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Transient reduction of 5-methylcytosine and 5-hydroxymethylcytosine is associated with active DNA demethylation during regeneration of zebrafish fin

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Key Words: zebrafish, fin regeneration, 5-methylcytosine, 5-hydroxymethylcytosine, active DNA demethylation,

Abbreviations: 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine;

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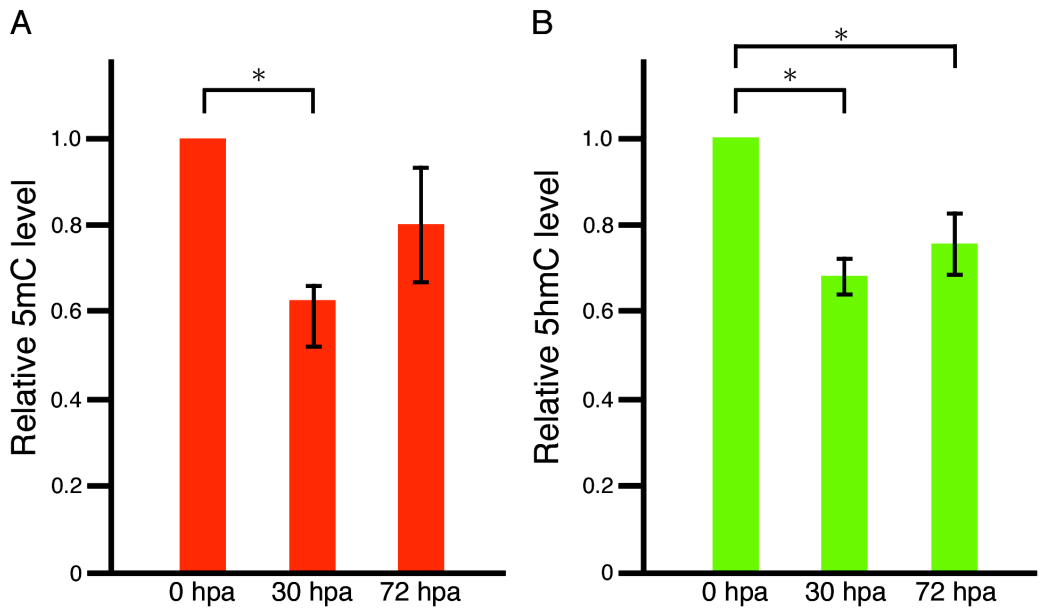

Fig. S1. Quantification of 5mC and 5hmC levels during regeneration of zebrafish fin

(A, B) Relative levels of 5mC and 5hmC at 30 and 72 hpa compared with those at 0 hpa. Average signal intensity for 5mC or 5hmC at 0 hpa is set as 1.0. * $p < 0.001$ by Student's *t*-test. Error bars represent the standard error.

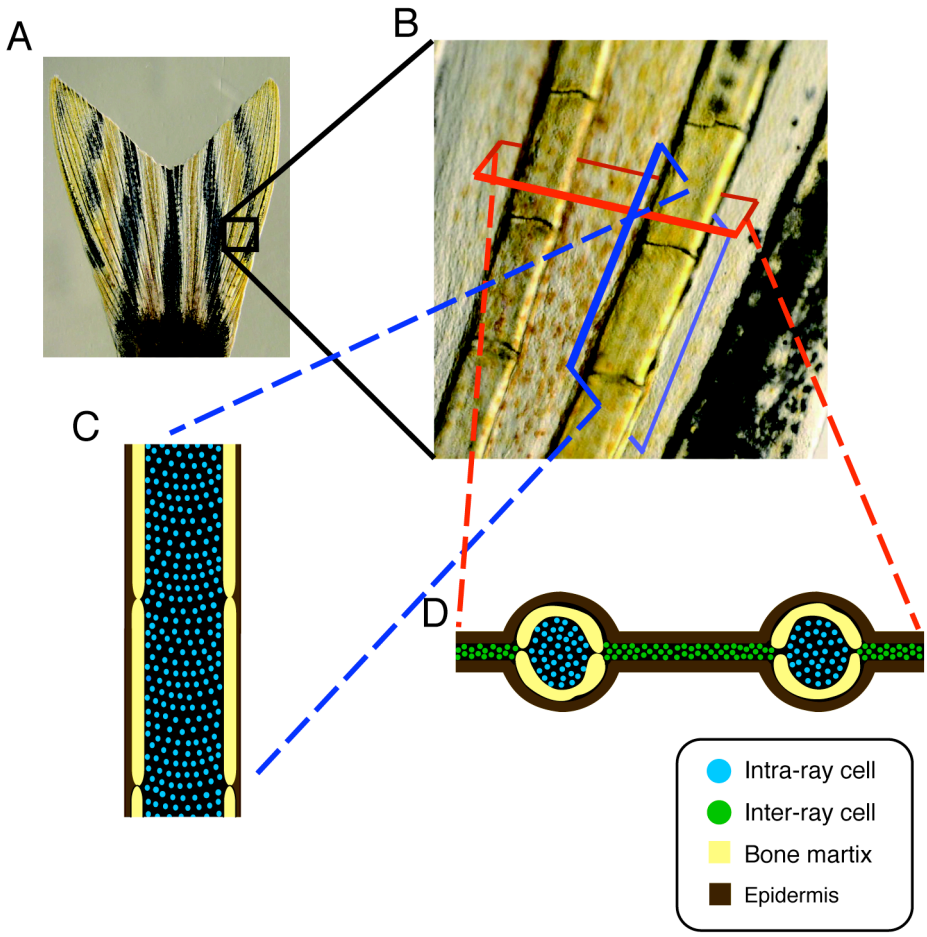

Fig. S2. Structure of adult zebrafish caudal fin

(A, B) A picture of adult zebrafish caudal fin (A) and an enlarged image of fin rays (B). (C) Longitudinal section of a fin ray. (D) Cross section of 2 fin rays.

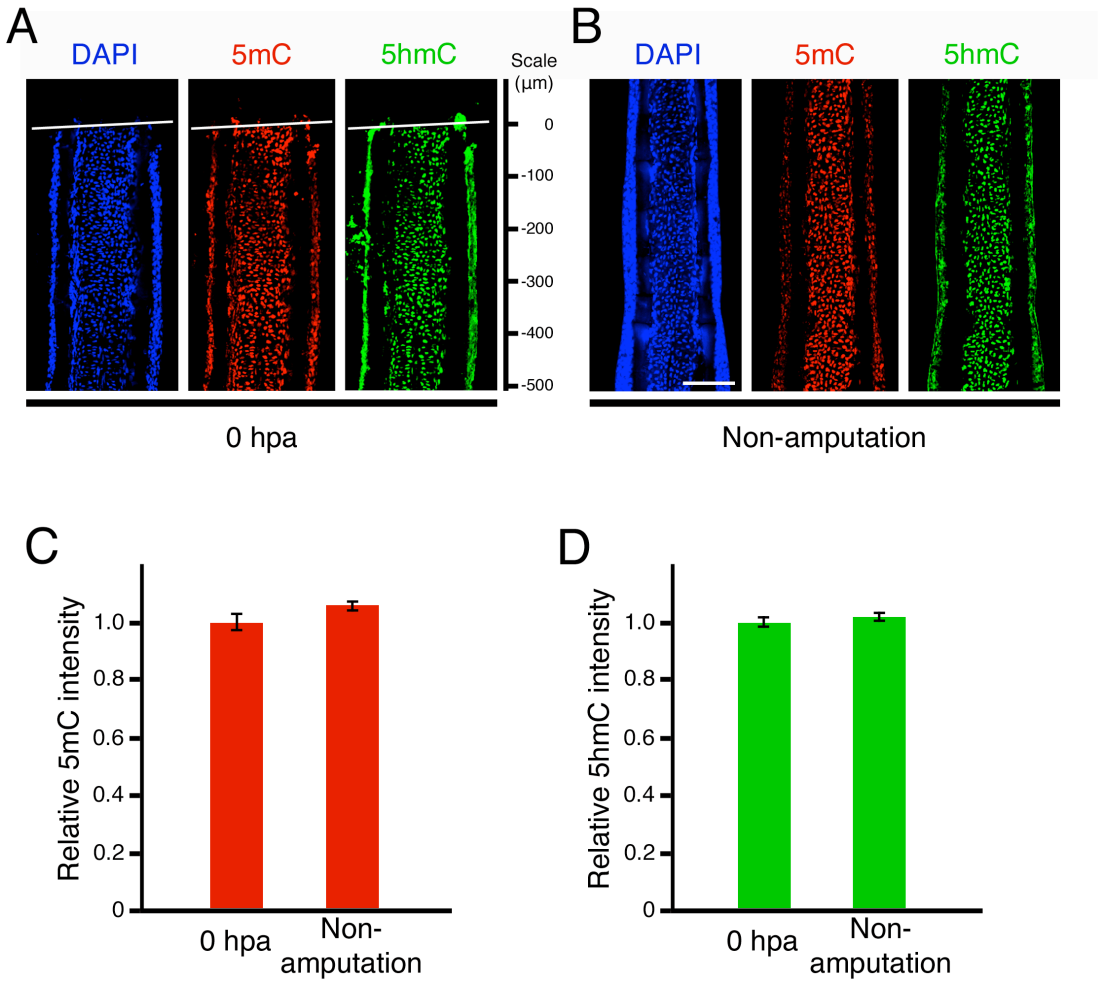

Fig. S3. The relative 5mC or 5hmC level did not differ between fins at 0 hpa and non-amputated ones

(A, B) Longitudinal sections of a fin at 0 hpa (A) and a non-amputated fin (B) that were immunohistochemically stained with antibodies against 5mC (red) and 5hmC (green). The fluorescent signals of DAPI (blue) indicate the presence of nuclei. The intensity of DAPI, 5mC, or 5hmC was shown at the approximate two-thirds position of the fins, which was a common amputation site in this study (B). (C, D) Quantification of the relative fluorescent signal of 5mC or 5hmC in fins at 0 hpa and in non-amputated fins. The relative level of 5mC

or 5hmC did not vary between non-amputated fins and those at 0 hpa. White lines indicate the amputation planes. Scale bars represent 100 μm. * $p < 0.001$ by Student's *t*- test. Error bars represent the standard error.

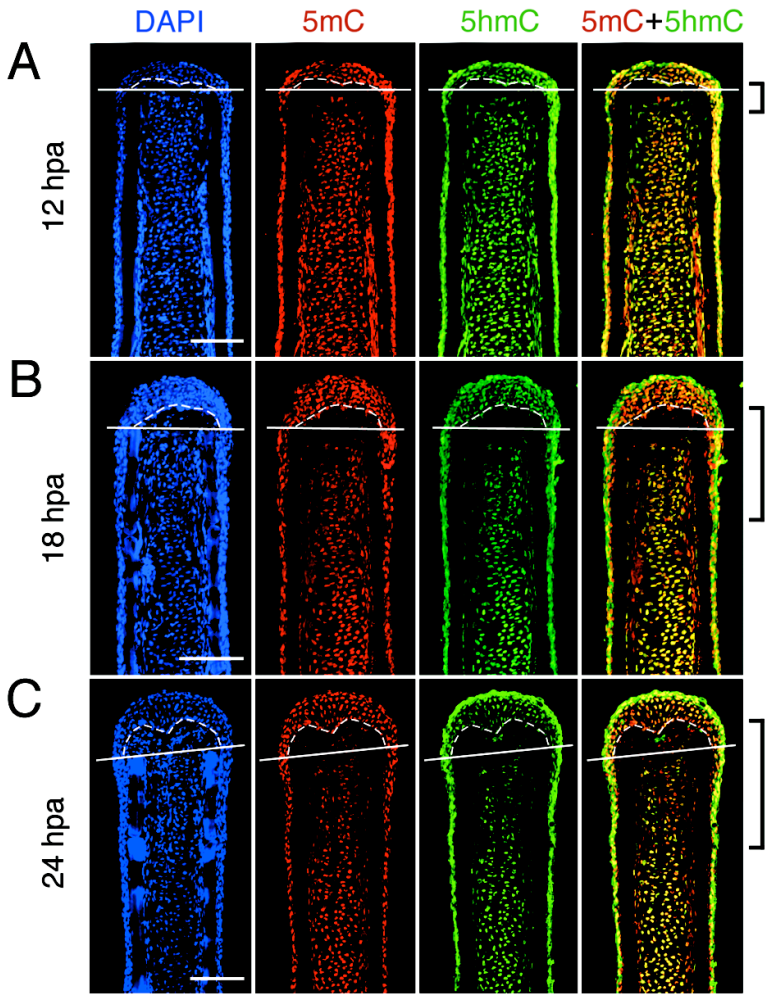

Fig. S4. Reduction of 5mC and 5hmC levels during regeneration of zebrafish fin

(A-C) Longitudinal sections of wild-type fin regenerates that were immunohistochmically stained with antibodies against 5mC (red) and 5hmC (green) at 12 (A), 18 (B), and 24 hpa (C). Blastema cells and cells adjacent to the amputation plane showed reduced levels of 5mC and 5hmC (brackets in A, B, C). The fluorescent signals of DAPI (blue) indicate the presence of nuclei. White lines indicate the amputation planes. Dashed lines outline the basement membrane, which shows the boundary between the epidermis and blastema. Scale bars represent 100 μm.

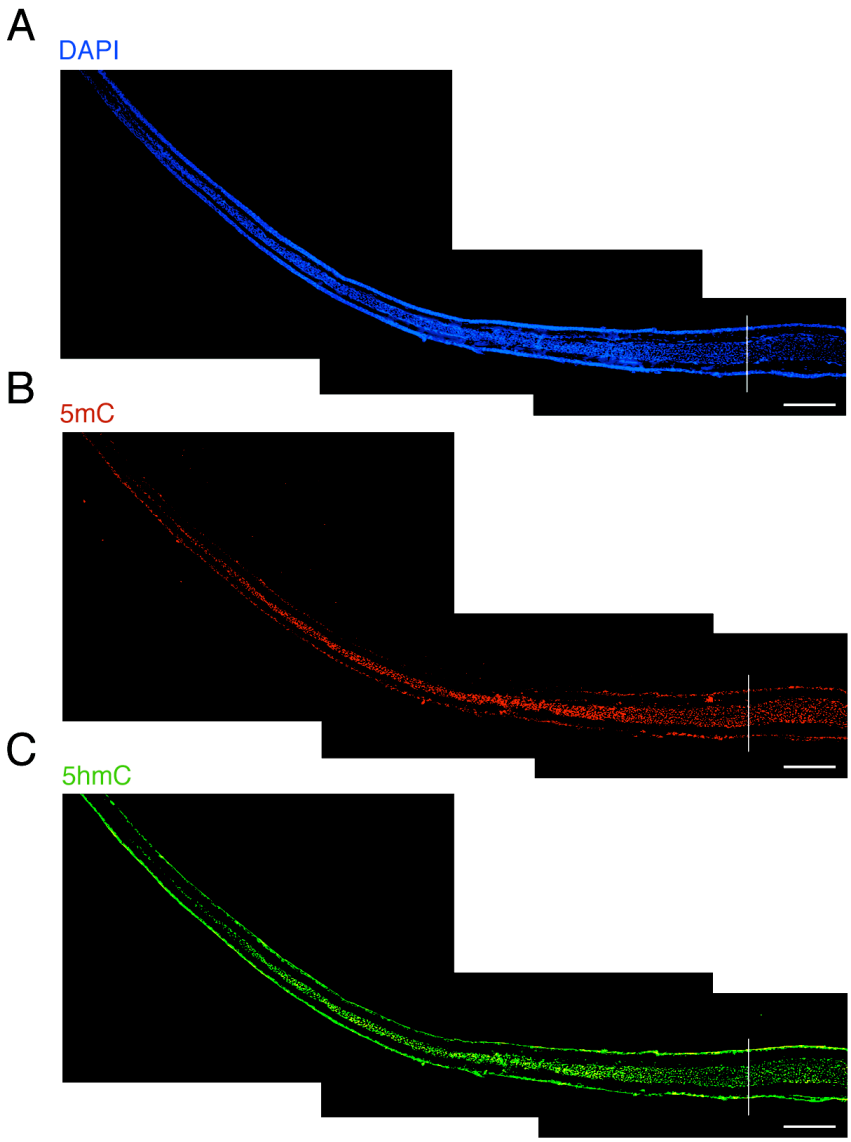

Fig. S5. Distributions of 5mC and 5hmC at 14 dpa

(A-C) Longitudinal sections of wild-type fin regenerates that were immunohistochmically stained with antibodies against 5mC (B) and 5hmC (C) at 14 dpa. The fluorescent signals of DAPI (A) indicate the presence of nuclei. White lines indicate the putative amputation planes. Scale bars represent 200 μm.

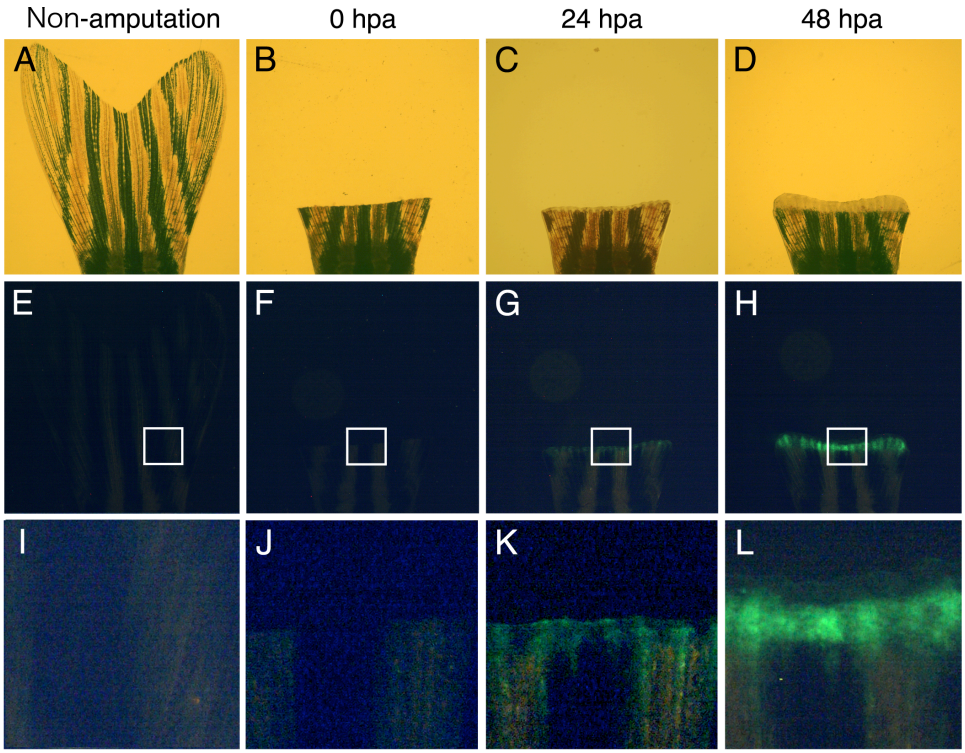

Fig. S6. The EGFP fluorescence of Tg(*ef1-α:EGFP*) transgenic fish was not detected before amputation or at 0 hpa.

(A-D) Bright field images of Tg(*ef1-α:EGFP*) transgenic fish fins before and after amputation. (E-L) Fluorescence images of Tg(*ef1-α:EGFP*) transgenic fish fins before and after amputation. The boxed areas in E, F, G, and H are shown enlarged in I, J, K, and L, respectively. No EGFP fluorescence was detected before amputation, or at 0 hpa (E, F, I, J). Scale bars represent 100 μm.

Supplementary Table : Primer sequences and PCR conditions

Gene	Sequence	Ref.	Annealing temperature
gadd45ba	forward: TCTCACAGTCGGCGTTTATG	1	56°C
	reverse: CGGCTCTCCTCACAGTAGGT		
gadd45bb	forward: GATCCACTTCACGCTCATCCA	2	56°C
	reverse: GGCAATAGAAGGCACCCACTG		
gadd45g	forward: CAACGACATCAACATCGTTCG	1	56°C
	reverse: TCAGCGTTCAGGCAGAGTAA		
apobec2b	forward: ACATACAAGGTGGAGCAGCAGAG		60°C
	reverse: AAATCCACAGGCCTCATCATGCG		
tdg	forward: ATGGATGAAAGGCTGTATGGATC	1	60°C
	reverse: TCCTCTGGATGTACAGGCAT		
mbd4	forward: CTTCTGCTCAGCGTTCACAACTC		60°C
	reverse: CATGGCTCTGTGCAGATCTTCAC		
tet2	forward: CACACCCAACTCTAAAACGGACAACAC	2	60°C
	reverse: ATGGTGGGGAAGCGTAAGAAGGA		
tet3	forward: GGACTGTCGTCTGGGCTGTAGGG	2	62°C
	reverse: GCCAGCAGCCGCACTTCTCTT		
parp1	forward: ATCAGACGTCTCTGTGGTGAGAC		60°C
	reverse: CTTGCAGCAGGCTATATCCTAGC		
actb1	forward: CCGTGACATCAAGGAGAAGCT		60°C
	reverse: TCATGGATACCGCAAGATTCC		
rpl13α	forward: TCTGGAGGACTGTAAGAGGTATGC	3	56°C
	reverse: AGACGCACAATCTTGAGAGCAG		
apobec2a	forward: TCAAGAACGTGGAGTACTCGTCC		58°C
	reverse: TTCCAAGTGTGTGCGTCGACTAG		
aid	forward: GACGGTGCAAGATTGTGTTAC		56°C
	reverse: TAAGTCATGACCGAGATCTGAAC		

References
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