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# Non-apoptotic caspase events and Atf3 expression underlie direct neuronal differentiation of adult neural stem cells

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1 *Research article*

2

3 **Non-apoptotic caspase events and Atf3 expression underlie direct neuronal**  
4 **differentiation of adult neural stem cells**

5

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7

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12

13

14 **Abstract**

15

16 Neural stem cells (NSCs) are key physiological components of adult vertebrate brains, generating  
17 neurons over a lifetime. In the adult zebrafish pallium, NSCs persist at long term through balanced fate  
18 decisions that include direct neuronal conversions, i.e., delamination and neurogenesis without a  
19 division. The characteristics and mechanisms of these events remain unknown. Here we reanalyze  
20 intravital imaging data of adult pallial NSCs and observe shared delamination dynamics between NSCs  
21 and committed neuronal progenitors. In a candidate approach for mechanisms predicting NSC  
22 decisions, we build an NSC-specific genetic tracer of Caspase3/7 activation (Cas3\*/Cas7\*) *in vivo* and  
23 show that non-apoptotic Cas3\*/7\* events occur in adult NSCs and are biased towards neuronal  
24 conversion under physiological conditions. We further identify the transcription factor Atf3 as  
25 necessary to express this fate. Finally, we show that the Cas3\*/7\*/Atf3 pathways are part of the processes  
26 engaged when NSCs are recruited for neuronal regeneration. These results provide evidence for the  
27 non-apoptotic caspase events occurring in vertebrate adult NSCs and link these events with the NSC  
28 fate decision of direct conversion, important for long-term NSC population homeostasis.

29

30

31 **Keywords**

32 Adult neural stem cells; telencephalon; pallium; zebrafish; direct fate conversion; apoptosis; caspase;

33 Atf3

34

35

## 36 Introduction

37

38 Stem cell (SC) fate decisions orchestrate adult organ maintenance and SC population renewal over a  
39 lifetime. These decisions often occur in link with SC division; as a result, much work has been devoted  
40 to understanding SC proliferation frequency and daughter cell fate control. However, it has also been  
41 reported in a few instances, notably in the brain and muscle, that a differentiated fate could be directly  
42 acquired from an adult SC in the absence of a division event under physiological conditions (Barbosa et  
43 al., 2015; Bjornson et al., 2012; Dray et al., 2015; Ismaeel et al., 2023; Mourikis et al., 2012). These events  
44 described as “direct conversion” have remained understudied. Their characteristics are not precisely  
45 described, and it remains largely unknown how they are controlled and what their physiological  
46 relevance may be.

47 In the adult vertebrate brain, neural stem cells (NSCs) are radial astroglia, which are mostly quiescent  
48 and divide at low frequency (Chaker et al., 2016; Lampada and Taylor, 2023; Obernier and Alvarez-  
49 Buylla, 2019; Urbán et al., 2019; Yeh et al., 2023). In mouse, NSCs are specialized SC that can generate  
50 differentiated neurons and astrocytes, both of which are molecularly distinct from NSCs  
51 (Beckervordersandforth et al., 2010; Dulken et al., 2017; Zywitzka et al., 2018). In zebrafish, NSCs co-  
52 express markers of mature astrocytes (e.g., Glial fibrillary acidic protein -Gfap-, or Glutamine synthase  
53 -GS-) and progenitors (e.g., *her4* genes) during their quiescence phase (Cosacak et al., 2019; Morizet et  
54 al., 2024). Upon division, they generate NSCs and/or committed neuronal progenitors (NPs) (negative  
55 for Gfap and/or GS) that differentiate into neurons (Dirian et al., 2014; Furlan et al., 2017; Kroehne et al.,  
56 2011; Mancini et al., 2023). In both models, genetic clonal tracing and/or intravital imaging also provided  
57 evidence for individual NSCs acquiring a neuronal fate while no cell division was detected. For  
58 example, in the mouse dentate gyrus, around 10% of clones issued from *Nestin:Cre*-mediated tracing of  
59 individual NSCs consist of single neurons after one month (Bonaguidi et al., 2011), suggesting a direct  
60 conversion -although the interpretation of these results can be confounded by cell death-. In the  
61 zebrafish pallium, intravital imaging reveals direct neuronal differentiation occurring from *her4.3*-  
62 positive or *gfap*-positive NSCs that do not express proliferation markers and/or have not divided since  
63 at least 14 days (4 imaging time points, 14-16 days) (Barbosa et al., 2015; Dray et al., 2015; Than-Trong  
64 et al., 2020). Longitudinal imaging in these cases avoids ambiguity as no cell death events were  
65 observed, but the occurrence of direct conversion events remains difficult to quantify at population scale  
66 as the duration of imaging is short relative to NSC division frequency. *her4:ERT2CreERT2*-mediated  
67 clonal tracing and modeling, performed on a dataset spanning over >530 days, predict that direct  
68 conversions represent 25% of fate decisions (Than-Trong et al., 2020), and are crucial to maintain  
69 homeostasis of the NSC population. Indeed, a long-term equilibrium of NSC numbers is observed *in*

70 *situ* and results from balancing NSC gains (through amplifying divisions) and losses (through  
71 symmetric neurogenic divisions and direct differentiation events) (Than-Trong et al., 2020).

72 In the line of our current work aiming to identify predictors and mechanisms of NSC fate decisions in  
73 vivo, we focused on NSC direct conversions and challenged whether they could be connected with non-  
74 apoptotic caspase events. Caspases are site-specific proteases of the programmed cell death system. In  
75 addition to the execution of cell death, however, caspases are increasingly recognized to participate in  
76 non-apoptotic events during development and homeostasis in a variety of tissues and organisms  
77 (reviewed in (Abdul-Ghani and Megeney, 2008; Burgon and Megeney, 2018). In particular, there is a  
78 frequent association of non-apoptotic caspase events with cell fate decisions, including in some SCs e.g.  
79 ES cells or hematopoietic SCs (Fujita et al., 2008; Janzen et al., 2008). In the nervous system, non-  
80 apoptotic caspase recruitments modulate dendritic pruning, developmental circuit maturation and  
81 axonal pathfinding, to cite a few (Unsain and Barker, 2015). In embryonic neurospheres in vitro,  
82 blocking these events limits neuronal differentiation and neurite extension (Fernando et al., 2005).  
83 Finally, in the embryonic peripheral nervous system in *Drosophila*, non-apoptotic caspase-mediated  
84 cleavage of signaling pathway effectors limit neural progenitor proliferation to control neuronal  
85 production (Colon-Plaza and Su, 2022; Kuranaga et al., 2006). Non-apoptotic caspase events involve  
86 activation of the direct caspase pathway converging onto the activation of effector Caspases 3 and 7  
87 (activated forms noted as Cas3\*, Cas7\*) by proteolytic cleavage, but can be tracked using Cas3\*/Cas7\*  
88 sensors. For example, in *Drosophila*, CasExpress and CaspaseTracker liberate Gal4 upon cleavage at the  
89 Cas3\*/Cas7\* canonical site DEVD, to drive lineage labeling when caspase activation is not followed by  
90 cell death (Ding et al., 2016; Tang et al., 2015). These studies revealed multiple cells surviving an early  
91 caspase event under physiological conditions. Non-apoptotic Cas3\* or Cas7\* can be seen as true death  
92 reversals (referred to as anastasis) (Sun et al., 2017; Tang and Tang, 2018). Rather than interrupted  
93 apoptosis, they may also signify the use of common pathways to trigger cell remodeling shared between  
94 apoptosis and cellular decisions. Whether non-apoptotic caspase events take place in adult NSCs is  
95 unknown.

96 In this paper, we proceed in two initially independent steps: first, we attempt to characterize the  
97 morphodynamic features of direct conversion events; second, we address whether non-apoptotic  
98 caspase events take place in NSCs in the adult vertebrate brain, and if so, whether these events encode  
99 specific fate decisions. We use as model the zebrafish adult pallium, the cartesian construction of which  
100 allows inferring the birthdate and origin of any neuron from its position at the time of analysis (Furlan  
101 et al., 2017). We generate an inducible, selective and stable Cre-mediated Cas3\*/Cas7\* sensor,  
102 *Cas<sup>CRE</sup>Atlas*, which allows long-term fate tracing of non-apoptotic Cas3\*/Cas7\* events occurring in NSCs.  
103 We find that such events do take place in NSCs and, further, that they are biased towards fate choices

104 of direct neuronal generation under physiological conditions. Combining gain- and loss-of-function  
105 experiments, we identify the stress-induced transcription factor Atf3 as necessary for this NSC fate in  
106 vivo. Finally, we show that Cas3\*/Cas7\* events are also induced in response to lesion. Together, these  
107 results highlight the occurrence of non-apoptotic caspase events in NSCs during pallial development  
108 and adult life, and demonstrate their relevance for a specific NSC fate decision involved in the  
109 physiological homeostasis of adult NSC populations.

110

## 111 **Results**

112

### 113 **Direct conversion events and post-division delaminations share morphodynamic features**

114 Using intravital imaging of the whole NSC population in the adult zebrafish pallium, NSC direct  
115 conversion events were previously defined as the loss of expression of the NSC marker Tg(*gfap:dTomato*)  
116 accompanied with delamination from the pallial ventricular layer in the absence of visible division  
117 during the preceding 4 imaging time points (14 to 16 days) (Than-Trong et al., 2020). The choice of this  
118 duration was based on the observation that, when a neurogenic division is visible in a movie, 85 +/- 10%  
119 of neuron-fated daughter cells visibly express their fate (loss of *gfap:dTomato*) within the 10-12 days  
120 post-division (Dray et al., 2021; Than-Trong et al., 2020). Such events are followed by expression of the  
121 neuronal differentiation marker HuC/D and the presence of a neuronal process (Barbosa et al., 2015).

122 To characterize the morphodynamic features of these events and determine whether and how they  
123 contrast with delamination and differentiation events occurring as a result of neurogenic divisions  
124 (hereafter referred to as “post-division delaminations”), we exploited the intravital imaging movies  
125 acquired in four 3-month post-fertilization (mpf) adults in the *Casper*;Tg(*gfap:ZO1-*  
126 *mKate2*);Tg(*deltaA:egfp*) background (Mancini et al., 2023). This dataset contains a total of 828 NSCs  
127 filmed over 39 to 43 days every 2 to 3 days in 4 pallial hemispheres (from 4 different fish) and reveals  
128 NSC apical surfaces (ZO1-mKate2) and a neurogenic fate (*deltaA* expression). We focused here on  
129 delaminations, which were not previously analyzed. We defined as “delamination termination” the first  
130 time point with no identifiable ZO1-mKate2-negative apical surface (within the resolution of our  
131 intravital two-photon imaging), i.e., the moment of apical closure (Fig.1A); this time point is then  
132 followed by the reestablishment of apicobasal boundaries between remaining cells. When no division  
133 is visible along the track, we refer to “time point 0” (tp0) as the first imaging time point of the movie  
134 (Fig.1A, top); in that case, we excluded delaminations occurring less than 4 time points (14-16 days)  
135 from the start of each movie, to avoid missing recent division events. When a division visible along the  
136 track, tp0 is the first time point post-division (Fig.1A, bottom). With these criteria, the dataset includes

137 83 delamination events. 54 of these delaminations terminated after at least 4 imaging time points (14-16  
138 days) without visible division, and 29 followed a visible division during the previous 3 imaging time  
139 points or less ( $\leq 13$  days). In the first category, the time from  $tp_0$  to delamination termination varies  
140 between 14 and 41 days. Two examples are illustrated in Fig.1B, displaying delamination events that  
141 occur without detectable division during the previous 23 days (example 1) and 35 days (example 2).

142 We next analyzed the measurable morphometric parameters of delaminations, comparing those  
143 occurring after at least 4 time points without division vs 3 time points or less post-division (red and  
144 black on Fig.1C-E, respectively). Cells in these two categories did not differ significantly in their apical  
145 area at the time point preceding delamination termination (on average,  $9.4 \mu\text{m}^2$ ,  $\pm 6.1 \mu\text{m}^2$  SD and  $9.3$   
146  $\mu\text{m}^2$ ,  $\pm 4.8 \mu\text{m}^2$  SD respectively) (Fig.1C), nor in their expression of *deltaA* (100% *deltaA<sup>pos</sup>* cells in both  
147 cases) (examples in Fig.1B, bottom). There was a moderate correlation between apical surface area at  
148 the onset of apical surface shrinkage ( $tp_{sh}$ ) and the duration of apical shrinkage during the delamination  
149 process (cells starting with a larger apical surface area taking longer to delaminate) (Fig.1D). When  
150 normalized over the duration of shrinkage until delamination termination, shrinkage rates appeared  
151 similar between the two categories ( $\sim 1 \mu\text{m}^2$  per time point) (Fig.1E). Together, these results characterize  
152 the quantitative and molecular features of the delamination process during adult pallial neurogenesis.  
153 They reveal its comparable dynamics irrespective of time post-division or apical surface area at the start.

154 The dataset does not allow to directly recognize direct conversions, as NPs can be misled for NSCs.  
155 Indeed, ZO1-mKate2 expressed by NSCs also surrounds intermingled NPs and makes them tractable.  
156 NSCs have on average larger apical surface areas than NPs (with medians at  $100 \mu\text{m}^2$  vs  $20 \mu\text{m}^2$ ,  
157 respectively), but the distributions are broad and overlap ((Mancini et al., 2023) and N. Dray, unpub.).  
158 To estimate the percentage of NSCs in our dataset, we considered the two possible NP configurations:  
159 (i) isolated NPs surrounded by NSCs, and (ii) NP clusters (e.g., an NP doublet generated by the  
160 symmetric neurogenic division of an NSC or an NP, which will appear as a single apical surface  
161 surrounded by ZO1-mKate) (Fig.S1A). To quantify NP clusters, we counted the number of ZO1-mKate  
162 surfaces tracked in intravital imaging that in fact contained adjacent NPs, by comparing our live data  
163 with immunohistochemistry for ZO1 on the same specimen fixed at the end of the film (Fig.S1A). The  
164 percentage of surrounded NP clusters increased as the ZO1-mKate surface area decreased (Fig.S1B), as  
165 logically expected from the fact that NPs have smaller apical surface areas than NSCs. With an apical  
166 surface area of  $30 \mu\text{m}^2$  or less at  $tp_{sh}$  (which is a large majority of recorded events, 80 over 83 events, 96%)  
167 (see below and Fig.1D), 21.4% of ZO1-mKate surfaces were NP clusters (Fig.S1B). To count isolated NPs,  
168 an NSC marker was necessary and we used fixed Tg(*gfap:dTomato*) pallia immunostained for Tomato  
169 and ZO1. With an apical surface area of  $30 \mu\text{m}^2$  or less, 29.3% of surfaces were isolated NPs (Fig.S1B).  
170 Together, we conclude that approximately half of the ZO1-mKate surfaces of  $30 \mu\text{m}^2$  or less recorded in

171 intravital imaging are NSCs (Fig.S1C). This makes it likely that the morphometric features measured  
172 above (Fig.1) also apply to NSC delaminations (referred to as direct conversions).

173

### 174 **Non-apoptotic Cas3\*/Cas7\*-mediated cleavage events take place during pallium development and** 175 **homeostasis**

176 In our search for predictive parameters of NSC decisions, including direct conversions, we next  
177 considered non-apoptotic Caspase events. To determine whether non-apoptotic proteolytic events at  
178 the DEVD Cas3\*/Cas7\* target site (Chéreau et al., 2003; Talanian et al., 1997) occur in the developing  
179 and adult zebrafish pallium, we designed a heritable lineage tracer of DEVD cleavage in NSCs. The  
180 driver transgenic line, Tg(*her4:mCD8-DEVD-V5-Cre*), produces a Cre recombinase (fused to a V5 tag)  
181 tethered to the plasma membrane via a DEVD site, expressed under control of the NSC *her4.3* regulatory  
182 elements (Yeo et al., 2007) (Fig.2A). When Tg(*her4:mCD8-DEVD-V5-Cre*) fish are crossed into the  
183 Tg(*βact:lox-stop-lox-hmg2bmCherry*) reporter background (Wang et al., 2011) (this double background is  
184 hereafter referred to as *Cas<sup>CRE</sup>Atlas*), non-apoptotic cleavage events at the DEVD site in *her4*-expressing  
185 cells are expected to trigger Cre-mediated recombination and the permanent expression of  
186 Hmg2bmCherry in all progeny cells. A Tg(*her4:mCD8-GSGC-V5-Cre*) driver, immune to Cas3\*/Cas7\*,  
187 was used as control.

188 To validate the approach, we first analyzed the expression of the *her4:mCD8-DEVD-V5-Cre* transgene  
189 and the inducibility and selectivity of *Cas<sup>CRE</sup>Atlas* tracing in the larval pallium at 6 days post-fertilization  
190 (dpf). Immunohistochemistry in the double transgenic Tg(*her4:mCD8-DEVD-V5-Cre*);Tg(*gfap:eGFP*)  
191 background showed that V5-Cre expression is faithfully restricted to NPs in the larval pallium (Fig.2B).  
192 Next, we used whole-mount immunohistochemistry to test whether Hmg2bmCherry-positive cells  
193 were present in the pallium of 6dpf *Cas<sup>CRE</sup>Atlas* versus control larvae. While Hmg2bmCherry events  
194 were rare in control pallia -possibly reflecting a low affinity of Cas3\*/Cas7\* for non-canonical sites, or  
195 cleavage of GSGC by another protease at low frequency-, Hmg2bmCherry-positive cells were easily  
196 detectable and significantly more numerous in *Cas<sup>CRE</sup>Atlas* pallia (Fig.2A',2C-E). Finally, we tested  
197 whether *Cas<sup>CRE</sup>Atlas* was responsive to activation of the caspase cascade. 4dpf larvae were transiently  
198 subjected to the apoptosis inducer Camptothecin (CPT) (Ikegami et al., 1999) injected into the hindbrain  
199 ventricle, then chased until 6dpf. CPT significantly increased the number of Hmg2bmCherry-positive  
200 cells in *Cas<sup>CRE</sup>Atlas* larvae compared to control DMSO injections (Fig.2F-I). These results together  
201 demonstrate the selective and sensitive tracing of non-apoptotic Caspase events by *Cas<sup>CRE</sup>Atlas* in the  
202 zebrafish pallium *in vivo*, and for the occurrence of such events under physiological conditions at least  
203 at larval stages.

204 The zebrafish pallium follows an outside-in neuronal generation pattern, whereby neurons generated  
205 at early stages end up in deep parenchymal locations whereas late-born neurons are positioned more  
206 superficially, close to the active NSC monolayer (Furlan et al., 2017). Tangential neuronal migration  
207 does not take place, and these properties together allow using the position of a neuron as a spatio-  
208 temporal signature to infer its generation time and approximative ventricular region of origin. We used  
209 these properties to characterize and interpret the pattern of Hmg2bmCherry cells at juvenile and adult  
210 stages in *Cas<sup>CRE</sup>Atlas* animals (Fig.2J and see below). At these stages, neurogenesis follows the sequence  
211 NSCs (*her4<sup>pos</sup>* or *gfap<sup>pos</sup>*; *Sox2<sup>pos</sup>*) > NPs (*her4<sup>neg</sup>* and *gfap<sup>neg</sup>*; *Sox2<sup>pos</sup>*) > neurons (Than-Trong et al., 2020).  
212 At 1.5mpf, a few deep groups of Hmg2bmCherry cells could be occasionally seen in control pallia  
213 (Fig.2L,L'), resulting from early non-specific activation events as described at 6dpf. In contrast, the  
214 Hmg2bmCherry pattern of *Cas<sup>CRE</sup>Atlas* 1.5mpf pallia was very different, with numerous small groups  
215 of labeled cells, some located in superficial pallial layers (Fig.2K,K'). Immunohistochemistry for Sox2 -  
216 which also labels some recently born neurons- was used as a landmark and revealed deeply located  
217 (old) Sox2-positive neurons as well as more superficial (recent) events, with staining of either freshly  
218 born neurons, NSCs or NPs (Fig.2K<sub>1</sub>,K<sub>2</sub>). We interpret this pattern as reflecting Cre recombination  
219 events that occurred at different time points (from older to more recent) in *her4*-expressing cells.

220

### 221 **The Cas3\*/Cas7\*-driven lineage is biased towards direct neuronal differentiation**

222 Cas3/Cas7 activation in *her4*-positive cells may reflect transient events unrelated to NSC decisions or  
223 may correlate with specific behaviors. To address this, we compared NSC fate in *Cas<sup>CRE</sup>Atlas* labeled  
224 clones with *her4*-mediated NSC fate tracing in the juvenile to young adult pallium (Fig.3A). Because  
225 neurons generated from pallial NSCs stack in age-related layers (Furlan et al., 2017), we established a  
226 temporal landmark across pallial depth, using a BrdU pulse at 1mpf, to label neurons born at that stage.  
227 This allowed to select *Cas<sup>CRE</sup>Atlas* events having occurred between 1mpf and the stage of analysis  
228 (2mpf): these events generated Hmg2bmCherry-positive cells located above the BrdU landmark  
229 (Fig.3B). In parallel, clonal recombination events were induced in *Tg(her4:ERT2CreERT2);Tg( $\beta$ act:lox-*  
230 *stop-lox-eGFP)* double transgenic animals just prior to the BrdU pulse, and analyzed at 2mpf as well.

231 We considered that Hmg2bmCherry-positive cells belong to the same clones when they were separated  
232 by less than a 2-cell diameter distance. This is based on previous observations documenting the absence  
233 of cell migration or death of *her4* progeny cells in the zebrafish pallium (Furlan et al., 2017; Webb et al.,  
234 2009). The composition of clones was assessed in 3D in whole-mount pallia using  
235 immunohistochemistry for *gfap:eGFP* (NSCs). GFP-negative cells include NPs and neurons and, for  
236 simplicity, were labeled as neurons (Fig.3C-C"). As expected, we found that unbiased clonal labeling  
237 generates a variety of clone types (Fig.3D). Specifically, single NSCs are interpreted as NSCs that



238 remained quiescent since the labeling pulse, NSC doublets as resulting from an amplifying NSC/NSC  
239 division (red in Fig.3D), neuron doublets from -most likely- a symmetric neurogenic division, and single  
240 neurons from a direct differentiation event (blue and yellow, respectively, in Fig.3D). The lineage tree  
241 of clones composed of 3 cells or more cannot be resolved, and these clones were simply classified as  
242 “attached” or “detached” depending on their link or not with the NSC layer (magenta and orange,  
243 respectively, in Fig.3D). They reflect ongoing, or terminated, productive neurogenic events,  
244 respectively. These fates together are in qualitative and quantitative agreement with the different  
245 division modes and fates reported in previous work (Than-Trong et al., 2020). We found Cas3<sup>\*</sup>Cas7<sup>\*</sup>  
246 events associated with most clone types; however, attached clones were virtually absent, while the  
247 proportion of single neurons was increased (Fig.3D). Thus, non-apoptotic Cas3<sup>\*</sup>/Cas7<sup>\*</sup> events in pallial  
248 NSCs correlate with a bias of NSC fate towards direct neuronal differentiation. Finally, this analysis  
249 allowed to estimate that, on average, 50 Cas<sup>CRE</sup>Atlas events occur during 4 weeks within an hemipallium  
250 (i.e., an area of approximately 2000-2500 her4<sup>pos</sup> NSCs).

251

### 252 **The Atf3 transcription factor, a mediator of anastasis, is expressed in a subset of scattered** 253 **delaminating cells at the adult pallial ventricle at any time**

254 We next aimed to identify molecular events involved in promoting the direct differentiation fate of  
255 pallial NSCs. The results above are correlative, but suggestive. Hence, we used a candidate approach  
256 searching among known mediators of the non-apoptotic caspase event known as anastasis (Sun et al.,  
257 2017; Tang et al., 2017). A common molecular signature of anastasis was identified in various  
258 mammalian cell types (Sun et al., 2017; Tang et al., 2017). The orthologs of many genes up-regulated at  
259 an early stage of anastasis fate reversal appeared expressed in a subset of pallial NSCs under  
260 physiological conditions, as revealed in our scRNAseq dataset (Morizet et al., 2024). Among these genes  
261 (Fig.S2A), we chose to focus on the transcription factor-encoding gene *atf3*, as one of the top genes  
262 induced in mammalian cells, and because its scRNAseq expression was detectable in a low number of  
263 pallial quiescent NSCs in a manner unlinked with clustering (Fig.4A). A low cell number is in agreement  
264 with the low frequency of NSC fate decisions at any given time, considering the slow dynamics of the  
265 NSC population (Dray et al., 2021; Than-Trong et al., 2020). Sparse expression was confirmed using  
266 whole-mount in situ hybridization (ISH), which revealed scattered *atf3*-positive cells across the pallial  
267 surface (Fig.4B). Some areas exhibited a higher concentration of *atf3*-positive cells (such as the posterior  
268 and dorsomedial pallium), but at small scale the *atf3* pattern is not exactly identical in each of these  
269 areas in the two hemispheres. Finally, we used the *Tg(gfap:eGFP)* background and fluorescent  
270 chromogenic ISH to address the morphology of *atf3*-positive cells. Cross-sections, or horizontal optical  
271 focus at progressively deeper z planes from the ventricular surface, showed that *atf3*-positive;GFP-

272 positive cells have a delaminating profile, with their nuclei often located in deeper positions than the  
273 majority of NSCs (Fig.4C,D). Two *atf3* transcripts were recovered in the adult pallium, corresponding  
274 to alternative splicing events predicted to encode long and short protein isoforms that differ in their N-  
275 terminus (Fig.4E). Our ISH used the *atf3-L* probe and does not distinguish between these isoforms.

276

### 277 **Atf3 is necessary for the direct neuronal differentiation of adult pallial NSCs, and impacts** 278 **physiological *Cas<sup>CRE</sup>Atlas* fate decisions**

279 Next, we combined gain- and loss-of-function experiments in adult pallial NSC *in vivo* to test whether  
280 Atf3 impacts NSC fate. Electroporation of a nlsGFP-encoding construct driven by the ubiquitous *pCMV*  
281 promoter upon injection into the cerebral ventricle highlights different fates at short time scale (2 days  
282 post-electroporation -dpe-): a large majority of ventricular cells (with a radial morphology and  
283 expressing Sox2, likely NSCs), and a minority of delaminating cells (with basally displaced nucleus and  
284 ventricular attachment, likely NPs) and of Sox2-negative parenchymal cells (interpreted as neurons)  
285 (Fig.5A-E). When electroporated under the same conditions, *pCMV:atf3-S-nlsGFP* had no effect on cell  
286 fate (Fig.5E) while *pCMV:atf3-L-nlsGFP* significantly increased parenchymal cells at the expense of  
287 ventricular cells (Fig.5D). We conclude that forced expression of Atf3-L leads to the direct delamination  
288 of ventricular cells. Because this is reminiscent of non-apoptotic Cas3\*-driven fates, we next assessed  
289 whether Atf3-L could induce *Cas<sup>CRE</sup>Atlas*. When electroporated into the *Cas<sup>CRE</sup>Atlas* double transgenic  
290 background, we observed that *pCMV:atf3-L-nlsGFP* indeed correlated with Hmg2bmCherry expression,  
291 although only in a small minority of GFP-positive cells at 7 dpe (Fig.5F, white arrows). Given the low  
292 number of electroporated cells, and the low number of ventricular *Cas<sup>CRE</sup>Atlas* cells, we believe that  
293 double positive cells are unlikely to result from chance. Their low occurrence is however puzzling. Cas3\*  
294 may be induced but at levels too low or too transient to generate enough productive Cre recombinase.  
295 Alternatively, Atf3 may require a specific context to act upstream of Cas3\* in the system studied here.  
296 It is to note that the hierarchical position of Atf3 relative to Cas3\* has been reported to vary (see  
297 Discussion).

298 We next aimed to test whether Atf3 was required for NSC direct differentiation/delamination. The very  
299 low number of *atf3*-expressing NSCs at any time, and our impossibility to track them with currently  
300 available tools, did not permit addressing this point in the general context of the entire NSC population.  
301 Thus, we more specifically determined whether Atf3 was necessary for direct neuronal differentiation  
302 downstream of Cas3\*, by testing the effect of blocking Atf3-L function *in vivo* in the *Cas<sup>CRE</sup>Atlas* double  
303 transgenic context. We designed a vivo-morpholino (MO) directed against the exon2-intron2 boundary  
304 of *atf3*, predicted to generate a truncated Atf3-L protein devoid of its DNA-binding and Leucine zipper  
305 domains (Fig.5G). This prediction was validated in embryos (Fig.S2B). This MO should not affect the

306 production of the Atf3-S isoform, the start codon of which is located 3' to the MO position. The *atf3* vivo-  
307 MO, or a control vivo-MO, were injected into the cerebral ventricle of *Cas<sup>CRE</sup>Atlas* double transgenic  
308 adults, and *Cas<sup>CRE</sup>Atlas* fates were analyzed after 7 days in whole-mount pallia (Fig.5H-J). Specifically,  
309 Hmg2bmCherry-positive clones were categorized when located within the first 1-2 cell rows below the  
310 ventricular surface (corresponding to the z-position of NSC-progeny cells generated during 7 days). We  
311 found that the *atf3* vivo-MO induced a significant increase in the proportion of clones composed of  
312 NSCs only, at the expense of the generation of single neurons (Fig.5K). These results together indicate  
313 that Atf3-L expression is necessary for lineage bias, either downstream of or in parallel to physiological  
314 non-apoptotic Cas3\* events.

315

### 316 **Experimentally induced Cas3/Cas7 activation events drive direct neuronal production from adult** 317 **NSCs in vivo**

318 The results above indicate that Cas3/Cas7 activation and Atf3-L are, together, linked with the specific  
319 NSC fate choice of direct neuronal differentiation under physiological conditions. To further address  
320 the relevance of this regulatory process, we first tested whether experimental stimulation of Cas3/Cas7  
321 activity in adult fish could modify NSC fate. CPT was used as an inducer. Incubation in CPT triggered  
322 Cas3\* induction and was followed by NP death at larval stages (as revealed by Tg(*ubi:secA5-mVenus*)  
323 reporter, in which Annexin 5-mVenus expression serves as a marker for cell death) (van Ham et al.,  
324 2010) (Fig.S3A-B<sub>i</sub>). In striking contrast, pallial Cas3\* cells were seen instead to delaminate when CPT  
325 was injected into the cerebral ventricle in adult animals (Fig.6A-C<sub>i</sub>'). To track their fate at longer term,  
326 CPT was applied to *Cas<sup>CRE</sup>Atlas* adults and clone types were assessed (Fig.6D-H). We found an increase  
327 of clones composed of single neurons (blue in Fig.6F',H) at the expense of clones made of single NSCs  
328 (red in Fig.6F',H), thus mimicking the fate observed for physiological *Cas<sup>CRE</sup>Atlas* events in the adult  
329 pallium.

330

### 331 **Non-apoptotic Cas3 activation events are seldom recruited upon NSC irradiation but contribute to** 332 **the generation of neurons during lesion repair**

333 Given that Cas3/Cas7 activation events can drive NSC fate change in the adult pallium, we next tested  
334 whether such events were recruited under challenged conditions known to impact NSC state or fate.

335 qNSCs have been described as radiation resistant, possibly thanks to an efficient mechanism of DNA  
336 repair (Barazzuol et al., 2019; Hellström et al., 2009; Mineyeva et al., 2019). However, radiation-induced  
337 differentiation was also described (Konirova et al., 2019; Schneider et al., 2013). We used Xray irradiation  
338 of live adults (3mpf) to further support this observation and test whether radiation resistance could also

339 be accompanied by a NSC fate change *in vivo*. Short (1-hour) treatment with a low radiation dose (5 Gy)  
340 induced  $\gamma$ H2AX-positive foci in NSC/NP nuclei, indicative of double strand DNA breaks and the  
341 recruitment of the repair machinery (Fig.7A-B'). This process was transient and completed by 2 hours  
342 post-treatment (Fig.S4) with no visible effect on NSC fate and was observed until very high irradiation  
343 doses (not shown). At 40 Gy, a low number of cells located very close to the ventricular surface turned  
344 positive for Cas3\* after a 24-hour chase (Fig.7C-D<sub>1</sub>). These cells displayed a delaminating profile with a  
345 cell body partly displaced into the parenchyme (Fig.7D<sub>2</sub>-D<sub>3</sub>) but sometimes keeping a ventricular  
346 attachment (Fig.7D<sub>2-2'</sub>) and were Sox2-negative. Although we did not ascertain cell survival at later chase  
347 times, these results suggest that, upon irradiation at high dose, a few Cas3\* events are induced and  
348 correlate with the first steps of neuronal commitment, namely parenchymal relocation and the loss  
349 of expression of the progenitor marker Sox2. The broad range of low to moderate irradiation schemes  
350 however lead to repair and do not recruit Cas3\*.

351 Mechanical lesions applied to the adult pallium lead to NSC recruitment into division for neuronal  
352 repair (Baumgart et al., 2012; Kishimoto et al., 2012; Kroehne et al., 2011; März et al., 2011), accompanied  
353 with a partial fate shift towards symmetric neurogenic divisions (Barbosa et al., 2015). Absolute  
354 numbers of direct differentiation events were not reported in the latter study. We tested whether *atf3*-  
355 positive or Cas3\*/Cas7\* events could be involved in the response to lesion. We found that *atf3* expression  
356 was induced around the lesioned ventricular zone starting at 1 day-post-lesion (dpl) and very  
357 prominently at 3dpl (Fig.7E-H, asterisk). It was also massively downregulated in the contralateral  
358 hemisphere at 3dpl, a phenomenon not reported yet for other lesion-responsive genes (Fig.7H, open  
359 arrowhead). Like under physiological conditions (Fig.4C1-D4), *atf3*-positive cells in lesioned pallia  
360 displayed a delaminating morphology (Fig.7I-J'). We next used *Cas<sup>CRE</sup>Atlas* to track the fate of  
361 Cas3\*/Cas7\* events in lesioned context. At 7dpl, *Cas<sup>CRE</sup>Atlas* fates recorded in lesioned hemispheres did  
362 not detectably differ, qualitatively and in proportion, from those recorded under physiological  
363 conditions (Fig.7K and Fig.5H-K), thereby contributing to reparative neurogenesis via their enhanced  
364 direct neuronal production.

365

## 366 Discussion

367 In this work, we focus on the adult NSC fate of direct neuronal conversion and bring together several  
368 observations in link with this fate. First, we provide a quantitative, morphometric and molecular  
369 characterization of delamination/neuronal differentiation events in the NSC/NP population of the adult  
370 pallium. This analysis suggests that direct NSC conversions follow a classical delamination process.  
371 Second, we demonstrate that non-apoptotic events of Cas3/Cas7 activation physiologically occur in  
372 NP/NSCs during development and homeostasis of the zebrafish pallium and, at adult stage, are

373 preferentially associated with the NSC fate choice of direct neuronal differentiation. We identify the  
374 transcription factor *Atf3* to be necessary downstream of or in parallel to *Cas3\**/*Cas7\** for the full  
375 expression of this fate. Third, we analyze whether *Cas3\**/*Cas7\** events can be recruited to trigger direct  
376 neuronal conversion under non-physiological conditions. We show that *Cas3\**/*Cas7\** events are induced  
377 in adult pallial NSCs in response to mechanical lesion and contribute to the formation of neurons for  
378 repair. Together, our results provide a first molecular insight, with lineage and functional tracking in  
379 vivo, into the important NSC fate decision of direct neuronal conversion. This process balances fate  
380 choices to preserve homeostasis of the pallial NSC population over a lifetime (Than-Trong et al., 2020).  
381 We propose that adding such a regulatory level beyond NSC division fate choices could add flexibility,  
382 in time and space, to the control of NSC numbers and neuronal production.

383 The existence of direct neuronal conversion of NSCs, i.e. their acquisition of a neuronal fate in the  
384 absence of division, can be postulated from clonal tracing in the adult mouse SGZ (Bonaguidi et al.,  
385 2011), and has been observed using intravital imaging in the adult zebrafish pallium (Barbosa et al.,  
386 2015; Dray et al., 2015; Than-Trong et al., 2020). The *Tg(gfap:ZO1-mKate);Tg(deltaA:egfp)* intravital  
387 imaging dataset allowed us to establish the morphodynamic and molecular characteristics of  
388 delaminations and, among those, of those occurring late post-division (at least 14-16 days and up to 41  
389 days in our movies). Our dataset includes NSCs and NPs that cannot be distinguished in intravital  
390 imaging but our estimations (Fig.S1) make it highly likely that NSCs are included among the recorded  
391 events, and our combined observations suggest that direct NSC conversions follow a classical  
392 delamination process. The fact that delaminating NSCs do not undergo apoptosis and convert into  
393 neurons, although not directly assessed here, is itself supported by a set of arguments from other works:  
394 apoptosis is not observed in the adult pallium under normal conditions (Barbosa et al., 2015; Webb et  
395 al., 2009), neurons are the sole parenchymal fate of pallial NSCs (Furlan et al., 2017; März et al., 2010),  
396 and delaminating NSCs can be traced to express neuronal markers (Barbosa et al., 2015).

397 Our analysis further suggests that *Cas<sup>CRE</sup>Atlas* captures a measurable fraction of these events, which are  
398 characterized by the expression of a specific molecular pathway involving *Cas3\**/*Cas7\** activation and  
399 *Atf3*. Several arguments support this conclusion. First, the intravital imaging dataset suggests a number  
400 of direct conversion events of the same order of magnitude as the number of *Cas<sup>CRE</sup>Atlas* clones  
401 quantified over the same period (NSC delaminations in intravital imaging: likely a few dozens of events  
402 in ~800 NSCs in 40 days; *Cas<sup>Cre</sup>Atlas*: 37-46 events in ~1000 NSCs in 56 days). Second, *Cas<sup>CRE</sup>Atlas*  
403 activation is driven in NSCs and generates persisting clones (Fig.3). Third, CPT treatment reveals that  
404 induction of the apoptosis cascade is sufficient to trigger NSC delamination (Fig.6), while *Atf3* blockade  
405 impairs the expression of the direct neuronal conversion fate (Fig.5G-K), together suggesting that a  
406 cascade involving *Cas3\** and/or *Cas7\** and *Atf3*, more than being a simple marker, actually drives the

407 neuronal conversion fate. Obviously, a missing step that would connect our morphometric description  
408 with the Cas3\*/Cas7\*/Atf3 cascade would be the direct tracking of the latter molecular events in an  
409 intravital imaging approach. This is however not possible with our current tools, notably because  
410 Hmg2B-mCherry needs several days to be directly visible by fluorescence, which would bypass the  
411 initial steps of the fate process. An NSC transcriptomic state possibly linked with direct conversions  
412 was recently proposed based on in silico analyses of single-cell RNAseq data (Mitic et al., 2024). This  
413 interpretation remains to be validated with lineage and functional assays in vivo, but its relationship  
414 with Cas3\*/Cas7\* and Atf3 could be interesting to assess.

415 Beyond this restriction, an important finding of our work is, in itself, the fact that non-apoptotic  
416 Cas3/Cas7 activation events are physiological components of NSC population fates: such events do  
417 occur in NPs of the developing pallium and in NSCs of the adult pallium, and bias NSC fates towards  
418 the direct generation of neurons (Fig.3). To our knowledge, this is the first demonstration of non-  
419 apoptotic Cas3/Cas7 activation events being associated with a specific SC fate choice in vivo. In this  
420 context, it is important to keep in mind that our work does not imply that this NSC fate is the  
421 consequence of a “rescue” of NSCs from death, but simply that this fate is associated with Cas3/Cas7  
422 activation. At the molecular level, the *Cas<sup>CRE</sup>Atlas* principle and the functional experiments of the present  
423 work provide some information on the effectors or facilitators of this process. The cleavage site of the  
424 *Cas<sup>CRE</sup>Atlas* construct is also recognized by Cas7\* and we cannot exclude a contribution of this effector  
425 Caspase to the *Cas<sup>CRE</sup>Atlas* signal. *casp7* is not expressed in the larval brain (Spead et al., 2018), arguing  
426 for a Cas3\*-specific cleavage at larval stages (Fig.2). However, transcription of both *casp3* and *casp7* is  
427 also detected in our scRNAseq dataset of quiescent NSC (Fig.S2A). Nevertheless, we observe a  
428 correlation between Cas3\* IHC and *Cas<sup>CRE</sup>Atlas* events in the number and spatial distribution in  
429 response to CPT (Fig.6), strongly suggesting that *Cas<sup>CRE</sup>Atlas* reads, at least in part, Cas3\*. Finally, when  
430 the production of the transcription factor Atf3 is abrogated, *Cas<sup>CRE</sup>Atlas* is still induced in NSCs, but its  
431 associated fate bias is abolished (Fig.4K), suggesting that Atf3 is either a mediator of non-apoptotic  
432 Cas3\*/Cas7\* events or a parallel and converging actor. There remain however several missing steps in  
433 our molecular understanding of this NSC fate. The relationship between non-apoptotic Cas3\*/Cas7\* and  
434 Atf3 is not a simple hierarchy, as overexpressed Atf3 is also capable to some extent of inducing  
435 *Cas<sup>CRE</sup>Atlas* (Fig.5F). This is reminiscent of previous observations, where Atf3 was described both  
436 upstream and downstream of Cas3\*, with highly context-dependent mechanistic links (Lu et al., 2007;  
437 Syed et al., 2005). Atf3 targets in adult NSCs remain to be identified, as well as Cas3\*/Cas7\* effectors,  
438 given that blocking Atf3 does not fully abolish direct neuronal differentiation (Fig.5F). Additionally, our  
439 work does not identify the functionally relevant Casp3\*/Casp7\* substrate the cleavage of which will bias  
440 NSC fate. We note that intense DNA damage, which can be caused by Caspase activation (upon

441 cleavage of the Caspase-activated DNase inhibitor) and trigger cell differentiation e.g. in muscle  
442 (Larsen et al., 2010), is here efficiently repaired in adult pallial NSCs (Fig.7), suggesting that other  
443 Caspase substrates are engaged in NSC fate. Along these lines, it would be interesting to search for NSC  
444 fate determinants containing Casp3\*/Casp7\* cleavage sites. Finally, and importantly, we note that  
445 *Cas<sup>CRE</sup>Atlas* is induced in NSCs following other endogenous fates, such as NSC-maintaining divisions  
446 (Fig.3D). Thus, Cas3\*/Cas7\* alone is not sufficient to encode the direct differentiation fate in NSCs and  
447 a further level of regulation must exist.

448 Our results also demonstrate that Cas3\*/Cas7\* events, and the associated fate of direct neuronal  
449 differentiation, can be engaged under non-physiological conditions of NSC stress (e.g. very high doses  
450 ionizing radiations) or NSC recruitment for neuronal repair (e.g. upon mechanical lesion). In the latter  
451 situation, direct neuronal conversion appears recruited without bias among other neurogenic fates  
452 (Fig.7K), and at present we did not identify a situation leading us towards the identification of  
453 Cas3\*/7\*/Atf3 induction pathways. Outside the brain, direct SC differentiation has been reported in  
454 adult mouse muscle satellite cells, at low levels under physiological conditions and much increased  
455 upon abrogation of Notch signaling or under conditions of regeneration, which disrupt MuSC  
456 quiescence (Bjornson et al., 2012; Ismaeel et al., 2023; Mourikis et al., 2012). Notch3 signaling is also a  
457 major gatekeeper of NSC quiescence in the adult pallium (Alunni et al., 2013; Chapouton et al., 2010)  
458 and whether abrogating this pathway and/or quiescence will result in mobilizing direct neuronal  
459 differentiation fates in addition to NSC activation for proliferation remains to be tested. It is to note that  
460 neuronal differentiation is not the “default” fate of perturbed NSCs, as, for example, adult zebrafish  
461 pallial NSCs will take a fate of differentiated astroglia (and not neurons) when Notch-induced stemness  
462 is abrogated (Than-Trong et al., 2018). Along the same lines, irradiation can drive the differentiation of  
463 proliferating neural progenitors towards the neuronal (Konirova et al., 2019) but also the astrocytic  
464 (Schneider et al., 2013) fates.

465 The association of caspase events with cell fate changes in a number of physiological contexts (Burgon  
466 and Megeney, 2018; Fujita et al., 2008; Janzen et al., 2008), or with cellular remodeling processes such as  
467 axonal degeneration or regeneration, dendritic pruning, axonal pathfinding, synaptic plasticity notably  
468 in the nervous system (Unsain and Barker, 2015), led to postulate *bona fide* non-apoptotic functions of  
469 the caspase pathway. Atf3 itself, in addition to a stress induced factor, is also seen as an adaptive  
470 response element driving cellular remodeling under stimulation by a number of signaling pathways  
471 (Rohini et al., 2018). Our work adds NSCs to the list of cells whose fate choices can be influenced by  
472 non-apoptotic caspase events. Neuronal differentiation and (neuro)epithelial delamination, such as  
473 undergone by delaminating NSCs in the adult pallium, both involve major cellular remodeling, such as  
474 massive changes in cytoskeletal architecture and intracellular trafficking, nuclear repositioning,

475 constriction of the apical and ciliary membranes, junction disassembly and abscission (Kasioulis and  
476 Storey, 2018; Kuijpers and Hoogenraad, 2011). Some of these are shared with the major cellular  
477 rearrangements that initiate apoptosis, which may go together with sharing core control pathways. It  
478 will of course also be interesting to determine whether the neurons issued from direct neuronal  
479 conversion of NSCs have specific structural features and identity.

480

## 481 **Materials and methods**

### 482 **Fish lines**

483 Wild-type (AB) and *Tg(GFAP:eGFP)* (Bernardos and Raymond, 2006), *Tg(ubi:SecA5)* (van Ham et al.,  
484 2010), *Tg(her4:ERT2CreERT2)* (Boniface et al., 2009), *Cas<sup>Cre</sup>Atlas* (see below), *Tg( $\beta$ actin:lox-stop-lox-hmg2B-*  
485 *mcherry)* (Wang et al., 2011) transgenic zebrafish were used. Embryos/larvae up to 5 dpf were  
486 maintained and staged as described (Kimmel et al., 1995). Adult zebrafish were maintained using  
487 standard fish-keeping protocols and in accordance with our Institute's Guidelines for Animal Welfare.

### 488 **Plasmids/vectors construction and transgenesis**

489 The *mCD8-DEVD-V5-Cre* (CDVC) construct was PCR-generated by fusing in frame the *mCD8-Diap1*  
490 region of the plasmid encoding *Drosophila Casexpress* DQVD (Ding et al., 2016) to a *V5-CRE*  
491 recombinase cassette and subcloned into the Tol2-kit vector *pME-MCS* to generate *pME-CDVC*. *pME-*  
492 *CDVC* was used to generate the transgenesis vector *pTol2-HCDVC* (*her4:mCD8-diap1-V5-CRE-SV40pA*)  
493 using the L/R recombinase reaction and the Tol2 vectors p302, p395, and the 5' vector *N11*. In the final  
494 product, the DEVD caspase site was mutagenized to GSGC to generate the control plasmid *pTol2-*  
495 *HCDVC\** by the Round-the-horn mutagenesis method ([https://openwetware.org/wiki/Round-the-](https://openwetware.org/wiki/Round-the-horn_site-directed_mutagenesis)  
496 [horn\\_site-directed\\_mutagenesis](https://openwetware.org/wiki/Round-the-horn_site-directed_mutagenesis)). Transgenic lines were made by injecting 1-cell embryos with a mix  
497 containing 60 ng/ $\mu$ l of plasmid and 60 ng/ $\mu$ l of *transposase* capped RNA.

498 The *atf3* cDNAs was amplified from reverse transcribed 16hpf embryo RNA and subcloned into *pSCA*.  
499 The *atf3* RNA probe was generated from the long version of the RNA (full coding sequence). The *atf3-*  
500 *P2A-GFP* constructs were generated with the Gibson method and subcloned into the *pCMV5* vector  
501 using the NEBuilder® HiFi DNA Assembly Cloning Kit.

### 502 **RT-PCR for the validation of efficiency of *atf3* vivoMO**

503 cDNA, extracted from 24hpf embryos, was amplified by RT-PCR using primers *atf3\_FL\_fwd* and  
504 *atf3\_FL\_rev* and the following cycles: 98°C, 1min; 98°C, 30 sec; the 35 cycles with 98°C, 10 sec; 63°C,  
505 30sec; 72°C, 20Sec; then 72°C, 2 min..

### 506 **Immunohistochemistry (IHC)**



507 Brains were dissected in 1X PBS at 4°C, their tela choroida was manually removed and the brains were  
508 directly transferred to a 4% paraformaldehyde solution in PBS for fixation. They were fixed overnight  
509 at 4°C under permanent agitation. After four washing steps in PBS, brains were dehydrated through 5  
510 to 10 minutes series of 25%, 50% and 75% methanol diluted in 0.1% tween-20 (Merck) PBS solution and  
511 kept in 100% methanol (Merck) at -20°C. Rehydration was performed using the same solutions, and  
512 then brains were processed for whole-mount immunohistochemistry (IHC). After rehydration, the  
513 telencephala were dissected out and subjected to an antigen retrieval step using Histo-VT One (Nacalai  
514 Tesque) for 1 hour at 65°C. Brains were rinsed three times for at least ten minutes in a 0.1% DMSO and  
515 0.1% Triton X-100 (Merck) PBS 1X solution (PBT) and then blocked with 4% normal goat serum in PBT  
516 (blocking buffer) 4 hours at RT on an agitator. The blocking buffer was later replaced by the primary  
517 antibody solution (diluted in blocking buffer), and the brains were kept overnight at 4°C on a rocking  
518 platform. The next day, brains were rinsed five to ten times over 24 hours at room temperature with  
519 PBT and incubated in a solution of secondary antibodies diluted in PBT overnight, in the dark, and at  
520 4°C on a rocking platform. In some instances, to be able to use the ZO1 dye-coupled antibody in the  
521 presence of another primary mouse mAb, secondary antibody free sites were blocked by incubation  
522 with 2% mouse serum in PBT for 1 hr before applying the dye-coupled ZO1 antibody. After three rinses  
523 in PBT over 4 hours, brains were transferred into PBS. Dissected telencephala were mounted in PBS on  
524 slides using 0.5 mm-thick holders. The slides were sealed using a glue gun.

525 Primary antibodies were used at a final concentration of 1:1000 for chicken anti-GFP and chicken anti  
526 BrdU, 1:500 Dye-coupled-ZO1, 1:300 Casp3a, 1:250 for DsRed, 1:200 for Sox2, ZO1, mAb anti GFP, 1:100  
527 for  $\gamma$ H2AX. Secondary antibodies were all used at a final concentration of 1:1000.

528 In situ hybridization was performed as described previously (Bosco et al., 2013; Chapouton et al., 2010;  
529 Ninkovic et al., 2005) except for the additional presence of 5% dextran-sulfate during the hybridization  
530 phase. For combined ISH and IHC, the ISH was developed using Fast-red. See Table S1 for detailed  
531 antibodies and probes used in this study.

### 532 **Whole-mount in situ hybridization (ISH) and immunohistochemistry (UHC)**

533 *In situ hybridization* was performed as described previously (Bosco et al., 2013; Chapouton et al., 2010;  
534 Ninkovic et al., 2005) except for the additional presence of 5% dextran-sulfate during the hybridization  
535 phase. For combined ISH and IHC, the ISH was developed using Fast-red (Sigma, F4648). See Table S1  
536 for detailed probes used in this study.

### 537 **Ventricular Micro-injections and Electroporation**

538 Micro-injections into the adult brain ventricle were performed on anaesthetized fish as described  
539 (Rothenaigner et al., 2011) except that DNA was injected at the midbrain midline to avoid damaging the

540 pallium. vivoMOs (Gene-tools) were injected at a concentration of 0.125 mM. Micro-injections into 4dpf  
541 larval hindbrain ventricle were performed on anesthetized larvae immobilized in 4% methyl-cellulose.  
542 For electroporation, plasmid DNA was diluted to 1µg/µl in 0.1 x PBS and injected into the ventricle.  
543 Electrodes (TWEEZERTRODES, 5mm Platinum) were placed on each side of the fish head. Fish were then  
544 administered two electric pulses (70 V, 50 ms width, 1,000 ms space).

#### 545 **Drug treatments**

546 4-Hydroxytamoxifen (4-OHT) Treatments and Bromodeoxyuridine Incorporation: 4-OHT (T176,  
547 Sigma) treatment was performed as previously described (Mosimann et al., 2011) on *her4:Ert2CreErt2*,  
548 *βactin:LoxSTOPloxhmgbmCherry*. Clonal recombination conditions at 1 mpf were 10 min with 0,5µM 4-  
549 OHT as in Than-Trong et al, 2020. They were followed by a 4h pulse of 1mM BrdU. *Cas<sup>Cre</sup>Atlas* were  
550 only treated with 1mM BrdU for 4h. Fish were then washed four times, transferred into fresh fish water,  
551 and grown as usual until 2-month-old.

552 Camptothecin treatment: CPT was dissolved as a 10mM stock in DMSO, aliquoted and stored frozen  
553 until use. Just before use, an intermediate solution was prepared in DMSO and further diluted 50 times  
554 in PBS (for injections) or fish water (for incubations) to reach the working concentration.

#### 555 **X-ray irradiation**

556 3-5 month old fish where placed into 2-liter cages inside an XRay irradiator (Gulmay CP160/10, 250kV,  
557 12mA) and irradiated for the length of time needed to reach the expected dose (5 Gy= 212 sec). Controls  
558 were placed for the same duration in the chamber but left untreated. Following irradiation, fish were  
559 kept in 6-liter cages in a 28°C incubator for the desired length of time. Fish were fed once a day and  
560 water changed daily if needed. Fish brains were then dissected, their tela choroida was removed, and  
561 the brains were then fixed for ISH or IHC.

#### 562 **Imaging and image analysis**

563 Images of whole-mount immunostained telencephali were acquired on a confocal microscope (LSM700  
564 and LSM700, Zeiss) using a 20X objective or a 40X oil objective (Plan-Apochromat 40x/1.3 Oil M27) and  
565 tile images of 4 to 8 z stacks were stitched with the ZEN2009 software. 3D renderings were generated  
566 using the Imaris® software (versions 8 and 9, Bitplane). Vertical plane images were extracted when  
567 needed. *Cas<sup>CRE</sup>Atlas* clones were resolved manually using 3D rendering and the slice mode and  
568 highlighted in different colors. For figures 7A-D and Supp 3, a nuclear mask was created in IMARIS  
569 using the sox2 nuclear staining in order to quantify nuclear H2AX staining. For figure 4D, single planes  
570 at different depth were extracted.

571 For dorsal whole-mount views of the telencephalon (ISH in blue), images were taken using a Nikon  
572 macrozoom.

573 **Statistics**

574 All experimental data were analyzed using Prism software and are expressed as mean  $\pm$  95% confidence  
575 interval (95% CI). Significance was set at  $p < 0.05$ . Comparison of proportions between experimental  
576 and control conditions (Figs. 3D, 5D-E, 6H, 7K) were performed via a contingency test based on a Chi2  
577 analysis. For all other comparisons, a Mann-Whitney test (non-parametric test, non-gaussian  
578 distribution) was used.

579

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597 Conceptualization: FR, LBC; Methodology: FR, ND, LBC; Funding acquisition: LBC; Project  
598 administration: LBC; Supervision: LBC; Writing – original draft: LBC; Writing – review & editing: FR,  
599 ND, LBC.

600 **Competing interests:**

601 Authors declare that they have no competing interests.

602 **Data availability:**

603 All data used in the analysis will be made available upon request.

604

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778 **Supplementary Materials**

779

780 Supplementary files include:

- 781 - Table S1: Tools and reagents
- 782 - Figures S1-S4
- 783 - Legends for supplementary figures

784

785 **Table S1: Tools and reagents**

786

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Chicken anti-GFP Antibody	Aves Labs	Cat#GFP-1020; RRID: AB_10000240
Mouse monoclonal (IgG2a) anti-GFP (=JL8)	Takara Bio	Cat#632381;RRID: AB_2313808
Mouse monoclonal (IgG1) anti-ZO1	Thermo Fisher Scientific	Cat#33-9100, RRID: AB_2533147
Mouse monoclonal (IgG2a) anti-V5 (SV5-Pk1)	Thermo Fisher Scientific	Cat#R960-25 ; RRID:AB_2556564
ZO1 647-coupled (ZO1-1A12)	Thermo Fisher Scientific	Cat#MA3-39100-A647 ; RRID:AB_2663167
ZO1 555-coupled (ZO1-1A12)		Cat#MA3-39100-A555 ; RRID:AB_2663168
ZO1 488-coupled (ZO1-1A12)		Cat#MA3-39100-A488 ; RRID:AB_2663169
Chicken anti BrdU	abcam	Ab92837 ; RRID:AB_10562139
Rabbit anti-DsRed	Takara	Cat# 632496, RRID:AB_10013483
Rabbit anti Casp3a	BD Pharmingen	Cat#559565; RRID:AB_397274
Mouse monoclonal (IgG1) anti- SOX2	Abcam	Cat#ab171380, RRID: AB_2732072
Rabbit Anti $\alpha$ H2AX.XS139ph	Genetex	CatGTX127342; RRID:AB_2833105
Rabbit Anti RFP	Takara	Cat632496; RRID:AB_10013483
Goat Anti-Chicken IgG(H+L) Alexa488 Conjugated	Thermo Fisher Scientific	Cat# A-11039, RRID:AB_142924
Goat anti-Mouse IgG1 Alexa546 conjugated	Thermo Fisher Scientific	Cat# A-21123, RRID:AB_141592)
Goat anti-Mouse IgG2a Alexa633 conjugated	Thermo Fisher Scientific	Cat# A-21136, RRID:AB_2535775
Goat Anti-Rabbit IgG(H+L) Alexa405 Conjugated	Thermo Fisher Scientific	Cat# A-31556, RRID:AB_221605
<b>Chemicals, peptides, and recombinant proteins</b>		
NEBuilder <sup>®</sup> HiFi DNA Assembly Cloning Kit	New England Biolabs	Cat#E5520S
Phusion High-Fidelity DNA polymerase	Thermo Fisher Scientific	Cat#F530S
Invitrogen <sup>™</sup> Clonase <sup>™</sup> Gateway <sup>™</sup> LR Clonase II Plus enzyme	Thermo Fisher Scientific	Cat#12538120
Paraformaldehyde, 16%	Thermo Fisher Scientific	Cat#043368.9M
Methanol 99,8%	Merck	Cat#322415
PBS	Thermo Fisher Scientific	Cat#BP399-20
Tween <sup>®</sup> 20	Merck	Cat#P9416
Antigen Retrieval (HistoVT One)	Nacalai Tesque	Cat#06380-05

Dimethyl sulfoxide	Merck	Cat#D8418
Triton™ X-100	Merck	Cat#X100
Goat serum donor herd	Merck	Cat#G6767
Normal Mouse serum	Abcam	Ab7486
Experimental models: Organisms/strains		
Zebrafish <i>Tg(gfap:eGFP)<sup>mi2001Tg</sup></i>	Zebrafish International Resource Center, Eugene, OR	ZDB-TGCONSTRUCT-070117-154
Zebrafish <i>Tg(gfap:dtTOMATO)<sup>nms17Tg</sup></i>	Zebrafish International Resource Center, Eugene, OR	ZFIN ID: ZDB-ALT-120215-4
<i>Tg(ubi:secA5-YFP)</i>	(van Ham et al., 2010)	Peterson R.T.
Oligonucleotides		
<i>mCD8-Diap1</i> Fwd	5'TAAGCAGGTACCAACATGGCCTCACCGTTGACC3'	
<i>mCD8-Diap1</i> Rev	5'GGTTAGGGATAGGCTTACCGGATCCCCTCATCTCTAGCG3'	
V5-CRE Fwd	5'CGCTAGAGATGAGGGGATCCGTAAGCCTATCCCTAAC3'	
V5-CRE Rev	5'TAAGCAACTAGTTCATCCAGCGTCCCCATCC3'	
Mut <i>Tol2-mCD8-CRE</i> Fwd	5'GGATGCGgctcacgaacgcgacc3'	
Mut <i>Tol2-mCD8-CRE</i> Rev	5'TGATCCaaaagcgataggtccataagacgg3'	
<i>atf3</i> FL Fwd	5'TAAGCAGGTACCGCATCAGGAGAGCATCACAA3'	
<i>atf3</i> FL Rev	5'TAAGCAACTAGTCCGTTAGCTTGTGTTGCTTGG3'	
<i>atf3</i> Sh Fwd	5'TAAGCAGGTACCCCCAAATTGATAGATGTTGTTATTTC3'	
<i>atf3-FLP2A_fwd</i>	5'tagtgaacctcagaattcagatctATGATGCTTCAGCACCTG3'	
<i>atf3-FLP2A_Rev</i>	5'gtctccagcctgcttcagcaggctgaagtagtagctccgctccCAGATGCCACAGCTGGTTG3'	
<i>P2AnlsGFP_fwd</i>	5'ctgctgaagcaggctggagacgtggaggagaacctggacctATGGCTCCAAA GAAGAAGCG3'	
<i>P2AnlsGFP_Rev</i> :	5'tatgatacgctggtaccagatctTACTTGTACAGCTCGTC3'	
<i>atf3-SHP2A_fwd</i>	5'tagtgaacctcagaattcagatctATGTTGTTATTTAGGCTGTCCC3'	
<i>atf3-SHP2A_rev</i> :	5'gtctccagcctgcttcagcaggctgaagtagtagctccgctccCAGATGCCACAGCTGGTTG3'	
<i>atf3 vivo</i> Fwd	5'CCGGCGACAACCTGTAACCTT3'	
<i>atf3 vivo</i> Rev	5'TGCTGCTGCAATTTTGTTC3'	
Plasmids		
<i>p5E-GFAP</i>	Gift from N. Cole	Addgene plasmid # 75024
<i>pCMV</i>		Tol2kit #382
<i>p3E-polyA</i>		Tol2kit #302
<i>pME-MCS</i>		Tol2kit #237
<i>pDestTol2CG2</i>		Tol2kit #395
<i>pattB-Ubi-CasExpress-DQVD</i>	Gift from D. Montell	(Mosimann et al., 2011)
<i>her4</i> promoter (fragment 2411pdb) in pDONR p4-p1R		(Furlan et al., 2017)
<i>atf3 Vivo</i> MO	5'GCCAGTAAATGAGTGGGTCTTACCT3'	
Standard control Vivo MO	5'AATGCTCAAGTTGCTTCTCAGATCC3'	
Software and algorithms		
Black Zen software	Carl Zeiss	RRID:SCR_018163
Imaris	Bitplane	RRID: SCR_007370
R Project for Statistical Computing	R Project for Statistical Computing	RRID:SCR_001905
Prism 8	GraphPad	RRID:SCR_002798



## Figure legends

### Figure 1. Intravital imaging permits the morphodynamic characterization of delamination events in the adult pallium.

**A.** Schematics of a delamination events observed using intravital imaging in *Tg(gfap:ZO1-mKate2)* 3mpf adults. Horizontal arrows: imaging time points (tp). In each series, the apical surface of a delaminating cell (\*) is depicted, with 4 non-delaminating neighbors (°) (apical view, red segments to ZO1-positive cellular interfaces). Delamination is considered terminated when no ZO1-negative apical domain is visible. **Top:** the tracking encompasses at least 4 imaging time points (tp) (14-16 days) without visible division prior to delamination termination. In that case, tp<sub>0</sub> is the first time point of the movie. Tracks with no division but delamination terminating less than 4 time points after the start of imaging are not considered. tp<sub>sh</sub> is the time point at which apical size shrinkage is initiated. **Bottom:** a division event takes place 3 tp or less (13 days or less) before delamination termination. In that case, tp<sub>0</sub> if the first time point post-division. **B. Top:** Snapshot series of two delamination events recorded from a *Casper;Tg(gfap:hZO1-mKate);Tg(deltaA:egfp)* 3mpf adult (fish named Outi, raw data in (Mancini et al., 2023)). Only the ZO1-mKate channel is shown (red). White arrows to delaminating cells, the first tp illustrated is the onset of apical area shrinkage (tp<sub>sh</sub>), the delamination termination tp is framed in red, the vertical time arrows indicate imaging tp (left) and corresponding days (right). The apical area of cells in examples 1 and 2 are 20.1 μm<sup>2</sup> and 22.9 μm<sup>2</sup>, respectively. **Bottom left insets:** Images including the *deltaA:eGFP* channel (cyan) at the onset of shrinkage. **C-E.** Quantified dynamic parameters of delaminations occurring after at least 4 time points (14-16 days) without division (red) and delaminations occurring 3 time points or less (≤ 13 days) post-division (black). **C.** Apical surface area at the tp preceding delamination termination. Mann Whitney: test, p-value: 0.661. **D.** Apical surface area at tp<sub>sh</sub> as a function of the time elapsed between tp<sub>sh</sub> and delamination termination. Linear regression, R squared = 0.256. **E.** Speed of apical size reduction (shrinkage speed), calculated from the tp<sub>sh</sub> until delamination termination. Mann Whitney: test, p-value: 0.849.

### Figure 2. The *Cas<sup>CRE</sup>Atlas* approach reveals non-apoptotic caspase-triggered events during pallium development and homeostasis.

**A.** The *Cas<sup>CRE</sup>Atlas* principle combines a NP/NSC-specific driver line (top and middle) expressing the Cre recombinase upon Cas3\*/Cas7\* cleavage with a reporter line (bottom) expressing *hmg2bmCherry* upon Cre recombination. mCD8: membrane anchor; V5: antigen segment (tag); DEVD: canonical Cas3\*/Cas7\* cleavage site; GSGC: mutated non-productive cleavage site; *her4* prom: *her4* regulatory elements. The *Tg(her4:mCD8-DEVD-V5-Cre);Tg(↯act;lox-stop-lox-hmg2bmCherry)* background is referred to as *Cas<sup>CRE</sup>Atlas*, and *Tg(her4:mCD8-GSGC-V5-Cre);Tg(bact;lox-stop-lox-hmg2bmCherry)* as *Control*. All analyses are conducted in double heterozygotes. **A'.** Schematic of

analyses in cross-sectioned or whole-mount pallia at 6dpf. **B.** Expression of the *Cas<sup>CRE</sup>* driver is confined to *gfap:eGFP*-positive cells. Cross sections through the pallium of *Tg(her4:mCD8-DEVD-V5-Cre);Tg(gfap:eGFP)* double transgenic larvae at 6dpf with triple immunostaining for GFP (NP/NSC), ZO1 (tight junctions at the apical surface of pallial ventricular cells) and V5 (transgene tag) (color coded), showing expression of V5 (membrane-anchored) and GFP in the same cells. **C-E.** Hmg2bmCherry whole-mount immunohistochemistry on *Cas<sup>CRE</sup>Atlas* (C) and *Control* (D) pallia at 6dpf (dorsal views, z projections, anterior left), and quantification of positive cells (E) (*Cas<sup>CRE</sup>Atlas*: n=4 brains, *Control*: n=10 brains, Mann-Whitney test). **F-I.** *Cas<sup>CRE</sup>Atlas* activity is induced by transient exposure to Camptothecin (CPT), an apoptosis inducer. **F.** Schematic of experiment: CPT (20 $\mu$ M) or DMSO are injected through the hindbrain into the neural tube ventricle in *Cas<sup>CRE</sup>Atlas* larvae at 4dpf, and Hmg2bmCherry is analyzed at 6dpf. **G-I.** Hmg2bmCherry whole-mount immunohistochemistry at 6dpf (dorsal views, z projections, anterior left) (G, DMSO; H, CPT) and quantification (I) (DMSO and CPT: n=6 brains each, Mann-Whitney test). **J-L'.** Hmg2bmCherry expression in *Cas<sup>CRE</sup>Atlas* (K) and *Control* (L) pallia at 1.5mpf. **J.** Schematic of analyses in whole-mount pallia at 1.5mpf. **K-L'.** Double whole-mount immunohistochemistry for Sox2 (NPs, NSCs and some freshly born neurons) and Hmg2bmCherry (K,K',L,L': dorsal views, z projections, anterior left, channels color-coded; K<sub>1</sub>,K<sub>2</sub>: cross sections at the levels indicated in K, dorsal up). Red arrows: Sox2-negative neurons, yellow arrow: Sox2-positive neurons, short yellow arrow: Sox2-positive NSC. Scale bars: B,C, D, G, H: 10 $\mu$ m, K-L', K<sub>1</sub>-2: 50 $\mu$ m.

**Figure 3. *Cas<sup>CRE</sup>Atlas*-driven clones in pallial NSCs display a biased fate.** **A.** Schematic of the experiment. *Tg(her4:ERT2CreERT2);Tg( $\beta$ act:lox-stop-lox-hmg2bmCherry);Tg(gfap:eGFP)* (top) and *Cas<sup>CRE</sup>Atlas;Tg(gfap:eGFP)* (bottom) triple transgenic fish are used to compare NSC fate between unbiased and *Cas3\**-driven clones, respectively. Left panels: temporal sequence of 4-OHT induction, BrdU pulse labeling, and analysis. Right panels: schematic pallial cross sections showing the relative positions of the NSC layer (cyan), BrdU-labeled neurons (magenta) and Hmg2bmCherry-positive clones (red) in each genotype. We focused on *Cas<sup>CRE</sup>Atlas* clones generated post-4 weeks-post-fertilization (wpf), i.e. situated between the layer of BrdU-positive neurons and the pallial ventricle. **B.** Cross section in a *Cas<sup>CRE</sup>Atlas;Tg(gfap:eGFP)* triple transgenic fish, processed for immunohistochemistry to reveal GFP (cyan, NSCs), BrdU (magenta) and Hmg2bmCherry (red) exemplifying the cartoon in A, bottom. Red arrows to Hmg2bmCherry-positive cells. **C-C''.** Whole-mount *Cas<sup>CRE</sup>Atlas;Tg(gfap:eGFP)* 2mpf pallium stained for GFP, BrdU and Hmg2bmCherry (dorsal view, anterior left). C,C': channels color-coded; C'': examples of several clones (C1-C3, indicated on C'), with color-coded segmentation, views from dorsal (C1-C3) and in optical cross sections (C1'-C3') -not all cells are visible in the latter case-. Clones in C1 are "attached" (magenta), clones in C2 are "single Neu" (1 clone, yellow) and "Neu doublets" (2 clones,

blue), clones in C3 are “single Neu” (2 clones, yellow) and “detached” (1 clone, orange); C’’: Imaris segmentation of unambiguously identifiable clones in the area shown in C’ (yellow dotted square). **D.** Quantification of the frequency of the different Hmg2bmCherry-positive clone types (represented schematically along the z axis; green triangles: NSCs; colored dots: Hmg2bmCherry-positive cells) generated from *her4*-positive NSCs between 4 and 8 wpf in the *Tg(her4:ERT2CreERT2);Tg(βact:lox-stop-lox-hmg2bmCherry);Tg(gfap:eGFP)* and *Cas<sup>CRE</sup>Atlas;Tg(gfap:eGFP)* backgrounds (black and gray bars, respectively) (n=5 brains for each genotype, 344 clones for unbiased clonal tracing (4-OHT treatment), n=360 clones for *Cas<sup>CRE</sup>Atlas* tracing). Statistical analysis performed by contingency Chi-square test, 95% confidence. Scale bars: B,B’: 15μm, C, C’, C’’: 30 μm, C3-8: 10μm.

**Figure 4. *atf3* is expressed in a subset of NSCs in the adult pallium.** **A.** *atf3*-expressing cells (orange dots, UMI values color-coded) positioned on the scRNAseq UMAP of adult quiescent NSCs (gray dots). The positions of NSCs closest to activation and/or neurogenesis commitment and NSCs in a deep/long quiescence phase are indicated (Morizet et al., 2023). **B.** Expression of *atf3* revealed by whole-mount ISH in the adult pallium (dorsal view, anterior left) with the *atf3-L* probe (E). Da, Dl, Dm, Dp: anterior, lateral, median and posterior regions of the pallium, respectively. Arrows to *atf3*-expressing cells in Dm and Dp. **C1-C4.** Optical cross sections of a *Tg(gfap:eGFP)* pallium stained in whole-mount for GFP (cyan), *atf3* transcripts (red) and DAPI (ventricular surface up). The sections are ordered from C1 to C4 along the anteroposterior axis. Red arrows to an *atf3*-positive, GFP-positive cell (red star to the nucleus). **D1-D4.** Optical horizontal sections of the same pallium as in C, ordered from D1 to D4 from superficial to deeper locations. Red arrows to the *atf3*-positive, GFP-positive cell. **E.** Atf3 protein isoforms predicted from adult pallial transcripts (L: long, S: short). The position of coding exons E2-E4 is indicated, as well as splice junctions for the L form (arrows). DBD: DNA-binding domain; LZ: leucine zipper domain. The yellow box indicates the different N-terminus of the S form due to alternative splicing. Scale bars: B: 100μm, C1-4 and D1-4: 5μm.

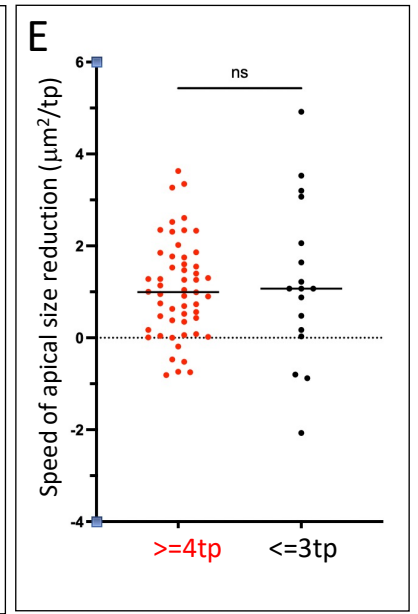
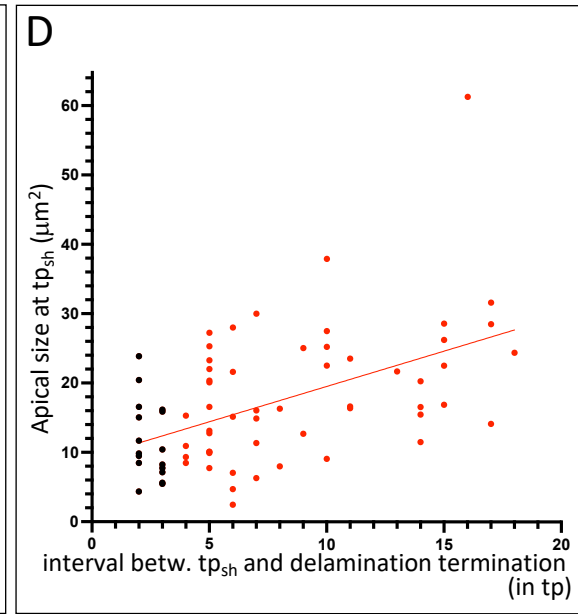
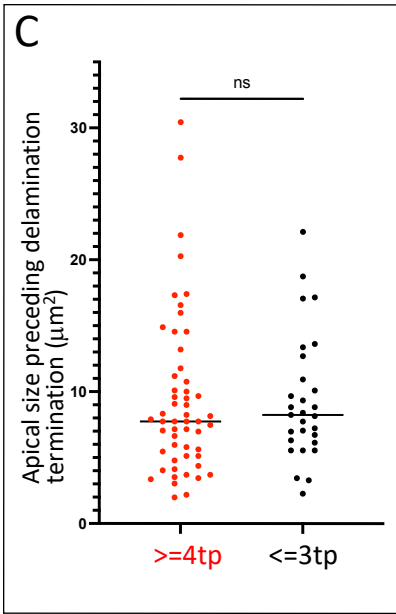
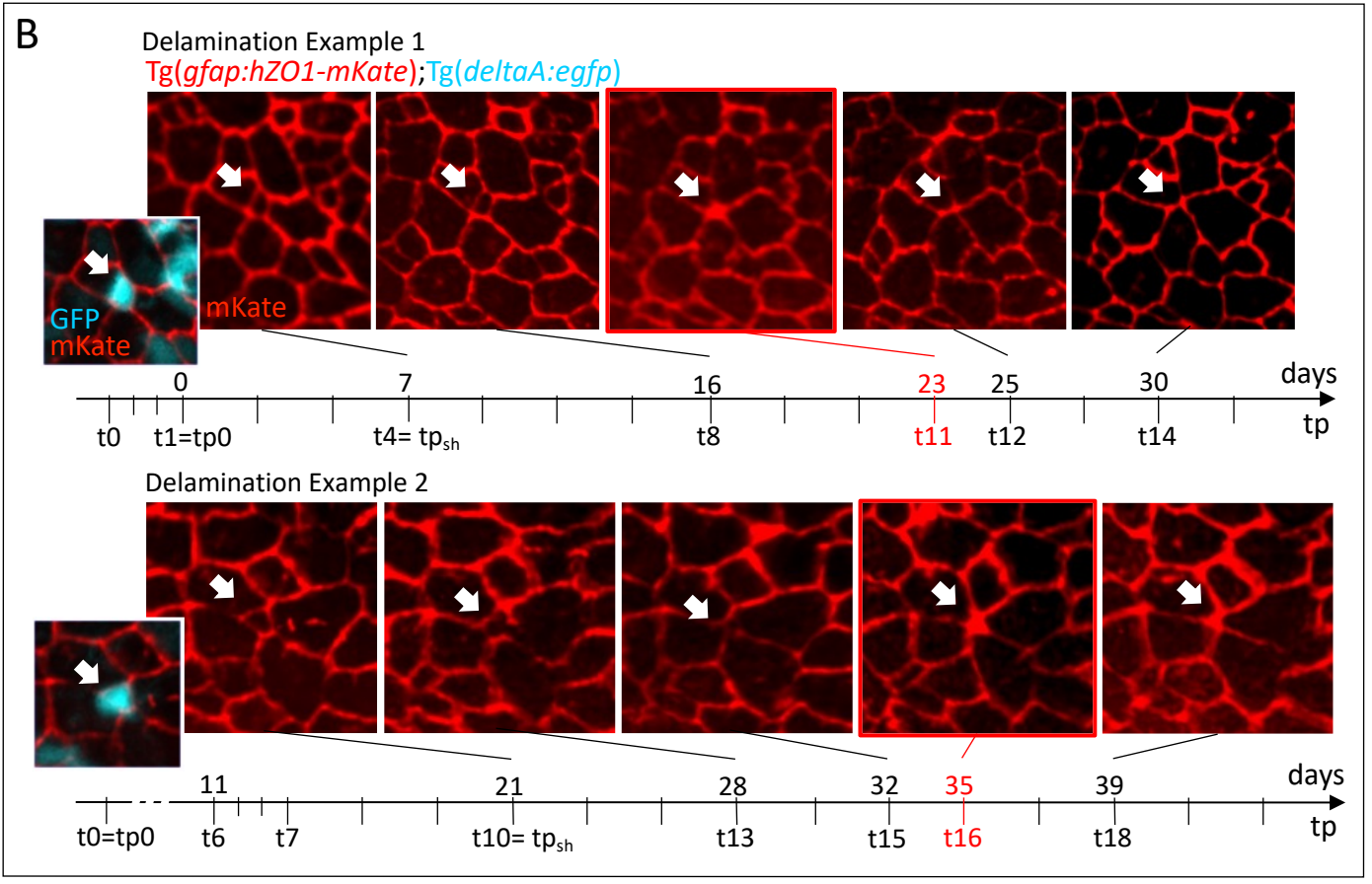
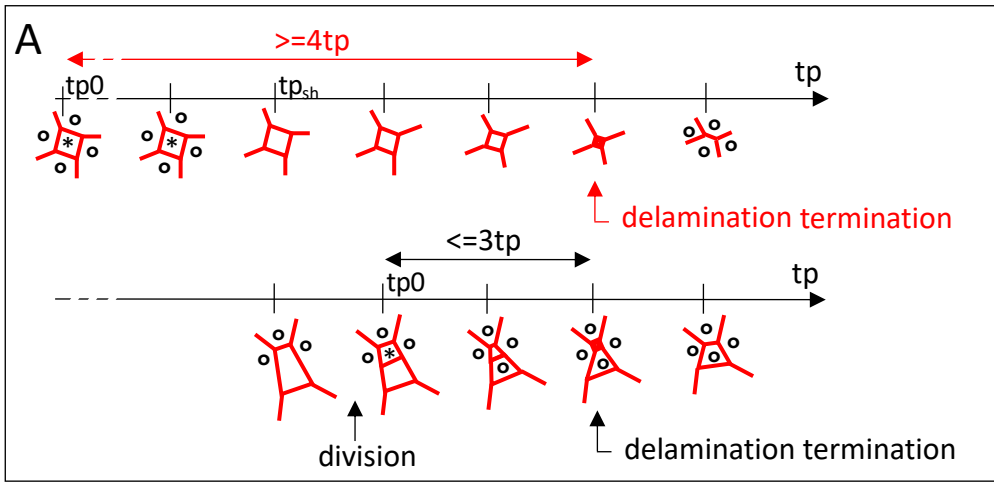
**Figure 5. Atf3 can drive NSC delamination and is needed for the expression of the *Cas<sup>CRE</sup>Atlas* fate bias.** **A-E.** Cell fates expressed by adult pallial ventricular cells at 2 days-post-electroporation (dpe). **A-C.** Visual categorization of the three possible fates, illustrated in whole-mount pallia immunostained for GFP (green, electroporated construct), ZO1 (magenta) and the progenitor marker Sox2 (cyan), and counterstained with DAPI (gray). A-C: dorsal (apical) views, A’-C’: cross sections as indicated in A-C. The arrowhead in B’ points to the apical attachment of a delaminating cell. **D,E.** Experimental scheme and quantification of cell fate categories upon electroporation of plasmids for *atf3-L-P2A-nlsGFP* versus *nlsGFP* (D) and *atf3-S-P2A-nlsGFP* versus *nlsGFP* (E). p values: Statistical analysis performed by Chi-

square test, 95% confidence D: *nlsGFP* n= 214 cells (4 hemipallia), *atf3-L-P2A-nlsGFP* n=69 (3 hemipallia), E: *nlsGFP* n= 62 (2 hemipallia), *atf3-S-P2A-nlsGFP* n=121 (3 hemipallia) **F-F''**. Some NSCs overexpressing *Atf3-F* in *Cas<sup>CRE</sup>Atlas* double transgenic adults are also Hmg2bmCherry-positive after 7 days of chase. Experimental scheme (left) and whole mount pallium processed for triple immunohistochemistry for ZO1 (magenta), GFP (cyan, electroporated construct) and Hmg2bmCherry (red). **F'** is a high magnification of **F** (dorsal views, anterior left) and **F''** a magnified cross section at the level indicated in **F'**. Double-positive cells indicated by white arrows. **G**. Exon-intron structure of *atf3* (top) and predicted proteins (bottom) produced upon splice blockade by *atf3-MO* (red bar). Positions of the different exons (E1-E4) and introns (I1-I3) are indicated, as well as the ATG positions for *Atf3-L* (L, arrow) and *Atf3-S* (S, arrowhead). The red arrowhead indicates the position of a double stop codon (\*\*). The color code is the same as in Fig.3C'''. **H-K**. Blocking *Atf3-L* function impairs the expression of the *Cas<sup>CRE</sup>Atlas* NSC fate bias. **H**. Experimental scheme. The *atf3-MO* was injected into the forebrain ventricle of *Cas<sup>CRE</sup>Atlas* double transgenic adults and Hmg2bmCherry clone types were quantified after 7 days. **I,J**. Whole-mount *Cas<sup>CRE</sup>Atlas* pallia immunostained for Hmg2bmCherry (red) and Sox2 (cyan) 7 days after injection of the control MO (**I**) or *atf3-MO* (**J**) (dorsal views). **K**. Quantification of clone types. "All NSCs" are the sum of "single NSCs" and "NSC doublets"; after this short chase, neurons we only observed as single neurons (the value for "Neu doublets" is null in both conditions). p values: Statistical analysis performed by contingency Chi-square test, 95% confidence, n=6 hemipallia per condition, CTL MO: n=82 clones, *atf3* MO: n=43 clones. Scale bars: A-C: 8  $\mu$ m, F-F': 50 $\mu$ m, F'': 10  $\mu$ m, I-J: 8  $\mu$ m.

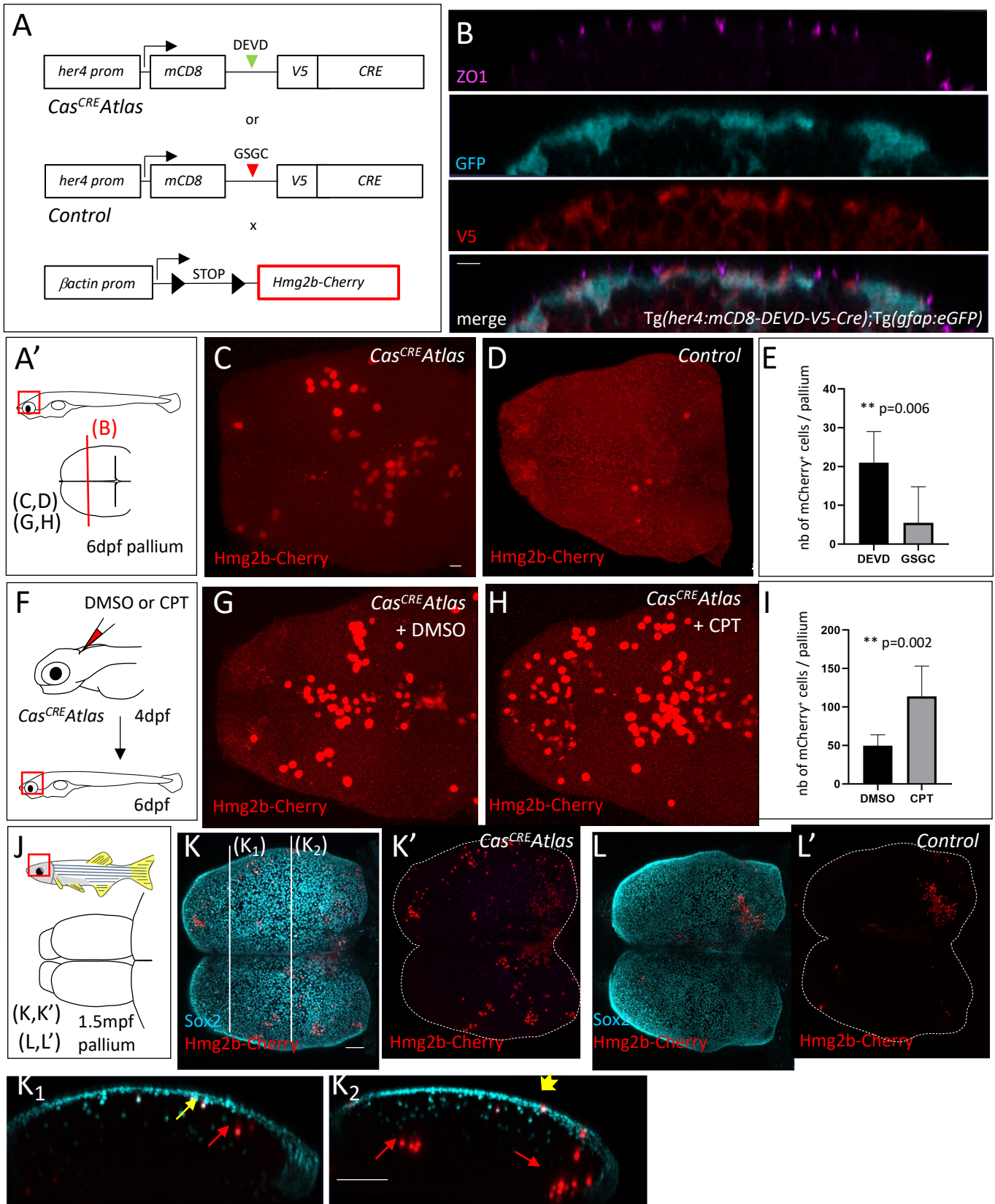
**Figure 6. Experimentally induced Cas3\* events drive direct neuronal differentiation in the adult pallium.** **A-C**. Induction of Cas3\* by CPT (C) compared to DMSO (B) injected into the cerebral ventricle (A), revealed by IHC in adult pallia at 2 days post-injection. **B,C**. Dorsal whole-mount views of the right hemisphere, anterior left, processed in IHC for ZO1 (cyan), Cas3\* (red) and Sox2 (magenta, only shown in C'). C<sub>1</sub> is a cross-section at the level indicated in B, red arrows to delaminating Cas3\*-positive cells. **D-G**. Experimental scheme and quantification of clone fate categories in *Cas<sup>CRE</sup>Atlas* adults upon intracerebral injection of CPT (F,F') compared to DMSO (E,F') at 7 days post-injection. **D**. Experimental scheme. **E,F**. Dorsal whole-mount views of the right hemisphere, anterior left, processed in IHC for ZO1 (cyan) and Hmg2bmCherry\* (red). **E',F'**. Segmentation of clones generated within 7 days (located at and immediately below the pallial ventricular surface), color-coded (red: NSCs, blue: parenchymal cells, identified as neurons). **G**. Quantification of Hmg2bmCherry-positive cells located within 2-3 cell rows from the ventricle at 7 days post-treatment. Statistical analysis performed by Welsh's t test: not significant (p=0.17). **H**. Quantification of clone types at 7 days post-treatment. Statistical analysis

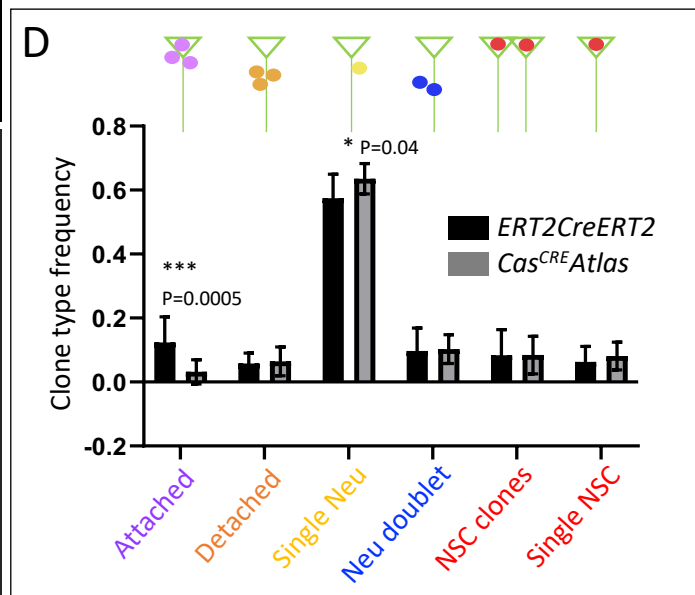
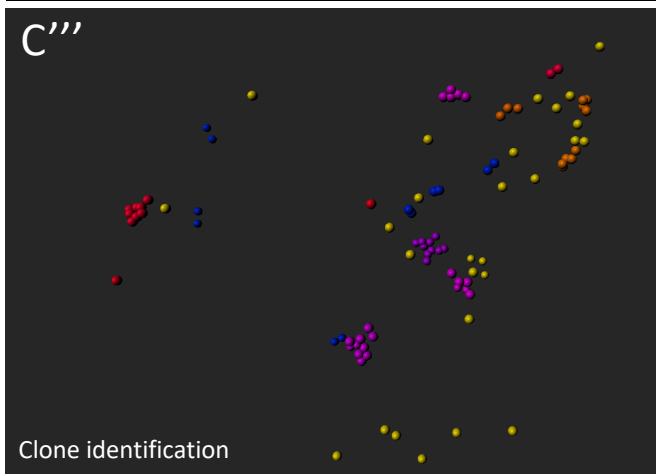
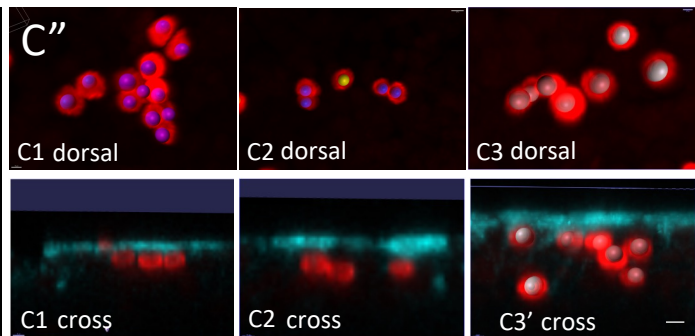
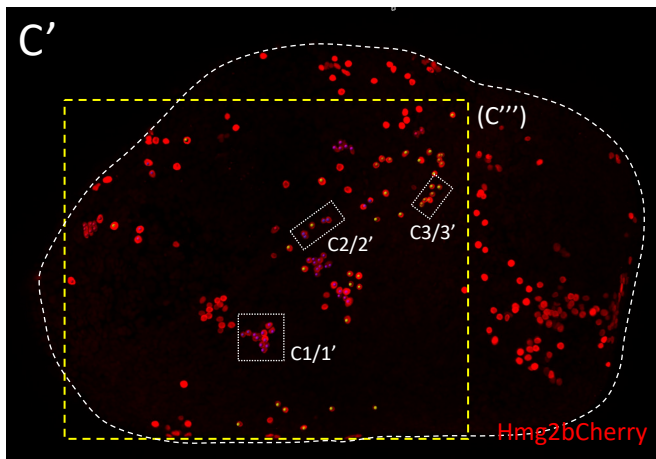
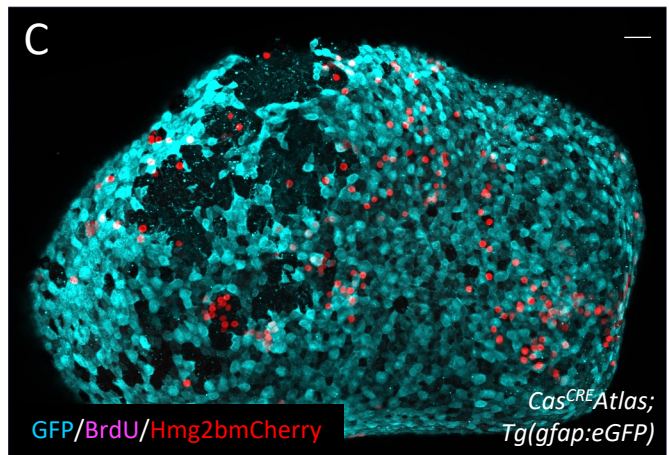
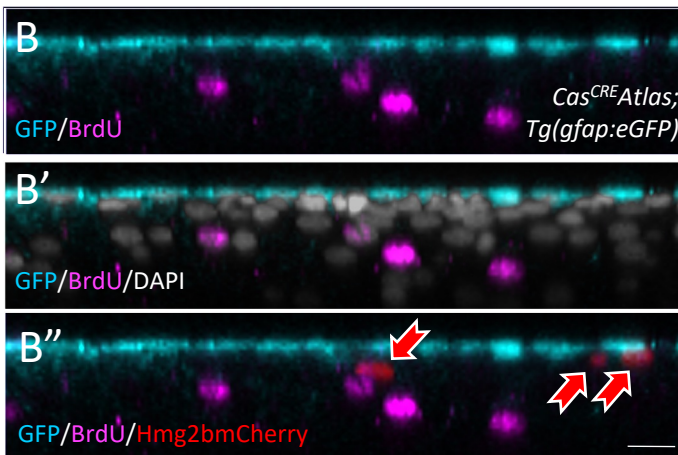
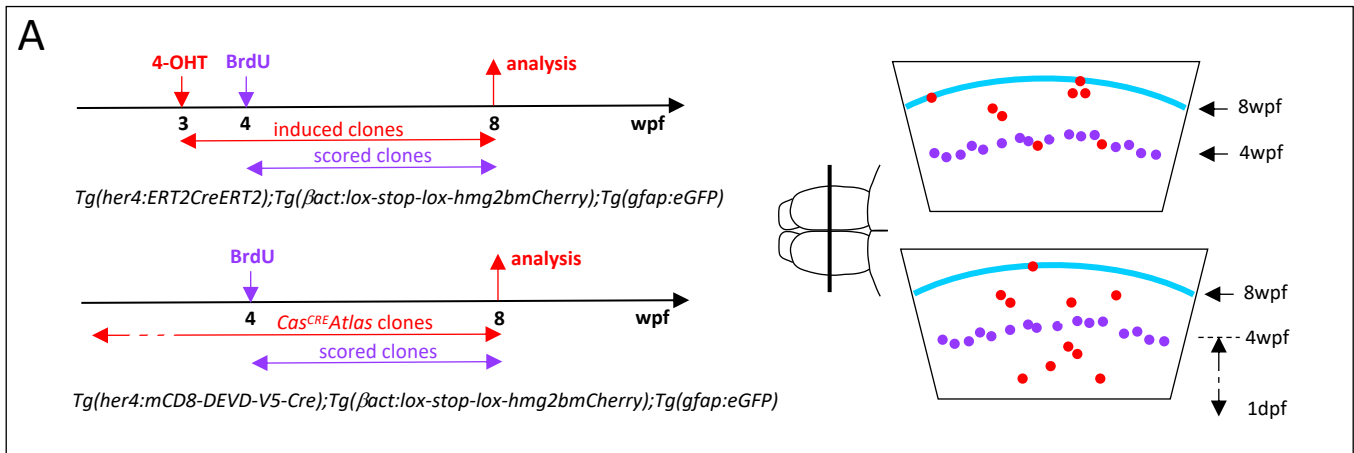
performed by contingency Chi-square test, 95% confidence. 4 hemipallia each condition. DMSO, n=37 clones, CPT n=89 clones. Scale bars: B,C,E,F,E',F',C1,C'1: 30 $\mu$ m.

**Figure 7. Differential recruitment of *atf3*-positive and Cas3\* events under irradiation versus mechanical lesion. A-D<sub>3</sub>.** Effect of X-rays on NSCs *in vivo*. **A-B'**.  $\gamma$ H2AX DNA repair foci (red) in NSCs/NPs (Sox2+, cyan) revealed by whole-mount IHC on adult pallia under control (A,A') or irradiation conditions (B,B') (5 Gy + 1hr chase). **C-D<sub>3</sub>**. Casp3\* induction (green) in NSCs/NPs (Sox2+, magenta) revealed by whole-mount IHC on adult pallia under control (C) or irradiation conditions (D) (40 Gy + 24hr chase). D<sub>1</sub> is a high magnification 3D rendering (Imaris) of the area boxed in D, and D<sub>2</sub>-D<sub>3</sub> are sections across Cas3\* cells along the dotted lines in D. The visible channels are color-coded. **E-K.** Induction of *atf3*+/*Cas3*\* events by mechanical lesion. **E-J.** *atf3* expression revealed by chromogenic ISH (F-H: blue, I-J': red) on whole-mount pallia 1 day (G, I, J) or 3 days (H) following stab-wound injury (E) compared to uninjured brains (F, I). Dorsal views, anterior left, only one hemisphere shown in I, J. Dotted lines: lesion trajectories; open arrowhead: downregulated *atf3* expression in the contralateral hemisphere at 3dpl. J' is a high magnification section across an *atf3*-positive cell along the dotted lines in J. The visible channels are color-coded. **K.** Experimental scheme (left) and quantification (graph) of the proportion of *Cas*<sup>CRE</sup>*Atlas* clone types in control versus lesioned hemispheres at 7dpl. Statistical analysis by contingency chi-square test: no significant difference between the proportion of the different *Cas*<sup>CRE</sup>*Atlas* clone types between lesioned and unlesioned hemispheres. Control 11 hemipallia, n=139 clones, Lesion 3 hemipallia, 43 clones. Scale bars: A,A', B, B': 10 $\mu$ m, C-C': 20 $\mu$ m, D1-D3, D': 10 $\mu$ m, F-H: 100 $\mu$ m, I-J: 30 $\mu$ m, J': 10 $\mu$ m.

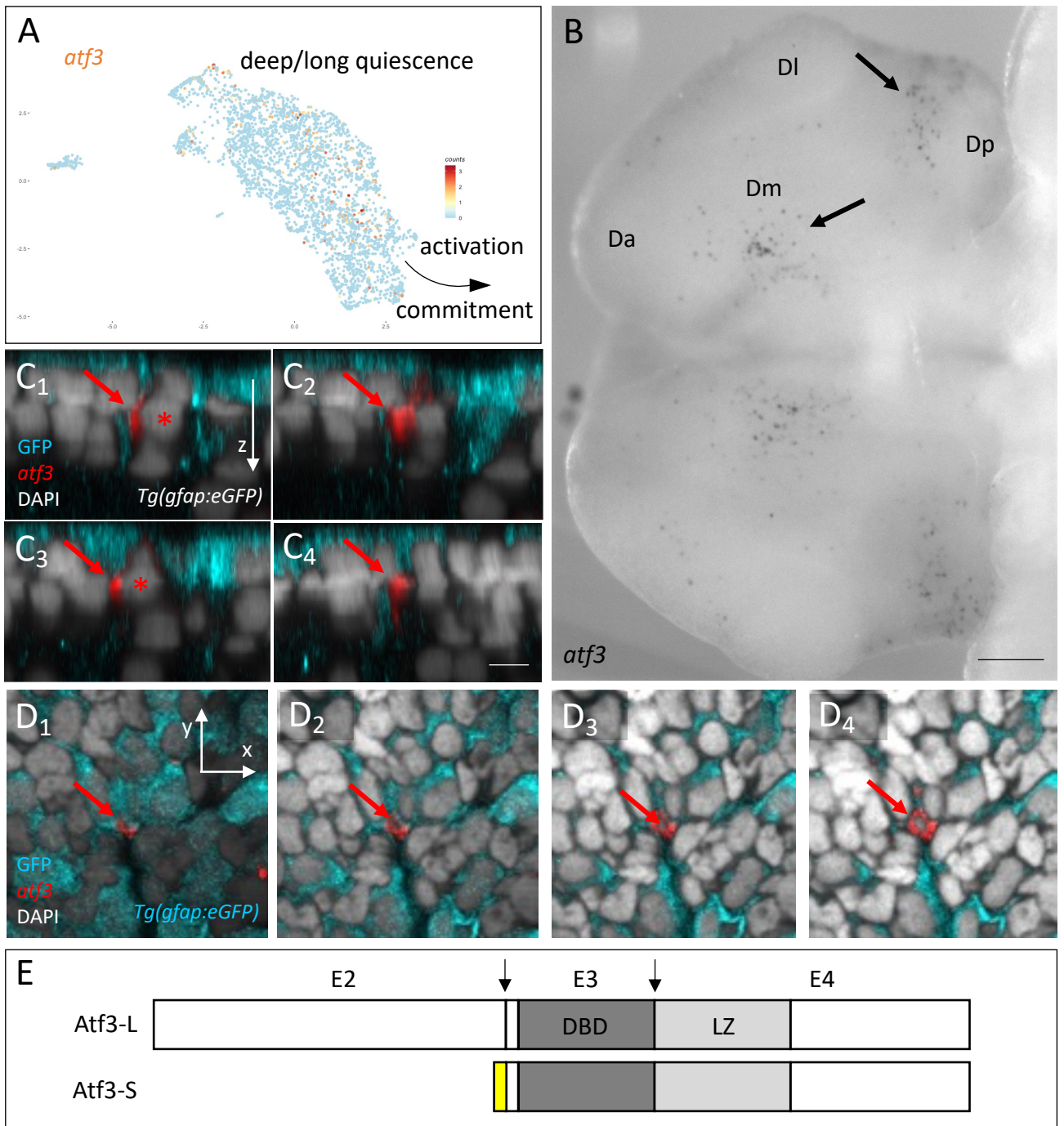


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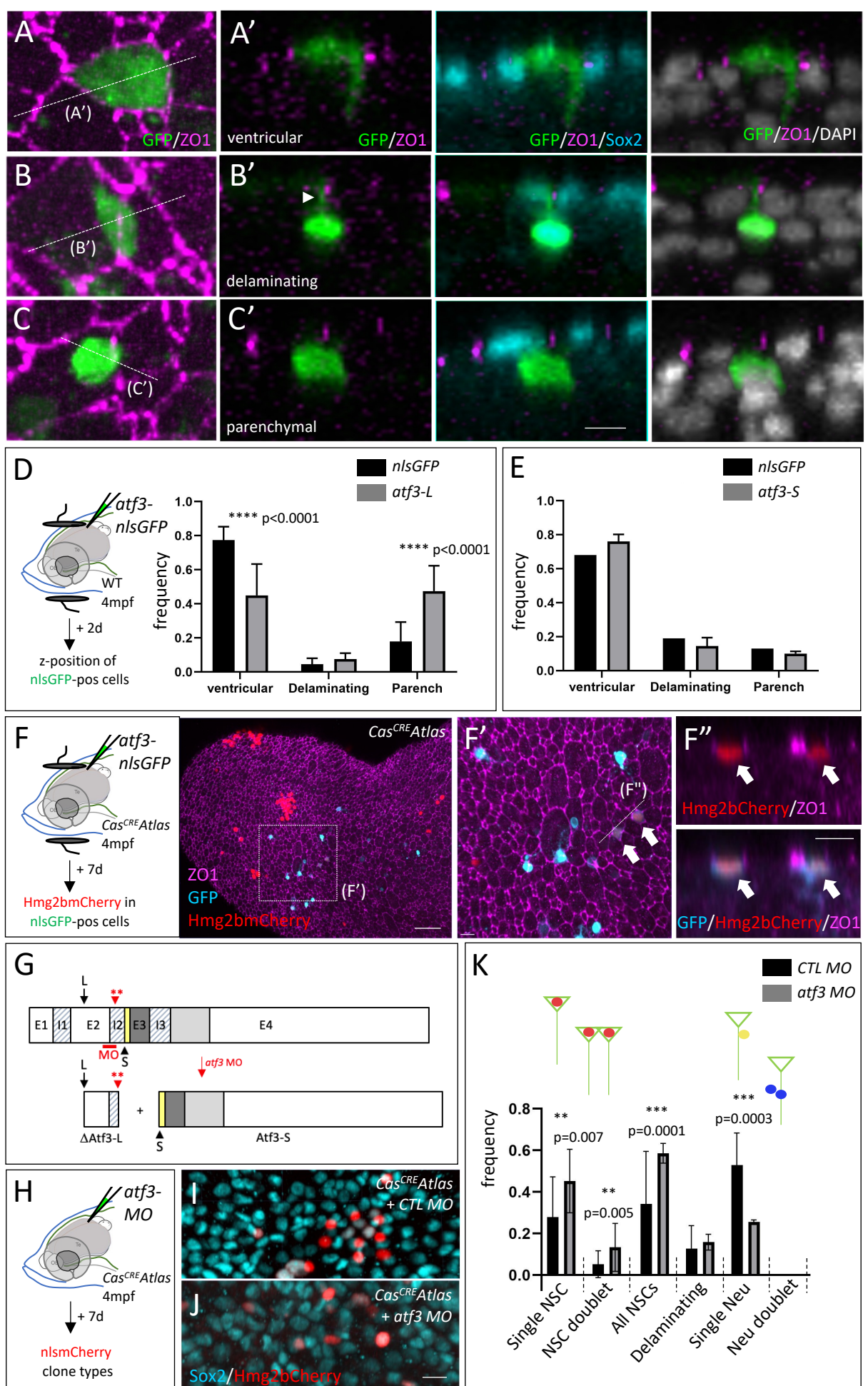


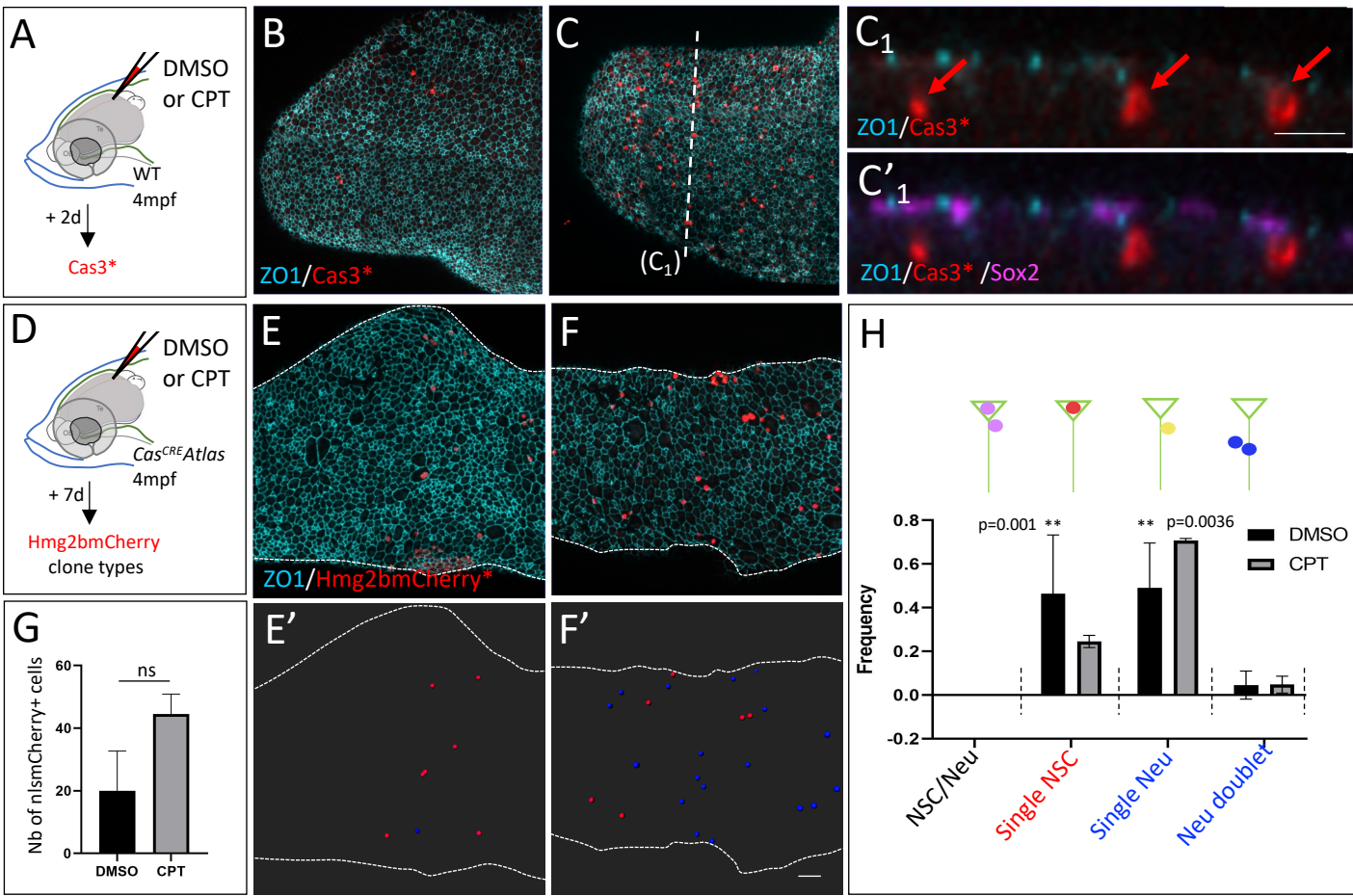


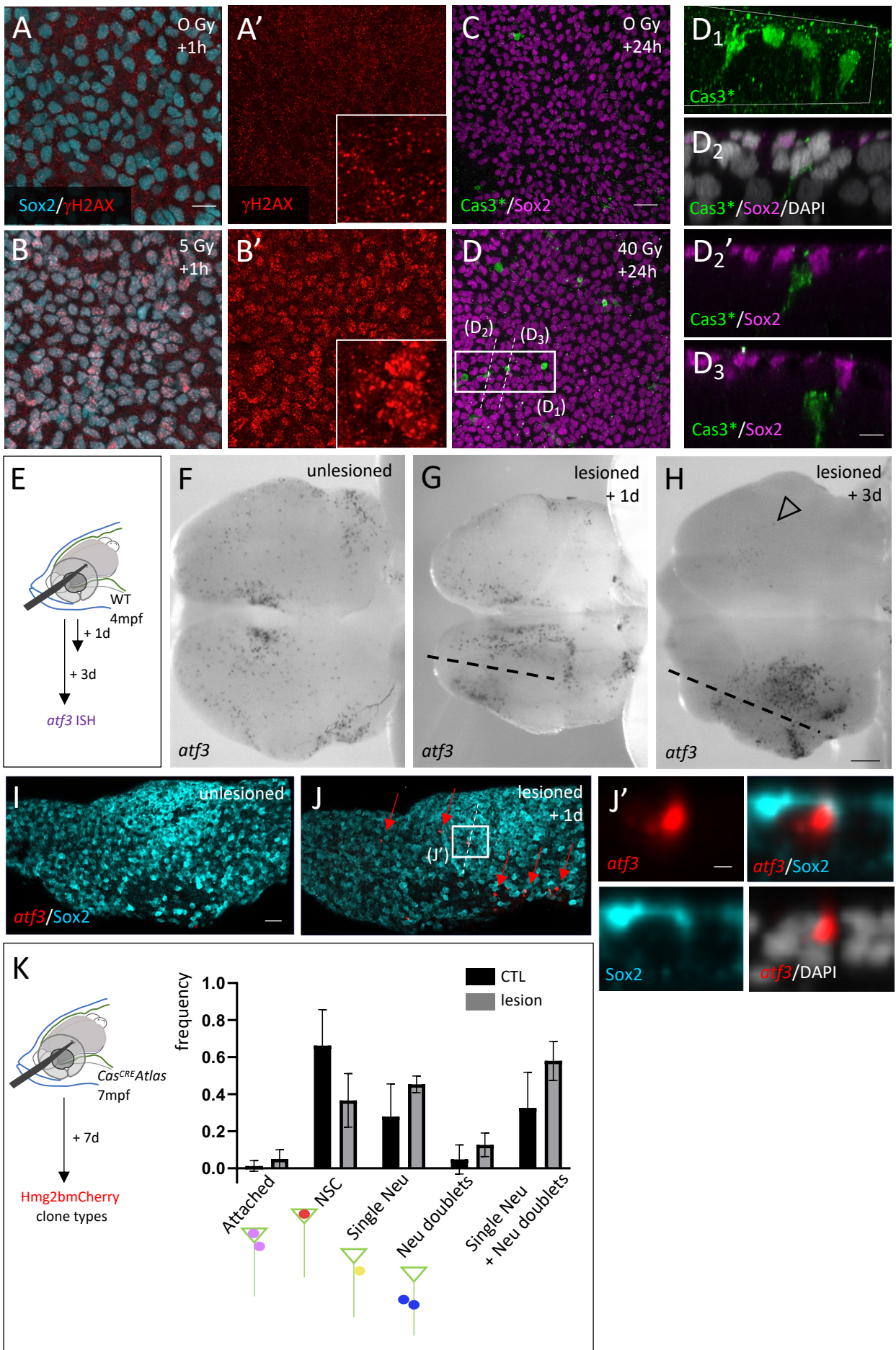




Rosa et al.  
Figure 4







## Supplementary Figures, legends

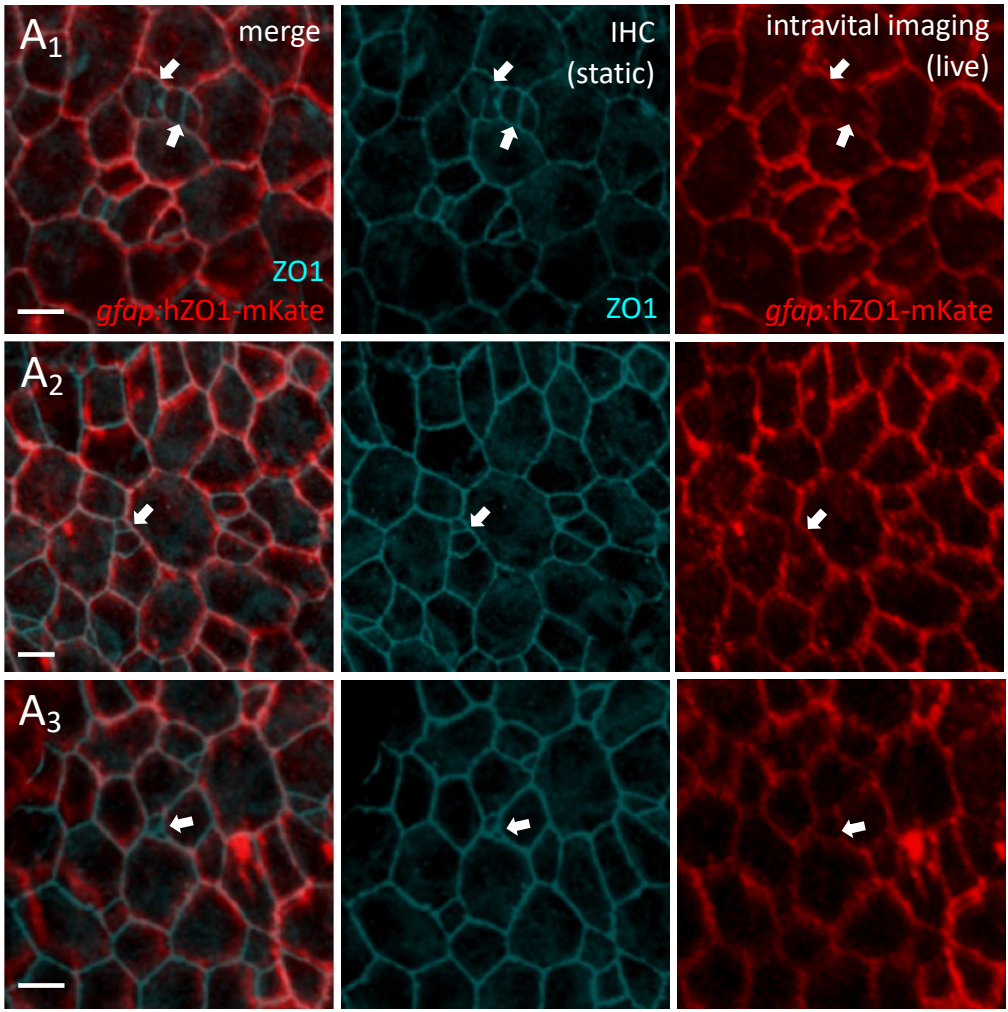
**Figure S1. Estimation of the proportion of ZO1-mKate apical surfaces corresponding to NPs in the intravital imaging approach. A<sub>1</sub>-A<sub>3</sub>.** Three examples of whole-mount overlays between ZO1-mKate fluorescence at a final imaging tp (red) and ZO1 immunohistochemistry (IHC) on the same brains following fixation (cyan) (Da pallial domain). The merge panel shows the unambiguous alignment of the fixed and live images, except for some adjacent NPs (white arrows), which cannot be recognized as distinct apical domains in the live image because expression of the ZO1-mKate fusion protein is driven by the NSC *gfap* regulatory elements. Scale bars: 10  $\mu\text{m}$ . **B.** Proportion of ZO1-mKate surfaces corresponding to NP clusters (cyan) versus single cells (white), estimated when comparing ZO1-mKate (live) and ZO1 (fixed). The percentage is estimated for different cut-offs of apical surface areas (in  $\mu\text{m}^2$ ). Da pallial domain, n=2 brains. **C.** Proportion of ZO1 surfaces corresponding to NPs isolated among NSCs, quantified on Tg(*gfap:dTomato*) pallia following ZO1 IHC. The percentage is estimated for different cut-offs of apical surface areas (in  $\mu\text{m}^2$ ). Dm pallial domain, n=2 brains. **D.** Compiled from B and C, identity of ZO1-mKate surfaces < 30  $\mu\text{m}^2$ . 48.3% are NSCs (white), 51.7% are NPs (isolated NPs - red- or NP groups -cyan-).

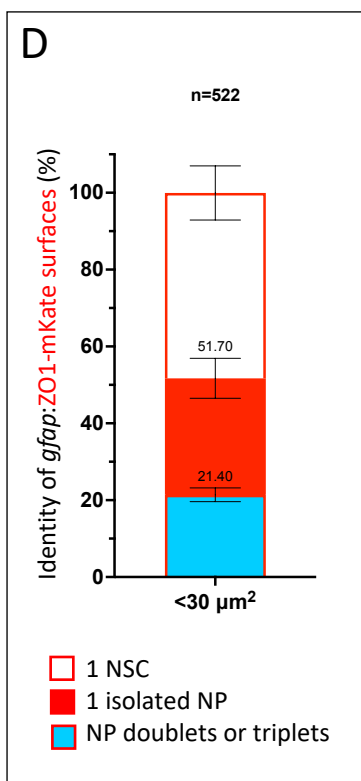
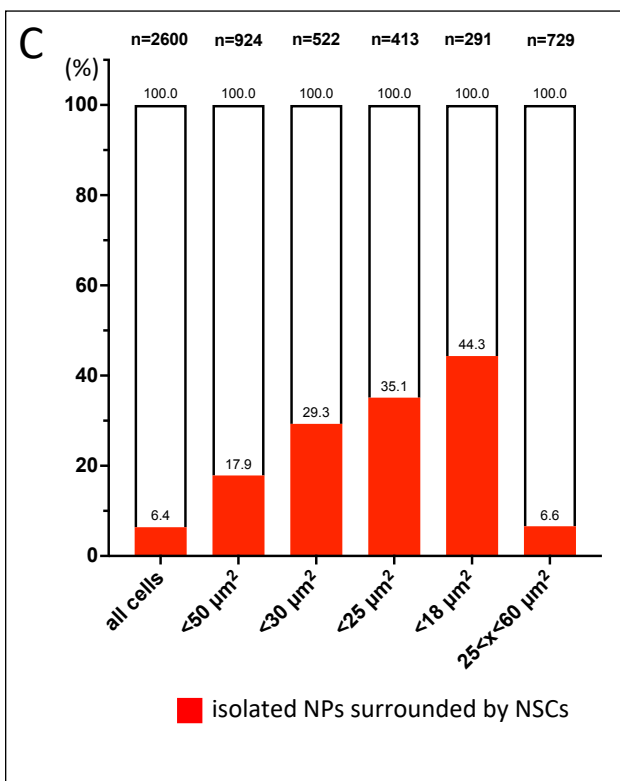
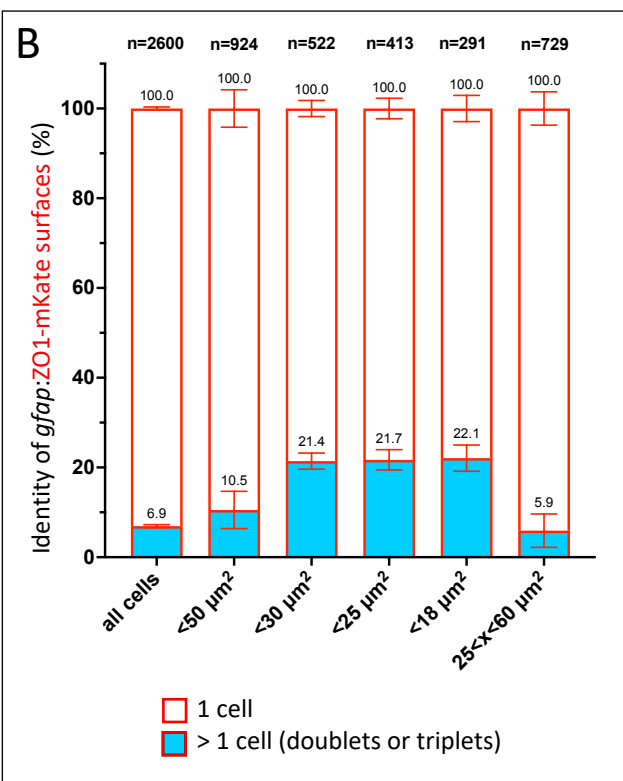
**Figure S2. Expression of apoptosis/anastasis mediators in zebrafish adult pallial NSCs and validation of the *atf3* MO. A.** Cells expressing the genes indicated (orange dots, UMI values color-coded) positioned on the scRNAseq UMAP of adult quiescent NSCs (gray dots). The candidate genes are taken from (Sun et al., 2017; Tang et al., 2017). The positions of NSCs closest to activation and/or neurogenesis commitment and NSCs in a deep/long quiescence phase are as in Fig.2A. **B.** RT-PCR for *atf3* in 24hpf embryos treated as follows: CTL: non-injected, MO: injected at the one-cell stage with 125mM of *atf3* vivoMO, CTL2: injected at the one-cell stage with 125mM of control vivoMO, H20: no RNA.

**Figure S3. Experimentally induced Cas3\* events drive NSC death in the larval pallium.** Tg(*ubi:secA5-mVenus*) 6dpf larvae were incubated overnight in DMSO (**A**) or CPT (**B**) (400nM). The brains were dissected and subjected to whole-mount IHC for Sox2 (magenta), Cas3\* (red) and mVenus (cyan). B<sub>1</sub> is an optical cross-section of B at the level indicated. Note that the mVenus staining is ventricular and affects Sox-positive NSC/NPs. Scale bars: A-A3''', B-B''': 15 $\mu\text{m}$ , B1: 10 $\mu\text{m}$

**Figure S4. Time course of the effect of X-rays on NSCs *in vivo*.**  $\gamma$ H2AX DNA repair foci (red) in NSCs/NPs (Sox2+, cyan) revealed by whole-mount IHC on adult pallia under control (A,A') or

irradiation conditions (B-C') (5 Gy + 1 or 2hr chase).  $\gamma$ H2AX DNA repair foci are prominent 1h after treatment but resolved after 2h. Scale bar: 10  $\mu$ m.









**B**

