SUPPLEMENTAL MATERIAL

Expanded Materials & Methods

Study design

In this study, we provide both genetic and chemical-genetic evidence to demonstrate that autophagy activation can reverse anthracycline-induced cardiac function decline. Using both a transgenic fish line that overexpresses atg7 and an atg7-knockout mutant line, we discovered therapeutic effects of atg7 overexpression in the later phase of AIC, which differs from its deleterious effects in the early phase. To translate this autophagy-based therapeutic strategy, we assessed autophagy-modulating drugs in a zebrafish eAIC model, confirmed the top-ranking drugs in a zebrafish aAIC model, and then validated the therapeutic efficacy of the top drugs in a mouse AIC model. Power analysis was performed by using PASS 11 software to determine approximate sample sizes for the animal heart function analysis.[39, 40] The sample size calculation based on assumption of an intended power of 80%(90%), an alpha error of 5%, at a reference normal ejection fraction of 50%-60% as well as applying model group of 35%-50%, and an equal sample ratio results. For all animal experiments, the animal groups were randomized, and the researchers were blind to the genotypes and treatments of animals. We did not separate male from female in the animal studies because we did not notice any difference during our experimentation. No animals were excluded from the analysis.

Animal husbandry

Wild-type adult zebrafish (Danio rerio) were maintained at 28.5°C in a light-dark cycle (14-h of light and 10 h of darkness). Zebrafish embryos were maintained in a 1-mm petri dish with E3 water at 28.5°C until 7 dpf, and then, they were maintained in a recycling water system. Animal density was maintained after 28 dpf at 15-20 fish per 3 liters. The atg7-mutant ($atg7^{sa10973}$) and cmlc2:creER zebrafish lines were purchased from the Zebrafish International Resource Center (ZIRC).

C57/BL6 mice were maintained in a 12-h light/12-h dark cycle from 6 AM to 6 PM. All animal experiments were approved by the Mayo Clinic College of Medicine Institutional Animal Care and Use Committee (A00002577-17) and Beijing University of Chinese Medicine Animal Care Committee (BUCM-4-2018101504-4068). Please see the Major Resources Table in the Supplemental Materials.

Zebrafish eAIC model, aAIC model and mouse AIC model

Zebrafish eAIC model: The protocol for establishing the eAIC model was based on a previous study.20 Zebrafish embryos 1 dpf were distributed in a 96-well plate after removal of the chorions, with each well containing 3 fish embryos in 200 μl of E3 water with either DMSO or 100 μmol/L doxorubicin hydrochloride (DOX; Sigma). The plates were covered with foil to avoid light exposure and incubated at 28.5°C. The medium was exchanged for fresh medium every day. Three days postfertilization, the zebrafish were phenotyped based on survival rate, heart rate, and fraction shortening.

Zebrafish aAIC model: The protocol for establishing the zebrafish aAIC model was modified based on a previous study.[16] The dose of 20 mg/kg DOX was determined experimentally and is comparable to the accumulative dose used in rodent AIC models (15-25 mg/kg).[16] DOX was injected intraperitoneally (IP) into anesthetized adult zebrafish using a syringe with a 34 G needle. The fish were positioned in the cavity of a sponge with their abdomen up. The needle penetrated the ventral midline between the pectoral fins, and with the needle pointing posteriorly, towards the tail, entered the abdomen and then continued to move under the silver skin. Because the skin is partially transparent, it is possible to closely monitor the movement of the needle, avoiding any physical damage to intestines and/or other internal organs.

Mouse AIC model: The protocol for establishing the mouse AIC model is based on a previous study.[12] Either tail vein injection (IV) or intraperitoneal injection (IP) was conducted in adult mice with DOX. Either a single injection of 20 mg/kg DOX or multiple injections of 5 mg/kg DOX weekly for 4 consecutive weeks was carried out. As a stock solution, DOX was dissolved in 0.9% saline as a 2 mg/ml solution. Based on the body weight of each mouse, the injection volume was calculated to reach the desired dose.

Quantification of the heart function in the eAIC model zebrafish

Embryos 3 dpf were anesthetized in tricaine (0.02%) (Argent Chemical Laboratories) for 2 min, placed on their lateral side, and held in place with 3% methyl cellulose (Sigma-Aldrich). Beating hearts were documented using a Zeiss Axioplan II microscope with a 10.0X lens. Ld and Ls, the length of the short axis of the ventricle at the end-diastolic stage and end-systolic stage, respectively, were measured using ImageJ software. Fraction shortening (FS) was calculated using the formula: [FS=(Ld−Ls)/Ld].[41] Tail blood flow was recorded by a high-speed camera and calculated by a customized analysis algorithm.[15, 42] We used a microscope to record a video of the end of the zebrafish tail vein for 30 seconds, and then, the video was played at 8%-10% original speed utilizing tracker software. We measured the red blood cell flow rate within a fixed time of 30 seconds. Each video clip was counted three times to obtain caudal vein velocity per unit time.

Quantification of the heart function in the aAIC model zebrafish

High-frequency echocardiography was carried out using a Vevo 3100 high-frequency imaging system (Fujifilm Visual Sonics Inc., Toronto, Canada) equipped with a 50-MHz linear array transducer (MX700). Acoustic gel (Aquasonic 100, Parker Laboratories Inc.) was applied over the surface of the transducer to provide adequate coupling with the tissue interface. Zebrafish were anesthetized with tricaine (0.16 mg/ml), placed ventral side up, and held in place on a soft-sponge stage. The MX700 transducer was positioned above the zebrafish to provide a sagittal imaging plane of the heart. B-mode images were acquired with an imaging field of view of 9.00 mm in the axial direction and 5.73 mm in the lateral direction, a frame rate of 123 Hz, medium persistence, and a transmit focus at the center of the heart. Images were quantified using the Vevo LAB workstation. Ejection fraction were measured from B-mode images. Cardiac contractility was quantified using ejection fraction (EF) [EF=(EDV−ESV)/EDV] or fractional shortening (FS) [FS=(Ld−Ls)/Ld], where EDV and ESV are the ventricular volumes at the end-diastolic stage and end-systolic stage, respectively.[43] Three to five independent cardiac cycles per fish were measured to determine average values.

Quantification of the heart function in the AIC model mice

Heart function and ventricular size were measured using a Vevo 2100 imaging system equipped with an MS400C scan head. Two-dimensionally directed M mode (2D M-mode) images of the left ventricle were acquired from the short axis view. Left ventricular internal diameters at end-diastole (LVEDD) and end-systole (LVESD) were measured. Fractional shortening (FS) was calculated using the formula (LVEDD – LVESD)/LVEDD (%). Ejection fraction (EF) was calculated using the formula (LVEDV – LVESV)/LVEDV (%).

Western blotting

Freshly dissected tissues were collected and mechanically homogenized in lysis buffer (Sigma-Aldrich) used for radioimmunoprecipitation assay containing proteinase inhibitor, phenylmethylsulfonyl fluoride, and stainless steel beads. Standard Western blotting protocol were followed. Samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a PVDF membrane (Millipore, CA, USA). The following primary antibodies were used: anti-LC3 antibody (1:3000; Cell Signaling Technology), anti-Atg7 antibody (1:1000; Cell Signaling Technology), anti-Top2b antibody(1:10000; Abcam), anti-actin (1:2000; Sigma-Aldrich), and anti-GAPDH (1:2000; Santa Cruz Biotechnology).

Generation of the Tg(βactin2: loxP-mCherry-stop-loxP-atg7-cerulean) transgenic line

The transgenic line was generated using the Tol2/Gateway system. The loxP-mCherry-stop-loxP fragment was inserted into the Kpn I/Eco RI sites of a pENTRI1a vector obtained from the Tol2Kit. [44] Full-length atg7 cDNA (ENSDARG00000102893.3) from a cDNA pool was amplified using forward primer 5’-GATGGCGGAATCCAGTCTGAAGC-3’ and reverse primer 5’ -GATGCTCTCGTCGTCACTCATGTCC-3’. A clone with the correct sequence of full-length atg7 was confirmed by Sanger sequencing, and the gene was then cloned into a pENTRI1-loxP-mCherry-stop-loxP vector. To generate the final construct, p5E-bactin2, pENTRI-loxP-mCherry-stop-loxP-atg7, p3E-cerulean-polyA and pDestTol2pA were combined using Gateway LR Clonase II Plus Enzyme (Thermo Fisher). Founder zebrafish fish (F0) were identified on the basis of mCherry fluorescence. Founder fish were also identified by genotyping using the forward primer 5’- CTTTTTACTGAGCCCTACGAC-3’ and the reverse primer 5’-CTTTCGTCTAAGCGGTACTCG-3’. F2- and F3-generation transgenic zebrafish were used for experiments.

Conditional expressional system

4HT (4-hydroxytamoxifen, Sigma-Aldrich) powder was dissolved in 100% ethanol to generate a 12.9 mM stock solution, which was stored at 20°C. The stock solution was diluted in aquarium water for different working concentrations. To activate a tissue-specific promoter,[45, 46] Tg(□act2:atg7);Tg(cmlc2:CreER) double-transgenic adult zebrafish were incubated in 400 ml of system water containing 1 μmol/L 4HT in a 1-liter mini tank for 24 h. Subsequently, the zebrafish were rinsed in fresh water and returned to the system.

Real-time quantitative PCR

Freshly dissected tissues were pooled for RNA extraction. RNA was extracted using TRIzol (Bio-Rad) following the manufacturer's instructions. cDNA was synthesized from 100 - 500 ng of RNA by using a Superscript III First-Strand Synthesis System (Invitrogen). Real-time reverse transcription PCR assays were performed in 96-well optical plates (Thermo Fisher Scientific) using an Applied Biosystems VAii 7 System (Thermo Fisher Scientific). Amplification was comprised of a 10 min initial denaturation/activation step at 95°C, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s, and a fluorescence measurement. Melting curve analysis was performed by monitoring fluorescence throughout incremental increases of temperature from 60°C to 95°C. Levels of gene expression were normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (gapdh) using –ΔΔCt (cycle threshold) values. The primers were 5'-ACTCAACGAGTACCGCTTAG -3' (sense) and 5'-CTCATTGGCTGCTTTGTCC 3' (antisense) for zebrafish atg7, 5'-TCAGATGGCAGAGTTTGGAG-3' (sense) and 5'- CGTGTATCTGGAGTGAAACTG-3' (antisense) for zebrafish nppa, 5'-GCAGGAATACACAATCCGC-3' (sense) and 5'-CGTGTATCTGGAGTGAAACTG-3' (antisense) for zebrafish nppb, 5'-AGAGGAGAAACTGGCACAGG -3' (sense) and 5'- CAAACTACCCACCAGCCAGT -3' (antisense) for zebrafish Top2b, 5'-CCACCCATGGAAAGTACAAG-3' (sense) and 5'-CTCTCTTTGCACCACCCTTA-3' (antisense) for zebrafish gapdh. In mouse studies, the primers were 5'- GACACTGTGCTGGTCTCCTTGC -3' (sense) and 5'-GTCCATACATCCGCTGAGGTTCAC-3' (antisense) for mouse Atg7 and 5'-TTCAACGGCACAGTCAAG-3' (sense) and 5'-TACTCAGCACCAGCATCA-3' (antisense) for mouse Gapdh.

Compounds for drug screening

All FAAs used in this study (the name, company and catalog number are listed in Table S1) were dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich). DMSO (0.1%) was used as a negative control. We also used the following autophagy activators: Tat-Beclin 1 peptide (Ana Spec),[47] rapamycin (Sigma-Aldrich) and the following autophagy inhibitors: bafilomycin A1 (Sigma-Aldrich) and 3-MA (Sigma-Aldrich).[48] Zebrafish embryos were incubated in E3 water containing compounds at the desired concentrations.

Deducing the concentration of drugs with the eAIC model to use in the aAIC model

Based on the eAIC model, we obtained the effective dose for each compound experimentally. We used the formula listed in online Fig VI B to deduce the corresponding concentration in aAIC.[49] We assumed that all the drugs added to 96-well plates were absorbed by the fish embryos (we arranged 3 embryos in each well with 0.2 ml of medium). The average weight of an adult zebrafish is approximately 0.3 g, and that of an embryo is 0.005 g. Thus, the weight ratio between adult fish and embryos is 60 (300/5).

Drug administration to adult fish and mice via oral gavage

To administer drugs, adult zebrafish were anesthetized with tricaine and then propped vertically in a damp sponge.[50, 51] The fish mouth was opened, and a soft catheter tube (Braintree Scientific) was inserted 1.0~1.5 cm deep to reach the fish stomach. In general, the insertion process was unobstructed; however, any obstructions encountered were circumvented by changing the insertion angle. The drug was released in the zebrafish when the syringe was depressed. The delivery volume was less than 1 µl/100 mg body weight.

To administer drugs to mice, a standard gavage protocol was used. [52] The doses of Spi (20 mg·kg^{-1}),[53] Pra (40 mg·kg^{-1}) [54] and Rapa (2.81 mg·kg^{-1}) [55] were determined based on previous studies. Mino (10.23 mg·kg^{-1}) was determined based on a conversion from human to mouse according to the FDA guideline (Online Fig. VI C). Given that these drugs are poorly soluble in water, we applied a 0.5% aqueous solution of sodium carboxymethylcellulose (CMC) as a vehicle, as described in a previous study.[56]

Histological analysis and immunofluorescence microscopy

Left ventricle tissue from each group was fixed with 4% paraformaldehyde, embedded with paraffin, and sectioned into about a 5 µm slice. Then slices were stained with hematoxylin-eosin (H&E). Specifically, following steps of deparaffination in xylene and ethanol (100%, 96%, 70%, 50%) solutions at room temperature, slides were washed in RNase-Free water and RNase-Free PBS. Slides were then incubated with Haematoxylin solution for 3 minutes followed by wash in RNase-Free water. Following bluing step for 3 minutes, slides were then washed in running tap water for 5minutes and counterstained with Eosin for 3 seconds. Sections were then washed in tap water and dehydrated (95%, 100% EtOH, Xylene) and mounted with xylene-based mounting medium to be imaged. Additionally, we detected the DNA double strand breaks (DSB) in myocardial tissue. The sections were blocked, permeated and incubated with antibody against γ-H2AX (anti–γ-H2AX, ab81299, Biolegend, US) for 24 h at 4℃ followed by incubation with horseradish peroxidase (HRP) conjugated secondary antibody for 30 min at room temperature. Finally, the slides were developed using diaminobenzidine (DAB). Nuclear staining by hematoxylin was performed as counterstaining. The tissue slides were also stained with TUNEL for detection of myocardium apoptosis. Briefly, tissue slides deparaffinized were treated with 20 μg/ml proteinase K for 20 min at 37℃, and then were rinsing in PBS for 3 times. The specimens were then incubated with TUNEL detection solution containing terminal deoxynucleotidyl transferase (TdT) and fluorescent labeling solution for 1 h at 37 °C. Dihydroethidium (DHE) staining were used to measure the production of ROS in cardiac sections. DHE was obtained from Sigma-Aldrich LLC (Shanghai, China). Frozen sections of the cardiac tissue were incubated with 5 μM DHE in a light-protected humidified chamber for 30 min at 37°C. All images were acquired under an optical microscope (Leica Microsystems GmbH).

Analysis of biomarkers in serum

Serum supernatants were extracted from abdominal aortic blood. The blood was poured into a collection tube and allowed to stand for 2 h at room temperature. Then, the blood was centrifuged for 15 min at 3000 r/min. The upper layer of serum was transferred to a new 1.5-ml Eppendorf tube. Lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) activity was quantified using an automatic biochemical analyzer (HITACHI 7080, Japan).

Electron Microscopy

1 mm^3 cardiac tissue was fixed with 2.5% (v/v) glutaraldehyde. After three washes with 0.1 mol/L sodium cacodylate buffer, tissue was post-fixed in 1% osmium tetroxide and 0.8% K3 [Fe(CN6)]/0.1 mol/L sodium cacodylate buffer for 1 hour (RT). Then followed by three rinses with water, specimens were then dehydrated with increasing dosages of ethanol, infiltrated with Embed-812 resin, and polymerized at a 60°C oven overnight. Blocks were then sectioned with a diamond knife on a Leica Ultracut UC7 ultramicrotome and collected onto copper grids, stained with 2% uranyl acetate in water and lead citrate. Finally, images were obtained using a Tecnai G2 spirit transmission electron microscope (FEI).

H9C2 cell culture system

GFP-mCherry-LC3 adenovirus transfection: According to the manufacturers' instructions (Hanbio Technology, Shanghai, China), H9C2 cell cultures were split one day before adenovirus transfection and reached 50-60% confluence after 24 h. Following the experimental protocol, H9C2 cells were incubated with adenovirus (multiplicity of infection=10) for approximately 8 h. Images were obtained using a laser confocal microscope (Leica Microsystems GmbH). To determine the colocalization efficiency of GFP with mCherry signals, ImageJ software was used.

Small interfering (si) RNA transfection: H9C2 cells were cultured in serum- and antibiotic-free siRNA transfection medium (Santa Cruz Biotechnology). Atg7 siRNA (5'-CTCGCCGAGCTCGCCCA-3') and Top2b siRNA (5'-GGUGCAAAACUUUGUAAUADTDT-3') were purchased from Hanbio Technology (Shanghai, China). Cells transfected with nonspecific scramble siRNA (NC siRNA) were used as controls. Lipofectamine 2000 (Thermo Fisher Scientific) was applied to perform siRNA transfection (100 nmol/L) according to the manufacturer's instructions. The cells were analyzed 48 h after transfection.

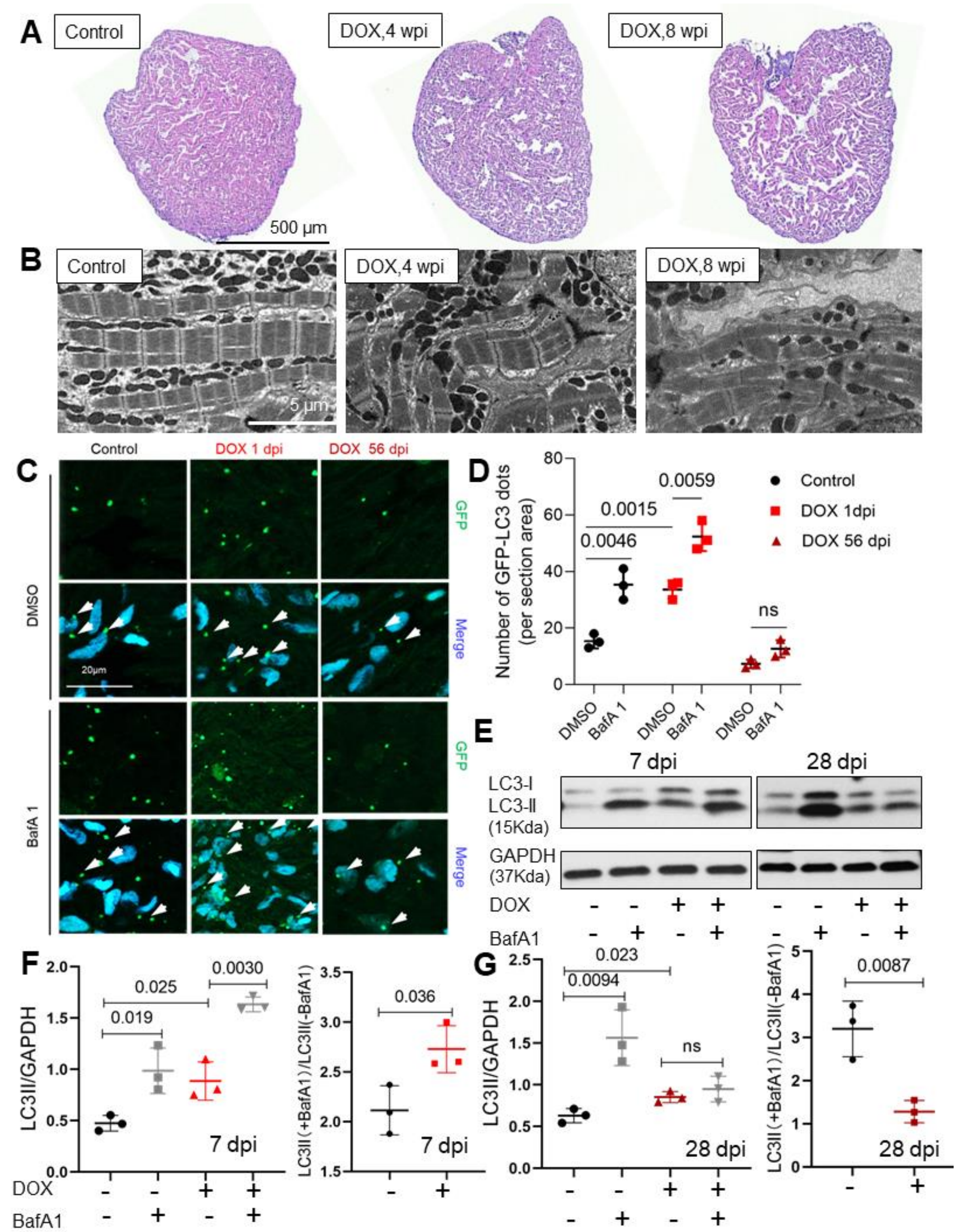
Tumor cell lines

MCF-7, U87 and HepG2 cells were obtained from China Infrastructure of Cell Line Resources (Chinese Academy of Medical Sciences, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, 11965–084) with 10% fetal bovine serum (FBS; Gibco, 16000-044) and 1% 100 U/mL penicillin/streptomycin (Corning, NYC, USA) in an incubator (Thermo, NYC, USA). CCK-8 was used to evaluate the interference of drugs on the anticancer effect of DOX.

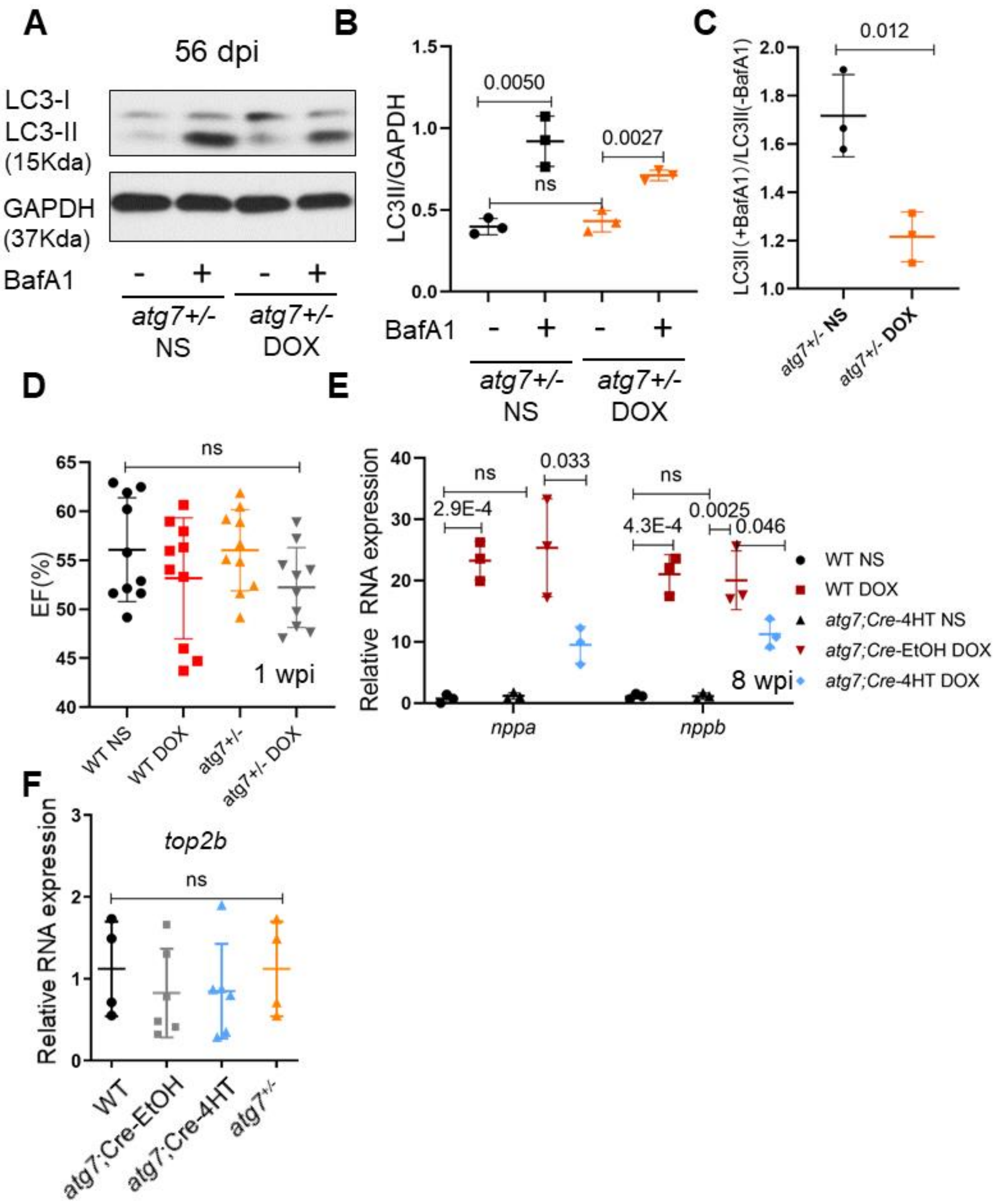
Statistical analyses

Graphs are presented as scatters or bar charts of means ± standard deviation (SD). All statistical analyses were conducted using R, SPSS 22.0 and Prism 8 (GraphPad) software. Unpaired two-tailed Student's t-test was used to compare two groups; one-way analysis of variance (ANOVA) (or Kruskal-Wallis test) followed by Tukey's post hoc test and Bonferroni post hoc test, was used for comparing three or more groups. All the data obtained from in vivo experiments was test for normal distribution using the Kolmogorov-Smirnov test. Data following a normal distribution was analyzed using unpaired t-test. In the case of data not-normally distributed or n too small ($n<6$) to test for normality, statistical significance was analyzed using Mann-Witney. Multiple testing correction was used for comparison of groups within ANOVA, Kruskal-Wallis test was used in the case of data not-normally distributed. Statistical test used to assess statistical significance is indicated in each figure legend with the precise p-value provided in the graphs where statistical significance was observed. P values less than 0.05 were considered statistically significant. Sample sizes can be found in the figure or figure legend. Power analysis was performed by using PASS 11 software to determine approximate sample sizes for the animal experiments.

Online Figures I - XI



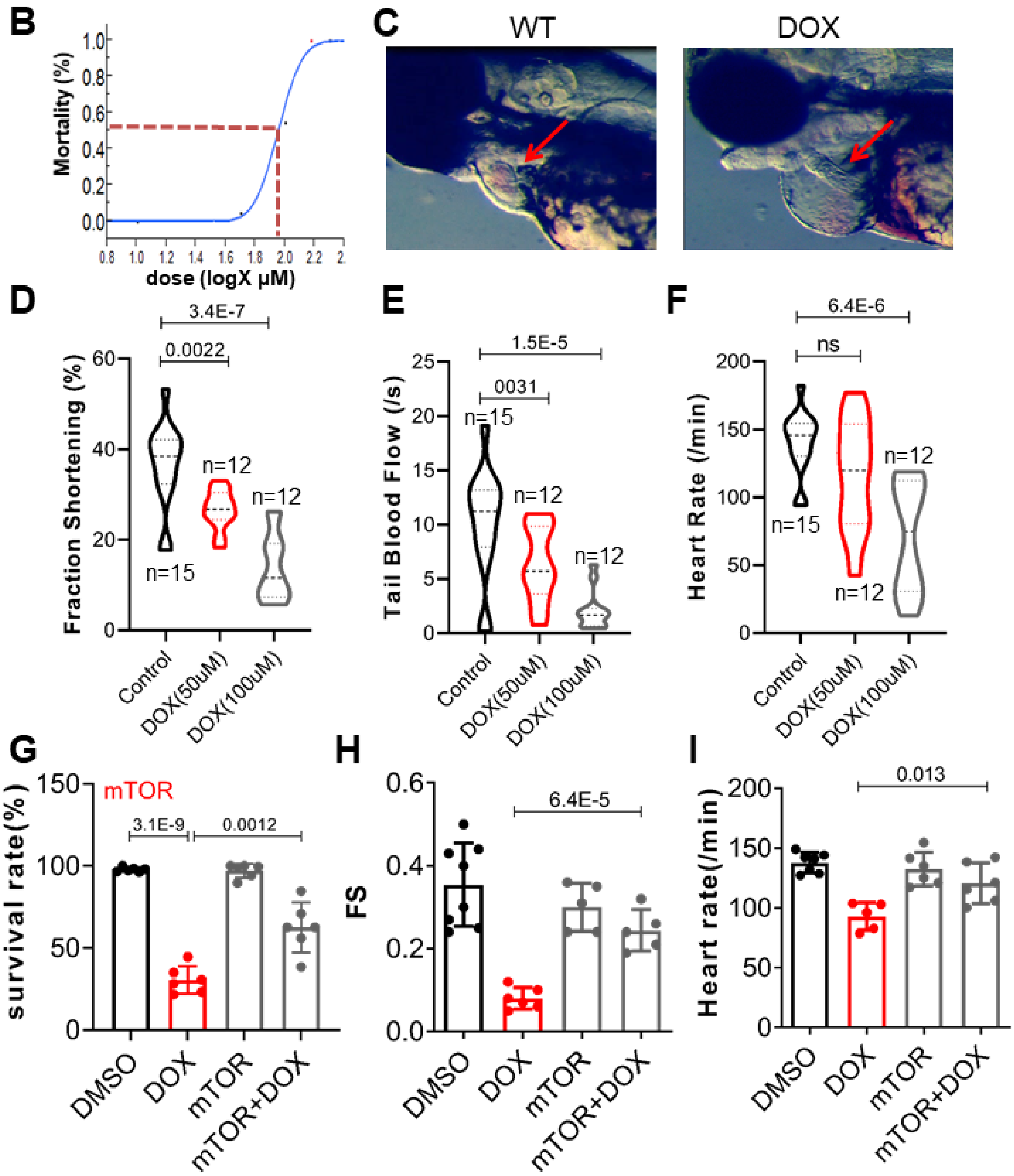
Online Fig. I. Supporting information on the zebrafish aAIC model. (A) HE staining and (B)Transmission electron microscopy (TEM) showed muscular disarray and myofibril loss in the fish ventricle compared with wild type control at 4 weeks and 8 weeks after DOX injection. (C) Representative images of sectioned ventricles of *Tg(GFP-Lc3)* zebrafish 1 and 56 dpi with or without BafA1 treatment. White arrows indicate LC3 aggregates. N=3 hearts/group. (D) Quantification of the GFP-LC3 aggregates in 1 dpi and 56 dpi. (E) Representative Western blot showing temporal changes in LC3-II protein expression in the hearts of adult zebrafish with AIC. Bafilomycin A1 (30 nM) was administered 4 h before the zebrafish were sacrificed. (F and G) Quantification of LC3-II and the ratio between hearts treated with and without BafA1 in (C), n=3 hearts/group. Bars represent the mean ± SD. Comparisons were performed by the Mann-Whitney test (two groups) or the Kruskal-Wallis test (multiple groups) followed by post hoc Tukey’s test.



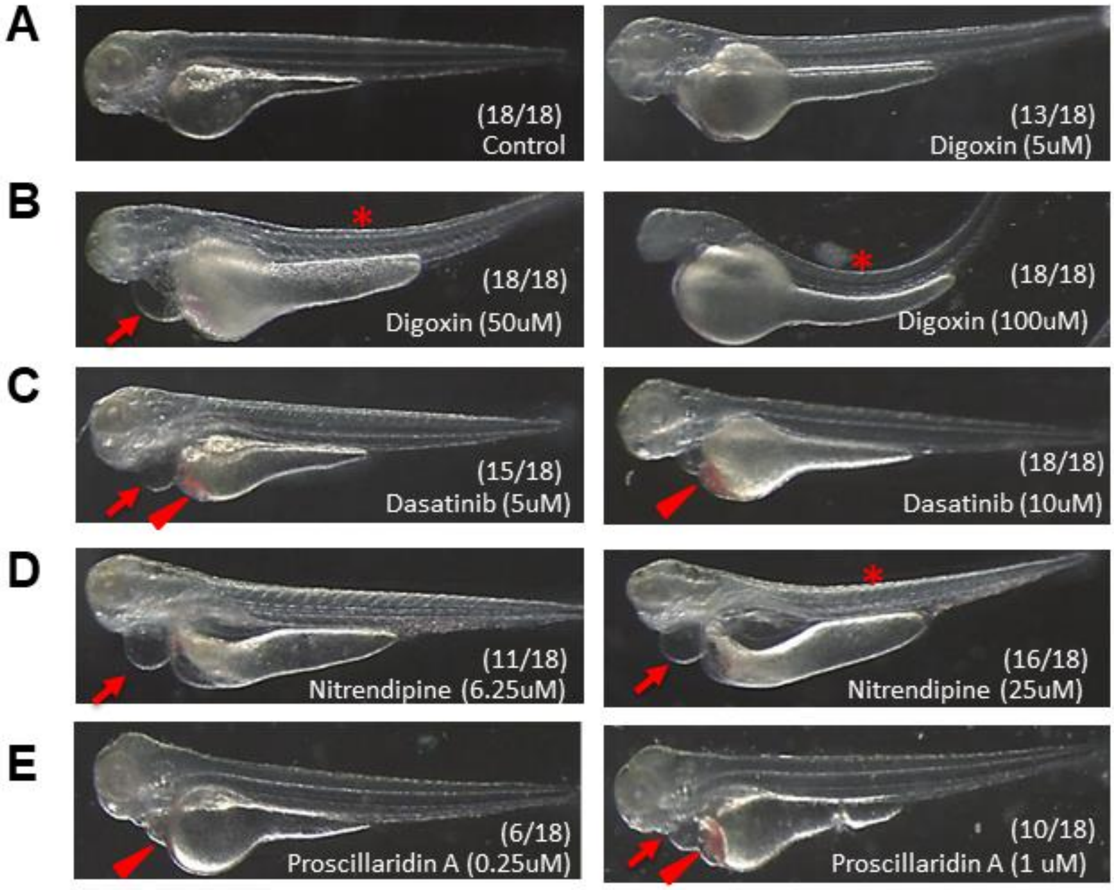
Online Fig. II. Supporting information on the atg7+/- and atg7 OE zebrafish. (A) Representative images of a Western blot showing the LC3 expression in the hearts atg7+/- zebrafish aAIC model at 56 days post DOX injection, in the absence or presence of 30 nM BafA1 for 4 h. (B and C) Quantification of the Western blot data in (A), n = 3 in each group. (D) *atg7+/-* did not affect cardiac function 1 week after injection with DOX. Ventricular ejection fraction of WT and *atg7+/-* zebrafish after induced DOX stress 1week post-injection (wpi) is shown (n=10). (E) Molecular markers are rescued by *atg7* OE. The levels of *nppa* and *nppb* gene transcript expression were assessed by quantitative RT-PCR 8 wpi. n=3 per group. (F)The expression level of top2b transcript were assessed by RT-PCR in either genetic manipulations of atg7. One-way ANOVA followed by Tukey's post hoc test was used in (D) and (F); Mann-Whitney test in (C); Kruskal-Wallis test was used followed by post hoc Tukey's test in (B) and (E). WT, wild type; DOX, doxorubicin.

A

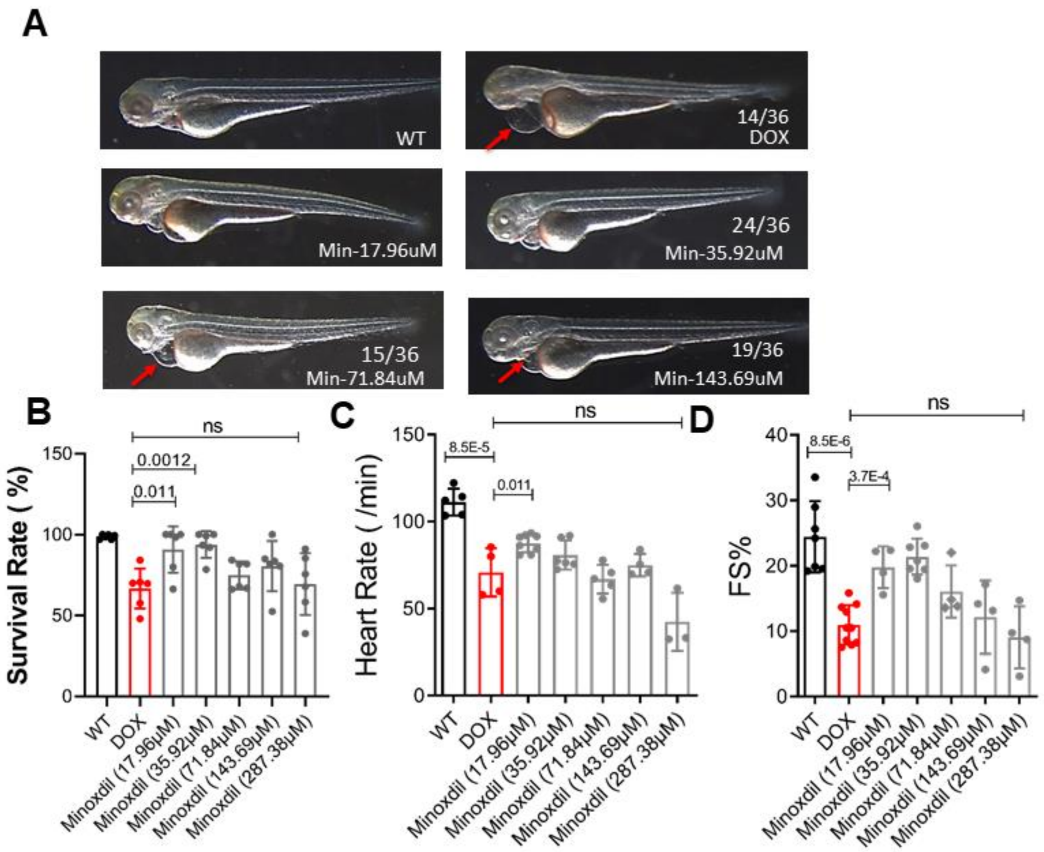
Days	N	LD50(μM)	Lower 95%	Upper 95%
2 dpf	90	194.83	178.08	235.15
3 dpf	93	99.380	75.99	112.04
4 dpf	88	54.31	44.46	63.10
5 dpf	90	33.99	23.59	45.22



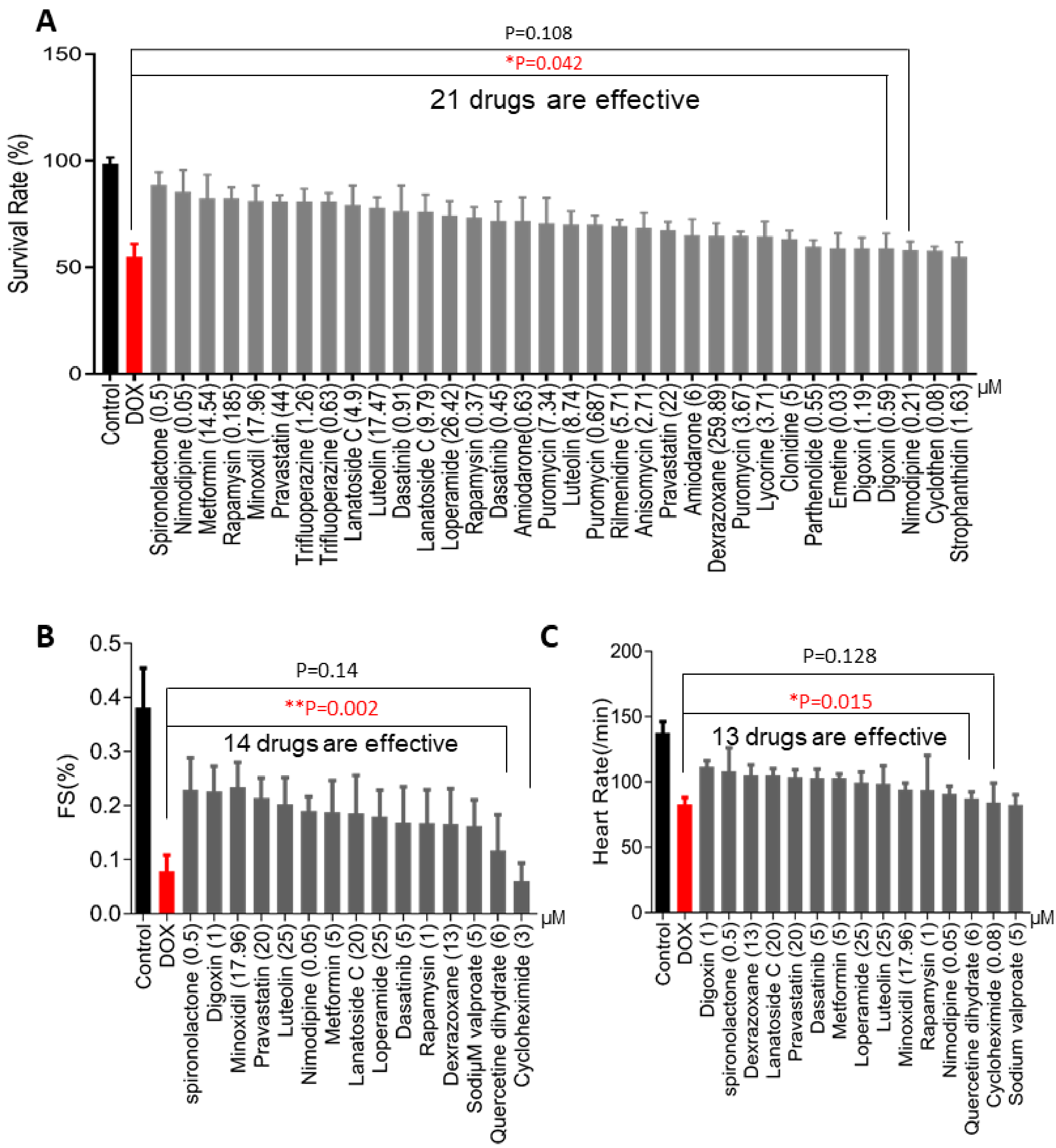
Online Fig. III. Establishment of an eAIC model with zebrafish embryos. (A) LD50 and the associated 95% confidence interval at the indicated timepoints. (B) Dose-mortality curve for DOX-treated zebrafish embryos. (C) Representative images of the fish embryos treated with or without DOX. Arrows indicate the location of the ventricle. (D) FS was reduced by the DOX-induced stress in a dose-dependent manner. (E) Tail blood flow was reduced by DOX-induced stress in a dose-dependent manner. (F) Heart rate was reduced by DOX-induced stress in a dose-dependent manner. (G to I) *mtor+/-* exerts therapeutic effects on eAIC, as indicated by the recovered survival rate, FS and heart rate. Kruskal-Wallis test was used followed by post hoc Tukey’s test.



Online Fig. IV. Digoxin, dasatinib, nitrendipine, and proscillaridin induce embryonic phenotypes in zebrafish. Representative embryonic phenotypes, including pericardial edema (arrow), blood accumulation (arrowhead), and curly body (*), are shown. Eighteen embryos were assessed for each drug at the indicated concentration. The number of fish with the indicated phenotypes are listed.

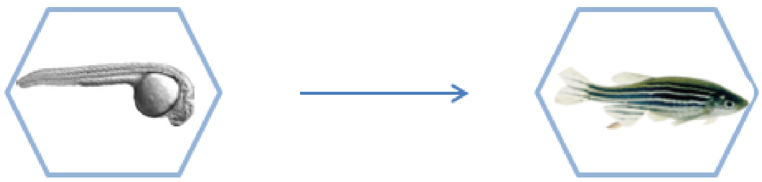


Online Fig. V. Assessing the therapeutic efficacy of a FAA using the eAIC models with minoxidil as an example. (A) Five concentrations (1/20, 1/40, 1/80, 1/160, and 1/320 of the LD50) of minoxidil were used to treat eAIC. The concentration at 35.92 µM (1/80 of the LD50) was the most effective (24/36). (B) Quantification of survival, heart rate and FS in the eAIC zebrafish treated with minoxidil at 5 different concentrations. One-way ANOVA followed by post hoc Bonferroni test.



Online Fig. VI. The therapeutic effects of FAAs on the eAIC model were ranked based on survival rate, fraction shortening (FS) and heart rate. Different concentrations of FAAs and their therapeutic effects as determined by the survival (A), FS (n=4-16) (B), and heart rate(n=3-9) (C) criteria, with 21, 14 and 13 FAAs considered effective, respectively; $P<0.05$ was the cutoff for significance.

A



Drugs	LD50 (μM)	e-ED (μM)
Spironolactone	10.11	0.5
Pravastatin	441.75	22
Minoxidil	5747.6	17.96
Rapamycin	3.67	0.185

Drugs	a-ED(mg/kg)
Spironolactone	2.78
Pravastatin	124.53
Minoxidil	50.11
Rapamycin	2.25

B

Formula

a-Dose(mg/kg) = e-dose(μM) × 0.2(ml)/3 × 60 × MW(g/mol)/0.3

e-dose(μM): dose in embryos

0.2(ml)/3: Volume /Number

60: Weight ratio 300/5(adult/embryo)

MW(g/mol): Drug

0.3: Weight Per fish: 0.3g

C

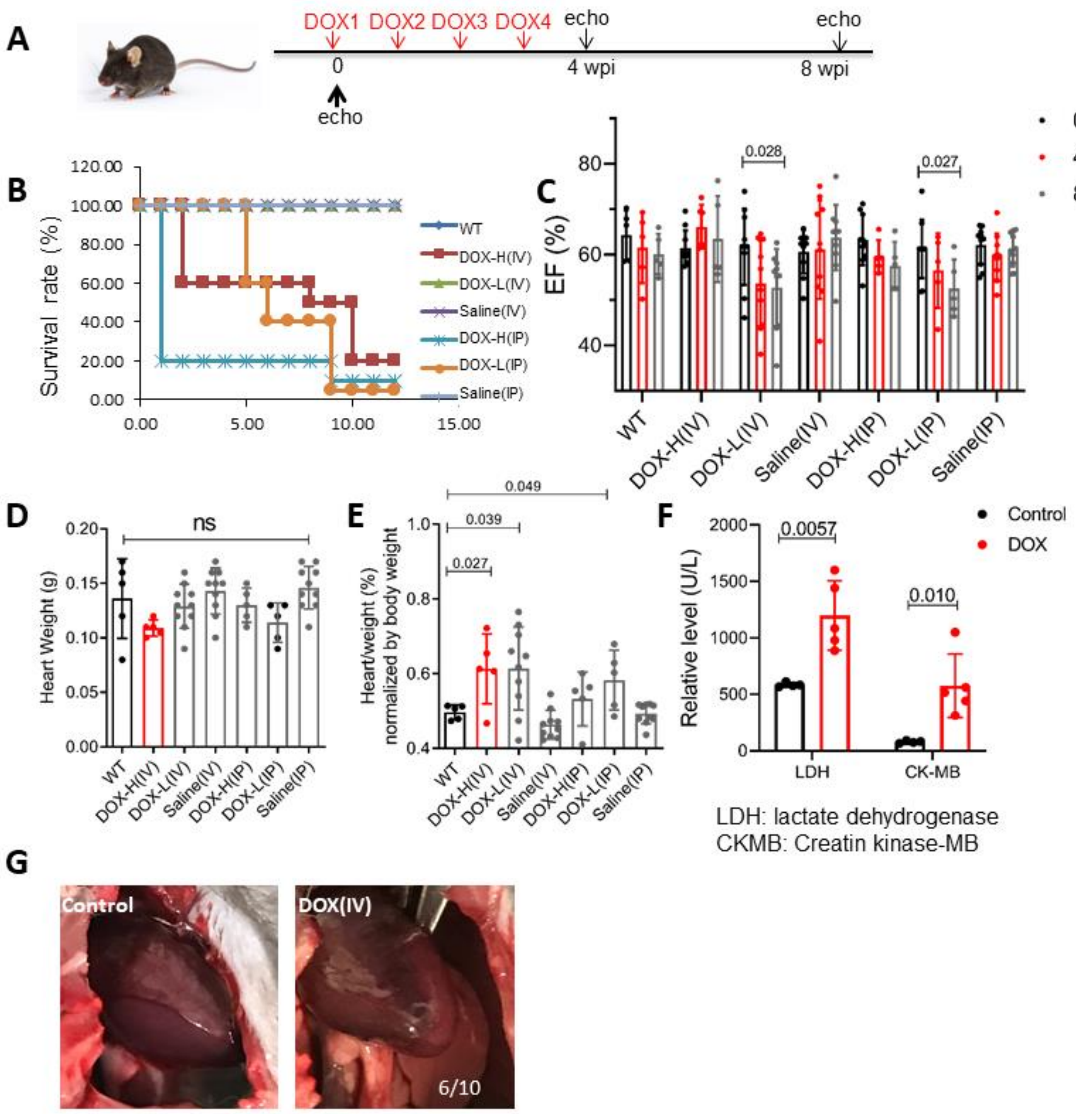
W_h=60kg W_m=20g Km_m=3 Km_h=37

ED_h(mg/Kg/day) = Dose for 60kg patient(mg/d) ÷ W_h
= 50mg/d ÷ 60 Kg

ED_m = ED_h × [Km_h / Km_m] = 10.23kg/kg

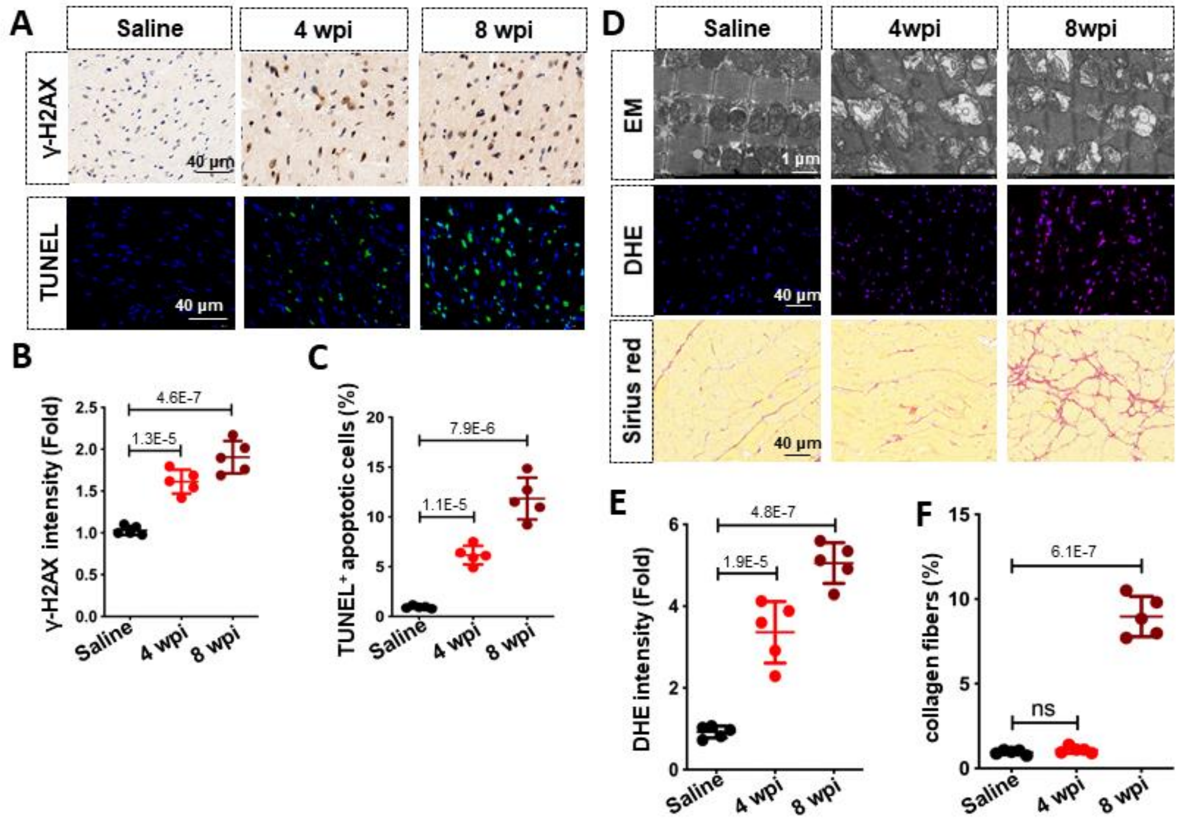
Notes: Human effective dose (ED) were obtained according to FDA guidelines: FDA reference ID3685475. W: weight; h:human; f: fish; Km: Factor for converting mg/kg dose to mg/ m^2 dose.

Online Fig. VII. Effective doses (EDs) for the top FAAs in the aAIC models were determined from the effective doses (EDs) in the eAIC. (A) While the LD50 and ED in the eAIC models (e-ED) were experimentally determined, the ED in the aAIC models (a-ED) was deduced using the formula shown in (B). (B) We assumed that all the drugs added to the 96-well plates (3 embryos in every well containing 0.2 ml of medium) were absorbed by the zebrafish embryos. The weight ratio between adult fish (0.3 g) and embryos (0.005 g) was 60 (300/5). ED: Effective Dose. (C) Drug dose conversion of minoxidil from human to mouse according to the FDA guideline.

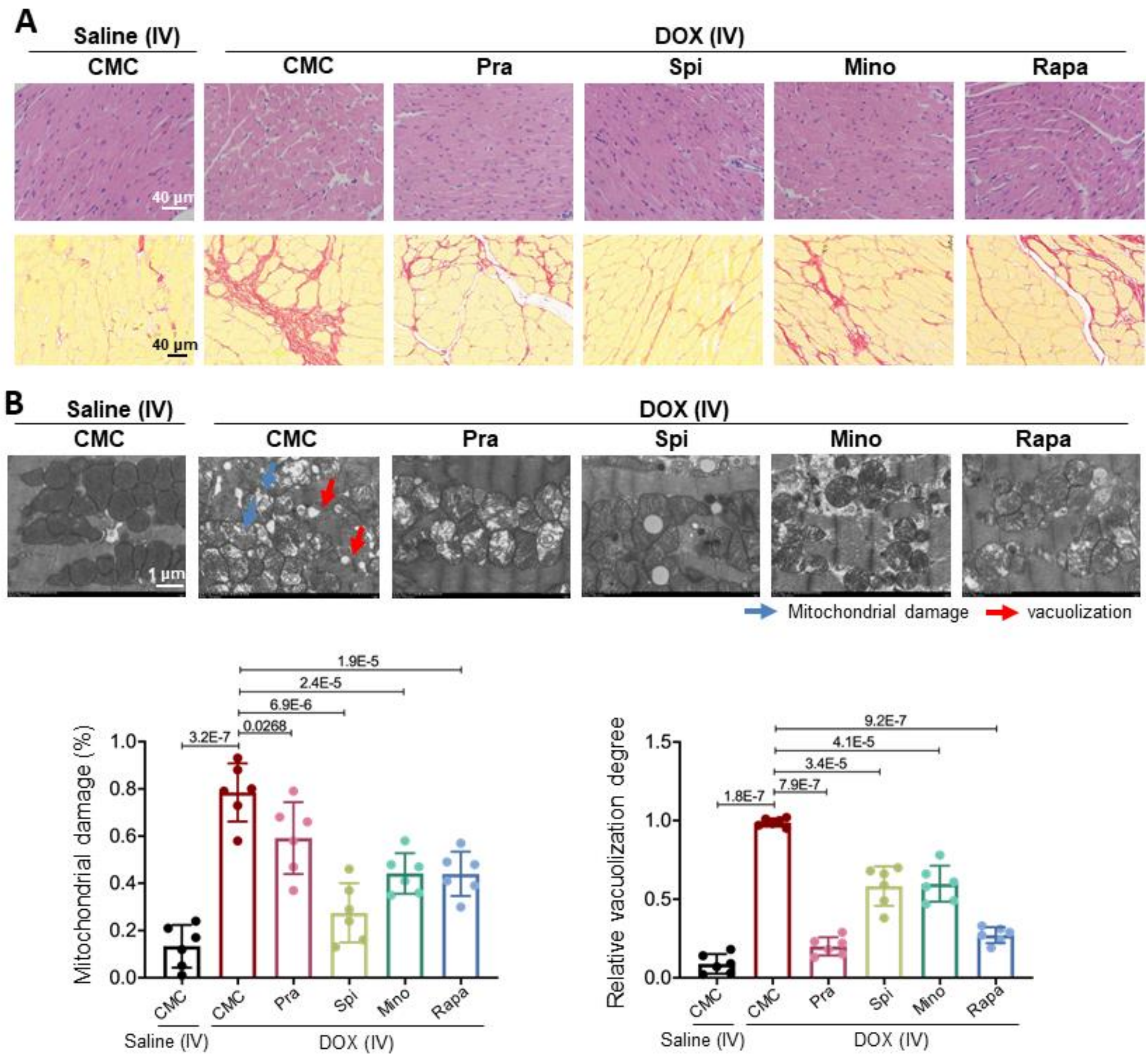


Online Fig. VIII. Generation of a mouse AIC model. (A) Schematics of the experimental procedure for establishing the AIC mouse model. Echocardiography was performed immediately before the first injection, 4 weeks after the first injection, and 8 weeks after the first injection. (B) Approximately 40% of the mice in the DOX-H(IV) group died 9 dpi, 80% of mice in the DOX-H(IP) group died at 1 week, 50% of mice in the DOX-L(IP) group died, and no mice died in the DOX-L(IV) group. N=5~10 per group. (C) EF (cube) of both DOX-L(IV) and DOX-L(IP) was deceased. (D and E) Heart weight was increased in the DOX-L(IP) group after normalization to body weight. N=5~10 per group. (F) LDH and CK-MB levels in serum were significantly increased in the DOX-L(IV) group. N=5~10 per group. (G) Heart structure was abnormal in the DOX(IV) group. DOX-H: single injection of high-dose DOX (20 mg/kg); DOX-L: four consecutive injections of low-dose DOX (5 mg/kg); IP: intraperitoneal injection; IV:

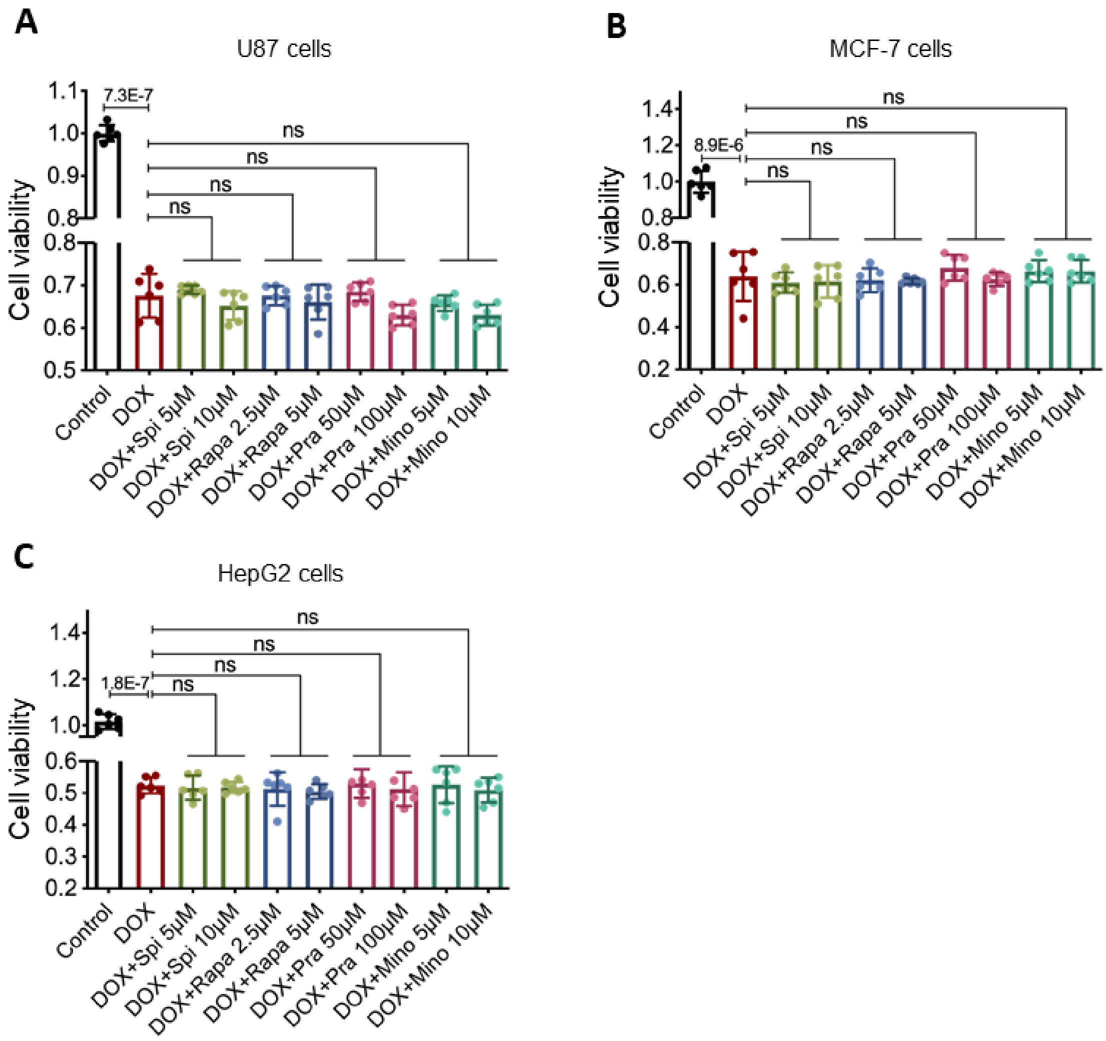
intravenous injection (tail vein). Comparisons were performed by the Mann-Whitney test (two groups) or the Kruskal-Wallis test (multiple groups).



Online Fig. IX. Histological and immunohistochemical analysis of the mouse AIC model. (A)The heart samples were stained with antibody against γ-H2AX (a marker for DSBs). TUNEL staining: Green color represents apoptotic cells via TUNEL staining; blue color represents cell nucleus stained by DAPI. (B and C) Quantification of (A). Scale car=40 μm. (D) Electron microscopy, DHE staining and Sirius red staining of mice hearts from saline group, 4 wpi group and 8 wpi group. Scale bar=1 μm (the first row). Scale bar=40 μm (the lower two rows). Analysis of DHE intensity (E) and collagen fibrosis (D). N=5 mice per group. Data are presented as mean ± SD. Kruskal-Wallis test was used followed by post hoc Tukey's test.



Online Fig. X. Four drugs rescued histological and ultrastructural abnormalities in hearts of the mouse AIC model. (A) Shown are images after HE staining (top panels) and sirius red staining (lower panels). Scale bar=40 μm. (B) Electron microscopy showed mitochondrial damage(blue arrows) and vacuolization (red arrows) by DOX can be rescued in drug treated hearts. Scale bar=1 μm. CMC: a 0.5% aqueous solution of sodium carboxymethylcellulose (CMC) was applied as a vehicle. Kruskal-Wallis test was used followed by post hoc Tukey's test.



Online Fig. XI. In three in vitro tumor models, none of the four FAA drugs affect DOX's antitumor activity. Shown are proliferative index in U87 (A) MCF-7 (B), and HepG2 cells (C). Kruskal-Wallis test was used followed by post hoc Tukey's test.

Online Table. I. LD50 for 37 FAAs.

Drug	Company	Catalogue number	LD50 (μM)	Reference
Dexrazoxane	Sigma-Aldrich	D1446	41582.16	7
Minoxidil	Sigma-Aldrich	M4145	5747.60	27
Carbamazepine	Sigma-Aldrich	C4024	1056.42	26
Metformin	Sigma-Aldrich	M0605000	581.6	26
Loperamide	Sigma-Aldrich	L4762	528.31	27
Pravastatin	Sigma-Aldrich	P4498	441.75	26
Quercetine	Millipore	551600-M	382.82	28
Luteolin	Sigma-Aldrich	L9283	174.74	28
Strophanthidin	Sigma-Aldrich	S6626	130.22	28
Verapamil	Sigma-Aldrich	676777	107.21	26,27,29
Strophantine Octahydrate	Fisher scientific	AC161732500	105.26	28
Clonidine	Sigma-Aldrich	1140393	101.66	26,27
Lanatoside C	Sigma-Aldrich	L2261	97.9	28
Lycorine	Sigma-Aldrich	L5139	74.15	28
Rilmenidine	Sigma-Aldrich	R134	57.12	26
Sodium valproate	Sigma-Aldrich	S0930000	56.72	26,27
Anisomycin	Sigma-Aldrich	A9789	54.2	28
Temozolomide	Sigma-Aldrich	T2577	39.97	27
Digoxin	Supelco	D6003	26.89	28
Digitoxigenin	Sigma-Aldrich	D9404	23.28	28
Nitrendipine	Sigma-Aldrich	N144	15.21	27
Trifluoperazine	Sigma-Aldrich	T8516	12.58	26,29

Amiodarone	Sigma-Aldrich	A8423	12.5	27,29
Parthenolide	Sigma-Aldrich	P0667	10.96	28
Spironolactone	Sigma-Aldrich	S3378	10.11	28
Dasatinib	Sigma-Aldrich	SML2589	9.07	28
Puromycin	Sigma-Aldrich	P8833	6.87	28
Kaempferol	Sigma-Aldrich	K0133	4.3	28
Nimodipine	Sigma-Aldrich	N149	4.28	27
Rapamycin	Sigma-Aldrich	R8781	3.67	26,27,29
Verapamil Hydrochloride	Sigma-Aldrich	V4629	2.38	28,29
Tat-beclin 1	AS-65467	AnaSpec	1.41	26,47
Cycloheximide	Sigma-Aldrich	C4859	0.76	28
Amrinone	Sigma-Aldrich	A9251	0.71	28
Emetine	Sigma-Aldrich	E2375	0.55	28
Proscillaridin A	Sigma-Aldrich	E2375	0.36	28
Helveticoside	Sigma-Aldrich	H2634	No available	28
Lithium	Sigma-Aldrich	499811	No available	26

The ordering information for 37 FAAs and references suggesting their identity as autophagy activators. The LD50 for each drug was determined experimentally, as exemplified by DOX (see Online Fig. II A-B).

Drugs	Survival	FS	Heart Rate	Phenotype	Score	Rank	Reported/ new drug
Spironolactone (0.5 µM)	1	1	2	NO	1.33	1	Reported(57)
Pravastatin (22 µM)	2	3	5	NO	3.33	2	Reported(54)
Metformin (14.54 µM)	4	7	7	NO	6.00	3	Reported(58)
Minoxidil (17.96 µM)	7	4	10	NO	7.00	4	New
Nimodipine (0.05 µM)	3	6	12	NO	7.00	5	New
Digoxin (1.19 µM)	13	2	1	YES (curly)	7.33	6	Reported(59)
Lanatoside C (4.9 µM)	10	10	4	NO	8.00	7	New
Rapamycin (0.185 µM)	6	9	11	NO	8.67	8	Reported(17)
Dexrazoxane (259.89 µM)	11	13	3	NO	9.00	9	Reported(60)
Luteolin (17.47 µM)	8	5	9	YES (edema)	9.33	10	Reported(61)
Trifluoperazine (1.26 µM)	5	8	16	NO	9.67	11	New
Dasatinib (0.91 µM)	9	11	6	YES (edema)	10.67	12	New
Loperamide (26.42 µM)	14	12	8	NO	11.33	13	New
Cycloheximide (0.08 µM)	15	16	14	NO	15.00	14	New
Quercetin (619.41 µM)	18	15	13	NO	15.33	15	Reported(62)
Puromycin (0.68 µM)	16	17	17	NO	16.67	16	New
Valproate (5 µM)	17	14	15	YES (blood)	17.33	17	New
Proscillaridin A (0.02 µM)	12	18	18	YES (edema)	18.00	18	New

Online Table. II. Ranking of the FAAs based on their therapeutic efficacy.

The rankings of the FAAs were based on survival rate, heart function, heart rate; whether FAAs induce the acquisition of embryonic phenotypes in zebrafish; and the overall rank based on a combinatory score using the formula: combinatory score=[(rank based on survival rate + rank based on FS+ rank based on heart rate)/3+Phenotype*2]. Information is presented on drugs that were previously reported in the literature as potentially effective for AIC and those that have been newly identified.