Data Supplement

Interdependent nuclear co-trafficking of ASPP1 and p53 aggravates cardiac ischemia/reperfusion injury

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Methods

Animals

To avoid the influence of estrogen fluctuations in the female mice, the male mice (C57BL/6 background) (7-8 weeks old, 22-25 g) were used to assure consistence of results in this study[39-41]. All mice were maintained in a temperature-controlled facility with 12 h light/dark cycle at 23±3°C and 30-70% humidity. All animal experiments were approved by the Ethic Committees of College of Pharmacy, Harbin Medical University (IRB3005821) and in accordance with the Guide for the Care and Use of Laboratory in Harbin Medical University. The experimenters were blind to treatment/genotype grouping information during the experiment and quantification. No mice were excluded from the study unless died. Group sizes were determined according to our previous experience with establishment of mouse model of myocardial ischemia and reperfusion[42]. Briefly, the number of mice in each group was determined based on power calculations for the primary parameter (infarct area) with mean differences and standard deviations taken from pilot data at power 80% with a standard level of significance of 0.05.

Generation of ASPP1 transgenic mice and knockout mice

Cardiomyocyte-specific ASPP1 overexpression transgenic (TG) mice and ASPP1 conventional knockout (KO) mice were generated by Cyagen Biosciences Co., Ltd (China). To generate ASPP1(TG) mice, the ASPP1 cDNA was amplified and cloned into a vector containing a murine α-myosin heavy chain (α-MHC) promoter. The transgenic expression vector was then injected into mouse fertilized eggs by microinjection. The ASPP1(KO) mice was constructed by CRISPR/Cas9 strategy. Briefly, gRNA1 (matching forward strand of gene: 5'-GAGTTACAGACATGTGGTGCTGG-3'), gRNA2 (matching reverse strand of gene: 5'-TCTAGCTTCTCTGTGGTACAGGG-3') and Cas9 expression plasmids were designed to delete the second exon of ASPP1. Genomic PCR of tail DNA was performed to detect genotype of offspring of ASPP1(TG) mice (forward: 5' -AGTGATGAACAAAGGCACCG-3', reverse: 5'-AGCCAGAAGTCAGATGCTCAAGG-3') and ASPP1(KO) mice (forward 1: 5'-TGTGGTTCCCCTGTCAAACTC-3', forward 2: 5'-GTTGAACTTAGGAAGGAGATGGC-3', reverse 1: 5'-CGTCCAGAAGAACTGAGCTAAC-3'). All mice were compared with non-transgenic or wild-type gender-matched littermates.

Construction of adeno-associated virus (AAV9) carrying p53 shRNA

To induce cardia-specific knockdown of p53, we commissioned Cyagen Biosciences Co., Ltd (China) to construct the shRNA of p53 (sense: 5'-GGACAGCCAAGUCUGUUAU-3', antisense: 5'-AUAACAGACUUGGCUGUCC-3') packaged by adeno-associated virus (AAV9). The AAV9 virus was injected into 6 weeks old mice through the tail vein at a dose of 1×10^{10} PFU per animal. Two weeks after injection, experimental interventions were carried out.

Cardiac ischemia/reperfusion injury

Cardiac I/R injury was induced by 45 min ischemia, followed by 24 h reperfusion. Briefly, male mice (7-8 weeks old, 22-25 g) were anesthetized with 2% avertin (0.1 ml/10 g) intraperitoneally (i.p.). The anesthetized mice were intubated and ventilated using a rodent ventilator with a tidal volume of 200 μl and a frequency of 110 breaths per minute (R415; RWD life science, China). Then, the skin surface of the left chest was disinfected and a thoracotomy through 3, 4 intercostal area was performed to expose the heart. The left anterior descending coronary artery (LAD) was occluded by tying a slipknot with 7-0 silk suture 1-2 mm from the lower edge of the left atrium. After 45 min, the slipknot was released to allow 24 h reperfusion.

For sham group mice, the operation followed the same procedure without ligation.

TTC staining

To determine the infarct size, we excised and sliced the cardiac tissue into 1 mm thick slices. Then rapidly incubated slices in 2% 2,3,5-triphenyltetrazolium chloride (TTC, Solarbio, China) at 37°C. After 15 min of incubation, the reaction was terminated by 4% paraformaldehyde (PFA). The infarct area was determined by stereomicroscope (Zeiss Stemi 508, Germany) and measured by computerized planimetry (Image pro-plus 6.0).

Echocardiography

To determine the cardiac function of mice (7-8 weeks old, 22-25 g), the M-mode echocardiography of heart were acquired by Vevo2100 Imaging System (VisualSonics, Toronto, Canada) equipped with a 10-MH2 phased-array transducer. Briefly, after removing the hair from the chest of mice using NairTM depilatory cream (Church & Dwight Co., Inc., Princeton, NJ, USA), the mice were smeared with medical ultrasonic couplant (Tianjin Yajie Medical Material Co., Ltd., Tianjin, China). Two-dimensional targeted M-mode traces were recorded from the parasternal short-axis view at the level of the mid-papillary muscles and from the parasternal long-axis view at the level of immediately under of the papillary muscle. A minimum of six consecutive cardiac cycles were obtained, and the left ventricular systolic diameter (LVID, s), left ventricular diastolic diameter (LVID, d), left ventricular end diastolic volume (LVEDV), and left ventricular end systolic volume (LVESV) were analyzed based on M-mode recordings. Finally, ejection fraction (EF) was calculated as EF = (LVEDV-LVESV)/LVEDV×100% and fractional shortening (FS) as (LVIDd-LVIDs)/LVIDd×100%. The data are presented as the average of measurements of three consecutive beats.

Isolation of adult mouse cardiomyocytes

Adult male mice (7-8 weeks old, 22-25 g) were anesthetized by intraperitoneal injection of 2% avertin (0.1 ml/10g body weight). After 15 minutes, hearts were rapidly separated, and the aorta was cannulated on a constant-flow Langendorf apparatus at 37℃. The heart was digested by perfusion with Tyrode's solution containing Type II collagenase (1 mg/ml), protease (0.02 mg/ml), and bovine serum albumin (BSA, 1mg/ml). Tyrode's solution contained (mM): NaCl 123, KCl 5.4, HEPES 10, NaH_2PO_4 0.33, $MgCl_2$ 1.0, and glucose 10; pH adjusted to 7.4 with NaOH. When the tissue turned softening, perfusion was stopped and the left ventricle was dissected and gently dispersed to obtain isolated cardiomyocytes. To obtain cardiomyocytes from ischemia/reperfusion region, we carefully dissected the free wall of left ventricle experienced ischemia/reperfusion based on the color (pale) and position (below the suture around the coronary artery). The cardiomyocytes were then equilibrated in Tyrode's solution with 200 μM $CaCl_2$ and 1% BSA. Cardiomyocytes were long rod-shape or rectangular under the microscope. All solutions were gassed with 95% O_2 and 5% CO_2 and warmed to 37±0.5℃.

Serum creatine kinase isoenzyme MB detection

Male mice (7-8 weeks old, 22-25 g) were anesthetized with 2% avertin (0.1 ml/10 g) intraperitoneally (i.p.). After anesthetization, blood was collected from the inferior vena cava and allowed to stand at room temperature for 1h. Then, centrifuged the blood at 1000 g for 20 min to obtain the serum. Serum creatine kinase isoenzyme MB (CKMB) was detected by mouse CKMB Elisa Kit (E-EL-M0355, Elabscience, China) according to the protocol. The finally optical density (OD) was read at 450 nm.

Neonatal mouse cardiomyocytes culture and treatment

Neonatal mice (1-3 days) were used to isolate primary neonatal mouse ventricular cardiomyocytes (NMVCs). Briefly, after the skin surface disinfection with 75% alcohol, mice hearts were collected in the clean bench. Then, ventricular tissues were isolated and digested by 0.25% trypsin (Beyotime, China). The obtained cells were centrifuged at 1500 g for 5 min and resuspended by high glucose DMEM (Biological Industries, Israel) complete medium containing 10% fetal bovine serum (Biological Industries, Israel) and 1% penicillin/streptomycin (Beyotime, China). After 2 h's incubation (5% CO_2, 95% humidified air, 37°C), NMVCs were isolated and incubated for another 48 h under the same condition. To induce hypoxia/reoxygenation (H/R) injury, NMVCs were incubated with hypoxic condition (5% CO_2, 95% N_2, 37°C) for 12 h, followed by common condition (5% CO_2, 95% humidified air, 37°C) for 24 h.

Cell transfection

ASPP1 cDNA were inserted into GV141 vector with T7 promoter and XhoI/KpnI by Shanghai Genechem Co., Ltd (China). Full length p53, N-terminal (the binding fragment of ASPP1 does not have NLS, 1-288 aa) of p53 and C-terminal (NLS of p53, 310-381 aa) of p53 cDNA were inserted into GV141 vector with T7 promoter and XhoI/KpnI, and were all tagged with flag epitope by Shanghai Genechem Co., Ltd (China). Transfection of plasmids was carried out by mixing with Lipofectamine™ 2000 reagent (Invitrogen, America). To induce gene knockdown, small interference RNAs (siRNAs) were designed by Suzhou Genepharma Co., Ltd (China). The sequences of siRNAs for mouse ASPP1 were: 5'-GCAAGAUCAUGAAUGGCAATT-3' and 5'-UUGCCAUUCAUGAUCUUGCTT-3' (siASPP1-1); 5'-GCUGCUGUGGGUCCUUAUATT-3' and 5'-UAUAAGGACCCACAGCAGCTT-3' (siASPP1-2); 5’-GCAAAGGGCCACCUCCCAUTT-3’ and 5’-AUGGGAGGUGGCCCUUUGCTT-3’ (siASPP1-3). The sequences of siRNAs for mouse p53 were: 5’-GGACAGCCAAGUCUGUUAUTT-3’ and 5’-AUAACAGACUUGGCUGUCCTT-3’ (sip53-1); 5’-GACCUAUCCUUACCAUCAUTT-3’ and 5’-AUGAUGGUAAGGAUAGGUCTT-3’ (sip53-2); 5’-CCACUUGAUGGAGAGUAUUTT-3’ and 5’-AAUACUCUCCAUCAAGUGGTT-3’ (sip53-3). The sequences of siRNAs for mouse ASPP2 were: 5’-GGACUAUACCCAAGAAUUATT-3’ and 5’-UAAUUCUUGGGUAUAGUCCTT-3’. The sequences of siRNAs for mouse iASPP were: 5’-GCAUGGGACUGAUGCACTT-3’ and 5’-GUGCAUCAGUCCCAUGCTT-3’. The sequences of siRNAs for mouse importin-β1 were: 5’-GGGAAGUCAAGAACUAUGUTT-3’ and 5’-ACAUAGUUCUUGACUUCCCTT-3’. The sequences of siRNAs for mouse E2F1 were: 5’-AUCUGACCACCAAACGCUUTT-3’ and 5’-AAGCGUUUGGUGGUCAGAUTT-3’. The sequences of siRNAs for mouse p63 were: 5’-CACAGACCACGCACAGAAUTT-3’ and 5’-AUUCUGUGCGUGGUCUGUGTT-3’ (sip63-1); 5’-AGAUGUUGCUGAAGAUCAATT-3’ and 5-UUGAUCUUCAGCAACAUCUTT-3’ (sip63-2); 5’-CAGUAUGUAGAAGAUCCUATT-3’ and 5’-UAGGAUCUUCUACAUACUGTT (sip63-3); The sequences of siRNAs for mouse p73 were: 5’-GGAACAGAAUUUACCACCATT-3’ and 5’-UGGUGGUAAAUUCUGUUCCTT-3’ (sip73-1); 5’-GCCUUUGGUUGACUCCUAUTT-3’ and 5’-AUAGGAGUCAACCAAAGGCTT-3’ (sip73-2); 5’-GCAUCUACCACCUGCAGAATT-3’ and 5’-UUCUGCAGGUGGUAGAUGCTT-3’ (sip73-3). The sequences of negative control (NC)/siRNA of control (siCTRL) were: 5’-UUCUCCGAACGUGUCACGUTT-3’ and 5’-

ACGUGACACGUUCGGAGAATT-3'. Transfection of siRNAs was performed by mixing with X-treme gene siRNA transfection reagent (Roche, Switzerland). Efficiency of small interfering RNA (siRNA) for ASPP2, iASPP, importin-β1 and E2F1 were shown in **Supplementary Figure 8**.

Serum lactate dehydrogenase detection

Serum lactate dehydrogenase (LDH) was determined by LDH Detection Kit (A020-1 Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. For in vitro assay, LDH levels of culture medium and cell lysates were detected. Relative cell death was calculated based on the ratio of released LDH into the medium. The finally OD of reaction was read at 450 nm.

TUNEL staining

The apoptosis of cells was determined by TUNEL assay (11684817910, Roche, Switzerland). The cells were fixed with 4% PFA at room temperature. After 1 h of fixation, blocking solution (3% H_2O_2: CH_3OH = 1: 9) was added and allowed to stand at room temperature for 10 min. To permeate the membrane of cells, permeabilization buffer (0.1% Triton X-100, 0.1% sodium citrate) was added and allowed to stand for 4 min at 4°C. The cells were then incubated with 50 μl TUNEL reaction mixture for 1 h at 37℃ without light. Finally, nuclei were labeled with 4,6-diamidino-2-phenylindole (DAPI) (Beyotime, China) for 15 min at room temperature without light. Photos were taken using a laser scanning confocal microscope (Handbuch LSM 880, Carl Zeiss, Germany).

JC-1 staining

Mitochondrial membrane potential (Δψm) was detected by Mitochondrial Membrane Potential

Assay Kit with JC-1 (Beyotime, China). To label the cells, 250 µl DMEM medium and 250 µL of JC-1 staining working solution (50 µl JC1 200× in 8 ml ddH_2O) were added and incubated at 37°C for 20 min. After incubation, cells were washed twice with pre-cooled JC-1 staining buffer (1×). Photos were taken using a laser scanning confocal microscope (Handbuch LSM 880, Carl Zeiss, Germany).

Caspase-3 activity assay

Caspase-3 activity assay Kit (ab39383, Abcam, America) were used to examine caspase-3 activity of cardiac tissues according to the manufacturer's instructions. Briefly, heart tissues were incubated with 50 ul lysis buffer on ice for 10 min, and then were add with 50 µl 2×reaction buffer (containing 10 mM DTT). DEVD-AFC substrate (5 µl, 1 mM) was mixed with each sample and allowed to stand at 37°C for 1-2 h. Samples were read in a fluorometer equipped with a 400-nm excitation filter and 505 nm emission filter.

Caspase-3 activity assay kit (5723, Cell Signaling Technology, America) was used to determine caspase-3 activity of cultured cardiomyocytes. Briefly, cells were incubated with lysis buffer on ice for 5 min, followed by 20 times of 3 s ultrasound/6 s pause cycle ultrasonication. Samples were obtained by centrifugation (10 min, 13000 g) at 4°C and then incubated with 20 µl substrate buffer at 37°C for 1-2 h without light. The samples were read in a fluorometer equipped with a 380 nm excitation filter and 460 nm emission filter.

Western blot

To obtain the total protein, tissue or cultured cells were lysed in RIPA buffer (Beyotime, China) containing 1% protease inhibitor (Roche, Switzerland) for 1 h in an ice bath. Protein samples were obtained by centrifugation (20 min, 13000 g) at 4°C. The samples were determined and quantified by BCA Protein Assay Kit (Beyotime, China). Then, protein samples (80 μg each) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7.5% - 12%) and transferred to nitrocellulose membranes. After 2 h of blocking in 5% milk, the nitrocellulose membranes were incubated with primary antibodies overnight at 4°C. After washing with PBST (0.05% Tween in phosphate-buffered saline), the membranes were incubated with the secondary anti-rabbit or anti-mouse (1:10000, LI-COR, Lincoln, USA) polyclonal antibody at room temperature for 50 min without light. The membranes were scanned and analyzed by Odyssey infrared scanning system (LI-COR, American). β-actin was used as an internal control. The antibodies used were ASPP1 (1:1000, A4355, Sigma, America), p53 (1:1000, 2524S, Cell Signaling Technology, America), Bcl2 (1:1000, 3498S, Cell Signaling Technology, America), Bax (1:5000, 60267-1-Ig, Proteintech, America), E2F1 (1:1000, A2067, ABclonal, China), Flag tag (1:500, 8146S, Cell Signaling Technology, America), p63 (1:500, A19652, ABclonal, China), p73 (1:500, A2670, ABclonal, China), and β-actin (1:5000, 66009-1-Ig, Proteintech, America).

Co-immunoprecipitation

To determine the interaction between proteins, Pierce™ CO-Immunoprecipitation Kit (Thermo fisher, America) was used. Cultured cells were lysed in lysis buffer containing 1% protease inhibitor (Roche, Switzerland) for 20 min in an ice bath. Protein samples were obtained by centrifugation (15 min, 13000 g) at 4°C. After incubated with control agarose resin for 1 h at a 4°C table concentrator, the final protein samples were obtained by centrifugation (1 min, 1000 g) at 4°C. The antibodies (10 μg) were pretreated by incubating with AminoLink Plus coupling resin for 1 h at room temperature, and then added to protein samples and incubate overnight at

4°C. After 3 times washing, co-immunoprecipitation products were obtained with elution buffer. The co-immunoprecipitation products were analyzed by Western blot. The antibodies used for co-immunoprecipitation were ASPP1 (HPA006394, Sigma-Aldrich, America) and p53 (A19585, ABclonal, China).

Real-time quantitative PCR

Total RNA samples of tissues and cells were extracted by TRIzol reagent (Invitrogen, Carlsbad, America). RNA samples were reverse transcribed using the Trans-Script All-in-one First-strand cDNA Synthesis Supermix for qPCR Kit (TransGen Biotech, China). Real-time quantitative PCR (qRT-PCR) was performed by SYBR Green Master (Roche, Switzerland). The relative RNA level was analyzed by using $2^{-\Delta\Delta ct}$ method, and β-actin was used as an internal control. The primer pairs were synthesized by Invitrogen and listed in **Table S6**.

Immunostaining

Cells were fixed with 4% PFA for 15 min at room temperature. Then, 0.5% Triton X-100 was added and allowed to stand for 1 h at room temperature. After 2 h of blocking with 10% normal goat serum at 37°C, cells were incubated with or without (negative control) primary antibodies primary antibodies overnight at 4°C. After washing with PBS, cells were incubated with the secondary antibody at room temperature for 1 h without light, successively. Finally, nuclei were labeled with DAPI (Beyotime, China) for 15 min at room temperature without light. Photos were taken using a laser scanning confocal microscope (Handbuch LSM 880, Carl Zeiss, Germany). The antibodies used for immunostaining assay were: ASPP1 (1:100, HPA006394, Sigma-Aldrich, America) followed by DyLight 488 (anti-rabbit) (1:1000, 35552, Thermo Fisher, America); p53 (1:100, AF1355, R&D, America) followed by DyLight 594 (anti-goat)

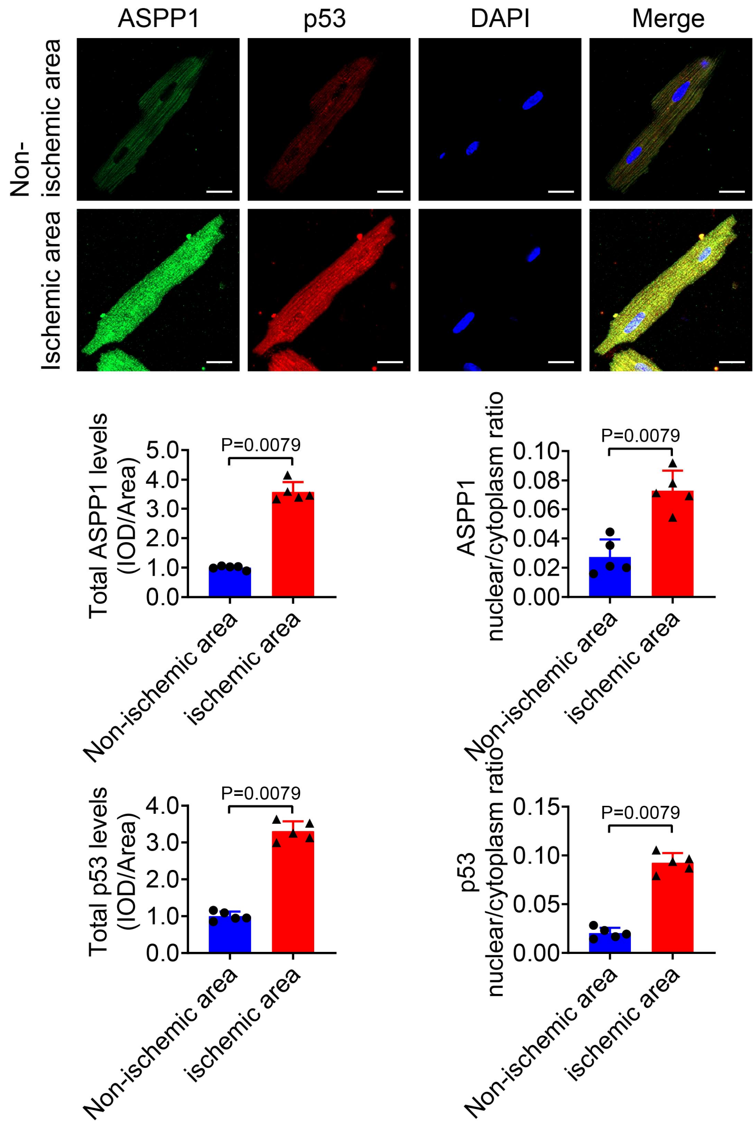
(1:500, A23430, AmyJet, China); ASPP2 (1:50, sc-53861, Santa, America), iASPP (1:50, sc-398566, Santa, America) and Flag tag (1:500, 8146S, Cell Signaling Technology, America) followed by DyLight 488 (anti-mouse) (1:1000, 35502, Thermo Fisher, America). p63 (1:50, sc-25268, Santa, America), p73 (1:50, sc-56190, Santa, America) followed by DyLight 594 (anti-mouse) (1:1000, 35510, Thermo Fisher, America). The fluorescent secondary antibody only (negative control) was used to validate antibody specificity and distinguish genuine target staining from background as presented in **Supplemental Figure 9**.

Statistical analysis

All statistical calculations were performed using Prism software (version 8.3.0, GraphPad, America). Data are expressed as mean ± SD. In data statistics, all data sets were tested for normality by D'Agostino & Pearson test ($n \geq 8$) and Shapiro-Wilk test ($n < 8$). For normally distributed data, two-tailed Student's *t* test was used to compare two groups; one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multi-comparison test was used to compare differences among multiple groups; statistical analyses comparing two genotypes (WT and ASPP1(TG) or WT and ASPP1(KO)), two manipulations (sham and I/R) was done using a two-way analysis of variance (ANOVA) followed by Tukey's post-hoc multi-comparison test. For non-normally distributed or small sample size ($n < 6$) data, the Mann-Whitney test (two-tailed) was used for two groups, and Kruskal-Wallis, followed by false discovery rate (FDR) method of Benjamini and Hochberg test was used for multiple groups. A value of $P < 0.05$ was considered statistically significant. No experiment-wide/across-test multiple test correction was applied and only within-test corrections were made. The representative image was selected from one of the repeated experiments that best matched the mean value. Detailed statistical analysis information including normalization procedures, precise *P* values, sample sizes, and named statistical tests is described in **Supplementary Table 7 and 8** in the Supplementary Materials.

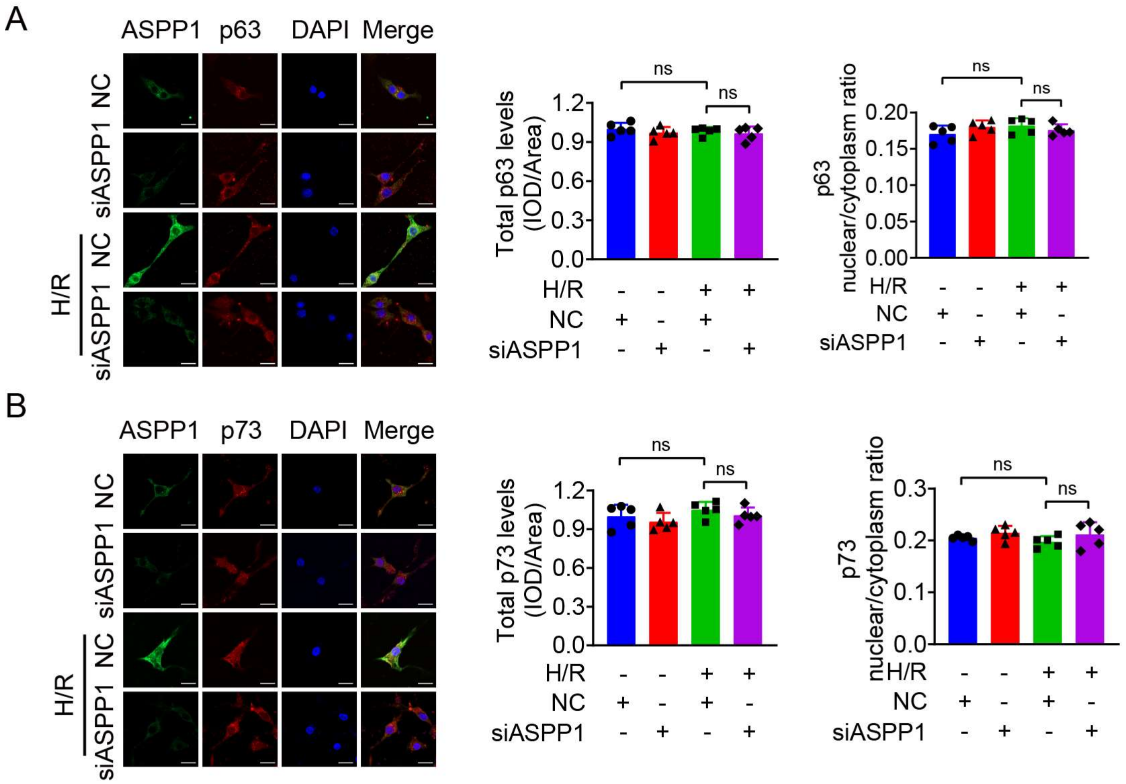
Supplementary Figures

Supplementary Figure 1



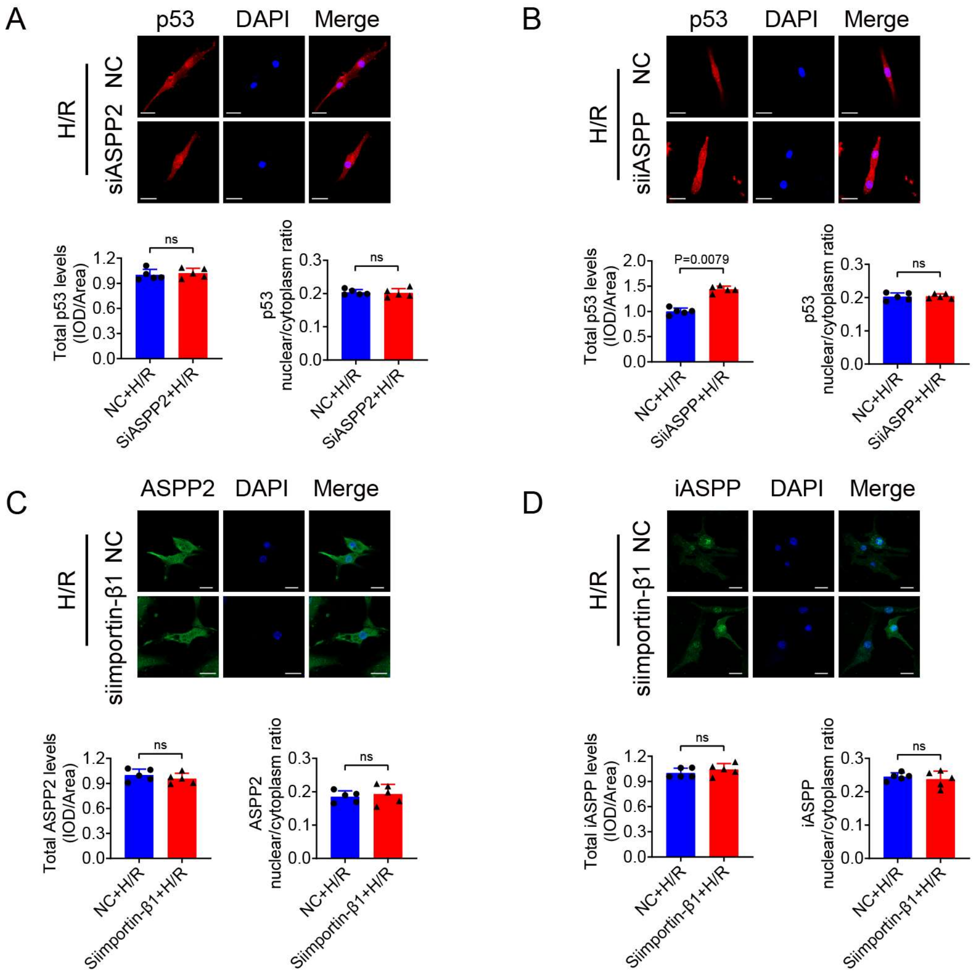
Supplementary Figure 1. Immunostaining assay was used to analyze the co-localization of ASPP1 and p53 in isolated adult cardiomyocytes of non-ischemic area and ischemic area from I/R mice (Mann-Whitney *U* test)**.** n = 5. Scale bar = 20 μm.

Supplementary Figure 2



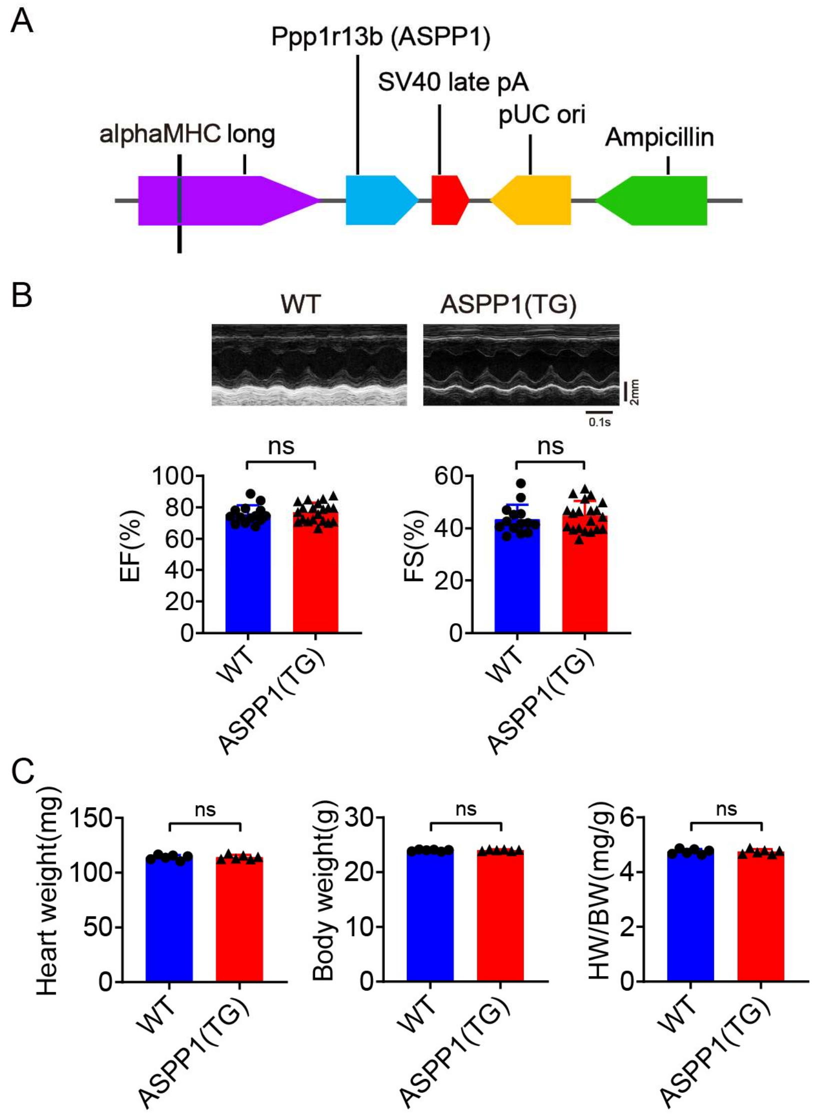
Supplementary Figure 2. The effects of ASPP1 knockdown on p63 (A) and p73 (B) nuclear translocation in NMVCs by immunostaining (Kruskal-Wallis, followed by false discovery rate (FDR) method of Benjamini and Hochberg test)**.** n = 5. Scale bar = 20 µm. ns, not significant.

Supplementary Figure 3



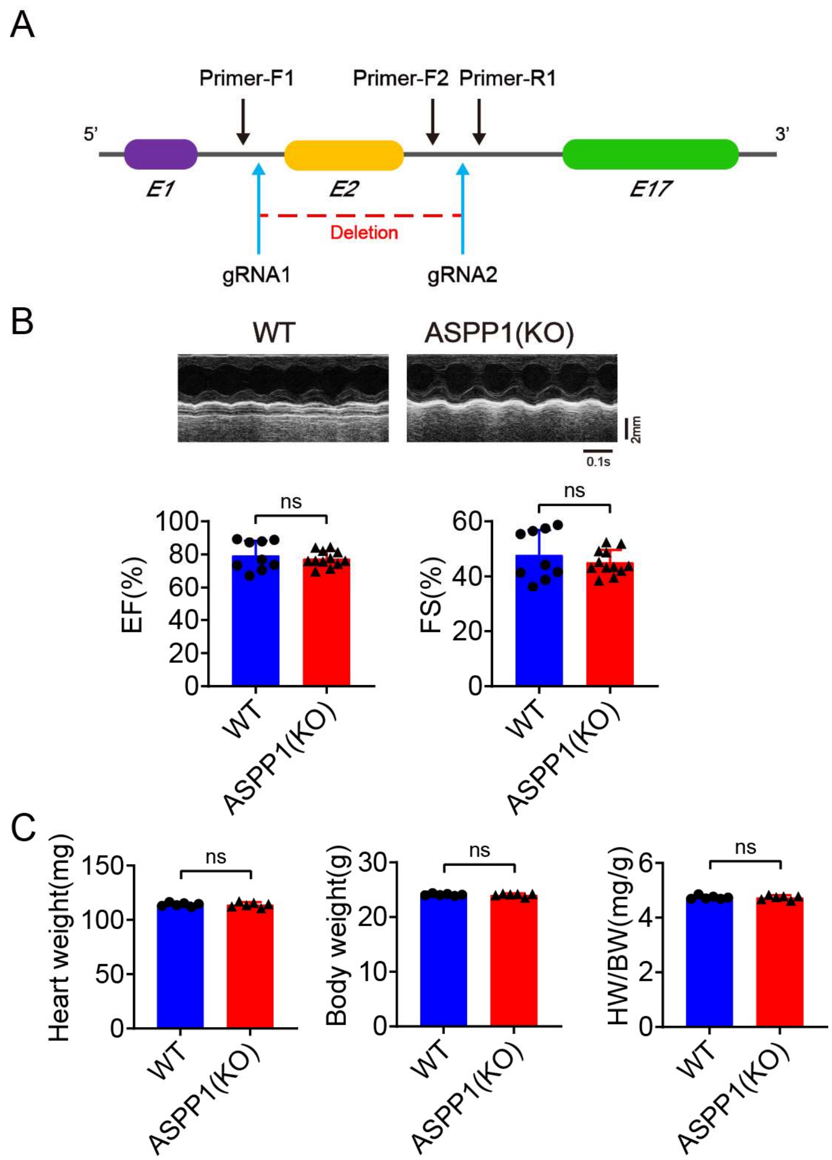
Supplementary Figure 3. The nuclear translocation of ASPP2 or iASPP is not coupled with p53. (A, B) Immunostaining was performed to analyze the effect of knockdown of ASPP2 and iASPP on p53 nuclear translocation in NMVCs (Mann-Whitney *U* test). n = 5. Scale bar = 20 µm. ns, not significant. (C, D) Effect of importin-β1 knockdown on ASPP2 and iASPP nuclear translocation in NMVCs (Mann-Whitney *U* test). n = 5. Scale bar = 20 µm. ns, not significant.

Supplementary Figure 4



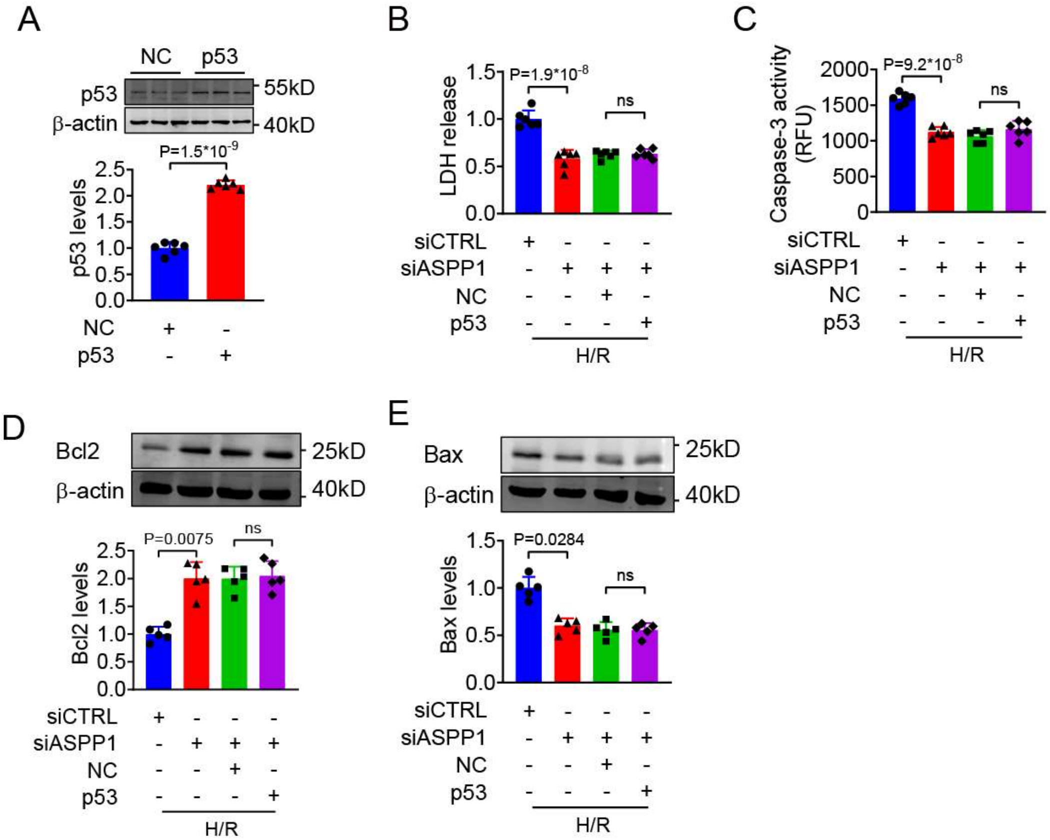
Supplementary Figure 4. Generation of ASPP1 transgenic overexpression mice. (A) Strategy for the generation of cardiomyocyte-specific ASPP1 overexpression transgenic mice. (B) Cardiac function of wild type (WT) and ASPP1 transgenic (TG) mice by echocardiography (EF, Student *t* test; FS, Mann-Whitney *U* test). n = 14 for WT, n = 20 for ASPP1 (TG) mice. ns, not significant. (C) Base line heart weight, body weight, and heart weight/body weight (HW/BW) of WT and ASPP1 (TG) mice (Student *t* test). n = 6. ns, not significant.

Supplementary Figure 5



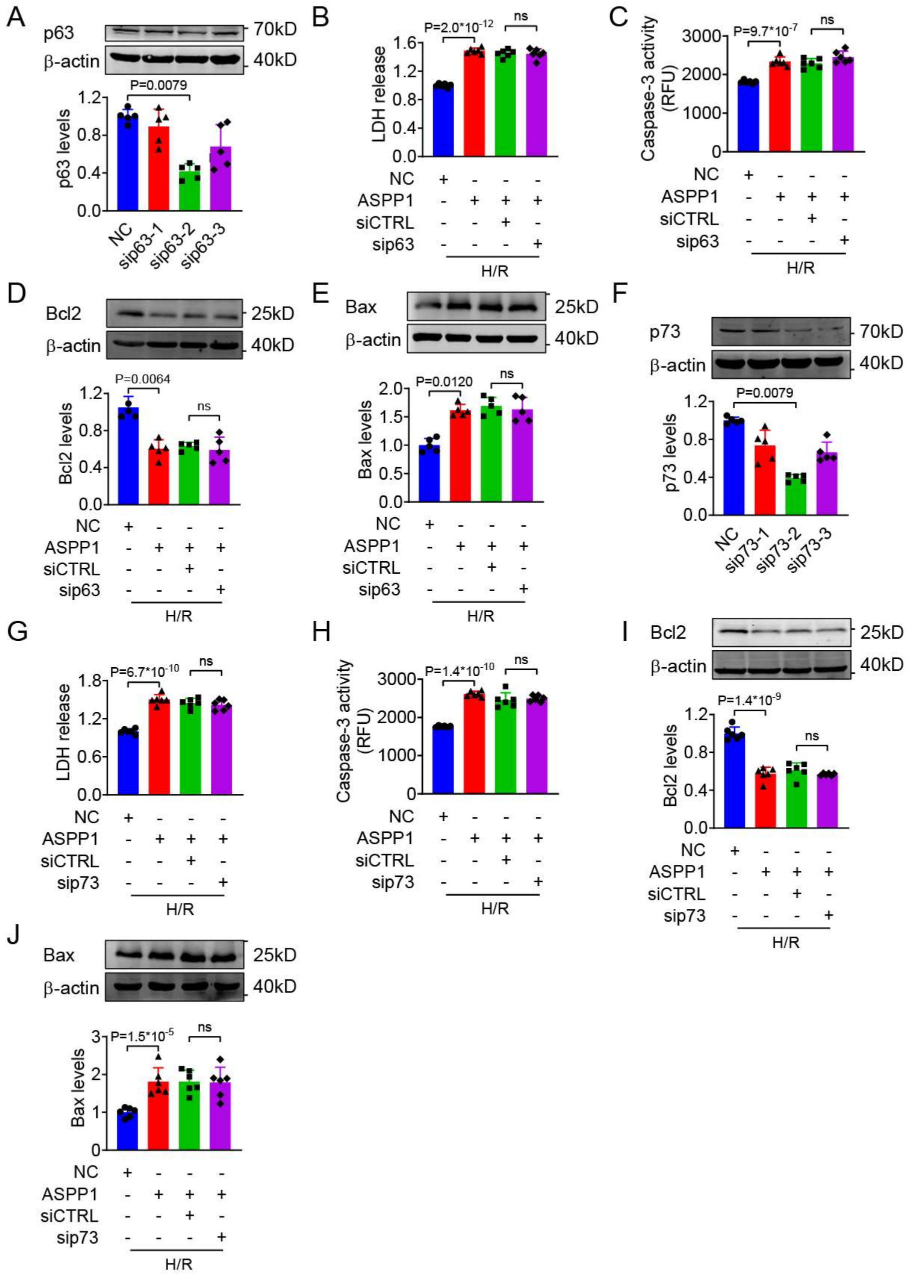
Supplementary Figure 5. Generation of ASPP1 knockout mice. (A) Strategy for the generation of ASPP1 knockout mice. (B) Cardiac function of WT and ASPP1 knockout (KO) mice by echocardiography (Student *t* test). n = 9 for WT, n = 12 for ASPP1(KO) mice. ns, not significant. (C) Base line heart weight (Student *t* test), body weight (Mann-Whitney *U* test), and heart weight/body weight (HW/BW) (Student *t* test) of WT and ASPP1 (KO) mice. n = 6. ns, not significant.

Supplementary Figure 6



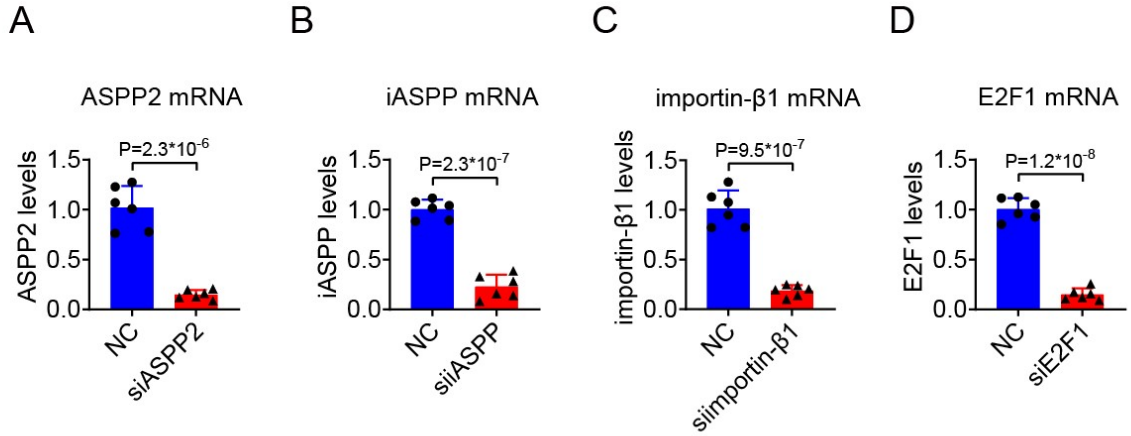
Supplementary Figure 6. Overexpression of p53 does not affect the protective effects of ASPP1 knockdown in NMVCs under H/R stimulation. (A) The efficiency of p53 overexpression plasmid in NMVCs by Western blot (Student *t* test). n = 6. (B) LDH release from NMVCs (One-way ANOVA, followed by Tukey post hoc multi-comparisons test). n = 6. ns, not significant. (C) Caspase-3 activity in NMVCs by ELISA assay (One-way ANOVA, followed by Tukey post hoc multi-comparisons test). n = 6. ns, not significant. (D-E) The protein levels of Bcl2 and Bax detected by Western blot (Kruskal-Wallis, followed by false discovery rate (FDR) method of Benjamini and Hochberg test). n = 5. ns, not significant.

Supplementary Figure 7



Supplementary Figure 7. p63 and p73 produced no effects on ASPP1 induced NMVCs injury under H/R stimulation. (A) The efficiency of small interfering RNA (siRNA) of p63 in NMVCs by Western blot (Mann-Whitney *U* test). n = 5. (B) LDH level in culture medium (One-way ANOVA, followed by Tukey post hoc multi-comparisons test). n = 6. ns, not significant. (C) Caspase-3 activity in NMVCs by ELISA assay (One-way ANOVA, followed by Tukey post hoc multi-comparisons test). n = 6. ns, not significant. (D, E) The protein levels of Bcl2 and Bax detected by Western blot (Kruskal-Wallis, followed by false discovery rate (FDR) method of Benjamini and Hochberg test). n = 5. ns, not significant. (F) The efficiency of small interfering RNA (siRNA) of p73 in NMVCs by Western blot (Mann-Whitney *U* test). n = 5. (G) Cell death of NMVCs by LDH release (One-way ANOVA, followed by Tukey post hoc multi-comparisons test). n = 6. ns, not significant. (H) Caspase-3 activity in NMVCs by ELISA assay (One-way ANOVA, followed by Tukey post hoc multi-comparisons test). n = 6. ns, not significant. (I, J) The protein levels of Bcl2 and Bax detected by Western blot (One-way ANOVA, followed by Tukey post hoc multi-comparisons test). n = 6. ns, not significant.

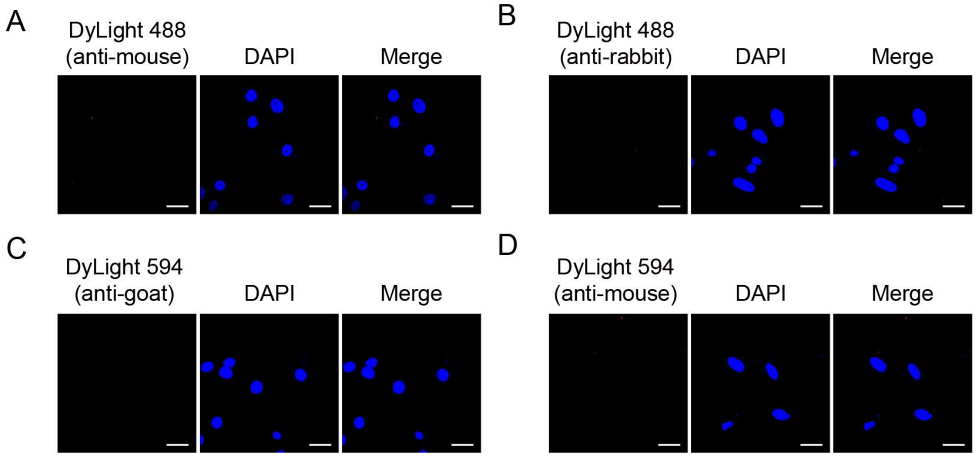
Supplementary Figure 8



Supplementary Figure 8. Efficiency of siRNA for ASPP2, iASPP, importin-β1 and E2F1.

(A) Efficiency of siASPP2 in NMVCs by qRT-PCR assay (Student *t* test). n = 6. (B) Efficiency of siiASPP in NMVCs by qRT-PCR assay (Student *t* test). n = 6. (C) Efficiency of siimportin-β1 in NMVCs by qRT-PCR assay (Student *t* test). n = 6. (D) Efficiency of siE2F1 in NMVCs by qRT-PCR assay (Student *t* test). n = 6.

Supplementary Figure 9



Supplementary Figure 9. Slices of NMVCs were permeabilized by 0.5% Triton X-100 with PBS and then blocked with 10% normal goat serum. (A-D) Slices of NMVCs were directly incubated with DyLight 488 (anti-mouse) (A), DyLight 488 (anti-rabbit) (B), DyLight 594 (anti-goat) (C) and DyLight 594 (anti-mouse) (D). Scale bar = 20 μm.

Supplementary Tables

Supplementary Table 1. Overexpression of ASPP1 does not affect cardiac function in physiological mice.

Group	WT (n=14)	ASPP1(TG) (n=20)
EF, %	75.51±5.751	76.98±6.021^{ns}
FS, %	43.47±5.552	44.77±5.638^{ns}
LVIDd, mm	3.25±0.29	3.16±0.35^{ns}
LVIDs, mm	1.85±0.29	1.75±0.32^{ns}
LVEDV, µl	44.42±11.97	40.36±11.54^{ns}
LVESV, µl	10.79±3.921	9.63±4.63^{ns}

The data are expressed as means ± SD. ns, not significant versus WT group. EF and LVIDs were analyzed by using nonpaired 2-tailed Student *t* test; LVIDd, LVEDV and LVESV were analyzed by using Mann-Whitney *U* test.

Abbreviations: EF, ejection fraction; FS, fractional shorting; LVIDd, left ventricular internal dimension at end diastole; LVIDs, left ventricular internal dimension at systole; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume.

Supplementary Table 2. Knockout of ASPP1 does not affect cardiac function in physiological mice.

Group	WT (n=9)	ASPP1(KO) (n=12)
EF, %	79.40±8.83	77.43±4.72^{ns}
FS, %	47.78±9.04	45.12±4.51^{ns}
LVIDd, mm	3.2±0.25	3.25±0.25^{ns}
LVIDs, mm	1.68±0.35	1.79±0.22^{ns}
LVEDV, µl	41.32±7.56	42.92±7.71^{ns}
LVESV, µl	8.74±4.39	9.78±3.15^{ns}

The data are expressed as means ± SD. ns, not significant versus WT group. They were analyzed by using nonpaired 2-tailed Student *t* test.

Supplementary Table 3. Overexpression of ASPP1 aggravates cardiac function in I/R mice.

Group	Sham+WT (n=9)	Sham+ASPP1 (TG) (n=9)	I/R+WT (n=9)	I/R+ASPP1(TG) (n=9)
EF, %	75.45±3.77	75.98±3.47	59.03±3.95 ($^{a}P=2.9*10^{-7}$)	42.15±7.40 ($^{b}P=1.6*10^{-7}$)
FS, %	43.10±3.61	43.45±2.99	30.63±2.62 ($^{a}P=6.1*10^{-8}$)	20.33±4.38 ($^{b}P=2.6*10^{-6}$)
LVIDd, mm	3.15±0.12	3.09±0.35	3.55±0.13 ($^{a}P=0.0088$)	3.64±0.41 (^{b}ns)
LVIDs, mm	1.79±0.11	1.75±0.26	2.46±0.17 ($^{a}P=1.4*10^{-6}$)	2.89±0.28 ($^{b}P=0.0013$)
LVEDV, µl	39.62±3.48	38.24±10.65	52.68±4.66 ($^{a}P=0.0091$)	56.73±15.50 (^{b}ns)
LVESV, µl	9.68±1.47	9.38±3.82	21.70±3.78 ($^{a}P=3.7*10^{-5}$)	32.35±7.66 ($^{b}P=0.0002$)

^{a}P values were compared with Sham+WT group; ^{b}P values were compared with I/R+WT group. ns, not significant. EF, FS, LVIDs, and LVESV were analyzed by using two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test. LVIDd and LVEDV were analyzed by using Kruskal-Wallis, followed by false discovery rate (FDR) method of Benjamini and Hochberg test. The data are expressed as means ± SD.

Supplementary Table 4. Knockout of ASPP1 improves cardiac function in I/R mice.

Group	**Sham+WT (n=11)**	**Sham+ASPP1 (KO) (n=11)**	**I/R+WT (n=11)**	**I/R+ASPP1(KO) (n=11)**
EF, %	75.62±3.40	78.43±8.12	58.24±4.64 ($^{a}P=6.5*10^{-9}$)	69.20±2.75 ($^{b}P=7.3*10^{-5}$)
FS, %	43.17±3.18	46.63±8.21	30.12±3.08 ($^{a}P=7.6*10^{-7}$)	37.71±2.08 ($^{b}P=0.0033$)
LVIDd, mm	3.11±0.24	3.16±0.30	3.61±0.24 ($^{a}P=0.0002$)	3.14±0.22 ($^{b}P=0.0002$)
LVIDs, mm	1.77±0.16	1.70±0.36	2.52±0.21 ($^{a}P=3.7*10^{-8}$)	1.96±0.18 ($^{b}P=1.4*10^{-5}$)
LVEDV, µl	38.53±6.49	40.22±8.39	55.03±8.66 ($^{a}P=0.0020$)	39.28±6.42 ($^{b}P=0.0002$)
LVESV, µl	9.39±2.06	9.03±4.28	23.07±4.75 ($^{a}P=3.4*10^{-10}$)	12.19±2.74 ($^{b}P=9.8*10^{-8}$)

^{a}P values were compared with Sham+WT group; ^{b}P values were compared with I/R+WT group. EF, FS, LVIDs and LVESV were analyzed by using two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test. LVIDd and LVEDV were analyzed by using Kruskal-Wallis, followed by false discovery rate (FDR) method of Benjamini and Hochberg test. The data are expressed as means ± SD.

Supplementary Table 5. AAV9-shp53 rescues the cardiac injury mediated by the transgenic overexpression of ASPP1.

Group	I/R+WT (n=7)	I/R+ASPP1(TG) (n=7)	I/R+ASPP1(TG)+AAV9-NC (n=7)	I/R+ASPP1(TG)+AAV9-shp53 (n=7)
EF, %	62.63±2.46	48.25±2.54 (^{a}P=7.3*10^{-9})	47.50±3.60	63.98±2.54 (^{b}P=5.1*10^{-10})
FS, %	33.11±1.85	23.79±1.51 (^{a}P=7.3*10^{-9})	23.38±2.09	34.11±1.82 (^{b}P=4.6*10^{-10})
LVIDd, mm	3.55±0.15	3.72±0.17 (^{a}P=ns)	3.77±0.19	3.59±0.14 (^{b}P=ns)
LVIDs, mm	2.37±0.08	2.84±0.15 (^{a}P=2.1*10^{-5})	2.89±0.19	3.36±0.14 (^{b}P=3.0*10^{-6})
LVEDV, µl	52.77±5.07	59.13±6.53 (^{a}ns)	61.06±7.33	54.06±5.23 (^{b}P=ns)
LVESV, µl	19.66±1.71	30.63±4.11 (^{a}P=6.1*10^{-5})	32.14±5.24	19.53±2.78 (^{b}P=8.0*10^{-6})

^{a}P values were compared with I/R+WT group; ^{b}P values were compared with I/R+ASPP1(TG)+AAV9-NC group. ns, not significant. EF, FS, LVIDs and LVESV were analyzed by using two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test. LVIDd and LVEDV were analyzed by using Kruskal-Wallis, followed by false discovery rate (FDR) method of Benjamini and Hochberg test. The data are expressed as means ± SD.

Supplementary Table 6. Sequences of mouse oligonucleotide primers used for real-time quantitative PCR

ASPP1	Forward	5'-CCACCAAGTCCCACATACCC-3'
	Reverse	5'-GGTGGCTGGTAGTTCTTAGGTG-3'
p53	Forward	5'-CTCTCCCCCGCAAAAGAAAAA-3'
	Reverse	5'-CGGAACATCTCGAAGCGTTTA-3'
ASPP2	Forward	5'-CAAGCCTGTGATAGCTGCTG-3'
	Reverse	5'-GGCTTCTAAGTCAGCATCGC-3'
iASPP	Forward	5'-TAGAGGCCCGTTTTGGACG-3'
	Reverse	5'-CCCGATCTAGGCTGCTGTAG-3'
Bax	Forward	5'-TGAAGACAGGGGCCTTTTTG-3'
	Reverse	5'-AATTCGCCGGAGACACTCG-3'
Puma	Forward	5'-AGCAGCACTTAGAGTCGCC-3'
	Reverse	5'-CCTGGGTAAGGGGAGGAGT-3'
Noxa	Forward	5'-GCAGAGCTACCACCTGAGTTC-3'
	Reverse	5'-CTTTTGCGACTTCCCAGGCA-3'
E2F1	Forward	5'-AGACCACCGACAGACCCGAT-3'
	Reverse	5'-AGCCGTTCCATAATGACCAG-3'
importin-β1	Forward	5'-AGCCTAGGGATTCAGGGTGT-3'
	Reverse	5'-CAGAGGGTATGGATCGTGCT-3'
β-actin	Forward	5'-GGCTGTATTCCCCTCCATCG-3'
	Reverse	5'-CCAGTTGGTAACAATGCCATGT-3'

Supplementary Table 7. Detailed statistical analysis information for all main and supplementary figures.

Figure		Groups (Sample size)	Normality test values	Statistical analysis	P value
1A	ASPP1 levels (Input)	Control (n=6)	0.1356	nonpaired 2-tailed Student *t* test	
		H/R (n=6)	0.2619		P= 0.000354003636749 *vs* Control
	p53 levels (Input)	Control (n=6)	0.8248	nonpaired 2-tailed Student *t* test	
		H/R (n=6)	0.1397		P=0.000636833131323 *vs* Control
	p53/ASPP1	Control (n=6)	0.0128	Mann-Whitney *U* test	
		H/R (n=6)	0.2218		P=0.002164502164502 *vs* Control
1B	ASPP1 levels (Input)	Control (n=6)	0.3884	nonpaired 2-tailed Student *t* test	
		H/R (n=6)	0.1944		P=0.000131112963606 *vs* Control
	p53 levels (Input)	Control (n=6)	0.5156	Mann-Whitney *U* test	
		H/R (n=6)	0.0146		P=0.002164502164502 *vs* Control
	ASPP1/p53	Control (n=6)	0.2275	nonpaired 2-tailed Student *t* test	
		H/R (n=6)	0.6580		P=0.001011151797024 *vs* Control
1C	Total ASPP1 levels	Control (n=5)	0.2164	Mann-Whitney *U* test	
		H/R (n=5)	0.3517		P=0.007936507936508 *vs* Control
	ASPP1 nuclear/cytoplasm ratio	Control (n=5)	0.5421	Mann-Whitney *U* test	
		H/R (n=5)	0.0538		P=0.007936507936508 *vs* Control
	Total p53 levels	Control (n=5)	0.1230	Mann-Whitney *U* test	
		H/R (n=5)	0.6936		P=0.007936507936508 *vs* Control
	p53 nuclear/cytoplasm ratio	Control (n=5)	0.6549	Mann-Whitney *U* test	
		H/R (n=5)	0.0916		P=0.007936507936508 *vs* Control
1D	Total ASPP1 levels	Sham (n=5)	0.1451	Mann-Whitney *U* test	
		I/R (n=5)	0.5901		P=0.007936507936508 *vs* Sham
	ASPP1 nuclear/cytoplasm	Sham (n=5)	0.2289	Mann-Whitney *U* test	
		I/R (n=5)	0.6468		P=0.007936507936508

	ratio				*vs* Sham
	Total p53 levels	Sham (n=5)	0.4280	Mann-Whitney *U* test	
		I/R (n=5)	0.8189		P=0.007936507936508 *vs* Sham
	p53 nuclear/cytoplasm ratio	Sham (n=5)	0.5889	Mann-Whitney *U* test	
		I/R (n=5)	0.2414		P=0.007936507936508 *vs* Sham
2A	Total p53 levels	NC (n=5)	0.6490	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1 (n=5)	0.2341		P=0.872600061 *vs* NC
		H/R+NC (n=5)	0.2234		P=0.005443982 *vs* NC
					P=0.008814655 *vs* ASPP1
		H/R+ASPP1 (n=5)	0.8586		P=0.006409444 *vs* NC
					P=0.010296549 *vs* ASPP1
					P=0.957371576 *vs* H/R+NC
	Nuclear p53 levels	NC (n=6)	0.3279	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1 (n=6)	0.1606		P=0.999575262 *vs* NC
		H/R+NC (n=6)	0.9921		P=0.000149212 *vs* NC
					P=0.000188613 *vs* ASPP1
		H/R+ASPP1 (n=6)	0.1188		P=1.40832E-09 *vs* NC
					P=1.65042E-09 *vs* ASPP1
					P=2.59424E-05 *vs* H/R+NC
	Cytoplasmic p53 levels	NC (n=6)	0.6742	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1 (n=6)	0.2486		P=0.8612093 *vs* NC
		H/R+NC (n=6)	0.3334		P=5.80237E-10 *vs* NC
					P=1.91148E-10 *vs* ASPP1
		H/R+ASPP1 (n=6)	0.1244		P=0.007177685 *vs* NC
					P=0.001211221 *vs* ASPP1
					P=2.53481E-07 *vs* H/R+NC
2B	Total p53 levels	NC (n=5)	0.7279	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		siASPP1 (n=5)	0.7529		P=0.872600061 *vs* NC
		H/R+NC (n=5)	0.3074		P=0.006409444 *vs* NC
					P=0.010296549 *vs* siASPP1
		H/R+siASPP1 (n=5)	0.4816		P=0.005443982 *vs* NC
					P=0.008814655 *vs*

					siASPP1
					P=0.957371576 *vs* H/R+NC
	Nuclear p53 levels	NC (n=6)	0.0608	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		siASPP1 (n=6)	0.4254		P=0.890271561 *vs* NC
		H/R+NC (n=6)	0.9167		P=3.79031E-07 *vs* NC
					P=1.50676E-06 *vs* siASPP1
		H/R+siASPP1 (n=6)	0.5781		P=0.163391288 *vs* NC
					P=0.476240517 *vs* siASPP1
					P=3.13601E-05 *vs* H/R+NC
	Cytoplasmic p53 levels	NC (n=6)	0.0857	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		siASPP1 (n=6)	0.4572		P=0.977118176 *vs* NC
		H/R+NC (n=6)	0.4090		P=5.6342E-08 *vs* NC
					P=2.80044E-08 *vs* siASPP1
		H/R+siASPP1 (n=6)	0.5957		P=2.26E-13 *vs* NC
					P=1.56E-13 *vs* siASPP1
					P=5.44347E-08 *vs* H/R+NC
2C	Total p53 levels	NC (n=6)	0.2033	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1 (n=6)	0.5668		P=0.989996387 *vs* NC
		H/R+NC (n=6)	0.1009		P=1.8229E-06 *vs* NC
					P=3.35579E-06 *vs* ASPP1
		H/R+ASPP1 (n=6)	0.6750		P=2.63546E-06 *vs* NC
					P=4.88699E-06 *vs* ASPP1
					P=0.9977131 *vs* H/R+NC
	p53 nuclear/cytoplasm ratio	NC (n=6)	0.0756	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1 (n=6)	0.0567		P=0.677318729 *vs* NC
		H/R+NC (n=6)	0.3180		P=2.17767E-05 *vs* NC
					P=0.000261169 *vs* ASPP1
		H/R+ASPP1 (n=6)	0.1446		P=3.1577E-11 *vs* NC
					P=1.37621E-10 *vs* ASPP1
					P=5.76212E-07 *vs* H/R+NC
2D	Total p53 levels	NC (n=6)	0.4379	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		siASPP1 (n=6)	0.7105		P=0.999486663 *vs* NC
		H/R+NC (n=6)	0.1367		P=2.02489E-08 *vs* NC
					P=2.44514E-08 *vs*

					siASPP1
		H/R+siASPP1 (n=6)	0.9552		P=3.90555E-08 *vs* NC
					P=4.73988E-08 *vs* siASPP1
					P=0.980091641 *vs* H/R+NC
	p53 nuclear/cytoplasm ratio	NC (n=6)	0.2868	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		siASPP1 (n=6)	0.9838		P=0.992785996 *vs* NC
		H/R+NC (n=6)	0.9954		P=1.67616E-06 *vs* NC
					P=2.88714E-06 *vs* siASPP1
		H/R+siASPP1 (n=6)	0.9115		P=0.98827785 *vs* NC
					P=0.999956262 *vs* siASPP1
					P=3.18814E-06 *vs* NC+H/R
2E		NC+H/R(n=5)	0.5499	Mann-Whitney *U* test	
		Sip53+H/R (n=5)	0.1132		P=0.007936507936508 *vs* NC+H/R
2F	ASPP1 nuclear/cytoplasm ratio	NC+H/R (n=5)	0.5499	Mann-Whitney *U* test	
		Siimportin-β1+H/R (n=5)	0.7922		P=0.007936507936508 *vs* NC+H/R
	p53 nuclear/cytoplasm ratio	NC+H/R (n=5)	0.9707	Mann-Whitney *U* test	
		Siimportin-β1+H/R (n=5)	0.6740		P=0.007936507936508 *vs* NC+H/R
2G	Bax levels	NC (n=9)	0.5396	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1 (n=9)	0.1089		P=0.999704054 *vs* NC
		H/R+NC (n=9)	0.5254		P=0.000355144 *vs* NC
					P=0.000462575 *vs* ASPP1
		H/R+ASPP1 (n=9)	0.6296		P=1.04E-13 *vs* NC
					P=1.04E-13 *vs* ASPP1
					P=4.637E-11 *vs* H/R+NC
	Puma levels	NC (n=9)	0.7920	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1 (n=9)	0.6469		P=0.998052082 *vs* NC
		H/R+NC (n=9)	0.7607		P=0.000444708 *vs* NC
					P=0.000728832 *vs* ASPP1
		H/R+ASPP1 (n=9)	0.9493		P=1.09E-13 *vs* NC
					P=1.12E-13 *vs* ASPP1
					P=1.35741E-10 *vs* H/R+NC

	Noxa levels	NC (n=9)	0.5163	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1 (n=9)	0.3974		P=0.993239184 *vs* NC
		H/R+NC (n=9)	0.3354		P=7.26598E-06 *vs* NC
					P=1.56771E-05 *vs* ASPP1
		H/R+ASPP1 (n=9)	0.3343		P=1E-13 *vs* NC
					P=1E-13 *vs* ASPP1
					P=1.31E-13 *vs* H/R+NC
2H	Bax levels	NC (n=8)	0.0089	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		siASPP1 (n=8)	0.2418		P=0.337355652 *vs* NC
		H/R+NC (n=8)	0.5982		P=9.69282E-06 *vs* NC
					P=0.000531195 *vs* siASPP1
		H/R+siASPP1 (n=8)	0.0006		P=0.028866319 *vs* NC
					P=0.220234759 *vs* siASPP1
					P=0.025181464 *vs* H/R+NC
	Puma levels	NC (n=9)	0.5755	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		siASPP1 (n=9)	0.2370		P=0.822204478 *vs* NC
		H/R+NC (n=9)	0.8387		P=7.1124E-11 *vs* NC
					P=9.349E-12 *vs* siASPP1
		H/R+siASPP1 (n=9)	0.4793		P=0.81599853 *vs* NC
					P=0.318340125 *vs* siASPP1
					P=6.20111E-10 *vs* H/R+NC
	Noxa levels	NC (n=8)	0.3229	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		siASPP1 (n=8)	0.0058		P>0.999999 *vs* NC
		H/R+NC (n=8)	0.5871		P=0.008303 *vs* NC
					P=0.001926 *vs* siASPP1
		H/R+siASPP1 (n=8)	0.6401		P>0.999999 *vs* NC
					P>0.999999 *vs* siASPP1
					P=0.005187 *vs* H/R+NC
3A	mRNA levels of ASPP1	Sham (n=6)	0.6304	nonpaired 2-tailed Student *t* test	
		I/R (n=6)	0.4433		P=8.44977E-05 *vs* Sham
	protein levels of ASPP1	Sham (n=6)	0.4787	Mann-Whitney *U* test	
		I/R (n=6)	0.0093		P=0.002164502164502 *vs* Sham
3B	mRNA levels of ASPP1	Control(n=9)	0.3273	nonpaired 2-tailed Student *t* test	
		H/R (n=9)	0.8890		P=1.30701E-06 *vs* Control
	protein levels of ASPP1	Control (n=6)	0.6238	nonpaired 2-tailed Student *t* test	
		H/R (n=6)	0.8123		P=6.75828E-05 *vs* Control

3C	E2F1 levels (*in vivo*)	Sham (n=6)	0.9006	nonpaired 2-tailed Student *t* test	
		I/R (n=6)	0.1460		P=4.32413E-07 *vs* Sham
	E2F1 levels (*in vitro*)	Control (n=6)	0.5126	nonpaired 2-tailed Student *t* test	
		H/R (n=6)	0.7646		P=1.90083E-07 *vs* Control
3D	mRNA levels of ASPP1	Control (n=5)	0.999	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R (n=5)	0.4379		P=0.004581111 *vs* Control
		H/R+NC (n=5)	0.0378		P=0.007481307 *vs* Control
					P=0.872504983 *vs* H/R
		H/R+E2F1(SI) (n=5)	0.9088		P=0.872504983 *vs* Control
					P=0.007481307 *vs* H/R
					P=0.011932078 *vs* H/R+NC
	protein levels of ASPP1	Control (n=6)	0.4088	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R (n=6)	0.1068		P=1.04794E-09 *vs* Control
		H/R+NC (n=6)	0.7878		P=1.63264E-10 *vs* Control
					P=0.170118127 *vs* H/R
		H/R+E2F1(SI) (n=6)	0.8604		P=0.04045555 *vs* Control
					P=3.84548E-08 *vs* H/R
					P=4.60338E-09 *vs* H/R+NC
3E		WT (n=6)	0.7438	Mann-Whitney *U* test	
		ASPP1(TG) (n=6)	0.0401		P=0.002164502164502 *vs* WT
3F	EF	WT+Sham (n=9)	0.3584	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=9)	0.6918		P=0.995724123 *vs* WT+Sham
		WT+I/R (n=9)	0.4626		P=2.86621E-07 *vs* WT+Sham
					P=1.51667E-07 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.3370		P=1.11E-13 *vs* WT+Sham
					P=1.08E-13 *vs* ASPP1(TG)+Sham
					P=1.63415E-07 *vs* WT+I/R
	FS	WT+Sham (n=9)	0.2560	two-way ANOVA analysis followed by	

		ASPP1(TG)+Sham (n=9)	0.7281	Tukey's post-hoc multi-comparison test	P=0.996340287 *vs* WT+Sham
		WT+I/R (n=9)	0.4289		P=6.09478E-08 *vs* WT+Sham
					P=3.37548E-08 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.2479		P=1.25E-13 *vs* WT+Sham
					P=1.16E-13 *vs* ASPP1(TG)+Sham
					P=2.59946E-06 *vs* WT+I/R
3G		WT+I/R (n=10)	0.2845	nonpaired 2-tailed Student *t* test	
		ASPP1(TG)+I/R (n=10)	0.9348		P=5.20283E-07 *vs* WT+I/R
3H	LDH	WT+Sham (n=10)	0.5771	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=10)	0.0596		P=0.971026736 *vs* WT+Sham
		WT+I/R (n=12)	0.8377		P=4.71E-13 *vs* WT+Sham
					P=4.71E-13 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=12)	0.7899		P=4.71E-13 *vs* WT+Sham
					P=4.71E-13 *vs* ASPP1(TG)+Sham
					P=5.16E-13 *vs* WT+I/R
	CKMB	WT+Sham (n=9)	0.5697	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG) +Sham (n=9)	0.1612		P=0.999436795 *vs* WT+Sham
		WT+I/R (n=9)	0.4889		P=1.01E-13 *vs* WT+Sham
					P=1.01E-13 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.1072		P=1E-13 *vs* WT+Sham
					P=1E-13 *vs* ASPP1(TG)+Sham
					P=3.67571E-10 *vs* WT+I/R
3I		WT+Sham (n=9)	0.6496	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=9)	0.8984		P=0.996034844 *vs* WT+Sham
		WT+I/R (n=9)	0.8501		P=1.65567E-06 *vs* WT+Sham

					P=3.12824E-06 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.3625		P=1.04E-13 *vs* WT+Sham
					P=1.05E-13 *vs* ASPP1(TG)+Sham
					P=4.73187E-09 *vs* WT+I/R
3J	Bcl-2	WT+Sham (n=6)	0.7153	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=6)	0.4573		P=0.626571604 *vs* WT+Sham
		WT+I/R (n=6)	0.0580		P=3.86021E-07 *vs* WT+Sham
					P=4.18363E-06 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=6)	0.0799		P=8.8617E-11 *vs* WT+Sham
					P=4.74575E-10 *vs* ASPP1(TG)+Sham
					P=0.000200635 *vs* WT+I/R
	Bax	WT+Sham (n=6)	0.6880	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=6)	0.5282		P=0.99931268 *vs* WT+Sham
		WT+I/R (n=6)	0.5124		P=0.001449762 *vs* WT+Sham
					P=0.001095024 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=6)	0.7617		P= 7.01575E-10 *vs* WT+Sham
					P=5.86235E-10 *vs* ASPP1(TG)+Sham
					P=1.24982E-06 *vs* WT+I/R
3K	Total p53 levels	WT+Sham (n=6)	0.4310	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=6)	0.2200		P=0.997301824 *vs* WT+Sham
		WT+I/R (n=6)	0.5514		P=7.63E-13 *vs* WT+Sham
					P=6.17E-13 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=6)	0.0895		P=1.4E-12 *vs* WT+Sham
					P=1.125E-12 *vs* ASPP1(TG)+Sham

					P=0.947037526 *vs* WT+I/R
	p53 nuclear/cytoplasm ratio	WT+Sham (n=6)	0.1752	two-way ANOVA analysis followed by Tukey’s post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=6)	0.8130		P=0.996998662 *vs* WT+Sham
		WT+I/R (n=6)	0.6513		P=4.90709E-06 *vs* WT+Sham
					P=3.25085E-06 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=6)	0.2234		P=3.214E-12 *vs* WT+Sham
					P=2.548E-12 *vs* ASPP1(TG)+Sham
					P=6.35812E-08 *vs* WT+I/R
4A		NC (n=6)	0.6724	nonpaired 2-tailed Student *t* test	
		ASPP1 (n=6)	0.1831		P=2.10846E-05 *vs* NC
4B		Control (n=6)	0.1600	one-way ANOVA analysis followed by Tukey’s post-hoc multi-comparison test	
		H/R (n=6)	0.5820		P=4.8E-14 *vs* Control
		H/R+NC (n=6)	0.9479		P=3.7E-14 *vs* Control
					P=0.898463348 *vs* H/R
		H/R+ASPP1 (n=6)	0.5761		P=2.3E-14 *vs* Control
					P=1.90757E-09 *vs* H/R
					P=5.64292E-09 *vs* NC+H/R
4C		Control (n=6)	0.4097	one-way ANOVA analysis followed by Tukey’s post-hoc multi-comparison test	
		H/R (n=6)	0.3212		P=3.306E-10 *vs* Control
		H/R+NC (n=6)	0.4716		P=3.96371E-10 *vs* Control
					P=0.999234872 *vs* H/R
		H/R+ASPP1 (n=6)	0.1196		P=2.3E-14 *vs* Control
					P=5.3965E-11 *vs* H/R
					P=4.5807E-11 *vs* NC+H/R
4D		Control (n=6)	0.5959	one-way ANOVA analysis followed by Tukey’s post-hoc multi-comparison test	
		H/R (n=6)	0.6259		P=3.1E-14 *vs* Control
		H/R+NC (n=6)	0.8878		P=3.1E-14 *vs* Control
					P=0.999999999804002 *vs* H/R
		H/R+ASPP1 (n=6)	0.2636		P=2.3E-14 *vs* Control
					P=7.59494E-08 *vs* H/R
					P=7.60583E-08 *vs* NC+H/R
4E		Control (n=6)	0.9673	one-way ANOVA	

		H/R (n=6)	0.5021	analysis followed by Tukey's post-hoc multi-comparison test	P=1.50351E-05 *vs* Control
		H/R+NC (n=6)	0.2270		P=8.99475E-06 *vs* Control
					P=0.994739552 *vs* H/R
		H/R+ASPP1 (n=6)	0.3283		P=1.16402E-09 *vs* Control
					P=0.000199025 *vs* H/R
					P=0.000344819 *vs* NC+H/R
4F		Control (n=6)	0.1602	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R (n=6)	0.7057		P=1.12672E-05 *vs* Control
		H/R+NC (n=6)	0.7665		P=4.83055E-06 *vs* Control
					P=0.976569466 *vs* H/R
		H/R+ASPP1 (n=6)	0.6032		P=1.8004E-11 *vs* Control
					P=4.38409E-07 *vs* H/R
					P=9.59008E-07 *vs* NC+H/R
4G		Control (n=6)	0.6700	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R (n=6)	0.7974		P=0.000302538 *vs* Control
		H/R+NC (n=6)	0.1783		P=0.000664013 *vs* Control
					P=0.985281237 *vs* H/R
		H/R+ASPP1 (n=6)	0.5723		P=2.78938E-08 *vs* Control
					P=0.000810792 *vs* H/R
					P=0.000368876 *vs* NC+H/R
5A		WT (n=6)	0.4983	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=6)	0.1535		P=8.10021E-07 *vs* WT
5B	EF	WT+Sham (n=11)	0.6023	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=11)	0.3526		P=0.5848123762 *vs* WT+Sham
		WT+I/R (n=11)	0.7810		P=6.5373E-9 *vs* WT+Sham
					P=1.33244E-10 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=11)	0.4097		P=0.02838704 *vs* WT+Sham
					P=0.000831822 *vs* ASPP1(KO)+Sham

					P=7.29144E-05 *vs* WT+I/R
	FS	WT+Sham (n=11)	0.4535	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=11)	0.3704		P=0.338366479 *vs* WT+Sham
		WT+I/R (n=11)	0.8341		P=7.6156E-07 *vs* WT+Sham
					P=3.46549E-09 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=11)	0.3639		P=0.050276088 *vs* WT+Sham
					P=0.00047368 *vs* ASPP1(KO)+Sham
					P=0.003278664 *vs* WT+I/R
5C		WT+I/R (n=15)	0.0304	Mann-Whitney *U* test	
		ASPP1(KO)+I/R (n=15)	0.8587		P=1.28935E-08 *vs* WT+I/R
5D		WT+Sham (n=13)	0.3464	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(KO)+Sham (n=13)	0.4114		P=0.9138197 *vs* WT+Sham
		WT+I/R (n=15)	0.0031		P=1.38128E-08 *vs* WT+Sham
					P=7.13507E-09 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=15)	0.0419		P=0.001250895 *vs* WT+Sham
					P=0.000840703 *vs* ASPP1(KO)+Sham
					0.01104994 *vs* WT+I/R
5E		WT+Sham (n=9)	0.1973	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=9)	0.5715		P=0.999955876 *vs* WT+Sham
		WT+I/R (n=9)	0.6686		P=1.01E-13 *vs* WT+Sham
					P=1.01E-13 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=9)	0.1669		P=1.09793E-08 *vs* WT+Sham
					P=1.25251E-08 *vs* ASPP1(KO)+Sham
					P=1.10357E-08 *vs* WT+I/R
5F		WT+Sham	0.5828	two-way ANOVA	

		(n=9)		analysis followed by Tukey’s post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=9)	0.7786		P=0.960849952 *vs* WT+Sham
		WT+I/R (n=9)	0.2676		P=3.38E-13 *vs* WT+Sham
					P=7.63E-13 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=9)	0.9520		P=0.000632509 *vs* WT+Sham
					P=0.002459429 *vs* ASPP1(KO)+Sham
					P=7.68615E-09 *vs* WT+I/R
5G	Bcl-2	WT+Sham (n=6)	0.9953	two-way ANOVA analysis followed by Tukey’s post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=6)	0.1698		P=0.895610801 *vs* WT+Sham
		WT+I/R (n=6)	0.4118		P=0.002780918 *vs* WT+Sham
					P=0.000562676 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=6)	0.8479		P=0.993548953 *vs* WT+Sham
					P=0.970899958 *vs* ASPP1(KO)+Sham
					P=0.001532373 *vs* WT+I/R
5H	Bax	WT+Sham (n=6)	0.3658	two-way ANOVA analysis followed by Tukey’s post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=6)	0.9307		P=0.999881886 *vs* WT+Sham
		WT+I/R (n=6)	0.4310		P=1.18067E-08 *vs* WT+Sham
					P=1.05531E-08 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=6)	0.1012		P=0.672950935 *vs* WT+Sham
					P=0.632018157 *vs* ASPP1(KO)+Sham
					P=8.25844E-08 *vs* WT+I/R
5I	Total p53 levels	WT+Sham (n=5)	0.4536	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(KO)+Sham (n=5)	0.4068		P=0.708281012 *vs* WT+Sham
		WT+I/R (n=5)	0.9847		P=0.032509445 *vs* WT+Sham

					P=0.011996214 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=5)	0.4668		P=0.004611783 *vs* WT+Sham
					P=0.001340641 *vs* ASPP1(KO)+Sham
					P=0.487130991 *vs* WT+I/R
	p53 nuclear/cytoplasm ratio	WT+Sham (n=5)	0.8126	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(KO)+Sham (n=5)	0.1154		P=0.830696011 *vs* WT+Sham
		WT+I/R (n=5)	0.6613		P=0.005443982 *vs* WT+Sham
					P=0.002759549 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=5)	0.3563		P=0.592980098 *vs* WT+Sham
					P=0.454260243 *vs* ASPP1(KO)+Sham
					P=0.02476849 *vs* WT+I/R
6A		NC (n=5)	0.1294	Mann-Whitney *U* test	
		siASPP1-1 (n=5)	0.1466		P=0.420634920634921 *vs* NC
		siASPP1-2 (n=5)	0.1541		P=0.222222222222222 *vs* NC
		siASPP1-3 (n=5)	0.5071		P=0.007936507936508 *vs* NC
6B		Control (n=6)	0.9741	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R (n=6)	0.1750		P=2.3E-14 *vs* Control
		H/R+NC (n=6)	0.7532		P=2.3E-14 *vs* Control
					P=0.992822613 *vs* H/R
		H/R+siASPP1 (n=6)	0.7040		P=0.001460505 *vs* Control
					P=2.5E-14 *vs* H/R
					P=2.5E-14 *vs* H/R+NC
6C		Control (n=6)	0.8078	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R (n=6)	0.5302		P=5.02505E-09 *vs* Control
		H/R+NC (n=6)	0.2386		P=3.40611E-09 *vs* Control
					P=0.994585774 *vs* H/R
		H/R+siASPP1 (n=6)	0.1536		P=0.046337371 *vs* Control
					P=7.28987E-07 *vs* H/R

					P=4.55034E-07 *vs* H/R+NC
6D		Control (n=6)	0.8956	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R (n=6)	0.8940		P=1.086E-12 *vs* Control
		H/R+NC (n=6)	0.7722		P=1.071E-12 *vs* Control
					P=0.99999926 *vs* H/R
		H/R+siASPP1 (n=6)	0.7299		P=5.83639E-05 *vs* Control
					P=1.96033E-09 *vs* H/R
					P=1.92296E-09 *vs* H/R+NC
6E		Control (n=6)	0.2446	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R (n=6)	0.3123		P=2.08378E-08 *vs* Control
		H/R+NC (n=6)	0.8922		P=1.82358E-09 *vs* Control
					P=0.445911189 *vs* H/R
		H/R+siASPP1 (n=6)	0.1606		P=0.054063753 *vs* Control
					P=3.50581E-06 *vs* H/R
					P=1.85878E-07 *vs* H/R+NC
6F		Control (n=5)	0.5790	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R (n=5)	0.3946		P=0.020593097944251 *vs* Control
		H/R+NC (n=5)	0.3312		P=0.009673458033440 *vs* Control
					P=0.667859103735774 *vs* H/R
		H/R+siASPP1 (n=5)	0.7611		P=0.708281012290605 *vs* Control
					P=0.042618870899495 *vs* H/R
					P=0.016331945415615 *vs* H/R+NC
6G		Control (n=5)	0.9964	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R (n=5)	0.1571		P=0.001933702 *vs* Control
		H/R+NC (n=5)	0.2645		P=0.008814655 *vs* Control
					P=0.630466582 *vs* H/R
		H/R+siASPP1 (n=5)	0.7379		P=0.708281012 *vs* Control
					P=0.006409444 *vs* H/R
					P=0.02476849 *vs* H/R+NC

7A		NC (n=5)	0.8589	Mann-Whitney *U* test	
		sip53-1 (n=5)	0.0844		P=0.007936507936508 *vs* NC
		sip53-2 (n=5)	0.2195		P=0.031746031746032 *vs* NC
		sip53-3 (n=5)	0.1345		P=0.007936507936508 *vs* NC
7B		H/R+NC (n=9)	0.0623	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=9)	0.4674		P=2.85061E-10 *vs* H/R+NC
		H/R+ASPP1+siCTRL (n=9)	0.4920		P=1.92158E-08 *vs* H/R+NC
					P=0.378632861 *vs* H/R+ASPP1
		H/R+ASPP1+sip53 (n=9)	0.1152		P=0.918781982 *vs* H/R+NC
					P=6.0045E-11 *vs* H/R+ASPP1
					P=3.53424E-09 *vs* H/R+ASPP1+siCTRL
7C		H/R+NC (n=5)	0.3365	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R+ASPP1 (n=5)	0.1790		P=0.008814655 *vs* H/R+NC
		H/R+ASPP1+siCTRL (n=5)	0.8961		P=0.02476849 *vs* H/R+NC
					P=0.708281012*vs* H/R+ASPP1
		H/R+ASPP1+sip53 (n=5)	0.4676		P=0.630466582 *vs* H/R+NC
					P=0.001933702 *vs* H/R+ASPP1
					P=0.006409444 *vs* H/R+ASPP1+siCTRL
7D		H/R+NC (n=6)	0.5123	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.7256		P=2.6032E-05 *vs* H/R+NC
		H/R+ASPP1+siCTRL (n=6)	0.6741		P=2.57442E-06 *vs* H/R+NC
					P=0.687786762 *vs* H/R+ASPP1
		H/R+ASPP1+sip53 (n=6)	0.2483		P=0.059018853 *vs* H/R+NC
					P=1.19602E-07 *vs* H/R+ASPP1
					P=1.73435E-08 *vs* H/R+ASPP1+siCTRL
7E		H/R+NC (n=6)	0.9905	one-way ANOVA	

		H/R+ASPP1 (n=6)	0.5199	analysis followed by Tukey's post-hoc multi-comparison test	P=1.10156E-06 *vs* H/R+NC
		H/R+ASPP1+siCTRL (n=6)	0.9617		P=3.83275E-06 *vs* H/R+NC
					P=0.563072735 *vs* H/R+ASPP1
		H/R+ASPP1+sip53 (n=6)	0.1651		P=0.483310244 *vs* H/R+NC
					P=5.03802E-06 *vs* H/R+ASPP1
					P=1.84754E-05 *vs* H/R+ASPP1+siCTRL
7F		H/R+NC (n=6)	0.0740	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.9345		P=2.60397E-08 *vs* H/R+NC
		H/R+ASPP1+siCTRL (n=6)	0.3855		P=4.42156E-07 *vs* H/R+NC
					P=0.414266597 *vs* H/R+ASPP1
		H/R+ASPP1+sip53 (n=6)	0.1799		P=0.533421157 *vs* H/R+NC
					P=2.98585E-07 *vs* H/R+ASPP1
					P=6.67017E-06 *vs* H/R+ASPP1+siCTRL
7J		H/R+NC (n=6)	0.5795	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.5605		P=2.38136E-10 *vs* H/R+NC
		H/R+ASPP1+NC(n=6)	0.0969		P=7.9355E-11 *vs* H/R+NC
					P=0.973265273 *vs* H/R+ASPP1
		H/R+ASPP1+Flag-p53-NT (n=6)	0.1157		P=0.004158301 *vs* H/R+NC
					P=1.05659E-06 *vs* H/R+ASPP1
					P=2.6117E-07 *vs* H/R+ASPP1+NC
		H/R+ASPP1+Flag-p53-CT (n=6)	0.5600		P=1.38582E-10 *vs* H/R+NC
					P=0.998244607 *vs* H/R+ASPP1
					P=0.997885494 *vs* H/R+ASPP1+NC
					P=5.31091E-07 *vs* H/R+ASPP1+Flag-p53-NT

7K		H/R+NC (n=6)	0.1228	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.9127		P=1.07415E-10 *vs* H/R+NC
		H/R+ASPP1+NC(n=6)	0.1655		P=2.8411E-11 *vs* H/R+NC
					P=0.941658765 *vs* H/R+ASPP1
		H/R+ASPP1+Flag-p53-NT (n=6)	0.2550		P=0.998126582 *vs* H/R+NC
					P=1.85408E-10 *vs* H/R+ASPP1
					P=4.7901E-11 *vs* H/R+ASPP1+NC
		H/R+ASPP1+Flag-p53-CT (n=6)	0.6447		P=1.79297E-10 *vs* H/R+NC
					P=0.998534455 *vs* H/R+ASPP1
					P=0.837443419 *vs* H/R+ASPP1+NC
					P=3.12431E-10 *vs* H/R+ASPP1+Flag-p53-NT
7L		H/R+NC (n=6)	0.5218	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.6630		P=1.76761E-09 *vs* H/R+NC
		H/R+ASPP1+NC(n=6)	0.9722		P=1.17218E-09 *vs* H/R+NC
					P=0.999545617 *vs* H/R+ASPP1
		H/R+ASPP1+Flag-p53-NT (n=6)	0.4472		P=0.998755172 *vs* H/R+NC
					P=1.0409E-09 *vs* H/R+ASPP1
					P=6.94997E-10 *vs* H/R+ASPP1+NC
		H/R+ASPP1+Flag-p53-CT (n=6)	0.4741		P=2.74926E-10 *vs* H/R+NC
					P=0.866652486 *vs* H/R+ASPP1
					P=0.93971395 *vs* H/R+ASPP1+NC
					P=1.66984E-10 *vs* H/R+ASPP1+Flag-p53-NT
7M		H/R+NC (n=6)	0.3079	one-way ANOVA analysis followed by Tukey's post-hoc multi-	
		H/R+ASPP1 (n=6)	0.2336		P=9.38838E-10 *vs* H/R+NC

		H/R+ASPP1+NC(n=6)	0.6420	comparison test	P=2.55664E-10 *vs* H/R+NC
					P=0.957972031 *vs* H/R+ASPP1
		H/R+ASPP1+Flag-p53-NT (n=6)	0.2790		P=0.464533339 *vs* H/R+NC
					P=2.90196E-08 *vs* H/R+ASPP1
					P=6.86022E-09 *vs* H/R+ASPP1+NC
		H/R+ASPP1+Flag-p53-CT (n=6)	0.5381		P=1.06292E-10 *vs* H/R+NC
					P=0.771385461 *vs* H/R+ASPP1
					P=0.988610037 *vs* H/R+ASPP1+NC
					P=2.59095E-09 *vs* H/R+ASPP1+Flag-p53-NT
8A		AAV9-NC (n=6)	0.1498	nonpaired 2-tailed Student *t* test	
		AAV9-shp53 (n=6)	0.7941		P=7.08908E-07 *vs* AAV9-NC
8B	EF	WT+I/R (n=7)	0.0964	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=7)	0.4951		P=7.26293E-09 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.2942		P=2.74458E-09 *vs* WT+I/R
					P=0.96010774 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=7)	0.2942		P=0.808847929 *vs* WT+I/R
					P=1.2712E-09 *vs* ASPP1(TG)+I/R
					P=5.06677E-10 *vs* ASPP1(TG)+I/R+AAV9-NC
	FS	WT+I/R (n=7)	0.1602	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=7)	0.3785		P=7.27796E-09 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.3644		P=3.16924E-09 *vs* WT+I/R
					P=0.974620984 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=7)	0.4521		P=0.737030875 *vs* WT+I/R
					P=9.9234E-10 *vs*

					ASPP1(TG)+I/R
					P=4.55216E-10 *vs* ASPP1(TG)+I/R+AAV9-NC
8C		WT+I/R (n=6)	0.4521	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=6)	0.6009		P=1.69895E-06 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=6)	0.5927		P=1.54881E-06 *vs* WT+I/R
					P=0.999962242 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=6)	0.9370		P=0.193897363 *vs* WT+I/R
					P=3.60309E-08 *vs* ASPP1(TG)+I/R
					P=3.32505E-08 *vs* ASPP1(TG)+I/R+AAV9-NC
8D		WT+I/R (n=10)	0.7606	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=10)	0.6424		P=1.6958E-08 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=10)	0.7573		P=3.27491E-08 *vs* WT+I/R
					P=0.99600497 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=10)	0.8147		P=0.97053238 *vs* WT+I/R
					P=4.66523E-09 *vs* ASPP1(TG)+I/R
					P=8.91365E-09 *vs* ASPP1(TG)+I/R+AAV9-NC
8E		WT+I/R (n=10)	0.3230	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=10)	0.4801		P=1E-15 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=10)	0.4691		P=1E-15 *vs* WT+I/R
					P=0.999940913 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=10)	0.5987		P=0.996316226 *vs* WT+I/R
					P<1E-15 *vs* ASPP1(TG)+I/R
					P<1E-15 *vs* ASPP1(TG)+I/R+AAV9-NC
8F		WT+I/R (n=10)	0.0558	one-way ANOVA analysis followed by	
		ASPP1(TG)+I/	0.3860		P=7.372E-12 *vs* WT+I/R

		R (n=10)		Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R+AAV9-NC (n=10)	0.7834		P=2.7912E-11 *vs* WT+I/R
					P=0.958881181 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=10)	0.1812		P=0.801594243 *vs* WT+I/R
					P=5.19E-13 *vs* ASPP1(TG)+I/R
					P=2.432E-12 *vs* ASPP1(TG)+I/R+AAV9-NC
8G		WT+I/R (n=6)	0.1142	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=6)	0.7255		P=1.86062E-06 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=6)	0.2360		P=2.67081E-06 *vs* WT+I/R
					P=0.997846099 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=6)	0.1402		P=0.457621629 *vs* WT+I/R
					P=1.08916E-07 *vs* ASPP1(TG)+I/R
					P=1.51002E-07 *vs* ASPP1(TG)+I/R+AAV9-NC
8H		WT+I/R (n=6)	0.7192	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=6)	0.9644		P=2.44309E-06 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=6)	0.1834		P=4.61814E-07 *vs* WT+I/R
					P=0.828929203 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=6)	0.5889		P=0.980224569 *vs* WT+I/R
					P=5.33597E-06 *vs* ASPP1(TG)+I/R
					P=9.66563E-07 *vs* ASPP1(TG)+I/R+AAV9-NC
S1	Total ASPP1 levels	Non-ischemic area (n=5)	0.2926	Mann-Whitney *U* test	
		Ischemic area (n=5)	0.0808		P=0.007936507936508 *vs* Non-ischemic area
	ASPP1 nuclear/cytoplasm	Non-ischemic area (n=5)	0.3337	Mann-Whitney *U* test	
		Ischemic area	0.9445		P=0.007936507936508

	ratio	(n=5)			*vs* Non-ischemic area
	Total p53 levels	Non-ischemic area (n=5)	0.6728	Mann-Whitney *U* test	
		Ischemic area (n=5)	0.7002		P=0.007936507936508 *vs* Non-ischemic area
	p53 nuclear/cytoplasm ratio	Non-ischemic area (n=5)	0.8457	Mann-Whitney *U* test	
		Ischemic area (n=5)	0.9890		P=0.007936507936508 *vs* Non-ischemic area
S2 A	Total p63 levels	NC (n=5)	0.8612	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		siASPP1 (n=5)	0.7951		P=0.21892122 *vs* NC
		H/R+NC (n=5)	0.0322		P=0.668929268 *vs* NC
					P=0.422678074 *vs* siASPP1
		H/R+siASPP1 (n=5)	0.1940		P=0.422678074 *vs* NC
					P=0.668929268 *vs* siASPP1
					P=0.708281012 *vs* H/R+NC
	p63 nuclear/cytoplasm ratio	NC (n=5)	0.3196	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		siASPP1 (n=5)	0.6856		P=0.199543244894338 *vs* NC
		H/R+NC (n=5)	0.1339		P=0.121113867656861 *vs* NC
					P=0.789268026134283 *vs* siASPP1
		H/R+siASPP1 (n=5)	0.5018		P=0.630466581587966 *vs* NC
					P=0.422678074170649 *vs* siASPP1
					P=0.285049407402629 *vs* H/R+NC
S2 B	Total p73 levels	NC (n=5)	0.1580	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		siASPP1 (n=5)	0.2203		P=0.422678074170649 *vs* NC
		H/R+NC (n=5)	0.5674		P=0.261651090588242 *vs* NC
					P=0.054319378127170 *vs* siASPP1
		H/R+siASPP1 (n=5)	0.4028		P=0.830696011306370 *vs* NC
					P=0.309823373372381 *vs* siASPP1
					P=0.363514722736453 *vs* H/R+NC
	p73	NC (n=5)	0.6926	Kruskal Wallis test with	

	nuclear/cytoplasm ratio	siASPP1 (n=5)	0.8762	FDR (Benjamini-Hochberg method)	P=0.285049407402629 *vs* NC
		H/R+NC (n=5)	0.8146		P=0.454260242566824 *vs* NC
					P=0.069159491941058 *vs* siASPP1
		H/R+siASPP1 (n=5)	0.4451		P=0.521245308114821 *vs* NC
					P=0.668929268252769 *vs* siASPP1
					P=0.164602236716164 *vs* H/R+NC
S3 A	Total p53 levels	H/R+NC (n=5)	0.1446	Mann-Whitney *U* test	
		H/R+SiASPP2 (n=5)	0.6318		P=0.547619047619048 *vs* H/R+NC
	p53 nuclear/cytoplasm ratio	H/R+NC (n=5)	0.1742	Mann-Whitney *U* test	
		H/R+SiASPP2 (n=5)	0.5772		P=0.547619047619048 *vs* H/R+NC
S3 B	Total p53 levels	H/R+NC (n=5)	0.8086	Mann-Whitney *U* test	
		H/R+SiiASPP (n=5)	0.8029		P=0.007936507936508 *vs* H/R+NC
	p53 nuclear/cytoplasm ratio	H/R+NC (n=5)	0.5613	Mann-Whitney *U* test	
		H/R+SiiASPP (n=5)	0.2121		P>0.999999999999999 *vs* H/R+NC
S3 C	Total ASPP2 levels	H/R+NC (n=5)	0.6547	Mann-Whitney *U* test	
		H/R+Siimportin-β1 (n=5)	0.6106		P=0.309523809523810 *vs* H/R+NC
	ASPP2 nuclear/cytoplasm ratio	H/R+NC (n=5)	0.5830	Mann-Whitney *U* test	
		H/R+Si importin-β1 (n=5)	0.6401		P=0.547619047619048 *vs* H/R+NC
S3 D	Total iASPP levels	H/R+NC (n=5)	0.0084	Mann-Whitney *U* test	
		H/R+Siimportin-β1 (n=5)	0.9961		P=0.547619047619048 *vs* H/R+NC
	iASPP nuclear/cytoplasm ratio	H/R+NC (n=5)	0.8803	Mann-Whitney *U* test	
		H/R+Si importin-β1 (n=5)	0.7667		P=0.841269841269841 *vs* H/R+NC
S4 B	EF	WT (n=14)	0.1793	nonpaired 2-tailed Student *t* test	
		ASPP1(TG) (n=20)	0.2993		P=0.480531939433960 *vs* WT
	FS	WT (n=14)	0.0379	Mann-Whitney *U* test	
		ASPP1(TG) (n=20)	0.3679		P=0.522479771269560 *vs* WT
S4 C	Heart	WT (n=6)	0.8228	nonpaired 2-tailed	

	weight	ASPP1(TG) (n=6)	0.4123	Student *t* test	P=0.807272802009156 *vs* WT
	Body weight	WT (n=6)	0.3561	nonpaired 2-tailed Student *t* test	
		ASPP1(TG) (n=6)	0.4245		P=0.591326096918382 *vs* WT
	Heart weight/body weight (HW/BW)	WT (n=6)	0.6636	nonpaired 2-tailed Student *t* test	
		ASPP1(TG) (n=6)	0.6200		P=0.946374698356178 *vs* WT
S5 B	EF	WT (n=9)	0.1256	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.8037		P=0.517412514137118 *vs* WT
	FS	WT (n=9)	0.0803	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.6661		P=0.385036128965754 *vs* WT
S5 C	Heart weight	WT (n=6)	0.9735	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=6)	0.9948		P=0.901440144573029 *vs* WT
	Body weight,	WT (n=6)	0.7287	Mann-Whitney *U* test	
		ASPP1(KO) (n=6)	0.0221		P=0.816017316017316 *vs* WT
	Heart weight/body weight (HW/BW)	WT (n=6)	0.1607	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=6)	0.6737		P=0.969474465338486 *vs* WT
S6 A		NC (n=6)	0.3171	nonpaired 2-tailed Student *t* test	
		p53 (n=6)	0.7327		P=1.53196E-09 *vs* NC
S6 B		H/R+siCTRL (n=6)	0.2223	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+siASPP1 (n=6)	0.0521		P=1.91774E-08 *vs* H/R+siCTRL
		H/R+siASPP1+NC (n=6)	0.1215		P=1.21756E-07 *vs* H/R+siCTRL
					P=0.71705067 *vs* H/R+siASPP1
		H/R+siASPP1+p53 (n=6)	0.3801		P=1.75269E-07 *vs* H/R+siCTRL
					P=0.597690229 *vs* H/R+siASPP1
					P=0.99706735 *vs* H/R+siASPP1+p53
S6 C		H/R+siCTRL (n=6)	0.8832	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+siASPP1 (n=6)	0.3306		P=9.20901E-08 *vs* H/R+siCTRL
		H/R+siASPP1+NC (n=6)	0.0705		P=1.35861E-08 *vs* H/R+siCTRL

					P=0.687113653 *vs* H/R+siASPP1
		H/R+siASPP1+ p53 (n=6)	0.5106		P=3.62933E-07 *vs* H/R+siCTRL
					P=0.876808818 *vs* H/R+siASPP1
					P=0.276985155 *vs* H/R+siASPP1+p53
S6 D		H/R+siCTRL (n=5)	0.9798	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R+siASPP1 (n=5)	0.3890		P=0.007526315166462 *vs* H/R+siCTRL
		H/R+siASPP1+ NC (n=5)	0.2819		P=0.010296548972126 *vs* H/R+siCTRL
					P=0.914864745735549 *vs* H/R+siASPP1
		H/R+siASPP1+ p53 (n=5)	0.7094		P=0.005443981805205 *vs* H/R+siCTRL
					P=0.914864745735549 *vs* H/R+siASPP1
					P=0.830696011306370 *vs* H/R+siASPP1+p53
S6 E		H/R+siCTRL (n=5)	0.8456	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R+siASPP1 (n=5)	0.6650		P=0.028412580599663 *vs* H/R+siCTRL
		H/R+siASPP1+ NC (n=5)	0.9171		P=0.003896500435959 *vs* H/R+siCTRL
					P=0.487130990687863 *vs* H/R+siASPP1
		H/R+siASPP1+ p53 (n=5)	0.3862		P=0.003283460986070 *vs* H/R+siCTRL
					P=0.454260242566823 *vs* H/R+siASPP1
					P=0.957371576490613 *vs* H/R+siASPP1+p53
S7 A		NC (n=5)	0.7107	Mann-Whitney *U* test	
		sip63-1 (n=5)	0.5432		P=0.309523809523810 *vs* NC
		sip63-2 (n=5)	0.4107		P=0.007936507936508 *vs* NC
		sip63-3 (n=5)	0.2928		P=0.031746031746032 *vs* NC
S7 B		H/R+NC (n=6)	0.7740	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.9736		P=1.967E-12 *vs* H/R+NC
		H/R+ASPP1+si	0.8743		P=8.287E-12 *vs* H/R+NC

		CTRL (n=6)			P=0.610792960863232 *vs* H/R+ASPP1
		H/R+ASPP1+si p63 (n=6)	0.4067		P=1.0805E-11 *vs* H/R+NC
					P=0.478230690462547 *vs* H/R+ASPP1
					P=0.995969851341333 *vs* H/R+ASPP1+siCTRL
S7 C		H/R+NC (n=6)	0.6125	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.1822		P=9.65924E-07 *vs* H/R+NC
		H/R+ASPP1+si CTRL (n=6)	0.6999		P=3.93556E-06 *vs* H/R+NC
					P=0.894283894086269 *vs* H/R+ASPP1
		H/R+ASPP1+si p63 (n=6)	0.3477		P=3.94909E-08 *vs* H/R+NC
					P=0.330687551391404 *vs* H/R+ASPP1
					P=0.100852016726795 *vs* H/R+ASPP1+siCTRL
S7 D		H/R+NC (n=5)	0.2759	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R+ASPP1 (n=5)	0.8245		P=0.006409443948789 *vs* H/R+NC
		H/R+ASPP1+si CTRL (n=5)	0.5411		P=0.013940092260532 *vs* H/R+NC
					P=0.789268026134283 *vs* H/R+ASPP1
		H/R+ASPP1+si p63 (n=5)	0.5998		P=0.004611783449109 *vs* H/R+NC
					P=0.914864745735550 *vs* H/R+ASPP1
					P=0.708281012290605 *vs* H/R+ASPP1+siCTRL
S7 E		H/R+NC (n=5)	0.5430	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		H/R+ASPP1 (n=5)	0.2189		P=0.011996214124711 *vs* H/R+NC
		H/R+ASPP1+si CTRL (n=5)	0.6893		P=0.002759548935304 *vs* H/R+NC
					P=0.630466581587966 *vs* H/R+ASPP1
		H/R+ASPP1+si p63 (n=5)	0.1430		P=0.011996214124711 *vs* H/R+NC
					P>0.999999999999999 *vs* H/R+ASPP1
					P=0.630466581587966

					vs H/R+ASPP1+siCTRL
S7 F		NC (n=5)	0.9008	Mann-Whitney *U* test	
		Sip73-1 (n=5)	0.7061		P=0.007936507936508 *vs* NC
		Sip73-2 (n=5)	0.1460		P=0.007936507936508 *vs* NC
		Sip73-3 (n=5)	0.0672		P=0.007936507936508 *vs* NC
S7 G		H/R+NC (n=6)	0.8799	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.2010		P=6.71988E-10 *vs* H/R+NC
		H/R+ASPP1+si CTRL (n=6)	0.8535		P=5.49217E-9 *vs* H/R+NC
					P=0.529595747282050 *vs* H/R+ASPP1
		H/R+ASPP1+si p73 (n=6)	0.4425		P=1.6802739E-8 *vs* H/R+NC
					P=0.201146220115563 *vs* H/R+ASPP1
					P=0.901961439951775 *vs* H/R+ASPP1+siCTRL
S7 H		H/R+NC (n=6)	0.7256	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.7135		P=1.44896E-10 *vs* H/R+NC
		H/R+ASPP1+si CTRL (n=6)	0.1807		P=5.71708E-09 *vs* H/R+NC
					P=0.093294084549919 *vs* H/R+ASPP1
		H/R+ASPP1+si p73 (n=6)	0.4395		P=2.19465E-09 *vs* H/R+NC
					P=0.270251911961957 *vs* H/R+ASPP1
					P=0.927688529265254 *vs* H/R+ASPP1+siCTRL
S7 I		H/R+NC (n=6)	0.4002	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.1229		P=1.41981E-09 *vs* H/R+NC
		H/R+ASPP1+si CTRL (n=6)	0.2016		P=6.40089E-09 *vs* H/R+NC
					P=0.769905954 *vs* H/R+ASPP1
		H/R+ASPP1+si p73 (n=6)	0.2811		P=1.13906E-09 *vs* H/R+NC
					P=0.99884115 *vs* H/R+ASPP1
					P=0.686259519 *vs*

					H/R+ASPP1+siCTRL
S7 J		H/R+NC (n=6)	0.5374	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		H/R+ASPP1 (n=6)	0.1487		P=0.001500717080863 *vs* H/R+NC
		H/R+ASPP1+si CTRL (n=6)	0.6262		P=0.001412806251957 *vs* H/R+NC
					P=0.001934746317545 *vs* H/R+ASPP1
		H/R+ASPP1+si p73 (n=6)	0.7065		P=0.999993118079006 *vs* H/R+NC
					P=0.999489377373111 *vs* H/R+ASPP1
					P=0.999034864743258 *vs* H/R+ASPP1+siCTRL
S8 A		NC (n=6)	0.3855	nonpaired 2-tailed Student *t* test	
		siASPP2 (n=6)	0.6649		P=2.32972E-06 *vs* NC
S8 B		NC (n=6)	0.3562	nonpaired 2-tailed Student *t* test	
		siiASPP (n=6)	0.6253		P=2.25397E-07 *vs* NC
S8 C		NC (n=6)	0.5567	nonpaired 2-tailed Student *t* test	
		siimportin-β1 (n=6)	0.5803		P=9.50083E-07 *vs* NC
S8 D		NC (n=6)	0.5631	nonpaired 2-tailed Student *t* test	
		siE2F1 (n=6)	0.3920		P=1.22798E-08 *vs* NC

Normality test values were analyzed by D'Agostino & Pearson test ($n \geq 8$) and Shapiro-Wilk test ($n < 8$).

Supplementary Table 8. Detailed statistical analysis information for all main and supplementary figures and tables.

Table		Groups (Sample size)	Normality test values	Statistical analysis	P value
S1	EF	WT (n=14)	0.1793	nonpaired 2-tailed Student *t* test	
		ASPP1(TG) (n=20)	0.2993		P=0.480531939433960 *vs* WT
	FS	WT (n=14)	0.0379	Mann-Whitney *U* test	
		ASPP1(TG) (n=20)	0.3679		P=0.522479771269560 *vs* WT
	LVIDd	WT (n=14)	0.1521	Mann-Whitney *U* test	
		ASPP1(TG) (n=20)	0.0065		P=0.344938235413373 *vs* WT
	LVIDs	WT (n=14)	0.3543	nonpaired 2-tailed Student *t* test	
		ASPP1(TG)	0.4092		P=0.396070566010893

		(n=20)			*vs* WT
	LVEDV	WT (n=14)	0.2083	Mann-Whitney *U* test	
		ASPP1(TG) (n=20)	0.0001		P=0.327372297262329 *vs* WT
	LVESV	WT (n=14)	0.9505	Mann-Whitney *U* test	
		ASPP1(TG) (n=20)	0.0130		P=0.241248546562209 *vs* WT
S2	EF	WT (n=9)	0.1256	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.8037		P=0.517412514137118 *vs* WT
	FS	WT (n=9)	0.0803	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.6661		P=0.385036128965754 *vs* WT
	LVIDd	WT (n=9)	0.6133	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.4343		P=0.652501422547523 *vs* WT
	LVIDs	WT (n=9)	0.1598	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.5917		P=0.404440482462218 *vs* WT
	LVEDV	WT (n=9)	0.6090	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.5397		P=0.639549860162757 *vs* WT
	LVESV	WT (n=9)	0.1694	nonpaired 2-tailed Student *t* test	
		ASPP1(KO) (n=12)	0.5466		P=0.534055415037592 *vs* WT
S3	EF	WT+Sham (n=9)	0.3584	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=9)	0.6918		P=0.995724123 *vs* WT+Sham
		WT+I/R (n=9)	0.4626		P=2.86621E-07 *vs* WT+Sham
					P=1.51667E-07 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.3370		P=1.11E-13 *vs* WT+Sham
					P=1.08E-13 *vs* ASPP1(TG)+Sham
					P=1.63415E-07 *vs* WT+I/R
	FS	WT+Sham (n=9)	0.2560	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=9)	0.7281		P=0.996340287 *vs* WT+Sham
		WT+I/R (n=9)	0.4289		P=6.09478E-08 *vs* WT+Sham

					P=3.37548E-08 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.2479		P=1.25E-13 *vs* WT+Sham
					P=1.16E-13 *vs* ASPP1(TG)+Sham
					P=2.59946E-06 *vs* WT+I/R
	LVIDd	WT+Sham (n=9)	0.0434	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(TG)+Sham (n=9)	0.9172		P=0.80552627 *vs* WT+Sham
		WT+I/R (n=9)	0.9790		P=0.008826852 *vs* WT+Sham
					P=0.004171542 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.5032		P=0.004171542 *vs* WT+Sham
					P=0.001863972 *vs* ASPP1(TG)+Sham
					P=0.80552627 *vs* WT+I/R
	LVIDs	WT+Sham (n=9)	0.6898	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=9)	0.3695		P=0.972320645 *vs* WT+Sham
		WT+I/R (n=9)	0.6847		P=1.41875E-06 *vs* WT+Sham
					P=4.16131E-07 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.7821		P=2.8659E-11 *vs* WT+Sham
					P=1.0496E-11 *vs* ASPP1(TG)+Sham
					P=0.001339017 *vs* WT+I/R
	LVEDV	WT+Sham (n=9)	0.0444	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(TG)+Sham (n=9)	0.7407		P=0.81427103 *vs* WT+Sham
		WT+I/R (n=9)	0.9633		P=0.009147538 *vs* WT+Sham
					P=0.004491451 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.1594		P=0.004186141 *vs* WT+Sham

					P=0.001943696 *vs* ASPP1(TG)+Sham
					P=0.796952561 *vs* WT+I/R
	LVESV	WT+Sham (n=9)	0.6872	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+Sham (n=9)	0.1966		P=0.999135733 *vs* WT+Sham
		WT+I/R (n=9)	0.5906		P=3.74868E-05 *vs* WT+Sham
					P=2.55036E-05 *vs* ASPP1(TG)+Sham
		ASPP1(TG)+I/R (n=9)	0.8047		P=9.3604E-11 *vs* WT+Sham
					P=6.786E-11 *vs* ASPP1(TG)+Sham
					P=0.0002187 *vs* WT+I/R
S4	EF	WT+Sham (n=11)	0.6023	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=11)	0.3526		P=0.5848123762 *vs* WT+Sham
		WT+I/R (n=11)	0.7810		P=6.5373E-9 *vs* WT+Sham
					P=1.33244E-10 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=11)	0.4097		P=0.02838704 *vs* WT+Sham
					P=0.000831822 *vs* ASPP1(KO)+Sham
					P=7.29144E-05 *vs* WT+I/R
	FS	WT+Sham (n=11)	0.4535	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(KO)+Sham (n=11)	0.3704		P=0.338366479 *vs* WT+Sham
		WT+I/R (n=11)	0.8341		P=7.6156E-07 *vs* WT+Sham
					P=3.46549E-09 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=11)	0.3639		P=0.050276088 *vs* WT+Sham
					P=0.00047368 *vs* ASPP1(KO)+Sham
					P=0.003278664 *vs* WT+I/R

	LVIDd	WT+Sham (n=11)	0.0005	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(KO)+ Sham (n=11)	0.0127		P=0.517314202 *vs* WT+Sham
		WT+I/R (n=11)	0.1982		P=0.000187324 *vs* WT+Sham
					P=0.002014853 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I /R (n=11)	0.6072		P=0.973511376 *vs* WT+Sham
					P=0.496064063 *vs* ASPP1(KO)+Sham
					P=0.00016408 *vs* WT+I/R
	LVIDs	WT+Sham (n=11)	0.2730	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(KO)+ Sham (n=11)	0.5167		P=0.913411653 *vs* WT+Sham
		WT+I/R (n=11)	0.7946		P=3.65627E-08 *vs* WT+Sham
					P=4.66246E-09 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I /R (n=11)	0.7598		P=0.262729716 *vs* WT+Sham
					P=0.073397222 *vs* ASPP1(KO)+Sham
					P=1.42267E-05 *vs* WT+I/R
	LVEDV	WT+Sham (n=11)	0.0020	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(KO)+ Sham (n=11)	0.0770		P=0.522728744 *vs* WT+Sham
		WT+I/R (n=11)	0.1889		P=0.000193802 *vs* WT+Sham
					P=0.00201633 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I /R (n=11)	0.7936		P=0.960278985 *vs* WT+Sham
					P=0.490855815 *vs* ASPP1(KO)+Sham
					P=0.000158879 *vs* WT+I/R
	LVESV	WT+Sham (n=11)	0.6914	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(KO)+ Sham (n=11)	0.7109		P=0.995742331 *vs* WT+Sham

		WT+I/R (n=11)	0.8212		P=3.3851E-10 *vs* WT+Sham
					P=1.70539E-10 *vs* ASPP1(KO)+Sham
		ASPP1(KO)+I/R (n=11)	0.9910		P=0.280370017 *vs* WT+Sham
					P=0.188776896 *vs* ASPP1(KO)+Sham
					P=9.78295E-08 *vs* WT+I/R
S5	EF	WT+I/R (n=7)	0.0964	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=7)	0.4951		P=7.26293E-09 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.2942		P=2.74458E-09 *vs* WT+I/R
					P=0.96010774 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=7)	0.2942		P=0.808847929 *vs* WT+I/R
					P=1.2712E-09 *vs* ASPP1(TG)+I/R
					P=5.06677E-10 *vs* ASPP1(TG)+I/R+AAV9-NC
	FS	WT+I/R (n=7)	0.1602	one-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=7)	0.3785		P=7.27796E-09 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.3644		P=3.16924E-09 *vs* WT+I/R
					P=0.974620984 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=7)	0.4521		P=0.737030875 *vs* WT+I/R
					P=9.9234E-10 *vs* ASPP1(TG)+I/R
					P=4.55216E-10 *vs* ASPP1(TG)+I/R+AAV9-NC
	LVIDd	WT+I/R (n=7)	0.0094	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(TG)+I/R (n=7)	0.8406		P=0.110893751 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.0397		P=0.054916854 *vs* WT+I/R
					P=0.744920237 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-	0.8943		P=0.794651096 *vs* WT+I/R

		shp53 (n=7)			P=0.182232341 *vs* ASPP1(TG)+I/R
					P=0.097063866 *vs* ASPP1(TG)+I/R+AAV9-NC
	LVIDs	WT+I/R (n=7)	0.4686	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=7)	0.3149		P=2.08833E-05 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.5595		P=3.86787E-06 *vs* WT+I/R
					P=0.896138371 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=7)	0.8664		P=0.999503074 *vs* WT+I/R
					P=1.59497E-05 *vs* ASPP1(TG)+I/R
					P=2.97812E-06 *vs* ASPP1(TG)+I/R+AAV9-NC
	LVEDV	WT+I/R (n=7)	0.0160	Kruskal Wallis test with FDR (Benjamini-Hochberg method)	
		ASPP1(TG)+I/R (n=7)	0.8462		P=0.122457658 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.0385		P=0.059299781 *vs* WT+I/R
					P=0.732783996 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=7)	0.9036		P=0.832607885 *vs* WT+I/R
					P=0.182472223 *vs* ASPP1(TG)+I/R
					P=0.094011536 *vs* ASPP1(TG)+I/R+AAV9-NC
	LVESV	WT+I/R (n=7)	0.5922	two-way ANOVA analysis followed by Tukey's post-hoc multi-comparison test	
		ASPP1(TG)+I/R (n=7)	0.2667		P=6.07924E-05 *vs* WT+I/R
		ASPP1(TG)+I/R+AAV9-NC (n=7)	0.4642		P=9.46072E-06 *vs* WT+I/R
					P=0.871480293 *vs* ASPP1(TG)+I/R
		ASPP1(TG)+I/R+AAV9-shp53 (n=7)	0.8841		P=0.999885245 *vs* WT+I/R
					P=5.14096E-05 *vs* ASPP1(TG)+I/R
					P=8.03653E-06 *vs* ASPP1(TG)+I/R+AAV9-NC

Normality test values were analyzed by D'Agostino & Pearson test ($n \geq 8$) and Shapiro-Wilk test ($n < 8$).