Appendix Materials Online

CONTENTS

Appendix Supplementary materials and methods

Cell culture and MALAT1 gene knockdown

Retinal Müller (glial) cells line, rMC-1, was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO) at 37°C and 5% CO_2 in a humidified incubator.

Two individual siRNAs (siRNA MALAT1-1 and siRNA MALAT1-2) and negative control siRNA was purchased from Shanghai GenePharma Co., Ltd (China). Target sequences for MALAT1 siRNAs were as follows: siRNA1, 5'-GCAGTTTAGGAGATTGTAAAG-3' and siRNA2, 5'- GCAGTTTAGAAGAGTCTTTAG-3'. The negative siRNA was a scrambled sequence with no homology within the mouse, rat, or human genome. siRNA duplexes were transfected into cells twice (48 h) within a gap of 24 h at the final concentration of 100 nM using lipofectamine RNAi max reagent (Life Technologies) according to the manufacturer's instruction.

MALAT1 shRNA adenoviral construction

Three different MALAT1 RNAi target sequences were as follows:

5'-AAAGTTAAGGCAGAATGCCTTTGAA-3';

5'-CCACATTTCCAAATATGCATGTTGA-3';

5'-CATGCTGGAGCAGCTAGCATGTGAT-3'.

A scrambled RNAi with no homology within the mouse and rat genome was used as a control (5'-CCGATCTGACATGACTGCG-3'). The adenoviruses harboring these RNAi constructs were generated using the BLOCK-iT™ Adenoviral RNAi Expression System (Life Technologies) according to manufacturer's instruction. Packaging and amplification of Adenovirus was conducted in HEK293T cells.

Intravitreal injection

The mice/ rats 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 received about 3 μl (for mice) or 6 μl (for rats) adenovirus. Injections were administered slowly over approximately one minute and monitored with a stereo microscope. Injections were conducted once a week for the required time periods.

MTT assay

Cell viability was detected using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium-bromide assay (MTT). Briefly, the primary RGCs or rMC-1 cells were plated at a density of 1×10^4 cells per well in 96-well plates. Following exposure to stress, they were incubated with MTT (0.5 mg/ml) at 37^oC for 3 h. 100 mM DMSO solution was finally added to dissolve formazan crystals. The absorbance was detected at 570 nm wavelength using a microplate reader (Molecular Devices).

Measurement of mitochondrial membrane potential (Δψm)

The primary RGCs or rMC-1 cells were incubated with the JC-1 fluorescent dye (Molecular Probes) at 37 °C for 1.5 h to detect mitochondrial membrane potential. The fluorescence intensity was detected using a microplate reader (Molecular Devices) (excitation at 485 nm, emission spectra between 530-620 nm). In the control cells, an intact Δψm allows the JC-1 dye in a delocalized positive charge, showing red fluorescence. In apoptotic cells, Δψm collapse allows JC-1 dye in the cytoplasm, showing green fluorescence. Mitochondrial depolarization was shown as the decrease in the red-to-green fluorescence intensity ratio.

Quantitative reverse transcription-PCR (qRT-PCR)

Total RNAs were extracted using TRIzol reagent (Invitrogen). RNA quality and purity was controlled spectrophotometrically and estimated by electrophoresis. Reverse transcriptions were performed using a SuperScript First-Strand Synthesis Kit (Takara) to produce total cDNAs. Quantitative PCRs were performed using cDNA templates on a PikoReal Real-Time PCR System (Thermo Scientific). For quantitative PCR reactions, 30-50 μg cDNA was added to a 20 μl PCR reaction mixture containing 10 μl of 2× Power SYBR Green PCR Master Mix and 0.5 μl of each primer (20 pmol). Comparative C_t method was used to determine the change of target gene expression in the test samples relative to the control samples.

Hoechst staining

After exposure to specific stress, the primary RGCs or rMC-1 cells were fixed

in 4% formaldehyde for 15 min at room temperature and permeabilized with Triton-X 100 for additional 10 min. After PBS wash, these cells were stained with Hoechst 33342 (100 μg/ml) for 10 min. The excess staining was removed by a series of PBS wash. The stained nuclei were observed using an Olympus IX-73 microscope.

Calcein-AM and propidium iodide (PI) double staining

Calcein-AM and PI double staining was used to discriminate viable and dead cells. After exposure to specific stress, the primary RGCs or rMC-1 cells were fixed in 4% formaldehyde for 15 min, and then these cells were stained with calcein-AM (10 μmol/l) for 15 min. After washing with PBS for three times, these cells were stained with PI (10 μmol/l) for 10 min. The viable cells were observed using a 490 nm excitation filter, while the dead cells were observed using a 545 nm excitation filter.

Immunoblot and immunoprecipitation

The primary RGCs or rMC-1 cells were lysed using cell lysis buffer. Protein concentrations of the extracts were detected using bicinchoninic acid assay (Pierce). 50 μg protein was used for immunoprecipitation or loaded per lane, subjected to SDS-PAGE, transferred onto PVDF membranes, and then blotted with the specific antibody.

RNA pull-down assay

Biotin-labeled RNAs were transcribed using the Biotin RNA Labeling Mix (Roche) and T7 RNA polymerase (Promega), treated with RNase-free DNase I

(Promega), and then purified with RNeasy Mini Kit (QIAGEN). Five μg biotinylated RNA was heated at 95°C for 5 min, put on ice for 5 min, and then placed at room temperature for 20 min to allow the formation of the secondary structure. The folded RNA was then mixed with cell extracts for 2 h. 50 μl washed Streptavidin agarose beads (Invitrogen) were added to each binding reaction for 1.5 h incubation. Beads were washed, treated with RNase, and dissolved in SDS buffer. The retrieved proteins were detected using western blots or resolved in the gradient gel electrophoresis for mass spectrometry identification.

Histological and morphometric study

Paraffin sections (10 μm thick) were cut through the optic nerve and stained with hematoxylin and eosin. The sections were observed using a microscope (Olympus IX-73; Olympus) equipped with Plan Fluor objectives connected to a DP80 camera (Olympus). Three photographs were taken at 40× magnification for each stained nerve (one each of the proximal, central, and distal portion of the optic nerve).

Electron microscopy

Ultra-thin cross-sections of the nerves were cut at a distance beyond approximately 0.5 mm from the optic nerve head. For electron microscopy observation, the optic nerves were fixed in 2.5% glutaraldehyde for 3 h, washed with 0.1 M phosphate buffer (pH 7.4), followed by 0.1 M cacodylate buffer (pH 7.4), and post-fixed for 1 h in 1% osmium tetroxide. The segments were then washed with cacodylate buffer (pH 7.4) and distilled water, stained in 1% uranyl

acetate for 24 h, dehydrated in graded acetone, infiltrated with resin, and polymerized for 48 h. Ultrathin cross-sections were collected on copper grids and contrasted in uranyl acetate and lead citrate. The ultrathin cross-sections were observed using JEOL 1010 transmission electron microcopy (Tokyo, Japan).

Spectral-domain optical coherence tomography (OCT) measurement

Retinal nerve fiber layer (RNFL) thickness was detected longitudinally in vivo using spectral-domain optical coherence tomography (SD-OCT) (Spectralis; Heidelberg Engineering GmbH). The experimental mice were placed on a custom-built imaging stage. RNFL thickness was determined from a circular B-scan (12° diameter) centered on the optic disc. The B-scan consisted of 1536 A-scans and an average of 100 individual sweeps to reduce speckle noise. Follow-up scans were collected using the eye tracking software. SD-OCT data was exported for performing retinal layer segmentations and deriving thickness values using custom software.

Visual evoked potential (VEP) recording

Visual evoked potentials were recorded by a single investigator in a blinded manner with stainless steel electrodes (Nihon Kohden NE 223 S, Nihon Kohden Corporation, Tokyo 161, Japan), which were placed 0.5 cm in front of (reference electrode) and behind bregma (active electrode). A ground electrode was placed on the tail of animal. After overnight dark-adaptation, animals were anesthetized with chloral hydrate (400 mg/kg) intraperitoneally. A photic stimulator was used to provide the flash stimulus at a distance of 15 cm. Repetition rate of flash stimulus

was 1 Hz and flash energy was 0.1 J. The averaging of 100 responses was accomplished with the averager of Biopac MP100 data acquisition equipment (Biopac System Inc., Santa Barbara, CA 93117, USA). Analysis time was 300 ms. Frequency bandwidth of the amplifier was 1Hz-100Hz. The gain was selected as 20-50 μV/div. Peak latencies of the components were detected from the stimulus artifact to the peak in ms. Amplitudes were detected as the voltage between successive peaks.

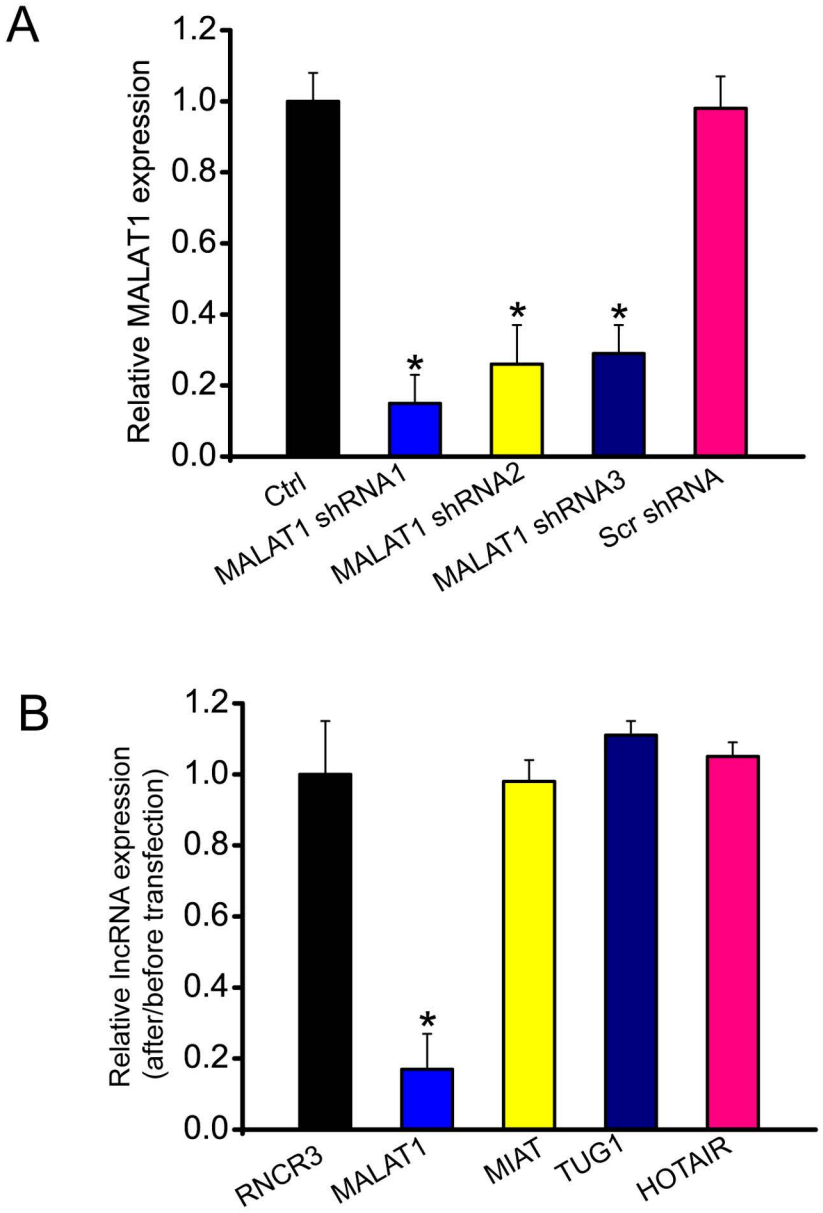
Patients in cerebrospinal fluid (CSF) study

The AD group included patients with probable AD with a high level of evidence of AD pathophysiological process and patients with mild cognitive impairment with a high likelihood of underlying AD process. Participants underwent detailed clinical and laboratory evaluations. The medical history was obtained from the informants. AD diagnosis was based on the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders association (NINCDS-ADRDA) criteria. The control group included these patients with other neuropsychiatric conditions without dementia. The clinical diagnoses (including neuroimaging and neuropsychology) were combined with the results of the following CSF biomarkers: $A\beta_{1-42}$, t-tau, and p-tau. No patients were included in the study with a completely negative AD biomarker pattern. Control subjects had all biomarkers within normal ranges. CSF t-tau, p-tau, and $A\beta_{1-42}$ concentration was determined using ELISAs.

Patients in aqueous humor (AH) study

Approximately 100 µl of AH was collected from each patient using a 30-gauge needle inserted through the peripheral cornea. The needle did not contact any iris or lens tissue during the sample collection. AH samples were centrifuged to remove cellular components at 300 g for 15 min, and its aqueous phase was gently collected. Aqueous humor was collected from the patients with primary open-angle glaucoma (POAG) and age-matched cataract patients without glaucoma or any other ocular neurodegenerative diseases. The diagnosis of glaucoma was based on characteristic alterations in the appearance of the optic nerve head and visual field in the absence of alternative causes of optic neuropathy.

Supplementary Figure 1



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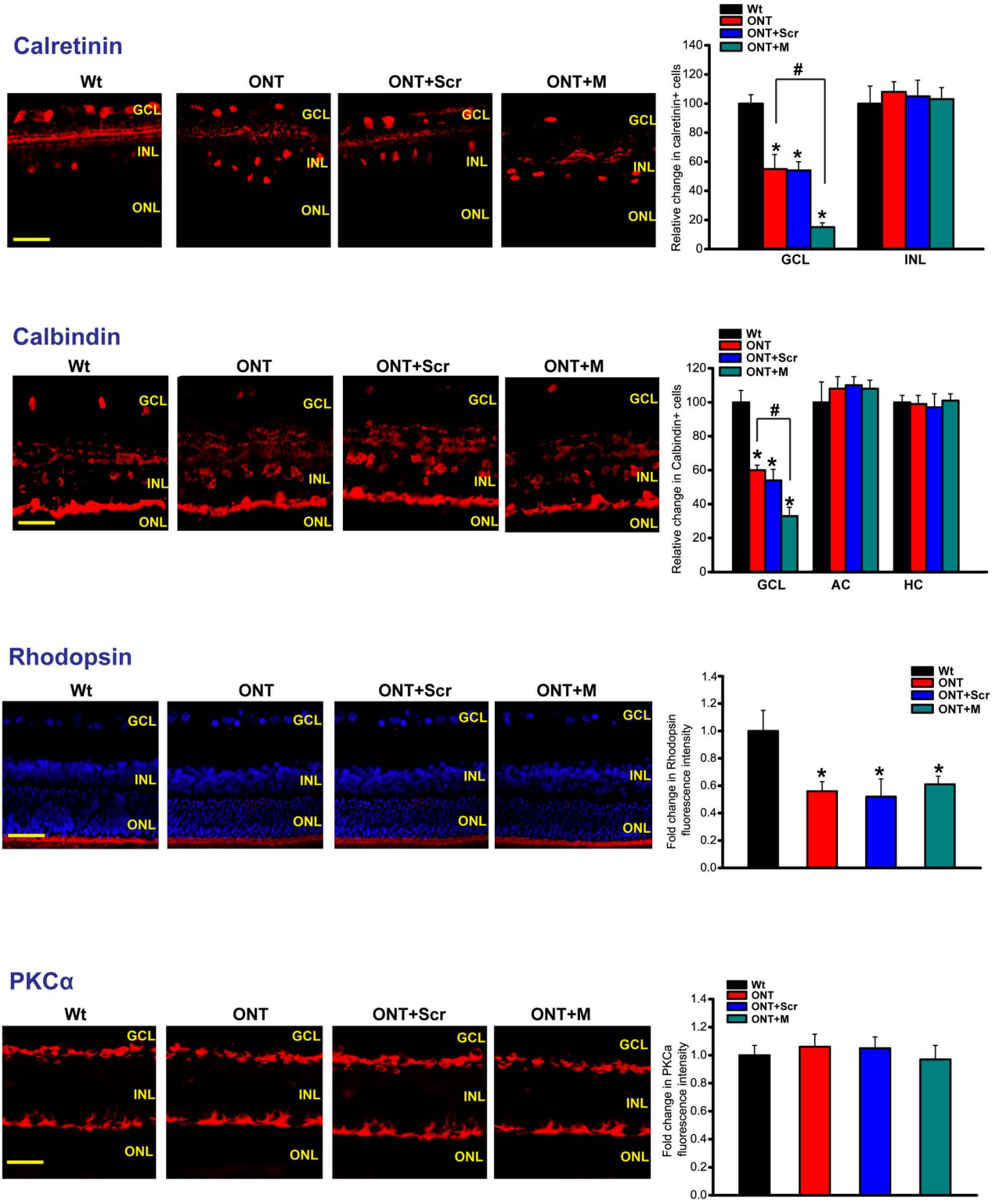

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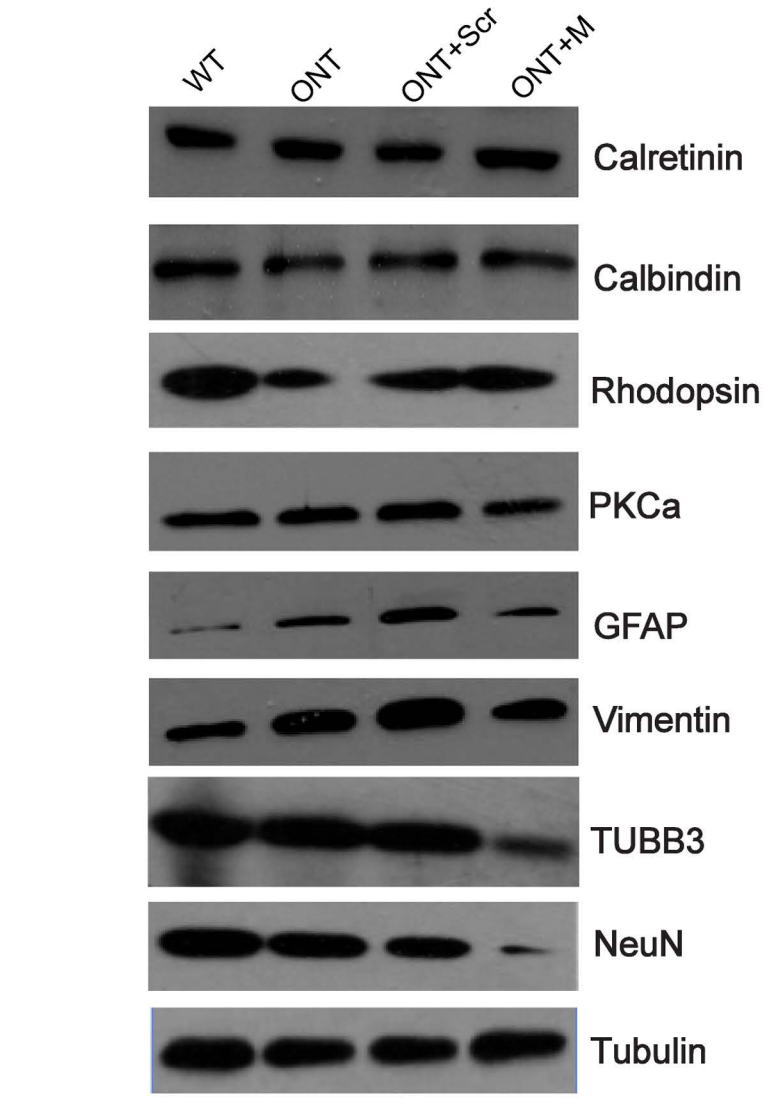

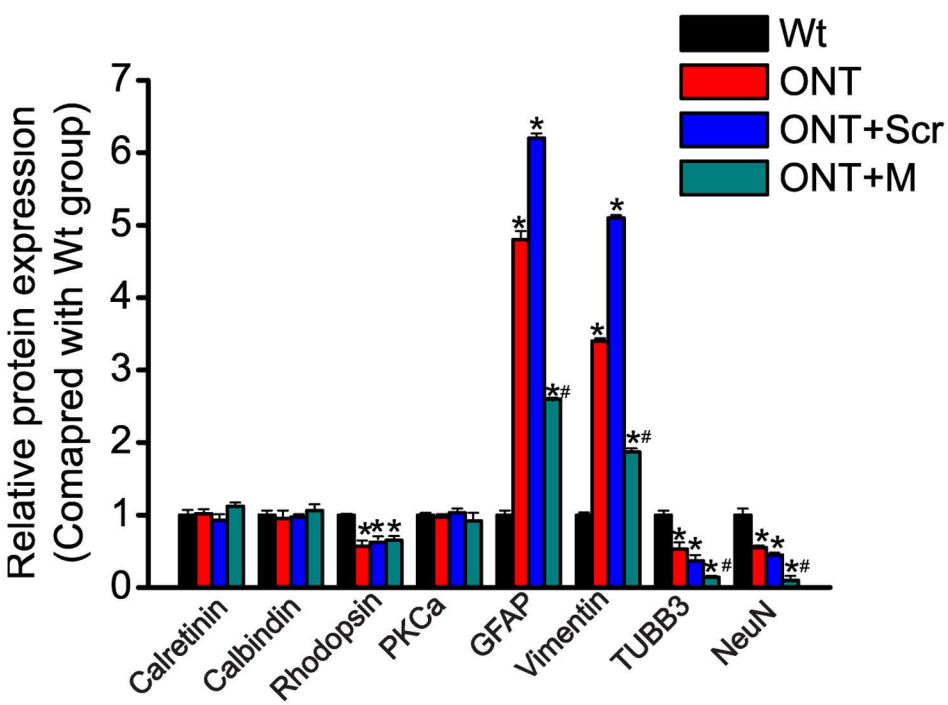

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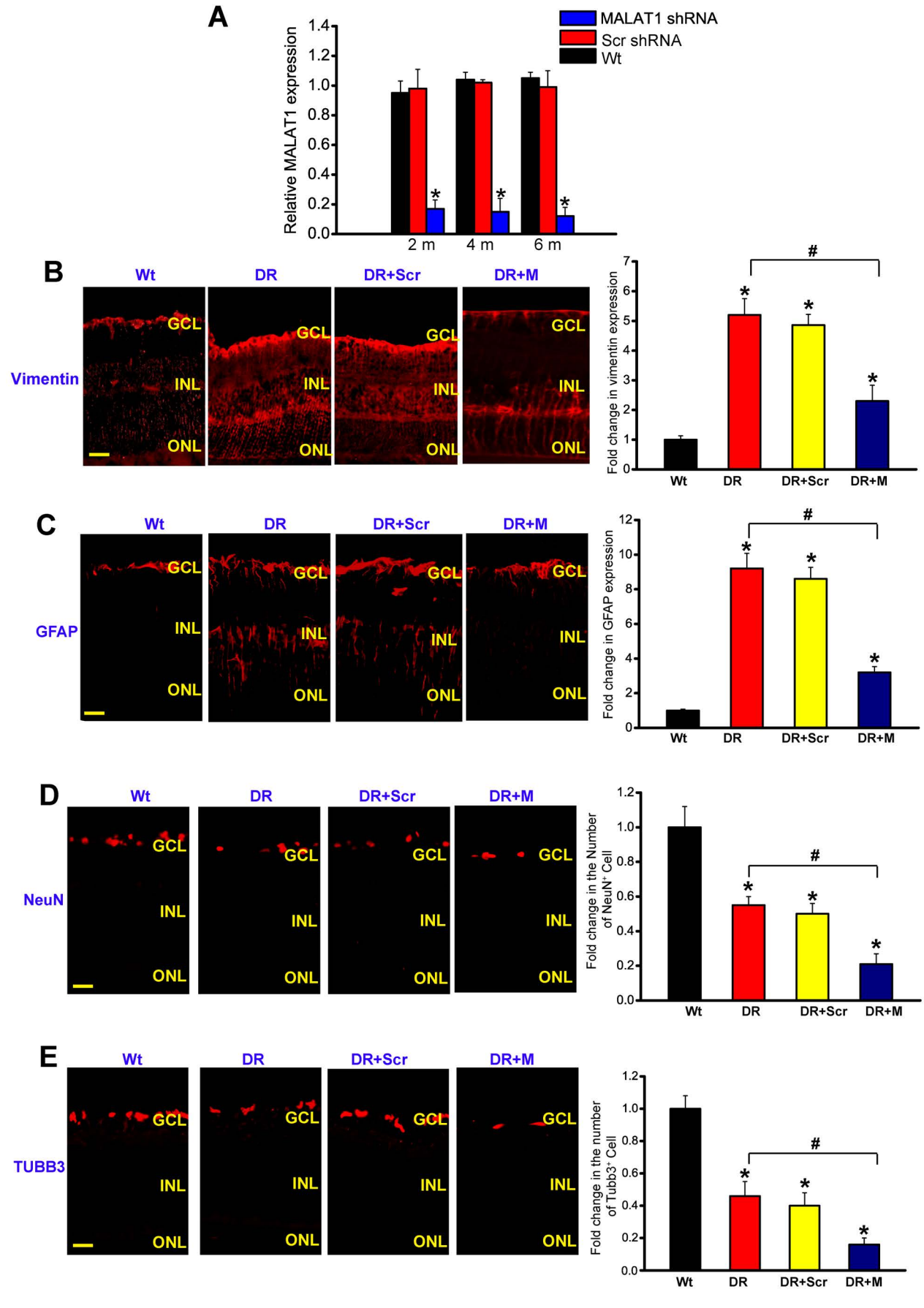

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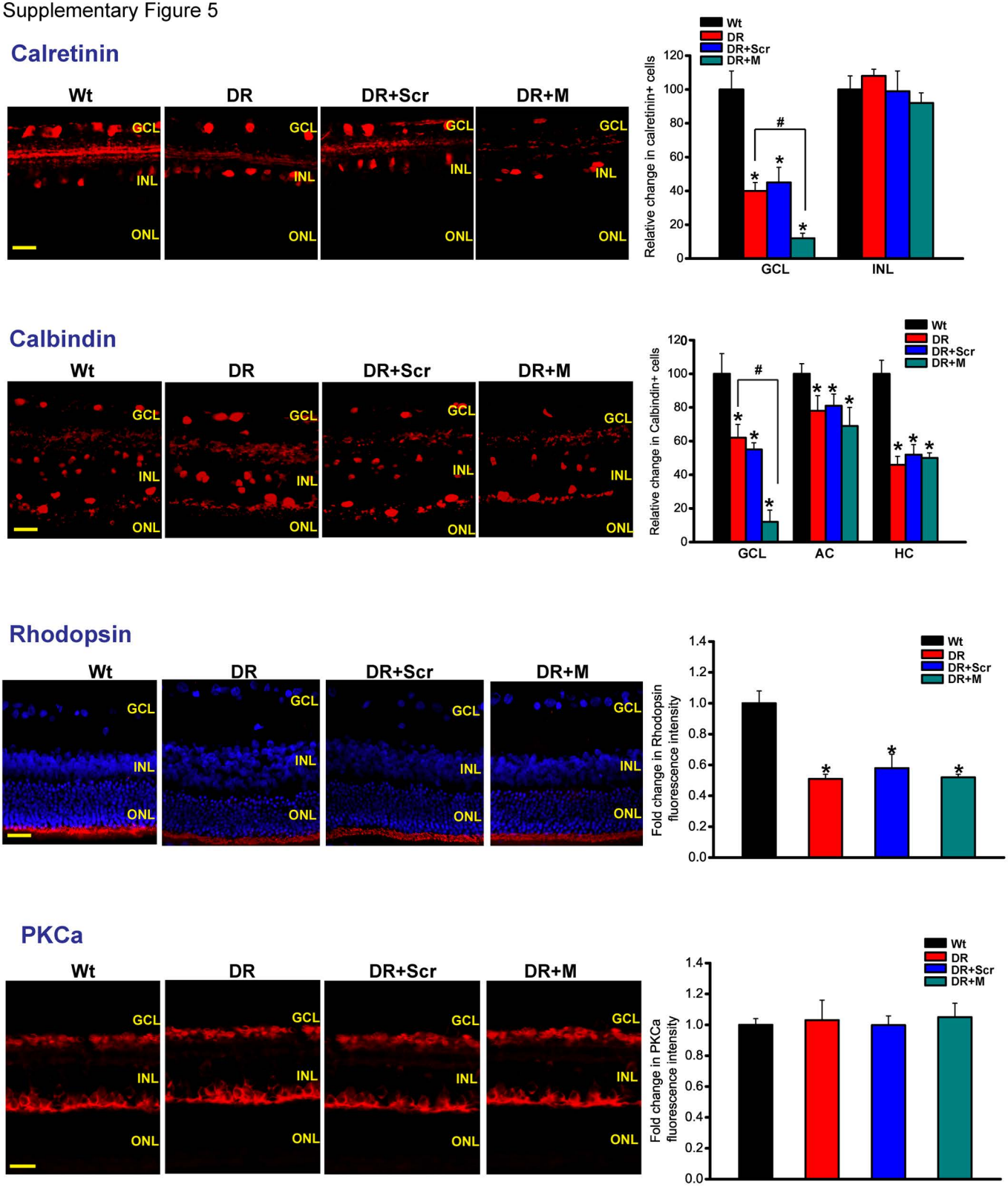

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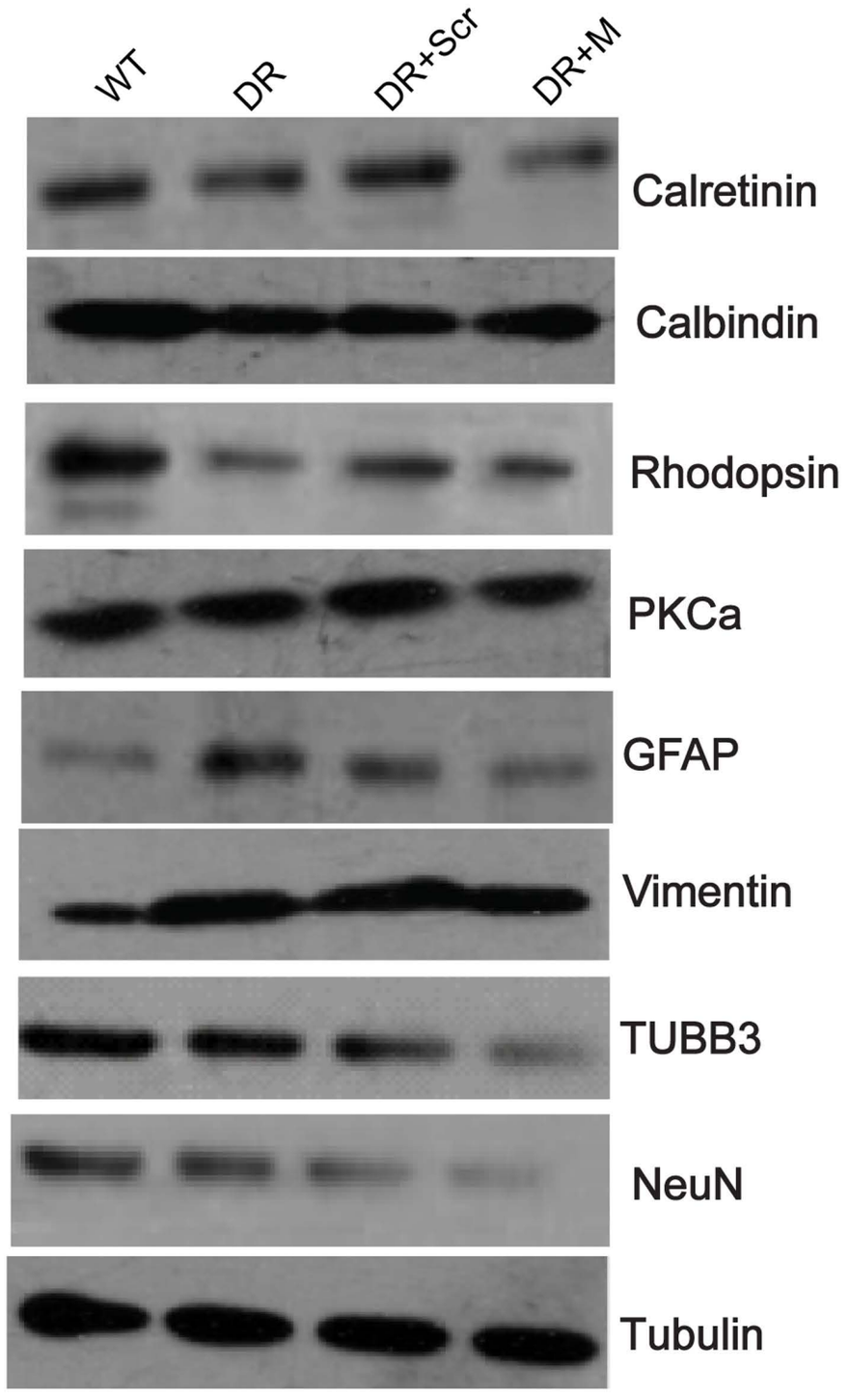
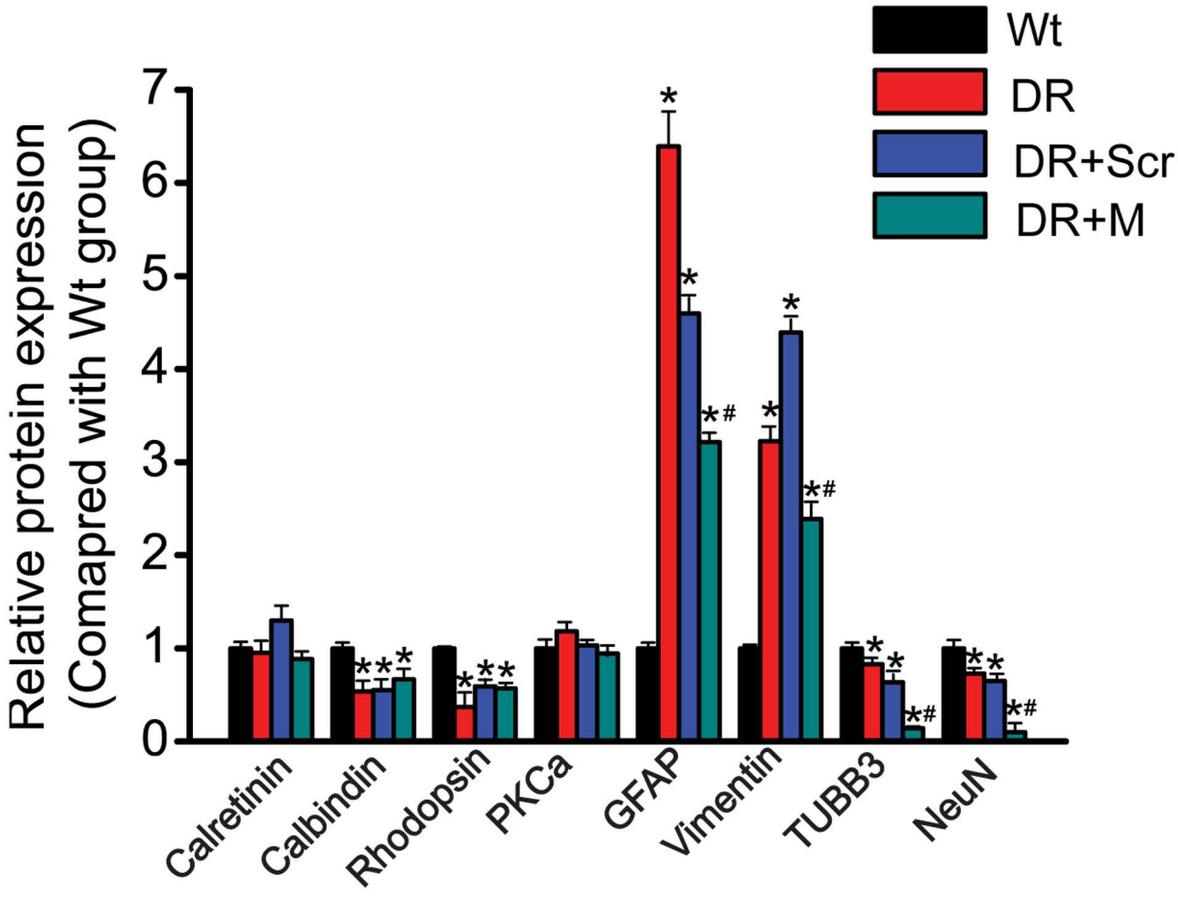
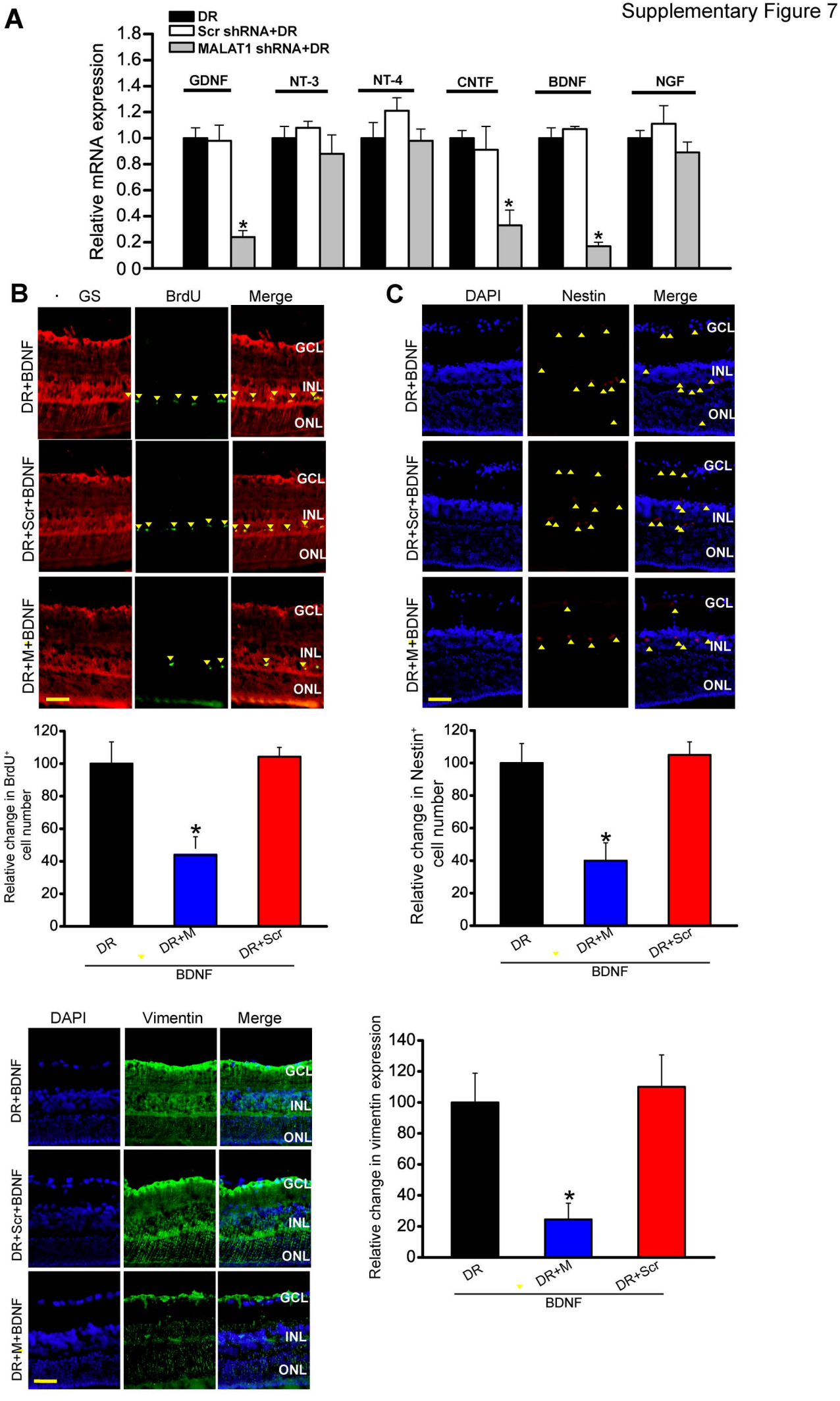
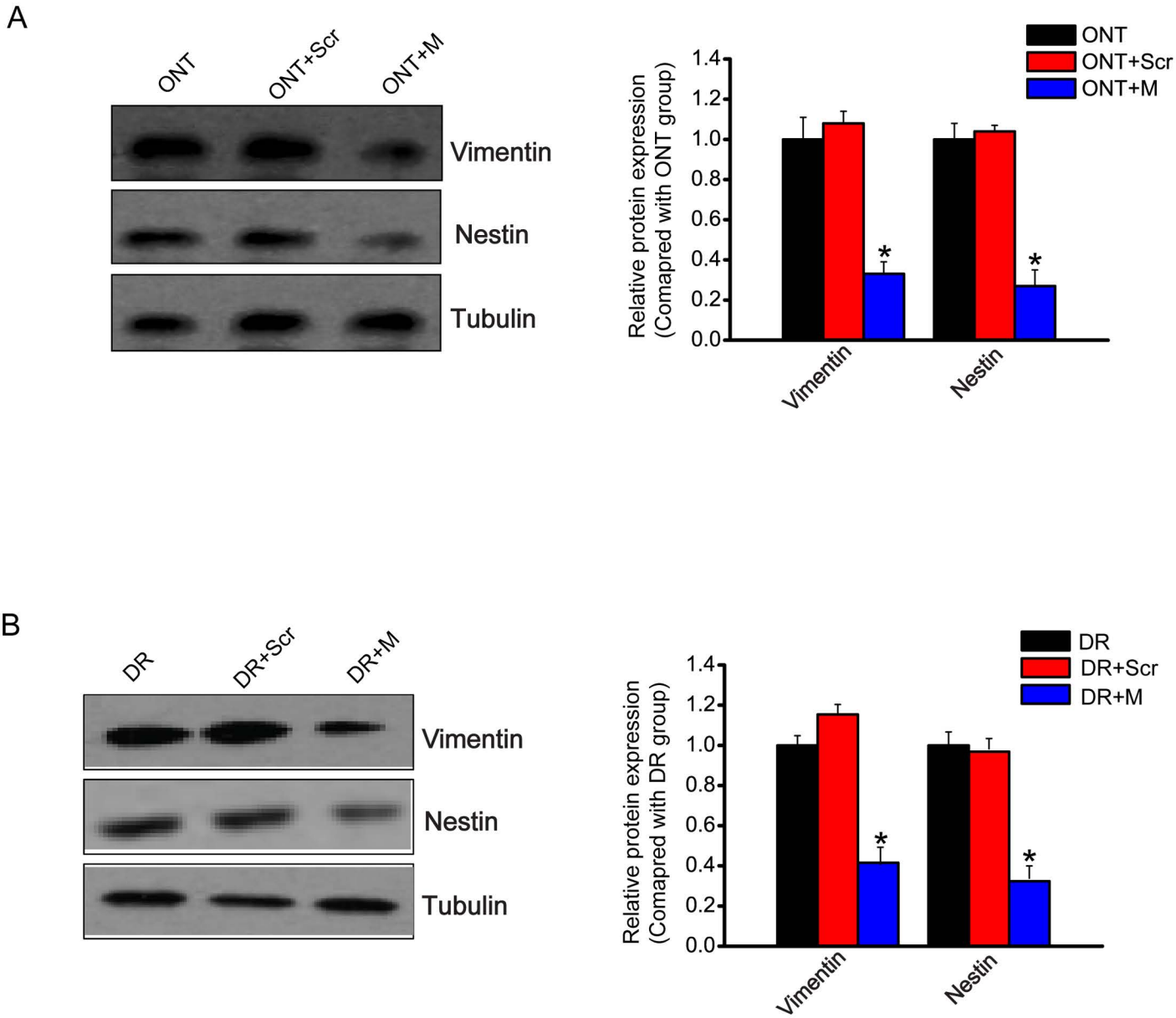
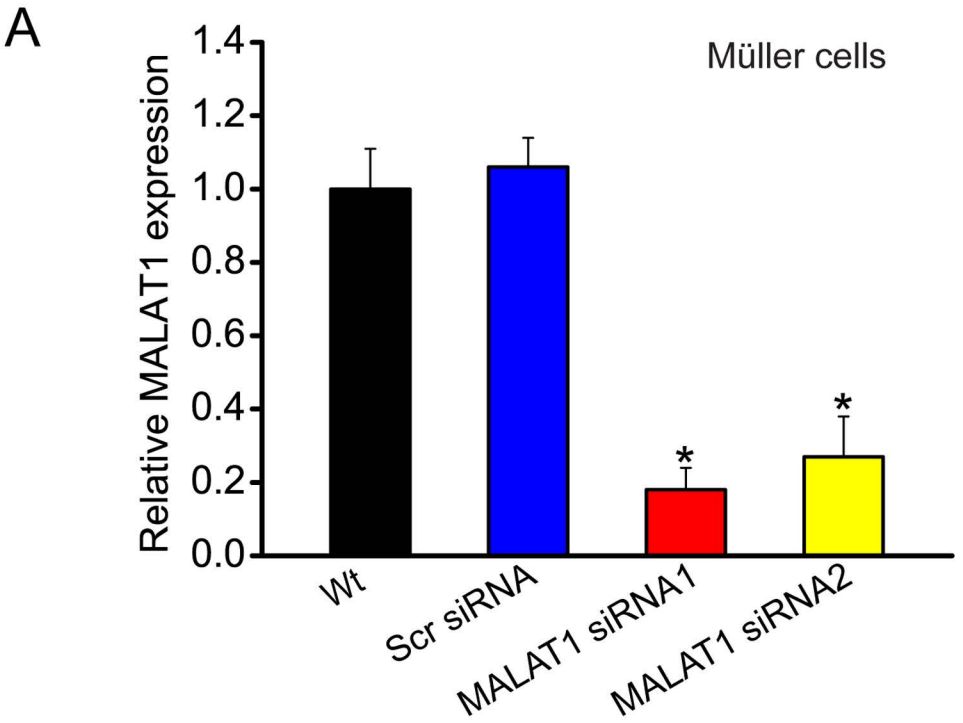


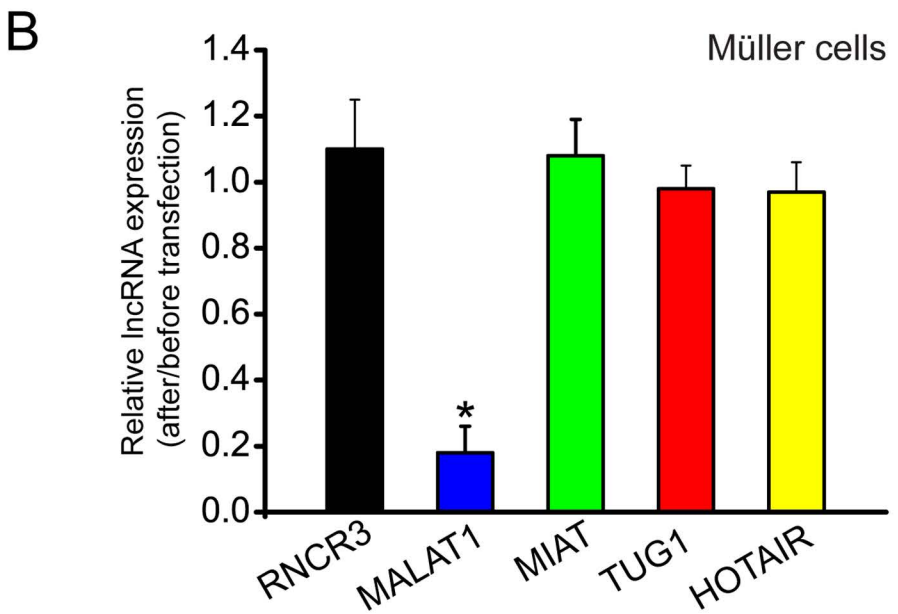



A
DR
Scr shRNA+DR
MALAT1 shRNA+DR
Relative mRNA expression
GDNF
NT-3
NT-4
CNTF
BDNF
NGF
B
GS
BrdU
Merge
DR+BDNF
DR+Scr+BDNF
DR+M+BDNF
GCL
INL
ONL
Relative change in BrdU+ cell number
DR
DR+M
DR+Scr
BDNF
C
DAPI
Nestin
Merge
Relative change in Nestin+ cell number
D
DAPI
Vimentin
Merge
Relative change in vimentin expression

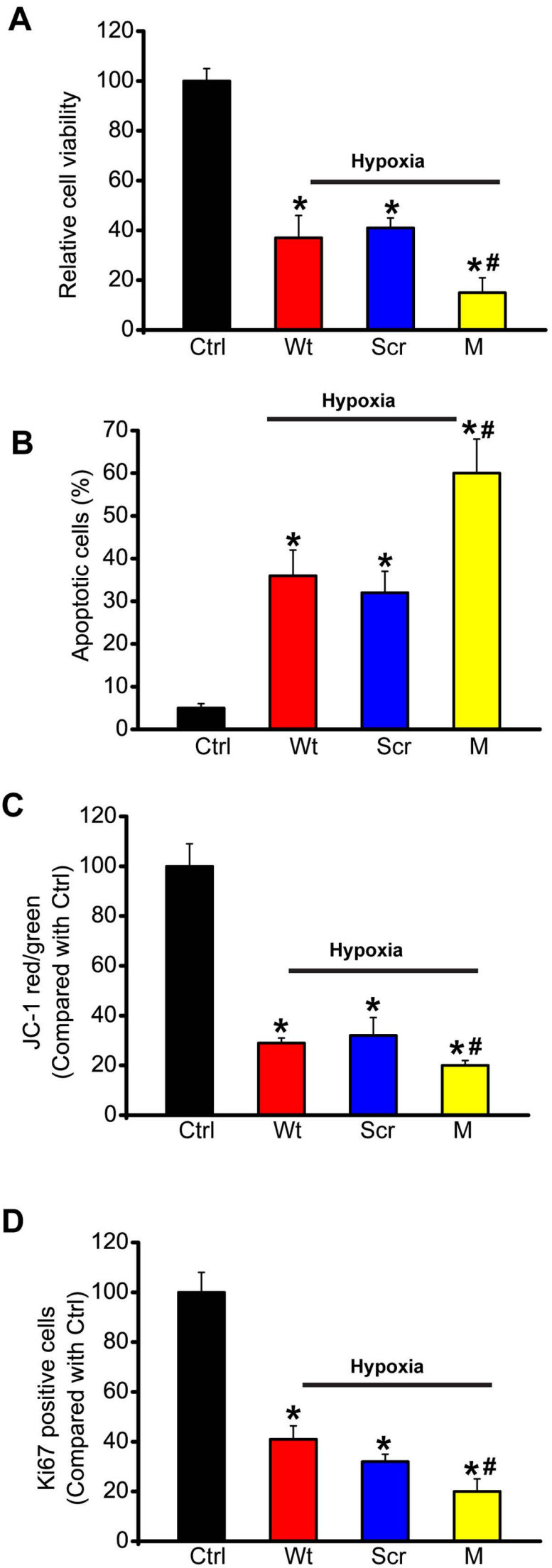
Supplementary Figure 8

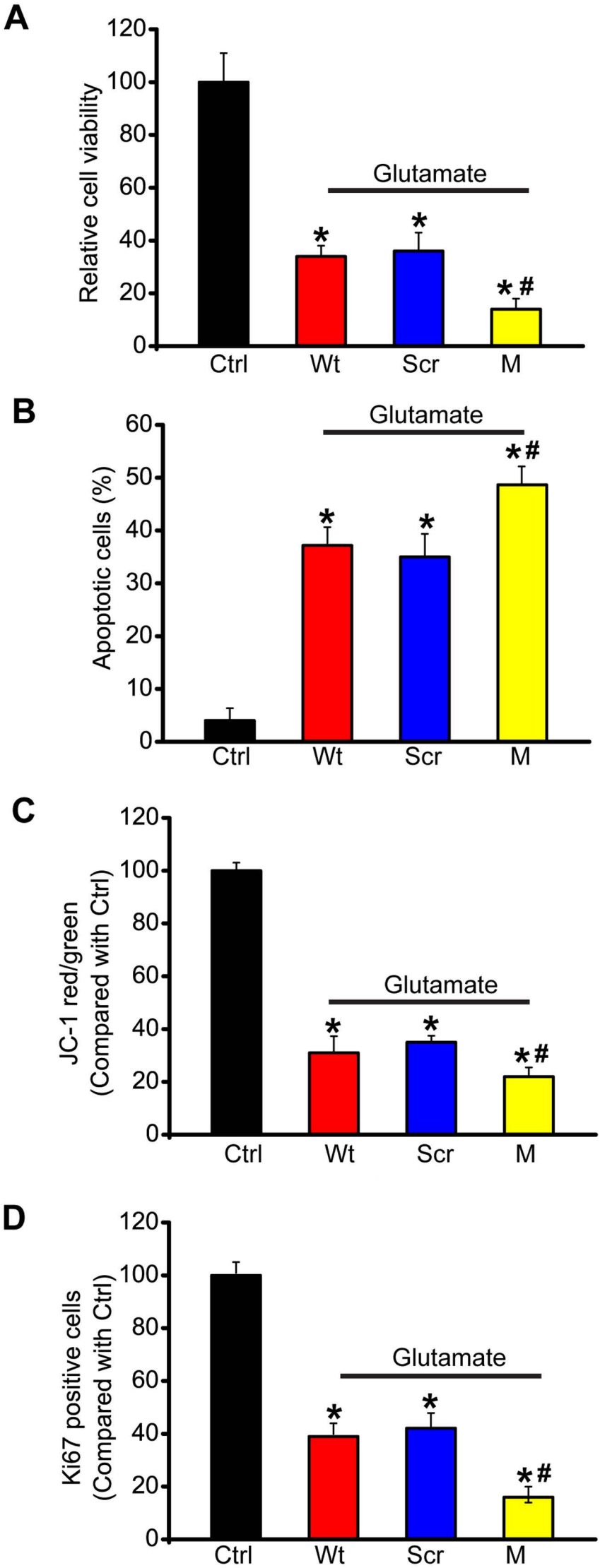


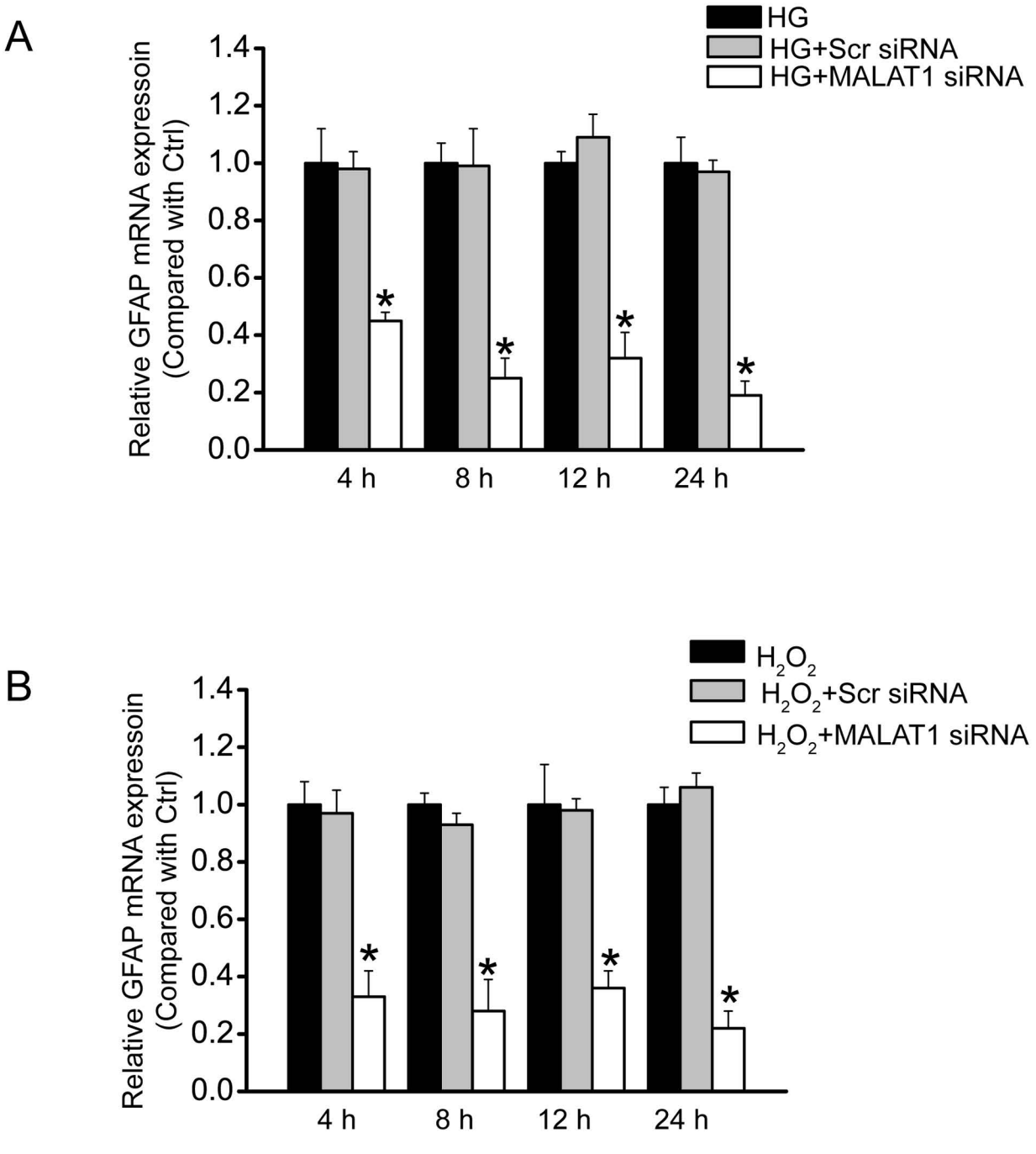

A
Müller cells
Relative MALAT1 expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Wt
Scr siRNA
MALAT1 siRNA1
MALAT1 siRNA2


B
Müller cells
Relative lncRNA expression
(after/before transfection)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
RNCR3
MALAT1
MIAT
TUG1
HOTAIR

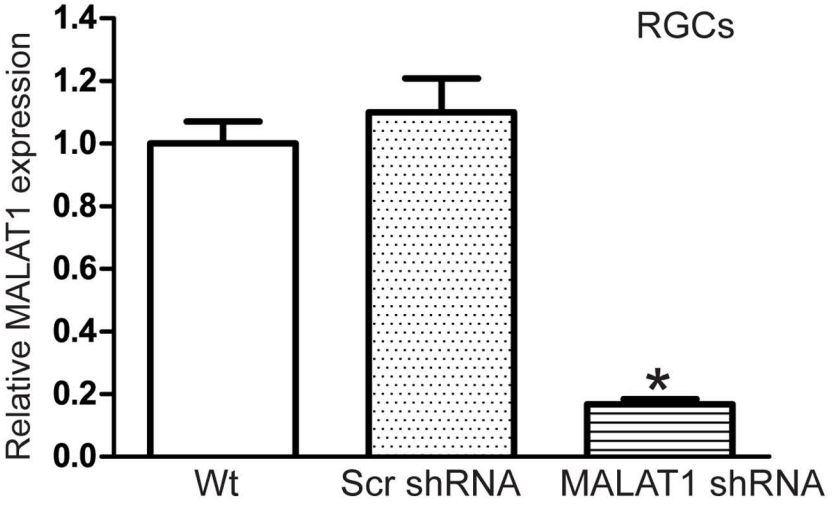
Supplementary Figure 10

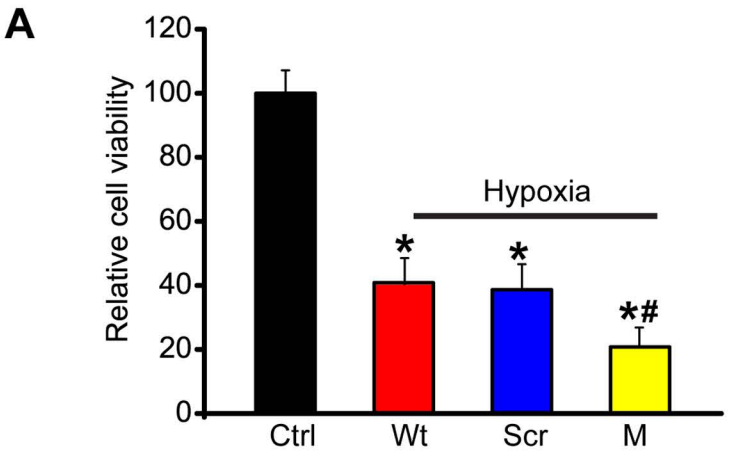


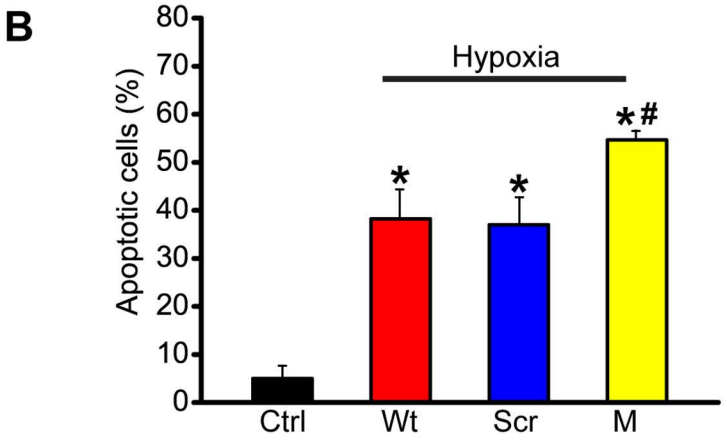

A
Relative cell viability
120
100
80
60
40
20
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M
B
Apoptotic cells (%)
60
50
40
30
20
10
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M
C
JC-1 red/green
(Compared with Ctrl)
120
100
80
60
40
20
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M
D
Ki67 positive cells
(Compared with Ctrl)
120
100
80
60
40
20
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M

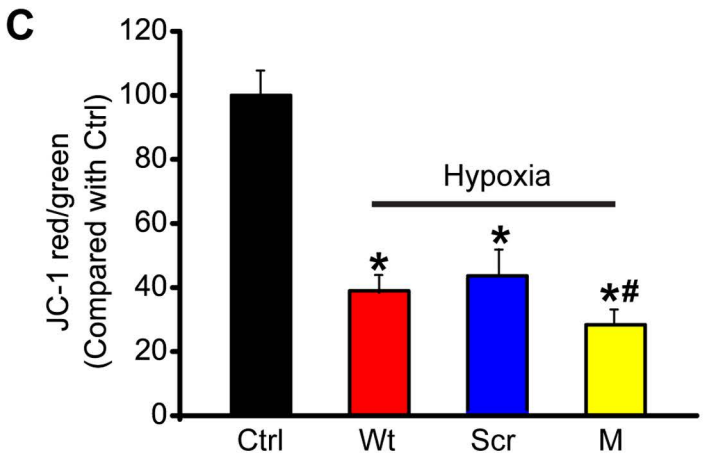

A
HG
HG+Scr siRNA
HG+MALAT1 siRNA
Relative GFAP mRNA expressoin
(Compared with Ctrl)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
4 h
8 h
12 h
24 h
B
H2O2
H2O2+Scr siRNA
H2O2+MALAT1 siRNA
Relative GFAP mRNA expressoin
(Compared with Ctrl)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
4 h
8 h
12 h
24 h

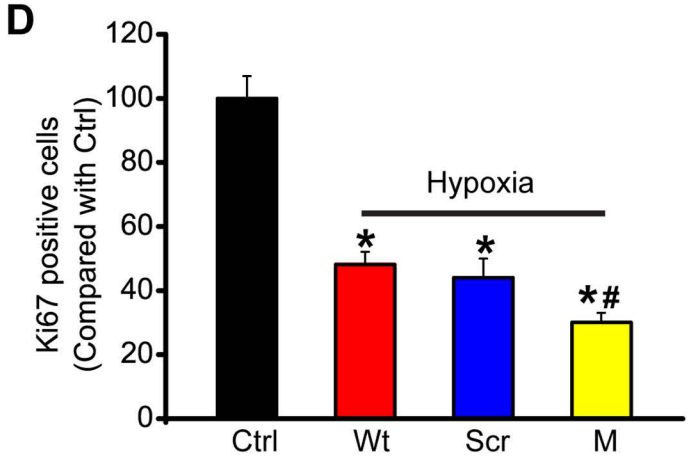
Supplementary Figure 13

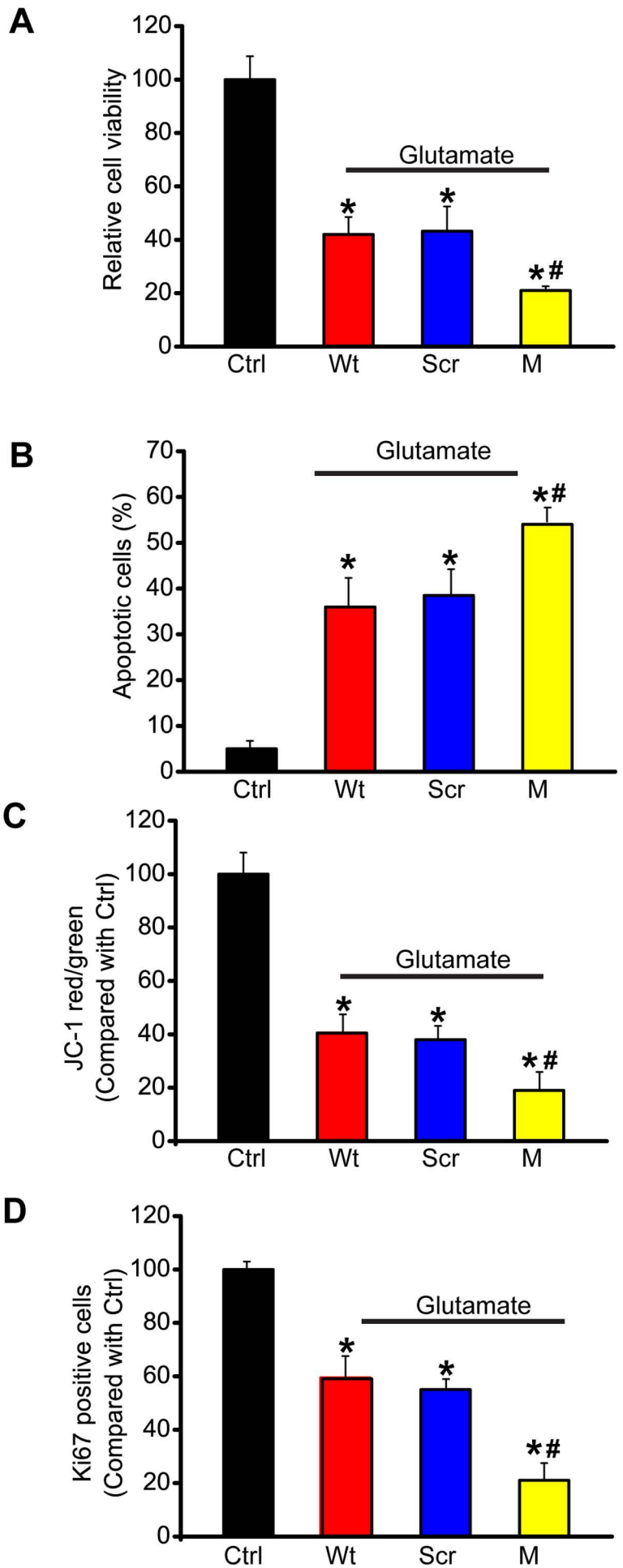



A
Relative cell viability
120
100
80
60
40
20
0
Hypoxia
*
*
*#
Ctrl
Wt
Scr
M

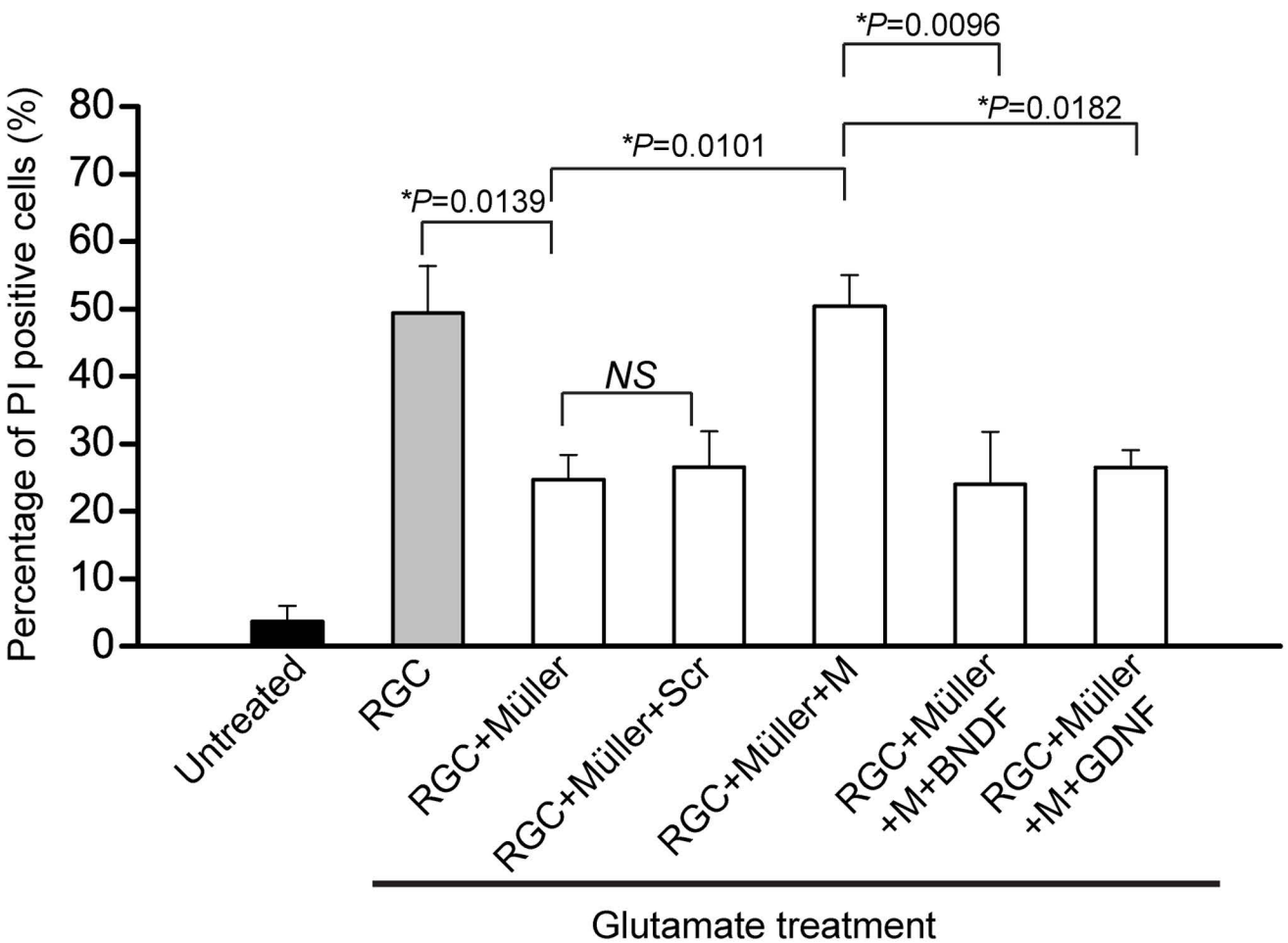

B
Apoptotic cells (%)
80
70
60
50
40
30
20
10
0
Hypoxia
*
*
*#
Ctrl
Wt
Scr
M

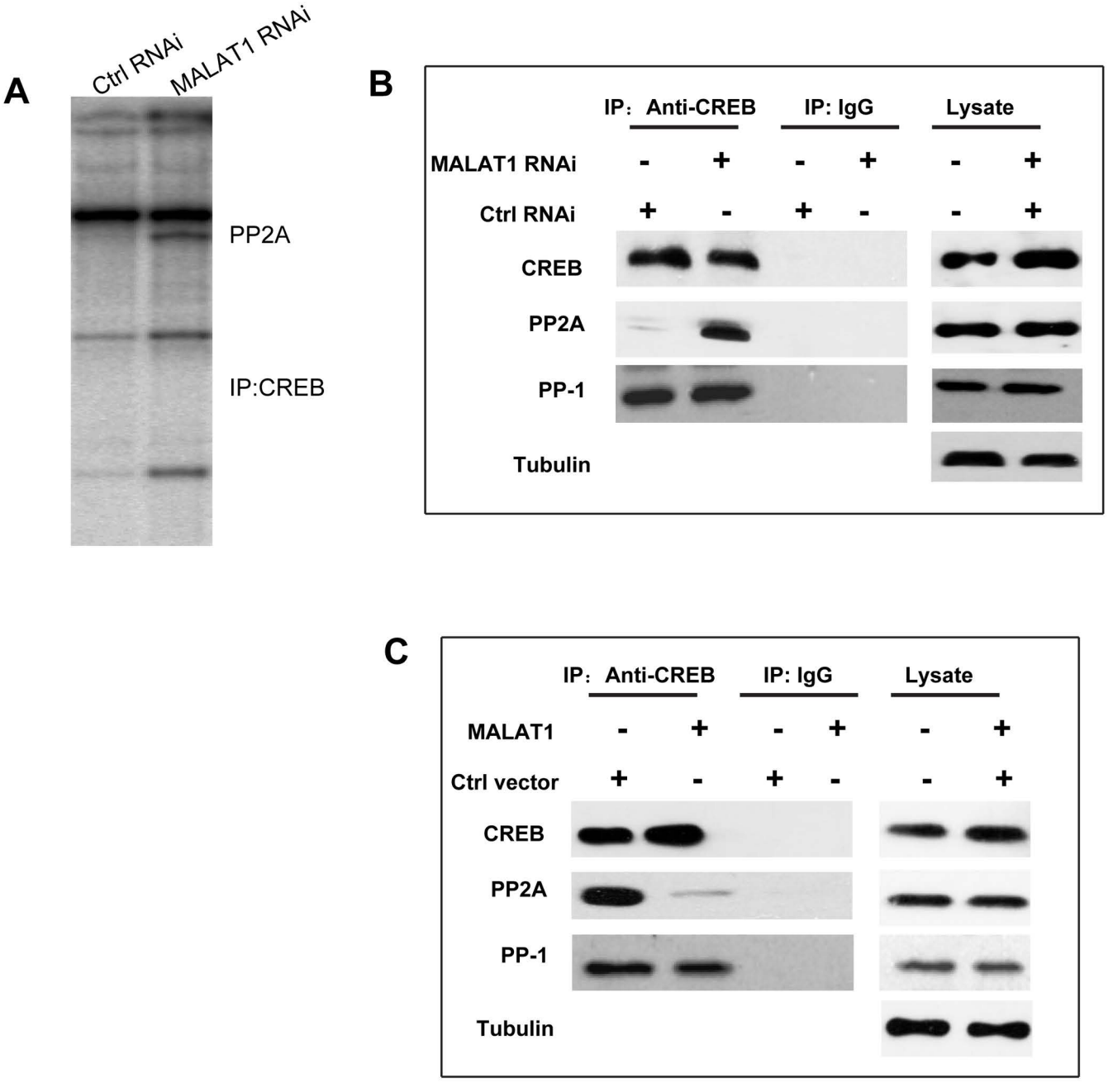

C
JC-1 red/green
(Compared with Ctrl)
120
100
80
60
40
20
0
Hypoxia
*
*
*#
Ctrl
Wt
Scr
M

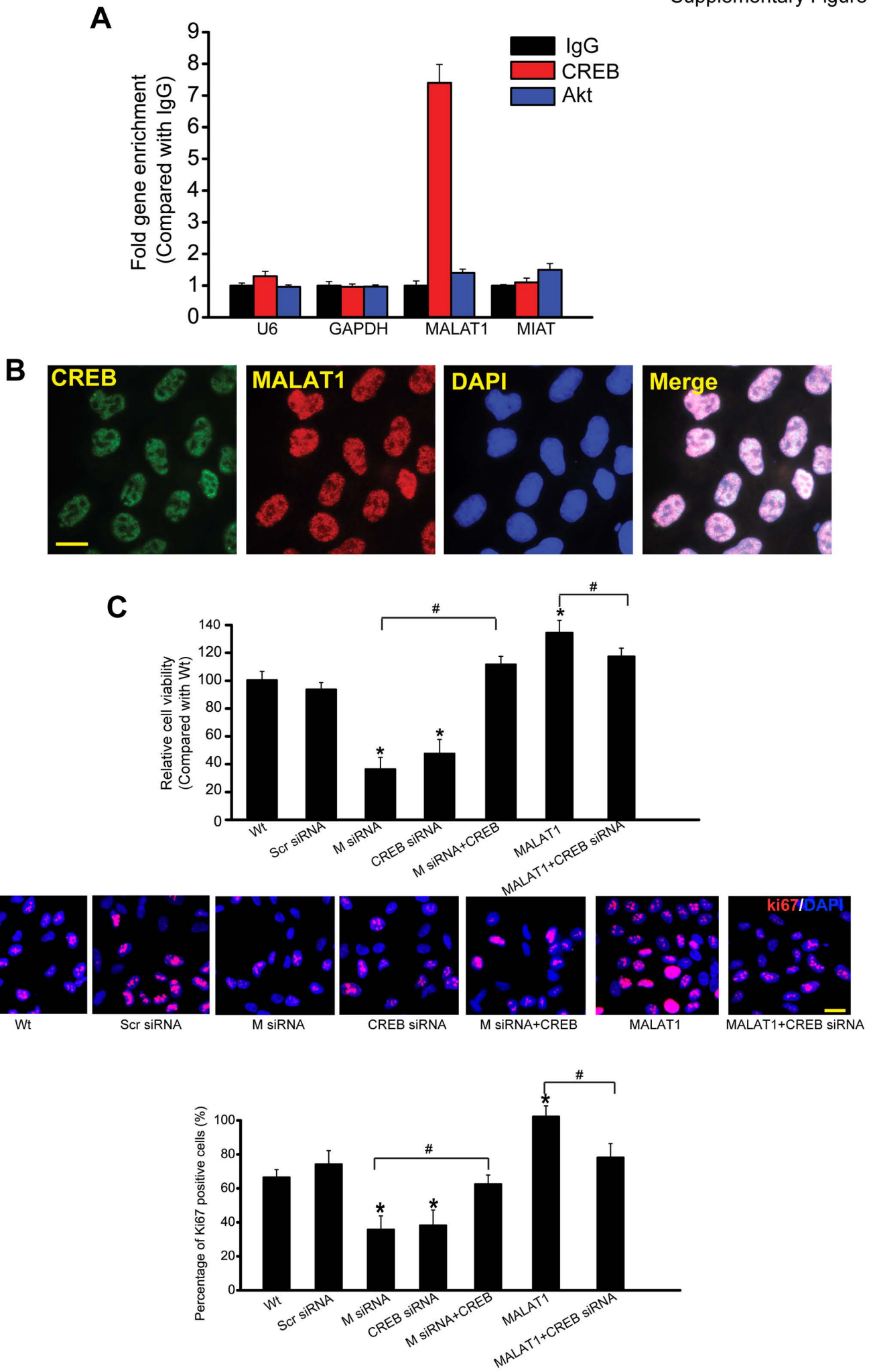

D
Ki67 positive cells
(Compared with Ctrl)
120
100
80
60
40
20
0
Hypoxia
*
*
*#
Ctrl
Wt
Scr
M

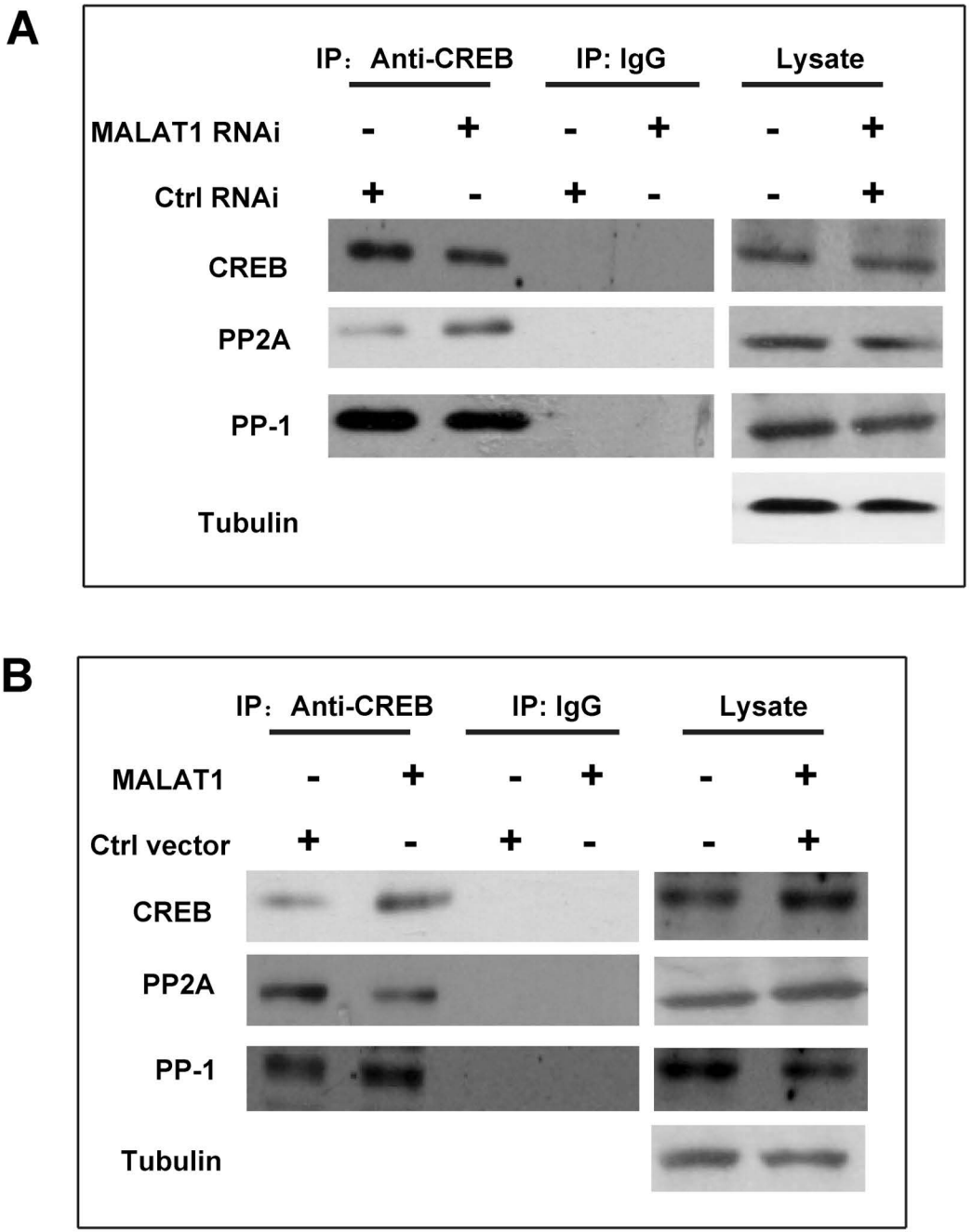

A
Relative cell viability
120
100
80
60
40
20
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M
B
Apoptotic cells (%)
70
60
50
40
30
20
10
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M
C
JC-1 red/green
(Compared with Ctrl)
120
100
80
60
40
20
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M
D
Ki67 positive cells
(Compared with Ctrl)
120
100
80
60
40
20
0
Glutamate
*
*
*#
Ctrl
Wt
Scr
M

Supplementary Figure 16




A
Ctrl RNAi
MALAT1 RNAi
PP2A
IP:CREB
B
IP: Anti-CREB
IP: IgG
Lysate
MALAT1 RNAi
Ctrl RNAi
CREB
PP2A
PP-1
Tubulin
C
IP: Anti-CREB
IP: IgG
Lysate
MALAT1
Ctrl vector
CREB
PP2A
PP-1
Tubulin


A
Fold gene enrichment (Compared with IgG)
IgG
CREB
Akt
U6
GAPDH
MALAT1
MIAT
B
CREB
MALAT1
DAPI
Merge
C
Relative cell viability (Compared with Wt)
Wt
Scr siRNA
M siRNA
CREB siRNA
M siRNA+CREB
MALAT1
MALAT1+CREB siRNA
D
ki67/DAPI
Wt
Scr siRNA
M siRNA
CREB siRNA
M siRNA+CREB
MALAT1
MALAT1+CREB siRNA
Percentage of Ki67 positive cells (%)
Wt
Scr siRNA
M siRNA
CREB siRNA
M siRNA+CREB
MALAT1
MALAT1+CREB siRNA


A
IP：Anti-CREB
IP: IgG
Lysate
MALAT1 RNAi
Ctrl RNAi
CREB
PP2A
PP-1
Tubulin
B
IP：Anti-CREB
IP: IgG
Lysate
MALAT1
Ctrl vector
CREB
PP2A
PP-1
Tubulin

Appendix supplementary figure legend

Appendix Fig S1: MALAT1 shRNA injection reduces MALAT1 but not other lncRNAs expression

(A) The mouse retinas were injected with scrambled shRNA (Scr), MALAT1 shRNA-1, MALAT1 shRNA-2, MALAT1 shRNA-3, or left untreated (Ctrl) for 48 h. qRT-PCRs were performed to detect MALAT1 levels [n=5 independent experiments; analyzed by Mann-Whitney U-test; $^{*}P$=0.0112 (MALAT1 shRNA-1), $^{*}P$=0.0169 (MALAT1 shRNA-2), $^{*}P$=0.0217 (MALAT1 shRNA-3)]. "*" indicated significant difference compared with Ctrl group. (B) The mouse retinas were injected with MALAT1 shRNA-1 for 48 h. qRT-PCRs were performed to compare the expression change of long non-coding RNAs, including MALAT1, RNCR3, MIAT, HOTAIR, and TUG1, before and after transfection [n=5 independent experiments; analyzed by Mann-Whitney U-test; $^{*}P$=0.0114(MALAT1)].

Appendix Fig S2: MALAT1 knockdown does not affect retinal horizontal cells, amacrine cells, photoreceptors, and bipolar cells in ONT retinas

Four-month old male C57Bl/6J mice were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then ONT models were established. All shRNA vectors were injected once a week. Two weeks after ONT, retinal slices were immunolabeled for the marker proteins, including, calretinin, calbindin, rhodopsin, and PKCα. Scale bar, 100 μm. Each experimental group had 6 animals. Quantification analysis data were pooled from

three independent experiments. *P*-values were calculated by Mann-Whitney U-test. Calretinin, **P*=0.0268 (ONT), **P*=0.0212 (ONT+Scr), **P*=0.0106 (ONT+M), # *P*=0.0115; Calbindin, **P*=0.0379 (ONT), **P*=0.0305 (ONT+Scr), **P*=0.0199 (ONT+M), # *P*=0.0315; Rhodopsin, **P*=0.0318 (ONT), **P*=0.0276 (ONT+Scr), **P*=0.0373 (ONT+M). AC, amacrine cell; HC, horizontal cell; GCL, ganglion cell layer; INL, inner nuclear layer; RGC, retinal ganglion cell; ONL, outer nuclear layer; ONT, optic nerve transection; Scr, scrambled shRNA; M, MALAT1 shRNA. "*" indicated significant difference compared with Wt group. "#" indicated significant difference between the marked groups.

Appendix Fig S3: MALAT1 knockdown reduces vimentin, GFAP, NeuN and TUBB3 expression in ONT retinas

Four-month old male C57Bl/6J mice were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then ONT models were established. All shRNA vectors were injected once a week. Two weeks after ONT, total proteins were extracted from ONT and wild-type (Wt) retinas. Western blots were performed to detect protein expression. Tubulin was detected as the internal control. The data was expressed as the relative change compared with Wt group [n=5 independent experiments; analyzed by Mann-Whitney U-test; Rhodopsin: **P*=0.0232 (DR), **P*=0.0280 (DR+Scr), **P*=0.0319 (DR+M); GFAP: **P*=0.0098 (DR), **P*=0.0019 (DR+Scr), **P*=0.0151 (DR+M), #*P*=0.0204 (DR+M); Vimentin: **P*=0.0126 (DR), **P*=0.0060 (DR+Scr), **P*=0.0185 (DR+M), #*P*=0.0232 (DR+M); TUBB3: **P*=0.0382 (DR), **P*=0.0317

(DR+Scr), *P=0.0243 (DR+M), #P=0.0264 (DR+M); NeuN: *P=0.0255 (DR), *P=0.0202 (DR+Scr), *P=0.0124 (DR+M), #P=0.0233 (DR+M)]. The data was expressed as the relative change compared with Wt group. "*" indicated significant difference compared with Wt group. "#" indicated significant difference between the marked groups.

Appendix Fig S4: MALAT1 knockdown affects retinal reactive gliosis and RGC survival in diabetic retinas

(A) Three-month old male SD rats were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA viral vector for the indicated time periods. MALAT1 levels were detected using qRT-PCRs [n=3 independent experiments; analyzed by Mann-Whitney U-test; *P=0.0212 (2 m), *P=0.0183 (4 m), *P=0.0175 (6 m)]. (B-E) Three-month old male SD rats were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then diabetic models were established. Each experimental group had 5 animals. All shRNA vectors were injected once a week. Six months after diabetes induction, retinal slices were immunolabeled for marker proteins, including vimentin, GFAP, NeuN, and TUBB3. Quantification analysis data were pooled from three independent experiments. P-values were calculated by Mann-Whitney U-test. Vimentin: *P=0.0047 (DR), *P=0.0097 (DR+Scr), *P=0.0176 (DR+M), #P=0.0218; GFAP: *P=0.0018 (DR), *P=0.0032 (DR+Scr), *P=0.0349(DR+M), #P=0.0118; NeuN: *P=0.0320 (DR), *P=0.0277 (DR+Scr), *P=0.0176 (DR+M), #P=0.0241; TUBB3: *P=0.0280 (DR), *P=0.0211

(DR+Scr), *P=0.0124(DR+M), #P=0.0283. Scale bar, 100 μm. GCL, ganglion cell layer; INL, inner nuclear layer; RGC, retinal ganglion cell; ONL, outer nuclear layer; DR, diabetic retinopathy; Scr, scrambled shRNA; M, MALAT1 shRNA. "*" indicated significant difference compared with the control group. "#" indicated significant difference between the marked groups.

Appendix Fig S5: MALAT1 knockdown does not affect retinal horizontal cells, amacrine cells, photoreceptors, and bipolar cells in diabetic retinas

Three-month old male SD rats were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then diabetic models were established. All shRNA vectors were injected once a week. Six months after diabetes induction, retinal slices were immunolabeled for the marker proteins, including calretinin, calbindin, rhodopsin, and PKCα. Each experimental group had 5 animals. Quantification analysis data were pooled from three independent experiments. *P*-values were calculated by Mann-Whitney U-test. Calretinin: *P=0.0211(DR), *P=0.0283 (DR+Scr), *P=0.0086 (DR+M), # P=0.0229; Calbindin: GCL, *P=0.0409 (DR), *P=0.0361 (DR+Scr), *P=0.0107 (DR+M), # P=0.0277; AC, *P=0.0385 (DR), *P=0.0401 (DR+Scr), *P=0.0321 (DR+M); HC, *P=0.0198 (DR), *P=0.0263 (DR+Scr), *P=0.0224 (DR+M); Rhodopsin, *P=0.0201 (ONT), *P=0.0301 (ONT+Scr), *P=0.0233 (ONT+M). Scale bar, 100 μm. AC, amacrine cell; HC, horizontal cell; GCL, ganglion cell layer; INL, inner nuclear layer; RGC, retinal ganglion cell; ONL, outer nuclear layer; DR, diabetic retinopathy; Scr, scrambled shRNA; M, MALAT1 shRNA. "*" indicated

significant difference compared with Wt group. "#" indicated significant difference between the marked groups.

Appendix Fig S6: MALAT1 knockdown reduces vimentin, GFAP, NeuN and TUBB3 expression in diabetic retinas

Three-month old male SD rats were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then diabetic models were established. All shRNA vectors were injected once a week. Six months after diabetes induction, total proteins were extracted from the diabetic retinas and wild-type (Wt) retinas. Western blots were performed to detect protein expression. Tubulin was detected as the internal control [n=5 independent experiments; analyzed by Mann-Whitney U-test; Calbindin: **P*=0.0216 (DR), **P*=0.0265 (DR+Scr), **P*=0.0382 (DR+M); Rhodopsin: **P*=0.0102 (DR), **P*=0.0286 (DR+Scr), **P*=0.0264 (DR+M); GFAP: **P*=0.0039 (DR), **P*=0.0103 (DR+Scr), **P*=0.0179 (DR+M), $^{\#}P$=0.0179 (DR+M); Vimentin: **P*=0.0185 (DR), **P*=0.0076 (DR+Scr), **P*=0.0225 (DR+M), $^{\#}P$=0.0285 (DR+M); TUBB3: **P*=0.0452 (DR), **P*=0.0381 (DR+Scr), **P*=0.0179 (DR+M), $^{\#}P$=0.0228 (DR+M); NeuN: **P*=0.0284 (DR), **P*=0.0252(DR+Scr), **P*=0.0174 (DR+M), $^{\#}P$=0.0164 (DR+M)]. The data was expressed as the relative change compared with Wt group. "*" indicated significant difference compared with Wt group. "#" indicated significant difference between the marked groups.

Appendix Fig S7: MALAT1 knockdown affects Müller cell activation in diabetic retinas

(A) Three-month old male SD rats were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then diabetic models were established. All shRNA vectors were injected once a week. Six months after diabetes induction, the effect of MALAT1 knockdown on neurotrophic factor expression was determined in the diabetic retinas. For each determination, mRNA level in DR group was normalized to a value of 1.0. Each experimental group had 5 animals. n=3 independent experiments; analyzed by Mann-Whitney U-test; *P=0.0129 (GDNF), *P=0.0185 (CNTF), *P=0.0095 (BDNF). "*" indicated significant difference compared with DR group. GNDF, glial cell line-derived neurotrophic factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; CNTF, ciliary neurotrophic factor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor. (B) Three-month old rats were received an intravitreous injection of Ad-Scr shRNA or MALAT1 shRNA, or left untreated for 1 week, and then diabetic models were established for 6-month induction. All shRNA vectors were injected once a week. BrdU (50 mg/kg) was injected into the diabetic retinas 1-month before sample collection. Meanwhile, BDNF (1 μg/μl) were intraocularly injected into DR, DR+Scr shRNA, or ONT+MALAT1 shRNA rats. BrdU-labeled cells in the INL were double labeled (arrowheads) with glutamine synthetase (GS). Each experimental group had 5 animals. Quantification analysis showed that MALAT1 knockdown affected Müller glia proliferation (n=3 independent experiments; analyzed by Mann-Whitney U-test; P=0.0040). (C, D) Three-month old male SD rats were received an intravitreous injection of

scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then diabetic models were established for 6-month induction. All shRNA vectors were injected once a week. Each experimental group had 5 animals. Immunohistochemical analysis was performed to detect nestin (C; n=3 independent experiments; analyzed by Mann-Whitney U-test; *P*=0.0025) or vimentin (D; n=3 independent experiments, analyzed by Mann-Whitney U-test; *P*=0.0008) expression. Scale bar, 100 μm. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. "*" indicated significant difference compared with DR group.

Appendix Fig S8: MALAT1 knockdown reduces vimentin and nestin expression

(A) Four-month old male C57Bl/6J mice were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then ONT models were conducted. All shRNA vectors were injected once a week. Two weeks after ONT, the total protein was extracted. Western blots were performed to detect vimentin and nestin expression. Tubulin was detected as the internal control. The data was expressed as the relative change compared with ONT group. "*" indicated significant difference compared with ONT group (n=3 independent experiments; analyzed by Mann-Whitney U-test; Vimentin: *P*=0.0225, Nestin, *P*=0.0198). (B) Three-month old male SD rats were received an intravitreous injection of scrambled (Scr) shRNA or MALAT1 shRNA, or left untreated for 1 week, and then diabetic models were established. All shRNA

vectors were injected once a week. Six months after diabetes induction, the total protein was extracted, and western blots were performed to detect vimentin and nestin expression. Tubulin was detected as the internal control. The data was expressed as relative change compared with DR group. "*" indicated significant difference compared with DR group (n=3 independent experiments; analyzed by Mann-Whitney U-test; Vimentin: P=0.0285, Nestin, P=0.0241).

Appendix Fig S9: MALAT1 siRNA transfection reduces MALAT1 but not other lncRNAs expression in Müller cells

(A) Müller cells were transfected with scrambled siRNA (Scr), MALAT1 siRNA1, MALAT1 siRNA2, or left untreated (Wt) for 48 h. qRT-PCRs were performed to detect MALAT1 levels. The data was shown as the relative change compared with Wt group. "*" indicated significant difference compared with Wt group [n=5 independent experiments; analyzed by two-sided Student's t-test; *P=0.0138 (MALAT1 siRNA1), *P=0.0216 (MALAT1 siRNA2)]. (B) Müller cells were transfected with MALAT1 siRNA for 48 h. qRT-PCRs were performed to compare the expression change of long non-coding RNAs, including MALAT1, RNCR3, MIAT, HOTAIR, and TUG1, before and after transfection [n=3 independent experiments; analyzed by Mann-Whitney U-test; *P=0.0128 (MALAT1)].

Appendix Fig S10: MALAT1 knockdown affects Müller cell function upon hypoxia stress in vitro

(A-D) Müller cells were transfected with scrambled siRNA (Scr), MALAT1 siRNA, or left untreated (Wt), and then exposed to $CoCl_2$ (200 μm) to mimic hypoxia

stress for 48 h. The group without any treatment was taken as the control (Ctrl) group. Cell viability was detected using MTT method. The data was expressed as the relative change compared with Ctrl group [A; n=5 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0187 (Wt), **P*=0.0213 (Scr), **P*=0.0136 (M), #*P*=0.0202]. Apoptotic cells were analyzed using Hoechst staining and quantitated [B; n=5 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0156 (Wt), **P*=0.0241 (Scr), **P*=0.0069 (M), #*P*=0.0112]. Müller cells were incubated with JC-1 probe at 37 °C for 30 min, centrifuged, washed, transferred to a 96-well plate (100 000 cells per well), and assayed using a fluorescence plate reader [C; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0195 (Wt), **P*=0.0228 (Scr), **P*=0.0162 (M), #*P*=0.0425]. Ki67 immunofluorescence staining and quantitative analysis was performed to detect Müller cell proliferation [D; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0284 (Wt), **P*=0.0220 (Scr), **P*=0.0190 (M), #*P*=0.0391]. "*" indicated significant difference compared with Ctrl group. "#" indicated significant difference between the marked groups.

Appendix Fig S11: MALAT1 knockdown affects Müller cell function upon excitatory toxicity stress in vitro

(A-D) Müller cells were transfected with scrambled siRNA (Scr), MALAT1 siRNA, or left untreated, and then exposed to glutamate (3 mM) for 48 h. The group without any treatment was taken as the control (Ctrl) group. Cell viability was

detected using MTT method. The data was expressed as the relative change compared with Ctrl group [A; n=5 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0132 (Wt), **P*=0.0199 (Scr), **P*=0.0086 (M), #*P*=0.0319]. Apoptotic cells were analyzed using Hoechst staining and quantitated [B; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0320 (Wt), **P*=0.0391 (Scr), **P*=0.0272 (M), #*P*=0.0444]. Müller cells were incubated with JC-1 probe at 37 °C for 30 min, centrifuged, washed, transferred to a 96-well plate (100, 000 cells per well), and assayed using a fluorescence plate reader [C; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0354 (Wt), **P*=0.0403 (Scr), **P*=0.0325 (M), #*P*=0.0451]. Ki67 immunofluorescence staining and quantitative analysis was performed to detect Müller cell proliferation [D; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0312 (Wt), **P*=0.0385 (Scr), **P*=0.0206 (M), #*P*=0.0428]. "*" indicated significant difference compared with Ctrl group. "#" indicated significant difference between the marked groups.

Appendix Fig S12: MALAT1 knockdown down-regulates GFAP expression in vitro

Müller cells were transfected with scrambled (Scr) siRNA, MALAT1 siRNA, or left untreated, and then exposed to high glucose (HG, 30 mM, A) or H_2O_2 (50 μm, B) for the indicated time periods. The group only treated with high glucose or H_2O_2 was taken as the control (Ctrl) group. qRT-PCRs were performed to detect GFAP mRNA levels. GFAP levels were shown as the relative change compared with Ctrl

group [n=5 independent experiments; analyzed by two-sided Student's *t*-test; HG :**P*=0.0371 (4 h), **P*=0.0221 (8 h), **P*=0.0273 (12 h), **P*=0.0197 (24 h) ; H_2O_2 :**P*=0.0336 (4 h), **P*=0.0271 (8 h), **P*=0.0353 (12 h), **P*=0.0165 (24 h)].

Appendix Fig S13: MALAT1 shRNA transfection reduces MALAT1 levels in primary RGCs

Primary RGCs were transfected with scrambled (Scr) shRNA, MALAT1 shRNA, or left untreated (Wt) for 48 h. qRT-PCRs were performed to detect MALAT1 levels. The data was shown as relative expression change compared with Wt group. "*" indicated significant difference compared with Wt group (n=5 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0015).

Appendix Fig S14: MALAT1 knockdown affects RGC function upon hypoxia stress in vitro

(A-D) Primary RGCs were transfected with scrambled (Scr) shRNA, MALAT1 shRNA, or left untreated (Wt), and then exposed to $CoCl_2$ (200 μm) to mimic hypoxia stress for 48 h. The group without any treatment was taken as the control (Ctrl) group. Cell viability was detected using MTT method. The data was expressed as the relative change compared with Ctrl group [A; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0292 (Wt), **P*=0.0286 (Scr), **P*=0.0118 (M), $^{\#}P$=0.0319]. (B) Apoptotic cells were analyzed using Hoechst staining and quantitated [B; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0232 (Wt), **P*=0.0270 (Scr), **P*=0.0097 (M), $^{\#}P$=0.0386]. Primary RGCs were incubated with JC-1 probe at 37 °C for 30 min,

centrifuged, washed, transferred to a 96-well plate, and assayed using a fluorescence plate reader [C; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0161 (Wt), **P*=0.0217 (Scr), **P*=0.0077 (M), #*P*=0.0432]. Ki67 staining and quantitative analysis was performed to detect RGC proliferation [D; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0299 (Wt), **P*=0.0275 (Scr), **P*=0.0183 (M), #*P*=0.0391]. "*" indicated significant difference compared with Ctrl group. "#" indicated significant difference between the marked groups.

Appendix Fig S15: MALAT1 knockdown affects RGC function upon excitatory toxicity stress in vitro

(A-D) Primary RGCs were transfected with scrambled shRNA (Scr), MALAT1 shRNA, or left untreated, and then exposed to glutamate (3 mM) for 48 h. The group without any treatment was taken as the control (Ctrl) group. Cell viability was detected using MTT method. The data was expressed as the relative change compared with Ctrl group [A; n=5 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0281 (Wt), **P*=0.0305 (Scr), **P*=0.0185 (M), #*P*=0.0290]. Apoptotic cells were analyzed using Hoechst staining and quantitated [B; n=8 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0365 (Wt), **P*=0.0301 (Scr), **P*=0.0232 (M), #*P*=0.0263]. Primary RGCs were incubated with JC-1 probe at 37 °C for 30 min, centrifuged, washed, transferred to a 96-well plate, and assayed using a fluorescence plate reader [C; n=5 independent experiments; analyzed by two-sided Student's *t*-test; **P*=0.0302

(Wt), *P=0.0267 (Scr), *P=0.0192 (M), $^{\#}P$=0.0386]. Ki67 staining and quantitative analysis was performed to detect RGC proliferation [D; n=8 independent experiments; analyzed by two-sided Student's *t*-test; *P=0.0329 (Wt), *P=0.0305 (Scr), *P=0.0155 (M), $^{\#}P$=0.0121]. "*" indicated significant difference compared with Ctrl group. "#" indicated significant difference between the marked groups.

Appendix Fig S16: MALAT1 knockdown in Müller cells affects RGC function upon excitatory toxicity stress

Primary RGCs were co-cultured with Müller cells. Müller cells were transfected with MALAT1 siRNA (M) or scrambled siRNA (Scr), and then treated with or without BDNF (1 μg/μl) or GDNF (1 μg/μl). After these treatments, the experimental groups were treated with glutamate (3 mM) for 48 h. PI staining and quantitative analysis was performed to detect the dead or dying RGCs (n=5 independent experiments; analyzed by one-way ANOVA with Bonferroni *post hoc*). "*" indicated significant difference between the marked groups; *NS*, no significant difference.

Appendix Fig S17: MALAT1 intervention affects CREB-PP2A, but not CREB-PP-1 interaction in Müller cells

(A) PAGE gel resolution of immunoprecipitated CREB and CREB-associated proteins from Müller cells after transfection with MALAT1 siRNA or control siRNA. Different bands were analyzed by mass spectrometry. (B, C) Immunoblot detection of CREB, PP2A, and PP-1 in CREB-immunoprecipitated or IgG-immunoprecipitated complex from the lysates of Müller cells treansfected

with control siRNA or MALAT1 siRNA (B) or overexpressed MALAT1 or vector (C). Tubulin was detected as the internal control.

Appendix Fig S18: MALAT1 regulates RGC function through CREB signaling

(A) qPCR detection of the indicated RNAs retrieved by immunoglobulin G (IgG), CREB- or Akt-specific antibody in the RIP assay within RGCs. (B) Co-localization analysis: RNA-FISH assay of MALAT1 combined with immunofluorescence detection of CREB in the primary RGCs. Scale bars, 20 μm. (B, C) Primary RGCs were transfected with scrambled (Scr) siRNA, MALAT1 (M) siRNA, MALAT1 siRNA (M) plus CREB, CREB siRNA, MALAT1, MALAT1 plus CREB siRNA, or left untreated for 48 h. MTT assay (C) and Ki67 staining (D) was performed to detect the change of cell viability and cell proliferation (n=5 independent experiments; analyzed by Kruskal-Wallis test). The data was shown as relative change compared with the untreated wild-type (Wt) group. DAPI, blue; Ki67, red. "*" indicated significant difference compared with Wt group. "#" indicated significant difference between the marked groups. C: $^{*}P$=0.0210 (M siRNA); $^{*}P$=0.0236 (CREB siRNA); $^{*}P$=0.0361 (MALAT1); $^{\#}P$=0.0237 M siRNA versus M siRNA+CREB; $^{\#}P$=0.0421 MALAT1 versus MALAT1+CREB siRNA. D: $^{*}P$=0.0211 (M siRNA); $^{*}P$=0.0279 (CREB siRNA); $^{*}P$=0.0307 (MALAT1); $^{\#}P$=0.0228 M siRNA versus M siRNA+CREB; $^{\#}P$=0.0245 MALAT1 versus MALAT1+CREB siRNA.

Appendix Fig S19: MALAT1 intervention affects CREB-PP2A, but not CREB-PP-1 interaction in primary RGCs

(A, B) Immunoblot detection of CREB, PP2A, and PP-1 in CREB-immunoprecipitated or IgG-immunoprecipitated complex from the lysates of primary RGCs transfected with scrambled (Ctrl) siRNA or MALAT1 siRNA (A) or overexpressed MALAT1 or vector (B). Tubulin was detected as the internal control.

Appendix tables S1 & S2 &S3

Appendix Table S1: Demographic and clinical characteristics of subjects included in Alzheimer's disease study

	Control	AD
Sample size	10	12
female/male	4/6	5/7
Age	60±6	65±10
CSF $A\beta_{1-42}$, pg/ml	1456±389	690±114*
CSF t-tau, pg/ml	235±34	735±51*
CSF p-tau, pg/ml	35±14	92±17*
MMSE score	28.8±2.6	19.8±3.5*

Note: Aβ, Amyloid-β; AD, Alzheimer's disease; CSF, Cerebrospinal fluid; p-tau, Phosphorylated tau; t-tau, Total tau; MMSE, Mini-Mental State Exam. Data was shown as means ± SEM. * $P< 0.05$ vs. control.

Appendix Table S2: Demographic and clinical characteristics of subjects included glioma study

	Glioma by Grade	
	Grade 3	Grade 4
Sample size	4	6
female/male	2/2	2/4
Age	45±4	51±6
KPS	<90 1	<90 3
	⩾90 3	⩾90 3

Abbreviations: KPS, Karnofsky performance scale.

Appendix Table S3: Characteristics of cataract and POAG patients enrolled in this study

Characteristic	Cataract	POAG
Sample size	30	30
female/male	13/17	14/16
Age	60.5±5.6	63.2±8.1
Preoperative IOP, mm Hg	12.7 ± 2.3	27.3 ± 4.5*

Abbreviations: POAG, primary open-angle glaucoma.

The patients having history of cancer, asthma, diabetes mellitus, cardiovascular diseases, and ocular diseases other than cataract or POAG were excluded. Data was shown as means ± SEM. * $P< 0.05$ vs. Cataract.