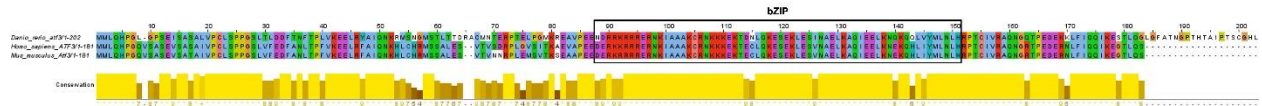


1 **ATF3 Overexpression is Associated with Cardiac Hypertrophy and Electrical Dysfunction**
2 **Accompanied by Enhanced Cardiac Cell Proliferation in Zebrafish**

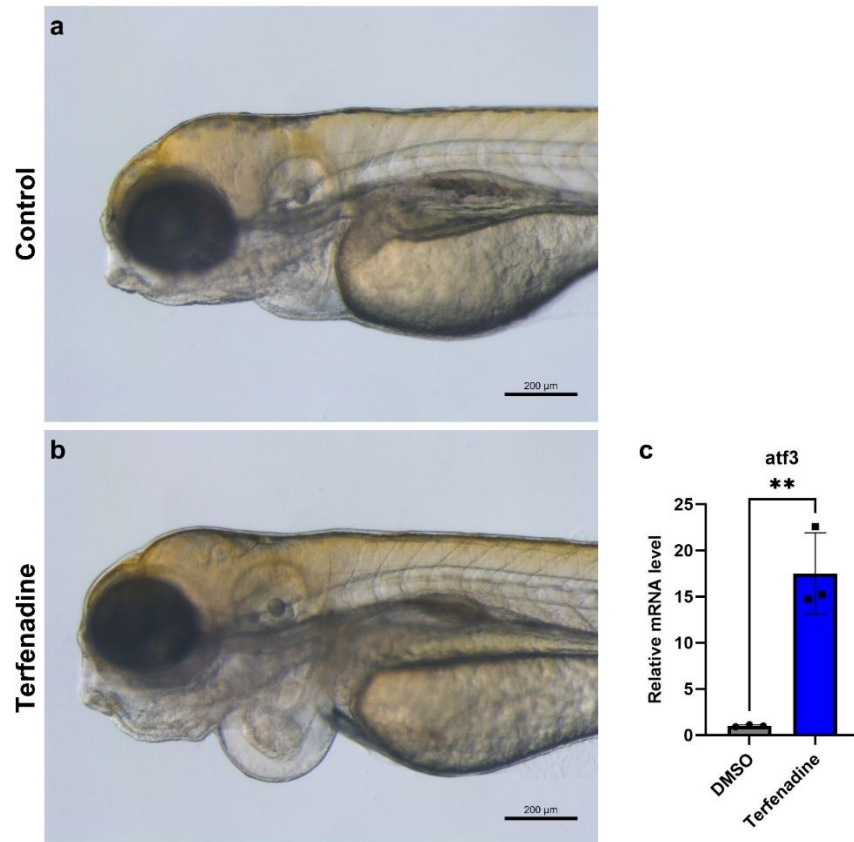
3 Eunmi Kim, Jinho Kim, Hyun-Yi Moon, Ji Yeon Kim, Myong-Ho Jeong, Geun-Young Kim,
4 Seung Hee Lee, Chul-Hong Kim, Jung-Woong Kim, Won-Ho Kim

5



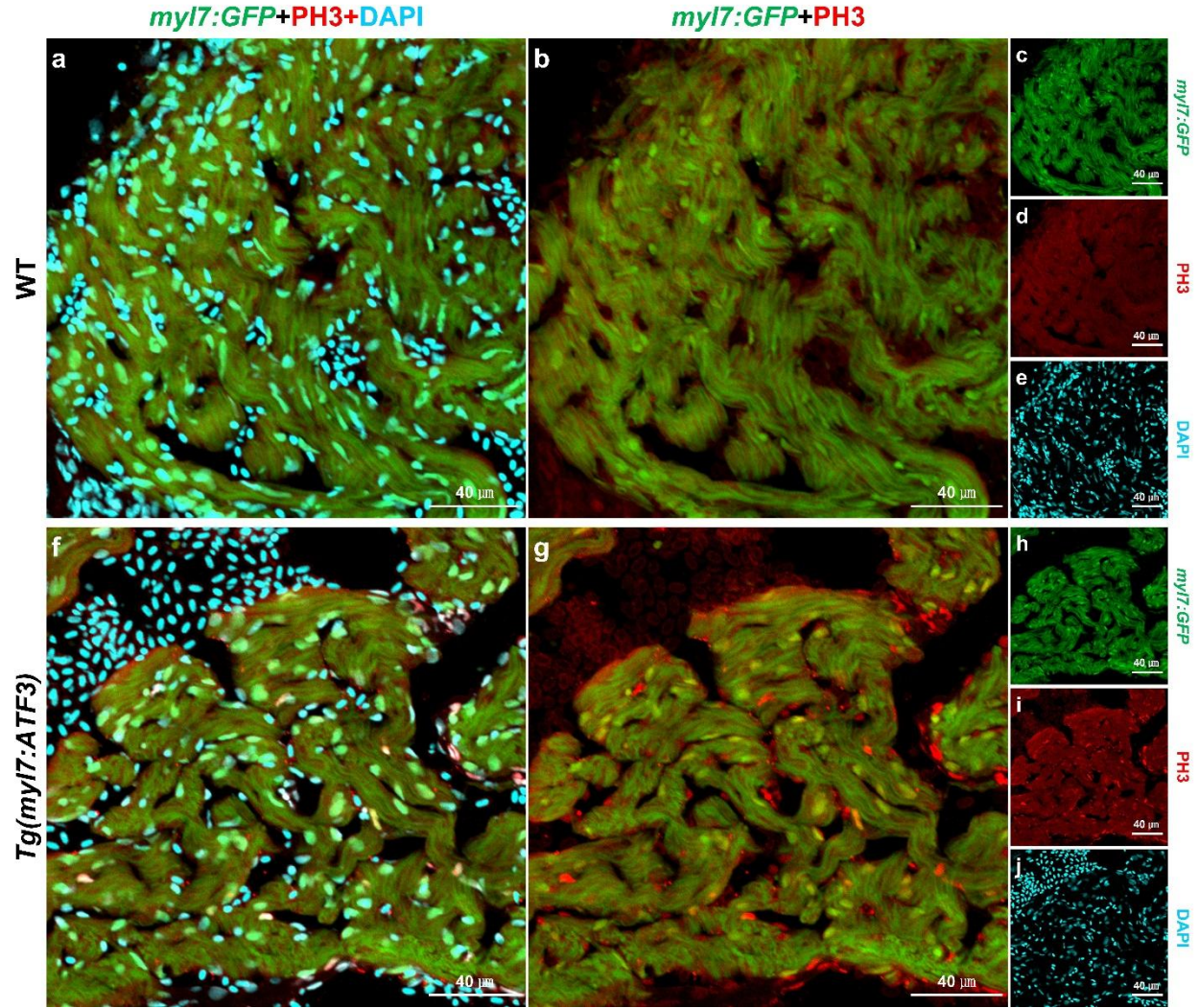
Supplementary Figure S1. Sequence alignment of ATF3 orthologs

Amino acid sequence alignment of ATF3 proteins from *Homo sapiens* (ENSP00000344352), *Mus musculus* (ENSMUSP00000027941), and *Danio rerio* (ENSDARP00000027550), generated using Clustal Omega (EMBL-EBI) and visualized in Jalview (v2.11.5.0). Conserved residues are indicated in color, and the basic leucine zipper (bZIP) domain (box; approximately residues 89–151 in human ATF3) is evident across species. Percentage ID (calculated in Jalview) was 95% between human and mouse and 71% between human and zebrafish.



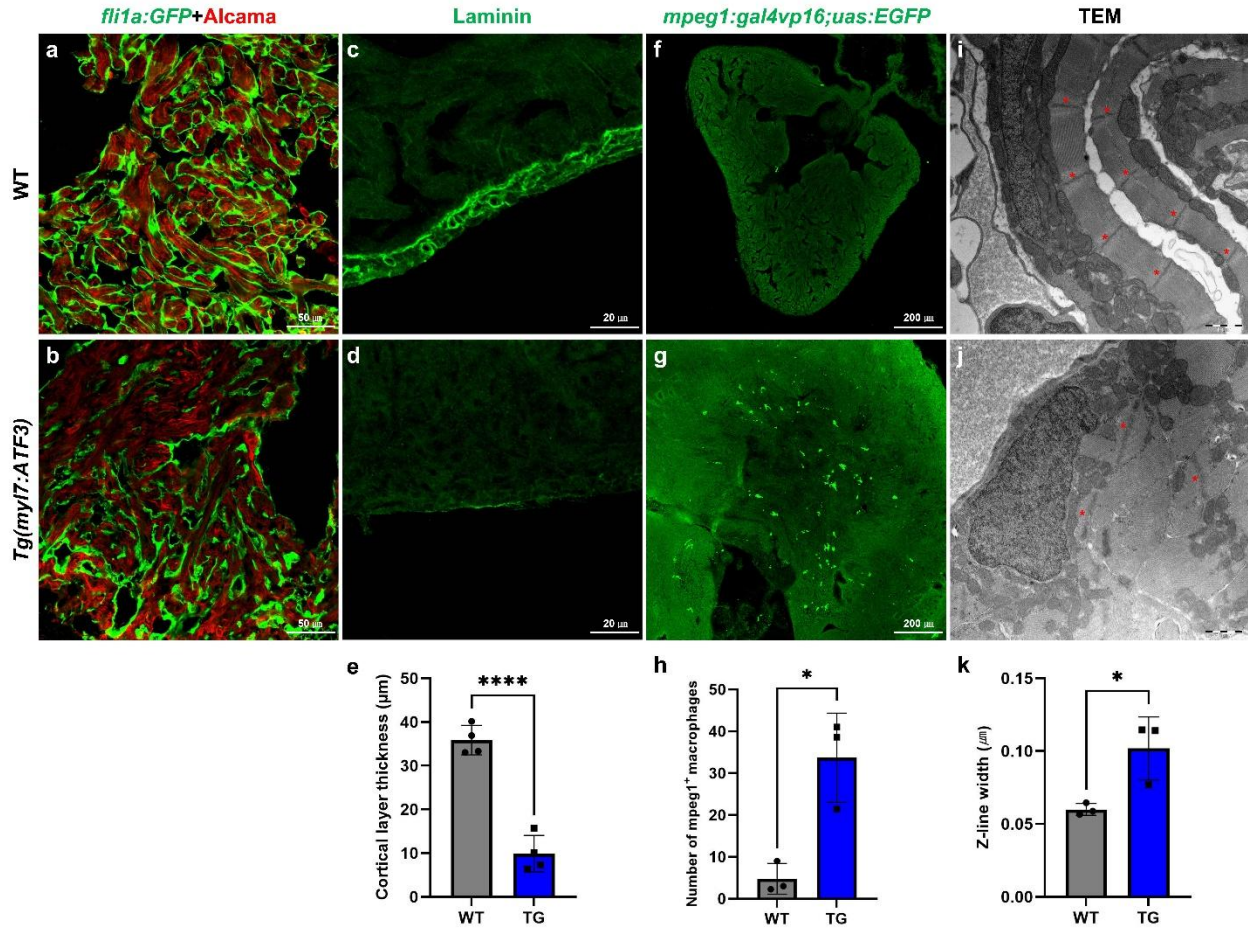
Supplementary Figure S2. Terfenadine-induced cardiac stress increases endogenous *atf3* expression in zebrafish larvae

(a, b) Representative bright-field images show cardiac enlargement and pericardial edema in terfenadine-treated larvae compared with DMSO controls at 4 dpf. (c) Quantitative PCR analysis demonstrates a significant increase in *atf3* mRNA levels in terfenadine-treated zebrafish relative to controls. Data are presented as the mean $\pm$ SD (n = 3 pooled biological replicates per group, each composed of 40 larvae) and analyzed using an unpaired two-tailed t-test; **P < 0.01.



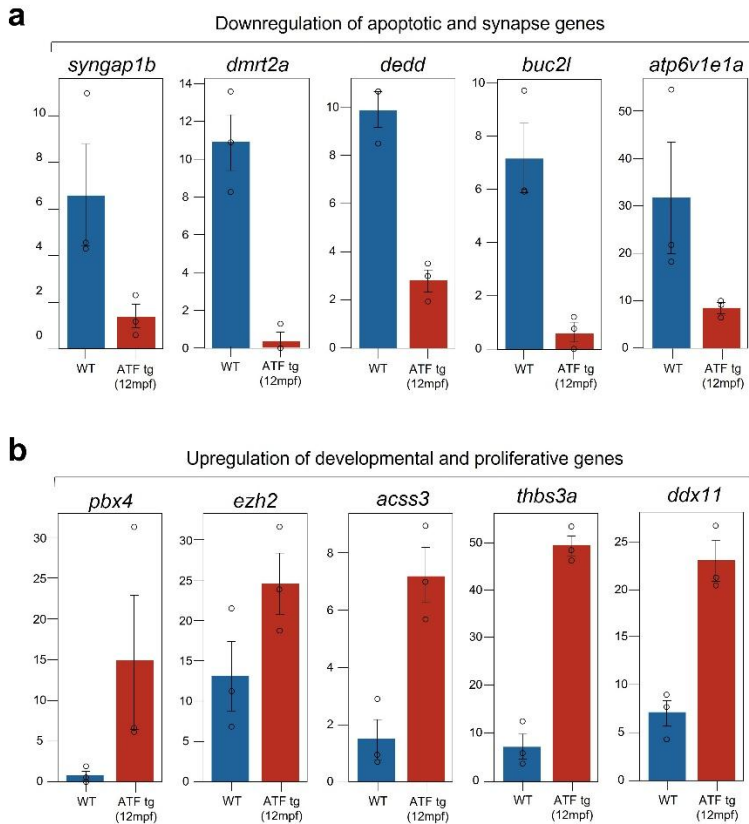
Supplementary Figure S3. Increased proliferating cells in *Tg(myl7:ATF3);myl7:GFP* zebrafish hearts revealed by PH3 and *myl7:GFP* co-staining

(a, b) Confocal images of adult control zebrafish hearts (*Tg(myl7:GFP)*) show cardiomyocytes labeled with *myl7:GFP* (green), proliferating cells labeled with PH3 (red), and nuclei counterstained with DAPI (cyan). (a) Merged image of *myl7:GFP*, PH3, and DAPI; (b) *myl7:GFP* and PH3 two-channel merge. (c–e) Single-channel images from the same field in (a): *myl7:GFP* (c), PH3 (d), and DAPI (e). (f, g) Corresponding merged images in *Tg(myl7:ATF3);myl7:GFP* hearts. (f) Merged *myl7:GFP*/PH3/DAPI; (g) *myl7:GFP*/PH3 two-channel merge. (h–j) Single-channel images from the same field in (f): *myl7:GFP* (h), PH3 (i), and DAPI (j). *Tg(myl7:ATF3)* hearts show increased PH3⁺ nuclei compared with the WT.

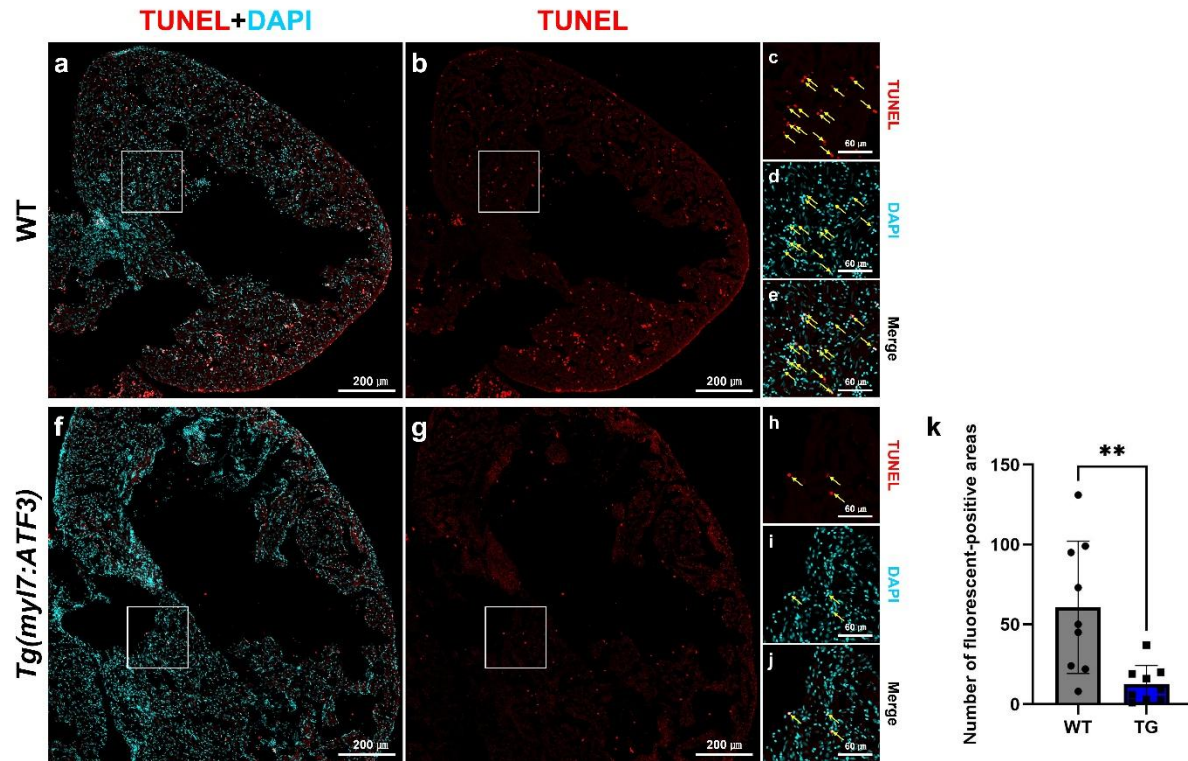


Supplementary Figure S4. Comparison of myocardium and cardiomyocyte structures and inflammation in WT and *Tg(myf7:ATF3)* zebrafish

(a, b) Alcama antibody staining reveals abnormal endocardial structure in *Tg(fli1a:GFP)* and *Tg(myf7:ATF3);Tg(fli1aGFP)* zebrafish at 4 mpf (n = 3 hearts per group). (c, d) Laminin antibody staining demonstrates damage to the cortical myocardium in *Tg(myf7:ATF3)* zebrafish at 12 mpf. (e) Quantification of Laminin⁺ cortical myocardium thickness (n = 4 hearts per group) (f, g) *Tg(mpeg1:gal4vp16;uas:EGFP)* staining shows increased macrophage infiltration in *Tg(myf7:ATF3)* zebrafish hearts at 12 mpf. Quantification of macrophage infiltration within the ventricular region is shown in (h) (n = 3 hearts per group). (i, j) Transmission electron microscopy images reveal disrupted myofibril structure in *Tg(myf7:ATF3)* zebrafish cardiomyocytes at 12 mpf. Quantification of z-line width (asterisk) of sarcomeres is shown in (k) (n = 3 hearts per group). Data are presented as the mean ± SD and are analyzed using an unpaired two-tailed t-test. *P < 0.05, ****P < 0.0001. mpf, months post-fertilization



Supplementary Figure S5. Expression levels of selected DEGs in adult zebrafish hearts
(a, b) Expression levels of selected DEGs in the 12 mpf group. **(a)** Apoptotic and synapse-related genes (*syngap1b*, *dmrt2a*, *dedd*, *buc2l*, and *atp6v1e1a*). **(b)** Developmental and proliferative genes (*pbx4*, *ezh2*, *acss3*, *thbs3a*, and *ddx11d*). Each dot represents an individual sample, and error bars indicate the SD. DEG, differentially expressed gene



Supplementary Figure S6. Apoptotic cell signals in *Tg(myl7:ATF3)* hearts revealed by the TUNEL assay

Representative fluorescence images of TUNEL staining in adult zebrafish hearts from WT (a–e) and *Tg(myl7:ATF3)* (f–j). (a, b, f, g) DAPI + TUNEL and TUNEL-only images at low magnification. (c–e, h–j) display high-magnification views corresponding to the boxed regions in (a, b) and (f, g), respectively, showing DAPI, TUNEL, and merged channels. (k) Quantification of TUNEL-positive area per $\times 100$ field (based on representative images in a, b, f, g). Data are presented as the mean $\pm$ SD (n = 3 hearts per group; three ventricular sections analyzed per heart). Statistical analysis was performed using an unpaired two-tailed t-test; **P < 0.01.