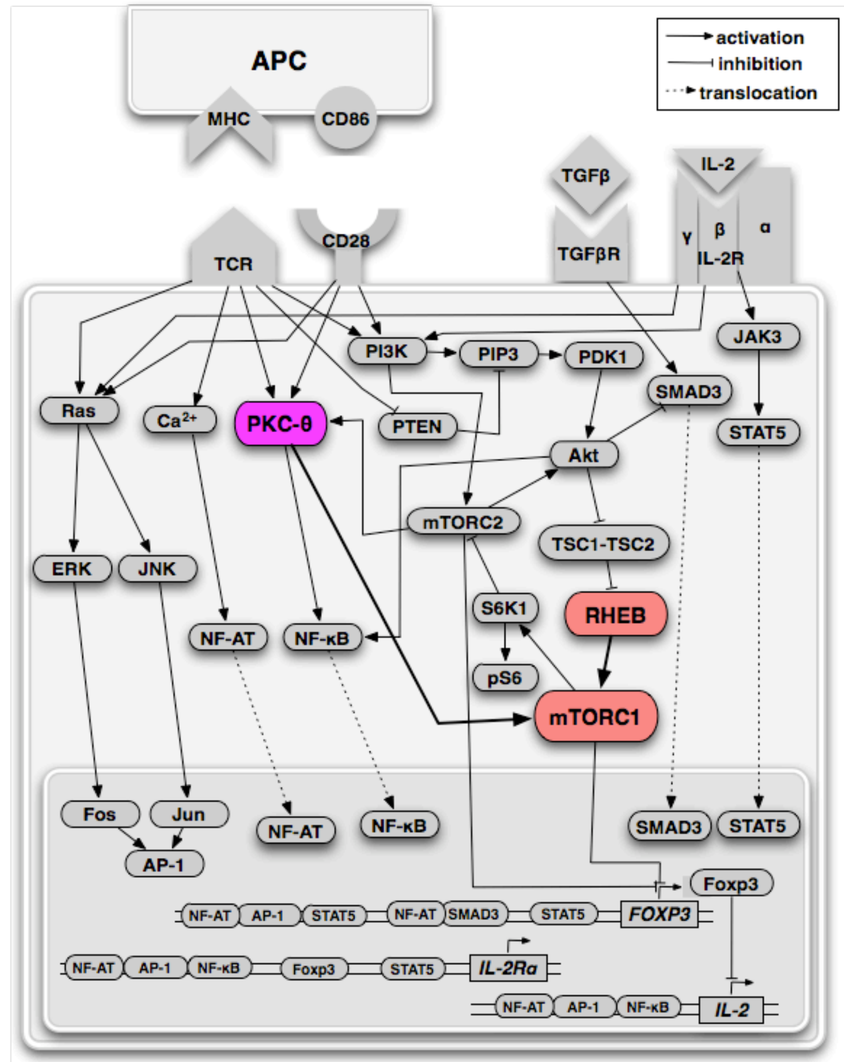
mTORC1’ = Rheb and (or?) PKC-θ



Logical modeling decisions

- Choice between AND and OR:

PKC-θ / Rheb	0	1
0		
1		



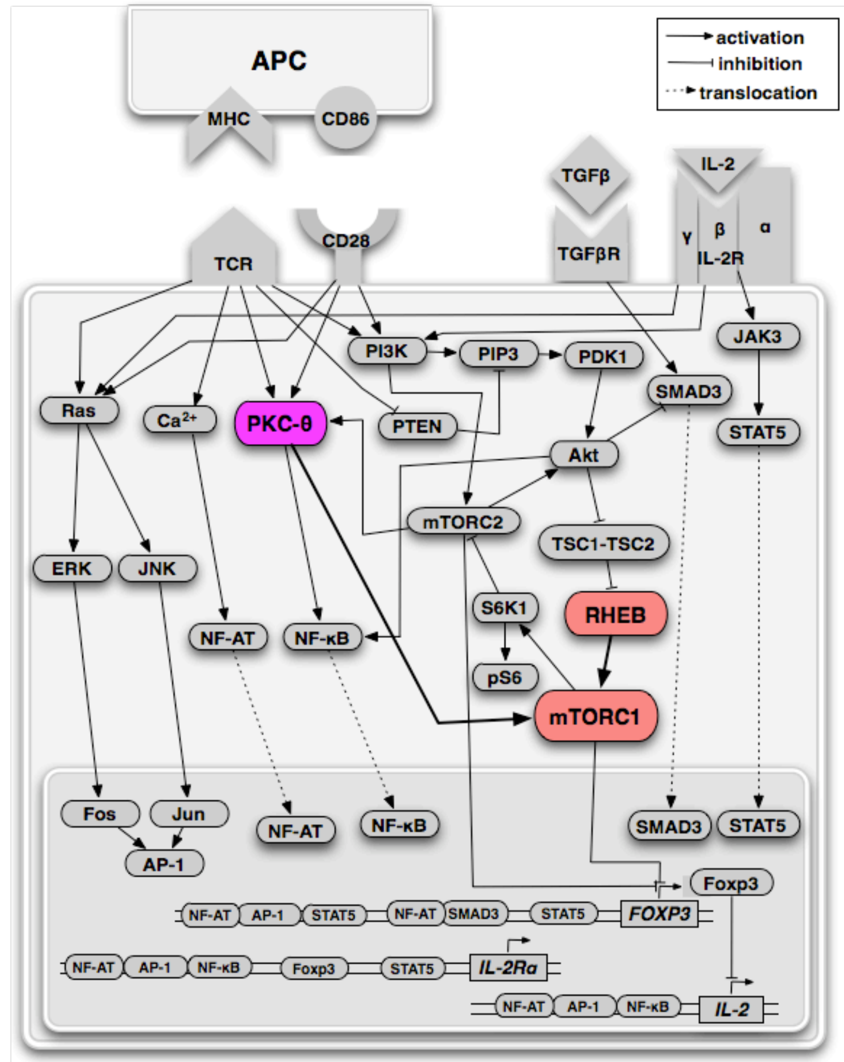
Logical modeling decisions

- Choice between AND and OR:

Rheb \ PKC-θ	0	1
0	0	0
1	0	1

mTORC1' = Rheb and PKC-θ

'and' rule means both are necessary for activation

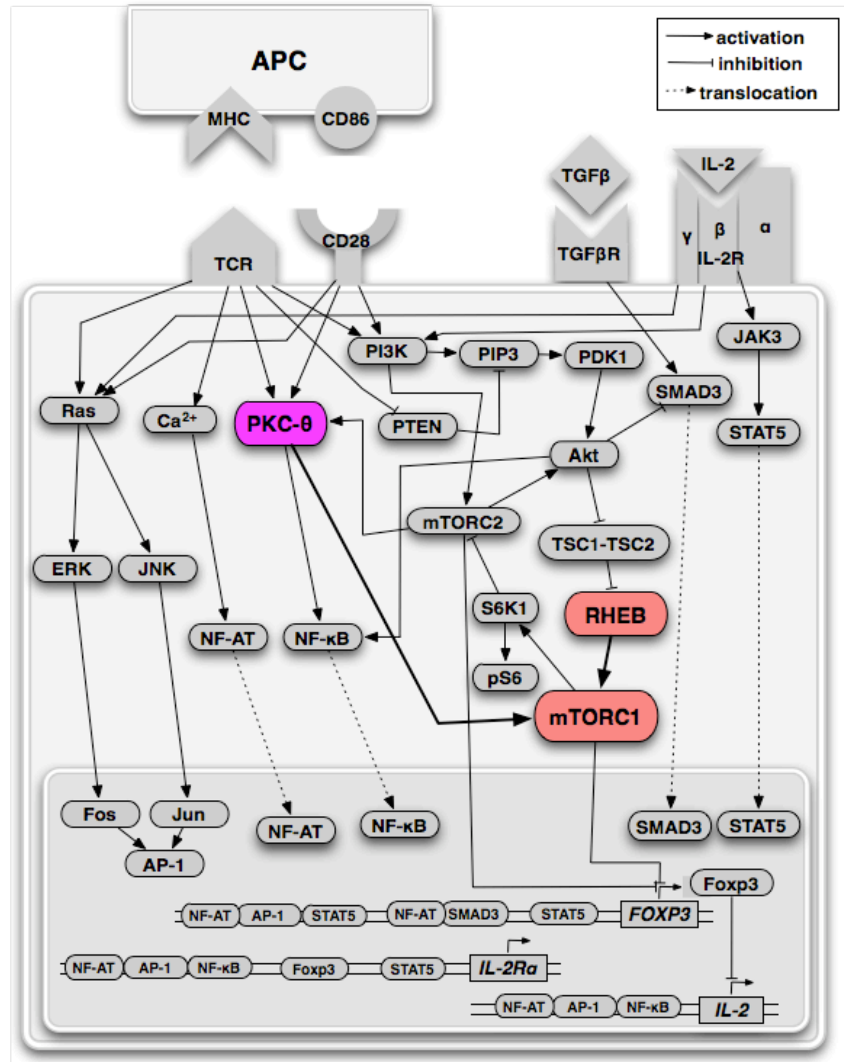


Logical modeling decisions

- Choice between AND and OR:

Rheb \ PKC-θ	0	1
0	0	0
1	1	1

mTORC1' = Rheb



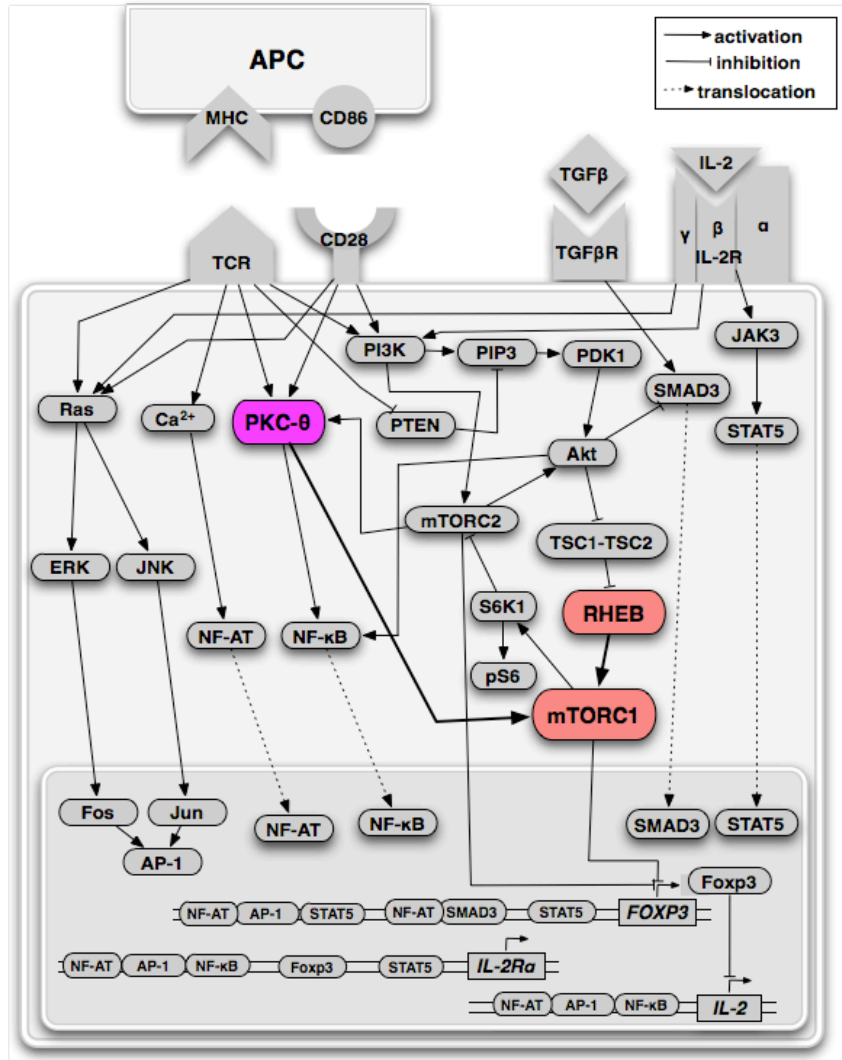
Logical modeling decisions

- Choice between AND and OR:

Rheb \ PKC-θ	0	1
0	0	1
1	1	1

mTORC1' = Rheb or PKC-θ

'or' rule means either one is sufficient for activation



Simulation setup

- Simulation:
  - For given initial conditions, computes system trajectory
  - Usually 20-40 steps to reach steady state

FOXP3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NFKAPPAB	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AP1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TSC	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AKT	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
IP3	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TCR_HIGH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PS6	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
IL2_EX	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
STAT5	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CD132	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S6K1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TCR	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TIME1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TIME2	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TCR_LOW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PIP	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CD28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PI3K	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TGFBETA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CD25	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MTOR	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SMAD3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NFAT	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CD122	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MTORC2	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MTORC1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PDK1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PTEN	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IL2R	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
IL2	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TIME	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RHEB	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

- Scenarios (initial conditions and rules)
  - Simulated 300 times
  - Results show the percentage of being equal '1' across all runs

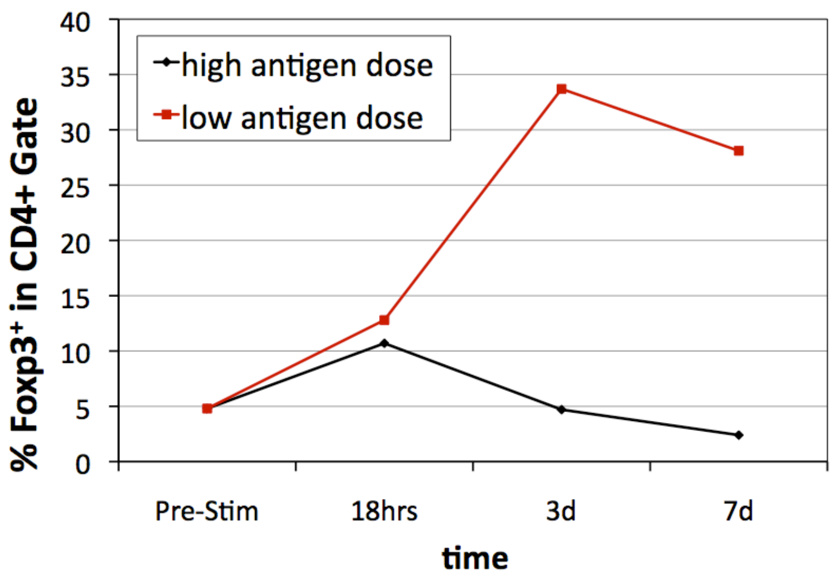
Model Validation

- Three main scenarios:
  1. High vs. Low antigen dose
  2. High antigen dose, then removed
  3. High antigen dose, then Akt or mTOR inhibitors added

Results are still preliminary.

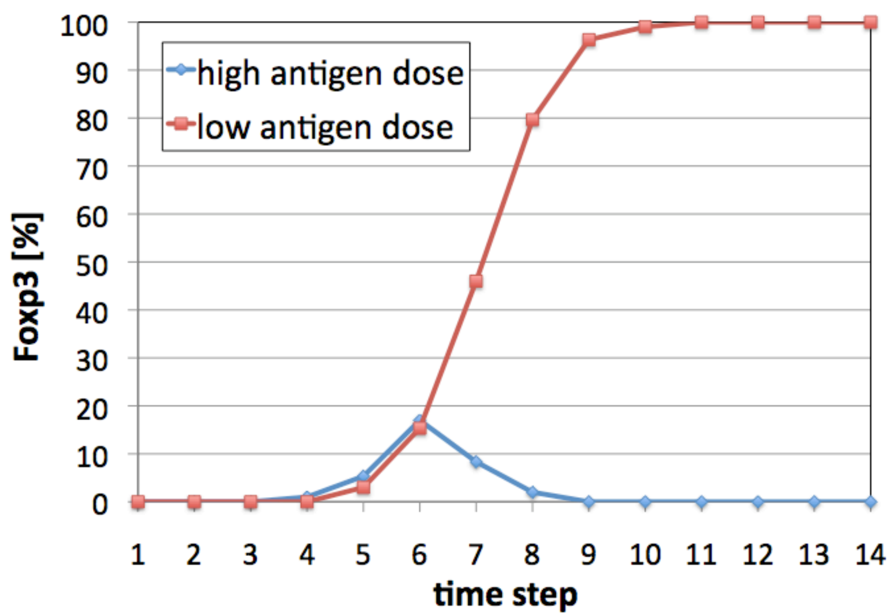
Antigen Dose Dependence

Experimental data



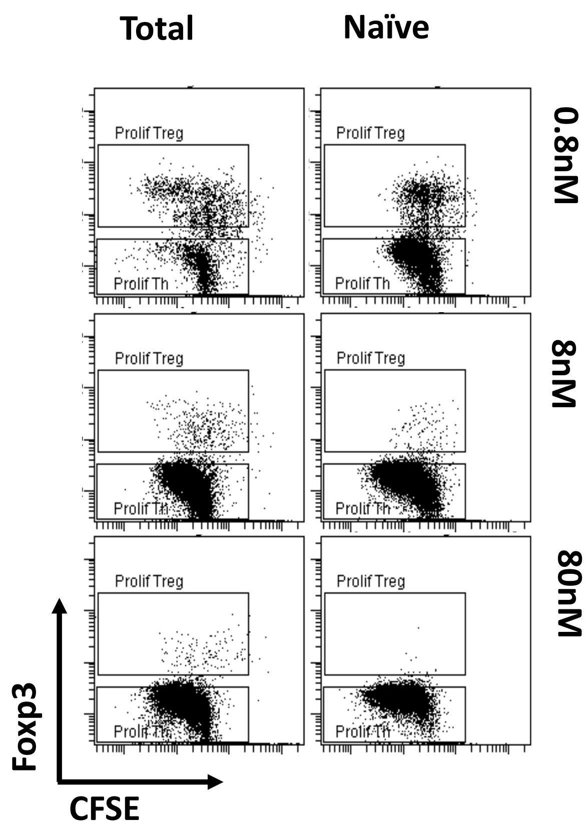
Source: Turner *et al.*, The Journal of Immunology, 2009, 183, 4895-4903.

Logical model results



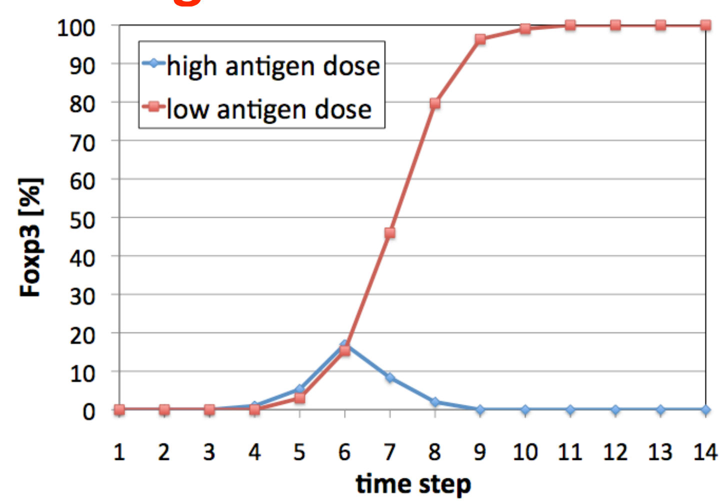
Antigen Dose Dependence

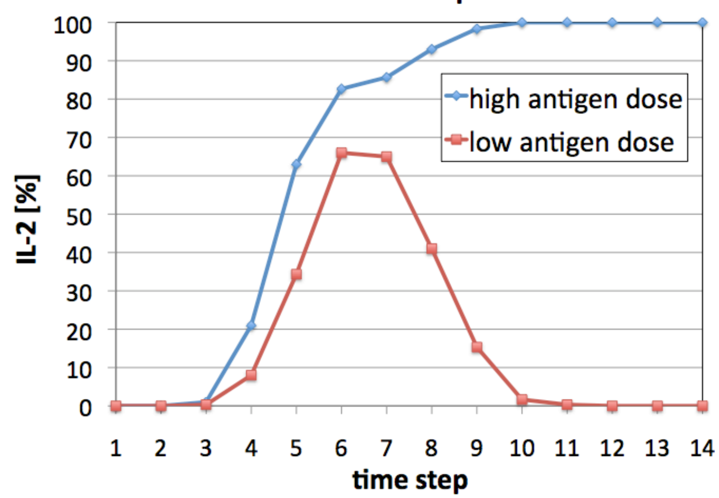
Experimental data



Source: Turner *et al.*, The Journal of Immunology, 2009, 183, 4895-4903.

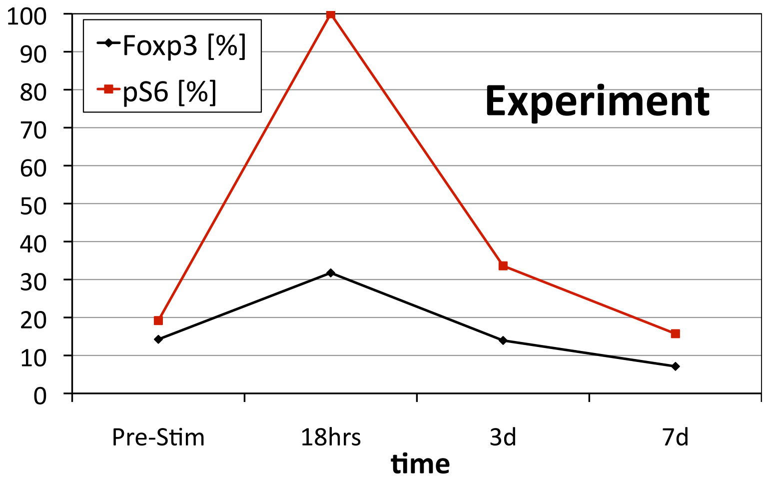
Logical model results

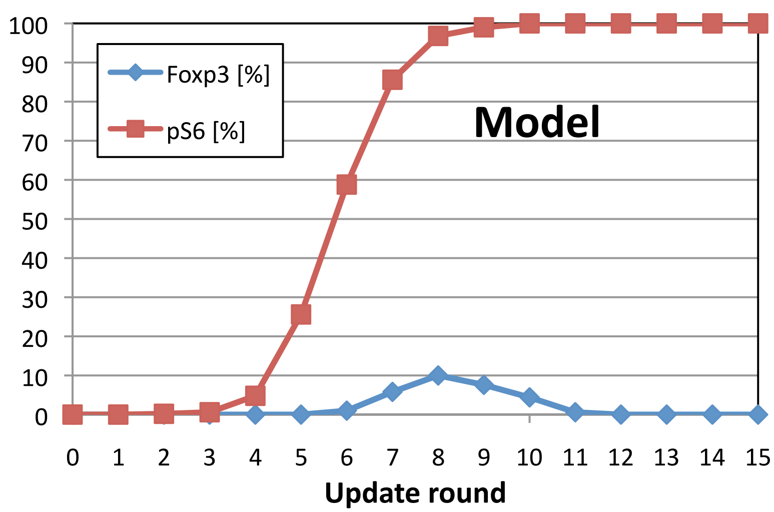




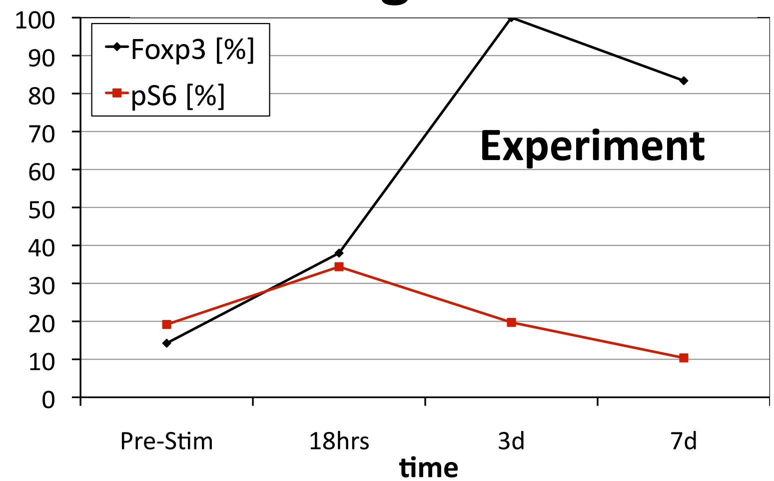
Foxp3 vs. pS6

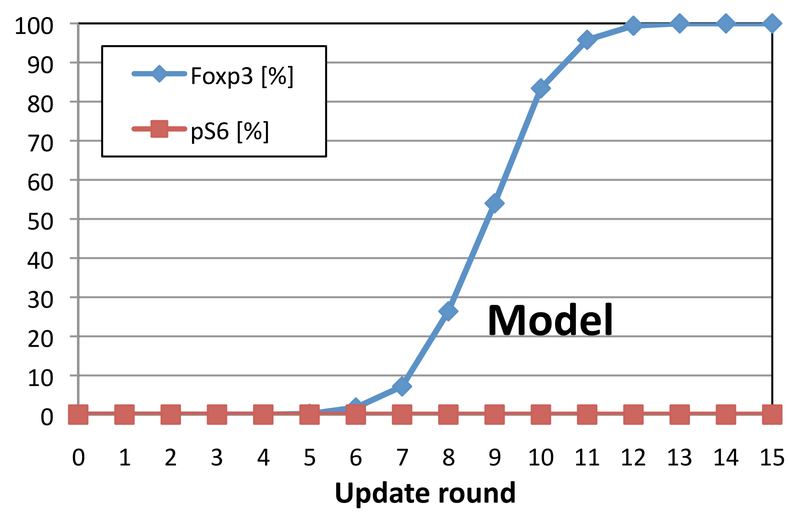
High Antigen Dose





Low Antigen Dose



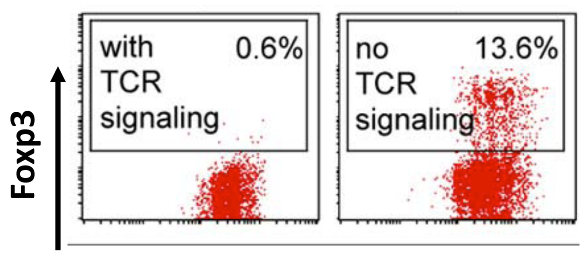


Antigen Removal

Experimental data

Source: Sauer *et al*., PNAS 105:7797, 2008.

Remove TCR after 18 hrs



Antigen Removal

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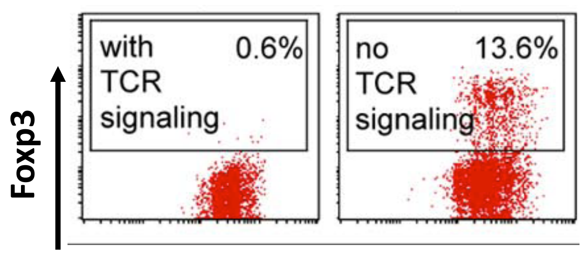
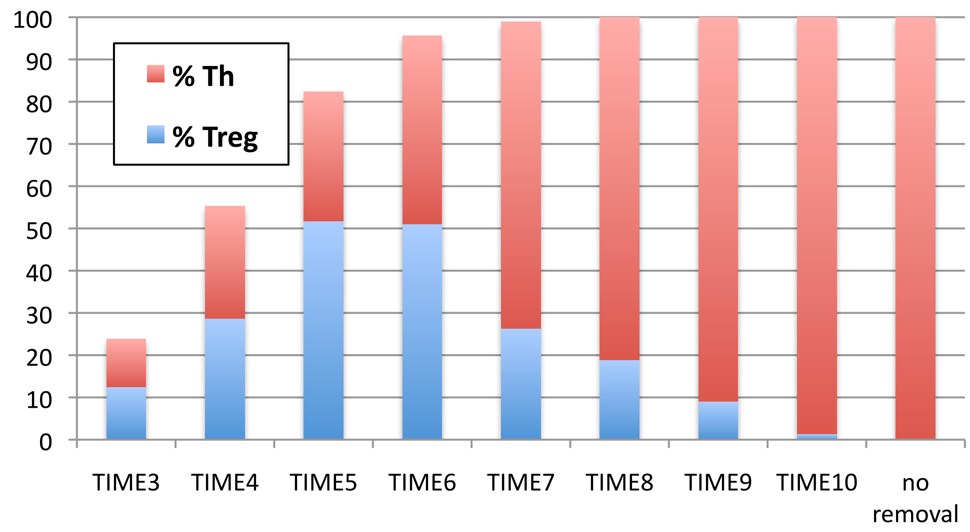
Remove TCR after 18 hrs



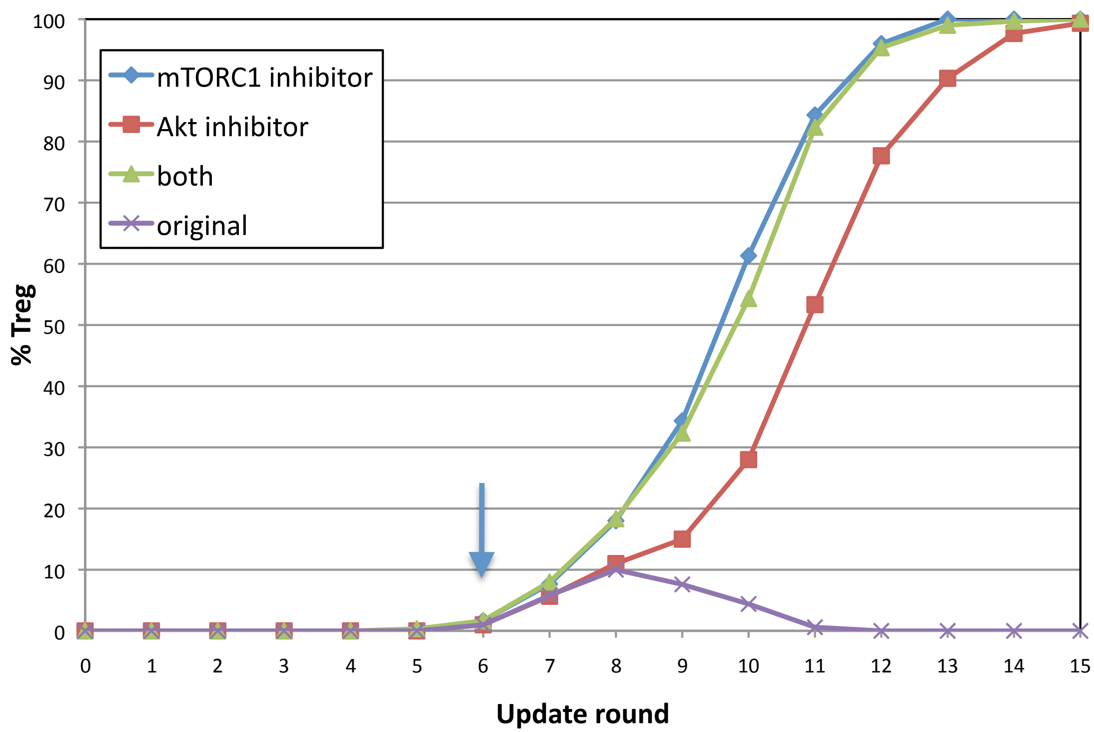
Logical model results



Akt and mTOR inhibitors

Experimental data

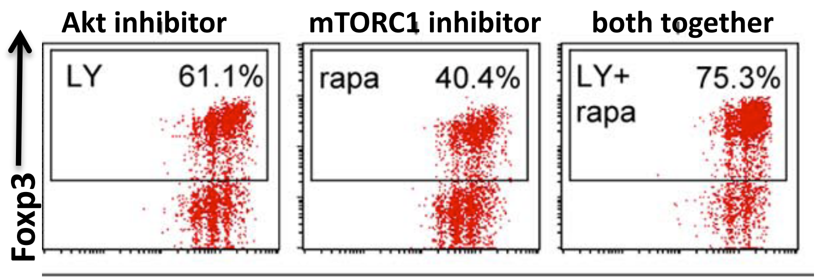
Source: Sauer *et al.*, PNAS 105:7797, 2008.

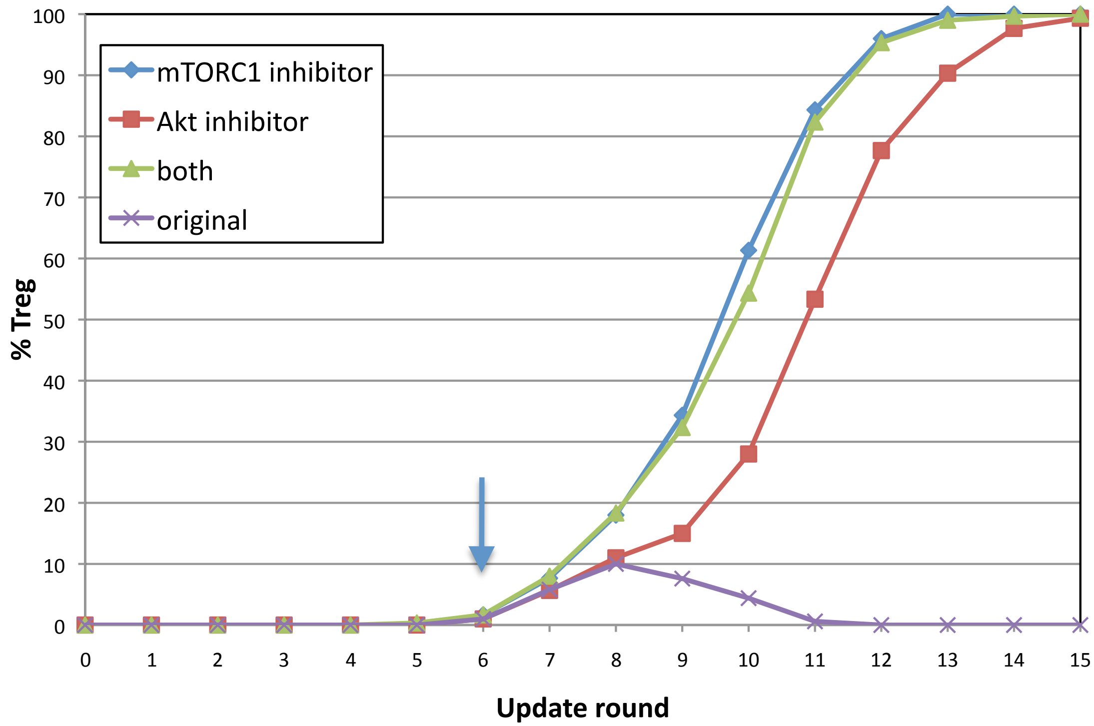


Akt and mTOR inhibitors

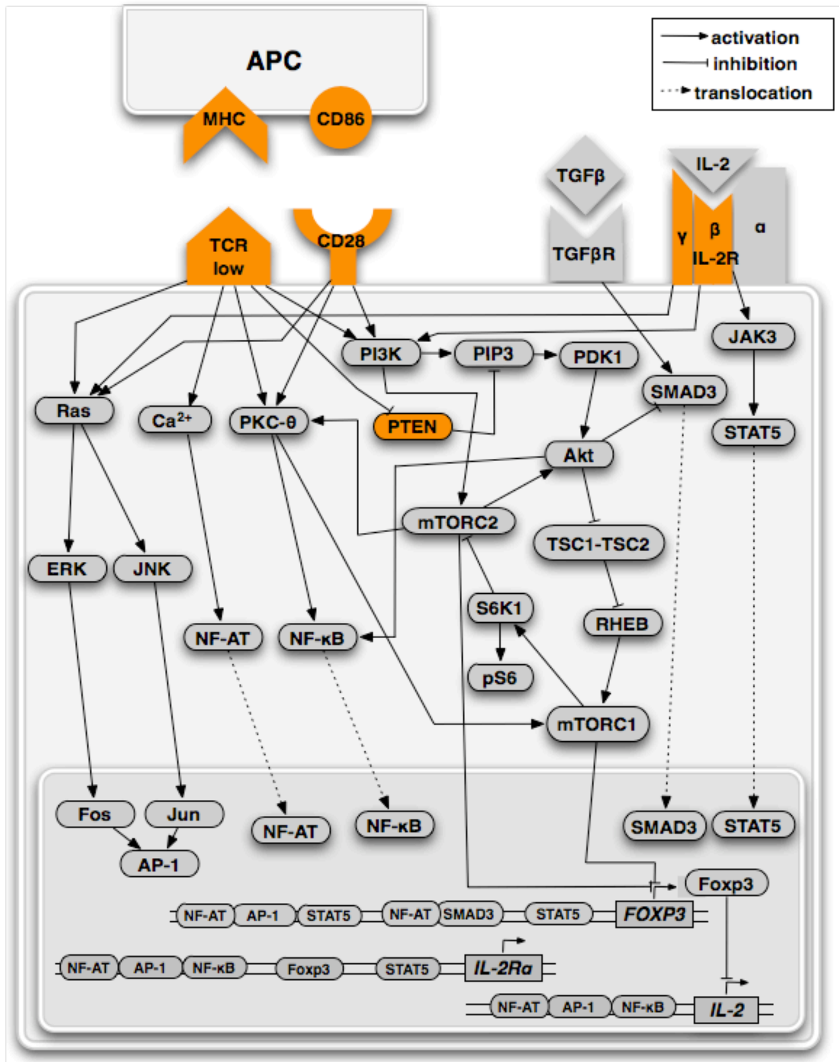
Experimental data

Source: Sauer *et al.*, PNAS 105:7797, 2008.

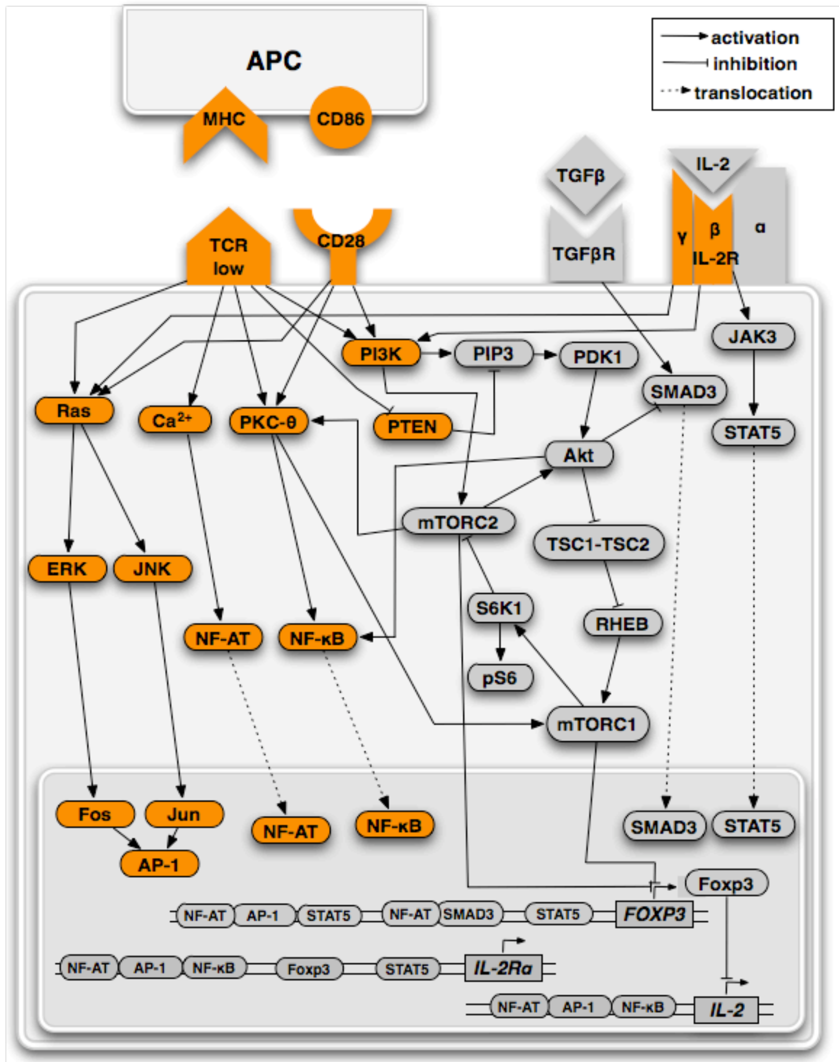




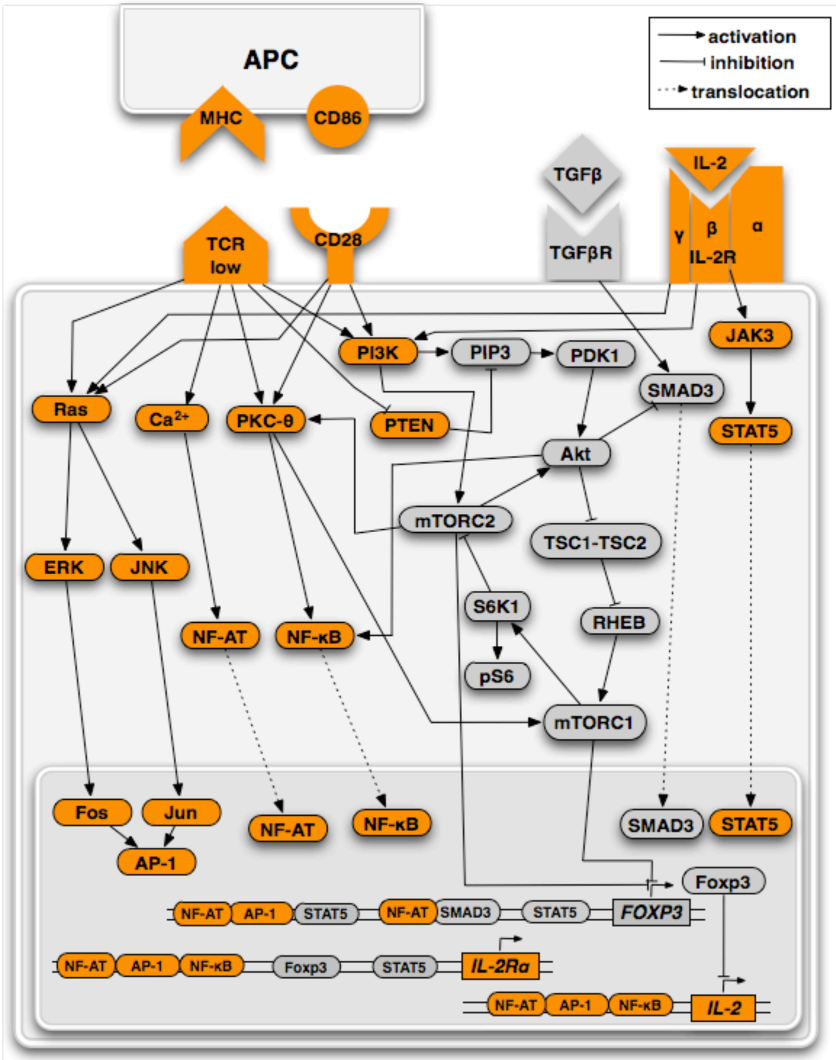
Low Antigen Trajectory



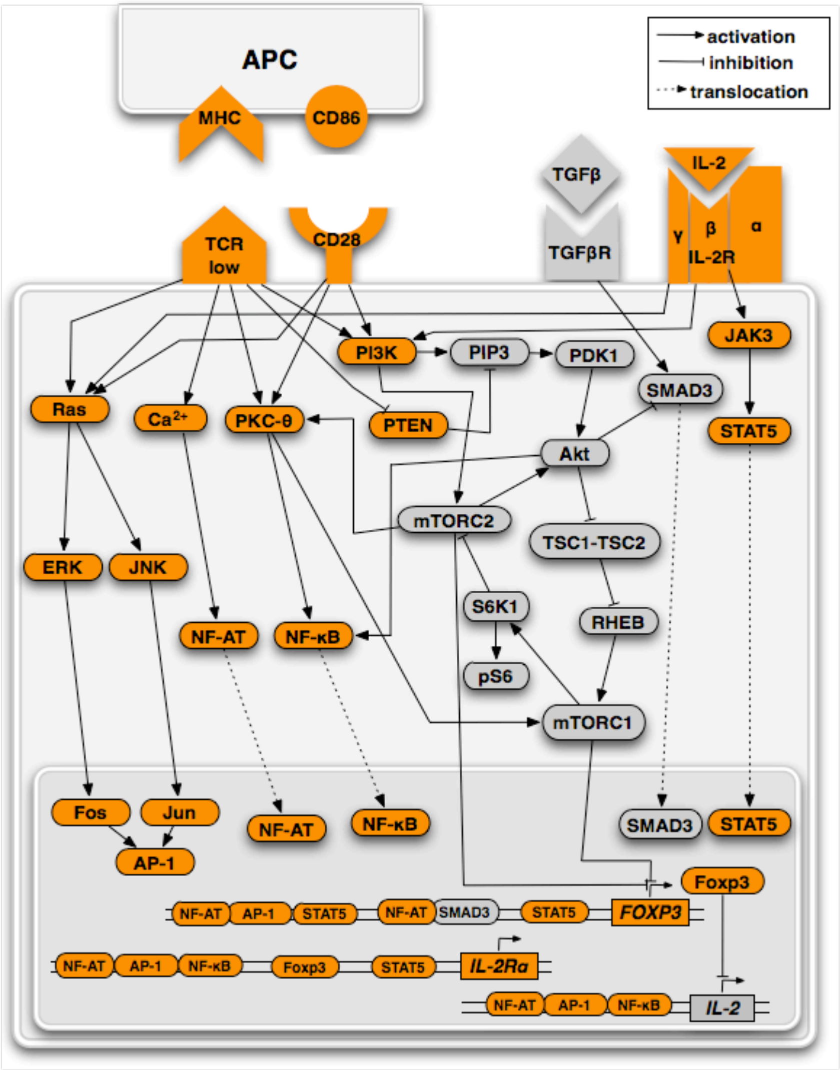
Low Antigen Trajectory



Low Antigen Trajectory

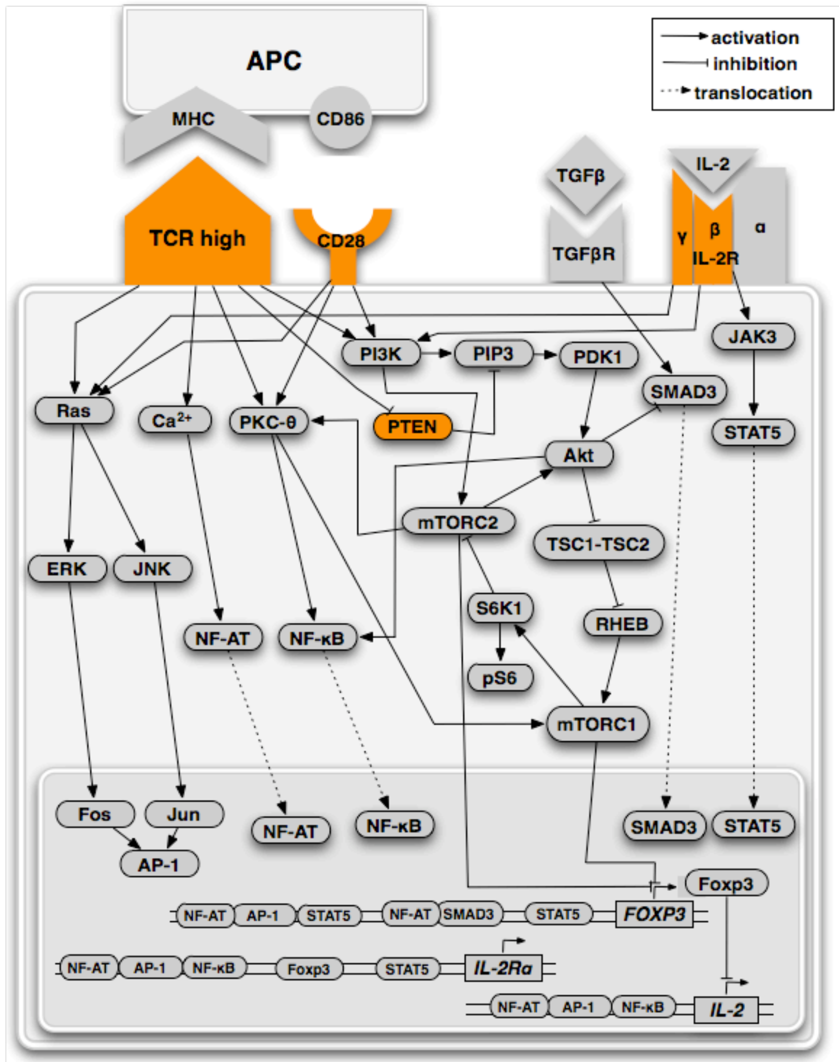


Low Antigen Trajectory



Low dose steady state

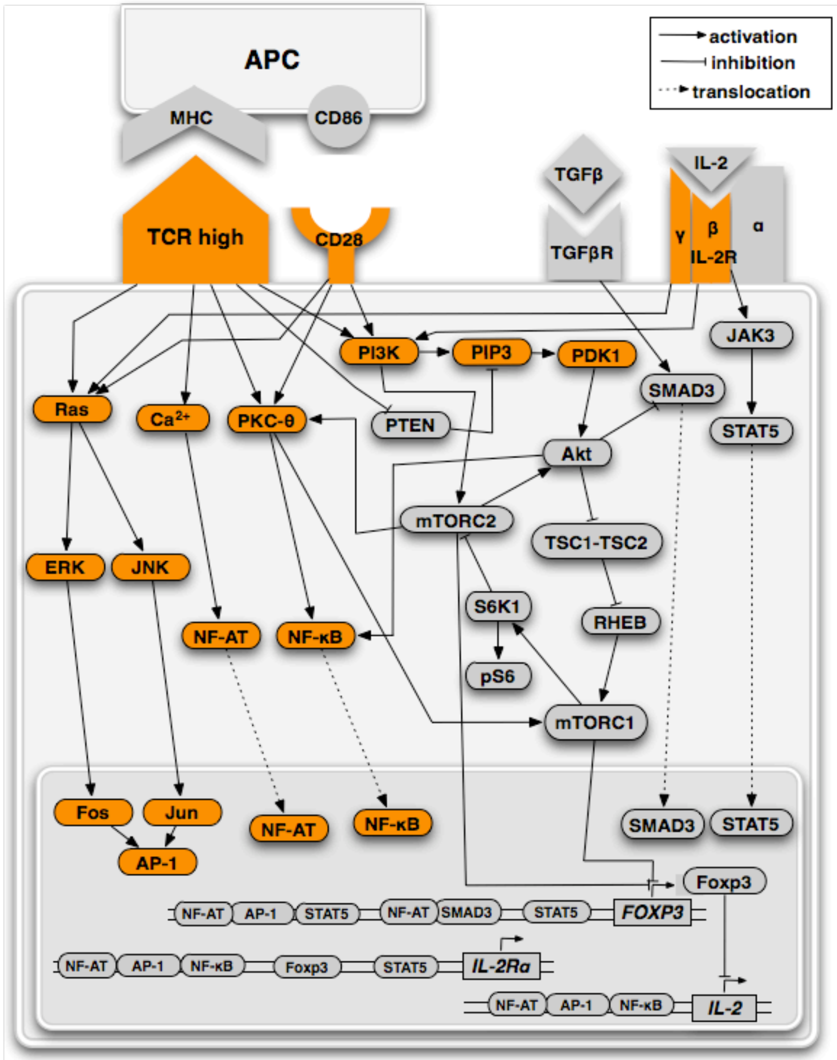
High Antigen Trajectory



High Antigen Trajectory

Suppression of PTEN allows signal to reach Akt/mTOR axis.

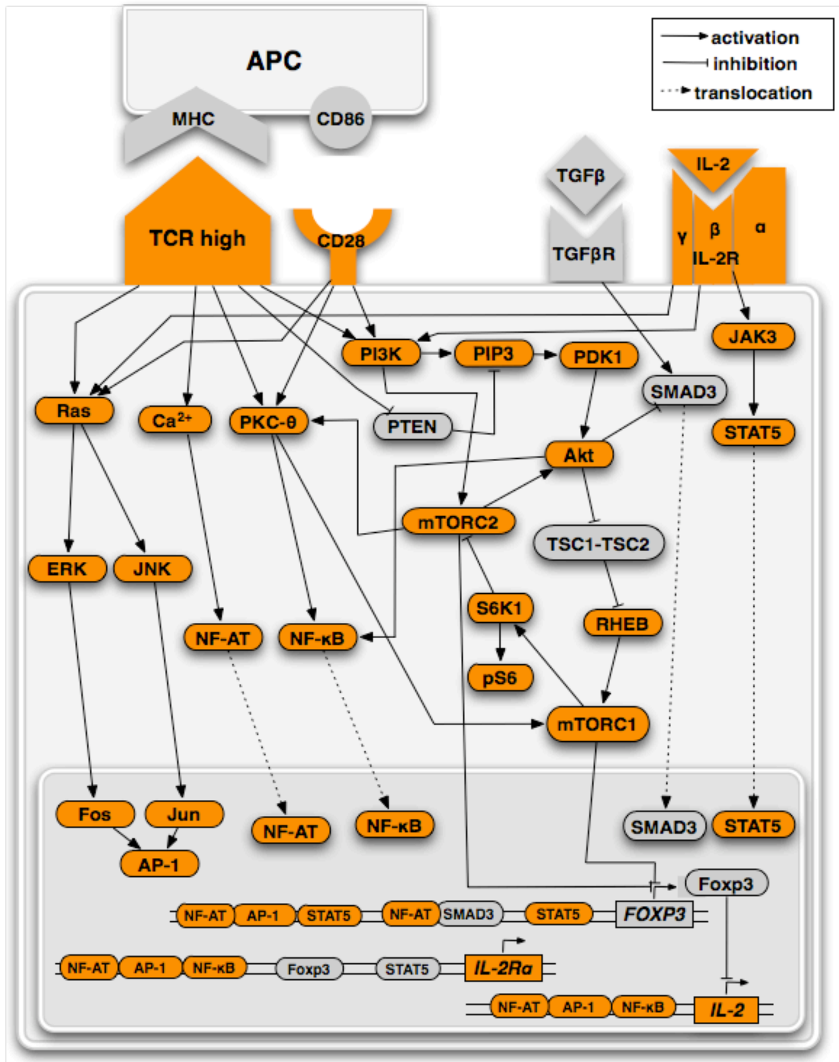
Could PIP3 level be a good early predictor of cell fate?



High Antigen Trajectory

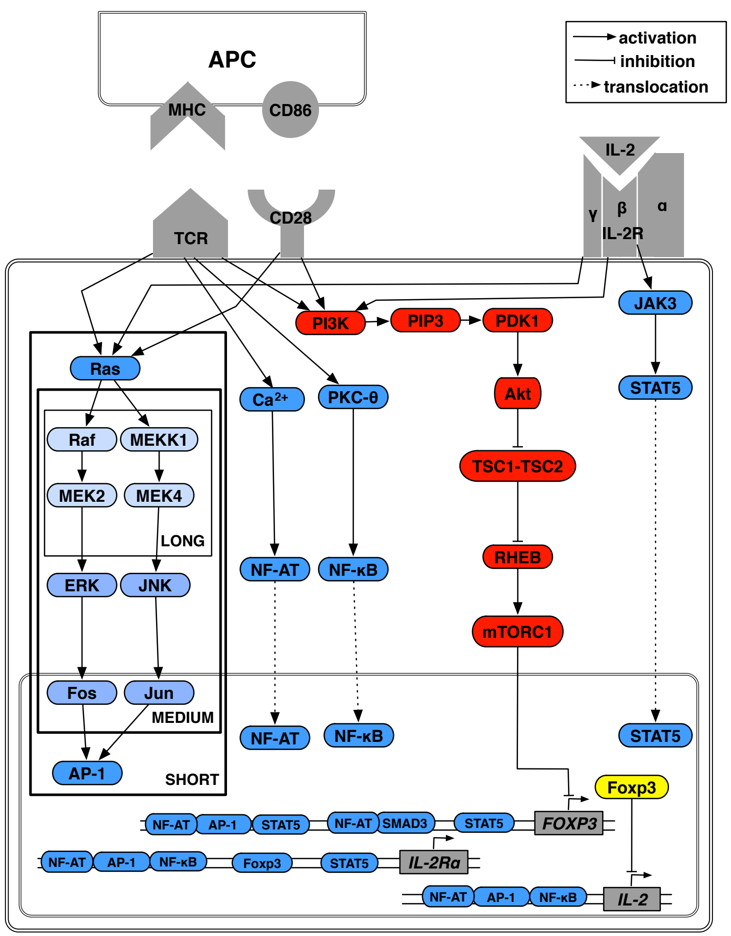
Notice that mTORC1 is activated at same time as STAT5.

If STAT5 activation happens first, Foxp3 expression can happen transiently before mTOR suppression occurs.

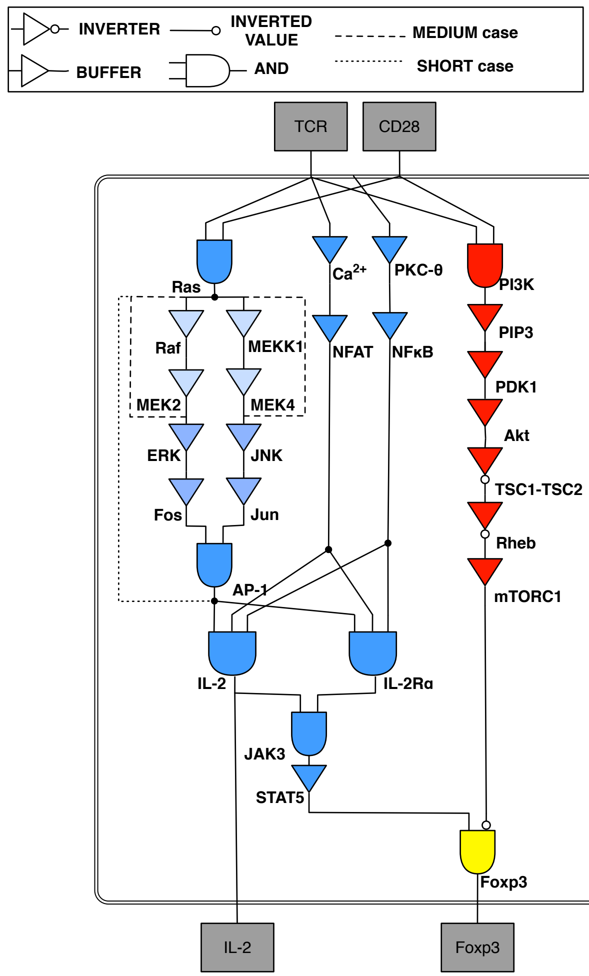


STAT5 vs. mTOR

Network Diagram

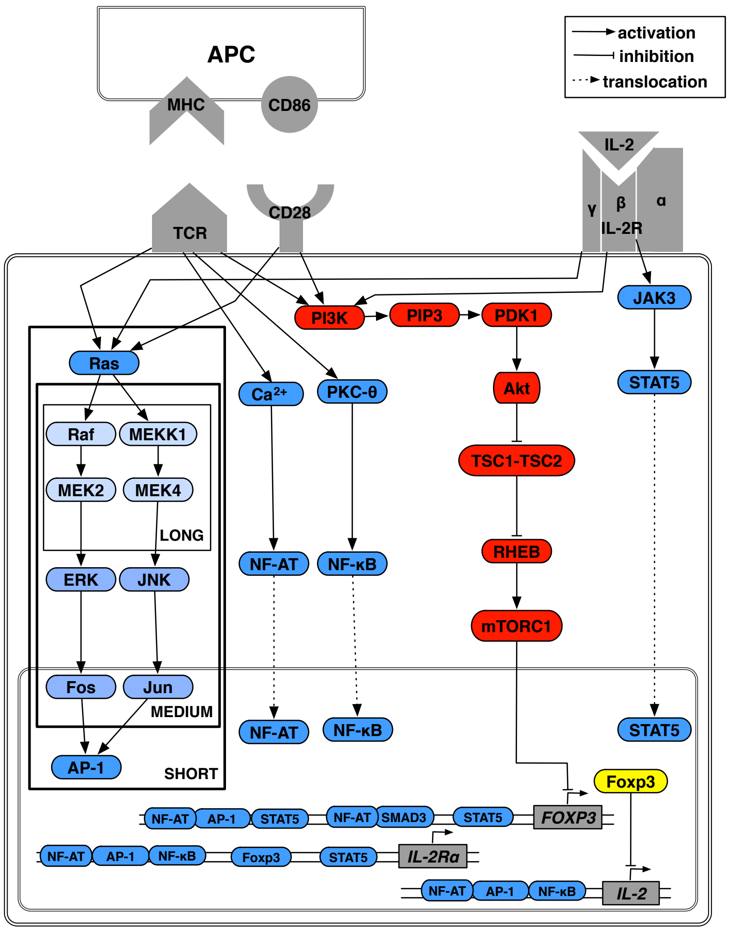


Circuit Diagram

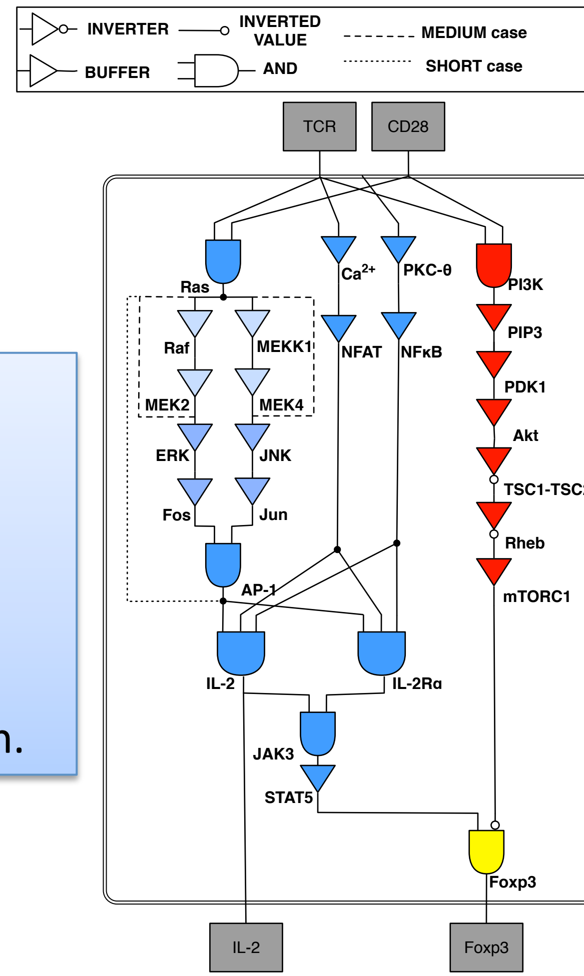


STAT5 vs. mTOR

Network Diagram



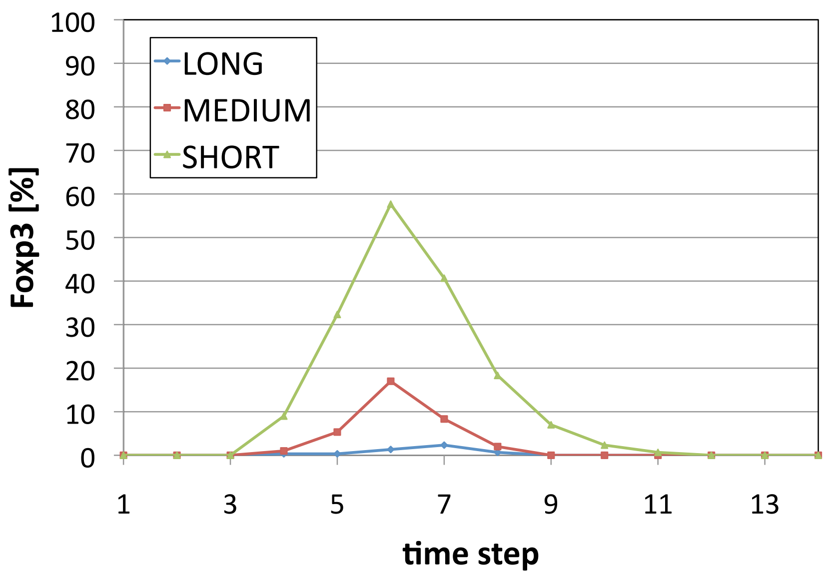
Circuit Diagram



Intermediate events may be very fast.

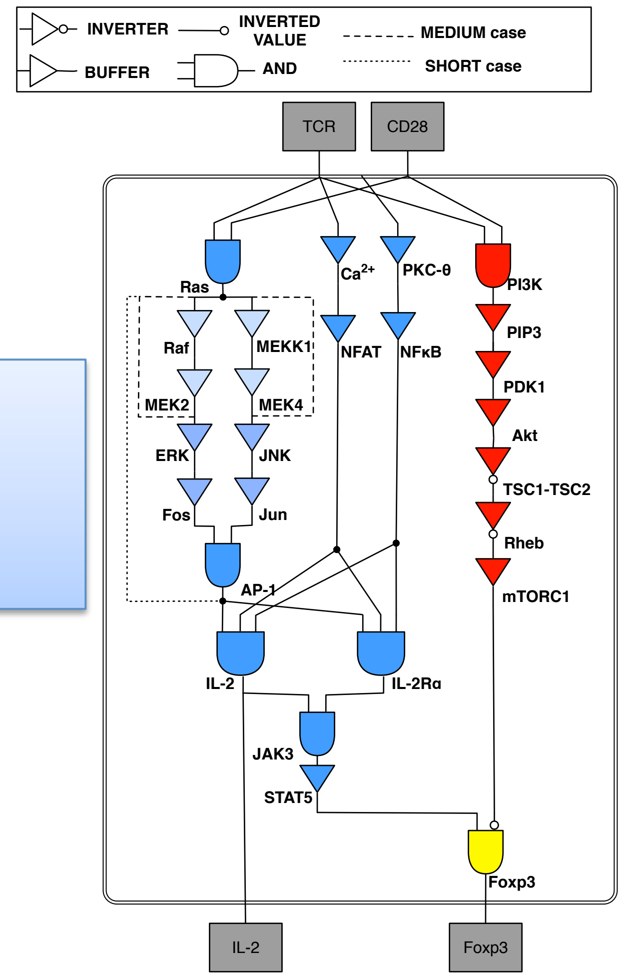
Test effect of varying the "buffer" length.

STAT5 vs. mTOR



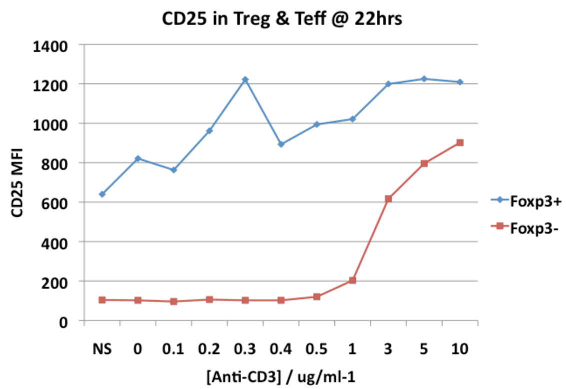
Longer buffer means STAT5 wins race less often.

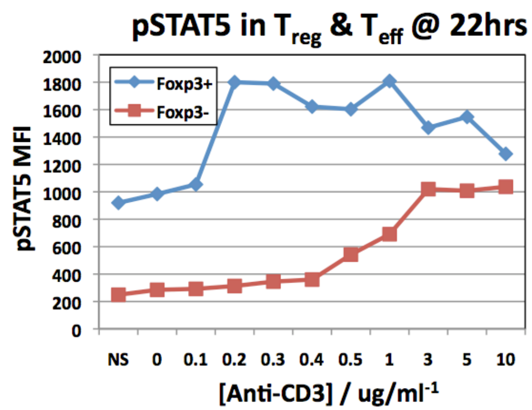
Circuit Diagram



Role of CD25->STAT5->Foxp3

- This pathway drives *transient* Foxp3 expression at high Ag dose and *sustained* expression at low dose (in the model).
- Experiments suggest that both CD25 expression and pSTAT5 remain low in Foxp3^{-} cells.





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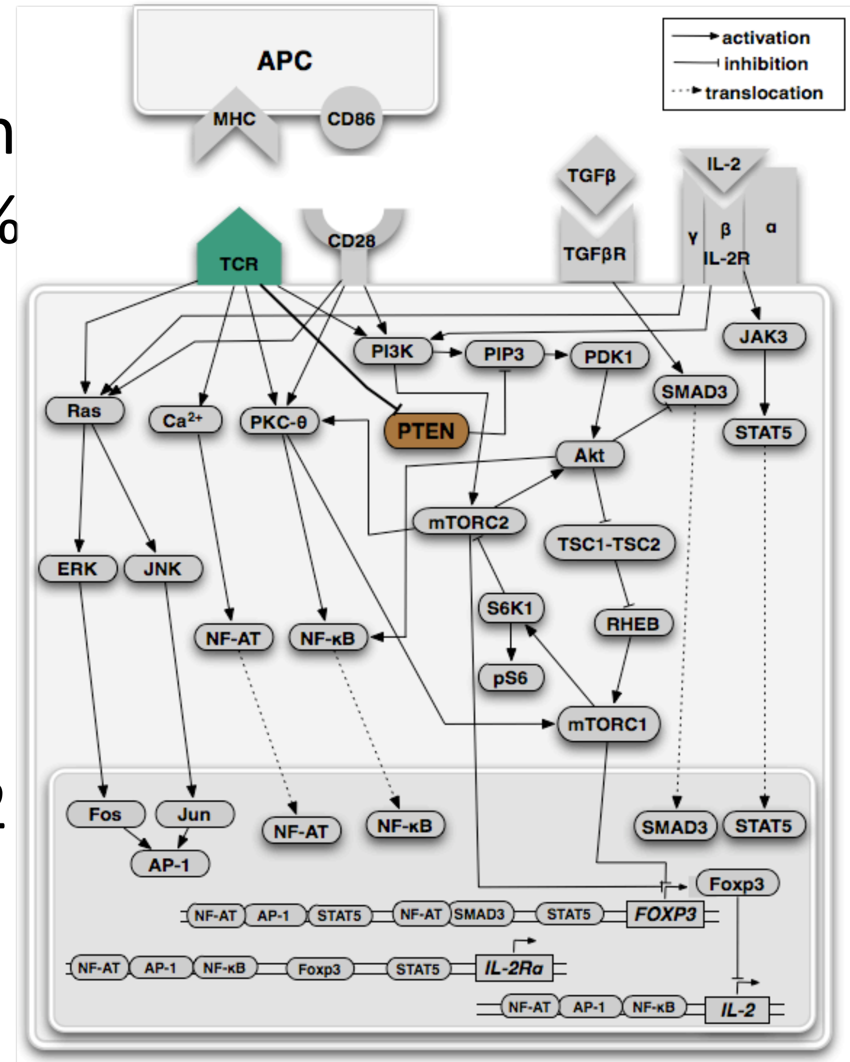
- This pathway drives *transient* Foxp3 expression at high Ag dose and *sustained* expression at low dose (in the model).
- Experiments suggest that both CD25 expression and pSTAT5 remain low in Foxp3^{-} cells.
- Implies weak TCR stimulation may not be enough to drive CD25. *Could Foxp3 be driving CD25 instead?*

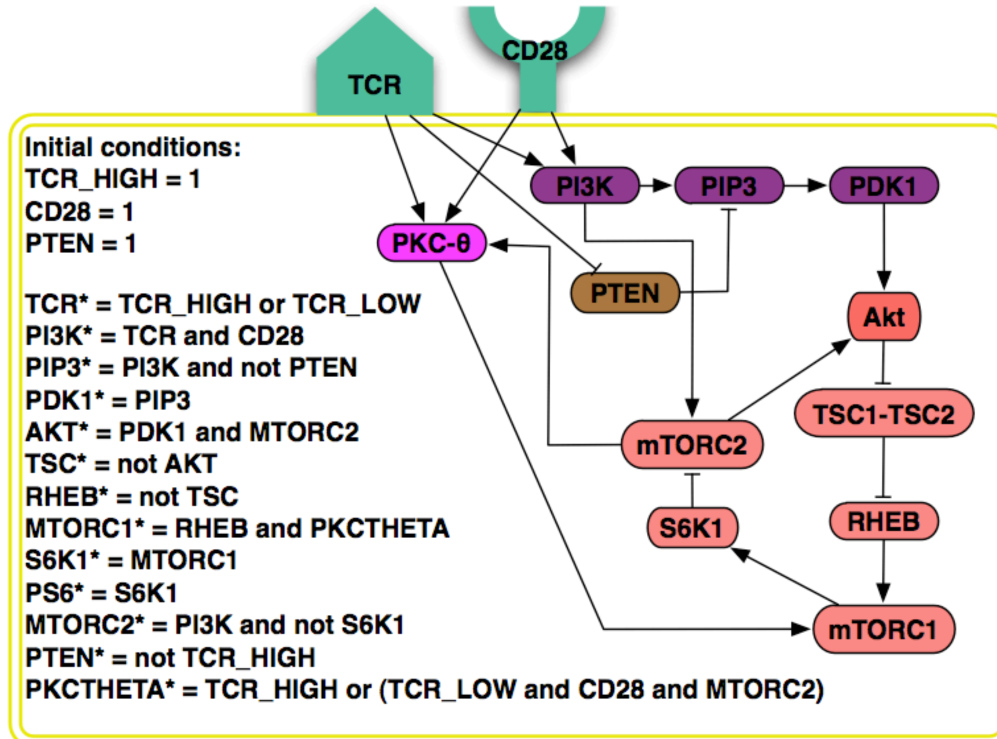
PTEN regulation

- PTEN blocks mTOR activation at low dose resulting in 100% Treg – not observed.
- Kinetics of PTEN / PIP3 could be very informative.
- Interplay with kinetics of CD25 / Foxp3 expression.
- PI3K activity increased by IL2 signaling and may partially overcome PTEN block.



Complex Interaction between mTORC1 and mTORC2

- mTORC2 activation still unclear:
  - Possible activation by PI3K or PIP3
  - Negative feedback from mTORC1 through S6K1
- **Oscillations** for high antigen dose



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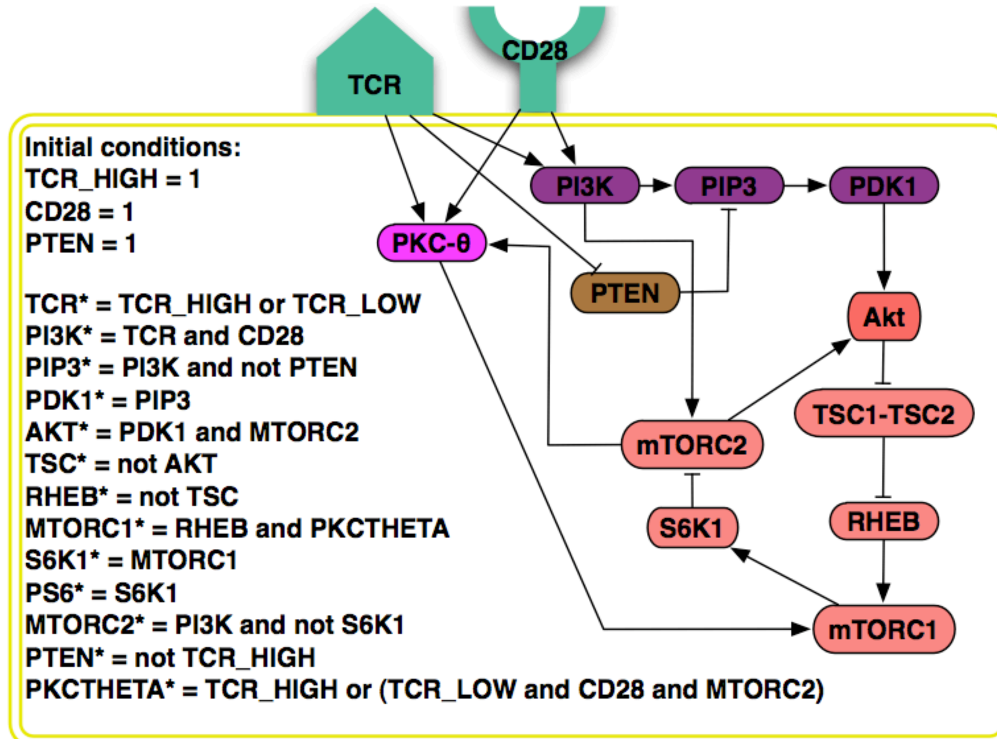
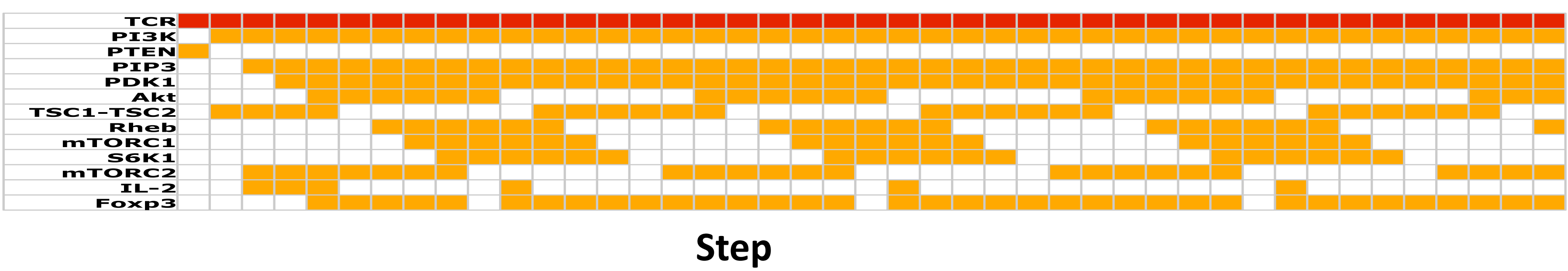
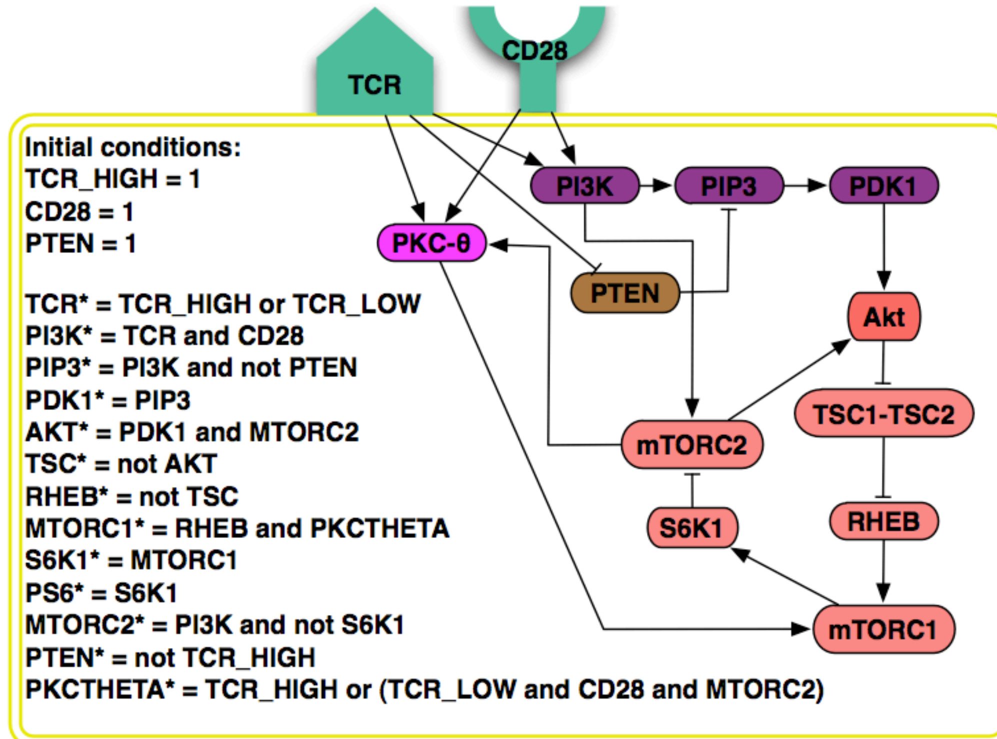
  - Negative feedback from mTORC1 through S6K1
- **Oscillations** for high antigen dose





Complex Interaction between mTORC1 and mTORC2

- mTORC2 activation still unclear:
  - Possible activation by PI3K or PIP3
  - Negative feedback from mTORC1 through S6K1
- **Oscillations** for high antigen dose
- ***Solved by using three levels for PI3K.***



mTOR role in Foxp3 expression

- Links between mTORC1 and mTORC2 and the Foxp3 expression are not well understood
  - Early mTORC1 signaling helps increase Foxp3 expression (through chromatin remodeling)
  - Prolonged mTORC1 signaling inhibits Foxp3
  - mTORC2 activation takes longer than mTORC1 activation
  - pS6 as a readout of mTORC1 activity decreases after 18 hours
  - Both mTORC1 and mTORC2 are necessary for Foxp3 inhibition

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- **Further Experiments**: correlation between levels of mTORC1 and mTORC2 and the level of Foxp3 expression

Conclusions

- Logical modeling approach allows collaborative model development.
- Preliminary model reproduces dependence of outcome on antigen dose and duration.
- Model focuses attention on several key elements
  - Relative kinetics of CD25 / Foxp3 expression
  - Role of differential PTEN regulation
  - Possible role of Smad3
  - Negative feedback between mTORC1 and mTORC2
  - mTORC1/2 regulation of Foxp3

Future modeling steps

- Experimenting with three instead of two levels
  - Increase in number of variables is not significant in terms of simulation runtime
- Modeling of population of cells
- Exploration of the system's sensitivity