Supplemental Data

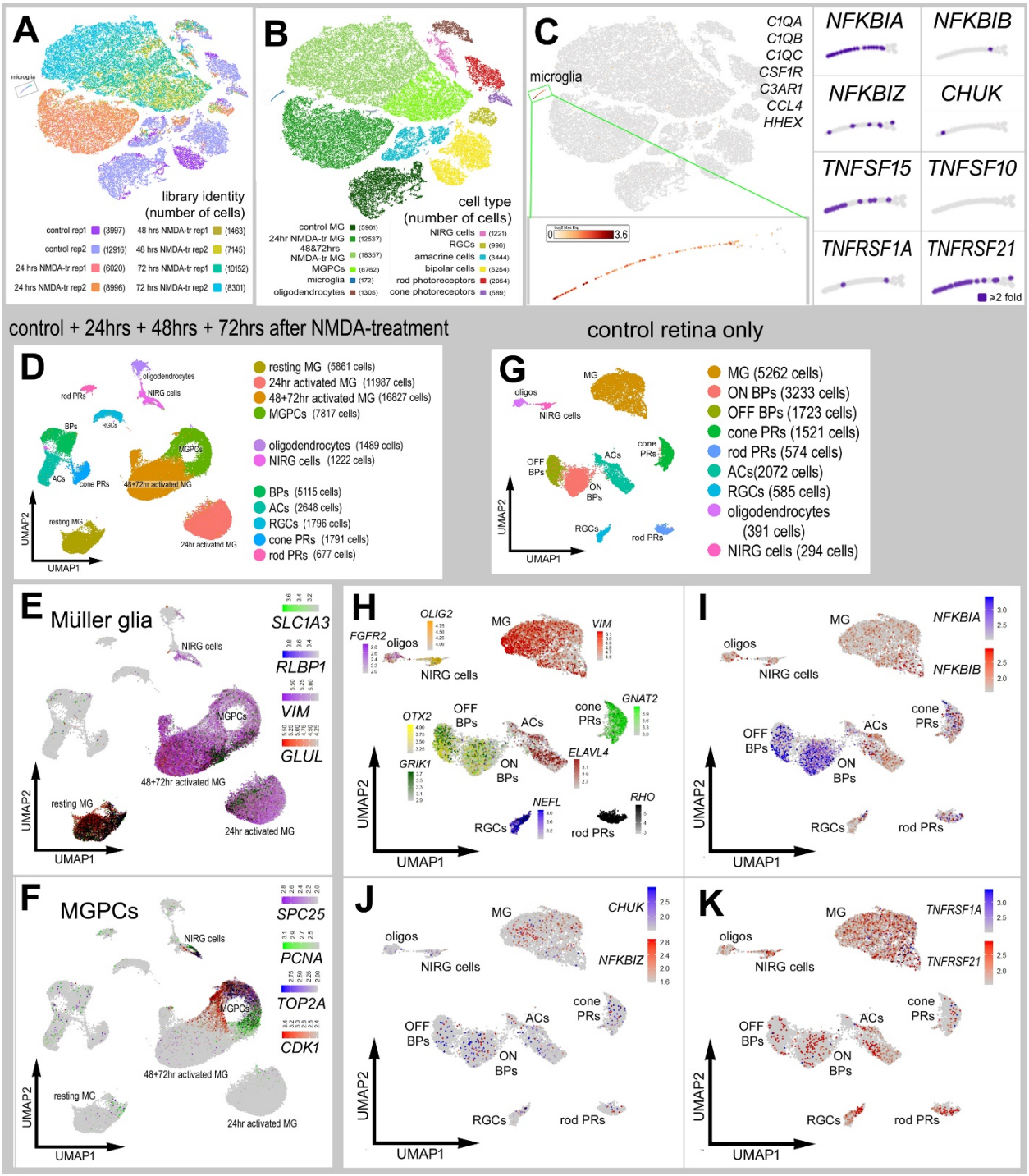

Figure S1: Expression NF-κB signaling components in saline- and NMDA-treated retinas. tSNE and UMAP plots show distinct clustering of different retinal cell types from control and NMDA-damaged retinas. Numbers of cells captured within each library (**A**) and numbers of cells within each clustered cell types (**B, D, G**) are listed in parentheses. Each dot represents one cell. Clusters of different types of retinal cells were identified based on collective expression of different cell-distinguishing markers as described in the Methods (**B, D, G, H**). Microglia were identified based on collective expression *of C1QA, C1QB, C1QC, CSF1R, C3AR1, CCL4, and HHEX* (**C**). tSNE plots for expression (≥2-fold; purple dots) of *NFKBIA, NFKBIB, NFKBIZ, CHUK, TNFSF15, TNFSF10, TNFRSF1A,* and *TNFRSF21* are shown for the microglial (**C**). UMAP plots show collective expression of Müller glial (*SLC1A3, RLBP1, VIM,* and *GLUL)* (**E**) and MGPCs (*SPC25, PCNA, TOP2A,* and *CDK1*) (**F**). UMAP plots of cells from only control retinas are illustrated in (**H-K**). Different retinal cell clusters were identified, in part, based on distinct patterns of expression for *FGFR2, OLIG2, VIM, OTX2, GRIK1, ELAVL4, GNAT2, NEFL,* and *RHO* (**H**). UMAP plots in **I-K** illustrate scattered expression of NF-κB components (*NFKBIA, NFKBIA, NFKBIZ* and *CHUK*) and TNF receptors (*TNFRSF1A* and *TNFRSF21*) in retinal neurons and glia.

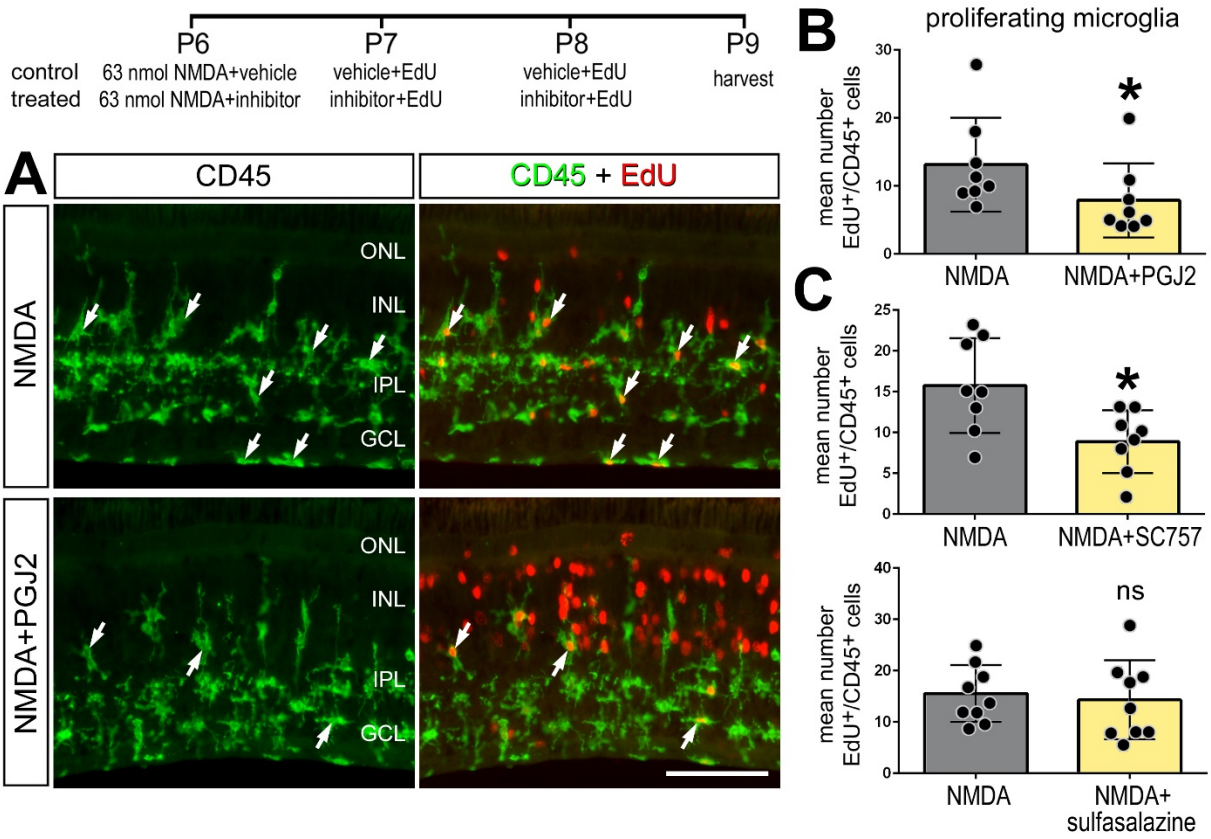

Figure S2: Inhibition of NF-κB after damage results in decreased microglia proliferation. Retinas were treated with NMDA plus vehicle or NMDA plus NF-κB inhibitors (PGJ2 or SC757) at P6, followed by 2 consecutive daily treatments with vehicle or inhibitor on P7 and P8. Edu was added to injections at P7-P8, and retinas were harvested at P9. Retinal sections were labeled for CD45 (green; **A**) and Edu (red; **A**). Histograms in **B** and **C** represent mean number (± SD and individual data points) of proliferating microglia. Arrows represent proliferating microglia in **A**. Calibration bars in **A** represent 50 µm. Abbreviations: ONL – outer nuclear layer, INL – inner nuclear layer, IPL – inner plexiform layer, GCL – ganglion cell layer.

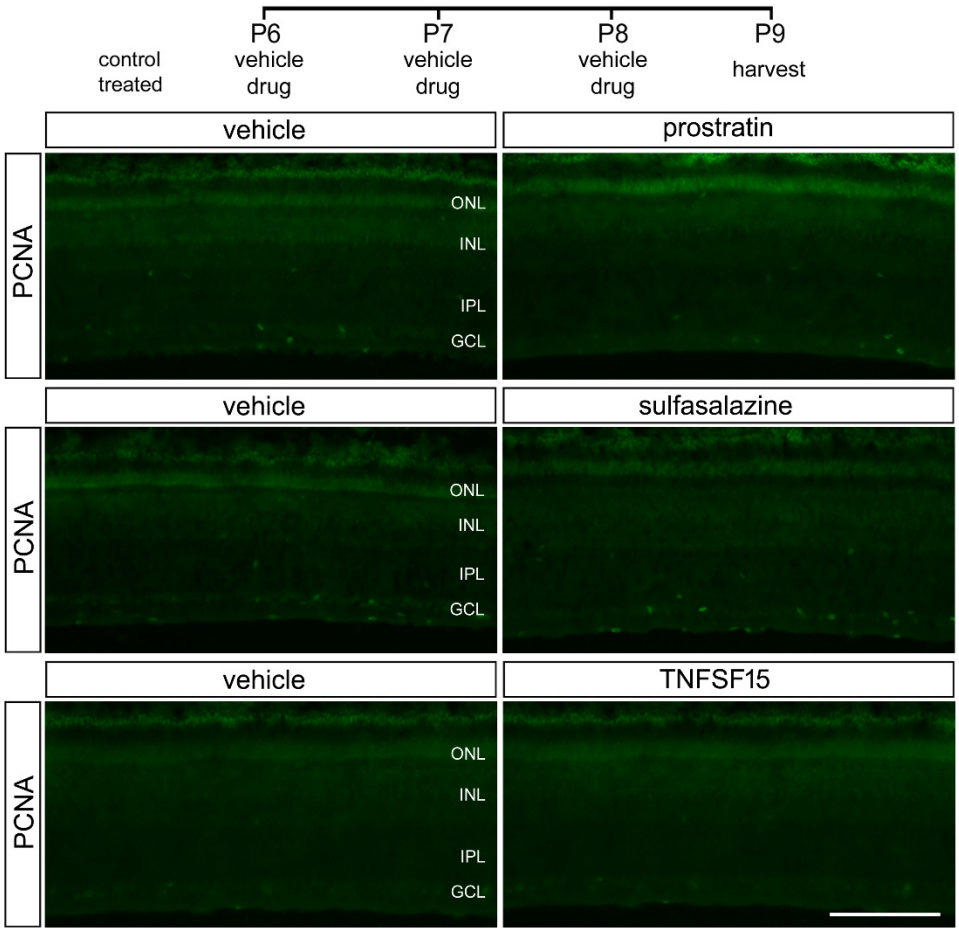

Figure S3: Activation or inhibition of NF-κB without damage or growth factor treatment fails to stimulate proliferation. Treatment of undamaged retinas with prostratin, sulfasalazine, or TNFSF15. Right eyes received 3 consecutive daily injections of prostratin, sulfasalazine, or TNFSF15 while left eyes were treated with vehicle control from P6 to P8. Retinas were harvested on P9. Retinal sections were labeled for PCNA (green) and show no proliferating Müller glia.

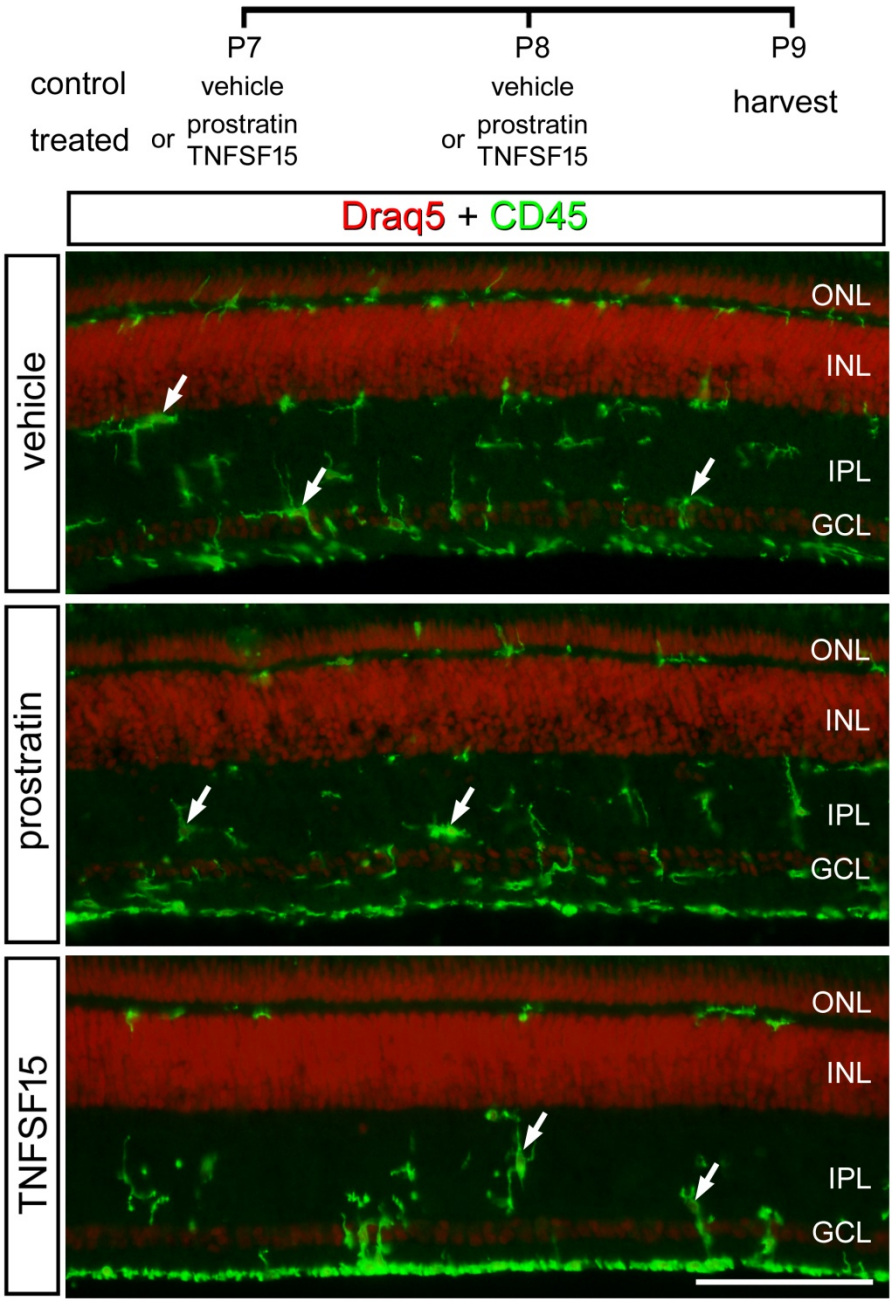

Figure S4: Microglia in retinas treated with prostratin or TNFSF15. Eyes were treated with vehicle, prostratin or TNFSF15 at P7 and P8, and retinas harvested at P9. Sections of the retina were labeled with DRAQ5 (red nuclei) and antibodies to CD45 (green). Arrows indicate microglia. The calibration bar represents 50 µm. Abbreviations: ONL – outer nuclear layer, INL – inner nuclear layer, IPL – inner plexiform layer, GCL – ganglion cell layer.

Table S1. Antibodies, sources and working dilutions

Antibody	Dilution	Host	Clone/Catalog number	Source
Sox2	1:1000	Goat	KOY0418121	R&D
Sox9	1:2000	Rabbit	AB5535	Millipore
CD45	1:200	Mouse	HIS-C7	Prionics
Pax6	1:1000	Rabbit	Poly19013	BioLegend
Neurofilament	1:100	Mouse	RT97	DSHB
Phospho-Histone H3 (PHH3)	1:120	Rabbit	06-570	Millipore
Brn3	1:50	Mouse	MAB1585	Chemicon
HuC/D	1:150	Mouse	A21271	Invitrogen
Glutamine Synthetase	1:2000	Mouse	610517	BD Transduction Laboratories
IkB-alpha	1:500	Rabbit	10268-1-AP	Proteintech
β-actin	1:1000	Rabbit	4970	Cell Signaling Technology