SUPPLEMENTAL MATERIAL

LEGENDS TO SUPPLEMENRAL FIGURES

Figure S1. Identification of *ATF6α*- and *ATF6β*-knockout medaka

Structure of the *ATF6α* gene consisting of 16 exons and sequencing patterns of a part of exon 5 obtained with wild-type fish as well as the *K149X*-homozygote (left), and structure of the *ATF6β* gene consisting of 17 exons and sequencing patterns of a part of exon 6 obtained with wild-type fish as well as the *S143X*-homozygote (right) are shown. Exon 5 of the *ATF6α* gene was amplified from the mutant library with specific primers designed using information provided by the Ensemble genome browser (http://www.ensembl.org/Oryzias_latipes/Info/Index), and the resulting PCR products were directly sequenced, revealing that one PCR fragment contained a nonsense mutation (TAG) in addition to the wild-type sequence (AAG coding for K149, left); the sequencing patterns of genomic DNA prepared from wild-type fish and homozygotes produced later are shown for simplicity. *in vitro* fertilization was conducted using frozen stock of the corresponding cryopreserved sperm and eggs from wild-type female fish, resulting in production of *ATF6α*-heterozygotes (N2). Male *ATF6α*-heterozygotes were backcrossed against wild-type female fishes seven more times to eliminate mutations other than K149X, obtaining *ATF6α*-heterozygotes (N9). Similarly, exon 6 of the *ATF6β* gene was amplified and the resulting PCR products were subjected to high resolution melting curve analysis as described in MATERIALS and METHODS. Sequencing of fragments showing altered melting points revealed that one PCR fragment contained a nonsense mutation (TAG) in addition to the wild-type sequence (TCG coding for S143right) *ATF6β*-heterozygotes (N2) were obtained by *in vitro* fertilization.

ATF6α-heterozygotes (N9) were crossed with female *ATF6β*-heterozygotes (N2) to obtain male *ATF6α* (N9)- and *ATF6β* (N2)-double heterozygotes, which were then crossed with a wild-type female transgenic line carrying the PBiP-*EGFP* reporter gene (Ishikawa *et al.*, 2011). Resulting *ATF6α* (N10)- and *ATF6β* (N3)-double heterozygotes carrying the PBiP-*EGFP* reporter gene were in-crossed to obtain *ATF6α/β*-double hetero, *ATF6α*-single knockout, and *ATF6β*-single knockout medaka, each carrying the PBiP-*EGFP* reporter gene.

Fig. S2 Identification of BiP mutant medaka

Structure of the *BiP* gene and sequencing patterns of a part of exon 1 obtained with wild-type fish as well as the *BiP(S38P)*-homozygote are shown. No nonsense mutations were found when exons 1-3 were amplified from the mutant library, but a missense mutation in the *BiP* coding region (TCT -> CCT, S38P) was obtained. By *in vitro* fertilization and backcrossing against wild-type female fishes, *BiP(S38P)*-heterozygotes (N10) were obtained.

Fig. S3 Monitoring of physiological ER stress occurring during early embryonic development

An embryo at 1 dpf (stage 15-20) and a different embryo at 2 dpf (stages 20-24) of *ATF6α/β*-double hetero carrying the P$_{BiP}$-*EGFP* reporter gene were analyzed by fluorescent microscopy. Note that three different exposure lengths were employed, 10000 ms (stages 15-17, top two panels), 1000 ms (stages 15-20, middle two panels), and 500 ms (stages 20-24, bottom two panels).

Fig. S4 Effect of morpholino-mediated knockdown on the level of target mRNA

Total RNA was prepared from wild-type embryo at 1dpf, into which control morpholino (left, A, B and C) or morpholino targeting *Col8a1-2* (right, A), *LamB1-1* (right, B) or *LamC1* (right, C) had been microinjected at the one-cell stage, and then subjected to quantitative RT-PCR to determine the level of *Col8a1-2* (A), *LamB1-1* (B) or *LamC1* (C) mRNAs relative to *β-actin* mRNA (n=2). Means with standard deviations (error bars) are shown.

Fig. S5 Effect of *ATF6α/β*-double knockout on gene expression

(A) Expression levels of various medaka homologues of genes known to be involved in notochord formation, Brachyury (Schulte-Merker *et al.*, 1994), laminins (Parsons *et al.*, 2002), components of COPI transport vesicles (Coutinho *et al.*, 2004), lysyl oxidases (Gansner *et al.*, 2007), collagens (Gansner *et al.*, 2008; Pagnon-Minot *et al.*, 2008) and fibrillins (Gansner *et al.*, 2008) in zebrafish; as well as Sox5 and

Sox6 transcription factors in mice (Smits and Lefebvre, 2003) are shown as in Fig. 5C.

(B) Embryos at 2 dpf of wild-type fishes were treated with various concentrations of tunicamycin for 24 h. Total RNA was extracted and analyzed by northern blot hybridization, as in Fig. 1D.

(C) Embyos at 1 dpf of wild-type fishes were treated with various concentrations of tunicamycin for 24 h and then subjected to whole mount *in situ* hybridization to detect *Brachyury* mRNA.

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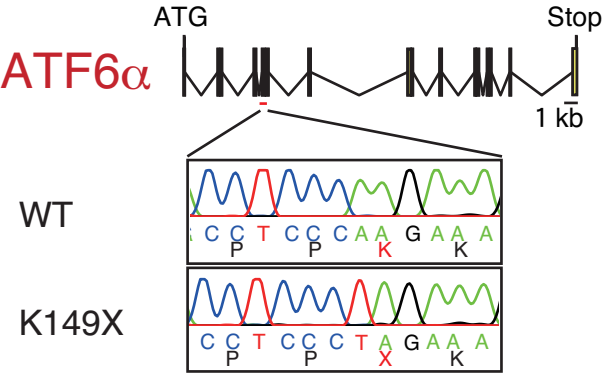
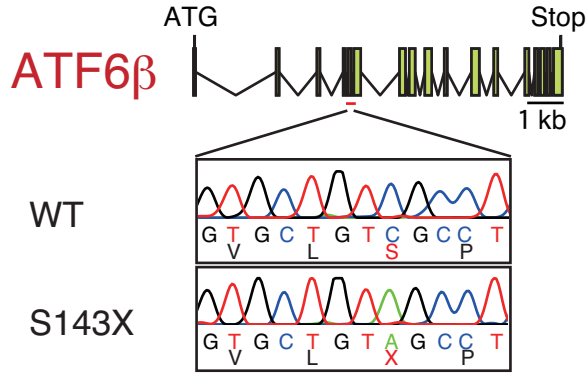
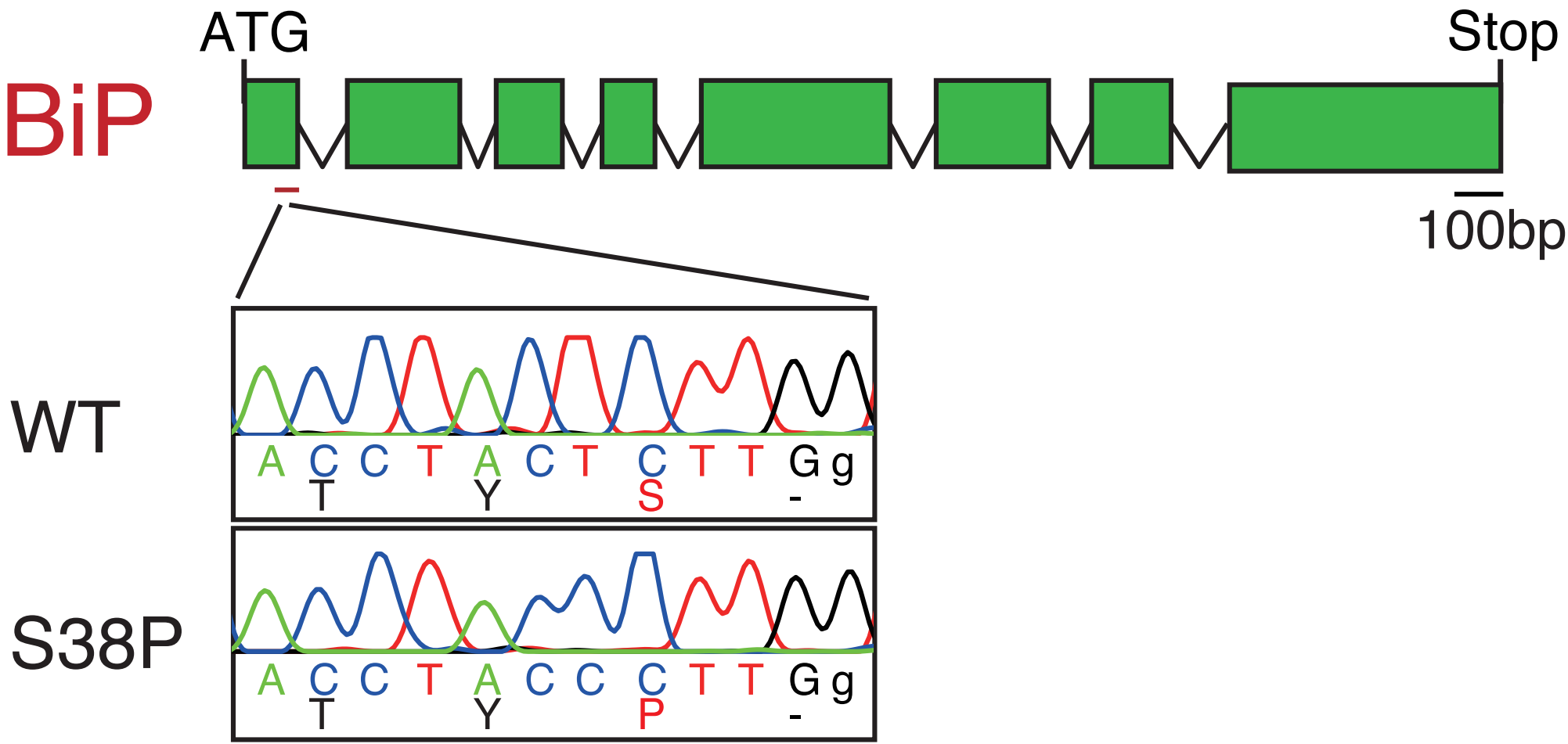
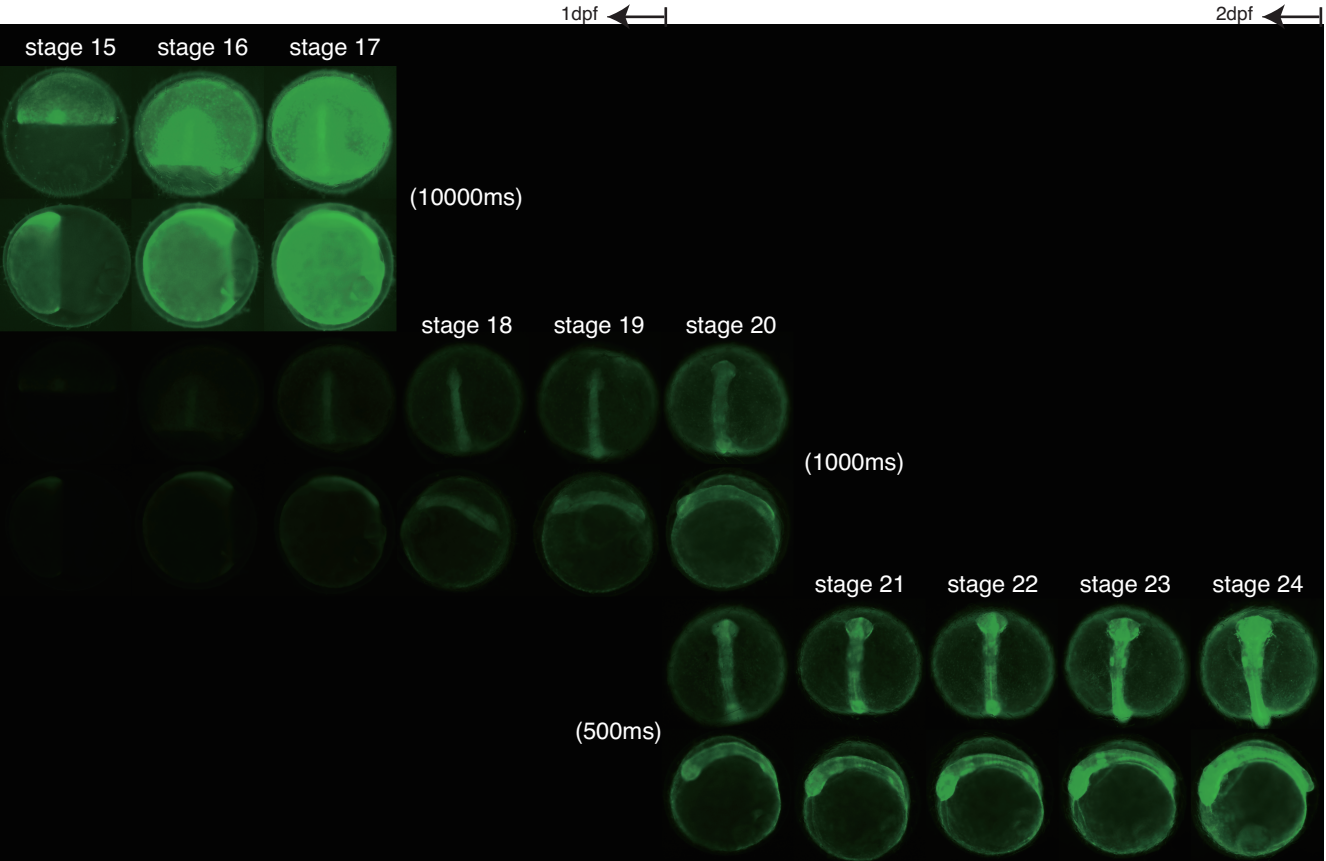
Supplemental Figure 1





Supplemental Figure 2

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2dpf
stage 15
stage 16
stage 17
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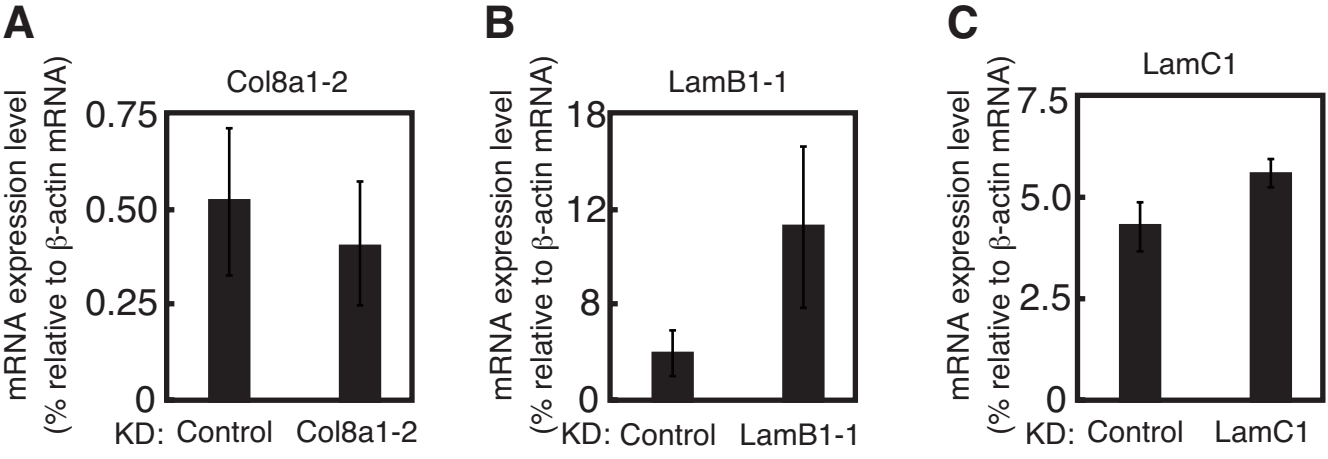
stage 19
stage 20
(1000ms)
stage 21
stage 22
stage 23
stage 24
(500ms)

Supplemental Figure 4



Supplemental Figure 5