

Supporting information Figure 1:

Standard protocol for sensitization, tolerance induction and allergen challenge.

Mice were sensitized to ovalbumin (OVA) by repetitive intraperitoneal injections of OVA adsorbed to aluminiumhydroxide (alum) on days 0, 7 and 14. Tolerance induction was conducted by three subcutaneous injections of OVA on three alternate days beginning at least 14 days after sensitization. Control animals received PBS injections instead. Mice were challenged 7 to 10 days after the last injection by OVA aerosol three times 72 hours apart. Animals were sacrificed and analyzed 24 hours after the last aerosol challenge.



IL-10 dependent beneficial effect of allergen specific TI in C57BL/6 wild type mice.

(A-D) Sensitized C57BL/6 wildtype mice treated with IL-10R blocking or isotype control antibody and (E) IL-10 knock-out mice (IL-10^{-/-}) were either sham treated (sham) or tolerized (TI) by s.c. allergen injections and analysed 24 hours after final allergen challenge as described in the *Materials and methods*. (A,E) Immune cells in BAL fluid were counted on Diff-Quick stained cytospins. (B) Serum levels of allergen-specific IgE and IgG1 were measured by ELISA. (C) IL-5 and IL-13 release by allergen-stimulated LN cells was measured by ELISA. (D) PAS-stained lung sections. Images shown are representative of 2 independent experiments. (A-E) Data are shown as mean+SEM (n=3-8 mice/group and experiment) and are pooled from (A-C) 7 or (E) 2 independent experiments. * p<0.05, *** p<0.001, ns: not significant; Mann- Whitney U test.



Supporting information Figure 3: Flow cytometry gating strategy for determination of GFP+ immune cells.

(A) Definition of distinct cell populations. (B) Analysis of GFP expression by comparing wildtype and IL-10 reporter mice (Vert-X).



Supporting information Figure 4:

IL-10 reporter expression (GFP) in different cell types of mediastinal LNs upon initiation of tolerance.

Sensitized transcriptional IL-10 reporter mice (Vert-X) were either sham treated (sham) or tolerized (TI) by s.c. allergen injections as described in the *Materials and methods*. Different cell populations of mediastinal LNs were analysed 132 hours after initiation of therapy, i.e. 36 hours after final injection by flow cytometry for reporter expression (GFP). Percent GFP+ cells per indicated cell type (upper panel) and mean fluorescence intensity (lower panel). Data are shown as mean +SEM (n=3-6 mice/group and experiment) and are pooled from 4 independent experiments. * p<0.05, ** p<0.01, *** p<0.001, ns: not significant Mann-Whitney U test.



Supporting information Figure 5:

IL-10 expression levels in immune cells of lung tissue and BAL during the elicitation phase of allergic airway inflammation.

Sensitized transcriptional IL-10 reporter mice (Vert-X) were sham treated (sham) or tolerized (TI) and allergen challenged by aerosol treatment as described in the *Materials and methods*. (A) Percentage of GFP expression (upper panel) and mean fluorescence intensity (MFI, lower panel) of immune cells in lung tissue before and 24 hours after the last allergen challenge. Significance levels for the comparison of tolerized and sham treated mice are displayed above a bracket. Significance levels for the comparison before and after allergen challenge are only displayed in the right panel above each column. (B) T cell composition within BAL cells after allergen challenge. (C) GFP expression of different BAL cell populations after allergen challenge. Data are shown as mean+SEM (n=16-24 mice/group) and are pooled from 4 independent experiments. * p<0.05, ** p<0.01, *** p<0.001, ns: not significant



Supporting information Figure 6:

Cell type specific deletion of the IL-10 gene in IL-10^{FL/FL}CD4-Cre⁺, IL-10^{FL/FL}CD19-Cre⁺, IL-10^{FL/FL}CD19-Cre⁺ and IL-10^{FL/FL}CD11c-Cre⁺ mice.

Southern blot analysis of DNA extracted from different FACSsorted spleen cell populations after Pstl digest. (A-C) CD3⁺ T cells and CD19⁺ B cells from (A) IL-10^{FL/FL}CD4-Cre⁺, (B) IL-10^{FL/FL}CD19-Cre⁺ and (C) IL-10^{FL/FL}CD4-Cre⁺CD19-Cre⁺ mice. (D) CD11c^{high}MHCII⁺ DCs and CD19⁺ B cells from IL-10^{FL/FL}CD11c-Cre⁺ mice. Data are representative of three animals.

D: deleted allele, FL: loxP-flanked allele, T: T cells, B: B cells.



Supporting information Figure 7: Lack of T and B cell derived IL-10 does not prevent tolerance induction.

Sensitized T and B cell specific IL-10 deficient mice (IL-10^{FL/FL}CD4-Cre+CD19-Cre+) and control mice (IL-10^{FL/FL}Cre) were sham treated (sham) or tolerized (TI) by s.c. injections, allergen challenged by aerosol treatment and analysed 24 hours later as described in the *Materials and methods*. (A) Immune cells in BAL fluid were counted on Diff-Quick stained cytospins.(B) Serum levels of allergen-specific IgE and IgG1 were measured by ELISA. (C) IL-5 and IL-13 release by allergen-stimulated LN cells was measured by ELISA. Data are shown as mean +SEM (n=3-10 mice/group and experiment) and are pooled from from (A) 5 or (B,C) 4 independent experiments. * p<0.05, ** p<0.01, *** p<0.001; ns: not significant, Mann-Whitney U test.



Supporting information Figure 8: Serum level of total IgE in mice with DC specific IL-10 deficiency

Sensitized DC specific IL-10 deficient mice (IL- $10^{FL/FL}CD11c$ -Cre⁺) and control mice (IL- $10^{FL/FL}Cre^{-}$) were sham treated (sham) or tolerized (TI) by s.c. injections, allergen challenged by aerosol treatment and analysed as described in the *Materials* and methods. (A) Kinetic of total IgE levels and (B) total IgE 24 h after challenge (final) was determined by ELISA. Data are shown as mean+SEM (n=6-7 mice/group and experiment) and are pooled from 3 independent experiments. ** p<0.01, Mann-Whitney U test.



Supporting information Figure 9: Lack of macrophage/neutrophil or mast cell derived IL-10 does not prevent tolerance induction.

Sensitized macrophage/neutrophil (IL-10^{FL/FL}LysM-Cre⁺) or mast cell (IL-10^{FL/FL}Mcpt5-Cre⁺) specific IL-10 deficient mice and control mice (IL-10^{FL/FL}Cre⁻) were sham treated (sham) or tolerized (TI) by s.c. injections, allergen challenged by aerosol treatment and analysed 24 hours later as described in the *Materials and methods*. (A) Immune cells in BAL fluid were counted on Diff-Quick stained cytospins. (B) Serum levels of allergen-specific IgE and IgG1 were measured by ELISA. (C) IL-5 and IL-13 release by allergen-stimulated LN cells was measured by ELISA. Data (n=5-6 mice/group) of 1 experiment are shown as mean+SD. LysM: IL-10^{FL/FL}LysM-Cre⁺ mice, Mcpt: IL-10^{FL/FL}Mcpt5-Cre⁺mice, * p<0.05, ** p<0.01, ns: not significant, Mann-Whitney U test.



Supporting information Figure 10:

Composition of significant immune cell populations in IL-10^{FL/FL}Vav-Cre⁺ mice and litter mate controls.

Axillary lymph nodes (LN), spleen, lung tissue and blood of naïve, 5 week old IL-10^{FL/FL}Vav-Cre⁺ mice and IL-10^{Wt/FL}Vav-Cre⁺ were analyzed by flow cytometry for total T cells (CD3⁺), B cells (CD19⁺) and dendritic cells (DCs, CD11c⁺MHCII⁺) (left panel), as well as for CD4⁺ and CD8⁺T cells (middel panel) and CD4⁺CD25⁺ T cells (right panel). Data are shown as mean+SEM (n=2-3 mice/group and experiment) and are pooled from from 2 independent experiments. ** p<0.01, Mann-Whitney U test.