Functional diversity among dermal dendritic cell subsets for CD8$^+$ T cell activation in the skin

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Abstract

Upon epicutaneous antigen challenge, dermal dendritic cells (dDCs) form perivascular clusters in the skin and play essential roles for efficient activation of effector T cells. In the steady state, murine skin contains two subsets of dDCs; XCR1$^+$ dDCs (conventional DC1: cDC1) and CD301b$^+$ dDCs (cDC2). Furthermore, in inflammatory state, monocyte-derived DCs (moDCs) appear in the skin. All dDC subsets compose the perivascular clusters. However, the relative contribution and the functional differences of each dDC suset for CD8$^+$ T cell activation in the skin remain unclear. Using a contact hypersensitivity (CHS) model, we tried to dissect the role of each dDC subset for CD8$^+$ T cell activation in the skin. We employed XCR1-diphteria toxin receptor knocked-in (DTR) mice and CD301b-DTR mice to deplete cDC1 and cDC2, respectively. To deplete moDCs, anti-CCR2 antibody was used. Bone marrow chimeric CD11c-DTR mice were also used to deplete all subsets of dDCs. IFN-γ and granzyme B (GrzB) production from CD8$^+$ T cells were evaluated as the parameters of CD8+ T cell activation in the skin. Depletion of both dDC subsets completely abrogated the production of IFN-γ and GrzB, confirming the essentialness of dDCs for CD8$^+$ T cell activation in the skin. Depletion of cDC1 did not affect these parameters. On the other hand, although depletion of cDC2 did not affect IFN-γ production, GrzB production from CD8$^+$ T cells was significantly attenuated. Conversely, depletion of moDCs led to impaired IFN-γ production with normal GrzB production. Taken together, our data revealed that both cDC2 and moDCs work as antigen presenting cells in the skin, and that moDCs play dominant roles for the induction of IFN-γ while cDC2 for GrzB production from CD8$^+$ T cells. These results indicate the functional diversity of dDC subsets in the skin, which may contribute to the fine tuning of the cutaneous immune responses.

Introduction

1) Dermal DC clusters are essential for efficient antigen presentation to CD8$^+$ T cells in the skin.
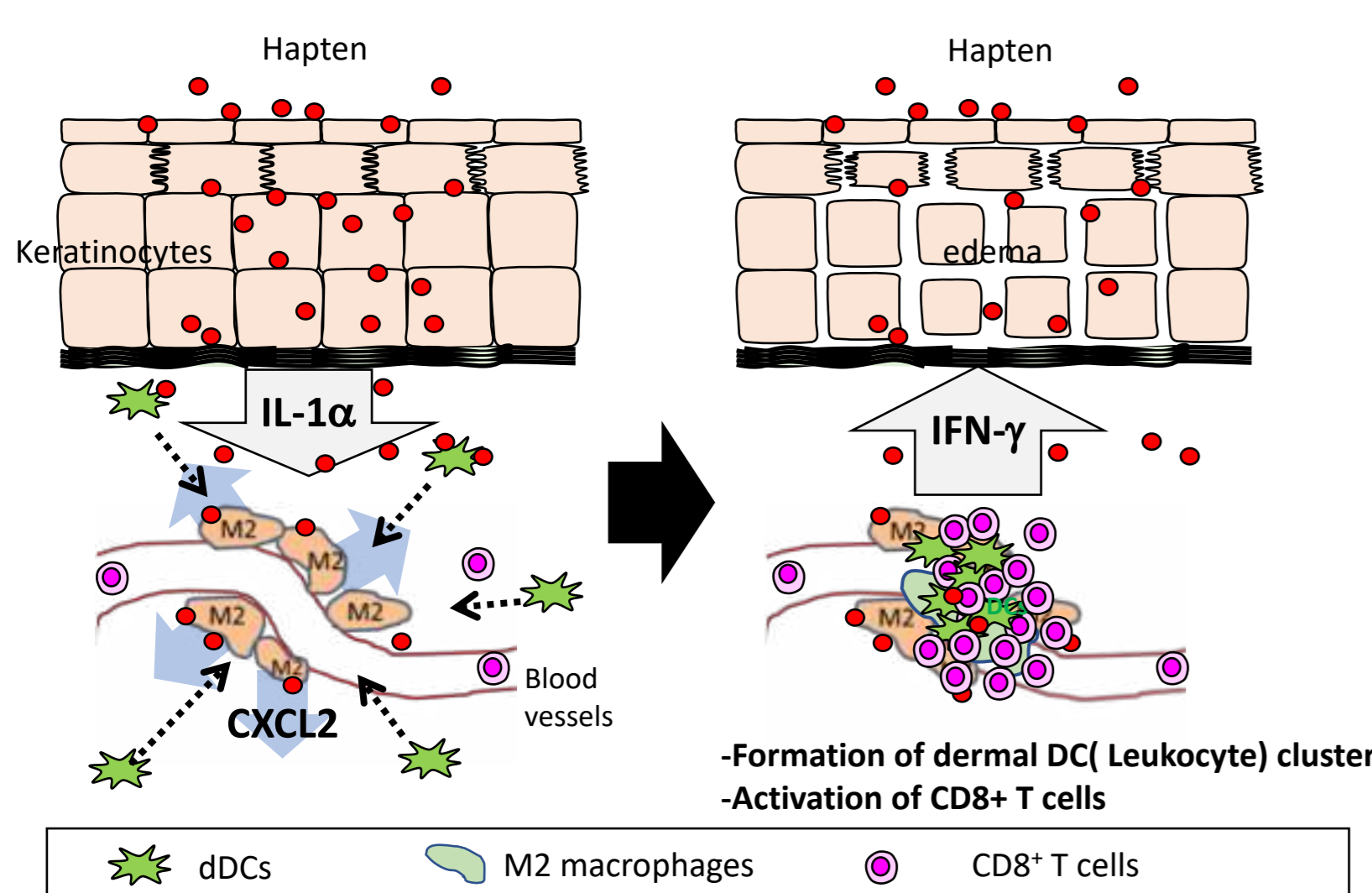
2) Dermal DC subsets in the skin
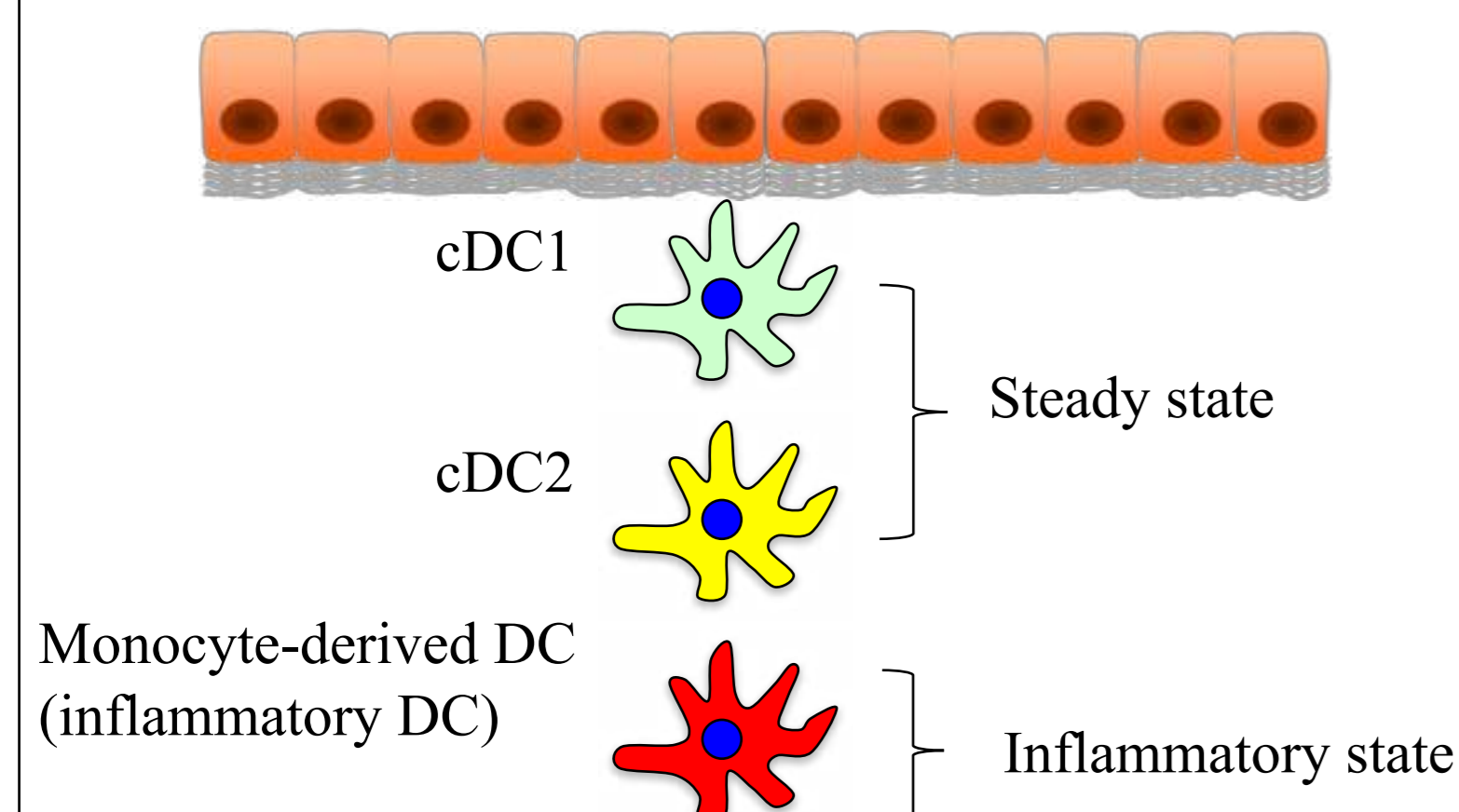
Questions

Which dermal DC subsets are responsible for antigen presentation to CD8+ T cells in the skin?

Aim

To dissect the role of each dDC subset for antigen presentation to CD8+ T cells in the skin.

Methods

1-Fluoro-2,4-dinitrobenzene (DNFB) was used as hapten. Mice were sensitized with 25 μl of 0.5% (wt/vol) DNFB in 4:1 acetone/olive oil (vol/vol) to their shaved abdomens, and then challenged with 20 μl of 0.3% (wt/vol) DNFB on both sides of each ear 5 days after sensitization. Ear thickness was measured before and 24 hrs after challenge. C57BL/6 WT mice, XCR1-DTR mice, bone marrow chimeric CD11c-DTR mice, and CD301b-DTR mice were used to deplete cDC1 and/or cDC2.. Anti-CCR2 antibody (MC-21) was used to deplete Mo-DCs. For immunohistochemistry, ear skin of CD11c-YFP was stained with anti-CD11b antibody and anti-XCR1 antibody.

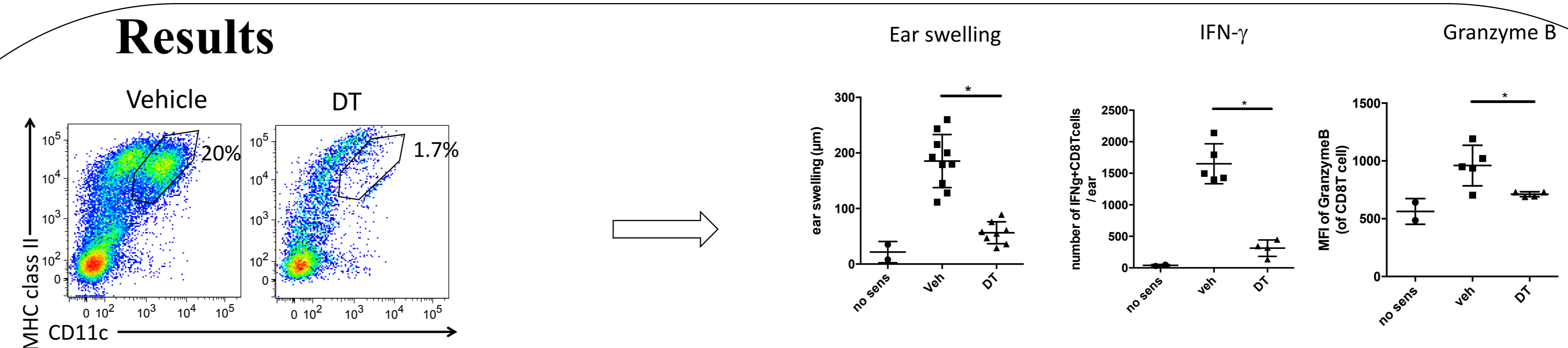**Strategy to deplete each DC subset in the skin**
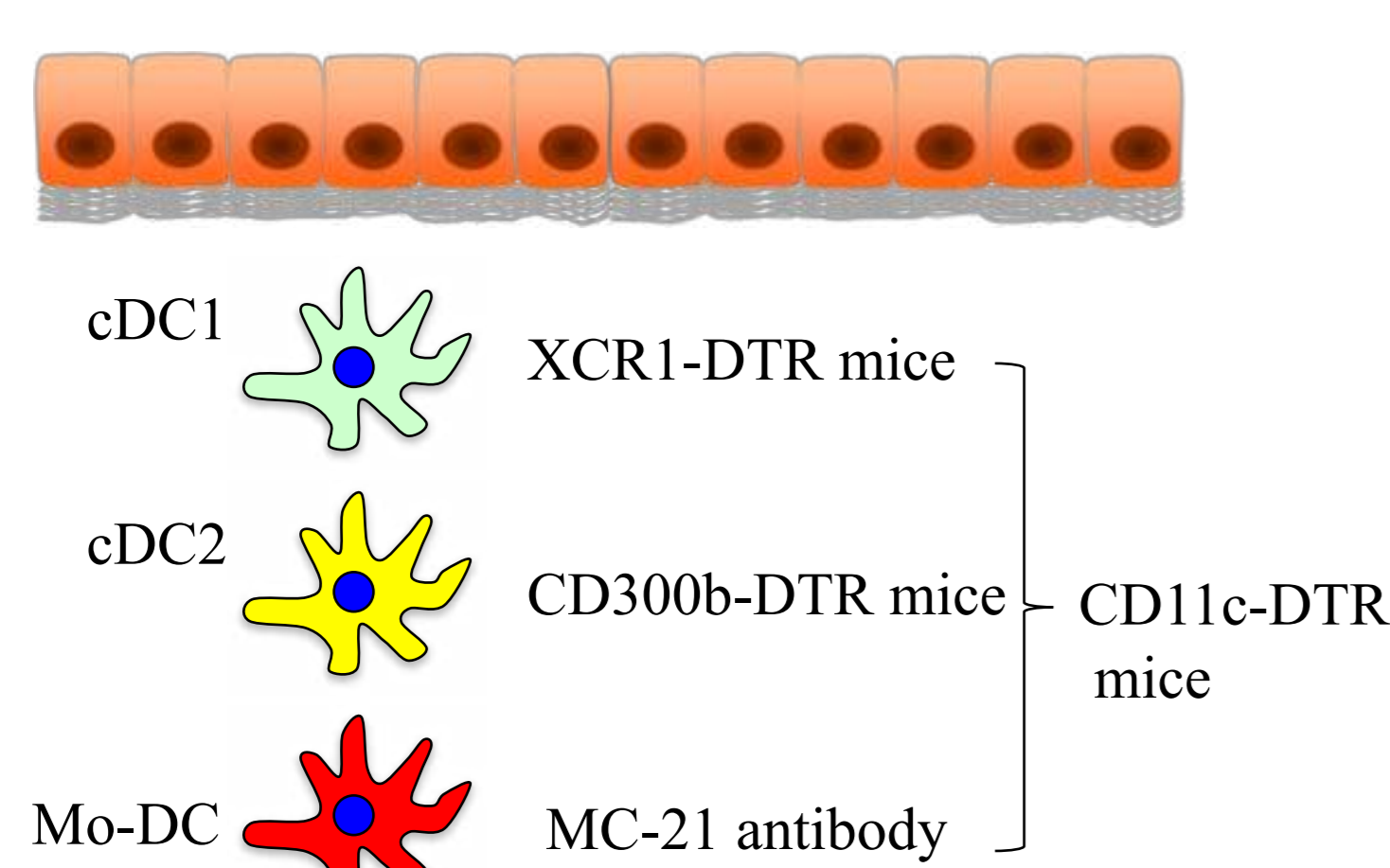
Results

Figure 1. Flow cytometry analysis of dDCs in the skin, and CHS responses in CD11c-DTR mice.
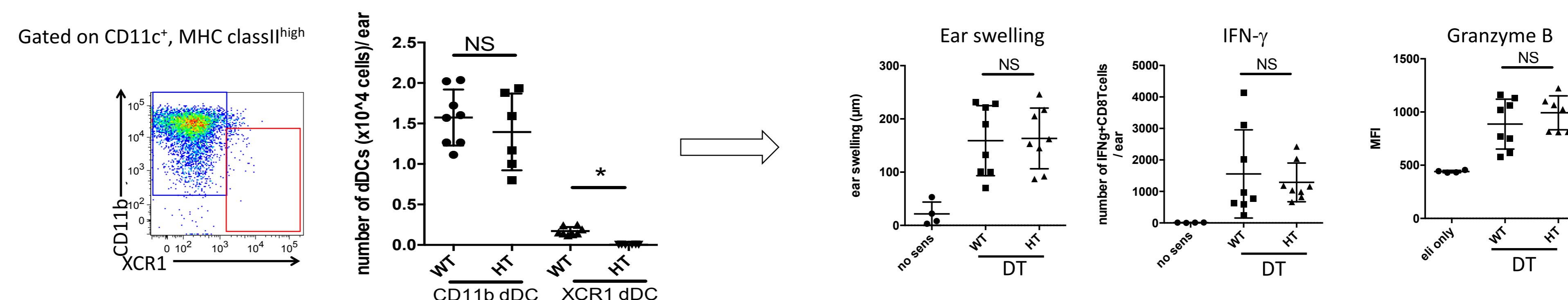

Figure 2. Flow cytometry analsys of dDCs in the skin of DT-treated XCR1-DTR mice, and their CHS responses.
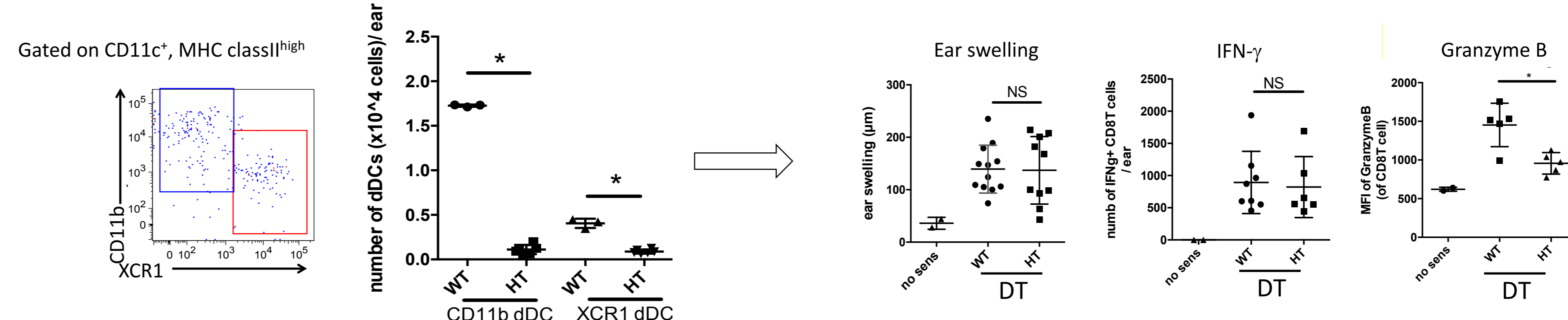

Figure 3. Flow cytometry analysis of dDCs in CD300b-DTR mice, and their CHS responses.
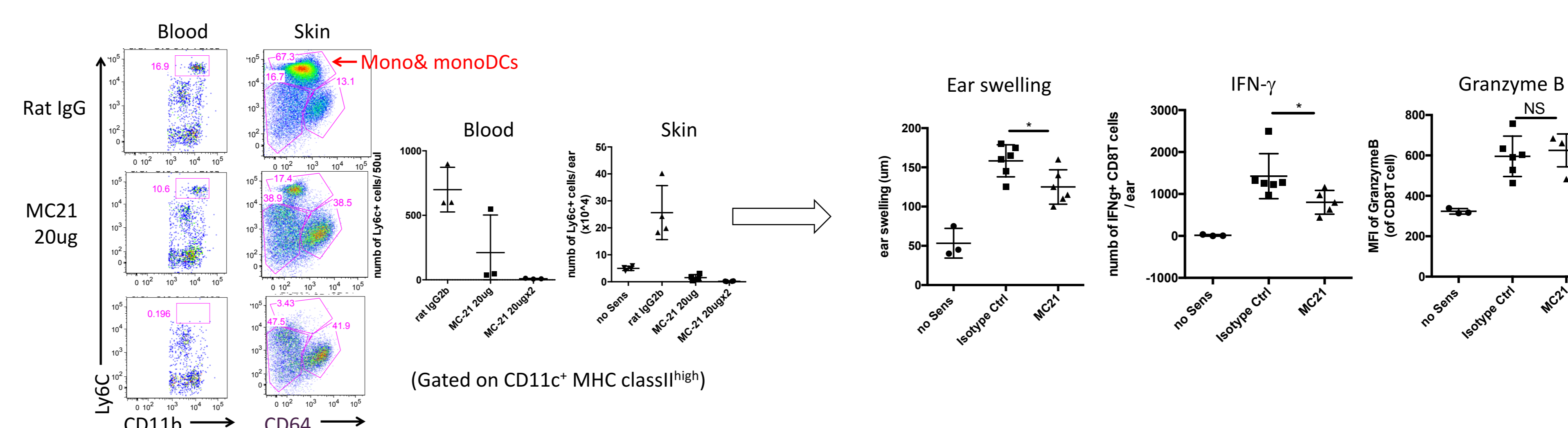

(Gated on CD11c$^+$ MHC classII^{high})

Figure 4. Effect of MC-21 antibody on dDC subsets in the skin and CHS responses.
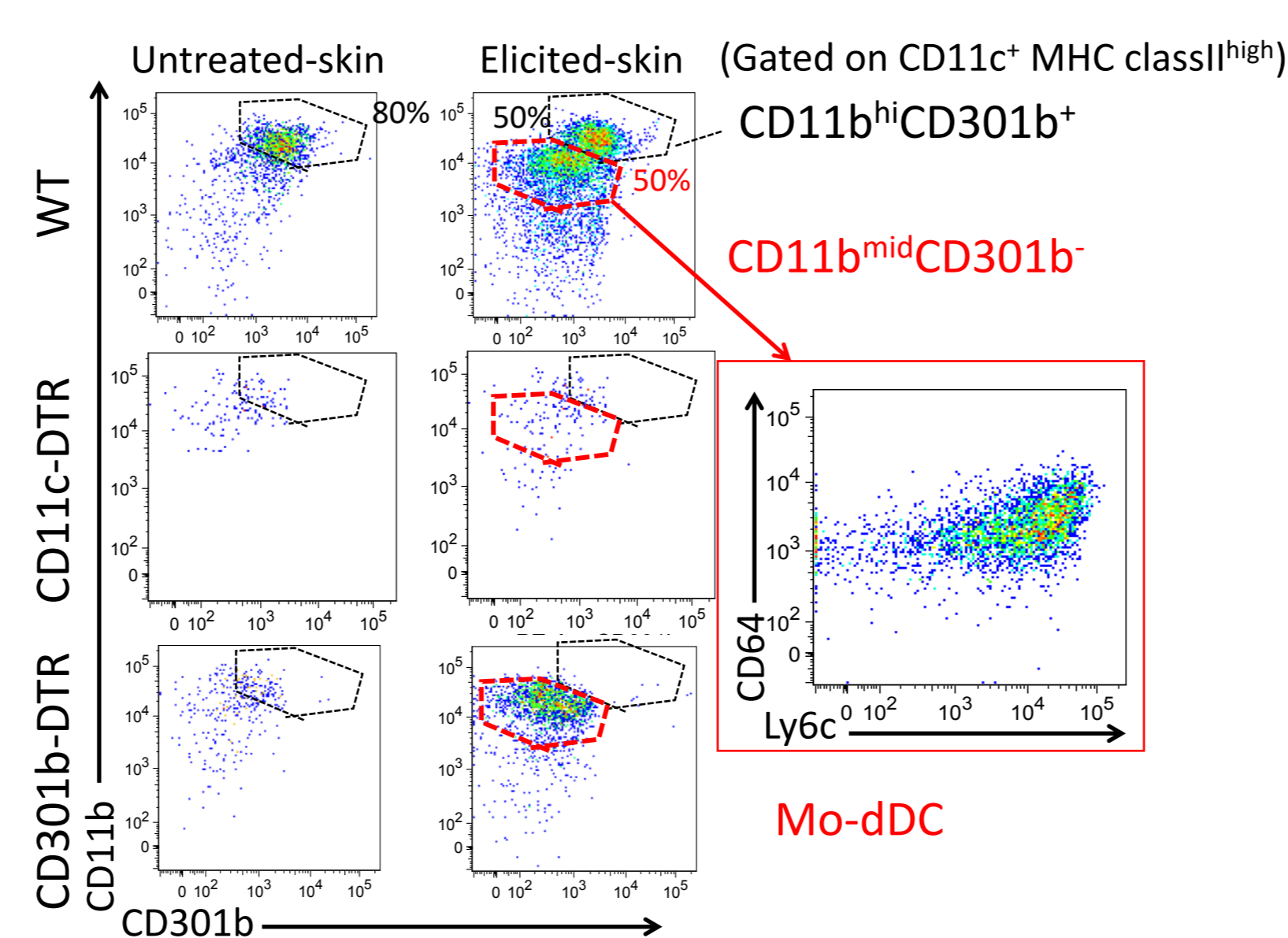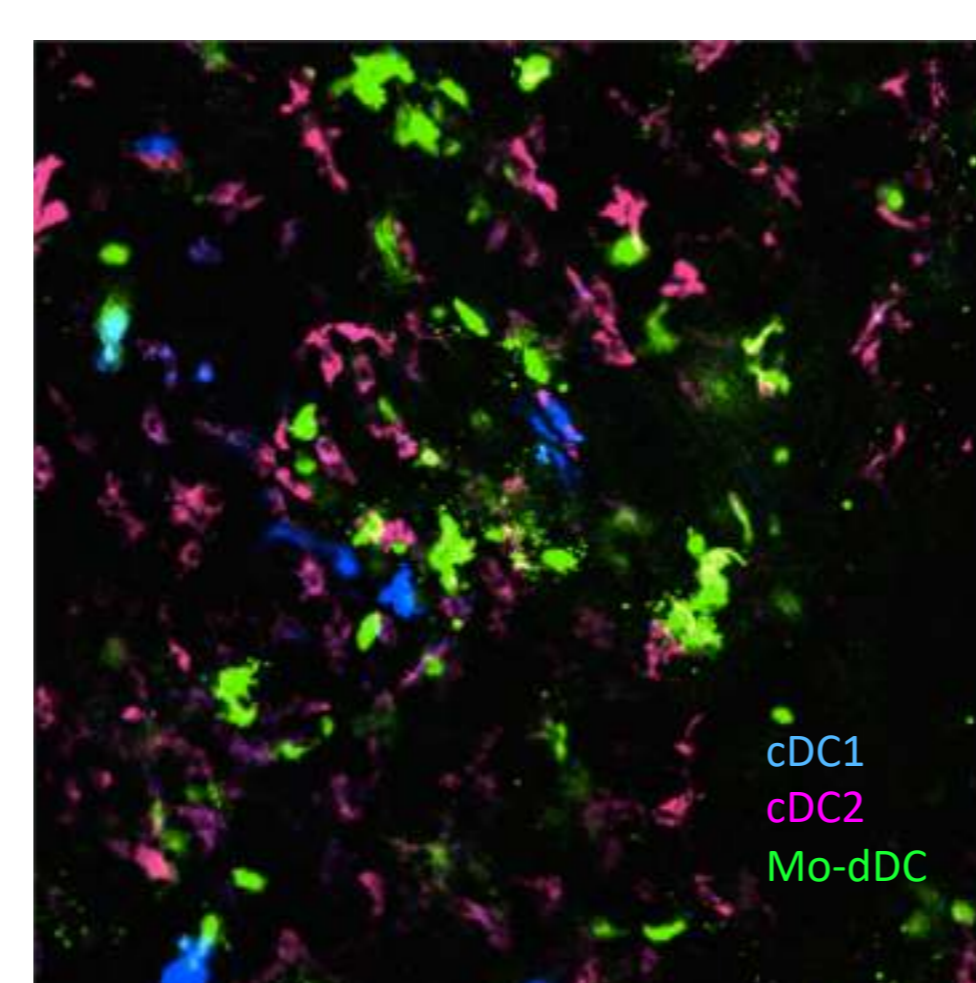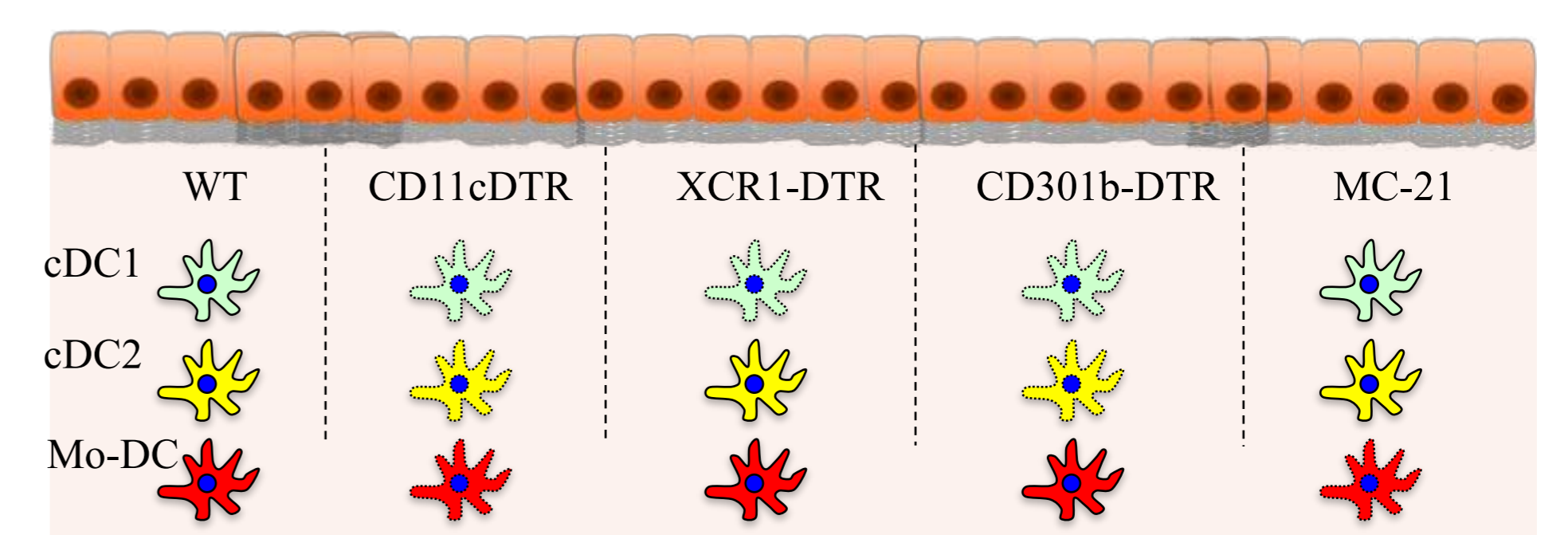

Figure 5. Flow cytometic analysis of Mo-dDCs in the skin of WT, CD-11cDTR mice and CD301b-DTR mice.

Figure 6. Immuno-histochemistry of dDC subsets in the skin during the elicitation phase of CHS.

Summary and Conclusion

1. cDC2 induce granzyme B production in CD8$^+$ T cells in the skin.

2. Mo-DCs induce IFN-γ production in CD8$^+$ T cells in the skin.

Acknowledgement

COI: None