Supplementary methods

OVA-alum model of allergic airway inflammation

Mice were sensitized to OVA (Worthington Biochemical) via intraperitoneal injection of OVA/alum (10 µg OVA grade V, Worthington Biochemical Corp., adsorbed to 1 mg aluminium hydroxide; Thermo Scientific Pierce Imject Alum) on days 0 and 7, and were then challenged with OVA aerosols on days 19-21, delivered from a jet nebulizer delivering 1%OVA in PBS for 30 minutes. Twenty-four hours after the last OVA exposure, BAL was performed with 3x1 ml of Ca^{2+} and Mg^{2+} -free PBS (Invitrogen) supplemented with 0.1 mM sodium EDTA, followed by lung resection and storage in OCT freezing medium. To measure cytokine levels, mediastinal LN cells were plated in round bottom 96 well plates ($1x10^6$ cells/ml) and restimulated with OVA (10 µg/ml) for 4 days. Then supernatants were collected and levels of IL-4, IL-5, IL-13, and IFN-γ were assayed by ELISA (R&D systems).

Migration assay in vitro

Experiments were performed in triplicate using 24-well Transwell chambers. Buffer or different concentrations of ATP were added into the lower compartment wells. PBS-pulsed WT and *Cd39*$^{-/-}$ BMDC (10^5 cells/well) were added to the upper compartment and incubated at 37°C for 90 min in a humidified atmosphere. After removing the cells from the upper compartment migrated DC on the lower chamber were fixed in methanol and stained with hematoxylin. Results are shown as chemotatic index, calculated as the number of cells in the lower chamber containing the different stimuli divided by the number of cells in the chamber containing medium alone.

Role of *Cd39* expression on BMDC on the activation of OVA-specific naive in a primary immune response

To address the relevance of *Cd39*$^{-/-}$ null expression on the primary activation of naive T cells, naive OVA-specific CD4+ T cells (1x10^{7}) purified from non-manipulated OTII TCR Tg (H-2d) were adoptively transferred into C57Bl/6 mice. Briefly, LNs and spleen were collected from OT-II mice and homogenized, and 10 x 10^{6} live cells were injected intravenously in the lateral tail vein of C57Bl/6 mice (day -2). On day 0, the mice received an intratracheal injection of OVA- or PBS-pulsed DC (1x10^{6}), generated from bone marrow of WT and *Cd39*$^{-/-}$ mice. On day 4, MLNs were collected, homogenized, and LN cells (200,000 cells/well in triplicates) were resuspended in RPMI 1640 containing 5% fetal calf serum and antibiotics and placed in 96-well plates. Four days later, supernatants were harvested and analysed for IL-4, IL-5, IL-13, and IFN-γ concentrations.

A mouse model of AAI induced by adoptive transfer of bone marrow derived DCs

BMDC generated form WT and *Cd39*$^{-/-}$ were pulsed overnight with 100 µg/ml LPS-low OVA (Worthington Biochemicals) or vehicle (PBS). After antigen pulsing, non-adherent DCs were collected and washed to remove free OVA and resuspended in PBS at a concentration of 12,5x10^{6} cells/ml. On day 0 WT and *Cd39*$^{-/-}$ mice were anesthetized were anesthetized with ketamine/ xylazine and 1x10^{6} vehicle-WT-DCs, OVA-WT-DCs, vehicle-*Cd39*$^{-/-}$ DCs or OVA-*Cd39*$^{-/-}$ DCs were instilled through the opening vocal cords, as described [4]. On days 10-12, mice were exposed to OVA aerosols (30 min). Twenty-four hours after the last OVA exposure, BAL was

performed with 3x1 ml of Ca_2+ and Mg_2+ -free HBSS (Invitrogen) supplemented with 0.1mM sodium EDTA, followed by collection of MLNs and lung resection and storage in OCT freezing medium. To measure cytokine levels, mediastinal LN cells were plated in round bottom 96 well plates ($1x10^6$ cells/ml) and re-stimulated with OVA (10 µg/ml) for 4 days. Then supernatants were collected and the levels of IL-4, IL-5, IL-13, and IFN-γ were assayed by ELISA (R&D systems).

Synapse formation - time lapse microscopy.

BMDCs generated form WT and *Cd39*$^{-/-}$ bone marrow cells and were pulsed overnight with 100 µg/ml LPS-low OVA (Worthington Biochemicals) or vehicle (PBS). Next day, non-adherent DCs were collected, washed to remove free OVA and stained with FITC-labelled anti-MHCII antibody (clone: M5/114.15.2, eBiosciences). Stained DCs ($1x10^5$) were cultured on µ-Dish 35mm glass bottom flasks (Ibidi, Munich, Germany) for four hours. $CD4^+$-T-cells were isolated from OT-II mice spleen by negative isolation method with magnetic beads (MACS 130-095-248, Miltenyi). CD4+T-cells were stained with an anti-TCR antibody (eBiosciences) followed by streptavidin-Cy5 (Invitrogen) and cells were added ($5x10^5$) to the DC flasks and co-cultured for 2 hours until imaging. Phenol red free DMEM with 10% FBS were used as culture medium to minimize background florescence. Images were taken every 5 minutes, from 6-9 different locations of the imaging flask, on a ZEISS Cell Observer inverted microscope with incubation chamber (37 C, 5% CO_2). Axiovision software (Zeiss) was used for image acquisition. Images from green (FITC) and red (Cy5) channels were overlaid and every double positive signal was considered as an immune synapse. Additional to fluorescent images, wide-field images were crosschecked to morphologically confirm T-cells and dendritic cells that are in

interaction. These immune synapses were quantified for each time point and acquisition position. Each immune synapse was individually tracked for the length of the interaction using Imaris Software (Bitplane AG, Zurich, Switzerland). Immune synapse duration was calculated by multiplying the number of concomitant images (in which the interaction is observed) with 5 min (time lapse between each acquisition).

Statistical Analysis

Values for all measurements are expressed as mean ±SEM. Unless stated otherwise; groups were compared using ANOVA, followed by Bonferroni comparison test. Probability values of $p < 0.05$ were regarded as significant.