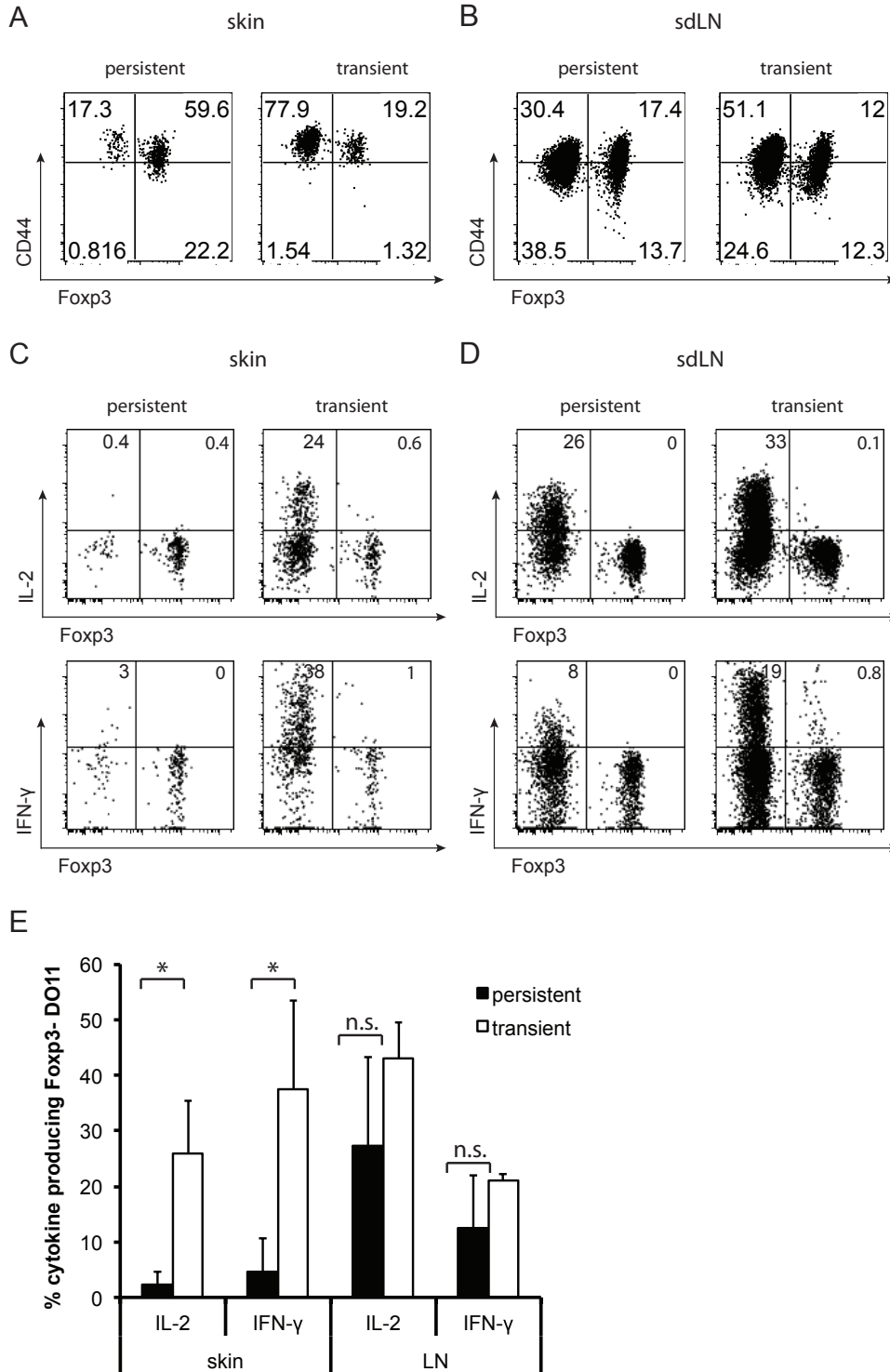


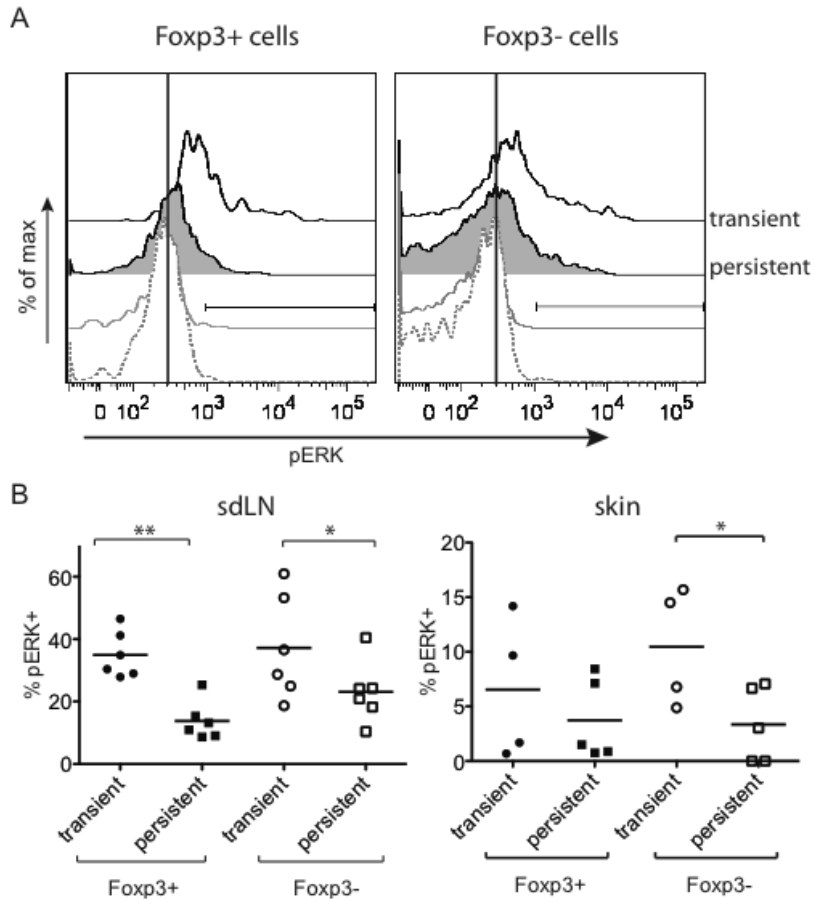
Supplemental Figure 1



SUPPLEMENTAL FIGURE 1. Persistent antigen expression leads to unresponsiveness of activated Teff cells. 0.8×10^6 DO11/Rag^{-/-} cells were adoptively transferred to

K5/TGO/TCR $\alpha^{-/-}$ and Ova-expression was either induced transiently or persistently. SdLNs and skin single cell suspensions were prepared between day 60–82 post transfer. **(A, B)** Cells were stained for CD4, KJ1-26, CD44 and Foxp3. Representative FACS data showing expression of CD44 by DO11 cells 62 days post-transfer in skin (A) and sdLNs (B). **(C, D)** Cells were re-stimulated *ex vivo* with Ova-pulsed BM-DCs and analyzed for Foxp3, IL-2 and IFN- γ expression. Representative FACS data showing cytokine production by DO11 cells 62 days post-transfer in skin (C) and sdLN (D). **(E)** Cumulative data from two independent experiments harvested between day 60–82 post transfer. Data are gated on donor Foxp3⁻ DO11 cells. (Error bars show SD; total n = 3-4 animals/condition)

Supplemental Figure 2



SUPPLEMENTAL FIGURE 2. Persistent Ag expression results in reduced phospho-ERK induction. CD90.1⁺DO11⁺Rag^{-/-} cells were adoptively transferred into K5/TGO/TCR α ^{-/-} recipients. Recipient mice were either fed with dox for 7 days and then switched back to regular feed (transient expression) or kept on dox feed for the entire time of the experiment (persistent expression). Single cell suspensions of skin or sdLNs were prepared between day 56–71 post-transfer, stimulated with anti-CD3 *ex vivo*. For stimulation cells were brought up at 2×10^7 cells/ml, rested in RPMI for 30 min at 37°C, stimulated with anti-CD3 (25 μ g/ml) (145-2C11) and cross-linking goat anti hamster antibody (50 μ g/ml). After stimulation for 90 s cells were fixed with 1.6% paraformaldehyde (Electron Microscopy Sciences), permeabilized with ice-cold methanol, stained with p-p44/42 MAPK (Erk1/2)

(Thr202/Tyr204, 197G2; Cell Signaling Technologies) and analyzed by flow cytometry. Data are gated on the CD4⁺ CD90.1⁺ KJ-126⁺ B220⁻ Foxp3⁺ or Foxp3⁻ DO11 T cell population. **(A)** Representative histograms showing phospho-ERK in stimulated and unstimulated LN cells (grey lines). **(B)** Percentage of phospho-ERK positive DO11 T cells upon *ex vivo* stimulation in either the Foxp3⁺ or Foxp3⁻ population in sdLNs (left) and skin (right) after stimulation. Data pooled from two independent experiments. Each symbol represents an individual animal.