Müller Glia regenerative potential is maintained throughout life despite neurodegeneration and gliosis in the ageing zebrafish retina

AUTHORS

Raquel R. Martins[1,2], Mazen Zamzam[3], Mariya Moosajee[4,5,6,7], Ryan Thummel[3], Catarina M. Henriques[1,2§], Ryan B. MacDonald[4§]

Affiliations:

1. Bateson Centre, University of Sheffield, Sheffield, S10 2TN, UK.
2. Department of Oncology and Metabolism, University of Sheffield, Sheffield, S10 2TN UK.
3. Department of Ophthalmology, Visual and Anatomical Sciences, Wayne State University School of Medicine, Detroit, MI 48201, USA.
4. Institute of Ophthalmology, University College London, London, EC1V 9EL, UK.
5. Moorfields Eye Hospital NHS Foundation Trust, London, EC1V 2PD, UK
6. Great Ormond Street Hospital for Children NHS Foundation Trust, London, WC1N 3JH, UK
7. The Francis Crick Institute, London, NW1 1AT, London

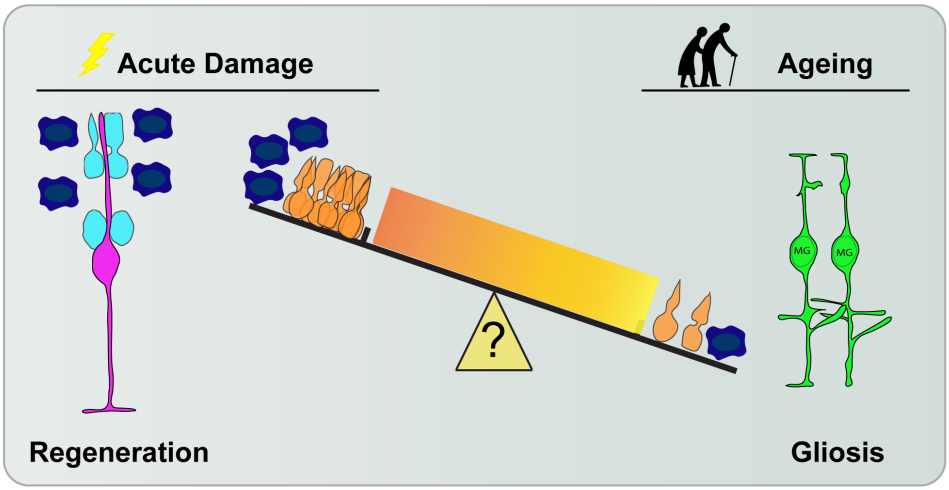
§Co-corresponding authors: c.m.henriques@sheffield.ac.uk and ryan.macdonald@ucl.ac.uk

ABSTRACT

Ageing is a significant risk factor for degeneration of the retina. Harnessing the regenerative potential of Müller glia cells (MG) in the retina offers great promise for the treatment of blinding conditions. Yet, the impact of ageing on MG regenerative capacity has not yet been considered. Here we show that the zebrafish retina undergoes telomerase-independent age-related neurodegeneration. Yet, this progressive neuronal loss in the ageing retina is insufficient to stimulate the MG regenerative response. Instead, age-related neurodegeneration leads to MG gliosis and loss of vision, similarly to humans. Nevertheless, gliotic MG cells retain Yap expression and the ability to regenerate neurons after acute light damage. Therefore, we identify key differences in the MG response to acute versus chronic damage in the zebrafish retina and show that aged gliotic MG can be stimulated to repair damaged neurons in old age.

KEY WORDS: Retina, Müller glia, Ageing, Proliferation, Degeneration, Gliosis, Zebrafish, Telomerase, Regeneration

SUMMARY



Our data suggest there are key differences between mechanisms driving regeneration in response to acute damage versus age-related chronic damage. It may be that either the number of cells dying in natural ageing is not enough to stimulate MG to proliferate, or the low number of microglia and respective signals released are not sufficient to trigger MG proliferation. Importantly, we show that gliotic MG cells can be stimulated to repair damaged neurons in old zebrafish retina.

INTRODUCTION

The physiology and structure of the healthy human eye is well known to degrade with age. As the population is ageing worldwide, the identification and potential treatment of the underlying cellular and molecular dysfunctions in the aged retina will be critical for global health going forward [1]. Hallmarks of the ageing retina include tissue thinning, neuronal loss and reduced visual function, especially in the macula [2-7]. Further, ageing is a significant risk factor for degeneration and disease in the retina, such as age-related macular degeneration or primary open angle glaucoma[6-8]. Retinal degeneration is often a process that plays out over months or years, whereby neurons gradually die leading to dysfunction [5,6]. Regenerative-based therapies, such as stimulating endogenous Müller Glia (MG) to regenerate, offer great promise for the treatment of various types of blindness resulting from the loss of retinal neurons. Thus, determining the effects of ageing on the regenerative potential of the retina is an important consideration for the efficacy of such treatments going forward.

The principal cells tasked with maintaining the retina throughout life are the MG cells. MG provide retinal neurons with a myriad of support functions, including trophic support, neurotransmitter recycling and energy metabolism [9]. While these functions are critical for healthy retinal function, MG also have a prominent role during disease and after neuronal insult. In the mammalian retina, neuronal damage results in a MG gliotic response, involving the up-regulation of stress proteins, proliferation and morphology changes. This response is thought to be neuroprotective initially, but may ultimately culminate in dysfunction and death via loss of metabolic support or tissue integrity [10]. However, in “lower vertebrates”, such as fish and amphibians, the MG cells have the capacity to regenerate the retina after neuronal damage [11]. Intensive efforts have been made to uncover the molecular mechanisms regulating these endogenous regenerative responses in the retinas of lower vertebrates [12-17]. In the zebrafish retina, after acute damage, MG undergo a brief reactive gliosis-like phase that transitions into a regenerative response: a de-differentiation and proliferation of the MG progenitor cell to specifically generate new neurons and restore vision [18-20]. While the mammalian retina lacks significant regenerative capacity [21,22], re-introduction of key molecular signals into MG has shown great promise to stimulate the genesis of new neurons in mice after damage [23-25]. These molecular mechanisms are largely studied in the young adult zebrafish using acute injury models, such as those generated by intense light or toxins [14,26]. In contrast to acute injury, damage to the retina caused by degenerative disease or in ageing manifest themselves over much longer timescales and result in the gradual accumulation of DNA damage and cell death[7]. As such, chronic degeneration may significantly differ from injury models, especially in its capacity to induce a regenerative response from glia. While multiple acute injuries do not appear to reduce the overall regenerative response of MG[26], the

87 effects of natural chronic age-related damage on the MG regenerative potential has not been
88 determined.

89 The zebrafish is an emerging ageing model, as it displays key human-like hallmarks of ageing
90 in most tissues, including the retina [27,28]. These include an age-related decrease in cell density
91 and increased cell death, suggesting chronic degeneration of the retina with increasing age
92 [27]. Depending on the tissue, hallmarks of ageing in the zebrafish include telomere shortening,
93 DNA damage, decreased proliferation and apoptosis or senescence [29-31]. Accordingly, the
94 telomerase mutant (*tert*$^{-/-}$) zebrafish model is now an established accelerated model of ageing
95 [29-31], where regeneration is known to be impaired in tissues such as the heart [32]. The *tert*$^{-/-}$
96 model therefore offers the possibility of studying key aspects of ageing in a shorter time period,
97 and allows for the identification of telomerase-dependent mechanisms of ageing, potentially
98 involved in retinal regeneration [29-31].

99 Here, we used the zebrafish retina as a model study the impact of ageing on the maintenance
100 of neuronal structure and regenerative capacity of MG cells. We show that naturally aged and
101 telomerase deficient retinas display known hallmarks of retinal ageing, including tissue
102 thinning, accumulation of DNA damage and neuronal death. However, this accumulation of
103 chronic damage does not stimulate MG to proliferate or replace lost neurons. Instead, and in
104 contrast to acute injury, ageing leads to a gliotic response in MG and loss of vision,
105 recapitulating hallmarks of human retinal degeneration with advancing age. Yet, gliotic MG in
106 the old retinas retain the expression of Hippo signalling, and thereby their potential for
107 regeneration. After acute light damage, gliotic MG cells maintain their regenerative potential
108 to old ages, and are therefore are capable of regenerating neurons throughout life. We
109 therefore identify key differences in the MG regenerative response to acute versus chronic
110 damage, a key consideration in potential therapies aiming to stimulate endogenous
111 regenerative mechanisms to treat human retinal disease.

112

113 **RESULTS**

114 **The aged zebrafish retina displays neurodegeneration independently of telomerase**

115 Similar to humans, the zebrafish retina consists of three nuclear layers separated by two
116 synaptic plexiform layers. The nuclear layers consist in the outer nuclear layer (ONL),
117 containing photoreceptors; the inner nuclear layer (INL) containing bipolar cells (BCs),
118 amacrine cells (ACs), horizontal cells (HCs) and MG; and the ganglion cell layer (GCL) mainly
119 containing retinal ganglion cells (RGCs) (see diagram in **Figure 1A**). The zebrafish retina is
120 considered specified and functional by 73 hours post-fertilisation (hpf) [33]. The retina continually
121 grows at the periphery due to proliferation of the ciliary marginal zone (CMZ) [17], a population

122 of progenitors thought to contribute to retinal growth throughout life. Thus, the central region
123 of the adult retina can be considered the "oldest", as it is largely generated during early
124 developmental stages, and where there is very little proliferation to generate new neurons [28].
125 In contrast, the peripheral region of the retina is continually expanding at the margins due with
126 the newest born cells being found adjacent to the CMZ.

127 It has recently been shown that the ageing zebrafish retina shows some degeneration with
128 advancing age [27,28]. Telomerase is known to be important for proliferation [30,31] and
129 regeneration in the zebrafish [32].[32]; however, its specific role in the ageing retina remains
130 unclear. Here, we characterised key hallmarks of ageing, (i.e DNA damage response and
131 apoptosis) in the central and the peripheral retina (**Fig 1; Supplementary Fig 1**) throughout
132 the zebrafish lifespan in naturally aged wildtype (WT) and telomerase mutants (*tert*$^{-/-}$). The
133 naturally aged WT central retina displays accumulation of DNA damage, as evidenced by the
134 presence of strong γH2AX nuclear foci, a molecular marker of the DNA damage response
135 (**Fig 1B, B'**). This is accompanied by a significant increase in cell death, as assessed by the
136 early cell death labelling terminal deoxynucleotidyl transferase dUTP nick end labelling
137 (TUNEL) (**Fig 1C, C'**). Increased DNA damage and cell death with ageing occur in a
138 telomerase-independent manner, as no differences are identified between genotypes at any
139 time-point analysed. Further, retinal thinning is a likely consequence of increased cell death
140 and is a known hallmark of human retinal ageing [34]. Accordingly, we show that zebrafish
141 central retina progressively thins with ageing, consistent with previous studies [27,28]. Moreover,
142 as for increased DNA damage and cell death, we show that this occurs largely independently
143 of telomerase (**Fig 1D**).

144 We next sought to determine whether this cell death and retinal thinning is caused by the
145 specific loss of any particular neuronal type. We used immunohistochemistry with several
146 molecular markers specific for different neuronal populations throughout the lifespan of
147 zebrafish (**Table 1**), in the presence and absence of telomerase (*tert*$^{-/-}$*).* As expected, there is
148 an overall reduction in neuron numbers with ageing in the zebrafish retina (**Fig 2A-C**).
149 Importantly, this neuronal loss is not due to a decreased number of RGCs in the GCL (**Fig**
150 **2D**), but due to a decreased number of HuC/D-expressing ACs (**Fig 2E**) and PKC-expressing
151 BCs in the INL layer (**Fig 2F**). Additionally, in the aged zebrafish retina, BCs display
152 disorganised axon terminals in the IPL (**Fig 2A, B**). None of these neurodegenerative
153 phenotypes are affected by depletion of telomerase, as there are no differences between WT
154 and *tert*$^{-/-}$ at any of the tested ages. The RGCs, ACs and BCs are neurons that come together
155 to make connections in the major synaptic neuropil, called the inner plexiform layer (IPL; **Fig**
156 **1A**). Thus, degeneration observed in these neuronal populations is expected to affect
157 connectivity in the IPL. Accordingly, we confirmed thinning of the synaptic IPL layer due to a

158 reduction and disorganisation of Ribeye A positive pre-synaptic terminals with ageing (**Fig**
159 **2G**). The loss of photoreceptor integrity is one of the key features of human retina ageing and
160 disease [35] and we show that rod photoreceptor outer segments in the zebrafish retina undergo
161 dramatic structural changes with ageing (**Fig 2H**), confirming previous results [27]. These
162 structural changes are accompanied by disruption of the tight junction protein zonula
163 occludens (ZO-1) (**Fig 2I**), a marker for the outer limiting membrane thought to be involved in
164 photoreceptor degeneration [28]. These defects are also not accelerated in the *tert*$^{-/-}$ at any age
165 tested, suggesting this is likely a telomerase-independent phenomenon.

166

167 **MG do not proliferate in response to chronic retinal neurodegeneration with ageing**

168 It is well established that MG in the zebrafish retina proliferate and regenerate lost neurons
169 after acute injury [11-13]. Although the progressive age-related retinal neurodegeneration would
170 suggest a lack of regenerative response by MG, it remains unclear whether these
171 degenerations are being counteracted, at least to some degree, by proliferation in the aged
172 retina. To test this, we used an EDU pulse-chase strategy to identify any cell divisions in the
173 retina throughout lifespan, which would be indicative of potential regeneration by MG. To
174 characterise the steady state regenerative capacity of the central and peripheral CMZ until old
175 age, we carried out a 3-day pulse of EdU followed by 0- and 30-days chase, in young and old
176 WT and *tert*$^{-/-}$ zebrafish (**Fig 3A, B**). We observe few EdU-positive cells and, instead of a
177 compensatory proliferation response, we detect even less EdU-positive cells with ageing,
178 suggesting overall reduced proliferative capacity in the central retina (**Fig 3C**). These levels
179 are further reduced after a 30-days chase (**Fig 3D**), suggesting that there are very few cells
180 proliferating in the aged central retina. In the peripheral retina, where the proliferative CMZ
181 resides, we see double the EdU-retaining cells in comparison to the central retina at 0-days
182 chase (**Fig 3C**). Nevertheless, as in the central retina, proliferation in the peripheral retina (**Fig**
183 **3 C and D**) decrease with ageing, suggesting that there is no compensatory proliferation from
184 the CMZ in response to the increased cell death with ageing. This also suggests that the CMZ
185 has a finite proliferative capacity and the retina of the zebrafish does not continue to grow at
186 the same rate throughout life. Finally, removing telomerase has no further effect on the already
187 low and decreasing levels of proliferation with ageing in the retina. These results suggest that
188 there is no compensatory proliferation in response to neurodegeneration in the aged zebrafish
189 retina.

190 As proliferation of MG is the primary source of neurons after acute injury in fish [12,36], we sought
191 to determine whether the EDU+ cells was indeed compensatory proliferation specifically within
192 these cells. We co-labelled cells with the MG specific marker glutamine synthetase (GS), and

193 counted the GS-positive; EdU-positive cells. We detect very few GS-positive; EdU-positive
194 cells in the central retina in both WT and *tert*$^{-/-}$, at any of the time-points characterised
195 throughout their lifespan (**Fig 3E, E'**), suggesting MG are not proliferating in the central retina
196 at old ages. We also do not observe PCNA expression in MG or signs of MG de-differentiation
197 to progenitors[19] in old age evidenced by a lack of Pax6 expression (**Supplementary Fig 2**).
198 Thus, contrary to what has been reported to occur in response to acute damage[11-13], our data
199 show that age-related chronic cell death does not trigger proliferation of MG cells. Finally, rods
200 can originate from rod-specific progenitors [37,38], which are found in the ONL and are derived
201 from MG that slowly divide in the WT retina [15,36,39,40]. To test whether there is any
202 compensatory proliferation with ageing occurring from rod-specific progenitors found
203 scattered throughout the ONL of the retina [37,41], we quantified levels of EdU positive cells in
204 the ONL. Although we observe EDU+ cells in the central ONL of 5 months WT (likely to be
205 rod precursors dividing), there are few detected in >30 months old WT. Once again, *tert*$^{-/-}$
206 mutants have no further effect (**Fig 3F**). Together, our data show that there is no compensatory
207 proliferation in response to age-related degeneration in the zebrafish retina, by any of the
208 known sources of regeneration and neurogenesis: CMZ, rod precursor cells or MG.

209

210 **Zebrafish vision declines with ageing, independently of telomerase**

211 Loss of visual acuity and contrast sensitivity with advancing age in humans has been well
212 documented [6,7]. We report the presence of retinal neuron loss and tissue thinning in the aged
213 retina, largely independently of telomerase. To determine whether the molecular and structural
214 changes we observe have a pathological consequence on the retinal function, we tested
215 whether zebrafish have impaired vision with ageing. Visual testing in the zebrafish has been
216 used to screen for mutants with defects in retinal development and function [42-45], but are yet
217 to be tested in the context of ageing. The optokinetic response (OKR) is a well-established
218 assay to measure innate visual responses and provides readout of visual acuity [42,46] (**Fig 4A**).
219 Our results show a decreased number of eye rotations per minute in aged fish (**Fig 4B, B'**
220 **and videos 1 and 2**), suggesting that zebrafish vision declines with ageing. As for most of the
221 hallmark phenotypes of ageing described so far, (**Supplementary Table 1**), telomerase is not
222 a limiting factor for visual acuity, since the *tert*$^{-/-}$ zebrafish do not display an accelerated
223 reduced visual acuity at 5 months or 12 months of age, close to the end point of *tert*$^{-/-}$ life (**Fig**
224 **4B, B' and videos 3 and 4**). As such, it does not appear that telomerase anticipates any of
225 the hallmarks of zebrafish retina ageing tested here, unlike in other tissues[29].

226 **The zebrafish retina shows signs of gliosis with ageing**

227 As we do not observe a MG regenerative response to neurodegeneration and vision loss, we
228 wondered whether the retina was undergoing a gliotic response to damage. In the mammalian
229 retina gliosis is a hallmark of retinal damage [47], but also commonly observed in the aged retina
230 [48]. MG respond to damage or injury in most, if not all, retinal degenerative diseases [10]. The
231 characteristic mammalian response is gliosis, whereby MG change shape and up-regulate
232 structural proteins like glial fibrillary acidic protein (GFAP) to promote a neuroprotective effect
233 [9,10]. In the zebrafish retina, the initial regenerative response to acute damage is similar to
234 mammalian gliosis [18,49], and gliosis can be aggravated if MG proliferation is blocked [19]. Here,
235 we identified MG using the well-described GS immunolabelling and characterised MG
236 reactivity using the gliosis markers GFAP, Vimentin and Cralbp [50,51]. We do not observe an
237 overall loss of MG cell number in the central with ageing (**Fig 5 A, A'**), in contrast to the
238 observed neuronal death observed in this region at the same stages (see **Fig 2**). Despite this,
239 there is a significant change in the morphology of the MG cells in the WT aged retina.
240 Aberrations in the aged MG cells include disruptions in the radial morphology along the
241 synaptic IPL and basal lamina (**Fig 5B-D**), which are known hallmarks of gliosis in retina
242 degeneration [48]. Qualitative assessment further shows that while all young fish display long
243 and aligned MG basal processes, 100% of the old fish show morphological disorganisation, a
244 hallmark of gliosis. We also observed MG shape changes and changes in Vimentin and Cralbp
245 expression at levels old ages in MG (Figure 5E-H). Thus, similarly to humans, chronic
246 neurodegeneration with ageing elicits a MG gliotic response in the zebrafish retina rather than
247 regeneration.

248 Microglia, the innate immune cells found in the retina (reviewed in [52]), are also key players in
249 maintaining tissue homeostasis throughout life and part of the gliosis process. They are
250 activated in many neurodegenerative diseases, with increased number at sites of damage,
251 including in the photoreceptor layer in many forms of retinal degeneration (reviewed in [48]). As
252 we do not observe regeneration in the aged zebrafish retina in response to neuronal death,
253 we asked whether there are alterations in the number of microglia (4C4-positive cells) found
254 in the tissue. In contrast to what is observed in acute damage paradigms [13,25], we observe few
255 microglia in the central retina, with no increase with ageing. Furthermore, microglia did not
256 appear to be localised to regions of neuronal death, such as in photoreceptors or the INL
257 (**Supplementary Fig 3)**.

258

259

260

261 **Yap expression is maintained in MG throughout life**

262 As we observe hallmarks of gliosis in MG cells and a lack of MG proliferation in response to
263 age-associated chronic damage, we next asked whether gliotic MG have altered molecular
264 profiles making them unable to proliferate in response to damage in the aged retina. Tgfβ
265 signaling has been shown to differ between zebrafish regeneration and mouse gliosis
266 response after acute damage, with Tgfβ3 being upregulated in regeneration and Tgfβ1 and 2
267 in gliosis[53]. However, we do not observe any difference in expression of any of the Tgfβ
268 isoforms in MG cells in the chronically aged zebrafish retina (**Supplementary Fig 4**).
269 Hippo/Yap signaling has been identified as a signaling pathway that is altered after neuron
270 degeneration and injury [54-56] within MG cells, and is required for MG regeneration[55]. Yap
271 expression is also affected in reactive MG cells after damage[56]. We used antibodies against
272 Yap, a downstream effector of Hippo signaling, to determine expression in MG throughout life.
273 Yap is expressed in MG from 5dpf all the way through to end of life, including in gliotic MG
274 cells (**Fig 6**). Yap is repressed from the nucleus after damage, suggesting that Yap activity
275 may be required for the maintenance of MG proliferative capacity[56]. Consistent with this we
276 do not observe any Yap positive nuclei at any stage suggesting that MG cells may retain the
277 molecular composition to regenerate in response to retinal damage throughout lifespan.

278

279 **Müller glia retain the ability to regenerate after acute damage until old age**

280 Studies on retinal regeneration typically focus on the young adult retina and have not taken
281 old age into account for the regenerative response. Several acute damage paradigms induce
282 retinal neuron death and elicit a regenerative response in zebrafish [36,49,57,58]. Chronic damage
283 in the adult retina, specifically between 9-18 months of age, results in MG continuously re-
284 entering the cell cycle to proliferate and regenerate lost neurons, although they do show signs
285 of chronic activation at later timepoints [49]. However, it remains unclear whether MG retain their
286 ability to proliferate and regenerate the retina throughout life, and whether gliotic MG, such as
287 those often occurring in retinal disease or old age, also retain proliferative capacity to replace
288 damaged neurons. As MG maintain Yap expression into old ages, we hypothesised that MG
289 maintain the ability to regenerate in response to acute damage in the aged retina, despite not
290 doing so after chronic degeneration. To test this, we used the light-damage model where aged
291 *albino* zebrafish, at three stages of their lifecycle, were treated with light to elicit photoreceptor
292 damage. To detect increased proliferation of MG in response to damage, a 3-day pulse of
293 BrdU was performed, followed by a 28-days chase period (**Fig 7A**). After a 3-day pulse, BrdU
294 should label both MG and their daughter cells, the newly formed progenitors (**Fig 7B**).
295 However, since the BrdU staining dilutes in the rapidly dividing progenitor cells and MG only
296 divide once, after a 28-days chase, the majority of the BrdU staining is retained in MG (**Fig
297 7B**). Our results show that light-lesion in aged retinas leads to a loss of photoreceptors, which

298 is accompanied by a strong increase in microglia in the ONL, typical of photoreceptor
299 degeneration in this damage model (**Fig 7C, C'**). Moreover, there are no differences in the
300 incorporation of BrdU with ageing on any layer, at 72 hours post-light damage (hpL), when the
301 initial regenerative response is mounted, or at 28 dpL, when the number of MG that initially
302 re-entered the cycle can be identified **(Fig 7D, D')**. Both the unaltered immediate timing of MG
303 response and the overall capacity to regenerate each neuronal layer are maintained with
304 increased age. Finally, MG in the aged *albino* background also show a characteristic gliosis
305 phenotype similar to WT (**Supplementary Fig 5**). Together these results suggest that the
306 regenerative response remains intact throughout zebrafish lifespan and the molecular and
307 morphological alterations in gliotic MG does not impact regenerative capacity in old age.

308

309 **DISCUSSION**

310 Using a combination of cell labelling strategies throughout adulthood into old age, we show
311 that the zebrafish retina retains its potential to regenerate in response to acute damage into
312 old age. Therefore, the lack of compensatory proliferation in response to chronic, age-
313 associated cell death in the ageing zebrafish retina is not due to a loss of capacity for MG to
314 proliferate *per se*, but likely due to the absence or insufficient levels of the required stimuli for
315 MG engagement. Indeed, contrasting to acute damage, we show that age-related retinal
316 damage is insufficient to trigger compensatory proliferation by any of the known sources of
317 regeneration and neurogenesis, namely the CMZ, rod precursor cells and MG. Moreover, we
318 show that MG's inability to heal the aged retina is not due to telomerase-dependent
319 proliferative limits. Overall, instead of regeneration, zebrafish retina ageing leads to a gliotic
320 response and loss of vision, reminiscent of human retinal ageing.

321

322 **Telomerase-dependent and -independent hallmarks of zebrafish retinal ageing**

323 Previous work suggested that telomerase and telomere length are important for human retinal
324 health. In particular, the retinal pigment epithelium (RPE) has been reported to have shorter
325 telomeres than the neural retina, and it accumulates senescence over-time [59]. Accordingly,
326 reactivation of telomerase has been described to ameliorate symptoms of age-associated
327 macular disease [60,61] and telomerase activators are currently in clinical trials (e.g.
328 NCT02530255). However, retina homeostasis requires more than RPE maintenance. It
329 requires a steady-state level of proliferation of different cell types involved in multiple aspects
330 of retina function. As in other proliferative tissues [29-31], it would be expected that telomerase
331 levels would influence zebrafish retina homeostasis. We therefore hypothesised that the
332 zebrafish retina would degenerate in a telomerase-dependent manner, leading to vision loss.

333 However, our data suggest that most age-related changes in the zebrafish retina, including
334 vision loss, are largely telomerase independent, since depletion of telomerase (*tert*$^{-/-}$) does not
335 accelerate or exacerbate any of these phenotypes. Telomere dysfunction is known to affect
336 mostly highly proliferative tissues, reviewed in [62] and what our data show is that, in the region
337 most affected by ageing phenotypes, the central retina, there is very little proliferation to start
338 with. This suggests that replicative exhaustion of telomeres is not a limiting factor in the age-
339 associated degeneration of the central retina. Instead, it is likely that chronic exposure to
340 damaging agents such as oxidising UV radiation is the key driver of degeneration in the retina,
341 as was proposed in the "Wear and Tear Theory" (reviewed in [6]). However, we cannot exclude
342 that telomere-associated damage may still be a contributing factor to the observed increased
343 levels of DNA damage and cell death in the central retina. Telomeres are known to be
344 damaged by oxidative stress and can act as sinks of DNA damage, irrespectively of length
345 and levels of telomerase [63,64]. In fact, this is a likely contributor to RPE damage with ageing
346 [65,66], potentially explaining why depleting telomerase has no effect on the proliferation of the
347 ONL, where photoreceptors reside, nor does it accelerate vision loss.

348

349 **Chronic vs. acute damage in MG responses to natural ageing**

350 Our results show that zebrafish develop vision loss with ageing and that this is underpinned
351 by retinal neurodegeneration and gliosis. This could seem counter-intuitive, given that it is well
352 established that the zebrafish retina is capable of regenerating after acute damage. Therefore,
353 the lack of regeneration after age-related cell death observed in this study suggest that there
354 are critical differences between chronic ageing and repair after an acute injury. Further
355 supporting this, we show that this lack of regeneration in the context of ageing is not due to
356 an intrinsic inability of MG to proliferate *per se*, as we show they are still capable of doing so
357 in response to an acute injury in old animals. Several tissues in the zebrafish retain the ability
358 to regenerate throughout the lifespan of the zebrafish, including the fin and the heart [67,68].
359 Likewise, the optic nerve crush paradigm has shown that there is successful recovery and
360 function in the zebrafish retinotectal system [69], suggesting that regeneration is still possible at
361 advanced age in the retina. The regenerative capacity of vertebrate tissues tends to decrease
362 after repeated injury and as animals advance in age. This is likely due to reduced progenitor
363 cell proliferation potential and subsequent differentiation [70-73]. In the context of the central
364 nervous system, this is aggravated by gliosis, a reactive change in glial cells in response to
365 damage. In the mammalian retina, MG cells undergo gliosis in response to damage and in
366 many retina degenerative diseases [10]. However, in the zebrafish retina MG cells undergo the
367 initial reactive gliotic response after acute damage but quickly shift to the regenerative
368 pathway. Here we show that while we observe morphology alterations in MG cells in response

369 to age-related neurodegeneration, MG in the aged retina retain their ability to regenerate in
370 response to acute damage of photoreceptors, providing evidence that regenerative
371 mechanisms in tissues that have been damaged or degeneration remains possible.

372

373 It is unclear if alternative damage paradigms will also lead to similar regenerative response
374 and potential in the aged zebrafish retina. It also remains unclear what is the consequence of
375 the gliotic response observed on retinal neurons in the aged retina, as gliosis is classically
376 thought of as "Janus-faced", with both pro- and anti- neuroprotective functions [10]. Importantly,
377 the kinetics of these MG morphology and molecular changes relative to neurodegeneration
378 may provide further clues to the precise cellular breakdown in the retina causing widespread
379 neuronal death and dysfunction. For instance, do the MG cells react first, thereby abandoning
380 their neuronal support functions and precipitating neuronal damage, or do they purely respond
381 to neuronal degeneration. Nevertheless, despite neuronal loss with ageing, MG cells maintain
382 their numbers throughout ageing, suggesting that MG may be protected from age related
383 death caused by the accumulation of DNA damage or free radicals. It remains unclear if there
384 is a molecular mechanism inferring this protection or if MG gliosis is in itself protective to cell
385 death. However, despite gliosis we show MG retain the ability to regenerate lost neurons after
386 acute damage, which is critical for the potential to use regeneration as a therapy for damage
387 in human retina in ageing or degenerative disease.

388

389 **Our proposed model: A molecular "tipping point" required to stimulate regeneration in**
390 **ageing**

391 Regeneration studies so far have relied on several damage paradigms, including phototoxic
392 [49] and ouabain induced lesions [74], which induce rapid cell death post-insult. While acute
393 damage models are suitable to explore the cellular and molecular mechanisms underpinning
394 the regenerative potential of the retina, they do not allow testing whether this regenerative
395 response is also occurring with natural ageing in the retina. "Natural ageing" and associated
396 stress-induced neuronal death play out over months or even years and we now show that they
397 do not elicit the same regenerative response. This may be because the slow degeneration is
398 not producing a strong enough signal in a short amount of time to induce MG to undergo the
399 cellular and molecular process of regeneration (**Fig 8**). It has been shown that the level of cell
400 death can induce a differential response of MG cells. Whilst a large amount of rod death
401 causes a regenerative response, small amounts do not [26,75]. Furthermore, there may be
402 distinct differences in signals released after apoptosis or necrosis in ageing vs damage
403 models[76,77]. In support of this concept, recent work suggests that there may be key signalling

404 differences underpinning the difference between a “regenerative” or a “reparative” response
405 to injury [53]. Thus, we propose that a molecular signal, or expression changes of such signal,
406 will regulate the “tipping point” required to elicit a MG regenerative response in ageing.
407 Research in the context of acute damage paradigms in the zebrafish retina has uncovered
408 many of the molecular mechanisms regulating this regenerative response (reviewed in [19, 37]).
409 For instance, proliferation appears to be a key mechanism for the initiation of the regenerative
410 response as blocking proliferation after damage in the zebrafish retina results in MG gliosis,
411 and not regeneration, similar to humans [16]. Moreover, age-associated degeneration of retinal
412 neurons and their synapses, as we observed to occur in this study, may result in a loss of
413 neurotransmitter release, such as GABA, which has been shown to facilitate the initiation of
414 MG proliferation [78]. Recently, it has been suggested that electrical stimulation may promote
415 MG proliferation and expression of progenitor markers [81]. Thus, dysregulation of
416 neurotransmitters upon neurodegeneration could inhibit the key molecular pathways
417 regulating regeneration. Alternatively, the initial inflammatory response has also been shown
418 to be determinant for the repair process. Upon light-induced retinal damage, overexpression
419 and subsequent release of TNF by apoptotic photoreceptors seems to induce MG proliferation
420 [18,79]. After acute damage, there is also an increased number of microglia in the retina [74,80] and
421 they have been shown to be essential for retinal regeneration [81,82]. In contrast, microglia
422 numbers do not increase with ageing and therefore may compromise the regenerative
423 response. Nonetheless, it is important to consider that the available immunohistochemistry
424 techniques to identify microglia number has a few limitations. Namely, there may be a spike
425 in microglia number in the aged retina that is rapidly resolved and missed at our timepoints or
426 microglia may undergo apoptosis similar to retinal neurons. Thus, we cannot exclude the
427 possibility that microglial cells are involved in the observed retinal degenerations or play a
428 critical role in the lack of a regenerative response observed in the aged retina. Identifying this
429 signal will be critical to stimulate regeneration in humans as using acute light damage is
430 unlikely to be therapeutically feasible. Finally, our data suggest that, as YAP expression is
431 maintained throughout lifespan, manipulating the Hippo/Yap pathways in the aged retina may
432 be a suitable model to treat retina degenerations into old age.

433

434 **Conclusions**

435 Our work demonstrates that, in the context of age-induced neuronal degeneration, the MG in
436 the zebrafish retina react by undergoing a response more akin to gliosis, rather than
437 regeneration. This resembles what occurs in the aged human retina as well as in many human
438 retinal degenerative diseases. Importantly, we identify key differences between chronic versus

439 acute damage and show that gliotic MG cells can be stimulated to repair damaged neurons in
440 the old zebrafish retina.

441

442 **MATERIALS AND METHODS**

443 Zebrafish husbandry

444 Zebrafish were maintained at 27-28ºC, in a 14:10 hour (h) light-dark cycle and fed twice a day.
445 The OKR was performed in the UCL Institute of Ophthalmology and the phototoxic lesions
446 and regeneration experiments were performed at Wayne State University School of Medicine
447 (USA). All other experiments were performed in the University of Sheffield. All animal work
448 was approved by local animal review boards, including the Local Ethical Review Committee
449 at the University of Sheffield (performed according to the protocols of Project Licence 70/8681)
450 and the Institutional Animal Care and Use Committee at Wayne State University School of
451 Medicine (performed according to the protocols of IACUC-19-02-0970).

452

453 Zebrafish strains, ages and sex

454 Three strains of adult zebrafish (*Danio rerio*) were used for these studies: wild-type (WT; AB
455 strain), *tert*$^{-/-}$ (*tert*$^{AB/hu3430}$) and *albino* (*slc45a2*$^{b4/b4}$). t*ert*$^{-/-}$ zebrafish is a premature model of
456 ageing and therefore, age and die earlier than the naturally aged zebrafish. While *tert*$^{-/-}$ fish
457 have a lifespan of 12-20 months, WT fish typically die between 36-42 months of age [29,30]. In
458 order to study age-related phenotypes in the zebrafish retina, here we used young (5 months)
459 WT and *tert*$^{-/-}$ fish, alongside with middle aged (12 months) WT fish, and old WT and *tert*$^{-/-}$ fish.
460 'Old' was defined as the age at which the majority of the fish present age-associated
461 phenotypes, such as cachexia, loss of body mass and curvature of the spine. These
462 phenotypes develop close to the time of death and are observed at >30 months of age in WT
463 and at >12 months in *tert*$^{-/-}$ [29,30]. In addition, we used adult *albino* zebrafish for retinal
464 regeneration studies (described in detail below). Importantly, none of the animals included in
465 this study displayed visible morphological alterations in the eyes (e.g. cataracts). Whenever
466 possible, males where chosen to perform the experiments.

467

468 OKR assay

469 Fish were anaesthetised in 4% tricaine methanesulfonate (MS-222; Sigma-Aldrich) and
470 placed in a small bed-like structure made of sponge, located inside a small petri dish
471 containing fish water. During the experiment, fish were maintained still by the strategic use of
472 needles that sustained the sponges close to the fish so that the fish could not move. The petri

473 dish was then placed inside a rotation chamber with black and white striped walls (8mm-thick
474 stripes). After fish recovered from anaesthesia, the trial began, and the walls of the chamber
475 started rotating at 12rpm (for 1min to the left side followed by 1min to the right side). Eye
476 movements were recorded using a digital camera throughout the experiment. After the
477 experiment, the number of eye rotations per minute was measured by video observation and
478 manually counting. The counting was performed blindly by two independent researchers. In
479 the end, the results were normalised for the WT young from the same day / batch, in order to
480 control for different days of experiments.

481

482 Intense light-damage paradigm with BrdU incorporation

483 A photolytic damage model in adult *albino* zebrafish was utilised to destroy rod and cone
484 photoreceptors and elicit a regenerative response [26]. Briefly, adult *albino* zebrafish were dark-
485 adapted for 10 days prior to a 30 min exposure to ~100,000 lux from a broadband light source.
486 Next, fish were exposed to ~10,000 lux of light from four, 250 W halogen lamps for 24 hrs.
487 Following 24 hrs of light treatment, fish were transferred to a 1L solution containing 0.66 g of
488 NaCl, 0.1 g Neutral Regulator (SeaChem Laboratories, Inc. Stone Mountain, GA, USA), and
489 1.5 g BrdU (5mM; B5002; Sigma-Aldrich) for 48 hrs. This timeframe for BrdU incubation was
490 based on previous studies in order to label MG cell-cycle re-entry [49]. Following a 48 hour
491 incubation in BrdU, the fish were split into two groups: one group was euthanised by an
492 overdose of 2-Phenoxyenthanol and eyes were processed for immunohistochemistry as
493 described below; the second group returned to normal husbandry conditions for an additional
494 25 days (or 28 days after light onset) prior to euthanasia and tissue collection. During this
495 time, BrdU incorporation dilutes in actively dividing cells, allowing for clear visualization of the
496 number of MG in the INL that only divide a single time. It also serves as an indirect measure
497 of regenerative capacity (i.e. an equal loss of BrdU-positive cells at 28 dpL between
498 experimental groups would indicate similar numbers of progenitor cell divisions earlier in the
499 regenerative process).

500

501 Tissue preparation: paraffin-embedded sections and cryosections

502 Adult fish were culled by overdose of MS-222, followed by confirmation of death. Whole fish
503 or dissected eyeballs were then processed for paraffin-embedded sections or for cryosections,
504 as follows:

505 Paraffin-embedded sections. Whole fish were fixed in in 4% paraformaldehyde (PFA) buffered
506 at pH 7.0, at 4ºC for 48-72h, decalcified in 0.5M EDTA at pH 8.0 for 48-72h, and embedded

507 in paraffin by the following series of washes: formalin I (Merck & Co, Kenilworth, NJ, USA) for
508 10min, formalin II for 50min, ethanol 50% for 1h, ethanol 70% for 1h, ethanol 95% for 1h
509 30min, ethanol 100% for 2h, ethanol 100% for 2h 30min, 50:50 of ethanol 100% : xilol for 1h
510 30min, xylene I for 3h, xylene II for 3h, paraffin I for 3h and paraffin II for 4h 30min. Paraffin-
511 embedded whole fish were then sliced in sagittal 4µm-thick or coronal 16µm-thick sections,
512 using a Leica TP 1020 cryostat.

513 Cryopreservation and cryosections. Dissected eyeballs were fixed in 4% PFA at 4ºC, overnight
514 (ON). Then, they were washed in cold 1x PBS and immersed in 30% sucrose in phosphate-
515 buffered saline (PBS), ON at 4ºC, for cryopreservation. Single cryopreserved eyeballs were
516 then embedded in mounting media – optimal cutting temperature compound (OCT, VWR
517 International), snap-frozen at −80°C, and stored at -20ºC until cryosectioning. Cryosections
518 were sliced at a 13µm thickness using a Leica Jung Frigocut cryostat or a Leica CM1860
519 cryostat.

520

521 Immunohistochemistry (IHC)

522 Before immunofluorescence staining, cryosections were hydrated in PBS at room temperature
523 (RT) for 10min, and paraffin-embedded sections were deparaffinised and hydrated as follows:
524 histoclear (Scientific Laboratory Supplies, Wilford, Nottingham, UK) 2x for 5min, followed by
525 ethanol 100% 2x for 5min, ethanol 90% for 5min, ethanol 70% for 5min, and distilled water 2x
526 for 5min. After antigen retrieval in 0.01M citrate buffer at pH 6.0 for 10min, the sections were
527 permeabilised in PBS 0.5% Triton X-100 for 10min and blocked in 3% bovine serum albumin
528 (BSA), 5% Goat Serum (or Donkey Serum), 0.3% Tween-20 in PBS, for 1h. The slides were
529 then incubated with the primary antibody at 4°C ON. After washes in PBS 0.1% Tween-20 (3x
530 10min) to remove excess to primary antibody, the sections were incubated with secondary
531 antibody at RT for 1h. Finally, the slides were incubated in 1µg/ml of 4′,6-diamidino-2-
532 phenylindole (DAPI, Thermo Fisher Scientific) at RT for 10min, washed in PBS 1x, and
533 mounted with vectashield (Vector Laboratories, Burlingame, CA, USA). The primary and
534 secondary antibodies used in this study are described in Table 1 and Table 2, respectively.

535

536 Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining

537 In paraffin-embedded sections, TUNEL was performed using the *In Situ Cell Death Detection*
538 *Kit*, *Fluorescein* (Merck & Co), following the manufacturer's instructions. Briefly, after
539 deparaffinisation, hydration, antigen retrieval and permeabilization, as described above, the

540 slides were incubated in enzyme and label solution (1:10) at 37ºC for 1h. The slides were then
541 washed in 1x PBS (2x 10min) before blocking and incubation with primary antibody.

542

543 5-Ethynyl-2'-deoxyuridine (EDU) labelling

544 EdU labelling was detected using the Click-iT® EdU Imaging Kit (Thermo Fisher Scientific),
545 following manufacturer's instructions. Briefly, fish were injected with 5µl of 10mM EdU diluted
546 in dimethyl sulfoxide (DSMO), by intraperitoneal (IP) injection, for 3 consecutive days (3-day
547 pulse). In order to differentiate proliferating cells and low-proliferative EdU-retaining cells, the
548 fish were separated into two groups: 0-day chase and 30-days chase groups. The fish from
549 the first group were culled 2h30min after the last injection of Edu, whereas the fish from the
550 second group were culled 30 days after the last injection of EdU. After culling, whole fish were
551 processed for paraffin-embedded sections as described above. In order to detect EdU
552 labelling in paraffin-embedded sections, the slides were deparaffinised, hydrated, underwent
553 antigen retrieved, were permeabilised and washed in 1x PBS. The slides were incubated in
554 freshly made EdU-labelling solution (per 1ml of solution: 860 1x Click-iT®EdU reaction buffer,
555 40 $CuSO_4$, 2.5 Alexa Fluor® 647 azide working solution and 100 10x EdU reaction buffer
556 additive) at RT for 30min. Finally, the slides were washed in 1x PBS before blocking and
557 incubation with primary antibody (ON, at 4ºC). The incubation in secondary antibody was
558 performed as previously described.

559

560 Imaging and quantifications

561 Paraffin-embedded sections were imaged by epifluorescence microscopy, using a DeltaVision
562 microscope with a 40x oil objective. Cryosections were imaged by laser scanning confocal
563 imaging, using a Leica SP5 microscope, Nikon A1 Confocal microscope or a Zeiss 900
564 Airyscan 2 confocal, with a 40x oil objective. In either paraffin or cryosections, multiple 0.2-
565 0.6µm thick z-stacks were acquired in order to capture the whole retina region. For each
566 staining, a total of 4 images were taken per retina, 2 from central and 2 from peripheral retinal.
567 The central retina was defined to be the centre point between opposing CMZs (A minimum of
568 ~1000µm from the periphery), while images of the peripheral retina contain the limits of the
569 retina, including the CMZ. The peripheral retina was defined as the tissue directly adjacent to
570 the CMZ.

571

572 In order to quantify the alteration in the staining patterns, a z-projection was generated and
573 three boxes of 100x100µm were drawn in each field of view. The total number of positive cells

was then manually counted for each labelling. Rhodopsin, ZO1, ribeye and GFAP staining were an exception to this, for which a qualitative assessment was performed instead. To do so, structural and morphological defects were identified as follows. For the rhodopsin staining, as young WT retinas usually display long and aligned outer segments, all short and/or misaligned outer segments were considered defective. For the ZO1 staining, the average number of breaks in the ZO1-labelled membrane per animal was quantified. The average of breaks in the WT young animals was used as a reference, and any fish presenting an average number of breaks bellow this average was considered to present defects in the membrane. For the ribeye staining, young retinas usually present two distinguished layers of pre-synaptic ribbons. Thus, staining where the two layers of pre-synaptic ribbons are not distinguished, was considered defective. GFAP staining usually reveals long and aligned MG processes in young WT retinas, and therefore short and/or misaligned MG processes are considered gliotic. Through this qualitative assessment it was calculated the percentage of fish per group presenting structural and morphological defects. Finally, raw images were used for quantification purposes. The images were then processed with Adobe Illustrator 21.0.2 for display purposes.

Statistical analysis

Statistics were performed using the GraphPad Prism v7.00. Normality was assessed by Shapiro-Wilk test. For normally distributed data unpaired t-test was used to compare 2 data points and one-way ANOVA followed by Bonferroni post-hoc test was used to compare more than 2 data points. For non-normally distributed data, Mann-Whitney test and Kruskal-Wallis test followed by Dunn's post-hoc test were used instead. Two-way ANOVA was used in order to compare more than 2 data points in 2 groups different groups (genotypes). Chi-square was performed to analyse structure and morphological changes in the retina based on qualitative assessment, having into account the number of animals per group displaying defects versus not displaying defects. A critical value for significance of $p<0.05$ was used throughout the analysis.

604 **Table 1. Primary antibodies used for immunostaining.**

Antibody, species and type	Dilution factor	Catalogue number; Company, City, Country
yH2AX rabbit polyclonal	1:500	GTX127342; GeneTex, Irvine, CA, USA
p53 rabbit polyclonal	1:200	AS-55342s; Labscoop, Little Rock, AR, USA
PCNA mouse monoclonal	1:500	NB500-106; Novus Biologicals, Littleton, CO, USA
PCNA rabbit polyclonal	1:50	GTX124496; GeneTex, Irvine, CA, USA
7.4.C4 (4C4) mouse monoclonal	1:100	A gift from A. McGown
HuCD mouse monoclonal	1:100	A21271; Thermo Fisher Scientific, Waltham, MA, USA
PKCβ1 rabbit polyclonal	1:100	Sc-209; Santa Cruz, Dallas, TX, USA
Ribeye rabbit polyclonal	1:10,000	A gift from Teresa Nicholson
GFAP rabbit polyclonal	1:200	Z0334, Agilent DAKO, Santa Clara, CA, USA
GFAP mouse monoclonal	1:100	zrf1, ZIRC
Glutamine Synthase (GS) mouse monoclonal	1:150	mab302, Merck, Kenilworth, NJ, USA
Rhodopsin rabbit polyclonal	1:5,000	A gift from David Hyde
BrdU rat	1:200	OBT0030A, Accurate Chemical & Scientific, Westbury, NY, USA

Yap mouse monoclonal	1:200	Sc-101199; Santa Cruz, Dallas, TX, USA
Cralbp rabbit polyclonal	1:200	15356-1-AP; Proteintech, Rosemont, Illinois, USA
Vimentin mouse monoclonal	1:500	60330-1-IG; Proteintech, Rosemont, Illinois, USA
Pax6 rabbit monoclonal	1:200	12323-1-AP Proteintech, Rosemont. Illinois, USA
Tgfb1 rabbit polyclonal	1:100	Ab215715, Abcam plc. Cambridge, UK
Tgfb2 rabbit polyclonal	1:100	Ab36495, Abcam plc. Cambridge, UK
Tgfb3 rabbit polyclonal	1:100	Ab215715, Abcam plc. Cambridge, UK

605
606

607 **Table 2. Secondary antibodies used for immunostaining.**

Antibody, species and type	Dilution factor	Catalogue number; Company, City, Country
Goat anti-rabbit IgG Alexa Fluor® 488	1:500	A11008;Invitrogen, Carlsbad, CA, USA
Goat anti-rat IgG Alexa Fluor® 488	1:500	A11006; Thermo Fisher Scientific, Waltham, MA, USA
Goat anti-rabbit IgG Alexa Fluor® 568	1:500	10032302; Thermo Fisher Scientific, Waltham, MA, USA
Donkey anti-rabbit IgG Alexa Fluor® 647	1:500	A31573; Thermo Fisher Scientific, Waltham, MA, USA
Goat anti-mouse IgG Alexa Fluor® 488	1:500	A11001; Thermo Fisher Scientific, Waltham, MA, USA
Goat anti-mouse IgG Alexa Fluor® 568	1:500	10348072; Thermo Fisher Scientific, Waltham, MA, USA
Goat anti-mouse IgG Alexa Fluor® 647	1:500	A21235; Thermo Fisher Scientific, Waltham, MA, USA

608

609

610 **ACKNOWLEDGEMENTS**

611 The authors would like to thank Alex McGowen for 4c4 and Teresa Nicholson for RibeyeA for
612 their generous gifts of antibodies. Part of this work was supported by grants from the National
613 Institutes of Health (NEI R01EY026551 and R21EY031526 to RT). Histology and imaging core
614 resources were supported by vision core grants (P30EY04068) and an unrestricted grant from
615 Research to Prevent Blindness to the Department of Ophthalmology, Visual and Anatomical
616 Sciences (RT). This work was also generously funded by a University of Sheffield PhD
617 studentship to RRM, a Sheffield University Vice Chancellor's Research Fellowship and a
618 Wellcome Trust/Royal Society Sir Henry Dale Fellowship (UNS35121) to CMH and a
619 Welcome Trust Seed Award (210152/Z/18/Z) and a BBSRC David Phillips Fellowship
620 (BB/S010386/1) to RBM.

621

622 **COMPETING INTERESTS**

623 The authors declare no competing interests.

624

625 **REFERENCES**

626

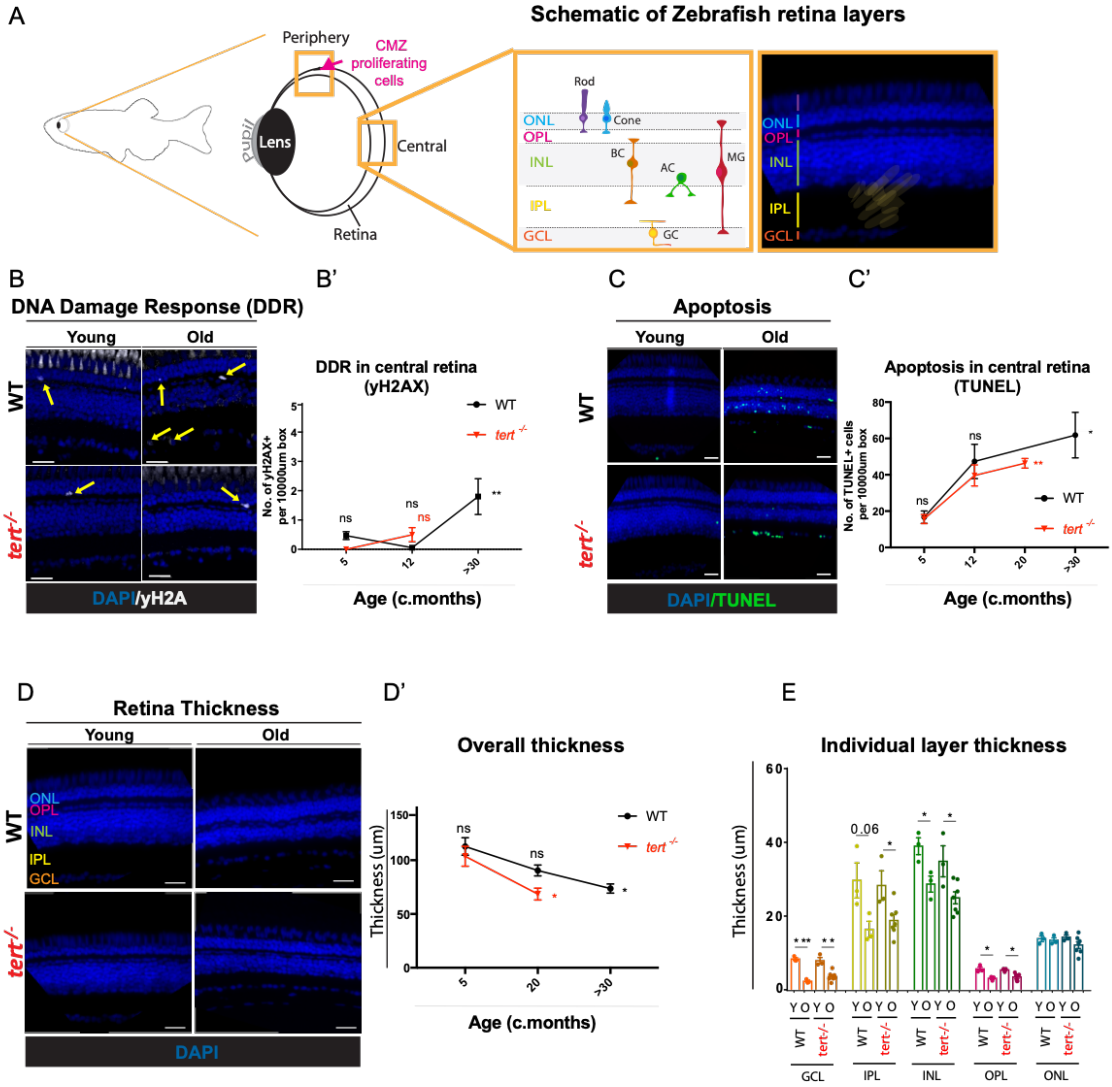
627 1. Chakravarthy, U. *et al.* Clinical risk factors for age-related macular degeneration: a
628 systematic review and meta-analysis. *BMC Ophthalmol* **10**, 31 (2010).
629 2. FJ, S. AGE NORMS OF REFRACTION AND VISION. *Arch Ophthalmol* **43**, 466–481
630 (1950).
631 3. Weale, R.A. Senile changes in visual acuity. *Trans Ophthalmol Soc U K* **95**, 36-8
632 (1975).
633 4. Arden, G.B. & Jacobson, J.J. A simple grating test for contrast sensitivity: preliminary
634 results indicate value in screening for glaucoma. *Invest Ophthalmol Vis Sci* **17**, 23-32
635 (1978).
636 5. Skalka, H.W. Effect of age on Arden grating acuity. *Br J Ophthalmol* **64**, 21-3 (1980).
637 6. Marshall, J. The ageing retina: physiology or pathology. *Eye (Lond)* **1 (Pt 2)**, 282-95
638 (1987).
639 7. Salvi, S.M., Akhtar, S. & Currie, Z. Ageing changes in the eye. *Postgrad Med J* **82**,
640 581-7 (2006).
641 8. Al-Ubaidi, M.R., Naash, M.I. & Conley, S.M. A perspective on the role of the
642 extracellular matrix in progressive retinal degenerative disorders. *Invest Ophthalmol*
643 *Vis Sci* **54**, 8119-24 (2013).
644 9. Reichenbach, A. & Bringmann, A. New functions of Muller cells. *Glia* **61**, 651-78
645 (2013).
646 10. Bringmann, A. *et al.* Cellular signaling and factors involved in Muller cell gliosis:
647 neuroprotective and detrimental effects. *Prog Retin Eye Res* **28**, 423-51 (2009).
648 11. Goldman, D. Muller glial cell reprogramming and retina regeneration. *Nat Rev*
649 *Neurosci* **15**, 431-42 (2014).
650 12. Fausett, B.V. & Goldman, D. A role for alpha1 tubulin-expressing Muller glia in
651 regeneration of the injured zebrafish retina. *J Neurosci* **26**, 6303-13 (2006).
652 13. Fischer, A.J., McGuire, C.R., Dierks, B.D. & Reh, T.A. Insulin and fibroblast growth
653 factor 2 activate a neurogenic program in Muller glia of the chicken retina. *J Neurosci*
654 **22**, 9387-98 (2002).
655 14. Lenkowski, J.R. & Raymond, P.A. Muller glia: Stem cells for generation and
656 regeneration of retinal neurons in teleost fish. *Prog Retin Eye Res* **40**, 94-123 (2014).
657 15. Raymond, P.a., Barthel, L.K., Bernardos, R.L. & Perkowski, J.J. Molecular
658 characterization of retinal stem cells and their niches in adult zebrafish. *BMC*
659 *developmental biology* **6**, 36 (2006).
660 16. Thummel, R., Kassen, S.C., Montgomery, J.E., Enright, J.M. & Hyde, D.R. Inhibition
661 of Muller glial cell division blocks regeneration of the light-damaged zebrafish retina.
662 *Dev Neurobiol* **68**, 392-408 (2008).
663 17. Wan, J. & Goldman, D. Retina regeneration in zebrafish. *Curr Opin Genet Dev* **40**,
664 41-47 (2016).
665 18. Thomas, J.L., Ranski, A.H., Morgan, G.W. & Thummel, R. Reactive gliosis in the
666 adult zebrafish retina. *Exp Eye Res* **143**, 98-109 (2016).
667 19. Thummel, R. *et al.* Characterization of Muller glia and neuronal progenitors during
668 adult zebrafish retinal regeneration. *Exp Eye Res* **87**, 433-44 (2008).
669 20. Eastlake, K. *et al.* Comparison of proteomic profiles in the zebrafish retina during
670 experimental degeneration and regeneration. *Sci Rep* **7**, 44601 (2017).
671 21. Jadhav, A.P., Roesch, K. & Cepko, C.L. Development and neurogenic potential of
672 Muller glial cells in the vertebrate retina. *Prog Retin Eye Res* **28**, 249-62 (2009).
673 22. Ooto, S. *et al.* Potential for neural regeneration after neurotoxic injury in the adult
674 mammalian retina. *Proc Natl Acad Sci U S A* **101**, 13654-9 (2004).
675 23. Jorstad, N.L. *et al.* Stimulation of functional neuronal regeneration from Muller glia in
676 adult mice. *Nature* **548**, 103-107 (2017).
677 24. Karl, M.O. *et al.* Stimulation of neural regeneration in the mouse retina. *Proc Natl*
678 *Acad Sci U S A* **105**, 19508-13 (2008).

679 25. Jorstad, N.L. *et al.* STAT Signaling Modifies Ascl1 Chromatin Binding and Limits
680 Neural Regeneration from Muller Glia in Adult Mouse Retina. *Cell Rep* **30**, 2195-
681 2208 e5 (2020).
682 26. Thomas, J.L., Nelson, C.M., Luo, X., Hyde, D.R. & Thummel, R. Characterization of
683 multiple light damage paradigms reveals regional differences in photoreceptor loss.
684 *Exp Eye Res* **97**, 105-16 (2012).
685 27. Van Houcke, J. *et al.* Extensive growth is followed by neurodegenerative pathology in
686 the continuously expanding adult zebrafish retina. *Biogerontology* **20**, 109-125
687 (2019).
688 28. Fu, J., Nagashima, M., Guo, C., Raymond, P.A. & Wei, X. Novel Animal Model of
689 Crumbs-Dependent Progressive Retinal Degeneration That Targets Specific Cone
690 Subtypes. *Invest Ophthalmol Vis Sci* **59**, 505-518 (2018).
691 29. Carneiro, M.C. *et al.* Short Telomeres in Key Tissues Initiate Local and Systemic
692 Aging in Zebrafish. *PLoS genetics* **12**, e1005798 (2016).
693 30. Henriques, C.M., Carneiro, M.C., Tenente, I.M., Jacinto, A. & Ferreira, M.G.
694 Telomerase is required for zebrafish lifespan. *PLoS Genet* **9**, e1003214 (2013).
695 31. Anchelin, M. *et al.* Premature aging in telomerase-deficient zebrafish. *Dis Model*
696 *Mech* **6**, 1101-12 (2013).
697 32. Bednarek, D. *et al.* Telomerase Is Essential for Zebrafish Heart Regeneration. *Cell*
698 *Rep* **12**, 1691-703 (2015).
699 33. Easter, S.S., Jr. & Nicola, G.N. The development of vision in the zebrafish (Danio
700 rerio). *Dev Biol* **180**, 646-63 (1996).
701 34. Eriksson, U. & Alm, A. Macular thickness decreases with age in normal eyes: a study
702 on the macular thickness map protocol in the Stratus OCT. *Br J Ophthalmol* **93**,
703 1448-52 (2009).
704 35. Lo, S.N.a.A.C.Y. Protecting the Aging Retina. in *Neuroprotection* (ed. Ho, R.C.-
705 C.C.a.Y.-S.) (2019).
706 36. Bernardos, R.L., Barthel, L.K., Meyers, J.R. & Raymond, P.A. Late-stage neuronal
707 progenitors in the retina are radial Muller glia that function as retinal stem cells. *J*
708 *Neurosci* **27**, 7028-40 (2007).
709 37. Johns, P.R. & Fernald, R.D. Genesis of rods in teleost fish retina. *Nature* **293**, 141-2
710 (1981).
711 38. Julian, D., Ennis, K. & Korenbrot, J.I. Birth and fate of proliferative cells in the inner
712 nuclear layer of the mature fish retina. *J Comp Neurol* **394**, 271-82 (1998).
713 39. Otteson, D.C., D'Costa, A.R. & Hitchcock, P.F. Putative stem cells and the lineage of
714 rod photoreceptors in the mature retina of the goldfish. *Dev Biol* **232**, 62-76 (2001).
715 40. Nelson, S.M., Frey, R.A., Wardwell, S.L. & Stenkamp, D.L. The developmental
716 sequence of gene expression within the rod photoreceptor lineage in embryonic
717 zebrafish. *Dev Dyn* **237**, 2903-17 (2008).
718 41. Stenkamp, D.L. Development of the Vertebrate Eye and Retina. *Prog Mol Biol Transl*
719 *Sci* **134**, 397-414 (2015).
720 42. Brockerhoff, S.E. *et al.* A behavioral screen for isolating zebrafish mutants with visual
721 system defects. *Proc Natl Acad Sci U S A* **92**, 10545-9 (1995).
722 43. Neuhauss, S.C. *et al.* Genetic disorders of vision revealed by a behavioral screen of
723 400 essential loci in zebrafish. *J Neurosci* **19**, 8603-15 (1999).
724 44. Gross, N.E., Aizman, A., Brucker, A., Klancnik, J.M., Jr. & Yannuzzi, L.A. Nature and
725 risk of neovascularization in the fellow eye of patients with unilateral retinal
726 angiomatous proliferation. *Retina* **25**, 713-8 (2005).
727 45. Lessieur, E.M. *et al.* Ciliary genes arl13b, ahi1 and cc2d2a differentially modify
728 expression of visual acuity phenotypes but do not enhance retinal degeneration due
729 to mutation of cep290 in zebrafish. *PLoS One* **14**, e0213960 (2019).
730 46. Tappeiner, C. *et al.* Visual acuity and contrast sensitivity of adult zebrafish. *Front*
731 *Zool* **9**, 10 (2012).
732 47. Bringmann, A. & Wiedemann, P. Muller glial cells in retinal disease. *Ophthalmologica*
733 **227**, 1-19 (2012).

734 48. Telegina, D.V., Kozhevnikova, O.S. & Kolosova, N.G. Changes in Retinal Glial Cells
735 with Age and during Development of Age-Related Macular Degeneration.
736 *Biochemistry (Mosc)* **83**, 1009-1017 (2018).
737 49. Ranski, A.H., Kramer, A.C., Morgan, G.W., Perez, J.L. & Thummel, R.
738 Characterization of retinal regeneration in adult zebrafish following multiple rounds of
739 phototoxic lesion. *PeerJ* **6**, e5646 (2018).
740 50. Garcia, M. & Vecino, E. Role of Muller glia in neuroprotection and regeneration in the
741 retina. *Histol Histopathol* **18**, 1205-18 (2003).
742 51. Verardo, M.R. *et al.* Abnormal reactivity of muller cells after retinal detachment in
743 mice deficient in GFAP and vimentin. *Invest Ophthalmol Vis Sci* **49**, 3659-65 (2008).
744 52. Cragnolini, A.B. *et al.* Regional brain susceptibility to neurodegeneration: what is the
745 role of glial cells? *Neural Regen Res* **15**, 838-842 (2020).
746 53. Conedera, F.M. *et al.* Diverse Signaling by TGFbeta Isoforms in Response to Focal
747 Injury is Associated with Either Retinal Regeneration or Reactive Gliosis. *Cell Mol*
748 *Neurobiol* (2020).
749 54. Hamon, A. *et al.* Retinal Degeneration Triggers the Activation of YAP/TEAD in
750 Reactive Muller Cells. *Invest Ophthalmol Vis Sci* **58**, 1941-1953 (2017).
751 55. Hamon, A. *et al.* Linking YAP to Muller Glia Quiescence Exit in the Degenerative
752 Retina. *Cell Rep* **27**, 1712-1725 e6 (2019).
753 56. Rueda, E.M. *et al.* The Hippo Pathway Blocks Mammalian Retinal Muller Glial Cell
754 Reprogramming. *Cell Rep* **27**, 1637-1649 e6 (2019).
755 57. Cameron, D.A. Cellular proliferation and neurogenesis in the injured retina of adult
756 zebrafish. *Vis Neurosci* **17**, 789-97 (2000).
757 58. Hanovice, N.J. *et al.* Regeneration of the zebrafish retinal pigment epithelium after
758 widespread genetic ablation. *PLoS Genet* **15**, e1007939 (2019).
759 59. Drigeard Desgarnier, M.C. *et al.* Telomere Length Measurement in Different Ocular
760 Structures: A Potential Implication in Corneal Endothelium Pathogenesis. *Invest*
761 *Ophthalmol Vis Sci* **57**, 5547-5555 (2016).
762 60. Dow, C.T. & Harley, C.B. Evaluation of an oral telomerase activator for early age-
763 related macular degeneration - a pilot study. *Clin Ophthalmol* **10**, 243-9 (2016).
764 61. Rowe-Rendleman, C. & Glickman, R.D. Possible therapy for age-related macular
765 degeneration using human telomerase. *Brain research bulletin* **62**, 549-53 (2004).
766 62. Henriques, C.M. & Ferreira, M.G. Consequences of telomere shortening during
767 lifespan. *Curr Opin Cell Biol* **24**, 804-8 (2012).
768 63. Fumagalli, M. *et al.* Telomeric DNA damage is irreparable and causes persistent
769 DNA-damage-response activation. *Nat Cell Biol* **14**, 355-65 (2012).
770 64. Hewitt, G. *et al.* Telomeres are favoured targets of a persistent DNA damage
771 response in ageing and stress-induced senescence. *Nat Commun* **3**, 708 (2012).
772 65. Honda, S., Hjelmeland, L.M. & Handa, J.T. Oxidative stress--induced single-strand
773 breaks in chromosomal telomeres of human retinal pigment epithelial cells in vitro.
774 *Invest Ophthalmol Vis Sci* **42**, 2139-44 (2001).
775 66. Wang, J. *et al.* Photosensitization of A2E triggers telomere dysfunction and
776 accelerates retinal pigment epithelium senescence. *Cell Death Dis* **9**, 178 (2018).
777 67. Itou, J., Kawakami, H., Burgoyne, T. & Kawakami, Y. Life-long preservation of the
778 regenerative capacity in the fin and heart in zebrafish. *Biol Open* **1**, 739-46 (2012).
779 68. Pinto-Teixeira, F. *et al.* Inexhaustible hair-cell regeneration in young and aged
780 zebrafish. *Biol Open* **4**, 903-9 (2015).
781 69. Van Houcke, J. *et al.* Successful optic nerve regeneration in the senescent zebrafish
782 despite age-related decline of cell intrinsic and extrinsic response processes.
783 *Neurobiol Aging* **60**, 1-10 (2017).
784 70. Collins, C.A., Zammit, P.S., Ruiz, A.P., Morgan, J.E. & Partridge, T.A. A population of
785 myogenic stem cells that survives skeletal muscle aging. *Stem Cells* **25**, 885-94
786 (2007).
787 71. Janzen, V. *et al.* Stem-cell ageing modified by the cyclin-dependent kinase inhibitor
788 p16INK4a. *Nature* **443**, 421-6 (2006).

72. Nave, K.A. Neuroscience: An ageing view of myelin repair. *Nature* **455**, 478-9 (2008).
73. Kirschner, D.A. *et al.* Rapid assessment of internodal myelin integrity in central nervous system tissue. *J Neurosci Res* **88**, 712-21 (2010).
74. Mitchell, D.M., Sun, C., Hunter, S.S., New, D.D. & Stenkamp, D.L. Regeneration associated transcriptional signature of retinal microglia and macrophages. *Sci Rep* **9**, 4768 (2019).
75. Montgomery, J.E., Parsons, M.J. & Hyde, D.R. A novel model of retinal ablation demonstrates that the extent of rod cell death regulates the origin of the regenerated zebrafish rod photoreceptors. *J Comp Neurol* **518**, 800-14 (2010).
76. Bergmann, A. & Steller, H. Apoptosis, stem cells, and tissue regeneration. *Sci Signal* **3**, re8 (2010).
77. Nelson, C.M. *et al.* Tumor necrosis factor-alpha is produced by dying retinal neurons and is required for Muller glia proliferation during zebrafish retinal regeneration. *J Neurosci* **33**, 6524-39 (2013).
78. Rao, M.B., Didiano, D. & Patton, J.G. Neurotransmitter-Regulated Regeneration in the Zebrafish Retina. *Stem Cell Reports* **8**, 831-842 (2017).
79. Iribarne, M., Hyde, D.R. & Masai, I. TNFalpha Induces Muller Glia to Transition From Non-proliferative Gliosis to a Regenerative Response in Mutant Zebrafish Presenting Chronic Photoreceptor Degeneration. *Front Cell Dev Biol* **7**, 296 (2019).
80. Mitchell, D.M., Lovel, A.G. & Stenkamp, D.L. Dynamic changes in microglial and macrophage characteristics during degeneration and regeneration of the zebrafish retina. *J Neuroinflammation* **15**, 163 (2018).
81. Conedera, F.M., Pousa, A.M.Q., Mercader, N., Tschopp, M. & Enzmann, V. Retinal microglia signaling affects Muller cell behavior in the zebrafish following laser injury induction. *Glia* **67**, 1150-1166 (2019).
82. White, D.T. *et al.* Immunomodulation-accelerated neuronal regeneration following selective rod photoreceptor cell ablation in the zebrafish retina. *Proc Natl Acad Sci U S A* **114**, E3719-E3728 (2017).

821 **FIGURES**



822

823 **Figure 1. Zebrafish retina ageing is characterised by increased DNA damage, cell death,**
824 **and tissue thinning over-time, independently of telomerase.** (A) Schematic figure of the
825 zebrafish retina highlighting the periphery, including Ciliary Marginal Zone (CMZ) and central
826 retina, with respective layers and cell types (inset). (B-C) The central retina immunolabeled
827 with (B) γH2AX (DNA damage, in white) and (C) TUNEL (apoptotic cells, in green) in both WT
828 and *tert*$^{-/-}$, at young (5 months) and old ages (>30 months in WT and 12 or 20 in *tert*$^{-/-}$). Scale
829 bars: 20µm. (B) Yellow arrows highlight γH2AX^{+} cells. (B'-C') Quantifications of the number of
830 (B') γH2AX^{+} cells and (C') TUNEL^{+} cells per area (10 000µm^{2}). Error bars represent the SEM.
831 N=3-4. (D) Central retina thinning in both WT and *tert*$^{-/-}$, at young (5 months) and old ages
832 (>30 months in WT and 20 months in *tert*$^{-/-}$). Scale bars: 20µm. (D', D'') Quantifications of the
833 central retina thickness cells per area (10 000µm^{2}), (D') in the overall retina and (D'') per layer
834 of the retina. Error bars represent the standard error of the mean (SEM). N=3. P-value: *
835 <0.05; ** <0.01; *** <0.001.

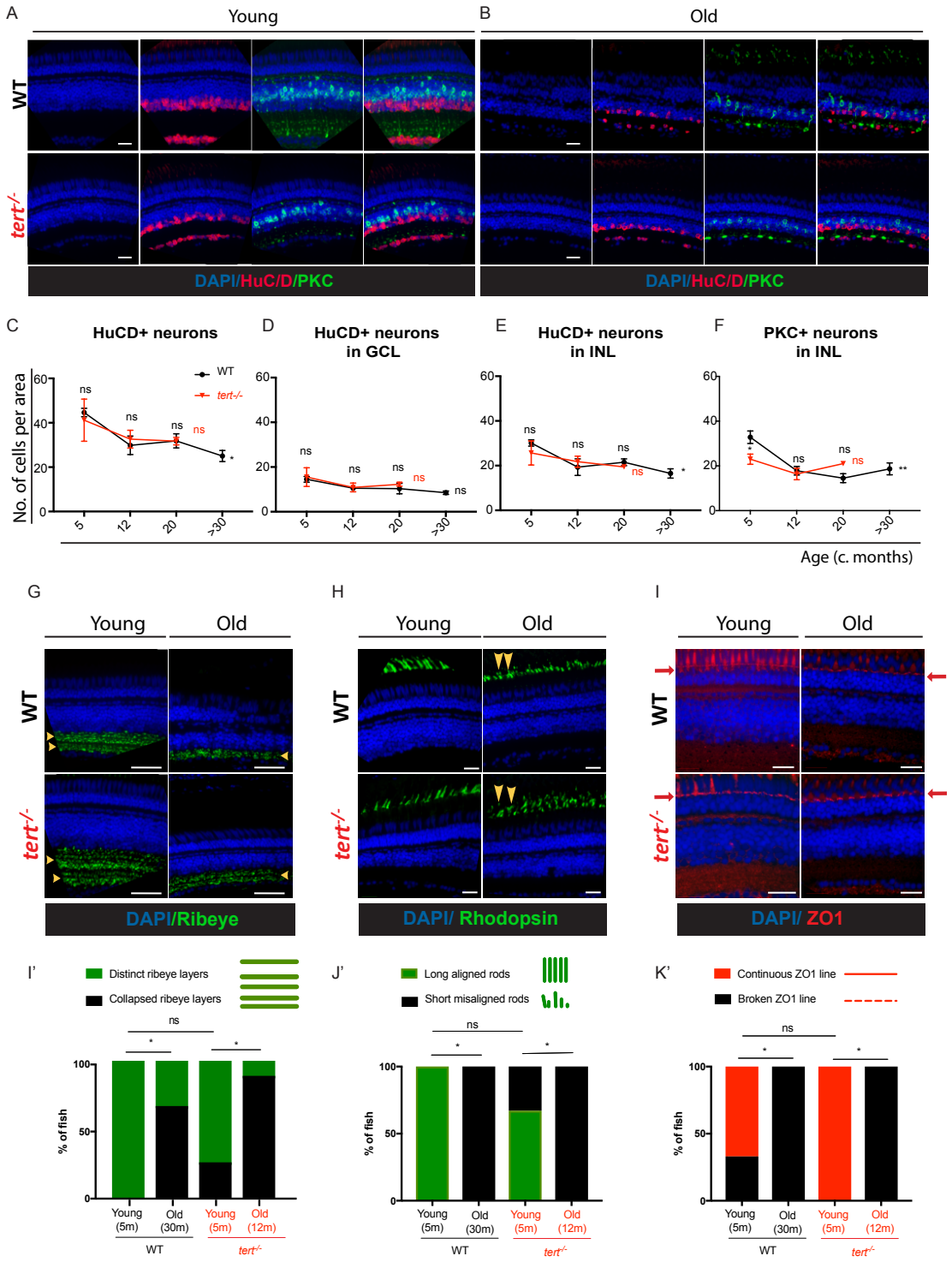
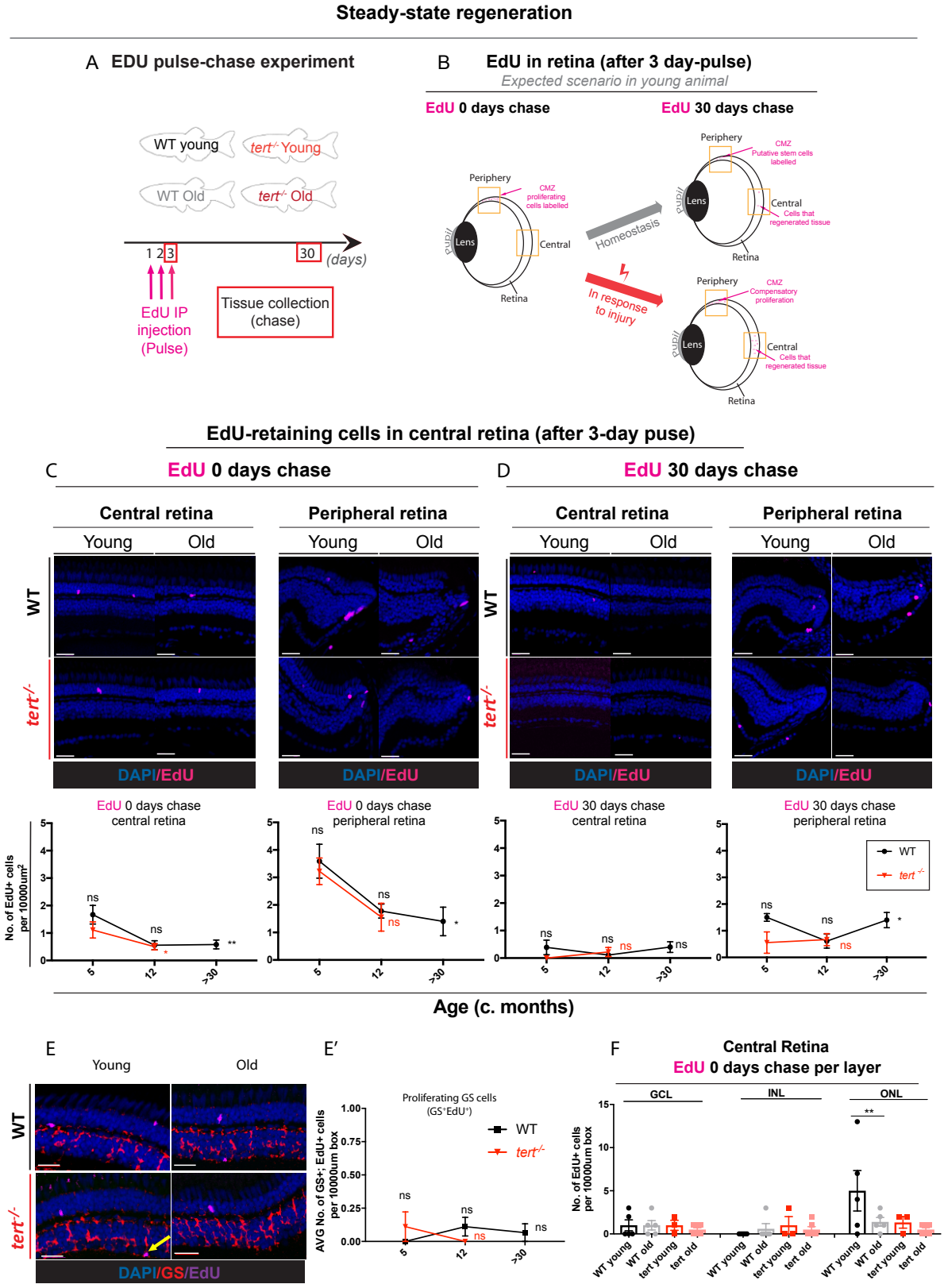


Figure 2: The zebrafish retina undergoes neurodegeneration with ageing, independently of telomerase. The central retina immunolabeled with HuC/D and PKC (amacrine in magenta and bipolar cells in green, respectively), in both WT and *tert*$^{-/-}$, at (A) young (5 months) and (B) old ages (>30 months in WT and 12 months in *tert*$^{-/-}$). Scale bars: 20µm. (C-F) Quantifications of the number of HuC/D-positive neurons per area (10 000µm^2) (C) in the overall retina, (D) in the GCL (ganglion cells) and (E) in the INL (amacrine cells). (F) Quantifications of the number of PKC-positive neurons cells per area (10 000µm^2) in the INL

844 (bipolar cells). Error bars represent the SEM. N=3-4. (G-I) The central retina immunolabeled
845 with (G) ribeyeA (pre-synaptic ribbons, in green), (H) rhodopsin (photoreceptors outer
846 segments, in green), and (I) ZO1 (outer limiting membrane, in red), in both WT and *tert*$^{-/-}$, at
847 young (5 months) and old ages (>30 months in WT and 12 months in *tert*$^{-/-}$). Scale bars: 20µm.
848 (G'-I') Quantification of the percentage of fish presenting defects in (G') ribeye (collapsed
849 ribeye layers), (H') rhodopsin (short and misaligned outer segments), and (I') ZO1 (broken
850 outer limiting membrane). Error bars represent the SEM. (G') N=4-9, (H', I') N=3-6. P-value: *
851 <0.05; ** <0.01; *** <0.001.

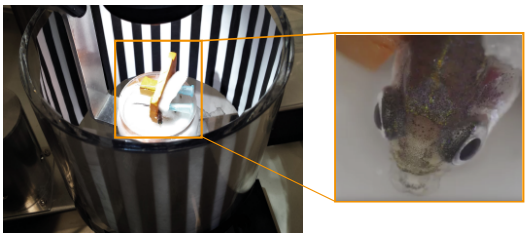


852

853 **Figure 3. Aged zebrafish retina does not show signs of regeneration in response to**
854 **spontaneous cell death and neuronal loss.** (A) Schematic figure of the experimental
855 design: 3-day pulse of EdU, by IP injection, followed by 0- or 30-day chase; (B) and respective

hypothesised result. We anticipate that in a healthy young fish, the retina has some cells proliferating in the CMZ, which, over-time, will replace older cells in the central retina. In the case of injury, we anticipate that there will be elevated levels of proliferation in peripheral and central retina to replace the death cells. (C-D) The central and the peripheral retina immunolabeled with EdU (in purple), at (C) 0- and (D) 30-days chase, in both WT and *tert*$^{-/-}$, at young (5 months) and old ages (>30 months in WT and 12 in *tert*$^{-/-}$). Scale bars: 20µm. Graphs show quantifications of the number of EdU-retaining cells per area (10 000µm^2), in the overall central and peripheral retina at (C) 0- and (D) 30-days chase. (E) The central retina immunolabeled with GS (Müller glia, in red) after a 3-day pulse of EdU, by IP injection, at 0-days chase, in both WT and *tert*$^{-/-}$, at young (5 months) and old ages (>30 months in WT and 12 in *tert*$^{-/-}$). Scale bars: 20µm. (E') Quantifications of the number of GS$^+$; EdU$^+$ cells per area (10 000µm^2). Error bars represent SEM. N=3-6. CMZ: ciliary marginal zone. (F) Quantifications of the number of EdU-retaining cells per area (10 000µm^2), per layer of the retina, at 0-days chase. Error bars represent SEM. N=3-6. P-value: * <0.05; ** <0.01; *** <0.001.

A **OptoKinetic Response (OKR)**



A'

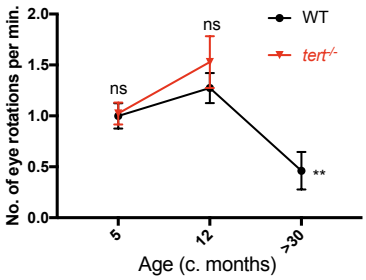


Figure 4: Zebrafish vision declines with ageing, independently of telomerase. (A) OKR assay was performed by immobilising the fish in between soft sponges, inside a petri dish containing water, placed in the centre of a rotation chamber. The walls of the rotation chamber had 0.8 mm-thick black and white stripes and the chamber was maintained at a constant velocity of 12 rpm throughout the experiment. (A') The number of eye rotations per minute was manually quantified by video observation. Error bars represent the SEM. N=5-8. P-value: * <0.05; ** <0.01; *** <0.001.

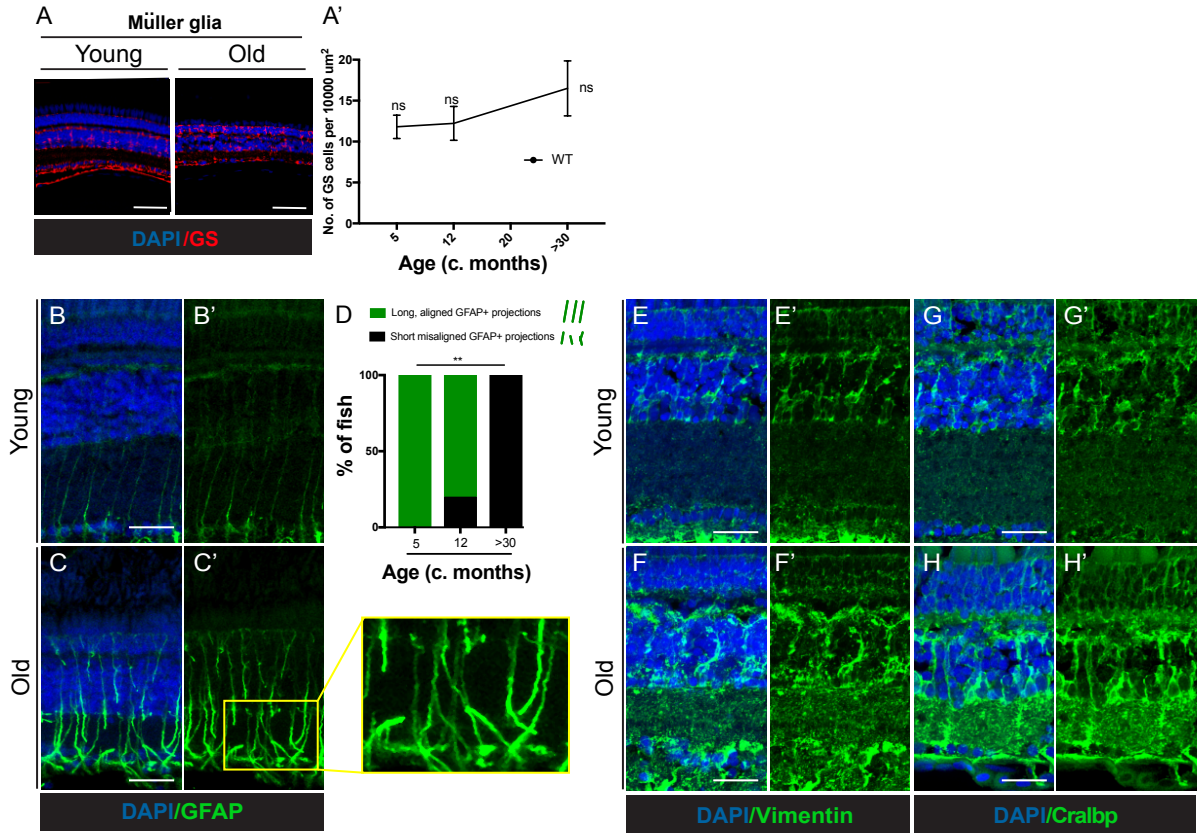


Figure 5. The zebrafish retina shows signs of gliosis with ageing. (A) The central retina immunolabeled with GS (Müller glia, in red) in WT fish, at young (5 months) and old ages (>30 months). Scale bars: 20µm. (A') Quantification of the number of GS-positive cells (MG), per area (10 000 µm^2). Error bars represent the SEM. N=3. (B,C) The central retina immunolabeled with GFAP (MG in green), in WT at young (5 months) and old ages (>30 months). (D) Quantifications of the percentage of fish presenting disorganised MG processes (gliosis-phenotype; inset) N = 6. P-value: * <0.05; ** <0.01; *** <0.001 (E,F) The central retina immunolabeled with Vimentin showing disorganised MG and upregulation of expression. (G,H) The central retina immunolabeled with Cralbp showing disorganised MG and upregulation of expression. Scale bars: 20µm.

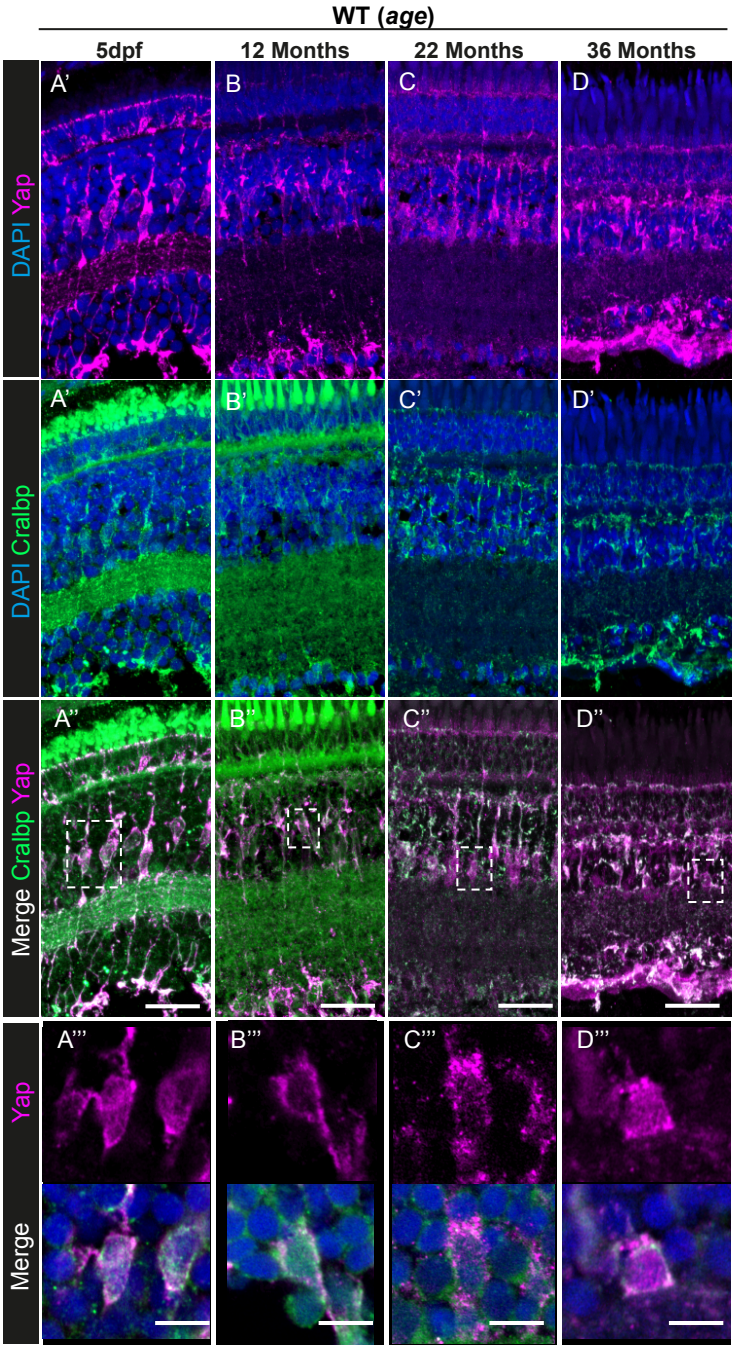


Figure 6. Yap expression is observed in the retinal MG throughout zebrafish lifespan. (A-D) The central retina immunolabeled with Yap (in magenta) and (A'-D') Cralbp (MG cells, in green) in WT zebrafish at different ages (5 dpf, 12 months, 22 months, and 36 months). Scale bars: 20μm. (A'''-D''') A single z-section from stacks showing a merge of DAPI, Yap and Cralbp staining with Yap expression specifically in MG cells at all stages. Scale bars: 3μm.

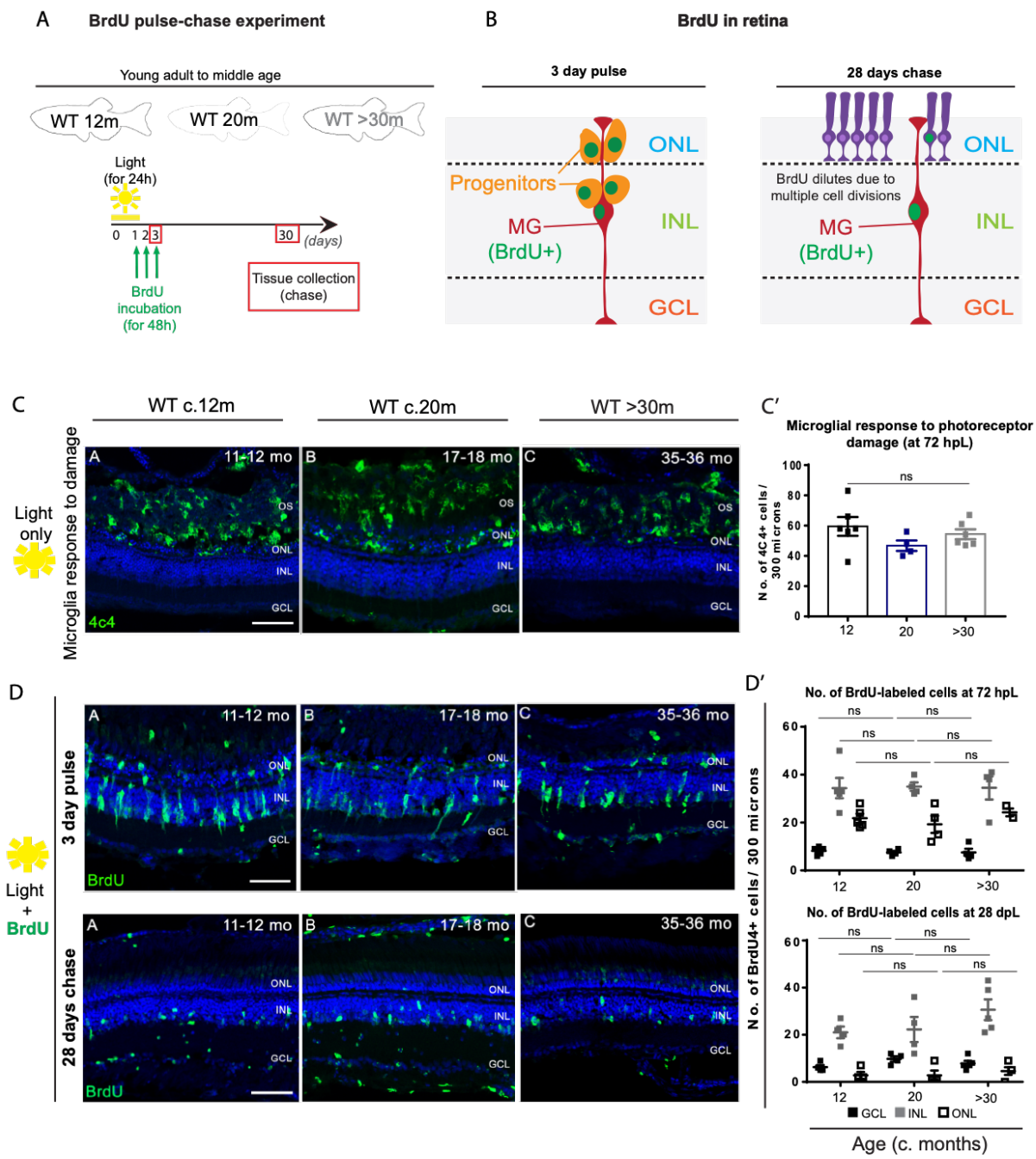


Figure 7. Zebrafish MG regenerative capacity upon acute damage is maintained until old age. (A) Schematic image of the experimental design and (B) expected results. Fish were light-treated for 24 hours and BrdU incorporation occurred between 24-72 hrs (green), which allowed for BrdU to be washed out the proliferating progenitors, leaving only MG which re-entered the cell cycle to be labelled. (C) The central retina labelled with 4C4 (microglia, in green), after light treatment, in young (c. 12 months), middle aged (c. 20 months) and old (>30 months) *albino* zebrafish. The majority of the microglia response to damage occurs within the outer segments (OS) and nuclear layer (ONL) of the retina, where a debris field is present due to photoreceptor degeneration. (C') Quantification of the number of microglial cells which

915 responded to photoreceptor damage in the different aged groups. (D, top) The central retina
916 72 hours after light treatment onset, immunolabeled with BrdU (proliferation, in green). (D').
917 Quantification of the number of cells proliferating in the ganglion cell layer (GCL, represented
918 in black squares), inner nuclear layer (INL, represented in grey squares), and outer nuclear
919 layer (ONL, represented in white squares) in each age group. (D, bottom) The central retina
920 28 days after light treatment onset. (D'). Quantification of the number of BrdU+ cells observed
921 in the GCL, INL, and ONL of each aged group. Error bars represent the SEM. N=4-5. ns: non-
922 significant. Scale bars: 20μm.
923
924
925
926
927
928
929
930
931
932
933
934

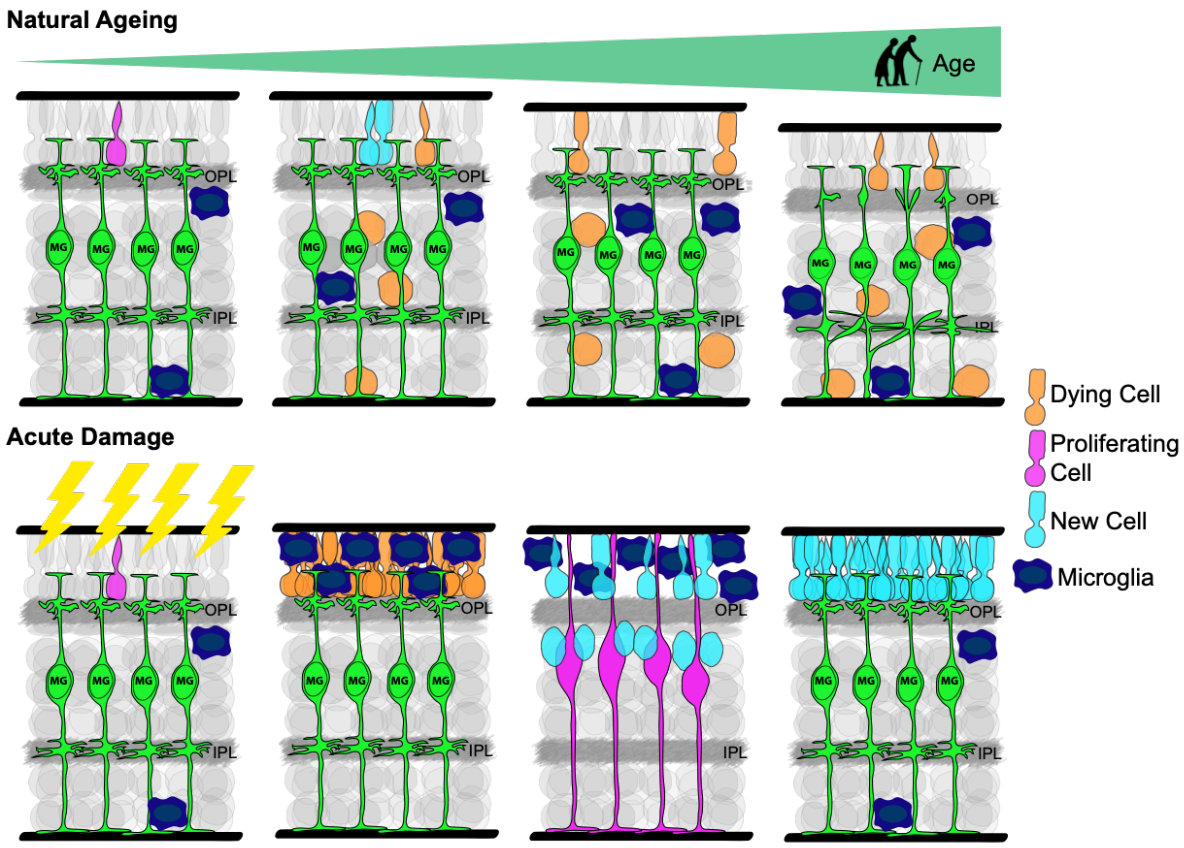
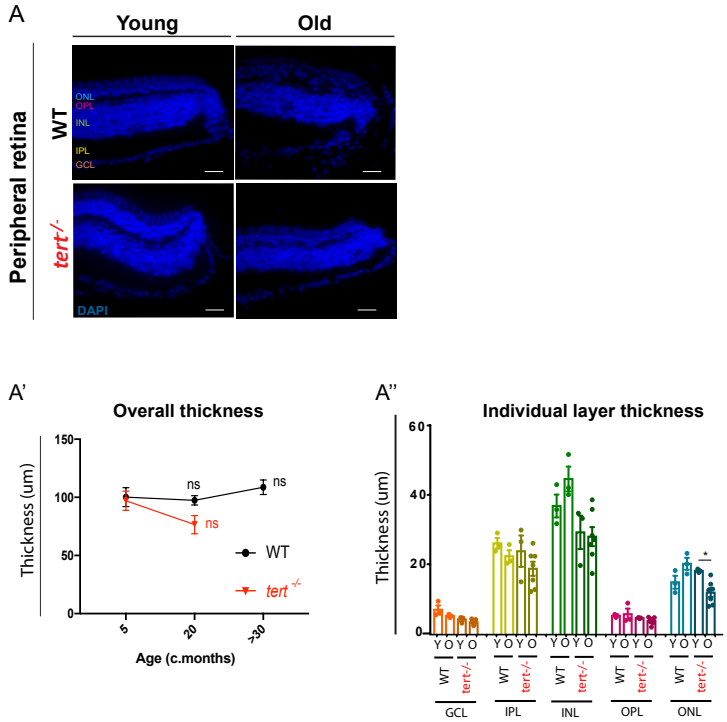


Figure 8: Our proposed model: A molecular "tipping point" required to stimulate regeneration in ageing. Schematic figure with the working model. Top: In natural ageing, zebrafish retina undergoes degeneration characterised by increased cell death and neuronal loss (represented in orange). Our current findings show that the proliferation levels are low in young ages and decrease even more with advancing age (represented in magenta). Moreover, the number of microglia, a key play in retina regeneration, is maintained stable throughout lifespan (represented in dark blue). The lack of MG proliferation in response to chronic, age-related damage seems to lead to retina thinning with ageing, where death cells are not replaced. In fact, instead of proliferating in response age-related chronic damage, zebrafish MG cells undergo a gliotic-like phenotype, similarly to humans. Despite maintaining Yap expression until old ages, MG cells do not stimulate regeneration of the aged zebrafish retina. Bottom: In contrast with age-associated chronic damage, in response to acute damage where there is a great number of dying cells, MG proliferates, generating new neuronal cells to replace the dying ones (represented in light blue). Here, we show that zebrafish gliotic MG cells retain their regenerative capacity in response to acute light damage until old ages (>30 months). These findings highlight key differences in the MG response to chronic versus acute damage and show that gliotic MG cells can be stimulated to repair damaged neurons in the old zebrafish retina.

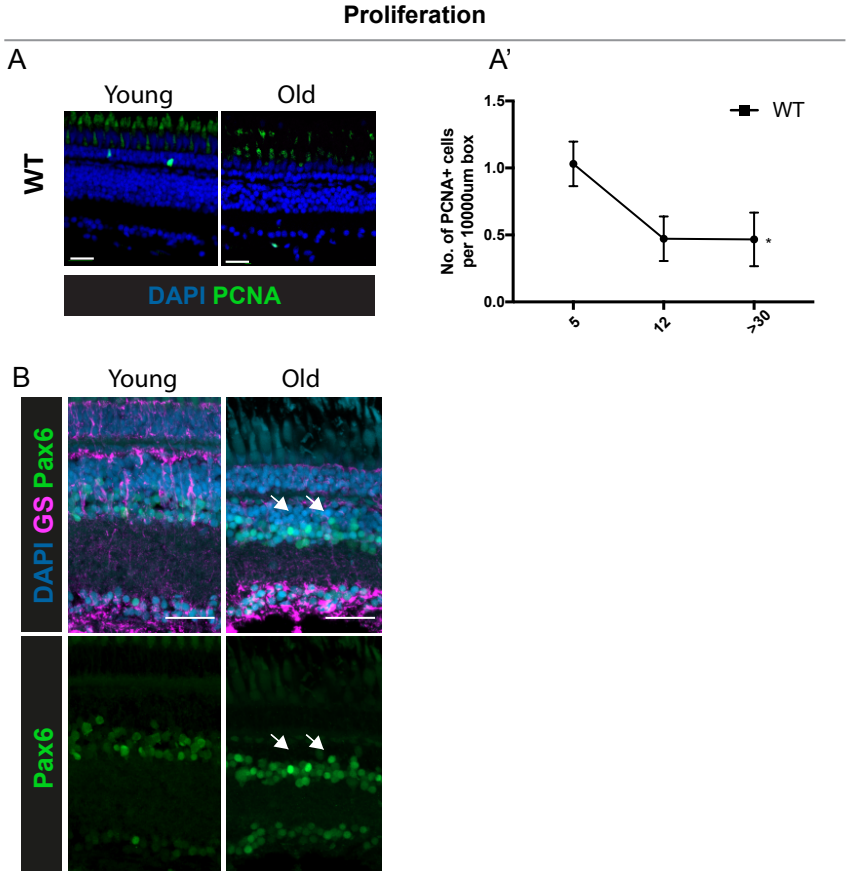
955 **Supplementary Data**

956 **Supplementary Table 1: Summary of the phenotypes observed in the aged zebrafish**
957 **retina, and which phenotypes are telomerase-dependent or independent.**

Phenotype of WT aged		Accelerated in tert$^{-/-}$?		Likely to be telomerase dependent?
		At 5 months	At 12 months (end life for tert$^{-/-}$)	
Visual impairment		No	No	No
Thinning of the central retina	GCL	No	N/A	No
	IPL	No	N/A	No
	INL	No	N/A	No
	OPL	No	N/A	No
	ONL	No	N/A	No
Neuronal degeneration in central retina	Rods morphology	No	N/A	No
	ZO-1 membrane	No	N/A	No
	Pre-synaptic ribbons (Ribeye)	No	N/A	No
	BCs (PKC in INL)	Yes	No	No
	ACs (HuCD in INL)	No	No	No
	GCs (HuCD in GCL)	No	No	No
Hallmarks of cellular ageing in central retina	Apoptosis	No	No	No
	DNA damage/ senescence	No	No	No
	Proliferation	No	No	No

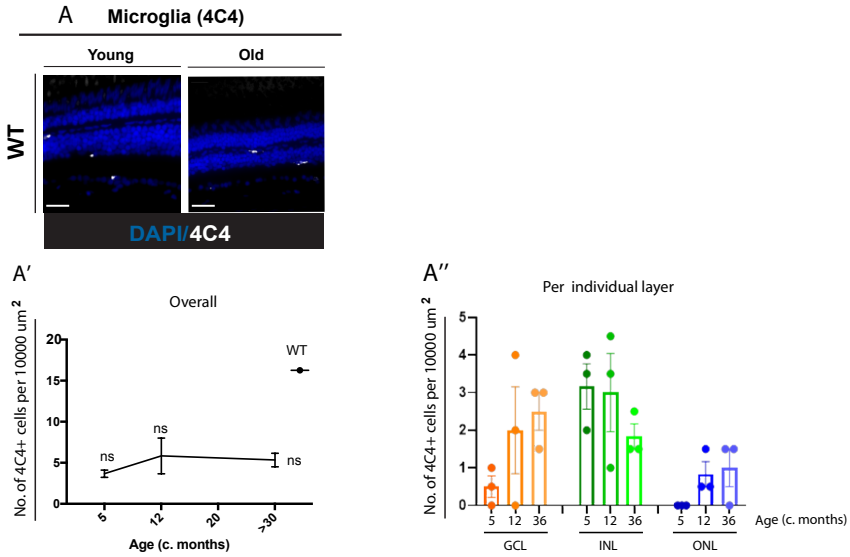


Supplementary Fig 1: Key hallmarks of ageing in the zebrafish peripheral retina. (A) Peripheral retina thickness in both WT and *tert*$^{-/-}$, at young (5 months) and old ages (>30 months in WT and 20 months in *tert*$^{-/-}$). Scale bars: 20µm. (A'-A'') Quantifications of the peripheral retina thickness cells per area (10 000µm^2), (A') in the overall retina and (A'') per layer of the retina. GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer. Error bars represent the SEM. N=3. P-value: * <0.05; ** <0.01; *** <0.001.

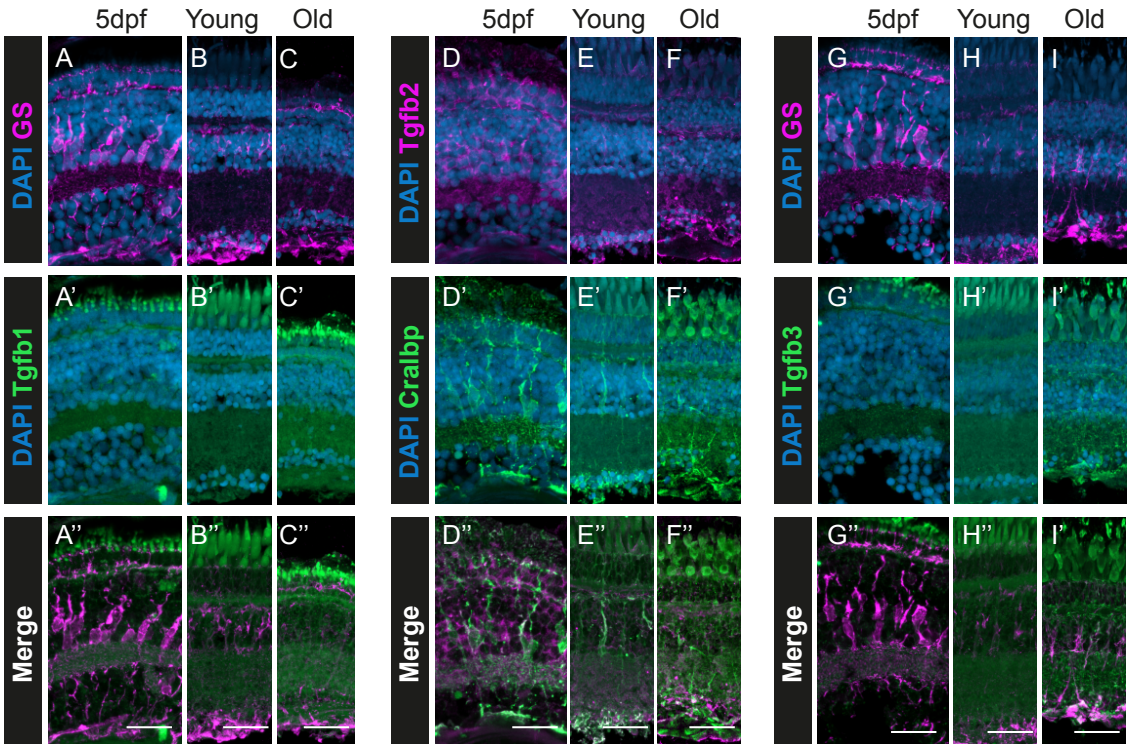


967

968 **Supplementary Figure 2: Lack of MG proliferation and dedifferentiation in the aged**
969 **zebrafish retina.** (A) The central retina immunolabeled with PCNA (cells proliferating, in
970 green) in WT at young (5 months) and old ages (>30 months). Scale bars: 20µm. (A')
971 Quantifications of the number of $PCNA^+$ cells per area (10 000μm^2). Error bars represent the
972 SEM. N=6-12. P-value: * <0.05; ** <0.01; *** <0.001. (B) We do not observe Pax6+ MG cells
973 or progenitors in the aged zebrafish retina (arrows). The central retina co-immunolabeled with
974 GS (MG cells, in magenta) and Pax6 (in green). N= 5.



Supplementary Figure 3: Microglia do not increase in number or distribution in the ageing zebrafish retina. (A) The central retina immunolabeled with 4C4 (microglia, in white) in WT zebrafish, from young (5 months) to old ages (>30 months). Scale bars: 20µm. (A', A'') Quantification of the number of 4C4-positive cells (microglia) per area (10 000 µm2) in the (A') overall retina and (A'') per layer of the retina. GCL: ganglion cell layer; INL: Inner nuclear layer; ONL: outer nuclear layer. Error bars represent the SEM. N=3. P-value: * <0.05; ** <0.01; *** <0.001.



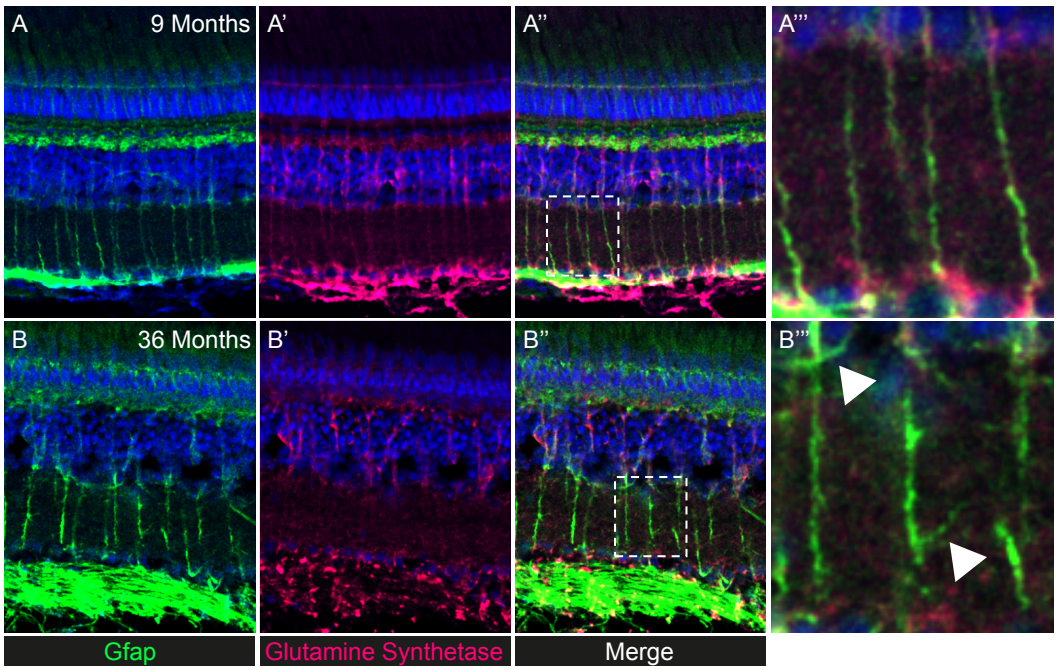
987

988 **Supplementary Figure 4: Expression of Tgfβ1, 2 and 3 in the zebrafish retina with**
989 **ageing.** We do not observe Tgfβ expression in MG cells in the old retina. The central retina
990 co-immunolabeled with (A-C) GS (MG cells, in magenta) and (A'-C') Tgfβ1 (in green); (D-F)
991 Tgfβ2 (in magenta) and (D'-F') Cralbp (MG cells, in green); and GS (MG cells, in magenta)
992 and Tgfβ3 (in green), in WT zebrafish, from young to old ages. Scale bars: 20μm.

993

994

995



Supplementary Figure 5: Mutant *albino* zebrafish display gliosis with ageing in MG similar to AB wildtype zebrafish. The central retina co-immunolabeled with (A, B) GFAP (reactive MG, in green) and (A', B') glutamine synthetase (MG, in magenta) in *albino* zebrafish at (A) 9 and (B) 36 months of age. (A''', B''') Insets of (A'', B'') merge staining showing disorganisation of MG processes in the IPL.