Supplementary MATERIALS AND METHODS

Mouse monoclonal antibodies to β-actin (clone AC15) and GAPDH (clone 6C5) were used as loading controls in western blotting. As expected, they provided single protein bands located at 42 kDa and 35 kDa, respectively.

Mouse anti-calretinin (clone 6B8.2) was used to label amacrine cells and RGCs, as has previously been shown in rat retina\(^1\). Specificity within the present study was confirmed by the characteristic pattern of labelling observed.

Mouse anti-PKCα (clone MC5) is a widely used marker of rod bipolar cells in various species including rat\(^2\)\(^,\)\(^3\) and marmoset\(^4\). Western blotting of rat retinal extracts reveals a single protein band of the expected molecular weight, 80 kDa\(^5\).

Mouse anti-glutamine synthetase (clone GS-6) was used to label Müller cells, as has been previously shown in rodent retinas\(^6\)\(^,\)\(^7\). It recognises a single protein band of 45 kDa on immunoblots from rat brain (see manufacturer’s data sheet) and in mouse retina\(^8\). The same antibody was also used to label optic nerve oligodendrocytes\(^9\)\(^,\)\(^10\).

HEK-293T, NIH3T3 and rMC-1 cells were cultured under standard conditions as described\(^11\). Treatments with hypoxia (<1% oxygen) or dimethyloxalyl glycine (DMOG) were for 16 h.

Primary Müller cells were derived and cultured as described\(^12\).

Immunocytochemistry (Fig TK2 C) was performed as previously described\(^13\). In brief, primary Müller cells cultured on coverslips were fixed with neutral-buffered formalin for 15 minutes, washed with PBS and incubated in PBS containing 0.1% Triton X-100. Cells were then washed and blocked with 3.3% horse serum (HS). Visualization of antigens was performed using the same procedure as double labeling fluorescent immunohistochemistry in the MATERIALS AND METHODS section, with DAPI staining. Primary antibodies used were anti-PKM2 antibody (Cell
Signaling Technology, 3198S) (1:250 in PBS-HS) and anti-vimentin antibody (DAKO) (1:5000 in PBS-HS).

Western blotting (Fig TK1 and TK2 A, B) was performed using whole cell extracts in 20 mM HEPES pH 7.8, 0.42 M NaCl, 0.5% Igepal, 25% glycerol, 0.2 mM EDTA, 1.5 mM MgCl₂ with 1 mM DTT, 1 mM PMSF, 1x protease inhibitor cocktail and 100 μM dipyridyl made from near-confluent cells. Proteins were separated on 7.5% SDS-PAGE, transferred to nitrocellulose membranes, blocked with 10% skim milk for 1 h, incubated overnight with a primary antibody diluted in 2% skim milk, and incubated with an appropriate horseradish peroxidase conjugated secondary antibody in 2% skim milk for 1 h before developing using enhanced chemiluminescence. Primary antibodies were anti-PKM2 antibody (Cell Signaling Technology, 3198S) (1:1000), anti-PKM2 antibody (Novus Biologicals, NBP1-48351) (1:500) and anti-beta actin antibody (abcam, ab8227) (1:2000).

References


