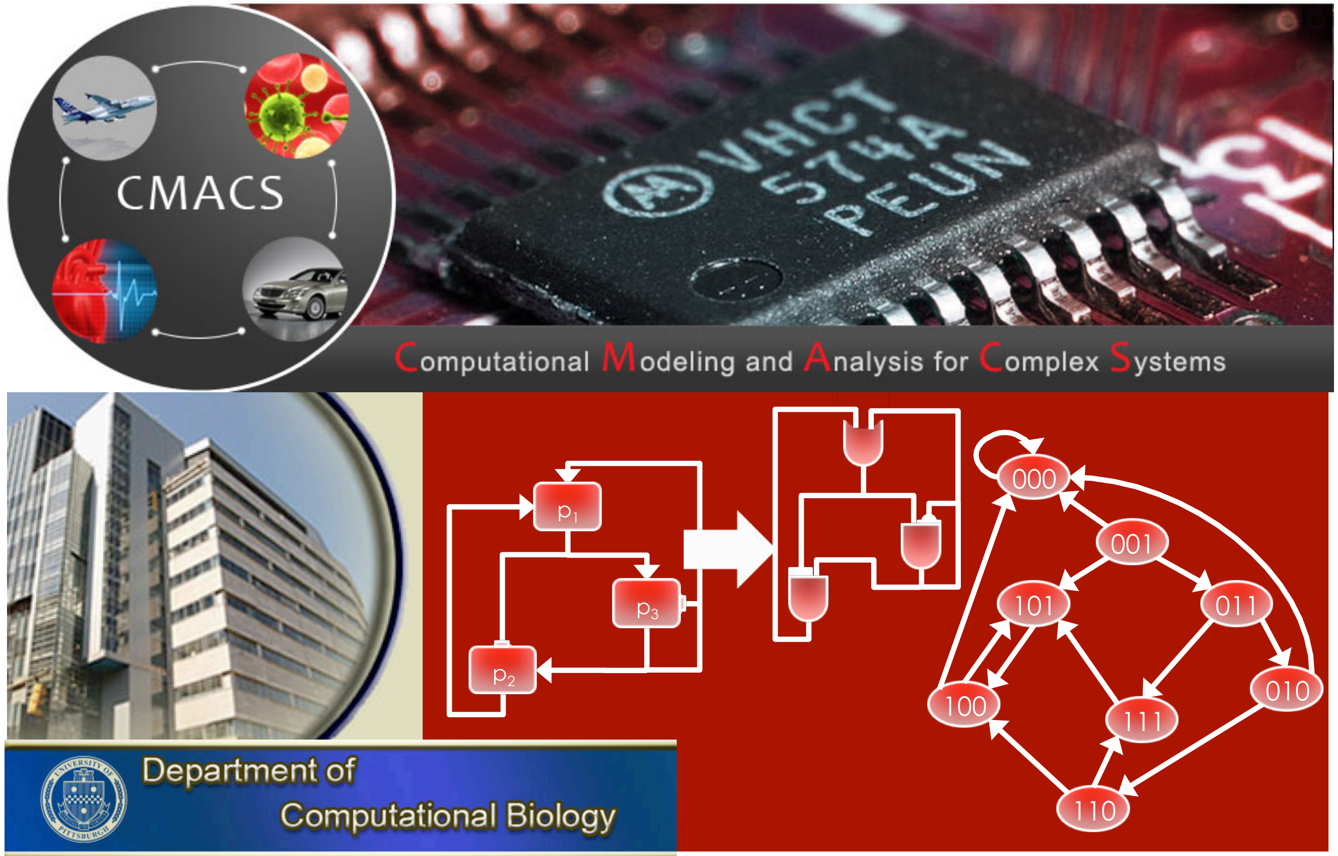
Virtual PI Meeting

April 2012



Timing Matters in T Cell Differentiation

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University of Pittsburgh

Acknowledgements

- **Faeder Lab:**
  - Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh
    - John Sekar, James Faeder
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  - Department of Immunology, School of Medicine, University of Pittsburgh
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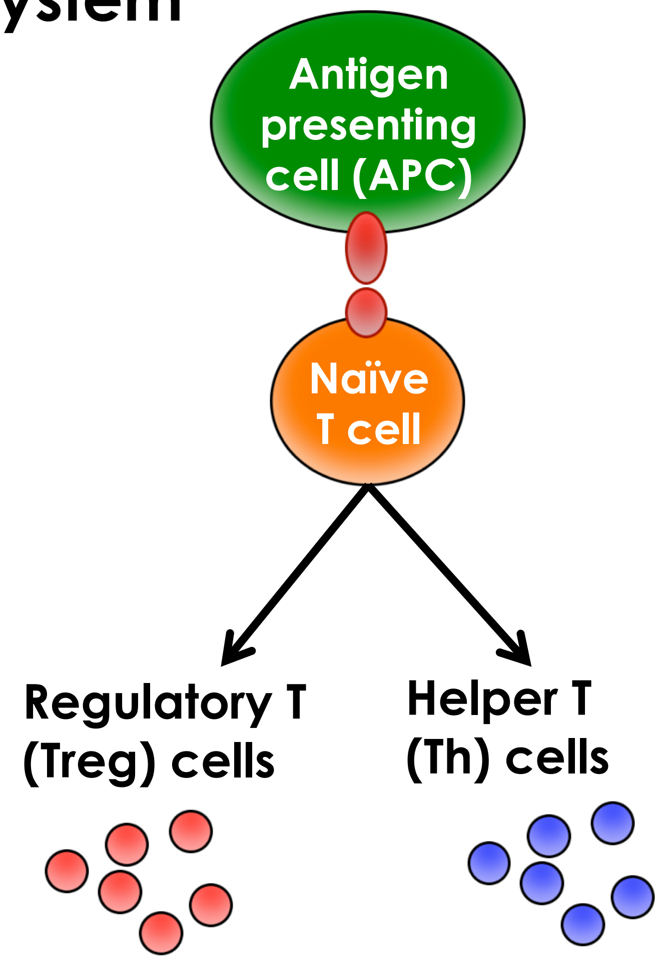
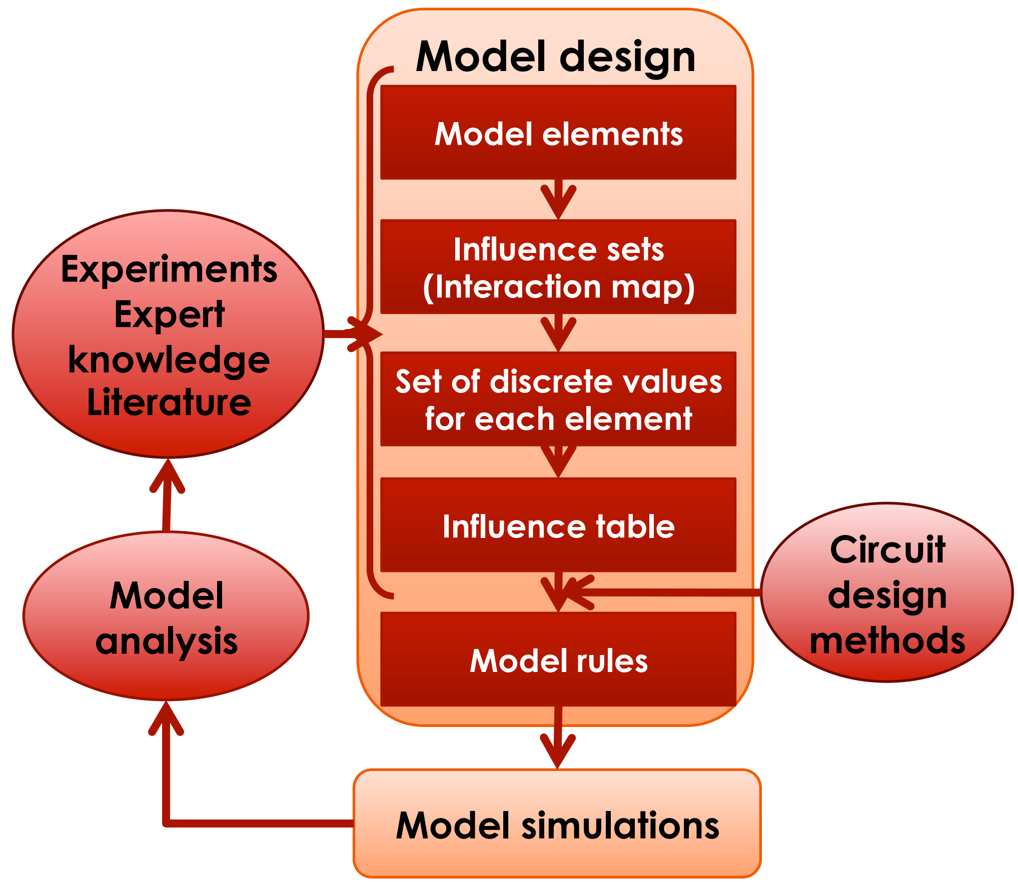
    - Paolo Zuliani, Haijun Gong, Edmund Clarke
    - Deepa Sathaye, Alexander Moser
- **Funding:**
  - NSF (Expeditions in Computing)
  - NIH

Outline

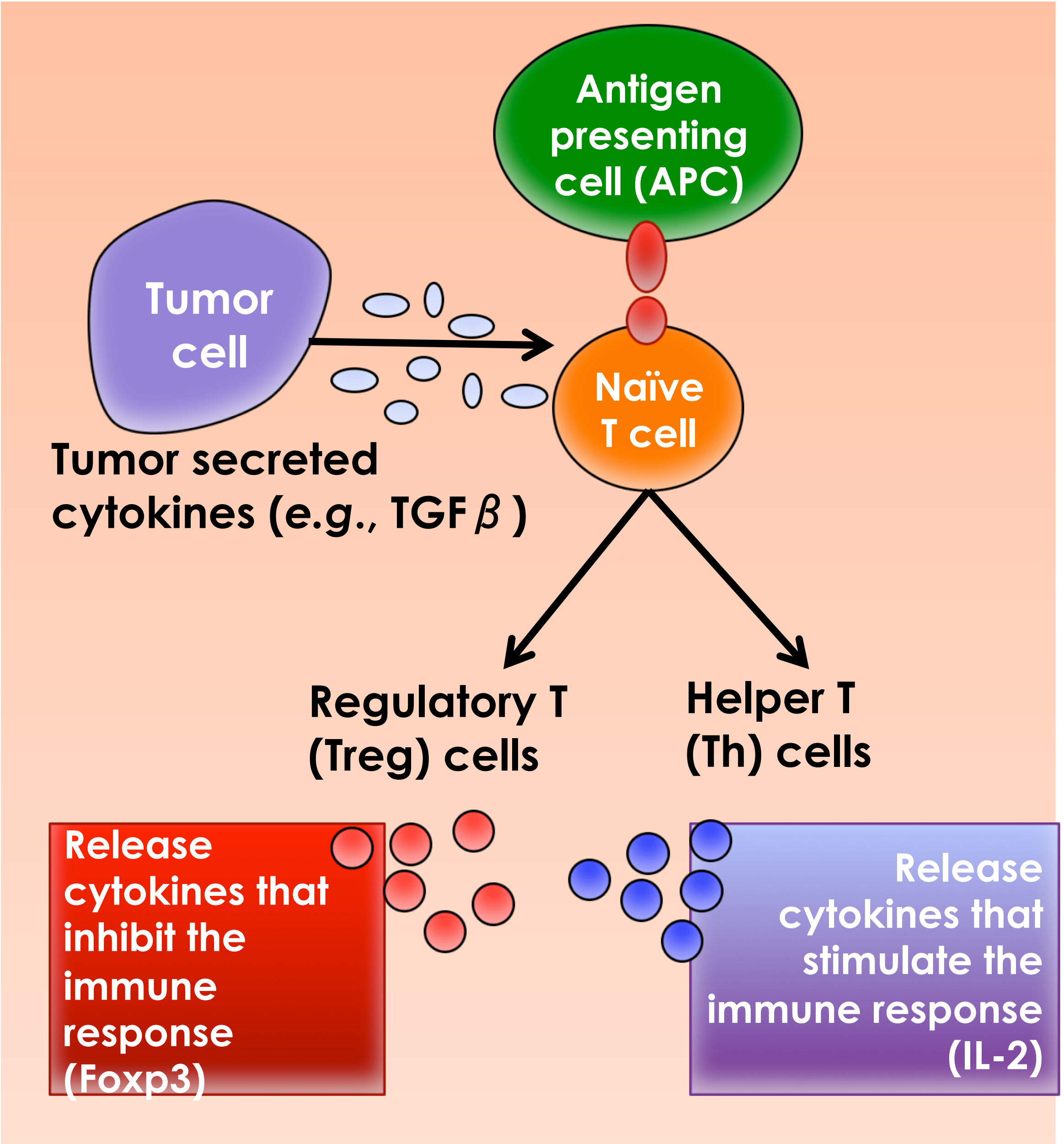
System



Methodology



T cell differentiation



- T cell subpopulation ratios are critical for numerous immune and auto-immune pathologies
- Determinants of the peripheral T cell differentiation are not completely understood

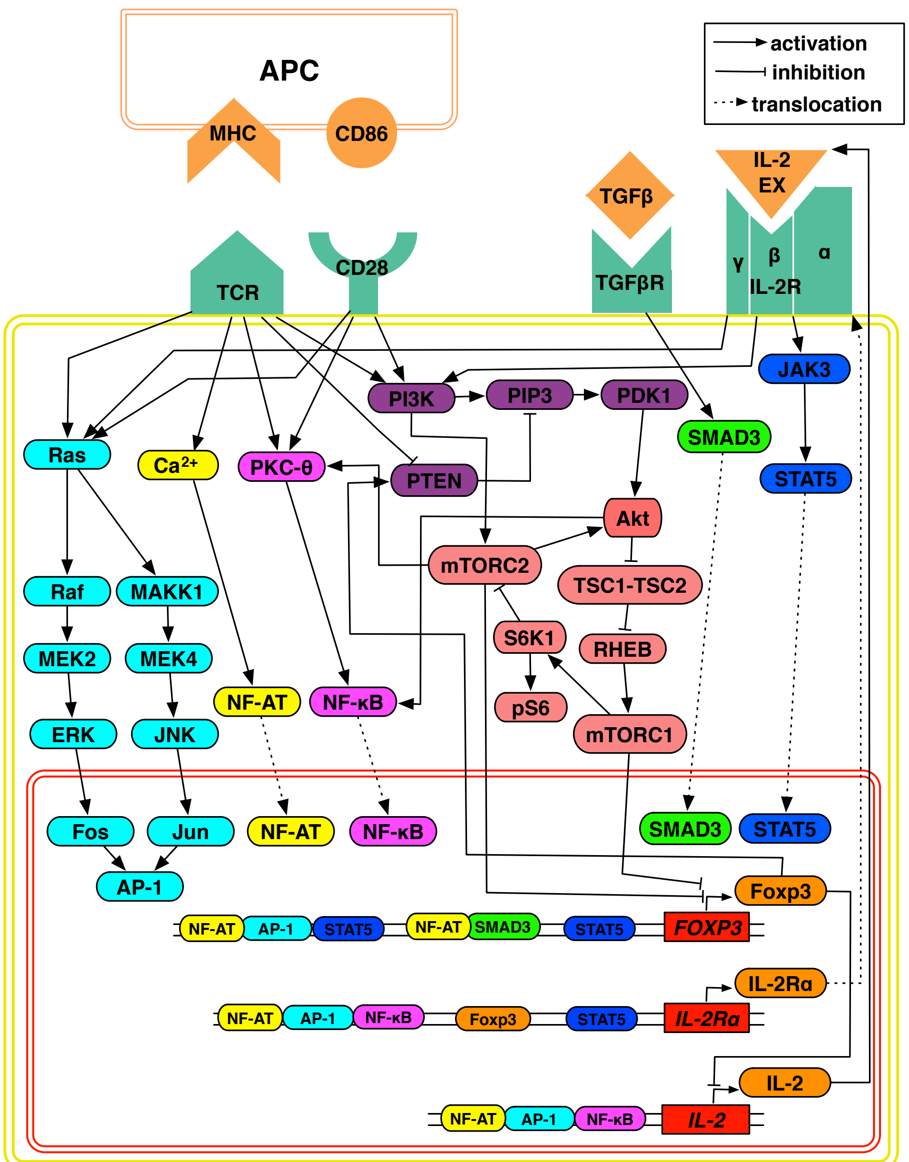
Questions to address

- How can we predict the antigen dose that will induce Treg versus Th?
- Are there signaling cascades in T cells that are critical in this cell fate decision?
- Can we use modeling to identify the critical factors?
- Many clinical trials involving DC vaccines do not take antigen dose into account
- Could also be important for the *ex vivo* expansion of Treg for therapeutic purposes

Modeling goals

- Determine whether known mechanisms are *sufficient* to explain experimental observations
- Suggest *additional experiments* to identify missing mechanisms and clarify areas of *uncertainty*
- Identify *early markers* of the response

Network model



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:

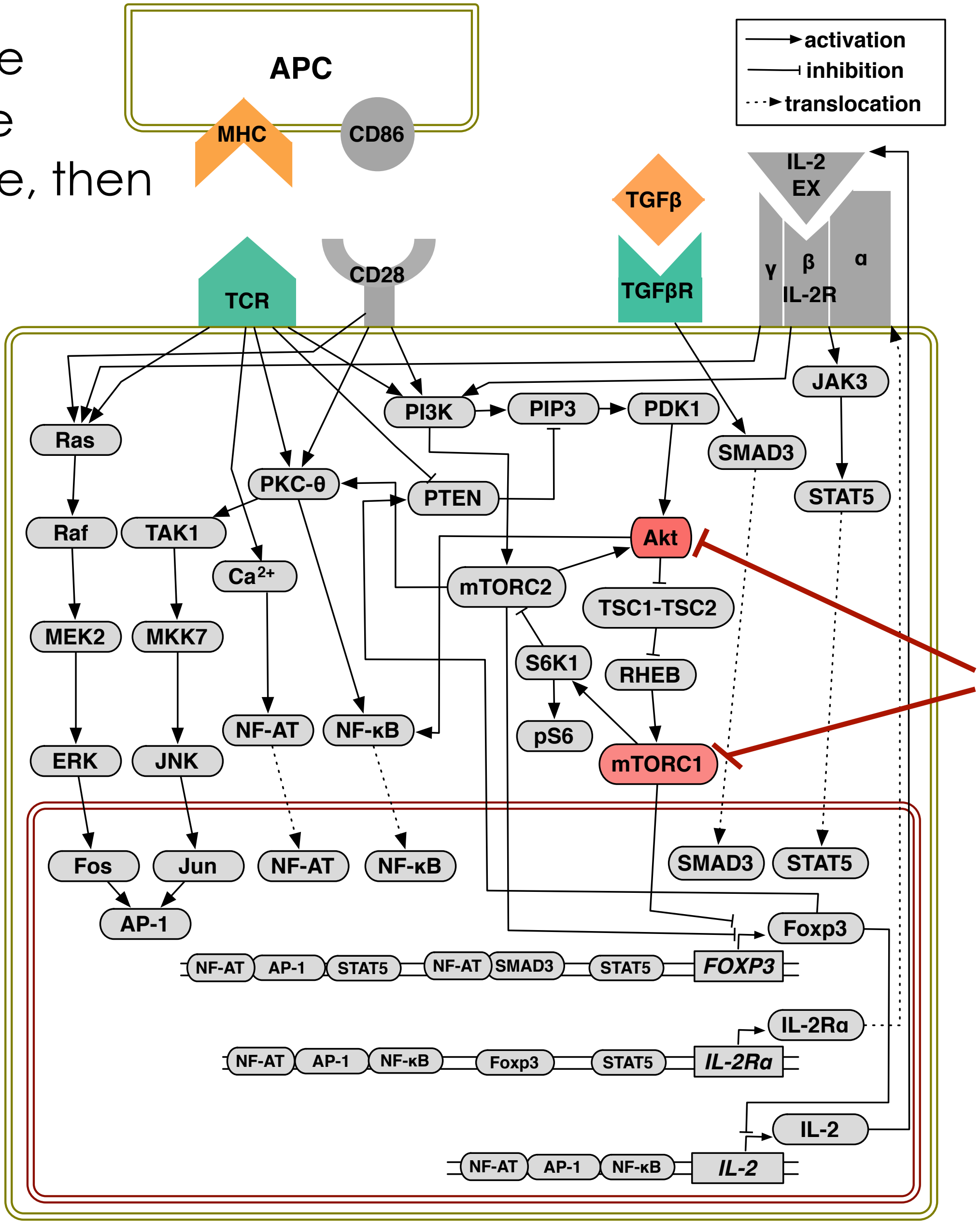
- IL-2, CD25, Foxp3

Other important elements:

- PTEN, PI3K, PIP3, PDK1,
- Akt, mTORC1, mTORC2, TSC1-TSC2, Rheb, S6K1, pS6

Five scenarios

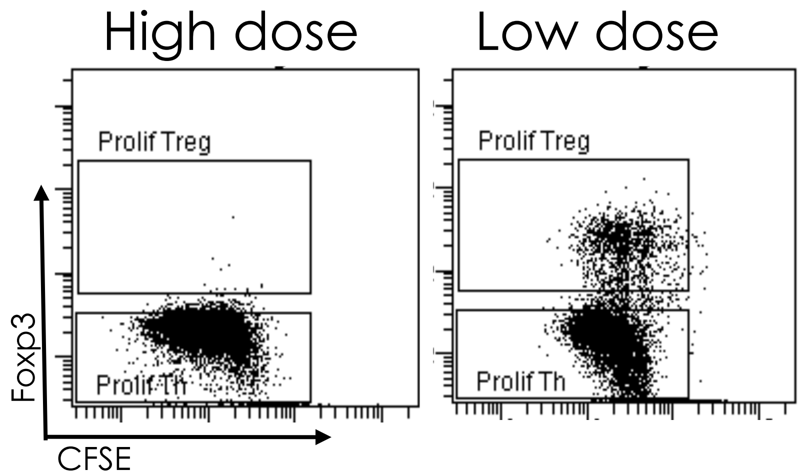
1. High antigen dose
2. Low antigen dose
3. High antigen dose, then removed



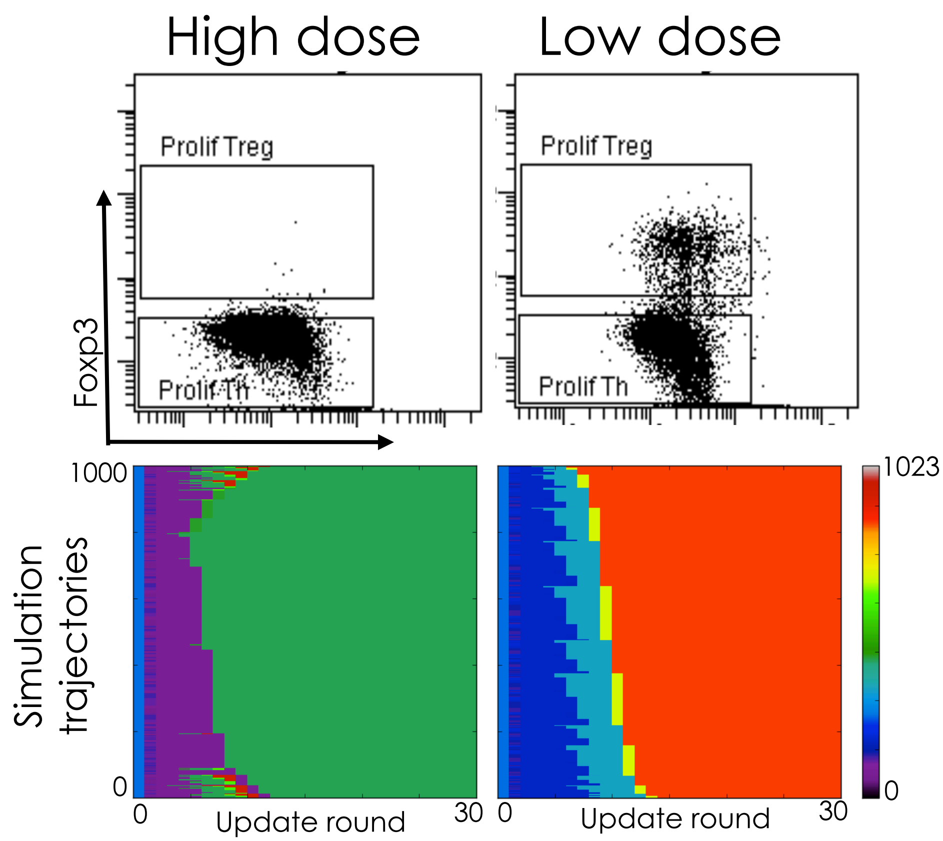
4. High antigen dose and TGF β

5. High antigen dose, then inhibitors added

Scenarios 1 and 2: High and Low antigen dose

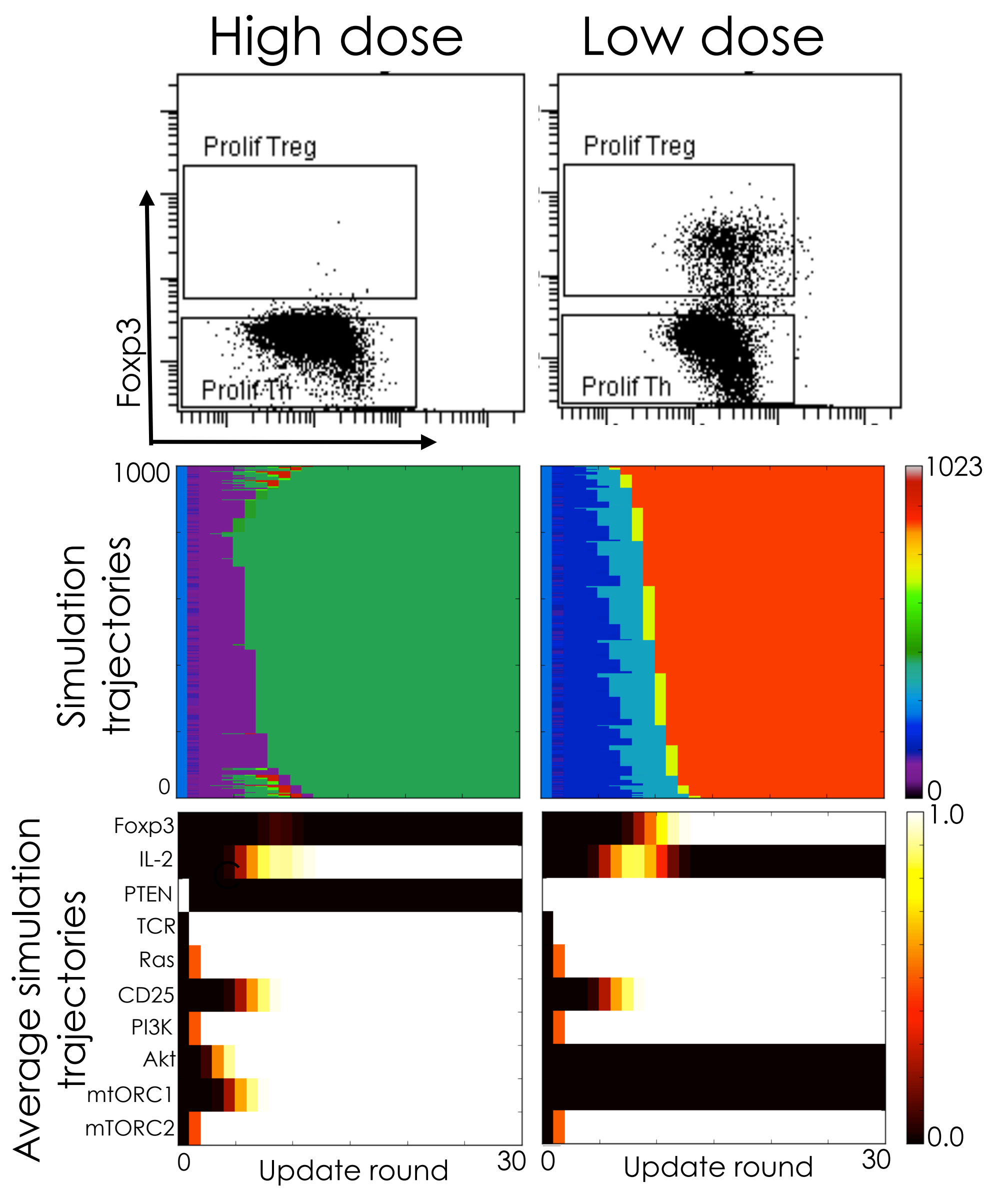


Scenarios 1 and 2: High and Low antigen dose



Source: N. Miskov-Zivanov et al., in preparation.

Scenarios 1 and 2: High and Low antigen dose



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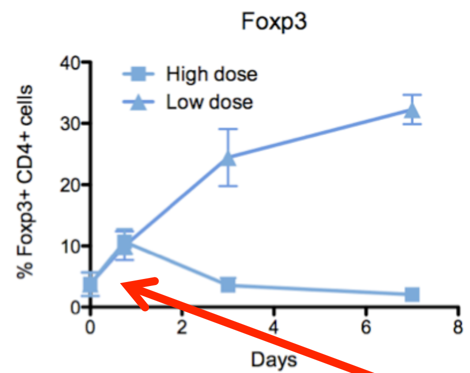
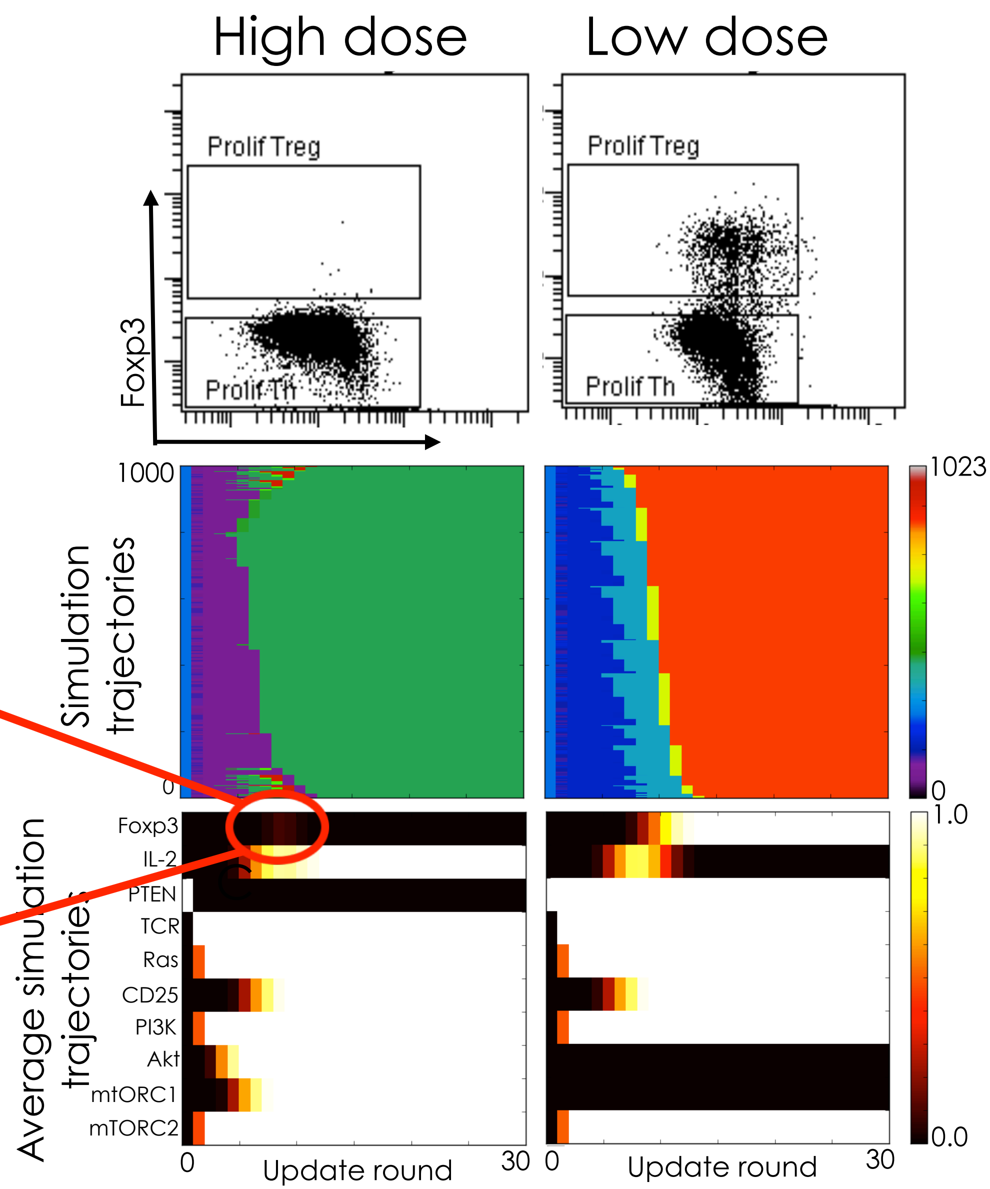
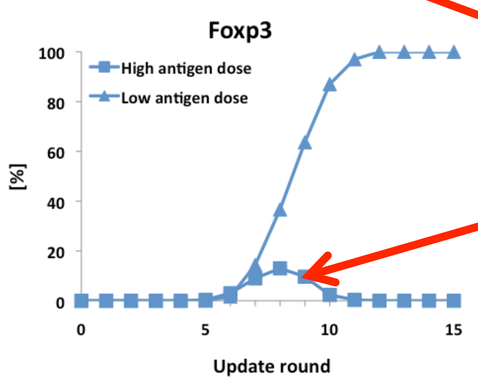
Scenarios 1 and 2: High and Low antigen dose

Experiments





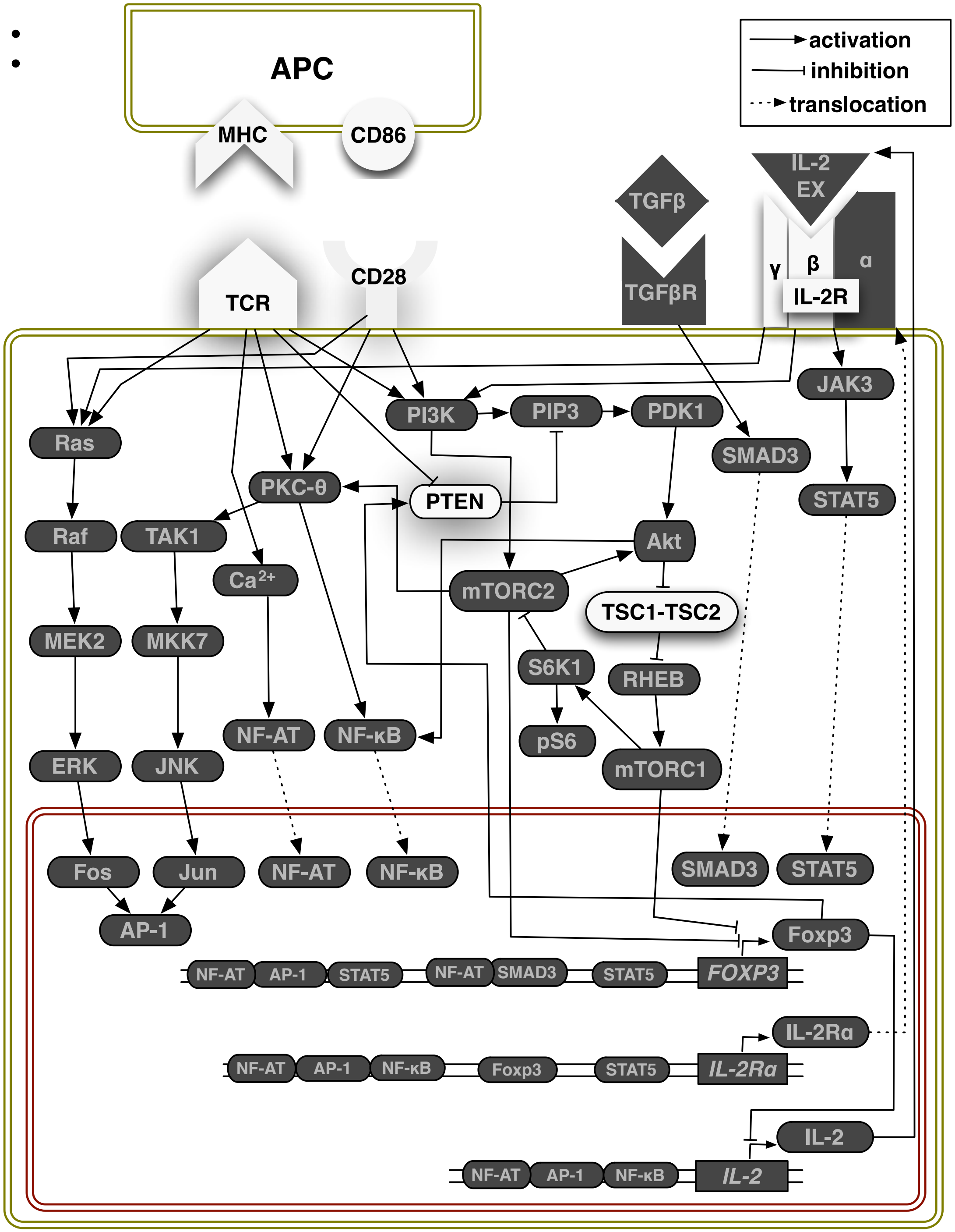


Simulations

Source: N. Miskov-Zivanov et al., in preparation.

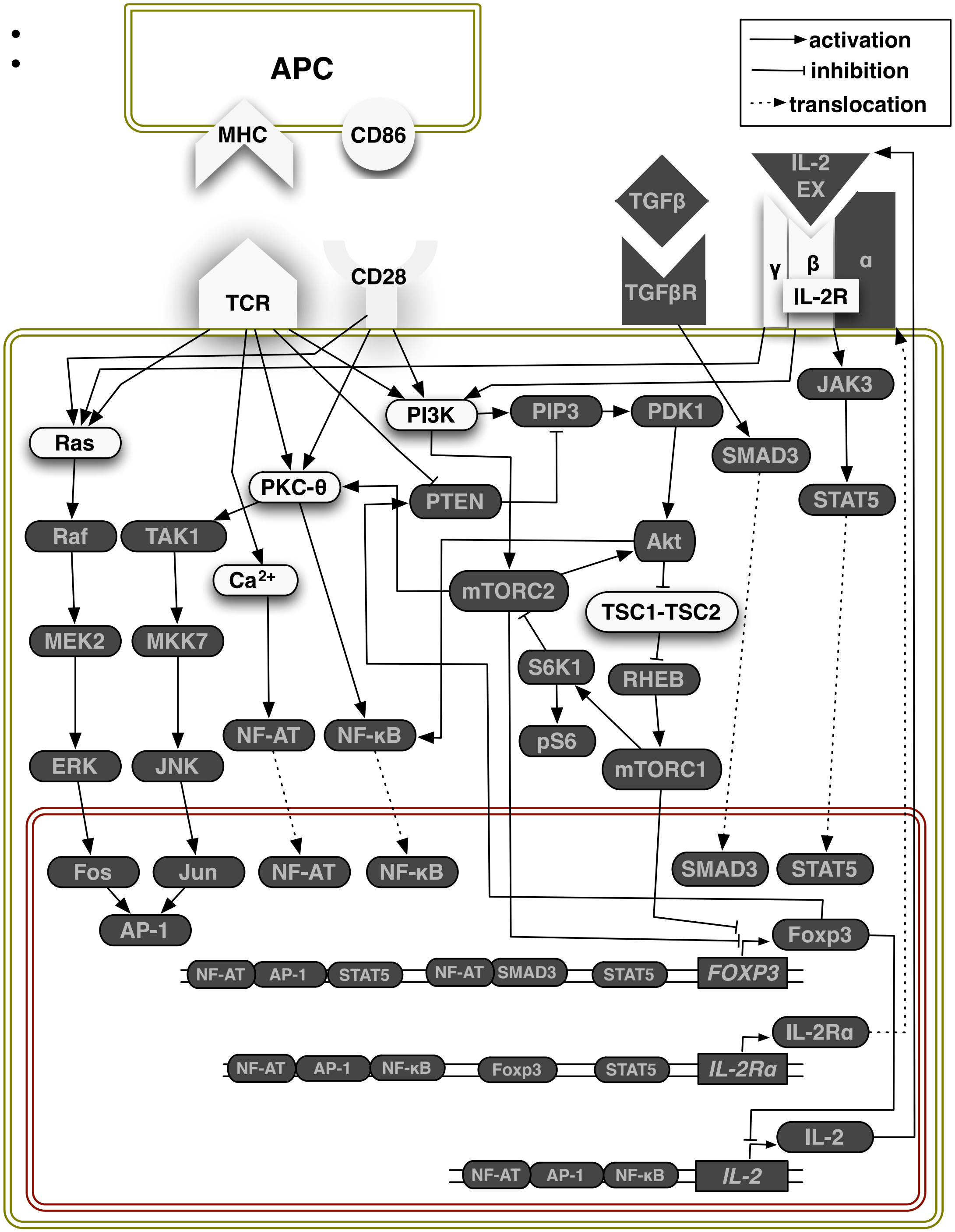
Scenario 1: High antigen dose

	value = ON (1)
	value = OFF (0)



Scenario 1: High antigen dose

(white)	value = ON (1)
(black)	value = OFF (0)



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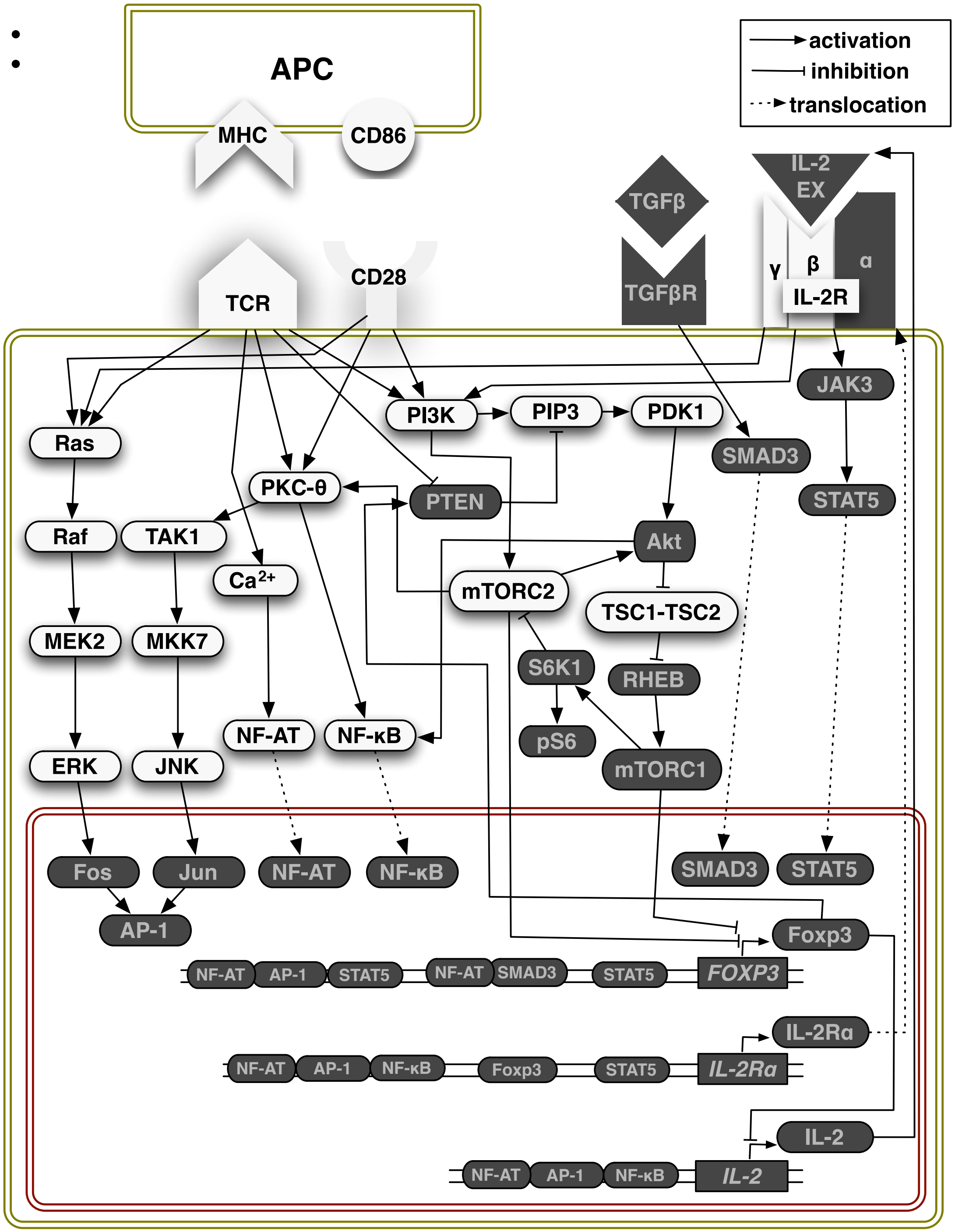

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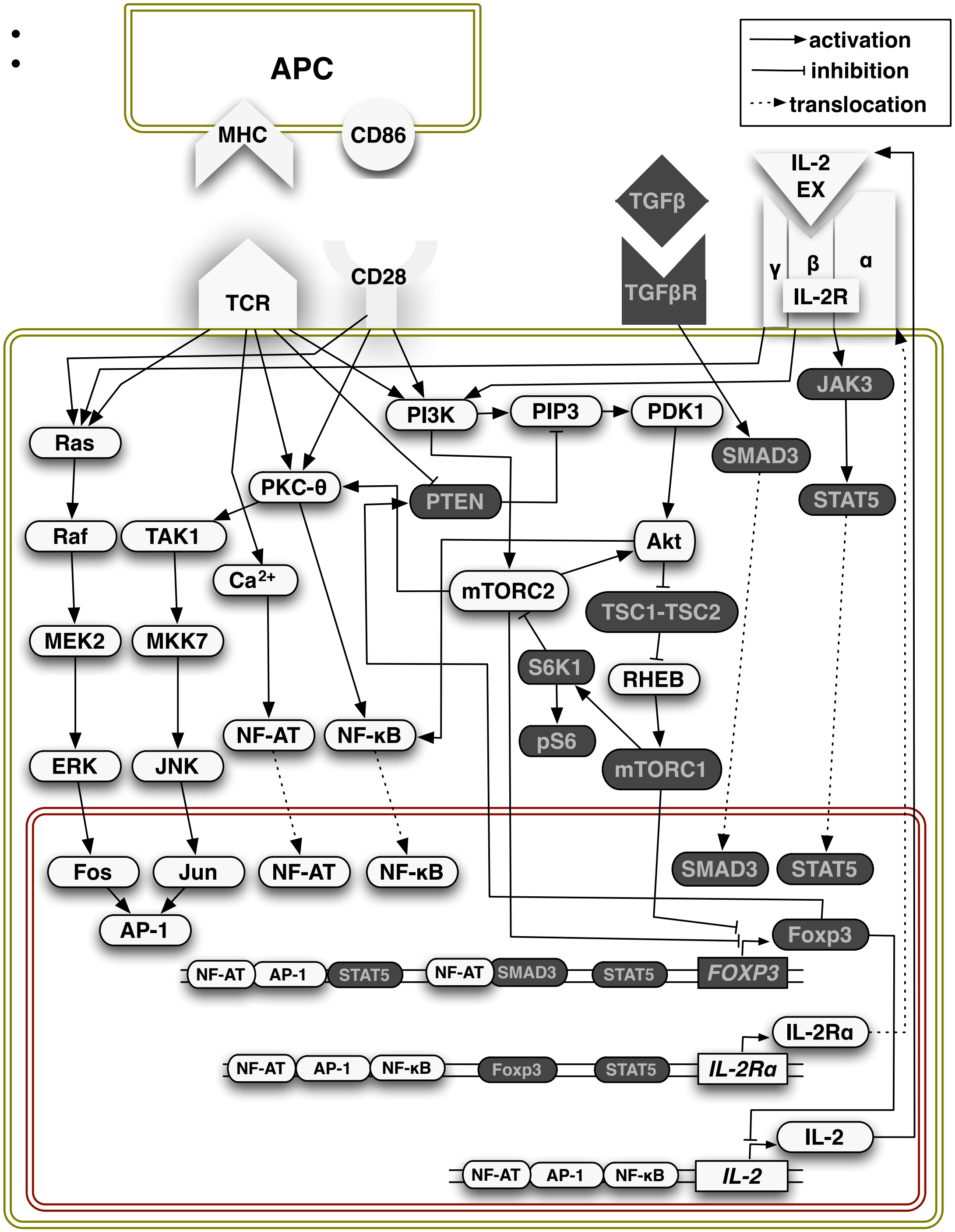

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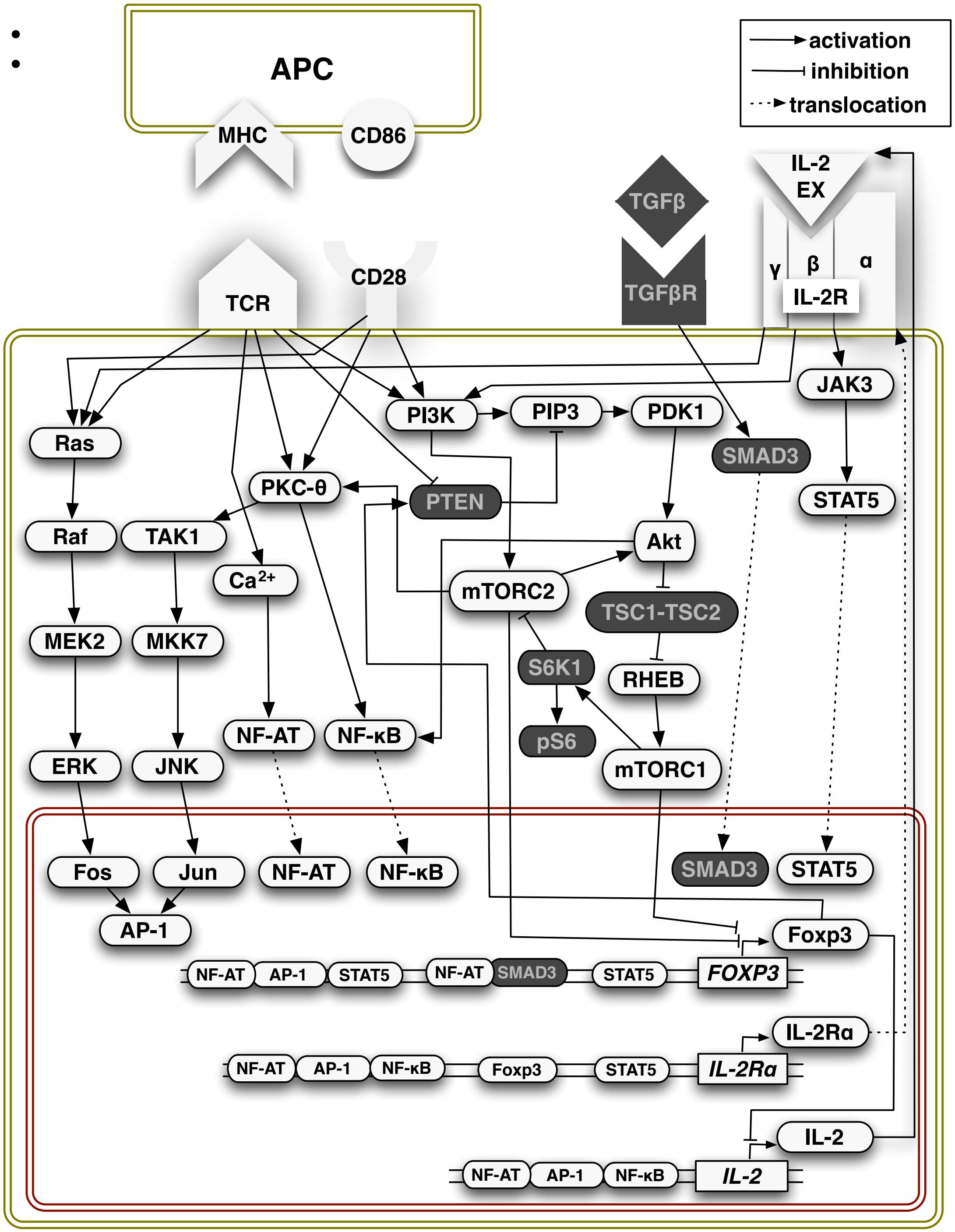

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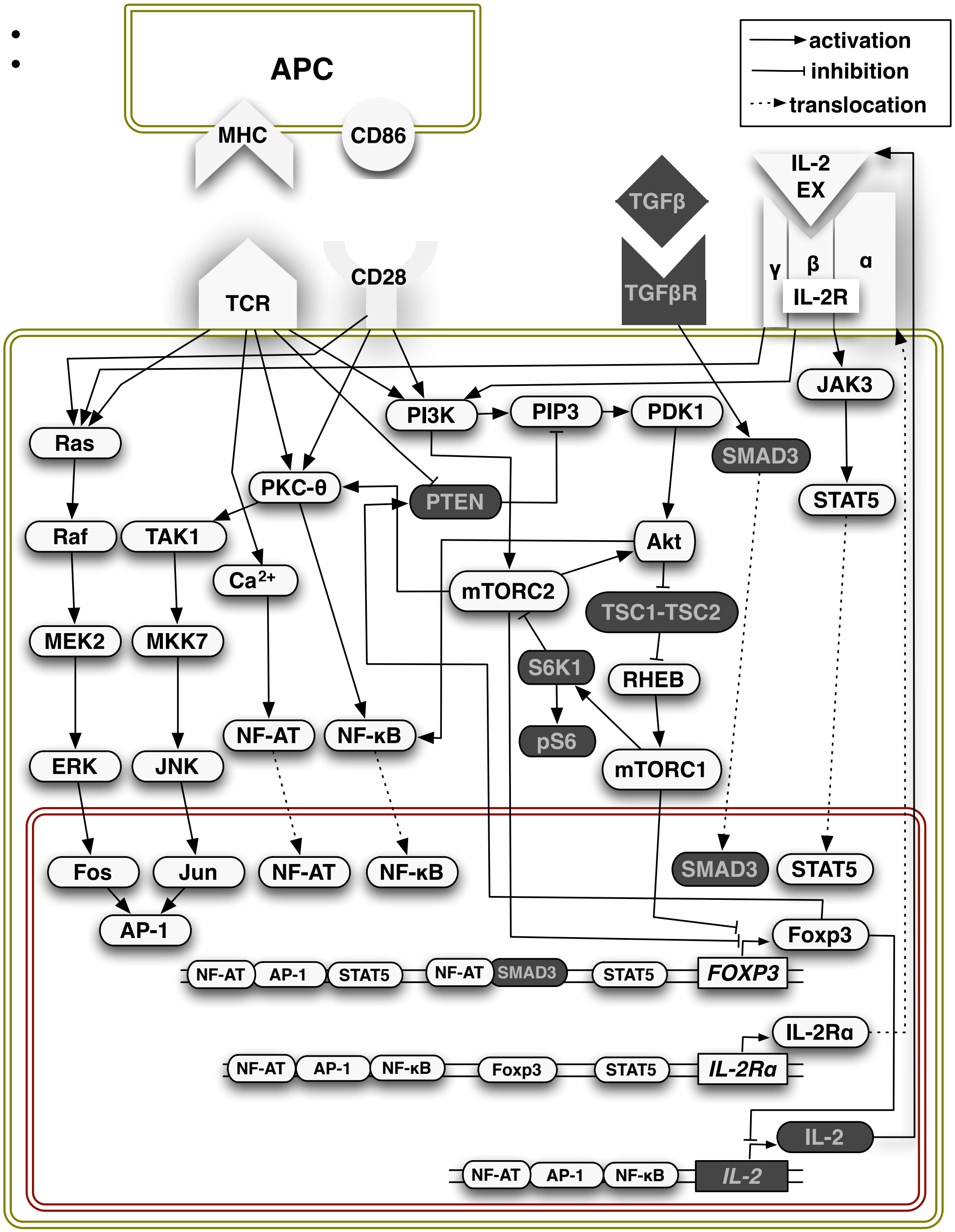

Scenario 1: High antigen dose

ON	value = ON (1)
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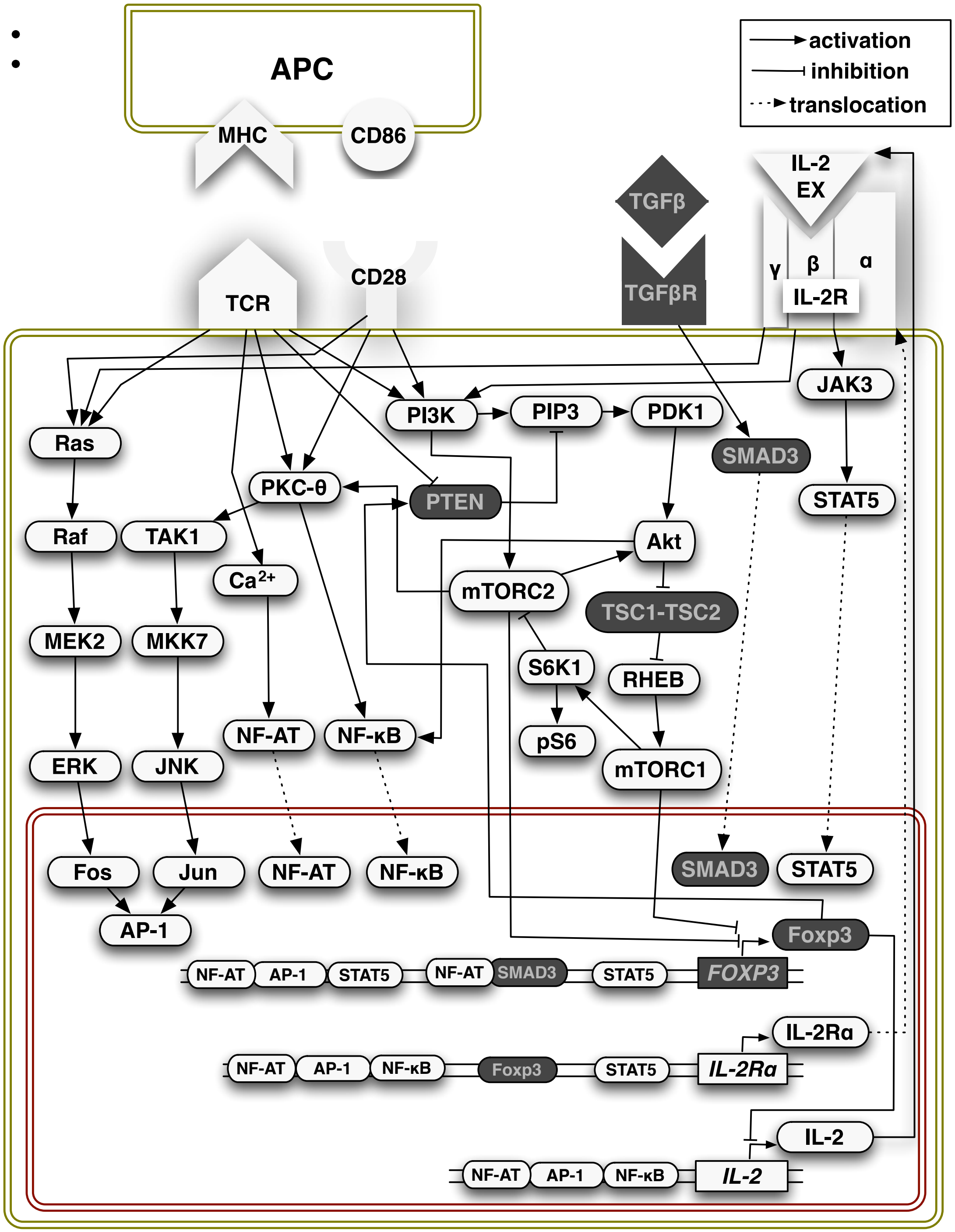
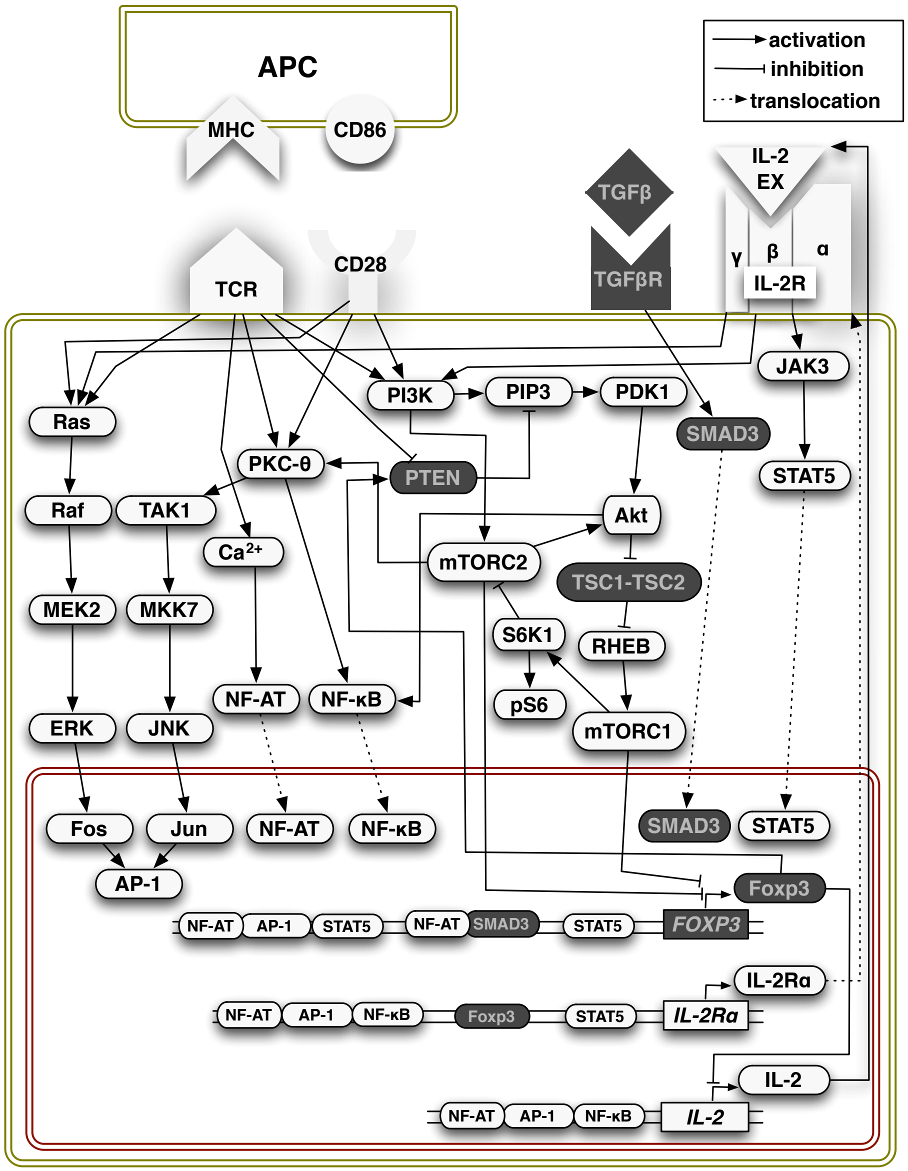
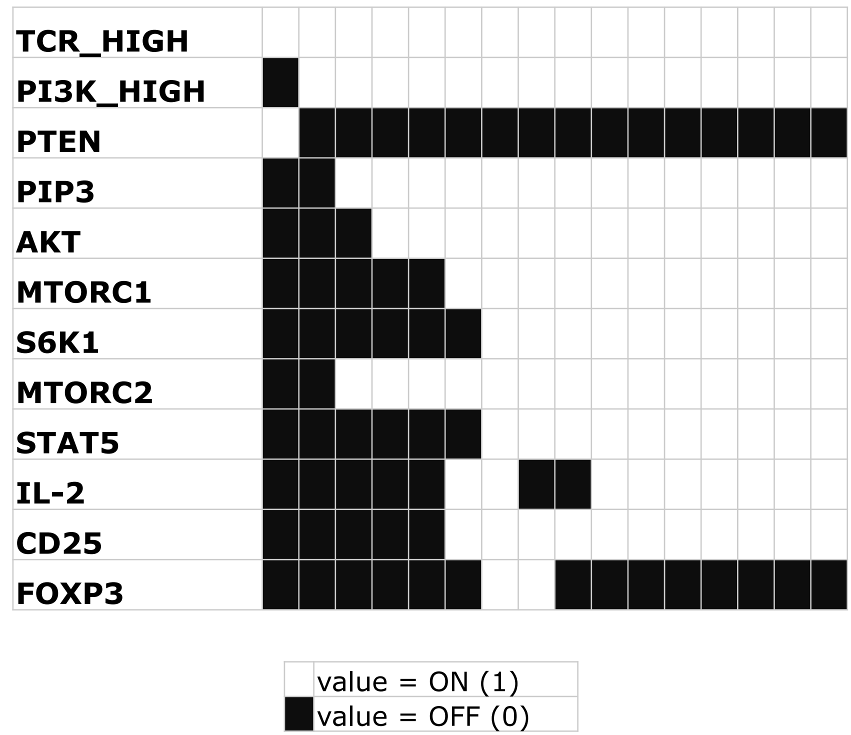


Scenario 1: High antigen dose trajectory



Trajectory example



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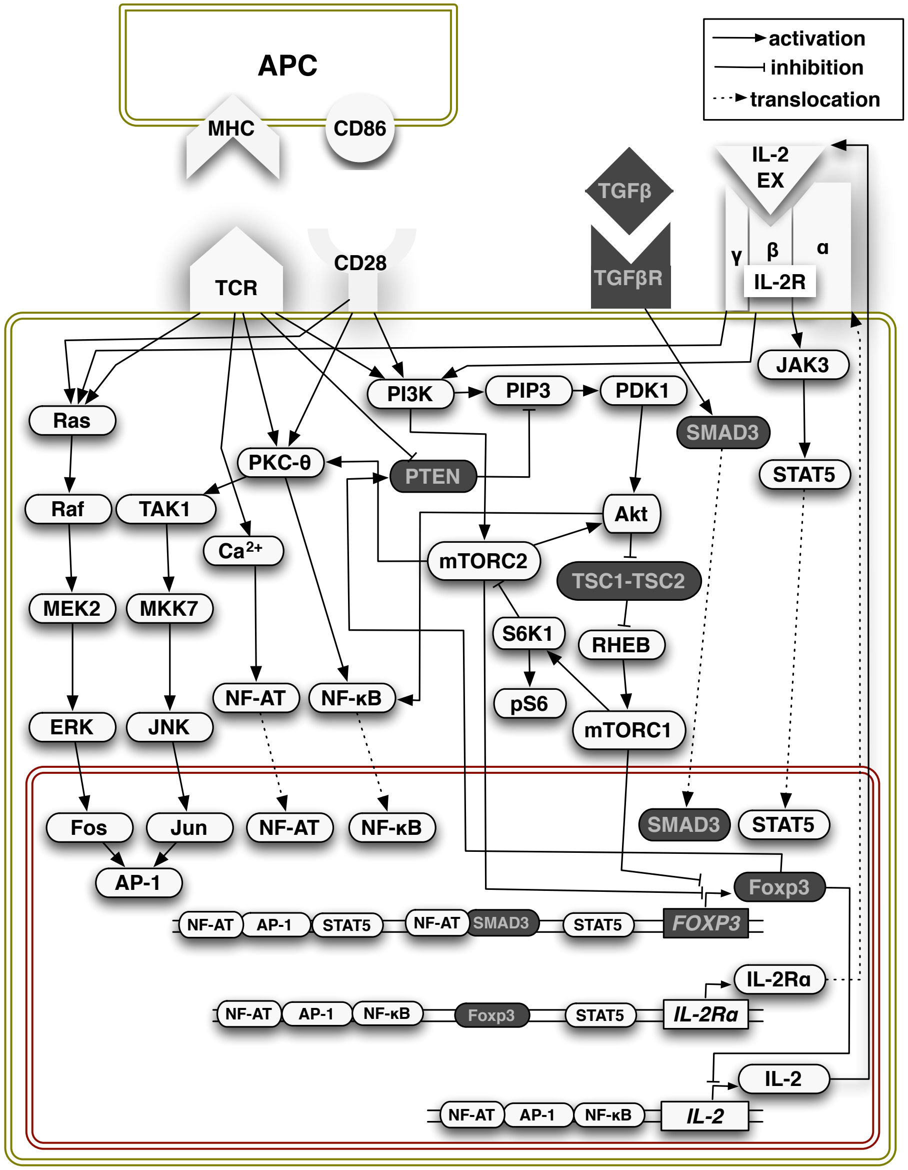

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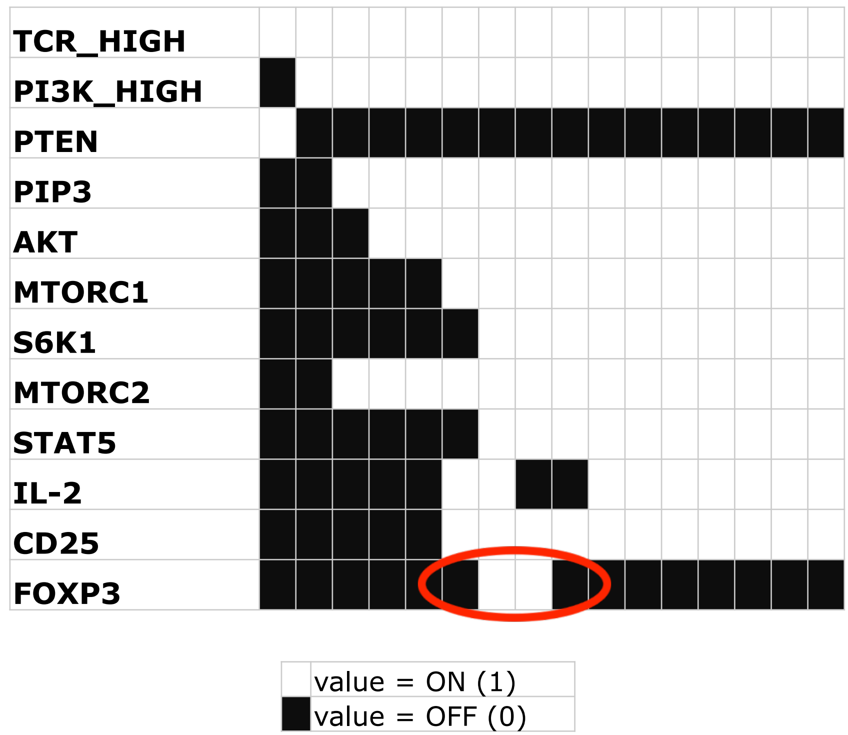
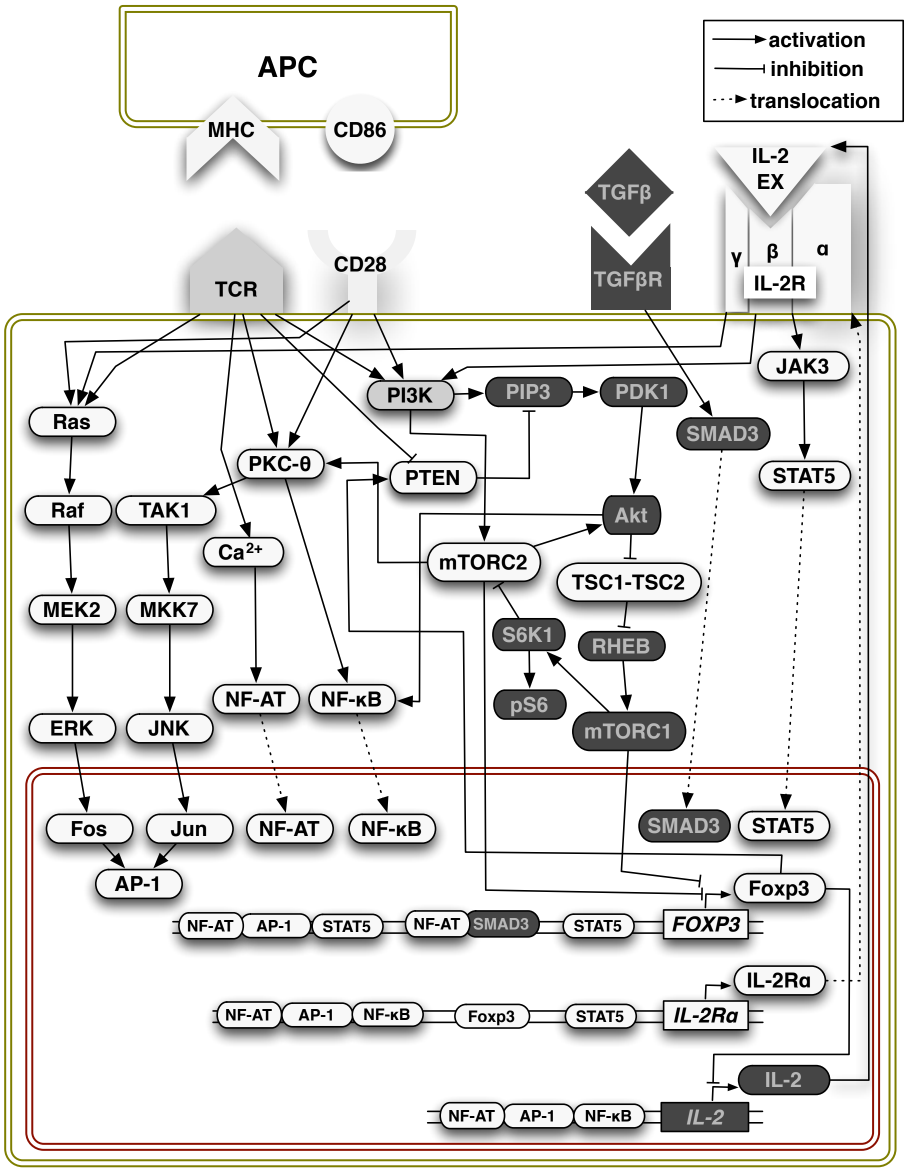


Scenario 2: Low antigen dose trajectory



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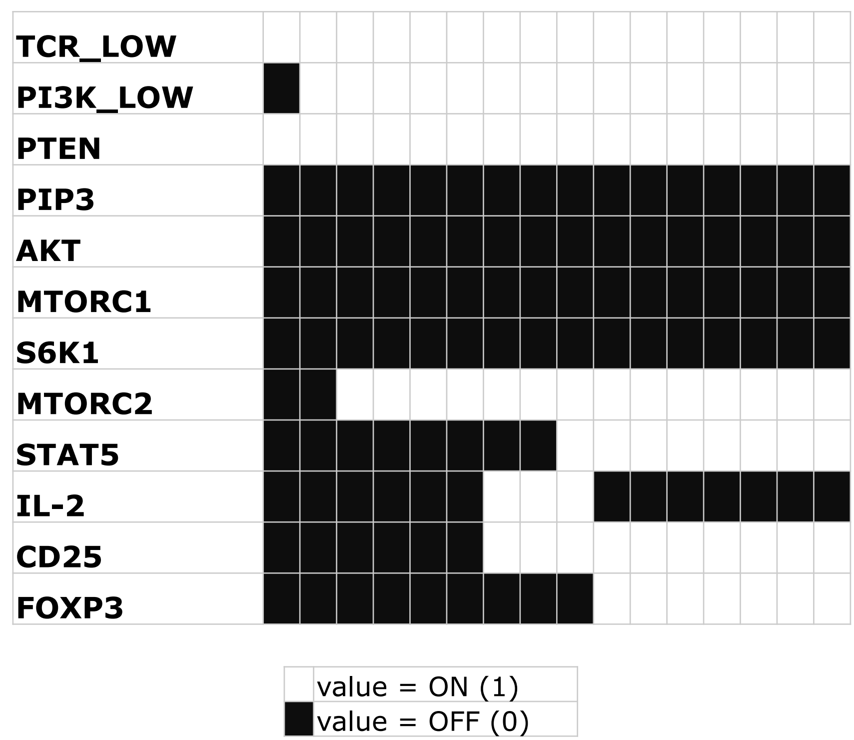

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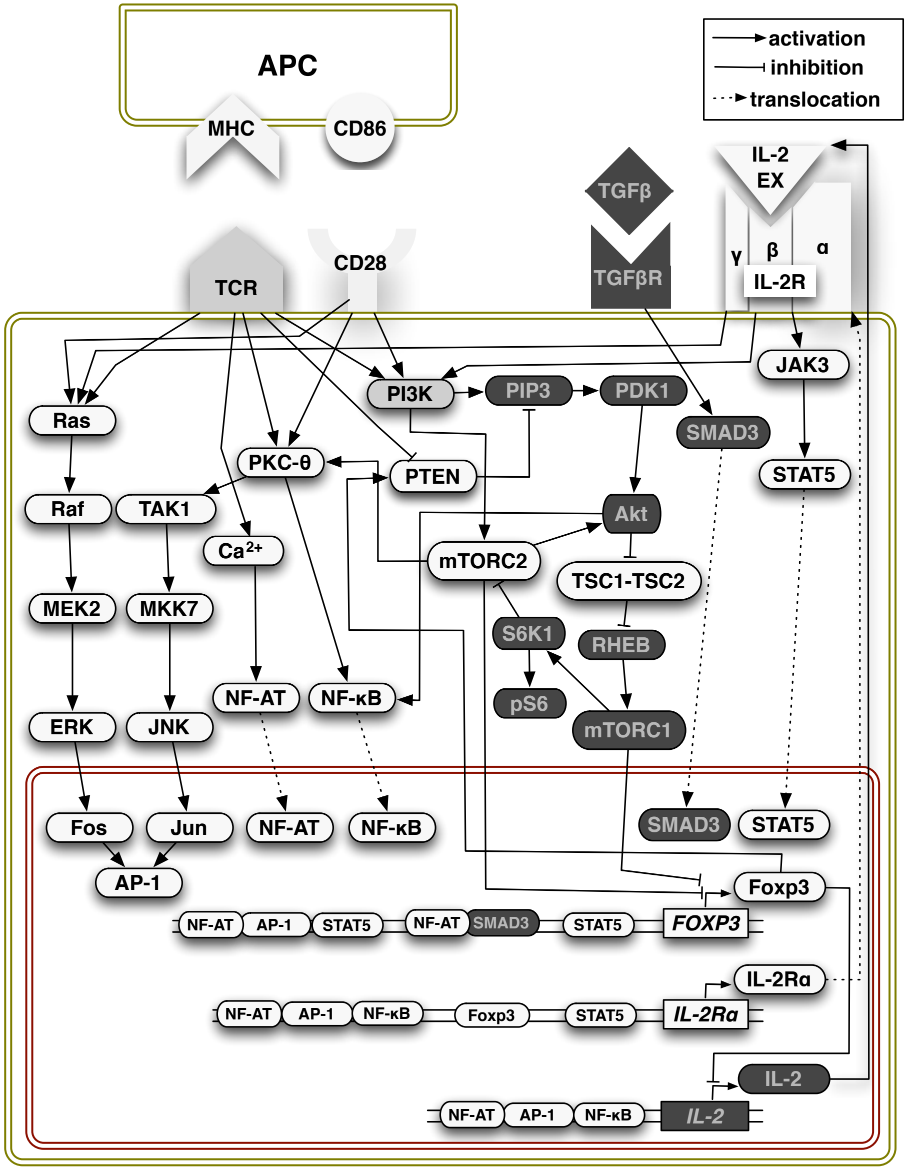

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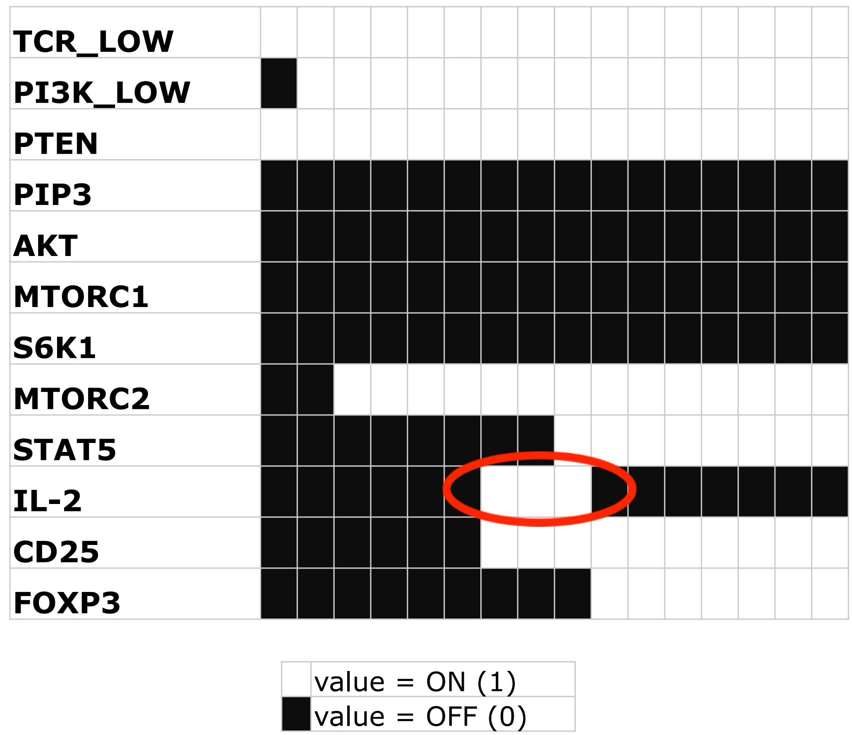
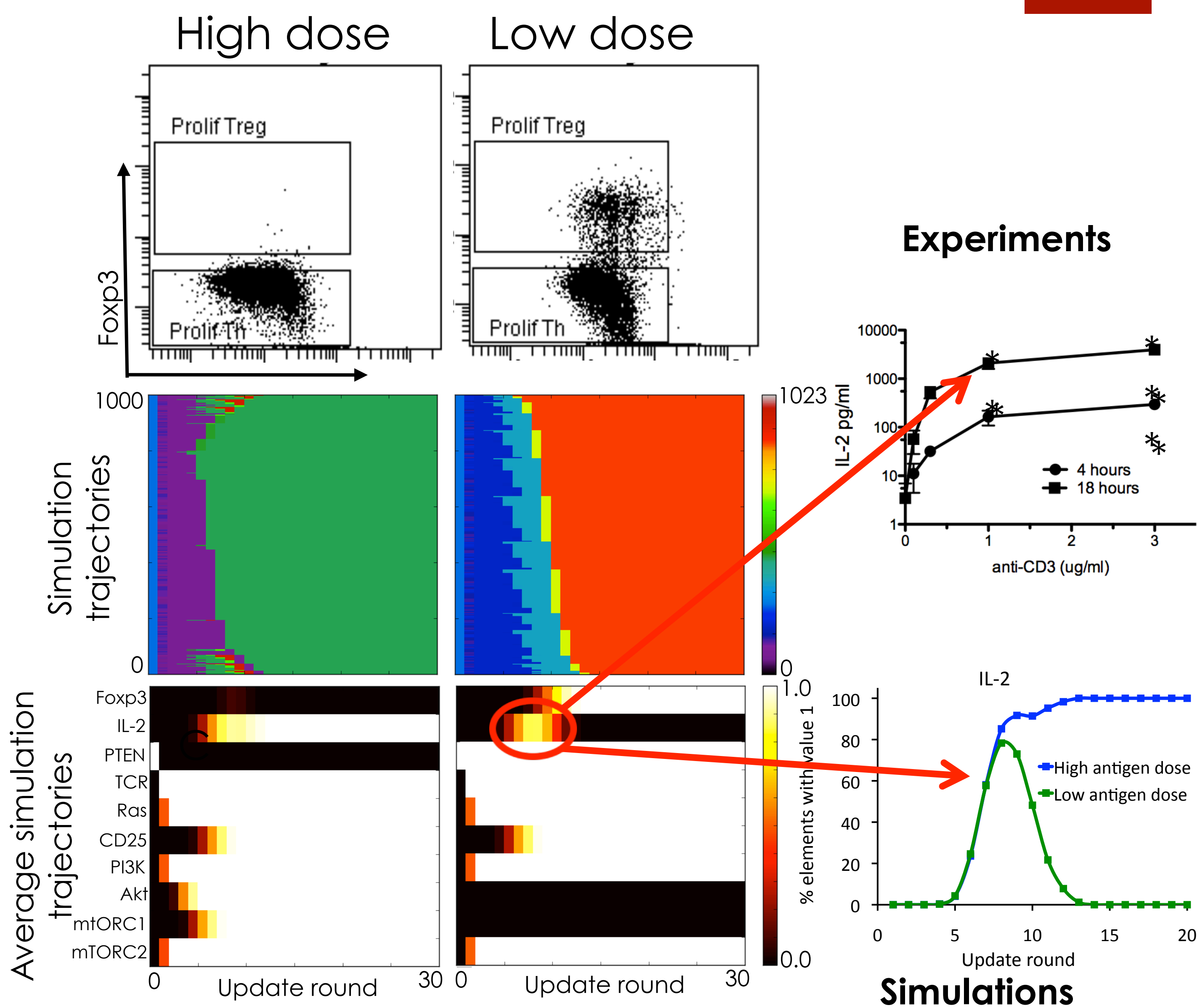


Scenarios 1 and 2: High and Low antigen dose



Source: N. Miskov-Zivanov et al,. in preparation.

Model does not capture low dose scenario

- Model simulations of low antigen dose result in 100% Foxp3+ cells, in experiments no more than 50% Foxp3+ cells

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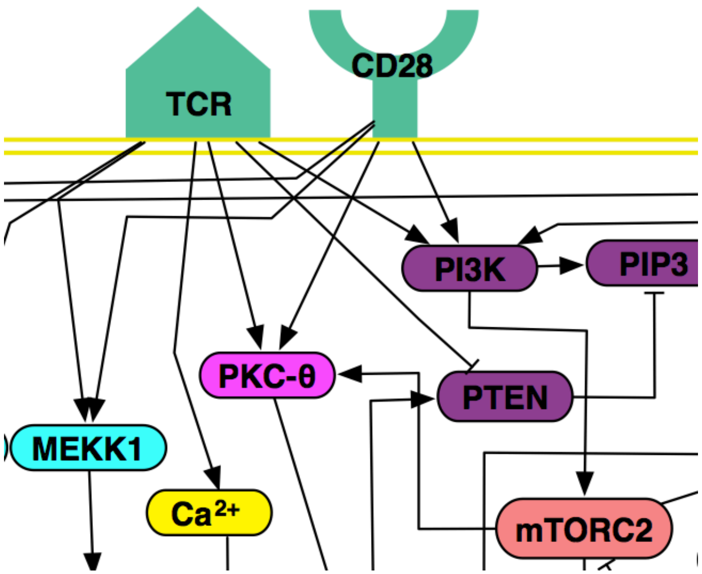

Low dose antigen is modeled as a change in rules:

PKCTHETA* = TCR_HIGH or (TCR_LOW and CD28 and MTORC2)

PI3K_LOW* = (TCR_LOW and CD28) or (PI3K_LOW and IL2_EX and IL2R)

PI3K_HIGH* = (TCR_HIGH and CD28) or (PI3K_HIGH and IL2_EX and IL2R)

PTEN* = (not TCR_HIGH and PTEN) or (not TCR_HIGH and FOXP3)

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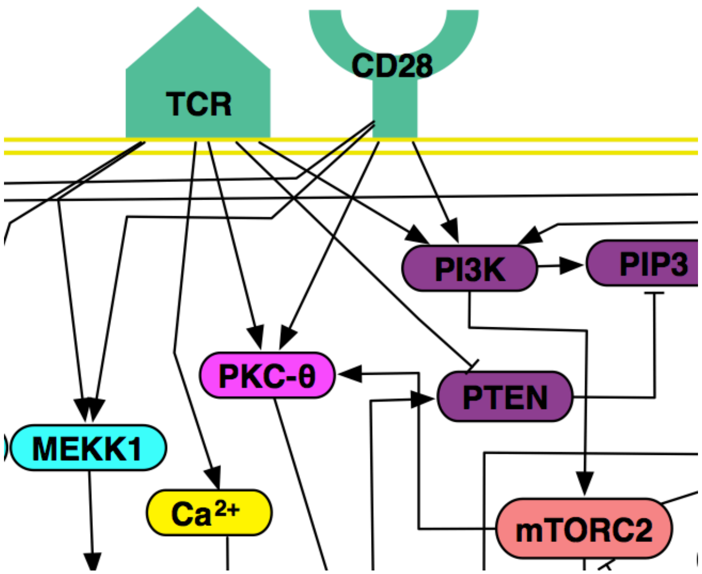

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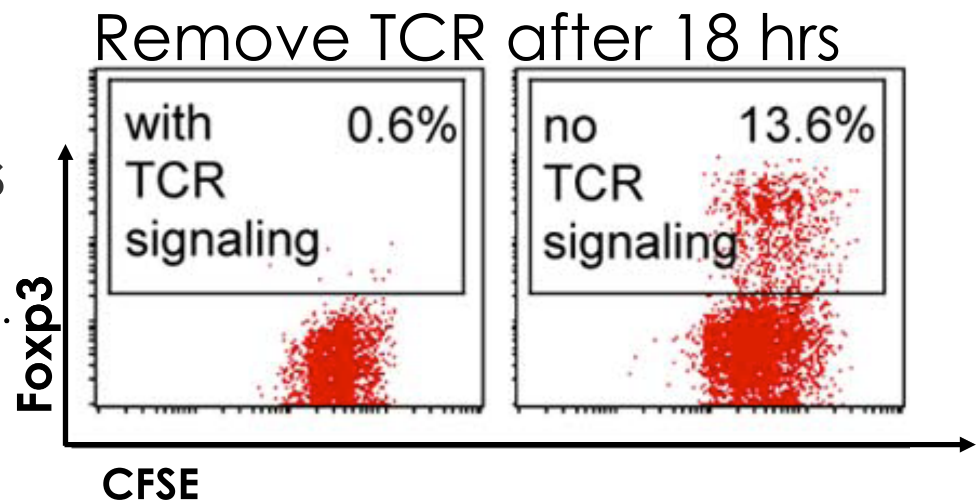
A more dynamic analysis of TCR signal strength necessary: duration of stimulation

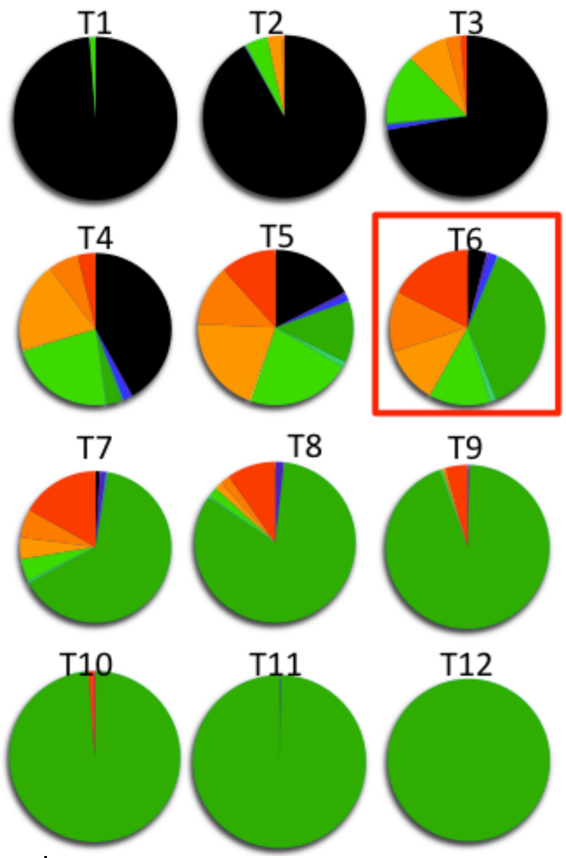
Analysis of duration of stimulation

Experiments

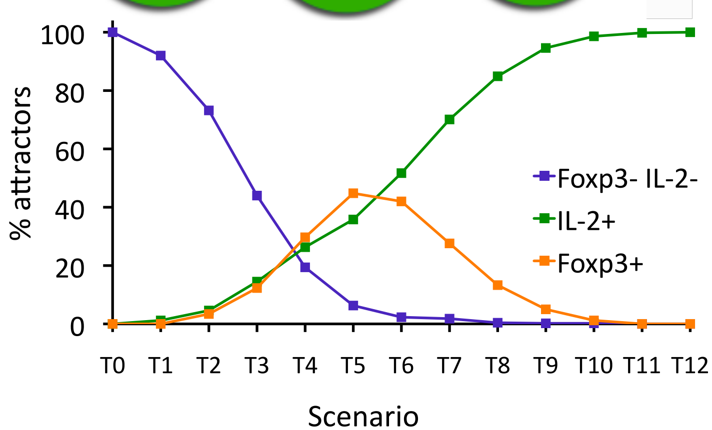
Source: Sauer *et al.*, PNAS 105:7797, 2008.



Scenario 3:
Antigen removal at rounds 1-12 (T1-T12)



Attractors	T6											No removal	
	A1	*A2*	*A3*	*A4*	*A5*	*A6*	*A7*	*A8*	*A9*	*A10*	*A11*	*HD*	*LD*
Foxp3	■	■	■	■	■	■	■					■	
IL-2	■	■	■					■	■	■	■		■
PTEN	■	■			■	■	■					■	
TCR	■	■	■	■	■	■	■	■	■	■	■		
Ras	■		■	■		■	■	■	■				
CD25	■		■	■			■						
PI3K	■		■	■			■		■	■			
Akt	■		■	■			■	■	■	■	■		■
mTORC1	■		■	■			■	■	■	■	■		■
mTORC2	■		■	■			■		■	■			
Attractor size	40	6	17	3	374	13	127	1	118	126	175	1000	1000



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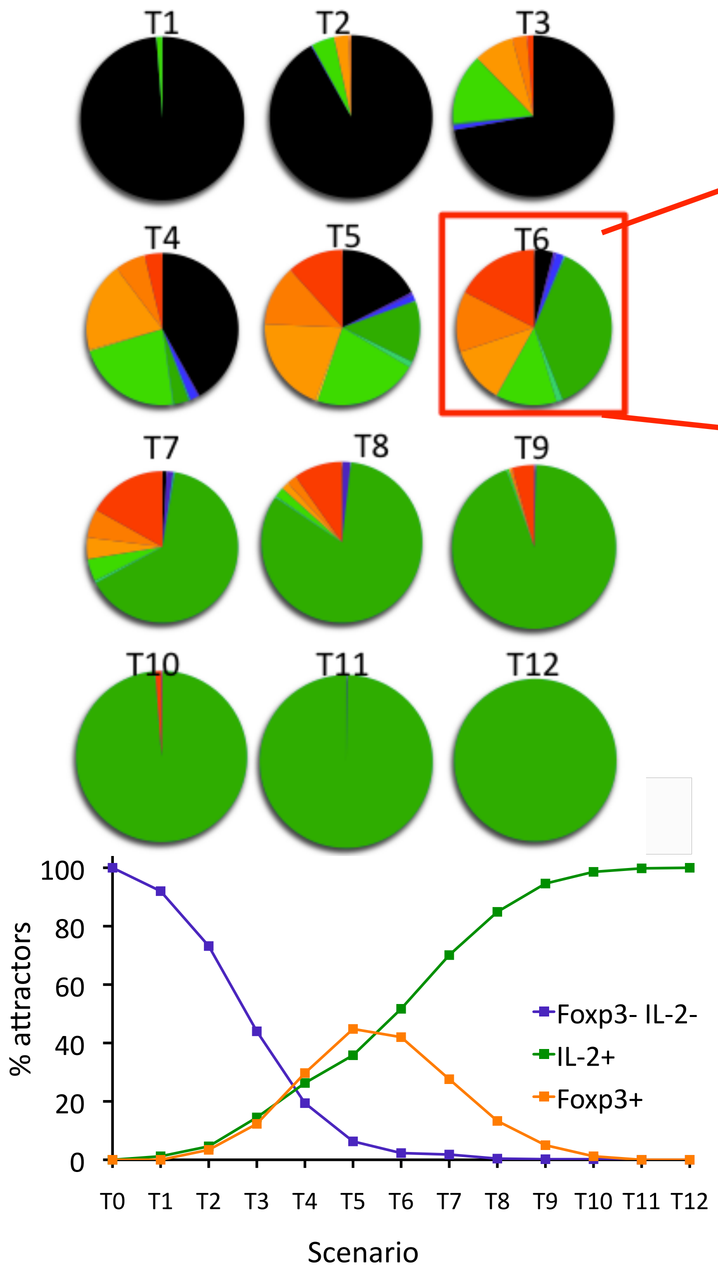
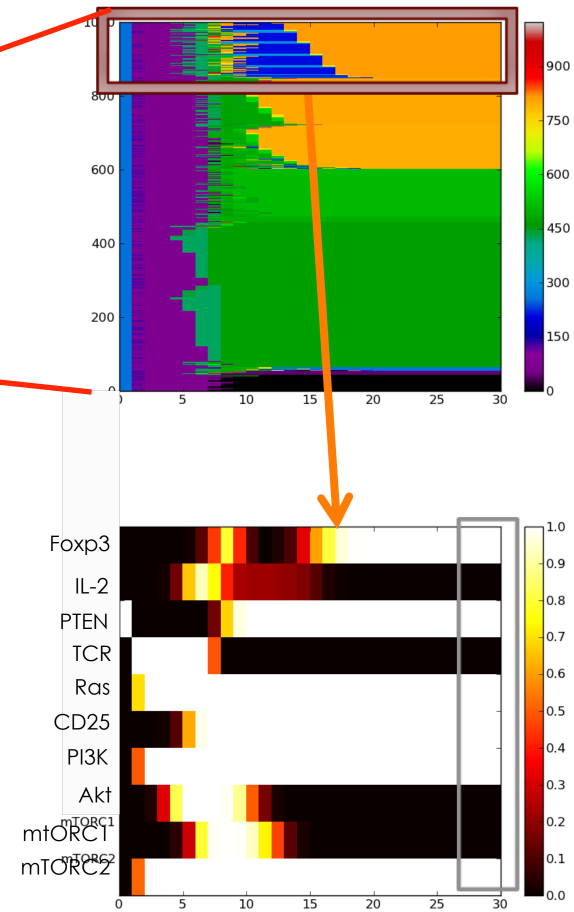




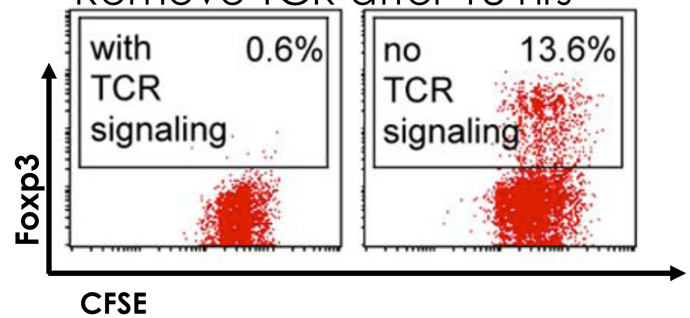
1000 simulation trajectories

Average trajectories for attractor 1010111001

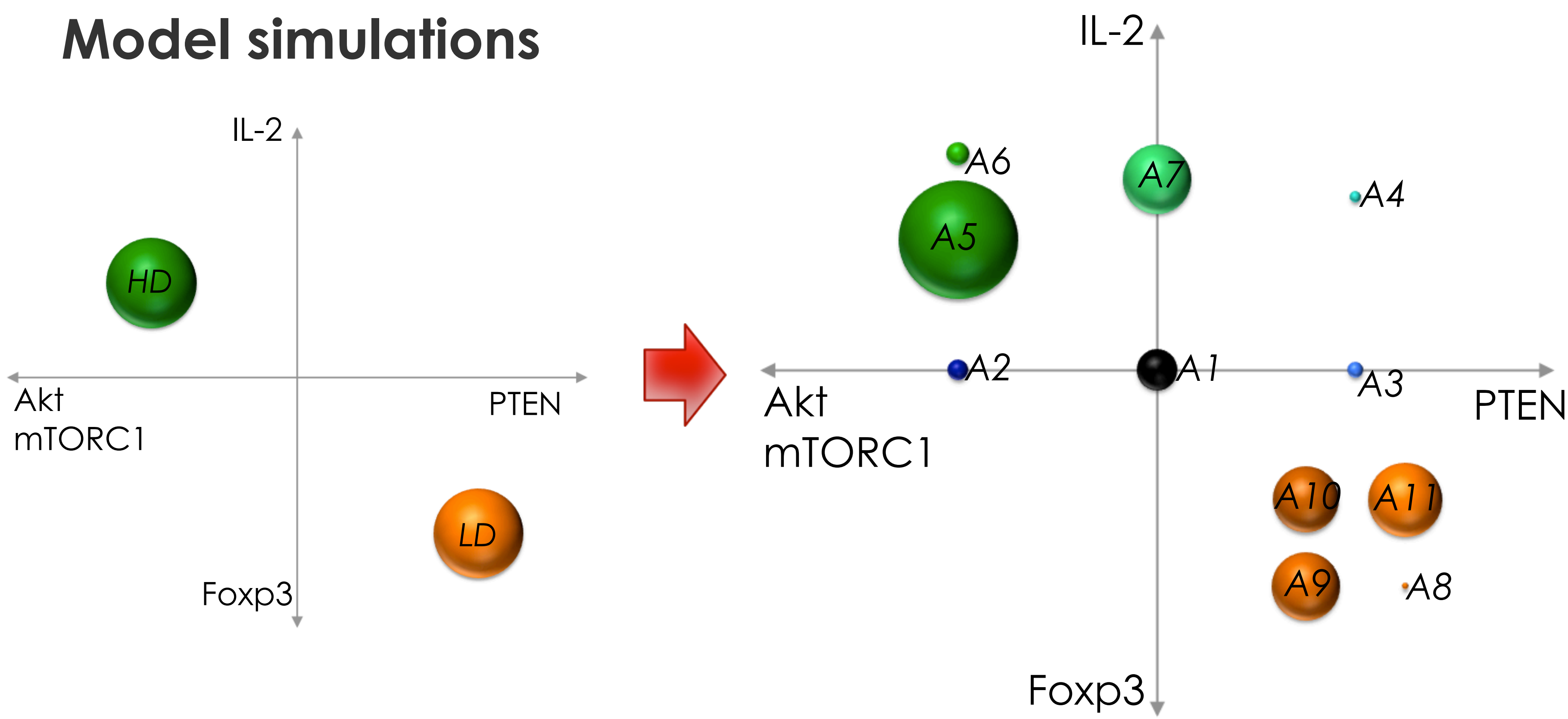
Scenario 3: Antigen removal at round 6 (T6)

Experiments

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



Model simulations



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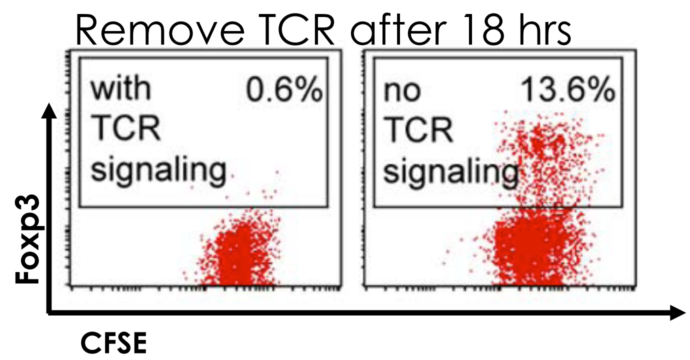
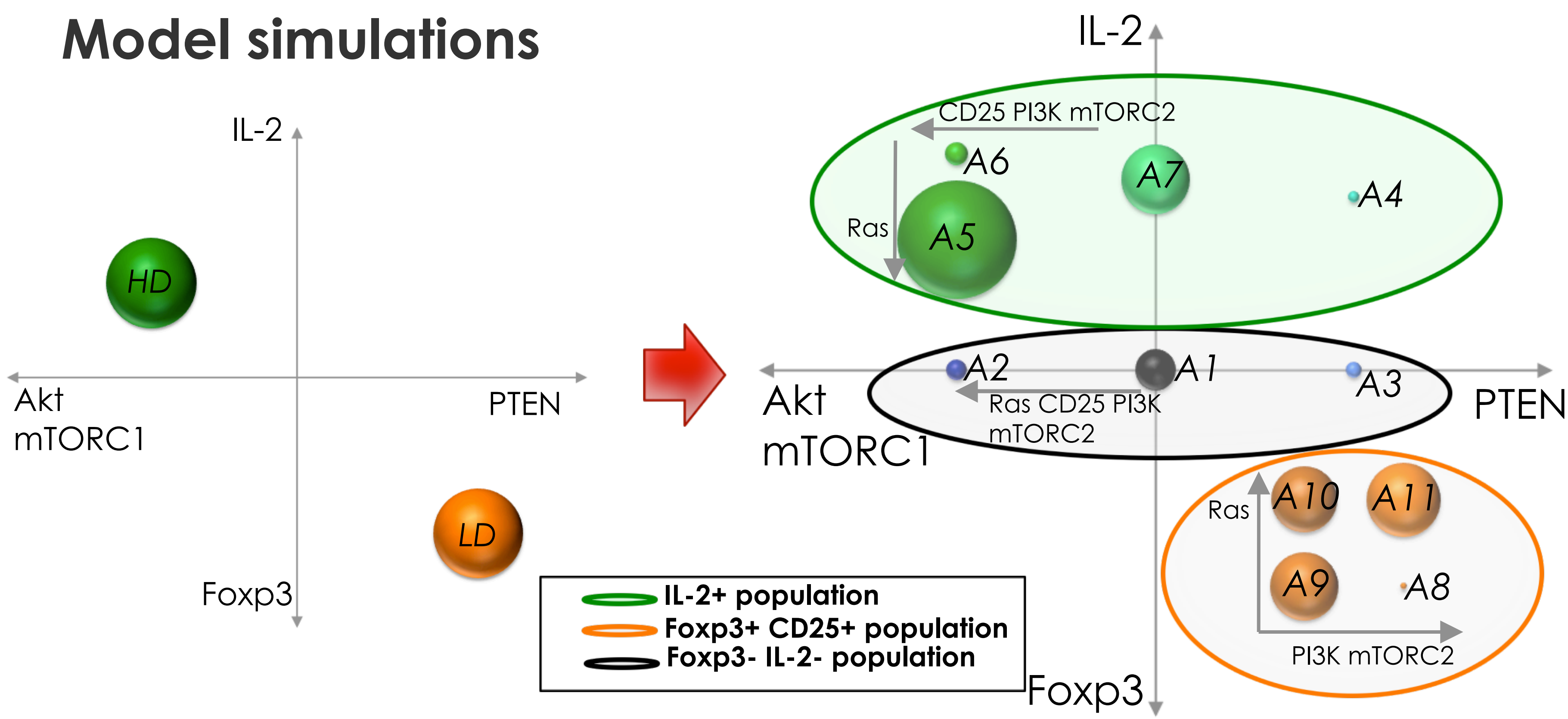
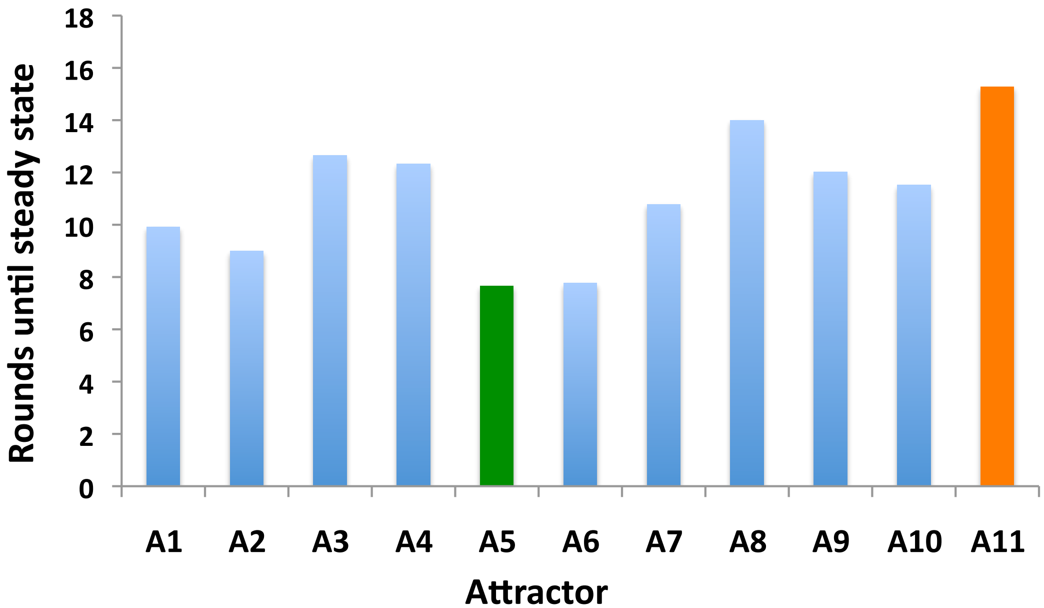


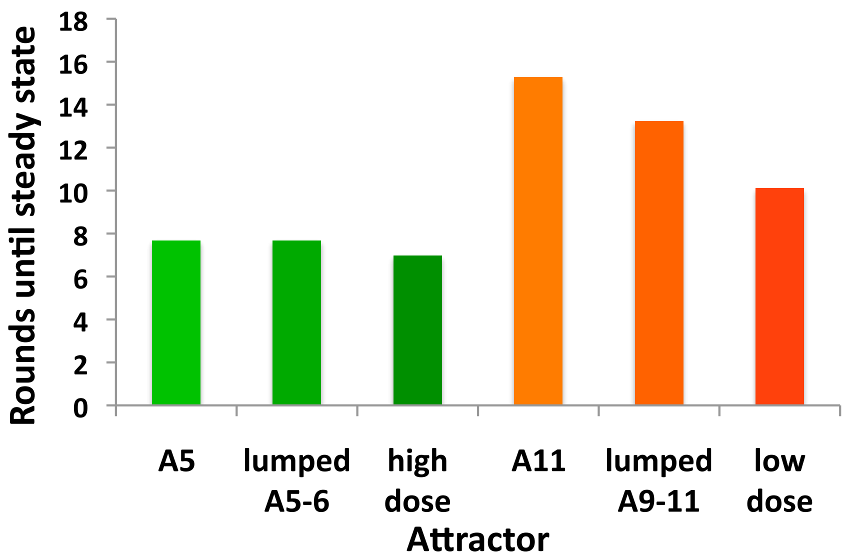
Model simulations

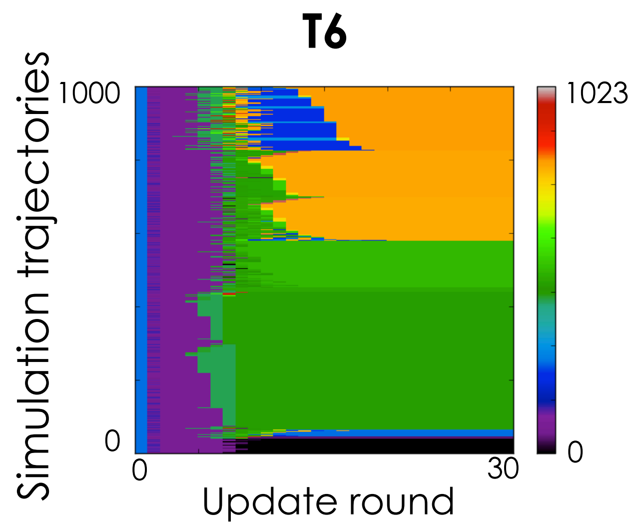


Time to reach steady state (T6, HD, LD)



- Treg cells take longer to differentiate than Th cells





Model checking

- SPIN provides yes/no answers

SPIN					
High dose			**Low dose**		
Specification	**LTL Formula**	**Verified**	**Specification**	**LTL Formula**	**Verified**
Does Foxp3 become false forever?	`!(<>[]!foxp3)`	Yes	Does Foxp3 become true forever?	`!(<>[]foxp3)`	Yes
Does ps6 become true forever?	`!(<>[]ps6)`	Yes	Does pS6 stay false forever?	`!([]!ps6)`	Yes
Does PIP3 become true forever?	`!(<>[]pip3)`	Yes	Does IL-2 become true but eventually become false forever?	`!(<>il2 && <>[] !il2)`	Yes
Does Akt become true forever?	`!(<>[]akt)`	Yes	Is PTEN always true?	`!([]pten)`	Yes
Does mtTORC1 become true forever?	`!(<>[]mtorc1)`	Yes	Does mTORC become true forever?	`!(<>[]mtorc)`	Yes
Does S6K1 become true forever?	`!(<>[]s6k1)`	Yes	Does CD25 become true forever?	`!(<>[]cd25)`	Yes
Does STAT5 become true forever?	`!(<>[]stat5)`	Yes	Does STAT5 become true forever?	`!(<>[]stat5)`	Yes
Does CD25 become true forever?	`!(<>[]cd25)`	Yes	**High dose + TGFβ**		
Does IL-2 become true forever?	`!(<>[]il2)`	Yes	Does IL-2 become true forever?	`!(<>[]il2)`	Yes
Does PTEN become true forever?	`!(<>[]pten)`	Yes	Does PTEN become false forever?	`!(<>[]!pten)`	Yes

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Probabilistic model checking

- PRISM allows for analyzing transient behavior

PRISM					
High dose			**Low dose**		
Specification	**LTL Formula**	**Prob.**	**Specification**	**LTL Formula**	**Prob.**
Does Foxp3 eventually become false forever?	`P=?[FG!foxp3] ]`	1	Does pS6 stay true?	`P=? [G ps6]`	0
What is the probability that Foxp3 never becomes true?	`P=?[G!foxp3]`	0.765098	Does mTORC2 ever get to true?	`P=?[F mtorc2]`	1
Does ps6 become true forever?	`P=? [F G ps6] ]`	1	Does PTEN ever get to false?	`P=? [ F !pten ]`	0
Are IL-2 and PTEN ever both true?	`P=?[F((pten)&(il2))]`	0.000468	**High dose + TGFβ**		
What is the probability that IL-2 is false before Foxp3 becomes true	`P=?[(!il2)U(foxp3)]`	0	Does IL-2 become true?	`P=? [ F il2 ]`	0.019584
PRISM – behavior over time					
What is the behavior of Foxp3 over time?	`P=? [G[t,t] foxp3]`	Figure	What is the behavior of IL-2 over time?	`P=? [G[t,t] il2]`	Figure

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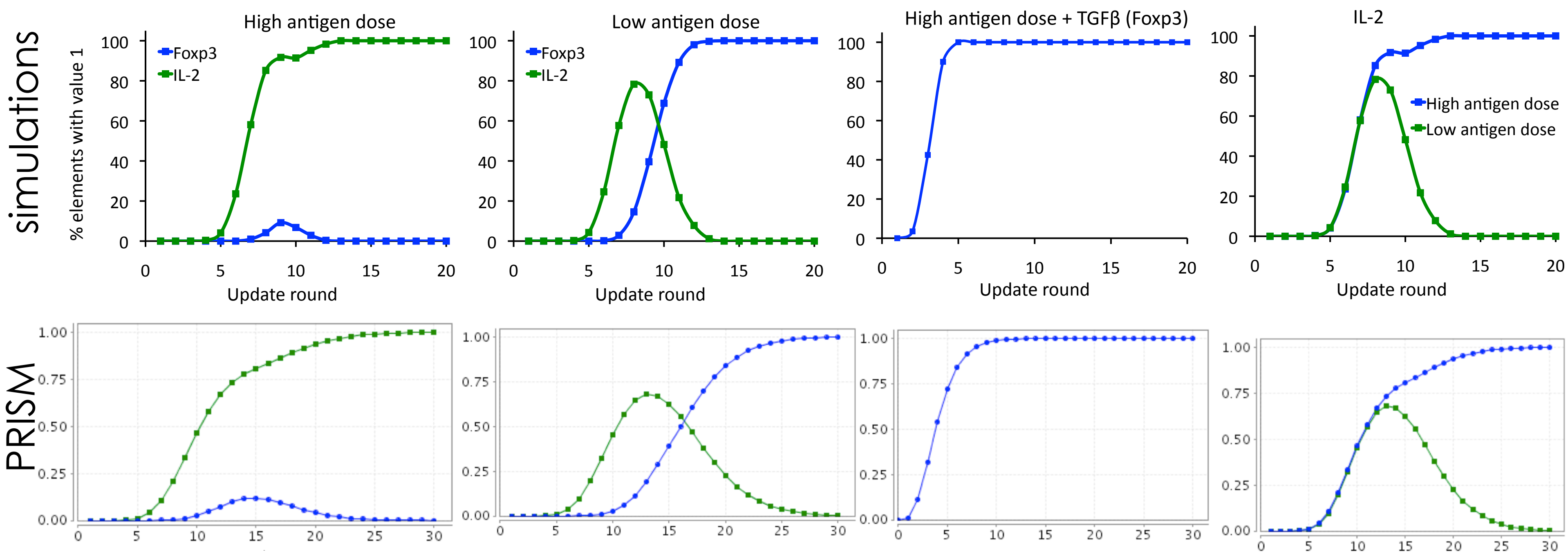
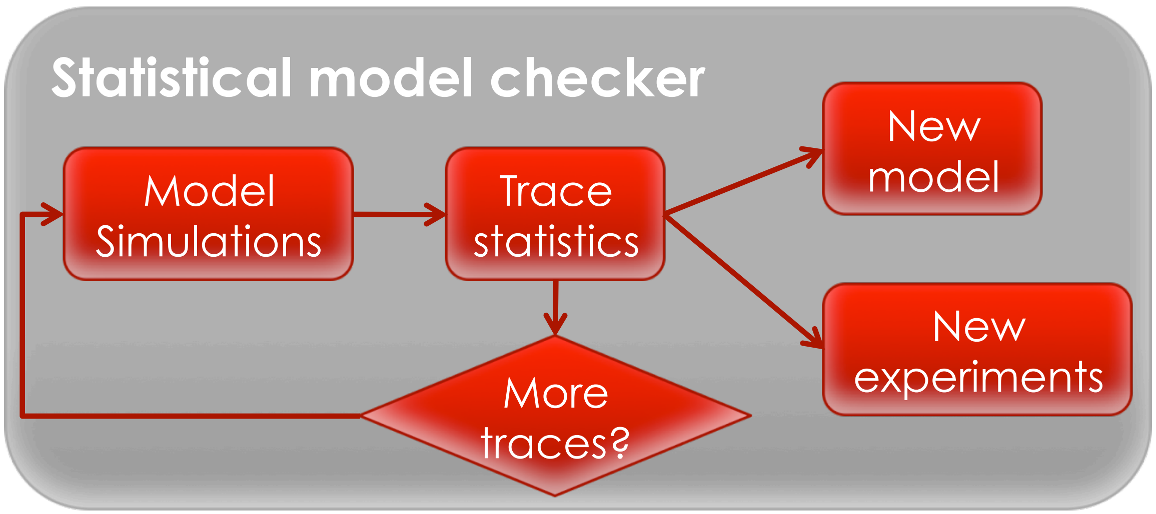
Probabilistic model checking

- PRISM allows for analyzing transient behavior



Statistical model checking



Statistical model checking

- Low antigen dose scenario:

  1. Does IL-2 always go to 1?

  Property: F[20] (IL2 == 1)

  Test: BEST 0.001 0.999

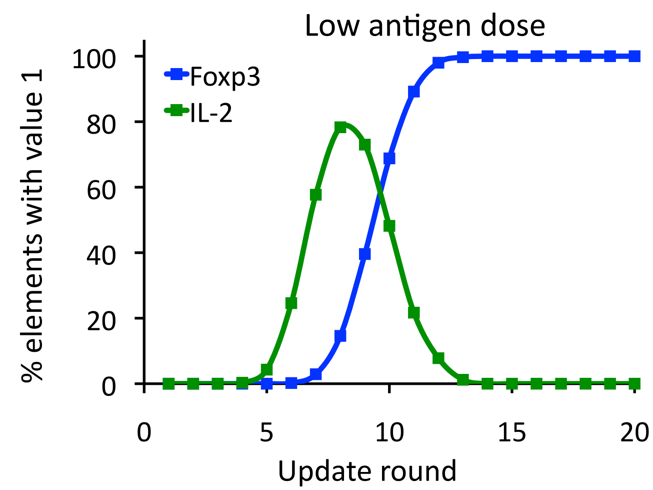
  Result: estimated probability close to 1

  2. Probability that IL-2 stays at 0 before Foxp3 becomes 1?

  Property: (IL2 == 0) U[15] (FOXP3 == 1)

  Test: BEST 0.0001 0.999

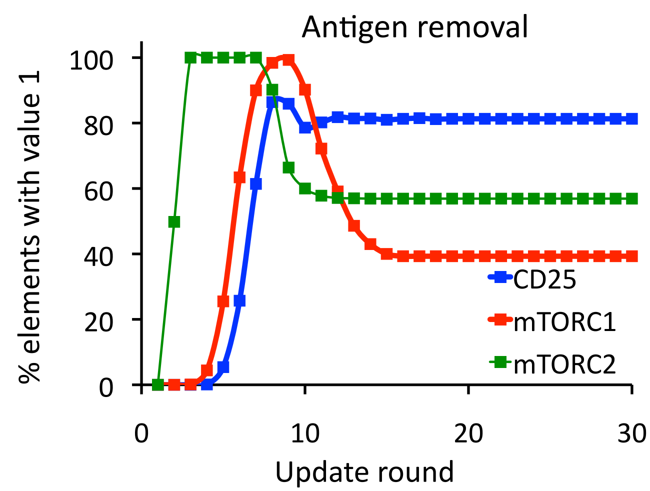
  Result: estimated probability = 0.00147
  rare event



Statistical model checking

- High antigen dose + antigen removal scenario:
  - Studies of relative timing on mTOR vs. CD25/ STAT5 pathway

	Property	Probability estimate and sample size	Elapsed time [s]
1	G^{7} ~(MTORC1 = 1 & MTORC2 = 1)	estimate = 0.0188048 samples = 200,160	1,946
2	F^{7} (MTORC1 = 1 & MTORC2 = 1)	estimate = 0.980884 samples = 2,352	23
3	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{18} (CD25 = 1)))	estimate = 0.60104 samples = 25,968	253
4	F^{28} (MTORC1 == 1 & MTORC2 == 1 & CD25 == 0 & (F^{1} (CD25 == 1)))	estimate = 0.592195 samples = 26,160	254
5	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{1} (G^{17} (CD25 = 1))))	estimate = 0.39669 samples = 25,920	254



Paolo Zuliani

Conclusion

- **Logical modeling approach allows development of comprehensive models of cell fate**
  - Model of peripheral T cell differentiation recapitulates a wide range of experimental observations
  - Circuit analysis reveals key elements of the mechanism for Foxp3 expression
  - Timing is critical for Treg differentiation:
    - Treg cells take longer to differentiate than Th cells
    - Race between Foxp3 activating and inhibiting pathways
    - Feedback between Foxp3 and PTEN

Conclusion

- **Model checking**
  - Allows for more efficient studies of the model
  - Probabilistic model checking:
    - Provides transient results that match simulations
  - Statistical model checking:
    - Further analysis of of transient behavior
    - Provides insights into timing relationships between elements

Next steps

- Analyze different removal scenarios using model checking
- Expansion of the model (keep up with fast pace of developments in the field)
- Develop a model for several cell types
- Develop population models that embed intracellular circuitry

Thank you!