Obesity Potentiates T_H2 Immunopathology via Dysregulation of PPAR γ

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Abstract

How obesity affects immune function is not well understood. Clinically, obesity is strongly associated with severe T_H2 immunopathology¹⁻³, though the physiological, cellular, and molecular underpinnings of this association remain obscure. Here, we demonstrate that obese mice are susceptible to severe atopic dermatitis (AD), a major manifestation of T_H2 immunopathology and disease burden in humans^{4,5}. Mechanistically, we show that dysregulation of the nuclear hormone receptor (NHR) PPAR γ (peroxisome proliferator-activated receptor gamma) in T cells is a causal link between obesity and the increased T_H2 immunopathology. We find that PPAR γ oversees a cellular metabolic transcriptional program that restrains nuclear gene expression of the chief T_H2 priming and effector cytokine interleukin-4 (IL-4). Accordingly, thiazolidinediones (TZDs), potent PPAR γ agonists, robustly protect obese mice from T_H2 immunopathology. Collectively, these findings establish PPAR γ as a molecular link between obesity and T_H2 immune homeostasis and identify TZDs as novel therapeutic candidates for T_H2 immunopathology. Fundamentally, these findings demonstrate that shifting physiologic metabolic states can shape the tone of adaptive immune responses to modulate differential disease susceptibility.

Obesity is a metabolic state that is characterized by several pathophysiological processes, imposing severe disease burden in the world today⁶⁻⁸. The immune system plays a fundamental role in restraining and potentiating much of this disease burden; however, little is known about how obesity shapes the immune system to drive

immunopathology. Recent epidemiological and clinical studies have uncovered associations between obesity and AD, a prominently T_H2 -driven immunopathology in humans^{2,9}, and the striking co-increase in worldwide incidence of obesity and AD, suggest the two seemingly independent disease entities may be related^{6,8,10}. To assess the relationship between obesity and AD, we challenged obese mice fed high-fat diet (HFD) with a well-characterized experimental model of MC903-induced AD on the ear^{11,12} (Figure 1a). Strikingly, the HFD mice, compared to control lean mice, displayed an overall increased inflammatory response, as measured grossly by a ~3-fold increase in change in ear thickness (Figure 1b). Consistently, physical examination of the MC903-treated ears revealed prominent erythema and scale which was increased in the HFD mice, hallmarks of dermal inflammation (Figure 1c). Under histological evaluation, the obese mice exhibited increased epidermal acanthosis, hyperkeratosis, and spongiosis, hallmark characteristics of human AD. Impressively, the dermis was significantly expanded with leukocytic inflammation (dashed line), which was relatively diminished in the lean mice (Figure 1d).

Since atopic disease is classically driven by T_H2 immunity¹³⁻¹⁵, we sought to further characterize the infiltrating T cells induced by our experimental model of AD. Flow cytometric analyses revealed that the $CD4^+$ conventional T cell (T_{conv}), regulatory T cell (T_{reg}), and $CD8^+$ T cell populations were all increased in the obese mice (Figure 1e); however, the T_{conv} cells were the predominant T cell population and were significantly increased in their IL-4-competent (~8.2 fold increase) but not $IFN\gamma$ -competent populations compared to the lean mice (Figure 1f), an indicator of an increased T_H2 -polarized

response. Of note, the link between obesity and T_H2 immunopathology is dose-dependent as mice fed short-term HFD also display an increased inflammatory response upon induction of experimental AD, though to a lesser extent compared to the conventional HFD obese mice (Extended Data Figure 1a-f).

Nuclear hormone receptors (NHRs) are sensors of shifting physiological states^{16,17}, and we wondered how NHRs were expressed in different types of T_H subsets and if any NHRs were specifically upregulated in T_H2 cells. We surveyed the expression of NHRs in *in vitro* differentiated T_H1, T_H2, and T_H17 cells and identified *Pparg* (and its heterodimeric partner *Rxra*) as highly and differentially expressed in T_H2 cells (Extended Data Figure 2a). Further focused validation evaluating end-point and time-course gene expression as well as protein expression clearly identified PPAR γ as differentially and highly expressed in T_H2 cells (Extended Data Figure 2b-d). Further, a differential analysis of genes involved in transcriptional regulation across *in vitro* differentiated T_H1, T_H2, and T_H17 cells revealed *Pparg* expression to be highly specific to T_H2 cells (Extended Data Figure 2e.) Consistent with our experiments, PPAR γ has recently been demonstrated as a transcription factor important for regulating transcriptional networks in T_H2 cells, though its physiological relevance and functional importance is not well understood¹⁸⁻²⁰.

PPAR γ and its ligands are master regulators of adipogenesis²¹⁻²⁴. It is highly expressed in adipocytes and several other cell types (including immune cells such as fat-resident T_{reg} cells^{25,26}) found within adipose tissue. Since obesity-associated dysregulation of PPAR γ activity in adipose tissue is an established cause of adipose tissue insulin resistance, we hypothesized that PPAR γ may be expressed *in vivo* in T_H2

cells and similarly undergo obesity-associated dysregulation to promote a maladaptive T_H2 immune responses in obese mice^{22,24,27}. Indeed, by sorting on the infiltrating $CD4^+$ T_{conv} and T_{reg} cells of lean mice, we were able to detect an appreciable 2-fold increase in *Pparg* in the T_{conv} but not T_{reg} cells compared to their respective splenic populations (Extended Data 2f).

These findings collectively motivated us to model T-cell specific $PPAR\gamma$ dysregulation *in vivo* by creating T-cell specific $PPAR\gamma$ -deficient mice (*Cd4^{Cre} x Pparg^{fl/fl}*, $PPAR\gamma$ TKO.) Importantly, T cell-specific loss of $PPAR\gamma$ does not elicit any overt systemic inflammation associated with T cell dysregulation. $PPAR\gamma$ TKO mice have normally sized spleens and lymph nodes (Extended Data Figure 3a,b) and equivalent splenic and lymph node T_{reg} ($CD25^+$ $Foxp3^+$) and activated ($CD62L^{lo}$ $CD44^{hi}$) $CD4^+$ T cell populations compared to their control (*Cd4^{Cre} x Pparg^{+/+}*) counterparts (Extended Data Figure 3c,d). Additionally, $PPAR\gamma$ TKOs exhibit normal thymic T cell development as measured by $CD4$ and $CD8$ expression of thymic T cells (Extended Data Figure 3e). However, remarkably, when challenged with experimental AD under normal chow conditions, the $PPAR\gamma$ TKO mice demonstrated a strikingly heightened T_H2 -driven immune response with a ~2.7-fold increase in change in ear thickness and ~60% increase in absolute ear thickness compared to control mice (Figure 2a,b). This heightened gross pathology was consistent with the histological examination which recapitulated many features of the wild-type obese mice including the most striking histologic feature – lymphocytic expansion of the dermis (Figure 2c, dashed line). Further flow cytometric analysis of the infiltrating immune cells demonstrated a ~3-fold increase in IL-4-competent $CD4^+$ T_{conv} cells (Figure 2d) with

similar numbers of IFN γ -competent CD4⁺ T_{conv} cells (Figure 2e). By contrast, when obese PPAR γ TKO and controls were challenged with experimental AD, the differences in gross and histological pathology were largely diminished (Figure 2a-c). Additionally, flow cytometric analyses revealed no appreciable difference between obese PPAR γ TKO and control mice in IL-4-competent T_{conv} cells and IFN γ -competent T_{conv} cells (Figure 2d,e). These results suggest that HFD-induced obesity is largely epistatic to T cell-specific genetic deficiency of *Pparg* in controlling T_H2 immune homeostasis, linking the obesity-associated susceptibility to T_H2 immunopathology with dysregulation of PPAR γ in T cells.

Our *in vivo* studies revealed PPAR γ as a T cell-intrinsic restraint on T_H2 immune responses. Although the transcriptomic and cistromic biology of PPAR γ has been studied extensively in the context of macrophages and dendritic cells²⁸⁻³¹, the genomic regulation of PPAR γ in T cells is less studied. Intriguingly, a recent study utilizing genome-wide CRISPR knock-out screens included PPAR γ as part of the core transcriptional factors regulating T_H2 activation and identity²⁰; however, the functional role of PPAR γ activation or deficiency on T_H2 cells has not been advanced. To gain mechanistic insights into how PPAR γ may be regulating T_H2 immune responses and restraining T_H2-driven immunopathology, we conducted transcriptomic analyses of *in vitro* differentiated T_H2 cells from wild-type or PPAR γ TKO mice with or without treatment with the PPAR γ -agonist rosiglitazone (Rosi) to experimentally activate the PPAR γ transcriptional program. 1071 genes were differentially expressed, and hierarchical clustering revealed clear clusters of Rosi-responsive genes that were dependent on PPAR γ – the Rosi Up Cluster (455 genes) and Rosi Down Clusters I (171 genes) and II (361 genes) (Figure 3a). To complement

transcriptomic analyses, we empirically identified the DNA binding sites for PPAR γ in T_H2 cells via ChIP-Seq. The top DNA-binding motif discovered was the canonical PPAR-response element (PPRE, Figure 3b.) 2534 binding sites mapping to 1563 genes were identified, of which 215 genes were members of the Rosi Up or Down I and II clusters (Figure 3c). Unexpectedly, ontological analyses of these 215 genes identified only one consensus GO term: response to organic cyclic compounds – a parent term for a large group of metabolism-related ontologies (Figure 3d). In contrast, when similar analyses were applied to the entirety of the genes found in the Rosi Up and Rosi Down clusters, the ontologies were exclusively immunological for the Rosi Down Clusters and a mix of metabolic and immunological for the Rosi Up Cluster (Figure 3d). Taken together, this suggests that PPAR γ activation directly activates genes involved in cellular metabolism whose collective expression and function then alters the expression of the genes associated with immunological processes. Congruent with this model, several metabolism genes markedly induced by Rosi treatment exhibited high PPAR γ ChIP peak scores, whereas several key immunological genes induced by Rosi treatment had largely diminished or absent PPAR γ ChIP peak scores (Figure 3e). Importantly, *IL4*, the chief T_H2 priming and effector cytokine, was found in the Rosi Down II Cluster and had an absent PPAR γ ChIP peak score (Figure 3f), as was the case with all cytokines found in the Rosi Down Clusters. This Rosi-induced, PPAR γ -dependent decrease in *IL4* was further confirmed by analysis of targeted gene expression, intracellular staining, and secretion (Extended Data Figure 4a-c).

Metabolic regulation of immune function occurs in a highly context and cell-type specific manner³²⁻³⁴. To further understand the mechanism by which PPAR γ -activated metabolic reprogramming could control immunological function in T_H2 cells, we examined genes in the Rosi Up Cluster that were also direct targets of PPAR γ . We found genes whose consensus activities would promote *de novo* lipogenesis (*Plin2*, *Gpam*, *Dgat1*), fatty acid oxidation (*Pdk4*, *Cpt1a*, *Acaa2*, *Eci2*, *Slc25a20*, *Acadl*, *Acs15*), and mitochondrial mass (*Etfb*, *Mrpl45*, *Mrpl1*), which would likely manifest in mitochondrial substrate usage of fatty acids (Extended Data Figure 4d,e). Indeed, follow-up studies showed a clear Rosi-induced, PPAR γ -dependent increase in mitochondrial mass (Extended Data Figure 4f-h) as well as mitochondrial oxidation of fatty acids (Extended Data Figure 4i,j) with no increase in cellular glucose uptake (Extended Data Figure 4k) or in mitochondrial oxidation of glucose-derived carbons (Extended Data Figure 4l). Collectively, these studies argue for PPAR γ as a core T_H2 transcription factor enforcing mitochondrial oxidative capacity required to restrain over-exuberant T_H2 effector function.

Due to the robust ability of Rosi to decrease T_H2 effector function and enforce lipid catabolic pathways in a PPAR γ -dependent fashion *in vitro*, we hypothesized that perhaps Rosi treatment could be used to protect obese mice from exacerbated AD. Remarkably, mice treated with Rosi had substantially reduced disease, as measured by a ~40% reduction in increase in ear thickness (Figure 4a,b), marked reduction in lymphocytic infiltration (Figure 4c), and a significant decrease in IL-4 competent T_{conv} cells (Figure 4d), effects that were largely dependent on T cell-specific PPAR γ . Interestingly, we did observe effects of Rosi that were not dependent on T cell-specific PPAR γ , including a

modest increase in disease severity (Figure 4a-c) and a reduction in IFN γ -competent T_{conv} cells (Figure 4e), possibly related to T cell-extrinsic mechanisms of PPAR γ action, which has been shown to be expressed in several skin cell types like adipocytes, sebocytes, hair follicles, and keratinocytes³⁵.

Taken together, our studies provide a physiological, cellular, and molecular basis for the clinically observed association between obesity and T_H2 immunopathology, establishing obesity-associated dysregulation of PPAR γ in T cells as an important driver of obesity-associated T_H2 immunopathology. Leveraging mouse genetics, we identify loss-of-function of PPAR γ specifically in T cells as a key mechanistic step in the potentiation of T_H2 immunopathology in the obese state. Unexpectedly, we find that PPAR γ serves as a brake on effector function by controlling mitochondrial metabolism. Further, we demonstrate that the PPAR γ agonists, TZDs, commonly prescribed as insulin-sensitizers for treatment of Type-2 diabetes, could be used to treat T_H2 immunopathology, a novel indication. Fundamentally, we demonstrate that distinct physiological metabolic states can alter cellular metabolic states to shape immune responses in specific ways to modulate disease susceptibility.

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Conflict of Interest Disclosures. A.M. is a co-founder of Arsenal Biosciences and Spotlight Therapeutics. A.M. serves as on the scientific advisory board of PACT Pharma, is an advisor to Trizell, and was a former advisor to Juno Therapeutics. The Marson Laboratory has received sponsored research support from Juno Therapeutics, Epinomics, Sanofi and a gift from Gilead. R.L.G. is a consultant and has equity interest in MatriSys Biosciences and Sente Inc. All other authors deny any conflicts of interest.

Materials and Methods

Mice. All mice were housed in the specific pathogen-free facilities at The Salk Institute for Biological Studies or purchased from The Jackson Laboratory. PPAR γ TKO mice were generated by crossing CD4Cre¹ transgenic mice and *Pparg*^{fl/fl} (ref. 2) mice. We used the *Foxp3*^{Thy1.1} (ref. 3) reporter mice when isolating T_{reg} and T_{conv} CD4⁺ cells from spleen and ear for subsequent RNA analysis. Mice within The Salk Institute for Biological Studies received autoclaved normal chow (MI laboratory rodent diet 5001, Harlan Teklad), irradiated HFD (60 kcal% fat, Research Diets), irradiated HFD mixed with rosiglitazone (15 mg kg⁻¹ of food, Research Diets) or DMSO (Research Diets), or ND (10 kcal% fat, Research Diets). All mice used for studies were male. All procedures involving animals were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) and Animal Resources Department (ARD) of the Salk Institute for Biological Studies.

MC903-induced experimental atopic dermatitis. Mice were anesthetized via isoflurane and MC903 solution (0.1 mM in ethanol, 10 μ L/ear, R&D Systems) was applied to mouse ears daily for 9-12 days. Ear thickness was assessed utilizing a micrometer (Mitutoyo, #227-211). Upon harvest, ears were dissected from the mice and prepared for histological analyses or flow cytometry.

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, 20mg/mL BSA, 20mg/mL penicillin, 20mg/mL streptomycin) containing 2 mg ml⁻¹ Collagenase D (Roche) at 37°C with intermittent shaking for 2 hrs. The suspension was then passed through a 100- μ m 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 subjected to FACS analysis directly or first stimulated with PMA and ionomycin in the presence of brefeldin A (GolgiPlug; BD) for 5 hrs at 37 °C for subsequent intracellular cytokine staining and FACS analysis.

Histological analyses. Sections (4 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: CD4 (RM4-5), CD8 (53-6.7), CD25 (7D4), CD44 (IM7), CD45.2 (104), CD62L (MEL-14), and IFN-γ (XMG1.2); eBioscience: Foxp3 (FJK-16s), IL-4 (11B11), IL-13 (eBio13A), TCRβ (H57–597). For intracellular staining, cells were treated with fixation and permeabilization reagents from BD or eBioscience (for Foxp3 staining) and labeled with appropriate antibodies before being analyzed on a BD FACS Aria Cell Sorter. Data were analyzed using the BD FACS Aria instrument (Becton Dickinson) and FlowJo software (FlowJo LLC).

***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). Naïve (CD25⁻ CD44^{hi} CD62L^{lo}) CD4⁺ T cells were sorted by flow cytometry from the bead purified CD4⁺ T cells. The naïve 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-hamster IgG antibody (200ng/mL; MP Biomedicals) with the addition of soluble anti-CD3 (1µg/mL; 145-2C11) and anti-CD28 (1µg/mL; 37.51) from Bio X Cell. Polarizing conditions for different T helper subsets are as

following: T_H1: hIL2 (100U/ml; PeproTech), mIL-12 (20ng/ml; PeproTech) and anti-IL-4 (5μg/ml; Bio X Cell); T_H2: hIL2 (100U/ml; PeproTech), mIL-4 (20ng/ml; Biolegend), anti-IFN-γ and anti-IL-12 (5μg/ml; Bio X Cell); T_H17: mIL-6 (20ng/ml; Biolegend), hTGF-β (2ng/ml; PeproTech), anti-IFN-γ and anti-IL-12 (5μg/ml; Bio X Cell). When indicated, Rosi was dissolved in DMSO and added to a final concentration of 10μM on Day 0 and Day 3 of 4-5 day cultures.

Primers for qPCR.

Il4 – For: AGGAGCCATATCCACGGATGCGA, Rev: TGTTCTTCGTTGCTGTGAGGACGT;

Il13 – For: CACAGAAGACCAGACTCCCC, Rev: GTTGGTCAGGGAATCCAGGG;

Pparg – For: CACAATGCCATCAGGTTTGGG, Rev: GAAATGCTTTGCCAGGGCTC;

Gata3 – For: CTTCCCACCCAGCAGCCTGC, Rev: CGGTACCATCTCGCCGCCAC;

Tbx21 – For: GTCGCGCTCAGCAACCACCT, Rev: CGGCCACGGTGAAGGACAGG;

Rorc – For: CCGGACATCTCGGGAGCTGC, Rev: CGGCGGAAGAAGCCCTTGCA;

Gapdh – For: CAAGGTCATCCATGACAACTT, Rev: GGCCATCCACAGTCTTCTGG;

Hprt – For: GTCATGCCGACCCGCAGTCC, Rev: GGCCACAATGTGATGGCCTCCC;

Col – For: TGCTAGCCGCAGGCATTAC, Rev: GGGTGCCCCAAAGAATCAGAAC;

Ndufv1 – For: CTTCCCCACTGGCCTCAAG, Rev: CCAAAACCCAGTGATCCAGC.

Antibodies for western blot. Rabbit anti-PPAR_γ mAb (81B8, Cell Signaling), mouse anti-NDUFB8 mAb (20E9DH10C12, Invitrogen), mouse anti-Tubulin (DM1A, Sigma).

ChIP-Seq library generation. Naïve CD4⁺ T cells were activated and polarized in T_H2 conditions. On day 1 and 2, T cells were transduced with a retroviral vector expressing TY1-tagged *Pparg*. On day 4, transduced T cells were harvested for ChIP as described previously⁴ utilizing a TY1

antibody (Sigma, SAB4800032). ChIP-sequencing libraries were constructed and sequenced (50 bp single end reads) as described previously⁴. 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 previously⁴. Visualization of ChIP-Seq results was achieved by uploading custom tracks onto the UCSC genome browser.

RNA-Seq library generation and sequencing analysis. Total RNA was extracted from T_H2 cells ~96 hours 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 34.1M reads per sample. Read alignment and junction finding was accomplished using STAR⁵ and differential gene expression with Cuffdiff²⁶, 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 bias⁷. Metascape⁸ was utilized for functional annotation of genes. Morpheus (<https://software.broadinstitute.org/morpheus>) was employed for hierarchical clustering. Circos (<https://circos.ca>) was employed to make Circos plots⁹.

IL-4 ELISA. Naïve CD4⁺ T cells were differentiated *in vitro* in T_H2 polarizing conditions as described above in 24 well plates. 96 hours after initial activation, cells were collected, washed twice with PBS, and resuspended in T_H2 polarizing medium without IL-4 in 24 well plates. The cells were cultured for an additional 48 hours and then the conditioned media was collected to

quantitate IL-4 content by ELISA (BioLegend, Mouse IL-4 ELISA MAX Standard Sets) using absorbance at 490nm as a readout.

Metabolic phenotyping via extracellular flux analysis. Mitochondrial substrate dependency and maximal respiration levels were determined by assessing oxygen consumption rate (OCR). OCR was measured using a 96 well extracellular flux analyser (Seahorse Bioscience). In brief, dead cells were removed from T cell cultures using Ficoll-Paque Premium 1.084 (17-5446-02; GE). 4×10^5 live cells per well (generally 5 wells per sample) were spun onto a Cell Tak (Corning)-coated Seahorse plate and rested for 1 hour in a CO₂ free incubator at 37 °C before commencing measurements using the Seahorse instrument. As indicated in figures, the following drugs were added into the wells (concentration is final concentration in wells): Oligomycin A (10 μ M, Sigma), FCCP (5 μ M, Sigma), Rotenone (7.5 μ M, Sigma), Antimycin A (7.5 μ M, Sigma), Etomoxir (100 μ M, Sigma), UK5099 (2 μ M, Sigma).

Fatty acid oxidation assay. PPAR γ TKO and control naïve CD4⁺ T cells were activated and polarized in T_H2 conditions for 5 days. Rosi or DMSO was added to a final concentration of 10 μ M on Day 0 and Day 3. On day 5, 5 million live cells were resuspended directly in 1 mL of loading media (DMEM, low glucose containing 2% fatty-acid free BSA (Sigma A6003), 0,25 mM carnitine (Sigma C-0158), and 2 μ Ci/reaction ³H-palmitic acid (American Radiolabeled Chemicals, Inc, ART 129)). Reaction mix was loaded onto a 24 well plate (200 μ L per well). The cells were incubated for 2 hours and supernatant was collected from each well. The cells were harvested and washed with PBS three times and lysed in 0.1N NaOH to determine protein concentration for normalization. 100 μ L of supernatants were treated with 100 μ L of 10% trichloroacetic acid (TCA). After mixing and 15 min incubation at room temperature, samples were centrifuged at maximum

speed for 10 min. The resultant supernatant was transferred to a new microtube and treated with 100 μ L of 5% TCA and 40 μ L of BSA (10% BSA in TE buffer). After mixing and 15 min incubation at room temperature, samples were centrifuged at maximum speed for 10 min. ~300 μ L of resulting supernatant was transferred to a new microtube and 750 μ L chloroform:methanol (2:1 mix) and 300 μ L KCl:HCl (2M each, 3.6g KCl in 20 mL water, 4 mL conc. HCl) were added to the reaction. After mixing and 15 min incubation at room temperature, samples were centrifuged at maximum speed for 10 min. ~600 μ L of the resulting supernatant was collected and added to scintillation counting tubes containing 5 mL Ecolume (MP Biomedicals). Samples were mixed and subjected to measurement of radiation via scintillation counter (3 H program).

Glucose uptake assay. PPAR γ TKO and control naïve CD4 $^+$ T cells were activated and polarized in T $_H$ 2 conditions for 5 days. Rosi or DMSO was added to a final concentration of 10 μ M on Day 0 and Day 3. 5 million cells per sample were resuspended in 1 ml of KRBH (Krebs-Ringer bicarbonate HEPES) buffer at pH of 7.4 (120 mM NaCl; 4 mM KH $_2$ PO $_4$; 1 mM MgSO $_4$; 0.75 mM CaCl $_2$; 30 mM HEPES; 10 mM NaHCO $_3$) and equally distributed into 5 wells of a 24 well plate. Cells were incubated for 30 min in 37 °C incubator, and then treated with 50 μ L/well of loading buffer (0.5mM 2-DG (2-Deoxy-D-glucose) and 0.5Ci (3H-2DG)). Negative control wells were additionally treated with 10 μ L/well of 1.5 mM cytochalasin B in DMSO to suppress active glucose uptake and control for non-specific uptake and binding of 3 H-2-DG. Cells were incubated for 1 hour at 37 °C and then washed three times with PBS. Cells were lysed in 300 μ L of 0.1 N NaOH. 200 μ L of cell lysis/sample were collected for radioactivity measurements via scintillation counter. The remaining amount was utilized to conduct the Bradford assay to quantify protein concentration. All radioactivity counts were normalized to sample protein concentration, and the negative control counts were further subtracted from experimental sample measurements.

Statistical analyses. Statistical analyses were performed with Prism 8 (GraphPad). *P* values were calculated using two-tailed unpaired or paired Student's *t*-test. 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. No animals were excluded from the statistical analysis, with the exception of exclusions due to technical errors, and the investigators were not blinded in the studies. Appropriate statistical analyses were applied, assuming a normal sample distribution, as specified in the figure legends. No estimate of variance was made between each group. All *in vivo* experiments were conducted with at least two independent cohorts. All *in vitro* T_H differentiation experiments were conducted at least three times. ChIP-Seq and RNA-Seq experiments were conducted using multiple biological samples (as indicated in figure legends).

Methods References

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Figure 1. Obesity provokes increased T_H2 immunopathology. **a**, Scheme of MC903-induced atopic dermatitis disease model used in this study. Black arrows indicate application of MC903 or EtOH administration to ear. **b**, Change in ear thickness during development of atopic dermatitis as indicated in **a**. **c**, Representative pictures of ears at Day 11. **d**, Representative images of hematoxylin and eosin stained histology of ears at Day 11. Scale bars, 100 μ m. **e**, Total T_{conv} , T_{reg} , and $CD8^+$ T cell number from whole ear at Day 11. **f**, Total $CD4^+$ $IL4^+$ and $IFN\gamma^+$ cell numbers from whole ear at Day 11. HFD, High fat diet; ND, Normal diet; D, Day. $n=5$ for ND and HFD for all comparisons. Data are mean $\pm$ s.e.m. ** $P < 0.01$, *** $P < 0.001$, Students t -test.

Figure 2. Obesity is epistatic to T cell-specific PPAR γ deficiency in potentiating atopic disease. **a**, Change in ear thickness during development of atopic dermatitis with mice fed ND or HFD. **b**, Absolute ear thickness at Day 11. Dotted line indicates average ear thickness at Day 0 of all mice in the study. **c**, Representative images of hematoxylin and eosin stained histology of ears at Day 11. Scale bars, 100 μ m. **d,e**, Total IL-4 $^{+}$ T $_{conv}$ cells (**d**) and IFN γ^{+} T $_{conv}$ cells (**e**) from whole ear at Day 11. HFD, High fat diet; ND, Normal diet; Control, CD4 Cre ; PPAR γ TKO, CD4 Cre PPAR $\gamma^{fl/fl}$; n=5 for all groups in figure, except (**e**) where 3 PPAR γ TKO were on ND and 4 PPAR γ TKO were on HFD. Data are mean $\pm$ s.e.m. * P < 0.05, ** P < 0.01, *** P < 0.001, Student's t -test.

Figure 3. PPAR γ directly controls T_H2 cellular metabolism. **a**, Hierarchical clustering of differentially expressed genes between T_H2 cells sufficient or deficient in *Pparg* treated with rosiglitazone (Rosi) or dimethyl sulfoxide (DMSO) (cells pooled from 4 mice, same data set used in **c-f**). **b**, Top scoring DNA motif of PPAR γ ChIP-Seq peaks in T_H2 cells via de novo analysis (cells pooled from 4 mice, same data set used in **c-f**). **c**, Circos plot representing gene lists from the Rosi Up, Rosi Down I, and Rosi Down II Clusters along with the peak lists from the PPAR γ ChIP-Seq of T_H2 cells. Purple arcs link identical gene names from two different lists. **d**, Bar graph representing P values of gene ontology clusters with $P < 10^{-10}$ of indicated gene lists. Each cluster is labeled with the most representative gene ontology term for that cluster. **e,f**, Selected FPKM and corresponding PPAR γ ChIP peak values of genes from the Rosi Up cluster (**e**) and Rosi Down clusters (**f**) that are metabolic or immunological in function.

Figure 4. Pharmacological activation of PPAR γ protects against obesity-associated T $_H$ 2 immunopathology in a T cell PPAR γ -dependent manner. **a**, Change in ear thickness during development of atopic dermatitis with mice fed HFD diet or HFD with Rosi. **b**, Absolute ear thickness at Day 11. Dotted line indicates average ear thickness at Day 0 of all mice in the study. **c**, Representative images of hematoxylin and eosin stained histology of ears at Day 11. Scale bars, 100 μ m. **d,e**, Total IL-4 $^{+}$ T $_{conv}$ cells (**d**) and IFN γ^{+} T $_{conv}$ cells (**e**) from whole ear at Day 11. PPAR γ TKO, CD4 Cre PPAR $\gamma^{fl/fl}$; n=5 for all groups, except (**e**) where 4 control mice were treated with HFD diet or HFD with Rosi. Data are mean $\pm$ s.e.m. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, Student's t -test.

Extended Data Figure 1. Short-term HFD provokes modestly increased T_H2 immunopathology, though muted compared to long-term HFD. **a**, Scheme of MC903-induced atopic dermatitis disease model used in this study. Black arrows indicate application of MC903 or EtOH administration to ear. **b**, Change in ear thickness during development of atopic dermatitis as indicated in **a**. **c**, Representative images of hematoxylin and eosin stained histology of ears at Day 11. Scale bars, 200 μ m. **d**, Total T_{conv} , T_{reg} , and $CD8^+$ T cell number from whole ear at Day 11. **e**, Total $CD4^+$ $IL4^+$ and $IFN\gamma^+$ cell numbers from whole ear at Day 11. HFD, High fat diet; ND, Normal diet; D, Day. $n=5$ for ND and HFD for all comparisons. Data are mean $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's t -test.

Extended Data Figure 2. PPAR γ is differentially expressed in *in vitro* differentiated TH2 cells and in MC903-challenged infiltrating T_{conv} cells *in vivo*. **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 TH1, TH2, and TH17 cells. NHR genes that are differentially expressed in TH2 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 TH1, TH2, and TH17 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 TH1, TH2, and TH17 cells (cells pooled from 3 mice before inducing differentiation) **e**, FPKM values of genes that are differentially expressed in TH2 cells and involved in transcriptional regulation. Position of *Pparg* is marked with a red dot. **f**, *Foxp3*^{Thy1.1} mice were challenged with MC903-induced atopic dermatitis and T_{reg} and T_{conv} cells were sorted from spleen and ear and assayed for *Pparg* expression, relative to *Gapdh* (cells pooled from 4 mice in biological duplicates). Data are mean $\pm$ s.e.m. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, #*P* = .055, Student's *t*-test.

Extended Data Figure 3. PPAR γ TKO mice display neither overt systemic inflammation nor altered T cell development. **a**, Picture of control and PPAR γ TKO spleens and lymph nodes. **b**, Spleen weights. **c,d**, T_{reg} (**c**) and activated T_{conv} (**d**) cell frequency in spleen and LN. **e**, Developing T cell subsets in thymus. n=3 mice per group. LN, lymph node. Student's *t*-test.

Extended Data Figure 4. PPAR γ restrains IL-4 expression and drives a fatty acid catabolic program in TH2 cells. **a**, Relative expression (using *Hprt* expression as housekeeping gene) of *Il4* (n=3 per group). **b,c**, Intracellular protein expression via flow cytometry (**b**) and secretion via ELISA from conditioned media (**c**) of IL-4 (n=3 per group). **d**, PPAR γ direct targets as identified by ChIP that are upregulated upon PPAR γ activation (found in the Rosi Up clusters.) **e**, Graphical representation of the phenotypic change in the mitochondrial metabolism of TH2 cells upon PPAR γ activation. **f**, Mitochondrial DNA (*CoI*) to nuclear DNA (*Ndufv1*) ratio (n=3 per group). **g**, Maximal respiration after treatment with carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) (n=3 per group). **h**, Western blot of NDUF8 (protein located in inner mitochondrial membrane) and tubulin at 96 hours in *in vitro* differentiated TH2 cells (cells pooled from 3 mice before inducing differentiation; D, DMSO; R, Rosi, 10 μ M). **i,l**, Mitochondrial substrate dependency of fatty acids (**i**) and glucose (**l**) measured by OCR after treatment with etomoxir and UK5099, respectively (OCR, oxygen consumption rate; n=5 per group). (**j,l**) Rates of beta-oxidation of exogenous fatty acids (**j**) and glucose uptake (**l**) (CPM, counts per minute, normalized to sample protein concentration; n=5 per group). All experiments performed with *in vitro* differentiated TH2 cells with cells pooled from 4 mice before inducing differentiation. Data are mean $\pm$ s.e.m. For box and whisker plots, whiskers plot min to max. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, Student's *t*-test.