



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/162714/>

Version: Submitted Version

Article:

Martins, R.R., Zamzam, M., Moosajee, M. et al. (Submitted: 2020) Age-related degeneration leads to gliosis but not regeneration in the zebrafish retina. bioRxiv. (Submitted)

<https://doi.org/10.1101/2020.06.28.174821>

© 2020 The Author(s). For reuse permissions, please contact the Author(s).

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1 **Age-related degeneration leads to gliosis but not regeneration in the zebrafish retina**

2

3

4

5 **AUTHORS**

6 Raquel R. Martins^{1,2}, Mazen Zamzam³, Mariya Moosajee^{4,5,6,7}, Ryan Thummel³, Catarina M.
7 Henriques^{1,2§}, Ryan B. MacDonald^{4§}

8

9 **Affiliations:**

- 10 1. Bateson Centre, University of Sheffield, Sheffield, S10 2TN, UK.
11 2. Department of Oncology and Metabolism, University of Sheffield, Sheffield, S10 2TN
12 UK.
13 3. Department of Ophthalmology, Visual and Anatomical Sciences, Wayne State
14 University School of Medicine, Detroit, MI 48201, USA.
15 4. Institute of Ophthalmology, University College London, London, EC1V 9EL, UK.
16 5. Moorfields Eye Hospital NHS Foundation Trust, London, EC1V 2PD, UK
17 6. Great Ormond Street Hospital for Children NHS Foundation Trust, London, WC1N
18 3JH, UK
19 7. The Francis Crick Institute, London, NW1 1AT, London

20

21

22

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

24

25

26 **ABSTRACT**

27 Ageing is a significant risk factor for degeneration of the retina. Harnessing the regenerative
28 potential of Müller glia cells (MG) in the retina offers great promise for the treatment of
29 blinding conditions, such as age-related macular degeneration. Yet, the impact of ageing on
30 their regenerative capacity has not yet been considered. Here we show that MG retain their
31 ability regenerate after acute damage in the aged zebrafish retina. Despite this, we observe
32 chronic age-related neurodegeneration in the retina, which is insufficient to stimulate MG
33 proliferation and regeneration. Instead of regeneration, ageing leads to a gliotic response
34 and loss of vision, recapitulating hallmarks of human retinal degeneration with age.
35 Therefore we identify key differences in the MG regenerative response to acute versus
36 chronic damage, a key consideration for stimulating endogenous regenerative mechanisms
37 to treat human retinal disease.

38

39 **KEY WORDS:** retina, Müller glia, ageing, proliferation, degeneration, gliosis, zebrafish,
40 telomerase, regeneration

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61 INTRODUCTION

62 The physiology and structure of the healthy human eye is well known to degrade with age.
63 Hallmarks of ageing in the retina include tissue thinning, neuronal loss and reduced visual
64 function, especially in the macula ¹⁻⁶. Ageing is a significant risk factor for degeneration of
65 the retina, such as age-related macular degeneration (ARMD) or primary open angle
66 glaucoma⁵⁻⁷. As the population is ageing worldwide, the identification and potential treatment
67 of the underlying cellular and molecular dysfunctions in the aged retina will be critical for
68 global health going forward ⁸. Retinal degeneration is often a process that plays out over
69 months or years, whereby neurons gradually die leading to dysfunction ^{5,6}. Regenerative-
70 based therapies, such as stimulating endogenous MG to regenerate, offer great promise for
71 the treatment of various types of blindness that are caused by the loss of retinal neurons.
72 Thus, determining the effects of ageing on the regenerative potential of the retina is an
73 important consideration for the efficacy of such treatments going forward.

74

75 The principal cells tasked with maintaining the retina throughout life are the Müller glia (MG).
76 MG provide retinal neurons with a myriad of support functions, including trophic support,
77 neurotransmitter recycling and energy metabolism ⁹. While these functions are critical for
78 healthy retinal function, MG also have a prominent role during disease and after neuronal
79 insult. In the mammalian retina, neuronal damage results in a MG gliotic response, involving
80 the up-regulation of stress proteins, proliferation and morphology changes. This response is
81 thought to be neuroprotective initially, but may ultimately culminate in dysfunction and death
82 via loss of metabolic support or tissue integrity ¹⁰. In the zebrafish retina after acute damage
83 MG undergo a brief reactive gliosis-like phase that transitions into a regenerative response:
84 a de-differentiation and proliferation of the MG progenitor cell to specifically generate new
85 neurons and restore vision ¹¹⁻¹³. Intensive efforts have been made to uncover the molecular
86 mechanisms regulating these endogenous regenerative responses in the retinas of lower
87 vertebrates ¹⁴⁻¹⁹. While the mammalian retina lacks significant regenerative capacity ^{20,21}, re-
88 introduction of key molecular signals into MG has shown great promise to stimulate the
89 genesis of new neurons in mice after damage ²²⁻²⁴. These molecular mechanisms are largely
90 studied in the zebrafish using acute injury models, such as those generated by intense light
91 or toxins ^{16,25}. Multiple acute injuries do not appear to reduce the overall regenerative
92 response of MG; however, there does appear to be chronic inflammation after successive
93 rounds of injury ²⁶.

94

95 In contrast to acute injury, damage to the retina caused by degenerative disease or in
96 ageing manifest themselves over much longer timescales and result in the gradual

97 accumulation of DNA damage and cell death⁶. As such, chronic degeneration may
98 significantly differ from injury models, especially in its capacity to induce a regenerative
99 response from glia. The zebrafish is an emerging ageing model, as it displays key human-
100 like hallmarks of ageing in most tissues, including the retina^{27,28}. These include an age-
101 related decrease in cell density and increased cell death, suggesting chronic degeneration of
102 the retina with increasing age²⁷. Depending on the tissue, hallmarks of ageing also include
103 telomere shortening, DNA damage, decreased proliferation and apoptosis or senescence²⁹⁻
104 ³¹. Accordingly, the telomerase mutant (*tert*^{-/-}) zebrafish model is now an established
105 accelerated model of ageing²⁹⁻³¹, where regeneration is known to be impaired in tissues
106 such as the heart³². The *tert*^{-/-} model therefore offers the possibility of studying key aspects
107 of ageing in a shorter amount of time, and it allows the identification of telomerase-
108 dependent mechanisms of ageing, potentially involved in retinal regeneration²⁹⁻³¹.

109

110 Here, we used the well-established zebrafish retina as a model study the impact of ageing
111 on retinal regenerative capacity, with a particular focus on proliferation and maintenance of
112 neuronal structure and function^{19,33-37}. We show that MG retain their regenerative potential
113 into old age in response to acute damage, and are therefore capable of proliferating to
114 regenerate neurons dying throughout life. However, despite this potential, naturally aged and
115 telomerase deficient retinas display known hallmarks of retinal ageing, including tissue
116 thinning, accumulation of DNA damage and neuronal death. We show, by PCNA staining
117 and EDU labelling, that MG in fact do not proliferate in response to this chronic neuronal
118 degeneration. Instead, and in contrast to acute injury, ageing leads to a gliotic response and
119 loss of vision, recapitulating hallmarks of human retinal degeneration with age. We therefore
120 identify key differences in the MG regenerative response to acute versus chronic damage, a
121 key consideration in potential therapies aiming to stimulate endogenous regenerative
122 mechanisms to treat human retinal disease.

123

124 **RESULTS**

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

126 Studies on retinal regeneration typically focus on the young adult retina, and do not take old
127 age into account for the regenerative response. Several acute damage paradigms induce
128 retinal neuron death and elicit a regenerative response in zebrafish^{26,38-40}. Chronic damage
129 in the adult retina, specifically between 9-18 months of age, results in MG continuously re-
130 entering the cell cycle to proliferate and regenerate lost neurons, although they do show
131 signs of chronic activation at later timepoints²⁶. However, it remains unclear if MG retain

132 their ability to proliferate and regenerate the retina throughout life. To test this, we used the
133 light-damage model where aged zebrafish, at three stages of their lifecycle, were treated
134 with light to elicit photoreceptor damage. To detect increased proliferation of MG in response
135 to damage, a 3-day pulse of BrdU was performed, followed by a 28-days chase period (**Fig**
136 **1A**). After a 3-day pulse, BrdU should label both MG and their daughter cells, the newly-
137 formed progenitors (**Fig 1B**). However, since the BrdU staining dilutes in the rapidly-dividing
138 progenitor cells and MG only divide once, after a 28-days chase, the majority of the BrdU
139 staining is retained in MG (**Fig 1B**). Our results show that light-lesion in aged retinas leads to
140 a loss of photoreceptors, which is accompanied by a strong increase in microglia in the ONL,
141 typical of photoreceptor degeneration in this damage model (**Fig 1C, C'**). Moreover, there
142 are no differences in the incorporation of BrdU with ageing on any layer, at 72 hours post-
143 light damage (hpL), when the initial regenerative response is mounted, or at 28 dpL, when
144 the number of MG that initially re-entered the cycle can be identified (**Fig 1D, D'**). Both the
145 unaltered immediate timing of MG response and the overall capacity to regenerate each
146 neuronal layer are maintained with increased age. Together these results suggest that the
147 regenerative response remains intact throughout zebrafish lifespan.

148

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

150 Similar to humans, the zebrafish retina consists of three nuclear layers separated by two
151 synaptic plexiform layers. The nuclear layers consist in the outer nuclear layer (ONL),
152 containing photoreceptors; the inner nuclear layer (INL) containing bipolar cells (BCs),
153 amacrine cells (ACs), horizontal cells (HCs) and MG; and the ganglion cell layer (GCL)
154 mainly containing retinal ganglion cells (RGCs) (see diagram in **Figure 2A**). The macula is a
155 specialised region of the human retina thought to be the region central for vision ⁴¹. It
156 remains unclear if the zebrafish has a true macula, however the central vision in fishes does
157 focus on a particular zone within the retina⁴². A recent report suggests molecular and cellular
158 specialised zones critical for visual acuity ⁴³, so it is possible that a specialised “macula-like”
159 zone is present in the central region of the zebrafish retina. This central region can be
160 considered the “oldest” part of the retina, as it is generated at early developmental stages,
161 and where there is usually very little proliferation to generate new neurons ²⁸. In contrast to
162 the peripheral region of the retina, where the proliferative ciliary marginal zone (CMZ)
163 resides¹⁷, which is a population of progenitors found in the peripheral retina that is thought to
164 contribute to retinal growth throughout life.

165

166 Given that the zebrafish retina maintains its regenerative potential until old ages, then we
167 hypothesised that MG would regenerate dying neurons lost from the central retina until old
168 ages, thereby counter-acting any age-related neurodegeneration. If this were the case, then
169 removing telomerase, known to be important for proliferation^{30,31} and regeneration in
170 zebrafish³², should reduce MG's ability to proliferate, thereby accelerating retinal
171 degeneration. However, despite the MG's regenerative potential, the naturally aged wild type
172 (WT) central retina displayed hallmarks of age-related retinal degeneration. Cells in the aged
173 aged zebrafish retina accumulate DNA damage, as evidenced by the presence of strong
174 γ H2AX nuclear foci, a molecular marker of the DNA Damage Response (**Fig 2B, B'**). This
175 was accompanied by a significant increase in cell death, as assessed by the early cell death
176 labeling terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (**Fig 2C, C'**).
177 Increased DNA damage and cell death with ageing occur in a telomerase-independent
178 manner, as no differences are identified between genotypes at any time-point analysed.
179 Retinal thinning is a likely consequence of increased cell death and is a known hallmark of
180 human retinal ageing⁴⁴. Accordingly, we show that zebrafish central retina progressively
181 thins with ageing, consistent with previous studies^{27,28}. Moreover, as for increased DNA
182 damage and cell death, we show that this occurs largely independently of telomerase
183 (**Supplementary Fig 1A**).

184

185 We next sought to determine whether the cell death, and resulting retinal thinning, is specific
186 to any particular neuronal type. We used immunohistochemistry with several molecular
187 markers specific for different neuronal populations throughout the lifespan of zebrafish, in
188 the presence and absence of telomerase (*tert*^{-/-}). As expected, there was an overall
189 reduction in neuron numbers with ageing in the zebrafish retina (**Fig 2D**). Importantly, this
190 neuronal loss is not due to a decreased number of RGCs in the GCL (**Fig 2F**), but due to a
191 decreased number of HuC/D-expressing ACs (**Fig 2G**) and PKC-expressing BCs in the INL
192 layer (**Fig 2H**). Additionally, in the aged zebrafish retina, BCs display disorganised axon
193 terminals in the IPL. The RGCs, ACs and BCs are neurons that come together to make
194 connections in the major synaptic neuropil, called the inner plexiform layer (IPL). Thus,
195 degeneration of these neuronal populations is expected to affect connectivity in the IPL.
196 Accordingly, we confirmed alterations in the synaptic IPL layer, namely a reduction and
197 disorganisation of pre-synaptic terminals with ageing (**Supplementary Fig 1B**). None of
198 these neurodegenerative phenotypes were affected by depletion of telomerase, as there
199 were no differences between WT and *tert*^{-/-} at any of the tested ages.

200 The loss of photoreceptor integrity is one of the key features of human retina ageing and
201 disease⁴⁵ and we show that rod photoreceptor outer segments in the zebrafish retina

202 undergo dramatic structural changes with ageing (**Supplementary Fig 1C**), confirming
203 previous results ²⁷. These structural changes are accompanied by disruption of the tight
204 junction protein zonula occludens (ZO-1) (**Supplementary Fig 1D**), a marker for the outer
205 limiting membrane thought to be involved in photoreceptor degeneration ²⁸. These defects
206 are also not accelerated in the *tert*^{-/-} at any age tested, suggesting this is likely a telomerase-
207 independent phenomenon. Together, these data suggest that, despite maintaining
208 regenerative potential into old age, MG does not completely maintain the central retina in the
209 context of age-related damage.

210

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

212 While the progressive age-related retinal neurodegeneration would suggest a lack of
213 regenerative response by MG, it remains unclear whether these degenerations are being
214 counteracted, at least to some degree, by proliferation in the retina. To test this, we used an
215 EDU pulse-chase strategy to identify any cell divisions in the retina, which would be
216 indicative of potential regeneration. To characterise the steady state regenerative capacity of
217 the central and peripheral CMZ until old age, we carried out a 3-day pulse of EdU followed
218 by 0- and 30-days chase, in young and old WT and *tert*^{-/-} zebrafish (**Fig 3A, B**). We observe
219 few EdU-positive cells and, instead of a compensatory proliferation response, we detect
220 even less EdU-positive cells with ageing, suggesting overall reduced proliferative capacity in
221 the central retina (**Fig 3C**). These levels are further reduced after a 30-days chase (**Fig 3D**),
222 suggesting that there are very few cells proliferating in the aged central retina. In the
223 peripheral retina, where the proliferative CMZ resides, we see double of the EdU-retaining
224 cells than in the central retina at 0-days chase, (**Fig 3C**). Nevertheless, as in the central
225 retina, proliferation in the peripheral retina (**Fig 3 C and D**) decreases with ageing,
226 suggesting that there is no compensatory proliferation in response to the increased cell
227 death with ageing. Removing telomerase has no further effect on the already low and
228 decreasing levels of proliferation with ageing in the retina. Reduced proliferation with ageing
229 is therefore unlikely to be the driving mechanism for retinal degeneration.

230

231 As proliferation of MG is the primary source of neurons after injury in fish ^{14,38}, we sought to
232 determine whether there was any compensatory proliferation occurring specifically within
233 this cell population. We co-labelled cells with the MG specific marker glutamine synthetase
234 (GS), and counted the GS-positive; EdU-positive cells. We detected very few GS-positive;
235 EdU-positive cells in the central retina in both WT and *tert*^{-/-}, at any of the time-points
236 characterised throughout their lifespan (**Fig 3E, E'**), suggesting MG are also not proliferating

237 in the central retina at old ages. Thus, contrary to acute damage, our data show that age-
238 related chronic cell death does not trigger proliferation of MG cells. Finally, rods can
239 originate from rod-specific progenitors^{46,47}, which are found in the ONL and are derived from
240 MG that slowly divide in the WT retina^{17,38,48,49}. To test whether there was any compensatory
241 proliferation with ageing occurring from rod-specific progenitors found scattered throughout
242 the ONL of the retina^{36,46}, we quantified levels of EdU positive cells in the ONL. Although we
243 observe EDU+ cells in the central ONL of 5 months WT (likely to be rod precursors dividing),
244 there are few detected in >30 months old aged WT. Once again *tert*^{-/-} mutants had no further
245 effect (**Fig 3F**). Together, our data show that there is no compensatory proliferation in
246 response to age-related degeneration in the zebrafish retina, by any of the known sources of
247 regeneration and neurogenesis: CMZ, rod precursor cells or MG.

248

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

250 In the mammalian retina gliosis is a hallmark of retinal damage⁵⁰, but also commonly
251 observed in the aged retina⁵¹. Microglia are the innate immune cells found in the retina
252 (reviewed in⁵². Microglia are also key players in maintaining tissue homeostasis throughout
253 life and part of the gliosis process. They are activated in many neurodegenerative diseases,
254 with increased number at sites of damage, including in the photoreceptor layer in many
255 forms of retinal degeneration (reviewed in⁵¹. As we do not observe regeneration in the aged
256 zebrafish retina in response to neuronal death, we asked whether there are alterations in the
257 number of microglia (4C4-positive cells) found in the tissue. In contrast to what is observed
258 in acute damage paradigms (Fig 1B; ref) we observed very few microglia in the central retina
259 with no increase with ageing (**Fig 4A, A'**).

260

261 MG respond to damage or injury in most, if not all, neurodegenerative diseases¹⁰. The
262 characteristic mammalian response is gliosis, whereby MG change shape and up-regulate
263 structural proteins like GFAP to promote a neuroprotective effect^{9,10}. In the zebrafish retina,
264 the initial regenerative response to acute damage is similar to mammalian gliosis^{11,26}, and
265 gliosis can be aggravated if MG proliferation is blocked¹². Here, we identified MG using the
266 well-described GS immunolabelling and characterised MG reactivity using the gliosis marker
267 GFAP^{53,54}. Interestingly, we do not observe an overall loss of MG with ageing (**Fig 4 B, B'**),
268 in contrast to the observed neuronal death observed at the same stages (**Fig 2**). Despite
269 this, there is a significant change in the morphology of the MG cells in the WT aged retina.
270 Aberrations in the aged MG cells include disruptions in the radial morphology along the
271 synaptic IPL and basal lamina (**Fig 4C, C'**), which are known hallmarks of gliosis in retina

272 degeneration⁵¹. Qualitative assessment further shows that while all young fish display long
273 and aligned MG basal processes, 100% of the old fish show morphological disorganisation,
274 a hallmark of gliosis. Thus, similarly to humans, chronic neurodegeneration with ageing
275 elicits a gliotic response in the zebrafish retina rather than the regeneration typically
276 observed after acute injury.

277

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

279 Loss of visual acuity and contrast sensitivity with advancing age in humans has been well
280 documented^{5,6}. To determine whether the molecular and structural changes we observe
281 have a pathological consequence on the retinal function, we tested whether zebrafish have
282 impaired vision with ageing. Visual testing in the zebrafish has been used to screen for
283 mutants with defects in retinal development and function⁵⁵⁻⁵⁸, but had yet to be tested in the
284 context of ageing. The optokinetic response (OKR) is a well-established assay to measure
285 innate visual responses and provides readout of visual acuity^{55,59} (**Fig 5A**). Our results show
286 a decreased number of eye rotations per minute in aged fish (**Fig 5B, B' and videos 1 and**
287 **2**), suggesting that zebrafish vision declined with ageing. As for most of the hallmark
288 phenotypes of ageing described so far, (**Supplementary Table 1**), telomerase is not a
289 limiting factor for visual acuity, since the *tert*^{-/-} zebrafish do not display an accelerated
290 reduced visual acuity at 5 months or 12 months of age, close to the end point of *tert*^{-/-} life
291 (**Fig 5B, B' and videos 3 and 4**).

292

293 **DISCUSSION**

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

305

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

307 Previous work suggested that telomerase and telomere length are important for human
308 retinal health. In particular, the retinal pigment epithelium (RPE) has been reported to have
309 shorter telomeres than the neural retina, and it accumulates senescence over-time⁶⁰.
310 Accordingly, reactivation of telomerase has been described to ameliorate symptoms of AMD
311^{61,62} and telomerase activators are currently in clinical trials (e.g. NCT02530255). However,
312 retina homeostasis requires more than RPE maintenance. It requires a steady-state level of
313 proliferation of different cell types involved in multiple aspects of retina function. As in other
314 proliferative tissues²⁹⁻³¹, it would make sense that telomerase levels would influence
315 zebrafish retina homeostasis. We therefore hypothesised that the zebrafish retina would
316 degenerate in a telomerase-dependent manner, leading to vision loss. However, our data
317 suggest that most age-related changes in the zebrafish retina, including vision loss, are
318 largely telomerase independent, since depletion of telomerase (*tert*^{-/-}) does not accelerate or
319 exacerbate any of these phenotypes. Telomere dysfunction is known to affect mostly highly
320 proliferative tissues, reviewed in⁶³ and what our data show is that, in the region most
321 affected by ageing phenotypes, the central retina, there is very little proliferation to start with.
322 This suggests that replicative exhaustion of telomeres is not a limiting factor in the age-
323 associated degeneration of the central retina. Instead, it is likely that chronic exposure to
324 damaging agents such as oxidising UV radiation is the key driver of degeneration in the
325 retina, as was proposed in the “Wear and Tear Theory” (reviewed in⁶). However, we cannot
326 exclude that telomere-associated damage may still be a contributing factor to the observed
327 increased levels of DNA damage and cell death in the central retina. Telomeres are known
328 to be damaged by oxidative stress and can act as sinks of DNA damage, irrespectively of
329 length and levels of telomerase^{64,65}. In fact, this is a likely contributor to RPE damage with
330 ageing^{66,67}, potentially explaining why depleting telomerase has no effect on the proliferation
331 of the ONL, where photoreceptors reside, nor does it accelerate vision loss.

332

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

334 Our results show that zebrafish develop vision loss with ageing and that this is underpinned
335 by retinal neurodegeneration and gliosis. This could seem counter-intuitive, given that it is
336 well established that the zebrafish retina is capable of regenerating after acute damage.
337 Therefore, the lack of regeneration after age-related cell death observed in this study
338 suggest that there are critical differences between chronic ageing and repair after an acute
339 injury. Further supporting this, we show that this lack of regeneration in the context of ageing
340 is not due to an intrinsic inability of MG to proliferate *per se*, as we show they are still
341 capable of doing so in response to an acute injury in old animals. Several tissues in the
342 zebrafish retain the ability to regenerate throughout the lifespan of the zebrafish, including

343 the fin and the heart^{68,69}. Likewise, the optic nerve crush paradigm has shown that there is
344 successful recovery and function in the zebrafish retinotectal system⁷⁰, suggesting that
345 regeneration is still possible at advanced age in the retina. The regenerative capacity of
346 vertebrate tissues tends to decrease after repeated injury and as animals advance in age.
347 This is likely due to reduced progenitor cell proliferation potential and subsequent
348 differentiation⁷¹⁻⁷⁴. In the context of the central nervous system, this is aggravated by gliosis,
349 a reactive change in glial cells in response to damage. In the mammalian retina, MG cells
350 undergo gliosis in response to damage and in many retina degenerative diseases¹⁰.
351 However, in the zebrafish retina MG cells undergo the initial reactive gliotic response after
352 acute damage but quickly shift to the regenerative pathway. Here we show that while we
353 observe morphology alterations in MG cells in response to age-related neurodegeneration,
354 MG in the aged retina retain their ability to regenerate in response to acute damage of
355 photoreceptors. It is unclear if alternative damage paradigms will also lead to similar
356 regenerative response and potential in the aged zebrafish retina. It also remains unclear
357 what is the consequence of the gliotic response observed on retinal neurons in the aged
358 retina, as gliosis is classically thought of as “Janus-faced”, with both pro- and anti-
359 neuroprotective functions¹⁰. Importantly, the kinetics of these MG morphology and
360 molecular changes relative to neurodegeneration may provide further clues to the precise
361 cellular breakdown in the retina causing widespread neuronal death and dysfunction. That is
362 the MG cells react first, thereby abandoning their neuronal support functions and
363 precipitating neuronal damage, or do they purely respond to neuronal degeneration. Despite
364 the gliosis detected in the MG with ageing, their numbers are maintained, despite neuron
365 loss, suggesting that MG may be protected from age related death caused by the
366 accumulation of DNA damage or free radicals. It remains unclear if there is a molecular
367 mechanism inferring this protection or if MG gliosis is in itself protective to cell death.

368

369 **Our proposed model: A molecular “tipping point” required to stimulate regeneration** 370 **in ageing**

371 Regeneration studies so far have relied on several damage paradigms, including phototoxic
372²⁶ and ouabain induced lesions⁷⁵, which induce rapid cell death post-insult. While acute
373 damage models are suitable to explore the cellular and molecular mechanisms underpinning
374 the regenerative potential of the retina, they do not allow testing whether this regenerative
375 response is also occurring with natural ageing in the retina. “Natural ageing” and associated
376 stress-induced neuronal death play out over months or even years and we now show that
377 they do not elicit the same regenerative response. This may be because the slow
378 degeneration is not producing a strong enough signal in a short amount of time to induce

379 MG to undergo the cellular and molecular process of regeneration (**Fig 6; proposed**
380 **model**). It has been shown that the level of cell death can induce a differential response of
381 MG cells. Whilst a large amount of rod death causes a regenerative response, small
382 amounts do not^{25,76}. Furthermore, there may be distinct differences in signals released after
383 apoptosis or necrosis in ageing vs damage models^{77,78}. In support of this concept, recent
384 work suggests that there may be key signalling differences underpinning the difference
385 between a “regenerative” or a “reparative” response to injury⁷⁹. Thus, we propose that a
386 molecular signal, or expression changes of such signal, will regulate the “tipping point”
387 required to elicit a MG regenerative response in ageing. Research in the context of acute
388 damage paradigms in the zebrafish retina has uncovered many of the molecular
389 mechanisms regulating this regenerative response (reviewed in^{19, 37}). For instance,
390 proliferation appears to be a key mechanism for the initiation of the regenerative response
391 as blocking proliferation after damage in the zebrafish retina results in MG gliosis, and not
392 regeneration, similar to humans¹⁸. Moreover, age-associated degeneration of retinal
393 neurons and their synapses, as we observed to occur in this study, may result in a loss of
394 neurotransmitter release, such as GABA, which has been shown to facilitate the initiation of
395 MG proliferation⁸⁰. Recently, it has been suggested that electrical stimulation may promote
396 MG proliferation and expression of progenitor markers⁸¹. Thus, dysregulation of
397 neurotransmitters upon neurodegeneration could inhibit the key molecular pathways
398 regulating regeneration. Alternatively, the initial inflammatory response has also been shown
399 to be determinant for the repair process. Upon light-induced retinal damage, overexpression
400 and subsequent release of TNF by apoptotic photoreceptors seems to induce MG
401 proliferation^{11,82}. After acute damage, there is also an increased number of microglia in the
402 retina^{75,83} and they have been shown to be essential for retinal regeneration^{84,85}. In
403 contrast, microglia numbers do not increase with ageing and therefore may compromise the
404 regenerative response. Nonetheless, it is important to consider that the available
405 immunohistochemistry techniques to identify microglia number has a few limitations.
406 Namely, there may be a spike in microglia number in the aged retina that is rapidly resolved
407 and missed at our timepoints or microglia may undergo apoptosis similar to retinal neurons.
408 Thus, we cannot exclude the possibility that microglial cells are involved in the observed
409 retinal degenerations or play a critical role in the lack of a regenerative response observed in
410 the aged retina.

411

412 **Conclusions**

413 Our work demonstrates that, in the context of age-induced neuronal degeneration, the MG in
414 the zebrafish retina react by undergoing a response more akin to gliosis, rather than

415 regeneration. This resembles what happens in the aged human retina. Importantly, we
416 identify lack of proliferation and increasing gliosis as a key difference in MG response after
417 acute damage compared to the damage done through chronic aging.

418

419 **MATERIALS AND METHODS**

420 Zebrafish husbandry

421 Zebrafish were maintained at 27-28°C, in a 14:10 hour (h) light-dark cycle and fed twice a
422 day. The OKR was performed in the UCL Institute of Ophthalmology and the phototoxic
423 lesions and regeneration experiments were performed at Wayne State University School of
424 Medicine (USA). All other experiments were performed in the University of Sheffield. All
425 animal work was approved by local animal review boards, including the Local Ethical Review
426 Committee at the University of Sheffield (performed according to the protocols of Project
427 Licence 70/8681) and the Institutional Animal Care and Use Committee at Wayne State
428 University School of Medicine (performed according to the protocols of IACUC-19-02-0970).

429

430 Zebrafish strains, ages and sex

431 Three strains of adult zebrafish (*Danio rerio*) were used for these studies: wild-type (WT; AB
432 strain), *tert*^{-/-} (*tert*^{AB/hu3430}) and *albino* (*slc45a2*^{b4/b4}). *tert*^{-/-} zebrafish is a premature model of
433 ageing and therefore, age and die earlier than the naturally aged zebrafish. While *tert*^{-/-} fish
434 have a lifespan of 12-20 months, WT fish typically die between 36-42 months of age^{29,30}. In
435 order to study age-related phenotypes in the zebrafish retina, here we used young (5
436 months) WT and *tert*^{-/-} fish, alongside with middle aged (12 months) WT fish, and old WT and
437 *tert*^{-/-} fish. 'Old' was defined as the age at which the majority of the fish present age-
438 associated phenotypes, such as cachexia, loss of body mass and curvature of the spine.
439 These phenotypes develop close to the time of death and are observed at >30 months of
440 age in WT and at >12 months in *tert*^{-/-}^{29,30}. In addition, we used adult *albino* zebrafish for
441 retinal regeneration studies (described in detail below). Importantly, none of the animals
442 included in this study displayed visible morphological alterations in the eyes (e.g. cataracts).
443 Whenever possible, males were chosen to perform the experiments.

444

445 OKR assay

446 Fish were anaesthetised in 4% tricaine methanesulfonate (MS-222; Sigma-Aldrich) and
447 placed in a small bed-like structure made of sponge, located inside a small petri dish
448 containing fish water. During the experiment, fish were maintained still by the strategic use

449 of needles that sustained the sponges close to the fish so that the fish could not move. The
450 petri dish was then placed inside a rotation chamber with black and white striped walls
451 (8mm-thick stripes). After fish recovered from anaesthesia, the trial began, and the walls of
452 the chamber started rotating at 12rpm (for 1min to the left side followed by 1min to the right
453 side). Eye movements were recorded using a digital camera throughout the experiment.
454 After the experiment, the number of eye rotations per minute was measured by video
455 observation and manually counting. The counting was performed blindly by two independent
456 researchers. In the end, the results were normalised for the WT young from the same day /
457 batch, in order to control for different days of experiments.

458

459 Intense light-damage paradigm with BrdU incorporation

460 A photolytic damage model in adult *albino* zebrafish was utilised to destroy rod and cone
461 photoreceptors and elicit a regenerative response ²⁵. Briefly, adult *albino* zebrafish were
462 dark-adapted for 10 days prior to a 30 min exposure to ~100,000 lux from a broadband light
463 source. Next, fish were exposed to ~10,000 lux of light from four, 250 W halogen lamps for
464 24 hrs. Following 24 hrs of light treatment, fish were transferred to a 1L solution containing
465 0.66 g of NaCl, 0.1 g Neutral Regulator (SeaChem Laboratories, Inc. Stone Mountain, GA,
466 USA), and 1.5 g BrdU (5mM; B5002; Sigma-Aldrich) for 48 hrs. This timeframe for BrdU
467 incubation was based on previous studies in order to label MG cell-cycle re-entry ²⁶.
468 Following a 48 hour incubation in BrdU, the fish were split into two groups: one group was
469 euthanised by an overdose of 2-Phenoxyethanol and eyes were processed for
470 immunohistochemistry as described below; the second group returned to normal husbandry
471 conditions for an additional 25 days (or 28 days after light onset) prior to euthanasia and
472 tissue collection. During this time, BrdU incorporation dilutes in actively dividing cells,
473 allowing for clear visualization of the number of MG in the INL that only divide a single time.
474 It also serves as an indirect measure of regenerative capacity (i.e. an equal loss of BrdU-
475 positive cells at 28 dpL between experimental groups would indicate similar numbers of
476 progenitor cell divisions earlier in the regenerative process).

477

478 Tissue preparation: paraffin-embedded sections and cryosections

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

482 Paraffin-embedded sections. Whole fish were fixed in in 4% paraformaldehyde (PFA)
483 buffered at pH 7.0, at 4°C for 48-72h, decalcified in 0.5M EDTA at pH 8.0 for 48-72h, and
484 embedded in paraffin by the following series of washes: formalin I (Merck & Co, Kenilworth,
485 NJ, USA) for 10min, formalin II for 50min, ethanol 50% for 1h, ethanol 70% for 1h, ethanol
486 95% for 1h 30min, ethanol 100% for 2h, ethanol 100% for 2h 30min, 50:50 of ethanol 100% :
487 xilol for 1h 30min, xylene I for 3h, xylene II for 3h, paraffin I for 3h and paraffin II for 4h
488 30min. Paraffin-embedded whole fish were then sliced in sagittal 4µm-thick or coronal
489 16µm-thick sections, using a Leica TP 1020 cryostat.

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

497

498 Immunohistochemistry (IHC)

499 Before immunofluorescence staining, cryosections were hydrated in PBS at room
500 temperature (RT) for 10min, and paraffin-embedded sections were deparaffinised and
501 hydrated as follows: histoclear (Scientific Laboratory Supplies, Wilford, Nottingham, UK) 2x
502 for 5min, followed by ethanol 100% 2x for 5min, ethanol 90% for 5min, ethanol 70% for
503 5min, and distilled water 2x for 5min. After antigen retrieval in 0.01M citrate buffer at pH 6.0
504 for 10min, the sections were permeabilised in PBS 0.5% Triton X-100 for 10min and blocked
505 in 3% bovine serum albumin (BSA), 5% Goat Serum (or Donkey Serum), 0.3% Tween-20 in
506 PBS, for 1h. The slides were then incubated with the primary antibody at 4°C ON. After
507 washes in PBS 0.1% Tween-20 (3x 10min) to remove excess to primary antibody, the
508 sections were incubated with secondary antibody at RT for 1h. Finally, the slides were
509 incubated in 1µg/ml of 4',6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific) at RT
510 for 10min, washed in PBS 1x, and mounted with vectashield (Vector Laboratories,
511 Burlingame, CA, USA). The primary and secondary antibodies used in this study are
512 described in Table 1 and Table 2, respectively.

513

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

515 In paraffin-embedded sections, TUNEL was performed using the *In Situ Cell Death*
516 *Detection Kit, Fluorescein* (Merck & Co), following the manufacturer's instructions. Briefly,
517 after deparaffinisation, hydration, antigen retrieval and permeabilization, as described above,
518 the slides were incubated in enzyme and label solution (1:10) at 37°C for 1h. The slides
519 were then washed in 1x PBS (2x 10min) before blocking and incubation with primary
520 antibody.

521

522 5-Ethynyl-2'-deoxyuridine (EDU) labelling

523 EdU labelling was detected using the Click-iT® EdU Imaging Kit (Thermo Fisher Scientific),
524 following manufacturer's instructions. Briefly, fish were injected with 5µl of 10mM EdU
525 diluted in dimethyl sulfoxide (DSMO), by intraperitoneal (IP) injection, for 3 consecutive days
526 (3-day pulse). In order to differentiate proliferating cells and low-proliferative EdU-retaining
527 cells, the fish were separated into two groups: 0-day chase and 30-days chase groups. The
528 fish from the first group were culled 2h30min after the last injection of Edu, whereas the fish
529 from the second group were culled 30 days after the last injection of EdU. After culling,
530 whole fish were processed for paraffin-embedded sections as described above. In order to
531 detect EdU labelling in paraffin-embedded sections, the slides were deparaffinised,
532 hydrated, underwent antigen retrieval, were permeabilised and washed in 1x PBS. The
533 slides were incubated in freshly made EdU-labelling solution (per 1ml of solution: 860 1x
534 Click-iT®EdU reaction buffer, 40 CuSO₄, 2.5 Alexa Fluor® 647 azide working solution and
535 100 10x EdU reaction buffer additive) at RT for 30min. Finally, the slides were washed in 1x
536 PBS before blocking and incubation with primary antibody (ON, at 4°C). The incubation in
537 secondary antibody was performed as previously described.

538

539 Imaging and quantifications

540 Paraffin-embedded sections were imaged by epifluorescence microscopy, using a
541 DeltaVision microscope with a 40x oil objective. Cryosections were imaged by laser
542 scanning confocal imaging, using a Leica SP5 microscope or a Nikon A1 Confocal
543 microscope, with a 40x oil objective. In both cases, multiple 0.2-0.6µm thick z-stacks were
544 acquired in order to capture the whole section. For each staining, a total of 4 images were
545 taken per retina, 2 from central and 2 from peripheral retina. The central retina was defined
546 to be the centre point between opposing CMZs (A minimum of ~1000µm from the periphery),
547 while images of the peripheral retina contain the limits of the retina, including the CMZ. The
548 peripheral retina was defined as the tissue directly adjacent to the CMZ.

549

550 In order to quantify the staining, after a z-projection was generated, three boxes of
551 100x100µm were drawn in each field of view. The total number of positive cells was then
552 manually counted for each labelling. Rhodopsin, ZO1, ribeye and GFAP staining were an
553 exception to this, for which a qualitative assessment was performed instead. To do so,
554 structural and morphological defects were identified as follows. For the rhodopsin staining,
555 as young WT retinas usually display long and aligned outer segments, all short and/or
556 misaligned outer segments were considered defective. For the ZO1 staining, the average
557 number of breaks in the ZO1-labelled membrane per animal was quantified. The average of
558 breaks in the WT young animals was used as a reference, and any fish presenting an
559 average number of breaks below this average was considered to present defects in the
560 membrane. For the ribeye staining, young retinas usually present two distinguished layers of
561 pre-synaptic ribbons. Thus, staining where the two layers of pre-synaptic ribbons are not
562 distinguished, was considered defective. GFAP staining usually reveals long and aligned
563 MG processes in young WT retinas, and therefore short and/or misaligned MG processes
564 are considered gliotic. Through this qualitative assessment it was calculated the percentage
565 of fish per group presenting structural and morphological defects. Finally, raw images were
566 used for quantification purposes. The images were then processed with Adobe Illustrator
567 21.0.2 for display purposes.

568

569 Statistical analysis

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

580

581

582

Table 1. Primary antibodies used for immunostaining.

Antibody, species and type	Dilu tion fact or	Catalogue number; Company, City, Country
yH2AX rabbit polyclonal	0	1:50 GTX127342; GeneTex, Irvine, CA, USA
p53 rabbit polyclonal	0	1:20 AS-55342s; Labscoop, Little Rock, AR, USA
PCNA mouse monoclonal	0	1:50 NB500-106; Novus Biologicals, Littleton, CO, USA
PCNA rabbit polyclonal	0	1:50 GTX124496; GeneTex, Irvine, CA, USA
7.4.C4 (4C4) mouse monoclonal	0	1:10 A gift from A. McGown
HuCD mouse monoclonal	0	1:10 A21271; Thermo Fisher Scientific, Waltham, MA, USA
PKC β 1 rabbit polyclonal	0	1:10 Santa Cruz, Dallas, TX, USA
Ribeye rabbit polyclonal	0	1:10,000 A gift from Teresa Nicholson
GFAP rabbit polyclonal	0	1:200 Z0334, Agilent DAKO, Santa Clara, CA, USA
GFAP mouse monoclonal	0	1:100 zrf1, ZIRC
Glutamine Synthase (GS) mouse monoclonal	0	1:150 mab302, Merck, Kenilworth, NJ, USA
Rhodopsin rabbit	0	1:5,000 A gift from David Hyde
BrdU rat	0	1:200 OBT0030A, Accurate Chemical & Scientific, Westbury, NY, USA

583

584

585

Table 2. Secondary antibodies used for immunostaining.

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

586

587

588 **ACKNOWLEDGEMENTS**

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

599

600

601 **COMPETING INTERESTS**

602 The authors declare no competing interests.

603

604 **REFERENCES**

605

- 606 1. FJ, S. AGE NORMS OF REFRACTION AND VISION. *Arch Ophthalmol* **43**, 466–481
607 (1950).
- 608 2. Weale, R.A. Senile changes in visual acuity. *Trans Ophthalmol Soc U K* **95**, 36-8
609 (1975).
- 610 3. Arden, G.B. & Jacobson, J.J. A simple grating test for contrast sensitivity: preliminary
611 results indicate value in screening for glaucoma. *Invest Ophthalmol Vis Sci* **17**, 23-32
612 (1978).
- 613 4. Skalka, H.W. Effect of age on Arden grating acuity. *Br J Ophthalmol* **64**, 21-3 (1980).
- 614 5. Marshall, J. The ageing retina: physiology or pathology. *Eye (Lond)* **1 (Pt 2)**, 282-95
615 (1987).
- 616 6. Salvi, S.M., Akhtar, S. & Currie, Z. Ageing changes in the eye. *Postgrad Med J* **82**,
617 581-7 (2006).
- 618 7. Al-Ubaidi, M.R., Naash, M.I. & Conley, S.M. A perspective on the role of the
619 extracellular matrix in progressive retinal degenerative disorders. *Invest Ophthalmol*
620 *Vis Sci* **54**, 8119-24 (2013).
- 621 8. Chakravarthy, U. *et al.* Clinical risk factors for age-related macular degeneration: a
622 systematic review and meta-analysis. *BMC Ophthalmol* **10**, 31 (2010).
- 623 9. Reichenbach, A. & Bringmann, A. New functions of Muller cells. *Glia* **61**, 651-78
624 (2013).
- 625 10. Bringmann, A. *et al.* Cellular signaling and factors involved in Muller cell gliosis:
626 neuroprotective and detrimental effects. *Prog Retin Eye Res* **28**, 423-51 (2009).
- 627 11. Thomas, J.L., Ranski, A.H., Morgan, G.W. & Thummel, R. Reactive gliosis in the
628 adult zebrafish retina. *Exp Eye Res* **143**, 98-109 (2016).
- 629 12. Thummel, R. *et al.* Characterization of Muller glia and neuronal progenitors during
630 adult zebrafish retinal regeneration. *Exp Eye Res* **87**, 433-44 (2008).
- 631 13. Eastlake, K. *et al.* Comparison of proteomic profiles in the zebrafish retina during
632 experimental degeneration and regeneration. *Sci Rep* **7**, 44601 (2017).
- 633 14. Fausett, B.V. & Goldman, D. A role for alpha1 tubulin-expressing Muller glia in
634 regeneration of the injured zebrafish retina. *J Neurosci* **26**, 6303-13 (2006).
- 635 15. Fischer, A.J., McGuire, C.R., Dierks, B.D. & Reh, T.A. Insulin and fibroblast growth
636 factor 2 activate a neurogenic program in Muller glia of the chicken retina. *J Neurosci*
637 **22**, 9387-98 (2002).
- 638 16. Lenkowski, J.R. & Raymond, P.A. Muller glia: Stem cells for generation and
639 regeneration of retinal neurons in teleost fish. *Prog Retin Eye Res* **40**, 94-123 (2014).
- 640 17. Raymond, P.a., Barthel, L.K., Bernardos, R.L. & Perkowski, J.J. Molecular
641 characterization of retinal stem cells and their niches in adult zebrafish. *BMC*
642 *developmental biology* **6**, 36 (2006).
- 643 18. Thummel, R., Kassen, S.C., Montgomery, J.E., Enright, J.M. & Hyde, D.R. Inhibition
644 of Muller glial cell division blocks regeneration of the light-damaged zebrafish retina.
645 *Dev Neurobiol* **68**, 392-408 (2008).
- 646 19. Wan, J. & Goldman, D. Retina regeneration in zebrafish. *Curr Opin Genet Dev* **40**,
647 41-47 (2016).
- 648 20. Jadhav, A.P., Roesch, K. & Cepko, C.L. Development and neurogenic potential of
649 Muller glial cells in the vertebrate retina. *Prog Retin Eye Res* **28**, 249-62 (2009).
- 650 21. Ooto, S. *et al.* Potential for neural regeneration after neurotoxic injury in the adult
651 mammalian retina. *Proc Natl Acad Sci U S A* **101**, 13654-9 (2004).
- 652 22. Jorstad, N.L. *et al.* Stimulation of functional neuronal regeneration from Muller glia in
653 adult mice. *Nature* **548**, 103-107 (2017).

- 654 23. Karl, M.O. *et al.* Stimulation of neural regeneration in the mouse retina. *Proc Natl*
655 *Acad Sci U S A* **105**, 19508-13 (2008).
- 656 24. Jorstad, N.L. *et al.* STAT Signaling Modifies Ascl1 Chromatin Binding and Limits
657 Neural Regeneration from Muller Glia in Adult Mouse Retina. *Cell Rep* **30**, 2195-
658 2208 e5 (2020).
- 659 25. Thomas, J.L., Nelson, C.M., Luo, X., Hyde, D.R. & Thummel, R. Characterization of
660 multiple light damage paradigms reveals regional differences in photoreceptor loss.
661 *Exp Eye Res* **97**, 105-16 (2012).
- 662 26. Ranski, A.H., Kramer, A.C., Morgan, G.W., Perez, J.L. & Thummel, R.
663 Characterization of retinal regeneration in adult zebrafish following multiple rounds of
664 phototoxic lesion. *PeerJ* **6**, e5646 (2018).
- 665 27. Van Houcke, J. *et al.* Extensive growth is followed by neurodegenerative pathology in
666 the continuously expanding adult zebrafish retina. *Biogerontology* **20**, 109-125
667 (2019).
- 668 28. Fu, J., Nagashima, M., Guo, C., Raymond, P.A. & Wei, X. Novel Animal Model of
669 Crumbs-Dependent Progressive Retinal Degeneration That Targets Specific Cone
670 Subtypes. *Invest Ophthalmol Vis Sci* **59**, 505-518 (2018).
- 671 29. Carneiro, M.C. *et al.* Short Telomeres in Key Tissues Initiate Local and Systemic
672 Aging in Zebrafish. *PLoS genetics* **12**, e1005798 (2016).
- 673 30. Henriques, C.M., Carneiro, M.C., Tenente, I.M., Jacinto, A. & Ferreira, M.G.
674 Telomerase is required for zebrafish lifespan. *PLoS Genet* **9**, e1003214 (2013).
- 675 31. Anchelin, M. *et al.* Premature aging in telomerase-deficient zebrafish. *Dis Model*
676 *Mech* **6**, 1101-12 (2013).
- 677 32. Bednarek, D. *et al.* Telomerase Is Essential for Zebrafish Heart Regeneration. *Cell*
678 *Rep* **12**, 1691-703 (2015).
- 679 33. Richardson, R., Tracey-White, D., Webster, A. & Moosajee, M. The zebrafish eye-a
680 paradigm for investigating human ocular genetics. *Eye (Lond)* **31**, 68-86 (2017).
- 681 34. Gestri, G., Link, B.A. & Neuhauss, S.C. The visual system of zebrafish and its use to
682 model human ocular diseases. *Dev Neurobiol* **72**, 302-27 (2012).
- 683 35. Angueyra, J.M. & Kindt, K.S. Leveraging Zebrafish to Study Retinal Degenerations.
684 *Front Cell Dev Biol* **6**, 110 (2018).
- 685 36. Stenkamp, D.L. Development of the Vertebrate Eye and Retina. *Prog Mol Biol Transl*
686 *Sci* **134**, 397-414 (2015).
- 687 37. Lahne, M., Nagashima, M., Hyde, D.R. & Hitchcock, P.F. Reprogramming Muller Glia
688 to Regenerate Retinal Neurons. *Annu Rev Vis Sci* (2020).
- 689 38. Bernardos, R.L., Barthel, L.K., Meyers, J.R. & Raymond, P.A. Late-stage neuronal
690 progenitors in the retina are radial Muller glia that function as retinal stem cells. *J*
691 *Neurosci* **27**, 7028-40 (2007).
- 692 39. Cameron, D.A. Cellular proliferation and neurogenesis in the injured retina of adult
693 zebrafish. *Vis Neurosci* **17**, 789-97 (2000).
- 694 40. Hanovice, N.J. *et al.* Regeneration of the zebrafish retinal pigment epithelium after
695 widespread genetic ablation. *PLoS Genet* **15**, e1007939 (2019).
- 696 41. Provis, J.M., Penfold, P.L., Cornish, E.E., Sandercoe, T.M. & Madigan, M.C.
697 Anatomy and development of the macula: specialisation and the vulnerability to
698 macular degeneration. *Clin Exp Optom* **88**, 269-81 (2005).
- 699 42. Baden, T., Euler, T. & Berens, P. Understanding the retinal basis of vision across
700 species. *Nat Rev Neurosci* **21**, 5-20 (2020).
- 701 43. Yoshimatsu, T., Schroder, C., Nevala, N.E., Berens, P. & Baden, T. Fovea-like
702 Photoreceptor Specializations Underlie Single UV Cone Driven Prey-Capture
703 Behavior in Zebrafish. *Neuron* (2020).
- 704 44. Eriksson, U. & Alm, A. Macular thickness decreases with age in normal eyes: a study
705 on the macular thickness map protocol in the Stratus OCT. *Br J Ophthalmol* **93**,
706 1448-52 (2009).
- 707 45. Lo, S.N.a.A.C.Y. Protecting the Aging Retina. in *Neuroprotection* (ed. Ho, R.C.-
708 C.C.a.Y.-S.) (2019).

- 709 46. Johns, P.R. & Fernald, R.D. Genesis of rods in teleost fish retina. *Nature* **293**, 141-2
710 (1981).
- 711 47. 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 48. 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 49. 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 50. Bringmann, A. & Wiedemann, P. Muller glial cells in retinal disease. *Ophthalmologica*
719 **227**, 1-19 (2012).
- 720 51. Telegina, D.V., Kozhevnikova, O.S. & Kolosova, N.G. Changes in Retinal Glial Cells
721 with Age and during Development of Age-Related Macular Degeneration.
722 *Biochemistry (Mosc)* **83**, 1009-1017 (2018).
- 723 52. Cragolini, A.B. *et al.* Regional brain susceptibility to neurodegeneration: what is the
724 role of glial cells? *Neural Regen Res* **15**, 838-842 (2020).
- 725 53. Garcia, M. & Vecino, E. Role of Muller glia in neuroprotection and regeneration in the
726 retina. *Histol Histopathol* **18**, 1205-18 (2003).
- 727 54. Verardo, M.R. *et al.* Abnormal reactivity of muller cells after retinal detachment in
728 mice deficient in GFAP and vimentin. *Invest Ophthalmol Vis Sci* **49**, 3659-65 (2008).
- 729 55. Bockerhoff, S.E. *et al.* A behavioral screen for isolating zebrafish mutants with visual
730 system defects. *Proc Natl Acad Sci U S A* **92**, 10545-9 (1995).
- 731 56. Neuhauss, S.C. *et al.* Genetic disorders of vision revealed by a behavioral screen of
732 400 essential loci in zebrafish. *J Neurosci* **19**, 8603-15 (1999).
- 733 57. Gross, N.E., Aizman, A., Brucker, A., Klanicnik, J.M., Jr. & Yannuzzi, L.A. Nature and
734 risk of neovascularization in the fellow eye of patients with unilateral retinal
735 angiomatous proliferation. *Retina* **25**, 713-8 (2005).
- 736 58. Lessieur, E.M. *et al.* Ciliary genes *arl13b*, *ahi1* and *cc2d2a* differentially modify
737 expression of visual acuity phenotypes but do not enhance retinal degeneration due
738 to mutation of *cep290* in zebrafish. *PLoS One* **14**, e0213960 (2019).
- 739 59. Tappeiner, C. *et al.* Visual acuity and contrast sensitivity of adult zebrafish. *Front*
740 *Zool* **9**, 10 (2012).
- 741 60. Drigeard Desgarnier, M.C. *et al.* Telomere Length Measurement in Different Ocular
742 Structures: A Potential Implication in Corneal Endothelium Pathogenesis. *Invest*
743 *Ophthalmol Vis Sci* **57**, 5547-5555 (2016).
- 744 61. Dow, C.T. & Harley, C.B. Evaluation of an oral telomerase activator for early age-
745 related macular degeneration - a pilot study. *Clin Ophthalmol* **10**, 243-9 (2016).
- 746 62. Rowe-Rendleman, C. & Glickman, R.D. Possible therapy for age-related macular
747 degeneration using human telomerase. *Brain research bulletin* **62**, 549-53 (2004).
- 748 63. Henriques, C.M. & Ferreira, M.G. Consequences of telomere shortening during
749 lifespan. *Curr Opin Cell Biol* **24**, 804-8 (2012).
- 750 64. Fumagalli, M. *et al.* Telomeric DNA damage is irreparable and causes persistent
751 DNA-damage-response activation. *Nat Cell Biol* **14**, 355-65 (2012).
- 752 65. Hewitt, G. *et al.* Telomeres are favoured targets of a persistent DNA damage
753 response in ageing and stress-induced senescence. *Nat Commun* **3**, 708 (2012).
- 754 66. Honda, S., Hjelmeland, L.M. & Handa, J.T. Oxidative stress--induced single-strand
755 breaks in chromosomal telomeres of human retinal pigment epithelial cells in vitro.
756 *Invest Ophthalmol Vis Sci* **42**, 2139-44 (2001).
- 757 67. Wang, J. *et al.* Photosensitization of A2E triggers telomere dysfunction and
758 accelerates retinal pigment epithelium senescence. *Cell Death Dis* **9**, 178 (2018).
- 759 68. Itou, J., Kawakami, H., Burgoyne, T. & Kawakami, Y. Life-long preservation of the
760 regenerative capacity in the fin and heart in zebrafish. *Biol Open* **1**, 739-46 (2012).
- 761 69. Pinto-Teixeira, F. *et al.* Inexhaustible hair-cell regeneration in young and aged
762 zebrafish. *Biol Open* **4**, 903-9 (2015).

- 763 70. Van Houcke, J. *et al.* Successful optic nerve regeneration in the senescent zebrafish
764 despite age-related decline of cell intrinsic and extrinsic response processes.
765 *Neurobiol Aging* **60**, 1-10 (2017).
- 766 71. Collins, C.A., Zammit, P.S., Ruiz, A.P., Morgan, J.E. & Partridge, T.A. A population of
767 myogenic stem cells that survives skeletal muscle aging. *Stem Cells* **25**, 885-94
768 (2007).
- 769 72. Janzen, V. *et al.* Stem-cell ageing modified by the cyclin-dependent kinase inhibitor
770 p16INK4a. *Nature* **443**, 421-6 (2006).
- 771 73. Nave, K.A. Neuroscience: An ageing view of myelin repair. *Nature* **455**, 478-9 (2008).
- 772 74. Kirschner, D.A. *et al.* Rapid assessment of internodal myelin integrity in central
773 nervous system tissue. *J Neurosci Res* **88**, 712-21 (2010).
- 774 75. Mitchell, D.M., Sun, C., Hunter, S.S., New, D.D. & Stenkamp, D.L. Regeneration
775 associated transcriptional signature of retinal microglia and macrophages. *Sci Rep* **9**,
776 4768 (2019).
- 777 76. Montgomery, J.E., Parsons, M.J. & Hyde, D.R. A novel model of retinal ablation
778 demonstrates that the extent of rod cell death regulates the origin of the regenerated
779 zebrafish rod photoreceptors. *J Comp Neurol* **518**, 800-14 (2010).
- 780 77. Bergmann, A. & Steller, H. Apoptosis, stem cells, and tissue regeneration. *Sci Signal*
781 **3**, re8 (2010).
- 782 78. Nelson, C.M. *et al.* Tumor necrosis factor-alpha is produced by dying retinal neurons
783 and is required for Muller glia proliferation during zebrafish retinal regeneration. *J*
784 *Neurosci* **33**, 6524-39 (2013).
- 785 79. Conedera, F.M. *et al.* Diverse Signaling by TGFbeta Isoforms in Response to Focal
786 Injury is Associated with Either Retinal Regeneration or Reactive Gliosis. *Cell Mol*
787 *Neurobiol* (2020).
- 788 80. Rao, M.B., Didiano, D. & Patton, J.G. Neurotransmitter-Regulated Regeneration in
789 the Zebrafish Retina. *Stem Cell Reports* **8**, 831-842 (2017).
- 790 81. Enayati, S. *et al.* Electrical Stimulation Induces Retinal Muller Cell Proliferation and
791 Their Progenitor Cell Potential. *Cells* **9**(2020).
- 792 82. Iribarne, M., Hyde, D.R. & Masai, I. TNFalpha Induces Muller Glia to Transition From
793 Non-proliferative Gliosis to a Regenerative Response in Mutant Zebrafish Presenting
794 Chronic Photoreceptor Degeneration. *Front Cell Dev Biol* **7**, 296 (2019).
- 795 83. Mitchell, D.M., Lovel, A.G. & Stenkamp, D.L. Dynamic changes in microglial and
796 macrophage characteristics during degeneration and regeneration of the zebrafish
797 retina. *J Neuroinflammation* **15**, 163 (2018).
- 798 84. Conedera, F.M., Pousa, A.M.Q., Mercader, N., Tschopp, M. & Enzmann, V. Retinal
799 microglia signaling affects Muller cell behavior in the zebrafish following laser injury
800 induction. *Glia* **67**, 1150-1166 (2019).
- 801 85. White, D.T. *et al.* Immunomodulation-accelerated neuronal regeneration following
802 selective rod photoreceptor cell ablation in the zebrafish retina. *Proc Natl Acad Sci U*
803 *S A* **114**, E3719-E3728 (2017).
- 804
- 805
- 806
- 807

808 **Figures & legends**

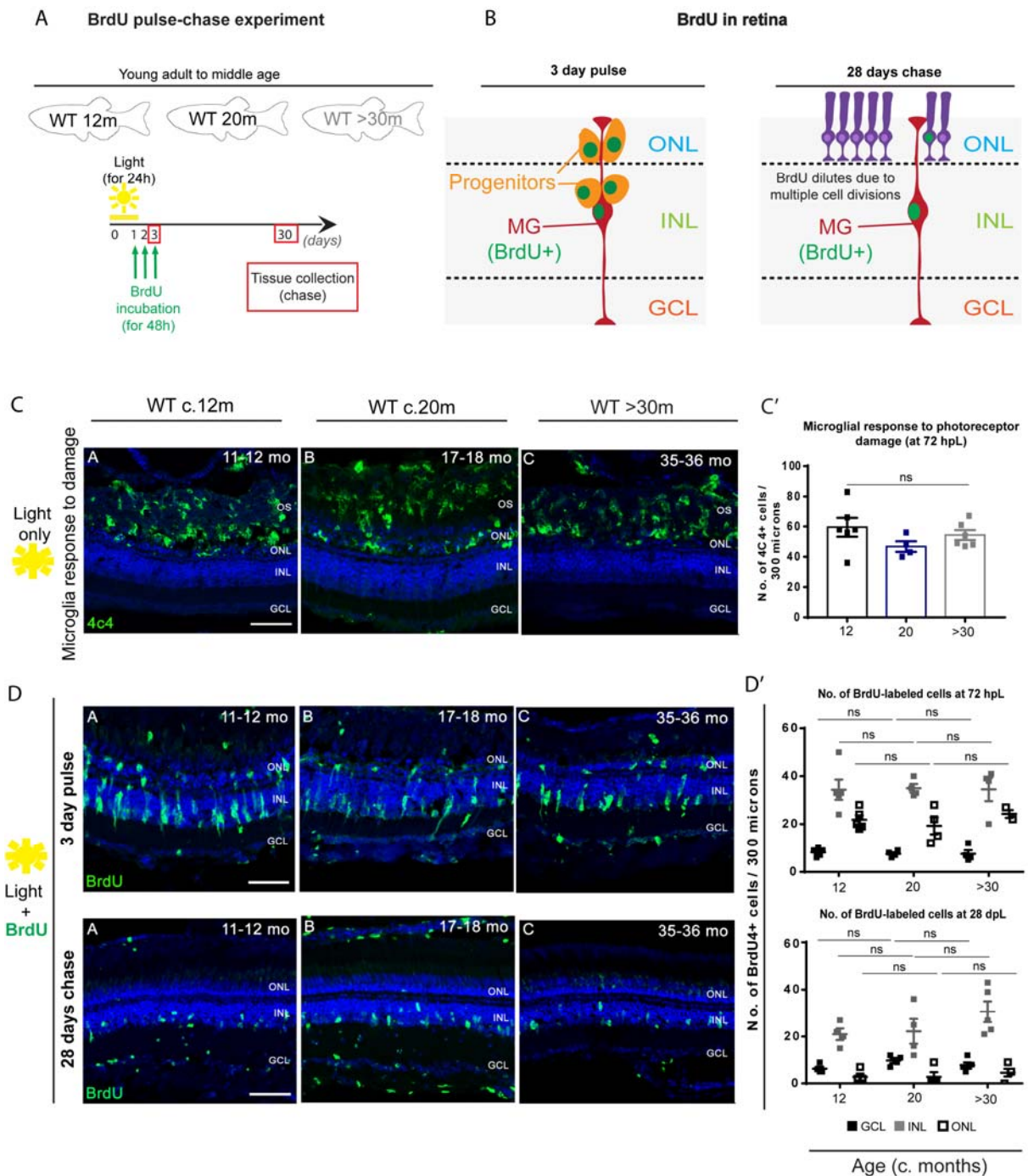


Figure 1. Zebrafish MG regenerative capacity upon acute damage is maintained until old ages.

(A) Schematic image of the experimental design. (B) Schematic image of the 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) Representative images of the central retina labelled with 4C4 (microglia, in green), after light onset, in young (12 months), middle aged (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 responded to photoreceptor damage in the different aged groups (young represented in black, middle aged in blue and old in grey). (D) Representative images of the central retina 72 hours after light onset, immunolabeled with BrdU (proliferation, in green). (D'). Quantification of the number of cells proliferating in the ganglion cell layer (GCL, represented in black squares), inner nuclear layer (INL, represented in grey squares), and outer nuclear layer (ONL, represented in white squares) in each age group. (D) Representative images of the central retina 28 days after light onset. (D'). Quantification of the number of BrdU+ cells observed in the GCL, INL, and ONL of each aged group. Error bars represent the standard error of the mean (SEM). N=4-5. Stats: One-way ANOVA followed by Turkey post-hoc tests.

809

810

811

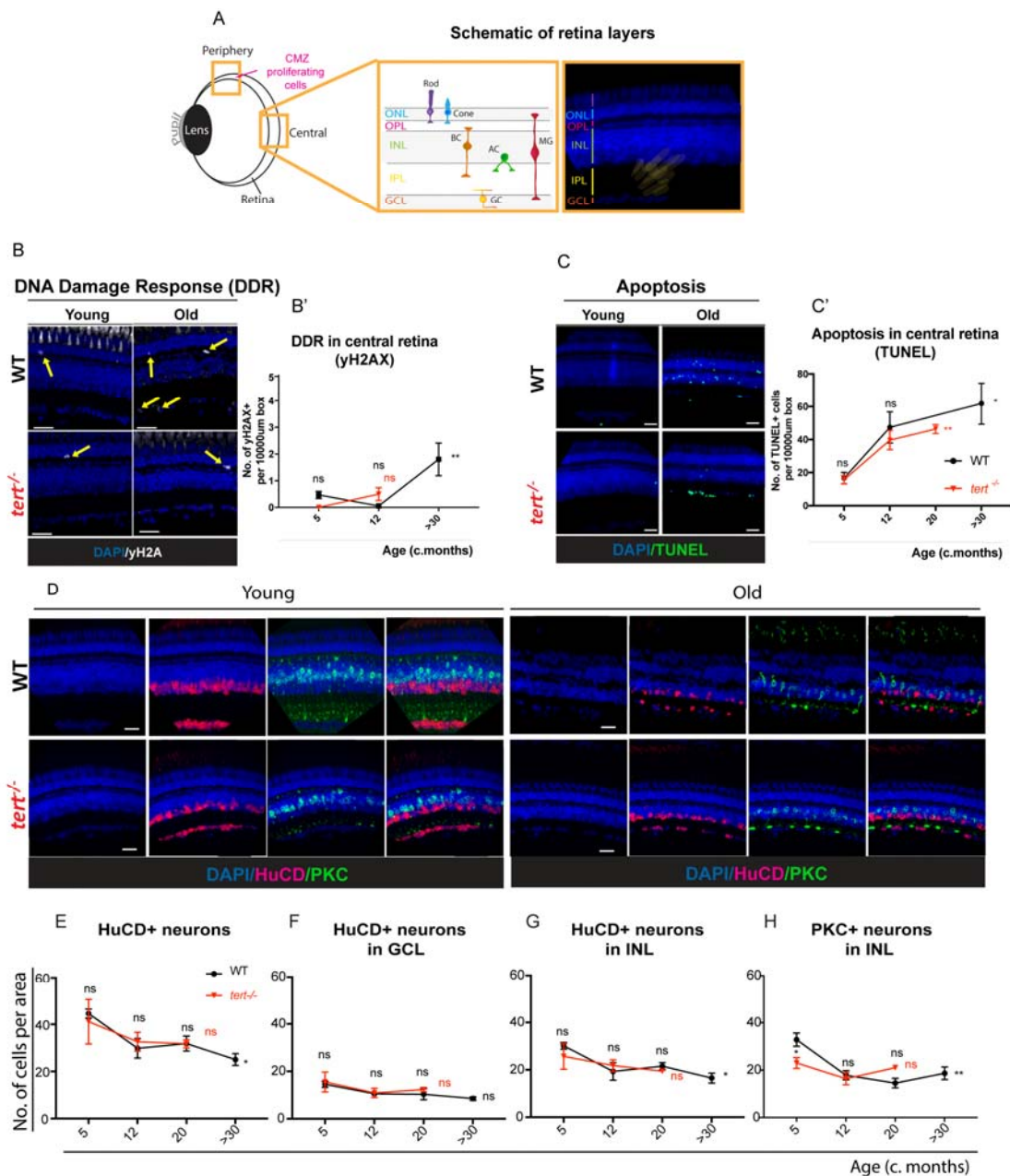


Figure 2. The ageing zebrafish retina displays neurodegeneration, independently of telomerase. (A) Schematic figure of the zebrafish retina with respective layers and cell types. (B-C) The central retina immunolabeled with (B) γ H2AX (DNA damage, in white) and (C) TUNEL (apoptotic cells, in green) in both WT and *tert*^{-/-}, at young (5 months) and old ages (>30 months in WT and 12 or 20 in *tert*^{-/-}). Scale bars: 20 μ m. (B) Yellow arrows highlight γ H2AX⁺ cells. (B'-C') Quantifications of the number of (B') γ H2AX⁺ cells and (C')

TUNEL⁺ cells. (D) The central retina immunolabeled with HuC/D and PKC (amacrine in magenta and bipolar cells in green, respectively), in both WT and *tert*^{-/-}, at young (5 months) and old ages (>30 months in WT and 12 months in *tert*^{-/-}). Scale bars: 20μm. (E-H) Quantifications of the number of HuCD-positive neurons (E) in the overall retina, (F) in the GCL (ganglion cells) and (G) in the INL (amacrine cells). (H) Quantifications of the number of PKC-positive neurons in the INL (bipolar cells). (B', C', E-H) Error bars represent the SEM. N=3-4. The quantifications were performed per area of the retina (100 000 μm²). Differences between genotypes are indicated above each timepoint, whereas differences over-time in each genotype are indicated in the end of the lines, in black WT, in red *tert*^{-/-}. P-value: * <0.05; ** <0.01; *** <0.001.

812

813

814

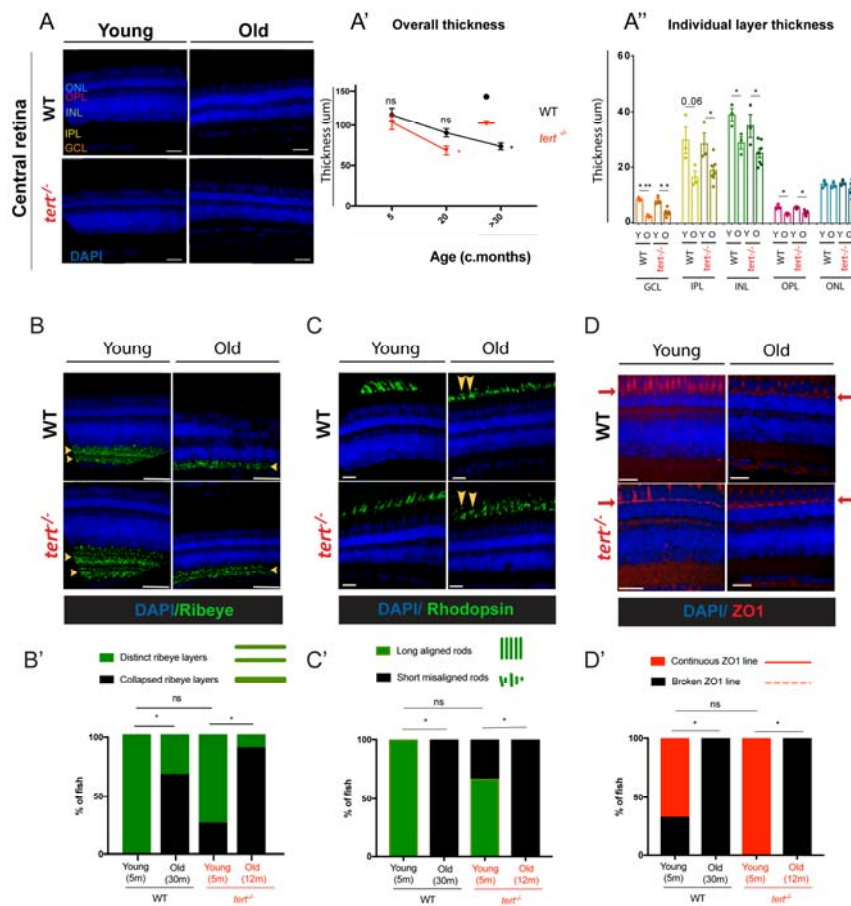
815

816

817

818

819



Supplementary Figure 1: Hallmarks of ageing in the zebrafish retina. (A) Central retina thinning 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') Quantifications of the central retina thickness, (A') in the overall retina and (A'') per layer of the retina. Error bars represent the SEM. N=3. (B-D) The central retina immunolabeled with (B) rhodopsin (photoreceptors outer segments, in green), (C) ZO1 (outer limiting membrane, in red) and (D) ribeye (pre-synaptic ribbons, in green), in both WT and *tert*^{-/-}, at young (5 months) and old ages (>30 months in WT and 12 months in *tert*^{-/-}). Scale bars: 20µm. (B'-D') Quantification of the percentage of fish presenting defects in (B') rhodopsin (short and misaligned outer segments), (C') ZO1 (broken outer limiting membrane) and (D') ribeye (collapsed ribeye layers). Error bars represent the SEM. (B'-C') N=3-6; (D') N=4-9. Differences between genotypes are indicated above each time-point, whereas differences over-time in each genotype are indicated in the end of the lines, in black WT, in red *tert*^{-/-}. (B'-D') Qui-square. P-value: * <0.05; ** <0.01; *** <0.001.

820

821

design: 3-day pulse of EdU, by IP injection, followed by 0- or 30-day chase; (B) and respective hypothesised result. We anticipated 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 in order 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 WT at young (5 months), middle (12 months) and old ages (>30 months). Scale bars: 20 μ m. Graphs below show quantifications of the number of EdU-retaining cells per area (100 000 μ m²), in the overall central and peripheral retina at 0-days chase (C) and in the overall central and peripheral retina at 30-days chase (D). (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 WT at young (5 months) and old ages (>30 months). Scale bars: 20 μ m. (E') Quantifications of the number of GS⁺; EdU⁺ cells per area (100 000 μ m²). Error bars represent SEM. N=3-6. CMZ: ciliary marginal zone. Statistics: Two-way ANOVA (WT vs *tert*^{-/-} at young versus old ages) and one-way ANOVA (WT and *tert*^{-/-} over-time). F) Quantifications of the number of EdU-retaining cells per area (100 000 μ m²), per layer of the retina, at 0-days chase. Error bars represent SEM. N=3-6. Differences between genotypes are indicated above each timepoint, whereas differences over-time in each genotype are indicated in the end of the lines, in black WT, in red *tert*^{-/-}. P-value: * <0.05; ** <0.01; *** <0.001.

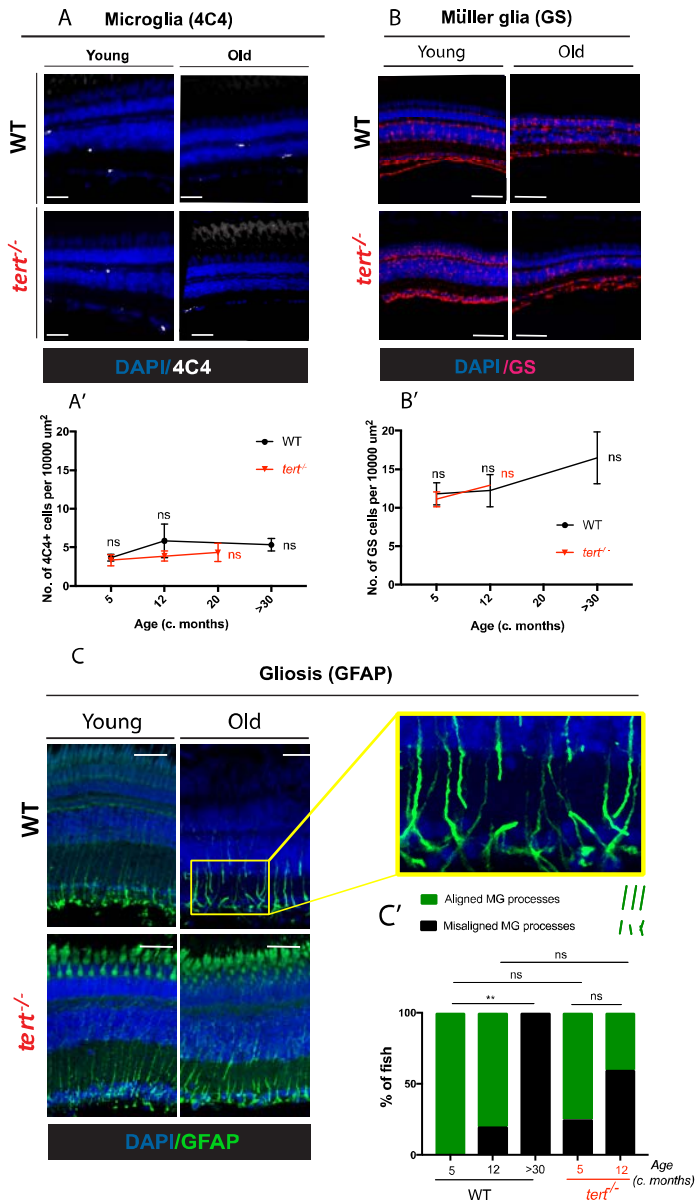


Figure 4. The zebrafish retina shows signs of gliosis with ageing, independently of telomerase. (A-B) The central retina immunolabeled with (A) 4C4 (microglia, in white) and (B) GS (Müller glia, in red), in WT, at young (5 months) and old ages (>30 months). Bars: 20μm. (A'-B') Quantification of the number of (A') 4C4-positive cells (microglia) and (B') GS-positive cells (MG), per area (100 000 μm²). Error bars represent the SEM. N=3. (C) The central retina immunolabeled with GFAP (reactive MG, in green), in WT at young (5 months), middle (12 months) and old ages (>30 months). Bars: 20μm. (C') Quantifications of the percentage of fish presenting disorganised MG processes (gliosis-phenotype). N= 3-6. Differences between genotypes are indicated above each time-point, whereas

differences over-time in each genotype are indicated in the end of the lines, in black WT, in red *tert*^{-/-}. (C) chi-square. P-value: * <0.05; ** <0.01; *** <0.001.

822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855

856

A Vision: OptoKinetic Response OKR

857

858

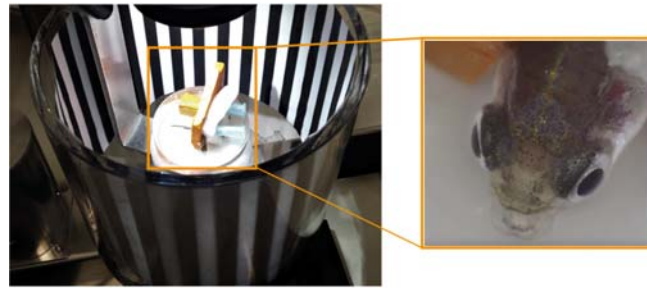
859

860

861

862

863



A'

864

865

866

867

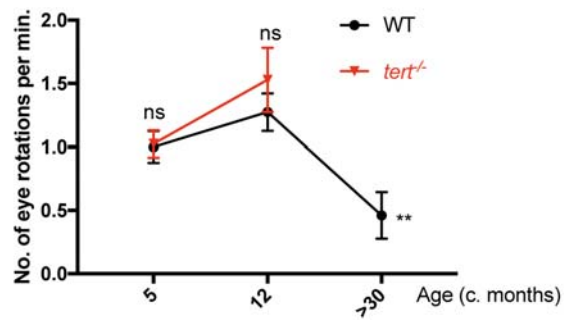
868

869

870

871

872



873

Figure 5: Zebrafish vision declines with ageing, independently of telomerase. (A-B)

874

OKR assay was performed by immobilising the fish in between soft sponges, inside a petri

875

dish containing water, placed in the centre of a rotation chamber. The walls of the rotation

876

chamber had 0.8 mm-thick black and white stripes and the chamber was maintained at a

877

constant velocity of 12 rpm throughout the experiment. (B') The number of eye rotations per

878

minute was manually quantified by video observation. Error bars represent the SEM. N=5-8.

879

Differences between genotypes are indicated above each timepoint, whereas differences

880

over-time in each genotype are indicated in the end of the lines, in black WT, in red *tert*^{-/-}. P-

881

value: * <0.05; ** <0.01; *** <0.001.

882

883

884

885

886

887

888

889

890

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

891

892 **Supplementary Table 1: Summary of the phenotypes observed in the aged zebrafish**

893 **retina, and which phenotypes are telomerase-dependent or independent.**

894

895

Natural Ageing

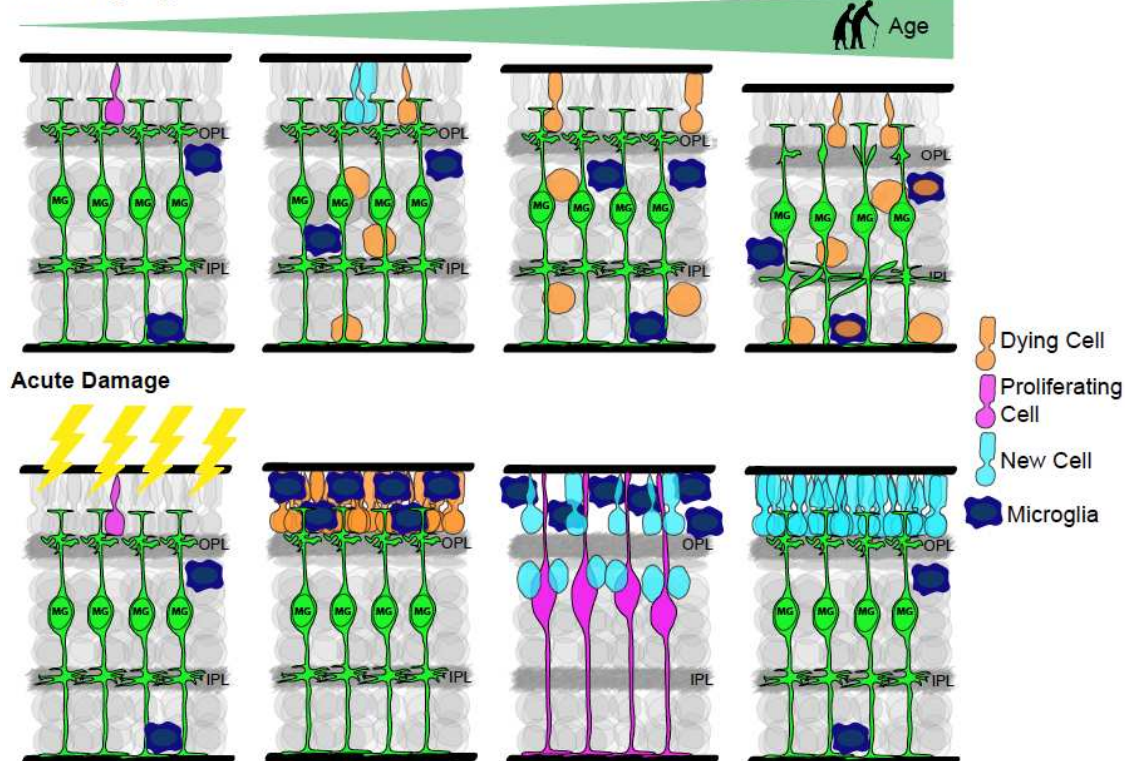


Figure 6: Our proposed model: A molecular “tipping point” required to stimulate regeneration in ageing. While zebrafish can regenerate the retina upon acute damage until late ages, its regenerative capacities do not prevent cell death, degeneration and consequent retina thinning with natural ageing. Schematic figure with the working model. In natural ageing, zebrafish retina undergoes degeneration characterised by increased cell death and neuronal loss. 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, with an increased number of microglia dying. 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. Middle: In contrast, in response to acute damage where there is a great number of dying cells followed by recruitment of microglia, MG proliferates, generating new neuronal cells, which replace the dying ones. Our current findings show that zebrafish is able to regenerate its retina in response to acute damage until late ages (>30 months), suggesting that the lack of regeneration in response to chronic damage is not due to MG’s inability of proliferate.

896