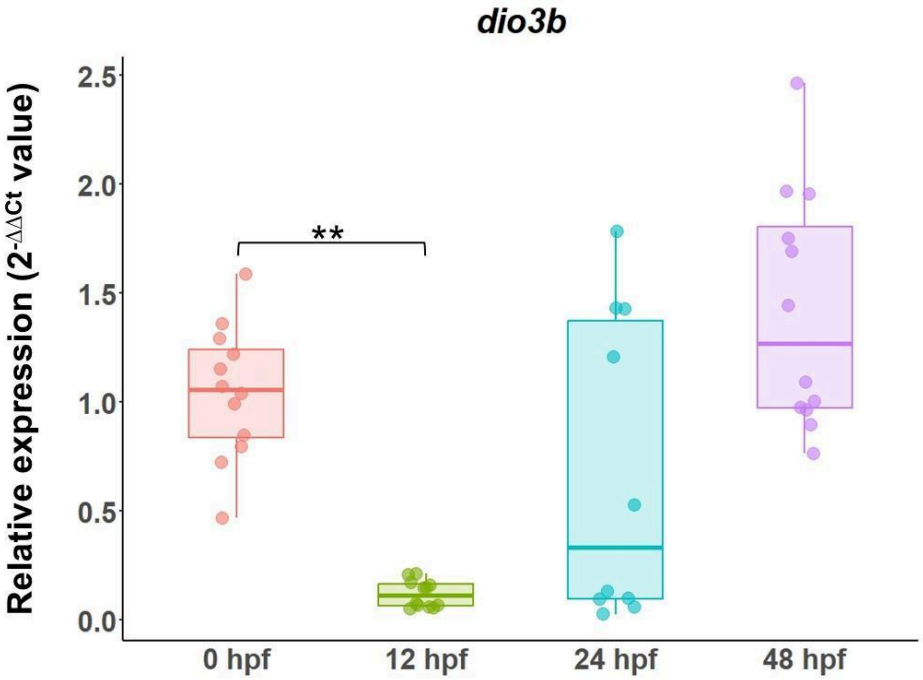
Condition	Sample	T3 concentration (nM)
Control (untreated wild-type embryos)	1	1.01
	2	1.20
	3	1.09
T3	1	1.36
	2	1.26
dio3b crispants	1	1.02
	2	1.20
	3	1.07

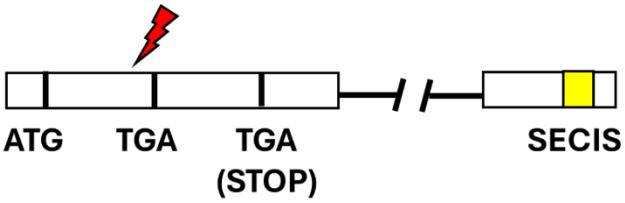
Analysis performed through ELISA assay in extracts of pools of 100 embryos.

Supplementary Table 1. Concentrations of T3 in 24 hpf embryos.



Supplementary Figure S1. Temporal expression of *dio3b* during early zebrafish development. *dio3b* expression was analyzed by qPCR in wild-type zebrafish at 0, 12, 24, and 48 hpf. Expression at 0 hpf was used as the calibrator to calculate relative expression levels at subsequent time points. Normality and homogeneity of variances were evaluated using the Shapiro-Wilk and Levene's tests, respectively. As the data did not meet the assumptions of parametric tests, statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's multiple comparisons test ($p \leq 0.01$**). n = 6 pools of 10 embryos per condition, measured in duplicate.

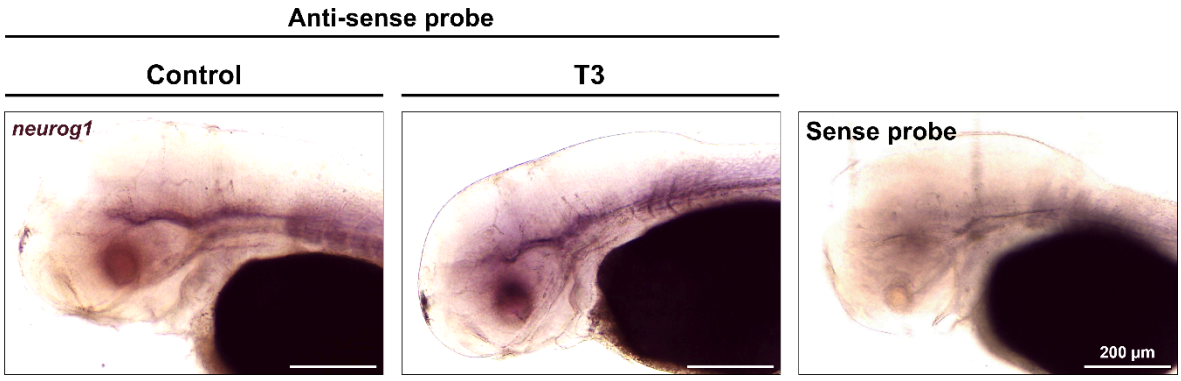
a



b

WT	GAGCGCGCGGCTCGGCGGAGCGGGCGCCCAAC
Crisp 1	GAGCGCGCGGCTCGCCCGCTGCAGGGACACACA
Crisp 2	GAGCGCATCCTGGATTACGCGCGCGGCCGGAGAC
Crisp 3	GAGCGCGCGGCTCGGCTGAACGCCCCCCCACAC
Crisp 4	GAGCGCGCGGCTCGGTGCTGAGCGCCCCCCCAA

Supplementary Figure S2. Representation of the target site for genome editing of the *dio3b* gene. (**a**) Schematic representation of the zebrafish *dio3b* gene indicating the CRISPR/Cas9 target site (red bolt) located in exon 1, upstream of the in-frame selenocysteine codon TGA. ATG = start codon; TGA (STOP) = stop codon; SECIS = selenocysteine-insertion sequence. (**b**) Nucleotide sequence of the target region in wild-type (WT) larvae and in individual crispant larvae. The PAM sequence is highlighted in pink.



Supplementary Figure S3. Representative whole-mount *in situ* hibridisation (WISH) for *neurog1*. Comparison of embryos hybridized with the *neurog1* anti-sense probe and the corresponding sense probe (negative control).