

ORIGINAL ARTICLE

Experimental Models of Allergic Disease

Short-term high-fat diet feeding protects from the development of experimental allergic asthma in mice

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Abstract

Background: A close association between obesity and asthma has been described. The nature of this association remains elusive, especially with respect to allergic asthma. Controversial findings exist regarding the impact of short-term high-fat diet (HFD) feeding on the development of allergic asthma.

Objective: To delineate the impact of short-term HFD feeding on the development of experimental allergic asthma.

Methods: Female C57BL/6J mice were fed with a short-term HFD or chow diet (CD) for 12 weeks. Allergic asthma was induced by intraperitoneal OVA/alum sensitization followed by repeated OVA airway challenges. We determined airway hyper-responsiveness (AHR) and pulmonary inflammation by histologic and flow cytometric analysis of immune cells. Furthermore, we assessed the impact of HFD on dendritic cell (DC)-mediated activation of T cells.

Results: Female mice showed a mild increase in body weight accompanied by mild metabolic alterations. Upon OVA challenge, CD-fed mice developed strong AHR and airway inflammation, which were markedly reduced in HFD-fed mice. Mucus production was similar in both treatment groups. OVA-induced increases in DC and CD4⁺ T-cell recruitment to the lungs were significantly attenuated in HFD-fed mice. MHC-II expression and CD40 expression in pulmonary CD11b⁺ DCs were markedly lower in HFD-fed compared to CD-fed mice, which was associated in vivo with a decreased T helper (Th) 1/17 differentiation and Treg formation without impacting Th2 differentiation.

Conclusions/clinical relevance: These findings suggest that short-term HFD feeding attenuates the development of AHR, airway inflammation, pulmonary DC recruitment and MHC-II/CD40 expression leading to diminished Th1/17 but unchanged Th2 differentiation. Thus, short-term HFD feeding and associated metabolic alterations may have protective effects in allergic asthma development.

KEYWORDS

allergy, asthma, dendritic cells, high fat diet, metabolism, overweight, T cells

1 | INTRODUCTION

Obesity and asthma are chronic diseases with an increasing worldwide prevalence that constitute a significant healthcare burden.^{1,2} Previous studies suggest that obesity serves as a risk factor for the development, severity and weaker responsiveness to standard asthma medication.³ In the late-onset non-allergic phenotype of obesity-associated asthma (LONA), airway hyperresponsiveness (AHR) is considered a consequence of lung compression and structural lung modifications due to increased body weight.^{4,5} Furthermore, animal models show that LONA is associated with T helper (Th)1- and Th17 aberrant immune responses.^{6,7} However, next to LONA, an early-onset allergic form of obesity-associated asthma has been described (EOA),⁸ which is characterized by a more classical allergen-driven eosinophilia and Th2 differentiation without body weight-induced modulations.⁹ EOA prevails until the body weight reaches obesity, which then worsens the development of Th2 immunity and asthma disease phenotype.¹⁰

In clinical asthma research, the majority of studies focused on the impact of obesity on asthma development, considering children with a body mass index (BMI) $\geq 85\%$ percentile or adults with a BMI $\geq 25 \text{ kg/m}^2$. However, they did not distinguish between mild body weight gain and manifest obesity (ie BMI $\geq 30 \text{ kg/m}^2$).¹¹ As a consequence, potential effects of mild body weight gain on the development of allergic asthma in comparison with normal weight are ill-defined. Importantly, two recent meta-analyses suggest that short-term HFD feeding, in contrast to obesity, may have protective effects and might be considered as a phenotype distinct from obesity.^{12,13}

Similar to human allergy studies, mouse models of experimental asthma with gene targeting-associated or HFD-induced increases in body weight have shown that the severity of allergic asthma is increased when the body weight increased between 160% and 250% as compared with CD-fed mice.^{6,14} In contrast, a lower but still substantial body weight increase in the range of 120% showed only a limited impact on allergic asthma development.¹⁵ These findings support the view that mild and strong increases in body weight may have distinct impacts on asthma induction and development.

To delineate the impact of short-term HFD feeding on allergic asthma immunopathogenesis, we used a mouse model of experimental allergic asthma in the context of short-term HFD feeding and associated mild body weight gain (ie 175% vs 150% increase). We observed that this pre-obese state decreased the severity of AHR and pulmonary inflammation in allergic asthma, characterized by decreased numbers of pulmonary conventional (c) DCs and monocyte-derived (mo) DCs, lower expression of MHC-II and CD40 in such cDCs and a decreased frequency of IFN $^+$ and interleukin (IL)-17 $^+$ but not IL-13 $^+$ CD44 $^+$ T effector cells. Our data suggest that short-term HFD feeding, in contrast to obesity, has a protective impact on the development and severity of allergic asthma.

2 | MATERIALS AND METHODS

2.1 | Mice and feeding

Female C57BL/6J mice (Janvier, France) were maintained in a specific pathogen-free facility at the University of Lübeck. Starting at the age of 4 weeks over a period of 12 weeks, mice were offered free access to either chow containing 12 kJ% from fat, 64 kJ% from carbohydrates and 24 kJ% from proteins (CD; Altromin 1324) or HFD containing 42 kJ% from fat, 43 kJ% from carbohydrates and 15 kJ% from proteins (HFD; Ssniff EF R/M acc. TD88137 mod.). This study was reviewed and approved by the Schleswig-Holstein state authorities [V242-66285/2015 (79-6/15)].

2.2 | Reagents

Antibody lists can be found in supplemental material. Ovalbumin (OVA) (grade V), aluminium potassium sulphate (alum) and acetyl- β -methyl-choline (methacholine) were all from Sigma-Aldrich.

2.3 | Experimental ovalbumin (OVA)-driven allergic asthma model

The OVA-induced asthma model was performed as described previously¹⁶ with minor modifications. Briefly, mice after 12 weeks of CD or HFD feedings were immunized by intraperitoneal (i.p.) injection with PBS/alum (1 mg) or OVA/alum (150 μg /1 mg) on days 0 and 7 and challenged intratracheally (i.t.) with 1.5% OVA (in 50 μL PBS) on days 14, 16, 18 and 20. After 24 hours, airway hyperresponsiveness (AHR) was determined and mice were sacrificed for tissue harvest and further analysis.

2.4 | Measurement of AHR

Anaesthetized mice were prepared and mechanically ventilated using a FlexiVent (SciReq) system, aerosolized with different concentrations of acetyl- β -methyl-choline (methacholine, Sigma-Aldrich), and the airway resistance was measured as described previously.¹⁷

2.5 | Collection of bronchoalveolar lavage fluid and differential cell counting

Bronchoalveolar lavage (BAL) samples were obtained as previously described.¹⁶ BAL cells were washed, counted and identified by flow cytometry, following the gating strategy displayed in Figure S1A. Cell numbers were calculated using cell-specific frequencies in BAL and expressed as total cell counts/mL.

2.6 | Lung histology

Lung histological staining, detection and quantification of mucus cell content were done as described previously,¹⁷ either with haematoxylin

and eosin (H&E) or with periodic acid-Schiff (PAS) to evaluate inflammatory influx in the tissue and mucus production, respectively. For the latter, the mucus positiveness of all the airways was evaluated by light microscopic observation and the percentages of PAS-positive airways were established to quantify mucus production as described.^{16,17}

2.7 | Lung and adipose tissue cell isolation and flow cytometric analysis

The right lung lobes and the visceral adipose tissue (VAT) were harvested and digested using Liberase TL and DNase I as described previously.^{16,17} Cell numbers were assessed by manual enumeration, and cell suspensions were analysed by flow cytometry using specific gating strategies (Figure S1C-F). To determine *in vivo* T-cell polarization, lung cell suspensions were adjusted to 1×10^6 cells/mL and rested overnight. On the next day, cell suspensions were restimulated for four hours (50 ng/mL phorbol 12-myristate 13-acetate, PMA, 500 ng/mL ionomycin, both from eBioscience) in the presence of Golgi apparatus inhibitors (2 μ mol/L monensin, 3 μ g/mL brefeldin, both from eBioscience). Cells were fixed and permeabilized using a Foxp3 staining kit (R&D Systems) following manufacturer's instructions. Cells were subsequently stained for Interleukin (IL)-10, IL-13, IL-17, interferon (IFN)- γ and Foxp3 and analysed by flow cytometry. Phenotypic characterization of cells was performed on a BD LSRII or ARIA III flow cytometer (BD Bioscience) using the gating strategies published previously and displayed in Figure S1E.

2.8 | Determination of MHC-II and CD40 costimulatory molecule expression in naïve pulmonary DC subsets after HFD or CD feeding

Lung cells from mice fed for 12 weeks with CD or HFD were isolated and seeded 1×10^6 cells/mL in 24-well plate. After 24-hour stimulation with PBS or OVA (1 μ mol/L), dendritic cells were stained and characterized as SiglecF⁺lineage⁻CD11c⁺MHC-II⁺ by flow cytometry, and the expression of MHC-II and CD40 in DCs (gating in Figure S1F) was evaluated.

2.9 | Metabolic parameters

Body weight and food consumption were measured weekly. After sacrificing the mice, the perigonadal fat depots, representative for the VAT, were removed and weighted. Serum leptin and insulin concentrations were measured using a mouse metabolic kit (Meso Scale Discovery K15124C-1) following the manufacturer's instructions. Of note, a dip in body weight coincided with the beginning of the airway challenging phase in all groups of mice due to the repeated narcosis and challenges every other day, triggering a typical loss in weight gain.

2.10 | Co-culture of pulmonary cDCs with OVA-T-cell receptor (TCR) transgenic CD4⁺T cells

After staining with a live/dead viability dye (APC-eF780; eBioscience), living SiglecF⁺lineage⁻CD11c⁺MHC-II⁺ DCs were sorted. After

overnight rest in medium supplemented with 20 ng/mL GM-CSF, cells (1×10^5 /well) were co-cultured, in the presence of 1 μ mol/L OVA, with CD4⁺ T cells (2.5×10^5) from OVA-TCR transgenic OTII mice isolated from spleen by magnetic selection using a CD4 isolation kit (Miltenyi Biotec) according to the manufacturer's instructions and labelled with carboxyfluorescein diacetate succinimidyl ester (1 μ mol/L) (CFSE Molecular Probes). At days 4 and 9, cells were harvested, stained with an anti-CD4-PeCy7 Ab (eBioscience) and assessed for cell proliferation by flow cytometry on a BD LSRII (BD Bioscience), and levels of IL-10, IL-13 and IFN- γ in the supernatant were assessed by ELISA according to manufacturer's instructions (DuoSet ELISA kits; R&D Systems). The ELISA sensitivities were 31 pg/mL for IL-10 and IFN- γ and 62.5 pg/mL for IL-13. Furthermore, at day 9, cell survival was evaluated by staining cells with a live/dead viability dye (APC-eF780; eBioscience).

2.11 | Statistical analysis

Results are expressed as mean $\pm$ standard error mean (SEM). Statistical analysis was performed using the GraphPad Prism version 5.03 for Windows (GraphPad Software, USA). Dot plots were chosen to provide information about distribution of values. Normal distribution of data was tested using the Kolmogorov-Smirnov and D'Agostino-Pearson tests. Statistical differences of two normally distributed groups were analysed by unpaired t test. Comparisons involving multiple groups were analysed by ANOVA followed by Tukey's test. Differences in non-normally distributed groups were tested using a Mann-Whitney U (two groups), ANOVA on ranks (multiple groups) followed by a Dunn's multiple comparison test, or as grouped analysis using a 2-way ANOVA followed by Bonferroni post-test. The respective statistical test is indicated in the figure legend.

In case of comparison of two groups, asterisk or pound (*, #) was used to indicate the p-value. In case of comparison of more than two groups, for example the PBS controls and the OVA-treated groups within each diet group are shown (eg in CD/PBS and HFD/PBS vs CD/OVA and HFD/OVA), * indicates significant difference between the OVA group and the respective PBS control (eg CD/OVA vs CD/PBS), and # indicates significant difference between the OVA groups of both diets (eg CD/OVA vs HFD/OVA). A P-value < 0.05 was considered statistically significant and marked with * or #, and P-value < 0.01 was considered highly statistically significant and marked with ** or ## and in case of P < 0.001 with *** or ###.

3 | RESULTS

3.1 | Short-term HFD feeding drives systemic and organ-specific metabolic alterations

Mice upon short-term HFD feeding over a period of 12 weeks showed a mild increase in body weight compared to CD-fed littermates (Figures 1A, S2A), also characterized by an increased VAT mass (Figure 1B). Alongside, the frequency of F4/80⁺CD11c⁺ adipose tissue macrophages increased in VAT of HFD-fed compared

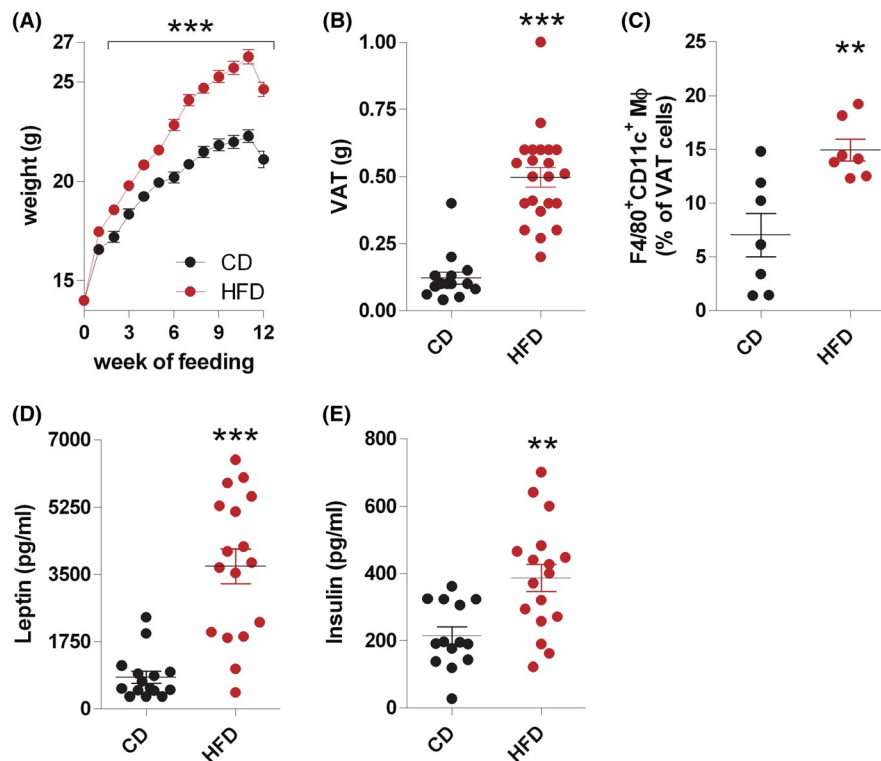


FIGURE 1 Short-term HFD feeding is associated with systemic and local metabolic alterations. A, Kinetic of body weight gain in response to 12 wk feeding with CD- (black circles) or HFD-fed mice (red circles); $n = 28$ –36 out of 5 independent experiments, 2-way ANOVA/Bonferroni. B, Changes in perigonadal fat depots, which are representative for visceral adipose tissue (VAT), 12 wk after either HFD or CD feeding; $n = 15$ –22 out of 5 independent experiments, Mann-Whitney U test. (C) Frequency of F4/80⁺CD11c⁺ macrophages in VAT stromal vascular cells 12 wk after HFD or CD feeding; $n = 7$ out of 2 independent experiments, t test. D, Serum leptin and E, serum insulin levels (not fasted) in HFD- and CD-fed mice after 12 wk treatment; $n = 15$ –17 out of 3 independent experiments, t test. Values shown are the mean $\pm$ SEM. Asterisks show significant differences between HFD- and CD treatment groups; ** $P < 0.01$; *** $P < 0.001$

to CD-fed mice pointing towards the development of a pro-inflammatory environment (Figure 1C). Furthermore, serum leptin and insulin concentrations were increased following short-term HFD feeding as compared to CD feeding (Figure 1D,E). The total amount of serum cholesterol as well as the HDL- and VLDL/LDL-cholesterol subfractions increased upon short-term HFD feeding (Figure S2B). Serum triglycerides were not affected by the feeding (Figure S2B). Additionally, H&E histology analysis of liver tissue revealed lipid accumulation in HFD but not CD mice (Figure S2C). Finally, analysis of the gut microbiota in representative mice before and after HFD and CD feeding revealed a significant change in the microbiota composition between the groups. In HFD mice, we observed an increased abundance of Proteobacteria associated with a decreased abundance of Firmicutes compared to CD mice. (Figure S3A). In order to define our model more precisely, we compared body weight development, mouse-adjusted body mass index (BMI), epididymal adipose tissue mass (VAT), pro-inflammatory macrophage accumulation in adipose tissue, serum lipids as well as changes in leptin and insulin concentration and hepatic lipid accumulation to reported values from models of obesity found in the literature. We chose both, a genetically induced obesity, as consequence of deficiency in the leptin gene (*ob/ob* mice)^{18–24} and HFD-fed female C56BL/6 wild-type mice housed under thermoneutral conditions reaching obesity including

associated metabolic disturbances to serve as proper positive controls²⁵ (Table 1). As a positive control reference for the serum lipids, we chose ApoE-deficient mice (ApoE^{−/−} mice)²⁶ (Table 1). We verified that all for these parameters, short-term HFD-fed mice show consistent changes in-between baseline and literature-reported obesity and can reliably be considered as mildly metabolically challenged.

3.2 | Short-term HFD feeding results in decreased AHR and airway inflammation

After 9 weeks of feeding, we immunized the two diet groups (while the feeding continued) i.p. with OVA/alum, while control groups received PBS/alum. Then, in week twelve, mice received four airway challenges (OVA vs PBS) every other day. Twenty-four hours after the final OVA airway challenge, three major hallmarks of asthma, that is airway hyperresponsiveness (AHR), inflammatory cell infiltration and mucus production, were evaluated. In CD/PBS and HFD/PBS mice, AHR remained low upon methacholine provocation but increased significantly in CD/OVA and HFD/OVA mice (Figure 2A). However, HFD/OVA mice showed a significant reduction in airway resistance compared to CD/OVA mice (Figure 2A). An evaluation of AHR in relative values normalized to baseline was performed to rule out that the feeding or the body weight itself may change the lung resistance.

TABLE 1 Comparison of cellular and metabolic parameters between chow diet (CD), short-term high-fat diet (HFD) feeding and literature reports of obesity

Mouse model	Body weight ^c (g)	Body weight gain ^c (g)	Body weight gain ^c (%)	Adjusted body mass index (BMI) (100g/cm ²)	VAT (g)	VAT mass index (100g/cm ²)	VAT macrophages (% of VAT cells)	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL/VLDL (mg/dL)	Triglycerides (mg/dL)	Serum leptin (pg/mL)	Serum insulin (pg/mL)	Liver lipid accumulation
CD-fed mice	20.4 ± 0.39	7.3 ± 1.65	52.6 ± 12.8	0.12 ± 0.02	0.12 ± 0.02	22.4 ± 0.39	7 ± 2.0	82.9 ± 8.3	56.8 ± 8.5	32.7 ± 4.3	127.6 ± 14.1	827.5 ± 156.3	215.3 ± 26.1	None
HFD-fed mice	24.6 ± 0.37	10.6 ± 2.04	76.5 ± 17.3	0.5 ± 0.04	0.5 ± 0.04	26.4 ± 0.48	15 ± 1 (twice increase vs CD)	177.2 ± 13.1	123.0 ± 9.3	53.1 ± 4.7	116.7 ± 5.6	3710 ± 452.4 (4.5 times increase)	387.5 ± 40.0 (1.8 times increase vs CD)	Mild
Obese mice ^a	18 ± 0.5 ²⁵	18 ± 0.5 ²⁵	200 vs 125 ⁶	2 times increase vs lean mice ³⁶	2 times increase vs lean mice ³⁶	>30 [in humans] ⁶⁰	28 ± 2.5 ²⁵ , 5-6 times increase vs lean mice ³⁶	200 ²⁵	50 ³⁵	60-100 ³⁵	140 ²⁵	4-6 times higher (human) ^{61,62}	1.5 to 4 times versus lean controls ⁶³ , 2000 pg/mL ⁶⁴	Strong ²⁵
ob/ob mice ^b	45 ± 3.6 ¹⁸ to 53 ¹⁹	39.8 ²⁰	175 ²⁰	0.7-1.5 ^{21,22}	0.7-1.5 ^{21,22}	NA	8-10 times increase (F4/80 mRNA expression) vs lep ^{ob} -heterozygous mice ²¹	134 ± 10 ²⁴	107 ± 11 ²⁴	NA	57.3 ± 3 ²⁴	NA	5000 ²¹	Strong ²³
ApoE ^{-/-} mice ^d								600 ²⁶	NA	NA	150 ²⁶			

Notes: If applicable data are shown as mean ± SEM.

^aDue to data from literature according to indicated reference, only diet-induced obesity was taken in account.

^bGenetically induced obesity model due to complete deficiency of leptin signalling.

^cEquivalent to 12-wk feeding.

^dUsed for positive control for serum lipids only.

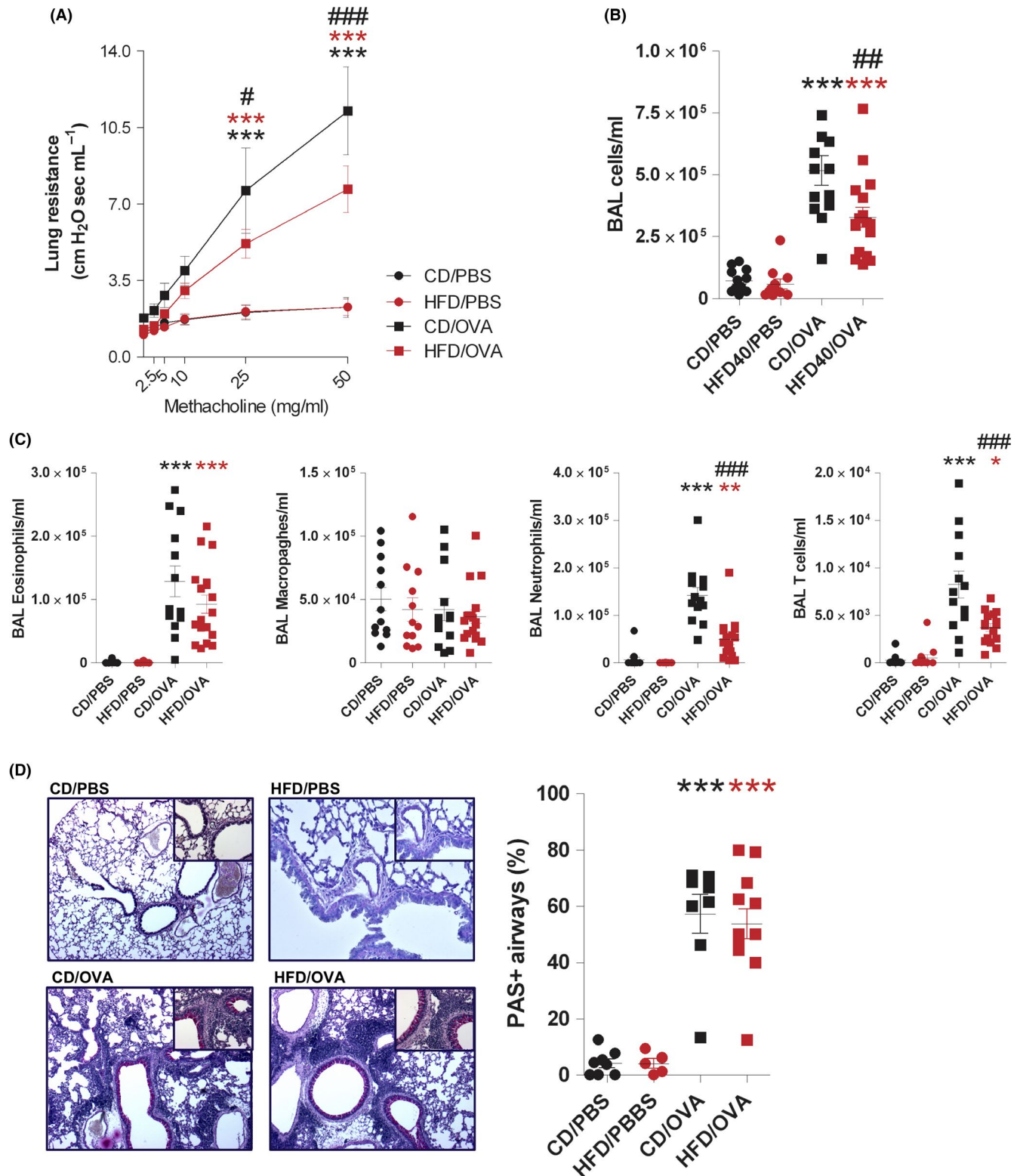
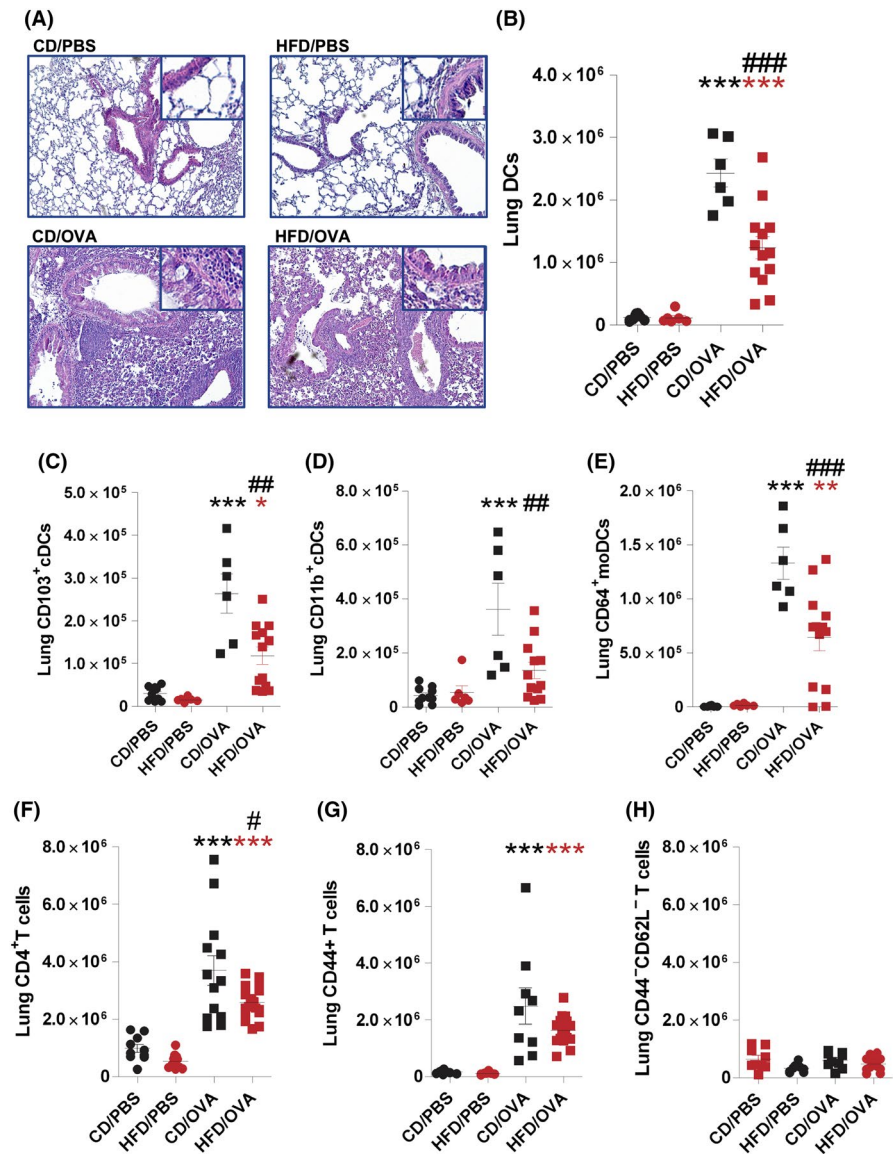


FIGURE 2 Short-term HFD feeding reduces AHR and airway inflammation. A, Methacholine-induced AHR in PBS (circles) or OVA-treated (squares) mice fed with either HFD (red) or CD (black); $n = 8$ – 16 out of 6 independent experiments, 2-way ANOVA/Bonferroni. B, Total number of cells in bronchoalveolar fluid (BAL) from PBS or OVA-treated mice fed with either HFD or CD; $n = 11$ – 17 out of 3 independent experiments, ANOVA/Tukey's. C, Numbers of eosinophils, macrophages, neutrophils and CD4⁺ T cells (from left to right) in BAL of mice fed with either HFD or CD; $n = 11$ – 17 out of 6 independent experiments, ANOVA/Tukey's. D, Histologic examination (PAS staining; 100×) of lung tissue from CD/PBS, CD/OVA, HFD/PBS and HFD/OVA mice including digitally zoomed areas (upper right); (right) frequency of PAS-positive airways in the different treatment groups, all airways within a given histologic sample were considered; $n = 5$ – 11 from 3 independent experiments, ANOVA/Tukey's. Values shown are the mean $\pm$ SEM, asterisks indicate significant differences between the PBS and OVA treatment groups; the pound sign (#) indicates significant differences between CD and HFD OVA-treated groups. * or # $P < 0.05$; ** or ## $P < 0.01$; *** or ### $P < 0.001$

FIGURE 3 Short-term HFD feeding is associated with decreased numbers of CD103⁺ and CD11b⁺ conventional DC and monocyte-derived (mo)DCs as well as total T cells. A, Histologic examination (H&E staining; 100×) of lung tissue from CD/PBS, CD/OVA, HFD/PBS and HFD/OVA mice including digitally zoomed areas (upper right). B, Total numbers of pulmonary CD11c⁺MHC-II⁺ DCs, (C-E) numbers of (C) CD103⁺ conventional (c)DCs, (D) CD11b⁺ cDCs and (E) CD11b⁺CD64⁺ monocyte-derived (mo) DCs. F-H, Numbers of (F) CD3⁺CD4⁺ T cells, (G) CD3⁺CD4⁺CD44⁺CD62L⁻ effector T cells and (H) CD3⁺CD4⁺CD44⁻CD62L⁺ naïve T cells in PBS or OVA-treated CD/OVA and HFD/OVA mice. (B-H) n = 8-19 out of 6 independent experiments, ANOVA/Tukey's. Values shown are the mean ± SEM, Asterisks show significant differences between PBS and OVA treatment groups; pound sign (#) indicates significant differences between CD and HFD OVA-treated groups. * or # indicates $P < 0.05$; ** or ## $P < 0.01$; *** or ### $P < 0.001$



This analysis recapitulated that HFD/OVA mice showed a significant reduction in airway resistance compared to CD/OVA mice (Figure S3B). Furthermore, we found significantly more immune cells in the airways of CD/OVA and HFD/OVA than in PBS controls. However, the immune cell accumulation was significantly lower in HFD/OVA compared to CD/OVA mice (Figure 2B), mainly due to a marked and significant reduction of neutrophils and CD4⁺ T cells in HFD/OVA compared to CD/OVA, whereas eosinophil and macrophage numbers were similar in both groups (Figure 2C). In histological analysis, we detected in both HFD/OVA and CD/OVA mice around 60% of the airways showing PAS-positive reactions, suggesting comparable mucus production in the two groups (Figure 2D). Altogether, our data show that short-term HFD feeding is associated with reduced AHR and airway inflammation but has no impact on mucus production.

To rule out that diet-specific features rather than the metabolic challenge itself are involved, we applied another diet containing 60% energy from fat (HFD60). Mice receiving HFD60 for a short period demonstrated an increased body weight similarly to the otherwise used HFD (Figure S4A). In addition, upon OVA-induced

allergic asthma, HFD60-fed mice presented with an attenuation of AHR as well as decreases in BAL neutrophils and T cells similar to the otherwise used short-term HFD feeding (Figure S4B-D). These data suggest that a mild increase in body weight does not, per se, increase the severity of allergic asthma and induce a pulmonary compression or other mechanical interferences with thorax mobility. Instead, the observed decrease in asthma severity may be due to modulations of the inflammatory recruitment and/or functions of immune cells.

3.3 | Short-term HFD feeding is associated with decreased numbers of CD103⁺ cDCs, CD11b⁺ cDCs and moDCs in the lungs of OVA-treated mice

H&E staining of lung sections from all treatment groups revealed a strong inflammatory cell infiltration into the lung tissue upon OVA-induced allergic asthma (Figure 3A). Furthermore, we observed a similar and significant increase in total pulmonary cell numbers, lung neutrophils,

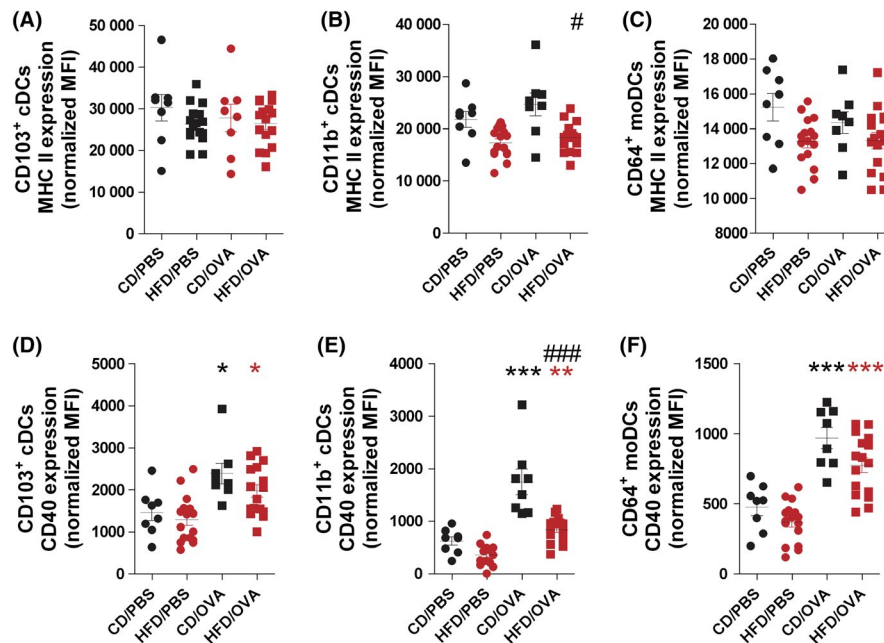


FIGURE 4 Changes of MHC-II and CD40 expression in lung DC subtypes in response to short-term HFD feeding. MHC-II (A-C) and CD40 (D-F) surface expression displayed as mean fluorescence intensity (MFI) in (A,D) CD103⁺ cDCs, (B,E) CD11b⁺ cDCs and (C,F) CD64⁺ moDCs. (A-D) $n = 8-16$ out of 2 independent experiments, ANOVA/Tukey's; values shown are the mean $\pm$ SEM, Asterisks show significant differences between PBS and OVA treatment groups; pound sign (#) indicates significant differences between CD and HFD OVA-treated groups. * or # indicates $P < 0.05$; ** or ## $P < 0.01$; *** or ### $P < 0.001$

eosinophils and macrophages upon OVA treatment in the CD and HFD groups when compared to PBS controls (Figure S5A-D). In contrast, we observed significantly lower numbers of all lung DCs (Figure 3B) of multiple subtypes, that is CD103⁺, CD11b⁺ cDCs and CD11b⁺CD64⁺ moDC in HFD/OVA as compared to CD/OVA (Figure 3C-E). Taken together, our data suggest that HFD feeding decreases the recruitment of cDCs and moDCs but not of eosinophils, alveolar macrophages and neutrophils to the lungs of OVA-challenged mice.

3.4 | Short-term HFD feeding results in reduced recruitment of CD4⁺ T cells to the lung tissue

In both OVA-treated groups, CD4⁺ T-cell numbers increased compared to the respective PBS control mice. However, the numbers of T cells were significantly lower in HFD/OVA as compared with CD/OVA mice (Figure 3F). Surprisingly, the numbers of CD44⁺CD62L⁺ naïve and CD44⁺CD62L⁻ effector T cells were similar in HFD/OVA and CD/OVA (Figure 3G,H) suggesting rather a general decrease in T-cell accumulation in HFD mice than a specific reduction in effector T cells.

3.5 | Correlation analysis of most relevant parameters indicates pulmonary DCs and T cells to be involved in decreasing AHR and airway inflammation in short-term HFD feeding

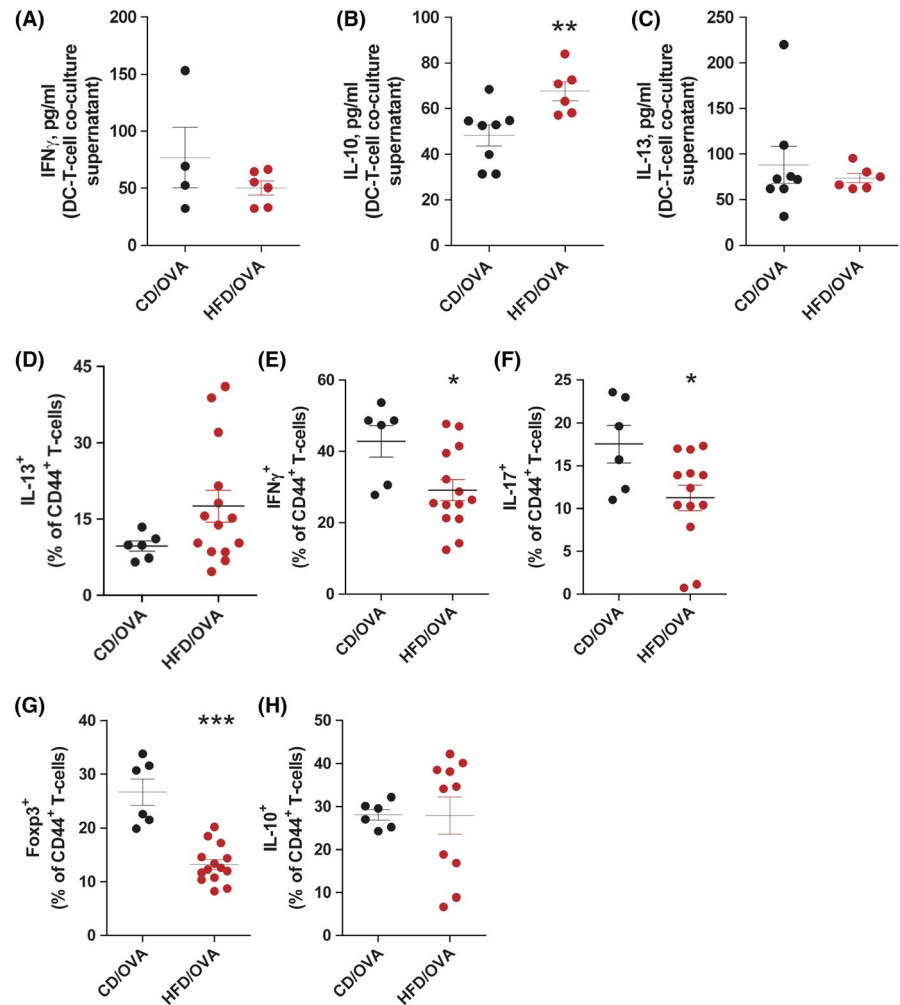
We considered, for deeper statistical analysis, the final body weight, VAT weight, BMI, serum leptin and serum insulin, as well as VAT-macrophage frequency, as most relevant metabolic parameters

and BAL cell number, BAL neutrophils and T cells as well as lung DCs and T cells as most relevant immunological parameters (Figure S6A-B). A Spearman's correlation analysis revealed that the metabolic parameters body weight, VAT weight, BMI, serum leptin and serum insulin were positively correlated to each other but negatively correlated to lung DCs and T-cell numbers (Figure S6C). The airway hyperresponsiveness of the lung at 50 mg/mL methacholine (AHR) negatively correlated to the metabolic variables leptin and insulin. In contrast, AHR positively correlated to DCs and T cells in lung tissue. In summary, the correlation data indicate that the increases in metabolic parameters collectively have a reducing effect on AHR as central asthma readout in our model. In addition, simple linear regression models show that the most suitable parameters to predict AHR were lung DCs, T cells and BAL cell numbers, followed by BAL T cells and neutrophils (data not shown). In conclusion, this correlation analysis suggests that DC and T cells are central for the alterations observed in AHR and airway inflammation of metabolically challenged mice.

3.6 | Short-term HFD feeding results in reduced MHC-II and CD40 expression on pulmonary CD11b⁺ cDCs

It is well documented that MHC-II and the costimulatory molecule CD40 are critical for DC-dependent T-cell activation.²⁷ To further assess a role of the diet on DC function, we first analysed MHC-II expression on naïve lung cDCs and moDCs from HFD/OVA and CD/OVA animals upon ex vivo OVA stimulation. Interestingly, CD11b⁺

FIGURE 5 Short-term HFD feeding suppresses the differentiation of Th1, Th17 and Treg but not of Th2 cells. A-C, Concentration of (A) IFN- γ , (B) IL-10 and (C) IL-13 in co-culture supernatants of sorted lung CD11c⁺MHC-II⁺ DCs from OVA-induced CD or HFD mice with CFSE-labelled OVA-specific transgenic T cells; (A) $n = 4-6$ out of 2 independent experiments, (B-C) $n = 6-8$ out of 2-3 independent experiments, (A-C) Mann-Whitney U test. (D-H) Frequency of (D) IL-13⁺, (E) IFN- γ ⁺, (F) IL-17⁺, (G) Foxp3⁺ or (H) IL-10⁺ T cells within CD3⁺CD4⁺CD44⁺ effector T cells in CD/OVA and HFD/OVA mice; $n = 6-14$ out of 3 independent experiments, Mann-Whitney U test. Values shown are the mean $\pm$ SEM, asterisks indicate significant differences, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. For (D-H), intracellular cytokines were stained and analysed



cDCs isolated from mice after HFD feeding expressed lower levels of MHC-II at their surface as compared to CD11b⁺ cDCs from CD-fed mice. In contrast, MHC-II expression was unchanged on CD103⁺ cDCs and moDCs (Figure 4A-C). Similar to MHC-II, ex vivo stimulation with OVA led to a significantly lower CD40 surface expression in CD11b⁺ cDCs but not moDCs and CD103⁺ cDCs from allergic HFD-fed as compared to CD-fed mice (Figure 4D-F). CD11b⁺ cDCs play important roles in T-cell proliferation and differentiation and are critical for the induction of maladaptive Th2 immunity in experimental allergic allergy.²⁸ Our findings of reduced MHC-II and CD40 expression on CD11b⁺ cDCs indicate that the reduced allergic phenotype upon short-term HFD feeding may result from an impaired potency of such DCs to activate CD4⁺ T cells.

3.7 | Pulmonary DCs from HFD-fed mice exhibit an impaired potency to drive Th1 and Th17 cell differentiation

To directly assess the impact of HFD feeding on pulmonary DC-driven T-cell activation, we co-cultured sorted pulmonary DCs from CD/OVA or HFD/OVA mice with CFSE-labelled OVA-specific TCR

transgenic OTII cells and evaluated T-cell proliferation and polarization by flow cytometry (Figure S7A). First, we observed that neither the frequency of T cells undergoing proliferation at day 4, nor T-cell survival at day 9 were affected by HFD feeding as compared to CD (Figure S7B-C). Second, cytokine analysis of co-culture supernatants revealed that, although the concentrations were very low, IL-10 concentration in HFD/OVA was higher than in CD/OVA co-culture, while IFN- γ and IL-13 concentrations were similar in HFD/OVA or CD/OVA co-cultures (Figure 5A-C). The concentration of IL-17 in co-culture supernatant was too low to be detected. However, these data indicate that Th-associated cytokine production is modulated by the diet in allergic mice.

To further test these observations, we stimulated pulmonary cells from HFD/OVA and CD/OVA isolated 24 hours after the final OVA challenge ex vivo with PMA and ionomycin and performed intracellular cytokine staining of CD4⁺CD44⁺ T cells. In line with the unchanged IL-13 concentration in DC-T-cell co-cultures, the frequency of IL-13⁺ T cells was similar in HFD/OVA and CD/OVA mice (Figure 5D). In contrast, the frequency of IFN- γ ⁺ T cells was lower in mice treated with HFD/OVA than with CD/OVA, mirroring the lower IFN- γ supernatant concentration observed in vitro (Figure 5A vs E).

In addition, the frequencies of IL-17⁺ and Foxp3⁺ regulatory T cells (Tregs) were lower in HFD/OVA than CD/OVA mice (Figure 5F,G), while no difference could be observed in the frequency of IL-10⁺ T cells in the two groups (Figure 5H).

These data demonstrate that short-term HFD feeding selectively protects, in part, from DC-driven Th1 and Th17 but not from Th2 differentiation and reduces Treg formation. Mechanistically, the reduced MHC-II and CD40 expression in CD11b⁺ cDCs upon HFD may trigger these changes in T-cell differentiation.

4 | DISCUSSION

The goal of our study was to assess a potential impact of short-term HFD feeding on the development of allergic asthma. For this purpose, we induced an experimental allergic asthma in short-term HFD-fed mice showing a mild body weight gain. We found that such short-term HFD feeding protected from the development of allergy-driven AHR, neutrophilic airway inflammation, accumulation of CD11b⁺ cDCs and mDCs and consequently maladaptive Th1 and Th17 immune responses.

Consistent with previously reported models of diet-induced body weight gain associated with robust metabolic alterations,^{15,29} the HFD mice developed a mild increase in body weight, mainly due to an expansion of VAT mass, together with an accumulation of pro-inflammatory VAT macrophages, development of mild insulinaemia and leptinaemia, hypercholesterolaemia and an increased storage of hepatic lipids. These observations point towards the initiation of pro-inflammatory alterations,³⁰ although the levels of leptin in HFD mice were still below levels reported for obese mouse models.^{31,32} Furthermore, the microbiota changed towards an increased abundance of Proteobacteria and a decreased abundance of Firmicutes, in line with previously reported microbiota changes in the context of HFD-associated systemic inflammation.³³ Reports about mouse models of obesity show a huge variety of changes in body weight and parameters of the metabolic system. There is no clear definition of 'obesity' or 'overweight' in mice, so the manifestation of alterations with respect to body weight and metabolic changes should be explicitly stated and thoroughly compared with matching experimental models.³⁴ For this purpose, we compared the metabolic parameters from our model with reports in the literature about obesity models including genetically induced obesity and atherosclerosis models^{18-24,26,35} as well as a model of female obesity caused by HFD feeding and thermoneutral housing.^{25,26} This comparison revealed that changes in our experimental model of short-term HFD-fed mice fall within the ranges seen in CD-fed mice and mice commonly reported to be obese. Altogether, these observations underline that the short-term HFD feeding induced a mild increase in body weight accompanied by the initiation of metabolic changes, mimicking an early phase of metabolic challenge without reaching a morbid obesity phenotype. Thus, this model rather reflects changes described in overweight individuals.^{12,13} However, the current study exhibit limitations, such as the lack of a deeper characterization

of environmental factors, a missing functional elucidation of the impact of microbiome alterations and the lack of other aeroallergens, such as house dust mite, which is also in humans responsible for allergic lung diseases. Future studies are needed to address these issues. In addition, a positive group displaying obesity, which would be handled in parallel within the reported model, is desirable. However, female C57BL/6J mice are known to be resistant to HFD-induced obesity.^{25,36} The known models of obesity in female C57BL/6J mice, such as the discussed ob/ob mice¹⁸⁻²⁴ or female mice housed in thermoneutral conditions,^{25,26} have altered immune and adipokine-associated mechanisms, which are relevant for our model of allergic asthma, and thus are not considered appropriate positive controls.

We found that short-term HFD feeding in OVA mice reduced neither airway nor tissue eosinophil numbers. In contrast, in obese mice (>35 g body weight), experimental allergen- or diesel particulate-induced allergic asthma was associated with a lower airway eosinophilia,^{14,37-39} but a higher recruitment of tissue-associated eosinophils as compared to lean mice.¹⁵ In addition, HFD-driven increases in leptin concentration impaired the recruitment of eosinophils into the airways.⁴⁰ Therefore, our data suggest that the changes in leptin concentration in our HFD/OVA group are not sufficient to modulate pulmonary and airway eosinophilia.

Our study demonstrates that short-term HFD feeding leads to a marked reduction of AHR in HFD/OVA compared to CD/OVA mice. However, PBS controls in both feeding groups did not augment AHR upon methacholine provocation indicating that short-term HFD feeding and mild body weight gain alone are not sufficient to induce a non-allergic form of asthma as opposed to obesity.^{6,7} While hyperinsulinaemia can aggravate the severity of AHR in obesity models, we found a decreased maximal response to methacholine upon HFD as compared with CD. In line, a broad statistical analysis revealed a negative correlation between AHR and insulin, but not body weight, supporting the idea that short-term HFD feeding-associated hyperinsulinaemia may act differently on the airways as compared to hyperinsulinaemia in obesity. Furthermore, we saw no change in mucus production, which is frequently associated with obesity-related hyperinsulinaemia and increased levels of adipokines.⁴¹ In addition, the correlation matrix suggested that the attenuation of AHR and airway inflammation is a consequence of the induced metabolic alterations acting via changes in the lung DCs and T-cell compartment. Altogether, our findings demonstrate that short-term HFD feeding-associated changes have a distinct impact on the development of allergen-driven airway reactivity and inflammation. Finally, since a change in the composition and energy of the HFD feeding did not translate into changes in the allergic asthma phenotype, our data indicate that the protective impact is independent of nutrient specificities of the diet.

Compared to CD, allergic HFD mice showed a marked decrease in airway neutrophils, but were equally abundant in the lung tissue of the two groups, suggesting that HFD controls the recruitment of neutrophils into the airways but not into the lung tissue. The mechanisms involved in the translocation of neutrophils from the lung tissue into the

alveolar space are still incompletely understood. However, IL-17, partly secreted by Th17 cells, is a known trigger of neutrophilic inflammation in experimental OVA-induced and human allergic asthma,⁴² and Th17 immunity has been associated with the severity of allergic asthma, through a synergism of IL-17 and IL-13.^{43,44} Interestingly, we observed a decreased T-cell infiltration into the airways in HFD mice, together with a decreased frequency of pulmonary Th17 cells, which may explain the observed reduction in airway neutrophils. Alternatively, obesity, in acute lung injury, suppresses neutrophil chemotaxis through the downregulation of chemotactic receptors such as CXCR2.⁴⁵ Also, leptin acts either as a neutrophil chemoattractant, directly or through the release of TNF- α and CXCL1 by peritoneal cells,⁴⁶ or as a suppressor of C5a- or FMLP-induced chemoattraction.⁴⁷ Short-term HFD-fed mice in our model display dyslipidaemia. Dyslipidaemia is reported to reduce neutrophil recruitment to the airspace in LPS- or bacteria-induced lung injury^{35,48}; Accordingly, changes in serum lipids may also be involved in the decrease in airway neutrophils of short-term HFD-fed mice. Nonetheless, it remains uncertain why the neutrophils reacted differently in airways and lung tissue in this model. Other factors, such as an elevation in blood neutrophils of HFD mice or an impact of experimental procedures, may also be relevant for the apparent discrepancy and cannot fully rule out.^{45,49}

A deeper characterization of lung immune cells revealed that cDCs, moDCs and T cells were recruited in lower numbers to the lungs of allergic HFD-fed mice as compared to CD mice. Interestingly, pulmonary DCs from CD or HFD-fed mice were equally potent to expand T cells with Th2 polarization profile, which depends on CD86/CD80 costimulation,⁵⁰ suggesting the CD86/CD80 axis not to be affected in our model. In contrast, CD11b⁺ cDCs express lower numbers of MHC-II and the costimulatory molecule CD40 upon HFD feeding compared to CD. Importantly, CD40 is necessary for DC functions^{27,51} and the initiation of antigen-specific T-cell activation and polarization towards Th1, Th17 and Tregs.^{27,52} Moreover, increases in CD40 ligand have been linked to allergic asthma in humans and correlated with obesity-associated inflammation.^{53,54} While leptin positively regulates CD40 expression in mouse bone marrow DCs and the DC cell line DC2.4,⁵⁵ our data suggest that this is not necessarily the case in pulmonary DC subsets. The finding of impaired DC-T-cell crosstalk suggests that CD40 is a target for short-term HFD feeding and may provide a functional link to the protection from allergic asthma development. In addition, we report a Th2/Th17-associated immune signature *in vivo* characterized by a high frequency of IL-13⁺ and IL-17⁺ T cells, although IFN- γ ⁺ T cells were also present. However, the impact of obesity on IFN- γ ⁺ and Th1 immune response in allergic asthma models is still contradictory.^{14,15,40} Of note, in a model of non-allergic asthma Th1 polarization has been shown to be affected by insulin resistance.⁵⁶ However, our data support the idea that mild body weight gain may favour a weaker IFN- γ ⁺ T-cell frequency together with a lower Th17 activation.

Previous studies related to obesity-associated asthma suggest that AHR develops as a result of adipokine-induced dysregulation of airway contractility, lung fibrosis, changes in collagen and elastin expression, and non-Th2 inflammation.^{40,57-59} In contrast, we found that

short-term HFD feeding-induced metabolic alterations in OVA-driven experimental allergic asthma are associated with protection from AHR development, pulmonary inflammation, altered DC activity and decreased Th1/Th17 differentiation. This may exemplify a link between metabolism and the inflammatory system in terms of chronic disorders and accompanying overweight, although environmental factors other than the diet may also be at play. Future studies, utilizing a recently demonstrated experimental model that allows for induction of severe obesity in female mice,^{25,26} without genetic modification, would allow direct comparison of lean, overweight and obese conditions on the development of allergic asthma. Our findings point towards a complex functional link between metabolism and the development of pulmonary allergy. In fact, future studies are needed to assess whether this protective effect also applies to overweight humans.

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CONFLICT OF INTEREST

The authors have no actual or potential conflicting interests regarding this article.

AUTHOR CONTRIBUTIONS

TS, AW, FE, KQ, JD, SDe, ZA, IL, SDi, CS, JK and YL participated in generation, analysis and interpretation of data. AS, AK, ZA, JK, MEMF and TV provided materials and technical support and participated in critical review of the manuscript. TS and YL participated in the conception and design of the study and wrote the manuscript. All authors have reviewed the manuscript and approved the final version.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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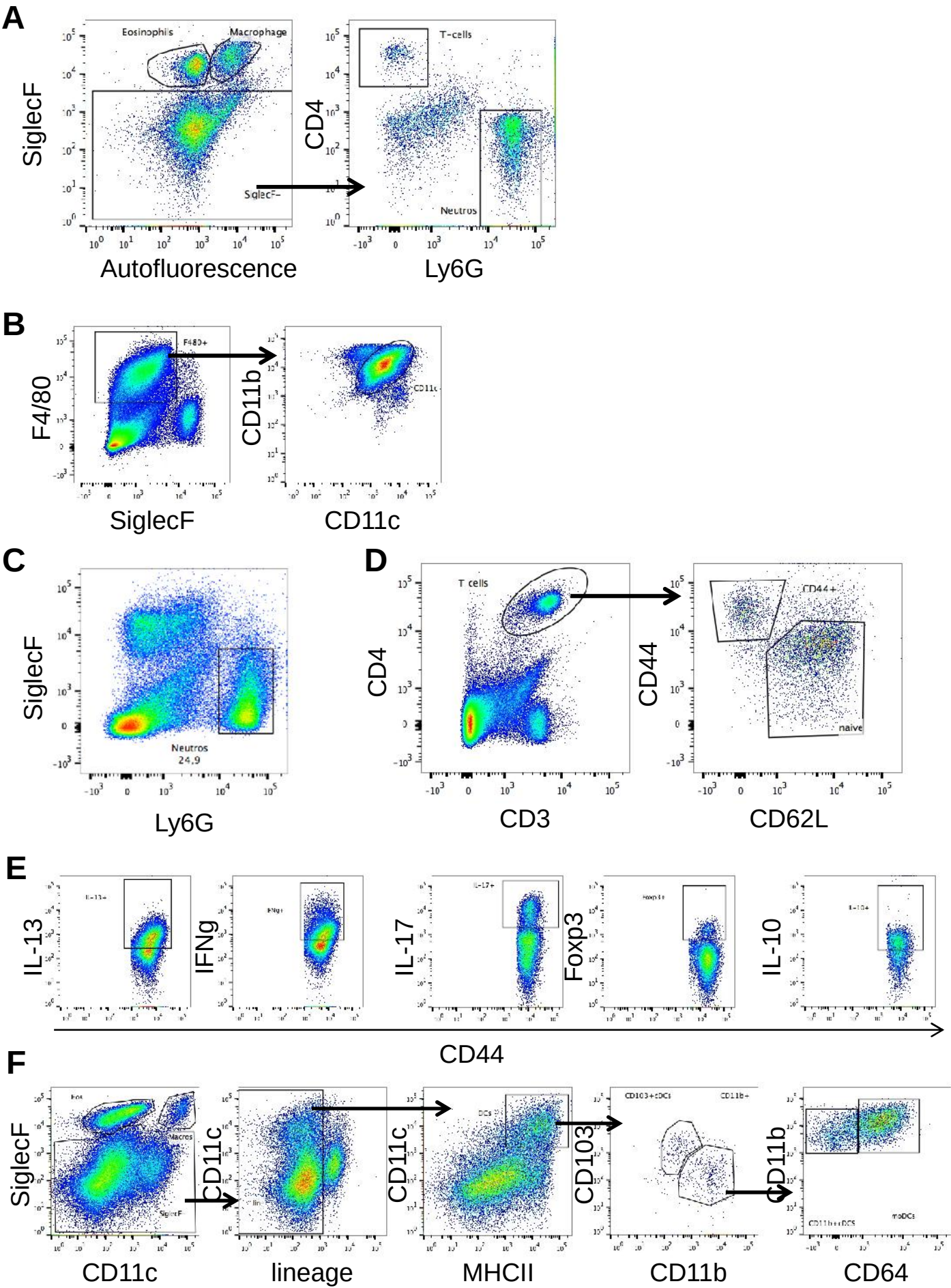
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SUPPORTING INFORMATION

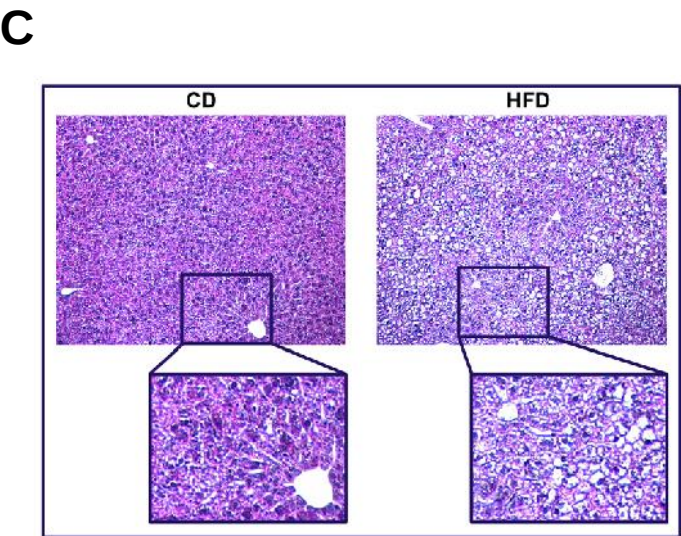
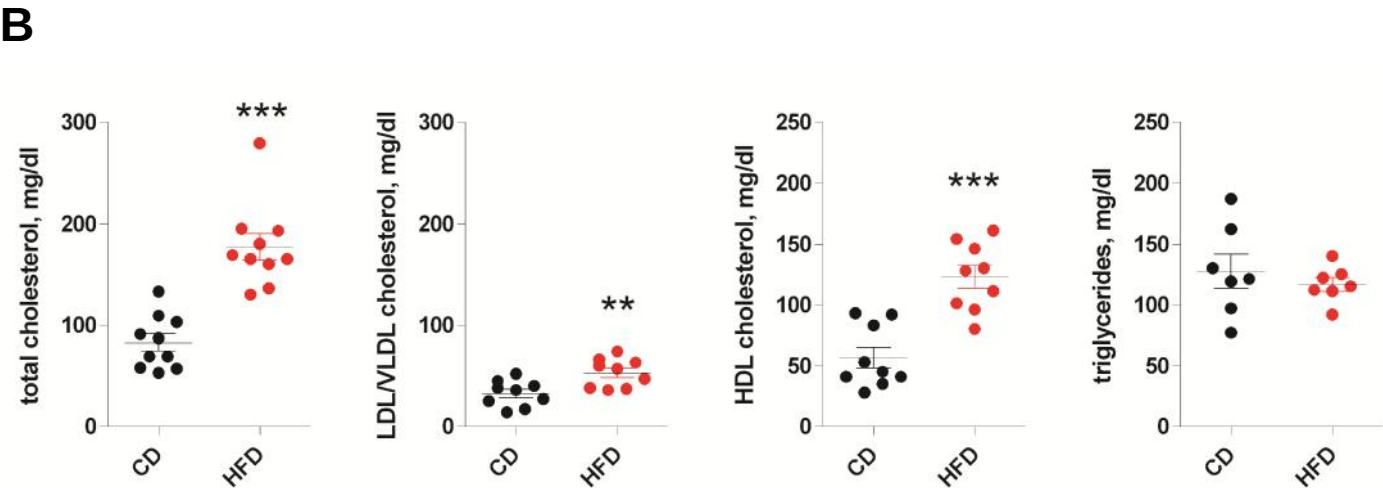
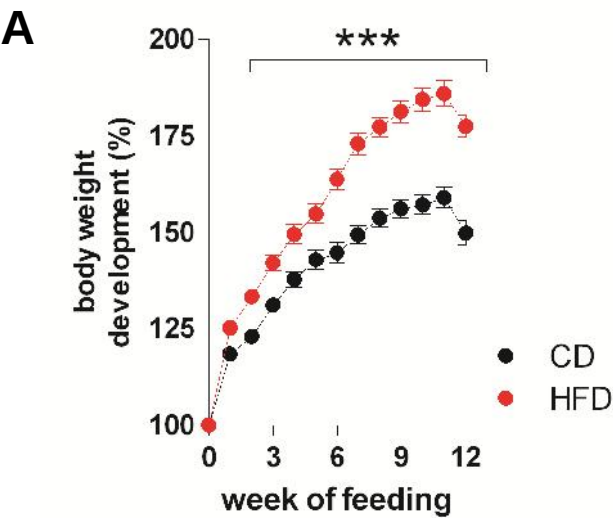
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary Figure 1

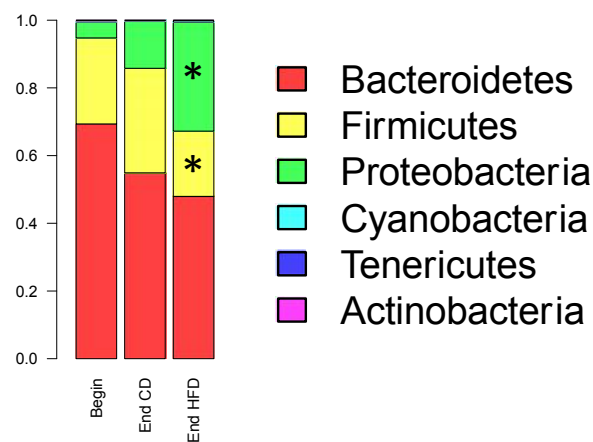


Supplementary Figure 2

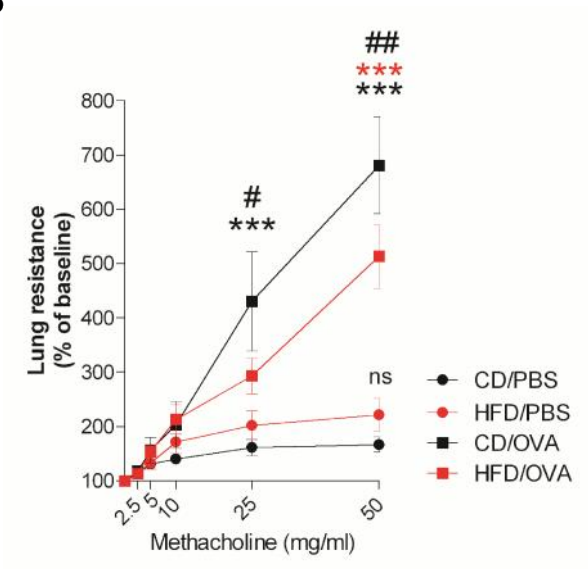


Supplementary Figure 3

A

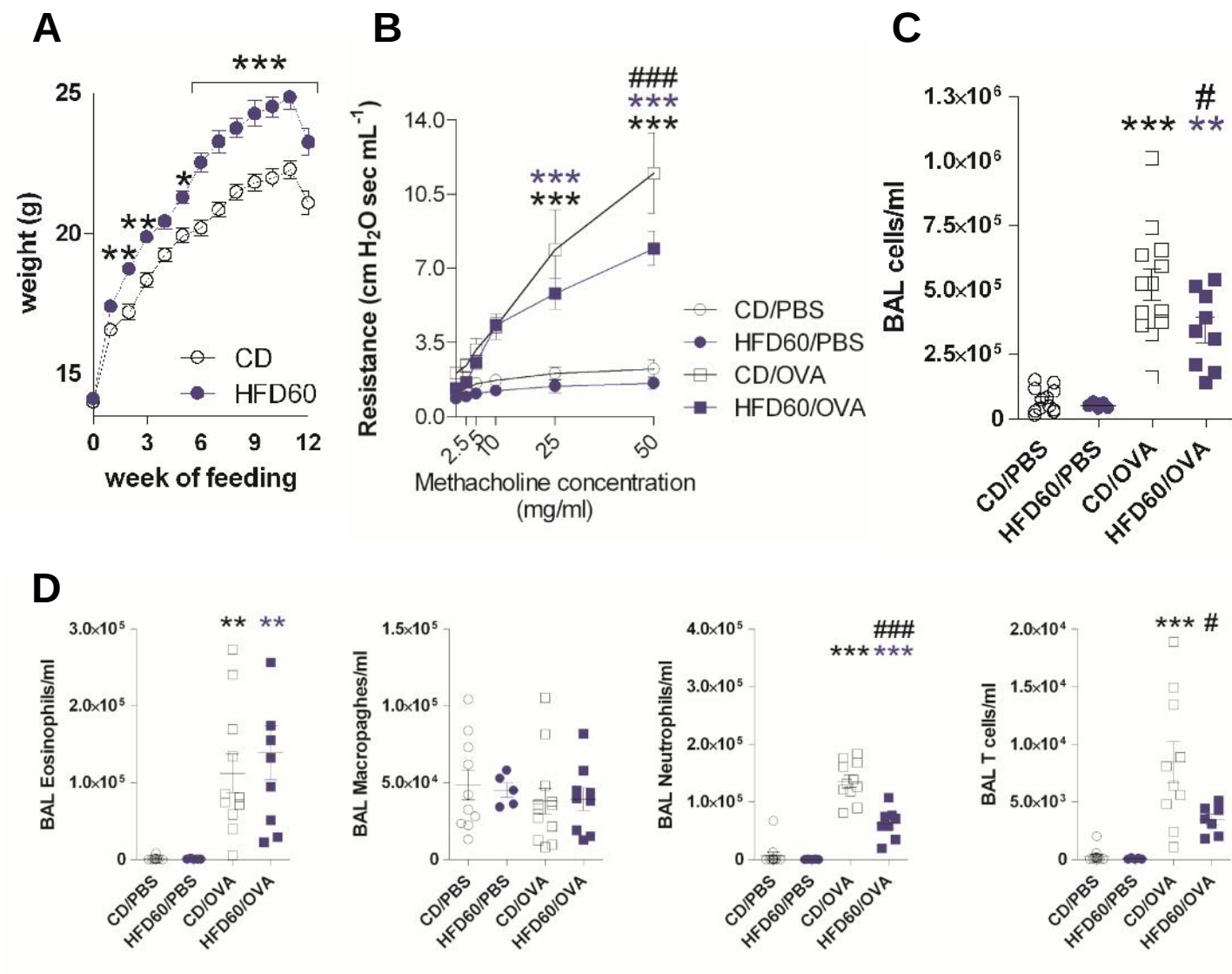


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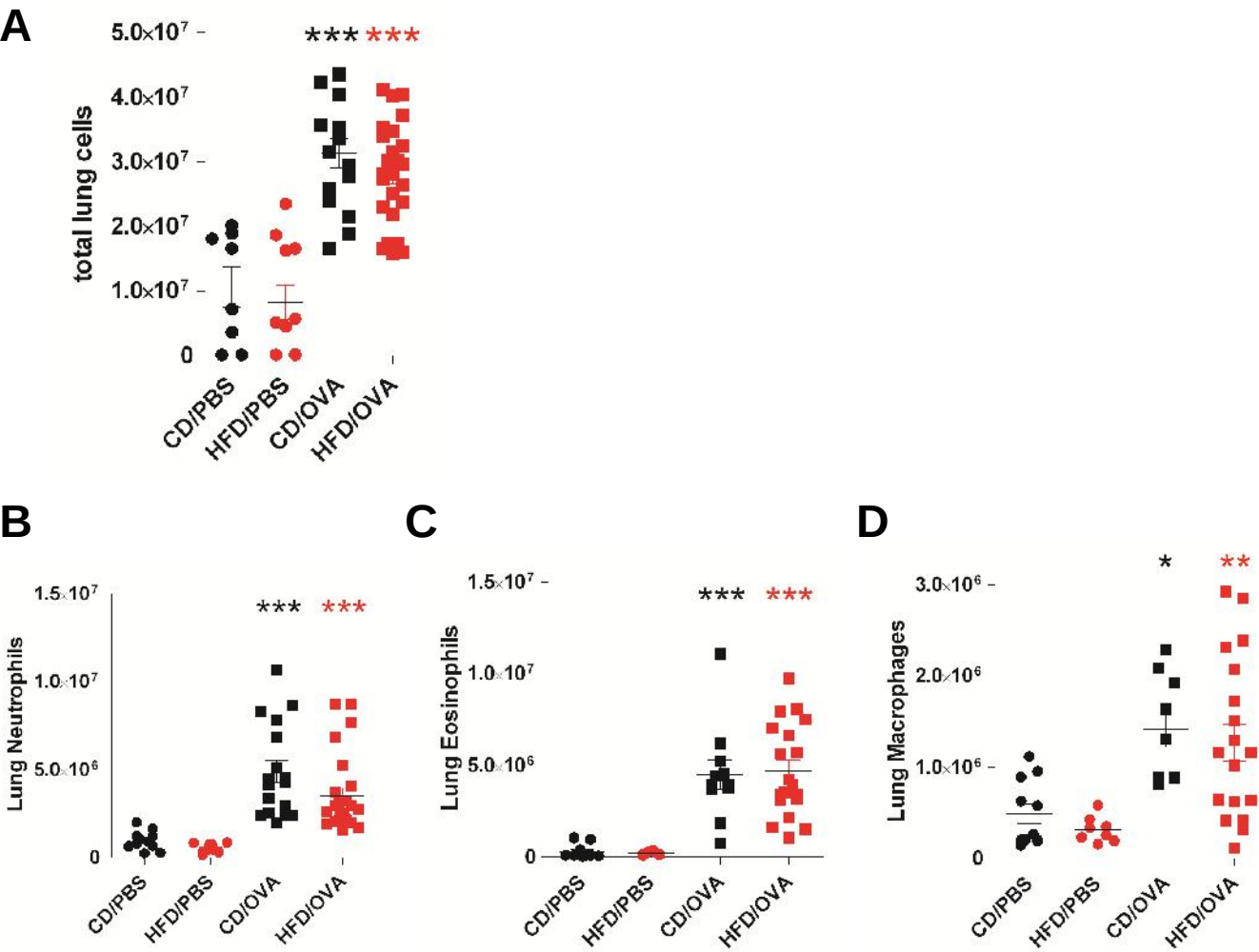


Supplementary Figure 4

HFD60 feeding



Supplementary Figure 5



Supplementary Figure 6

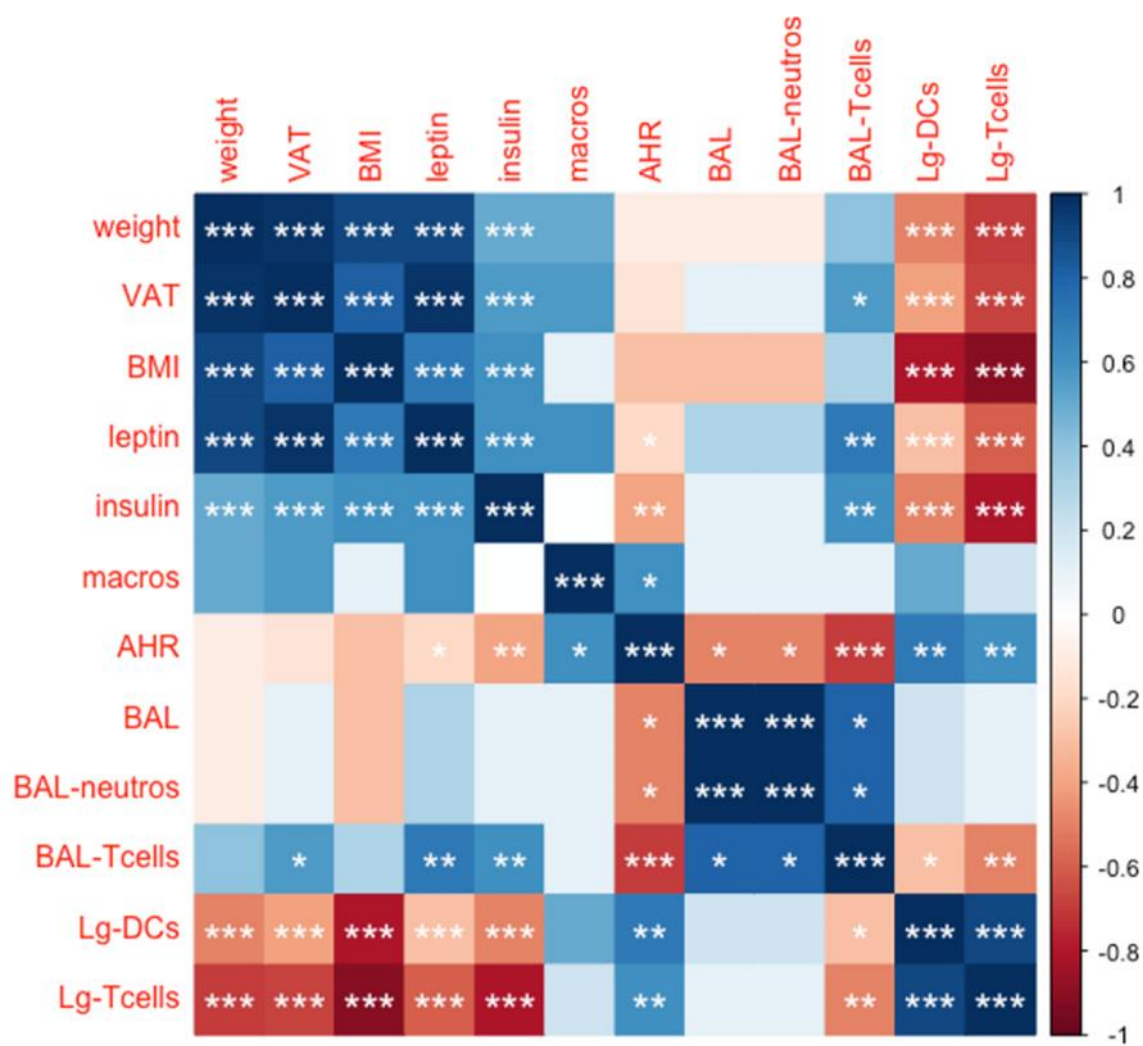
A

	body weight (g)	weight VAT (g)	BMI (100g/cm2)	leptin (pg/ml)	insulin (pg/ml)	Macro- phages (% of VAT cells)	AHR (Resis- tance 50 mg/ml MC)	BAL cells/ml	BAL neutro- phils/ml	BAL Tcells/ml	number of Lg-DCs	number of Lg-Tcells
Min.	17,4	0,05	20,12	319,9	27,8	1,4	0,9967	14286	0	0	53760	35964
1st Qu.	20,65	0,115	22,47	533,2	189,5	7,17	2,2357	82309	169,6	113,8	104310	612352
Median	23,65	0,4	24,34	1483,8	320,1	12,4	5,3088	210417	17174,3	1989,4	335580	1089000
Mean	22,96	0,3589	24,86	2272	315,4	10,98	5,9625	290622	39123,7	3113,4	822177	1427727
3rd Qu.	24,9	0,55	27,42	3987,1	426,9	14,32	8,7603	410161	56190,8	4054,3	1365630	2382750
Max.	28,8	1	31,24	6485,3	700,5	19,2	19,3143	1457944	255840	24648	2786400	5751000

B

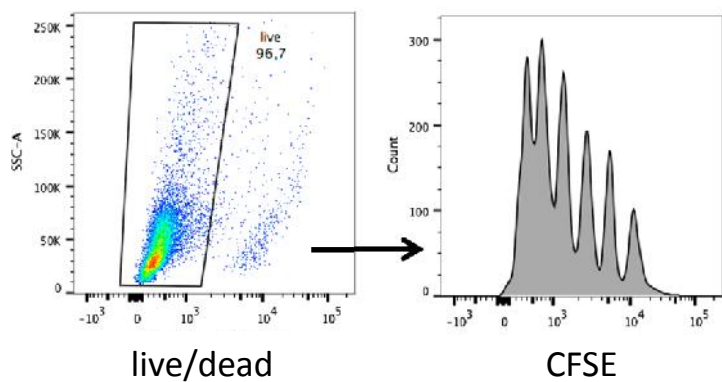


C

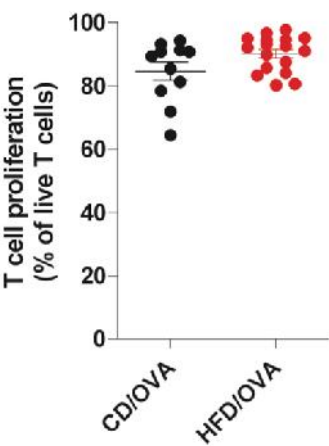


Supplementary Figure 7

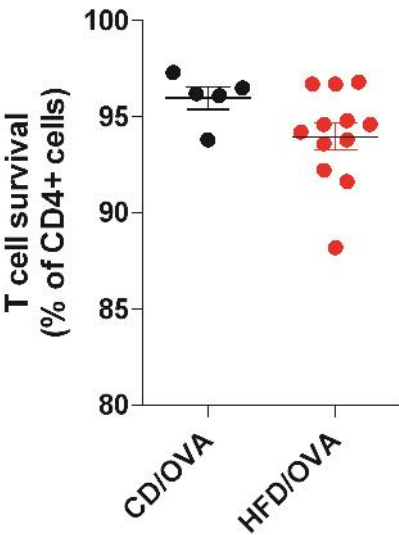
A



B



C



Supplementary methods

Alternative high fat diet

Besides the chow (CD) and the high fat diet, a second dietary intervention (HFD60) was used. In contrast to HFD, HFD60 contained 60 kJ% from fat, 21 kJ% from carbohydrates, and 19 kJ% from proteins (Ssniff E15741-34, EF acc. D12492 (II) mod.). HFD60 contained more energy (60 kJ% vs. 42 kJ%) from fat than HFD, 20% less calories from carbohydrates (HFD60), and had ten times less cholesterol (290 mg/kg vs. 2.071 m/kg).

OTII mice were purchased from Charles River and held in internal breeding.

Antibodies

The following antibodies were used. Brilliant Violet (BV) 421- or PE-labeled Abs against SiglecF (E50-2440), V450-labeled Ab against CD4 (RM4-5), and V450- or allophycocyanin (APC)-labeled Abs against Ly6G (1A8) from BD Biosciences. EFluor (eF)450-labeled Abs against CD19 (1D3), CD3e (145-2C11), CD49b (DX5), FITC-labeled Ab against CD11c, APC-labeled Ab against CD11c (N418), Foxp3 (FJK-16s), and CD40 (IC10), Per-CP–Cy5.5–labeled Ab against CD3 (17A2) and PE-cyanine (Cy)7-labeled Ab against CD4 (RM4-5), IL-13 (eBio13A), IL-17 (eBio17B7), IL-10 (JES5-16E3), and F4/80 (BM8) from eBioscience (Affimetrix). BV510-labeled Ab against CD11b (M1/70), PE-labeled Ab against CD64 (X54-5/7.1), APC-eF780–labeled Ab against MHC class II (M5/144.15.2), IFN γ (XMG1.2), and CD62L (MEL-14), BV421-labeled Ab against CD44 (IM7), and Peridinin chlorophyll protein–cyanine 5.5 (Per-CP–Cy5.5)-labeled Ab against CD103 (2E7) from BioLegend. All flow cytometric investigations were performed using a BD LSRII or an ARIA III flow cytometer.

Flow cytometric analyses and gating strategies

BAL cells were identified using surface markers for eosinophils (Siglec F), macrophages (Siglec F/Autofluorescence), T cells (Siglec F/CD4), and neutrophils (Siglec F/Ly6G)

(**Suppl. Figure 1A**). In visceral adipose tissue, macrophages were identified as F4/80⁺CD11c⁺ (**Suppl. Figure 1B**). In lung tissues, neutrophils were identified as Siglec F⁻ Ly6G⁺ (**Suppl. Figure 1C**). T cells were defined as CD3⁺CD4⁺ cells. Naïve and effector T cells could be discriminated based on their CD62L and CD44 expression (**Suppl. Figure 1D**). The intracellular cytokine staining in CD3⁺CD4⁺CD44⁺ cells included antibodies for IL-13, IFN γ , IL-17, Foxp3, and IL-10. Combinations used were IL-13+IFN γ , IL-17+Foxp3, and IL-10 only (**Suppl. Figure 1E**). Lung cells were further discriminated between eosinophils (SiglecF⁺), macrophages (SiglecF⁺CD11c^{hi}) and SiglecF⁻ cells (**Suppl. Figure 1F**). Subsequently, lineage-positive cells (T cells (CD3e⁺), B cells (CD19⁺), neutrophils (Ly6G⁺), and NK cells (CD49b⁺)) were excluded and CD11c⁺MHCII⁺ cells gated as dendritic cells (DCs). DCs were subdivided into 2 subsets of cells: CD103⁺ conventional (c)DC and CD11b⁺ DCs. The latter was further divided into two cell populations based on the expression of CD64: CD64⁺ monocyte(mo)-derived DCs, and the CD64⁻CD11b⁺ cDCs. In DC subtypes MHC II and CD40 surface expression was determined.

Blood lipid analysis

Blood lipids were detected in plasma samples, collected for all four genotypes. Total cholesterol, high-density lipoprotein (HDL) cholesterol, very low and low -density lipoprotein (VLDL/LDL) cholesterol was measured using the HDL and LDL/VLDL Assay Kit (Biochain). Triglycerides were analyzed using the Triglyceride Colorimetric Assay Kit (Cayman chemical). Lipids were measured according to the manufacture's instruction.

Liver histology

Histological assessment of liver tissue was performed like described previously (1). Briefly, the whole liver was rinsed with phosphate buffered saline and the part for histopathological analysis was fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with H&E using standard procedures.

Intestinal microbiota analysis

Samples were collected directly upon defecation and immediately frozen at -80°C. Microbial genomic DNA was extracted from each fecal sample. DNA extraction, 16s rRNA PCR and gene sequencing for intestinal microbiota analysis was performed as previously described (2).

Sequence processing: Raw reads were demultiplexed with CASAVA version 1.8.2 and merged using fastq_mergepairs from VSEARCH version 2.0.2 with a maximum number of mismatches of 12 and the merged reads (contigs) between 270 and 330 bp long (3). Subsequently, contigs were quality filtered using fastq_filter command (USEARCH version 8.1.1861) with expected numbers of errors set to 0.5. Finally, chimeras were identified by uchime_ref (USEARCH) with the RDP Gold database version 9 as reference database and were removed from the contig set (4). Chimera free sequences were dereplicated (VSEARCH) and clustered into operational taxonomic units (OTUs) at 97% similarity level using UPARSE (as implemented in the USEARCH suite) (5). Taxonomy was assigned to the genus level for the OTUs using SILVA database v123 and Mothur version 1.38.0 with 80% bootstrap support (6).

Statistical analyses: For each individual 8,100 contigs were randomly chosen to normalize for different contig numbers between the individuals. Singletons were removed from the OTU table leaving 588 OTUs for downstream analysis. All statistical analyses were performed using R version 3.2.

T cell proliferation assay and survival

CD4⁺ T cells isolated from spleen by magnetic selection (Miltenyi Biotec) from OTII mice were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE, Molecular Probes) according to manufacturer's instructions. T cell proliferation was assessed by flow cytometry

at day (d)4 and 9. Survival at d9 was checked by incubation with a live/dead viability dye (APC-eF780; eBioscience).

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Supplementary Figures

Suppl. Figure 1

Gating strategies

(A) shows the gating strategy applied for cytometric analysis to identify BAL cells; (B) gating strategy of lineage negative VAT cells to identify F4/80⁺CD11c⁺ macrophages and (C) lung neutrophils; (D) shows the gating strategy applied for cytometric analysis of lung CD4⁺CD3⁺T cells, CD44⁺ effector T cells, and CD44⁻CD62L⁺ naïve T cells; (E) shows the gating of IL-13⁺, IFN γ ⁺, IL-17⁺, Foxp3⁺, and IL-10⁺ effector T cells in the respective staining, and (F) the strategy to identify lung eosinophils, macrophages, and lung DCs with the respective subtypes.

Suppl. Figure 2

Liver histology

(A) Kinetic of relative bodyweight gain (in %) in response to 12 weeks feeding with CD- (black circles) or HFD-fed mice (red circles); n=28-36 out of 5 independent experiments, 2-way ANOVA/Bonferroni. (B) Serum lipids (total cholesterol, LDL/VLDL-cholesterol, HDL-cholesterol, and triglycerides) after 12 weeks feeding with CD- (black circles) or HFD-fed mice (red circles); n=7-10 out of 3 independent experiments; t-test. Values shown are the mean $\pm$ SEM, Asterisks show significant differences between CD and HFD OVA-treated groups. **/** indicate $p < 0.01/0.001$ using t test in normally distributed. (C) Representative pictures of H&E stained (amplification 100x) liver tissue of HFD and CD fed CD/PBS, CD/OVA, HFD/PBS, and HFD/OVA with respective digitally zoomed area. HFD mice showed lipid accumulation in contrast to CD mice.

Suppl. Figure 3

Microbiome analysis

HFD mice showed an altered intestinal microbiota on phyla level; Firmicutes were decreased and Proteobacteria increased as compared to CD feeding, data from 4-8 animals of 1 representative feeding.

Suppl. Figure 4

Alternate HFD feeding

Mice were fed for a period of 12 weeks with an alternate high fat diet (HFD) containing 60% of calories derived from fat (HFD60). (A) HFD60 mice displayed a higher bodyweight during the feeding. (B) AHR was lower in HFD60/OVA mice as compared to CD/OVA mice. In both diets without allergen exposure (PBS controls) AHR was low. (C-D) Cells in bronchoalveolar lavage (BAL) were enumerated as indicator for airway inflammation, HFD60/OVA mice showed less BAL cells than CD/OVA. In addition, BAL T cells and neutrophils were lower in HFD60/OVA as compared to CD/OVA1; n=5-13 out of 2 independent experiments. Values shown are the mean $\pm$ SEM, Asterisks show significant differences between PBS and OVA treatment groups; Pound sign (#) indicates significant differences between CD and HFD OVA-treated groups. * or # indicate $p < 0.05$; ** or ## $p < 0.01$; *** or ### $p < 0.001$.

Suppl. Figure 5

Additional lung cell analysis

(A) shows the numbers of total lung cells in CD/PBS, CD/OVA, HFD/PBS, and HFD/OVA. Flow cytometric analysis of digested lung tissue to identify (B) lung neutrophils, (C) lung eosinophils, (D) lung alveolar macrophages, and (E) Methacholine-induced AHR in PBS (circles) or OVA-treated (squares) mice fed with either HFD (red) or CD (black) in relation to baseline; n=8-16 out of 6 independent experiments. Values shown are the mean $\pm$ SEM, asterisks show significant differences between PBS and OVA treatment groups; * indicates $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, 2-way ANOVA/Bonferroni.

Suppl. Figure 6

Correlation analysis of most relevant parameters

(A) Summary of most relevant parameters evaluated for correlation. Minimum (Min), maximum (Max), median, mean, first (1st Qu.), and third quartiles (3rd Qu.) are presented for bodyweight, VAT weight, BMI, leptin, insulin, macrophages in VAT, BAL cells, BAL neutrophils, BAL T cells, AHT (lung resistance at 50 mg/ml methacholine), lung DCs, and lung T cells. (B) Plot of most relevant factors. (C) Correlation matrix with most relevant parameters including p-value (* indicates $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$), the color indicates correlation coefficient from -1 (dark blue) to +1 (dark red).

Suppl. Figure 7

T cell proliferation and survival analysis

(A) Gating strategy to analyze (B) proliferation (d4) and (C) survival (d9) of T cells in a co-culture with pulmonary DCs derived from OVA treated CD and HFD B6 mice. Values shown are the mean $\pm$ SEM, n=5-17 from 3 independent experiments.