

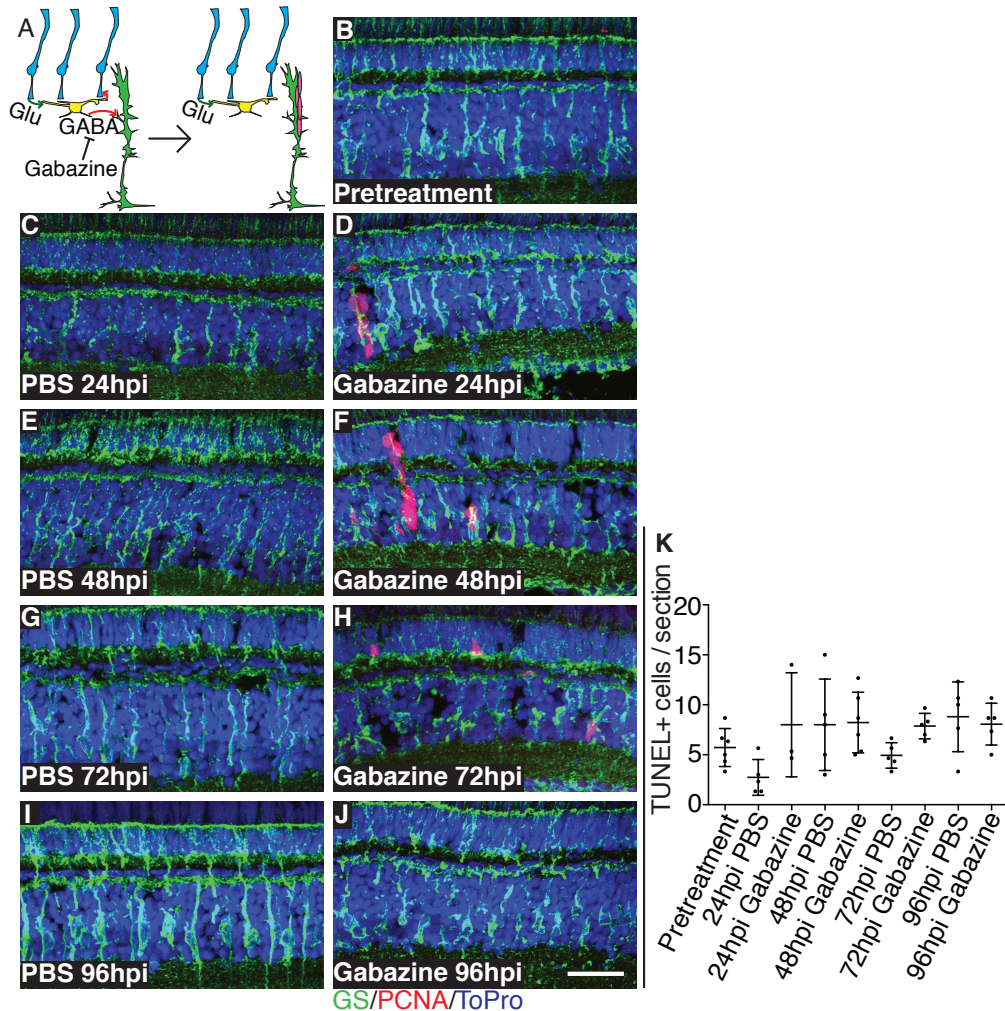
**Stem Cell Reports, Volume 8**

**Supplemental Information**

**Neurotransmitter-Regulated Regeneration in the Zebrafish Retina**

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1 **Supplemental Information**

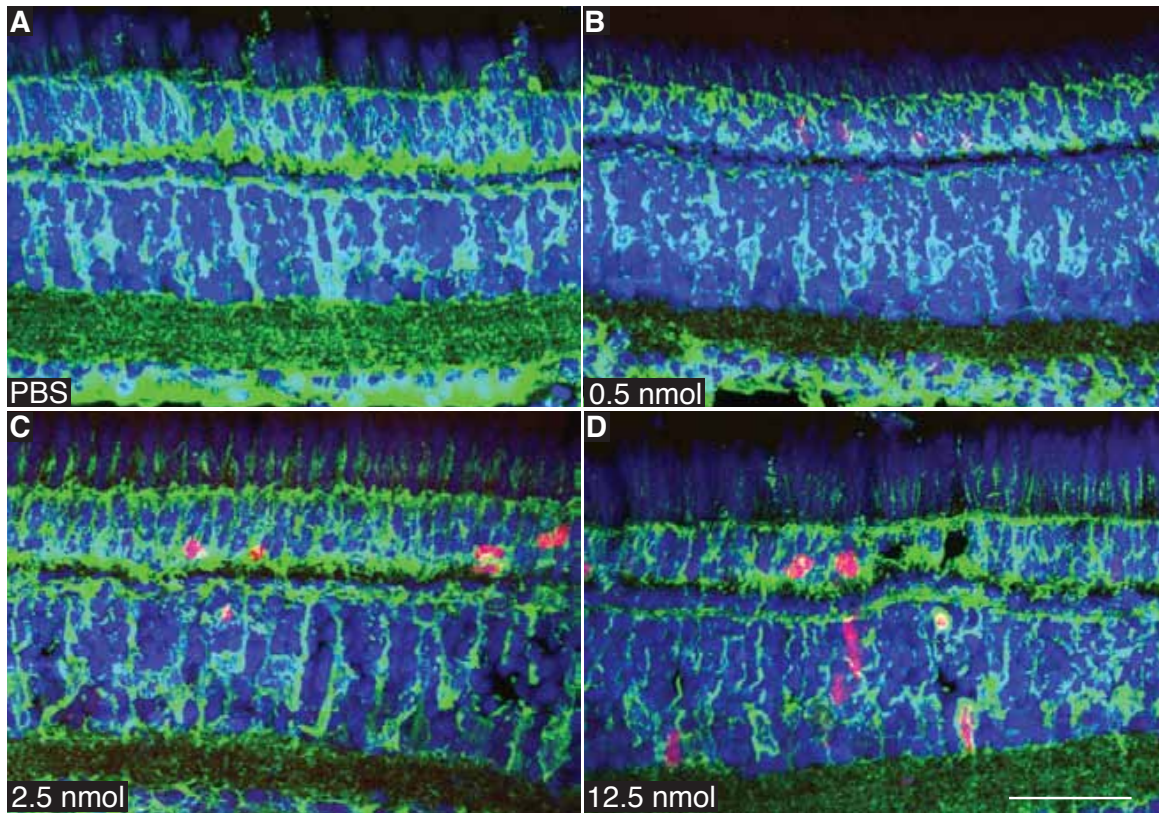


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3 **Supplemental Figure S1. Gabazine injection causes time dependent**

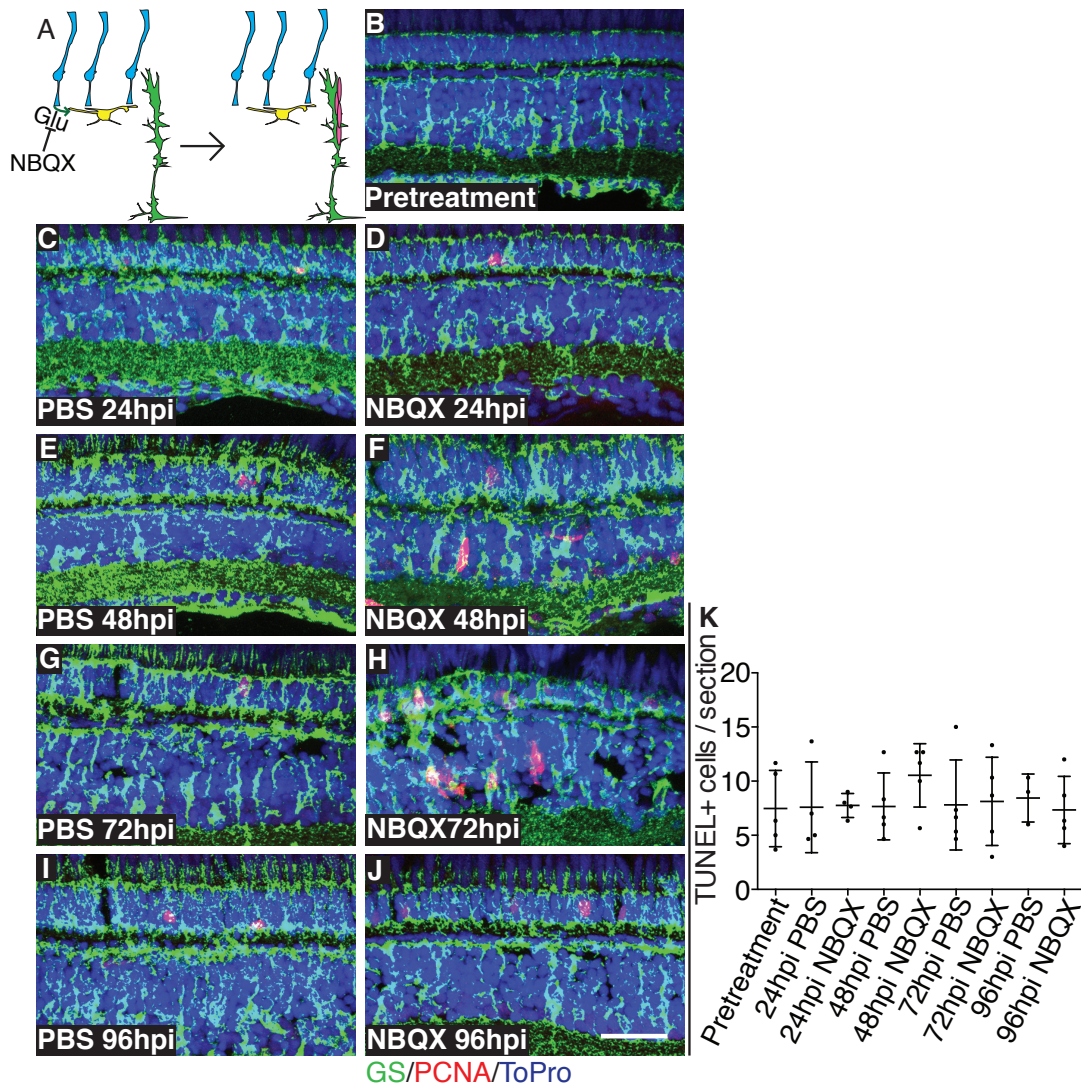
4 **spontaneous proliferation in undamaged retinas, related to Figure 1. Model**

5 illustrating predicted effects of gabazine on MG proliferation (A). Eyes were  
 6 injected with PBS or 12.5 nmol of gabazine and allowed to recover. Eyes were  
 7 removed before injection (B) as well as at 24hpi (C,D), 48hpi (E (same as Figure 1B),  
 8 F), 72hpi (G,H), or 96hpi (I,J) and proliferation assessed by PCNA staining. Scale bar  
 9 is 100µm. Apoptosis was also measured before injection (n=6) and after PBS or  
 10 gabazine injection at 24hpi (n=5, 3), 48hpi (n=5, 6), 72hpi (n=5, 5), and 96hpi (n=5,  
 11 5) by TUNEL (K). A one-way ANOVA was used; Error bars = SD.

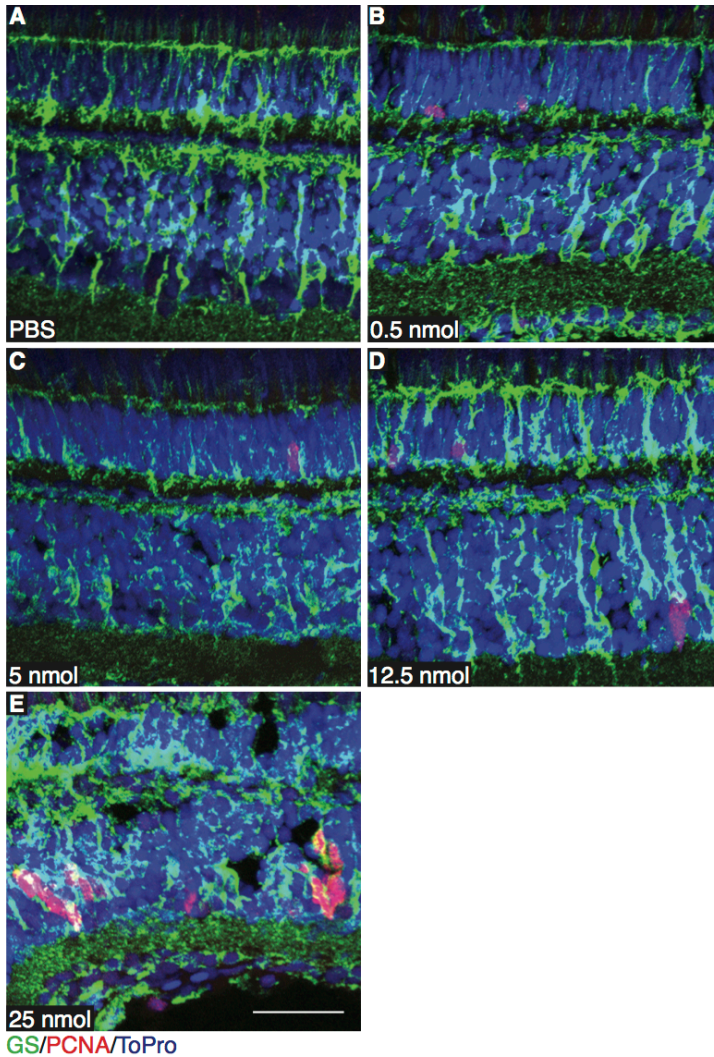


GS/PCNA/ToPro

12  
13 **Supplemental Figure S2. Gabazine induced spontaneous proliferation is dose**  
14 **dependent, related to Figure 1.** WT fish were injected with PBS (A), 0.5 nmol (B),  
15 2.5 nmol (C), or 12.5 nmol (D) and proliferation measured at 48hpi by PCNA  
16 staining. Representative images are small portions of entire retina. Representative  
17 images are small portions of total retina sections. Scale bar is 100 $\mu$ m.



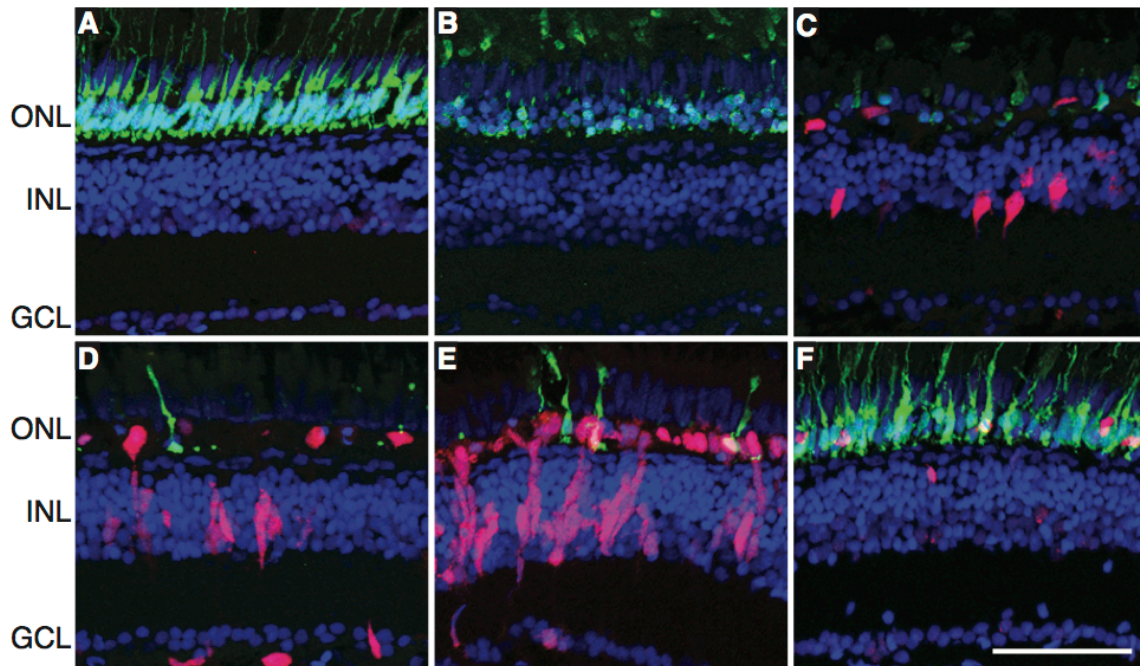
18  
 19 **Supplemental Figure S3. NBQX injection causes time dependent spontaneous**  
 20 **proliferation in undamaged retinas, related to Figure 2.** Model illustrating  
 21 predicted effects of NBQX injections on MG proliferation (A). Eyes were injected  
 22 with 25 nmol of NBQX or PBS and allowed to recover. Eyes were removed before  
 23 injection (B) as well as at 24hpi (C, D), 48hpi (E, F), 72hpi (G (same as Figure 2B),  
 24 H), or 96hpi (I, J) and proliferation assessed by PCNA staining. Scale bar is 100µm.  
 25 Apoptosis was also measured before injection (n=5) and after PBS or NBQX  
 26 injection at 24hpi (n=4, 4), 48hpi (n=5, 5), 72hpi (n=5, 5), and 96hpi (n=3, 5) by  
 27 TUNEL (K). A one-way ANOVA was used; Error bars = SD.



28

GS/PCNA/ToPro

29 **Supplemental Figure S4. NBQX induced spontaneous proliferation is dose**  
30 **dependent, related to Figure 2.** WT fish were injected with PBS (A), 0.5 nmol (B),  
31 5 nmol (C), 12.5 nmol (D), or 25 nmol (E) and proliferation measured at 72hpi by  
32 PCNA staining. Representative images are small portions of total retina sections.  
33 Representative images are small portions of total retina sections. Scale bar is  
34 100 $\mu$ m.

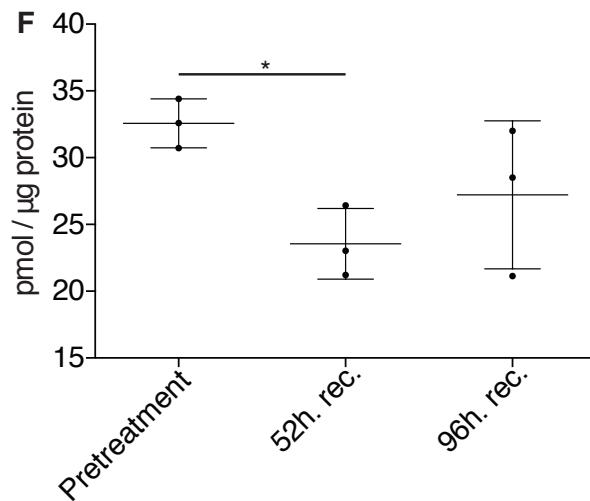
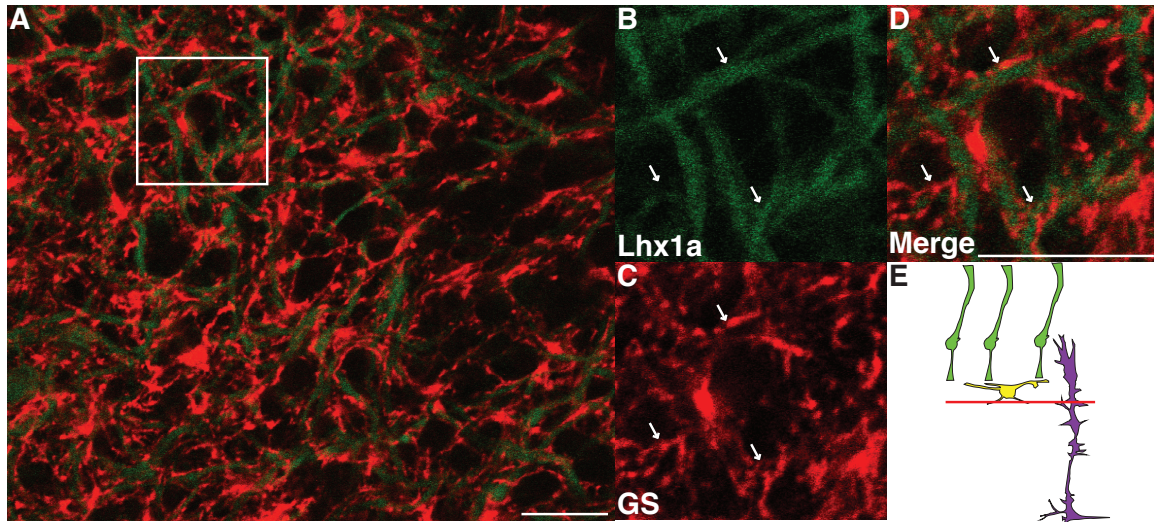


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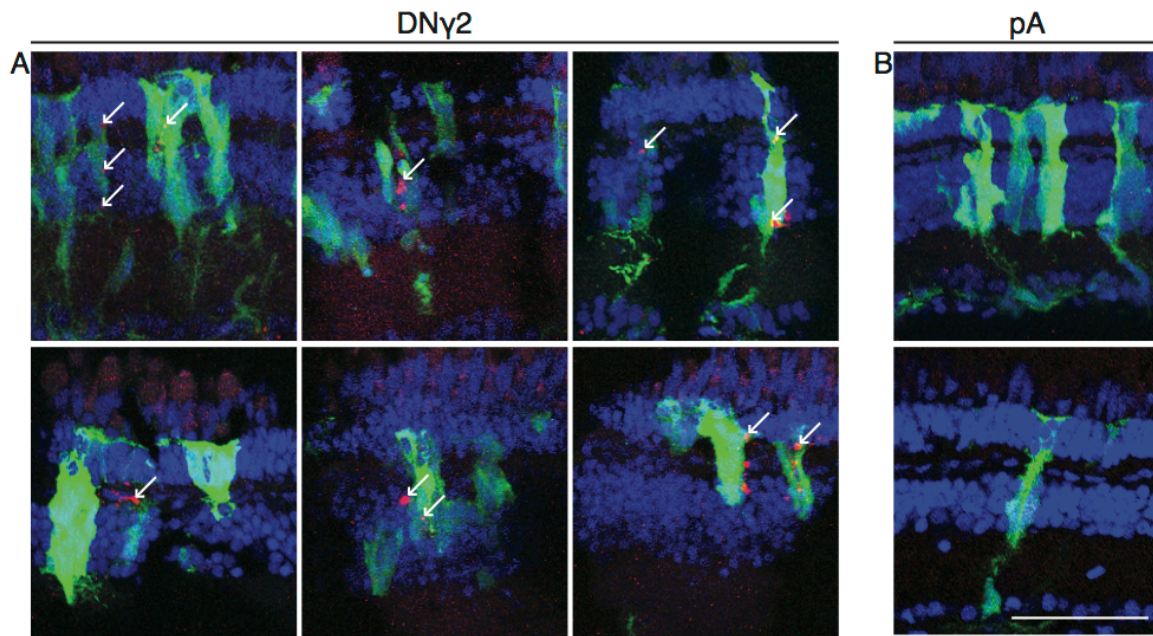
Rods/PCNA/ToPro

**Supplemental Figure S5. Timeline of regeneration in *Tg(zop:nfsb-EGFP)<sup>nt19</sup>***

37 **after MTZ treatment, related to Figure 3.** *Tg(zop:nfsb-EGFP)<sup>nt19</sup>* fish were placed  
 38 in egg water containing 10mM Metronidazole and treated for 24 hours, then  
 39 returned to normal egg water to recover. Eyes were removed and proliferation  
 40 assessed by PCNA staining. Times of recovery observed were pretreatment (A), 0h.  
 41 recovery (B), 52h. recovery (C), 72h. recovery (D), 96h. recovery (E), and 28 days  
 42 recovery (F). Scale bar is 100µm.



43  
 44 **Supplemental Figure S6. MG processes surround HC processes in INL, related**  
 45 **to Figure 4.** *Tg(lhx1a:EGFP)<sup>pt303</sup>* retinas were removed and stained for GS and  
 46 prepared for flat mount imaging. The region of the INL where MG and HCs overlap  
 47 (red line in E) was imaged. Arrows indicate regions of close association between  
 48 MG and HCs. Scale bar is 100μm. *Tg(zop:nfsb-EGFP)<sup>nt19</sup>* fish were treated with MTZ  
 49 and allowed to recover. Whole retinas were removed at indicated time points and  
 50 subjected to HPLC. Levels of GABA were measured (F, related to section *MG are*  
 51 *poised to detect changes in GABA levels*). A one-way ANOVA was used; n=3 biological  
 52 replicates for each timepoint; Error bars = SD; \* = p<0.05.



Tuba/DN $\gamma$ 2/ToPro

53  
 54 **Supplemental Figure S7. Expression of DN $\gamma$ 2 in proliferating cells, related to**  
 55 **Figure 5.** *Tg(tuba1a:GFP)* fish were electroporated with a construct containing  
 56 either a GFAP:mCh-DN $\gamma$ 2 or GFAP:mCh-pA and allowed to recover for 96 hours,  
 57 after which retinas were stained for GFP and mCherry to measure co-localization.  
 58 Representative images of DN $\gamma$ 2 (A) and pA (B) are small portions of entire retinas.  
 59 Arrows indicate co-localization of mCh and GFP. Scale bar is 100 $\mu$ m.