Figure S1. Model elements

Elements included in our network model, and references to related work that show relevance of elements for the T cell differentiation.

Element	Definition	References
TCR	T cell receptor	(*1*)
CD28	CD28 receptor	(*2*)
Ras	Ras	(*3, 4*)
Raf	Raf kinase, effector of Ras	(*3, 4*)
TAK1	mitogen-activated protein kinase kinase kinase 7	(*3, 4*)
MEK2	mitogen-activated protein kinase kinase 2	(*3, 4*)
MKK7	mitogen-activated protein kinase kinase 7	(*3, 4*)
ERK	MAPK 1/elk related kinase	(*3, 4*)
JNK	Jun kinase	(*5*)
Fos	c-Fos proto-oncogene	(*5*)
Jun	c-Jun proto-oncogene	(*5*)
AP-1	Fos/Jun complex	(*5*)
Ca^{2+}	Calcium	(*6*)
NF-AT	Nuclear factor of activated T cells	(*6*)
PKC-θ	Protein kinase C-θ	(*7-9*)
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, 2	(*10, 11*)
PI3K	Phosphoinositide-3-kinase	(*12*)
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate	(*12*)
PDK1	3-phosphoinositide dependent protein kinase-1	(*12, 13*)
PTEN	Phosphatase and tensin homolog	(*14*)
Akt	Akt (Protein kinase B)	(*15, 16*)
TSC1-TSC2	Tuberous sclerosis protein 1 and 2	(*13, 15*)
RHEB	Ras homolog enriched in brain	(*13, 15*)
mTORC1	Mammalian target of rapamycin 1	(*13, 15*)
S6K1	Ribosomal protein S6 kinase	(*13, 15*)
mTORC2	Mammalian target of rapamycin 2	(*13, 15*)
pS6	Ribosomal protein S6	(*13, 15*)
SMAD3	SMAD family member 3	(*17*)
IL-2 ex	Interleukin 2, exogenous	(*18*)
Foxp3	Forkhead box P3	(*19*)
IL-2	Interleukin 2	(*20, 21*)
IL-2Rα (CD25)	Interleukin 2 receptor α	(*20, 21*)
IL-2Rβ (CD122)	Interleukin 2 receptor β	(*20, 21*)
IL-2Rγ (CD132)	Interleukin 2 receptor γ	(*20, 21*)
TGF-β	Transforming growth factor β	(*22*)
TGF-βR	Transforming growth factor β receptor	(*17*)
JAK3	Janus kinase 3	(*21*)
STAT5	Signal transducer and activator of transcription 5	(*21*)

Figure S2. Model rules

Description of logic rules in the model with supporting references.

	Logical rule	Description and references
1	TCR' = TCR_LOW or TCR_HIGH	*Overall activation of TCR, model related.* T cell receptor-related variable (TCR) can have value '1' if either low or high antigen dose is present. This specific variable is used in the model when low and high antigen dose have identical effects.
2	RAS' = (TCR and CD28) or (RAS and IL2_EX and IL2R)	Ras is activated by signaling from TCR/CD28 (*23, 24*). Once signaling through IL-2R is established, it can help keep Ras active (*25*)
3-6	RAF' = RAS, MEK2' = RAF, ERK' = MEK2, FOS' = ERK **	Activation of Fos. Although variables carry names of specific molecules, since this is a path that does not have any cross talk with other paths, the only aspect of it that matters in the model is the number of steps, as shown in Figure 2E. (*26*) (*27*)
7	PKCTHETA' = TCR_HIGH or (TCR_LOW and CD28 and MTORC2)	PKC-θ is activated by signaling from TCR (*9*), if antigen dose presented to the cell is high. If the dose is low, additional activation signals come from CD28 and mTORC2 (*28*).
8	TAK1' = PKCTHETA	TAK1 is activated by PKC-θ (*29, 30*).
9-11	MKK7' = TAK1, JNK' = MKK7, JUN' = JNK	Activation of Jun. (*31*) (*5, 32*)
12	AP1' = FOS and JUN	AP-1 is activated when both Fos and Jun are active (*32*)
13-14	CA' = TCR, NFAT' = CA	Ca^{2+} is activated by TCR, and NFAT is activated by Ca^{2+} (*33, 34*)
15	NFKAPPAB' = PKCTHETA or AKT	NFκB is primarily activated by PKC-θ (*8*), but it can also be activated by Akt (*11, 30, 35*).
16	IL2' = ((AP1 and NFAT and NFKAPPAB) or IL2) and not FOXP3	AP-1, NFκB and NFAT are the transcription factors necessary for IL-2 transcription. To be able to implement to distinguish between the cells that only transiently express IL-2 and those that actually secret higher amount of IL-2, we include a positive feedback in IL-2 rule that will allow IL-2 to remain in the "on" state if it is active for two or more rounds (*18*).
17	IL2R' = CD25 and CD122 and CD132	IL-2 receptor is assumed to be active if all three parts are expressed on the surface of the cell: IL-2Rα (CD25), IL-2Rβ (CD122) and IL-2Rγ (CD132) (*21, 36*).
18-20	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) PI3K' = PI3K_LOW or PI3K_HIGH	TCR signaling activates PI3K/Akt/mTOR network (*37*); activation by CD28 (*38*), downstream of IL-2 receptor (*39*). PI3K is represented using two Boolean variables to avoid oscillations in the mTORC1/mTORC2 loop (as described in the Results section). For PI3K to be activated, each PI3K variable in the model requires the corresponding TCR variable (low or high) and CD28 variable to have value '1'. Once active, PI3K can remain active if the signal from IL-2R reaches PI3K before it switches to "off" state."
21-23	PIP3_LOW' = PI3K_LOW and not PTEN PIP3_HIGH' = PI3K_HIGH and not PTEN PIP3' = PIP3_LOW or PIP3_HIGH	PIP3 is activated by PI3K and it is inhibited by PTEN (*14*).
24	PDK1' = PIP3	PDK1 connected directly to PIP3 (*40*).
25	AKT' = PDK1 and MTORC2	Akt is activated after phosphorylation by both PDK1 (*15*) and mTORC2 (*41, 42*).
26-27	TSC' = not AKT, RHEB' = not TSC	Akt is a negative regulator of TSC1-TSC2, which is a negative regulator of Rheb (*13, 15, 43*).
28	MTORC1' = RHEB **	mTORC1 is activated by Rheb (*15, 43*).
29	MTORC2' = PI3K_HIGH or (PI3K_LOW and not S6K1) **	mTORC2 is activated by high level of PI3K when a high dose of antigen is present, or with low level of PI3K when there is no inhibition by S6K1.Link from PI3K to mTORC2 (*41*), inhibition by S6K1 (*13*), (*44*)
30	MTOR' = MTORC1 and MTORC2 **	*Overall activation of mTOR, model related*
31-32	S6K1' = MTORC1, PS6' = S6K1	S6K1 is activated by mTORC1 and pS6 is activated by S6K1 (*13, 43*).
33	SMAD3' = TGFBETA	Smad3 propagates signal from the TGFβ receptor (*45, 46*).
34	JAK3' = IL2R and IL2_EX	JAK3 is activated by IL-2R signal once IL-2 (IL-2 exogenous) binds to the receptor (*39, 45*)
35	STAT5' = JAK3 **	STAT5 is activated by JAK3 (*45*).
36	FOXP3' = (((NFAT and AP-1 and STAT5) or STAT5) and not mTOR) or (NFAT and SMAD3)	Foxp3 is activated either by binding of STAT5 to its promoter (*36, 47*), or by binding of NFAT and Smad3 to its promoter (*48*). Activation by STAT5 can be inhibited by mTOR, while TGFβ signaling through Smad3 prevails over mTOR inhibition.
37	CD25' = (AP1 and NFAT and NFKAPPAB) or STAT5 or Foxp3	IL-2Rα (CD25) is activated by the same transcription factors as IL-2, or by STAT5, or Foxp3 (*19, 49, 50*).
38	PTEN' = (not TCR_HIGH and PTEN) or (not TCR_HIGH and FOXP3)	PTEN is at high levels in naïve T cells, and is inhibited by the signaling from TCR. Foxp3 promotes PTEN activity (*51, 52*). Also, once active after removal of the signal from TCR, PTEN can stay active (*53*).
39	IL2_EX' = IL2 or IL2_EX	We include IL-2 exogenous (IL-2_EX) in the model to be able to distinguish between IL-2 that is secreted outside of the cell and IL-2 expression inside the cell. Since we simulate single cell, once IL-2 is expressed, it is assumed to stay outside of the cell in the form of IL-2 exogenous

** We also model additional delay for those elements.

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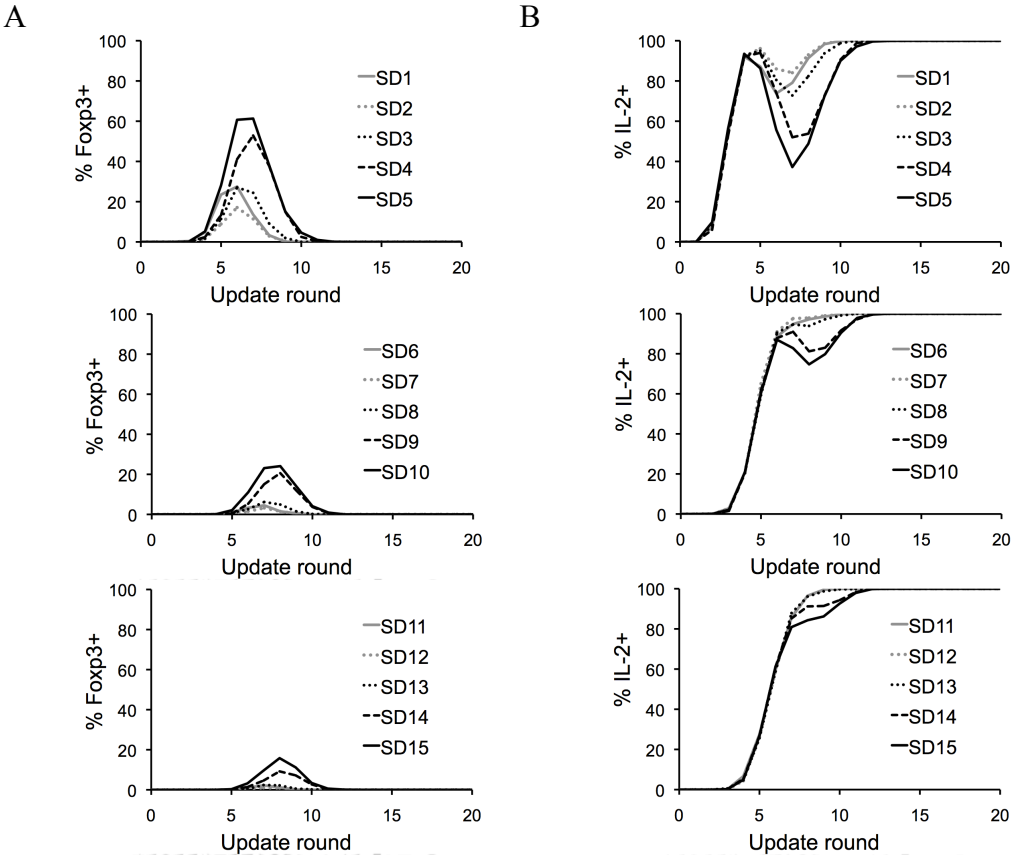
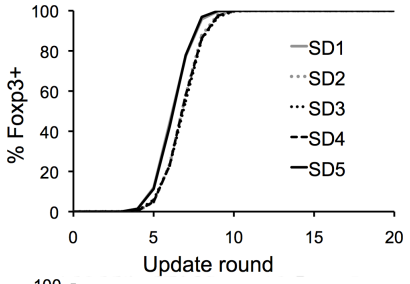
Figure S3. Foxp3 and IL-2 behavior for different delay setups in high and low Ag dose scenarios

We studied Foxp3 and IL-2 response to changes in model delays for setups S_{D1}-S_{D15} (Figure 2D), in high and low Ag dose scenarios. The results show that there is no qualitative difference in the behavior and steady state of these elements. For the high Ag dose scenario, we observe differences in transients, the magnitude and the time of the peak of transients. As expected, when the delay on Akt/mTOR pathway is much longer than the delay on MAPK pathway, the transient of Foxp3 in high Ag dose scenario is much higher (Panel A, top). Delaying MAPK pathway not only reduces the transient magnitude, but also shifts the peak to a later round (Panel A, middle and bottom). In contrast to the high Ag dose scenario, the only effect of delays observed in the low Ag dose scenario is shifting the IL-2 transient to a later round with the increase in MAPK pathway delay (Panel D). The plots show the percentage of trajectories with Foxp3+ (left) or IL-2+ (right) within 1000 trajectories. (A) Foxp3 behavior in high Ag dose scenario. (B) IL-2 behavior in high Ag dose scenario. (C) Foxp3 behavior in low Ag dose scenario. (D) IL-2 behavior in low Ag dose scenario.



C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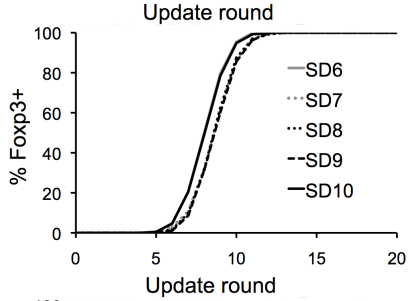
Update round
SD1
SD2
SD3
SD4
SD5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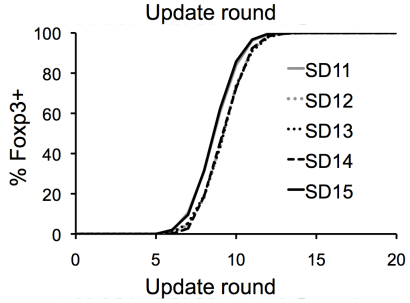
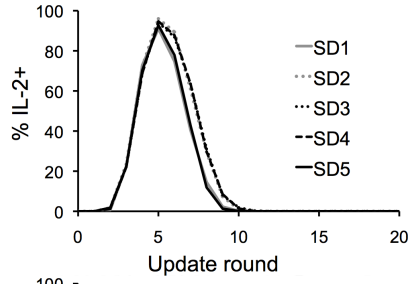
Update round
SD6
SD7
SD8
SD9
SD10


% Foxp3+
Update round
SD11
SD12
SD13
SD14
SD15

D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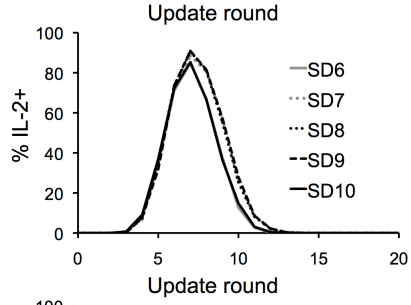
Update round
SD1
SD2
SD3
SD4
SD5


% IL-2+
Update round
SD6
SD7
SD8
SD9
SD10

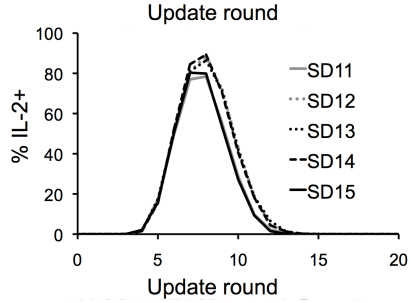

% IL-2+
Update round
SD11
SD12
SD13
SD14
SD15

Figure S4. Initial element values for several scenarios

Element	High Ag dose	Low Ag dose	High Ag dose + TGF- β
TCR*	2	1	2
CD28	1	1	1
Ras	0	0	0
Raf	0	0	0
TAK1	0	0	0
MEK2	0	0	0
MKK7	0	0	0
ERK	0	0	0
JNK	0	0	0
Fos	0	0	0
Jun	0	0	0
AP-1	0	0	0
Ca^{2+}	0	0	0
NF-AT	0	0	0
PKC-θ	0	0	0
NF-κB	0	0	0
PI3K*	0	0	0
PIP3*	0	0	0
PDK1	0	0	0
PTEN	1	1	1
Akt	0	0	0
TSC1-TSC2	1	1	1
RHEB	0	0	0
mTORC1	0	0	0
S6K1	0	0	0
mTORC2	0	0	0
pS6	0	0	0
SMAD3	0	0	0
IL-2 ex	0	0	0
Foxp3	0	0	0
IL-2	0	0	0
IL-2Rα (CD25)	0	0	0
IL-2Rβ (CD122)	1	1	1
IL-2Rγ (CD132)	1	1	1
TGF-β	0	0	1
TGF-βR	0	0	1
JAK3	0	0	0
STAT5	0	0	0

*These elements are encoded with two variables.

Figure S5. Computation of numbers for heat maps

(A) Decimal numbers from trajectory heat maps are computed according to Gray code, which always switches only a single bit between two consecutive decimal values (e.g., (0010 in Gray code) = (3 in decimal) and (0110 in Gray code) = (4 in decimal)), while binary code computes corresponding decimal value according digit position (e.g., (0110 in binary) = (0*2^3 + 1*2^2 + 1*2^1 + 0*2^0 = 6 in decimal)). (B) Examples of encoding two different states in terms of ten element values (Foxp3, IL-2, PTEN, TCR, Ras, CD25, PI3K, Akt, mTORC1, mTORC2).

A

Decimal (used in heat maps)	Gray code (used to compute decimal numbers)	Binary code
0	0000	0000
1	0001	0001
2	0011	0010
3	0010	0011
4	0110	0100
5	0111	0101
6	0101	0110
7	0100	0111
8	1100	1000
9	1101	1001
10	1111	1010
11	1110	1011
12	1010	1100
13	1011	1101
14	1001	1110
15	1000	1111

B

FOXP3	IL-2	PTEN	TCR	RAS	CD25	PI3K	AKT	MTORC1	MTORC2	Decimal assuming Gray code	Decimal assuming binary code
1	0	1	1	1	1	1	0	0	1	849	761
1	0	1	0	1	1	1	0	0	1	814	697

Figure S6. System trajectories for different delay setups in high and low Ag dose scenarios

The heat maps are created following the method described in Materials and Methods. Each heat map shows 1000 simulation trajectories, with each row being a single trajectory from round 0 (initial state), until round 30. The heat maps are shown here for 15 delay setups, S_{D1}-S_{D15} (Figure 2D). The effect of delays on the overall system behavior, both transients and the time to reach steady state can be seen from the changes in colors. Increasing the delay on the MAPK pathway (top to bottom) reduces the transient behavior (red and orange points on the heat map), while increasing the delay on the Akt/mTOR pathway (left to right) increases the percentage of trajectories with transient, as well as the duration of transient. The effect of delays on the overall system behavior in the low Ag dose scenario is only seen in the time when the switch to steady state occurs.

(A) Heat maps in high Ag dose scenario. (B) Heat maps in low Ag dose scenario.

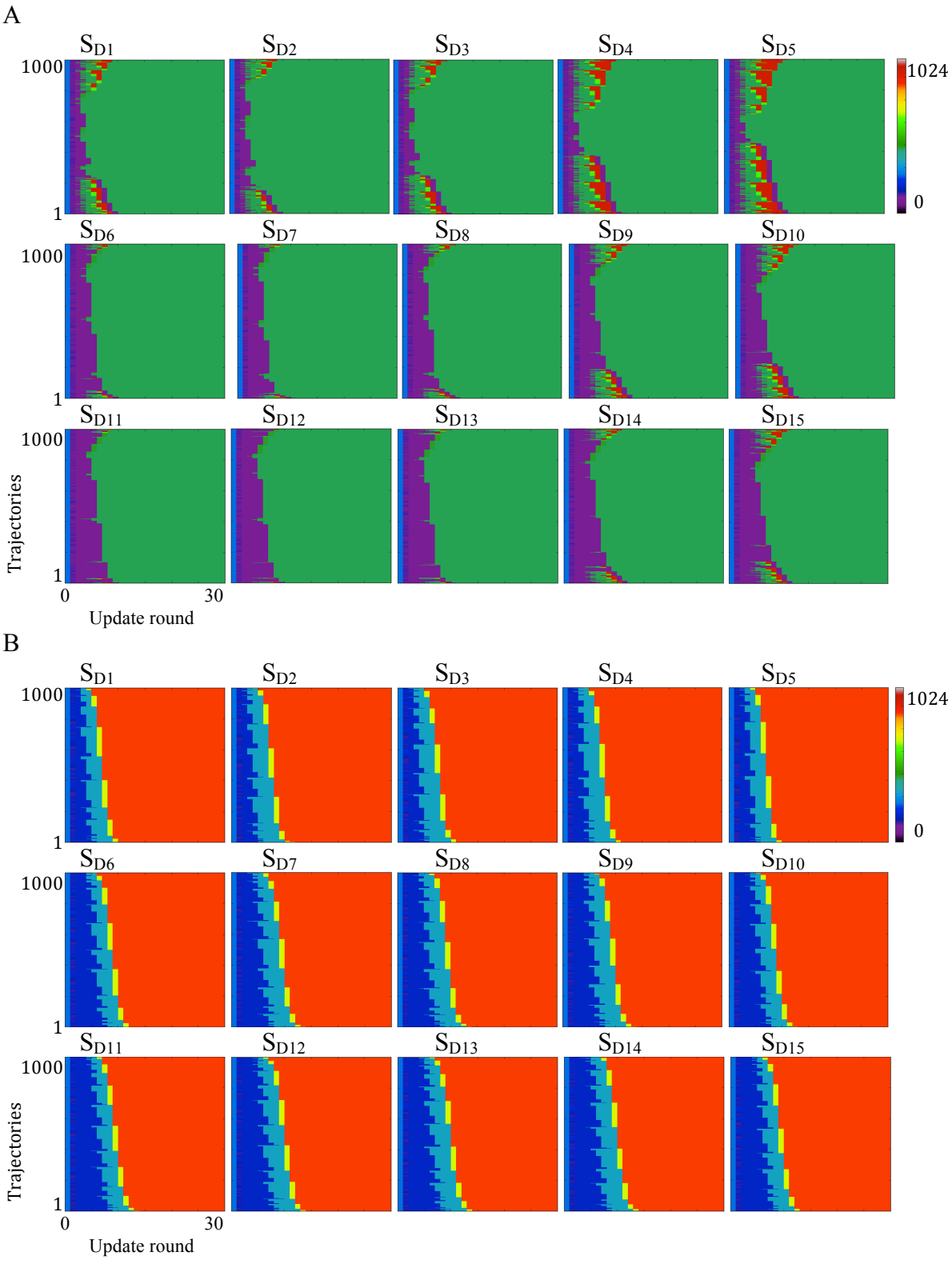

A
S_{D1}
S_{D2}
S_{D3}
S_{D4}
S_{D5}
S_{D6}
S_{D7}
S_{D8}
S_{D9}
S_{D10}
S_{D11}
S_{D12}
S_{D13}
S_{D14}
S_{D15}
1000
1
1024
0
Trajectories
0
30
Update round
B
S_{D1}
S_{D2}
S_{D3}
S_{D4}
S_{D5}
S_{D6}
S_{D7}
S_{D8}
S_{D9}
S_{D10}
S_{D11}
S_{D12}
S_{D13}
S_{D14}
S_{D15}
1000
1
1024
0
Trajectories
0
30
Update round

Figure S7. Attractor A1-A11 occurrence frequency in scenarios T1-T12

This figure shows the differences in attractor presence for different antigen removal scenarios (x axis).

The attractors are grouped according to three main phenotypes, Foxp3- IL-2-, IL-2+ and Foxp3+.

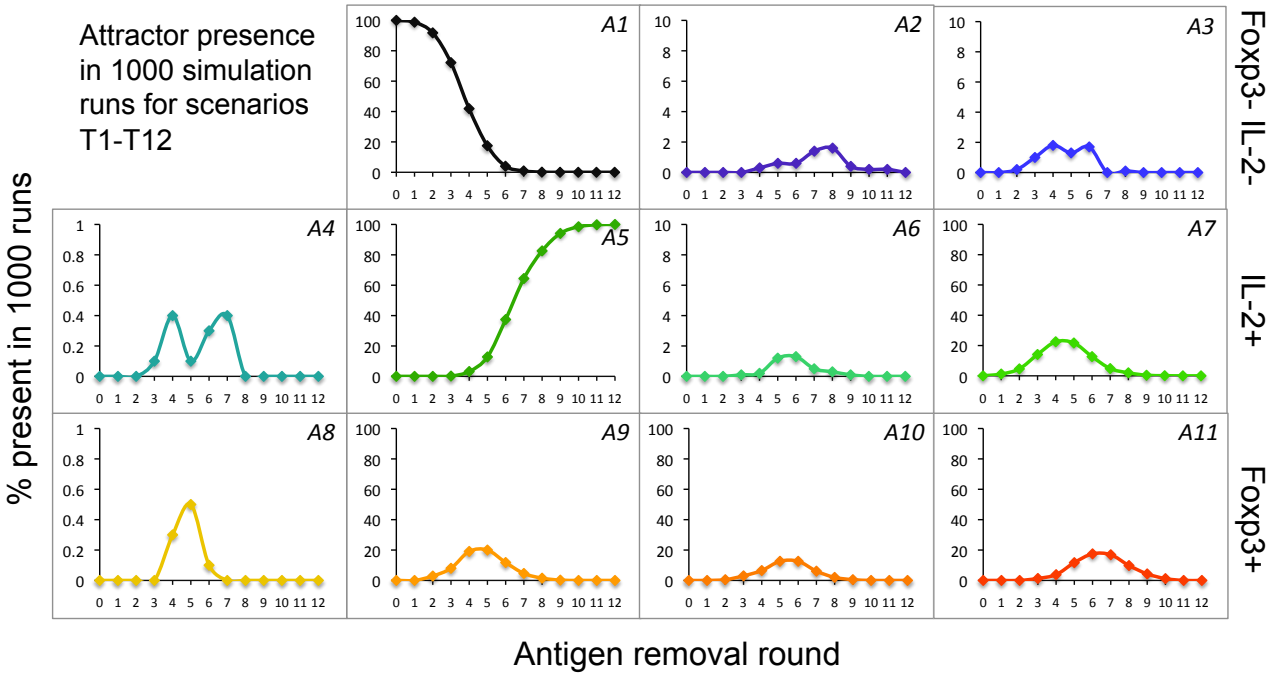
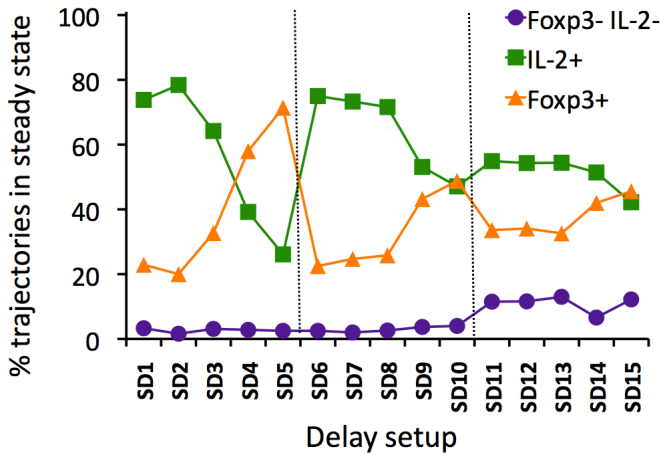


Figure S8. Analysis of effects of different delay setups for high Ag dose + Ag removal scenario

In the scenario of high Ag dose + Ag removal at round 6 (T6), we study the effect of delays on phenotype distribution in steady state, and on transient system behavior for 15 delay setups, S_{D1}-S_{D15} (Figure 2D). Changing the delays alters the relative proportions of Th and Treg cells but all setups result in a mixed Th/Treg population. The results show that the percentage of Foxp3- IL-2- phenotype always remains low, and that it only slightly increases in the last five delay setups that have the longest MAPK pathway. The effect on the ratio between Foxp3+ and IL-2+ phenotypes is much stronger when MAPK delay is small compared to the Akt/mTOR pathway delay (S_{D1}-S_{D5}). The heat maps are created following the method described in Materials and Methods. Each heat map shows 1000 simulation trajectories, with each row being a single trajectory from round 0 (initial state), until round 30. (A) Changes in distribution of Foxp3-IL-2-, Foxp3+, and IL-2+ phenotypes for the 15 delay setups. (B) Heat maps of system trajectories in scenario T6, for the 15 delay setups.

A



B

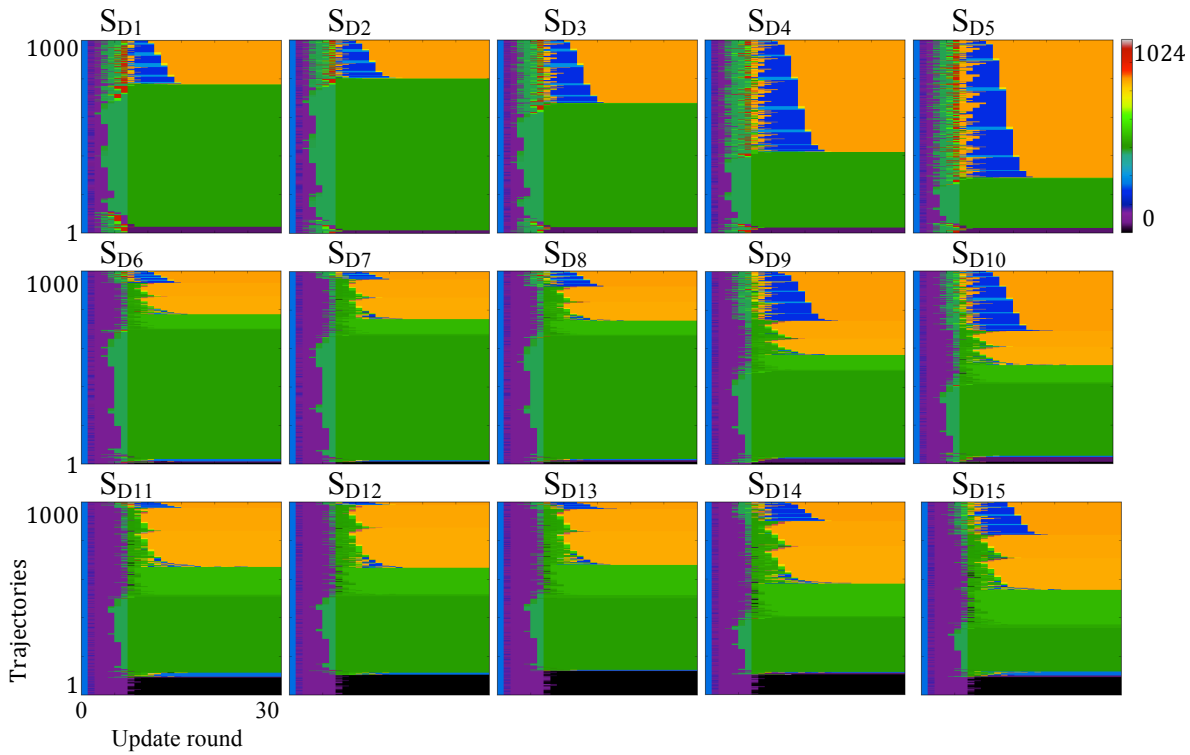
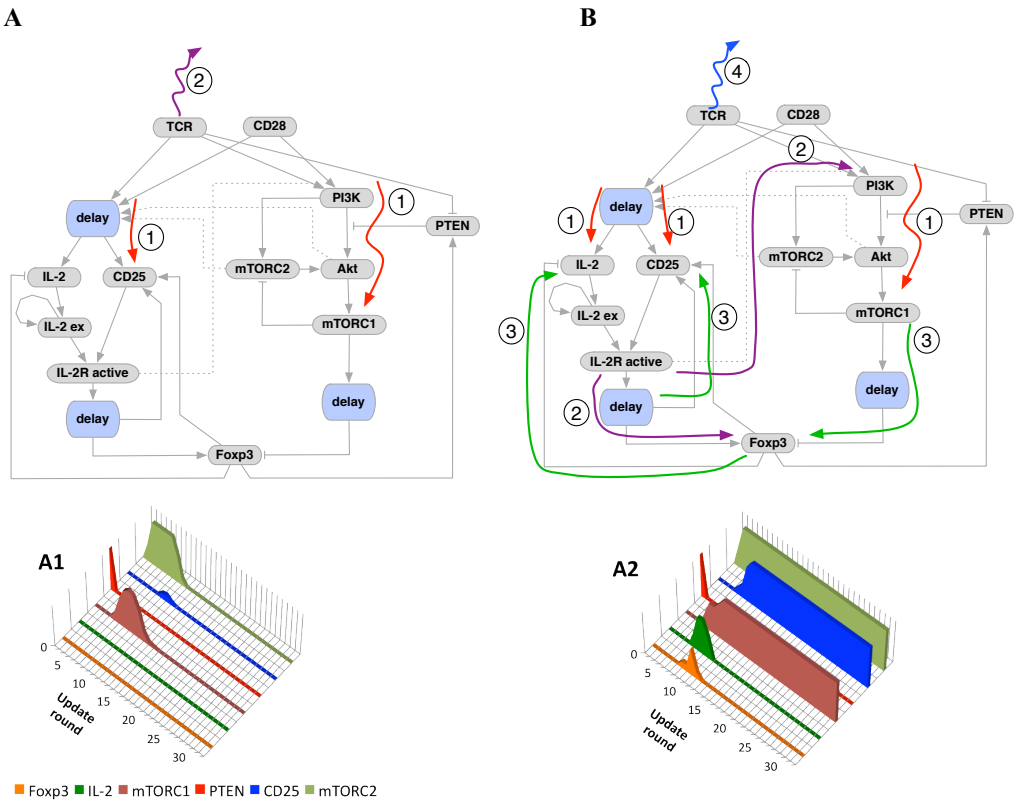

S_{D1}
S_{D2}
S_{D3}
S_{D4}
S_{D5}
S_{D6}
S_{D7}
S_{D8}
S_{D9}
S_{D10}
S_{D11}
S_{D12}
S_{D13}
S_{D14}
S_{D15}
1000
1
1024
0
Trajectories
0
30
Update round

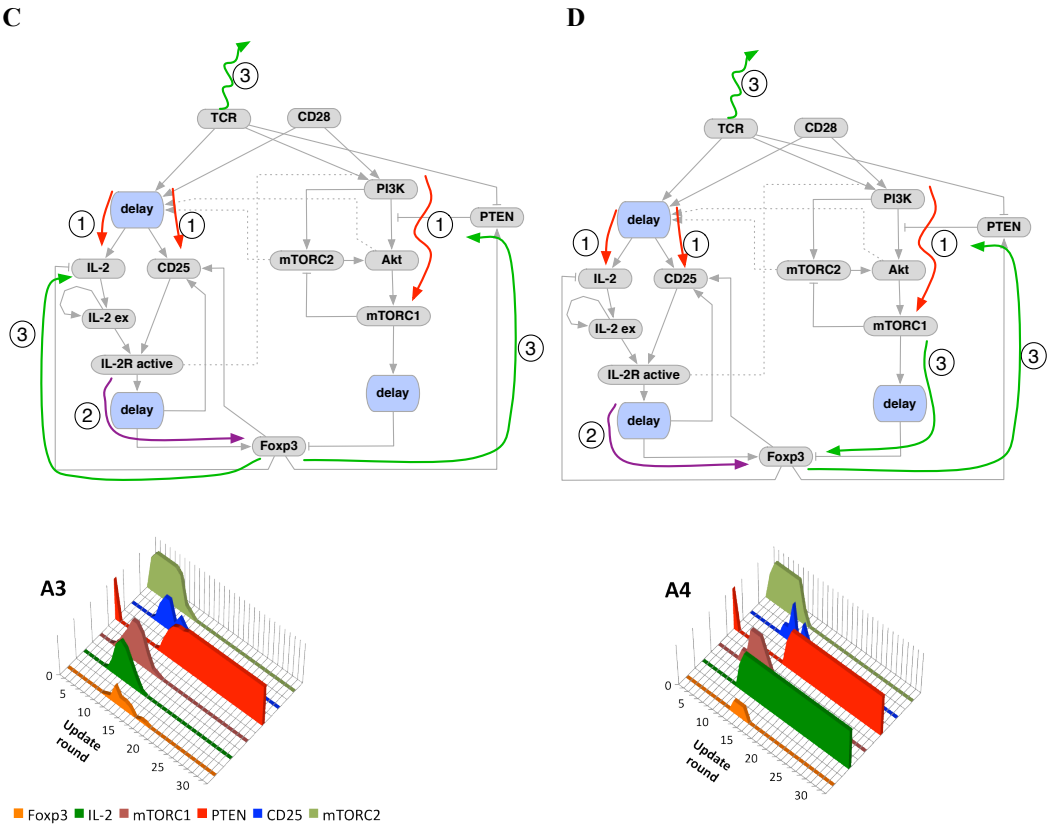
Figure S9. Signal propagation and element trajectories of different attractors in scenario T6

This figure shows signaling paths, feedback and feed-forward loops critical for fate decision in scenario T6 (see Figure 6), and element trajectories from initial to steady state, averaged across simulations leading to a specific attractor.



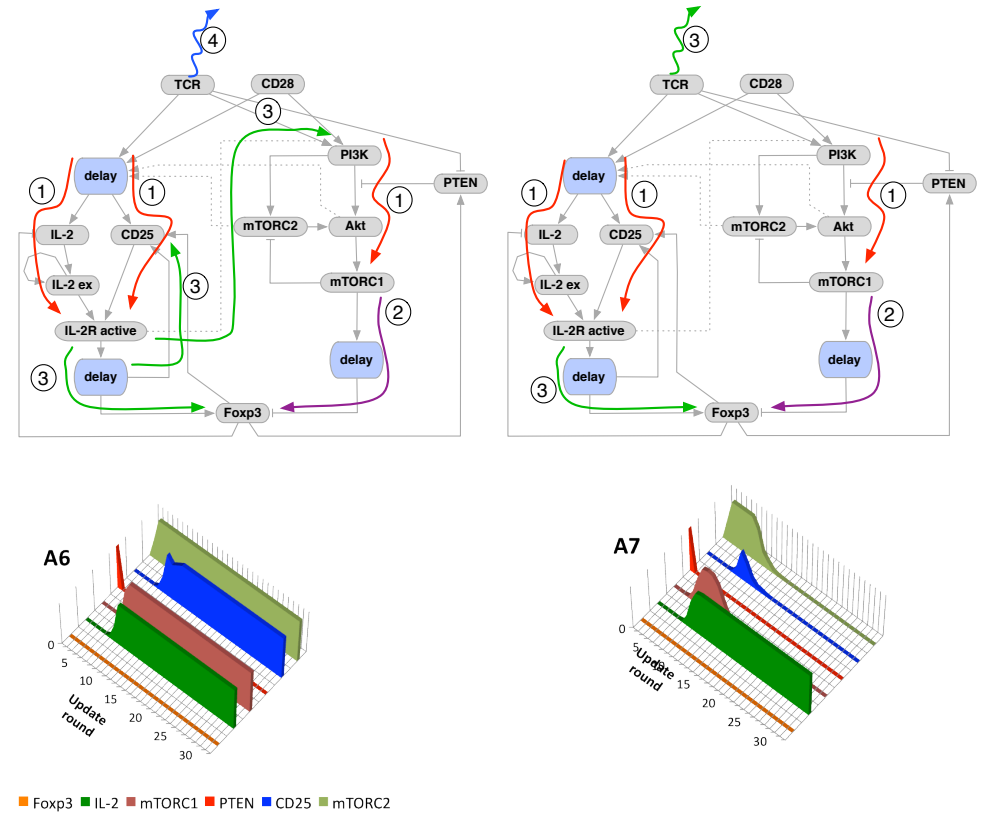
(A) Attractor A1, 40 trajectories: CD25 is activated on a small number of paths (1), mTORC1 and mTORC2 are activated (1), but antigen removal occurs early enough (2) to prevent any other element activation, and also leads to switching CD25, mTORC1 and mTORC2 back to the "off" state (2). Propagation of activation is slow, while propagation of the TCR "off" signal occurs quickly.

(B) Attractor A2, 6 trajectories: IL-2, CD25, mTORC1 and mTORC2 are activated early (1). Fast activation of positive branch of global iFFL leads to STAT5 activation and expression of Foxp3 (2), as well as the regulation of PI3K/Akt/mTORC1 path by IL-2R signaling (2). This is followed by activation of positive feedback between CD25 and STAT5 (3) and negative feedback from Foxp3 to IL-2 (3). At the same time, the inhibiting effect from mTORC1 on Foxp3 takes place (3). Finally, antigen removal (4) does not allow IL-2 to switch back to "on" state once Foxp3 is "off".



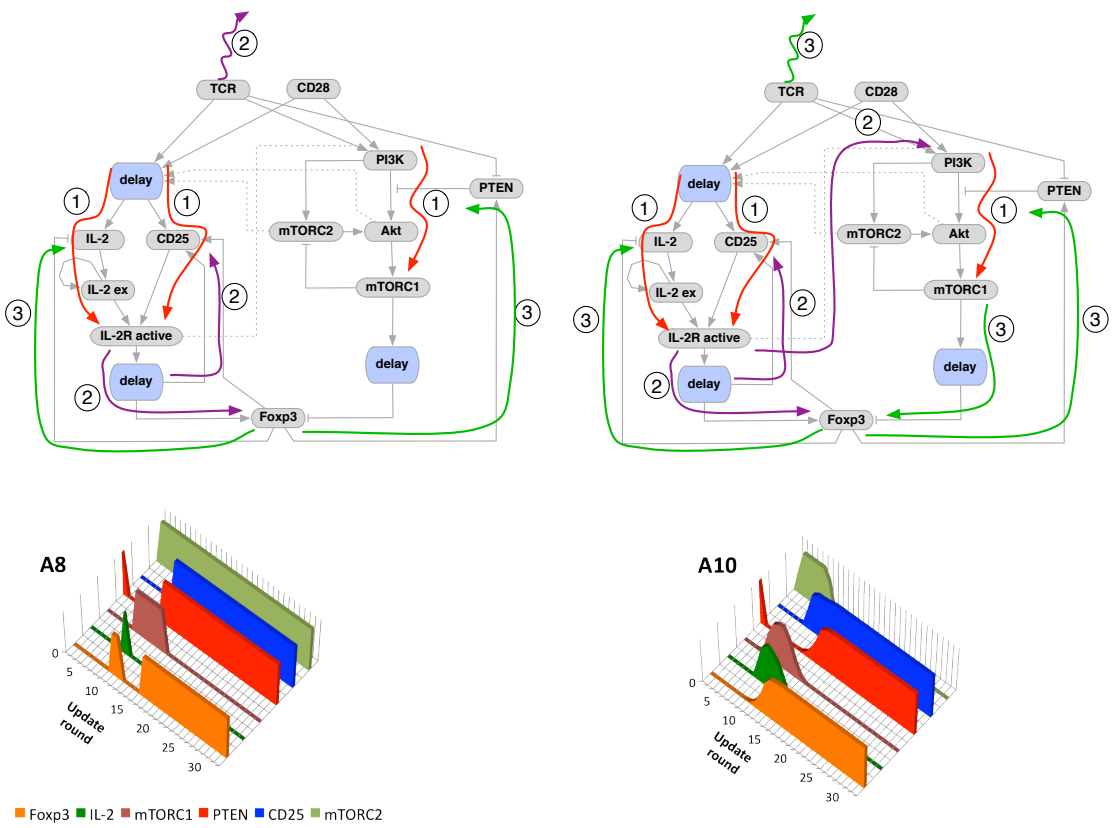
(C) Attractor A3, 17 trajectories: IL-2, CD25, mTORC1 and mTORC2 are activated early (1). Fast activation of positive branch of global iFFL leads to STAT5 activation and expression of Foxp3 (2), which is followed by negative feedback from Foxp3 to IL-2 (3). The feedback between CD25 and STAT5 is not established due to delay: activation of CD25 propagates to STAT5, but CD25 is switched "off" due to antigen removal (3), before STAT5 activation feeds back to it. Active STAT5 will briefly re-activate CD25, but eventually both CD25 and STAT5 are turned to "off" state. Foxp3 is expressed long enough to activate PTEN when antigen is removed (3). Since IL-2R and STAT5 are not active, Foxp3 is permanently switched to "off" state, as well as the PI3K/Akt/mTOR pathway.

(D) Attractor A4, 3 trajectories: Very similar to A3, except that Foxp3 is active very briefly and therefore it does not inhibit IL-2, allowing for longer expression and secretion of IL-2. Delayed negative branch of iFFL leading to Foxp3 inhibition, timely feedback from Foxp3 to PTEN, delayed feedback between CD25 and STAT5, and delayed feedback from Foxp3 to IL-2 are critical for this attractor.



(E) Attractor A6, 13 trajectories: Very similar to A5, the only difference is in Ras (not shown), which is “on” in attractor A5, and “off” in A6. IL-2, CD25, mTORC1 and mTORC2 are activated early (1). On most paths, negative branch of iFFL is activated faster, leading to inhibition of Foxp3 by mTOR (3). Activation of most of the paths occurs early enough, such that antigen removal (4) does not affect already established cell fate. Feedback between CD25 and STAT5 is not delayed, and thus, CD25 and STAT5 are active in steady-state. IL-2R positively regulates PI3K pathway, thus mTOR remains active after antigen removal.

(F) Attractor A7, 127 trajectories: IL-2, CD25, mTORC1 and mTORC2 are activated early (1). On most paths, inhibition of Foxp3 by mTOR (2) takes place before its activation (3). Antigen removal occurs early enough (3), switching “off” the PI3K/Akt/mTOR path, as well as preventing activation of positive feedback between CD25 and STAT5.

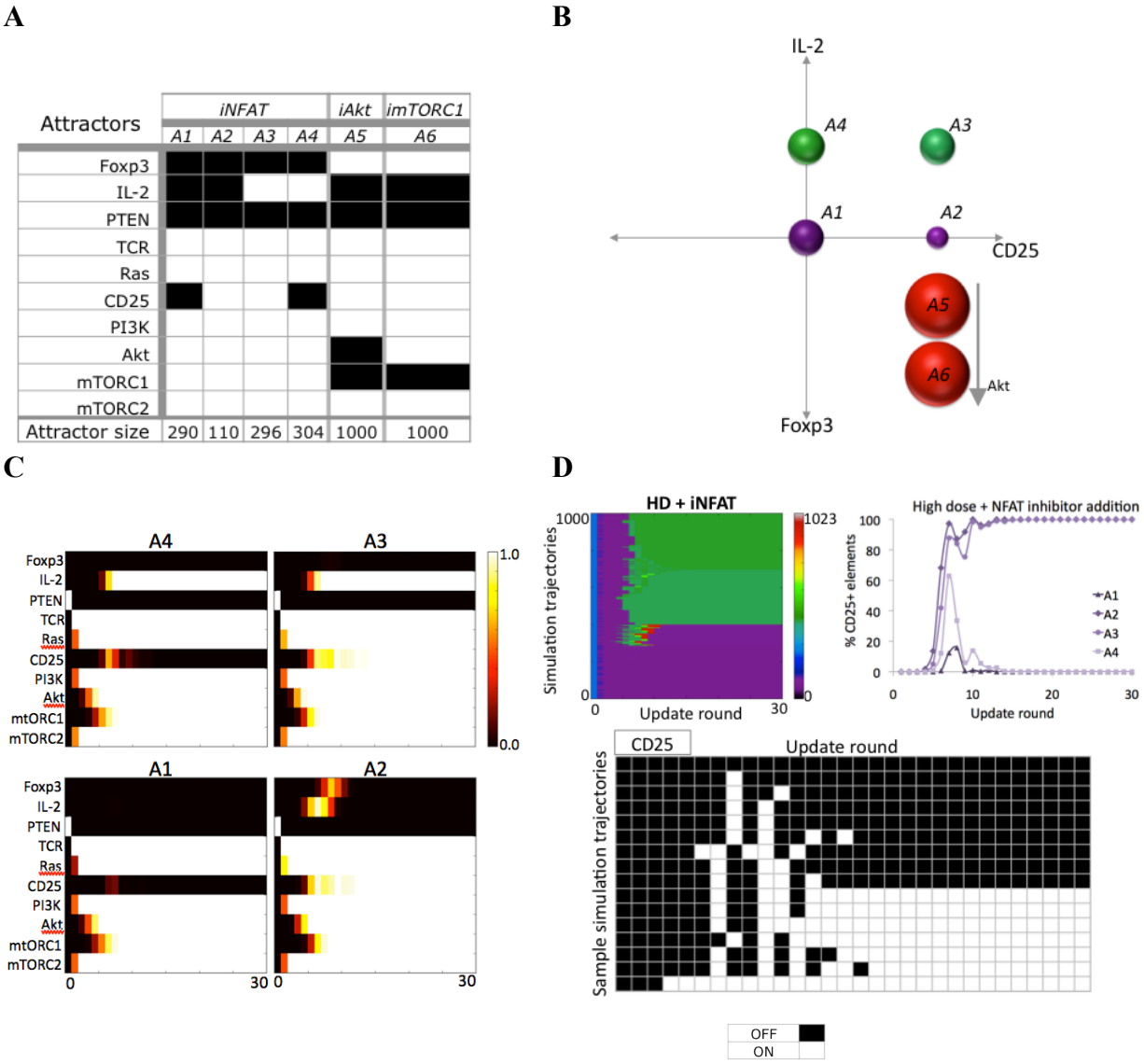


(G) Attractor A8, 1 trajectory: Very similar to attractor A11, the only difference being in activation of Ras (not shown), which is active in A11, but not in A8. IL-2, CD25, mTORC1 and mTORC2 are activated early (1). Propagation of activation is quick, positive feedback between CD25 and STAT5 is activated (2), and positive regulation of Foxp3 (2) in the iFFL occurs faster than negative regulation (3), leading to inhibition of IL-2 (3). This also allows for PI3K/Akt/mTORC1 path to remain active (2). Foxp3 expressed on time to activate PTEN when antigen is removed (3). At the same time, the inhibiting effect from mTORC1 takes place (3), leading to brief inactivation of Foxp3. However, PTEN eventually inhibits mTOR, leading to re-activation of Foxp3.

(H) Attractors A9, 118 trajectories, and A10, 126 trajectories: The only difference is in activation of Ras (not shown), which is active in A10, but not in A9. IL-2, CD25, mTORC1 and mTORC2 are all activated first (1). Early activation of STAT5, leads to activation of STAT5/CD25 positive feedback (2), and expression of Foxp3 (2), which then inhibits IL-2 (3). Positive regulation of PI3K/Akt/mTOR pathway by IL-2R does not occur on time to keep mTORC2 in "on" state. Foxp3 activates PTEN when antigen is removed (3), mTORC1 is inhibited by PTEN before it affects Foxp3.

Figure S10. Transient behavior and phenotypes in the presence of inhibitors and high Ag dose

In this analysis, all attractors are added at simulation round 6.



(A) 4 attractors are observed when NFAT inhibitor is added (iNFAT case, attractors A1-A4), and a single attractor is observed for adding either Akt (iAkt, attractor A6) or mTORC1 (imTORC1, attractor A6) inhibitor; steady state values of ten elements (Foxp3, IL-2, PTEN, TCR, Ras, CD25, PI3K, Akt, mTORC1, mTORC2) in these attractors, and the number of simulations trajectories ending in each attractor for 1000 simulation runs. (B) Attractors, sizes of attractors and their relationships with respect to key differentiating elements. (C) Trajectories of 10 elements (Foxp3, IL-2, PTEN, TCR, Ras, CD25, PI3K, Akt, mTORC1, mTORC2), averaged across all simulation runs leading to a given attractor (A1-A4). (D) Heat map of all 1000 simulation trajectories (top left). In all

four attractors resulting from addition of NFAT inhibitor CD25 oscillates several times before reaching the steady value (top right). Oscillations are not resulting from averaging across simulations: oscillations exist within a single run, shown here for a sample of trajectories (bottom).

Figure S11. Analysis of effects of switching model elements OFF

We analyzed effects of switching individual model elements OFF on the transient behavior and steady-state values of Foxp3, IL-2 PTEN, CD25, mTORC1, and mTORC2, for the following scenarios: high Ag dose (HD), low Ag dose (LD), high Ag dose + Ag removal at round 6 (T6), and high Ag dose + TGF-β (HD + TGF-β).

In high Ag dose scenario, given two model elements, $Ei \in$ {26 model elements that are turned OFF (see tables A to D)} and $Ej \in$ {Foxp3, IL-2 PTEN, CD25, mTORC1, mTORC2}, there are four different effects that can be observed from turning OFF a model element *Ei*: (*i*) it does not have an effect on an element *Ej*, (*ii*) it switches the element *Ej* ON (if *Ei* is its negative regulator), (*iii*) it switches the element *Ej* OFF (if *Ei* is its necessary positive regulator), and (*iv*) does not change qualitatively element *Ej*, but it changes its behavior in time (slows down or speeds up the transient, increases or decreases transient magnitude), or its average steady state value. In low Ag dose scenario, since PTEN plays a key role, turning PTEN OFF results in oscillation of all elements *Ej*, due to the negative feedback loop between Akt, mTORC1 and mTORC2.

In the original T6 scenario, average steady-state value for many elements is different from OFF ('0') and ON ('1'). Turning OFF elements on Akt/mTOR and CD25/STAT5 pathways affects the transient behavior of elements *Ej*, as well as their average steady-state value. The only element that exhibits slightly different behavior in this scenario is mTORC2, which either has a similar transient behavior like in the original case, but returns to OFF in steady-state (when turning OFF elements on MAPK pathway, NFAT, NFκB, or CD25/STAT5 pathway), or it follows exactly the same behavior as in the original scenario. We also analyzed the effect of timing in turning OFF NFAT, Akt, and mTORC1, which effectively represents addition of inhibitors to the system. We observed that the timing does not affect the results of adding Akt and mTORC1 inhibitor, while adding NFAT inhibitor (which is activated early in simulations by TCR) can result in different phenotypes, if added at later rounds (see fig. S10 for more details).

A

Response	High Ag dose (HD) + switching OFF element:																								
	CD28	PKC-θ	Ras	AP1	Jun	Fos	ERK	JNK	NFAT	NFκB	IL-2	CD25	STAT5	JAK3	Foxp3	PI3K_HIGH	PI3K_LOW	PTEN	PIP3_HIGH	PIP3_LOW	PDK1	Akt	mTORC1	mTORC2	S6K1	Smad3	Original
Foxp3	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,1	=,=	=,=	-,1	=,=	-,1	-,1	-,1	-,1	=,=	=,=	✓,0
IL-2	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	=,=	=,=	=,=	∧,1	∨,0	=,=	=,=	∨,0	=,=	∨,0	∨,0	∨,0	∨,0	=,=	=,=	✓,1
PTEN	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	✓,0
CD25	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	=,=	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	✓,1
STAT5	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	✓,1
mTORC1	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	-,0	=,=	-,0	-,0	-,0	=,=	=,=	=,=	✓,1
mTORC2	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	✓,1

B

Response	Low Ag dose (LD) + switching OFF element:																								
	CD28	PKC-θ	Ras	AP1	Jun	Fos	ERK	JNK	NFAT	NFκB	IL-2	CD25	STAT5	JAK3	Foxp3	PI3K_HIGH	PI3K_LOW	PTEN	PIP3_HIGH	PIP3_LOW	PDK1	Akt	mTORC1	mTORC2	S6K1	Smad3	Original
Foxp3	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	=,=	-,0	∨,∞	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	✓,1
IL-2	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧,1	∧,1	∧,1	∧,1	=,=	-,0	∨,∞	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	✓,0
PTEN	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	=,=	=,=	=,=	=,1	=,=	=,=	-,1
CD25	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	=,=	=,=	=,=	=,=	-,0	∨,∞	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	✓,1
STAT5	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	=,=	=,=	-,0	∨,∞	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	✓,1
mTORC1	-,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
mTORC2	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	∨,∞	∨,∞	∨,∞	=,=	=,=	=,=	-,0	=,=	=,=	✓,1

Legend:

The first component in each table entry describes element transient behavior between initial state and final simulated state, the second component describes average element value in steady-state across 1000 simulations.

Transient:

✓: Single or double transient exists in the original case

= : Transient behavior same as in Original for the given scenario

- : No transient

∧ : Transient exists, higher than original

∨ : Transient exists, lower than original

Steady-state value:

[0,1] : Steady-state value is in this interval

= : Steady state value same as in Original for the given scenario

∞ : Oscillatory behavior, not reaching steady-state

d : Delayed transient when compared to original case

£ : Takes very long to reach steady state (e.g., at round 19)

€ : Increases to 0.8, then decreases and settles at steady state value 0.4 at round 20

¥ : Oscillates early and then settles at steady-state at round 16

C

Response	HD + Ag removal (T6) + switching OFF element:																								
	CD28	PKC-θ	Ras	AP1	Jun	Fos	ERK	JNK	NFAT	NFκB	IL-2	CD25	STAT5	JAK3	Foxp3	PI3K_HIGH	PI3K_LOW	PTEN	PIP3_HIGH	PIP3_LOW	PDK1	Akt	mTORC1	mTORC2	S6K1	Smad3	Original
Foxp3	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧, 0.6	=,=	∨, 0.3	∧, 0.6	=,=	∧, 0.6	∧, 0.6	∧, 0.75	∧, 0.6	=,=	=,=	✓, 0.5[£]
IL-2	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧, 0.9	∧, 0.9	∧, 0.9	∧, 0.9	∨, 0.2	=,=	=,=	∨, 0.2	=,=	∨, 0.2	∨, 0.2	∨, 0.15	∨, 0.2	=,=	=,=	✓, 0.4[€]
PTEN	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧, 0.65	=,=	-,0	∧, 0.65	=,=	∧, 0.65	∧, 0.65	∧, 0.75	∧, 0.65	=,=	=,=	✓,0.5
CD25	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∨,0	-,0	∨,0	∨,0	=,=	∨, 0.6	=,=	=,=	∨, 0.6	=,=	∨, 0.6	∨, 0.6	=,=	∨, 0.6	=,=	=,=	✓, 0.7[¥]
STAT5	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	=,=	∨, 0.6	=,=	=,=	∨, 0.6	=,=	∨, 0.6	∨, 0.6	=,=	∨, 0.6	=,=	=,=	✓,0.7
mTORC1	-,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∨,0	∧, 0.4	-,0	=,=	∧, 0.4	-,0	=,=	-,0	-,0	-,0	-,0	=,=	=,=	✓, 0.25
mTORC2	-,0	=,0	=,0	=,0	=,0	=,0	=,0	=,0	=,0	=,0	=,0	=,0	=,0	=,0	=,=	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	✓,0.4

D

Response	HD + TGF-β + switching OFF element:																								
	CD28	PKC-θ	Ras	AP1	Jun	Fos	ERK	JNK	NFAT	NFκB	IL-2	CD25	STAT5	JAK3	Foxp3	PI3K_HIGH	PI3K_LOW	PTEN	PIP3_HIGH	PIP3_LOW	PDK1	Akt	mTORC1	mTORC2	S6K1	Smad3	Original
Foxp3	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	∨,0	✓,1
IL-2	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧,1	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧,1	-,0
PTEN	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	✓,0
CD25	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	d,1	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	d,1	✓,1
STAT5	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧,1	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	-,0	∧,1	-,0
mTORC1	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	-,0	=,=	-,0	-,0	-,0	-,0	=,=	=,=	✓,1
mTORC2	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	=,=	=,=	=,=	=,=	=,=	-,0	=,=	=,=	✓,1

E

Response	HD + Inhibitor addition at different times											
	iNFAT				iAkt				imTORC1			
	$T_{iNFAT}0$*	$T_{iNFAT}3$	$T_{iNFAT}6$	$T_{iNFAT}9$	$T_{iAkt}0$	$T_{iAkt}3$	$T_{iAkt}6$	$T_{iAkt}9$	$T_{imTORC1}0$	$T_{imTORC1}3$	$T_{imTORC1}6$	$T_{imTORC1}9$
Foxp3	No activation	No activation	Small transient	Higher transient	Always switches to 1, with transient starting later T0->T9				Always switches to 1, with transient shifted later T0->T9			
IL-2	No activation	No activation	Steady state 0.6	Steady state 0.9	Always switches back to 0, with transient starting later T0->T9				Always switches to 0, with transient starting later T0->T9			
PTEN	No effect				No effect				No effect			
CD25	No activation	No activation	Steady state 0.4	Steady state 0.95	No effect				No effect			

Figure S12. Analysis of effects of changing initial conditions

We analyzed the effects of randomizing initial values of model elements. We study three different cases: R1 –initial values of model elements (not including stimulations and genes, the latter being set to OFF) are random, R2 - initial values of model elements (not including stimulations, including genes) are random, and R3 - initial values of all model elements are random. Cases R1 and R2 are simulated for four different scenarios, high Ag dose (HD) stimulation, high Ag dose + TGF-β (HD + TGF-β), low Ag dose (LD) stimulation, and no stimulation present. In case R3 all elements are set to random, so we consider only one scenario here. The heat maps show 2000 system trajectories. The HD (first column) and HD + TGF-β (second column) scenarios steady states are not affected by random initial conditions, and only show some delay in reaching steady state. The largest impact of randomizing initial element values is observed in the LD (third column) scenario, where randomizing initial values results in a mixed population of phenotypes in steady state. In the case of no stimulation (fourth column, first and second row), most of the trajectories show no activation, although there is a small percentage of trajectories (orange and green) that result in activation.