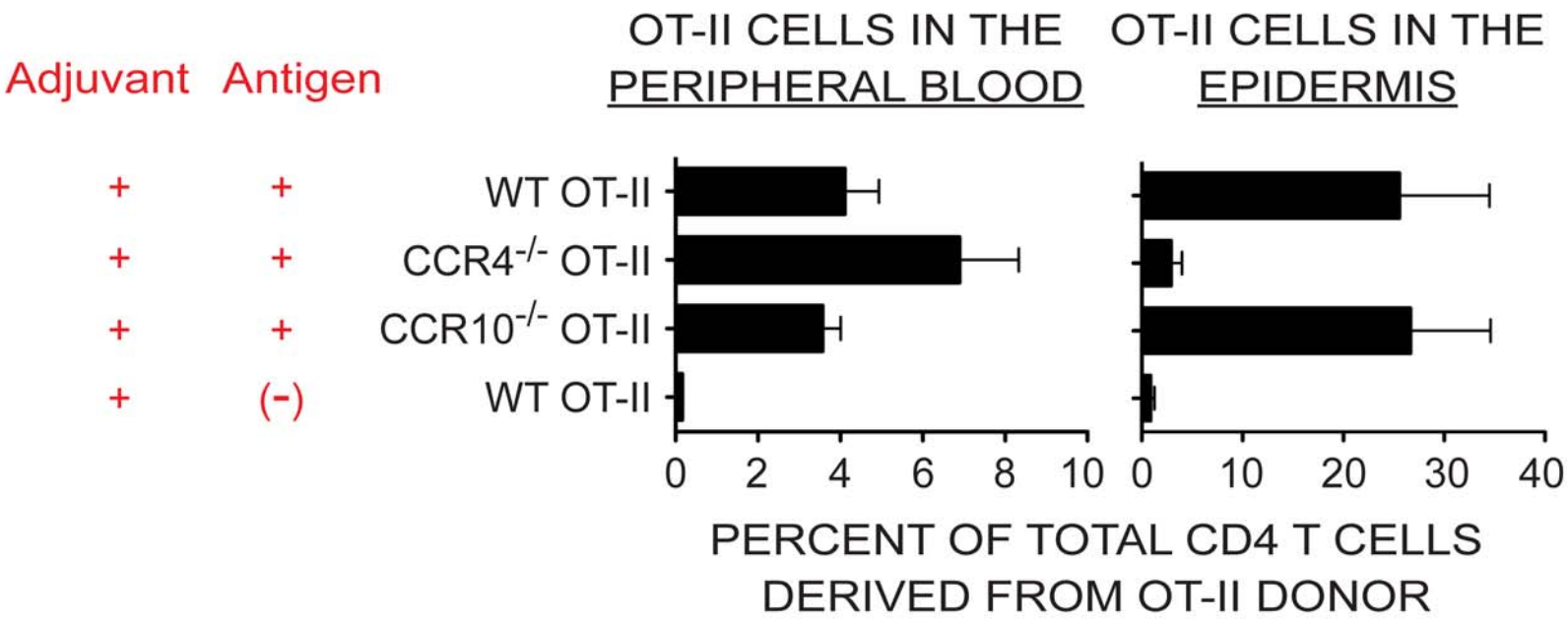

SUPPLEMENTAL FIGURE 1

Roles of CCR4 and CCR10 in Epidermal CD4 T Cell Homing In Torso (Back) Skin Consistent with That seen for Ear Skin in Fig 4. CCR10 has previously been suggested to have a role in homing of CD4 T cells to the epidermis. However, the results of our ear skin inflammation model did not supportan this hypothesis. We therefore considered the possibility that ear skin may not be representative of "normal" skin in mice, that is, the skin that covers most of the mouse's body. To investigate this, we looked at trafficking of OT-II CD4 T cells to the epidermis of the skin on the upper back of the mouse.

Briefly, animals were injected with 5e6 WT OT-II, CCR4$^{-/-}$ OT-II, or CCR10$^{-/-}$ OT-II cells, as described in the methods. WT OT-II cells were used as a positive homing control, CCR4$^{-/-}$ OT-II cells as a negative homing control. One day later, animals were shaved on the back with electric shears, and Nair was applied to a quarter(coin)-sized area on the mouse's back, then removed after ~1min. The area was then washed with cotton balls soaked in warm water to remove residual Nair and dried with fresh cotton balls. The denuded area was gently tape-stripped and treated with acetone (as described for ear skin protocol). 50ul of a 1.0mg/mL Cholera Toxin solution (50ug/mouse) and 50ul of a 3.33mg/mL $OVA_{323-339}$ solution (111ug/mouse) was applied to the skin, and both were distributed evenly with with a small artists paintbrush. After six days, the animals were sacrificed, and blood and back skin were harvested. Fat was mechanically removed from the skin, and epidermis was separated from other skin components as described in the Methods. Cell suspensions for FACS analysis were prepared as described in the Methods. Some WT OT-II recipients were treated with adjuvant (CT) only, as a negative control for those that received antigen plus adjuvant (indicated in red on the figure).

The peripheral blood of CCR4$^{-/-}$ OT-II recipients contained an appreciably higher proportion of OT-II cells than blood from WT OT-II or CCR10$^{-/-}$ OT-II recipients. This is consistent with the relative paucity of CCR4$^{-/-}$ OT-II cells from skin.

The results in skin were consistent with those observed in the ear skin inflammation model. WT OT-II and CCR10$^{-/-}$ OT-II cells accumulated similarly well in the epidermis, while CCR4$^{-/-}$ OT-II cells accumulated poorly.

Thus, we-conclude that our observation of CCR10 as an un-necessary component of antigen-specific CD4 T cell homing to CT inflamed skin is consistent between ear and torso skin. N was at least 4 individual mouse experiments for all data above.