Supplemental material

Supplemental figure S1

Beta-*Gal* histochemistry on a retinal section from a mouse *Chx10-Cre; R26R;Dicer*^{flox}*/ Dicer*^{flox}, P35 animal. The R26R allele is activated in cells that have at any time during their development expressed the site-specific recombinase cre (Soriano, 1999). Importantly, once the R26R allele has been activated in a cell, all descendants of the cell will express LacZ. LacZ positve cells (cells that have been exposed to Cre) that were also homozygous for the Dicer conditional allele alternated with Cre negative (wild type cells) in the retina. onl: outer nuclear layer. ipl: inner plexiform layer.

Supplemental figure S2

In situ hybridization on wt (A) and CKO (B) retinal sections (B) probed for miR-183 a microRNA highly expressed in the retina, by means of a digoxigenin labeled LNA probe (MiRCURY detection, from Exiqon). Protocol was the same as described in Methods except for the hybridization step, carried on at room temperature. The dark blue staining, indicative of the reaction product, is concentrated in photoreceptor inner segments of the wt retina (arrows in A) and in the outer half of the inner nuclear layer. In the *Dicer* CKO retina of this 7 month-old animal (B) staining is patchy, with alternating positive and negative areas (brackets). onl: outer nuclear layer; inl: inner nuclear layer; ipl: inner plexiform layer; gcl: ganglion cell layer.

Supplemental figure 3

Antibody labeling of Dicer CKO retinal sections at P2. A: Phosphohistone 3 antibodies showed dividing cells appropriately positioned at the outer aspect of the developing retina, within and outside the columns of Cre+, GFP+, Dicer-/- clones of

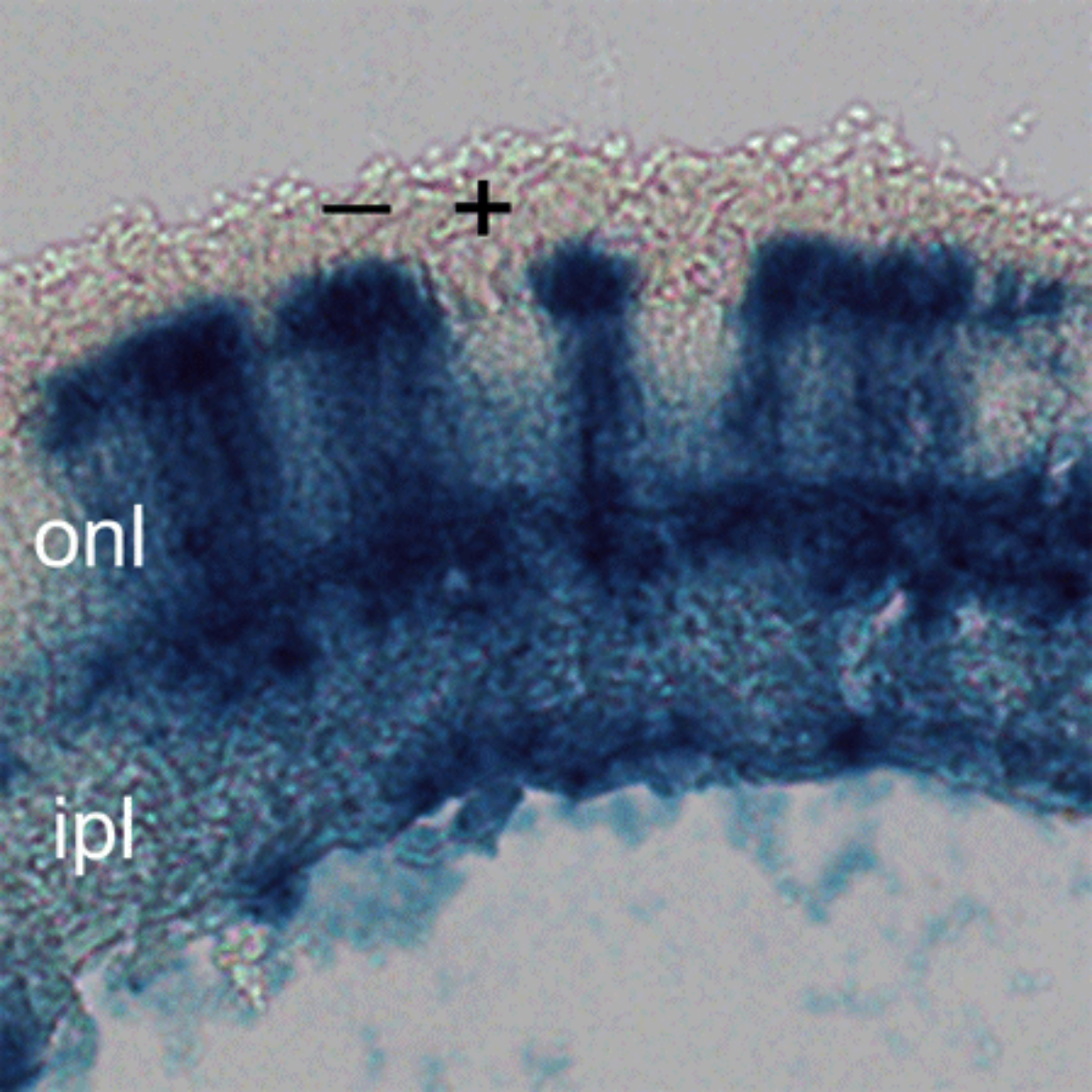
cells (in green). nz: neuroblastic zone. B: Calbindin D antibodies (red) showed differentiating horizontal cells, correctly positioned in the outer retina, and appropriately located amacrine and ganglion cells in the inner retina. C: At P2 GABA-ergic amacrines are differentiating. An antibody against the vesicular GABA transporter (vGAT) labeled the developing inner plexiform layer in mutants as expected (red signal). D: the vesicular glutamate transporter (vGLUT 1) showed developing synaptic terminals of photoreceptors as bright puncta (arrowheads). E: recoverin antibodies (red) showed photoreceptors (Ph) differentiating at the outer retinal margin, both within and outside the columns of Cre+ neuroblasts (green). Inset: developing photoreceptors with elongated shape. F: S-cone opsin antibodies (red). Inset: developing cone with asymmetric shape, typical of the phase of active elongation of cilia.

Supplemental figure S4

Morphology of the outer retina in Dicer CKO mice at P2. A. Light micrograph of semitin section. Dividing cells (m) abut the outer retinal margin (A and inset) in a well known pattern described for wild type animals. In addition, differentiating horizontal cells, recognized by their pale cytoplasm and elongated shape, interrupt the neuroblastic zone (nz) to assume a tangential position (arrows) at the border of the developing opl (dashed line). Pycnotic (dying) cells (p in the inset) in the outer retina, as previously reported at this developmental stage.

B: Adherens junctions of the outer limiting membrane in fully mature retinas are composed of several proteins including ZO-1 and ZO-2. Antibodies against ZO-1 showed a highly specific pattern of staining in the Dicer CKO retina at P2. Staining precisely underlied the developing junctional complex at the outer margin of the tissue (B). A similar, albeit weaker, pattern of staining is produced with atypical PKC

antibodies, which recognize proteins participating in the so-called subapical region of the photoreceptors. Positive staining in mutants was suggestive that the polarity of these cells was correct (not shown). The development of adherens junctions at the appropriate apical location was confirmed by EM (C). These junctions can be recognized from their electron dense appearance and thickening of the adjoining membranes. They form a continuous ribbon joining developing photoreceptors and neuroblasts (C). D: Appropriate photoreceptor orientation in the CKO retina was confirmed by the identification of cilia (c) growing from the outer aspects of cells positioned at the interface with the pigment epithelium. Arrowheads point to adherens junctions.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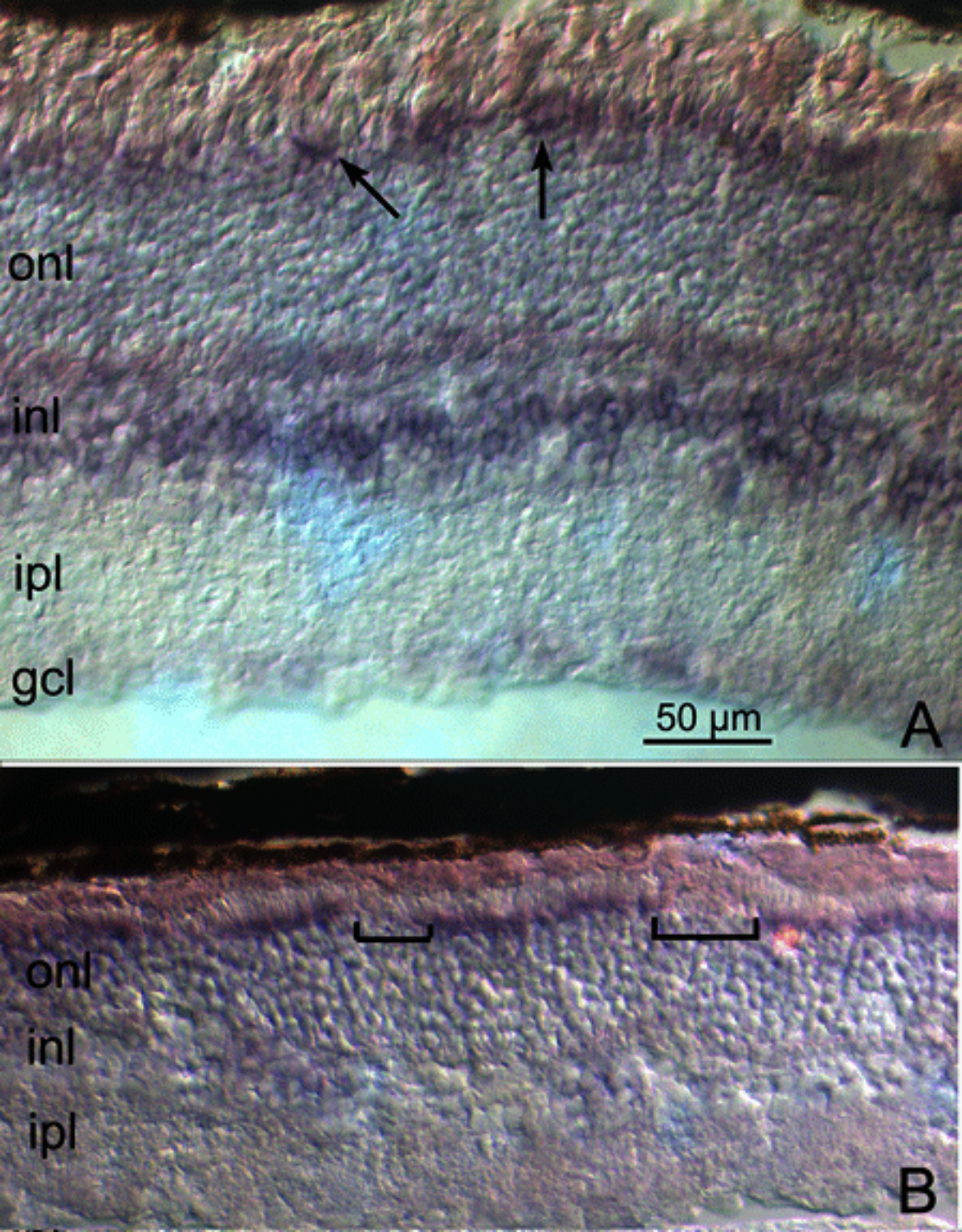
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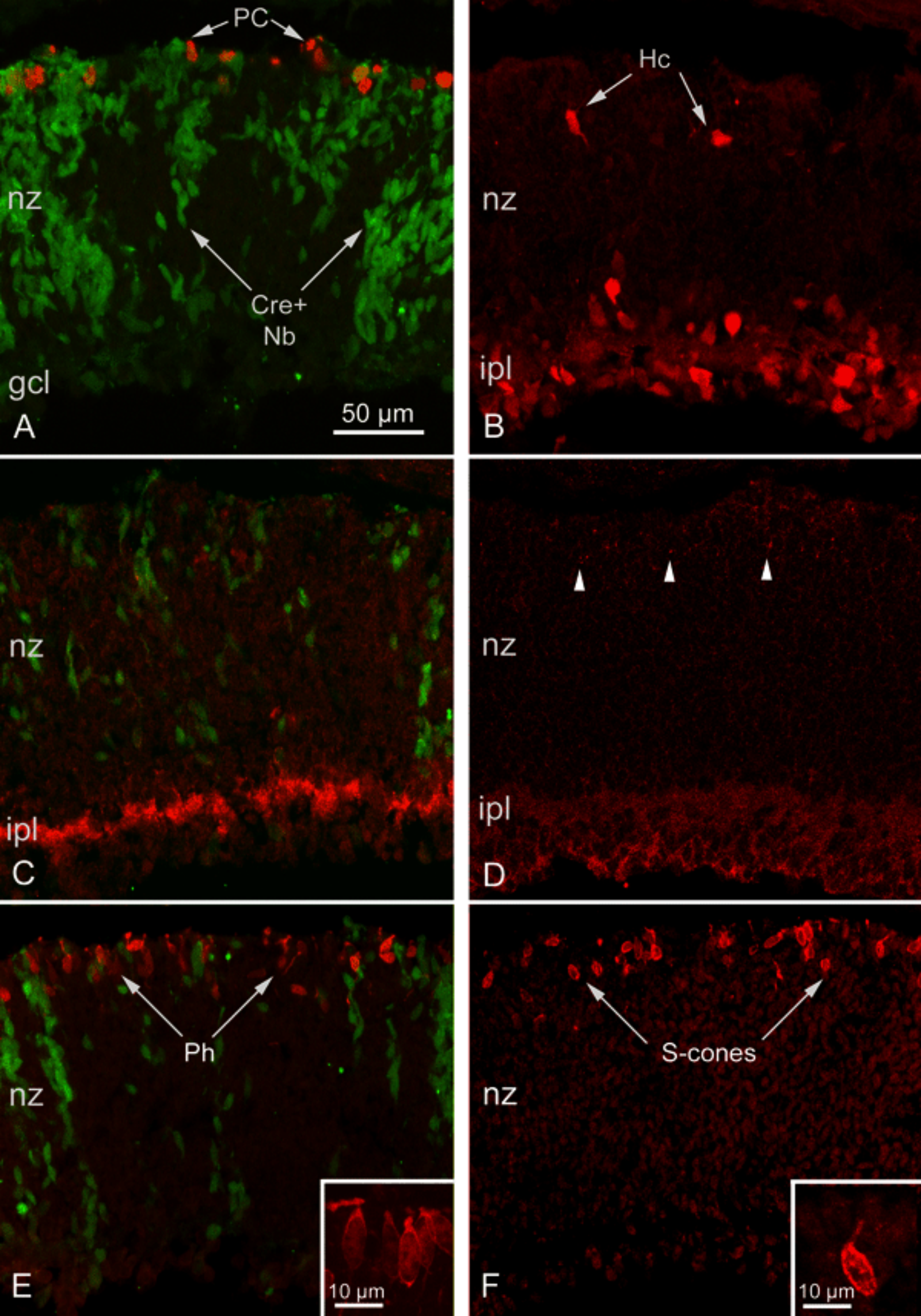
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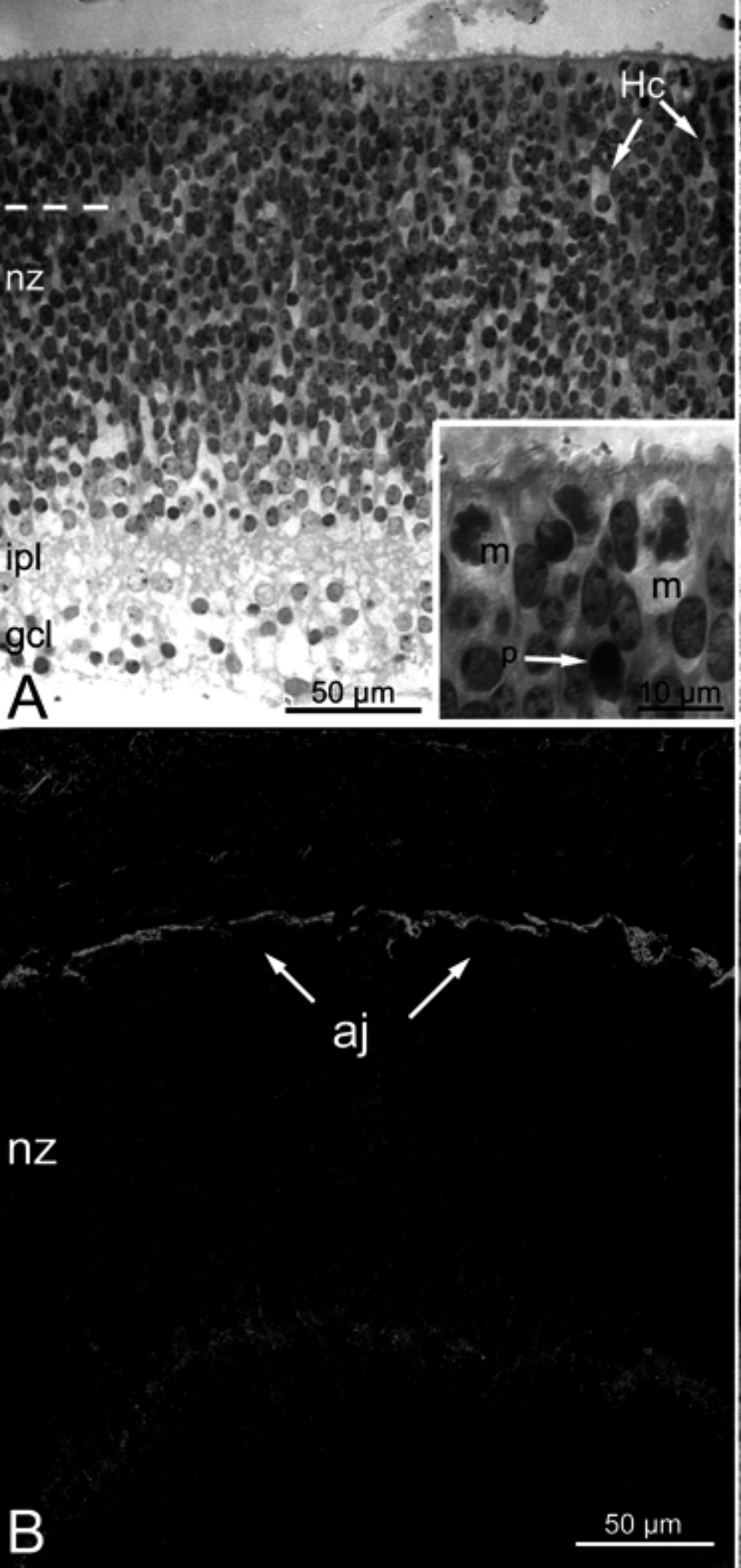
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