

Supplementary Materials for  
**Parental mutations influence wild-type offspring via  
transcriptional adaptation**

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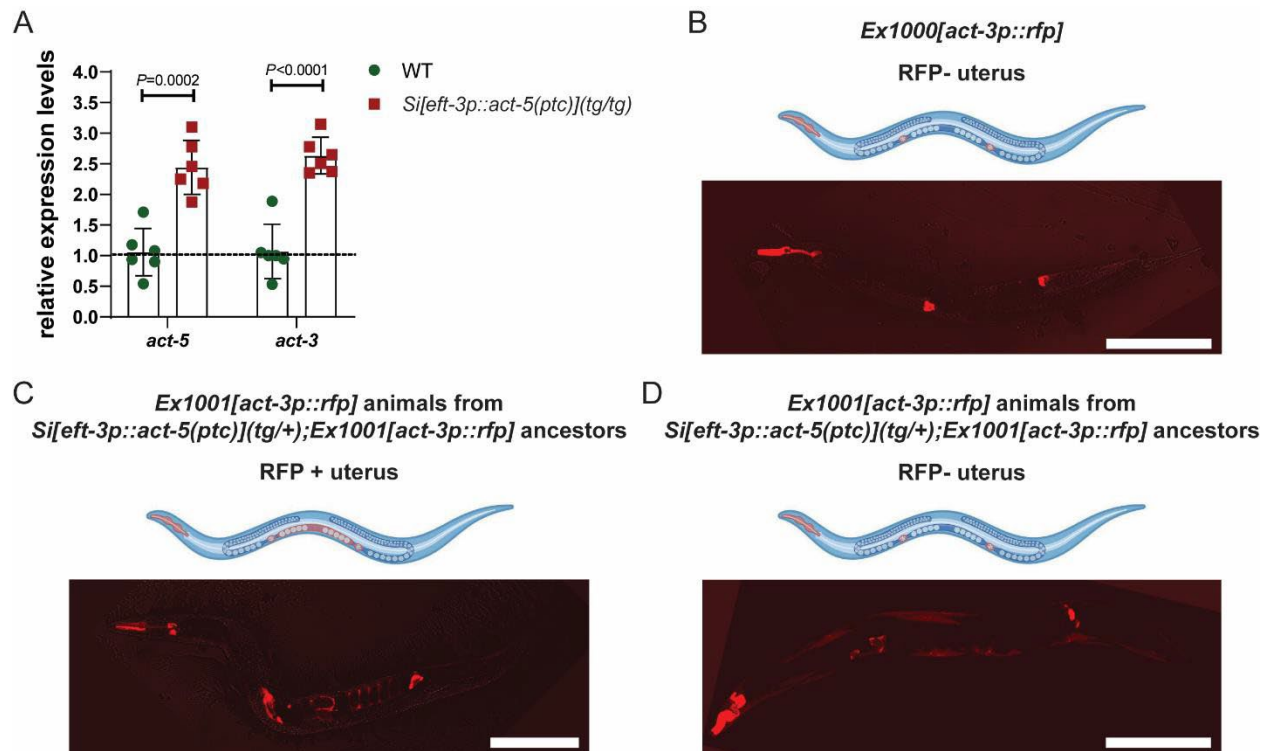
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**The PDF file includes:**

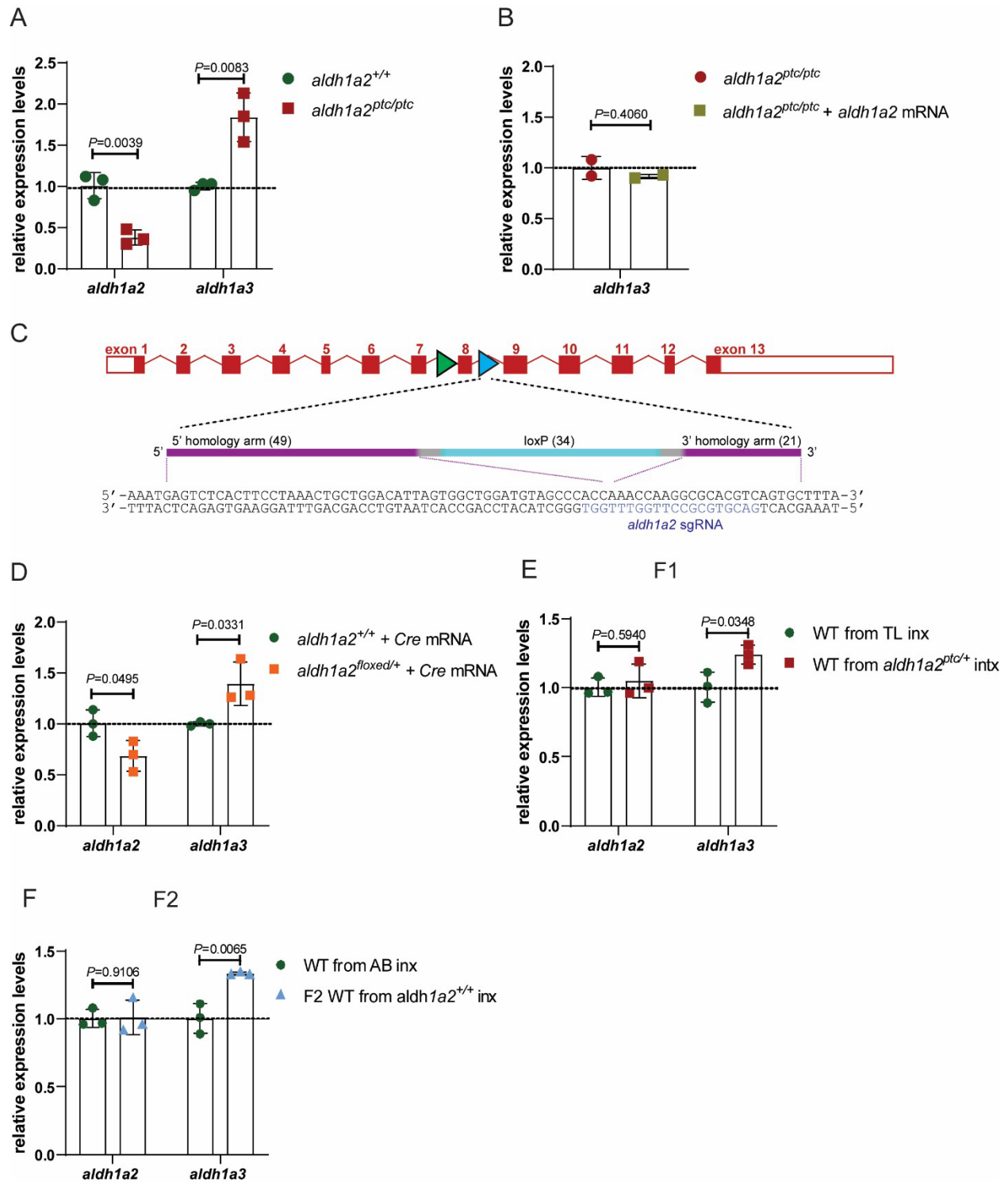
Figs. S1 to S9  
Legends for tables S1 and S2

**Other Supplementary Materials for this manuscript includes the following:**

Tables S1 and S2

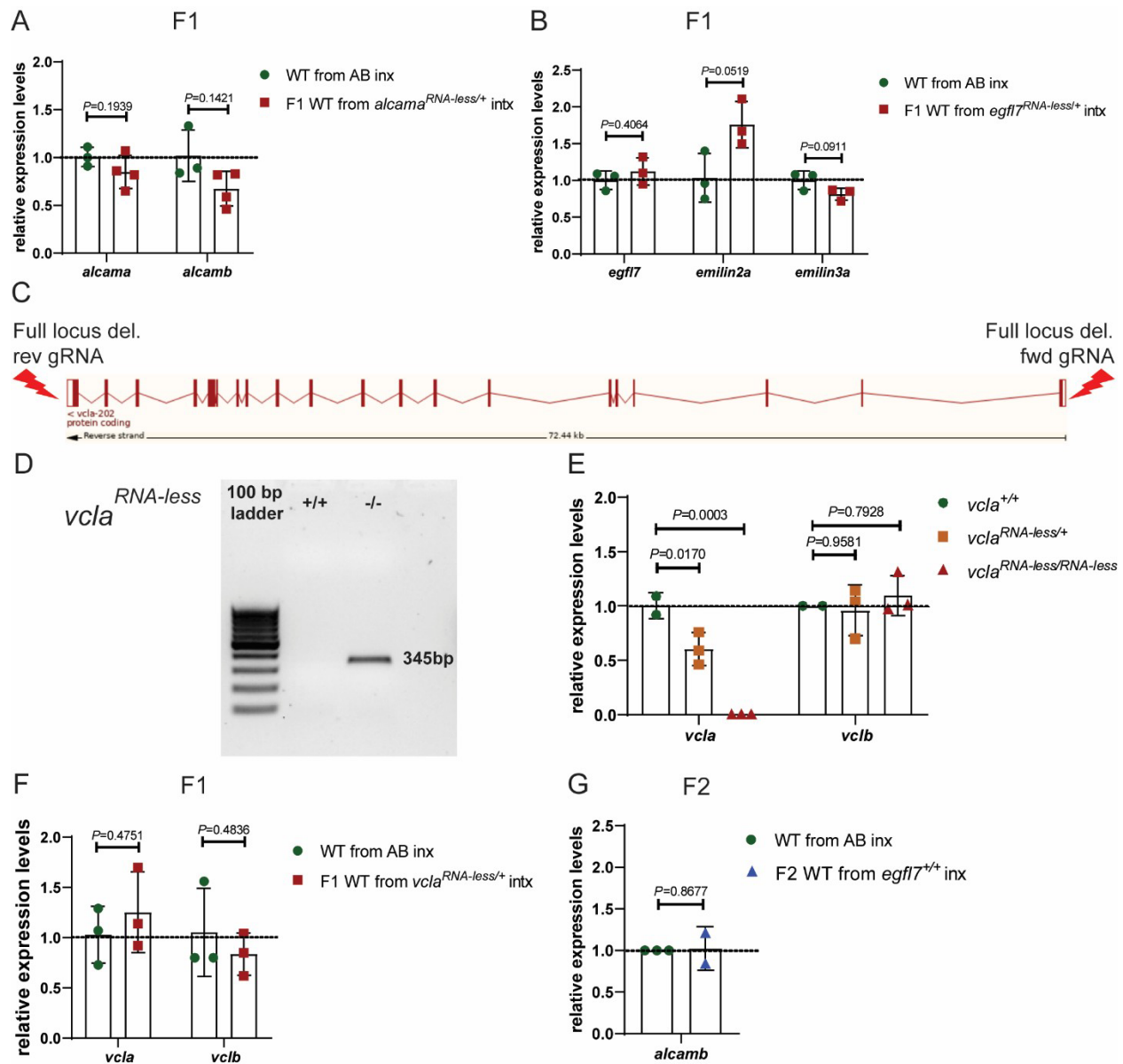


**Fig. S1. Overexpression of *act-5(ptc)* transgene induces transcriptional adaptation and transgenerational inheritance of transcriptional adaptation in *C. elegans*.** (A) Relative mRNA levels of *act-5* and *act-3* in wild-type and *Si[eft-3p::act-5(ptc)](tg/tg)* nematodes.  $n = 6$  biologically independent samples. Data are mean  $\pm$  s.d., and a two-tailed Student's *t*-test was used to calculate *P* values. *Ct* values are listed in Table S1. (B) Representative picture of RFP expression in the pharynx and spermatheca of *Ex1000[act-3p::rfp]* adult control nematodes. (C) Representative picture of RFP expression in the pharynx, spermatheca, and uterus of *Ex1001[act-3p::rfp]* adult wild-type offspring from *Si[eft-3p::act-5(ptc)](tg/+);Ex1001[act-3p::rfp]* nematodes. (D) Representative picture of RFP expression in the pharynx and spermatheca of *Ex1001[act-3p::rfp]* adult wild-type offspring from *Si[eft-3p::act-5(ptc)](tg/+);Ex1001[act-3p::rfp]* nematodes that have lost the ectopic uterus expression. Scale bars: 200  $\mu$ m. Illustrations in panels B to D were created with BioRender.com.

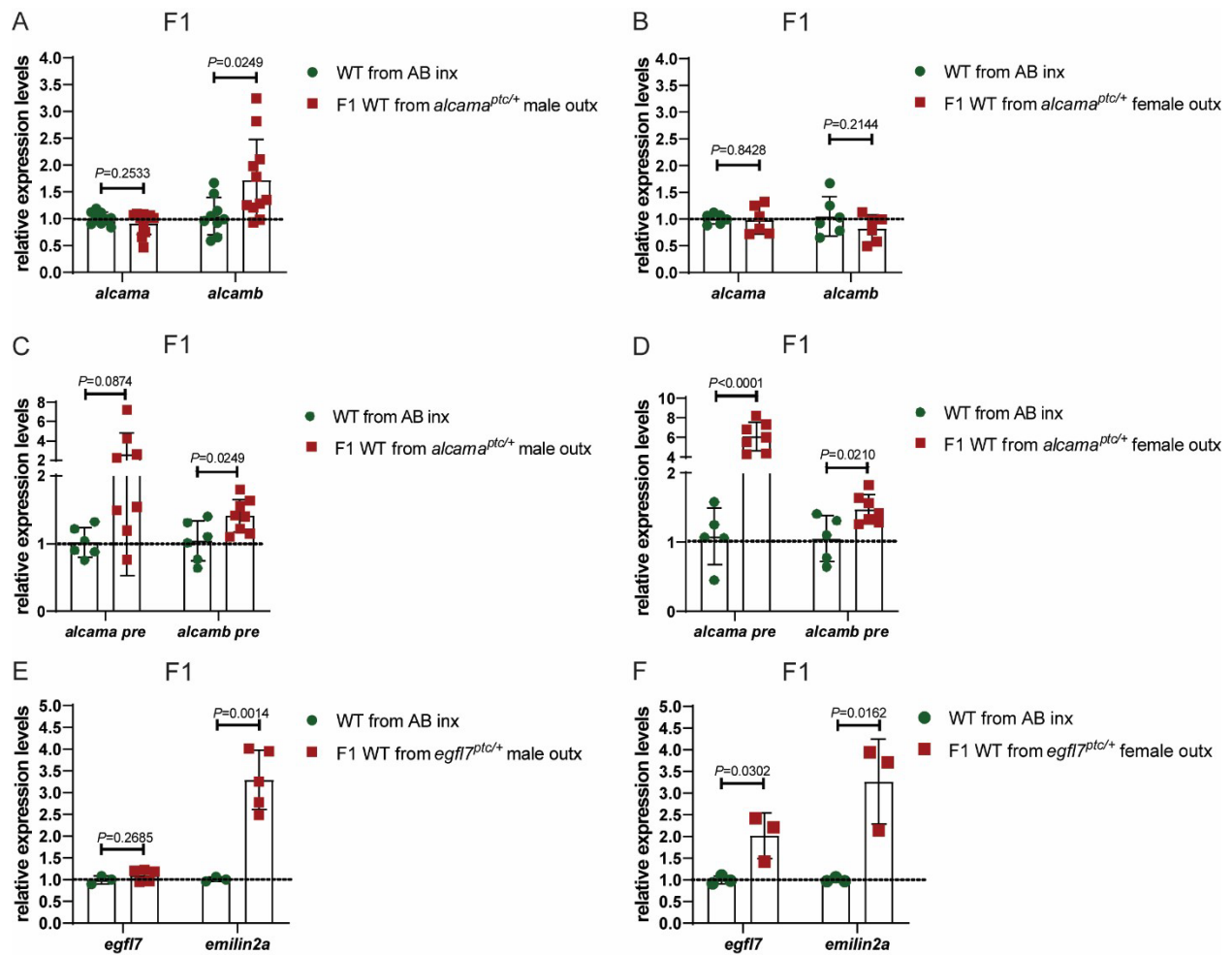


**Fig. S2. *ald1a2*<sup>ptc</sup> and *ald1a2*<sup>floxed</sup> alleles are new zebrafish models of transcriptional adaptation and transgenerational inheritance of transcriptional adaptation.** (A) Relative mRNA levels of *ald1a2* and *ald1a3* in 24 hpf *ald1a2*<sup>ptc/ptc</sup> embryos when compared with *ald1a2*<sup>+/+</sup> siblings. (B) Relative mRNA levels of *ald1a3* in 24 hpf *ald1a2*<sup>ptc/ptc</sup> embryos when compared with *ald1a2*<sup>ptc/ptc</sup> embryos injected with wild-type *ald1a2* mRNA. (C) Schematic view of *ald1a2*<sup>floxed</sup> allele based on Burg et al. (34), with the previously integrated loxP site shown as a green triangle and the additional loxP site shown as a cyan triangle in intron 8 of the *ald1a2* locus; sequence of the recovered loxP integration shown below. (D) Relative mRNA levels of *ald1a2* and *ald1a3* in 24 hpf *ald1a2*<sup>floxed/+</sup> embryos injected with Cre mRNA

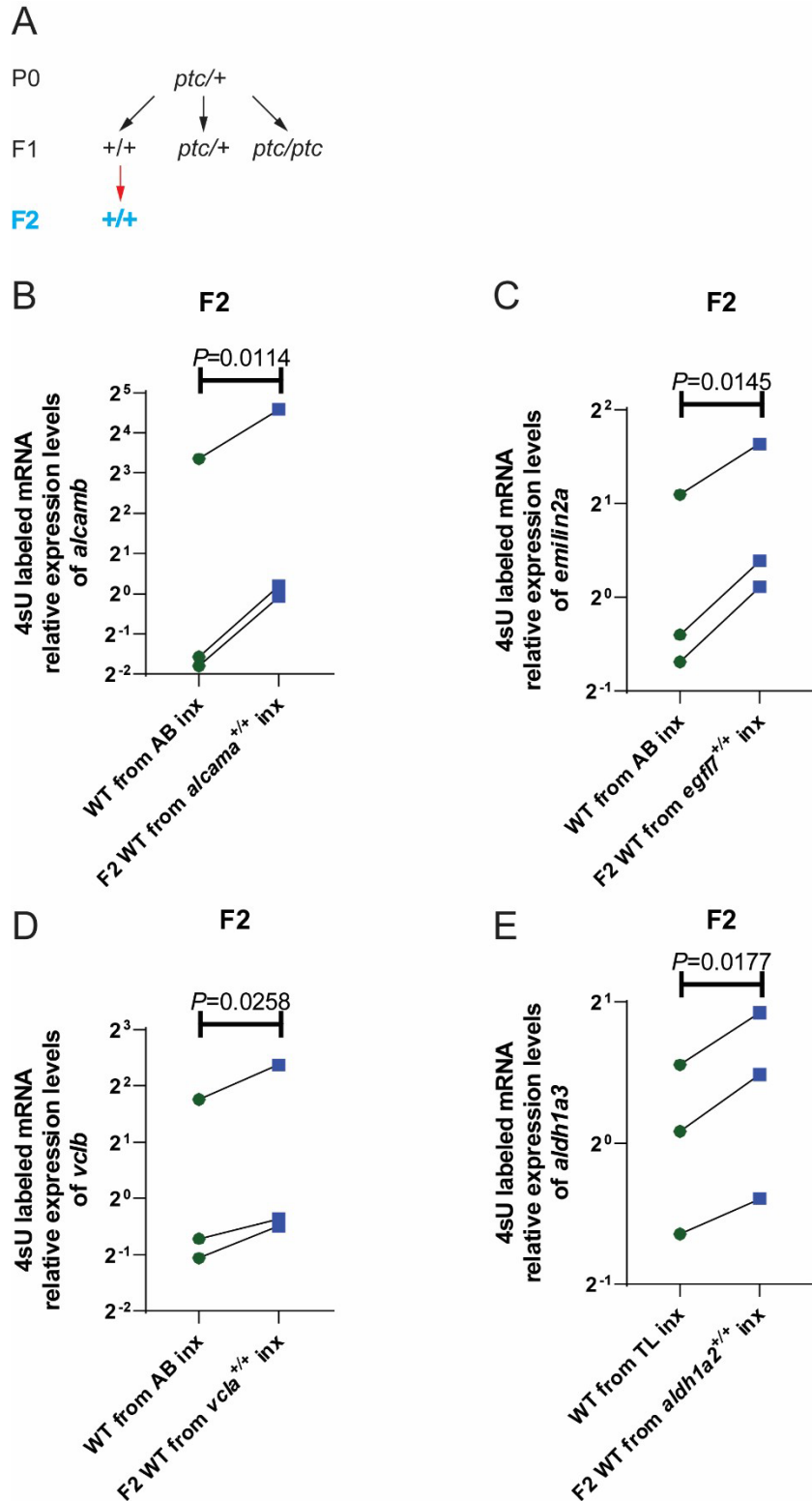
compared with *aldh1a2*<sup>+/+</sup> siblings injected with *Cre* mRNA. **(E)** Relative mRNA levels of *aldh1a2* and *aldh1a3* mRNA in 24 hpf wild-type embryos from TL incrosses (inx) and from *aldh1a2*<sup>ptc/+</sup> intercrosses (intx). **(F)** Relative mRNA levels of *aldh1a2* and *aldh1a3* in 24 hpf wild-type embryos obtained from TL incrosses and F1 *aldh1a2*<sup>+/+</sup> (i.e., wild types from *aldh1a2*<sup>ptc/+</sup> intercrosses) incrosses.  $n \geq 2$  biologically independent samples. Control expression levels were set at 1. Data are mean  $\pm$  s.d., and a two-tailed Student's *t*-test was used to calculate *P* values. *Ct* values are listed in Table S1.



**Fig. S3. Intergenerational inheritance of transcriptional adaptation is not observed in wild-type offspring obtained from RNA-less alleles.** (A) Relative mRNA levels of *alcama* and *alcamb* in 28 hpf wild-type embryos from AB incrosses and *alcama*<sup>RNA-less/+</sup> intercrosses. (B) Relative mRNA levels of *egfl7*, *emilin2a*, and *emilin3a* mRNA in 24 hpf wild-type embryos from AB incrosses and *egfl7*<sup>RNA-less/+</sup> intercrosses. (C) Schematic view of *vcla*<sup>RNA-less</sup> allele. (D) Representative image of gel providing evidence for the genomic deletion in the *vcla*<sup>RNA-less</sup> allele. (E) Relative mRNA levels of *vcla* and *vclb* in 24 hpf *vcla*<sup>RNA-less/RNA-less</sup> embryos when compared with *vcla*<sup>+/+</sup> and *vcla*<sup>RNA-less/+</sup> siblings. (F) Relative mRNA levels of *vcla* and *vclb* mRNA in 24 hpf wild-type embryos from AB incrosses and *vcla*<sup>RNA-less/+</sup> intercrosses. (G) Relative mRNA levels of *alcamb* in 24 hpf wild-type embryos from AB incrosses and F1 *egfl7*<sup>+/+</sup> (i.e., wild types from *egfl7*<sup>ptc/+</sup> intercrosses) incrosses.  $n \geq 2$  biologically independent samples. Control expression levels were set at 1. Data are mean  $\pm$  s.d., and a two-tailed Student's *t*-test was used to calculate *P* values. *Ct* values are listed in Table S1.



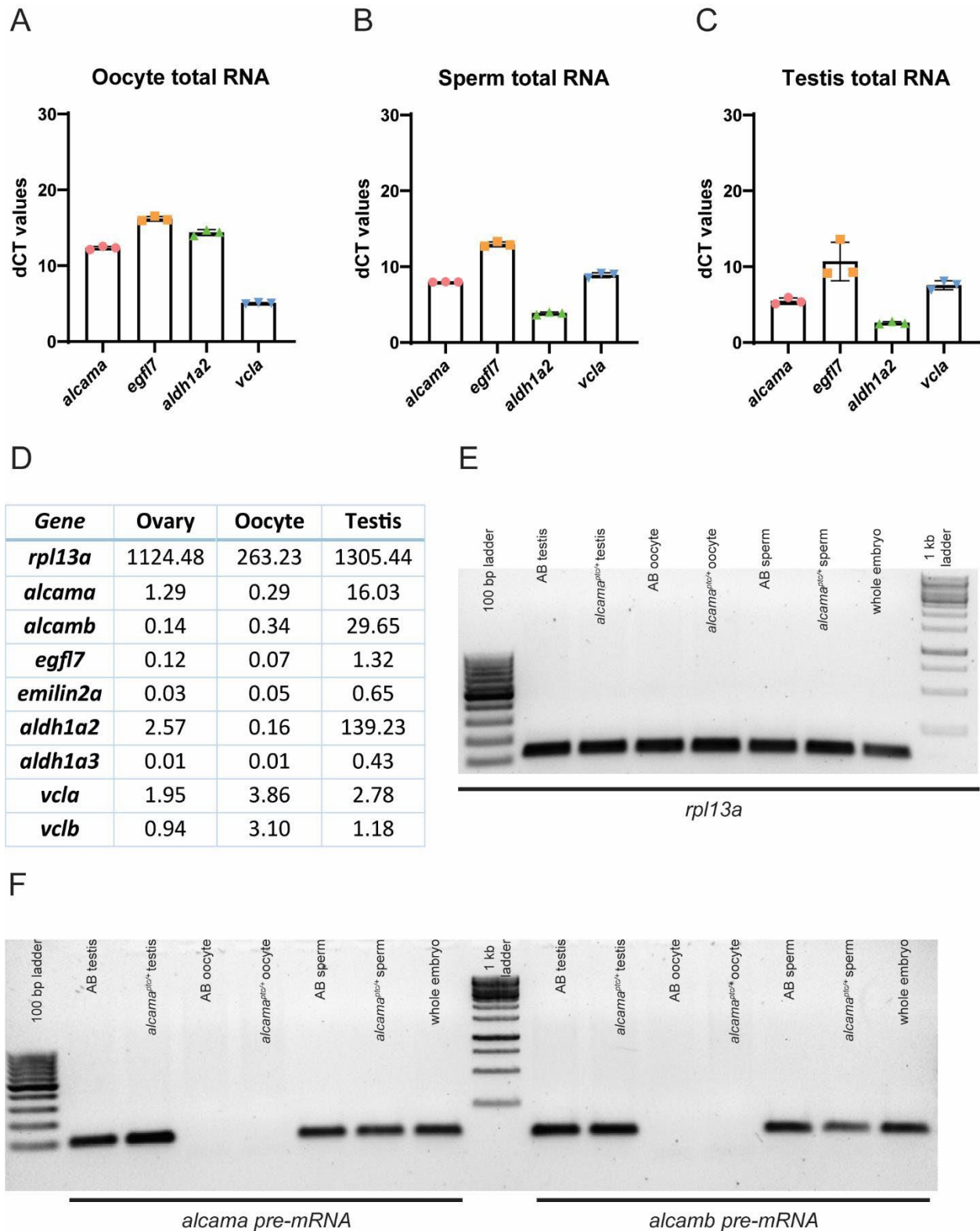
**Fig. S4. Gender regulation of intergenerational inheritance of transcriptional adaptation.** (A) Relative mRNA levels of *alcama* and *alcamb* in 28 hpf wild-type embryos from AB incrosses (inx) and *alcama*<sup>ptc/+</sup> male outcrosses (outx). (B) Relative mRNA levels of *alcama* and *alcamb* in 28 hpf wild-type embryos from AB incrosses and *alcama*<sup>ptc/+</sup> female outcrosses. (C) Relative pre-mRNA levels of *alcama* and *alcamb* in 6 hpf wild-type embryos from AB incrosses and *alcama*<sup>ptc/+</sup> male outcrosses. (D) Relative pre-mRNA levels of *alcama* and *alcamb* in 6 hpf wild-type embryos from AB incrosses and *alcama*<sup>ptc/+</sup> female outcrosses. (E) Relative mRNA levels of *egfl7* and *emilin2a* in 24 hpf wild-type embryos from AB incrosses and *egfl7*<sup>ptc/+</sup> male outcrosses. (F) Relative mRNA levels of *egfl7* and *emilin2a* in 24 hpf wild-type embryos from AB incrosses and *egfl7*<sup>ptc/+</sup> female outcrosses.  $n \geq 3$  biologically independent samples. Control expression levels were set at 1. Data are mean  $\pm$  s.d., and a two-tailed Student's *t*-test was used to calculate *P* values. *Ct* values are listed in Table S1.



**Fig. S5. Transgenerational inheritance of transcriptional adaptation models display increased transcription of the adapting gene.** (A) Genetic crosses used to obtain F2 wild-type offspring from heterozygous zebrafish. Black arrows indicate heterozygous intercrosses (*inx*); red arrow indicates subsequent wild-type incrosses (*inx*). (B) qPCR analysis of 4sU-labelled *alcamb* mRNA levels in 28 hpf wild-type embryos from AB incrosses and from F1 *alcama*<sup>+/+</sup> (i.e., wild types from *alcama*<sup>ptc/+</sup> intercrosses) incrosses. (C) qPCR analysis of 4sU-labelled *emilin2a* mRNA levels in 24 hpf wild-type

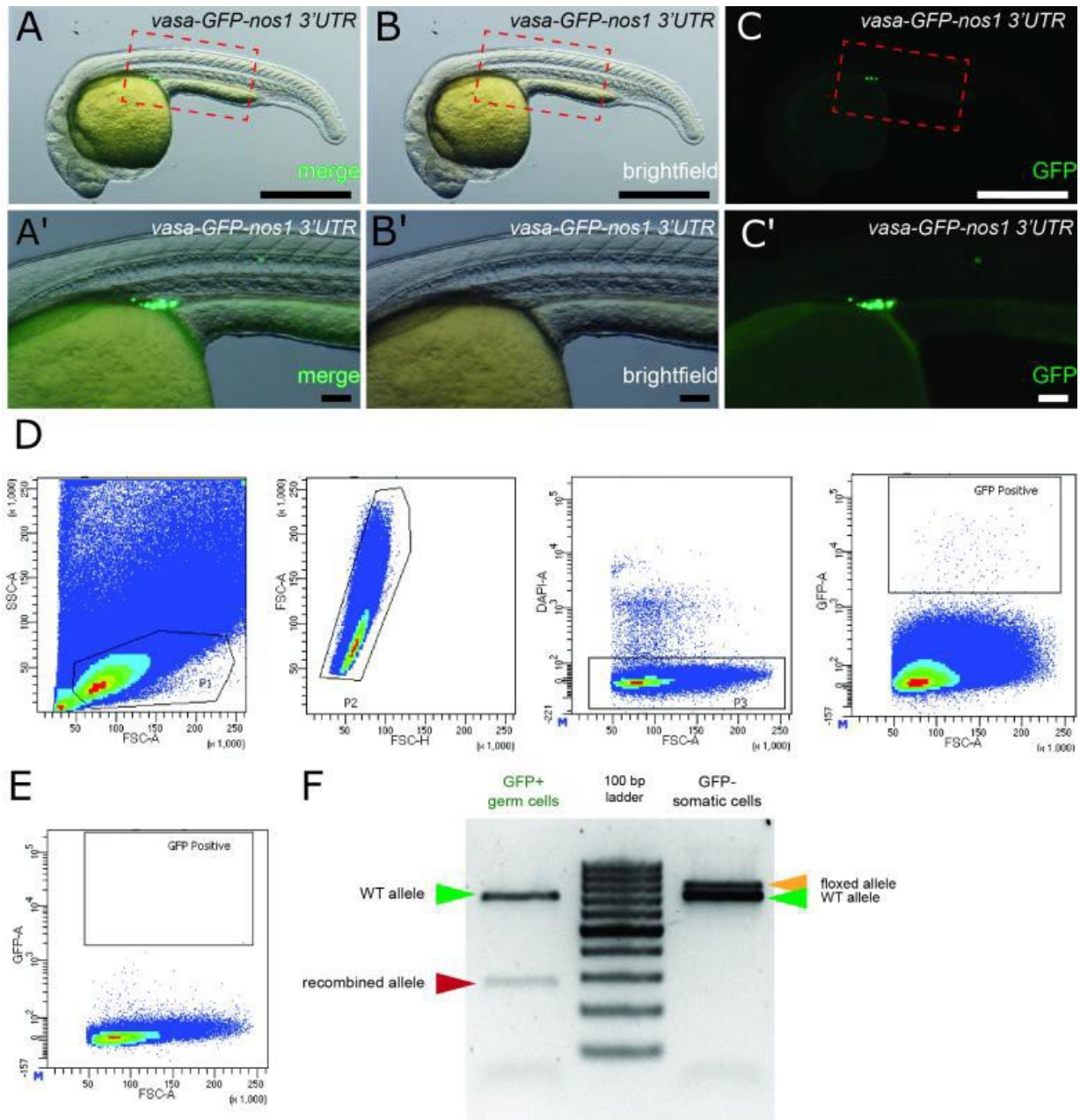
embryos from AB incrosses and from F1 *egfl7*<sup>+/+</sup> (i.e., wild types from *egfl7*<sup>ptc/+</sup> intercrosses) incrosses. (D) qPCR analysis of 4sU-labelled *vclb* mRNA levels in 24 hpf wild-type embryos from AB incrosses and from F1 *vcla*<sup>+/+</sup> (i.e., wild types from *vcla*<sup>ptc/+</sup> intercrosses) incrosses. (E) qPCR analysis of 4sU-labelled *aldh1a3* mRNA levels in 24 hpf wild-type embryos from TL incrosses and from F1 *aldh1a2*<sup>+/+</sup> (i.e., wild types from *aldh1a2*<sup>ptc/+</sup> intercrosses) incrosses. *n* = 3 biologically independent samples. Control expression levels were set at 1. A two-tailed paired *t*-test was used to calculate *P* values. *Ct* values are listed in Table S1.



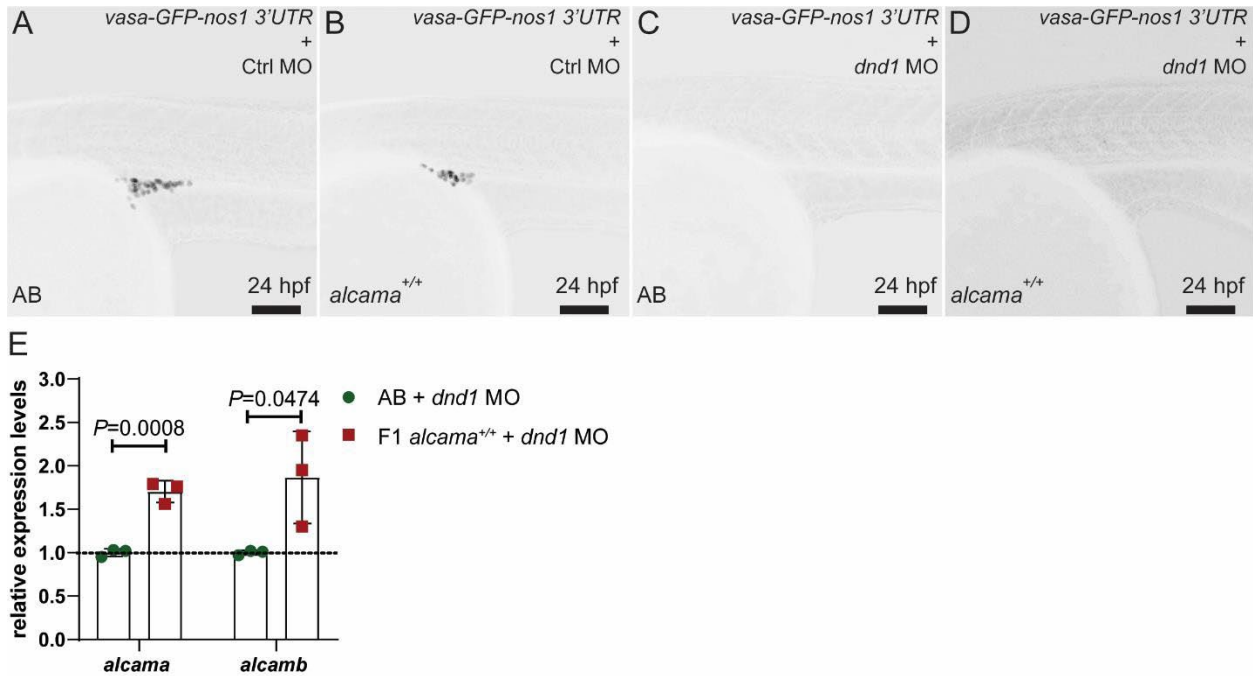


**Fig. S6. Mutated genes in transgenerational inheritance of transcriptional adaptation models are expressed in germ cells.** (A) qPCR analysis of *alcama*, *egfl7*, *aldh1a2*, and *vcla* mRNA levels from oocytes of wild-type zebrafish. (B) qPCR analysis of *alcama*, *egfl7*, *aldh1a2*, and *vcla* mRNA levels from sperm of wild-type zebrafish. (C) qPCR analysis of *alcama*, *egfl7*, *aldh1a2*, and *vcla* mRNA levels from testes of wild-type zebrafish. (D) Transcripts per million for *rpl13a*,

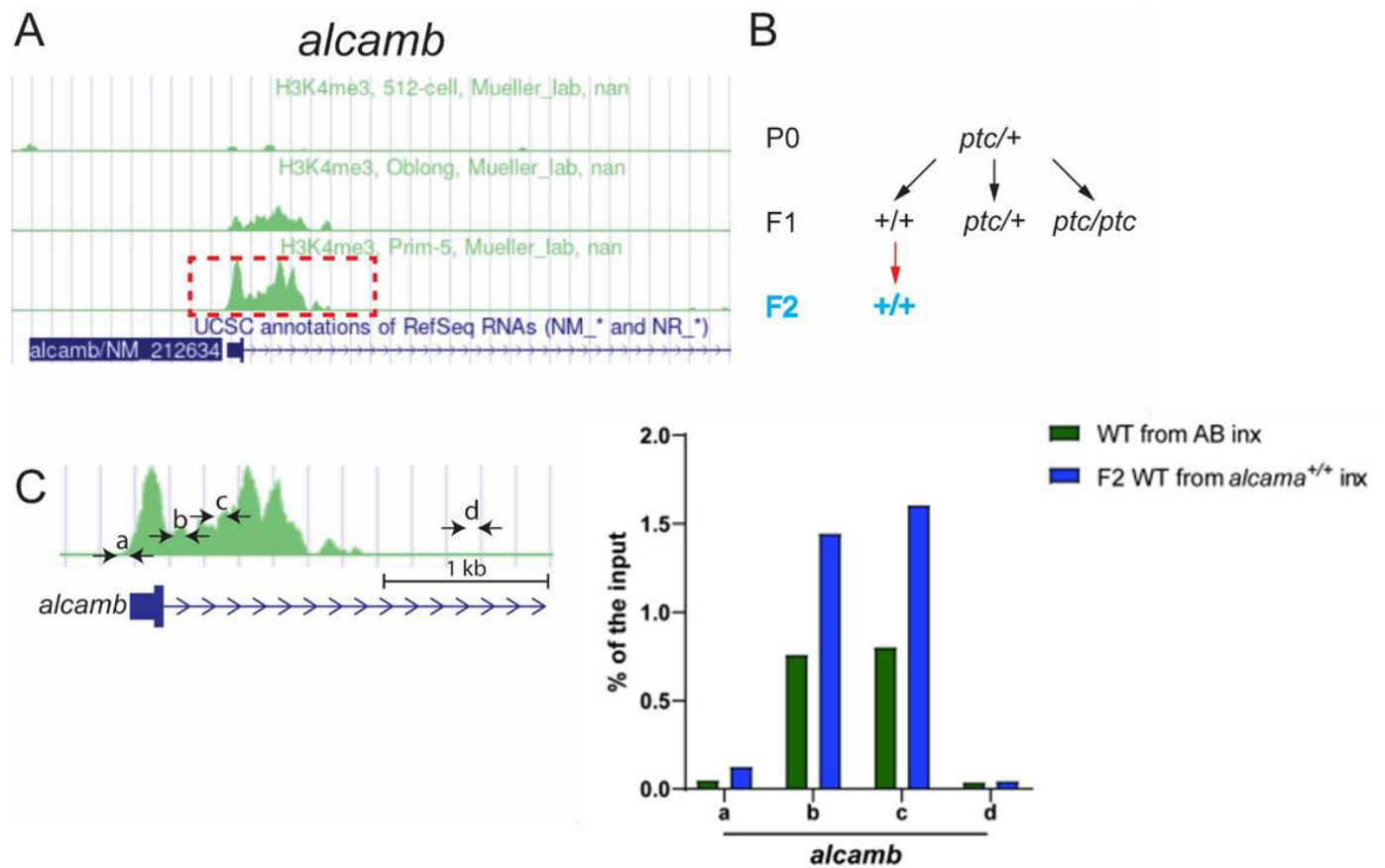
*alcama*, *alcamb*, *egfl7*, *emilin2a*, *aldh1a2*, *aldh1a3*, *vcla*, and *vclb* in the indicated tissues, as obtained from the GSE111882 transcriptome dataset. (E) Gel electrophoresis of qPCR products of the housekeeping gene *rpl13a* from total RNA samples of testes, oocytes and sperm obtained from wild-type and *alcama*<sup>ptc/+</sup> zebrafish with total RNA sample obtained from 6 hpf wild-type embryos as control. (F) Gel electrophoresis of qPCR products of *alcama pre-mRNA* and *alcamb pre-mRNA* from total RNA samples of testes, oocytes and sperm obtained from wild-type and *alcama*<sup>ptc/+</sup> zebrafish with total RNA sample obtained from 6 hpf wild-type embryos as control.



**Fig. S7. *aldh1a2<sup>floxed</sup>* allele shows germline-specific recombination upon injection of *vasa-Cre-nos1 3'UTR* mRNA.** (A to C) Lateral views of 24 hpf wild-type embryos injected with *vasa-GFP-nos1 3'UTR* mRNA. Scale bars: 1000  $\mu$ m. (A' to C') Higher magnification images of red boxed area in panels A, B and C, respectively. Scale bars: 100  $\mu$ m. (D) FACS contour plots of cells isolated from 24 hpf embryos from *aldh1a2<sup>floxed/+</sup>* intercrosses (intx) injected with *vasa-GFP-nos1 3'UTR* mRNA plus *vasa-Cre-nos1 3'UTR* mRNA; GFP+/DAPI- cells were isolated. (E) Negative control FACS contour plots of cells isolated from 24 hpf embryos from *aldh1a2<sup>floxed/+</sup>* intercrosses. (F) *aldh1a2<sup>floxed</sup>* allele displays germline-specific recombination upon injection of *vasa-Cre-nos1 3'UTR* mRNA. Green arrowheads point to wild-type allele; yellow arrowhead points to *aldh1a2<sup>floxed</sup>* allele; red arrowhead points to recombined *aldh1a2<sup>floxed</sup>* allele.



**Fig. S8. Intergenerational inheritance of transcriptional adaptation is detected in somatic cells.** (A, B) Lateral views of 24 hpf wild-type embryos from AB incrosses (inx) and from *alcama*<sup>ptc/+</sup> intercrosses (intx) injected with 100 pg *vasa-GFP-nos1 3'UTR* mRNA plus 0.6 ng control MO. Scale bars: 100 μm. (C, D) Lateral views of 24 hpf wild-type embryos from AB incrosses and from *alcama*<sup>ptc/+</sup> intercrosses injected with 100 pg *vasa-GFP-nos1 3'UTR* mRNA plus 0.6 ng *dnd1* MO. Scale bars: 100 μm. (E) Relative mRNA levels of *alcamb* in 28 hpf AB embryos and F1 *alcama*<sup>+/+</sup> (i.e., wild types from *alcama*<sup>ptc/+</sup> intercrosses) embryos injected with 100 pg *vasa-GFP-nos1 3'UTR* mRNA plus 0.6 ng *dnd1* MO.  $n = 3$  biologically independent samples. Control expression levels were set at 1. Data are mean  $\pm$  s.d., and a two-tailed Student's *t*-test was used to calculate *P* values. *Ct* values are listed in Table S1.



**Fig. S9. Transgenerational inheritance of transcriptional adaptation in zebrafish is associated with H3K4me3 histone marks.** (A) Peaks of H3K4me3 ChIP-seq at the *alcamb* locus at different stages (obtained from DANIO-CODE). Red boxed area was enlarged in (C) to show the relative location of the ChIP-qPCR primers marked by black arrows. (B) Genetic crosses used to obtain F2 wild-type offspring from heterozygous zebrafish. Black arrows indicate heterozygous intercrosses (inx); red arrow indicates subsequent wild-type incrosses (inx). (C) Chromatin immunoprecipitation coupled with qPCR analysis of H3K4me3 occupancy near exon 1 of *alcamb* in high stage (3.3 hpf) wild-type embryos from incrosses of F1 *alcama*<sup>+/+</sup> (i.e., wild types from *alcama*<sup>ptc/+</sup> intercrosses), compared with wild-type embryos from AB incrosses. Green peaks in *alcamb* locus represent prim-5 stage (24 hpf) H3K4me3 ChIP-seq data obtained from DANIO-CODE. Scale bar: 1 kb. *Ct* values are listed in Table S1.

**Table S1: Ct values for Fig. 2-5 and Fig. S1-S9.**

**Table S2: Used oligos for qPCR primers, genotyping primers, gRNA for mutagenesis, and primers for cloning.**