Supporting Information

Azithromycin protects retinal glia against oxidative stress-induced morphological changes, inflammation, and cell death

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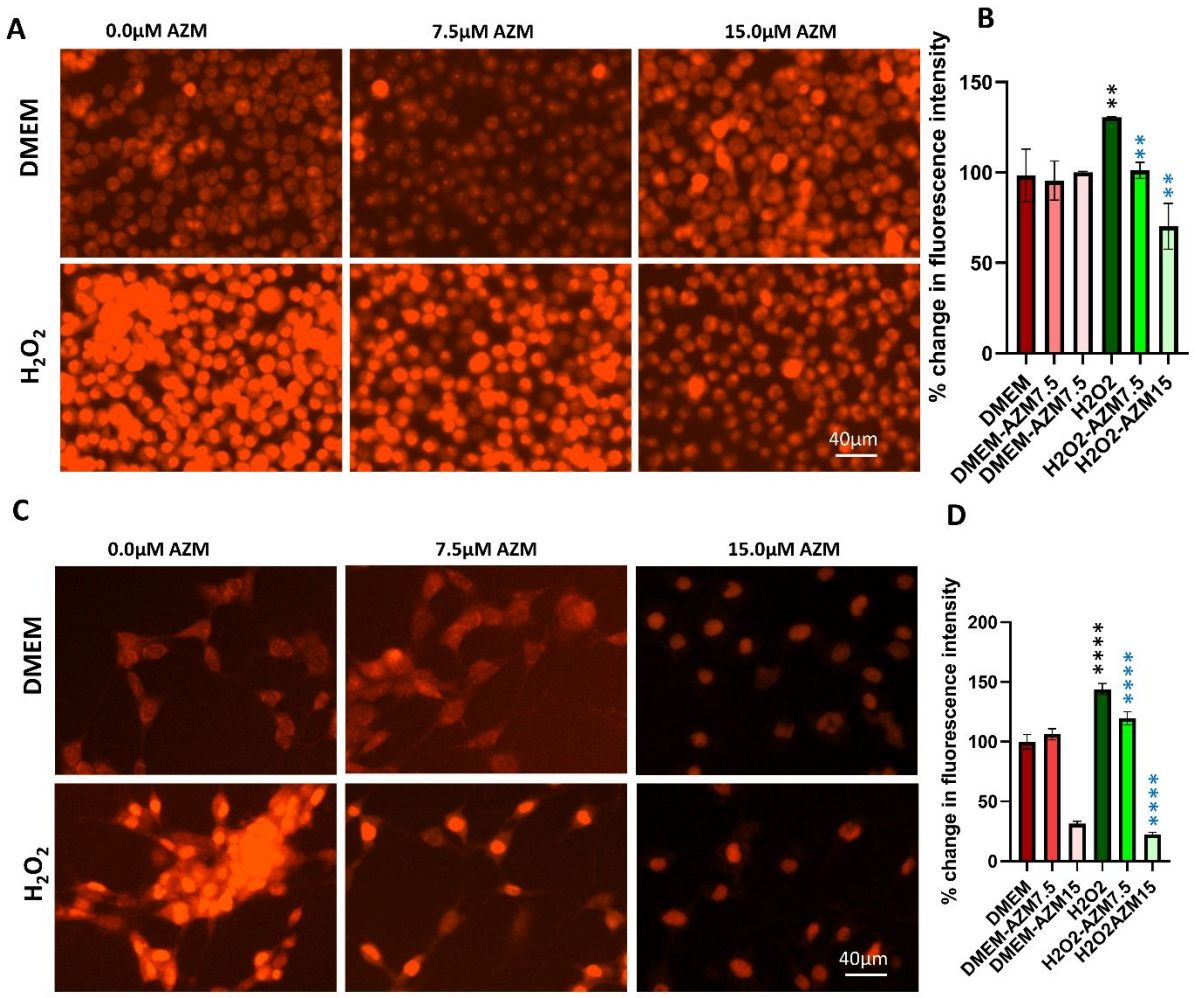

Figure S1. The intracellular reactive oxygen species (ROS) was determined by DHE staining in control and 300μM of H_2O_2 treated BV-2 microglia and MIO-M1 Müller glial cells *in vitro* in the presence and absence of azithromycin (AZM). AZM mitigates H2O2-induced intracellular ROS in BV-2 microglia and MIO-M1 Müller glial cells *in vitro*. (A) Representative images of DHE staining show increased fluorescence intensity in H_2O_2 induced BV-2 microglial cells and 30min of pretreatment with AZM shows comparatively reduced fluorescence indicating prevention of H_2O_2 induced oxidative stress formation by AZM. (B) Spectrofluorometric measurement for the levels of ROS significant increase in ROS by H_2O_2 treatment but not in AZM pretreated group in BV-2 cells. (C) Representative images of DHE staining show increased fluorescence intensity in H_2O_2 induced MIO-M1 microglia cells and 30min of pretreatment with AZM shows comparatively reduced fluorescence indicating prevention of H_2O_2 induced oxidative stress formation by AZM. (D) Spectrofluorometric measurement for the levels of ROS significant increase in ROS by H_2O_2 treatment but not in AZM pretreated group in MIO-M1 cells. Red bars indicated fluorescence quantified in control cells and faint red AZM treated cells, green bars indicated H_2O_2 treated cells and faint green AZM pretreated H_2O_2 treated cells. All the results presented as mean ±SD, one-way ANOVA was performed with Turkey's multiple comparisons, **p<0.01,****p<0.0001, black stars as compared to DMEM, and blue stars are as compared to the H_2O_2.

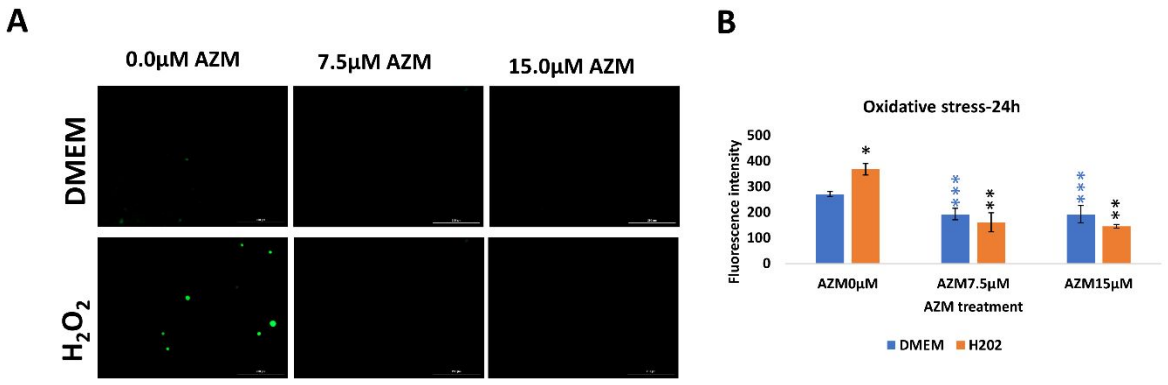

Figure S2. Effect of 300µM of H_2O_2 and AZM on Microglia cell death and oxidative stress at the 24h timepoint. (A) Oxidative stress by DCFDA and (B) Quantification of fluorescence intensity determined from DCFDA staining at 24h. All the results were presented as mean ±SD, one-way ANOVA was performed with Turkey's multiple comparisons. * $p<0.05$, ** $p<0.01$, ***$p<0.001$, ****$p<0.0001$. The black stars are as compared to DMEM and the blue stars are as compared to the H_2O_2

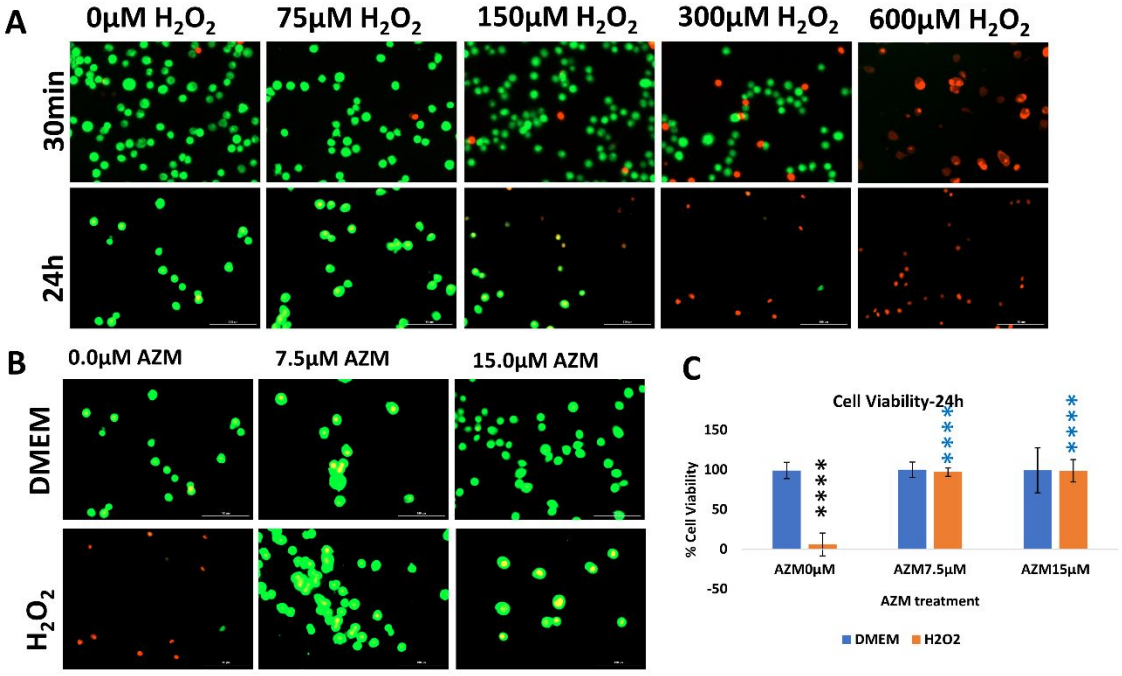

Figure S3. Effect of 300µM of H_2O_2 on Microglia cell death. (A) Effect of concentrations and time of incubation on microglia cell death. Row-1 30min and row-2 at 24h. (B) Inhibition of Cell death by azithromycin at 24h time point (AO/PI) staining and (C) Quantification of % cell viability at 24h timepoints. All the results presented as mean ±SD, one way ANOVA was performed with Turkey's multiple comparisons, ****$p<0.0001$ black stars are as compared to DMEM and blue stars are as compared to the H_2O_2

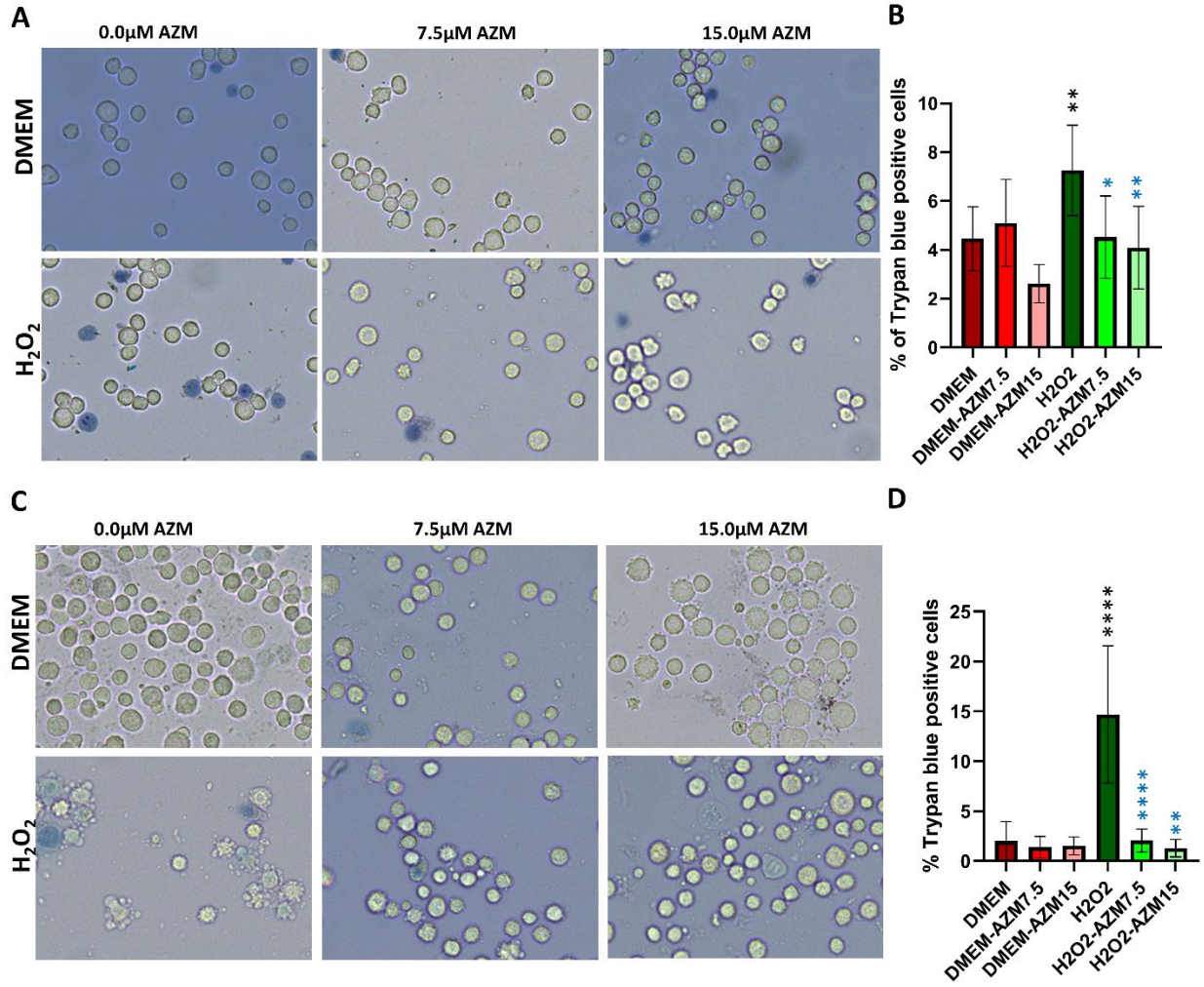

Figure S4. Live and dead cells were visualized by Trypan blue staining. Trypan blue is a hydrophobic dye that only entered to dead cells and provided blue color (A) Representative images of Trypan blue staining showed there was no change in the numbers of live and dead BV-2 cells in the AZM pretreatment group as compared to the control. H_2O_2 treatment increased the number of dead BV-2 microglial cells and 30min of pretreatment with AZM showed a comparatively reduced number of 300µM of H_2O_2 induced dead cells by AZM. (B) Quantification of the percentage of trypan blue positive cells per total number of microglia (8 images). The result indicated there was no significant change in the number of dead to the total number of cells in control and in AZM pretreated cells. H_2O_2 treatment significantly increased the number of dead BV-2 microglial cells and 30min of pretreatment with AZM showed a significant reduction in the number of H_2O_2 induced dead cells. (C) Representative images of Trypan blue staining showed there was no change in the numbers of live and dead MIO-M1cells in AZM pretreatment group as compared to the control. H_2O_2 treatment increased the number of dead MIO-M1 cells and 30min of pretreatment with AZM showed a comparatively reduced number of H_2O_2 induced dead cells by AZM. (D) Quantification of the percentage of trypan blue positive cells per total number of MIO-M1 cells (8 images). The results indicated that there was no significant change in the number

of dead to the total number of cells in control and AZM pretreated cells. H_2O_2 treatment significantly increased the number of dead MIO-M1 cells and 30min of pretreatment with AZM showed a significant reduction of the number of H2O2-induced dead cells. All the results presented as mean ±SD, one-way ANOVA was performed with Turkey's multiple comparisons, * $p< 0.05$, **$p< 0.01$, ****$p<0.0001$, black stars as compared to DMEM and blue stars are as compared to the H_2O_2.