

## Supplementary figure legend

### **Figure S1: cZNF609 silencing does not affect retinal amacrine cells, photoreceptors, and bipolar cells in EVL-treated retinas**

Three months after EVL, retinal slices were immunolabeled for the marker proteins, including Calretinin (A), Rhodopsin (B), and PKC $\alpha$  (C). Quantitative analysis showed that cZNF609 silencing did not affect retinal amacrine cells, photoreceptors, and bipolar cells (n=5, \* $P$ <0.05 versus Scr shRNA). Scale bar, 100  $\mu$ m. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; Scr, scrambled shRNA.

### **Figure S2: cZNF609 silencing regulates retinal reactive gliosis and RGC survival in microbeads-injected retinas**

Two months after microbead injection, retinal slices were stained with GFAP (A) , TUJ1 (B), Calretinin (C), Rhodopsin (D), and PKC $\alpha$  (E) (n=5, \* $P$ <0.05 versus Scr shRNA). Quantitative analysis showed that cZNF609 silencing affected retinal reactive gliosis and RGC survival, but did not affect retinal amacrine cells, photoreceptors, and bipolar cells in microbeads-injected retinas. Scale bar, 100  $\mu$ m. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; Scr, scrambled shRNA.

### **Figure S3: cZNF609 silencing indirectly regulates RGC function in vitro**

(A) RGCs were transfected with scrambled (Scr) shRNA, cZNF609 shRNA1, cZNF609 shRNA2, or left untreated (Ctrl) for 24 h. qRT-PCRs were conducted to detect cZNF609 expression (n=4, \* $P$ <0.05 versus Ctrl). (B) The viability of RGCs was detected using MTT method. The data was expressed as relative change

compared with Ctrl group (n=4, \* $P$ <0.05 versus Ctrl). (C) Ki67 staining was conducted to detect RGC proliferation (n=4, \* $P$ <0.05 versus Ctrl group). Scale bar: 20  $\mu$ m. (D) RGCs were co-cultured with wild-type Müller cells (Ctrl), scrambled (Scr) shRNA-transfected Müller cells, cZNF609 shRNA1-transfected Müller cells, cZNF609 shRNA2-transfected Müller cells, and then exposed to glutamate (3 mM) for 24 h. PI staining and quantitative analysis was conducted to detect apoptotic RGCs (n=4, \* $P$ <0.05 versus Müller cell group). All data were from at least three independent experiments.

**Figure S4: cZNF609 silencing affects Müller cell activation in vivo**

(A) Two months after microbead injection, retinal slices were stained with PCNA and GS antibody to detect Müller cell proliferation. (B) Retinal slices were stained with the progenitor marker, Nestin, to determine the effect of cZNF609 silencing on the reactivation of stem and progenitor properties of glial cells. Scale bar, 100  $\mu$ m. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; Scr, scrambled shRNA; n=5; \* $P$ <0.05 versus Scr shRNA.

**Figure S5 : cZNF609-miR-615-METR N crosstalk occurs in Müller cells**

(A) Müller cells were transfected with miR-615 mimic, scrambled (Scr) miRNA mimic, or left untreated (Ctrl) for 24 h. qRT-PCRs were conducted to detect METRN expression (n=4, \* $P$ <0.05 versus Ctrl group). (B) Müller cells were co-transfected LUC-METR N with or without miR-615 mimic or scrambled (Scr) miRNA mimic. Luciferase activity was detected by the dual luciferase assay 24 h after transfection (n=4, \* $P$ <0.05 versus Ctrl group). (C) Müller cells were transfected with scrambled

(Scr) shRNA, cZNF609 shRNA, or left untreated (Ctrl) for 24 h. qRT-PCRs were conducted to detect METRN expression (n=4, \* $P < 0.05$  versus Ctrl group). (D) Müller cells were treated as shown for 24 h. Ki67 staining and quantitative analysis was conducted to detect cell proliferation. \* $P < 0.05$  versus Ctrl group; # $P < 0.05$  cZNF609+vector group versus cZNF609+METRN group.

## **Supplementary methods**

### **Intravitreal injection**

The injections were performed under the general anesthetic condition by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (75 mg/kg). Before virus injection, a 30-gauge needle was used to perforate the sclera to form a tunnel about 1.5 mm behind the limbus. About 5  $\mu$ L ( $1 \times 10^{12}$  vg/mL) of AAV was injected into the vitreous through the tunnel, followed by 1 min holding in place and slow withdrawing. The antibiotic ointment was used to prevent ocular infection.

### **MTT assay**

3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl-tetrazolium-bromide assay (MTT) was used to detect cell viability. In brief, Müller cells or RGCs were seeded in 96-well plates at a density of  $1 \times 10^4$  cells per well. After the specific stress treatment and transfection, they were incubated with MTT (0.5 mg/mL) for 3 h at 37°C. The formazan crystals were dissolved by DMSO solution (100 mM). Finally, the absorbance was detected by a microplate reader (Molecular Devices) at 570 nm wavelength.

### **Propidium iodide (PI) staining**

Cell apoptosis was detected by PI staining. After the required treatment, the cells were fixed in 4% PFA for 15 min, and then stained with PI (10  $\mu\text{mol/l}$ ) for 15 min at room temperature. Finally, these cells were stained with DAPI (0.5  $\mu\text{g/ml}$ ) to show cell nuclei.

### **Quantitative real-time PCR**

Total RNAs from the rat retinas or clinical samples were isolated by the Trizol reagent (Life Technologies, Carlsbad, CA), followed by the reverse transcription to cDNAs using the PrimeScript™ RT Master Mix (Takara, Dalian, China). Quantitative real-time PCRs were performed by SYBR Green PCR kit (Toyobo, Osaka, Japan). Relative gene expression was calculated by the  $2^{-\Delta\Delta\text{Ct}}$  method.

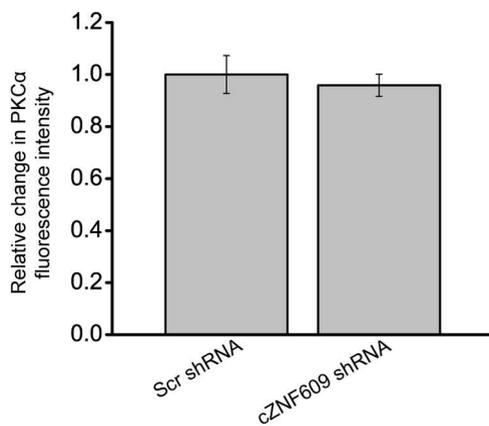
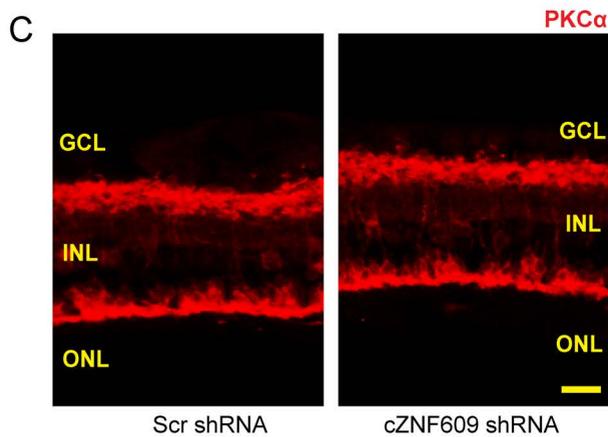
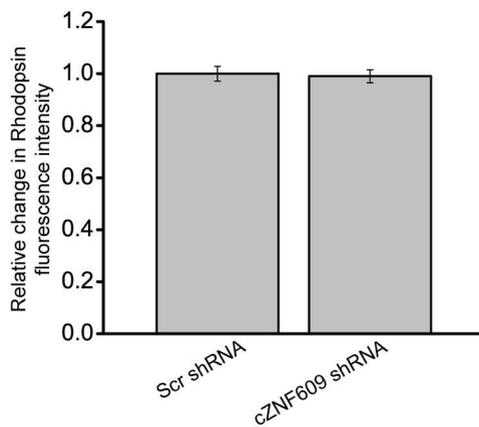
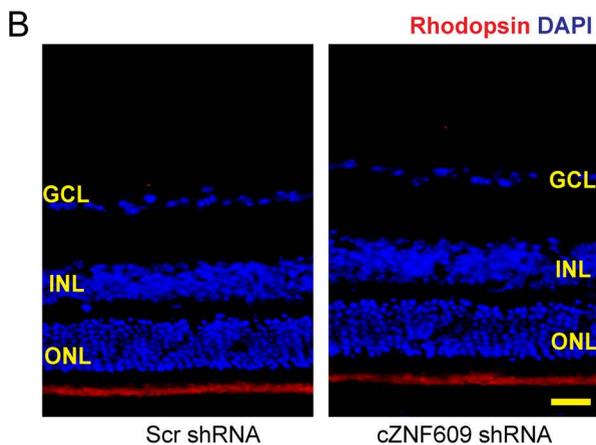
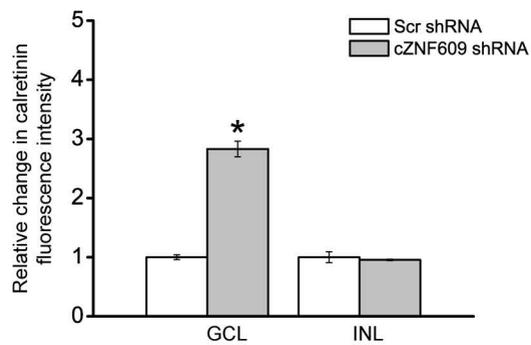
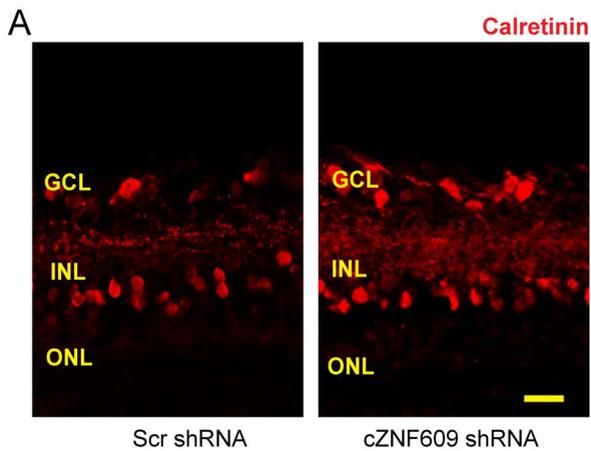
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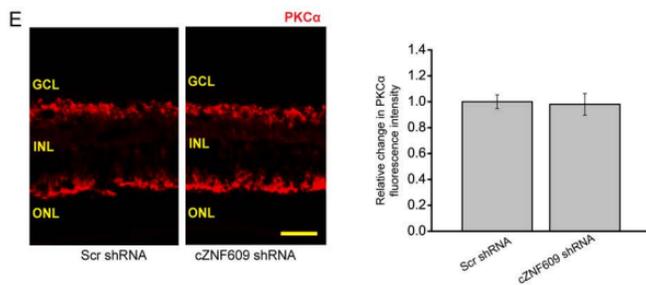
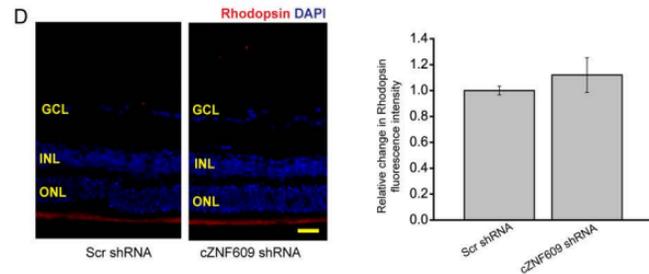
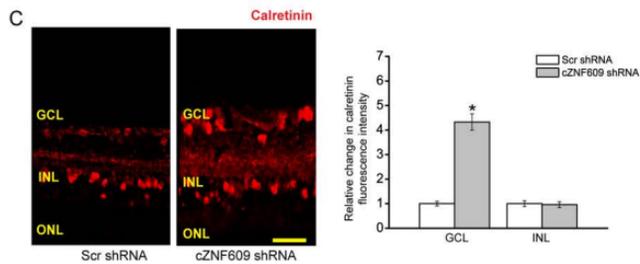
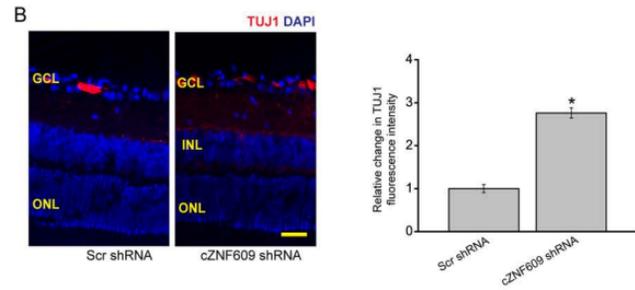
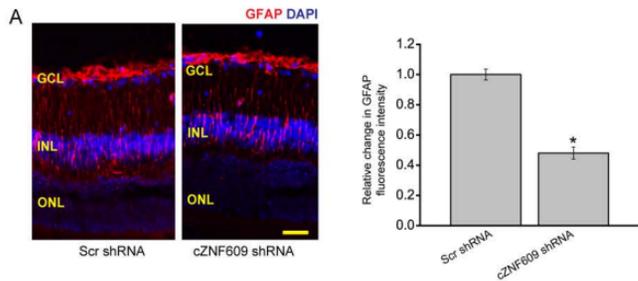
Cell proliferation was detected by Ki67 staining. After the required treatment, cells were fixed with 4% PFA, permeabilized with 0.25% Triton X-100, and then blocked with 5% BSA in PBS. These cells were incubated with Ki67 antibody overnight at 4°C, and then incubated with the secondary antibody for 3 h at room temperature. They were stained with DAPI (0.5  $\mu\text{g/ml}$ ) to show cell nuclei.

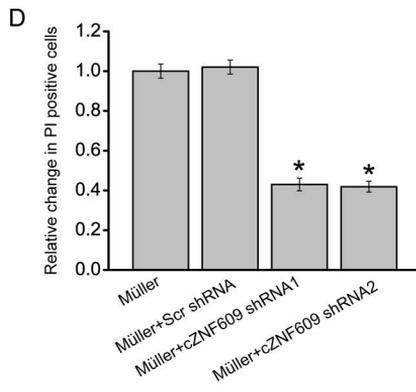
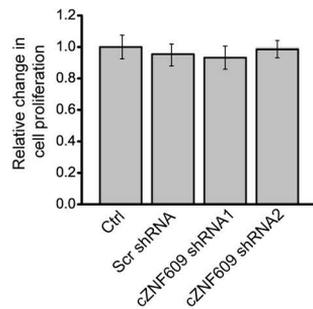
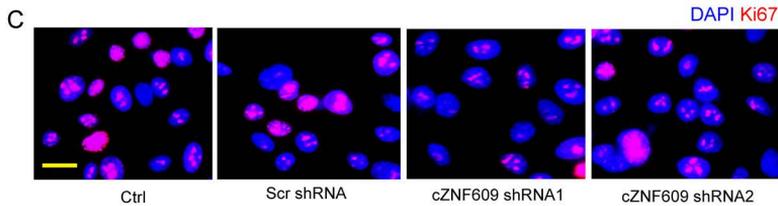
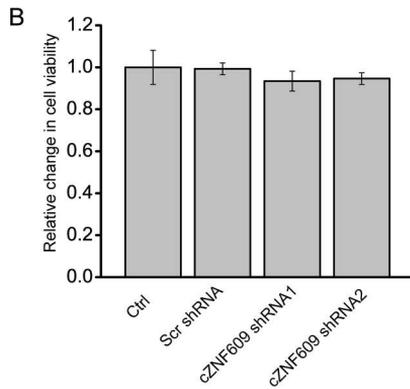
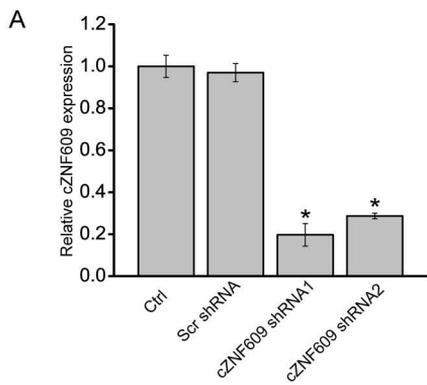
### **Luciferase reporter assay**

The 3'-untranslated regions (UTR) of METRN or the full sequence of cZNF609 was cloned into the downstream of the firefly luciferase gene in pGL3 vector (Promega). Cells were seeded in 96-well plates at a density of  $1 \times 10^4$  cells per well before transfection. The cells were co-transfected with a mixture of firefly luciferase

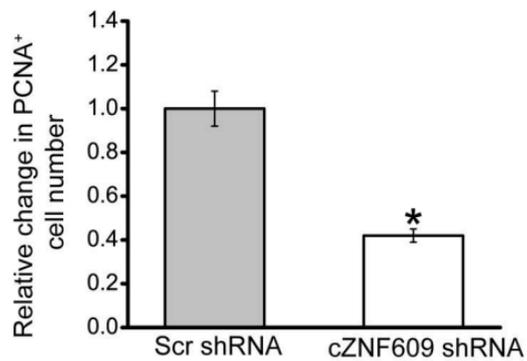
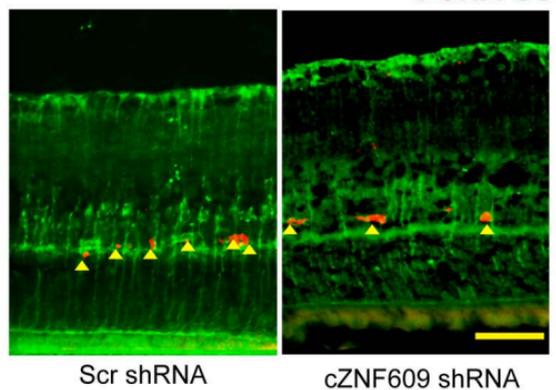
reporter vectors, Renilla luciferase (RL) reporter vectors (pRL-TK), and miRNA mimics. After 24 h transfection, the luciferase activity was detected by the dual luciferase reporter assay system (Promega).



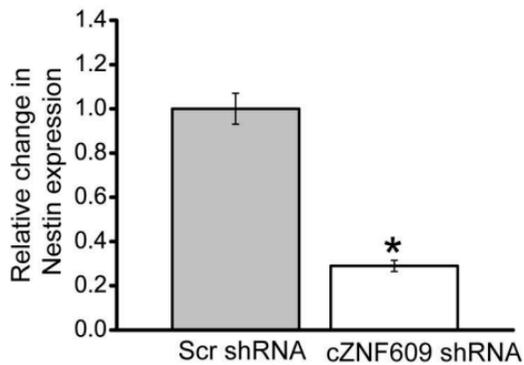
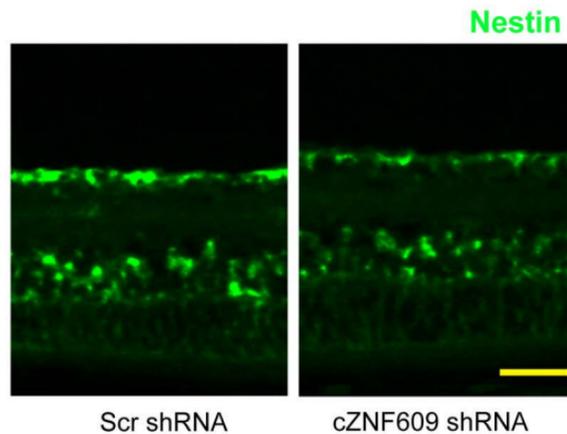


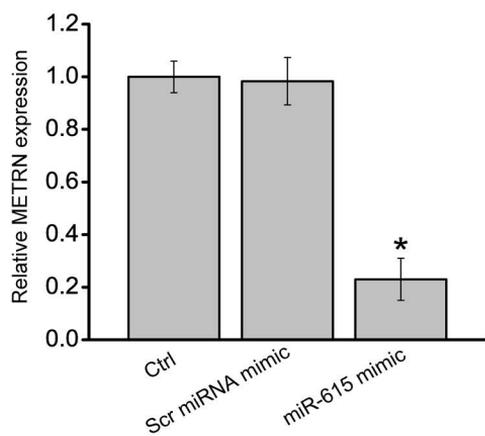
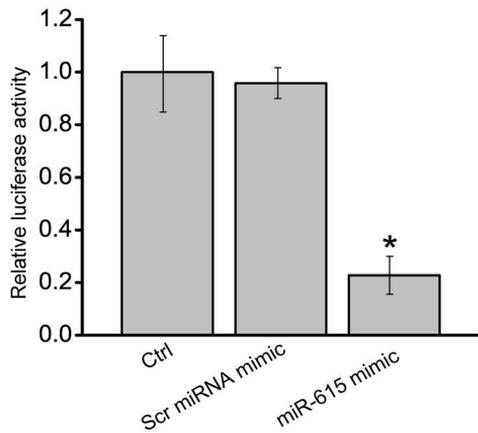
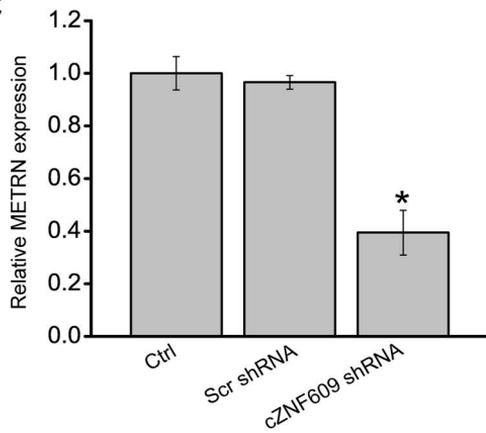


A



B



**A****B****C****D**