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Supplementary Materials for

Glycolysis links reciprocal activation of myeloid cells and endothelial cells in the retinal angiogenic niche

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Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/12/555/eaay1371/DC1)

Data file S1. Raw data (provided as separate Excel file).

Materials and Methods

Chemicals and reagents

Collagen type 1 (rat tail) was obtained from BD Biosciences). α-Cyano-4-Hydroxycinnamic Acid, dimethyl sulfoxide (DMSO) and lactate (lactic acid, L6402) (*14*) were purchased from Sigma. Calcein-AM, L-glutamine, and penicillin/streptomycin were from Gibco, and 4, 6,-Diamidino-2-Phenylindole (DAPI) was from Invitrogen (Life Technologies). The glycolysis inhibitor 2-deoxy-D-glucose (2-DG) was from EMD Millipore. BMS 303141 and anacardic acid were from Tocris Bioscience. Recombinant murine IL-4 and recombinant murine and human M-CSF were from PeproTech. DNase I was from Roche.

Mouse generation and breeding

Animals were used according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and in accordance with the protocol approved by the Institutional Animal Care and Use Committee at Augusta University. The floxed *Pfkfb3* (*Pfkfb3*$^{flox/flox}$) mice were generated by Xenogen Biosciences Corporation(*6*). Cell-specific inactivation of *Pfkfb3* in macrophages or in endothelial cells (ECs) was achieved by cross-breeding *Pfkfb3*$^{flox/flox}$ mice with *Lysm*-Cre transgenic mice (The Jackson Laboratory, stock no. 004781) or *Cdh5*-Cre transgenic mice (The Jackson Laboratory, stock no. 006137) to generate *Pfkfb3*$^{flox/flox}$;*Lysm*-Cre (*Pfkfb3*$^{\Delta M\phi}$) and *Pfkfb3*$^{flox/flox}$;*Cdh5*-Cre (*Pfkfb3*$^{\Delta EC}$) mice, respectively. *Pfkfb3*$^{\Delta M\phi}$ mice were also crossed with *Pfkfb3*$^{\Delta EC}$ mice to ultimately obtain macrophage and endothelial cell *Pfkfb3* double-knockout mice (*Pfkfb3*$^{\Delta M\phi\Delta EC}$). The floxed *Pkm2* (*Pkm2*$^{flox/flox}$) mice from The Jackson Laboratory (stock no. 024048) were crossed with *Lysm*-cre transgenic mice to obtain *Pkm2*$^{flox/+}$;*Lysm*-Cre heterozygous breeders, and ultimately *Pkm2*$^{flox/flox}$;*Lysm*-Cre homozygous-knockout mice (*Pkm2*$^{\Delta M\phi}$) and littermate-control mice (*Pkm2*$^{+/+}$;*Lysm*-Cre, *Pkm2*^{WT}). All of the mice were on a C57BL/6J background.

Mouse model of Oxygen-induced Retinopathy (OIR)

The OIR model was described previously (*26*). Briefly, seven-day-old (postnatal day (P) 7) mouse pups along with their foster/nursing mothers were exposed to 75% oxygen for 5 days to induce vaso-obliteration. At P12, the mice were returned to room air (RA, 21% oxygen) to induce retinal neovascularization, which was maximal at P17. Age-matched mice kept in RA served as the RA controls. Underdeveloped neonatal mice with very low body weight (less than 5 grams at P17) were excluded. Areas of avascular region and neovascular tufts were quantified as a percentage of total retinal areas using Adobe Photoshop by researchers who were blinded to experimental conditions as previously described (*46*).

Laser-capture microdissection of retinal vessels

Retinal vessels/macrophages were microdissected with laser capture in retinal cross sections from RA and OIR mice at P17, as described previously (*2, 47*). Briefly, eyes were embedded in OCT, cyrosectioned under RNase-free conditions into 10-μm sections, and collected on RNase-free polyethylene naphthalate glass slides (11505189, Leica). Sections were dehydrated with a gradient of 70%, 90%, and 100% ethanol washes and stained with isolectin B4 (*Griffonia simplicifolia*, Invitrogen,1:50 in 1 mM $CaCl_2$). Retinal vessels/macrophages were microdissected with a Leica LMD 6000 system (Leica Microsystems) and collected directly into RNA stabilizing buffer from the RNeasy Micro kit (Qiagen). RNA was extracted from microdissected tissues using the RNeasy Micro kit (Qiagen), and qRT-PCR was performed with the generated cDNA.

Cell isolation, culture and treatments

Bone marrow-derived macrophage (BMDM) isolation and treatment

Freshly isolated femurs and tibias from WT C57BL/6 or *Pfkfb3*$^{M\varphi}$ mice were flushed with RPMI-1640 medium (HyClone). Cells were harvested and passed through a 70-μm strainer. The acquired cells were plated at a density of 2×10^6/mL and cultured in RPMI 1640 medium (HyClone), supplemented with 10% heat-inactivated FBS (Life Technologies), 20% L929 conditioned medium and 1% penicillin-streptomycin for 7 days to induce macrophage differentiation. The macrophages

were cultured for another 24 hours in RPMI 1640 medium supplemented with 5% heat-inactivated FBS and 10 ng/mL recombinant mouse M-CSF (PeproTech) prior to treatment with lactate or IL-4. For endothelial cell (EC)-conditioned medium (CM) treatment, the macrophages were cultured for another 24 hours in 1 mL complete RPMI 1640 medium and 1 mL complete VCBM per well in a sterile six-well tissue culture plate after 7-day differentiation, and then incubated with 0.5 mL complete RPMI 1640 medium and stimulated with 1 mL VCBM, Nx-REC-CM or Hx-REC-CM. For hypoxic experiments, BMDMs were placed in a modular incubator chamber (Thermo Fisher Scientific) at 37°C with 0.5% O_2. The cells were harvested at 6 and 12 hours and used in subsequent experiments for Western blot and qRT-PCR.

Isolation and culture of mouse retinal microglia

Retinal microglia were isolated and cultured according to protocols described previously by us with some modifications (*48*). Briefly, twelve-day-old (postnatal day (P) 12) mouse pups were euthanized and their eyes enucleated. The retinas were dissected free from the underlying retinal pigment epithelium (RPE) layer, rinsed with pre-cooled PBS, transferred into 2% dispase (Sigma, St Louis, MO) in HBSS containing 5% Fetal Bovine Serum and 10 mM HEPES, and placed in an incubator at 37 °C for 30 minutes. Dispase activity was terminated by washing the retinas in 10 mL DMEM/F12 (D8437-500ML, Sigma) plus 10% FBS (Gibco/Invitrogen). The retinas were transferred into culture medium consisting of DMEM/F12 with 10% FBS and 50 ng/mL recombinant murine M-CSF and subsequently triturated several times with a transfer pipette. The dissociated cells were then transferred into 75 cm^2 flasks and left to grow in a humidified atmosphere of 5% CO_2 at 37 °C. Retinal cells were allowed to grow in 75 cm^2 flasks for at least 5 days and then were replenished with additional culture medium, until the mixed culture had grown confluent. For purification, after washing with PBS, the cells were incubated with Versene Solution (Thermo Fisher Scientific) containing 5% FBS for 1 hour at 4°C and detached by vigorous pipetting. The resultant cell suspension was then placed in 75 cm^2 flasks and cells allowed to adhere in an incubator at 37°C for 30 minutes. Afterward, suspended and loosely adhering cells were removed by gently shaking the

flasks. The detached cells, composed of ~94% microglia (identifiable by FACS analysis of $CD11b^{+}CX3CR1^{+}$ double-positive cells), were then cultured in a 12-well-plate (precoated with 10 μg/ml Poly-D-lysine (A-003-E, sigma)) in the same culture medium.

Isolation of mouse retinal macrophages/microglia (MRMs) and endothelial cells (MRECs)

Isolation of MRMs and MRECs was performed according to protocols described previously with some modifications (*31*). Specifically, eyes from one or two litters of RA and OIR mice were enucleated and hemisected. The retinas were dissected out and kept in pre-cooled RPMI 1640 (HyClone) or DMEM (Life Technologies). Retinas were pooled, rinsed with pre-cooled PBS buffer, quickly minced into small pieces in a 1.5 mL tube using eye scissors, and digested with papain (Worthington, 20 IU/mL in Earle's balanced salt solution (EBSS)), 2% dispase (Sigma) or collagenase type II (Worthington, 2 mg/mL in serum-free DMEM, Corning, NY, USA) containing 200 IU/ml DNase I for 30 min at 37°C with gentle shaking. Tissue was dissociated by gentle pipetting. The cell digestion suspensions were transferred and passed through 70-μm and 40-μm nylon filters (Corning) to ensure a single-cell suspension. F4/80, CD11b or CD31-positive cells were then isolated using anti-F4/80, anti-CD11b or anti-CD31-MicroBeads (Miltenyi Biotec Inc) based on the manufacturer's protocol. To increase the purity of MRMs, leukocyte populations can be enriched by Percoll gradient (Sigma) before magnetic bead selection. Positively selected cells were stained with F4/80, CD11b or CD31 monoclonal antibodies (mAb), and purity was confirmed by flow cytometry. Purified MRMs or MRECs were collected and analyzed by qRT-PCR or flow cytometry.

Human primary retinal endothelial cells (RECs) and treatment

Human RECs were obtained from Cell Biologics (Cat. No. H-6065) and used between passages 3-8. The type of cells and absence of pathogen contamination were confirmed by the supplier. RECs were cultured in VCBM (ATCC) supplemented with Microvascular Endothelial Cell Growth Kit-BBE (ATCC) or in EBM-2 supplemented with EGM-2 (Lonza) and 1% penicillin/streptomycin (Gibco).

For experiments requiring hypoxia, Human RECs were placed in a modular incubator chamber (Thermo Fisher Scientific) at 37°C with 0.5% O_2.

Preparation of REC-CM

Human RECs were maintained with complete VCBM (ATCC). When reaching about 80% confluence, cells were cultured with fresh complete VCBM in 75 cm^2 BD Falcon flasks at 37°C under normoxia (21% O_2) or hypoxia (0.5% O_2) conditions. Supernatants were collected on days 2 and 3. Conditioned medium (CM) from each of the flasks was transferred to a 50 mL BD Falcon tube and centrifuged at 1,500 r.p.m. for 10 min to remove cellular debris. The CM was sterile filtered using a 0.22 μm filter (Fisherbrand, Fisher Scientific) with a 12 ml syringe barrel. The fractionation of hypoxic REC-CM (Hx-REC-CM) was achieved using Amicon Ultra - 15 centrifugal filters (Ultracel-3K, Millipore). The CM fraction that was >3 kDa remained above the filter, and the <3kDa CM fraction passed through to the lower chamber. The >3 kDa fraction was resuspended in complete VCBM to the pre-filtration volume. Growth medium (VCBM) not exposed to cultured cells was also prepared as above and served as control medium.

Preparation of BMDM-CM

BMDMs were stimulated with Hx-REC-CM or VCBM in a humidified air atmosphere at 37°C overnight, the culture medium was aspirated, and the cells were rinsed thoroughly and cultured with fresh growth medium (RPMI 1640 complete medium) for an additional 24 hours. The CM was centrifuged, sterile filtered (0.22 μm filter), aliquoted and stored at -80°C. BMDM-CM was used at equivalent dilutions in EC growth medium. Macrophage-free medium with the same composition and conditions served as control medium.

Isolation of adipose stromal vascular fraction (SVF) cells

Epididymal adipose tissues were collected from 12-week high fat diet (HFD)-fed C57BL/6J mice after a 4-hour fast. The tissue was minced on ice and then digested on a shaker at 37°C for 45 min in

DMEM medium (A14430-01, Gibco) containing 1 mg/mL collagenase D (COLLD-RO, Sigma-Aldrich), 10 mg/mL BSA (A7030, Sigma-Aldrich), 0.5 mM $CaCl_2$ and 15 mM HEPES. After centrifugation at 500 g for 5 min at 4°C, the SVF pellets were incubated with red blood cell lysing buffer (R7757, Sigma-Aldrich) for 1 min at room temperature, filtered through a 40 μm filter and centrifuged at 500 g for 5 min at 4°C.

Flow cytometry analysis

$CD11b^+$ cells, whole retinal cells from the retinas of RA and OIR mice or SVF cells from HFD-induced obese mice were resuspended in FACS buffer (138 mM NaCl, 2.67 mM KCl, 8.06 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 0.1% BSA, 0.02% NaN3, pH 7.4) and incubated in 100 μL FACS buffer containing 2 μL Fc block (553141, BD Biosciences) for 15 min at room temperature, and then stained with PerCP-Cy5.5 anti-mouse CD11b (2 μg/mL, 561114, BD Biosciences), PE anti-mouse F4/80 (3 μg/mL, 565410, BD Biosciences), FITC anti-mouse CD206 (6 μg/mL, 141703, Biolegend), APC anti-mouse CD11c (3 μg/mL, 550261, BD Biosciences), APC anti-mouse CD86 (3 μg/mL, 558703, BD Biosciences), CD163 monoclonal antibody (TNKUPJ), eBioscience™ Super Bright 600, (3 μg/mL, 63-1631-80, Thermo fFsher Scientific), BV510 hamster anti-mouse CD80 (3 μg/mL, 740130, BD Biosciences), PerCP-Cy™5.5 rat anti-mouse Ly-6G (2 μg/mL, 560602, BD Biosciences), APC rat anti-CD11b (3 μg/mL, 553312, BD Biosciences) and the matching control isotype IgGs (BD Biosciences) for 30 min at 4°C. For staining intracellular antigens, after washing, cells were fixed and permeabilized using eBioscience™ Intracellular Fixation & Permeabilization Buffer Set (88-8824-00, Thermo Fisher Scientific), and then stained with Arginase 1 monoclonal antibody (A1exF5), eBioscience™ PE-Cyanine7, (5 μg/mL, 25-3697-80, Thermo Fisher Scientific) and IL-1 beta (Pro-form) monoclonal antibody (NJTEN3), or eBioscience™ eFluor 450, (5 μg/mL, 48-7114-80, Thermo Fisher Scientific) for 30 min at room temperature. The cells were then washed with 1 mL FACS buffer, centrifuged at 500g for 5 min at 4°C, and resuspended in 0.5 mL FACS buffer and analyzed using FACSCalibur or LSR II 5/FACSCanto (BD Biosciences) and FlowJo

(TreeStar). The compensation and gates were set using isotype controls, single-stained and fully stained.

RNA interference

Human RECs were transfected at 80% confluence with 30 nM siRNAs targeting human *PFKFB3* (si*PFKFB3*, Cat. No. L-006763-00-0005; Dharmacon) or with a non-targeting negative control (si*CTRL*, Cat. No. D-001810-10-05; Dharmacon) using Lipofectamine RNAiMax reagent (Cat. No 13778-150; Invitrogen) according to the manufacturer's protocol. Six hours later, the medium was changed to complete growth medium, and cells were maintained for an additional 42 hours before further experiments. BMDMs were transfected with control nontargeting siRNAs or specific mouse *Mmp9*, or *Il1b* siRNAs (Santa Cruz Biotechnology); MMP-9 siRNA (m), Cat. No. sc-29401; or IL-1β siRNA (m), Cat. No. sc-39616) using HiPerFect transfection reagent (Qiagen) according to the manufacturer's instructions. Briefly, siRNAs and HiPerFect reagents (1:1 ratio, pmol of siRNA:μ l of HiPerFect reagents) were dissolved in Opti-MEM medium and incubated for 5 min at room temperature. The siRNA- HiPerFect reagent complexes were then added to macrophages and incubated at 37 °C for 6 hours. The complexes were then removed, and fresh complete media was added to the cells. The cells were cultured for an additional 48 hours before treatment.

REC spheroid sprouting assay

Human RECs (500 cells) were incubated overnight in 25 % VCBM (25 % VCBM complete medium + 75 % VCBM basal medium) containing 0.25 % (w/v) methylcellulose (Sigma-Aldrich) to form spheroids as described previously (*31*). After 24 hours, spheroids were harvested and embedded in 0.5 ml collagen solution in pre-warmed 24-well plates, with a final concentration of rat type I collagen (BD Biosciences) at 1.5 mg/mL. The spheroid-containing gels were rapidly transferred into a humidified incubator (37°C, 5% CO_2). After the gels had solidified, 0.5 mL control medium or BMDM-conditioned medium were added per well. After 24 hours, cells were imaged using a Zeiss

LSM 780 Inverted Confocal Microscope (Carl Zeiss). The number of sprouts and cumulative length of sprouts per spheroid were quantified from 10 spheroids for each condition using Image J.

Aortic ring and choroidal sprouting assay

Aortae from 4-week-old male C57BL/6J mice were dissected and cut into approximately 1-mm long rings. Retinal pigment epithelium (RPE)/choroid/sclera complex (also referred to as "choroid explant") from 3-week-old wild-type (C57BL/6J) male mice was dissected and cut into ~ 0.5 mm × 0.5 mm pieces . Aortic rings or choroid explants were then embedded into growth factor-reduced Matrigel (354230, Corning, NY, USA) and cultured in EBM-2 medium (Lonza) in a 24-well-plate. At days 4 and 6, 0.5 mL control medium or BMDM-conditioned medium was added per well, and MMP9 antibody (10 µg/mL, IM09L, EMD Millipore) or IL-1 beta antibody (10 µg/mL, AF-401-NA, R&D Systems) was introduced into the culture medium. On day 8, the tissue and sprouting ECs were stained with Calcein AM. Endothelial sprouts were imaged using a Zeiss LSM 780 Inverted Confocal Microscope (Carl Zeiss). The sprouting area was quantified with Adobe Photoshop.

Co-culture of aortic explants and BMDMs

Aortae from 4-week-old male C57BL/6J mice were dissected and cut into approximately 1 mm length rings. Each aortic ring was opened and then embedded into growth factor-reduced Matrigel (BD Bioscience). Co-culture was performed with a transwell system, with the aortic explant cultured at the bottom of the well before the addition of pretreated BMDMs to the top of the transwells. BMDMs were pretreated with REC-CM or VCBM overnight. At day 4, the pretreated BMDMs were added into the transwell on the top of the wells with the aortic explants growing at the bottom. Aortic explant sprouting was imaged at day 8 and quantified. Alternatively, at day 8, the aortic segments were removed from the matrix, and the matrix was digested with 1 mg/mL collagenase D (Cat. No. 11088882001, Sigma) for 10 min at 37°C. The subsequent cell suspensions were centrifuged, and the cells were lysed in RIPA buffer for Western blot analysis.

Capillary tube network formation

Growth factor-reduced Matrigel (BD Bioscience) was placed in 96-well tissue culture plates (50 μL/well) and allowed to form a gel at 37°C for at least 30 min. Human RECs were added to each well (1×10^4 cells per well) in 0.1 mL control medium or BMDM-conditioned medium. After 8 hours, the endothelial tubule formation was observed and photographed using a Zeiss LSM 780 Inverted Confocal Microscope (Carl Zeiss) after staining with Calcein AM. Cumulative tube length was quantified using Image J. Branch points were manually counted.

Fluorescence immunostaining in whole-mount retinas

Eyes were enucleated and fixed in 4% paraformaldehyde for 2 hours at room temperature. The intact retinas were collected, blocked and permeabilized in PBS containing 10% goat serum, 3% BSA, 1% Triton-X-100 and 0.2% Tween 20 for 1 hour. Samples were then incubated with primary antibodies against rabbit PFKFB3 (1:200, ab181861, Abcam), rabbit GLUT1 (1:100, ab115730, Abcam), rabbit PKM2 (1:100, 4053, Cell Signaling Technology), rabbit ARG1 (1:100, AV45673, Sigma), rabbit IL1B (1:200, ab9722, Abcam), rabbit FGF2 (1:100, ab8880, Abcam), rabbit Laminin (1:100, ab11575, Abcam), rabbit Collagen IV (1:200, 2150-1470, Bio-Rad), mouse CD11c (1:100, ab11029, Abcam), rat F4/80 (1:100, Abcam, ab6640), rabbit IBa1 (1:400, Sakura Finetek, 019-19741), FITC-CD206 (1:50, Biolegend, 141703), rat CD31 (1:25, Invitrogen, DIA-310), rabbit ERG antibody (1:200, Abcam, Clone number: EPR3864), and Alexa-488, Alexa-594 or Alexa-647-labeled *Griffonia simplicifolia* isolectin B4 (10 μg/mL, Invitrogen, 121411, 121413 or I32450, Carlsbad, CA, USA) overnight at 4 °C, followed by incubation with fluorescence-conjugated cross-adsorbed secondary antibody (1:500, Molecular Probes, Life Technologies) for 1 hour, and then counterstained with 4',6-diamidino-2-phenylindol (DAPI) (Invitrogen). Retinas were flat mounted on microscope slides in mounting medium (Vectashield; Vector Laboratories) and examined by confocal microscopy (Zeiss 780; Carl Zeiss). For all immunofluorescence experiments, parallel groups of tissues were stained with only secondary antibody as negative controls.

Immunofluorescence staining of BMDMs

BMDMs were plated in Falcon® CultureSlides (Corning) and stimulated for 6 h with Hx-REC-CM. Cells were subsequently fixed with 4% PFA for 30 minutes at room temperature and permeabilized with 0.5% Triton X-100 in PBS for 15 minutes. After rinsing with PBS 3 times, the cells were blocked with 10% goat serum at room temperature for 1 hour. Cells were then incubated with primary antibodies against rabbit Ac-H3 (06-599, EMD Millipore) and mouse H3 (14269, Cell Signaling Technology), or rabbit Ac-H4 (06-866, EMD Millipore) and mouse H4 (2935, Cell Signaling Technology) overnight at 4°C, followed by the application of Alexa Fluor 594-conjugated goat anti-rabbit secondary antibody (1:500 dilution, Invitrogen). The slides were counterstained with DAPI (Invitrogen) and mounted in mounting medium (Vectashield), then were subjected to confocal microscopy (Zeiss 780; Carl Zeiss).

Permeability assay

For visualizing retinal vascular permeability, 1 mL PBS containing 50 mg/mL fluorescein isothiocyanate-dextran (FITC-dextran, 70K Dalton; Sigma-Aldrich) was injected into the left ventricle of anesthetized OIR mice. After 5 min, eyes were enucleated immediately and fixed in 4% PFA for 1 hour. Retinas were dissected, stained with Alexa-594-labeled isolectin B4 (20μg/ml, Invitrogen, 4 hours, RT), washed with PBS, flat-mounted and imaged using a confocal microscope (Zeiss 780; Carl Zeiss).

qRT-PCR analysis

Total RNA of cells and tissues were extracted using Trizol Reagent (Invitrogen) RNeasy Micro kit (Cat. No. 74004, Qiagen). The resultant sample of total RNA was utilized as a template for reverse transcription using the iScriptTM cDNA synthesis kit (Cat. No. 170-8891, Bio-Rad) or QuantiTect Reverse Transcription Kit (Cat. No. 205311, Qiagen). qRT-PCR was performed on a StepOne Plus System (Applied Biosystems) using Power SYBR GreenMaster Mix (Cat. No. 4367659, Life Technologies) with the respective gene-specific primers listed in Table S1. Quantification of relative

gene expression was calculated with the efficiency-corrected $2^{-\triangle\triangle CT}$ method using *Rpl13a* or *18S ribosomal RNA* as the internal control, and data were presented as fold change relative to control groups.

Protein extraction and Western blot

For histone Western blots, BMDMs were lysed in 4× SDS lysis buffer or using EpiQuik Total Histone Extraction Kit (OP-0006-100, EpiGentek). For all other Western blots, cells or tissues were lysed in RIPA buffer (Sigma) supplemented with 1% proteinase inhibitor cocktail (Pierce). After centrifugation of cell lysates, protein was quantified with the BCA assay and then loaded onto an 8-13% SDS-PAGE gel. Primary antibodies used in this study were as follows: rabbit PFKFB3 (1:1000, ab181861, Abcam), rabbit Ac-H3 (1:3000, 06-599, EMD Millipore), rabbit Ac-H4 (1:1000, 06-866, EMD Millipore), mouse anti-H3 (1:2000, 14269, Cell Signaling Technology), mouse anti-H4 (1:2000, 2935, Cell Signaling Technology), and mouse β-actin (1:4000, 3700, Cell Signaling Technology). Images were taken with the ChemiDoc MP system (Bio-Rad), and band densities were quantified using Image Lab software (Bio-Rad).

Chromatin immunoprecipitation (ChIP)

BMDMs (8×10^6) were plated in 10 cm tissue culture plates and stimulated for 6 h with Hx-REC-CM. ChIP was performed as described (*51*), using acetylated H3 (06-599, EMD Millipore), acetylated H4 (06-866, EMD Millipore), or IgG (Santa Cruz) antibodies (*27*). Fold enrichment was calculated as ChIP signals divided by IgG control and normalized to input. ChIP primer sequences are provided in Table S2.

Metabolic measurements

As described previously (*31*), cells were seeded onto Seahorse XF96 polystyrene tissue culture plates (Seahorse Bioscience), and incubated at 37°C overnight. To avoid differences due to unequal cell numbers and growth rates, all measurements were made starting with confluent cells by seeding

5×10^4 per well. The next day, the medium was changed to XF Base Medium (Seahorse Bioscience) supplemented with 25 mM glucose, 2 mM glutamine, and 1 mM pyruvate, pH adjusted to 7.4 with 0.1 M NaOH, and then the plate was incubated for 1 hour in a non-CO_2 incubator at 37°C. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured with an XF^e96 extracellular flux analyzer (Seahorse Bioscience). Inhibitors and activators were used in these tests at the following concentrations: oligomycin (2 μM), FCCP (1 μM), antimycin A (0.5 μM) and rotenone (0.5 μM).

Fructose 2,6-bisphosphate (F-2,6-P2) assay

F-2,6-P2 was detected with the previously described method (*35*). Mouse retinas were homogenized with 0.05 M NaOH and heated for 5 min at 80°C. After cooling, the samples were centrifuged and the supernatants was neutralized with acetic acid. The mixture was centrifuged, and the concentration of F-2,6-P2 in the supernatant were measured by the stimulation of PPi-PFK assayed in the presence of 0.5 mM pyrophosphate and 1 mM fructose 6-phosphate in the assay mixture as shown in the table below. Measurement was conducted at 340 nm with Synergy H1 Hybrid Reader (BioTek). The values have been normalized using protein concentration.

Reaction system for F-2,6-P2 assay:

Substrate	Initial concentration	Final concentration
Tris-HCl (pH7.5)	50 mM	50 mM
NADH	20 mM	0.2 mM
DTT	1 M	5 mM
F6P	200 mM	1 mM
$MgCl_2$	1 M	2 mM
Aldolase	700 U/mL	0.7 U/mL
GDH	450 U/mL	0.45 U/mL
TIM	1200 U/mL	0.6 U/mL

Measurement of pyruvate, lactate and Acetyl-Coenzyme A (Ac-CoA)

The amount of pyruvate, lactate and Ac-CoA of BMDMs, retinal ECs and macrophages, as well as of whole retina samples were determined using Pyruvate Assay Kit (Cat. No. MAK071, Sigma-Aldrich), Lactate Assay Kit (Cat. No. MAK064, Sigma-Aldrich) and Ac-CoA Assay Kit (Cat. No. MAK039, Sigma-Aldrich), according to the manufacturer's instructions.

WST-1 proliferation assay

BMDMs were seeded at 8×10^3 cells per well in 96-well plates. The cells were incubated in RPMI 1640 medium supplemented with 5% heat-inactivated FBS and 10 ng/mL M-CSF for 48 hours, and proliferation was assessed by WST-1 assay (Sigma-Aldrich, Cat. No. 5015944001).

Ki-67 staining and BrdU incorporation analysis

Human RECs or BMDMs were treated with BrdU labeling reagent (Invitrogen) for 24 hours. Following BrdU treatment, cells were fixed with 4% PFA for 15 min, permeabilized in PBS containing 0.5% Triton-X-100 for 15 min, treated with 2N HCl for 30 min, blocked with 10% goat serum for 1 hour, and then incubated with a mouse monoclonal anti-BrdU antibody (1:200, Abcam, Cat. No. ab6326) and rabbit anti-Ki-67 antibody (1:200, Abcam, Cat. No. ab16667) overnight at 4 °C, followed by incubation with fluorescence-conjugated secondary antibody (1:500, Life Technologies) for 1 hour. The cells were then immersed in ProLong Gold mounting medium with DAPI (Invitrogen) to visualize the nuclei. Images were obtained using an upright confocal microscope (Zeiss 780; Carl Zeiss). The number of Ki-67 or BrdU-positive cells was counted in six non-overlapping and randomly selected microscopic fields per slide.

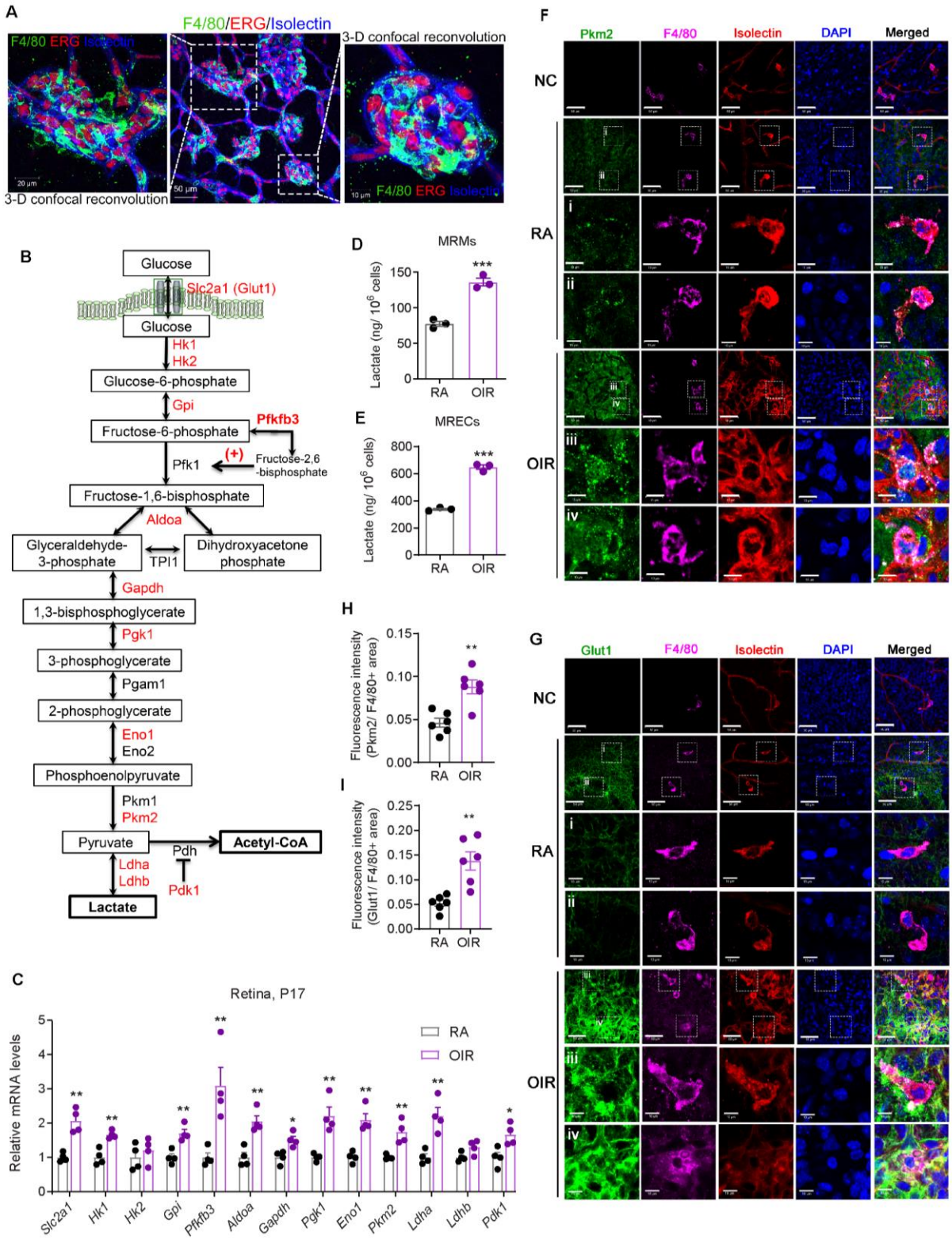


Fig. S1. Retinal macrophages/microglia are hyperglycolytic in the pathological angiogenic vascular niche in a mouse model of OIR. (**A**) Triple IHC staining of F4/80 (green), ERG (red), and isolectin B4 (blue) on the sections of the P17 OIR retinas. (**B**) Schematics showing the glycolytic pathway and associated enzymes and metabolites. (**C**) qRT-PCR analysis of the mRNA expression of glycolytic genes in P17 RA or P17 OIR retinas (n = 4). (**D-E**) The amount of intracellular lactate

of mouse retinal macrophages/microglia (MRMs) (**D**) or endothelial cells (MRECs) (**E**) isolated from RA and OIR mice at P17. (**F**-**G**) Immunofluorescent staining of Pkm2 (**F**) and Glut1 (**G**) on sections of P17 RA and P17 OIR retinas. Representative Pkm2 or Glut1 (geen), F4/80 (pink), isolectin B4 (red), DAPI (nuclei, blue) staining, and merged images captured with confocal fluorescent microscopy. NC, negative control. Scale bar: 50 μm (1st, 2nd and 5th rows) and 10 μm (3rd, 4th, 6th and 7th rows). n = 6 mice for each group. (**H-I**) The fluorescence intensity of Pkm2 (**H**) and Glut1 (**I**) staining were calculated by Image J software. Data are represented as means ± SEM. *$P < 0.05$; **$P < 0.01$ vs RA, by two-tailed unpaired Student's t-test.

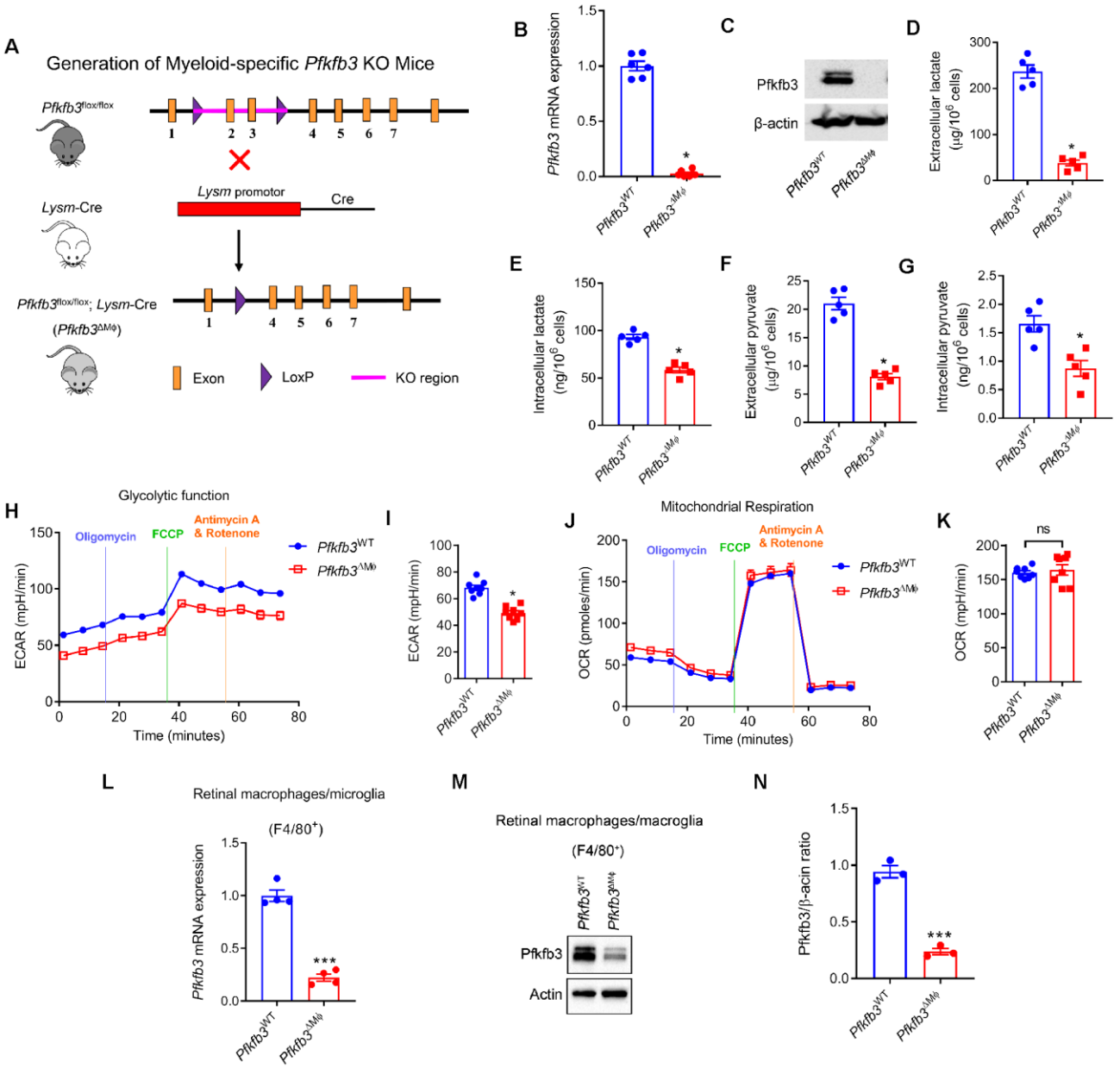


Fig. S2. Generation of myeloid-specific *Pfkfb3* KO mice. (**A**) Schematic illustration of the generation of myeloid-specific *Pfkfb3* KO mice. (**B-C**) qRT-PCR (**B**) and Western blot (**C**) analysis of mRNA and protein expression of Pfkfb3 in bone-marrow-derived macrophages (BMDMs) cultured with bone marrow of wild-type (*Pfkfb3*^{WT}) and myeloid-specific *Pfkfb3* knockout (*Pfkfb3*$^{\Delta M\Phi}$) mice, respectively. *n* = 6. (**D-E**) The amount of intracellular and secreted lactate of BMDMs (*n* = 5). (**F-G**) The amount of intracellular and secreted pyruvate of BMDMs (*n* = 5). (**H**) ECAR profile showing glycolytic function in BMDMs. (**I**) Quantification of glycolytic function parameters from **H**. *n* = 8. (**J**) OCR profile of BMDMs. (**K**) Quantification of mitochondrial respiration function parameters from **J**. *n* = 8. (**L**) qRT-PCR analysis of mRNA and protein

expression of *Pfkfb3* in mouse retinal macrophages/microglia (MRMs) isolated from OIR mice at P17 using anti-F4/80 microbeads. $n = 4$. (**M-N**) Western blot analysis (**M**) and quantification (**N**) of protein expression of Pfkfb3 in MRMs isolated from OIR mice at P17. $n = 3$. $^{*}P < 0.05$; $^{***}P < 0.001$ vs control group. Data are represented as means ± SEM. Statistical significance was determined by two-tailed unpaired Student's *t*-test.

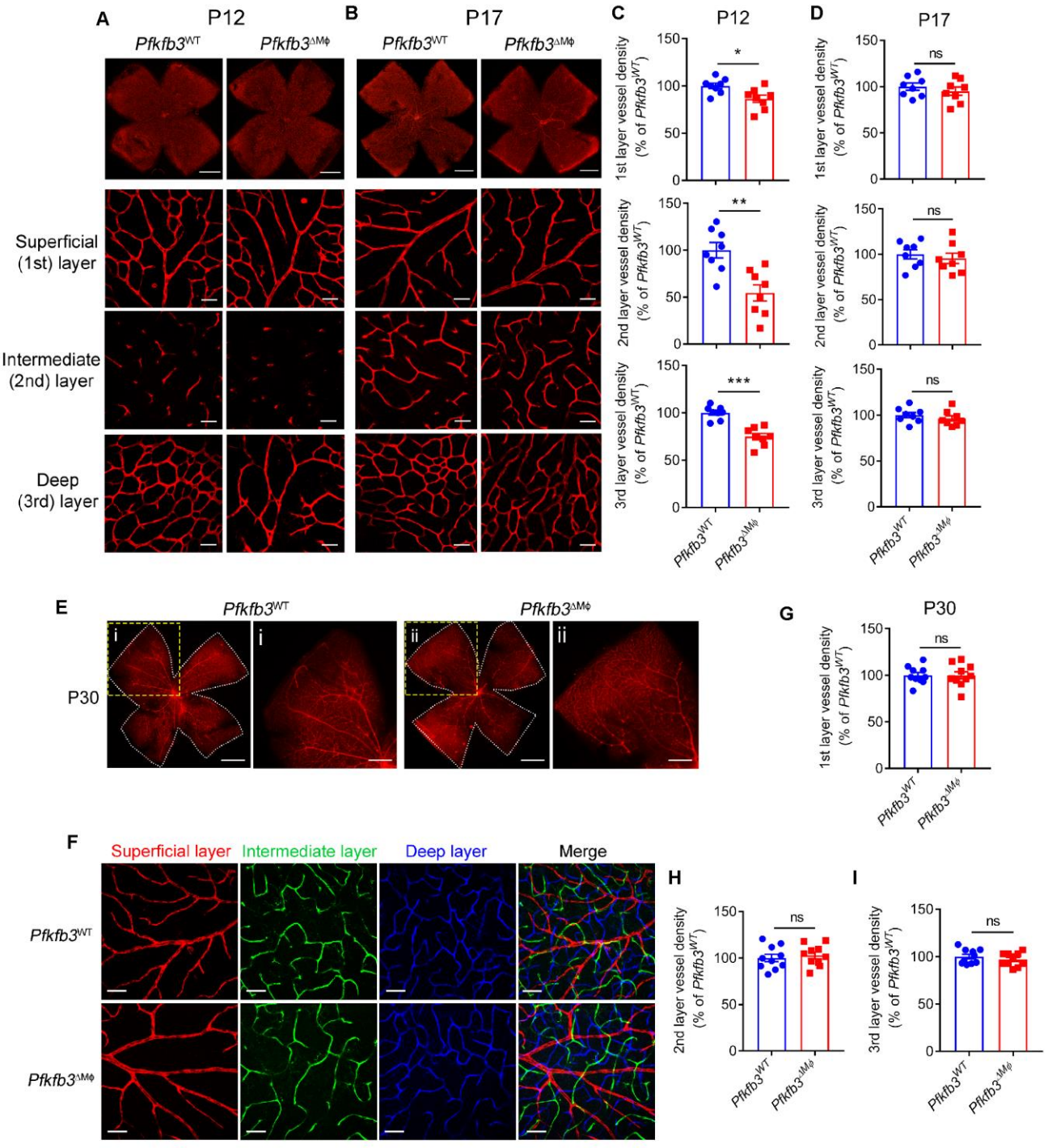


Fig. S3. Effect of myeloid *Pfkfb3* deficiency on the development of retinal vasculature in mice.

The development of retina vasculature of from *Pfkfb3*^{WT} or *Pfkfb3*$^{\Delta M\phi}$ mice at postnatal day (P) 12 (**A**), P17 (**B**), and P30 (**E**) in room air was visualized by isolectin B4 staining in whole-mount retinas. Scale bars, 1000 μm for original images and 500 μm for enlarged images. The retinal vasculatures of the superficial, intermediate and deep vascular layers were examined at P12 (**A**), P17 (**B**) and P30 (**F**) by isolectin B4 staining of whole-mount retinas. The distributions of three retinal vascular layers are

displayed in distinct confocal planes. Scale bar, 50 μm. Quantification of the vascular density (vessel coverage area per field) in the superficial layer, intermediate layer, and deep layer at P12 (**C**, $n = 8$), P17 (**D**, $n = 8$) and P30 (**G-I**, $n = 10$). Data are mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs *Pfkfb3*^{WT}, by two-tailed unpaired Student's *t*-test. ns, not significant.

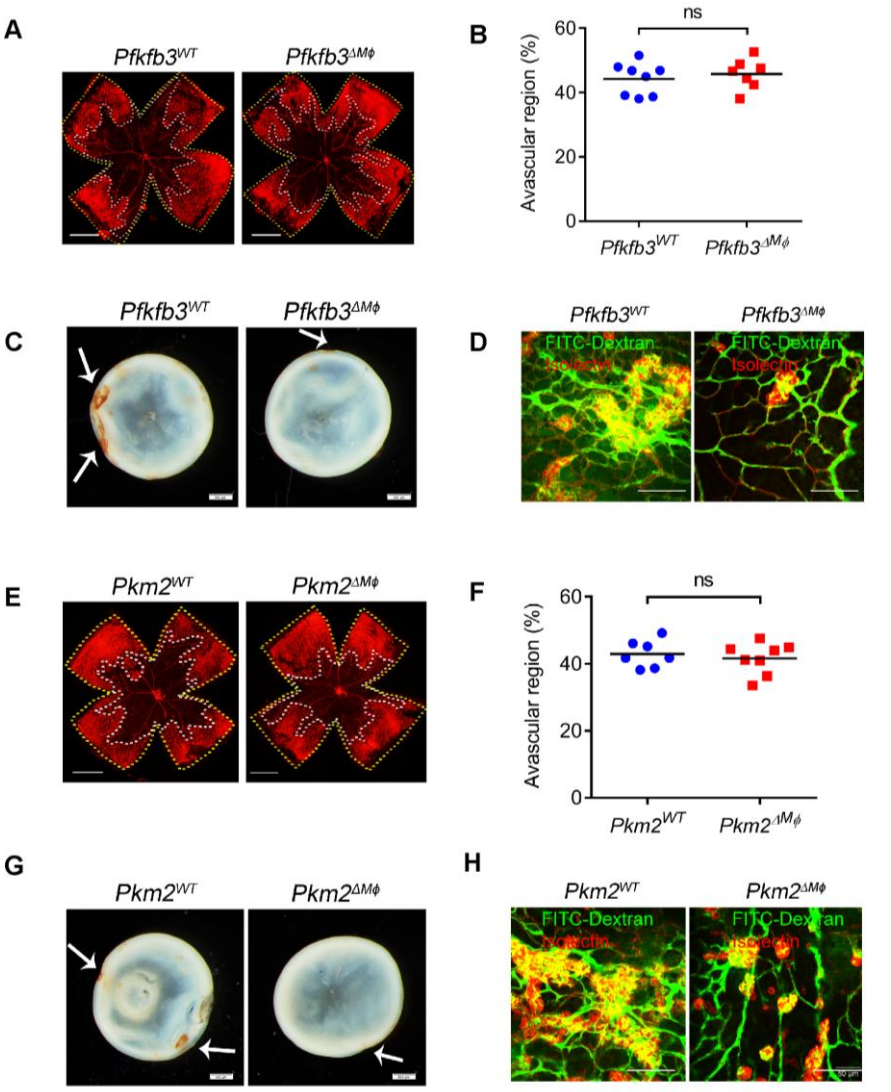


Fig. S4. Effect of myeloid *Pfkfb3* or *Pkm2* deficiency on retinal vaso-obliteration at P12 in OIR. Retinal blood vessels of *Pfkfb3*^{WT}, *Pfkfb3*$^{\Delta M\phi}$ (**A**), *Pkm2*^{WT}, and *Pkm2*$^{\Delta M\phi}$ (**E**) mice visualized by isolectin B4 staining of OIR retinas at P12. The entire retinal surface is shown by the yellow dotted line. The vascular area is indicated by the white dotted line. Scale bar: 1000 μm. (**B** and **F**) Avascular area (%) was quantified as a percentage of the whole retinal surface (n = 7-8 for each group). (**C** and **G**) Representative images of the whole retina after dissection. White arrows point to visible hemorrhages in WT OIR mice at P17, which are absent in *Pfkfb3*$^{\Delta M\phi}$ and *Pkm2*$^{\Delta M\phi}$ mice. Scale bar, 500 μm. (**D** and **H**) Integrity of the blood-retinal barrier in P17-OIR retinas examined with FITC-dextran (green) perfusion and isolectin B4 (red) staining. Scale bar, 100 μm. Data are mean ± SEM. Statistical significance was determined by two-tailed unpaired Student's *t*-test. ns, not significant.

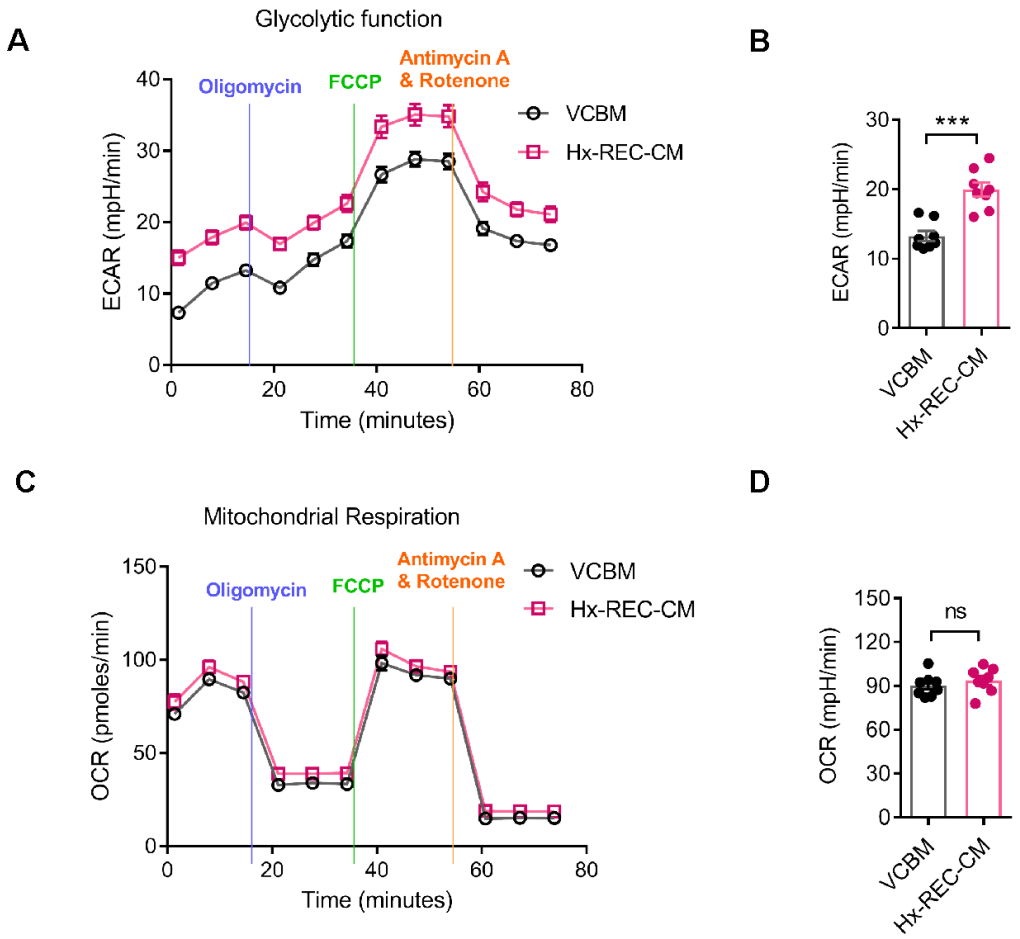


Fig. S5. Effect of hypoxic REC-CM on glycolytic function and mitochondrial respiration function of BMDMs. (**A**) ECAR profile showing glycolytic function of BMDMs pretreated with control medium (VCBM), or hypoxic REC-conditioned medium (Hx-REC-CM) for 12 h. (**B**) Quantification of glycolytic function parameters from **A**. $n = 8$. ***$P < 0.001$ vs VCBM. (**C**) OCR profile of BMDMs. (**D**) Quantification of mitochondrial respiration function parameters from **G**. n = 8. ns, not significant.

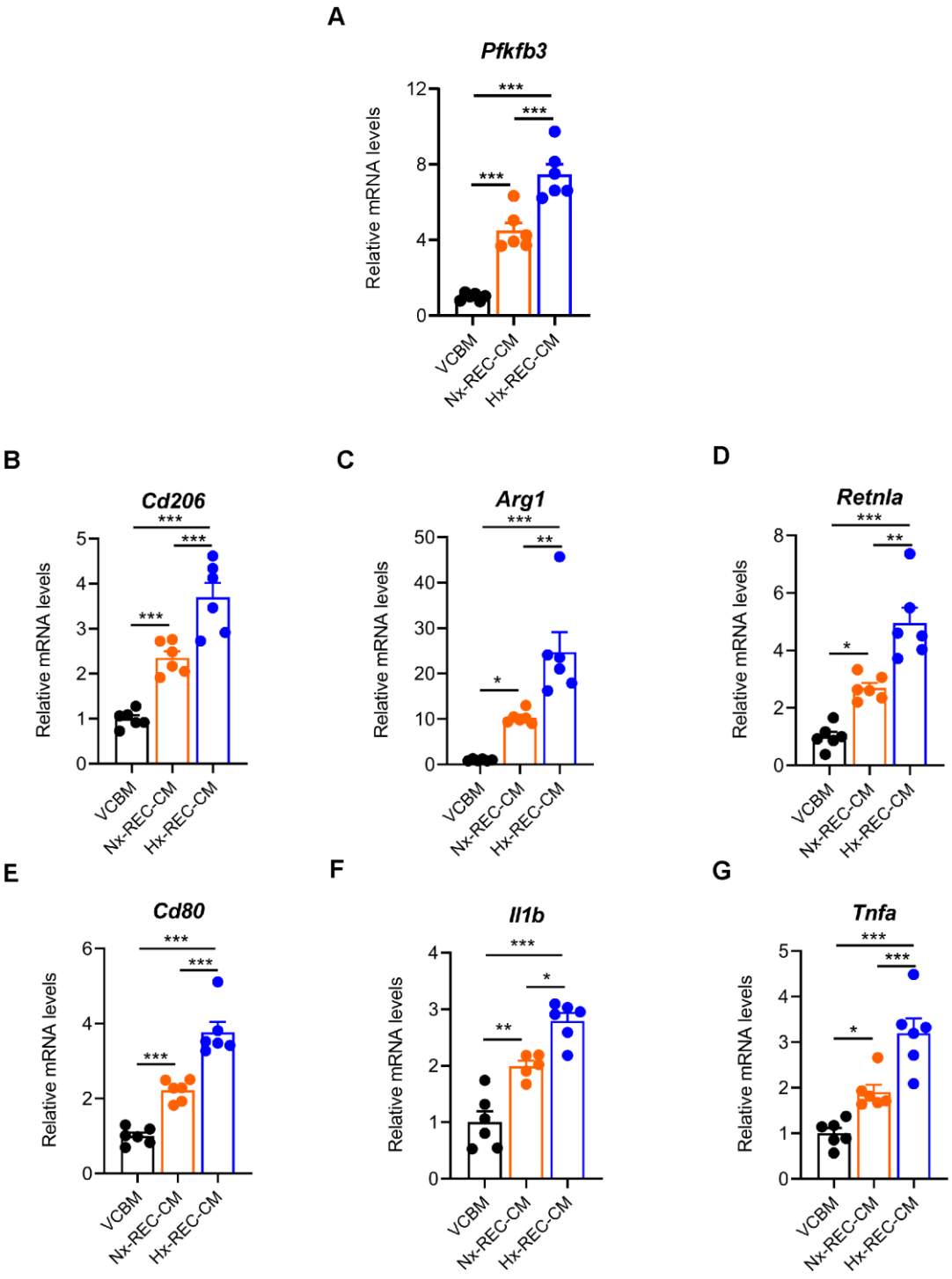


Fig. S6. Effect of hypoxic REC-CM on retinal microglia glycolysis and polarization. (**A-G**) qRT-PCR analysis of the mRNA expression of *Pfkfb3* (**A**) and M1/M2 markers (**B-G**) in mouse retinal microglia exposed to control medium (VCBM), normoxic REC-conditioned medium (Nx-REC-CM) or hypoxic REC-conditioned medium (Hx-REC-CM) for 12 h (n = 5-6). *P < 0.05 vs VCBM. Data are mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001, by one-way ANOVA followed by Bonferroni test.

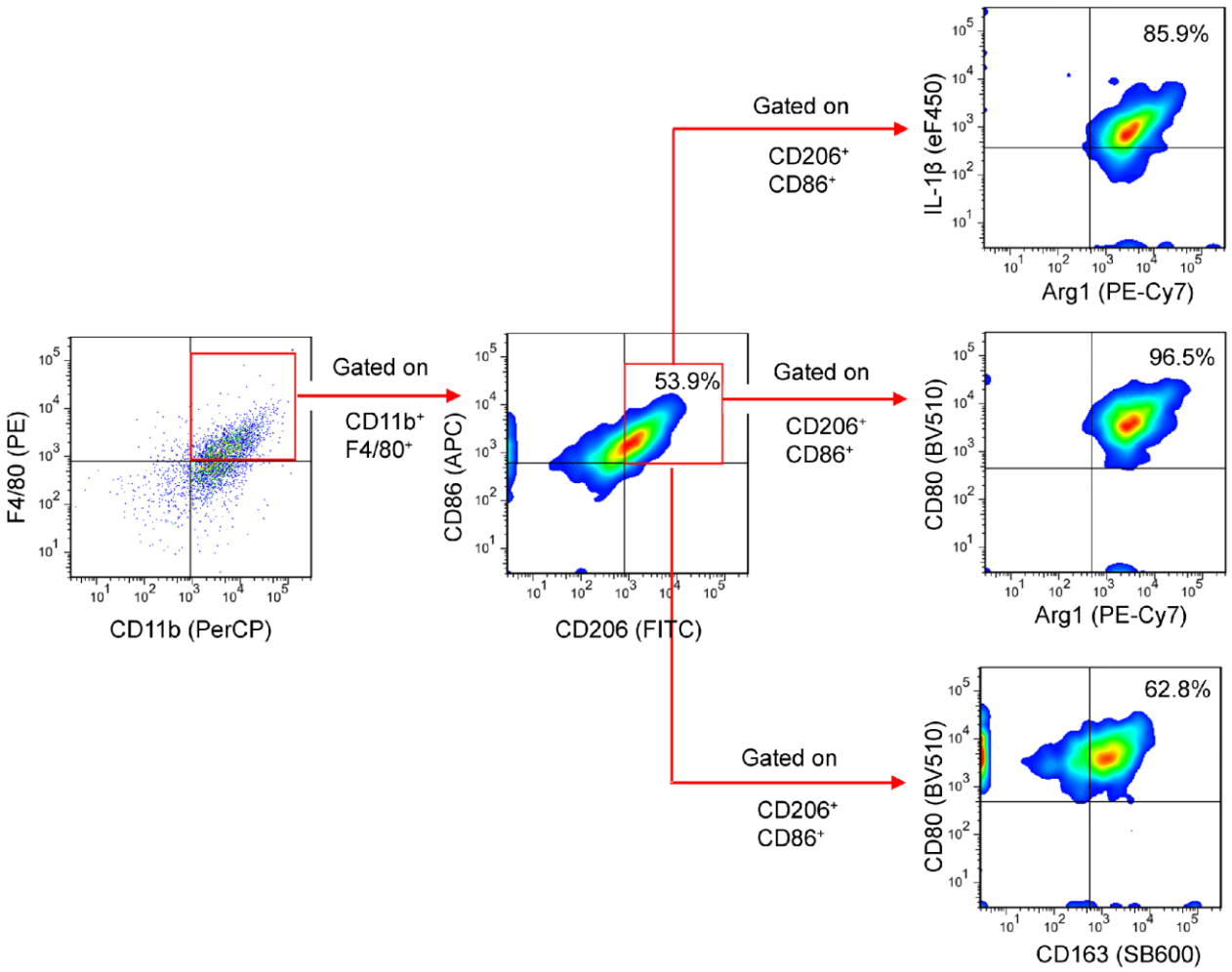


Fig. S7. Macrophages/microglia exhibit mixed M1/M2-like phenotype in OIR retinas. $CD11b^+$ cells were isolated from mouse OIR retinas at P17 using anti-CD11b microbeads. The selected $CD11b^+$ cells were collected and analyzed simultaneously for both cell surface markers (CD11b, F4/80, CD86, CD206, CD80 and CD163) and intracellular antigens (Arg1 and IL-1β) by flow cytometry. $CD11b^+F4/80^+CD86^+CD206^+$ cells also highly expressed CD80, IL-1β, CD163 and Arg1 in OIR retinas at P17. n = 4.

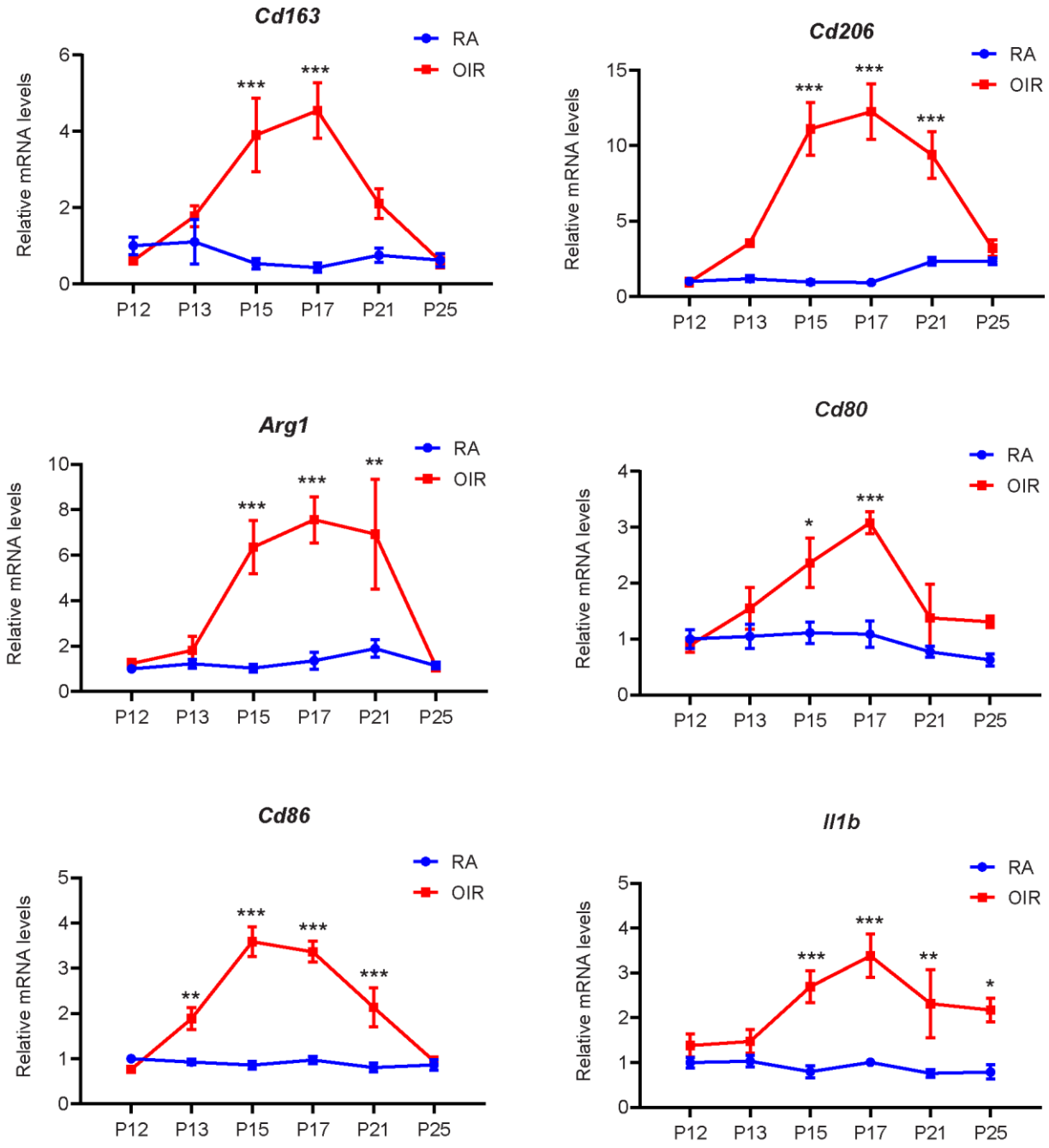


Fig. S8. Expression of M1/M2 markers in retinal macrophages/microglia in OIR retinas at different time points. F4/80^{+} cells were isolated from mouse RA and OIR retinas at different time points from P12 to P25 using anti-F4/80 microbeads. qRT-PCR analysis was performed for the mRNA expression of M1/M2 markers in mouse retinal F4/80^{+} cells (n = 3). *P < 0.05 vs VCBM. Data are mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001 vs RA, determined by two-way ANOVA followed by Bonferroni test.

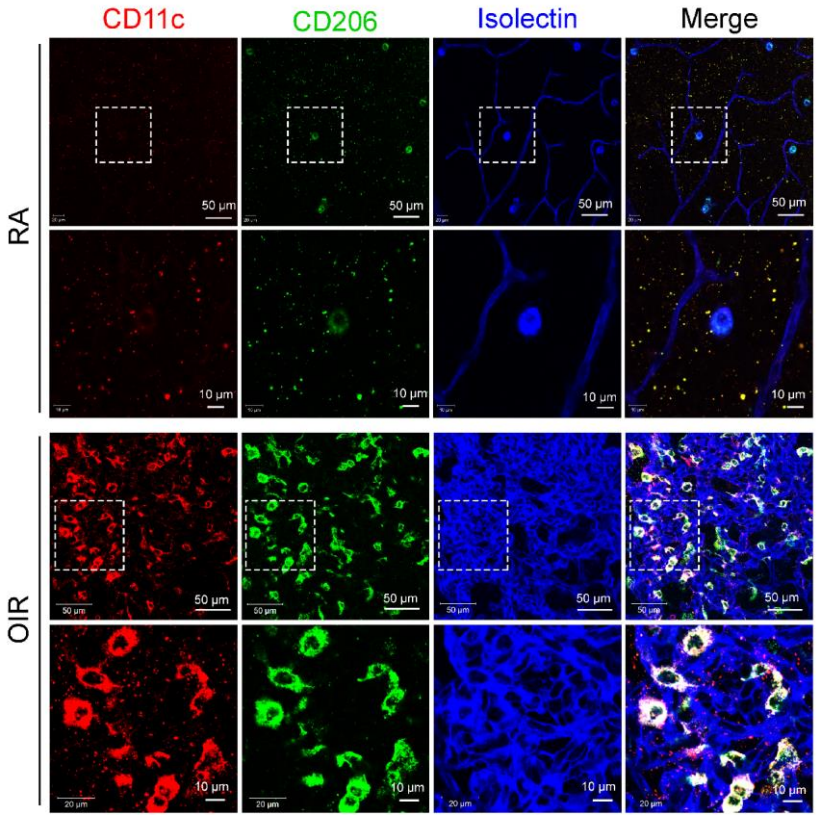


Fig. S9. Immunofluorescent staining shows the colocalization and expression of CD11c and CD206 within the pathological neovascular tufts of OIR retina at P17. Representative CD11c (red), CD206 (green), isolectin B4 (blue), and merged images were captured with confocal fluorescent microscopy. *n* = 5 mice for each group.

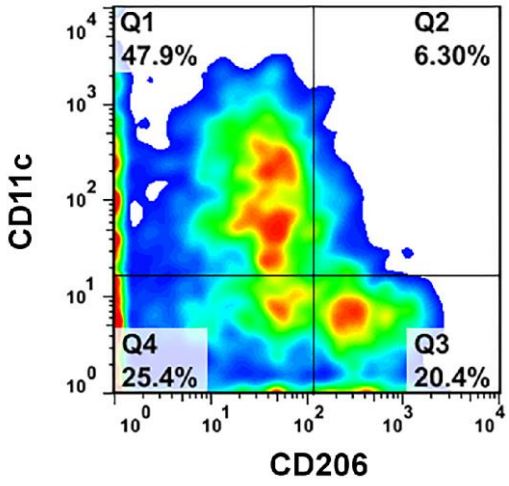


Fig. S10. Mixed M1/M2 population of macrophages is not found in adipose tissue from mice fed an HFD. Representative flow cytometric plots of gated $CD11b^{+}F4/80^{+}$ cells showing $CD11c^{+}CD206^{-}$ (M1-like macrophages), $CD11c^{-}CD206^{+}$ (M2-like macrophages) and $CD11c^{+}CD206^{+}$ (mixed M1/M2-like macrophages) fractions and the percentage of $CD11c^{+}CD206^{+}$ cells among stromal vascular fractionated cells isolated from epididymal white adipose tissue of wild-type mice after 12 weeks of high-fat-diet feeding (n = 4 mice).

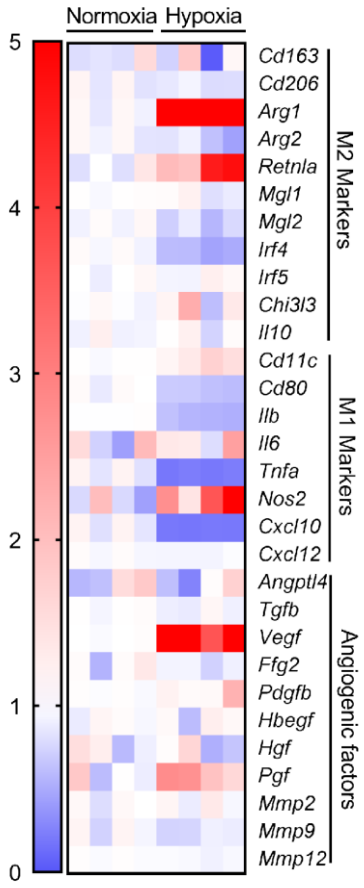


Fig. S11. Hypoxia alone is not sufficient to cause a mixed M1/M2-like phenotype in macrophages. Heatmap displaying the fold changes of gene expression detected by qRT-PCR in BMDMs exposed to hypoxia (0.5% O_2) or normoxia (21% O_2) for 12 h. n = 4.

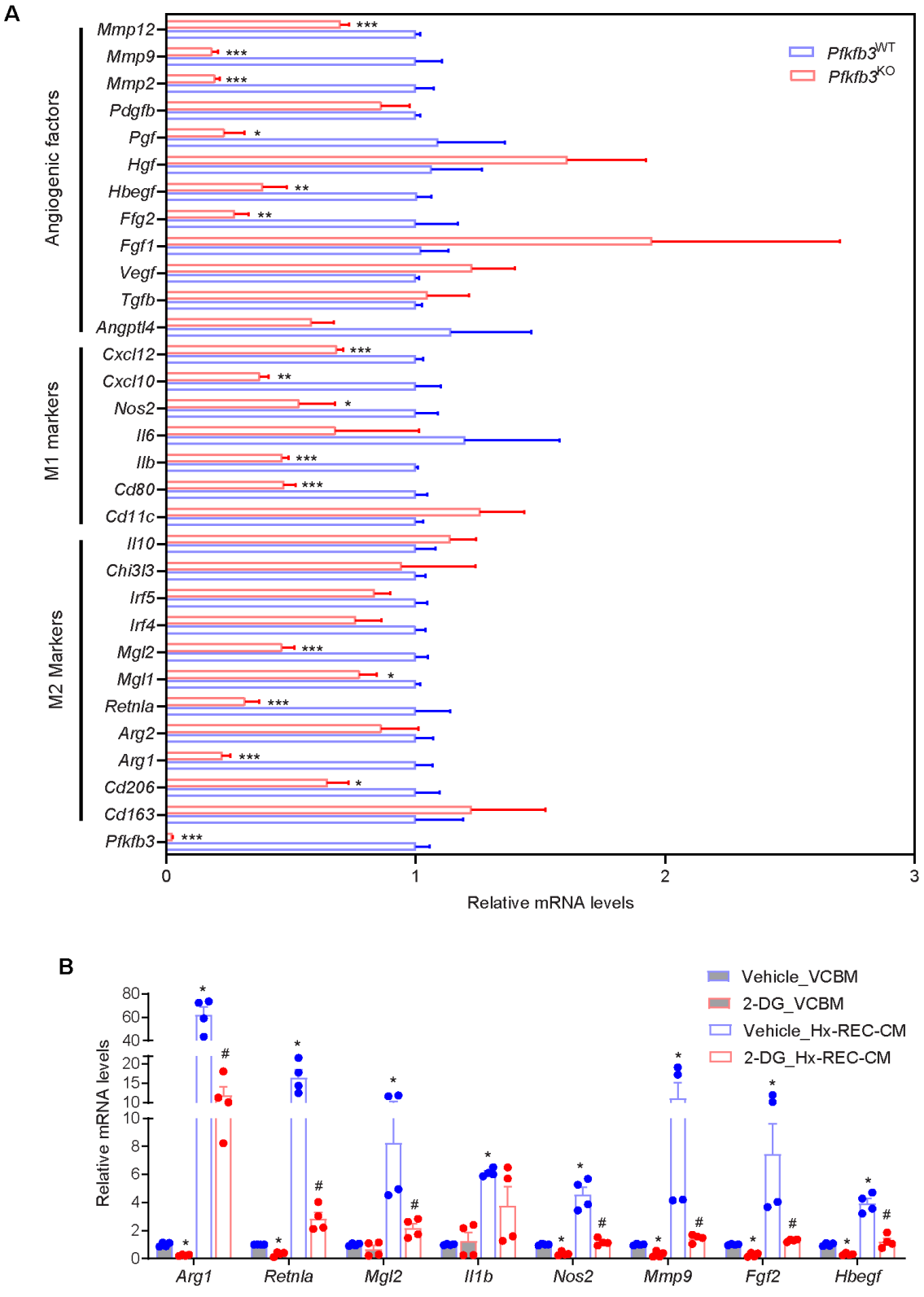


Fig. S12. Effect of glycolysis on macrophage M1/M2 polarization. (**A**) qRT-PCR analysis of gene expression in *Pfkfb3*^{WT} or *Pfkfb3*$^{\Delta M\phi}$ BMDMs (n = 4). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs *Pfkfb3*^{WT}. (**B**) qRT-PCR analysis of gene expression in WT BMDMs exposed to VCBM or Hx-REC-

CM for 12 h, with or without 2-DG (2 mM) treatment (n = 4). *P < 0.05 vs VCBM_vehicle. Data are mean ± SEM. $^{\#}P$ < 0.05 vs Hx-REC-CM_vehicle. Statistical significance was determined by Student's t-test (for **A**) one-way ANOVA followed by Bonferroni test (for **B**).

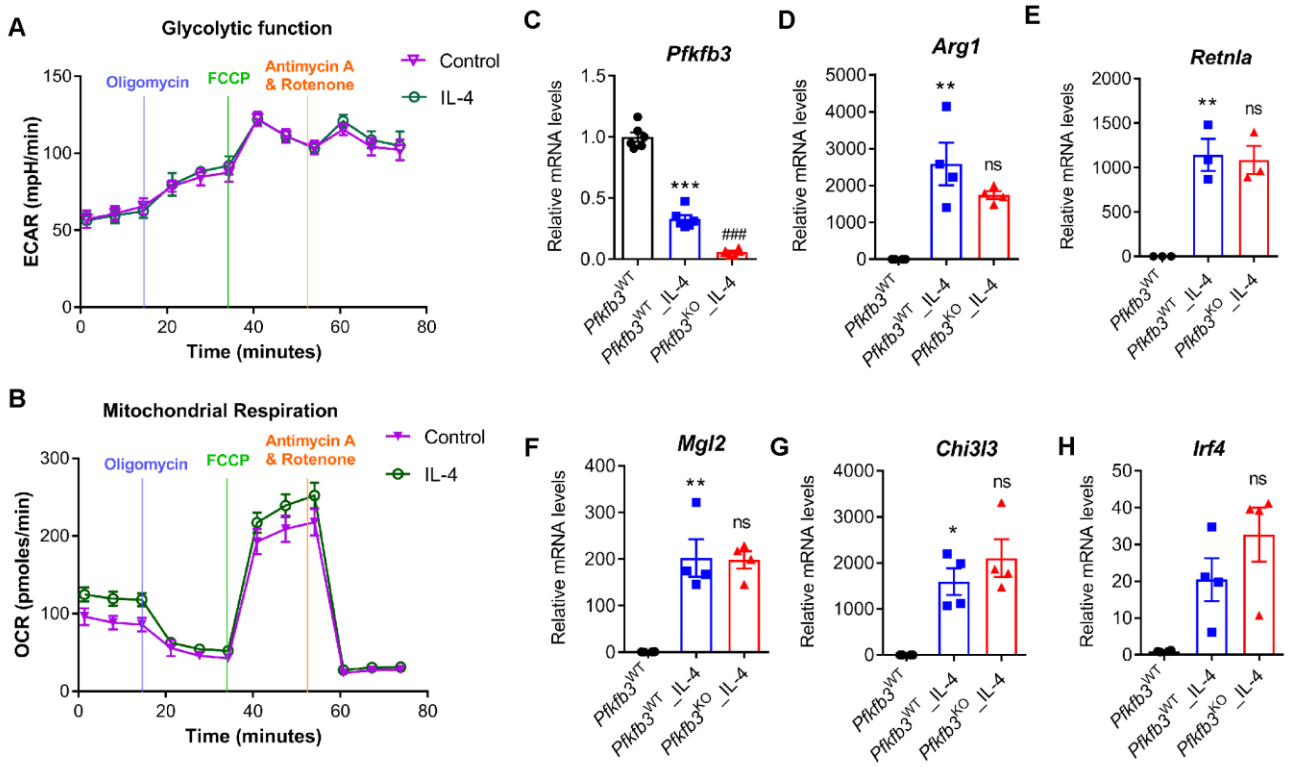
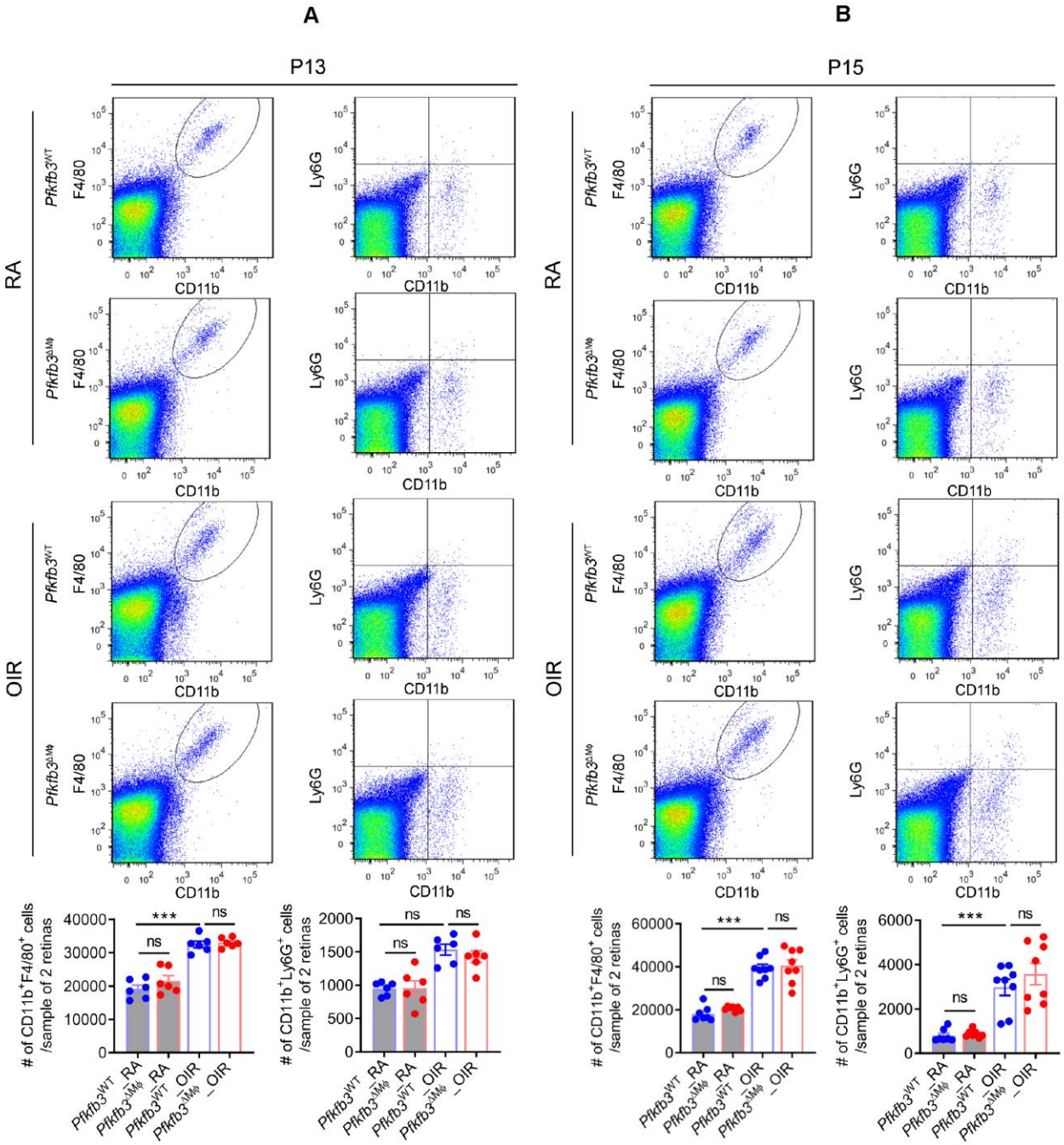


Fig. S13. Effect of *Pfkfb3* KO on IL-4–induced macrophage M2 polarization. (**A**) ECAR profile showing glycolytic function in bone-marrow-derived macrophages (BMDMs) treated with 20 ng/mL IL-4 for 12 h. *n* = 8. (**B**) OCR profile showing mitochondrial respiration function in BMDMs treated with 20 ng/mL IL-4 for 12 h. *n* = 8. (**C**-**H**) BMDMs cultured with bone marrow of wild-type (*Pfkfb3*^{WT}) or myeloid-specific *Pfkfb3* knockout (*Pfkfb3*$^{\Delta M\phi}$) mice were treated with 20 ng/mL IL-4 for 12 h. qRT-PCR analysis was performed for the mRNA expression of *Pfkfb3* and M2 genes in BMDMs (*n* = 3-6). **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs *Pfkfb3*^{WT}; ns, not significant, $^{\#\#\#}P$ < 0.001 vs *Pfkfb3*^{WT}_IL-4. Data are mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Bonferroni test.


A
P13
B
P15
RA
OIR
Pfkfb3WT
Pfkfb3ΔMΦ
F4/80
Ly6G
CD11b
# of CD11b+F4/80+ cells /sample of 2 retinas
# of CD11b+Ly6G+ cells /sample of 2 retinas
***
ns
Pfkfb3WT_RA
Pfkfb3ΔMΦ_RA
Pfkfb3WT_OIR
Pfkfb3ΔMΦ_OIR

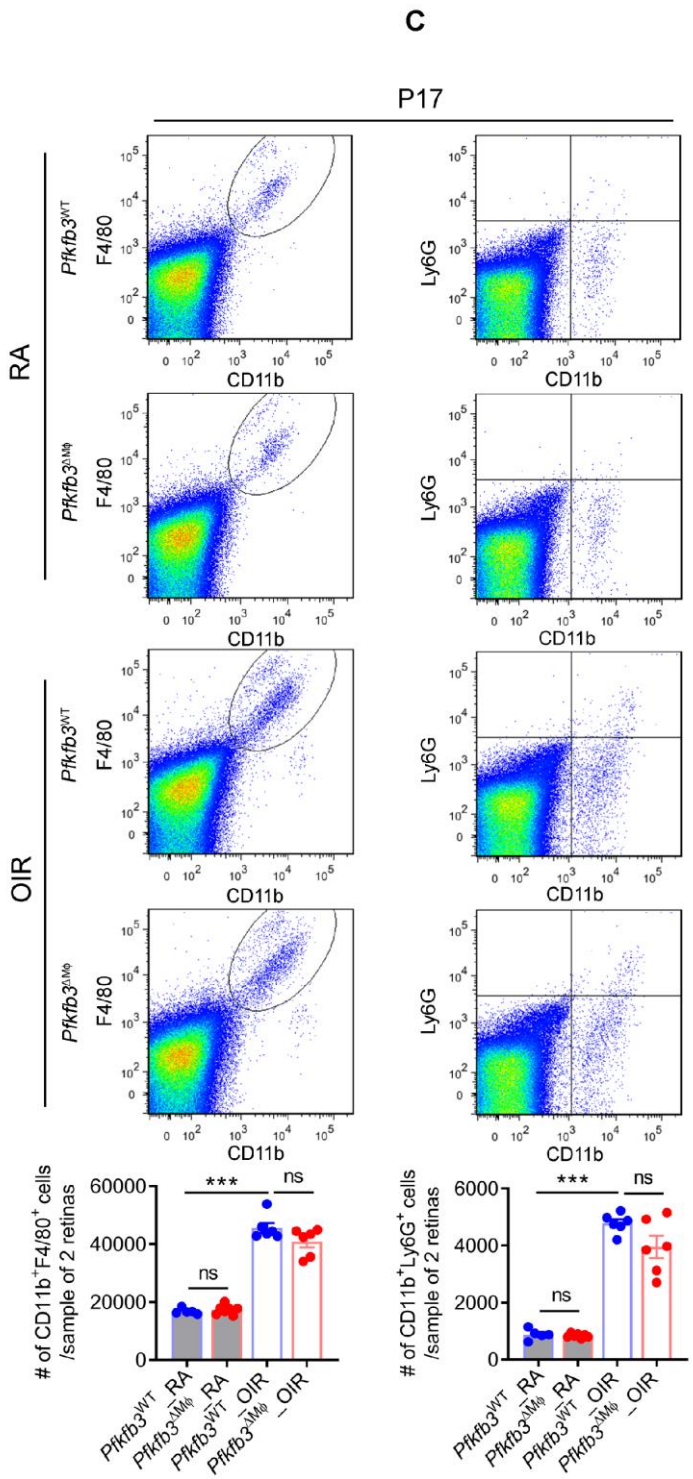


Fig. S14. Effect of *Pfkfb3* KO on the number of macrophages and neutrophils in RA or OIR retinas. Representative flow cytometric plots of $CD11b^{+}F4/80^{+}$ cells (macrophages/microglia) or $CD11b^{+}Ly6G^{+}$ cells (neutrophils) in RA and OIR retinas collected at (**A**) P13, (**B**) P15, and (**C**) P17 from *Pfkfb3*^{WT} and *Pfkfb3*$^{\Delta M\phi}$ mice. The number of retinal macrophages/microglia and neutrophils were comparable between genotypes either in RA or OIR at all points analyzed; n = 6 (total of 12

retinas per condition; each “n” comprises 2 retinas). ***$P < 0.001$. ns, not significant. Data are mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Bonferroni test.

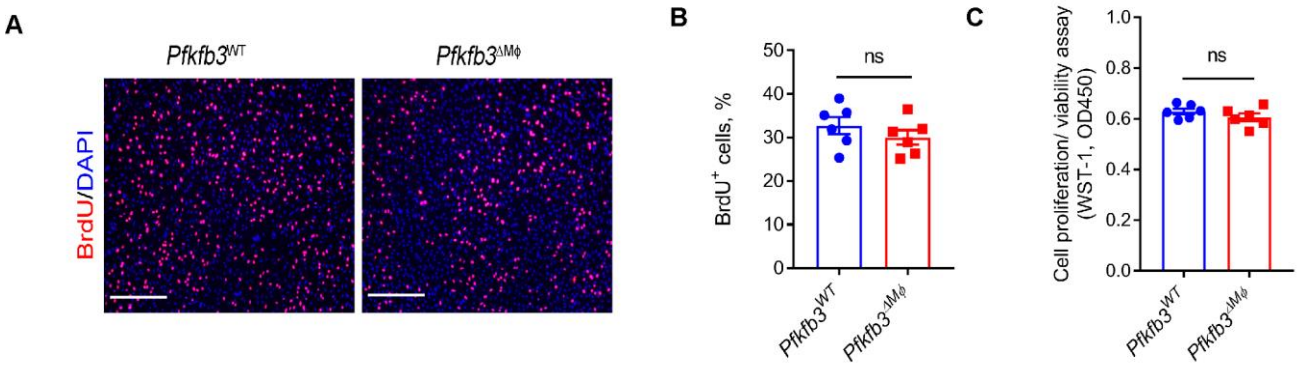


Fig. S15. Effect of *Pfkfb3* KO on macrophage proliferation and viability. (**A-B**) Bromodeoxyuridine (BrdU) staining of bone-marrow-derived macrophages (BMDMs) from wild-type (*Pfkfb3*^{WT}) or myeloid-specific *Pfkfb3* knockout (*Pfkfb3*$^{\Delta M\phi}$) mice. $n = 6$. Scale bars, 100 μm. (**C**) Cell proliferation and viability measured by WST-1 assay. $n = 6$. Data are mean ± SEM. Statistical significance was determined by two-tailed unpaired Student's *t*-test. ns, not significant.

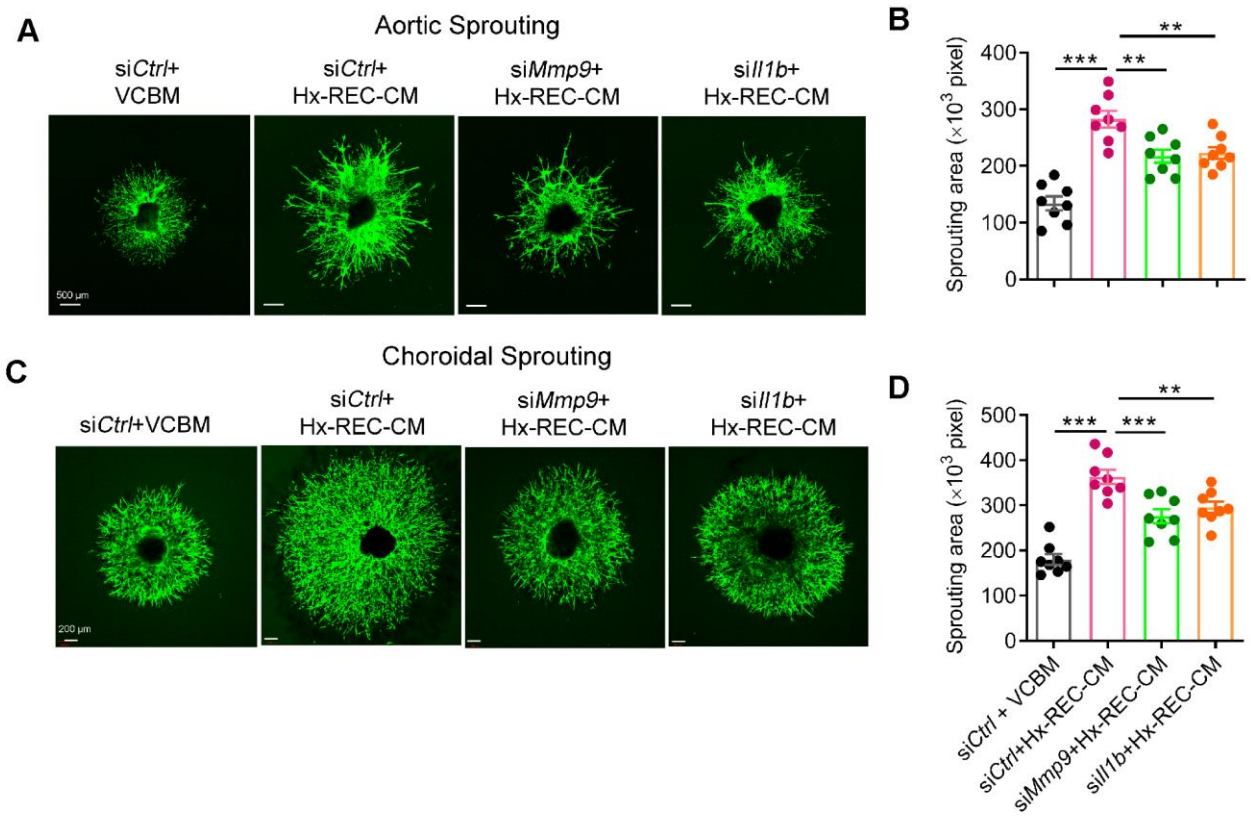


Fig. S16. Hx-REC-CM–treated macrophages promote EC sprouting though increased secretion of proinflammatory and proangiogenic factors. BMDMs were transfected with siRNAs targeting mouse *Mmp9* (si*Mmp9*), mouse *Il1b* (si*Il1b*) or with a non-targeting negative control (si*Ctrl*). 48 hours after transfection, WT BMDMs were stimulated with VCBM or Hx-REC-CM. After 12 h, the medium was changed to fresh RPMI 1640 complete medium for an additional 24 h, and then the conditioned medium (CM) was collected for aortic ring and choroidal explant culture. Macrophage-free medium with the same composition and conditions served as control medium. Representative images of sprouting aortic rings (**A**) and choroids (**C**) exposed to CM from WT BMDMs pre-treated with VCBM or Hx-REC-CM. Sprouting areas were quantified (**B** and **D**). $n = 8$. $^{**}P < 0.001$, $^{***}P < 0.001$. Data are mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Bonferroni test.

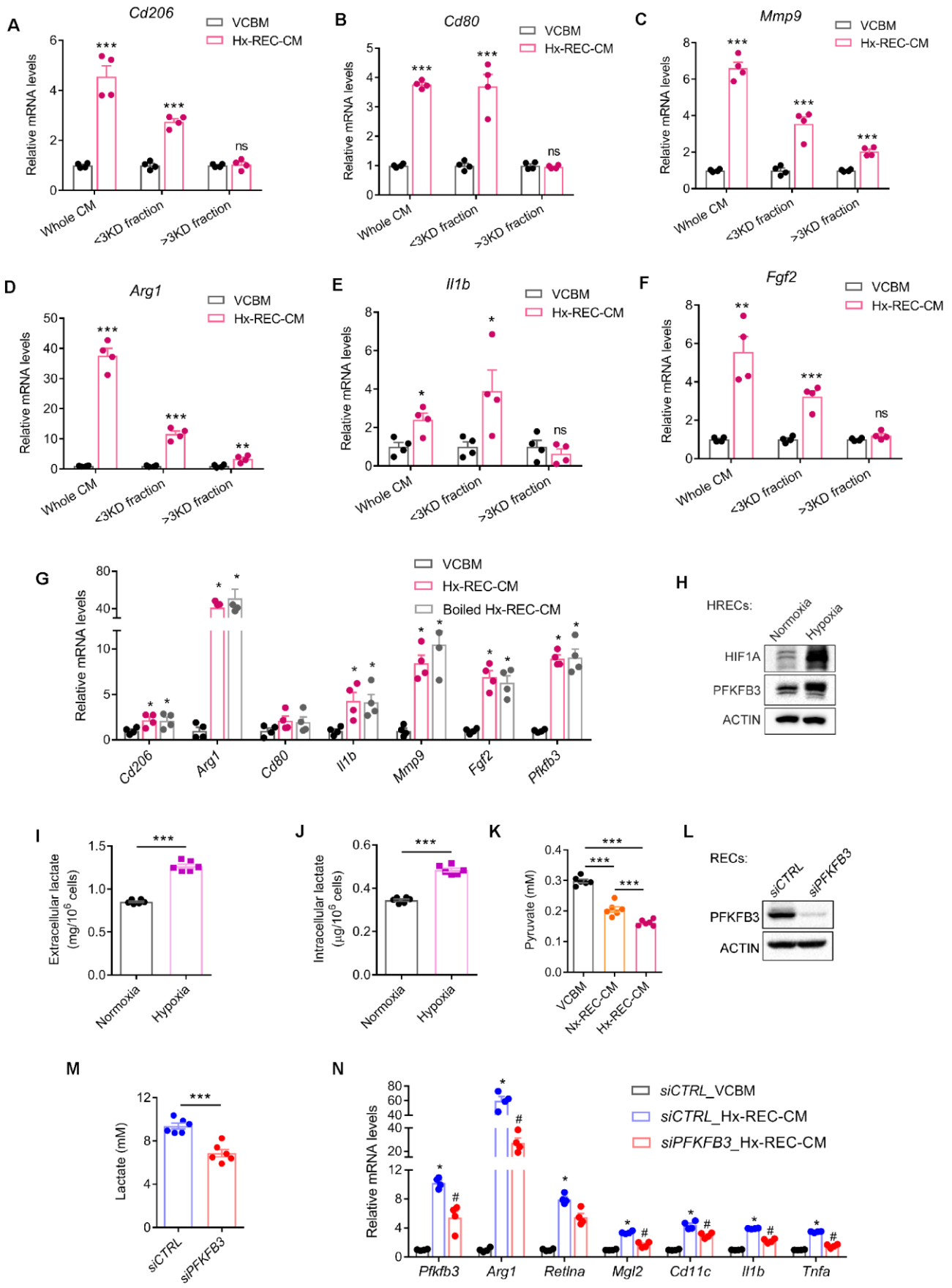


Fig. S17. REC-secreting metabolites are sufficient to induce macrophage glycolysis and activation. (**A-F**) Control (VCBM) or hypoxic REC-conditioned medium (Hx-REC-CM) was used

unfractionated (whole) or as >3 kDa or <3 kDa fractions to stimulate BMDMs as follows. qRT-PCR analysis of gene expression in BMDMs. n =4. *P < 0.05; **P < 0.01; ***P < 0.001 vs VCBM. (**G**) qRT-PCR analysis of gene expression in BMDMs stimulated with boiled (100 °C) or non-boiled Hx-REC-CM for 12 h. n = 4. *P < 0.05 vs VCBM. (**H**) Western blot analysis of PFKFB3, HIF1A and β-actin in Human RECs (HRECs) exposed to normoxia (21% O_2) or hypoxia (0.5% O_2) for 12 h. n = 5. (**I**-**J**) The amount of extracellular and intracellular lactate of HRECs under normoxia or hypoxia for 24 h. n = 6. ***P < 0.001. (**K**) Pyruvate concentration in the Hx-REC-CM or control medium. n = 6. ***P < 0.001. (**L**) Western blot analysis of Pfkfb3 and β-actin in HRECs transfected with siRNA targeting human *PFKFB3* (*siPFKFB3*) or with a non-targeting negative control (*siCTRL*) under hypoxia conditions (0.5% O_2). n = 3. (**M**) The amount of secreted lactate of HRECs were determined. n = 6. ***P < 0.001. (**N**) qRT-PCR analysis of the mRNA expression of *Pfkfb3* and macrophage polarization genes in BMDMs stimulated with control medium (VCBM) or conditioned medium from HRECs transfected with *siPFKFB3* or *siCTRL* under hypoxia conditions (0.5% O_2) (Hx-REC-CM). *P < 0.05 vs VCBM. $^{\#}P$ < 0.05 vs *siCTRL*-Hx-REC-CM. Data are mean ± SEM. Statistical significance was determined by two-tailed unpaired Student's *t*-test (for **A-F, I-J** and **M**) and one-way ANOVA followed by Bonferroni test (for **G, K** and **N**).

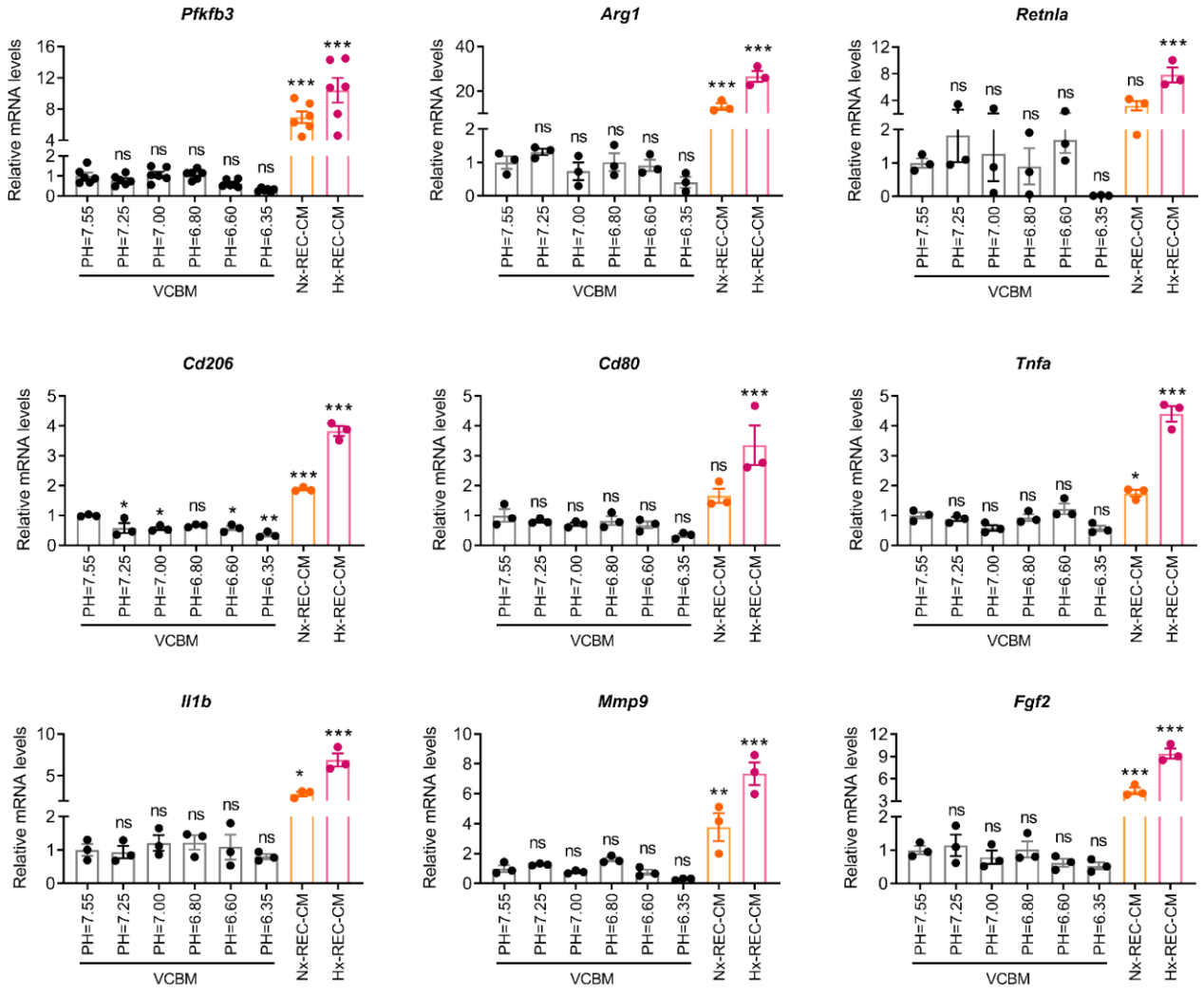


Fig. S18. Effect of acidic pH on macrophage activation. The pH of VCBM not exposed to cultured cells, Nx-REC-CM and Hx-REC-CM are 7.55, 7.28 and 7.16, respectively. VCBM was titrated to a range of pHs using sterile HCl. qRT-PCR analysis of gene expression in WT BMDMs exposed to VCBM with different pHs as indicated, Nx-REC-CM or Hx-REC-CM for 12 h, (n = 3-6). *P < 0.05 vs VCBM_vehicle. Data are mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001 vs VCBM (PH = 7.55); ns, not significant. Statistical significance was determined by one-way ANOVA followed by Bonferroni test.

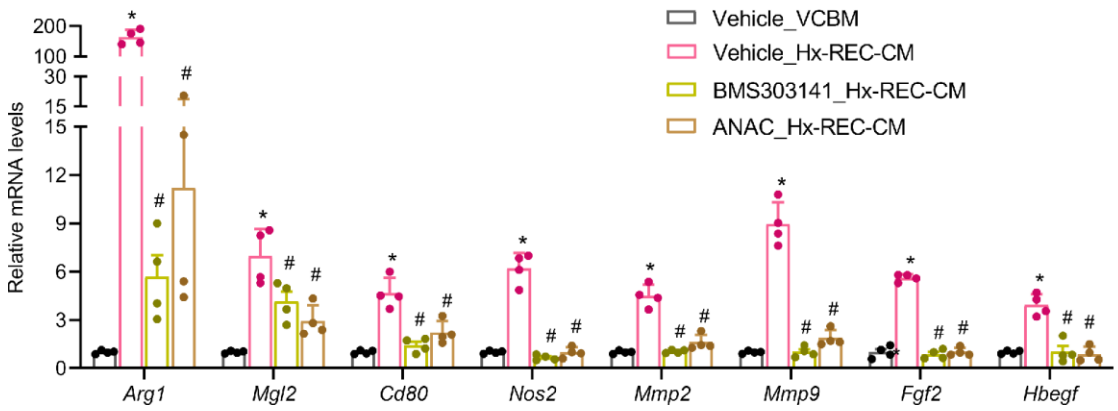


Fig. S19. Effect of histone acetylation inhibition on the Hx-REC-CM–induced expression of macrophage polarization markers and proangiogenic factors. BMDMs were pretreated with BMS303141 (10 μM) or Anacardic acid (ANAC) (20 μM) for 16 h, and then were stimulated with VCBM or Hx-REC-CM for an additional 12 h in the presence of BMS303141 and ANAC. qRT-PCR analysis of gene expression in BMDMs (n = 4). Data are mean ± SEM. *P < 0.05 vs vehicle _VCBM. $^{\#}P$ < 0.05 vs Hx-REC-CM_vehicle. Statistical significance was determined by one-way ANOVA followed by Bonferroni test.

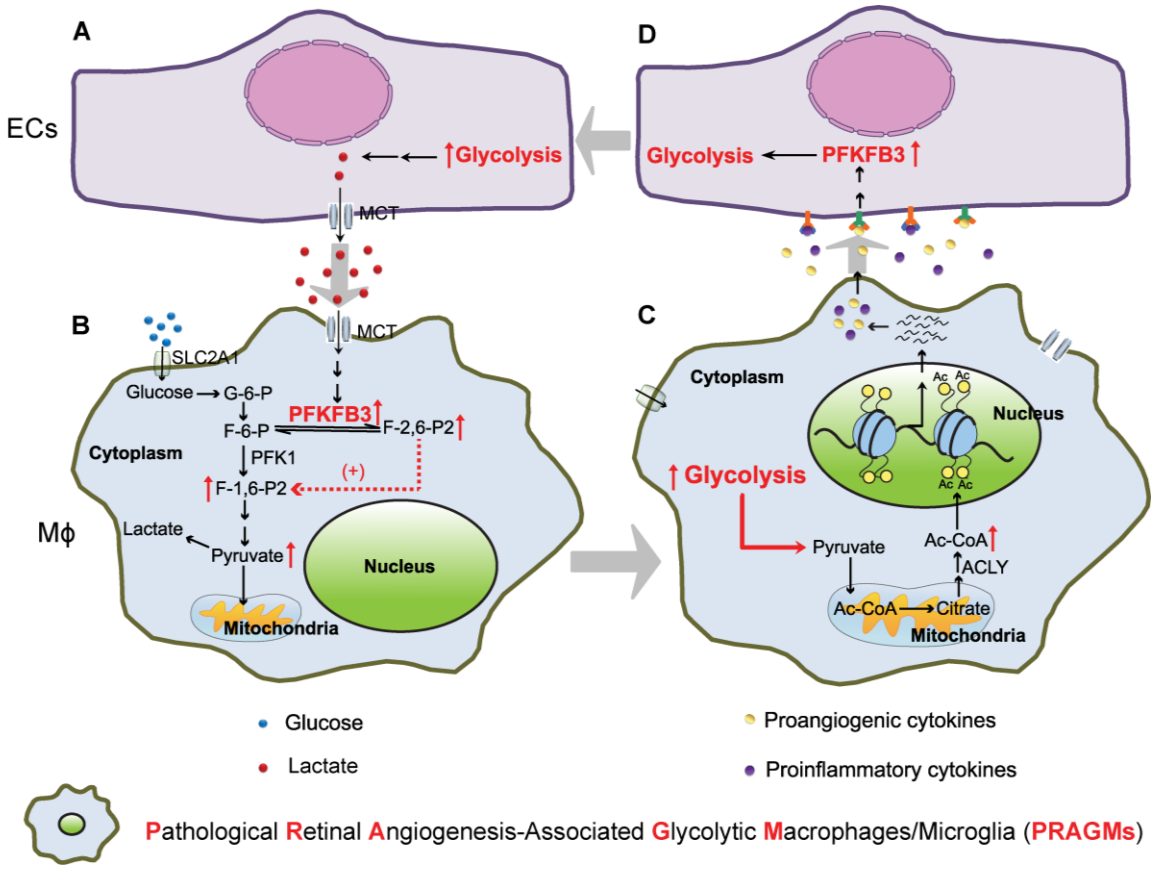


Fig. S20. Schematic diagram illustrating the proposed mechanisms for PFKFB3-driven glycolysis-mediated reciprocal activation between macrophages/microglia and ECs in the retinal angiogenic niche. (**A**) Retinal ECs are hyper-glycolytic and release large amount of glycolytic metabolites, especially lactate. Lactate released by ECs enters macrophages/microglia and elevates glycolysis of macrophages/microglia (**B**). Glycolytic products such as pyruvate are further processed in mitochondria to provide more citrate and Ac-CoA; the latter enhances histone acetylation and subsequently increases gene expression of proinflammatory and angiogenic cytokines (**C**). Eventually, these cytokines released from macrophages/microglia are able to elevate EC glyolysis and stimulate EC proliferation and sprouting (**D**).

Table S1. Primer sets used for qRT-PCR. (F: forward primer; R: reverse primer)

Target gene	Sequence
mouse *Rpl13a*	F:5'- GAGGTCGGGTGGAAGTACCA -3'
	R:5'- TGCATCTTGGCCTTTTCCTT -3'
mouse*18S ribosomal RNA*	F: 5'- CTTAGAGGGACAAGTGGCG -3'
	R: 5'- ACGCTGAGCCAGTCAGTGTA -3'
mouse *Slc2a1*	F: 5'- GCAGTTCGGCTATAACACTGG -3'
	R: 5'- GCGGTGGTTCCATGTTTGATTG -3'
mouse *Hk1*	F: 5'- AACGGCCTCCGTCAAGATG -3'
	R: 5'- GCCGAGATCCAGTGCAATG -3'
mouse *Hk2*	F: 5'- ATGATCGCCTGCTTATTCACG -3'
	R: 5'- CGCCTAGAAATCTCCAGAAGGG -3'
mouse *Gpi*	F: 5'- CTCAAGCTGCGCGAACTTTTT -3'
	R: 5'- GGTTCTTGGAGTAGTCCACCAG -3'
mouse *Pfkfb1*	F: 5'- TACGAAGCTCACACGCTATCT -3'
	R: 5'- GCCTCTCGTCGATACTGACCTA -3'
mouse *Pfkfb2*	F: 5'- ACATGCTCATGGGCTTCCTAT -3'
	R: 5'- GTTGAGGTAGCGTGTTAGTTTCT -3'
mouse *Pfkfb3*	F: 5'- GATCTGGGTGCCCGTCGATCACCG -3'
	R: 5'- CAGTTGAGGTAGCGAGTCAGCTTC -3'
mouse *Pfkfb4*	F: 5'- TCTGGATGCCGTACAGCAATG -3'
	R: 5'- GTGTCGGACAGTTAGTCATGC -3'
mouse *Aldoa*	F:5'- AGTCCACCGGAAGCATTGC -3'
	R:5'- CAGCCCCTGGGTAGTTGTC -3'
mouse *Gapdh*	F:5'- AGGTCGGTGTGAACGGATTTG -3'

	R:5'- GGGGTCGTTGATGGCAACA -3'
mouse *Pgk1*	F:5'- ATGTCGCTTTCCAACAAGCTG -3'
	R:5'- GCTCCATTGTCCAAGCAGAAT -3'
mouse *Eno1*	F:5'- TGCGTCCACTGGCATCTAC -3'
	R:5'- CAGAGCAGGCGCAATAGTTTTA -3'
mouse *Pdk1*	F:5'- GGACTTCGGGTCAGTGAATGC -3'
	R:5'- TCCTGAGAAGATTGTCGGGGA -3'
mouse *Ldha*	F:5'- ACATTGTCAAGTACAGTCCACAC -3'
	R:5'- TTCCAATTACTCGGTTTTTGGGA -3'
mouse *Ldhb*	F:5'- TGCGTCCGTTGCAGATGAT-3'
	R:5'- TTTCGGAGTCTGGAGGAACAA-3'
mouse *Cd80*	F:5'- ACCCCCAACATAACTGAGTCT -3'
	R:5'- TTCCAACCAAGAGAAGCGAGG -3'
mouse *Arg1*	F:5'- CAGAAGAATGGAAGAGTCAG -3'
	R:5'- CAGATATGCAGGGAGTCACC -3'
mouse *Fgf2*	F:5'- GCGACCCACACGTCAAACTA -3'
	R:5'- TCCCTTGATAGACACAACTCCTC -3'
mouse *Pdgfb*	F:5'- CATCCGCTCCTTTGATGATCTT -3'
	R:5'- GTGCTCGGGTCATGTTCAAGT -3'
mouse *Chi3l3*	F:5'- GGAGTAGAGACCATGGCACTGAAC -3'
	R:5'- GACTTGCGTGACTATGAAGCATTG -3'
mouse *Cxcl10*	F:5'- GAGCCTATCCTGCCCACG -3'
	R:5'- GGAGCCCTTTTAGACCTT -3'
mouse *Nos2*	F:5'- CAGCTGGGCTGTACAAACCTT -3'
	R:5'- CATTGGAAGTGAAGCGTTTCG -3'

mouse *Mgl1*	F:5'- CAGAATCGCTTAGCCAATGTGG -3'
	R:5'- TCCCAGTCCGTGTCCGAAC -3'
mouse *Mgl2*	F:5'- TTCAAGAATTGGAGGCCACT -3'
	R:5'- CAGACATCGTCATTCCAACG -3'
mouse *Retnla*	F:5'- CCCTGCTGGGATGACTGCTA -3'
	R:5'- TCCACTCTGGATCTCCCAAGA -3'
mouse *Irf4*	F:5'- TCCGACAGTGGTTGATCGAC -3'
	R:5'- CCTCACGATTGTAGTCCTGCTT -3'
mouse *Irf5*	F:5'- GGTCAACGGGGAAAAGAAACT -3'
	R:5'- CATCCACCCCTTCAGTGTACT -3'
mouse *Mmp2*	F:5'- CCTGGACCCTGAAACCGTG -3'
	R:5'- TCCCCATCATGGATTCGAGAA -3'
mouse *Pkm2*	F:5'- AGGATGCCGTGCTGAATG -3'
	R:5'- TAGAAGAGGGGCTCCAGAGG -3'
mouse *Arg2*	F:5'- TCCTCCACGGGCAAATTCC -3'
	R:5'- GCTGGACCATATTCCACTCCTA -3'
mouse *Il6*	F:5'- GTTCTCTGGGAAATCGTGGA -3'
	R:5'- TGTACTCCAGGTAGCTATGG -3'
mouse *Il10*	F:5'- GCTATGCTGCCTGCTCTTACT -3'
	R:5'- CCTGCTGATCCTCATGCCA -3'
mouse *Fizz1*	F:5'- CAAGACTATGAACAGATGGGCCT -3'
	R:5'- AGGAGATTGATGGGAGAGGACA -3'
mouse *Fgf1*	F:5'- CCCTGACCGAGAGGTTCAAC -3'
	R:5'- GTCCCTTGTCCCATCCACG -3'
mouse *Pgf*	F:5'- GTCTGCTGGGAACAACTCAACA -3'

	R:5'- CACCTCATCAGGGTATTCATCCA -3'
mouse *Hgf*	F:5'- ATGTGGGGGACCAAACTTCTG -3'
	R:5'- GGATGGCGACATGAAGCAG -3'
mouse *Hbegf*	F:5'- CGGGGAGTGCAGATACCTG -3'
	R:5'- TTCTCCACTGGTAGAGTCAGC -3'
mouse *Vegf*	F:5'- TCACCAAAGCCAGCACATAGGAGA -3'
	R:5'- TTTCTCCGCTCTGAACAAGGCTCA -3'
mouse *Mmp9*	F:5'- TTGAAGTCTCAGAAGGTGGAT -3'
	R:5'- GCAGGAGGTCGTAGGTCAC -3'
mouse *Mmp12*	F:5'- AAAGTGGGTTGTAGCATTGC -3'
	R:5'- AGAAGGCAGACCAGGACAC -3'
mouse *Il1b*	F:5'- TGTCTTGGCCGAGGACTAAGG -3'
	R:5'- TGGGCTGGACTGTTTCTAATGC -3'
mouse *Tnfa*	F:5'- ACGGCATGGATCTCAAAGAC -3'
	R:5'- AGATAGCAAATCGGCTGACG -3'
mouse *Tgfb1*	F:5'- ATGGTGGACCGCAACAAC -3'
	R:5'- CCAAGGTAACGCCAGGAA -3'
mouse *Cxcl12*	F:5'- CCAAGAGTACCTGGAGAAAGC -3'
	R:5'- AGTTACAAAGCGCCAGAGCA -3'
mouse *Cd163*	F:5'- GGTGGACACAGAATGGTTCTTC -3'
	R:5'- CCAGGAGCGTTAGTGACAGC -3'
mouse *Cd206*	F:5'- TCTTTGCCTTTCCCAGTCTCC -3'
	R:5'- TGACACCCAGCGGAATTTC -3'
mouse *Cd11c*	F:5'- TCGTTGGCCTCTAACGAGCTTTCT -3'
	R:5'- AGGATAACATGGAAGCACGGACCA -3'

Table S2. Primer sets used for ChIP. (F: forward primer; R: reverse primer)

mouse *Arg1*	F:5'- GTG AAC TGG ACG GAT GAA TAA -3'
	R:5'- CGA ATG GAA GAA TGA CAG AGT -3'
mouse *Retlna*	F:5'- GTG TGT GTG GTG TGT GTT A -3'
	R:5'- CTA CAT GAA GGT ACC TGG AAA G -3'
mouse *Mgl2*	F:5'- GCA GGA AGC AAA TGA AGA TAA G -3'
	R:5'- CAT ATT CCT CTT TCT AGA CCC TTC -3'
mouse *Hbegf*	F:5'- TGCAAGAGGGAGTACGGAAC -3'
	R:5'- CTGCGCCTACCACTGTTCC -3'
mouse *Nos2*	F:5'- TCCCTAGTGAGTCCCAGTTTTGA -3'
	R:5'- CTGGTCGCCCGTCCAAGG -3'
mouse *Fgf2*	F:5'- TCAGGACAGAGGTGCAGACAATC -3'
	R:5'- AATCTCCAGTCCCGTAGAGCACA -3'
mouse *Il1b*	F:5'- CTCAATGGACAGAATATCAACCAACA -3'
	R:5'- ACAGGACAGGTATAGATTCTTTCCTTTG -3'
mouse *Mmp2*	F:5'- CAA GCC AAG GGA TAG AGG ACA -3'
	R:5'- GCA AGG ATA ATC TGG AAA GGA GG -3'
mouse *Mmp9*	F:5'- GCCTGCTGGAGCTAGGGGTTTG -3'
	R:5'- GAGTGCAGCCTGGAGCCCATC -3'
mouse *Rpl13a*	F:5'- TTCCCCAAAACCATAGCCCC -3'
	R:5'- CCTGAGTCGAGTCCTCCTGT -3'

Data file S1. Raw data (provided as separate Excel file).