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## A layered erythromyeloid ontogeny ensures timely supply of erythrocytes and macrophages during development

Ramy Elsaid, Aya Mikdache, Patricia Diabangouaya, Gwendoline Gros, Pedro P Hernández

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1 A layered erythromyeloid ontogeny ensures timely supply of erythrocytes and  
2 macrophages during development

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4 Ramy Elsaid<sup>1,\*</sup>, Aya Mikdache<sup>1</sup>, Patricia Diabangouaya<sup>1,#</sup>, Gwendoline Gros<sup>1,#</sup> and Pedro P.  
5 Hernández<sup>1,\*</sup>.

6  
7 <sup>1</sup>Institut Curie, PSL Research University CNRS UMR 3215, INSERM U934, 26 Rue d'Ulm, 75248 Paris Cedex 05,  
8 France.

9  
10 # These authors contributed equally to this work.

11  
12 \*Corresponding author. Email: [pedro.hernandez-cerda@curie.fr](mailto:pedro.hernandez-cerda@curie.fr) (P.P.H.) and [ramy.elsaid@curie.fr](mailto:ramy.elsaid@curie.fr) (R.E.).

13  
14 Short running title: Layered erythromyeloid ontogeny in zebrafish

15  
16 Highlights

17  
18 Primitive hematopoietic progenitors give rise and sustain embryonic erythromyeloid lineages.

19  
20 Definitive hematopoietic progenitors show a late contribution to macrophages and  
21 erythrocytes during development.

22  
23 Embryos show a layered ontogeny of macrophages that ensures their abundance for a timely  
24 contribution to tissue homeostasis and repair.

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26 Selective ablation of definitive macrophages does not impair tail fin regeneration during early  
27 larval developmental stages.

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49 **Abstract**

50

51 In all organisms studied, from flies to humans, blood cells emerge in several sequential waves  
52 and from distinct hematopoietic origins. However, the relative contribution of these  
53 ontogenetically distinct hematopoietic waves to embryonic blood lineages and to tissue  
54 regeneration during development is yet elusive. Here, using a lineage-specific ‘switch and  
55 trace’ strategy in the zebrafish embryo, we report that the definitive hematopoietic progeny  
56 barely contributes to erythrocytes and macrophages during early development. Lineage  
57 tracing further show that ontogenetically distinct macrophages exhibit differential  
58 recruitment to the site of injury based on the developmental stage of the organism. We  
59 further demonstrate that primitive macrophages can solely maintain tissue regeneration  
60 during early larval developmental stages after selective ablation of definitive macrophages.  
61 Our findings highlight that the sequential emergence of hematopoietic waves in embryos  
62 ensures the abundance of blood cells required for tissue homeostasis and integrity during  
63 development

64 **Keywords**

65 Ontogeny; Hematopoiesis; Lineage tracing; Regeneration; Zebrafish

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## 81 Introduction

82 Hematopoiesis is a complex biological process by which all mature blood lineages are  
83 generated. In mammals, hematopoiesis originates from distinct blood progenitors that arise  
84 during development resulting in a layered organization of the immune system (Elsaid et al.,  
85 2020). In mice, the first wave, also called primitive wave, starts in the extra-embryonic  
86 microenvironment of the yolk sac (YS) around embryonic day (E) 7 (Palis et al., 1999; Kingsley  
87 et al., 2004; Palis et al., 2001; Soares-da-Silva et al., 2021). Around E8.25, the YS also harbors  
88 endothelial-derived multipotent erythro-myeloid progenitors (EMPs) which represent the  
89 first definitive wave of hematopoiesis (Hoeffel et al., 2015; Gomez Perdiguero et al., 2015;  
90 Bertrand et al., 2005; Gentek et al., 2018; Frame et al., 2016). At E9.5, other intra-embryonic  
91 definitive hematopoietic progenitors emerge in the major arteries and they are the main  
92 source of adult hematopoietic stem cells (HSCs) in the bone marrow (BM) (Cumano et al.,  
93 1996; Medvinsky and Dzierzak, 1996; Busch et al., 2015). These progenitors emerge through  
94 an endothelial-to-hematopoietic transition mechanism (EHT) (Bertrand et al., 2010; Boisset  
95 et al., 2010; Kissa and Herbomel, 2010b). At E10.5, the fetal liver (FL), the main hematopoietic  
96 organ during embryonic development, harbors progenitors of dual origin derived from both  
97 definitive and primitive hematopoietic progenitors (McGrath et al., 2015a). As in mammals,  
98 zebrafish hematopoiesis also consists of multiple waves that emerge at distinct anatomical  
99 locations (Elsaid et al., 2020). In the zebrafish embryo, primitive hematopoiesis emerges intra-  
100 embryonically around 11 hours post-fertilization (hpf) in the rostral blood island (RBI) and the  
101 posterior lateral mesoderm (PLM) and generates primitive erythrocytes and myeloid cells  
102 (Elsaid et al., 2020; Herbomel et al., 2001). Recently, the RBI was shown to be major source  
103 of embryonic microglia (Ferrero et al., 2018; Xu et al., 2015). Other definitive or endothelial-  
104 derived hematopoietic waves are also produced in the developing organism (Elsaid et al.,



105 2020). One of them is a transient wave that emerges from the posterior blood island (PBI) at  
106 24-30 hpf via EHT and gives rise to EMPs like those in mammals (Bertrand et al., 2007; Ferrero  
107 et al., 2018; Tian et al., 2017). At 36 hpf, other waves of HSCs and HSC-independent  
108 progenitors emerge from the dorsal aorta (DA) via EHT (Bertrand et al., 2010; Kissa and  
109 Herbomel, 2010b). These DA-derived hematopoietic progenitors then migrate to the caudal  
110 hematopoietic tissue (CHT) (Murayama et al., 2006). By 4 days post-fertilization (dpf),  
111 hematopoietic stem and progenitor cells migrate to the kidney marrow, the BM counterpart  
112 in zebrafish, where they reside and differentiate to all blood cell lineages, establishing the  
113 adult hematopoietic system (Murayama et al., 2006). Therefore, due to its similarity to  
114 mammalian hematopoiesis, the zebrafish is an excellent model to study the ontogeny of  
115 different hematopoietic waves during development.

116 Although it is well characterized that blood cells are produced in sequential and overlapping  
117 waves (Elsaid et al., 2020), little is known about their contribution to different hematopoietic  
118 lineages during development. However, recent studies in mice and in zebrafish suggest that  
119 immune and blood cells might have distinct origins throughout life (Hoeffel et al., 2015;  
120 Ferrero et al., 2018; Xu et al., 2015; Gentek et al., 2018; Gomez Perdiguero et al., 2015; Soares-  
121 da-Silva et al., 2021). Independent reports support the notion that definitive hematopoiesis  
122 sustains embryonic blood lineages (Tian et al., 2017; Ulloa et al., 2021; Patel et al., 2022;  
123 Stachura and Traver, 2011). However, primitive hematopoietic progenitors give rise also to  
124 the erythromyeloid lineage and it is yet unknown to what extent these progenitors contribute  
125 to embryonic blood lineages. This is mainly due to the lack of specific markers for primitive  
126 hematopoietic progenitors in mammals (Elsaid et al., 2020; Ferrero et al., 2018; Xu et al.,  
127 2015; McGrath et al., 2015b).

128 The difficulty in accurately tracing the output of each wave in mammalian animal models has  
129 hindered the full understanding of immune cell ontogeny. Recent studies in zebrafish showed  
130 that embryonic and larval microglia originate from the RBI while adult microglia and other  
131 adult macrophages arise from the ventral wall of dorsal aorta (VDA) (Xu et al., 2015; He et al.,  
132 2018; Ferrero et al., 2018). Despite their elegant experimental designs, only microglia and  
133 adult macrophages origins were tested, without exploring the contribution of primitive  
134 hematopoiesis to the embryonic/larval erythromyeloid lineage during tissue homeostasis and  
135 regeneration.

136 In this study, we used the unique strengths of the zebrafish embryo to perform temporarily  
137 resolved lineage tracing combined with live imaging to investigate the embryonic  
138 erythromyeloid lineage ontogeny *in vivo*. We unveiled that embryonic erythrocyte and  
139 macrophage lineages originate from primitive hematopoietic progenitors. In contrast,  
140 definitive hematopoietic waves contribution to the erythromyeloid lineage occurred only at  
141 the larval developmental stage. We further showed that upon tissue injury only primitive, but  
142 not definitive, macrophages are recruited to the site of injury during embryonic development.  
143 While both ontogenetically distinct macrophage populations were recruited to the site of  
144 injury at early larval developmental stages, primitive macrophages were recruited to the  
145 damage site earlier than their definitive counterparts. Our results also suggest that primitive  
146 macrophages maintain tissue regeneration during early larval developmental stages after  
147 selective ablation of definitive macrophages. Combined, our study reveals that the sequential  
148 emergence of hematopoietic waves ensures the abundance of macrophages and erythrocytes  
149 required for tissue homeostasis and integrity during development.

150

151 Results and discussion

152 Erythrocytes originate from primitive hematopoietic progenitors during embryonic  
153 development.

154 To set up a strategy to distinguish between primitive and definitive hematopoietic progenitors  
155 based on their tissue of origin, we used an endothelial-specific tamoxifen-inducible transgenic  
156 line *Tg(fli1a:CreERT2)* (Sánchez-Iranzo et al., 2018) in combination with different  
157 hematopoietic lineage-specific switch and trace lines (Carney and Mosimann, 2018). All  
158 definitive hematopoietic progenitors, including HSCs and EMPs, are generated as early as 24  
159 hpf from endothelial progenitors (Bertrand et al., 2010; Kissa and Herbomel, 2010a; Tian et  
160 al., 2017; Henninger et al., 2017; Ulloa et al., 2021; Bertrand et al., 2007). Thus, our inducible  
161 labeling strategy at 24 hpf will label exclusively definitive hematopoietic progenitors as they  
162 emerge without labeling primitive hematopoietic progenitors. To assess the efficiency of our  
163 labeling strategy, we combined the *Tg(fli1a:CreERT2)* line with two different independent  
164 transgenic lines, the lymphocyte-specific *Tg(lck:loxP-DsRedx-loxP-GFP)* (Tian et al., 2017;  
165 Langenau et al., 2004) line (supplemental Figure 1A) and the *Tg(coro1a:loxP-DsRedx-loxP-*  
166 *GFP)* line which labels all leukocytes (Xu et al., 2015; Li et al., 2012) (supplemental Figure 1D).  
167 We found that 80% of thymocytes in the thymus, which have only a definitive hematopoietic  
168 origin (Tian et al., 2017; Elsaid et al., 2021), were labelled using either the *Tg(lck:loxP-DsRedx-*  
169 *loxP-GFP)* (supplemental Figure 1B-C) or the *Tg(coro1a:loxP-DsRedx-loxP-GFP)* lines  
170 (supplemental Figure 1E-F), indicating the high labelling efficiency of our system.

171 To determine the origin of erythrocytes during embryonic development and early larval  
172 stages, we combined the *Tg(fli1a:CreERT2)* line with the erythrocyte-specific *Tg( $\alpha/\beta_{a2}$ -*  
173 *globin:loxP-DsRedx-loxP-GFP)* line (referred to hereafter as globin:switch) (Tian et al., 2017;

174 Ganis et al., 2012). In double transgenic zebrafish, 4-OH-tamoxifen (4-OHT) induces cre  
175 recombination and removes the DsRed cassette, leading to permanent GFP expression in  
176 *fli1a*<sup>+</sup>-derived erythroid progeny (Figure 1A). To determine the contribution of definitive  
177 hematopoietic progenitors to erythropoiesis during early stages of development, we exposed  
178 embryos to either 4-OHT or ethanol (EtOH) starting at 24 hpf to label the aortic-endothelium-  
179 derived definitive hematopoietic progenitors (Bertrand et al., 2010; Kissa and Herbomel,  
180 2010a; Tian et al., 2017), and then monitored GFP<sup>+</sup> definitive erythrocytes via live imaging  
181 (Figure 1B and supplemental video 1). Primitive hematopoietic progenitors robustly  
182 generated erythrocytes from early embryonic stages (Figure 1B). In contrast, GFP<sup>+</sup> definitive  
183 erythrocytes started to emerge after 2 dpf, accumulated in the CHT region starting at 4 dpf  
184 (Figure 1C) and their contribution to the erythrocyte pool was not appreciable until later larval  
185 developmental stages (~21 dpf, Figure 1C-F).

186 Using this labeling strategy, we demonstrated that under physiological conditions, definitive  
187 hematopoietic progenitors barely contribute to erythropoiesis during early stages of  
188 development. These results indicate that erythrocytes emerge in a layered organization  
189 during embryonic and early larval stages as in mammals (Soares-da-Silva et al., 2021), and  
190 that primitive hematopoietic progenitors generate a sufficient number of erythrocytes to  
191 sustain embryonic survival and tissue homeostasis (Palis, 2014).

## 192 **Embryonic macrophages originate exclusively from primitive hematopoietic progenitors**

193 To assess the contribution of definitive hematopoietic progenitors to the embryonic  
194 macrophage pool, we marked the latter by combining the *Tg(fli1a:CreERT2)* line with the  
195 macrophage-specific *Tg(mpeg1:LoxP-DsRedx-LoxP-GFP-NTR)* line (referred to hereafter as

196 mpeg1:switch) (Lin et al., 2019; Ellett et al., 2011). As aforementioned, 4-OHT-induced cre  
197 recombination leads to permanent GFP expression in *fli1a*<sup>+</sup>-derived macrophages progeny  
198 (Figure 2A). Using this system, embryonic microglia cells, which are known to be of primitive  
199 hematopoietic origin in zebrafish (Xu et al., 2015; Ferrero et al., 2018), were not labelled,  
200 indicating the precision of this labelling system (supplemental figure 2A-B). While primitive  
201 macrophages contributed robustly to the embryonic macrophage pool, we found that  
202 definitive hematopoietic progenitors minimally contribute to macrophages during early  
203 developmental stages, akin to erythrocytes ontogeny (Figure 2B). We showed further that  
204 GFP<sup>+</sup> definitive macrophages started to emerge after 2 dpf and gradually increased in  
205 numbers in the CHT region (Figure 2B and E) with a delayed modest contribution to the  
206 macrophage pool in the periphery starting after 4 dpf (Figure 2B-D). GFP<sup>+</sup> definitive  
207 macrophages contributed robustly to peripheral macrophages by 16 dpf, suggesting a distinct  
208 differentiation kinetics of primitive and definitive macrophages throughout development  
209 (Figure 2C).

210 Altogether, our lineage tracing experiments show that aortic endothelium derived  
211 hematopoietic progenitors are barely contributing to embryonic erythroid and myeloid  
212 lineages (Figures 1 and 2). These data demonstrate that erythromyeloid lineages are mainly  
213 sustained by primitive hematopoietic progenitors during early developmental stages under  
214 physiological conditions.

215

216 **Ontogenically distinct macrophages are differentially recruited to the site of injury throughout**  
217 **development.**

218 It has been recently reported that distinct macrophage subpopulations play different roles to

219 promote tail regeneration after amputation (Wynn and Vannella, 2016; Morales and Allende,  
220 2019). However, whether ontogenetically distinct macrophages display different functions  
221 and recruitment behaviors to the site of injury remains poorly understood. Our tracing  
222 experiments suggested that the definitive hematopoietic progenitors barely contribute to the  
223 macrophage pool during early developmental stages under homeostasis. Therefore, we  
224 sought to analyze if macrophages of distinct origins are recruited in a differential manner to  
225 the damage site at different developmental stages. To track ontogenetically distinct  
226 macrophages recruitment upon injury, we used the same strategy applied to trace them  
227 under homeostasis (Figure 3A). We analyzed macrophages recruitment to the damaged site  
228 in embryos (2 dpf) and larvae (5 dpf) since our lineage tracing experiments showed a different  
229 abundance of ontogenetically distinct macrophages at these developmental stages (Figure 2).  
230 We observed that only primitive macrophages were recruited to the site of injury in embryos  
231 upon tail amputation (Figure 3B-C and supplemental Figure 3A). We then analyzed  
232 macrophage recruitment to the damage site in larvae, where both primitive and definitive  
233 macrophage populations coexist in the periphery. We did not observe differences in the  
234 recruitment of both primitive and definitive macrophages to the damage site 24 hours post  
235 amputation (hpa) with a slight increase in the number of definitive macrophages 48 hpa  
236 (Figure 3D-E and supplemental Figure 3B). Altogether, these results suggest that primitive  
237 macrophages can alone maintain embryonic tissue homeostasis while both macrophage  
238 populations are recruited to the damaged site and contribute to tail fin regeneration in the  
239 larvae.

240 Previous reports showed that the first recruited macrophages play an important role in  
241 proper tail fin regeneration (Morales and Allende, 2019; Nguyen-Chi et al., 2017; Petrie et al.,  
242 2014), therefore, we further analyzed which macrophage population is recruited first to the

243 site of injury. We found that primitive macrophages are recruited earlier than their definitive  
244 counterparts (Figure 3F-G). Since our observation of a delay in the recruitment of definitive  
245 macrophages could be the result of differences in the number of primitive and definitive  
246 macrophages in the periphery at the time of injury, we attempted to normalize their numbers.  
247 Data was normalized by dividing the number of each recruited macrophage subpopulation by  
248 their total number in the tail of the same larvae (Morales and Allende, 2019). After  
249 normalization, we still observed that primitive macrophages are recruited earlier compared  
250 to their definitive counterparts (Figure 3G). These data indicate that primitive macrophages,  
251 as the first arrivals to the damage site, may have a specific role in the regeneration process  
252 after amputation during development. Collectively, these results suggest that a layered  
253 ontogeny of macrophages ensures their abundance for a timely contribution to tissue  
254 homeostasis and regeneration.

255

256 [Primitive macrophages solely maintain tissue regeneration during early larval developmental](#)  
257 [stages after selective ablation of definitive macrophages.](#)

258 To evaluate the contribution of ontogenetically distinct macrophages to the regeneration of  
259 the damaged tissue, we used the aforementioned strategy to trace and deplete macrophages  
260 in a wave-specific manner during the regeneration process (Figure 4A). We used the *mpeg1*  
261 switch line *Tg(mpeg1:loxP-dsRed-loxP-eGFP-NTR)* that allows selective ablation of  
262 macrophages based on their origin. In this line, the expression of bacterial nitroreductase  
263 (NTR) is under the control of the *mpeg1* promoter (Ellett et al., 2011). Thus, the NTR will be  
264 expressed exclusively in the traced macrophages, thereby, traced macrophages could be  
265 ablated by metronidazole (MTZ) treatment (Curado et al., 2008). Following this approach, we  
266 evaluated the contribution of definitive macrophages selectively upon tissue injury. To assess

267 the efficiency of the NTR-MTZ system, we quantified the number of primitive and definitive  
268 macrophages in the tail after treatment with either DMSO or MTZ for 48 hours from 4 to 6  
269 dpf. We found that while primitive macrophages were not affected by the MTZ treatment,  
270 definitive macrophages were significantly reduced after 48 hours of MTZ treatment (Figure  
271 4B-C).

272 We next performed tail fin amputation in 5 dpf larvae that was treated with either DMSO or  
273 MTZ from 4-6 dpf to ensure ablation of definitive macrophages through the regeneration  
274 process. To assess if tail fin regeneration is affected when definitive macrophages are ablated,  
275 we evaluated tail fin regeneration after amputation by measuring the tail fin area. While  
276 primitive macrophage numbers and recruitment were not affected during the regeneration  
277 process, definitive macrophages were significantly reduced during the first 48 hpa but  
278 recovered by 72 hpa (Figure 4D-G and supplemental Figure 3C-D). We then performed tail fin  
279 regeneration analysis and observed no differences in the regenerated tail fin area between  
280 larvae treated with either DMSO or MTZ (Figure 4H-I). Our results, thus, indicate that primitive  
281 macrophages recruitment to the damage site is not affected and tail fin regeneration is not  
282 impaired when definitive macrophages are depleted. Taken together, these data further  
283 emphasize that primitive macrophages are sufficient to ensure a timely tissue regeneration  
284 and to compensate the absence of other distinct macrophage populations during early  
285 developmental stages.

286 Our study shows that the embryonic hematopoietic system follows a layered strategy to  
287 provide a timely supply of innate immune cells and erythrocytes. Under steady-state  
288 conditions, we found that the contribution of definitive hematopoietic progenitors to  
289 embryonic erythromyeloid lineage is limited. This is of particular interest as none of the



290 lineage tracing strategies performed in mice, despite their elegant designs, are specific to  
291 definitive hematopoietic waves. Our results also complement the recently reported  
292 observations that HSCs have a delayed contribution to the lymphomyeloid lineage during  
293 development (Ulloa et al., 2021). Together, our observations indicate that the embryonic  
294 erythromyeloid lineage is sustained by primitive hematopoietic progenitors under  
295 homeostasis.

296 To investigate the temporal contribution of the macrophage lineage upon tissue injury, we  
297 analyzed macrophage recruitment to the damaged tissue during embryonic and early larval  
298 developmental stages. We found that primitive macrophages are the only ones recruited to  
299 the site of injury during embryonic stages, however, both primitive and definitive macrophage  
300 populations were recruited at early larval developmental stages. This difference in  
301 recruitment behaviour between primitive and definitive macrophages could be due to that  
302 the primitive macrophages are more sensitive to damage sensing and/or a consequence of  
303 the immaturity of definitive macrophages at early developmental stages.

304 Furthermore, as timely recruitment of macrophages to the damage site is important to ensure  
305 proper tail fin regeneration (Wynn and Vannella, 2016; Morales and Allende, 2019; Nguyen-  
306 Chi et al., 2017; Petrie et al., 2014), we found that the primitive macrophages are the first  
307 responders after tail fin injury showed by their earlier recruitment than their definitive  
308 counterparts. Thus, we propose that during development, primitive macrophages are more  
309 prone to sense signals from the damage site and migrate toward it earlier than their definitive  
310 counterparts. These intriguing results suggest that embryos and early larvae adopt a layered  
311 ontogeny of macrophages to ensure their abundance for a timely contribution to tissue  
312 regeneration.

313 A major finding of our study is the demonstration that primitive macrophages can solely  
314 maintain tissue regeneration during development. This observation was supported by  
315 selectively ablating the definitive macrophage pool and analyzing the tail fin area of  
316 amputated larvae. We found that the number of recruited primitive macrophages to the site  
317 of injury was not altered and tail fin regeneration was not impaired in definitive macrophage-  
318 ablated larvae. Together, these results suggest that primitive macrophages are sufficient to  
319 ensure tissue regeneration and to compensate the absence of other distinct macrophage  
320 populations during critical early developmental stages.

321 In conclusion, our study provides insights into the ontogeny of the erythromyeloid lineage  
322 during embryonic/early larval developmental stages. Our findings support the notion that  
323 embryos show a sequential emergence of hematopoietic waves to ensure the abundance of  
324 macrophages required for tissue homeostasis and regeneration during a crucial  
325 developmental time window. In line with our results, it has been recently reported in mice  
326 (Gomez Perdiguero et al., 2015; Patel et al., 2022; Yokomizo et al., 2022; Fanti et al., 2023)  
327 and in zebrafish (Tian et al., 2017; Ulloa et al., 2021) that embryonic and adult HSCs do not  
328 give rise to several blood subtypes at steady state, upon ablation of mature blood cells, and  
329 in response to immune challenge. These observations indicate that ensuring a rapid response  
330 to stress to maintain tissue homeostasis in an HSC-independent manner might be a conserved  
331 mechanism between species throughout their life.

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## 340 **Materials and Data availability**

341

342 This study did not generate new unique reagents. The data sets generated during the  
343 current study are available from the corresponding authors upon request.

344

## 345 **Materials and methods**

346

347 **Zebrafish.** Embryonic and adult zebrafish (*Danio rerio*) were maintained at 28°C on a 14-hour  
348 light/10 hour dark cycle. The collected embryos were raised in fish water containing  
349 0.01% methylene blue to prevent fungal growth. All fish are housed in the fish facility of our  
350 laboratory, which was built according to the local animal welfare standards. All animal  
351 procedures were performed in accordance with French and European Union animal welfare  
352 guidelines and were evaluated by the ethic committee of the Curie Institute (Paris, France)  
353 and approved.

354

355 **Transgenic lines.** The following lines of the AB strain were used: *Tg(fli1a: CreER<sup>T2</sup>)* (Sánchez-  
356 Iranzo et al., 2018), *Tg(mpeg1:LoxP-DsRedx-LoxP-GFP-NTR)* (Lin et al., 2019), *Tg(coro1a:loxP-*  
357 *DsRedx-loxP-GFP)* (Xu et al., 2015), *Tg(globin:loxP-DsRedx-loxP-GFP)* (Tian et al., 2017) and  
358 *Tg(lck:loxP-DsRedx-loxP-GFP)* (Tian et al., 2017).

359

360 **Fluorescence Microscopy.** Zebrafish embryos, larvae and adults were anesthetized with  
361 0.01% tricaine (A5040, Sigma) and mounted in 2.5% methylcellulose in 35-mm imaging  
362 dishes (MatTek) as described previously (Renaud et al., 2011). Fluorescent imaging was  
363 performed with either Zeiss Axio Zoom V.16 upright microscope with an AxioCam HRm Zeiss  
364 camera and Zeiss Zen 3.3 software or with Leica thunder imaging system with Leica LAS-X  
365 software. Fluorescence was detected with dsRed and green fluorescent protein (GFP) filters.

366

367 **Image Analysis.** All images were analyzed using FIJI software (Schindelin et al., 2012).

368

369 **CreER-loxP Cell Labelling (Lineage Tracing).** Zebrafish embryos at 24 hpf were treated with  
370 5 µM 4-OHT (H7904, Sigma) for 24 hours. Controls were incubated in the equivalent amount  
371 of Ethanol solution during the same period. Light exposure was avoided by using foil to cover  
372 the plates as 4-OHT is light sensitive. After treatment, embryos were washed with fresh  
373 embryo medium and placed back in incubator. From 5 dpf, larvae were transferred to the fish  
374 facility nursery where they were kept and fed. Embryos were raised for further analysis at  
375 different developmental stages.

376

377 **Metronidazole treatment and cell ablation.** The *Tg(fli1CreER<sup>T2</sup>;mpeg1:loxP-DsRedx-loxP-*  
378 *GFP-NTR)* embryos were immersed in system water containing 10 mM metronidazole  
379 (M1547, Sigma) for 48 hours, which caused an acute depletion of GFP-NTR<sup>+</sup> cells. The medium  
380 containing the MTZ was changed every 24 hours. Controls were incubated in the equivalent  
381 amount of DMSO solution during the same period. Light exposure was avoided by using foil  
382 to cover the plates as MTZ is light sensitive. Zebrafish were further analyzed by fluorescence  
383 imaging at different developmental time points.

384

385 **Tail Fin Amputation.** 2 and 5 dpf larvae were anesthetized with 0.01% tricaine and amputated  
386 with a sterile scalpel. The transection was performed by using the posterior section of the

387 ventral pigmentation gap in the tail fin as a reference, and immediately after amputation  
388 larvae were incubated in either E3 medium, or in MTZ in case of macrophage ablation at 28°C.  
389

390 **Quantification of Tail Fin Regeneration.** At 24, 48 and 72 hours post amputation or dpa (6,7-  
391 and 8-days post fertilization, respectively), larvae were mounted in 2.5% methylcellulose, and  
392 regenerating tail fins were imaged in bright field. Tail fin area was measured from the anterior  
393 section of the ventral tail fin gap to the end of the regenerating fin, as previously described (Pei  
394 et al., 2016), using Fiji software. Tail fin areas were calculated and expressed in square  
395 micrometers ( $\mu\text{m}^2$ ).

396  
397 **Quantification of Tail Macrophages.** Macrophages were quantified based on their location in  
398 the tail (periphery vs CHT) (Figure 2B) in non-amputated larvae. In amputated larvae,  
399 recruited macrophages to the site of injury were quantified. Normalization of recruited  
400 macrophage numbers was obtained by dividing the number of recruited macrophage  
401 subpopulation by their total number in the tail of the same larvae (periphery, CHT and  
402 recruited macrophages) (Morales and Allende, 2019).

403  
404 **Quantification and statistical analysis.** Statistical analyses were performed by the GraphPad  
405 Prism software (Prism 9). All experiments with only two groups and one dependent variable  
406 were compared using an unpaired t-test with Welch's correction. Two-way ANOVA with  
407 Tukey's multiple comparison was used for this analysis. Statistical data show mean  $\pm$  s.e.m.  
408 Each dot plot value represents an independent embryo, and every experiment was conducted  
409 three times independently. The corrected total cell fluorescence (CTCF) was calculated using  
410 the formula 'Integrated density whole - (area whole embryo x mean fluorescence  
411 background)'. This formula is loosely based on a method described for calculating cell-  
412 fluorescence (Ulloa et al., 2021).

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426

427

## 428 **Authors contribution**

429

430 Conceptualization: R.E. and P.P.H.; Methodology and data collection: R.E., A.M., P.D. and  
431 G.G.; Writing - Original Draft: R.E.; Writing - Review & Editing: R.E. and P.P.H.; Funding  
432 Acquisition: P.P.H.

433 **Conflict of Interest**

434

435 The authors declare no competing interests.

436

437 **References**

438

439 Bertrand, J.Y., N.C. Chi, B. Santoso, S. Teng, D.Y.R. Stainier, and D. Traver. 2010.

440 Haematopoietic stem cells derive directly from aortic endothelium during

441 development. *Nature*. 464:108–111. doi:10.1038/nature08738.

442 Bertrand, J.Y., A. Jalil, M. Klaine, S. Jung, A. Cumano, and I. Godin. 2005. Three pathways to

443 mature macrophages in the early mouse yolk sac. *Blood*. 106:3004–3011.

444 doi:10.1182/blood-2005-02-0461.

445 Bertrand, J.Y., A.D. Kim, E.P. Violette, D.L. Stachura, J.L. Cisson, and D. Traver. 2007.

446 Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in

447 the zebrafish embryo. *Development*. 134:4147–4156. doi:10.1242/dev.012385.

448 Boisset, J.-C., W. van Cappellen, C. Andrieu-Soler, N. Galjart, E. Dzierzak, and C. Robin. 2010.

449 In vivo imaging of haematopoietic cells emerging from the mouse aortic

450 endothelium. *Nature*. 464:116–120. doi:10.1038/nature08764.

451 Busch, K., K. Klapproth, M. Barile, M. Flossdorf, T. Holland-Letz, S.M. Schlenner, M. Reth, T.

452 Höfer, and H.-R. Rodewald. 2015. Fundamental properties of unperturbed

453 haematopoiesis from stem cells in vivo. *Nature*. 518:542–546.

454 doi:10.1038/nature14242.

455 Carney, T.J., and C. Mosimann. 2018. Switch and Trace: Recombinase Genetics in Zebrafish.

456 *Trends Genet*. 34:362–378. doi:10.1016/j.tig.2018.01.004.

457 Cumano, A., F. Dieterlen-Lievre, and I. Godin. 1996. Lymphoid potential, probed before

458 circulation in mouse, is restricted to caudal intraembryonic splanchnopleura. *Cell*.

459 86:907–916. doi:10.1016/s0092-8674(00)80166-x.

460 Curado, S., D.Y.R. Stainier, and R.M. Anderson. 2008. Nitroreductase-mediated cell/tissue

461 ablation in zebrafish: a spatially and temporally controlled ablation method with

462 applications in developmental and regeneration studies. *Nat Protoc*. 3:948–954.

463 doi:10.1038/nprot.2008.58.

464 Ebisuya, M., and J. Briscoe. 2018. What does time mean in development? *Development*.

465 145:dev164368. doi:10.1242/dev.164368.

466 Ellett, F., L. Pase, J.W. Hayman, A. Andrianopoulos, and G.J. Lieschke. 2011. mpeg1 promoter

467 transgenes direct macrophage-lineage expression in zebrafish. *Blood*. 117:e49–e56.

468 doi:10.1182/blood-2010-10-314120.

469 Elsaid, R., S. Meunier, O. Burlen-Defranoux, F. Soares-da-Silva, T. Perchet, L. Iturri, L. Freyer,

470 P. Vieira, P. Pereira, R. Golub, A. Bandeira, E.G. Perdiguero, and A. Cumano. 2021. A

471 wave of bipotent T/ILC-restricted progenitors shapes the embryonic thymus

- 472 microenvironment in a time-dependent manner. *Blood*. 137:1024–1036.  
473 doi:10.1182/blood.2020006779.
- 474 Elsaid, R., F. Soares-da-Silva, M. Peixoto, D. Amiri, N. Mackowski, P. Pereira, A. Bandeira, and  
475 A. Cumano. 2020. Hematopoiesis: A Layered Organization Across Chordate Species.  
476 *Front. Cell Dev. Biol.* 8:606642. doi:10.3389/fcell.2020.606642.
- 477 Fanti, A.-K., K. Busch, A. Greco, X. Wang, B. Cirovic, F. Shang, T. Nizharadze, L. Frank, M.  
478 Barile, T.B. Feyerabend, T. Höfer, and H.-R. Rodewald. 2023. Flt3- and Tie2-Cre  
479 tracing identifies regeneration in sepsis from multipotent progenitors but not  
480 hematopoietic stem cells. *Cell Stem Cell*. 0. doi:10.1016/j.stem.2022.12.014.
- 481 Ferrero, G., C.B. Mahony, E. Dupuis, L. Yvernogeu, E. Di Ruggiero, M. Miserocchi, M. Caron,  
482 C. Robin, D. Traver, J.Y. Bertrand, and V. Wittamer. 2018. Embryonic Microglia Derive  
483 from Primitive Macrophages and Are Replaced by cmyb-Dependent Definitive  
484 Microglia in Zebrafish. *Cell Reports*. 24:130–141. doi:10.1016/j.celrep.2018.05.066.
- 485 Frame, J.M., K.H. Fegan, S.J. Conway, K.E. McGrath, and J. Palis. 2016. Definitive  
486 Hematopoiesis in the Yolk Sac Emerges from Wnt-Responsive Hemogenic  
487 Endothelium Independently of Circulation and Arterial Identity. *Stem Cells*. 34:431–  
488 444. doi:10.1002/stem.2213.
- 489 Ganis, J.J., N. Hsia, E. Trompouki, J.L.O. de Jong, A. DiBiase, J.S. Lambert, Z. Jia, P.J. Sabo, M.  
490 Weaver, R. Sandstrom, J.A. Stamatoyannopoulos, Y. Zhou, and L.I. Zon. 2012.  
491 Zebrafish globin Switching Occurs in Two Developmental Stages and is Controlled by  
492 the LCR. *Dev Biol*. 366:185–194. doi:10.1016/j.ydbio.2012.03.021.
- 493 Gentek, R., C. Ghigo, G. Hoeffel, M.J. Bulle, R. Msallam, G. Gautier, P. Launay, J. Chen, F.  
494 Ginhoux, and M. Bajénoff. 2018. Hemogenic Endothelial Fate Mapping Reveals Dual  
495 Developmental Origin of Mast Cells. *Immunity*. 48:1160–1171.e5.  
496 doi:10.1016/j.immuni.2018.04.025.
- 497 Gomez Perdiguero, E., K. Klapproth, C. Schulz, K. Busch, E. Azzoni, L. Crozet, H. Garner, C.  
498 Trouillet, M.F. de Bruijn, F. Geissmann, and H.-R. Rodewald. 2015. Tissue-resident  
499 macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*.  
500 518:547–551. doi:10.1038/nature13989.
- 501 He, S., J. Chen, Y. Jiang, Y. Wu, L. Zhu, W. Jin, C. Zhao, T. Yu, T. Wang, S. Wu, X. Lin, J.Y. Qu, Z.  
502 Wen, W. Zhang, and J. Xu. 2018. Adult zebrafish Langerhans cells arise from  
503 hematopoietic stem/progenitor cells. *eLife*. 7:e36131. doi:10.7554/eLife.36131.
- 504 Henninger, J., B. Santoso, S. Hans, E. Durand, J. Moore, C. Mosimann, M. Brand, D. Traver,  
505 and L. Zon. 2017. Clonal fate mapping quantifies the number of haematopoietic stem  
506 cells that arise during development. *Nat Cell Biol*. 19:17–27. doi:10.1038/ncb3444.
- 507 Herbomel, P., B. Thisse, and C. Thisse. 2001. Zebrafish Early Macrophages Colonize Cephalic  
508 Mesenchyme and Developing Brain, Retina, and Epidermis through a M-CSF  
509 Receptor-Dependent Invasive Process. *Developmental Biology*. 238:274–288.  
510 doi:10.1006/dbio.2001.0393.



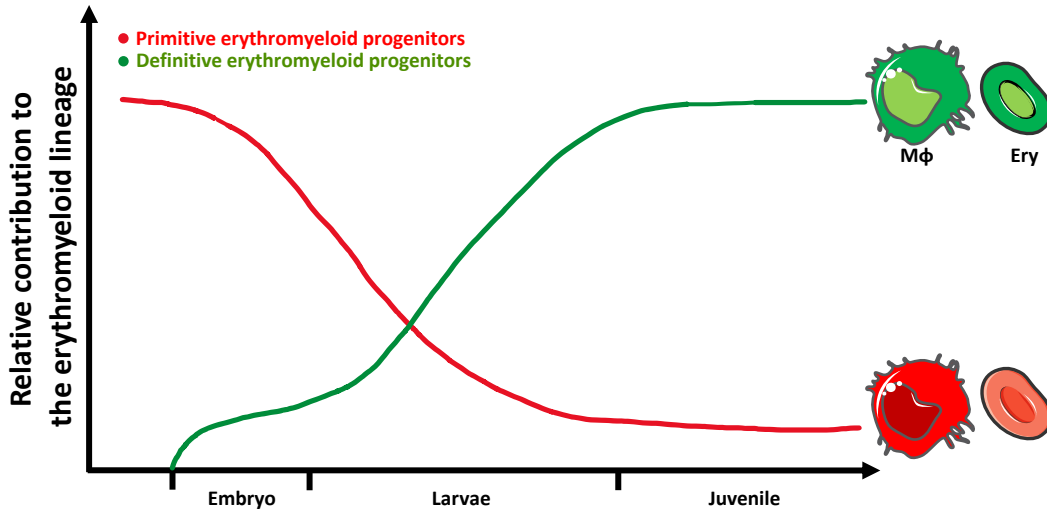
- 511 Hoeffel, G., J. Chen, Y. Lavin, D. Low, F.F. Almeida, P. See, A.E. Beaudin, J. Lum, I. Low, E.C.  
512 Forsberg, M. Poidinger, F. Zolezzi, A. Larbi, L.G. Ng, J.K.Y. Chan, M. Greter, B. Becher,  
513 I.M. Samokhvalov, M. Merad, and F. Ginhoux. 2015. C-Myb(+) erythro-myeloid  
514 progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages.  
515 *Immunity*. 42:665–678. doi:10.1016/j.immuni.2015.03.011.
- 516 Kingsley, P.D., J. Malik, K.A. Fantauzzo, and J. Palis. 2004. Yolk sac-derived primitive  
517 erythroblasts enucleate during mammalian embryogenesis. *Blood*. 104:19–25.  
518 doi:10.1182/blood-2003-12-4162.
- 519 Kissa, K., and P. Herbomel. 2010a. Blood stem cells emerge from aortic endothelium by a  
520 novel type of cell transition. *Nature*. 464:112–115. doi:10.1038/nature08761.
- 521 Kissa, K., and P. Herbomel. 2010b. Blood stem cells emerge from aortic endothelium by a  
522 novel type of cell transition. *Nature*. 464:112–115. doi:10.1038/nature08761.
- 523 Langenau, D.M., A.A. Ferrando, D. Traver, J.L. Kutok, J.-P.D. Hezel, J.P. Kanki, L.I. Zon, A.T.  
524 Look, and N.S. Trede. 2004. In vivo tracking of T cell development, ablation, and  
525 engraftment in transgenic zebrafish. *Proc Natl Acad Sci U S A*. 101:7369–7374.  
526 doi:10.1073/pnas.0402248101.
- 527 Li, L., B. Yan, Y.-Q. Shi, W.-Q. Zhang, and Z.-L. Wen. 2012. Live Imaging Reveals Differing  
528 Roles of Macrophages and Neutrophils during Zebrafish Tail Fin Regeneration. *J Biol*  
529 *Chem*. 287:25353–25360. doi:10.1074/jbc.M112.349126.
- 530 Lin, X., Q. Zhou, C. Zhao, G. Lin, J. Xu, and Z. Wen. 2019. An Ectoderm-Derived Myeloid-like  
531 Cell Population Functions as Antigen Transporters for Langerhans Cells in Zebrafish  
532 Epidermis. *Developmental Cell*. 49:605–617.e5. doi:10.1016/j.devcel.2019.03.028.
- 533 McGrath, K.E., J.M. Frame, K.H. Fegan, J.R. Bowen, S.J. Conway, S.C. Catherman, P.D.  
534 Kingsley, A.D. Koniski, and J. Palis. 2015a. Distinct Sources of Hematopoietic  
535 Progenitors Emerge before HSCs and Provide Functional Blood Cells in the  
536 Mammalian Embryo. *Cell Rep*. 11:1892–1904. doi:10.1016/j.celrep.2015.05.036.
- 537 McGrath, K.E., J.M. Frame, and J. Palis. 2015b. Early hematopoiesis and macrophage  
538 development. *Semin Immunol*. 27:379–387. doi:10.1016/j.smim.2016.03.013.
- 539 Medvinsky, A., and E. Dzierzak. 1996. Definitive hematopoiesis is autonomously initiated by  
540 the AGM region. *Cell*. 86:897–906. doi:10.1016/s0092-8674(00)80165-8.
- 541 Morales, R.A., and M.L. Allende. 2019. Peripheral Macrophages Promote Tissue  
542 Regeneration in Zebrafish by Fine-Tuning the Inflammatory Response. *Front*  
543 *Immunol*. 10:253. doi:10.3389/fimmu.2019.00253.
- 544 Murayama, E., K. Kissa, A. Zapata, E. Mordelet, V. Briolat, H.-F. Lin, R.I. Handin, and P.  
545 Herbomel. 2006. Tracing hematopoietic precursor migration to successive  
546 hematopoietic organs during zebrafish development. *Immunity*. 25:963–975.  
547 doi:10.1016/j.immuni.2006.10.015.

- 548 Nguyen-Chi, M., B. Laplace-Builhé, J. Travnickova, P. Luz-Crawford, G. Tejedor, G. Lutfalla, K.  
549 Kissa, C. Jorgensen, and F. Djouad. 2017. TNF signaling and macrophages govern fin  
550 regeneration in zebrafish larvae. *Cell Death Dis.* 8:e2979–e2979.  
551 doi:10.1038/cddis.2017.374.
- 552 Palis, J. 2014. Primitive and definitive erythropoiesis in mammals. *Frontiers in Physiology.* 5.
- 553 Palis, J., R.J. Chan, A. Koniski, R. Patel, M. Starr, and M.C. Yoder. 2001. Spatial and temporal  
554 emergence of high proliferative potential hematopoietic precursors during murine  
555 embryogenesis. *Proc Natl Acad Sci U S A.* 98:4528–4533.  
556 doi:10.1073/pnas.071002398.
- 557 Palis, J., S. Robertson, M. Kennedy, C. Wall, and G. Keller. 1999. Development of erythroid  
558 and myeloid progenitors in the yolk sac and embryo proper of the mouse.  
559 *Development.* 126:5073–5084. doi:10.1242/dev.126.22.5073.
- 560 Patel, S.H., C. Christodoulou, C. Weinreb, Q. Yu, E.L. da Rocha, B.J. Pepe-Mooney, S. Bowling,  
561 L. Li, F.G. Osorio, G.Q. Daley, and F.D. Camargo. 2022. Lifelong multilineage  
562 contribution by embryonic-born blood progenitors. *Nature.* 606:747–753.  
563 doi:10.1038/s41586-022-04804-z.
- 564 Pei, W., K. Tanaka, S.C. Huang, L. Xu, B. Liu, J. Sinclair, J. Idol, G.K. Varshney, H. Huang, S. Lin,  
565 R.B. Nussenblatt, R. Mori, and S.M. Burgess. 2016. Extracellular HSP60 triggers tissue  
566 regeneration and wound healing by regulating inflammation and cell proliferation.  
567 *npj Regen Med.* 1:1–11. doi:10.1038/npjregenmed.2016.13.
- 568 Petrie, T.A., N.S. Strand, C. Tsung-Yang, J.S. Rabinowitz, and R.T. Moon. 2014. Macrophages  
569 modulate adult zebrafish tail fin regeneration. *Development.* 141:2581–2591.  
570 doi:10.1242/dev.098459.
- 571 Renaud, O., P. Herbomel, and K. Kissa. 2011. Studying cell behavior in whole zebrafish  
572 embryos by confocal live imaging: application to hematopoietic stem cells. *Nat*  
573 *Protoc.* 6:1897–1904. doi:10.1038/nprot.2011.408.
- 574 Sánchez-Iranzo, H., M. Galardi-Castilla, A. Sanz-Morejón, J.M. González-Rosa, R. Costa, A.  
575 Ernst, J. Sainz de Aja, X. Langa, and N. Mercader. 2018. Transient fibrosis resolves via  
576 fibroblast inactivation in the regenerating zebrafish heart. *Proc. Natl. Acad. Sci.*  
577 *U.S.A.* 115:4188–4193. doi:10.1073/pnas.1716713115.
- 578 Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C.  
579 Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D.J. White, V. Hartenstein, K. Eliceiri, P.  
580 Tomancak, and A. Cardona. 2012. Fiji: an open-source platform for biological-image  
581 analysis. *Nat Methods.* 9:676–682. doi:10.1038/nmeth.2019.
- 582 Soares-da-Silva, F., L. Freyer, R. Elsaid, O. Burlen-Defranoux, L. Iturri, O. Sismeiro, P. Pinto-  
583 do-Ó, E. Gomez-Perdiguero, and A. Cumano. 2021. Yolk sac, but not hematopoietic  
584 stem cell-derived progenitors, sustain erythropoiesis throughout murine embryonic  
585 life. *J Exp Med.* 218:e20201729. doi:10.1084/jem.20201729.



- 586 Stachura, D.L., and D. Traver. 2011. Chapter 4 - Cellular Dissection of Zebrafish  
587 Hematopoiesis. *In* Methods in Cell Biology. H.W. Detrich, M. Westerfield, and L.I.  
588 Zon, editors. Academic Press. 75–110.
- 589 Tian, Y., J. Xu, S. Feng, S. He, S. Zhao, L. Zhu, W. Jin, Y. Dai, L. Luo, J.Y. Qu, and Z. Wen. 2017.  
590 The first wave of T lymphopoiesis in zebrafish arises from aorta endothelium  
591 independent of hematopoietic stem cells. *Journal of Experimental Medicine*.  
592 214:3347–3360. doi:10.1084/jem.20170488.
- 593 Ulloa, B.A., S.S. Habbsa, K.S. Potts, A. Lewis, M. McKinstry, S.G. Payne, J.C. Flores, A. Nizhnik,  
594 M. Feliz Norberto, C. Mosimann, and T.V. Bowman. 2021. Definitive hematopoietic  
595 stem cells minimally contribute to embryonic hematopoiesis. *Cell Reports*.  
596 36:109703. doi:10.1016/j.celrep.2021.109703.
- 597 Wynn, T.A., and K.M. Vannella. 2016. Macrophages in Tissue Repair, Regeneration, and  
598 Fibrosis. *Immunity*. 44:450–462. doi:10.1016/j.immuni.2016.02.015.
- 599 Xu, J., L. Zhu, S. He, Y. Wu, W. Jin, T. Yu, J.Y. Qu, and Z. Wen. 2015. Temporal-Spatial  
600 Resolution Fate Mapping Reveals Distinct Origins for Embryonic and Adult Microglia  
601 in Zebrafish. *Developmental Cell*. 34:632–641. doi:10.1016/j.devcel.2015.08.018.
- 602 Yokomizo, T., T. Ideue, S. Morino-Koga, C.Y. Tham, T. Sato, N. Takeda, Y. Kubota, M.  
603 Kurokawa, N. Komatsu, M. Ogawa, K. Araki, M. Osato, and T. Suda. 2022.  
604 Independent origins of fetal liver haematopoietic stem and progenitor cells. *Nature*.  
605 609:779–784. doi:10.1038/s41586-022-05203-0.
- 606

## Steady state



## Caudal fin Injury

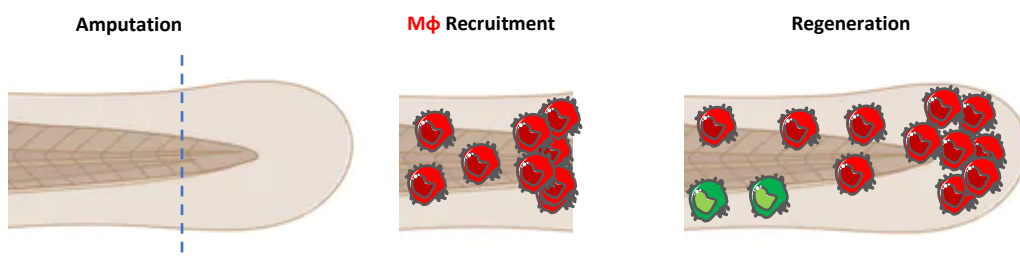
During embryonic stages

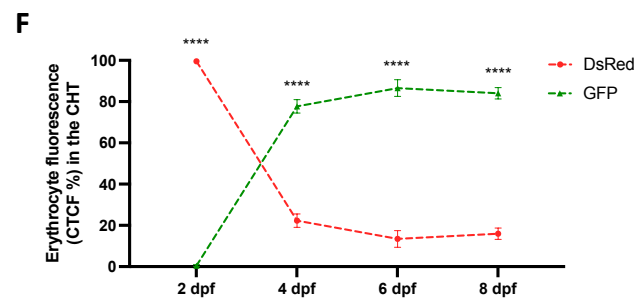
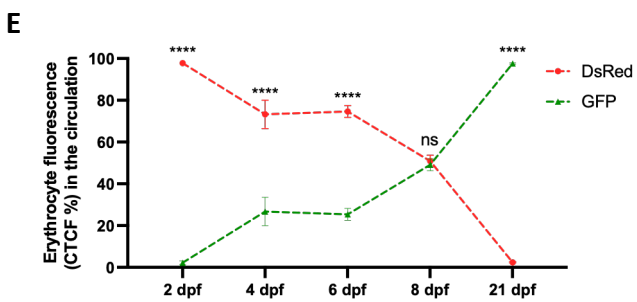
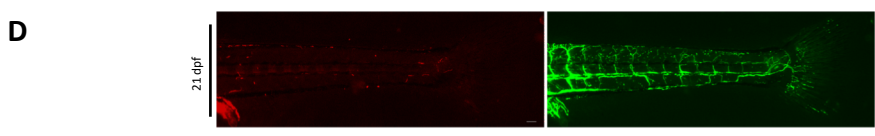
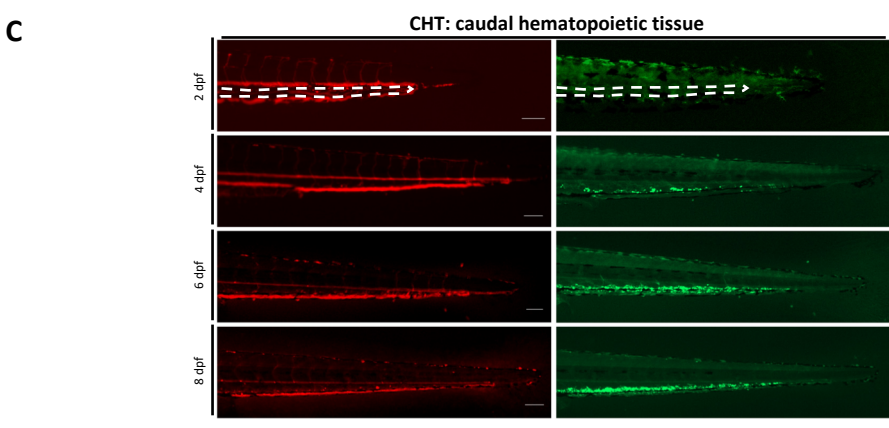
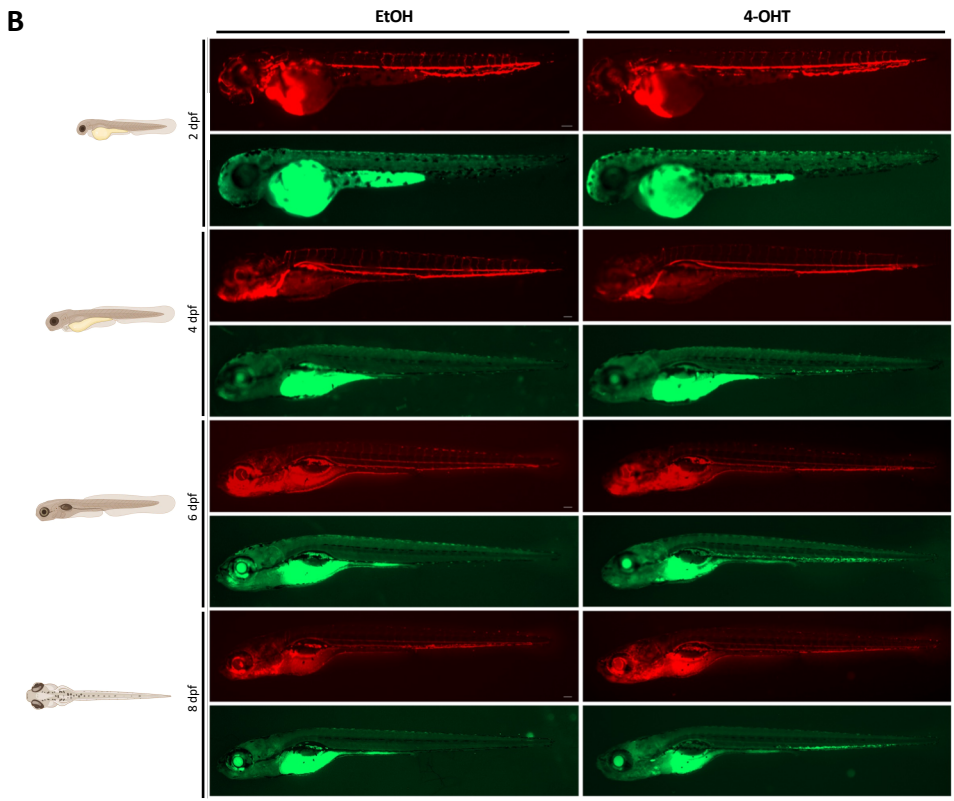
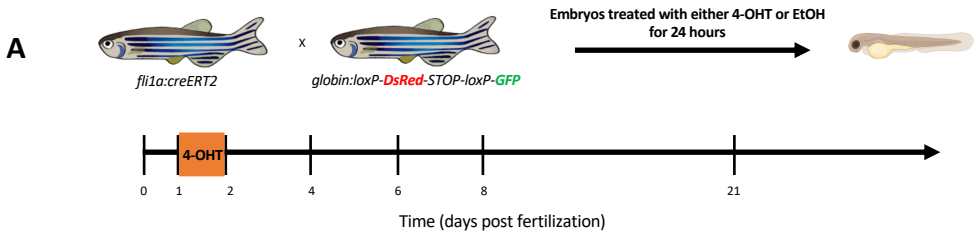


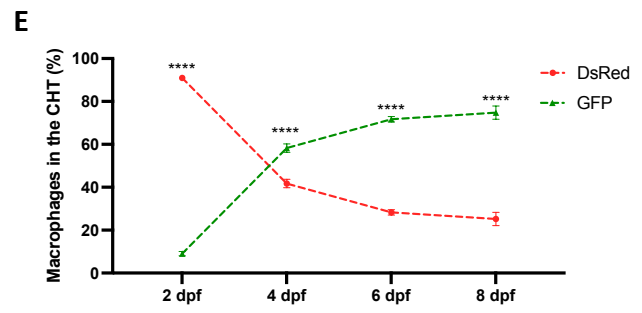
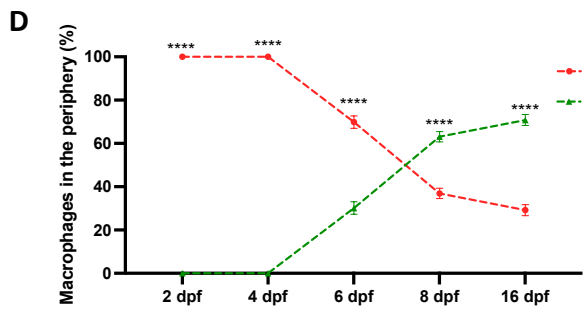
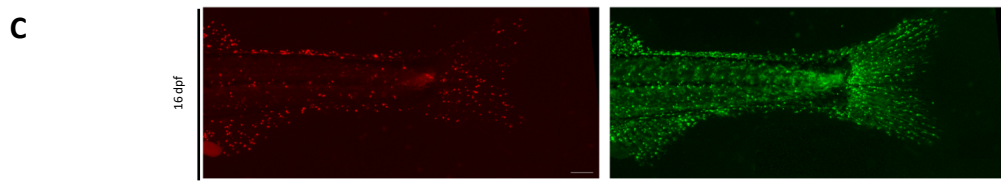
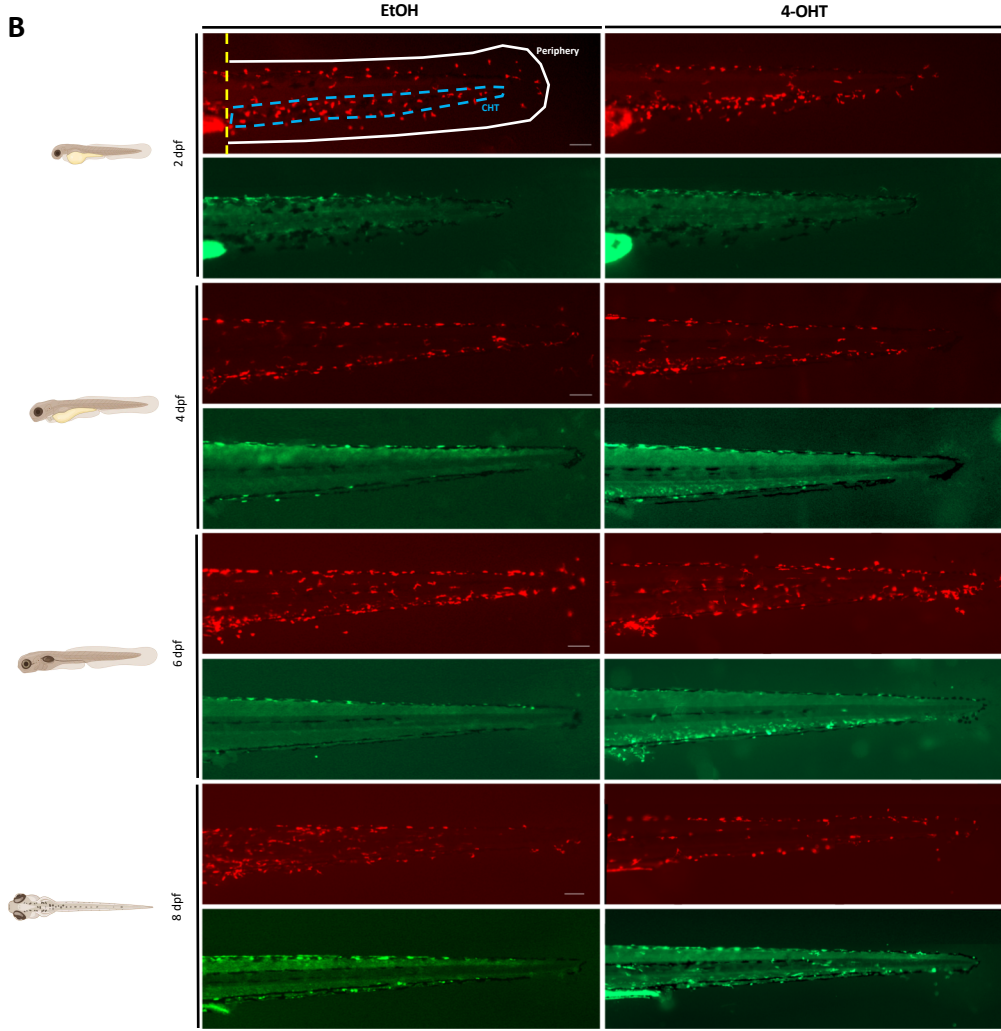
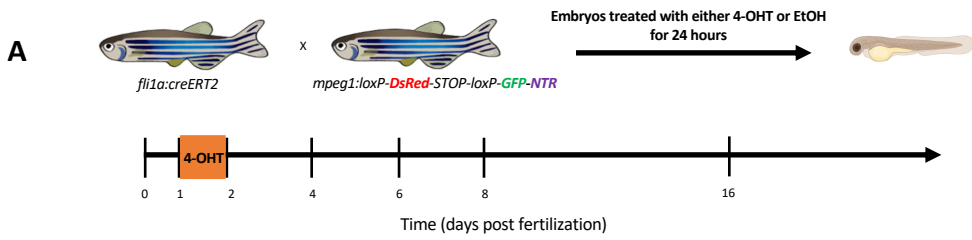
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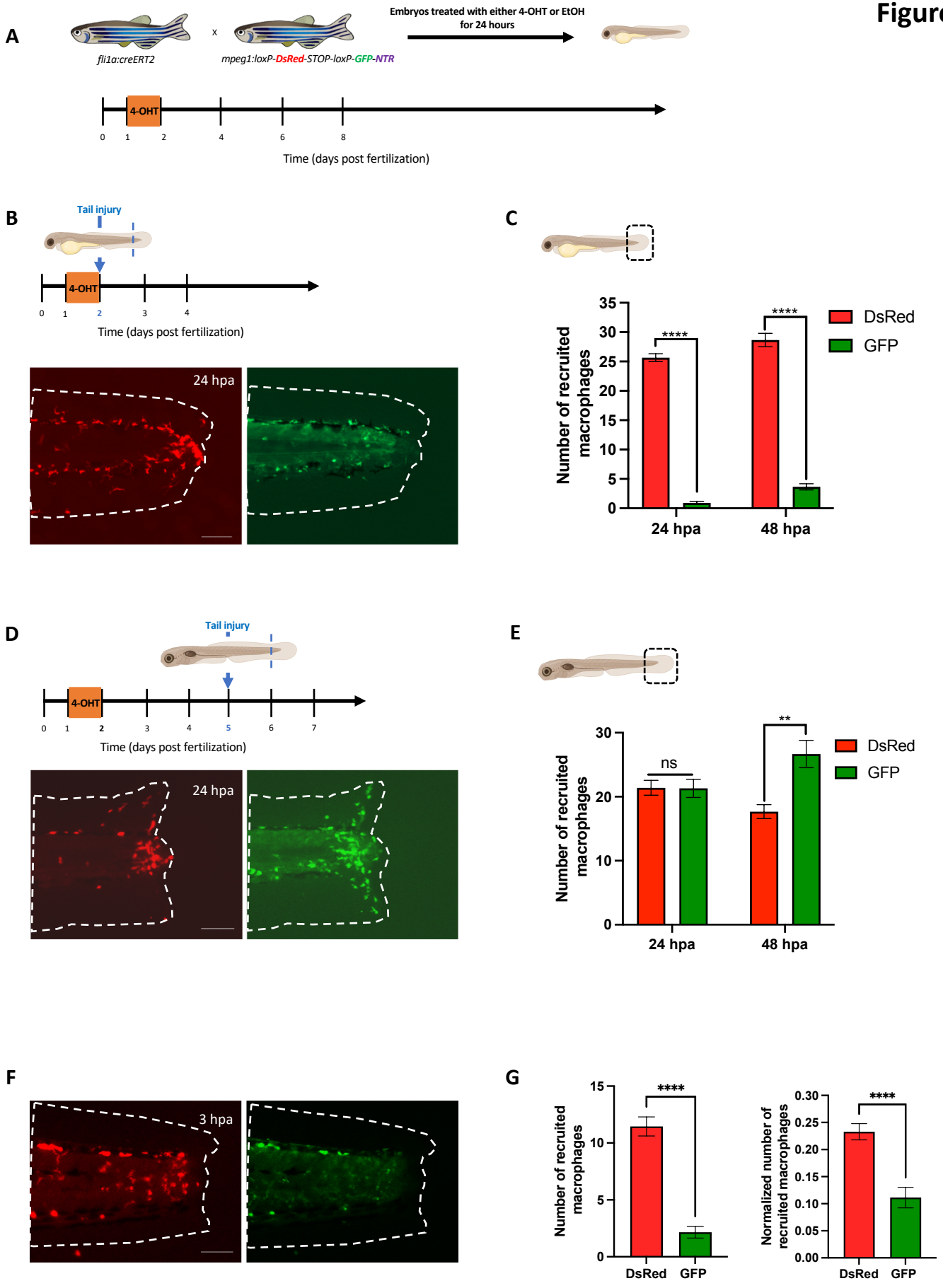


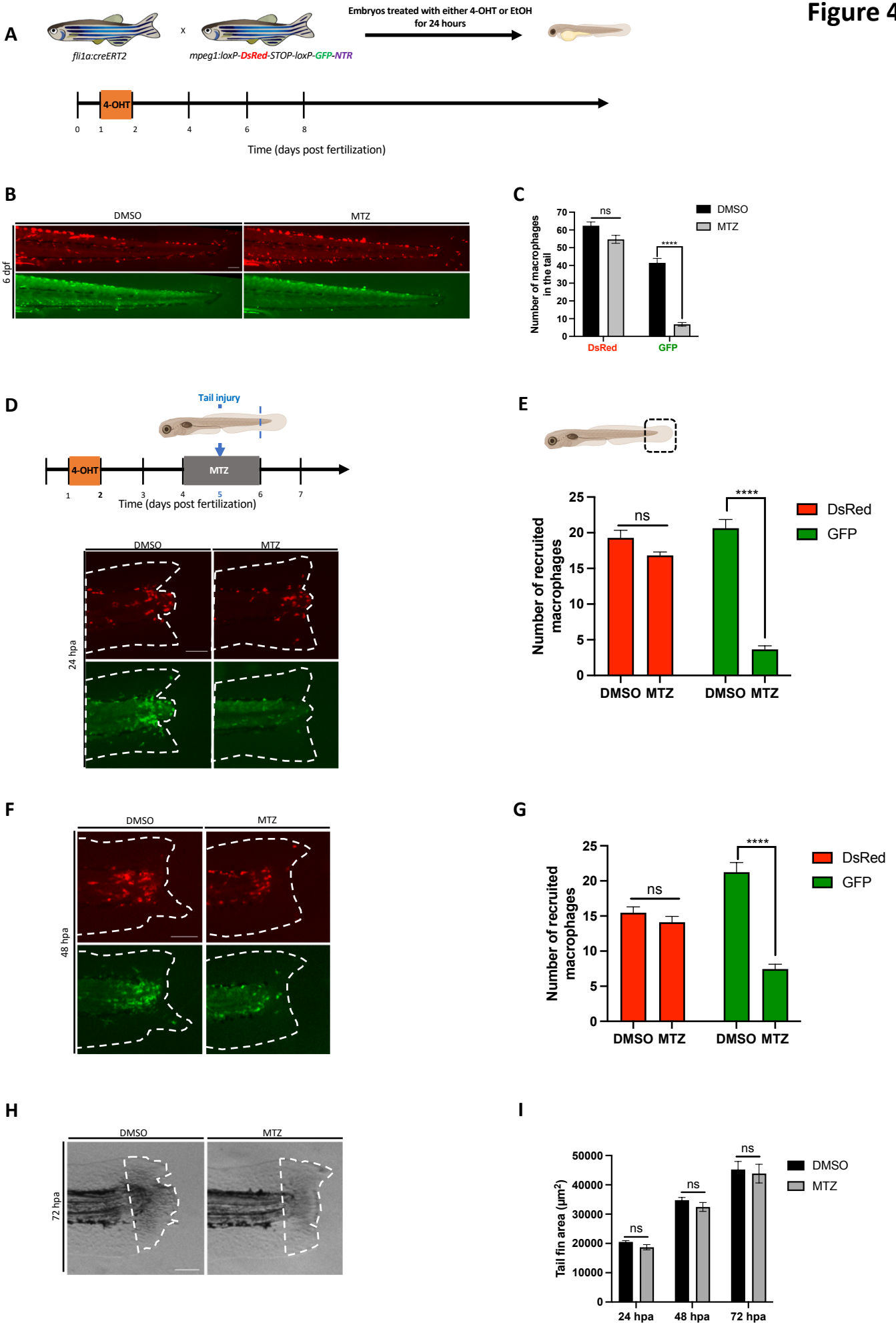
During early larval developmental stages upon ablation of definitive macrophages



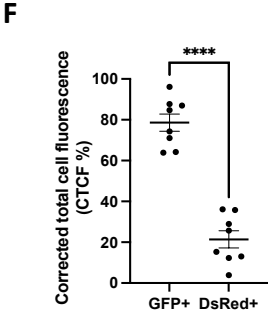
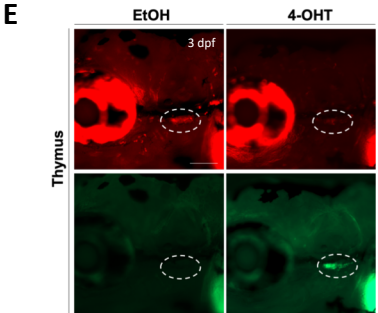
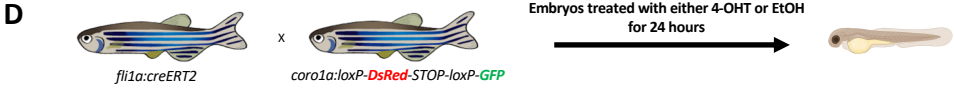
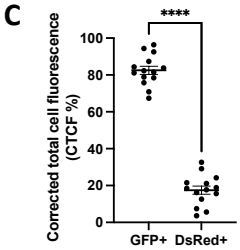
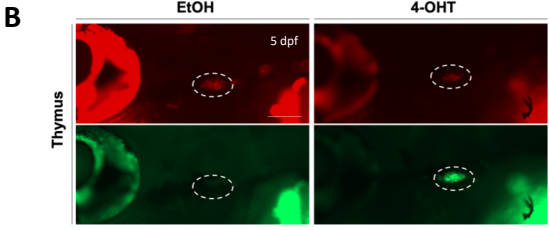




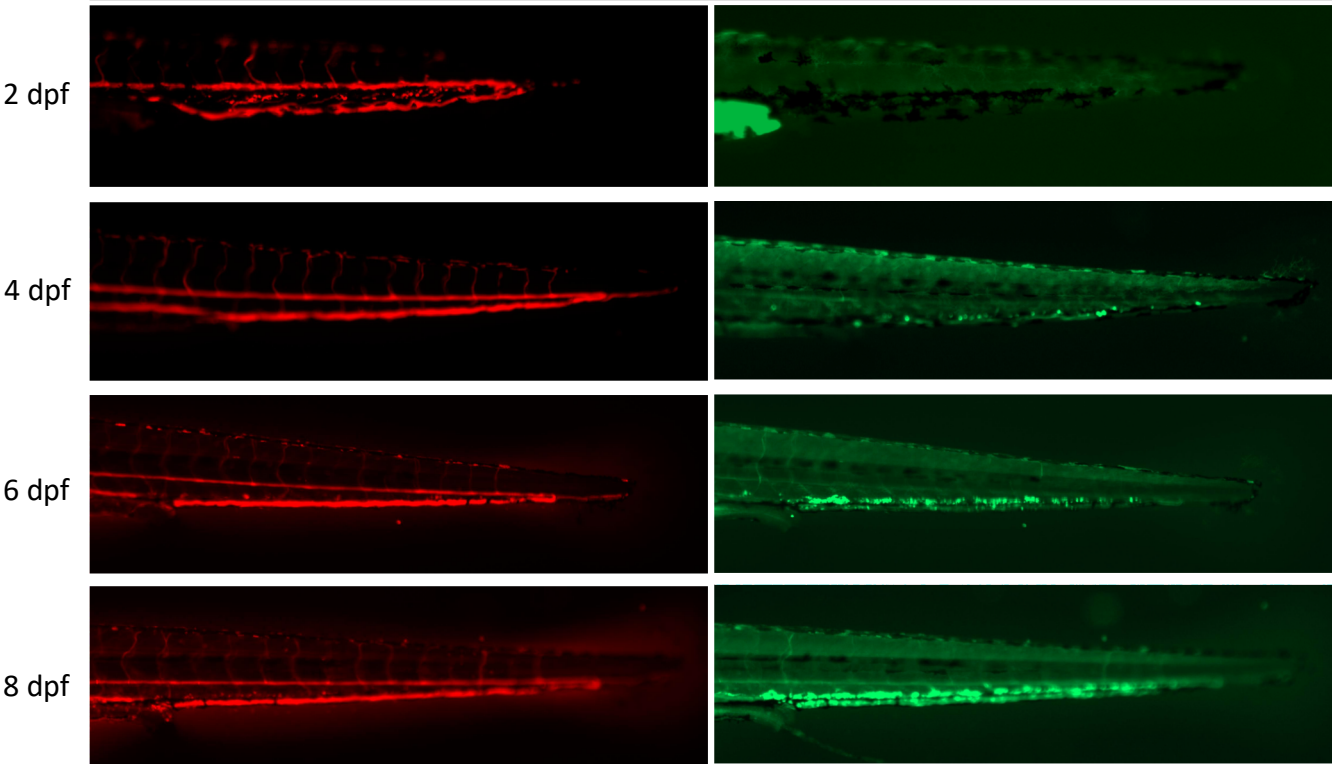




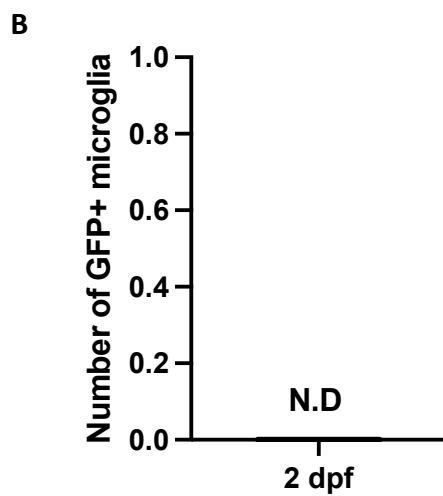
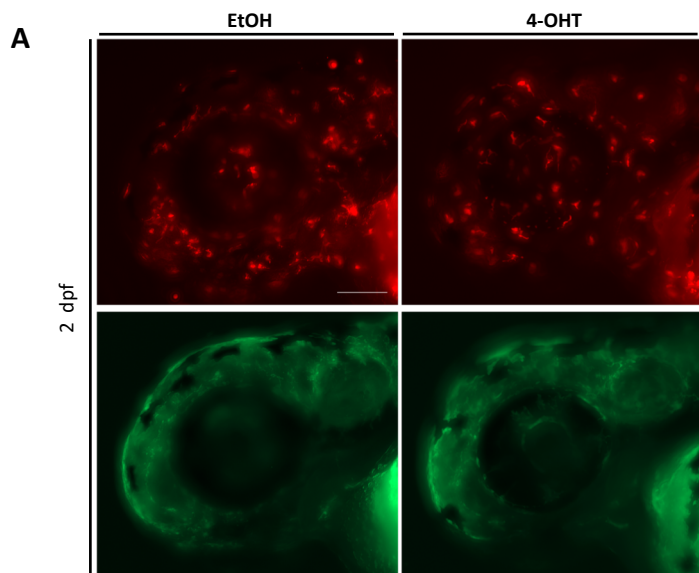




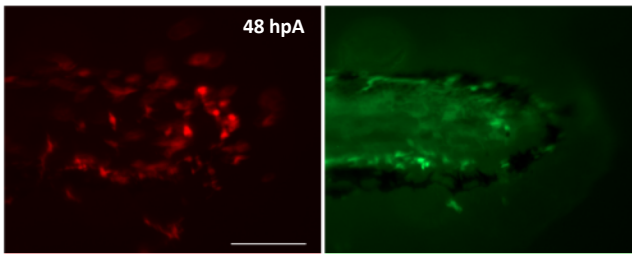
CHT: caudal hematopoietic tissue



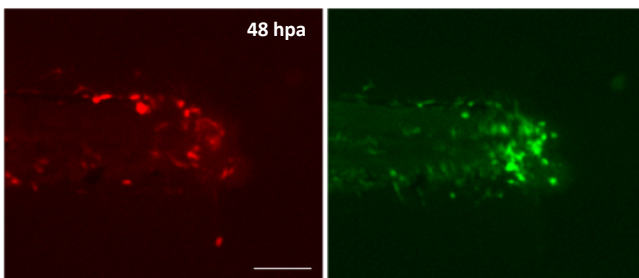




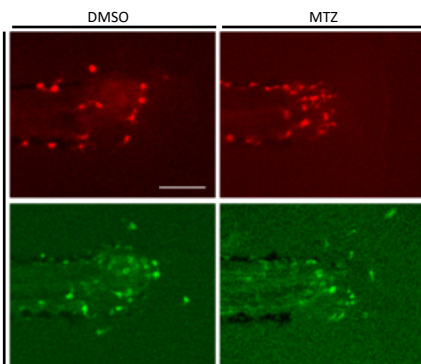
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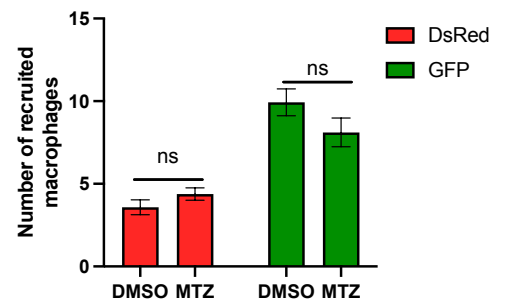
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D



**Figure 1: Tracing the contribution of definitive hematopoietic waves to the erythroid lineage in the embryo and early larvae.**

- (A) Scheme of the 4-OHT-inducible transgenic lines used to assess the contribution of definitive hematopoietic waves to the erythroid lineage.
- (B) Fluorescent images of EtOH non-switched controls (right) and 4-OHT-induced (left) *Tg(fli1a:creERT2;globin:Switch)* embryos and larvae (2 dpf-8 dpf). Scale bars, 100  $\mu$ m. Representative images are shown with quantification of erythrocytes in circulation in (E).
- (C) Fluorescent images of 4-OHT-induced *Tg(fli1a:creERT2;globin:Switch)* embryos and larvae (2-8 dpf) in the CHT region. Non-switched primitive erythrocytes (right) and definitive erythrocytes (left). Scale bars, 100  $\mu$ m. Representative images are shown with quantification of erythrocytes in the CHT in (F).
- (D) Fluorescent images of 4-OHT-induced *Tg(fli1a:creERT2;globin:Switch)* (21 dpf) in the circulation. Scale bar, 100  $\mu$ m. Non-switched primitive erythrocytes (right) and definitive erythrocytes (left). Representative images are shown with quantification of erythrocytes in the circulation in (E).
- (E) Quantification of DsRed and GFP fluorescence intensity percentage in the circulation was measured over a time course of 2-21 dpf. (2 dpf n=6; 4 dpf n=4; 6 dpf n=5; 8 dpf n=4; 21 dpf n=5). Mean  $\pm$  SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> corrected total cell fluorescence (CTCF) percentage at each time point is shown. Two-way ANOVA with Sidak's multiple comparison was used for this analysis. \*\*\*\*p  $\leq$  0.0001.
- (F) Quantification of DsRed and GFP fluorescence intensity percentage in the CHT was measured over a time course of 2-8 dpf. (2 dpf n=6; 4 dpf n=4; 6 dpf n=5; 8 dpf n=4). Mean  $\pm$  SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> corrected total cell fluorescence (CTCF) percentage at each time point is shown. Two-way ANOVA with Sidak's multiple comparison was used for this analysis. \*\*\*\*p  $\leq$  0.0001.

**Figure 2: Tracing the contribution of definitive hematopoietic waves to macrophages in the embryo and early larvae.**

- (A) Scheme of the 4-OHT-inducible transgenic lines used to assess the contribution of definitive hematopoietic waves to the macrophage lineage.
- (B) Fluorescent images of EtOH non-switched controls (right) and 4-OHT-induced (left) *Tg(fli1a:creERT2;mpeg1:Switch)* embryos and larvae (2 dpf-8 dpf). Macrophages were considered as peripheral macrophages (white area) or CHT-resident macrophages (blue area). Scale bars, 100  $\mu$ m. Representative images are shown with quantification of macrophages in the periphery in (D) and the CHT in (E).
- (C) Fluorescent images of 4-OHT-induced *Tg(fli1a:creERT2;mpeg1:Switch)* (16 dpf) in the periphery. Scale bar, 500  $\mu$ m. Non-switched primitive macrophages (right) and definitive macrophages (left). Representative images are shown with quantification of macrophages in the periphery in (D).
- (D) Quantification of DsRed and GFP macrophage number in the periphery was measured over a time course of 2-16 dpf. (2 dpf n=6; 4 dpf n=11; 6 dpf n=7; 8 dpf n=4; 16 dpf n=3). Mean  $\pm$  SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number at each time point is shown. Two-way ANOVA with Sidak's multiple comparison was used for this analysis. \*\*\*\*p  $\leq$  0.0001.
- (E) Quantification of DsRed and GFP macrophage number in the CHT was measured over a time course of 2-8 dpf. (2 dpf n=6; 4 dpf n=11; 6 dpf n=7; 8 dpf n=4). Mean  $\pm$  SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number at each time point is shown. Two-way ANOVA with Sidak's multiple comparison was used for this analysis. \*\*\*\*p  $\leq$  0.0001.

**Figure 3: Ontogenetically distinct macrophages exhibit different migration behavior and recruitment after tissue injury during early developmental stages.**

- (A) Scheme of the 4-OHT-inducible transgenic lines used to assess the contribution of ontogenetically distinct hematopoietic waves to the macrophage lineage upon tissue injury.
- (B) Diagram showing the amputation plan, tail fin of *Tg(fli1a:creERT2;mpeg1:Switch)* were amputated at 2 dpf and macrophages recruitment to the site of injury was analyzed. Representative images are shown at 24 hpa. Scale bar: 100 $\mu$ m.
- (C) Diagram showing the counting region in the larvae. Quantification of DsRed and GFP macrophage number at the site of injury at 24 hpa (n=14) and 48 hpa (n=12). Mean  $\pm$  SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number at each time point is shown. \*\*\*\*p  $\leq$  0.0001.

(D) Diagram showing the amputation plan, tail fin of *Tg(fli1a:creERT2;mpeg1:Switch)* were amputated at 5 dpf and macrophages recruitment to the site of injury was analyzed. Representative images are shown at 24 hpa. Scale bar: 100µm.

(E) Diagram showing the counting region in the larvae. Quantification of DsRed and GFP macrophage number at the site of injury at 24 hpa (n=10) and 48 hpa (n=6). Mean ± SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number at each time point is shown. \*\*\*\*p ≤ 0.0001.

(F) Tail fin of *Tg(fli1a:creERT2;mpeg1:Switch)* were amputated at 5 dpf and macrophages recruitment to the site of injury was analyzed. Representative images are shown at 3 hpa. Scale bar: 100µm.

(G) Quantification of DsRed and GFP macrophage number at the site of injury at 3 hpa (n=13). Mean ± SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number at each time point is shown (left). Quantification was normalized by the number of total macrophages in the tail of the respective larvae (the sum of peripheral, CHT and recruited macrophages of distinct origins). \*\*p ≤ 0.01; \*\*\*\*p ≤ 0.0001.

**Figure 4: Selective ablation of definitive macrophages does not impair tail fin regeneration in early larvae.**

(A) Diagram showing the macrophage ablation and tail fin amputation plan.

(B) Switched *Tg(fli1a:creERT2;mpeg1:Switch)* larvae were treated with DMSO as a control, or metronidazole (MTZ) to ablate definitive macrophages. Treatments were performed from 4 to 6 dpf. Representative images are shown 48 hours after treatments. Scale bars: 100 µm.

(C) Quantification of DsRed and GFP macrophage number in the tail region 48 hours after DMSO (n=9) or MTZ (n=9) treatments. Mean ± SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number is shown. \*\*\*\*p ≤ 0.0001.

(D) Switched *Tg(fli1a:creERT2;mpeg1:Switch)* larvae were treated with DMSO as a control, or metronidazole (MTZ) to ablate definitive macrophages. Treatments were performed from 4 to 6 dpf and tail fins were amputated at 5 dpf. Representative images are shown 24 hours after amputation. Scale bars: 100 µm.

(E) Quantification of DsRed and GFP recruited macrophage number in the tail region 24 hours after amputation. Larvae were treated at 4 dpf with either DMSO (n=11) or MTZ (n=20) for 48 hours. Mean ± SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number is shown. \*\*\*\*p ≤ 0.0001.

(F) Switched *Tg(fli1a:creERT2;mpeg1:Switch)* larvae were treated with DMSO as a control, or metronidazole (MTZ) to ablate definitive macrophages. Treatments were performed from 4 to 6 dpf and tail fins were amputated at 5 dpf. Representative images are shown 48 hours after amputation. Scale bars: 100 µm.

(G) Quantification of DsRed and GFP recruited macrophage number in the tail region 48 hours after amputation. Larvae were treated at 4 dpf with either DMSO (n=17) or MTZ (n=9) for 48 hours. Mean ± SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> macrophage number is shown. \*\*\*\*p ≤ 0.0001.

(H) Representative images of regenerating tail fins of larvae at 72 hours after amputation. Larvae were treated with either DMSO or MTZ. Scale bars: 100 µm.

(I) Tail fin area quantification of regenerating tail fins at 24 hpa, 48 hpa and 72 hpa in larvae treated with either DMSO or MTZ (24 hpa: DMSO n=21, MTZ n=20; 48 hpa: DMSO n=17, MTZ n=9; 72 hpa: DMSO n=14, MTZ n=9).

**Sup. Figure 1: labeling strategy and the labeling efficiency of using thymocytes as a positive read out with *lck:switch* and *coro1a:switch* transgenic lines.**

(A) Scheme of the 4-OHT-inducible transgenic lines used to assess the efficiency of the labelling strategy using the *Tg(fli1a:creERT2;lck:Switch)* double transgenic line.

(B) Fluorescent images of EtOH non-switched controls (right) and 4-OHT-induced (left) *Tg(fli1a:creERT2;lck:Switch)* thymocytes at 5 dpf larvae. Representative images are shown. Scale bars, 100 µm.

(C) Quantification of DsRed and GFP fluorescence intensity percentage in the thymic region was measured at 5 dpf (n=14). Mean ± SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> corrected total cell fluorescence (CTCF) percentage is shown. \*\*\*\*p ≤ 0.0001.

(D) Scheme of the 4-OHT-inducible transgenic lines used to assess the efficiency of the labelling strategy using the *Tg(fli1a:creERT2;coro1a:Switch)* double transgenic line.

(E) Fluorescent images of EtOH non-switched controls (right) and 4-OHT-induced (left) *Tg(fli1a:creERT2;coro1a:Switch)* thymocytes at 3 dpf embryos. Representative images are shown. Scale bars, 100 µm.

(F) Quantification of DsRed and GFP fluorescence intensity percentage in the thymic region was measured at 3 dpf (n=8). Mean ± SEM of the DsRed<sup>+</sup> and GFP<sup>+</sup> corrected total cell fluorescence (CTCF) percentage is shown. \*\*\*\*p ≤ 0.0001.

**Supp videos related to figure 1.** Fluorescence imaging of 4-OHT-induced *Tg(fli1a:creERT2;globin:Switch)* embryos and larvae over a time course of 2- 8 dpf. Videos are assembled from different Z-stacks projections at each defined developmental stage.

**Supp Fig.2. Quantification of GFP+ microglia in the head region at 2 dpf embryos. related to Fig.2**

(A) Fluorescent images of EtOH non-switched controls (right) and 4-OHT-induced (left)

*Tg(fli1a:creERT2;mpeg1:Switch)* head regions in the developing embryos at 2 dpf. Representative images are shown Scale bar: 100µm.

(B) Quantification of GFP+ macrophage number in the head at 2 dpf (n=6). Mean ± SEM of the and GFP+ macrophage number is shown.

**Supp fig 3. Ontogenetically distinct macrophages recruitment after tail injury at different developmental stages. related to fig 3**

(A) tail fin of *Tg(fli1a:creERT2;mpeg1:Switch)* were amputated at 2 dpf and macrophages recruitment to the site of injury was analyzed. Representative images are shown at 48 hpA. Scale bar: 100µm.

(B) tail fin of *Tg(fli1a:creERT2;mpeg1:Switch)* were amputated at 5 dpf and macrophages recruitment to the site of injury was analyzed. Representative images are shown at 48 hpA. Scale bar: 100µm.

(C) Switched *Tg(fli1a:creERT2;mpeg1:Switch)* larvae were treated with DMSO as a control, or metronidazole (MTZ) to ablate definitive macrophages. Treatments were performed from 4 to 6 dpf and tail fins were amputated at 5 dpf. Representative images are shown 72 hours after amputation. Scale bars: 100 µm.

(D) Quantification of DsRed and GFP recruited macrophage number in the tail region 72 hours after amputation. Larvae were treated at 4 dpf with either DMSO (n=14) or MTZ (n=9) for 48 hours. Mean ± SEM of the DsRed+ and GFP+ macrophage number is shown.