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Supporting Information

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Bifunctional MXene-Augmented Retinal Progenitor Cell Transplantation for Retinal Degeneration

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Supporting Information includes:

S1. Supporting Figures S1~S15.

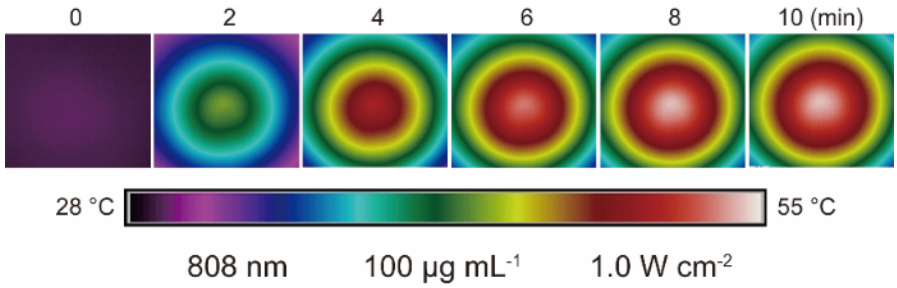

Figure S1. Thermal images of Nb_2C MXene (100 μg mL^{-1}) under irradiation by an 808 laser at a power density of 1.0 W cm^{-1}.

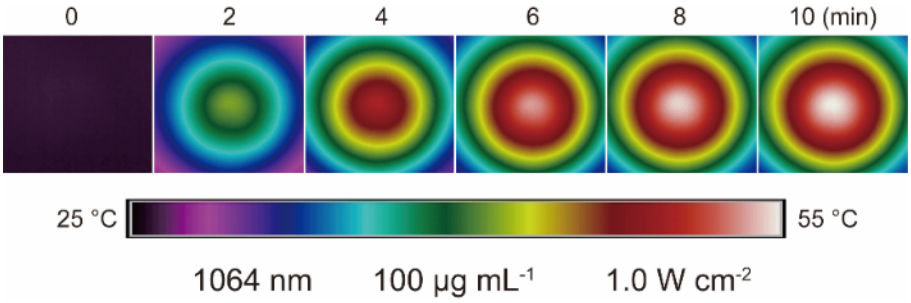

Figure S2. Thermal images of Nb_2C MXene (100 μg mL^{-1}) under irradiation by a 1064 laser at a power density of 1.0 W cm^{-1}.

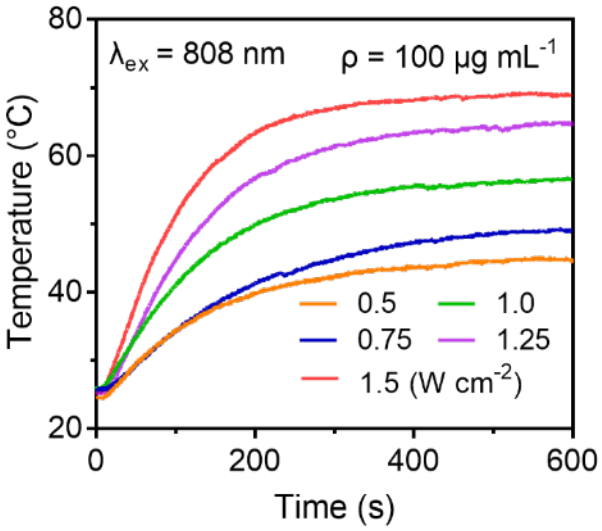

Figure S3. Photothermal profile of Nb_2C MXene (100 μg mL^{-1}) under irradiation by an 808 laser at different power densities.

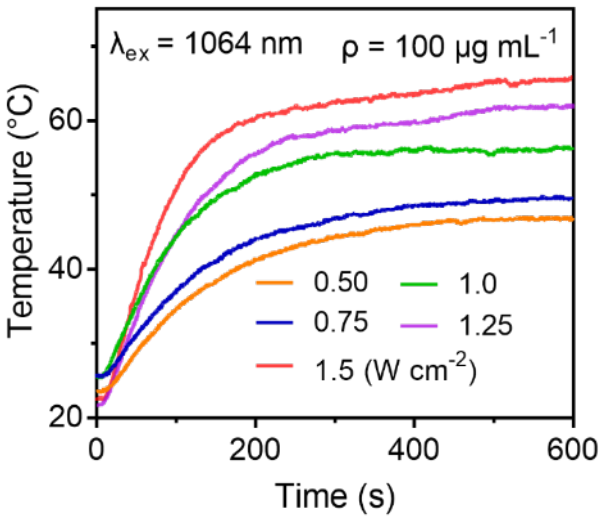

Figure S4. Photothermal profile of Nb_2C MXene (100 μg mL^{-1}) under irradiation by a 1064 laser at different power densities.

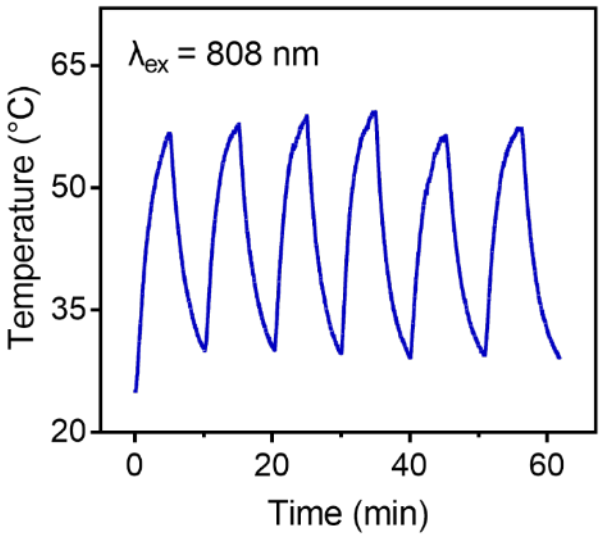

Figure S5. Photothermal curves of Nb_2C aqueous solution for six on/off irradiation cycles by an 808 nm laser (1 W cm^{-2}).

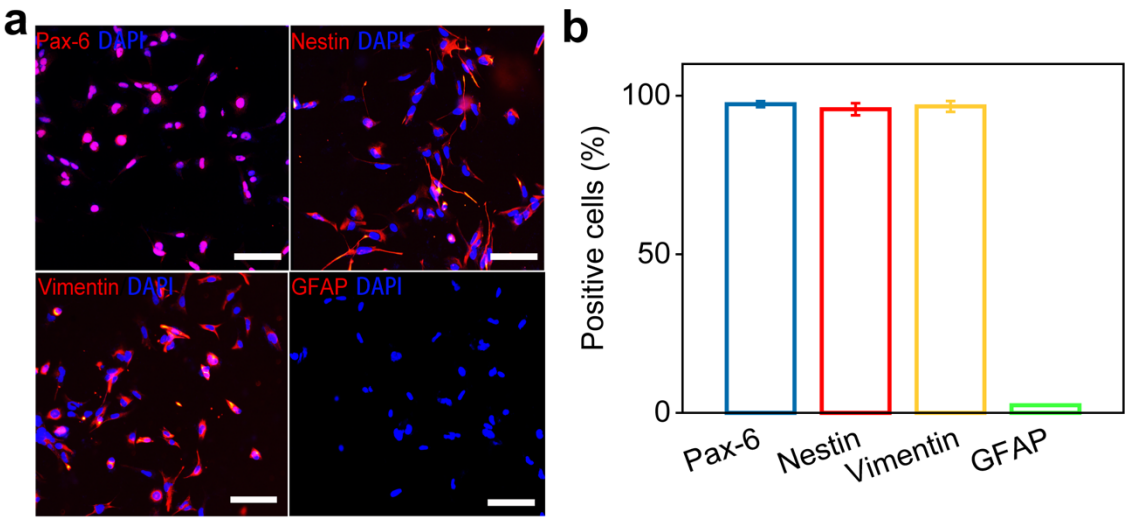

Figure. S6 Identification of specific markers of RPCs. a) Representative images of Pax-6, Nestin, Vimentin and GFAP-positive cells were taken after immunocytochemistry staining. Scale bars: 50 μm. b) Quantitative analysis of the positive ratio of Pax-6-, Nestin-, Vimentin-, and GFAP-expressed cells. n=6 independent experiments, data presented as mean ± SD.

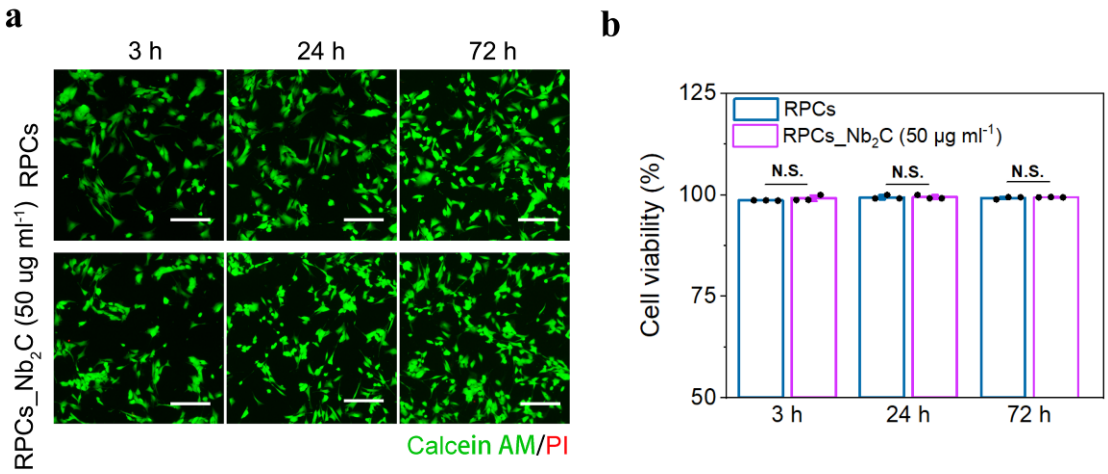

Figure S7. Effects of 2D Nb_2C MXene (50 μg ml^{-1}) on cell viability by a) live/dead staining after treatment for 3 h, 24 h and 72 h, and b) the survived cells were further calculated using Image J software. Living cells (green): calcein AM staining, dead cells (red): PI staining. Scale bars: 100 μm. n=3, not significant (N.S.) > 0.05 by two-tailed Student's t-test, data presented as mean ± SD.

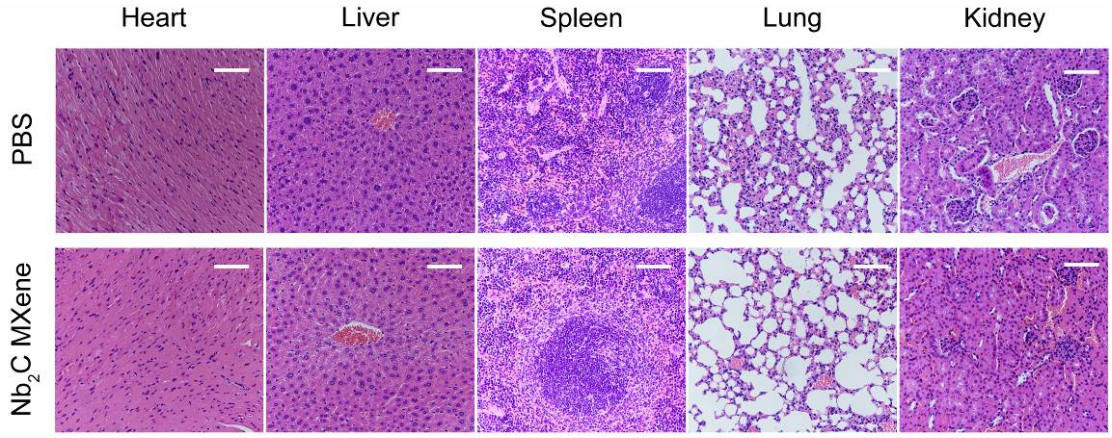

Figure S8. Representative H&E staining images of organic tissues, including heart, liver, spleen, lung and kidney were taken in Nb_2C MXene-injected healthy mice 2-weeks post-administration. Scale bars: 100 μm.

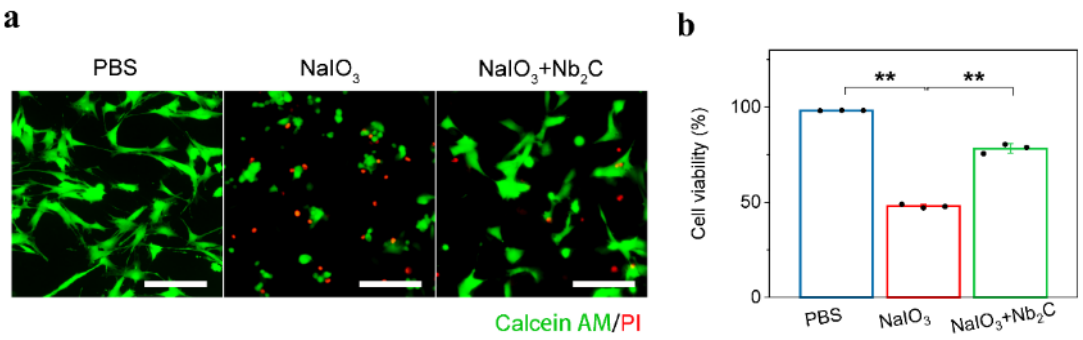

Figure S9. RPCs treated with PBS, $NaIO_3$ (10 mM), and $NaIO_3$ + Nb_2C under differentiation condition for 24 h, then a) representative live/dead RPCs were imaged, and b) the survived cells were further calculated using Image J software. Scale bars: 100 μm. n=3, one-way ANOVA with Bonferroni correction, **P < 0.01, data presented as mean ± SD.

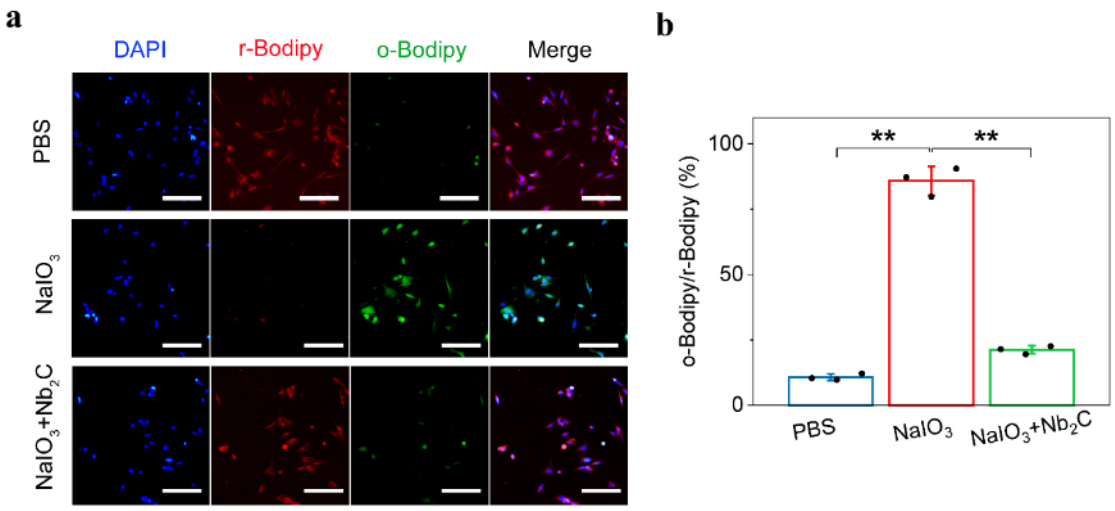

Figure S10. RPCs treated with PBS, $NaIO_3$, and $NaIO_3$ + Nb_2C MXene under differentiation condition for 24 h, then a) lipid peroxidation in RPCs was determined using C11-BODIPY probe and b) relative fluorescence intensity of oxidized-Bodipy was further calculated. Blue: cell nuclei, red: reduced-Bodipy, green: oxidized-Bodipy. Scale bars: 100 μm. n=3, one-way ANOVA with Bonferroni correction, **P < 0.01, data presented as mean ± SD.

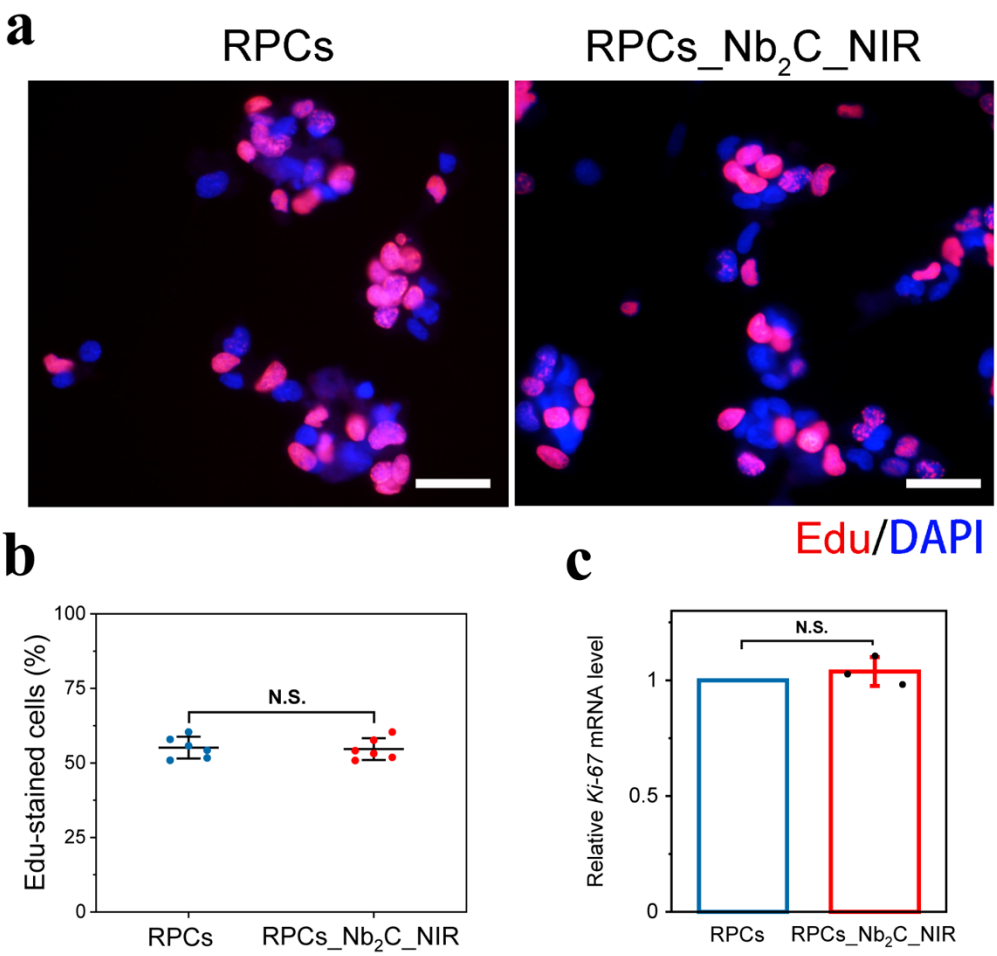

Figure S11. After 3 days of Nb_2C_NIR treatment under proliferation culture, a) proliferation capability of RPCs was subject to Edu-staining assay, and b) Edu-positive cell percentage was calculated by Image J software. Scale bars: 50 μm. n=6, not significant (N.S.) > 0.05 by two-tailed Student's t-test. c) Gene expression level of Ki-67 (a cell proliferation marker) was additionally detected. n=3, not significant (N.S.) > 0.05 by two-tailed Student's t-test, data presented as mean ± SD.

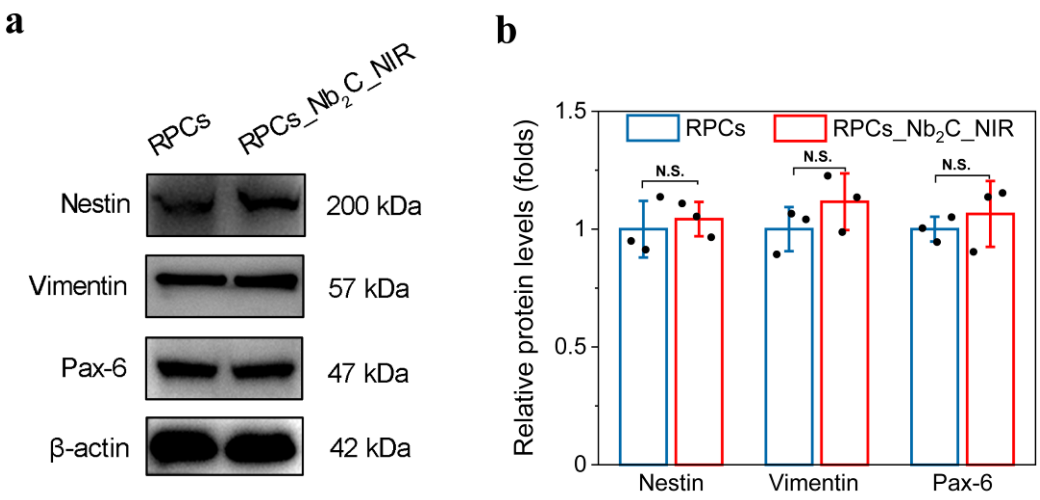

Figure S12. After 3 days of Nb_2C_NIR treatment under proliferation culture, stemness of RPCs was evaluated by detecting a) the protein expression levels and d) quantitative analysis of retinal progenitor-related markers (Nestin, Vimentin and Pax-6). n=3, not significant (N.S.) > 0.05 by two-tailed Student's t-test, data presented as mean ± SD.

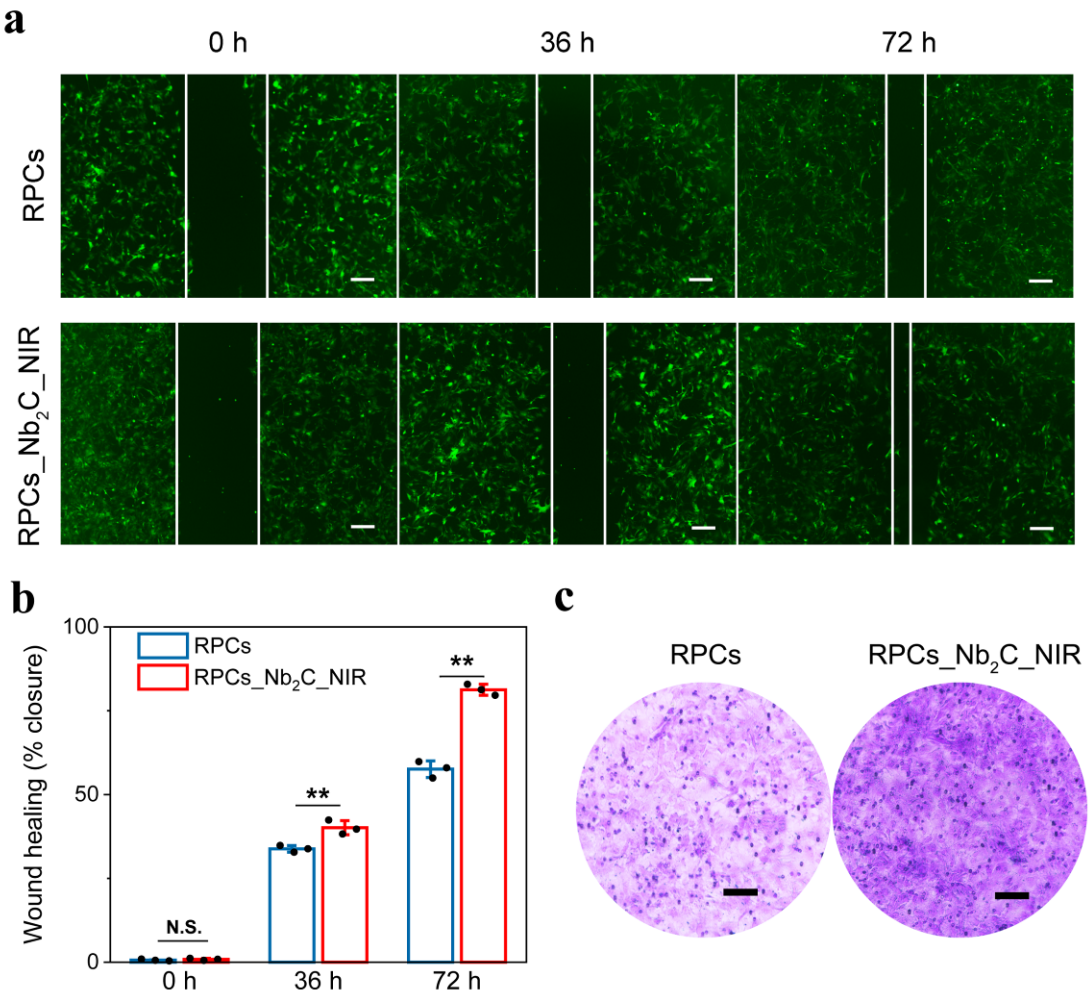

Figure S13. a) Representative imaging and b) quantitative analysis of cell horizontal migration across the wound areas (indicated between two white lines) after scratch for 36 h and 72 h. Scale bars: 100 μm. n=3, **P < 0.01, not significant (N.S.) > 0.05 by two-tailed Student's t-test. c) Transwell assay demonstrated the vertical migration of Nb_2C_NIR treated RPCs by 1% crystal violet staining. Scale bars: 100 μm, data presented as mean ± SD.

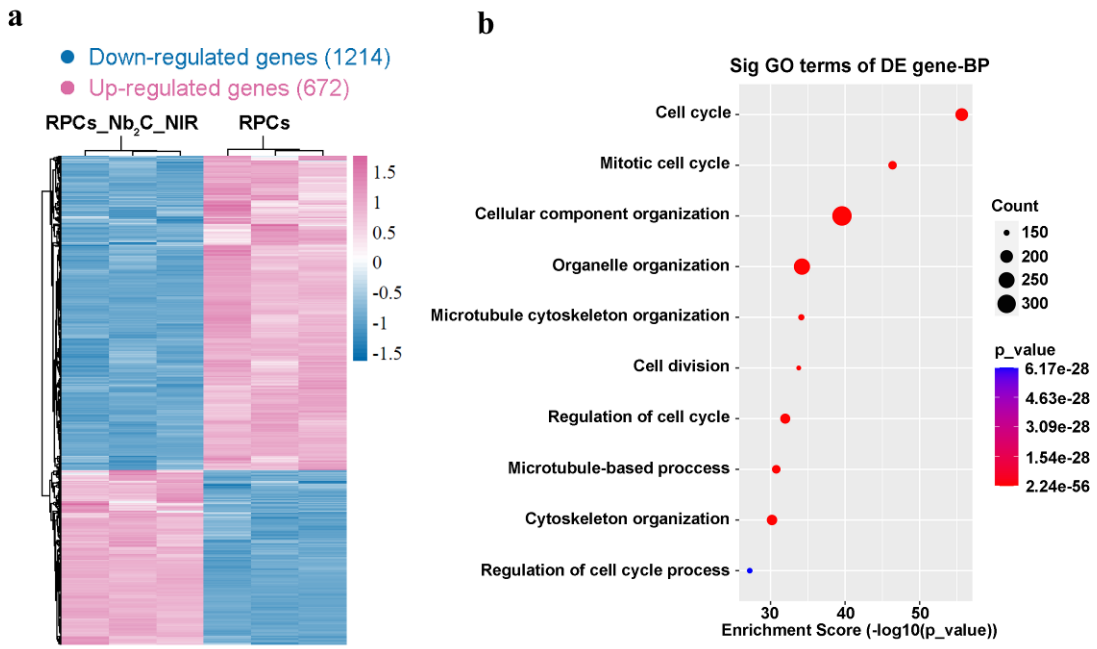

Figure S14. a) Heatmap showed differentially expressed genes across Nb_2C_NIR treated RPCs (RPCs_Nb_2C_NIR) against PBS-treated RPCs (RPCs). Red: upregulated genes, blue: downregulated genes. b) Top ten of significant GO terms by enrichment analysis of differentially downregulated genes involving biological process.

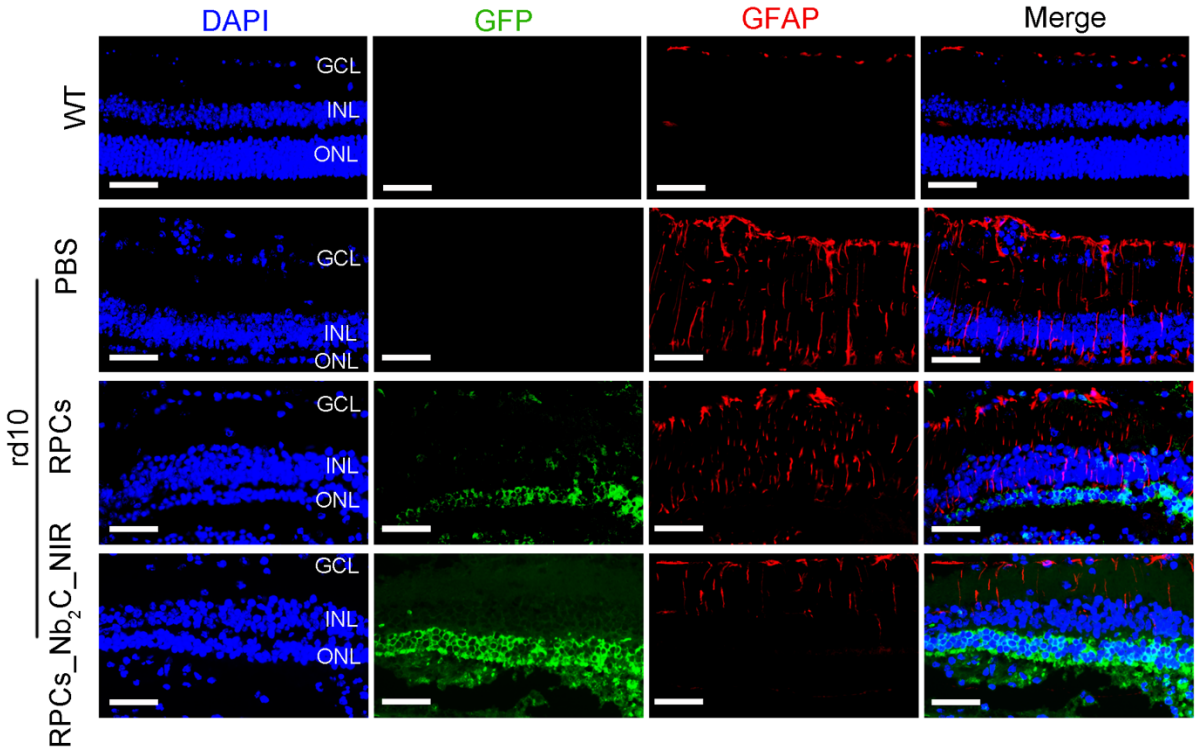

Figure. S15 Immunostaining of GFAP in WT and rd10 retina 8 weeks after transplantation of PBS, GFP^+ RPCs or GFP^+ RPCs_Nb_2C_NIR. Scale bars: 50 μm. n=6 independent experiments. GFAP (red): glial fibrillary acidic protein. WT: wild type. ONL: outer nuclear layer, INL: inner nuclear layer, GCL: ganglion cell layer.

S2. Supporting Tables S1~S2

Table S1. Primer sequences for qPCR analysis

Genes	Accession number	Forward (5-3′)	Reverse (5-3′)	Annealing temperature (°C)	Product size (base pairs)
Ki-67	X82786	cagtactcggaatgcagcaa	cagtcttcaggggctctgtc	60	170
IL-6	NM_031168.1	aggagtggctaaggaccaaga	ataacgcactaggtttgccga	60	100
MCP-1	NM_011333.3	acctgctgctactcattcacc	attccttcttggggtcagca	60	148
Caspase-3	NM_004346	catggaagcgaatcaatggact	ctgtaccagaccgagatgtca	60	139
β3-tubulin	NM_023279	cgagacctactgcatcgaca	cattgagctgaccagggaat	60	152
Rhodopsin	NM_145383	tcaccaccaccctctacaca	tgatccaggtgaagaccaca	60	216
PKC-α	NM_011101	cccattccagaaggagatga	ttcctgtcagcaagcatcac	60	212
GFAP	NM_010277	agaaaaccgcatcaccattc	tcacatcaccacgtccttgt	60	184
β-actin	NM_007393	agccatgtacgtagccatcc	ctctcagctgtggtggtgaa	60	152

Table S2. Primary antibodies used in western blot, immunocytochemistry (ICC) and/or immunofluorescence analysis

Antibodies	Animal	Vendor	Western blot	ICC	Immunofluorescence
β-actin	Mouse	Proteintech	Yes (1:5000)	No	No
PKC-α	Rabbit	Cell Signaling Technology	Yes (1:800)	Yes (1:200)	Yes (1:200)
β3-tubulin	Rabbit	Sigma-Aldrich	Yes (1:800)	Yes (1:200)	No
GFAP	Mouse	Sigma-Aldrich	Yes (1:800)	Yes (1:200)	Yes (1:200)
Rhodopsin	Mouse	Sigma-Aldrich	Yes (1:800)	Yes (1:200)	Yes (1:200)
Recoverin	Rabbit	Proteintech	No	No	Yes (1:200)
RBPMS	Rabbit	Proteintech	No	No	Yes (1:200)
Caspase-3	Mouse	Proteintech	Yes (1:800)	No	No
MCP-1	Mouse	Santa Cruz Biotechnology	Yes (1:800)	No	No
IL-6	Rabbit	Abcam	Yes (1:800)	No	No
CD68	Rabbit	Abcam	No	No	No
Nestin	Rabbit	Sigma-Aldrich	Yes (1:800)	Yes (1:200)	No
Vimentin	Rabbit	Sigma-Aldrich	Yes (1:800)	Yes (1:200)	No
Pax-6	Rabbit	Sigma-Aldrich	Yes (1:800)	Yes (1:200)	No
p-Erk	Rabbit	Proteintech	Yes (1:800)	No	No
Erk	Mouse	Proteintech	Yes (1:800)	No	No
p-Akt	Rabbit	Proteintech	Yes (1:800)	No	No
Akt	Rabbit	Abcam	Yes (1:800)	No	No