

SUPPLEMENTAL MATERIAL

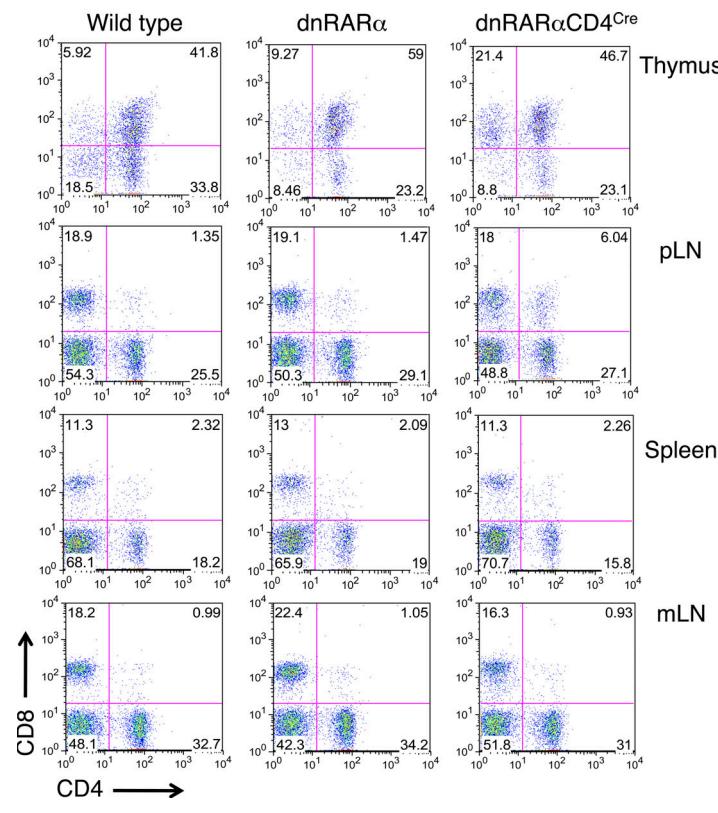
Pino-Lagos et al., <http://www.jem.org/cgi/content/full/jem.20102358/DC1>

Figure S1. Overexpression of the dnRAR- α gene in the T cell compartment. Thymus, pLNs, spleen, and mesenteric LNs (mLN) were removed from WT C57BL/6, dnRAR- α , and dnRAR- α CD4 Cre mice and stained for CD4 and CD8 to analyze by flow cytometry T cell distribution in the different organs. Representative staining of two independent experiments is shown.

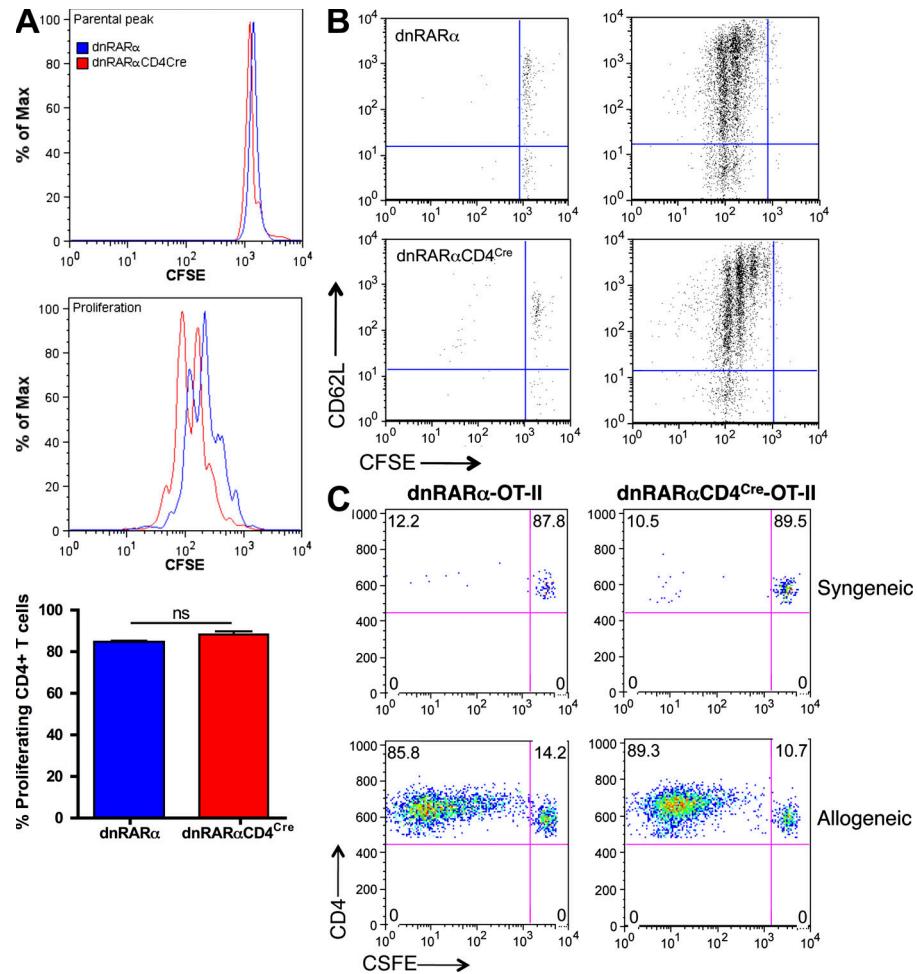


Figure S2. Overexpression of the dnRAR- α in the T cell compartment does not alter CD4+ T cell proliferation in vitro. Splenic CD4+ T cells were isolated from dnRAR- α and dnRAR- α CD4 Cre and labeled with CFSE. CFSE-labeled CD4+ T cells were plated alone or together with T cell-depleted splenocytes (APC) plus α -CD3 for 3 d. Proliferation was analyzed by flow cytometry. (A) CFSE dilution and percentage of cells proliferating are depicted. Error bars indicate SEM. ns, nonstatistical difference. (B) CFSE dilution and CD62L expression on the CD4+ T cell population are shown. (C) For in vivo analysis, C57BL/6 recipient mice received either CFSE-labeled dnRAR- α -OT-II-Ly5.2 or dnRAR- α CD4 Cre -OT-II-Ly5.2 cells, and the next day, transplantation of B6 or actin-OVA skin grafts was performed. After 5 d, skin dLNs were removed, and CFSE dilution was analyzed by flow cytometry. $n = 3$ mice per group. Representative staining of at least two independent experiments is shown.

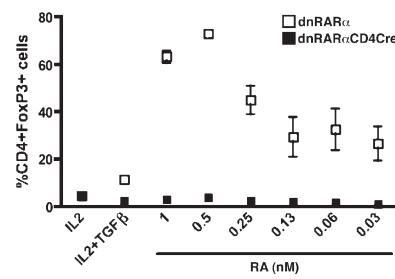


Figure S3. Disruption of RAR- α signaling on CD4+ T cells blocks RA-mediated FoxP3 conversion. Splenic CD4+ T cells isolated from dnRAR- α and dnRAR- α CD4 Cre were stimulated polyclonally (α -CD3 plus α -CD28) under the conditions indicated. After 4 d in culture, intracellular staining for FoxP3 was performed, and its expression was analyzed by flow cytometry. Data are representative of two independent experiments. Error bars indicate SEM.

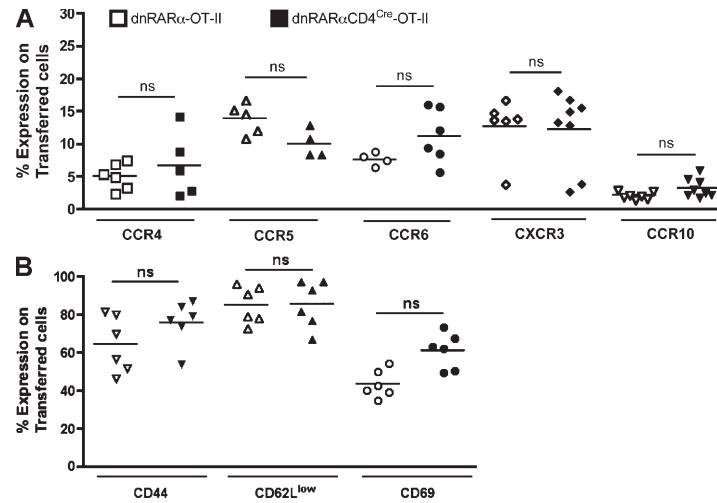


Figure S4. Disruption of RAR- α signaling on CD4 $^{+}$ T cells does not alter the expression of activation markers and chemokine receptors. (A and B) dnRAR- α -OT-II-Ly5.2 or dnRAR- α CD4 Cre -OT-II-Ly5.2 cells were transferred into RAG $^{-/-}$ mice, which received syngeneic (B6) or allogeneic (actin-OVA) skin grafts 1 d after T cell transfer. After 7 d, dLNs were removed, and cell suspension was prepared. Cell surface staining for chemokine receptors (A) and activation markers (B) was analyzed by flow cytometry. $n = 2$ –4 mice per group. Graphs depict pooled data from two independent experiments. ns, nonstatistical difference.

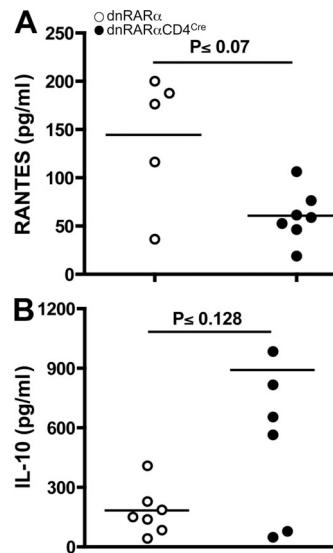


Figure S5. Disruption of RAR- α signaling on CD4 $^{+}$ T cells skews T cell polarity toward the Th2 phenotype. (A and B) dnRAR- α -OT-II-Ly5.2 (open circles) or dnRAR- α CD4 Cre -OT-II-Ly5.2 (closed circles) cells were transferred into RAG $^{-/-}$ mice, which received an allogeneic (actin-OVA) skin graft 1 d after T cell transfer. After 7 d, dLN CD4 $^{+}$ T cells were isolated and co-cultured with ISQ-pulsed DCs for 3 d. Supernatant was harvested and analyzed for cytokine/chemokine production using Luminex. $n = 2$ –3 mice per group. Graphs depict pooled data from three independent experiments.