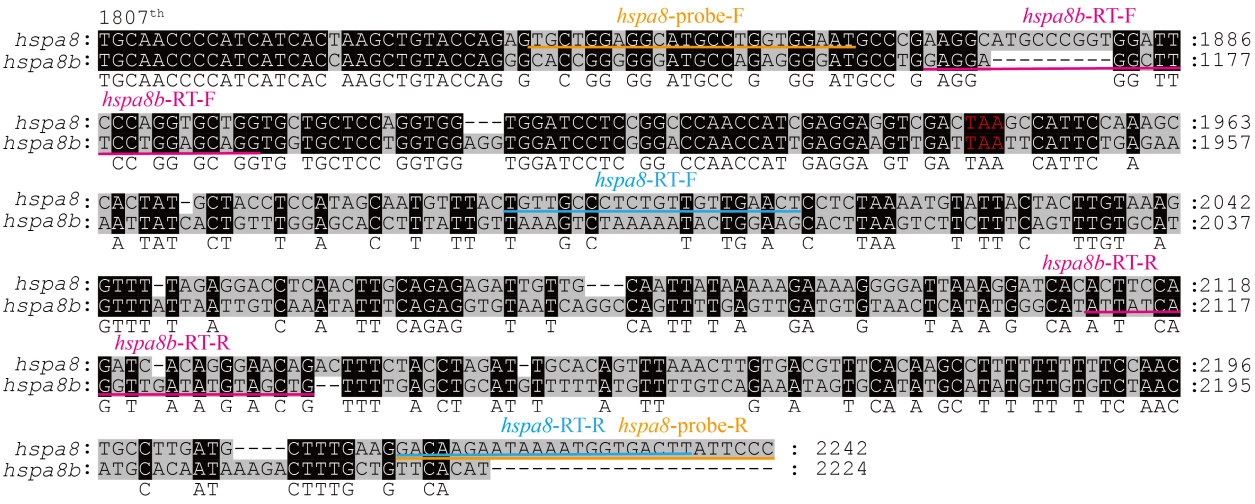
Fig.S1

A



B

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Fig. S1. Sequence alignment. (A) Comparison of the sequence of partial open-reading frame and 3′ UTR region from *hspa8* and *hspa8b*. The specific primers for RT-PCR analysis and riboprobes generation were underlined. (B) Amino acid sequence alignment of human HSPA8, mouse HSPA8, and zebrafish Hspa8 and Hspa8b. The epitope that anti-HSPA8 antibody was against is highlighted with a red box. Accession numbers are: human HSPA8 NP_006588.1, mouse HSPA8 NP_112442.2, and zebrafish Hspa8 NP_001103873.1 and Hspa8b NP_001186941.1.

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Fig.S2

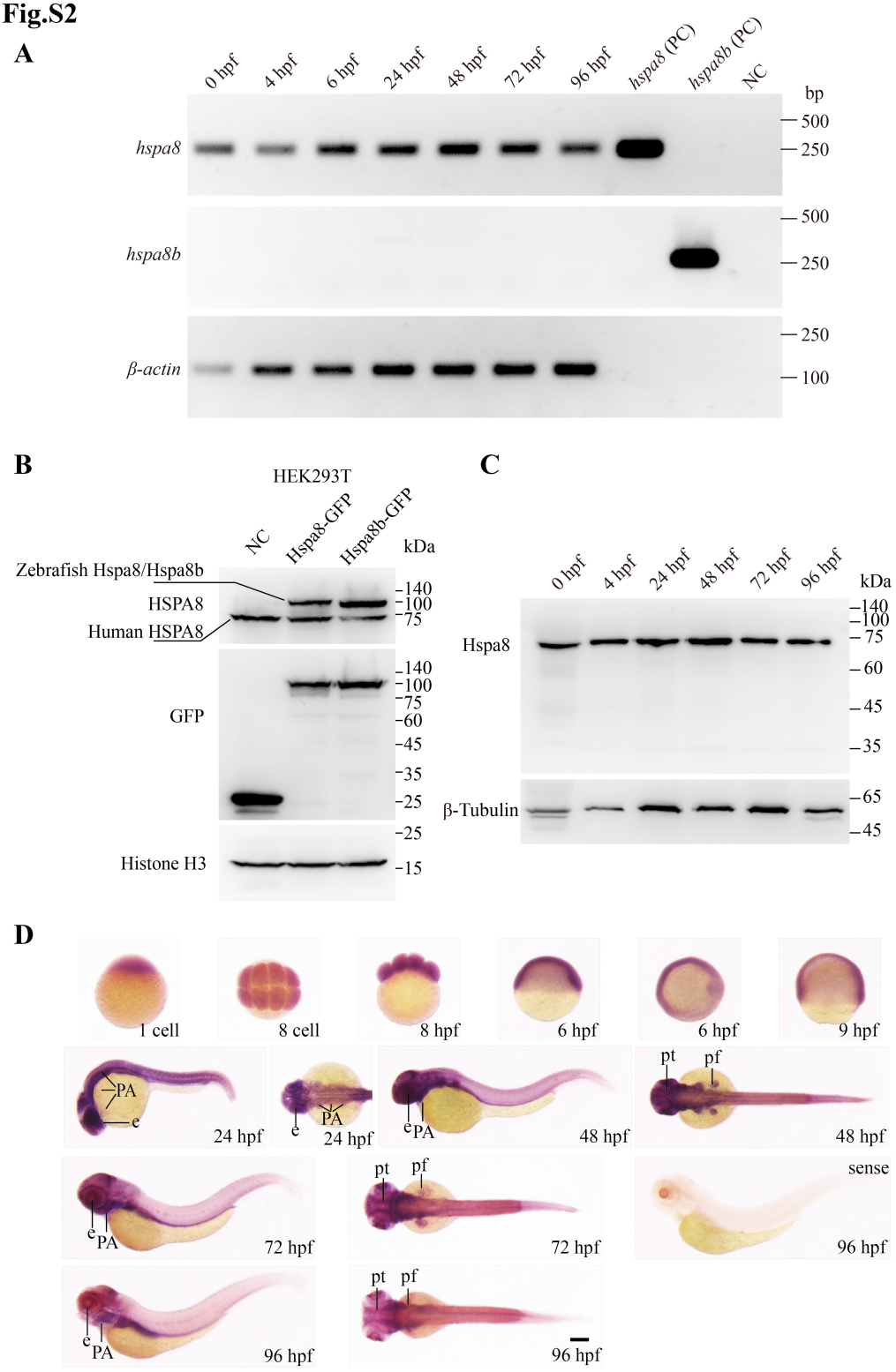

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Fig. S2. Spatiotemporal expression pattern of zebrafish *hspa8*. (A) RT-PCR analysis of zebrafish *hspa8* and *hspa8b* mRNAs at the indicated embryonic stages. Numbers indicate different developmental stages as hours post fertilization (hpf). *β-actin* was used as an internal control. PC, positive control; NC, negative control. (B) Antibody validation. Plasmids encoding GFP-tagged Hspa8 or Hspa8b were transfected into HEK293T cells. After 24h, the cell extracts were subjected to western blot analysis with an anti-GFP antibody or an anti-HSPA8 antibody. (C) Hspa8 protein level in the indicated embryonic stages. Numbers indicate different developmental stages as hours post fertilization (hpf). β-Tubulin was used as an internal control. (D) Whole-amount *in situ* hybridization analysis of zebrafish *hspa8* mRNA at the indicated stages. Panels represent the dorsal, top, or lateral views with animal pole up or anterior to the left. e, eye; PA, pharyngeal arches; pf, pectoral fin; pt, posterior tectum. Scale bar = 200 μm.

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Fig.S3

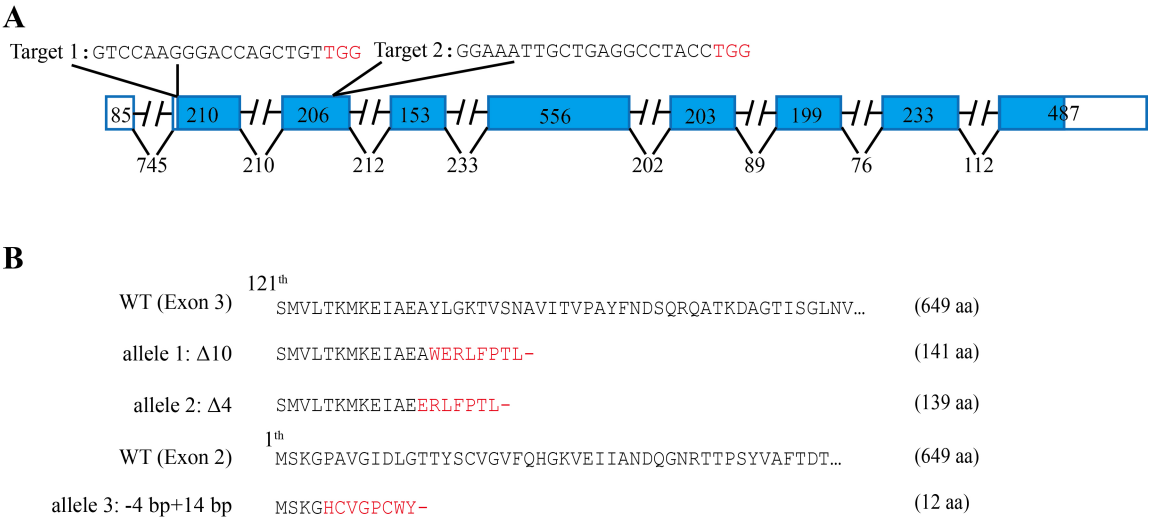

Fig. S3. Generation of *hspa8* mutants using the CRISPR/Cas9 system. (A) Schematic representation of the *hspa8* locus. Exons are shown as boxes, while introns are shown as lines. Diagram showing the CRISPR/Cas9 target DNA sequence of zebrafish *hspa8*. Protospacer adjacent motif (PAM) region is shown in red. (B) The predicted protein sequences of WT and mutant *hspa8* alleles (allele1, allele2, and allele3). The protein sequences of WT and mutants are shown.

Fig.S4

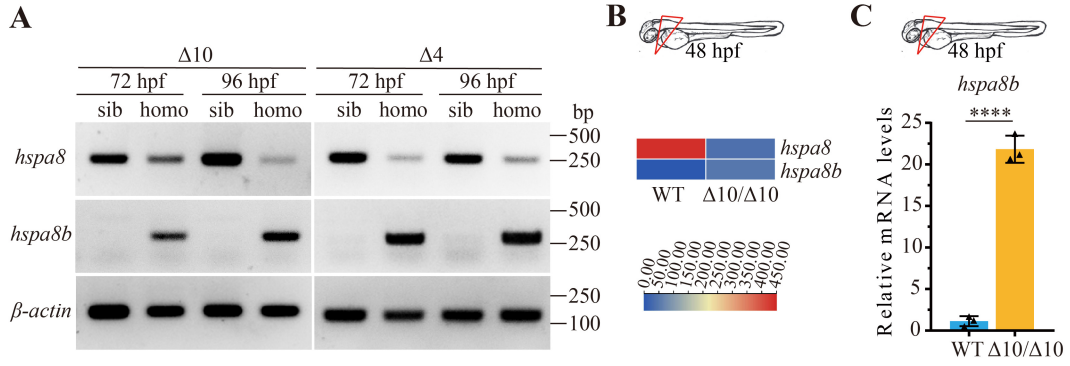

Fig. S4. Depletion of Hspa8 increases the transcription of *hspa8b*. (A) The mRNA levels of *hspa8* or *hspa8b* in siblings and *hspa8* mutants as indicated by semi-quantitative RT-PCR analysis. *β-actin* was used as an internal control. Similar results were obtained from three experiments. (B) Heatmaps of transcripts show the expression of *hspa8* and *hspa8b* in the PAs of WT sibling and *hspa8* mutant embryos at 48 hpf. (C) Relative mRNA levels of *hspa8b* in the PAs of WT sibling and *hspa8* mutant embryos at 48 hpf, as indicated by qRT-PCR analysis. Values are represented as means ±S.D. ($n = 3$). **** $p < 0.001$. Unpaired *t*-test, two-tailed.

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Fig.S5

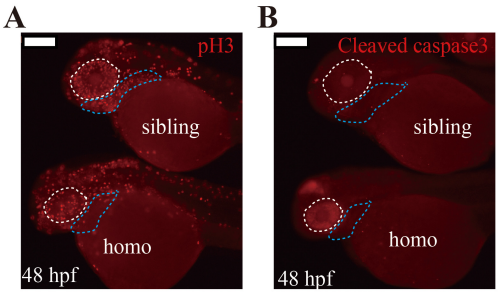

Fig. S5. Detection of cell proliferation or apoptosis in the anterior structures of zebrafish embryos. (A) Representative image of pH3-positive cells in the anterior structures of siblings and *hspa8*$^{\Delta 10/\Delta 10}$ mutants at 48 hpf. Embryos were immunostained with an anti-pH3 antibody. (B) Representative image of apoptotic cells in the anterior structures of siblings and *hspa8*$^{\Delta 10/\Delta 10}$ mutants at 48 hpf. Embryos were immunostained with an anti-cleaved caspase 3 antibody. The white and blue dashed lines define the eyes and pharyngeal regions, respectively. Scale bar = 200 µm.

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Fig. S6

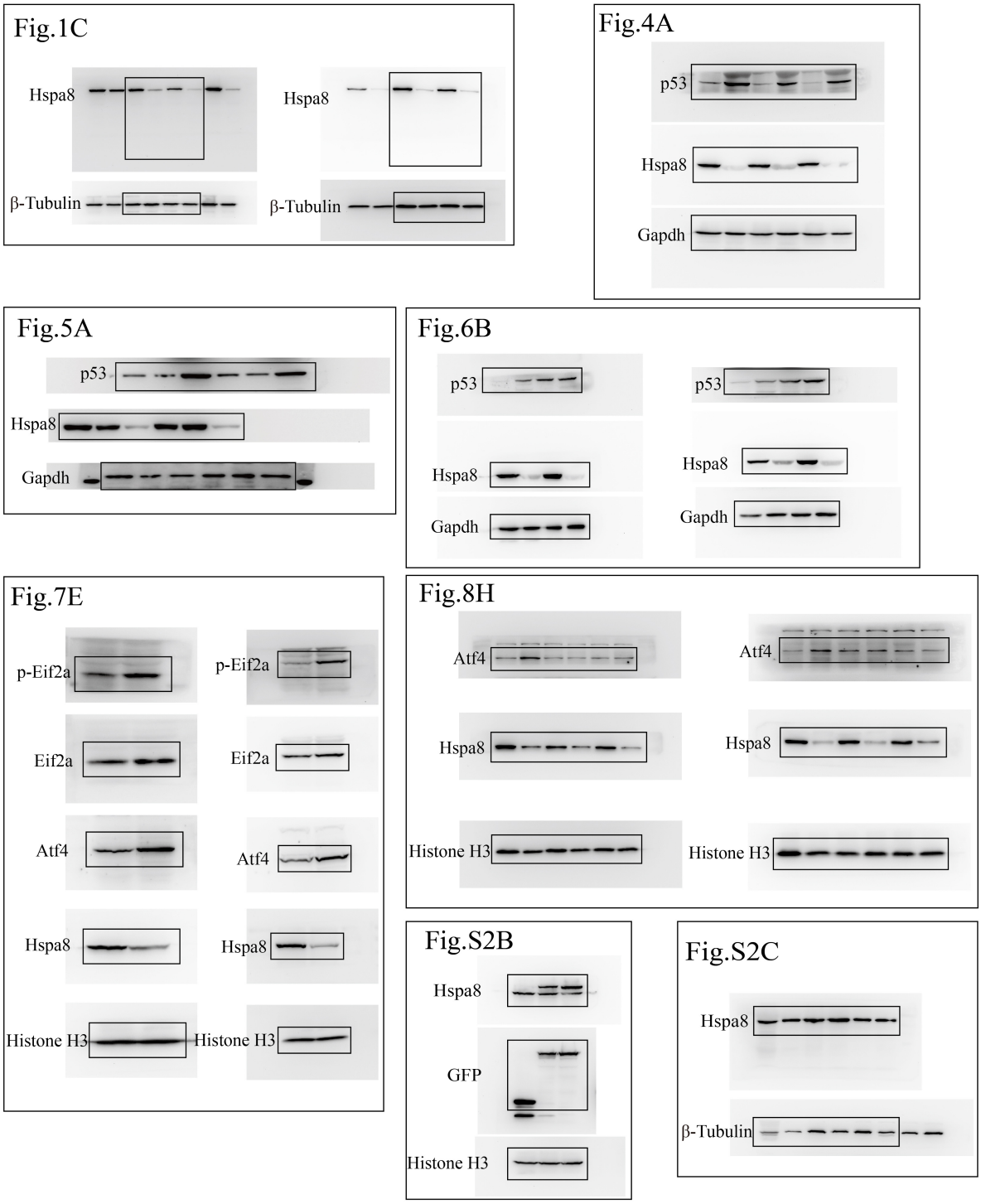

Fig. S6. Blot transparency.

Table S1. List of downregulated and upregulated genes in the pharyngeal arch region of Hspa8-depleted embryos at 48 hpf

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