**Supplementary materials and methods**

**Quantitative real-time PCR**

Total RNAs were extracted using Trizol reagent (Invitrogen, Carlsbad, CA), and then reversely transcribed to cDNAs using the reverse transcription kit (Takara, Dalian, China). Quantitative real-time PCRs were conducted using SYBR Green PCR kit (Toyobo, Osaka, Japan) in the Applied Biosystems 7300 Real Time PCR system (Applied Biosystems, Foster City, CA). The $2^{-\Delta\Delta Ct}$ method was used to determine relative gene expression. For the absolute quantification, the purified PCR product amplified from cDNA corresponding to cZNF609 transcitp was serially diluted to generate a standard curve.

**Intravitreal injection**

At the beginning of diabetes induction, C57BL/6 mice (8-week old, male) were anesthetized by i.p. injection (IP) of 20 mg/kg ketamine and 6 mg/kg xylazine. About 1 μL ($1\times10^{10}$ viral particles/mL) of adeno-associated virus (AAV) containing cZNF609 shRNA or scrambled shRNA was delivered into the vitreous cavity using a 33-gauge needle. To maximize virus delivery, these mice were injected every two weeks.

The pups of C57BL/6 mice at P5 were anesthetized with 2% isoflurane, and a drop of 0.5% proparacaine was administered as a topical local anesthesia. A fine glass micropipette connected to a 10 μl Hamilton glass syringe was inserted through the incision in the cornea and slid between the iris and the lens into the posterior chamber of the eye. Each eye was received about 0.5 μl adeno-associated virus. Injections were

administered slowly over approximately one minute and monitored using a stereo microscope.

**MTT assay**

Cell viability assays were conducted using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Briefly, the treated cells were incubated with MTT for 3 h at 37 ℃. DMSO solution was then added to dissolve formazan crystals after medium removal. The absorbance was detected at 570 nm wavelength using a microplate reader (Molecular Devices).

**Propidium iodide (PI) staining**

Cell apoptosis was determined using PI staining. The cells were washed twice in cold 1 × PBS, and then stained with PI for 15 min at room temperature in the dark. Finally, these cells were stained with DAPI (0.5 μg/ml) to visualize cell nuclei.

**EdU assay**

Cell proliferation was detected using EdU (5-ethynyl-2’-deoxyuridine) DNA Cell Proliferation Kit (RiboBio, Guangzhou, China). Briefly, HUVECs were seeded in each well of 96-well plates, and transfected with the corresponding siRNA or vector for 48 h. These cells were incubated with EdU (50 mM) for 2 h, fixed with 4% paraformaldehyde, and stained using Apollo Dye Solution for the proliferating cells. Cell nuclei were stained with Hoechst 33342.

**Tube formation assay**

The formation of capillary-like structures was assessed in a 24-well plate using growth factor-reduced Matrigel (BD Biosciences). After the specific treatment,

HUVECs ($1\times10^5$ cells/well) were plated onto Matrigel (300 μl/well). After 24-h culture, these cells were observed using a bright-field microscope. The tube length was quantified using Image J software.

**Cell migration assay**

Transwell migration assays were performed in a modified Boyden-type blind well chamber. HUVECs were seeded into the upper well, and allowed to invade through the transwell plate for 8 h. Non-migrated cells from the upper surface of the filter were scraped off with a cotton bud. The cells on the inserts were fixed with 4% paraformaldehyde, stained with crystal violet, and counted by a light microscope. Cell migration was expressed as fold change of the number of migrating cells through transwell plate.

**Caspase-3 activity assay**

Caspase-3 activity was determined by the caspase-3 activity kit (Beyotime, China). After the required treatment, HUVECs were homogenized in the reaction buffer (1% NP-40, 20 mM Tris-HCl (pH 7.5), 137 mM Nad and 10% glycerol) containing 10 ml caspase-3 substrate (Ac-DEVD-pNA) (2 mM). Lysates were incubated with caspase-3 substrate at 37°C for 1.5 h. Samples were detected using a microplate reader (Molecular Devices) at 405 nm.

**Immunofluorescence experiment**

HUVECs or flat-mounted retinas were fixed in 4% paraformaldehyde (PFA), permeabilized with 0.1% Triton X-100 for 15 min, and blocked in a solution containing 5% BSA in PBS. These cells were incubated with the primary antibody

overnight at 4°C, and then incubated with the secondary antibody conjugated with FITC or Cy3 (Invitrogen) for 3 h at room temperature. DAPI (0.5 μg/ml) was stained to visualize cell nuclei.

**RNA fluorescent *in situ* hybridization (RNA-FISH)**

HUVECs were fixed in 4% PFA, and dehydrated following serial incubations in 70%, 80%, 95%, and 100% ethanol. Fluorescent probes were heat denatured and incubated with HUVECs at 37°C overnight in a humidified chamber. Then, slides were washed with saline sodium citrate buffers. Cell nuclei were counterstained with DAPI. Cy3-labeled sense (negative control) and antisense probes were used for RNA-FISH analysis.

**Dual luciferase activity assay**

The 3′-UTR or mutant 3′-UTR of IGF2, Tie2, MEF2A or cZNFP609 containing the putative target site for miR-615-5p was inserted into the downstream of the luciferase gene in the pGL3-control vector (Promega, Madison, WI, USA). HUVECs were plated at $2 \times 10^5$ cells/well in 24-well plates. Two hundred nanograms of pGL3-vector containing corresponding gene sequence were transfected in combination with miR-615 mimic. The luciferase activity was measured 24 h after transfection using the Dual Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to *Renilla luciferase* activity for each transfected well.

**Biotin-coupled miRNA capture**

The 3’ end biotinylated miR-615-5p or control mimic RNA were transfected into

HUVECs at the concentration of 20 nM for 24 h. The biotin-coupled RNA complex was pull-downed by incubating cell lysates with streptavidin-coated magnetic beads (Life Technologies). cZNF609 abundance in the bound fraction was detected by qRT-PCRs.

**RNA binding protein immunoprecipitation assay (RIP)**

HUVECs were washed in ice-cold PBS, lysed in 500 μl buffer (20 mM Tris-CL, pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 0.5% NP-40, and 5μg/ml aprotinin), and then incubated with 5 μg of the primary antibody (Ago2 or IgG) at 4℃ for 3 h. About 30 μl protein A-Sepharose was added to each sample, and the mixtures were incubated at 4℃ for 3 h. The pellets were washed with PBS and re-suspended in Trizol reagent (Invitrogen). The precipitated RNA was then subjected for qRT-PCR analysis.

**PCR primer, siRNA sequence, shRNA target sequence and FISH probe sequence**

| | | |
|---|---|---|
| Primer sequence | | |
| cZNF609 for human | Forward | 5’-CAGCGCTCAATCCTTTGGGA-3’ |
| | Reverse | 5’-GACCTGCCACATTGGTCAGTA-3’ |
| ZNF609 mRNA for human | Forward | 5’-TTTTGTTCCATTGGCGTTGCT-3’ |
| | Reverse | 5’-AATAACCAGCAGCCCTCACA-3’ |
| GAPDH mRNA for human | Forward | 5’-AAGACGGGCGGAGAGAAACC-3’ |
| | Reverse | 5’-CGTTGACTCCGACCTTCACC-3’ |
| cZNF609 for mouse | Forward | 5’- CAGCGCTCAATCCTTTGGGA-3’ |
| | Reverse | 5’-GACCTGCCTCCTTGGTCAGAA-3’ |
| ZNF609 mRNA for mouse | Forward | 5’-ATGTTCTGACCAAGGAGGCAG-3’ |
| | Reverse | 5’-TGTCTCAACCGGGTTTGCAT-3’ |
| GAPDH mRNA for mouse | Forward | 5’-CTACTCGCGGCTTTACGGG-3’ |
| | Reverse | 5’-AGGGCTGCAGTCCGTATTTA-3’ |
| siRNA sequence | | |
| cZNF609 siRNA1 | | 5’- GUCAAGUCUGAAAAGCAAUGA-3′ |
| cZNF609 siRNA2 | | 5’- GCACAAGUAGUGACUGCAA-3′ |
| cZNF609 siRNA3 | | 5’- GGAGUAAGAGUGGCAAAGA-3′ |
| shRNA target sequence | | |
| cZNF609 shRNA1 | | 5’- GUCAAGUCUGAAAAGCAAUGA-3′ |
| cZNF609 shRNA2 | | 5’- GCACAAGUAGUGACUGCAA-3′ |
| cZNF609 shRNA3 | | 5’- GGAGUAAGAGUGGCAAAGA-3′ |
| FISH probe sequence | | |
| Negative control | | GCTAAAGTCAAGTCTGAAAAGCAATGATGT TGTCCACTGG |
| cZNF609 | | CCAGTGGACAACATCATTGCTTTTCAGACTT GACTTTAGC |

**Supplementary figure legend**

**Figure S1: cZNF609 gene homology comparison**

cZNF609 homology analysis in human and mouse genome was conducted using Basic Local Alignment Search Tool (BLAST).

**Figure S2: High glucose treatment does not affect ZNF609 mRNA expression *in vivo* and *in vitro***

(A) HUVECs were incubated with the medium containing 5 mM glucose, 30 mM glucose, or 30 mM mannitol for the indicated time points. The group treated with mannitol was taken as the osmolar control. qRT-PCRs were conducted to detect ZNF609 mRNA expression (n=4, **P*<0.05 versus 5 mM glucose). (B) qRT-PCRs were conducted to detect ZNF609 mRNA expression in the retinas of C57BL/6 mice after 2, 4, and 6-months diabetes mellitus induction (n=6 animals per group, **P*<0.05 versus non-DM). (C) Neonatal C57BL/6 mice were exposed to 75% oxygen from P7 to P12, and then returned to room air. qRT-PCRs were conducted to compare ZNF609 mRNA expression between un-treated (Ctrl) and OIR retinas (n=6 animals per group, **P*<0.05 versus Ctrl). All data were from at least three independent experiments.

**Figure S3: cZNF609 shRNA injection significantly down-regulates cZNF609 expression**

(A) Diabetic C57BL/6 mice (8-week old, male) were received an intravitreous injection of shRNA1 (shRNA targeting the backsplice sequence of cZNF609), shRNA2 (shRNA targeting the sequence only existed in ZNF609 linear transcript), shRNA3 (shRNA targeting the sequence shared by the linear and circular ZNF609

transcript), or left untreated (Ctrl). qRT-PCRs were conducted to detect the expression of cZNF609 and ZNF609 mRNA after 30-day shRNA injection (n=5, *$P$<0.05). (B) Diabetic C57BL/6 mice (8-week old, male) were received an intravitreous injection of scrambled (Scr) shRNA, cZNF609 shRNA, or left untreated (Ctrl) for the indicated time points. qRT-PCRs were conducted to detect cZNF609 expression at the indicated time periods (n=5, *$P$<0.05 versus Ctrl group).

**Figure S4: cZNF609 silencing has no effect on normal retinal vascular development**

(A) Retinal whole mounts of cZNF609 silencing mice and control mice at P5 and P7 were stained with isolectin B4 for retinal vasculature. Quantification of retinal vascularized area, vascular branch point, and tip cell number was then conducted. (B and C) A representative image for isolectin B4 staining for retinal vasculature at P5 was shown. Scale bar, 200 μm (B), Scale bar, 50 μm (C). (D) Retinal vasculature was stained with isolectin B4 and NG2 to detect pericyte coverage. Scale bar, 50 μm (E) Staining of retinal slices from cZNF609 silencing mice and the matched control mice were conducted using hematoxylin-eosin at P17. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; Scale bar: 50 μm.

**Figure S5: cZNF609 silencing by siRNA3 but not siRNA2 affects endothelial cell function *in vitro***

HUVECs were transfected with scrambled siRNA (Scr), siRNA targeting the sequence only in ZNF609 linear transcript (siRNA2), siRNA targeting the sequence shared by both linear and circular ZNF609 transcript (siRNA3), or left untreated (Ctrl)

for 48 h. Cell viability was detected using MTT method. Result was shown as relative change compared with Ctrl group (A, n=4, *$P$<0.05). Cell proliferation was detected using EdU detection kits (Ribobio, Guangzhou, China) to analyze EdU incorporation during DNA synthesis (B, n=4, *$P$<0.05). Transwell assay and quantification analysis was conducted to detect the migration of HUVECs (C, n=4, *$P$<0.05). HUVECs were seeded on the matrigel matrix. The tube-like structures were observed 24 h after cell seeding. Average length of tube formation for each field was statistically analyzed (D, n=4, *$P$<0.05). All data were from at least three independent experiments.

**Figure S6: cZNF609 silencing affects endothelial cell apoptosis upon stress *in vitro***

(A) HUVECs were transfected with scrambled siRNA (Scr), cZNF609 siRNA, or left untreated, and then treated with or without $CoCl_2$ (200 μm) to mimic hypoxic stress for 48 h. (B) HUVECs were transfected with scrambled siRNA (Scr), cZNF609 siRNA, or left untreated, and then treated with or without $H_2O_2$ (100 μm) for 48 h. Apoptotic cells were analyzed using PI staining and quantified (n=4, *$P$<0.05). Scale bar: 20 μm. Data were from at least three independent experiments.

**Figure S7: cZNF609 silencing by siRNA1 decreases caspase 3 activity upon stress *in vitro***

(A) HUVECs were transfected with scrambled siRNA (Scr), cZNF609 siRNA1, or left untreated, and then treated with or without $H_2O_2$ (100 μm) for 48 h. Caspase 3 activity was determined using a commercial kit (n=4, *$P$<0.05). (B) HUVECs were transfected with scrambled siRNA (Scr), cZNF609 siRNA1, or left untreated, and

then treated with or without $CoCl_2$ (200 μm) to mimic hypoxic stress for 48 h. Caspase 3 activity was determined using a commercial kit (n=4, *$P$<0.05). Data were from at least three independent experiments.

**Figure S8: cZNF609 silencing by siRNA3 but not siRNA2 decreases cell apoptosis upon stress *in vitro***

(A) HUVECs were transfected with scrambled siRNA (Scr), cZNF609 siRNA2, cZNF609 siRNA3, or left untreated (Ctrl), and then treated with or without $H_2O_2$ (100 μm) for 48 h. Apoptotic cells were analyzed using PI staining and quantified (n=4, *$P$<0.05). (B) HUVECs were transfected with scrambled siRNA (Scr), cZNF609 siRNA2, cZNF609 siRNA3, or left untreated (Ctrl), and then treated with or without $CoCl_2$ (200 μm) for 48 h. Apoptotic cells were analyzed using PI staining and quantified (n=4, *$P$<0.05). (C and D) HUVECs were transfected with scrambled siRNA (Scr), cZNF609 siRNA2, cZNF609 siRNA3, or left untreated, and then treated with $H_2O_2$ (100 μm, C) or $CoCl_2$ (200 μm, D) for 48 h. Caspase 3 activity was detected using a commercial kit. Data were from at least three independent experiments.

**Figure S9: cZNF609 overexpression affects endothelial cell function under basal condition and high glucose condition *in vitro***

(A-D) HUVECs were transfected with pcDNA3.0 (vector), pcDNA3.0-cZNF609, or left untreated (Ctrl) for 48 h. Cell viability was detected using MTT assays (A, n=4, *$P$<0.05 versus Ctrl group). Cell proliferation was detected using EdU detection kits (Ribobio, Guangzhou, China) to analyze the incorporation of EdU during DNA

synthesis (B, n=4, *$P$<0.05 versus Ctrl group). Transwell assay and quantification analysis was conducted to detect the migration of HUVECs (C, n=4, *$P$<0.05 versus Ctrl group). HUVECs were seeded on the matrigel matrix. The tube-like structures were observed 24 h after cell seeding. Average length of tube formation for each field was statistically analyzed (D, n=4, *$P$<0.05 versus Ctrl group). (E and F) HUVECs were transfected with pcDNA3.0 (vector), pcDNA3.0-cZNF609, or left untreated (Ctrl) for 48 h, and then treated with $H_2O_2$ (100 μm, E) or $CoCl_2$ (200 μm, F) for 48 h. Caspase 3 activity was determined using a commercial kit. All data were from at least three independent experiments.

**Figure S10: Prediction of cZNF609-interacting miRNAs**

CircNet database was used to predict potential miRNAs which can bind to cZNF609.

**Figure S11: miR-615-5p regulates endothelial cell function upon hypoxia stress *in vitro***

HUVECs were transfected with scrambled mimic (Scr), miR-615-5p mimic, miR-615-5p mimic plus cZNF609 siRNA, or left untreated, and then treated with or without $CoCl_2$ (200 μm) for 48 h (n=4, *$P$<0.05). "$^{\#}$" indicated significant difference between the marked groups. Apoptotic cells were analyzed using PI staining and quantified. Scale bar: 20 μm. Data were from at least three independent experiments.

**Figure S12: miR-615-5p/cZNF609 interaction is involved in regulating endothelial cell function *in vitro***

(A) HUVECs were transfected with scrambled mimic (Scr), miR-615-5p mimic, miR-615-5p mimic plus cZNF609 siRNA, or left untreated, and then treated with or

without $H_2O_2$ (100 μm) for 48 h. Caspase 3 activity was determined using a commercial kit (n=4, *$P$<0.05). "#" indicated significant difference between the marked groups. (B) HUVECs were transfected with scrambled mimic (Scr), miR-615-5p mimic, miR-615-5p mimic plus cZNF609 siRNA, or left untreated scrambled siRNA (Scr), and then treated with or without $CoCl_2$ (200 μm) to mimic hypoxic stress for 48 h. Caspase 3 activity was determined using a commercial kit (n=4, *$P$<0.05). "#" indicated significant difference between the marked groups. Data were from at least three independent experiments.

**Figure S13: cZNF609 overexpression rescues the effect of miR-615-5p mimic transfection on HUVEC function *in vitro***

(A and B) HUVECs were treated as shown, and then treated with or without $H_2O_2$ (100 μm) or $CoCl_2$ (200 μm) for 48 h. Caspase 3 activity was determined using a commercial kit (n=4, *$P$<0.05). (C and D) HUVECs were treated as shown, transwell assays and martrigel assays were conducted to detect HUVEC migration and tube formation ability (n=4, *$P$<0.05). "#" indicated significant difference between the marked groups. All data were from at least three independent experiments.

**Figure S14: miR-615-5p inhibitor transfection affects HUVEC function *in vitro***

(A and B) HUVECs were transfected with scrambled inhibitor (Scr), miR-615-5p inhibitor, or left untreated, and then treated with or without $H_2O_2$ (100 μm) or $CoCl_2$ (200 μm) for 48 h. Caspase 3 activity was determined using a commercial kit (n=4, *$P$<0.05). (C and D) HUVECs were transfected with scrambled inhibitor (Scr), miR-615-5p inhibitor, or left untreated (Ctrl). Transwell assays and martrigel assays

were conducted to detect HUVEC migration and tube formation ability (n=4, **P*<0.05). All data were from at least three independent experiments.

**Figure S15: miR-615-5p mimic transfection has no effect on FGF2, NOTCH1, CXCR4, LRP5, and LRP6 expression**

HUVECs were transfected with miR-615-5p mimic, scrambled (Scr) miRNA mimic, or left untreated (Ctrl) for 48 h. qRT-PCRs were conducted to detect FGF2, NOTCH1, CXCR4, LRP5, and LRP6 expression (n=4, **P*<0.05).

**Figure S16: MEF2A but not Tie2 or IGF2 overexpression rescues cZNF609 silencing-mediated effects on HUVEC function**

(A and B) HUVECs were treated as shown for 48 h. Cell migration and tube formation was detected using transwell assay and matrigel assay, and then quantification analysis was conducted (n=4, **P*<0.05, $^{\#}$*P*<0.05). "*" indicated significant difference compared with Ctrl group. "$^{\#}$" indicated significant difference between the marked groups. *NS* indicated no significant difference. (C and D) HUVECs were treated as shown for 48 h. Cell apoptosis was determined using PI staining and caspase 3 activity kit. "*" indicated significant difference compared with the group only treated with $CoCl_2$ to mimic hypoxic stress. "$^{\#}$" indicated significant difference between the marked groups (n=4, **P*<0.05, $^{\#}$*P*<0.05).

**Figure S17: Detection of miR-615-5p expression in clinical samples**

(A) qRT-PCRs were performed to detect miR-615-5p expression in the fibrovascular membranes of diabetic patients (n=15) and idiopathic epiretinal membranes of non-diabetic patients (n=10) (**P*<0.05 versus non-diabetic controls). (B) qRT-PCRs

were conducted to detect miR-615-5p expression in EDTA-plasma obtained from diabetic patients (n=30) and non-diabetic controls (n=30) (*$P$<0.05 versus non-diabetic controls). (C) miR-615-5p expression in EDTA-plasma obtained from the patients with CAD (n=15) and healthy volunteers (HC; n=10) was determined by qRT-PCRs. (D) miR-615-5p expression in EDTA-plasma obtained from patients with hypertension (n=30) and healthy volunteers (HC; n=30) was determined by qRT-PCRs.

**Figure S18: Detection of cZNF609 and miR-615-5p expression abundance in mouse retina**

qRT-PCRs were conducted to detect relative expression abundance of cZNF609 and miR-615-5p in mouse retinas (n=4).

Fig. S1

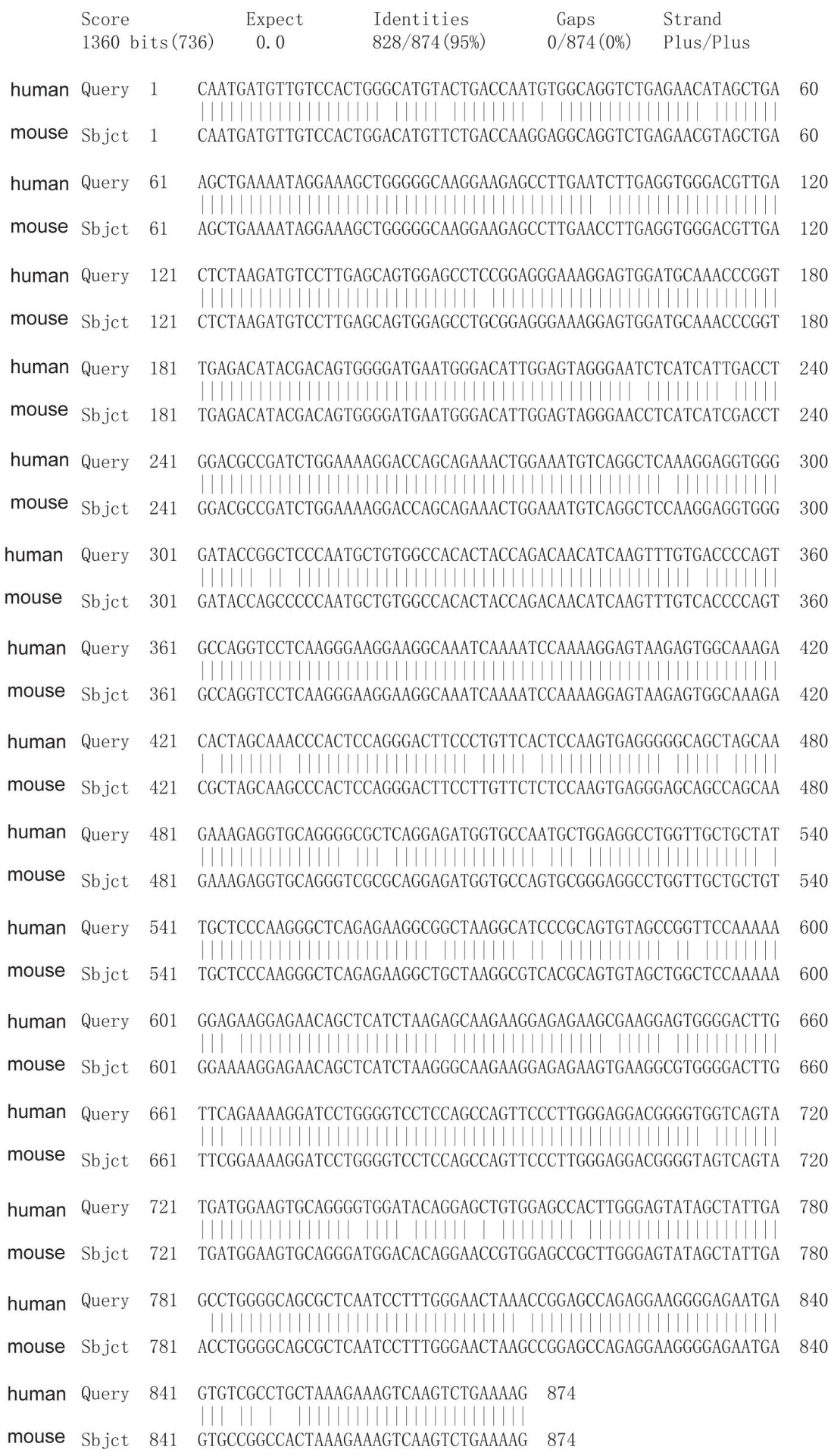
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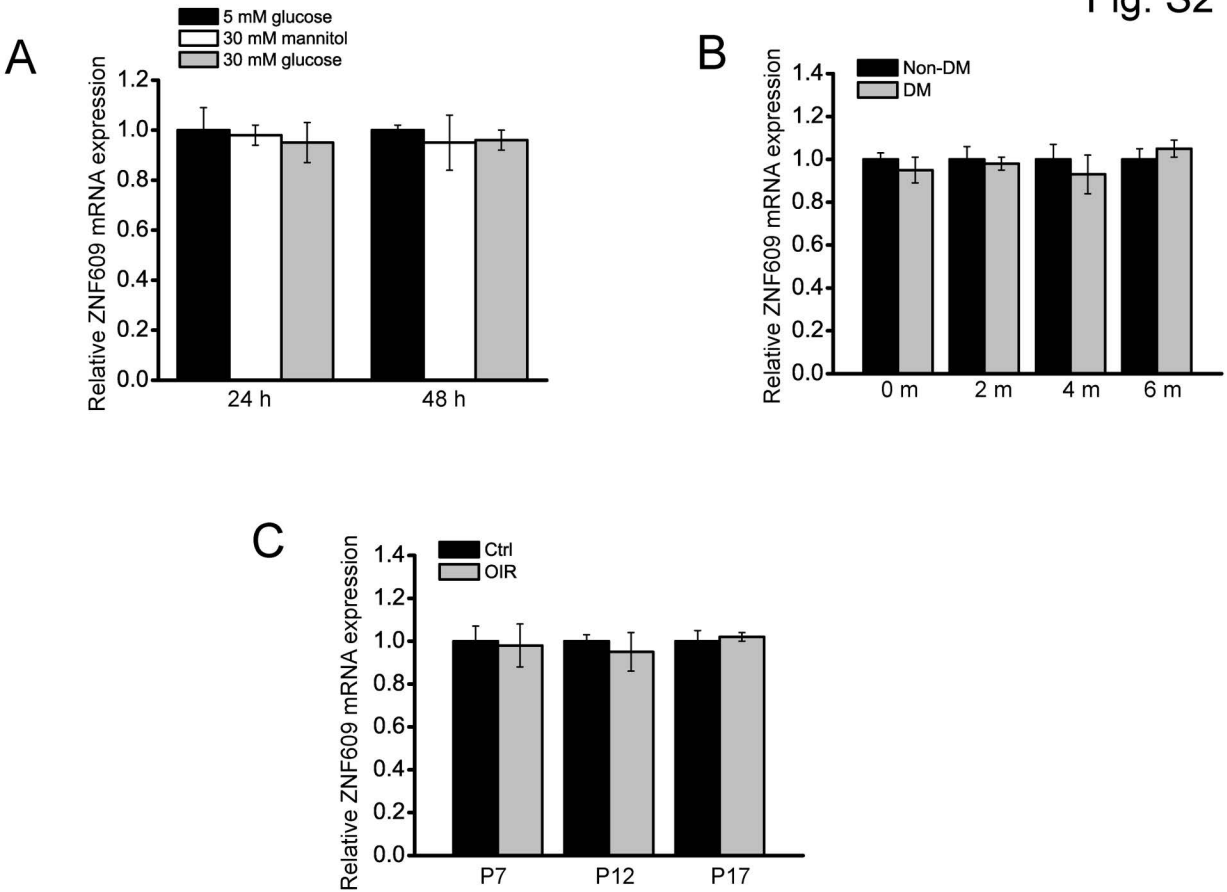
Fig. S2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0.6
0.4
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0.0
24 h
48 h
B
Non-DM
DM
Relative ZNF609 mRNA expression
1.4
1.2
1.0
0.8
0.6
0.4
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0 m
2 m
4 m
6 m
C
Ctrl
OIR
Relative ZNF609 mRNA expression
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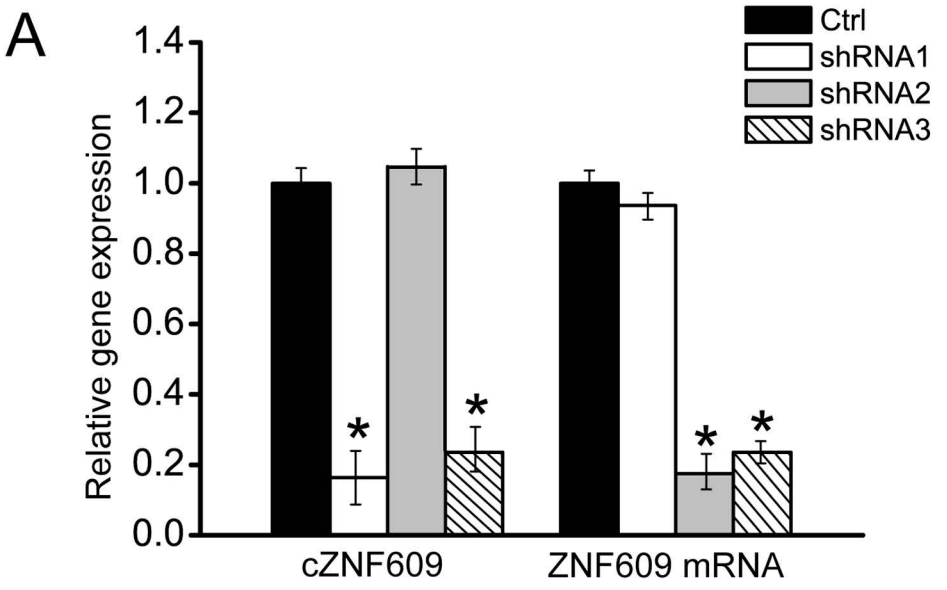
0.4
0.2
0.0
P7
P12
P17

Fig. S3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shRNA3
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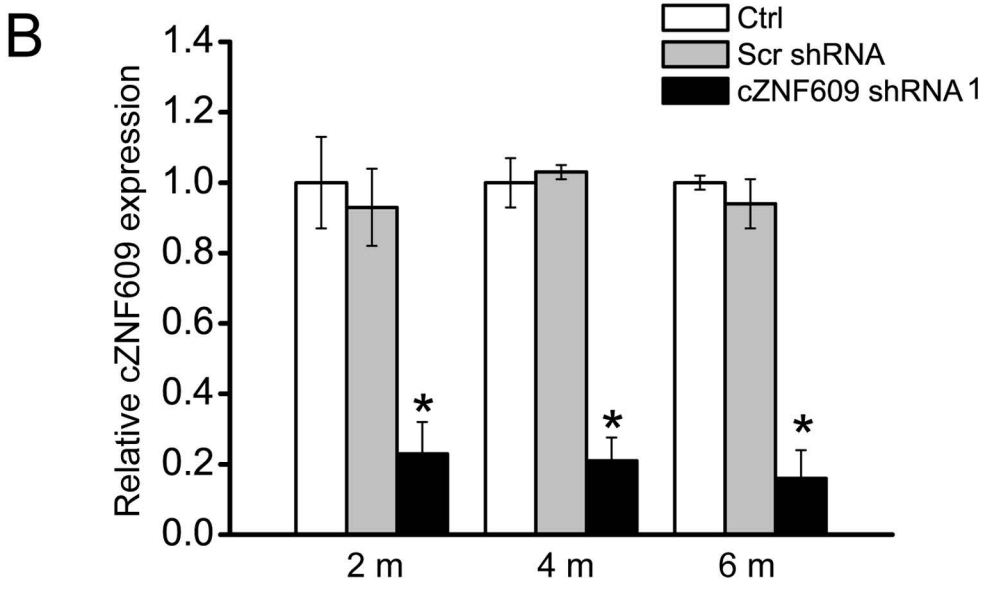
*
*
*
*
cZNF609
ZNF609 mRNA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Scr shRNA
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1.4
1.2
1.0
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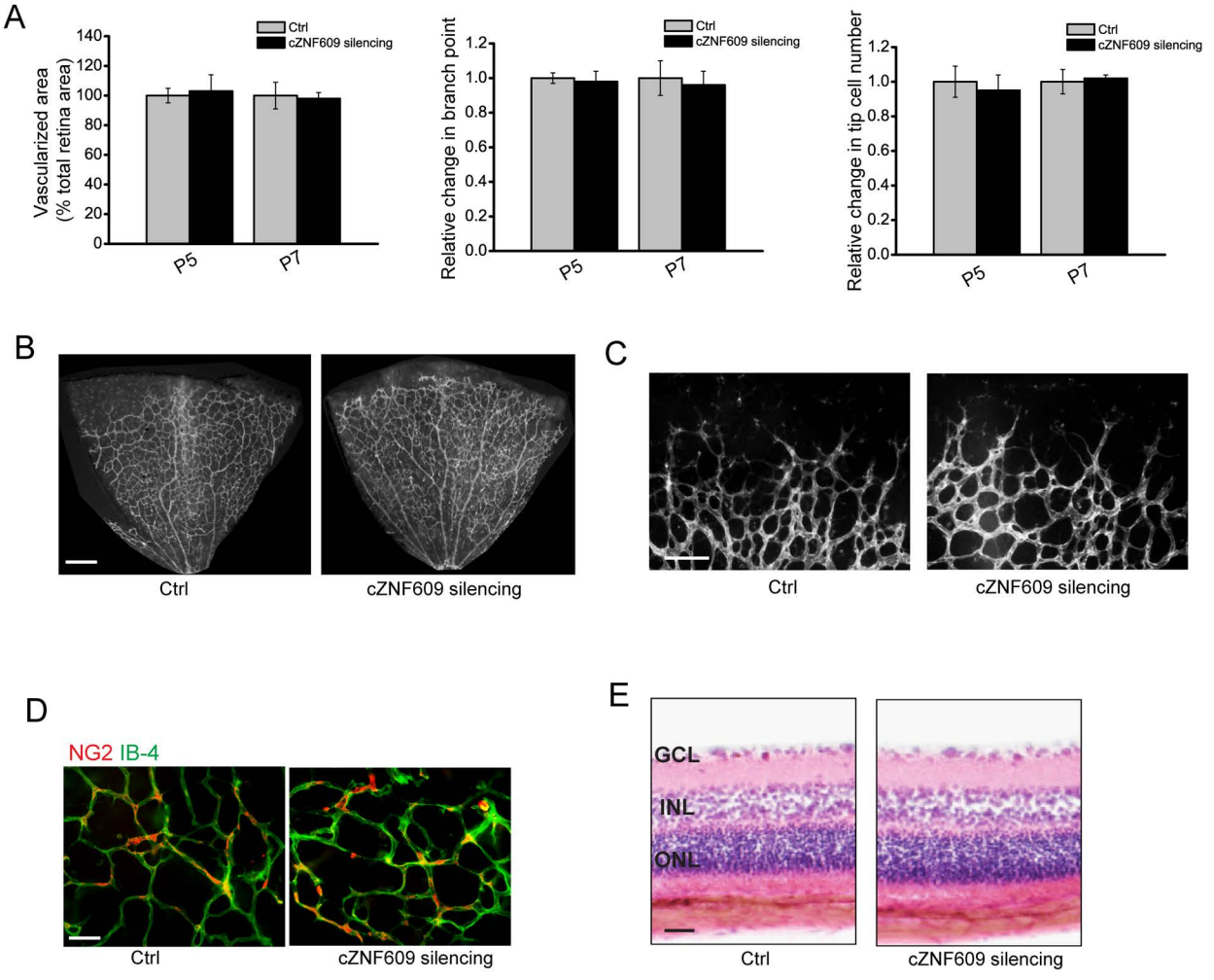
0.2
0.0
*
*
*
2 m
4 m
6 m

Fig.4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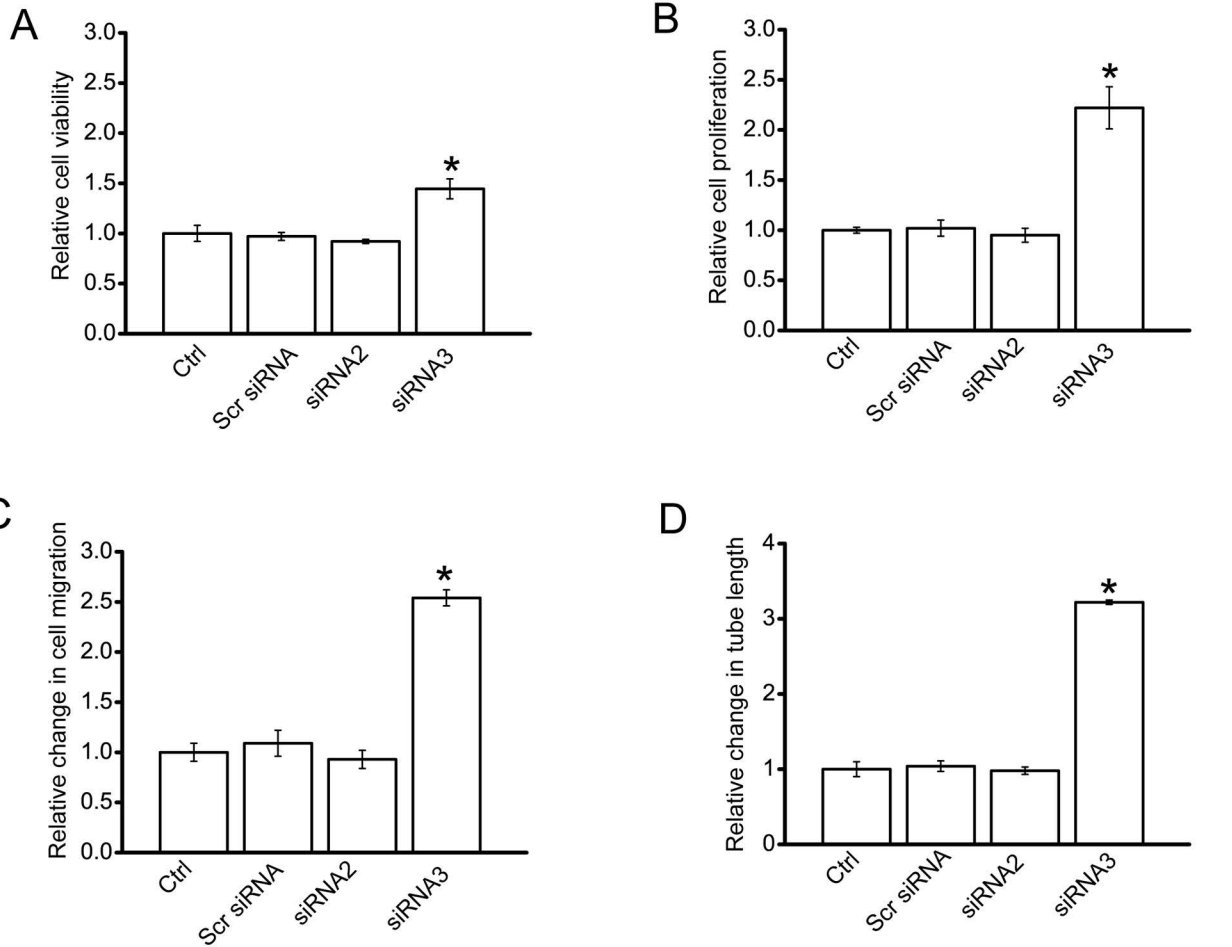
Vascularized area (% total retina area)
Ctrl
cZNF609 silencing
P5
P7
Relative change in branch point
Relative change in tip cell number
B
Ctrl
cZNF609 silencing
C
D
NG2 IB-4
E
GCL
INL
ONL

Fig. S5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2.0
1.5
1.0
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siRNA3
B
Relative cell proliferation
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2.5
2.0
1.5
1.0
0.5
0.0
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siRNA2
siRNA3
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Relative change in cell migration
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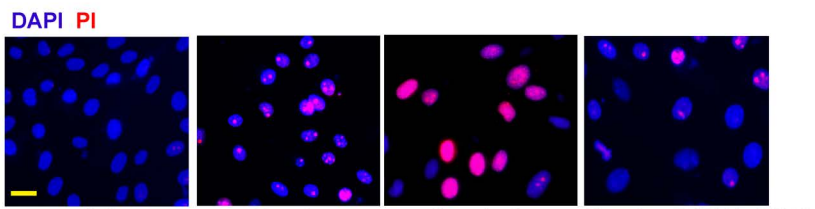
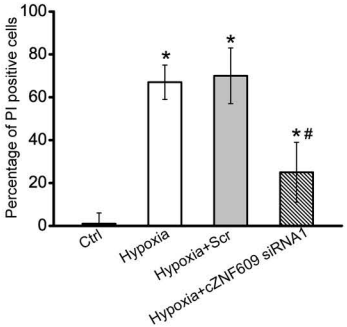
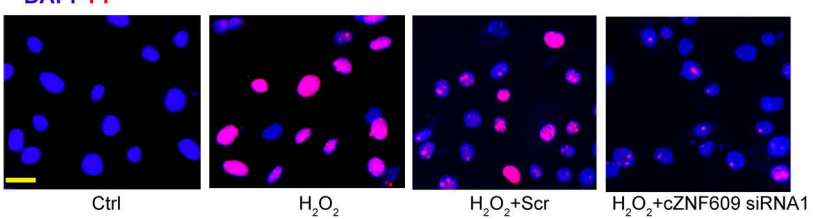
2.5
2.0
1.5
1.0
0.5
0.0
*
Ctrl
Scr siRNA
siRNA2
siRNA3
D
Relative change in tube length
4
3
2
1
0
*
Ctrl
Scr siRNA
siRNA2
siRNA3

A


DAPI PI
Ctrl
Hypoxia
Hypoxia+Scr
Hypoxia+cZNF609 siRNA1


Percentage of PI positive cells
100
80
60
40
20
0
*
*
*#
Ctrl
Hypoxia
Hypoxia+Scr
Hypoxia+cZNF609 siRNA1

B


DAPI PI
Ctrl
H2O2
H2O2+Scr
H2O2+cZNF609 siRNA1

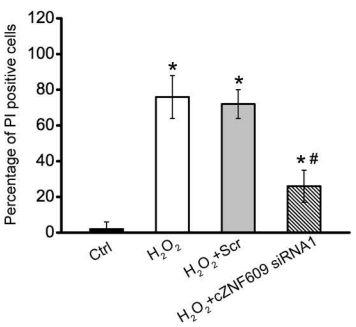
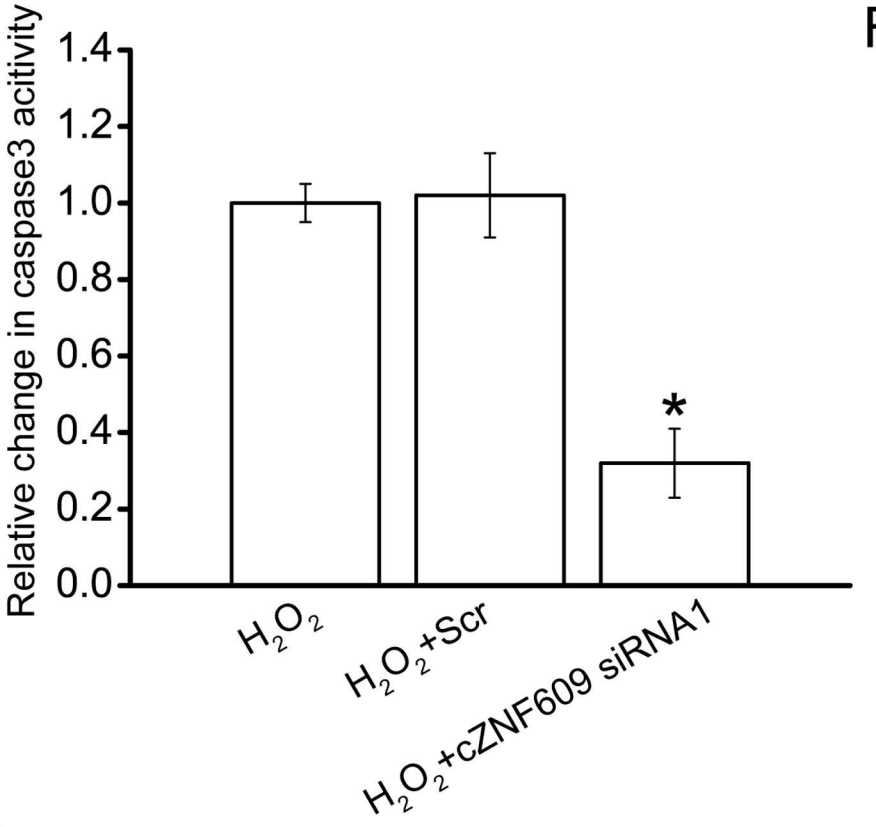

Percentage of PI positive cells
120
100
80
60
40
20
0
*
*
*#
Ctrl
H2O2
H2O2+Scr
H2O2+cZNF609 siRNA1

Fig. S7

A


Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
H2O2
H2O2+Scr
H2O2+cZNF609 siRNA1

B

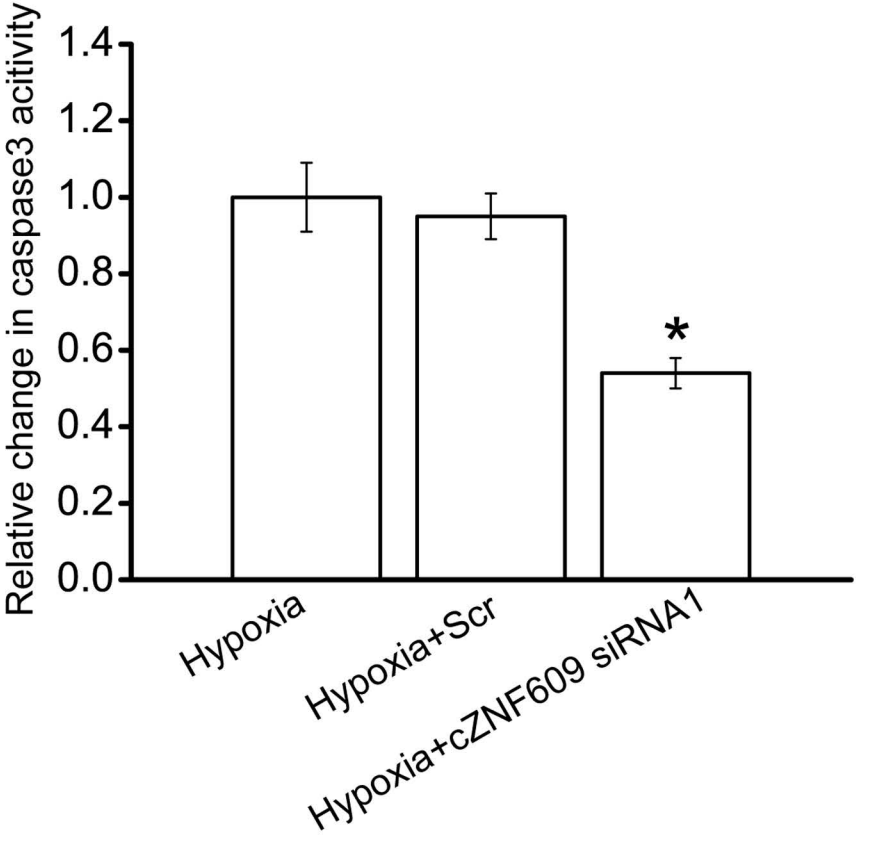

Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Hypoxia
Hypoxia+Scr
Hypoxia+cZNF609 siRNA1

Fig. S8

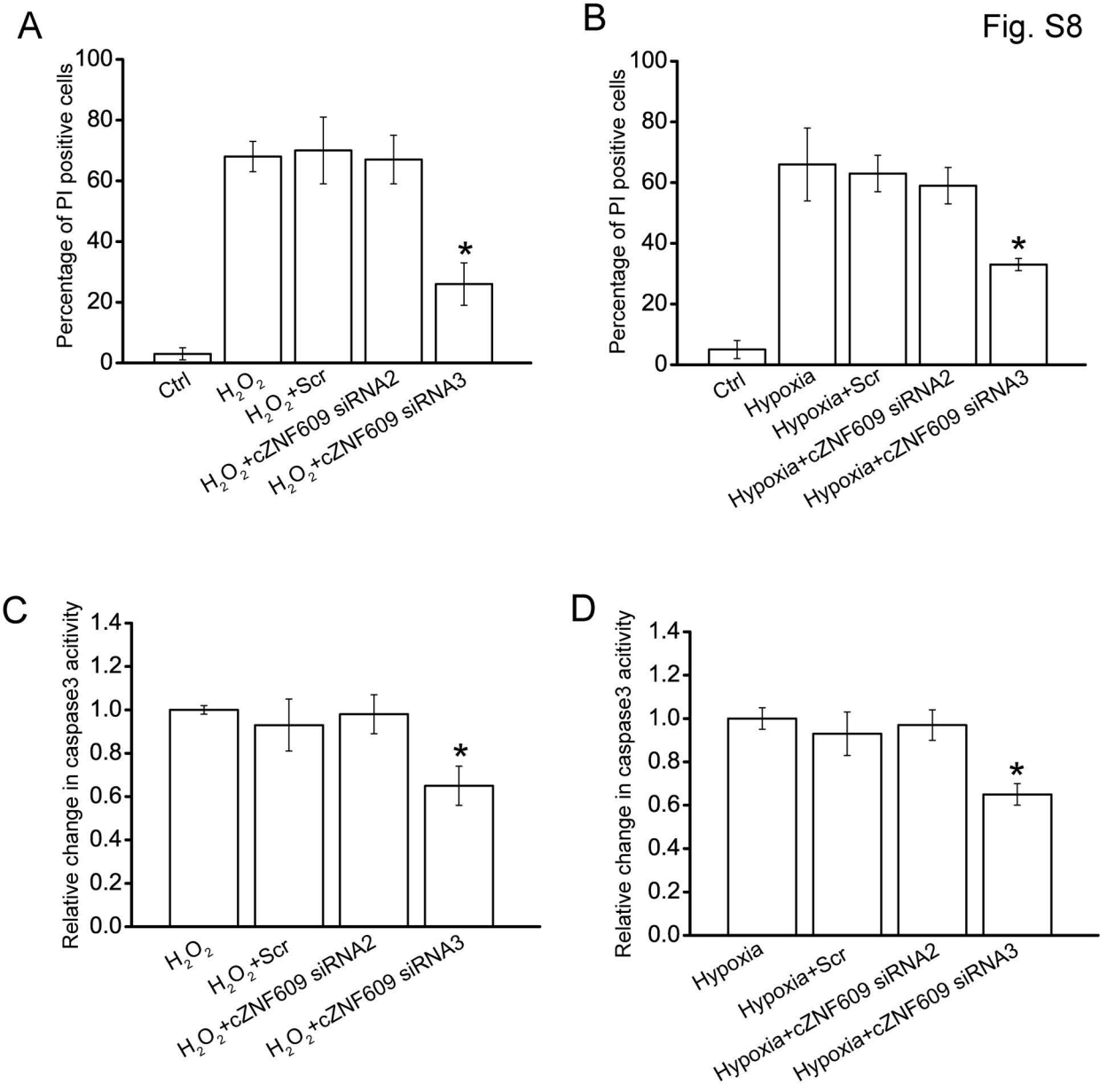

A
Percentage of PI positive cells
100
80
60
40
20
0
*
Ctrl
$H_2O_2$
$H_2O_2$+Scr
$H_2O_2$+cZNF609 siRNA2
$H_2O_2$+cZNF609 siRNA3
B
Percentage of PI positive cells
100
80
60
40
20
0
*
Ctrl
Hypoxia
Hypoxia+Scr
Hypoxia+cZNF609 siRNA2
Hypoxia+cZNF609 siRNA3
C
Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
$H_2O_2$
$H_2O_2$+Scr
$H_2O_2$+cZNF609 siRNA2
$H_2O_2$+cZNF609 siRNA3
D
Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Hypoxia
Hypoxia+Scr
Hypoxia+cZNF609 siRNA2
Hypoxia+cZNF609 siRNA3

Fig. S9

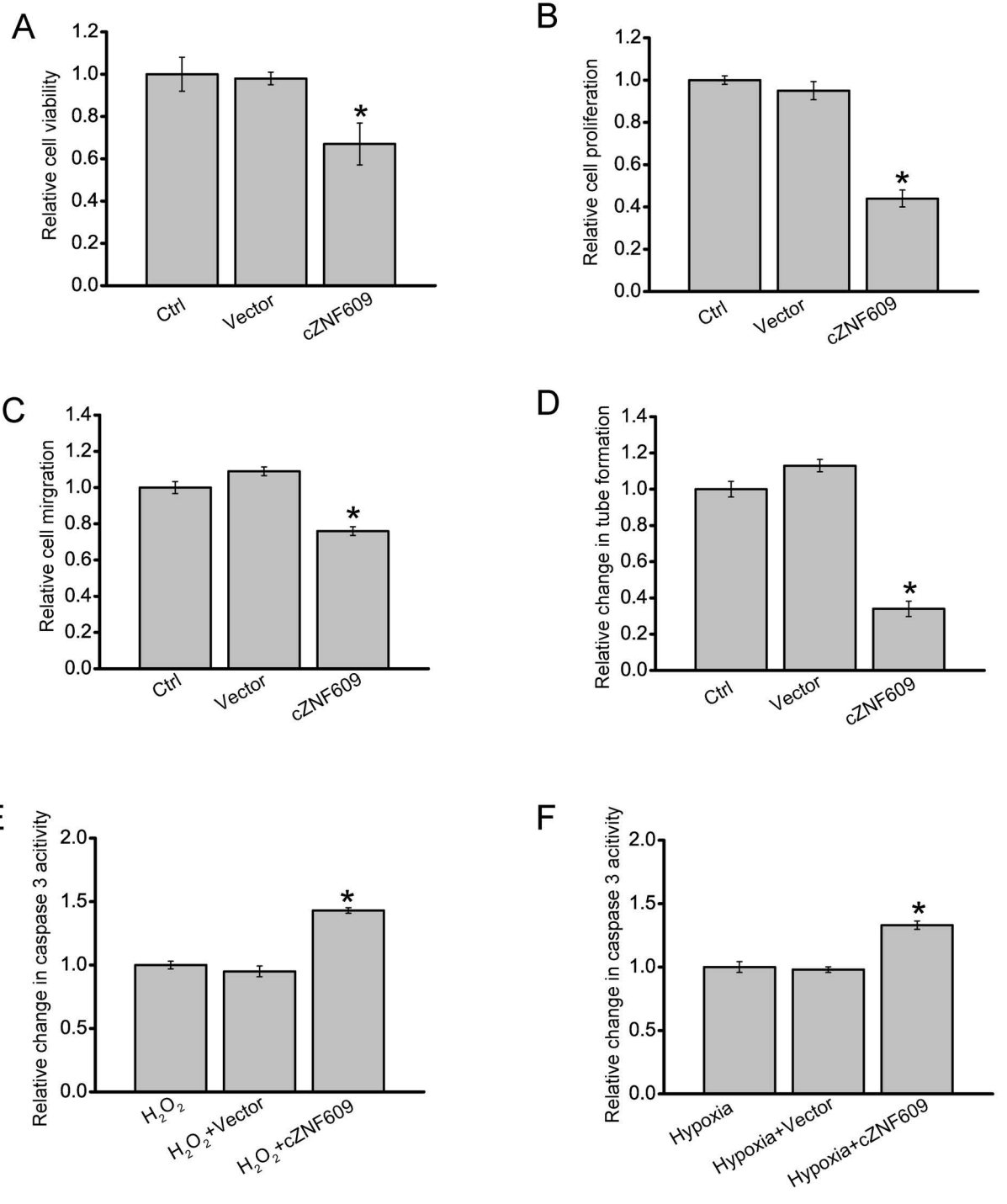

A
Relative cell viability
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ctrl
Vector
cZNF609
B
Relative cell proliferation
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ctrl
Vector
cZNF609
C
Relative cell mirgration
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ctrl
Vector
cZNF609
D
Relative change in tube formation
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ctrl
Vector
cZNF609
E
Relative change in caspase 3 acitivity
2.0
1.5
1.0
0.5
0.0
*
H2O2
H2O2+Vector
H2O2+cZNF609
F
Relative change in caspase 3 acitivity
2.0
1.5
1.0
0.5
0.0
*
Hypoxia
Hypoxia+Vector
Hypoxia+cZNF609

Fig. S10

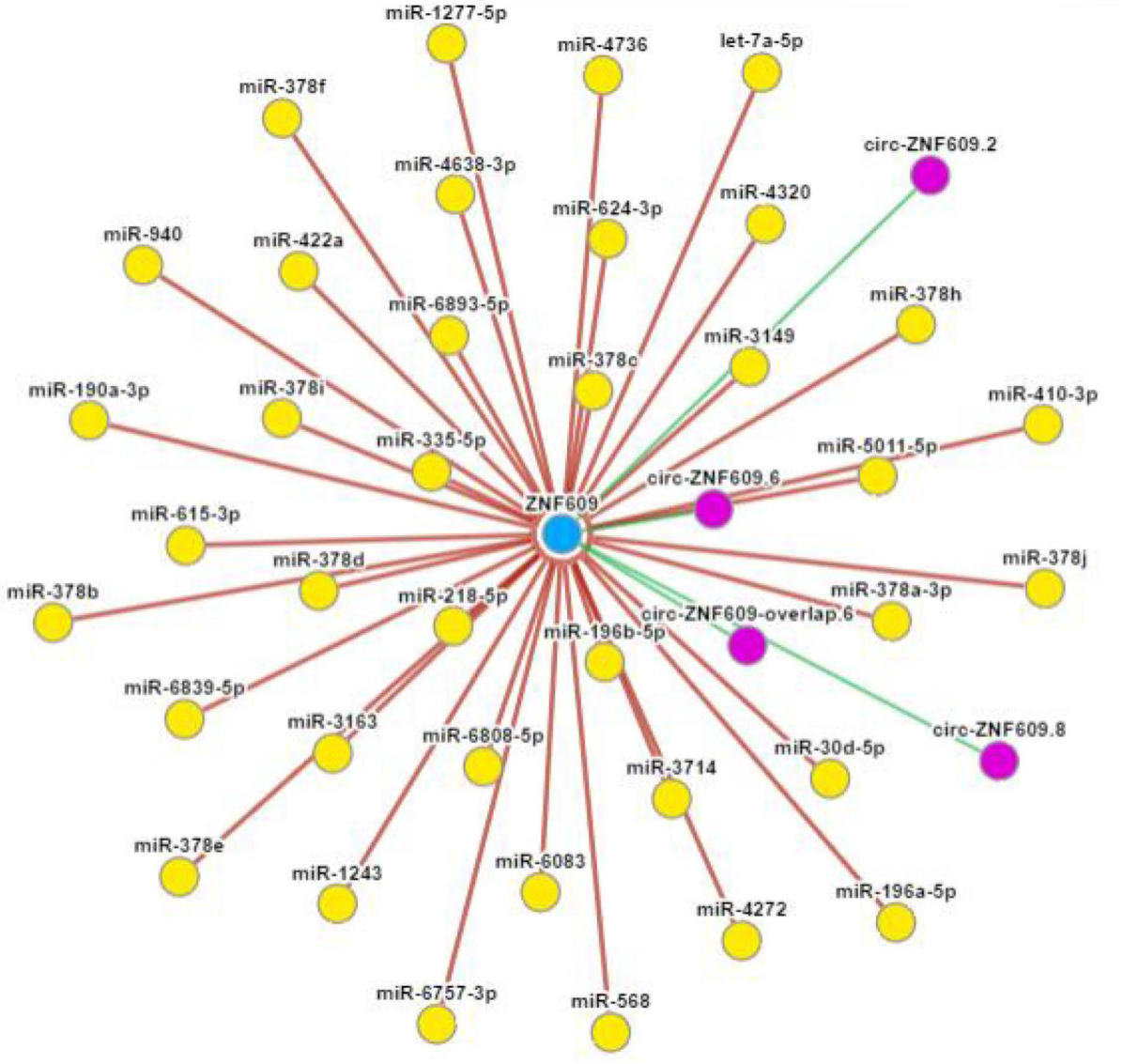

miR-1277-5p
miR-4736
let-7a-5p
miR-378f
circ-ZNF609.2
miR-4638-3p
miR-4320
miR-624-3p
miR-940
miR-422a
miR-6893-5p
miR-378h
miR-3149
miR-378c
miR-190a-3p
miR-378i
miR-410-3p
miR-335-5p
miR-5011-5p
circ-ZNF609.6
ZNF609
miR-615-3p
miR-378d
miR-378j
miR-378b
miR-218-5p
miR-378a-3p
circ-ZNF609-overlap.6
miR-196b-5p
miR-6839-5p
miR-3163
miR-6808-5p
circ-ZNF609.8
miR-30d-5p
miR-3714
miR-378e
miR-1243
miR-6083
miR-196a-5p
miR-4272
miR-6757-3p
miR-568

Fig. S11

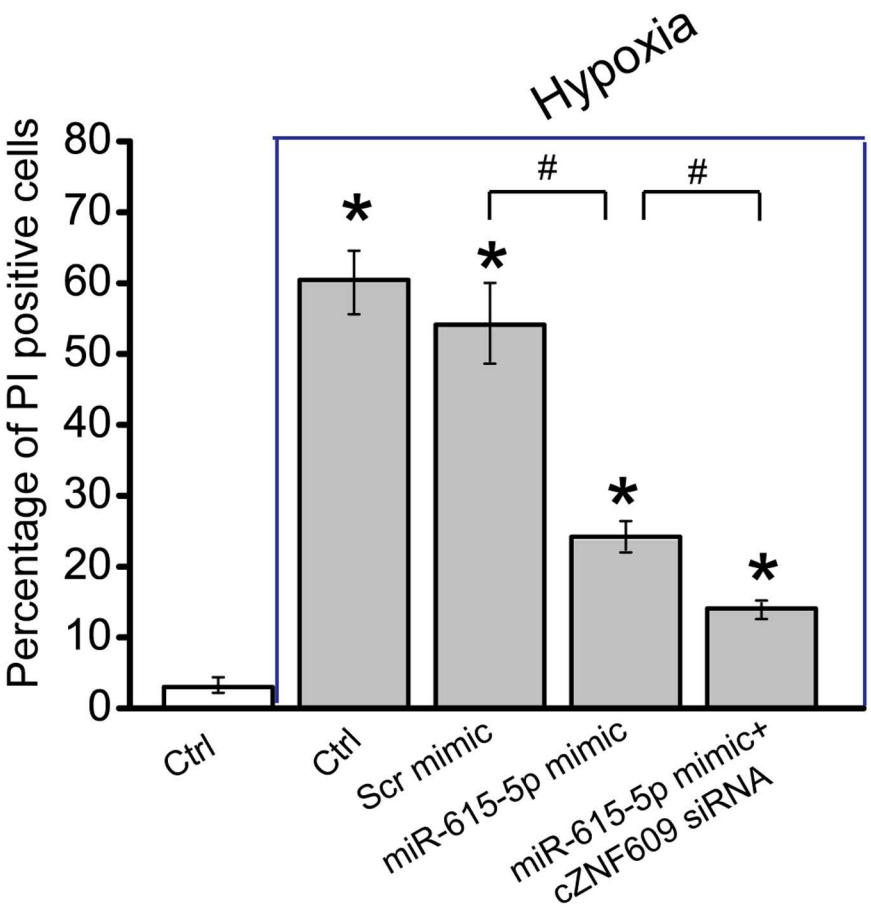
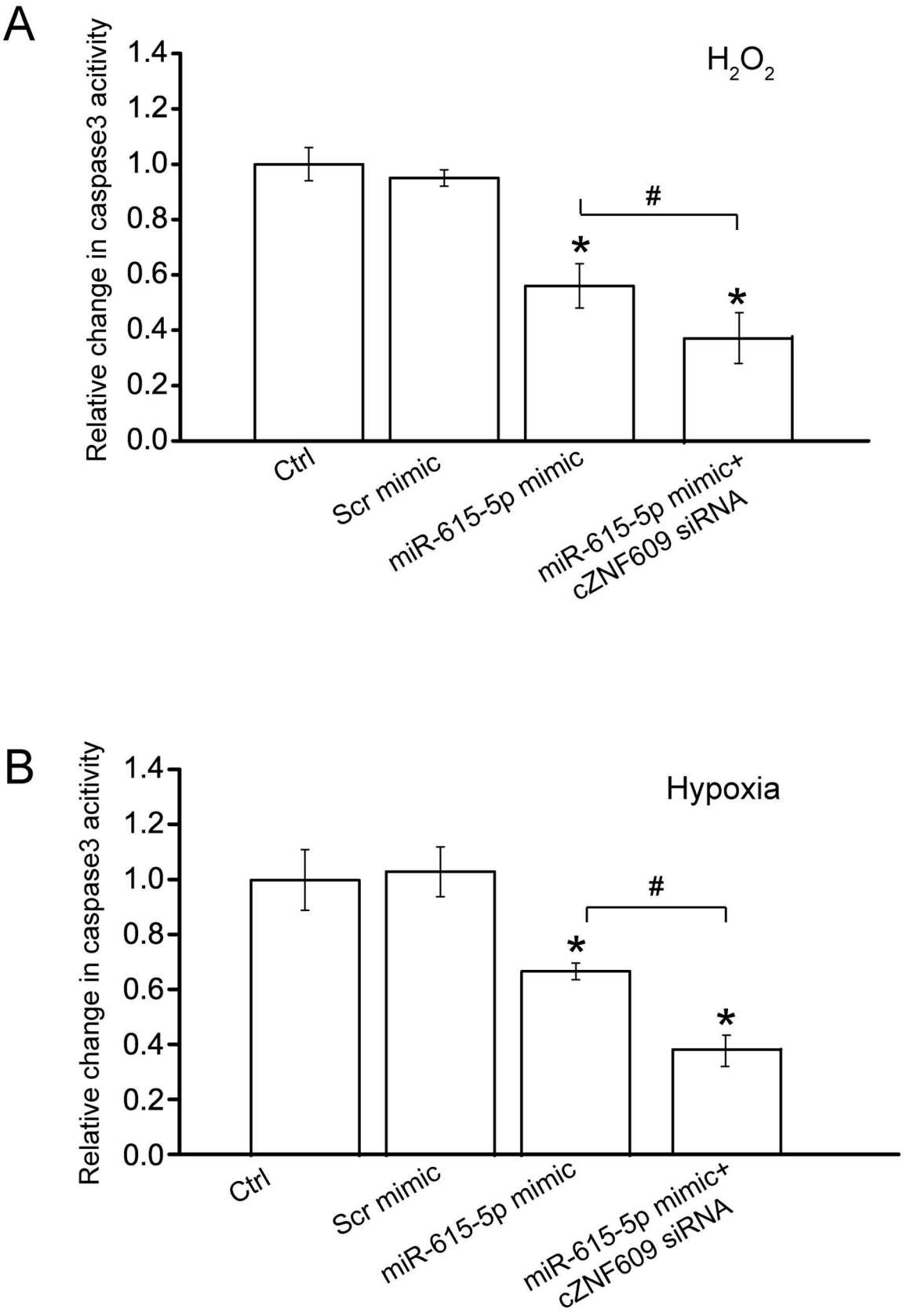

Hypoxia
Percentage of PI positive cells
80
70
60
50
40
30
20
10
0
*
*
*
*
#
#
Ctrl
Ctrl
Scr mimic
miR-615-5p mimic
miR-615-5p mimic+
cZNF609 siRNA

Fig. S12


A
Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
$H_2O_2$
#
*
*
Ctrl
Scr mimic
miR-615-5p mimic
miR-615-5p mimic+
cZNF609 siRNA
B
Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Hypoxia
#
*
*
Ctrl
Scr mimic
miR-615-5p mimic
miR-615-5p mimic+
cZNF609 siRNA

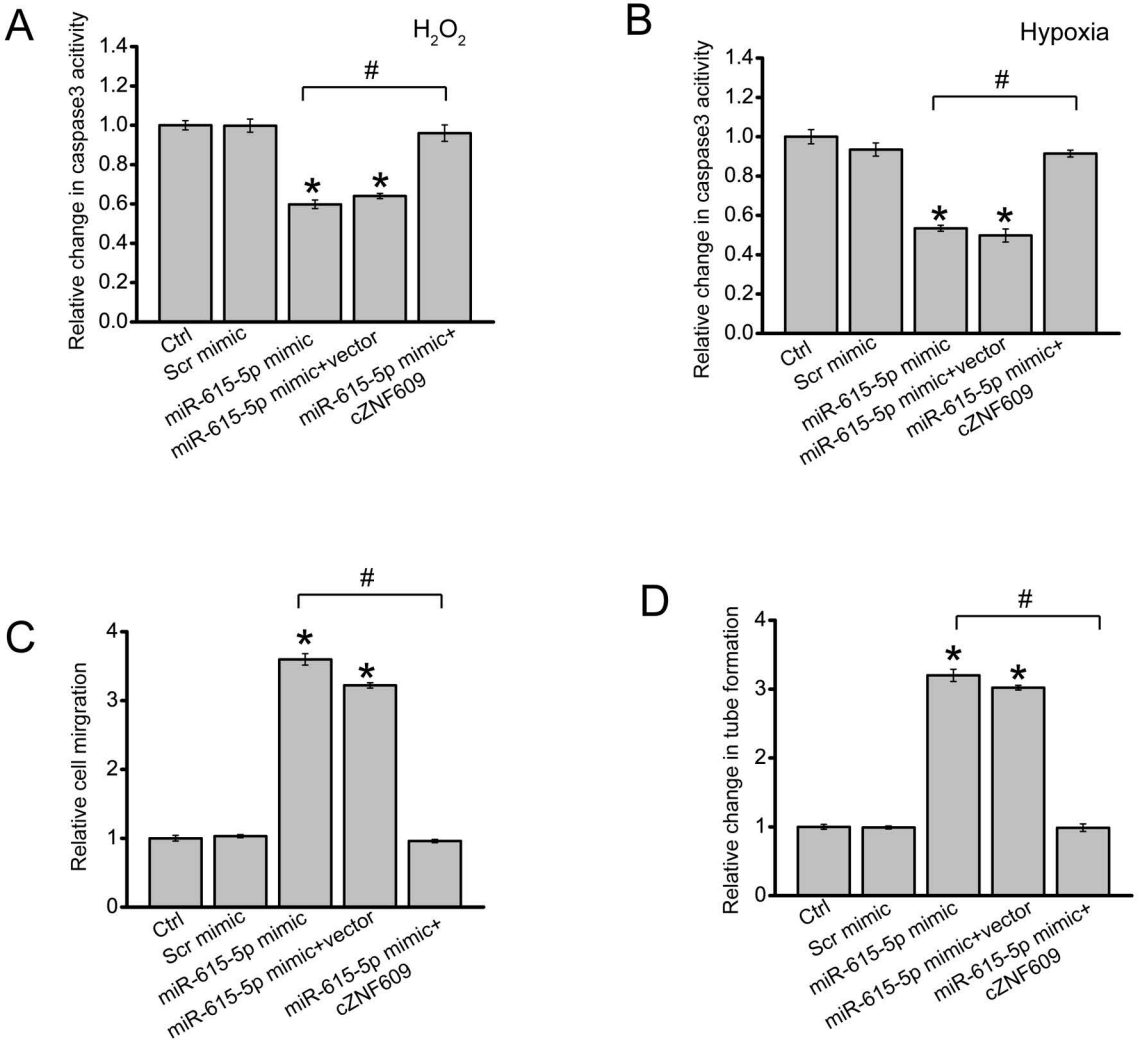
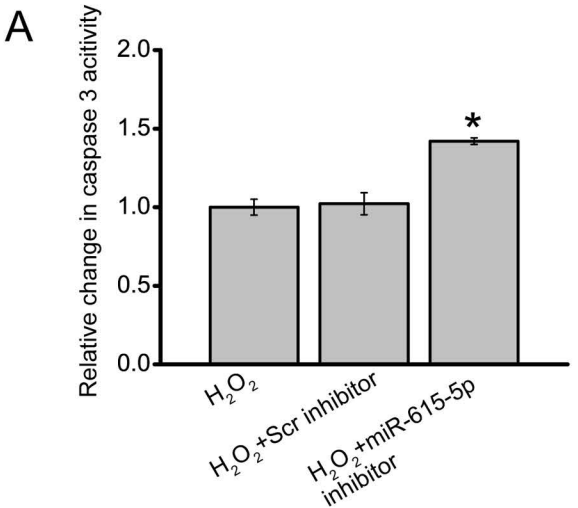
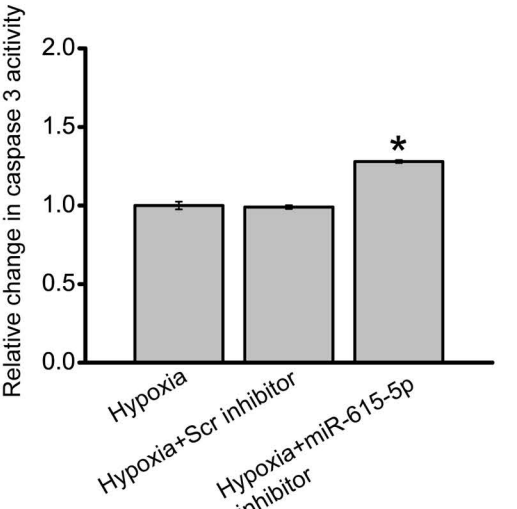
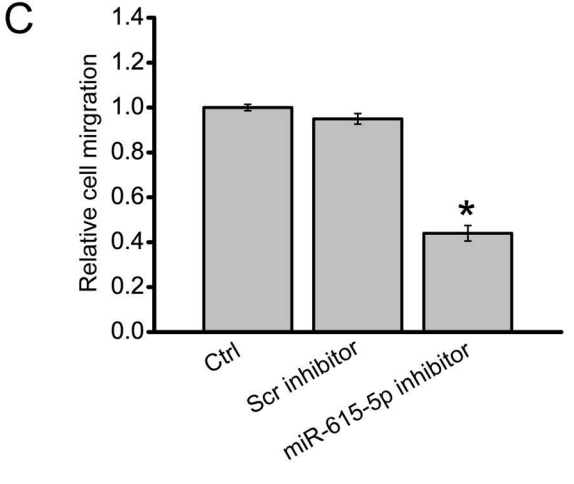

A
$H_2O_2$
Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
#
*
*
Ctrl
Scr mimic
miR-615-5p mimic
miR-615-5p mimic+vector
miR-615-5p mimic+ cZNF609
B
Hypoxia
Relative change in caspase3 acitivity
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
#
*
*
Ctrl
Scr mimic
miR-615-5p mimic
miR-615-5p mimic+vector
miR-615-5p mimic+ cZNF609
C
Relative cell mirgration
4
3
2
1
0
#
*
*
Ctrl
Scr mimic
miR-615-5p mimic
miR-615-5p mimic+vector
miR-615-5p mimic+ cZNF609
D
Relative change in tube formation
4
3
2
1
0
#
*
*
Ctrl
Scr mimic
miR-615-5p mimic
miR-615-5p mimic+vector
miR-615-5p mimic+ cZNF609

Fig. S14


A
Relative change in caspase 3 acitivity
2.0
1.5
1.0
0.5
0.0
*
$H_2O_2$
$H_2O_2$+Scr inhibitor
$H_2O_2$+miR-615-5p inhibitor


B
Relative change in caspase 3 acitivity
2.0
1.5
1.0
0.5
0.0
*
Hypoxia
Hypoxia+Scr inhibitor
Hypoxia+miR-615-5p inhibitor


C
Relative cell mirgration
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ctrl
Scr inhibitor
miR-615-5p inhibitor

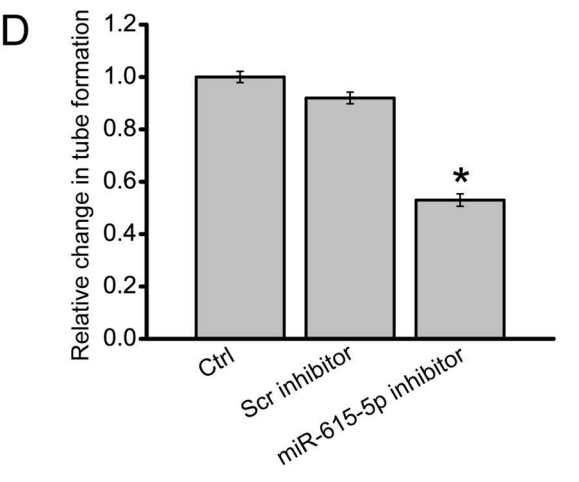
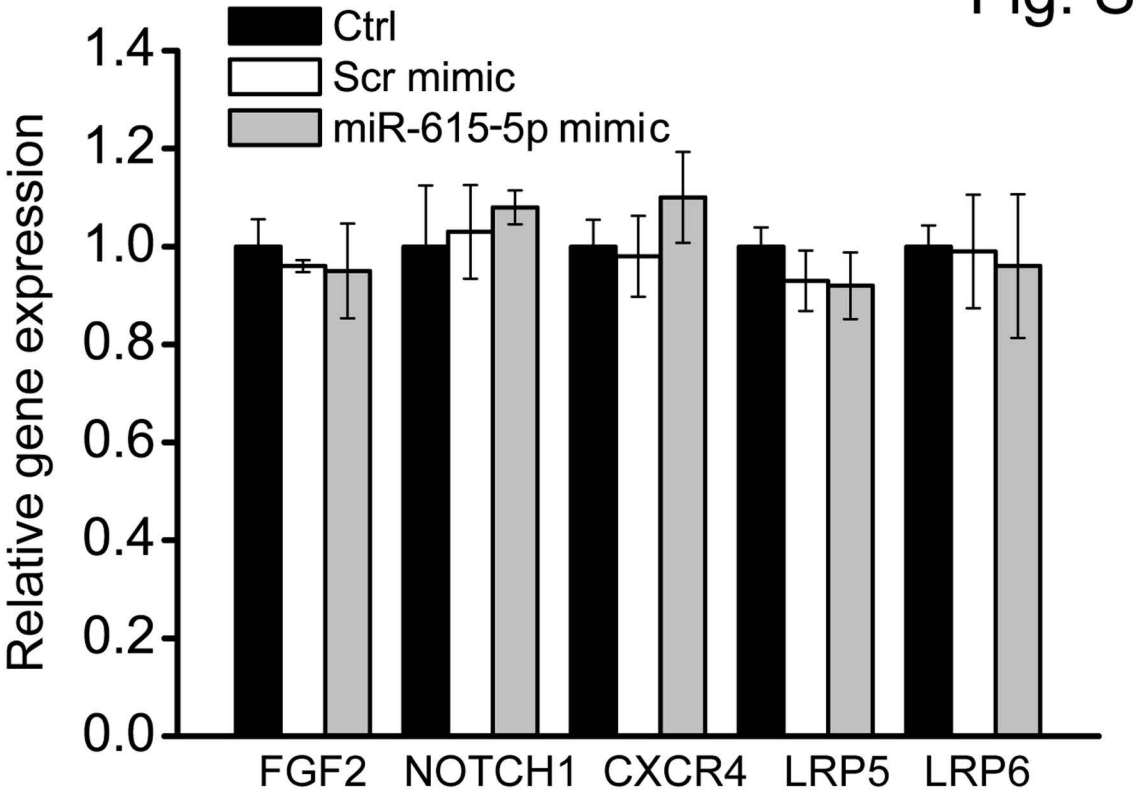
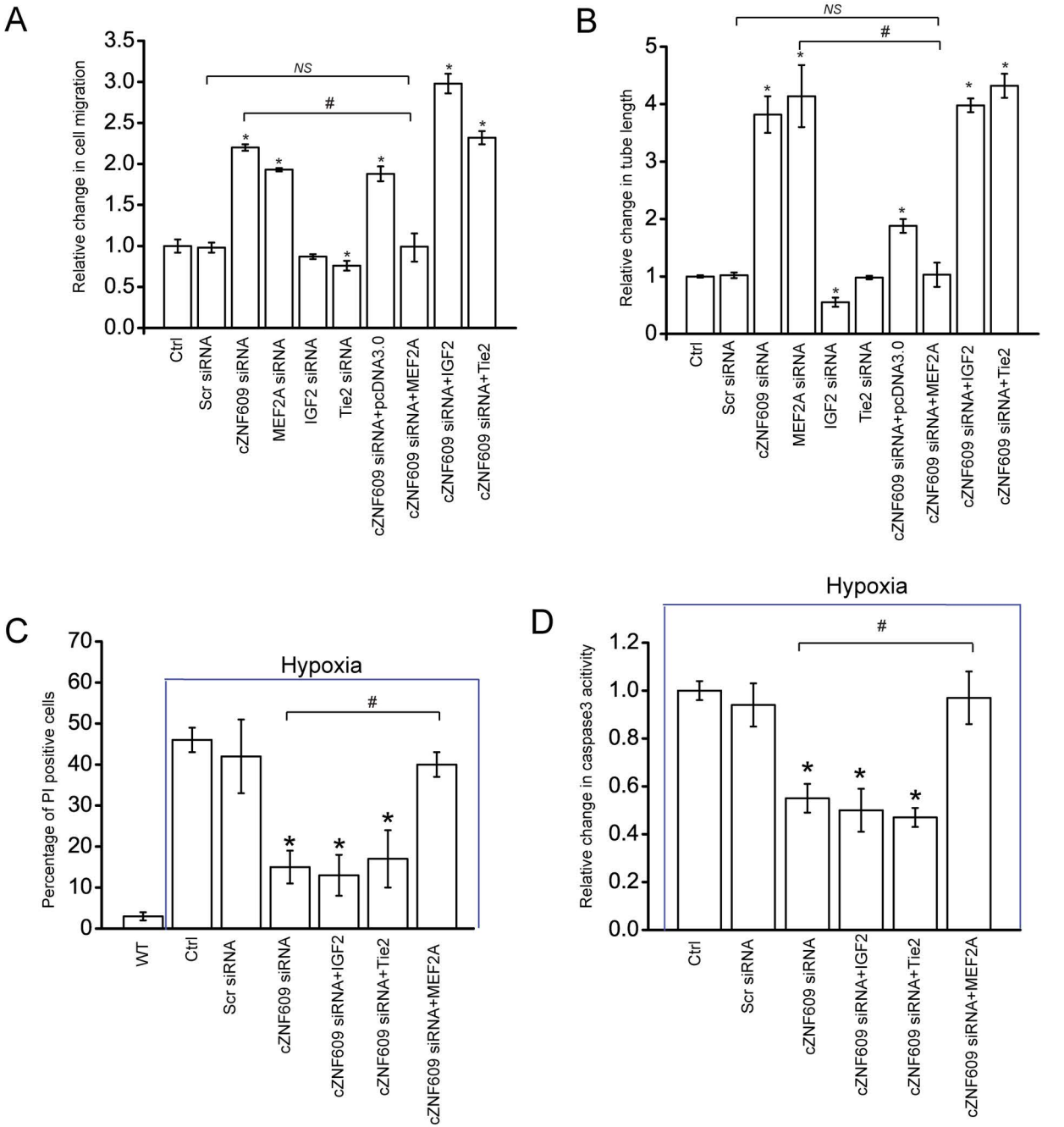

D
Relative change in tube formation
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ctrl
Scr inhibitor
miR-615-5p inhibitor

Fig. S15


Ctrl
Scr mimic
miR-615-5p mimic
Relative gene expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
FGF2
NOTCH1
CXCR4
LRP5
LRP6


A
Relative change in cell migration
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
NS
#
*
Ctrl
Scr siRNA
cZNF609 siRNA
MEF2A siRNA
IGF2 siRNA
Tie2 siRNA
cZNF609 siRNA+pcDNA3.0
cZNF609 siRNA+MEF2A
cZNF609 siRNA+IGF2
cZNF609 siRNA+Tie2
B
Relative change in tube length
5
4
3
2
1
0
NS
#
*
Ctrl
Scr siRNA
cZNF609 siRNA
MEF2A siRNA
IGF2 siRNA
Tie2 siRNA
cZNF609 siRNA+pcDNA3.0
cZNF609 siRNA+MEF2A
cZNF609 siRNA+IGF2
cZNF609 siRNA+Tie2
C
Percentage of PI positive cells
70
60
50
40
30
20
10
0
Hypoxia
#
*
WT
Ctrl
Scr siRNA
cZNF609 siRNA
cZNF609 siRNA+IGF2
cZNF609 siRNA+Tie2
cZNF609 siRNA+MEF2A
D
Relative change in caspase3 acitivity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Hypoxia
#
*
Ctrl
Scr siRNA
cZNF609 siRNA
cZNF609 siRNA+IGF2
cZNF609 siRNA+Tie2
cZNF609 siRNA+MEF2A

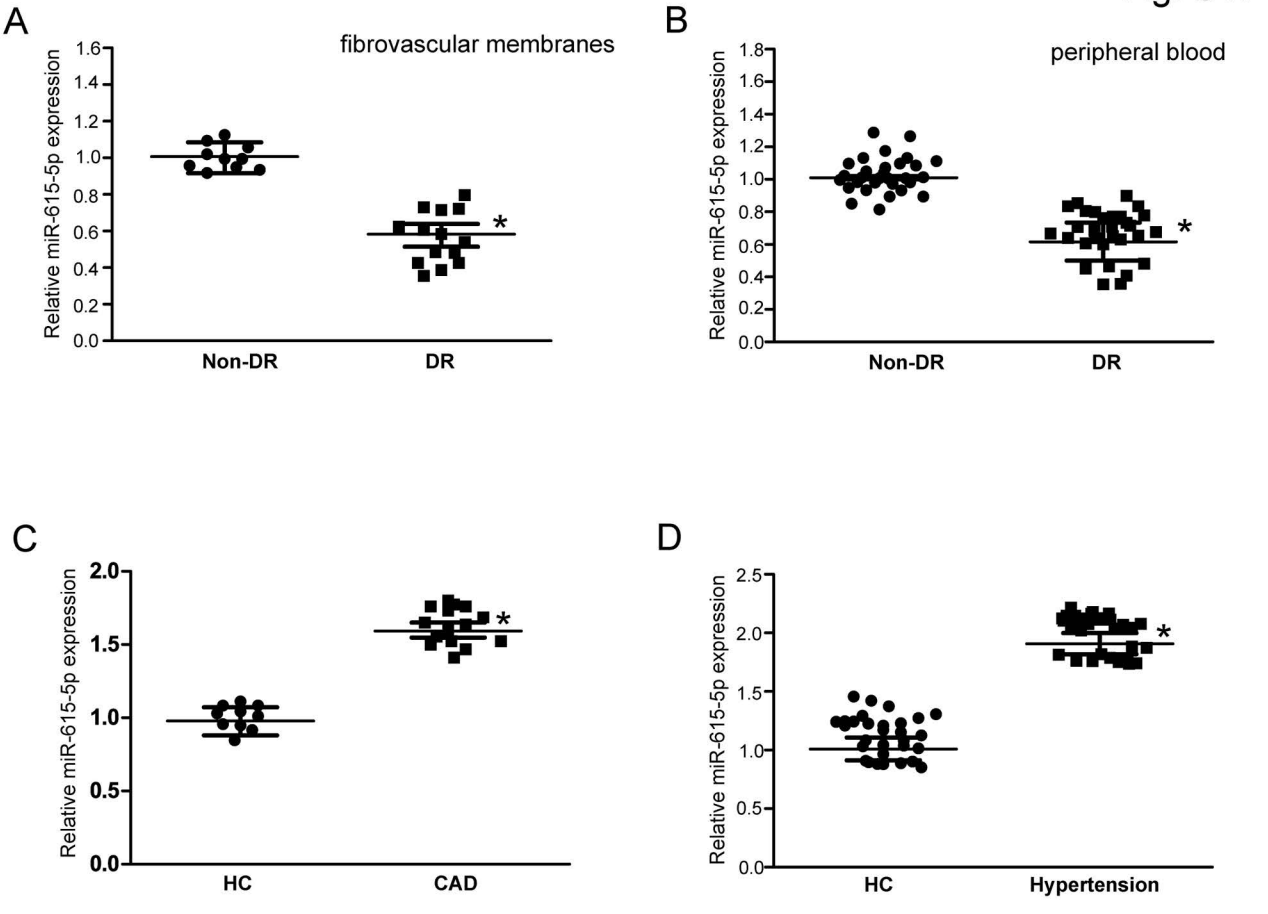

A
fibrovascular membranes
Relative miR-615-5p expression
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Non-DR
DR
B
peripheral blood
Relative miR-615-5p expression
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Non-DR
DR
C
Relative miR-615-5p expression
2.0
1.5
1.0
0.5
0.0
*
HC
CAD
D
Relative miR-615-5p expression
2.5
2.0
1.5
1.0
0.5
0.0
*
HC
Hypertension

Fig. S18

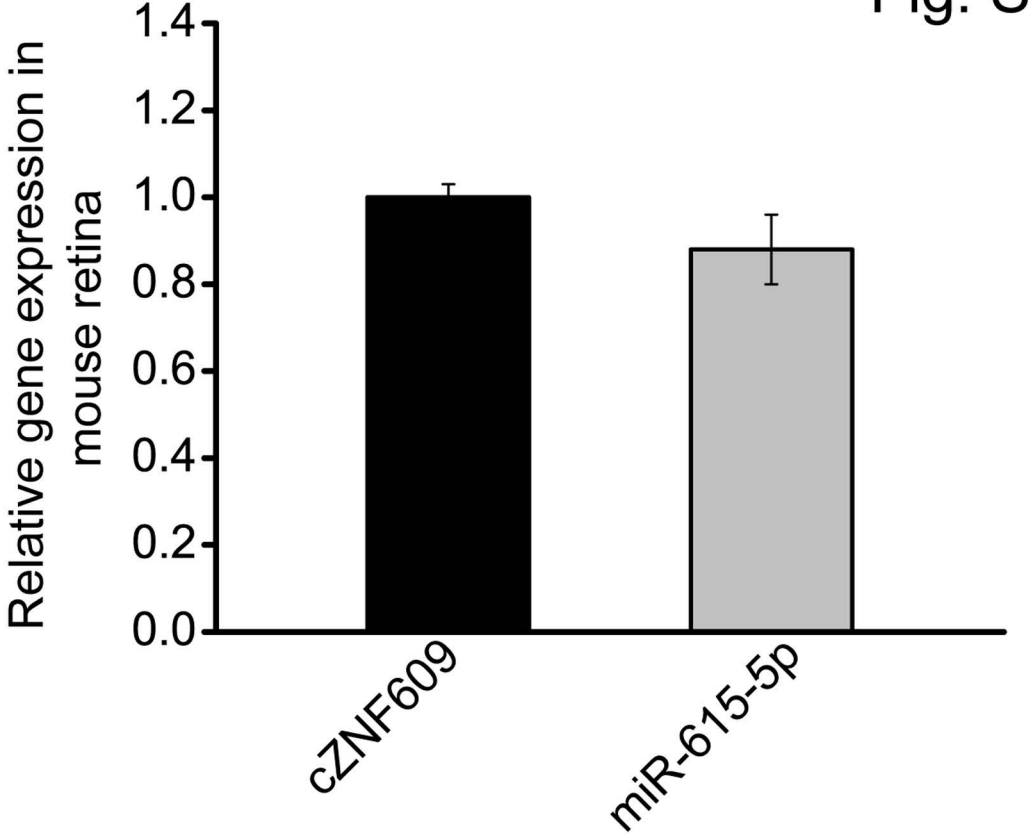

Relative gene expression in mouse retina
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
cZNF609
miR-615-5p

**Table S1: Potential miRNAs targeting cZNP609 predicted by Circular RNA Interactome database**

| miRNA | #Sites |
|---|---|
| hsa-miR-1200 | 1 |
| hsa-miR-1203 | 1 |
| hsa-miR-1224-3p | 2 |
| hsa-miR-1225-3p | 1 |
| hsa-miR-1231 | 1 |
| hsa-miR-1233 | 1 |
| hsa-miR-1236 | 1 |
| hsa-miR-1247 | 1 |
| hsa-miR-1248 | 2 |
| hsa-miR-1272 | 1 |
| hsa-miR-1273 | 1 |
| hsa-miR-1280 | 1 |
| hsa-miR-1290 | 1 |
| hsa-miR-142-3p | 1 |
| hsa-miR-145 | 1 |
| hsa-miR-149 | 1 |
| hsa-miR-324-5p | 1 |
| hsa-miR-337-3p | 1 |
| hsa-miR-338-3p | 1 |
| hsa-miR-339-3p | 2 |
| hsa-miR-361-3p | 1 |
| hsa-miR-384 | 1 |
| hsa-miR-432 | 1 |
| hsa-miR-487a | 1 |
| hsa-miR-492 | 1 |
| hsa-miR-502-5p | 1 |
| hsa-miR-503 | 1 |
| hsa-miR-556-5p | 1 |
| hsa-miR-558 | 1 |
| hsa-miR-578 | 1 |
| hsa-miR-615-5p | 1 |
| hsa-miR-621 | 1 |
| hsa-miR-623 | 1 |

| miRNA | #Sites |
|---|---|
| hsa-miR-629 | 1 |
| hsa-miR-644 | 1 |
| hsa-miR-646 | 1 |
| hsa-miR-648 | 1 |
| hsa-miR-654-3p | 1 |
| hsa-miR-663b | 1 |
| hsa-miR-766 | 1 |
| hsa-miR-767-3p | 1 |
| hsa-miR-767-5p | 1 |
| hsa-miR-890 | 1 |
| hsa-miR-892b | 1 |
| hsa-miR-942 | 2 |

**Table S2 Prediction of miR-615-p target genes by TargetScan**

| Target gene | Representative transcript | Gene name |
|---|---|---|
| LRRC73 | ENST00000372441.1 | leucine rich repeat containing 73 |
| BLOC1S1 | ENST00000549147.1 | biogenesis of lysosomal organelles complex-1, subunit 1 |
| SMARCE1 | ENST00000377808.4 | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1 |
| TIE2 | ENST00000615002 | transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)<br>TEK receptor tyrosine kinase |
| MEF2A | ENST00000354410.5 | myocyte enhancer factor 2A |
| TM9SF2 | ENST00000376387.4 | transmembrane 9 superfamily member 2 |
| ARFGAP1 | ENST00000370275.4 | ADP-ribosylation factor GTPase activating protein 1 |
| MYLK2 | ENST00000375994.2 | myosin light chain kinase 2 |
| PSMD11 | ENST00000261712.3 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 11 |
| MAPT | ENST00000344290.5 | microtubule-associated protein tau |
| FBXW2 | ENST00000608872.1 | F-box and WD repeat domain containing 2 |
| KIAA0556 | ENST00000261588.4 | KIAA0556 |
| SHANK3 | ENST00000414786.2 | SH3 and multiple ankyrin repeat domains 3 |
| IGF2 | ENST00000381395.1 | insulin-like growth factor 2 (somatomedin A) |
| GBX2 | ENST00000551105.1 | gastrulation brain homeobox 2 |

**Table S3. Demographic and clinical features of study subjects for fibrovascular membrane collection**

| Disease | Fibrovascular membranes | |
|---|---|---|
| | Idiopathic macular holes | Proliferative diabetic retinopathy |
| Number (case) | 10 | 12 |
| Age (years) | 59 ± 4.9 | 53 ± 9.2 |
| Sex (M/F) | 5/5 | 7/5 |
| Total cholesterol | 199.8 ± 26.8 | 196.3 ± 42.8 |
| Creatinine (mg/dL) | 1.93 ± 0.96 | 1.62 ± 0.76 |
| Triglyceride (mg/dL) | 82.2 ± 23.2 | 131.4 ±35. 3 |
| Glycosylated hemoglobin | 4.32 ±1.21 | 7.98±1.86 |

**Table S4: Demographic and clinical features of study subjects for peripheral blood collection**

| Disease | Non-diabetic | Diabetic |
|---|---|---|
| Number (case) | 30 | 30 |
| Age (years) | 54 ± 5.7 | 58 ±3.4 |
| Sex (M/F) | 12/18 | 13/17 |
| Total cholesterol | 194.8 ± 19.2 | 197.3 ±32.6 |
| Creatinine (mg/dL) | 1.63 ± 0.37 | 1.83 ± 0.54 |
| Triglyceride (mg/dL) | 86.3 ± 15.4 | 148.5 ± 27.4 |
| Glycosylated hemoglobin | 3.71 ±1.03 | 8.13±1.21 |

**Table S5: Characteristics of study cohort for coronary artery disease study**

| | Healthy volunteers (n=10) | Patients with CAD (n=15) |
|---|---|---|
| Gender (Male/Female) | 5/5 | 8/7 |
| Age (years) | 36±6.4 | 61.3±5.3 |
| Stable CAD for at least 4 months | 0 | 15 |
| Hypertension | 0 | 2 |
| Active smoker | 0 | 3 |
| Adipositas (BMI > 25) | 2 | 2 |
| Diabetes mellitus | 0 | 3 |
| Total cholesterol (mg/dl) | 176±26.7 | 181±31.9 |
| LDL cholesterol (mg/dl) | 104±22.6 | 109±33.2 |
| Triglycerides (mg/dl) | 75.5± 21.2 | 122.4 ± 32.8 |
| HDL cholesterol (mg/dl) | 64.43 ±15.44 | 50.39 ± 20.92 |
| Concurrent medication: | | |
| Beta - blocker | 0 | 5 |
| Aspirin / Clopidogrel | 0 | 15 |
| ACE-Inhibitor/ATRB | 0 | 15 |
| Diuretics | 0 | 5 |
| Statin therapy | 0 | 2 |

**Table S6: Demographic and clinical features of normotensive controls and hypertensive patients**

| Parameters | Normotensive (n=30) | Hypertensive patients (n=30) |
|---|---|---|
| Gender, F/M | 17/13 | 12/18 |
| Age, y | 49.29±5.16 | 59.28±7.39 |
| Body weight, kg | 76.72±7.18 | 70.43±10.25 |
| systolic blood pressure (SBP), mm Hg | 118.53±7.15 | 164.29±6.37* |
| diastolic blood pressure (DBP), mm Hg | 68.91±6.38 | 87.51±6.16* |
| Serum Na, mEq/L | 128.17±4.26 | 129.55±8.16 |
| Serum K, mEq/L | 4.18±0.53 | 4.76±0.54 |
| Serum Cre, mg/dL | 0.69±0.17 | 0.59±0.22* |
| Plasma AGT,μg/mL | 31.36±4.16 | 20.82±4.93 |
| UNa/UCre, mEq/g | 117.52±16.49 | 123.51±6.27 |
| UK/UCre, mEq/g | 48.36±7.21 | 26.16±4.48* |
| UPro/UCre, mEq/g | 0.13±0.08 | 0.36±0.12* |

Note: Cre indicates creatinine; UNa/UCre, urinary sodium:creatinine ratio; UK/UCre, urinary potassium: creatinine ratio; UPro/UCre, urinary protein:creatinine ratio; *$P$<0.05 versus normotensive.