SUPPLEMENTAL MATERIAL

Supplemental Methods

Human and Animal Subject Information:

Human hearts from healthy donors and patients with heart failure were obtained from the tissue bank maintained by the Division of Cardiology at the University of Colorado reviewed and approved by the Colorado Multiple Institutions Review Board. All research involving animals complied with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of University of Colorado.

Plasmids and Cloning

pBabe-X GFP, LacZ, GATA4, Hand2, Mef2c, Tbx5, miR-1, and miR-133 were generated previously [48]. *Trdn-as* and *TRDN-AS* were cloned from cDNA of WT mouse heart or WT hiPSC-CM and later subcloned to pBluescript II SK (+), pBabe-X, pCDNA3.1, pENTR2b, pAd/CMV/V5-DEST and pAAV-MCS vectors. Human cardiac triadin *Trisk32* (ENSG00000186439.14) was cloned from WT hiPSC-CM and subcloned to pBabe-X and pAAV-MCS vectors. pBabe-X or pAd/CMV/V5-DEST-MS2 and pBabe-X or pAd/CMV/V5-DEST-*Trdn-as*-MS2 were subcloned from pCDNA3.1 (-) + SLNCR1 + 12x MS2 bs, a gift from Carl Novina (Addgene plasmid # 86828; http://n2t.net/addgene:86828; RRID: Addgene_86828). pBabe-X or pAd/CMV/V5-DEST-MCP were subcloned from pCDNA3.1 (-) + FLAG-NLS-MS2-eGFP, a gift from Carl Novina (Addgene plasmid # 86827; http://n2t.net/addgene:86827; RRID: Addgene_86827).

5' and 3’ RACE

The 5' and 3’ RACE was performed using the SMARTer RACE 5'/3' Kit (Takara 634858), according to the manufacturer’s instructions. The first-strand cDNA was synthesized using RNA from WT mouse heart tissue or hiPSC-CMs. RACE PCR products were purified by gel extraction and cloned to the pCR-2.1 vector using the TOPO TA Cloning Kit (Invitrogen 450641). At least 10 colonies were picked from each gel band for sequencing to confirm the 5’/3’ end sequence.

Transfection

Cell transfection was performed using Polyethylenimine (PEI, Sigma 408727), or Lipofectamine 3000 (Invitrogen L3000001), FuGENE 6 (Promega E2692) reagents according to instructions provided by manufacturers. Cells were harvested 48 hours post transfection for immunoblotting or immunoprecipitation.

***In vitro* transcription**

MAXIscript™ T7 Transcription Kit (Invitrogen AM1312) was used with 1ug DNA PCR template including T7 promoter sequence for in vitro transcription by following the manufacturer's instruction. RNA products were treated with RNase-free DNase I, proteinase K, and purified with LiCl precipitation.

Isolation of Mouse Embryonic Fibroblasts (MEFs)

Embryonic fibroblasts were isolated as described previously [48]. C57BL/6 pregnant mice with embryos at E13 were purchased from Charles River's laboratory, embryos at E14.5 were harvested, and their internal organs and head were removed. The body below the liver was minced into fine pieces. Minced embryos were incubated with 0.25% trypsin/1 mM EDTA, Phenol Red (Gibco 25200056) for 40 min at 37 °C with 5% CO_2. Cells were cultured in DMEM/High Glucose (HyClone SH30022.02) containing 10% fetal bovine serum (FBS; Gemini 100106), 1.1% Penicillin Streptomycin (Gibco15140-122), and 1.1% GlutaMAX supplement (Gibco 35050079) medium. After 72 hours, MEFs were collected and frozen for later use.

Generation of retroviruses for cardiac reprogramming

Generation of retroviruses and cardiac reprogramming were previously described [48]. Briefly, 12 μg of retroviral plasmid DNA was transfected using FuGENE 6 (Promega E2692) into Platinum E cells (Cell Biolabs RV-101) that were plated on a 10-cm tissue culture dish at a density of 3×10^6 cells per dish, 24 h before transfection. 24 hours after transfection, viral medium was collected and filtered through a 0.45-μm cellulose filter. The filtered viral medium was mixed with polybrene (Sigma TR-1003-G) to a final

concentration of 6 μg/mL before infection. MEFs were infected with retroviruses carrying reprogramming factors, GATA4, Hand2, Mef2C, Tbx5, miR-1, and miR-133. Transduced cells were cultured in in induction medium composed of DMEM/High Glucose:Medium 199 (4:1, HyClone:Gibco), 10% FBS (Gemini), 5% horse serum (Gemini), antibiotics (Gibco), 1X non-essential amino acids (Gibco), 1X essential amino acids (Gibco), 1X B-27 (Gibco), 1X insulin–selenium–transferin (Gibco), 1X MEM vitamin solution (Gibco) and 1X sodium pyruvate (Gibco), 0.5 μM A-83-01 (Tocris 2939) starting at 72 hours post-infection. Induction medium was changed every 2 days.

Generation of recombinant AAVs

Plasmid DNA of pAAV-expression vector, pAAV-DJ, and pHelper (Cell Biolabs VPK-400-DJ) were transfected (1:1:1 ratio) using FuGENE 6 (Promega E2692) into Lenti-X 293T cells (ATCC CRL-11268) grown in DMEM/High glucose (Hyclone SH30022.02) supplemented with 10% FBS (Gemini 100106), 1.1% Penicillin–Streptomycin (Gibco15140-122) and 1.1% GlutaMAX supplement (Gibco 35050079). 72 hours after transfection, cells were harvested with 1X PBS and 0.5 M EDTA. The cells were lysed by 3X freeze and thaw cycles, the lysate was treated with Benzonase Nuclease (Sigma E1014-25KU) at a final concentration of 50 units per mL for 1 h at 37 °C. Supernatant was collected by centrifugation. AAVs were purified with HiTrap heparin columns (GE Healthcare 17040601) and titered by serial dilution followed by infection of HEK293 cells.

Generation of adenovirus

Adenoviruses were generated using the ViraPower Adenoviral Expression System (Invitrogen K493000) according to the manufacturer's instructions. The gene of interests were first cloned into pENTR2B vector (Invitrogen A10463) and later cloned to pAd/CMV/V5-DEST Vector (Invitrogen V49320) with LR Clonase II Enzyme (Invitrogen 11791100) using in vitro recombination. All constructs were linearized and transfected into the 293A cell line (Invitrogen R70507) with Lipofectamine 3000 Transfection Reagent (Invitrogen L3000001). Transfected cells were re-plated into 10 cm dishes to produce crude adenoviral lysate in approximately 10 days. Crude adenoviral lysates were then purified and amplified in 293A cells in 24 to 48 hours to produce the F1 virus. The F1

adenoviruses were titered with Sea-Plaque–agarose assay. Adenoviral infection efficiency into cardiomyocytes or other cell types were tested with optimal MOI based on adenoviral-GFP reporter infection efficiency.

Transcriptomic analysis

Total RNA was extracted using the TRIzol reagent (Invitrogen 15596026) and chloroform. The aqueous layer containing RNA was transferred to a new tube and precipitated using isopropanol, while glycogen (Thermo Fisher R0561) was used to facilitate precipitation if cell number was limited. RNA was pelleted by centrifugation, washed with 70% ethanol, and dissolved in nuclease free H_2O after drying. RNA was treated with DNase I and purified with LiCl precipitation (Invitrogen AM9480). RNA quality was confirmed via analysis on Tapestation (Agilent Technologies, Santa Clara, CA).

Real-Time PCR: cDNA was synthesized using the iScript Reverse Transcription Supermix (Biorad 1708891). qPCR was performed using SYBR PowerUP master mix (Applied Biosystems 4309155) on the StepOne Real-Time PCR System (Applied Biosystems). qPCR primer sequences are listed in **Supplemental Table S1**.

Microarray: A DNA microarray were performed by the Genomics and Microarray Core Facility at the University of Colorado Anschutz Medical Campus using the GeneChip® WT PLUS Reagent Kit (Affymetrix, 2013). The data were analyzed with Affymetrix Microarray Suit version 5.0. The microarray data generated in this study was deposited to the GEO Repository as GSE185370.

RNA-seq: Total RNA was purified from total RNA using oligo(dT) magnetic beads, then fragmented. Library preparation and RNA-seq was performed by the BGI Group (Shenzhen, CN). RNA-seq was performed at a read depth of 40 million reads, using paired-end sequencing. FastQC was used for the raw RNA-seq reads for the control of sequence quality, GC content, the presence of adaptor sequences and duplicated reads and ensure homogeneity of sequencing reads between samples. The reads passed quality control were aligned to GRCm38/mm10 mouse reference genome with the splice-aware STAR aligner v2.7. The read counts were generated from the aligned reads with featureCounts function in the RsubRead package. The read counts were normalized with

DESeq2 R package. The RNA-seq data generated in this study was deposited to the GEO Repository as GSE185522.

Fluorescence in situ Hybridization (FISH) and Single molecule FISH (smFISH)

Conventional RNA Fluorescence in situ hybridization was performed with minor modifications of a previously described FISH protocol [49]. Briefly, Digoxigenin-11-UTP (Sigma 3359247910) labeled RNA sense or antisense probes approximately 200 nt long were produced through in vitro transcription with the MAXIscript T7 Transcription Kit (Invitrogen AM1312). After DNase (Invitrogen AM2238) treatment, probes were purified using LiCl (Invitrogen AM9480) precipitation and stored at -80 °C. Cells were fixed in 4% paraformaldehyde at room temperature for 15 min and permeabilized with 0.2% Triton-X in 1X DPBS. 100-200 ng/mL DIG labeled RNA probes were used in the hybridization mix, containing 50% formamide, 2X SSPE, 1 mg/mL tRNA, 5X Denhardt's Solution (Invitrogen 750018), and 5 units/50 µL RNase OUT (Invitrogen LS10777019). Hybridization was performed in a humidity chamber for 12-16 h at 68 °C. After hybridization, cells were washed with 50% formamide-diluted DPBS at 68°C 6 times, for a total of 1 hour. Cells were then switched to DPBS and blocked with 1X Roche blocking solution in 1X DPBS at room temperature for 30 min. Anti-Digoxigenin-POD (Roche 11 207 733 910) antibody was used at a dilution of 1:600, diluted in 1X blocking solution at room temperature for 1 hour. Cells were then washed with DPBS 3 times. Cells were then incubated with secondary antibody Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) (1:800, Invitrogen A-11029) in 1X blocking solution for 45 minutes at room temperature. Nuclei were then stained with Hoechest 33342 (Invitrogen 62249) for 10 min at room temperature in DPBS. Digoxigenin labeled probe was detected using the Cyanine 3 Plus Amplification Reagent (Perkin Elmer NEL744001KT), 1:600 in 1X Plus Amplification Diluent (Perkin Elmer NEL744001KT) at room temperature for 10 min. Primer sequences to generate DNA templates for in vitro transcribed probes are listed in **Supplemental Table S2**.

smFISH was performed with the Stellaris RNA FISH system (LGC Biosearch Technologies). A set of 30 oligonucleotides anti-sense to *Trdn-as* in **Supplemental Table S2**, individually labeled with Quasar570, were designed with LGC Biosearch Technologies' Stellaris RNA FISH Probe Designer. Briefly, cells were cultured in a

chamber slide with a removable 12 well silicone chamber for cell culture (ibidi 81201) and fixed in 3.7% formaldehyde in 1X PBS 10 min at room temperature. After DPBS wash, cells were permeabilized with 0.1% Triton X-100 in 1X PBS for 5 minutes at room temperature. Immunofluorescence staining was then performed with 1:400 diluted SRSF1 (Invitrogen 32-4500) or with 1:200 diluted SRSF10 primary antibody (Invitrogen PA5-72926) in 1X PBS for 1 hour at room temperature. Cells were then washed with 1X PBS three times, followed by incubation with secondary antibody Alexa Fluor 488 Goat Anti-Mouse IgG (H+L) (Invitrogen A-11029) or Alexa Fluor 488 Goat Anti-Rabbit IgG (H+L) (Invitrogen A-11034) diluted 1:800 in 1X PBS for 1 hour at room temperature. After washing with 1X PBS three times, cells were incubated with fixation buffer again for 10 min at room temperature and washed with 1X PBS an additional three times. For smFISH, cells were incubated in 1X Wash Buffer A with 10% formamide (Biosearch Technologies Cat# SMF-WA1-60) at room temperature for 2-5 minutes, then a humidified chamber for hybridization was assembled with cells incubated in 10% formamide containing Hybridization Buffer (Biosearch Technologies Cat# SMF-HB1-10). 1 µL of probe stock solution (125nM) was used in 100 µL of Hybridization Buffer and cells were then incubated in the dark at 37 °C for 16 hours. After hybridization, cells were washed with wash buffer A in the dark at 37 °C for 30 minutes twice, with Hoechst 33342 (Invitrogen 62249) stain added to the second wash for nuclear staining. Cells were then washed with Wash Buffer B (Biosearch Technologies Cat# SMF-WB1-20) at room temperature for 2-5 minutes. Glass slides were then sealed using ProLong Gold Antifade Mounting reagent (Thermo Fisher P10144), after removing the silicone chamber and replacing it with a coverslip. Slides were then cured for 24 to 48 hours in the dark at room temperature prior to imaging. For imaging, a standard cooled CCD camera equipped Olympus IX81 DSU was used with deconvolution and Micro-Manager Software Version 2.0-gamma (ImageJ).

Immunocytochemistry

Cells were fixed in 2% paraformaldehyde for 15 min on ice and then washed with DPBS (Coring 45000-434) three times at room temperature. The cells were permeabilized in 0.5% Triton X-100 diluted in in PBS for 20 min at room temperature. Cells were then blocked in DPBS with 10% FBS for 30 min at room temperature, followed by incubation with primary

antibodies against cTnT (Thermo Fisher Scientific MS-295-P, 1:400), SRSF1 (Invitrogen 32-4500, 1:400), SRSF10 (1:200, Invitrogen PA5-72926), or FLAG (UBPBio Y1101, 1:400) in 10% FBS (Gemini 100106) in DPBS for 1 hour at room temperature. Cells were washed with DPBS three times, then incubated with the secondary antibody (Alexa Fluor 555 Molecular Probes A21422, 1:1,000) and Hoechst 33342 (Invitrogen 62249, 1:5,000) for 30 min at room temperature.

Western Blot

Cells were washed with ice-cold 1XDPBS (Corning 45000-434) twice. Whole cell extracts were harvested with ice-cold lysis buffer (150 mM NaCl, 50 mM Tris-Cl pH 7.4, 1 mM EDTA, 1% Triton, and freshly added protease inhibitors (Complete mini tablet (Roche11836153001) and 1 mM phenylmethylsulphonyl fluoride (PMSF, Roche 10837091001)). Protein concentration was determined by BCA assay (Thermo scientific 23227) and 10 to 30 µg protein lysates were loaded in a 10% polyacrylamide gel and later transferred to a PVDF membrane (110 mAmp, 16 hours, 4 ˚C). For dephosphorylation of protein extracts, 30µg total lysate was treated with 5U of Antarctic Phosphatase (New England Biolabs M0289S) in 1X phosphatase buffer at 37 ˚C for 30 min before loaded in the polyacrylamide gel. The following primary antibodies were used: anti-Trdn (Rabbit, Invitrogen PA5-63535, 1:1000), anti-SRSF1 (Mouse, Invitrogen 32-4500, 1:1000), anti-SRSF10 (Rabbit, Invitrogen PA5-72926, 1:500), anti-Calnexin (Sigma C4731-25UL, 1:4000), anti-Flag HRP (Sigma A8592-0.2mg, 1:4000), and anti-GAPDH (Life Technologies AM4300, 1:5000). Following primary antibody incubation, membranes were incubated with the following secondary antibodies: Goat Anti-Mouse IgG (H+L; Southern Biotech, 1031-05, 1:2,000) or Goat Anti-Rabbit IgG (Life Technologies, 65–6120, 1:2,000). Signal intensities were quantified by densitometry using ImageJ software.

Electrophoretic mobility shift assay (EMSA) for detecting RNA-RNA interaction

Single-stranded RNAs were generated using MAXIscript™ T7 Transcription Kit (Invitrogen AM1312) and purified as described above. EMSA was performed as previously described [50]. Briefly, in vitro transcribed (IVT) RNAs were refolded by incubating for 1 min at 90 ˚C in water, followed by chilling on ice for 2 min. Single or

combination of IVT RNAs were then incubated in 1x TMN buffer (20 mM Tris–acetate, pH 7.6, 100 mM NaOAc, 5 mM $Mg(OAc)_2$) at 37 ˚C for 15 min before loaded in 0.8% agarose/TBE gel for electrophoresis. The gel was stained with SYBR Gold nucleic acid gel stain (Invitrogen S11494) according to the manufacturer's protocol. Stained gel was illuminated and photographed by using Alpha Innotech FluorChem 8900 gel imaging system.

RNA immunoprecipitation (RIP)

For adherent cells, cells were washed with DPBS (Corning) twice, scraped into Eppendorf tubes, and pelleted by centrifugation. Whole cell extracts were prepared with ice-cold lysis buffer (150 mM NaCl, 50 mM Tris-Cl pH 7.4, 1 mM EDTA, 1% Triton, and freshly added inhibitors (Complete mini tablet (Roche),1 mM phenylmethylsulphonyl fluoride (PMSF), RNaseOUT (1:200) (Invitrogen LS10777019), TCEP (1:500) (Thermo Fisher 77720) and Halt Protease and Phosphatase inhibitor cocktail to 1X (Thermo Fisher 78443)). For nuclear extraction, the NE-PER kit (Thermo Fisher 78835) was used according to manufacturer's instructions, using the same inhibitors as for whole cell lysates. Lysate concentrations were determined using the BCA protein assay. To perform the RNA immunoprecipitation, 250 to 500 µg (for nuclear extracts) or 1 mg (for whole cell extracts) protein was diluted in RIP buffer (150 mM KCl, 25 mM Tris pH 7.4, 5 mM EDTA, 0.5 mM DTT,0.5% NP-40) for each immunoprecipitation (IP).

For the in vitro transcribed RNA binding assay, RNA was thawed and denatured at 90 ºC for 2 minutes, then incubated on ice for 2 minutes. 1 µg RNA was added to 10 µL RNA structure buffer (10 mM Tris-HCl pH 7, 100 mM KCl, 10 mM $MgCl_2$) and incubated at room temperature for 15 minutes. RNA was then added to IP lysates, and 2% of the IP volume was saved for input samples.

For each IP, 4% of total input was saved. 2.5 µg Anti-SRSF1 (Invitrogen #32-4500), 5 µg Anti-FLAG (UBPBIO Y-1101), 5 µg Anti-GFP (UBPBIO Y-1031), or 5 µg mouse IgG (Santa Cruz sc-2025) were added to each IP and rotated at 4 ºC for 2 hours. 20 µL Protein G Dynabeads (Invitrogen 10004D) was then added to each IP and rotated at 4 ºC for 1 hour. Beads were separated on a magnetic rack, and solution was gently aspirated. Beads were then washed with 500 µL cold RIP buffer 4 times. After the final wash, the

beads were harvested by adding 1 mL TRIzol for RNA extraction or adding 40 µL 1X Laemmli sample buffer to the beads and boiled for 15 minutes for protein SDS-PAGE.

For RIP from heart tissue, hearts were rinsed with sterile PBS to remove excess blood from samples. Tissue was finely minced into ~1mm chunks with a razor blade. Tissue chunks were then stored in RNAlater Reagent (Sigma R0901-100ML) and incubated overnight at 4 ºC. After sample preservation in RNAlater tissue pieces were removed from RNAlater, briefly dried on a tissue, and nuclei were isolated using the Nuc101-1KT kit (Sigma NUC101-1KT) with Nuclear EZ lysis buffer supplemented with freshly added protease, phosphatase, and RNase inhibitors. Nuclei were pelleted by centrifugation, the supernatant was removed by aspiration, and 500 µL Nuclear lysis buffer (Thermo Fisher Scientific 78835) supplemented with freshly added inhibitors was added to pelleted nuclei. Nuclei were resuspended completely by pipetting. For complete lysis, the nuclei solution was incubated on ice for 40 minutes, vortexing every 10 minutes, followed by centrifugation at 11000xg for 20 minutes at 4 ºC. The supernatant was collected in a new tube and concentration was measured by BCA assay. Immunoprecipitations were then performed as described above with 2mg protein per IP and double the antibody and Dynabeads volumes. Additionally, IP samples were rotated at 4 ºC overnight.

Isolation of mouse cardiomyocytes

Neonatal mouse cardiomyocytes (NMCMs) were isolated as previously described [46]. Briefly, pups were rinsed quickly in 70% ethanol solution and decapitated. Hearts were removed from 1- to 3-day-old mouse pups and ventricles were minced in CBFFHH buffer (NaCl 8 g/L, KCl 0.4 g/L, $MgSO_4$•7 H_2O 0.2 g/L, glucose 1 g/L, KH_2PO_4 0.06 g/L, Na_2HPO_4 0.09 g/L, HEPES 4.766 g/L, pH 7.5) supplemented with Heparin (500 µL in 50 mL CBFFHH, Henry Schein, Cat# 1184434). Minced tissue was digested in CBFFHH trypsin digestion buffer (300 mg trypsin/200 mL CBFHH buffer). Isolation was performed in a 50 mL tube, gently stirred with a small magnetic stir, and cells were collected in a 2-hour window of repeated digestion followed by pre-plating for 45 min at 37 °C, 1% CO_2 to separate non-myocytes. NMCMs which did not attach during pre-plating were collected and plated on gelatin-coated plates or Laminin (Corning 354232) coated 12 well silicone

chamber plates (Ibidi 81201) in MEM (Hanks' Balanced Salts, Gibico 11575032) containing 5% bovine calf serum and vitamin B12. After 24 hours, the medium was changed to DMEM/199 medium (Life Technologies 11150-059) supplemented with 2% horse serum and 1% penicillin/streptomycin.

Adult mouse cardiomyocytes (AMCMs) were isolated as described previously [26, 27]. Mice were anesthetized with isoflurane and killed via cervical dislocation. The chest was then opened, the heart removed rapidly and placed in ice-cold oxygenated perfusion buffer (120 mM NaCl, 15 mM KCl, 0.6 mM Na_2HPO_4, 0.6 mM KH_2PO_4, 1.2 mM $MgSO_4$, 10 mM HEPES, 10 mM creatine monohydrate, 30 mM Taurine, 5.6 mM D-glucose, 4.6 mM $NaHCO_3$, and 10 mM BDM, pH 7.4, filtered using a 0.40 μm filter prior to use). The heart was washed briefly in perfusion buffer, trimmed of fat, and was then rapidly cannulated (within 3-5 minutes of removing the heart) using a blunted 20-gauge needle and was mounted on a perfusion flow apparatus with a heating pump (VWR) that used gravity to drive oxygenated buffer heated to 37 °C into the heart. The buffer reservoir was placed approximately three feet above the cannulation needle and heating apparatus. The heart was initially perfused for 3 min using perfusion buffer, followed by calcium-free digestion buffer (perfusion buffer with 2.4 mg/mL collagenase II) for 3 min and then by digestion buffer supplemented with 28 nM $CaCl_2$ for 6–10 min. After this time, the heart was removed from the cannulating needle and placed in calcium-containing digestion buffer. The atria and right ventricle were quickly removed, and the ventricle was dissociated using pipette. Digestion was then halted by mixing the dissociated cells with stopping buffer (perfusion buffer supplemented with 10% FBS, 12.5 μM $CaCl_2$ and 2mM ATP). Myocytes then were filtered through 200-μm mesh into a 50-ml conical tube followed by incubation at RT to allow the pellet to form. The cell pellet was washed three times for 3 minutes each wash in perfusion buffer containing increasing concentrations of $CaCl_2$ (100 μM, 400 μM, and 900 μM respectively). Myocytes were then visually inspected under a microscope, and only isolations resulting in more than 60% rod-shaped myocytes were used. Myocytes were resuspended in plating medium (perfusion buffer supplemented with 2.5% FBS, 2% penicillin/streptomycin, 1.4 μM $CaCl_2$, and 2 mM ATP) and plated on dishes coated with 9–10 μg/mL Laminin (Corning). After incubation in a cell-culture incubator for at least 1 h in plating medium, myocytes were then washed with

PBS and incubated in myocyte culture medium (MEM supplemented with 0.2% (wt/vol) BSA, 10 mM HEPES, 4 mM $NaHCO_3$, 10 mM creatine, 0.5% insulin-selenium-transferrin, 25 μM Blebbistatin, pH 7.4) at 37°C with 2% CO_2. Prior to and during isolation, buffers were bubbled with oxygen to supply cells with oxygen during isolation.

Analysis of Ca^{2+} transients

For Ca^{2+} transient analysis using the IonOptix system, AMCMs were loaded with the ratiometric calcium dye Fura-2-AM (F2AM, Invitrogen F1221). F2AM was stored in aliquots at a stock concentration of 1 mg/mL in DMSO at -20°C. Cardiomyocytes were washed 1X in warm filtered 1X Tyrode's solution (140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4, 37°C). For SR calcium load experiments, NES buffer (137 mM NaCl, 5.9 mM KCl, 1.2 mM CaCl2, 1.2 mM MgCl2, 11.6 mM HEPES, 1.2 mM dextrose) was used instead of Tyrode's. Cells were then incubated in F2AM diluted 1:500 with Pluronic F127 (Invitrogen P3000MP, to enhance F2AM loading) in 1X prewarmed Tyrode's solution, for 10 minutes at 37°C. During incubation, cells were protected from light by wrapping in foil. Cells were then washed 3 times with 1 mL Tyrode's solution, and then incubated in 2 mL 1X Tyrode's solution at 37°C for 30 min in the dark to allow optimal Fura-2 loading. Ca^{2+} transients were recorded using 1 Hz field stimulation at 40V, both at baseline and after 100 nM isoproterenol (ISO) treatment. For ISO treatment, cells were incubated with ISO for 10 minutes prior to recording Ca^{2+} transients. SR Ca^{2+} content under adrenergic stimulation was measured in the presence of 10 mM Caffeine and 100 nM ISO. Cells were kept in Tyrode's solution or NES buffer while acquiring data. Approximately 40 to 60 transients were recorded per cardiomyocyte assessed, while for determination of SR calcium load, a single caffeine-induced transient was measured per cell. Transients were recorded from each plate for a total of 30 minutes. Transients analysis was performed using IonWizard (IonOptix) as previously described [51].

For Ca^{2+} analysis with the GCaMP system, AMCMs were infected with adenoviral-GCaMP6f (SignaGen Laboratories SL101144, MOI=5) after isolation and plating. After 72-hour culture with medium changes every 24 hours, cells in 48-well plates were imaged both at baseline and after 100 nM ISO treatment using a 3i Marianas Spinning Disk

Confocal with the microscope stage heated to 37°C. Time-lapse imaging data was exported with Slidebook6 (3i) and later processed to ImageJ-FIJI for signal intensity analysis. The raw time-lapse intensity data were further analyzed with by MATLAB for analysis of Ca^{2+} transient amplitude.

Subcellular fractionation of heart tissue and cardiomyocytes

hiPSC-CMs were pelleted and washed with PBS twice. Nuclear and cytosol fractions of hiPSC-CMs were prepared with NE-PER Nuclear and Cytosolic Extraction Kit (Thermo Fisher Scientific 78835) in the presence of inhibitors (Complete mini tablet,1 mM PMSF, RNaseOUT (1:200)), according to the manufacturer's instruction. Mouse heart tissue was homogenized with Bullet Blender® Homogenizer (Adaptas Solutions BBX24) in 1XPBS added with inhibitors (Complete mini tablet,1 mM PMSF, RNaseOUT (1:200)). Nuclear and cytosolic fractions were prepared with Nuclei EZ Prep Kit (Sigma Cat# NUC101-1KT). The nuclear and cytosol fractions were harvested in 1ml of TRIzol reagent (Invitrogen 15596026). RNA was extracted and treated with DNase I and purified with LiCl precipitation (Invitrogen AM9480). cDNA was synthesized using the iScript Reverse Transcription Supermix (Biorad 1708891). qPCR was performed using SYBR PowerUP master mix (Applied Biosystems 4309155) on the StepOne Real-Time PCR System (Applied Biosystems). qPCR primer sequences are listed in **Supplemental Table S1**.

The cytoplasmic, soluble nuclear and chromatin fractions of adult mouse cardiomyocytes (AMCMs) isolated from wild type mice were prepared with Subcellular Protein Fractionation Kit (Thermo Fisher Scientific 78840) based on the volume of the cell pellet following the manufacturer's instruction. Each fraction was harvested in 1ml of TRIzol reagent (Invitrogen 15596026). RNA was extracted and treated with DNase I and purified with LiCl precipitation (Invitrogen AM9480). cDNA was synthesized using the iScript Reverse Transcription Supermix (Biorad 1708891). qPCR was performed using SYBR PowerUP master mix (Applied Biosystems 4309155) on the StepOne Real-Time PCR System (Applied Biosystems). qPCR primer sequences are listed in **Supplemental Table S1**.

hiPSC culture and hiPS-CM differentiation

This project utilized cardiomyocytes differentiated from the CUSO-2 hiPSC line [52] generated in Song lab from NHDF-Ad-Der Fibroblast cells, CC-2511, Lonza) to study the LncRNA function. Cardiomyocyte differentiation was performed as described previously [52], and induction efficiency was evaluated by flow cytometry on cells stained with cardiac troponin T. Briefly, when hiPSCs reached ~85% confluence, single cell dissociation was performed using Accumax (Innovative cell technologies AM105) at 37°C for 5 min and replated to a 24-well plate pre-coated with MatriGel (Corning 354277). Cells were cultured in 1 mL of mTeSR1 per well for 4 days before induction with daily media changes. At day 0, medium was changed to RPMI1640 (Life Technologies 11875093) with B27 supplement minus insulin (Life Technologies A1895601) plus 8 μM ChIR99021 (Cayman 252917-06-9). On day 1 (24h), medium was changed to RPMI1640 with B27 supplement minus insulin. On day 3 (72h), media was replaced with combined medium (made by combining 0.5 mL of conditioned cell medium, with 0.5 mL fresh RPMI1640/B27 supplement minus insulin, supplemented with 5 μM IWP2 (Tocris 3533/10). On day 5, media was replaced with fresh RPMI1640/B27 supplement minus insulin. On day 7, medium was replaced with RPMI1640/B27 supplement (Life Technologies 11875093/A1895601). On day 20, hiPSC-CMs were enriched by lactate selection, using glucose free DMEM supplemented with 4 mM lactate, for 5 days. Cells were then maintained in RPMI1640/B27 medium with medium changed every 3 days.

Trdn-as knockout mice

The C57BL/6J CRISPR knockout (KO) mouse line was generated from wild type C57BL/6J embryos at the Genetics Core Facility of National Jewish Health by CRISPR-Cas9 mediated deletion of exons 2 and 3 of *Trdn-as* using the following sgRNAs: 5’ TTTGCTTTTTGGGTAATCTCTGG 3’ and 5’ CTTGAGCTAGCCTTATACATAGG 3’. Two founder lines were later bred in the animal facility at the University of Colorado Anschutz Medical Campus with wild type C57BL/6J animals purchased from Jackson Labs to produce individual homozygous KO animals. KO mice were genotyped with primers specific for the deleted region, 5’ AGAGCTAGACAGTGGTCTCTT 3’, 5’ TCCTTAGGTTTGCTCTTCTCATC 3’, and 5’ CTGTTTGTCTTCTACACTTATCCTTTAC

3’. Top candidate sgRNA off-target loci were sequenced to ensure no additional mutations were induced by the CRISPR KO strategy (**Supplemental Table S3**).

Mouse exercise on the treadmill

The mouse treadmill study was performed as previously described [53]. Briefly, mice were trained on a treadmill (Columbus Instruments, incline 5.8°) for three days to adapt to treadmill exercise. A speed of 15m/min was initially used for 3 min and increased to 25m/min gradually over 1 min, with less than 5 min total exercise time per day. For the treadmill test, mice ran on the treadmill with a warm-up speed of 5 m/min for 4 min, followed by 15 m/min for 1 min, and 25m/min for 1 min. During every subsequent 1 min interval, the speed was increased by 3 m/min until the mouse was exhausted. Exhaustion was defined as the inability of the mouse to return to running within 10 s of direct contact with an electric-stimulus grid. Running time was measured and running distance was calculated.

Echocardiography and Electrocardiography measurements

To study roles of *Trdn-as* in cardiac function, we performed echocardiography on WT and knockout mice at the age of 5 months to 12 months. After weighing the animals, anesthesia was induced with 3% isoflurane and then maintained with 1.5% isoflurane during the echocardiography procedure. The animals was laid supine on a warming pad to maintain body temperature at 37 degrees Celsius. The chest was shaved and covered with warm ultrasound gel. Echocardiography images were obtained while the probe was held gently against the chest.

Baseline electrocardiogram (ECG) were recorded using easyTEL + M_EPTA™ transmitters (emka Technologies) (**Table S4**). Mice were anesthetized under general gaseous anesthesia (2-2.5% isoflurane/O2). Anesthetized mice were placed on a heating pad and chest hair was removed with depilatory cream. The electrodes were placed subcutaneously in the region of the right shoulder (negative lead) and near the lower left chest (positive lead). ECG recordings were collected for 5-10 min at a sampling rate of 2000 Hz. At least 500 PQRST cycles were analyzed for each mouse. QT duration was defined as the interval between the first deviation from isoelectric PR interval (Q wave)

until the return of the ventricular repolarization to the isoelectric baseline [54]. QT was corrected with the formula of Mitchell *et al.* [55] using the average RR interval for that mouse. To examine ECG features under adrenergic stimulation, we performed Lead II ECG studies in WT and *Trdn-as* knockout animals (>6-month-old-male) as described previously (Sysa-Shah et al., 2015). ECGs were recorded from 1.5% isoflurane-anesthetized mice. ECG data was collected at baseline for 3 minutes and 30 minutes after isoproterenol treatment (i.p. injection of isoproterenol at a dose of either 0.1 mg/kg or 1 mg/kg). ECG signals were amplified with an Animal Bioamp (ADInstruments) connected to a Powerlab amplifier (ADInstruments) and later analyzed offline using Labchart 7 Pro software (ADInstruments) as described previously [56].

Histological Analysis

Mouse hearts were removed, washed, fixed for 48 hours in 10% buffered formalin (Fisher Scientific SF100-4) and transferred to 70% ethanol. Fixed heart tissue was then paraffin embedded and transversely sectioned (5 μm). For H&E staining of heart sections, a previously described protocol was used (http://stmichaelshospitalresearch.ca/staff-services/research-facilities/facilities/histology-core/routine-he-stain/). ImageJ Software was used to calculate the fibrotic and nonfibrotic surface area in each image captured with a 20X objective and approximately 150 images per heart were used for data analysis.

Statistical Analysis

All data are presented as mean ± SE, mean + SE, mean ± SD, or mean + SD, as indicated in figure legends. GraphPad Prism was used for statistical analyses. Unless otherwise stated, statistical analysis was performed using ANOVA followed by a Tukey post hoc test for multiple comparisons. If only two groups were compared, a Student's *t* test (2 tailed, unequal variance) was performed instead. Data were regarded as significant at $p < 0.05$.

Supplemental Tables S1-S4

Table S1. qPCR primers.

Primers	Primer Sequence (5' -> 3')
human and mouse 18S rRNA Forward	GCCGCTAGAGGTGAAATTCTTA
human and mouse 18S rRNA Reverse	CTTTCGCTCTGGTCCGTCTT
Trdn MT-1 (junction)-F	TGACAAAGAGATGCCAGCTG
Trdn MT-1 (junction)-R	CCCCATCTGAGGTTTAATTTTAGTC
Trdn MT-2-F	GAAGGATGACAAAGAGATGCC
Trdn MT-2-R	GCTTAGTACAGACAAAGCTGAC
Trdn MT-3-F	AGGACAAAGCCCAGTTATGC
Trdn MT-3-R	ACTAAGTACTTGCCTTCAAGGG
Trdn95-F	AAGACTCCAAAGATGTCCCAC
Trdn95-R	AACCCATAGCCATTGTACCC
Trdn Exon 7-8-F	TGATGGCAAAAGAGGACAAGA
Trdn Exon 7-8-R	GGTGTTTTCGGAGTTTCTTTGG
Trdn Exon 11-F	GAAAAAGAAGAGAAGAAGAAAATGG
Trdn Exon 11-R	TCCCAGCAACACTATCAATTAGG
mGAPDH-F	GCAGTGGCAAAGTGGAGATTG
mGAPDH-R	GGAGATGATGACCCTTTTGGCTCC
Trdn- as-F	TCTCATGTGCCGAGGAAAAG
Trdn-as-R	CTGAAGATGAAGCCCCGAAG
mU1 snRNA-RT-F	GCTCACCCATTGCACTTTG
mU1 snRNA-RT-R	CCCACTACCACAAATTATGCAG
Trdn intron 8-F	TTTAAAATGGTCACAAAGGGCC
Trdn intron 8-R	ACAGAGCAGACCTTGTGTTC
Trdn- as exon 1-F	CCTGGACGATTCCTCTTTCAG
Trdn- as exon 1-F	TTATAGCTAGGCAGCACTTGAC
Trdn- as exon 3-F	TTTCAAGCTCCAGACATCCG

Trdn- as exon 3-F	TCCACACTCTCACCTACACA
Malat1-F	CATGGCGGAATTGCTGGTA
Malat1-R	CGTGCCAACAGCATAGCAGTA
TRDN-AS-F	CCCCAATCCTGCAAACAATG
TRDN-AS-R	CTGTGTTTTCCATTCTCTGCC
hU1SnRNA-F	AGGCTTATTCATTGTACTCCGG
hU1SnRNA-R	CAAATTATGCAGTCGAGTTTCCC

Table S2. FISH and smFISH probes.

Probes	Sequence
TRDN-AS dig-antisense 1	TAATACGACTCACTATAGGGAGA
TRDN-AS dig-antisense 2	TGAATTGGCTATGTGACCCTAC
Trdn-as smFISH-1	tgggctggtctttgagatgc
Trdn-as smFISH-2	tgtccaagaagctcttgttg
Trdn-as smFISH-3	ctgaagatgaagccccgaag
Trdn-as smFISH-4	tgaagtgaactctcctttgg
Trdn-as smFISH-5	aagatggtgttctcacctga
Trdn-as smFISH-6	tctctgagagacctgagata
Trdn-as smFISH-7	caaatgctctcctgtgaact
Trdn-as smFISH-8	aaaagctctgttgcttctgc
Trdn-as smFISH-9	ctcttgcagcaaaggtacac
Trdn-as smFISH-10	tgtctggctagagcttattt
Trdn-as smFISH-11	tttgctttttaccatttcgt
Trdn-as smFISH-12	gtactgcattctgatgatgc
Trdn-as smFISH-13	tgtatccaggactcactgtg
Trdn-as smFISH-14	tgccctatttaaaatcctct
Trdn-as smFISH-15	tcaaggacttctacctgttt
Trdn-as smFISH-16	tgagggatttttatgcttcc

Trdn-as smFISH-17	gctctttaaggacttctacc
Trdn-as smFISH-18	tgtttcgctgtgttttatgt
Trdn-as smFISH-19	ggtttttgctcaattccat
Trdn-as smFISH-20	gattctaccttccttttaga
Trdn-as smFISH-21	caaggtgaccccaattttta
Trdn-as smFISH-22	atggagagtcctctagagga
Trdn-as smFISH-23	tctcaggtcccacacaattg
Trdn-as smFISH-24	tgaccagagggacttgtgac
Trdn-as smFISH-25	ctgtctcagaaactgtccta
Trdn-as smFISH-26	cggatgtctggagcttgaaa
Trdn-as smFISH-27	ctccttttgcagggaagatg
Trdn-as smFISH-28	gcaaagtccttccaggaagg
Trdn-as smFISH-29	cagatatctggtgttcggag

Table S3. On- and Off-targets of CRISPR in Mouse knockout line 1 and line 2

Chromosome position	CRISPR quality score	Nearest gene	Note	Sequence changed?
sgRNA-1: CTTGAGCTAGCCTTATACATAGG				
chr10: +32945994	72	*Trdn-as*	Intron	Yes
chr12: +20932964	3.0	*Gm30124*	57kb upstream	No
chr9: -16126239	1.7	*Fat3*	Intron	No
chr4: -69646693	0.8	*Cdk5rap2*	570kb downstream	No
chr1: +16397025	0.8	*Stau2*	Intron	No
chr5: +103464755	0.6	*Mapk10*	Intron	No
sgRNA-2: TTTGCTTTTTGGGTAATCTCTGG				

chr10: -32942159	63	*Trdn-as*	Intron	Yes
chr17: -76372005	4.8	*Gm4710*	484kb downstream	No
chr19: -54874628	1.5	*Sorcs1*	4196kb upstream	No
chr1: -144423341	1.4	*Gm37796*	1668kb upstream	No
chr1: +13320268	1.3	*Ncoa2*	Intron	No
chr18: -76834768	1.0	*Gm31933*	202kb downstream	No

Table S4. Mouse ECG parameters at baseline.

	RR	*HR*	*PR*	*QRS*	*QT*	*QTc*
WT	133 ± 4	453 ± 5	40 ± 3	11.4 ± 0.6	31.3 ± 1	27.2 ± 0.8
KO	135 ± 5	449 ± 18	38 ±5	10.7 ± 0.3	33.3 ± 0.8	28.8 ± 0.5
p-value	0.8851	0.8629	0.8385	0.1537	0.1368	0.1207

All data are presented as mean ± SEM. RR, time between two successive ventricular depolarizations; HR, heart rate; PR, P-R interval; QRS, QRS complex duration; QT, Q-T duration; QTc, corrected QT. P values for comparing WT and KO mice were calculated by Student's *t*-test (n=6 mice per group).

Supplemental Figures S1-S10 and Figure Legends

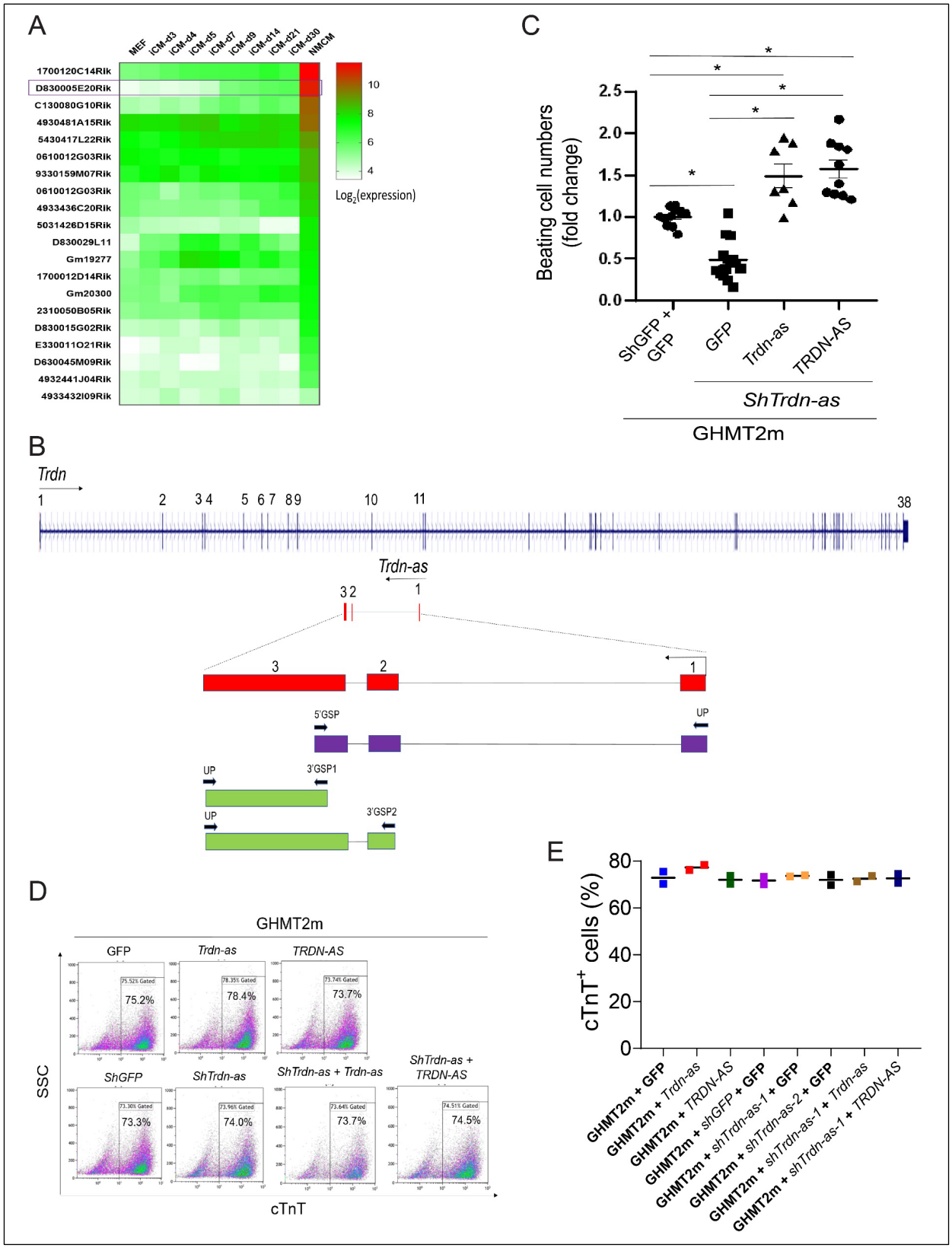


Figure S1. *Trdn-as* is required for generation of beating iCMs. Related to **Figure 1.** **A**) Expression of *Trdn-as* (D830005E20Rik, ENSMUST00000217716.1) and other genes during reprogramming of MEFs into iCMs by RNA microarray analysis. **B**) The *Trdn-as* transcript in the mouse heart was determined by 5' and 3' RACE using primers specific to *Trdn-as* exons 2 and 3. Genomic structure of the mouse *Trdn* gene from the UCSC genome browser. Arrow denotes transcription direction. 38 exons of *Trdn* are numbered and shown as boxes, and introns as lines. Arrows indicate transcription direction. GSP, *Trdn-as* specific primer. UP, universal primer. **C**) The effect of *Trdn-as* or *TRDN-AS* on spontaneous contraction of iCMs. MEFs were transduced with retroviral vectors carrying GHMT2m and short hairpin RNA for GFP (shGFP) or *Trdn-as* (sh*Trdn-as*). The sh*Trdn-as* targets the 3' end of *Trdn-as*, which was not included in its over-expression construct. *GFP*, *Trdn-as*, or *TRDN-AS* was also expressed in indicated GHMT2m-transduced MEFs. Spontaneously beating iCMs were quantified 15 days post-infection. Each dot/square/triangle represent one reprogramming experiment. One-way ANOVA with Tukey's post test, *p<0.05. **D-E**) Flow cytometry analysis for expression of cardiac troponin T (cTnT), a myocyte marker in MEFs expressing various factors. MEFs were infected with retroviruses encoding indicated factors and analyzed at day 7. The percentage of cells positive for cTnT in each group is shown in ***D***, and is quantified in *E*. n = 2 independent experiments. Data are presented as mean.

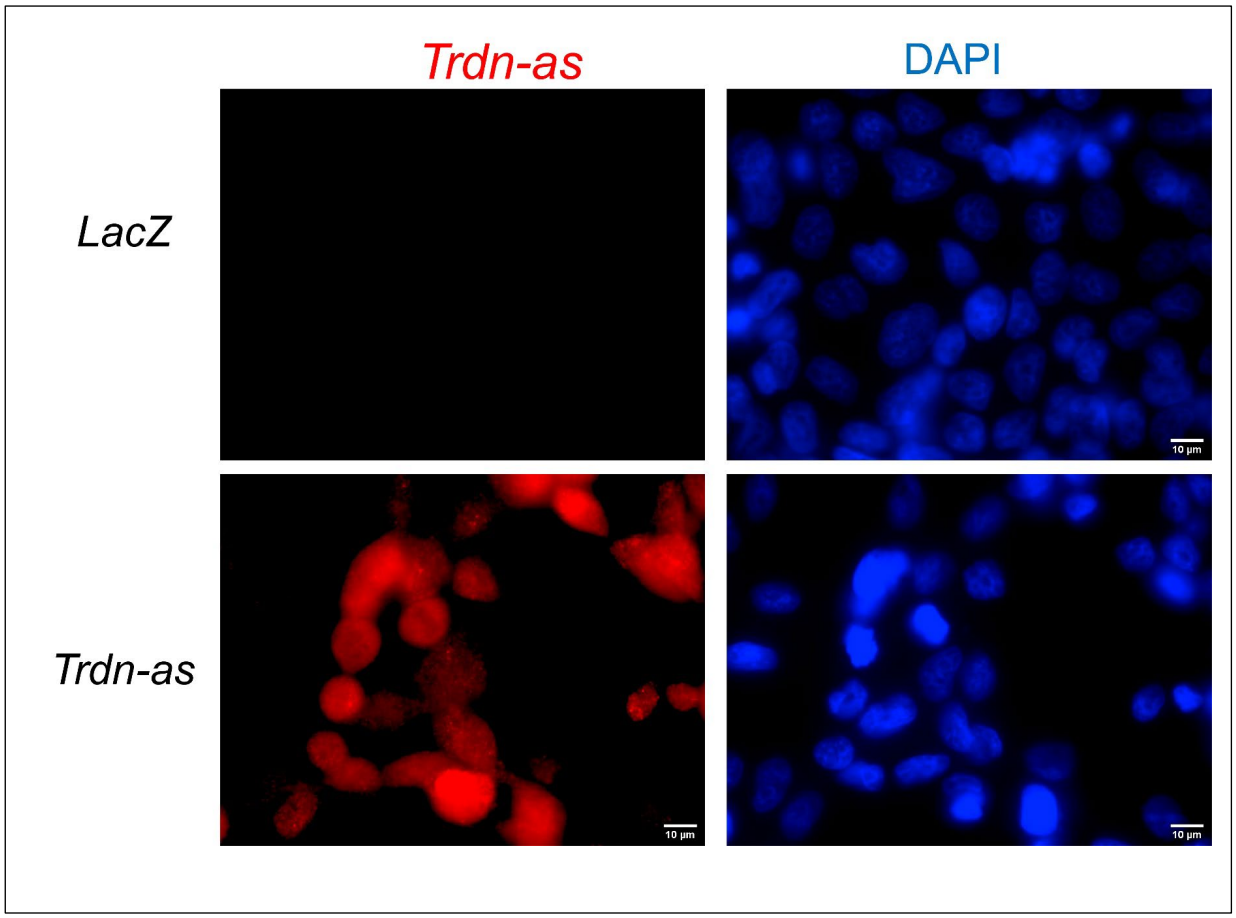


Figure S2. Specificity of smFISH probes for *Trdn-as*. Related to **Figure 1.** HeLa cells were transfected with *LacZ* or *Trdn-as* expression vectors. smFISH was performed 48 hours post-transfection.

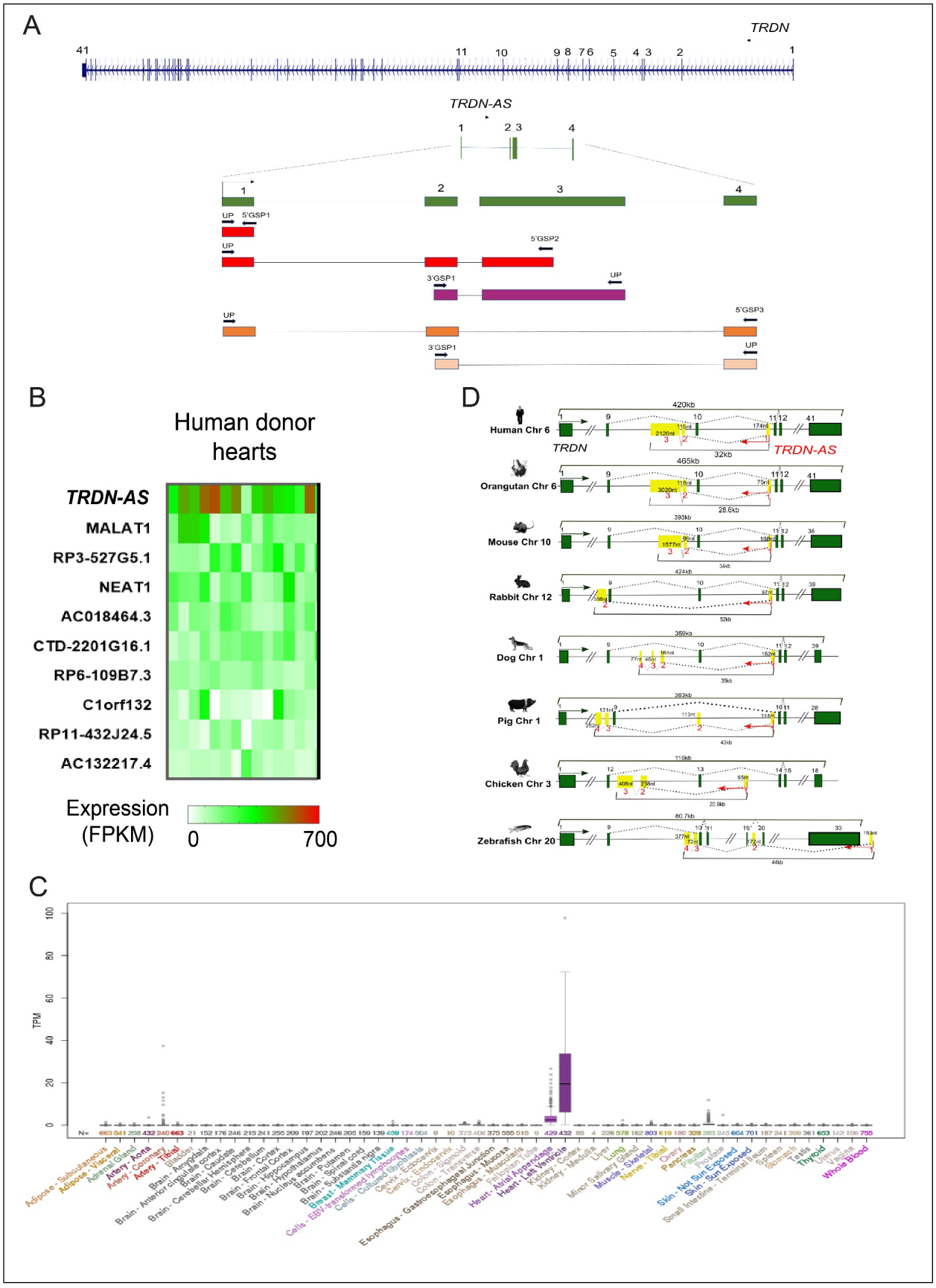

A
TRDN
41
11
10
9 8 7 6
5
4 3
2
1
TRDN-AS
1
2 3
4
UP
5'GSP1
5'GSP2
3'GSP1
5'GSP3
B
Human donor hearts
TRDN-AS
MALAT1
RP3-527G5.1
NEAT1
AC018464.3
CTD-2201G16.1
RP6-109B7.3
C1orf132
RP11-432J24.5
AC132217.4
Expression (FPKM)
0
700
D
Human Chr 6
TRDN
TRDN-AS
Orangutan Chr 6
Mouse Chr 10
Rabbit Chr 12
Dog Chr 1
Pig Chr 1
Chicken Chr 3
Zebrafish Chr 20
C
TPM
N=

Figure S3. *TRDN-AS* is cardiac-specific and conserved in genomic position across species. Related to **Figure 1. A**) The *TRDN-AS* transcript in hiPSC-CMs was determined by 5' and 3' RACE using primers specific to exons 1, 2, and 3 of *TRDN-AS* (ENSG00000235535). Arrows denote the direction of gene transcription. GSP, *TRDN-AS* specific primer. UP, universal primer. **B**) RNA-seq analysis of non-coding RNAs in human hearts. Expression of *TRDN-AS* in 14 healthy human donors by Sweet *et al.* (Sweet et al., 2018). A list of top 10 lncRNAs with the highest expression levels in healthy hearts is shown. **C**) Expression of *TRDN-AS* in 54 tissues from GTEx RNA-seq of 17382 samples, 948 donors (GTEx Consortium V8, August 2019). **D**) LncRNA genomic positions across species.

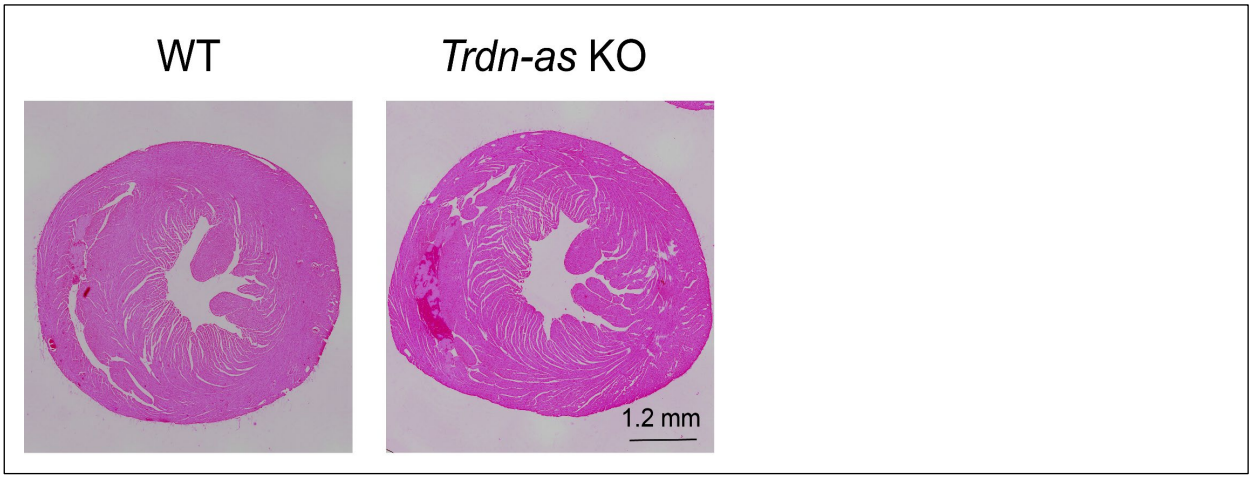
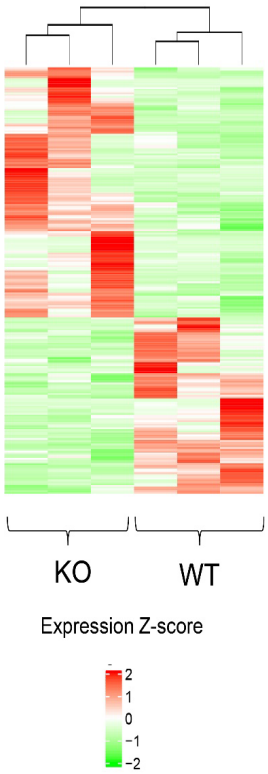


Figure S4. Hematoxylin and eosin (H&E) analysis of transverse sections of WT and *Trdn-as* knockout (KO) hearts at the age of 12 months. Related to **Figure 2**. Note: slight dilation of left ventricle is observed in the KO heart.

A



B

	GO terms	P values
Up-regulated genes in KO hearts	negative regulation of adenylate cyclase-inhibiting adrenergic receptor signaling pathway involved in heart process (GO:0140194)	0.01
	negative regulation of calcium ion transmembrane transporter activity (GO:1901020)	0.01
	regulation of calcium ion transmembrane transport (GO:1903169)	0.02
Down-regulated genes in KO hearts	positive regulation of heart growth (GO:0060421)	0.01
	positive regulation of ryanodine-sensitive calcium-release channel activity (GO:0060316)	0.03

Figure S5. Dendrogram cluster and GO analysis of RNA-seq illustrating significantly differentially expressed genes in WT and *Trdn-as* KO adult mouse hearts. Related to **Figure 3**. Total RNA was harvested from 3 WT and 3 KO adult mice, followed by deep sequencing. FPKM (fragments per kilobase of exon per million mapped fragments) > 50 was used to filter transcripts. Fold change between |WT and KO| > 1.5 and FDR (false discovery rate) adjusted $p < 0.05$ were set as criteria to determine differentially expressed genes (DEGs). A) A heatmap of DEGs between WT and *Trdn-as* KO mice**. B**) Gene Ontology (GO) analysis of genes differentially expressed between WT and *Trdn-as* KO hearts.

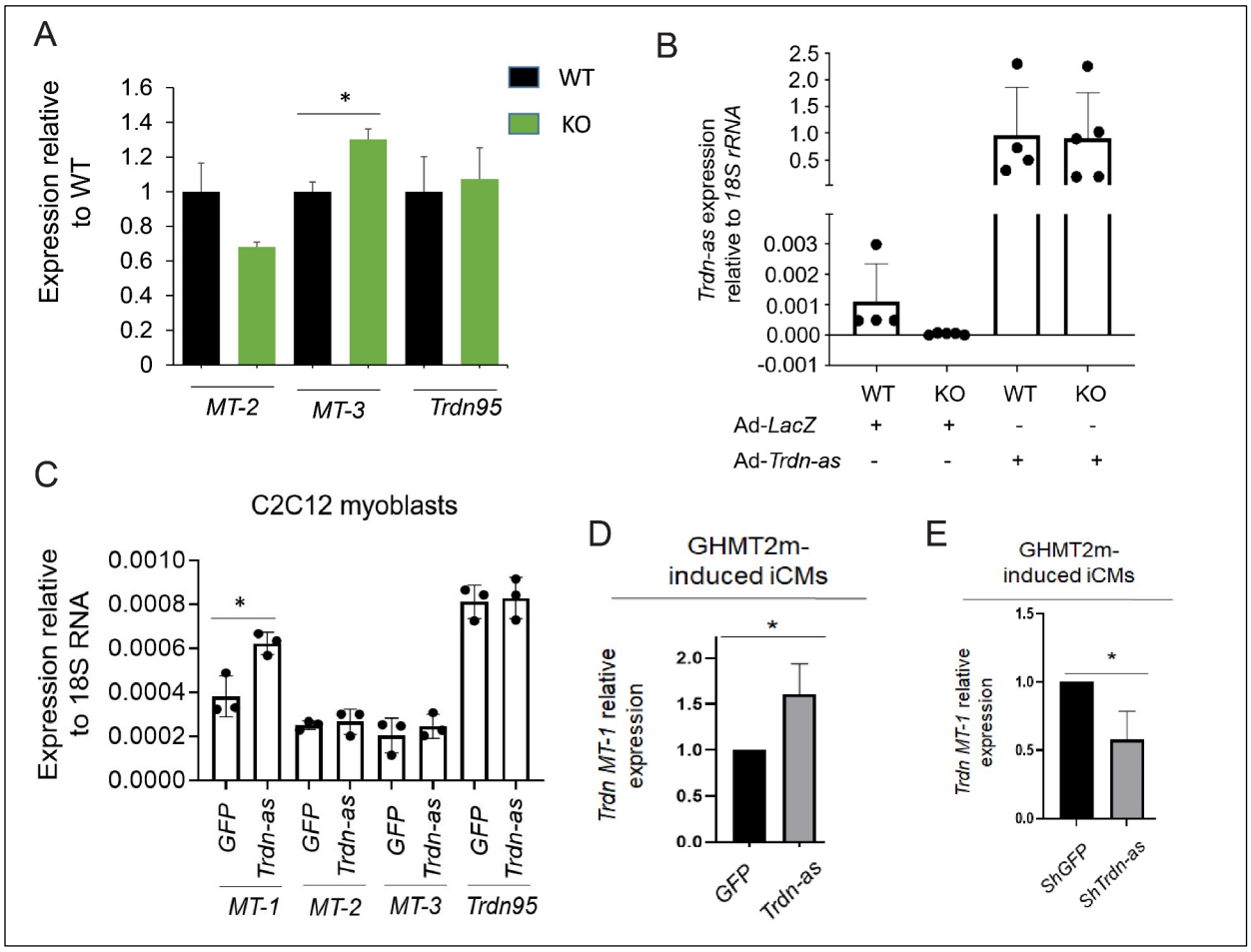


Figure S6. *Trdn-as* is sufficient to increase cardiac triadin levels in various types of cells. Related to **Figure 4. A**) Expression of triadin isoforms in WT or KO hearts by qPCR. Total RNA was extracted from adult mouse hearts. Expression of triadin isoforms were quantified by qPCR using paired primers amplifying specific isofroms of triadin. N = 5 animals per each group. Data are presented as mean + SD. **B**) qPCR analysis of *Trdn-as* expression in WT and KO AMCMs infected with adenoviruses carrying either *LacZ* or *Trdn-as* 48 hours post-infection. One dot represents one mouse. Data are presented as mean + SD. **C**) *Trdn-as* was transfected into C2C12 myoblasts. *Trdn* isoforms were quantified 48 hours post-transfection using primers specifically targeting the triadin isoforms *MT-1*, *MT-1*, *MT-3*, and *Trdn95*. N = 3 independent experiments. Data are presented as mean ± SD. **D**) MEFs were transduced with retrovirus vectors carrying GHMT2m plus either GFP or *Trdn-as*. *Trdn MT-1* was quantified 15 days post-transduction. N = 3 independent experiments. Data are presented as mean + SD. **E**)

MEFs were transduced with retrovirus vectors carrying GHMT2m plus either shGFP or sh*Trdn-as*. *Trdn MT-1* was quantified 15 days post-transduction. N = 3 independent experiments. Data are presented as mean + SD. *p < 0.05, One-way ANOVA with Tukey's post test in **A** and **C**, and Student's *t*-test in **D** and **E**.

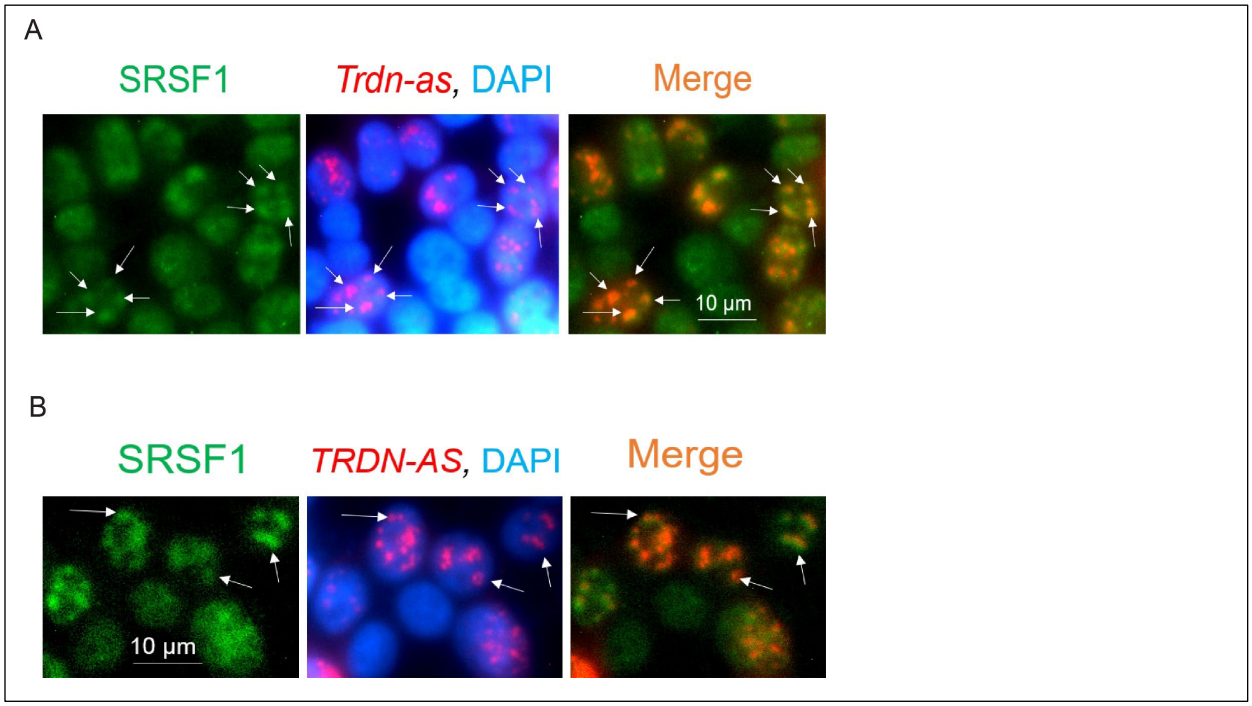
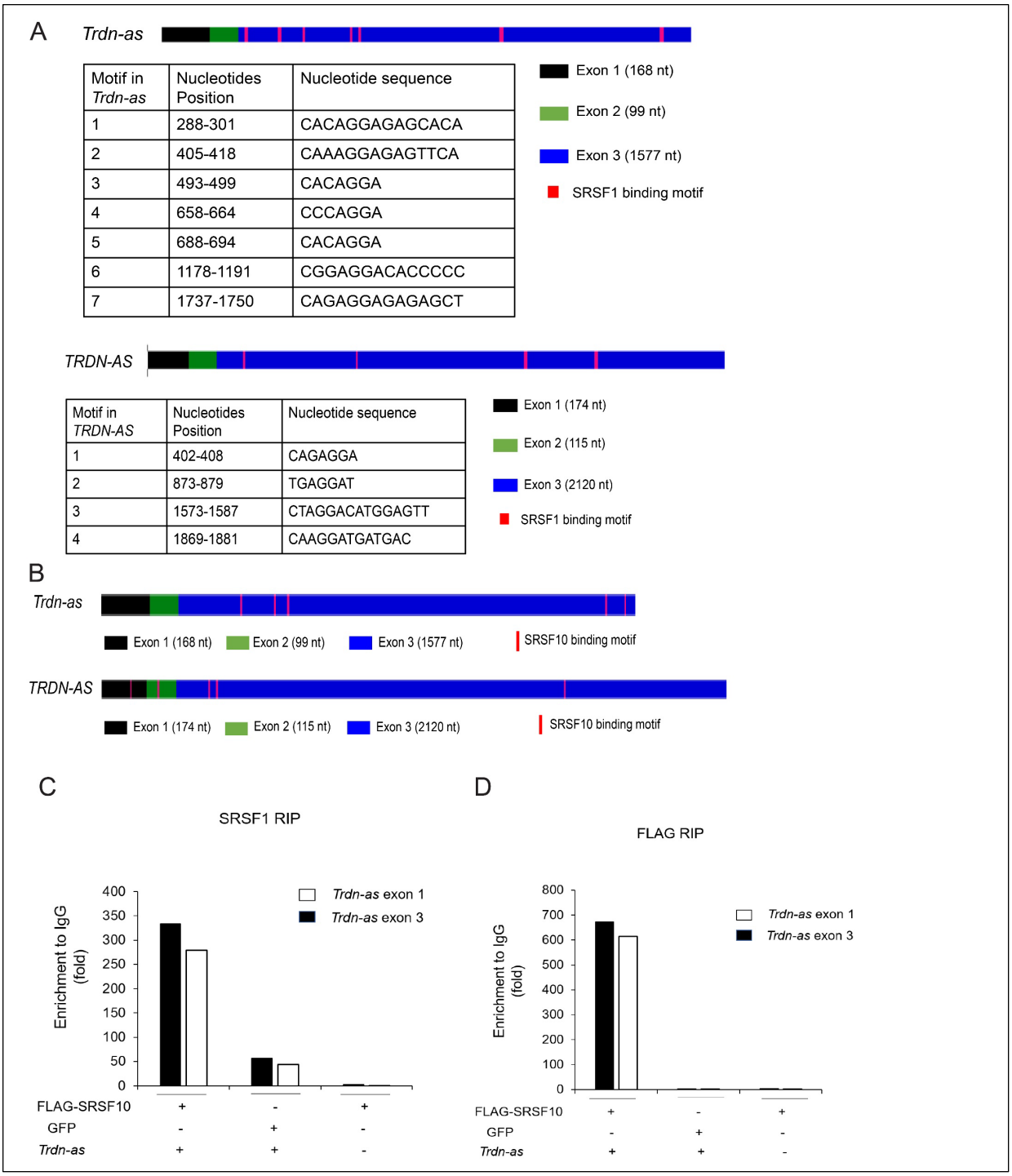


Figure S7. *Trdn-as* or *TRDN-AS* colocalizes with SR splicing factor SRSF1 in HEK293 cells. Related to **Figure 6.** HEK293 cells were transfected with *Trdn-as* (**A**) or *TRDN-AS* (**B**) expression constructs. 48 hours later, cells were fixed for FISH to detect *Trdn-as* or *TRDN-AS* and immunostaining for SRSF1.



Motif in *Trdn-as*	Nucleotides Position	Nucleotide sequence
1	288-301	CACAGGAGAGCACA
2	405-418	CAAAGGAGAGTTCA
3	493-499	CACAGGA
4	658-664	CCCAGGA
5	688-694	CACAGGA
6	1178-1191	CGGAGGACACCCCC
7	1737-1750	CAGAGGAGAGAGCT

Motif in *TRDN-AS*	Nucleotides Position	Nucleotide sequence
1	402-408	CAGAGGA
2	873-879	TGAGGAT
3	1573-1587	CTAGGACATGGAGTT
4	1869-1881	CAAGGATGATGAC

Figure S8. Interactions of *Trdn-as* with SR splicing factors SRSF1 and SRSF10. Related to **Figure 7. A-B**) SRSF1 and SRSF10 binding motifs within *Trdn-as* and *TRDN-AS* transcripts were predicted with SFmap (http://sfmap.technion.ac.il/), and according to previous studies [55], respectively. Most of predicted SRSF1 or SRSF10 binding motifs are

within regions encoded by lncRNA exon 3. Positions and sequences of SRSF1 binding motifs are shown. **C-D**) HEK293 cells were transfected with various combinations of expression constructs encoding *Trdn-as*, FLAG-SRSF10, or GFP. RIPs with antibodies against SRSF1 or FLAG were conducted 48 hours post-transfection. Levels of *Trdn-as* associated with SR splicing factors were quantified using paired primers to specifically amplify *Trdn-as* exon 1 or exon 3.

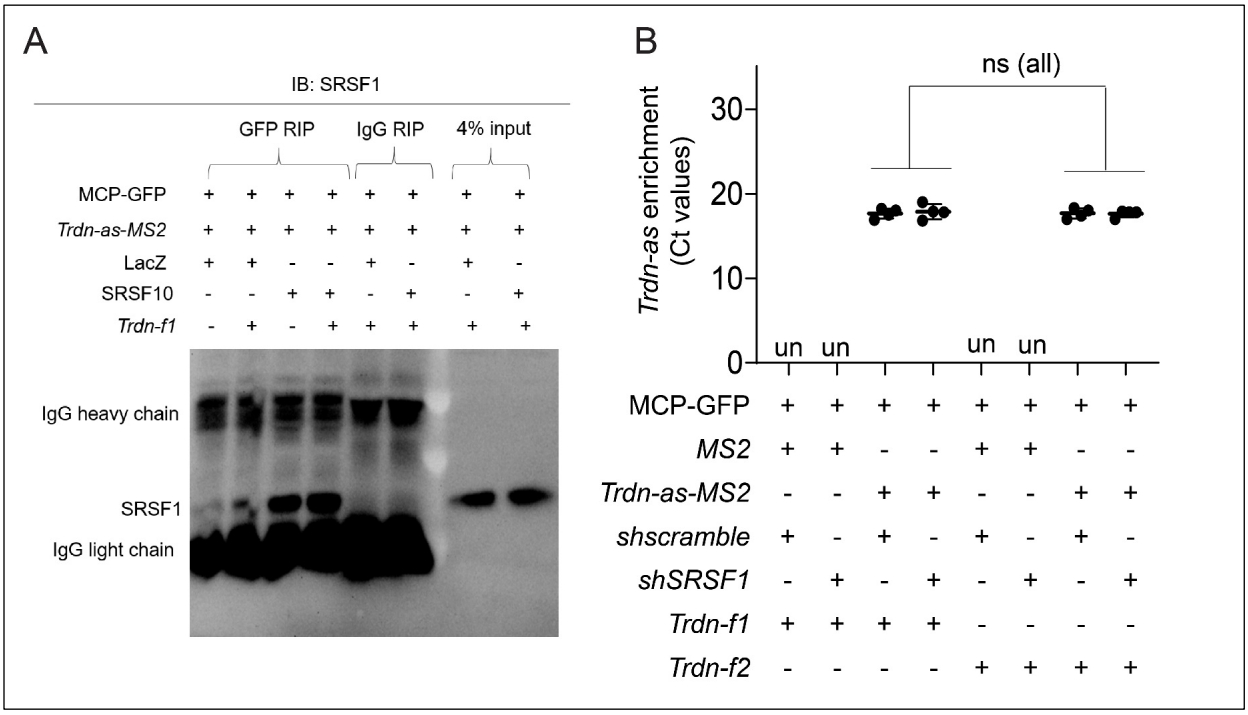


Figure S9. Interactions of *Trdn-as* with SR splicing factors were detected using the MS2-MCP system in HEK293 cells. Related to **Figure 7.** HEK293 cells were transfected with various combinations of expression plasmids encoding MCP-GFP, LacZ, *shSRSF1*, *shscramble*, SRSF10, *MS2*, or *Trdn-as-MS2*. 48 hours later, nuclear extracts of HEK293 cells were incubated with *in vitro* transcribed *Trdn* pre-mRNA, *Trdn-f1*, *Trdn-f2*. Trdn-as-MS2 associated complexes were precipitated by the GFP antibody. **A**) Immunoblotting for SRSF1, following RIP with GFP antibody or IgG, shows interaction between *Trdn-as* and SRSF1. **B**) qPCR analysis of *Trdn-as* levels in complexes precipitated by the anti-GFP antibody. N = 4 independent experiments. Data are presented as mean ± SD. One-way ANOVA with Tukey's post test. ns, not significant. un, undetectable.

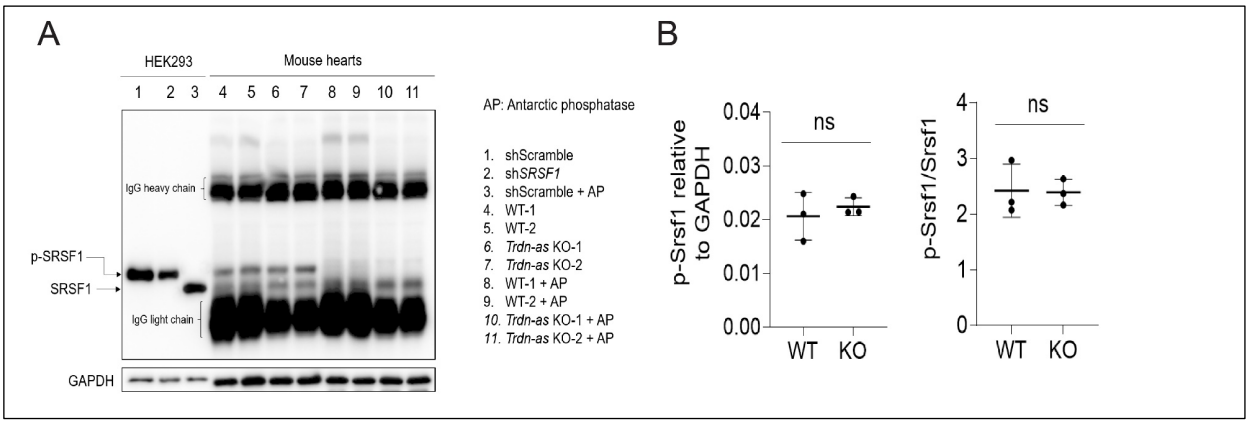


Figure S10. Phosphorylation of SR splicing factor 1 in WT and *Trdn-as* KO hearts. Related to **Figure 8. A-B**) Immunoblot assays extracts from HEK293 cells treated with *shScramble* or *shSRSF1*, WT and *Trdn-as* KO hearts. The antibody specificity was determined using shRNA knockdown assays. Treatment with phosphatase was used to determine immunoblotting bands representing phosphorylated or non-phosphorylated SRSF1. Quantification of phosphorylated SRSF1 (p-SRSF1) is shown in **B**. N = 3 mice at the age of 12 months per group. Data are presented as mean ± SD. Student *t*-test, ns, not significant.