Heme-mediated inhibition of Bach1 regulates the liver specificity and transience of the Nrf2-dependent induction of zebrafish heme oxygenase 1

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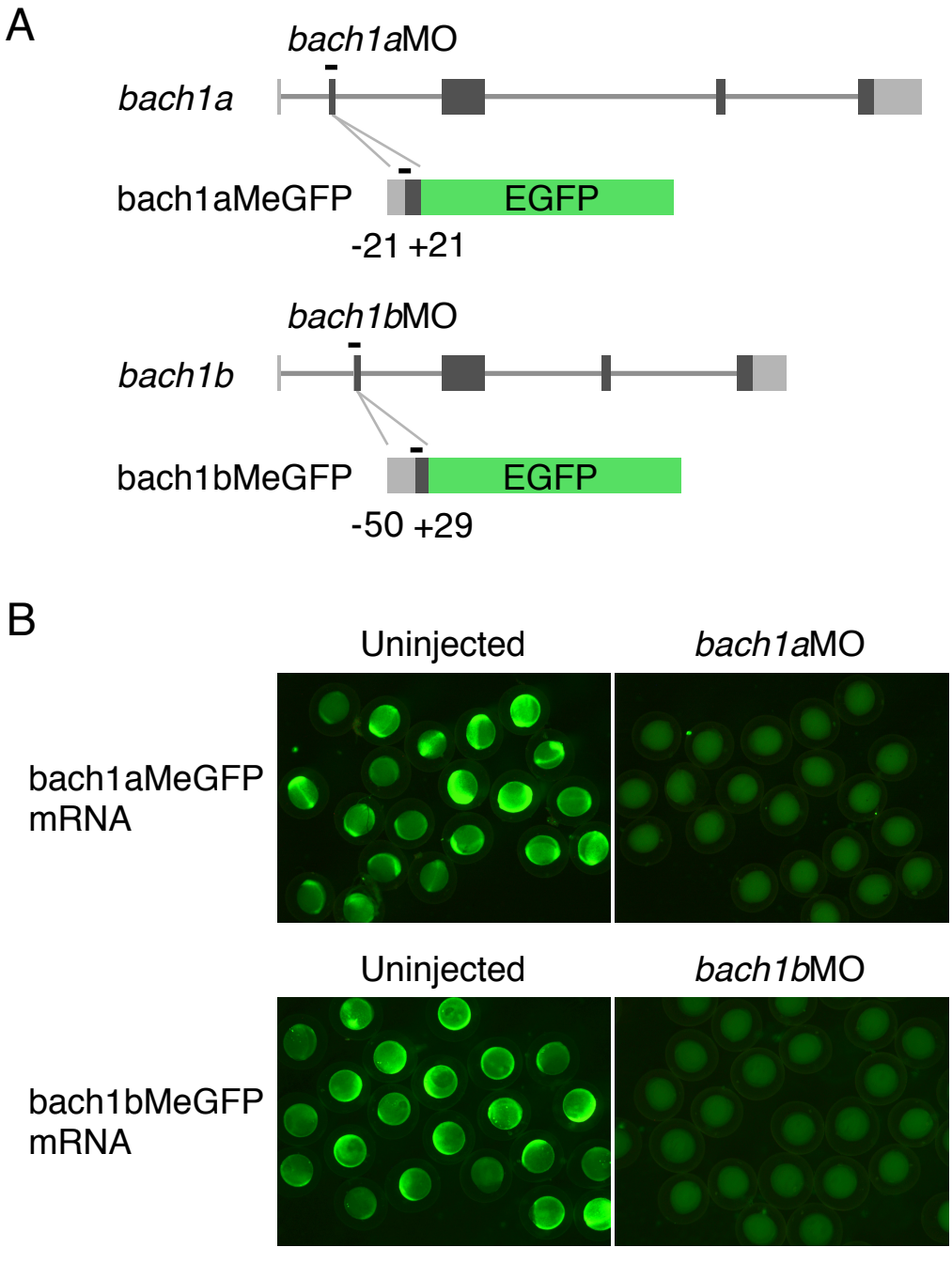

Figure S1 Specific translational inhibition by *bach1a*MO and *bach1b*MO. (A) Schematic diagrams of the GFP reporter constructs that contain the target sites for *bach1a*MO and *bach1b*MO. *bach1a*MO and *bach1b*MO were designed to knock down the translation of *bach1* and *bach1b* mRNA, respectively. The boxes are exons, lines are introns, and dark gray indicates the ORF of the *bach1a* and *bach1b* genes. (B) mRNA for bach1aMeGFP or bach1bMeGFP (150 pg) was injected into one-cell stage embryos, with or without *bach1a*MO and *bach1b*MO (1 pmole), respectively. The GFP expression was evaluated after 10 hours.

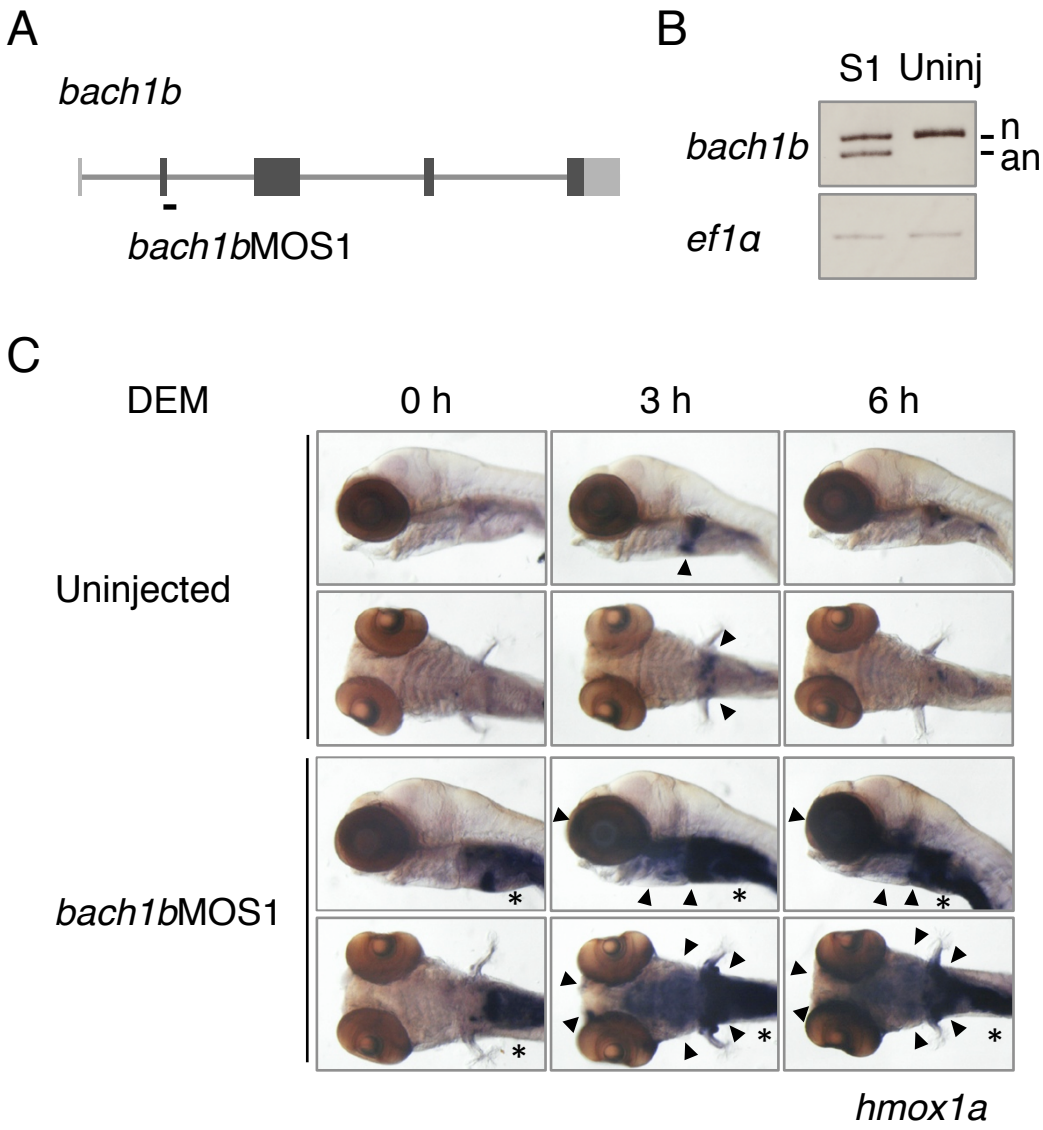

Figure S2 Confirmation of the ectopic and prolonged *hmox1a* induction by the *bach1b* knockdown using splice-blocking morpholino. (A) A schematic diagram of the binding sites for *bach1b*MOS1. *bach1b*MOS1 was designed to knock down the splicing of *bach1b* mRNA. The boxes are exons, lines are introns, and dark gray indicates the ORF of the *bach1b* gene. (B) The splice-blocking activity of *bach1b*MOS1 was evaluated by RT-PCR analysis. RNA isolated from embryos injected with or without *bach1b*MOS1 was analyzed. "n" and "an" indicate RT-PCR products corresponding to normal and abnormal-size *bach1b* mRNA, respectively. The amount of cDNA used for RT-PCR was standardized by the *ef1α* expression. (C) The expression of *hmox1a* was analyzed in 5-dpf larvae injected with or without *bach1b*MOS1 (1 pmol) and treated with 100 μM DEM for the indicated times. The ectopic and prolonged induction of *hmox1a* was observed similar to that in the larvae injected with the *bach1b*MO (see Fig. 4). The arrowheads indicate the *hmox1a* induction in the nose, gills and liver. The asterisks denote the basal expression in the intestine.

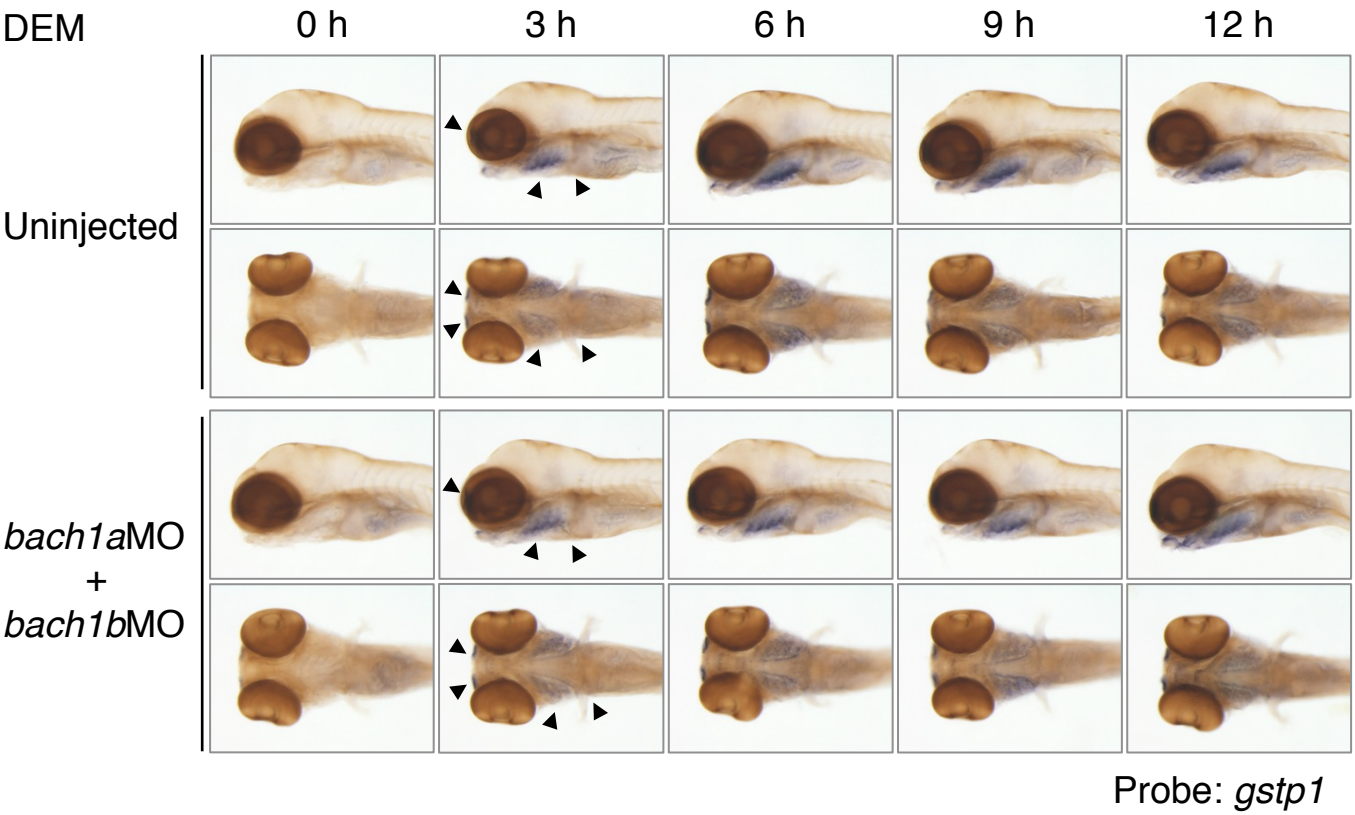

Figure S3 *gstp1* induction profile in *bach1a-bach1b* double knocked-down larvae. The expression of *gstp1* was analyzed in 5-dpf larvae co-injected with or without *bach1a*MO/*bach1b*MO (1 pmol each) and treated with 100 μM DEM for the indicated times. The induction profiles were identical between *bach1a-bach1b* double knocked-down larvae and uninjected control. The arrowheads indicate the *hmox1a* induction in the nose, gills and liver.

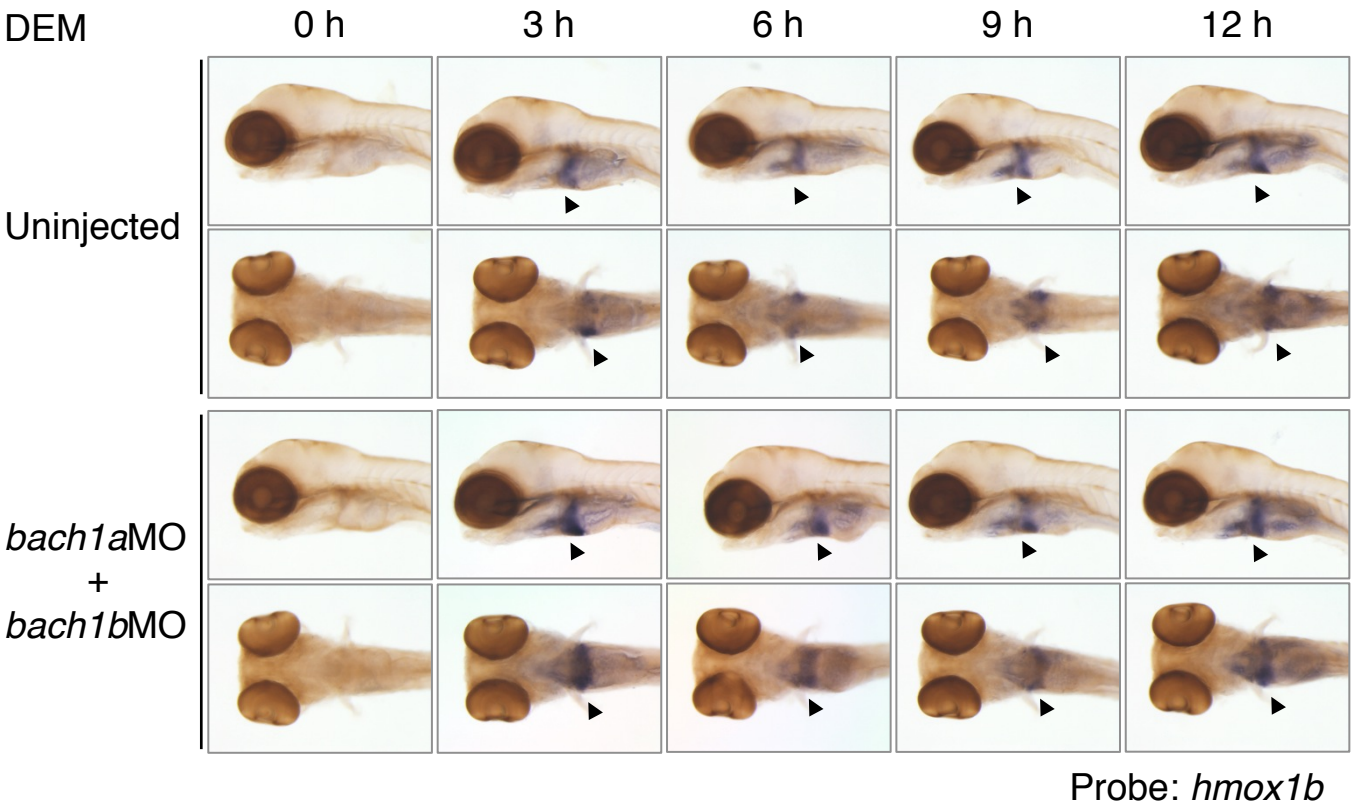

Figure S4 *hmox1b* induction profile. The expression of *hmox1b* was analyzed in 5-dpf larvae co-injected with or without *bach1a*MO/*bach1b*MO (1 pmol each) and treated with 100 μM DEM for the indicated times. The induction profiles were liver-specific and prolonged in both *bach1a-bach1b* double knocked-down larvae and uninjected control. The arrowheads indicate the induction in the liver and gills.

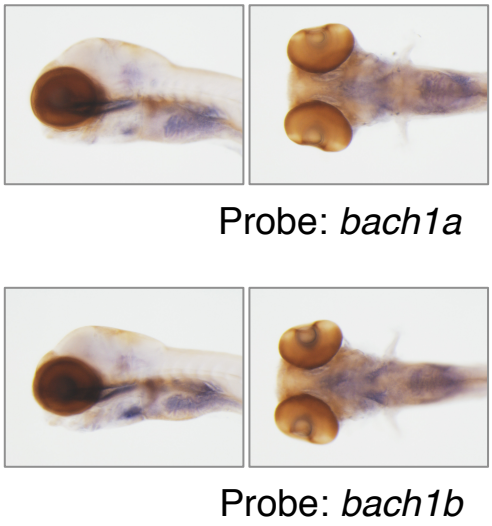

Figure S5 The ubiquitous expression of two zebrafish Bach1 genes. *bach1a* and *bach1b* expression was analyzed in 5-dpf larvae, and was found to be ubiquitous.

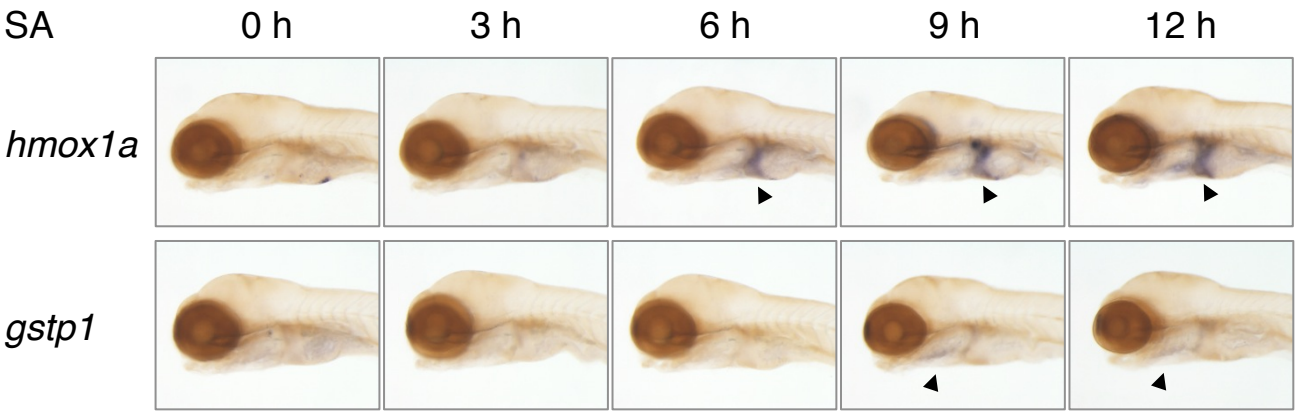

Figure S6 Weak induction of *hmox1a* and *gstp1* by SA treatment. The expression of *hmox1a* and *gstp1* was analyzed in 5-dpf larvae treated with 0.5 mM SA for the indicated times. *hmox1a* was induced in the liver after 6 hours, while *gstp1* was induced in the gills after 9 hours. It should be noted that SA did not induce both *hmox1a* and *gstp1* during 3-hour treatment. The arrowheads indicate the induction in the liver and gills.

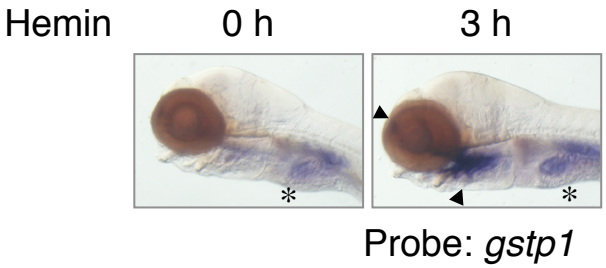

Figure S7 The *gstp1* induction by hemin treatment. The expression of *gstp1* was analyzed in 5-dpf larvae treated with 100 μM hemin for three hours by a WISH analysis. The arrowheads indicate the *gstp1* induction in the nose, gills and liver. The asterisks denote the basal expression in the intestine.

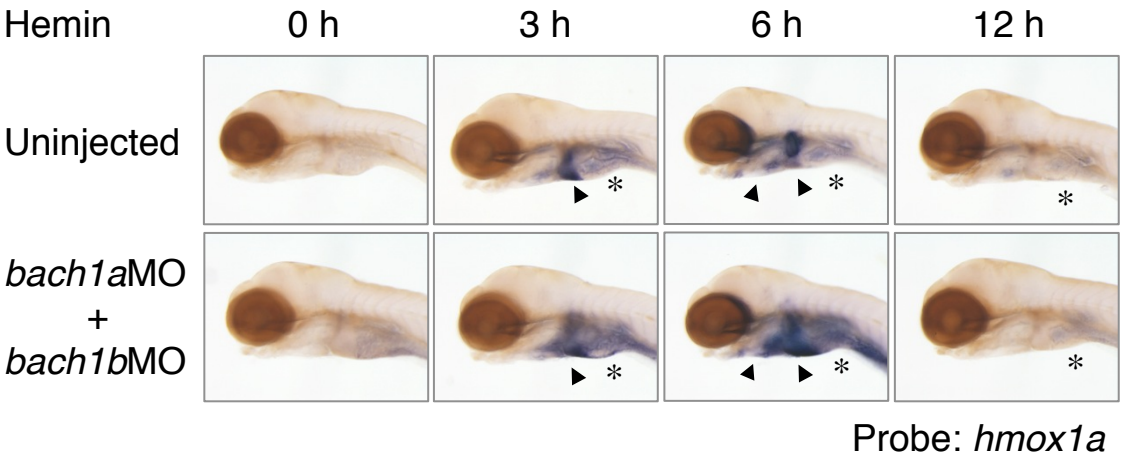

Figure S8 Ectopic and prolonged induction of *hmox1a* by hemin treatment. The expression of *hmox1a* was analyzed in 5-dpf larvae treated with 100 μM hemin for the indicated times. *hmox1a* was induced in the gills and nose as well as in the liver. The arrowheads indicate the *hmox1a* induction in the nose, gills and liver. The asterisks denote the basal expression in the intestine.

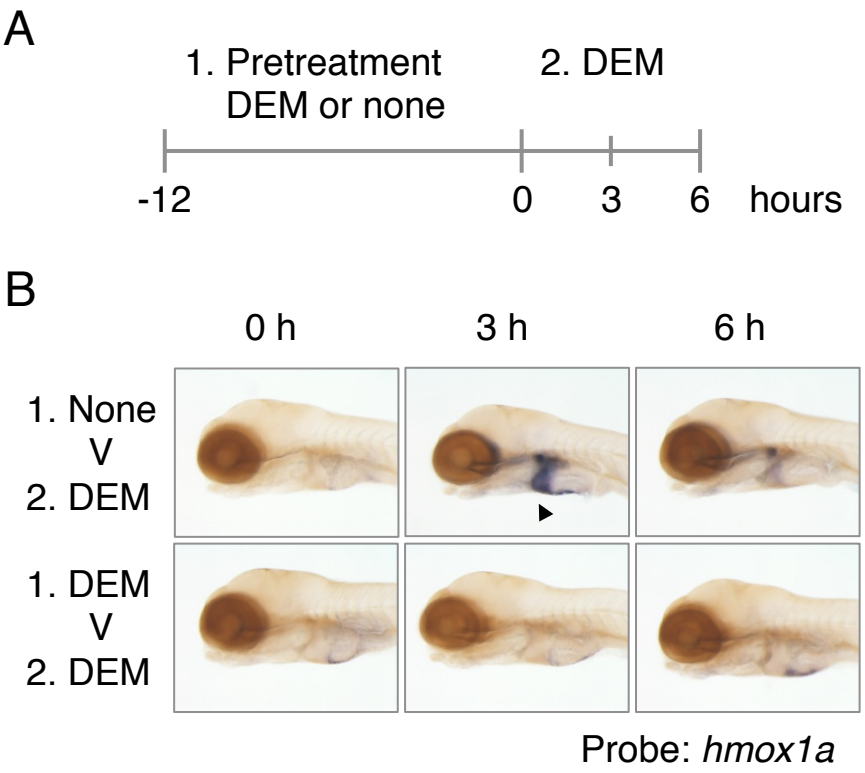

Figure S9 The effect of DEM pretreatment on *hmox1a* expression profile. The expression of *hmox1a* was analyzed in 5-dpf larvae treated with 100 μM DEM for the indicated times, after either a 12-hour pretreatment with or without 100 μM DEM. *hmox1a* was not induced after a 12-hour DEM pretreatment. The arrowhead indicates the *hmox1a* induction in the liver.

Table S1 Oligonucleotide primers for plasmid construction

Plasmids	Primer sequences
pCS2nrf1b	5’-GGGGATCCGCCATGCTTTACTTGAAAAAGTACTTC
	5’-GGTCTAGACTCACTTCTTTTTGTCCTTCTG
pCS2cfos	5’-GGGGATCCACCATGATGTTTACCAGCCTTAACG
	5’-GGCTCGAGTCAAAGAGTGAGGAGGGTTG
pCS2bach1b	5’-GCATCGATACCGCCATGTCGGTGGAAAGCTCAAAG
	5’-GGTCTAGACTATTTGTCTGTTTCAGGTC
pKSbach1a	5’-GGGGATCCACACTGCGAAACTTCACTTCAC
	5’-GGCTCGAGTGCTTCGTTCATTGCTGCTATC
pKSbach1b	5’-CCGGATCCCACGGGACAGCGAGTC
	5’-CCGTCGACGAGTTTCTGGATTTCACACTC
pKShmox1b	5’-GGGGATCCATGCTGAGCTACCAGAGGG
	5’-GGCTCGAGTCTCAACAGTACAAATGTGCCG
pCS2bach1bMeGFP	5’-GGGGATCCGTATCAAATCCAACTTATTAC
	5’-GGGGATCCACGCGTTTAAATGACTTTGAGCTTTCC

Table S2 Oligonucleotide primers for RT-PCR analyses

Genes	Primer sequences
hmox1a	5’-GGAATTCATGGACTCCACCAAAAGCAAAG
	5’-GGTCGACTTAAAAAGCGTAAACTCCCATGC
gstp1	5’-CTAGGAGCAGCTTTGAAACGCAC
	5’-TGGCCAGAACATTTTCAAGC
prdx1	5’-GCCCGCGAGTTCACTTTC
	5’-GCTTCCATCCGGCTGGAC
fthl	5’-TACGACCGCGACTGCGAG
	5’-TGGCTGCAGATGATCCGA
gclc	5’-CCAAGAAACATGCTGACCAC
	5’-GTCAGAGTGCTGAATCTTGG
ef1α	5’-GCCCCTGCCAATGTA
	5’-GGGCTTGCCAGGGAC
bach1a	5’-AAACCACAGCCAAGCAAACC
	5’-TGAAGAGGAAGGCAACTGAGG
bach1b	5’-AAGTCCAGAGGAAATGCTGC
	5’-CAGACAGTTGAGACCGGAG
bach1b (splicing)	5’-GCATCGATACCGCCATGTCGGTGGAAAGCTCAAAG
	5’-GGTGATTTGTCTTCATCAGTG