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SUPPLEMENTAL MATERIAL

Barrett et al., http://www.jem.org/cgi/content/full/jem.20100793/DC1

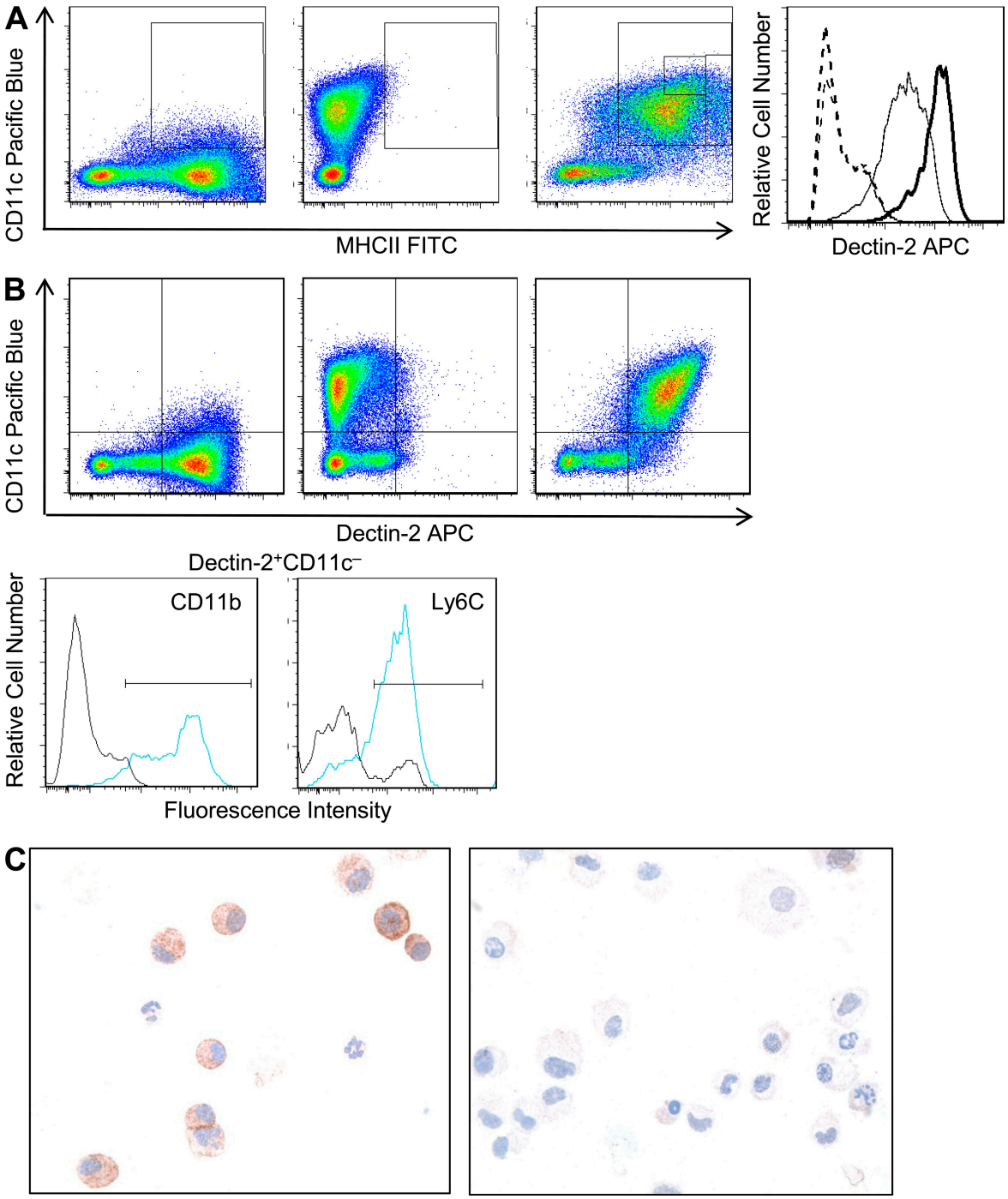

Figure S1. Dectin-2 expression in GM-CSF–expanded BM cultures. Day-7 BMCs$^{GM\text{-}CSF}$ were harvested and stained for the cell surface expression of CD11c, MHCII, and Dectin-2 and analyzed by flow cytometry. (A) Left, a representative histogram is shown of CD11c and MHCII expression, as gated by the isotype controls. Gating on a CD11c^{hi}MHCII^{low} and a CD11c^{+}MHCII^{hi} subset is also indicated (small box and small rectangle, respectively). MHCII staining was performed with saturating antibody concentrations. Right, Dectin-2 expression on the CD11c^{hi}MHCII^{low} subset (thick black line) and the CD11c^{+}MHCII^{hi} subset (thin black line) is shown, as well as the isotype control staining for each population (thick dashed and thin dashed lines, respectively). (B) Top, a representative histogram is shown of CD11c and Dectin-2 expression, as gated by the isotype controls. Bottom, CD11b and Ly6C expression on the Dectin-2^{+}CD11c^{-} population. (C) Dectin-2 expression on cytospin preparations from day-7 BMCs$^{GM\text{-}CSF}$ detected by immunocytochemistry. Positive staining is indicated in brown. Left, anti–Dectin-2. Right, rat IgG2a control. Results are representative of four cultures from two independent experiments.

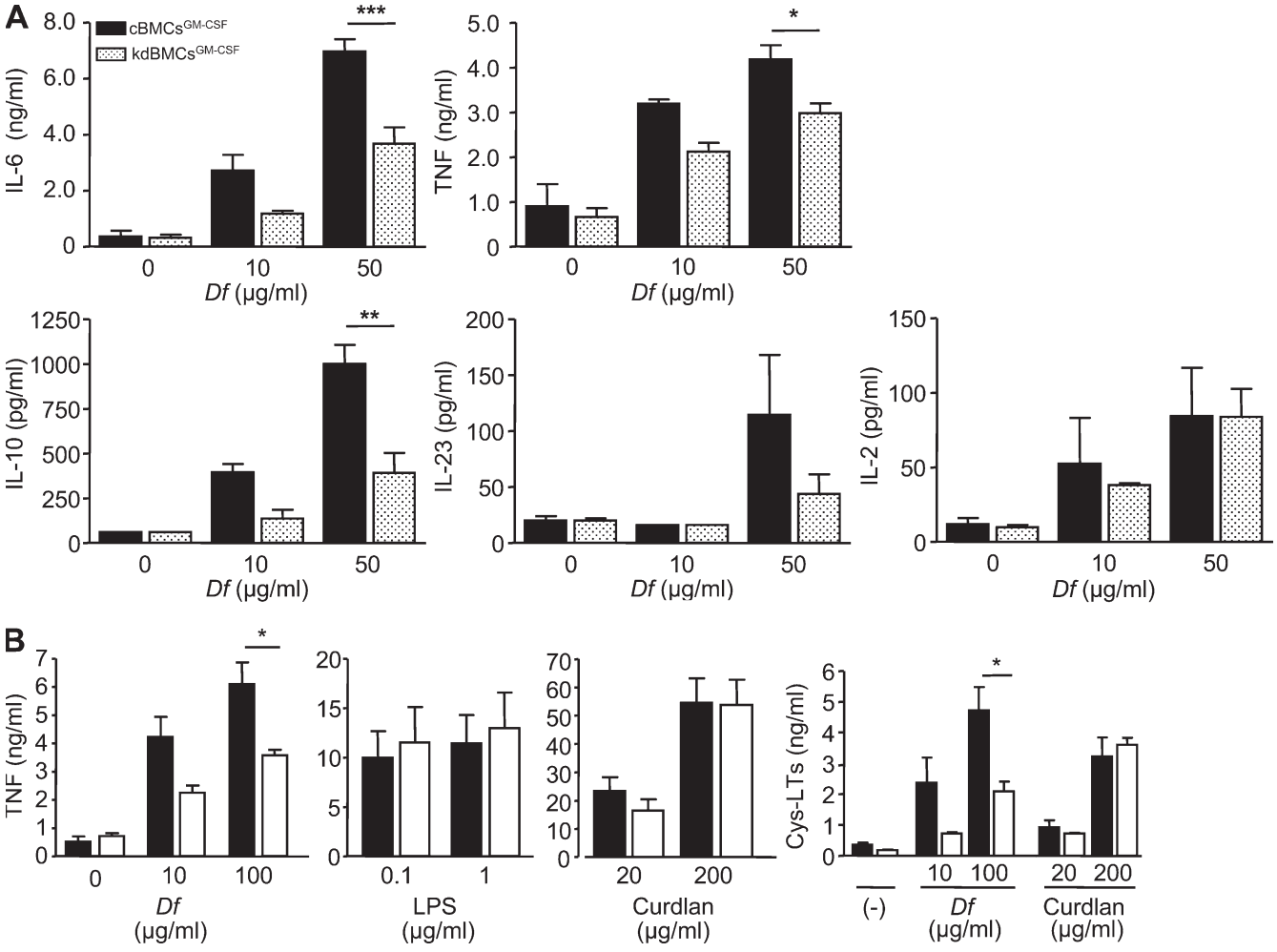

Figure S2. Cys-LT and cytokine production by BMCs$^{GM\text{-}CSF}$ after lentiviral knockdown. BMCs$^{GM\text{-}CSF}$ were infected with viral particles containing Dectin-2 shRNA (kdBMCs$^{GM\text{-}CSF}$), an empty vector control (cBMCs$^{GM\text{-}CSF}$), or a nontargeting vector control (ntcBMC$^{GM\text{-}CSF}$), selected with puromycin, and harvested at day 7. (A) BMCs$^{GM\text{-}CSF}$ were stimulated for 30 h with *Df* at the indicated concentrations, and cytokines in the supernatant were measured by ELISA. Results are means ± SEM (n = 5–6 mice per group) from three independent experiments. *, P = 0.01; **, P = 0.004; ***, P = 0.002. Significance was determined with an unpaired Student's t test. (B) Left, TNF production from cBMCs$^{GM\text{-}CSF}$ (black) or kdBMCs$^{GM\text{-}CSF}$ (white) in response to *Df*, curdlan, or LPS at 4h. Results are means ± SEM (n = 5–6) from three independent experiments. *, P = 0.04. Significance was determined with an unpaired Student's t test. Right, Cys-LT production from cBMCs$^{GM\text{-}CSF}$ (black) or kdBMCs$^{GM\text{-}CSF}$ (white) in response to 10 and 100 µg/ml *Df* or 20 and 200 µg/ml curdlan at 60 min. Results are means ± SEM (n = 4–5) from two independent experiments. *, P = 0.02. Significance was determined with an unpaired Student's t test.

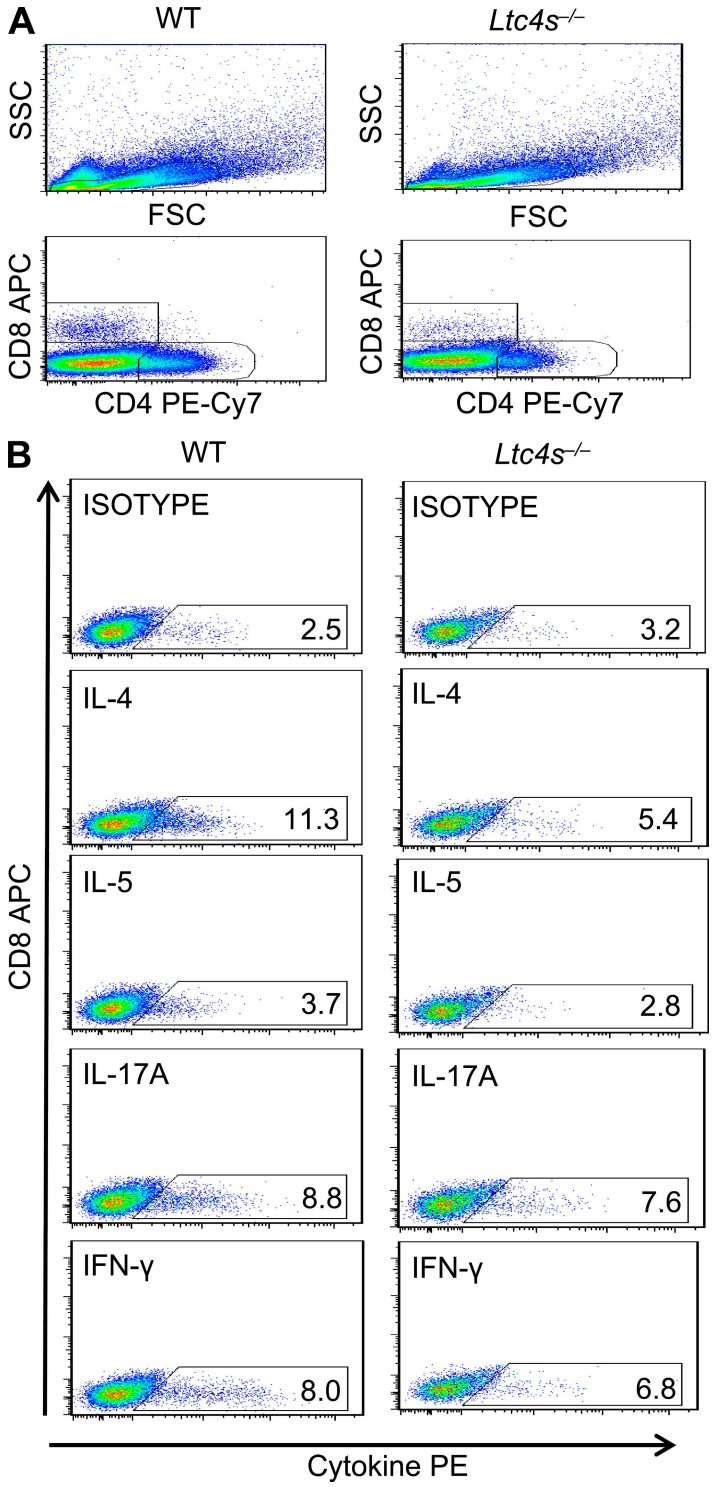

Figure S3. ***Df*-elicited CD4⁺ Th2 cell recruitment to the lung.** WT mice were sensitized with saline-pulsed or *Df*-pulsed WT or *Ltc4s*$^{-/-}$ BMCs$^{GM\text{-}CSF}$ and challenged with *Df* as described in the Fig. 4 legend. Pulmonary mononuclear cells were isolated and stimulated with 50 ng/ml PMA and 1 µM ionomycin for 2 h. 2.5 µM monensin was added and cells were stimulated for an additional 8 h, fixed, permeabilized, stained for cell surface expression of CD4 and CD8 and for intracellular expression of IL-4, IL-5, IL-17A, and IFN-γ, and analyzed by flow cytometry. (A) Cell size (top) and CD4 and CD8 expression (bottom) from mice sensitized with *Df*-pulsed WT (left) or *Ltc4s*$^{-/-}$ (right) BMCs$^{GM\text{-}CSF}$. (B) Intracellular cytokine staining in CD4⁺ cells in the lymphocyte gate from mice sensitized with *Df*-pulsed WT (left) or *Ltc4s*$^{-/-}$ (right) BMCs$^{GM\text{-}CSF}$ with the percentage of cytokine-positive cells noted.

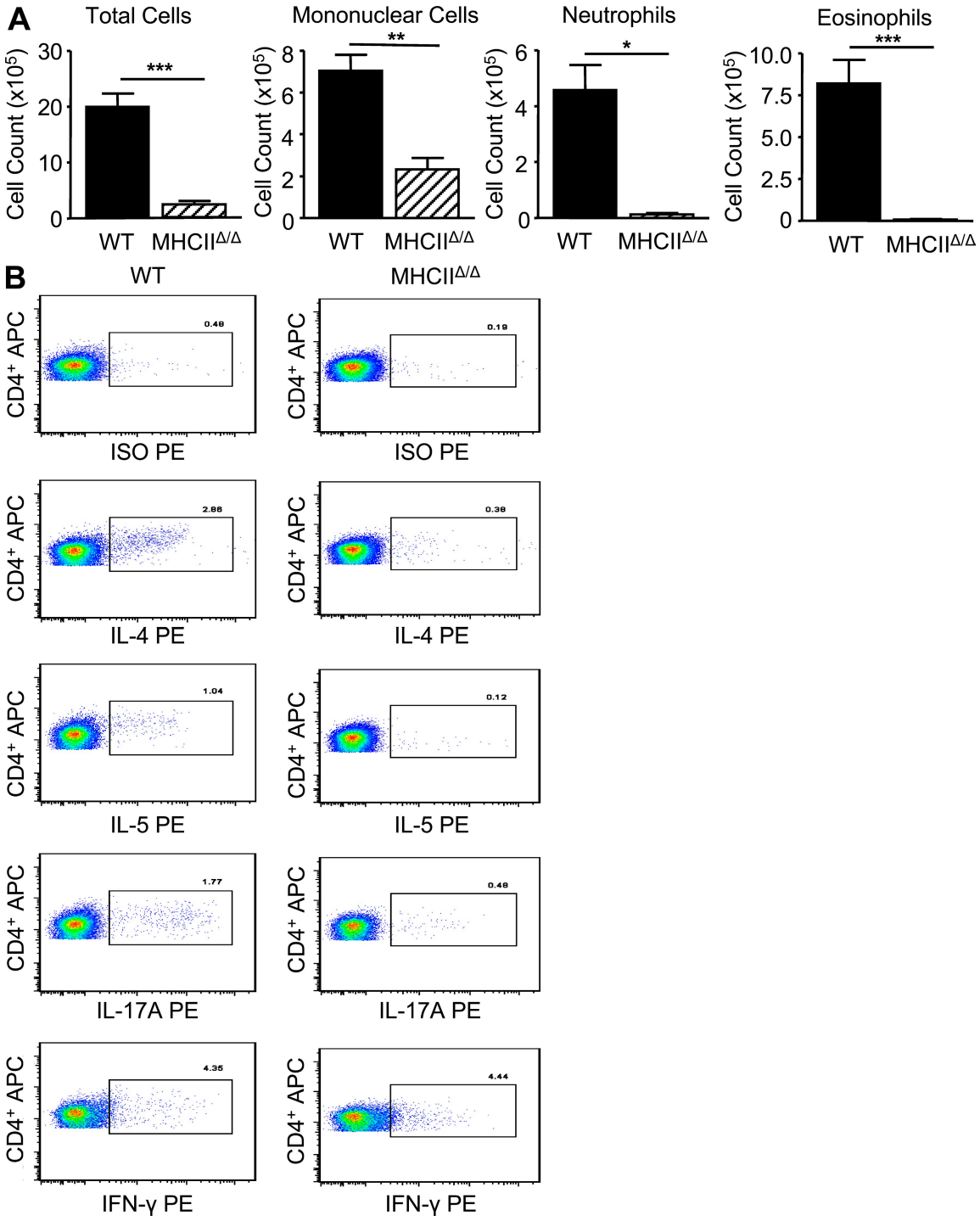

Figure S4. BMCs$^{GM\text{-}CSF}$ MHCII expression is required for *Df*-elicited pulmonary inflammation and CD4$^+$ T cell cytokine generation. WT C57BL/6 mice were sensitized with intranasal administration of 10^4 *Df*-pulsed WT or MHCII$^{\Delta/\Delta}$ BMCs$^{GM\text{-}CSF}$, challenged with 10 µg *Df* on day 22, day 24, and day 26, and analyzed on day 27. (A) Cells from the BAL fluid were counted and 400 cells/slide in cytospins were counted for specific cell types. Results are means ± SEM (n = 8–10 mice per group) from two experiments. *, P = 0.0005; **, P = 0.0002; ***, P = 0.0001. Significance was determined with an unpaired Student's t test. (B) Parabronchial lymph node cells were isolated, stimulated as in Fig. S3 for a total of 6 h, stained for cell surface expression of CD4 and CD8 and for intracellular expression of IL-4, IL-5, IL-17A, and IFN-γ, and analyzed by flow cytometry. Representative histograms show intracellular cytokine staining on CD4$^+$ cells in the lymphocyte gate from WT recipients of *Df*-pulsed WT (left) or MHCII$^{\Delta/\Delta}$ (right) BMCs$^{GM\text{-}CSF}$.

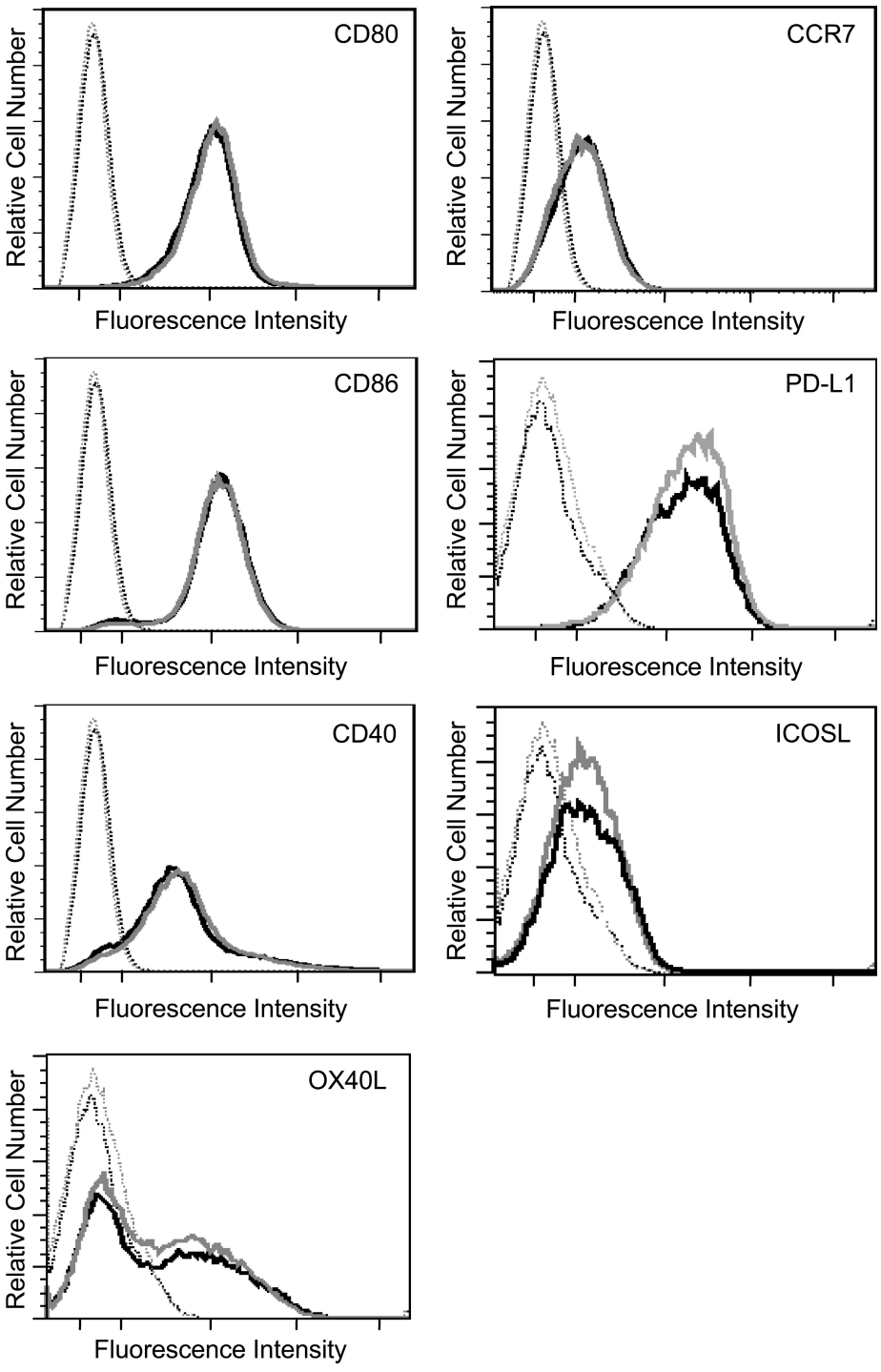

Figure S5. WT and *Ltc4s*$^{-/-}$ BMCs$^{GM\text{-}CSF}$ have comparable cell surface expression of costimulatory markers. WT and *Ltc4s*$^{-/-}$ BMCs$^{GM\text{-}CSF}$ were grown to day 7, pulsed with 100 µg/ml *Df* for 24 h, harvested, and stained for the cell surface expression of CD11c, MHCII, CD80, CD86, CD40, OX40L, CCR7, PD-L1, and ICOSL, and analyzed by flow cytometry. Representative histograms are shown of costimulatory marker expression on CD11c^{+}MHCII^{+} BMCs$^{GM\text{-}CSF}$. Specific stainings are shown for WT (thick black lines) and *Ltc4s*$^{-/-}$ (thick gray lines) BMCs$^{GM\text{-}CSF}$, and isotype control stainings are shown for WT (dotted black lines) and *Ltc4s*$^{-/-}$ (dotted gray lines) BMCs$^{GM\text{-}CSF}$.

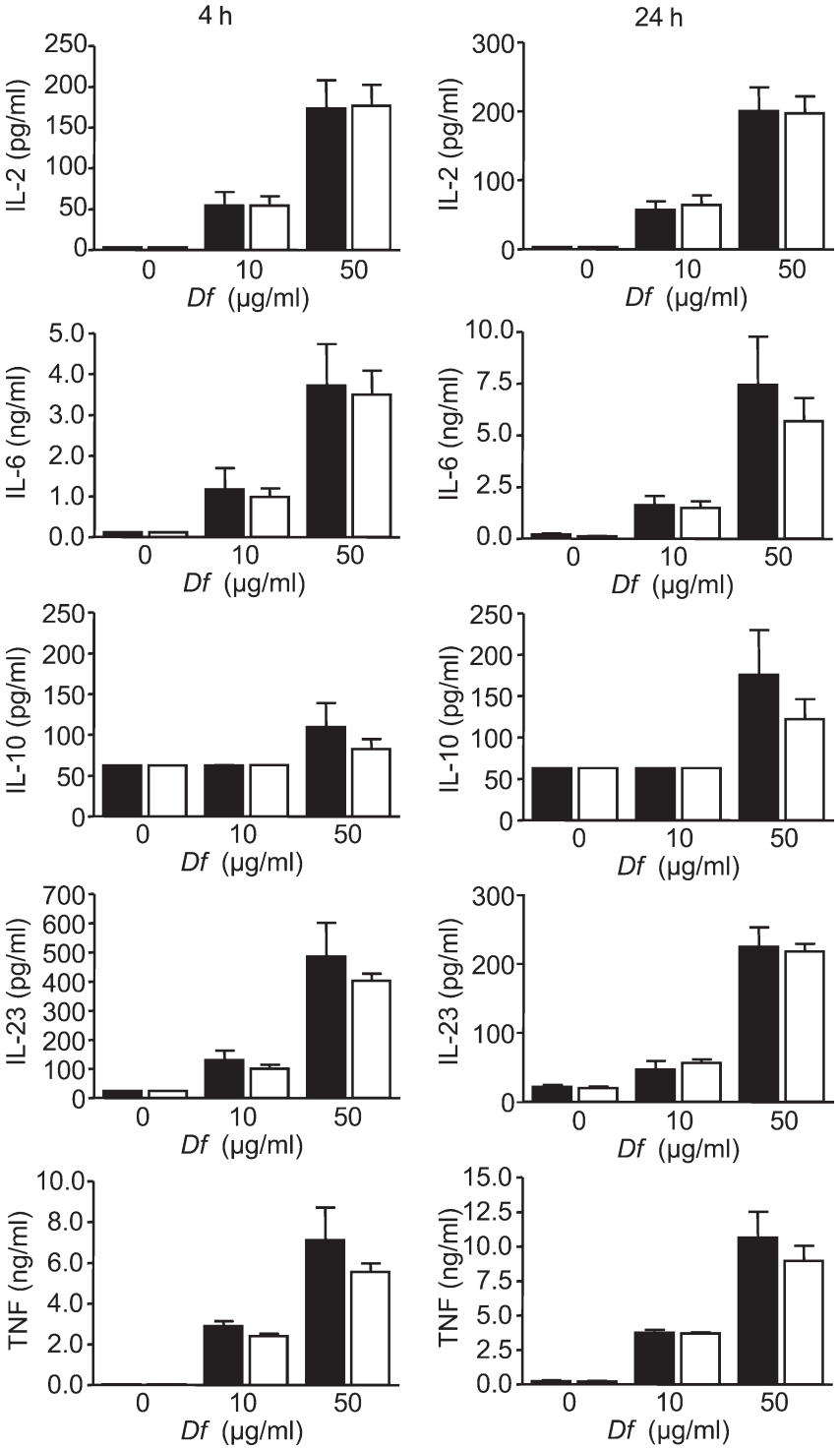

Figure S6. WT and *Ltc4s*$^{-/-}$ BMCs$^{GM\text{-}CSF}$ generate comparable concentrations of *Df*-elicited cytokines at 4 and 24 h. WT (black bars) and *Ltc4s*$^{-/-}$ (white bars) BMCs$^{GM\text{-}CSF}$ were grown to day 7 and stimulated with *Df* at the indicated concentrations for either 4 or 24 h, and cytokines in the supernatants were measured by ELISA. Results are means ± SEM (n = 4 mice per group) from two independent experiments.

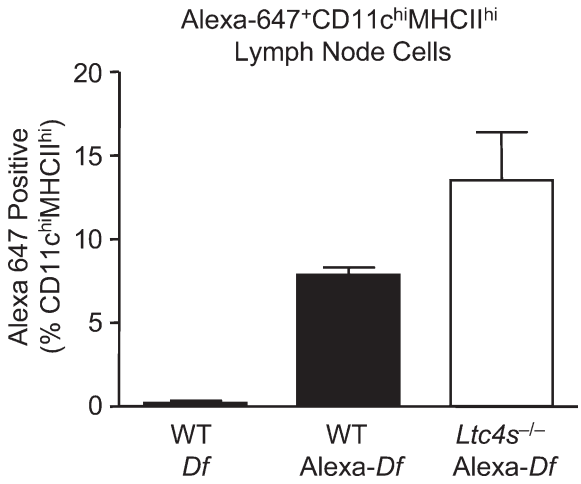

Figure S7. WT and *Ltc4s*$^{-/-}$ BMCs$^{GM\text{-}CSF}$ show comparable migration to the lung-draining lymph nodes. Day-7 BMCs$^{GM\text{-}CSF}$ were stimulated with 100 µg/ml of unlabeled *Df* or Alexa Fluor 647–*Df* for 18 h, and 10^6 cells were transferred intranasally into WT recipients. 24 h later, the draining lymph nodes were harvested and digested, and single cell suspensions were stained for CD11c-PE-Cy7 and MHCII-PE and analyzed by flow cytometry. The percentage of CD11c^{hi}MHCII^{hi} cells that were Alexa Fluor 647 positive is shown. Results are means ± SEM (n = 4–5 mice per group) from two independent experiments.