Supplementary Figure Legends

Supplementary Figure S1 PPARγ regulates alternative macrophage activation. **a-b,** LsyM-cre mediated recombination of the floxed PPARγ genes. (a) Efficiency of deletion was quantified by Q-PCR using genomic DNA from BMDM and peritoneal elicited macrophages (PECs). (b) Immunoblot analysis for PPARγ in control and Mac-PPARγ KO mice. Total of 70 μg of whole cell lysates from macrophages were subjected to electrophoresis and immunoblotted for PPARγ with combination of H-100 and E-8 antibodies (Santa Cruz). NS: non-specific. **c,** Reduced expression of arginase I (Arg1) mRNA in PPARγ-deficient macrophages. **d,** Direct binding of PPARγ/RXR heterodimers to the identified PPRE in the arginase I enhancer (nucleotides 3771 and 3758 upstream of the transcription initiation site). **e,** Pro-inflammatory cytokine secretion in control and PPARγ-deficient macrophages. **f**, Pharmacological activation of PPARγ by rosiglitazone increases β-oxidation of fatty acids in macrophages. **g,** IL-4 fails to induce genes in fatty acid metabolism pathways in PPARγ-deficient macrophages. **h**, Fatty acid uptake in control and Mac-PPARγ KO macrophages. BMDMs were treated with vehicle or IL-4 (10 ng/ml) for 24 hours prior to assaying for uptake of fatty acids. **i,** PPARγ is required for mitochondrial biogenesis during alternative activation. Macrophages were stained with MitoTracker Red and analyzed by fluorescence microscopy (60X).

Supplementary Figure S2 The proximal IL-4 signaling pathway is intact in PPARγ-deficient macrophages. **a**, Cell surface expression of IL-4Rα in BMDM from control and Mac-PPARγ KO mice. Open histogram- untreated macrophages; gray shaded histogram- macrophages pre-treated with IL-4 (10 ng/ml) for 48 hours. **b**, Intracellular staining for phospho-STAT6. BMDMs were treated with vehicle or IL-4 (20 ng/ml) for 30 minutes prior to intracellular

staining for phospho-STAT6. Open histogram- untreated macrophages; gray shaded histogram- macrophages stimulated with IL-4. Note equivalent levels of cell surface IL-4Rα and intracellular phospho-STAT6 in control and PPARγ-deficient macrophages.

Supplementary Figure S3 Decreased susceptibility of Mac-PPARγ KO mice to cutaneous Leishmaniasis, as assessed by cell number in draining popliteal lymph nodes of control and Mac-PPARγ KO mice (n=5).

Supplementary Figure S4 Adipocyte mass, morphology and function in Mac-PPARγ KO mice. **a**, Epididymal fat pad mass after 19 weeks of HFD. **b,** Serum leptin levels in control and Mac-PPARγ KO mice. **c,** Adipocyte morphology (100x) after H-E stain. **d,** Adipocyte cell size distribution was measured using dark-field images. Note, no qualitative or quantitative difference in adipocyte cell size was observed between control and Mac-PPARγ KO mice. **e**, Q-PCR analysis of 3T3-L1 adipocytes co-cultured with BMDM from control and Mac-PPARγ KO mice. BMDMs (250,000/well) were added to day 8 adipocytes differentiated in 6-well plates. Macrophages and adipocytes were co-cultured for 4 days prior to harvesting for RNA analysis.

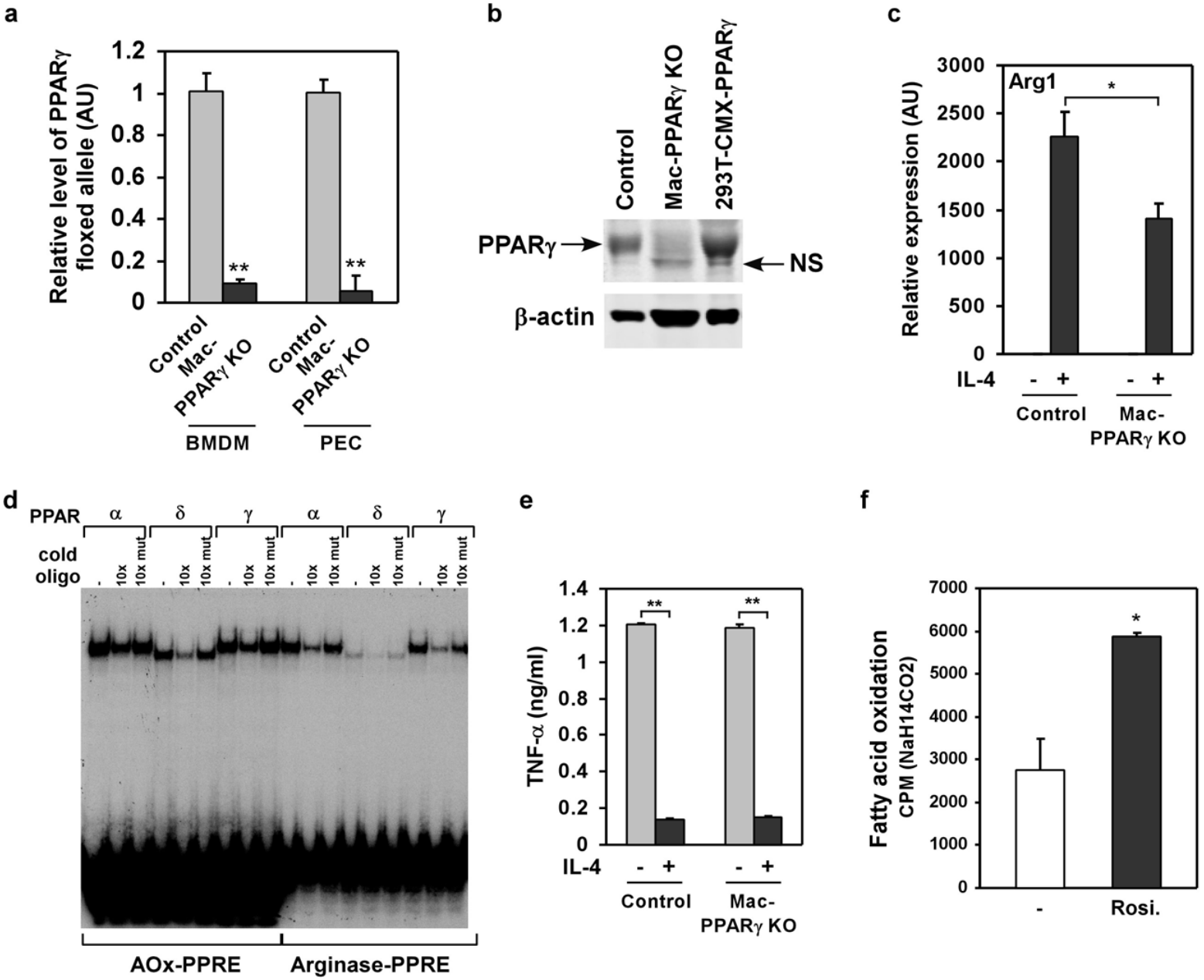
Supplementary Figure S5 Requirement for Th2 cytokine signaling in maturation of alternatively activated ATMs. **a**, Q-PCR analysis for macrophage markers in WAT of chow fed control and Mac-PPARγ KO mice (n=5/genotype). **b-d**, Decreased expression of alternatively activated macrophage signature in WAT of lean STAT6 KO (b), lean Mac-IL-4Rα KO (c) and obese STAT6 KO (d) mice (n=4-5/gentotype). **e**, Infusion of recombinant IL-4 promotes alternative activation of ATMs. Control and Mac-PPARγ KO mice maintained on HFD for 14-15 weeks were injected with IL-4 and epididymal fat pads were analyzed by Q-PCR for expression of markers for alternative activation (n=3-5/genotype).

Emr1, F4/80; *Cd68*, macrosialin; *Arg1*, arginase I; *Mrc1*, mannose receptor; *Clec7a*, dectin-1; *Retnla*, resistin like alpha; *Chi3l3*, Ym-1; *Nos2*, inducible nitric oxide synthase; *IL-6*, interleukin-6; *Il1rn*, IL-1 receptor antagonist; *Jag1*, jagged1.

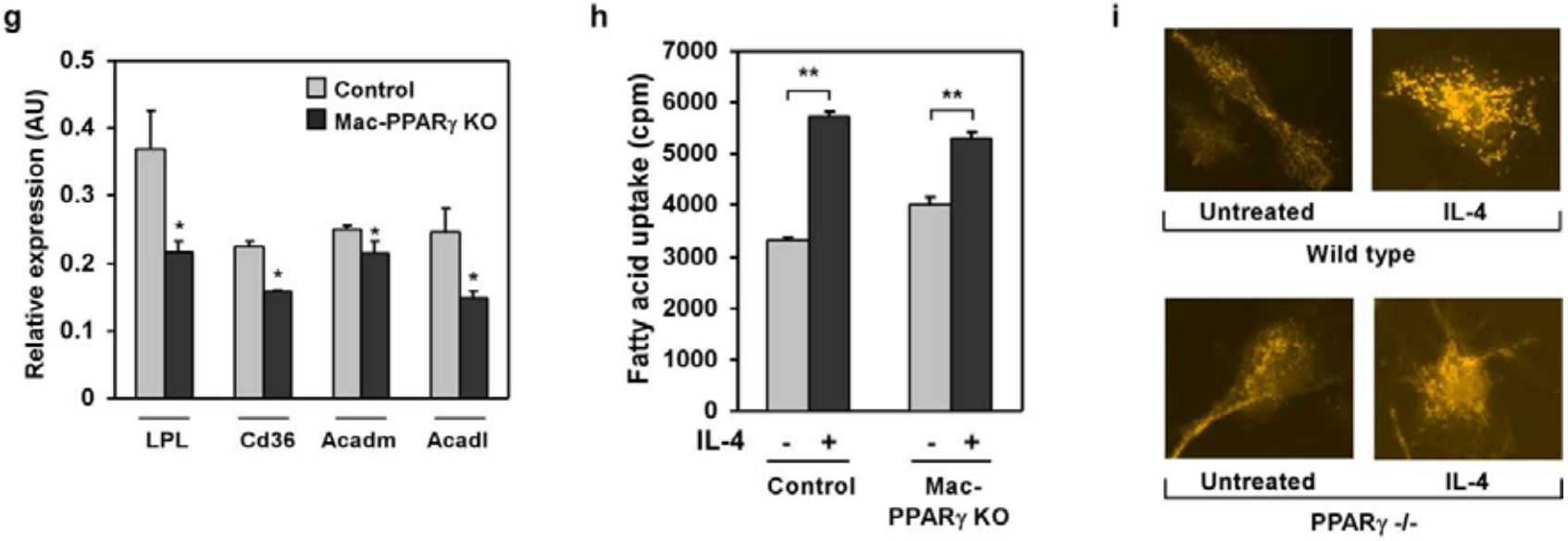
Supplementary Figure S6 Liver gene expression and lipid content of control and Mac-PPARγ KO mice. **a-b**, Hepatic macrophage content and activation is not significantly different in Mac-PPARγ KO mice. Relative transcript levels of macrophage-specific genes in liver (a). Immunohistochemical analysis for macrophages (F4/80 staining) in livers of control and Mac-PPARγ KO mice (b). **c**, Hepatic triglyceride content of control and Mac-PPARγ KO mice (n=5/genotype). **d**, Representative sections of liver stained with H&E. Note absence of significant lipid accumulation or fibroinflammatory lesions in livers of Mac-PPARγ KO mice. **e**, Relative transcript levels of genes important in nutrient uptake, fatty acid synthesis, β-oxidation and cholesterol synthesis, and of transcriptional regulators controlling these pathways in control and Mac-PPARγ KO mice.

Supplementary Table S1 Serum profiles of control and Mac-PPARγ KO fed HFD for 18 weeks

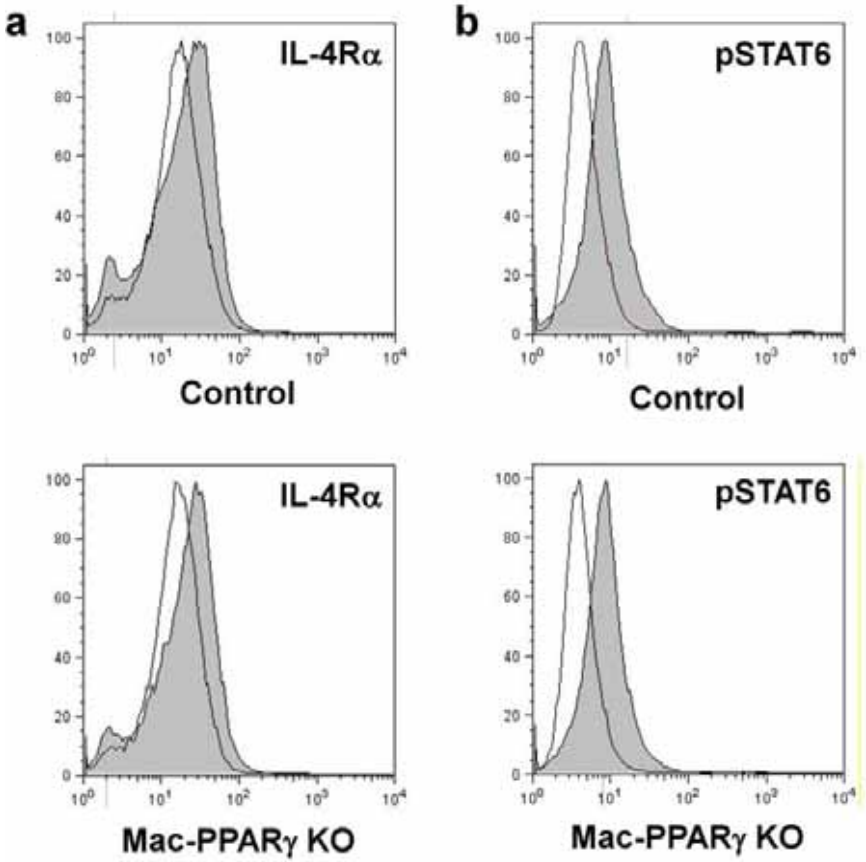
	Control	Mac-PPARγ KO
Resistin (ng/ml)	16.0 ± 1.6	16.3 ± 1.2
Total Cholesterol (mg/dl)	134.0 ± 11.8	120.4 ± 5.9
Triglycerides (mg/dl)	44.9 ± 4.1	41.2 ± 5.9



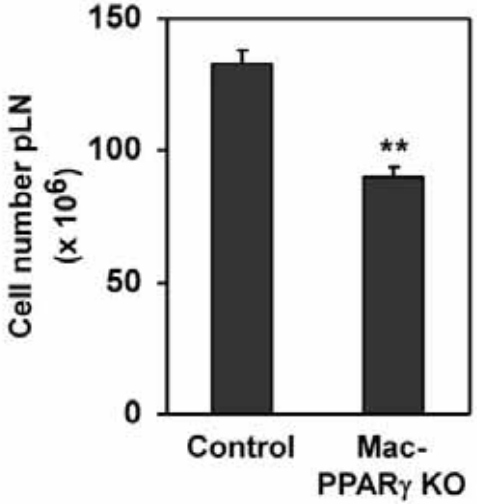
Supplementary Fig. S1



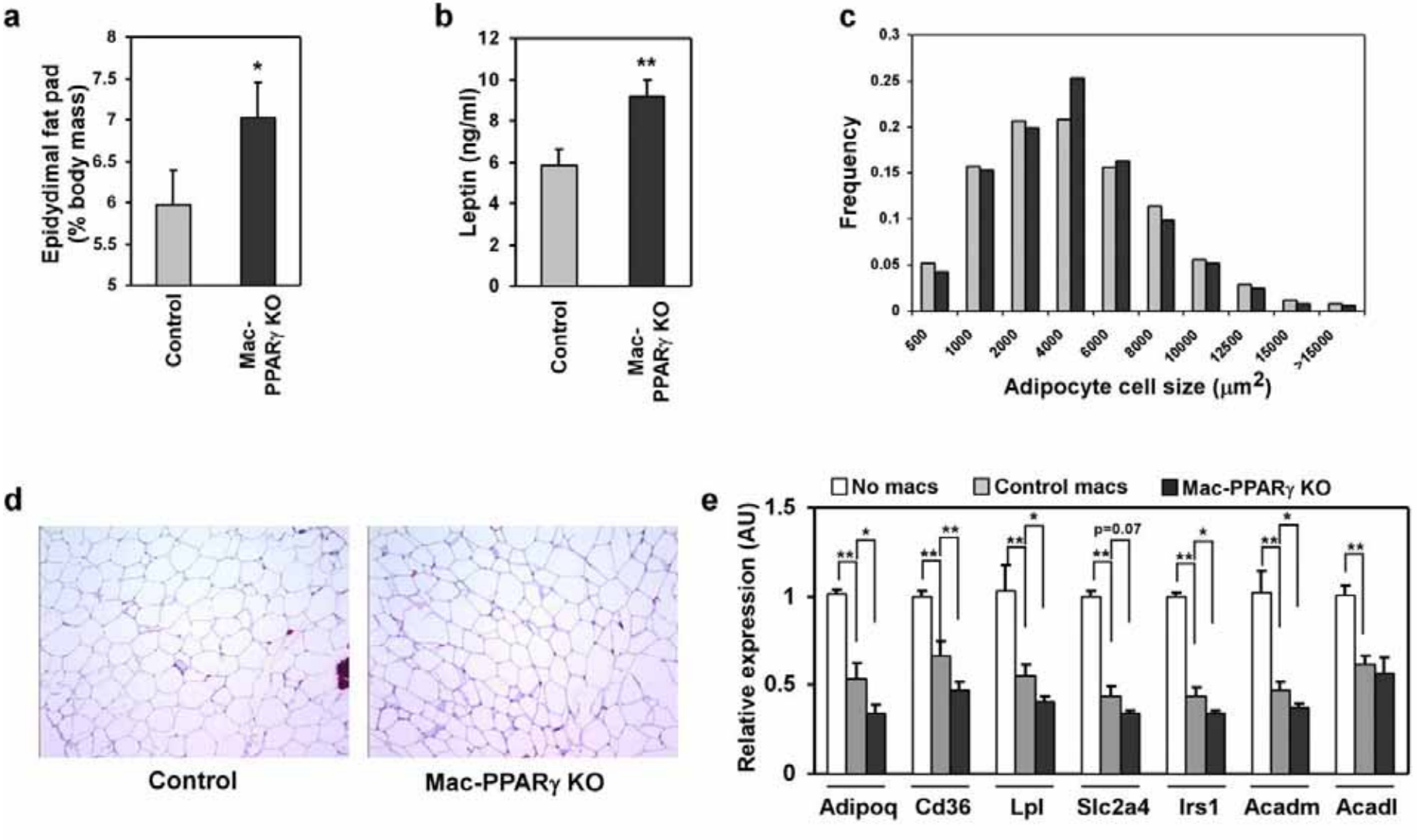
Supplementary Fig. S1



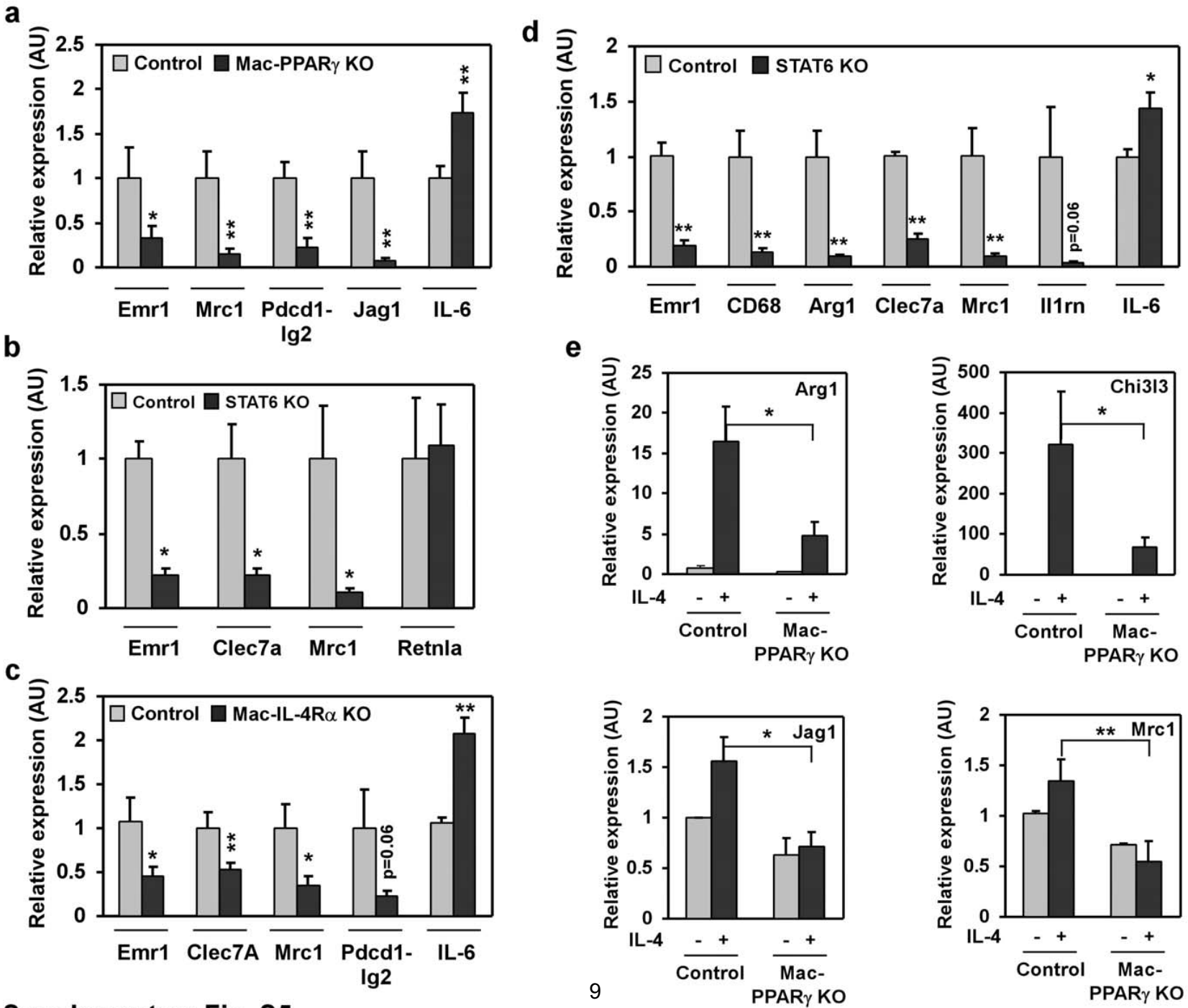
Supplementary Fig. S2



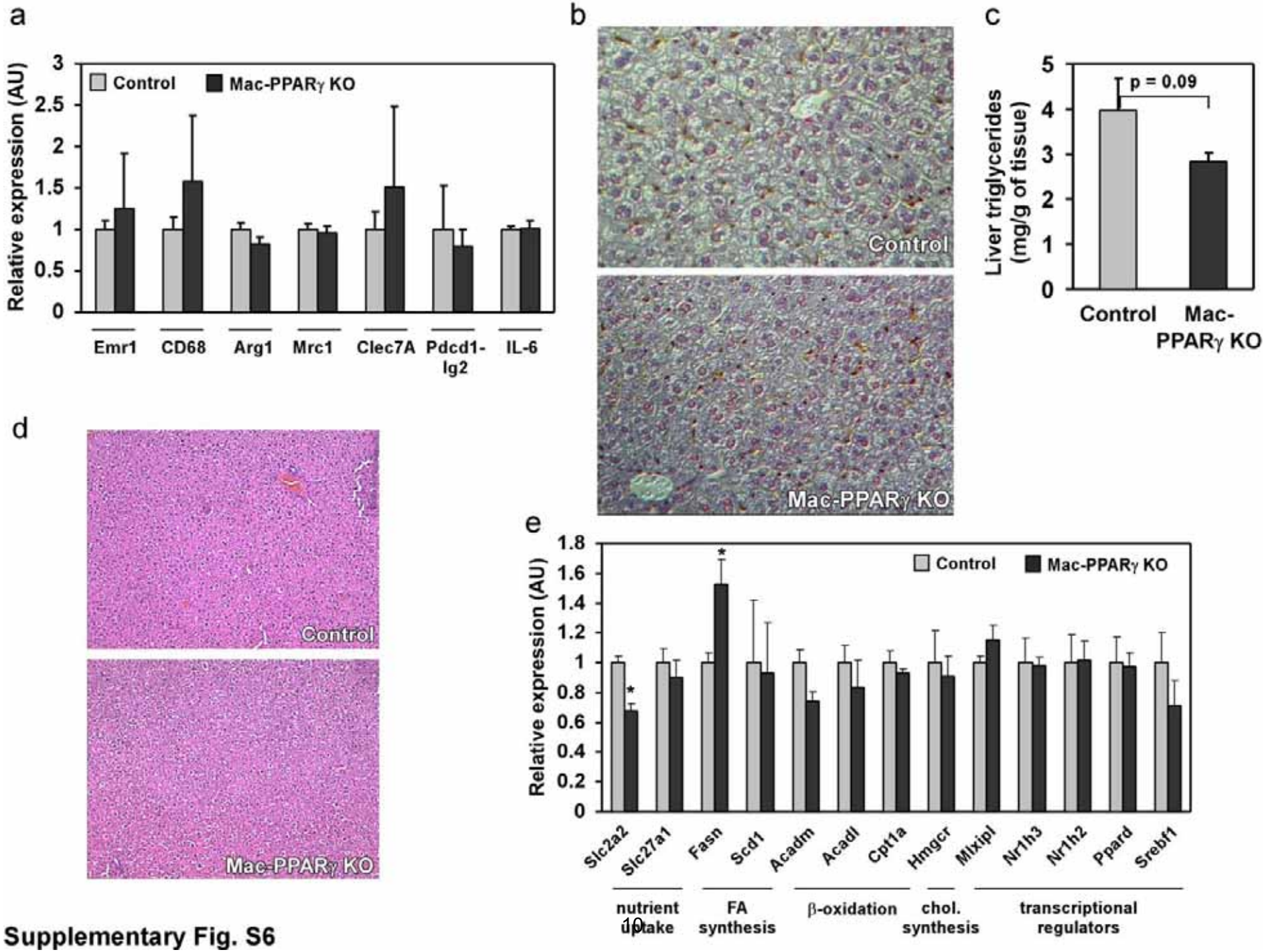
Supplementary Fig. S3



Supplementary Fig. S4



Supplementary Fig. S5



Supplementary Fig. S6

Supplementary Methods

Animals. Balb/c and STAT6 null mice were obtained from Jackson laboratory. As described previously, Mac-IL-4Rα KO mice were generated by inter-breeding IL-4Rα$^{f/f}$; LysM^{Cre} with IL-4Rα$^{-/-}$ mice to generate IL-4Rα$^{f/-}$ (control) and IL-4Rα$^{f/-}$; LysM^{Cre} (Mac-IL-4Rα KO) mice[1]. To activate ATMs with IL-4, mice were injected with IL-4 complexed with anti-IL-4 antibody, a procedure that has been shown to extend the half-life of this cytokine *in vivo* [2]. Briefly, soluble complexes of IL-4 (2 μg) with anti-IL-4 antibody (10 μg) were injected intraperitoneally into obese recipient mice (fed HFD for 14-15 weeks) on day 1 and 4, and epididymal fat pads were harvested on day 5 for Q-PCR analysis

Immunoblotting and flow cytometry. Total cellular proteins were subjected to electrophoresis and immunoblotted for PPARγ (1:200, H-100 and E-8, Santa Cruz)[3]. For flow cytometry, BMDMs were treated with vehicle or IL-4 (10 ng/ml) for 48 hours, harvested in PBS and resuspended in PBS containing 2% FCS and 0.2mM EDTA. Macrophages were blocked with 200μg/ml normal mIgG, stained with PE-conjugated IL-4Rα antibody (BD Pharmingen) and analyzed on FACSCalibur. Macrophages of both genotypes were stimulated with IL-4 (20 ng/ml) or vehicle for 30 minutes, and stained for phoshpo-STAT6 as described previously[4,5]. Briefly, 1 x10^6 cells were resuspended in media, fixed in formaldehyde, permeablized in MeOH, and resuspended in 100μl 0.5% BSA in PBS. Subsequently, 1 x10^6 cells were resuspended in 100μl, stained with 20μl pSTAT6-Alexa647 (BD Pharmingen) and analyzed on FACSCalibur.

Fatty acid uptake and triglyceride quantification. Uptake of fatty acids was monitored in BMDMs that had been pre-treated with vehicle or IL-4 (10 ng/ml) for 24 hours. Cells were subsequently incubated with fatty acid-free BSA conjugated ^{14}C-oleic acid for 30 minutes, washed extensively and incorporated

radioactivity was quantified in cellular lysates[6]. Hepatic lipids were extracted using the Folch method, resuspended in PBS containing 5% Triton X-100, and hepatic triglycerides were quantified using colorimetric assays (Sigma).

.

References

1. Herbert, D. R. et al. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* **20**, 623-35 (2004).
2. Finkelman, F. D. et al. Anti-cytokine antibodies as carrier proteins. Prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J Immunol* **151**, 1235-44 (1993).
3. Akiyama, T. E. et al. Conditional disruption of the peroxisome proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. *Mol Cell Biol* **22**, 2607-19 (2002).
4. Krutzik, P. O., Clutter, M. R. & Nolan, G. P. Coordinate analysis of murine immune cell surface markers and intracellular phosphoproteins by flow cytometry. *J Immunol* **175**, 2357-65 (2005).
5. Krutzik, P. O., Hale, M. B. & Nolan, G. P. Characterization of the murine immunological signaling network with phosphospecific flow cytometry. *J Immunol* **175**, 2366-73 (2005).
6. Vats, D. et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* **4**, 13-24 (2006).