SUPPLEMENTAL MATERIAL

METHODS

Echocardiography

Transthoracic echocardiography was performed on both saline and ISO treated mice before treatment and 21 days after pump implantation. Echocardiography was performed by a single operator who was blinded to the mouse strains and treatment groups using the Vevo 770 high-resolution ECHO system equipped with a 35 MHz transducer. First, chest fur was removed using a depilatory lotion (Nair). Mice were lightly anesthetized with vaporized isoflurane (2.5% for induction, 1.0% for maintenance) in oxygen and lightly restrained in a supine position on a heated pad to maintain body temperature at 37° C. Continuous EKG monitoring was done throughout the imaging studies and heart rates were maintained between 500 and 600 beats per minute. The probe was placed along the short axis of the left ventricle with the papillary muscles providing a guide for the proper depth. 2D images were captured to measure internal wall dimensions during both systole and diastole, as well as providing another measure of the heart rate. Saved images were analyzed by a single operator who was blinded to the experimental design using the Vevo 2100 software. The LV chamber dimensions and posterior wall thickness (PWT) were obtained from M-mode images; LV systolic function was also assessed from these measurements by calculating ejection fraction (EF) and fractional shortening (FS).

Heart weight, body weight, and tibia length collection

After 21 days of saline or isoproterenol treatment, mice from each strain (n=20 per strain) were sacrificed and their body weights were recorded. The heart was removed and, after PBS perfusion, weighed. Additionally, the right tibia of each mouse was removed and measured with a caliper.

Histological analyses (Trichrome staining, scar size measurement, immunohistochemistry)

Freshly isolated tissues were fixed in 4% paraformaldehyde (Electron Microscopy Sciences) in PBS (Fisher Scientific) overnight in 4°C. Afterwards, the tissues were washed with PBS and immersed in a 30% sucrose solution (Sigma) in PBS in 4°C overnight. The hearts were then embedded in Tissue- Tek OCT Compound (Sakura) and transferred to a bath of 2-Methylbutane (Fisher Scientific) on dry ice. Frozen tissues were sectioned at 7 µm thickness using a cryostat (Leica) and stored at -80°C.

Masson's trichrome staining (Sigma) was performed according to the manufacturer's instructions and images were taken of the entire cross-section of the heart using bright-field microscopy (Leica). To assess the degree of cardiac fibrosis, NIH ImageJ software was used by comparing the area of tissue stained blue (collagen) to the total tissue area (10-12 randomly chosen sections per heart, n=7-8 hearts per strains).

For immunohistochemistry, slides were dried at room temperature for 20 minutes, washed 3 times for 10 minutes each in PBS, permeabilized in PBS containing 0.25% Triton X-100 (Fisher Scientific) for 10 minutes at room temperature followed by washing in PBS-T (PBS containing 0.05% Tween-20 (Fisher)) twice for 5 minutes. The tissue slides were incubated with blocking buffer (10% normal goat serum (Sigma) in PBS-T) for 30 minutes at room temperature. The tissue slides were then incubated with primary antibodies **(Supplementary Table I)** diluted in blocking buffer overnight at 4 °C followed by 1 hour at room temperature. After washing three times for 10 minutes with PBS-T, the tissue slides were incubated with the secondary antibody **(Supplementary Table I)** for 1 hour at room temperature, washed three times for 10 minutes with PBS-T, and then mounted with DAPI-containing mounting media (Vector). The immunostained slides were observed and analyzed using a fluorescent microscope (LEICACTR6500, Leica) and a confocal microscope (LSM880, Zeiss).

Isolation of cardiac fibroblasts from murine hearts

Mice were injected with heparin (SAGENT Pharmaceuticals) prior to being euthanized. After euthanasia, the hearts were dissected and perfused with Hanks' Balanced Salt Solution (HBSS). They were cut into small pieces and digested with Liberase Blendzyme TH and TM (Roche) in Medium 199 with DNAse I (Invitrogen) and polaxamer in 37°C for 1h. Cells were passed through a 70μm cell strainer (BD Falcon) and centrifuged. The cell pellet was re-suspended in staining buffer (3% FBS in HBSS) containing antibodies targeting an established panel of surface markers: exclusion of hematopoietic ($CD45^-$[1:200], $Ter119^-$ [1:400]), macrophage ($CD11b^-$ [1:400]), and endothelial ($CD31^-$ [1:400]) lineages, followed by the inclusion of $Thy1^+$ (1:200) cells (hereafter referred to as $Thy1^+HE^-$) **(Supplementary Table I)**. The cells were incubated in the dark for 30 minutes at room temperature. The cells were then sorted by a BD FACSAria II flow cytometer.

BrdU detection by flow cytometry and immunohistochemistry

BrdU (1mg) was injected intraperitoneally on the day of pump implantation. Mice were supplied BrdU in their drinking water (1 mg/mL) for 21 days. The water was changed every two days. Intracellular staining for BrdU was performed in accordance to the instructions of the BD Pharmingen™ BrdU Flow Kit. In short, cells were fixed and permeabilized in Cytofix/Cytoperm Buffer (BD), followed by incubation in Cytoperm Permeabilization Buffer plus (BD) and DNase treatment (BD). The cells were then exposed to fluorescent anti-BrdU antibody, washed, resuspended in staining buffer, and analyzed using a BD FACSAria II flow cytometer. For immunohistochemistry, slides were dried at room temperature for 20 minutes, washed 3 times for 10 minutes each in PBS, permeabilized in PBS containing 0.5% Triton X-100 (Fisher Scientific) for 10 minutes at room temperature, and pre-treated with 2M HCl for 30 minutes at 37°C. After brief washing with PBS, the slides were incubated with blocking buffer (10% normal goat serum (Sigma) in PBS) for 30 minutes at room temperature. The slides were then incubated with anti-BrdU antibody (Abcam) and primary antibodies to mark fibroblasts and activated fibroblasts **(Supplementary Table I)** in 4°C overnight, followed by 15 minutes in 37°C. After washing three

times for 10 minutes with PBS-T, the tissue slides were incubated with the secondary antibody **(Supplementary Table I)** for 1 hour at room temperature, washed three times for 10 minutes with PBS-T, and then mounted with mounting media (Vector). The slides were then imaged by an LSM880 confocal microscope (Zeiss).

Quantitative RT-qPCR

Total RNA from $Thy1^{+}HE^{-}$ cells from control and ISO-treated hearts were extracted using TRIzol® LS Reagent (Ambion) and RNeasy MinElute Cleanup Kit (Qiagen) according to the manufacturers' instructions. The concentration and quality of extracted RNA were measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). cDNA was synthesized using an *iScript*™ cDNA Synthesis Kit (Bio-Rad). For quantitative RT-qPCR, we used an *iTaq*™ *Universal SYBR*® *Green supermix* (Bio-RAD), amplified cDNA and gene-specific primers **(Supplementary Table IV)** on a *CFX96 real-time* PCR detection system (Bio-Rad). PCR conditions included initial denaturation at 95°C for 2 minutes and 10 seconds, 39 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, extension at 72 °C for 30 seconds, followed by a final extension at 72°C for 10 minutes. The mean cycle threshold (Ct) values from triplicate measurements were used to calculate relative gene expressions, with normalizations to GAPDH as an internal control. We used the $\Delta\Delta$CT method to analyze relative gene expression in treated samples compared to non-treated samples. Technical replicates (n=3) and biological replicates (n=7-9) were performed for each strain and condition.

Transverse Aortic Constriction (TAC)

Adult mice weighing 25±5 g were randomly divided into sham and TAC groups (n=4-6 per group) Animals were anesthetized by an intraperitoneal injection of ketamine/xylazine (100 mg/10 mg/kg). Endotracheal intubation was performed using a blunt 20-gauge needle that was then

connected to a volume-cycled rodent ventilator (SAR-830/P; CWE, Inc.) with a tidal volume of 0.2 ml and a respiratory rate of 120/min. The chest was entered in the second intercostal space at the top left aortic arch, the transverse aorta was isolated, and aortic constriction was performed by tying a 7-0 nylon suture ligature against a 27-gauge blunt needle. The needle was then removed to yield a constriction 0.4 mm in diameter. In sham-operated control mice, the entire procedure was identical except that aortic constriction was not performed. The chest tube was used to evacuate the pneumothorax, and it was removed once negative pressure was re-established. The chest was closed in layers using 5-0 Vicryl sutures. Ventilation was maintained until sufficient spontaneous breathing occurred, followed by extubation and removal of the chest tube. The whole surgical procedure was performed under aseptic conditions.

Plasma collection and Enzyme-linked immunosorbent assay (ELISA)

Blood was collected from euthanized mice after saline and ISO treatment into Lithium Heparin Blood Collection Tubes (BD). Plasma was separated from blood using Ficoll-Paque PLUS (GE Healthcare), following the manufacturer's instructions. ELISAs were conducted following the manufacturer's instructions. All samples were measured in duplicate and the calculated concentrations were multiplied by the dilution factor to determine the final concentration. The study was approved by an IRB (12-001164) and participants gave written informed consent.

Supplementary Table I: Antibodies

Antibody	Catalog number	Vendor
Collagen 1 (1:100)	ab34710	Abcam
αSMA (1:100)	A2547	Sigma-Aldrich
PDGFRα (1:100)	Sc-338	Santa Cruz Biotechnology
Periostin (1:100)	AF2955	R&D Systems
LTBP2 (1:200)	Gift from Dr. Marko Hyytiäinen from the University of Helsinki, Finland	
DDR2 (1:100)	MAB25381	R&D Systems
pHH3 (1:50)	ab47297	Abcam
BrdU (1:50)	ab6326	Abcam
Anti-Mouse CD 90.1 APC-eFluor 780	47-0900-82	eBioscience
Anti-Mouse CD 90.2 APC-eFluor 780	47-0900-82	eBioscience
Anti-Mouse CD31 PE-Cyanine7	25-0311-81	eBioscience
Anti-Mouse CD11b PE-Cyanine7	25-0112-81	eBioscience
Anti-Mouse CD45 PE-Cyanine5	48-0451-82	eBioscience
TER-119 PE-Cyanine7	25-5921-81	eBioscience
Donkey Anti-Rabbit Alexa Fluor 647	A-31573	Invitrogen
Rabbit Anti-Mouse Alexa Fluor 647	A-21239	Invitrogen
Goat Anti-Rabbit Alexa Fluor 594	A-11037	Invitrogen
Goat Anti-Rabbit Alexa Fluor 488	A-21206	Invitrogen
Goat Anti-Mouse Alexa Fluor 488	A-11001	Invitrogen

Supplementary Table II: RT-qPCR primer sequences

Gene	Forward Primer	Reverse Primer
Gapdh	TTGTCTCCTGCGACTTCAAC	GTCATACCAGGAAATGAGCTTG
Ltbp2	AACAGCACCAACCACTGTATC	CCTGGCATTCTGAGGGTCAAA

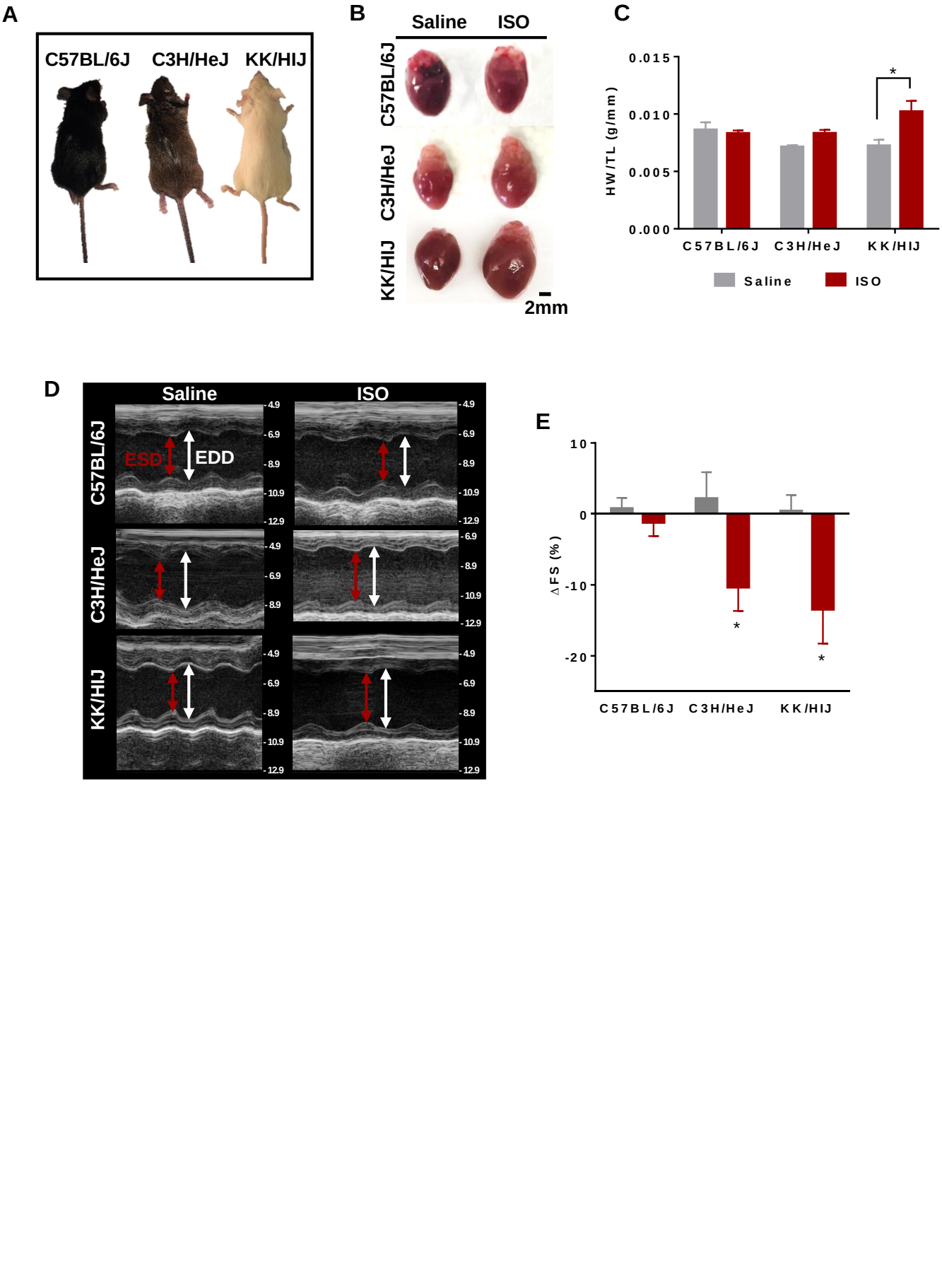
Supplementary Table III: ELISA kits

Marker	Species	Catalog number	vendor
LTBP2	Mouse	MBS2885029	MyBioSource
LTBP2	Human	MBS2882493	MyBioSource

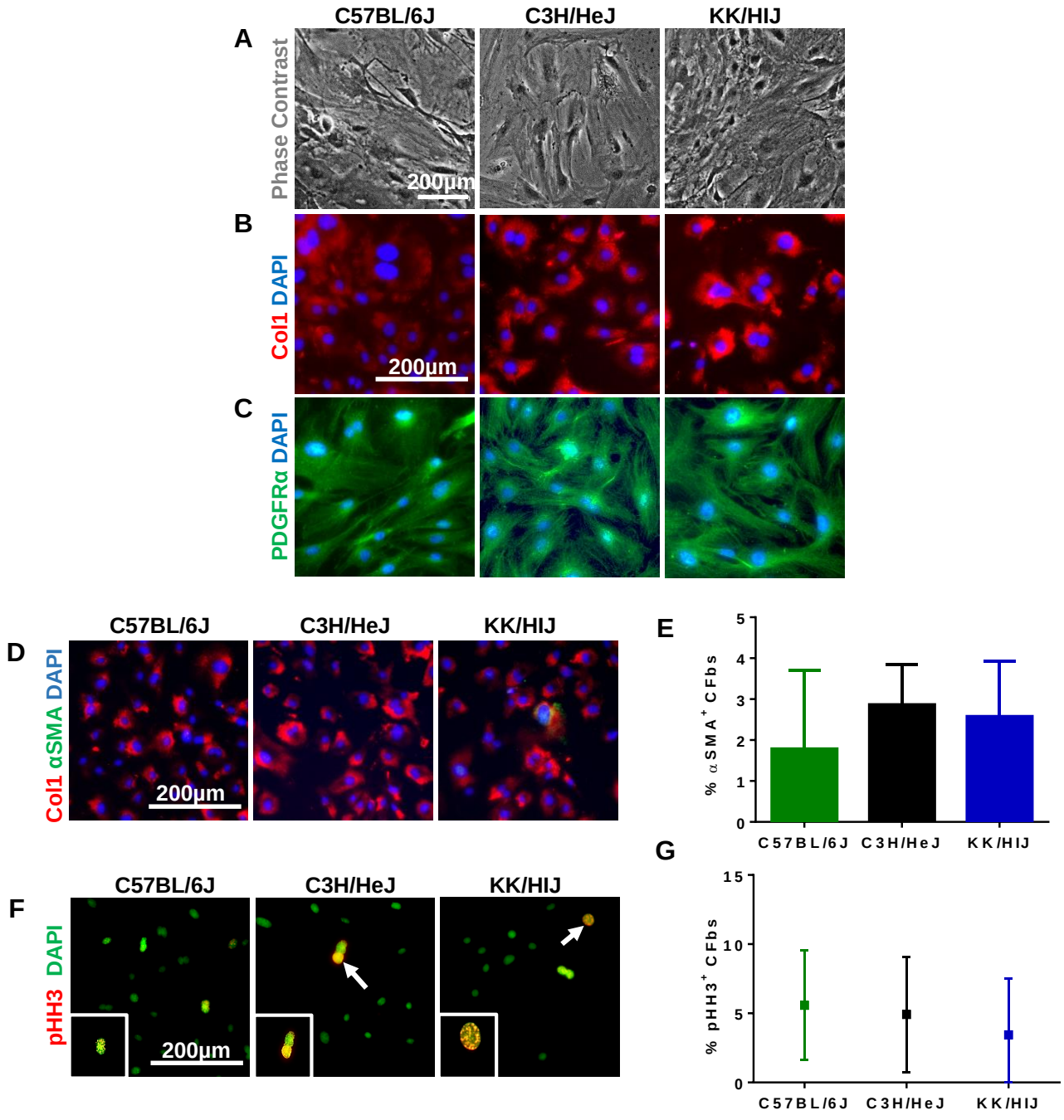
Supplementary Table IV: Characteristics of human subjects

	Age (year)	Gender	Etiology	EF (%)	Median BNP (pg/ml)	DM	h/o HTN	Afib
Healthy individuals	50±10	50% Male 50% Female	N/A	≥55	NA	0%	0%	0%
HF-1	56	Male	Non-ischemic	15%	1719	N	h/O	N
HF-2	48	Male	Non-ischemic	20%	1244	Y	Y	Y
HF-3	59	Female	Ischemic	25%	716	N	N	N
HF-4	70	Male	Ischemic	25%	737	Y	N	N
HF-5	60	Female	Non-ischemic	25%	696	N	N	N
HF-6	64	Male	Ischemic	30%	567	Y	Y	Y
HF-7	53	Male	Ischemic	20%	541	Y	Y	N
HF-8	67	Male	Ischemic	25%	710	Y	Y	N
HF-9	57	Male	Ischemic	20%	707	N	N	N
HF-10	69	Male	Ischemic	25%	1107	Y	Y	N
HF-11	50	Male	Non-ischemic	25%	1671	N	Y	N
HF-12	52	Female	Non-ischemic	35%	554	N	N	N
HF-13	49	Male	Non-ischemic	30%	498	N	N	N
HF-14	59	Male	Ischemic	20%	870	Y	N	N
HF-15	62	Female	Ischemic	25%	1104	Y	Y	N
HF-16	49	Male	Non-ischemic	25%	1009	N	N	N
HF-17	53	Male	Non-ischemic	20%	947	N	N	N
HF-18	57	Female	Ischemic	25%	894	Y	Y	N

Supplementary Figure I



Supplementary Figure II



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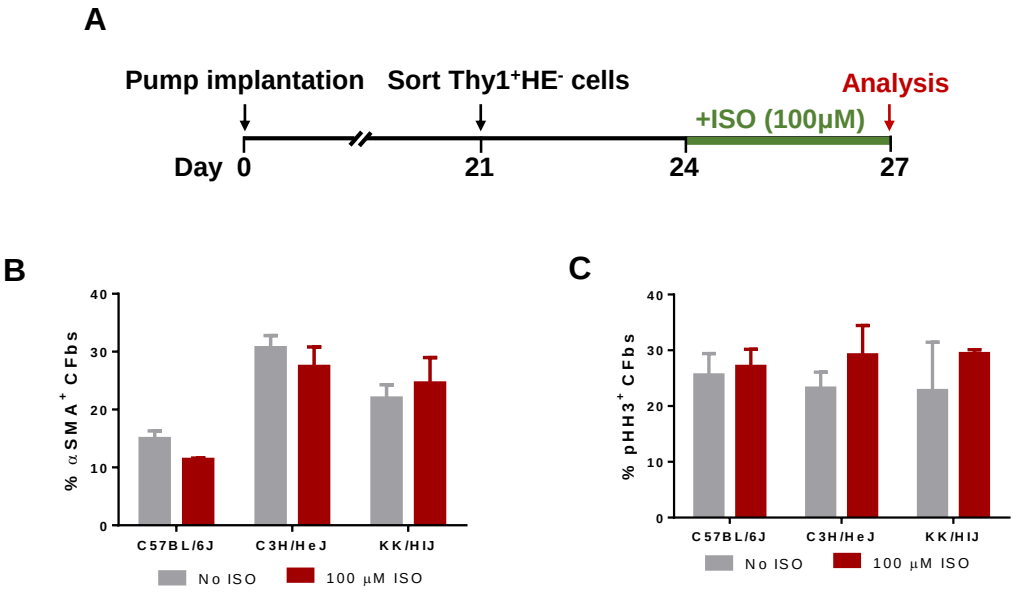
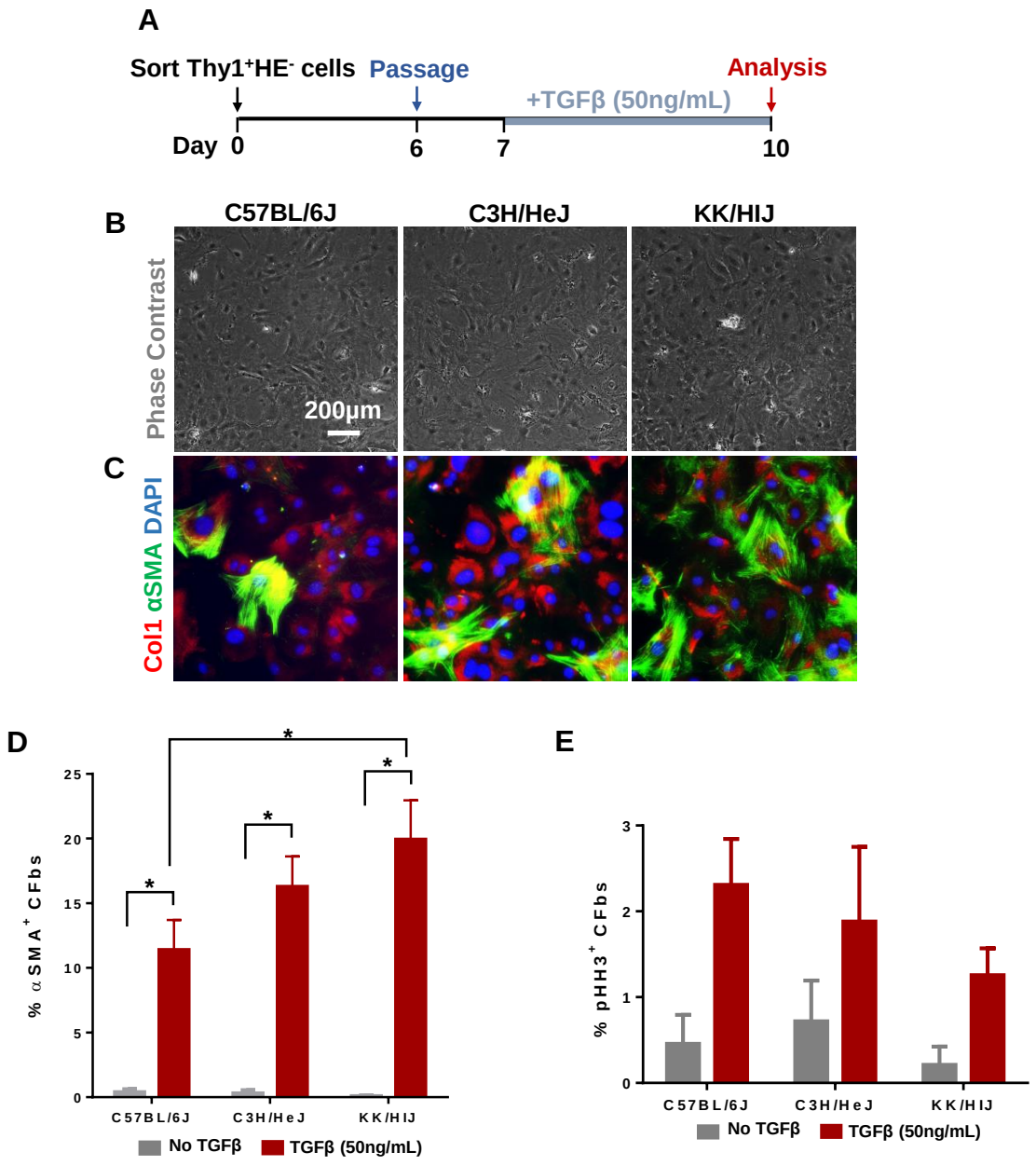
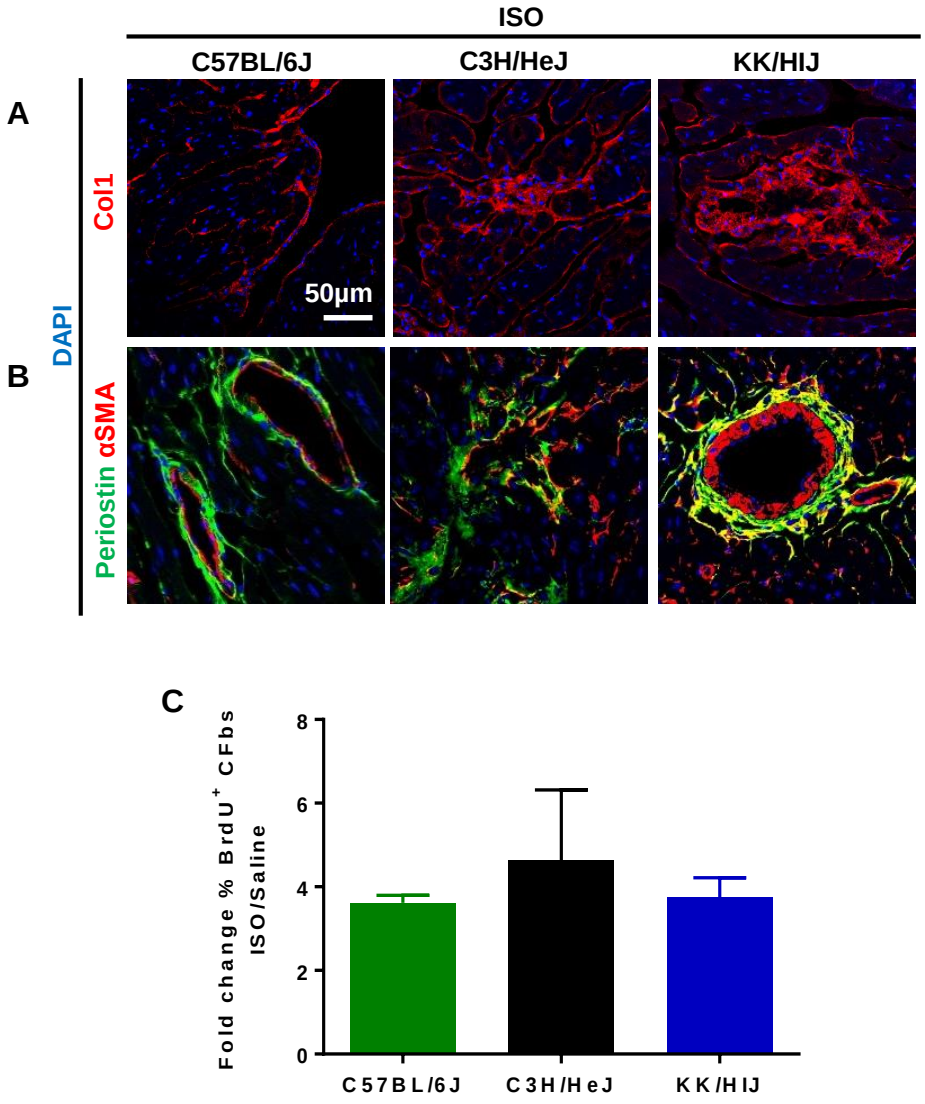


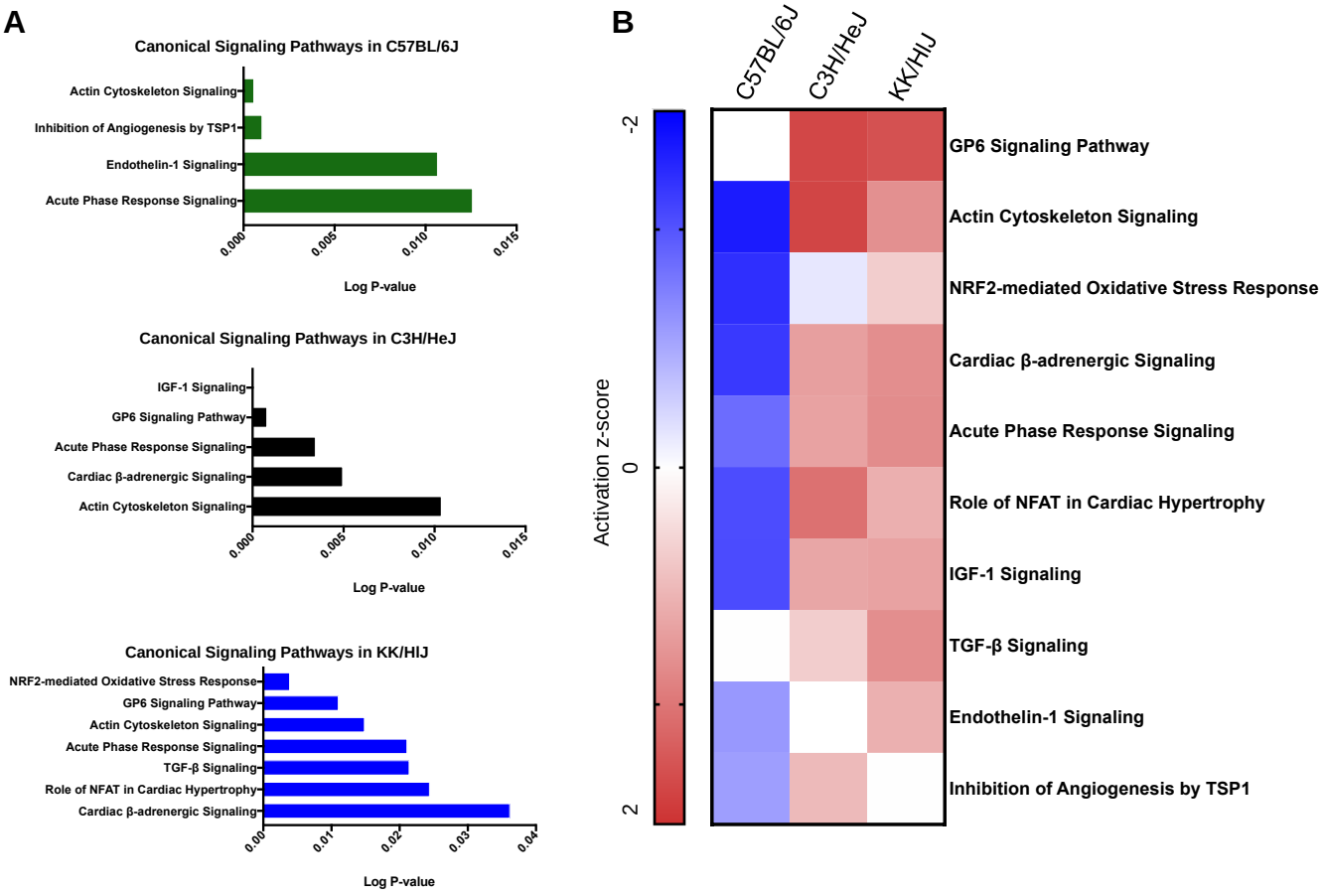
Supplementary Figure IV



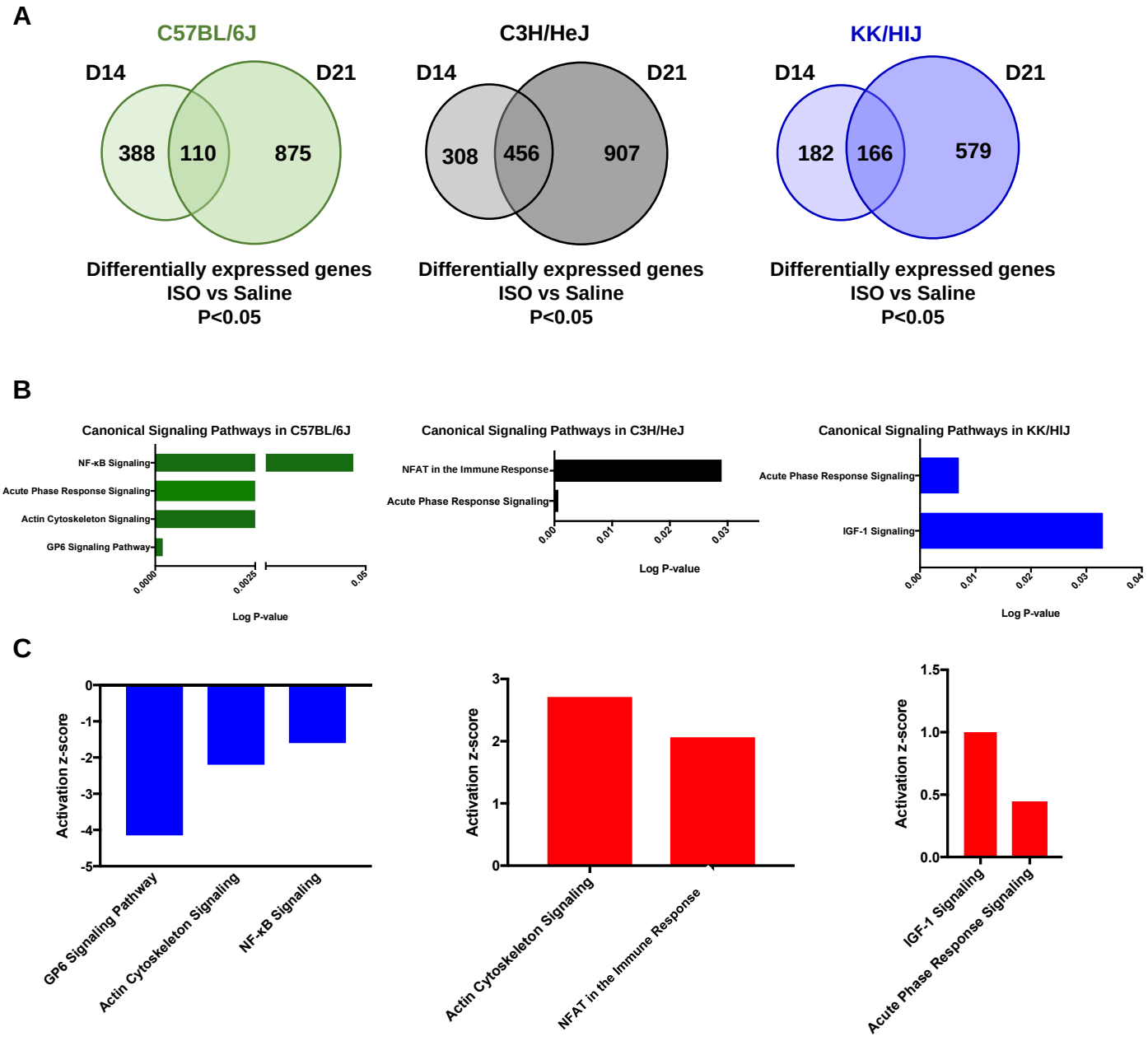
Supplementary Figure V



Supplementary Figure VI



Supplementary Figure VII



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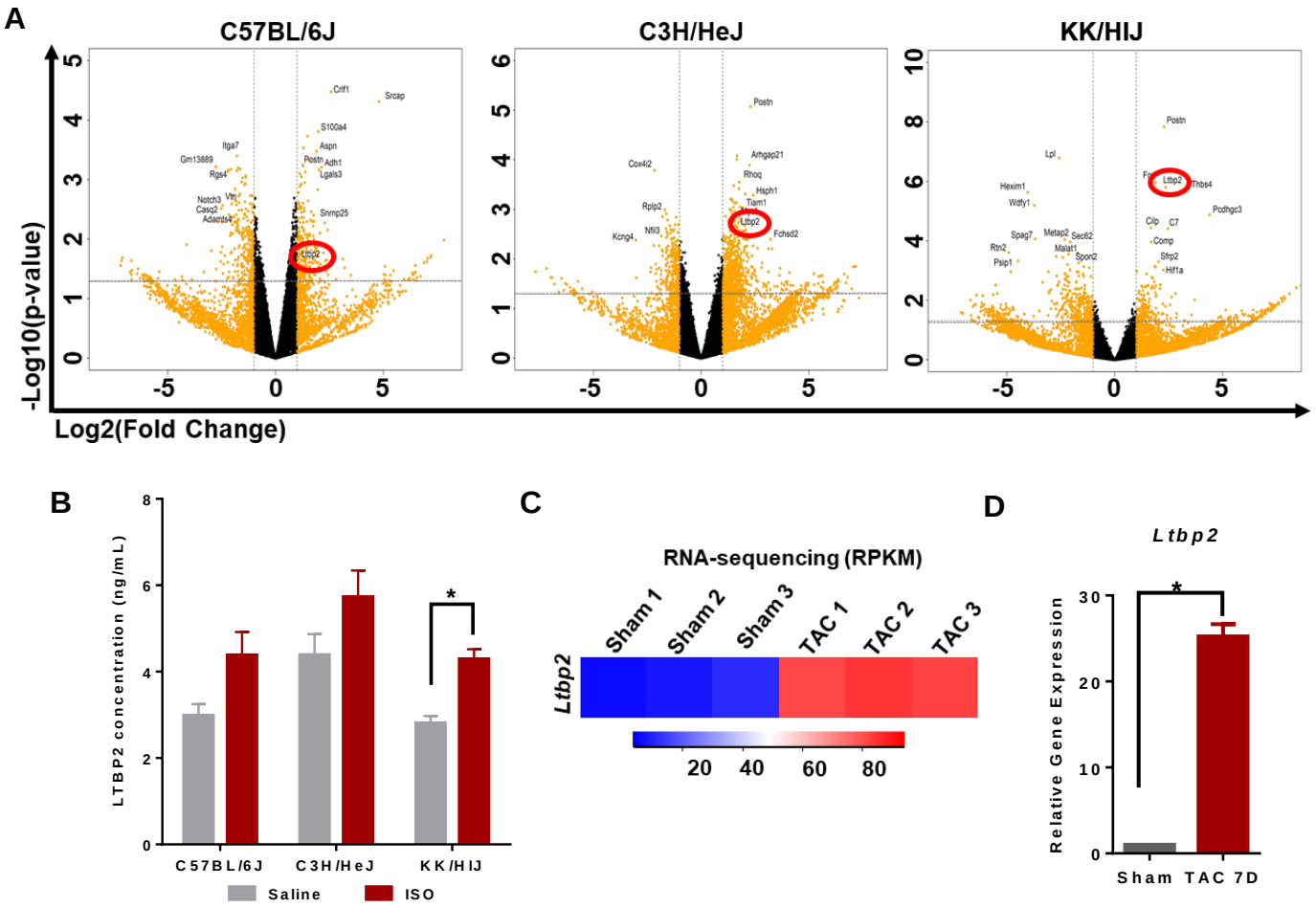
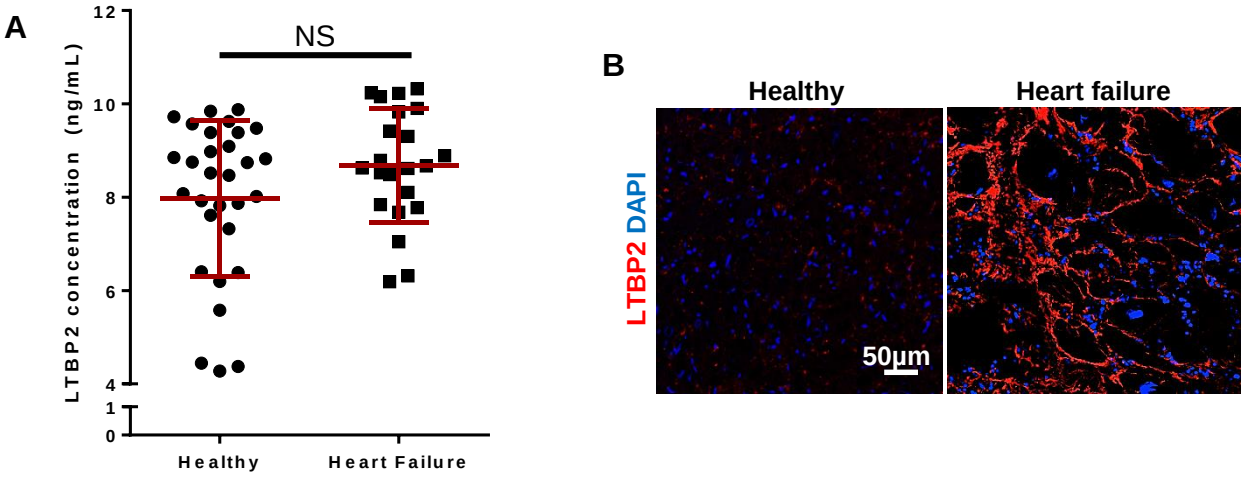


Supplementary Figure IX



Supplementary Figure I. **Severity of cardiac hypertrophy and cardiac dysfunction varies among select strains in response to ISO treatment. (A)** Representative images of adult, female mice (8-12 weeks) from all three strains: C57BL/6J, C3H/HeJ, and KK/HIJ. **(B)** Whole heart images of the different strains after 21 days of saline or ISO treatment (n=6 mice/condition). **(C)** Heart weight/tibia length (HW/TL) ratios after saline or ISO treatment (n=20 hearts/strain). **(D)** Representative M-mode echocardiographic images of hearts from animals following 21 days of saline or ISO treatment (n=20 mice/strain). **(E)** Fractional shortening (FS) was measured by echocardiography in both saline and ISO groups across the different strains (n=20 mice/strain). ESD: end-systolic dimension. EDD: end-diastolic dimension. Data presented as mean ± SEM. Two-way ANOVA, *P < 0.05. Scale bar: 2mm.

Supplementary Figure II. Strain-specific CFbs exhibit similar characteristics in culture. **(A)** Phase contrast images of $Thy1^{+}HE^{-}$ cells from the hearts of the three strains that were isolated by FACS and expanded in culture. Cultured $Thy1^{+}HE^{-}$ cells expressed fibroblast associated markers Collagen1 (Col1) (red) **(B)** and PDGFRα (green) **(C)**. Activated CFbs were marked by co-expression of αSMA (green) and Col1 (red) **(D)**. Rare, activated CFbs were observed in the absence of any stimuli. The bar graph demonstrates the percentage of $Col1^{+}\alpha SMA^{+}$ cells relative to all $Col1^{+}$ cells (n=5 wells/strain/condition) **(E)**. Proliferating CFbs were identified by nuclear staining for phospho-Histone H3 (pHH3) (red) **(F)**. The percentage of $pHH3^{+}$ nuclei was measured relative to total nuclei (green) (n=5 wells/strain/condition) **(G)**. DAPI was used to stain nuclei. Data presented as mean ± SEM. Student t-test. Scale bar: 200μm.

Supplementary Figure III. *In vitro* culture of CFbs isolated from ISO-treated mice exhibit minimal responses. **(A)** Schematic outlining culture of CFbs from mice that have been treated with ISO prior to CFb isolation. *In vitro* ISO treatment had no significant effect on the percentage of αSMA^{+} **(B)** and $pHH3^{+}$ **(C)** cardiac fibroblasts isolated from ISO-treated mice. Data presented as mean ± SEM. Two-way ANOVA.

Supplementary Figure IV. Treatment of CFbs from different strains with TGFβ induces differential responses. (A) Schematic of *in vitro* TGFβ treatment. **(B)** Phase contrast images of CFbs from different strains after TGFβ treatment. **(C)** Immunocytochemistry for Col1 (red) and αSMA (green) was used to identify activated fibroblasts. **(D)** CFb activation was quantified as a percentage of $Col1^{+}\alpha SMA^{+}$ cells relative to all $Col1^{+}$ cells. **(E)** Quantification of CFb proliferation is presented as a percentage of $pHH3^{+}$ nuclei of total nuclei. DAPI was used to stain nuclei. Scale bar: 200μm. Data presented as mean ± SEM. Two-way ANOVA, *P<0.05

Supplementary Figure V. Levels of CFb activation after ISO treatment correlate with extent of collagen deposition, while CFb proliferation does not. (A) Heart sections after ISO treatment were stained for Col1 (red) to visualize extracellular matrix deposition. **(B)** Immunohistochemistry of ISO-treated heart sections stained for periostin (green) and αSMA (red), which both mark activated fibroblasts. **(C)** Fold change of proliferation rates in response to ISO were compared across the different strains. Each ISO-treated group was normalized to their respective control groups within each strain (n=12/strain/condition). DAPI was used to stain nuclei. Data presented as mean ± SEM. One-way ANOVA. Scale bar: 50μm.

Supplementary Figure VI. CFbs from select strains are enriched for various canonical pathways after 21 days of ISO treatment. (A) Significantly enriched pathways from Ingenuity Pathway Analysis (IPA) in each strain after 21 days of ISO treatment. **(B)** IPA was used to generate a list of activated canonical pathways in CFbs treated with ISO compared to saline. Pathways were selected based on the trends that correlated with the extent of fibrosis seen *in vivo*.

Supplementary Figure VII. CFbs from all strains display unique gene expression changes between 14 and 21 days of ISO treatment. (A) Venn diagrams showing number of differentially expressed genes in CFbs from mice that had been treated with ISO for 14 or 21 days compared to CFbs from saline-treated mice. IPA was also used to determine activation

scores **(B)** and canonical pathways that were significantly enriched for **(C)** within each strain between 14 and 21 days of ISO treatment.

Supplementary Figure VIII. LTBP2 is upregulated in multiple injury models in mice. (A) Volcano plots derived from RNA-sequencing data from all three strains depicting differentially expressed genes 21 days after ISO treatment compared to saline-treated. *Ltbp2* is indicated by the red circles. **(B)** LTBP2 protein levels in mouse plasma samples after ISO treatment, measured by ELISA (n=3/strain/condition). RNA-sequencing **(C)** and RT-qPCR analysis **(D)** of *Ltbp2* gene expression in CFbs isolated from C57BL/6J, 7 days post-sham or TAC operation. (n=3/mice/condition). RPKM: Reads Per Kilobase of transcript, per Million mapped reads. TAC: transverse aortic constriction. Data presented as mean ± SEM. Student t-test, *P < 0.05.

Supplementary Figure IX. LTBP2 expression is upregulated in human heart failure patients. (A) Quantification of LTBP2 levels in plasma samples from healthy individuals and heart failure patients by ELISA. **(B)** Heart sections from healthy and failing human hearts stained for LTBP2 (red). DAPI was used to stain nuclei. Data presented as mean ± SD. Student t-test, NS: non-significant. Scale bar: 50μm.