

Fig. S1. The homozygous *GBT411/dnajb6b* mutant exerted deleterious effects on the *bag3^{e2/e2}* mutant

Images of the *bag3^{e2/e2}; dnajb6b^{-/-}* double mutant fish compared to the *bag3^{e2/e2}* or *dnajb6b^{-/-}* single mutants and WT controls at 6 days post-fertilization (dpf). Scale bar, 1 mm.

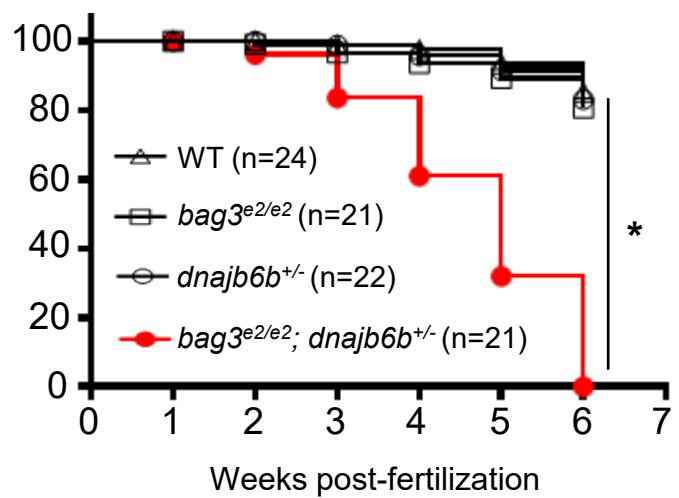


Fig. S2. The heterozygous GBT411/dnajb6b mutant exerted deleterious effects in survival on the *bag3^{e2/e2}* mutant

Survival curves of *bag3^{e2/e2}*; *dnajb6b^{+/−}* double mutant fish compared to single mutants and WT controls. * N=21-24. * P<0.05, Log rank test.

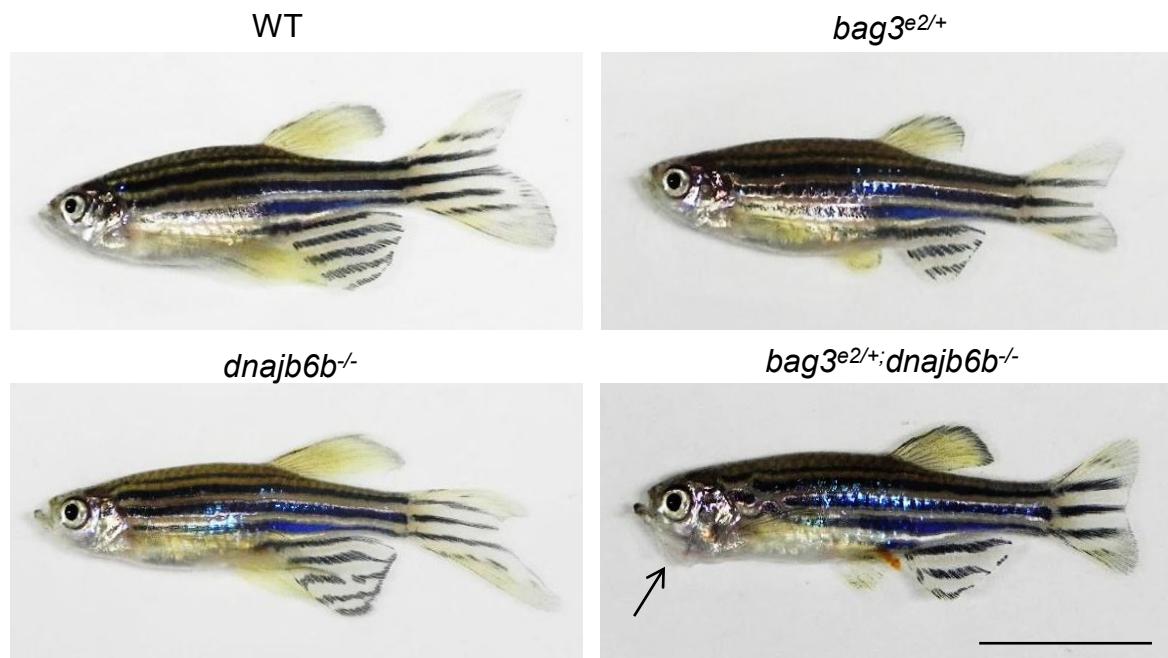


Fig. S3. Genetic interaction between *bag3^{e2/+}* and *dnajb6b^{-/-}* mutant fish Representative images of *bag3^{e2/+}; dnajb6b^{-/-}* double mutant fish and their corresponding single mutant controls at 3 months of age. The arrow points to unusual jaw protruding phenotype. Scale bar: 1 cm.

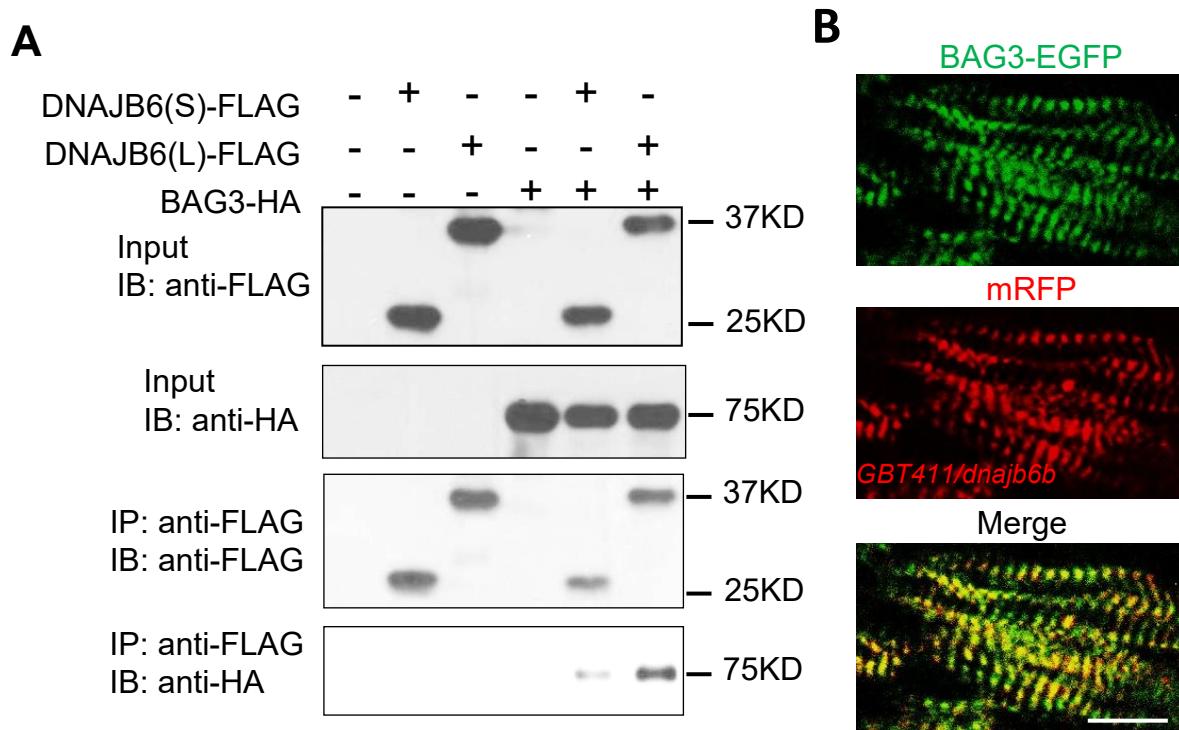


Fig. S4. DNAJB6 protein interacts with BAG3 *in vitro*

A, Western blot analysis of the *in vitro* protein pull-down assay indicated a direct protein-protein interaction between the human DNAJB6(L) and BAG3 proteins and between the human DNAJB6(S) and BAG3 proteins in the HEK293 cells. IP: immunoprecipitation. IB: immunoblot.

B, Fluorescent images of adult heart sections from *GBT411/dnajb6b* mutant after crossed with the *Tg(cmhc2:BAG3-EGFP)* transgenic fish. Scale bar, 10 µm.

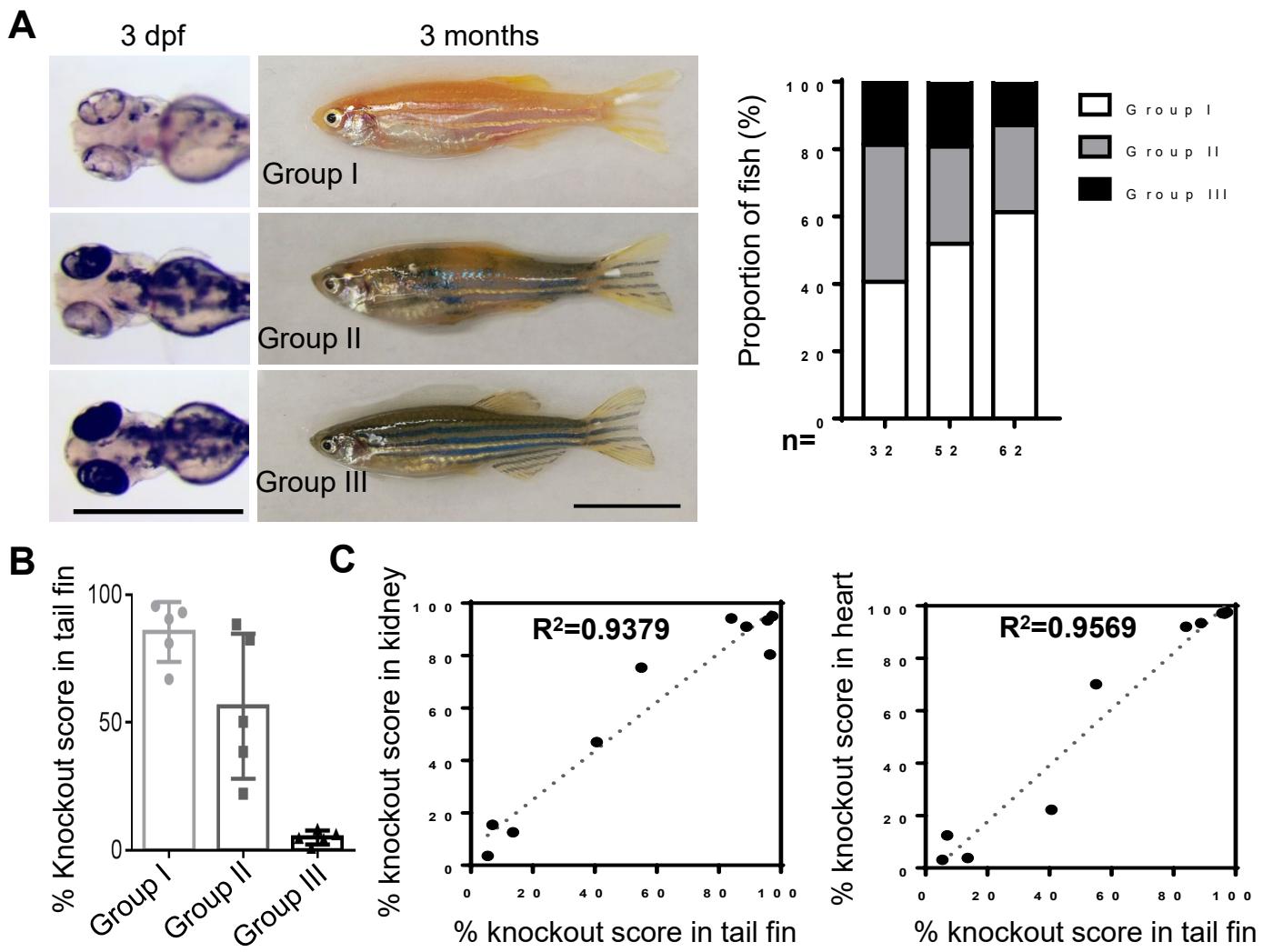


Fig. S5. Effective gene knockout by microhomology-mediated end-joining (MMEJ)-based *tyrosin* single guide RNA sustained from embryonic to adult stages

A-B, Representative images and quantification of *tyrosine* (*tyr*) microhomology-mediated end-joining (MMEJ)-based single guide RNA (sgRNA) injected fish at 3 dpf and 3 months categorized from high to low knockout (KO) score (group I to group III). Scale bar in left panels, 1mm, right panels, 1 cm. Three bars in the bar graph in (A) represent results from three independent injections of *tyr* sgRNA. **C**, Correlation of KD score calculated using genomic DNA extracted from the tail fin, heart or kidney, respectively.

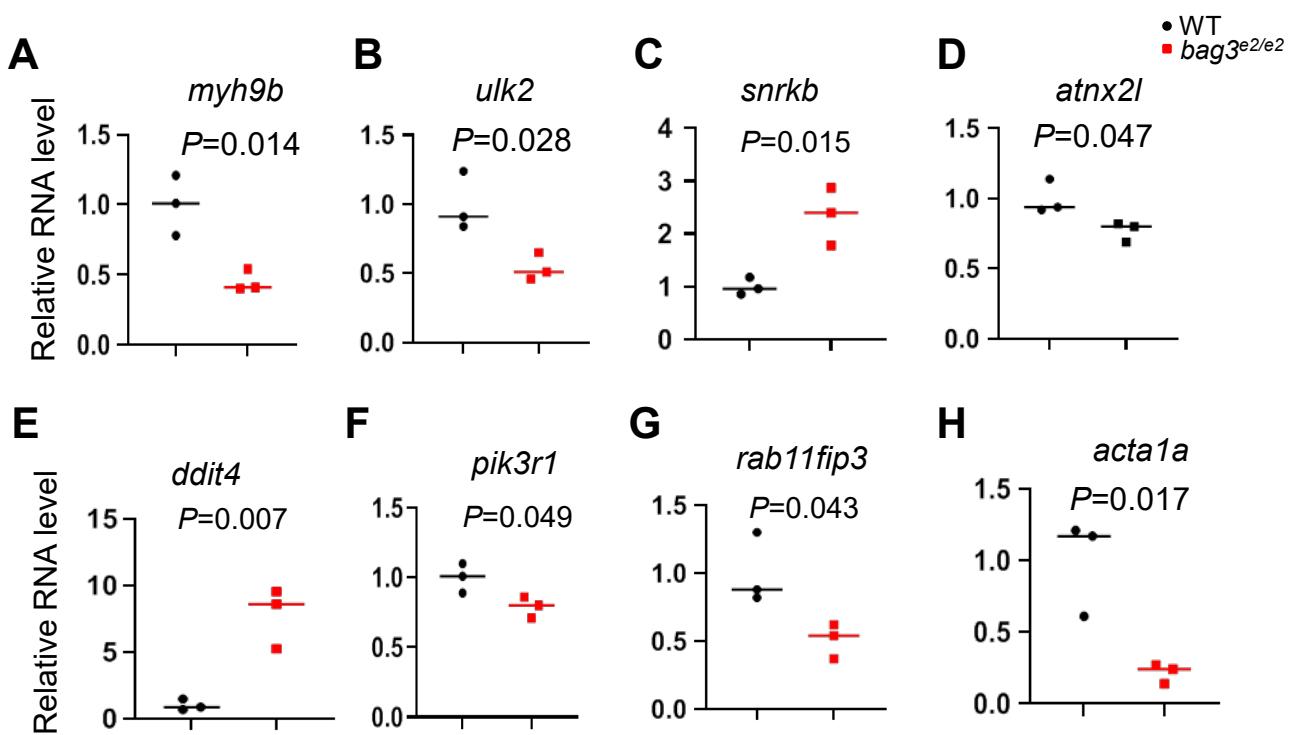


Fig. S6. Quantitative RT-PCR results of 8 differentially expressed proteostasis/autophagy genes in the *bag3^{e2/e2}* cardiomyopathy model

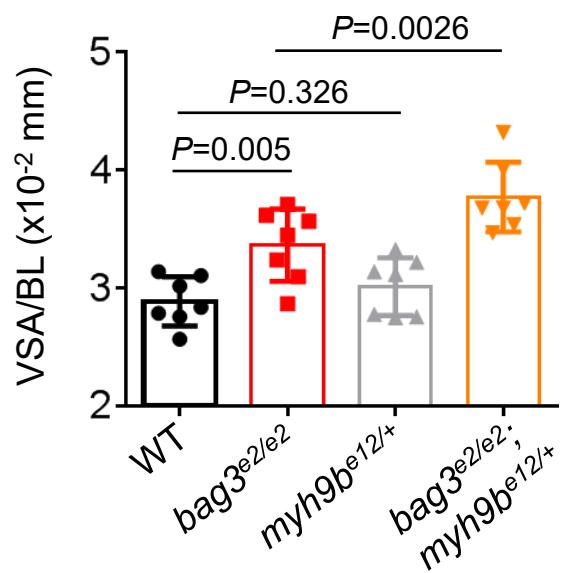


Fig. S7. The *bag3^{e2/e2};myh9b^{e12/+}* double mutant fish displayed enlarged ventricular chamber size

Quantification of the ventricular surface area (VSA) normalized to the body length (BL) of fish at 6 months. N=6, one-way ANOVA.

Table S1. Primers for genotyping PCR to identify the *bag3^{e2/e2}* and gene-break transposon (GBT) mutants

Primer name	Primer Sequence
RP2-5'-LTR	5'-GCATGAACTCCTGATGACG-3'
RP2-3'-LTR	5'-GTACAGTAATCAAGTAAAATTACTCA-3'
GBT002/sorbs2b-F	5'-GGGTCGGCATGCTTCATCACTG-3'
GBT002/sorbs2b-R	5'-AATCCAGCAGACCGAACAAACGC-3'
GBT136/ano5a-F	5'-AGGGTCTTGTGGATGTCTGTGAATG-3'
GBT136/ano5a-R	5'-CTGAGGTCTCTGCACAACCTATTAGTGT-3'
GBT411/dnajb6b-F	5'-TGTGATAACATCACAGCACC-3'
GBT411/dnajb6b-R	5'-ACGATGAAAATGAGCTGAAGC-3'
GBT419/rxraa-F	5'-ATATCGCTTAGTCGCTCACTTTAT-3'
GBT419/rxraa-R	5'-TATATACACGAAAATCCTCCACACA-3'
bag3-F	5'-CGGCGTATAAAGAATTGCTGG-3'
bag3-R	5'-GTGAAGTAGGTGAGCAAGAC-3'

Table S2. Quantitative RT-PCR primers used to validate differentially expressed (DE) genes

Gene	Primer Sequence
<i>acta1a</i>	F: 5'-CAGCTTACTTCAGGACTCACG-3' R: 5'-TACCTCTCTGCTCTGAGCCTC-3'
<i>ddit4</i>	F: 5'-AGCCATCAGACTTGAGGATAC-3' R: 5'-GGGTCGAAAAACTCAGATTGG-3'
<i>atnx2l</i>	F: 5'-ACCCGAGACACTTCACAG-3' R: 5'-GAUTGGAGTCATAGGTGGAC-3'
<i>pik3r1</i>	F: 5'-AACTCCTAACCGAGACCTC-3' R: 5'-AGCACTGTCTGACATCCAG-3'
<i>snrkb</i>	F: 5'-CGG ACGCATGAAGATTTCAG-3' R: 5'- CATATAGACCAGCTATCTGCC-3'
<i>ulk2</i>	F: 5'-AAGACTGATTGGGAGGTAG-3' R: 5'- GCTGCAATCTGCTGTAGAAAG-3'
<i>myh9b</i>	F: 5'-ATCTACTCAGAGGAGATCGTG-3' R: 5'- GCTGCTCTGGTCTTCTG-3'
<i>rab11fip3</i>	F: 5'-AGGCATCAGTGCAATCAGC-3' R: 5'-GTCGTCTCCATCTGTGAGT-3'

Table S3. PCR primers for quantifying knockout efficiency of predicted MMEJ-inducing sgRNAs

Gene	Primer Sequence
<i>atxn2l</i>	F: 5'-CCTGCCTAATCGAACAAATCG-3' R: 5'-GTGTGAGAGTGAATGTGAGAG-3'
<i>myh9b</i>	F: 5'-GAATGTGACCGATTCACCC-3' R: 5'-CCTCAAAGATCTCAAAGCCG-3'
<i>ulk2</i>	F: 5'-CTGAAGGCTGTCTCAGATTG-3' R: 5'-ACTTTAGGCATTCAACCGTAG-3'
<i>snrkb</i>	F: 5'-CGGACGCATGAAGATTTCAG-3' R: 5'-CATATAGACCAGCTATCTTGCC-3'
<i>rab11fip3</i>	F: 5'-AGGCATCAGTGCAATCAGC-3' R: 5'-GTCGTCTCCATCTGTGAGTG-3'
<i>pik3r1</i>	F: 5'-AACTCCTAACCGAGACCTC-3' R: 5'-AGCACTGTCTGACATCCAG-3'
<i>ddit4</i>	F: 5'-GATAACTGATCATGCAGGACTG-3' R: 5'-TGCACACCTGAATCTGAGG-3'
<i>acta1a</i>	F: 5'-CACACACCTTCTACAATGAG-3' R: 5'-ACATCAGAGTTTCGATCAGC-3'
<i>dnajb6b</i>	F: 5'-CACAGTGAGCCTGAAATGATG-3' R: 5'-AGCTGCACACAGATTAACATAC-3'
<i>mtor</i>	F: 5'-AGGTGCAGCCATTCTTGAT-3' R: 5'-ATGTCATACCTCTCCCTCCATAC-3'
<i>tryosinase</i>	F: 5'-GCGTCTCACTCTCCTCGACTCTC-3' R: 5'-GTAGTTCCGGCGCACTGGCAG-3'