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Obesity alters pathology and treatment response in inflammatory disease

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Additional information

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Abstract

Decades of work have elucidated cytokine signalling and transcriptional pathways that control T cell differentiation and have led the way to targeted biologic therapies that are effective in a range of autoimmune, allergic and inflammatory diseases. Recent evidence indicates that obesity and metabolic disease can also influence the immune system^{1–7}, although the mechanisms and effects on immunotherapy outcomes remain largely unknown. Here, using two models of atopic dermatitis, we show that lean and obese mice mount markedly different immune responses. Obesity converted the classical type 2 T helper (T_H2)-predominant disease associated with atopic dermatitis to a more severe disease with prominent T_H17 inflammation. We also observed divergent responses to biologic therapies targeting T_H2 cytokines, which robustly protected lean mice but exacerbated disease in obese mice. Single-cell RNA sequencing coupled with genome-wide binding analyses revealed decreased activity of nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) in T_H2 cells from obese mice relative to lean mice. Conditional ablation of PPAR γ in T cells revealed that PPAR γ is required to focus the in vivo T_H response towards a T_H2-predominant state and prevent aberrant non-T_H2 inflammation. Treatment of obese mice with a small-molecule PPAR γ agonist limited development of T_H17 pathology and unlocked therapeutic responsiveness to targeted anti-T_H2 biologic therapies. These studies reveal the effects of obesity on immunological disease and suggest a precision medicine approach to target the immune dysregulation caused by obesity.

Emerging clinical data of multiple immunological diseases including atopy and asthma indicate that obese patients have more severe disease and exhibit resistance to therapies that are effective in lean patients^{8–13}, although the mechanisms underlying these observations remain unclear. To gain mechanistic insights into the immunopathological effects of obesity, we employed a well-characterized mouse model of atopic dermatitis^{14,15} (AD). We challenged obese mice fed high-fat diet (HFD) and lean controls with the vitamin D₃ analogue MC903 to induce AD on the ear (Fig. 1a). The obese mice displayed a markedly increased inflammatory response, evidenced by an approximately twofold to fourfold increase in ear thickness relative to lean mice that received the same MC903 treatment (Fig. 1b). The MC903-treated ears of the obese mice had more severe erythema and scale, hallmarks of dermal inflammation (Fig. 1c). Histological evaluation demonstrated greater expansions of the epidermal and dermal layers in obese mice, along with a marked increase in leukocytic infiltration (Fig. 1d, dashed line). Of note, studies modulating duration of the HFD (Extended Data Fig. 1a–d) and using monogenic models of obesity on normal or HFD (Extended data Fig. 1e–j) suggested that the increased ear thickness and inflammation seen in obesity was at least partially dependent on HFD. Further, we observed a persistent inflammatory effect of obesity, even after weight loss (Extended Data

Fig. 1k–m). Additionally, obesity increased disease severity, including after weight loss, in a second model of AD (Extended Data Fig. 2a–g), involving sensitization to ovalbumin (OVA) followed by serial tape-stripping and exposure to a mixture of OVA and papain (TOP). Finally, this increased inflammatory response was not limited to atopic diseases of the skin, as challenging lean and obese mice in an experimental model of allergic airway disease (ovalbumin sensitization and challenge) yielded increased cellular infiltration across multiple immune subsets in the bronchial–alveolar lavage fluid of obese mice and increased CD4⁺ and CD8⁺ T cells in the draining lymph node of obese mice (Extended Data Fig. 2h–j). Together, these results show that obesity exacerbates multiple mouse models of atopic disease.

The marked difference in atopic disease severity between lean and obese mice prompted us to profile the infiltrating T cell populations in the AD lesions using flow cytometry. Numbers of conventional CD4⁺ T (T_{conv}) cells were significantly increased in the obese mice, with trending increases in the regulatory T (T_{reg}) and CD8⁺ T cell populations, suggestive of an overall increased inflammatory response (Extended Data Fig. 2k). Intracellular staining for cytokine competence revealed trending approximately 3.9- and 1.7-fold increases in CD4⁺ T cells from obese mice positive for the T_H2 cytokines IL-4 and IL-13, respectively, in this cohort; however, we also observed an unexpected and prominent 6.5- and 11.5-fold increase in CD4⁺ T cells positive for the T_H17 cytokines IL-17A and IL-17F, respectively (Fig. 1e, Extended Data Fig. 3a, b). We observed similar increases in T_H17 cytokines in obese mice relative to lean mice challenged with TOP (Extended Data Fig. 4a, b) and in a model of allergic airway disease (Extended Data Fig. 4c).

More prominent T_H17 inflammation may also occur in obese humans with allergic disease. Using a previously collected dataset of targeted serum proteomes of patients with AD, we identified two markers of cutaneous T_H17 inflammation^{16,17} that correlated positively with body mass index (BMI) (Extended Data Fig. 5a–c). Separately, an analysis of lean, overweight, obese and morbidly obese individuals with asthma from a large, longitudinal, multi-centre study (SARP)¹⁸ demonstrated a clear decrease in sputum eosinophil percentage, a defining marker of the T_H2-high endotype¹⁹, as a function of BMI (Extended Data Fig. 5d, e).

Although previous studies have demonstrated increased disease severity in obesity in T_H17-dependent disease models such as multiple sclerosis and colitis^{20,21}, our findings were surprising as experimental AD is classically a T_H2-driven model of cutaneous autoimmunity, and suggested that obesity may shift the T_H-responsive landscape away from T_H2-directed inflammation towards an aberrant T_H17 or unfocused inflammatory response. To further characterize this shift, we systematically profiled the lesional T_{conv} cells using single-cell RNA-sequencing (scRNA-seq) (Fig. 1f). Analysis revealed four T_{conv} cell populations (annotated here as naive-like, T_H1, T_H2 and T_H17) and a cluster of cycling cells along with associated RNA velocity^{22,23} transcriptional trajectories visualized with uniform manifold approximation and projection (UMAP)^{24,25} (Fig. 1f; Methods). The gene-expression heatmaps of lineage-defining transcription factors (*Gata3*, *Tbx21* and *Rorc*), lineage-defining effector cytokines and cytokine receptors (*Il4*, *Il13*, *Ifng*, *Il17a*, *Il17f*, *Il22* and *Il23r*) and other prominent markers of activation, quiescence and memory (*Ccr7*, *S1pr1*,

Sell, *Klf2* and *Bcl2*) validated these population assignments (Fig. 1f, g, Extended Data Fig. 6a–c). Cells from lean and obese mice showed marked differences in distribution across the cell populations (Fig. 1h). In particular, we observed clear differences in the degree of T_H17 differentiation between lean and obese mice (Fig. 1h, i). A proportion of the T_{conv} cells from the lean mice were assigned to an ‘early’ position (expressing the T_H17 lineage-defining transcription factor *Rorc*, but not the effector cytokines *Il17a*, *Il17f* or *Il22*, or the cytokine receptor *Il23r*). By contrast, a majority of the T_H17 cells from the obese mice were assigned to a ‘late’ position (expressing both *Rorc*, the effector cytokines and the cytokine receptor *Il23r*) (Fig. 1f–i). Of note, the emergence of these more-mature-appearing T_H17 cells in the inflamed skin of obese animals probably depended on egress of lymphocytes from secondary lymphoid organs, as blocking lymphocyte egress from secondary lymphoid organs with FTY720 (an agonist of the sphingosine 1-phosphate receptor) decreased disease severity in obese mice and caused a marked increase in CD4⁺ T cells expressing IL-17A and IL-17F in the draining lymph node (Extended Data Fig. 7a–c). Together, these findings demonstrate that obesity alters the T_H-response in AD and asthma to include substantial T_H17 inflammation in addition to the canonical T_H2 inflammation found in lean mice.

Biologic treatments blocking the signalling of the T_H2 effector cytokines IL-4 and IL-13 have proved remarkably successful in managing T_H2-driven severe allergic diseases such as asthma, sinusitis and AD^{26–29}. Owing to the aberrant T_H17 inflammation in AD in obese mice, we questioned to what extent neutralizing IL-4 and IL-13 would limit AD in these animals. We treated lean and obese mice with anti-IL-4 and anti-IL-13 neutralizing antibodies (anti-IL-4/IL-13) while eliciting experimental AD. As expected, anti-IL-4/IL-13 treatment strongly protected lean mice from AD (Fig. 1j–l). Notably, anti-IL-4/IL-13 treatment was not just ineffective in obese mice, it also worsened disease in both MC903-induced AD (Fig. 1j–l) and the TOP AD model (Extended Data Fig. 8a–d). Additionally, anti-IL-4/IL-13 treatment of obese mice with MC903-induced AD resulted in the emergence of small pustules, consistent with severe AD (Fig. 1m, arrows). Flow cytometric analyses of cytokine-competence demonstrated decreased IL-13-positive and IFN γ -positive CD4⁺ T cells after anti-IL-4/IL-13 treatment in lean but not obese mice (Fig. 1n). Whereas the small numbers of IL-7A-positive and IL-17F-positive CD4⁺ T cells tended to be even further decreased in lean mice after anti-IL-4/IL-13 treatment, the numbers of IL-17F-positive cells were increased in obese mice (with the IL-17A-positive and IFN γ -positive cells also trending higher in this cohort), suggesting that anti-IL-4/IL-13 T_H2 blockade exacerbates non-T_H2 inflammation in the obese animals and worsens disease.

The nature of a T_H host response to a given immune challenge can shape the course of disease. Our observation of increased T_H17 inflammation in the more severe manifestation of AD in obese mice led us to hypothesize that this immunological misfiring in obesity was an important causal step in disease pathophysiology and the negative response to anti-IL-4/IL-13 treatment. We aimed to identify a factor *in vivo* whose function was to protect the dominant T_H2 response to experimental AD in lean mice. Such a factor likely would be expressed in T_H2 cells and have dysregulated or decreased activity in obesity, and its T cell-specific ablation in lean mice might enable the emergence of non-T_H2 inflammation in AD and lead to worsened disease upon anti-IL-4/IL-13 treatment. We focused on the nuclear hormone receptor (NHR) superfamily, a class of transcription

factors that can be sensitive to systemic changes in physiologic and metabolic state. Among T_H1, T_H2 and T_H17 cells, PPAR γ ^{30–33} (and its heterodimeric partner RXR α) stood out among NHRs as highly and differentially expressed in T_H2 cells relative to T_H1 and T_H17 cells (Extended Data Fig. 9a–e). PPAR γ is a transcription factor that was recently demonstrated to be important for regulating transcriptional networks in T_H2 cells³⁴. However, its physiological, in vivo relevance and functional role are not well understood^{35–41}, with different studies demonstrating seemingly contradictory results regarding the effect of PPAR γ activation or repression on T_H2-associated diseases. Of note, one study demonstrated a role for PPAR γ agonists in blunting the T_H17 response and protecting mice against experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis, even though expression of *Pparg* in T_H17 cells was not demonstrated⁴² (Extended Data Fig. 9a–e).

We assessed how obesity affects gene expression downstream of PPAR γ in T cells. We performed chromatin immunoprecipitation with sequencing (ChIP–seq) of PPAR γ in T_H2 cells to identify its genome-wide binding sites (Extended Data Fig. 9f, g, Supplementary Information Table 1). We then interrogated our scRNA-seq dataset to determine whether the expression of the genes composing this PPAR γ cistrome was altered in the lesional T cell subsets of lean and obese mice. In lean mice, genes in the PPAR γ cistrome were most highly expressed in T_H2 cells, as expected (Fig. 2a, b). However, expression of these genes in obese mice was decreased in T_H2 cells and the related naive-like cells (and to a lesser degree in T_H1 cells) compared with the corresponding subsets from lean mice (Fig. 2b), suggesting decreased PPAR γ activity in T cells in obesity. By contrast, there was no change in PPAR γ cistrome gene expression in T_H17 and cycling cells between lean and obese mice. Together, these findings suggest that PPAR γ activity is impaired in T cells in obesity. We hypothesized that PPAR γ may act to preserve T_H2 inflammatory predominance and that in vivo PPAR γ deficiency could unfocus T_H2 responses in favour of other T_H cell responses.

We tested this hypothesis by generating T cell-specific PPAR γ -deficient mice (*Cd4^{cre}Pparg^{fl/fl}* (PPAR γ TKO)). T cell-specific loss of PPAR γ did not elicit overt systemic inflammation associated with spontaneous T cell dysregulation; PPAR γ -TKO mice had normally sized spleens and lymph nodes (Extended Data Fig. 10a, b) and equivalent splenic and lymph node T_{reg} (CD25⁺FOXP3⁺) and activated (CD62L^{lo}CD44^{hi}) CD4⁺ T cell populations compared with controls (*Cd4^{cre}Pparg^{+/+}*) (Extended Data Fig. 10c, d). Additionally, PPAR γ -TKO mice exhibited normal thymic T cell development, as measured by CD4 and CD8 expression of thymic T cells (Extended Data Fig. 10e). When challenged with experimental MC903 AD, the PPAR γ -TKO lean mice developed more severe disease, similar but less extreme to that observed in wild-type obese mice (approximately 80% increase in change in ear thickness compared with control mice) (Fig. 2c, d). Histological examination in PPAR γ -TKO mice also recapitulated many features of AD in obese *Pparg*-sufficient mice including the most distinctive histologic feature—leukocytic expansion of the dermis (Fig. 2e, dashed line). Flow cytometric analyses of cytokine competence revealed that the PPAR γ -TKO mice had an increase in IL-17A- and IL-17F-positive CD4⁺ T cells while additionally demonstrating an increase in IL-13-positive CD4⁺ T cells and a trending increase in IL-4- and IFN γ -positive CD4⁺ T cells in this cohort (Fig. 2f). Supplementing the flow cytometry data with single-cell RNA-seq of lesional T_{conv} cells provided a

more holistic picture, revealing that PPAR γ -TKO mice exhibited a generally increased inflammatory response across all T_H subtypes with a clear loss of T_{H2} selectivity (Fig. 2g). The T_{conv} cells from control mice largely overlapped with the T_{H2} cluster (Fig. 2g, merge), whereas cells from the PPAR γ -TKO mice were more distributed among the naive-like, T_{H1} and T_{H17} clusters, and comparatively diminished in the T_{H2} cluster. Similar to what we observed in obese relative to lean *Pparg*-sufficient mice, the T_{H17} cells from the PPAR γ -TKO mice were also further along their differentiation trajectory compared with the relatively few T_{H17} cells from control mice (Fig. 2g, h).

Given that T cell-specific ablation of PPAR γ was associated with the emergence of non-T_{H2} inflammation and more severe disease in AD, we next investigated whether treatment with anti-IL-4/IL-13 would worsen AD in PPAR γ -TKO mice. Indeed, anti-IL-4/IL-13 treatment exacerbated AD (Fig. 2i–k), with the emergence of small pustules (Fig. 2l, arrows) along with trending increases in IL-17A, IL-17F and IFN γ -positive lesional CD4⁺ T cells in treated PPAR γ -TKO mice in this cohort (Fig. 2m). Of note, T_{reg} cell-specific ablation of PPAR γ ⁴³ (*Foxp3^{cre}Pparg^{fl/fl}*) did not lead to a significant difference in AD development and severity relative to controls (*Foxp3^{cre}Pparg^{+/+}*, Extended Data Fig. 10f–j). Together, these results strongly support the role of PPAR γ as a focusing factor crucial to the in vivo T_{H2} response, serving to maintain T_{H2} responses against competing T_H programs during an inflammatory challenge – a role not previously identified by in vitro investigations of PPAR γ in T_{H2} cells (Fig. 2n).

Thiazolidinediones (TZDs) are an FDA-approved class of potent PPAR γ agonists that are used as insulin-sensitizing medications to manage type 2 diabetes. Having observed that T cell-specific ablation of *Pparg* causes the aberrant emergence of non-T_{H2} inflammation and conversion of anti-IL-4/IL-13 treatment from therapy to anti-therapy, we hypothesized that pharmacological enforcement of PPAR γ action via TZD treatment in obese mice may promote T_{H2} selective responses and suppress T_{H17} inflammation, reduce disease severity and perhaps even restore the therapeutic efficacy of anti-IL-4/IL-13 treatment. We treated obese control or PPAR γ -TKO mice with rosiglitazone (the archetypal TZD) or DMSO and challenged the obese mice with AD. Rosiglitazone treatment significantly reduced AD severity in obese control mice, as measured by an approximately 40% reduction in the ear thickness increase (Fig. 3a) and a marked reduction in leukocytic infiltration (Fig. 3b, dashed line). These effects were dependent on T cell-specific PPAR γ expression. In the PPAR γ -TKO mice, rosiglitazone modestly increased disease severity (Fig. 3a, b), possibly related to T cell-extrinsic mechanisms of PPAR γ action. Of note, although rosiglitazone treatment did not protect PPAR γ -TKO mice from AD, its insulin-sensitizing effects were intact (Extended Data Fig. 11a–d), which argues for distinct therapeutic mechanisms of action for rosiglitazone in restoring organismal insulin sensitivity and protection from AD in obesity.

Next, we investigated the effect of rosiglitazone treatment on the T_H response profile in AD. We performed scRNA-seq to analyse the distribution of lesional T_{conv} cell states from obese control or PPAR γ -TKO mice, with or without rosiglitazone treatment (Fig. 3c, Extended Data Fig. 12a–c). We observed that T_{conv} cells from obese control mice were distributed across the naive-like, T_{H1}, T_{H2} and T_{H17} populations. Rosiglitazone treatment

of obese control mice seemed to collapse their CD4⁺ T cell compartment to the T_H2 cluster, a finding that was partially observed in the obese PPAR γ -TKO mice (Fig. 3d). Further, rosiglitazone treatment strongly reduced the T_H17 population in the obese control mice (Fig. 3d), although the rare T_H17 cells present were somewhat more differentiated along the T_H17 pathway relative to the T_H17 cells from mice treated with DMSO (Fig. 3e). Additionally, PPAR γ activity as measured by collective expression of the PPAR γ T cell cistrome was increased by rosiglitazone in the T_H2 and, to a lesser extent, T_H17 cells (Fig. 3f, g, Supplementary Information Table 1), suggesting that rosiglitazone may activate PPAR γ in T_H2 and T_H17 cells in vivo in concert with the consequent suppression of T_H17 differentiation. Notably, the rosiglitazone-induced increase in PPAR γ cistromic activity required PPAR γ expression in T cells, and rosiglitazone treatment did not affect the degree of differentiation along the T_H17 pathway among the T_H17 cells in PPAR γ -TKO mice (Fig. 3e–g). Finally, we hypothesized that if rosiglitazone treatment decreases immunological misfiring in obesity, enforcing a T_H2-predominant immune response in AD, then it may be able to restore the efficacy of anti-IL-4/IL-13 treatment. Using regimens of rosiglitazone treatments in obese mice that did not on their own reduce AD severity, rosiglitazone indeed improved the outcomes of anti-IL-4/IL-13 for treatment of AD in obese mice (Fig. 3h–k, Extended Data Fig. 13a–c).

Obesity is a systemic pathophysiological state that is increasing in prevalence and incidence worldwide^{44–46}, yet how obesity changes the architecture of immune responses is not well understood. Here, by studying the pathology of allergic inflammation in lean and obese mice with matched genetic backgrounds, we demonstrate that obese mice develop more severe disease associated with immunological ‘misfiring’⁴⁷, converting an effective, targeted immunological therapy to an anti-therapy (Fig. 3l). Our demonstration that a PPAR γ agonist can help to enforce an ‘on-target’ immunological response and restore efficacy for a targeted immunological therapy that would otherwise be harmful in this setting suggests a new strategy for precision immunotherapies to overcome altered pathology resulting from high-fat diet and obesity.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-022-04536-0>.

Methods

Mice

Mouse studies were conducted at two separate academic research institutions: The Salk Institute for Biological Studies, USA and University of California, San Francisco (UCSF), USA. Mice were housed in specific pathogen-free facilities. When required, mice were purchased from The Jackson Laboratory. By default, mice within The Salk Institute for Biological Studies were fed with autoclaved normal chow (MI laboratory rodent diet 5001, Harlan Teklad) whereas mice fed at UCSF were given irradiated PicoLab Rodent Diet 20

5053. For experiments requiring the use of special diets, at the Salk Institute for Biological Studies and UCSF, diets were irradiated and purchased from Research Diets. The HFD used was 60 kcal% fat (D12492), the control normal diet was 10 kcal% fat (D12450J). Rosiglitazone was provided at 15 mg kg⁻¹ of food. Unless specified, mice treated with rosiglitazone began treatment at the introduction of high-fat diet feeding. Mice designated as obese in this study were fed high-fat diet for at least 9 weeks, starting at an age of 6–7 weeks, and weighed at least 38 g (as an average within a cage). During the high-fat withdrawal studies, the high-fat diet (Research Diets, D12492) was replaced with control normal diet (D12450J), and the always obese and always lean control groups were fed with the corresponding high-fat diet or control normal diet from Research Diets. To definitively identify T_{conv} cells for FACS analyses, we used the *Foxp3^{Thy1.1}* (ref. 48) reporter mice. PPAR γ -TKO mice were generated by crossing *Cd4^{cre}* (ref. 49) transgenic mice and *Pparg^{fl/fl}* (ref. 50) mice. PPAR γ T_{reg} cKO mice were generated by crossing *Foxp3^{cre}* (*Foxp3-Ires-YFP-cre*)⁵¹ and *Pparg^{fl/fl}* mice. All mice used for studies were male. All procedures involving animals were performed in accordance with protocols approved by the respective Institutional Animal Care and Use Committee (IACUC) of the Salk Institute for Biological Studies and UCSF.

MC903-induced experimental atopic dermatitis

Mice were anaesthetized with isoflurane and MC903 solution (0.1 mM in ethanol, 10 μ l per ear, R&D Systems) was applied to mouse ears daily for 9–13 days. Ear thickness was assessed with a micrometer (Mitutoyo, 227–211). After collection, ears were dissected from the mice and prepared for histological analyses or flow cytometry.

TOP-induced experimental atopic dermatitis

Mice were injected intraperitoneally with an OVA-Imject Alum mixture (OVA (Sigma-Aldrich); Imject Alum Adjuvant (Thermo Fisher); mixture made per manufacturer instructions) on day -7. Seven days later, on day 0, mice were subjected to a treatment of tape-stripping (Shurtape, HP-500, 3 tape strips on both sides of the ear) and application of OVA-papain in PBS (250 μ g OVA and 125 μ g papain (Sigma-Aldrich, P3375) per 25 μ l per mouse), which was repeated on days 2, 4 and 6. Ear thickness was assessed with a micrometer (Mitutoyo, 227–211). Upon collection on day 8, ears were dissected from the mice and prepared for histological analyses or flow cytometry.

Reanalyses of atopic dermatitis patient cohort

Peripheral blood serum from patients with moderate-to-severe AD ($n = 59$) were previously analysed by Olink Proseek multiplex assay using inflammation I, cardiovascular disease/CVD II, and CVD III multiplex panels. The proteins that were identified previously as differentially expressed genes from non-lesional keratinocytes treated with IL-13 or IL-17A were then correlated with patient BMI. Correlations between BMI and protein expression levels were assessed using Spearman correlation coefficients, and data are presented in scatter plots.

OVA-induced acute asthma

Mice were sensitized on days 0, 7 and 14 by intraperitoneal injection of 50 µg of OVA (Sigma-Aldrich) emulsified in 1 mg of aluminium potassium sulfate. One week after the last sensitization, mice were intranasally challenged on 3 consecutive days with 100 µg OVA in 40 µl saline. Twenty-four hours after the last OVA challenge, mice were sacrificed for downstream analyses including cell counting of bronchoalveolar lavage fluid and flow cytometric analyses of the lung-draining lymph nodes.

Reanalyses of asthma patient cohort

Clinical data were originally collected as part of the NHLBI Severe Asthma Research Program⁵² and reanalysed for correlation with patient BMI. One-way ordinary ANOVA followed by a test of linear trend was used to determine statistical significance.

FTY720 treatments

Mice were injected intraperitoneally, daily with FTY720 (1 mg kg⁻¹) or water starting one day before initiation of the MC903 challenge through conclusion of challenge.

Antibody treatments

The following antibodies were used: anti-mouse IL-4 (BioXCell, 11B11), anti-mouse IL-13 (InvivoGen, 8H8), and isotype control (IgG1, BioXCell, MOPC-21). The antibodies were injected intraperitoneally at days 0, 3, 6, and 8 of the MC903-induced AD challenge at a dose of 0.25 mg per mouse for anti-IL-4, 0.1 mg per mouse for anti-IL-13, and 0.35 mg per mouse for isotype control (IgG1). For the TOP-AD model, the antibodies were injected intraperitoneally at days 0, 2, 4 and 6 with the same dosage as for the MC903-AD model. Injection volumes never exceeded 150 µl.

Single-cell suspension of ear-infiltrating immune cells

Dissected ears were minced into fine pieces (1–2 mm³), and digested in a stromal vascular isolation buffer (HBSS with calcium and magnesium, 20 mg ml⁻¹ BSA, 20 mg ml⁻¹ penicillin, 20 mg ml⁻¹ streptomycin) containing 2 mg ml⁻¹ Collagenase D (Roche) at 37 °C with intermittent shaking for 2 h. The suspension was then passed through a 100-µm mesh to remove undigested clumps and debris. The flow-through was centrifuged at 400 RCF for 10 min. The pellet containing the stromal vascular fraction was washed once in 10 ml RPMI, and the resultant isolated cells were prepared for FACS analysis directly or first stimulated with PMA and ionomycin in the presence of brefeldin A (GolgiPlug; BD) for 5 h at 37 °C for subsequent intracellular cytokine staining and then prepared for FACS analysis.

Histological analyses

Sections (5 µm) of fixed tissues were stained with haematoxylin and eosin according to standard procedures. Histopathological analyses were conducted on blinded samples for severity and extent of inflammation and morphological changes by a pathologist.

Flow cytometry

The following antibodies were used. Biolegend: CD3 (145-2C11), CD4 (RM4-5), CD8 (53–6.7), CD25 (7D4), CD44 (IM7), CD45.2 (104), CD62L (MEL-14), CD90.1 (Thy1.1, OX-7) and IFN- γ (XMG1.2), IL-17F (9D3.1C8), TCR γ/δ (GL3); eBioscience: FOXP3 (FJK-16s), IL-4 (11B11), IL-13 (eBio13A), IL-17A (eBio17B7). We used the eBioscience Fixable Viability Dye eFluor780 or DAPI to distinguish live from dying or dead cells. For intracellular staining, cells were treated with fixation and permeabilization reagents from eBioscience and labeled with appropriate antibodies before being analysed. Data were analysed using a LSRII, LSRFortessa, or FACS Aria instrument (Becton Dickinson) and FlowJo software (FlowJo LLC). Cells were sorted on a BD FACS Aria cell sorter.

scRNA-seq

scRNA-seq was performed in two batches on separate samples with the Chromium Single Cell 3' Reagent Kit, v3 chemistry (10x Genomics, PN-1000092) following the manufacturer's protocol. The same procedure was followed for both Chromium runs unless otherwise specified. In brief, cells were sorted by FACS (sorting on live, CD45.2⁺TCR γ/δ ⁻CD4⁺ cells on a BD FACS Aria cell sorter), resuspended at 500–900 cells per μ l in PBS + 1% FBS, and loaded into the Chromium Controller (10x Genomics, PN-1000202) for an anticipated targeted recovery of 3,000–6,000 cells depending on sample recovery from FACS. We performed 11 cycles of PCR for cDNA amplification after GEM recovery, and 25% of each cDNA sample was carried into transcriptome library preparation. We performed 13 cycles of PCR to introduce Chromium i7 multiplex indices (10x Genomics, PN-120262). cDNA was diluted 1:5 in buffer EB and quantified by Bioanalyzer DNA High Sensitivity (Agilent, 5067–4626) and Qubit dsDNA High Sensitivity (Thermo Fisher, Q32854) reagents. Samples were pooled equally and sequenced on a HiSeq 4000 (Illumina) using read parameters 28 \times 8 \times 98.

Raw fastq files were mapped to the mouse transcriptome ('refdata-cellranger-mm10-3.0.0') using Cell Ranger (10x Genomics, version 3.0.2) and 'filtered_feature_bc_matrix.h5' files were loaded into Scanpy (version 1.6.0)⁵³ for processing. For batch 1 (lean, obese, *Cd4^{cre}* and PPAR γ TKO), 2,500–4,500 cell barcodes were recovered per sample, totalling 12,597 cells before filtering. For batch 2 (control-DMSO, control-Rosi, PPAR γ TKO-DMSO and PPAR γ TKO-Rosi), 3,100–5,500 cell barcodes were recovered per sample, totalling 15,867 cells before filtering. Cells with fewer than 200 detected genes, more than 6,000 detected genes, and/or mitochondrial read fraction higher than 25% were removed from the complete dataset. After filtering, 12,034 and 15,658 cells from batches 1 and 2, respectively, were carried through for subsequent analyses. Counts were log normalized and scaled before regressing out total UMI counts and mitochondrial fraction per cell. Principal component analysis (PCA) was performed on highly variable genes, and the first 20 PCA components were used to construct a UMAP^{24,25}. Cell clustering was performed using the Leiden algorithm⁵⁴ (version 0.7.0, resolution 0.9). Two (batch 1) or three (batch 2) small clusters identified as contaminating antigen presenting cells were excluded from downstream analyses. Additionally, *Foxp3*-expressing cells were excluded from analyses in this study. We performed Gaussian kernel density estimation (Scanpy 'embedding_density') to visualize the distribution of samples in UMAP space. Continuous

probability density curves were generated using Seaborn (version 0.10.0) kdeplot function with 'common_norm=False' to account for differences in sample number. Mann-Whitney U statistics were calculated using Scipy.stats (version 1.4.1) mannwhitneyu function. The PPAR γ activity score was calculated with the score_genes function (Scanpy) using filtered genes associated with the top 50% of peaks annotated by HOMER (see 'ChIP-seq library generation') compared against a random, size-matched control gene set.

RNA velocity

Cellranger bam files were processed with Velocity (version 0.17.17) using the default Cell Ranger mm10 gtf reference file (version 3.0.0) with repeat masking (UCSC). Output files were loaded into scVelo (version 0.2.2) (<https://scvelo.readthedocs.io>) and merged with the gene-expression anndata object from Scanpy. Low abundance genes (less than 30 total counts) were filtered from the merged dataset. Cell moments were calculated considering 30 nearest neighbors and 30 principal components and used to estimate RNA velocities using default settings. The 'T_H17-ness' metric was inferred from (1) the directionality of RNA Velocity vectors along the UMAP1 coordinate (*x*-axis) and (2) a composite T_H17 score calculated from *Il17a*, *Il17f*, *Il22* and *Il23r* expression using the score_genes function (Scanpy).

In vitro CD4⁺ T cell differentiation

CD4⁺ T cells were isolated from the spleen and lymph nodes using the EasySep Mouse CD4 Positive Selection Kit II (Stemcell Technologies). Naive (CD25⁻CD44^{hi}CD62L^{lo}) CD4⁺ T cells were sorted by flow cytometry from the bead purified CD4⁺ T cells. The naive CD4⁺ T cells were resuspended in Click's medium (Irvine Scientific) at 1 million cells per ml, and then plated on day 0 in 24 well plates coated with goat anti-hamster IgG antibody (200 ng ml⁻¹; MP Biomedicals) with the addition of soluble anti-CD3 (1 μ g ml⁻¹; 145-2C11) and anti-CD28 (1 μ g ml⁻¹; 37.51) from Bio X Cell. Polarizing conditions for different T helper subsets are as following: T_H1: human IL-2 (100 U ml⁻¹; PeproTech), mouse IL-12 (20 ng ml⁻¹; PeproTech) and anti-IL-4 (5 μ g ml⁻¹; Bio X Cell); T_H2: human IL-2 (100 U ml⁻¹; PeproTech), mouse IL-4 (20 ng ml⁻¹; Biolegend), anti-IFN- γ and anti-IL-12 (5 μ g ml⁻¹; Bio X Cell); T_H17: mouse IL-6 (20 ng ml⁻¹; Biolegend), human TGF- β (2 ng ml⁻¹; PeproTech), anti-IFN- γ and anti-IL-12 (5 μ g ml⁻¹; Bio X Cell).

RNA-seq library generation and sequencing analysis

Total RNA was extracted from T_H1, T_H2 and T_H17 cells approximately 96 h after initiation of in vitro differentiation. RNA-sequencing libraries were prepared from 100 ng total RNA (TrueSeq v2, Illumina) and single-end sequencing was performed on the Illumina HiSeq 2500, using bar-coded multiplexing and a 100 bp read length, yielding a median of 34.1 million reads per sample. Read alignment and junction finding was accomplished using STAR⁵⁵ and differential gene expression with Cuffdiff 2 (ref. ⁵⁶), utilizing UCSC mm10 as the reference sequence. Transcript expression was calculated as gene-level relative abundance in fragments per kilobase of transcript per million mapped fragments and employed correction for transcript abundance bias⁵⁷.

Primers for quantitative PCR.—*Pparg*: forward, CACAATGCCATCAGGTTTGGG; reverse: GAAATGCTTTGCCAGGGCTC. *Gata3*: forward, CTTCCACCCAGCAGCCTGC, reverse: CGGTACCATCTCGCCGCCAC. *Tbx21*: forward, GTCGCGCTCAGCAACCACCT, reverse: CGGCCACGGTGAAGGACAGG. *Rorc*: forward, CCGGACATCTCGGGAGCTGC, reverse: CGGCGGAAGAAGCCCTTGCA. *Hprt*: forward, GTCATGCCGACCCGCAGTCC, reverse: GGCCACAATGTGATGGCCTCCC.

Antibodies for western blot

Rabbit anti-PPAR γ mAb (81B8, Cell Signaling), mouse anti-tubulin (DM1A, Sigma).

ChIP-seq library generation

Naive CD4⁺ T cells were activated and polarized in T_H2 conditions. On days 1 and 2, T cells were transduced with a retroviral vector expressing TY1-tagged *Pparg*. On day 4, transduced T cells were collected for ChIP as described previously⁵⁸ using a TY1 antibody (Sigma, SAB4800032). ChIP-seq libraries were constructed and sequenced (100 bp single end reads) as described previously¹³. Short DNA reads were demultiplexed using Illumina CASAVA v1.8.2. Reads were aligned against the mouse mm10 reference genome using the Bowtie2 aligner with standard parameters that allow up to 2 mismatches per read. Peak calling, motif analyses, and other data analysis were performed using HOMER, a software suite for ChIP-seq analysis as described previously⁵⁸. Visualization of ChIP-seq results was achieved by uploading custom tracks onto the UCSC genome browser.

Glucose tolerance test

Fasting was induced for 6 h. Glucose (1.5 g kg⁻¹, intraperitoneally) was injected and serum was collected for glucose and insulin measurements. Blood glucose was monitored using a Nova Max Plus glucometer. Serum insulin levels (Ultra Sensitive Insulin, Crystal Chem) were measured by ELISAs.

Statistical analyses

Statistical analyses were performed with Prism 9 (GraphPad). *P* values were calculated using Welch's *t*-test unless otherwise noted. Mice cohort size was designed to be sufficient to enable statistical significance to be accurately determined. When applicable, mice were randomly assigned to treatment or control groups. Experimental data was not excluded from the statistical analyses, except for exclusions due to technical errors or loss of confidence in appropriate controls. The investigators were not blinded in the studies unless otherwise stated. Appropriate statistical analyses were applied, assuming a normal sample distribution, unless otherwise specified. No estimate of variance was made between each group. All in vivo experiments were conducted with at least two independent cohorts unless otherwise specified. All in vitro T_H differentiation experiments were conducted at least three times. Unless otherwise noted, data from one instance of the repeated experiments are represented in this manuscript. Bulk and single-cell RNA-seq experiments and ChIP-seq experiments were conducted once using multiple biological samples per group (as indicated in figure legends).

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

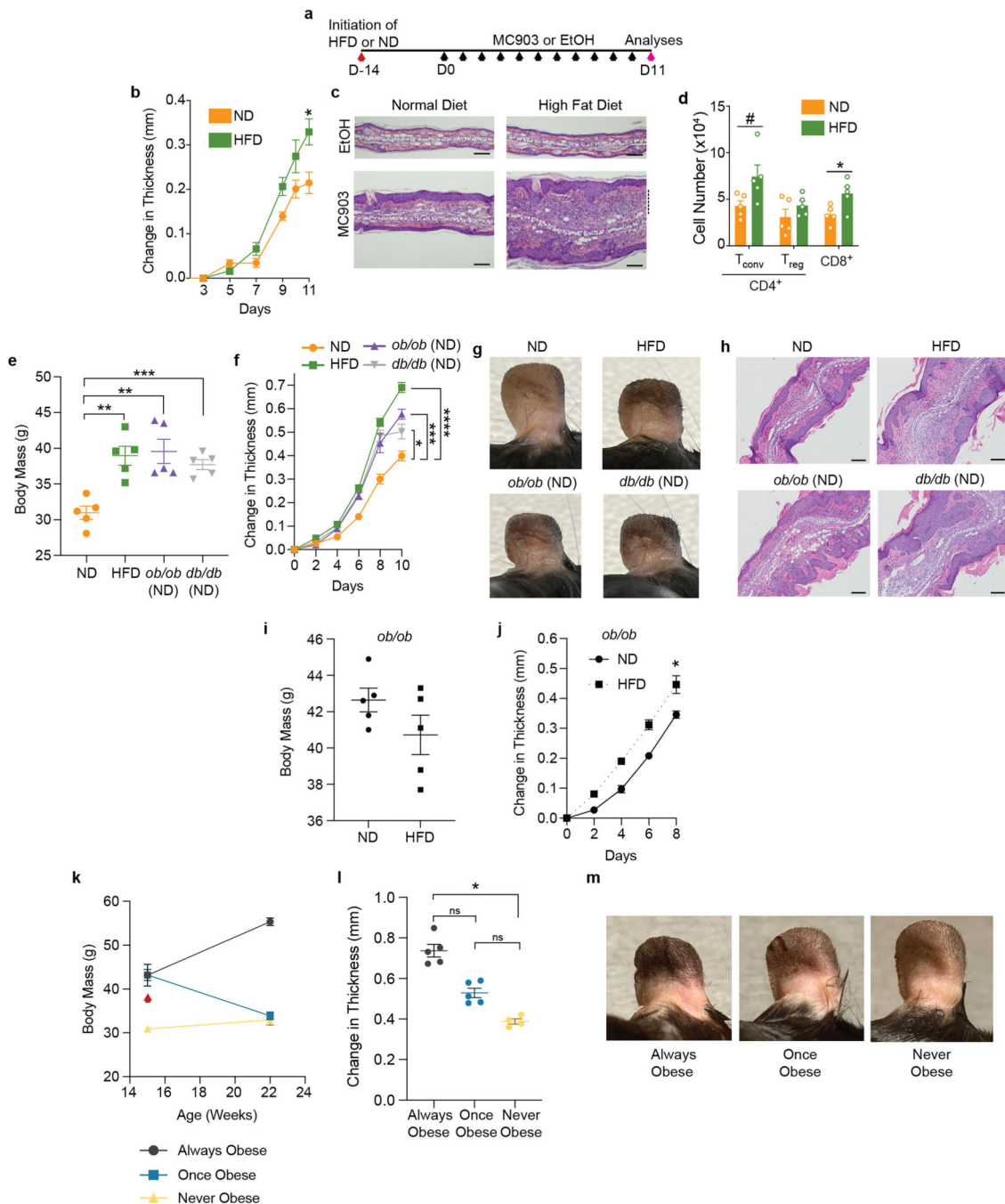
Data availability

The bulk RNA-sequencing and ChIP-seq data can be accessed in the NCBI Sequence Read Archive under the accession PRJNA553761. Additionally, the ChIP-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under the accession code GSE189216. The single cell-RNA sequencing data used in this study are deposited in the GEO under the accession code GSE189476. Other relevant data are available from the corresponding authors upon reasonable request. Source data are provided with this paper.

Code availability

Code used in this manuscript can be provided by reasonable request to the corresponding authors.

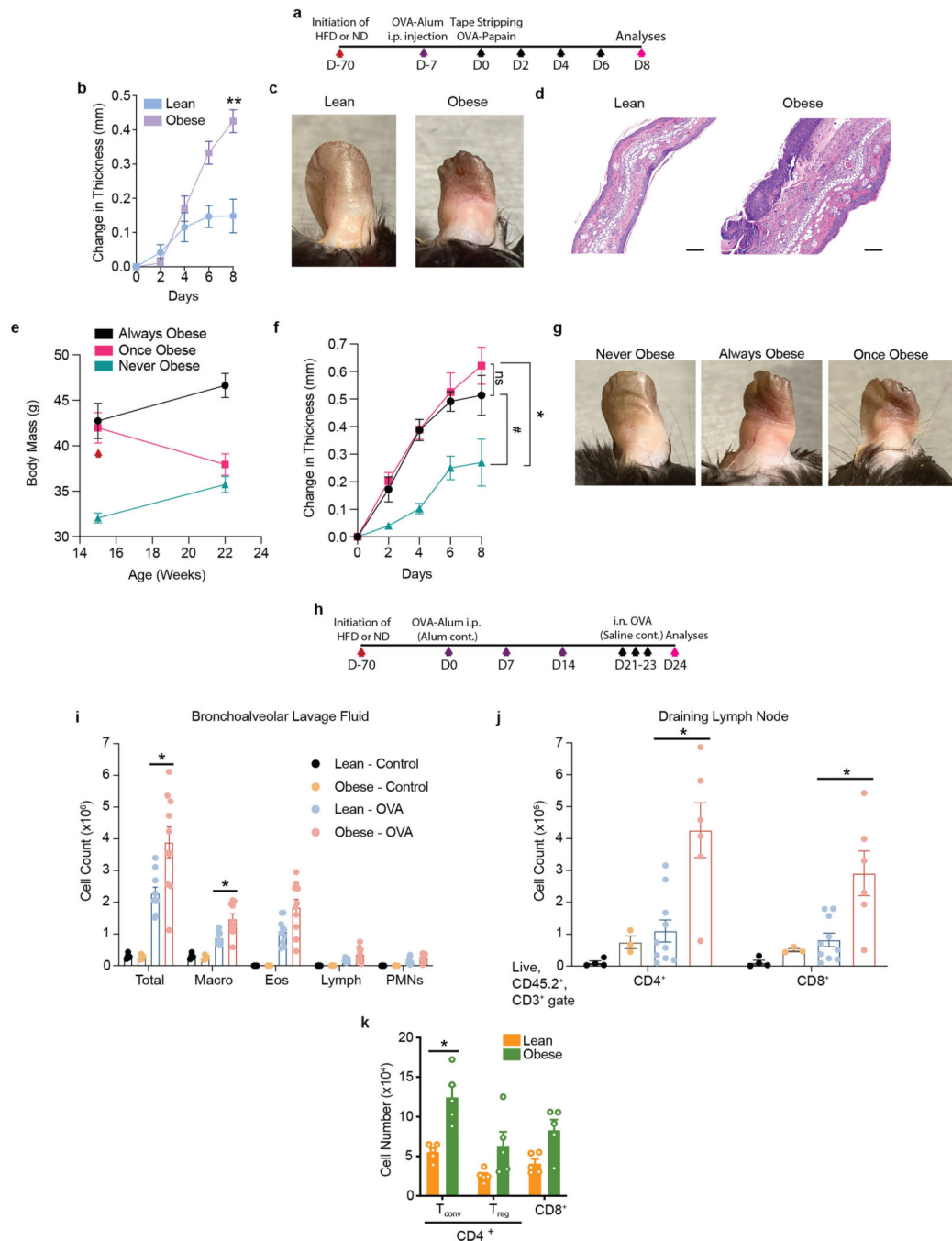
Extended Data



Extended Data Fig. 1 | Diet-induced and monogenic models of obesity provoke increased disease severity upon MC903-AD challenge.

a. Scheme of MC903-induced atopic dermatitis disease model where animals are fed either high fat diet (HFD) or normal diet (ND) for two weeks before initiating MC903 treatments. Black arrows indicate MC903 or EtOH administration to ear. **b.** Change in ear thickness during AD development as in (a). **c.** Representative H&E ear histology, Day 11. Scale bars, 100 μ m. Dashed line, leukocytic expansion of the dermis. **d.** Total T_{conv} , T_{reg} , and $CD8^+$ T

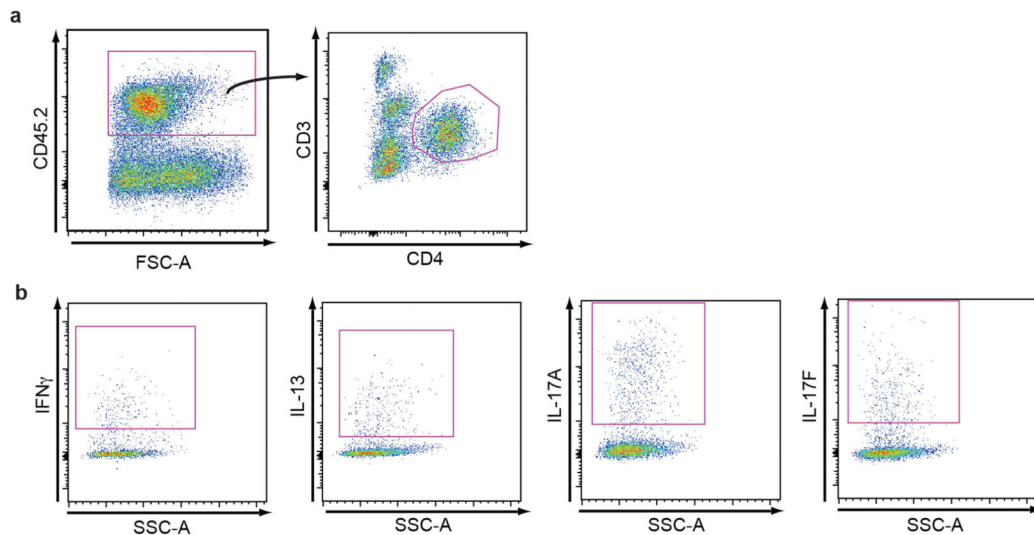
cell number from whole ear, Day 11. **e-h**, Body mass (**e**), change in ear thickness during AD challenge (**f**), representative images at Day 10 of challenge (**g**), and representative H&E ear histology, Day 10 (**h**) of ND-fed mice, HFD-fed obese mice, or ND-fed *ob/ob* or *db/db* mice. **i, j**, Body mass at Day 0 (**i**) and change in ear thickness upon AD challenge (**j**) of 9 week old *ob/ob* mice fed ND or HFD for three weeks. **k-m**, Timeline of body mass (**k**), change in ear thickness (**l**), and representative images at Day 10 of challenge (**m**) of always obese (AO), once obese/now lean (OO), and never obese (NO) mice. Red arrow (**k**) indicates replacement of HFD with control lean diet for OO mice. $n = 5$ for all groups in (**b-m**) except 4 never obese (NO) mice were used in (**l**) and (**m**). Data are mean \pm s.e.m. Peak values were tested with Welch's *t*-test in (**b, j**) and ordinary one-way ANOVA with all groups tested against ND in (**f**). Ordinary one-way ANOVA with all groups tested against ND in (**e**) and with all groups tested against all groups in (**l**). *P* values were adjusted for multiple comparisons in (**d**) using Holm-Šídák method and Šídák's multiple comparisons test in (**e, f, l**). ns – not significant, [#]*P* < 0.07, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Extended Data Fig. 2 | Increased inflammation in obesity in multiple models of allergic inflammatory disease.

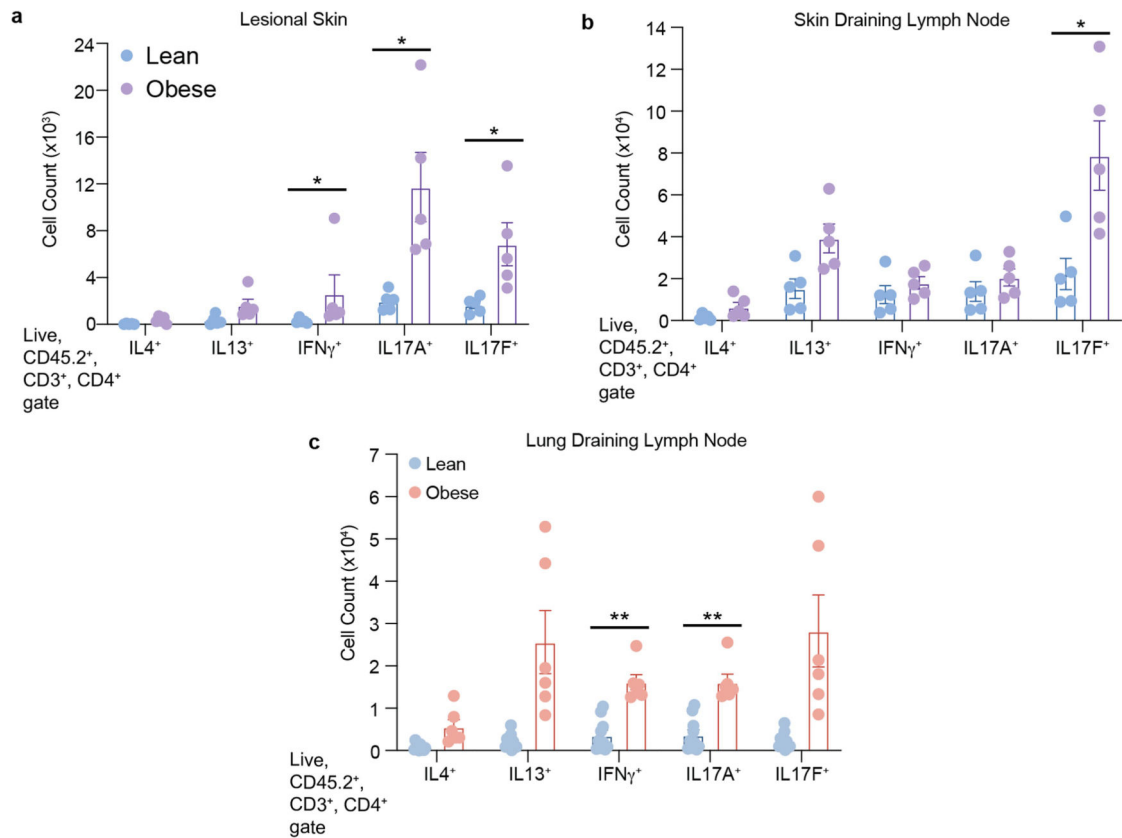
a, Scheme of TOP (Tape stripping followed by Ova-Papain exposure)-induced atopic dermatitis disease model with either high fat diet (HFD) or normal diet (ND). Black arrows indicate tape stripping followed by OVA-papain administration. **b**, Change in ear thickness during AD development. **c**, Representative pictures of ears at Day 8 of challenge. **d**, Representative H&E ear histology, Day 8. Scale bars, 100 μ m. **e–g**, Timeline of body mass (**e**), change in ear thickness (**f**), and representative pictures of ears (**g**) at Day 8 of

challenge of always obese (AO), once obese/now lean (OO), and never obese (NO) mice. Red arrow (e) indicates replacement of HFD with control lean diet for OO mice. h, Scheme of OVA-Alum allergic asthma model. Purple arrows indicate OVA-Alum i.p. injection. i, Total immune cell numbers from bronchoalveolar lavage fluid upon sacrifice on Day 24. j, Total CD4⁺ and CD8⁺ T cell numbers from lung draining lymph node, Day 24. k, Total T_{conv}, T_{reg}, and CD8⁺ T cell numbers from whole ear of lean and obese mice challenged with MC903-AD, Day 11. Asthma model was conducted once. Macro, macrophages; Eos, eosinophils; Lymph, lymphocytes; PMNs, polymorphonuclear leukocytes; n = 5 for (b–g); n = 10 for Lean-OVA and Obese-OVA, n = 5 for Lean-Control, and n = 4 for Obese-Control in (i); n = 10 for Lean-OVA, n = 6 for Obese-OVA, n = 4 for Lean-Control, and n = 3 for Obese-Control in (j). n = 5 for all groups in (k). Data are mean ± s.e.m. Peak values were tested with Welch's *t*-test in (b) and ordinary one-way ANOVA with preselected followup tests as indicated in (f). *P* values were adjusted for multiple comparisons using Šídák's multiple comparisons test in (f) and the Holm-Šídák method in (i–k) where only the Lean-OVA and Obese-OVA groups were compared in (i, j). #*P* < 0.12, **P* < 0.05, ***P* < 0.01.



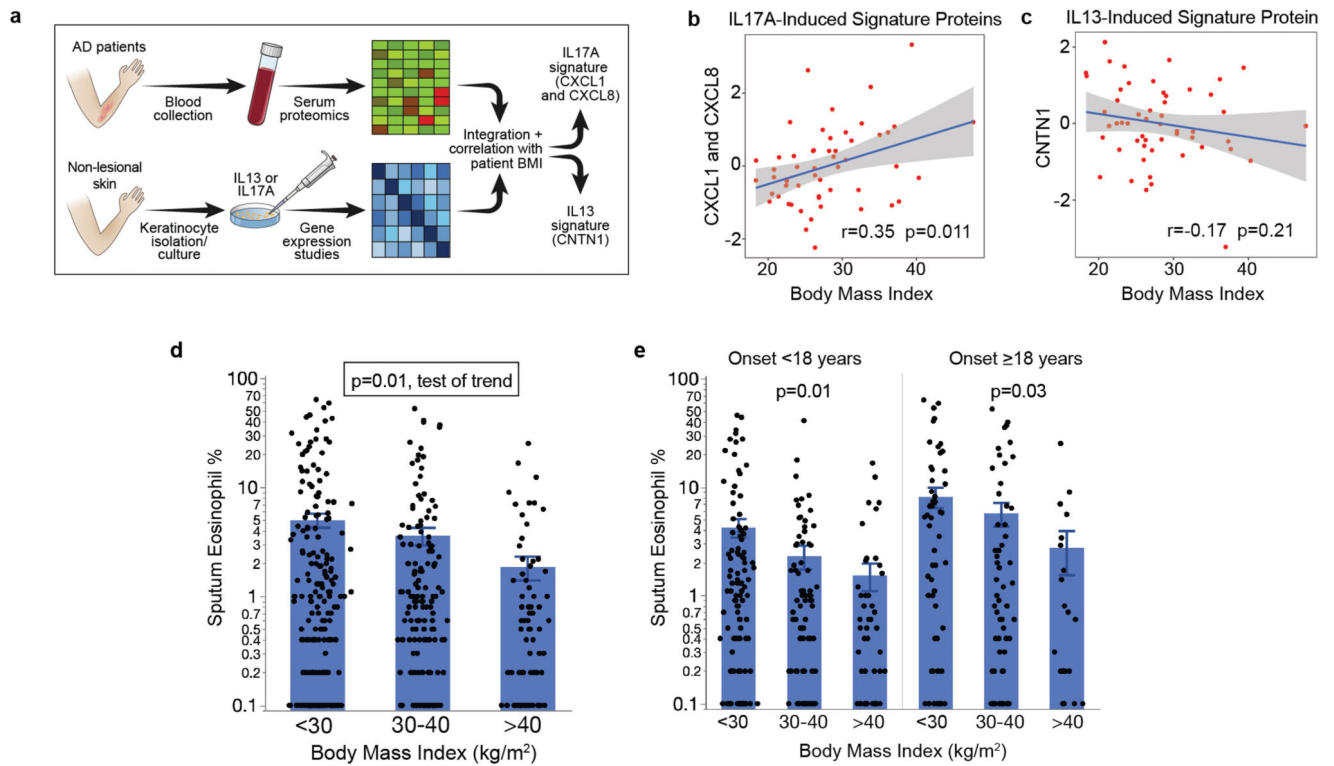
Extended Data Fig. 3 | Selected flow cytometry gating strategies for evaluating cytokine-competence.

a, b, Different T cell subsets were identified through the use of distinct antibody cocktails. Here, using obese mice, we show how the skin-resident/infiltrating hematopoietic cells can be analyzed by flow cytometry to identify CD4⁺ T cells (**a**) and selected cytokine competence of those CD4⁺ T cells (**b**).



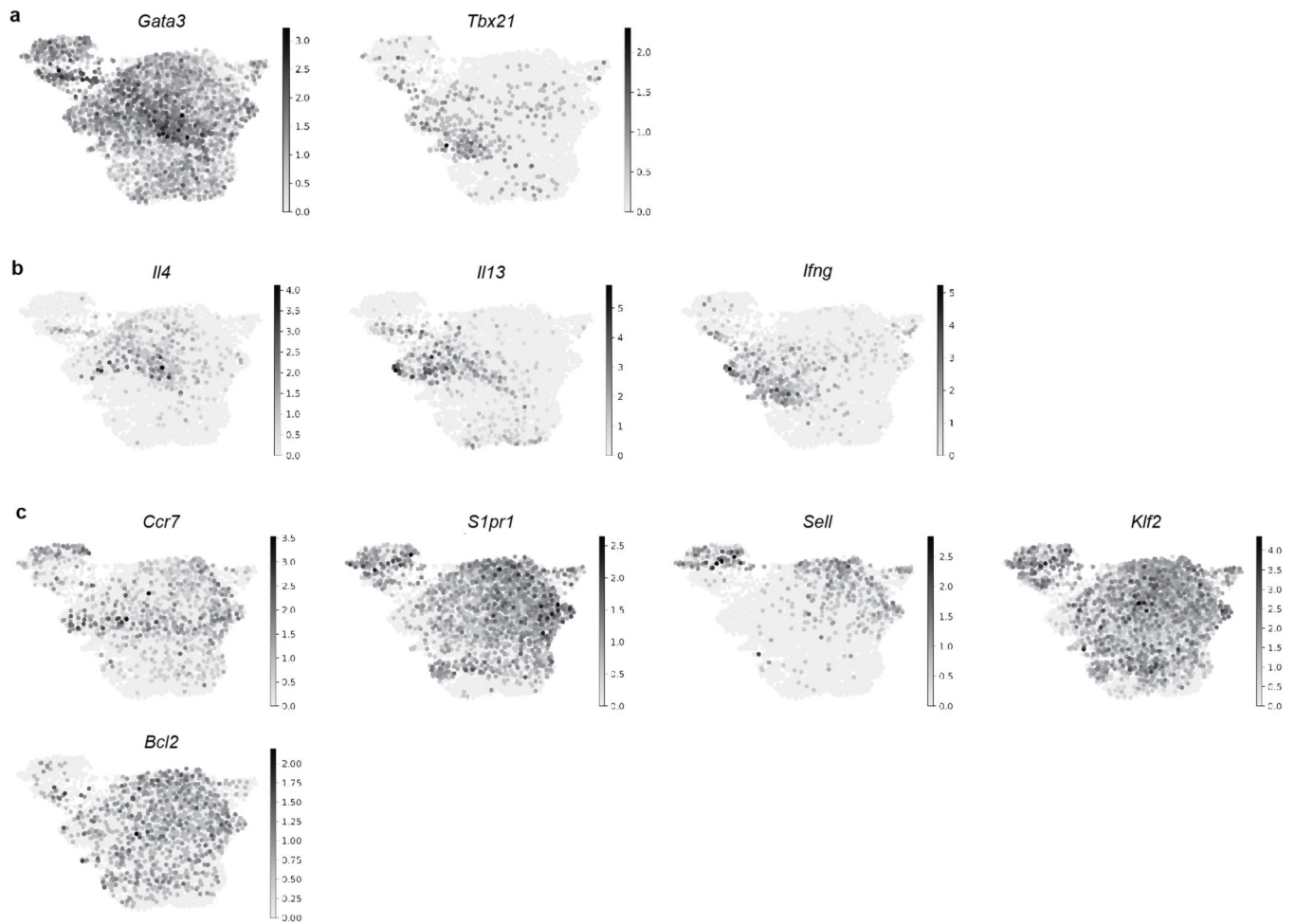
Extended Data Fig. 4 |. Multiple models of allergic inflammatory disease demonstrate increased TH17-driven inflammation in obesity.

a, b, Total IL4⁺, IL13⁺, IFN γ ⁺, IL17A⁺, and IL17F⁺ cell numbers of CD4⁺ T cells from whole ear (**a**) or ear skin draining lymph node (**b**) at Day 8 of mice challenged with TOP-induced AD. **c**, Total IL4⁺, IL13⁺, IFN γ ⁺, IL17A⁺, and IL17F⁺ cell numbers of CD4⁺ T cells from bronchoalveolar lavage fluid upon sacrifice on Day 24 of mice challenged with experimental allergic airway disease (ovalbumin sensitization and challenge). $n = 5$ for all groups in (**a, b**); $n = 10$ for Lean and $n = 6$ for Obese in (**c**). Mann-Whitney tests were conducted in (**a, b**) and P values were adjusted for multiple comparisons using Holm-Šidák method in (**a-c**). * $P < 0.05$, ** $P < 0.01$.

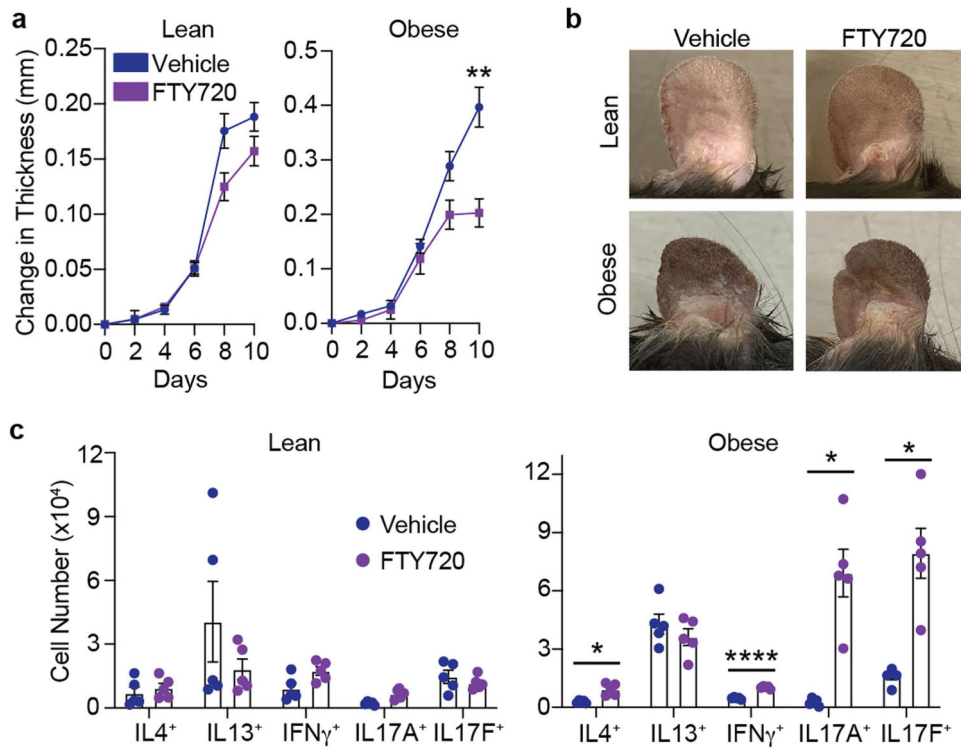


Extended Data Fig. 5 | Evidence of increased T_H17 inflammation or decreased T_H2 -associated pathology in obese patients with allergic disease.

a, Scheme demonstrating workflow and integration of human AD patient serum proteomics with cytokine-induced gene expression studies of human keratinocytes (KCs) correlated with patient BMI. **b**, **c**, Scatterplots depicting the serum protein levels from AD patients of genes specifically induced by IL17A (**b**) or IL13 (**c**) in human KCs versus patient BMI. $n = 59$ moderate-to-severe AD patients for scatter plots in (**b**, **c**). r and p values for scatter plots in (**b**, **c**) were obtained using Spearman rank correlation. **d**, **e**, Sputum eosinophil percentage from human severe asthma patients across a range of BMIs, on their first clinical baseline visit as part of SARP, represented irrespective of age of onset (**d**) or broken down by age of onset (**e**). $n = 272$, 211 , and 95 for patients with BMI <30 , $30-40$, and >40 , respectively in (**d**). $n = 113$, 83 , and 52 (pediatric-onset patients) and $n = 70$, 66 , and 23 (adult-onset patients) with BMI <30 , $30-40$, and >40 , respectively in (**e**). P value from test of linear trend (post-test after statistically significant ordinary one-way ANOVA) in (**d**, **e**).

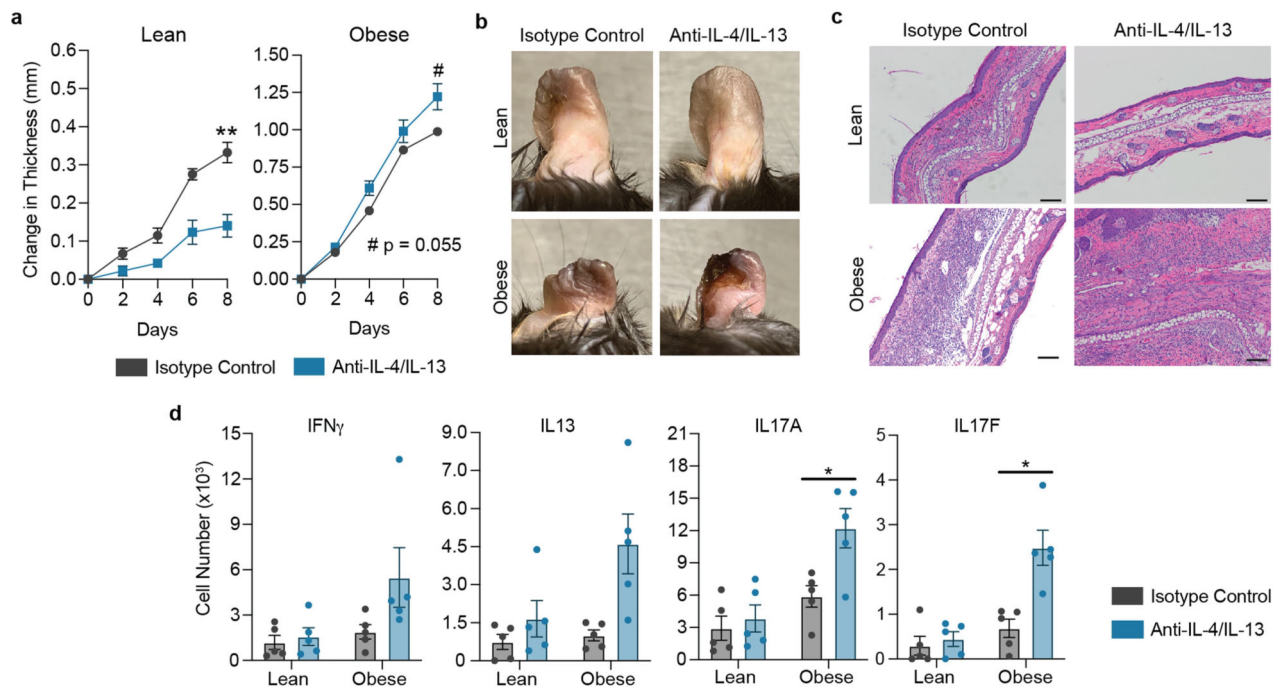


Extended Data Fig. 6 |. Gene heatmaps from scRNA-Seq data used in Fig. 1 and Fig. 2. Heatmaps of transcription factors (a), cytokines (b), and markers of activation, quiescence, and memory (c) overlain on UMAP plot from Fig. 1f to assign names to clusters. Grayscale indicates gene expression, with the highest expressing cells in black.



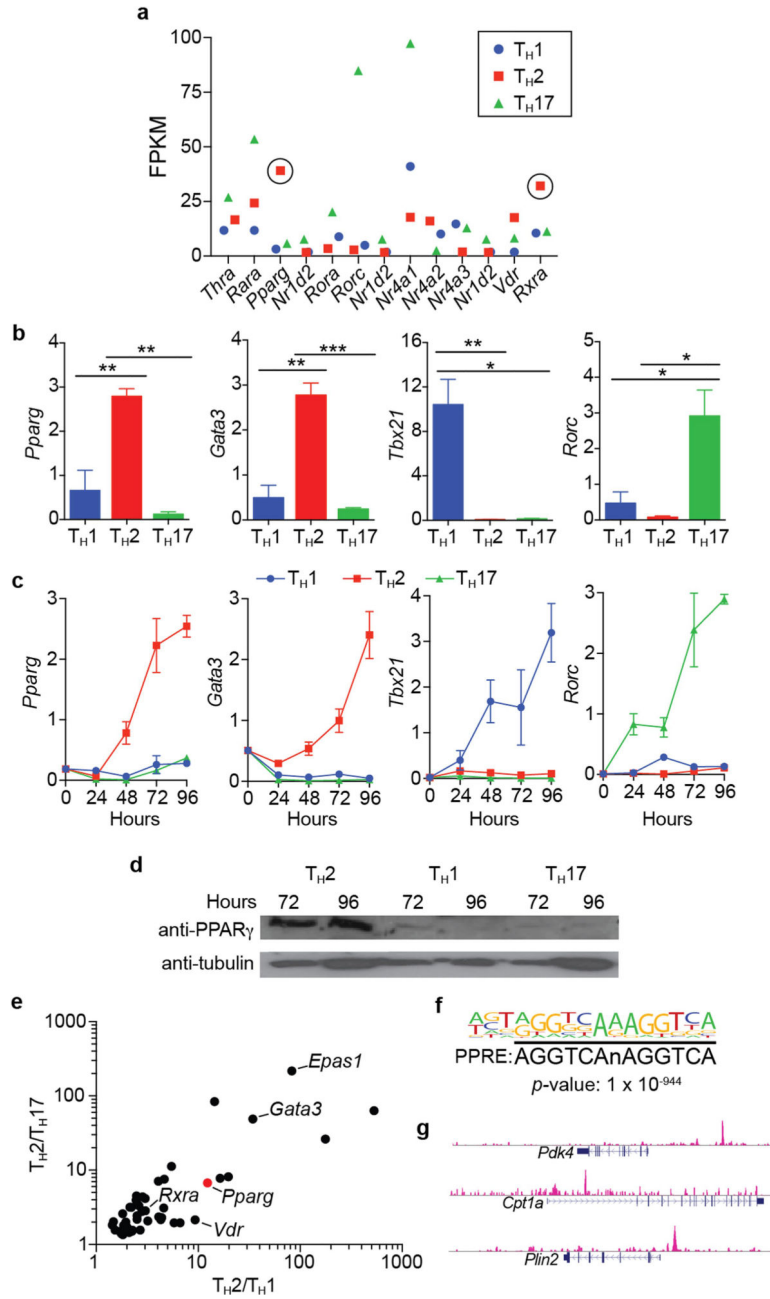
Extended Data Fig. 7 | Blocking lymphocyte egress from secondary lymphoid organs during AD challenge reduces disease severity and TH17 inflammation in the lesions of obese mice.

a. Change in ear thickness during development of MC903-AD of lean or obese mice treated with vehicle (water) or FTY720. **b.** Representative pictures of ears at Day 10 of challenge. **c.** Total IL4⁺, IL13⁺, IFN γ ⁺, IL17A⁺, and IL17F⁺ CD4⁺ T cell numbers assessed by flow cytometry from draining lymph node of lean (left) and obese (right) mice treated with vehicle or FTY720. $n = 5$ for all groups in (a–c). Data are mean \pm s.e.m. Peak values were tested with Welch's t -test in (a). P values were adjusted for multiple comparisons using Holm-Šídák method in (c). * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.



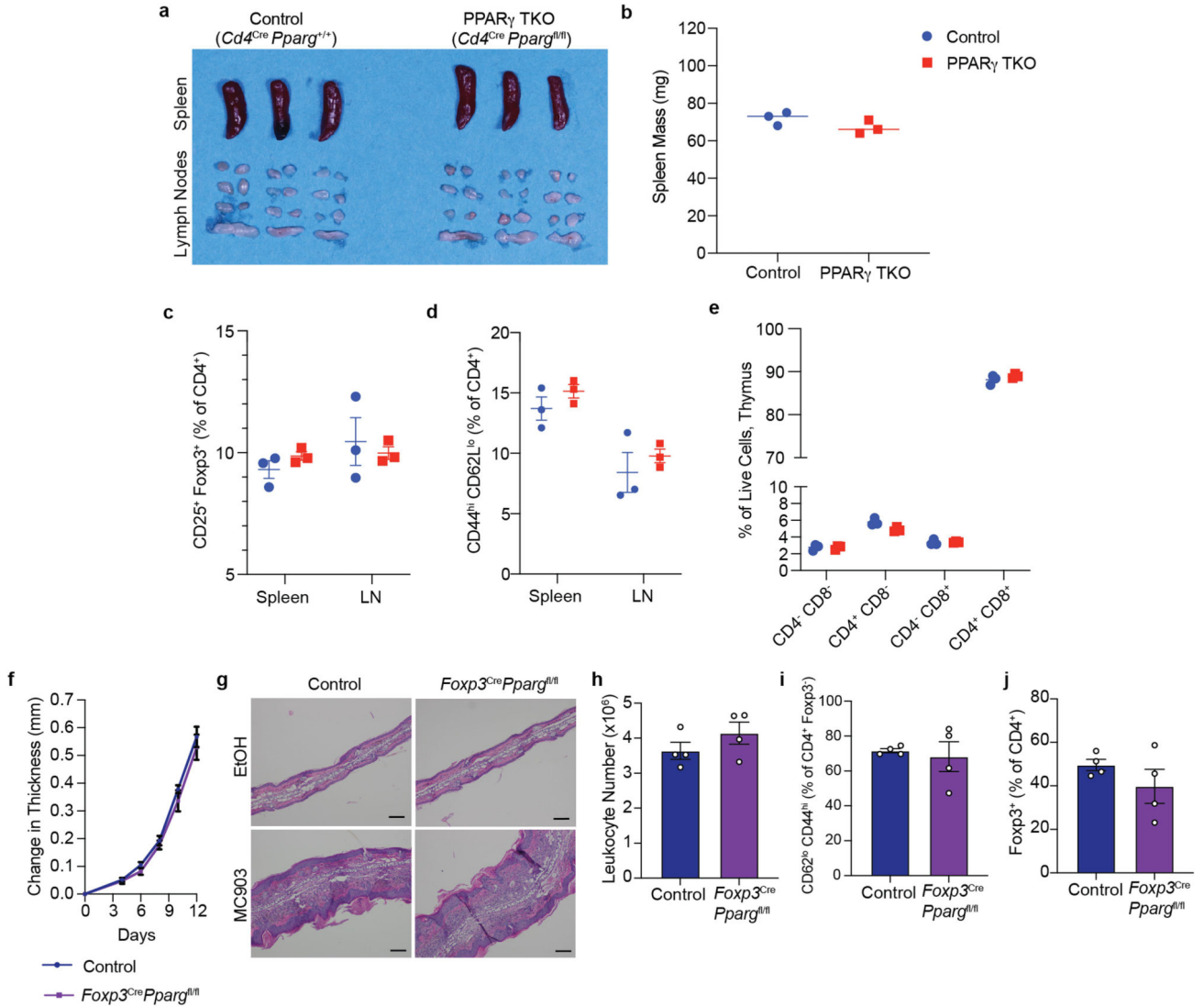
Extended Data Fig. 8 | Targeted anti-IL-4/IL-13 blockade is ineffective in obese mice challenged with TOP.

a. Change in ear thickness during development of TOP-AD of lean or obese mice treated with anti-IL-4/IL-13 or IgG1 isotype control. **b.** Representative pictures of ears at Day 8 of challenge. **c.** Representative images of H&E-stained histology of ears at Day 8. Scale bars, 100 μ m. **d.** Lesional number of CD4⁺ T cells with the indicated cytokine competence as measured by intracellular cytokine staining via flow cytometry from lean and obese mice treated with anti-IL-4/IL-13 or isotype control. $n = 5$ for all groups in (**a–d**). Data are mean \pm s.e.m. Peak values were tested with Welch's t -test in (**a**). P values were adjusted for multiple comparisons using Holm-Šídák method in (**d**). # $P < 0.06$, * $P < 0.05$, ** $P < 0.01$.



Extended Data Fig. 9 | PPAR γ is differentially expressed in *in vitro* differentiated T_H2 cells.
a, Fragments per kilobase of transcripts per million mapped reads (FPKM) values of nuclear hormone receptor (NHR) superfamily genes differentially expressed in *in vitro* differentiated T_H1 , T_H2 , and T_H17 cells. NHR genes that are differentially expressed in T_H2 cells are encircled (cells pooled from 4 mice before inducing differentiation in triplicate, same data set used in (e)). **b**, **c**, Relative expression (using *Hprt* expression as housekeeping gene) of indicated genes in *in vitro* differentiated T_H1 , T_H2 , and T_H17 cells (cells pooled from 4 mice before inducing differentiation in triplicate). **b**, Gene expression determined at Hour 120 post induction of differentiation. **c**, Time course of gene expression from Hours 0–96 post induction of differentiation. **d**, Western blot of PPAR γ and tubulin at Hours 72 and 96 in

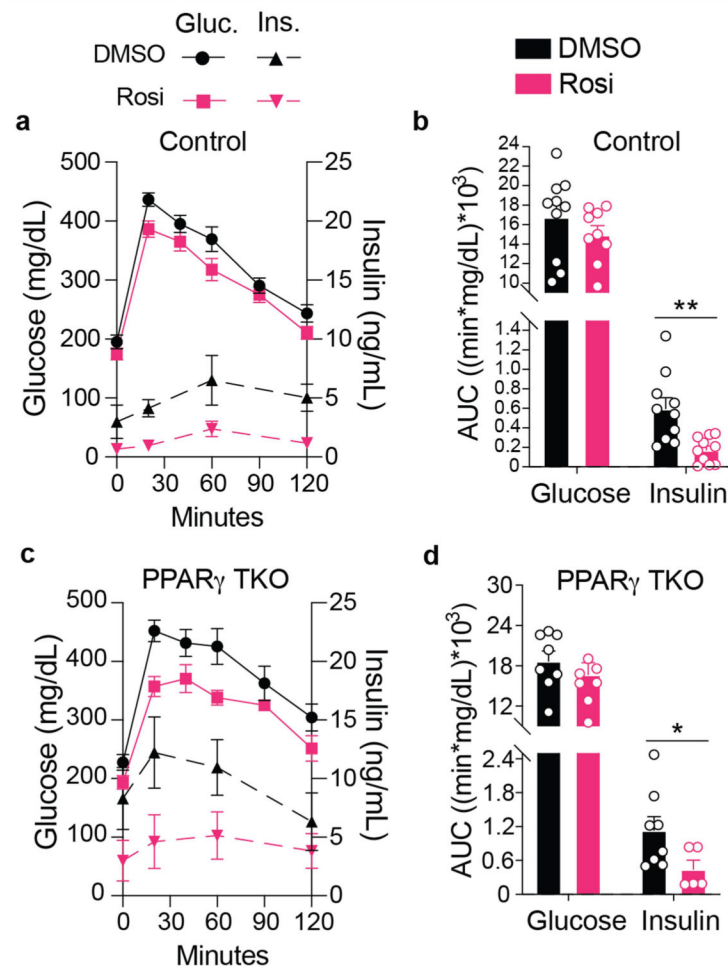
in vitro differentiated T_H1, T_H2, and T_H17 cells (cells pooled from 3 mice before inducing differentiation). **e**, FPKM values of genes that are differentially expressed in T_H2 cells and involved in transcriptional regulation. Position of *Pparg* is marked with a red dot. **f**, Top scoring DNA motif of PPAR γ ChIP-Seq peaks in *in vitro* differentiated T_H2 cells via *de novo* analysis (cells pooled from 4 mice). **g**, Visualization of PPAR γ ChIP-Seq experiment utilizing UCSC genome browser across following genomic loci: *Pdk4*, *Cpt1a*, *Plin2*. Data are mean \pm s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test.



Extended Data Fig. 10 | PPAR γ TKO mice display neither overt systemic inflammation nor altered T cell development, and PPAR γ T_{reg} cKO (*Foxp3^{Cre} Pparg^{fl/fl}*) mice do not display an overt difference in AD severity relative to controls.

a–e, Comparison between PPAR γ TKO and Control (*Cd4^{Cre} Pparg^{+/+}*) mice. **a**, Picture of control and PPAR γ TKO spleens and lymph nodes. **b**, Spleen mass. **c**, **d**, T_{reg} (**c**) and activated T_{conv} (**d**) cell frequency in spleen and LN. **e**, Developing T cell subsets in thymus. **f–j**, Comparison between PPAR γ T_{reg} cKO and Control (*Foxp3^{Cre} Pparg^{+/+}*) mice. **f**, Change in ear thickness during development of atopic dermatitis. **g**, Representative images

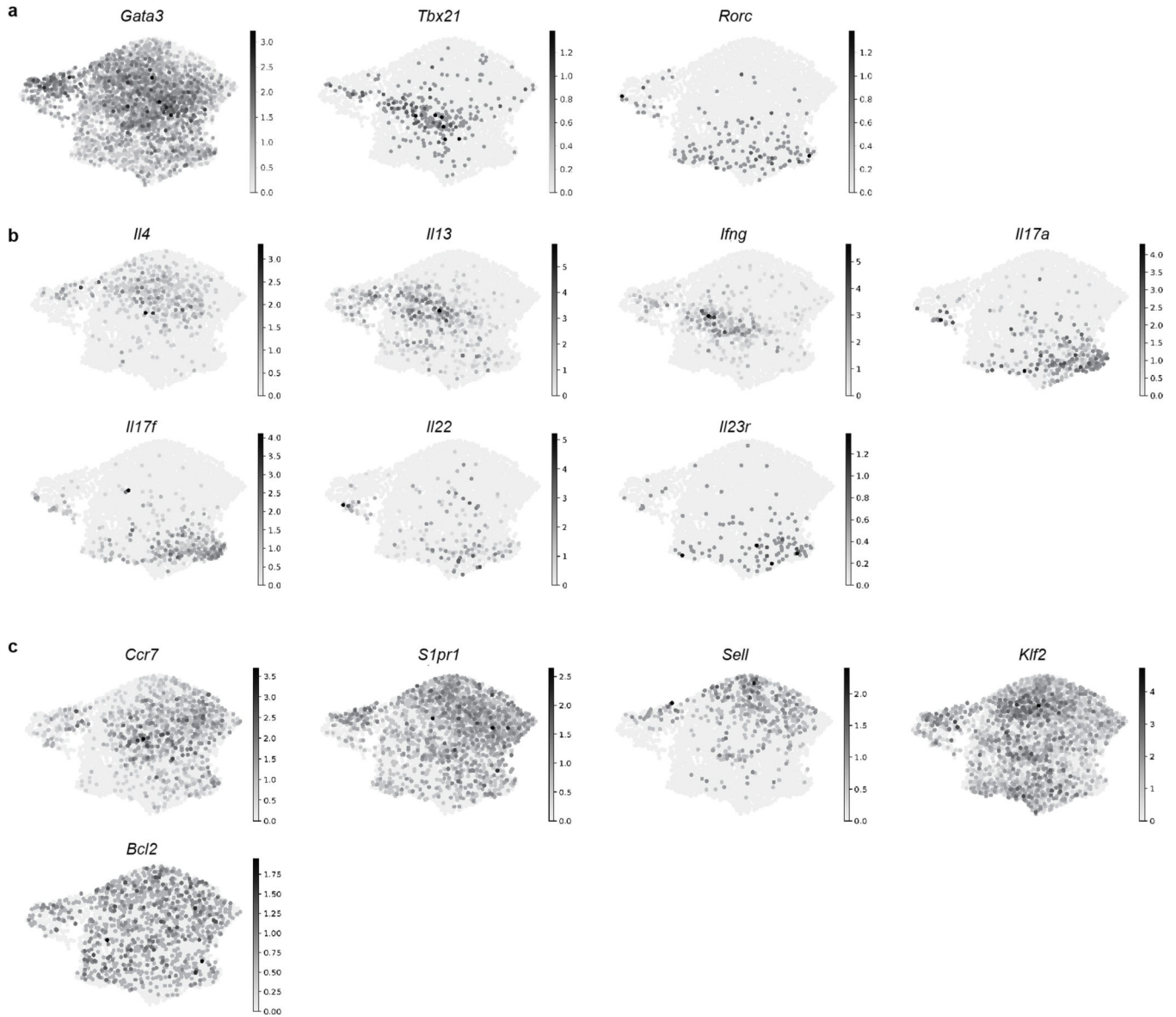
of H&E-stained histology of ears at Day 10. Scale bars, 100 μ m. Dashed line, leukocytic expansion of the dermis. **h**, Absolute lesional leukocyte number assessed by flow cytometry at Day 13. **i**, Lesional activated T_{conv} cells as a percentage of T_{conv} cells assessed by flow cytometry at Day 13. **j**, Lesional T_{reg} cells as a percentage of CD4⁺ T cells assessed by flow cytometry at Day 13. Control, *Foxp3*^{Cre} *Pparg*^{+/+}. LN, lymph node. For (**a–e**), n = 3 mice per group. For (**f–i**), n = 4 mice per group. Data are mean \pm s.e.m.



Extended Data Fig. 11 | PPAR γ in T cells is dispensable for the insulin-sensitizing action of TZDs.

a–d, Glucose tolerance tests (GTTs) of Control (**a, b**) or PPAR γ TKO (**c, d**) mice that have been treated with or without Rosi. Glucose and insulin traces (**a, c**) and corresponding area under the curves (AUCs) (**b, d**) are presented. Control, *Cd4*^{Cre}; PPAR γ TKO, *Cd4*^{Cre} *Pparg*^{fl/fl}; For (**a–d**) n = 10 for all Control mice with exception in (**a, b**) where n = 9 for the glucose measurements for mice treated with Rosi; n = 8 for PPAR γ TKO mice with exception in (**c, d**) where n = 7 for the PPAR γ TKO Rosi-treated mice that were sampled for the serum glucose measurements and n = 5 for the PPAR γ TKO Rosi-treated mice that were sampled for the serum insulin measurements. Gluc., Glucose; Ins., Insulin; Rosi,

Rosiglitazone. This experiment was conducted once with a weight- and age-matched cohort. Data are mean \pm s.e.m. * $P < 0.05$, ** $P < 0.01$.



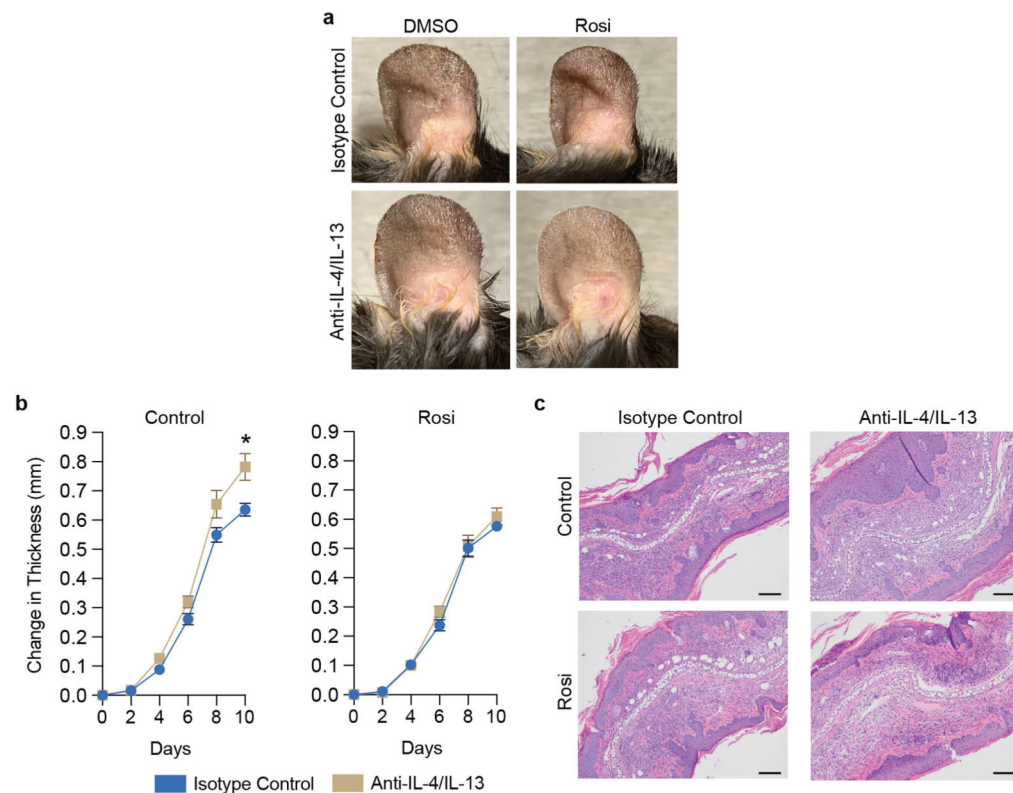
Extended Data Fig. 12 | Gene heatmaps from scRNA-Seq data used in Fig. 3. Heatmaps of transcription factors (a), cytokines/cytokine receptors (b), and markers of activation, quiescence, and memory (c) overlain on UMAP plot from Fig. 3c to assign names to clusters. Grayscale indicates gene expression, with the highest expressing cells in black.

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Extended Data Fig. 13 | Pictures of the ears of obese DMSO- or Rosi-HFD fed mice challenged with AD while treated with anti-IL-4/IL-13 or IgG1 isotype control (a), and initiating Rosi treatment at AD-challenge onset prevents worsening of disease upon treatment with anti-IL-4/IL-13 in obese mice (b, c).

a, Representative pictures of ears at Day 10. **b**, Change in ear thickness during development of MC903-AD of obese mice treated with Rosi or DMSO with anti-IL-4/IL-13 or IgG1 isotype control. **c**, Representative images of H&E-stained histology of ears at Day 10. Scale bars, 100 μ m. $n = 5$ for all groups in (a–c). Rosi-mixed HFD introduced four weeks prior to initiation of experimental AD in (a) and upon AD-challenge onset in (b, c). Data are mean \pm s.e.m. Peak values were tested with Welch's t -test in (b). * $P < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Competing interests

E.G.Y. has received institutional research funds from and is a consultant for Abbvie, Almirall, Amgen, Asana Biosciences, AstraZeneca, Boehringer-Ingelheim, Cara Therapeutics, Celgene, Elli Lilly, Galderma, Glenmark/Ichnos Sciences, Janssen, Kyowa Kirin, Leo Pharma, Pfizer, Regeneron, and UCB. E.G.Y. has received institutional research funds from AnaptysBio, Innovaderm, KAO, Kiniksa, Novan, Novartis, and Ralexar. E.G.Y. is a consultant for Arena, Aslan Pharmaceuticals, Bristol-Myers Squibb, Connect Pharma, EMD Serono, Evidera, Incyte, Pandion Therapeutics, RAPT Therapeutics, Sanofi, SATO Pharmaceutical, Siolta Therapeutics, Target Pharma Solutions, and Ventyx Biosciences. R.L.G. is a consultant and has equity interest in MatriSys Biosciences and Sente Inc. C.J.Y. is a Scientific Advisory Board member for and holds equity in Related Sciences and ImmunAI, a consultant for and holds equity in Maze Therapeutics, and a consultant for TRex Bio. C.J.Y. has received research support from Chan Zuckerberg Initiative, Chan Zuckerberg Biohub, and Genentech. A.M. is a compensated cofounder, member of the boards of directors and a member of the scientific advisory boards of Spotlight Therapeutics and Arsenal Biosciences. A.M. is a cofounder, member of the boards of directors and a member of the scientific advisory board of Survey Genomics. A.M. is a compensated member of the scientific advisory board of NewLimit. A.M. owns stock in Arsenal Biosciences, Spotlight Therapeutics, NewLimit, Survey Genomics, PACT Pharma, and Merck. A.M. has received fees from PACT Pharma, Juno Therapeutics, Trizell, Vertex, Merck, Amgen, Genentech, AlphaSights, 23andMe, ALDA, Rupert Case Management and Bernstein. A.M. is an investor in and informal advisor to Offline Ventures and a client of EPIQ. The Marson lab has received research support from Juno Therapeutics, Epinomics, Sanofi, GlaxoSmithKline, Gilead and Anthem.

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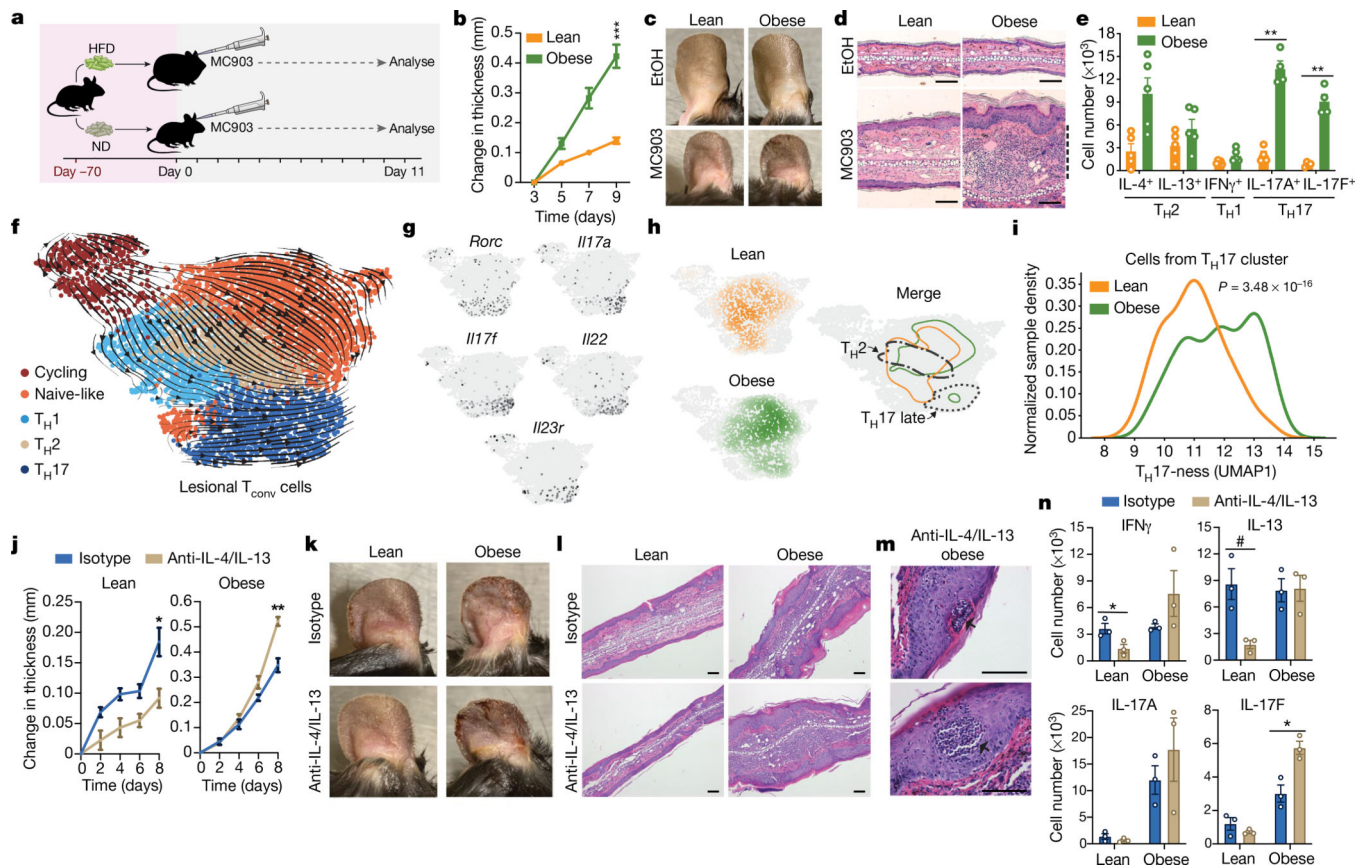


Fig. 1 | Obesity converts a classically T_H2 -driven inflammatory disease to a more severe T_H17 -driven disease that is worsened upon anti- T_H2 antibody treatment.

a, MC903-induced AD model with either high-fat diet (HFD) or normal diet (ND). **b**, Change in ear thickness during development of AD. **c**, Representative images of ears of mice treated with ethanol (EtOH) or MC903, on day 11. **d**, Representative haematoxylin and eosin (H&E) ear histology on day 11. Dashed line, leukocytic expansion of dermis. Original magnification, $\times 200$. **e**, Total cytokine-competent $CD4^+$ T cell numbers from whole ear, on day 11. **f**, RNA velocity visualization of transcriptional trajectories in UMAP space with Leiden clustering of scRNA-seq of T_{CONV} cells from AD-challenged ears on day 10. **g**, Gene-expression heat maps of *Rorc*, *Il17a*, *Il17f*, *Il22* and *Il23r*, with the highest-expressing cells in black. **h**, Distribution of T_{CONV} cells in UMAP space (from **f**) from lean and obese mice after AD challenge. Contour lines are set at identical thresholds. Borders of T_H2 and T_H17 late clusters are outlined. T_H17 late contour outlines cells in the top 50th percentile of *Il17a*, *Il17f*, *Il22* and *Il23r* expression. **i**, T_H17 cluster continuous probability density curves, scaled by sample. **j–l**, Change in ear thickness (**j**), with representative images from day 10 (**k**) and representative H&E ear histology from day 11 (**l**; original magnification, $\times 100$) of lean or obese mice with induced AD treated with anti-IL-4/IL-13 or isotype control. **m**, Representative epidermal pustules (arrows) in obese mice with induced AD and treated with anti-IL-4/IL-13. Original magnification, $\times 400$. **n**, Number of lesional $CD4^+$ T cells with indicated cytokine competence (detected by intracellular cytokine staining and flow cytometry) from lean and obese mice treated with anti-IL-4/IL-13 or isotype control. Scale

bars, 100 μm . $n = 5$ (**b–e**) except $n = 4$ for IL-17A and IL-17F CD4⁺ T cell measurements (**e**); $n = 1$ (pooled from 5 mice per group) for scRNA-seq in **f–i**; $n = 5$ (**j–m**) except $n = 4$ for lean mice with isotype control (**j**); $n = 3$ (pooled from 1–2 mice per sample) (**n**). Data are mean \pm s.e.m. Peak values were tested with Welch's t -test (**b, j**). P values adjusted for multiple comparisons using Holm-Šídák method (**e**); Mann-Whitney U test (**i**). # $P < 0.06$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

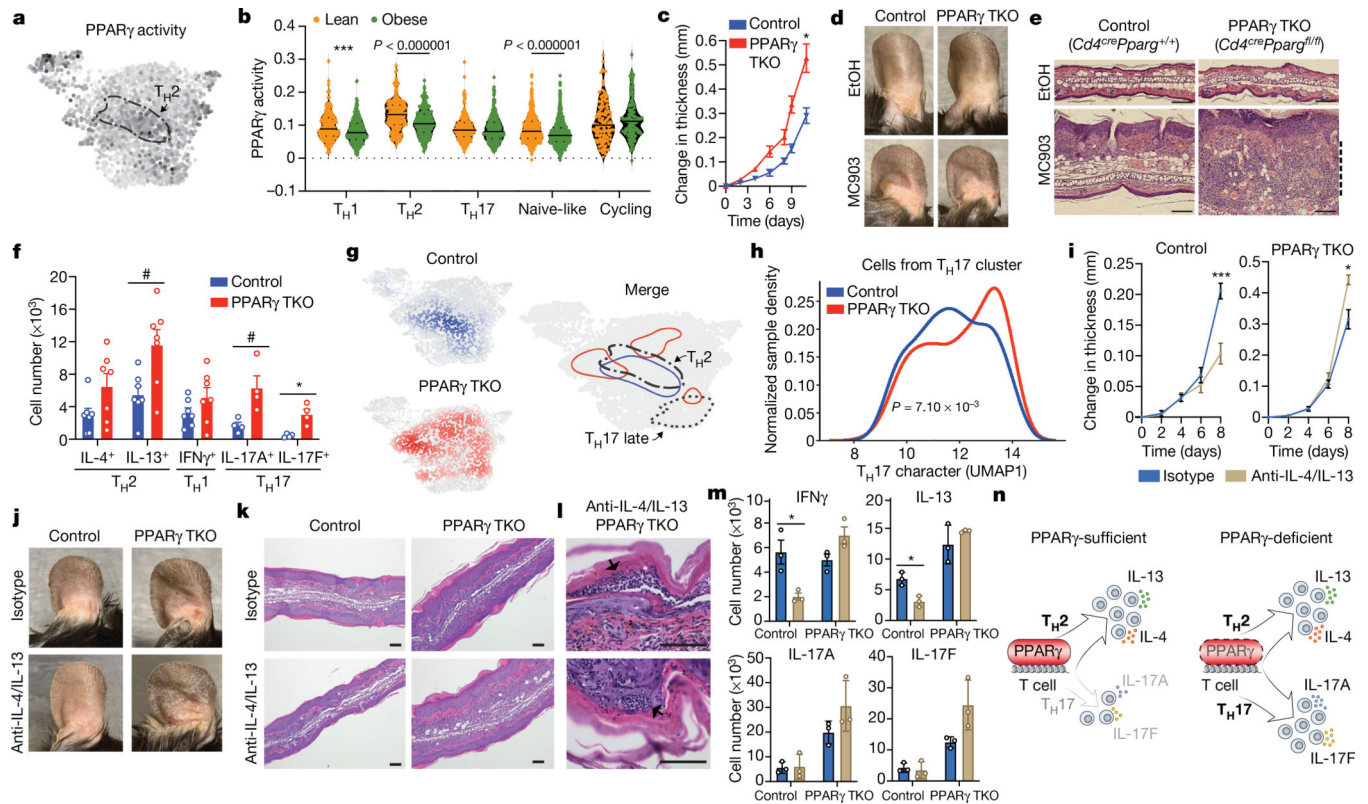


Fig. 2 | Mice with T cell-specific PPAR γ deficiency largely phenocopy obese PPAR γ -sufficient mice upon experimental AD challenge.

a, Heat map of PPAR γ activity (ChIP-seq cistrome expression) using UMAP visualization of scRNA-seq data of T_{conv} cells from Fig. 1f. **b**, Violin plots representing T cell-PPAR γ cistrome expression across distinct Leiden clusters from lean and obese mice. **c**, Change in ear thickness during MC903-induced AD development of PPAR γ -TKO or control mice. **d**, Representative images of ears of mice as in **c** on day 10. **e**, Representative H&E ear histology of mice as in **c** on day 10. Dashed line highlights leukocytic expansion of dermis. Original magnification, $\times 200$. **f**, Total cytokine-competent CD4⁺ T cell numbers assessed by flow cytometry from whole ear of mice as described in **c** on day 11. **g**, Distribution of T_{conv} cells in UMAP space (Fig. 1f) from lean control and PPAR γ -TKO mice after AD challenge. Contour lines are set at identical thresholds. Borders of T_{H2} and T_{H17} late clusters are outlined. T_{H17} late contour defined as in Fig. 1h. **h**, T_{H17} cluster continuous probability density curves of control and PPAR γ TKO, scaled by sample. **i–k**, Change in ear thickness (**i**), representative images from day 10 (**j**), and representative H&E ear histology from day 11 (**k**; original magnification, $\times 100$), of control or PPAR γ -TKO mice with induced AD and treated with anti-IL-4/IL-13 or isotype control. **l**, Representative epidermal pustules (arrows) of PPAR γ -TKO mice with induced AD and treated with anti-IL-4/IL-13. Original magnification, $\times 400$. **m**, Lesional CD4⁺ T cell numbers with indicated cytokine competence. **n**, Proposed PPAR γ function as focusing factor for T_{H2} responses in vivo. Control, *Cd4^{cre}*; PPAR γ TKO, *Cd4^{cre}Pparg^{fl/fl}*. Scale bars, 100 μ m. $n = 5$ (**c–e**); $n = 7$ (**f**) except $n = 4$ for IL-17A/F-competent T cells; $n = 1$ (pooled from 5 mice per group) for scRNA-seq (**g**, **h**), run with Fig. 1 samples (batch 1 in Methods, ‘scRNA-seq’); $n = 6$ (**i–l**) except $n = 5$

for PPAR γ TKO + isotype control; $n = 3$ (each pooled from 2 mice) (**m**). Data are mean \pm s.e.m. Only peak values were tested with Welch's t -test in (**c**, **i**). P values adjusted for multiple comparisons using Holm-Šídák method (**f**). Mann-Whitney U test (**h**). [#] $P < 0.07$, * $P < 0.05$, *** $P < 0.001$.

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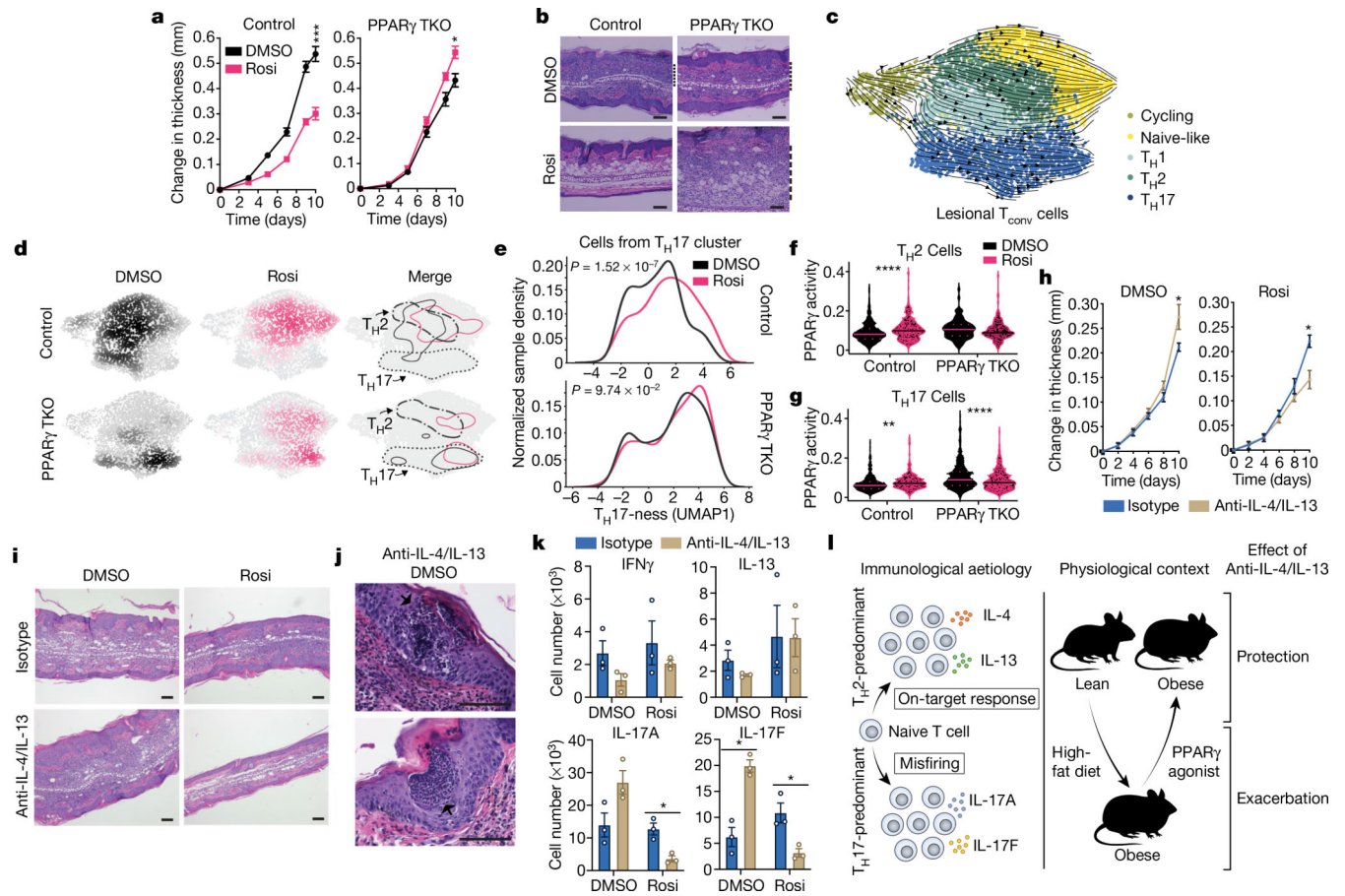


Fig. 3 | Treatment with PPAR γ agonist reduces TH17-inflammation and restores efficacy of anti-TH2 antibody treatment in obese mice challenged with AD.
a, Change in ear thickness during AD development of obese PPAR γ -TKO or control mice treated with rosiglitazone (Rosi) or DMSO. **b**, Representative H&E ear histology on day 10 of cells treated as in **a**. **c**, RNA velocity visualization of transcriptional trajectories in UMAP space with Leiden clustering of scRNA-seq data of T_{CONV} cells from AD-challenged ears on day 10. **d**, Distribution of T_{CONV} cells in UMAP space from **c** isolated from obese control or PPAR γ -TKO mice treated with DMSO or rosiglitazone after AD challenge. Contour lines set at identical thresholds. Borders of TH2 and TH17 clusters are outlined. **e**, TH17 cluster continuous probability density curves of obese control and PPAR γ -TKO mice fed with DMSO or rosiglitazone-mixed HFD, scaled by sample. **f**, **g**, Violin plots representing T cell-PPAR γ activity (ChIP-seq cistrome expression) in TH2 (**f**) and TH17 (**g**) cells from obese control or PPAR γ -TKO mice treated with rosiglitazone or DMSO, using scRNA-seq data from **c**. **h**, **i**, Change in ear thickness (**h**) and representative H&E ear histology on day 10 (**i**; original magnification, $\times 100$) of obese mice with induced AD fed DMSO- or rosiglitazone-mixed HFD, treated with anti-IL-4/IL-13 or isotype control. **j**, Representative epidermal pustules (arrows) of obese B16 mice with induced AD fed with DMSO-mixed HFD and treated with anti-IL-4/IL-13. Original magnification, $\times 400$. **k**, Lesional CD4⁺ T cell number with indicated cytokine competence. **l**, Model of PPAR γ agonists as immunopathological modifiers in obese mice to enable targeted therapy against a

classically T_H2-driven disease. Control, *Cd4^{cre}* (except for scRNA-seq, when wild-type B16 mice co-housed with the PPAR γ -TKO mice were used). Scale bars, 100 μ m. Rosiglitazone-mixed HFD was introduced four weeks before initiation of experimental AD (**h–k**). $n = 5$ (**a, b, h–j**); $n = 1$ (pooled from 5 mice per group) for scRNA-seq (**d, e**); $n = 3$ (each pooled from 2 mice) (**k**). Data are mean \pm s.e.m. Peak values tested with Welch's *t*-test (**a, h**). Mann–Whitney *U* test (**e**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.