Model checking for studying timing in T cell differentiation

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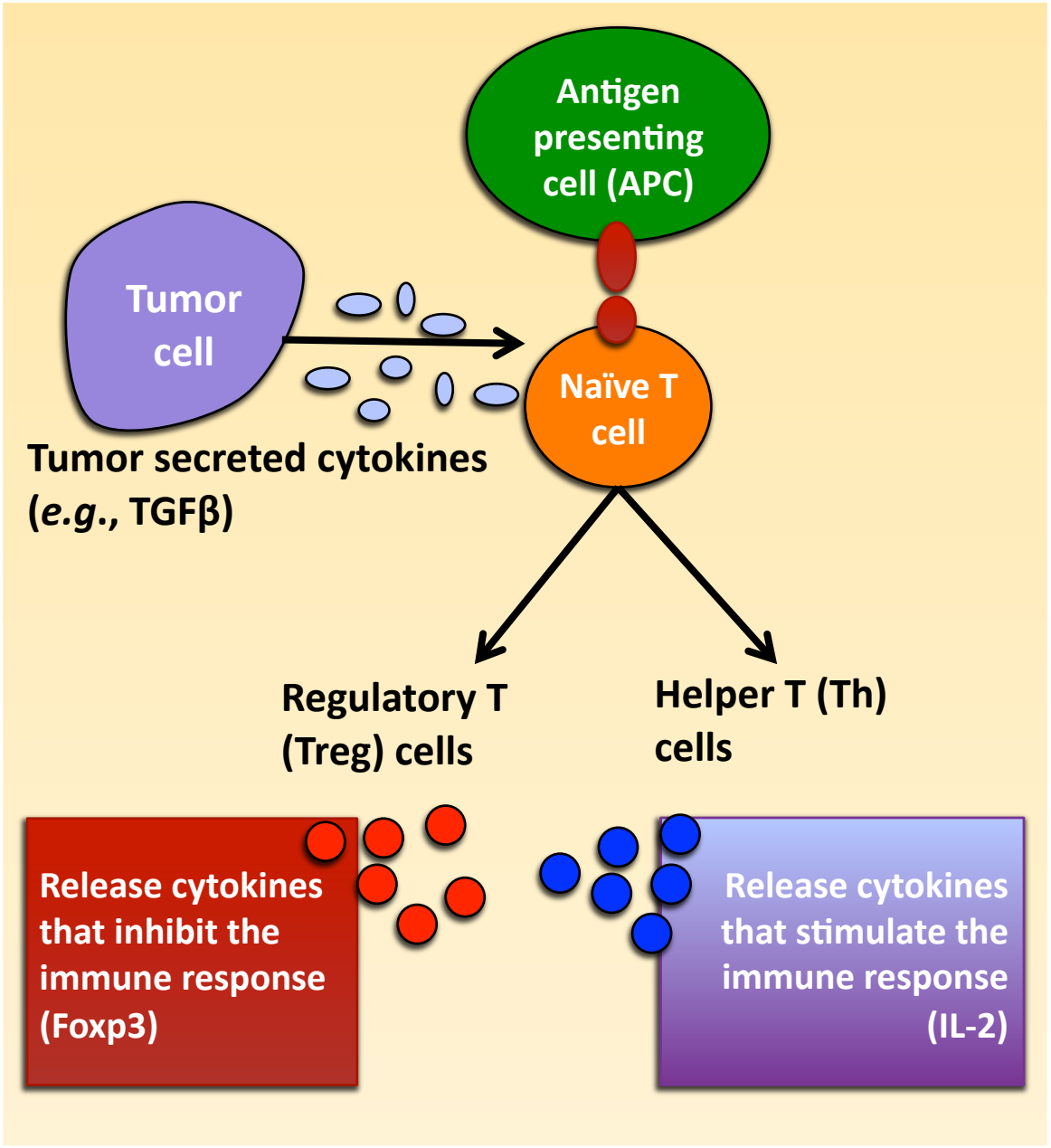
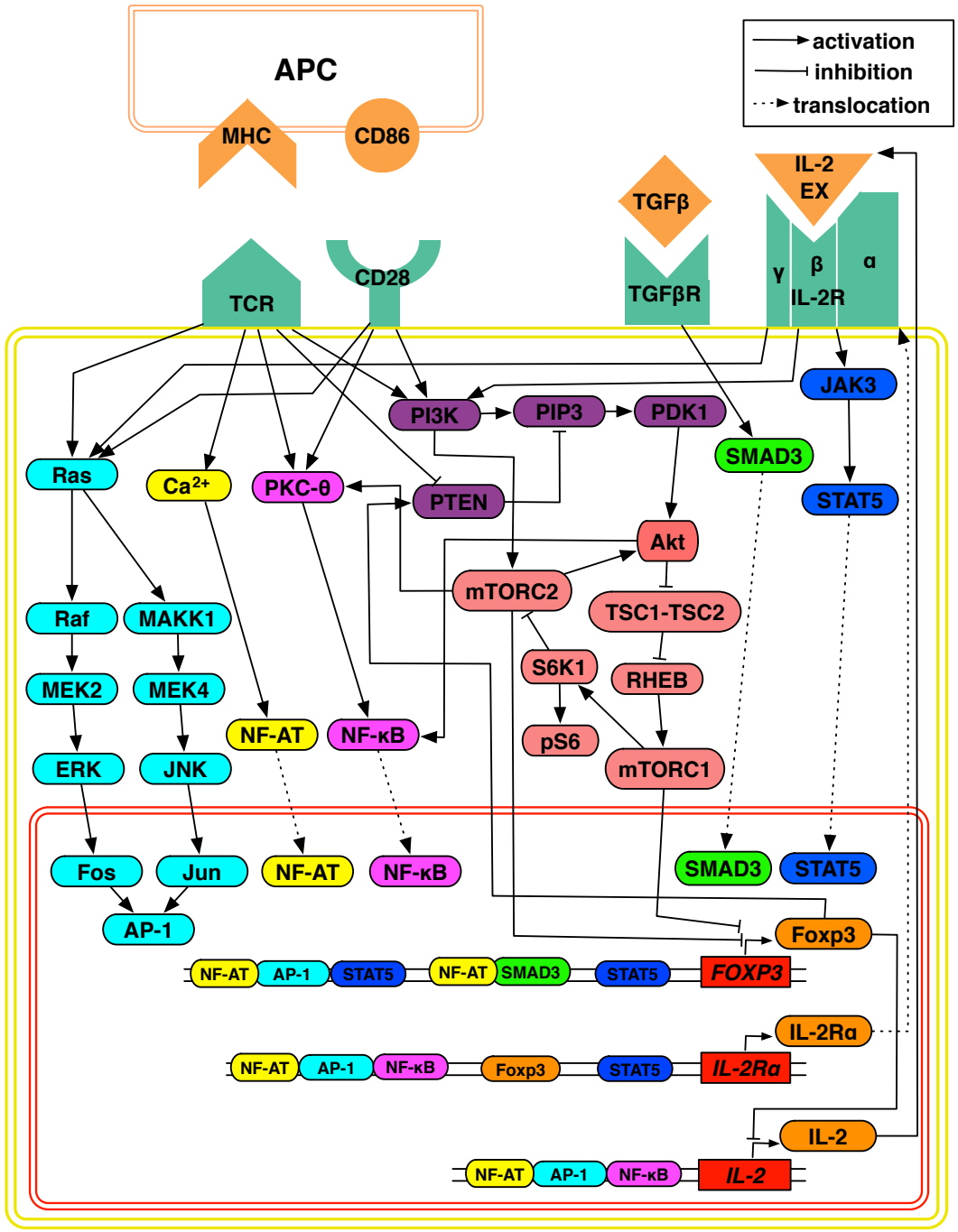
T cell differentiation



- T cell subpopulation ratios are critical for numerous immune and auto-immune pathologies
- Modeling goals:
  - Determine whether known mechanisms are sufficient to explain experimental observations
  - Find signaling cascades in T cells critical for cell fate decision
  - Suggest additional experiments to identify missing mechanisms
  - 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

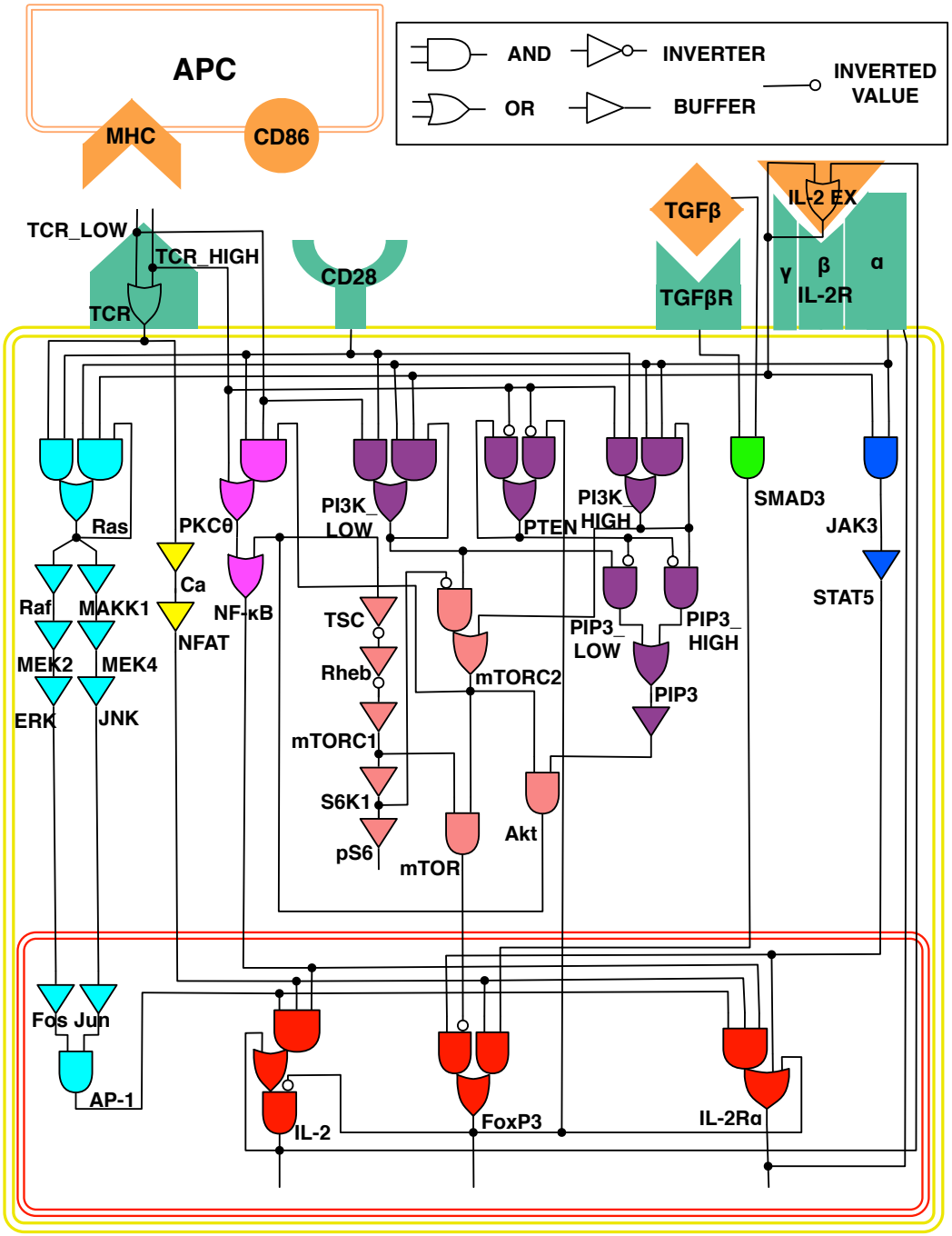
Other important elements:

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

Miskov-Zivanov et al., *Science Signaling,* 2013.

Modeling approach

- **States of elements** in the signaling network are described using a discrete variables:
  - Element inactive or absent (value 0)
  - Element active or present (values 1, 2,… for different levels of activity)
- **Interactions between elements**:
  - Described with logic functions
  - Next state is computed from the states of its regulators

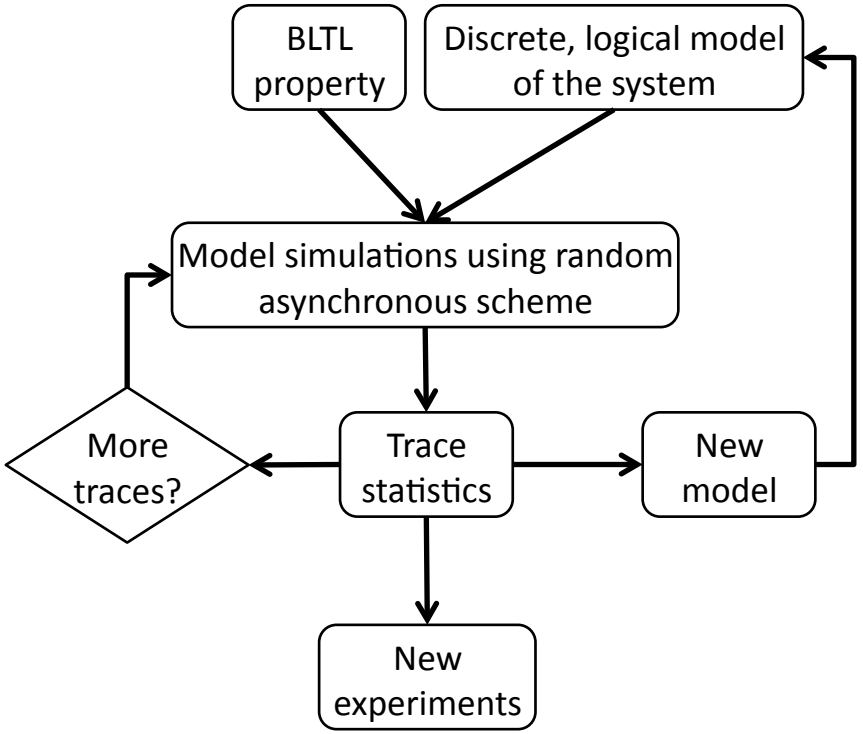


Circuit model

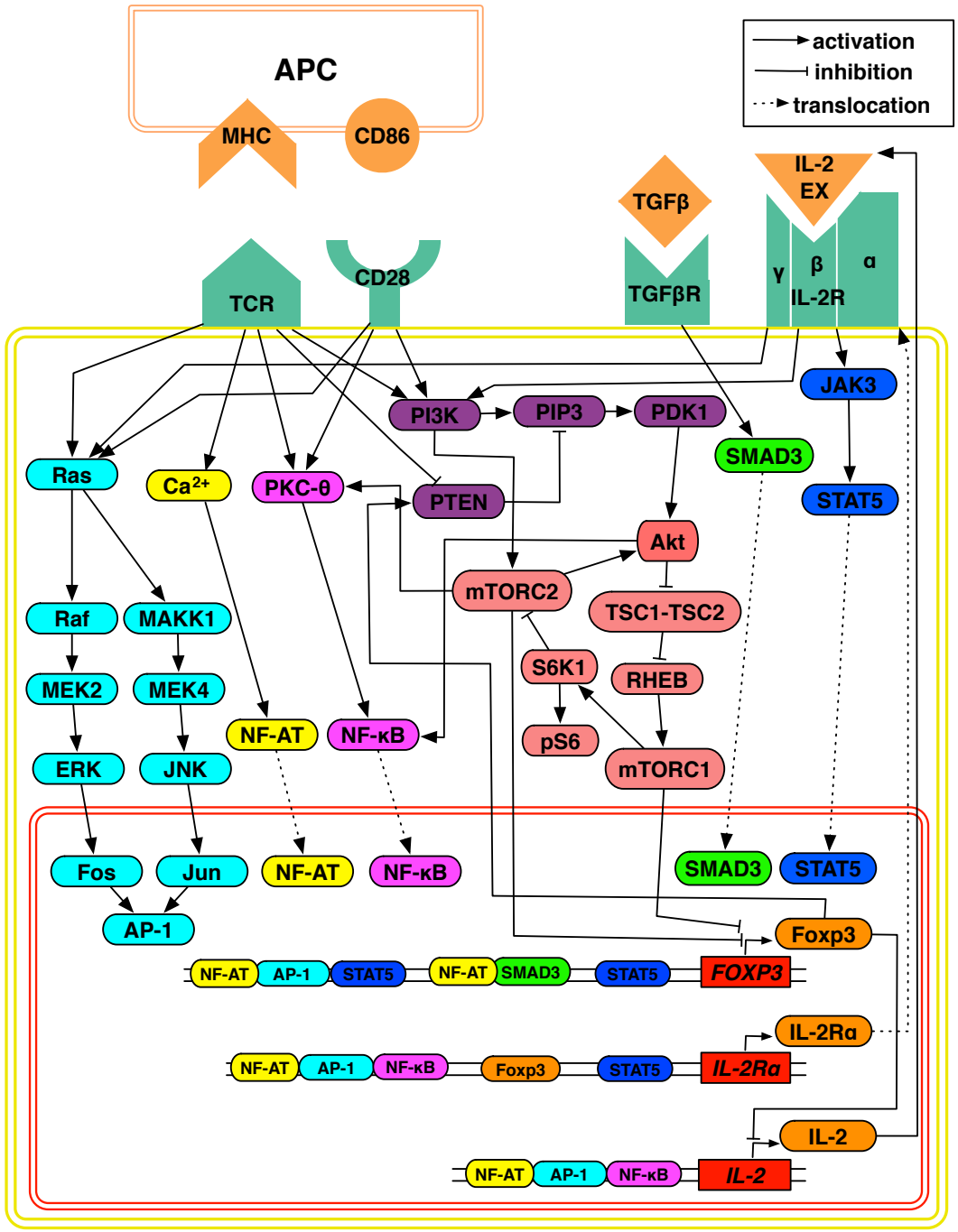
- Discrete, logical model
- Simulated using Random Order Asynchronous approach
  - Variables updated one at a time in random order
  - Stochastic
- BooleanNet tool used for simulations (http://code.google.com/p/booleannet/)

Miskov-Zivanov et al., *Science Signaling,* 2013.

Analysis framework with model checking



- Combine BooleanNet simulation tool with a parallel statistical model checker
  - Verification of BLTL properties performed efficiently and automatically on a multi-core system (32 cores)
- Statistical model checking treats the verification problem for stochastic systems as a statistical inference problem
  - Uses randomized sampling to generate traces (or simulations) from the system model
  - Uses model checking methods and statistical analysis on those traces

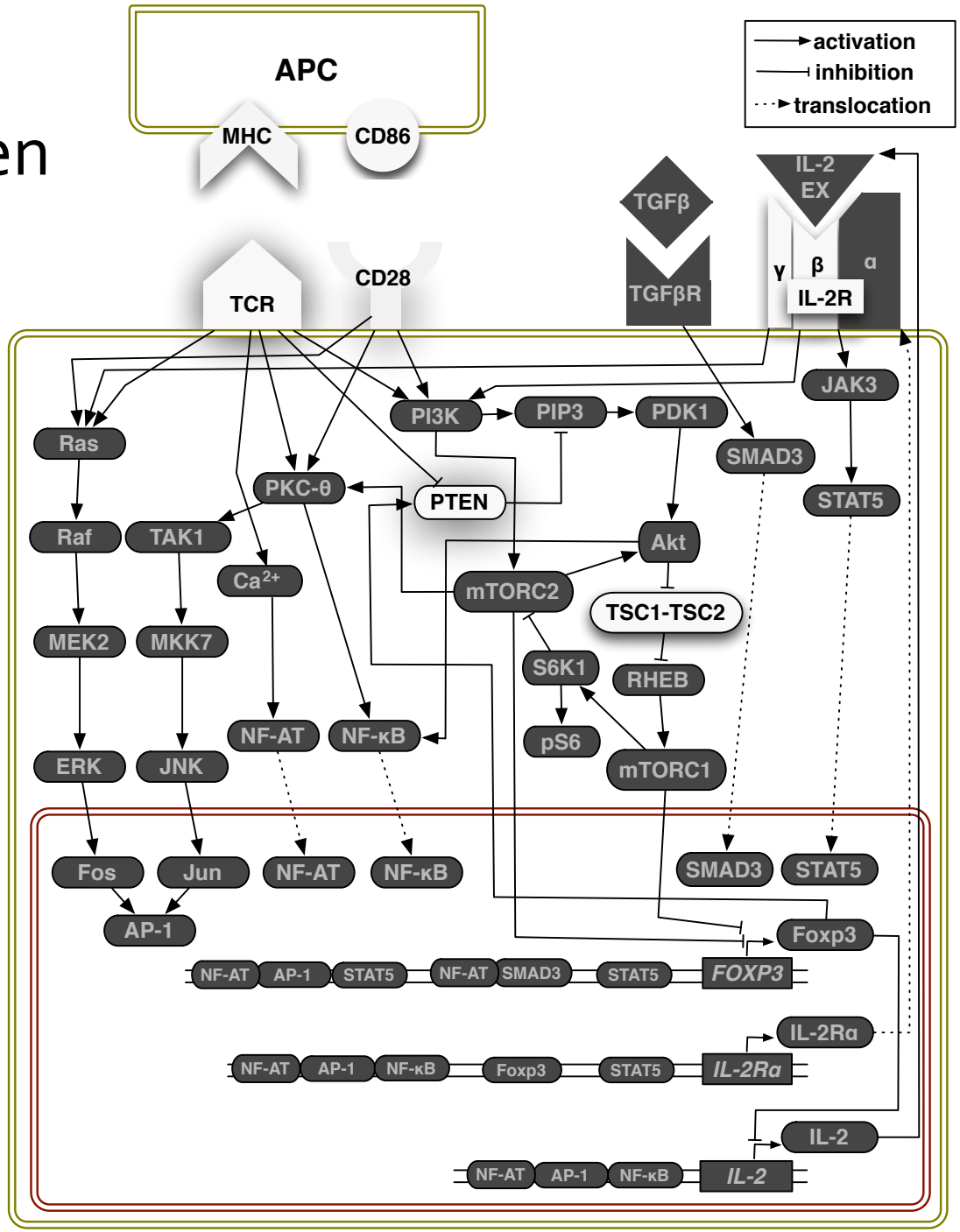


Scenarios

1. High antigen dose
2. Low antigen dose
3. High antigen dose, then removed
4. High antigen dose and TGFβ

Scenario 1: High antigen dose

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



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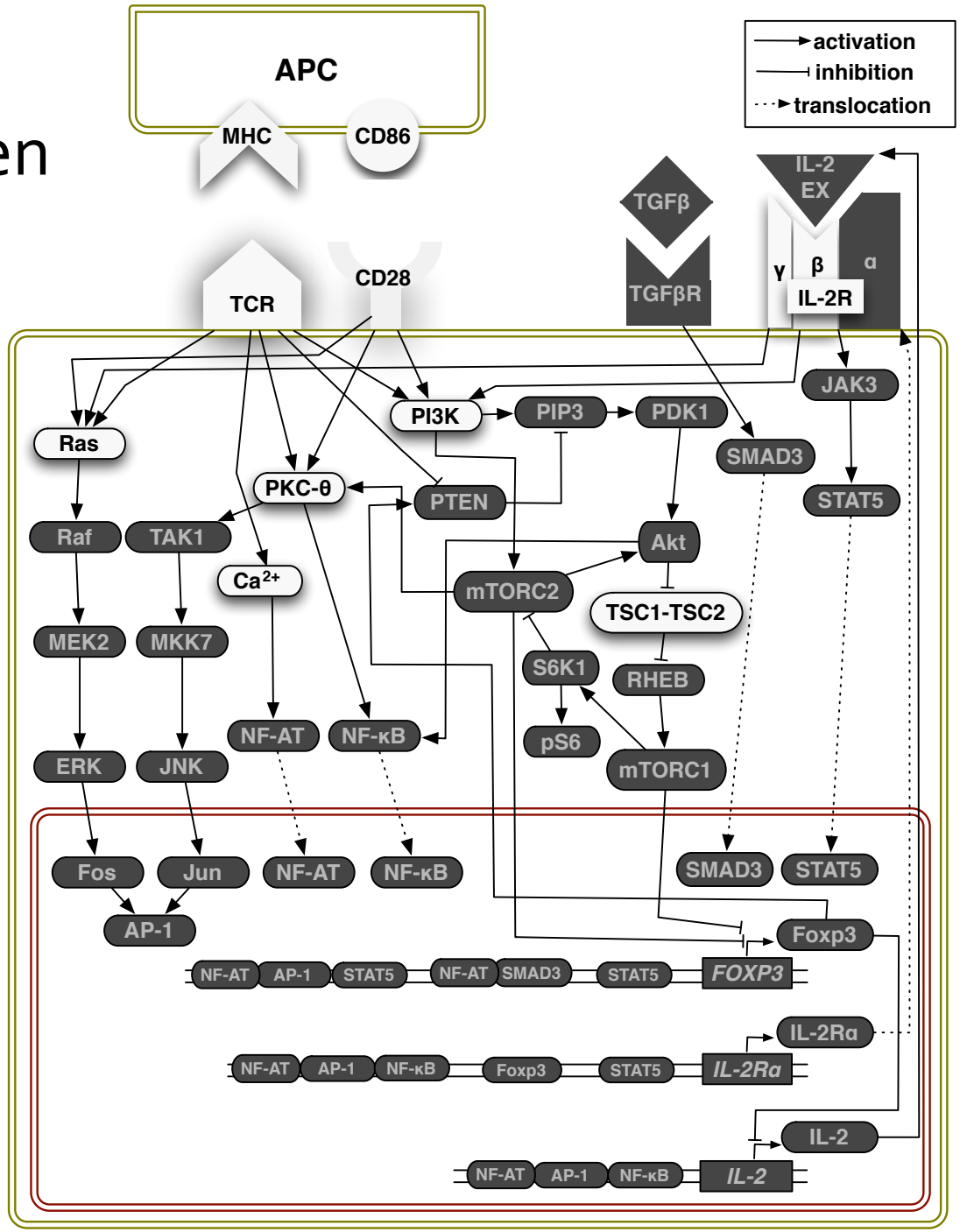

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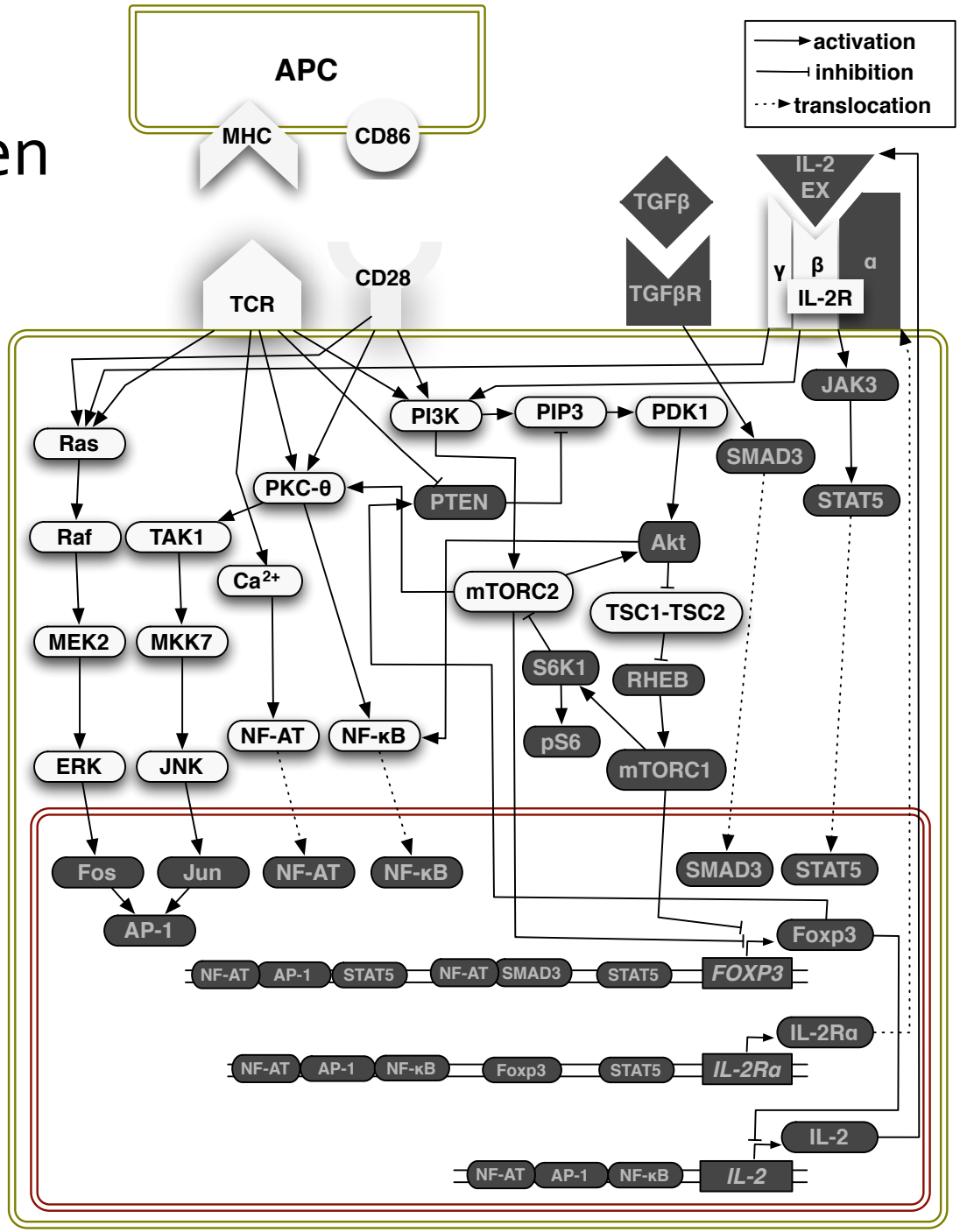

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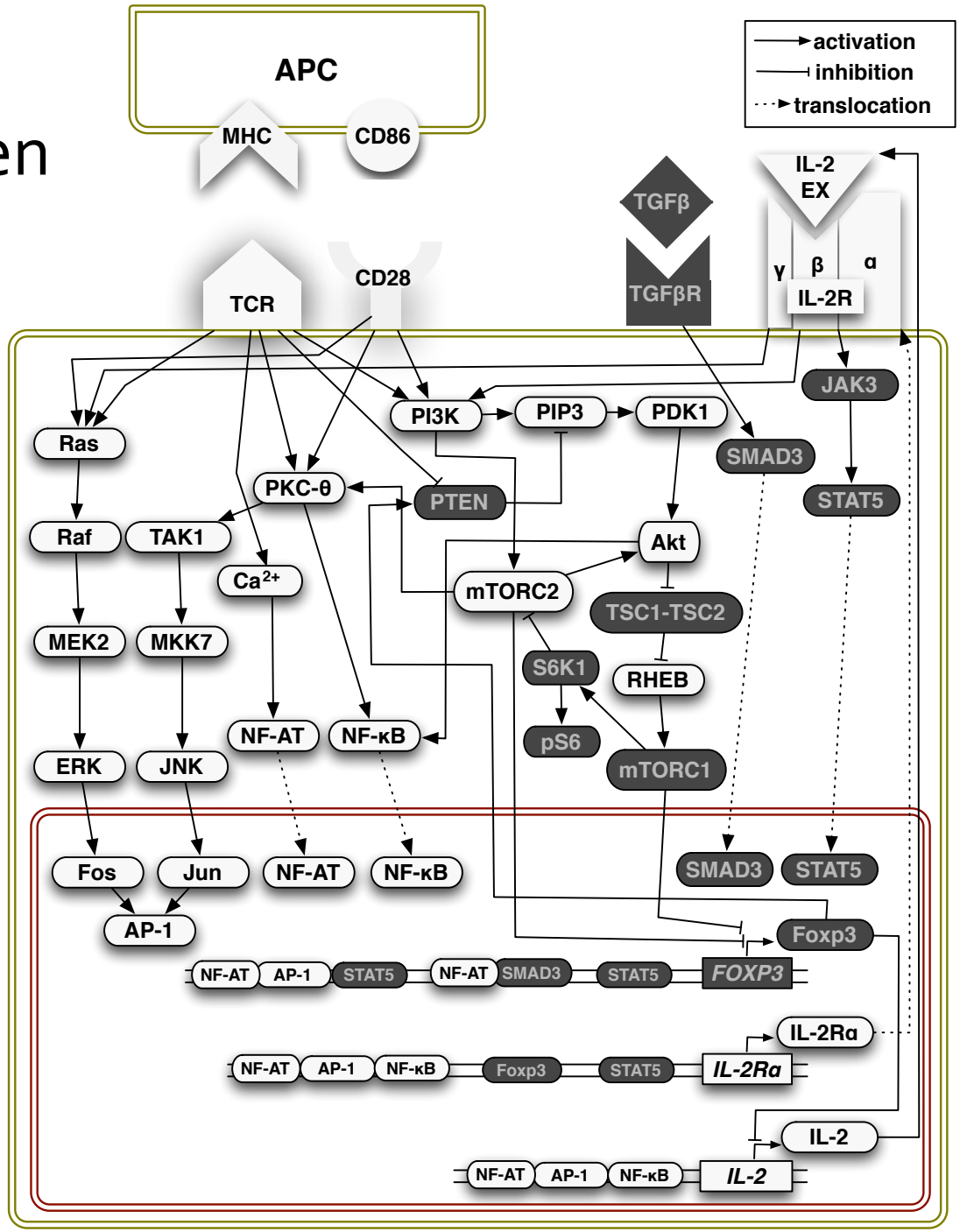

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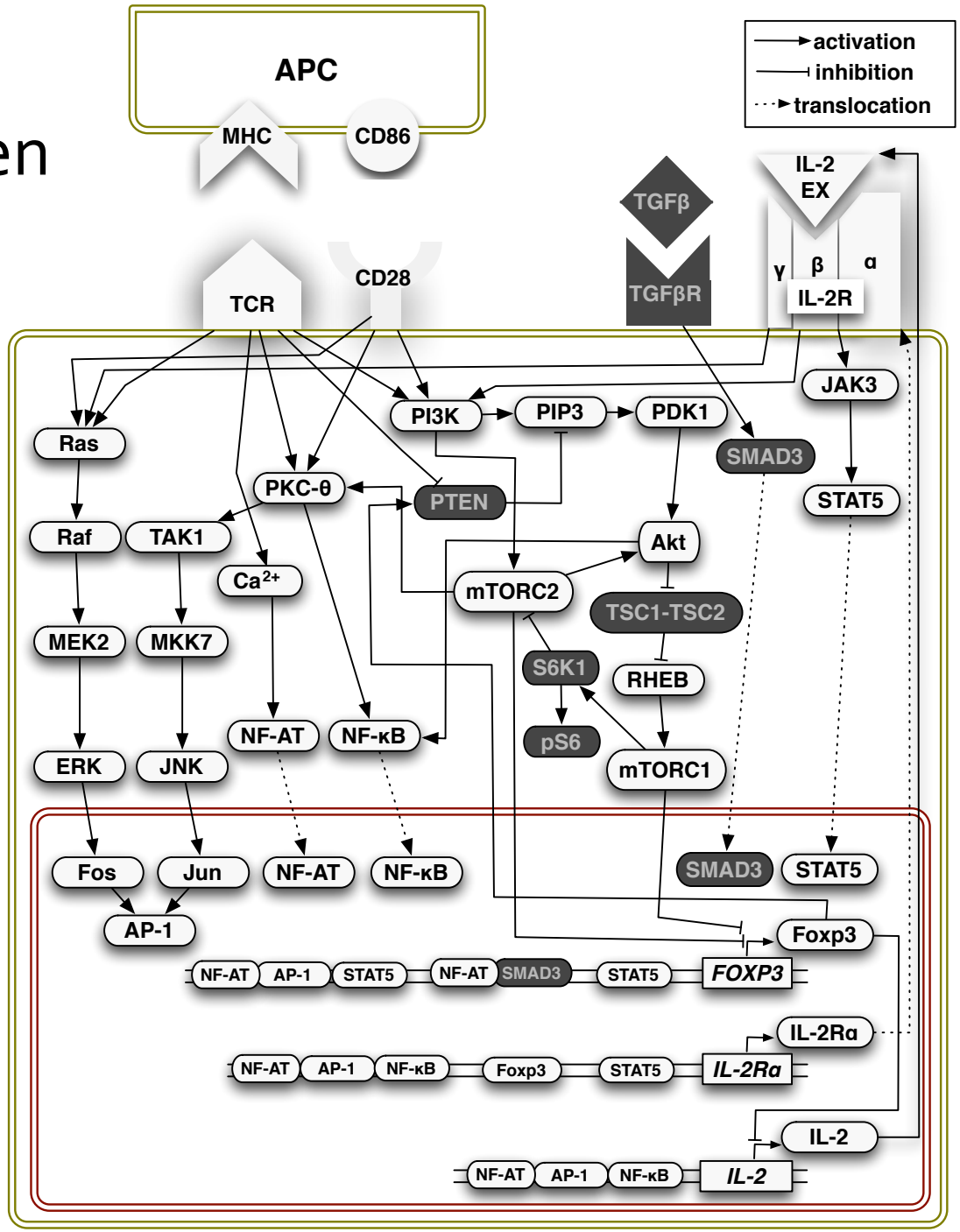

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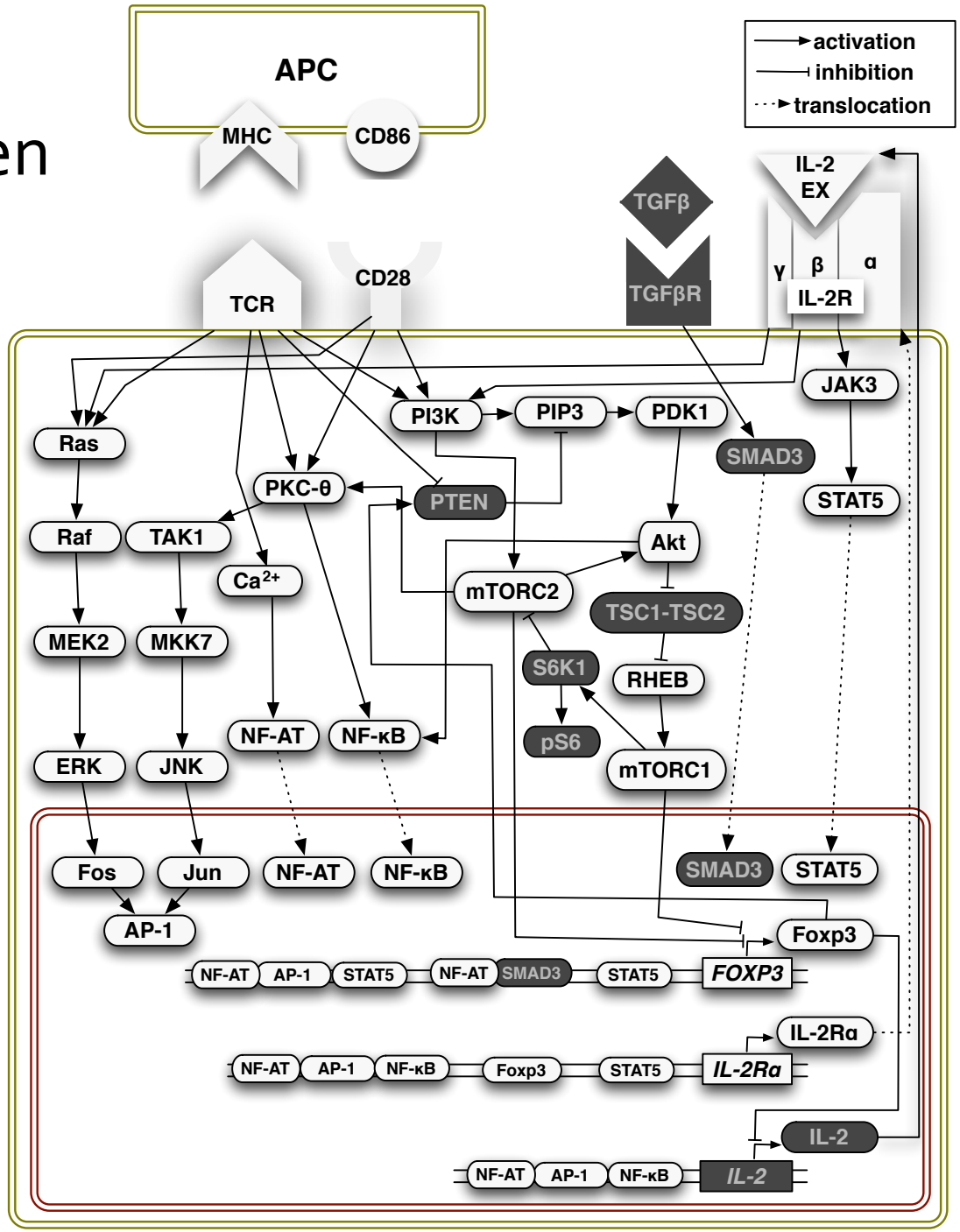

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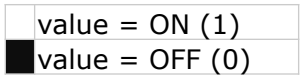
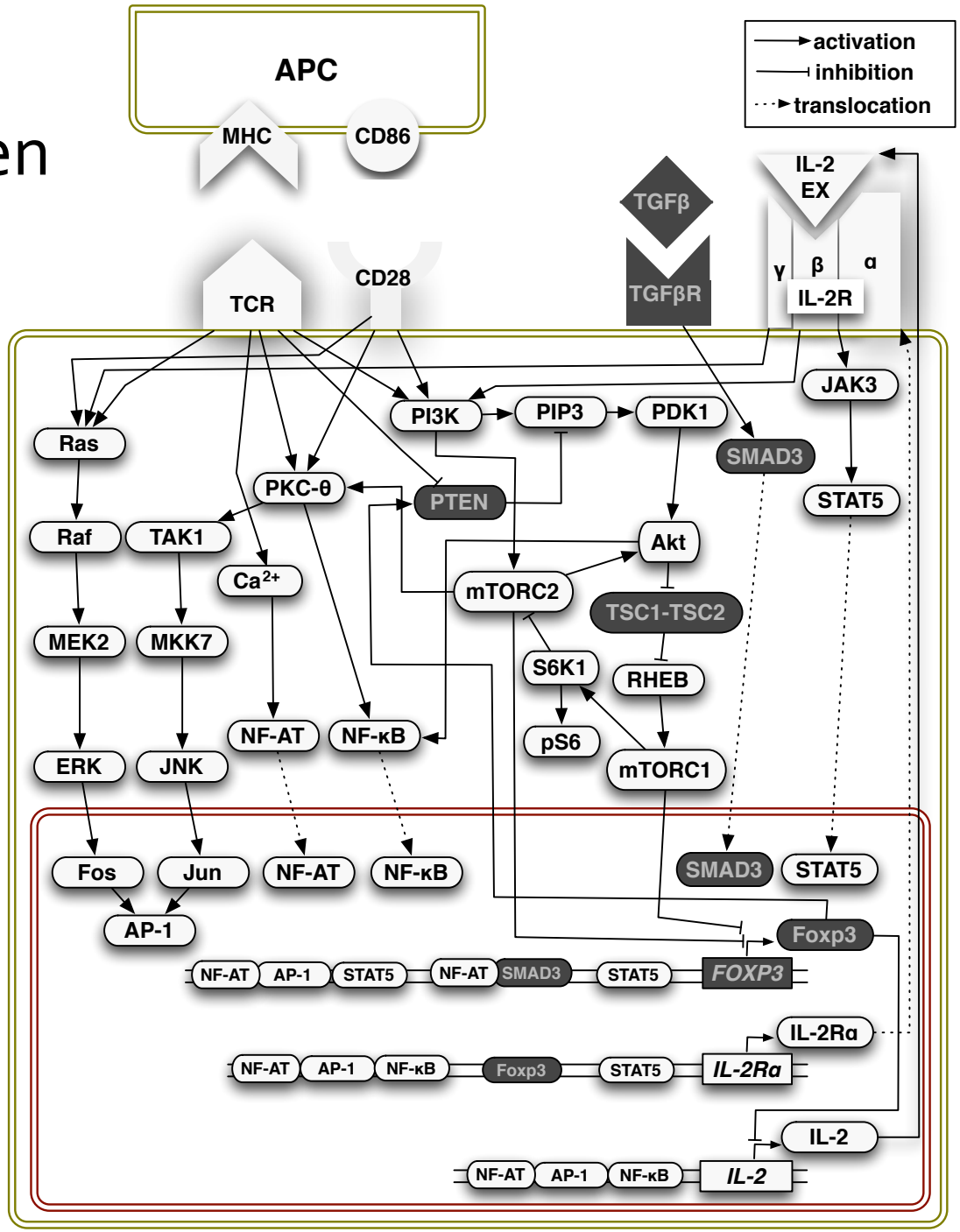
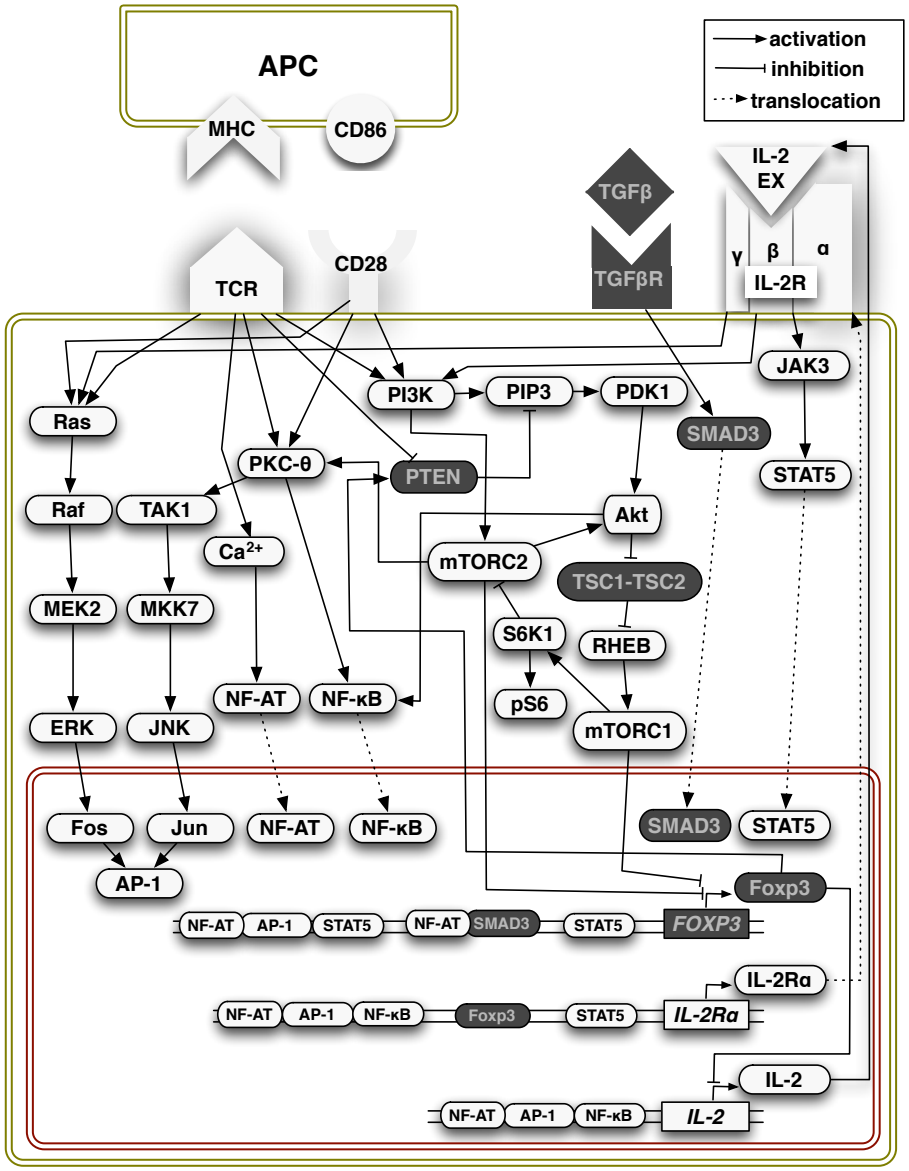
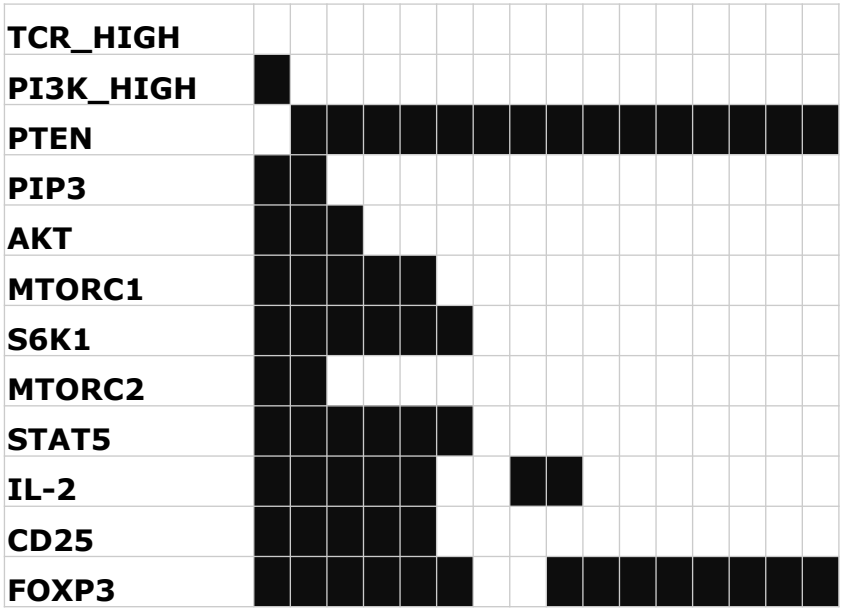




Scenario 1: High antigen dose trajectory



Trajectory example



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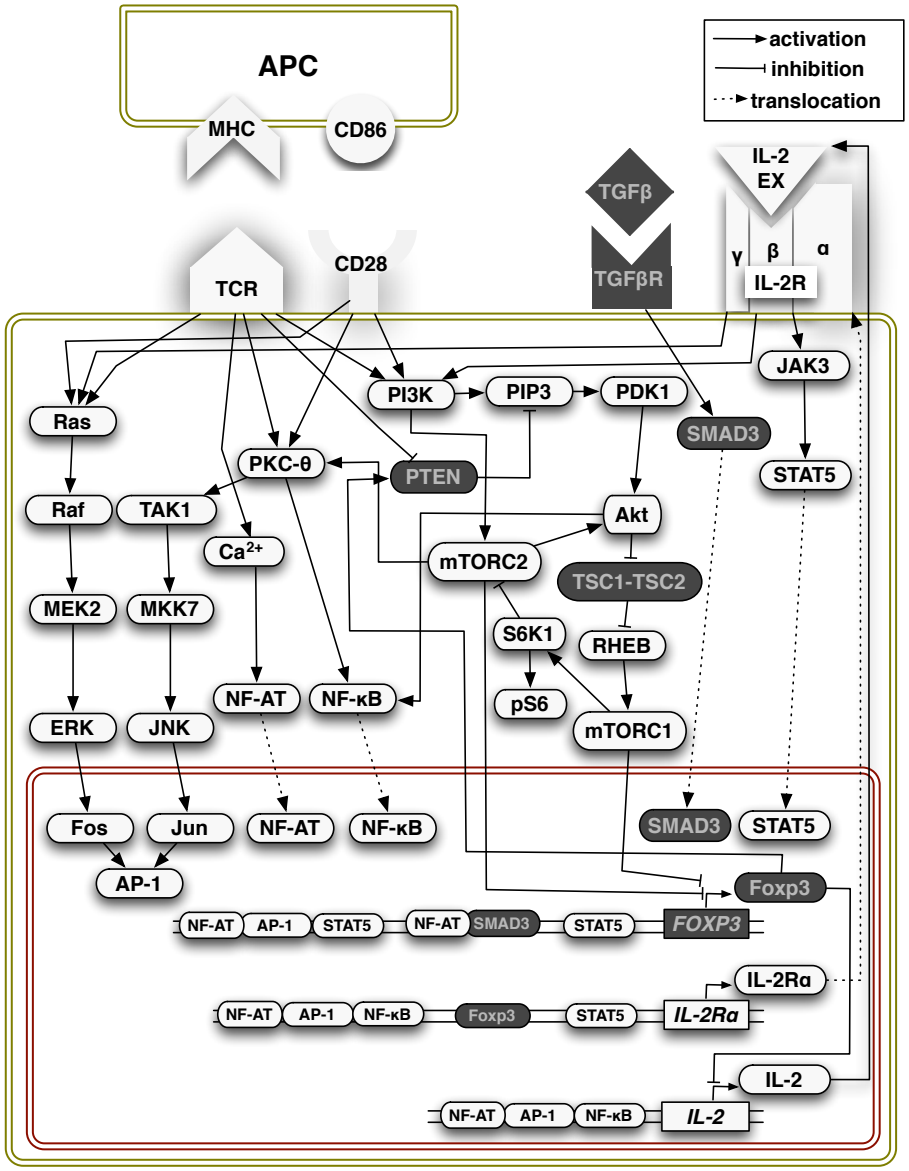
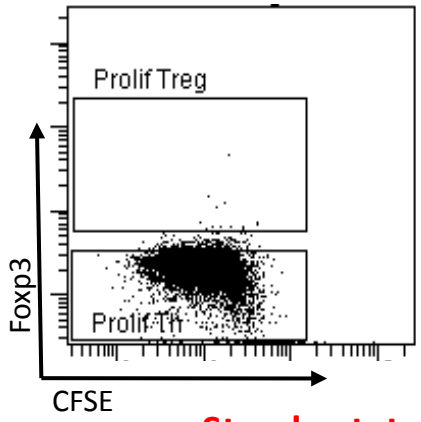
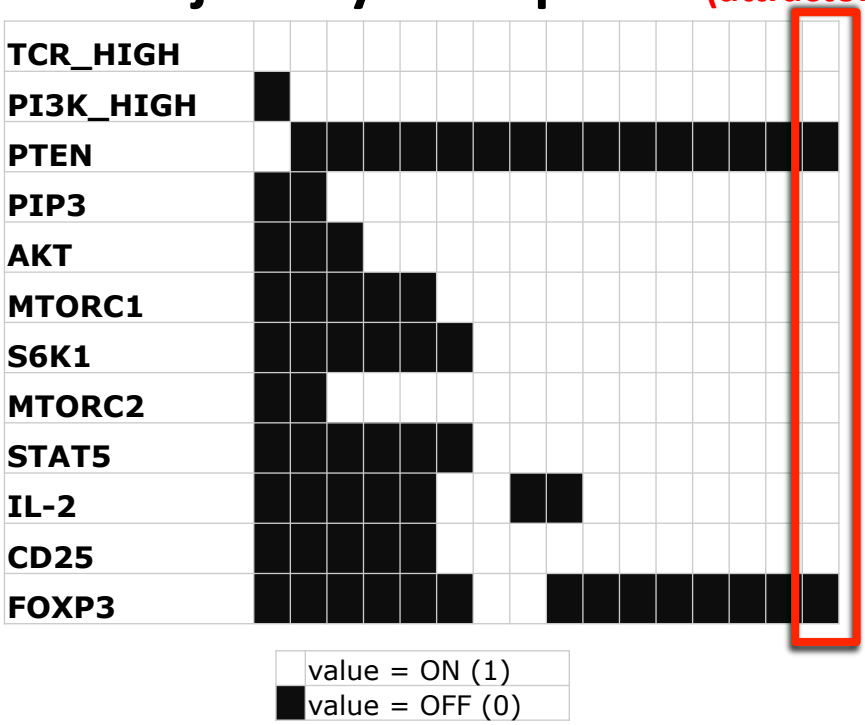


High dose



Steady-state (attractor)

Trajectory example



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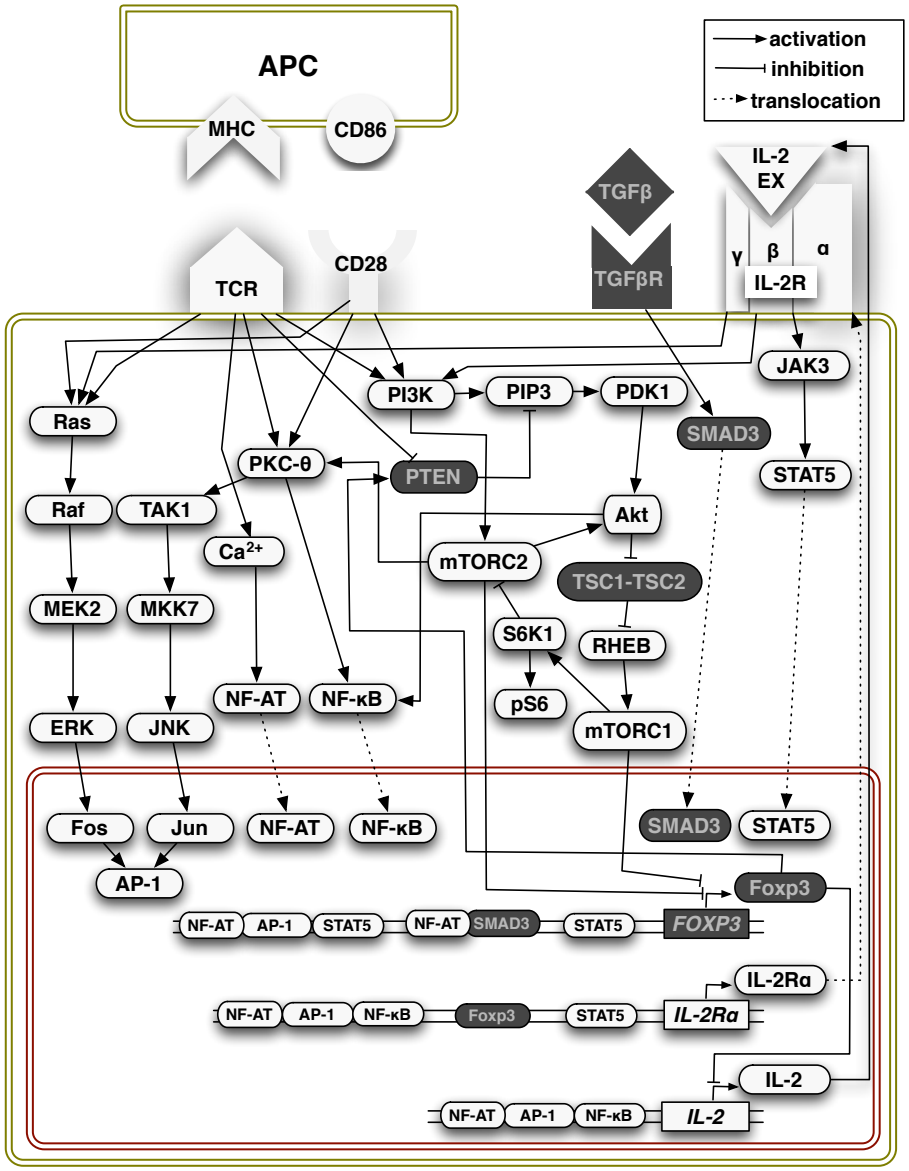

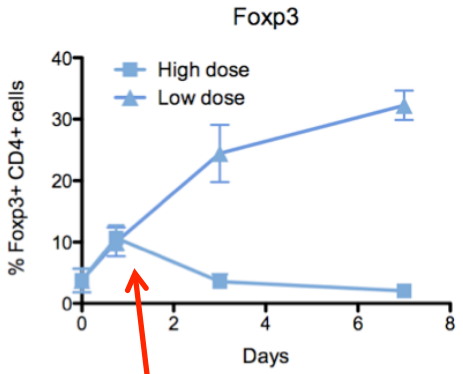

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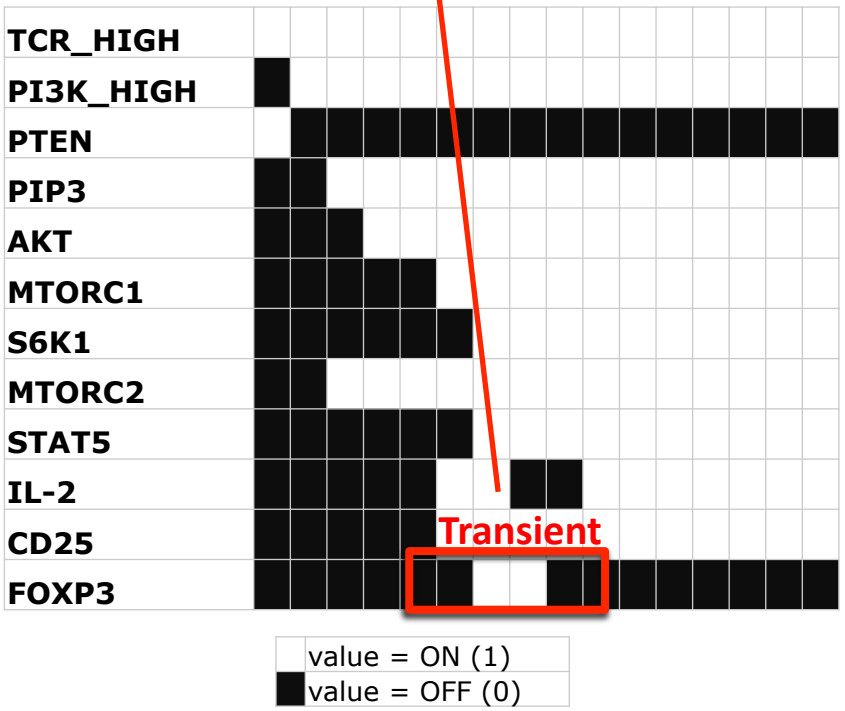
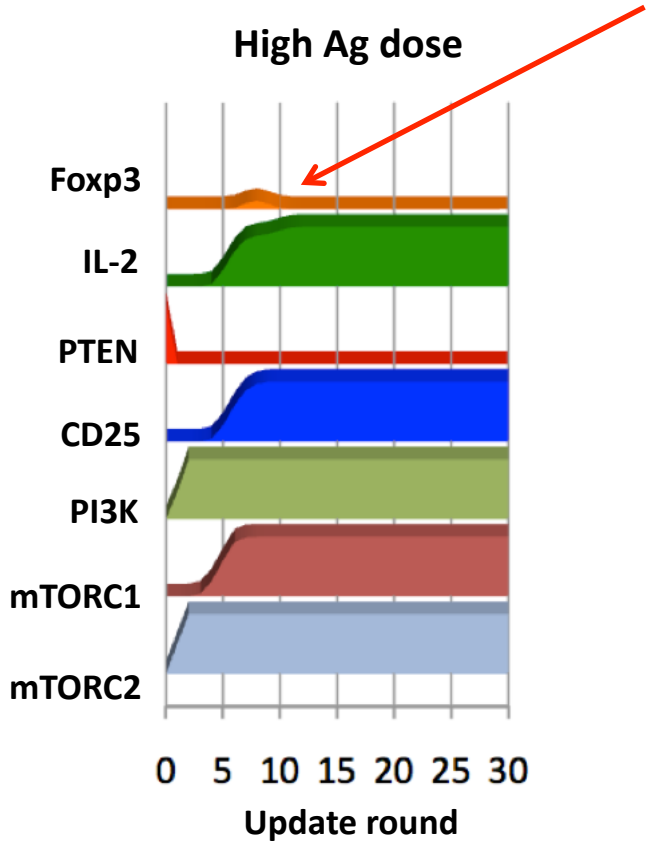


High antigen dose scenario

Simulation:
average element trajectories



Magnitude of transient is 0.1-0.15, which means that at maximum 15% trajectories have Foxp3=1 in the same round.

High antigen dose scenario

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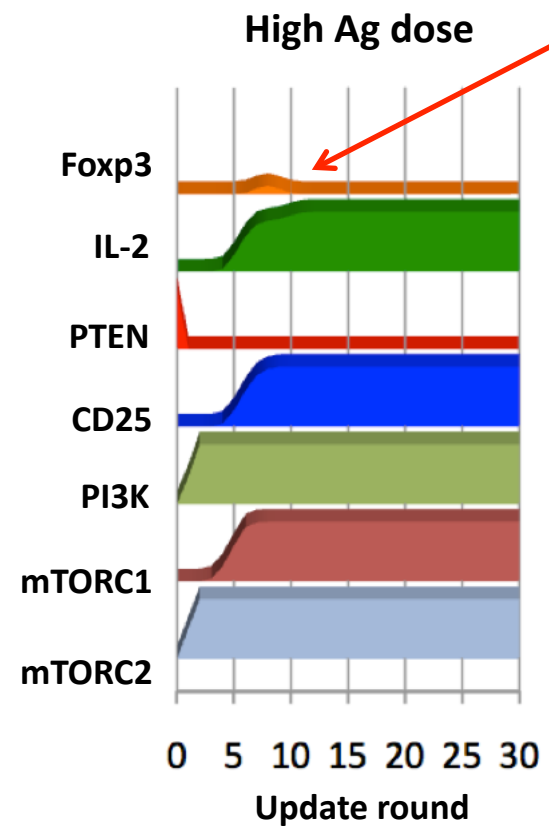

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How often Foxp3 increases to 1? How often it remains 0?

High antigen dose scenario

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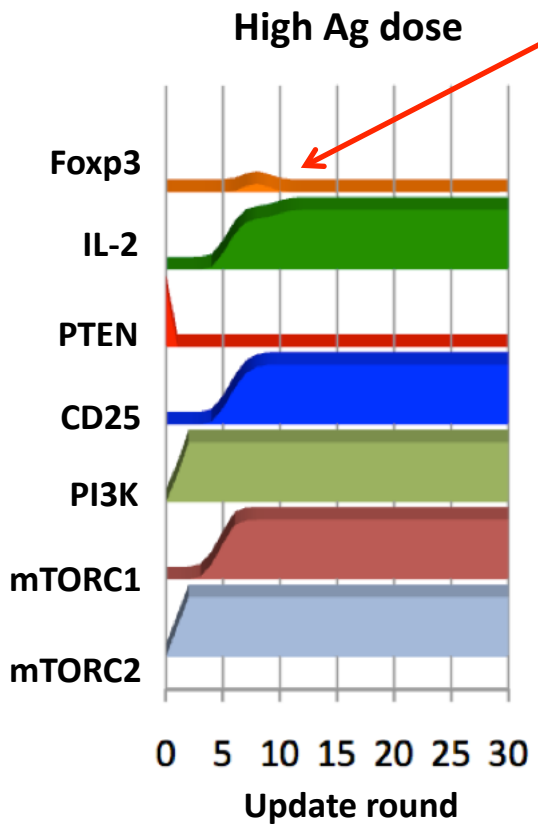
average element trajectories



Magnitude of transient is 0.1-0.15, which means that at maximum 15% trajectories have Foxp3=1 in the same round.

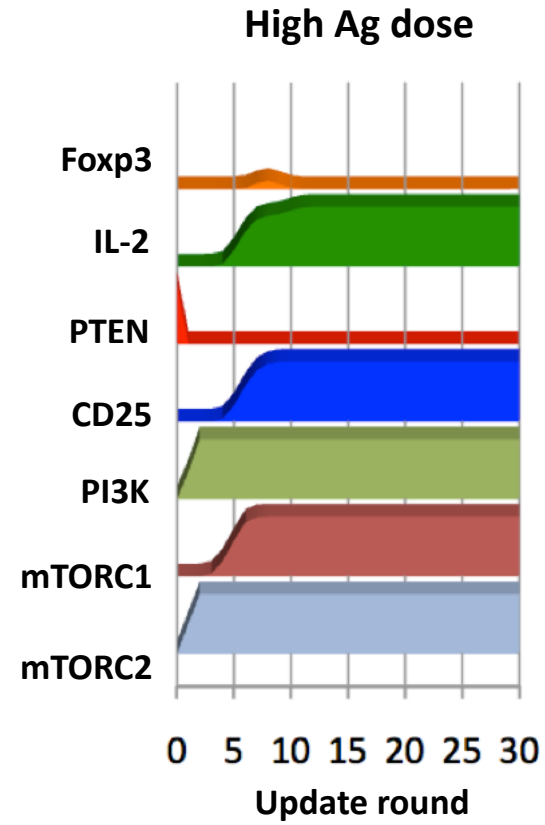
How often Foxp3 increases to 1? How often it remains 0?

Probability of Foxp3 becoming 1 is higher than the peak value in simulations -> Foxp3 transiently increases on a larger number of trajectories.

#	Property	Probability estimate	Success count	Sample size	Elapsed time [s]
P1	F^{29} (FOXP3 == 1); F^{10} (FOXP3 == 1 & F^{19} (FOXP3 == 0))	0.237494	2857	12032	120
P2	F^{10} G^{2} (FOXP3 == 1)	0.0415313	10970	264160	2704
P3	F^{10} G^{1} (FOXP3 == 1)	0.119089	830	6976	73
P4	F^{20} G^{9} (FOXP3 == 0 & IL2 == 1 & PTEN == 0 & CD25 == 1 & PI3K == 1 & MTORC1 == 1 & MTORC2 == 1)	0.996124	256	256	2

High antigen dose scenario

Simulation:
average element trajectories



Foxp3 increase to 1 often lasts only one round.

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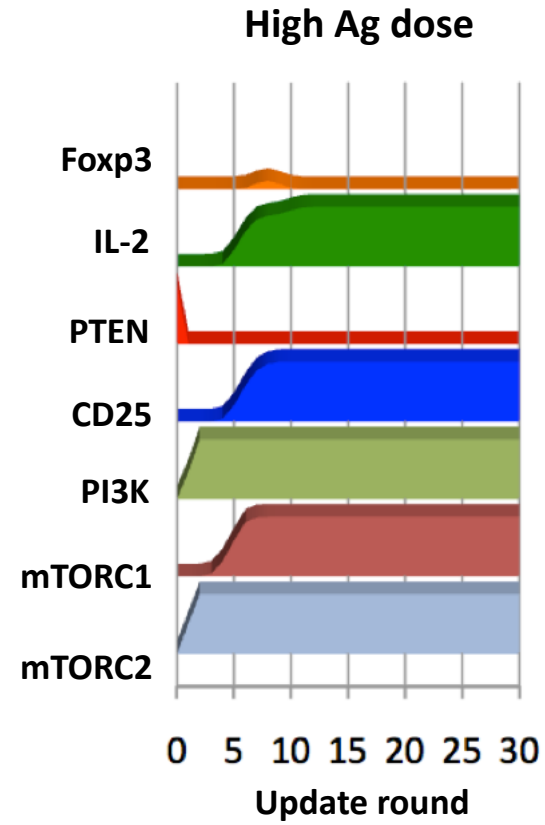

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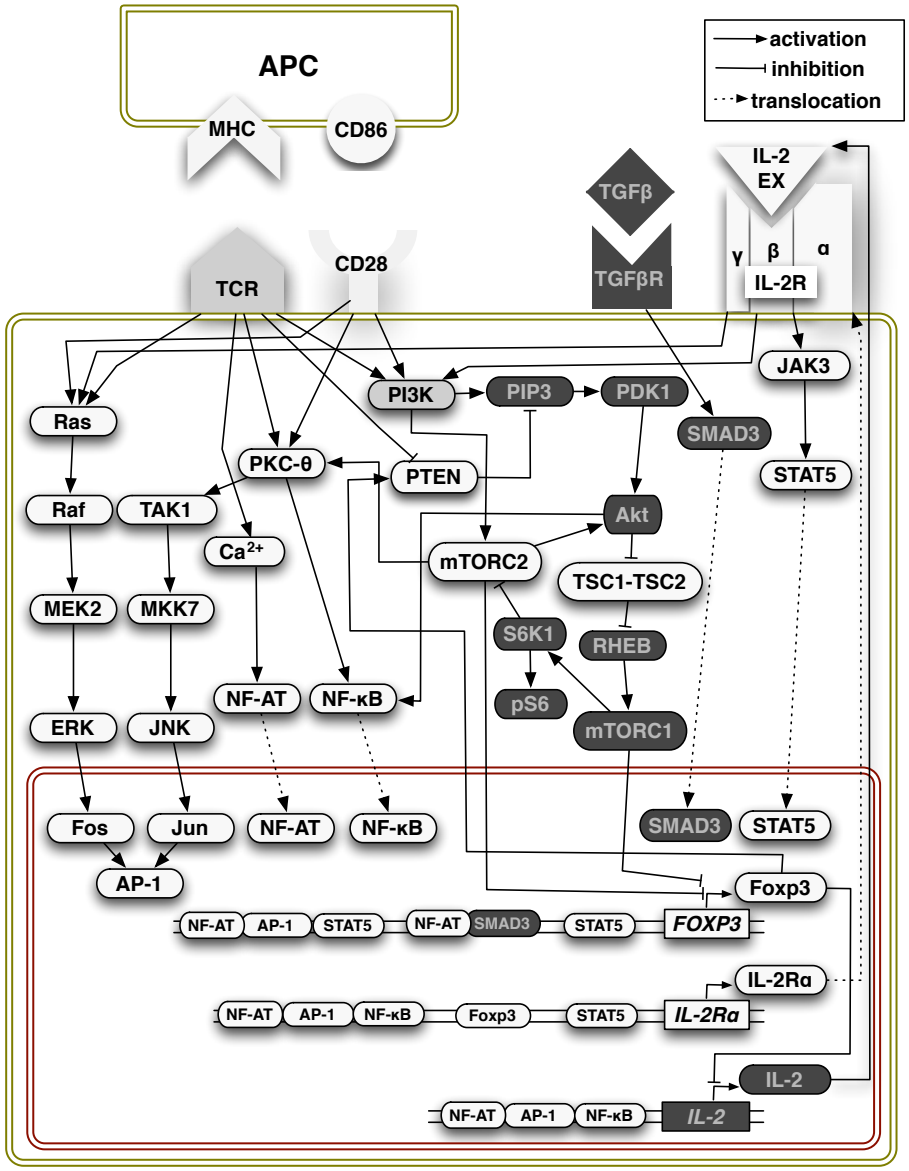
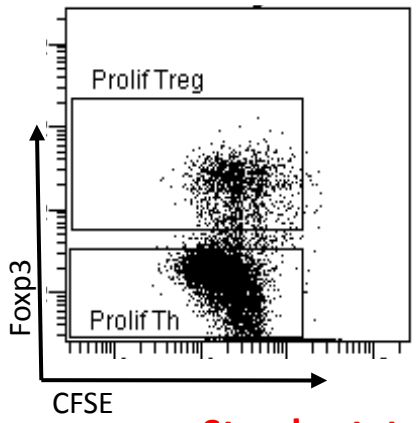
All samples reach same steady state

Scenario 2: Low antigen dose trajectory

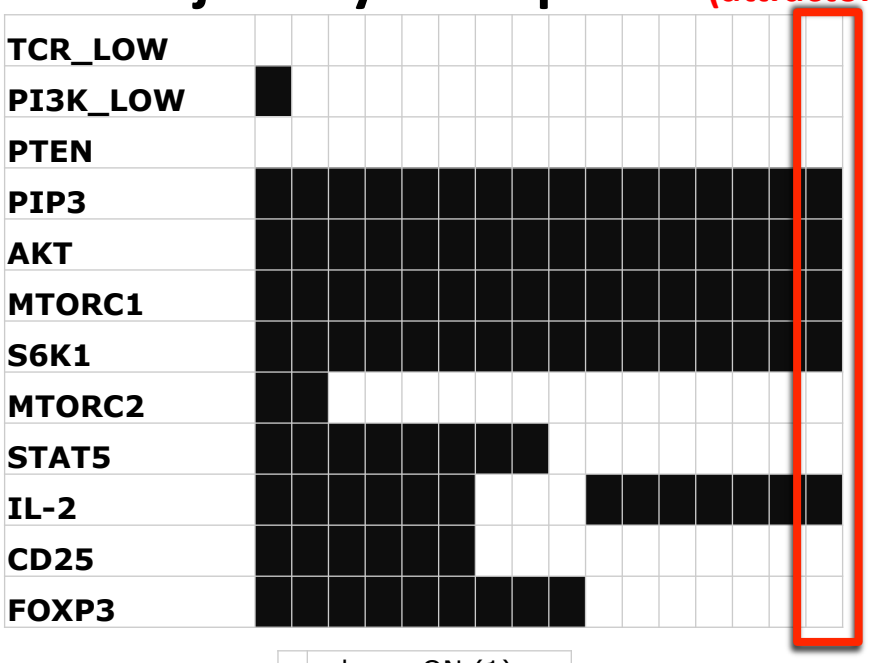


Low dose



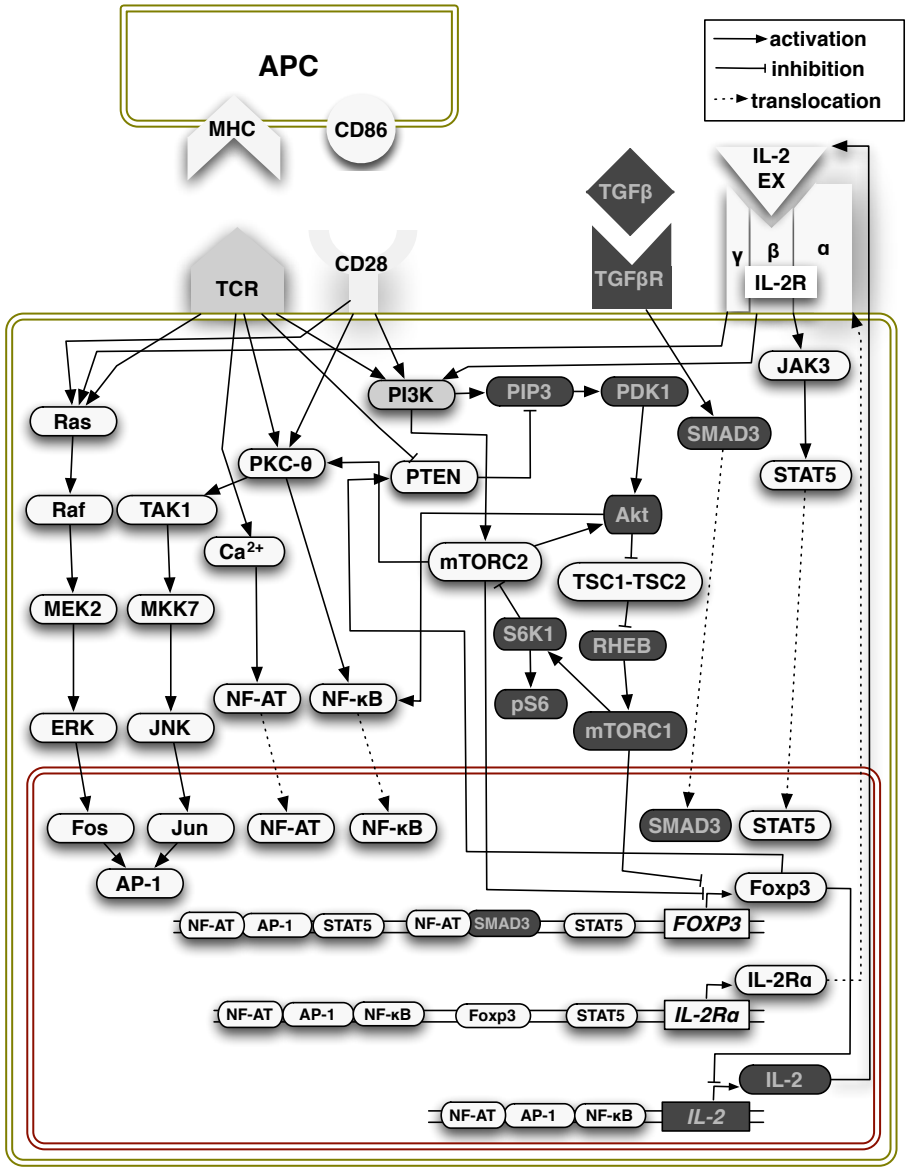
Steady-state (attractor)

Trajectory example



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

Scenario 2: Low antigen dose trajectory



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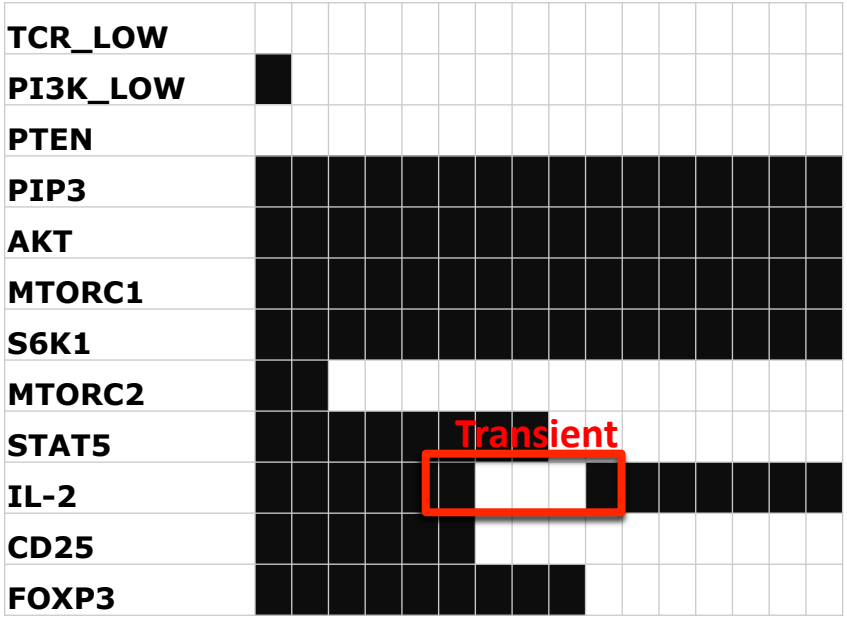

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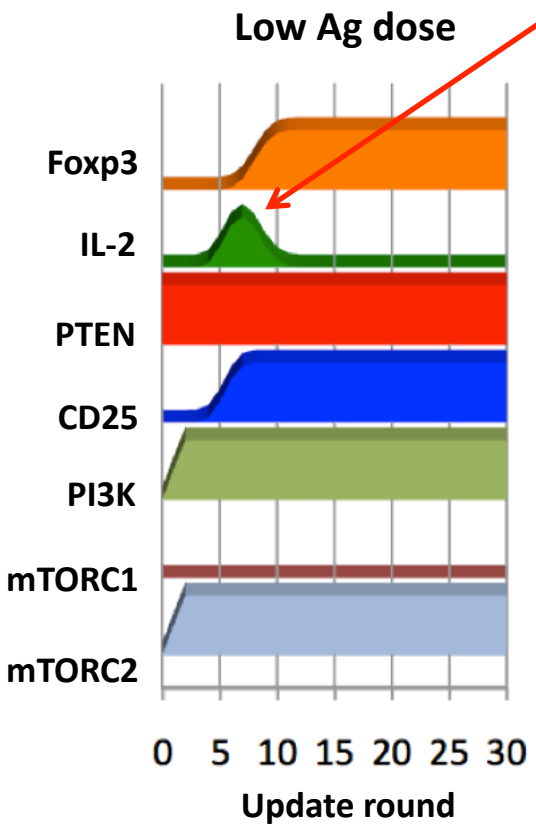
Low antigen dose scenario

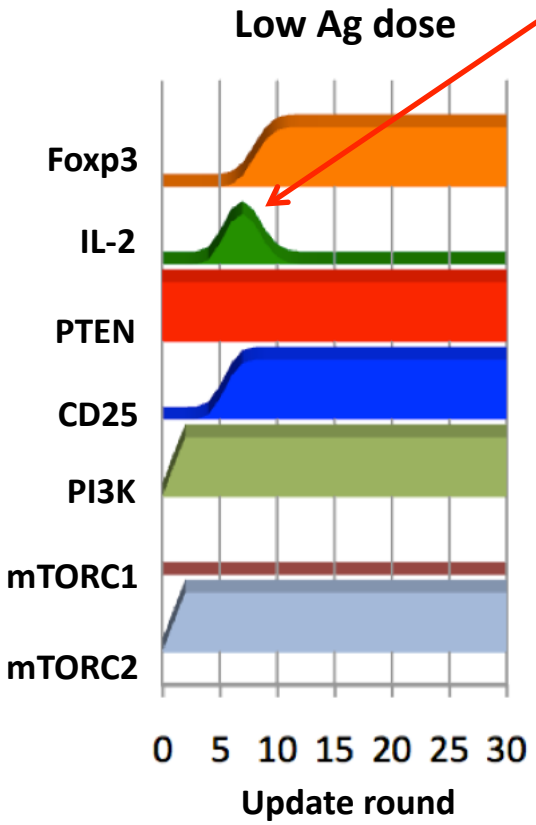
Simulation:
average element trajectories



Magnitude of transient is ~0.8, which means that at maximum 80% trajectories have IL-2=1 in the same round.

Low antigen dose scenario

Simulation:
average element trajectories



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IL-2 transiently becomes 1 in all samples

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P6	F^{10} G^{2} (IL2 == 1)	0.781024	8873	11360	115
P7	F^{10} G^{1} (IL2 == 1)	0.979681	1349	1376	14
P8	F^{20} G^{9} (FOXP3 == 1 & IL2 == 0 & PTEN == 1 & CD25 == 1 & PI3K == 1 & MTORC1 == 0 & MTORC2 == 1)	0.996124	256	256	2

Low antigen dose scenario

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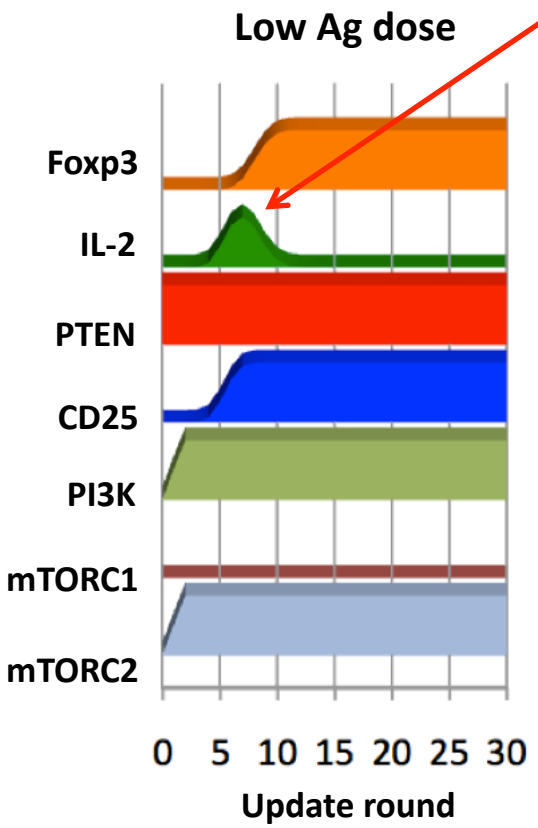
average element trajectories



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IL-2 transiently becomes 1 in all samples

IL-2 transient lasts longer than the Foxp3 transient.

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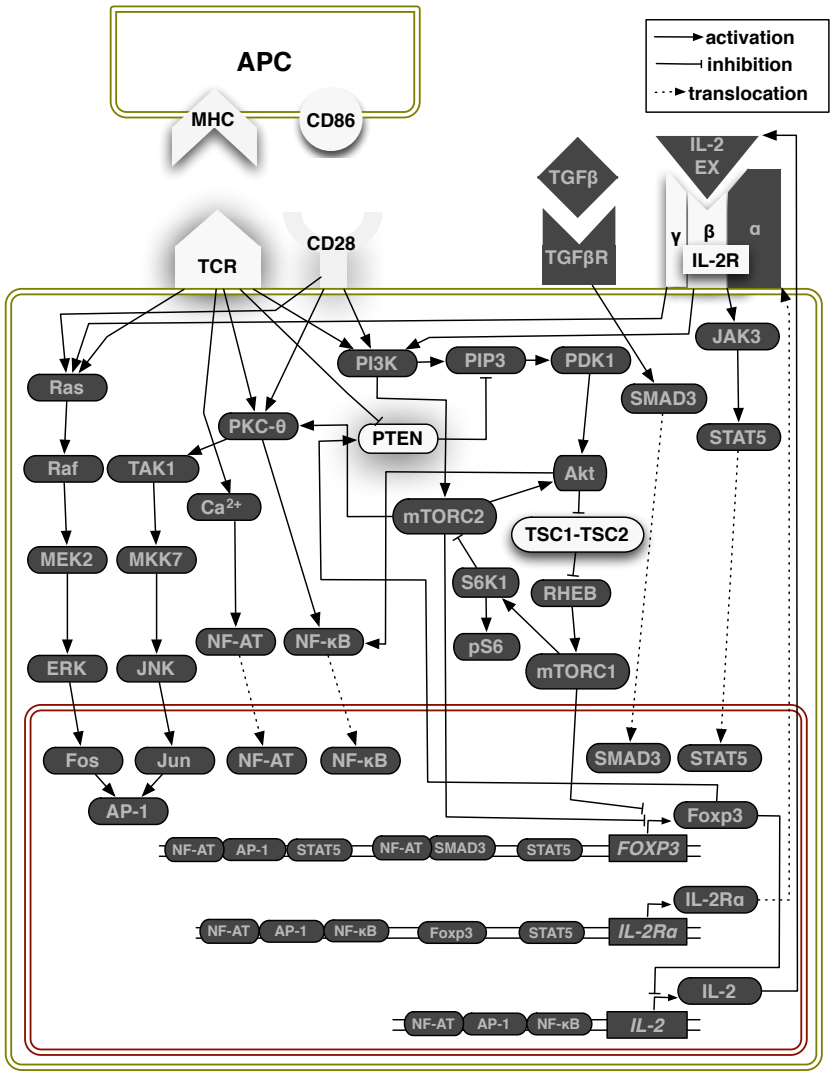
Scenario 3: Antigen removal at rounds 1-12 (T1-T12)

Initial state



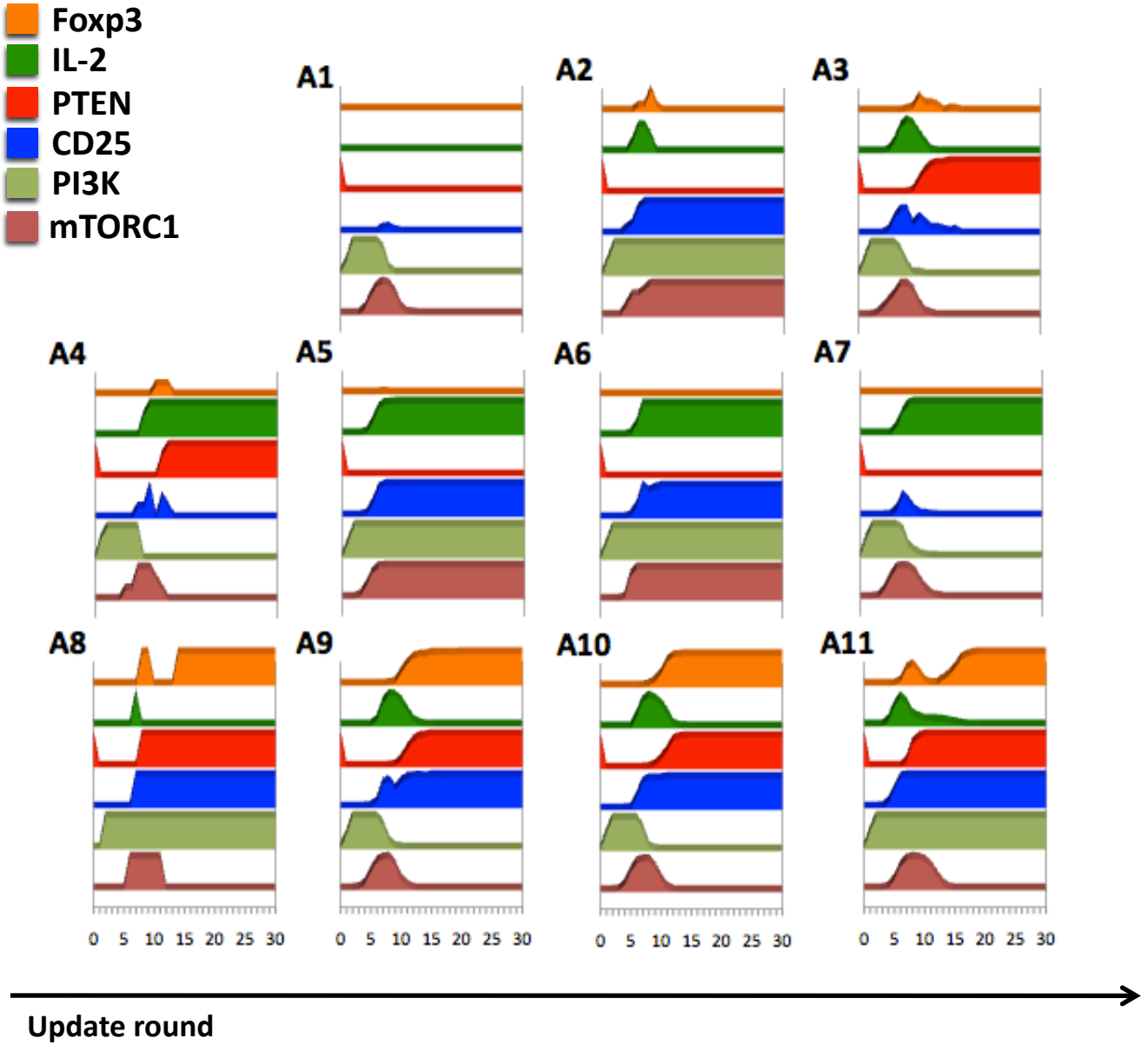
Steady-states (attractors)

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

	High Ag dose + Ag removal at T6											No removal	
Attractors	*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 frequency	40	6	17	3	374	13	127	1	118	126	175	1000	1000

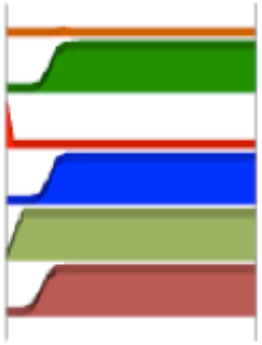
Miskov-Zivanov et al., *Science Signaling,* 2013.

Antigen removal scenario

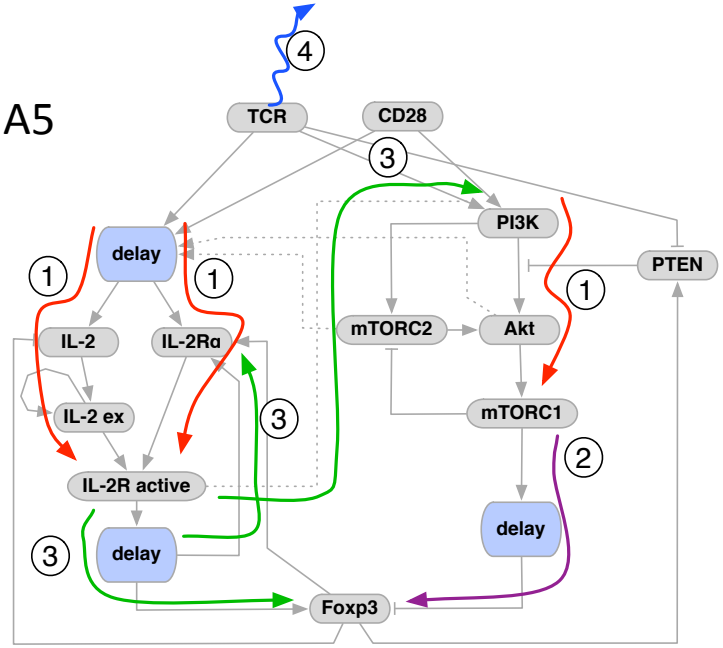


Order of events is important for differentiation

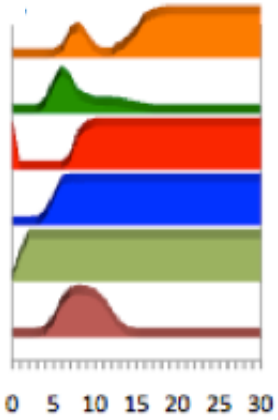
Average element trajectories leading to A5

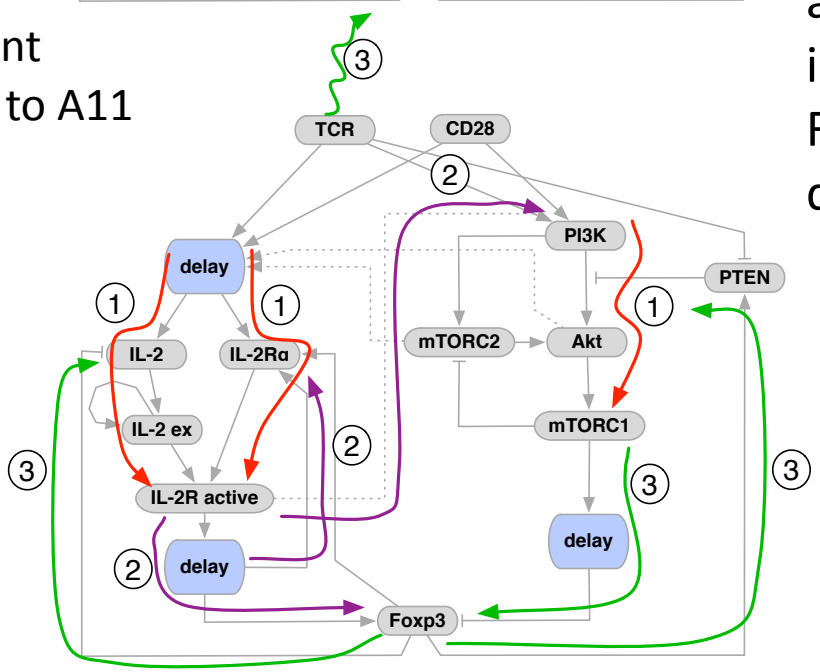


Foxp3
IL-2
PTEN
CD25
PI3K
mTORC1



Average element trajectories leading to A11



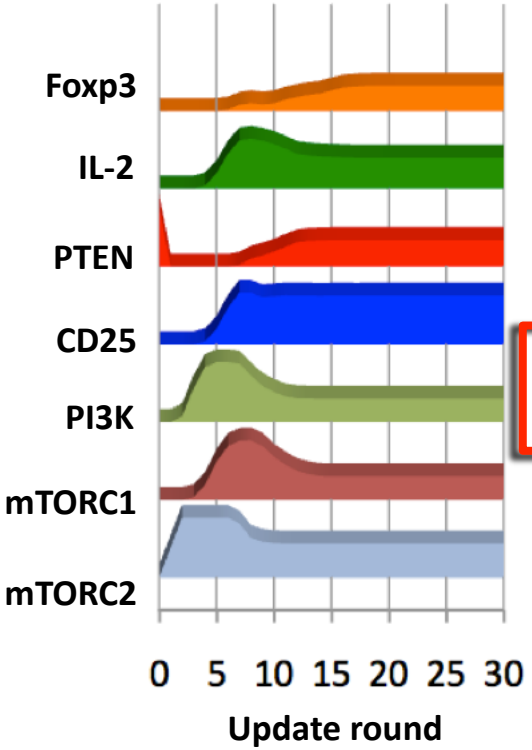


Relative timing on pathways leading to activation (IL-2R) vs. inhibition (mTOR) of Foxp3 critical for fate decision.

Antigen removal scenario – timing matters

Simulation:
average element trajectories

High Ag dose + Ag removal



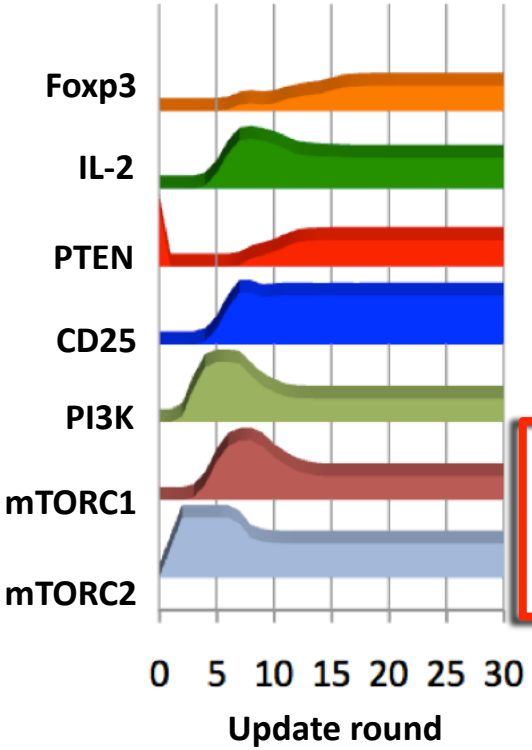
Both mTORC1 and mTORC2 will become 1 with high probability by round 7.

#	Property	Probability estimate	Success count	Sample size	Elapsed time [s]
P9	G^{7} ~(MTORC1 = 1 & MTORC2 = 1)	0.019567	46	2400	38
P10	F^{7} (MTORC1 = 1 & MTORC2 = 1)	0.982159	2201	2240	34
P11	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{18} (CD25 = 1)))	0.600977	15616	25984	407
P12	F^{28} (MTORC1 == 1 & MTORC2 == 1 & CD25 == 0 & (F^{1} (CD25 == 1)))	0.590649	15461	26176	405
P13	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{1} (G^{17} (CD25 = 1))))	0.405376	10585	26112	404
P14	F^{10} (MTORC1 == 1) & F^{15} G^{10} (PTEN == 1)	0.197865	3409	17232	175
P15	F^{25} G^{4} (FOXP3 == 1 & PTEN == 0)	2.893e-05	0	34560	350
P16	F^{2} G^{26} (FOXP3 == 0 & PTEN == 0)	0.550633	608	1104	11
P17	F^{2} G^{25} (PTEN == 0) & F^{10} (FOXP3 == 1)	0.0361264	143	3984	41

Antigen removal scenario – timing matters

Simulation:
average element trajectories

High Ag dose + Ag removal



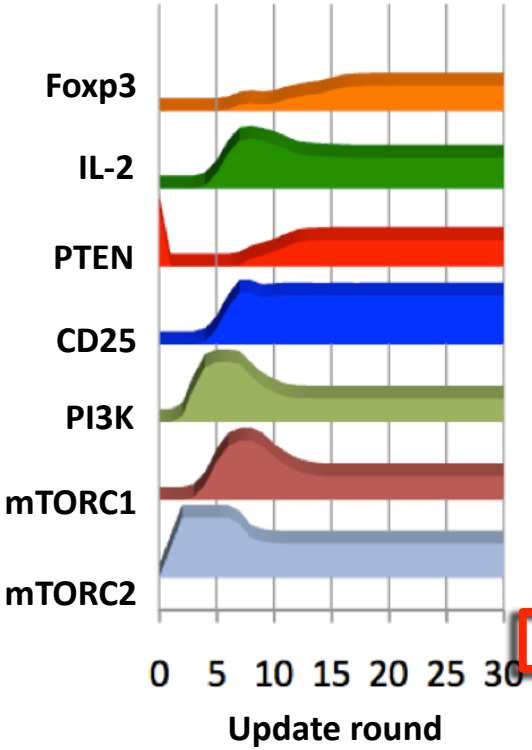
There is a significant probability that trajectories leading to CD25=1 in steady state will initially have both mTORC1 and mTORC2 at level 1 and CD25 at level 0.

#	Property	Probability estimate	Success count	Sample size	Elapsed time [s]
P9	G^{7} ~(MTORC1 = 1 & MTORC2 = 1)	0.019567	46	2400	38
P10	F^{7} (MTORC1 = 1 & MTORC2 = 1)	0.982159	2201	2240	34
P11	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{18} (CD25 = 1)))	0.600977	15616	25984	407
P12	F^{28} (MTORC1 == 1 & MTORC2 == 1 & CD25 == 0 & (F^{1} (CD25 == 1)))	0.590649	15461	26176	405
P13	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{1} (G^{17} (CD25 = 1))))	0.405376	10585	26112	404
P14	F^{10} (MTORC1 == 1) & F^{15} G^{10} (PTEN == 1)	0.197865	3409	17232	175
P15	F^{25} G^{4} (FOXP3 == 1 & PTEN == 0)	2.893e-05	0	34560	350
P16	F^{2} G^{26} (FOXP3 == 0 & PTEN == 0)	0.550633	608	1104	11
P17	F^{2} G^{25} (PTEN == 0) & F^{10} (FOXP3 == 1)	0.0361264	143	3984	41

Antigen removal scenario – timing matters

Simulation:
average element trajectories

High Ag dose + Ag removal



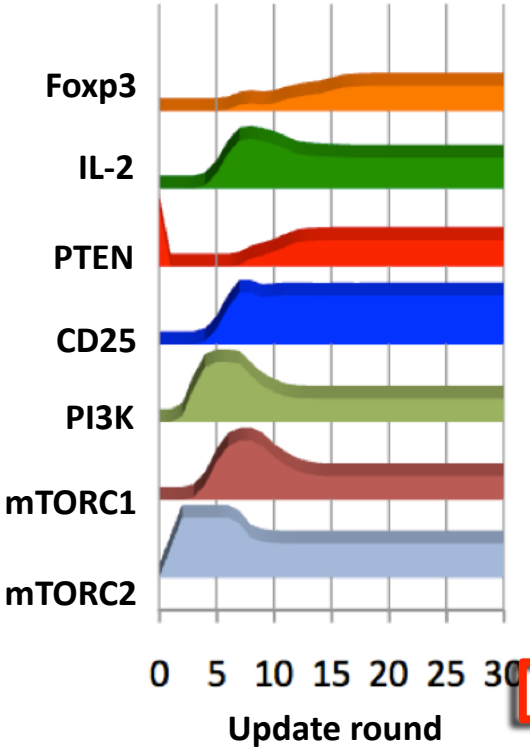
There is a small number of cases where mTORC1 becomes 1 by round 10, and PTEN is 1 in steady state.

#	Property	Probability estimate	Success count	Sample size	Elapsed time [s]
P9	G^{7} ~(MTORC1 = 1 & MTORC2 = 1)	0.019567	46	2400	38
P10	F^{7} (MTORC1 = 1 & MTORC2 = 1)	0.982159	2201	2240	34
P11	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{18} (CD25 = 1)))	0.600977	15616	25984	407
P12	F^{28} (MTORC1 == 1 & MTORC2 == 1 & CD25 == 0 & (F^{1} (CD25 == 1)))	0.590649	15461	26176	405
P13	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{1} (G^{17} (CD25 = 1))))	0.405376	10585	26112	404
P14	F^{10} (MTORC1 == 1) & F^{15} G^{10} (PTEN == 1)	0.197865	3409	17232	175
P15	F^{25} G^{4} (FOXP3 == 1 & PTEN == 0)	2.893e-05	0	34560	350
P16	F^{2} G^{26} (FOXP3 == 0 & PTEN == 0)	0.550633	608	1104	11
P17	F^{2} G^{25} (PTEN == 0) & F^{10} (FOXP3 == 1)	0.0361264	143	3984	41

Antigen removal scenario – timing matters

Simulation:
average element trajectories

High Ag dose + Ag removal



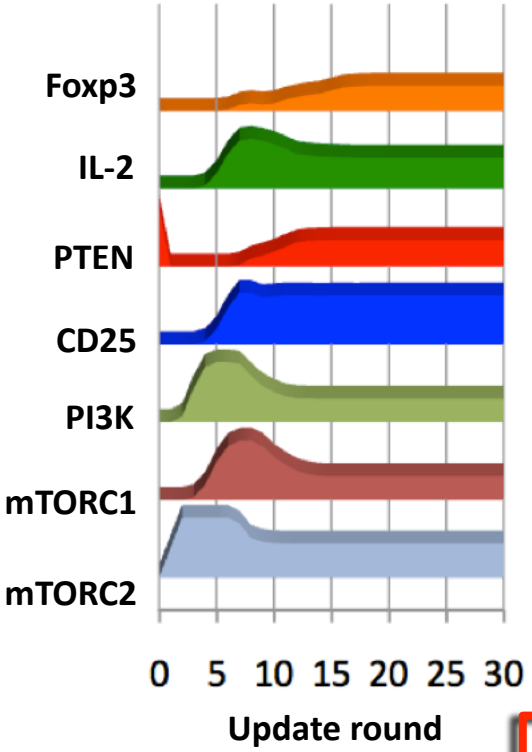
There are probably no cases in which Foxp3 is 1 and PTEN is 0 in steady state.

#	Property	Probability estimate	Success count	Sample size	Elapsed time [s]
P9	G^{7} ~(MTORC1 = 1 & MTORC2 = 1)	0.019567	46	2400	38
P10	F^{7} (MTORC1 = 1 & MTORC2 = 1)	0.982159	2201	2240	34
P11	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{18} (CD25 = 1)))	0.600977	15616	25984	407
P12	F^{28} (MTORC1 == 1 & MTORC2 == 1 & CD25 == 0 & (F^{1} (CD25 == 1)))	0.590649	15461	26176	405
P13	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{1} (G^{17} (CD25 = 1))))	0.405376	10585	26112	404
P14	F^{10} (MTORC1 == 1) & F^{15} G^{10} (PTEN == 1)	0.197865	3409	17232	175
P15	F^{25} G^{4} (FOXP3 == 1 & PTEN == 0)	2.893e-05	0	34560	350
P16	F^{2} G^{26} (FOXP3 == 0 & PTEN == 0)	0.550633	608	1104	11
P17	F^{2} G^{25} (PTEN == 0) & F^{10} (FOXP3 == 1)	0.0361264	143	3984	41

Antigen removal scenario – timing matters

Simulation:
average element trajectories

High Ag dose + Ag removal



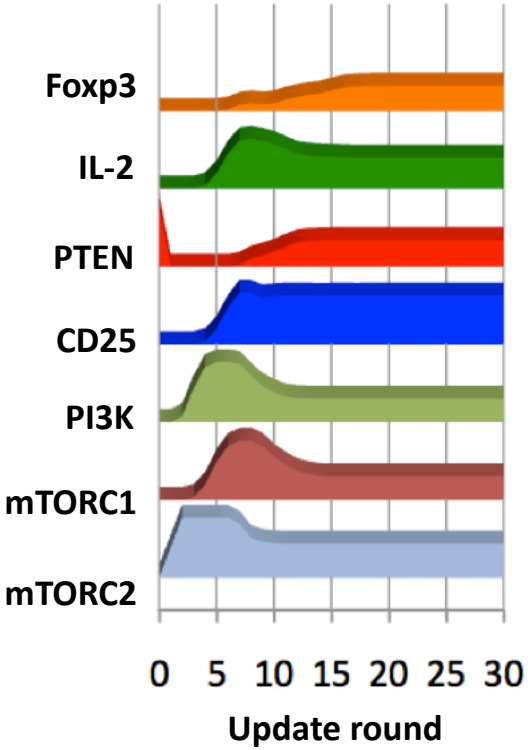
There is a significant number of cases in which both Foxp3 and PTEN are 0 early and remain 0 until steady state.

#	Property	Probability estimate	Success count	Sample size	Elapsed time [s]
P9	G^{7} ~(MTORC1 = 1 & MTORC2 = 1)	0.019567	46	2400	38
P10	F^{7} (MTORC1 = 1 & MTORC2 = 1)	0.982159	2201	2240	34
P11	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{18} (CD25 = 1)))	0.600977	15616	25984	407
P12	F^{28} (MTORC1 == 1 & MTORC2 == 1 & CD25 == 0 & (F^{1} (CD25 == 1)))	0.590649	15461	26176	405
P13	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{1} (G^{17} (CD25 = 1))))	0.405376	10585	26112	404
P14	F^{10} (MTORC1 == 1) & F^{15} G^{10} (PTEN == 1)	0.197865	3409	17232	175
P15	F^{25} G^{4} (FOXP3 == 1 & PTEN == 0)	2.893e-05	0	34560	350
P16	F^{2} G^{26} (FOXP3 == 0 & PTEN == 0)	0.550633	608	1104	11
P17	F^{2} G^{25} (PTEN == 0) & F^{10} (FOXP3 == 1)	0.0361264	143	3984	41

Antigen removal scenario – timing matters

Simulation:
average element trajectories

High Ag dose + Ag removal



There is a small number of cases in which PTEN is 0 early and remains 0 until steady state and Foxp3 becomes 1 by round 10.

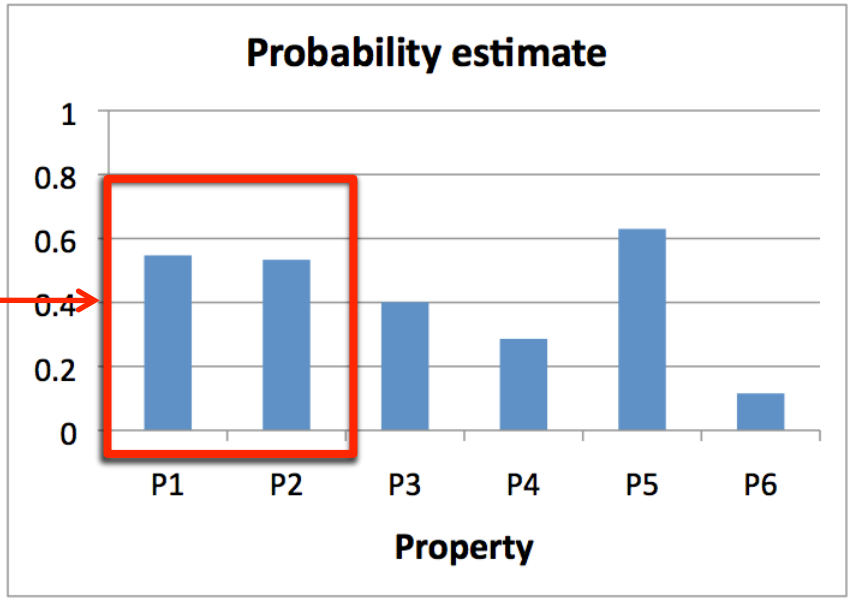
#	Property	Probability estimate	Success count	Sample size	Elapsed time [s]
P9	G^{7} ~(MTORC1 = 1 & MTORC2 = 1)	0.019567	46	2400	38
P10	F^{7} (MTORC1 = 1 & MTORC2 = 1)	0.982159	2201	2240	34
P11	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{18} (CD25 = 1)))	0.600977	15616	25984	407
P12	F^{28} (MTORC1 == 1 & MTORC2 == 1 & CD25 == 0 & (F^{1} (CD25 == 1)))	0.590649	15461	26176	405
P13	F^{10} (MTORC1 = 1 & MTORC2 = 1 & CD25 = 0 & (F^{1} (G^{17} (CD25 = 1))))	0.405376	10585	26112	404
P14	F^{10} (MTORC1 == 1) & F^{15} G^{10} (PTEN == 1)	0.197865	3409	17232	175
P15	F^{25} G^{4} (FOXP3 == 1 & PTEN == 0)	2.893e-05	0	34560	350
P16	F^{2} G^{26} (FOXP3 == 0 & PTEN == 0)	0.550633	608	1104	11
P17	F^{2} G^{25} (PTEN == 0) & F^{10} (FOXP3 == 1)	0.0361264	143	3984	41

Attractor analysis

	High Ag dose + Ag removal at T6											No removal	
Attractors	*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 frequency	40	6	17	3	374	13	127	1	118	126	175	1000	1000

Occurrence of specific states on trajectories leading to a given attractor

Foxp3 is activated late (at round 13) on trajectories leading to attractor A3

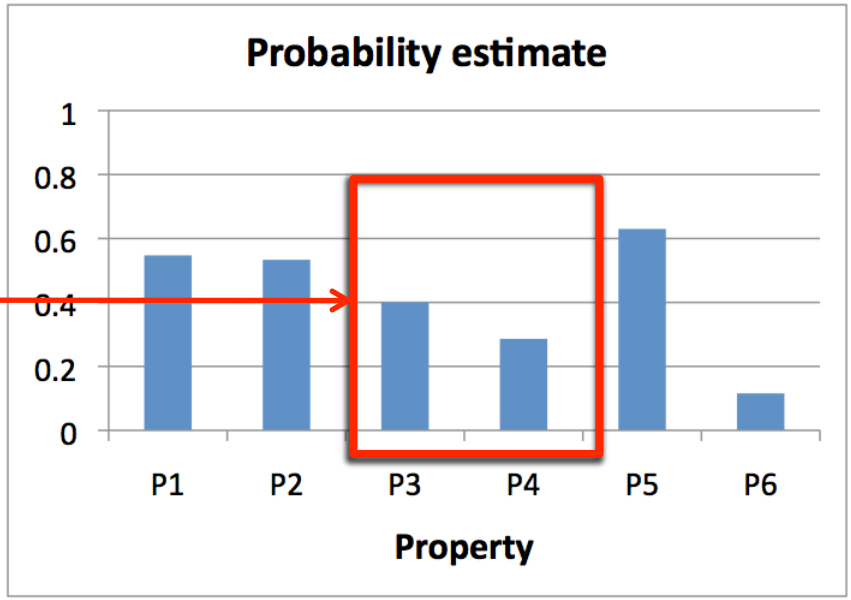


Attractor analysis

	High Ag dose + Ag removal at T6											No removal	
Attractors	*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 frequency	40	6	17	3	374	13	127	1	118	126	175	1000	1000

Occurrence of specific states on trajectories leading to a given attractor

Foxp3 is activated late (at round 13) on trajectories leading to attractor A4

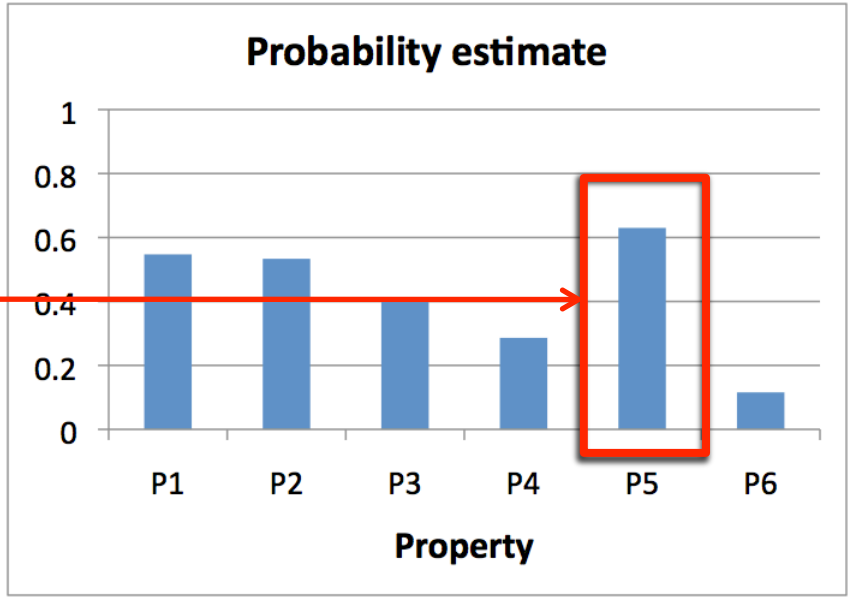


Attractor analysis

	High Ag dose + Ag removal at T6											No removal	
Attractors	*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 frequency	40	6	17	3	374	13	127	1	118	126	175	1000	1000

Occurrence of specific states on trajectories leading to a given attractor

mTORC1 is activated early (at round 5) and before CD25 gets activated on trajectories leading to attractor A7

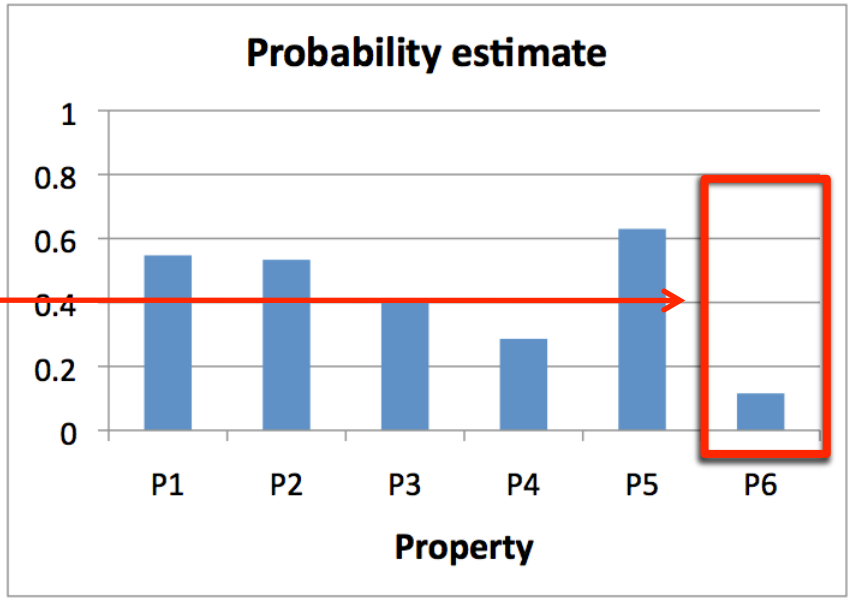


Attractor analysis

	High Ag dose + Ag removal at T6											No removal	
Attractors	*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 frequency	40	6	17	3	374	13	127	1	118	126	175	1000	1000

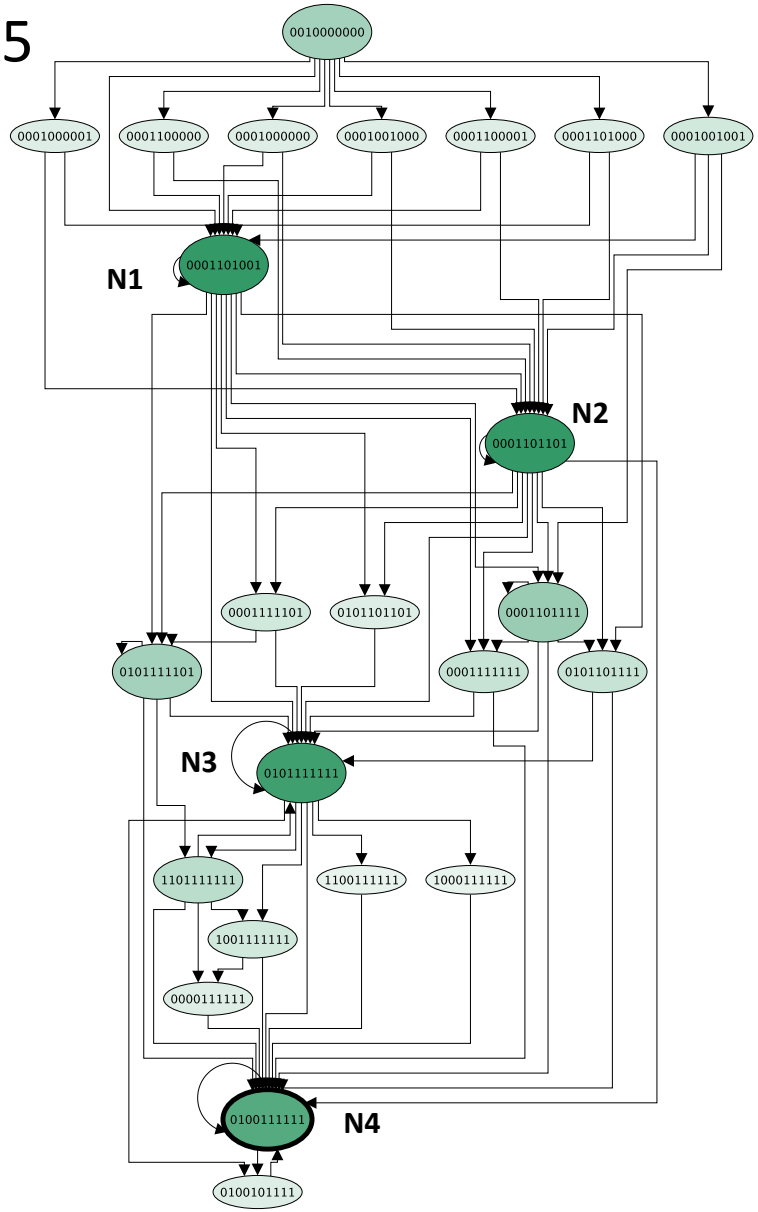
Occurrence of specific states on trajectories leading to a given attractor

IL-2 and CD25 are often not both activated as early as round 4 in A11.

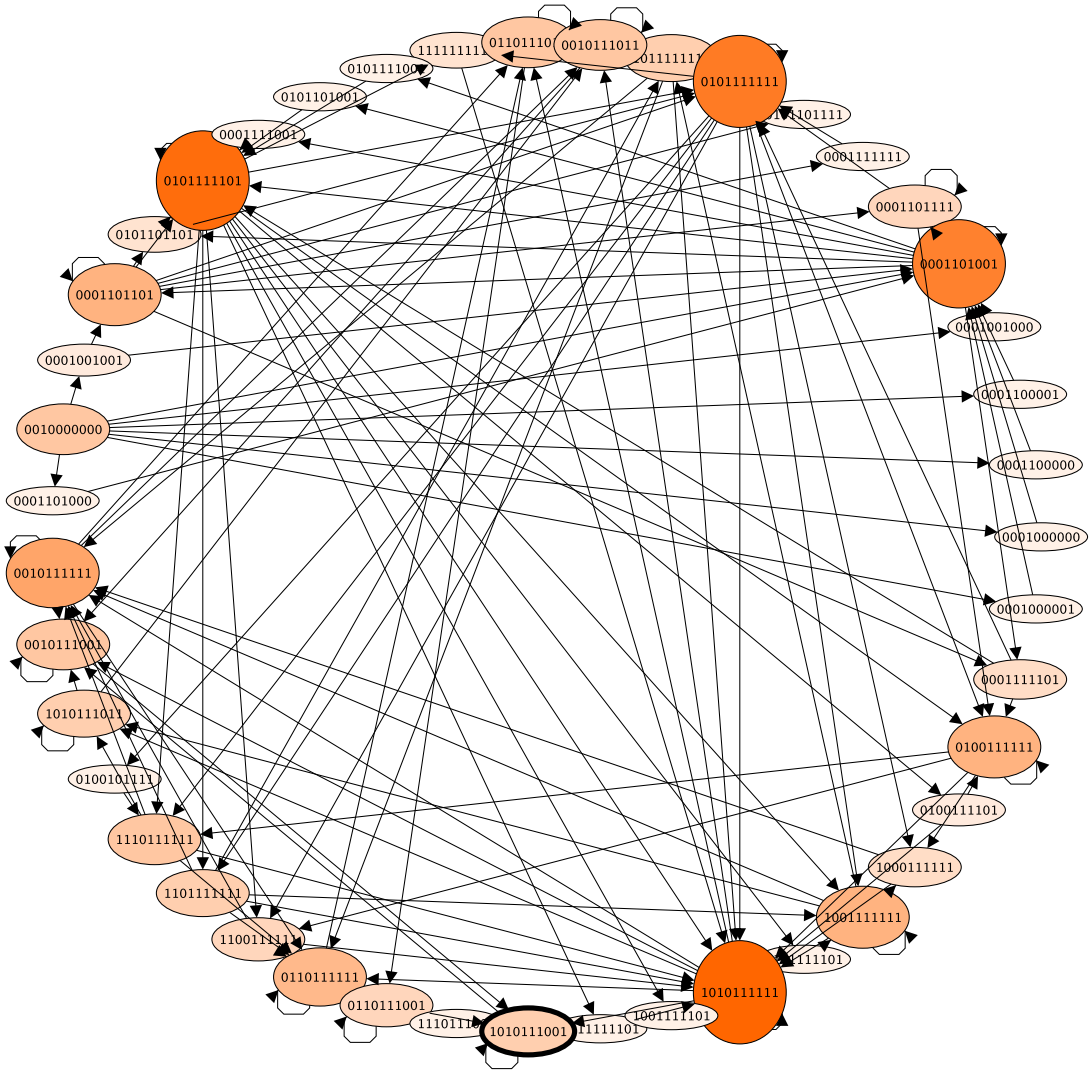


Frequent nodes

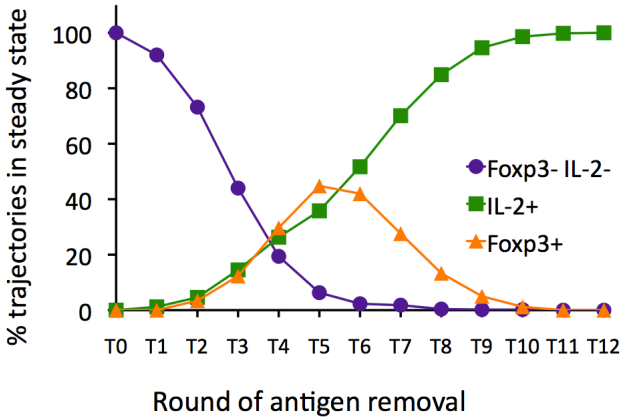
A5

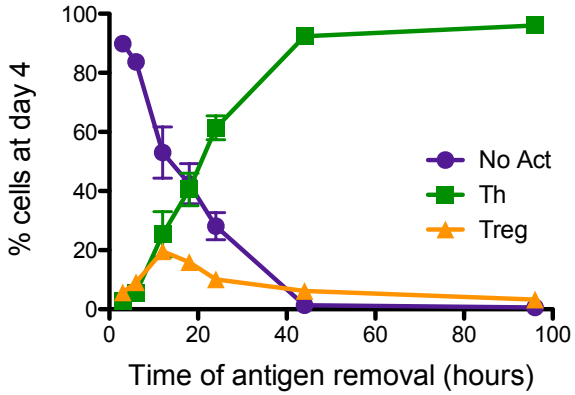


A11



Frequent nodes

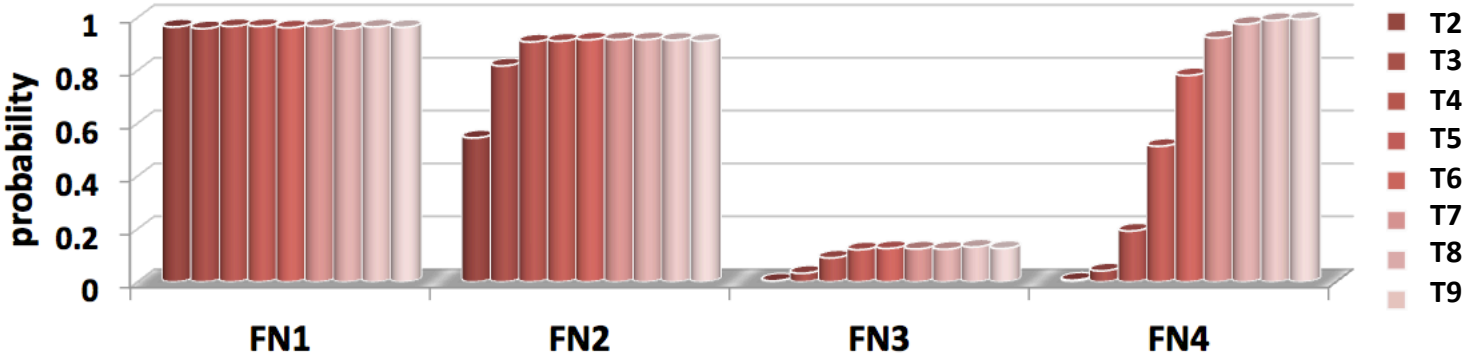




Miskov-Zivanov et al., *Science Signaling,* 2013.

Frequent node #	Element values						
	Foxp3	IL-2	PTEN	CD25	Akt	mTORC1	mTORC2
FN1	0	0	0	0	1	0	1
FN2	0	0	0	0	1	1	1
FN3	0	0	0	0	0	0	1
FN4	0	1	0	1	1	1	1

Antigen removal at different rounds:
round 2 (T2)
round 3 (T3)
…
round 9 (T9)



Conclusion

- Model of peripheral T cell differentiation
  - Recapitulates a wide range of experimental observations
  - Circuit analysis reveals key elements and mechanisms for Foxp3 expression
  - Timing is critical for Treg differentiation
- Statistical model checking is an efficient approach for:
  - Studying transient behavior of the system
  - Relationships between elements in time

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- **Faeder Lab:**
  - Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh
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    - Edmund Clarke
    - Paolo Zuliani (now at Newcastle University, UK)
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    - Lawrence Kane
    - Michael Turner
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