Supporting Information

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SI Materials and Methods

Induction of Diabetes in Mice. Male C57BL/6J mice and PPARα KO mice (8 wk old) received daily i.p. injections of STZ (50 mg·kg^{-1}·day^{-1} in 10 mM citrate buffer, pH 4.5) for 5 d to induce diabetes.

Immunohistochemistry. Slides were incubated with an anti-PPARα antibody (SC-9000, 1:200 dilution; Santa Cruz Biotechnologies) and anti-GFAP antibody (G3893, 1:200 dilution; Sigma-Aldrich). The slides were then incubated with Cy3- or FITC-labeled secondary antibodies (Jackson ImmunoResearch Europe) for 1 h and mounted with Mounting Medium for Fluorescence with DAPI (Vector Laboratories). Human eye sections were stained with the anti-PPARα antibody only and processed as above.

Intravitreal Injection. Rats were anesthetized with a 10:50 mix of ketamine (100 mg/mL) and xylazine (20 mg/mL). The pupils were dilated with topical application of phenylephrine [2.5% (wt/vol)] and tropicamide (1%). A sclerotomy was created ∼0.5 mm posterior to the limbus with a blade, and a glass injector (∼33 gauge) connected to a syringe filled with 5 μL of adenovirus-expressing green fluorescence protein (Ad-GFP) (2.5 × 10^{9} ifu/mL) or the same titer of adenovirus expressing PPARα (Ad-PPARα) was introduced through the sclerotomy into the vitreous cavity.

Retina Trypsin Digestion Assay. Dissected retinas were digested with pepsin [5% (wt/vol) pepsin in 0.2% hydrochloric acid] for 30 min, and then with trypsin (1% trypsin in 0.2 M NaF and 0.1 M Tris, pH 7.8) for 2 h. The isolated retinal vasculature was then stained with PAS and hematoxylin. Images of retinal vasculature were captured at eight random fields (1.08 mm^{2}) in each retina. Pericytes and acellular capillaries in the field were counted and averaged within each group.

Endothelial Cell Migration Assay. Human retinal capillary endothelial cells (HRCECs) were cultured in six-well plates to confluence. HRCECs were pretreated with 25 μM fenofibrate, with the same volume of DMSO as vehicle control, or infected with Ad-GFP (control) or Ad-PPARα at multiplicity of infection (MOI) of 20 for 24 h. The HRCEC monolayer was scratched by a plastic pipette tip to generate a no-cell zone. After the scratch, the cells were cultured in normal culture medium. Images of no-cell area were taken at 0 and 8 h postscratching in at least four nonoverlapping areas per well. Wound closure was quantified by measuring the width of the no-cell zone using Image J (NIH) software.

Cell Viability Assay. The viability of HRCECs was determined by trypan blue dye exclusion assay. Briefly, the cells were plated at a density of 1× 10^{4} in 24-well plates and treated with 25 μM fenofibrate with the same volume of DMSO as control, or infected with Ad-PPARα (MOI = 20), with Ad-GFP (MOI = 20) as control for 24 h. The cells were then cultured in growth medium for 48 h and collected. An aliquot of cell suspension was mixed with an equal volume of 0.4% trypan blue (Life Technologies), and the viable cells were counted under a microscope. Cell viability was analyzed and expressed as % of live cells (without blue staining) in total cells.

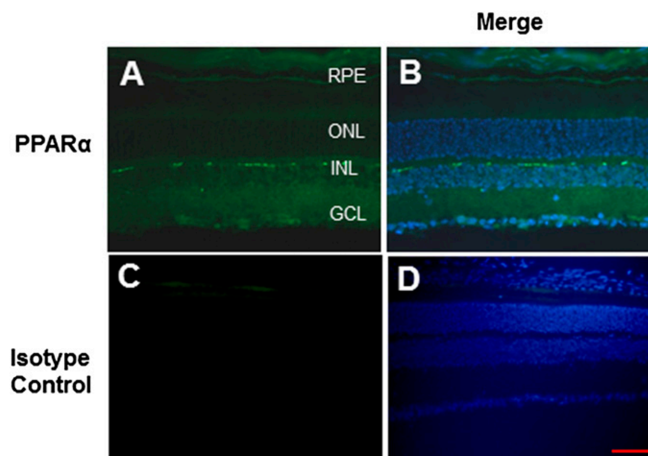

Fig. S1. Expression of PPARα in the mouse retina. Retinal sections from C57/BL6 mice were stained with an anti-PPARα antibody (*A* and *B*) or isotype control (*C* and *D*). The nuclei were counterstained with DAPI; shown are merged signals of PPARα (green) and DAPI staining (blue). (Scale bar, 20 μm.) RPE, retinal pigment epithelium; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.

Fig. S2. PPARα knockout enhanced the diabetes-induced overexpression of retinal VEGF, TNF-α, and ICAM-1 in the retina. At the age of 8 wk, diabetes was induced in PPARα KO mice and WT mice by STZ injections. At 12 wk after the onset of diabetes, the same amount (100 μg) of retinal proteins from each mouse was used for ELISA specific for VEGF (*A*), TNF-α (*B*), and ICAM-1 (*C*), and the results were compared in age-matched nondiabetic WT mice (WT), diabetic WT mice (WT+STZ), nondiabetic PPARα KO mice (KO), and diabetic PPARα KO mice (KO+STZ) (mean ± SD, $n = 4$, *$P < 0.05$, compared with diabetic WT mice).

Fig. S3. PPARα overexpression reduced the up-regulation of retinal VEGF, TNF-α, and ICAM-1 expression in diabetic rats. Diabetic rats with 8 wk of STZ-induced diabetes received an intravitreal injection of Ad-PPARα or Ad-GFP (control). ELISA was performed 4 wk after the injection. The same amount (100 μg) of retina protein from each rat was used for ELISA analysis of VEGF (*A*), TNF-α (*B*), and ICAM-1 (*C*) (mean ± SD, $n = 4$, *$P < 0.05$, **$P < 0.01$, compared with Ad-GFP-treated control).

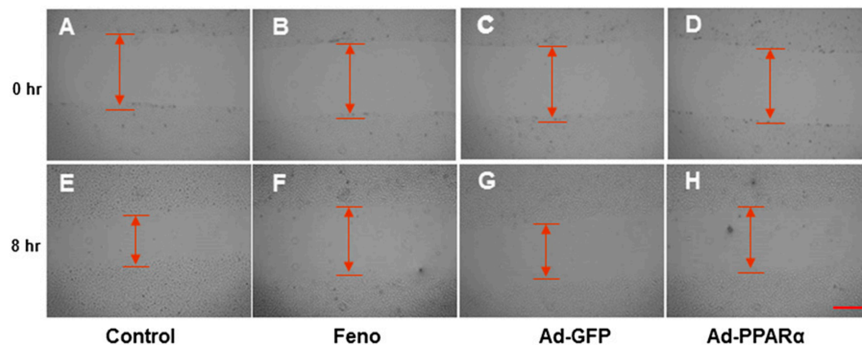

Fig. S4. PPARα overexpression significantly inhibited endothelial cell migration. HRCECs were exposed to 25 μM fenofibrate, with DMSO as control or infected with Ad-PPARα at MOI of 20, with Ad-GFP at MOI of 20 as control, for 24 h and then subjected to in vitro scratch-wound healing assay. Images captured at 0 and 8 h after the scratch using phase-contrast microscope. Representative images from DMSO control group (*A* and *E*), fenofibrate treatment group (*B* and *F*), Ad-GFP treatment group (*C* and *G*), and Ad-PPARα treatment group (*D* and *H*). (Scale bar: 500 μm.)

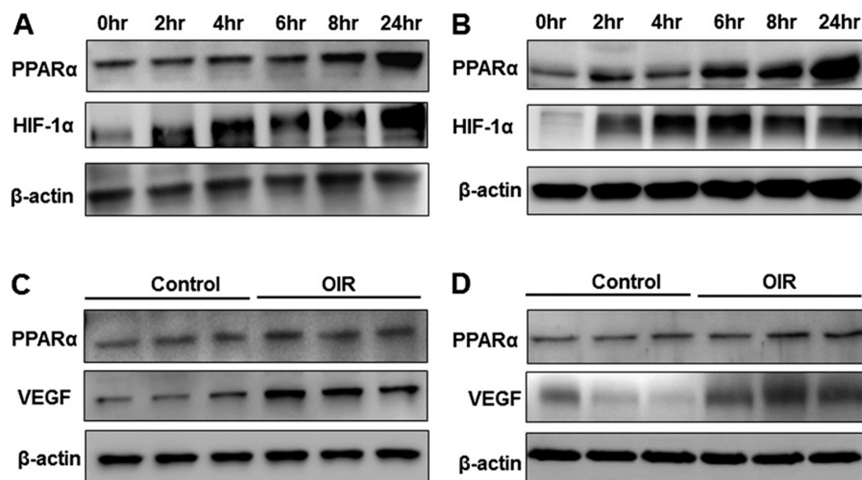

Fig. S5. No changes in PPARα expression in hypoxia-treated retinal cells and in the retinas of OIR mice and rats. (*A* and *B*) hTERT RPE (*A*) and rMC-1 (*B*) cells were exposed to 100 μM $CoCl_2$. Total cell lysates were used for Western blot analysis of PPARα and HIF-1α at the indicated time points of the $CoCl_2$ treatment. (*C* and *D*) Mice (*C*) and rats (*D*) were exposed to 75% O_2 from age of postnatal day 7 (P7) to P12. At P16, the retinas were dissected and homogenized. The same amount (50 μg) of retinal proteins was used for Western blot analysis of PPARα and VEGF in the retinas from normoxia control and OIR mice (*C*) and normoxia control and OIR rats (*D*).

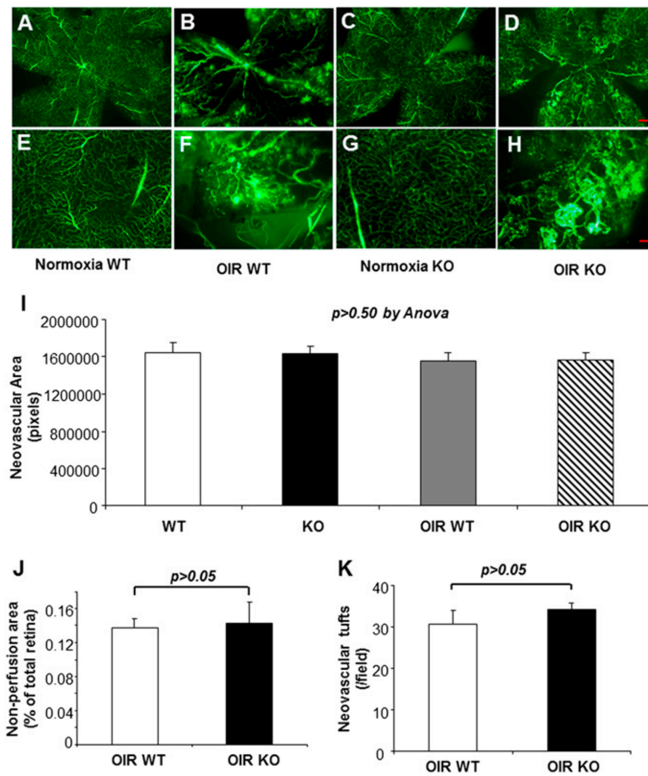

Fig. S6. PPARα KO mice with OIR showed no significant difference in retinal neovascularization compared with OIR WT mice. (*A–H*) PPARα KO and WT mice were exposed to 75% O_2 from P7 to P12. At P18, fluorescein angiography was performed with high molecular weight fluorescein-dextran in the whole-mounted retina of normoxia WT mice (*A* and *E*), OIR WT mice (*B* and *F*), normoxia PPARα KO mice (*C* and *G*), and OIR PPARα KO mice (*D* and *H*). [Scale bars: 100 μm (*A–D*) and 25 μm (*E–H*).] (*I–K*) Total neovascular areas (*I*), nonperfused areas (*J*), and neovascular bulbs per filed (*K*) were measured using software (NIH Image J) in retinal angiographs. The results showed no significant differences in these parameters among these groups (mean ± SD, $n = 6$, analyzed by ANOVA).

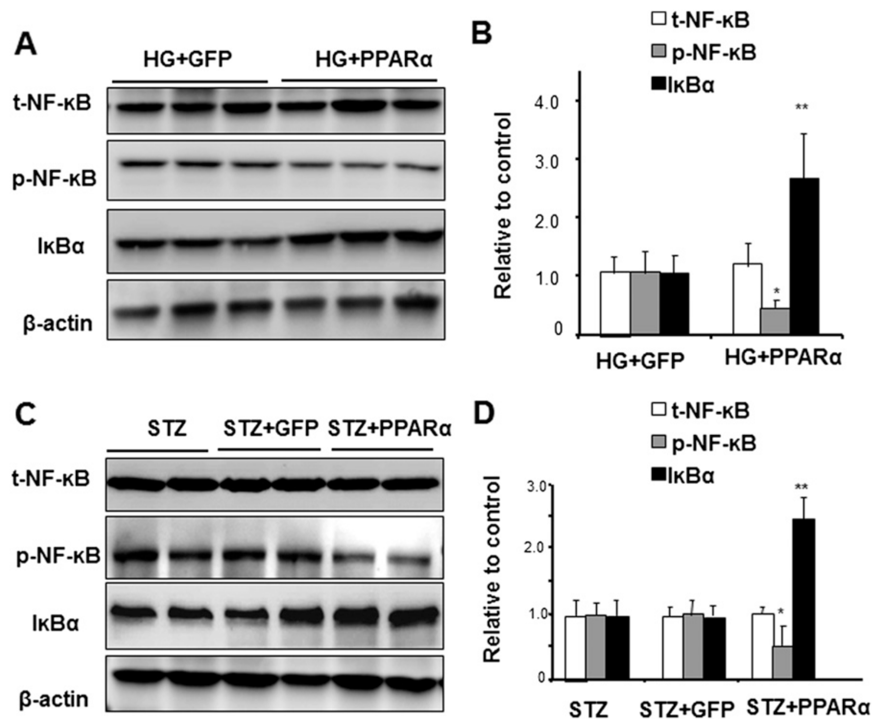

Fig. S7. PPARα overexpression inhibited NF-κB signaling in high glucose-treated retinal cells and in the retina of diabetic rats. (*A* and *B*) hTERT RPE cells were exposed to 30 mM D-glucose for 48 h and then infected with Ad-PPARα at MOI of 20, with Ad-GFP at the same MOI as control. Twenty-four hours following the infection, total cell lysates were used for Western blot analysis of total NF-κB, phospho-NF-κB, and IκBα (*A*). The results were semiquantified by densitometry and normalized by β-actin levels (*B*) (mean ± SD, $n = 3$, $*P < 0.05$, $**P < 0.01$). (*C* and *D*) Diabetic rats with 8 wk of STZ-induced diabetes received an intravitreal injection of Ad-PPARα or Ad-GFP (control). Four weeks after the injection, the same amount (50 μg) of retina proteins was used for Western blot analysis of total NF-κB, phospho-NF-κB, and IκBα (*C*). These protein levels were semiquantified with densitometry and normalized by β-actin levels (*D*) (mean ± SD, $n = 4$, $*P < 0.05$, $**P < 0.01$, compared with the Ad-GFP–treated group).