

1 **Title** Loss of cell cycle control renders cells nonresponsive to local extrinsic differentiation cues

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3 **Running Title** Cell-autonomous behavior of cell cycle mutants

4

5 **Key words** neural progenitors, retina, extrinsic signals, proliferation, differentiation, cell cycle  
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7

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21

22

23 **Abstract**

24 **Objective and approaches:** Aberrantly proliferating cells are linked to a number of diseases  
25 including cancers and developmental defects. To determine the extent to which local extrinsic  
26 signals contribute to or ameliorate mutant cell behaviors, we examined survival and differentiation  
27 of mutant cells in wild-type retinal environments by generating chimeric zebrafish embryos  
28 comprised of unlabeled host cells and GFP-labeled neural progenitor donor cells. In addition, we  
29 examined the fate of retinal progenitor cells when *cdkn1c*, a cyclin dependent kinase inhibitor,  
30 was induced in clones within wild-type and *hdac1* mutant retinæ.

31 **Results:** We found that seven of the ten mutants examined exhibited apoptosis when grafted into  
32 wild-type tissue, with cells from two slowly cycling mutants, *elys* and *emi1*, noticeably  
33 differentiating in a wild-type environment. Observations of the one hyperproliferative mutant,  
34 *hdac1*, revealed that these mutant cells did not appear to die or differentiate but instead survived  
35 and formed tumor-like rosettes in a wild-type environment. Ectopic expression of *cdkn1c* was  
36 unable to force cell cycle exit and differentiation of the majority of *hdac1* mutant cells.

37 **Conclusions:** Together, these results suggest that although a wild-type environment rarely  
38 encourages cell cycle exit and differentiation of neural progenitors with cell cycle defects, wild-  
39 type survival signals may enable hyperproliferative progenitor cells to persist instead of die.

40

41 **Introduction**

42 Strict control of proliferation, cell cycle exit, and differentiation underlies the formation,  
43 maintenance, and repair of nervous tissues of appropriate size and composition (Morales and  
44 Mira, 2019; Schmidt et al., 2013; Urbán and Guillemot, 2014). Mutations in genes that control  
45 these processes are linked to cancer and overgrowth syndromes (Fruman et al., 2017;  
46 Santamaria and Ortega, 2006). Proliferation is intrinsically-controlled through the precisely timed  
47 synthesis and degradation of cyclins, proteins that activate cyclin-dependent kinases (CDKs) to  
48 propel the cell through sequential phases of DNA replication, growth, and mitosis. Cyclin-  
49 dependent kinase inhibitors (CKIs), cell cycle phase specific ubiquitin ligases, and other  
50 regulatory proteins also govern cell cycle progression, ensuring sufficient growth, accurate DNA  
51 replication, and equal chromosome segregation during each phase of the cell cycle (Murray,  
52 2004; Santamaria and Ortega, 2006). Cells employ a variety of intrinsic mechanisms to ensure  
53 that the decision to proceed from one phase of the cell cycle to the next is appropriate. For  
54 example, cells transitioning from DNA replication to chromosome segregation survey their DNA  
55 for damage, either satisfying a molecular checkpoint and passing into the next phase or activating  
56 checkpoint controls that initiate DNA repair or, if the damage is extensive, cell death (Murray and  
57 Carr, 2018).

58

59 In addition to the well-studied checkpoint control that is shared by all cells, neural progenitors and  
60 neurons themselves require additional cell cycle control mechanisms to ensure appropriate  
61 proportions of distinct types of neurons and glia (Frade and Ovejero-Benito, 2015). For example,  
62 differentiated neurons in the vertebrate central nervous system have been found to contain  
63 chromosomal abnormalities including tetraploidy consistent with proliferation defects, suggesting  
64 that differentiation rather than death of defective cells is possible in particular tissues (e.g.,  
65 (Zupanc et al., 2009); reviewed in (Frade and Ovejero-Benito, 2015)). Furthermore, several  
66 studies of zebrafish eyes have shown that a differentiated retinal environment can support survival  
67 and differentiation of aberrantly proliferating cells that typically die (Cervený et al., 2010; Link et  
68 al., 2000). In some contexts, differentiation is not incompatible with continued proliferation as  
69 horizontal cells in the retina have been shown to proliferate and contribute additional neurons to  
70 the developing retinal circuitry (Godinho et al., 2007). These observations raise the possibility of  
71 central nervous system (CNS)-specific environmental control over proliferation and differentiation  
72 decisions.

73

74 Extrinsic input into proliferation and differentiation decisions, especially in multicellular organisms,  
75 is not unexpected. In the vertebrate retina, Müller glia can be stimulated to re-enter proliferation  
76 and support retinal growth and regeneration in response to a number of extrinsic factors including  
77 insulin growth factors, FGF, and  $TNF\alpha$  (e.g., Conner et al., 2014; Wan et al., 2014). In addition to  
78 secreted proteins, exogenous teratogens such as ethanol have been shown to perturb  
79 proliferation and differentiation, especially in the developing nervous system, by impinging on  
80 extrinsically-regulated signaling pathways (e.g., Kashyap et al., 2007; Muralidharan et al., 2018).  
81 Extrinsically-regulated signaling pathways are also known to stimulate cell cycle exit and  
82 differentiation, with pathways like Notch-Delta balancing proliferation and differentiation decisions  
83 of neighboring cells through lateral-inhibition type mechanisms (Louvi and Artavanis-Tsakonas,  
84 2006). Recent analysis of medaka retinae provide evidence that Notch pathway activation  
85 appears to be limited to a subset of progenitor cells and is required for generating eyes with  
86 appropriate proportions of neurons and glia (Pérez Saturnino et al., 2018). In addition, locally  
87 secreted molecules can encourage cell cycle exit and differentiation of cycling progenitors during  
88 development. For example, the spatial and temporal pattern of Hedgehog pathway activity, in  
89 both invertebrate and vertebrate eyes, ensures timely cell cycle exit and differentiation of retinal  
90 progenitors (García-Morales et al., 2019; Locker et al., 2006; Masai et al., 2005; Neumann and  
91 Nüsslein-Volhard, 2000). In addition, retinoic acid (RA) can promote cell cycle exit and neuronal  
92 differentiation, biasing cells toward various neuronal fates both in vivo (eg., Hyatt et al., 1996;  
93 Stevens et al., 2011; Valdivia et al., 2016) and in tissue culture (e.g., Estephane and Anctil, 2010;  
94 Kelley et al., 1999). Whether these extrinsic signals could be used to force cells to differentiate  
95 rather than die or undergo hyperproliferation in disease contexts is an open question.

96  
97 To systematically examine whether wild-type extrinsic signals could promote survival and  
98 differentiation of aberrantly cycling cells, this study first identified and assessed an arbitrary  
99 sample of 10 mutant zebrafish lines (see Table 1). It then generated chimeric retinae to assess  
100 behaviors of those mutant cells in a wild-type context. Finally, it investigated whether ectopic  
101 expression of an intrinsic cyclin dependent kinase inhibitor, *Cdkn1c*, which is linked to cell cycle  
102 exit and differentiation, could force differentiation of hyperproliferative cells in developing retinae.  
103 The data presented here confirm that neurotypical cell proliferation and differentiation often  
104 require both intrinsic factors and extrinsic cues. In addition, the data indicate that cells carrying  
105 mutations in genes encoding the cell cycle machinery do not appear to be susceptible to  
106 differentiation cues from the local environment.

## 107 108 **Results and Discussion**

109 To test whether a wild-type environment could encourage the differentiation of neuronal  
110 progenitor cells with impaired cell cycle control, we created chimeric zebrafish embryos containing  
111 mutant cells in wild-type retinae. We examined all retinae at 3 days post-fertilization (dpf) and  
112 found that out of the ten strains that we tested, nearly all cell cycle deficient cells failed to alter  
113 their behavior in a wild-type environment (Table 1). Consistent with previous reports, retinal  
114 progenitor cells (RPCs) in *cdk1*, *dtl*, *ele*, *emi1*, *elys*, *gins2*, *mcm5*, *ssrp1a*, and *rbbp6* deficient  
115 cells appeared to die by apoptosis (Table 1; references therein) as we observed pyknotic nuclei  
116 throughout the developing retinal neuroepithelium at 48 and 72 hours post fertilization (hpf) of all  
117 embryos (data not shown). When mutant cells were transplanted into wild-type hosts, the only  
118 clear evidence of mutant cell differentiation was found in chimeric retinae containing *emi1* or *elys*  
119 homozygous mutant cells (Figure 1C-H). All of the other mutant cells exhibit blebbing and  
120 fragmentation when integrated into wild-type retinae, consistent with cell-autonomous apoptosis  
121 (e.g., Figure 1I-J; Table 1).

122  
123 Previous reports have shown that zebrafish embryos carrying homozygous mutations in *emi1*  
124 (also known as *fbxo5*) and *elys* still exhibit some neuronal differentiation (Cervený et al., 2010;

125 Riley et al., 2010; Zhang et al., 2008), potentially due to maternal inheritance of these mRNAs or  
126 stability of the protein. For example, a small but significant fraction of *emi1* mutant RPCs still  
127 express the neurogenic gene *atoh7*, exit the cell cycle, and differentiate into retinal ganglion cells  
128 in *emi1* mutant eyes (Figure 1A-B). The same has been shown for *elys* mutants (Cervený et al.,  
129 2010; Davuluri et al., 2008). Interestingly, we found that all *elys* mutant cells transplanted into  
130 wild-type eyes appeared to differentiate (Figure 1F, n = 19 transplants;(Cervený et al., 2010))  
131 whereas *emi1* mutant cells transplanted into wild-type eyes either survived and differentiated  
132 (Figure 1G, n = 10/34 transplants) or appeared to quiesce or be lost due to cell death (Figure 1H,  
133 n = 24/34 transplants). Because we controlled for the number of cells transplanted into each  
134 embryo, survival and differentiation of *emi1* cells in a wild-type environment were possibly  
135 influenced by the location of the transplanted cells, slight differences in age of the host embryo at  
136 time of analysis, or stochastic fluctuations in gene expression in the transplanted cells or host  
137 embryos.

138  
139 The difference in susceptibility of *emi1* and *elys* mutant cells to differentiation factors from the  
140 wild-type environment may be explained by the distinct functions of these mutated genes. For  
141 example, the *emi1* gene encodes a protein that directly participates in the cell cycle by acting as  
142 both a substrate for and inhibitor of the anaphase promoting complex (APC/C) (Cappell et al.,  
143 2018), whereas the *elys* gene encodes a large scaffold protein required for nuclear pore formation  
144 and possibly chromatin organization (Rasala et al., 2006). It is therefore tempting to speculate  
145 that differences in epigenetic regulation may underlie the complete differentiation of *elys* cells  
146 transplanted into a wild-type retina. It is also important to note that of all the mutants we tested,  
147 only *elys* does not carry a mutation in a gene directly linked to cell cycle progression or regulation.

148  
149 Our transplant studies also confirmed previous reports that mutations in histone deacetylase 1,  
150 *hdac1*, are linked to cell autonomous hyperproliferation in the retina ((Stadler et al., 2005;  
151 Yamaguchi et al., 2005), Figure 1K-L). When we examined *hdac1* mutant cells integrated into  
152 wild-type chimeric retinæ at 3 dpf, a point at which apoptotic cells are found scattered throughout  
153 the *hdac1* mutant retinæ (Supplemental Figure 1), we did not observe pyknotic nuclei or cell  
154 blebbing, two other key hallmarks of apoptosis. This finding raises the possibility that a wild-type  
155 retinal environment supports the survival of these hyperproliferative cells but it does not alter their  
156 uncontrolled proliferation.

157  
158 Intrigued by the survival and continued proliferation of *hdac1* mutant cells in wild-type embryos,  
159 we further analyzed wild-type retinæ containing *hdac1* mutant clones at 4 dpf. Wild-type cells  
160 transplanted into a wild-type environment appeared to differentiate as expected and wild-type  
161 cells in an *hdac1* environment also exhibited hallmarks of differentiation, forming clones that  
162 contained cells with typical photoreceptor and interneuron morphologies (Figure 2A,C). Small  
163 clones of wild-type cells did not appear to force neighboring mutant cells to differentiate (Figure  
164 2C). Interestingly, we found that *hdac1* mutant cells formed rosettes in wild-type retinæ,  
165 reminiscent of early tumor formation (Figure 2B). Not only do these rosettes persist in wild-type  
166 eyes, but they also appear to disrupt adjacent retinal architecture, breaching the outer boundary  
167 of the apical surface (Figure 2B'') and interrupting the inner plexiform layer. Moreover, these  
168 clones of *hdac1* mutant cells appear to disrupt the lamination and possibly differentiation of  
169 neighboring wild-type cells (Figure 2B''). In 9 of 14 chimeric wild-type retinæ containing *hdac1*  
170 mutant cells, we observed similar phenotypes of disrupted lamination and apical boundaries as  
171 shown in **Figure 2B''**, raising the possibility that persistently cycling cells can alter the organization  
172 of wild-type tissues. We believe that these studies establish the chimeric retina approach as a  
173 potentially powerful way to study and understand the effects of nascent tumors on surrounding  
174 neuroepithelial tissues.

175

176 A previous study showed that *hdac1* mutant retinæ are likely hyperproliferative because they fail  
177 to express key cell cycle exit genes including cyclin dependent kinase inhibitors (Yamaguchi et  
178 al., 2005). We asked whether *hdac1* mutant cells could be forced to exit the cell cycle and  
179 differentiate by inducing expression of *cdkn1c* (also known as *p57*) in *hdac1* mutant retinæ.  
180 Contrary to previous reports showing that over-expression of a different cyclin dependent kinase  
181 inhibitor, *cdkn1b* (also known as *p27*), could induce cell cycle exit and differentiation of *hdac1*<sup>-/-</sup>  
182 retinal progenitor cells (e.g., Ohnuma et al., 1999; Yamaguchi et al., 2005), we found that mosaic  
183 expression of *cdkn1c* from a heat-shock-inducible promoter from 26-28 hpf was rarely associated  
184 with differentiation of *hdac1* mutant RPCs (Figure 3C-E). Specifically, clones expressing *cdkn1c*  
185 always revealed morphologies typical of retinal neurons, most obviously photoreceptors, bipolar  
186 cells, and ganglion cells in wild-type eyes (e.g., Figure 3C; Supplemental Figure 2C) whereas  
187 nearly all *cdkn1c*-positive cells in *hdac1* mutant eyes appeared neuroepithelial (e.g., Figure 3D;  
188 Supplemental Figure 2B). In contrast to our observations of *hdac1*<sup>-/-</sup> wholemount eyes, our  
189 observations of thin cryosections of eyes with clones of *cdkn1c*-expressing cells revealed a small  
190 minority of *hdac1*<sup>-/-</sup> clones (2 clones out of 26 clones in 9 eyes) that contained some cells with  
191 neuronal morphologies (Supplemental Figure 2A). These same eyes, as well as most other eyes,  
192 contained clones that did not exhibit neuronal morphology. Based on the GFP intensity in the  
193 *hdac1*<sup>-/-</sup> cells that appeared to differentiate, it is possible that either extremely high levels of  
194 Cdkn1c and/or slight differences in developmental timing of *cdkn1c* expression may support  
195 retinal progenitor cell differentiation in the *hdac1*<sup>-/-</sup> background.

196  
197 To further explore how Cdkn1c activation altered *hdac1* and wild-type cells, we used precisely  
198 timed heat-shock to induce expression of either GFP alone or Cdkn1c and GFP in 28 hpf embryos  
199 and then measured the size of clones by counting the number of nuclei in GFP-labelled retinal  
200 clones in 60 hpf embryos. Based on clone size, wild-type retinal progenitor cells appeared to be  
201 more likely to exit the cell cycle and differentiate when *cdkn1c* was induced, but *hdac1* clones  
202 with and without *cdkn1c* appeared similar. Specifically, we found that control (GFP only) clones  
203 in wild-type retinæ contained an average of 34.5 nuclei whereas *cdkn1c*-expressing clones in  
204 wild-type retinæ contained an average of only 23.3 nuclei per clone (Figure 2E; n=22 clones, 8  
205 eyes for each wild-type sample; p=0.0174, Welch's t-test, 95% CI). These data suggest that high  
206 levels of *cdkn1c* can encourage cell cycle exit and differentiation of proliferating retinal progenitor  
207 cells. In contrast, control clones in *hdac1* mutant retinæ contained an average of 19.6 nuclei and  
208 were not much larger than *cdkn1c*-expressing clones in *hdac1* mutant retinæ, which had an  
209 average of 15.4 nuclei (Figure 2E; n=23 clones, 9 eyes for each *hdac1*<sup>-/-</sup> sample; p=0.1008,  
210 Welch's t-test, 95% CI). Interestingly, clone size in *hdac1* embryos did not differ from *cdkn1c*-  
211 positive clones in wild-type retinæ, suggesting that *hdac1*-linked hyperproliferation is not linked  
212 to an increase in cell cycle speed but a failure to ever exit the cell cycle. Taken together with our  
213 observations of limited differentiation in *hdac1* mutant cells containing induced *cdkn1c*, these data  
214 support the idea that Hdac1 activity likely contributes to expression of a number of gene products  
215 that ultimately work together to efficiently promote cell cycle exit and differentiation.

216  
217

## 218 **Conclusions and Limitations**

219 Based on our analyses of cell cycle mutant cells in wild-type retinal environments, we suggest  
220 that cell cycle machinery and/or cell cycle control components are intrinsically required for cells  
221 to respond to local extrinsic differentiation cues. We observed that nearly all cells carrying  
222 mutations in genes directly controlling the cell cycle were not encouraged to differentiate by a  
223 wild-type environment. The only mutant with direct links to cell cycle control that appeared to have  
224 both autonomous and non-autonomous behaviors in a wild-type environment was *emi1*. A recent  
225 study shows that cell cycle progression, and by extension, cell cycle exit, can be differentially  
226 influenced by levels of Emi1 protein, with low levels of Emi1 linked to quiescence and high levels

227 linked to S-phase entry and robust DNA replication (Cappell et al., 2018). We speculate that small  
228 fluctuations in *emi1* mRNA is one possible explanation for the variable behavior we observe when  
229 *emi1* mutant cells are transplanted into wild-type retinæ.

230 In this study and in previous studies (Cervený et al., 2010), we observed that cells carrying  
231 mutations that likely have secondary effects on cell cycle progression (e.g., *elys*) can reliably  
232 survive and differentiate in a wild-type environment. The gene that is mutated in *elys* encodes a  
233 key component of the nuclear pore complex that possibly links nuclear pore assembly, nucleus  
234 size and DNA replication with chromatin organization (Gillespie et al., 2007; Jevtić et al., 2019;  
235 Zierhut et al., 2014). Similar to *elys* mutants, cells carrying a mutation in the chromatin-remodeling  
236 gene *brg1* can be encouraged to exit the cell cycle and differentiate in a wild-type environment  
237 (Gregg et al., 2003; Link et al., 2000). We therefore propose that global changes in genome  
238 organization underlie the phenomenon of environmentally enforced differentiation.

239 Not all types of genome organization defects are equivalent, however, as *hdac1* mutants  
240 appeared to act in a cell-autonomous manner, continuing to proliferate in a wild-type environment.  
241 We were, however, unable to follow the long-term fate of the *hdac1* mutant transplants past 4 dpf  
242 and so were unable to establish whether these clusters of cells evolve into full-blown retinal  
243 tumors. It would also have been interesting to further explore how the surrounding wild-type  
244 tissues and cells alter their organization and behavior.

245  
246 Our observations and those previously examining the effect of *hdac1* mutation in the CNS  
247 (Schultz et al., 2018; Stadler et al., 2005; Yamaguchi et al., 2005) run counter to the vast collection  
248 of data showing that pharmacological inhibition of histone deacetylase (HDAC) activity prevents  
249 over-proliferation, especially of cancerous cells (e.g., Li and Seto, 2016). Both laboratory and  
250 clinical studies provide evidence that inhibition of HDAC activity blocks proliferation and often  
251 promotes apoptosis of proliferating, oncogenic cells. In differentiated brain tissue, however,  
252 inhibition of HDAC activity is linked to neuronal survival, especially in neurodegenerative disease  
253 models (e.g., Didonna and Opal, 2015). Histone deacetylases not only remove acetyl groups from  
254 histones but are also known to deacetylate other targets including the tumor suppressor p53.  
255 Interestingly, one study provides evidence that maintenance of acetylation at specific lysine  
256 residues in p53 prevents its association with chromatin specifically in neurons (Brochier et al.,  
257 2013). Whether this type of regulation for p53 occurs only in differentiated neurons or also in  
258 neuronal progenitors, such as the RPCs examined in this study, is unknown.

259  
260

## 261 **Methods**

### 262 **Zebrafish lines**

263 Eggs were collected by natural spawning, raised at either 25°C or 28.5°C in E3 embryo medium  
264 (Nüsslein-Volhard, C. and Dahm, R., 2002) and staged according to Kimmel et al., 1995. After  
265 gastrulation and before 24 hours post fertilization, embryos were cultured in 0.003%  
266 phenylthiourea (PTU, Sigma) in E3 to prevent pigment formation. Lines used in this study and  
267 references are listed in Table 1. Adult zebrafish were cared for with protocols approved by the  
268 Reed College IACUC.

269

### 270 **Cell transplants**

271 Similar to previously published studies (e.g., (Cervený et al., 2010; Turner et al., 2019), donor  
272 embryos were injected at the 1-cell stage with ~20 ng of GFP mRNA synthesized from linearized  
273 pCS2-GFP or membrane-targeted RFP mRNA synthesized from linearized pCS2-mCherry with  
274 the T7 mMessage mMachine kit (Ambion) according to manufacturer's instructions. Host and  
275 donor embryos were grown at 28.5°C until sphere stage (approximately 4 hours post-fertilization)  
276 and then 10-15 fluorescently labelled cells were removed from donor embryos and transplanted  
277 into the animal pole of unlabelled host embryos. Donor and host embryos were incubated overnight

278 at 28.5°C. All embryos were screened and E3 was exchanged for PTU in E3. Donors were  
279 identified by visual inspection and by PCR and restriction digest mediated genotyping. Genotyping  
280 protocols for each line can be found at Zebrafish International Resource Center (ZIRC.org) and  
281 in relevant references (see Table 1). For *gins2* experiments, 1-cell stage embryos were first  
282 injected with ~1 nl of 1 mM *gins2* morpholino (Gene Tools, Philomath, OR; 5'-  
283 GGGGTGAGTCAATTTATAATCTAC-3'), a dose that phenocopies *gins2*<sup>-/-</sup> mutants (Varga et  
284 al., in preparation) and then injected with ~10 ng of membrane-targeted RFP mRNA.  
285

### 286 Heat-shock inducible expression of *cdkn1c*

287 *cdkn1c* was amplified from cDNA using the following primers:

288 P57 forward: 5'-ATGGCAAACGTGGACGTATCAAGC-3'

289 P57 reverse: 5'-GCATGAAATTGCAAACCAAACCTT-3'.

290 PCR product was cloned into the pCRII vector (TOPO-TA kit; Invitrogen), generating pCRII-  
291 *cdkn1c*. pCRII-*cdkn1c* was digested with EcoRI and then ligated into EcoRI-cut, shrimp alkaline  
292 phosphatase treated pSGH2 (Bajoghli et al., 2004), generating pKC040 to enable the expression  
293 of both the green fluorescent protein (GFP) and *cdkn1c* from bidirectional heat-shock elements.  
294 15 ng/μl of either pSGH2 or pKC040 plasmid were injected into single-cell staged embryos and  
295 incubated at 25°C. To induce expression of GFP or GFP and *cdkn1c*, 28 hpf embryos were  
296 incubated at 38°C for 30 minutes, transferred to E3 with PTU, incubated until 3.5 dpf, and then  
297 fixed with 4% PFA for immunohistochemistry.  
298

### 299 Immunohistochemistry, imaging, and analysis

300 After fixation, wholemount embryos were either subjected to immunohistochemistry as previously  
301 described (Cervený et al., 2010) or were cryoprotected in 15% and then 30% sucrose before  
302 being embedded in Optimal Cutting Temperature (OCT) resin and cut into 30 μm thick sections  
303 that were collected on charged glass slides (Polysciences, 24216) and stained with the following  
304 antibodies: beta-catenin (mouse, 1:250 dilution; Sigma, C7207); GFP (chicken, 1:250 dilution,  
305 Abcam, ab139709); RFP (rabbit, 1:500 dilution, MBL, PM005); PH3 (rabbit, 1:300 dilution,  
306 Millipore, 06-570); ZO-1 (mouse 1:100, Invitrogen, 339100). Nuclei were counterstained with  
307 DAPI (1 μg/ml from a 1 mg/ml stock in DMSO; Sigma) or sytox orange (1:10,000 dilution,  
308 Invitrogen).

309 All images were captured on a Nikon A1+ confocal with a long working distance 25X, 1.1 NA  
310 water immersion lens. To quantify clone size, stacks of confocal images were converted to Imaris  
311 (Bitplane) files and distinct clones were first manually contoured to generate distinct *cdkn1c* and  
312 GFP-positive surfaces. Surfaces were masked, generating a channel containing DAPI nuclei in  
313 each surface. Images were then batch processed to automatically count nuclei per clone. Clone  
314 sizes were exported to a spreadsheet, then graphed and statistically analyzed using JMP Pro 14  
315 (Scintilla).  
316

### 317 Figure Legends

318 **Figure 1.** Comparison of differentiation behavior of wild-type, *emi1*<sup>-/-</sup>, and *elys*<sup>-/-</sup> neuronal  
319 progenitors in developing zebrafish retinae.

320 A-B Lateral maximum intensity projection of 50 hpf retinae from *Tg[atoh7:GFP]* embryos showing  
321 progression of neurogenic gene expression (green) stained with sytox orange (red) to highlight  
322 nuclei.

323 C-D Lateral views of maximum intensity projection of 3 dpf chimeric wild-type retinae containing  
324 GFP-labelled wild-type (C) or *emi1* mutant (D) cells and immunostained for phosphohistone H3  
325 (PH3, red).

326 E-H Representative images of frontal cryosections of 3 dpf chimeric wild-type retinae containing  
327 GFP-labelled wild-type (E), *elys* mutant (F), or *emi1* mutant (G, H) cells immunostained for GFP  
328 (green cells from donor embryo) and B-catenin (red cell boundaries and plexiform layers).

329 I-J Lateral views of single z-slice of ventral-nasal region of 3 dpf chimeric wild-type retinæ  
330 containing wild-type (I) or *gins2* morphant (H) cells labeled with membrane-targeted RFP.  
331 K-L Representative images of frontal cryosections of 3 dpf chimeric wild-type retinæ containing  
332 GFP-labelled wild-type (K) or *hdac1* mutant (L) cells immunostained for GFP (green cells from  
333 donor) and B-catenin (red) to mark cell boundaries and plexiform layers.

334  
335 **Figure 2.** *hdac1* mutant cells form rosette-like structures that invade surrounding tissues when  
336 transplanted into WT retinæ and alter lamination and organization of adjacent wild-type cells.  
337 A-C Frontal cross-sections of 4 dpf chimeric retinæ when GFP-expressing wild-type (A, C) and  
338 *hdac1* mutant (B) cells were transplanted into wild-type (A, B) or *hdac1* mutant (C) host eyes  
339 immunostained for ZO-1 (purple) and GFP (green).  
340 B'-B'' Same cross section as in B, but showing transplanted cells (green) and DAPI-stained nuclei  
341 (red). Boxed region ~2X zoom shown in B''. Arrow indicates wild-type cells disrupting the inner  
342 plexiform layer within this chimeric retina and *hdac1*<sup>-/-</sup> clone outlined with dashed line.

343  
344 **Figure 3.** Forced expression of *cdkn1c* does not significantly alter proliferative behavior of *hdac1*  
345 mutant cells.

346 A-D Lateral views of maximum intensity z-projections showing nuclei (red) and heat-shock  
347 induced GFP (A-B, green) or *cdkn1c*-positive clones (C-D, green) in wholemount 60 hpf embryos  
348 with genotype indicated.

349 E Box plot overlaid on all data points for number of nuclei per clone in GFP and *cdkn1c* containing  
350 clones in either *hdac1* and wild-type sibling retinæ. NS, not significant with p-value of p=0.1008;  
351 \*\*, significant with p-value of 0.0174; Welch's t-test.

352  
353 **Supplemental Figure 1.** Retinal progenitor cells undergo apoptosis in *hdac1* mutant retinæ.  
354 Wild-type siblings (left) and *hdac1* mutants (right) were fixed at ~3.5 dpf and then probed with  
355 activated caspase 3 antibody. Both representative images are maximum intensity projections.  
356 Note puncta scattered throughout *hdac1* mutant but not in wild-type.

357  
358 **Supplemental Figure 2.** Inducing high levels of *cdkn1c* rarely trigger neuronal differentiation in  
359 *hdac1* retinal progenitor cells.

360 Frontal cross-sections of retinæ from same experiment shown in Figure 3; *cdkn1c*-positive clones  
361 were detected by immunohistochemistry (green) and nuclei stained with DAPI (red). *hdac1* mutant  
362 cells in *cdkn1c*-expressing clones (green) generally exhibit neuroepithelial morphology (A, B)  
363 consistent with proliferation and rarely show neuronal phenotypes (B, arrow heads) whereas age-  
364 matched wild-type sibling cells within *cdkn1c*-expressing clones (green) always exhibit neuronal  
365 morphology and lamination (C).

366  
367 **Declarations:**

368 **Ethics approval:** Adult zebrafish were bred and cared for with protocols approved by the Reed  
369 College IACUC.

370 **Consent for publication:** All authors have read, provided feedback, and approved the  
371 manuscript.

372 **Availability of data and material:** All data of this study are presented in this manuscript and  
373 any fish lines or other materials are available from the corresponding author or, with regard to  
374 mutant fish lines, from the Zebrafish International Resource Center (ZIRC; zebrafish.org).

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380 MV, KLC, AVD, IT, HB, OH, and DBL performed experiments and made figures. MV supplied the  
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386

### 387 **References**

- 388 Amsterdam, A., Nissen, R.M., Sun, Z., Swindell, E.C., Farrington, S., Hopkins, N., 2004.  
389 Identification of 315 genes essential for early zebrafish development. *Proc. Natl. Acad.*  
390 *Sci. U. S. A.* 101, 12792–12797. <https://doi.org/10.1073/pnas.0403929101>
- 391 Bajoghli, B., Aghaallaei, N., Heimbucher, T., Czerny, T., 2004. An artificial promoter construct  
392 for heat-inducible misexpression during fish embryogenesis. *Dev. Biol.* 271, 416–430.  
393 <https://doi.org/10.1016/j.ydbio.2004.04.006>
- 394 Brochier, C., Dennis, G., Rivieccio, M.A., McLaughlin, K., Coppola, G., Ratan, R.R., Langley,  
395 B., 2013. Specific acetylation of p53 by HDAC inhibition prevents DNA damage-  
396 induced apoptosis in neurons. *J. Neurosci. Off. J. Soc. Neurosci.* 33, 8621–8632.  
397 <https://doi.org/10.1523/JNEUROSCI.5214-12.2013>
- 398 Cappell, S.D., Mark, K.G., Garbett, D., Pack, L.R., Rape, M., Meyer, T., 2018. EMI1 switches  
399 from being a substrate to an inhibitor of APC/CCDH1 to start the cell cycle. *Nature* 558,  
400 313–317. <https://doi.org/10.1038/s41586-018-0199-7>
- 401 Cervený, K.L., Cavodeassi, F., Turner, K.J., de Jong-Curtain, T.A., Heath, J.K., Wilson, S.W.,  
402 2010. The zebrafish flotte lotte mutant reveals that the local retinal environment promotes  
403 the differentiation of proliferating precursors emerging from their stem cell niche. *Dev.*  
404 *Camb. Engl.* 137, 2107–2115. <https://doi.org/10.1242/dev.047753>
- 405 Conner, C., Ackerman, K.M., Lahne, M., Hobgood, J.S., Hyde, D.R., 2014. Repressing notch  
406 signaling and expressing TNF $\alpha$  are sufficient to mimic retinal regeneration by inducing  
407 Müller glial proliferation to generate committed progenitor cells. *J. Neurosci. Off. J. Soc.*  
408 *Neurosci.* 34, 14403–14419. <https://doi.org/10.1523/JNEUROSCI.0498-14.2014>
- 409 Davuluri, G., Gong, W., Yusuff, S., Lorent, K., Muthumani, M., Dolan, A.C., Pack, M., 2008.  
410 Mutation of the zebrafish nucleoporin elys sensitizes tissue progenitors to replication  
411 stress. *PLoS Genet.* 4, e1000240. <https://doi.org/10.1371/journal.pgen.1000240>
- 412 Didonna, A., Opal, P., 2015. The promise and perils of HDAC inhibitors in neurodegeneration.  
413 *Ann. Clin. Transl. Neurol.* 2, 79–101. <https://doi.org/10.1002/acn3.147>
- 414 Estephane, D., Anctil, M., 2010. Retinoic acid and nitric oxide promote cell proliferation and  
415 differentially induce neuronal differentiation in vitro in the cnidarian *Renilla koellikeri*.  
416 *Dev. Neurobiol.* 70, 842–852. <https://doi.org/10.1002/dneu.20824>
- 417 Frade, J.M., Ovejero-Benito, M.C., 2015. Neuronal cell cycle: the neuron itself and its  
418 circumstances. *Cell Cycle Georget. Tex* 14, 712–720.  
419 <https://doi.org/10.1080/15384101.2015.1004937>
- 420 Fruman, D.A., Chiu, H., Hopkins, B.D., Bagrodia, S., Cantley, L.C., Abraham, R.T., 2017. The  
421 PI3K Pathway in Human Disease. *Cell* 170, 605–635.  
422 <https://doi.org/10.1016/j.cell.2017.07.029>
- 423 García-Morales, D., Navarro, T., Iannini, A., Pereira, P.S., Míguez, D.G., Casares, F., 2019.  
424 Dynamic Hh signalling can generate temporal information during tissue patterning.  
425 *Development* 146, dev176933. <https://doi.org/10.1242/dev.176933>

- 426 Gillespie, P.J., Khoudoli, G.A., Stewart, G., Swedlow, J.R., Blow, J.J., 2007. ELYS/MEL-28  
427 chromatin association coordinates nuclear pore complex assembly and replication  
428 licensing. *Curr. Biol.* CB 17, 1657–1662. <https://doi.org/10.1016/j.cub.2007.08.041>
- 429 Godinho, L., Williams, P.R., Claassen, Y., Provost, E., Leach, S.D., Kamermans, M., Wong,  
430 R.O.L., 2007. Nonapical symmetric divisions underlie horizontal cell layer formation in  
431 the developing retina in vivo. *Neuron* 56, 597–603.  
432 <https://doi.org/10.1016/j.neuron.2007.09.036>
- 433 Gregg, R.G., Willer, G.B., Fadool, J.M., Dowling, J.E., Link, B.A., 2003. Positional cloning of  
434 the young mutation identifies an essential role for the Brahma chromatin remodeling  
435 complex in mediating retinal cell differentiation. *Proc. Natl. Acad. Sci. U. S. A.* 100,  
436 6535–6540. <https://doi.org/10.1073/pnas.0631813100>
- 437 Hyatt, G.A., Schmitt, E.A., Fadool, J.M., Dowling, J.E., 1996. Retinoic acid alters photoreceptor  
438 development in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 93, 13298–13303.  
439 <https://doi.org/10.1073/pnas.93.23.13298>
- 440 Jevtić, P., Schibler, A.C., Wesley, C.C., Pegoraro, G., Misteli, T., Levy, D.L., 2019. The  
441 nucleoporin ELYS regulates nuclear size by controlling NPC number and nuclear import  
442 capacity. *EMBO Rep.* 20. <https://doi.org/10.15252/embr.201847283>
- 443 Kashyap, B., Frederickson, L.C., Stenkamp, D.L., 2007. Mechanisms for persistent  
444 microphthalmia following ethanol exposure during retinal neurogenesis in zebrafish  
445 embryos. *Vis. Neurosci.* 24, 409–421. <https://doi.org/10.1017/S0952523807070423>
- 446 Kelley, M.W., Williams, R.C., Turner, J.K., Creech-Kraft, J.M., Reh, T.A., 1999. Retinoic acid  
447 promotes rod photoreceptor differentiation in rat retina in vivo. *Neuroreport* 10, 2389–  
448 2394.
- 449 Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of  
450 embryonic development of the zebrafish. *Dev. Dyn. Off. Publ. Am. Assoc. Anat.* 203,  
451 253–310. <https://doi.org/10.1002/aja.1002030302>
- 452 Koltowska, K., Apitz, H., Stamatakis, D., Hirst, E.M.A., Verkade, H., Salecker, I., Ober, E.A.,  
453 2013. Ssrp1a controls organogenesis by promoting cell cycle progression and RNA  
454 synthesis. *Dev. Camb. Engl.* 140, 1912–1918. <https://doi.org/10.1242/dev.093583>
- 455 Li, Y., Seto, E., 2016. HDACs and HDAC Inhibitors in Cancer Development and Therapy. *Cold*  
456 *Spring Harb. Perspect. Med.* 6. <https://doi.org/10.1101/cshperspect.a026831>
- 457 Link, B.A., Fadool, J.M., Malicki, J., Dowling, J.E., 2000. The zebrafish young mutation acts  
458 non-cell-autonomously to uncouple differentiation from specification for all retinal cells.  
459 *Dev. Camb. Engl.* 127, 2177–2188.
- 460 Locker, M., Agathocleous, M., Amato, M.A., Parain, K., Harris, W.A., Perron, M., 2006.  
461 Hedgehog signaling and the retina: insights into the mechanisms controlling the  
462 proliferative properties of neural precursors. *Genes Dev.* 20, 3036–3048.  
463 <https://doi.org/10.1101/gad.391106>
- 464 Louvi, A., Artavanis-Tsakonas, S., 2006. Notch signalling in vertebrate neural development. *Nat.*  
465 *Rev. Neurosci.* 7, 93–102. <https://doi.org/10.1038/nrn1847>
- 466 Masai, I., Yamaguchi, M., Tonou-Fujimori, N., Komori, A., Okamoto, H., 2005. The hedgehog-  
467 PKA pathway regulates two distinct steps of the differentiation of retinal ganglion cells:  
468 the cell-cycle exit of retinoblasts and their neuronal maturation. *Dev. Camb. Engl.* 132,  
469 1539–1553. <https://doi.org/10.1242/dev.01714>
- 470 Morales, A.V., Mira, H., 2019. Adult Neural Stem Cells: Born to Last. *Front. Cell Dev. Biol.* 7,  
471 96. <https://doi.org/10.3389/fcell.2019.00096>

- 472 Muralidharan, P., Sarmah, S., Marrs, J.A., 2018. Retinal Wnt signaling defect in a zebrafish fetal  
473 alcohol spectrum disorder model. PLOS ONE 13, e0201659.  
474 <https://doi.org/10.1371/journal.pone.0201659>
- 475 Murray, A.W., 2004. Recycling the cell cycle: cyclins revisited. Cell 116, 221–234.
- 476 Murray, J.M., Carr, A.M., 2018. Integrating DNA damage repair with the cell cycle. Curr. Opin.  
477 Cell Biol. 52, 120–125. <https://doi.org/10.1016/j.ceb.2018.03.006>
- 478 Neumann, C.J., Nüsslein-Volhard, C., 2000. Patterning of the zebrafish retina by a wave of  
479 sonic hedgehog activity. Science 289, 2137–2139.  
480 <https://doi.org/10.1126/science.289.5487.2137>
- 481 Nüsslein-Volhard, C., Dahm, R., 2002. Zebrafish: A practical approach. Oxford University  
482 Press, Oxford, UK.
- 483 Ohnuma, S., Philpott, A., Wang, K., Holt, C.E., Harris, W.A., 1999. p27Xic1, a Cdk inhibitor,  
484 promotes the determination of glial cells in Xenopus retina. Cell 99, 499–510.
- 485 Pérez Saturnino, A., Lust, K., Wittbrodt, J., 2018. Notch signalling patterns retinal composition  
486 by regulating *atoh7* during post-embryonic growth. Development 145, dev169698.  
487 <https://doi.org/10.1242/dev.169698>
- 488 Rasala, B.A., Orjalo, A.V., Shen, Z., Briggs, S., Forbes, D.J., 2006. ELYS is a dual  
489 nucleoporin/kinetochore protein required for nuclear pore assembly and proper cell  
490 division. Proc. Natl. Acad. Sci. U. S. A. 103, 17801–17806.  
491 <https://doi.org/10.1073/pnas.0608484103>
- 492 Rhodes, J., Amsterdam, A., Sanda, T., Moreau, L.A., McKenna, K., Heinrichs, S., Ganem, N.J.,  
493 Ho, K.W., Neuberger, D.S., Johnston, A., Ahn, Y., Kutok, J.L., Hromas, R., Wray, J., Lee,  
494 C., Murphy, C., Radtke, I., Downing, J.R., Fleming, M.D., MacConaill, L.E., Amatruda,  
495 J.F., Gutierrez, A., Galinsky, I., Stone, R.M., Ross, E.A., Pellman, D.S., Kanki, J.P.,  
496 Look, A.T., 2009. Emi1 maintains genomic integrity during zebrafish embryogenesis and  
497 cooperates with p53 in tumor suppression. Mol. Cell. Biol. 29, 5911–5922.  
498 <https://doi.org/10.1128/MCB.00558-09>
- 499 Riley, B.B., Sweet, E.M., Heck, R., Evans, A., McFarland, K.N., Warga, R.M., Kane, D.A.,  
500 2010. Characterization of harpy/Rca1/emil mutants: patterning in the absence of cell  
501 division. Dev. Dyn. Off. Publ. Am. Assoc. Anat. 239, 828–843.  
502 <https://doi.org/10.1002/dvdy.22227>
- 503 Ryu, S., Holzschuh, J., Erhardt, S., Ettl, A.-K., Driever, W., 2005. Depletion of minichromosome  
504 maintenance protein 5 in the zebrafish retina causes cell-cycle defect and apoptosis. Proc.  
505 Natl. Acad. Sci. U. S. A. 102, 18467–18472. <https://doi.org/10.1073/pnas.0506187102>
- 506 Sansam, C.L., Cruz, N.M., Danielian, P.S., Amsterdam, A., Lau, M.L., Hopkins, N., Lees, J.A.,  
507 2010. A vertebrate gene, *ticrr*, is an essential checkpoint and replication regulator. Genes  
508 Dev. 24, 183–194. <https://doi.org/10.1101/gad.1860310>
- 509 Santamaria, D., Ortega, S., 2006. Cyclins and CDKS in development and cancer: lessons from  
510 genetically modified mice. Front. Biosci. J. Virtual Libr. 11, 1164–1188.
- 511 Schmidt, R., Strähle, U., Scholpp, S., 2013. Neurogenesis in zebrafish – from embryo to adult.  
512 Neural Develop. 8, 3. <https://doi.org/10.1186/1749-8104-8-3>
- 513 Schultz, L.E., Haltom, J.A., Almeida, M.P., Wierson, W.A., Solin, S.L., Weiss, T.J., Helmer,  
514 J.A., Sandquist, E.J., Shive, H.R., McGrail, M., 2018. Epigenetic regulators Rbbp4 and  
515 Hdac1 are overexpressed in a zebrafish model of RB1 embryonal brain tumor, and are  
516 required for neural progenitor survival and proliferation. Dis. Model. Mech. 11.  
517 <https://doi.org/10.1242/dmm.034124>

- 518 Stadler, J.A., Shkumatava, A., Norton, W.H.J., Rau, M.J., Geisler, R., Fischer, S., Neumann,  
519 C.J., 2005. Histone deacetylase 1 is required for cell cycle exit and differentiation in the  
520 zebrafish retina. *Dev. Dyn. Off. Publ. Am. Assoc. Anat.* 233, 883–889.  
521 <https://doi.org/10.1002/dvdy.20427>
- 522 Stevens, C.B., Cameron, D.A., Stenkamp, D.L., 2011. Plasticity of photoreceptor-generating  
523 retinal progenitors revealed by prolonged retinoic acid exposure. *BMC Dev. Biol.* 11, 51.  
524 <https://doi.org/10.1186/1471-213X-11-51>
- 525 Turner, K.J., Hoyle, J., Valdivia, L.E., Cervený, K.L., Hart, W., Mangoli, M., Geisler, R., Rees,  
526 M., Houart, C., Poole, R.J., Wilson, S.W., Gestri, G., 2019. Abrogation of Stem Loop  
527 Binding Protein (Slbp) function leads to a failure of cells to transition from proliferation  
528 to differentiation, retinal coloboma and midline axon guidance deficits. *PloS One* 14,  
529 e0211073. <https://doi.org/10.1371/journal.pone.0211073>
- 530 Urbán, N., Guillemot, F., 2014. Neurogenesis in the embryonic and adult brain: same regulators,  
531 different roles. *Front. Cell. Neurosci.* 8. <https://doi.org/10.3389/fncel.2014.00396>
- 532 Valdivia, L.E., Lamb, D.B., Horner, W., Wierzbicki, C., Tafessu, A., Williams, A.M., Gestri, G.,  
533 Krasnow, A.M., Vleeshouwer-Neumann, T.S., Givens, M., Young, R.M., Lawrence,  
534 L.M., Stickney, H.L., Hawkins, T.A., Schwarz, Q.P., Cavodeassi, F., Wilson, S.W.,  
535 Cervený, K.L., 2016. Antagonism between Gdf6a and retinoic acid pathways controls  
536 timing of retinal neurogenesis and growth of the eye in zebrafish. *Dev. Camb. Engl.* 143,  
537 1087–1098. <https://doi.org/10.1242/dev.130922>
- 538 Varga, M., Csályi, K., Bertényi, I., Menyhárd, D.K., Poole, R.J., Cervený, K.L., Kövesdi, D.,  
539 Barátki, B., Rouse, H., Vad, Z., Young, R.M., Hawkins, T.A., Stickney, H.L.,  
540 Cavodeassi, F., Schwarz, Q.P., Wilson, S.W., n.d. The GINS complex is required for the  
541 survival of rapidly proliferating retinal and tectal progenitors during zebrafish  
542 development. *Prep.*
- 543 Wan, J., Zhao, X.-F., Vojtek, A., Goldman, D., 2014. Retinal injury, growth factors, and  
544 cytokines converge on  $\beta$ -catenin and pStat3 signaling to stimulate retina regeneration.  
545 *Cell Rep.* 9, 285–297. <https://doi.org/10.1016/j.celrep.2014.08.048>
- 546 Yamaguchi, M., Tonou-Fujimori, N., Komori, A., Maeda, R., Nojima, Y., Li, H., Okamoto, H.,  
547 Masai, I., 2005. Histone deacetylase 1 regulates retinal neurogenesis in zebrafish by  
548 suppressing Wnt and Notch signaling pathways. *Dev. Camb. Engl.* 132, 3027–3043.  
549 <https://doi.org/10.1242/dev.01881>
- 550 Zhang, L., Kendrick, C., Jülich, D., Holley, S.A., 2008. Cell cycle progression is required for  
551 zebrafish somite morphogenesis but not segmentation clock function. *Dev. Camb. Engl.*  
552 135, 2065–2070. <https://doi.org/10.1242/dev.022673>
- 553 Zhou, W., Liang, I.-C., Yee, N.S., 2011. Histone deacetylase 1 is required for exocrine  
554 pancreatic epithelial proliferation in development and cancer. *Cancer Biol. Ther.* 11,  
555 659–670. <https://doi.org/10.4161/cbt.11.7.14720>
- 556 Zierhut, C., Jenness, C., Kimura, H., Funabiki, H., 2014. Nucleosomal regulation of chromatin  
557 composition and nuclear assembly revealed by histone depletion. *Nat. Struct. Mol. Biol.*  
558 21, 617–625. <https://doi.org/10.1038/nsmb.2845>
- 559 Zupanc, G.K.H., Wellbrock, U.M., Sîrbulescu, R.F., Rajendran, R.S., 2009. Generation, long-  
560 term persistence, and neuronal differentiation of cells with nuclear aberrations in the  
561 adult zebrafish brain. *Neuroscience* 159, 1338–1348.  
562 <https://doi.org/10.1016/j.neuroscience.2009.02.014>  
563

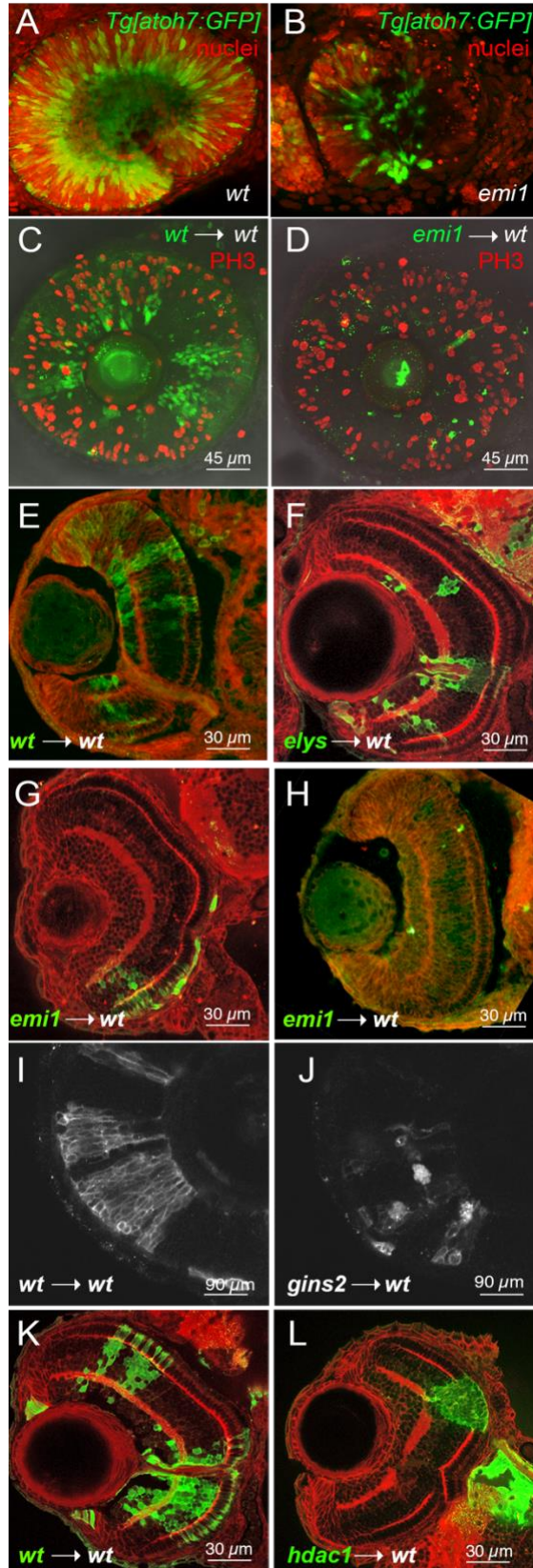
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**Table 1. Cell cycle mutants examined by chimeric analysis in the zebrafish retina**

mutant	phenotype linked to cell cycle defect	phenotypes of mutant cells transplanted into WT retinae	Reference
<i>cdk1<sup>hi3235Tg</sup></i>	stall in G1, G1/S, S phases, apoptosis	apoptosis	(Amsterdam et al., 2004); this study
<i>dtl<sup>hi3627Tg</sup></i>	arrest in late S/early G2, apoptosis	apoptosis	(Sansam et al., 2010); this study
<i>ele<sup>ty77e</sup> (slbp1)</i>	stall in G1/S, apoptosis	apoptosis	(Turner et al., 2019)
<i>emi1<sup>hi2648Tg</sup> (fbxo5)</i>	primarily arrest in G2/M, apoptosis.	some differentiation but also apoptosis	(Rhodes et al., 2009; Riley et al., 2010; Zhang et al., 2008); this study
<i>flo<sup>ti262c</sup> (ELYS)</i>	cycle slowly, stalling in either G1/S or G2/M	survival and differentiation	(Cerveny et al., 2010; Davuluri et al., 2008); this study
<i>gins2</i>	Delayed/prolonged S phase, apoptosis	apoptosis	(Varga et al., in preparation.)
<i>hdac1<sup>hi1618Tg</sup></i>	unable to exit the cell cycle; hyperproliferate and do not differentiate	survival and proliferation	(Yamaguchi et al., 2005; Zhou et al., 2011); this study
<i>mcm5<sup>m850</sup></i>	prolonged S phase, apoptosis	apoptosis	(Ryu et al., 2005); this study
<i>cmp<sup>s819</sup> (ssrp1a)</i>	arrest in S phase, apoptosis.	quiescence and apoptosis	(Koltowska et al., 2013); this study
<i>rbbp6<sup>hi2993Tg</sup></i>	predicted to arrest in G1/S, apoptosis	apoptosis	(Amsterdam et al., 2004); this study

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**Figure 1. Comparison of differentiation behavior of wild-type, *emi1*<sup>-/-</sup>, and *elys*<sup>-/-</sup> neuronal progenitors in developing zebrafish retinæ.**

A-B Lateral maximum intensity projection of 50 hpf retinæ from *Tg[atoh7:GFP]* embryos showing progression of neurogenic gene expression (green) stained with sytox orange (red) to highlight nuclei.

C-D Lateral views of maximum intensity projection of 3 dpf chimeric wild-type retinæ containing GFP-labelled wild-type (C) or *emi1* mutant (D) cells and immunostained for phosphohistone H3 (PH3, red).

E-H Representative images of frontal cryosections of 3 dpf chimeric wild-type retinæ containing GFP-labelled wild-type (E), *elys* mutant (F), or *emi1* mutant (G, H) cells immunostained for GFP (green cells from donor embryo) and B-catenin (red cell boundaries and plexiform layers).

I-J Lateral views of single z-slice of ventral-nasal region of 3 dpf chimeric wild-type retinæ containing wild-type (I) or *gins2* morphant (J) cells labeled with membrane-targeted RFP.

K-L Representative images of frontal cryosections of 3 dpf chimeric wild-type retinæ containing GFP-labelled wild-type (K) or *hdac1* mutant (L) cells immunostained for GFP (green cells from donor) and B-catenin (red) to mark cell boundaries and plexiform layers.

Figure 1

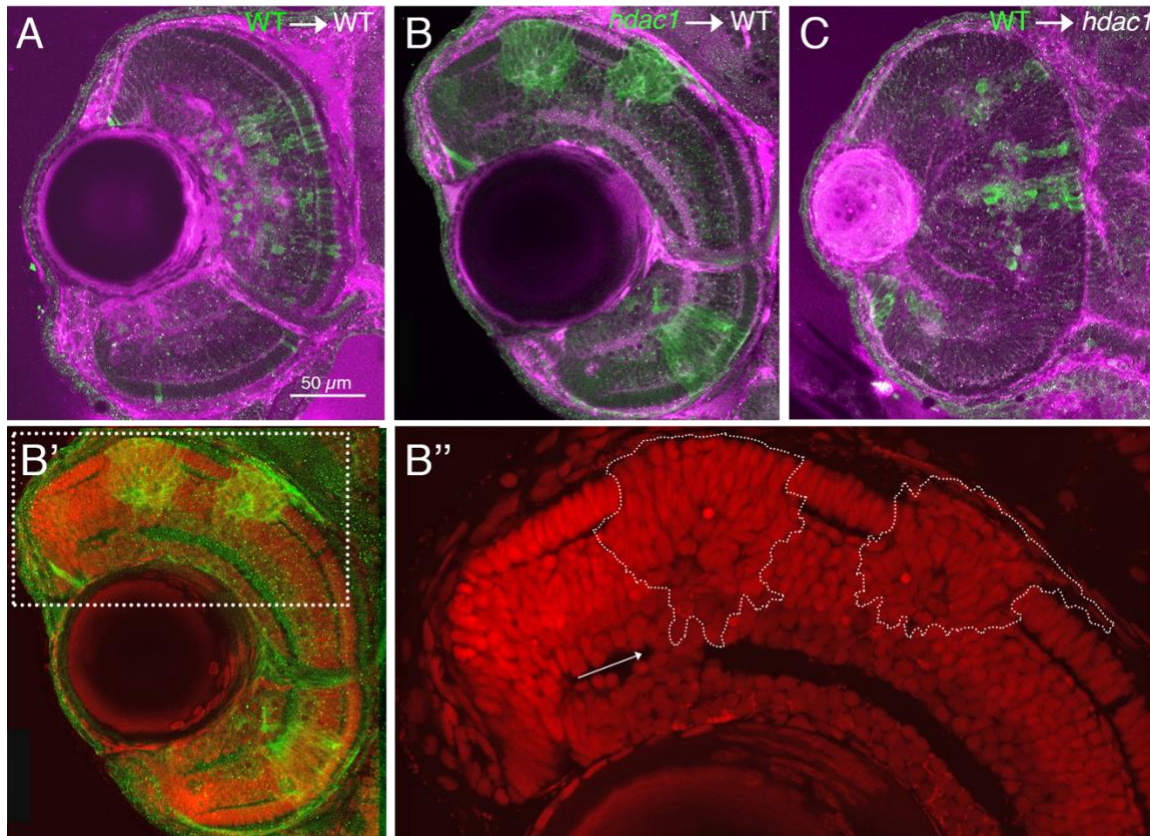
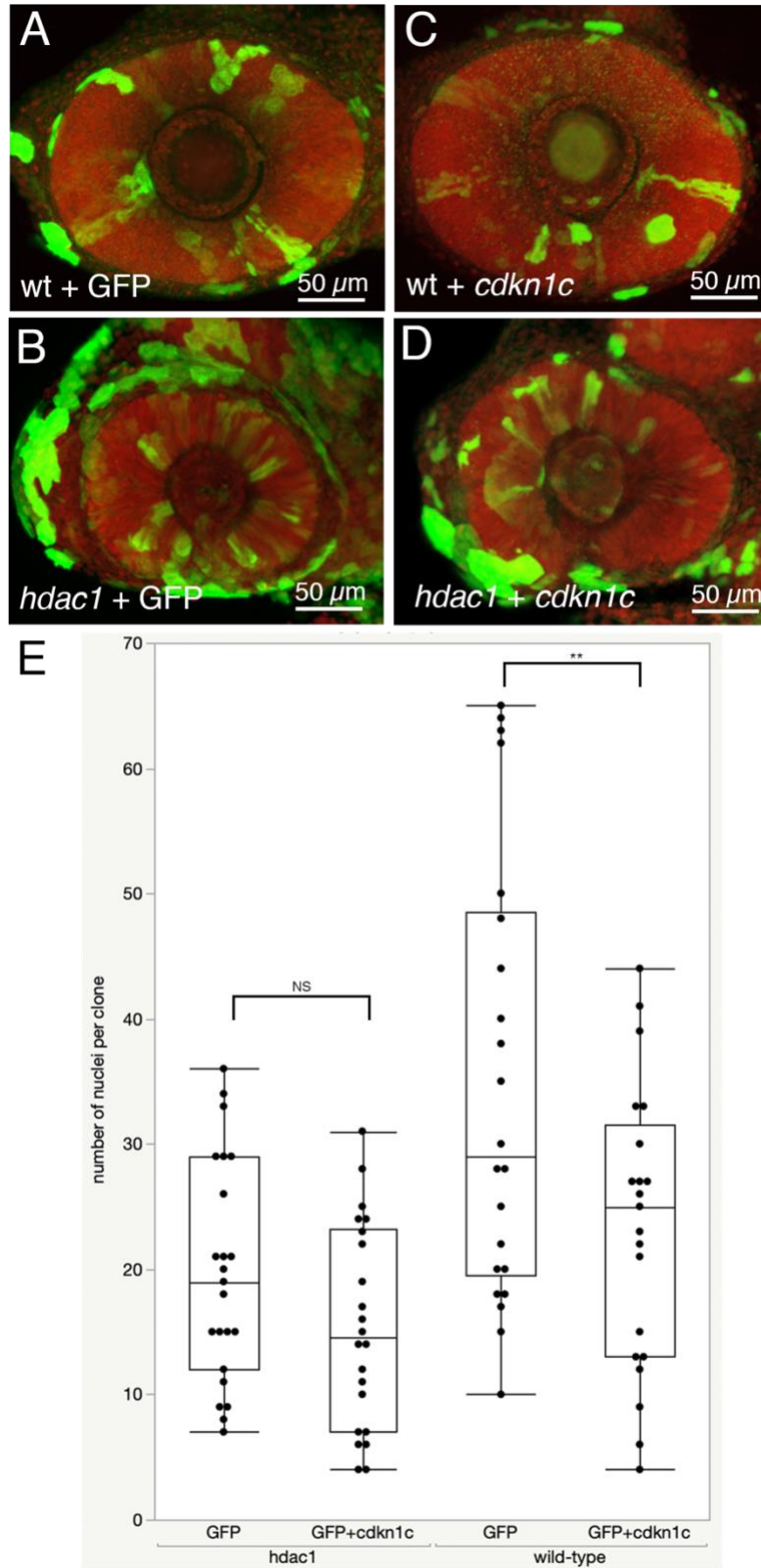


Figure 2

**Figure 2. *hdac1* mutant cells form rosette-like structures that invade surrounding tissues when transplanted into WT retinæ and alter lamination and organization of adjacent wild-type cells.**

A-C Frontal cross-sections of 4 dpf chimeric retinæ when GFP-expressing wild-type (A, C) and *hdac1* mutant (B) cells were transplanted into wild-type (A, B) or *hdac1* mutant (C) host eyes immunostained for ZO-1 (purple) and GFP (green).

B'-B'' Same cross section as in B, this time showing transplanted cells (green) and DAPI-stained nuclei (red). Boxed region ~2X zoom shown in B''. Arrow indicates wild-type cells disrupting the inner plexiform layer within this chimeric retina and *hdac1*<sup>-/-</sup> clone outlined with dashed line.



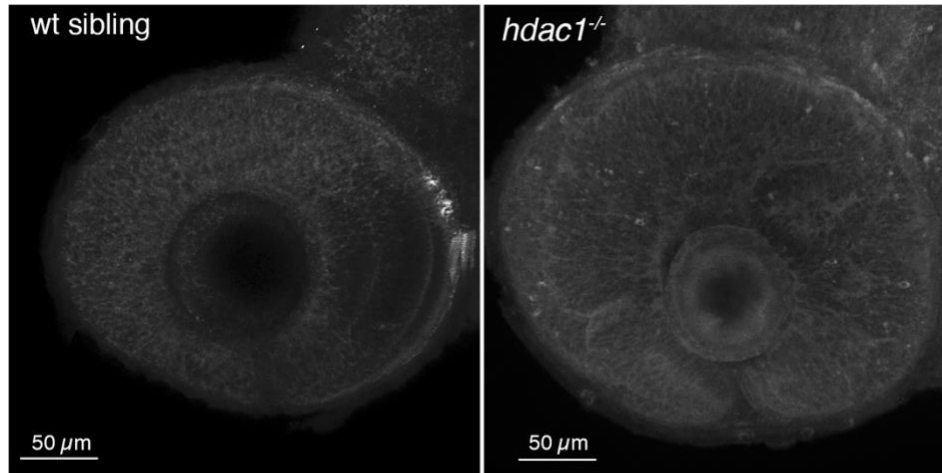
**Figure 3. Forced expression of *cdkn1c* does not significantly alter proliferative behavior of *hdac1* mutant cells.**

A-D Lateral views of maximum intensity z-projections showing nuclei (red) and heat-shock induced GFP (A-B, green) or *cdkn1c*-positive clones (C-D, green) in wholemount 60 hpf embryos with genotype indicated.

E Box plot overlaid on all data points for number of nuclei per clone in GFP and *cdkn1c* containing clones in either *hdac1* and wild-type sibling retinæ. NS, not significant with p-value of  $p=0.1008$ ; \*\*, significant with p-value of 0.0174; Welch's t-test.

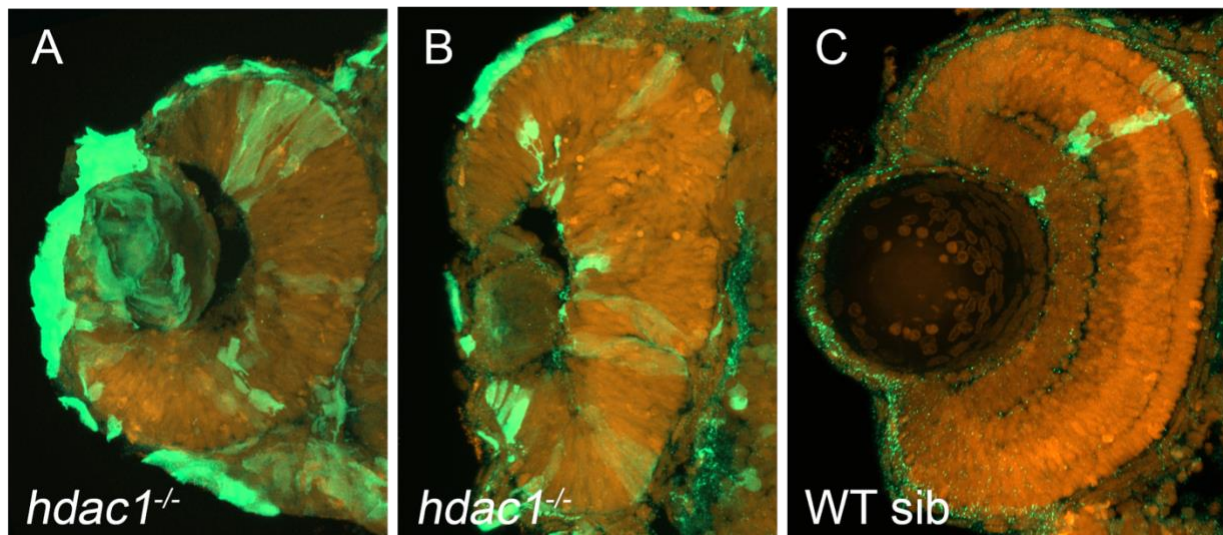
Figure 3





Supplemental Figure 1

**Supplemental Figure 1.** Retinal progenitor cells undergo apoptosis in *hdac1* mutant retinæ. Wild-type siblings (left) and *hdac1* mutants (right) were fixed at ~3.5 dpf and then probed with activated caspase 3 antibody. Both representative images are maximum intensity projections. Note puncta scattered throughout *hdac1* mutant but not in wild-type.



supplemental figure 2

**Supplemental Figure 2.** Inducing high levels of *cdkn1c* rarely trigger neuronal differentiation in *hdac1* retinal progenitor cells.

Frontal cross-sections of retinæ from same experiment shown in Figure 3; *cdkn1c*-positive clones were detected by immunohistochemistry (green) and nuclei stained with DAPI (red). *hdac1* mutant cells in *cdkn1c*-expressing clones (green) generally exhibit neuroepithelial morphology (A, B) consistent with proliferation and rarely show neuronal phenotypes (B, arrow heads) whereas age-matched wild-type sibling cells within *cdkn1c*-expressing clones (green) always exhibit neuronal morphology and lamination (C).