**p53 regulates the cardiac transcriptome**

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**Supplementary Information containing:**

- **Figure S1:** Transcriptomic analysis of p53 target genes in p53KO post-Tam.
- **Figure S2:** GO gene sets that regulate cardiac differentiation and hypertrophy in p53KO.
- **Figure S3:** Factors involved in the regulation of mt biogenesis in p53-deficient cardiomyocytes.
- **Supplementary Materials and Methods**

Supplementary Figure S1

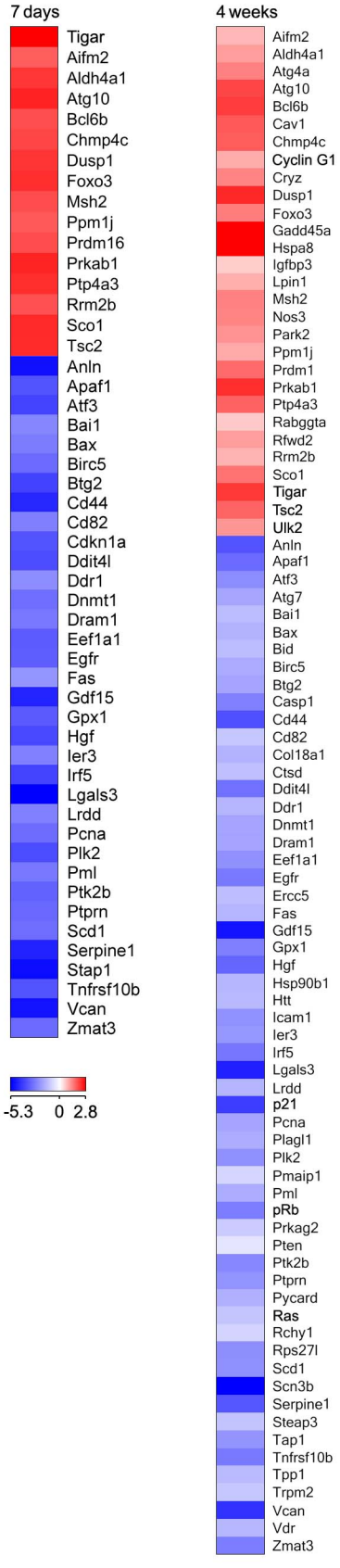


**Figure S1:** Heat map examining the impact of genomic modifications in p53KO at 7d post-Tam of validated p53-target genes.
$Log_2$ expression values. *n*=3 biological replicates. *P*<0.01. Fold change >1.3 or <-1.3.

Supplementary Figure S2

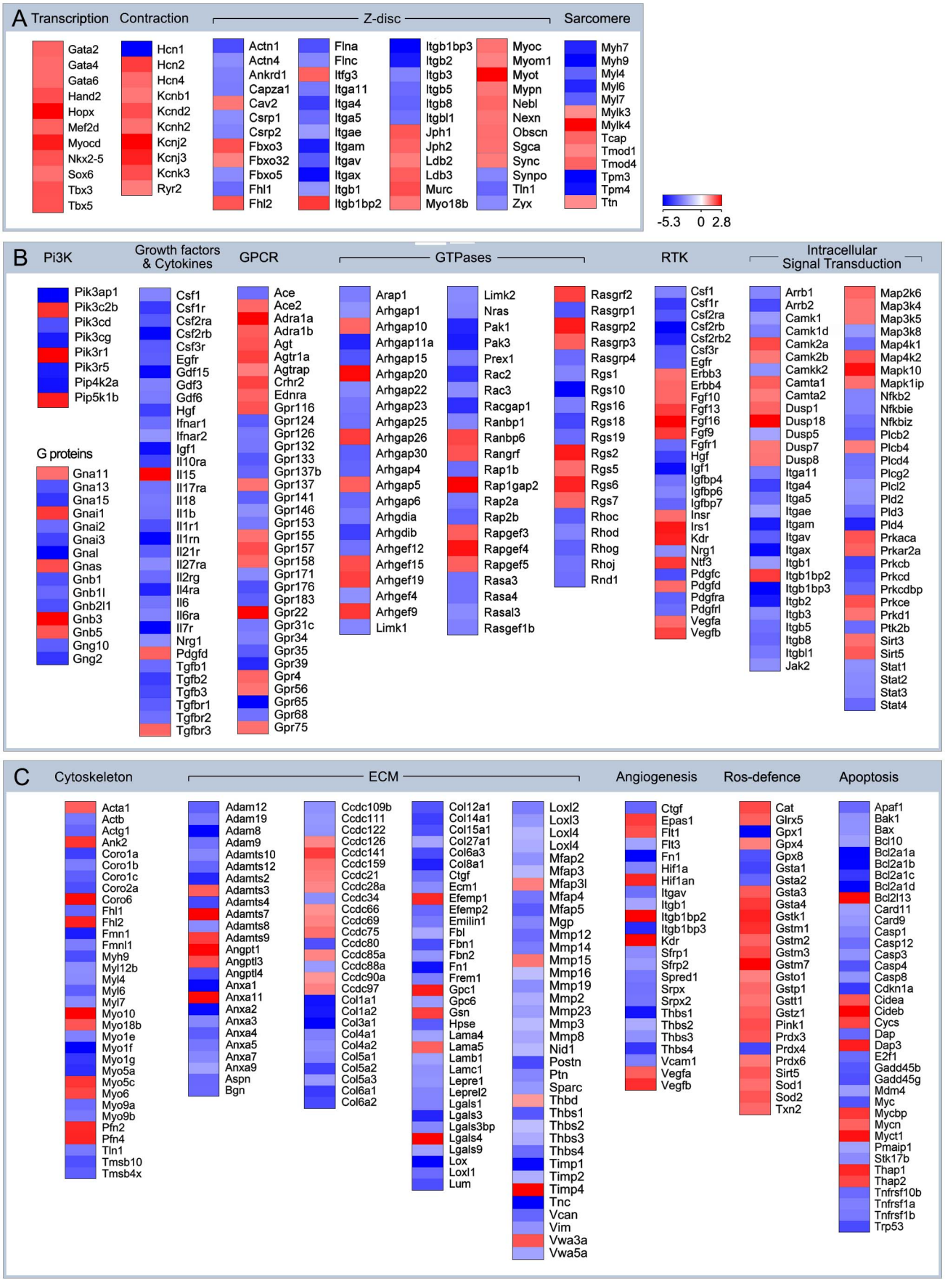


**Figure S2:** (A-C) GO gene sets regulating cardiac hypertrophy in p53KO at 7 days post-Tam.

Supplementary Figure S3

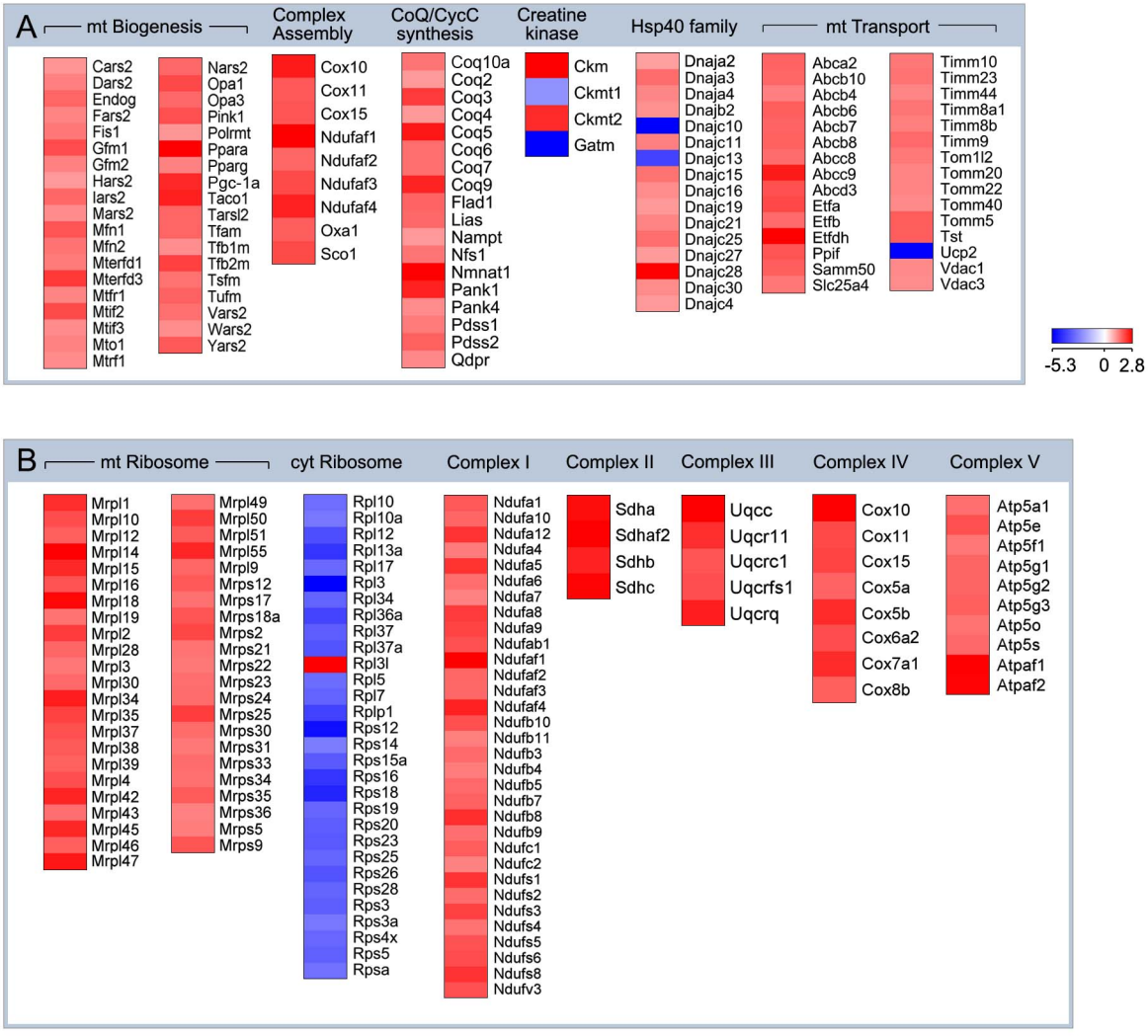


**Figure S3:** (A and B) Heat maps representations showing enrichment of induced (red) and repressed (blue) genes involved in the regulation of mt biogenesis, mt electron complex assembly and coenzyme Q/C synthesis, mt Hsp40 homologs and mt transporters (A), and ribosomal subunits , respiratory chain complexes and ATP synthase subunits (B) as determined by GO analysis. Selected genes are shown and explained in the results section. Shown are subsets of most abundantly enriched or down-regulated genes (rows) when comparing p53 mutant hearts (columns) post-Tam versus vehicle control tissue samples. Selected enriched regulatory genes in each pathway are shown and discussed. $Log_2$ expression values. *n*=3 biological replicates. *P*<0.01 vs. -Tam. Fold change >1.3 or <-1.3. Right, symbols of selected genes significantly induced (red) and repressed (blue) upon genetic modification of p53.

**Supplementary Materials and Methods**

**Cardiac-specific *p53$^{fl/fl}$;mcm* conditional mutant mice**

All animal usage in this study was in accordance with approved institutional animal care guidelines of the UHN (AUP 1815/1379, Canadian Council in Animal Care). All animals used in this study were 10 weeks old (22-26g) at the beginning of experimentation. All experiments used isogenic littermate controls of matched age and sex.

The *p53$^{fl/fl}$* mice (01XH9) were obtained from Frederick (Rockville, MD 20852 USA). This strain was previously backcrossed into a C57BL/6J background for 7 generations. The *mcm* strain (Tg(Myh6-cre/Esr1*)1Jmk/J) was from Jackson (Bar Harbor, ME 04609 USA). In these mice, the cardiac muscle $\alpha$-myosin heavy chain 6 promoter drives the expression of Cre (c) recombinase fused to two mutant (m) estrogen-receptor ligand-binding domains (*mcm*) when exposed to Tam. We crossed *mcm* transgenic mice on a C57BL/6J background with mice carrying the conditional alleles *p53$^{fl/fl}$* to obtain *p53$^{fl/fl}$;mcm* animals referred to as p53KO throughout this work.

Because Tam and Cre expression can be toxic to cells, we included vehicle- and Tam-injected wild-type C57BL/6J mcm and *p53$^{fl/fl}$* mice in all initial analyses of the corresponding mutants. We found that mice of these experimental groups were phenotypically indistinguishable from vehicle-injected *p53$^{fl/fl}$*;mcm control animals used in this study, as judged by heart body weight ratios, ventricular fibrosis and fractional shortening.

After weaning, experimental male mice were housed in groups of 3-5 animals in mechanically ventilated cages (600 cm$^2$; changed fortnightly) environmentally enriched by bedding and nesting materials and crawl tubes. Animals were held in a temperature-controlled environment

at 19-22°C on a diurnal 12 hour light cycle. Mice were provided free access to standard nonmedicated pelleted laboratory rodent chow (Harlan) and tap water ad libitum from a portable water faucet.

DNA isolated from fresh tail snips was employed for genotyping. Samples were incubated in 300 μl of 50 mM NaOH for 2h, 80°C while rocking. Then, samples were neutralized with 25 μl 1 M HCl, 700 μl $H_2O$, vigorously vortexed, centrifuged for 13,000 rpm for 10 min and stored at 4°C. We used 0.5 μl of DNA solution per PCR reaction and the following primers: $p53^{fl/fl}$ forward 5'-CACAAAAACAGGTTAAACCCAG-3'; $p53^{fl/fl}$ reverse 5'-AGCACATAGGAGGCAGAGAC-3'. Mcm forward 5'-AGGTGGACCTGATCATGGAG-3'; mcm reverse 5'-ATACCGGAGATCATGCAAGC-3'. PCR amplicons: *p53* wild-type allele, 288 bp. *p53* LoxP allele 370bp. Mcm transgene, 440 bp. We performed PCR analysis with Quanta Accustart Geltrac with GelDye (no. 95136-04K; VWR) and Platinum Blue Supermix (no. 12580-023; Invitrogen).

An ethanol-peanut oil emulsion of 4-Hydroxytamoxifen (Tam; H6278, Sigma-Aldrich) was prepared by completely dissolving 100 mg Tam in 5 ml highly purified ethanol (ACS reagent grade, anhydrous, absolute; no. 6590-32; Ricca Chemical, Fisher) while vortexing vigorously for 5-8 min. Peanut oil (32ml) (P2144; Sigma-Aldrich) was added and the emulsion was again vigorously vortexed for 2 min. Then, the emulsion was sonicated on ice at highest output for 10-30 sec until it became translucent, aliquoted and stored at -20°C for several months. Shortly before usage, Tam was melted in a 37°C water bath, briefly vortexed, and 200-250 μl were immediately injected intraperitoneally into conscious mice. Vehicle control mice were intraperitoneally injected with an ethanol-peanut oil emulsion lacking Tam. Animals were injected daily between 5-6 pm for four consecutive days (43.2 mg/kg cumulative dosage).

Homologous recombination was completed 5-6 days after the last Tam-injection. Heart-specific deletion of *p53* was determined by PCR (forward CACAAAAAACAGGTTAAACCCAG; reverse AGCACATAGGAGGCAGAGAC) of DNA isolated from LV tissue samples of wild-type, vehicle injected control p53KO, and p53KO mice at 7 days post-Tam (amplicon size 370 bp, loxP allele; 289 bp, wild-type allele).

**Microarray Analysis**

RNA samples were processed for analysis by Affymetrix Mouse Gene 1.0 ST expression arrays at the Centre for Applied Genomics (The Hospital for Sick Children, Toronto, Canada). Processing of probe level data and all subsequent analyses were performed using GeneSpring (Version 13.2; Agilent Technologies Inc.). Total RNA from mouse left ventricular tissues was isolated with Trizol reagent (no. 15596026; Thermo Fisher Scientific). Phase lock Gels (no. 826754; VWR) were employed to eliminate interphase-protein contaminations. RNA quality was assessed by 260/280 and 260/230 absorption ratios employing a Nanodrop spectrophotometer (NanoDrop; Thermo Fisher Scientific), and an Agilent Bioanalyzer at the Microarray Facility, Centre for Applied Genomics, The Hospital for Sick Children (Toronto). RNA samples were processed for analysis by Affymetrix Mouse Gene 1.0 ST expression arrays at the Centre for Applied Genomics. Processing of probe level data and all subsequent analyses were performed using GeneSpring (Version 13.2; Agilent Technologies Inc.). Genome wide data from the gene expression microarrays were normalized, filtered on expression (-5.6 - 3.4) in the normalized data, filtered on on Error-CV < 50.0 percent, and filtered for genes with significant expression levels ($\log_2$ fold change $\pm 1.3$; $P<0.05$) plus statistical significance according to the

*t* test with Benjamini-Hochberg correction ($log_2$ fold change ±1.3; $P$<0.01) in Tam-treated (7 days and 4 weeks) *p53$^{fl/fl}$;mcm*, compared with vehicle injected control *p53$^{fl/fl}$;mcm* (*n*=3) employing GeneSpring. GSEA and GO term analysis of differentially expressed genes were done with GeneSpring. All microarray data were submitted to the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress; accession number E-MTAB-5441).

**Echocardiography**

Echocardiography in anesthetized mice (2% isoflurane, 98% oxygen) was performed using a 15-MHz linear ultrasound transducer (Vivid7; GE). Body temperature was maintained at 37 °C. M-mode measurements of the LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD) were made from short-axis views at the level of the papillary muscle. LVEDD was measured at the time of the apparent maximal LV diastolic dimension, whereas LVESD was measured at the time of the most systolic excursion of the posterior wall. LV fractional shortening (FS) was calculated as follows: FS = (LVEDD − LVESD)/LVEDD × 100%.

**Antibodies**

We used primary antibodies directed against the following proteins: Cox4i1 (ab16056), Mef2a (ab32866), Ndufa10 (ab96464), Opa1 (ab42354; Abcam), Pparα (PAB11321), Pparγ (PAB8500; Abnova), Pgc-1α (3934-100; Biovision), normal rabbit IgG (2729), Pln (8495), Serca2 (9580), Sod2 (13194), Sirt (5490; Cell Signaling), p53 (BML-SA293-0050; Enzo), Cpt1b (LS-C12435; Lifespan), Esrrα/β (NBP1-47254; Novus), α-actinin, sarcomeric (A7811), Npm1 (B0556; Sigma-

Aldrich), Essrγ (Proscience; 27-388), Gata4 (560327; Becton Dickinson), normal mouse IgG (sc-2025; SantaCruz).

We used the following secondary antibodies for immunoblotting: horseradish peroxidase conjugated sheep anti-mouse IgG (RPN4201, GE Life Sciences) and horseradish peroxidase conjugated goat anti-rabbit IgG (RPN4301, GE Life Sciences) combined with an enhanced chemiluminescence system (Luminata Crescendo; WBLUR0100, Millipore). We used the following secondary antibodies for immunofluorescence microscopy: (Alexa Fluor 488-goat anti-rat IgG, A-11006; Alexa Fluor 555-goat anti-rabbit IgG, A-21429; Alexa Fluor 555-goat anti-mouse IgG, A-2142; Alexa Fluor 647-goat anti-mouse IgG, A-20990 (Thermo Fisher Scientific).

**Immunofluorescence microscopy and morphometric analyses**

Mice were euthanized without anesthesia by cervical dislocation between 9-11am. Anticoagulant was not administered. Hearts were quickly excised and rinsed in 20 ml ice-cold PBS, pH 7.4 without $Ca^{2+}$/$Mg^{2+}$ (no. 10010023; Thermo Fisher Scientific), fixed in 4% PBS-buffered formalin (10 ml) for 50 min at room temperature with constant agitation and incubated in 0.3 M glycine in PBS (pH 7.4; 10 ml) at 4°C for 3-5d. After embedding hearts in Tissue-Tek OCT Compound (Sakura, Finetek; VWR) sections were cut at 10 μm thickness using a cryostat (HM525 NX; Thermo Fisher Scientific), and mounted on histological slides (Superfrost Plus Microslides; no. 48311-703; VWR). Samples were co-stained with cardiac-specific anti-sarcomeric α-actinin after permeabilization. Specimen were permeabilized in 1% Triton X-100 (X100; Sigma-Aldrich) in TBS (20 mM Tris, 150 mM NaCl), pH 7.6 for 60 minutes at room temperature. Samples were incubated with primary antibodies in TBS/1% Triton X-100, for 16-

20h at room temperature without agitation. Thereafter, specimen were briefly rinsed with TBS/1% Triton X-100. For visualization, samples were incubated with Alexa Fluor secondary antibodies, diluted 100-fold in TBS/1% Triton X-100 for 1h in the dark. Specimen were briefly rinsed with TBS/1% Triton X-100, and nuclear DNA was visualized with 4,6-diamidino-2-phenylindole (Dapi; 1 μg/ml in PBS) (D9542; Sigma-Aldrich). ProLong Diamond antifade reagent (no. 36965; Thermo Fisher Scientific) was applied, and samples were sealed with a coverslip. For determination of cardiomyocyte cell size and ventricular remodeling, ventricular samples were stained with sarcomeric α-actinin and Alexa Fluor 488-conjugated wheat germ agglutinin (WGA) (W11261; Thermo Fisher Scientific). Cross-dimensions of adult cardiomyocytes and fibrotic area were determined by planimetry of immunofluorescence microphotographs using ImageJ 1.51d (National Institutes of Health, Bethesda, MD, https://imagej.nih.gov/ij/). After recording, simple adjustments and assembly of entire and cropped microphotographs were performed employing Adobe Photoshop CS6.

**Cell fractionation, total heart tissue extracts and isolation of mitochondria**

Subcellular cell fractions were prepared using the NE-PER Kit (no. 78833; Pierce) supplemented with phosphatase inhibitors (1 mM $Na_3VO4$, 20 mM NaF, 10 mM β-glycerophosphate; Sigma) and with a protease inhibitor cocktail containing AEBSF, Aprotinin, Bestatin, E64, Leupeptin and Pepstatin (no. P2714; Sigma). Ventricular specimen were mechanically homogenized in RIPA Buffer (no. 9806; Cell Signaling) composed of 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1% NP-40, 1% sodium deoxy cholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM $Na_3VO_4$, 1 mg/ml leupeptin, phosphatase and protease inhibitors.

Mechanochemical assisted isolation of mitochondria from adult mouse ventricular tissues was performed using extraction buffer A (10 mM HEPES, pH 7.5; 200 mM mannitol; 70 mM sucrose; 1 mM EGTA) supplied with the Mitochondrial Isolation Kit (MITOISO1; Sigma) according to the manufacturer's instructions. Isolated mitochondria derived from one heart were resuspended in 200 μl storage buffer (10 mM HEPES, pH 7.5, containing 250 mM sucrose, 1 mM ATP, 80 μM ADP, 5 mM sodium succinate, 2 mM $K_2HPO_4$, 1 mM DTT) (MITOISO1; Sigma), and used directly in mitochondrial complex activity assays, or aliquoted and stored at -80°C for Western blot analysis. The protein concentration was approximately 3.0-4.0 μg/μl as determined by fluorometry (Qubit 2.0 Fluorometer; no. Q33217; Thermo Fisher Scientific).

**Mitochondrial electron transport complex I and III function**

The relative *activities* of mt *complex* I/III *were determined employing* the Complex I Enzyme Activity Assay Kit (MS141; MitoSciences), and the MitoTox OXPHOS Complex III Activity Kit (ab109905; Abcam) according to the manufacturer's procedures except that mt extracts (100 μl/well; (3-4 μg/μl total protein) derived from isolated mt were immobilized on 96-well plates for 1 hour at 4 $^{o}$C. Absorbance at 600 nm was recorded at room temperature for 60 min at 2 min intervals using a spectrophotometer (FlexStation 3; Molecular Devices). The rate between two time points (Δabsorbance at 600nm/min) was calculated in the most linear range of decline (5-20 min) for DCPIP absorbance: Rate (mOD/min) = Absorbance 1 - Absorbance 2/Time (min). The activity of immunocaptured complexes was calculated as the mean of 4 independent measurements obtained with immunocaptured enzyme minus the rate obtained without immunocaptured enzyme. Complex activities in vehicle-treated samples were set as 100%.

**Immunoprecipitations and immunoblotting**

For immunoprecipitation (IP) assays, cellular extracts in supplemented RIPA Buffer were incubated with antibodies or normal rabbit IgG (7.5 μg) covalently linked to protein A agarose beads (Seize X Protein A IP Kit; no. 26149; Pierce) for 3h at 4°C. Immunocomplexes were washed twice with lysis buffer and boiled in SDS sample buffer (no. 7722; Cell Signaling). Protein samples were resolved by SDS-PAGE employing 4-12% and 3-8% NuPAGE pre-cast gels (Life Technologies), and PVDF membranes (iBlot; Life Technologies). The following secondary antibodies were employed for chemiluminescence detection of proteins: horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (no. 7074; Cell Signaling), HRP-conjugated anti-mouse IgG (no. 7076; Cell Signaling), and Luminata Crescendo (WBLUR0100, Millipore).

**Oligonucleotide precipitation and ChIP assays**

We identified potential p53 binding elements (BE) in the p21, Cpt1b, Gata4, Nkx2-5 and Pgc-1α mouse gene promoters by *in silico* promoter analysis employing Ensembl (http://useast.ensembl. org/index.html) and TFSEARCH software (version 1.3; http://www.cbrc.jp/research/db/ TFSEARCH.html). The threshold score in TFSEARCH was set to 65% (Nkx2-5), 60% (p21, Cpt1b) and 50% (Gata4, Pgc-1α). The following oligonucleotides were employed in oligonucleotide precipitation experiments. The wild type p53 BE are underlined. Mutations (mt) introduced in mt.p53 BE are bolded. Nucleotide sequence of the p53 BE in the mouse Gata4 gene promoter (Gene ID 14463) located in intron 1 at -2806 bp to -2792 bp upstream of the Gata4 coding sequence: wt.p53 BE-Gata 5'-

AGAAAAGTCTAGGCCAGGCCGGACTAGACTAGACTTAAGT-3', mt.p53 BE-Gata 5'-AGAAAAGTCTAG**ACTGT**GCCGGACTAGACTAG ACTTAAGT-3'. Nucleotide sequence of the p53 BE in the mouse Nkx2-5 gene promoter (Gene ID 18091) located at -1007 bp to -988 bp upstream of the Nkx2-5 coding sequence: wt.p53 BE-Nkx 5'-GCCCCGTGGAGTAGAAGCCCAG GAAGGCCCTGGAGGATCG-3', mt.p53 BE-Nkx 5'-GCCCCGTGGAGT**GCAGT**CCCAGGAAGGCCCTGGA GGATCG-3'. Nucleotide sequence of the p53 BE in the mouse p21 gene promoter (Gene ID 12575) located at −2157 bp to −2137 bp upstream of the p21 coding sequence: wt.p53 BE-p21 5'-GGAGTCCTCTGGTCATGCCCCTCAAGGTGAAAGACGAGAGA-3'; mt.p53 BE-p21 5'-GGAGTCCTC TGG**GGAGT**CCCCTCAAGGTGAAAGACGAGAGA-3'. Nucleotide sequence of p53 BE in the mouse Cpt1b gene promoter (Gene ID 12895) located at −1605 bp to −1586 upstream of Cpt1b bp coding sequence: wt.p53 BE-Cpt1b 5'-CCTCGTTCCCTAGGTACTCCCTGGCATCTCACTTTTT CTG-3'; mt.p53 BE-Cpt1b 5'-CCTCGTTCCCTAG**CCATG**CCCTGGCATCTCACTTTTTCTG-3'. Nucleotide sequence of p53 BE in the mouse Pgc-1$\alpha$ gene promoter (Gene ID 19017) located at −238 bp to −219 upstream of Pgc-1$\alpha$ bp coding sequence: wt.p53 BE-Cpt1b 5'-ACTGAGGCAGAGGGCTGCCT TGGAGTGACGTCAGGAGT-3'; mt.p53 BE-Cpt1b 5'-ACTAGGCAGAG**AATAC**CCTTGGAGTGACGTCA GGAGT-3'.

For oligonucleotide precipitation lysates, LV tissue samples were mechanically disintegrated and lysed in 10 mmol/L HEPES (pH 7.9), 100 mmol/L KCl, 2 mmol/L $MgCl_2$, 10% glycerol, 1 mmol/L DTT, 0.5% NP40. Samples (250 μg total protein) were precleared with ImmunoPure streptavidin agarose beads (30 μl; Pierce) for 2 hours and incubated with 5 μg of 3'-biotinylated double stranded oligonucleotides (Qiagen) and 10 μg poly(dI-dC; P4929, Sigma) overnight. After collection of DNA-bound proteins with streptavidin-agarose beads for 2 hours, samples were

eluted with 1x SDS sample buffer and subjected to immunoblotting employing anti-p53 antibodies.

For chromatin immunoprecipitation (ChIP) analysis, LV tissues were fixed in 1% formaldehyde for 30 min at room temperature. ChIP assays were carried out employing p53 antibodies (5 μg/sample) and the Agarose ChIP kit (Pierce) according to the manufacturer's specifications. We used normal mouse IgG (sc-2025; SantaCruz) as negative controls in the IP reaction. For qPCR analysis, we used 1.0 μl from a 50 μl DNA extraction and 21-25 cycles of amplification. ChIP qPCR primers to p53 BE in the p21, Cpt1b, Gata4, Nkx2-5 and Pgc-1α gene loci were designed employing Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3 plus.cgi/). PCR primers to p53BE in p21 (product size 161 bp): p53BE-p21 forward 5'-GGTGGAGTGCTGGGATTAAA-3', p53BE-p21 reverse 5'-TTTGAATTCAGACCCTTT T GG-3'. PCR primers to p53BE in Cpt1b (product size 140 bp): p53BE-Cpt1b forward 5'-AGAGCCGTGGATAT TTTGGA-3', p53BE-Cpt1b reverse 5'-GACCACAGACCCCAGAAAA-3'. PCR primers to p53BE in Gata4 (product size 192 bp): p53BE-Gata forward 5'-GGTTCCAGAAAAGCTGTGGA-3', p53BE-Gata reverse 5'-TTCTCCTGGGTGGATTCTTG-3'. PCR primers to p53BE in Nkx2-5 (product size 161 bp): p53BE-Nkx2-5 forward 5'-GGACTGAAAATGTCCCTCCA-3', p53BE-Nkx2-5 reverse 5'-TGGGGAATTG ACTCTTCCTG-3'. PCR primers to p53BE in Pgc-1α (product size 205 bp): p53BE-Pgc-1α forward 5'-GCGGTTACTTCACTGAGGCAGA-3', p53BE-Pgc-1α reverse 5'-AGAGTGAACTGAAGGCACCTG-3'.

**Reverse transcription and quantitative real time PCR assays**

We carried out two-step reverse transcriptase (RT) and quantitative real-time polymerase chain reactions (qPCR) on a LightCycler 480 (Roche; TMDT Core Facility) for mRNA analysis. Total RNA from mouse and human ventricular cardiac specimen was isolated with Trizol reagent (no. 15596026; Thermo Fisher Scientific), and Phase lock Gels (no. 826754; VWR) were employed to eliminate interphase-protein contaminations. We used 1.0 μg total RNA in a 20 μl reaction for first-strand cDNA synthesis employing the SensiFast cDNA synthesis kit (BIO-65053; Bioline). For qPCR, we employed 4.0 μl first-strand synthesis product, diluted 5-fold with MilliQ-grade water and the Quanta Accustart II PCR Supermix (no, 95136-04; VWR) with EvaGreen dye (no. 31000; VWR). Relative quantification of transcript levels was performed using the ΔΔCt method with normalization to beta-2 microglobulin (B2m) employing the data analysis module (Roche Light Cycler 480 SW). We used the following primers: ANF forward 5'-CATCACCCTGGGCTTCTTCCT-3', reverse 5'-GGGCTCCAATCCTGTCAATC-3'; BNP forward 5'-GCGGCATGGATCTCCTGAAGG-3', reverse 5'-CCCAGGCAGAGTCAGAAACT G-3'; α-MHC forward 5'-CCAATGAGTACCGCGTGAA-3', reverse 5'-ACAGTCATGCCGGGATGAT-3'; β-MHC forward 5'-ATGTGCCGGACCTTGGAA-3', reverse 5'-CCTCG GGTTAGCTGAGAGATCA-3'; GAPDH forward 5'-ATG TTCCAGTATGACCCACTCACG-3', reverse 5'-GAAGACACCAGTAGACTCCACGACA-3'; B2m forward 5'-GGATTGGCTGTGAGTTCAGG-3', reverse 5'-GGTCAGTGAGACAAGCACCA-3'; Cpt1b forward 5'-GCACACCAGGCAGTAGCTTT-3', reverse 5'-CAGGAGTTGATTCCAGACAGGTA-3'; Cox4i1 forward 5'-ATTGGCAAGAGAGCCATTTCT AC-3', reverse 5'-CACGCCGATCAGCGTAAGT-3'; Essrα forward 5'-CTCAGCTCTCTACCCAAACGC-3', reverse 5'-CCGCTTGGTGATCTCACACTC-3'; Essrγ forward 5'-AAGATCGACACATTGATTCCAGC-3', reverse 5'-CATGGTTGAACTGTAACTCCCAC-3'; Gata4 forward 5'-CCCTACCCAGCCTACATGG-3', reverse 5'- ACATATCGAGATTGGGGTGTCT-3'; Mef2a forward 5'-CAGGTGGTGGCAGTCTTGG-3',

reverse 5’-TGCTTATCCTTTGGGCATTCAA-3’; Ndufa10 forward 5’-ACCTTTCACTACCTGCGGATG-3’, reverse 5’-TACCCAGGGGCATACTTGC-3’; Opa1 forward 5’-CGACTTTGCCGAGGATAGCTT-3’, reverse 5’-CGTTGTGAACACACTGCTCTTG-3’; Pgc-1$\alpha$ forward 5’-TATGGAGTGACATAGAGTGTGCT-3’, reverse 5’-CACTTCAATCCACCCAGAAAG-3’; Ppar$\alpha$ forward 5’-AGAGCCCCATCTGTCCTCTC-3’, reverse 5’-ACTGGTAGTCTGCAAAACCAAA-3’; Ppar$\gamma$ forward 5’-TCGCTGATGCACTGCCTATG-3’, reverse 5’-GAGAGGTCCACAGAGCTGATT-3’; Pln forward 5’-AAAGTGCAATACCTCACTCGC-3’, reverse 5’-GGCATTTCAATAGTGGAGGCTC-3’; Serca2 forward 5’-GAGAACGCTCACACAAAGACC-3’, reverse 5’-CAATTCGTTGGAGCCCCAT-3’; Sirt3 forward 5’-ATCCCGGACTTCAGATCCCC-3’, reverse 5’-CAACATGAAAAAGGGCTTGGG-3’; Sod2 forward 5’-CAGACCTGCCTTACGACTATGG-3’, reverse 5’-CTCGGTGGCGTTGAGATTGTT-3’.

**Statistical analyses**

We used factorial design analysis of variance (ANOVA) or $\tau$-tests to analyze data as appropriate employing GraphPad InStat (version 3.0) and GraphPad Prism (GraphPad Software, version 5.0; La Jolla, CA 92037 USA). Significant ANOVA values were subsequently subjected to simple main effects analyses or *post hoc* comparisons of individual means using the Tukey method as appropriate. We considered $P$ values of $< 0.05$ as significant.