Supplement

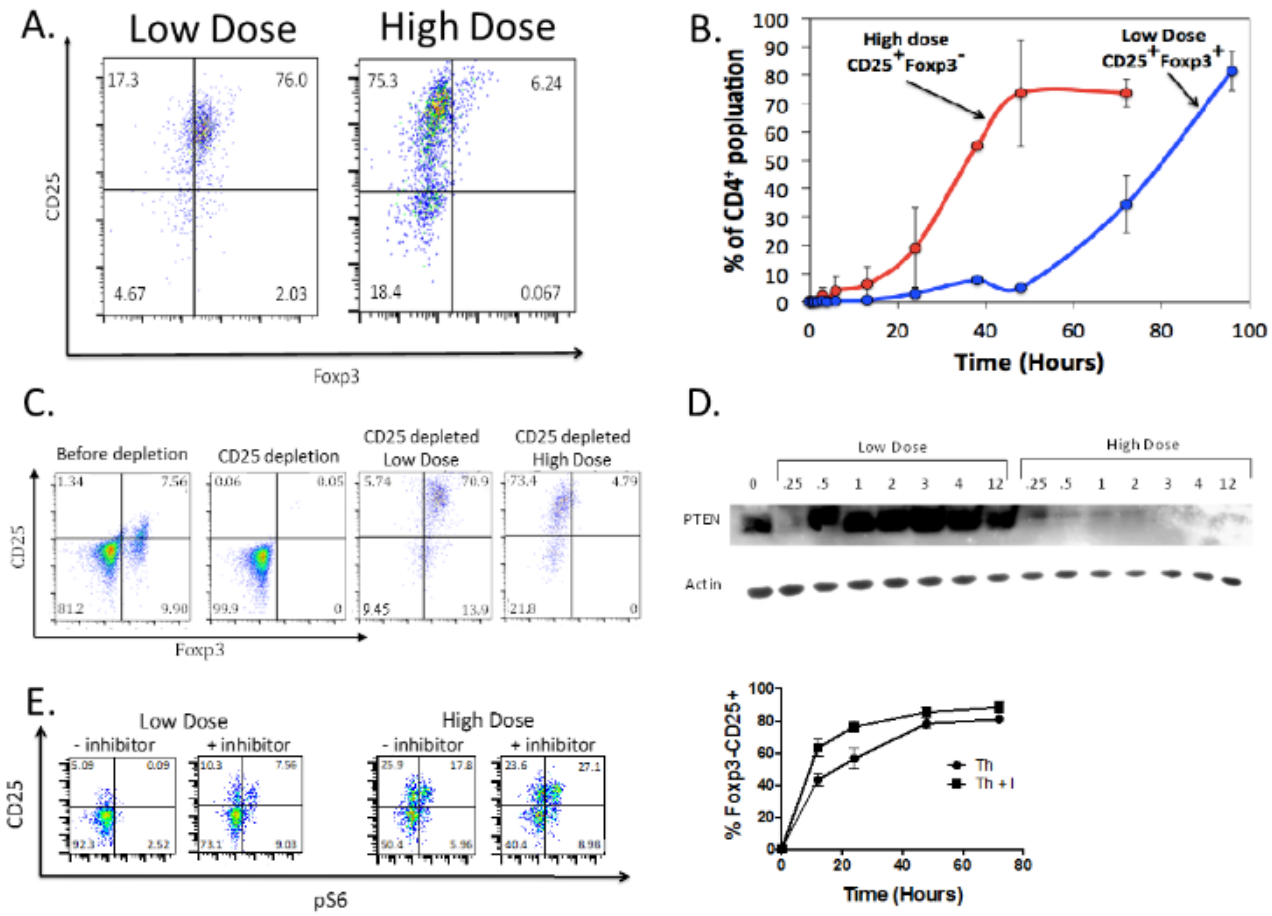

Figure S1. Low dose stimulation promotes Treg induction, while high dose stimulation promotes Th induction and PTEN inhibition enhances Th induction.

$CD4^+$ T cells were stimulated under low and high dose stimulation and gated $CD3^+CD4^+$ were stained for Foxp3 and CD25 after 72 and 96 hours of stimulation **(A and B)**. Low dose stimulation yields mostly $CD25^+Foxp3^+$ cells, which are defined as Treg (left panel A). High dose stimulation yielded mostly $CD25^+Foxp3^-$ cells, which are defined at Th (right panel A). **(B)** A time course of Th and Treg development under high (red line) and low (blue line) conditions. Results shown represent the mean ± SEM of three similar experiments **(C)** CD25 depletion was performed to remove nTregs from the sample. Flow cytometry of CD25 and Foxp3 expression on gated $CD3^+$ $CD4^+$ T cells before (far left panel) and after (left panel) depletion and following stimulation with low and high dose anti-CD3 (right and far right panels. **(D)** Following CD25 depletion, $CD4^+$ T cells were activated under low and high dose stimulation and samples were analyzed for total PTEN. The results in C and D are representative of two similar experiments. **(E)** $CD4^+$ T cells were activated under high and low dose stimulation with or without a PTEN inhibitor (SF1670, 10 μM) and gated $CD3^+CD4^+$ T cells were stained for pS6 and CD25 to follow activation at twelve hours of activation (left and center panels). High dose stimulation in the presence or absence of PTEN inhibitor resulted in the generation of similar numbers of Th cells (right panel). Results shown represent the mean ± SEM of three similar experiments

	Logical Rule	Description and Reference
1	TCR' = TCR_LOW or TCR_HIGH	TCR signaling can be induced by high or low antigen doses.
2	CK2' = TCR_HIGH	CK2 is activated under high antigen doses (26)
3	MEK1' = TCR	MEK1 is activated under both high and low antigen doses (32)
4	PIP3' = (TCR_LOW or TCR_HIGH) and not PTEN_active	PIP2 is converted to PIP3 under both high and low antigen doses. It is converted back to PIP2 by PTEN catalytic activity
5	MTORC2' = (TCR_LOW or TCR_HIGH) and not AKT	MTORC2 is activated under both high and low antigen doses. It is deactivated through effectors, downstream of AKT (31)
6	AKT' = PIP3 and MTORC2	AKT is activated in the presence of PIP3 and MTORC2 (11)
7	PTEN_total' = FOXO1 and not NEDD4	PTEN levels increase due to transcription by FOXO1 (this paper). PTEN is ubiquitinated by NEDD4, leading to proteasomal degradation (19).
8	PTEN_active' = PTEN_total and MEK1 and not CK2	PTEN is activated through localization to the membrane induced by MEK1 (30) It is deactivated through phosphorylation by CK2 (25).
9	FOXO1' = not AKT	FOXO1 is deactivated through phosphorylation by AKT (27)
10	NEDD4’ = TCR_HIGH	NEDD4 is activated by TCR signaling.

Figure S2. *Update rules for extended logical model including FoxO1.* A minimized version of the model as diagramed in Figure 3E was specified as a Boolean model using the logical rules shown here. These rules are used to determine the next state of each element in the system as a logical function of its inputs, which are binary variables that take the values 0, representing the inactive state, or 1, representing the active state. The logical functions used in the rules are constructed using standard operators such as AND, OR, and NOT. For example, mTORC2 activation is described by the following rule: MTORC2′ = (TCR_LOW or TCR_HIGH) and not AKT, meaning that mTORC2 is activated when either TCR_LOW or TCR_HIGH input is present and AKT is inactive. At the initial time of the simulation, all variables are set to inactive except PTEN_total, FoxO1, and either TCR_LOW or TCR_HIGH depending on the stimulation scenario. The system is then allowed to evolve in time according to the General Asynchronous update scheme (15). One simulation unit is the mean firing interval of a single Boolean update rule in the system. For the simulations performed here, the logical rules were translated into the BioNetGen rule-based modeling language using an automated tool called Boolean2BNGL included in the BioNetGen package (14) (http://bionetgen.org). Both the Boolean model rules and the derived BioNetGen language file are available online at http://bionetgen.org/PTEN_model. The BioNetGen model was using a modified version Gillespie’s Direct Stochastic Simulation Algorithm (16). Note: reference numbers refer to the references in the main text.