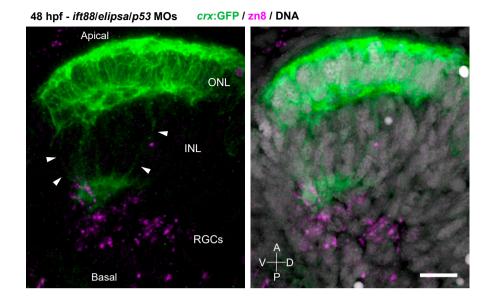


SUPPLEMENTARY MATERIAL

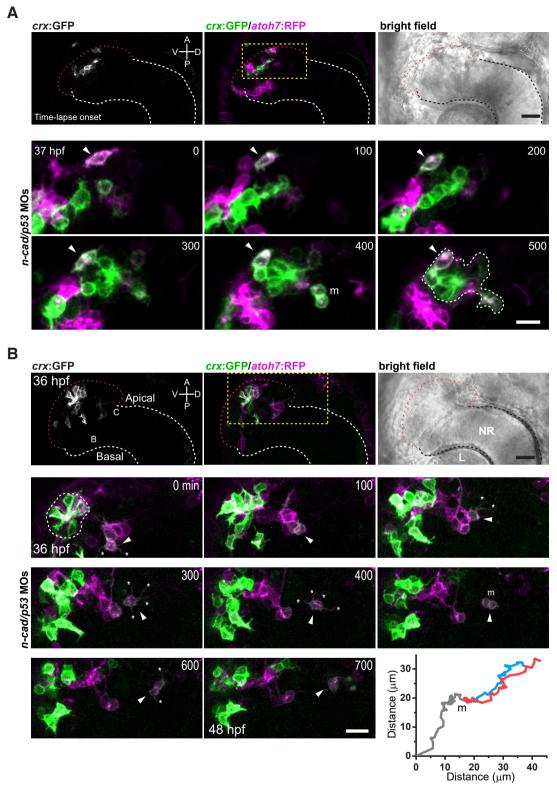
corresponding to:

Photoreceptor progenitor dynamics in the zebrafish embryo retina and its modulation by primary cilia and N-cadherin

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Suppl. Fig. S1. Extension of aberrant basal processes by photoreceptor progenitors in Elipsa/IFT88 morphant retina. Maximum intensity projections of a confocal stack (35 slices, separated by 0.5 μm) showing the anterior ventral region of the retina from a crx:GFP transgenic embryo, labeled to highlight the RGCs (zn8 antibody) and nuclei (methyl green). Arrowheads: basal processes; INL: inner nuclear layer; ONL: outer nuclear layer; RGCs: retinal ganglion cells. Scale bar: 10 μm.



Suppl. Fig. S2. Photoreceptor progenitors dynamics in crx:GFP/atoh7:RFP double transgenic embryos, upon N-cadherin knock-down: two examples. (A) Several disperse cells coalesce to form a single rosette. One of these cells starts expressing high levels of atoh7:RFP and to gradually increase crx:GFP expression with time (arrowhead). m: photoreceptor progenitor undergoing mitosis, just before the daughter cells join the rosette. (B) Dynamics of an isolated photoreceptor progenitor (arrowheads) from the same time-lapse shown in Figure 10. This progenitor is initially detected as a double-labeled cell, around the central retina and in close association with cells strongly expressing only RFP (most probably RGCs). Eventually, the photoreceptor separates from these cells, displaying several dynamic cell processes (asterisks), until it divides at time point 500 min (cell marked "m"). The upper row shows a lower magnification of the embryo eye and head at the beginning of the time-lapse; the yellow squared area corresponds to the time sequence below. A tracking of the cell and its daughters is represented in the graph at the lower right corner. White dashed lines (A-B): limit of the neural retina; red dashed lines: apical limit of the aberrant anterior-ventral area of the retina.

Suppl. Videos are available at: https://doi.org/10.1387/ijdb.200113fz

Suppl. Video 1. Confocal time-lapse experiment (maximum intensity projection) from a crx:EGFP-CAAX (crx:GFP) transgenic embryo showing a photoreceptor progenitor since it starts expressing detectable GFP at approximately 38.5 hpf until it integrates to the outer nuclear layer where it divides symmetrically to give rise to two daughter cells that remain there. 3D stacks of confocal sections at 1 µm separation were taken every 10 min for around 17 hours in total. During this time, the cell is seen to detach a basal process (see Supplementary Video 2), translocate its body apically, and display a high cortical activity including the extension and retraction of multiple tangential and basal processes. This video is further analyzed in Fig. 2A.C-D. as well as in Supplementary Video 2.

Suppl. Video 2. Confocal time-lapse experiment (maximum intensity projection of a few sections in each photogram) from a crx:GFP transgenic embryo showing the same photoreceptor progenitor as in Supplementary Video 1. A color palette of 16 colors was used to highlight very low fluorescence, like that from the thin basal process that appears to be attached to the basal retinal surface at the initial part of the time-lapse, and detaches between 140 and 150 min. See Fig. 2D for further reference.

Suppl. Video 3. Confocal time-lapse experiment (maximum intensity projection) from a crx:GFP transgenic embryo showing a group of photoreceptor progenitors already localized at the future ONL. A lateral and a top (from apical) view are shown. These cells continue displaying a high surface activity all along the time-lapse, which includes the extension and retraction of multiple cell processes (mostly tangential and basal). One cell just divided at the beginning of the time-lapse and both daughter cells remain apical. This video is further analyzed in Fig. 2B.

Suppl. Video 4. Confocal time-lapse experiment (maximum intensity projection) from an atoh7: Gap-EGFP (atoh7:GFP) transgenic embryo, showing a cell that can be identified as a photoreceptor progenitor because it remains at an apical position, has no long basal process and progressively reduces GFP expression. This cell continuously extends and retracts many cell processes, including many tangential and a few directed basally.

Suppl. Video 5. Confocal time-lapse experiment (maximum intensity projection) from an crx:GFP transgenic embryo, showing a photoreceptor progenitor translocating its cell body while profusely extending tangential processes at its apical process tip (see Fig. 3A).

Suppl. Video 6. Animation through a confocal stack at high magnification, of a tangential view of a 36 hpf retina from a crx:GFP (green) transgenic embryo, labeled with phalloidin (Factin, magenta) and methyl green (nuclei, blue). A photoreceptor progenitor is seen, which has a prominent apical protrusion squeezed between two mitotic cells. Some short tangential processes extend from this protrusion. See details in Fig. 3B.

Suppl. Video 7. Animated 3D reconstruction from a confocal stack at high magnification, of the apical retina from a 36 hpf crx:GFP (green) transgenic embryo, labeled with phalloidin (Factin, magenta) and methyl green (nuclei, blue). A photoreceptor progenitor is seen, which presents a cell protrusion that extends along the retinal apical surface. The cell body of this cell also bulges towards the sub-retinal space, apparently displacing the nuclei of two overlaying RPE cells.

Suppl. Video 8. Animated 3D reconstruction from a confocal stack at high magnification, of the apical retina from a 40 hpf crx:GFP (green) transgenic embryo, labeled with phalloidin (F-actin, magenta). The photoreceptor progenitor in the center extends many tangential processes radially, some of which appear to contact a neighboring cell, also crx:GFP-positive. See details in Fig. 3B.

Suppl. Video 9. Confocal time-lapse experiment (maximum intensity projection) showing the retinas from two crx:GFP transgenic embryos: control, on the left, and morphant for Elipsa/IFT88 on the right. Recorded time was 17 hours (36-53 hpf), with images taken every 10 min. Although in both cases photoreceptor progenitors accommodate at the apical retina to form an outer nuclear layer, the ones in the morphant present relatively long basal processes that remain highly active all along the time-lapse. See Fig. 6B and C, as well as Supplementary Video 10.

Suppl. Video 10. Detail view from the confocal time-lapse experiment (maximum intensity projection) in Supplementary Video 9, showing the behavior of a small group of photoreceptor progenitor in an Elipsa/IFT88 MO-injected embryo. Two cells are particularly evident, displaying very active basal processes that extend and retract. Some other short processes are also evident, either tangential to the retinal surface or extending from the lateral-basal membrane. See Fig. 6C.

Suppl. Video 11. Detail view from a confocal time-lapse experiment (maximum intensity projection), from an Elipsa/IFT88 morphant showing a photo-receptor progenitor dividing normally and stopping basal process extension just during M phase.

Suppl. Video 12. Confocal time-lapse experiment showing crx:GFP-positive photoreceptor progenitors joining to form a rosette in an N-cadherin morphant embryo. See details in Fig. 9B.

Suppl. Video 13. High magnification from the time-lapse image analyzed in Fig. 9 and Supplementary Video 12, showing an isolated crx:GFP-positive photoreceptor progenitor extending multiple and highly dynamic neurite-like cell processes in an N-cadherin morphant embryo. See details in Fig. 9C.

Suppl. Video 14. Confocal time-lapse experiment showing crx:GFP/atoh7:RFP double-labeled photoreceptor progenitors joining to form a rosette (dotted line in last photogram) in an N-cadherin morphant embryo. Asterisks: cell processes. See details in Supplementary Fig. 2A.

Suppl. Video 15. Detail view from a confocal time-lapse experiment (maximum intensity projection) from an N-cadherin morphant, showing a double-labeled atoh7:RFP/crx:GFP cell detaching from a group of atoh7:RFP-positive cells to migrate while acquiring a "stage 2-like" conformation with several dynamic neurite-like processes.