Supplementary Material

Downregulation of FKBP5 promotes atrial arrhythmogenesis

Xiaolei Wang*, Jia Song*, Yue Yuan*, Luge Li, Issam Abu-Taha, Jordi Heijman, Liang Sun, Shokoufeh Dobrev, Markus Kamler, Liang Xie, Xander H.T. Wehrens, Frank T. Horrigan, Dobromir Dobrev, Na Li

*These authors contribute equally

Expanded Materials & Methods

Animal studies

All studies involving mice were performed according to protocols approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine and conformed to the Guide for the Care and Use of Laboratory Animals published by National Institutes of Health. Whole body knockout *Fkbp5*$^{-/-}$ mice were purchased from the Jackson Laboratory (Strain #:017989). The *Fkbp5*$^{floxl/flox}$ mice were generated by crossbreeding the *Fkbp5*$^{tm1a(KOMP)Wtsi}$ mice (purchased from Knockout Mouse Project) with FLP transgenic mice (The Jackson Laboratory). Afterwards, the Fkbp5flox/flox mice were crossbred with inducible CM-specific Cre mice (*Myh6*$^{MCM/+}$) to generate *Myh6*$^{MCM/+}$;*Fkbp5*$^{flox/flox}$ mice, of which the offspring maintained the Mendelian ratio. At the age of 6 weeks, *Myh6*$^{MCM/+}$;*Fkbp5*$^{flox/flox}$ (cKD) and *Myh6*$^{+/+}$; *Fkbp5*$^{flox/flox}$ (as control, Ctl) were injected with tamoxifen (50mg/kg, i.p.) for 5 days to induce the CM-specific knockdown of *Fkbp5*. For interventional studies, two weeks after tamoxifen injections, cKD mice were injected with either 17-AAG (50mg/Kg in saline, i.p.) or vehicle (100μL/20g body weight, i.p.), once every two days for 2 weeks. We used mice that were matched for age (2- to 5-month-old), sex, and genetic background whenever possible. Mice were housed in AAALAC approved facilities, with standard room temperature and a fixed 12:12hr light/dark cycle. All strains were maintained on a C57Bl6/J genetic background.

Cell culture and transfection

HEK293T cells were seeded in 6-well plates and cultivated in DMEM-medium (Gibco) with 10% FBS. Plasmids were transferred using Lipofectamine 2000 in DMEM-medium to the HEK293T cells. 48 hours after transfections, GFP were directly visualized using a Zeiss Axio observer fluorescence microscopy. For hypoxia treatment: H9C2 cells were incubated in hypoxia chamber (2% O_2) for 3 hours before the harvest of cell lysates. The cells incubated in normoxia condition for the same duration were used as control (Ctl). For $CoCl_2$ treatment: H9C2 cells

were treated with CoCl2 solutions at a final concentration of 100 μm. After treatment, the cells were collected and prepared for next assays. For HSP90 or proteasome inhibition, cells were incubated with 17-AAG (ApexBio A4054) or MG-132 (ApexBio A2585) dissolved in medium for 3 hours, prior to harvest for Western blots.

Programmed intracardiac stimulation

Programmed intracardiac stimulation was performed to assess AF-inducibility as previously described.[20] Briefly, AF was induced by an overdrive pacing protocol, starting with 2-second burst pacing at a cycle length (CL) of 40-ms and decreasing in each successive burst by a 2-ms decrement to a CL of 10-ms. AF was defined as the occurrence of rapid, fragmented atrial electrograms with irregular R-R intervals lasting at least 1-second. To determine whether AF-inducibility was reproducible, mice were subjected to the same atrial burst-pacing protocols for 3 times, and only the mice in which AF could be evoked by burst-pacing in at least 2 out of 3 attempts were considered as AF-positive. The incidence of inducible AF was calculated as the percentage of the AF-positive mice divided by the total number of mice studied. The experimenter was blinded to genotype or viral gene transfer status of mice.

Quantitative PCR (qPCR)

RNA was extracted from the heart tissue using the Trizol reagent (Invitrogen). For reverse transcription, 1 μg of total RNA was used in a final reaction volume of 20 μL containing 4 μL iScript Reverse transcription Supermix (Bio-Rad 1708841). After reverse transcription, the cDNA was three times diluted. PCR was performed in duplicate for 40 cycles using 1ul of the volume of the cDNA in a total volume of 20 μL that included 10 μL of PowerUP SYBR Green Master Mix (Thermo Fisher A25742). mRNA was quantified by real-time PCR analysis using the QuantStudio5 (Thermo Fisher). Thermocycler conditions were 40 cycles of denaturation at 95oC for 15 seconds and annealing and extension step of 60oC for 60 seconds. The ΔCT

method was used to quantify all relative mRNA levels using Rpl7 as the reference and internal standard. All the primers used for qPCR are listed in **Supplementary Table S12.**

Sarcolemmal and cytosolic protein isolation

To isolate the sarcolemmal fraction, cells or atrial tissue were homogenized in the buffer containing (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 150 mM NaCl, with added including 1mM PMSF, PIC, and PhosStop) with a homogenizer at 20,000 rpm, followed by centrifugation at 600g for 5 minutes to remove debris and nuclei. Subsequently, sarcolemmal fraction was pelleted at 100,000g for 30 minutes at 4°C, while the supernatant contained the cytosolic fraction. The pellet containing the membrane fraction was then resuspended in 100 - 250 mL buffer containing (1% Triton X-100, 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 150 mM NaCl including 1mM PMSF, PIC and PhosStop) and incubated at 4°C for 30 minutes, followed by centrifugation at 16,000g for 10 minutes at 4°C. The supernatant contains the enriched sarcolemmal proteins. Sarcolemmal- and cytosolic-proteins were frozen in liquid N2 and stored at -80°C.

Western Blotting

Cell lysates or atrial and ventricular homogenates were treated with RIPA buffer on ice for extraction. A total of 60 μg protein for each sample was reduced with β-mercaptoethanol for 30 minutes. Proteins were separated using 10% polyacrylamide gels for 1 hour and transferred onto PVDF membrane overnight at 4°C. Blocking was done with 5% BSA in TBS buffer. Primary antibodies against CD68 (Abcam, ab201973, 1:1000), Collagen I (Abcam, ab34710; 1:1000), MMP9 (Santa Cruz, sc-393859; 1:1000), αSMA (Invitrogen, MA1-06110; 1:1000), NCX1 (Abcam, ab2869; 1:500), FKBP5 (Abcam, ab126715; 1:1000), RyR2 (Invitrogen, PA5-77717; 1:1000), Cav1.2 (Alomone, ACC-003; 1:1000), SERCA2a (Invitrogen, MA3-919; 1:1000), PMCA (Invitrogen, MA3-914; 1:1000), HIF-1α (CST, 36169; 1:1000), GAPDH (CST, 97166; 1:1000), Gb (Santa Cruz, sc-378; 1:500), Caveolin-3 (Abcam, ab87770; 1:1000),

Histone H3 (CST, 3638; 1:1000) were added to the membrane and incubated at 4°C overnight, respectively. After washing 3 times with TBST, fluorescent-linked secondary antibodies were added according to the species of the primary antibodies (Invitrogen, A32729; 1:10000, and Invitrogen, A32735; 1:10000). After that, wash the membrane 3 times with TBST. The membranes were analyzed in a LiCor Odyssey scanner.

Chromatin Immunoprecipitation (ChIP)

ChIP assay was performed following the instruction of Simple ChIP Plus Enzymatic Chromatin IP Kit (Cell Signaling Technology, #9005). To measure the HIF-1α enrichment, chromatins were isolated from the formaldehyde cross-linked cardiac tissue (cardiac tissues in the oxygenated KB buffer (normoxia) or the KB buffer pre-treated in hypoxia chamber (2% O_2 overnight) for 2 hours before crosslink.), which were then digested to produce 0.2- to 1-kb DNA fragments by Micrococcal Nuclease. Nucleus were sonicated using a VirTis Virsonic 100 Ultrasonic Homogenizer/Sonicator at setting 6 with a 1/8-inch probe. Lysates were clarified by centrifugation at 9,400g in a microcentrifuge for 10 minutes at 4°C. Transfer supernatant to a new tube and diluted as desired. The supernatant was diluted, portion of which was taken out as input. The remaining supernatant was separated uniformly into 2 new tubes. One tube was incubated with HIF-1α antibody (CST Cat#D1S7W; 1:1000) overnight at 4°C, the other was used as negative control. 30 μL of Protein G Magnetic Beads were added to each immunoprecipitation (IP) reaction and incubate for 2 hours at 4°C with rotation. Pellet protein G magnetic beads in each IP and input by placing the tubes in a magnetic separation rack. Protein G magnetic beads were washed by adding 1 mL of low salt washing buffer to the beads and incubating at 4°C for 5 minutes with rotation for 3 times. 1 mL of high salt washing buffer was added to the beads and incubated at 4°C for 5 minutes with rotation. The beads were collected and. 150 μL 1X ChIP Elution Buffer was added to each IP and Input samples. The supernatant was collected after incubating at 65°C for 15 minutes. After reversal of protein-DNA cross-links, the DNA is purified using DNA purification spin columns. The purified

DNA was resuspended in double-distilled water, and enriched DNA fragments were quantified by qPCR with the primers listed in Supplementary Table S4. Input samples were used for normalization in qPCR results.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed following the instruction of Electrophoretic mobility shift assay (EMSA kit (Invitrogen E33075). The HIF-1α protein was extracted from the HEK293 cells as previous description. The 5X binding buffer (Component E) contains: 750mM KCl, 0.5 mM dithiothreitol, 0.5mM EDTA, 50 mM Tris, pH7.4. The increments of HIF-1α were 20 ng, 50 ng and 100 ng. 60 ng DNA probe was added and incubated in room temperature for 30 minutes. At the end of incubation period, add 2 μL of 6X loading solution (Component D) for each 10 μL of reaction mixture. The DNA-protein complex was separated by electrophoresis using nondenatured polyacrylamide gel. Once electrophoresis stopped, the gel was placed in a clean plastic staining container, adding 1XSYBR GREEEN EMSA staining solution to cover the gel for 20 minutes and protected from light. Afterwards, gel was washed with distilled H_2O for 10 seconds, followed with imaging under 300 transillumination UV.

Histology

Heart samples were fixed by 4% PFA, embedded in paraffin, and cut into 5 μm sections. Picrosirius Red and Masson's trichrome were performed on paraffin embedded sections then examined under ordinary polychromatic light to determine extent of cardiac fibrosis. For each section, 4 to 6 images were acquired from randomly selected fields. Image analysis was conducted using FIJI. The region of interest was outlined with Polygon selection tool and was saved in ROI manager tool. Under the setting of RGB stack type, threshold was adjusted to reflect total tissue area (including both blue and red area) and the fibrosis area (red-only area in Picrosirius Red staining, or blue-only area in Masson Trichrome staining). The threshold level was consistent in each image. The percentage of fibrosis was calculated as the ratio of

fibrosis area to total tissue area. The representative image for each group was selected based on the mean value.

Immunocytochemistry

Isolated mouse cardiomyocytes were placed on glass slides pre-coated with 20 μg/mL of laminin (37°C, 30 minutes). After 1 hour, a gentle wash with PBS was applied to remove unattached cells, followed by fixation using 4% PFA, washing the chambers with 500 μL of 1X PBS 3 times, permeabilizing with 0.1% Triton X, washing the chambers with 500 μL of 1X PBS 3 times and blocking with 10% natural goat serum (NGS) to further prepare cells to receive antibody treatment. After blocking, cells were incubated with primary antibody at 4°C overnight. On the following day, after washing off excess primary antibody with PBS, cells were incubated with a complimentary secondary antibody for 1 hour at room temperature. Following a quick PBS wash, mounting medium containing a nuclear DAPI stain was applied to each slide and a coverslip was gently placed. The slides were sealed with nail polish and allowed to set for at least 30 minutes prior to imaging. Images were acquired using the Zeiss Axio observer fluorescence microscopy. The primary antibodies were used: HIF-1α (CST Cat#D1S7W; 1:100). Secondary antibody only staining was performed to validate the antibody specificity.

Echocardiography

To characterize ventricular function, echocardiography was performed under general anesthesia (inhalation of 2% isoflurane in 100%, 0.8 - 1.2 L/min) using VisualSonics Vevo 2100 Ultrasound.

Optical mapping

Optical mapping was performed as previously described.[18] Briefly, hearts were dissected and cannulated via the aorta and retrograde perfused and superfused with Tyrode's solution (2–5 mL/min). To load hearts with the voltage-sensitive dye, di4-ANEPPS (Invitrogen, 0.1 mmol/L,

1 mL) was slowly injected into a drug-port over 10 min period. Afterwards, blebbistatin (Sigma–Alrich, 6.8 mmol/L, 0.1 mL) was delivered to heart via the drug-port to eliminate motional artifacts. The emitted fluorescence Vm-signal was long-passed (>700nm) and acquired via MiCAM CMOS camera (SciMedia, USA) at the sampling rate of 1 kHz and pixel size of 100 mm/pixel. Right atrial pacing and surface ECG was recorded by PowerLab 26 T stimulator (AD Instruments, Australia). Atrial-effective-refractory period (AERP) was assessed with S1-S2- pacing at a CL of 100 ms. A5 ms pulse width and 5 V pulse amplitude were applied. Mapping results were analyzed by ElectroMap, an established open-source software.[32] For 10 Hz pacing, activation maps were generated based on the depolarization midpoint value. Conduction velocity was calculated by multi-vector method for entire right atrium. Action potential duration at 20%, 50%, 70%, and 90% repolarization was calculated for RA. For each value, the average of 10 consecutive beats at 10 Hz pacing were calculated for each mouse.

Adeno-associated virus (AAV) production and delivery

Adeno-associated virus 9 (AAV9) was used to express an atrial CM-selective Cre (AAV9-ANF-Cre). The adenoviral helper plasmid pAdDeltaF6 (PL-F-PVADF6) and the AAV9 packaging vector pAAV2/9 (PL-T-P0008-R2) were obtained from Puresyn Inc. AAV9 were packaged in HEK293T cells by the triple transfection method and purified by CsCl density gradient centrifugation as described. 6 weeks old *Fkbp5*$^{flox/flox}$ mice were injected with one dose of AAV9-ANF-Cre virus or AAV9-ANF-Flag ($5x10^{11}$ GC/mouse) via retro-orbital route. For the intervention study, 6 weeks old *Fkbp5*$^{-/-}$ mice of both sexes were injected with one dose of AAV9-ANF-FKBP5 or AAV9-ANF-Flag virus ($1x10^{12}$ GC/mouse) via retro-orbital route.

Supplementary Figures

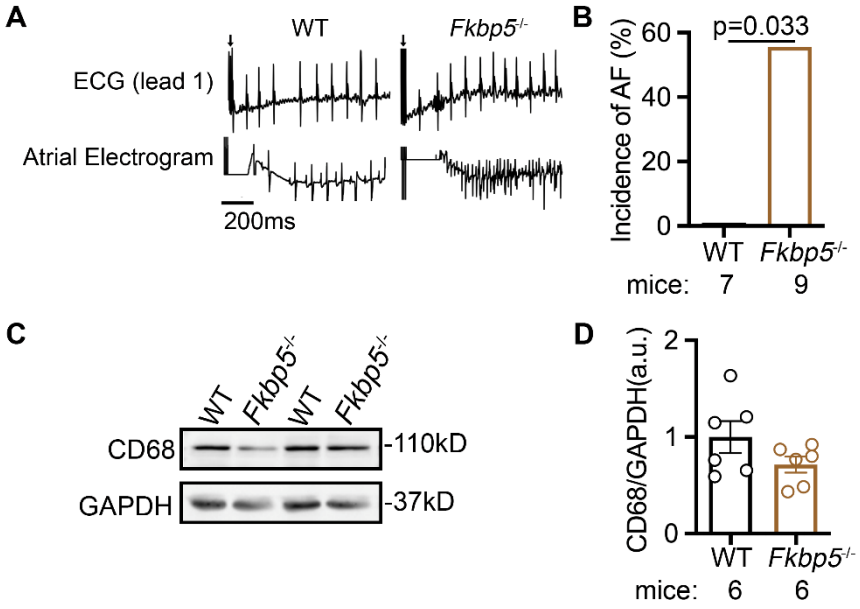

Figure S1. Enhanced AF inducibility in FKBP5 whole-body knockout mice. (**A**) Representative recordings of surface ECG (lead 1) and atrial electrogram in wildtype (WT) and *Fkbp5*$^{-/-}$ mice, indicative of sinus rhythm in WT and pacing-induced AF in *Fkbp5*$^{-/-}$ mice. Arrows indicated the end of pacing protocol. (**B**) Incidence of inducible AF. (**C**) Representative Western blots and (**D**) quantification of CD68 protein level normalized to GAPDH. p-value was determined by Fisher's exact test in **B**.

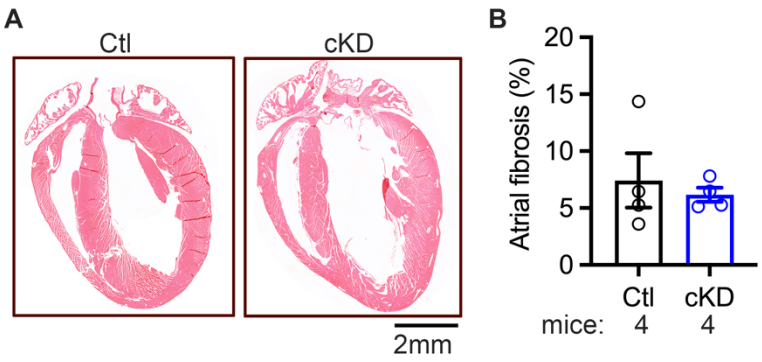

Figure S2. (**A**) Representative images of Masson's Trichome staining in cardiac sections of control (Ctl) and FKBP5-cKD (cKD) mice. (**B**) Quantification of atrial fibrosis in Ctl and cKD mice.

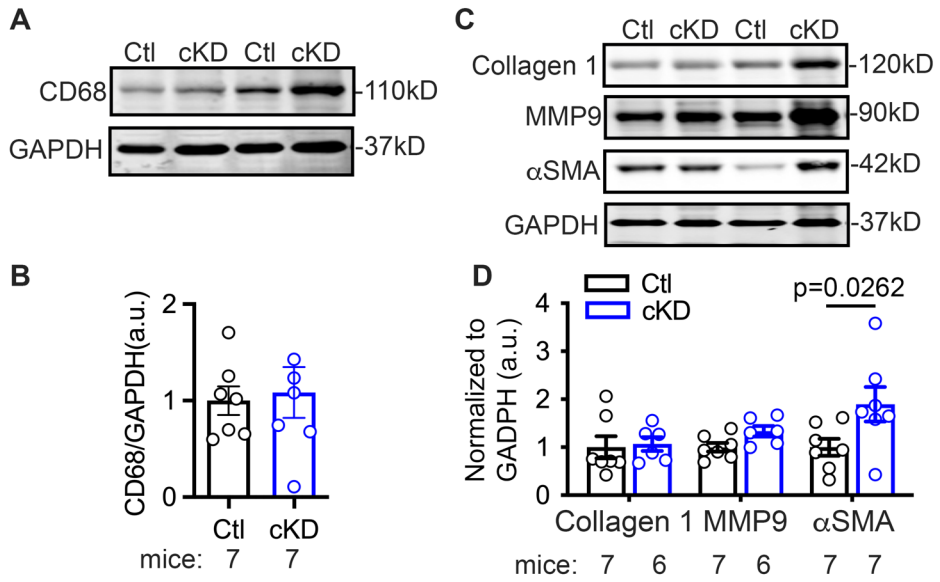

Figure S3. (**A-B**) Representative Western blots (**A**) and quantification (**B**) of macrophage marker CD68 in atria of Ctl and cKD mice. (**C-D**) Representative Western blots (**C**) and quantification (**D**) of profibrotic markers (collagen I, MMP9, and αSMA) in atria of Ctl and cKD mice. *p*-value was determined with Mann-Whitney test in **D**.

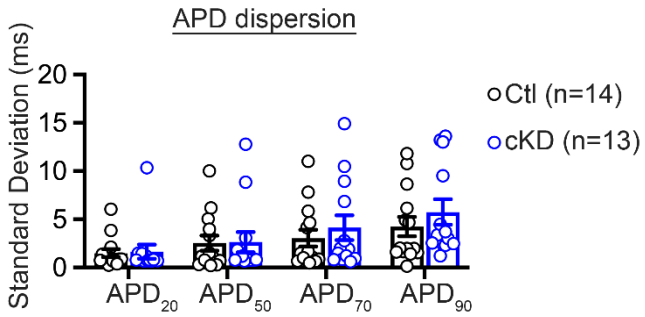

Figure S4. Unchanged APD dispersion determined as the standard deviation.

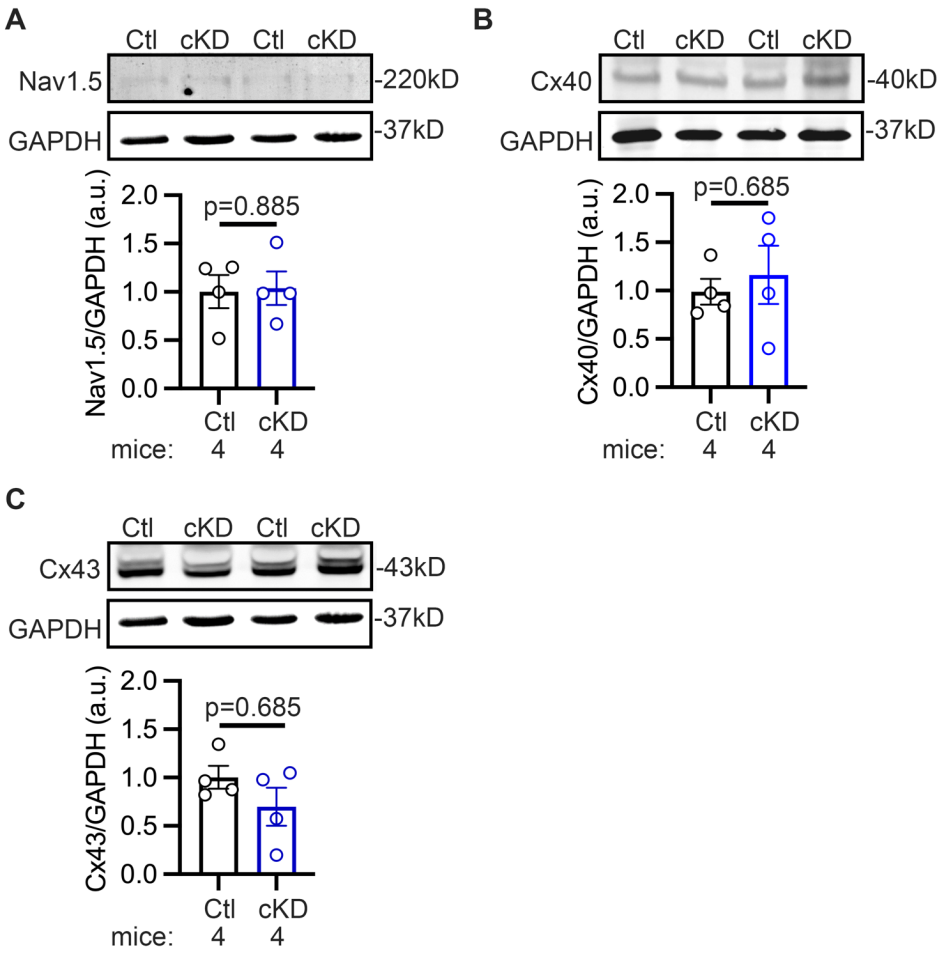

Figure S5. Unchanged Nav1.5, connexin 40 (Cx40), and connexin 43 (Cx43) in atria of cKD mice. *p*-values were determined with Mann-Whitney test.

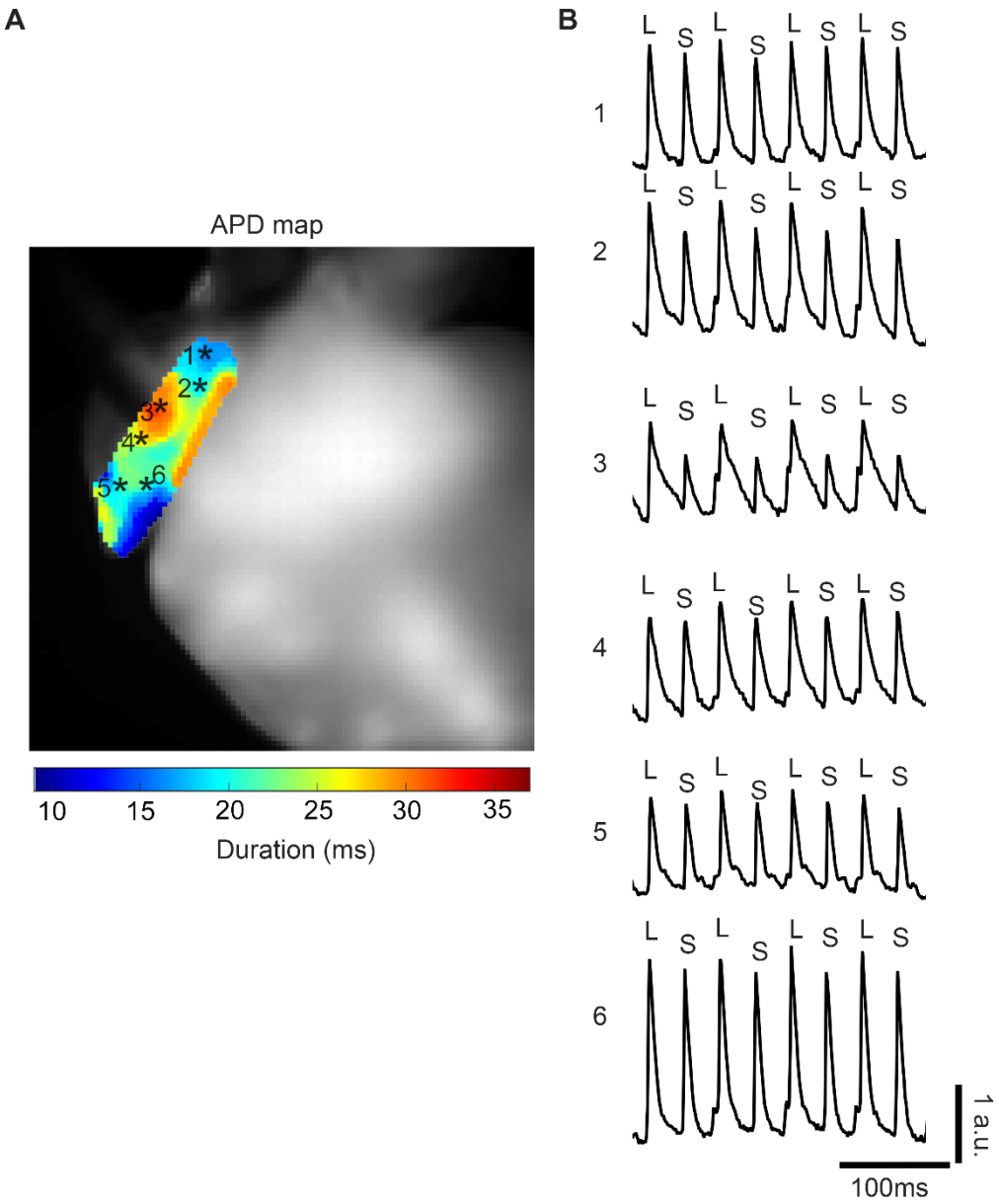

Figure S6. Spatial characterization of APD alternans in atrium of FKBP5-cKD mouse.

(**A**) APD map in right atrium of a FKBP5-cKD mouse at 15 Hz pacing. (**B**) Traces of APD alternans at 6 regions (*) on the APD map with different APD durations.

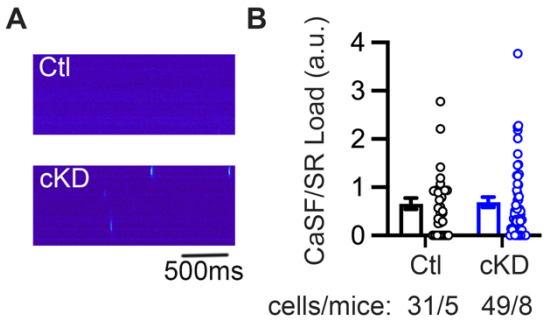

Figure S7. (**A**) Representative images of Ca^{2+} spark recording in atrial cardiomyocytes of control (Ctl) and FKBP5-cKD mice. (**B**) Quantification of the Ca^{2+} spark frequency (CaSF) normalized to sarcoplasmic reticulum (SR) load.

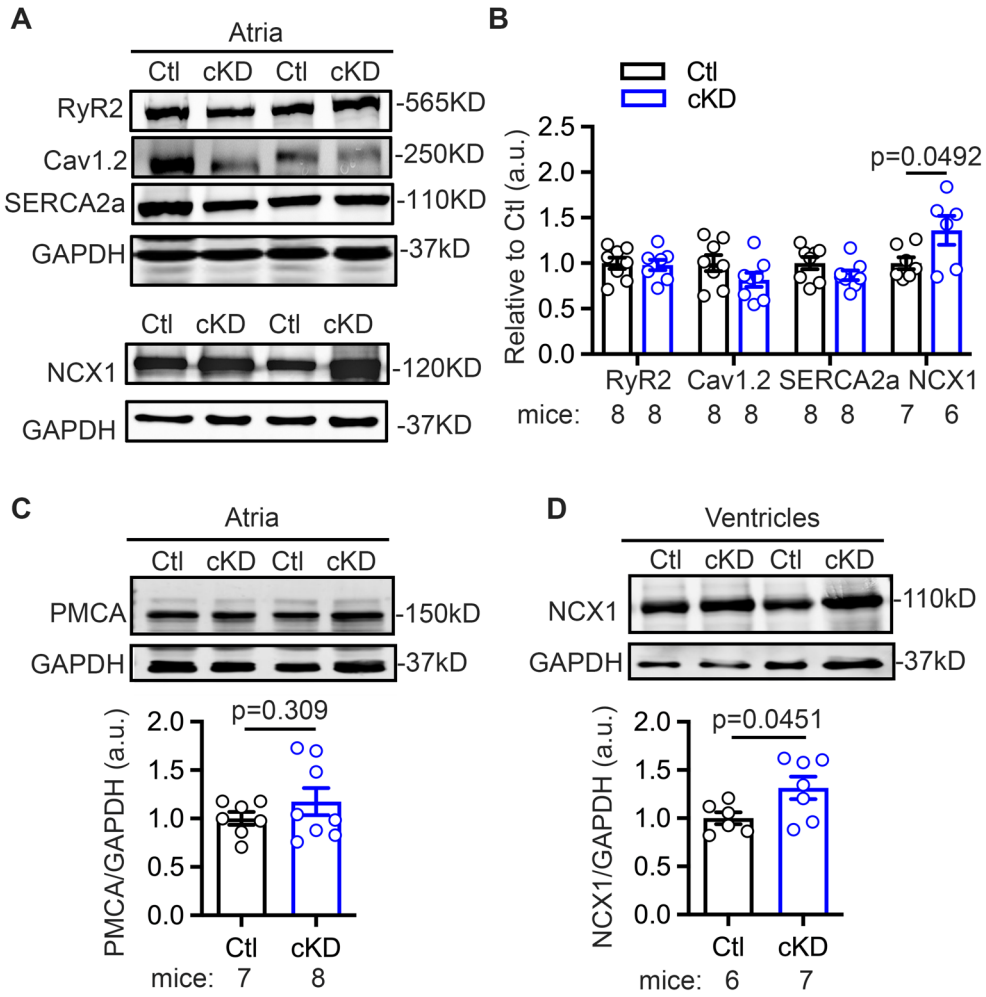

Figure S8. (**A**) Representative Western blots of major Ca^{2+} handling proteins in atrial tissue. (**B**) Quantification of relative protein levels in atria of Ctl and cKD mice. (**C**) Representative Western blots and quantification of PMCA protein levels in atria of Ctl and cKD mice. (**D**) Representative Western blots and quantification of NCX1 protein levels in ventricles of Ctl and cKD mice. RyR2, ryanodine receptor type-2; Cav1.2, alpha-subunit of L-type Ca^{2+} channel; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a; NCX1, Na^{+}/Ca^{2+} exchanger 1; PMCA, plasma membrane Ca^{2+} ATPase. *p*-values were determined with Shapiro-Wilk test and unpaired Student's t-test in **B**, **C** and **D**.

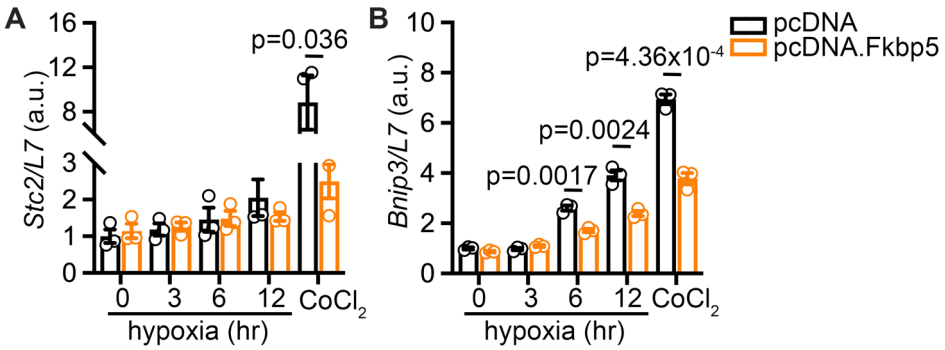

Figure S9. mRNA levels of HIF-1α targets reduced by FKBP5 overexpression. (**A**) *Stc2* and (**B**) *Bnip3* in H9C2 cells transfected with pcDNA (as control) or pcDNA.Fkbp5 after hypoxia challenge. $CoCl_2$ treated cells served as positive control. *p*-values were determined with Shapiro-Wilk test and unpaired Student's t-test.

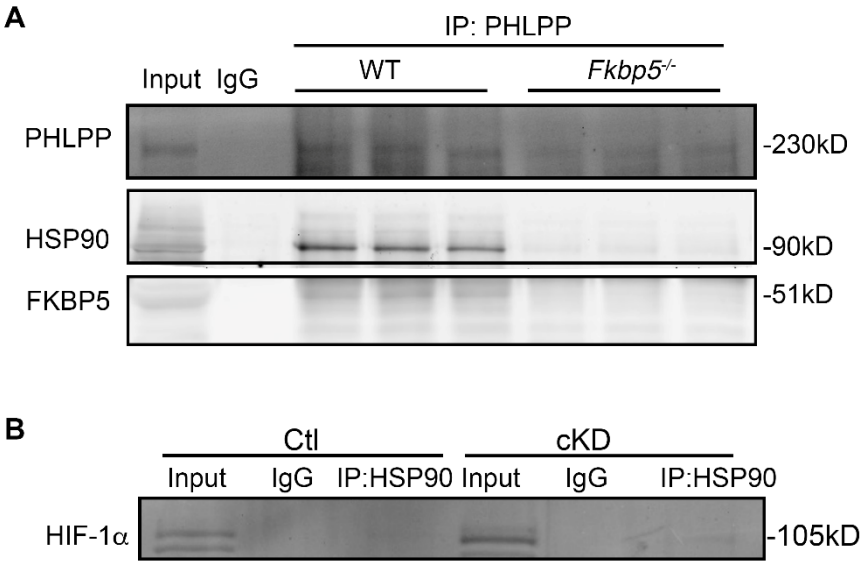

Figure S10. Reduced interaction between HSP90 and FKBP5, and increased interaction between HSP90 and NCX1 due to the FKBP5-deficiency. (**A**) Co-immunoprecipitation of PHLPP, HSP90 and FKBP5 in atrial tissue of wildtype (WT) and *Fkbp5* whole-body knockout (*Fkbp5*$^{-/-}$) mice. (**B**) Co-immunoprecipitation of HSP90 and NCX1 in atrial tissue of control (Ctl) and FKBP5-cKD mice.

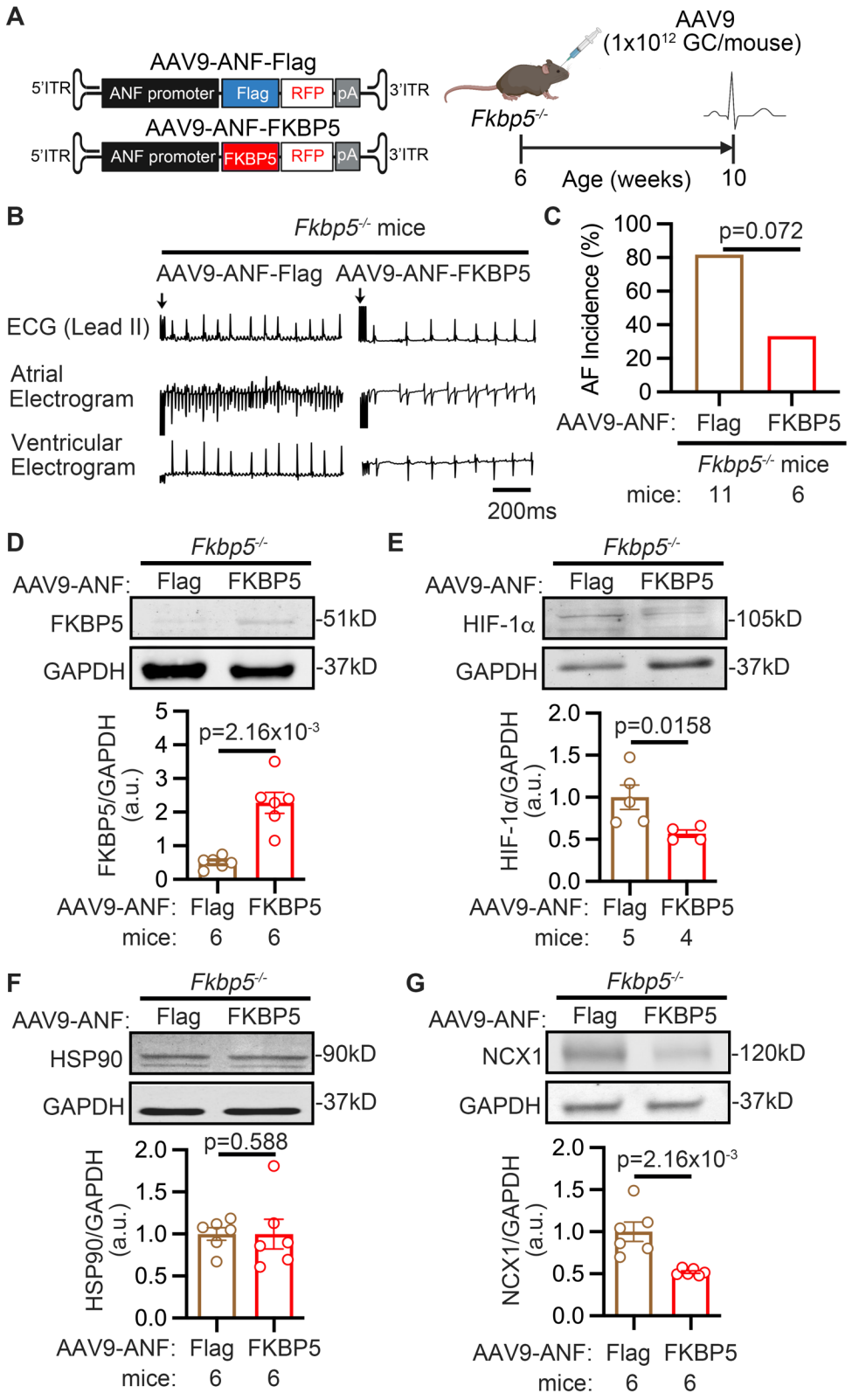

Figure S11. Atrial CM-selective expression of FKBP5 in Fkbp5-/- reduced AF inducibility. (**A**) Schematic diagram showed the strategy to restore FKBP5 level in an atrial CM-selective manner using AAV9-ANF-FKBP5 virus injected *Fkbp5*$^{-/-}$ mice. AAV9-ANF-Flag virus injected *Fkbp5*$^{-/-}$ mice used as control. (**B**) Representative recording and (**C**) the incidence of pacing-induced AF in mice. (**D-G**) Representative Western blots and quantification of FKBP5 (**D**), HIF-1α (**E**), HSP90 (**F**), and NCX1 (**G**) in atria of Fkbp5-/- mice. *p*-values were determined with Fisher's exact test in **C**, Mann-Whitney test in **D**, **E**, **F**, and **G.**

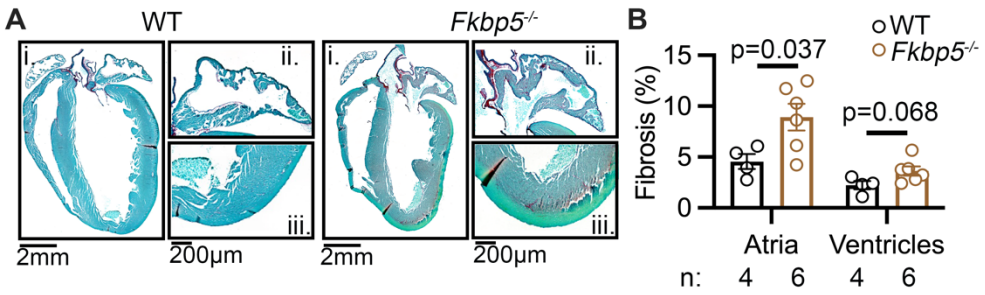

Figure S12. Increased cardiac fibrosis in whole-body *Fkbp5*$^{-/-}$ mice. (**A**) Representative Picrosirius Red staining in whole hearts (i), atria (ii) and ventricles (iii) of Ctl and cKD mice. (**B**) The quantification of fibrosis areas in atria and ventricles of Ctl and cKD mice. *p*-values were determined with Mann-Whitney test in **B.**

Supplementary Tables

Table S1. Patient Characteristics for Figure 1A.

	NSR	pAF	P-value	Statistical Test Applied
Patients, n	12	12		
Gender, M/F	7/5	8/4	>0.999	Fisher's exact test
Age, y	75.9 ± 2.2	72.8 ± 2.2	0.315	Unpaired t test
Body mass index, kg/m$^{2\$}$	27.9 ± 1.4	29.9 ± 1.6	0.347	Unpaired t test
CAD, n (%)	2 (17)	3 (25)	>0.999	Fisher's exact test
AVD/MVD, n (%)	5 (42)	5 (42)	>0.999	Fisher's exact test
CAD+AVD/MVD, n (%)	5 (42)	4 (33)	>0.999	Fisher's exact test
Hypertension, n (%)	8 (67)	10 (83)	0.640	Fisher's exact test
Diabetes, n (%)	4 (33)	5 (42)	>0.999	Fisher's exact test
Hyperlipidemia, n (%)	6 (50)	7 (58)	>0.999	Fisher's exact test
LVEF, %§	59.6 ± 3.9	56.6 ± 3.4	0.560	Unpaired t test
LA, mm$^{\#}$	45.6 ± 4.0	50.0 ± 3.3	0.684	Mann Whitney test
Digitalis, n (%)	0 (0)	0 (0)	>0.999	Fisher's exact test
ACEi OR ARB, n (%)	7 (58)	5 (42)	0.684	Fisher's exact test
β-Blockers, n (%)	7 (58)	8 (67)	>0.999	Fisher's exact test
Dihydropyridines, n (%)	4 (33)	3 (25)	>0.999	Fisher's exact test
Diuretics, n (%)	4 (33)	8 (67)	0.220	Fisher's exact test
Nitrates, n (%)	1 (8)	0 (0)	>0.999	Fisher's exact test
Lipid-lowering drugs, n (%)	6 (50)	7 (58)	>0.999	Fisher's exact test

NSR, normal sinus rhythm as control; pAF, paroxysmal atrial fibrillation; CAD, coronary artery disease; AVD, aortic valve disease; MVD, mitral valve disease; LVEF, left ventricular ejection fraction; LA, left atrial diameter; ACEi, angiotensin converting enzyme; ARB, angiotensin receptor blocker; §Data were not available for 1 CTL. $^{\#}$Data were not available for 7 NSR, and 6 pAF. Continuous variables are presented as mean ± SEM.

Table S2. Patient Characteristics for Figure 1B.

	CTL	cAF	P-value	Statistical Test Applied
Patients, n	15	12		
Gender, M/F	9/6	8/4	>0.999	Fisher's exact test
Age, y	74.6±3.1	71.2±2.2	0.080	Mann Whitney test
Body mass index, kg/m^2	27.3±0.9	24.8±1.0	0.089	Unpaired t test
CAD, n (%)	4 (27)	2 (17)	0.662	Fisher's exact test
AVD/MVD, n (%)	7 (47)	5 (42)	>0.999	Fisher's exact test
CAD+AVD/MVD, n (%)	4 (27)	5 (42)	0.448	Fisher's exact test
Hypertension, n (%)	11 (73)	9 (75)	>0.999	Fisher's exact test
Diabetes, n (%)	4 (27)	2 (16)	0.662	Fisher's exact test
Hyperlipidemia, n (%)	7 (47)	9 (75)	0.239	Fisher's exact test
LVEF, %	59.7±3.4	55.9±2.9	0.411	Unpaired t test
LA, mm$^{\#}$	42.1±3.6	46.7±5.3	0.617	Mann Whitney test
Digitalis, n (%)	0 (0)	3 (25)	0.075	Fisher's exact test
ACEi OR ARB, n (%)	6 (40)	6 (50)	0.707	Fisher's exact test
β-Blockers, n (%)	8 (53)	8 (67)	0.696	Fisher's exact test
Dihydropyridines, n (%)	6 (40)	2 (17)	0.236	Fisher's exact test
Diuretics, n (%)	5 (33)	6 (50)	0.452	Fisher's exact test
Nitrates, n (%)	2 (13)	2 (17)	>0.999	Fisher's exact test
Lipid-lowering drugs, n (%)	7 (47)	5 (42)	>0.999	Fisher's exact test

CTL, sinus rhythm as control; cAF, chronic atrial fibrillation; CAD, coronary artery disease; AVD, aortic valve disease; MVD, mitral valve disease; LVEF, left ventricular ejection fraction; LA, left atrial diameter; ACEi, angiotensin converting enzyme; ARB, angiotensin receptor blocker; $^{\#}$Data were not available for 8 CTL, and 9 cAF. Continuous variables are presented as mean±SEM

Table S3. Patient Characteristics for Figure 1C.

	CTL	cAF	P-value	Statistical Test Applied
Patients, n	8	8		
Gender, M/F	5/3	3/5	0.619	Fisher's exact test
Age, y	63.5±3.3	71.6±5.6	0.234	Unpaired t test
Body mass index, kg/m^2	26.4±1.0	25.2±1.4	0.524	Unpaired t test
CAD, n (%)	2 (25)	1 (12)	>0.999	Fisher's exact test
AVD/MVD, n (%)	4 (50)	4 (50)	>0.999	Fisher's exact test
CAD+AVD/MVD, n (%)	2 (25)	3 (38)	>0.999	Fisher's exact test
Hypertension, n (%)	8 (100)	6 (75)	0.467	Fisher's exact test
Diabetes, n (%)	3 (38)	2 (25)	>0.999	Fisher's exact test
Hyperlipidemia, n (%)	3 (38)	1 (12)	0.569	Fisher's exact test
LVEF, %[§]	61.1±6.1	52.7±3.2	0.236	Mann Whitney test
LA, mm[#]	n.a.	43.0±2.5	n.a	n.a
Digitalis, n (%)	0 (0)	4 (50)	0.077	Fisher's exact test
ACEi OR ARB, n (%)	4 (50)	6 (75)	0.608	Fisher's exact test
β-Blockers, n (%)	5 (62)	6 (75)	>0.999	Fisher's exact test
Dihydropyridines, n (%)	2 (25)	0 (0)	0.467	Fisher's exact test
Diuretics, n (%)	1 (12)	3 (38)	0.569	Fisher's exact test
Nitrates, n (%)	2 (25)	1 (12)	>0.999	Fisher's exact test
Lipid-lowering drugs, n (%)	2 (25)	1 (12)	>0.999	Fisher's exact test

CTL, sinus rhythm as control; cAF, chronic atrial fibrillation; CAD, coronary artery disease; AVD, aortic valve disease; MVD, mitral valve disease; LVEF, left ventricular ejection fraction; LA, left atrial diameter; ACEi, angiotensin converting enzyme; ARB, angiotensin receptor blocker; [§]Data were not available for 1 cAF. [#]Data were not available for 8 CTL, and 5 cAF. Continuous variables are presented as mean±SEM

Table S4. Patient Characteristics for Figure 4l.

	CTL	cAF	P-value	Statistical Test Applied
Patients, n	8	8		
Gender, M/F	7/1	4/4	0.282	Fisher's exact test
Age, y	64.5±3.8	64.6±6.0	0.986	Unpaired t test
Body mass index, kg/m^2	27.0±1.4	27.1±1.0	0.943	Unpaired t test
CAD, n (%)	4 (50)	1 (12)	0.282	Fisher's exact test
AVD/MVD, n (%)	2 (25)	1 (12)	>0.999	Fisher's exact test
CAD+AVD/MVD, n (%)	2 (25)	6 (75)	0.132	Fisher's exact test
Hypertension, n (%)	4 (50)	4 (50)	>0.999	Fisher's exact test
Diabetes, n (%)	2 (25)	4 (50)	0.608	Fisher's exact test
Hyperlipidemia, n (%)	5 (62)	3 (38)	0.619	Fisher's exact test
LVEF, %	59.0±6.3	57.8±3.9	0.518	Mann Whitney test
LA, mm[#]	41.6±3.7	44.8±4.8	0.651	Mann Whitney test
Digitalis, n (%)	0 (0)	3 (38)	0.200	Fisher's exact test
ACEi OR ARB, n (%)	3 (38)	2 (25)	>0.999	Fisher's exact test
β-Blockers, n (%)	5 (62)	4 (50)	>0.999	Fisher's exact test
Dihydropyridines, n (%)	1 (13)	4 (50)	0.282	Fisher's exact test
Diuretics, n (%)	3 (38)	4 (50)	>0.999	Fisher's exact test
Nitrates, n (%)	0 (0)	0 (0)	>0.999	Fisher's exact test
Lipid-lowering drugs, n (%)	5 (62)	2 (25)	0.315	Fisher's exact test

CTL, sinus rhythm as control; cAF, chronic atrial fibrillation; CAD, coronary artery disease; AVD, aortic valve disease; MVD, mitral valve disease; LVEF, left ventricular ejection fraction; LA, left atrial diameter; ACEi, angiotensin converting enzyme; ARB, angiotensin receptor blocker; [#]Data were not available for 3 CTL, and 3 cAF. Continuous variables are presented as mean±SEM

Table S5. Patient Characteristics for Figure 5A.

	CTL	cAF	P-value	Statistical Test Applied
Patients, n	6	6		
Gender, M/F	5/1	5/1	>0.999	Fisher's exact test
Age, y	70.7±3.4	78.8±1.9[‡]	0.024	Mann Whitney test
Body mass index, kg/m^2[$]	28.5±2.1	26.5±1.3	0.615	Mann Whitney test
CAD, n (%)	2 (33)	3 (50)	>0.999	Fisher's exact test
AVD/MVD, n (%)	1 (17)	2 (33)	>0.999	Fisher's exact test
CAD+AVD/MVD, n (%)	3 (50)	1 (17)	0.546	Fisher's exact test
Hypertension, n (%)	6 (100)	6 (100)	>0.999	Fisher's exact test
Diabetes, n (%)	4 (67)	3 (50)	>0.999	Fisher's exact test
Hyperlipidemia, n (%)	4 (67)	4 (67)	>0.999	Fisher's exact test
LVEF, %[§]	46.2±5.3	52.3±5.1	0.574	Mann Whitney test
LA, mm[#]	n.a.	n.a	n.a	n.a
Digitalis, n (%)	0 (0)	2 (33)	0.454	Fisher's exact test
ACEi OR ARB, n (%)	1 (17)	2 (33)	>0.999	Fisher's exact test
β-Blockers, n (%)	3 (50)	4 (67)	>0.999	Fisher's exact test
Dihydropyridines, n (%)	0 (0)	1 (17)	>0.999	Fisher's exact test
Diuretics, n (%)	3 (50)	3 (50)	>0.999	Fisher's exact test
Nitrates, n (%)	0 (0)	0 (0)	>0.999	Fisher's exact test
Lipid-lowering drugs, n (%)	3 (50)	6 (100)	0.182	Fisher's exact test

CTL, sinus rhythm as control; cAF, chronic atrial fibrillation; CAD, coronary artery disease; AVD, aortic valve disease; MVD, mitral valve disease; LVEF, left ventricular ejection fraction; LA, left atrial diameter; ACEi, angiotensin converting enzyme; ARB, angiotensin receptor blocker; [#]Data were not available for 6 CTL, and 6 cAF. Continuous variables are presented as mean±SEM.

Table S6. ECG parameters in FKBP5-cKD mice.

	Ctl (n=16)	cKD (n=24)	P-value
RR (ms)	110.9 ± 2.36	109.2 ± 2.05	0.595
HR (bpm)	544.8 ± 11.6	553.9 ± 10.0	0.564
PR (ms)	35.8 ± 0.90	37.7 ± 0.57	0.184
QRS (ms)	10.1 ± 0.29	10.6 ± 0.33	0.102
QT (ms)	24.4 ± 1.16	25.9 ± 1.12	0.359
QTc (ms)	23.3 ± 1.28	24.9 ± 1.14	0.354
SNRT (ms)	138.9 ± 8.48	136.3 ± 7.61	0.821
cSNRT (ms)	39.6 ± 5.35	35.0 ± 6.86	0.635
AVNERP (ms)	50.6 ± 1.26	48.4 ± 1.49	0.311

AVNERP, atrioventricular node effective refractory period; HR, heart rate; SNRT, sinus node recovery time; cSNRT, corrected sinus node recovery time. *p*-values were determined with unpaired Student's t-test.

Table S7. Incidence of pacing-induced VT in FKBP5-cKD mice.

Pacing protocol	Ctl		cKD	
	positive/total	Incidence	positive/total	Incidence (%)
S1(90ms)-S2	1/13	7.7%	0/19	0%
S1(70ms)-S2	0/13	0%	0/19	0%
S1(90ms)-S2(40ms)-S3	0/13	0%	0/19	0%
S1(40ms)-burst	0/13	0%	1/19	5.3%

Table S8. Echocardiography parameters in FKBP5-cKD mice.

	Ctl (n=11)	cKD (n=20)	P-value
Heart Rate (BPM)	492.5 ± 14.1	485.3 ± 10.4	0.685
ESD (mm)	2.2 ± 0.10	2.6 ± 0.13	0.070
EDD (mm)	3.7 ± 0.11	3.8 ± 0.12	0.393
Volume,s (μL)	17.0 ± 2.01	25.6 ± 3.40	0.086
Volume,d (μL)	56.8 ± 4.26	62.8 ± 4.24	0.371
Stroke Volume (mL)	39.8 ± 1.66	37.2 ± 2.60	0.422
EF (%)	71.0 ± 1.25	61.4 ± 2.60	0.004
FS (%)	39.9 ± 0.95	33.0 ± 1.77	0.013
Cardiac Output (mL/min)	19.5 ± 1.19	18.1 ± 1.06	0.402
LVAWd (mm)	0.9 ± 0.03	0.9 ± 0.06	0.741
LVAWs (mm)	1.3 ± 0.06	1.2 ± 0.10	0.786
LVPWd (mm)	0.8 ± 0.02	0.8 ± 0.05	0.905
LVPWs (mm)	1.1 ± 0.05	1.1 ± 0.05	0.581

EDD, left-ventricular end-diastolic diameter; ESD, left-ventricular end-systolic diameter; EF, ejection fraction; FS, fractional shortening; LVAWd, left-ventricular end-diastolic anterior wall thickness; LVAWs, left-ventricular end-systolic anterior wall thickness; LVPWd, left-ventricular end-diastolic posterior wall thickness; LVPWs, left-ventricular end-systolic posterior wall thickness. *p*-values were determined with unpaired Student's t-test.

Table S9. Echocardiography parameters in FKBP5-cKD with 17-AAG mice.

	Vehicle (n=5)	17-AAG (n=5)	P-value
Heart Rate (BPM)	420.8 ± 42.9	454.4 ± 27.7	0.179
ESD (mm)	2.8 ± 0.35	2.5 ± 0.19	0.144
EDD (mm)	4.1 ± 0.26	3.8 ± 0.18	0.058
Volume,s (μL)	30.8 ± 9.41	23.3 ± 4.38	0.144
Volume,d (μL)	76.3 ± 11.11	63.2 ± 6.63	0.053
Stroke Volume (mL)	45.5 ± 2.98	39.9 ± 6.03	0.099
EF (%)	60.4 ± 6.41	63.0 ± 6.03	0.515
FS (%)	32.0 ± 4.24	33.7 ± 4.29	0.536
Cardiac Output (mL/min)	19.1 ± 2.34	18.1 ± 2.48	0.498
LVAWd (mm)	1.1 ± 0.22	0.9 ± 0.17	0.237
LVAWs (mm)	1.6 ± 0.28	1.5 ± 0.22	0.568
LVPWd (mm)	1.0 ± 0.25	1.1 ± 0.28	0.957
LVPWs (mm)	1.3 ± 0.37	1.5 ± 0.19	0.284

EDD, left-ventricular end-diastolic diameter; ESD, left-ventricular end-systolic diameter; EF, ejection fraction; FS, fractional shortening; LVAWd, left-ventricular end-diastolic anterior wall thickness; LVAWs, left-ventricular end-systolic anterior wall thickness; LVPWd, left-ventricular end-diastolic posterior wall thickness; LVPWs, left-ventricular end-systolic posterior wall thickness. *p*-values were determined with Shapiro-Wilk test and unpaired Student's t-test.

Table S10. ECG parameters in FKBP5-cKD with 17-AAG mice.

	Vehicle (n=5)	17-AAG (n=5)	P-value
RR (ms)	139.5 ± 12.45	143.2 ± 12.68	0.843
HR (bpm)	447.0 ± 54.93	410.0 ± 33.70	0.794
PR (ms)	39.0 ± 2.67	40.2 ± 1.56	0.680
QRS (ms)	10.2 ± 0.69	9.3 ± 0.68	0.399
QT (ms)	22.6 ± 1.40	19.3 ± 0.88	0.077
QTc (ms)	21.7 ± 1.61	17.7 ± 0.95	0.059
SNRT (ms)	179.3 ± 15.11	135.2 ± 9.39	0.036
cSNRT (ms)	35.0 ± 20.79	22.0 ± 2.80	0.468
AVNERP (ms)	51.8 ± 1.72	54.0 ± 2.02	0.456

AVNERP, atrioventricular node effective refractory period; HR, heart rate; SNRT, sinus node recovery time; cSNRT, corrected sinus node recovery time. *p*-values were determined with Shapiro-Wilk test and unpaired Student's t-test.

Table S11. ECG parameters in FKBP5-/- mice receiving AAV9-ANF-FKBP5 virus.

	Fkbp5-/- mice		
	AAV9-ANF-Flag (n=10)	AAV9-ANF-FKBP5 (n=6)	P-value
RR (ms)	118.40 ± 4.55	121.47 ± 4.91	0.672
HR (bpm)	516.1 ± 18.8	498.2 ± 19.9	0.545
PR (ms)	36.8 ± 1.09	37.2 ± 2.29	0.872
QRS (ms)	8.86 ± 0.53	8.30 ± 0.66	0.530
QT (ms)	21.0 ± 0.94	20.84 ± 0.34	0.770
QTc (ms)	20.0 ± 1.07	20.01 ± 0.68	0.999
SNRT 90 (ms)	151.5 ± 5.49	143.7 ± 10.68	0.481
cSNRT 90 (ms)	31.3 ± 5.03	22.2 ± 10.10	0.383

HR, heart rate; SNRT, sinus node recovery time; cSNRT, corrected sinus node recovery time.

p-values were determined with Shapiro-Wilk test and unpaired Student's t-test.

Table S12. Primers used in this study.

qPCR Primers:	
Bnip3-F	CACCCGAAGCGCACAGCTACTCT
Bnip3-R	TTGTCAGACGCCTTCCAATGTAG
Stc2-F	TTGTGGAGATGATTCATTTCAAG
Stc2-R	CTGTTCACACTGAGCCTGGACGC
Slc8a1-F	GTTTGCCTTCGTCCCACCTAC
Slc8a1-R	GTCACGGAATCTTTCAGACCAAT
Rpl7-F	ATCATCTGCATGGAGGATCTAAT
Rpl7-R	TCATCCGTCTAATAAGCCTGTTT
Fkbp5-F	TGAGGGCACCAGTAACAATGG
Fkbp5-R	AACATCCCTTTGTAGTGGACAT
ChIP-qPCR primers:	
P1-F	TAAAAACTTTCCTGGGAGGCTGC
P1-R	TTTATTTTGTTGGTGCCTGTATC
P2-F	AGAGTTCTTTTGATGCTCCTTTC
P2-R	AAAGTCATCCTTAACTACACGGC
P3-F	TGGCCGTGTAGTTAAGGATGACT
P3-R	GGACAAGCTGGTATATGGATGCC
P4-F	CCAGCTTGTCCTTTTTAATTTAT
P4-R	TTTATTGCTTCTCACTAAGGGTA
P5-F	TTCTAAATCCTTTTTTCTCACCC
P5-R	AAAGCAGAAGGCCAGAGATGTCA
P6-F	TGGTAAGCAGCCAGAATCAAGCA
P6-R	AATATGCTTTAGATTGGCAGTCG
EMSA probes:	
P5-F	TTAGCTTAACACACCTGCCACCTAATAA
P5-R	TTATTAGGTGGCAGGTGTGTTAAGCTAA
M5-F (mutant)	TTAGCTTAACAATTCCGGCACCTAATAA
M5-R (mutant)	TTATTAGGTGCCGGAATTGTTAAGCTAA
P6-F	GCATATTTGTAAGCGTGTGTAGTGGCAG
P6-R	CTGCCACTACACACGCTTACAAATATGC
M6-F (mutant)	GCATATTTGTAATTCCGGGTAGTGGCAG
M6-R (mutant)	CTGCCACTACCCGGAATTACAAATATGC
Plasmid construction:	
pcDNA3.1-FBP5-GFP-F	ATAGCTAGCATGACTACTGATGAGGGCACCAG
pcDNA3.1-FKBP5-GFP-R	ATAGCGGCCGCTACATGGCCTTTGGCCTTTC
pcDNA3.1-FKBP5-F	ATAGCTAGCATGACTACTGATGAGGGCACCAG
pcDNA3.1-FKBP5-R	ATAGCGGCCGCTACATGGCCTTTGGCCTTTC