1.0×10^7 cells were administered i.v. into naïve mice 2 days before being sensitized with HDM. They were then challenged with HDM a week later and sacrificed four days later.

Isolation of draining (mediastinal) lymph node cells

Draining (mediastinal) lymph nodes were made into single cell suspensions. The tissue was mechanically dissociated and passed through a filter to remove debris. Red blood cells were lysed with ammonium chloride-potassium lysing buffer after which the single cell suspension was stained for flow cytometry.

Isolation of lung cells

The lungs were perfused with 25 U/mL of heparin (Sigma Aldrich) in PBS and minced with scissors. Tissue dissociation was achieved by incubating the tissue for 30 minutes at 37℃ in 5% complete media with 150 U/mL collagenase I (Invitrogen). The digest was passed through a filter to remove debris, and red blood cells were lysed with ammonium chloride-potassium lysing buffer after which the single cell suspension was stained for flow cytometry.

Production of bone marrow-dendritic cells (BMDCs)

BMDCs were generated as previously described (40). Bone marrow was flushed from the femurs and tibias of mice, and the cells were cultured in 10% complete DMEM supplemented with 20 ng/mL GM-CSF (Shenandoah Biotechnology). On day 3 and 6, the media was replenished with 20 ng/mL GM-CSF. On day 8, the suspension cells were harvested, and 5×10^5 BMDCs were cultured in 24-well plates with OVA (percent viability: 91.9 ± 3.0), OVA-IC (percent viability: 93.1 ± 3.1), or HDM (percent viability: 91.7 ± 2.1) at a concentration of 25 μg/mL.

Quantitative PCR (qPCR)

RNA was isolated from cells using an RNeasy Micro Kit according to the manufacturer's protocol (Qiagen) and quantified by nanodrop (Thermo Scientific). cDNA synthesis was done using the Superscript III Reverse Transcriptase kit according to the manufacturer's protocol (Invitrogen). cDNA samples were amplified with the Power Sybr Green PCR master mix (Applied Biosystems) and run on a ABI 7300 cycler (Bio-Rad). Primer sequences used are available in the supplementary materials.

Statistical analysis

All statistical analyses were performed with GraphPad Prism software, and a P-value less than 0.05 was considered significant (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns = not significant). Experiments with two groups were analyzed using an unpaired Student's two-tailed *t* test. Experiments with greater than two groups were analyzed with a one-way ANOVA and post-hoc Tukey test. Error bars represent the SEM.

Supplementary Figure 1: HDM-mediated Th2 inflammation requires both a sensitization and challenge with HDM. WT mice were sensitized on day 0 with PBS or HDM and challenged on day 7 with PBS or HDM as indicated. On day 11 the mice were sacrificed. Airway inflammation was assessed by determining the number of eosinophils (left panel) and $CD4^+$ T

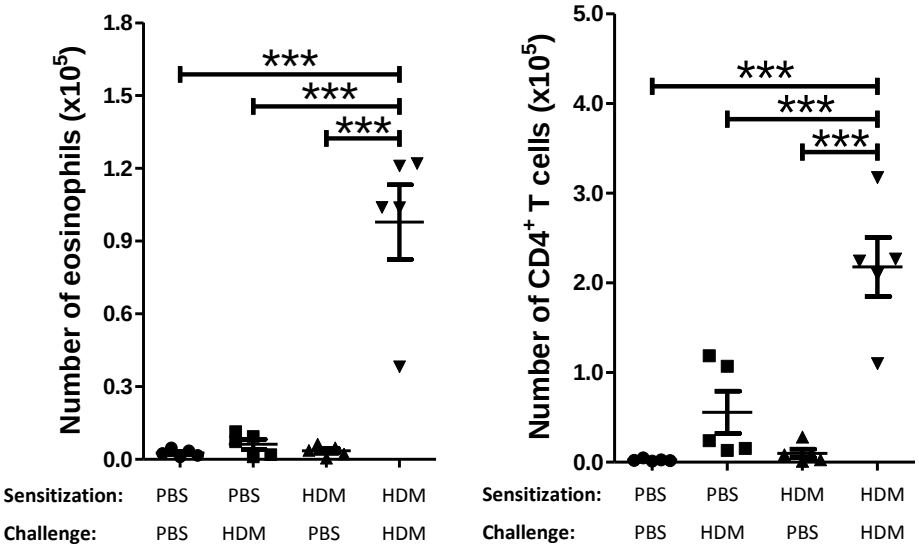
cells (right panel) in the BAL by flow cytometry. Data represent the mean ± SEM (***, $P < 0.001$).

Supplementary Figure 2: Analysis of activation markers on BMDCs. (A) WT BMDCs were untreated (red) or treated with OVA (blue), OVA-IC (green), or HDM (orange) overnight. Expression of MHCII and CD86 were assessed on $CD11c^+CD11b^+$ cells. (B) WT or $FcR\gamma^{-/-}$ BMDCs were treated overnight with HDM and expression of MHCII, CD86, and CD40 were assessed on $CD11c^+CD11b^+$ cells. Fluorescence minus one (FMO – red) compared to HDM-treated groups (blue). Data are representative of three independent culture sets.

Supplementary Figure 3: HDM-induced IL-33 upregulation in BMDCs is TLR4 dependent. BMDCs were generated from WT and $TLR4^{-/-}$ mice and treated with OVA or HDM overnight before assessing for IL-33 mRNA expression normalized to β2M mRNA expression. Data represent the mean ± SEM from at least three independent culture sets (**, $P < 0.01$).

Supplementary Figure 4: HDM-mediated Th2 inflammation is dependent on T cells. (A) WT or $Rag^{-/-}$ mice were sensitized and challenged with HDM. (B) Naïve $Rag^{-/-}$ mice received vehicle control or nylon wool non-adherent T cells i.v. 2 days before being sensitized and challenged with HDM. (A) and (B) Airway inflammation was assessed by determining the number of total cells (left panel) and eosinophils (right panel) in the BAL. Data represent the mean ± SEM (**, $P < 0.01$; ***, $P < 0.001$).

Supplementary Figure 5: IC-mediated Th2 inflammation is antigen-specific. α-OVA or α-BSA serum was administered i.v. to naïve mice. On day 1, the mice were challenged i.t. with OVA or BSA as indicated in the graphs under antigen (Ag). On day 8, 9, and 10, mice that received α-OVA were challenged with OVA, and mice that received α-BSA were challenged with BSA. On day 11 the mice were sacrificed. (A) Airway inflammation was assessed by determining the number of eosinophils (left panel) and $CD4^+$ T cells (right panel) in the lung by flow cytometry. (B) Representative H&E sections of lung tissue from treated mice. Black bars = 100 μm. Data represent the mean ± SEM (*, $P < 0.05$).



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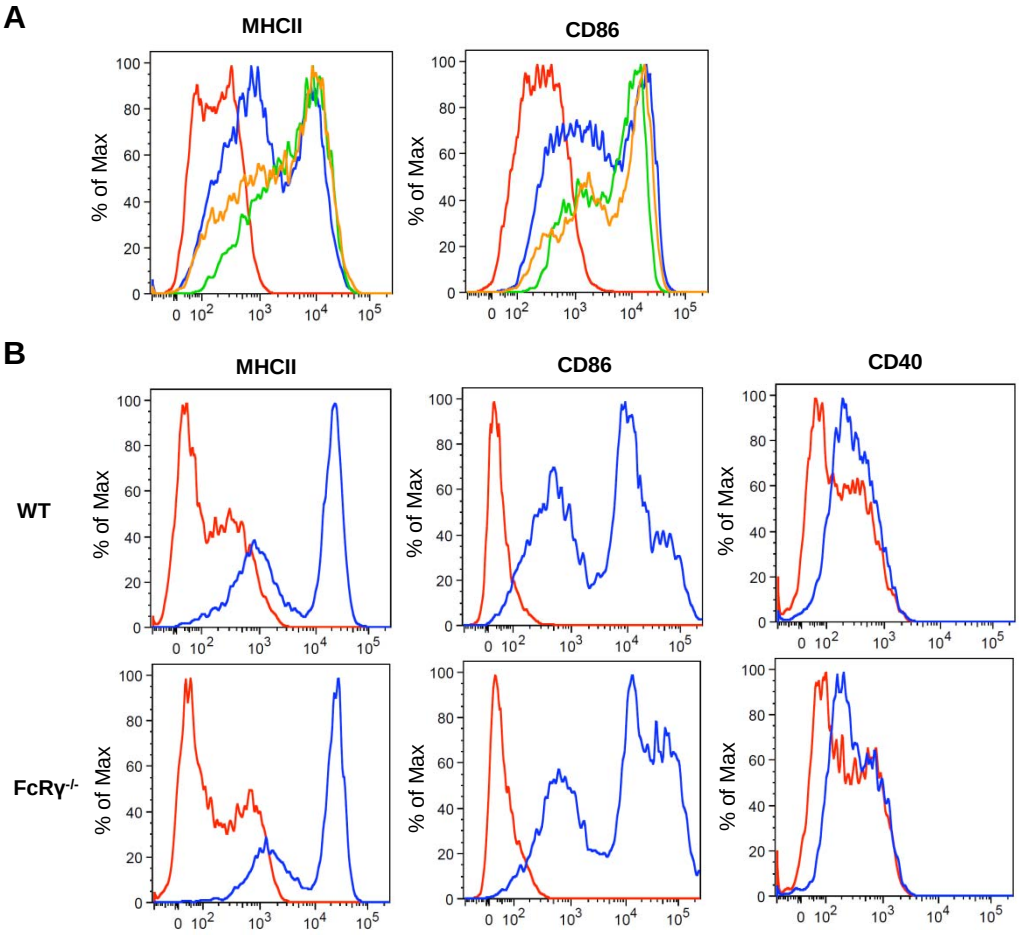

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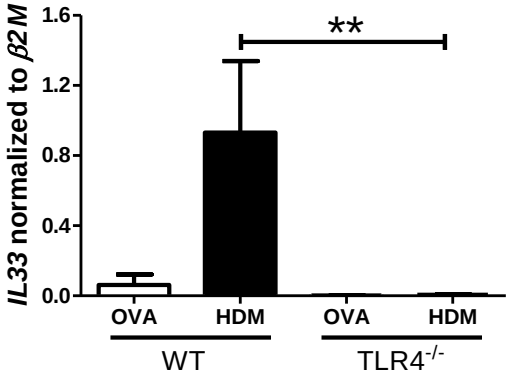

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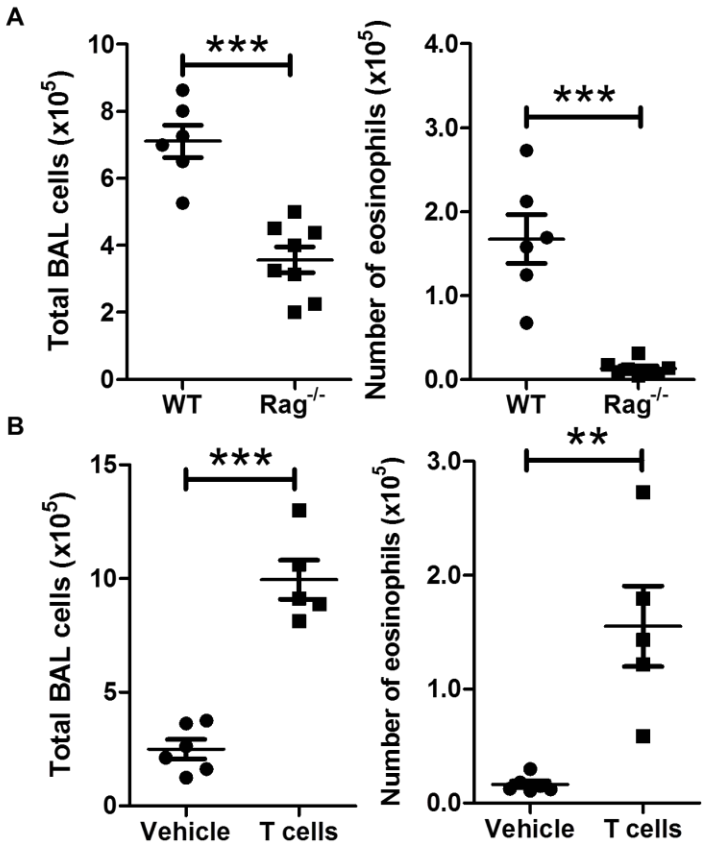

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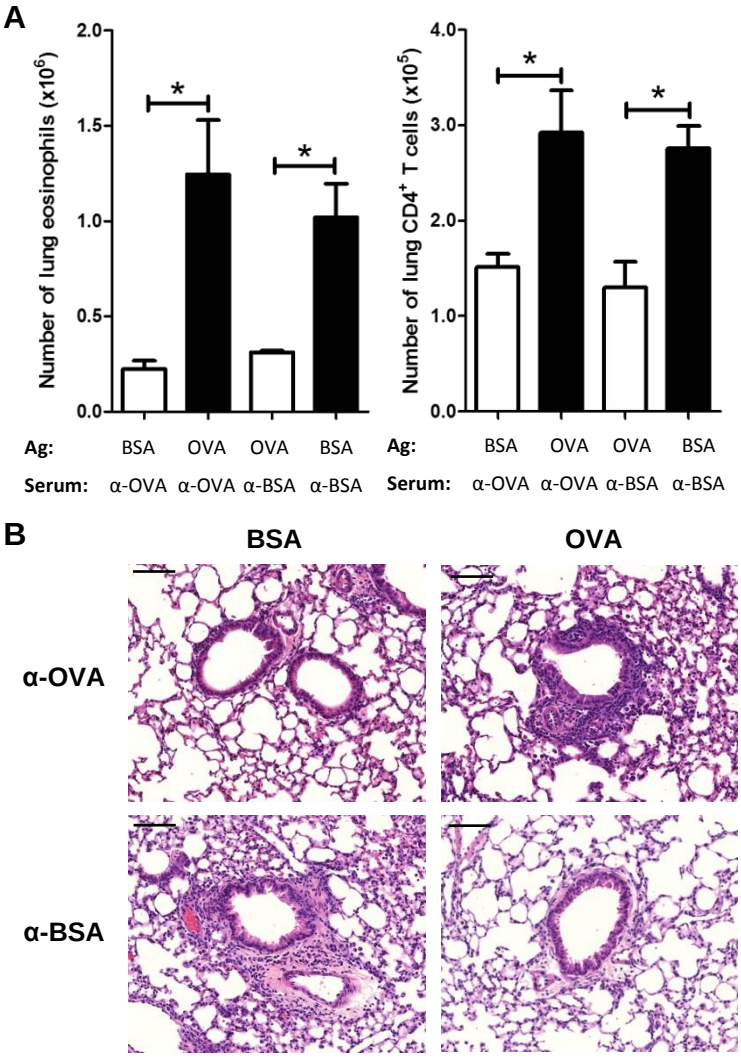

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