

**Targeting the Notch and TGF- β signaling pathways to prevent retinal fibrosis *in vitro*
and *in vivo***

Jiawen Fan^{1,2}, Weiyong Shen^{1*}, So-Ra Lee¹, Ashish Easow Mathai¹, Rui Zhang¹, Gezhi Xu²
and Mark C. Gillies^{1*}

¹The University of Sydney, Save Sight Institute, Discipline of Ophthalmology, Sydney
Medical School, Sydney, New South Wales, Australia.

² Department of Ophthalmology and Vision Sciences and Key Laboratory of Myopia of State
Health Ministry, Eye and ENT Hospital, Shanghai Medical College, Fudan University,
Shanghai, People's Republic of China.

***Correspondence should be addressed to:**

Weiyong Shen

Save Sight Institute

The University of Sydney

Sydney 2000, Australia

E-mail: weiyongshen1@gmail.com

Mark C. Gillies

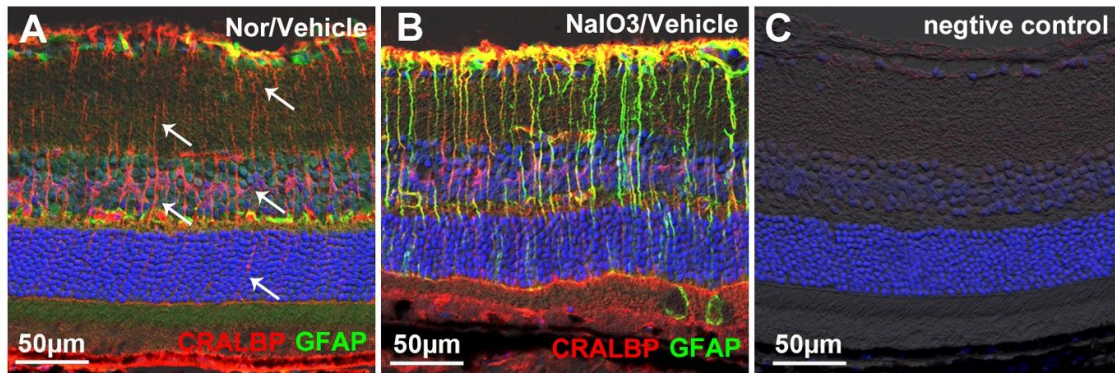
Save Sight Institute

The University of Sydney

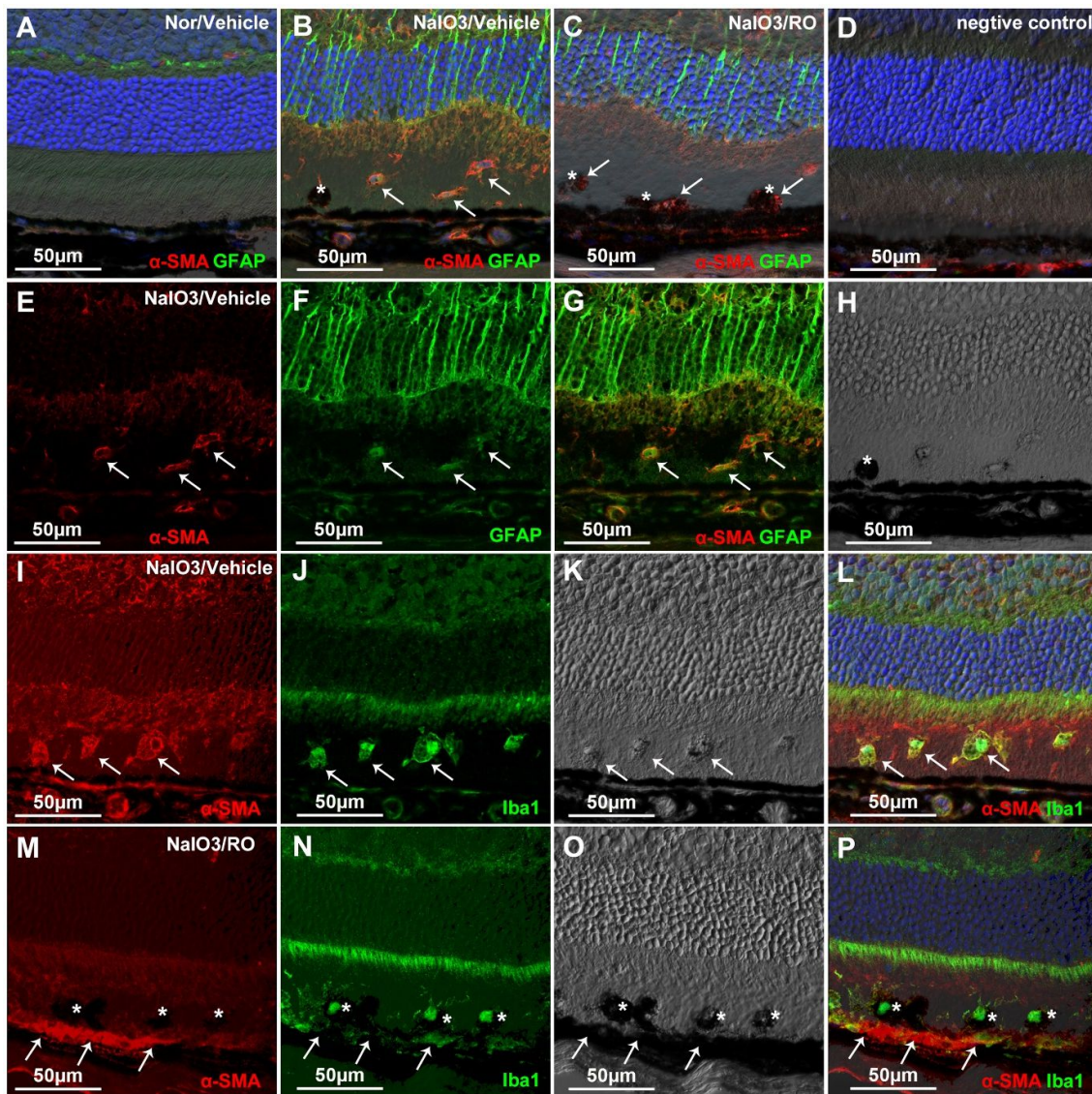
Sydney 2000, Australia

E-mail: mark.gillies@sydney.edu.au

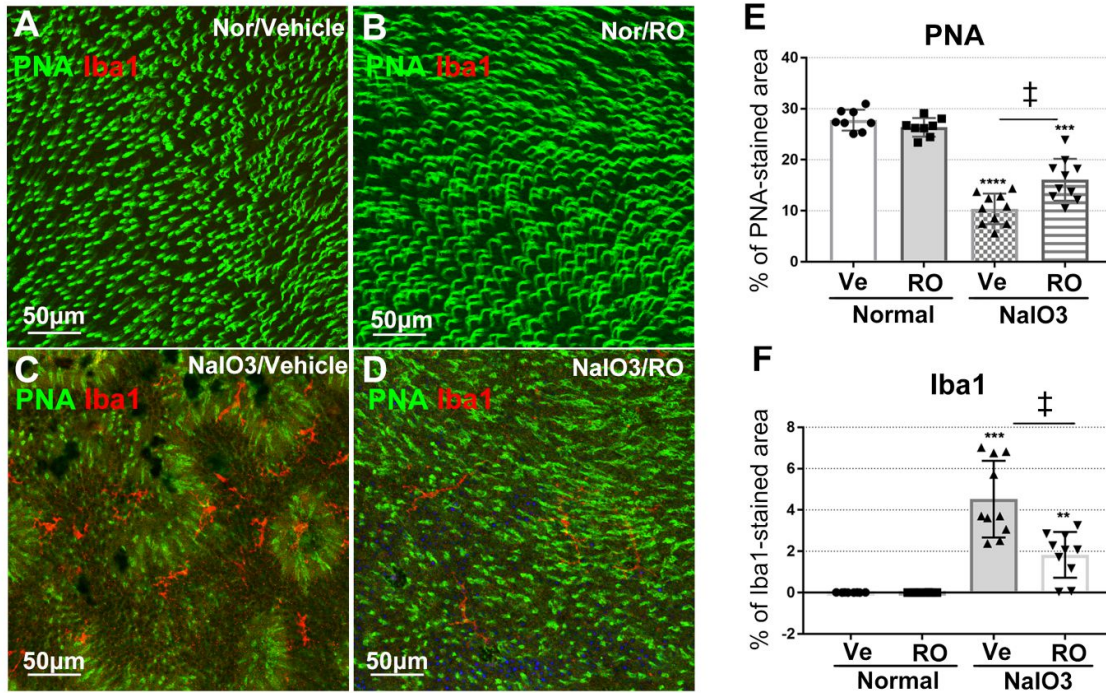
Supplementary Figures



Supplementary Figure 1. Phenotypic characterisation of Müller cells in normal and NaIO₃-damaged retinas. (A, B): Double label IHC for CRALBP (red, arrows) and GFAP (green) showing reduced immunoreactivity for CRALBP in gliotic Müller cells after NaIO₃-induced retinal injury (B) compared with the normal retina (A). Nor/Vehicle, normal eye receiving vehicle injection. **(C):** Negative control without primary antibodies.



Supplementary Figure 2. Myofibroblast formation in subretinal space after chemically-induced retinal damage. (A-C): Double label IHC on retinal frozen sections using antibodies against α -SMA (a marker of myofibroblast, red) and GFAP (a marker of Müller cell gliosis, green). **(D):** Negative control without primary antibodies. **(E-H):** Separated staining of Fig. S1B to show cells double labelled for GFAP and α -SMA, indicating that some cells in the subretinal space may derive from Müller cells. **(I-P):** Double label IHC using antibodies against α -SMA (red) and Iba1 (a marker of microglia, green) in NaIO₃-damaged retinas. The pigment-laid cells (asterisks in B, C, G and O) may be attributed to necrotic RPE cells phagocytosed by macrophages. Nor/Vehicle, normal eye receiving vehicle injection.



Supplementary Figure 3. Intravitreal injection of RO4929097 reduces photoreceptor degeneration and microglial infiltration into the outer retina. (A-D): Staining of retinal flatmounts using fluorescently labelled PNA (green) and an antibody against Iba1 (a marker of microglia, red). **(E and F):** Quantitative analyses of PNA- and Iba1-stained areas in retinal flatmounts with photoreceptors facing up. *** $P < 0.001$ and **** $P < 0.0001$, vs normal mice receiving vehicle or RO. ‡ $P < 0.01$, paired t-tests between RO and Ve treated groups. $N = 10$ /group. Multiple comparison correction were calculated using one-way ANOVA followed by Tukey's multiple comparison test, $P < 0.0001$ in **(E)**, $P = 0.002$ in **(F)**. Nor/Vehicle, normal eye receiving vehicle injection.