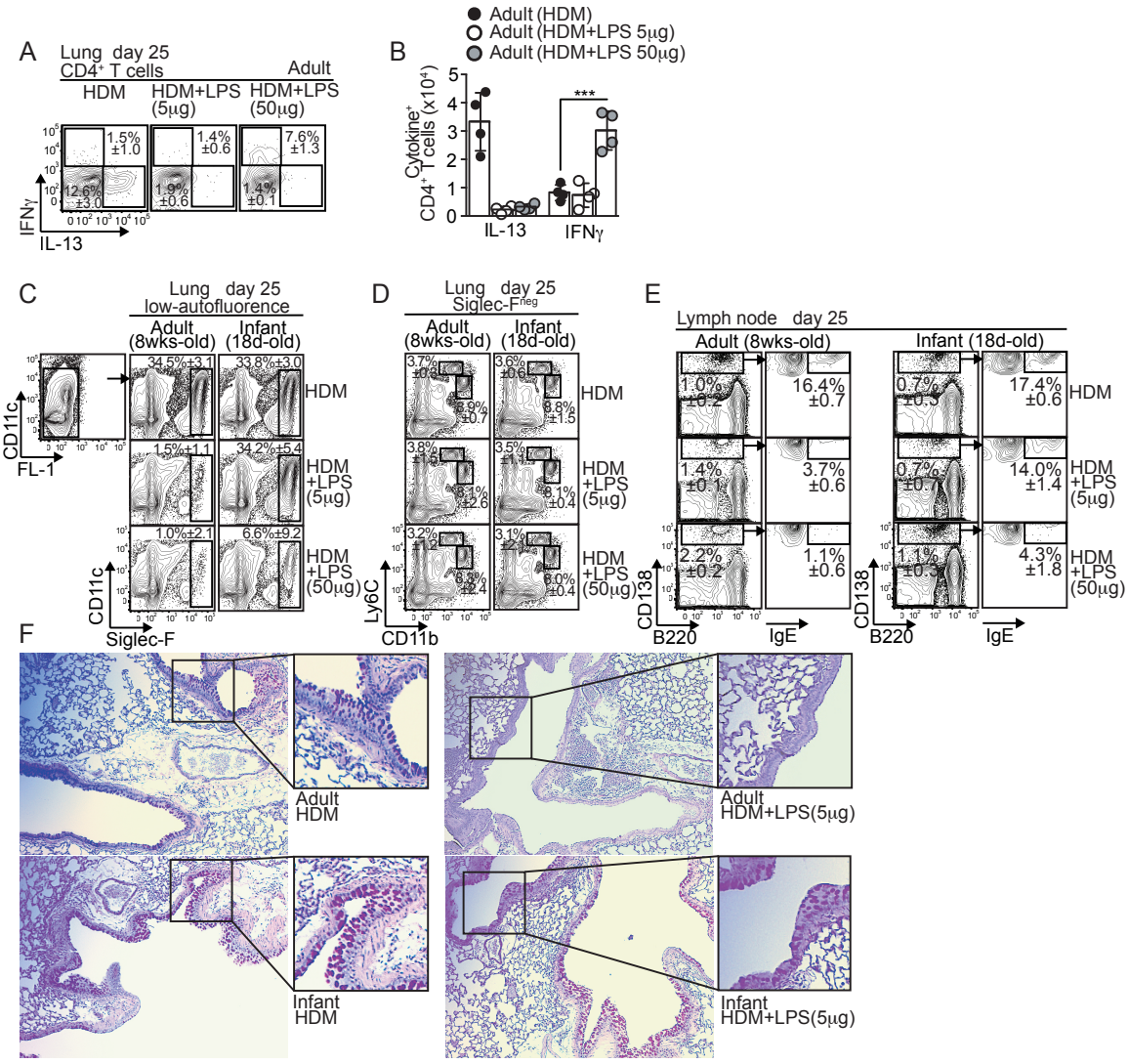

Figure S1. Related to Figure 1. Low-dose LPS sensitization in 18d-old infants does not prevent HDM-induced asthma features in adult life. B6 adult and 18d-old infant mice were i.n treated with 50μg of HDM plus different concentrations of LPS for 3 consecutive days starting on day 1. On day 20, mice were i.n challenged with 50μg of HDM daily for 3 days and analyzed on day 25. (**A-B**) Frequencies (**A**) and numbers (**B**) of IL-13- and IFNγ-expressing $CD4^+$ T cells in the lungs determined by ICS after restimulation for 4 h. (**C-D**) Frequencies of eosinophils (autofluorescence^{lo} $CD11c^{lo}$ Siglec-F^+) (**C**), neutrophils (Siglec-F^-CD11b^{hi}-$Ly6C^{int}$) and monocytes (Siglec-$F^-CD11b^{int}Ly6C^{hi}$) (**D**) in the lungs. (**E**) Frequencies of IgE^+ ACS in the mLN. (**F**) Lung sections stained with Periodic Acid-Schiff (PAS) to identify globlet cells. *P < 0.05 **P < 0.01 ***P < 0.001 (unpaired Student's t test). Data are representative of at least two independent experiments (mean and S.D. of 4-5 mice per group).

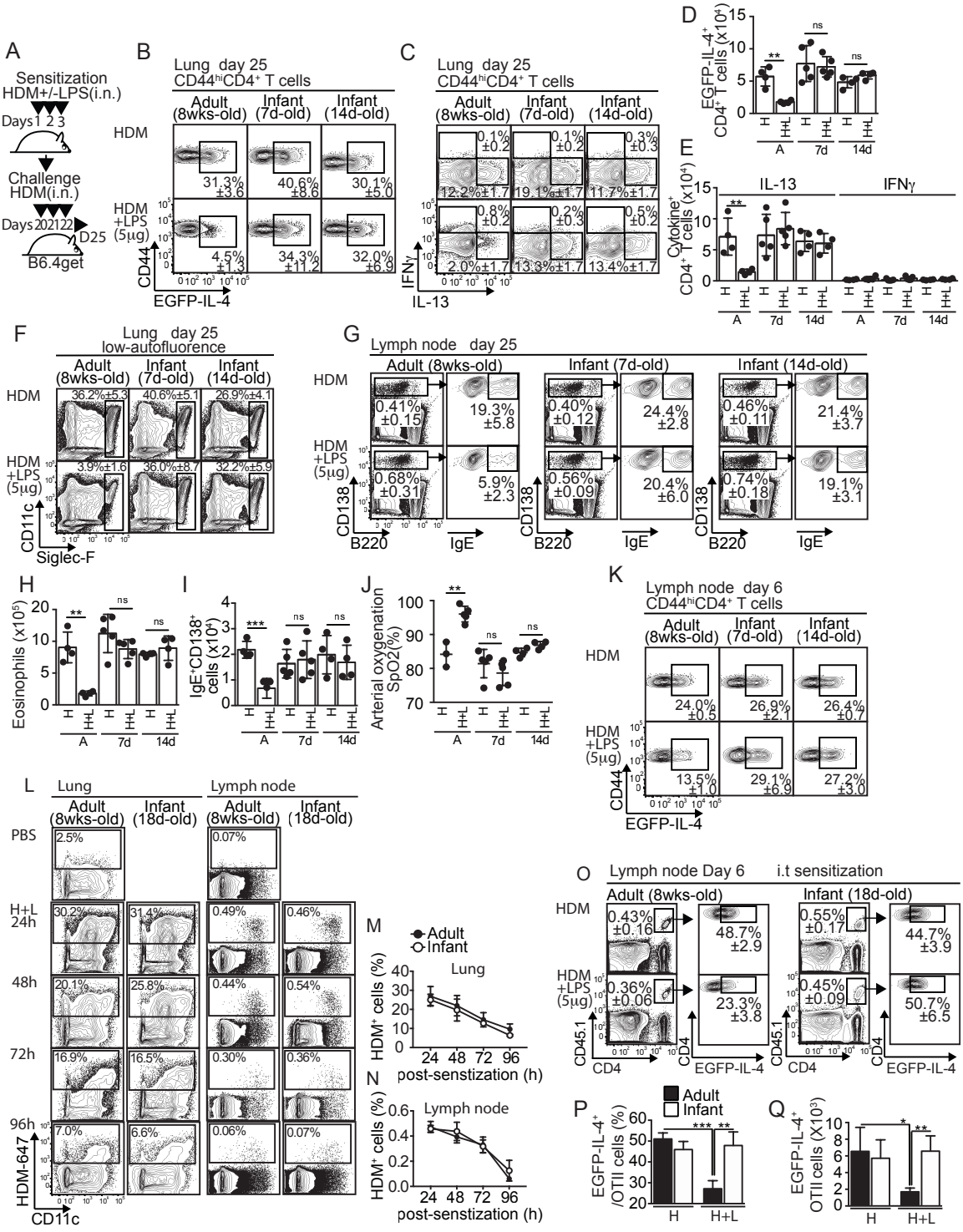

Figure S2. Related to Figure 1. Low-dose LPS sensitization in 7 to 18d-old infants does not prevent HDM-induced Th2 priming and development of Th2 cell-mediated asthmatic features later in life. (**A-J**) B6.4get adults, 7d-old neonates and 14d-old infants were i.n treated with 50µg of HDM+/-5µg of LPS for 3 consecutive days starting on day 1. On day 20, mice were i.n challenged with 50µg of HDM daily for 3 days and analyzed on day 25. Frequencies (**B-C**) and numbers (**D-E**) of IL-4-expressing (EGFP$^+$) CD4$^+$ T cells (**B, D**) and IL-13- and IFNγ-expressing CD4$^+$ T cells (**C, E**) in the lungs. Frequencies (**F**) and numbers (**H**) of eosinophils in the lungs. Frequencies (**G**) and numbers (**I**) of IgE$^+$ ACS in the mLN. SpO2 as determined by pulse-oximetry (**J**). (**K**) B6.4get adults, 7d-old neonates and 14d-old infants were i.n treated with 50µg of HDM+/-5µg of LPS for 3 consecutive days starting on day 1 and analyzed on day 6. Frequencies of IL-4-expressing (EGFP$^+$) CD4$^+$T cells in the mLN. (**L-N**) B6 adult and 18d-old infant mice were i.n treated with Alexa Fluor 647-labeled HDM+5µg of LPS on day 1 and analyzed at different time points. Frequencies of HDM-bearing cells in the lungs (**L-M**) and mLNs (**L, N**). (**O-Q**) Adult and 18d-old infant naïve B6 mice were adoptively transferred with 25x10^3 CD45.1$^+$ OTII.4get cells at day 0, i.t treated with 50µg of HDM+5µg of OVA+/-5µg of LPS for 3 consecutive days starting on day 1 and analyzed by flow cytometry on day 6. Frequencies (**O-P**) and numbers (**Q**) of donor IL-4-expressing (EGFP$^+$) OTII T cells in the mLN of recipient mice. *P < 0.05 **P < 0.01 ***P < 0.001 (unpaired Student's t test). Data are representative of two independent experiments (mean and S.D. of 3-5 mice per group).

Figure S3. Related to Figure 2. Kinetic analysis of specific CD4+ T cells and mDCs in the mLN after low-dose LPS sensitization. (**A-D**) Adult and 18d-old infant naïve B6 mice were adoptively transferred with 25x10³ CD45.1+ OTII.4get cells at day 0, i.n treated with 50μg of HDM+5μg of OVA+/-5μg of LPS for 3 consecutive days starting on day 1 and analyzed by flow cytometry on days 4,5 and 6. Frequencies of donor OTII T cells (**A**) and frequencies of GATA-3 and T-bet-expressing cells (**B**), MFI of GATA-3 expression (**C**) and MFI of T-bet expression (**D**) in donor OTII T cells from mLN of recipient mice. (**E-K**) B6 adult and 18d-old infant mice were i.n treated with HDM+/-5μg of LPS (**E-G**) or with Alexa Fluor 647-labeled HDM+/-5μg of LPS (**H-K**) for 3 consecutive days and analyzed at different time points. Frequencies and numbers of CD103+ mDCs (**E-F**), CD11b+ mDCs (**E,G**), HDM-bearing mDCs (**H-I**), HDM-bearing CD103+ mDCs (**H,J**) and HDM-bearing CD11b+ mDCs (**H,K**). Data are representative of three independent experiments (mean ± SD of 4 mice per time point).

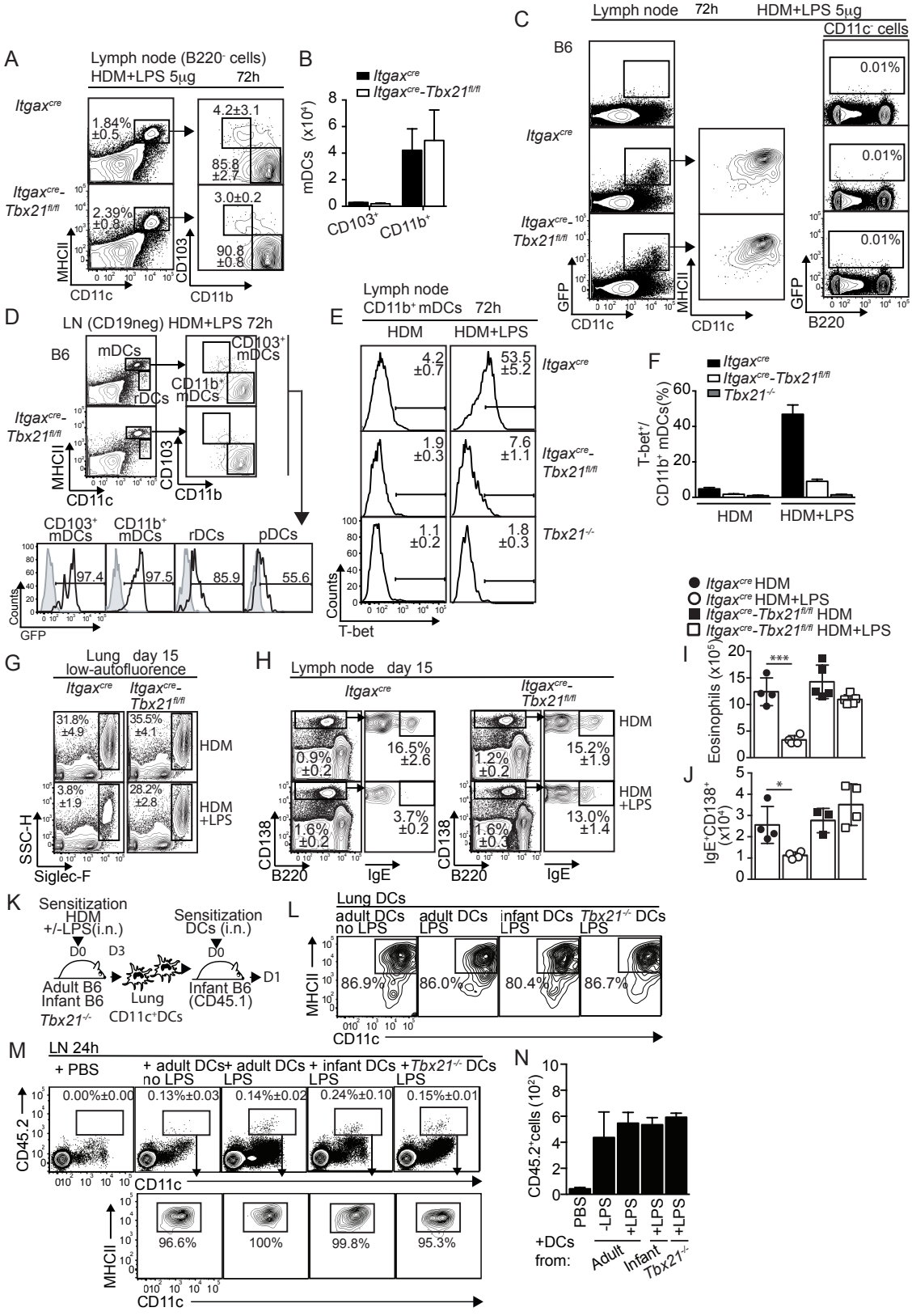

Figure S4. Related to Figure 4. Analysis of DCs in *Itgax*^{cre}-*Tbx21*$^{fl/fl}$ mice and analysis of lung mDCs in recipient mice after i.n. transference. (**A-F**) *Itgax*^{cre}-*Tbx21*$^{fl/fl}$ and control mice were i.n. HDM or HDM+5µg of LPS sensitized on day 1 and analyzed on day 4. Frequencies (**A**) and numbers (**B**) of CD103$^+$ mDCs and CD11b$^+$ mDCs in the mLN. Expression of GFP in total mLN cells and in CD11c$^-$ mLN cells and expression of CD11c and MHCII in GFP$^+$ cells from mLN (**C**). Expression of GFP in gated CD103$^+$ mDCs, CD11b$^+$ mDCs, rDCs and pDCs (Siglec-H+, CD11cl^o, B220$^+$ CD19$^-$). Gray filled overlays indicate control staining from B6 mice (**D**). Frequencies of T-bet expression in CD11b$^+$ mDCs from mLN (**E-F**). (**G-J**) *Itgax*^{cre}-*Tbx21*$^{fl/fl}$ and control mice were i.n treated with 50µg of HDM+/-5µg of LPS for 3 consecutive days starting on day 1. On day 10, mice were i.n challenged with 50µg of HDM daily for 3 days and analyzed on day 15. Frequencies (**G**) and numbers (**I**) of eosinophils in the lungs. Frequencies (**H**) and numbers (**J**) of IgE$^+$ ACS in the mLN. (**K-N**) Day 3 CD11c$^+$ lung mDCs from HDM/OVA+/-LPS-treated adult and infant B6 mice or *Tbx21*$^{-/-}$ mice were purified and i.n. transferred into B6 (CD45.1$^+$) infant mice (K). Frequencies of CD11c$^+$MHCII^{hi} lung mDCs in purified lung preparations before transference (**L**). Frequencies (**M**) and numbers (**N**) of donor mDCs in the mLN 24h after transference. *P < 0.05 ***P < 0.001 (unpaired Student's t test). Data are representative of at least two independent experiments (mean and S.D. of 3-5 mice per group).

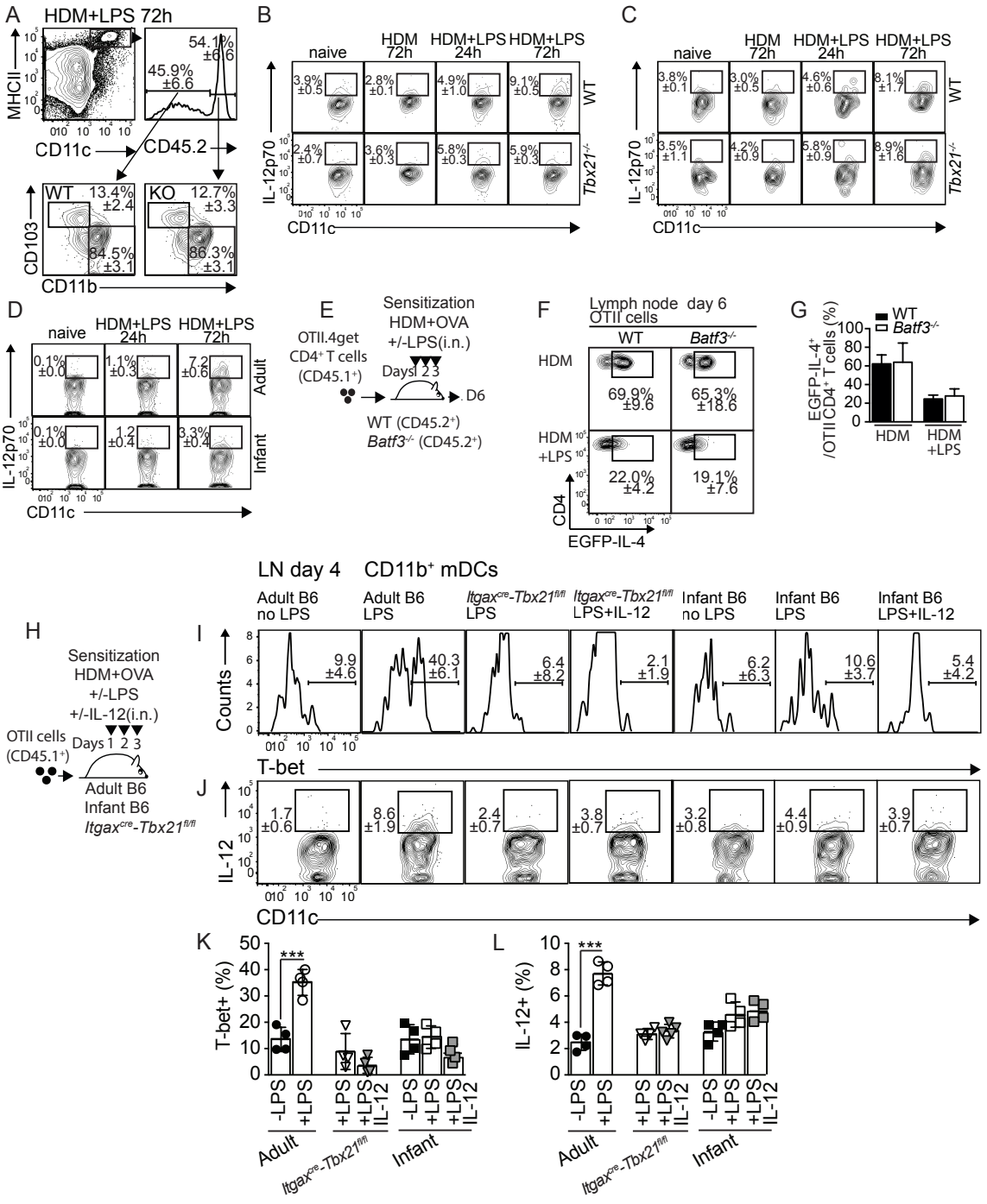

Figure S5. Related to Figure 5. *Tbx21*$^{-/-}$ and infant CD11b^{+} mDCs are intrinsically impaired in their ability to produce IL-12 after low-dose LPS exposure. (**A-C**) B6 (CD45.1^{+}) mice were irradiated and reconstituted with 1:1 mixture of BM from B6 (CD45.1^{+}) and *Tbx21*$^{-/-}$ (CD45.2^{+}) donors. Frequency of WT and *Tbx21*$^{-/-}$ CD11b^{+} and CD103^{+} mDCs in the mLN of day 3-HDM+LPS-treated reconstituted chimeric mice (**A**). Frequencies of IL-12p70-expressing WT and *Tbx21*$^{-/-}$ CD11b^{+} mDCs (**B**) and CD103^{+} mDCs (**C**) in the mLN of HDM+/-LPS-treated reconstituted chimeric mice. (**D**) Frequency of IL-12p70-expressing CD11b^{+} mDCs in the mLN of HDM+/-LPS-treated adult and infant B6 mice. (**E-G**) *Batf3*$^{-/-}$ and control mice were adoptively transferred with 25x10^{3} CD45.1^{+} OTII.4get cells at day 0 and i.n treated with 50µg of HDM+5µg of OVA+/-5µg of LPS for 3 consecutive days starting on day 1 (**E**). Frequencies of IL-4-expressing (EGFP^{+}) cells in donor OTII T cells from the mLN on day 6 (**F-G**). (**H-L**) Adult and 18d-old infant B6 mice or *Itgax*^{cre}-*Tbx21*$^{fl/fl}$ mice were i.n treated with 50µg of HDM+5µg of OVA+/-5µg of LPS+/-150ng of rIL-12p70 for 3 consecutive days starting on day 1 (**H**). Frequencies of T-bet (**I,K**) and IL-12p70 (**J,L**) expression in CD11b^{+} mDCs from mLN on day 4. ***P < 0.001 (unpaired Student's t test). Data are representative of two independent experiments (mean and S.D. of 3-4 mice per group).

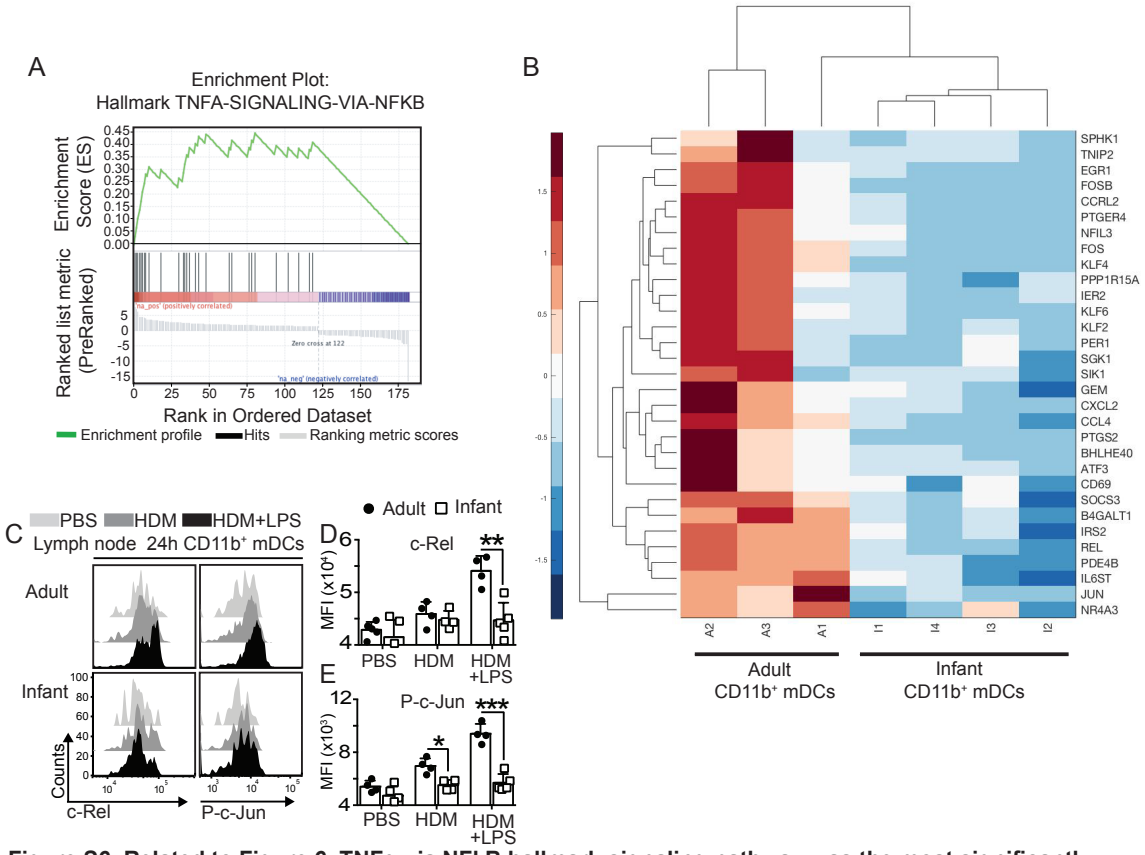

Figure S6. Related to Figure 6. TNFα-via NFkB hallmark-signaling pathway was the most significantly down-regulated pathway in infant CD11b⁺ mDCs after low-dose LPS sensitization. (**A-B**) CD11b⁺ mDCs were sorted from the mLN of adult and 18d-old infant mice 24 after HDM+LPS^{lo} sensitization and RNA-seq was performed. (**A**) GSEA showing enrichment in the TNF-α via NFkB hallmark-signaling pathway in adults vs. infant CD11b⁺ DCs (adjusted p-value <0.05). (**B**) Heat map displaying the expression of the TNFα-via NFkB hallmark signaling pathway genes that are differentially expressed in adult CD11b⁺ mDCs relative to infant CD11b⁺ mDCs. Replicates for each cell type were obtained from three or four independent experiments. (**C-E**) B6 adult and 18d-old infant mice were i.n treated with HDM+/-5µg of LPS. Expression c-Rel (**C,D**) and P-c-Jun (**C,E**) in CD11b⁺ mDCs from the mLN on day 1. *P < 0.05 **P < 0.01 ***P < 0.001 (unpaired Student's t test). Data are representative of two independent experiments (mean and S.D. of 4-5 mice per group).

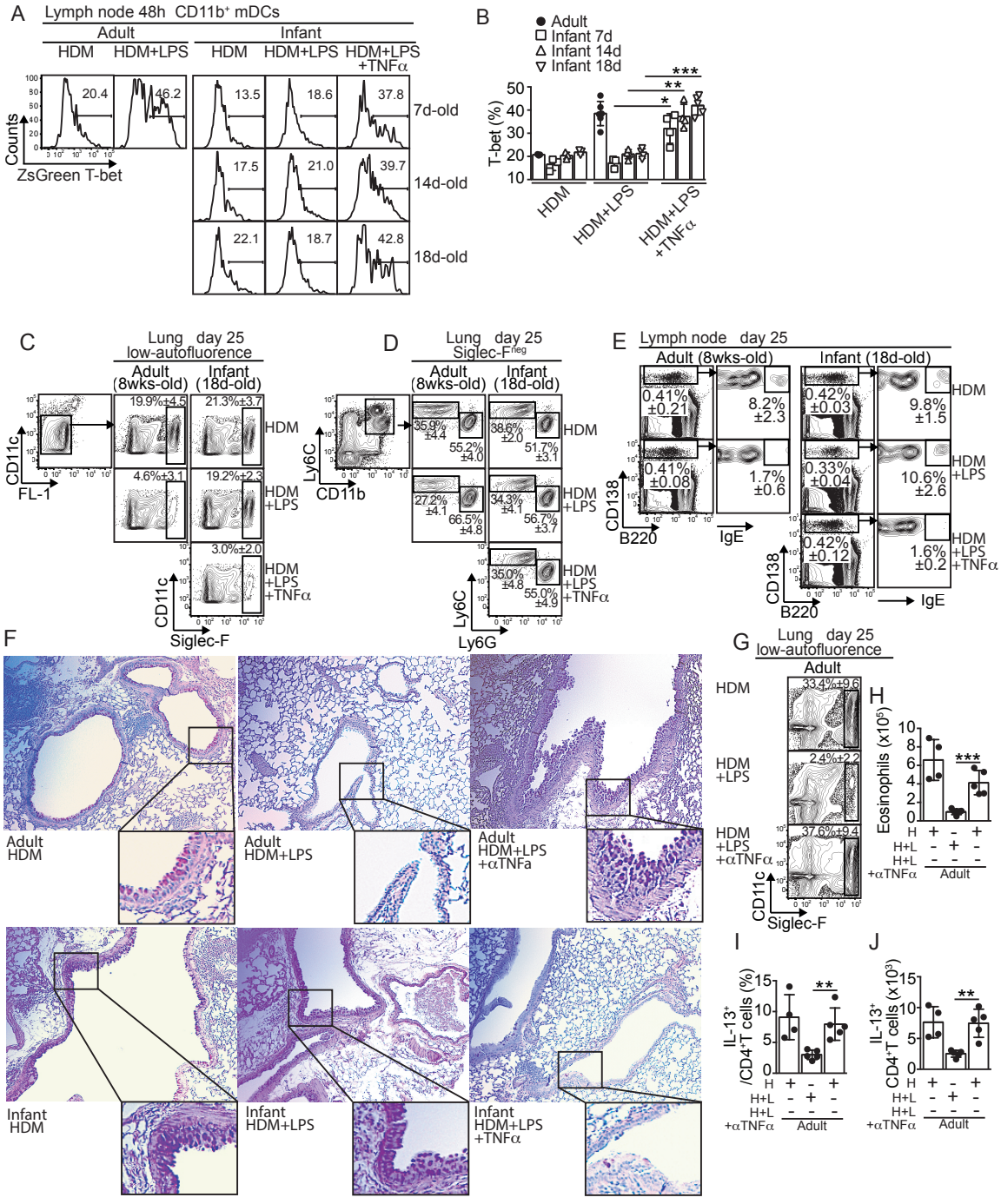

Figure S7. Related to Figure 7. Asthma features in the presence and absence of TNF-α. (**A-B**) T-bet-ZsGreen reporter adults, 7d-old neonates, 14d-old infants and 18d-old infants were i.n treated with HDM+/-5μg of LPS+/-30ng of TNF-α. Frequencies of T-bet-expressing (ZsG+) mLN CD11b$^+$ mDCs on day2. (**C-J**) B6 adult and 18d-old infant mice were i.n treated with HDM+/-5μg of LPS+/-30μg of TNF-α. Some adult mice also receive 250μg of anti-TNFα (i.p.). On day 20, mice were i.n challenged with HDM and analyzed on day 25. Frequencies of eosinophils (autofluorescencelo CD11c^{lo} Siglec-F$^+$) (**C, G**), neutrophils (Siglec-F$^-$CD11b^{hi}Ly6C^{int}) and monocytes (Siglec-F$^-$CD11b^{int}Ly6C^{hi}) (**D**) and numbers of eosinophils (**H**) in the lungs. Frequencies of IgE$^+$ ACS (**E**) in the mLN. Lung sections stained with PAS to identify globlet cells (**F**). Frequencies (**I**) and numbers (**J**) of IL-13-expressing CD4$^+$ T cells in the lungs determined by ICS after restimulation for 4 h. *P < 0.05 **P < 0.01 ***P < 0.001 (unpaired Student's t test). Data are representative of two independent experiments (mean and S.D. of 3-5 mice per group).