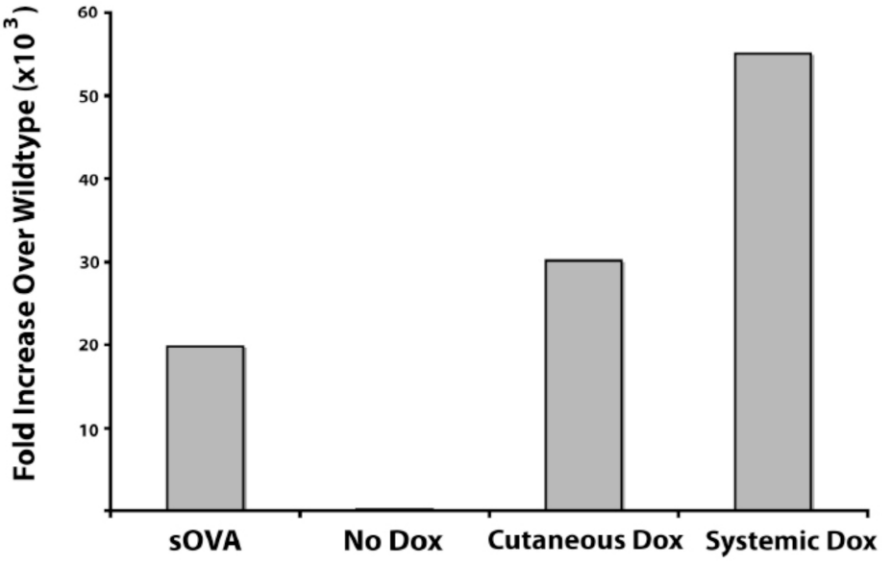
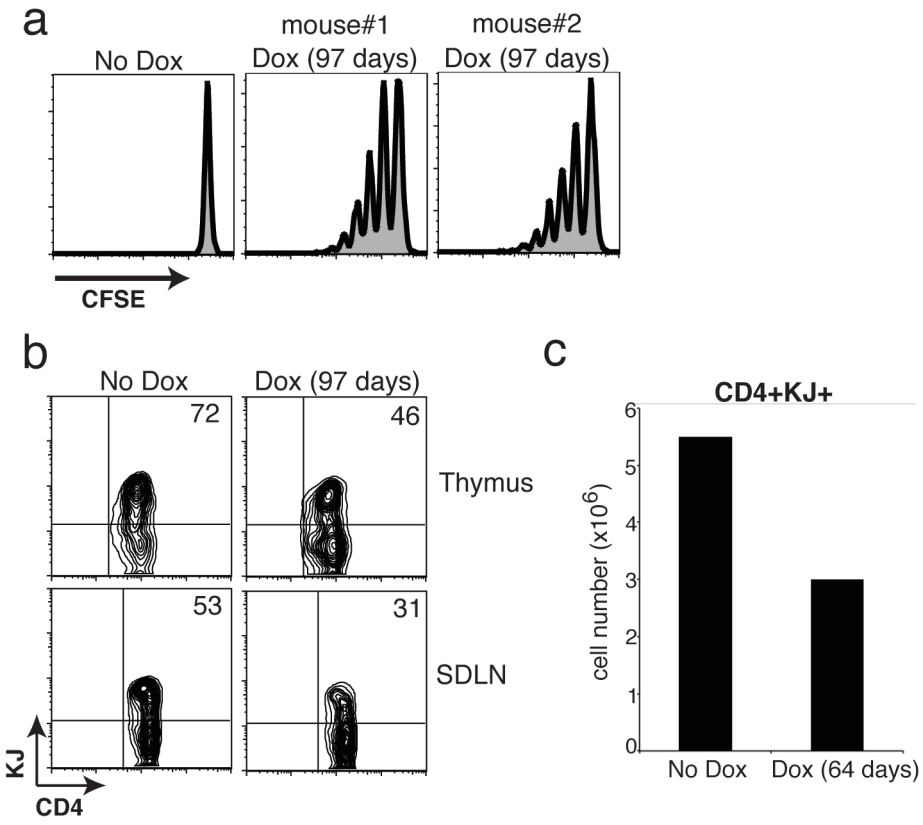
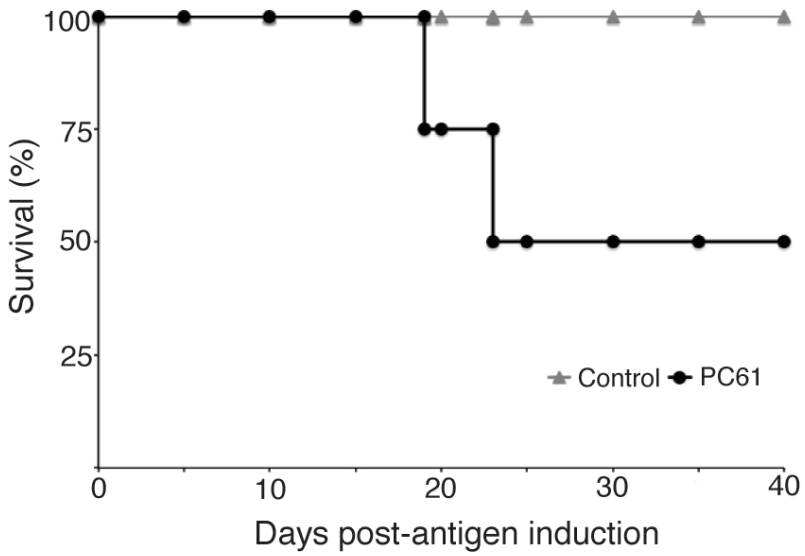


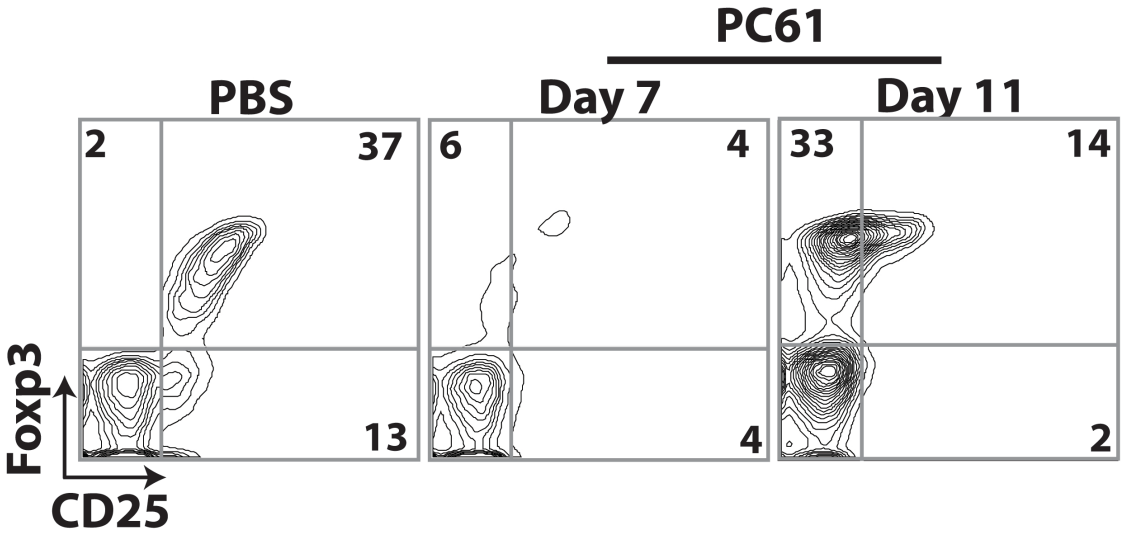
Supplementary Figure 1. Expression of Ova in the epidermis after induction with doxycycline. Ova-specific RT-PCR of epidermal cell suspensions isolated from K5/TGO mice after treatment with topical doxycycline ("Cutaneous Dox") or maintenance on doxycycline chow ("Systemic Dox"). Data are normalized to background expression from epidermal cells isolated from wildtype Balb/c mice. sOVA group represents liver tissue harvested from mice constitutively expressing a soluble for of Ova (positive control). Results are representative of 2 replicate experiments.



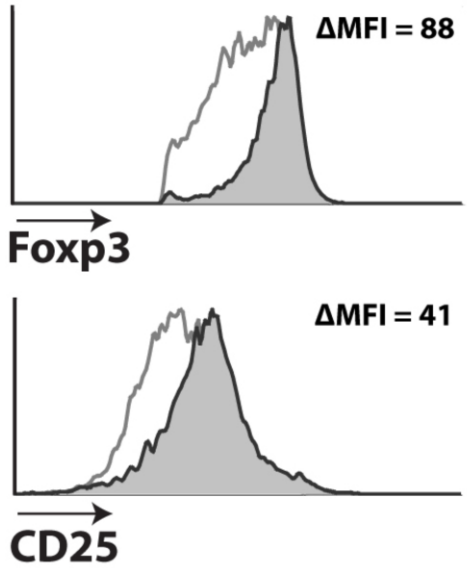
Supplementary Figure 2. **Expression of Ova in the periphery and continued generation of Ova-specific T cells in K5/TGO/DO11 mice maintained on doxycycline**. (**a**) Flow cytometry of CFSE-labeled DO11 cells adoptively transferred into K5/TGO/DO11 mice that were either left untreated (No dox) or maintained on doxycycline for 97 days. The 2 right panels (Dox (97 days)) are results from two separate mice, both of whom had completely resolved skin disease at the time of adoptive transfer. (**b**) Flow cytometry of thymocytes and SDLN cells isolated from K5/TGO/DO11 mice maintained on doxycycline for 97 days or left untreated. Cells are pre-gated on CD4 single-positive cells. (**c**) Numbers of SDLN $CD4^{+}KJ^{+}$ cell in SDLNs from mice maintained on doxycycline for 64 days or left untreated. Results are representative of 3 replicate experiments with 2-4 mice/group.



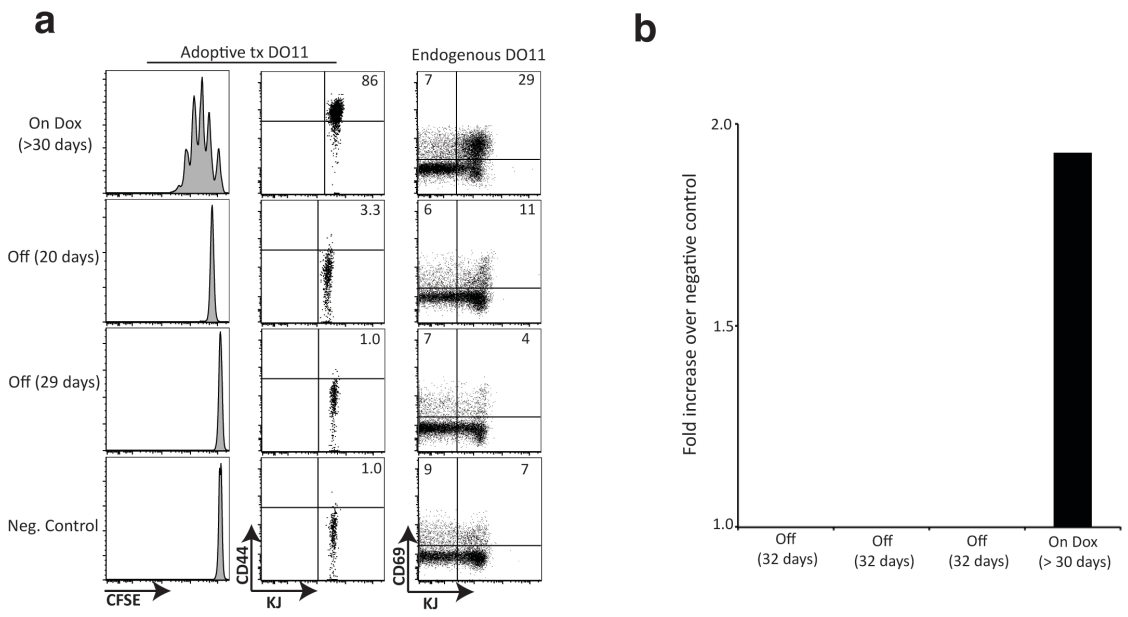
Supplementary Figure 3. Depletion of Treg cells prior to antigen induction results in non-resolving lethal skin inflammation. Survival of K5/TGO/DO11 mice treated i.p. with 2 doses of PC61 monoclonal antibody or isotype control antibody at 10 and 3 days prior to beginning doxycycline. Results are representative of 3 replicate experiments with 3-4 mice/group.



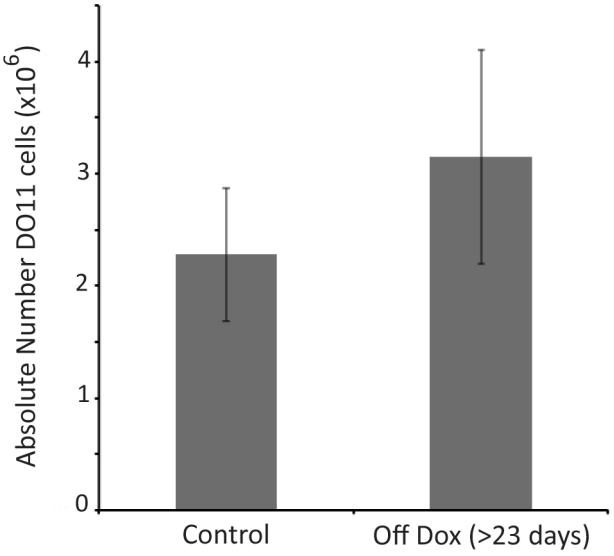
Supplementary Figure 4. **Anti-CD25 antibody treatment depletes nTreg cells**. Flow cytometry of SDLN cells isolated from TGO/DO11 at 7 days and 11 days after i.p. injection of a single dose of anti-CD25 antibody (PC61; 0.5mg/mouse). Cells are gated on $CD4^{+}KJ^{+}$ cells. Results are representative of 3 replicate experiments.



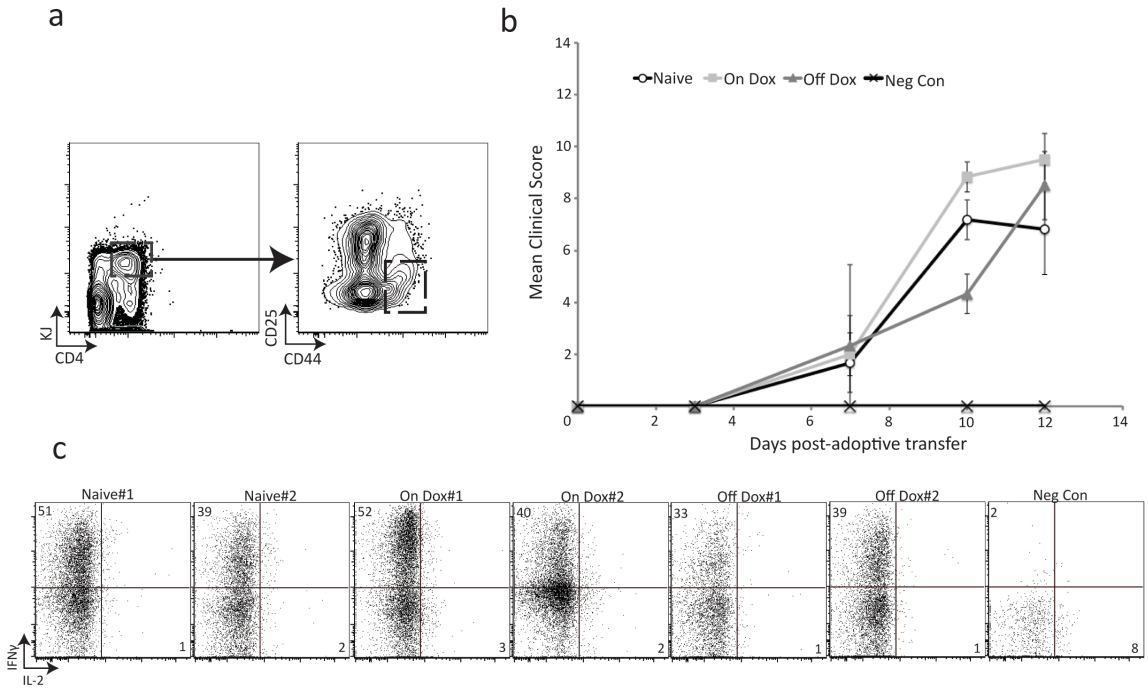
Supplementary Figure 5. Treg cells from K5/TGO/DO11 mice that have resolved skin inflammation express higher levels of Foxp3 and CD25. Flow cytometry of SDLN cells isolated from K5/TGO/DO11 mice left untreated (unshaded) or treated with doxycycline for 63 days (shaded). Mice treated with doxycycline for 63 days had completely resolved skin inflammation. Cells are gated on $CD4^{+}KJ^{+}$ cells. Results are representative of 3 replicate experiments.



Supplementary Figure 6. Antigen expression is effectively 'turned off' by 30 days after discontinuing doxycycline. (**a**) LN cells from thy1.1+ DO11.10 mice were labeled with CFSE and adoptively transferred into K5/TGO/DO11 mice maintained on doxycycline for greater than 30 days (positive control), mice that had been off of doxycycline chow for 20 or 29 days, and TGO/DO11 mice (negative control). CFSE dilution and CD44 expression was assayed by flow cytometry 3 days after adoptive transfer. Endogenous DO11 cells from the same K5/TGO/DO11 mice were assayed for CD69 expression by flow cytometry. Adoptively transferred DO11 cells are gated on Thy1.1+CD4+KJ+ cells and endogenous DO11 cells are gated on Thy1.1-CD4+KJ+ cells. (**b**) Ova-specific RT-PCR of skin harvested from K5/TGO/DO11 mice maintained on doxycycline for greater than 30 days ("On dox", positive control) and mice that had been on doxycycline for >30 days and off for 32 days, and TGO/DO11. Three individual mice are shown. CT values for Ova mRNA were normalized to β-actin controls from each sample. Data shown are β-actin-normalized values expressed as fold increases over mean single transgene (TGO/DO11) negative controls. Results are from 2-3 mice/group.



Supplementary Figure 7. DO11 T cells return to baseline numbers after cessation of doxycycline. Absolute numbers of CD4+KJ+ cells isolated from SDLNs of TGO/DO11 mice (Control) or K5/TGO/DO11 mice that had been on doxycycline for 30 days and subsequently taken off doxycycline for 24 or 30 days (“>23 days”). Results shown are means of 4 TGO/DO11 mice and 4 K5/TGO/DO11 mice (2 K5/TGO/DO11 mice had been off doxycycline for 24 days and 2 K5/TGO/DO11 mice had been off doxycycline for 32 days). Error bars represent standard deviation of samples within each group.



Supplementary Figure 8. Antigen-experienced DO11 T cells induce skin disease when adoptively transferred into antigen-expressing hosts. To test whether effector T cells are rendered functionally unresponsive after exposure to tissue antigen, antigen-experienced DO11 cells were isolated from K5/TGO/DO11 mice that were maintained on doxycycline for >100 days (and had resolved disease) or antigen-experienced DO11 cells from K5/TGO/DO11 mice that were on doxycycline for >50 days and off doxycycline for >50 days. Sorted cells were adoptively transferred into Ova-expressing K5/TGO/TCRα$^{-/-}$ hosts. K5/TGO/TCRα$^{-/-}$ mice were utilized as hosts because these mice do not have endogenous Treg cells, allowing for specific examination of cell-intrinsic anergy of adoptively transferred effector T cells. (**a**) Gating strategy for cells sorted from antigen-experienced mice. (**b**) Clinical disease upon adoptive transfer of antigen-experienced DO11 effector cells into antigen-expressing hosts. Approximately 6.5x10^5 CD4+KJ+CD44+CD25- SDLN cells from K5/TGO/DO11 mice that were maintained on doxycycline for >100 days (On Dox) or from K5/TGO/DO11 mice that were on doxycycline for >50 days and off doxycycline for >50 days (Off Dox) were adoptively transferred into K5/TGO/TCRα$^{-/-}$ hosts. As a positive control, 6.5x10^5 CD4+KJ+CD25- SDLN cells from antigen-naïve K5/TGO/DO11 mice (Naïve) were transferred into K5/TGO/TCRα$^{-/-}$ hosts, and as negative control 'On Dox' cells were transferred into TGO/TCRα$^{-/-}$ hosts (Neg Con). Recipient mice were started on doxycycline 1 day prior to adoptive transfer. (**c**) Intracellular cytokine staining from SDLNs harvested at 14 days after adoptive transfer. Two replicate mice from each group are shown. Gated on Live CD4+KJ+Foxp3- cells. Results are from 3 mice/group. Error bars represent standard deviation of samples within each group.