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Effect of selectively knocking down key metabolic genes in Müller glia on photoreceptor health

Running title: Glucose metabolism in Müller glia

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W.S. and M.C.G. wrote the paper; N.L.B., J.D. and J.B.H. edited the paper. W.S. and M.C.G. designed research; W.S., S.R.L., A.E.M., M.X.Y., V.P., R.Z., N.L.B. and C.L.R. performed research and analysed data. W.S., L.Z., J.D., and M.C.G contributed to funding acquisition. P.S., Y.H. and S.F. contributed to research materials.

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ABSTRACT

The importance of Müller glia for retinal homeostasis suggests that they may have vulnerabilities that lead to retinal disease. Here, we studied the effect of selectively knocking down key metabolic genes in Müller glia on photoreceptor health. Immunostaining indicated that murine Müller glia expressed insulin receptor (IR), hexokinase 2 (HK2) and phosphoglycerate dehydrogenase (PHGDH) but very little pyruvate dehydrogenase E1 alpha 1 (PDH-E1α) and lactate dehydrogenase A (LDH-A). We crossed Müller glial cell-CreER (MC-CreER) mice with transgenic mice carrying a floxed IR, HK2, PDH-E1α, LDH-A or PHGDH gene to study the effect of selectively knocking down key metabolic genes in Müller glia cells on retinal health. Selectively knocking down IR, HK2 or PHGDH led to photoreceptor degeneration and reduced electroretinographic responses. Supplementing exogenous L-serine prevented photoreceptor degeneration and improved retinal function in MC-PHGDH knockdown mice. We unexpectedly found that the levels of retinal serine and glycine were not reduced but, on the contrary, highly increased in MC-PHGDH knockdown mice. Moreover, dietary serine supplementation, while rescuing the retinal phenotypes caused by genetic deletion of PHGDH in Müller glial cells, restored retinal serine and glycine homeostasis probably through regulation of serine transport. No retinal abnormalities were observed in MC-CreER mice crossed with PDH-E1α- or LDH-A-floxed mice despite Cre expression. Our findings suggest that Müller glia do not complete glycolysis but use glucose to produce serine to support photoreceptors. Supplementation with exogenous serine is effective in preventing photoreceptor degeneration caused by PHGDH deficiency in Müller glia.

Key words: Müller glia, photoreceptor, glucose metabolism, glycolysis, serine metabolism.

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Main Points:

- Müller glia use glucose to produce serine to support photoreceptors.
- The lactate shuttle hypothesis may not reflect the metabolic relationship between Müller glia and photoreceptors in the retina.

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1. INTRODUCTION

The retina, one of the most metabolically active tissues in the body, meets its energy demands by extracting glucose and oxygen from the retinal and choroidal circulations. Retinal cells use glucose as the principal energy substrate to maintain the ion gradients across cell membranes, transport neurotransmitters at synapses and replenish photoreceptor outer segments, all of which are important for retinal health (Hurley, Lindsay, & Du, 2015; Toft-Kehler, Skytt, & Kolko, 2018).

Phototransduction occurs in the photoreceptor outer segments. Since photoreceptors shed around 10% of their outer segments every day, vertebrate photoreceptors need enough energy and metabolites to constantly renew outer segments. Disturbance of outer retinal metabolism is a potential cause of photoreceptor degeneration (Ivanovic et al., 2011; C. L. Poitry-Yamate, Poitry, & Tsacopoulos, 1995; Poitry, Poitry-Yamate, Ueberfeld, MacLeish, & Tsacopoulos, 2000; Punzo, Kornacker, & Cepko, 2009; Punzo, Xiong, & Cepko, 2012). Photoreceptor degeneration, including the loss of photoreceptor outer segments, is found in many blinding diseases such as age-related macular degeneration (Fisher & Ferrington, 2018), diabetic macular edema (Mori et al., 2016) and macular telangiectasia type 2 (Issa et al., 2013). The specific mechanisms which lead to photoreceptor degeneration in many retinal diseases are poorly understood.

Similar to the "Warburg effect" in cancer cells (Warburg, 1956), the outer retinal energy metabolism is dominated by aerobic glycolysis even when O_2 is abundant (Hurley et al., 2015; Ng et al., 2015). Previous studies indicate that the retina uses around 80-90% of glucose for aerobic glycolysis (Hurley et al., 2015; Toft-Kehler et al., 2018). Systemic inhibition of glycolysis induced photoreceptor degeneration (Liang et al., 2008). Genetic perturbations of glycolytic genes in rods resulted in shortening of outer segments (Chinchore, Begaj, Wu, Drokhlyansky, & Cepko, 2017), indicating that rod photoreceptors rely on glycolysis for the biogenesis of their outer segments. However, whether

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photoreceptors also require metabolic support from Müller glia to maintain their health remains largely unexplored.

The close anatomic relationship between Müller glia and photoreceptors indicates that Müller glia may provide important metabolites to support photoreceptors (Hurley et al., 2015; Reichenbach & Bringmann, 2013). Müller glial microvilli form a dense network to ensheath photoreceptor inner segments where mitochondria are concentrated. It has been proposed that Müller glia function as a "astrocyte-neuron-lactate shuttle (ANLS)" by which Müller glia metabolize glucose to produce lactate to fuel photoreceptors (C. L. Poitry-Yamate et al., 1995; Reichenbach & Bringmann, 2013; Winkler, Arnold, Brassell, & Puro, 2000). This concept has been challenged by recent observations that Müller glia do not express any isoform of pyruvate kinase, the enzyme that catalyzes the final step of glycolysis (Agarwal et al., 2007; Hurley et al., 2015; Kanow et al., 2017; Lindsay et al., 2014). A better understanding how Müller glia provide metabolic support for photoreceptors may provide new insights into the mechanisms of photoreceptor degeneration in conditions such as macular telangiectasia type 2, a poorly understood disorder characterized by loss of Müller glia and photoreceptor degeneration (Issa et al., 2013; Powner et al., 2010; Powner et al., 2013).

We have previously generated transgenic mice in which a 3kb fragment of the retinaldehyde-binding protein 1 (Rlbp1) was used as a cell-specific promoter to drive Müller glial cell-specific gene targeting (here termed as MC-CreER mice) (Shen et al., 2012; Vazquez-Chona, Clark, & Levine, 2009). We found that selective ablation of Müller glia by crossing MC-CreER mice with a transgenic line carrying an attenuated form of the diphtheria toxin fragment A gene leads to photoreceptor degeneration, indicating that Müller glia are critical for photoreceptor survival (Shen et al., 2012). Here, we studied the effect of selectively knocking down key metabolic genes in Müller glia on photoreceptor health by crossing MC-CreER mice with transgenic mice carrying floxed insulin receptor (IR), hexokinase 2 (HK2), pyruvate dehydrogenase E1α (PDH-E1α), lactate dehydrogenase

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A (LDH-A) or phosphoglycerate dehydrogenase (PHGDH) gene. Since we found that Müller glia may use glucose to produce serine to support photoreceptors, we also studied the effect of supplementing exogenous L-serine on photoreceptor degeneration caused by knocking down PHGDH in Müller glia.

2. Materials and Methods

2.1 Animals

This study was conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research and approved by the University of Sydney Animal Ethics Committee. All animals were housed in pathogen-free facilities on a 12hr light/dark cycle and were fed a standard rodent chow. Transgenic mice expressing inducible Cre recombinase under control of the retinaldehyde-binding protein 1 (Rlbp1) promoter were used to manipulate gene expression in Müller glial cells (here termed as MC-CreER mice) as described previously (Shen et al., 2012). MC-CreER mice were crossed into Rosa-LacZ reporter mice (Jackson laboratories, ME; Stock# 003474) to confirm the Müller glia-specific gene targeting as described previously (Shen et al., 2012). To analyse the Cre induced LacZ expression, offspring carrying the Rlbp1 promoter and the LacZ reporter gene received daily intraperitoneal injection of TMX (3mg per dose, dissolved in sunflower oil) at 6-8 weeks of age for 4 days. To localize the LacZ reporter gene expression, eyes were enucleated 4 days after TMX treatment and fixed in 0.25% glutaraldehyde for 1 hour. Cryosections were produced for X-gal (5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside) staining to detect β-gal activity as described previously (Shen et al., 2012).

We next crossed MC-CreER mice with transgenic mice carrying a floxed metabolic gene including IR, HK2, LDH-A, PDH-E1α or PHGDH to study the effect of selectively knocking down key metabolic genes in Müller glia on retinal health. Mice that harbor loxP sites flanking exon 4 of the IR gene or exon 8 of the PDH-E1α gene, were purchased from Jackson Laboratories ($IR^{FL/FL}$ mice: JAX

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stock# 006955. PDH-E1α$^{FL/FL}$ mice: JAX stock#017443). Mice that harbor LoxP sites at intron 3 and intron 10 of the HK2 gene were obtained from the European Mouse Mutant Archive (HK2$^{FL/FL}$ mice, deposited by Dr Eija Pirinen). Mice that harbor loxP sites flanking exon 2 of the lactate dehydrogenase A (LDH-A) or exon 3 of the PHGDH gene, were generated as described previously (Xie et al., 2014; Yang et al., 2010). We conducted genotyping using tail DNAs to identify mice carrying the Rlbp1-CreER construct and homozygous for the floxed genes (Table 1). Mice carrying the Rlbp1-CreER construct but not a floxed gene were used as controls. We performed daily intraperitoneal injection of tamoxifen (100mg/kg, dissolved in sunflower oil) at 1-2 months age for 4 consecutive days to induce Cre expression as described previously (Shen et al., 2012; Shen et al., 2014; Shen, Zhu, Lee, Chung, & Gillies, 2013). All transgenic mice used in this study have a C57BL/6J × CBA background.

2.2 Genotyping transgenic mice

Transgenic animals were screened by PCR using two sets of primers unique to the mouse Rlbp1 gene to identify mice that carry the Rlbp1-CreER construct (Shen et al., 2012). The primers that we used to genotype the Rlbp1-CreER construct and floxed genes are listed in Table 1.

2.3 Primary culture of mouse Müller cells and confirmation of target gene knockdown *in vitro*

We isolated Müller glia from MC-CreER mice (controls) and those crossed with mice carrying floxed IR or HK2 gene (hereafter referred to as MC-IRKO or MC-HKKO) at one months of age. Briefly, retinas were collected from 6-8 eyeballs and placed in Dulbecco's modified Eagle's medium (DMEM, Gibco) without fetal bovine serum (FBS). The collected eyes were kept in the dark at 4 °C for overnight. The next day, retinas were isolated and digested with pre-warmed TrypLE (ThermoFisher) solution at 37 °C for 45 min. After digestion, retinas were cut into small pieces (around 1×1 mm) and transferred into T25-cell culture flasks (Corning) for culturing in DMEM supplemented with 10% FBS (Sigma Aldrich) and 1% penicillins-streptomycin (Invitrogen). We

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performed immunocytochemistry on Müller glia at passage one (P1) to confirm their identity using a panel of antibodies (Abs) against Müller glial markers including glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS). Knockdown of IR or HK2 *in vitro* was conducted by treating Müller glia at P1 in DMEM containing 1μM 4-hydroxy tamoxifen (4-OHT, the active metabolite of tamoxifen) for 24 hrs. Changes in expression of target proteins were quantitatively analyzed by Western blots.

2.4 Cellular uptake of fluorescently-labelled glucose (2-NBDG) in Müller glia isolated from MC-CreER (control) and MC-IRKO mice

The cellular uptake of 2-NBDG was studied using an assay kit from Abcam (Cat# ab235976). Briefly, primary Müller glia were isolated from MC-CreER and MC-IRKO mice as described above. The isolated Müller glia were seeded into a 96-well plate at a density of $1x10^4$ cells/well in DMEM media containing 25mM glucose, 10% FBS and 1% penicillins-streptomycin until they reached 80% confluence. Cells were then treated with a DMEM medium containing 1 μM of 4-hydroxytamoxifen (4-OHT), 5 mM glucose, 10% FBS and 1% penicillins-streptomycin for overnight. The next day, cells were washed with PBS and incubated in 100 μl of DMEM media containing 100 μg/ml of 2-NBDG and 10% FBS for 2 hours. Cells treated with 4-OHT and incubated in media without 2-NBDG were used as blank controls. The plate was centrifuged at 400 x *g* at room temperature for 5 minutes and supernatants were aspirated. The cellular uptake of 2-NBDG was measured using a Tecan Safire2 fluorescence multi-well plate reader (Tecan, Switzerland) at excitation/emission= 485/535 nm. At the end of the treatment, cells were lysed with 30 μl of RIPA buffer containing proteinase inhibitors at 4°C for 30 mins. The results were expressed as fluorescent reading per mg of cellular protein.

2.5 Immunostaining of frozen sections and retinal wholemounts

Eyes were enucleated, fixed in 4% paraformaldehyde and embedded in optimal cutting temperature compound. Frozen sections were incubated with Abs (Table 2) at +4°C overnight, the bound Abs were detected with corresponding secondary Abs conjugated with Alexa Fluor 488 or 594 (1:1000; Invitrogen) using a confocal laser scanning microscope.

For immunostaining on retinal wholemounts, dissected eye cups were fixed in 4% paraformaldehyde for one hour and then placed in PBS at +4°C. The next day, retinas were isolated, permeabilized and incubated in a solution containing peanut-agglutinin (PNA) conjugated with Alexa Fluor 488 (10µg/ml, Invitrogen) or an Ab against cone arrestin (1:500, Millipore#AB15282) for cone photoreceptors or ionized calcium binding adaptor molecule 1 (Iba1, 1:400, Wako#019-19741) for microglia. Retinal wholemounts were counterstained with Hoechst and confocal images were photographed from the posterior retina around the optic nerve and quantitatively analysed as previously described (Shen et al., 2012; Shen et al., 2014; Shen et al., 2013).

2.6 Western blots

Proteins were extracted from cultured primary Müller glia and the whole retinas and their concentrations determined by detergent compatible protein assay. Equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis then transferred to a polyvinylidene difluoride (PVDF) membrane. The PVDF membranes were probed with primary Abs (Table 2) and then incubated with secondary Abs conjugated with horseradish peroxidise. Protein bands were visualised using the G:Box BioImaging system and quantified using the GeneTools image scanning and analysis package. Protein expression was normalised to α/β-tubulin (rabbit polyclonal, 1:2000; Cell Signalling #2148), which served as a loading control.

2.7 Colour fundus photography and electroretinography (ERG)

Retinal photography was performed using a Micron IV mouse fundus camera (Phoenix Research Laboratories, Pleasanton, CA, USA). Full-field scotopic ERG was performed to assess retinal function as previously described (Moxon-Lester, Takamoto, Colditz, & Barnett, 2009; Shen et al., 2012). Briefly, mice were dark adapted overnight, anesthetized by intraperitoneal injection of a cocktail consisting of ketamine (50mg/kg, Parnell Laboratories, New Zealand) and medetomidine (1mg/kg Pfizer Animal Health, USA) and the pupils were dilated with 1% tropicamide and 2.5% phenylephrine. Flash stimuli for mixed rod and cone responses were provided by a photographic flash unit (Mecablitz 60CT4, Metz, Zirndorf, Germany) or the Phoenix Ganzfeld ERG to study electroretinographic responses over a range of stimulus intensities. Photopic ERGs were recorded using the Phoenix Ganzfeld ERG system immediately after scotopic recordings as described previously with slight modifications (Rodriguez et al., 2018). In brief, the mouse eye was exposed to constant green light (504 nm) at a light intensity of 1.9 log cd/m^2 for 10 min to saturate rod photoreceptors. Three series of recordings were then performed with green light flashes at 1.3, 1.9 log cd/m^2 (both with 5 flashes, 1ms flash duration, 20 s delay interstimulus interval) and 3.1 log cd/m^2 (3 flashes, 1ms flash duration, 60 s delay interstimulus interval) to study the function of M-cone photoreceptors. The a-wave amplitude was measured from the baseline to the trough of the a-wave response and the b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave. Mice received an intraperitoneal injection of atipamezole (2.5mg/kg, Pfizer Animal Health, USA) immediately after procedures of ERG, to reverse the anesthetic.

2.8 Supplementation of L-serine into drinking water

As we found that selectively knocking down PHGDH in Müller glia led to photoreceptor degeneration, we also tested whether supplementing exogenous L-serine protects photoreceptors from degeneration in MC-CreER:PHGDH$^{Fl/FL}$ mice (hereafter referred to as MC-PHGDH KO mice). We added L-serine (Sigma, Cat# S4500) into drinking water to produce a final concentration of 2.4mg/ml. Mice were provided with drinking water enriched with L-serine or un-supplemented for 4

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weeks, starting immediately after the last tamoxifen injection. Water bottles were changed once a week until termination of the study.

Analyses of retinal metabolites using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS)

Changes in metabolites in the glycolytic pathway, serine and glycine were analyzed with GC MS as described previously (Du, Linton, & Hurley, 2015; Grenell et al., 2019; Yam et al., 2019). Briefly, retinas were isolated within one minute after euthanasia by cervical dislocation and snap frozen in liquid nitrogen. Samples were stored at -80°C for analysis of metabolites in glycolysis and the TCA cycle using mass spectrometry. Retinas were homogenized with 20µl of 80% methanol, placed on dry ice for 30 min and centrifuged for 15 min at 15000rpm, 4℃. Supernatants were transferred to a glass insert and mixed with 0.1 mM myristic-d_{27} acid as an internal standard and then dried under vacuum at 4℃. For GC-MS, the dried metabolites were derivatized with methoxyamine hydrochloride N-tert-butyldimethylsilyl- N-methyltrifluoroacetamide before analyzed by a 7890/5977B GC/MS system (Agilent Technologies) with an Agilent DB-5MS column as previously described (Grenell et al., 2019). LC-MS was performed with a Shimadzu LC Nexera X2 UHPLC coupled with a QTRAP 5500 LC MS (AB Sciex) as previously described (Grenell et al., 2019; Wang et al., 2019). An ACQUITY UPLC BEH Amide analytic column (2.1 X 50 mm, 1.7 µm, Waters) was used for chromatographic separation. The mobile phase was (A) water with 10 mM ammonium acetate (pH 8.9) and (B) acetonitrile/water (95/5) with 10 mM ammonium acetate (pH 8.2). The gradient elution was 95%–61% B in 6 min, 61%–44% B at 8 min, 61%–27% B at 8.2 min, and 27%–95% B at 9 min. The column was equilibrated with 95% B at the end of each run. The collision gas was N2. The ion source conditions in positive and negative mode were as follows: curtain gas (CUR) = 25 psi, collision gas (CAD) = high, ion spray voltage (IS) = 3800/- 3800 V, temperature (TEM) = 500°C, ion source gas 1 (GS1) = 50 psi, and ion source gas 2 (GS2) = 40 psi. Each metabolite was tuned with standards for optimal transitions and ^{13}C-nicotinic acid (Toronto

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Research Chemicals) was used as the internal standard. The extracted MRM peaks were integrated using MultiQuant 3.0.2 software (AB Sciex).

2.9 Statistical analysis

Results are expressed as mean ± SEM. SPSS 17.0 for Windows version software was used for the statistical analysis. Data were analyzed using unpaired student t-test and one-way ANOVA with a post hoc Bonferroni's correction to analyze significant differences among groups. A p value <0.05 was regarded as statistically significant.

3. RESULTS

3.1 Knocking down IR in Müller glia led to photoreceptor degeneration and Müller cell gliosis

Immunofluorescent studies indicated that IRα was expressed in the retinal pigment epithelium, photoreceptor inner segments, the outer plexiform layer, the inner nuclear layer, the inner plexiform layer and the ganglion cell layer (Figure 1). Müller glia were identified by an antibody to cellular retinaldehyde-binding protein (CRALBP, Figure 1b). Double labelling for IRα and CRALBP indicated that Müller glia expressed IRα (Figure 1c-f, arrows).

MC-CreER mice were crossed with Rosa-LacZ reporter mice (Maxwell, Maxwell, & Glode, 1987; Wu, Wu, & Capecchi, 2006) to study the Müller glia-specific gene targeting (Figure 2a). TMX given at 6w of age induced LacZ expression in cells with the typical morphology of Müller glia showing radial processes extending from the inner limiting membrane (ILM) to the outer limiting membrane (OLM) and cell bodies in the inner nuclear layer (INL) (Figure 2a, arrows). No LacZ expression was observed in the retinal pigment epithelium (RPE) or any other retinal cells (Figure 2a).

We next crossed MC-CreER mice with $IR^{FL/FL}$ mice to study the effect of selectively knocking down IR in Müller glia on retinal health. We studied changes in IR *in vitro* since the virtual universal

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expression of IR by various populations of retinal cells makes whole retinal assays relatively insensitive to Müller glia-specific changes. We isolated Müller glia from MC-CreER (control) and MC-IRKO mice and confirmed that the isolated cells expressed Müller glial markers including GFAP and GS (Figure 2b,c) and the two subunits of insulin receptors (IRs) including IRα and IRβ (Figure 2d,e). We next treated the isolated Müller glia with 1μM 4-OHT, the active metabolite of tamoxifen, for 24 hrs. Western blot analysis indicated that 4-OHT treatment significantly reduced the expression of IRα and IRβ in Müller glia isolated from MC-IRKO mice compared to those isolated from control mice (Figure 2f). We also studied the effect of knocking down IR on uptake of fluorescently labelled glucose (2-NBDG) in Müller glia isolated from MC-CreER control and MC-IRKO mice (Figure 2g). We found that treatment of Müller glia isolated from MC-IRKO mice with 1μM 4-OHT resulted in significant reduction in uptake of 2-NBDG compared with control Müller glia receiving the same treatment (Figure 2g).

We conducted double label immunohistochemistry (IHC) on frozen retinal sections to study the effects of knocking down IR in Müller glia on photoreceptor health (Figure 3a-h). The normal Müller glia expressed IRα regularly (Figure 3a1,a2), aligning noticeably with PNA-stained photoreceptor apical processes (Figure 3b-d). Immunostaining for CRALBP and GFAP indicated that 4 doses of TMX in MC-IRKO mice induced depletion of IRα in Müller glia (Figure 3e1,e2; arrows). Double-label IHC indicated that loss of IR in Müller glia was accompanied by loss of photoreceptor apical processes in the corresponding region (Figure 3f-h). Knocking down IR in Müller glia also caused reactive Müller cell gliosis as evidenced by increased expression of GFAP but immunostaining for CRALBP did not show obvious loss of Müller glia (Figure 3i-l).

Western blots were used to study changes in photoreceptors and Müller glia 4 weeks after knocking down IR (Figure 3m,n). The levels of red/green opsin and recoverin were significantly reduced

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(Figure 3m), with significant upregulation of GFAP while the level of GS expression remained unchanged in MC-IRKO mice compared with control mice (Figure 3n).

3.2 Knocking down HK2 in Müller glia also resulted in photoreceptor degeneration

Immunostaining for HK2 indicated that HK2 was expressed in photoreceptor inner segments, the outer plexiform layer, the inner nuclear layer and the ganglion cell layer (Figure 4a). Double-label immunostaining for HK2 and CRALBP to mark Müller glia indicated that Müller glia expressed HK2 (Figure 4c-f, arrows).

As described above for knocking down IR in Müller glia, we crossed MC-CreER mice with $HK2^{FL/FL}$ mice to study the effect of knocking down HK2 in Müller glia on photoreceptor health. Immunocytochemistry confirmed that primary Müller glia expressed HK2 (Figure 4j-m). Western blot analysis indicated that 4-OHT treatment caused significant reduction in HK2 expression in MC-HKKO mice compared with those isolated from MC-CreER mice (Figure 4n).

TMX treatment in MC-HKKO mice resulted in reduced HK2 expression in the inner nuclear layer when compared with MC-CreER mice (Figure 5a-e). The reduced expression of HK2 in Müller glia was more obvious in higher power images after double-labelling the retina for HK2 and CRALBP (Figure 5f1,f2). Double-label IHC for HK2 and PNA indicated that loss of HK2 in Müller glia was accompanied by loss of photoreceptor apical processes in the corresponding region (Figure 5g-l). Western blots indicated that knocking down HK2 in Müller glia caused significant reduction in photoreceptor-associated proteins including Gαt and IRBP (Figure 5k), along with overexpression of GFAP while the level of GS expression remained unchanged (Figure 5l), suggesting that, similar to knocking down of IR, knocking down HK2 in Müller glia also caused photoreceptor degeneration and reactive retinal gliosis.

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3.3 Müller glia expressed little PDH-E1α and LDH-A and there were no retinal abnormalities in MC-CreER mice crossed with PDH-E1α$^{FL/FL}$ or LDH-A$^{FL/FL}$ mice

Glycolysis involves nine distinct enzymatic reactions through which glucose is converted to pyruvate. PDH-E1α is a key element in the PDH complex which converts pyruvate to acetyl-CoA that is used by the tricarboxylic acid (TCA) cycle. LDH-A and LDH-B catalyze the inter-conversions of pyruvate and L-lactate with concomitant inter-conversion of NADH and NAD^+. A previous study indicates that the direction of flux is determined by reaction thermodynamics (i.e. Keq and levels of lactate, pyruvate, NADH, NAD+ and pH) but not by isoform-dependent kinetic parameters or regulation (Quistorff & Grunnet, 2011). We found that PDH-E1α was strongly expressed in the RPE, the photoreceptor inner segments, the outer plexiform layer, the inner nuclear layer, the inner plexiform layer and the ganglion cell layer (Figure 6a-f). LDH-A was expressed in photoreceptor inner segments, some cells in the inner nuclear layer and the ganglion cell layer but not in the RPE (Figure 6g-l). However, high power microscopy of the inner nuclear layer indicated that Müller glia expressed very little PDH-E1α (Figure 6d-f, arrows) or LDH-A (Figure 6j-l, arrows).

We crossed MC-CreER mice with PDH-E1α$^{FL/FL}$ or LDH-A$^{FL/FL}$ mice to study whether there were retinal changes after treating the offspring with tamoxifen (Figure 7). We did not observe morphological evidence of photoreceptor damage 4 weeks after TMX treatment in MC-CreER:PDH-E1α$^{FL/FL}$ (Figure 7b) or MC-CreER:LDH-A$^{FL/FL}$ mice (Figure 7c) when compared with MC-CreER mice (Figure 7a), although immunostaining confirmed that Cre was expressed in Müller glia in these mice (Figure 7d-i). Western blot analysis indicated that the levels of expression of photoreceptor-associated proteins including Gαt and IRBP and the Müller cell gliotic marker GFAP, did not change in MC-CreER mice crossed with PDH-E1α$^{FL/FL}$ (Figure 7j) or LDH-A$^{FL/FL}$ mice (Figure 7k). Metabolic analysis of metabolites using mass spectrometry indicated that there were no significant changes in glycolytic metabolites in the retina of MC-IRKO and MC-HKKO mice compared with MC-CreER controls (Supplementary Figure 1).

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3.5 Photoreceptor degeneration in MC-IRKO and MC-HKKO mice was accompanied by impaired electroretinographic responses

Staining of retinal wholemounts with fluorescently labelled PNA indicated that there was a significant degeneration of photoreceptor apical processes in MC-IRKO and MC-HKKO mice but not in MC-CreER mice crossed with PDH-E1α$^{FL/FL}$ or LHD-A$^{FL/FL}$ mice (Figure 8a-f). Electroretinography conducted 4 weeks after TMX injection found significant reduction in the amplitudes of a and b waves of scotopic ERG in MC-IRKO and MC-HKKO mice after stimulating the retina with various intensities of green light (Figure 8g-h).). However, we only detected a significant reduction in the amplitude of b wave response of photopic ERG in these mice after stimulation with the maximum intensity of green light (Figure 8i). These results indicate that knocking down IR or HK2 may affect rods more severely than cones.

3.6 Knocking down PHGDH in Müller glia also led to photoreceptor degeneration and impaired retinal function

Since we found that Müller glia expressed little PDH-E1α and LDH-A and there was no retinal abnormality in MC-CreER mice crossed with PDH-E1α$^{FL/FL}$ or LDH-A$^{FL/FL}$ mice, we next studied whether Müller glia use another metabolic pathway to support photoreceptors. We found that Müller glia strongly expressed phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme which catalyzes the first step in the *de novo* synthesis of serine and glycine (Figure 9a1-a3). Crossing MC-CreER mice with PHGDH-floxed mice led to successful knockdown of PHGDH, as revealed by immunostaining of retinal sections (Figure 9b,c) and western blots using retinal proteins (Figure 9d). Results from PNA staining and colour fundus photography indicated that knocking down PHGDH in Müller glia led to disruption of photoreceptor apical processes (Figure 9b,c) and the development of yellowish deposits in the fundus (Figure 9e,f). Measurements of scotopic ERG indicated that both a-

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and b-wave responses were significantly attenuated 4 weeks after knocking down PHGDH in Müller glia (Figure 9g,h).

IHC for cone arrestin found that knocking down IR, HK2 or PHGDH led to reduced number of cone arrestin-positive cells and decreased thickness of the outer nuclear layer (ONL) but only knockdown of PHGDH resulted in statistically significant changes compared with MC-CreER control mice, indicating that knocking down PHGDH produced more profound effect on cone photoreceptor health than knockdown of IR or HK2 (Supplementary Figure 2).

3.7 L-serine treatment prevented photoreceptor degeneration in MC-PHGDH knockdown mice

We next studied whether supplementing exogenous L-serine prevents photoreceptor degeneration in MC-PHGDH KO mice (Figures 10, 11). Immunostaining of retinal wholemounts showed significant reduction in cone arrestin-stained cone photoreceptor outer segments 4 weeks after tamoxifen treatment in MC-PHGDH KO mice while supplementation of exogenous L-serine in drinking water significantly prevented photoreceptor degeneration (Figure 10a-c,g).

We also performed immunostaining for Iba1 on retinal wholemounts to examine microglial infiltration in the outer retina. Microglia were not detected in the outer retina of control mice (Figure 10d). Photoreceptor damage resulting from knocking down PHGDH in Müller glia was associated with microglial infiltration in the outer retina (Figure 10e) while supplementation of exogenous L-serine significantly reduced microglial infiltration (Figure 10f,h).

Western blot results indicated that knockdown of PHGDH in Müller glia significantly reduced the expression of photoreceptor-associated proteins, including photoreceptor G protein transducin subunit α (Gαt) and interphotoreceptor retinoid binding protein (IRBP), along with increased expression of retinal stress markers including GFAP and heat-shock protein 60 (Hsp60) (Figure

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11a,b). Consistent with our observations that supplementation of exogenous L-serine prevented the loss of cone arrestin-stained photoreceptor outer segments (Figure 10a-c,g), Western blot results indicated that supplementing L-serine prevented the loss of Gαt and IRBP and reduced the levels of retinal stress markers including GFAP and Hsp60 (Figure 11a,b). ERG responses indicated that supplementation of exogenous L-serine into drinking water also significantly improved the amplitudes of both a- and b-waves in MC-PHGDH KO mice (Figure 11c,d).

We performed mass spectrometry to study changes in the levels of retinal serine and glycine in MC-CreER mice and MC-PHGDH KO mice receiving normal drinking water or L-serine supplement for 8 weeks (Figure 12). Surprisingly, we found that the retinal levels of serine and glycine were dramatically increased but not decreased after knocking down PHGDH in Müller glia, while treatment with L-serine reduced their levels to those of normal control mice (Figure 12a,b). Double label IHC for PHGDH and Iba1 indicated that loss of PHGDH in Müller glia led to infiltration of activated microglia and macrophages, both of which strongly expressed PHGDH (Figure 12c-h). The increased serine and glycine after knocking down PHGDH might be partially attributed to the infiltration of activated microglia and macrophages.

Since RPE cells also strongly express PHGDH, which potentially makes them an alternative source for serine and glycine after knocking down PHGDH in Müller glia, we next studied changes in sideroflexin 1 (SFXN1), a mitochondrial serine transporter (Kory et al., 2018), in the retina of MC-PHGDH KO mice receiving normal drinking water or treated with L-serine (Supplementary Figures 3 and 4). Double-label IHC found that neither Müller glia nor RPE cells expressed SFXN1 (Supplementary Figure 3). Double label IHC for PHGDH and SFXN1 indicated that loss of PHGDH in Müller glia resulted in increased immunostaining of SFXN1 in photoreceptor inner segments while L-serine treatment reduced SFXN1 immunoreactivity in this structure (Supplementary Figure 4).

4. DISCUSSION

We have identified a mechanism by which Müller glia may use glucose to produce serine to support photoreceptors to maintain the retinal health. We provide *in vivo* evidence that the ANLS hypothesis may not accurately reflect the metabolic relationships between Müller glia and photoreceptors in the mouse retina. We found that Müller glia expressed genes for IR, HK2 and PHGDH, selective knockdown of which specifically in Müller glia led to photoreceptor degeneration. Supplementation of exogenous L-serine into drinking water effectively prevented photoreceptor degeneration in MC-PHGDH KO mice. Our results support a hypothesis that Müller glia may use glucose to produce serine to support photoreceptors (Figure 13) (Sinha, Ikelle, Naash, & Al-Ubaidi, 2020).

Insulin, IRs and insulin-sensitive glucose transporters are required for insulin-stimulated glucose metabolism in the brain (Bingham et al., 2002; Clarke, Mudd, Boyd, Fields, & Raizada, 1986; Hopkins & Williams, 1997; McCall et al., 1997; Mueckler, 1994; Ngarmukos, Baur, & Kumagai, 2001; Pardridge, Eisenberg, & Yang, 1985). IRs have been found throughout the human brain (Hopkins & Williams, 1997) including the endothelium of the blood-brain barrier (BRB), thus allowing receptor-mediated active transport of insulin and glucose into the brain (Pardridge et al., 1985). Insulin can be produced by neuronal cells in response to stimulation via potassium and calcium ions (Clarke et al., 1986). However, most insulin in the brain is thought to originate from systemic circulation. Insulin can cross the BRB, penetrating to the circumventricular organs including the arcuate and ventromedial nuclei of the hypothalamus (Clarke et al., 1986). Insulin-sensitive glucose transporters (GLUTs), including GLUT1 and GLUT 4, have been found on the endothelium of the BRB and on glial cells in various brain studies (McCall et al., 1997; Mueckler, 1994; Ngarmukos et al., 2001). More importantly, it has been reported that suppression of endogenous insulin production in humans leads to a significant reduction in global brain glucose uptake as measured by 18-fluorodeoxyglucose and positron emission tomography, suggesting that

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basal insulin has a role in regulating global glucose uptake in the human brain (Bingham et al., 2002).

The importance of glucose metabolism in Müller glial cells for retinal health is not well defined. IRs are widely expressed in the retina including the nerve fibre layer, ganglion cells, retinal neurons in the inner nuclear layer, Müller glial cells, inner segments of rods and cones and the retinal pigment epithelium (Naeser, 1997). Among various isoforms of GLUTs, GLUT1 is the major isoform that is highly expressed in the retinal pigment epithelium, the inner segments of photoreceptors, Müller glial cells, the inner plexiform layer, vascular endothelial and ganglion cells (Kanow et al., 2017; Kumagai, Glasgow, & Pardridge, 1994). Our current study confirmed that IRs are expressed in Müller glial cells (Figure 1, a-f). More importantly, we provided evidence that selectively knocking down insulin receptors in Müller glial cells led to reduced uptake of fluorescently labelled glucose *in vitro* (Figure 2, d-g) and photoreceptor degeneration *in vivo* (Figure 3, a-h, m; Figure 8, b, f-i). These results together suggest that insulin receptor-mediated glucose metabolism in Müller glial cells is importance for the maintenance of retinal homeostasis.

There has been a debate on whether Müller glia function like astrocytes in the ANLS to support photoreceptors (Hurley et al., 2015; Toft-Kehler et al., 2018). Initial studies conducted in avascular guinea pig retinas suggested a lactate shuttle between Müller glia and photoreceptors (C. L. Poitry-Yamate et al., 1995; C. L. Poitry-Yamate & Tsacopoulos, 1992; Poitry et al., 2000). The investigators compared the production of lactate from isolated Müller glia *versus* a complex containing both Müller glia and photoreceptors and found that the Müller glia-photoreceptor complexes released less lactate into the medium than what was released from Müller glia alone (C. L. Poitry-Yamate et al., 1995; C. L. Poitry-Yamate & Tsacopoulos, 1992; Poitry et al., 2000). They reasoned that less lactate released from the Müller glia-photoreceptor complexes was because lactate produced by Müller glia was shuttled to photoreceptors for oxidative phosphorylation. However, recent studies have reported that

Müller glia lack key glycolytic enzymes such as the M1 and M2 isoforms of pyruvate kinase (Kanow et al., 2017; Lindsay et al., 2014; Rueda et al., 2016), indicating that Müller glia may not be able to catalyze the conversion of phosphoenolpyruvate to pyruvate in the final step of glycolysis. A recent study also found that Müller glia express low levels of enzymes involved in oxidative phosphorylation (Rueda et al., 2016). Consistent with these findings, we found that Müller glia express very little LDH-A and PDH-E1α, the two enzymes that catalyze the conversion of pyruvate to lactate and acetyl-CoA respectively (Figure 6), indicating that Müller glia may not be able to produce much lactate and acetyl-CoA to fuel photoreceptors. Three independent groups have reported that photoreceptors express little LDH-B, the enzyme which is also involved in the inter-conversions between lactate and pyruvate (Casson et al., 2016; Chinchore et al., 2017; Rueda et al., 2016). We did not observe any retinal abnormalities in MC-CreER mice crossed with LDH-A$^{FL/FL}$ (Figure 7c,k; Figure 8e,f) although Cre was expressed in Müller glia in these mice (Figure 7g-i). These collective observations suggest that Müller glia have limited ability to complete aerobic glycolysis and that there is no lactate shuttle from Müller glia to photoreceptors. We also found no abnormalities in MC-CreER mice crossed with PDH-E1α$^{FL/FL}$ mice (Figure 7b,j; Figure 8d,f), indicating that Müller glia may not be able to use photoreceptor-derived lactate for mitochondrial oxidative phosphorylation. Future research is warranted to generate transgenic mice carrying the Müller glia-specific promoter and homozygous for a GFP reporter and the floxed gene, thus allowing us to directly use GFP as a readout to quantitatively study photoreceptor degeneration in the region with target protein depletion in Müller glia.

We observed strong immunoreactivities for IR, HK2, PDH-E1α and LDH-A in photoreceptor inner segments (Figure 1a, Figure 4a, Figure 6a,g), suggesting that photoreceptors have the capacity for glycolysis and oxidative phosphorylation. Our observations are consistent with recent findings that photoreceptors express abundant enzymes for glycolysis and oxidative phosphorylation (Casson et al., 2016; Kanow et al., 2017; Rueda et al., 2016) and that selective perturbations of glycolytic

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enzymes such as LDH-A or PKM2 in rods caused shortening of photoreceptor outer segments (Chinchore et al., 2017). HK catalyzes the phosphorylation of glucose to glucose-6-phosphate. Four homologous HK isoforms have been found in mammalian tissues including HK-1, HK-2, HK-3 and HK4 (also glucokinase) and photoreceptor inner segments express high levels of HK1 and HK2 (Rueda et al., 2016). A recent study reported that knocking down HK2 in rods caused reduction in electroretinographic responses without affecting photoreceptor structure or survival (Petit et al., 2018). Photoreceptors that lost HK2 survived by shifting their metabolism from aerobic glycolysis to oxidative phosphorylation (Petit et al., 2018). The high capacity for both glycolysis and oxidative phosphorylation in photoreceptors would likely make them less dependent on Müller glia to shuttle metabolites for ATP production as proposed by the ANLS hypothesis.

Single-cell RNASeq analysis of the retinas of chicks, mice and zebrafish has recently been reported (Hoang et al., 2020). We have used their publicly available database (https://proteinpaint.stjude.org/F/2019.retina.scRNA.html) to analyze the gene expression profiles of IR, HK1, HK2, PHGDH, PKM, LDH-A, LDH-B and PDH-E1α in 17 types of retinal cells in the mouse retina (Supplementary Figures 5 and 6). We found that photoreceptors strongly express key enzymes for glycolysis, including HK2, PKM and LDH-A, which is consistent with previous studies that glucose metabolism in photoreceptors is dominated by aerobic glycolysis (Casson, Chidlow, Han, & Wood, 2013; Chinchore et al., 2017; Hurley et al., 2015; Kanow et al., 2017; Ng et al., 2015). The single-cell RNASeq data also suggest that Müller glia express moderate levels of IR, HK1, HK2 and PHGDH but little PDH-E1α (Supplementary Figure 6), which is consistent with our hypothesis that Müller glia use glucose to produce 3-phosphoglycerate which is then catalyzed by PHGDH for the *de novo* biosynthesis of serine to support photoreceptors (Figure 13) (Sinha et al., 2020). However, we also noted that the gene expression profiles of PKM and LDH-A in Müller glia and PDH-E1α in photoreceptors revealed by the single-cell RNASeq analysis are different from the profiles of protein expression observed by us (Figure 6) and others (Casson et al., 2016;

Chinchore et al., 2017; Kanow et al., 2017; Lindsay et al., 2014; Rajala et al., 2018). IHC conducted by different groups indicate that Müller glia express very little PKM1, PKM2 and LDH-A (Chinchore et al., 2017; Kanow et al., 2017; Lindsay et al., 2014; Rueda et al., 2016) but the single-cell RNASeq data suggest that Müller glia express moderate or high levels of these genes. We observed strong immunostaining of PDH-E1α in the photoreceptor inner segments (Figure 6a-c) but the single-cell RNASeq data indicate photoreceptors express very low levels of PDH-E1α. These discrepancies highlight the importance of conducting functional studies for understanding how Müller glia and photoreceptors use glucose in the intact retina.

Whether Müller glia can take up glucose at all is also controversial and depends on how it is studied (Kanow et al., 2017; C. Poitry-Yamate et al., 2013; C. Poitry-Yamate & Tsacopoulos, 1991). It was previously reported that only Müller glia took up radioactively labelled D-glucose in guinea pig retinas (C. Poitry-Yamate & Tsacopoulos, 1991). This finding was recently reproduced in rat retinas using Positron Emission Tomography (PET) to image a stable isotope of fluorine after an intravenous injection of ^{18}fluorodeoxy-D-glucose (C. Poitry-Yamate et al., 2013). The investigators observed high uptake and phosphorylation of ^{18}fluorodeoxy-D-glucose in Müller glial end feet and the inner synaptic cell layer, suggesting Müller glia can take up glucose. However, a recent study questioned the capacity of Müller glia for taking up any glucose at all (Kanow et al., 2017). Fluorescently-labelled D-glucose was reported to enter the retina primarily through photoreceptors in mouse and zebrafish, supporting that photoreceptors are the main user of glucose in the retina (Kanow et al., 2017). These discrepancies highlight the need of using cell-specific approaches to study how disruption of glucose metabolism in Müller glia and photoreceptors affects the retinal health (Toft-Kehler et al., 2018).

We found that Müller glia express IR, HK2 and PHGDH. IR and HK2 were expressed by most cells, including Müller glia in the inner nuclear layer (Figure 1a-f, Figure 4a-f), while PHGDH was predominantly expressed by astrocytes, Müller glia and RPE cells (Figure 9a1-a3, Supplementary

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Figure 5). We confirmed the expression of IR and HK2 in Müller glia *in vitro* (Figure 2d-e, Figure 4l-m) and demonstrated that they were successfully knocked down in Müller glia isolated from MC-IRKO (Figure 2f) or MC-HKKO mice (Figure 4n) after treating them with 4-OHT. We also demonstrated that knocking down IR in isolated Müller glia led to reduced uptake of fluorescently-labelled glucose (2-NBDG) *in vitro* (Figure 2g). Results from *in vivo* studies indicate that selectively knocking down IR, HK2 or PHGDH in Müller glia led to loss of photoreceptor apical processes and reduced electroretinographic responses (Figures 3, 5, 8, 9). These collective findings indicate that Müller glia may use glucose to produce serine to support photoreceptors.

Müller glia may produce serine to support photoreceptors in multiple ways. The *de novo* serine biosynthesis is through a four-step process involving enzymes including PHGDH, phosphoserine aminotransferase, phosphoserine phosphatase and serine hydroxy-methyltransferase (Amelio, Cutruzzola, Antonov, Agostini, & Melino, 2014). PHGDH, the rate-limiting enzyme of this pathway, controls the metabolic flux of this process. Human Müller glia express PHGDH and *de novo* synthesis of serine is important for Müller glia survival under stress, possibly through maintaining mitochondrial function and generating glutathione and NADPH to counteract reactive oxygen species (Zhang et al., 2018). Serine has been reported to contribute to the biosynthesis of sphingolipids to regulate tissue homeostasis in the central nervous system including photoreceptors (Acharya et al., 2003; Hwang et al., 2017). Serine and glycine are also major sources of methyl groups for one-carbon unit metabolism which provides important metabolites for the biosynthesis of precursor proteins, nucleotides and phospholipids (Amelio et al., 2014), all of which are important to maintain photoreceptor health.

We found that neither Müller glia nor RPE cells express the mitochondrial serine transporter SFXN1 (Kory et al., 2018), indicating that these two types of cells produce serine but may not use it (Supplementary Figure 3). Strong immunostaining of SFXN1 was observed in photoreceptor inner

segments, some inner retinal neurons, the inner plexiform and ganglion cell layers, consistent with a recent study that serine might be predominantly used by neurons in the brain (Rivell, Petralia, Wang, Mattson, & Yao, 2019). We found loss of PHGDH in Müller glia increased immunostaining of SFXN1 in photoreceptor inner segments and that L-serine treatment reduced SFXN1 immunoreactivity in this structure (Supplementary Figure 4), suggesting that stressed photoreceptors may increase uptake of serine released by the RPE to adapt to the loss of PHGDH in Müller glia.

The ability of exogenous L-serine in preventing photoreceptor degeneration in MC-PHGDH-KO mice (Figures 10,11) suggests that supplementation of exogenous serine may be used to prevent photoreceptor degeneration in macular disease in which Müller glia are defective, for example macular telangiectasia type 2 (MacTel). Loss of macular Müller glia is a striking and consistent pathological feature observed in *post* mortem specimens of eyes with MacTel (Powner et al., 2010; Powner et al., 2013). Selectively knocking out Müller glia in transgenic murine retinas reproduced some characteristic features of MacTel, including photoreceptor degeneration (Shen et al., 2012).

An important question that remains to be answered is what energy substrates Müller glia use to maintain their health and function if they cannot complete glycolysis and oxidative phosphorylation. A recent study found that Müller glia express high levels of glutamate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate thiokinase, GABA transaminase and phosphate transferring kinases (Rueda et al., 2016). The authors hypothesized that Müller glia may utilize anaplerosis and cataplerosis to replenish TCA cycle intermediates to generate GTP and phosphate, thereby transferring kinases to produce ATP to meet their energy demand (Rueda et al., 2016). The presence of the mitochondrial enzyme glutamate dehydrogenase in Müller glia enables this pathway to produce energy (Rueda et al., 2016), i.e., oxidative deamination of glutamate to α- ketoglutarate by glutamate dehydrogenase provides an anaplerotic input to the TCA cycle intermediates. There is evidence that the enzymes for fatty acid β-oxidation are more preferentially expressed in Müller glial mitochondria

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than retinal neurons (Atsuzawa et al., 2010). Future research is warranted to study whether Müller glia are able to use fatty acid β-oxidation and ketone bodies to generate acetyl-CoA for ATP production to meet their energy demand (Puchalska & Crawford, 2017).

In summary, our findings suggest that the lactate shuttle hypothesis may not accurately reflect the metabolic relationship between Müller glia and photoreceptors in the outer retina. Our *in vivo* experiments suggest that Müller glia may use glucose to produce serine to support photoreceptors. There are several mechanisms by which this may occur (Figure 13), including (1) support for sphingolipid biosynthesis in photoreceptors, (2) conversion of serine to glycine for the biosynthesis of glutathione as an antioxidant to protect photoreceptors and (3) provision of methyl groups for one-carbon unit metabolism to synthetize precursor proteins, nucleotides and phospholipids in photoreceptors (Simon, Prado Spalm, Vera, & Rotstein, 2019; Sinha et al., 2020). Further research is warranted to dissect the complex relationship between Müller glia and photoreceptors.

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Table 1. Primers used to genotype Rlbp1-CreER mice and those carrying floxed metabolic genes

Gene	**Forward/Reverse**	**Primers**
Rlbp1 (primer 1)	Forward	CCT CAC AGC GGT CAC TTT TGG TAG
	Reverse	GGA GGG CAG GCA GGT TTT GGT G
Rlbp1 (primer 2)	Forward	CAC AGC GGT CAC TTT TGG TAG ATA
	Reverse	GCG CAG CAG GGT GTT GTA GG
IR	Forward	GAT GTG CAC CCC ATG TCT G
	Reverse	CTG AAT AGC TGA GAC CAC AG
HK2	Forward	TGT CTT GGC TCA GAT GTG AC
	Reverse	CCC CTT CGC TTG CCA TTA C
LDH-A	Forward	CTC GCT TGC CTT ATG GGT TC
	Reverse	TGG CAG TCA AGT CTC CAA GAA G
Pdha1	Forward	CGT CTG TTG AGA GAG CAG CA
	Reverse	CGC ACA AGA TAT CCA TTC CA
PHGDH	Forward	CAT GAG GAA CTG AAC TGA AGG ATT GA
	Reverse	CAA GGA GGC TCA CAC ATC CCA GAA C

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Table 2. Antibodies used for immunohistochemistry (IHC), immunocytochemistry (ICC) and western blots (WB)

Antibody	Source and Catalogue No.	Dilution (IHC or ICC)	Dilution (WB)
Cone arrestin	Millipore, #AB15282	1:500	1:2000
CRALBP	Millipore, #MAB302	1:100	Nil
Cre	Millipore, #69050	1:200	1:5000
	Millipore, #3120	1:500	1:1000
Gαt	BD, #610589	Nil	1:2000
GFAP	Dako #Z0334	1:250	Nil
	Abcam, #ab53554	Nil	1:500
GS	Chemicon International, #MAB302	1:100	1:1000
HK2	Cell Signaling Technology, #2867	1:100	1:1000
HSP60	Cell Signaling Technology, #4870	Nil	1:1000
Iba-1	Wako, #019-19741	1:500	Nil
IRα	Santa Cruz, #sc-710	1:50	1:1000
IRβ	Santa Cruz, #sc-711	1:50	1:1000
IRBP	Abcam, #101456	Nil	1:2000
LDH-A	Novus Biologicals, #NBP1-48336	1:100	1:2000
PDH-E1α	Sapphire, #GTX104015	1:200	1:1000
PHGDH	Frontier Institute, #3PGDH-GP-Af198	1:500	Nil
	Millipore, #ABS571	1:200	1:5000
Red/green opsin	Millipore, #AB5405	1:200	1:1000
SFXN1	Proteintec, #12296-1-AP	1:100	Nil

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FIGUREURES AND LEGENDS

FIGURE 1. Expression of insulin receptor (IR) in the mouse retina. (a-f) Immunostaining for IRα and cellular retinaldehyde-binding protein (CRALBP). (a) IRα was expressed in the retinal pigment epithelium (RPE), photoreceptor inner segments (IS), the outer plexiform layer (OPL), the inner nuclear layer (INL), the inner plexiform layer and the ganglion cell layer (GCL). (b) Müller glial cells (MCs) were identified by an antibody (Ab) to CRALBP. (c) IRα and CRALBP expression merged. (d-f) High power images of the INL. Arrows in c-f point to Müller glia that express IRα. (g-i) Negative controls in which the primary Abs were omitted but with the same secondary Abs used as (a-f).

FIGURE 2. Generation of transgenic mice for selectively knocking down IR in Müller glia. (a) Expression of LacZ reporter gene in Müller glia (arrows) but not in other retina cells in Müller glial cell-CreER (MC-CreER) mice crossed with Rosa-LacZ Cre reporter mice 4 days after tamoxifen (TMX) treatment. (b-e) Immunocytochemistry showing primary murine Müller glia express IRα and IRβ. (b,c) Primary murine Müller glia stained with Abs against glial fibrillary acidic protein (GFAP, b) or glutamine synthetase (GS, c). (d,e) Murine Müller glia express IRα (d) and IRβ (e). (f) Knockdown of IRα in Müller glia isolated from MC-CreER mice crossed with IR-floxed ($IR^{FL/FL}$) mice. Western blots confirmed that both IRα and IRβ were successfully knocked down in Müller glia isolated from MC-CreER:$IR^{FL/FL}$ (MC-IRKO) mice after treatment of isolated murine Müller glia with 1μM of 4-hydroxy tamoxifen (4-OHT) for 24hrs. **P<0.01, n=4/group. (g) Reduced uptake of fluorescently-labelled glucose (2-NBDG) in 4-OHT treated-Müller glia isolated from MC-IRKO mice. *P<0.05, n=12/group.

FIGURE 3. Selectively knocking down IR led to photoreceptor degeneration and reactive Müller cell gliosis. (a-h) Staining of retinal sections from control (a-d) and Müller glia-IR knockdown (MC-IRKO, e-h) mice 4 weeks after tamoxifen treatment. (a1-a2, e1-e2) Double labelling for IRα (red)

and CRALBP (green). Arrows in a1 and a2 point to IRα expression in Müller glia in the normal retina while arrows in (e2) point to depletion of IRα in Müller glia identified by CRALBP in MC-IRKO mice. (b-d, f-h) Double label IHC for IRα and peanut-agglutinin (PNA) in MC-CreER mice (control) and MC-IRKO mice to correlate knockdown of IRα in Müller glia with photoreceptor degeneration. The arrows in (d) point to IRα-positive Müller glia in the inner nuclear layer while loss of IRα in this region corresponded to shortened photoreceptor apical processes (f-h). (i-l) Double labelling for CRALBP and GFAP. Müller glia in MC-IRKO mice showed increased immunoreactivity for GFAP (k,i) compared with control mice (i,j). (i,j) Western blot analyses indicated reduced expression of red/green opsin (**P<0.01), along with overexpression of GFAP (**P<0.01) while GS was not changed significantly in MC-IRKO mice compared with control mice. N=8/group.

FIGURE 4. Expression of hexokinase 2 (HK2) in the mouse retina and generation of transgenic mice for selectively knocking down HK2 in Müller glia. (a-f) Double labelling for HK2 and CRALBP. (a) HK2 was expressed in photoreceptor inner segments (IS), the outer plexiform layer (OPL), the inner nuclear layer (INL) and the ganglion cell layer (GCL). (b) Müller glia were identified by CRALBP. (c) HK2 and CRALBP expression merged. (d-f) High power images of the INL. Arrows in (c-f) point to Müller glia that express HK2. (g-i) Negative controls by omitting the primary antibodies but with corresponding secondary antibodies as used in (a-f). (j-m) Immunocytochemistry using primary mouse Müller glia. (j,k) The identity of Müller glia was confirmed by immunostaining for GFAP (j) and GS (k). (l) Expression of HK2 in primary mouse Müller glia. (m) Negative control by omitting primary antibody against HK2 but with the corresponding secondary antibody as used in (l). (n) Müller cell-CreER (MC-CreER) mice were crossed with HK2-floxed ($HK^{FL/FL}$) to selectively knock down HK2 in Müller glia. Western blots confirmed that HK2 was successfully knocked down in Müller glia isolated from MC-CreER:$HK2^{FL/FL}$ (MC-HKKO) mice after treatment with 1μM of 4-OHT for 24hrs. **P<0.01, n=4/ group.

FIGURE 5. Selectively knocking down HK2 led to photoreceptor degeneration and upregulation of GFAP. (a-f) Double label IHC for HK2 (red) and CRALBP (green) on retinal sections from control (a-c) and Müller glia-HK2 knockdown mice (MC-HKKO, d-f) 4 weeks after tamoxifen treatment. (c1-c2, f1-f2) Higher power images of the INL in (a,c) and (e,g) to demonstrate HK2 expression in Müller glia in the normal retina (arrows in c1 and c2) and reduction of HK2 expression in Müller glia identified by CRALBP in H2 (arrows) in MC-HKKO mice (f1, f2). (g-i) Double label IHC for HK2 and PNA in MC-CreER mice (control) and MC-HKKO mice indicated that loss of HK2 in the middle of the inner nuclear layer was associated with shortened photoreceptor apical processes (j-l). (m,n) Western blots indicated that knocking down HK2 in Müller cells led to reduced expression of photoreceptor-associated proteins including photoreceptor-associated G protein transducin subunit α (Gαt, *P<0.05) and interphotoreceptor retinoid-binding protein (IRBP, **P<0.01) and overexpression of GFAP (**P<0.01) while the expression of GS was not significantly affected. N=4/group.

FIGURE 6. Expression of pyruvate dehydrogenase E1 alpha 1 (PDH-E1α) and lactate dehydrogenase A (LDH-A) in the mouse retina. (a-f) Double label IHC for PDH-E1α (green) and CRALBP (red). (a) PDH-E1α was expressed in the retinal pigment epithelium (RPE), photoreceptor inner segments (IS), the outer plexiform layer (OPL), some cells in the inner nuclear layer (INL), the inner plexiform layer and the ganglion cell layer (GCL). (b) Müller glia were identified by CRALBP. (c) PDH-E1α and CRALBP expression merge. (d-f) Higher power images of the INL indicated that Müller glia expressed little PDH-E1α (arrows). (g-l) Double label immunostaining for LDH-A (green) and CRALBP (red). (g) LDH-A was expressed in photoreceptor inner segments (IS), some cells in the inner nuclear layer (INL) and the ganglion cell layer (GCL). (h) Müller glia were identified by CRALBP. (i) LDH-A and CRALBP expression merge. (j-l) Higher power images of the INL showing Müller glia expressed little LDH-A (arrows). (m-o) Negative controls by omitting the primary antibodies but with the same secondary antibodies as used in (a-l).

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FIGURE 10. Supplementation with exogenous L-serine prevented photoreceptor degeneration and reduced microglial activation in MC-PHGDH KO mice. (a-f) Immunostaining for cone arrestin (a-c) and ionized calcium binding adaptor molecule 1 (Iba1, a marker of microglia, d-f) in retinal wholemounts prepared from control and MC-PHGDH KO mice receiving normal drinking water or that enriched with L-serine (400mg/kg BW) immediately after tamoxifen treatment for 6 weeks. (g-i) Double label IHC for PHGDH (green) and cone arrestin (red) on frozen sections from MC-Cre mice (g) and MC-PHGDH KO mice receiving normal drinking water (h) or water enriched with L-serine (i). (j,k) Quantitative analyses of cone arrestin (g) and Iba1 (h) staining in retinal wholemounts as described in (a-f). **P<0.01, vs Ctl and *P<0.05 vs MC-PHGDH KO receiving normal drinking water. N=6-7/group.

FIGURE 11. Supplementation with exogenous L-serine prevented loss of photoreceptor-associated proteins and upregulation of retinal stress markers along with improved retinal function in MC-PHGDH KO mice. (a,b) Western blot analyses of changes in photoreceptor-associated proteins

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including Gαt and IRBP and retinal stress markers including GFAP and heat-shock protein 60 (Hsp60) 6 weeks after treatment with L-serine in MC-PHGDH KO mice. **P<0.01, vs Ctl and †P<0.05 and ‡P<0.01, both vs MC-PHGDH KO receiving normal drinking water, n=6-8/group. (c,d) Scotopic Phoenix ERG performed in control and MC-PHGDH KO mice receiving normal drinking water or water enriched with L-serine for 4 weeks. KO=MC-PHGDH KO, **P<0.01, vs normal control and †P<0.05 and ‡P<0.01, vs MC-PHGDH KO receiving normal drinking water. N=7-10/group.

FIGURE 12. Changes in retinal serine and glycine in MC-PHGDH KO mice receiving normal drinking water or that enriched with L-serine for 8 weeks, starting immediately after the last dose of TMX injection. (a,b) Metabolic analysis by mass spectrometry indicated that the levels of serine and glycine were dramatically increased after knocking down PHGDH in Müller glia, while treatment with L-serine reduced their levels comparable to normal control mice. **P<0.01, vs control (ctl); P<0.05, vs MC-PHGDH KO mice receiving normal drinking water. N=8/group. (c-h) Double labelling for PHGDH and Iba1 indicated that knocking down PHGDH in Müller glia led to infiltration of activated microglia (arrows, f-h) and macrophages (arrowheads, f-h), both of which strongly expressed PHGDH. The arrows in (c-e) point to a resting microglial cell which weakly expressed PHGDH in the control retina. INL=inner nuclear layer.

FIGURE 13. Proposed mechanisms for the metabolic support from Müller glia for photoreceptors. Müller glia express low levels of pyruvate kinase, LDH-A and PDH, which makes them unable to produce much lactate and pyruvate as energy substrates to fuel photoreceptors. Instead, Müller glia use glucose to produce 3-phosphoglycerate (3PG) which is catalysed by PHGDH for the *de novo* biosynthesis of serine to support photoreceptors. There are several mechanisms by which this may occur, including (1) support for sphingolipid biosynthesis in photoreceptors, (2) conversion of serine to glycine for the biosynthesis of glutathione as an antioxidant to protect photoreceptors and (3)

provision of methyl groups for one-carbon unit metabolism to synthetize precursor proteins, nucleotides and phospholipids in photoreceptors (Simon et al., 2019; Sinha et al., 2020). IR=insulin receptor, HK=hexokinase, G-6-P=glucose-6-phosphatase, PEP=phosphoenolpyruvate, PKM=pyruvate kinase muscle isozyme, SFXN1=sideroflexin 1, TCA=tricarboxylic acid. The reactions that occur in photoreceptors are labelled in blue.

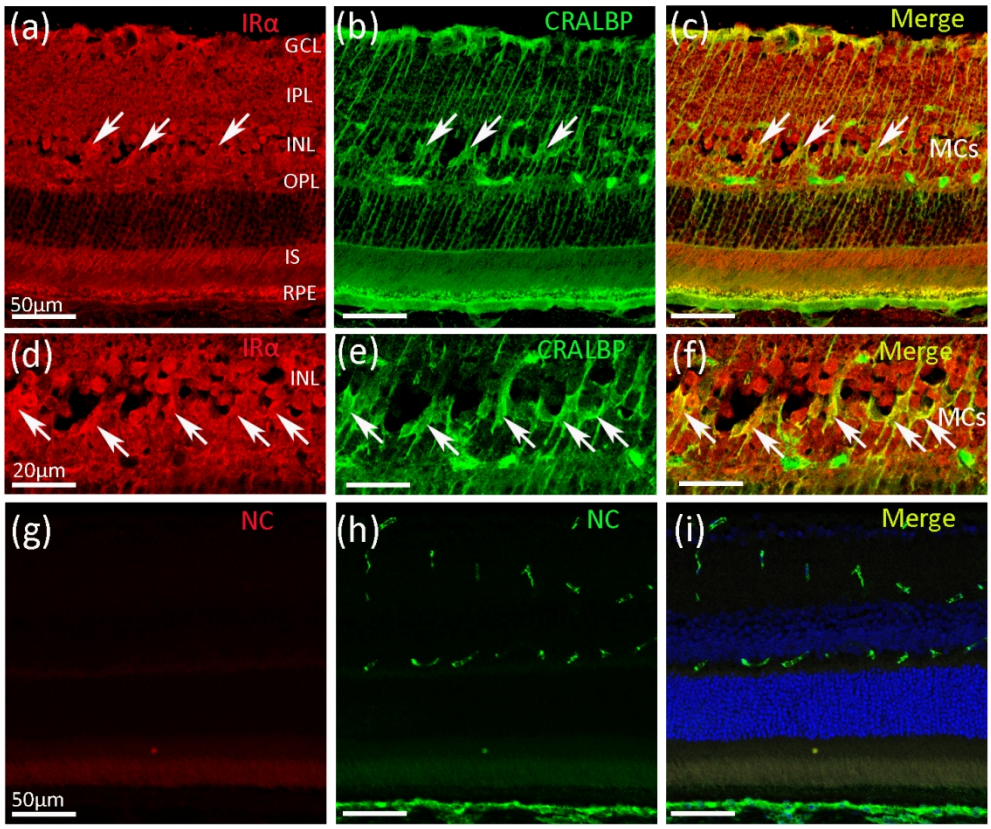

FIGURE 1. Expression of insulin receptor (IR) in the mouse retina. (a-f) Immunostaining for IRα and cellular retinaldehyde-binding protein (CRALBP). (a) IRα was expressed in the retinal pigment epithelium (RPE), photoreceptor inner segments (IS), the outer plexiform layer (OPL), the inner nuclear layer (INL), the inner plexiform layer and the ganglion cell layer (GCL). (b) Müller glial cells (MCs) were identified by an antibody (Ab) to CRALBP. (c) IRα and CRALBP expression merged. (d-f) High power images of the INL. Arrows in c-f point to Müller glia that express IRα. (g-i) Negative controls in which the primary Abs were omitted but with the same secondary Abs used as (a-f).

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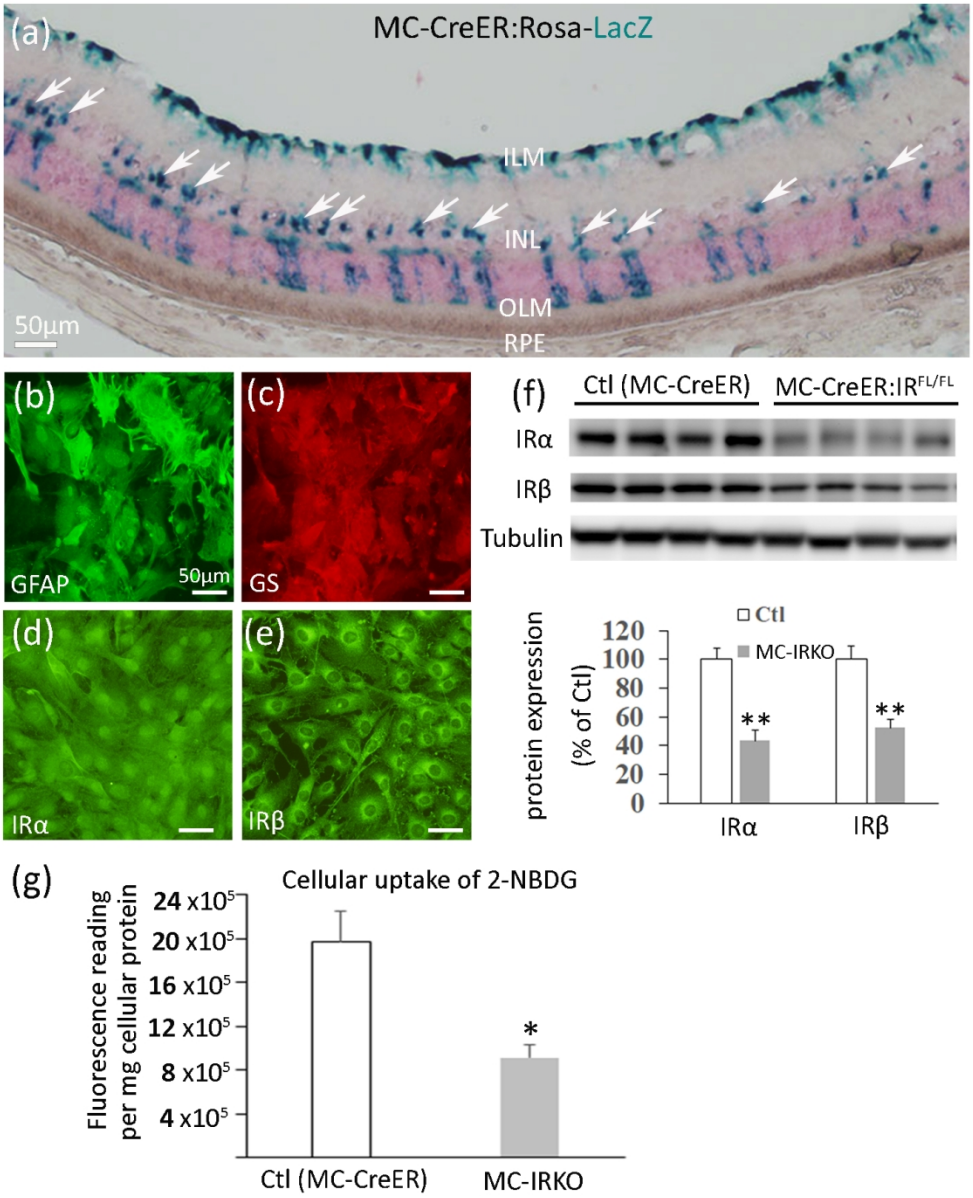

FIGURE 2. Generation of transgenic mice for selectively knocking down IR in Müller glia. (a) Expression of LacZ reporter gene in Müller glia (arrows) but not in other retina cells in Müller glial cell-CreER (MC-CreER) mice crossed with Rosa-LacZ Cre reporter mice 4 days after tamoxifen (TMX) treatment. (b-e) Immunocytochemistry showing primary murine Müller glia express IRα and IRβ. (b,c) Primary murine Müller glia stained with Abs against glial fibrillary acidic protein (GFAP, b) or glutamine synthetase (GS, c). (d,e) Murine Müller glia express IRα (d) and IRβ (e). (f) Knockdown of IRα in Müller glia isolated from MC-CreER mice crossed with IR-floxed (IRFL/FL) mice. Western blots confirmed that both IRα and IRβ were successfully knocked down in Müller glia isolated from MC-CreER:IRFL/FL (MC-IRKO) mice after treatment of isolated murine Müller glia with 1□M of 4-hydroxy tamoxifen (4-OHT) for 24hrs. **P<0.01, n=4/group. (g) Reduced uptake of fluorescently-labelled glucose (2-NBDG) in 4-OHT treated-Müller glia isolated from MC-IRKO mice. *P<0.05, n=12/group.

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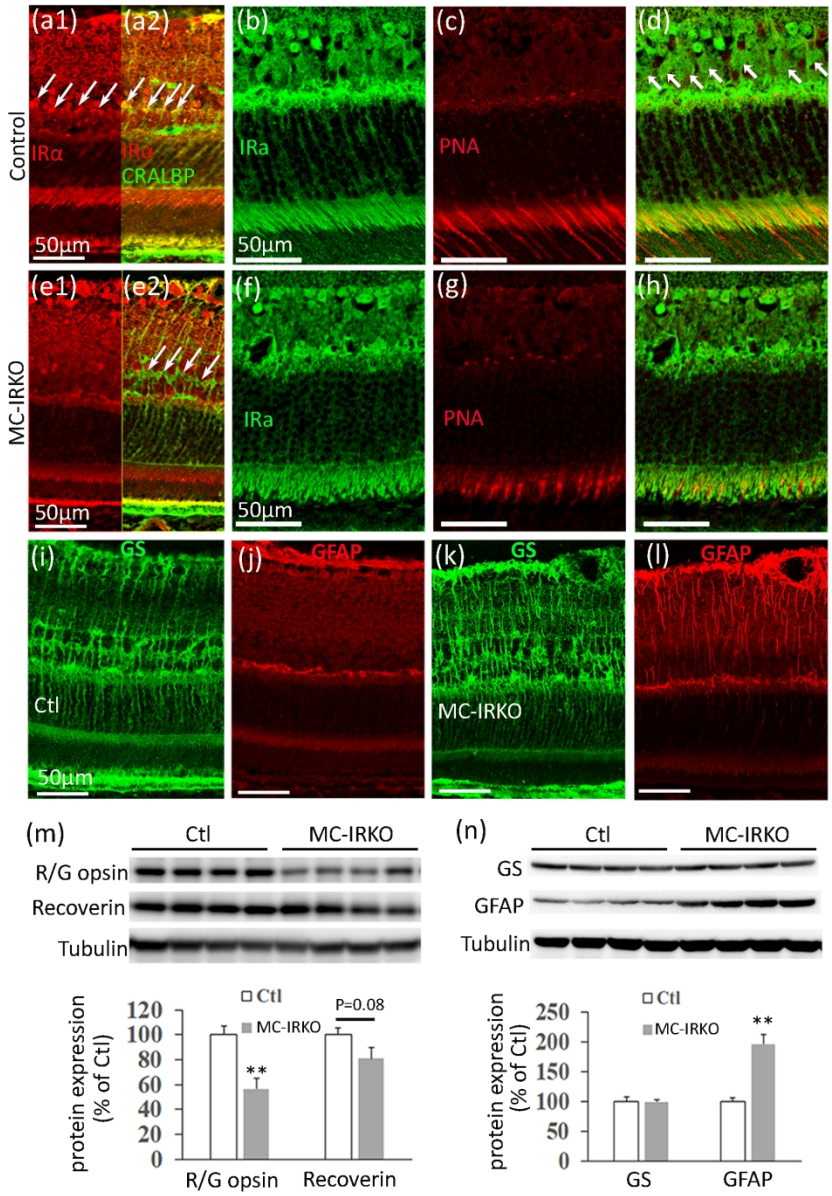

FIGURE 3. Selectively knocking down IR led to photoreceptor degeneration and reactive Müller cell gliosis. (a-h) Staining of retinal sections from control (a-d) and Müller glia-IR knockdown (MC-IRKO, e-h) mice 4 weeks after tamoxifen treatment. (a1-a2, e1-e2) Double labelling for IRα (red) and CRALBP (green). Arrows in a1 and a2 point to IRα expression in Müller glia in the normal retina while arrows in (e2) point to depletion of IRα in Müller glia identified by CRALBP in MC-IRKO mice. (b-d, f-h) Double label IHC for IRα and peanut-agglutinin (PNA) in MC-CreER mice (control) and MC-IRKO mice to correlate knockdown of IRα in Müller glia with photoreceptor degeneration. The arrows in (d) point to IR□-positive Müller glia in the inner nuclear layer while loss of IR□ in this region corresponded to shortened photoreceptor apical processes (f-h). (i-l) Double labelling for CRALBP and GFAP. Müller glia in MC-IRKO mice showed increased immunoreactivity for GFAP (k,i) compared with control mice (i,j). (i,j) Western blot analyses indicated reduced expression of red/green opsin (**P<0.01), along with overexpression of GFAP (**P<0.01) while GS was not changed significantly in MC-IRKO mice compared with control mice. N=8/group.

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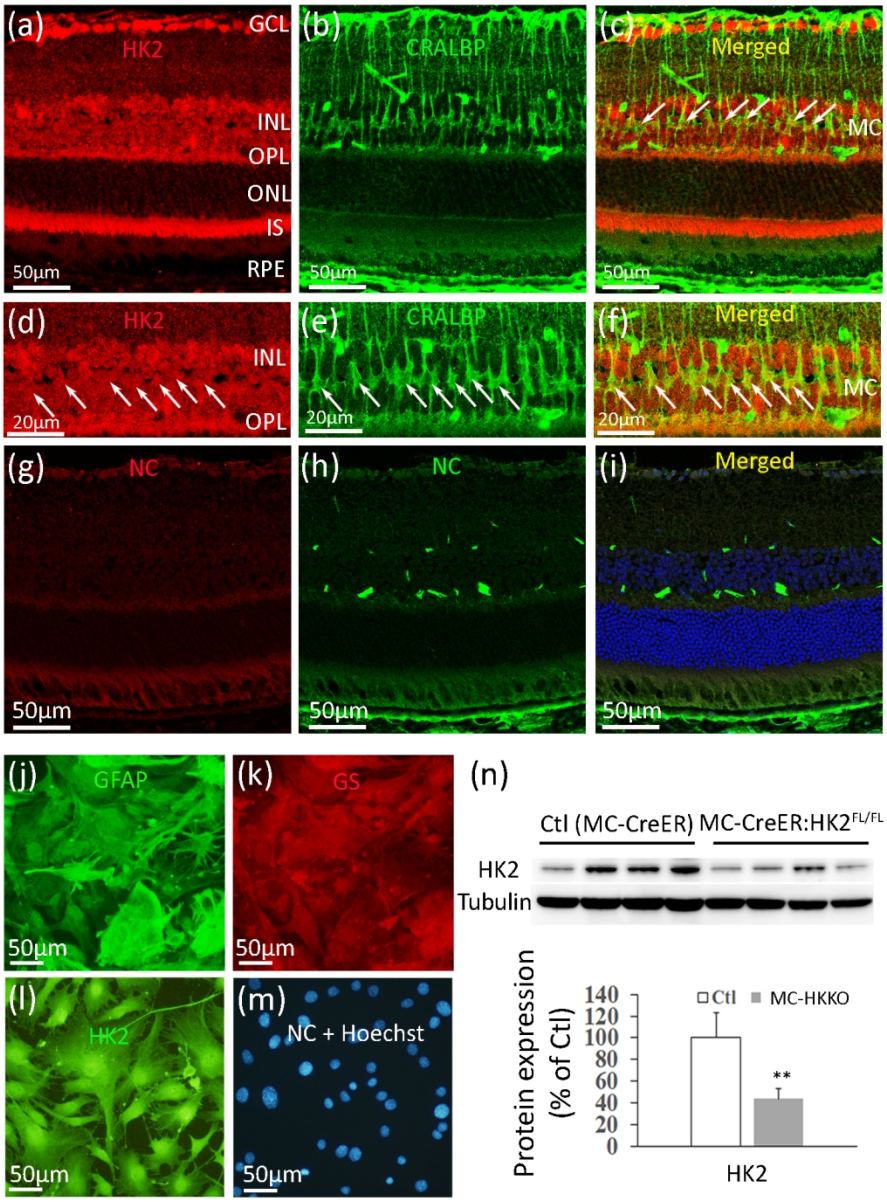

FIGURE 4. Expression of hexokinase 2 (HK2) in the mouse retina and generation of transgenic mice for selectively knocking down HK2 in Müller glia. (a-f) Double labelling for HK2 and CRALBP. (a) HK2 was expressed in photoreceptor inner segments (IS), the outer plexiform layer (OPL), the inner nuclear layer (INL) and the ganglion cell layer (GCL). (b) Müller glia were identified by CRALBP. (c) HK2 and CRALBP expression merged. (d-f) High power images of the INL. Arrows in (c-f) point to Müller glia that express HK2. (g-i) Negative controls by omitting the primary antibodies but with corresponding secondary antibodies as used in (a-f). (j-m) Immunocytochemistry using primary mouse Müller glia. (j,k) The identity of Müller glia was confirmed by immunostaining for GFAP (j) and GS (k). (l) Expression of HK2 in primary mouse Müller glia. (m) Negative control by omitting primary antibody against HK2 but with the corresponding secondary antibody as used in (l). (n) Müller cell-CreER (MC-CreER) mice were crossed with HK2-floxed (HKFL/FL) to selectively knock down HK2 in Müller glia. Western blots confirmed that HK2 was successfully knocked down in Müller glia isolated from MC-CreER:HK2FL/FL (MC-HKKO) mice after treatment with 1□M of 4-OHT for 24hrs. **P<0.01, n=4/group.

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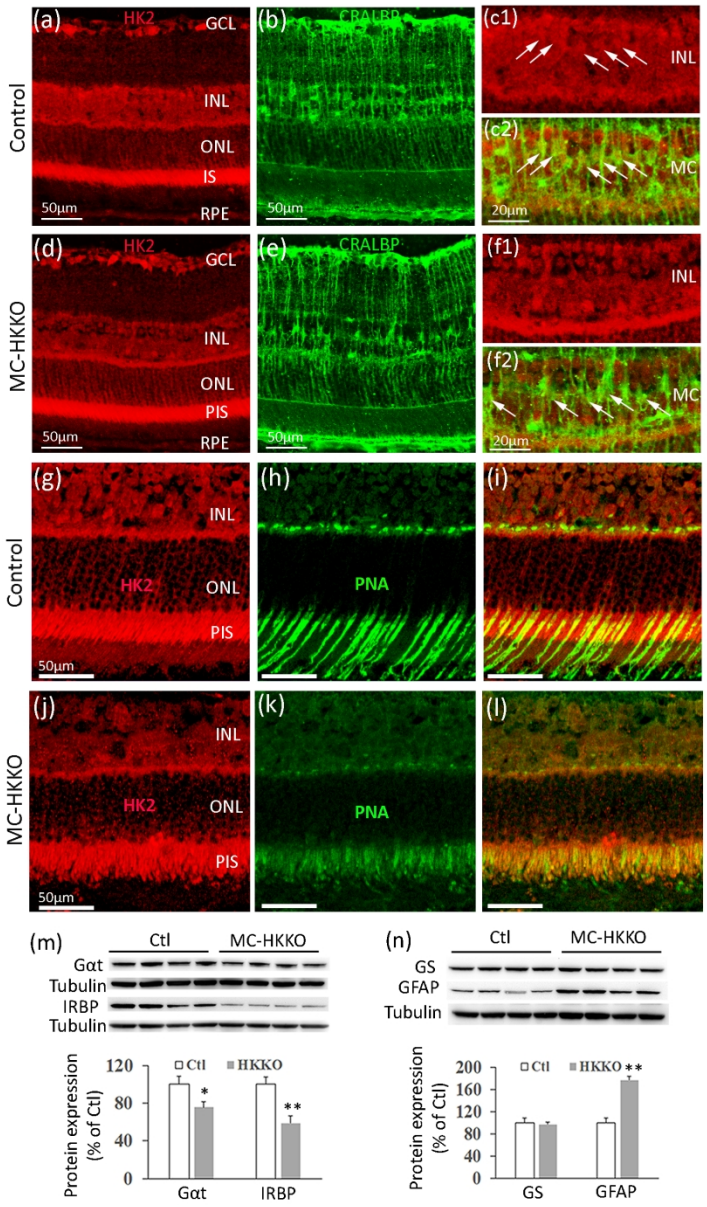

FIGURE 5. Selectively knocking down HK2 led to photoreceptor degeneration and upregulation of GFAP. (a-f) Double label IHC for HK2 (red) and CRALBP (green) on retinal sections from control (a-c) and Müller glia-HK2 knockdown mice (MC-HKKO, d-f) 4 weeks after tamoxifen treatment. (c1-c2, f1-f2) Higher power images of the INL in (a,c) and (e,g) to demonstrate HK2 expression in Müller glia in the normal retina (arrows in c1 and c2) and reduction of HK2 expression in Müller glia identified by CRALBP in H2 (arrows) in MC-HKKO mice (f1, f2). (g-i) Double label IHC for HK2 and PNA in MC-CreER mice (control) and MC-HKKO mice indicated that loss of HK2 in the middle of the inner nuclear layer was associated with shortened photoreceptor apical processes (j-l). (m,n) Western blots indicated that knocking down HK2 in Müller cells led to reduced expression of photoreceptor-associated proteins including photoreceptor-associated G protein transducin subunit α (Gαt, *P<0.05) and interphotoreceptor retinoid-binding protein (IRBP, **P<0.01) and overexpression of GFAP (**P<0.01) while the expression of GS was not significantly affected. N=4/group.

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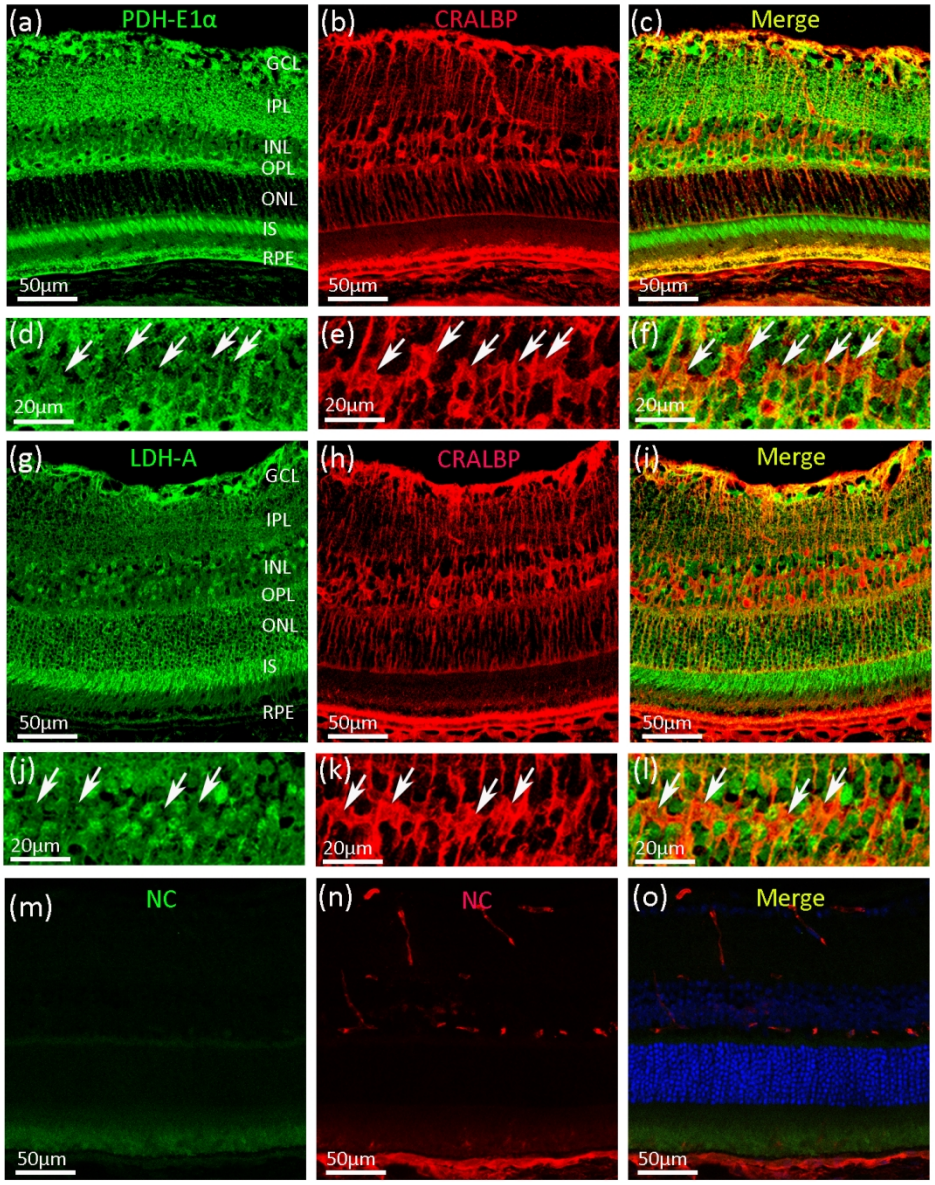

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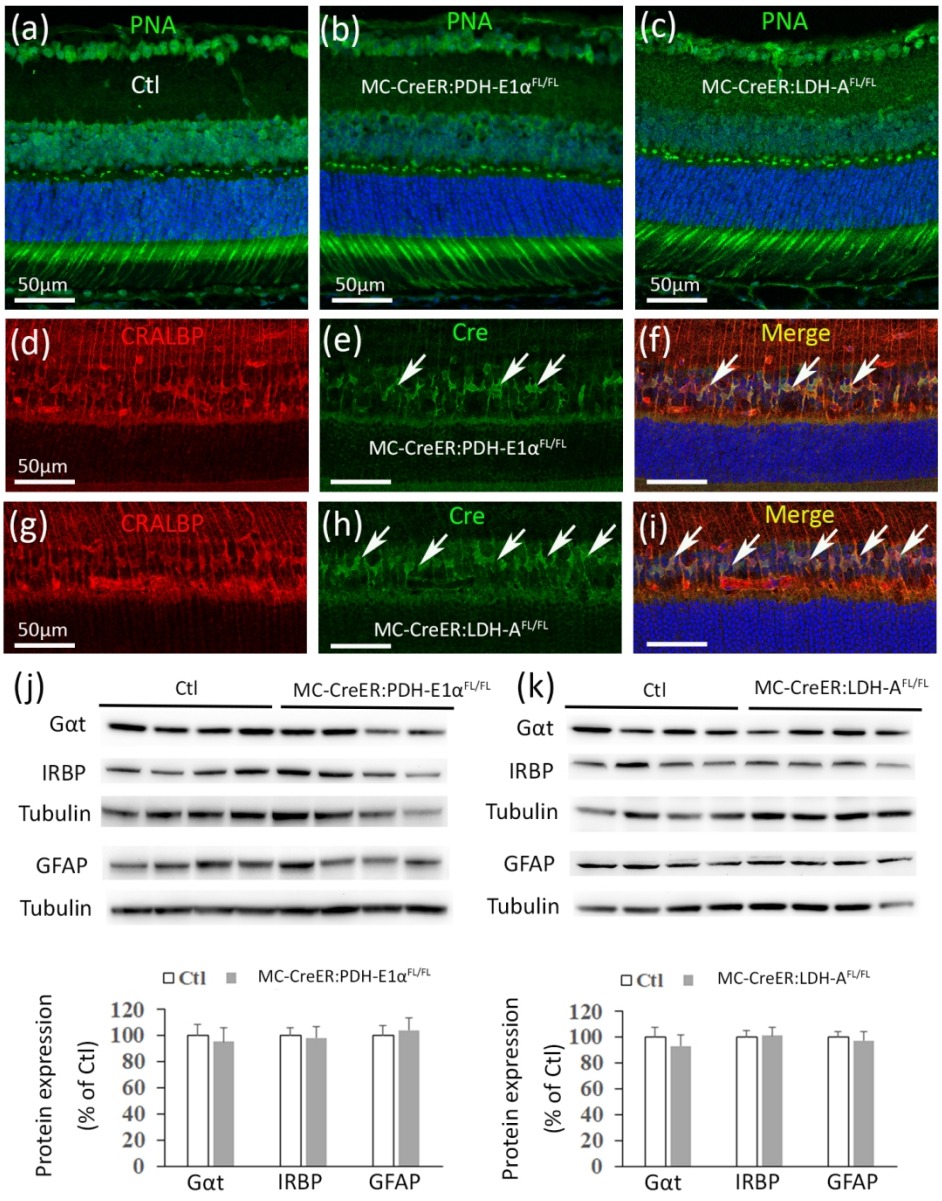

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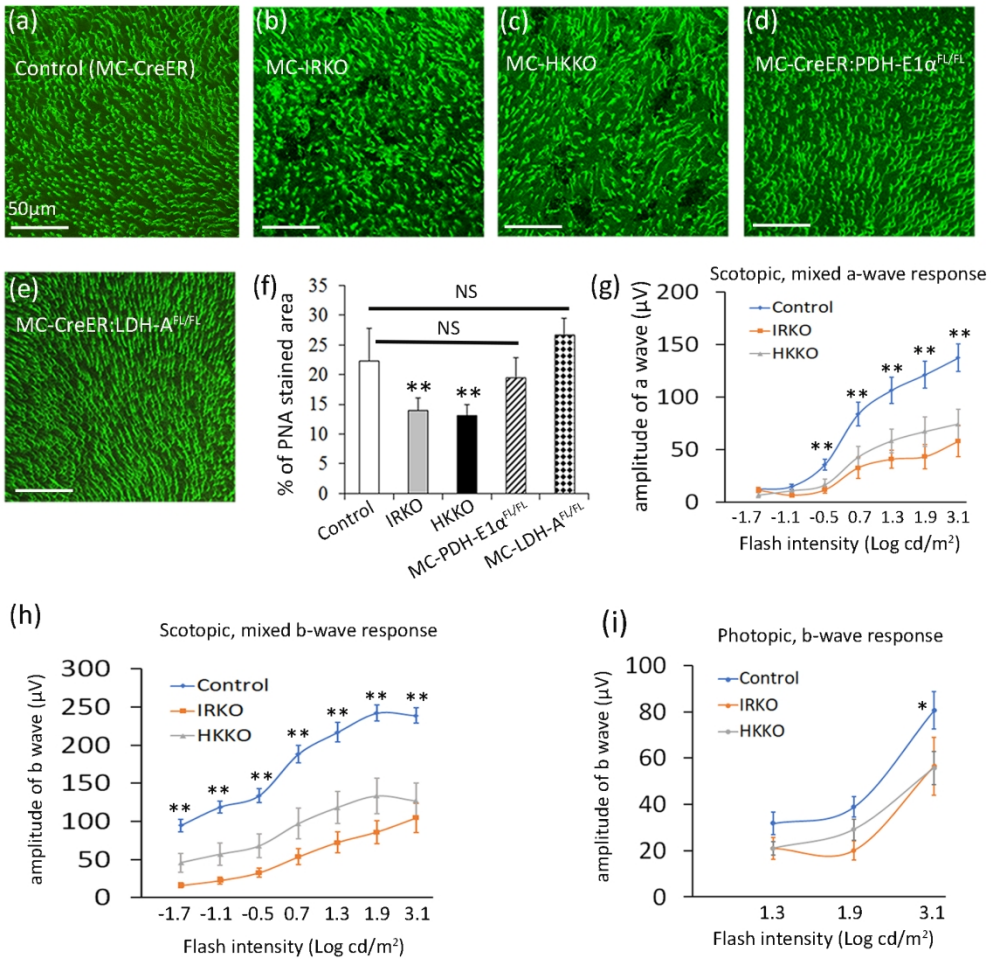

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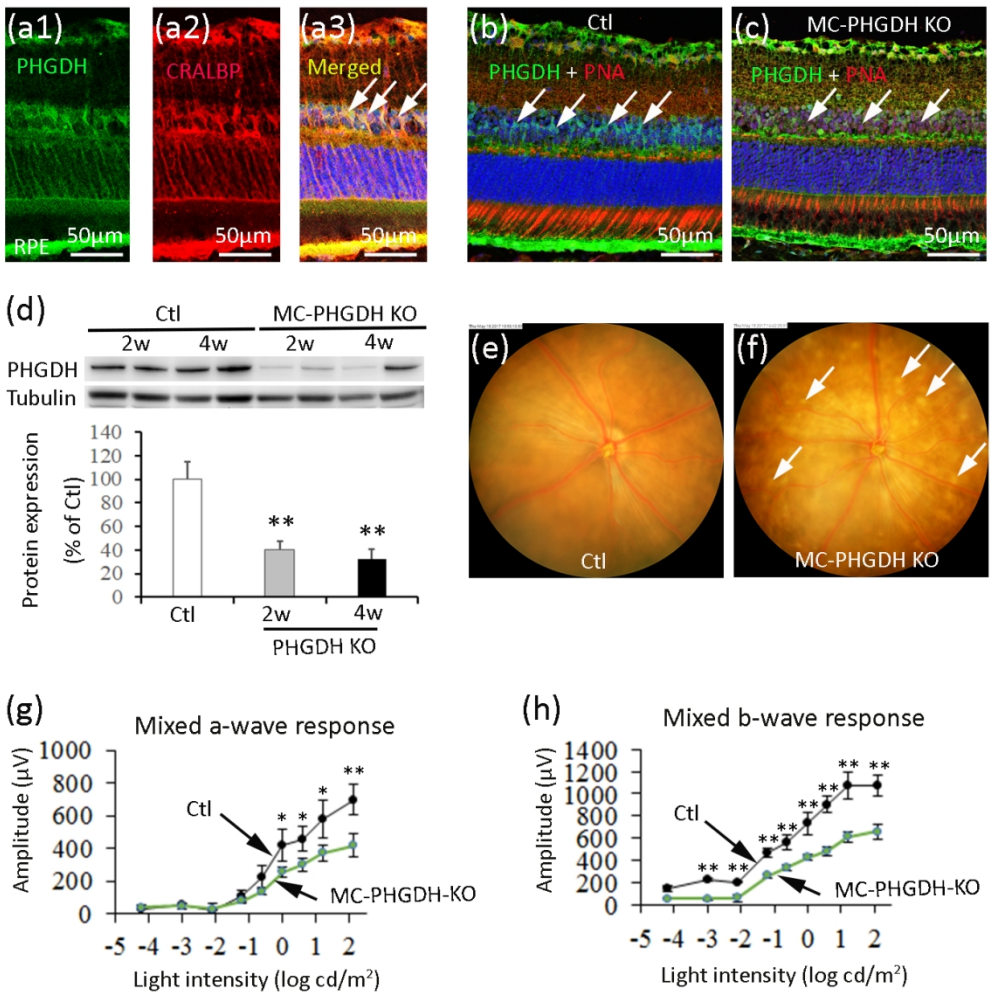

FIGURE 9. Müller glia expressed PHGDH and selectively knocking it down also led to photoreceptor degeneration and reduced electroretinographic responses. (a1-a3) Double label immunostaining indicated that Müller glia (arrows in a3) and the retinal pigment epithelium (RPE) strongly express PHGDH. (b,c) Labelling of retinas for PHGDH and PNA indicated that knocking down PHGDH in Müller glia led to degeneration of photoreceptor apical processes (c). Note: the regular expression of PHGDH in Müller glia in (b) aligned with PNA-stained photoreceptor apical processes while knocking down PHGDH in Müller glia was accompanied by disruption of photoreceptor apical processes in (c). The arrows in (c) point to regions in the INL where PHGDH was depleted. (d) Western blots confirmed PHGDH knockdown 2 and 4 weeks after tamoxifen treatment. **P<0.01, vs Ctl, n=4-8/group. (e,f) Color fundus photography showing yellowish deposits in the retina 4 weeks after knocking down PHGDH in Müller glia (arrows in f). (g,h) Knocking down PHGDH in Müller glia resulted in abnormal electroretinography characterized by reduced amplitudes of both a (g) and b (h) waves in scotopic ERG. *P<0.05 and **P<0.01, vs Ctl (MC-CreER), n=3-6/group. Scotopic full filed ERG was recorded as described previously (Moxon-Lester et al., 2009).

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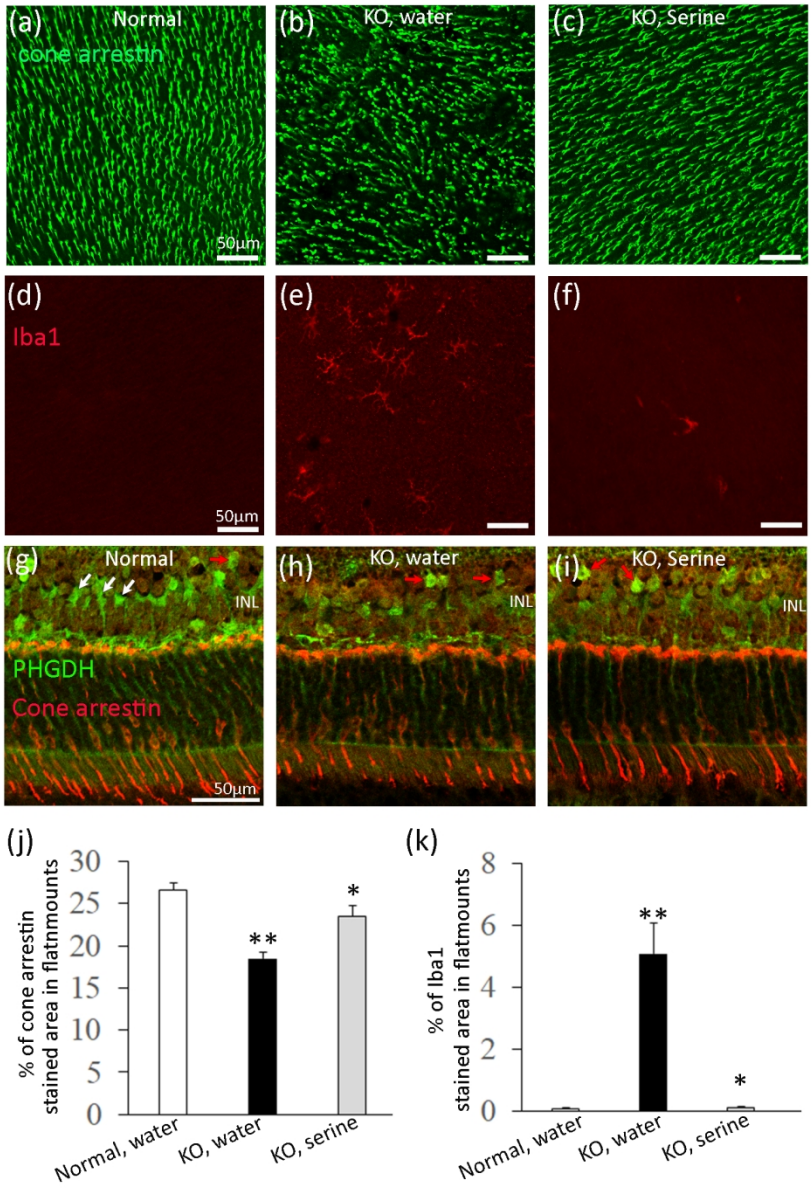

FIGURE 10. Supplementation with exogenous L-serine prevented photoreceptor degeneration and reduced microglial activation in MC-PHGDH KO mice. (a-f) Immunostaining for cone arrestin (a-c) and ionized calcium binding adaptor molecule 1 (Iba1, a marker of microglia, d-f) in retinal wholemounts prepared from control and MC-PHGDH KO mice receiving normal drinking water or that enriched with L-serine (400mg/kg BW) immediately after tamoxifen treatment for 6 weeks. (g-i) Double label IHC for PHGDH (green) and cone arrestin (red) on frozen sections from MC-Cre mice (g) and MC-PHGDH KO mice receiving normal drinking water (h) or water enriched with L-serine (i). (j,k) Quantitative analyses of cone arrestin (g) and Iba1 (h) staining in retinal wholemounts as described in (a-f). **P<0.01, vs Ctl and *P<0.05 vs MC-PHGDH KO receiving normal drinking water. N=6-7/group.

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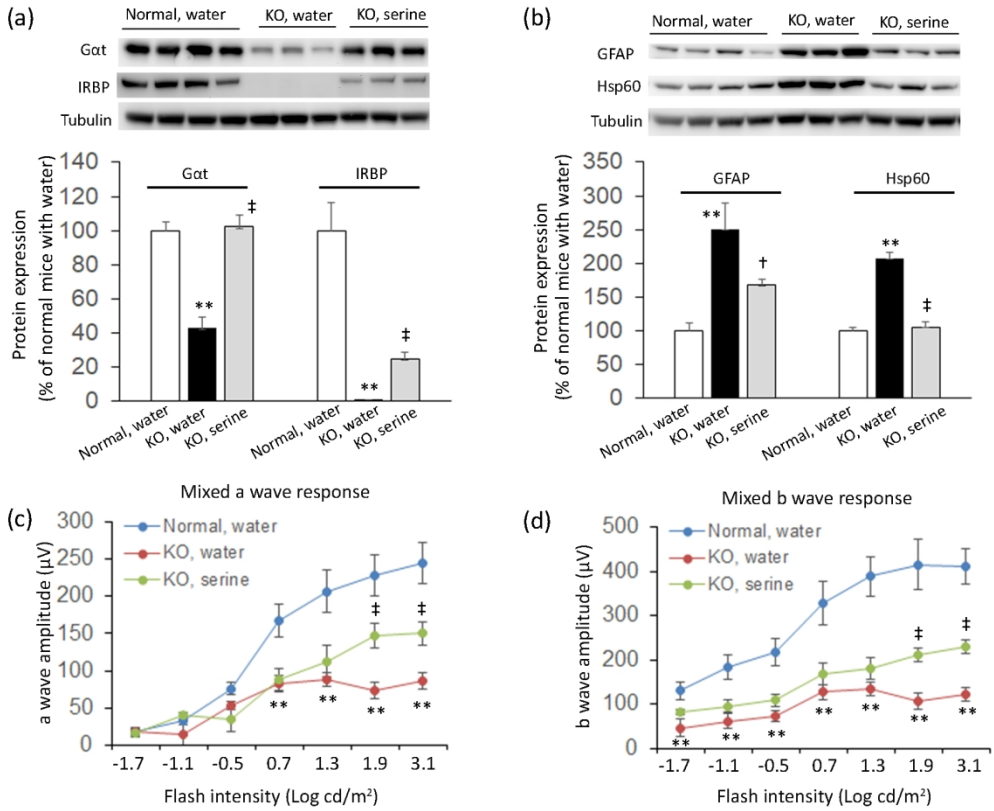

FIGURE 11. Supplementation with exogenous L-serine prevented loss of photoreceptor-associated proteins and upregulation of retinal stress markers along with improved retinal function in MC-PHGDH KO mice. (a,b) Western blot analyses of changes in photoreceptor-associated proteins including Gαt and IRBP and retinal stress markers including GFAP and heat-shock protein 60 (Hsp60) 6 weeks after treatment with L-serine in MC-PHGDH KO mice. **P<0.01, vs Ctl and †P<0.05 and ‡P<0.01, both vs MC-PHGDH KO receiving normal drinking water, n=6-8/group. (c,d) Scotopic Phoenix ERG performed in control and MC-PHGDH KO mice receiving normal drinking water or water enriched with L-serine for 4 weeks. KO=MC-PHGDH KO, **P<0.01, vs normal control and †P<0.05 and ‡P<0.01, vs MC-PHGDH KO receiving normal drinking water. N=7-10/group.

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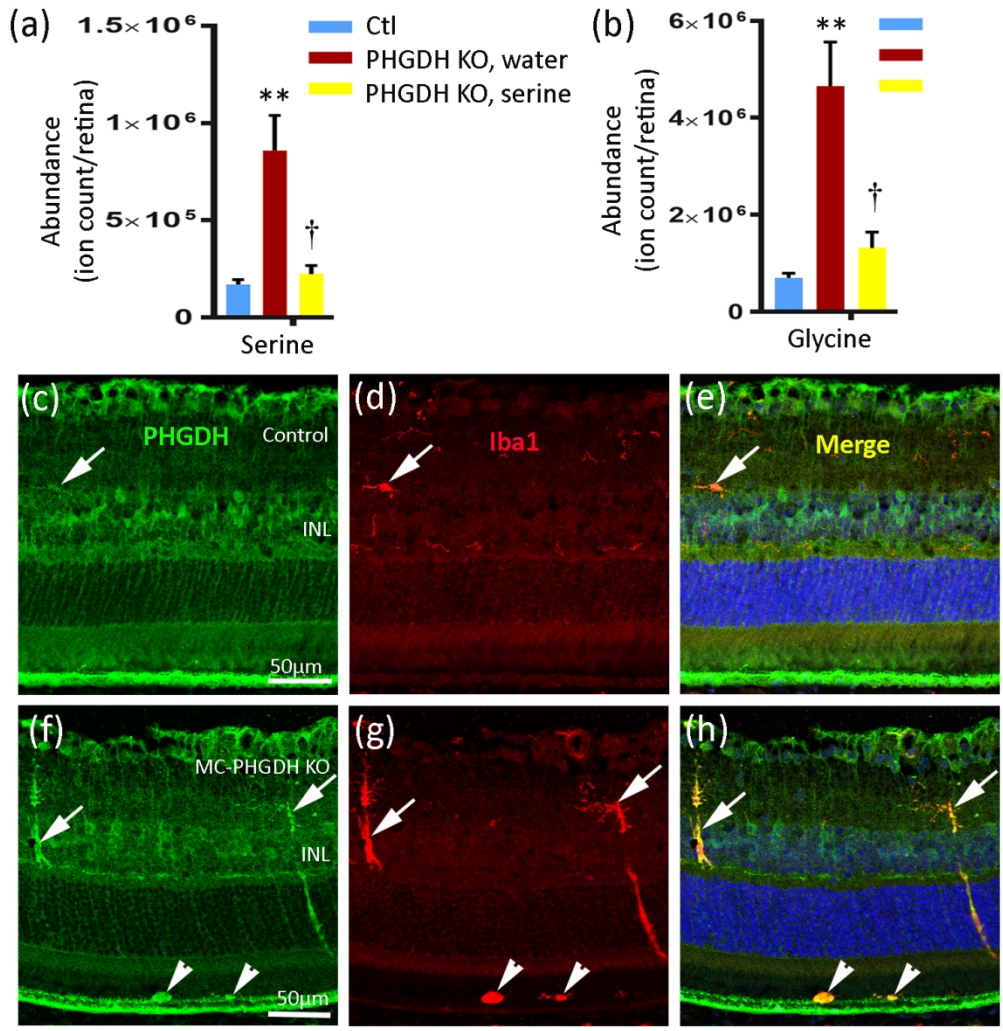

FIGURE 12. Changes in retinal serine and glycine in MC-PHGDH KO mice receiving normal drinking water or that enriched with L-serine for 8 weeks, starting immediately after the last dose of TMX injection. (a,b) Metabolic analysis by mass spectrometry indicated that the levels of serine and glycine were dramatically increased after knocking down PHGDH in Müller glia, while treatment with L-serine reduced their levels comparable to normal control mice. **P<0.01, vs control (ctl); P<0.05, vs MC-PHGDH KO mice receiving normal drinking water. N=8/group. (c-h) Double labelling for PHGDH and Iba1 indicated that knocking down PHGDH in Müller glia led to infiltration of activated microglia (arrows, f-h) and macrophages (arrowheads, f-h), both of which strongly expressed PHGDH. The arrows in (c-e) point to a resting microglial cell which weakly expressed PHGDH in the control retina. INL=inner nuclear layer.

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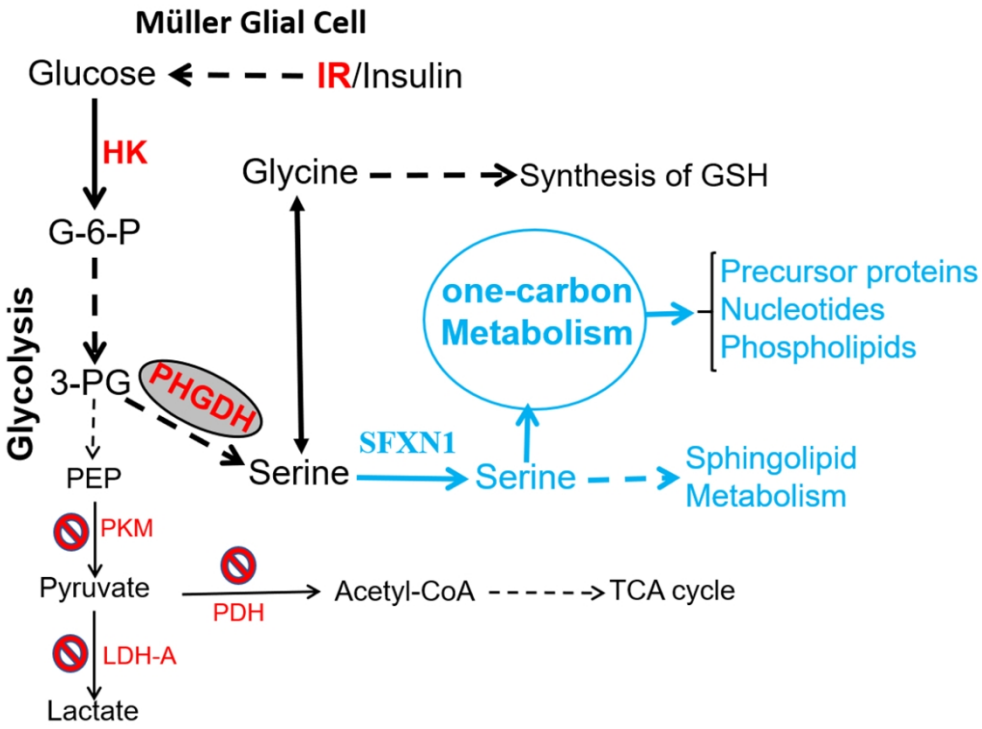

FIGURE 13. Proposed mechanisms for the metabolic support from Müller glia for photoreceptors. Müller glia express low levels of pyruvate kinase, LDH-A and PDH, which makes them unable to produce much lactate and pyruvate as energy substrates to fuel photoreceptors. Instead, Müller glia use glucose to produce 3-phosphoglycerate (3PG) which is catalysed by PHGDH for the de novo biosynthesis of serine to support photoreceptors. There are several mechanisms by which this may occur, including (1) support for sphingolipid biosynthesis in photoreceptors, (2) conversion of serine to glycine for the biosynthesis of glutathione as an antioxidant to protect photoreceptors and (3) provision of methyl groups for one-carbon unit metabolism to synthetize precursor proteins, nucleotides and phospholipids in photoreceptors (Simon et al., 2019; Sinha et al., 2020). IR=insulin receptor, HK=hexokinase, G-6-P=glucose-6-phosphatase, PEP=phosphoenolpyruvate, PKM=pyruvate kinase muscle isozyme, SFXN1=sideroflexin 1, TCA=tricarboxylic acid. The reactions that occur in photoreceptors are labelled in blue.

99x74mm (300 x 300 DPI)

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Effect of selectively knocking down key metabolic genes in Müller glia on photoreceptor health

Running title: Glucose metabolism in Müller glia

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Supplementary Materials and Methods

Analyses of retinal metabolites using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS)

Changes in metabolites in the glycolytic pathway, serine and glycine were analyzed with GC MS as described previously (Du, Linton, & Hurley, 2015; Grenell et al., 2019; Yam et al., 2019). Briefly, retinas were isolated within one minute after euthanasia by cervical dislocation and snap frozen in liquid nitrogen. Samples were stored at -80°C for analysis of metabolites in glycolysis and the TCA cycle using mass spectrometry. Retinas were homogenized with 20µl of 80% methanol, placed on dry ice for 30 min and centrifuged for 15 min at 15000rpm, 4℃. Supernatants were transferred to a glass insert and mixed with 0.1 mM myristic-d_{27} acid as an internal standard and then dried under vacuum at 4℃. For GC-MS, the dried metabolites were derivatized with methoxyamine hydrochloride N-tert-butyldimethylsilyl- N-methyltrifluoroacetamide before analyzed by the 7890/5977B GC/MS system (Agilent Technologies) with an Agilent DB-5MS column as previously described (Grenell et al., 2019). LC-MS was performed with a Shimadzu LC Nexera X2 UHPLC coupled with a QTRAP 5500 LC MS (AB Sciex) as previously described (Grenell et al., 2019; Wang et al., 2019). An ACQUITY UPLC BEH Amide analytic column (2.1 X 50 mm, 1.7 µm, Waters) was used for chromatographic separation. The mobile phase was (A) water with 10 mM ammonium acetate (pH 8.9) and (B) acetonitrile/water (95/5) with 10 mM ammonium acetate (pH 8.2). The gradient elution was 95%–61% B in 6 min, 61%–44% B at 8 min, 61%–27% B at 8.2 min, and 27%–95% B at 9 min. The column was equilibrated with 95% B at the end of each run. The collision gas was N2. The ion source conditions in positive and negative mode were as follows: curtain gas (CUR) = 25 psi, collision gas (CAD) = high, ion spray voltage (IS) = 3800/- 3800 V, temperature (TEM) = 500°C, ion source gas 1 (GS1) = 50 psi, and ion source gas 2 (GS2) = 40 psi. Each metabolite was tuned with standards for optimal transitions and ^{13}C-nicotinic acid (Toronto Research Chemicals) was used as the internal standard. The extracted MRM peaks were integrated using MultiQuant 3.0.2 software (AB Sciex).

Supplementary Figures and Legends

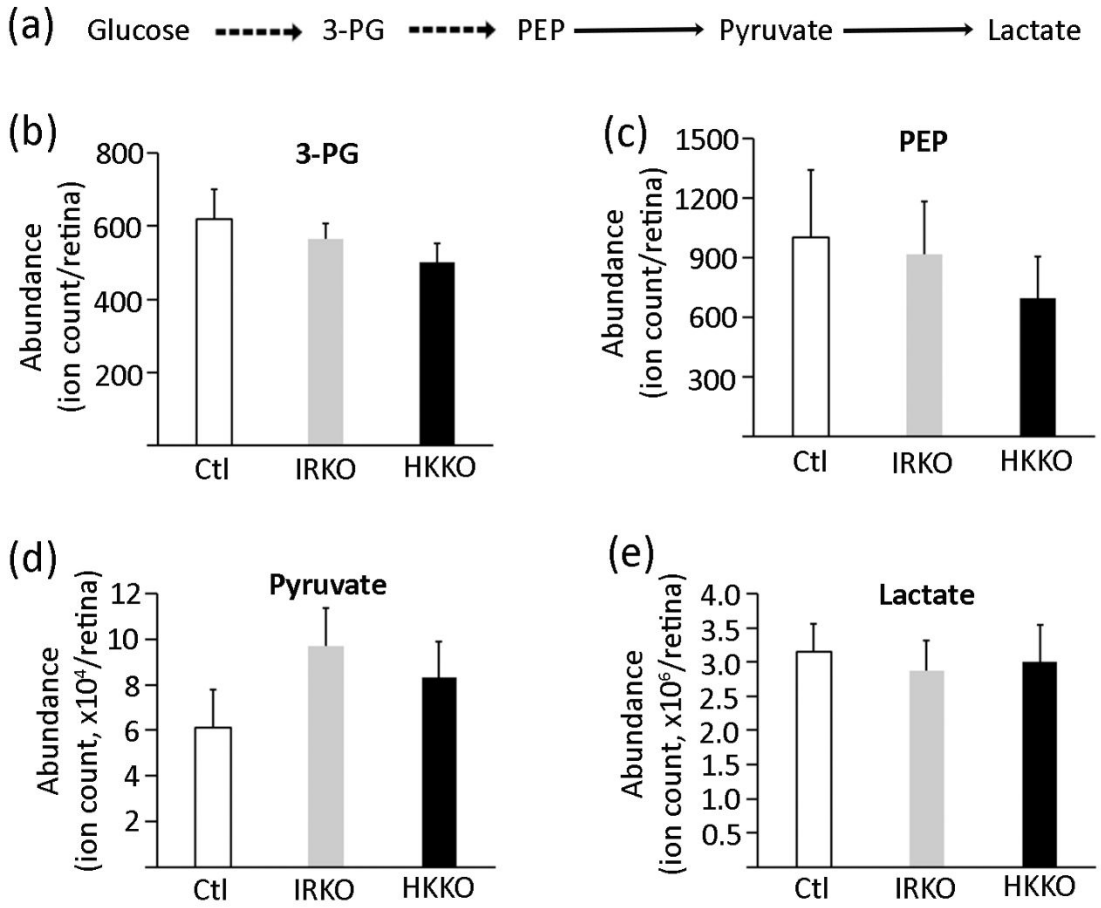

Figure _1_ SuppInfo. Selectively knocking down IR or HK2 in Müller glia did not affect the levels of retinal metabolites in glycolysis. (a) Schematic of glucose metabolism through glycolysis. 3-PG=3-phosphoglycerate, PEP=phosphoenolpyruvate. (b-e) Analyses of glycolytic metabolites by GC-MS and LC-MS in retinas isolated from control, MC-IRKO and MC-HKKO mice 4 weeks after TMX treatment (see materials and methods for details). There was no statistical difference among the 3 groups for each metabolite. N=8/group.

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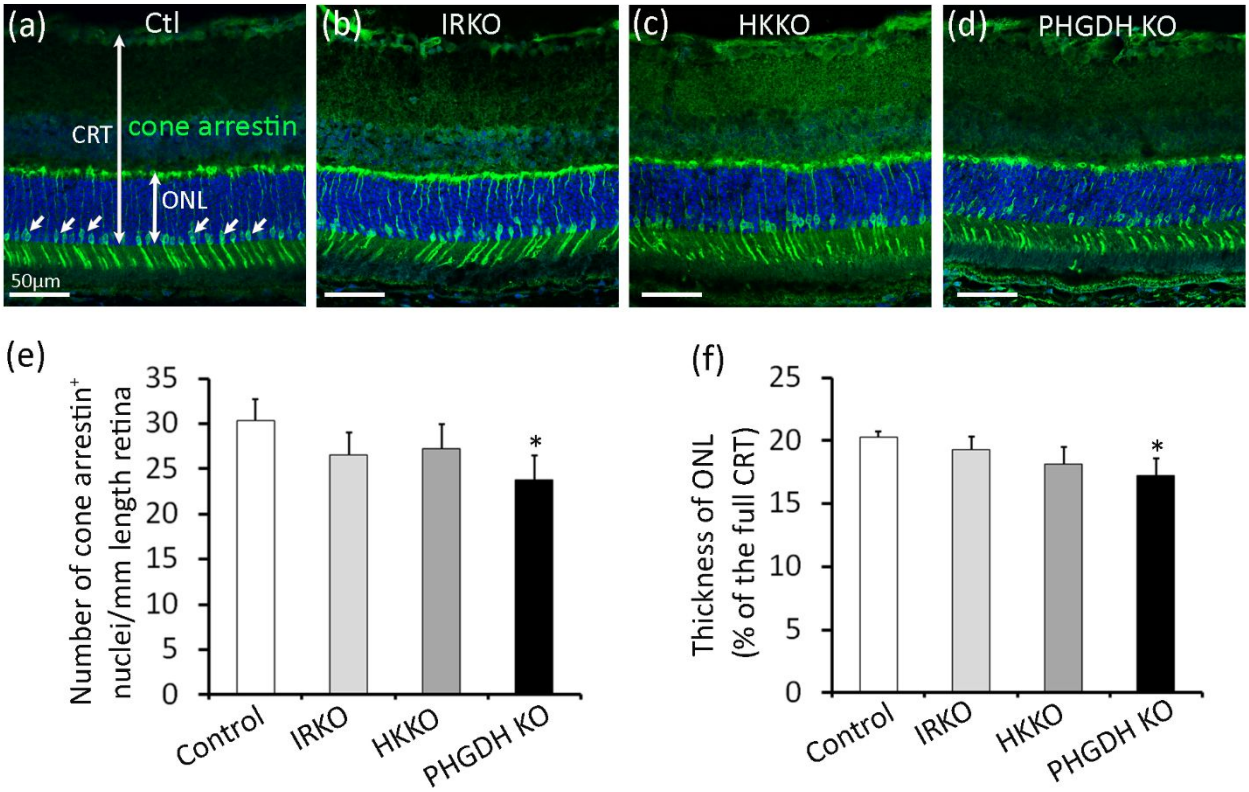

Figure _2_ SuppInfo. Changes in cone photoreceptors and the outer nuclear layer (ONL) 4 weeks after knocking down IR, HK2 or PHGDH in Müller glia. (a-d) Immunostaining for cone arrestin (green) in MC-CreER control (a), MC-IRKO (b), Mc-HKKO (c) and MC-PHGDH KO (d) mice 4 weeks after TMX treatment. (e) Quantitative analyses of the number of cone arrestin-positive cells in the ONL as indicated by the arrows in (a). (f) Changes in the thickness of the ONL after normalization to the thickness of the central retinal thickness (CRT). *P<0.05, n=6 in control, MC-IRKO and MC-HKKO groups and n=9 in MC-PHGDH KO group, respectively.

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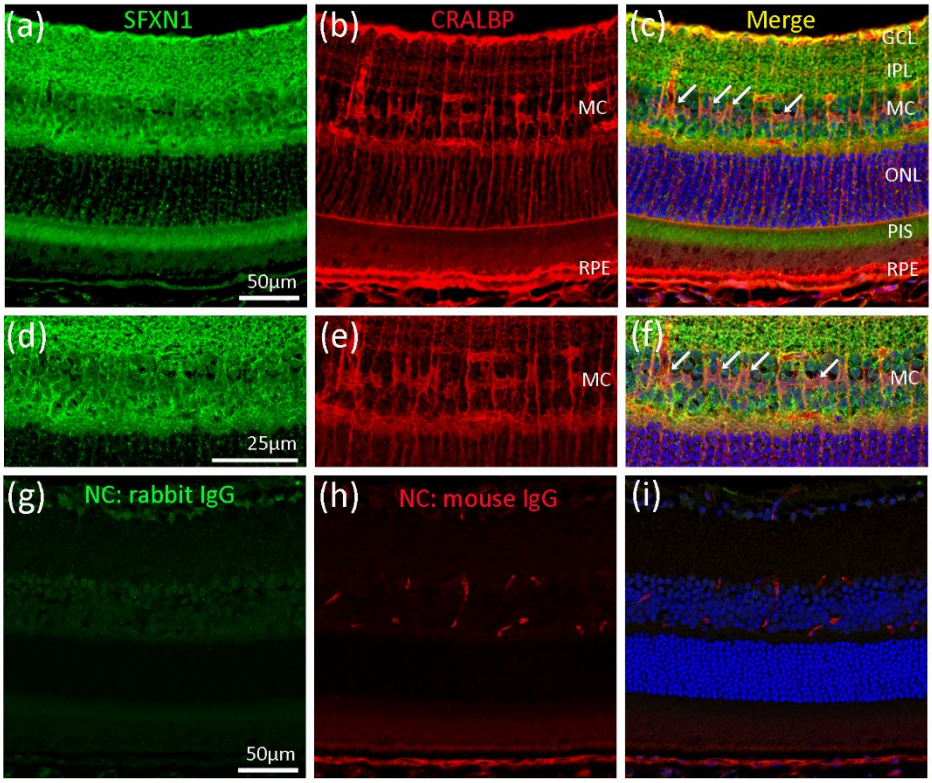

Figure _3_ SuppInfo. Expression of sideroflexin 1 (SFXN1), a mitochondrial serine transporter (Kory et al., 2018), in the mouse retina. (a-f) Double label IHC for SFXN1 and CRALBP indicated that SFXN1 was not expressed by Müller glia (arrows in c and f) and the retinal pigment epithelium (RPE) but strongly expressed in photoreceptor inner segments (PIS), some neurons in the inner nuclear layer (INL), the inner plexiform layer and the ganglion cell layer (GCL. (d-f) Higher power images of the INL in (a-c). (g-i) Negative controls in which the primary Abs were omitted but with the same secondary Abs as used in (a-f).

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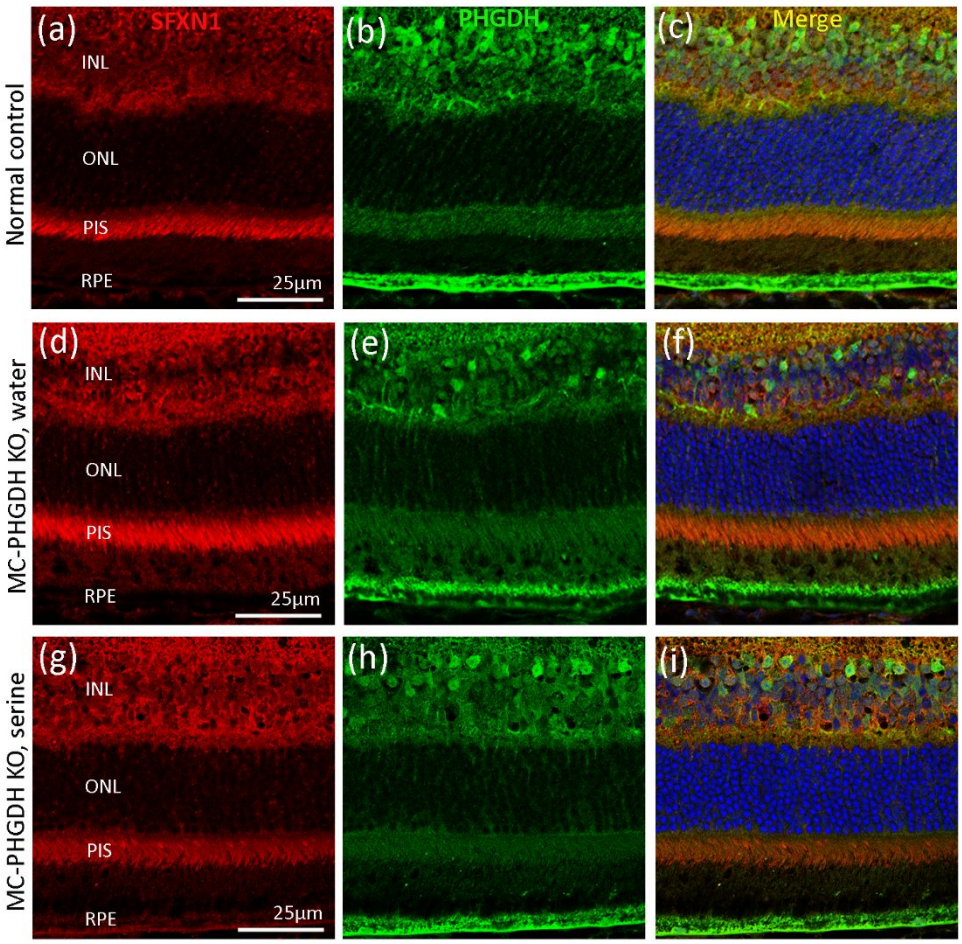

Figure _4_ SuppInfo. Knocking down PHGDH in Müller glia led to increased expression of SFXN1 in photoreceptor inner segments (PIS) while this effect was reduced by L-serine treatment. (a-i) Double label IHC for SFXN1 (red) and PHGDH (green) in the retinas of normal mice (a-c) and MC-PHGDH KO mice receiving normal drinking water (d-f) or treated with L-serine for 8 weeks (g-i). INL=inner nuclear layer, ONL=outer nuclear layer, RPE=retinal pigment epithelium.

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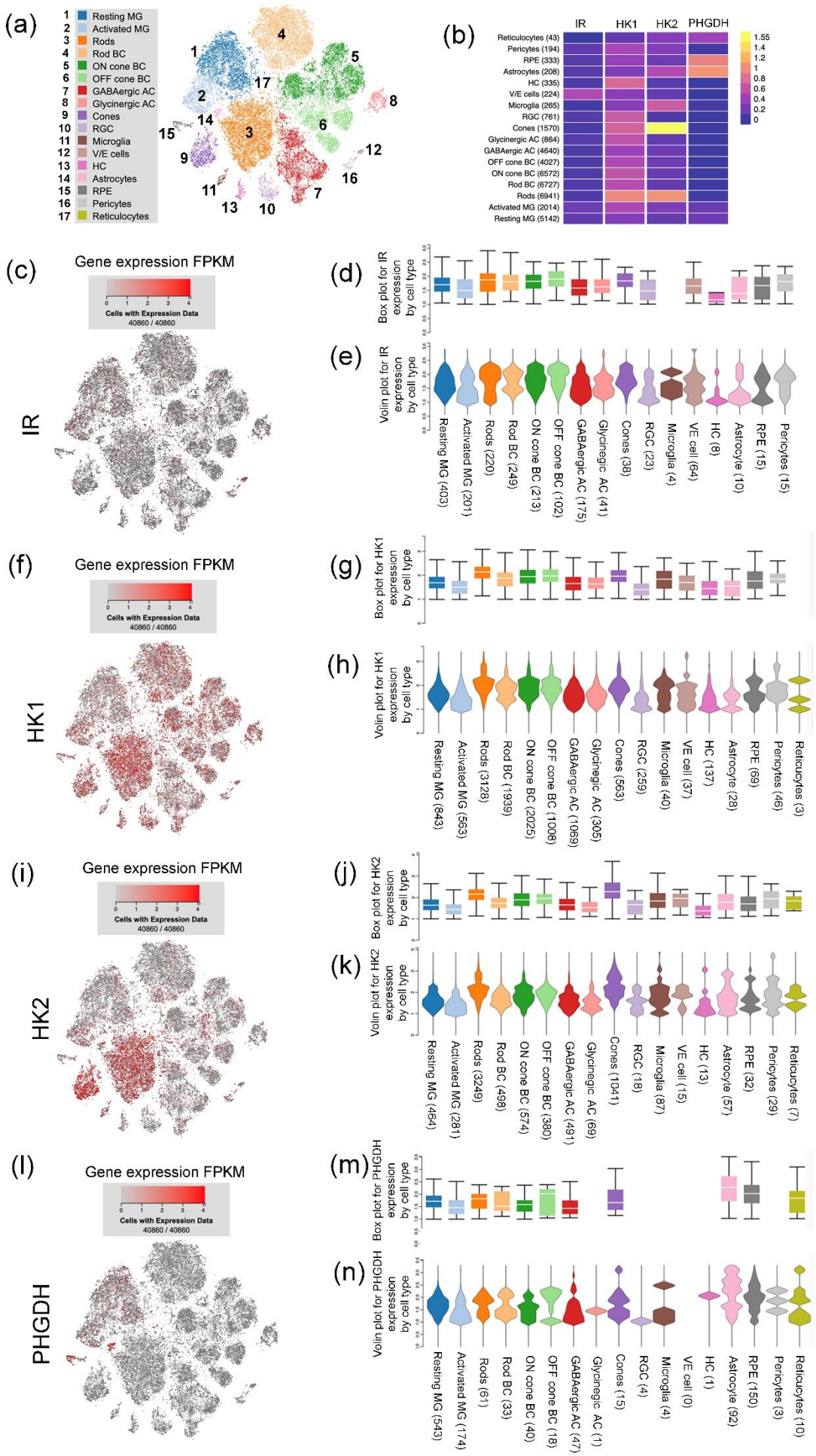

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Figure _5_ SuppInfo. Single-cell RNA-seq analyses of gene expression profiles of IR, HK1, HK2 and PHGDH in the adult mouse retina using a publicly available database deposited at https://proteinpaint.stjude.org/F/2019.retina.scRNA.html (Hoang et al., 2020). (a) 17 types of retinal cells including resting and activated Müller glia were included in the single-cell RNA-seq analysis. (b) Heatmap for expression of IR, HK1, HK2 and PHGDH in these 17 types of retinal cells. (c,f,i,l) Stochastic neighbor embedding (t-SNE) plots of gene expression distribution in each single cell type as indicated in (a). (d,e,g,h,j,k,m,n) Levels of expression of IR, HK1, Hk2 and PHGDH in different types of retinal cells presented with box plots (d,g,j,m) and violin plots (e,h,k,n). FPKM: Fragments per kilo base per million mapped reads.

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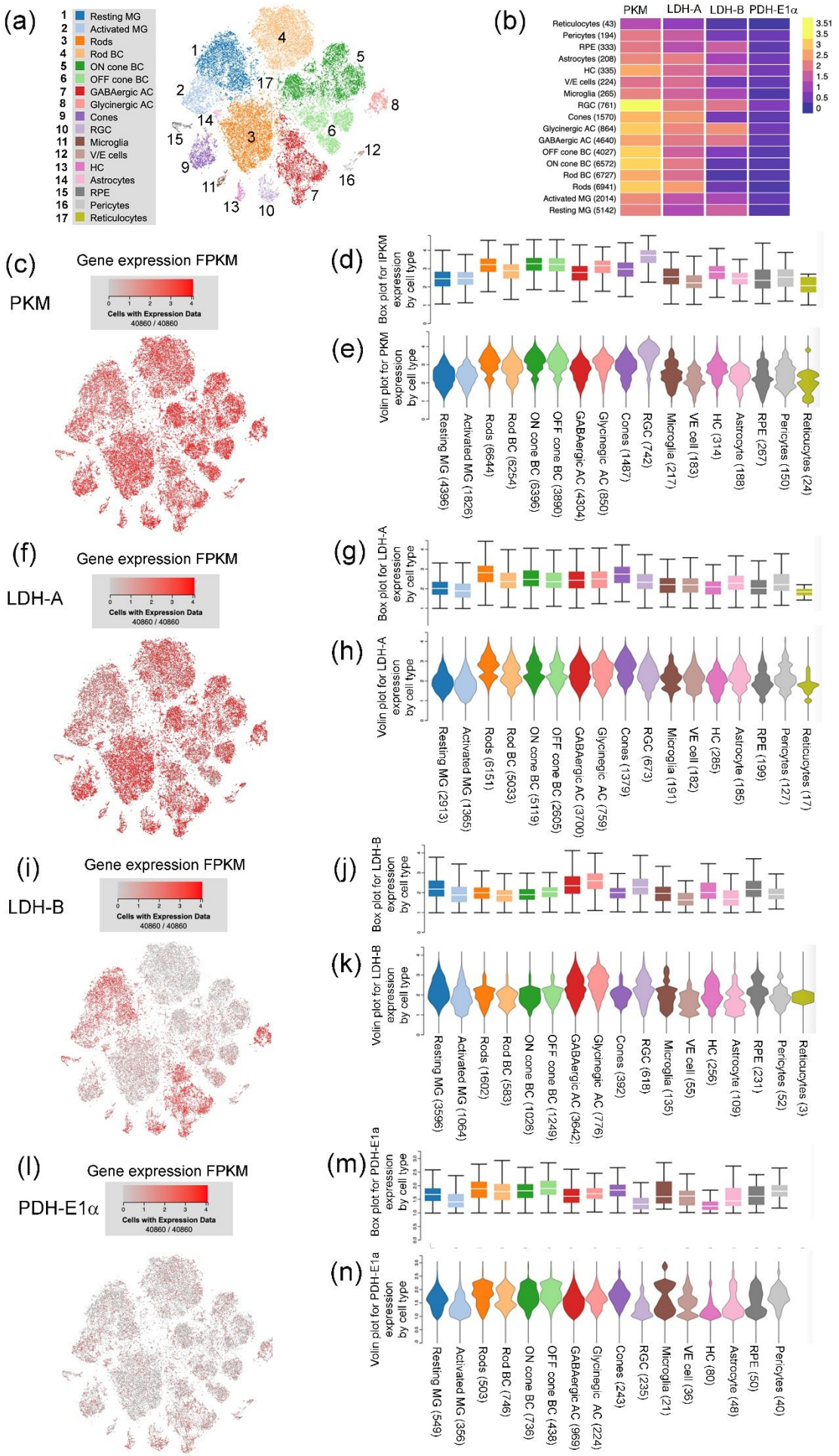

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Figure _6_ SuppInfo. Single-cell RNA-seq analyses of gene expression profiles of pyruvate kinase M isoform (PKM), LDH-A, LDH-B and PDH-E1α in the adult mouse retina using a publicly available database deposited at https://proteinpaint.stjude.org/F/2019.retina.scRNA.html (Hoang et al., 2020). (a) 17 types of retinal cells including resting and activated Müller glia were included in the single-cell RNA-seq analysis. (b) Heatmap for expression of PKM, LDH-A, LDH-B and PDH-E1α in different types of retinal cells. (c,f,i,l) Stochastic neighbor embedding (t-SNE) plots of gene expression distribution in each single cell type as indicated in (a). (d,e,g,h,j,k,m,n) Levels of gene expression in different types of retinal cells presented with box plots (d,g,j,m) and violin plots (e,h,k,n). FPKM: Fragments per kilo base per million mapped reads.

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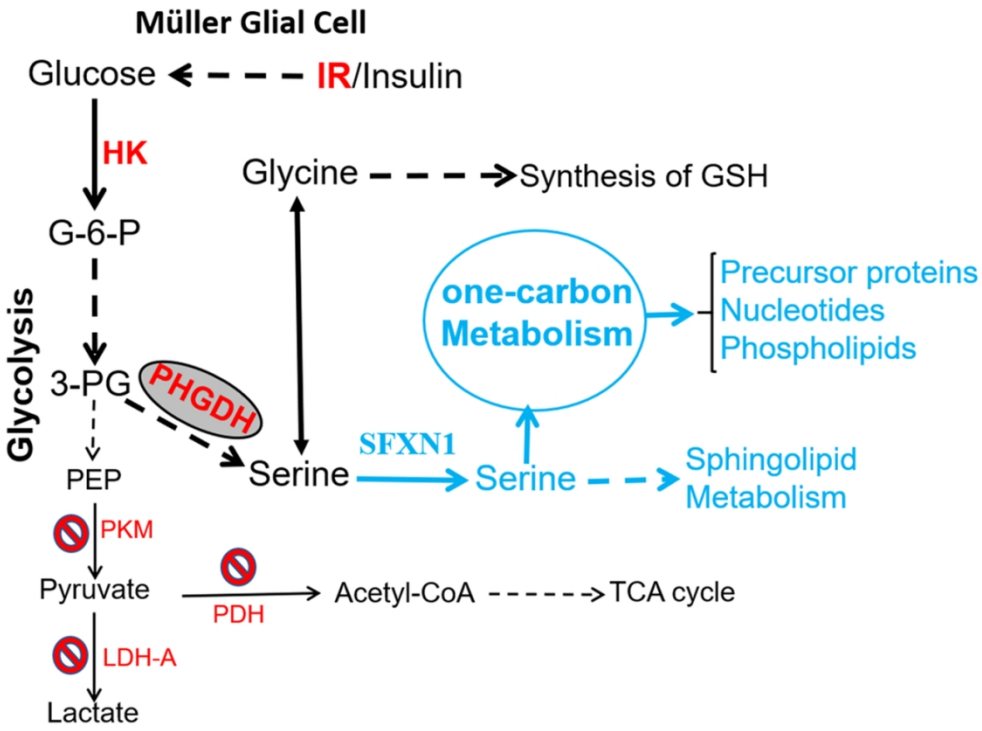

Table of Contents Image: Müller glia do not complete glycolysis but use glucose to produce serine to support photoreceptors in the retina.

56x42mm (600 x 600 DPI)

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