Epigenetic Regulation of Mitochondrial Fission and Cardiac Fibrosis via sFRP3 Promoter Methylation

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Data Supplementary

Materials and methods

Animals[1]

The in vivo assay received approval from the Animal Care and Use Committee of Anhui Medical University. Furthermore, all experimental procedures and animal care strictly adhered to the institutional ethics guidelines for animal experiments. The C57BL/6 mice(male, 8-10 weeks old) were accommodated in a specific, opportunistic pathogen-free facility with six mice per cage. They were kept under a 12-hour light-dark cycle with ad libitum access to food and water. Subsequently, the mice were randomly allocated into four groups: vehicle, ISO, AAV9-NC, and AAV9-POSTN-shDNMT3A. Except for the vehicle group, mice in each group received Isoproterenol (ISO) via subcutaneous injection (5 mg/kg/day) for a duration of 2 weeks[2]. One week following the treatment, the mice were subjected to infection with adeno-associated virus containing shDNMT3A, The mice were injected with AAV9-NC or AAV9-POSTN-shDNMT3A (200 u/mouse) via the tail vein. Following an additional three weeks, the mice were anesthetized with isoflurane, and anesthesia was sustained through continuous isoflurane administration via a nose cone, utilizing an isoflurane anesthesia machine (E-Z Anesthesia, Euthanex Corp., PA, USA). Subsequently, the hearts of mice in each group were dissected. Sections of heart tissue were either rapidly frozen in liquid nitrogen and preserved at -80° C or fixed in 10% formalin. Additionally, some sections were frozen in optimal cutting temperature (OCT) compound (Sakura Finetek U.S.A., Inc., Torrance, CA) for histological analysis. Blood samples were collected in EDTA-containing tubes for plasma isolation, which was then stored at -80° C.

Echocardiography[3]

Prior to heart dissection, cardiac hemodynamics were assessed using ultrasound with a Vevo2100 (VeVo 2100 Imaging System; VisualSonics, Toronto, ON, Canada). The mouse was anesthetized using 2% isoflurane inhalation and underwent transthoracic echocardiography. Echocardiographic M-mode tracings in the short-axis view were utilized, with at least six cardiac cycles analyzed to calculate various parameters, including left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), percent fractional shortening (FS), and ejection fraction (EF).

Histopathology[4]

Histopathological analysis of mouse tissues was conducted at the Core Experimental Facility at The Second Hospital of Anhui Medical University. Following paraffin embedding, heart specimens were sectioned into 5 um thick slices. After deparaffinization, the sections were stained individually using Sirius Red staining and Masson's trichrome (MT) staining to identify collagen, as well as Masson's trichrome staining to detect fibrosis. For each staining procedure, six images per heart were captured using a microscope.

Immunofluorescence[5]

The total cells on the slides or frozen tissue sections underwent permeabilization using 0.3% Triton X-100 following fixation in 4% paraformaldehyde. Subsequently, fetal bovine serum was employed for blocking. The cells were then incubated overnight with POSTN and SFRP3 at 4℃. Next, the slides underwent a 1-hour incubation with anti-rabbit Alexa Fluor 488 (Jackson Immunoresearch, West Grove, PA, USA) at room temperature. Nuclei were counterstained with DAPI. Each sample was examined using a fluorescence microscope (DMI4000B, Leica). The counting of positive cells was performed randomly at ×40 magnification.

Primary cardiac fibroblasts isolation and culture[6]

Cardiac myocytes and cardiac fibroblasts were isolated from the hearts of neonatal mice. Hearts from newborn C57BL/6 mice (2–3 days old) were rapidly excised, washed in pre-cooled PBS solution, and then mechanically sheared and fully digested at 37° C for 7-8 minutes using a mixture of trypsin (0.16%, Beyotime Biotechnology, Shanghai, China) and type II collagenase (0.67%, Biofrox). To halt the digestion, pre-cooled DMEM medium containing 10% FBS was added when the tissue started to loosen. Cells isolated from the tissue were collected and cultured in DMEM medium with a glucose concentration of 5.5 mM and 10% FBS in an incubator at 37° C with 5% CO2 for 120 minutes. Postn (1:200, proteintech, Wuhan, China)-positive cells were identified as cardiac fibroblasts (CFs). Once the cells reached 80% confluence, they were passaged at a 1:1 ratio, and cells from the 2nd to 4th passages were used for subsequent experiments.

Adeno-associated viruses, plasmid, siRNA and transfection[7]

The acute knockout of DNMT3A in heart was achieved by the injection of adeno-associated virus, following the manufacturer's instructions. The mice were injected with AAV9-NC or AAV9-POSTN-shDNMT3A (200 u/mouse) via the tail vein. Following an additional three weeks. The mice were then anesthetized and underwent transthoracic echocardiography.

The plasmid-based SFRP3 overexpression vector and an empty vector were provided by GenePharma (Shanghai, China). Cardiac fibroblasts were infected with the plasmid following the manufacturer's instructions.

The siRNA of SFRP3 or DNMT3A were constructed by GenePharma (Shanghai, China), and used to individually transfect cells.

The plasmid and siRNA were transfected using Lipofectamine 2000 (Invitrogen), following the manufacturer's instructions. The sequences for the lentivirus, plasmid, and siRNA are provided in the supplementary Table.

5-Ethynyl-2′-deoxyuridine (EdU) staining[8]

DNA synthesis was assessed using the Cell-Light EdU DNA Cell Proliferation Kit (RiboBio, Guangzhou, China) to evaluate cell proliferation. Following the manufacturer's instructions, a total of 1×10^4 cells were seeded in each well of a 24-well plate. After a 3-hour incubation with 50 μM EdU, the cells were fixed using 4% paraformaldehyde and then stained with Apollo Dye Solution. The count of EdU-positive cells was determined and normalized by the total number of cells stained with Hoechst 33342. These experiments were repeated thrice for accuracy.

Cell migration assays[9]

Cell migration capability was evaluated through both wound healing and transwell assays.

In the wound healing assay, cells were seeded in a 6-well plate and allowed to reach 100% confluence. A 200 μL yellow pipette tip was used to create a distinct wound in the cell monolayer. Images were captured using an optical microscope system at 0 hours, 24 hours, and 48 hours to monitor the closure of the wound.

For the transwell migration assay, cells were placed in the upper chambers with 100 μL of serum-free medium, while the lower chamber was filled with medium containing 10% FBS. After 24 hours of incubation, the cells remaining on the bottom surface of the upper chamber were stained with a 0.1% crystal violet solution for 30 minutes and then imaged.

Cell Counting Kit-8 assay(CCK-8)[10]

For the CCK8 assay, a CCK8 Cell Proliferation Kit (Beyotime, Beijing, China) was used according to the manufacturer's instructions. Cells were seeded into a 96-well plate at 5 × 10 3 cells per well with 100 uL medium and cultured in a incubator at 37 ° C with 5% CO 2 , and CCK8 solution was added (10 uL per well) 2 h before measuring the absorbance at 450 nm.

Acridine Orange assay[11]

The assessment of apoptotic potential was conducted using an Acridine Orange assay. Glass coverslips were prepositioned within a 24-well culture plate. Transfected cell suspensions were then seeded into each well. The cells were fixed with 95% ethanol for 15 minutes and allowed to air-dry slightly. Subsequently, 5 μL of Acridine Orange (100 mg/L in PBS) and 5 μL of Ethidium Bromide (100 mg/L in PBS) were added, gently mixed, and lightly tapped. The percentage of apoptotic cells was determined by counting 100 cells across 4 different fields of view.

Western blotting[12]

Proteins were extracted from both cells and heart tissue homogenates using RIPA lysis buffer (Beyotime, China) and quantified using a BCA protein assay kit (Thermo Scientific, USA). Equal amounts of protein were loaded and separated on SDS-PAGE gels, then transferred onto PVDF membranes with a pore size of 0.45 μm using a constant current of 200mA for a minimum of 40 minutes. Following the transfer, the membranes were blocked with 5% skim milk powder and subsequently incubated with a primary antibody overnight at 4℃, followed by a secondary antibody. Finally, the blots were detected using an enhanced chemiluminescence kit (Millipore, Billerica, USA), and images were captured using a BioSpectrum 600 Imaging System (UVP, CA, USA).

Quantitative real time reverse transcription (RT-qPCR)[13]

The extraction of total RNA was carried out using Trizol, followed by reverse transcription using the Evo M-MLV RT premix for qPCR (Accurate Biotechnology, Hunan, China). Subsequently, cDNA amplification was performed in accordance with the manufacturer's instructions using the SYBR Green Premix Pro Taq HS qPCR kit II (Accurate Biotechnology, Hunan, China). Amplification of samples was conducted for 40 cycles utilizing a StepOnePlus Real-Time PCR system. Quantitative RT-PCR analysis employed GAPDH as an endogenous control and followed the $2^{-\Delta\Delta CT}$ method.The primer sequences are provided in the supplementary Table.

Bisulfite sequencing PCR(BSP)[14]

To validate DNA methylation, targeted bisulfite sequencing was employed. 1 μg of genomic DNA was subjected to sodium bisulfite treatment. The converted DNA was subsequently purified and utilized as the template for PCR amplification. The bisulfite-treated DNA underwent PCR amplification using the specified primers. The resulting fresh PCR products were purified and then subjected to sequencing. The primer sequences are provided in the supplementary Table.

Methylation-specific polymerase chain reaction(MSP)[14]

Methylation analysis of the SFRP3 promoter region was conducted via Methylation-Specific PCR (MSP). Genomic DNA was extracted using the TIANamp Genomic DNA Kit(Tiangen, China). Subsequently, DNA underwent bisulfite conversion using the EZ DNA MethylationGold™ Kit (Zymo Research, USA). Two sets of primers were employed to amplify the SFRP3 gene's promoter region: one set was specific to the methylated sequence, and the other targeted the unmethylated sequence. MSP amplification for all genes was carried out and optimized using the Touchdown PCR protocol and RePure-A machine (BIO-GENER, China). The resulting reaction products were subjected to electrophoresis on agarose gels (2%) for analysis. The primer sequences are provided in the supplementary Table.

Chromatin immunoprecipitation analysis (ChIP) and methyl-DNA immunoprecipitation(MeDIP)[14]

Chromatin immunoprecipitation analysis was conducted using the SimpleChIP Plus Sonication Chromatin IP Kit (Cell Signaling Technology, USA), and Methylated DNA Immunoprecipitation Kit (Abcam, USA). ChIP is a method employed to identify DNA fragments bound to specific proteins through co-precipitation with chromatin and subsequent polymerase chain reaction (PCR). The DNA and proteins were cross-linked and then sonicated to break them into smaller fragments. The DNA fragments of interest were isolated using antibodies specific to DNMT3A, 5mc, or IgG and subsequently purified for PCR amplification using ChIP-specific SFRP3 primers. The resulting reaction products were analyzed through electrophoresis on agarose gels(2%). The primer sequences are provided in the supplementary Table.

Co-immunoprecipitation assays[15]

The cells from various groups were lysed using an IP/CO-IP kit (Absin, China). Pre-cleared lysates were subjected to immunoprecipitation using 3 μg of specified antibodies, with IgG serving as a control, during an overnight incubation at 4° C. Subsequently, the immunoprecipitates were coupled with protein A/G agarose (Absin, China) for an additional 4 hours at 4° C. The immunocomplexes underwent 3 washes with a washing buffer, and the bound proteins were eluted by boiling. Following elution, the proteins in each group were subjected to analysis through immunoblotting.

Periostin(POSTN)-promoter, AAV9 adeno-associated virus construction

DNMT3A cardiac fibroblast-specific AAV9 were generated with a periostin promoter added before DNMT3A sequence to specifically target periostin+ cells. The sequence of the promoter is listed as below:

ATTCTTTCAAGCTAACAATCTTTTTTTTTTTTTTAAAGTGGCCTCAGTCAAAGACA
CTAAAGATCACCGAGTCTTGCATAGAGTTTCCATTTACAGGACTAGAGAAAGCTA
GTGGAGACACAGATCGGGTGCGGAGGTAGTGAGAAGCACTTTTCCTAAGAAGG
TGCAGGGTTGACTCCAAGGCTTGGCTGGGTTATAAGAGTTACATGTATTATTTAT
TCTATATGTAAGCAACTTTTGAGCTCATGTGCCATGGCAACCTATGGACCGCATG
TTAATATAGAAGCATTTTAAAATTAGTGATACAATCAAGACCAAGGGCATCCTGCT
TATGGTTTGTGTGCACAGGCTTACAGAGTGCAGAGTCCGCGAGGAGTCCCAGG
GACTGCTGGAGTTTGAGGTTGGTTTCACAGTGGTGAGTAAGCGTGGCAGTGTA
ATGACCTCATGGTCTCCCGAGGCCAGATAACAGAGAACTGCCTATAAATCAGCA
TGCCGCGGCTAGAGAGAAACGGCCCTGTTTCTCAGACACACTATCTCTCTTCA
GCTACATAATGAACCATTTCTTTCTCAGTAATGACTTACATCTCTGGGTCAGACTT
TGCAGCCCTGGAAAGTCGGACTTCATTTTCATGATTTCCGTCATCTTCCCGACT
GGTAGGAAAATTGCAGGGGTCAGTAGTGTCAGCATAGTTTCACAGAGCTGAAG
AGAAAGGGCCCTGTGTGGAGAGCGACTTTTGATGAGAGCCCCGGAAGAGAGT
GTGCCCTTCCGGGGATTTTTTTCCCAGTCTCTTCTACAACTTCAGACATCTATCC
AGGATTTGGGTTAAATGCCCCTGTGATTTCTCTTCTCCGTGTTCTGCTGTGGAG
TGATTTAAGTGCAATCAGATCAAACCAGGAAAGTAACTGAGCTCAGAGACACAG
AGTGTGGTGGCAGAGACAGAAGGCAGAGAGATCCCTAAACTCAGAATCAGCTC
TTTTCGCAATGTAAACCTATAGAAGTGAAAAACGGGCTCACCATGATTGAAAACA
AATAGGAGACAGAGTTCAGATTGCTCAGAACCCAGGAGATTTCCAGGGACAGC
CCAGGGCTGCTGGTGCTTCTGTAAGGCCATCGCAAGCTTCAGGTTGGCCCAG
CGCCCCCTCCCACAGCCTTGCTCCCTCCCACAGCCCAGAGCTATATAAACTCA
GCTCTCCAGAGCACAGGCCAGATCTCTTCCTGGACGGAGCTCAGGGCTGAA

Human samples[16]

Samples for histopathology, immunofluorescence, western blotting, RT-qPCR, BSP, MSP, and CHIP were collected from Sinus Rhythm subjects(n=31) and Atrial Fibrillation patients(n=39) at the Second Hospital of Anhui Medical University. With consent, samples were acquired from the left atrial appendage (LAA) tissue of patients with Sinus Rhythm and Atrial Fibrillation during heart valve replacement surgery. Patients were carefully matched for gender and age. The excised heart tissue samples were promptly collected and preserved at -80° C.This study obtained approval from the Ethics Committee of Anhui Medical University. The inclusion criteria for the Atrial Fibrillation group comprised the following conditions: (a) Presence of atrial fibrillation confirmed by ECG and Holter monitoring, (b) Patients with heart failure whose cardiac function had improved to NYHA class I or II after treatment, and (c) Willingness to provide informed consent for surgery and participate in follow-up.For the control group, the inclusion criteria were as follows: (a) Maintenance of normal blood lipid levels, blood pressure, and blood glucose within the standard ranges, along with normal heart function and pulmonary artery pressures, (b) Matched age and sex with the atrial fibrillation group.

MitoTracker Red staining

Cells were sliced into 24-well plates at 30% density and grouped according to requirements. After washing with PBS, mitochondria were labeled with 50 nM fluorescent probe MitoTracker-Red for 30min. Other staining and cell fixation washing were performed according to conventional procedures followed by counterstaining of nuclei with DAPI for 10 min. Finally. confocal fluorescence microscopy was used for imaging.

Analysis of the mitochondrial[17]

The National Health software, ImageJ, along with the "Mitochondrial Morphology" plugin developed by Ruben K Dagda, Ph.D, was used to evaluate the length and quantity of mitochondria in cells. To accurately identify mitochondrial segments, the images were processed through background subtraction, median filtering, thresholding, and binarization techniques in ImageJ (NIH). The particle counting subroutine was then utilized to count continuous mitochondrial structures. The count was normalized to the total mitochondrial area in pixels to determine the mitochondrial fragmentation count (MFC) for each cell. Each experiment was repeated at least three times, with 16 to 25 cells quantified per condition.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 9.0. Each sample represents an independent experiment. Prior to analysis, normality and variance homogeneity tests were performed on all datasets. To compare two groups, Student's t-test was employed, while for comparisons involving multiple groups, a one-way analysis of variance (ANOVA) was utilized, followed by Tukey's multiple comparisons test. Statistical significance was defined at $P < 0.05$. The n in the figure legends represents the number of biological replicates.

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Table S1. Baseline Characteristics of Healthy Control and Atrial fibrillation patients

Characteristics	Healthy Control	AF Patients	P Value
Cases (n)	31	39	-
Age (years)	63.26	67.33	-
Male (%)	17(54.84%)	22(56.41%)	-
BMI(kg/m2)	24.36±4.01	23.79±3.76	P=0.3946
Heart rates (/min)	80.55±17.72	91.03±27.31	P=0.1014
SBP (mm Hg)	126.18±17.83	121.04±23.17	P=0.1781
DBP (mm Hg)	80.55±14.83	77.45±16.97	P=0.0741
TC (mmol/L)	4.17±0.92	4.01±0.88	P=0.1929
Triglycerides(mmol/L)	1.59±0.63	1.71±1.01	P=0.0831
HDL (mmol/L)	1.03±0.25	1.17±0.31	P=0.1472
LDL (mmol/L)	2.71±0.61	2.48±0.43	P=0.2870
Creatinine (umol/L)	81.36±23.91	94.72±45.09	P=0.1880
BUN (umol/L)	7.01±2.43	7.31±2.91	P=0.0289
uric acid (umol/L)	358.12±121.78	339±113.69	P=0.1700
Cystatin c (mg/L)	1.12±0.29	1.56±0.47	P=0.0371
LV (mm)	45.58±5.13	51.12±8.13	P=0.0159
LA(mm)	34.10±5.81	43.16±7.12	P<0.0001
RV (mm)	20.84±2.17	24.26±4.13	P=0.0019
RA Major (mm)	42.18±3.45	51.87±7.57	P<0.0001
RA Minor (mm)	33.46±3.77	41.14±6.98	P<0.0001
IVS (mm)	9.32±1.34	10.69±1.69	P=0.0069
LVPW (mm)	8.81±1.18	9.87±1.26	P=0.0052
LVEF(%)	63.28±3.81	53.12±10.81	P<0.0001
LVFS(%)	34.01±2.31	29.91±1.87	P<0.0001

Table S2. Sequences of primers

		Target genes	Forward primer	Reverse primer
RT-qPCR				
	Human			
		SFRP3	5'-CGGACGGAGCTGATTTTCCT-3'	5'-TTACAGCGTTCACTGCTTGC-3'
		SFRP1	5'-TGGCCCGAGATGCTTAAGTG-3'	5'-GACACACCGTTGTGCCTTG-3'
		SFRP2	5'-CAGGACAACGACCTTTGCAT-3'	5'-ACATACCTTTGGAGCTTCCTCG-3'
		SFRP4	5'-GAGTGGCGCTCAAGGATGAT-3'	5'-TGTCCTGAACTGTTCTCCGC-3'
		SFRP5	5'-GCTCCAGTGACTTTGTGGTC-3'	5'-CTCCAATCAGCTTCCGGTCC-3'
		DKK1	5'-GACAACTACCAGCCGTACCC-3'	5'-CCTGCAGGCGAGACAGATTT-3'
		WIF1	5'-ACCTGCCATGAACCCAACAA-3'	5'-AGGCTGGCTTCGTACCTTTT-3'
		AXIN2	5'-AGGCCCTGCTGTAAAAGAGAG-3'	5'-AGTTCCTCTCAGCAATCGGC-3'
		STOT	5'-CAACAAGACCATGAACCGGG-3'	5'-GTACTCGGACACGTCTTTGGT-3'
		DNMT1	5'-ACAGAAAAGGAATGTGTGAAGGA-3'	5'-CAGGTAGCCCTCCTCGGATA-3'
		DNMT3A	5'-GGCCATACGGTGGAGCC-3'	5'-TGTTGAGCCCTCTGGTGAAC-3'
		DNMT3B	5'-AGTAAACCTAGCTCGGCGAT-3'	5'-TCCCTTCATGCTTTCCTGCC-3'
		β-actin	5'-ACAGAGCCTCGCCTTTGCC-3'	5'-GATATCATCATCCATGGTGAGCTGG-3'
	Mouse			
		SFRP3	5'-TGCGAGCCCATTCTCATCAA-3'	5'-TCCATAGGAAAATCCGCTCCG-3'
		SFRP1	5'-CAAGCGAGTTTGCACTGAGG-3'	5'-CCGCTTCAGCTCCTTCTTCT-3'
		SFRP2	5'-GAAGCTCCCAAGGTGTGTGA-3'	5'-TCACTTTGATTTTCAGTGCGAAGT-3'
		SFRP4	5'-ATGAGTGGCGTTCAAGGATGA-3'	5'-TGAAGCCTCTCTTCCCACTG-3'
		SFRP5	5'-ACCAAGATCTGTGCCCAGTG-3'	5'-TCGGTCCCCGTTGTCTATCT-3'
		DKK1	5'-TGGTAATGACCACAACGCCG-3'	5'-GGAGCCTTCTTGTCCTTTGGT-3'
		WIF1	5'-CTGTGCTCTAAGCCCGTCTG-3'	5'-CTCTCGACACTGGCACTTGT-3'
		AXIN2	5'-CGCCAGCGGATCAATGAGAG-3'	5'-GAGCGTCTCACTGAGTTTGGA-3'
		STOT	5'-AAGCCTTCAGGAATGATGCCA-3'	5'-TACTCGGACACATCTTTGGCG-3'
		POSTN	5'-GAAGTGATCCACGGAGAGCC-3'	5'-CCTCCTGTGGAAATCCTGGT-3'
		Collagen I	5'-CGATGGATTCCCGTTCGAGT-3'	5'-GAGGCCTCGGTGGACATTAG-3'
		PCNA	5'-CCTGTGCAAAGAATGGGGTG-3'	5'-TCTCTATGGTTACCGCCTCC-3'
		Fascin	5'-ACTCTGGATGCCAACCGTTC-3'	5'-TGCCCGTGGAGTCTTTGATG-3'
		Beclin1	5'-CAGGAACTCACAGGAGCCATT--3'	5'-CTCCCCGATCAGAGTGAAGC-3'
		DNMT1	5'-AGAGCTGTTCTGTCGTCTGC-3'	5'-TTCCAAGTCTTTGAGCCGCC-3'
		DNMT3A	5'-ACACGGTAACCAGCACGG-3'	5'-GCGGCTCTGCCCTCG-3'
		DNMT3B	5'-TGTGCCAGACCTTGGAAACC-3'	5'-TGTCTCCCTTCATTGTTTCCTGA-3'
		β-actin	5'-GCAGGAGTACGATGAGTCCG-3'	5'-ACGCAGCTCAGTAACAGTCC-3'

Table S2. Sequences of primers

		Target genes	Forward primer	Reverse primer
RT-qPCR				
	Mouse			
		Drp1	5'-CCTCAGATCGTCGTAGTGGGA-3'	5'-GTTCCTCTGGGAAGAAGGTCC-3'
		Fis1	5'-AGAGCACGCAATTTGAATATGCC-3'	5'-ATAGTCCCGCTGTTCCTCTTT-3'
		Mfn2	5'-GGTGGAAATACAGGGCTACAG-3'	5'-ACACTCAGGAAGCAGTTGG-3'
		Opa1	5'-ATACTGGGATCTGCTGTTGG-3'	5'-AAGTCAGGCACAATCCACTT-3'
		MFF	5'-ATGTACTTGAGTGGCAGCCT-3'	5'-TCACGACGAACCAGGAAGAA-3'
		MID49	5'-TCGATTGGTGCTAGGTGTG-3'	5'-GTCATCCTCATCTGGAGGG-3'
		MID51	5'-TCTATGATGATCTGCAGGTGG-3'	5'-ATGACCACAGGTTCTGCTC-3'
		Mfn1	5'-GTAGACAGCCCAGGTACAG-3'	5'-AACAAAGACATCAGCATCAAGG-3'
		TFAM	5'-AAAGCTAAACACCCAGATGC-3'	5'-TTCTGCTTCTGGTAGCTCC-3'
		NRF1	5'-TACTCTGCATCTCACCCTC-3'	5'-ACTTTCGCACCACATTCTC-3'
MSP/CHIP				
	Human			
		MSP-SFRP3(M)	5'-AAATATTTTTAGGAAGGAGGGTTTC-3'	5'-GAAAATAAAATAAACGCCCGAA-3'
		MSP-SFRP3(U)	5'-AAATATTTTTAGGAAGGAGGGTTTT-3'	5'-CCAAAAATAAAATAAACACCCAAA-3'
		CHIP-Primer 1	5'-CCAAAGACGGGTTCCCTAGT-3'	5'-CAGGGACGTCTGTGCCTCT-3'
		CHIP-Primer 2	5'-CTGCACCCTTCTCTCCCATTA-3'	5'-GCAGAAGCATCAGACTTCGCA-3'
		CHIP-Primer 3	5'-GTGTGCGTGATCTAGGGGAG-3'	5'-GTCCCACGAGCTTTACCGAG-3'
	Mouse			
		MSP-SFRP3(M)	5'-GTTTTTAGGAGGTTGTTATTTTTGC-3'	5'-CCGACTCTAAATCCATAAACGAA-3'
		MSP-SFRP3(U)	5'-TGTTTTTAGGAGGTTGTTATTTTTGT-3'	5'-CCCCAACTCTAAATCCATAAACA-3'
		CHIP-Primer 1	5'-GGGTTTGCTGTGAGGAAAGAG-3'	5'-TTTTCAGGGGACTGGAGTGT-3'
		CHIP-Primer 2	5'-ATCTCTGTGGTGCGTTGAGA-3'	5'-CCACCTCGGCCGACAAGA-3'
Transfection				
		siRNA-DNMT3A	5'-CAUGUCAUGACAGCGAUGATT-3'	5'-UCAUCGCUGUCAUGACAUGTT-3'
		siRNA-DNMT3B	5'-GUACCACACUCUGGAGAAATT-3'	5'-UUUCUCCAGAGUGUGGUACTT-3'
		siRNA-SFRP3	5'-CCGGAACAAUUACAACUAUTT-3'	5'-AUAGUUGUAAUUGUUCCGGTT-3'

Table S3. Reagents and antibodies

Name	Manufacturers	Location	Article Number
BCA kit	Beyotime	Shanghai, China	P0012
CCK-8	Beyotime	Shanghai, China	C0038
RT-PCR kit	Accurate Biology	Hunan, China	AG11705, AG11718
Edu assay kit	Ribobio	Guangzhou, China	R11053.10
Co-IP kit	Absin	Beijing, China	abs955
MethylationGold™ Kit	ZYMO	Orange County, California	D5005
Methylated DNA Immunoprecipitation kit	Abcam	Cambridge, UK	ab117133
SimpleChIP® Chromatin IP Kit	Cell Signaling Technology	Danvers, Massachusetts	56383
TGF-β1	Peprotech	Rocky Hill, New Jersey	1020209
TRIzol	Invitrogen	Carlsbad, California	15596018
Lipofectamine 2000	Invitrogen	Carlsbad, California	11668019
Antifade Mounting Medium with DAPI	Beyotime	Shanghai, China	P0131
anti-SFRP3 antibody	Proteintech	Wuhan, China	67081-1-Ig
anti-SFRP1 antibody	Proteintech	Wuhan, China	26460-1-AP
anti-SFRP2 antibody	Proteintech	Wuhan, China	12189-1-AP
anti-SFRP4 antibody	Proteintech	Wuhan, China	15328-1-AP
anti-SFRP5 antibody	Abcam	Cambridge, UK	ab230425
anti-DKK1 antibody	Proteintech	Wuhan, China	21112-1-AP
anti-WIF1 antibody	Proteintech	Wuhan, China	67904-1-Ig
anti-AXIN2 antibody	Proteintech	Wuhan, China	20540-1-AP
anti-SOST antibody	Proteintech	Wuhan, China	21933-1-AP
anti-POSTN antibody	Proteintech	Wuhan, China	66491-1-Ig
anti-Collagen I antibody	Proteintech	Wuhan, China	66761-1-Ig
anti-PCNA antibody	Proteintech	Wuhan, China	10205-2-AP
anti-DNMT1 antibody	Proteintech	Wuhan, China	24206-1-AP
anti-DNMT3A antibody	Proteintech	Wuhan, China	20954-1-AP
anti-DNMT3B antibody	Proteintech	Wuhan, China	26971-1-AP
anti-Fascin antibody	Proteintech	Wuhan, China	14384-1-AP
anti-Beclin1 antibody	Proteintech	Wuhan, China	11306-1-AP
anti-WNT1 antibody	Proteintech	Wuhan, China	27935-1-AP
anti-β-catenin antibody	Proteintech	Wuhan, China	17565-1-AP
anti-β-actin antibody	Proteintech	Wuhan, China	66009-1-Ig
anti-Mouse IgG	Proteintech	Wuhan, China	B900620
anti-Rabbit IgG	Proteintech	Wuhan, China	30000-0-AP
Goat anti-Rabbit IgG / Alexa Fluor® 488	ZSGB-BIO	Beijing, China	ZF-0516
Goat anti-Mouse IgG / Alexa Fluor® 594	ZSGB-BIO	Beijing, China	ZF-0513

Figure Legends:

Supplementary Figure 1

(A)The expression of SFRP3, SFRP1, SFRP2, SFRP4, SFRP5, DKK1, WIF1, AXIN2 and STOT in AF or SR patient heart tissues were analyzed by western blotting for indicated protein levels (n = 6).

(B)Hearts tissues from AF or SR patient were embedded in paraffin, and tissue sections were evaluated by Immunofluorescence staining. Immunofluorescence assays were performed to evaluate SFRP3 and POSTN expression in human heart tissue, Nuclei were stained with DAPI (n = 6).

(C)Hearts tissues from AF or SR patient were embedded in paraffin, and tissue sections were evaluated by Immunofluorescence staining. Immunofluorescence assays were performed to evaluate cTnT and SFRP3 expression in human heart tissue, Nuclei were stained with DAPI (n = 6), $P > 0.05$ with Student's *t*-test.

(D)Hearts tissues from AF or SR patient were embedded in paraffin, and tissue sections were evaluated by Masson's trichrome staining(n = 6) and Sirius red staining (n = 6).

(E)The expression of POSTN and type I collagen in AF or SR patient heart tissues were analyzed by western blotting for indicated protein levels (n = 6).

(F)The expression of WNT1 and β-catenin in AF or SR patient heart tissues were analyzed by western blotting for indicated protein levels (n = 6).

(G) Correlation scatter plot of RNA expression levels of sFRP3 and cardiac function, Pearson's correlation test was used (n = 20).

"ns" indicates findings that were not significant ($P > 0.05$). The "n" in the figure legends indicates the number of biological replicates.

Supplementary Figure 2

(A)The expression of SFRP3, SFRP1, SFRP2, SFRP4, SFRP5, DKK1, WIF1, AXIN2 and STOT in ISO induced experimental cardiac fibrosis tissues were analyzed by western blotting for indicated protein levels (n = 6).

(B)Hearts tissues from ISO induced experimental were embedded in paraffin, and tissue sections were evaluated by Immunofluorescence staining. Immunofluorescence assays were performed to evaluate SFRP3 and POSTN expression in mice heart tissue, Nuclei were stained with DAPI (n = 6).

(C)Hearts tissues from ISO induced experimental were embedded in paraffin, and tissue sections were evaluated by Immunofluorescence staining. Immunofluorescence assays were performed to evaluate cTnT and SFRP3 expression in mice heart tissue, Nuclei were stained with DAPI (n = 6), $P > 0.05$ with Student's *t*-test.

(D)Hearts tissues from ISO induced experimental were embedded in paraffin, and tissue sections were evaluated by Masson's trichrome staining(n = 6) and Sirius red staining (n = 6).

(E)The expression of POSTN and type I collagen in ISO induced experimental cardiac fibrosis tissues were analyzed by western blotting for indicated protein levels (n = 6).

(F)The expression of WNT1 and β-catenin in ISO induced experimental cardiac fibrosis tissues were analyzed by western blotting for indicated protein levels (n = 6).

(G)Correlation scatter plot of RNA expression levels of sFRP3 and cardiac function, Pearson's correlation test was used (n = 20).

"ns" indicates findings that were not significant ($P > 0.05$). The "n" in the figure legends indicates the number of biological replicates.

Supplementary Figure 3

(A)The expression of SFRP3 in TGF-β1 induced cardiac fibroblasts and cardiomyocytes were analyzed by western blotting for indicated protein levels (n = 6).

(B)The expression of SFRP3 in TGF-β1 induced cardiac fibroblasts and cardiomyocytes were analyzed by RT-qPCR for indicated mRNA levels (n = 6), *P* value with Student's *t*-test.

(C)The expression of POSTN, type I collagen and PCNA in TGF-β1 induced cardiac fibroblasts were analyzed by western blotting for indicated protein levels (n = 6).

(D)The expression of POSTN, type I collagen and PCNA in TGF-β1 induced cardiac fibroblasts were analyzed by RT-qPCR for indicated mRNA levels (n = 6), *P* value with Student's *t*-test.

(E)EdU assay to detect TGF-β1 induced the proliferation of cardiac fibroblasts (n=6).

(F)CCK-8 assay to detect TGF-β1 induced the proliferation of cardiac fibroblasts (n=6), *P* value with Student's *t*-test.

(G)The scratch assay was performed to test TGF-β1 induced the migration ability of cardiac fibroblasts (n=6).

(H)Transwell migration assay detect TGF-β1 induced the migration ability of cardiac fibroblasts (n=6).

(I) The expression of DNMT3A and sFRP3 in TGF-β1 induced Cardiomyocytes treatment with siRNA-DNMT3A were analyzed by western blotting for indicated protein levels (n = 6).

"ns" indicates findings that were not significant ($P > 0.05$). The "n" in the figure legends indicates the number of biological replicates.

Supplementary Figure 4

(A)The expression of DNMT1, DNMT3A and DNMT3B in AF or SR patient heart tissues were analyzed by western blotting for indicated protein levels (n = 6).

(B)The expression of DNMT1, DNMT3A and DNMT3B in ISO induced experimental cardiac fibrosis tissues were analyzed by western blotting for indicated protein levels (n = 6).

(C)The expression of DNMT1, DNMT3A and DNMT3B in AF or SR patient heart tissues and ISO induced experimental cardiac fibrosis tissues were analyzed by RT-qPCR for indicated mRNA levels (n = 6), $P \leq 0.001$ with Student's *t*-test.

(D)The expression of DNMT1, DNMT3A and DNMT3B in TGF-β1 induced cardiac fibroblasts were analyzed by western blotting for indicated protein levels (n = 6).

(E)The expression of DNMT1/DNMT3A/DNMT3B and SFRP3 in TGF-β1 induced cardiac fibroblasts treatment with siRNA-DNMT1/siRNA-DNMT3A/siRNA-DNMT3B were analyzed by western blotting for indicated protein levels (n = 6).

(F)The expression of SFRP3 in TGF-β1 induced cardiac fibroblasts treatment with siRNA-DNMT3A/5-azadC were analyzed by RT-qPCR for indicated mRNA levels (n = 6), $P \leq 0.001$ with Student's *t*-test.

(G)DNMT3A binds to the SFRP3 promoter in human heart tissues. ChIP products were amplified by PCR. The ChIP assay revealed that DNMT3A could bind to the SFRP3 promoter (n = 6), $P \leq 0.001$ with Student's t-test.

(H) The human and mice heart DNA GAPDH as a loading control.

"ns" indicates findings that were not significant ($P > 0.05$). The "n" in the figure legends indicates the number of biological replicates.

Supplementary Figure 5

(A)Line graphs reflecting body weight changes of mice injected with Vector and mice injected with AAV9-DNMT3A (n=6), ns not significant; analyzed by two-way analysis of variance (*ANOVA*).

(B)Line graphs reflecting daily water intake changes of mice injected with Vector and mice injected with AAV9-DNMT3A(n=6), ns not significant; analyzed by two-way analysis of variance (*ANOVA*).

(C)Line graphs reflecting daily food intake changes of mice injected with Vector and mice injected with AAV9-DNMT3A(n=6), ns not significant; analyzed by two-way analysis of variance (*ANOVA*).

(D)The expression of type I collagen and POSTN in ISO induced experimental treatment with AAV9-DNMT3A were analyzed by western blotting for indicated protein levels (n = 6).

(E)The expression of DNMT3A in ISO induced experimental treatment with AAV9-DNMT3A were analyzed by western blotting for indicated protein levels (n = 6).

(F)Hearts tissues from ISO induced experimental treatment with AAV9-DNMT3A, the mice heart tissues were embedded in paraffin, and tissue sections were evaluated by Masson's trichrome staining(n = 6) and Sirius red staining (n = 6).

(G)The expression of SFRP3 in ISO induced experimental treatment with AAV9-DNMT3A were analyzed by western blotting for indicated protein levels (n = 6).

(H)Hearts tissues from ISO induced experimental treatment with AAV9-DNMT3A, the mice heart tissues were embedded in paraffin, and tissue sections were evaluated by Immunofluorescence staining. Immunofluorescence assays were performed to evaluate SFRP3 and Postn expression in mice heart tissue, Nuclei were stained with DAPI (n = 6).

Supplementary Figure 5

(I)Hearts tissues from ISO induced experimental treatment with AAV9-DNMT3A, the mice heart tissues were embedded in paraffin, and tissue sections were evaluated by Immunofluorescence staining. Immunofluorescence assays were performed to evaluate DNMT3A and Postn expression in mice heart tissue, Nuclei were stained with DAPI (n = 6), P value with Student's t-test.

(J)Hearts tissues from ISO induced experimental treatment with AAV9-sFRP3, the mice heart tissues were embedded in paraffin, and tissue sections were evaluated by Masson's trichrome staining(n = 6) and Sirius red staining (n = 6), P value with Student's t-test.

(K)Transthoracic echocardiography was performed on mice ISO induced experimental treatment with AAV9-sFRP3 to assess cardiac function (n = 6), P value with Student's t-test.

(L)The expression of sFRP3, POSTN, Collagen I, WNT1 and β-catenin in ISO induced experimental treatment with AAV9-sFRP3 were analyzed by western blotting for indicated protein levels (n = 6), P value with Student's t-test.

(M) The expressions of sFRP3 and DNMT3A in TGF-β1-induced cardiac fibroblasts treated with siRNA-DNMT3A and siRNA-sFRP3 were analyzed by western blotting for the indicated proteins (n = 6), *ANOVA* and Student's t-tests were used.

"ns" indicates findings that were not significant ($P > 0.05$). The "n" in the figure legends indicates the number of biological replicates.

Supplementary Figure 6

(A) The expression of POSTN, type I collagen, PCNA, Fascin and Beclin1 in TGF-β1 induced cardiac fibroblasts and cardiomyocytes were analyzed by RT-qPCR for indicated mRNA levels (n = 6), *P* value with Student's *t*-test.

(B) The expression of sFRP3, PCNA and Fascin in TGF-β1 induced TGF-β1 induced cardiac fibroblasts treatment with sFRP3 overexpression were analyzed by RT-qPCR for indicated mRNA levels (n = 6), *P* value with Student's *t*-test.

(C) The expression of sFRP3, PCNA and Fascin in TGF-β1 induced TGF-β1 induced cardiac fibroblasts treatment with siRNA-sFRP3 were analyzed by RT-qPCR for indicated mRNA levels (n = 6), *P* value with Student's *t*-test.

(D) The expression of Beclin1 in TGF-β1 induced cardiac fibroblasts treatment with sFRP3 overexpression were analyzed by western blotting for indicated protein levels (n = 6), $P > 0.05$ with Student's t-test.

(E) Acridine Orange assay to detect TGF-β1 induced treatment with SFRP3 overexpression the autophagy level of cardiac fibroblasts (n=6), $P > 0.05$ with Student's t-test.

(F) The expressions of type I collagen, POSTN, and fascin in TGF-β1-induced cardiac fibroblasts treated with siRNA-sFRP3 were analyzed by RT-qPCR for the indicated mRNAs (n = 6), $P \leq 0.001$ with Student's t-test.

(G) The expression of type I collagen, POSTN and Fascin in TGF-β1 induced treatment with siRNA-sFRP3 in cardiac fibroblasts were analyzed by western blotting for indicated protein levels (n = 6), *P* value with Student's *t*-test.

(H) The expressions of POSTN and fascin in TGF-β1-induced cardiac fibroblasts treated with siRNA-sFRP3 and siRNA-DNMT3A were analyzed by western blotting for the indicated proteins (n = 6), *ANOVA* and Student's t-tests were used.

Supplementary Figure 6

(I) Transwell migration assay detect TGF-β1 induced treatment with siRNA-sFRP3 and siRNA-DNMT3A the migration ability of cardiac fibroblasts (n=6), *ANOVA* and Student's t-tests were used.

(J) EdU assay to detect the proliferation of TGF-β1-induced cardiac fibroblasts treated with siRNA-sFRP3 and siRNA-DNMT3A (n = 6), *ANOVA* and Student's t-tests were used.

(K) EdU assay to detect the proliferation of TGF-β1-induced cardiac fibroblasts treated with siRNA-DNMT3A (n = 6), $P \leq 0.001$ with Student's t-test.

(L) The expressions of WNT1, β-catenin, POSTN, and fascin in TGF-β1-induced cardiac fibroblasts treated with siRNA-DNMT3A were analyzed by western blotting for the indicated proteins levels (n = 6), $P \leq 0.001$ with Student's t-test.

(M) Transwell migration assay to detect the migration ability of TGF-β1-induced cardiac fibroblasts treated with siRNA-DNMT3A (n = 6), $P = 0.001934$ with Student's t-test.

(N) EdU assay to detect TGF-β1 induced treatment with DNMT3A overexpression the proliferation of cardiac fibroblasts (n=6), $P \leq 0.001$ with Student's *t*-test.

(O) The expression of sFRP3, WNT1, β-catenin, POSTN and Fascin in TGF-β1 induced treatment with DNMT3A overexpression in cardiac fibroblasts were analyzed by western blotting for indicated protein levels (n = 6), $P \leq 0.001$ with Student's *t*-test.

(P) Transwell migration assay detect TGF-β1 induced treatment with DNMT3A overexpression the migration ability of cardiac fibroblasts (n=6), $P = 0.003996$ with Student's t-test.

"ns" indicates findings that were not significant ($P > 0.05$). The "n" in the figure legends indicates the number of biological replicates.

Supplementary Figure 7

(A) The expressions of β-catenin in TGF-β1-induced cardiac fibroblasts treated with sFRP3 overexpression and IWR-1 were analyzed by western blotting for the indicated proteins (n = 6).

(B) The expressions of AXIN1 in TGF-β1-induced cardiac fibroblasts treated with sFRP3 overexpression and IWR-1 were analyzed by western blotting for the indicated proteins (n = 6), P value with Student's t-test.

(C) The expressions of β-catenin in TGF-β1-induced cardiac fibroblasts treated with siRNA-sFRP3 and IWR-1 were analyzed by western blotting for the indicated proteins (n = 6).

(D) The expressions of AXIN1 in TGF-β1-induced cardiac fibroblasts treated with siRNA-sFRP3 and IWR-1 were analyzed by western blotting for the indicated proteins (n = 6), P value with Student's t-test.

(E) The expressions of Drp1 in TGF-β1-induced cardiac fibroblasts treated with sFRP3 overexpression and MDIVI1 were analyzed by western blotting for the indicated proteins (n = 6).

(F) The Relative GTPase cativity in TGF-β1-induced cardiac fibroblasts treated with sFRP3 overexpression and MDIVI1 were analyzed by GTPase Activity Assay Kit (n = 6), P value with Student's t-test.

(G) The expressions of Drp1 in TGF-β1-induced cardiac fibroblasts treated with siRNA-sFRP3 and MDIVI1 were analyzed by western blotting for the indicated proteins (n = 6).

(H) The Relative GTPase cativity in TGF-β1-induced cardiac fibroblasts treated with siRNA-sFRP3 and MDIVI1 were analyzed by GTPase Activity Assay Kit (n = 6), P value with Student's t-test.

(I) Cardiac fibroblasts treated with siRNA-sFRP3 and sFRP3 overexpression after TGF-β1-induced were co-stained with mitotracker and PCNA. Measure mitochondrial length, mitochondrial fragmentation and the PCNA expression of different groups(n = 6).

"ns" indicates findings that were not significant ($P > 0.05$). The "n" in the figure legends indicates the number of biological replicates