

ALLERGY

PPAR- γ promotes type 2 immune responses in allergy and nematode infection

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A hallmark of immunity to worm infections and many allergies is a strong type 2 immune response. This is characterized by the production of cytokines interleukin-5 (IL-5) and IL-13 by adaptive T helper 2 (T_H2) cells and/or type 2 innate lymphoid cells. Peroxisome proliferator activated receptor- γ (PPAR- γ) is typically regarded as an anti-inflammatory factor. We report that T_H2 cells express high levels of PPAR- γ in response to the allergen house dust mite and after infection with the parasite *Heligmosomoides polygyrus*. Mice lacking PPAR- γ in T cells failed to effectively differentiate into IL-5- and IL-13-secreting cells and, hence, did not develop T_H2 cell-associated pathologies, including goblet cell metaplasia and eosinophilia, in response to allergen challenge. Furthermore, these mice could not mount protective immune responses to nematode infection. In addition, mice lacking PPAR- γ in T cells had greatly reduced frequencies of T_H2 cells in visceral adipose tissue. Mechanistically, PPAR- γ appeared to promote the expression of the IL-33 receptor on the surface of T_H2 cells. These results pinpoint PPAR- γ as a factor that drives type 2 responses in allergy, worm infection, and visceral adipose tissue.

INTRODUCTION

Naïve CD4 T cell activation initiates the differentiation of these cells into mature, functionally specialized T helper (T_H) cell subsets. To date, a number of T_H cell subsets have been delineated, including T_H1, T_H2, T_H17, and suppressive regulatory T cells (T_{regs}) (1, 2). Analogous to adaptive T_H cells, innate lymphoid cells (ILCs) have been shown to develop from a common bone marrow precursor into ILC1, ILC2, and ILC3, which share features of T_H1, T_H2, and T_H17 cells, respectively (3). Type 2 immune responses, which are mediated by T_H2 cells and ILC2, are essential for immunity to large extracellular pathogens such as worms. T_H2 cells and ILC2 are characterized by the production of cytokines interleukin-4 (IL-4), IL-5, and IL-13, which promote macrophage functions, epithelial cell activation, and B cell immunoglobulin E (IgE) secretion (1). Although type 2 responses are beneficial in some contexts, these responses are heavily implicated in allergies such as asthma, whose incidence has risen markedly in the past few decades (4).

The differentiation of naïve CD4 T cells into functional T_H2 cells depends on many environmental cues and cell-intrinsic changes. Typically, IL-4 from the extracellular environment promotes activation of signal transducer and activator of transcription 6 (STAT-6), which rapidly induces the master transcriptional regulator of T_H2 cells, GATA-3 (1, 5). Together, GATA-3 and STAT-6 promote changes to the chromatin architecture of differentiating cells, support proliferation, and promote the transcription of effector cytokine genes, including IL-4, IL-5, and IL-13 (1, 6, 7).

An important function of GATA-3 is to promote the expression of the IL-33 receptor (IL-33R) on the surface of differentiating T_H2 cells (8). The IL-33R, which is a hallmark of T_H2 cells, is composed of a common IL-1RAP chain and the subunit ST2 and signals through MyD88 to promote nuclear factor κ B activation and stimulate the production of effector cytokines (1, 9). Expression of the IL-33R on the surface of T_H2 cells is critical for the function of these cells (10, 11), and single-nucleotide polymorphisms (SNPs) in the *IL33* or *IL1RL1* (ST2) genes are strongly associated with an increased risk of asthma (12) and heart disease (13). A recent study suggested that the acquisition of full effector functions by T_H2 cells and ILC2 depended on their migration to organs (such as the lung) and exposure to cytokines (such as IL-33, IL-25, and thymic stromal lymphopoietin) (14). Thus, the priming lymph node is not sufficient for fulminant T_H2 cell responses, and the elucidation of tissue-specific factors that regulate responsiveness to the cytokine IL-33, for example, could lead to substantial improvements in the treatment of many inflammatory disorders.

The nuclear receptor peroxisome proliferator activated receptor- γ (PPAR- γ) is a master regulator of adipocyte differentiation and a potent modulator of lipid metabolism (15). Studies over the past few decades have demonstrated that PPAR- γ suppresses the secretion of proinflammatory cytokines by epithelial cells (16, 17), promotes type 2 macrophage development (18), and enhances T_{reg} accumulation at inflammatory sites such as the visceral adipose tissue (VAT) (19). This strong anti-inflammatory function has suggested that agonists of PPAR- γ , known as thiazolidinediones (TZDs), may be useful for the treatment of various disorders (20). To this end, TZDs have repeatedly shown strong therapeutic efficacy in preclinical models of asthma (21, 22), and this has led to early-phase clinical trials in this setting (23).

In this study, we identified PPAR- γ as a gene selectively up-regulated in T_H2 cells after exposure to house dust mite (HDM) extracts or the nematode *Heligmosomoides polygyrus*. In contrast to the known anti-inflammatory function of PPAR- γ , T_H2 cells were dependent on PPAR- γ for their differentiation into IL-5- and IL-13-producing effector cells. PPAR- γ expression in CD4 T cells promoted pathogenic T_H2

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cell responses to HDM and protective type 2 immunity to *H. polygyrus*. Mice lacking PPAR- γ in T cells were unable to effectively up-regulate the IL-33R subunit ST2. These results identify PPAR- γ as a crucial amplifier of type 2 immune responses.

RESULTS

T_H2 cells preferentially express PPAR- γ

We recently performed whole-genome expression profiling on T_H cells isolated from the lungs of mice sensitized and challenged with HDM extracts (24). A strong up-regulation in the expression of *Pparg* and several known PPAR- γ target genes, including *Retnla*, *Arg1*, *Chi3l3*, and *Chi3l4*, was observed (25), implying that this nuclear receptor was driving the response (Fig. 1A). Because HDM induces both T_H2 and T_H17 cell populations in the mouse lung, we sought to clarify which subset was expressing PPAR- γ . The surrogate markers ST2 and CCR6 were used to identify T_H2 and T_H17 cells, respectively, from the CD4⁺ T cell population (10, 26). *Pparg* mRNA was preferentially expressed by the ST2⁺ cell fraction ($P < 0.001$) expressing high levels of IL-5 (Fig. 1, B to E). T_H2 cells also play a critical protective role in immunity to helminth infection (27). To examine whether a similar pattern of PPAR- γ expression was observed in mice infected with the nematode *H. polygyrus*, we purified T_H2 (ST2⁺), T_H17 (CCR6⁺), and T_H1

cells [which express the chemokine receptor CXCR3 (28)] from the mesenteric lymph nodes (mesLN) of infected mice. Quantitative polymerase chain reaction (PCR) confirmed that ST2⁺ cells expressed the most *Il5* ($P < 0.001$), CCR6⁺ cells expressed the most *Il17a* ($P < 0.001$), and CXCR3⁺ cells expressed the highest levels of *Ifng* mRNA ($P < 0.05$) (Fig. 1, H to J). Levels of *Pparg* mRNA were again more abundantly expressed in T_H2 cells after worm infection ($P < 0.001$) (Fig. 1G). Thus, PPAR- γ appears to be preferentially expressed by T_H2 cells in the two models where type 2 immunity is important. We further characterized the expression of PPAR- γ after classical T_H cell differentiation cultures. Similarly, *Pparg* mRNA was most highly expressed in cells cultured under T_H2 cell differentiation conditions (Fig. 1K), and this expression appeared dependent on the master transcriptional regulator GATA-3 because loss of one allele of *Gata3* reduced *Pparg* mRNA levels significantly ($P = 0.0286$) (Fig. 1L). These results demonstrate that PPAR- γ is a T_H2 cell-specific factor in multiple contexts.

PPAR- γ expression in T cells drives pathogenic type 2 immune responses to HDM

To determine the effect of PPAR- γ on T_H2 cell function, we crossed mice in which LoxP sites were engineered into the *Pparg* gene with mice expressing the *Lck-Cre* transgene. Mice in which T cells were targeted for deletion of the *Pparg* gene (*Lck-Cre*⁺*Pparg*^{fl/fl}) are termed *Pparg*^{Lck Δ}

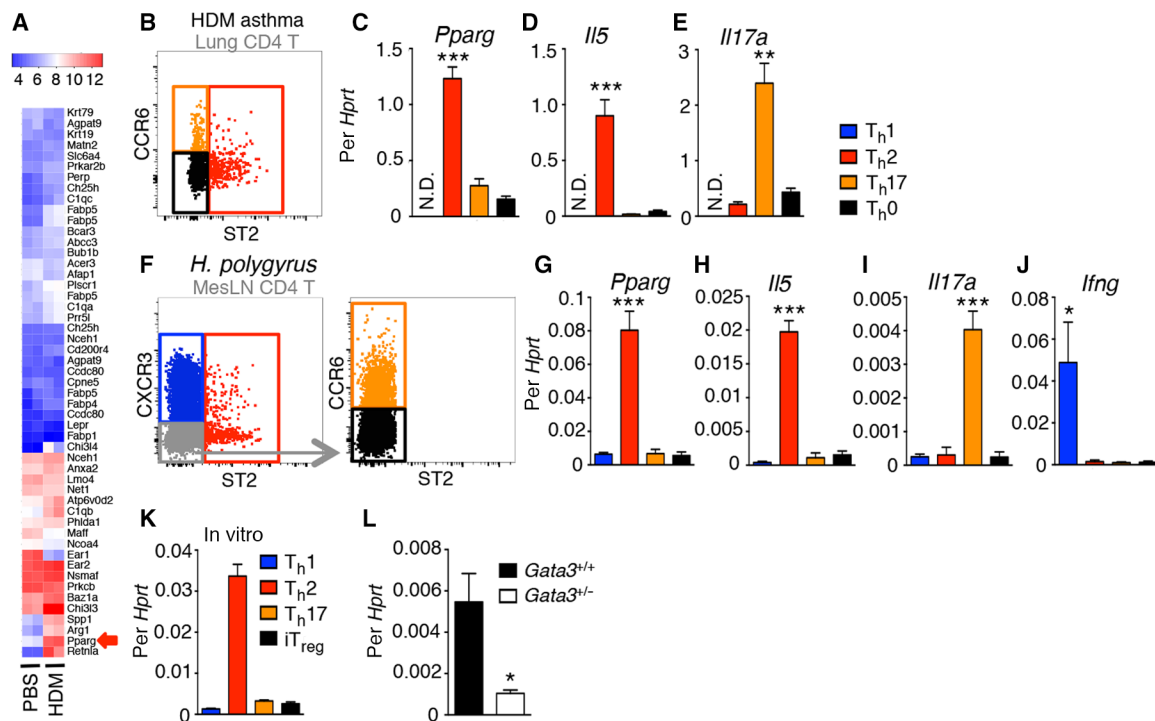


Fig. 1. PPAR- γ is expressed specifically in T_H2 cells. (A) Genome-wide expression analysis of CD4⁺ T cells (CD4⁺CD45⁺F4/80⁺) purified from the lungs of naïve PBS-treated or HDM-sensitized and HDM-challenged mice on day 15. Clustering analysis of PPAR- γ (indicated by a red arrow) and a number of its targets is depicted. Values are log₂-transformed. (B to E) *Il5*, *Il17a*, and *Pparg* mRNA levels in purified T_H2 (ST2⁺), T_H17 (CCR6⁺ST2⁺), and T_H0 cells from the lungs of HDM-sensitized and HDM-challenged mice. (F to J) *Il5*, *Il17a*, *Ifng*, and *Pparg* mRNA levels in purified T_H2 (ST2⁺), T_H17 (CCR6⁺ST2⁺CXCR3⁺), T_H1 (CXCR3⁺ST2⁺), and T_H0 cells from mesLN of *H. polygyrus*-infected mice 15 days after infection. Data are representative of two independent experiments with $n = 3$ to 4 mice in each experiment (means and SEM are depicted). N.D., not done. In (C) to (J), all subsets are compared with the T_H0 subset. (K) Naïve CD4 T cells were differentiated into T_H1, T_H2, T_H17, and T_{reg} cells, and the expression of *Pparg* mRNA was analyzed. (L) *Pparg* mRNA expression in WT *Gata3*^{+/+} and heterozygous *Gata3*^{+/-} T_H2 cells in vitro ($n = 4$ separate mice for each genotype from one of two representative experiments). (C) to (J) were analyzed using one-way ANOVA and Bonferroni's test for multiple comparisons to the T_H0 subset. Mann-Whitney *U* test was used in (L). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

here, and wild-type (WT) littermate control *Lck-Cre^{-/-}Pparg^{fl/fl}* mice are referred to as *Pparg⁺*. We first investigated the presence of alveolar macrophages in the airways because PPAR- γ is essential for their development (25). Normal levels of alveolar macrophages were observed in bronchoalveolar lavage (BAL) samples of *Pparg^{Lck Δ}* mice administered phosphate-buffered saline (PBS) (Fig. 2, A and B), suggesting that *Pparg* was not deleted in this myeloid lineage. To test whether *Pparg* was deleted in CD4 T cells, we activated naïve CD4 T cells in the presence of T_H2 cell differentiation factors and analyzed the expression of *Pparg* mRNA after 4 days. Levels of *Pparg* transcripts were reduced by about 90% in CD4 T cells from *Pparg^{Lck Δ}* mice, confirming that the *Pparg* gene was excised in the CD4 T cell lineage (Fig. 2C). To determine the impact of PPAR- γ deletion on T_H2 cell responses in vivo, we sensitized and challenged mice with HDM extracts and analyzed the composition of airway-infiltrating cells and mucus secretion, two hallmarks of the asthmatic response. After administration of HDM, *Pparg^{Lck Δ}* mice had reduced frequencies ($P = 0.0013$) and numbers ($P = 0.0004$) of airway eosinophils compared with control *Pparg⁺* mice (Fig. 2,

D to F). In addition, *Pparg^{Lck Δ}* mice had reduced infiltration of T cells into the airways but normal levels of alveolar macrophages and neutrophils ($P = 0.0149$) (Fig. 2G). Furthermore, periodic acid-Schiff staining of lung sections revealed a complete absence of mucus-secreting goblet cells lining the airways of *Pparg^{Lck Δ}* mice (Fig. 2H). This strongly suggested that PPAR- γ expression in CD4 T cells was driving the pathogenic hallmarks of the asthmatic response and that PPAR- γ was important for the differentiation of T_H2 cells.

To address T_H2 cell function directly, we analyzed the CD4⁺ T cell response to HDM in the lung and lung-draining mediastinal lymph node (medLN). Comparable numbers of total cells and CD4⁺CD44⁺ memory cells were present in the lung and medLN of *Pparg^{Lck Δ}* and control *Pparg⁺* mice after sensitization and challenge with HDM (Fig. 3, A to C). However, significantly fewer memory CD4 T cells in the lung produced T_H2 cytokines, including IL-5 and IL-13 (IL-5⁺IL-13⁺, $P = 0.0006$; IL-5⁺IL-13⁺, $P = 0.002$) (Fig. 3, D and E). The frequencies of IL-4⁺ and IL-17⁺ memory CD4 T cells in the lungs of mice were similar between *Pparg^{Lck Δ}* and control *Pparg⁺* mice (fig. S1). In the medLN, the frequency of memory CD4 T cells producing IL-5 and IL-13 in the medLN was similar between *Pparg^{Lck Δ}* and control *Pparg⁺* mice (Fig. 3F), suggesting that PPAR- γ specifically promoted the pathogenic functions of T_H2 cells in the effector tissue and was not important for the initial priming of this population. Thus, PPAR- γ was important for the differentiation of IL-5- and IL-13-secreting T_H2 cells but was not required for IL-4 production by CD4 T cells.

PPAR- γ expression promotes protective T_H2 cell-mediated immune responses to *H. polygyrus*

To determine whether PPAR- γ played a similar role in other type 2 immune responses, we infected *Pparg^{Lck Δ}* and control *Pparg⁺* mice with *H. polygyrus* and analyzed the response. The total number of lymphocytes and memory CD4 T cells in the mesLN was greatly increased in infected compared with noninfected mice but was not significantly reduced in worm-infected *Pparg^{Lck Δ}* mice 15 days after infection (Fig. 4A). Furthermore, the proportion of memory CD4 T cells secreting both IL-5 and IL-13 was significantly reduced in the mesLN of worm-infected *Pparg^{Lck Δ}* mice ($P = 0.0279$) (Fig. 4B), as was the frequency of eosinophils ($P = 0.0004$) (Fig. 4C and fig. S2). Similar to the HDM model, normal frequencies of CD4 T cells expressed the cytokines IL-4 and IL-17 (Fig. 4, D and E, and fig. S2). This suggested that PPAR- γ was required for the production of IL-5 and IL-13 but not for the production of IL-4 by T_H2 cells. After primary infection with *H. polygyrus*, levels of total IgE in the serum of control *Pparg⁺* mice were significantly higher than those in *Pparg^{Lck Δ}* mice (Fig. 4F). Although robust type 2 responses to *H. polygyrus* were observed in *Pparg⁺* mice, they showed no enhanced ability to clear the parasite 15 days after primary infection (Fig. 4G), in line with the persistent nature of this parasite after primary infection (29). To test whether T cell-derived PPAR- γ conferred protective immunity to *H. polygyrus*, we dewormed mice 2 weeks after primary infection and reinfected them 1 week later. Upon reinfection, *Pparg^{Lck Δ}* mice had a greatly impaired ability to control worm load ($P = 0.0051$) (Fig. 4G). Hence, PPAR- γ expression in T cells is important for T_H2 cell responses to *H. polygyrus* and promotes protective immunity to this pathogen.

Agonists of PPAR- γ induce ST2 expression on T_H2 cells in vitro

To investigate the role of PPAR- γ on T_H2 cell effector functions, we tested the impact of known PPAR- γ agonists in T_H2 cell differentiation cultures. One such agonist is the prostaglandin derivative 15d Δ 12,14-PGJ₂ (15d-PGJ₂) (30, 31). 15d-PGJ₂ is derived from PGD₂, which has

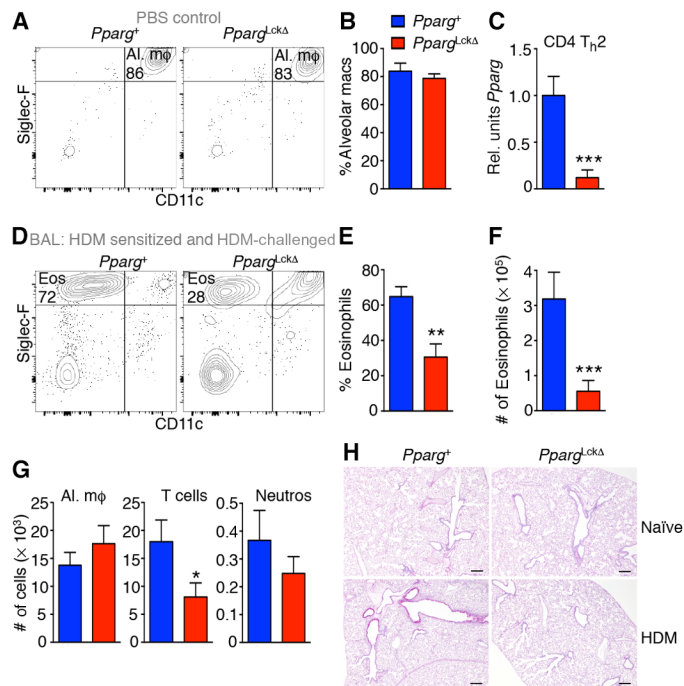


Fig. 2. PPAR- γ promotes pathogenic responses to HDM. (A) Representative plots of CD11c versus Siglec-F expression in BAL of naïve PBS-treated WT littermate control *Lck-Cre^{-/-}Pparg^{fl/fl}* (*Pparg⁺*, $n = 3$) and in *Lck-Cre^{-/-}Pparg^{fl/fl}* (*Pparg^{Lck Δ}* , $n = 4$) mice. (B) Frequency of alveolar macrophages (Al. m ϕ) shown in the top right quadrant of (A). (C) Relative expression of *Pparg* mRNA from naïve CD4 T cells activated under T_H2 cell differentiation conditions for 4 days (control *Pparg⁺*, $n = 6$; *Pparg^{Lck Δ}* , $n = 8$). (D) Representative plots of CD11c versus Siglec-F expression in BAL samples from control *Pparg⁺* and *Pparg^{Lck Δ}* mice sensitized and challenged with HDM on day 15 (frequency of Siglec-F⁺CD11c⁻ eosinophils is indicated). (E and F) Graphs of BAL eosinophil (Siglec-F⁺CD11c⁻) frequency and number in *Pparg^{Lck Δ}* ($n = 12$) compared with WT control mice (*Pparg⁺*, $n = 17$) sensitized and challenged with HDM (pooled from three independent experiments). (G) Graphs of the number of alveolar macrophages (Siglec-F⁺CD11c⁺), T cells (Siglec-F⁺CD11c⁻CD3⁺), and neutrophils (Siglec-F⁺CD11c⁻Gr-1⁺). (H) Representative periodic acid-Schiff stain of lung sections from naïve PBS-treated or HDM-sensitized and HDM-challenged mice ($n = 3$ in each HDM group; goblet cells in dark purple are seen lining the airways in HDM-treated *Pparg⁺* mice). Scale bar, 250 μ m. Mann-Whitney *U* test was used for all comparisons. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

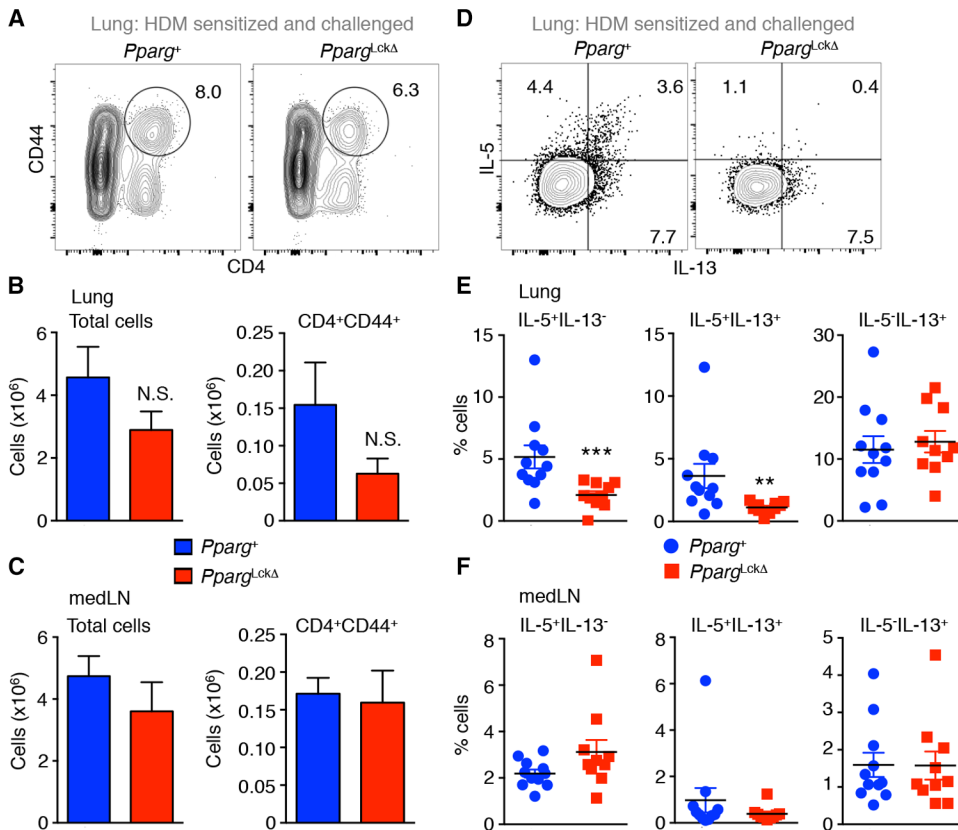


Fig. 3. *Pparg*^{LckΔ} mice fail to develop robust TH2 cell responses. (A) Representative plots of CD4 versus CD44 expression on lymphocytes from the lungs of WT control *Pparg*⁺ and *Pparg*^{LckΔ} mice sensitized and challenged with HDM on day 15. (B and C) Total cell number and CD4⁺CD44⁺ cell number in the lung (B) and the medLN (C) in control *Pparg*⁺ and *Pparg*^{LckΔ} mice after HDM sensitization and challenge. (D) Representative plots of IL-13 versus IL-5 expression from gated lung CD4⁺CD44⁺ cells in control *Pparg*⁺ and *Pparg*^{LckΔ} mice after restimulation for 3 hours with PMA and ionomycin. (E and F) Frequency of cytokine-producing cells in the lung (E) and medLN (F) (each symbol is an individual mouse) (*Pparg*^{LckΔ}, *n* = 10; *Pparg*⁺, *n* = 11, pooled from two independent experiments). N.S., not significant. Mann-Whitney *U* test was used for all comparisons. ***P* < 0.01 and ****P* < 0.001.

been postulated to potentiate inflammation during asthma, partly by stimulating the receptor CRTH2 (32). The addition of 15d-PGJ₂ did little to directly promote IL-5 or IL-13 production in TH2 cell differentiation cultures (Fig. 5A). However, 15d-PGJ₂ notably up-regulated transcription of the ST2 gene (*Il1rl1*) (Fig. 5B). This was intriguing because a recognized caveat of classical TH2 cell differentiation assays is the inefficient induction of ST2 on the surface of TH2 cells (33). The addition of 15d-PGJ₂ to TH2 cell differentiation cultures induced ST2 expression on about 10% of differentiating TH2 cells after 7 days of culture (*P* < 0.01) (Fig. 5, C and D). The synthetic PPAR-γ agonist pioglitazone (PIO) induced a similar increase in ST2 expression in differentiating TH2 cells (*P* < 0.05) (Fig. 5E), and the activity of 15d-PGJ₂ and PIO appeared to be PPAR-γ-dependent (15d-PGJ₂, *P* = 0.0002; PIO, *P* = 0.011) (Fig. 5F). These results imply that addition of PPAR-γ ligands to TH2 cell differentiation cultures may more accurately recapitulate physiological TH2 cell differentiation, by inducing IL-33R expression for instance.

CD4 T cells from *Pparg*^{LckΔ} mice express reduced levels of the IL-33R subunit ST2

To validate our findings from in vitro cell cultures, we analyzed the expression of ST2 on CD4 T cells in HDM-induced airway inflammation and after nematode infection. In the lungs of *Pparg*^{LckΔ} mice adminis-

tered HDM, the frequency of memory CD4 T cells expressing ST2 was considerably reduced (*P* = 0.0002) (Fig. 6, A and C), as was the mean fluorescence intensity of ST2 expression on ST2⁺ memory CD4 T cells (*P* < 0.0001) (Fig. 6E). These differences were not observed in the medLN (Fig. 6, B and D), reinforcing the notion that PPAR-γ activation is important for the promotion of pathogenic TH2 cells in the lung tissue directly (Fig. 3). Similarly, the frequency of ST2⁺ cells (*P* = 0.0007) and the intensity of ST2 expression (*P* = 0.0127) on memory CD4 T cells [CD4⁺CD44⁺Foxp3⁻ cells to exclude gut-associated T_{regs} (34)] were also reduced in the mesLN and Peyer's patches of *Pparg*^{LckΔ} mice in response to *H. polygyrus* infection (Fig. 6, F to I).

To verify that PPAR-γ was required in a cell-intrinsic fashion for pathogenic TH2 cell differentiation and that the effects observed were not due to alterations in non-CD4 T cells or an altered cytokine milieu, we created 1:1 chimeras of WT and *Pparg*^{LckΔ} cells. Congenic CD45.1⁺ mice were lethally irradiated and infused with bone marrow cells from WT (CD45.1⁺CD45.2⁺) and *Pparg*^{LckΔ} (CD45.2⁺ only) mice (fig. S3A). This experimental setup allowed us to differentiate between transferred WT, *Pparg*^{LckΔ}, and radioresistant host cells. After 8 weeks, chimeric mice were sensitized and challenged with HDM, and the TH2 cell response in the lung was analyzed. Very few memory CD4 T cells from the *Pparg*^{LckΔ} fraction produced both IL-5

and IL-13 compared with the WT cell fraction (*P* = 0.0156) (fig. S3B). Similar to the results in *Pparg*^{LckΔ} mice, the frequency and intensity of ST2 expression in memory CD4 T cells from the *Pparg*^{LckΔ} fraction were severely impaired compared with WT cells in the same mouse (fig. S3C). Furthermore, in control experiments to test for effects of the *Lck-Cre* transgene alone, HDM was administered to *Lck-Cre*⁺*Pparg*^{+/+} and *Lck-Cre*⁺*Pparg*^{+/+} littermate control mice. No difference in airway eosinophilia, frequency of ST2⁺ cells, or mean fluorescence intensity (MFI) of ST2 expression in the lung was observed (fig. S4). Together, this demonstrates that PPAR-γ instructs TH2 cell differentiation and the expression of ST2 in a cell-intrinsic manner and, furthermore, that PPAR-γ signals are crucial for the full up-regulation of ST2 on CD4 T cells in the lung.

Whole-genome expression arrays pinpoint genes affected by loss of PPAR-γ

To gain more mechanistic insight into what genes PPAR-γ may regulate, we performed whole-genome arrays of CD4⁺CD44⁺ST2⁺ cells from the lungs of mice sensitized and challenged with HDM. Expression arrays showed no difference in the expression of master transcriptional regulators, including *Foxp3*, *Gata3*, *Bcl6*, *Tbx21*, and *Rorc*, between cells sorted from control *Pparg*⁺ and *Pparg*^{LckΔ} mice (fig. S5). A total

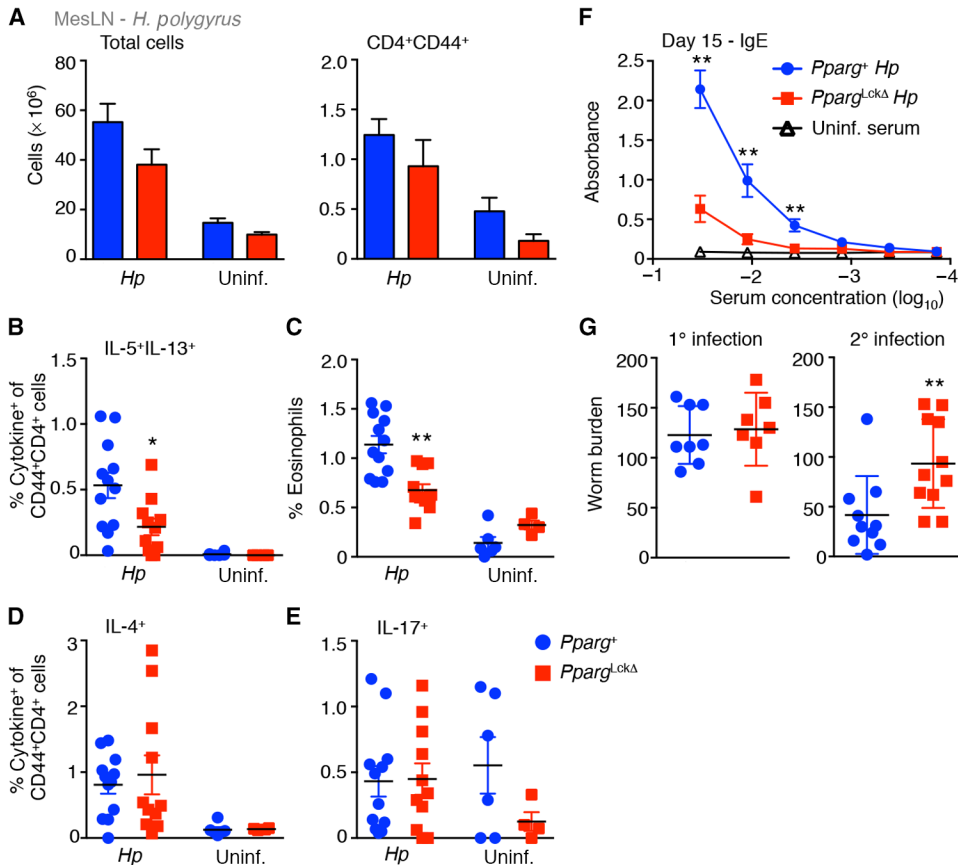


Fig. 4. PPAR- γ promotes type 2 immunity to *H. polygyrus*. Mice were infected with *H. polygyrus* (Hp), and responses in the mesLN and Peyer's patches were measured by flow cytometry 15 days after infection. (A) Total and CD4⁺CD44⁺ cell number in mesLN in littermate control *Pparg*⁺ (infected, *n* = 12; uninfected, *n* = 6) and *Pparg*^{LckΔ} mice (infected, *n* = 11; uninfected, *n* = 4) 15 days after *H. polygyrus* infection (pooled from two to four independent experiments). (B) The frequency of IL-5⁺IL-13⁺ cells among gated CD4⁺CD44⁺ cells in mesLN and Peyer's patches of control *Pparg*⁺ and *Pparg*^{LckΔ} mice of infected and uninfected mice. (C) Graph of eosinophil (Siglec-F⁺CD11c⁺) frequency 15 days after infection. (D and E) The frequency of IL-4⁺ and IL-17⁺ cells among gated CD4⁺CD44⁺ cells in mesLN and Peyer's patches of control *Pparg*⁺ and *Pparg*^{LckΔ} mice of infected and uninfected mice. (F) Serum concentration of total IgE in mice 15 days after infection, as measured by enzyme-linked immunosorbent assay (ELISA). One representative of two experiments is shown (*Pparg*⁺ Hp, *n* = 6; *Pparg*^{LckΔ} Hp, *n* = 4; uninfected, *n* = 3). (G) Viable adult worms per gut 15 days after primary infection with *H. polygyrus* (*n* = 6 to 8 mice pooled from two independent experiments) and 15 days after secondary infection (*n* = 10 to 11 mice pooled from two independent experiments). Mann-Whitney *U* test was used for all comparisons. **P* < 0.05 and ***P* < 0.01.

of 29 annotated genes appeared to be differentially expressed by at least twofold between CD4⁺CD44⁺ST2⁺ cells from *Pparg*⁺ and *Pparg*^{LckΔ} mice (Fig. 6J). These included a number of known T_H2 cell and asthma risk factors such as *Il5*, *Il13*, and *Ltb4r1*, as well as PPAR- γ target genes such as *Chi3l3*. The expression of several factors involved in carbohydrate synthesis, metabolite transport, or lipid storage, such as *Galnt3*, *Tgtp2*, *Slc7a8*, *Slc37a2*, and *Plin2*, was increased in PPAR- γ -proficient cells, whereas two small GTPases, *Tagap1* and *Ras2*, were reduced. To investigate whether the expression of some of these targets could be regulated by PPAR- γ , we analyzed the expression of *Galnt3*, *Plin2*, and *Ltb4r1* in T_H2 cells cultured in the presence or absence of 15d-PGJ₂. We observed notable increases in the expression of *Galnt3* and *Plin2* (*P* = 0.0025) (Fig. 6, K and L) after the addition of 15d-PGJ₂ to culture, whereas *Ltb4r1* mRNA showed no significant trend (Fig. 6M). These results suggest that although PPAR- γ may not directly induce the production of effector molecules, it may promote

the expression of a key set of metabolic regulators that support T_H2 cell functions in the lung.

PPAR- γ supports a population of resident T_H2 cells and ST2⁺ T_{regs} in VAT

The notable impact of PPAR- γ on ST2 expression in CD4 T cells prompted us to investigate VAT, which contains prominent populations of CD4 T cells and ILC2 that have been implicated in the regulation of glucose sensitivity (19, 35). *Pparg* mRNA was detectable in both CD4 T cells and ILC2 from VAT of *Pparg*⁺ mice (fig. S6A). Note that expression of *Pparg* mRNA was higher in purified macrophages from the VAT than in ILC2 or CD4 T cells. Likewise, in vitro-generated M2 macrophages expressed about eightfold more *Pparg* mRNA than differentiating T_H2 cells (fig. S6B). It is possible that the lower levels of *Pparg* mRNA in T_H2 cells reflect the fact that only a small proportion of differentiating T_H2 cells express this factor. This would explain why ST2 is only up-regulated in around 10% of cells in vitro after stimulation with 15d-PGJ₂. The lack of reliable antibodies for intracellular detection of PPAR- γ at the single-cell level makes this a difficult premise to test.

In *Pparg*^{LckΔ} mice, *Pparg* mRNA was detected at normal levels in purified ILC2 and macrophages, whereas it was completely absent from purified CD4 T cells (fig. S6A). Thus, *Pparg* was deleted from T cells but not from ILC2, despite reports of *Lck* expression in ILC2 (36). In line with their normal expression of *Pparg*, ILC2 were present at standard frequencies in VAT of *Pparg*^{LckΔ} mice (fig. S6, C and D). Furthermore, the frequency of ILC2 in response to *H. polygyrus* and

HDM was, for the most part, comparable between *Pparg*⁺ and *Pparg*^{LckΔ} mice (fig. S7), despite a moderate increase in the total frequency of ILC2 in the lungs of *Pparg*^{LckΔ} mice after HDM administration. In a short-term model of ILC2-mediated airway inflammation with recombinant (r) IL-33, airway eosinophilia and ILC2 responses were induced comparably in *Pparg*⁺ and *Pparg*^{LckΔ} mice (fig. S8). Hence, ILC2 responses were not greatly affected by the absence of PPAR- γ in T cells.

Because VAT CD4 T cells lacked *Pparg* mRNA, we further analyzed this population. Previous studies have shown that VAT T_{regs} express high levels of ST2 (19, 35). In VAT of *Pparg*^{LckΔ} mice, we observed a notable reduction specifically in ST2-expressing CD4 T cells both within the Foxp3⁺ T_{reg} compartment (*P* = 0.0051) and among conventional CD4⁺Foxp3⁺ cells (*P* = 0.0039) (Fig. 7, A and B). This reduction was not observed in other organs analyzed, including the thymus, spleen, medLN, lung, and colon (fig. S9), where ST2⁺ T_{regs} have been suggested to regulate tolerance to the gut microbiota (34). In response to HDM,

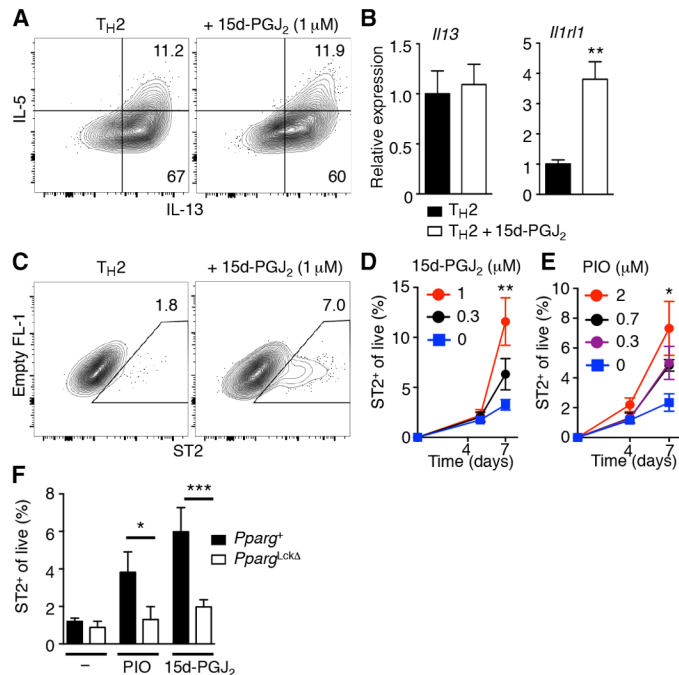


Fig. 5. Agonists of PPAR- γ promote ST2 expression in T_H2 cell differentiation cultures. Naïve CD4 T cells were cultured under T_H2 cell differentiation conditions in the presence or absence of 15d-PGJ₂ for 7 days. (A) Cytokine expression was analyzed by intracellular staining (ICS). Representative plots of IL-13 versus IL-5 expression. (B) *Il13* and *Il1rl1* mRNA expression was quantified after 7 days of culture in the presence or absence of 15d-PGJ₂ ($n = 6$ to 7 separate wells pooled and normalized to the level in T_H2 -only cultures). (C) Expression of ST2 versus fluorescence parameter 1 (FL-1) in representative plots. (D and E) Graphical representation of ST2 expression in the presence of the indicated doses of 15d-PGJ₂ (D) or PIO (E) over time ($n = 6$ to 8 separate wells over two to three independent experiments). (F) In separate experiments, naïve CD4 T cells from *Pparg*⁺ and *Pparg*^{LckΔ} mice were cultured under T_H2 cell differentiation conditions in the presence of 15d-PGJ₂ or PIO, and the frequency of ST2⁺ cells was quantified after 7 days ($n = 7$ to 11 separate wells over three independent experiments). (D) and (E) were analyzed using one-way ANOVA and Bonferroni's test for multiple comparisons to T_H2 cells in the absence of compounds. Mann-Whitney *U* test was used in (B) and (F). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

the overall numbers of T_{reg} s were comparable between *Pparg*⁺ and *Pparg*^{LckΔ} mice (fig. S10, A and B); however, a specific reduction in the frequency of ST2-expressing T_{reg} s was observed in *Pparg*^{LckΔ} mice ($P = 0.0159$) (fig. S10B). No such difference was observed in the *H. polygyrus* model (fig. S10C). Thus, ST2⁺ T_{reg} s in VAT are dependent on PPAR- γ , and responses of this population to the HDM allergen in the lung are also somewhat dependent on PPAR- γ .

To test the cytokine secretion potential of VAT CD4 T cells, lymphocytes were restimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin. The frequency of CD4 T cells that produced IL-5 in VAT of *Pparg*^{LckΔ} mice was significantly reduced compared with that in control mice ($P = 0.0056$), although there was a reciprocal increase in cells expressing interferon- γ (IFN- γ) ($P = 0.0314$) (Fig. 7, C and D). This suggests that PPAR- γ promotes a resident population of T_H2 cells in VAT, in addition to ST2⁺ T_{reg} s.

Type 2 cytokines, such as IL-4 and IL-5, and T_{reg} -derived IL-10 have been classically viewed to mediate beneficial metabolic effects, for instance, by promoting sensitivity to glucose (19, 37). However, we observed that aged *Pparg*^{LckΔ} mice were leaner (Fig. 7E) and exhibited

significantly greater control of plasma glucose levels after overnight fasting ($P = 0.0051$) (Fig. 7F), suggesting that ST2⁺ T_{reg} s and T_H2 cells may impair glucose sensitivity in the context of aging.

Together, our study identifies PPAR- γ as an important factor for the full effector differentiation of ST2⁺ IL-5- and IL-13-secreting T_H2 cells, as well as for responses of ST2⁺ T_{reg} s in VAT and in response to the allergen HDM.

DISCUSSION

PPAR- γ is typically regarded as an immunosuppressive factor. It serves to impair T cell priming by dendritic cells (38), suppress inflammatory chemokine and cytokine production by epithelial cells of the gut or airways (16, 17), promote the conversion of naïve CD4 T cells to T_{reg} s (39, 40), and maintain the T_{reg} population in VAT (19). Contrary to these well-described anti-inflammatory functions, we have demonstrated here that PPAR- γ has an important function in T_H2 cells, where it is critical for T_H2 cell-mediated allergic inflammation and immunity to worm infection. We found little evidence that PPAR- γ was required for IL-4 production by T_H2 cells and rather supported a population of IL-5⁺IL-13⁺ T_H2 cells, considered a pathogenic subset of cells, for instance, in the context of airway inflammation. In light of a recent study proposing that pathogenic T_H2 cells complete their differentiation in the lung tissue directly and depend on tissue-specific signals such as IL-33 (14), our results suggest that PPAR- γ agonists up-regulate the expression of ST2 on differentiating T_H2 cells in the lung and thereby promote their potential for IL-5 and IL-13 secretion. Thus, strategies to specifically target PPAR- γ in IL-5⁺IL-13⁺ T_H2 cells may effectively ameliorate airway inflammation.

Supportive of our observations in T_H2 cells, PPAR- γ was previously shown to promote M2 macrophage differentiation and to be induced by IL-4 receptor signaling and STAT-6 activation (18, 41). In dendritic cells and macrophages, PPAR- γ and STAT-6 have been shown to orchestrate a joint transcriptional program (42). This has made the association between PPAR- γ and the T_H2 cell lineage intuitive, yet it has remained unstudied to date. PPAR- γ may perform similar functions in T_H2 cells, for instance, by facilitating the binding of STAT-6 and/or GATA-3 to certain T_H2 gene loci and regulating changes in chromatin architecture. However, previous studies and our own results do not suggest that PPAR- γ is a strong inducer of effector T_H2 cytokine gene transcription. It is more likely that PPAR- γ regulates various “nonclassical” T_H2 cell target genes such as *Arg1* and *Chil3l3*. Furthermore, given the known interplay of PPAR- γ in lipid metabolism, it is enticing to hypothesize that PPAR- γ facilitates the requirements of T_H2 cell metabolism, for instance, by enhancing the expression of genes including *Galnt3* and *Plin2*.

One limitation of our study was an inability to accurately detect PPAR- γ protein. This has various implications. First, whether all T_H2 cells or only a subset of “pathogenic” T_H2 cells express PPAR- γ is still unclear. Gene expression analysis using single-cell RNA-sequencing approaches or following purification of pathogenic T_H2 cells using an IL-5 reporter strain may help clarify this point. Second, we were unable to reliably precipitate PPAR- γ from DNA and identify loci to which it had bound. Nonetheless, in adipocyte differentiation cultures, super-enhancers associated with PPAR- γ -responsive elements have been found at many loci, including an enhancer of *Il1rl1* (43). Thus, although we were unable to directly confirm the binding of PPAR- γ to this locus in T_H2 cells, the induction of ST2 protein after agonism of PPAR- γ suggests that this may be one transcriptional target.

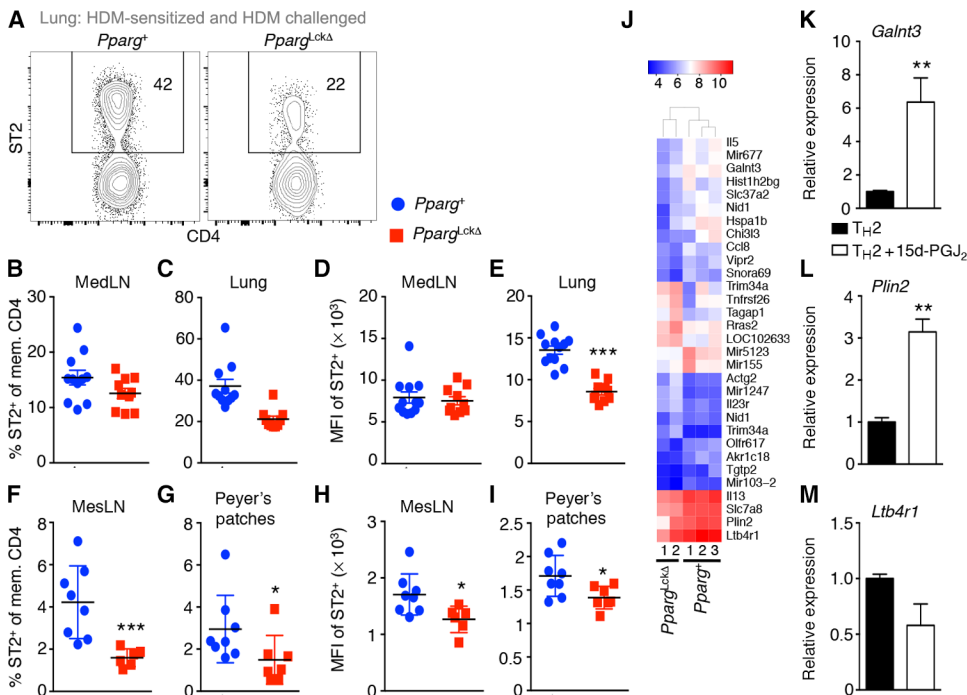


Fig. 6. PPAR- γ promotes the expression of ST2 in T_H2 cells in vivo. (A to E) Control $Pparg^+$ and $Pparg^{Lck\Delta}$ mice sensitized and challenged with HDM were analyzed for ST2 expression in medLN and the lung. (A) Representative plots of CD4 versus ST2 expression on gated lung CD4 $^+$ CD44 $^+$ cells. (B to E) Graphs of the frequency of ST2 $^+$ cells among memory CD4 $^+$ cells in the medLN (B), the lung (C), and the MFI of ST2 expression on ST2 $^+$ memory CD4 $^+$ cells (D and E). (F to I) Control $Pparg^+$ mice and $Pparg^{Lck\Delta}$ mice were infected with *H. polygyrus*, and ST2 expression on CD4 T cells was analyzed by flow cytometry 15 days after infection. Graphs of the frequency of ST2 $^+$ cells among CD4 $^+$ CD44 $^+$ Foxp3 $^-$ cells (F and G) and MFI of ST2 on ST2 $^+$ CD4 $^+$ CD44 $^+$ Foxp3 $^-$ cells (H and I) in the mesLN and Peyer's patches 15 days after infection with *H. polygyrus* ($n = 6$ to 8 mice pooled from two independent experiments). (J) ST2 $^+$ CD44 $^+$ CD4 $^+$ cells from the lungs of HDM-sensitized and HDM-challenged $Pparg^+$ and $Pparg^{Lck\Delta}$ mice were purified and analyzed for genome-wide mRNA expression. Heat map showing the 29 genes that were consistently up-regulated or down-regulated between groups. Values are log $_2$ -transformed. (K to M) *Galnt3*, *Plin2*, and *Ltb4r1* mRNA expression was quantified after 7 days of culture in the presence or absence of 15d-PGJ $_2$ ($n = 5$ to 7 separate wells pooled and normalized to the level in T_H2 -only cultures). Mann-Whitney *U* test was used for all comparisons. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Although we have demonstrated here a clear amplifying function for PPAR- γ in T_H2 cell-mediated responses, preclinical studies have repeatedly demonstrated that TZDs ameliorate airway inflammation and improve respiratory function in animal models. This effect has often been linked to inhibition of the inflammatory activity of airway epithelial cells or the priming function of dendritic cells (21, 22). The therapeutic effects of TZDs in small animal models even led to trials in asthmatic patients; however, these showed that agonists of PPAR- γ had limited benefits (23, 44). Our data suggest that activation of PPAR- γ may in fact exacerbate type 2-mediated airway inflammation in some cases. Although TZD use in patients with type 2 diabetes mellitus has proven useful, side effects of TZDs include edema, weight gain, and heart failure, which all testify to a potentially inflammatory function for PPAR- γ (20, 45). Note that SNPs in *IL1RL1* and *IL33* are strongly associated with heart disease and asthma (12, 13), and it is possible that the side effects of TZDs on cardiac function may be linked to the activation of this highly inflammatory ST2-IL-33 axis.

A recent area of contention has revolved around the impact of PPAR- γ agonists on cell populations in VAT. Although TZDs have been shown to improve glucose sensitivity (20), whether T_{reg} s were

the critical mediators of this effect or whether other cell populations in VAT were responsible was unclear (19, 46). We have shown here that PPAR- γ is expressed by both CD4 T cells and ILC2 and, furthermore, that this expression is important for the accumulation of T_H2 cells. Because both ILC2 and CD4 T cells in VAT have previously been shown to regulate metabolism, our results suggest that TZDs may also act on ILC2 and T_H2 cells to regulate glucose and insulin sensitivity. Recent single-cell RNA-sequencing analysis of human ILC populations also highlighted the fact that PPAR- γ was enriched in ILC2 from human tonsils, suggesting that PPAR- γ expression is a trait conserved not only between adaptive and innate type 2 lymphocytes but also across species (47). Another recent observation in the study by Bapat *et al.* (46) was that T_{reg} cells that accumulated with age impaired glucose sensitivity. This was odd because T_{reg} s have typically been thought to suppress inflammation and promote glucose sensitivity (19, 48), especially in the context of obesity. Our results support their assertion that PPAR- γ -dependent accumulation of T_{reg} s (and, potentially, T_H2 cells as well) may act to impair glucose sensitivity, in the setting of aging. Hence, TZDs are likely to target T_{reg} s, T_H2 cells, and ILC2 cells in VAT, but their therapeutic effects in the context of either aging or obesity may be disparate.

Last, in the *H. polygyrus* model of infection, we observed reduced levels of IL-5 $^+$ IL-13 $^+$ T_H2 cells and severely reduced serum IgE responses, despite normal IL-4 production by CD4 T cells. It is possible that the reduction in IgE reflects the reduction in CD4 T cell-derived IL-13, although we cannot rule out the possibility that IL-4 was reduced at earlier time points and that this affected IgE levels on day 15. Although IL-5, eosinophils, and IgE play a limited role in expulsion or control of *H. polygyrus* (29), it is clear that loss of PPAR- γ in T cells impaired immunity to secondary infection with this pathogen. The impact of PPAR- γ in other models of helminth infection where IL-5 and eosinophils can play a greater role is worth exploring (49, 50).

MATERIALS AND METHODS

Study design

The aim of this study was to characterize the role of PPAR- γ in type 2 immunity. To achieve this aim, we analyzed cellular, histological, and molecular components from age-matched littermate control WT and gene-targeted mice. Cages were randomly assigned to uninfected, infected, PBS-treated, or allergen-treated groups. Mice within one cage were always treated in the same fashion to avoid the risk of transmitting pathogens or allergens from exposed to unexposed mice. Power

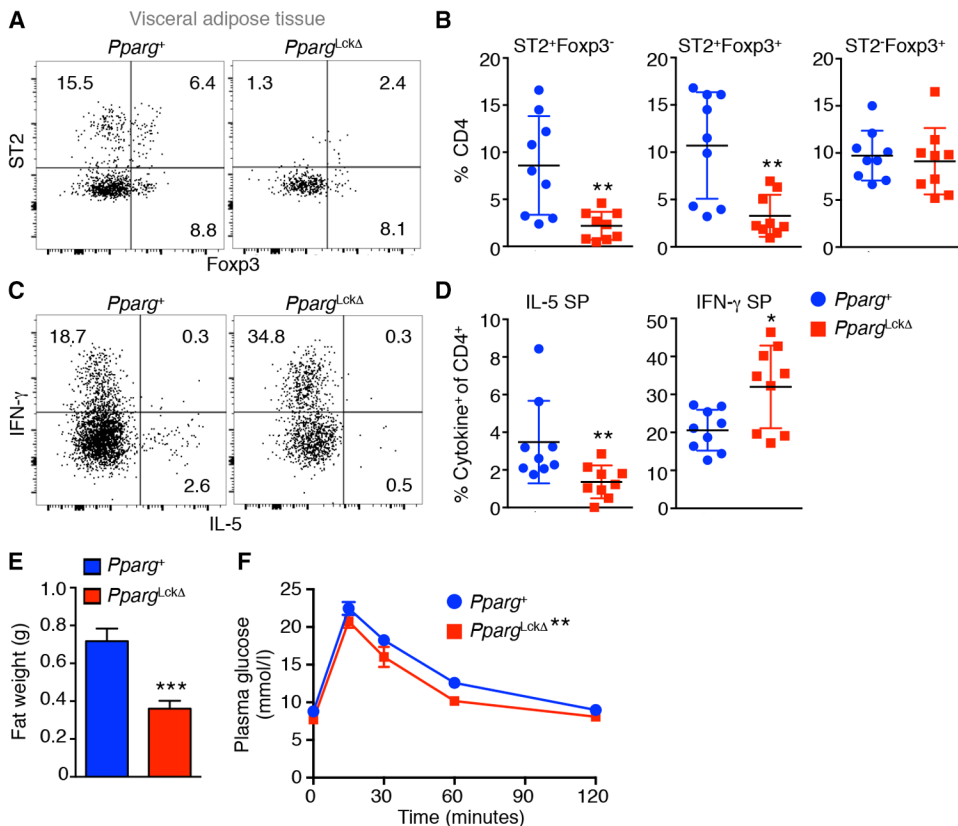


Fig. 7. PPAR- γ supports resident T_H2 cells in the VAT. Populations of CD4⁺ T cells in VAT of 12- to 20-week-old mice were analyzed by flow cytometry ($n = 9$ mice per group pooled from three experiments). (A) Representative dot plots of Fopx3 versus ST2 expression on gated CD4⁺ T cells from VAT of control *Pparg*⁺ and *Pparg*^{LckΔ} mice. (B) Quantification of ST2⁺Fopx3⁻, ST2⁺Fopx3⁺, and ST2⁺Fopx3⁺ CD4⁺ cells from VAT of *Pparg*^{LckΔ} mice. (C) Representative plots of IL-5 versus IFN- γ expression on gated CD4⁺ T cells from VAT of control *Pparg*⁺ and *Pparg*^{LckΔ} mice. (D) Quantification of IL-5 and IFN- γ single-positive (SP) CD4⁺ cells. (E) Weight of VAT from 20-week-old mice on a normal chow diet. (F) Glucose concentration in plasma after injection of glucose over 120 min ($n = 7$ to 10 mice pooled from two independent experiments). For statistical analysis, area under the curve was calculated. Mann-Whitney U test was used for all comparisons. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

calculations were performed on the basis of many previous studies that have been conducted by our groups in the context of allergen challenge and helminth infection. The assessment of worm burden and histological sections was performed in a blinded fashion. All experiments were conducted in accordance with the Stockholm Norra djurförsöksetiska nämnd.

Mice and generation of bone marrow chimeras

WT C57BL/6, *Lck-Cre*⁺, *Pparg*^{fl/fl}, and C57BL/6.CD45.1 congenic mice were bred and maintained at the Department of Microbiology, Tumor and Cell Biology, Karolinska Institute. *Gata3*^{+/+} and *Gata3*^{+/−} mouse spleens were transported overnight from Erasmus MC (Rotterdam, Netherlands) to Karolinska Institute (Stockholm, Sweden) for further analysis. For generation of bone marrow chimeras, C57BL/6 CD45.1 congenic mice were lethally irradiated with 2 $\times$ 5-Gy γ -irradiation 3 hours apart. The following day, mice were reconstituted intravenously with a 1:1 mixture of CD45.1⁺CD45.2⁺ WT and CD45.1[−]CD45.2⁺ *Pparg*^{LckΔ} bone marrow cells. Mice were kept on antibiotics for 3 weeks and used in experiments 8 weeks after irradiation.

Models of asthma and *H. polygyrus* infection House dust mite.

Four- to 5-week-old mice were anesthetized briefly with isoflurane. For intranasal sensitization, mice received 1 μ g of HDM in 40 μ l of PBS. One week after sensitization, mice were challenged for 5 consecutive days with 10 μ g of HDM administered intranasally under light anesthesia with isoflurane. Four days after the last administration, mice were sacrificed, and their organs were dissected for analysis. BAL was performed by two consecutive flushes of the lung with 1 ml of PBS. The medLN and spleen were dissected through 100 μ m sieves and analyzed by flow cytometry. Periodic acid-Schiff staining was performed at the Morphological Phenotype Analysis facility at Karolinska Institute.

Heligmosomoides polygyrus.

Three- to 4-week-old mice were infected with 200 L3 (third-stage *H. polygyrus* larvae), and euthanized 2 weeks after infection. For removal of worms, the mice were treated with pyrantel pamoate (1 mg/g; Fyranet vet) for 3 consecutive days, starting 2 weeks after infection. After anti-helminth treatment, mice were screened for eggs in feces. No eggs were shed when reinfection with 200 L3 was given 3 days after the last treatment with pyrantel pamoate. Mice were euthanized 2 weeks after reinfection.

T_H cell differentiation assays

For T_H cell differentiation, the spleen was harvested, and naïve CD4⁺ T cells were enriched with the Naïve CD4⁺ T Cell Isolation Kit (Miltenyi Biotec) or purified on

the basis of CD4, CD44, and CD25 using a FACS Aria Fusion (BD). All cell culture was performed in Iscove's modified Dulbecco's medium (IMDM) or RPMI supplemented with penicillin/streptomycin, glutamine, 2-mercaptoethanol (all from Invitrogen), and 8% heat-inactivated fetal calf serum (FCS) (Sigma). For all differentiation conditions, 1 $\times$ 10⁵ enriched naïve CD4⁺ T cells in 200 μ l of culture medium were plated in 96-well polystyrene round-bottom plate precoated with anti-CD3 (4 μ g/ml; 145-2C11, BD) and soluble anti-CD28 (1 μ g/ml; clone 37.51, BD). For T_H1 cell differentiation, the culture was conditioned in rmIL-12 (100 ng/ml; PeproTech) and anti-IL-4 (5 μ g/ml; 11B11, BD); to induce T_H2 , we added rmIL-4 (20 ng/ml; Sigma) and anti-IFN- γ (10 μ g/ml; XMG1.2, Bio X Cell) to the medium; T_H17 cells were differentiated in rhTGF- β (5 ng/ml; R&D), rmIL-6 (20 ng/ml; BD), and anti-IFN- γ (10 μ g/ml; XMG1.2, Bio X Cell); T_{reg} s were induced in rhTGF- β (5 ng/ml; R&D) and anti-IFN- γ (10 μ g/ml; XMG1.2, Bio X Cell).

For PIO or 15d-PGJ₂ (Sigma)-treated T_H2 cultures, various doses of the drugs were added to the culture on day 0, and cells were harvested for ST2 expression between days 4 and 7. For cultures that were maintained for 7 days, culture medium was replenished on days 4 and 6 with

the components of T_H2 differentiation at appropriate concentrations, as described above.

Restimulation (ICS, ELISA, and PMA)

Cell culture was performed in either RPMI-1640 or IMDM supplemented with penicillin/streptomycin, glutamine, 2-mercaptoethanol (all from Invitrogen), and 8% heat-inactivated FCS (Sigma). To analyze cytokine production, we added PMA (50 ng/ml) and 5 μ M ionomycin (both from Sigma) to cultures of total medLN, mesLN, VAT, Peyer's patches, or lung for 2.5 to 3 hours in the presence of Brefeldin A (Sigma). The BD Fixation/Permeabilization Kit was used for intracellular staining of cells for cytokines. The eBioscience Fixation Kit was used when intranuclear staining of Foxp3 was performed.

Quantitative PCR and array

RNA was obtained using the TRIzol isolation reagent (Thermo Scientific) and isolated according to the manufacturer's instructions. The RNA was reverse-transcribed using Invitrogen SuperScript (generations 3 and 4), and samples were analyzed by SYBR Green-based reverse transcription PCR on the Bio-Rad CFX384 machine.

For the microarrays in Fig. 1, $CD4^+$ T cells ($CD4^+CD45^+F4/80^-$) from the lung were purified from naïve PBS-treated or HDM-treated mice. Samples were analyzed on a GeneChip Mouse Gene 2.0 ST Array (Affymetrix). Data were deposited at the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) database (GSE72005). For the microarrays in Fig. 6, $ST2^+$ memory $CD4^+$ T cells were purified, and samples were analyzed on a GeneChip Mouse Gene 2.1 ST Array (Affymetrix). Only 29 annotated genes (31 probes) were consistently differentially expressed with at least a twofold difference between $Pparg^{Lck\Delta}$ and control $Pparg^+$ mice. T cell receptor, immunoglobulin, and unannotated genes were discarded from the analysis at this time. A full description is provided in the Supplementary Materials.

Statistical analysis

A nonparametric Mann-Whitney U test was used to compare two groups. One-way analysis of variance (ANOVA) and Bonferroni's post hoc test were used for multiple comparisons. For glucose tolerance testing, statistical significance was analyzed by Mann-Whitney U test after measuring the area under the curve in both groups of mice. Mean and SEM are shown in all graphs. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Normal expression of IL-4 and IL-17 by lung memory $CD4^+$ T cells after HDM sensitization and challenge.

Fig. S2. Representative profiles of mesLN cells after *H. polygyrus* and graphs of cytokine production in Peyer's patches.

Fig. S3. The effects of PPAR- γ on IL-5, IL-13, and ST2 expression are cell-intrinsic.

Fig. S4. Normal BAL eosinophilia and ST2 expression after HDM administration into control *Lck-Cre*⁺ WT mice.

Fig. S5. Sort strategy for whole-genome expression arrays.

Fig. S6. ILC2 in VAT express PPAR- γ but are not targeted for *Pparg* gene deletion in *Pparg*^{Lck Δ} mice.

Fig. S7. The effect of PPAR- γ on ILC2 frequency after HDM administration and *H. polygyrus* infection.

Fig. S8. Normal ILC2 responses in *Pparg*^{Lck Δ} mice.

Fig. S9. Normal frequencies of $ST2^+$ T_{reg} s at baseline in various organs.

Fig. S10. Analyzing the requirement for PPAR- γ in T_{reg} s in the HDM and *H. polygyrus* model.

Raw data (Excel)

Array data (Excel)

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PPAR- γ promotes type 2 immune responses in allergy and nematode infection

Ting Chen, Christopher A. Tibbitt, Xiaogang Feng, Julian M. Stark, Leona Rohrbeck, Lisa Rausch, Saikiran K. Sedimbi, Mikael C. I. Karlsson, Bart N. Lambrecht, Gunilla B. Karlsson Hedestam, Rudi W. Hendriks, Benedict J. Chambers, Susanne Nylén and Jonathan M. Coquet

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Editor's Summary Type 2 immunity on PPARAgonists to the anti-inflammatory molecule peroxisome proliferator activated receptor-g (PPAR-g) decrease airway inflammation in animal models but have had limited success in clinical trials. Now, Chen et al. examine the contribution of PPAR-g to T helper type 2 (TH2) immunity, which is critical for both allergy and parasite immune response. Mice that lacked PPAR-g did not develop allergic pathology and failed to protect against nematode infection. These mice lacked interleukin-5 (IL-5)- and IL-13-secreting cells and had reduced frequencies of TH2 cells in visceral adipose tissue. PPAR-g agonists up-regulated the IL-33 receptor ST2. Hence, PPAR-g may contribute to IL-5 and IL-13 production by TH2 cells, which highlights the need for caution in using PPAR-g agonists for TH2-mediated diseases.

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Supplementary Materials

PPAR- γ promotes type 2 immune responses in allergy and nematode infection

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Supplementary materials and methods

Organ Isolation

Thymus, spleen, mesLN and medLN were passed through 100 μ M filters with the end of a 3 mL syringe and PBS containing 2% heat inactivated FCS (FACS buffer). Spleens were also lysed of red blood cells with a hypotonic solution briefly before being pelleted and resuspended in FACS buffer. For bone marrow cell isolation, femurs and tibias of mice were flushed with a 25-gauge needle with medium. Cells were pelleted, red blood cells were lysed as above and resuspended in PBS before transfer into irradiated mice. For VAT, tissue was carefully dissected to avoid the gonads and lymph nodes. VAT was intensely chopped with dissection scissors and incubated in serum free medium containing LiberaseTM (Roche) for 30 minutes at 37°C, making sure to agitate the sample by passing through a Pasteur pipette every 10 minutes. The cell suspension was then passed through a 70 μ M filter, spun down and red blood cells were lysed before cells were eventually resuspended in FACS buffer.

Quantification of worm load

To determine the load of adult worms in mice, the small intestine was collected and cut open. The intestine was placed in a fine mesh, with the inside facing the mesh, in a tube containing medium (RPMI-1640 with 100 U/ml penicillin, 200 μ g/ml streptomycin and 500 μ g/ml gentamycin). The intestines were incubated at 37°C for 3-4 hours, allowing viable worms to migrate through the mesh into the medium. Free worms were collected from the tube and transferred to a 10cm-dish with medium and further incubated at 37°C for 1 hour to easily allow individual counting of worms.

Reagents, antibodies and flow cytometry

HDM extracts were obtained from Greer Laboratories. Pioglitazone and 15d Δ 12,14-PGJ₂ were purchased from Sigma. Fluorochrome-labelled and biotinylated antibodies from BD: CD3 (145-2C11), CD8 (53-6.7), CD4 (RM4-5), Siglec-F (E50-2440), Ly6C/G (R86-8C5), CD127 (SB-199), CXCR3 (CXCR3-173), IL-5 (TRFK5), NK1.1 (PK136), CD44 (IM7), CD19 (1D3), CD45.1 (A20), CD45.2 (104), CD90.2 (53-2.1), CCR6 (140706), IFN- γ (XMG1.2), E Bioscience: CD11c (N418), CD11b (M1/70), IL-13 (eBio13A), Biolegend: T1/ST2 (DIH9). Lineage⁻ cells when gating for ILC were identified as CD3⁻CD4⁻CD11c⁻CD19⁻CD11b⁻.

Stained samples were analyzed on a BD LSRII, LSRFortessa or FACSCalibur and subsequently using FlowJo (TreeStar) software. Sorting was performed on a BD FACSARIA Fusion or Dako Cytomation MoFlow.

Genome-wide expression arrays

RNA from CD4⁺ T cells was assessed with a Bioanalyzer 2100 (Agilent). With a WT Expression Kit (Ambion), 50 ng of total RNA per sample, 'spiked' with bacterial poly(A) RNA positive control (Affymetrix) was converted to double-stranded cDNA in a reverse-transcription reaction. Samples were fragmented and labeled with biotin in a terminal labeling reaction according to the Affymetrix WT Terminal Labeling Kit. A mixture of fragmented biotinylated cDNA and hybridization controls (Affymetrix) was hybridized on a GeneChip Mouse Gene 2.0 ST Array (Affymetrix). For analysis of raw probe signal intensities, chips were scanned with a GeneChip scanner 3000 (Affymetrix). Samples were subsequently analyzed and clustered in 'R' software. For microarrays in Figure 6, a similar protocol was used except that cDNA was hybridized on a GeneChip Mouse Gene 2.1 ST Array (Affymetrix).

List of PCR primers

PCR primers are listed in the Supplemental materials. PCR primers were as follows: *Hprt* 5' TGAAGAGCTACTGTAATGATCAGTCAAC, *Hprt* 3' AGCAAGCTTGCAACCTTAACCA, *Il4* 5' CTCATGGAGCTGCAGAGACTCTT, *Il4* 3' CATTTCATGGTGCAGCTTATCGA, *Il17a* 5' TGAAGGCAGCAGCGATCA, *Il17a* 3' GGAAGTCCTTGGCCTCAGTGT, *Il5* 5' AGCACAGTGGTGAAAGAGACCTT, *Il5* 3' TCCAATGCATAGCTGGTGATTT, *Pparg* 5' (1) CAAGAATACCAAAGTGCGATCAA, *Pparg* 5' (2) CAACAGCCTGACGGGGTCT, *Pparg* 3' GAGCAGGGTCTTTTCAGAATAATAAG, *Ifng* 5' GCCAAGTTTGAGGTCAACAAC, *Ifng* 3' AAATTCAAATAGTGCTGGCAGA. *Galnt3* 5' TTCTCTGCACCGGGACCTT, *Galnt3* 3' GGCGGGCAGCGCTTA, *Plin2* 5' CTCAGGAGGAGCTGGAGAT, *Plin2* 3' CGCCATCGGACACTTCCTTA, *Ltb4r1* 5' GGACCCTGGCACTAAGACA, *Ltb4r1* 3' CAGTAGAACAATGGGCAAC, *Pparg* 5' (2) was only used for comparing *Pparg* mRNA levels in WT control *Pparg*⁺ versus *Pparg*^{LckΔ} mice in Fig. 2B. This is due to the location of loxP sites in *Pparg*^{fl/fl} mice. *Pparg* 5' (1) primer was used in all other conditions.

Bone marrow macrophage differentiation

Bone marrow cells were cultured in DMEM containing Penicillin/Streptomycin, glutamine, 2-mercaptoethanol and 8% heat-inactivated FCS in the presence of 10 ng/ml M-CSF. Medium was changed every three days. rIL-4 and rIL-10 (both at 10 ng/ml) were added on day 7 for the differentiation of M2 macrophages and these were harvested for RNA one day later.

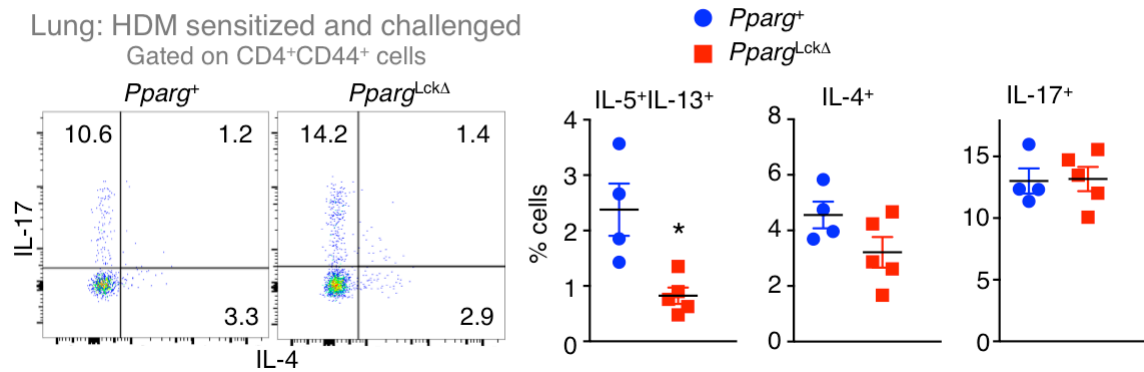
Enzyme-linked Immunosorbant Assay (ELISA)

Plates were coated with 2 µg/ml (100 µl/well) IgE capture antibody (BD Clone: R35-92) diluted in PBS overnight. Plates were blocked with 2% dry milk in PBS for 1h. Mouse serum was diluted in 2% dry milk in PBS and incubated for 2h at room temperature. IgE detection antibody (2 µg/ml, BD Clone R35-72) and Streptavidin-HRP (diluted 1:1000) were used to detect bound IgE. TMB was used as a substrate and the reaction stopped with 1M H₂SO₄. Absorbance at 450 nm was recorded.

Glucose tolerance test

5 month-old male littermates were fasted overnight and were injected i.p. with 2 g/kg glucose the following morning. Blood glucose was measured at 15, 30, 60 and 120 minutes after injection.

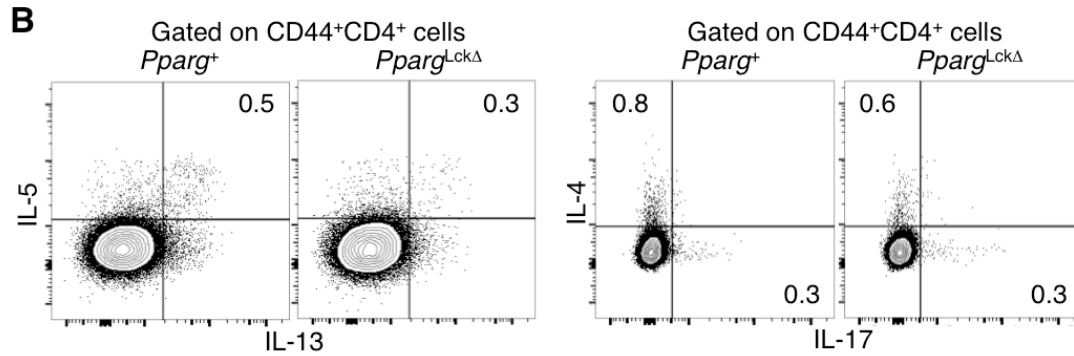
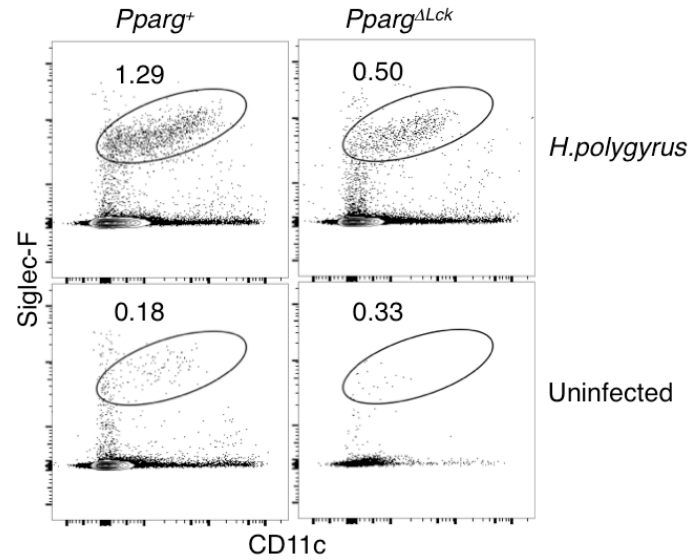
Supplementary Fig. 1



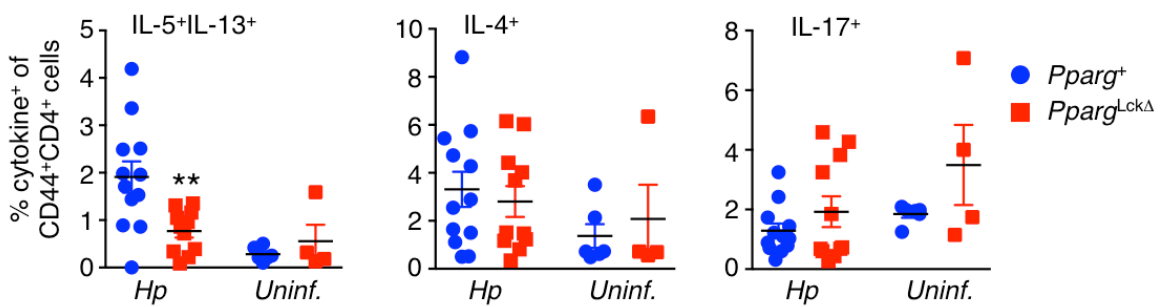
Supplementary Fig. 1. Normal expression of IL-4 and IL-17 by lung memory CD4 T cells following HDM sensitization and challenge. In an independent experiment, *Pparg*⁺ and *Pparg*^{LckΔ} mice were sensitized and challenged to HDM and secretion of cytokines by CD4 T cells was analysed by flow cytometry. Plots show IL-4 versus IL-17 expression on restimulated lung lymphocytes. Graphs depict a reduction in the frequency of IL-5⁺IL-13⁺ cells, but not IL-4⁺ and IL-17⁺ cells (*Pparg*⁺ (n=4) and *Pparg*^{LckΔ} mice (n=5)). Mann-Whitney U rank test was used to compare *Pparg*⁺ and *Pparg*^{LckΔ} groups. *p<0.05

Supplementary Fig. 2

A *H. Polygyrus* - mesLN



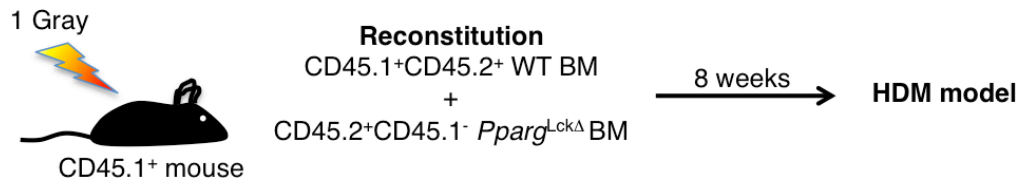
C *H. Polygyrus* - Peyer's Patches



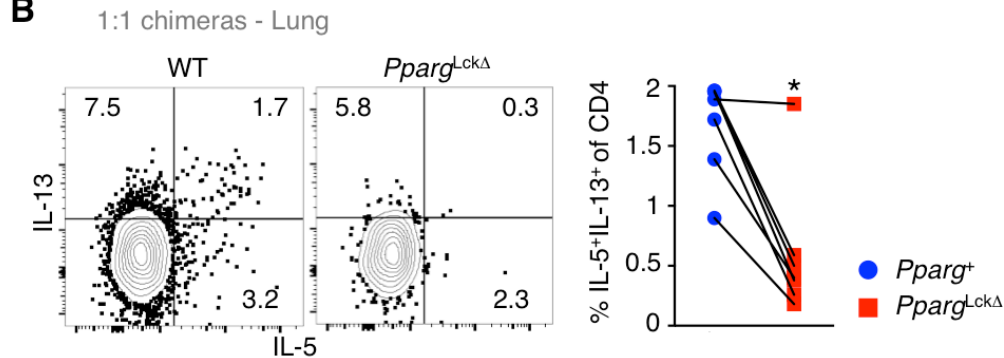
Supplementary Fig. 2. Representative profiles of mesLN cells after *H. polygyrus* and graphs of cytokine production in the Peyer's Patches. (A) Representative plots of CD11c versus Siglec-F expression in uninfected and infected mice. **(B)** Representative plots of IL-13 versus IL-5 and IL-17 versus IL-4 expression in gated memory CD4 T cells from mesLN following *H. polygyrus* infection. **(C)** Graphs of the frequency of cytokine producing cells among gated memory CD4 T cells in the Peyer's Patches following restimulation. Mann-Whitney U rank test was used to compare *Pparg*⁺ and *Pparg*^{ΔLckΔ} groups. *p<0.05

Supplementary Fig. 3

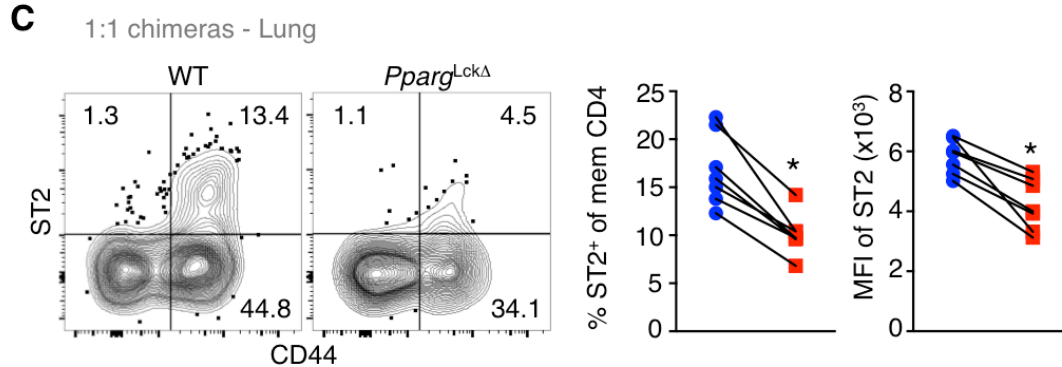
A



B

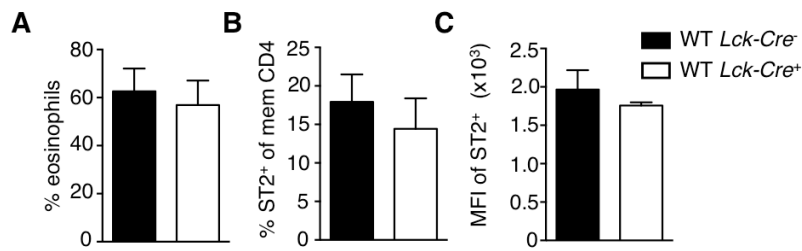


C



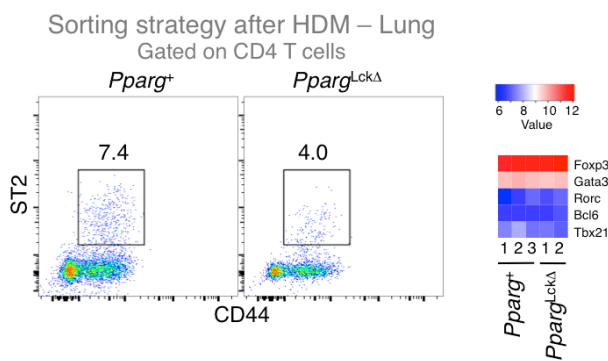
Supplementary Fig. 3. The effects of PPAR- γ on IL-5, IL-13 and ST2 expression are cell-intrinsic. (A) Recipient congenic CD45.1⁺CD45.2⁻ mice were lethally irradiated and reconstituted with WT (CD45.1⁺CD45.2⁺) and *Pparg*^{LckΔ} (CD45.2⁺CD45.1⁻) bone marrow cells at a 1:1 ratio. After 8 weeks, mice were sensitized and challenged with HDM and Th2 cell responses were measured by flow cytometry. (B) Representative plots of IL-5 versus IL-13 expression in WT and *Pparg*^{LckΔ} cell fractions. Graphs represent percentages of WT or *Pparg*^{LckΔ} cells producing IL-5 and IL-13. (C) Representative plots of CD44 versus ST2 expression in WT and *Pparg*^{LckΔ} CD4⁺ cell fractions in the lung. Graphs represent the percentages of WT and *Pparg*^{LckΔ} cells expressing ST2 among gated CD4⁺CD44⁺ cells and the intensity of ST2 expression on gated ST2⁺CD4⁺CD44⁺ cells. Linked symbols represent values within the one mouse. n=7 chimeric mice from two independent experiments. Wilcoxon signed rank test was used to compare paired values. *p<0.05

Supplementary Fig. 4



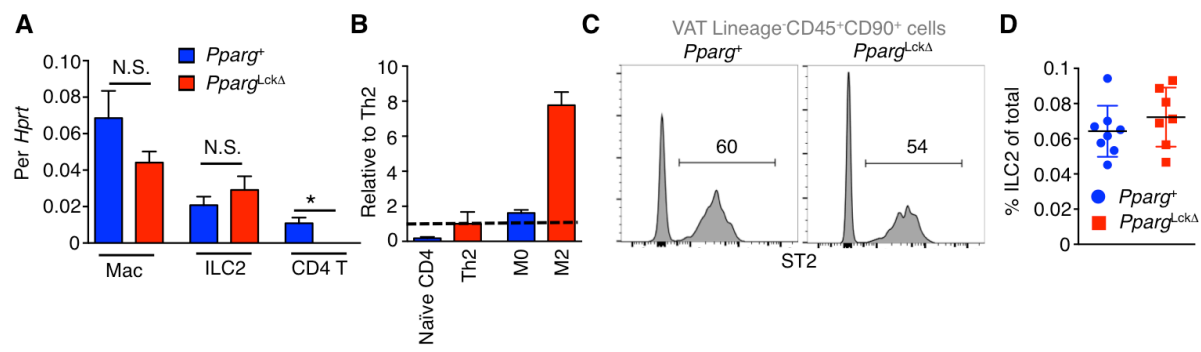
Supplementary Fig. 4. Normal BAL eosinophilia and ST2 expression following HDM administration into control *Lck-Cre*⁺ WT mice. WT *Lck-Cre*^{-/-} vs. WT *Lck-Cre*^{+/+} mice were sensitized and challenged with HDM and analyzed at 15 days post-sensitization. (A) The frequency of BAL eosinophils. (B) The percentage of ST2⁺ cells of CD4⁺CD44⁺ memory cells. (C) The MFI of ST2⁺ cells (n=5 WT *Lck-Cre*^{-/-} and 3 WT *Lck-Cre*^{+/+} mice).

Supplementary Fig. 5



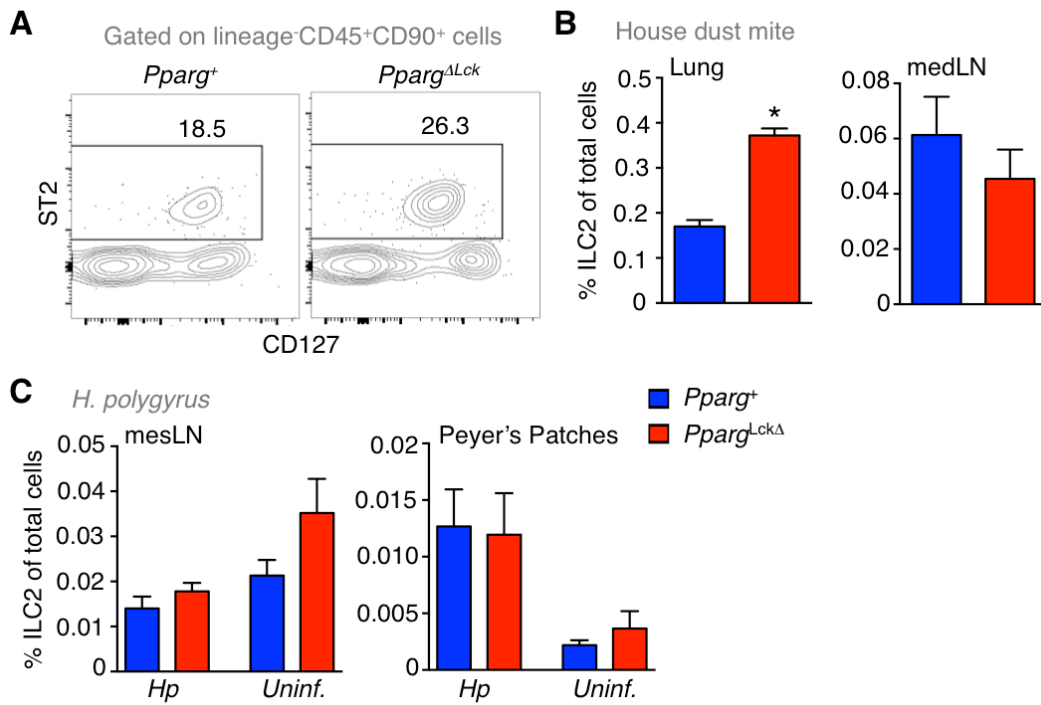
Supplementary Fig. 5. Sort strategy for whole genome expression arrays. ST2⁺ memory CD4 T cells (CD4⁺CD44⁺ST2⁺) were purified from the lungs of HDM-sensitized and -challenged *Pparg*⁺ and *Pparg*^{LckΔ} mice on day 15. Expression of the 'master' transcription factors is depicted by heat map.

Supplementary Fig. 6



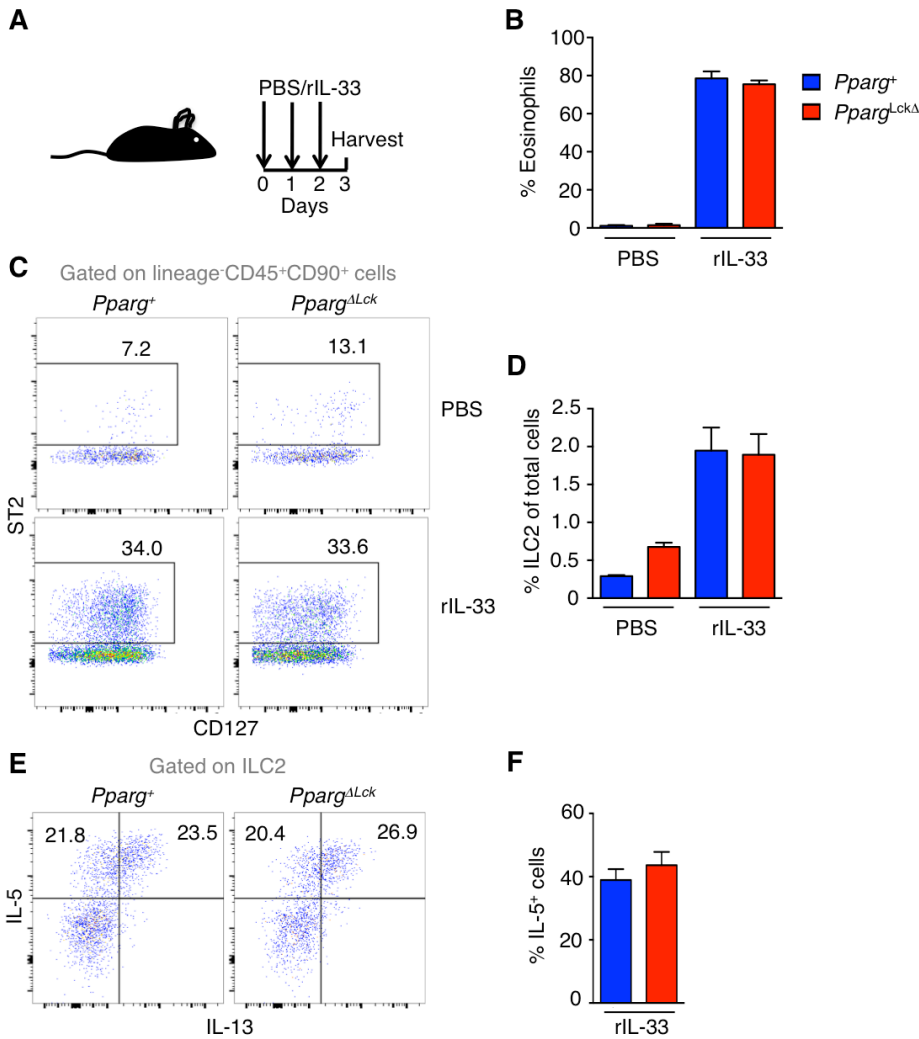
Supplementary Fig. 6. ILC2 in VAT express PPAR- γ but are not targeted for *Pparg* gene deletion in *Pparg*^{LckΔ} mice. (A) ILC2 (Lineage⁻CD4⁻CD45⁺CD90⁺ST2⁺), CD4 T (CD3⁺CD4⁺) cells and F4/80⁺ macrophages were purified from VAT of 5 month-old male *Pparg*⁺ and *Pparg*^{LckΔ} mice. RNA was isolated, reverse transcribed and analysed for levels of *Pparg* and *Hprt*. The graph depicts the expression of *Pparg* relative to *Hprt* (ILC2 and Mac: n=7 mice, CD4 T: n=4 mice). (B) To compare *Pparg* mRNA levels between M2 macrophages and Th2 cells, day 7 cultured macrophages were differentiated towards the type 2 subset with rhTGF- β and rIL-4 for 1 day. M2 macrophages expressed almost 8 times more *Pparg* mRNA than Th2 cells (n=3-5 wells in one representative experiment of two). (C) Representative histograms of ST2 expression in Lineage⁻CD4⁻CD45⁺CD90⁺ cells in *Pparg*⁺ and *Pparg*^{LckΔ} mice. (D) Graph of the percentage of ILC2 of total cells in VAT (n=8 *Pparg*⁺ mice, n=7 *Pparg*^{LckΔ} mice). Mann-Whitney U rank test was used to compare *Pparg*⁺ and *Pparg*^{LckΔ} groups. *p<0.05

Supplementary Fig. 7



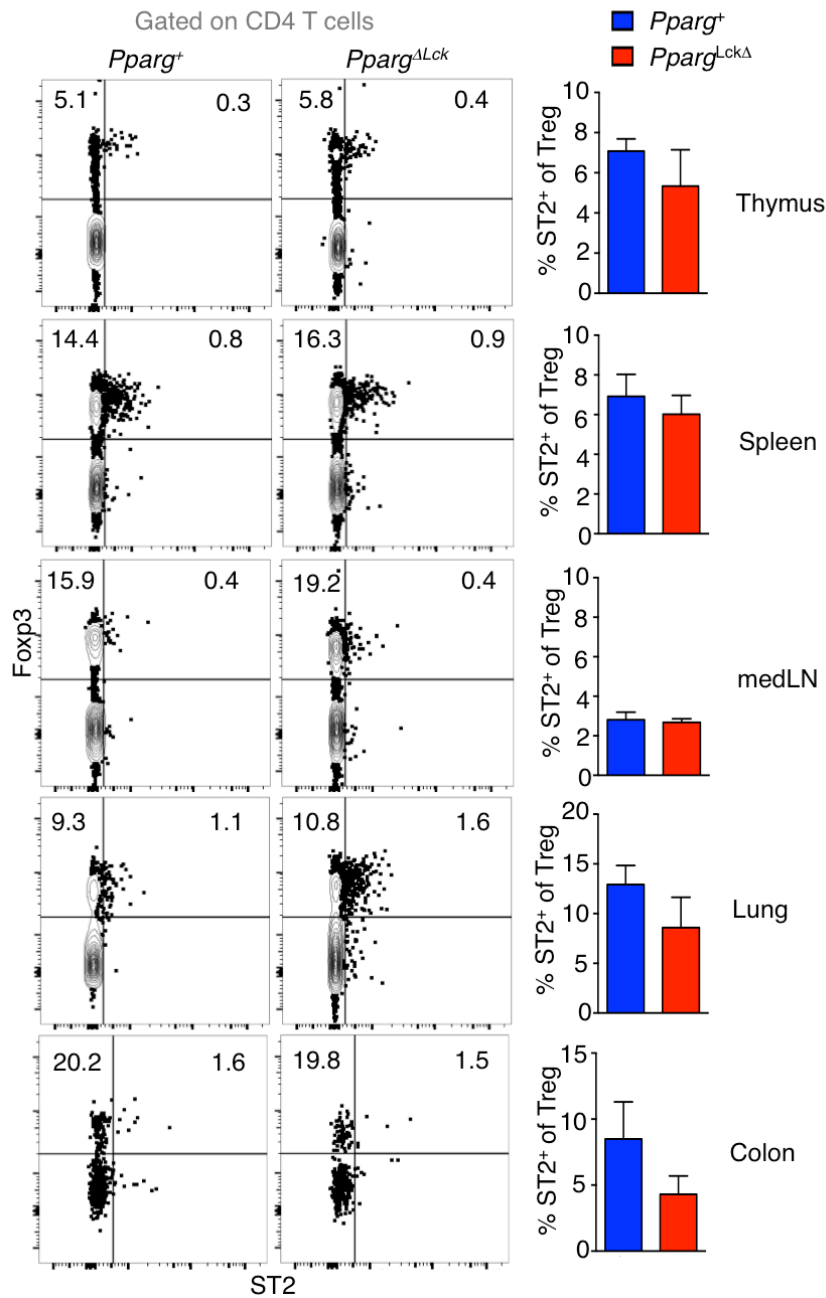
Supplementary Fig. 7. The effect of PPAR- γ on ILC2 frequency following HDM and *H. polygyrus*. (A) Representative plots of ST2 versus CD127 expression on ILCs (lineage⁻CD90⁺CD45⁺) in lung from *Pparg*⁺ control and *Pparg*^{LckΔ} mice sensitized and challenged with HDM after 15 days. (B) The frequency of ILC2 in lung and medLN as defined as percentage of total cells (*Pparg*^{LckΔ} n=5 and *Pparg*⁺ n=4). (C) Mice were infected with *H. polygyrus* and ILC2 frequency in the mesLN and Peyer's patches was analysed by flow cytometry at day 15 post-infection (*Hp* - *Pparg*^{LckΔ} n=5 and *Pparg*⁺ n=5; Uninfected - *Pparg*^{LckΔ} n=4 and *Pparg*⁺ n=6). Mann-Whitney U rank test was used to compare *Pparg*⁺ and *Pparg*^{LckΔ} groups. *p<0.05

Supplementary Fig. 8



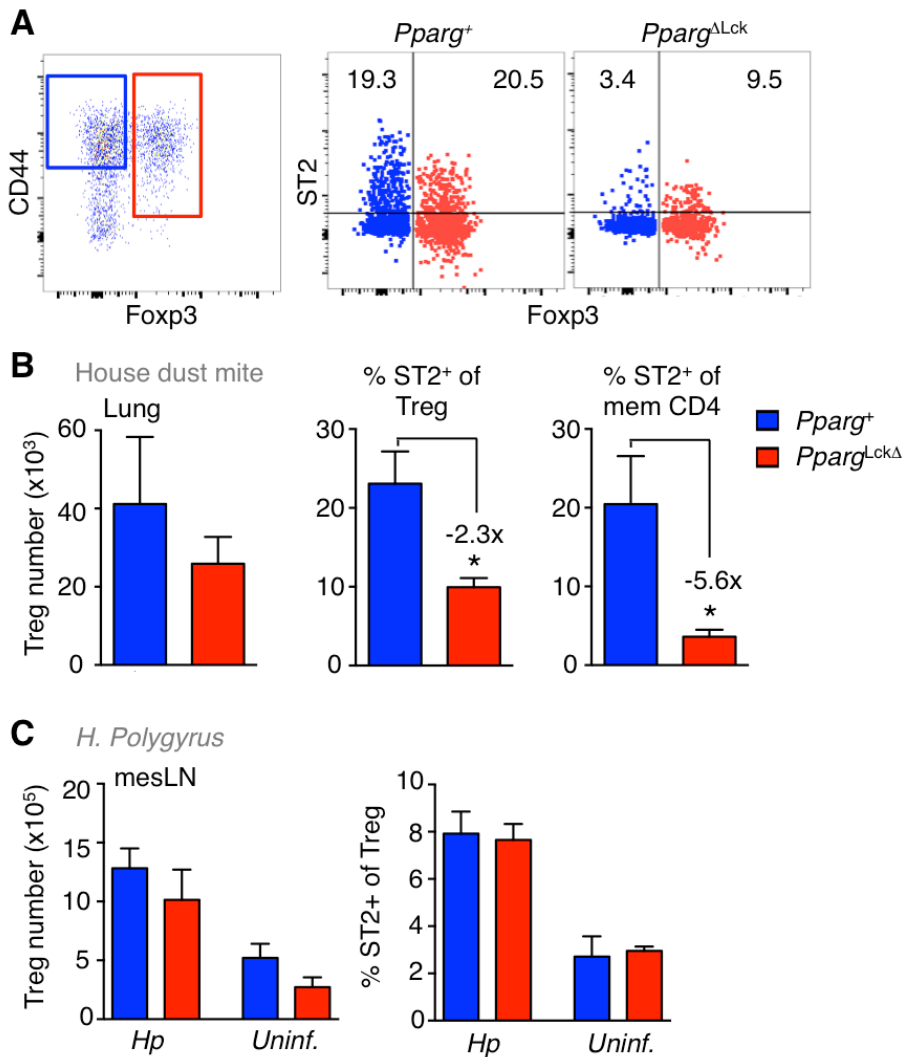
Supplementary Fig. 8. Normal ILC2 responses in $Pparg^{Lck\Delta}$ mice. (A) Schematic of rIL-33 administration (300 ng/day i.n.) (PBS - $Pparg^{Lck\Delta}$ n=4, $Pparg^{+}$ n=3, rIL-33 - $Pparg^{Lck\Delta}$ n=4, $Pparg^{+}$ n=3). (B) The frequency of eosinophils in the airways. (C) Representative plots of CD127 versus ST2 expression on gated lineage⁻CD45⁺CD90⁺ cells. (D) The frequency of ILC2 among total lung lymphocytes. (E) Representative plots of IL-13 versus IL-5 expression on gated ILC2 following restimulation. (F) The frequency of IL-5⁺ cells among gated ILC2.

Supplementary Fig. 9



Supplementary Fig. 9. Normal frequencies of ST2⁺ Treg at baseline in various organs. Representative plots of ST2 versus Foxp3 expression on gated CD4⁺ T cells in naïve *Pparg*⁺ control and *Pparg*^{LckΔ} mice are shown for thymus, spleen, medLN, lung and colon. Graphs indicate the frequency of ST2⁺ cells among gated Treg (CD4⁺Foxp3⁺) in the respective tissue (*Pparg*⁺ n=6-11 and *Pparg*^{LckΔ} n=4-8).

Supplementary Fig. 10



Supplementary Fig. 10. Analysing the requirement for PPAR- γ in Treg cells in the HDM and *H. polygyrus* models. (A,B) *Pparg*⁺ (n=4) and *Pparg*^{Lck Δ} (n=5) mice were sensitized and challenged with HDM in an independent experiment and the Treg cell response was analysed. (A) A representative gating strategy for Treg and memory CD4 T cells is shown (left-hand plot). Representative plots of Foxp3 versus ST2 expression on gated Treg (red dots) and memory CD4 T cells (blue dots) from *Pparg*⁺ and *Pparg*^{Lck Δ} mice are shown. (B) The left-hand graph depicts the total number of Foxp3⁺CD4⁺ Treg cells in the lung of mice. The middle and right-hand plots depict the frequency of ST2⁺ cells among gated Treg and memory CD4 T cells respectively. A reduction of approximately 2.3-fold in the frequency of ST2⁺ cells among Treg was observed in *Pparg*^{Lck Δ} mice. The reduction in the frequency of ST2⁺ cells in memory CD4 T cells was approximately 5.6-fold. (C) *H. polygyrus* infection. The left-hand graph depicts the total number of Foxp3⁺CD4⁺ Treg cells in the mesLN of *H.*

polygyrus-infected and uninfected mice (*Pparg*⁺ (infected n=12, uninfected n=6) and *Pparg*^{LckΔ} mice (infected n=10, uninfected n=4)). The right-hand graph depicts the frequency of ST2⁺ cells among gated Treg. The frequency of ST2⁺ cells among memory CD4 T cells is reported in Figure 6. Although ST2⁺ Treg are increased in frequency following infection with *H. polygyrus*, this does not appear to be dependent on PPAR-γ. Mann-Whitney U rank test was used to compare *Pparg*⁺ and *Pparg*^{LckΔ} groups. *p<0.05