Lung microbiota promotes tolerance to allergens in neonates via PD-L1

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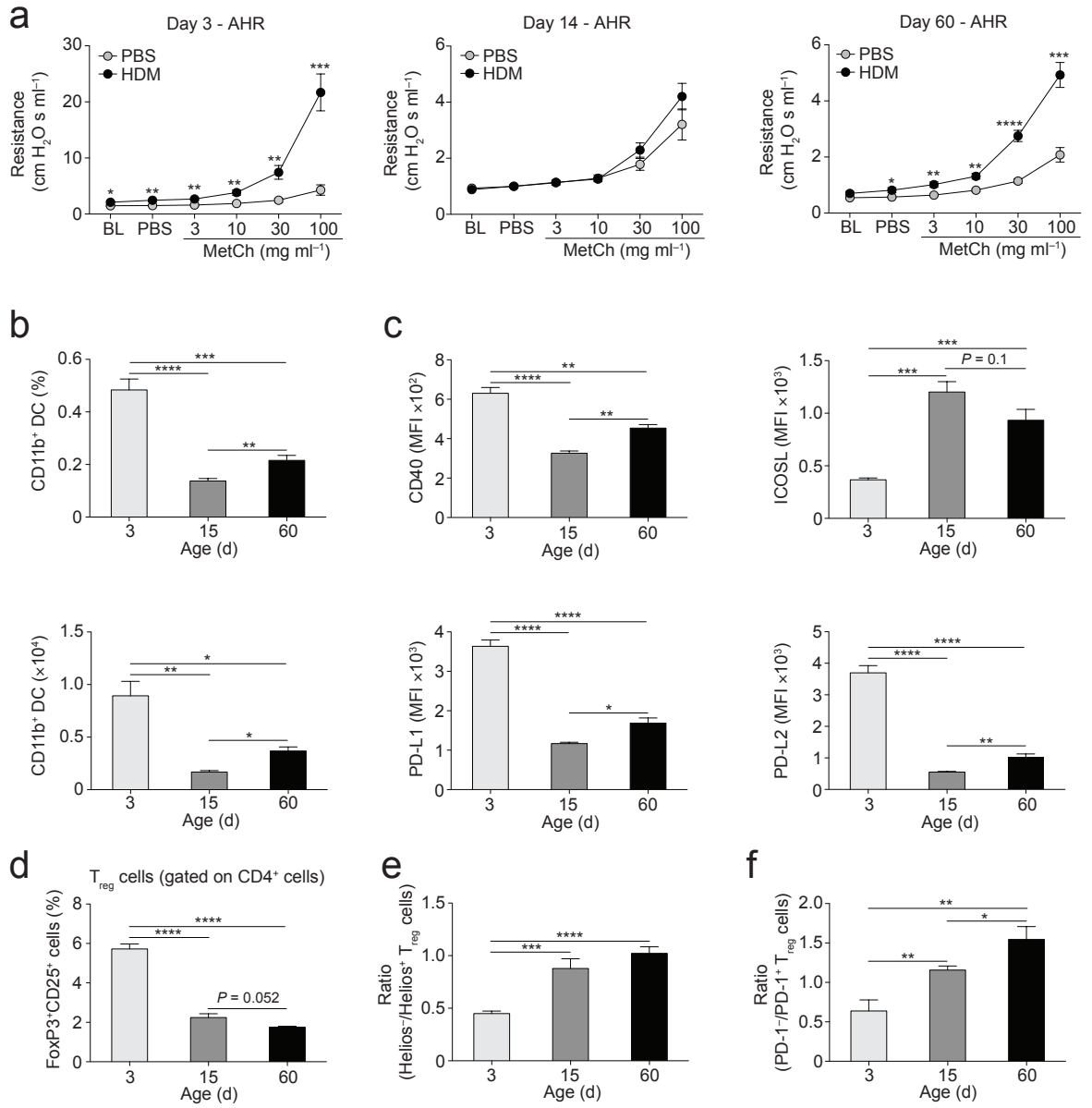
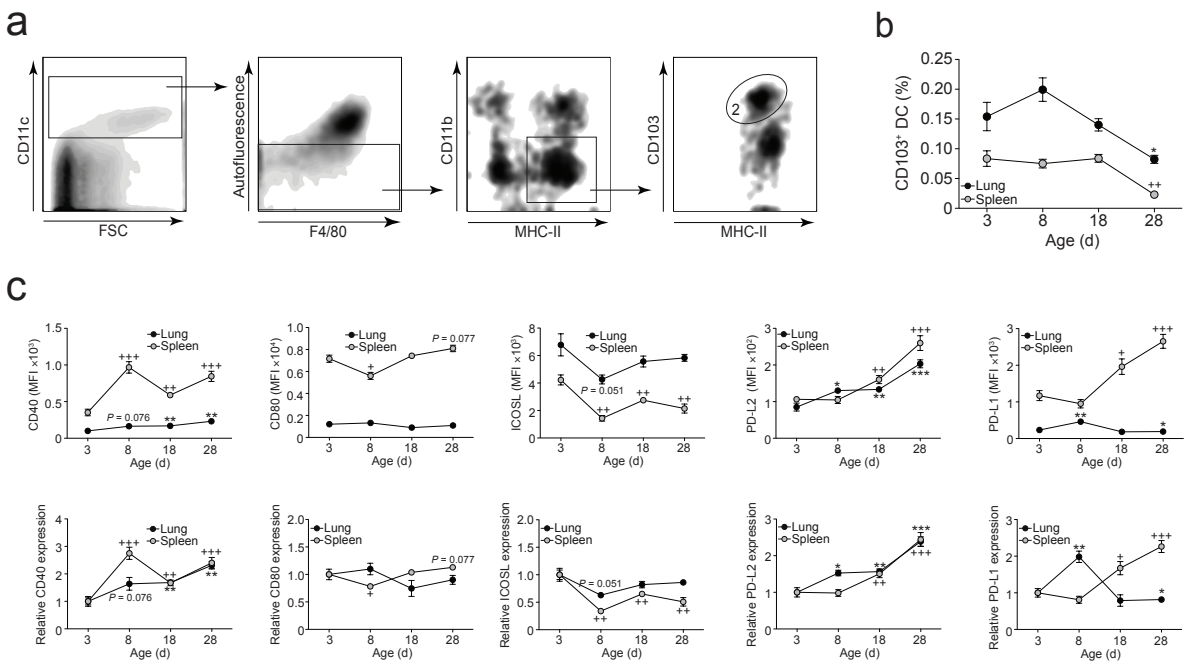


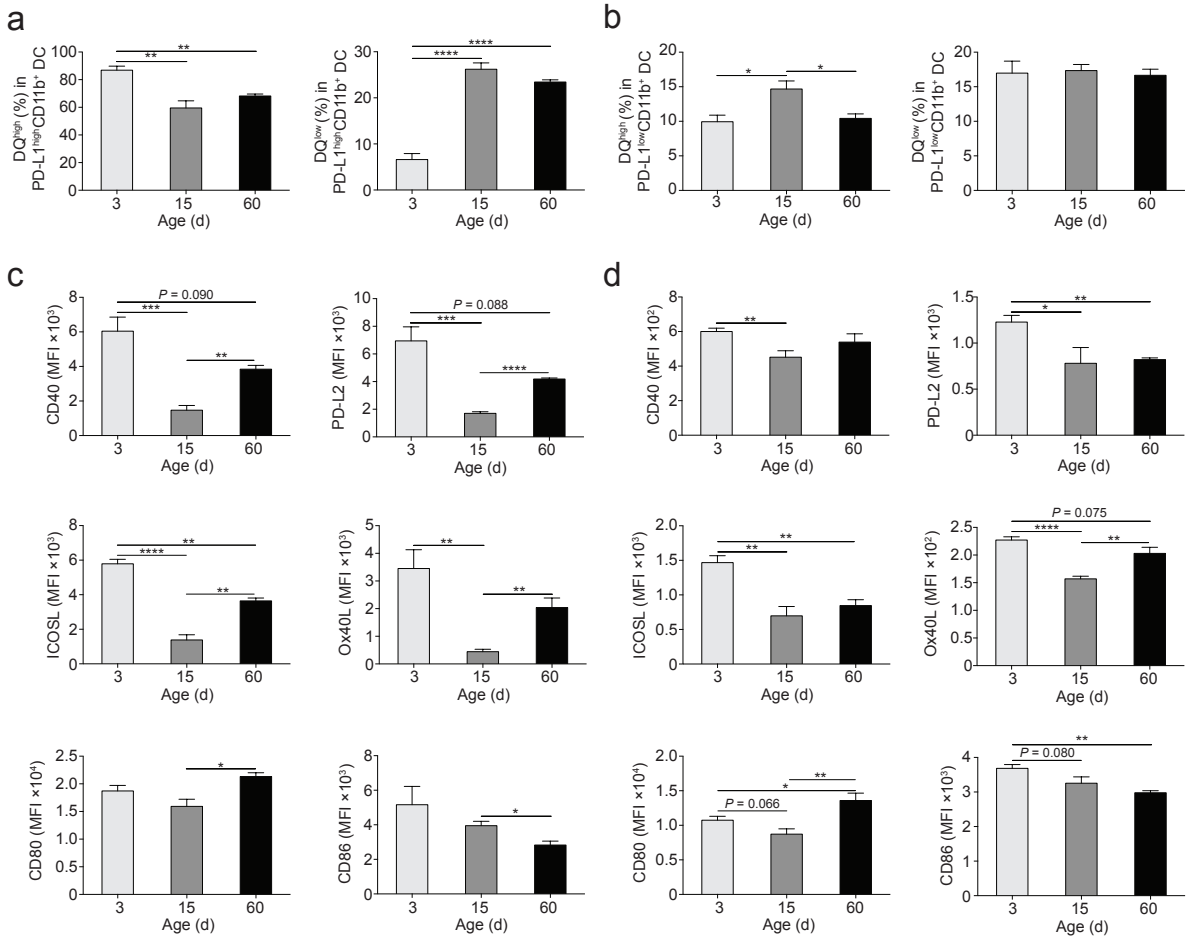
Supplementary Figure 1: Immunological parameters after neonatal and adult HDM exposure.

(**a**) Airway hyperresponsiveness (AHR) after HDM exposure of day three, day 14 and day 60 old mice as measured by airway resistance. (**b**) Frequencies and total number and (**c**) surface expression of CD40, ICOSL, PD-L1 and PD-L2 on $CD11b^+$ DCs. (**d**) Frequencies of T_{reg} cells in the $CD4^+$ T cell compartment, (**e**) their intracellular expression profile of Helios and (**f**) their surface expression pattern of PD-1. Results are representative of two independent experiments. Data are expressed as mean ± s.e.m. (n = 8 PBS-treated mice per timepoint and n = 11 day 3, n = 10 day 14 and n = 12 day 60 HDM-treated mice in **a**; n = 5 neonatal mice, n = 4 pre-weanling and adult mice in **b-f**). Statistical significance was determined with Student's t test. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.



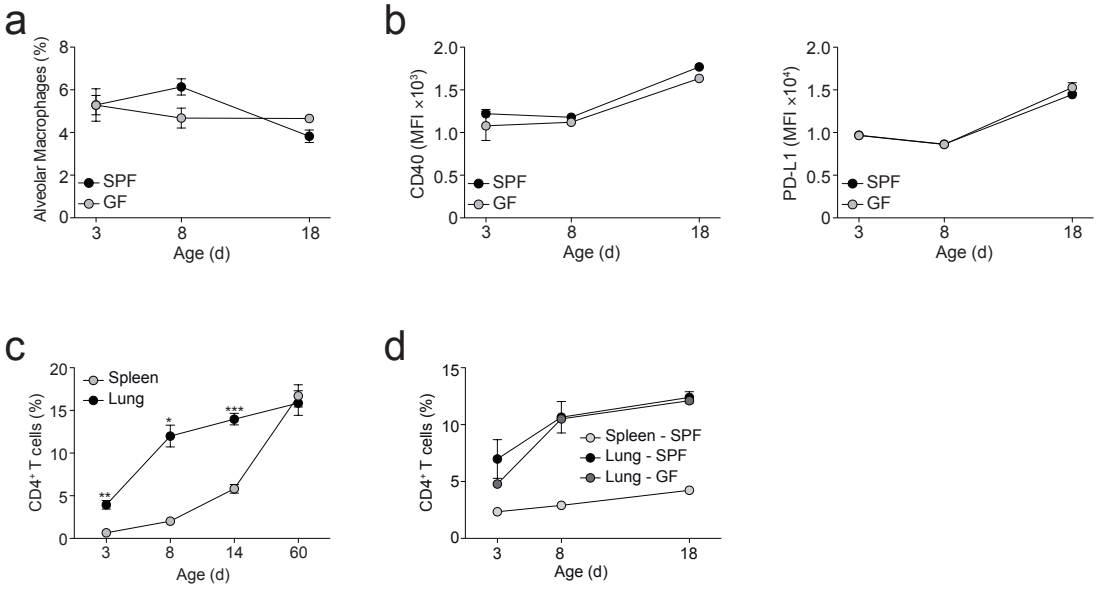
Supplementary Figure 2: PD-L1 is transiently expressed on CD103$^+$ DCs in the lung.

(**a**) Gating strategy to identify the CD103$^+$ lung DC population (2). (**b**) Frequency of CD103$^+$ in the lung and spleen of naïve BALB/c mice. (**c**) Surface expression of CD40, CD80, ICOSL, PD-L1, and PD-L2 on CD103$^+$ DC represented as total mean fluorescent intensity (MFI) and relative MFI to day three expression levels. Results are representative of two independent experiments. Data are expressed as mean ± s.e.m. (*n* = 4 day 3, *n* = 3 day 8, *n* = 5 day 18, and *n* = 4 day 28 old mice). Statistical significance was determined with Student's *t* test and is expressed in relation to day three (* for lung and $^+$ for spleen). **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001.



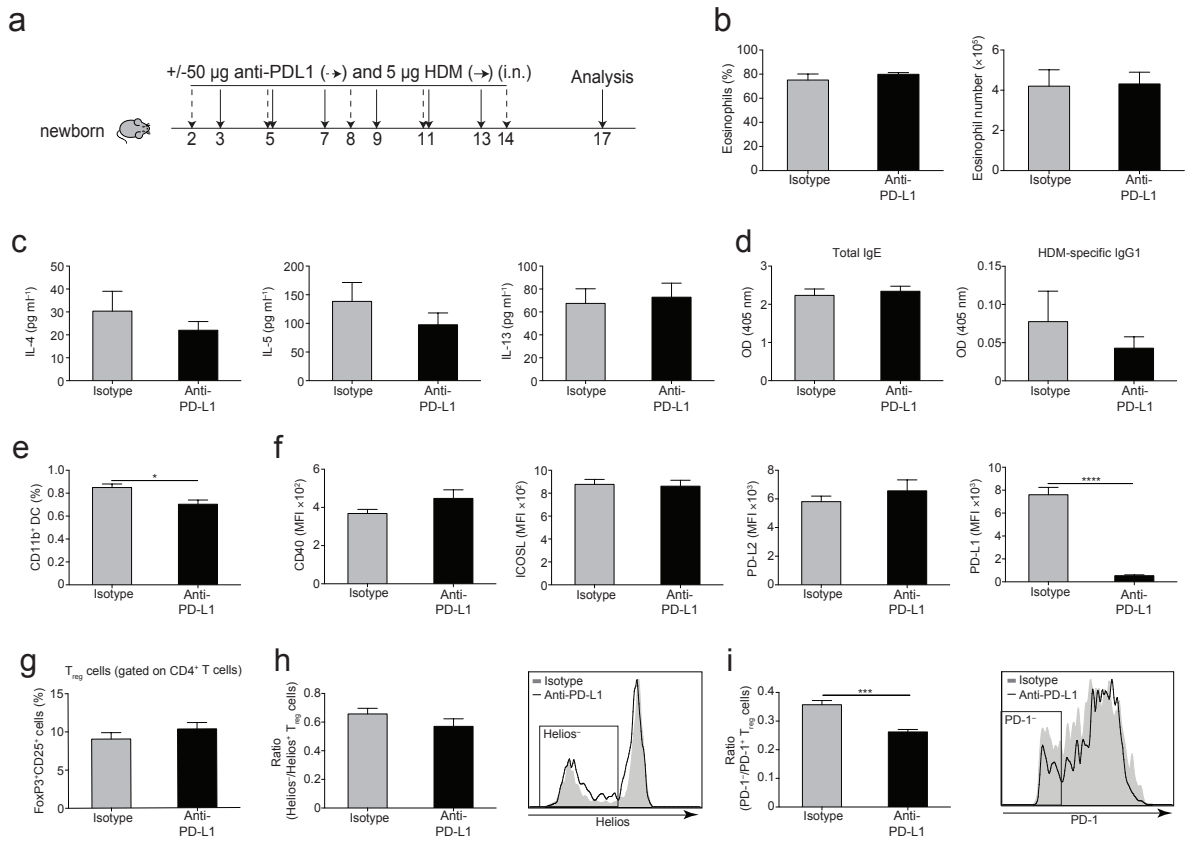
Supplementary Figure 3: PD-L1^{high} DCs have a higher antigen processing ability and are more activated at baseline.

(**a,b**) Measurement of antigen processing ability of (**a**) PD-L1^{high} and (**b**) PD-L1^{low} CD11b^{+} DCs in the lungs of naïve mice. (**c,d**) Surface expression of CD40, PD-L2, ICOSL, Ox40L, CD80 and CD86 on (**c**) PD-L1^{high} and (**d**) PD-L1^{low} CD11b^{+} DC represented as total mean fluorescent intensity (MFI). Results are representative of two independent experiments. Data are expressed as mean ± s.e.m. (n = 5 neonatal and pre-weanling mice and n = 3 adult mice). Statistical significance was determined with Student's t test. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.

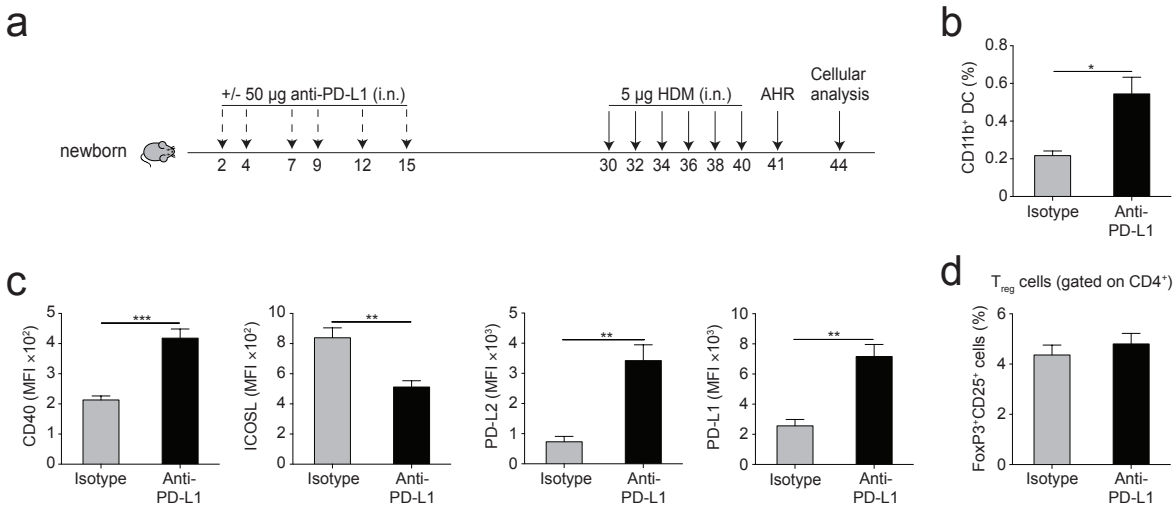


Supplementary Figure 4: Frequencies and phenotype of alveolar macrophages and CD4$^+$ T cells are not affected by the microbiota.

(a) Frequencies of alveolar macrophages and (b) their surface expression of CD40 and PD-L1 in germ-free and SPF mice. (c) Frequencies of CD4$^+$ T cells in the lung and spleen of SPF mice. (d) Frequencies of CD4$^+$ T cells in the lung and spleen of SPF mice as compared to the lung of germ-free mice. Results are representative of data generated in two independent experiments. Data are expressed as mean ± s.e.m. (*n* = 3 day 3, *n* = 5 day 8 and *n* = 4 GF and 5 SPF day 18 old mice).

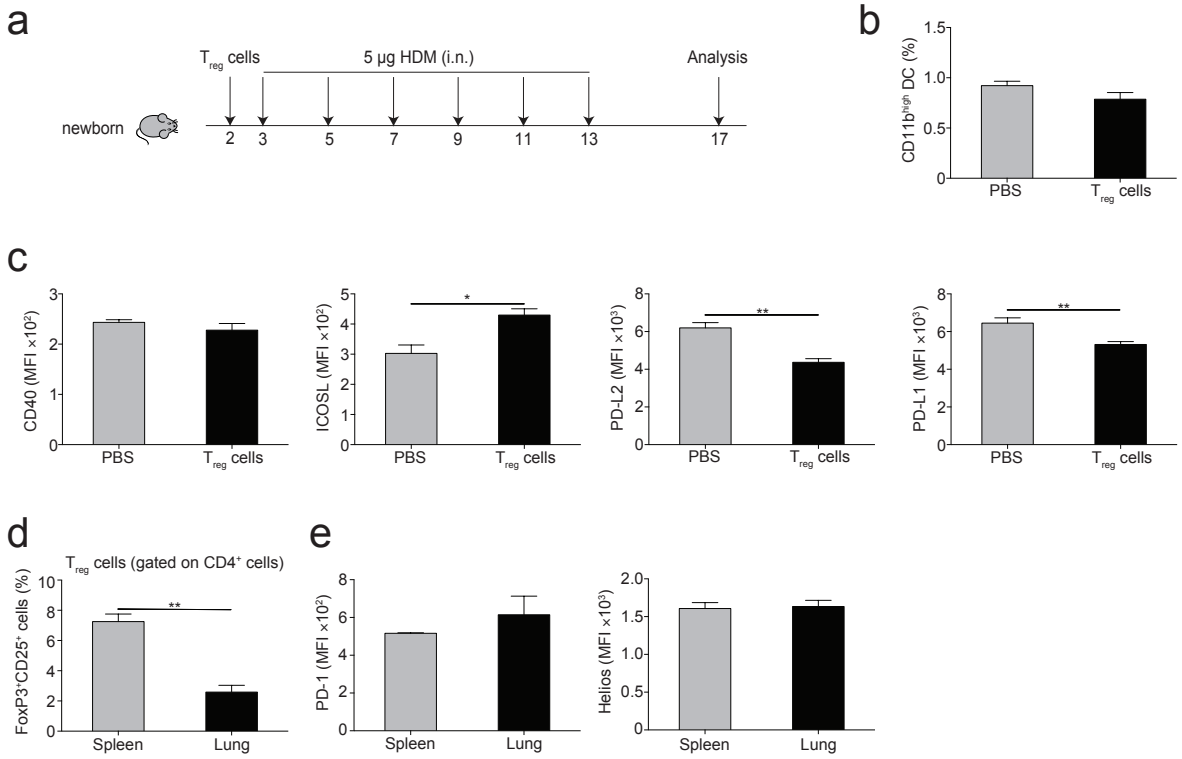


Supplementary Figure 5: Blockade of PD-L1 during neonatal HDM exposure has no impact on the severity of allergic airway inflammation.
(**a**) Representation of the experimental procedure. (**b**) Frequencies and total numbers of eosinophils infiltrating the BALF. (**c**) Concentration of IL-4, IL-5, and IL-13 in the BALF and (**d**) total IgE as well as HDM-specific IgG1 antibody levels in serum. (**e**) Frequencies of $CD11b^+$ DCs in the lung and (**f**) their surface expression of CD40, ICOSL, PD-L1, and PD-L2. (**g**) Frequencies of T_{reg} cells in the $CD4^+$ T cell compartment, (**h**) their intracellular expression profile of Helios and (**i**) their surface expression pattern of PD-1. Results are representative of data generated in two independent experiments. Data are expressed as mean ± s.e.m. (*n* = 6 mice/group). Statistical significance was determined with Student's *t* test. **P* ≤ 0.05, ****P* ≤ 0.001, *****P* ≤ 0.0001.



Supplementary Figure 6: DC phenotypes and T_{reg} cell frequencies following blockade of PD-L1.

(**a**) Representation of the experimental procedure. (**b**) Frequencies of $CD11b^+$ DCs in the lung and (**c**) their surface expression of CD40, ICOSL, PD-L1, and PD-L2. (**d**) Frequencies of T_{reg} cells in the $CD4^+$ T cell compartment. Results are representative of two independent experiments. Data are expressed as mean ± s.e.m. (*n* = 4 isotype-treated and *n* = 6 anti-PD-L1-treated mice). Statistical significance was determined with Student's *t* test. **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001.



Supplementary Figure 7: Adult T_{reg} cells are capable of decreasing neonatal allergic airway inflammation.

(**a**) Representation of the experimental procedure. (**b**) Frequencies of splenic and pulmonary T_{reg} cells in the $CD4^+$ T cell compartment, (**b**) their surface expression pattern of PD-1 and their intracellular expression profile of Helios. (**d**) Frequencies of $CD11b^+$ DCs in the lung and (**e**) their surface expression of CD40, ICOSL, PD-L1, and PD-L2. Results are representative of two to three independent experiments. Data are expressed as mean ± s.e.m. (*n* = 3 PBS-treated and *n* = 5 T_{reg} cell-treated mice in **b** and **d;** *n* = 2 spleen and *n* = 3 lung samples in **d** and **e**). Statistical significance was determined with Student's *t* test. **P* ≤ 0.05, ***P* ≤ 0.01.