Supplemental Material

Tead1 is required for maintaining adult cardiomyocyte function and its loss results in lethal dilated cardiomyopathy

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Supplemental Methods

Generation of *Tead1* flox mice

Briefly, the Tead1 targeting vector was constructed using PRPGS00097_A_H05 harboring two loxP sites encompassing a neomycin resistance cassette flanked by two FRT sites. Targeting arm sequences were isolated from C57BL/6N genomic DNA by PCR. The *Tead1* 5' targeting arm (5169 bp), *Tead1* 3' targeting arm (4773 bp), and *Tead1* knockout arm (2 kb) were cloned into the vector. The sequence-verified targeting vector was linearized and electroporated into C57Bl/6N-derived ES cells. Targeting of the mutant allele was screened through Southern blot analysis. Targeted ES cells were injected into blastocysts to generate chimeric mice. High-percentage chimeric males were bred with mice expressing FLPe recombinase to remove the neomycin resistance cassette to generate Tead1 loxP alleles [floxed exon: ENSMUSE00000203030]. The homologous recombination was verified by long range PCR using primer set #1 (forward: GCAGTGCTCTCAGGAGTGCTGAGTGCGAC; reverse: AGAAGCCACAGTGCCCTGGAAGTGT) and regular PCR using primer set #2 (forward: GCCTTCTGAGTGCTGGCATTAAAGG; reverse: same as #1reverse) (**Supplemental Figure 2**). Mice were genotyped using *Tead1*-flox-forward (GCCTTCTGAGTGCTGGCATTAAAGG) and reverse (AAGGCAGACTCCTTCATTGGAATGG) primers.

Antibodies for immunoblotting and immunofluorescence. The antibodies used for immunoblotting are listed below: TEAD1 [EPR3967(2)] (ab133533, Abcam; 1:2,000), GAPDH [FL-335] (sc-25778, Santa Cruz Biotechnology; 1:4,000), SERCA2 (4388S, Cell Signaling; 1:2,000), pPLN^{Ser16} (07-052, Millipore; 1:1,000-5,000), pPLN^{Thr17} (A010-13, Badrilla; 1:2,000), PLN (05-205, Millipore; 1:2,000-5,000), PP1c (1050-1, Epitomics; 1:5,000), I-1 (ab40877, Abcam; 1:5,000), HSP90 (4874S, Cell Signaling; 1:2,000). The antibodies for immunofluorescence are: cardiac Troponin T [1C11] (ab8295, Abcam; 1:500), TEAD1 ([clone 31] 610923, BD Biosciences; 1: 200).

Plasmids construction. For the construction of FUCGW-*hTEAD1*, full length human *TEAD1* gene (NM_021961.5) was amplified by PCR using primers with EcoRI restriction sites (F: CGgaattcATTGAGCCCAGCAGCTGGAG; R: CGgaattcTCAGTCCTTTACAAGCCTGT) from human embryonic kidney 293T cell cDNA template and then cloned into modified FUCGW lentiviral vector (a gift from Dr. Yi Li). For the construction of pGL3-basic-*I1*-1.2k luciferase reporter, the -2390~-1137bp 5' promoter region was amplified by PCR using primers with MluI and BglII restriction sites (F: CGacgcgtCAGCTCAGGGCATAAATAAAGGTC, R: GAagatctAGAGAAGGAGAAGGCCTATAGTGC) from a C57BJ/6 wild-type mouse genomic DNA template and cloned into pGL3-basic vector (Promega). For the construction of pGL3-basic-*Serca2a*-0.8k luciferase reporter, the -2303~-1574bp 5' promoter region was amplified as described above flanked with KpnI and BglII restriction sites.

Histology. For Masson's Trichrome staining, dissected hearts were immediately fixed in 10% formalin overnight at room temperature, followed by paraffin embedding. Coronal or cross sectioned tissues (5 μm) were deparaffinized in Histo-Clear (National Diagnostics), rehydrated and fixed in Bouin's Solution at 56°C for 1 hr. Following washes in deionized water, sections were sequentially stained with Weigerts' Iron Hematoxylin, Beibrich Scarlet-Acid Fuchsin solution, phosphotungstic/phosphomolybdic acid and Aniline blue (25088, Masson's Trichrome stain kit from Polysciences). Sections were dehydrated via ethanol series, cleared in Xylene and mounted in resin-based medium. Images were analyzed using a Zeiss Axioplan-2 imaging system.

For wheat germ agglutinin (WGA) staining of tissue sections, following antigen retrieval in boiling 10mmol/L sodium citrate (pH 6.0) for 30min, sections were blocked with 10% goat serum, followed by incubation of the sections with Rhodamine-WGA (RL-1022, Vector Lab) at 5μg/ml and mounted with ProLong® Goad Antifade Mountant (ThermoFisher). Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay was carried out

following the manufacturer's protocol using *In Situ* Cell Death Detection Kit (11684817910, Roche). For cytology staining, cells were washed with PBS and fixed in 10% formalin for 15 min at room temperature, incubated in 0.5% Triton X-100/PBS and blocked in 2% BSA/PBS. Cells were then incubated with primary antibodies as indicated, subsequently with Alexa Fluor conjugated anti-mouse IgG (Invitrogen, 1:200). Images were taken with a DeltaVision (Deconvolution) image restoration microscope or a Zeiss Axioplan-2 imaging system.

RNA extraction and real-time PCR. Total RNAs were extracted and cleaned up using RNeasy Mini Kit (QIAGEN) according to manufacturer's protocol. Complementary DNA were synthesized using q-Script cDNA Supermix kit (Quanta Biosci.). Realtime PCR was performed using Perfecta SYBR Green Supermix (Quanta Biosci.) in a Roche 480 Light Cycler machine. Relative mRNA expression levels were determined by normalization of target genes to 36B4 as internal control. Primer sequences are available upon request.

Microarray analyses. Microarray sample quality control and preparation including cRNA synthesis, labeling and RNA amplification, hybridization procedures were carried out by Genomic and RNA Profiling Core in the Baylor College of Medicine Advanced Technology Core Laboratories. Microarray profiling was performed using SurePrint G3 Mouse GE 8x60K Microarray Kit (Agilent Technologies). All analysis was corrected for multiple hypotheses testing, and effects were determined to be significant when there was a ≥1.5-fold increase/decrease relative to the control and they had an adjusted P value <0.05 and FDR <0.25.

Western blot analysis. Tissue or cell protein samples were prepared in ice-cold lysis buffer (100 mmol/L TrisHCl pH7.4, 10 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 10% glycerol, 0.5% deoxycholate, 1% Triton X-100, 0.1% SDS, 20mmol/L $Na_4P_2O_7$, 2 mmol/L Na_3VO_4, 1 mmol/L NaF, 1 mmol/L PMSF) supplemented with 1X protease inhibitor and phosphatase inhibitor (Roche) when necessary. Equal amount of protein samples were loaded and fractionated by SDS-PAGE, then transferred to 0.45 μm pore-size nitrocellulose membranes

(Millipore). Membranes were blocked in 5% non-fat milk-TBS (w/v), then incubated with indicated primary antibodies and HRP-conjugated secondary antibodies, enhanced chemiluminescence substrate (Thermo Sci.) was applied for detection. Band densities were quantified by ImageJ software.

Chromatin immunoprecipitation (ChIP) and luciferase reporter assay. Mouse genomic sequences were retrieved from Ensemble database (http://www.ensembl.org). Lasagna search (http://biogrid-head.engr.uconn.edu/lasagna_search/) was used to predict Tead1 binding sites. For ChIP assay, briefly, 120-150mg mouse heart ventricles were dissected, fixed in 1.5% formaldehyde and collected using SDS-lysis buffer. The chromatin was fragmented to sizes ranging from 200-1000bp by sonication, immunoprecipitated by 2µg normal mouse IgG or Tead1 antibody (610923, BD Biosciences) and protein A/G beads. Precipitated chromatin fragments were then purified. PCR was carried out for detection of the DNA enrichment.

The rat cardiomyoblast H9c2 cell line (CRL-1446, ATCC) was maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum at 37°C in 5% CO2. For luciferase reporter assay, H9c2 or neonatal cardiomyocytes were seeded in 24-well plates at 50-60% confluence, pGL3-*I1*-1.2k 200ng and pRL-null (Promega) 10ng were cotransfected using jetPRIME (Polyplus). After 48 hours transfection, cells were harvested and luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega), relative luciferase unit was determined by normalization to renilla activity as internal control.

Supplemental Tables

Supplemental Table 1. *mSerca2a* promoter regions harbor multiple Tead binding motifs.

gi|372099105:c122507225-122501226 Mus musculus strain C57BL/6J chromosome 5, GRCm38.p3 C57BL/6J						
Serca2a NM_009722						
Name	**Sequence**	**Position (0-based)**	**Strand**	**Score**	**p-value**	**E-value**
TEAD1(MA0090.1)	CACATTCCTCTG	3831	+	15.37	0	0
TEAD1(MA0090.1)	ACATTCCTCTG	3832	+	11.28	0	0
TEAD1(MA0090.1)	AGCATTCCACTT	340	-	9.95	0.000175	1.05
TEAD1(MA0090.1)	AACATTCCAGA	2278	+	8.38	0.0004	2.40
TEAD1(MA0090.1)	AGCATTGCACAG	1765	+	7.71	0.000675	4.0
TEAD1(MA0090.1)	TGAATTCCAGGC	1842	-	7.63	0.000725	4.3
TEAD1(MA0090.1)	AGCATTCCACT	341	-	6.94	0.000975	5.8
TEAD1(MA0090.1)	**TGGATTCCAGTT**	**3325**	-	6.94	0.000975	5.8

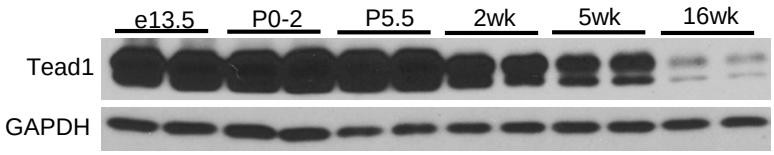
Supplemental Table 2. *mPpp1r1a (I-1)* promoter regions harbor multiple Tead binding motifs.

gi|372099095:c103542992-103537993 Mus musculus strain C57BL/6J chromosome 15, GRCm38.p3 C57BL/6J						
***Ppp1r1a* NM_021391**						
Name	**Sequence**	**Position (0-based)**	**Strand**	**Score**	**p-value**	**E-value**
TEAD1(MA0090.1)	GACATTCCTGAC	519	-	11.64	0	0

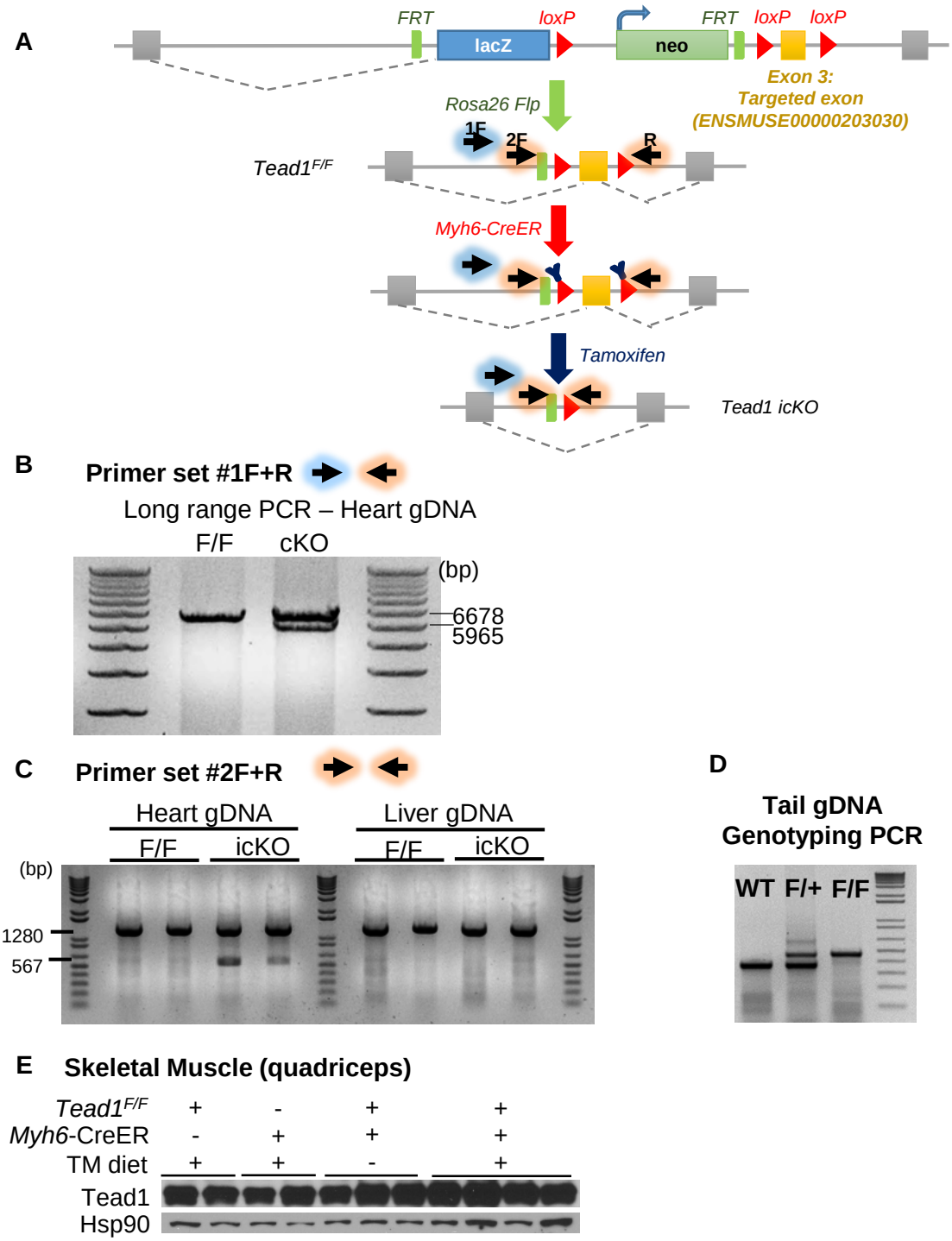
TEAD1(MA0090.1)	CACATCCCAGTG	3502	-	10.56	2.50E-05	0.125
TEAD1(MA0090.1)	TGCACTCCTGGG	2009	-	10.55	7.50E-05	0.37
TEAD1(MA0090.1)	CACATTGCACTG	3495	+	10.29	0.00015	0.75
TEAD1(MA0090.1)	TGAATTCCACTG	1065	+	9.21	0.00025	1.25
TEAD1(MA0090.1)	AACATTCCTACT	1681	+	9.14	0.000275	1.37
TEAD1(MA0090.1)	GACATTCCTGA	520	-	8.97	0.0003	1.5
TEAD1(MA0090.1)	CACACTCCAAAG	4450	+	8.01	0.000525	2.62
TEAD1(MA0090.1)	AACCTTCCTCTG	2212	-	7.94	0.000575	2.87
TEAD1(MA0090.1)	CAAATTCCAGCC	2239	+	7.69	0.000675	3.4
TEAD1(MA0090.1)	CCCATTCCACCT	2811	+	6.95	0.000975	4.9

Supplemental Table 3 (Correlated to Figure 7c). Clinical information for donors of the human heart failure tissues, listed in the order of the loading on the immunoblot.

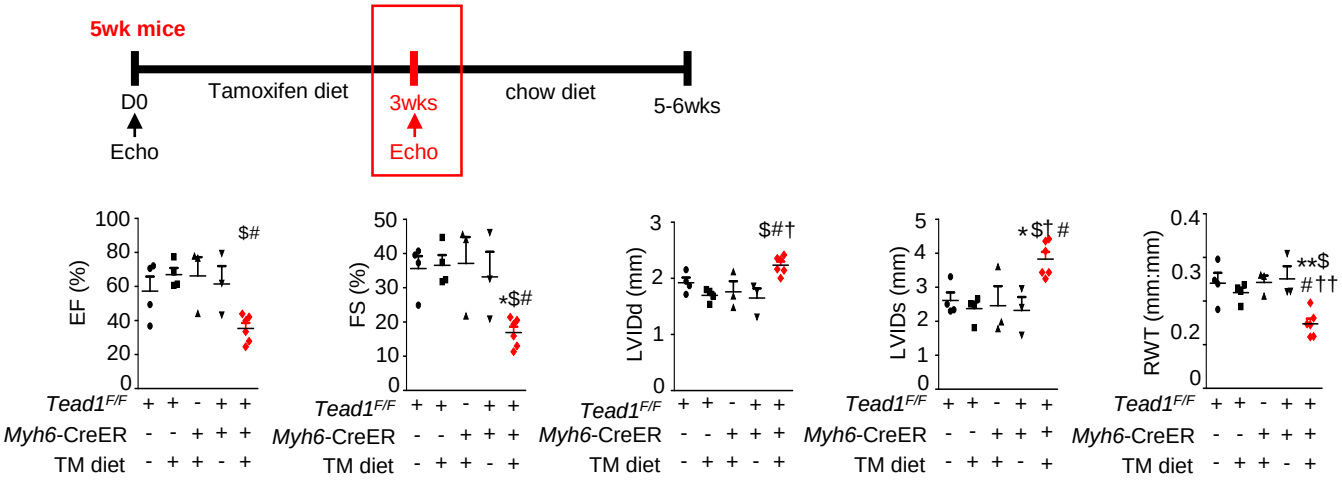
Donor ID	**Age**	**Gender**	**Diabetes**	**HTN**	**BMI kg/m^2**	**Diagnosis**
HR0110	44	F	No	Yes	53.1	DCM, not restrictive or hypertrophic CM
HR0117	44	M	Yes	Yes	39.4	DCM, not restrictive or hypertrophic CM
HR0120	40	M	No	Yes	33.3	Post VAD sample, originally dx DCM, not restrictive or hypertrophic CM
HR0082	60	F	Yes	Yes	26.9	DCM, not restrictive or hypertrophic CM



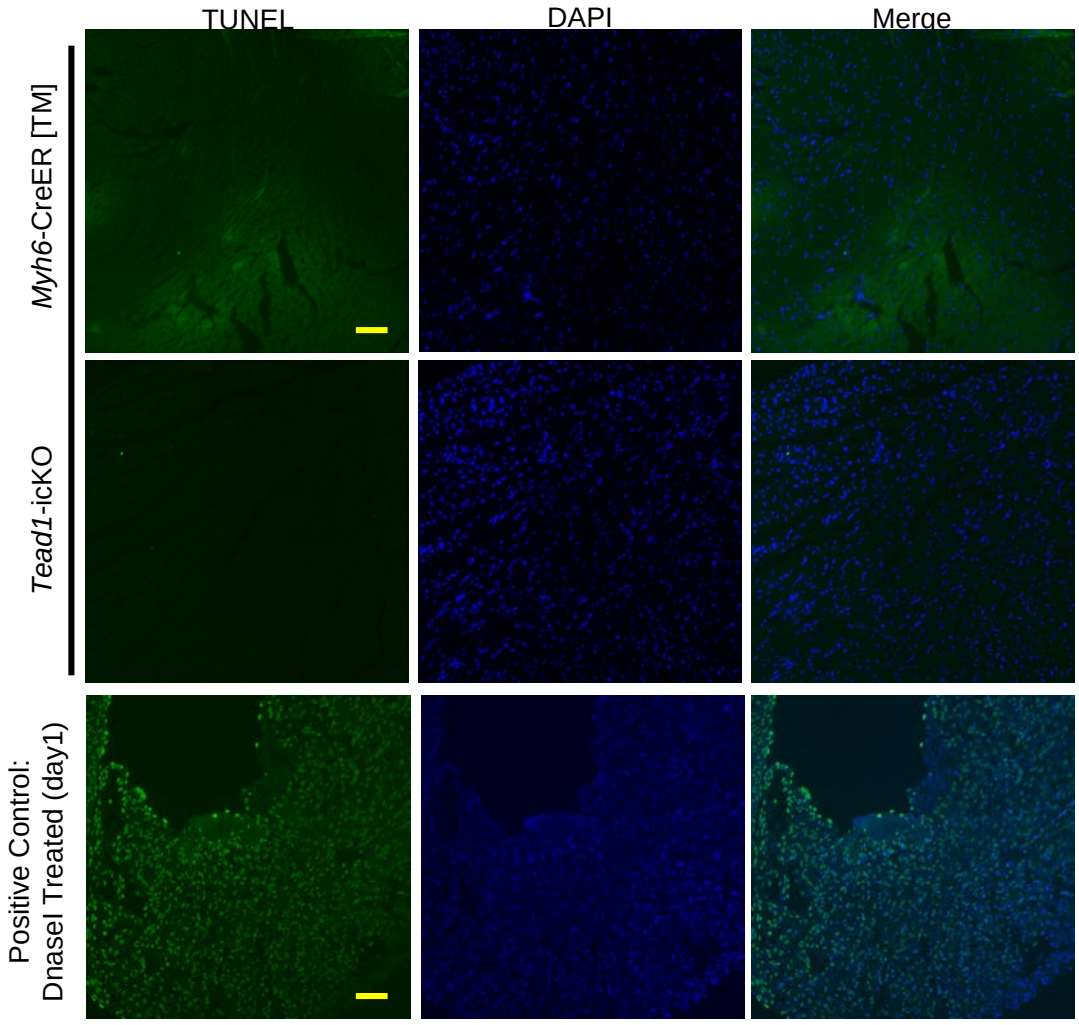
Supplemental Figure 1. Tead1 expression levels during development in mouse hearts. Western blot showing the expression of Tead1 during mouse heart development at embryonic day 13.5, postnatal day 0-2, day 5.5, 2-week-old, 5-week-old and 16-week-old. GAPDH served as loading control.



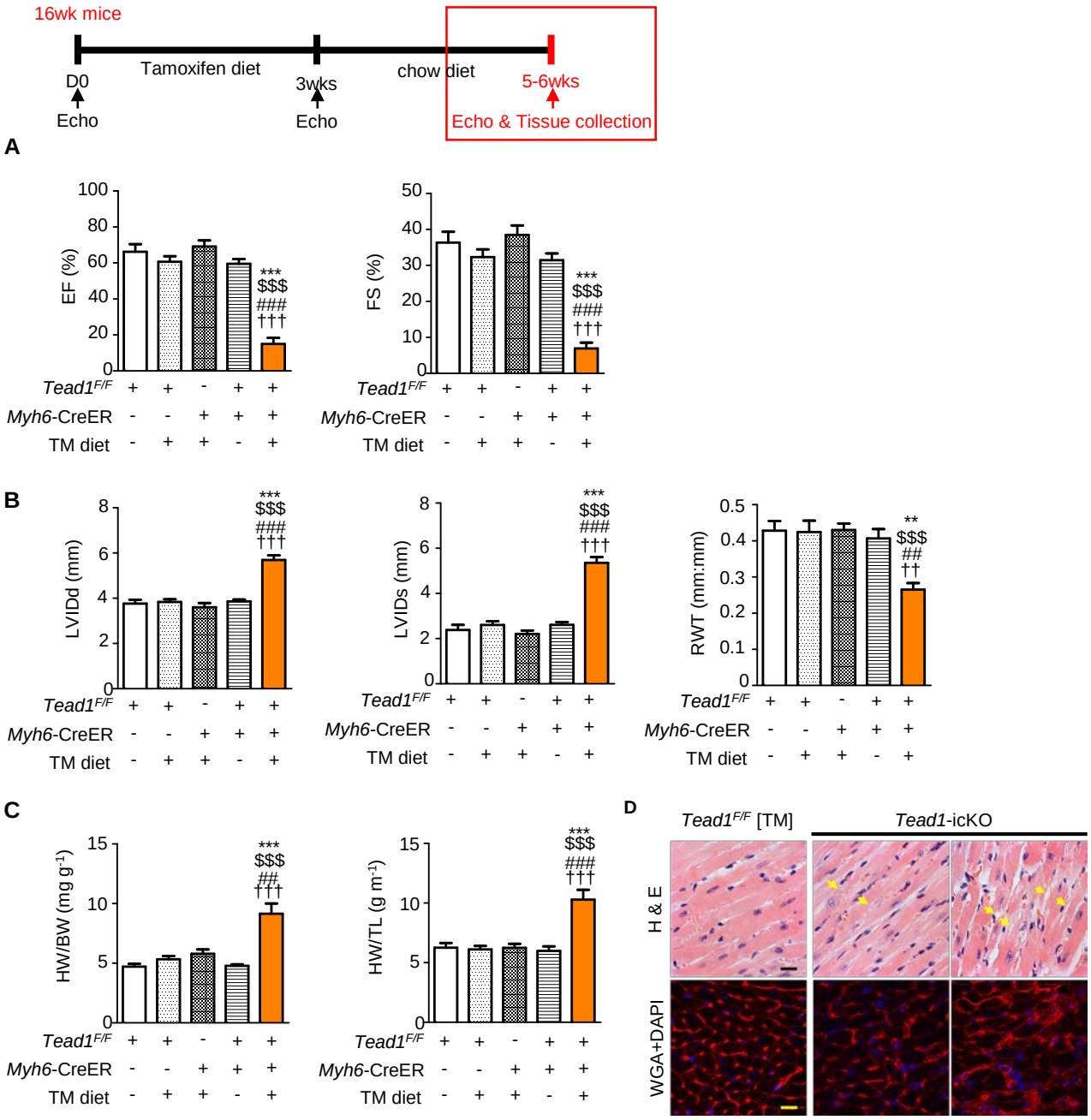
Supplemental Figure 2. Generation of *Tead1* flox mice and cardiomyocyte-specific Tead1 knockout mice. (**A**) Generation of *Tead1* conditional knockout allele, and tamoxifen-induced cardiomyocyte specific knockout mice models (icKO) using knockout-first strategy: promoter-driven selection cassette. (**B**) *Tead1* flox allele verified by long-range PCR in cKO hearts. (**C**) PCR showing the recombined fragment of the *Tead1* allele in hearts from male icKO. (**D**) PCR confirmation of WT, *Tead1*$^{F/+}$ and *Tead1*$^{F/F}$ mice genotyping. (**E**) Tead1 expression in skeletal muscle, by Western blotting, is not altered in *Tead1*-icKO mice.



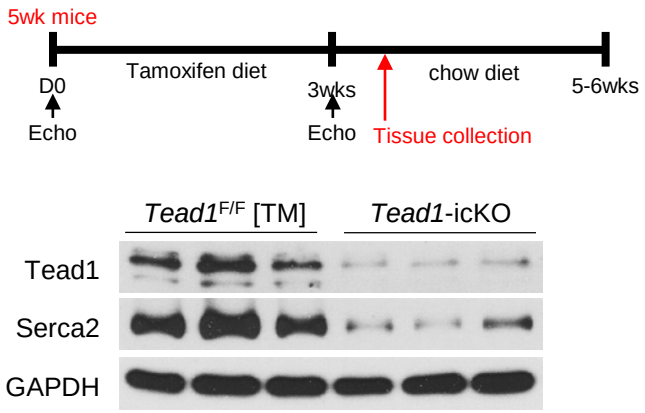
Supplemental Figure 3. Echocardiographic assessment at 3 weeks of TM-diet protocol. EF, ejection fraction; FS, fractional shortening; LVIDd, left ventricular internal diameter at end diastole; LVIDs, left ventricular internal diameter at end systole; RWT, relative wall thickness. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; data were analyzed by one-way ANOVA with Tukey's *post hoc* test. Comparisons between *Tead1* icKO mice (n=6) and *Tead*$^{F/F}$ chow diet mice (n=4), *Tead*$^{F/F}$ TM diet mice (n=4), *Myh6*-CreER TM diet mice (n=3) or *Tead*$^{F/F}$;*Myh6*-CreER chow diet mice (n=3) are indicated as *, $, # and † respectively. No significance was present among the four control groups.



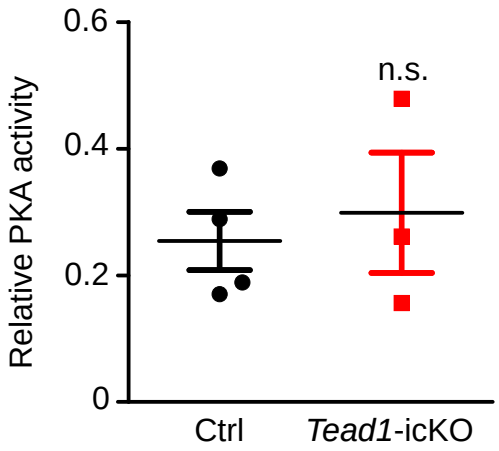
Supplemental Figure 4. Tead1 deletion did not increase apoptosis in the myocardium. Representative figures of the TUNEL staining in control and *Tead1*-icKO heart sections. Scale bars, 100μm.



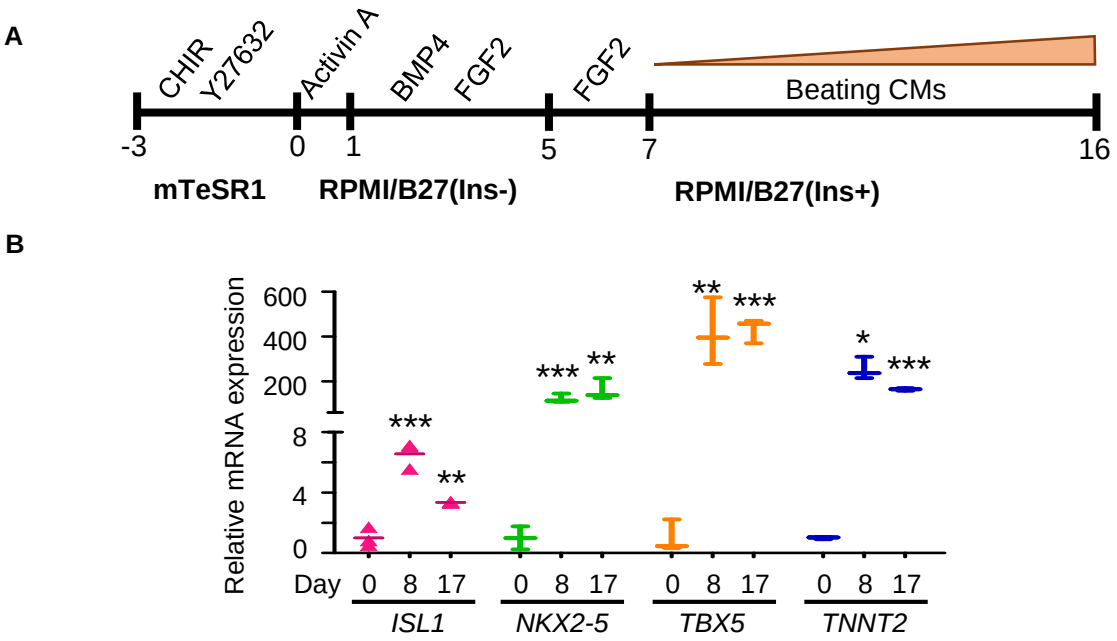
Supplemental Figure 5. Tead1 deletion in older (21-22 wk) mice (*Tead1* icKO) also led to severe dilated cardiomyopathy. (**A-B**) Echocardiographic assessment after six weeks of TM diet protocol. (**C**) Heart weight measurement normalized by body weight or tibial length. Values are shown as mean ± S.E.M. ***$P < 0.001$, **$P < 0.01$; data were analyzed by one-way ANOVA with Tukey's *post hoc* test. Comparisons between *Tead1* icKO mice (n=7-8, 4 males and 4-5 females) and *Tead*$^{F/F}$ chow diet mice (n=5, 3 males and 2 females), *Tead*$^{F/F}$ TM diet mice (n=9, 4 males and 5 females), *Myh6*-CreER TM diet mice (n=4-5, 2 males and 2-3 females) or *Tead*$^{F/F}$;*Myh6*-CreER chow diet mice (n=9, 3 males and 6 females) are indicated as *, $, # and † respectively. No significance was present among the four control groups. (**D**) H&E staining and WGA staining (red) showed disrupted cardiac muscle structures. Nuclei were stained with DAPI (blue). Yellow arrowheads indicating disrupted sarcomere structure and cellular vaculolation. Scale bar, 20μm.



Supplemental Figure 6. Tead1 is required for normal CM *Serca2* expression. Immunoblots from *Tead1*-icKO hearts after 3 weeks of TM-diet and 1 week chow.



Supplemental Figure 7. Protein Kinase A activity was unchanged in *Tead1*-icKO hearts. PKA activity in *Tead1*-icKO hearts (n=3) and ctrl hearts (n=4, 2 *Tead1*$^{F/F}$ TM diet and 2 *Myh6*-CreER TM diet).



Supplemental Figure 8. Differentiation of human induced pluripotent stem cells (hiPS) to cardiomyocytes. (**A**) Cardiomyocyte differentiation protocol. (**B**) mRNA expression levels of the cardiac maturation markers during the differentiation. See also synchronized contractions in these cells after differentiation in **Supplemental Movie 3**.