

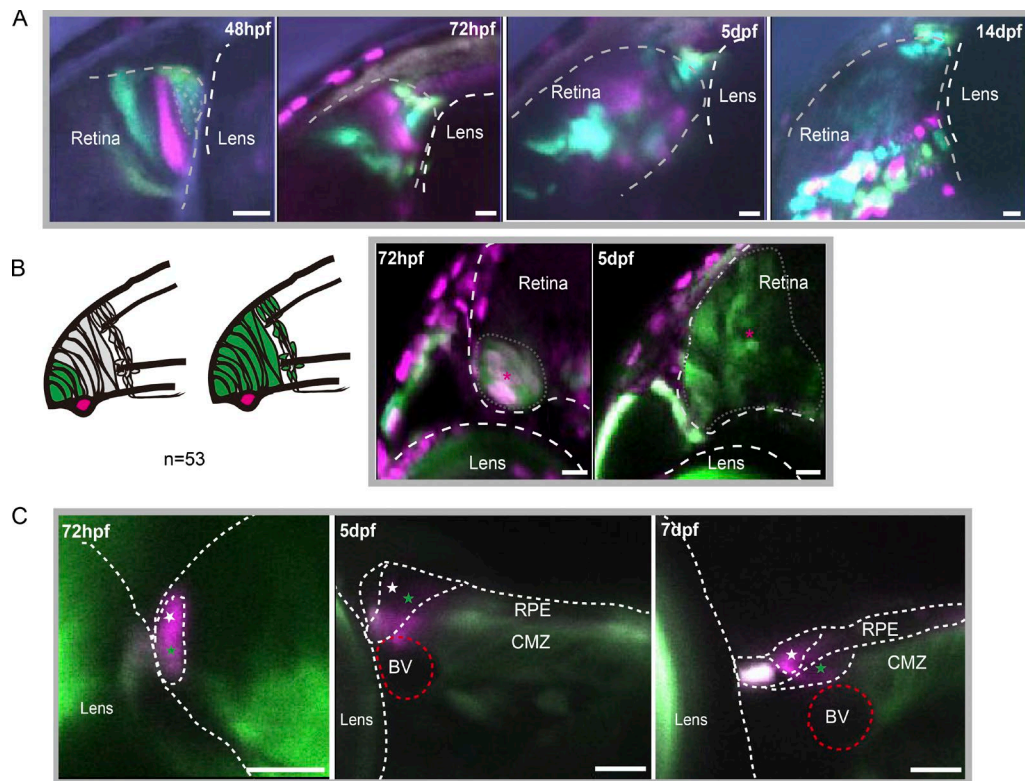
Tang et al., <https://doi.org/10.1083/jcb.201611057>

Figure S1. **Characterization of RSCs.** (A) Individual CMZ cells were marked in different colors stochastically through the *Cre*-dependent combination of *CFP*, *GFP*, and *tdTomato* and were followed from 72 hpf until 14 dpf. (B) If more than four CMZ tip cells were labeled, the clones occupied the entire CMZ. Magenta asterisks indicate traced cells. (C) Lineage tracing using photoconverted Kaede showed that the first or second position cells (in white and green asterisks) in the CMZ tip did not divide from 72 hpf until 7 dpf. BV, blood vessel. White dashed lines outline the boundary of retinas and lenses. Bars, 10  $\mu$ m.

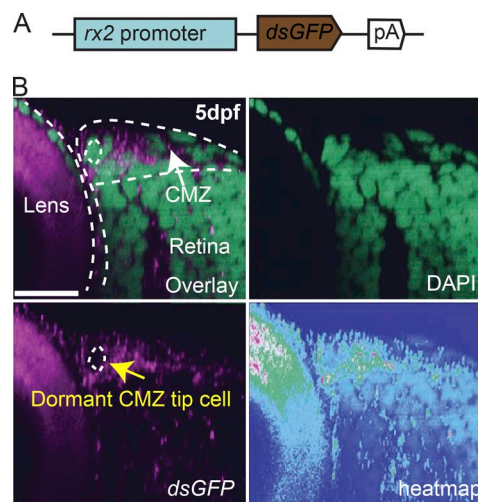


Figure S2. **Characterization of dormant CMZ tip cells.** (A) Schematic of the transgenic construct of *rx2:dsGFP*. (B) Immunostaining of *dsGFP* showed no expression of *rx2* in dormant CMZ tip cells (circled by yellow dashed lines and the yellow arrow) at the 5-dpf retina. The CMZ is indicated by the white arrow. White dashed lines outline the boundary of retinas and lenses. Bars, 20  $\mu$ m.

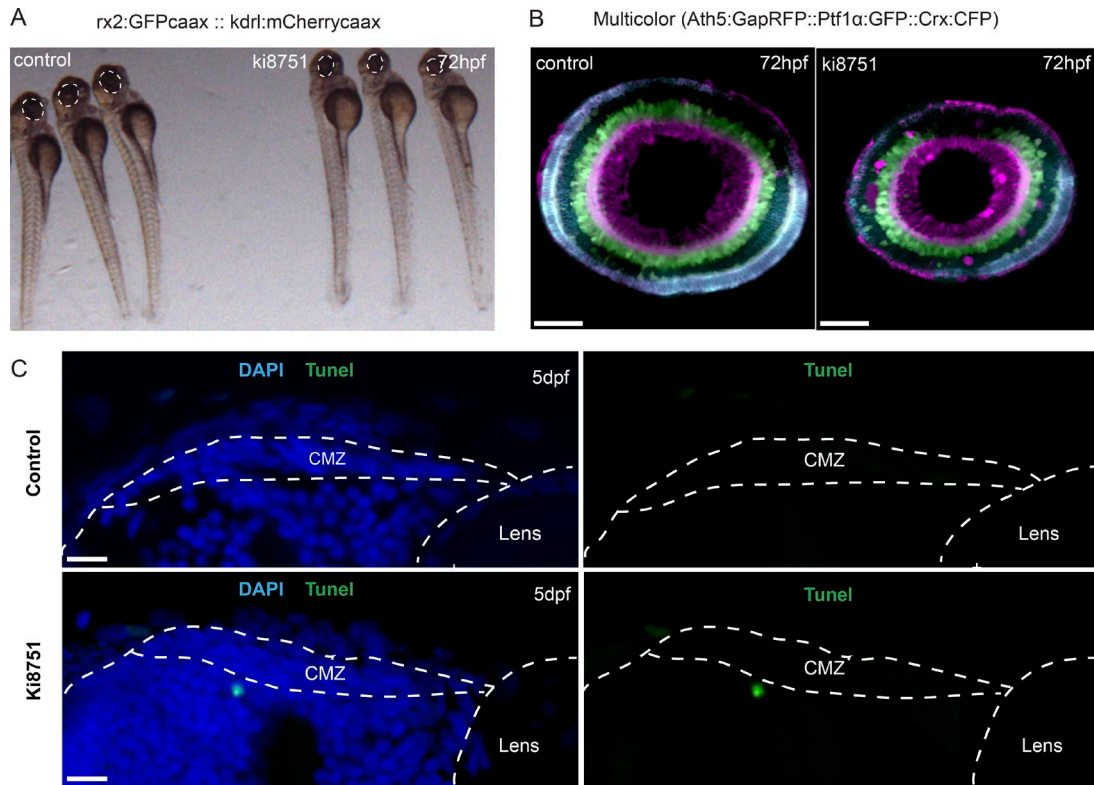


Figure S3. **The effects of Ki8751.** (A) Ki8751-treated embryos had smaller eyes compared with controls at 72 hpf, while the development of the entire body appeared normal. Eyes are indicated by white dashed circles. (B) Analysis of multicolor transgenic line *Tg(Ath5:GapRFP::Ptf1a:GFP::Crx:CFP)* showing Ki8751 treatment did not influence the development of the central retina. (C) TUNEL assays showing no obvious cell death in the eyes of both the control and Ki8751-treated embryos at 5 dpf ( $n = 18$  slices from six eyes). The white dashed lines outline the boundary of the CMZ and lenses. Bars: (B) 50  $\mu\text{m}$ ; (C) 10  $\mu\text{m}$ .

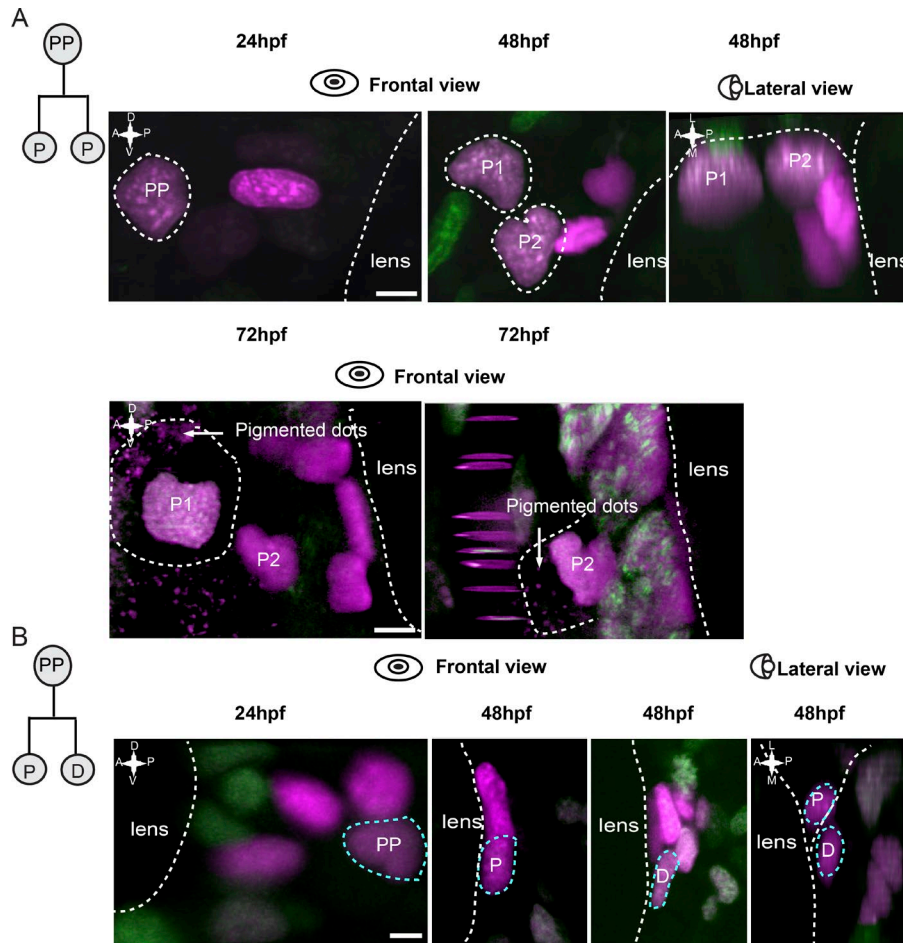


Figure S4. **Lineage analysis of pigmented progenitors.** (A) One PP gave rise to two pigmented cells (P) from 24 to 48 hpf. In the frontal view, the nature of pigmented cells was decided based on their characteristic cell shapes (indicated by the white dashed lines) and many pigmented precipitates (white arrows) inside and surrounding the pigmented cells. (B) An example showing one PP giving rise to one pigmented cell and the other dormant CMZ tip cell (D). A, anterior; D, dorsal; P, posterior; V, ventral. Bars, 5  $\mu$ m.

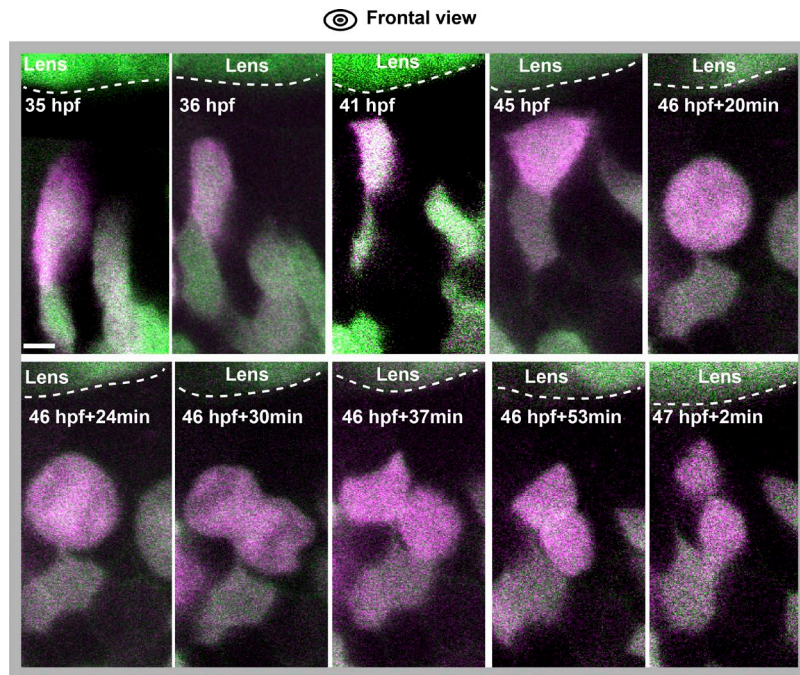
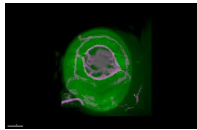
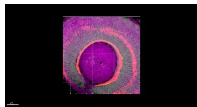


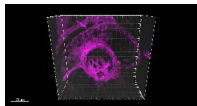
Figure S5. **Cell division of SPs.** Time lapse showing the cell division of an SP. The white dashed lines outline the boundary of lenses. Bar, 2.5  $\mu$ m.



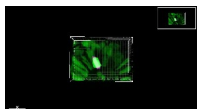
Video 1. **3D animation of an eye of the transgenic fish line *Tg(rx2:GFPcaax::kdr:mCherrycaax)*.** This video was shot at 10 frames per second.



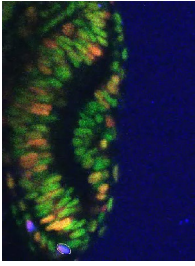
Video 2. **3D animation of the optical sections of the CMZ in the transgenic fish line *Tg(rx2:GFPcaax::kdr:mCherrycaax)*.** This video was shot at 15 frames per second.



Video 3. **3D animation of an eye in the transgenic fish line *Tg(kdr:mCherrycaax)*, which was treated with Ki8751.** This video was shot at 23 frames per second.



Video 4. **3D animation of peripheral cells located in the first and second layers (PPs and SPs, as in Fig. 4) in green and magenta.** This video was shot at 25 frames per second.



Video 5. **A time-lapse video showing that a photoconverted cell in the ML migrates into the most peripheral region of the optic cup at 24 hpf.** Green, H<sub>2</sub>BGFP; Red, H<sub>2</sub>BPSmOrange; Blue, photoconverted H<sub>2</sub>BPSmOrange. The cell is outlined with the white circle. This video was shot at seven frames per second.