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PEDF Peptides Promote Photoreceptor Survival in *rd10* Retina Models

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15 Highlights

- 16 • *Rd10* retinal explants are a valuable system to evaluate PEDF activity
- 17 • Neurotrophic PEDF peptides exhibit *rd10* photoreceptor-protective property
- 18 • PEDF peptides may prove useful as potential therapeutics for retinal degenerations

ABSTRACT

The purpose of the study is to evaluate the protective properties of PEDF peptide fragments on *rd10* mouse models of retinal degeneration *ex vivo*. Human recombinant PEDF and synthetic peptides were used. *Rd10* retinal explants as well as wild-type retinal explants treated with zaprinast to mimic the *rd10* photoreceptor cell death were employed. PEDF protein was intravitreally administered into *rd10* mice. Outer nuclear layer thickness measurements in retinal sections, TUNEL labeling in retinal explants, western blots and immunofluorescence with retinal samples were performed. PEDF protein levels in the RPE of *rd10* mice decreased with age (P15 - P25). Levels of PEDF receptor PEDF-R declined in the photoreceptor inner segments from *rd10* relative to wild-type mice at P25. PEDF administration increased the outer nuclear layer thickness of *rd10* retinas *in vivo* and decreased the number of TUNEL⁺ nuclei of photoreceptors in *rd10* retinal explant cultures, both relative to untreated controls. Peptides containing the PEDF neurotrophic region decreased the number of TUNEL⁺ photoreceptors in both *rd10* and zaprinast-induced cell death *ex vivo* models, while peptides without the neurotrophic region and/or lacking affinity for PEDF-R were ineffective in protecting photoreceptors. Thus, retinal explants are a valuable system to evaluate PEDF activity. Short peptides with the photoreceptor-protective property of PEDF may prove useful for the development of therapeutic agents for photoreceptor protection in retinal degenerations.

Retinitis Pigmentosa (RP) is a collection of different genetic diseases that lead to progressive photoreceptor degeneration and ultimately vision loss. More than 60 genes are implicated in molecular pathways towards photoreceptor cell death (Wang et al., 2005). Currently, there are no treatments available and, due to its vast diverse genetic background, a putative gene-based therapy seems challenging. Consequently, mutation-independent strategies to protect photoreceptors against continued damage and progressive vision loss are appealing (Guadagni et al., 2015). One of them is the use of survival, neurotrophic and neuroprotective factors that target common signaling pathways of cell death in the different types of RP.

Photoreceptor survival is highly dependent on the retinal pigment epithelium (RPE), a monolayer of hexagonal pigmented cells that exert physiological support and help maintaining retinal homeostasis (Strauss, 2005). Among the RPE-derived trophic factors essential for visual functions, pigment epithelium-derived factor (PEDF) encoded by the *SERPINF1* gene is highlighted. It is produced and secreted by RPE cells and deposited in the subretinal space, where it acts not only on neuroprotection, but on antiangiogenesis and immunomodulation for the retina (Becerra and Notario, 2013). *SERPINF1* gene overexpression or PEDF protein administration delay degeneration of photoreceptors and the neuroretina, attenuate angiogenesis and neovessel invasion, and prevent visual function loss in several animal models of hereditary retinopathies (Becerra and Notario, 2013; Polato and Becerra, 2016; Ortin-Martinez et al., 2014). PEDF promotes development, differentiation and survival of isolated photoreceptor, retinal ganglion cells and retinal progenitors in culture (Jablonski et al., 2000; Murakami et al., 2008; Rapp et al., 2014; Tanimoto et al., 2006; Unterlauff et al., 2012; Vigneswara et al., 2013; Winokur et al., 2017; Zhu et al., 2011). Given its multimodal nature, fragmentation of the PEDF polypeptide is ideal to study individual activities and develop agents relevant for use in the clinic. Structure-function relationships have revealed a distinct region on the PEDF polypeptide for neurotrophic action that is separated from that of its antiangiogenic activities (Becerra, 1997; Becerra,

2006). This neurotrophic region interacts with a receptor, PEDF-R, encoded by the *PNPLA2* gene, which is present in photoreceptor inner segments (Notari et al., 2006; Subramanian et al., 2013). Previously, we described amino acid positions within a short fragment of 17 amino acids within the neurotrophic region that are crucial for PEDF-R binding and retinal cell survival in culture and in the *rd1* mouse, an RP model (Kenealey et al., 2015). These short peptides have not yet been tested in other RP models.

Although the effectiveness of PEDF in protecting photoreceptors *in vivo* has been reported (Polato and Becerra, 2016), its actions on *rd10* mouse photoreceptors are yet unknown. The *rd10* mouse carries a spontaneous missense mutation in the phosphodiesterase 6B (*Pde6b*) gene and provides a reliable model for autosomal recessive RP in humans (Chang et al., 2002). Using *rd10* and *rd10*-mimicry retinal explant cultures, we tested the protective activity of synthetic peptides derived from PEDF, with and without a blocking peptide on degenerating photoreceptors. The protective effect of intravitreal human recombinant PEDF protein injections in the *rd10* mouse *in vivo* was also evaluated. The findings show that PEDF decreased the number of dying photoreceptors through binding its specific receptor PEDF-R. We discuss the advantage of the *rd10 ex vivo* model for PEDF assay and use of PEDF peptides as promising neuroprotective agents.

The *rd10* mouse model of retinal degeneration and wild type (C57Bl/6J, WT) control mice of the same background were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). All animals were housed and handled in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research, USA guidelines, European Union guidelines, and those of local ethics committees of the NIH and CSIC.

Recombinant human PEDF was purified as before (Stratikos et al., 1996). Human PEDF-derived peptides 34-mer, 44-mer, 17-mer, and 17-mer[R99A] and 17-mer[H105A] (17-mer with R99A or H105A

substitutions, respectively) and human PEDF-R-derived peptide P1 (blocking peptide) were synthesized as described {Kenealey, 2015 #19} (amino acid sequences indicated in Fig. 1N).

Rd10 mice were anesthetized with isoflurane and intravitreal injection was performed under an ophthalmoscope using a Hamilton syringe (Model 75-RN-SYR) with a 33-gauge removable needle. One eye received 2 μ l of PEDF (0.24 pmol) or derived peptide (0.24, 0.48 or 2 pmol) in PBS, whereas the contralateral eye was injected with PBS (2 μ L). Animals were euthanized and eyes processed for analysis. Alternatively, retinal layer thickness was determined by Optical Coherence Tomography (OCT) (Spectralis OCT adapted for mice imaging, Heidelberg Engineering, Germany). Both male and female mice were used for this study with at least four animals per group. Outer nuclear layer (ONL) and inner nuclear layer (INL) thickness were measured in whole-mounted retinas by using the "XZY acquisition mode" of a laser confocal microscope (TCS SP2; Leica Microsystems, Wetzlar, Germany). Serial optical cross-sections (50 μ m) were acquired with a 63X objective every 2.5 μ m and ONL/INL thickness ratio measured using FIJI software (Schindelin et al., 2012).

Retinal explants were prepared as described (Arroba et al., 2011). After enucleation, neuroretinas were dissected from the eyes free of other tissues, especially from RPE, which is main source of PEDF. Then they were whole-mounted with the photoreceptor side-down onto PTFE inserts (PICM03050, Millipore, MA, USA), and maintained in chemically-defined medium R16 (Caffe et al., 2001) for 48-72h at 37°C in a 5% CO₂ atmosphere. For *rd10* mimicry, zaprinast was included in the medium of WT retinal explants at 100 μ M (final concentration). For protein and peptide evaluation, the factors were added to the medium at indicated concentration for the entire culturing period. Afterward, the retinas were rinsed with fresh medium, fixed one hour with 4% paraformaldehyde (w/v) in 0.1 M phosphate buffer (pH 7.4) and processed for photoreceptor death detection.

Programmed cell death was determined by TUNEL staining. Fixed retinas were permeated for 2h with 2% Triton X-100 in PBS, incubated with 20 µg/mL proteinase K (Sigma) (10min, 37°C) and processed for TUNEL staining following the manufacturer's instructions (Promega, Madison, WI, USA). Retinas were counterstained with 1 µM DAPI and mounted with Fluoromount-G. Serial optical sections of the ONL were acquired with the TCS-SP2 microscope using a 63X objective every 1 µm in depth in four central fields around the optic nerve, avoiding the optic nerve head and peripheral regions.

RPE/choroid isolated from each eye was homogenized by sonication (5 pulses 1s each) in 65.5 µL of T-PER Tissue Protein Extraction Reagent (Thermo Scientific) with protease inhibitors (cOmplete™ Mini-EDTA free, Roche). Extracts were incubated at 4°C for 30 min and clarified by centrifugation (16000xg, 5 min, 4°C). Protein concentration was determined by Bicinchoninic Acid Assay. Proteins were subjected to western blot standard procedures with anti-PEDF (BioProductsMD #AB-PEDF1; 1:100,000 dilution) or anti-β-Actin (Santa Cruz Biotechnology sc-47778; 1:1000 dilution). Secondary antibodies were HRP-Anti-rabbit (Kindle Biosciences, R1006; 1:1000 dilution) and HRP-Anti-mouse (Kindle Biosciences, R1005; 1:1000 dilution). Immunoreactive bands were detected by chemiluminescence and their optical densities digitized and quantified using UN-SCAN-IT gel 6.1 (Silk Scientific Corporation).

For immunofluorescence, eyes were fixed (as described before), cryosectioned and labelled with antibodies against PEDF-R (PNPLA2, Millipore #ABD66; 1:100 dilution) and visual arrestin (Santa Cruz, sc-166383; 1:100 dilution).

Data size was estimated in accordance with previous literature, following the Reduction principle for animal research. At least 4 animals were analyzed in duplicate per experimental point. Retinas with microscopical damages due to manipulation were not included for analysis. Outliers from qPCR and western-blots, detected by Grubbs' test, were also excluded from further analysis. Valid data were checked for normality using both D'Agostino-Pearson omnibus normality tests and homoscedasticity

was determined by Fisher's test. Paired Student's T-test was performed for comparing treated vs untreated eyes within each individual. For grouped analysis two-way ANOVA was used, with Turkey's and Fisher's LSD post-test when interaction between variables was detected. Comparisons of dead cell numbers among treated retina explants (non-homoscedastic data) were analyzed using a multivariable linear regression with adjustment for repeated measures (grader within each condition/retina) and a variance components structure to account for correlations in the data. If previous literature allowed us to make a prediction about the result of the experiment, one-sided test was applied. Otherwise, tests were two-sided. All analyses were performed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com).

PEDF administration *in vivo* was performed before the peak of cell death (P25) in our animals (Corrochano et al., 2008) and the photoreceptor degeneration level was determined at P25 by measuring ONL/INL thickness ratios. Figures 1A-1B show representative images of retinal sections of PEDF-treated and control animals. Quantification demonstrated that PEDF treatment of *rd10* retina resulted in a greater ONL thickness than PBS injection (Fig. 1C). Thus, PEDF preserved *rd10* photoreceptor cell layer *in vivo*.

The protective effect of PEDF was confirmed in *rd10* retinal explants by evaluating photoreceptor cell death. Elevated levels of cell death were observed in control explants, while PEDF treatment decreased significantly the number of TUNEL-positive nuclei (Figs. 1D-1F). Thus, PEDF protected *rd10* photoreceptors from cell death *ex vivo*.

PDE6 chemical inhibition with zaprinast (Zhang et al., 2005) is widely used to mimic *rd10* phenotype in WT retina explants. It is a useful model that allows controlled triggering of the degeneration process and results suitable for screening drugs (Martinez-Fernandez de la Camara et al., 2015; Paquet-Durand et al., 2014). Therefore, we used this model for peptide screening. Retinal explants from WT mice at P22 were

150 treated with zaprinast and, where indicated, PEDF-derived peptides or PEDF. We tested neurotrophic
151 PEDF-derived 44-mer and 17-mer peptides (20 nM each), which have PEDF-R binding affinity (Kenealey
152 et al., 2015). PEDF protein (20 nM) was positive control, whereas peptides without PEDF-R affinity, 34-
153 mer and 17-mer[R99A], were negative controls. Peptide P1 (200 nM), which interferes with the
154 interactions to the PEDF-R receptor, was used as blocking peptide (Subramanian et al., 2013). In retinas
155 exposed to zaprinast, the mean number of TUNEL⁺ nuclei decreased significantly with PEDF and peptides
156 44-mer and 17-mer (Figs. 1G-1H). On the other hand, peptides 34-mer and 17-mer[R99A], and the
157 combination PEDF with a molar excess of blocking peptide P1 were not effective in protecting retinal
158 explants treated with zaprinast. Thus, PEDF peptides protected photoreceptors in the *rd10* mimicry
159 model *ex vivo* and involved PEDF-R as mediator.

160 Then we tested the effects of neurotrophic peptides 44-mer, 17-mer and 17-mer[H105A] in retinal
161 explants from *rd10* mice. Peptide 17-mer[H105A] has higher affinity for PEDF-R than 17-mer (Kenealey
162 et al., 2015). Among them, peptides 44-mer and 17-mer[H105A] diminished significantly the mean
163 number of TUNEL⁺ cells with respect to untreated *rd10* retinas (Figs. 1I-1J). Blocking the binding of these
164 peptides with molar excess of P1 peptide impaired the pro-survival effect. Accordingly, we selected 44-
165 mer and 17-mer[H105A] to intravitreally inject *rd10* mice and evaluate protective effects *in vivo*.
166 Treatments with increasing doses of peptide (0.24-2 pmol of peptides) showed that, while no significant
167 effects were found for lower concentrations of 17-mer[H105A] or 2 pmol of 44-mer (data not shown), 2
168 pmol of 17-mer[H105A] preserved the ONL thickness (Figure 1K-1M).

169 We determined the expression of the PEDF encoding gene in eyecups, given that RPE is its main source
170 (Becerra et al., 2004), and found that *Serpinf1* levels slightly increased with age in both WT and *rd10*
171 animals (data not shown). Interestingly, PEDF protein in pooled RPE/choroid samples decreased in *rd10*
172 with age (P15-P25) (Fig. 2A-2B), suggesting post-transcriptional downregulation of PEDF with age

associated with the *rd10* genotype. PEDF-R protein in retinal extracts did not exhibit differences with age (P15-P25) nor between WT and *rd10* when analyzed by western blotting (data not shown). PEDF-R distribution observed under the microscope by immunofluorescence was mainly detected in the photoreceptor inner segments and inner retina of WT and *rd10* animals (Fig. 2C and 2E). PEDF-R tended to accumulate in the outer plexiform layer at the studied age (P25) but total fluorescence (area under the curve) did not change between genotype (WT: 32885 ± 576 ; *rd10*: 32841 ± 516) (Fig 2D and 2F), in agreement with retinal PEDF-R levels determined by western blotting.

To our knowledge, this is the first study showing the effect of PEDF on photoreceptors of *rd10* mice and retina explants of *rd10* and *rd10* mimicry *ex vivo*. We conclude that human PEDF protein can protect mouse photoreceptors from death caused either by a mutation in the photoreceptor-specific gene *Pde6* or by inhibition of the PDE6 enzyme that is associated with RP in humans. This protective effect is specific to PEDF -full-length or derived peptides-, as it is not seen in the contralateral eye of the same animals that was mock-treated with vehicle. Given that PEDF displays no structural homology and no known functional relationship with the PDE6 protein, it is unlikely that an intraocular PEDF increase saves photoreceptors via functional complementation. More likely, PEDF interferes with the execution of the cell death program (Comitato et al., 2018). Interestingly, PEDF prevents photoreceptor death even when most of the residues are removed from the polypeptide, leaving short PEDF peptide fragments containing amino acid residue positions 98–114, which contain the neurotrophic domain. The action is exclusively facilitated by interactions within this 17-mer region and the cell-surface receptor PEDF-R, as it is not seen with the 17-mer[R99A] peptide with no affinity for PEDF-R and is blocked by a soluble ectodomain PEDF-R peptide with the ligand binding region (P1 peptide). These observations agree with those previously reported on serum starved-mediated R28 cell death, zaprinast-induced death of immortalized photoreceptor precursors (661W) cells and *rd1* mouse *in vivo* models (Comitato et al., 2018; Kenealey et al., 2015; Subramanian et al., 2013).

The protective outcome of a single intravitreal injection of 0.24 pmol (12 nanogram) of PEDF into the vitreous of the *rd10* mouse is detectable with a median ONL thickness of 137% relative to vehicle-injected contralateral eye (100%) by three days post-injection (Fig. 1C). In the case of the *Pde6*-mutated *rd1* mouse, a single intravitreal injection of 20 pmol (1 microgram) of PEDF increases the median ONL height to similar values (139%) relative to non-injected or mock-injected contralateral eye by three days post-injection (Cayouette et al., 1999). A previous dose-response study for the protective effects on *rd1* photoreceptors shows no improvement with lower PEDF doses (Kenealey et al., 2015). Therefore, the similarity in outcome with doses that are 83-fold lower in the present than in previous reports suggests higher protection efficacy of PEDF in the *rd10* than in the *rd1* mice. There are reports of protective effects of other compounds on *rd10* mice, such as with intravitreal injections of proinsulin (Isiegas et al., 2016), progestin, norgestrel (Jackson et al., 2016) or p75 NTR antagonists (Platon-Corchado et al., 2017); however, their long-term effects -including those of PEDF- on *rd10* are still unknown.

Comparison of cell survival efficacies between the *rd10* *ex vivo* models and other models shows that these *ex vivo* cultures are suitable and more sensitive to assay PEDF neuroprotective properties, as it significantly diminishes death of explanted *rd10* photoreceptors. In this case, the remaining TUNEL-positive nuclei in explants treated with 20 nM PEDF represented 27% of those untreated contralateral eye controls (Fig. 1F). We have previously reported that cultured retinal R28 cells induced to die by serum-starvation respond to 10 nM PEDF by decreasing the number of TUNEL⁺ cells to 17-50% relative to controls {Kenealey, 2015 #19}. In contrast, in the retinal degeneration slow (*rds*) mice with a mutation in the *Prph2* gene for peripherin unrelated to PDE6, we observed a higher percentage of remaining dead cells (61.5%-81.2% TUNEL⁺ photoreceptor nuclei) with a single intravitreal injection of 1 microgram (20 pmol) of PEDF *in vivo* relative to mock- and non-injections in the contralateral eye by three-days post-injection. (Cayouette et al., 1999). In *rd1* mice, TUNEL⁺ nuclei were 80% with 2 pmol (100 ng) and 50% with both 10 pmol and 20 pmol (0.5 – 1 microgram) of PEDF intravitreal injections relative to vehicle

injections in the contralateral eye by one-day post-injection (Kenealey et al., 2015). These observations lead us to conclude that *ex vivo rd10* culture assays prove sensitive for PEDF evaluation and even more than those *in vivo*, in which RPE is present.

At the biochemical level, upon PEDF binding, PEDF-R liberates fatty acids from phospholipids as secondary bioactive mediators, such as DHA, to exert neuroprotection and likely reducing intracellular calcium in the degenerating retina, which intersects with a cascade of reactions leading to cell survival (Subramanian et al., 2013; Pham et al., 2017; Comitato et al., 2018). In the treated *rd10* retinas, the 17-mer peptide region of PEDF is available to interact with the PEDF-R ectodomain (P1 peptide region) at the photoreceptor cell-surface interface to stimulate the production of bioactive lipids and interfere with the path leading to photoreceptor degeneration.

PEDF levels decrease during retinal degenerative processes, such as AMD, Diabetic Retinopathy or RP and in animal models of retinal degeneration. Some pathologic changes take place early in the RPE during retina degeneration, which may affect the production of neurotrophic factors for the retina. The findings reveal decreases in PEDF production in *rd10* RPE with age, likely due to an apparent post-transcriptional downregulation of PEDF associated with the onset of photoreceptor cell death previously observed in *rd10* mice (P18) (Arango-Gonzalez et al. 2014). Post-transcriptional downregulation in the RPE mediated by metalloprotease action on PEDF has been reported (Notari et al. 2005). With the degeneration process, the few PEDF molecules and remaining PEDF-R in the inner segments of photoreceptors would lessen opportunities for survival stimulation. The decreased endogenous PEDF levels associated with age suggested that administration of PEDF could compensate such defects in the *rd10* retinas. In the case of cultured retinal explants, the main source of PEDF -the RPE- is absent, offering the advantage of decreasing any background activity mediated by endogenous PEDF in the *ex vivo* system.

In summary, small PEDF neurotrophic peptides are efficient neurotrophic agents, and the *ex vivo* assay described here constitute a sensitive system for screening of PEDF and derived peptides for retina degeneration.

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FIGURE LEGENDS

Fig. 1. PEDF treatment protects mouse photoreceptor cells. (A - C) Intravitreal injections were performed with vehicle (PBS) in the left eye (A) and human recombinant PEDF (0.24 pmol) in the right eye of *rd10* mice at P22 (B). Representative retinal sections are shown in (A and B). Scale bar, 20 μ m. (C) Mice were sacrificed at P25 and photoreceptor preservation was evaluated by measuring the ONL/INL thickness ratio in 4 fields. Each data point corresponds to the mean of each eye. (D - F) Retinal explants from *rd10* mice at 23 days old were incubated in R16 medium either with PBS (explants from left eye; D) or with 20 nM of PEDF (right eye; E) for 48 h. (F) TUNEL staining was performed in whole-mounted retinas to quantify photoreceptor cell death in four fields and the number of TUNEL⁺ nuclei in the ONL was compared for each pair of eyes from the same mouse. Statistical analysis vs control * $p < 0.05$ (paired t-test; C and F). Scale bar, 20 μ m. (G and H) PEDF-derived peptides protect photoreceptors in a *rd10* mimicry *ex-vivo* model. Retinas from WT mice at P22 were cultured for 72 h in presence of 100 μ M zaprinast together with a series of peptides derived from the PEDF sequence at 20 nM, with or without blocking peptide P1 at 200 nM, as indicated. Photoreceptor cell death was analyzed by TUNEL assay in the ONL of whole-mounted retinas. (G) Representative images of TUNEL stained nuclei are shown; scale bar, 20 μ m. (H) Dying cells from the ONL in four fields close to the optic nerve were counted and plotted. Each data point corresponds to the mean of each retina. Statistical analysis: PBS vs PEDF * $p = 0.013$; PBS vs 44-mer * $p = 0.0062$; PBS vs 17-mer * $p = 0.0055$ (Fisher's LSD test). (I and J) PEDF-derived peptides protect photoreceptors in *rd10* explants. Retinas from *rd10* mice were incubated 48h in R16 medium in presence of a series of peptides derived from the PEDF sequence at 20 nM, with or without the blocking peptide P1 at 200 nM, as indicated. Photoreceptor cell death was analyzed by TUNEL assay in whole-mounted retinas. (I) Representative images of TUNEL stained nuclei of the ONL are shown; scale bar, 20 μ m. (J) Dying cells from the ONL in four fields close to the optic nerve were counted and plotted. Each data point corresponds to the mean of each retina. Statistical analysis; PBS vs 44-mer

* $p=0.0109$; PBS vs 17-mer[H105A] * $p=0.0145$ (Dunn's test). (K – M) 17-mer(H105A) peptide decreases ONL loss *in-vivo*. Intravitreal injections were administered to *rd10* mice at 22 days old with PBS in the right eye (K) and 2 pmol of a short peptide derived and modified from the PEDF sequence in the left eye (L). Retinas were analyzed by OCT 48h after the injection (K and L, representative OCT images with an image of the eye fundus and the optic nerve head -insets-) and retinal layers thickness was measured in 10 areas close to the optic nerve. (M) ONL/INL ratio was compared for each pair of eyes from the same mouse. Each bar represents the mean $\pm$ SD of ten fields from four mice. Statistical comparison OD vs OS * $p=0.0277$ (Paired t-test).

FIG. 2. PEDF and PEDF-R proteins in mouse RPE and retinas. (A) Photographs of western blots of protein extracts pooled from each WT and *rd10* mouse RPE/choroid at the indicated days of age ($n = 7 - 9$ per age, per genotype). A total of 10 μ g of proteins and purified mouse recombinant PEDF protein standard (10 ng) were subjected to Western blotting with antibody anti-PEDF and anti- β -Actin, as indicated. (B) Amount of PEDF protein per microgram of total protein in RPE extract. Each bar represents PEDF amount obtained by determining the relative density values of PEDF (adjusted to β -Actin) to the density measured for mouse PEDF standard. The plot was derived by averaging the values obtained from three exposures of the blot. (C-F) PEDF-R protein distribution in WT (C) and *rd10* (E) retinas at P25. Eye cryosections were labeled with antibodies against PEDF-R and visual arrestin, and counterstained with DAPI. Confocal images from three eyes were taken in fields close to the optic nerve head and the fluorescence intensity along the "Y" axis was analyzed for each fluorophore (D and F). Lines represent the mean of all the measurements from three eyes and each color corresponds to an antibody or fluorophore: red for PEDF-R, green for visual arrestin (photoreceptors) and blue for DAPI (nuclei). OS: outer segments; IS: inner segments; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer.

Figure 1.

Figure 2.