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Supplementary Materials for

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Dusp1 regulates thermal tolerance limits in zebrafish by maintaining mitochondrial integrity

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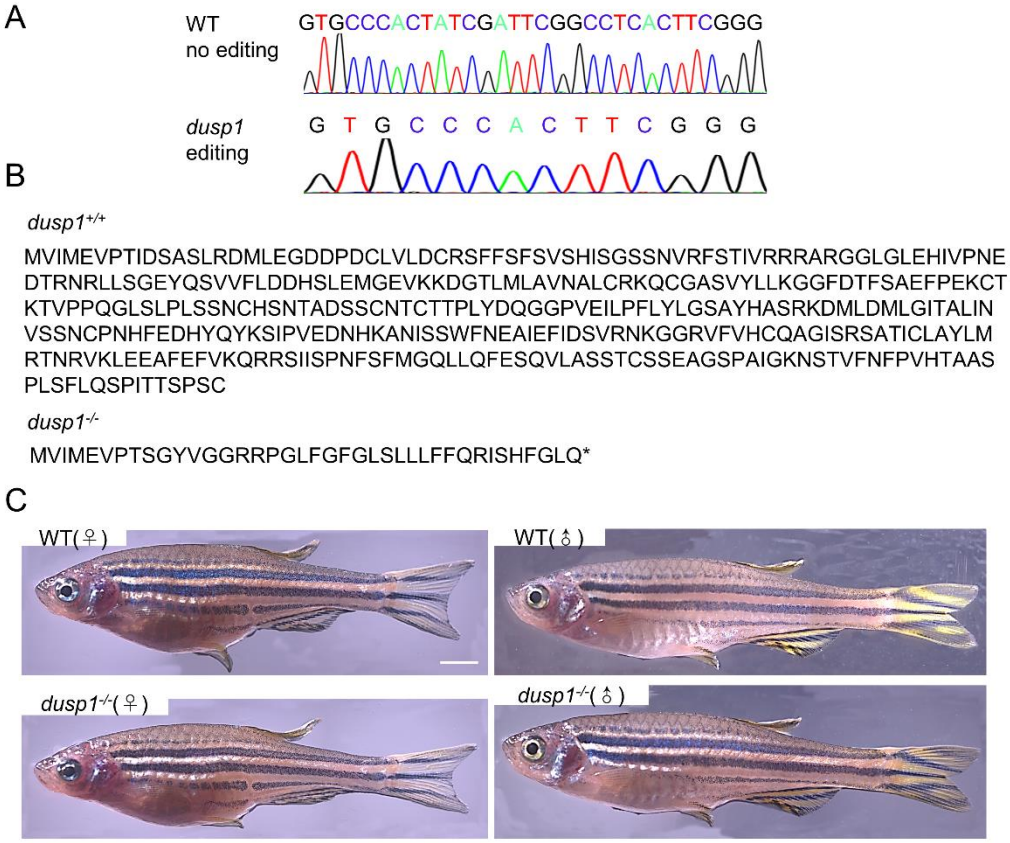
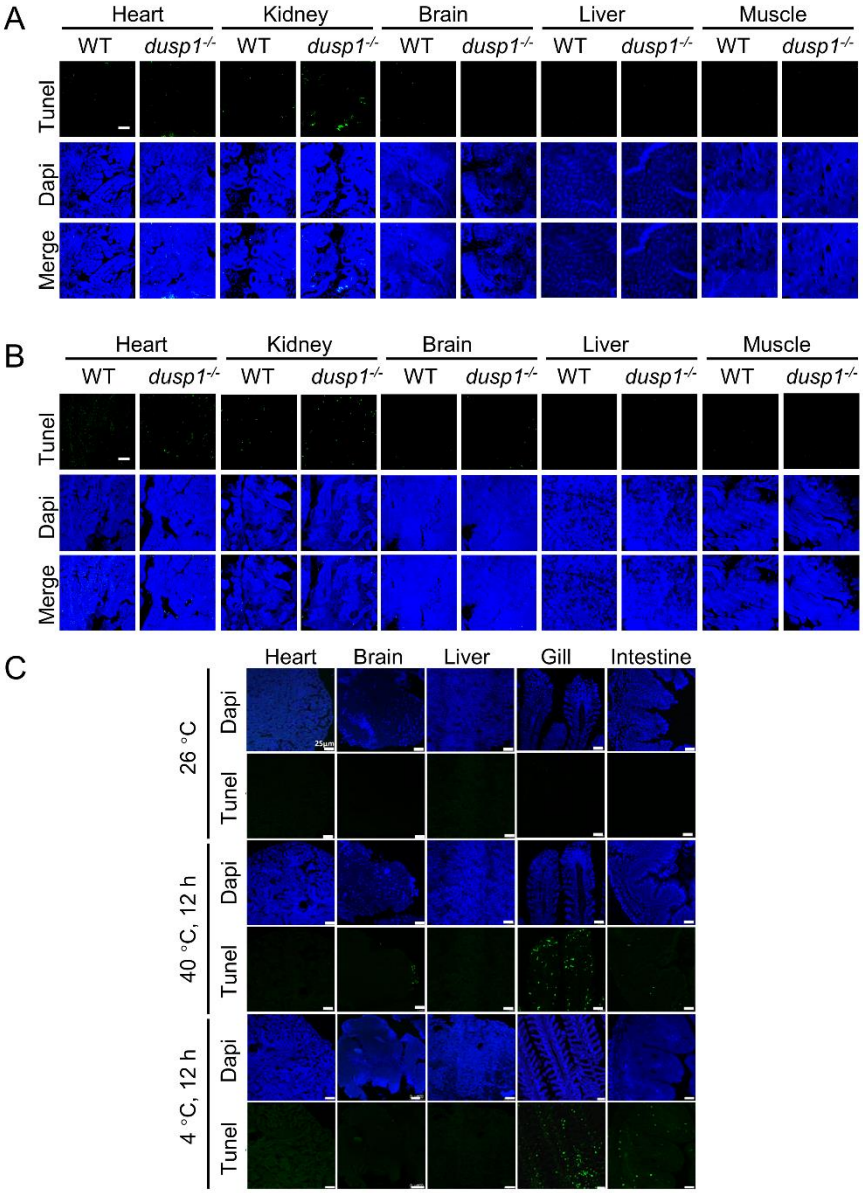


Supplementary Figure S1 Generation and characterization of zebrafish mutants lacking *dusp1* gene expression

A: Nucleotide sequences showing edited region in *dusp1*$^{-/-}$ fish.

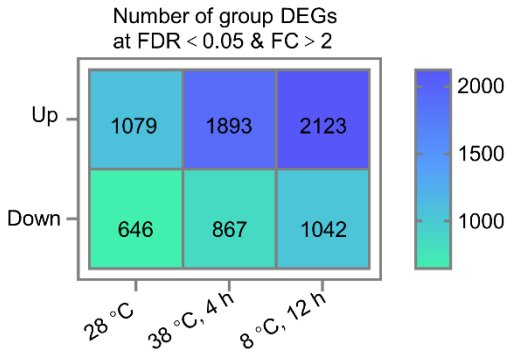
B: Amino acid sequences showing a nonsense mutation in exon 1 of *dusp1*$^{-/-}$. Asterisk indicates premature stop codon.

C: Morphological comparison of WT and *dusp1*$^{-/-}$ zebrafish at three months of age. Scale bar is 1 000 μm.

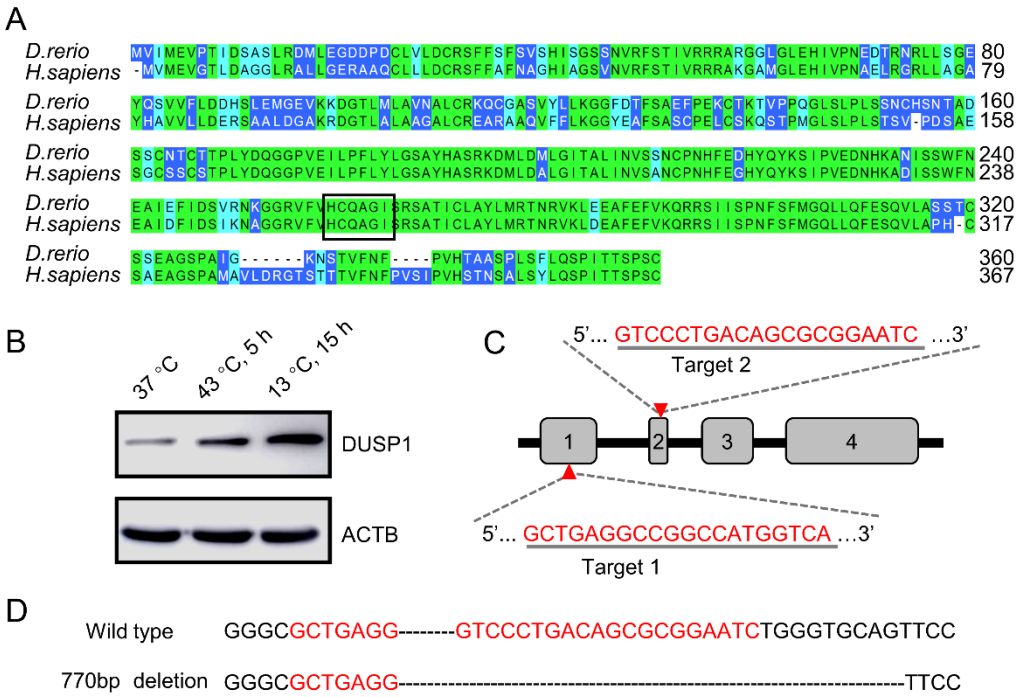


Supplementary Figure S2A-B TUNEL assay of five tissues from *dusp1*$^{-/-}$ and WT zebrafish exposed to 8 ℃ for 12 h (A) and 38 ℃ for 4 h (B) showing lower intensity in apoptotic signals in heart, kidney, brain, liver, and muscle than in the gill after the same treatments as shown in Figure 2C and Figure 2F of this study.

Figure S2C Apoptotic signals detected in five tissues of medaka at under hot (40 °C 12 h) and cold treatment (4 °C 12 h) showing gill is the most thermally sensitive tissue. Nucleus was counterstained with DAPI. Scale bar is 25 μm. Tissues from at least three fish were stained for apoptosis signals at each temperature-time point and three biological replicates were performed. Scale bar is 50 μm.



Supplementary Figure S3 Statistics of differentially expressed genes (DEGs) in gills between WT and *dusp1*$^{-/-}$ zebrafish under three temperature treatments



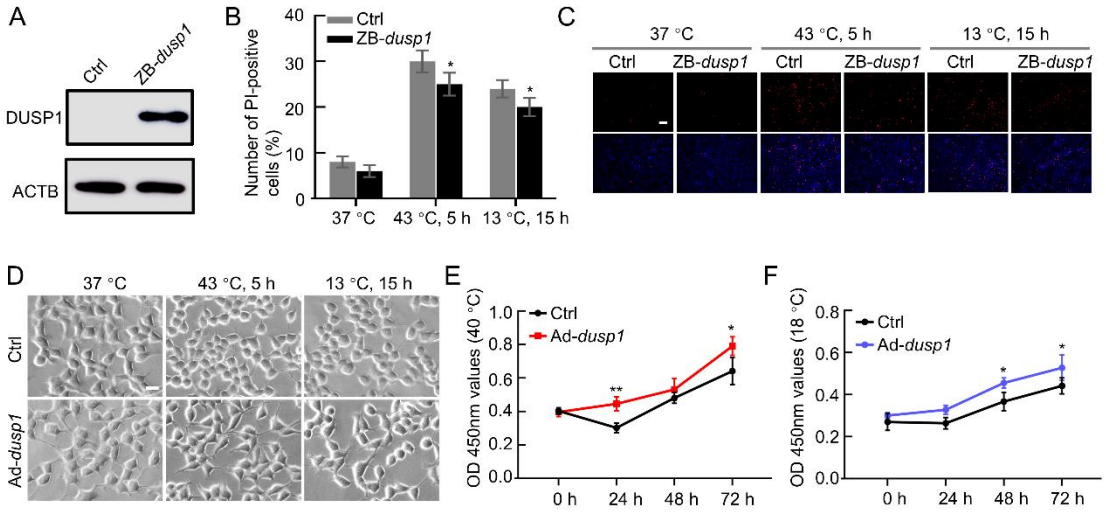
Supplementary Figure S4 Alignment of human and zebrafish Dusp1 protein sequences, temperature-induced expression of DUSP1 in HEK293T cells, and construction of DUSP1 mutant cell line

A: Alignment of human and zebrafish Dusp1 protein sequences. Black box denotes kinase catalytic site.

B: Western blot analysis of DUSP1 in 293T cells exposed to thermal stress, with ACTB as the loading control.

C: Schematic of scheme to target DUSP1 gene by CRISPR/Cas9 in HEK293T cells.

D: Sequencing analysis showing homozygous deletion of 770 base pairs of DUSP1 in HEK293T cell line.



Supplementary Figure S5 Overexpression of zebrafish dusp1 in HEK293T cells increased the cell survival rate under lethal temperature challenge

A: Western blot analysis confirming expression of transfected zebrafish DUSP1 protein in *DUSP1*$^{-/-}$ 293T cells.

B-C: Exogenous expression of zebrafish dusp1 reduced apoptosis in *DUSP1*$^{-/-}$ HEK293T cells. Scale bar is 100 μm.

D: Improved cell morphology in *DUSP1*$^{-/-}$ HEK293T cells expressing zebrafish dusp1 under thermal stress. Scale bar is 20 μm.

(E-F) OD450 values of *DUSP1*$^{-/-}$ HEK293T cells expressing zebrafish dusp1 measured by CCK8 assay, indicating improved viability.

Supplementary Figure S6 Alignment of sequences surrounding S616 site of human Drp1, indicating conservation of the site between fish and mammals