Expanded View Figures

Figure EV1. Validation of PACE.

A A scatter plot showing the reproducibility of the codon effects measured by PACE in two replicates.
B A scatter plot comparing the codon effects measured by PACE in the absence (x-axis) and in the presence of GFP MO (y-axis).
C A scatter plot comparing codon usage in the zebrafish genome (per thousand codons, x-axis) and the codon effects measured by PACE (y-axis).
D A scatter plot comparing CSCs (Bazzini *et al*, 2016, x-axis) and the codon effects measured by PACE (y-axis).
E, F Comparison of the codon effects measured by PACE (E) with the results of qRT–PCR (F). mRNA levels at 6 hpf relative to 2 hpf are shown. The experiments were repeated three times. Error bars show SD.

Data information: In (A–D), the regression line is shown in blue and the 95% confidence interval is shown in gray. *r* represents Pearson's correlation. The significance was calculated using Student's *t*-test.
Source data are available online for this figure.

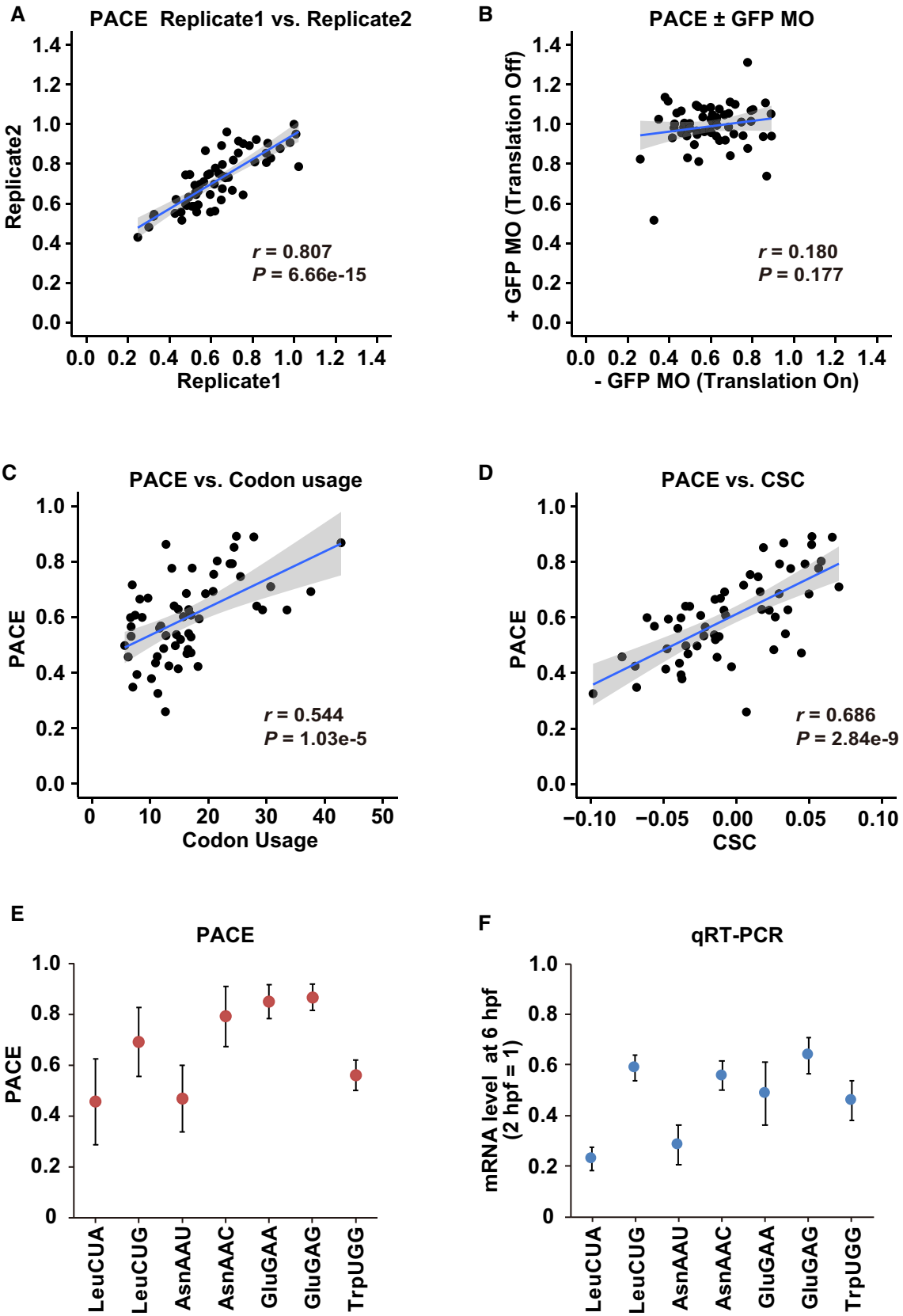


Figure EV1.

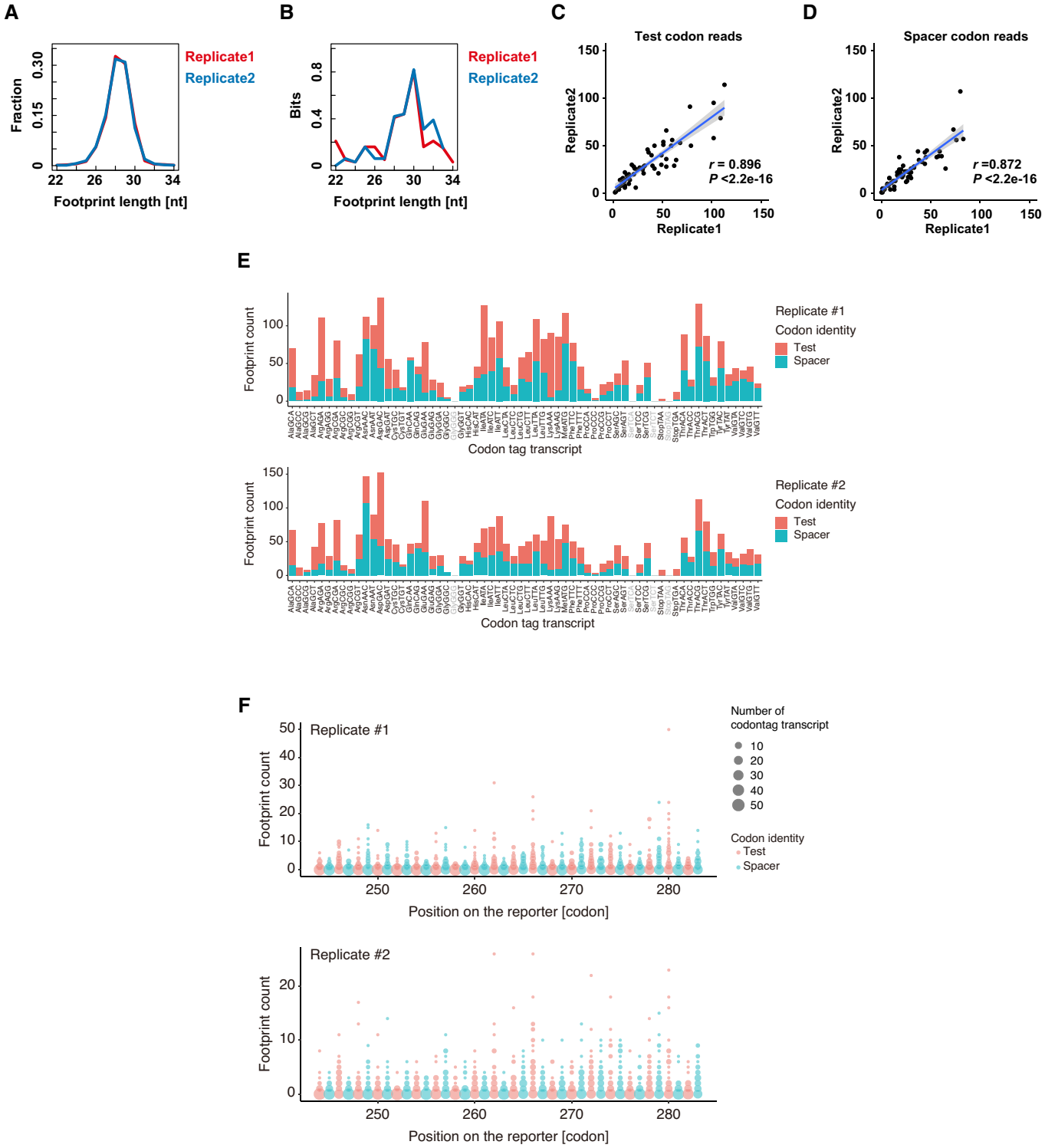


Fig EV2.

Figure EV2. Validation of ribosome footprint analysis.

A Distribution of the ribosome footprint lengths derived from codon-tag sequences. The results of the two replicates are plotted separately in red and blue.

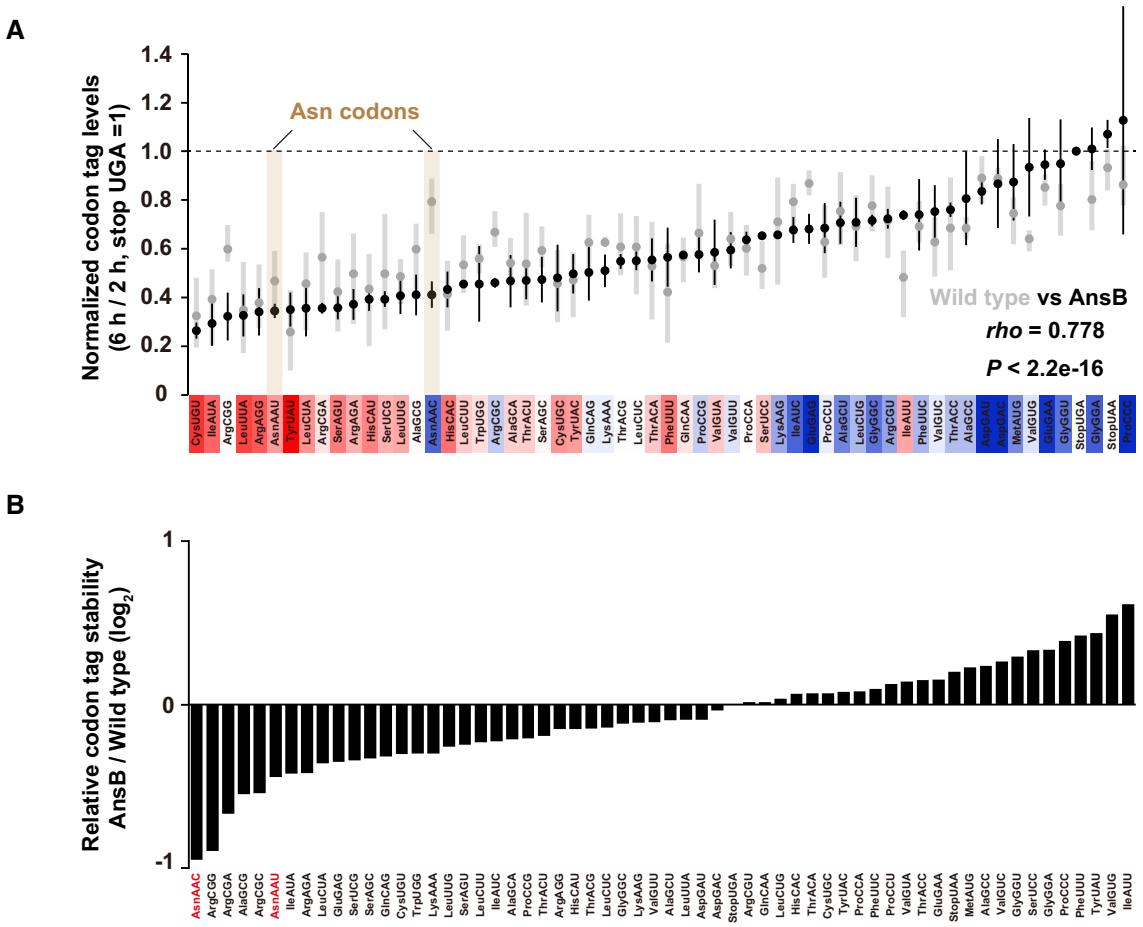
B Evaluation of the codon periodicity by calculating the relative entropy of information content (Kullback–Leibler divergence) of each reading frame using ribosome footprints aligned to the codon-tag reporter sequences. In this analysis, higher bits indicate the presence of periodicity in a particular reading frame. The results of the two replicates are plotted separately in red and blue.

C, D Scatter plots showing the reproducibility of ribosome footprint analysis using PACE reporters. Two replicates for test codon reads (C) and spacer codon reads (D) are shown. The regression line is shown in blue and the 95% confidence interval is shown in gray. *r*, Pearson's correlation. Significance was calculated by Student's *t*-test.

E Numbers of ribosome footprints with a test codon (pink) or a spacer codon (turquoise) in the corresponding A-site position are shown for each codon-tag transcript. The results of the two replicates are plotted separately.

F Distributions of the ribosome footprints along the codon-tag sequences. The size of the circles indicates the number of codon-tag transcripts that generated footprints at each codon-tag position. The y-axis indicates the number of counted footprints. The results of the two replicates are plotted separately.

Source data are available online for this figure.



D

Emsemble Gene ID	Gene	Fold change
ENSDARG00000016375	*asns*	7.41
ENSDARG00000016733	*psat1*	1.96
ENSDARG00000039269	*arg2*	1.94
ENSDARG00000000551	*slc1a4*	42.40
ENSDARG00000062821	*slc6a15*	25.46
ENSDARG00000009901	*slc38a5a*	9.34
ENSDARG00000020645	*slc7a3a*	5.03
ENSDARG00000036427	*slc3a2a*	2.31
ENSDARG00000045886	*slc38a2*	2.29

Figure EV3. PACE under asparagine deprivation conditions.

A Results of PACE in AnsB-expressing zebrafish embryos. Black circles show the relative stability of the reporter mRNAs (averages of two biological replicates) in AnsB-expressing embryos. Gray circles show the relative stability of reporter mRNAs in wild-type embryos presented in Fig 2B. The stability of a codon-tag reporter with a UGA stop codon is set to one. Error bars represent maximum and minimum data points. The relative effect of each codon on mRNA stability in wild-type embryos measured in Fig 2B is shown as a color gradation with red (destabilizing) to blue (stabilizing). *rho*, Spearman's correlation. Significance was calculated by Student's *t*-test.

B Relative change in codon-tag reporter stability between wild-type and AnsB-expressing embryos. PACE results in AnsB-expressing embryos were divided by PACE results in wild-type embryos and are shown as bar charts. Asn codons are indicated in red.

C Western blotting to detect phospho-eIF2α (Ser51) and pan-eIF2α at 6 hpf in the absence or in the presence of AnsB. Tubulin was detected as a loading control.

D A list of genes related to amino acid metabolism or transport and upregulated in AnsB-expressing embryos compared to wild-type embryos at 6 hpf. Fold changes assessed by RNA-Seq and ensemble gene ID are shown.

Source data are available online for this figure.

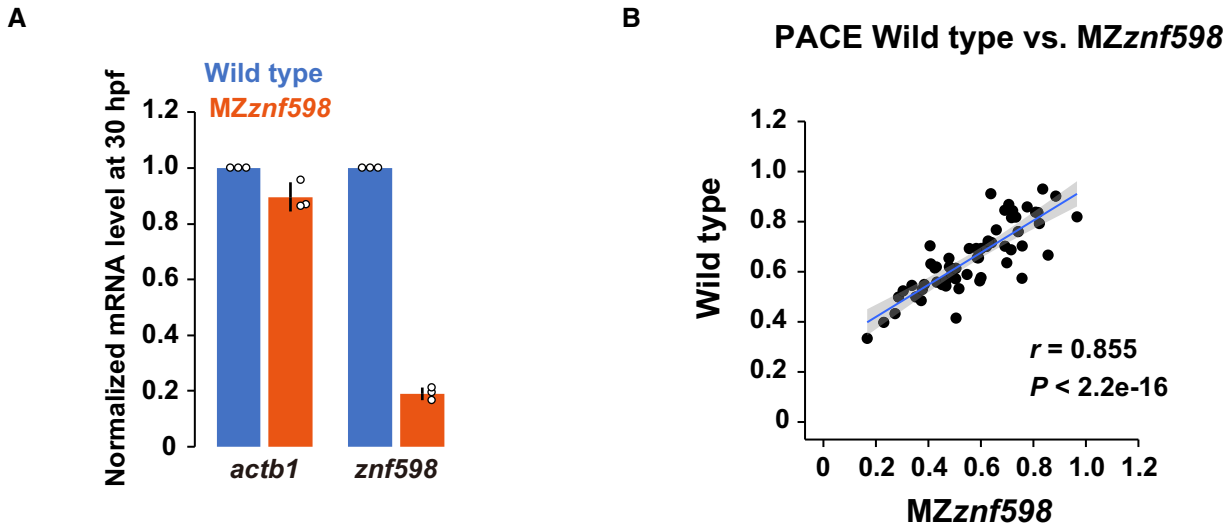


Figure EV4. Validation of MZ*znf598* mutant.

A qRT–PCR analysis of *actb1* and *znf598* mRNA levels in wild-type and MZ*znf598* embryos at 24 hpf. The results are normalized to 18S rRNA levels. The mRNA levels in wild type were set to one. The experiments were repeated three times. Error bars show SD. Individual data points are shown as dots.

B A scatter plot comparing the codon effects measured by PACE in MZ*znf598* (x-axis) and wild-type (y-axis) embryos. The regression line is shown in blue, and the 95% confidence interval is shown in gray. *r*, Pearson's correlation. Significance was calculated by Student's *t*-test.

Source data are available online for this figure.

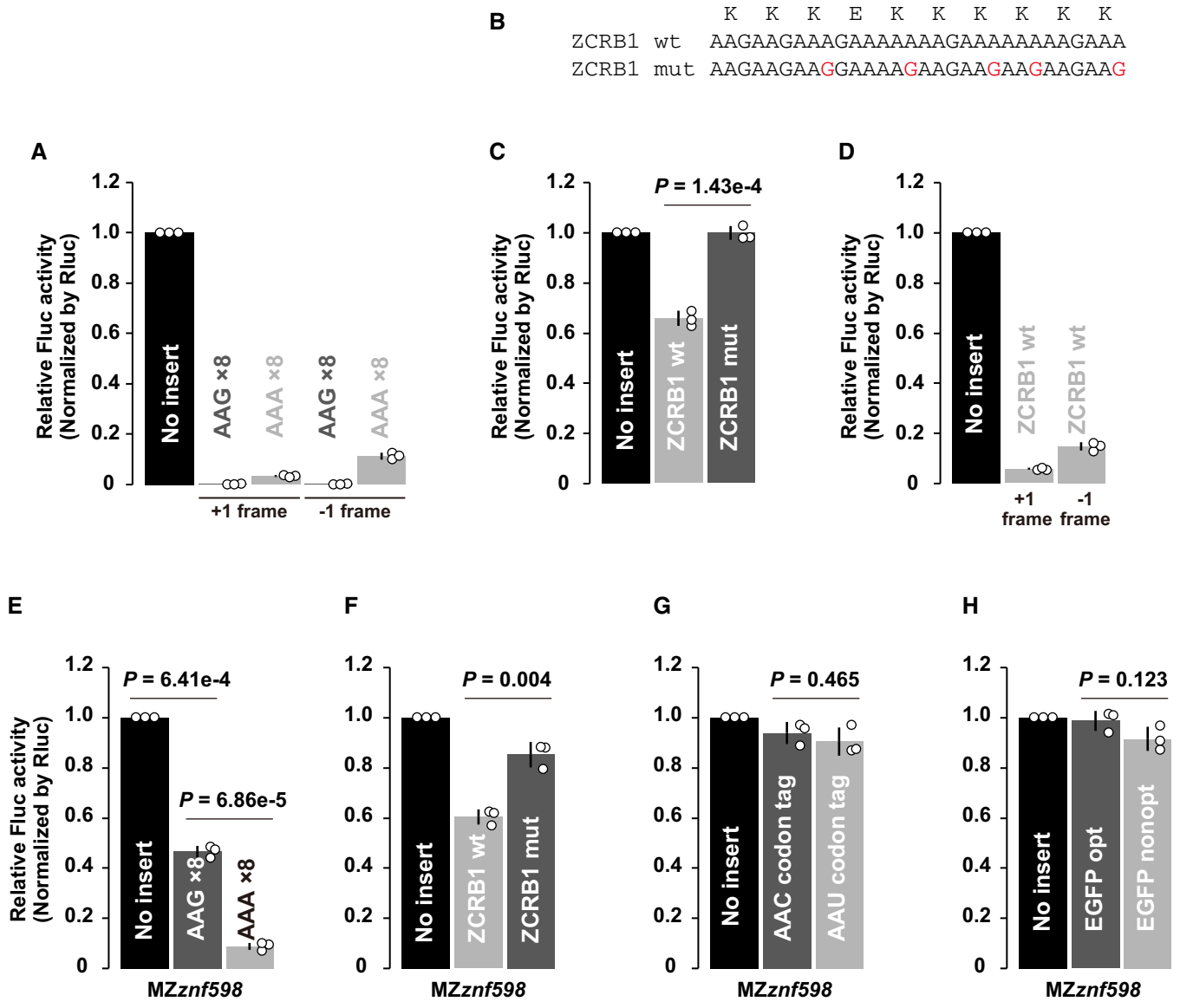


Figure EV5. Additional analysis of the translation elongation rate in zebrafish embryos.

A Results of the tandem ORF assay to detect frameshifting with Lys AAG×8 or Lys AAA×8 sequences. One or two guanine nucleotides (G or GG) were inserted after Lys AAG×8 or Lys AAA×8 sequences to detect protein products from +1 or −1 frame.

B Wild-type (wt) or mutated (mut) stalling sequences of human ZCRB1. Mutated nucleotides were indicated in red. The amino acid sequence is shown above.

C Results of the tandem ORF assay with wild-type (wt) or mutated (mut) stalling sequences of ZCRB1.

D Results of the tandem ORF assay to detect frameshifting with a ZCRB1-stalling sequence. One or two guanine nucleotides (G or GG) were inserted after the ZCRB1 sequence to detect protein products from +1 or −1 frame.

E Results of the tandem ORF assay with Lys AAG×8 or Lys AAA×8 sequences in MZ*znf598* embryos.

F Results of the tandem ORF assay with wild-type (wt) or mutated (mut) stalling sequences of ZCRB1 in MZ*znf598* embryos.

G Results of the tandem ORF assay with Asn AAC and Asn AAU codon tags in MZ*znf598* embryos.

H Results of the tandem ORF assay using EGFP ORFs with different codon optimalities in MZ*znf598* embryos.

Data information: In all experiments, normalized Fluc activity with no insert was set to one. All experiments were repeated three times, and the average Fluc activity is shown as bar charts. Error bars show SD. Individual data points are shown as dots. *P*-values were calculated using two-sided Student's *t*-test.
Source data are available online for this figure.