SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Materials and Methods

Procedure and Follow up of clinical study	Blood samples were collected in the stable phase during the first admission, and the serum human epididymis protein 4 (HE4) levels were measured using the CLIA method (Abbott). The coefficient of variation of HE4 among samples was less than 10%. The study primary endpoint was a composite of all-cause death, left ventricular assist device (LVAD) implantation, and hospitalization for heart failure (HF) events. Furthermore, composite of all-cause death and LVAD implantation were defined as secondary endpoint. Death, LVAD implantation and heart failure events were identified by searching the medical records and confirmed by direct contact with the patients, relatives, and caring physicians.
Echocardiography of clinical study	Left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), thickness of the interventricular septum, posterior ventricular wall and left atrial diameter were obtained from M-mode or two-dimensional images of parasternal long axis views. Left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic were (LVESV) were measured in apical 4-and 2-chamber windows by the Simpson method. Left ventricular (LV) ejection fraction (LVEF) was calculated by the modified Simpson method (Vivid 7®; GE-Vingmed Ultrasound).
Statistical analysis of Echo data	Univariable linear regression and logistic regression analysis for ΔLVEDVi (follow-up LVEDVi – baseline LVEDVi), ΔLVESVi (follow-up LVESVi – baseline LVESVi), and left ventricular reverse remodeling (LVRR) was performed using HE4 and other variables involved in LV remodeling. [12] Multivariable analysis was performed using the variables achieving significance at $p< 0.05$ on univariable analysis or clinically important variables to determine the factors associated with ΔLVEDVi, ΔLVESVi, and LVRR. LVRR was defined as the combined presence of: (1) an increase in LVEF of at least 10 points or a follow-up LVEF ≥ 50%; and (2) a decrease in LVEDDi of at least 10% or an LVEDDi ≤ 33 mm/m. [3]
CMR image acquisition and Image analysis	66 patients (76%) underwent cardiac magnetic resonance (CMR) and were checked for the presence of late gadolinium enhancement (LGE). All images were acquired using a 3.0 T scanner (Achieva 3.0 T X-series TX; Philips Medical Systems). We used electrocardiogram-gated cine imaging techniques with a segmented steady-state free precession sequence in the short and three

	long cardiac axes with LGE imaging as described previously. [24] Approximately 10 min after injection of 0.1 mmol/kg of a gadolinium-based contrast agent (Magnevist; Bayer Healthcare), we acquired two-dimensional inversion-recovery sequences, including the LV from base to apex. CMR images were independently analyzed by a cardiologist and a radiologist. Patients were then classified into LGE-positive or -negative groups. [24]
Mouse models and Procedures	Wild-type (WT) male mice on a BALB/cA background were used in this study. All procedures were performed in accordance with the Kumamoto University animal care guidelines, which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (publication No. 85-23, revised 1996). The study was approved by the Animal Research Ethics Committee of Kumamoto University (#A2019-122). WT male mice with BALB/cA background were purchased from Kyudo company (Saga, Japan). The mice were housed in a temperature- and humidity-controlled (24°C) room on a 12 h light/dark cycle. The 8-week-old mice were anesthetized with isofluran. The mouse myocardial infarction (MI) model was generated as previously described. [13, 14] Briefly, the trachea was cannulated with a polyethylene tube connected to a respirator (tidal volume, 0.6 mL: frequency, 110 breaths per minute). A left thoracotomy was performed between the fourth and fifth ribs. The pericardial tissue was removed, and the left anterior descending artery was visualized under a microscope and permanently ligated with 7-0 silk suture. Sham-operated mice underwent surgery but not left anterior descending artery ligation. At 4 weeks after MI surgery, mouse body weight, echocardiographic data, and urine output were analyzed prior to sacrifice. The mouse DCM model was generated using knock-in mice on the genetic background of BALB/cJ, in which three base-pairs coding for K210 in cTnT were deleted from the endogenous Tnnt2 gene as previously described.[15] 5 Homozygous mutant mice and WT mice were obtained by crossing heterozygous mutant mice, and were used as DCM and control models, respectively. MI surgery model and six-week old DCM model mouse were anesthetized with overdose isoflurane, and hearts, kidney, lung and liver were rapidly excised, and freeze clamped for subsequent analyses. *In vivo* analysis and post-euthanasia myocardial histological and molecular analyses were performed by investigators who were blinded to the experimental groups.

Echocardiography, in vivo	At 1 day before harvest, echocardiography was performed using the Xario system (Toshiba, Tokyo, Japan) with a 12-MHz linear array transducer. Heart rates and respiratory rates were continuously monitored. LV wall thickness and LV systolic and diastolic dimensions were measured in M-mode. LV percent fractional shortening were calculated. These analyses were performed by investigators who were blinded to the mice models.
Cell culture, harvest and incubation of neonatal rat cardiomyocytes and fibroblasts	Primary neonatal rat cardiomyocytes and fibroblasts were isolated from 2-day-old Wistar rats (Japan SLC, Inc). The hearts were harvested and minced and allowed to digest in 1 mg/ml Type II collagenase (Sigma Chemical Co.). After digestion, cardiomyocytes and fibroblasts were separated by Percoll density gradient centrifugation and incubated under 5% CO_2 and 37°C in 1 g/L glucose Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), ampicillin (10 U/µl), streptomycin (10 µg/µl), and amphotericin B (25 µg/ml).
Quantitative real time PCR analysis	RNA was extracted using a RNeasy Mini Kit (QIAGEN). cDNA synthesis was performed using PrimeScript RT Master mix (TAKARA) according to the manufacturer's directions. A quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was carried out for CoI1a1, CoI3a1, alpha smooth muscle actin (αSMA), plasminogen activator inhibitor-1 (PAI-1), fibroblast growth factor 2 (FGF2), smooth muscle protein 22 (SM22), periostin, fibronectin, transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). The reactions were carried out in technical duplicates. Primers were utilized with SYBR Green PCR Master Mix (BIO-RAD) in CFX384 Real-Time System (BIO-RAD). The data processing is based on a standard curve-based method for relative qRT-PCR. Measurements were standardized to expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S. For *in vivo* studies, qRT-PCR was carried out for HE4 and GAPDH. **Table S1** lists the primer sequences used in this study.
Western blot analysis	Cells were scraped and lysed with 1% SDS lysis buffer containing protease inhibitor cocktail (Thermo). The samples were centrifuged at 20400 g for 15 min. The supernatant was collected, and protein concentrations were determined using a Pierce BCA Protein assay Kit (Code: 23225, Thermo). After proteins were transferred to a PVDF Blotting membrane (GE Healthcare Life Sciences), the membrane was blocked with 100 mM Tris-HCl, pH 7.5, 0.9% NaCl, and 0.1% Tween 20 (TBST) containing 5% nonfat dry milk for 1 hour and then

	incubated with primary antibodies at 4°C overnight. The primary antibodies were as follows: anti-HE4 (ab200828, Abcam), anti-type I collagen (#84336S, 1, CST), anti-αSMA (ab5694, Abcam), anti-GAPDH (#2118, CST), ERK (#9102, CST), p-ERK (#4377, CST), Akt (#9272, CST), p-Akt (#9271, CST), Smad2/3 (#8685, CST), p-Smad2 (#18338, CST), p-Smad3 (#9520, CST), JNK (#9252, CST), p-JNK (#9251, CST), p38 (#9211, CST), p-p38 (#4511, CST). Membranes were then incubated with HRP-secondary antibodies for 1 hour at room temperature. Immunoreactive proteins were detected using ECL Prime (GE Healthcare UK Ltd.) with LAS-4000 Imaging system (FUJIFILM).
Immunofluorescence staining for fibroblast phenotyping in vitro	After 24 hours in culture, cells were fixed with 4% paraformaldehyde diluted in PBS for 20 minutes at room temperature. Further, cells were permeabilized with 0.1% Triton X-100. To assess the degree of differentiation, cells were double stained for F-actin using rhodamine-phalloidin (1:1000 dilution, P1951-.1MG, Sigma) and for α-smooth muscle actin, using an antibody against αSMA (1:500 dilution, #102M4804V, Sigma), to characterize stress fibers. The coverslips were mounted using Prolong Gold anti-fade with DAPI (1:1000, NX034, Dojindo). Fluorescence imaging was done using a confocal microscope TCS SP8 LS with 20X/0.4 objective. Degree of differentiation was evaluated by counting the number of cells positive for either F-actin or αSMA stress fibers in three randomly chosen images with a minimum of 80 cells counted per sample. Results from these 3 samples were averaged.

Table S1. Primer sequences used for quantitative real-time PCR.

	Forward Primer	Reverse Primer
HE4 (human)	CCCAATGATAAGGAGGGT	ATTTCATCTGGCCAGGAC
HE4 (mouse)	AACCAATTACGGACTGTGTGTT	TCGCTCGGTCCATTAGGCT
αSMA (rat)	GGGATCCTGACCCTGAAG	AGTGGTGCCAGATCTTTT
PAI-1 (rat)	ACATCCTGGAACTGCCCT	TGGTCATGTTGCTCTTCC
FGF2 (rat)	CGCCTGGAGTCCAATAAC	ACAGTATGGCCTTCTGTC
SM22 (rat)	GGAACAGGTGGCTCAATTCT	CCCAAAGCCATTACAGTCCT
collagen1a1 (rat)	GATGGACTCAACGGTCTC	GGCAGGAAGCTGAAGTCA
collagen3a1 (rat)	ATGCATGTTTCTCCGGTTT	CTCGGAATTGCAGAGACC
TGF-β1 (rat)	CGGACTACTACGCCAAAG	TTCCCGAATGTCTGACGT
TGF-β1 (human)	GCGTGCTAATGGTGGAAACC	GCTTCTCGGAGCTCTGATGT
Periostin (rat)	CAAACCACTTTCACGGACCT	TTGTTCACAGGCGCTAACAG
Fibronectin (rat)	CAGCCCCTGATTGGAGTC	TGGGTGACACCTGAGTGAAC
TNF-α (human)	GGACCTCTCTCTAATCAGCCC	TGAAGAGGACCTGGGAGTAGA
IL-6 (human)	TACATCCTCGACGGCATCTC	TGGCTTGTTCCTCACTACTCT
GAPDH (rat)	TCAAGAAGGTGGTGAAGCAG	AGGTGGAAGAATGGGAGTTG
18S (human)	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT
ANP (rat)	AGGCCATATTGGAGCAAATC	CATCTTCTCCTCCAGGTGGT
β-MHC (rat)	CTGGCACCGTGGACTACAAT	GCCCTTGTCTACAGGTGCAT

PCR, polymerase chain reaction; HE4, human epididymis protein 4; αSMA, alpha smooth muscle actin; PAI-1, plasminogen activator inhibitor-1; FGF2, Fibroblast growth factor 2; SM22, smooth muscle protein 22; TGF-β1, Transforming Growth Factor-β1; TNF-α, tumor necrosis factor-α; IL-6, Interleukin-6; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; ANP, atrial natriuretic peptides; β-MHC, β-myosin heavy chain

Table S2. Baseline characteristics of the control and DCM groups.

	Control (n = 59)	DCM (n = 87)	*p* value
HE4, pmol/L	44.1 [35.6-52.9]	59.65 [49.0-86.2]	<0.0001
Age, y	69 ± 3	60 ± 15	<0.0001
Male sex, n (%)	30 (51)	62 (71)	0.012
Body Mass Index, kg/m2	23.8 ± 3.9	23.8 ± 4.2	0.902
Systolic blood pressure on admission, mmHg	123 ± 17	114 ± 17.4	0.002
Hypertension, n (%)	36 (61)	30 (35)	0.002
Diabetes mellitus, n (%)	17 (29)	15 (17)	0.105
Dyslipidemia, n (%)	43 (73)	34 (40)	<0.0001
Current smoker, n (%)	14 (24)	14 (16)	0.250
Atrial fibrillation, n (%)	3 (5)	22 (25)	0.001
Non-Sustained ventricular tachycardia, n (%)	1 (2)	20 (23)	<0.0001
Ventricular fibrillation, n (%)	0 (0)	3 (3)	0.150
Prior HF hospitalizations, n (%)	0 (0)	31 (36)	<0.0001
Laboratory examination parameters			
White blood cell, /µL	6066 ± 1798.6	6387 ± 1938	0.313
Hemoglobin, g/dL	13.9 ± 1.64	14.2 ± 2.16	0.493
hs-cTnT, ng/mL	0.007 [0.003-0.010]	0.015 [0.009-0.029]	0.013
BNP, pg/mL	16.6 [9.9-29.8]	249.0 [72.7-654.3]	<0.0001
Albumin, g/dL	4.2 ± 0.33	3.9 ± 0.5	<0.0001
Serum sodium, mEq/L	140 ± 1.8	139 ± 2.6	0.011
Creatinine, mg/dL	0.71 ± 0.16	0.93 ± 0.27	<0.0001
eGFR, mL/min*m^2	76 ± 11.8	65 ± 15.4	<0.0001
T-bil, mg/dL	0.8 ± 0.29	1.0 ± 0.59	0.009

CRP, mg/ml	0.04 [0.02-0.08]	0.13 [0.05-0.36]	0.021
HbA1c (NGSP)	6.0 ± 1.00	5.8 ± 0.7	0.305
Electrocardiogram parameters			
Heart rate, bpm	68 ± 12.1	78 ± 18.5	0.001
CLBBB, n (%)	0 (0)	13 (15)	0.002
QRS duration, msec	99 ± 11.6	114.5 ± 29.3	<0.0001
Echocardiogram parameters			
LVEF, %	65 ± 4.6	33 ± 10.7	<0.0001
LVEDD, mm	44 ± 5.0	60 ± 8.7	<0.0001
LVESD, mm	27 ± 4.2	51 ± 10.2	<0.0001
Intraventricular septal thickness, mm	9.7 ± 1.5	9.3 ± 1.6	0.151
LV posterior wall thickness, mm	9.7 ± 1.6	10.0 ± 1.6	0.348
LVEDVi, ml/L/min/m^2	38 ± 15.0	97 ± 35.2	<0.0001
LVESVi, ml/L/min/m^2	13 ± 5.9	67 ± 31.6	<0.0001
LAD, mm	34 ± 5.1	42 ± 8.2	<0.0001

Data are number of patients (%), mean ± standard deviation (SD), and median (interquartile range).

DCM, dilated cardiomyopathy; HE4, human epididymis protein 4; HF, heart failure; hs-cTnT, high-sensitivity cardiac troponin T; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; T-bil, total bilirubin; CRP, c-reactive protein; CLBBB, complete left bundle branch block; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LV, left ventricular; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LAD, left atrium diameter

Table S3. Univariate and multivariate linear regression analyses of ΔLVEDVi.

	Univariate Analysis		Multivariate Analysis	
	β-coefficient	*p* Value	β-coefficient	*p* Value
Log (HE4), per 1 pmol/L increment	0.344	0.006	0.518	0.001
Age, per 1-year increment	0.086	0.499	-0.094	0.484
NYHA class ≥ III	-0.089	0.484		
Systolic blood pressure on admission, 1 mmHg increment	0.007	0.954		
Hypertension	-0.091	0.473		
Diabetes mellites	-0.029	0.818		
β-blocker on discharge	-0.209	0.098	-0.166	0.188
ACE-I or ARB on discharge	-0.092	0.469		
Log (BNP), per 1 pg/mL increment	-0.133	0.294	-0.194	0.168
Log (Creatinine), per 1 mg/dL increment	-0.033	0.794	-0.192	0.146
eGFR, per 1 mL/(min·m2) increment	-0.053	0.677		
Log (CRP), per 1 mg/m increment	0.107	0.407		
QRS duration, per 1 mm increment	-0.057	0.657		
CLBBB	0.104	0.412		
LVEF, per 1 % increment	0.299	0.017		
LVEDD, mm	-0.381	0.002	-0.359	0.009
LVESD, mm	-0.366	0.003		
LGE	0.039	0.778	0.091	0.777

LVEDVi, left ventricular end-diastolic volume index; HE4, human epididymis protein 4; NYHA, New York Heart Association; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; CRP, c-reactive protein; CLBBB, complete left bundle branch block; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LGE, late gadolinium enhancement

Table S4. Univariate and multivariate linear regression analyses of ΔLVESVi.

	Univariate Analysis		Multivariate Analysis	
	β-coefficient	*p* value	β-coefficient	*p* value
Log (HE4), per 1 pmol/L increment	0.344	0.006	0.508	0.001
Age, per 1-year increment	0.086	0.499	-0.072	0.592
NYHA class ≥ III	-0.089	0.484		
Systolic blood pressure on admission, 1 mmHg increment	0.007	0.954		
Hypertension	-0.091	0.473		
Diabetes mellites	-0.029	0.818		
β-blocker on discharge	-0.209	0.098	-0.153	0.227
ACE-I or ARB on discharge	-0.092	0.469		
Log (BNP), per 1 pg/mL increment	-0.133	0.294	-0.195	0.168
Log (Creatinine), per 1 mg/dL increment	-0.033	0.794	-0.175	0.192
eGFR, per 1 mL/(min*m2) increment	-0.053	0.677		
Log (CRP), per 1 mg/m increment	0.107	0.407		
QRS duration, per 1 mm increment	-0.057	0.657		
CLBBB	0.104	0.412		
LVEF, per 1 % increment	0.299	0.017		
LVEDD, mm	-0.381	0.002		
LVESD, mm	-0.366	0.003	-0.364	0.009
LGE	0.039	0.778	0.109	0.356

LVESVi, left ventricular end-systolic volume index; HE4, human epididymis protein 4; NYHA, New York Heart Association; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; CRP, c-reactive protein; CLBBB, complete left bundle branch block; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LGE, late gadolinium enhancement

Table S5. Univariate and multivariate logistic regression analyses of LVRR positive.

	Univariate Analysis		Multivariate Analysis	
	B	p value	B	p value
Log (HE4), per 1 pmol/L increment	-0.398	0.001	-0.615	<0.0001
Age, per 1 year increment	-0.209	0.094	-0.032	0.830
NYHA class ≥ III	-0.123	0.329		
Systolic blood pressure on admission, 1 mmHg increment	0.163	0.195		
Hypertension	0.150	0.235		
Diabetes mellites	0.072	0.569		
β-blocker on discharge	0.003	0.983	0.074	0.584
ACE-I or ARB on discharge	-0.070	0.585		
Log (BNP), per 1 pg/mL increment	0.058	0.646	0.419	0.008
Log (Creatinine), per 1 mg/dL increment	-0.109	0.387	0.241	0.096
eGFR, per 1 mL/(min*m2) increment	0.148	0.238		
Log (CRP), per1 mg/m increment	-0.151	0.239		
QRS duration, per 1 mm increment	-0.060	0.636		
CLBBB	-0.177	0.158		
LVEF, per 1 % increment	-0.050	0.694		
LVEDD, mm	-0.045	0.721	-0.260	0.068
LVESD, mm	-0.087	0.492		
LGE	0.133	0.329	0.026	0.844

LVRR, left ventricular reverse remodeling; HE4, human epididymis protein 4; NYHA, New York Heart Association; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; CRP, c-reactive protein; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LGE, late gadolinium enhancement

Table S6. Results of multivariate Cox regression analysis for the primary endpoint.

Factor	Multivariate Analysis		
	HR	95% CI	*p* value
Model 1			
Log HE4	7.91	3.49-17.94	<0.0001
Age (years)	0.97	0.95-1.00	0.074
Model 2			
Log HE4	5.07	2.25-11.43	<0.0001
NYHA class ≥ III	2.12	0.58-3.82	0.405
Model 3			
Log HE4	4.92	2.34-10.35	<0.0001
Systolic blood pressure (mmHg)	0.97	0.93-1.00	0.058
Model 4			
Log HE4	5.09	2.31-11.19	<0.0001
Prior HF hospitalizations (yes)	3.23	1.27-8.21	0.014
Model 5			
Log HE4	4.29	1.85-9.94	0.001
Sodium (mEq/L)	0.90	0.78-1.03	0.130
Model 6			
Log HE4	5.09	2.05-12.64	<0.0001
Log Creatinine	1.57	0.28-8.76	0.606

	HR	95% CI	P value
Model 7			
Log HE4	6.49	2.98-14.14	<0.0001
T-bil (mg/dL)	2.32	1.26-4.28	0.007
Model 8			
Log HE4	6.68	2.43-18.37	<0.0001
Log CRP	0.92	0.64-1.31	0.640
Model 9			
Log HE4	5.16	2.26-11.76	<0.0001
Log BNP	1.16	0.77-1.75	0.474
Model 10			
Log HE4	8.81	3.78-20.51	<0.0001
LVEDD (mm)	1.12	1.06-1.18	<0.0001
Model 11			
Log HE4	9.13	3.78-22.08	<0.0001
LGE (yes)	2.55	0.89-7.31	0.082

HR, hazard ratio; HE4, human epididymis protein 4; NYHA, New York Heart Association; HF, heart failure; T-bil, total bilirubin; CRP, c-reactive protein; BNP, B-type natriuretic peptide; LVEDD, left ventricular end-diastolic diameter; LGE, late gadolinium enhancement

Table S7. Parameters at harvest in BALB/cA WT and genetically induced HFrEF model mice (Homo).

	WT (n = 7)	Homo (n = 7)	*p* value
Age, week	6	6	1.000
Body weight, g	18.3 ± 1.15	17.1 ± 1.76	0.134
Heart rate, bpm	715 ± 43.6	681 ± 57.8	0.243
Echocardiogram parameters at 1 day before harvest			
LVEDD, mm	2.63 ± 0.39	4.74 ± 1.03	0.001
LVESD, mm	1.47 ± 0.42	3.91 ± 1.13	0.001
Intraventricular septal thickness, mm	0.59 ± 0.09	0.44 ± 0.05	0.005
LV posterior wall thickness, mm	0.63 ± 0.14	0.36 ± 0.05	0.001
%FS, %	44.6 ± 11.1	18.5 ± 8.93	<0.0001
Organ weight at harvest			
Heart/tibial length, mg/mm	7.07 ± 0.62	13.5 ± 3.63	0.003
Lung/tibial length, mg/mm	8.92 ± 0.80	13.9 ± 5.93	0.070
Kidney/tibial length, mg/mm	8.93 ± 0.62	8.36 ± 0.52	0.091

Values are mean ± SD. WT, Wild-type; HFrEF, heart failure with reduced ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LV, left ventricular; %FS, % fractional shortening

Table S8. Parameters at harvest 4 weeks after MI-induced HFrEF model mice in BALB/cA WT mice.

	Sham-operated (n = 7)	MI (n = 7)	*p* value
Age, week	12	12	1.000
Body weight, g	23.8 ± 0.93	23.8 ± 1.41	0.976
Heart rate, bpm	643 ± 59.0	677 ± 41.8	0.241
Echocardiogram parameters at 1 day before harvest			
LVEDD, mm	3.00 ± 0.59	3.71 ± 0.42	0.025
LVESD, mm	1.30 ± 0.51	2.69 ± 0.53	<0.0001
Intraventricular septal thickness, mm	0.63 ± 0.10	0.30 ± 0.10	<0.0001
LV posterior wall thickness, mm	0.64 ± 0.08	0.50 ± 0.12	0.021
%FS, %	58.0 ± 10.2	28.1 ± 7.32	<0.0001
Organ weight at harvest			
Heart/tibial length, mg/mm	8.28 ± 0.76	9.35 ± 1.38	0.103
Lung/tibial length, mg/mm	8.31 ± 0.38	8.58 ± 0.69	0.387
Liver/tibial length, mg/mm	63.5 ± 5.07	66.0 ± 6.72	0.443
Kidney/tibial length, mg/mm	12.3 ± 0.82	12.2 ± 2.03	0.927

Values are mean ± SD. MI, myocardial infarction; HFrEF, heart failure with reduced ejection fraction; WT, Wild-type; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LV, left ventricular; %FS, % fractional shortening

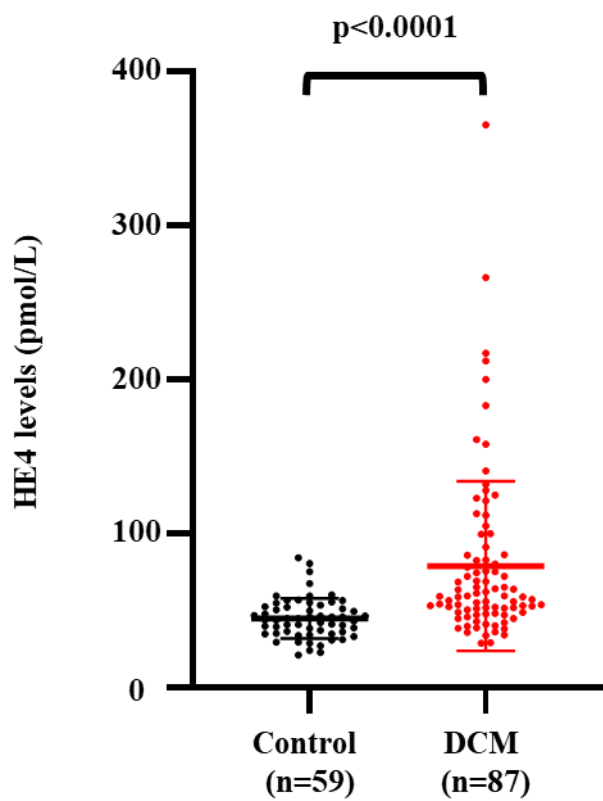**Figure S1. Flow chart of patient enrollment protocol in the present study.**

We excluded 9 patients because they were undergoing hemodialysis or had a tumor. According to the median value of HE4 (59.65 pmol/L), we divided all DCM patients into the high HE4 group (n = 43) and the low HE4 group (n = 44).

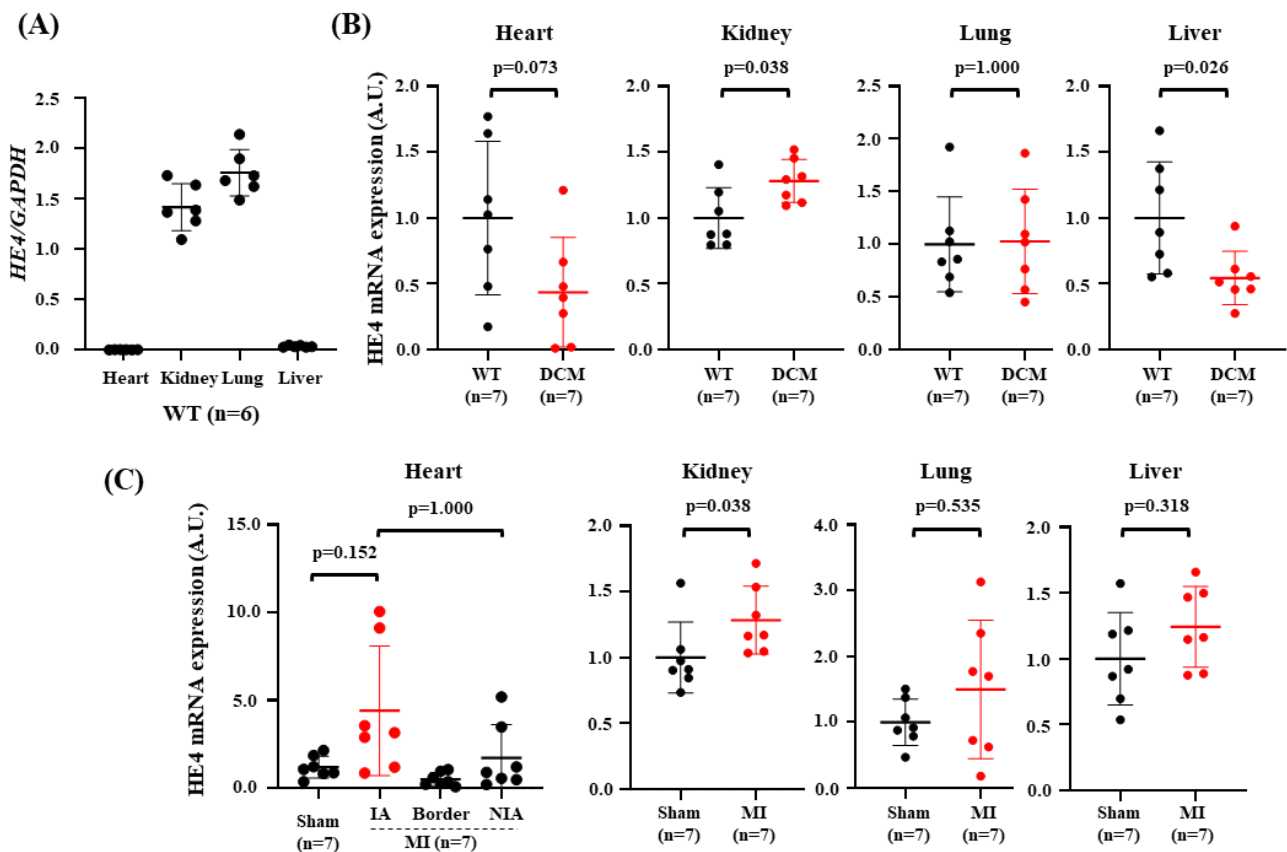HE4: human epididymis protein 4, DCM: dilated cardiomyopathy

Figure S2. Serum HE4 levels in the control and DCM group.

Unpaired t-tests were used to compare groups.

HE4: human epididymis protein 4, DCM: dilated cardiomyopathy

Figure S3. HE4 is upregulated at kidney tissue in situation of HFrEF.

(A) The expression profile of HE4 in the heart, kidneys, lungs, and liver of BALB/cJ WT mice (n = 6). GAPDH was used as an internal control. (B) Quantitative evaluation of HE4 mRNA expression normalized to GAPDH mRNA expression in each tissue from DCM model mice (n = 7) and their WT littermates (n = 7) using the standard curve-method. The ratio of DCM mice to WT mice is shown. (C) Quantitative evaluation of HE4 mRNA expression normalized to GAPDH in heart, kidneys, lungs, and liver from MI model mice (n = 7) and sham operated mice (n = 7) using standard curve-method. The ratio of MI to sham operated mice is shown.

HE4: human epididymis protein 4, WT: wild type, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, MI: myocardial infarction, IA: infarcted area, NIA: non-infarcted area

Figure S4. Overexpression of HE4 have no impact on the expression of inflammatory-related and fibrosis-related genes in HEK293T cells.
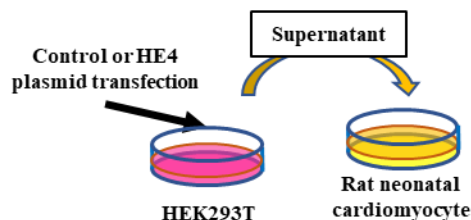

(A) Experimental scheme for HE4 overexpression. (B) qRT-PCR analysis in HEK293T cells transfected with control or HE4 plasmid. 18S was used as an internal control. (C) WB for HE4 in supernatant of control or HE4 plasmid transfected HEK293T. (D) qRT-PCR analysis in HEK293T cells transfected with control or HE4 plasmid. 18S was used as an internal control.

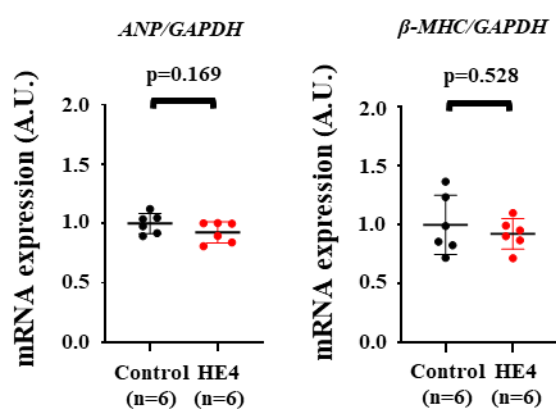Unpaired t-tests with Welch's correction were used to compare groups.

WB: western blotting, HE4: human epididymis protein 4, HEK293T: human embryonic kidney 293T, qRT-PCR: quantitative reverse-transcription polymerase chain reaction, TNF-α: tumor necrosis factor-α, TGF-β1: transforming growth factor-β1, IL-6: interleukin-6

Figure S5. The addition of the supernatant that contained HE4 show no elevations of hypertrophy-related genes expression in cardiomyocytes.
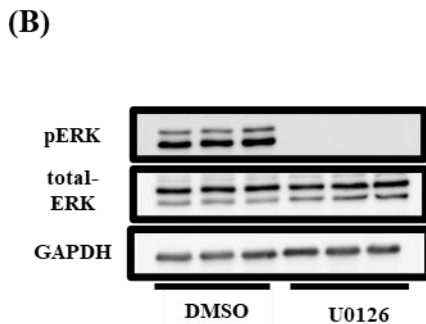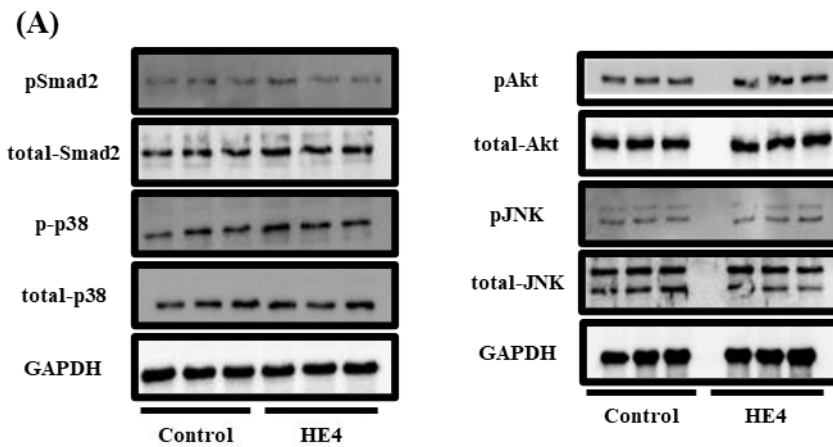
(A)

(B)

(A) Experimental scheme for HE4 overexpression and transfer to cardiomyocyte. (B) Cardiac hypertrophy-related genes were evaluated by qRT-PCR. The measurements were standardized to expression of the GAPDH.

Unpaired t-tests with Welch's correction were used to compare groups.

HE4: human epididymis protein 4, HEK293T: human embryonic kidney 293T, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, ANP: atrial natriuretic peptides, β-MHC: β-myosin heavy chain, qRT-PCR: quantitative reverse-transcription polymerase chain reaction

Figure S6. HE4 does not affect the activity of Smad2, p38 MAP kinase, Akt, and JNK

(A)

(B)

(A) WB for intracellular signaling other than ERK in cardiac fibroblasts treated with HEK293T culture medium. (B) WB for ERK in cardiac fibroblasts treated with HEK293T culture medium and U0126, MEK 1/2 inhibitor, or DMSO.

WB: western blotting, ERK: extracellular signal-regulated kinase, HE4: human epididymis protein 4, HEK293T: human embryonic kidney 293T, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, DMSO: dimethyl sulfoxide