Müller glia provide essential tensile strength to the developing retina

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To investigate the cellular basis of tissue integrity in a vertebrate central nervous system (CNS) tissue, we eliminated Müller glial cells (MG) from the zebrafish retina. For well over a century, glial cells have been ascribed a mechanical role in the support of neural tissues, yet this idea has not been specifically tested in vivo. We report here that retinas devoid of MG rip apart, a defect known as retinoschisis. Using atomic force microscopy, we show that retinas without MG have decreased resistance to tensile stress and are softer than controls. Laser ablation of MG processes showed that these cells are under tension in the tissue. Thus, we propose that MG act like springs that hold the neural retina together, finally confirming an active mechanical role of glial cells in the CNS.

Introduction

The nervous system is one of the softest tissues in the body (Franze et al., 2013; Swift et al., 2013), with a consistency similar to that of cream cheese. Yet it is also the most highly organized tissue in the body, composed of neurons and glial cells arranged into discrete layers. How this incredibly soft and complex tissue maintains its integrity throughout life, despite the many physical stresses imparted on it, remains a mystery. The retina, an outpocket of the central nervous system, is constantly exposed to physical stresses (Franze et al., 2011), which can lead to a tearing of the retinal tissue along the layers known as retinoschisis. The neurons of the retina do not appear to provide much structural support to the tissue, as various genetic manipulations have shown that any of the neuronal types can be completely eliminated from the tissue, either singly (Tomita et al., 2000; Kay et al., 2001; Green et al., 2003; Randlett et al., 2013) or in various combinations (Randlett et al., 2013), without reported structural consequences.

More than 150 years ago, Rudolf Virchow first described glial cells, which he likened to “Nervenkitt” or nerve-putty. Expecting them to provide mechanical support to neurons, he termed them “neuroglia” (after the Greek word for glue; Virchow, 1856). While glial cells are now appreciated for their many roles in neural development and function (Allen and Barres, 2009; Reichenbach and Bringmann, 2013; Kettenmann and Ransom, 2013), Virchow’s earliest speculation of their involvement in physical support persists in most modern textbooks of basic neuroscience, despite a lack of direct experimental proof. There is some evidence both for and against Virchow’s idea.

First, investigations into the biomechanical properties of glial cells revealed that they are twice as soft as their neighboring neurons, indicating that glia cannot provide a rigid structural framework that neurons can cling to (Lu et al., 2006). However, other studies showed that glial cells in the retina are responsive to mechanical signals (Lindqvist et al., 2010) and quickly recover their shape after deformation (Lu et al., 2006), suggesting that they might be able at least to withstand tensile stresses. Additionally, the elimination of myelin and oligodendrocytes decreases the tensile strength and stiffness of the spinal cord exposed to large deformations ex vivo (Shreiber et al., 2009). However, whether glial cells are mechanically important to neural tissue in vivo if it is exposed to physiologically relevant strains is currently unknown. To investigate the cellular mechanism of neural tissue integrity, it is therefore critical to remove the glial cells from the tissue, while leaving the neurons intact. Here, we eliminated Müller glia cells (MG) specifically from the zebrafish retina by manipulating Notch signaling at a critical time point for gliogenesis. Retinal tissue lacking glial cells was less capable of resisting stretch and had a strong tendency to rip apart. We provide evidence that MG act like taut springs that hold the neural tissue of the retina together.

Results and discussion

Zebrafish MG are generated by 48 h postfertilization (hpf)

The zebrafish retina, like all vertebrate retinas, consists of five major neuronal and one glial type, MG (Cajal, 1972). MG are...
the only cells to span the width of the mature tissue from the inner to the outer limiting membranes (Fig. 1 A). During retinogenesis, postmitotic cells are generated from retinal progenitor cells (RPCs) in a conserved histogenetic order, in which MG are among the last cells to be generated (Rapaport et al., 2004). To identify when MG first appear in the zebrafish retina, we tested several MG markers. Of these, an antibody against cis-retinaldehyde binding protein (Cralbp) proved to be the earliest marker for MG, showing expression on the apical side of the retina as early as 48 hpf (Fig. 1 B). MG cell bodies, labeled for Cralbp, first appeared in the inner nuclear layer (INL) at ∼60 hpf (Fig. 1 C) and obtained their mature morphology by 72 hpf (Fig. 1 D).

Notch signaling is critical for the specification of MG in several species (Dorsky et al., 1995; Furukawa et al., 2000; Bernardos et al., 2005). To assess whether this might be the case in fish, we first examined the dynamics of Notch signaling in retinal cells using the transgenic reporter line Tg(TP1:Venus-PEST) (Ninov et al., 2012). This transgene consists of the Notch responsive element (TP1), which controls the expression of the destabilized fluorescent protein, Venus-PEST (Aulehla et al., 2008; Parsons et al., 2009). Thus, we could rapidly visualize the changes in Notch activity over time. Notch was active in many cells within the developing retina at 35 hpf (Fig. 1 E). However, it became progressively restricted, and by 72 hpf Notch activity was restricted to MG at 72 hpf (Fig. 1 F). The Notch-positive cells in the mature retina are MG, as shown by the overlap in the TP1:Venus and GFAP:dTomato transgenic constructs (arrows). (K) The transplantation strategy to generate clones of Tg(TP1:Venus) (green) embryos into the ath5:gapRFP host (magenta). (L–P) Panels from a time-lapse movie of a clone in a living zebrafish retina (n = 6). (L) Notch activity is high in many cells in the clone at 38 hpf. Notch activity is progressively restricted until a single cell maintains Notch activity at the apical side of the retina at 42 hpf (M). Notch activity increases in this cell at 48 hpf (N), and at 55 hpf (O) it migrates basally and will position itself within the INL and begin to mature by 62 hpf (P). Figures always show the apical side of the retina at the top and basal at the bottom. OPL, outer plexiform layer; IPL, inner plexiform layer. Bars, 10 µm.
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...was present only in MG (Fig. 1, F–J). To see this progressive restriction at the level of a single clone, we transplanted individual blastomeres from Tg(TP1:VenusPEST) into Tg(ath5:gapRFP) embryos, where most retinal neurons are labeled with membrane RFP (Zolessi et al., 2006; Fig. 1 K). At ∼38 hpf, we saw many Notch-positive clusters of cells, representing individual clones in the host retina (Fig. 1 L and Video 1). As development progressed, however, most of these cells lost Notch activity, and by 48 hpf, we often found that only one cell within a clone retained strong Notch activity on the apical side of the retina (Fig. 1, M and N, arrowheads). At ∼55 hpf, Notch-positive cells migrated basally into the INL (Fig. 1 O), and by 62 hpf obtained an MG-like morphology (Fig. 1 P).

Specific elimination of MG from the developing retina

The data above suggest that MG arise at ∼48 hpf, which is after most other cell types in the zebrafish retina are generated (Hu and Easter, 1999; He et al., 2012). Therefore, to specifically block the formation of MG, we added the γ-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl (DAPT) to the embryo media just before this stage at ∼45 hpf. This treatment resulted in reduced Notch levels (Fig. 2, A and B). In such treated fish, there was no staining for MG-specific markers (Fig. 2, C and D; and Fig. S1; Randlett et al., 2013) or transgenes (Fig. 2, E and F) in the retina until at least 120 hpf. Exposing fish to DAPT even a few hours later (at ∼50 hpf) was too late to prevent MG specification in the central retina (Fig. 2, G and G'). We also manipulated the Notch pathway with a second γ-secretase inhibitor, Compound E, and by knocking down the Notch effector Su(H), using a translation blocking morpholino (Sieger et al., 2003). Consistent with DAPT treatments, these developing retinas also lacked MG (Fig. 2, H and I).

We found it surprising that embryonic retinas lacking MG due to DAPT treatment at 45 hpf showed an apparently normal cellular architecture of all the neuronal cell types in the retina, as clearly demonstrated in the Spectrum-of-fates zebrafish line (Fig. 2, J and K; Almeida et al., 2014). Retinas of treated embryos did not display any significant changes in cell death from 48 hpf to 120 hpf (Fig. 2, L and M). This was surprising because MG have many physiological functions in the retina (Bringmann et al., 2006; Reichenbach and Bringmann, 2013). Previous work has also shown that, when MG in the adult mouse retina are ablated by cell-specific killing, there are defects in retinal vascularization, structure, and function, including the wide-scale disorganization and apoptosis of photoreceptor cells (Shen et al., 2012; Byrne et al., 2013). In contrast, we saw only very modest effects on photoreceptor structure and...
The loss of MG results in retinoschisis

Strikingly, retinas lacking MG frequently showed a splitting of the neural retina, or retinoschisis, specifically in the ganglion cell layer (GCL) between ganglion cell bodies (Fig. 3; Fig. S2, A–D; and Videos 2 and 3). This retinoschisis varied in severity within the GCL between embryos (Fig. S2, A–D). There were occasional pigment granules within the neural layers and disorganization of the photoreceptor layer, but these defects were less penetrant than the ripping phenotype and did not correlate strongly with the retinoschisis, suggesting that these may be minor independent effects of interfering with Notch signaling (Fig. S3).

To determine when these rips first appeared, we examined MG-less retinas at a variety of stages. We did not find any evidence for ripping at 33 hpf and 48 hpf (Fig. S2, E–G); however, by 96 hpf, 50% of treated retinas exhibited retinoschisis (Fig. 3, A–D and F). The ripping was not a nonspecific effect of the pharmacological inhibitor, as we saw similar retinoschisis phenotypes in Su(H) morphants (Fig. 3, E and F) and Compound E–treated retinas (not depicted). Additionally, when exposure to DAPT treatment started at 72 hpf rather than at 45 hpf, MG started to develop in the central retina, and there was no retinoschisis even 2 d later (Fig. S2 H). As the knockdown of her4, which is a downstream Notch target specifically expressed only in MG and bipolar cells (Fig. S2 I), also resulted in a reduction of MG in the retina and a concomitant retinoschisis phenotype (~10% affected; Fig. S2 J) without interfering with Notch signaling in other neurons, it is likely that the observed phenotype is a direct consequence of the loss of MG rather than Notch signaling. While we cannot exclude the possibility that bipolar cells provide some physical support to the retina, this is unlikely, as their development appears unperturbed in the DAPT treatment or her4 knockdown, and their basal processes do not extend into the GCL where the ripping occurs.

The retinoschisis phenotype observed in our study is reminiscent of degenerative retinoschisis, a human condition characterized by the splitting of the retina into two layers (Samuels and Fuchs, 1952; Straatsma and Foss, 1973). A hereditary X-linked form of retinoschisis is characterized by intraretinal splitting, which is attributed to mutations in the Retinoschisin gene (Sauer et al., 1997). MG transport the secreted Retinoschisin protein to the inner retina (Reid and Farber, 2005), but MG-specific misexpression of Retinoschisin is not sufficient to rescue a mouse model of the disease (Byrne et al., 2014). However, the involvement of MG in this disease remains a matter of debate (Molday et al., 2012). In the zebrafish retina lacking MG, Retinoschisin protein expression appears to be normal (Fig. S2, K and L), indicating that the observed phenotype is likely more similar to the degenerative form of retinoschisis and not the genetic form of the disease.

MG act like taut springs, providing resilience and stiffness to the retina

To test whether MG provide mechanical resilience to the retina, we applied controlled tensile forces (i.e., stretch) to retinas with and without MG, and measured their resulting deformations using atomic force microscopy (AFM; Fig. 4 A; Franze, 2011). At 33 hpf and 48 hpf, when radially oriented RPCs are still present in the retina, treated and control retinas showed no difference in tissue deformation (Fig. 4 B). However, at 72 hpf, we found a significantly larger increase in tissue deformation, and thus a decrease in resistance to tensile forces, in retinas without MG (Fig. 4 C). Interestingly, control retinas did not deform significantly more at 72 hpf than they do at 33 hpf, suggesting that at early stages it is the radially oriented RPCs that are providing
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resilience to the retina, while at later stage, this tensile strength rests in MG, which are the only remaining cell type to span the tissue after RPCs are gone. These results indicated that MG can act like springs (which may be put under tension) that hold the tissue together. To test if MG are normally under tension within the tissue, we used laser ablations (Fig. 4 D; Williams et al., 2010) to cut the basal processes of MG cells. After basal process ablation, the cell bodies of MG rapidly moved apically away from the inner plexiform layer (Fig. 4 D). These results are consistent with the idea that MG are normally under tension in vivo, and that when their processes are cut, this tension is released, and MG cell bodies are pulled to the side of the retina to which they are still attached.

Because MG are under tension during retinal development, they should compact the neuronal components of the retina in the apicobasal dimension. Conversely, when MG are missing, this should result in an expansion or thickening of the tissue. Indeed, in the presence of MG, the GCL was significantly thinner than in their absence (31.0 ± 2.7 µm vs. 49.9 ± 2.3 µm; P < 0.005, n = 20 retinas per condition; Fig. 4, E and G). It is important to note that there was no increase in the number of cells within the GCL in the retina (300.9 ± 7.8 with DMSO vs. 305 ± 7.96 with DAPT; P > 0.05; nuclei per section in the GCL at 4 d postfertilization (dpf; n = 10 retinas per condition), further supporting our hypothesis. However, the expansion of the tissue only occurred within the GCL, and not in the other layers.

Figure 4. **MG act as springs in the retina to hold the neural retina together.** [A] To test the biophysical properties of retinas lacking MG, we used AFM to pull on the tissue. [B] Tissue deformation was not significantly different for measurements at 33 hpf or 48 hpf (mean ± SEM [error bars], n = 10 retinas). [C] At 72 hpf, retinas deformed significantly more when no MG were present in the tissue than controls (mean ± SEM [error bars], n = 10 retinas). [D] To test if MG are under strain within the retinal tissue, we applied a laser ablation technique to specifically target the apical or basal processes of GFAP:GFP MG in vivo. Ablating the apical process resulted in its retraction of the process and displacement of the cell body basally to the IPL. Ablation of the basal process resulted in its rapid retraction and in cell displacement apically in the INL. The merge is the overlay between each time point. Red lines delineate the regions of ablation. [E and F] Confocal images showing the expanded GCL labeled in the ath5:gapRFP;vsx1:GFP transgenic fish after DAPT treatment. [G] In the absence of MG, we noted a significant increase specifically in the thickness of the GCL (n = 20 retinas, mean ± SEM [error bars], P < 0.01). [H] Stiffness measurements at the time of treatment (~45 hpf), 72 hpf, and 96 hpf of control and DAPT-treated retinas (n = 20 retinas, mean ± SEM [error bars], P < 0.01). IPL, inner plexiform layer; ONL, outer nuclear layer. Bars, 15 µm.
Importantly, in the absence of MG, it is the GCL where the retina spontaneously ripped apart. Interestingly, in culture it has been shown that providing physical support to the inner, but not to the outer, surface of the retina prevents tissue collapse and increases cell survival (Taylor et al., 2014). Thus, our data suggest that tension along MG is required for the integrity of the GCL.

Central nervous system tissue stiffens when exposed to strain (Begonia et al., 2010). Retinal tissue that is compacted by MG cells under tension should thus be stiffer, i.e., resist compressive forces more, than retinal tissue devoid of MG. To test this hypothesis, we measured the apparent elastic modulus (a measure of elastic stiffness) of the tissue by pushing on the inner retinal surface with AFM. We found, as predicted, that retinas lacking MG are significantly softer than controls (Fig. 4 H). While control retinas became stiffer with increasing age, MG-less retinas did not (Fig. 4 H). This increase in retinal stiffness with age may therefore be due to the increasing compaction of developing neural cells (Koser et al., 2015), which are squeezed together by the spring-like properties of glial cells.

In conclusion, our results suggest that MG act like taut springs that protect the neural tissue from ripping apart by holding the neural layers together (Fig. 5). This physical function is initially performed by retinal neuroepithelial cells in the nascent tissue and then taken over by the MG once they are born and the neuroepithelial cells have differentiated into neurons. In the absence of the MG in the mature tissue, there are no cells spanning the tissue present, and it becomes susceptible to shear and tensile forces, eventually leading to ripping. Thus, the presence of MG likely makes the retina more resistant to deformation. This is a critical function, as the vertebrate retina is constantly exposed to dynamic shear forces and tension (Lu et al., 2006; Franze et al., 2011), especially along its inner surface (Marmor, 1993). How MG provide tensile strength is yet unclear, but both RPCs at early stages and MG at later stages abundantly express the intermediate filament Vimentin (Shaw and Weber, 1984; Jones and Schechter, 1987). Vimentin networks stiffen under load (Janmey et al., 1991). The expression of intermediate filaments may therefore be an adaptation of glial cells to optimize their mechanical function. Our results may help explain the similar ripping of retinai in mice deficient for GFAP and Vimentin induced by indirect mechanical stress (Lundkvist et al., 2004). The intermediate filaments in MG cells may thus provide structural support to the retina, much as collagen fibers do in the skin (Fratzl, 2008). It would therefore be interesting to examine whether glial cells elsewhere in the brain also provide mechanical resilience to neural structures. Our study demonstrates that at least one type of glial cell, the MG in the retina, do indeed act like “nerve putty” and physically support neurons in vivo.

Materials and methods

Zebrafish maintenance

Zebrafish were maintained and bred at 26.5°C. All animal work was approved by Local Ethical Review Committee at the University of Cambridge, and performed according to the protocols of project license PPL 80/2198 and to the institutional guidelines (IACUC) of the University of Washington.

Embryo manipulations

Embryos used for whole-mount imaging were treated with 0.003% phenylthiourea (Sigma-Aldrich) from 10 hpf to prevent pigmentation. Embryos used for sections were fixed with 4% paraformaldehyde and cryoprotected in 30% sucrose in 1× PBS overnight before cryosectioning. Morpholino injections for Su(H) (sequence: 5′-CGCCATCTTCACC AAATCTCTCTTAAA-3′; Sieger et al., 2003) and her4.3 (sequence: 5′-TTGATCCAGTGATGTAGAAGTCAT-3′; Pasini et al., 2004) were performed using a previously described morpholino oligonucleotide at similar concentrations. The control morpholino was injected at a similar concentration (sequence: 5′-CCTCTTACCTAGTTACATTAT-3′).

For blastomere transplantsations, high- to oblong-stage embryos were dechorionated by promerase digestion (Sigma-Aldrich) and placed in agarose molds, and between 5 and 30 blastomeres were transferred between embryos using a glass capillary connected to a 2 ml syringe.

Zebrafish embryos were continuously treated with DAPT (25–50 µM) or Compound E (10 µM) in 0.5% DMSO in E3 embryo media from ∼45 hpf up to 5 dpf. Control embryos were treated with 0.5% DMSO in E3 media. Media was changed daily.

Transgenic lines and constructs

Transgenic lines Tg(TP1:glob:VenusPEST)S940 (Ninov et al., 2012), Tg(Zeb1a:mCherry,Cre-ERT2) (Krohne et al., 2011), and Tg(her4.3:EGFP) (Yeo et al., 2007) were used to report on Notch signaling in the retina. Tg(gfap:GFPlacZ) (Bernardos and Raymond, 2006) and Tg(GFAP:dTomato) (a gift from S. Higashijima, National Institutes of Natural Sciences, Okazaki, Japan; Satou et al., 2012) were used to label glial cells in the retina. Tg(atoh7:gap43-mRFP1)z1 (Zolesi et al., 2006), Tg(vsx1:GFPlacZ) (Kimura et al., 2008), and Tg(crc:CFP; ptfla-Gal4:UAS-YFP; atoh7:gap43-mRFP1)z1 (Almeida et al., 2014) were used to label neuronal retinal cells. Tg(betaActin:H2B-GFP) (He et al., 2012) was used to label all nuclei in the retina. These transgenes are abbreviated hereafter as TPI:Venus, GFAP:dTomato, ath5:gapRFP, vsx1:GFPlacZ, GFAP:GFPlacZ, SoFa, H2B:GFP, Her4:Cherry, and her4:GFPlacZ.

Confocal imaging and immunostaining

Confocal imaging of live embryos was performed at ∼28°C and fixed embryos at ∼22°C, as described previously (Das et al., 2003). Laser scanning confocal imaging was performed using a microscope (FV1000; Olympus) with a 60× silicon oil immersion objective lens (1.3 NA) using the FV10-ASW software (Version 4.2; Olympus), or
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cryosections with an SP2 microscope (Leica) with a 63× (1.2 NA) water immersion objective lens using the LCS Leica Confocal Software (Leica). Image analysis was performed using ImageJ or Volocity Software (PerkinElmer).

Immunostaining was performed using Alexa Fluor-conjugated secondary antibodies (Invitrogen) and the following primary antibodies: mouse anti-glutamine synthase (GS; 1:50, mab302; Millipore), rabbit polyclonal anti-Crabp (antigen bovine CRALBP [UW55]; 1:100; a gift from J. Saari, University of Washington, Seattle, WA), mouse anti-α-FGFAP (1:100 zrf1; ZIRC), rabbit anti-carbonic anhydrase (1:200, ab108367; Abcam), rabbit anti-Harmonin (1:200, 2090-00-02; Sdx), rabbit anti-BLBP (1:200, ab32423; Abcam), rabbit anti-Sox2 (1:150, ab5603; Millipore), mouse anti-Vimentin (1:100, 40E-C; Developmental Studies Hybridoma Bank), rabbit anti-BLB (1:100, ab32423; Abcam), mouse anti-Rs1 (1:200, ab167579; Abcam), mouse anti-zpr1(1:100; ZIRC), and rabbit anti-rodopsin (1:100, ab3424; Abcam). Cryosections were taken at 12 µm thickness using a Jung Frigocut cryostat (Leica).

**TUNEL staining and nuclei counting**

Slides were stained with an in situ cell death detection kit, TMR Red from Roche for 1.5 h at 37°C. The slides were washed in 1x PBS for 5 min and mounted with Vectashield Hard Set medium with DAPI (#H-1500). Only sections containing the lens, corresponding to the central retina, were imaged and quantified. To count the number of cells in the GCL, DAPI+ nuclei were counted per section (12 µm thick) in DMSO control and DAPT experimental conditions at 4 dpf.

**Transmission electron microscopy**

Zebrafish embryos and larvae were fixed at 6 d after fertilization with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for several hours. After washing in buffer, they were postfixed in 1% OsO4 in cacodylate buffer and stained en bloc with 1% uranyl acetate. After immersion in a graded ethanol series, the animals were embedded in Araldite, sectioned, and stained with 1% lead citrate before imaging.

**AFM**

Embryos (33–96 hpf) were anesthetized with MS-222 (tricaine, 0.02%), the lens was removed, and the retina was microdissected in 1x Danieau’s solution (58 mM NaCl, 0.7 mM KCl, 0.4 mM MgSO4, 0.6 mM Ca(NO3)2, and 5 mM Hepes, pH 7.6), washed in 1x Danieau’s solution for 5 min, and mounted with the nerve fiber layer facing up on glass slides with adhesive Cell-Tak (BD). Polystyrene beads (diameter solution for 5 min, and mounted with the nerve fiber layer facing up on glass slides with adhesive Cell-Tak (BD). Polystyrene beads (diameter = 37 µm) were glued to tipless silicon cantilevers (ARROW-TL1-50, spring constant ~0.01 N/m; Nano World), coated with Cell-Tak and mounted on the AFM (CellHesion 200; JPK Instruments AG). Cantilevers were calibrated with the thermal resonance method, which is built into the AFM software. For tensile measurements, cantilevers were brought in contact with the retinae (with F = 2nN until the bead firmly adhered to the tissue (~30 s). Subsequently, the cantilever was retracted with F = −5nN, thus pulling on the retina for 5 s, and tissue deformation was measured. Retraction curves were smooth, indicating that there were no sudden rips (i.e., tearing) of the tissue during the experiment. Using the described AFM parameters, control and DAPT-treated retinae had triplicate measurements taken per sample (n = 20). Measurements of control and treated retinae were alternated to avoid bias.

Stiffness measurements were taken using the same AFM parameters and tissue preparations with the exception that beaded cantilevers (not treated with CellTak) were used to indent the samples and determine their resistance to the applied force (i.e., their apparent elastic modulus $K = E/(1 - \nu^2)$, where $E$ is the Young’s modulus and $\nu$ is the Poisson’s ratio). Control and treated retinae were prepared one at a time and measured alternatively to avoid bias. For each retina, 20 measure-

**Data analysis and statistics**

As all data were normally distributed (as determined by the Kolmogorov-Smirnov test), differences were tested using the two-tailed Student’s $t$ test, and an ANOVA test in the case of three or more groups. Newman-Keuls and Dunn’s tests were used as multiple-comparison post hoc tests. All values given in the text are mean values ± SEM.

**Online supplemental material**

Fig. S1 shows that additional MG markers are lost in DAPT-treated retinae. Fig. S2 shows that the retina before MG are generated and the severity of the retinoschisis phenotype without MG. Fig. S3 shows additional cellular defects in the retina lacking MG. Video 1 is a time lapse of Notch activity within clones in the embryonic zebrafish retina. Videos 2 and 3 are confocal stacks through the retina in control and DAPT-treated retina at 96 hpf. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201503115/DC1. Additional data are available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201503115.dv.

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