

SUPPLEMENTAL MATERIAL

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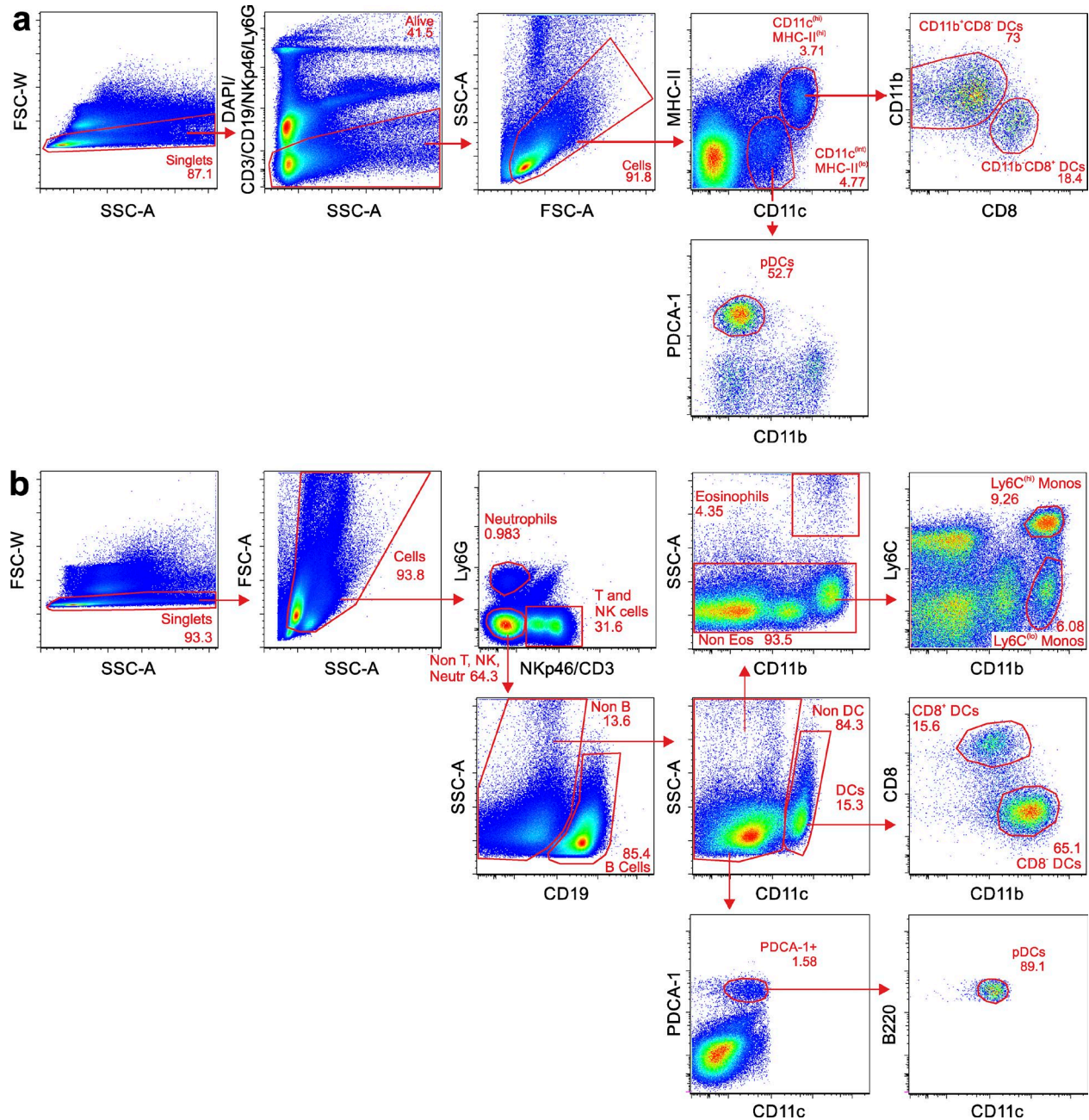


Figure S1. Gating strategy of DC subpopulations and other cells in mouse spleen. (a) Spleen single-cell suspensions were generated from C57BL/6 and the respective FcγR knockout mice by Collagenase D/DNase I digestion. Cells were stained with DAPI, CD11c, PDCA-1, CD11b CD8, MHC-II, Ly6G, CD3, CD19, CD335 (NKp46), and the corresponding FcγR antibodies. In all experiments, doublets were excluded by SSC-A/FSC-W gating followed by exclusion of T cells, B cells, NK cells, and neutrophils (lin⁻: CD3⁻CD19⁻Ly6G⁻NKp46⁻). (a) CD11c⁺ cells were further analyzed for CD11c^{high}MHC-II^{high}CD11b⁺CD8⁻ DCs (CD11b⁺CD8⁻ DCs), CD11c^{high}MHC-II^{high}CD11b⁺CD8⁺ DCs (CD11b⁺CD8⁺ DCs), and CD11c^{low}MHC-II^{low}CD11b⁺PDCA-1⁺ pDCs (pDCs). (b) For analysis of recombinant antibody uptake, whole splenocytes were isolated, labeled with the recombinant Ova-carrying antibodies, and incubated for 120 min at 37°C or on ice, respectively. After fixation, cells were stained with the secondary and cell identification antibodies αmslgG1, CD3, CD8, CD11b, CD11c, CD19, CD45R (B220), CD335 (NKp46), Ly6C, Ly6G, and PDCA-1. Pseudocolor dot plots show doublet exclusion by SSC-A/FSC-W gating followed by exclusion of debris and dead cells with gates set as Ly6G⁺ (neutrophils), NKp46⁺CD3⁺ (T and NK cells), and Ly6G⁻NKp46⁻CD3⁻ cells. From the latter ones, CD19⁺ cells (B cells) were further gated in a SSC-A/CD19 blot. CD19⁻ cells were depicted in a SSC-A/CD11c blot, in which CD11c^{high} cells were analyzed for CD8 (CD11c⁺CD8⁺) and CD11b (CD11c⁺CD8⁻) expression. From the SSC-A/CD11c⁻ gate, cells were further analyzed for CD11b expression in a SSC-A/CD11b gate. SSC-A^{high}/CD11b^{high} cells characterize eosinophils. All SSC-A^{low} cells were split up into Ly6C^{high}CD11b^{high} and Ly6C^{low}CD11b^{high} cells. pDCs were gated as CD19⁻, CD11c^{low}, PDCA-1⁺, and B220^{low}. FACS analysis was performed on a FACSCanto II, and data were analyzed using DIVA and FlowJo software. The experiment was repeated at least three times with similar results.

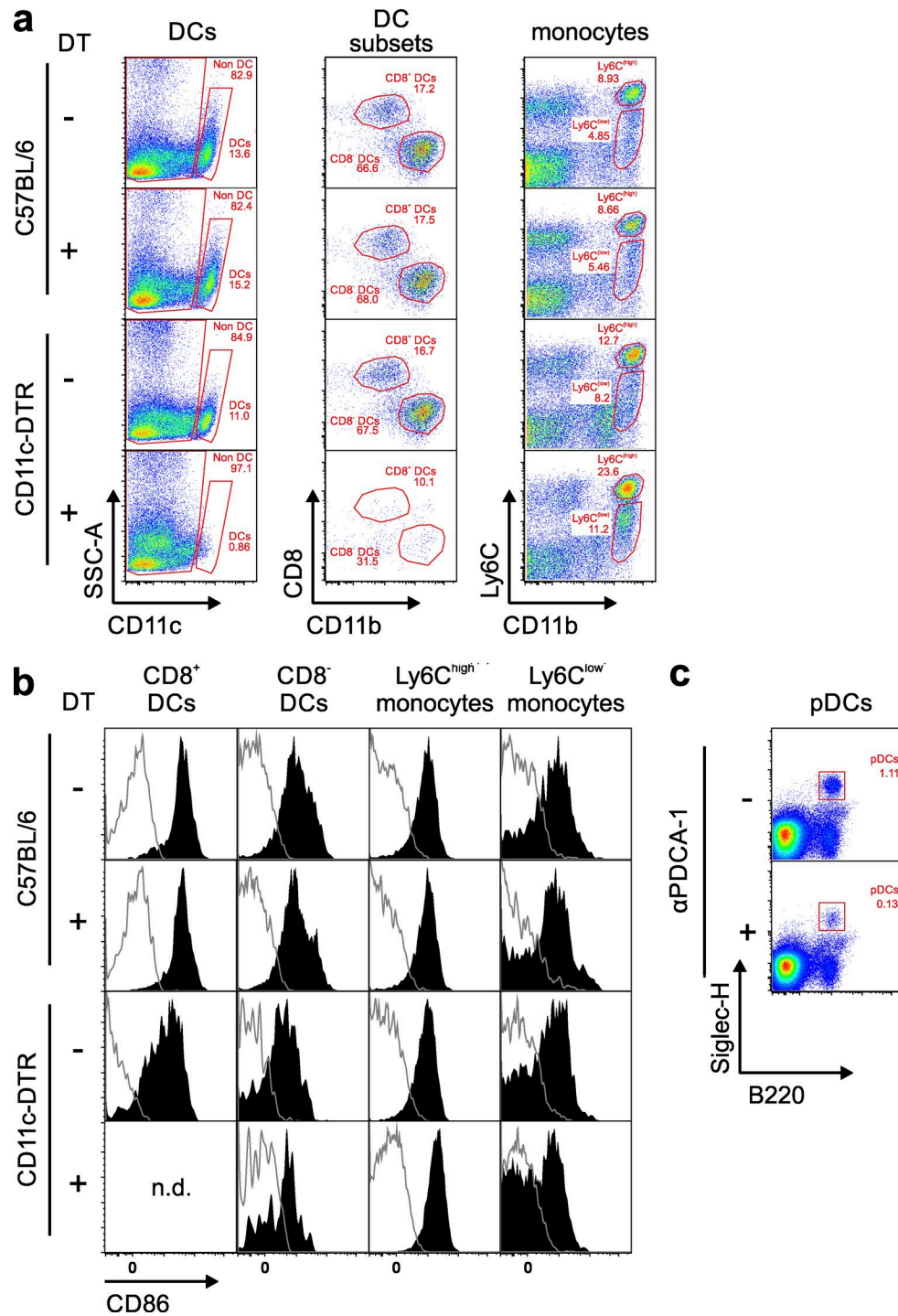


Figure S2. Depletion of cDCs by DT injection in CD11c-DTR transgenic mice, and pDC depletion by α PDCA-1 injection in C57BL/6 mice. CD11c-DTR transgenic and C57BL/6 mice were treated i.p. with 20 ng/g bodyweight DT or PBS. (a and b) 24 h later, splenocytes were isolated and analyzed for DC and monocyte subpopulations (a) and their activation status (b); gating was performed as shown in Fig. S1). n.d., not detected. (c) C57BL/6 mice were i.p. treated three times (72, 48, and 24 h before analysis) with 200 μ g α PDCA-1 antibody. Splenic single-cell suspensions were generated, and Siglec-H⁺B220⁺ pDC numbers were analyzed by FACS. The experiment was repeated three times with similar results.