

SUPPLEMENTAL MATERIAL

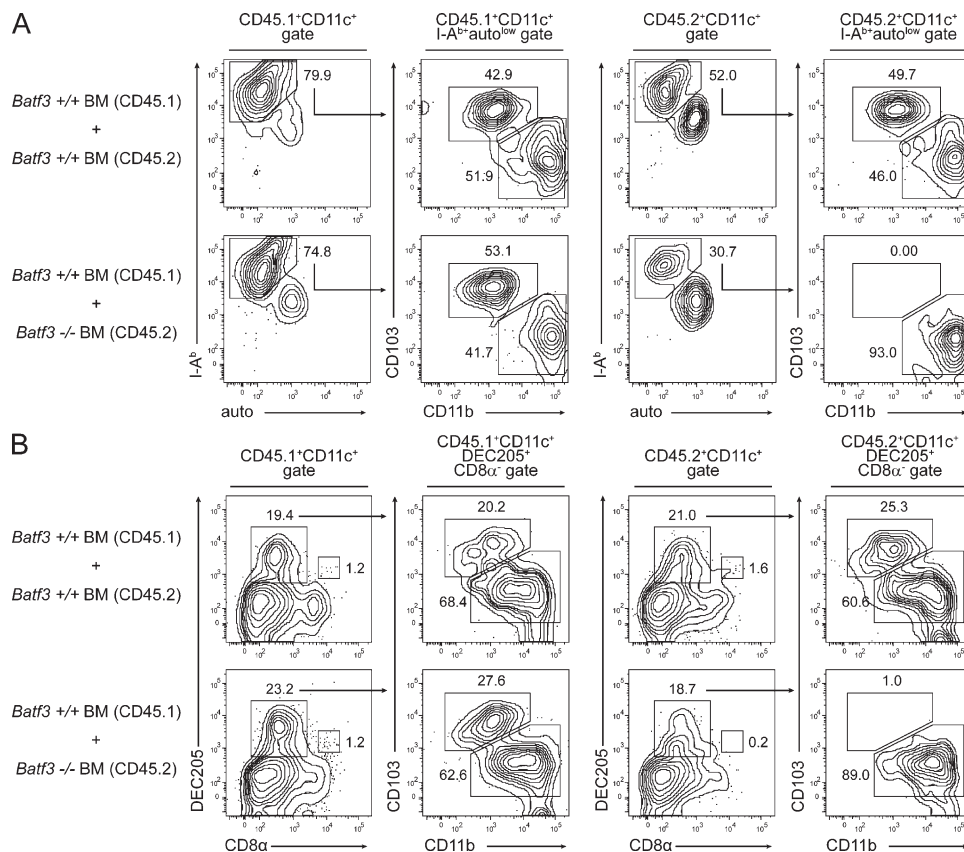
Edelson et al., <http://www.jem.org/cgi/content/full/jem.20091627/DC1>

Figure S1. Cell-intrinsic requirement for *Batf3* in the development of CD103⁺CD11b⁻ DCs. (A) Cell suspensions of lung digests from mixed BM chimeric mice were stained for CD45.1, CD45.2, CD11c, I-A^b, autofluorescence (FITC channel; auto), CD11b, and CD103 (representative data from a single experiment involving two mice per group). Live CD45.1⁺CD11c⁺ or CD45.2⁺CD11c⁺ cells, representing mixtures of lung macrophages and DCs, were initially gated, with the I-A^b⁺auto^{low} population (DCs) further analyzed for the discrimination of pulmonary DC subsets. Numbers represent the percentage of cells within the indicated gates. Note that in the bottom right plot, CD45.2⁺ DCs, representing those derived from *Batf3*^{-/-} BM cells, lack the CD103⁺CD11b⁻ DC population. (B) Cell suspensions of pooled SDLNs from mixed BM chimeric mice were stained for CD45.1, CD45.2, CD11c, DEC205, CD8α, CD11b, and CD103 (representative data from a single experiment involving two mice per group). Live CD45.1⁺CD11c⁺ or CD45.2⁺CD11c⁺ cells were initially gated. Numbers represent the percentage of cells within the indicated gates. Note that in the bottom right plots, CD45.2⁺ DCs, representing those derived from *Batf3*^{-/-} BM cells, lack both the CD8α⁺DEC205⁺ and CD8α⁻DEC205⁺CD103⁺CD11b⁻ DC populations.

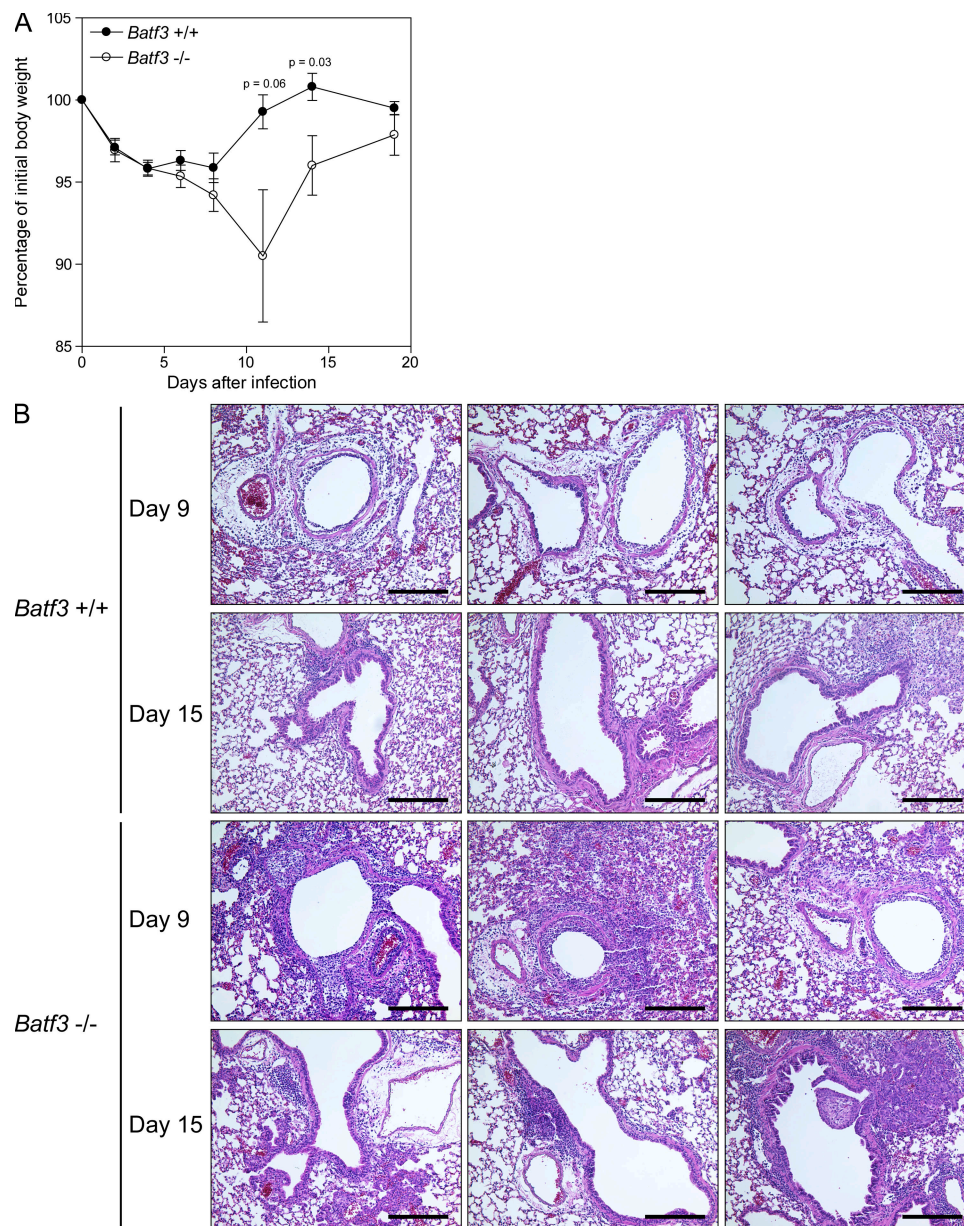


Figure S2. SeV infection of *Batf3*^{-/-} mice. *Batf3*^{+/+} and *Batf3*^{-/-} mice were infected with 100 PFU SeV intranasally (10 mice per genotype in one independent experiment). (A) Weight curves of infected mice followed for 19 d after infection. Values represent the mean and standard deviation. Note that three mice were killed at days 9 and 15 after infection during this experiment for assessment of lung histology, shown in B. At day 9, cells from lung digests were also used for flow cytometry to assess SeV-specific CD8 T cell responses (not depicted). (B) Light microscopy of H&E-stained sections of lungs from SeV-infected *Batf3*^{+/+} and *Batf3*^{-/-} mice at days 9 and 15 after infection. Note the increased airway inflammation in *Batf3*^{-/-} mice, extending to involve adjacent airspaces. Bars, 200 μ m.

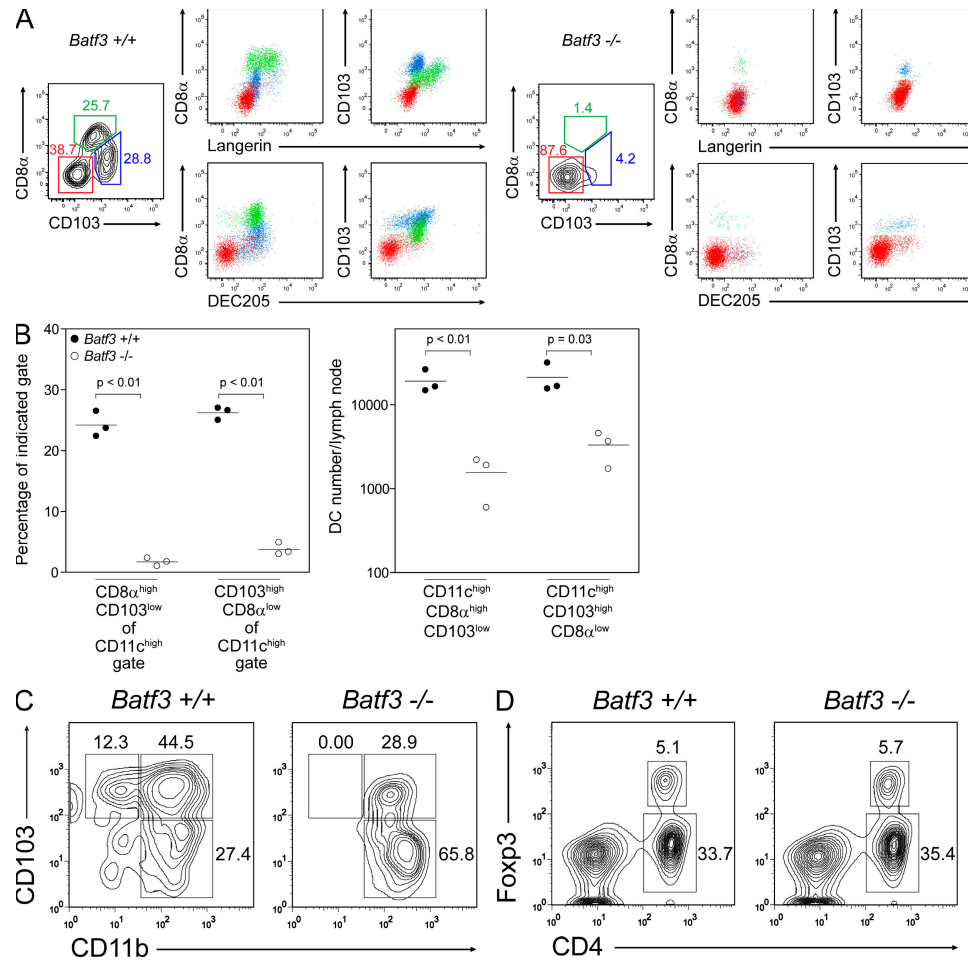


Figure S3. *Batf3*^{-/-} mice lack intestinal CD103⁺CD11b⁻ DCs in MLNs. (A) Cell suspensions of MLNs from *Batf3*^{+/+} and *Batf3*^{-/-} mice were stained for CD11c, CD103, CD8 α , intracellular Langerin, and DEC205 (representative data from a single experiment involving three mice per group). Live CD11c^{high} cells were gated. *Batf3*^{+/+} MLNs (left) showed three distinct populations: CD8 α ⁻CD103⁻ (red gate), CD8 α ⁺CD103^{low} (green gate), and CD103⁺CD8 α ^{low/-} (blue gate). Dot plot analysis of each of these populations is shown (staining for Langerin and DEC205), with the color of the dots corresponding to the original gated population. A similar analysis was performed for *Batf3*^{-/-} MLNs (right). Numbers represent the percentage of cells within the indicated gates. (B) Percentage and number of DCs per MLN for the CD8 α ⁺CD103^{low} (green gate) and CD103⁺CD8 α ^{low/-} (blue gate) populations as gated in A. Each point represents the MLN of a single mouse. Horizontal lines represent the mean. (C) Cell suspensions of MLNs from *Batf3*^{+/+} and *Batf3*^{-/-} mice were stained for CD11c, CD103, and CD11b (representative data from two independent experiments, each involving one mouse per group). Live CD11c^{high} cells were gated. Numbers represent the percentage of cells within the indicated gates. (D) Cell suspensions of MLNs from *Batf3*^{+/+} and *Batf3*^{-/-} mice were stained for CD4 and intracellular Foxp3 (representative data from a single experiment with two mice per group). Live cells were gated by forward and side scatter. Numbers represent the percentage of cells within the indicated gates.

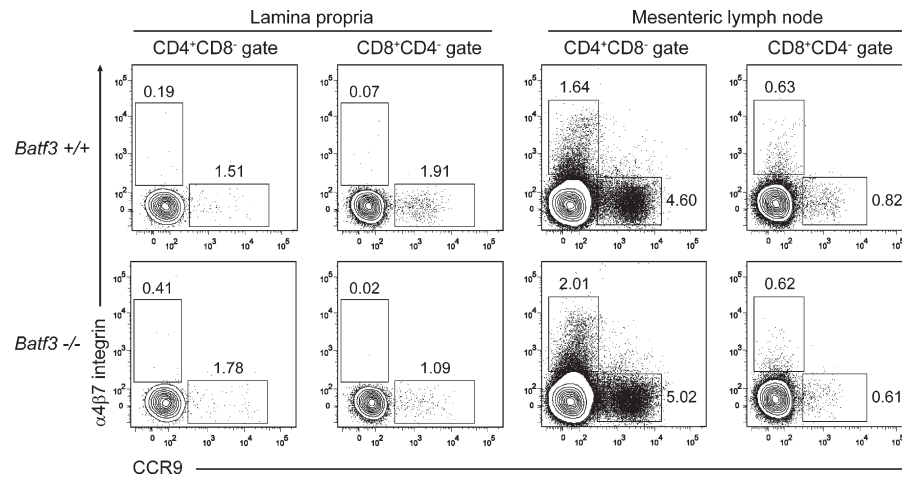


Figure S4. Homing receptor expression on intestinal T cells in *Batf3*^{-/-} mice. Cell suspensions of LP and MLNs from *Batf3*^{+/+} and *Batf3*^{-/-} mice were stained for CD4, CD8 α , α 4 β 7 integrin, and CCR9 (representative data from a single experiment involving two to three mice per group; an independent experiment was performed a second time for LP T cells). Numbers represent the percentage of cells within the indicated gates.

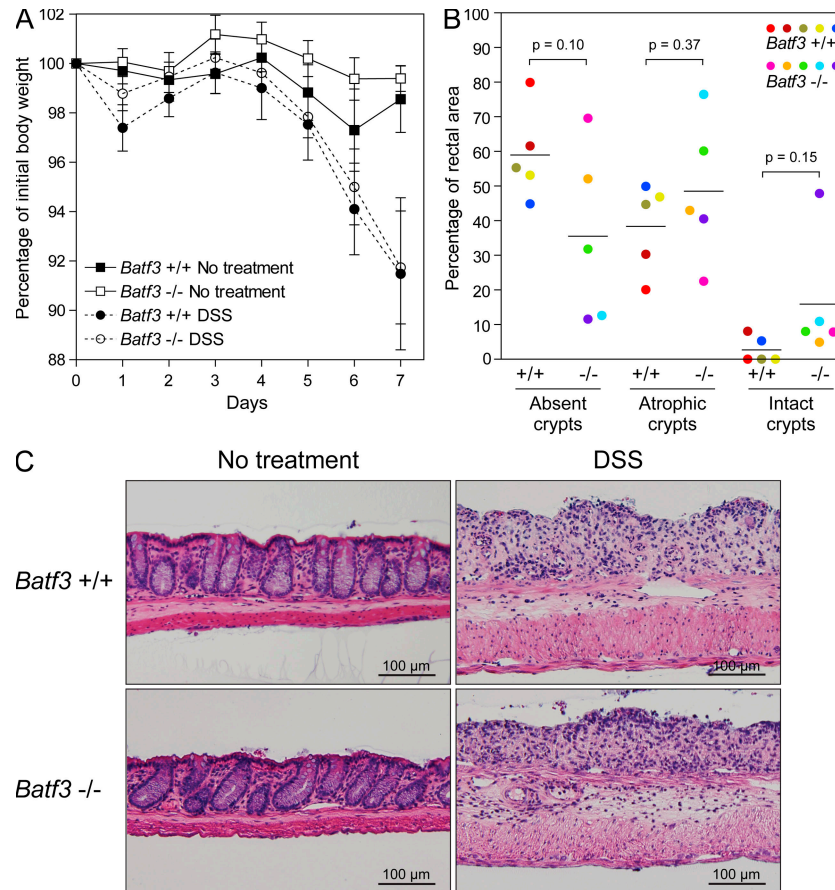


Figure S5. DSS-induced colitis in *Batf3*^{-/-} mice. (A) Weight curves of DSS-treated or untreated *Batf3*^{+/+} and *Batf3*^{-/-} mice followed for 7 d (five mice per group in a single experiment). Values represent the mean and standard deviation. (B) Morphometric analysis of the distal colons of *Batf3*^{+/+} and *Batf3*^{-/-} mice at day 7 of DSS treatment. Horizontal lines represent the mean percentage of rectal area showing absent, atrophic, or intact crypts. Each mouse is represented by a single color (three values per mouse, totaling 100%). (C) Light microscopy of H&E-stained sections of colon from DSS-treated and untreated *Batf3*^{+/+} and *Batf3*^{-/-} mice (day 7 after treatment). Colons of untreated mice show cross sections of intact crypt-surface units. Representative areas of colons of DSS-treated mice show a total loss of epithelium and ulcer base material in the mucosa (chronic inflammatory cells, blood vessels, and fibroblasts). Bars, 100 μ m.

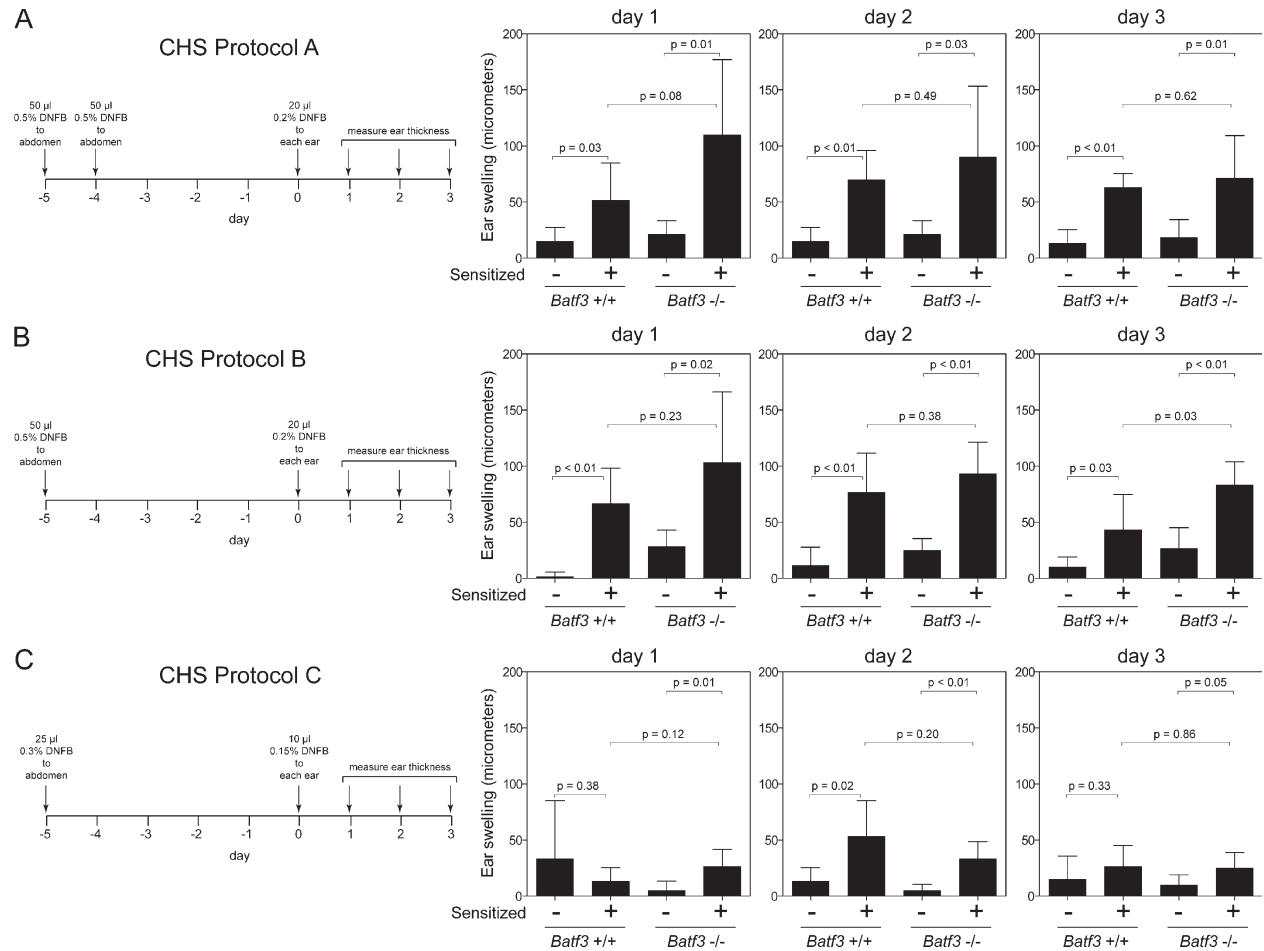


Figure S6. *Batf3*-deficient mice display a normal CHS response in three different CHS protocols. Sensitized and non-sensitized *Batf3*^{+/+} and *Batf3*^{-/-} mice were treated on their ears with DNFB to elicit a hapten-specific CHS response using three separate protocols (all in one independent experiment), varying both the number of sensitizing doses and the hapten concentration (A, B, and C). Ear swelling was measured at days 1–3 after elicitation. Bars represent the mean and standard deviation (three mice per group, six ears per group).

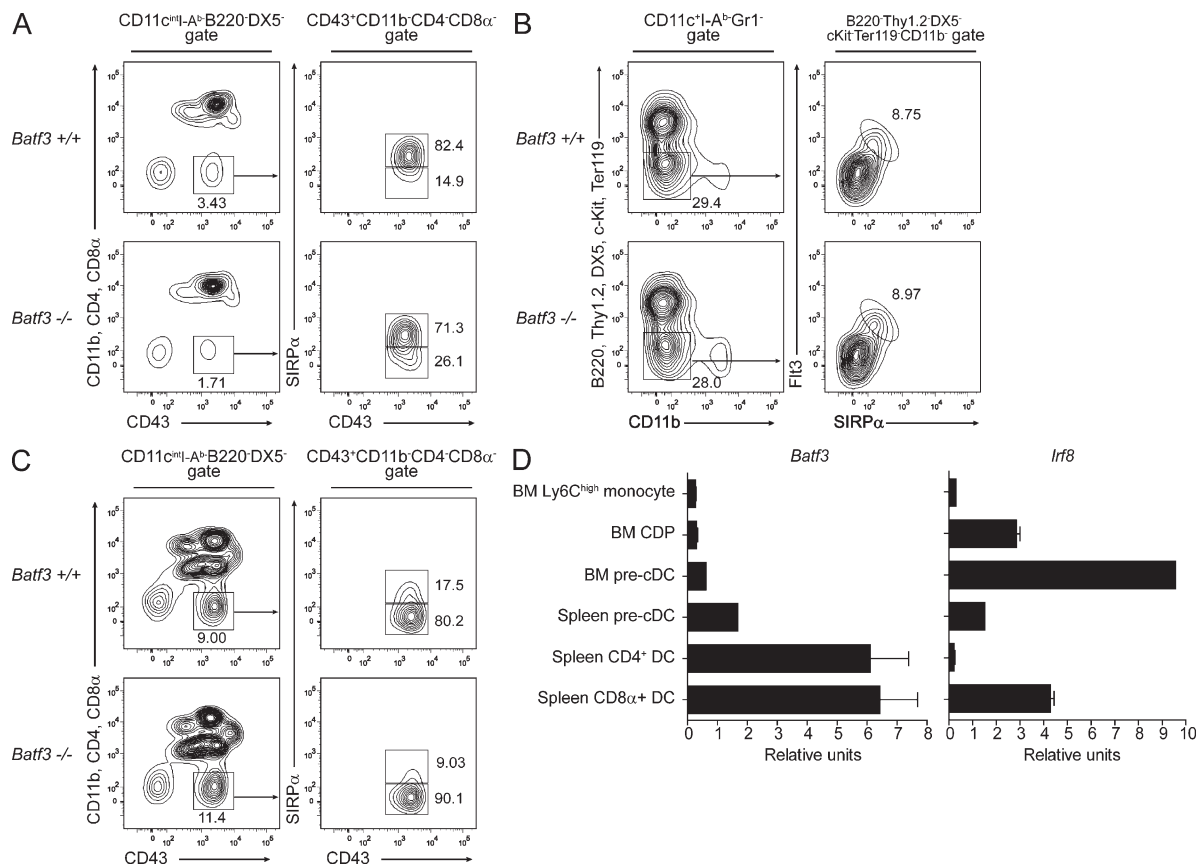


Figure S7. *Batf3*^{-/-} mice maintain a normal pre-cDC population. (A) BM cells from *Batf3*^{+/+} and *Batf3*^{-/-} mice were stained for CD11c, I-A^b, B220, DX5, CD11b, CD4, CD8α, CD43, and SIRPα (representative data from a single experiment involving two mice per group). Live CD11c^{intL}-A^b-B220-DX5⁻ cells were initially gated, with the CD11b⁺CD4⁺CD8α⁺CD43⁺ population further analyzed for the discrimination of pre-cDCs (SIRPα⁺CD43⁺). Numbers represent the percentage of cells within the indicated gates. (B) BM cells from *Batf3*^{+/+} and *Batf3*^{-/-} mice were stained for CD11c, I-A^b, Gr1, B220, Thy1.2, DX5, c-Kit, Ter119, CD11b, Flt3, and SIRPα (representative data from a single experiment involving two mice per group). Live CD11c⁺I-A^b-Gr1⁻ cells were initially gated, with the B220⁺Thy1.2⁺DX5⁺c-Kit⁺Ter119⁺CD11b⁻ population further analyzed for the discrimination of pre-cDCs (Flt3⁺SIRPα⁺). Numbers represent the percentage of cells within the indicated gates. (C) Spleen cells from *Batf3*^{+/+} and *Batf3*^{-/-} mice were stained for CD11c, I-A^b, B220, DX5, CD11b, CD4, CD8α, CD43, and SIRPα (representative data from a single experiment involving two mice per group). Live CD11c^{intL}-A^b-B220-DX5⁻ cells were initially gated, with the CD11b⁺CD4⁺CD8α⁺CD43⁺ population further analyzed for the discrimination of pre-cDCs (SIRPα⁺CD43⁺). Numbers represent the percentage of cells within the indicated gates. (D) Quantitative RT-PCR for *Batf3* and *Irf8* in the indicated cell populations from cells purified from WT mice. The assay was performed on two independently purified sets of each cell type, except for BM and spleen pre-cDCs, which were only sorted a single time. Bars represent the mean and standard deviation.