

Figure S1 | Related to Figure 1

(A) CD301b⁺ DCs as a percentage of total viable CD11c⁺ cells from the popliteal LN 24 hrs after Th0 or Th2-skewing immunization in male or female mice. (B & C) CCR7 expression compared to fluorescence minus one (FMO) on viable CD45⁺CD11c⁺CD301b⁺ cells from the immunization site (skin) or popliteal LN (dLN) 24 hrs after the indicated immunization. Bar graphs represent mean value of indicated data points, error bars represent SEM. Data shown is representative of 3 independent experiments with 2-6 individual mice per condition per experiment. * p<0.05, ** p<0.01, *** p<0.001, Student's T test.

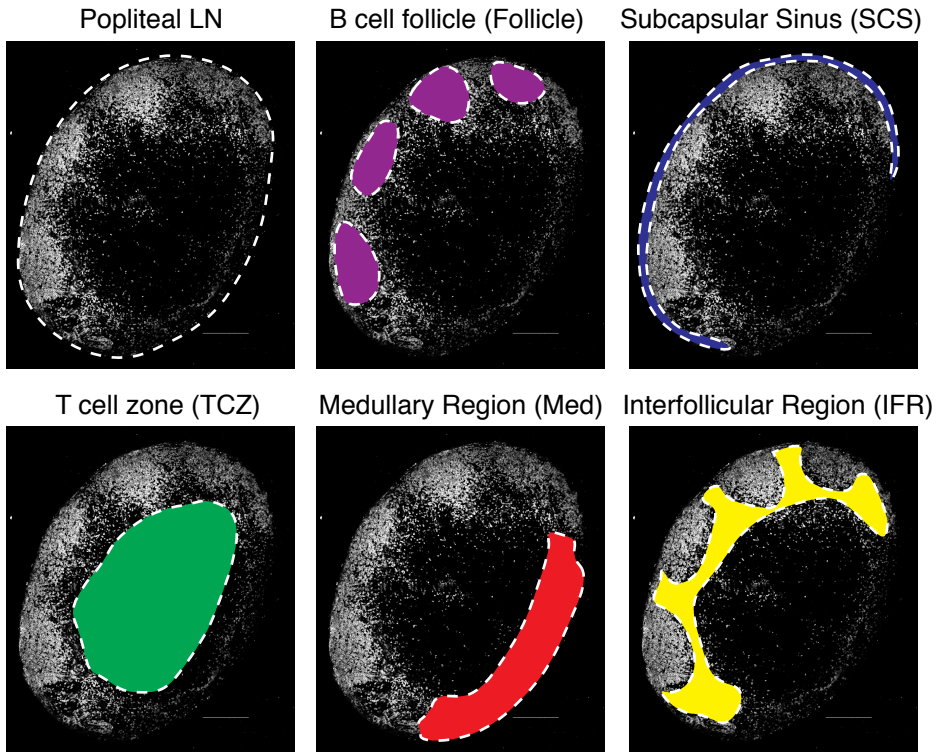


Figure S2 | Related to Figure 2.

Representative section of draining popliteal LN is shown (top left). Color regions indicated LN areas analyzed as determined by B220 staining (white) and LN architecture. Scale bars = 200 μm .

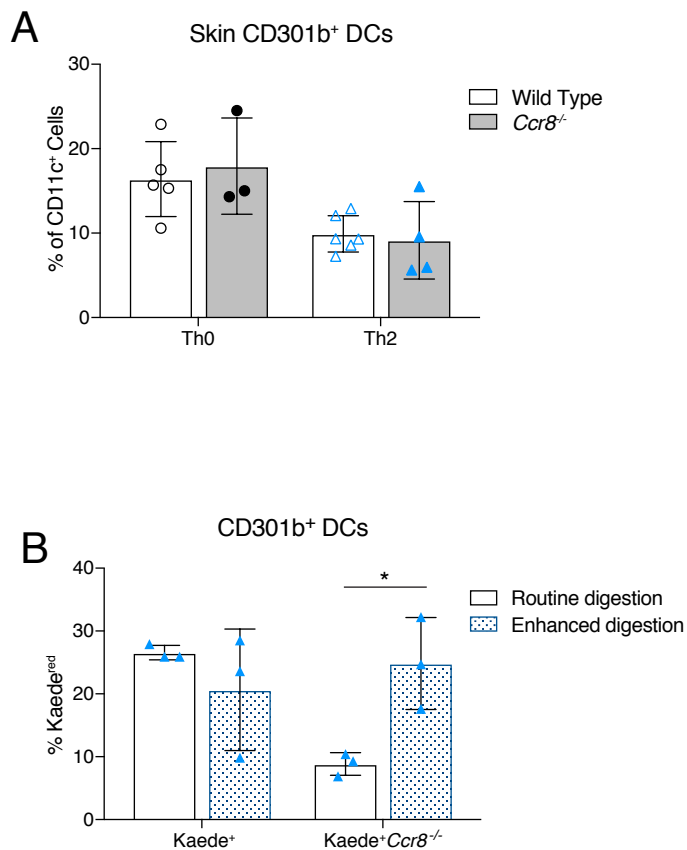


Figure S3 | Related to Figure 3.

(A) CD301b⁺ DCs as a percentage of total viable CD45⁺CD11c⁺ cells from the cutaneous immunization site (skin, footpad) 24 hrs after the indicated immunization of Wild Type or *Ccr8*^{-/-} mice. (B) Percentage of Kaede^{red} cells out of total CD11c⁺CD301b⁺ cells from the dLN 24 hours after photoconversion and Th2-skewing immunization of Kaede⁺ or Kaede⁺*Ccr8*^{-/-} mice. dLNs were harvested and divided for either routine enzymatic digestion (clear black bar) or enhanced enzymatic digestion (dotted blue bar). Bar graphs represent mean value of indicated data points, error bars represent SEM. Data shown is representative of 2 independent experiments with 2-3 individual mice per condition per experiment. * p<0.05, ** p<0.01, *** p<0.001, Student's T test.

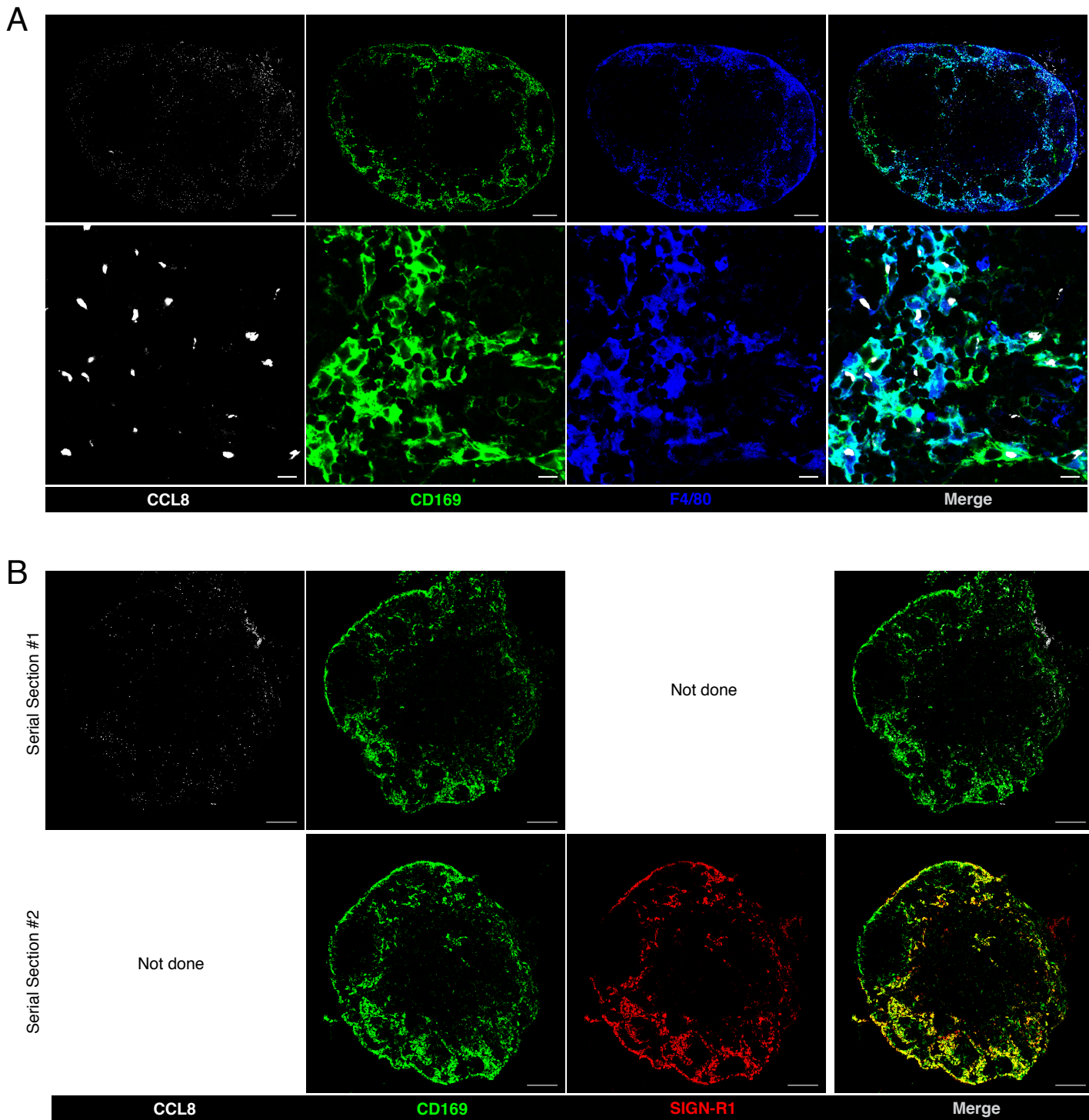


Figure S4 | Related to Figure 4.

(A) CCL8⁺ cells co-express CD169 and F4/80 on their surfaces. Top panels show CCL8 (white), CD169 (green) and F4/80 (blue) staining of entire LN, scale bar = 200 μ m. Bottom panels show zoom in of LN area of interest, scale bar = 10 μ m. (B) CD169⁺CCL8⁺ cells are colocalized with CD169⁺SIGNR1⁺ cells in serial sections. Top panels show CD169 (green) and CCL8 (white) in dLN 24 hrs after Th2-skewing immunization. Bottom panels are of serial section showing CD169 (green) and SIGN-R1 (red) staining in the same dLN. SIGN-R1 and CCL8 could not be done in the same sections due to antibody cross-reactivity and so are labeled “Not done” where appropriate. Scale bars = 200 μ m. Images shown are representative of two independent experiments with 3 mice in each experiment and images are one of six sections per mouse.

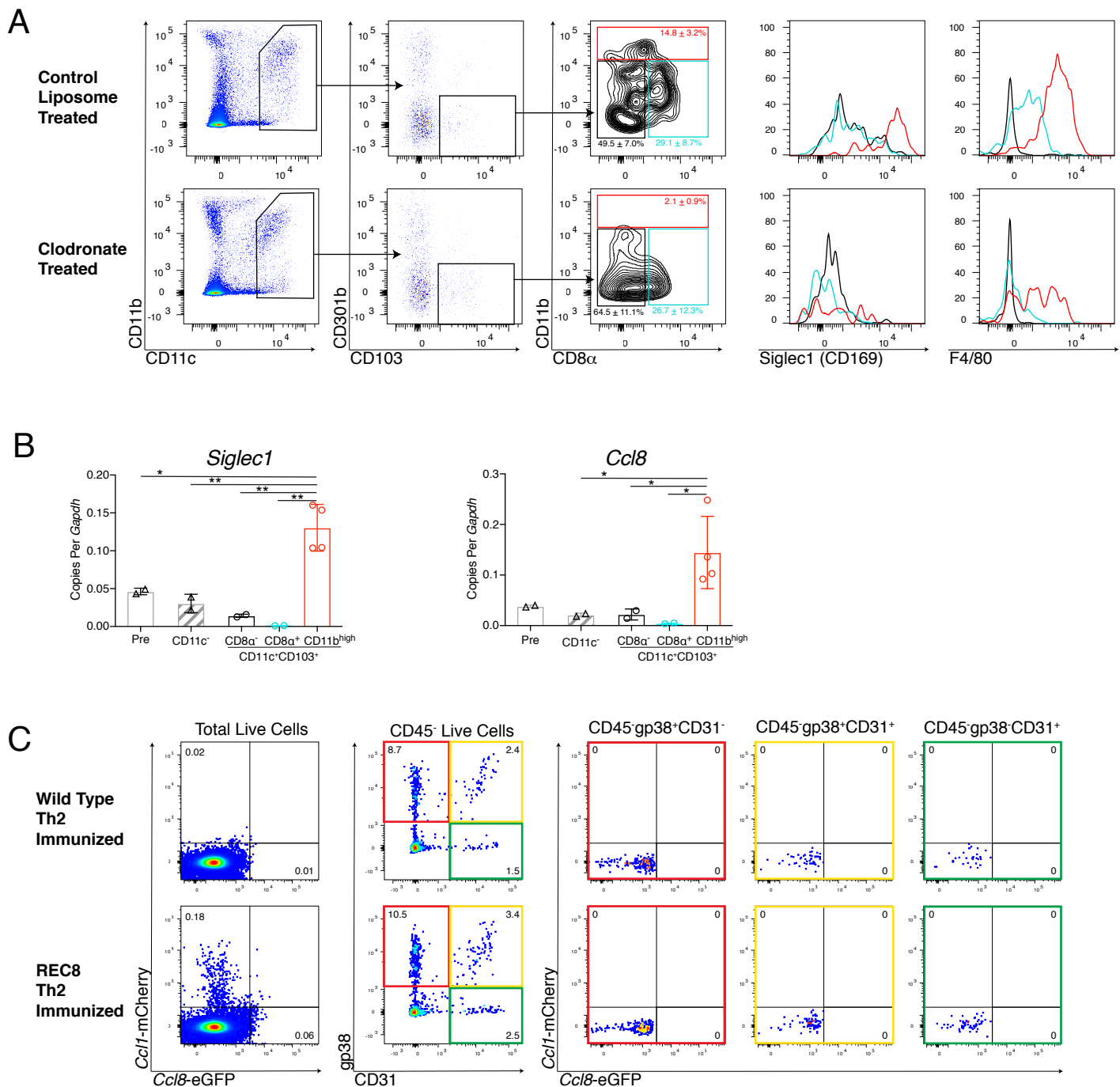


Figure S5 | Related to Figures 4 and 5.

(A) Flow cytometry showing gating scheme to identify CD11c⁺CD103⁺CD11b^{hi}CD169⁺ cells out of total viable singlets from the dLN of control liposome treated or macrophage-depleted, clodronate-treated mice. Colored histograms correspond to colored gates. Data are representative graphs, with percentages and standard deviations derived from 3-5 mice per group, one of four independent experiments shown. (B) QPCR analysis of *Siglec1* and *Ccl8* mRNA from total LN cells (Pre), CD11c⁻ cells, as well as CD11c⁺CD103⁺ cells from the three populations indicated in (A). (C) Flow cytometry of dLN from WT and REC8 mice revealed *Ccl1*-mCherry and *Ccl8*-eGFP expression in total live cells but not in CD45-gp38⁺CD31⁻ (red gate and plots, fibroblastic reticular cells), CD45-gp38⁺CD31⁺ (yellow gate and plots, lymphatic endothelial cells), and CD45-gp38⁻CD31⁺ (green gate and plots, blood endothelial cells). Values are percentages in gate taken from 2-4 independent replicates, one of three independent experiments shown. QPCR data presented as copies of indicated transcript over *Gapdh*. Bar graphs represent mean value of indicated data points, error bars represent SEM. * p<0.05, ** p<0.01, Student's T test.

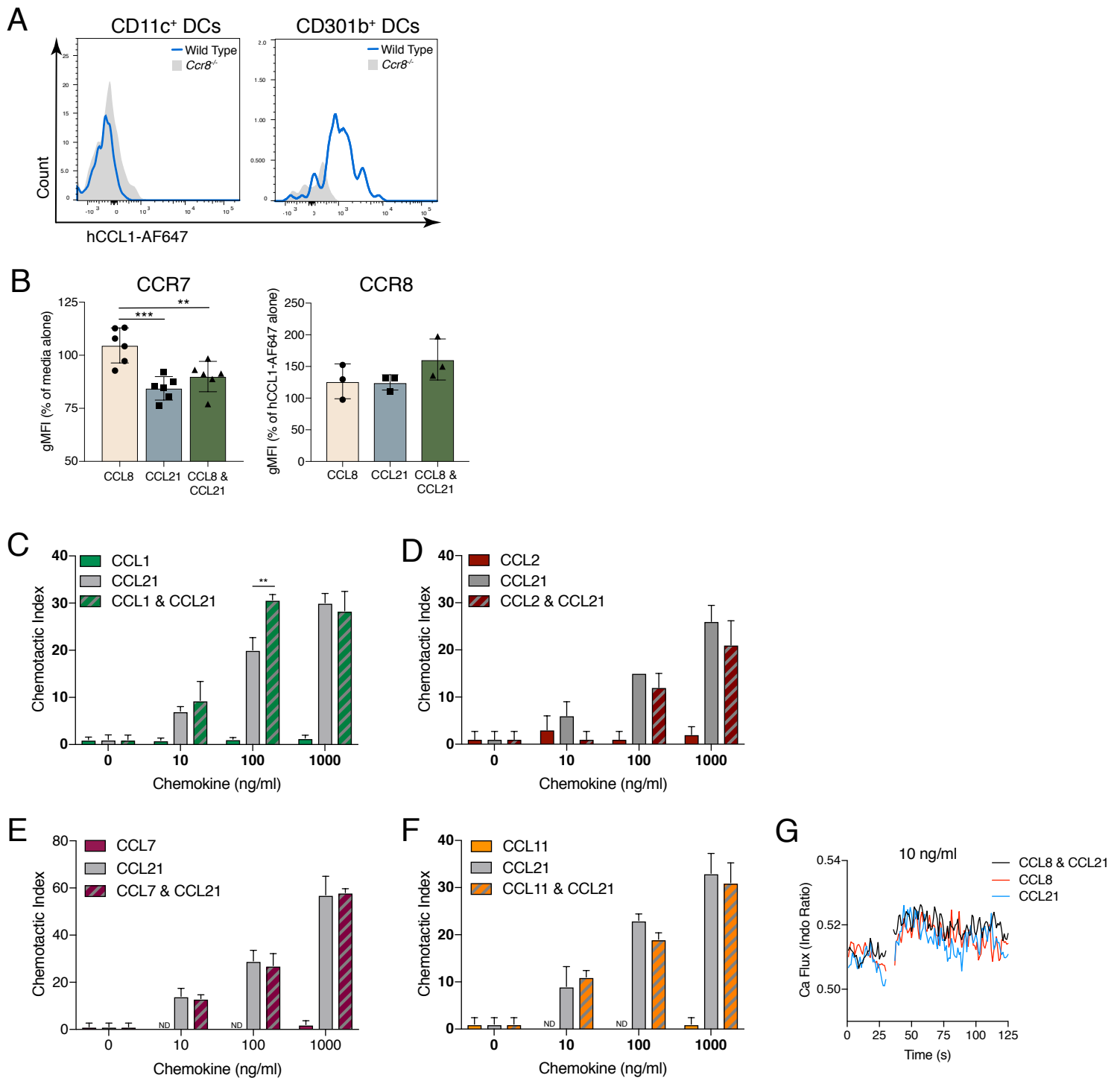


Figure S6 | Related to Figure 6.

(A) CCR8 expression as a measure of hCCL1-AF647 binding after overnight exposure of wild type (blue histogram) or *Ccr8*^{-/-} (shaded gray histogram) CD11c⁺ DCs or CD301b⁺ DCs to hCCL1-AF647. (B) CCR7 expression and hCCL1-AF647 binding of ex vivo CD301b⁺ DCs after 2 hours stimulation with the specified chemokine over that of the media control (gray shaded histogram). gMFI data shown is normalized to that of the media control. Transwell chemotaxis of CD301b⁺ DCs to (C) CCL1 (green bar), (D) CCL2 (red bar), (E) CCL7 (magenta bar), (F) CCL11 (orange bar), (C-F) CCL21 (gray bar), or combined respective chemokines (hatched bars). (G) Ca²⁺ flux expressed as the Indo-1 AM ratio from CD301b⁺ DCs stimulated ex vivo as indicated. In all cases cells were harvested from dLNs of mice 24 hrs after Th2-immunization. Representative graphs are shown from one of three (A, B, C) or one of two (D-G) independent experiments. Cells in (C-D) were pooled from 50 mice and cells in (G) were pooled from 12 mice. Bar graphs represent mean value of 2-3 data points/replicates per bar, error bars represent SEM. ** p<0.01, Student's T test.

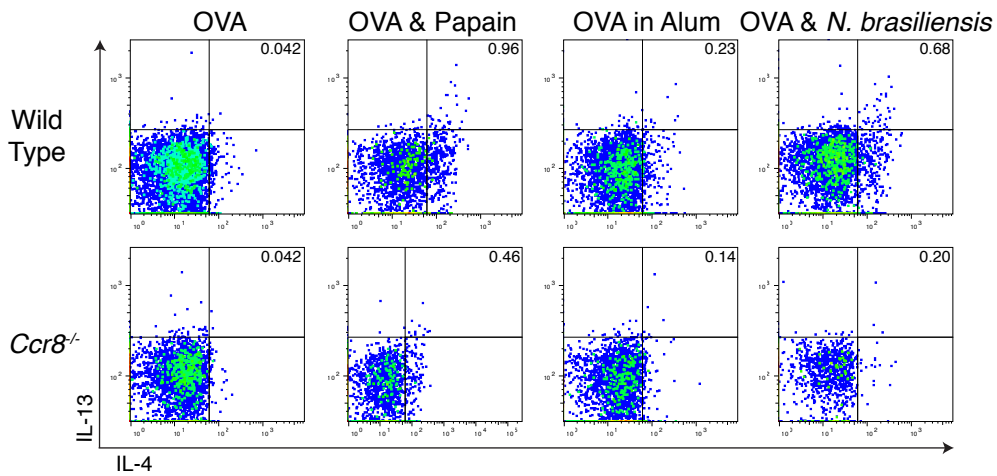


Figure S7 | Related to Figure 7.

Intracellular IL-4 and IL-13 staining of CD4⁺Thy1.1⁺ viable cells 4 days after OVA, OVA & Papain, or OVA in Alum immunization or 5 days after OVA & *N. brasiliensis* infection. Mice received 2.5-5x10⁵ naïve CD4⁺Thy1.1⁺ OTII cells prior to immunization. Representative plots are shown with percentage of cells in quadrants indicated. Data shown is representative of 3 independent experiments.