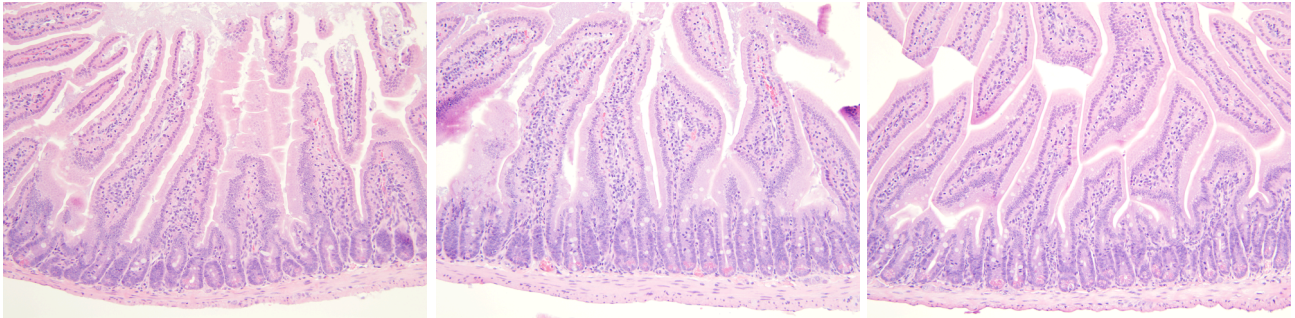


Supplementary Figure 1. Systemic immune response to oral immunization with OVA. (A) Protocol of oral immunization and EC challenge with OVA. **(B)** OVA specific serum antibody levels. Data represent the mean and standard errors of 6 mice per group. **(C)** Splenocyte proliferation to OVA. **(D)** Cytokine production by splenocyte cultures from mice orally immunized with OVA. Values in C and D represent individual mice and bars represent mean ($n=6-8$ mice per group). Student's t-test was used to determine statistical differences between each set of two groups. Similar results were obtained in 2 other independent experiments with 4 mice per group.

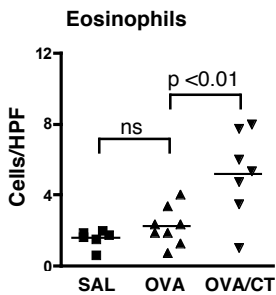
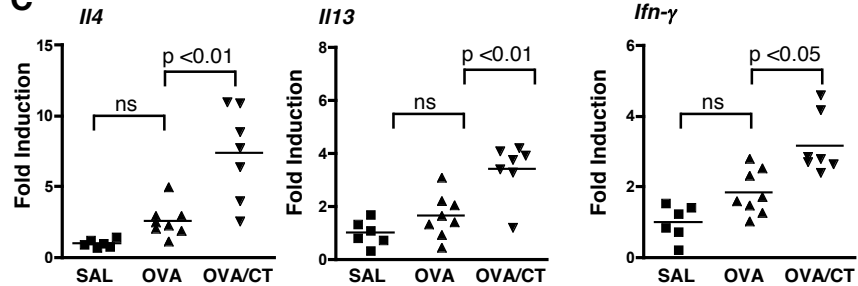
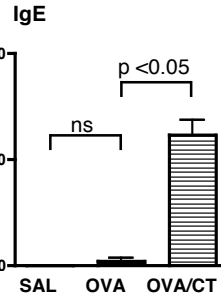
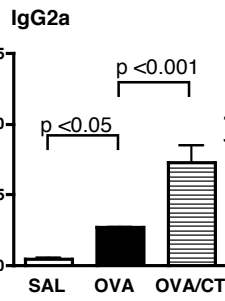
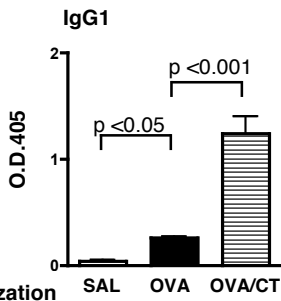
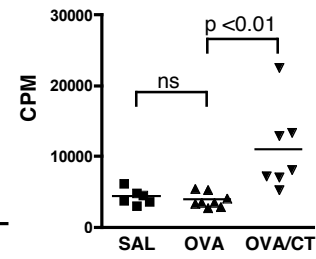
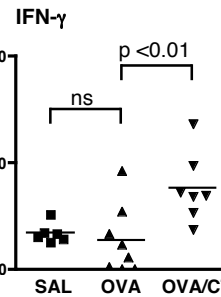
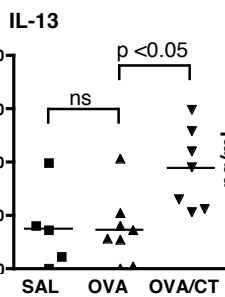
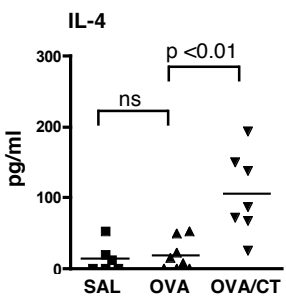
A

Oral immunization

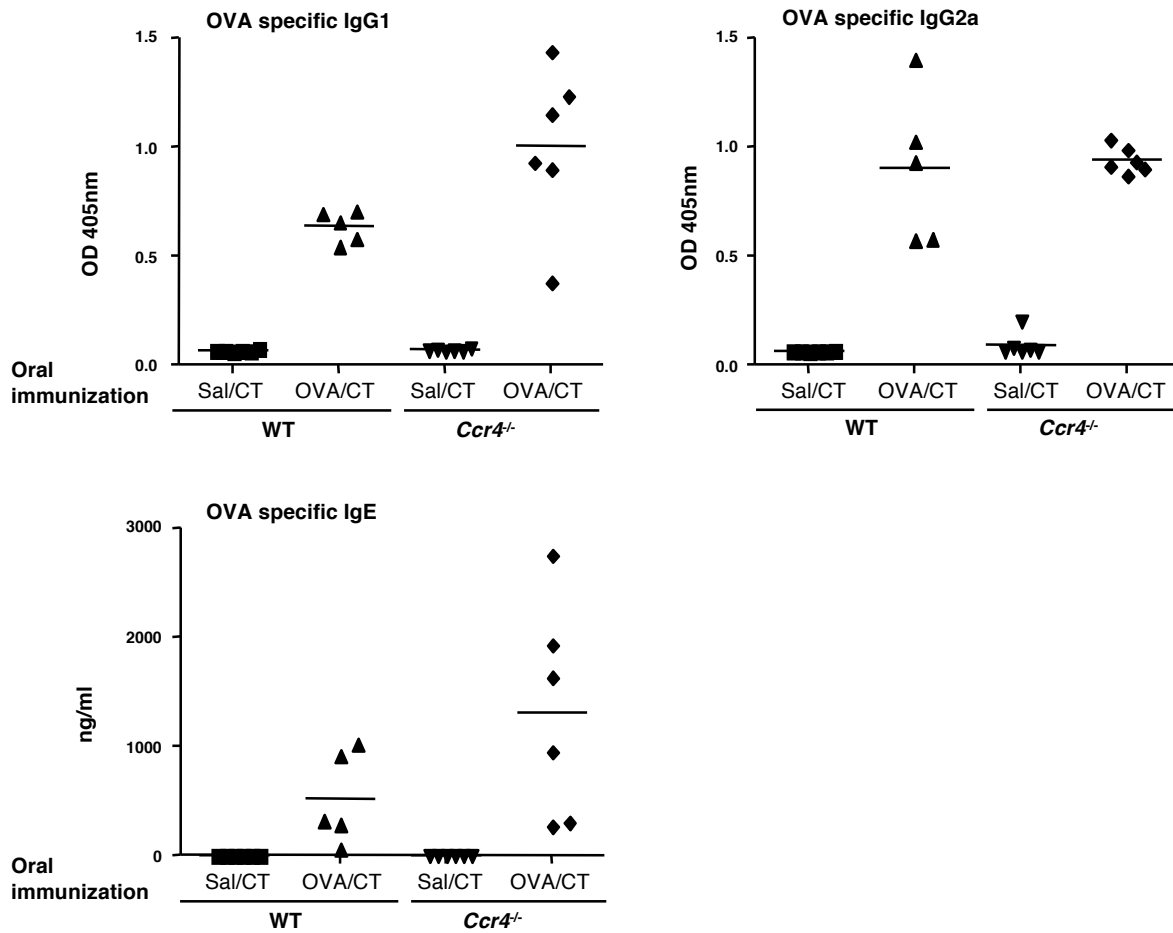
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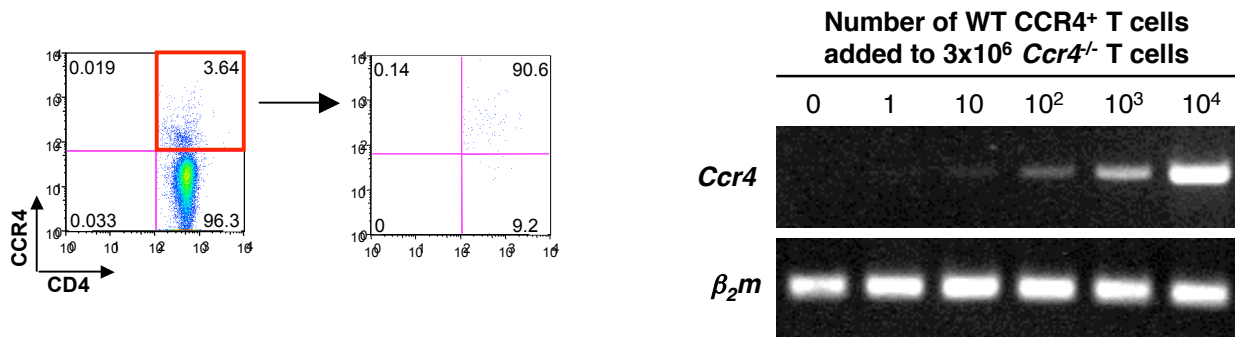
OVA/CT

B**C****D****E****F**

Supplementary Figure 2. Oral feeding with OVA in the absence of CT does not result in a local immune responses and elicits only a weak systemic immune response. (A-C) Representative photomicrographs of H&E sections of the small intestine (x200 magnification) (A), number of eosinophils (B), and relative cytokine mRNA expression (C) in the small intestine of mice orally fed with saline, OVA, and OVA/CT. The cytokine mRNA level for mice fed with saline was set at 1. **(D)** OVA specific serum antibody levels. **(E, F)** Proliferation (E) and cytokine production (F) by splenocytes stimulated with OVA. Values in B, C, E, and F represent individual mice and bars represent means. Columns in D represent the mean and standard errors of 6-8 mice per group. One-way ANOVA was used to determine statistical differences between groups. Similar results were obtained in another independent experiment with 3 mice per group.



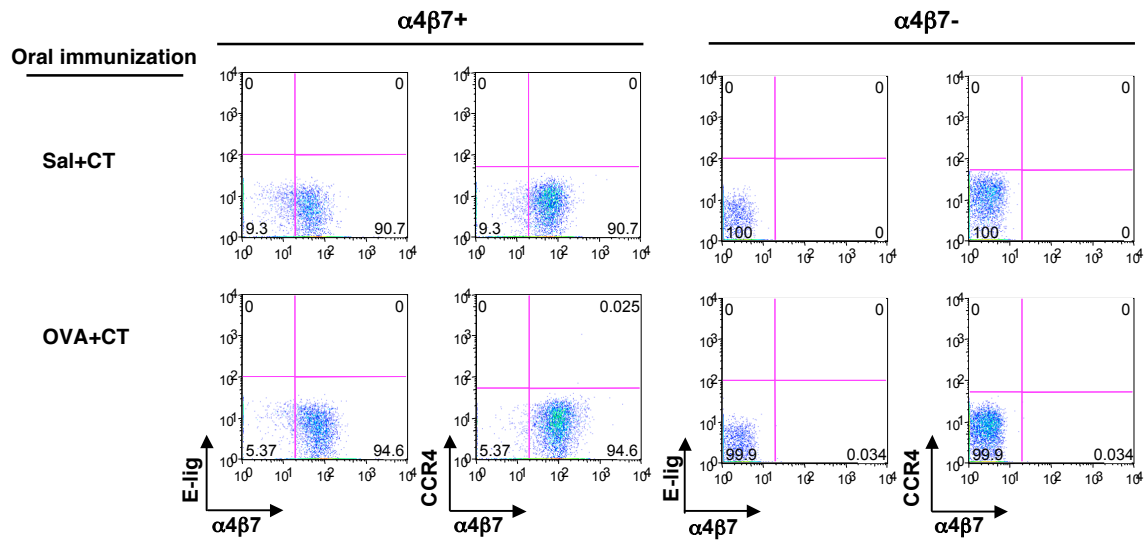
Supplementary Figure 3. OVA specific serum antibody levels in orally immunized *Ccr4*^{-/-} mice and WT C57BL/6 controls. Values represent individual mice and bars represent means of WT and *Ccr4*^{-/-} mice (n=5-6 mice per group). Similar results were obtained in another independent experiment with 4 mice per group.



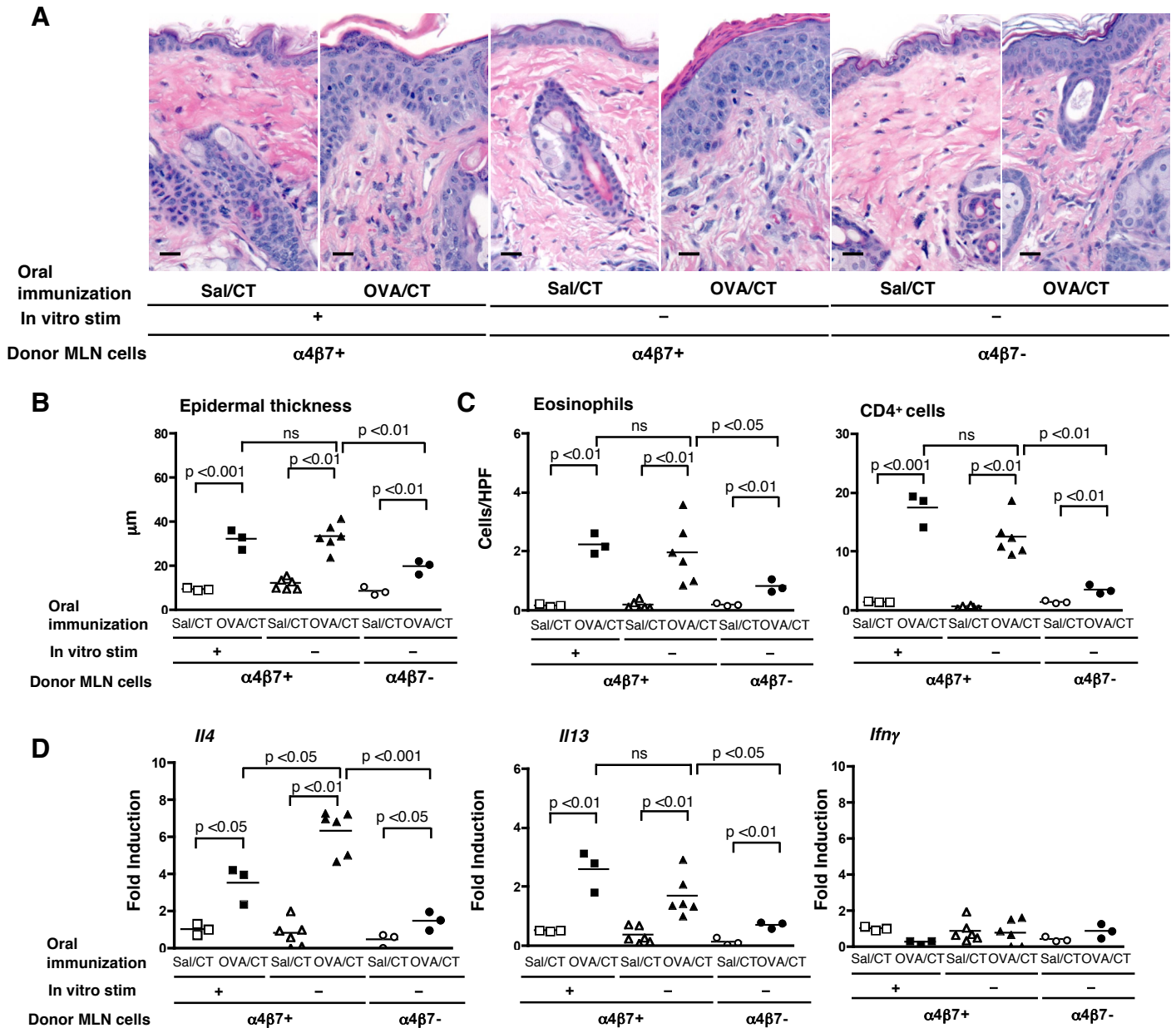
Supplementary Figure 4. Detection power of PCR analysis for CCR4 positive cells.

CCR4⁺ T cells from skin draining lymph node (DLN) of WT mice were sorted by FACS then increasing numbers of purified CCR4⁺ cells were added to 3x10⁶ purified T cells from *Ccr4*^{-/-} mice, a number of cells equal to that of purified $\alpha 4\beta 7$ ⁺ cells used in the adoptive transfer experiments. Total RNA was extracted from the cell mixture and RT-PCR was performed on 100 ng cDNA, an amount equal to that used to examine for *Ccr4* mRNA in purified $\alpha 4\beta 7$ ⁺ cells. Similar results were obtained in another independent experiment. Numbers in dot plots indicate the percentage of cells in each quadrant in purified CD4⁺ T cells after gating on CD4⁺ cells (>95% purity)(left) and in FACS sorted CD4⁺CCR4⁺ cells (right).

Purified MLN cells

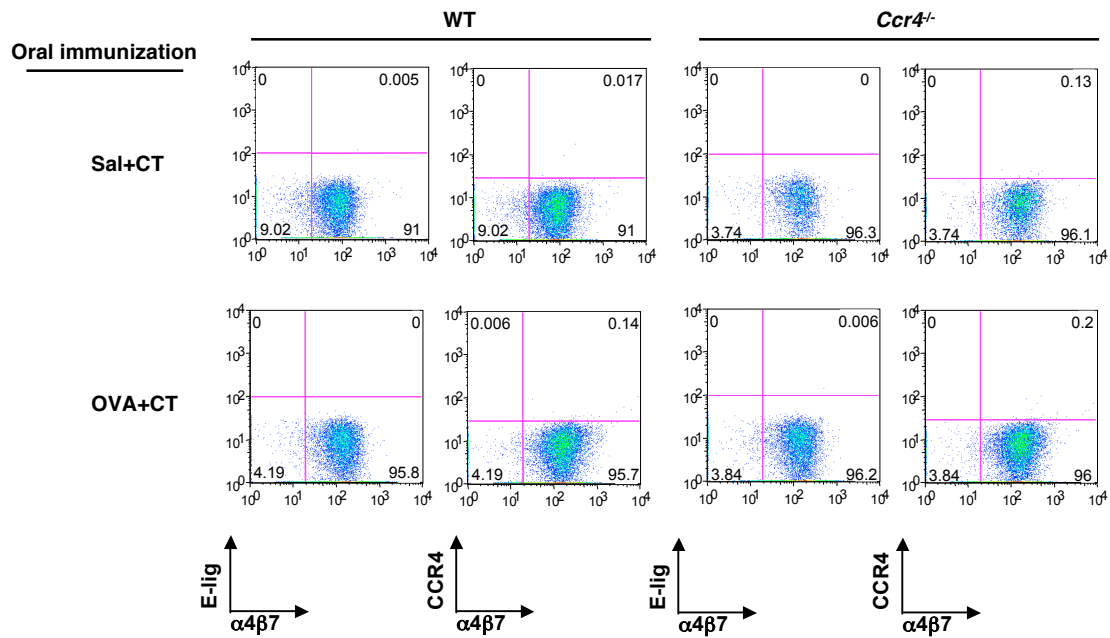


Supplementary Figure 5. Representative FACS analysis of purified $\alpha 4\beta 7^+$ and $\alpha 4\beta 7^-$ cells directly isolated from MLN of orally immunized mice. Representative of two experiments. Numbers indicate the percentage of cells in each quadrant in purified $CD4^+\alpha 4\beta 7^+$ or $CD4^+\alpha 4\beta 7^-$ cells.

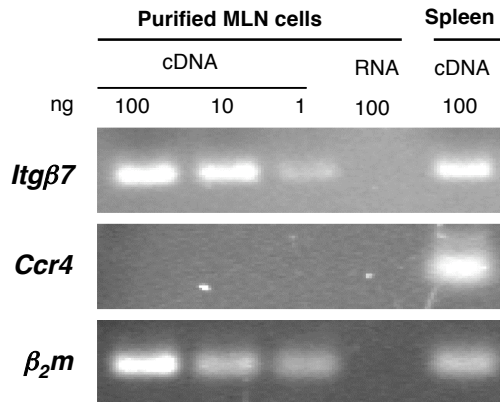


Supplementary Figure 6. Adoptive transfer of allergic skin inflammation by CD4⁺ $\alpha 4\beta 7^+$ and $\alpha 4\beta 7^-$ T cells directly isolated from MLN of orally OVA-immunized BALB/c mice. (A) Representative photomicrographs of H&E sections. Scale bars: 10 μ m. **(B)** Epidermal thickness. **(C)** Numbers per HPF of eosinophils and of CD4⁺ cells infiltrating the dermis. **(D)** Relative cytokine mRNA expression in OVA challenged skin. $\beta 2$ microglobulin (β_2m) was used as a housekeeping gene. The mean for OVA challenged skin of recipients of CD4⁺ $\alpha 4\beta 7^+$ T cells from MLN of donors orally immunized with saline was arbitrarily set at 1. Values represent individual mice and bars represent means. n=3 for recipients of in vitro OVA stimulated $\alpha 4\beta 7^+$ cells, n=6 for recipients of unstimulated $\alpha 4\beta 7^+$ cells, n=3 for recipients of unstimulated $\alpha 4\beta 7^-$ cells. One-way ANOVA was used to determine statistical differences between each set of two groups. Similar results were obtained in another independent experiment with 3 mice per group.

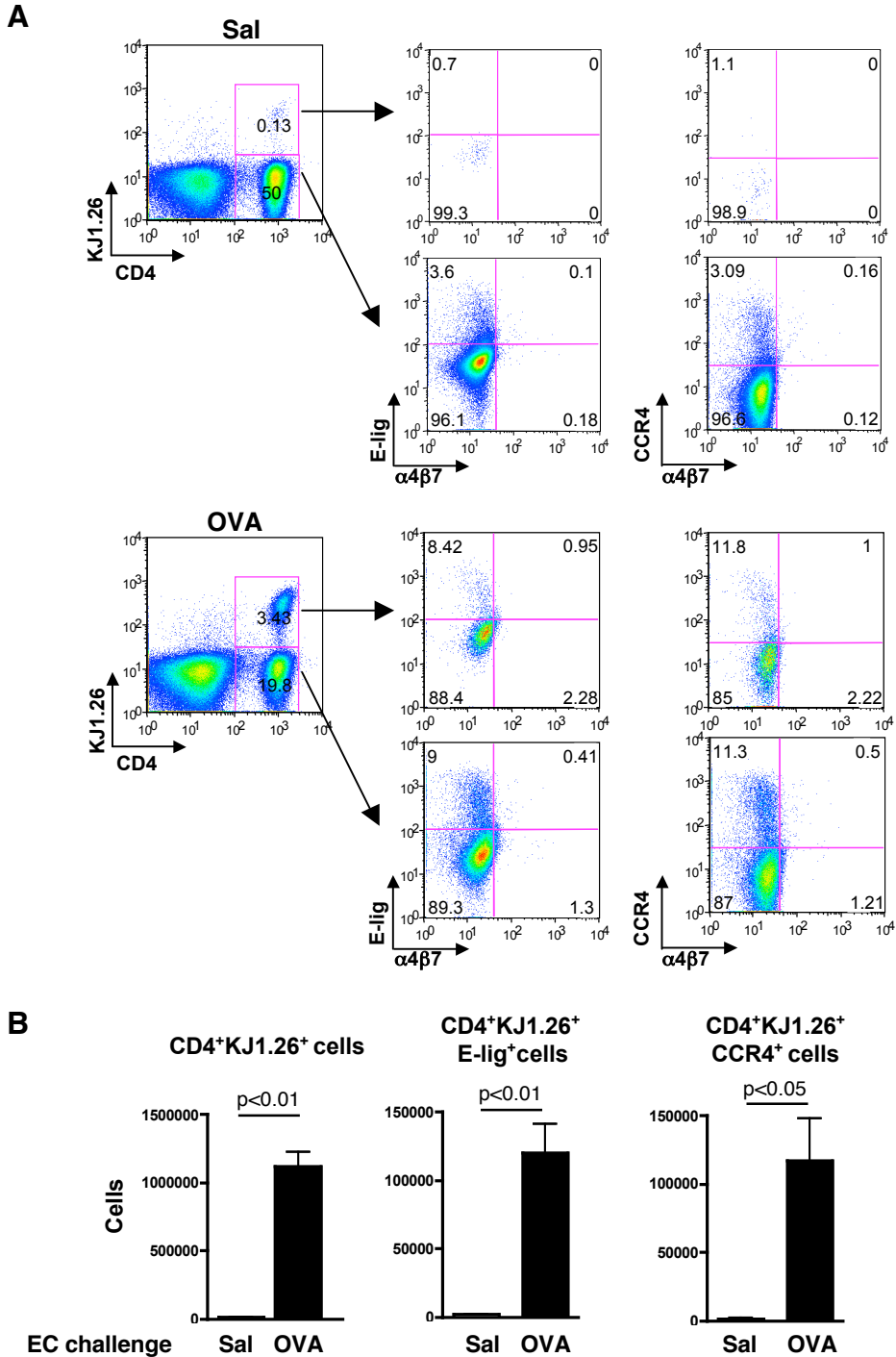
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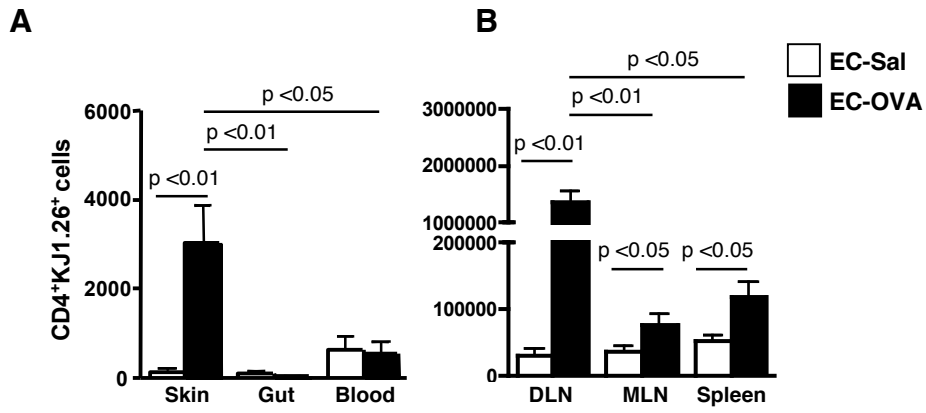
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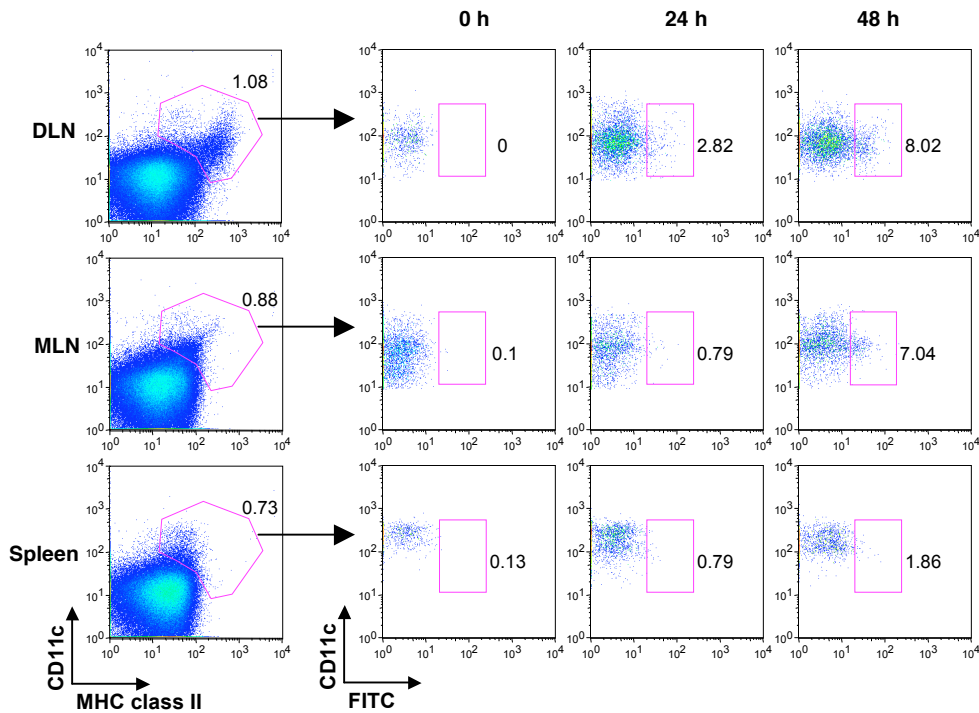
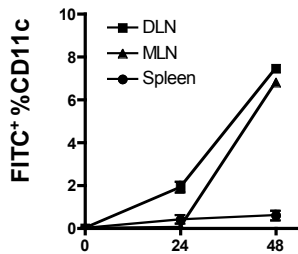
Supplementary Figure 7. Expression of gut and skin homing receptors on OVA stimulated purified $CD4^+\alpha4\beta7^+$ MLN T cells from C57BL/6 mice used in adoptive transfer. (A) FACS analysis of $\alpha4\beta7$, E-lig and CCR4 expression (representative of two experiments). Numbers indicate the percentage of cells in each quadrant in purified $CD4^+\alpha4\beta7^+$ cells. (B) RT-PCR analysis of $\beta7$ integrin (*Itgb7*) and *Ccr4* expression (representative of two experiments). Spleen cDNA was used as a positive control. RNA from purified MLN cells was used as a control for contamination by genomic DNA, and gave no detectable signal.



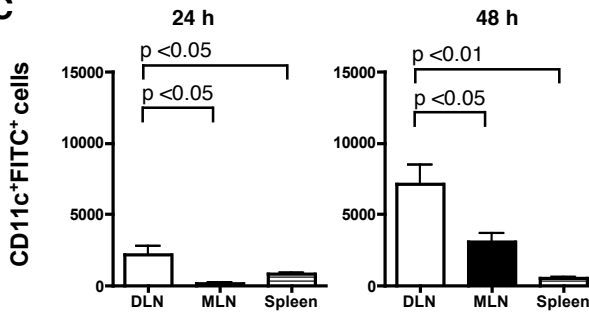
Supplementary Figure 8. Adoptively transferred CD4⁺ α 4 β 7⁺ DO11.10 effector T cells accumulate in DLN of antigen challenged skin sites of BALB/c recipients. (A) Representative FACS analysis of cells in DLN of OVA- or saline-challenged skin of recipients of DO11.10 MLN CD4⁺ α 4 β 7⁺ T cells. Numbers indicate the percentage of CD4⁺KJ1.26⁺ and CD4⁺KJ1.26⁻ cells in total DLN cells (left) and the percentage of cells in each quadrant after gating on each CD4⁺ population (middle and right). **(B)** Number of donor-derived cells in skin DLN of recipients of CD4⁺ α 4 β 7⁺ T cells from MLN of DO11.10 mice EC challenged with OVA (n=4) or saline (n=3). Data represent mean and standard errors. Two-tailed Student's t-test was used to determine statistical differences between each set of two groups. Similar results were obtained in 2 other independent experiments with 3 mice per group.



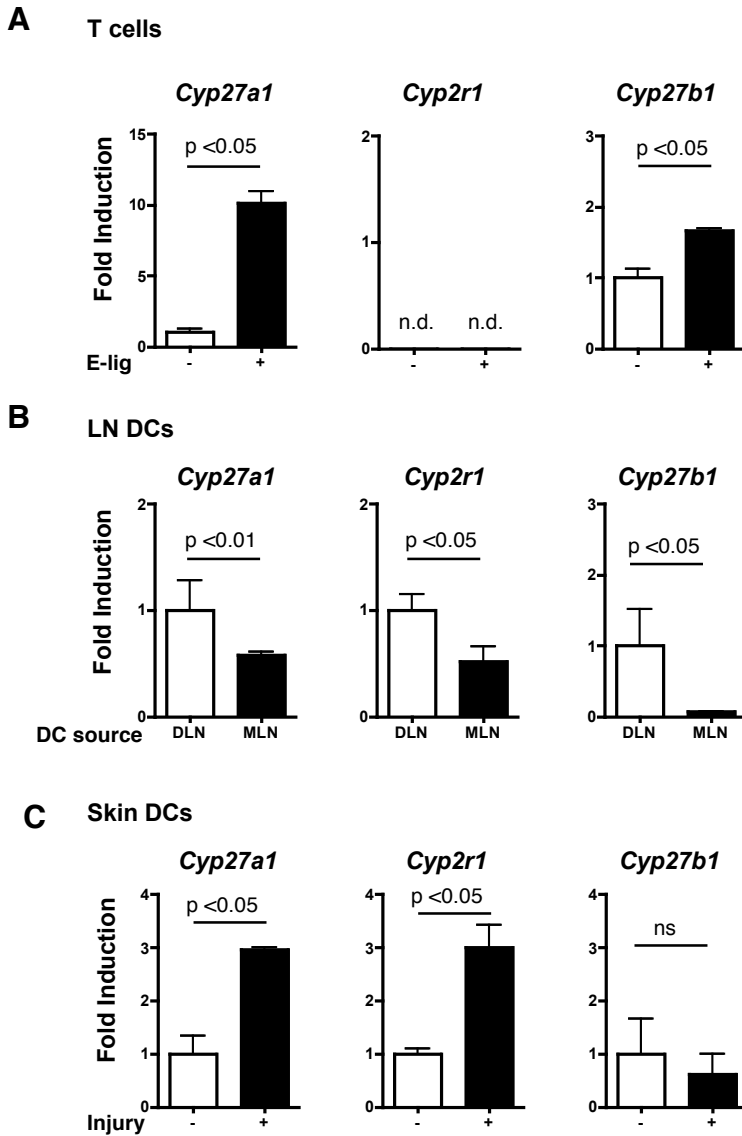
Supplementary Figure 9. Distribution of donor-derived cells in recipients of DO11.10 CD4⁺α4β7⁺ T cells from MLN. (A-B) Number of CD4⁺KJ1.26⁺ donor derived cells in skin, gut, and blood (A), DLN, MLN, and spleen (B). Columns represent the mean and standard errors of 3 mice per group. Similar results were obtained in another independent experiment with 3 mice per group.

A**B**

Time after OVA-FITC skin painting (hr)

C

Supplementary Figure 10. Migration of skin-derived DC loaded with OVA-FITC. OVA-FITC (0.5 mg/mouse) was painted on shaved and tape striped skin and DC trafficking to skin draining LNs (DLN), MLNs, and spleen was examined 24 hrs and 48 hrs after FITC painting. **(A)** Representative FACS analysis of CD11c⁺MHC class II⁺ DCs (left panel) and of FITC⁺ cells among CD11c⁺MHC class II⁺ gated cells in lymphoid organs (right panel). Numbers indicate the percentage of CD11c⁺MHC class II⁺ cells in total cells (left) and the percentage of FITC⁺ cells after gating on CD11c⁺MHC class II⁺ population (right). **(B)** Percentage of FITC⁺ cells among CD11c⁺MHC class II⁺ cells at various times after skin painting with OVA-FITC. **(C)** Number of CD11c⁺FITC⁺ cells in DLN, MLN, and spleen. Columns represent the mean and standard errors of 3 mice per group. Similar results were obtained in another independent experiment with 3 mice per group.



Supplementary Figure 11. Reprogrammed T cells and skin or skin DLN derived dendritic cells express increased levels of vitamin D3 metabolizing enzymes. (A) Relative mRNA expression of vit-D3 metabolizing enzymes in T cells. OVA-specific E-lig⁺ and E-lig⁻ cells were sorted from DLN of skin EC challenged with OVA obtained from recipients of $\alpha 4\beta 7^+$ T cells from DO11.10 donors. mRNA levels of the vit-D3 metabolizing 25-hydroxylases *Cyp27a1* and *Cyp2r1* and 1-hydroxylase *Cyp27b1* were examined by qPCR. The mean for E-lig⁻ cells was set at 1. n.d.= not detectable. **(B-C)** Relative mRNA expression of vit-D3 metabolizing enzymes in CD11c⁺DCs isolated from skin DLN and MLN (B), and from unmanipulated skin (-) and skin 6 hrs after tape stripping (+) (C). The mean for DCs from DLN was set at 1 in B, and DCs from unmanipulated skin was set at 1 in C. Columns represent the mean and standard errors of 3 mice per group. Similar results were obtained in another independent experiment with 3 mice per group.