JEM

Supplemental material

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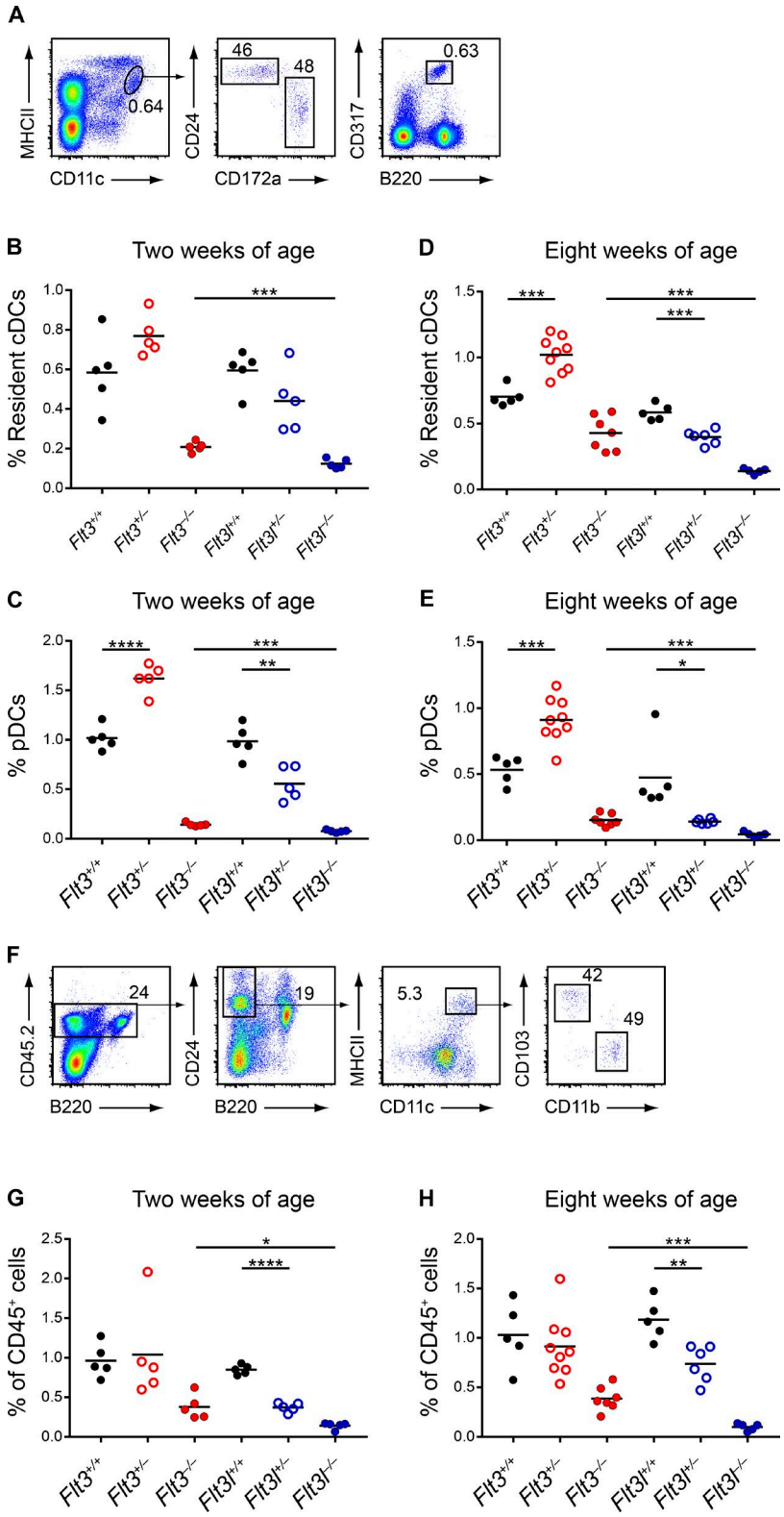

Figure S1. **DC deficiency in SLNs and lungs is more severe in *Flt3l*$^{-/-}$ mice than in *Flt3*$^{-/-}$ mice. (A)** Gating strategy used for identifying resident cDCs (CD11c^{+}MHCII^{int}) and pDCs (B220^{+}CD317^{+}) in the SLNs by flow cytometry. Numbers specify the percentage of cells within the indicated gates. **(B–E)** Summary data for the percentage of resident cDCs (B and D) and pDCs (C and E) in SLNs of mice of the indicated genotypes at 2-wk (B and C) or 8-wk (D and E) of age. Dots represent biological replicates; small horizontal lines indicate the mean. Data are pooled from 16 independent experiments (n = 5–9 mice per genotype). **(F)** Gating strategy used for identifying cDCs (CD45.2^{+}CD24^{+}B220^{-}CD11c^{+}MHCII^{+}) in the lung by flow cytometry. **(G and H)** Summary data for the percentage of cDCs among CD45^{+} cells in lungs of mice of the indicated genotypes at 2-wk (G) or 8-wk (H) of age. Dots represent biological replicates; small horizontal lines indicate the mean. Data are pooled from 16 independent experiments (n = 5–9 mice per genotype). *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001 using unpaired, two-tailed Student's t test.

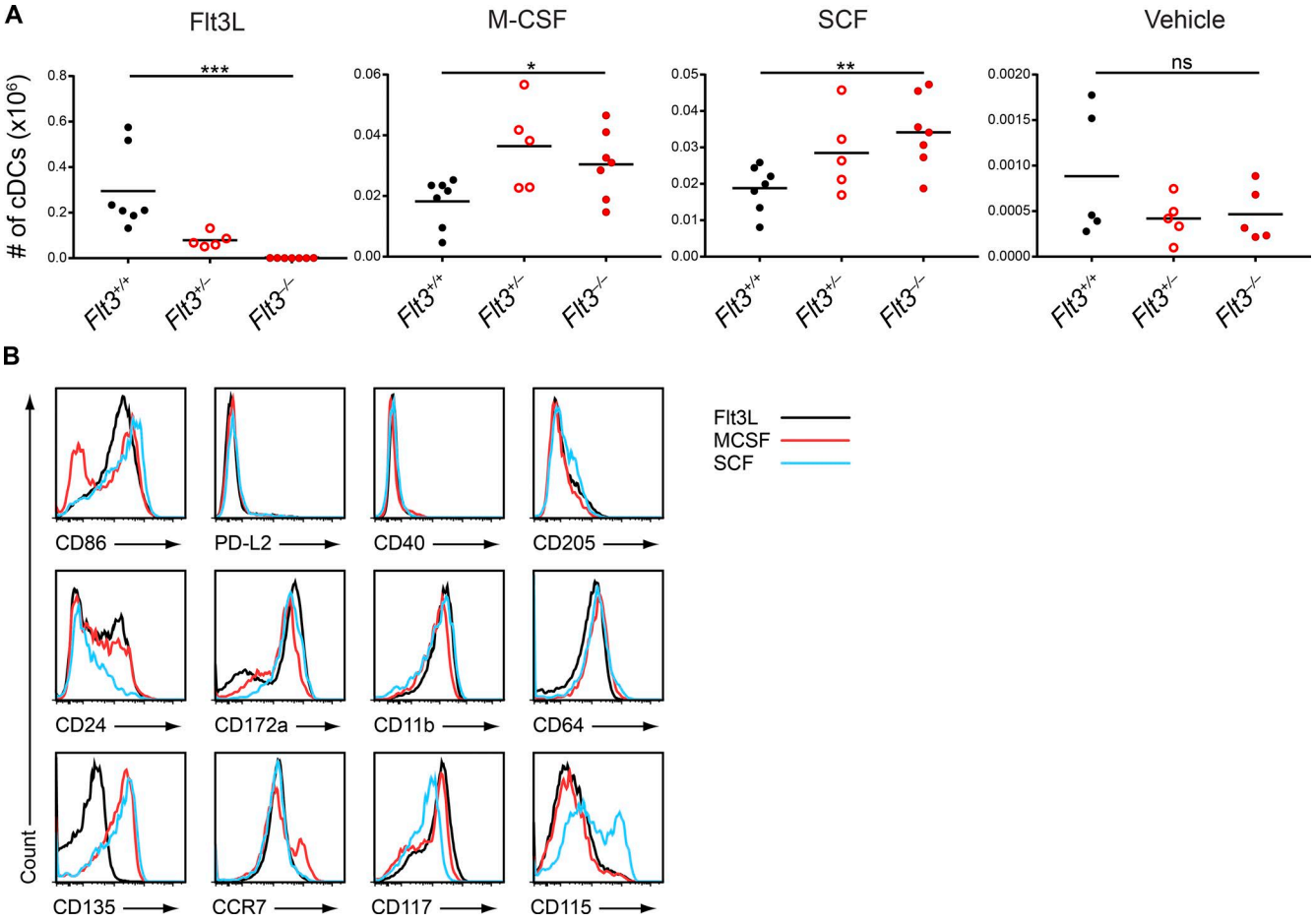

Figure S2. **cDCs from M-CSF and SCF cultures are phenotypically similar to cDCs from Flt3L cultures. (A and B)** BM cells from mice of the indicated genotypes were treated with vehicle, Flt3L, M-CSF, or SCF and cultured for 7 d. Live cells were subsequently analyzed by flow cytometry for the development of cDCs and for their surface marker expression. **(A)** Summary data for the number of cDCs in each culture are shown. Dots represent biological replicates; small horizontal lines indicate the mean. Data are pooled from five independent experiments (n = 5–7 mice per genotype). **(B)** cDCs ($B220^-F4/80^-CD11c^+MHCII^+$) from WT BM cultures were analyzed by flow cytometry for expression of surface markers. Shown are representative one-color histograms of the indicated marker in cDCs from the indicated cytokine cultures. Data are representative of two independent experiments (n = 4 mice). ns, not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ using unpaired, two-tailed Student's t test.

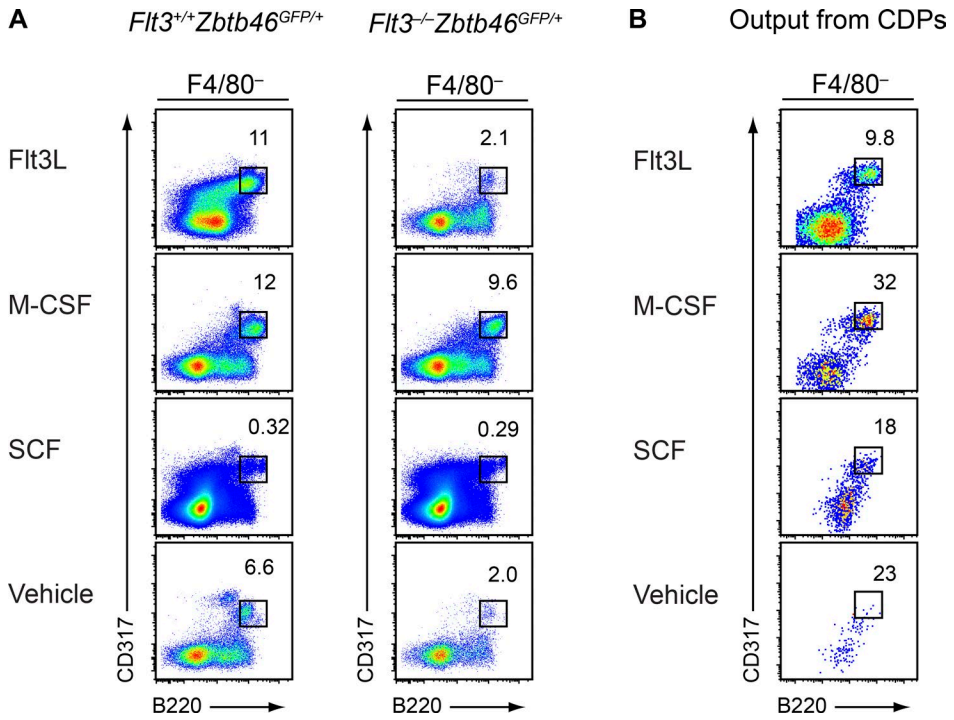

Figure S3. **M-CSF and SCF support pDC development from *Flt3*$^{-/-}$ BM. (A)** BM cells from mice of the indicated genotypes were treated with vehicle, Flt3L, M-CSF, or SCF and cultured for 7 d. Live cells were subsequently analyzed by flow cytometry to quantify pDC development. Shown are representative two-color histograms of live cells pregated as indicated above the plots. Numbers specify the percentage of cells within the indicated gates. Data are representative of five independent experiments (n = 5–7 mice per genotype). **(B)** CDPs (Lin$^-$CD117^{int}CD135$^+$CD115$^+$CD11c$^-$MHCII$^-$) were sorted from *Zbtb46*$^{GFP/+}$ mice and cultured with vehicle, Flt3L, M-CSF, or SCF for 5 d. Live cells were subsequently analyzed by flow cytometry to quantify pDC development. Shown are representative two-color histograms of live cells pregated as indicated above the plots. Numbers specify the percentage of cells within the indicated gates. Data are representative of three independent experiments (sorted cells from three to five mice pooled in each individual experiment).

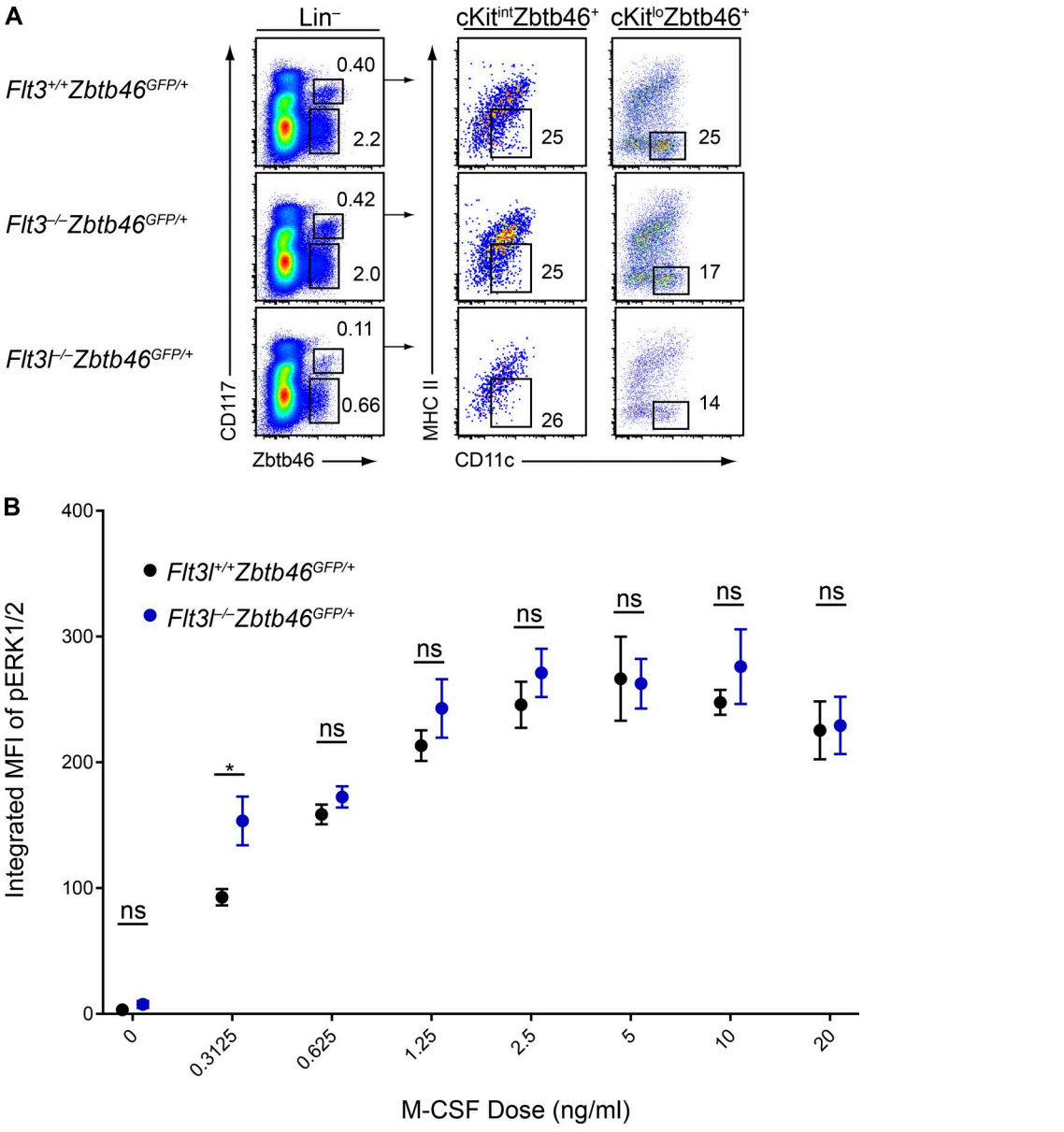

Figure S4. **Committed DC progenitors arise in *Flt3l*$^{-/-}$ BM and do not display increased sensitivity to M-CSF relative to WT progenitors. (A)** BM cells from mice of the indicated genotypes were analyzed by flow cytometry for DC progenitor populations; lineage markers include CD3, CD19, CD105, Ter119, and Ly-6G. Representative two-color histograms are shown of live cells pregated as indicated above the plots. Numbers specify the percentage of cells within the indicated gates. Data are representative of three independent experiments (n = 3–7 mice per genotype). **(B)** Serum-starved BM from mice of the indicated genotypes was treated with M-CSF and assayed for phosphorylated Erk1/2 (pErk1/2) by intracellular flow cytometry. Shown are summary data presented as the integrated MFI of pErk1/2 in pre-cDC2s from mice of the indicated genotypes stimulated with the indicated concentration of M-CSF. Dots indicate the mean from three independent experiments; error bars indicate the SEM (n = 3 mice per genotype). ns, not significant ($P > 0.05$); *, $P < 0.05$ using Student's t test.

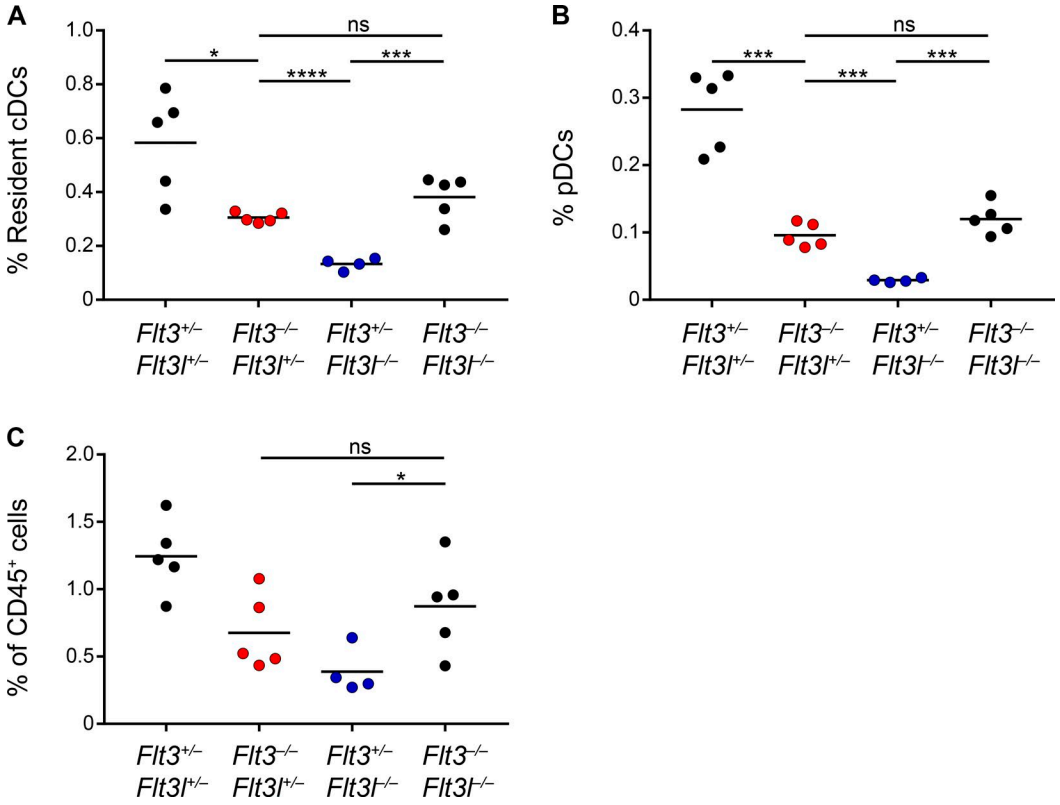

Figure S5. **Deletion of Flt3 in *Flt3l*$^{-/-}$ mice reverses the severe DC defect in SLNs and lungs. (A and B)** Summary data for the percentage of resident cDCs (A) and pDCs (B) determined as in Fig. S1 A in the SLNs of mice of the indicated genotypes. Dots represent biological replicates; small horizontal lines indicate the mean. Data are pooled from six independent experiments (n = 4–5 mice per genotype). **(C)** Summary data for the percentage of cDCs among CD45$^+$ cells determined as in Fig. S1 F from mice of the indicated genotypes. Dots represent biological replicates; small horizontal lines indicate the mean. Data are pooled from six independent experiments (n = 4–5 mice per genotype). *, P < 0.05; ***, P < 0.001; ****, P < 0.0001 using unpaired, two-tailed Student's t test.