Supplemental Materials and Methods

1. Chemicals

Trigonelline and brusatol were purchased from Toronto Research Chemicals (Toronto, Canada) and Sigma-Aldrich (St. Louis, MO), respectively.

2. Survival assay

A survival assay without feeding was performed as previously described [1]. In the chemical treatment, 20 each of 5-days-post-fertilization (dpf) larvae were placed in 3-cm culture dishes containing E3+ medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, and 0.1 mg/L methylene blue) with Nrf2 inhibitors. For feeding conditions, 40 each of 5 days-post-fertilization (dpf) larvae were transferred into a 1-L tank on a circulating system, and feeding with 20 mg of Otohime A (Marubeni Nisshin Feed, Tokyo, Japan) 6 times per day was started.

3. Eating analysis

For eating analysis, 50 of each larva in a 9-cm culture dish were fed 20 mg of Otohime A once a day for 1 h from 5 dpf. Whether they ate was determined by microscopic photographs of fixed larvae in 4% paraformaldehyde (PFA). Genotyping was performed after photographs were taken.

4. Histological analysis

Seven-dpf larvae were fixed in 4% PFA and paraffin-embedded, and serial slices of 1-μm thickness were then prepared. At room temperature, rehydrated paraffin sections were stained with 0.1% (m/v) Carrazi's hematoxylin for 20 min, differentiated with 0.5% hydrochloric acid-ethanol solution for 30 sec, and restained with 0.5% (m/v) eosin for 4 min. The stained sections were photographed with an Olympus DP73 digital camera attached to a Leica DMRD microscope. Genotypes were analyzed by use of genomic DNA isolated from tails cut from the fixed larvae.

5. Alcian blue staining

Seven-dpf larvae were fixed in 4% PFA for less than 2 h, washed with ethanol, and stained overnight with Alcian blue solution (0.02% Alcian blue, 60 mM $MgCl_2$, 70% ethanol) as previously described [2]. The samples were bleached with 1.5% H_2O_2/1% KOH solution, serial cleared with 20% glycerol, and stored at 4°C in 50% glycerol. Genotypes were analyzed after photographs were taken.

6. Gene expression analysis

Whole-mount *in situ* hybridization analysis was performed as described previously [3]. Genotypes were analyzed after photographs were taken. Quantitative reverse transcription PCR (qRT-PCR) was performed using a Quant Studio 5 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) with THUNDERBIRD Next SYBR qPCR Mix (Toyobo). The primers used are described below. The expression level of each gene was normalized to the level of *eef1a1l1*. For RNA-sequencing analysis (RNA-seq), total RNA was extracted from 5-dpf larvae using ISOGEN II (Nippon Gene, Tokyo, Japan) after genotyping with genomic DNA isolated from larval cut tails. RNA-seq library was constructed as previously described [4]. RNA-seq was performed by the Tsukuba i-Laboratory LLP (Tsukuba, Japan), and gene ontology analysis was carried out by means of DAVID 6.8 with GOTERM_BP_DIRECT (BP: biological processes) (https://david.ncifcrf.gov/home.jsp), after conversion of identified zebrafish genes into their human homologs by use of ZFIN (https://zfin.org/) or bioDBnet (https://biodbnet-abcc.ncifcrf.gov/) as previously described [4].

7. Statistical analysis

The survival data were calculated using the Kaplan–Meier method and analyzed using the log-rank test. The comparisons of gene expression levels between different genotypes were performed using one-way analysis of variance followed by a Bonferroni multiple comparisons test. Statistical analyses were performed by EZR [5] and Statistical Package for the Social Sciences (SPSS) version 23.

8. Regulation for animal experiments

All animal experiments were performed in accordance with the animal protocol approved by the Animal Experiment Committee of the University of Tsukuba. All methods were carried out in accordance with the Regulation for Animal Experiments in our university and Fundamental Guideline for Proper Conduct of

Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

9. Primers used in qRT-PCR

Gene	Forward primer (5'->3')	Reverse primer (5'->3')
eef1a1l1	CGTGGTAATGTGGCTGGAGA	CTGAGCGTTGAAGTTGGCAG
gss	GCCAGCAGCACTCTTTCATC	AACTGTGATTCTGGCTGACCC
gstp1.2	GAGCAGCTTTGAAACGCACTTC	CATTTGAGGTGGTTGGGCAGATC
lrata	TTTTGTGGATGGCTGGTTGA	GTTGGACCGATGCATTTGAA
prdx1	GTCCCACTGAGATCATCGCC	AACCACCTTGTTTTCGGGGT
rdh5	GCGATTCTGTGGGTTCTGG	CCAGAGTCACAACCGGTCAC
rdh8b	GGAGGGTCTCAGTCTGGATG	CCCATCACGCTGCTGATGAC
rgra	GGTTCTCTTCACCTGGCTCT	GCAGGTCCTCAGAGGCTCATA
rpe65a	GGGATGACGGAGAACTACTTT	CCTTTCTCTTCATCCGACTCG
txn	AGTTGGTGGTGGTGGACTTC	CACATCCACCTTTAGAAACACC

References

[1] Mukaigasa, K., Nguyen, L. T., Li, L., Nakajima, H., Yamamoto, M., Kobayashi, M. Genetic evidence of an evolutionarily conserved role for Nrf2 in the protection against oxidative stress. Molecular Cell. Biol. 32 (2012) 4455–4461. https://doi.org/10.1128/MCB.00481-12

[2] M.B. Walker, C.B. Kimmel. A two-color acid-free cartilage and bone stain for zebrafish larvae. Biotech. Histochem. 82 (2007) 23-28. https://doi.org/10.1080/10520290701333558

[3] H. Nakajima, Y. Nakajima-Takagi, T. Tsujita, S. Akiyama, T. Wakasa, K. Mukaigasa, H. Kaneko, Y. Tamaru, M. Yamamoto, M. Kobayashi. Tissue-restricted expression of Nrf2 and its target genes in zebrafish with gene-specific variations in the induction profiles. PLoS One 6 (2011) e26884. https://doi.org/10.1371/journal.pone.0026884

[4] V.T. Nguyen, L. Bian, J. Tamaoki, S. Otsubo, M. Muratani, A. Kawahara, M. Kobayashi. Generation and characterization of *keap1a*- and *keap1b*-knockout zebrafish. Redox Biol. 36 (2020) 101667. https://doi.org/10.1016/j.redox.2020.101667

[5] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 43 (2013) 452-458. https://doi.org/10.1038/bmt.2012.244