Supporting Information

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SI Materials and Methods

Mice and Surgeries. The 129S1/SvIm/J (PPARα$^{+/+}$) and 129S4/SvJae-*Ppara*^{tm1Gonz}/J (PPARα$^{-/-}$) mice were from The Jackson Laboratory. The 5B6 TCR Tg mice of the (SJL/J) strain were obtained from Jennifer Gommerman (University of Toronto, Toronto, Canada) through an agreement with Pfizer. Castration and sham surgeries and pellet implantations were conducted on 4–5-wk-old mice as described previously (1). Thirty-day release pellets of DHT (5 mg) or placebo were purchased from Innovative Research of America. Animal protocols were approved by the University Health Network in accordance with the guidelines of the Canadian Council on Animal Care.

EAE Induction, Recall Assays, and T Reg Staining. Mice (8–10 wk) were vaccinated (s.c.) with an emulsion (volume ratio, 1:1) of 100 μg of PLP p139-151 (2 mg/mL in 1× PBS) mixed with CFA (4 mg/mL heat-killed *Mycobacterium tuberculosis H37Ra*; Difco Laboratories) as described (2). PLP p139-151 was synthesized by the Stanford Pan Facility (Stanford, CA). Spleens and lymph nodes were harvested from mice 8 d postimmunization and were dissociated into a single-cell suspension. The recall proliferation and cytokine production by cultured cells in response to PLP p139-151 were measured (2). CD4$^+$FoxP3$^+$CD25$^+$ cells were stained using the mouse regulatory T-cell staining kit (88-8111-40; eBioscience). Mononuclear cells were isolated from spinal cords of EAE mice as described previously (3).

Proliferation and Cytokine Measurements. Proliferation was measured using a [^{3}H]thymidine incorporation assay (2). The levels of mouse and human cytokines in culture supernatants were measured using Ready-SET-Go mouse or human ELISA kits (eBioscience). Flow cytometric analysis of intracellular cytokine staining on mouse CNS mononuclear cells was conducted as described previously (3). For human intracellular cytokine staining, cells were stimulated with 10 ng/mL PMA (Sigma) and 750 nM ionomycin (Calbiochem) for 4 h in the presence of GolgiStop (BD Pharmingen) before staining with Cy7-APC-CD4 and LIVE/DEAD fixable aqua (Invitrogen). Cells were then fixed and permeabilized (BD reagents and protocols) before staining for human IL-4 (PE; clone 8D4-8; BD Pharmingen), human IFNγ (APC; clone B27; BD Pharmingen), or human IL-17A (PE; eBioscience). Data were collected using the LSRII (BD) and were analyzed using Flowjo software.

In Vitro Mouse T-Cell Culture. CD4$^+$ T cells (negative-selection) or CD11c$^+$ (positive-selection) cells were isolated from secondary lymphoid organs using magnetic beads (Miltenyi). For coculture experiments, naïve 5B6 TCR Tg cells (50,000) were cultured with half (25,000) the number of irradiated CD11c$^+$ cells in round-bottomed, 96-well plates with indicated concentrations of PLP p139-151. Alternatively, CD4$^+$ T cells (150,000/well) were cultured in 96-well flat-bottomed plates precoated with various quantities of anti-CD3 (clone 145-2C11) and anti-CD28 (clone 37.51) (eBioscience) in complete RPMI that contained either 10% (vol/vol) FCS (prepared as in ref. 4) or 1% syngeneic sera. For Th17-skewing experiments, complete media was further supplemented with 3 ng/mL TGF-β, 30 ng/mL IL-6, and 10 μg/ml of anti-IFNγ (clone XMG1.2; eBioscience). For PPAR ligand experiments, T cells were first preincubated with fenofibrate (Sigma; F6020; 1–10 μM), rosiglitazone (R2408; 1 μM), or DMSO vehicle (0.1%) in X-VIVO-15 (human) or X-VIVO-20 (mouse) media (Lonza) overnight before TCR and CD28 costimulation.

Human Subjects and Blood Collection. Blood samples (40–70 mL) were drawn from healthy men and women (aged 20–30 y) after obtaining informed consent with approval from the University Health Network Research Ethics Board (REB 09-0577-AE) or the Stanford University Institutional Review Board (protocol identification no. 97959). Ten milliliters of blood were collected in a clot tube for serum collection for hormone measurement. All participants were free of medications except for the use of oral contraceptives by ∼50% of women. Other exclusion criteria include smoking or drug use, recent infection (<4 wk), history of cancer, thyroid problems, autoimmune, inflammatory, neurological or cardiovascular conditions, bleeding disorders, anemia, recent surgery, or obesity (body mass index, >30 kg/m^2). Women provided a sample of blood during the self-reported follicular (self-reported) or luteal (21 d after the start of the last menstrual period, 28-d cycle) phase of the menstrual cycle. All blood draws were conducted in the morning hours with one man and one woman drawn per occasion.

Isolation and Culture of Human CD4$^+$ T Cells. PBMCs were isolated from heparinized blood using Ficoll-Paque density gradient centrifugation (GE Healthcare). CD14$^+$ monocytes and naïve (CD45RA$^+$) or total CD4$^+$ T cells were further fractionated using magnetic beads (Miltenyi). The purity of isolated CD4$^+$ T cells was >96%, as determined using flow cytometry. Naïve CD4$^+$ T cells (50,000/well) were cultured in serum-free X-VIVO-15 media (Lonza; supplemented with 2 mM L-glutamine and 100 U/mL penicillin) in 96-well plates with Dynabeads human T-activator anti-CD3 and anti-CD28. For hormone stimulation of CD4$^+$ T cells, CD4$^+$ T cells were cultured in complete RPMI (2) that contained 10% charcoal-stripped FCS (HyClone) with either 100 nM DHT (Wako; catalog no. 045-26071) or ethanol vehicle (0.01%). For T-cell and monocyte coculture experiments (Fig. S4*A*), CD4$^+$ T cells (50,000) were added, along with anti-CD3 (Clone OKT) anti-CD3 (eBioscience), to monocytes (50,000) that had been preactivated with peptidoglycan (Fluka; 77145; 1 μg/mL) for 5 h. Memory CD4$^+$CD45RO$^+$ Th17 cells were expanded according to a previously published protocol (5).

Hormone Assays. Testosterone and estradiol were quantified in serum by previously described RIA methods (6, 7). Before the RIAs, steroids are extracted with hexane:ethyl acetate (3:2), and testosterone and estradiol are separated by Celite column partition chromatography (John Morris Scientific). Appropriate tritiated internal standards are added to each sample before the extraction step to determine and correct for procedural losses. A highly specific antiserum is used in conjunction with an iodinated radioligand in each RIA. The assay sensitivities are 1.5 ng/dL and 2 pg/mL for testosterone and estradiol, respectively. The interassay CVs for testosterone 11%, 9%, and 10% at concentrations of 14.9 ng/dL, 32.8 ng/dL, and 105 ng/dL, respectively. For estradiol, the interassay CVs are 12%, 11%, and 10% at concentrations of 22 pg/mL, 66 pg/mL, and 183 pg/mL, respectively.

Real-Time PCR Detection of Human and Murine PPARα and PPARγ mRNAs. Total RNA was isolated using Absolutely RNA miniprep kit (Agilent Technologies). Transcript levels were measured either from (*i*) RNA using TaqMan one-step RT-PCR master mix (Applied Biosystems; 4309169) and primer probe sets; or (*ii*) cDNA that was reverse-transcribed from total RNA (Superscript II; Invitrogen) (2) before being amplified using Qiagen

QuantiTect SYBR Green PCR reagents and sequence-specific primers (3) (Table S3).

For TaqMan, the following primer/probes were used: mouse PPARα (Mm00440939_m1), mouse PPARγ (Mm01184322_m1), human PPARα (Hs00947538_m1), mouse β-actin (Mm00607939_s1), and human 18S rRNA (Hs03003631_g1) with the following cycling conditions: 48 °C for 30 min, 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 seconds and 60 °C for 60 s. For the SYBR method, the following cycling conditions were used: 95 °C for 15 min, followed by 50 cycles of 94 °C for 20 s, 55 °C for 30 s, and 72 °C for 30 s. All reactions were run using the Roche LightCycler480 real-time PCR machine.

ChIP of *PPARA* Promoter and *IFNG* CNS-6 and CNS-22 Regions. ChIP was performed to investigate the recruitment of AR to the human PPARα promoter. PBMCs (50×10^6) were obtained from each of three female donors, and these cells were stimulated with Dynabeads human T-activator (half of recommended strength) in the presence or absence of 100 nM DHT or vehicle for 16–18 h. Cells were fixed, and then ChIP was performed immediately according to the directions of the manufacturer (Cell Signaling; SimpleChIP Enzymatic Chromatin IP kit; no. 9002S) with the addition of a manual homogenization step (10 gentle, manual strokes using a tissue grinder) at the time of lysis in buffer A. The following rabbit antibodies were used for immunoprecipitation (IP): anti-androgen receptor (1:30 dilution; Millipore; PG-21, 06-680) or an anti-IgG negative control antibody raised in rabbit (1:500; Cell Signaling; no. 2729). DNA template was amplified using real-time PCR (cycling conditions as described above) or traditional PCR using the following conditions: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s. PCR primer sequences are detailed in Table S3.

In a second experiment, we investigated the abundance of acetylated histone-H4 and RelA at the CNS-6 and CNS-22 sites of the *IFNG*. T cells were isolated from five men for this purpose and were transfected with either PPARα siRNAs or nontargeting control siRNAs before stimulation with Dynabeads human T-activator for 8 h or 24 h. Cells from these donors were pooled ($15–20 \times 10^6$ T cells total) after the fixation step. Anti-acetylated H4 (1:25; Cell Signaling; no. 2594S), anti-RelA (p65 NFκB) (1:100; Cell Signaling; no. 8242S), and a negative control antibody raised in rabbit were used for IP. PCR was conducted similarly as above with the same cycling conditions as above, except with an annealing temperature set to 53 °C.

siRNA Transfection. Transfection of human (Human T Cell Nucleofector Kit) or mouse (Mouse T Cell Nucleofector Kit) $CD4^+$ T cells was conducted according to product directions and the Nucleofector device (Amaxa). Cells were transfected with ON-TARGETplus SMART pool siRNAs (Thermo Scientific) designed against: human PPARγ (L-003436-00-0005), human PPARα (L-003434-00-0005), mouse PPARα (L-040740-01), or mouse PPARγ (L-040712-00), along with a GFP expression vector provided in the kits. As a control, $CD4^+$ cells were transfected with nontargeting siRNAs (ON-TARGETplus control pool; D-001810-10-05). Human $CD4^+$ T cells were recovered from the electroporation vial and then allowed to rest overnight in Lymphocyte Growth Medium (LGM) (Lonza) supplemented with 10% FCS (HyClone), penicillin (100 U/mL), and 2 mM glutamine (both from Gibco). Mouse $CD4^+$ T cells were recovered from the electroporation vial and then allowed to rest overnight in supplemented Mouse T Cell Nucleofector Medium [containing 5% FCS (HyClone), 2 mM glutamine (Gibco), and Medium A and B]. The next day, some T cells were reserved for flow cytometric analysis of GFP expression and for PCR measurement of PPAR mRNA knockdown, whereas the rest were plated with stimuli for cytokine measurement. The level of PPAR mRNA knockdown was determined using real-time PCR or traditional RT-PCR.

Statistical Analyses. Data are presented as means ± SEM. When data were parametric (kurtosis and skewness of <2) and group variances were homogenous (Bartlett homogeneity test), a one-way ANOVA and Tukey post hoc test (for more than two groups) or a *t* test ($n = 2$ groups) were used to detect between-group differences. When data were nonparametric, ranks were compared among groups using a Kruskal—Wallis test and nonparametric test for multiple comparisons (for more than two groups) or a Mann–Whitney *U* test ($n = 2$ groups). For paired human data, a paired *t* test was used to compare differences between males and females. A value of $P \leq 0.05$ was considered significant.

1. Dunn SE, et al. (2007) Peroxisome proliferator-activated receptor (PPAR)alpha expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. *J Exp Med* 204:321–330.
2. Dunn SE, et al. (2006) Isoprenoids determine Th1/Th2 fate in pathogenic T cells, providing a mechanism of modulation of autoimmunity by atorvastatin. *J Exp Med* 203:401–412.
3. Dunn SE, et al. (2010) Peroxisome proliferator-activated receptor delta limits the expansion of pathogenic Th cells during central nervous system autoimmunity. *J Exp Med* 207:1599–1608.
4. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F (2007) Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 8:942–949.
5. Kebir H, et al. (2009) Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis. *Ann Neurol* 66:390–402.
6. Goebelsmann U, et al. (1979) Serum gonadotropin testosterone estradiol and estrone levels prior to and following bilateral vasectomy. *Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man*, eds Lepow IH, Crozier R (Academic Press, New York), p 165.
7. Probst-Hensch NM, et al. (1999) Aromatase and breast cancer susceptibility. *Endocr Relat Cancer* 6:165–173.

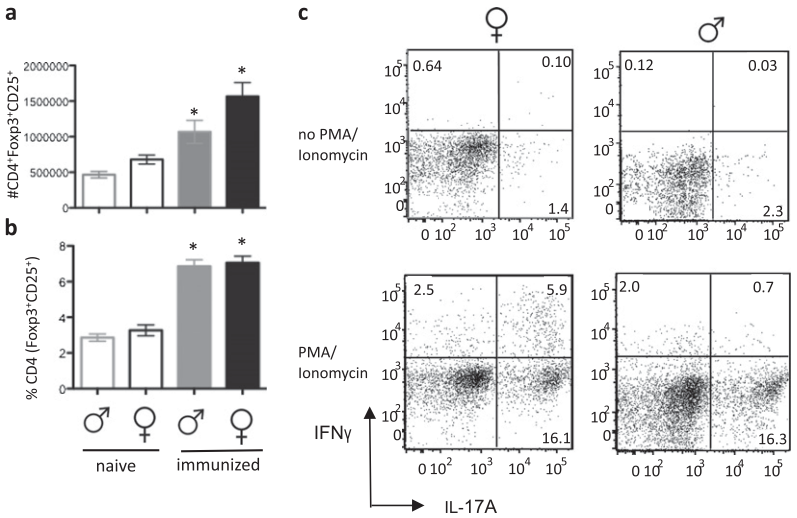

Fig. S1. Frequency of T regs in the periphery and frequencies of CNS-infiltrating Th1 and Th17 cells during EAE. Female and male SJL/J mice (n = 4/group) were immunized with PLP p139-151 in CFA. (*A* and *B*) Eight days later, individual spleens were harvested from mice, dissociated into a single-cell suspension, and stained using fluorochrome-conjugated antibodies (CD4-FITC, CD25-APC, and FoxP3-PE). (*A*) Mean ± SEM number of $FoxP3^+CD25^+CD4^+$ cells in unimmunized (naïve) and immunized mice. Results are of individual mice from one experiment. (*B*) Frequency of $FoxP3^+CD25^+$ cells in the live $CD4^+$ gate. *Significantly different ($P \leq 0.05$), as determined by a one-way ANOVA and Tukey post hoc test, from the naive mice of the same genotype. (*C*) Several mice immunized with PLP p139-151 and CFA were followed until 6 d after the onset of clinical signs. At this time, male and female mice with synchronized disease (EAE score 3) were perfused with PBS, and mononuclear cells were isolated from the spinal cord. Mononuclear cells were pooled within sex and were stimulated with PMA/ionomycin in the presence of GolgiStop for 4 h. These cells were then stained for CD4-FITC, IFNγ-PE, and IL-17-APC. Shown are the frequencies of IFNγ- and IL-17A-producing cells in the live $CD4^+$ T gate. Results in *C* are representative of two independent experiments.

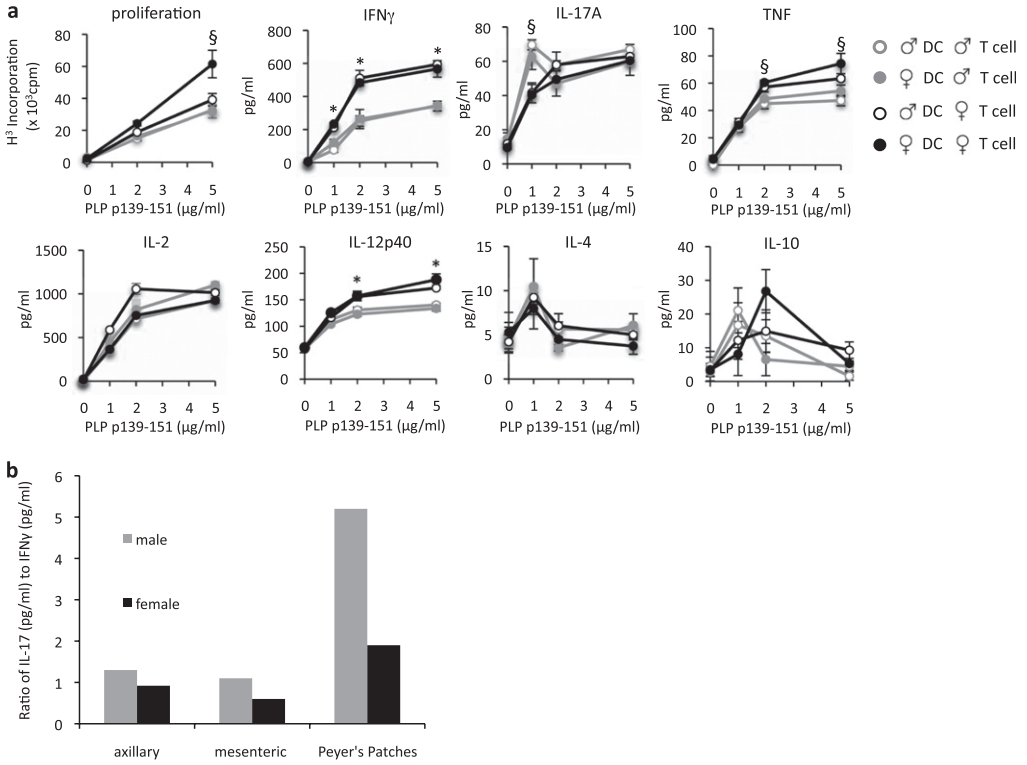

Fig. S2. Sex of T cell determines Th cytokine production. (*A*) Spleens were harvested from male or female SJL/J mice (8 wk old; n = 5/group; pooled) and were dissociated into a single-cell suspension. $CD11c^+$ cells were isolated, irradiated, and then cocultured with naïve 5B6 TCR transgenic $CD4^+$ T cells in the presence of PLP p139-151 at various concentrations. Values are means ± SEM picograms per milliliter of cytokine levels measured in triplicate cultures. Results are representative of two independent experiments. *Significantly different from cultures that contained male T cells ($P \leq 0.05$); §cultures that contained female APC and female T cells were significantly different from cultures that contained male APCs and male T cells ($P \leq 0.05$). These data were analyzed using a one-way ANOVA and Tukey post hoc test. (*B*) $CD4^+$ T cells were isolated from age-matched male and female SJL/J mice (n = 5/group; pooled) from the indicated secondary lymphoid organs and were stimulated in vitro with anti-CD3 and anti-CD28 (2 μg/mL). The productions of IL-17A and IFNγ were measured by ELISA. Shown is the ratio of production of IL-17A to IFNγ in male and female $CD4^+$ T-cell cultures.

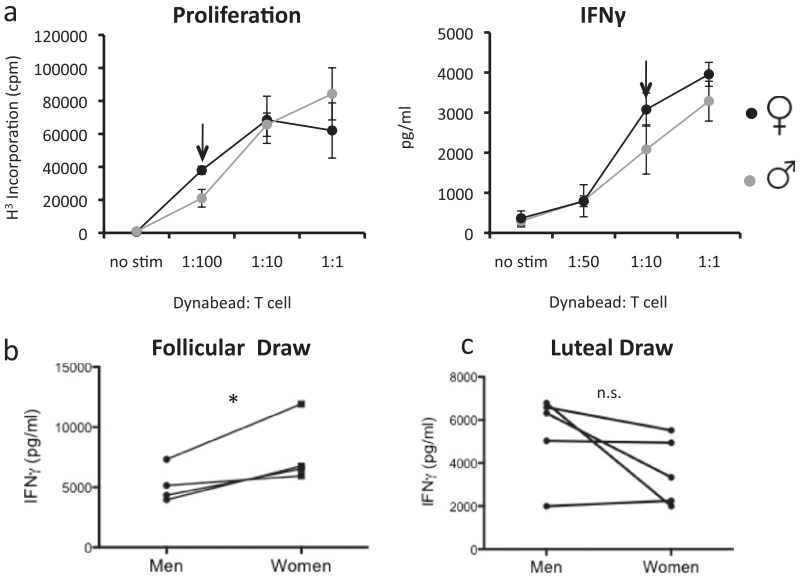

Fig. S3. Preliminary human T-cell experiments. Naïve $CD4^+$ T cells were isolated from peripheral blood of men and women (n = 4–5/group). Women were at the follicular (*A* and *B*) or luteal (*C*) phase of their cycle at the time of blood draw. $CD4^+$ cells were then cultured in X-VIVO-15 media with various concentrations of anti-CD3- and anti-CD28-coated Dynabeads that were prepared according to the instructions of the manufacturer. Proliferation was measured by [^{3}H]thymidine incorporation assay (cpm). IFNγ was measured in culture supernatants by ELISA. The arrows in *A* indicate the concentration of Dynabeads that were chosen for assays of proliferation and cytokine measurement. In *B* and *C*, * indicates a significant difference between men and women using a t test (two-tailed, $P \leq 0.05$). n.s., not significant.

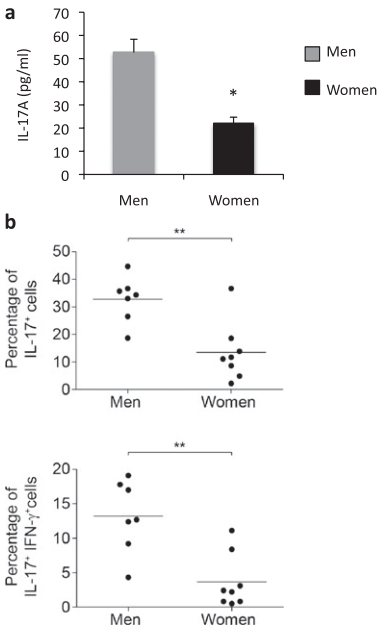

Fig. S4. Male T cells are more Th17-prone when cocultured with monocytes. (*A*) Human naïve $CD4^+$ T cells and $cd11b^+$ cells were isolated from peripheral blood of healthy men and women (n = 10/group). These cells were then cocultured together in X-VIVO-15 media that also contained peptidoglycan (1 μg/mL) and Dynabeads (1 bead:10 T cells). The concentration of IL-17A was measured in these cultures at 72 h of culture. *Significant ($P \leq 0.05$) difference, as determined by t test (two-tailed). (*B*) These cells are memory $CD4^+$ T cells that were isolated from the blood of healthy men and women according to the following protocol. T cells were cultured with autologous monocytes at a ratio of 2:1 and stimulated with anti-CD3 (2.5 μg/mL) in the presence of rhIL-23 (10 ng/mL) and neutralizing antibodies against IFNγ and against IL-4 (5 μg/mL). Cells were harvested on day 6 and analyzed by flow cytometry for IL-17 and IFNγ determination. **Significant difference ($P \leq 0.01$), as determined using a t test (two-tailed).

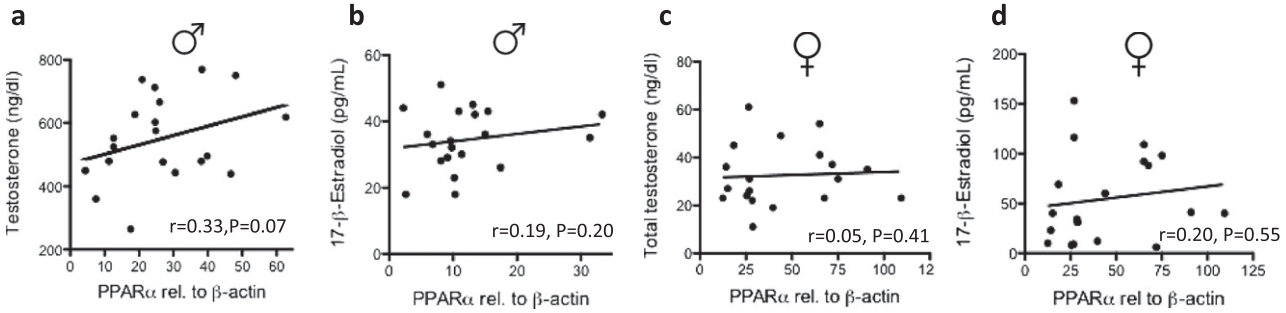

Fig. S5. Correlation of T-cell PPARα mRNAs with circulating testosterone and estradiol. Naïve CD4$^+$ T cells were freshly isolated from peripheral blood of healthy men (n = 20) and women (n = 20). The blood draw was conducted in the morning hours, when women were at the follicular phase of their cycle. Total RNA was isolated from these cells and was reverse-transcribed to cDNA. cDNAs were amplified using real-time PCR using primers specific for human PPARα and human β-actin. PPARα transcript levels were normalized to β-actin. Total testosterone and 17-β-estradiol were measured in the sera of the same individuals. (*A–D*) Correlations (Pearson) of PPARα mRNAs with circulating testosterone (*A* and *C*) and 17-β-estradiol (*B* and *D*) in men (*A* and *B*) and women (*C* and *D*).

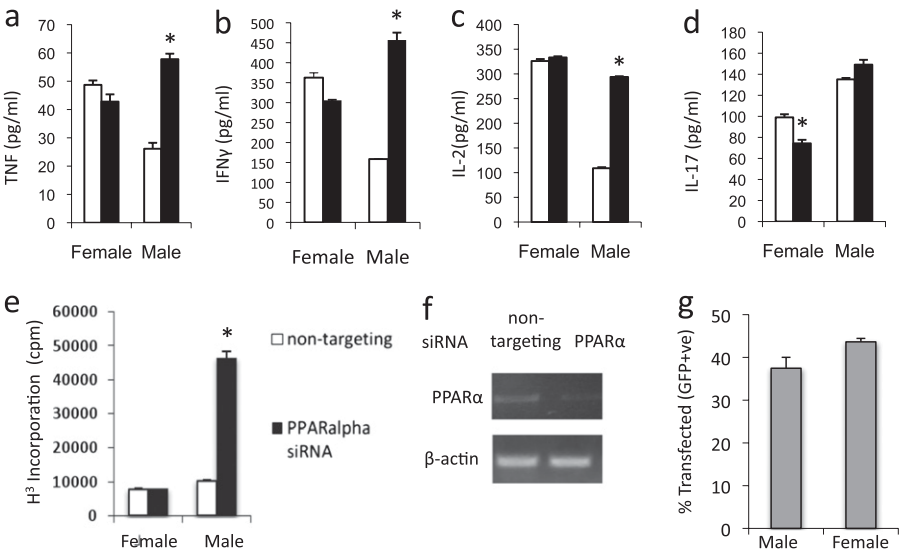

Fig. S6. Effect of PPARα siRNAs on murine CD4$^+$ T-cell proliferation and cytokine production. CD4$^+$ T cells were isolated from spleens and lymph nodes of SJL/J mice and were transfected with PPARα siRNAs or nontargeting siRNAs and then left to rest overnight. The next day, an aliquot of cells was reserved for assessment of PPARα mRNA expression by real-time PCR (*F*), and the frequency of GFP$^+$ cells was measured by flow cytometry (shown are frequencies of GFP$^+$ cells in PPARα siRNA samples in the live CD4$^+$ gate) (*G*). The remaining cells were then cultured (0.1 × 10^6 cell/well) with plate-bound anti-CD3 and anti-CD28 (0.1, 1, or 2 μg/mL). Proliferation (*E*) was measured by [^{3}H]thymidine incorporation (shown are cpms at 0.1 μg/mL), and cytokine levels were measured by ELISA (shown are cytokine levels at 1 or 2 μg/mL) (*A–D*). Values are means ± SE. Results are from one of three independent experiments. *Significantly different ($P \leq 0.05$) from the nontargeting siRNA group.

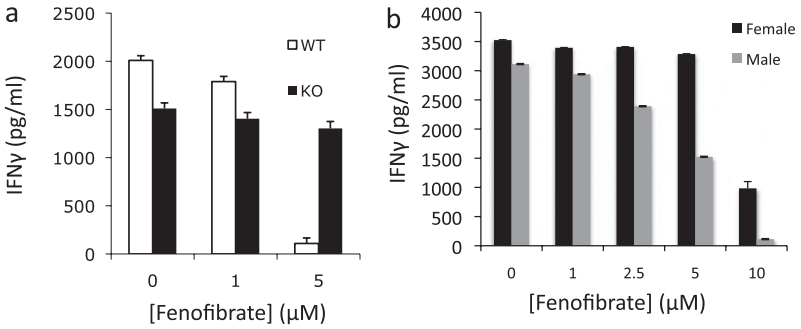

Fig. S7. Dose-dependent effect of fenofibrate on IFNγ production by murine CD4$^+$ T cells. (*A*) CD4$^+$ T cells were isolated from spleens of WT or PPARα$^{-/-}$ (KO) 129 male and were stimulated with plate-bound anti-CD3 and anti-CD28 in X-VIVO-20 serum-free media after overnight pretreatment with vehicle or fenofibrate. Note that beyond 5 μM, fenofibrate also reduced IFNγ production by KO cells. (*B*) CD3$^+$ T cells were taken from male or female SJL/J mice and were cultured in X-VIVO-20 media with fenofibrate or equal volume of vehicle. The next day, cells were transferred to 96-well plates, precoated with anti-CD3 and anti-CD28. IFNγ production was measured by ELISA. Values are means ± SEM. *A* is representative of three independent experiments. *B* is representative of two independent experiments.

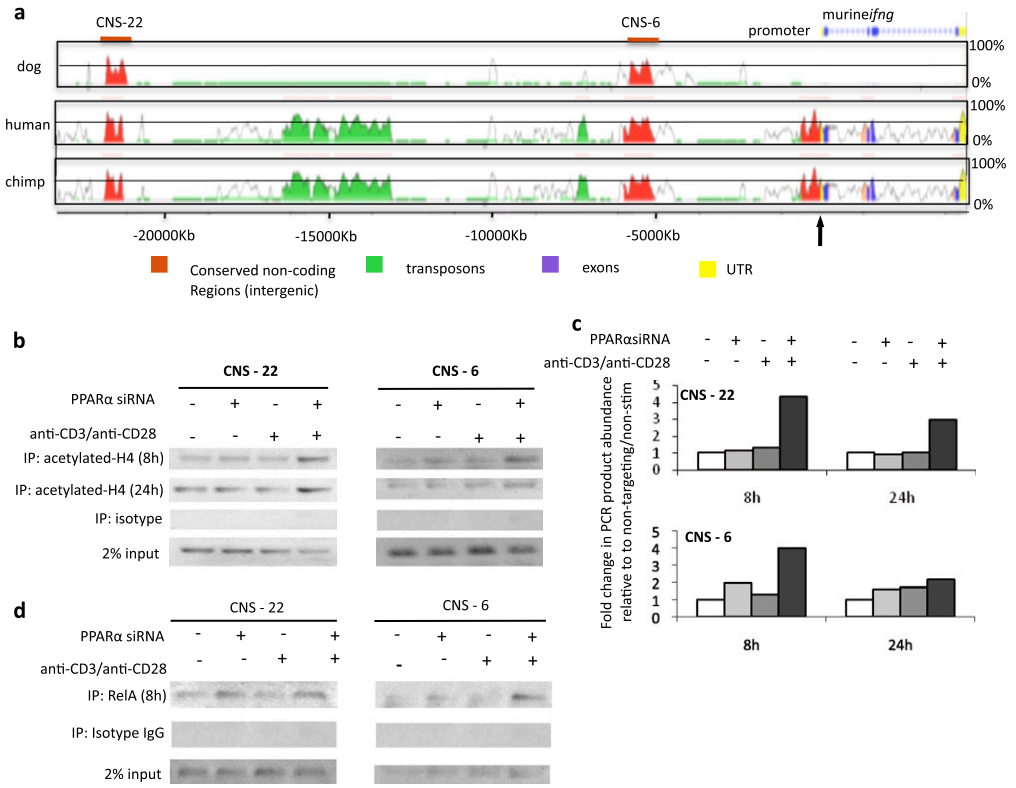

Fig. S8. Effects of PPARα at the *IFNG* locus. Transfection with PPARα siRNAs results in increased acetylation of histone H4 at conserved noncoding (CNS) sequences at the human IFNγ locus. (*A*) Alignment of the dog, human, and chimpanzee with the mouse genomic sequence upstream of *ifng*, highlighting the location of CNS-22 and CNS-6 sites. The arrow shows the start of *ifng*. This alignment was conducted using Vista tools (http://genome.lbl.gov/vista/index.shtml). (*B–D*) T cells from five healthy male donors were isolated and were transfected with either nontargeting (–) or PPARα-specific siRNAs (+), after which they were left to rest (–) or were stimulated (+) with anti-CD3 and anti-CD28 for 8 or 24 h. Cells were fixed and then pooled for ChIP using antibodies specific for acetylated-histone H4 (*B* and *C*), RelA (*D*), or a rabbit isotype control antibody (*B* and *D*). After reversal of cross-links, immunoprecipitated chromatin was amplified using primers that spanned the CNS-22 or CNS-6 regions. Representative gels of the PCR-amplified products of CNS-22 and CNS-6 regions post-ChIP are shown for acetylated-H4 (*B*) and RelA (*D*). (*C*) Average relative densitometric analysis of the 8- and 24-h experiments normalized first to input DNA and then to the nonstimulated, nontargeting control.

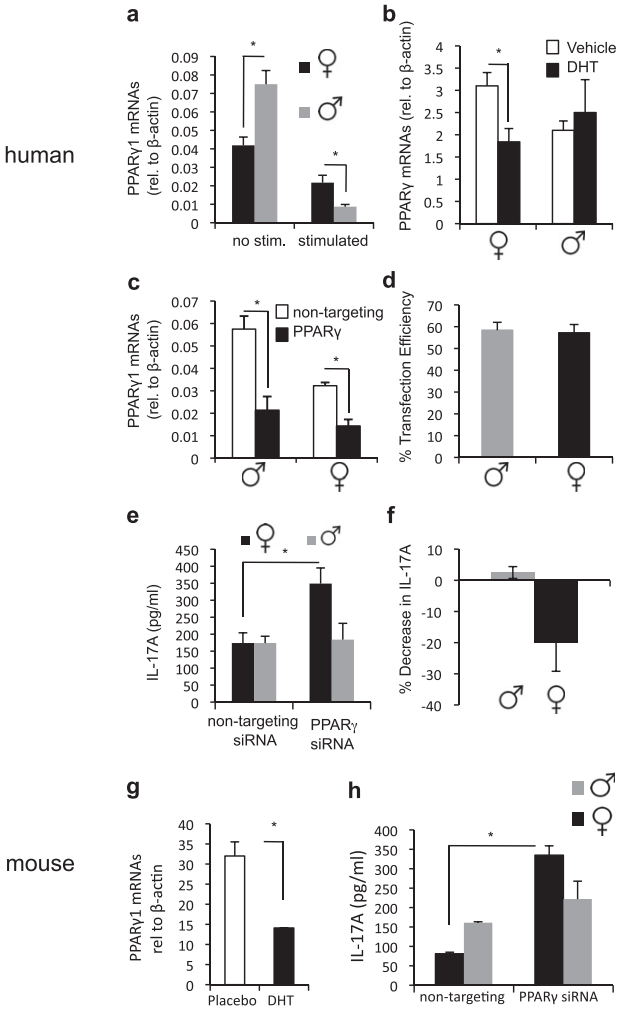

Fig. S9. PPARγ operates selectively in female T cells to repress IL-17A production. (*A*) PPARγ1 mRNAs were measured in naïve or activated CD4$^+$ T cells that were isolated from men or women (n = 10/group). (*B*) Mean PPARγ1 expression by CD4$^+$ T cells of men or women after in vitro treatment (72 h) with 100 nM DHT or ethanol vehicle in media containing charcoal-stripped FCS. (*C–E*) CD4$^+$ T cells were transfected with a GFP-expression construct, along with either PPARγ or nontargeting control siRNAs. The following day, transfection efficiency (GFP$^+$ T cells) was assessed by flow cytometry, and the level of knockdown was assessed by real-time PCR. Remaining cells were stimulated with anti-CD3 and anti-CD28 for 48 h in the presence of Th17-skewing cytokines. (*C*) Extent of PPARγ1 knockdown, as measured using real-time PCR. (*D*) Percentage of GFP-positive events in the live CD4$^+$ gate posttransfection. (*E*) Absolute IL-17A levels. (*F*) Percentage decrease in IL-17A relative to vehicle control after stimulation of CD4$^+$ T cells of men and women in the presence of 1 μM rosiglitazone (expressed as percentage change in IL-17A relative to vehicle control). Values are means ± SEM of individual donors. (*G*) PPARγ1 mRNA expression relative to β-actin by freshly isolated CD4$^+$ T cells from female 129 mice that were treated with a placebo or DHT pellet (5 mg pellet dose; n = 5/group). (*H*) IL-17A levels by male and female SJL/J T cells (n = 4/group) after transfection with either nontargeting or species-specific PPARγ siRNAs and stimulation with anti-CD3 and anti-CD28 in the presence of IL-6 and TGF-β. Results are representative of two experiments. *Significantly different ($P \leq 0.05$), as determined using a t test (two-tailed) or a one-way ANOVA and Tukey post hoc test.

Table S1. Proliferation and cytokine production by human naïve $CD4^+$ T cells

	Men	Women	*P* value
Proliferation	13,015 (1,098)	15,854 (1,909)	0.18
Cytokine Production			
IFNγ	3,324 (418)	4,874 (671)	0.0047*
IL-2	1,232 (111)	1,127 (178)	0.47
TNF	751 (121)	722 (79)	0.80
IL-10	102 (20)	107 (14)	0.73
IL-17A	55 (23)	35 (11)	0.37
IL-4	11 (4)	14 (5)	0.80
Frequency of $CD4^+$ T cells			
$IFN\gamma^+IL\text{-}17A^-$	22.3 (2.8)	31.3 (2.8)	0.0066*
$IFN\gamma^+/IL\text{-}17A^+$	0.8 (0.1)	1.1 (0.3)	0.37
$IFN\gamma\text{-}IL\text{-}17A^+$	4.4 (0.7)	4.1 (0.7)	0.71
$IFN\gamma^+IL\text{-}4^-$	19.4 (2.7)	25.3 (2.9)	0.019*
$IFN\gamma^+IL\text{-}4^-$	3.7 (0.6)	4.7 (0.8)	0.20
$IFN\gamma^-IL\text{-}4^+$	8.7 (1.2)	8.4 (1.1)	0.88
IFNγ (total)	23.1 (2.8)	32.5 (3.0)	0.005*
IL17A (total)	5.3 (0.7)	5.2 (0.8)	0.94
IL-4 (total)	12.4 (1.5)	13.2 (1.5)	0.65

Values are means (SE) (n = 25 for proliferation and cytokine production; n = 18 for intracellular cytokine analyses).
*Significant difference between men and women using a paired t test (two-tailed; $P \leq 0.05$).

Table S2. Effect of PPARα siRNA on Th cell cytokine production

	Nontargeting siRNA (pg/mL)	PPARα siRNA (pg/mL)	*P* value	*P* value (men vs. women)
IFNγ				
Men	643 (180)	987 (276)	0.024*	
Women	793 (140)	790 (125)	0.970	0.038*
IL-2				
Men	905 (201)	988 (215)	0.061	
Women	958 (138)	954 (150)	0.252	0.170
TNF				
Men	622 (131)	767 (175)	0.059	
Women	1,024 (330)	921 (249)	0.309	0.12
IL-17				
Men	15 (4)	25 (9)	0.18	
Women	46 (26)	31 (17)	0.19	0.79

*Significant (paired t test; two-tailed; $P < 0.05$).

Table S3. Human primer sequences used for SYBR real-time PCR

Primer names	Sequences (5′-3′)
Human PPARα FWD	TCGACTCAAGCTGGTG
Human PPARα REV	TTCCTGAGAGGATGACCC
Human PPARγ1 FWD	CTTGGGTCGGCCTCGAG
Human PPARγ1 REV	CATTACGGAGAGATCCACGGA
Human β-actin FWD	CCTCGCCTTTGCCGA
Human β-actin REV	TGGTGCCTGGGGCG
Human CNS-5 FWD	CTAATAGCAGATGTTTCACTGC
Human CNS-5 REV	CGTTTTGCACTTGTTTCCACAA
Human CNS-22 FWD	GCGATTTCCTTTTTTCTCAGGGTG
Human CNS-22 REV	CCAGGACAGAGGTGTCAAGCCA
Human PPARα promoter FWD	CTGACGCTCAGCGGTGTC
Human PPARα promoter REV	CTCAGCGGCTCCCACCTA
Human IFNγ FWD	TGGAGACCATCAAGGAAGAC
Human IFNγ REV	GCGTTGGACATTCAAGTCAG
Human IL-17A FWD	CCCCTAGACTCAGGCTTCCT
Human IL-17A REV	TCAGCTCCTTTCTGGGTTGT

FWD, forward; REV, reverse.