SUPPLEMENTARY MATERIALS

Pulmonary CD103^{+} dendritic cells prime Th2 responses to inhaled allergens

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This file includes:

Legends for Supplementary Figures 1 through 11

Supplementary Figure Legends

Supplementary Figure 1 $CD103^+$ DCs promote Th2 differentiation of naïve $CD4^+$ T cells. DCs were purified from lungs of C57BL/6 mice 16 h post-OVA inhalation and co-cultured with naïve $CD4^+$ T cells from OT-II mice. (**a**) Proliferation of T cells as inferred from T cell recovery after 5 d of co-culture. (**b**) Cytokines in supernatants of 5 d cultures, as measured by ELISA.

Supplementary Figure 2 $CD103^+$ DCs from BALB/c mice promote Th2 differentiation of naïve $CD4^+$ T cells. DCs were prepared from lungs of BALB/c mice 16 h post-OVA inhalation and co-cultured with naïve $CD4^+$ T cells from DO11.10 mice. (**a**) Proliferation of T cells as inferred from T cell recovery after 5 d of co-culture, and cytokines in supernatants of 5 d co-cultures. (**b**) Cytokines in supernatants of DO11.10 $CD4^+$ T cells after 5 d of co-culture and subsequent incubation in anti-CD3ε and anti-CD28-coated plates for 24 h. Cytokine levels assessed by ELISA.

Supplementary Figure 3 Effect of enzymatic digestion on lung resident DC function. DCs were prepared from lungs of OVA-treated mice, with or without digestion with Liberase TM, collagenase XI, Hyaluronidase and DNase. (**a**) $CD11b^{hi}$ and $CD103^+$ DCs recovered after flow cytometry-based sorting. (**b**) CD4 T cell proliferation in 5 d culture. (**c**) IL-4 production by T cells incubated for 24h with anti-CD3ε and CD28 antibodies after 5 d of culture. Data shown are from one of two independent experiments giving similar results.

Supplementary Figure 4 Effect of adjuvants on T cell priming by $CD103^+$ DCs. Lung $CD103^+$ DCs were prepared from C57BL/6 mice that received OVA (100 μg) together with either LPS (1 ng) or poly I:C (1 μg). These DCs were cultured for 5 d with naïve $CD4^+$ T cells from *Rag2*$^{-/-}$ OT-II mice. IFN-γ and IL-4 production by T cells were assessed following 24 h incubation with anti-CD3ε and -CD28 antibodies.

Supplementary Figure 5 Impaired allergic airway inflammation in mice lacking $CD103^+$ DCs. WT B6C3F1 and $CD103^+$ DC-deficient BXH2 mice were sensitized by oropharyngeal aspiration of OVA-LPS, and subsequently challenged by exposure to

aerosolized OVA. The number of total leukocytes and indicated subsets in BALF from unsensitized, challenged mice (open column) and from sensitized and challenged animals (filled column) are shown. *p*-value by Student's *t*-test is indicated (n=7).

Supplementary Figure 6 Notch ligand expression by lung CD11b^{hi} and CD103^{+} DCs. Lung DC subsets were purified from C57BL/6 mice 16 h after OVA-LPS instillation. mRNA levels of *Jagged 1*, *Jagged 2* and *Delta-like 4* in the indicated subsets were determined by quantitative real time PCR and normalized to GAPDH mRNA. Results of 3 independent experiments are shown.

Supplementary Figure 7 Cytokine production by lung CD11b^{hi} or CD103^{+} DCs. (**a, b**) Lung DC subsets were purified from naïve C57BL/6 mice, activated with PMA and ionomycin, and the culture supernatants analyzed for the indicated cytokines (**a**) and chemokines (**b**). (**c**) Intracellular IFN-γ staining of total non-autofluorescent CD11c^{hi} lung DCs. (**d**) Intracellular staining of CD11b^{hi} or CD103^{+} DCs with anti-IFN-γ antibodies (solid red line) or rat IgG_1 isotype control (dotted blue line) after stimulation with PMA and ionomycin. (**e**) Percentages of IFN-γ^{+} cells among each DC subset with or without PMA and ionomycin stimulation.

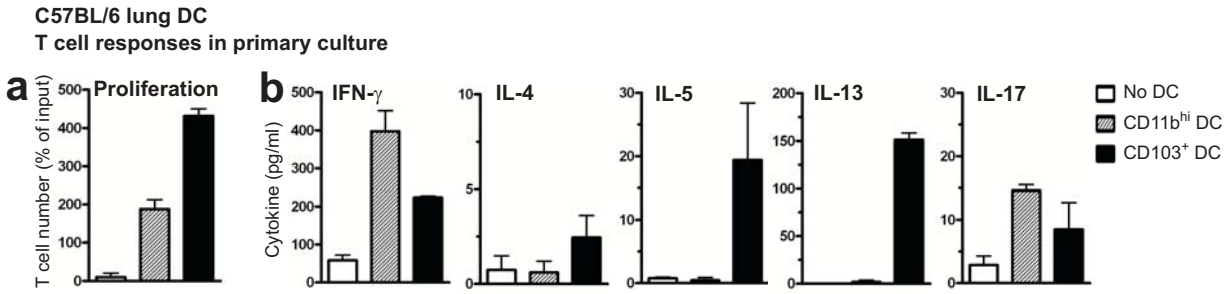
Supplementary Figure 8 Effect of candidate gene disruption on DC-mediated T cell differentiation. DCs from the indicated mutant and WT mice were co-cultured with MHC-matched, OVA-specific, naïve CD4^{+} T cells for 5 d. Following subsequent 24 h incubation of the T cells in anti-CD3ε-, CD28-coated plates, the indicated cytokines in supernatants were measured by ELISA. Mice bearing mutations in the following genes were tested; (a) IFN-γ (b) IL-4 (c) IL-2 (d) IL-9 (e) IL-10 (f) c-Kit.

Supplementary Figure 9 Cell surface molecule display on lung DC subsets. Lung DCs of naive C57BL/6 mice were stained with antibodies against the indicated molecules (solid line) or with isotype control antibodies (dotted line) and analyzed by flow cytometry. Display levels of costimulatory molecules (**a**) and myeloid markers (**b**) are shown. Similar results were obtained in two independent experiments.

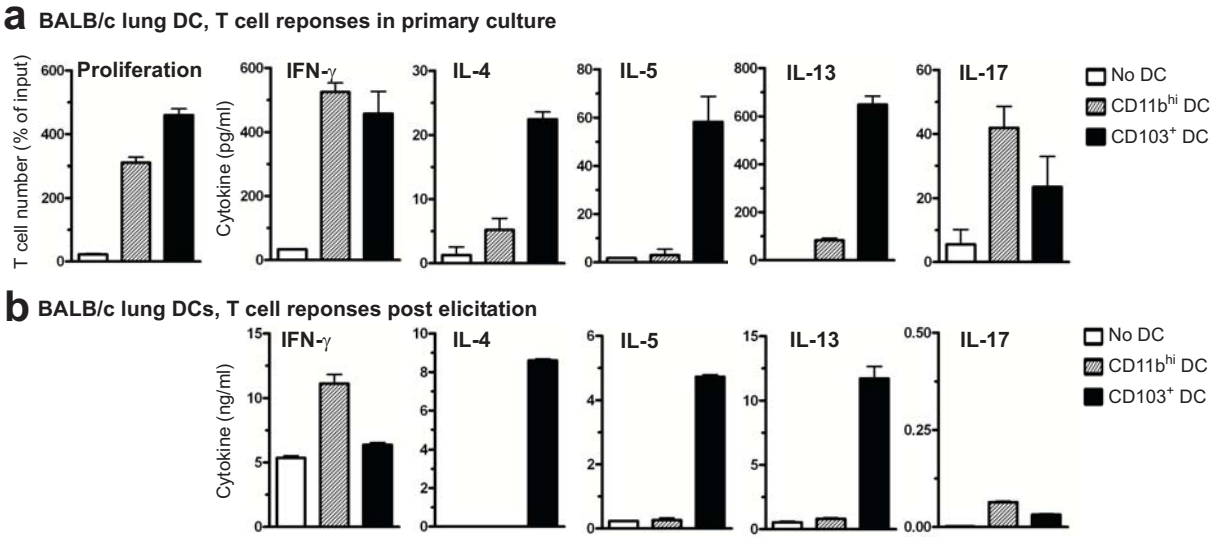
Supplementary Figure 10 Effect of TLR ligands on lung DCs. C57BL/6 mice received OVA alone, or OVA together with the indicated amounts of microbial products by intratracheal instillation. Shown are individual flow plots (**a**) and histograms of compiled data (**b**) for cell surface levels of CD86 and MHC class II I-A^b and for OVA uptake (solid lines). Shaded regions represent staining with isotype control antibodies. *p*-value by Student's *t*-test (n=2).

Supplementary Figure 11 Naïve $CD4^+$ T cell preparation. Flow cytometric analyses of T cells before and after sorting of naïve $CD4^+$ T cells. Non-naïve $CD4^+$ T cells were depleted from cells of pooled LNs and spleen by magnetic activated sorter (MACS) and antibodies against CD8α, CD8β, CD11b, CD11c, CD16/32, CD19, CD25, CD44, B220, CD49b, I-A, Ly6C/G.

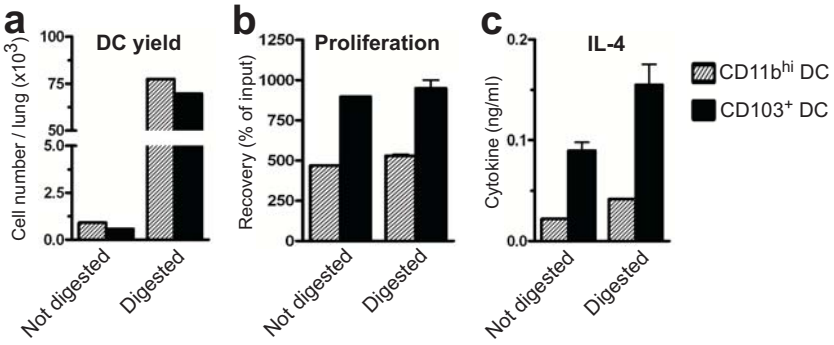
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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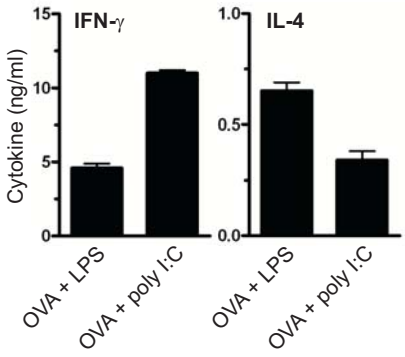

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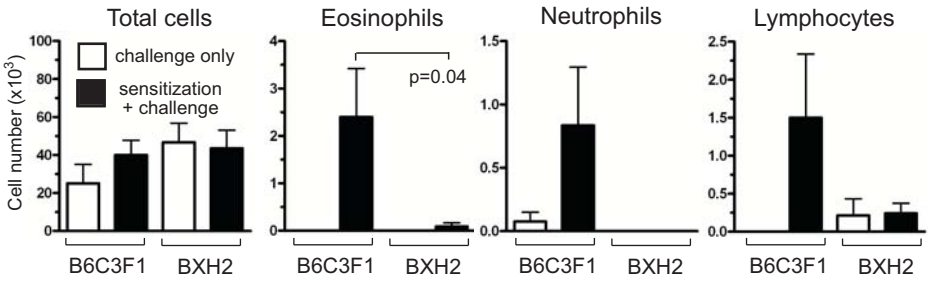

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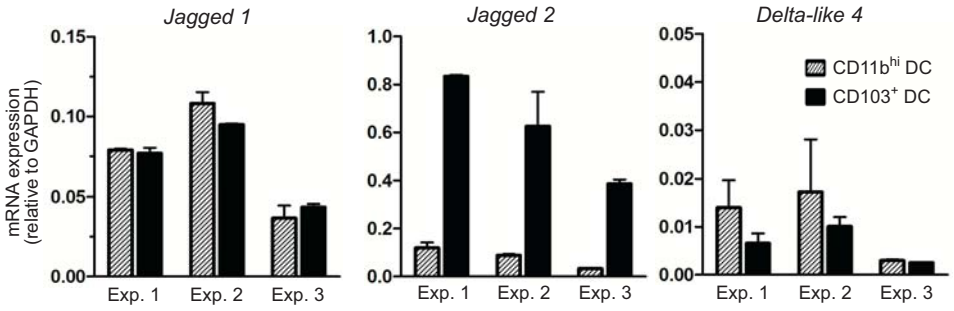

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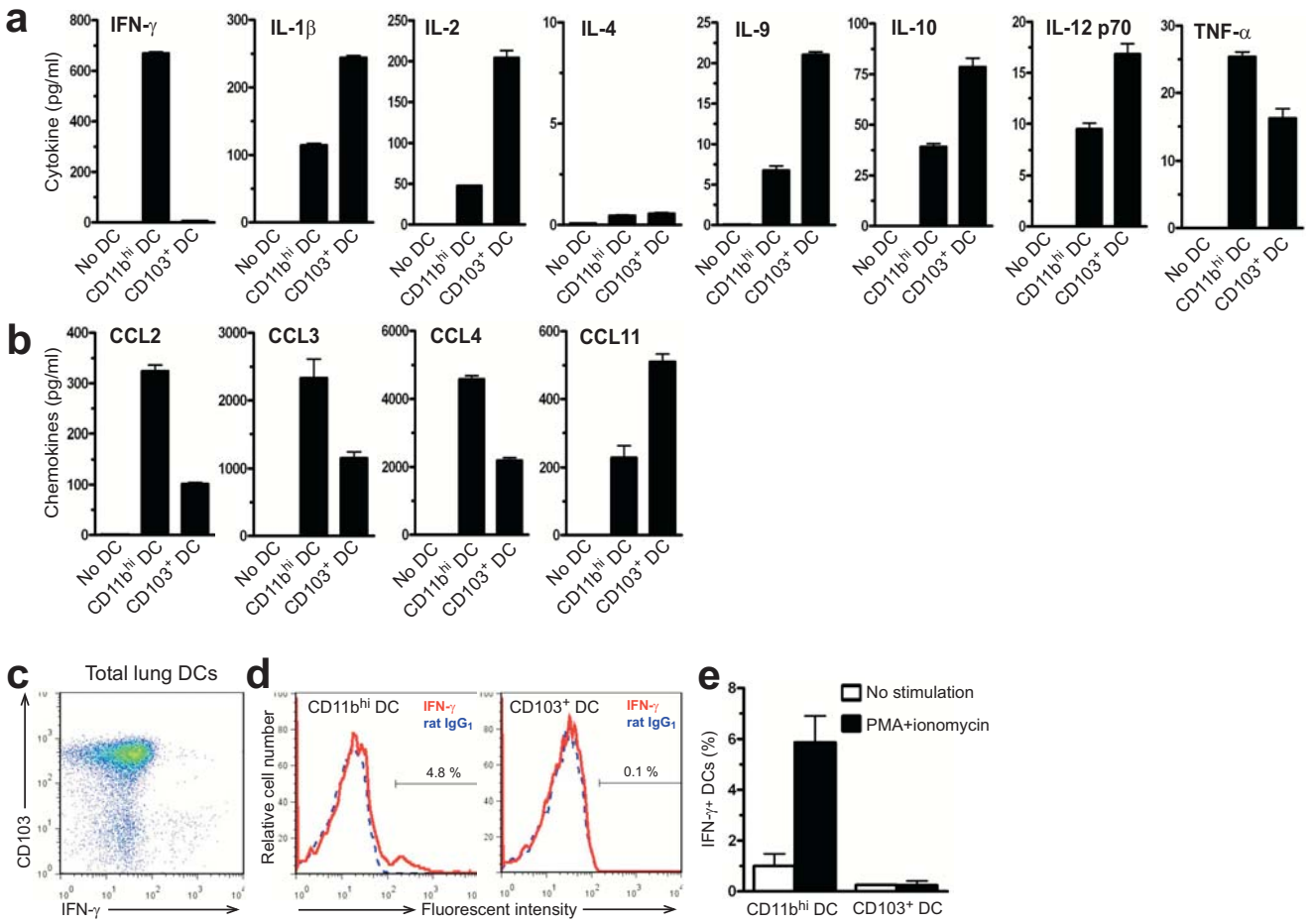

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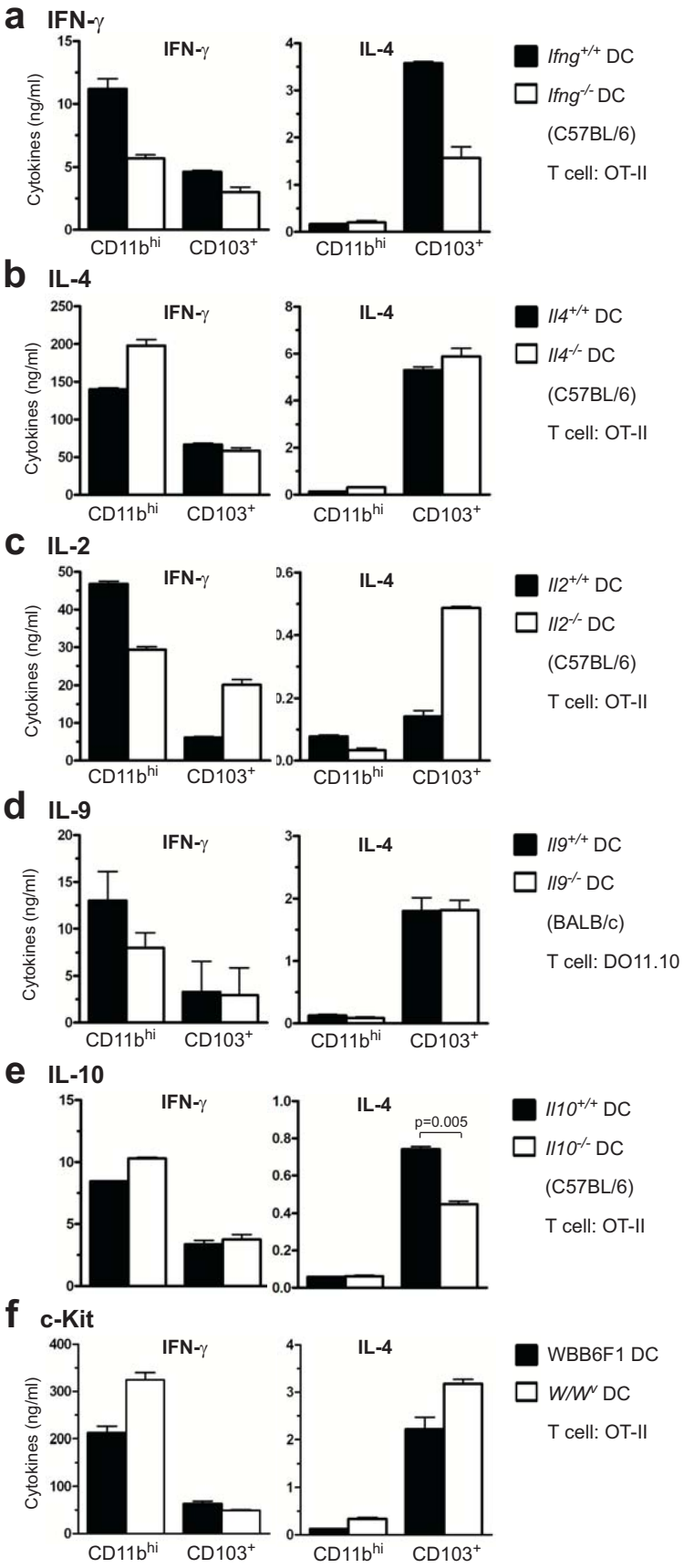

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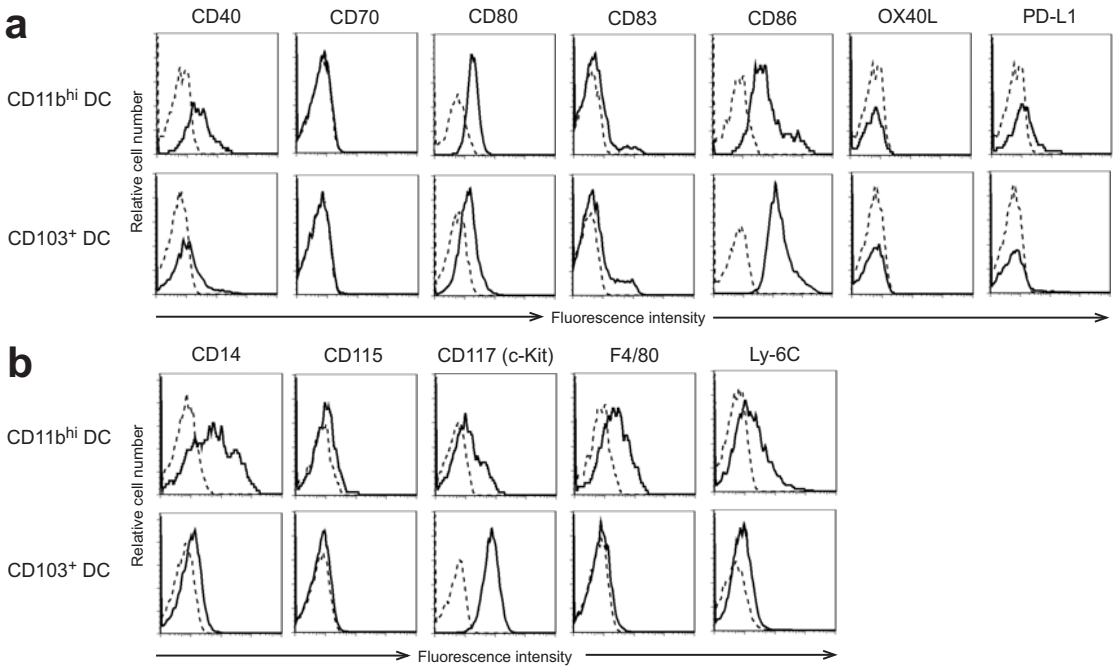
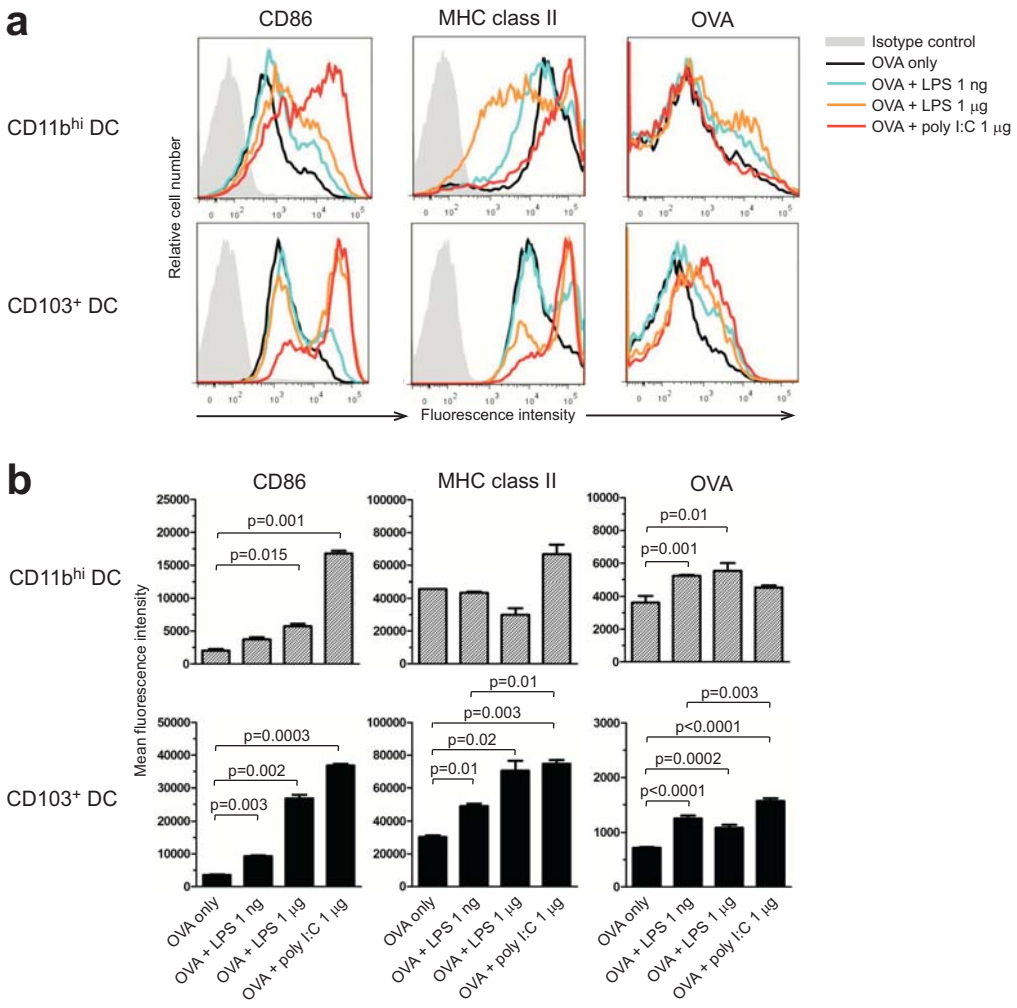


Supplementary Figure 10



Supplementary Figure 11