Expanded View Figures

Figure EV1. *In vivo* time-lapse recording of dividing BC progenitors in a *vsx1*:GFP retina.

A Confocal *in vivo* image of a 2 dpf *vsx1*:GFP retina. Expression of GFP, depicted using a “Fire” look-up table (LUT), follows the retinal differentiation gradient, appearing first in the ventro-nasal patch and subsequently in the nasal, dorsal, and temporal parts of the retina. Scale bar: 10 μm.

B Time-lapse images of the boxed region in (A). Left panels represent the raw levels of GFP fluorescence (Fire LUT). Right panels depict GFP fluorescence adjusted for better visualization (gray). Dashed circles mark mitotic events. Scale bar: 10 μm.

C Individual mitotic events marked in (B) at progressively later times during development. GFP fluorescence levels of the mitotic progenitors and that of six cells in their surround. Orange circles, apical mitotic divisions; cyan circles, non-apical mitotic divisions; gray squares, individual surrounding cells; black squares, average fluorescence of surrounding cells.
Figure EV1.

**A**

*vsx1:GFP*

**B**

**C**

Mitosis

Surrounding

Fluorescence (norm.)

Time (min)
Figure EV2. BC progenitors dividing in the laminated retina express Crx and ribeye α.

A. Confocal image of a cryostat section of a 2 dpf crx:mCFP retina immunostained with antibodies against Crx and pH3. Co-labeled cells are indicated by magenta dots. Scale bars: 10 μm.

B. In vivo image of a 2 dpf crx:mCFP retina. Expression of mCFP in the INL is largely limited to the "laminated" region (cyan bar above the figure panel) of the retina while only photoreceptors are labeled in the un laminated parts of the retina (orange bar above panel). One crx:mCFP+ progenitor (cyan arrowhead) can be seen in an early stage of division, which judged by transgene expression levels, belongs to the most differentiated cells in this intermediate part of the INL. Scale bar: 10 μm.

C. In vivo time-lapse images of a crx:mCFP+ progenitor undergoing mitotic division (pseudo-colored cyan) in the INL of a 2 dpf embryo. Ninety-seven such divisions were observed in four time-lapse recordings of a total of 32.1 h. Scale bar: 10 μm.

D. Contrast inverted confocal images of an eye from a 2 dpf ctbp2:mEGFP transgenic fish immunostained to visualize GFP (left panel) and processed for fluorescence in situ hybridization to detect expression of a ribeye α specific exon (right panel). Scale bar: 10 μm.

E. High magnification of boxed area in (D). Expression of the ctbp2:mEGFP transgene and endogenous ribeye α mRNA is restricted to the INL in the laminated region of the retina (cyan bar over figure panel). Dashed line indicates onset of expression. Scale bar: 10 μm.

F. Confocal images of a 2 dpf ctbp2:mEGFP retina labeled to detect GFP, pH3, and ribeye α mRNA. A ribeye α+ pH3+ non-apically dividing progenitor (magenta arrowheads, middle panel) can be seen in a laminated region of the retina (cyan bars above panels). Scale bar: 10 μm.

G. ctbp2:mEGFP+ progenitor cell undergoing mitotic division (pseudo-colored cyan) in the INL during an in vivo time-lapse recording of a 2 dpf retina. Eighty-seven such divisions were observed in two time-lapse recordings totaling 32.8 h. Scale bar: 10 μm.
Figure EV3. Centrosomes of BCs are clustered at the OPL.

A In vivo confocal images of a 2 dpf retina in which centrosomes (centrin 4 mRNA) and cellular membranes (BODIPY-Texas Red) are labeled. The outer part of the INL is largely devoid of centrosomes. The emergence of the OPL (region to the right of the arrowhead) coincides with the clustering of centrosomes in this location. Solid gray line indicates the apical surface. Orange and cyan bars above the images represent unlaminated and laminated parts of the retina, respectively. Scale bar: 10 μm.

B In vivo confocal images of a 2 dpf retina in which centrosomes (centrin 4 mRNA) and a subset of BCs (Q19) are labeled. BC somata are devoid of centrosomes. The relocation of BC centrosomes from the apical surface (solid gray line) to the OPL coincides with the retraction of the apical process of BCs (arrowhead). Orange and cyan bars above the images represent unlaminated and laminated parts of the retina, respectively. Scale bar: 10 μm.

C Confocal in vivo images of a 3 dpf retina with an isolated BC, in which the centrosome and cellular membranes are labeled with YFP and cerulean, respectively (plasmid encoding UAS:centrin4-YFP/UAS:memCerulean was injected into Q26). The centrosome (green) is localized to the dendritic tuft at the OPL rather than in the soma. Scale bar: 5 μm.

D Scheme illustrating distinct developmental steps (nucleokinesis, centrosome relocation, apical process retraction, transient arborizations of the apical process in the OPL and mitosis at the INL/OPL interface) for the vsx1+ progenitor from Fig 3D and E, observed by time-lapse imaging. Nucleokinesis trajectories of the cell are color coded to indicate the local cytoarchitecture of the retina when these events took place (orange: unlaminated; cyan: laminated).
Figure EV4. mOrange2-PCNA allows for studying HUA-induced delay of mitosis.

A Time-lapse imaging of vsx1.GFP retinas expressing mOrange2-PCNA was used to determine the time interval between the beginning of late S-phase and the beginning of M-phase for progenitors. In control fish, the average time for this interval was 142 ± 48 min (mean ± 2 standard deviations, SD, 38 cells from six fish, orange circles represent single cells). HUA-treated progenitors were significantly delayed compared to controls, with an average time interval of 534 ± 32 min (10 cells from four fish, P < 0.0001, Mann–Whitney U-test, cyan circles). The delay with which each of the 10 HUA-treated progenitors reached M-phase was calculated by subtracting the average "expected" time interval between S- and M-phase (obtained from control cells, orange diamond) from the observed time interval between S- and M-phase for each HUA-treated progenitor (cyan circles). An example of this calculation for the cell tracked in (B) is shown.

B Time-lapse confocal images of a HUA-treated vsx1.GFP retina expressing mOrange2-PCNA. Based on control experiments, the outlined cell (for which the delay in mitosis is depicted in A, cyan cell with a pink outline) would have been "expected" to undergo mitotic division around the 148 min time point (orange diamond, closest to the calculated mean "expected" time of 142 min), but entered M-phase at 693 min (cyan circle). Scale bar: 10 μm.
Figure EV4.
Figure EV5. Schematic comparison of “classical” and “uncoupled” models of neuronal development.

An idealized representation of the developmental trajectories of four progenitors tracked over time until they differentiate and reach maturity. The period of neurogenesis is the same for cells in the “classical” and “uncoupled” models. As compared to the “classical” model in which differentiation is initiated only after cell cycle exit, “uncoupling” neurogenesis and differentiation permits a cell population to reach maturity faster and in relative synchrony.