

1 The chromatin-remodeling enzyme Smarca5 regulates erythrocyte aggregation

2 via Keap1-Nrf2 signaling

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22 **Abstract**

23 Although thrombosis has been extensively studied using various animal models, however, our
24 understanding of the underlying mechanism remains elusive. Here, using zebrafish model, we
25 demonstrated that *smarca5*-deficient red blood cells (RBCs) formed blood clots in the caudal vein
26 plexus that mimics venous thrombosis. We further used the anti-thrombosis drugs to treat
27 *smarca5*^{zko1049a} embryos and found that a thrombin inhibitor, argatroban, partially prevented blood
28 clot formation in *smarca5*^{zko1049a}. To explore the regulatory mechanism of *smarca5* in RBC
29 homeostasis, we profiled the chromatin accessibility landscape and transcriptome features in
30 RBCs from *smarca5*^{zko1049a} and their siblings and found that both the chromatin accessibility at the
31 *keap1a* promoter and expression of *keap1a* were decreased. Keap1 is a suppressor protein of Nrf2,
32 which is a major regulator of oxidative responses. We further identified that the expression of
33 *hmox1a*, a downstream target of Keap1-Nrf2 signaling pathway, was markedly increased upon
34 *smarca5* deletion. Importantly, overexpression of *keap1a* or knockdown of *hmox1a* partially
35 rescued the blood clot formation, suggesting that the disrupted Keap1-Nrf2 signaling is
36 responsible for the venous thrombosis-like phenotypes in *smarca5* mutants. Together, our study
37 using zebrafish *smarca5* mutants not only characterizes a novel role for *smarca5* in blood clot
38 formation, but also provides a new venous thrombosis animal model to support drug screening and
39 pre-clinical therapeutic assessments to treat thrombosis.

40

41 **Introduction**

42 The erythrocytes, or red blood cells (RBCs), are highly differentiated cells produced during
43 erythropoiesis. Mature RBCs are characterized for their abundance of hemoglobin, which can
44 deliver oxygen to surrounding tissues. Importantly, the flexible structure of RBCs makes it
45 capable of traveling through all blood vessels including capillaries by deformation
46 (Rodriguez-Garcia et al., 2016). On the benefit of accumulated hemoglobin and the deformation
47 ability, RBCs are essential for organism development by facilitating tissue oxygen delivery and
48 transporting carbon dioxide into the respiration tissues. Moreover, RBCs participate in the
49 maintenance of thrombosis and hemostasis (Weisel & Litvinov, 2019).

50 Epigenetic regulation of RBC-related genes is fundamental for normal development and
51 maintenance of RBCs (Hewitt, Sanalkumar, Johnson, Keles, & Bresnick, 2014). In this process,
52 the regulation of chromatin accessibility is a prerequisite for gene transcription and is regulated by
53 chromatin remodelers. For instance, Brg1 could regulate α - and β -globin gene transcription in
54 primitive erythrocytes in mice (Bultman, Gebuhr, & Magnuson, 2005; Griffin, Brennan, &
55 Magnuson, 2008). The nucleosome remodeling and histone deacetylase (NuRD) is identified to
56 activate human adult-type globin gene expression (Miccio & Blobel, 2010).

57 Disorder of the gene regulation in RBCs will lead to cellular defects, thereby causing multiple
58 diseases, such as hemoglobinopathy-induced anemia, RBC lysis-induced hemolytic anemia and
59 thrombosis (Kato et al., 2018; Roumenina, Rayes, Lacroix-Desmazes, & Dimitrov, 2016; Weisel
60 & Litvinov, 2019). Among them, thrombosis is a leading cause of death worldwide (Wendelboe &
61 Raskob, 2016). In contrast to arterial thrombi, which are rich in platelets, the venous thrombi are
62 enriched in fibrin and RBCs (Mackman, 2008; Mackman, Bergmeier, Stouffer, & Weitz, 2020).
63 Moreover, venous thrombi can break off, travel and lodge in the lung, thereby causing pulmonary
64 embolism (Wolberg et al., 2015).

65 Currently, the ligature-based inferior vena cava models, free radical thrombosis models and
66 genetic knockout models are widely used in mice to study deep vein thrombosis (Diaz et al., 2019;
67 Grover & Mackman, 2019). These disease models are generated mainly through disrupting blood
68 flow, endothelium and blood coagulability. Taking advantage of the conserved hemostatic system
69 and the transparency of embryos, zebrafish has been also used to generate thrombosis models. For
70 instance, phenylhydrazine-treated zebrafish develop severe thrombosis in the caudal vein (Zhu et

71 al., 2016). Mechanistically, phenylhydrazine causes externalization of phosphatidylserine on
72 plasma of RBC membrane and generates oxidative radicals, thereafter, resulting in the thrombosis
73 formation. These studies in animal models shed light on the understanding and treatment of
74 vaso-occlusion phenotype in patients with RBC defects. However, the detailed kinetics and
75 underlying mechanism of thrombosis formation in these models are not fully explored.

76 In our previous study, genetic deletion of an epigenetic regulator-*smarca5* (*smarca5*^{zko1049a})
77 resulted in abnormal chromatin accessibility, and we observed disruption of hematopoietic
78 transcription factor binding in the genome, finally leading to defects in fetal hematopoietic stem
79 and progenitor cells (HSPCs) (Ding et al., 2021). However, whether the other hematopoietic cell
80 types are regulated by *smarca5* is unknown. Here, we develop a new zebrafish thrombosis model
81 with a deletion of *smarca5*, loss of which leads to the formation of blood clots in the caudal vein
82 plexus (CVP). We further present how exactly the change in the subcellular structure of
83 *smarca5*-deficient RBCs occurred using transmission electron microscopy (TEM), and uncovered
84 the disintegration of cristae in mitochondria in RBCs. To explore the regulatory mechanism of
85 *smarca5* in RBC homeostasis, we profiled the chromatin accessibility landscape and transcriptome
86 features by performing Assay for Transposase-Accessible Chromatin with high-throughput
87 sequencing (ATAC-seq) and RNA sequencing (RNA-seq) analyses in RBCs from *smarca5*^{zko1049a}
88 and their siblings. Mechanistically, loss of *smarca5* led to the decreased chromatin accessibility at
89 *keap1a* promoter and thus decreased transcriptional expression of *keap1a*. Keap1 is a suppressor
90 protein of Nrf2, which regulates the expression of oxidative response genes. A downstream target
91 of Keap1-Nrf2, *hmox1a*, showed a markedly increased expression upon *smarca5* deletion.
92 Moreover, overexpression of *keap1a* or knockdown of *hmox1a* partially rescued the blood clot
93 formation, supporting that the disrupted Keap1-Nrf2 signaling in *smarca5* mutants led to the blood
94 clot formation, thereby mimicking the venous thrombosis phenotypes. Collectively, our
95 *smarca5*-deficient zebrafish model may serve as a new venous thrombosis model for drug
96 screening in clinical therapy.

97

98 **Results**

99 ***smarca5*-deficient RBCs tend to form aggregates in the CVP.**

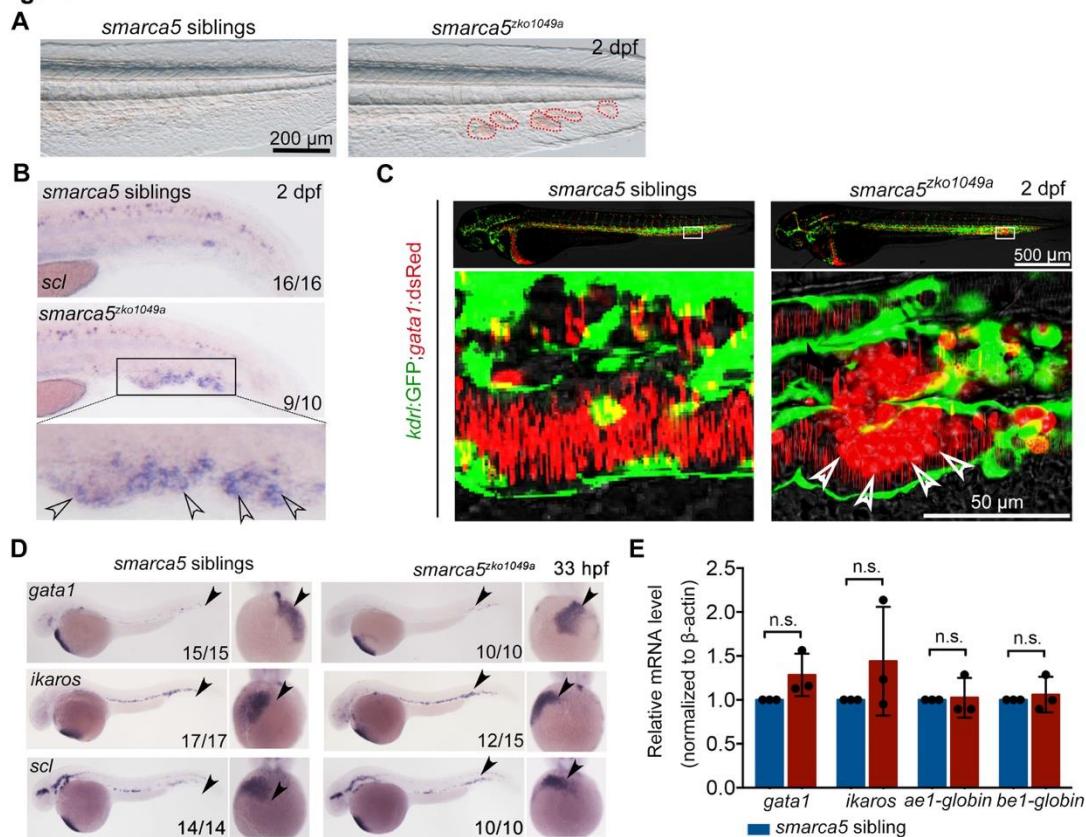
100 In our previously generated *smarca5*^{zko1049a} mutants (Ding et al., 2021), we observed that the
101 blood clots were formed in CVP at 2 dpf, which was not present in their sibling embryos (Figure
102 1A). Our whole mount *in situ* hybridization (WISH) data showed that *scl* was expressed in blood
103 clots, indicating that cells in the observed blood clots were primitive RBCs in *smarca5*^{zko1049a}
104 (Figure 1B). To directly observe the blood clot formation in the CVP, we used the transgenic line
105 (Tg) (*gata1*:dsRed/*kdr1*:GFP) to label RBCs and endothelial cells, in *smarca5*^{zko1049a} and in
106 siblings. Confocal imaging analysis showed that the blood clots were formed inside the blood
107 vessels (Figure 1C). Notably, there was no difference in the distribution of myeloid cells labelled
108 by Tg (*coro1a*:GFP) or Tg (*mpo*:GFP) in caudal hematopoietic tissue (CHT) between
109 *smarca5*^{zko1049a} and their siblings, and we did not observe accumulation of myeloid cells in the
110 blood clots of *smarca5*^{zko1049a} (supplemental Figure 1A).

111 To further determine whether *smarca5* is involved in the development of primitive hematopoiesis,
112 we examined the expression level of *gata1* and *pu.1*, which are the erythrocyte and myeloid
113 marker genes, respectively, in *smarca5*^{zko1049a} and their siblings. WISH and quantitative PCR
114 (qPCR) analyses showed that the expression level of *gata1* and *pu.1* was comparable between
115 *smarca5*^{zko1049a} and their siblings at 33 hours post fertilization (hpf) (Figure 1D-E and
116 supplemental Figure 1B-C). Moreover, the expression level of *ikaros* and *scl*, which are two
117 primitive erythrocyte markers, was normal (Figure 1D-E), as well as the expression of globin
118 genes in *smarca5*^{zko1049a} (Figure 1E). In addition, the myeloid markers *pu.1*, *lyz* and *mfap4* were
119 normally expressed in *smarca5*^{zko1049a} at 33 hpf and 2 days post fertilization (dpf) (supplemental
120 Figure 1B-C). Thus, the early development of primitive erythrocytes and myeloid cells, is not
121 affected upon the loss of *smarca5* in zebrafish embryos.

122 Taken together, these results show that *smarca5* is functionally required for normal behaviors of
123 primitive erythrocytes and the blood clotting is formed by erythrocytes in *smarca5*^{zko1049a}.

124

Figure1

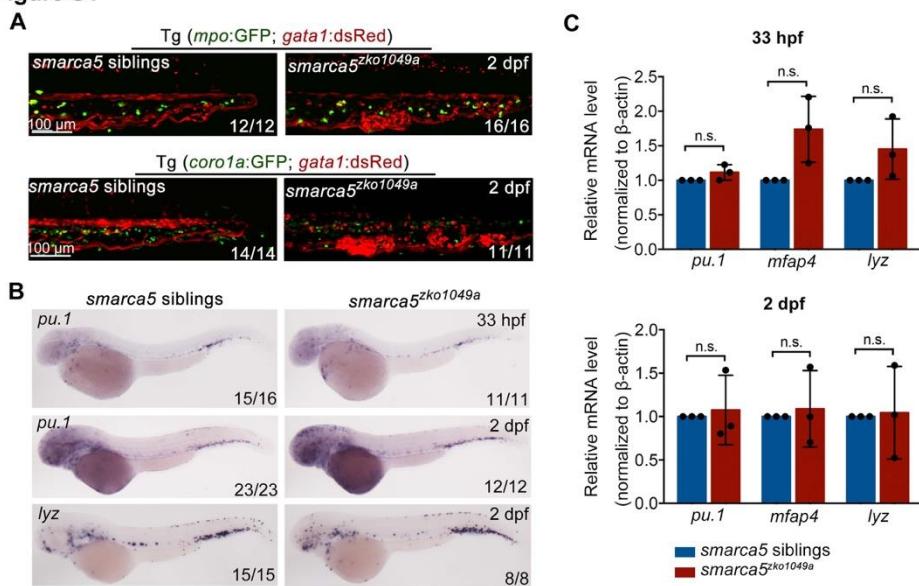


125

126 **Figure 1. Blood clots occur in *smarca5*^{ko1049a}.** (A) The bright-field of tail region in
 127 *smarca5*^{ko1049a} and their siblings at 2 days post fertilization (dpf). The areas circled by red dotted
 128 line show the blood clots in the caudal vein plexus. Scale bars, 200 μ m. (B) Expression of *scl* at 2
 129 dpf in *smarca5*^{ko1049a} and their siblings by WISH. The amplification region in the black
 130 rectangular box in CHT shows the enriched expression of *scl* in blood clots (indicated by arrow
 131 heads) in the caudal vein plexus. (C) The confocal imaging of transgenic line (Tg) (*kdr1*:GFP;
 132 *gata1*:dsRed) in *smarca5*^{ko1049a} and their siblings at 2 dpf. The amplification region in the white
 133 rectangular box in CHT shows the coagulation of red blood cells (RBCs) (indicated by arrow
 134 heads) in the caudal vein plexus. (D) Expression of *gata1*, *ikaros* and *scl* at 33 hours post
 135 fertilization (hpf) in *smarca5*^{ko1049a} and their siblings by WISH. (E) qPCR analysis showing the
 136 expression of *gata1*, *ikaros*, *ae1-globin* and *be1-globin* in *smarca5*^{ko1049a} and their siblings at 33
 137 hpf. The expression level of these genes in *smarca5* siblings was set at 1. Data are mean \pm s.d. (E).
 138 Asterisk presents statistical significance (n.s. not significant). *P* values were calculated by
 139 two-tailed unpaired Student's *t*-test.

140

Figure S1



141

142 **supplemental Figure 1. *smarca5* is dispensable for the development of primitive myeloid cells.**

143 (A) The confocal imaging of Tg (*mpo*:GFP; *gata1*:dsRed) and Tg (*coro1a*:GFP; *gata1*:dsRed) in
144 *smarca5*^{zko1049a} and their siblings at 2 dpf. (B) Expression of *pu.1* and *lyz* at 33 hpf and/or 2 dpf in
145 *smarca5*^{zko1049a} and their siblings by WISH. (C) qPCR analysis showing the expression of *pu.1*,
146 *mfap4* and *lyz* in *smarca5*^{zko1049a} and their siblings at 33 hpf and 2 dpf. The expression level of
147 these genes in *smarca5* siblings was set at 1. Data are mean \pm s.d. (C). Asterisk presents statistical
148 significance (n.s. not significant). *P* values were calculated by two-tailed unpaired Student's *t*-test.
149

150 **The blood clots are formed by RBC aggregation that mimics venous thrombosis.**

151 To visualize how *smarca5*-deficient RBCs formed blood clots in the CVP of *smarca5*^{zko1049a}, we
152 performed time lapse imaging using Tg (*gata1*:dsRed). We tracked the behavior of circulating
153 RBCs in siblings (supplemental Movie 1) and *smarca5*^{zko1049a} (supplemental Movie 2) from 36 hpf
154 to 2 dpf. The results showed that *smarca5*-deficient RBCs tended to clump in the CVP at around
155 40 hpf, after which these clots will migrate or break off under blood flow at the early stage. As the
156 blood clots formed with larger size, these clots will finally lodge in the vein (Figure 2A and
157 supplemental Movie 2). These results show that the clumping of RBCs precedes their
158 sequestration in CVP, suggesting that the formation of blood clots might be independent of
159 vascular niche.

160 To further explore whether the blood clots formed in *smarca5*^{zko1049a} were not resulted from the
161 abnormal niche environment, we performed parabiosis experiment using *smarca5*^{zko1049a} and their
162 siblings and found that the blood clots were observed in both *smarca5*^{zko1049a} and their siblings in
163 parabiosis pairs (Figure 2B). To label the RBCs in *smarca5*^{zko1049a} and their siblings, the Tg
164 (*gata1*:dsRed) or Tg (*gata1*:GFP) transgenic line was used, respectively. The results showed that
165 *smarca5*-deficient RBCs labelled by *gata1*:GFP aggregated both in *smarca5*^{zko1049a} and in their
166 siblings in parabiosis pairs (Figure 2C). Although several sibling RBCs labelled by *gata1*:dsRed
167 were found trapped in blood clots, the vast majority of *gata1*:dsRed⁺ cells were normally
168 circulating in blood stream both in *smarca5*^{zko1049a} and their siblings (Figure 2C). Overall, these
169 results indicate that the blood clots in *smarca5*^{zko1049a} are formed largely in RBC-autonomous
170 manner. To further explore whether thrombocytes participate in the formation of blood clots, we
171 detected the blood clots using Tg (*CD41*:GFP). The imaging data showed that no
172 *CD41*:GFP^{high}-labelled thrombocytes were present in the blood clots (Figure 2D).

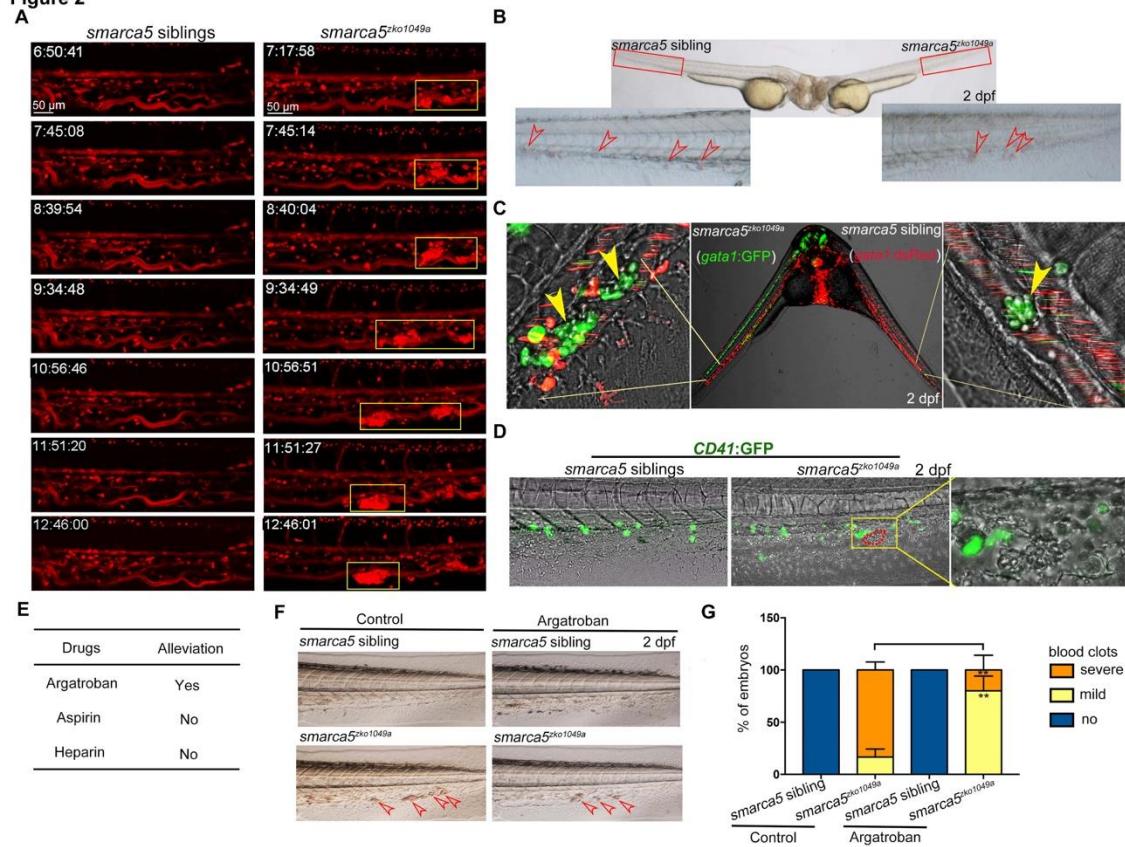
173 The CHT is a hematopoietic tissue critical for HSPC development. We thus wanted to know
174 whether the blood clots formed in *smarca5*^{zko1049a} could influence the structure of CHT, further
175 leading to HSPC defects. As observed previously, the structure of CHT was normal in
176 *smarca5*^{zko1049a} and the number of *cmyb*:GFP⁺ HSPCs in CHT at 2 dpf was comparable between
177 *smarca5*^{zko1049a} and their siblings (supplemental Figure 2A-B), indicating that the formation of
178 blood clots in *smarca5*^{zko1049a} is dispensable for HSPC development in CHT.

179 Zebrafish is a useful model to screen drugs for preclinical applications. In our *smarca5*-deficient

180 zebrafish model, we observed blood clots in veins, raising questions regarding whether there was
181 a thrombus-like phenotype. To this end, we tried to test the clinically used anti-thrombosis drugs
182 to treat *smarca5*^{zko1049a} embryos. We tested reagents including heparin, aspirin and argatroban that
183 have been reported to target thrombosis to examine whether the blood clots in *smarca5*^{zko1049a} can
184 be alleviated after chemical treatment. The embryos were incubated in aspirin or injected with
185 heparin or argatroban at 36 hpf and the phenotype was examined at 2 dpf. As a result, we found
186 that a direct thrombin inhibitor, argatroban, but not an antithrombin-dependent drug, heparin, or a
187 platelet aggregation inhibitor, aspirin, partially prevented blood clot formation in *smarca5*^{zko1049a} at
188 2 dpf (Figure 2E-G). These results suggest that the RBC clots in *smarca5*^{zko1049a} are more relevant
189 to venous thrombosis and the *smarca5*-deficient zebrafish model may serve as a venous
190 thrombosis model to screen drugs in preclinical setting.

191

Figure 2



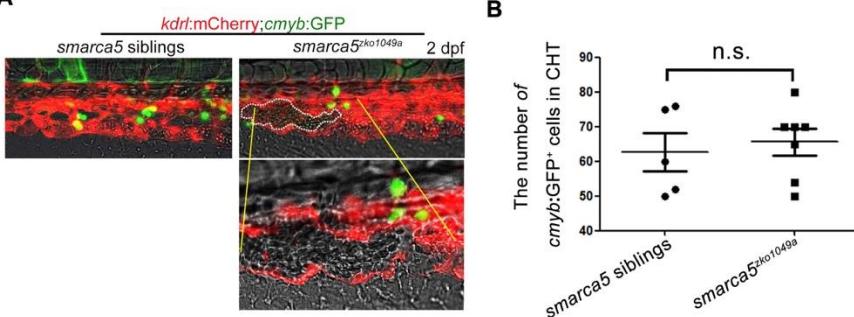
192

193 **Figure 2. The Blood clots formed by RBC aggregation mimics venous thrombosis.** (A) The
 194 snapshot of Tg (gata1:dsRed) in *smarca5*^{zko1049a} and their siblings from 36 hpf to 2 dpf. The
 195 yellow rectangular boxes in CHT show the formed blood clots *smarca5*^{zko1049a}. (B) The
 196 bright-field of parabiosis at 2 dpf between *smarca5*^{zko1049a} and their siblings. The amplification
 197 region in the red rectangular box in CHT shows the blood clots (indicated by arrow heads) in
 198 *smarca5*^{zko1049a} and their siblings. (C) The confocal imaging of parabiosis generated between
 199 *smarca5*^{zko1049a} and their siblings with Tg (gata1:GFP) and Tg (gata1:dsRed) background,
 200 respectively. The magnification in CHT shows the aggregation of *gata1:GFP*⁺ cells (indicated by
 201 arrow heads) in the caudal vein plexus. (D) The confocal imaging of Tg (CD41:GFP) in
 202 *smarca5*^{zko1049a} and their siblings at 2 dpf. The magnification in the yellow rectangular box in CHT
 203 shows the blood clots and the distribution of *CD41:GFP*⁺ cells. (E) Drugs used to examine
 204 whether the blood clots in *smarca5*^{zko1049a} can be alleviated. (F) The bright-field of tail region in
 205 *smarca5*^{zko1049a} at 2 dpf in control group and with argatroban treatment. The blood clots are
 206 indicated by arrow heads. (G) The quantification of blood clots phenotype in (F). Data are mean \pm
 207 s.d. (G). Asterisk presents statistical significance ($**p < 0.01$). *P* values were calculated by
 208 two-tailed unpaired Student's *t*-test.

209

Figure S2

A



210

211 **supplemental Figure 2. The RBC aggregation has no overt influence on the number of**
212 **HSPCs in the CHT.** (A) The confocal imaging of Tg (*kdrl:mCherry*; *cmyb:GFP*) in
213 *smarca5^{ko1049a}* and their siblings at 2 dpf. (B) The statistical data shows the number of *cmyb:GFP*⁺
214 cells in the CHT in (A). Data are mean \pm s.d. (B). Asterisk presents statistical significance (n.s. not
215 significant). *P* values were calculated by two-tailed unpaired Student's *t*-test.

216

217 **smarca5-deficient RBCs manifest disintegration of cristae in mitochondria.**

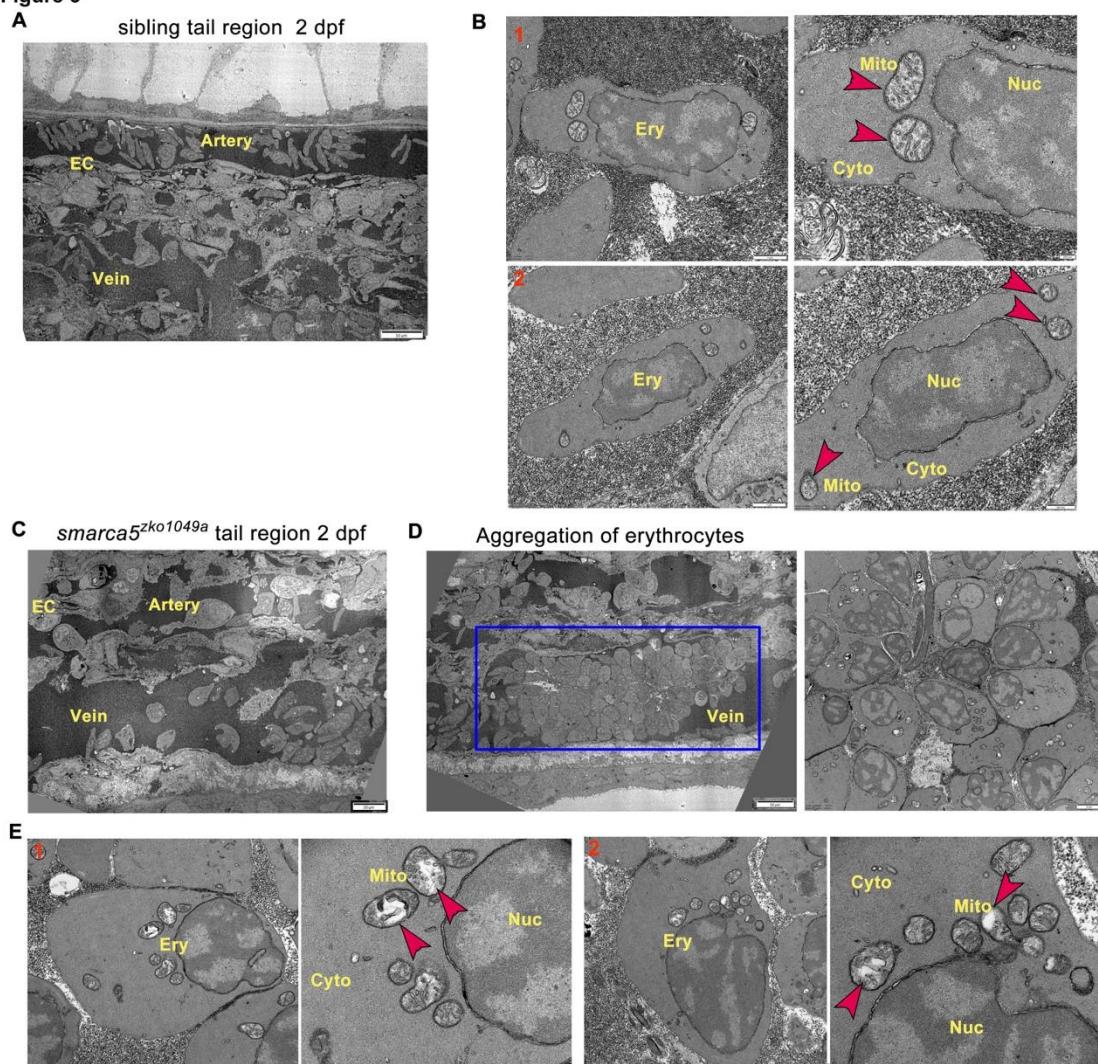
218 Both quantitative and qualitative changes in RBCs have been linked to thrombosis (Weisel &
219 Litvinov, 2019). To identify whether *smarca5* deletion will lead to the quantitative changes of
220 RBCs, we performed fluorescence activating cell sorter (FACS) analysis of the percentage of
221 *gata1*:dsRed⁺ cells in *smarca5*^{zko1049a} and their siblings. Deletion of *smarca5* did not lead to the
222 significant changes in RBC counts at 2 dpf (supplemental Figure 3A-B). These data suggest that
223 the blood clots in *smarca5*^{zko1049a} are formed by RBC aggregation with no overt cell number
224 change.

225 To explore whether there exist qualitative changes in *smarca5*-deficient RBCs, we performed
226 blood-smear and Giemsa-staining analysis. The results showed that the morphology of RBCs had
227 no obvious changes in *smarca5*^{zko1049a} (supplemental Figure 3C). And the statistical analysis
228 showed that the nucleocytoplasmic ratio was normal in *smarca5*-deficient RBCs (supplemental
229 Figure 3D), indicating that the differentiation of RBCs at 2 dpf was not evidently impaired upon
230 *smarca5* loss.

231 To further investigate the changes in subcellular structure of erythrocytes in *smarca5*^{zko1049a}, we
232 performed TEM analysis. Compared with *smarca5* sibling embryos in which the circulating RBCs
233 had normal organization in mitochondria (Figure 3A-B), we found that the *smarca5*-deficient
234 erythrocytes displayed disintegration of cristae in mitochondria while nuclear integrity was
235 preserved in *smarca5*^{zko1049a} (Figure 3C-E). We propose that the erythrocytes in *smarca5*^{zko1049a}
236 may have undergone cellular damages, such as oxidative stress, which could lead to the
237 disintegration of mitochondria (Lewerenz, Ates, Methner, Conrad, & Maher, 2018). Thus, the
238 morphological disruption in mitochondria suggests the disorder of cellular homeostasis in
239 erythrocytes after *smarca5* deletion.

240

Figure 3

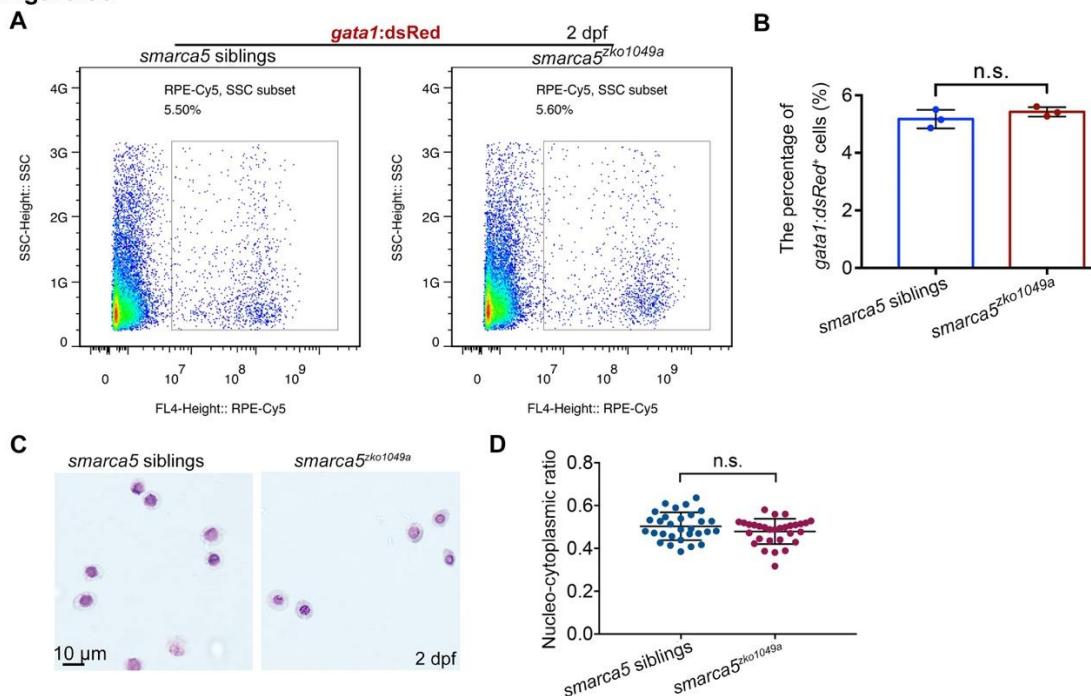


241

242 **Figure 3. TEM shows abnormal organelle morphology in *smarca5*-deficient RBCs.** (A) The
243 transmission electron microscopy (TEM) view of a longitudinal section through the artery and
244 vein plexus in sibling tail region at 2 dpf. (B) TEM view of erythrocytes in sibling embryos. The
245 red arrow heads indicate the characteristic pattern of organization in mitochondria. (C) TEM view
246 of a longitudinal section through the artery and vein plexus in *smarca5*^{zko1049a} tail region at 2 dpf.
247 (D) The blue rectangular box showing blood clots in the caudal vein plexus in *smarca5*^{zko1049a}. The
248 magnification of blood clots is shown (right). (E) TEM view of erythrocytes in *smarca5*^{zko1049a}.
249 The red arrow heads indicate the characteristic pattern of organization in mitochondria. The
250 disintegration of cristae in mitochondria is observed in *smarca5*-deficient RBCs. Ery, erythrocyte;
251 EC, endothelial cell; Mito, mitochondria; Nuc, nucleus; Cyto, cytoplasm.

252

Figure S3



253

254 **supplemental Figure 3. The morphology and number of RBCs has no obvious change in**
255 ***smarca5*^{zko1049a}.** (A) FACS of *gata1:dsRed*⁺ cells in wild type embryos and Tg (*gata1: dsRed*) in
256 *smarca5*^{zko1049a} and their siblings at 2 dpf. (B) The statistical data show the percentage of *gata1:*
257 *dsRed*⁺ cells in *smarca5*^{zko1049a} and their siblings in (A). (C) Blood-smear and Giemsa-staining
258 analysis in *smarca5*^{zko1049a} and their siblings at 2 dpf. Scale bar, 10 μ m. (D) Scatter plots showing
259 the nucleocytoplasmic ratio in RBCs from *smarca5*^{zko1049a} and their siblings. Data are mean \pm s.d.
260 (B and D). Asterisk presents statistical significance (n.s. not significant). *P* values were calculated
261 by two-tailed unpaired Student's *t*-test.

262

263 **Transcriptional dysregulation of genes related to erythrocyte function and homeostasis after**
264 ***smarca5* deletion.**

265 Smarca5 typically regulates nucleosome spacing, further affecting gene transcription (Clapier,
266 Iwasa, Cairns, & Peterson, 2017). To decipher how loss of Smarca5 affects the transcriptome,
267 RNA-seq was used to profile sorted erythrocytes labelled by *gata1*:dsRed from *smarca5*^{zko1049a}
268 and their siblings at 2 dpf, respectively (Figure 4A). Principal components analysis (PCA)
269 indicated clear separation of the *smarca5*^{zko1049a} and sibling samples (supplemental Figure 4A).
270 1,506 genes were upregulated and 633 genes were downregulated significantly (Log2(fold
271 change) > 1, adjusted *P*-value < 0.05) in *smarca5*-deficient erythrocytes compared to erythrocytes
272 from siblings (Figure 4B).

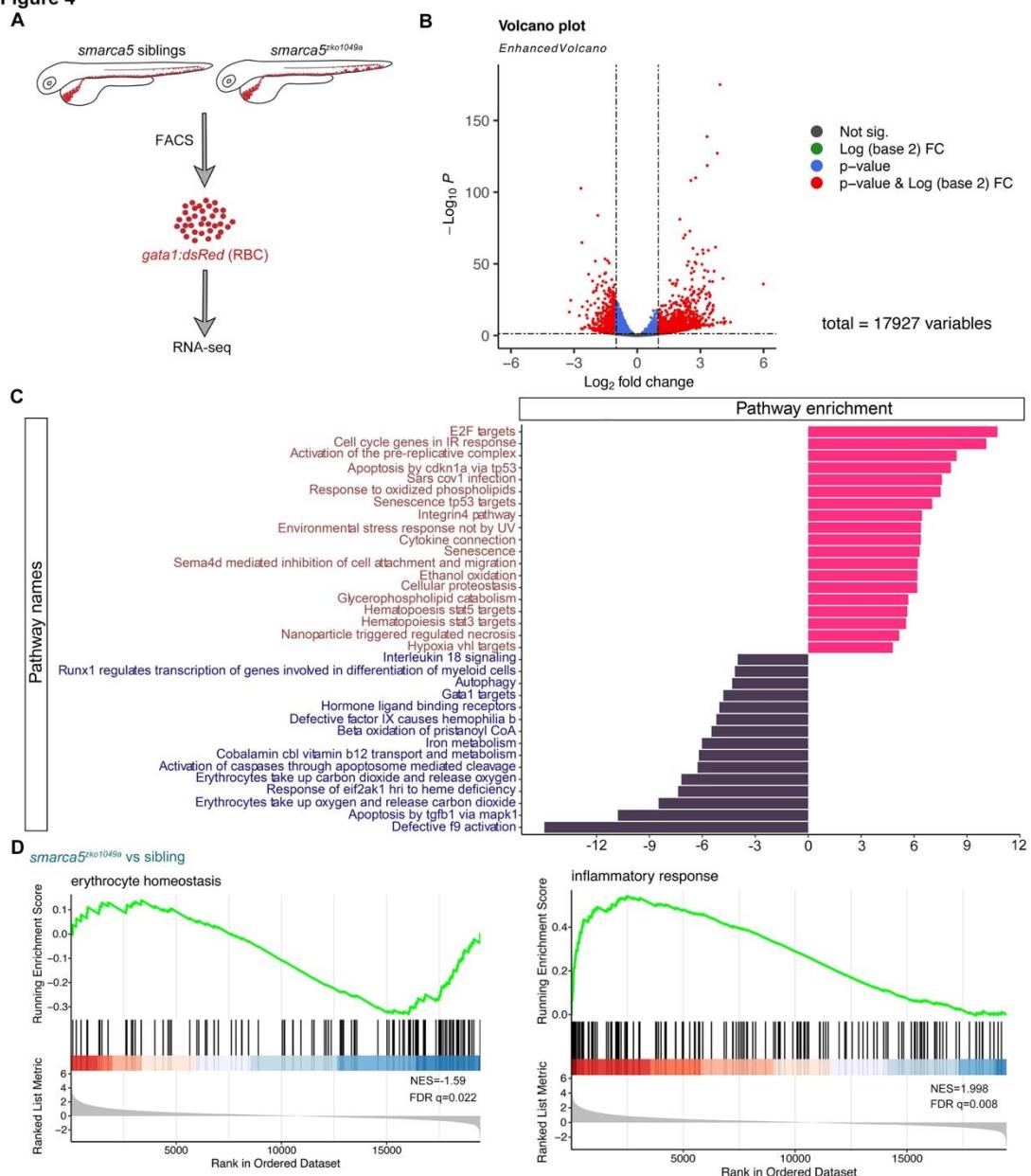
273 Gene set variation analysis (GSVA) revealed a strong enrichment of terms related to ‘Gata1
274 targets’, ‘autophagy’, ‘erythrocytes take up carbon dioxide and release oxygen’ and ‘erythrocytes
275 take up oxygen and release carbon dioxide’ in sibling erythrocytes; for *smarca5*^{zko1049a}, while the
276 ‘apoptosis’, ‘environmental stress response’, ‘senescence’ and ‘cell oxidation’ were markedly
277 increased (Figure 4C). The enrichment plots showed the decreased expression of genes related to
278 ‘erythrocyte homeostasis’ in *smarca5*^{zko1049a}, whereas the expression of genes related to
279 ‘inflammatory response’ was increased (Figure 4D). These results suggest that the disrupted
280 pathways in *smarca5*-deficient RBCs were highly related to erythrocyte function and cellular
281 homeostasis.

282 RBCs have specialized proteome, which is enriched in hemoglobin. We then focused on the
283 expression of hemoglobin complex related genes. The expression of genes related to the
284 hemoglobin complex, including *hbbe1.1*, *hbbe1.3*, *hbbe2*, and *hbae3*, was slightly decreased in
285 *smarca5*^{zko1049a} (supplemental Figure 4B). However, the level of hemoglobin detected by
286 O-dianisidine staining was comparable between *smarca5*^{zko1049a} and their siblings (supplemental
287 Figure 4C). Therefore, the slightly decreased expression of hemoglobin complex related genes
288 after *smarca5* deletion does not lead to obvious hemoglobinopathy in *smarca5*^{zko1049a} at 2 dpf.

289 In addition, we observed the persistent expression of *spi1a*, *spi1b*, *mfap4* and *lyz* markers
290 characteristic of myeloid cells in *smarca5*-defecient erythrocytes (supplemental Figure 4D).
291 Perturbation of the exquisite control by *smarca5* likely causes “hybrid” primitive erythrocytes that
292 resemble partial transcriptional properties of myeloid cells. One possible mechanism for this

293 phenotype is the regulation of SMARCA5 and CTCF at the enhancer of PU.1 (Dluhosova et al.,
294 2014), thereby blocking of *smarca5* leads to the upregulation of *pu.1* gene expression. However,
295 despite the inappropriate expression of myeloid genes in *smarca5*-deficient RBCs, the
296 development of myeloid lineage was not obviously impaired in *smarca5*^{zko1049a} manifested with
297 normal expression pattern of *pu.1* and *lyz* at 33 hpf and 2 dpf (supplemental Figure 1A-B),
298 suggesting the unaltered lineage choices at the primitive stage. To further explore whether the
299 inappropriate expression of myeloid genes in *smarca5*-deficient RBCs caused RBC aggregation,
300 we tried knockdown of *pu.1* in *smarca5*^{zko1049a}. The results showed that knockdown of *pu.1*
301 cannot rescue the RBC aggregation phenotype in *smarca5*^{zko1049a} (supplemental Figure 4E-F).
302 Taken together, *smarca5* deletion leads to the disrupted pathways related to erythrocyte function
303 and cellular homeostasis.
304

Figure 4

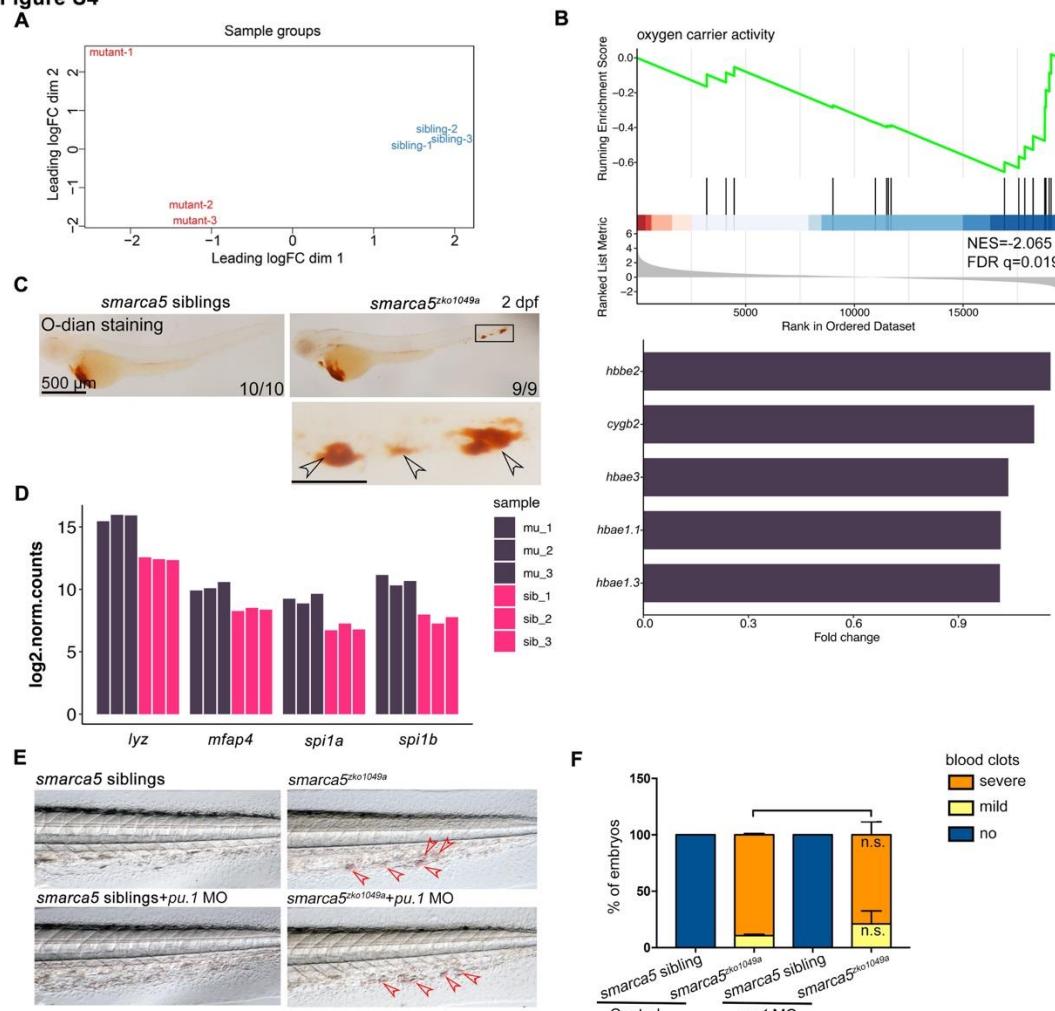


305

306 **Figure 4. Transcriptional disruption of genes related to erythrocyte function and**
 307 **homeostasis after smarca5 deletion.** (A) Schematic representation of the RBC RNA-seq
 308 workflow in *smarca5^{zko1049a}* and their siblings at 2 dpf. (B) Volcano plot showing differential
 309 expression genes between RBCs from *smarca5^{zko1049a}* and their siblings by Deseq2. $-\text{Log}_{10} P$,
 310 negative \log_{10} adjusted P-value. Adjusted P-value < 0.05 , \log_2 fold change > 1 . (C) Differential
 311 pathway enriched in RBCs from *smarca5^{zko1049a}* and their siblings by GSVA. The x axis represents
 312 the t values of GSVA scores. Two-sided unpaired limma-moderated t test. (D) Enrichment plots
 313 for the top pathways in the mutant RBCs by GSEA.

314

Figure S4



315

316 **supplemental Figure 4. RNA-seq analysis for RBCs in *smarca5*^{ko1049a} and their siblings.** (A)
317 Principal components analysis (PCA) plot of biological replicates by the transcriptional data. (B)
318 Enrichment plot for the pathway in sibling RBCs by GSEA (top) and bar plot showing the
319 pathway specific highly expressed genes (bottom). Fold change, log₂ fold change. The genes are
320 sorted by the values of log₂ fold change. (C) O-dianisidine staining in *smarca5*^{ko1049a} and their
321 siblings at 2 dpf. The amplification region in the black rectangular box in CHT shows the blood
322 clots (indicated by arrow heads) in *smarca5*^{ko1049a}. (D) Bar plot showing the gene counts of *lyz*,
323 *mfap4*, *spi1a* and *spi1b* in RBCs from *smarca5*^{ko1049a} and siblings. (E) The bright-field of tail
324 region in *smarca5*^{ko1049a} at 2 dpf in control group and with *pu.1* MO injection. The blood clots are
325 indicated by arrow heads. (F) The quantification of blood clots in (E). Data are mean \pm s.d. (F).
326 Asterisk presents statistical significance (n.s. not significant). *P* values were calculated by
327 two-tailed unpaired Student's *t*-test.

328

329 **Deletion of *smarca5* disrupts chromatin accessibility in RBCs.**

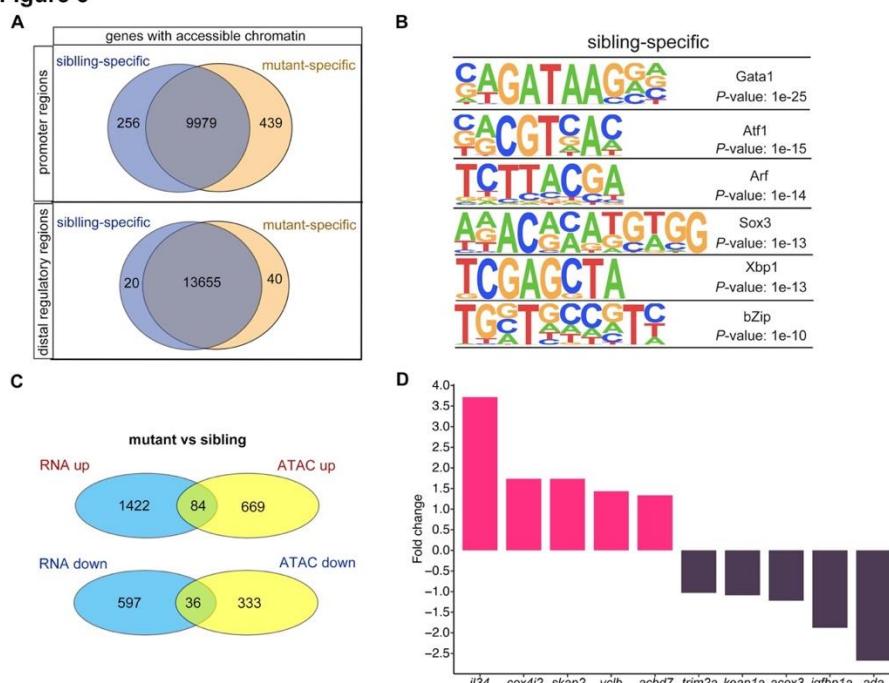
330 To explore the mechanism through which Smarca5 in regulating the chromatin accessibility in
331 RBCs, we performed the ATAC-seq in FACS-purified RBCs from *smarca5*^{zko1049a} and their
332 siblings at 2 dpf. Density heatmaps of mapped ATAC-seq reads showed that fragments less than
333 100 bp in length clustered immediately upstream of transcriptional start sites (TSSs) throughout
334 the zebrafish genome in both mutant and sibling RBC nuclei (supplemental Figure 5A-B). The
335 PCA analysis was performed for ATAC-seq samples and the results showed that the mutant
336 samples or sibling samples can be grouped together, respectively (supplemental Figure 5C). The
337 feature distributions of mutant-ATAC-seq peaks and sibling-ATAC-seq peaks across the genome
338 were identified by ChIPseeker (supplemental Figure 5D).

339 We then calculated the number of genes with changes in chromatin accessibility after *smarca5*
340 deletion (Figure 5A). The chromatin accessibility at promoters of 256 genes was decreased in
341 *smarca5*^{zko1049a}, while there were 439 genes with increased chromatin accessibility at promoters
342 after *smarca5* deletion. Next, we screened the motifs enriched in sibling RBC-specific accessible
343 chromatin regions. We found that the erythrocyte master regulator-Gata1 motif was on the top list
344 (Figure 5B). Thus, deletion of *smarca5* might affect the binding of hematopoietic transcription
345 factors in erythrocytes, such as Gata1. It has been reported that Smarca5 could interact with Gata1
346 in erythrocytes (Rodriguez et al., 2005). We propose that Smarca5 might be recruited by Gata1
347 and mediate the chromatin accessibility of Gata1 binding sites in target genes.

348 We further detected the genes in which the chromatin accessibility at promoters and their
349 transcription were both increased or decreased after *smarca5* deletion (Figure 5C). The results
350 showed that the chromatin accessibility and transcription of 84 genes, such as *il34*, *cox4i2*, *skap2*,
351 *vclb*, and *acbd7*, were increased, while the chromatin accessibility and transcription of 36 genes,
352 such as *trim2a*, *keap1a*, *skap2*, *acox3*, *igfbp1a* and *ada*, were decreased in *smarca5*-deficient
353 RBCs (Figure 5D). Taken together, *smarca5* deletion leads to the disrupted chromatin accessibility
354 and transcriptome in RBCs.

355

Figure 5



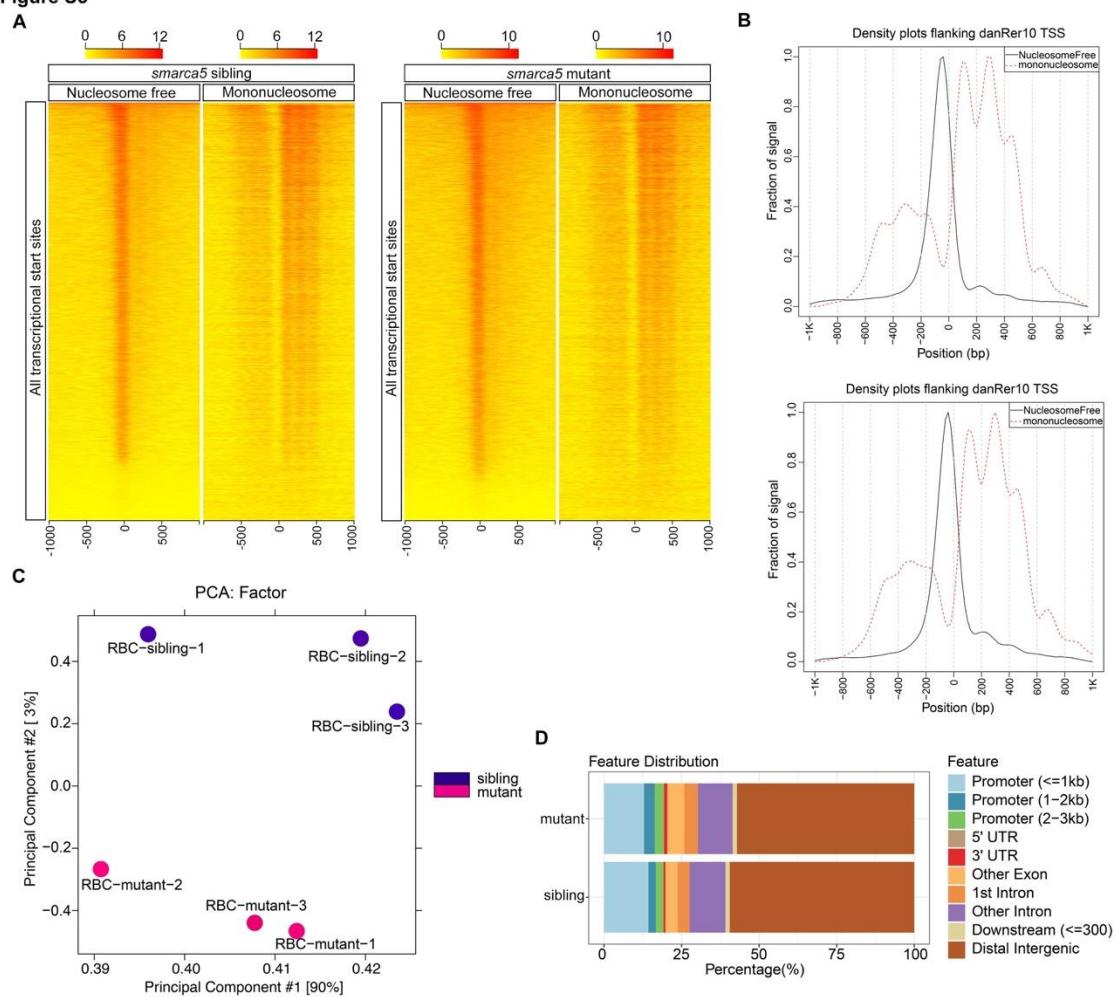
356

357 **Figure 5. Changes in chromatin accessibility in RBCs from *smarca5*^{zko1049a} and their siblings.**

358 (A) Venn plot showing the genes of sibling and mutant specific accessible chromatin regions at
359 promoter and distal regulator regions, respectively. (B) Motifs enriched in nucleosome free
360 regions (NFRs) with lost accessibility after *smarca5* deletion. (C) Venn plot showing the overlap
361 of genes with specific accessible chromatin regions and up-regulated expression in mutant (top)
362 and sibling (bottom) RBCs, respectively. Genes for ATAC-seq were assigned by differential
363 accessible regions. (D) Bar plot showing the selected sibling and mutant specific differential
364 expression genes. Fold change, \log_2 fold change.

365

Figure S5



366

367 **supplemental Figure 5. ATAC-seq analysis for RBCs in *smarca5*^{zko1049a} and their siblings.** (A)

368 Heatmaps showing density of mapped ATAC-seq reads from single biological replicates 1 kb up
 369 and downstream of transcriptional start sites (TSS) in danRer10. Separate heatmaps for fragments
 370 of nucleosome free (less than 100 bp) and mononucleosome (between 180 and 247 bp) are shown.
 371 (B) Density plots flanking danRer10 TSS from sibling and mutant RBCs for mapped reads shown
 372 in (A). (C) PCA plot of biological replicates using all ATAC-seq peaks. (D) Bar plot drew by
 373 ChIPseeker showing the feature distributions of mutant-ATAC-seq peaks and sibling-ATAC-seq
 374 peaks across the genome.

375

376 **keap1a acts as a downstream target of Smarca5 in RBC aggregation.**

377 Based on the screening results, the chromatin accessibility at *keap1a* promoters, which contains
378 Gata1 motif, was decreased in *smarca5*^{zko1049a} (Figure 6A). The transcription level of *keap1a*
379 detected by qPCR was also decreased in *smarca5*-deficient RBCs (Figure 6B). Given that *keap1*
380 was previously identified to correlate with human venous thrombosis (Akin-Bali, Eroglu, Ilk, Egin,
381 & Kankilic, 2020), we propose that *keap1a* may act as a downstream target of Smarca5 in RBCs.

382 Keap1-Nrf2 system is an evolutionarily conserved defense mechanism in oxidative stress (Itoh et
383 al., 1997; Itoh et al., 1999). In cytoplasm, Keap1 could anchor to Nrf2 to facilitate the Nrf2
384 degradation, while oxidative stress leads to the proteasomal degradation of Keap1 and release of
385 Nrf2 to the nucleus, thereafter activate the expression of oxidation defense factors. Both our
386 RNA-seq and qPCR analysis showed the downregulation of *keap1a* and as a downstream target of
387 Nrf2, *hmox1a* showed a markedly increase in gene expression upon *smarca5* deletion (Figure 6C),
388 suggesting the disruption of Keap1-Nrf2 signaling pathway. It is worthy of note that, although the
389 upregulated expression of Keap1-Nrf2 downstream targets can protect cells from oxidative
390 damage, the excessive activation of *hmox1a*, which catalyzes the degradation of heme to
391 biliverdin, carbon monoxide, and Fe²⁺, could even lead to the oxidative stress (Hassannia,
392 Vandenabeele, & Vanden Berghe, 2019). Thus, we propose that the unbalanced Keap1-Nrf2
393 signaling, especially the upregulation of *hmox1a*, could increase oxidative damage in
394 *smarca5*-deficient RBCs. We next performed functional validation of *keap1a* in *smarca5*^{zko1049a} by
395 overexpression of *hsp70:keap1a*-EGFP. Heat shock was performed at 24 hpf and 36 hpf, and the
396 phenotype was examined at 2 dpf. The results showed that overexpression of *keap1a* in
397 *smarca5*^{zko1049a} could partially rescue the blood clots phenotype (Figure 6D-E). In addition,
398 knockdown of *hmox1a*, the downstream target of Keap1-Nrf2, can also partially rescue the blood
399 clots phenotype in *smarca5*^{zko1049a} (Figure 6F-G), further supporting that the Keap1-Nrf2 signaling
400 pathway downstream of Smarca5 is essential for blood clot formation.

401 To further identify the conserved role of *SMARCA5* in mammalian erythrocyte homeostasis, we
402 used K562 cells (human erythroleukemic cells) to perform further analysis. Treatment of hemin
403 induced the hemoglobinization of most K562 cells, suggesting the efficient erythroid
404 differentiation (supplemental Figure 6A-B). We then knocked down *SMARCA5* in hemin-induced
405 K562 cells using *SMARCA5* short interfering RNA (siRNA) and the qPCR and western blot

406 analyses showed that both the RNA and protein levels of *SMARCA5* were decreased significantly
407 after si*SMARCA5*s (si*SMARCA5*-1, si*SMARCA5*-2 and si*SMARCA5*-3) transfection (supplemental
408 Figure 6C-D). In addition, the expression of *HMOX1* was obviously upregulated after *SMARCA5*
409 knockdown (supplemental Figure 6E), indicating the conserved role of *SMARCA5* in human
410 erythrocyte homeostasis.

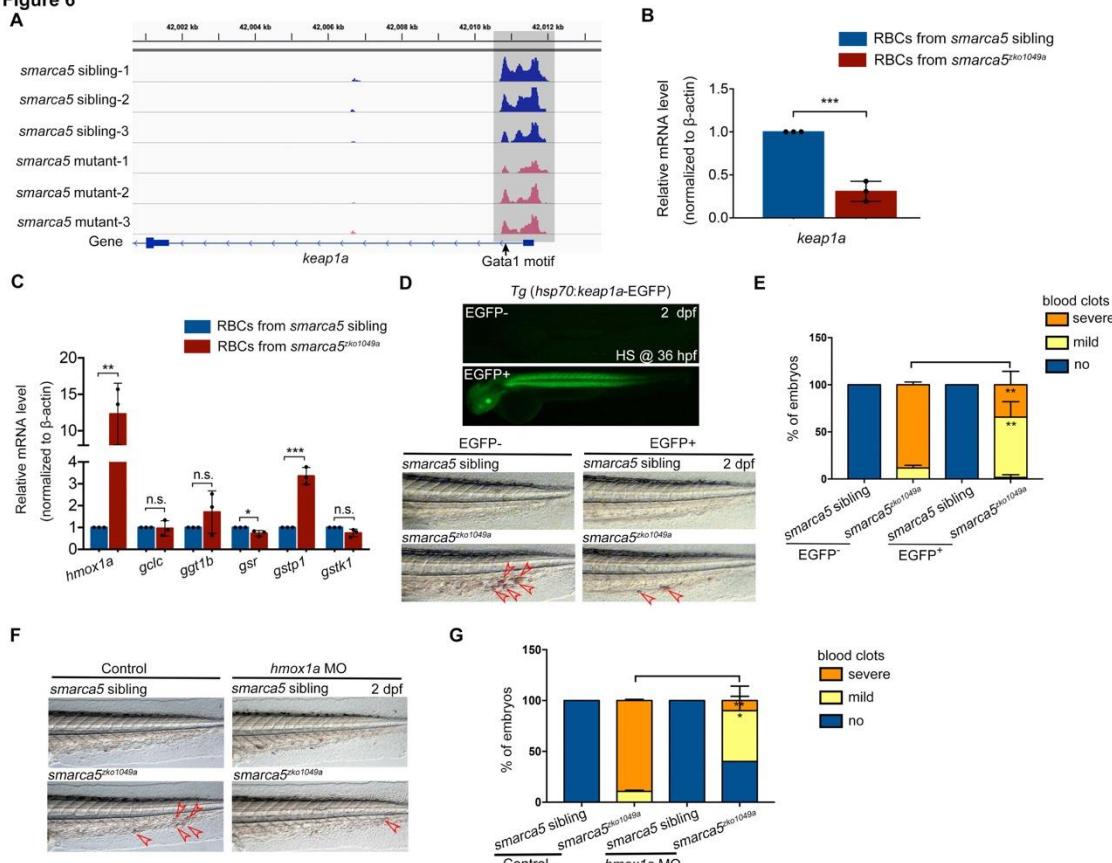
411 Considering the role of Keap1-Nrf2 signaling pathway in oxidative stress regulation, we further
412 asked whether the oxidative stress could be a trigger for blood clot formation in *smarca5* mutants.

413 Then, we used a free radical scavenger glutathione to determine the mechanisms of
414 *smarca5*-deficiency induced blood clots. We found that glutathione obviously prevented
415 thrombosis in *smarca5*^{zko1049a} (supplemental Figure 7A-B), implying that free radical generation
416 may play an important role in thrombosis in *smarca5*^{zko1049a}.

417 Taken together, loss of *smarca5* leads to the disruption of *keap1a* expression and excessive
418 activation of *hmox1a* in *smarca5*^{zko1049a}, which together contribute to the formation of blood clots.

419

Figure 6

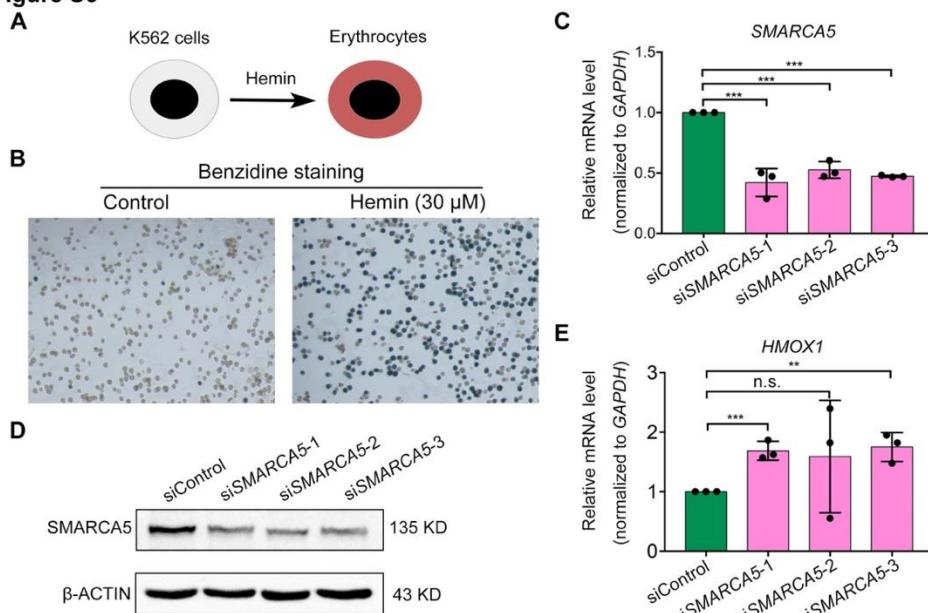


420

421 **Figure 6. Keap1- Nrf2 signaling pathway acts at downstream of Smarca5 in regulating RBC**
 422 **aggregation.** (A) The browser views showing the ATAC-seq peaks in *keap1a* promoter with in
 423 *smarca5^{ko1049a}* and their siblings. Gray box indicates the change of ATAC-seq peaks after *smarca5*
 424 deletion. The location of Gata1 motif at *keap1a* promoter is indicated by arrow. (B) qPCR analysis
 425 showing the expression of *keap1a* in RBCs from *smarca5^{ko1049a}* and their siblings at 2 dpf. (C)
 426 qPCR analysis showing the expression of *hoxox1a*, *gclc*, *ggt1b*, *gsr*, *gstp1* and *gstk1* in RBCs from
 427 *smarca5^{ko1049a}* and their siblings at 2 dpf. (D) The imaging of EGFP fluorescence in Tg
 428 (*hsp70:keap1a-EGFP*) embryos at 2 dpf. Heat shock was performed at 36 hpf. The bright-field of
 429 tail region in *smarca5^{ko1049a}* and their siblings, with or without Smarca5 overexpression at 2 dpf.
 430 (E) The quantification of blood clots phenotype in (D). (F) The bright-field of tail region in
 431 *smarca5^{ko1049a}* and their siblings, in control group and with *hoxox1a* MO injection. The blood clots
 432 are indicated by arrow heads. (G) The quantification of blood clots phenotype in (F). Data are
 433 mean \pm s.d. (B, C, E, G). Asterisk presents statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p <$
 434 0.001, n.s. not significant). P values were calculated by two-tailed unpaired Student's *t*-test.

435

Figure S6

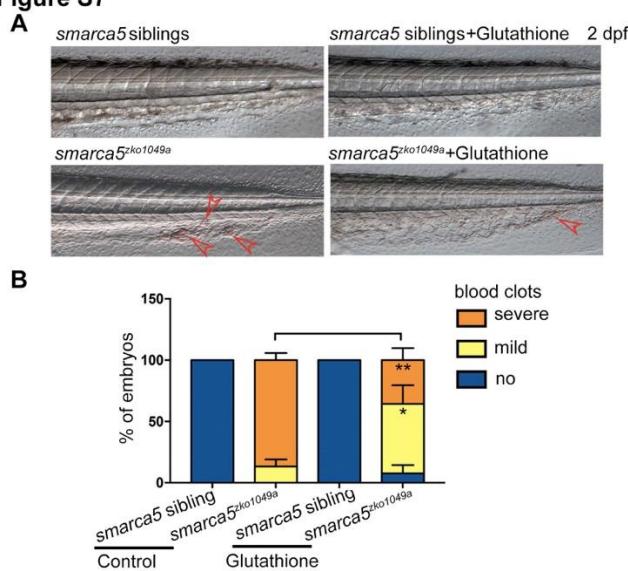


436

437 **supplemental Figure 6. The upregulation of *HMOX1* in hemin-induced K562 cells after**
438 **knockdown of *SMARCA5*.** (A) Schematic representation the induction of erythroid
439 differentiation of K562 cells using hemin. (B) Benzidine staining of K562 cells in control group
440 and with hemin treatment. (C) qPCR analysis showing the expression of *SMARCA5* in control
441 (siControl) and after *SMARCA5* knockdown (si*SMARCA5*-1, si*SMARCA5*-2, si*SMARCA5*-3). The
442 expression level of *SMARCA5* was normalized to *GAPDH*. (D) Western blot showing the protein
443 level of *SMARCA5* in control and after *SMARCA5* knockdown. (E) qPCR analysis showing the
444 expression of *HMOX1* in control and after *SMARCA5* knockdown. The expression level of
445 *HMOX1* was normalized to *GAPDH*. Data are mean \pm s.d. (C and E). Asterisk presents statistical
446 significance ($**p < 0.01$, $***p < 0.001$, n.s. not significant). *P* values were calculated by two-tailed
447 unpaired Student's *t*-test.

448

Figure S7



449

450 **supplemental Figure 7. The free radical generation may play a major role in RBC**
451 **aggregation in smarca5^{ko1049a}.** (A) The bright-field of tail region in smarca5^{ko1049a} at 2 dpf in
452 control group and with glutathione treatment. The blood clots are indicated by arrow heads. (B)
453 The quantification of blood clots phenotype in (A). Data are mean \pm s.d. (B). Asterisk presents
454 statistical significance ($*p < 0.05$, $**p < 0.01$). P values were calculated by two-tailed unpaired
455 Student's t -test.

456

457 **Discussion**

458 In this work, we develop a zebrafish thrombosis model with a deletion of an epigenetic
459 regulator-*smarca5*. The blood clots are formed in the CVP of *smarca5*^{zko1049a} and the erythrocytes
460 manifest disintegration of cristae in mitochondria. Further transcriptome and chromatin
461 accessibility analysis show that *keap1a* acts as a downstream target of Smarca5. Moreover, the
462 elevated expression of the downstream target of Keap1-Nrf2, *hmox1a*, leads to the aggregation of
463 *smarca5*-deficient RBCs. Together, these results demonstrate the protective role of Smarca5 in
464 regulating erythrocyte homeostasis and that the *smarca5* loss-of-function zebrafish mutant may
465 serve as a new thrombosis model to screen molecular drugs for clinical therapy.

466 Considering the conserved coagulation and anticoagulation signaling pathway, the zebrafish
467 model has been used to study the physiology of thrombosis (Hanumanthaiah, Day, &
468 Jagadeeswaran, 2002; Jagadeeswaran, Sheehan, Craig, & Troyer, 1999; Sheehan et al., 2001). The
469 ferric chloride and laser injury methods are widely used in zebrafish to generate thrombus in the
470 circulation (M. Gregory, Hanumanthaiah, & Jagadeeswaran, 2002). Phenylhydrazine-treated
471 zebrafish also develop thrombosis in the caudal vein (Zhu et al., 2016). Moreover, zebrafish is an
472 ideal model to explore novel players in thrombosis based on genetic manipulation. For example,
473 mutation of *anti-thrombin III* gene in zebrafish can mimic disseminate intravascular coagulation
474 (Liu et al., 2014). miR-126 was identified as a regulator of thrombi generation in zebrafish
475 (Zapilko et al., 2020). Importantly, the transparency of zebrafish embryo makes it feasible to
476 image the kinetics of thrombus formation. In our study, the *gata1*:dsRed labelled RBCs were
477 imaged during blood clot formation. Thus, the zebrafish thrombosis model is a great asset for
478 exploring the underlying mechanisms in thrombosis formation.

479 Unlike Brg1, which is essential for mouse erythrocyte development by regulating globin gene
480 expression (Bultman et al., 2005; Griffin et al., 2008), Smarca5 is required for primitive
481 erythrocyte homeostasis at the erythrocyte differentiation stage. Deletion of *smarca5* does not lead
482 to the gross changes in RBC morphology and viability, but specifically results in the RBC
483 aggregation phenotype. The mechanistic details for different chromatin remodelers functioning in
484 the different processes during erythropoiesis warrant further investigation.

485 Previous evidence suggests that chromatin remodeler NuRD is required to maintain lineage
486 fidelity during erythroid-megakaryocyte ontogeny (Gao et al., 2010; G. D. Gregory et al., 2010).

487 Our results show that, despite the normal lineage choice for primitive erythrocytes in
488 *smarca5*^{zko1049a}, the aberrant activation of myeloid genes occurred in RBCs after *smarca5* deletion.
489 The exquisite cell lineage control by *smarca5* may be due to the regulation of SMARCA5 at the
490 enhancer of PU.1 (Dluhosova et al., 2014).

491 Besides the conserved role of Keap1-Nrf2 system in oxidative stress, Keap1-Nrf2 is also
492 demonstrated to act as a regulator in cell development and differentiation across multiple tissues
493 and cell types. For instance, Keap1-Nrf2 signaling pathway is indispensable for hematopoietic
494 stem cell (HSC) lineage commitment in mice (Murakami, Shimizu, Romeo, Yamamoto, &
495 Motohashi, 2014). Knockout of *Keap1* in HSCs showed enhanced granulocyte-monocyte
496 differentiation ability at the expense of lymphoid and erythrocyte differentiation. And the
497 expression level of erythrocyte and lymphoid genes was decreased in *Keap1*-deficient HSCs.
498 Importantly, the abundance of Hmox1 is upregulated during erythrocyte differentiation, and
499 Hmox1 expression must be tightly regulated at appropriate level for efficient erythropoiesis
500 (Garcia-Santos et al., 2014). Overexpression of Hmox1 impairs hemoglobin synthesis, while lack
501 of Hmox1 leads to the enhancement of hemoglobinization. Here, we show that the disruption of
502 *keap1a* expression and excessive activation of *hmox1a* in *smarca5*^{zko1049a} contribute to RBC
503 aggregation. Besides the free radical generation, which may play an important role in RBC
504 aggregation in *smarca5*^{zko1049a}, we cannot rule out other possibilities that maybe also involved in
505 the observed phenotype, such as the regulation of Keap1-Nrf2 signaling pathway in erythrocyte
506 gene expression.

507 In summary, we have demonstrated, for the first time, that deletion of *smarca5* in zebrafish leads
508 to the formation of blood clots that mimics venous thrombosis by regulating the Keap1-Nrf2
509 signaling pathway in RBCs. These findings raise the possibility that zebrafish *smarca5* mutant
510 may serve as a new venous thrombosis model for drug screening and pre-clinical therapeutic
511 assessment.

512

513 **Methods**

514 **Zebrafish strains**

515 Zebrafish strains including Tubingen, Tg (*CD41*:GFP) (Lin et al., 2005), Tg (*gata1*:dsRed)
516 (Traver et al., 2003), Tg (*kdrl*:mCherry) (Bertrand et al., 2010), Tg (*mpo*:GFP) (Renshaw et al.,
517 2006), Tg (*coro1a*:GFP) (L. Li, Yan, Shi, Zhang, & Wen, 2012), *smarca5*^{zko1049a} heterozygous
518 mutants (Ding et al., 2021) were raised under standard conditions (28.5°C in system water). The
519 zebrafish embryos were raised in incubator at 28.5°C. The present study was approved by the
520 Ethical Review Committee of the Institute of Zoology, Chinese Academy of Sciences, China.

521

522 **Whole mount *in situ* hybridization (WISH)**

523 WISH was performed as previously described (Wang et al., 2011). The Digoxigenin-labelled RNA
524 probe genes including *gata1*, *ikaros*, *scl*, *pu.1*, and *lyz* were cloned from zebrafish cDNA and
525 ligated to the T-vector, then *in vitro* transcribed using T7 or SP6 polymerase.

526

527 **Quantitative PCR (qPCR)**

528 Total RNAs were extracted from *smarca5*^{zko1049a} and their sibling embryos using TRIzol reagent
529 (Life technologies, 15596018) or from sorted RBCs using QIAGEN RNeasy Mini Kit (Cat. No.
530 74104). The cDNA was reverse transcribed using M-MLV Reverse Transcriptase (Promega,
531 M1701). The detailed primers used for qPCR are listed in Supplementary file 1A.

532

533 **Morpholinos (MOs)**

534 The antisense MOs were purchased from GeneTools. The sequences of MOs were used as
535 previous described, these gene-specific MOs include *hmx1a* MO and *pu.1* MO. The detailed
536 sequence and dosage used in this work are listed in Supplementary file 1B.

537

538 **Parabiosis experiment**

539 Parabiosis experiment was performed by following the previous published procedures (Demy et
540 al., 2013; Hagedorn et al., 2016). Briefly, *smarca5*^{zko1049a} and their sibling embryos between the
541 128-cell blastula and 30% epiboly stages were removed out of chorions and gently transferred into
542 methylcellulose drop under fish water. Then, detach a few cells from each embryo at the contact

543 points using the pulled glass micropipette and move these two embryos contact each other
544 properly until they fusion together.

545

546 **O-dianisidine staining, Giemsa-staining and Benzidine staining**

547 *smarca5^{zko1049a}* and their sibling embryos at 2 dpf were stained with o-dianisidine staining solution
548 for 15 min in the dark as previously described (Detrich et al., 1995). The blood cells from
549 *smarca5^{zko1049a}* and their sibling embryos at 2 dpf were collected from heart and caudal vein and
550 attached to slides. The dried slides will be stained by Fast Giemsa Stain (Yeasen Biotech Co., Ltd,
551 CAT: 40751ES02) following the standard manufacturer's instructions. The K562 cells were
552 collected and washed once using PBS. Then the cells were suspended using 500 μ l PBS.
553 Subsequently, add 10 μ l 0.4% benzidine, 1 μ l 30% H₂O₂, and 1 μ l 5% sodium nitroferricyanide
554 dihydrate and incubate for 3 min, 5 min and 3 min, respectively. Then the cells were attached to
555 slides for further imaging.

556

557 **Chemical treatment**

558 Argatroban (Sigma, A0487), dissolved in DMSO (2 mg/ml), was injected into *smarca5^{zko1049a}* and
559 their sibling embryos at 36 hpf at the dosage of 4 nl/embryo. The control embryos were injected
560 with DMSO alone at the same dosage. Heparin (Sigma, H3393), dissolved in H₂O (2.5 mg/ml),
561 was injected into *smarca5^{zko1049a}* and their sibling embryos at 36 hpf at the dosage of 4 nl/embryo.
562 For aspirin treatment, the *smarca5^{zko1049a}* and sibling embryos at 36 hpf were incubated with
563 aspirin (Sigma, A2093) at the concentration of 5 μ g/ml. The *smarca5^{zko1049a}* and sibling embryos
564 at 36 hpf were incubated with Glutathione (Sigma, PHR1359) at the concentration of 0.5 mg/ml.

565

566 **Confocal microscopy**

567 Confocal microscopy was performed using Nikon confocal A1 laser microscope (Nikon) and
568 Andor high speed confocal (dragonfly, Belfast, UK). The embryos were embedded in 1.2% low
569 melting agarose.

570

571 **Generation of transgenic zebrafish**

572 For overexpression experiment, the full length CDS of *keap1a* was cloned into pDestTol2pA2

573 with a *hsp70* promoter and an EGFP reporter by DNA assembly (NEBuilder HiFi DNA Assembly
574 Master Mix, E2621S). The plasmids together with *tol2* mRNA were injected into zebrafish
575 embryos at 1-cell stage to generate Tg (*hsp70:flag-keap1a-EGFP*).

576

577 **Short interfering RNAs (siRNAs) and RNA interference**

578 Control and *SMARCA5* siRNAs were synthesized by GenePharma Corporation. The K562 cells
579 were maintained in RPMI-1640 medium supplemented with 10% FBS and stimulated with hemin
580 (Sigma, 51280, 30 µM) for 3 days to induce erythroid differentiation. Then, the hemin-induced
581 K562 cells were transfected with siRNAs using Lipofectamine RNAiMAX Reagent (Invitrogen,
582 13778-030) following the manufacturer's instructions. The detailed sequences are listed in
583 Supplementary file 1C.

584

585 **Western blotting**

586 The western blotting was performed to detect the protein level of SMARCA5 in K562 cells after
587 siRNA transfection. The antibodies used were as followings: anti-Smarca5 antibody (Santa Cruz,
588 H-300: sc-13054), anti-β-Actin antibody (Cell Signaling Technology, 4967).

589

590 **Flow cytometry**

591 The *smarca5^{zko1049a}* and their sibling embryos with Tg (*gata1:dsRed*) background at 2 dpf were
592 collected and washed by Ringers buffer. After digesting into single cell suspension using 0.5%
593 trypsin, the reaction was stopped by adding CaCl₂ up to 1 M and fetal calf serum up to 10%. Then
594 the cells were filtered through 300 Mesh nylon cell-strainer to make single cell suspension. The
595 RBCs (*gata1:dsRed*⁺) were sorted using MoFlo XDP (Beckman Coulter) and collected into PBS
596 containing 1% FBS.

597

598 **RNA-seq**

599 RNA-seq was performed in FACS-purified RBCs from *smarca5^{zko1049a}* and their siblings at 2 dpf.
600 50,000 RBCs were used per sample for RNA-seq experiments. The RNAs of sorted HSPCs were
601 isolated using QIAGEN RNeasy Mini Kit (Cat. No. 74104) following the standard manufacturer's
602 instructions. The mRNA libraries were constructed using NEBNext Ultra RNA Library Prep Kit

603 for Illumina and sequenced under Illumina HiSeq X Ten with pair end 150bp (PE150).

604

605 **Processing of RNA-seq analysis**

606 Raw RNA-seq reads data were trimmed using the fastp (Chen, Zhou, Chen, & Gu, 2018) (v2.4)
607 (parameter: with default parameters), and aligned to “*Danio rerio* GRCz10” cDNA reference
608 sequence using the STAR (Dobin et al., 2013) (v 2.7.7a) with the default parameters. Read counts
609 for each gene were quantified as the total number of reads mapping to exons using featureCounts
610 (Liao, Smyth, & Shi, 2014) (subread v1.5.3). DESeq2 (Love, Huber, & Anders, 2014) was applied
611 to perform differential expression analysis with raw counts quantified by featureCounts. We used
612 Benjamini-Hochberg adjusted P-value < 0.05 and log2 fold change > 1 as the threshold for
613 significant difference. Gene set enrichment analysis was performed using *GSEA* function in the
614 clusterProfiler (Yu, Wang, Han, & He, 2012) package (v 3.18.0). Gene set variation analysis was
615 performed by the GSVA (Hanzelmann, Castelo, & Guinney, 2013) package (v 1.38.0). The gene
616 sets we used were exported by the msigdbr package (v 7.2.1). The differences in pathway
617 activities scored between *smarca5*^{zko1049a} and their sibling RBCs were calculated with limma
618 (Ritchie et al., 2015) package (v 3.46.0).

619

620 **Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq)**

621 ATAC-seq was performed in FACS-purified RBCs from *smarca5*^{zko1049a} and their siblings at 2 dpf.
622 50,000 RBCs were used per sample for ATAC-seq library preparation using TruePrepTM DNA
623 Library Prep Kit V2 for Illumina (Vazyme, TD501) as previously described (Ding et al., 2021).
624 Firstly, wash the sorted RBCs using 1xPBST. Then, the cell pellet was lysed using 50 µl cold lysis
625 buffer (10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM MgCl₂ and 0.15% NP-40) for 5 min on ice.
626 Centrifuge and discard the supernatant to get the cell pellet (about 2 µl). Then the transposition
627 reaction system combining 5xTTBL (10 µl), TTE Mix (5 µl) and H₂O (33 µl) was added
628 immediately to the cell pellet and pipetted up and down gently for several times. After the
629 incubation at 37°C for 30 min, the DNA was extracted with chloroform-phenol. After the
630 purification, the DNA was amplified using TruePrepTM DNA Index Kit V2 for Illumina (Vazyme,
631 TD202). After the fragments length purification using VAHTSTM DNA Clean Beads (Vazyme,
632 N411), The DNA libraries are under sequencing under Illumina NovaSeq with pair end 150bp

633 (PE150).

634

635 **Processing of ATAC-seq analysis**

636 Raw ATAC-seq reads were trimmed using cutadapt (v 2.4) (parameter: -q 20 -m 20) and mapped
637 to the danRer10 reference genome using Bowtie2 (Langmead & Salzberg, 2012) (v 2.3.4.2)
638 (default parameters). Sorting, removal of PCR duplicates and conversion from SAM to BAM files
639 were performed using SAMtools (H. Li et al., 2009) (v 1.3.1). For quality assessment of
640 ATAC-seq libraries, we applied an R package ATACseqQC (Ou et al., 2018) (v 1.6.4) to check the
641 fragment size distributions, Transcription Start Site (TSS) enrichment scores, and plot heatmaps
642 for nucleosome positions. We employed deepTools2 (Ramirez et al., 2016) (v 2.5.7) to check the
643 reproducibility of the biological replicates and generated bigwig files from BAM output to
644 visualize mapped reads. Peaks were called using MACS2 (Zhang et al., 2008) (v2.1.2) (parameter:
645 --nomodel --nolambda --gsize 1.4e9 --keep-dup all --slocal 10000). Differentially accessible
646 regions were identified using an R package DiffBind (Ross-Innes et al., 2012) (v 2.10.0) with a
647 log₂ fold change threshold of 0.5, and Benjamini-Hochberg adjusted P-value < 0.1. Peak
648 annotation was performed by an R package ChIPseeker (Yu, Wang, & He, 2015) (v 1.18.0). We
649 identified the enriched de novo motifs across the whole genomic regions using the
650 findMotifsGenome.pl function of HOMER (Heinz et al., 2010) (parameter: -size 500 -len 8,10,12
651 -mask -dumpFasta).

652

653 **Transmission electron microscopy**

654 The tail region of *smarca5*^{zko1049a} and their siblings at 2 dpf were fixed with 2.5% (vol/vol)
655 glutaraldehyde and 2% paraformaldehyde in phosphate buffer (PB) (0.1 M, pH 7.4). After
656 washing with PB for four times, the tissues were immersed in 1% (wt/vol) OsO₄ and 1.5% (wt/vol)
657 potassium ferricyanide aqueous solution at 4°C for 1 hour. After washing, the tissues were
658 incubated in filtered 1% thiocarbohydrazide (TCH) aqueous solution (Sigma-Aldrich) at room
659 temperature for 30 min, followed by 1% unbuffered OsO₄ aqueous solution at 4°C for 1 hour and
660 1% UA aqueous solution at room temperature for 2 hours. The tissues were dehydrated through
661 graded alcohol (30%, 50%, 70%, 80%, 90%, 100%, 100%, 10 min each, at 4°C). Then, transfer
662 the tissues into pure acetone for 10 min (twice). Tissues were infiltrated in graded mixtures of

663 acetone and SPI-PON812 resin (21 ml SPI-PON812, 13 ml DDSA and 11ml NMA) (3:1, 1:1, 1:3),
664 then transfer the tissues into pure resin. Finally, the tissues were embedded in pure resin with 1.5%
665 BDMA and polymerized at 45°C for 12 hours, followed by at 60°C for 48 hours. The ultrathin
666 sections (70 nm thick) were sectioned with microtome (Leica EM UC6), and examined by a
667 transmission electron microscope (FEI Tecnai Spirit120kV).

668

669 **Image Analysis**

670 Raw image data were processed using ImageJ, photoshop CC 2018 and Adobe Illustrator CC
671 2018.

672

673 **Statistical Analysis**

674 All of the statistical analysis was performed for at least three independent biological repeats.
675 GraphPad Prism 6 was used to analyze the data. Data are mean \pm s.d. *P* values calculated by
676 two-tailed unpaired Student's *t*-test were used to indicate the significance if not clarified in figure
677 legends.

678

679 **DATA AVAILABILITY**

680 The accession number for the sequencing raw data in this paper is BioProject: PRJNA716463.
681 Source data of supplemental Figure 6D was provided, including the original files of the full raw
682 unedited blots (supplemental Figure 6-source data 2 and 4) and figures with the uncropped blots
683 with the relevant bands clearly labelled (supplemental Figure 6-source data 1 and 3).

684

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693

694 **Authorship Contributions**

695 Y.D. performed most of the experiments; Y.L. performed the bioinformatics analysis; Y.D., Y.L.,
696 Q.Z., and F.L. conceived the project, analyzed the data, and wrote the paper. All authors read and
697 approved the final manuscript.

698

699 **Conflict of Interest Disclosures**

700 The authors declare no competing interests.

701

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892

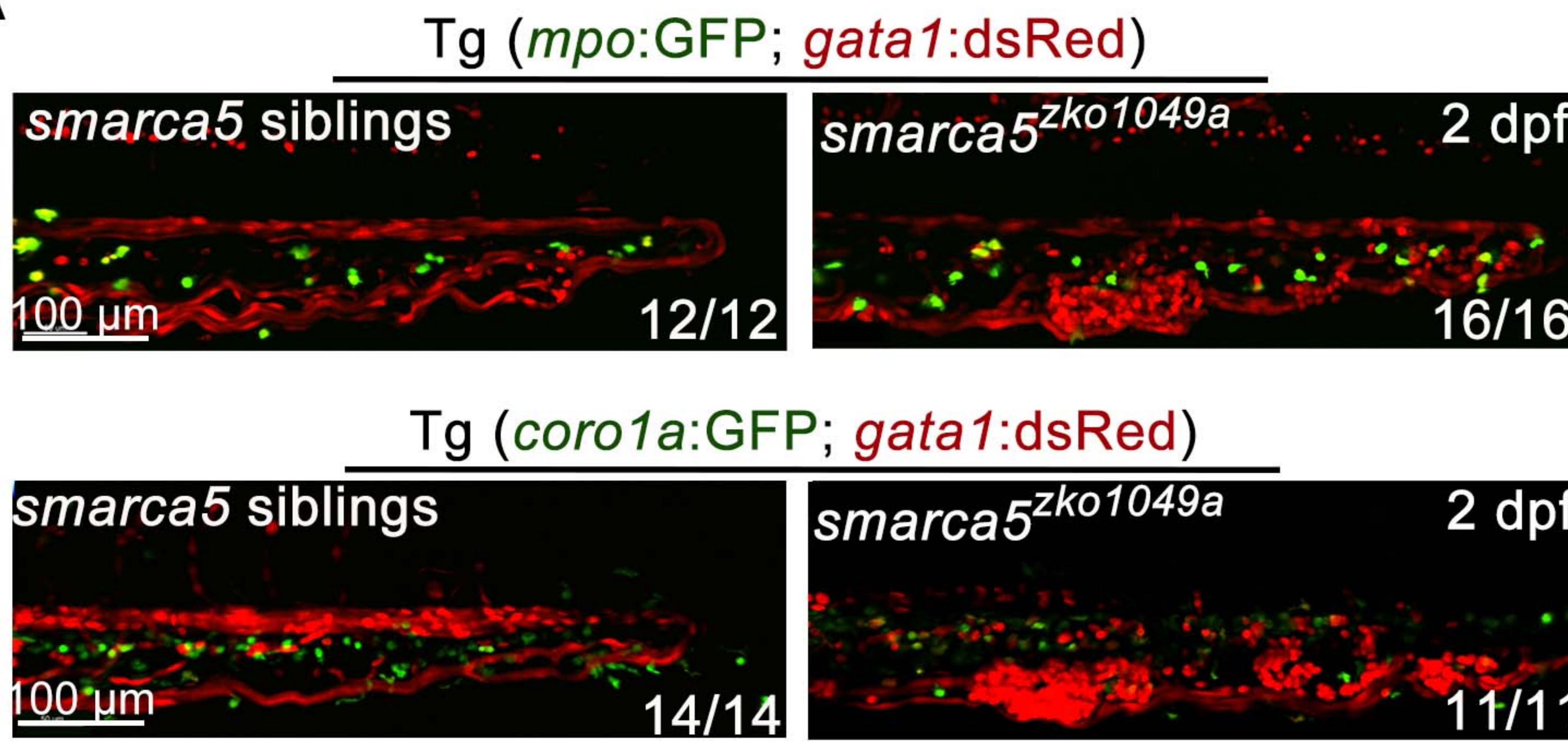
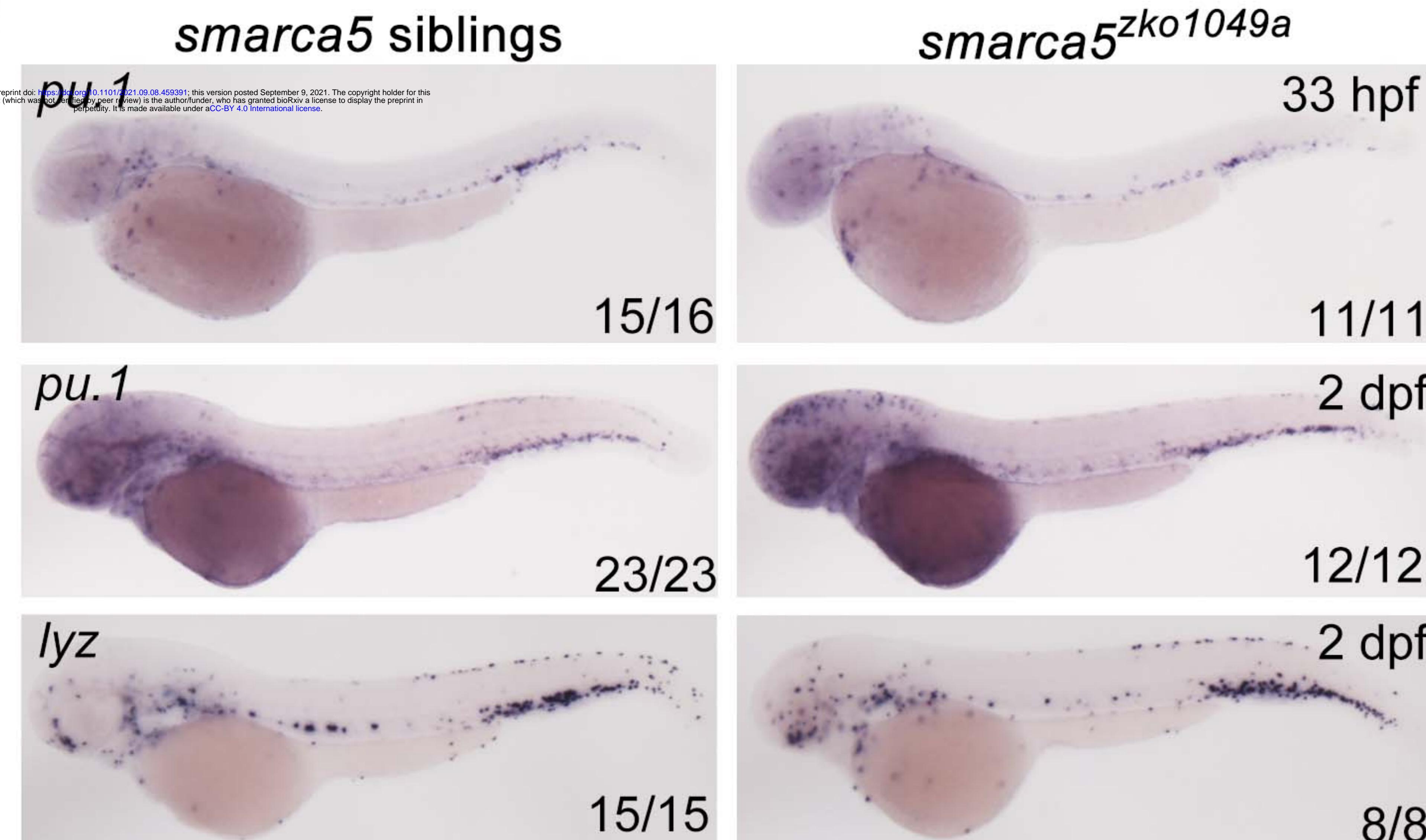
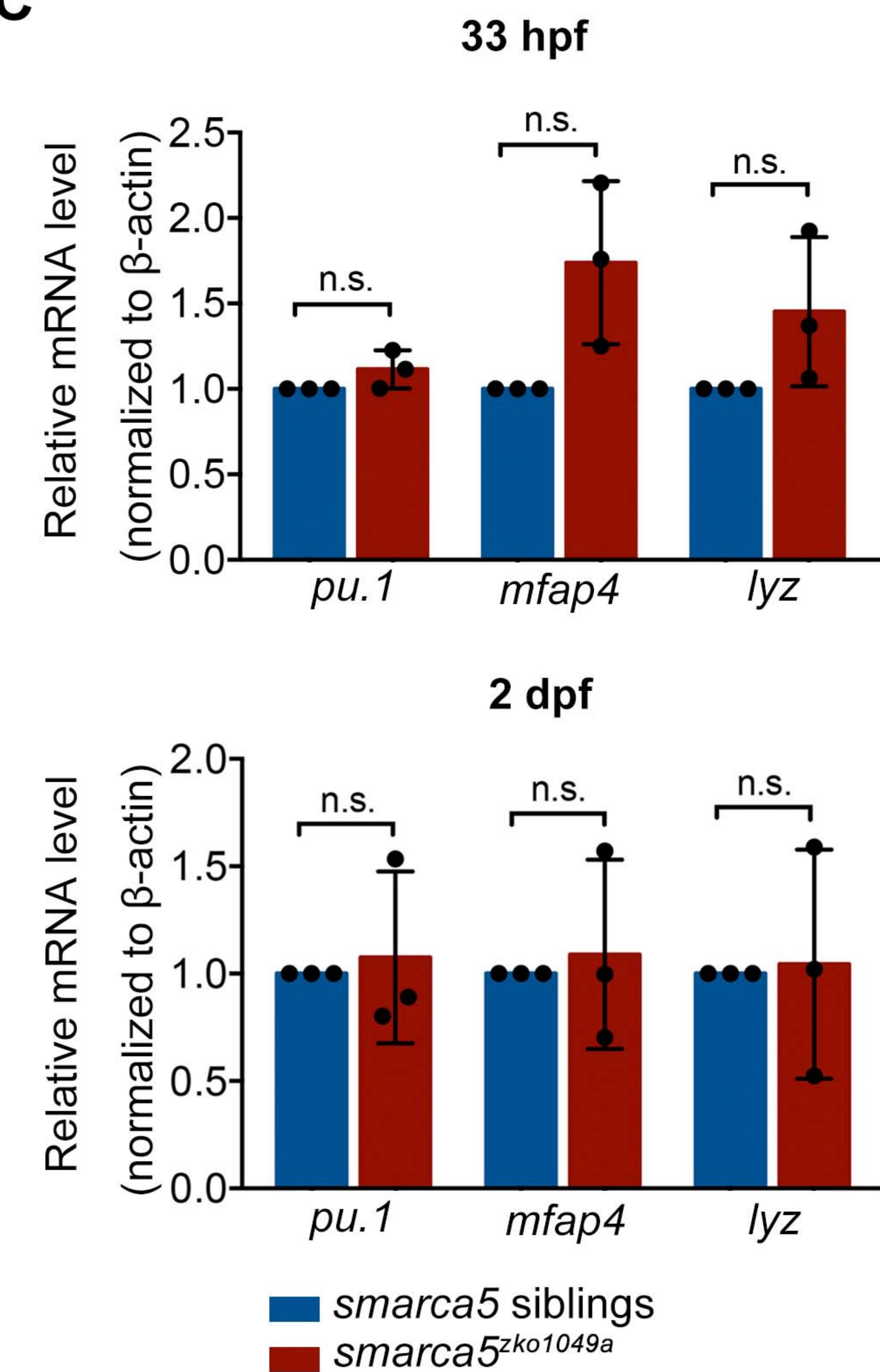
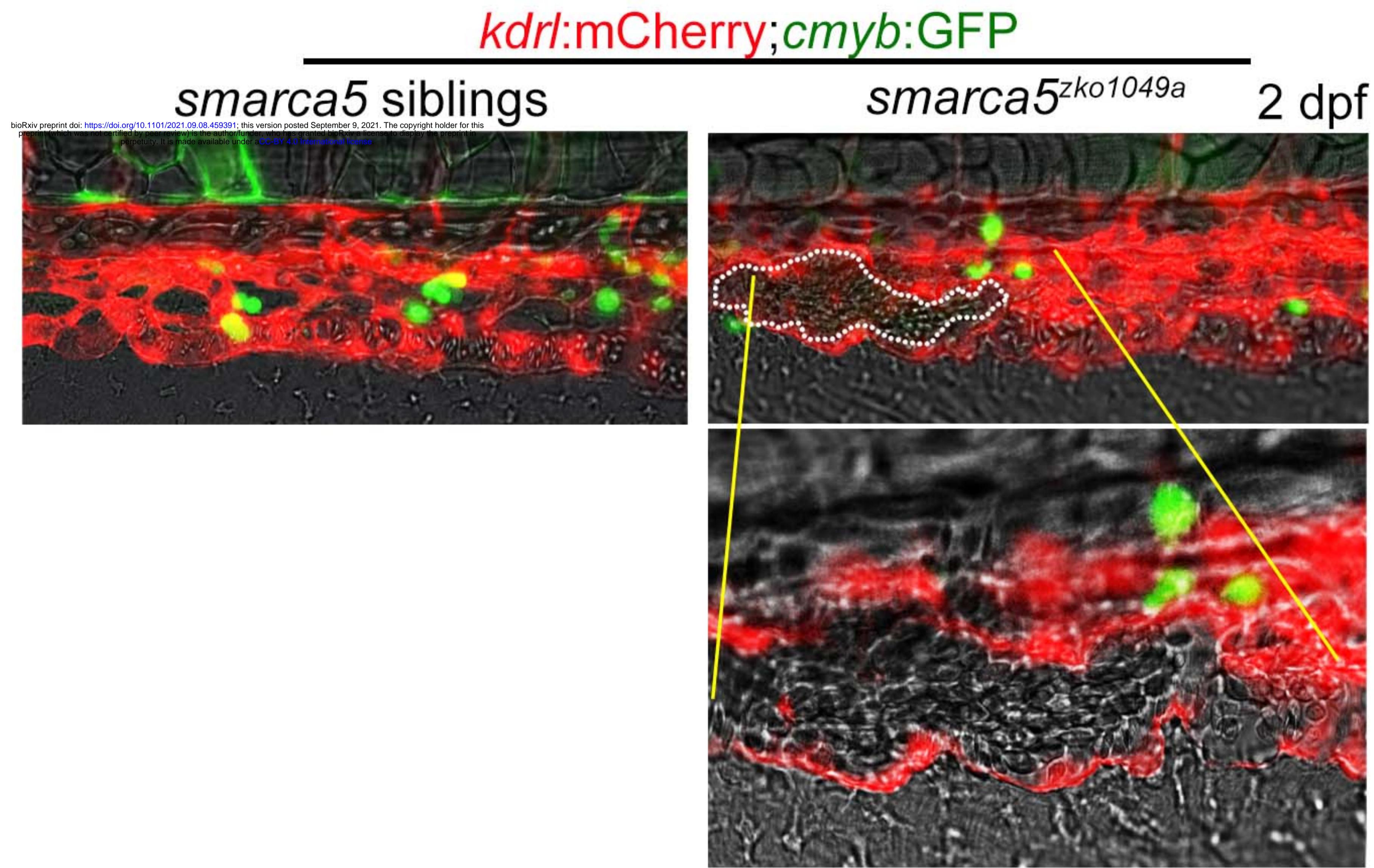
Figure S1**A****B****C**

Figure S2

A



B

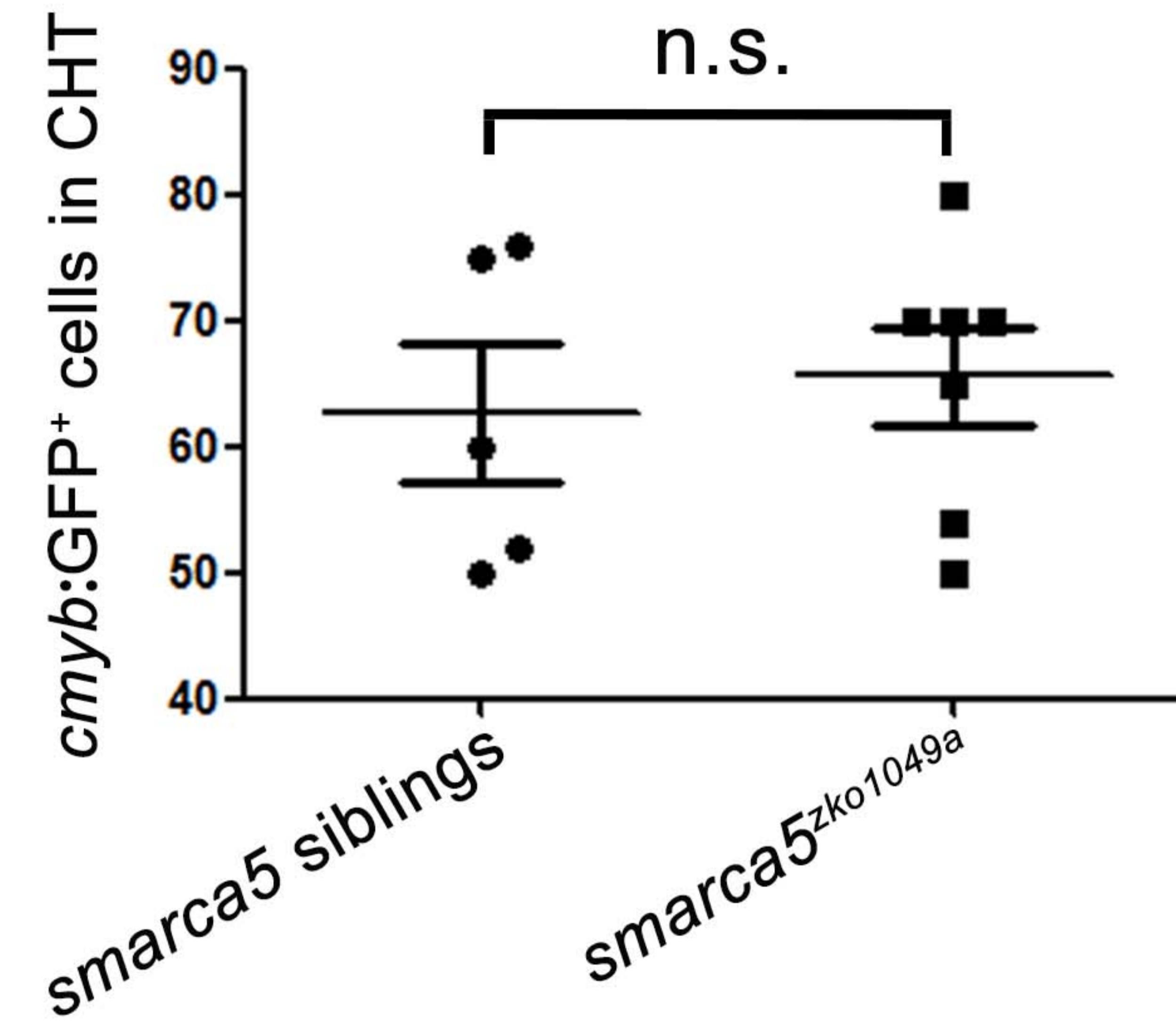


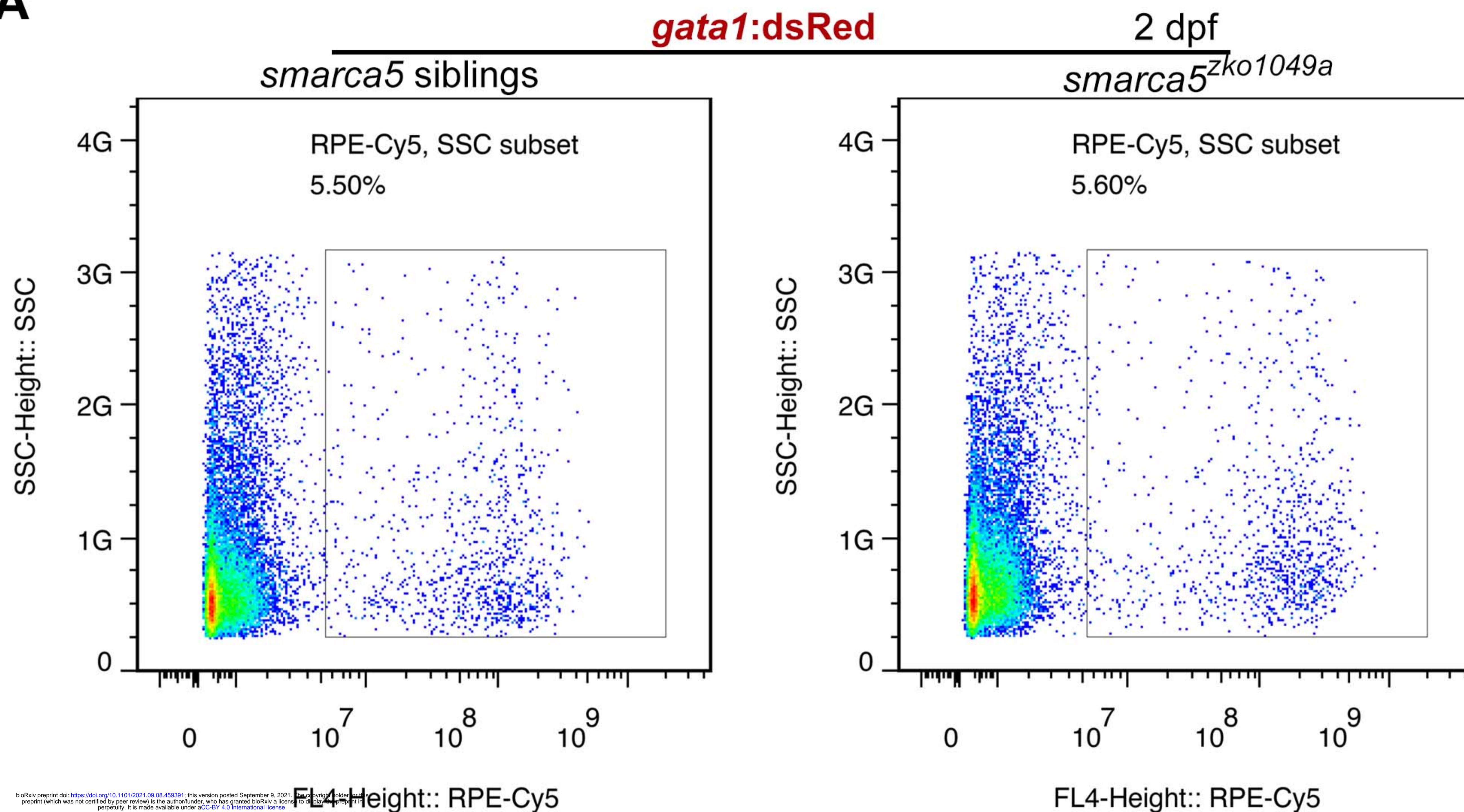
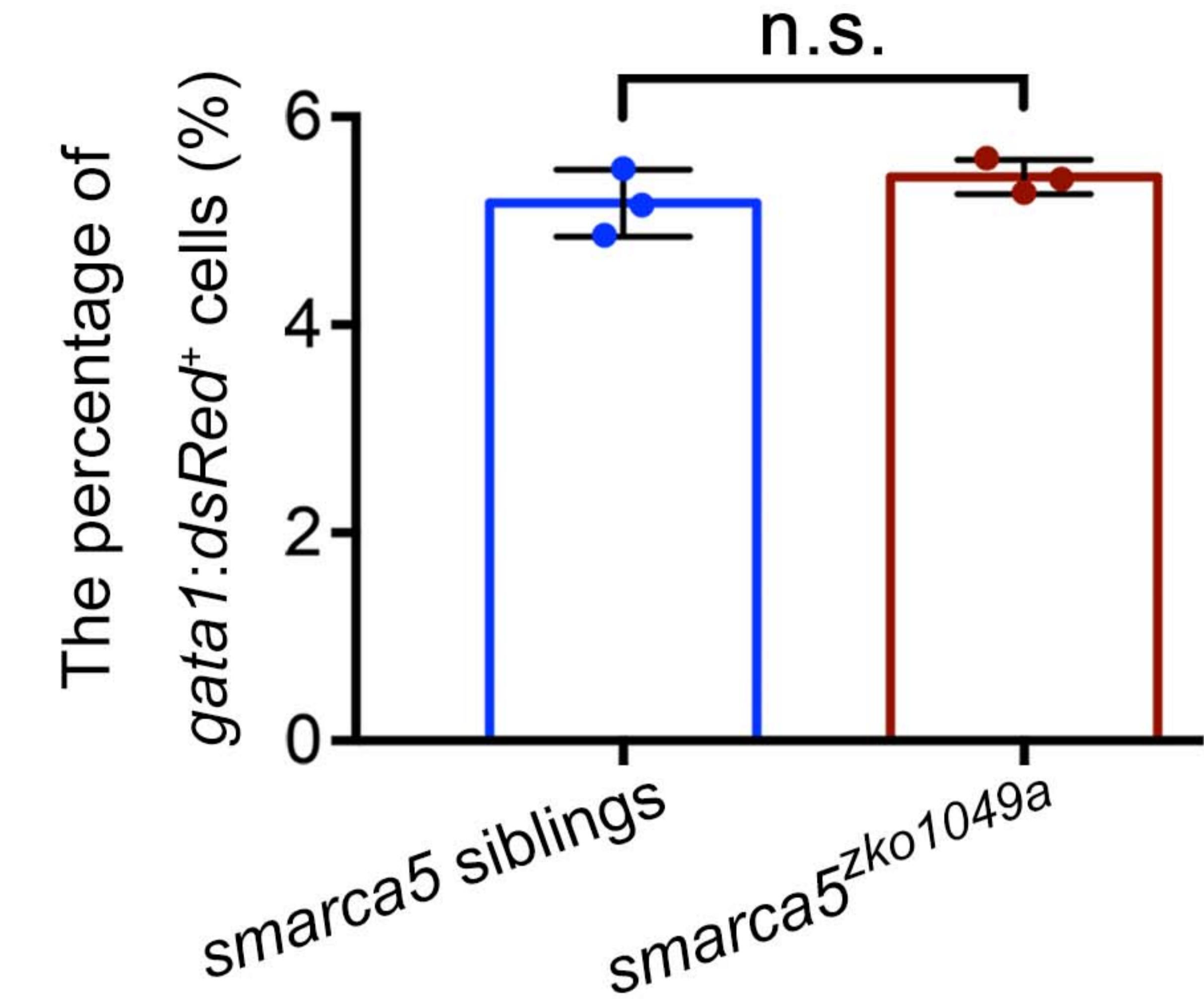
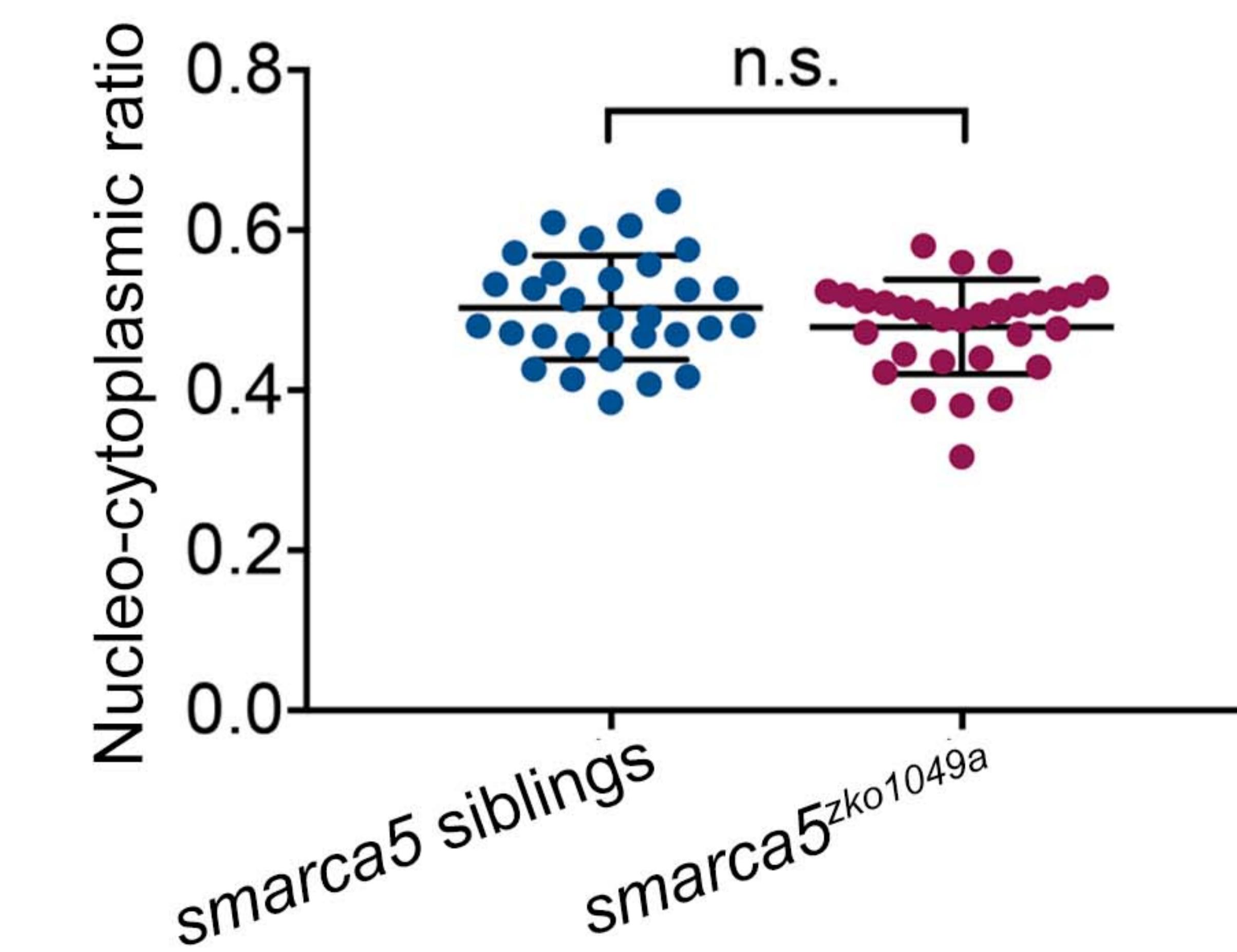
Figure S3**A****B****C****D**

Figure S4

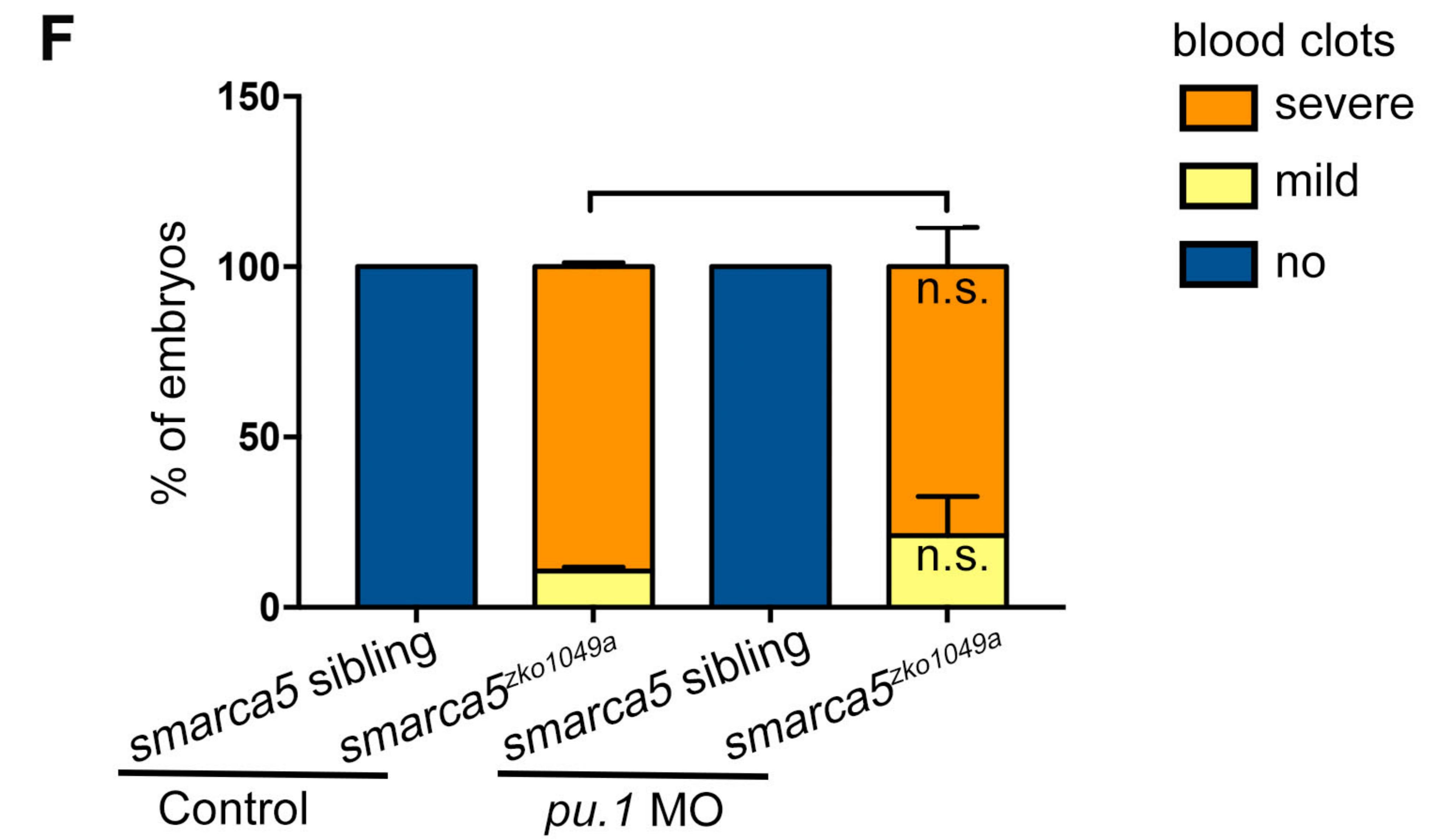
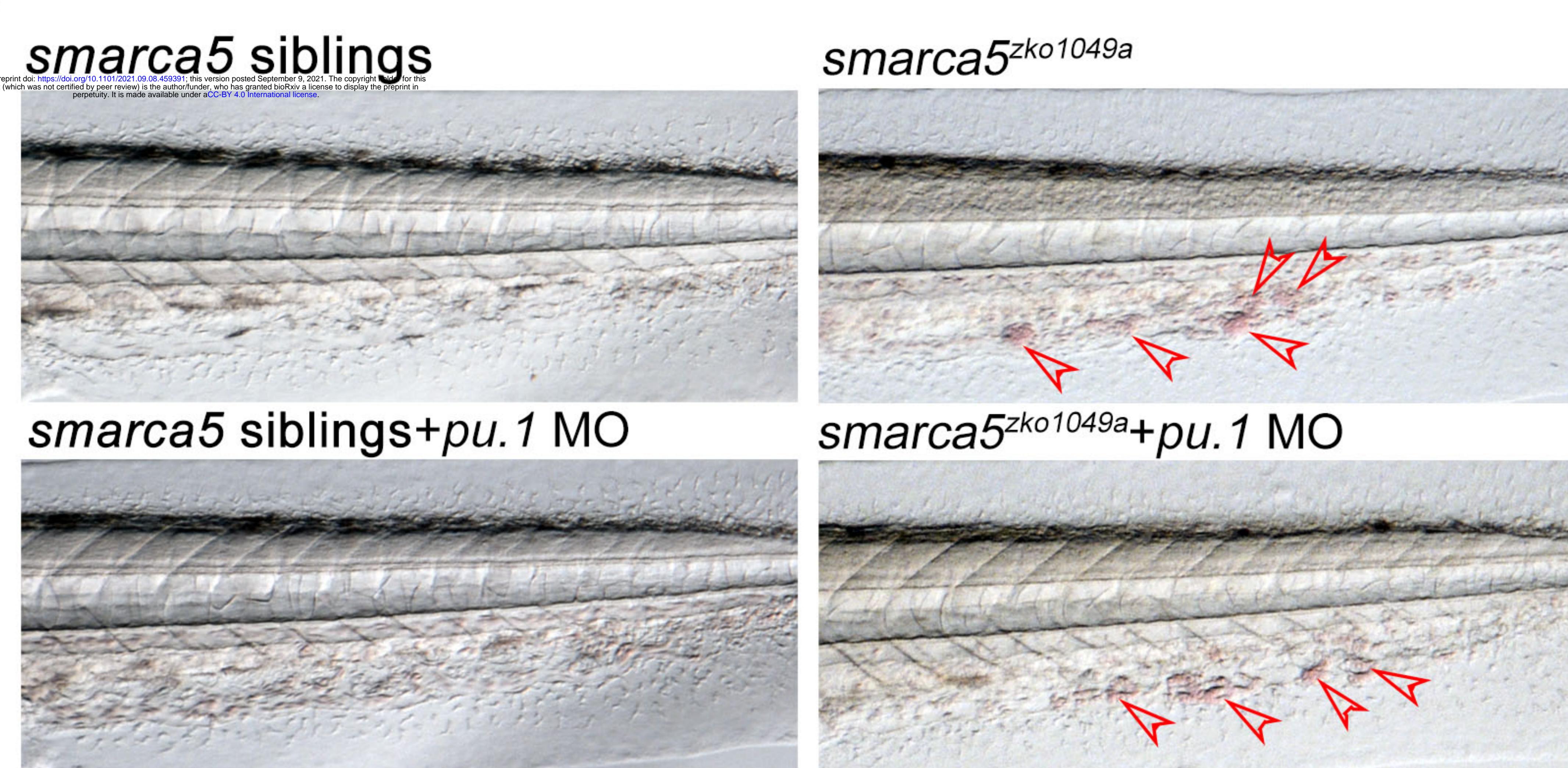
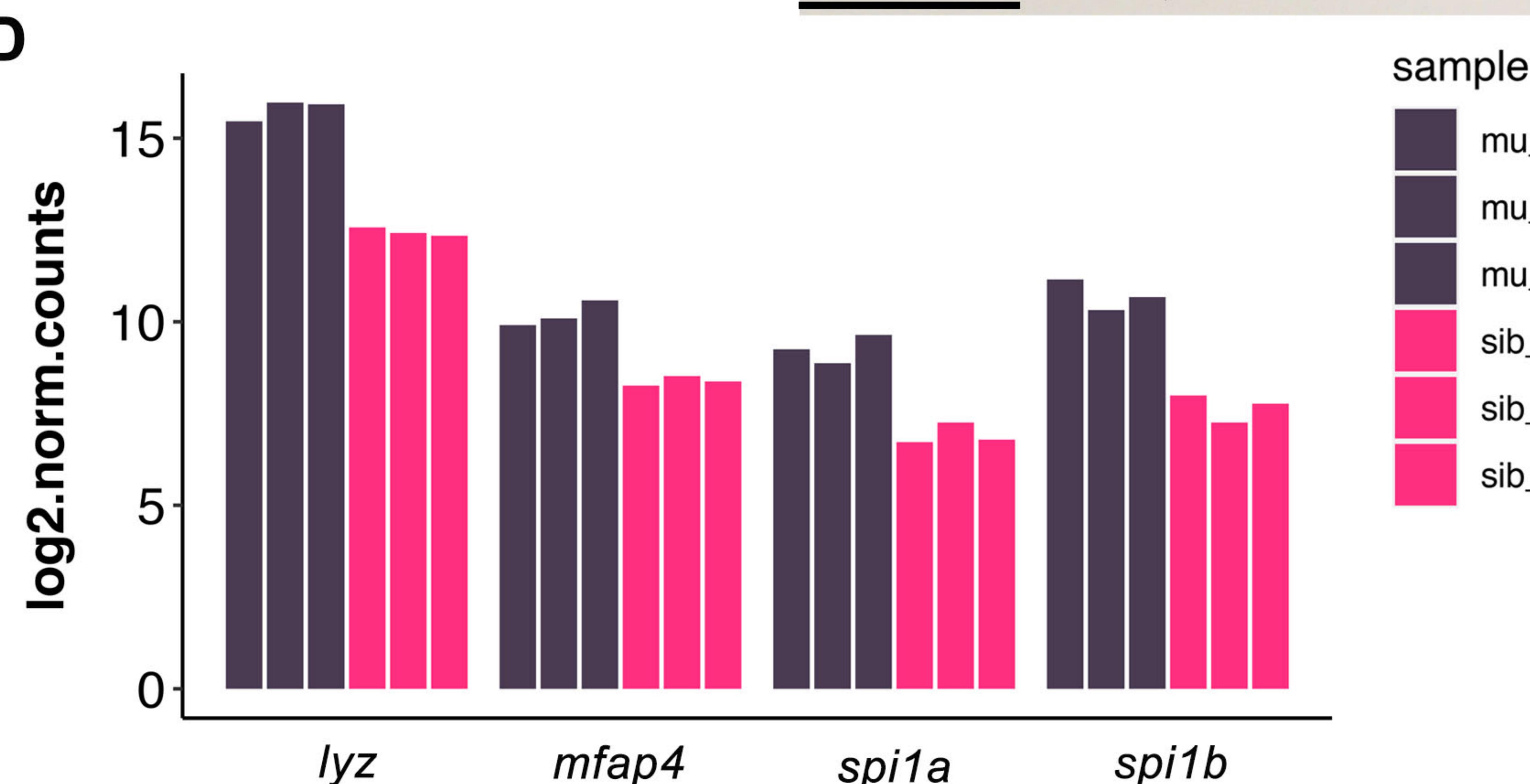
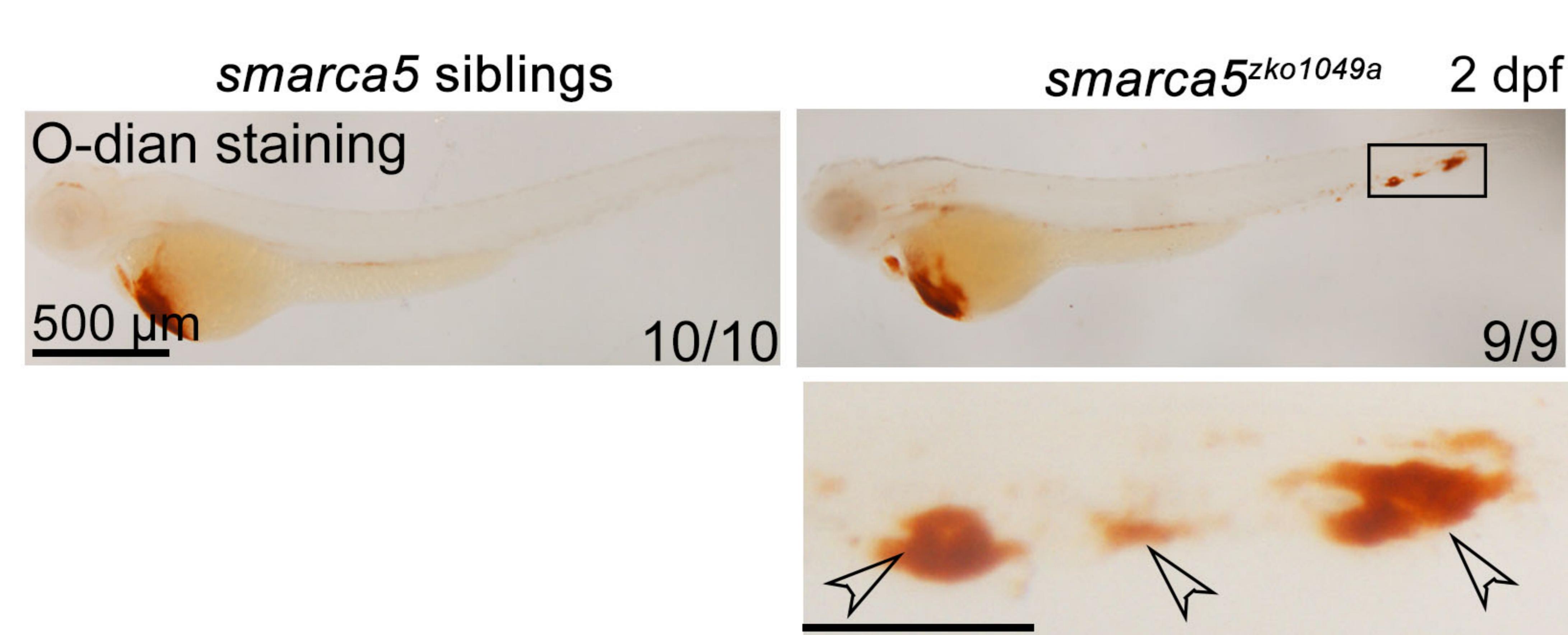
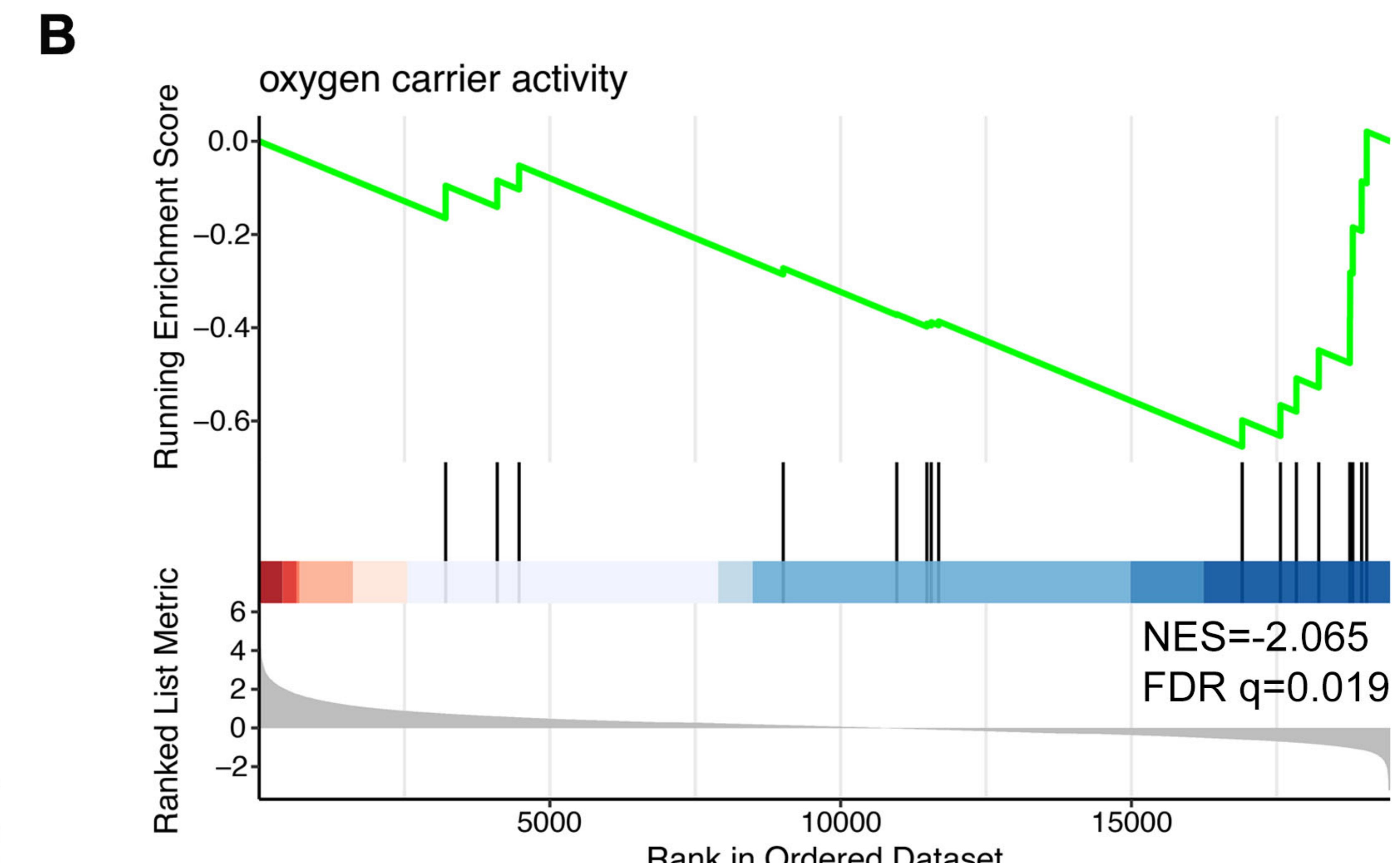
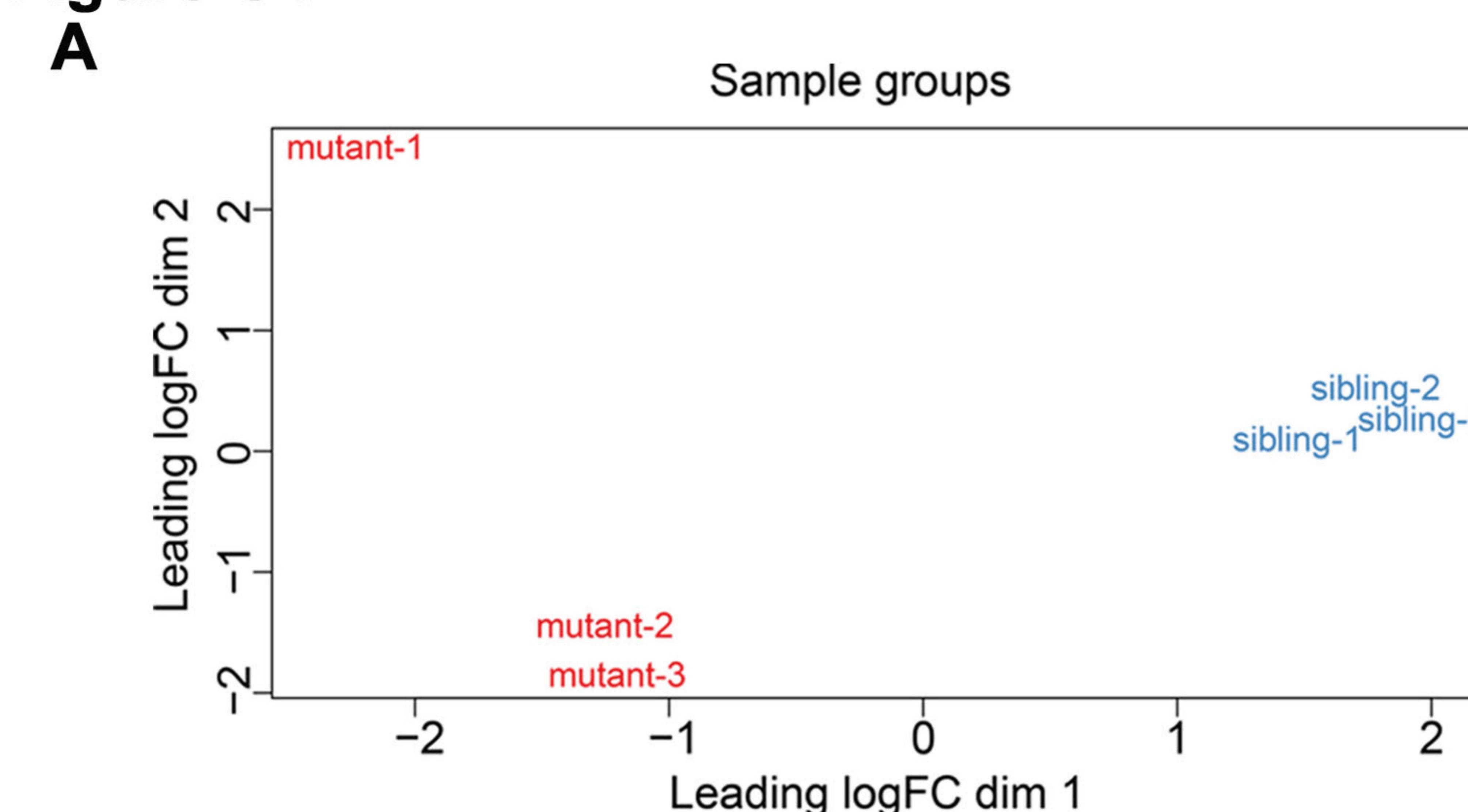


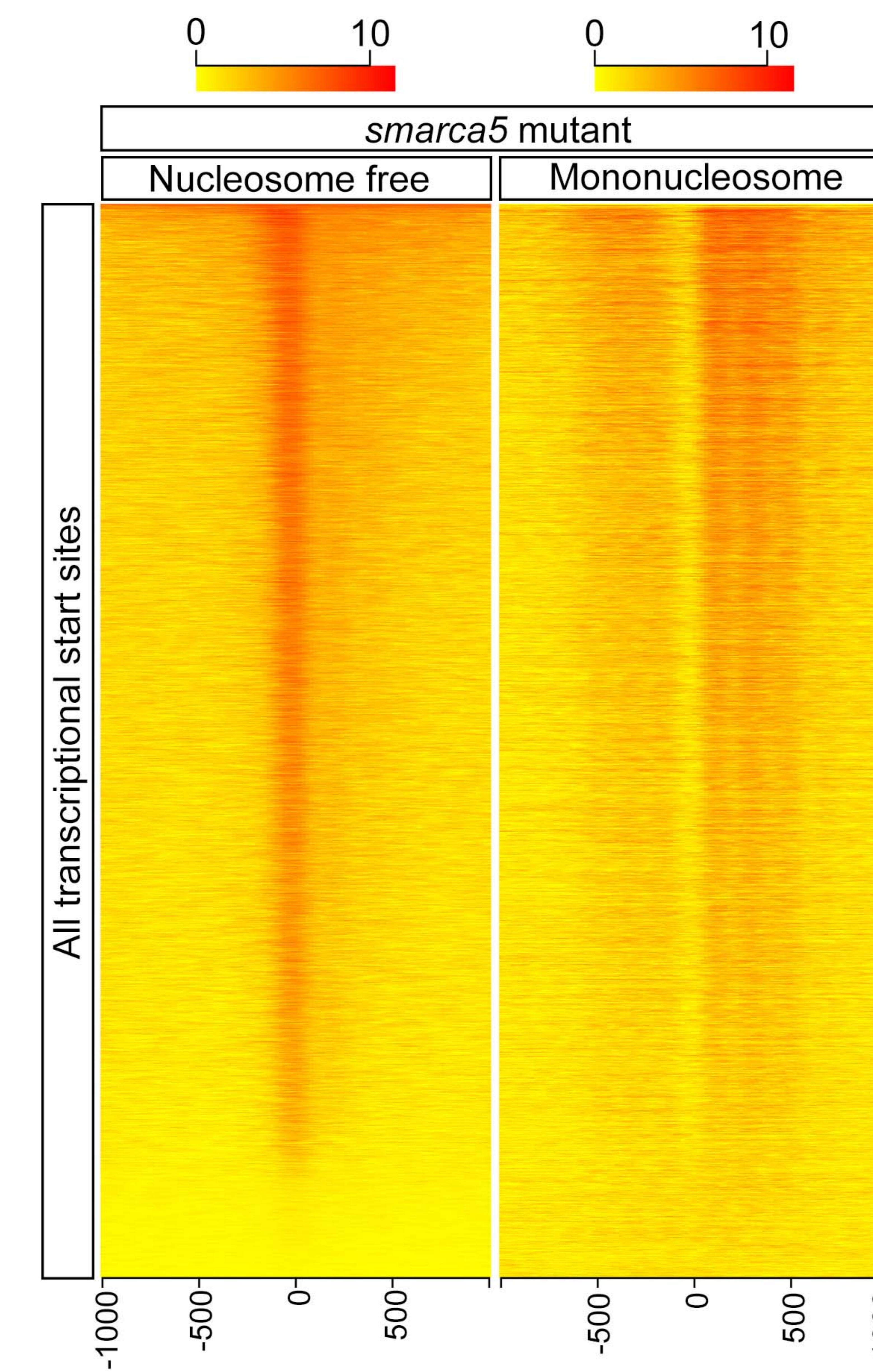
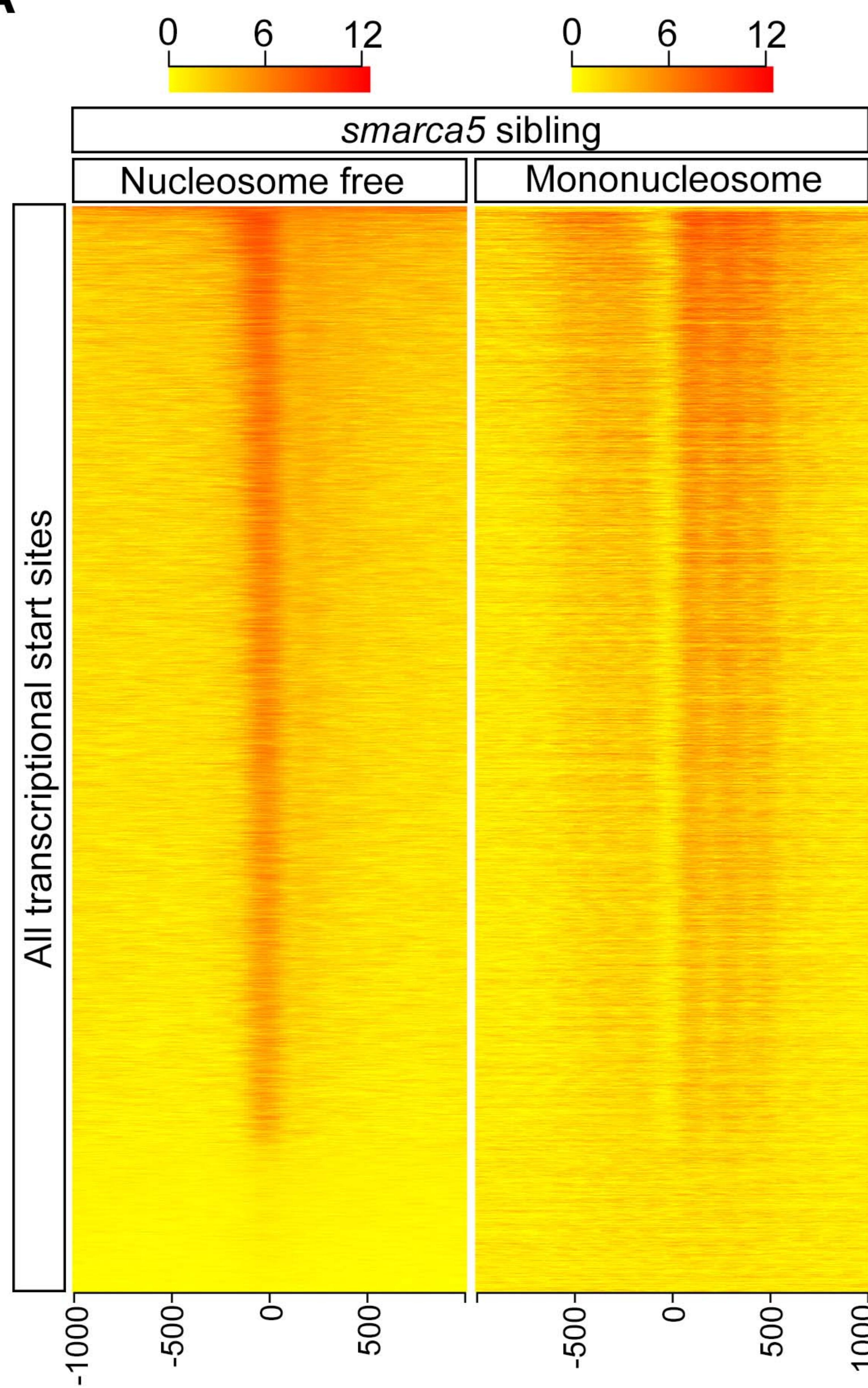
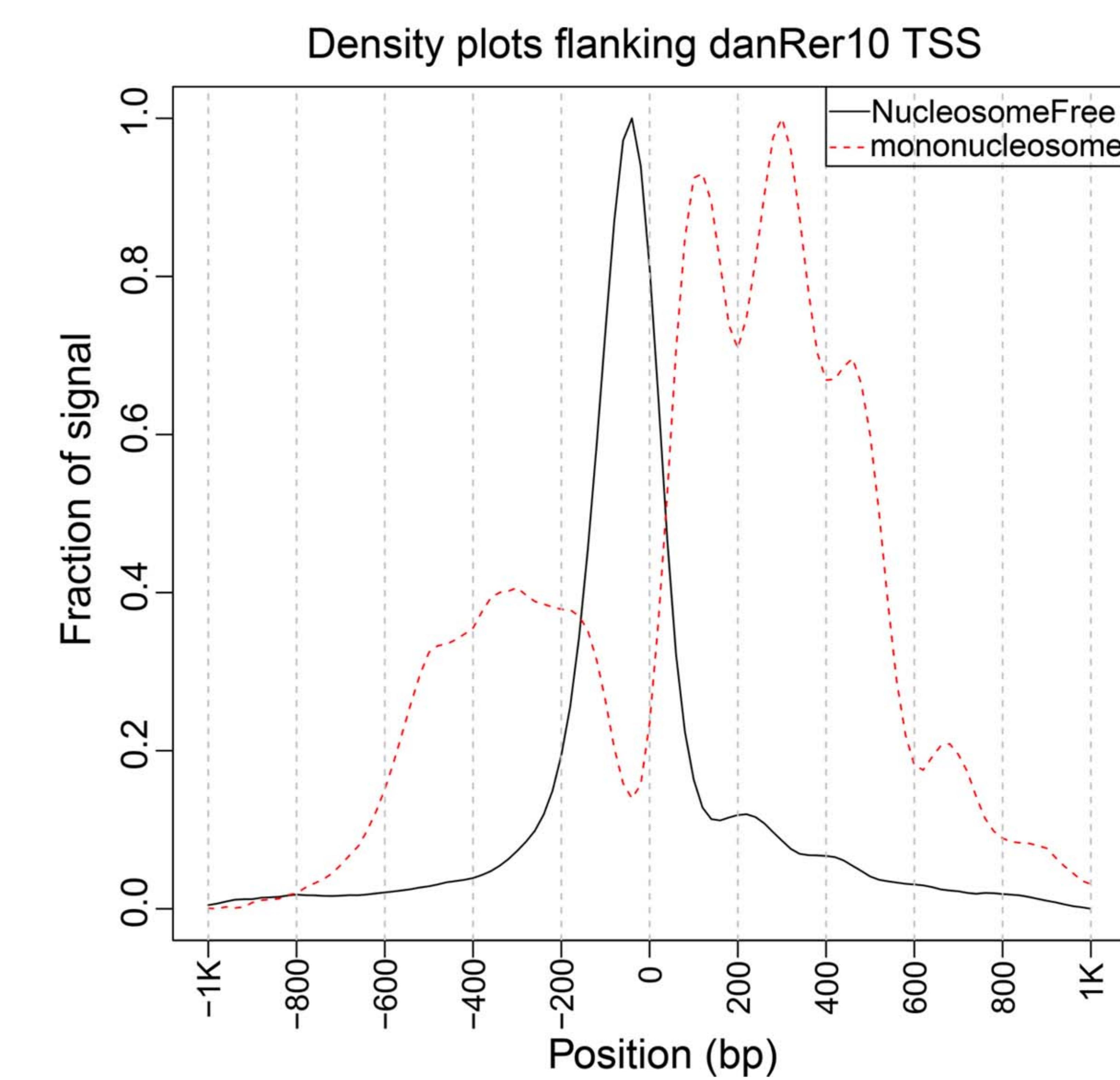
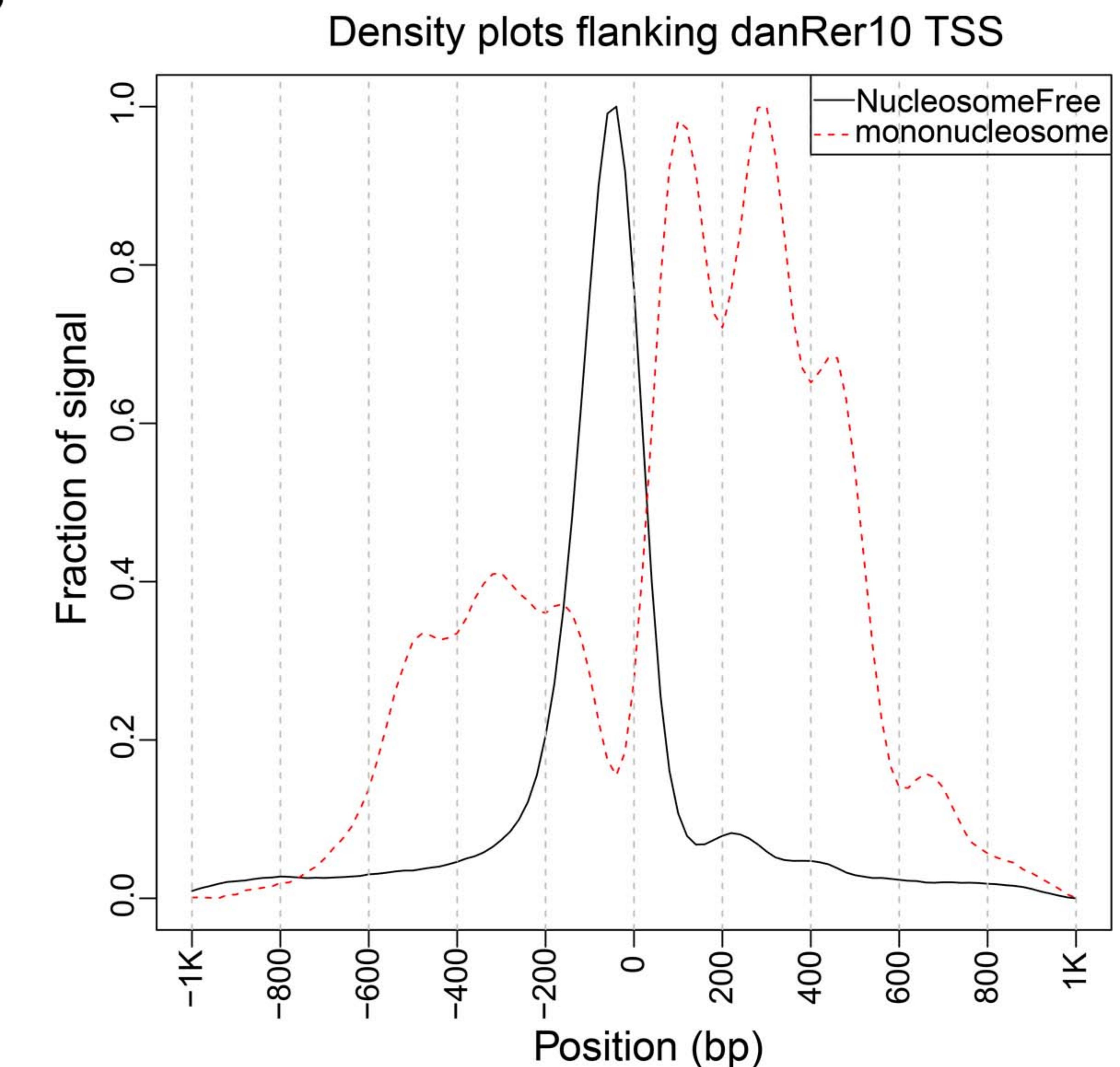
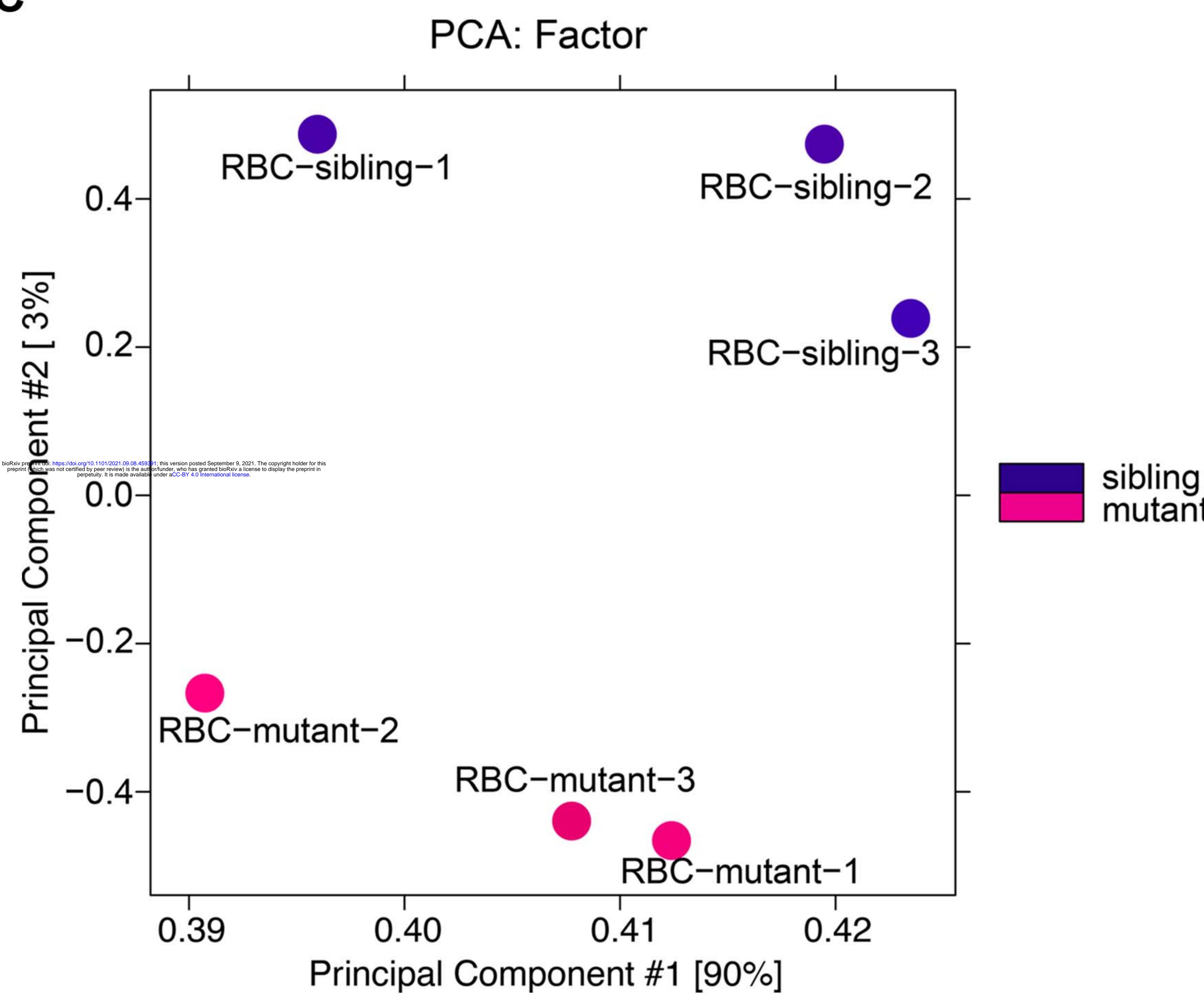
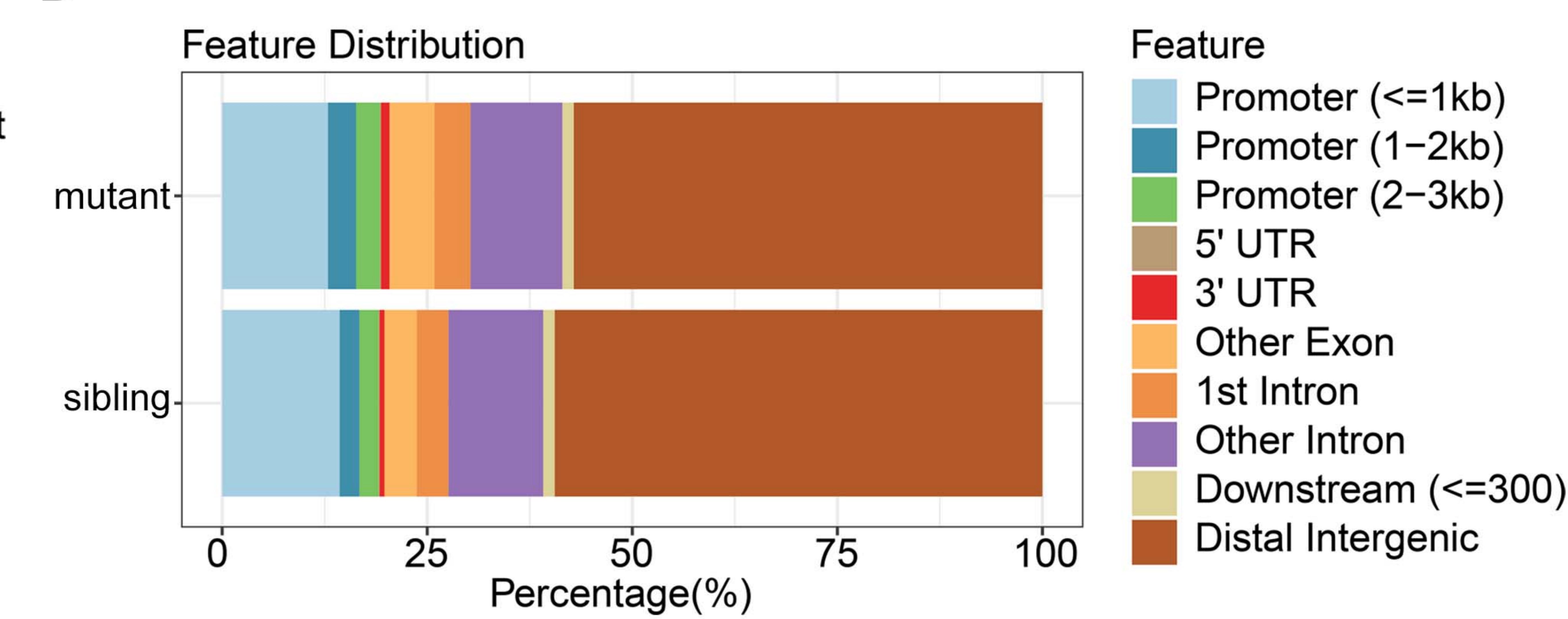
Figure S5**A****B****C****D**

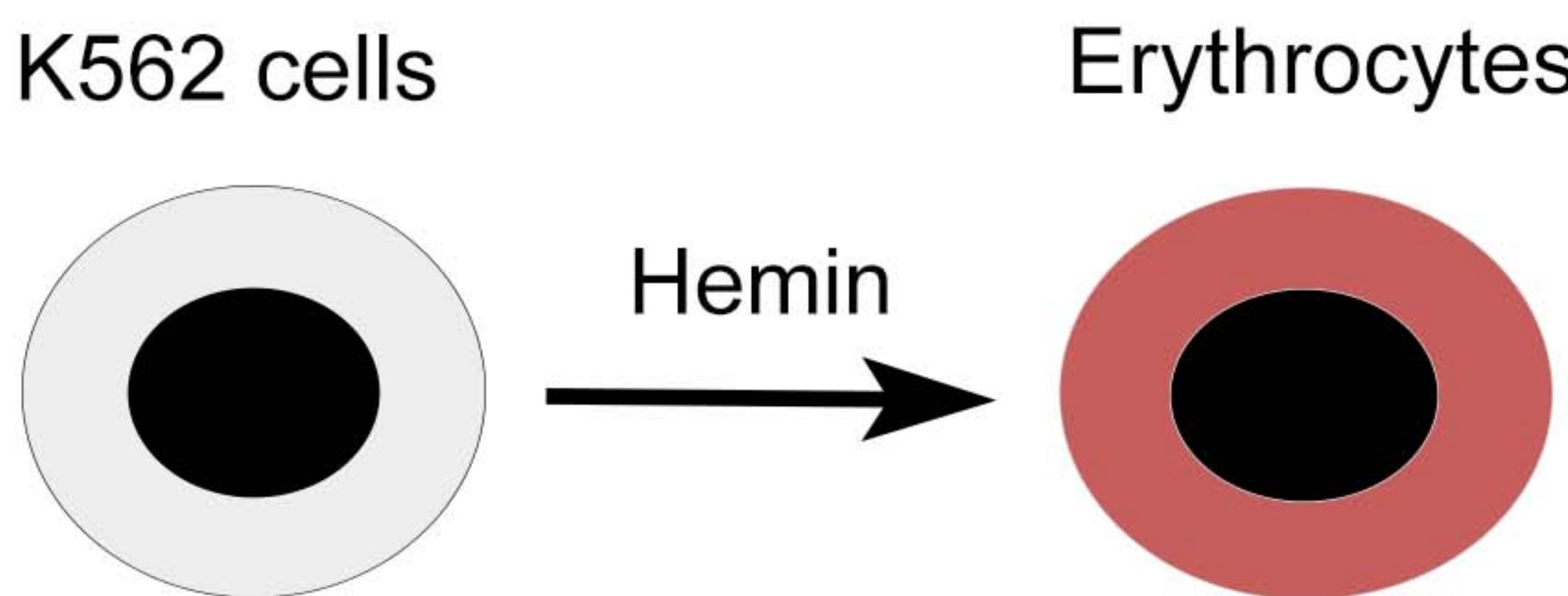
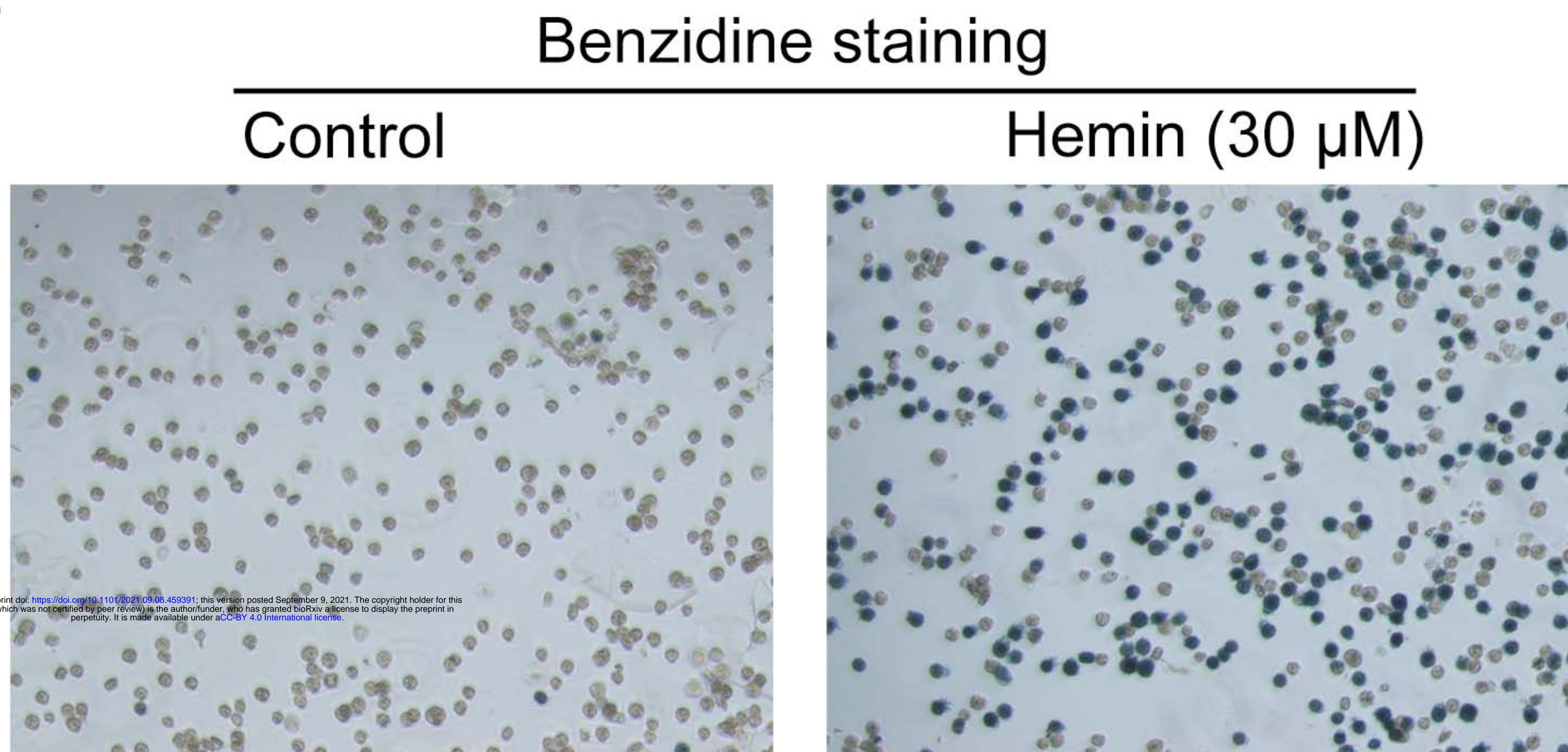
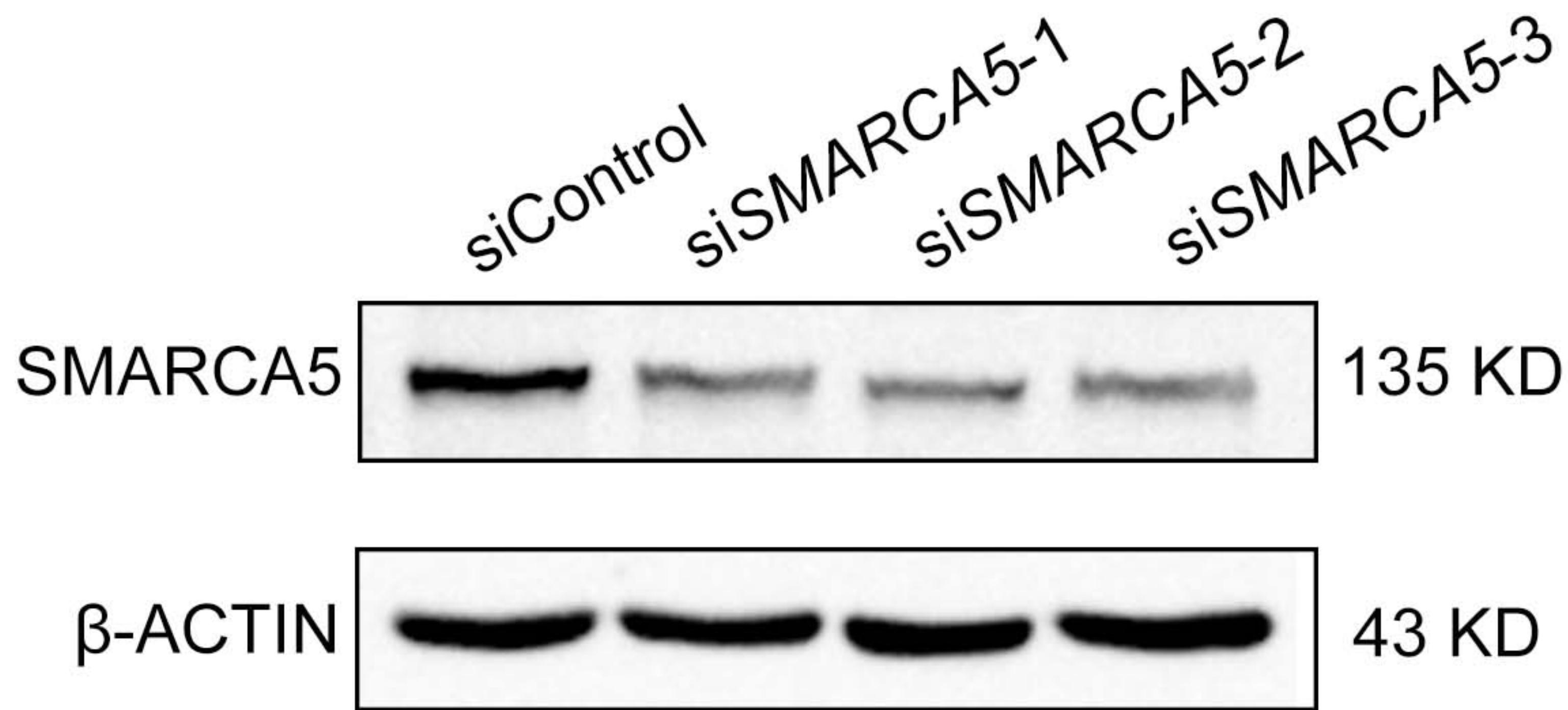
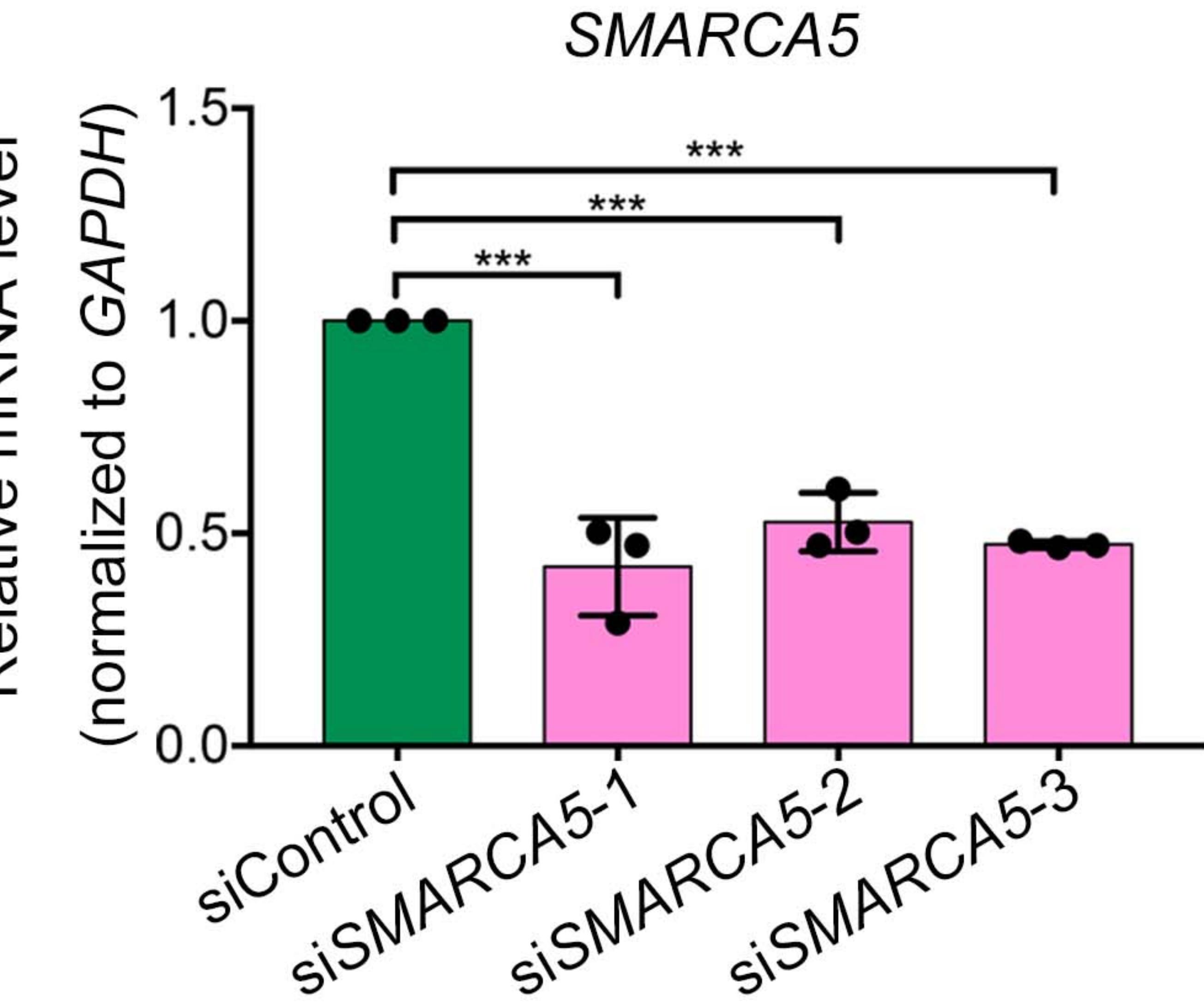
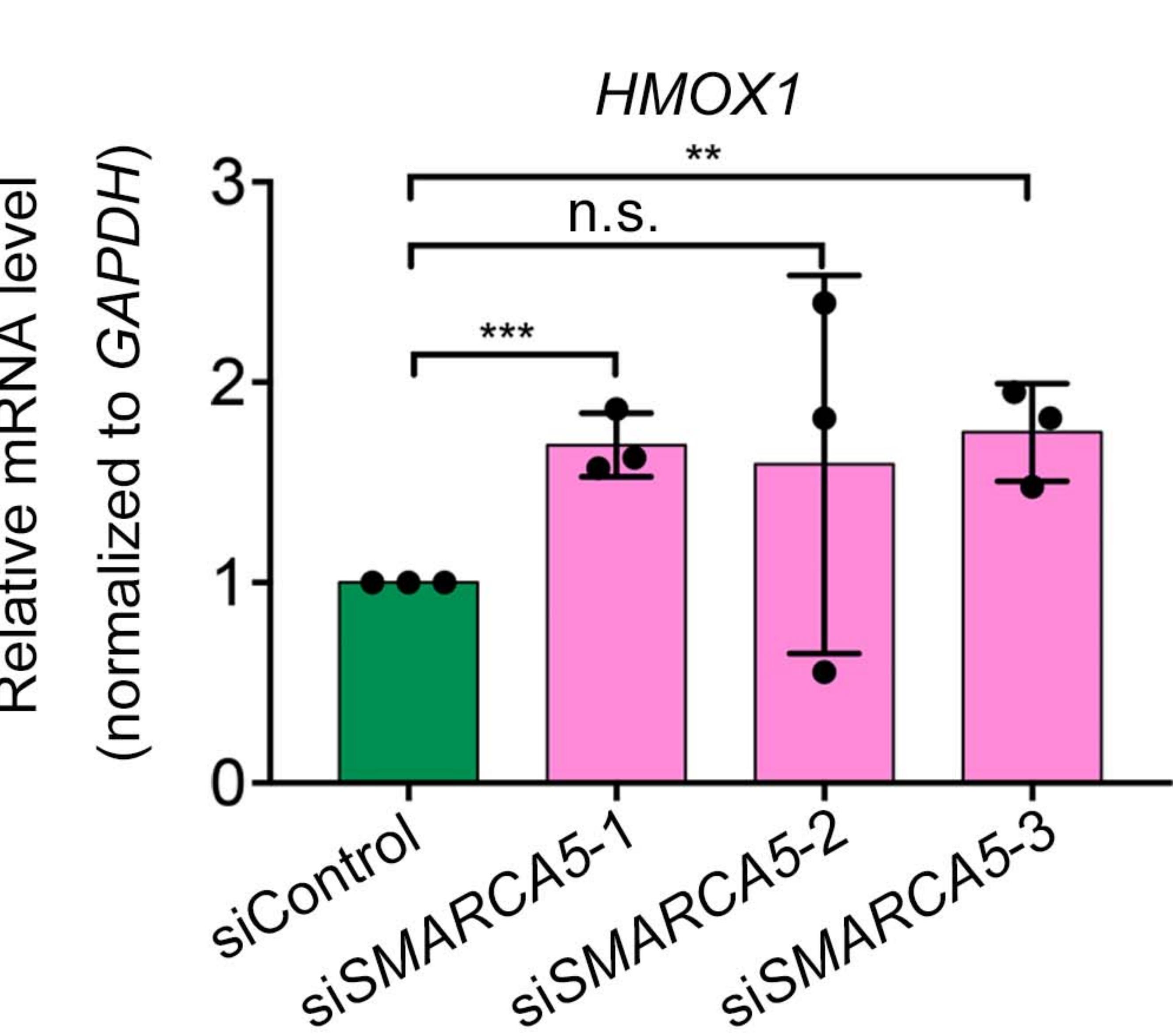
Figure S6**A****B****D****C****E**

Figure S7

A

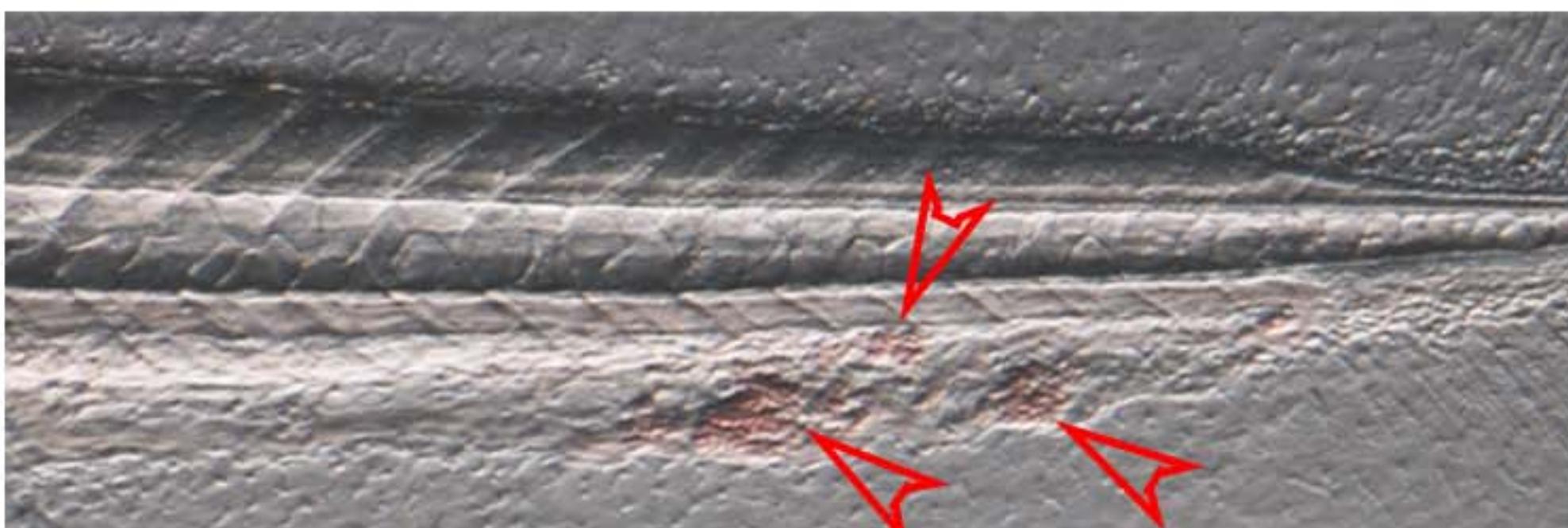
smarca5 siblings



smarca5 siblings+Glutathione 2 dpf



smarca5^{zko1049a}



smarca5^{zko1049a}+Glutathione



B

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