SUPPLEMENTAL METHODS

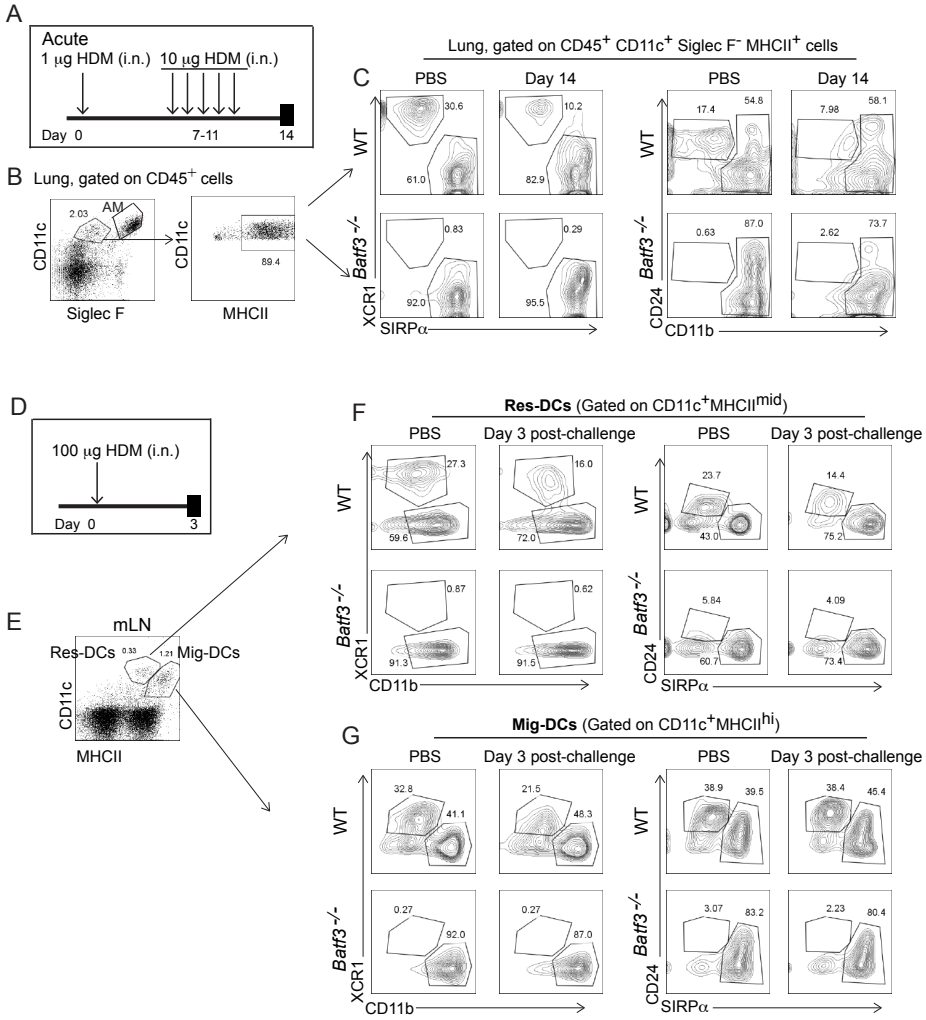
Cell isolation and restimulation

Lungs and mLN were collected in RPMI and enzyme digested. Single-cell suspensions were prepared from mLNs as described above, and cells were stained for cDC1 and cDC2 surface markers. Lungs were plated at $5x10^5$ cells/well in 96-well round-bottom plates and restimulated for 24 hours with or without 30 μg/mL HDM extract in the presence of Brefeldin A (5 μg/mL; Sigma-Aldrich) for the last 4 hours.

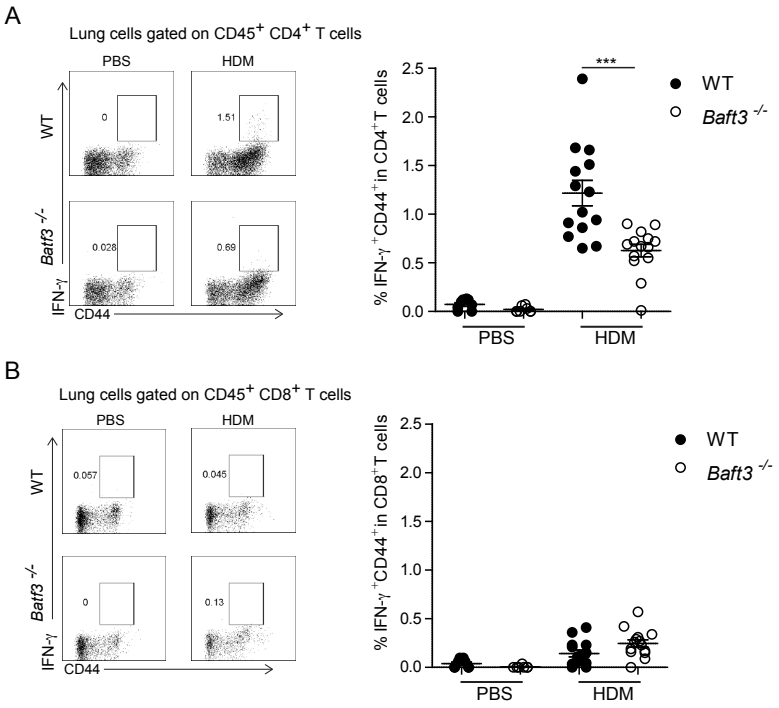
Flow cytometry

All staining reactions were performed at 4ºC with the appropriate antibody cocktail in cold PBS supplemented with 2.5 % FCS, 2 mM EDTA and 0.1% sodium azide. CD16/CD32 (2.4G2) (TONBO bioscience) was used to reduce non-specific binding. For detection of cDC1 and cDC2 in lung and mLNs, the following fluorochrome-labeled antibodies were used: CD45 (30-F11), Siglec-F (E50-2440), I-A/I-E (MHCII, [2G9]), CD11c (HL3), CD11b (M1/70), and CD103 (M290), all from BD Biosciences, SIRPα (CD172 [P84]) and CD24 (30-F1) from eBioscience, XCR1(ZET) from BioLegend, and CD8α (53.6-7) from TONBO Bioscience. Hoechst 33258 (0.1 μM) was used as a counterstain to exclude dead cells.
For detection of IFN-γ production by $CD4^+$ and $CD8^+$ T cells in the lung, cells were stained with CD45 (30-F11) purchased from BD Bioscience, CD4 (RM4-5) and CD8α (53.6-7) were from TONBO Bioscience; CD44 (IM7), and IFN-γ (XMG1.2) from eBioscience. For intracellular staining, cells were fixed for 10 min in 4% paraformaldehyde-PBS at RT, and staining was conducted for 40 min at 4°C in permeabilization buffer (1% BSA, 0.1 % saponin, and 0.2% sodium azide). Samples were processed with a BD FACSCanto flow

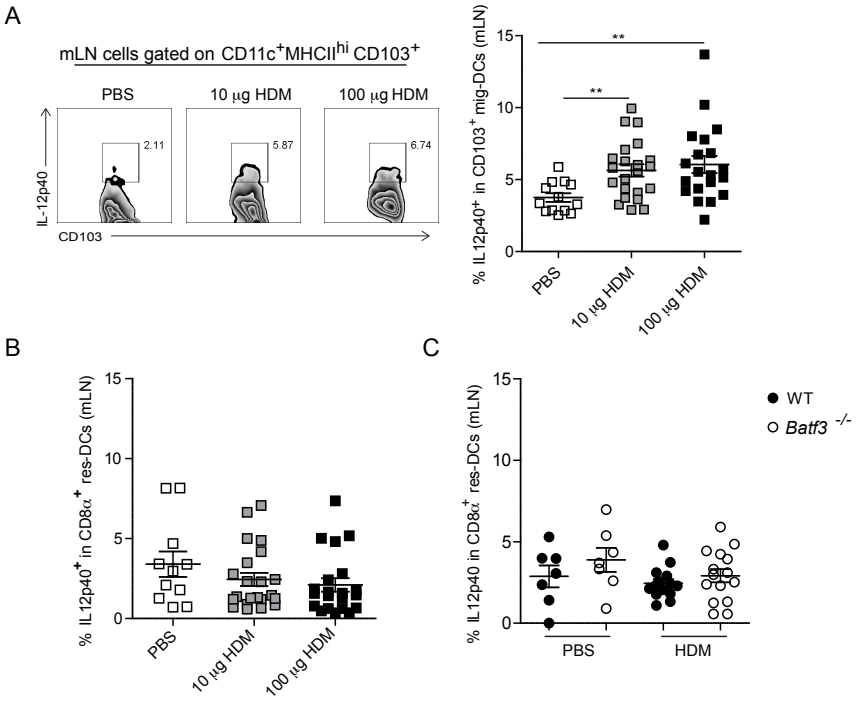
cytometer using FACSDiva software, and data were analyzed with FlowJo software (Tree Star, Ashland Or).



Supplemental Figure 1. Expression of XCR1, SIRPα, and CD24 on cDCs in lung and mLN following PBS or HDM i.n. administration. (**A**) Acute HDM sensitization and challenge regime. (**B**) Gating strategy for lung Batf3-dependent DCs. (**C**) WT and *Batf3*$^{-/-}$ mice were sensitized and challenged i.n. with HDM and lung cells stained for XCR1^{+}, SIRPα^{+}, CD24^{+} and CD11b^{+} DCs as indicated at day 14. (**D**) HDM exposure protocol to evaluate early DC response following allergen challenge. (**E**) Gating strategy to identify resident (res) (MHCII^{mid}) and migratory mig (MHCII^{hi}) DC populations in the mLN. (**F** and **G**) WT and *Batf3*$^{-/-}$ mice were sensitized and challenged i.n. with HDM (100 μg) and mLN cells stained for XCR1^{+}, SIRPα^{+}, CD24^{+} and CD11b^{+} DCs as indicated at day 3. Analysis of resident DC (res-DCs, **F**) and migratory DC (mig-DCs, **G**) is depicted. (**B, C, E-G**) Plots representative of two independent experiments (n=3-4 PBS, n=6-7 HDM, mice per experiment). i.n., intranasal; HDM, house dust mite; mLN, mediastinal lymph node.



Supplemental Figure 2. *Batf3*$^{-/-}$ mice display impaired IFN-γ production by lung effector CD4^{+} T cells upon HDM exposure. Lungs from WT and *Batf3*$^{-/-}$ mice sensitized and challenged with HDM were collected at day 14. (**A** and **B**) Representative plots (left) and percentages (right) showing intracellular IFN-γ staining in CD45^{+} CD4^{+} T cells (**A**) and CD45^{+} CD8^{+} T cells (**B**) upon restimulation of lung cells with HDM for 24 hours. Individual data and mean ± SEM from a pool of two independent experiments (n=7 PBS, n=14 HDM). ***p<0.001; Mann Whitney U test. HDM, house dust mite.



Supplemental Figure 3. CD103$^+$ mig-DCs but not CD8α$^+$ DCs enhance IL-12p40 production upon HDM challenge. Mice were challenged with the indicated dose of HDM i.n., mLNs collected 3 days later and cells stained for CD11c, MHC-class-II, CD103, CD8α and intracellular IL12-p40. (**A**) Left: Representative plots showing IL-12p40 staining in CD103$^+$ mig-DCs. Right: frequencies of IL-12p40 producing CD103$^+$ mig-DC (n=13 PBS, n=22 HDM). (**B**) Frequencies of IL-12p40-producing CD8α+ res-DC (n=11 PBS, n=22 HDM). (**C**) Frequencies of IL-12p40-producing CD8α$^+$ res-DC subsets in WT and *Batf3*$^{-/-}$ mice following exposure to 100 µg HDM (n=7 PBS, n=15 HDM). (**A-C**) Individual data and mean ± SEM from a pool of three (**A** and **B**) or two (**C**) independent experiments. ***P*< 0.01; Mann Whitney U test. HDM, house dust mite; mLN, mediastinal lymph node. HDM, house dust mite.