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Supplemental information

Hepatocyte-derived exosomal miR-27a activates hepatic stellate cells through the inhibition of PINK1-mediated mitophagy in MAFLD

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Supplemental Material: Materials and Methods

Animal experiments

A total of 110 male C57BL/6 mice (8 weeks old) were randomized into 3 groups (Shanghai SLAC Laboratory Animal Co., Ltd., Shanghai, China). (1) Forty mice were randomly fed either a low-fat diet (LFD: standard diet) or a high-fat diet (HFD: 60% fat, 20% protein, and 20% carbohydrate) for 8 weeks (Trophic Animal Feed High-tech Co., Ltd., Nantong, China). The cirrhotic mouse model was then established by intraperitoneal injection of carbon tetrachloride (CCl_4, 2μl/g bodyweight; Aladdin, Shanghai, China) twice a week during the last 6 weeks. Olive oil injections were used as a control. Then, 40 mice were divided to 4 subgroups (LFD, HFD, LFD+CCl_4, and HFD+CCl_4; each group n=10). (2) Thirty mice were fed a methionine-choline deficient diet (MCD: 40% carbohydrate, 10% fat, and deficient in methionine and choline) for 6 weeks (Trophic Animal Feed High-tech Co., Ltd.). They were randomized into 3 subgroups that were treated with CCl_4/olive oil and exosomes. Exosomes (30μg in 50μL) derived from the culture medium (incubated with 200μM palmitic acid [PA] or 3% bovine serum albumin [BSA] as vehicle control; Sigma-Aldrich, St. Louis, MO) of LO2 cells was intraperitoneally injected to MCD mice twice a week for 6 weeks. Then, 30 mice were divided into 3 subgroups (MCD, MCD+CCl_4, and MCD+CCl_4+Exo; each group n=10). (3) Forty mice were fed an HFD for 8 weeks and then treated with CCl_4 and various purified exosomes during the last 6 weeks. The exosomes (30μg in 50μL) were derived from the culture medium of LO2 cells with different pre-transfection treatments (50nM miR27a mimics [mi-miR] and miR negative control mimics [mi-NC] to over-express miR27a; 100nM miR27a inhibitors [in-miR] and miR negative inhibitors [in-NC] to knock-down miR27a; Shanghai GenePharma Co. Ltd., Shanghai, China; Table S3). Based on this, 40 HFD+CCl_4 mice were divided to 4 subgroups (Exo-mi-CN, Exo-mi-miR, Exo-in-CN, and Exo-in-miR; each group n=10).

The animal study was approved by the Institutional Animal Care and Use Committee of Shanghai General hospital.

Histological studies

Liver tissue sections are prepared and stained with hematoxylin-eosin (H&E), Sirius-red and oil red O (ORO) as described in our previous study.[1] Tissue morphology is observed through a light microscope (Leica Microsystems) and captured by the attached camera.

TEM of liver tissues

After different treatments, fresh liver tissues from mice are fixed with 2.5% glutaraldehyde (Sigma-Aldrich). Tissues are subsequently embedded, sectioned, and double stained with uranyl acetate and lead, citrate. Images were captured using a transmission electron microscope (JEOL).

Serum ALT activity

The serum activities of ALT are detected using ALT assay kits (Sigma-Aldrich) according to the manufacturer's protocol.

Isolation and culture of primary cells of mouse

Isolation and culture of hepatocytes (PHCs), hepatic stellate cells (PHSCs) and Kupffer cells (PKCs) were isolated from wide-type (WT) C57BL/6 mice as our previously study or according to the protocol described.[1-3] PHSCs are isolated using a gradient centrifugation method. After perfusing the livers with collagenase and pronase (Roche, Basel, Switzerland), the PHSCs are isolated by Nycodenz density gradient (Sigma-Aldrich) centrifugation. The PHSCs are subsequently cultured in DMEM supplemented with 100 mg/ml streptomycin, 100 IU/ml penicillin, and 10% fetal bovine serum (FBS; Gibco). PHCs and PKCs are isolated using a two-step collagenase digestion method. PHCs and PKCs are cultured with M199 or RPMI-1640 medium (Gibco) supplemented with 10% FBS, 100 mg/ml streptomycin and 100 IU/ml penicillin. After 1-7 days in culture, the cells are harvested for the subsequent experiments.

Cell line culture and treatment

LO2 cells (human HC cell line), AML12 cells (mice HC cell line), HepG2 and Huh7 cells (human HCC cell line), THP-1 cells (human macrophages cell line), LX2 cells (human HSC cell line) and EGI-1 cells (human cholangiocarcinoma cell line) are used in this study.

Cells are cultured in DMEM or RPMI-1640 medium with 10% FBS, 100 mg/ml streptomycin as well as 100 IU/ml penicillin. LX2 cells treated with transforming growth factor-β1 (TGFβ1 10ng/ml; Sigma-Aldrich) for 24-48 hours. To induce lipotoxic environment, LO2 or other hepatic cells are treated by 200-400μM PA or 3%BSA as the vehicle control for 24 hours.

Cell transfection

For cell transfection, 50nM miR27a mimics (mi-miR) and miR negative control mimics (mi-NC) is used to over-express miR27a, while 100nM miR27a inhibitors (in-miR) and miR negative inhibitors (in-NC) is used to knock-down miR27a expression. Besides, 100nM small interfering-RNA (si-RNA) against PINK1 (si-PINK1) and negative control si-RNAs (si-NC) is used to knock-down PINK1. All synthesized oligonucleotides in this study are showed (Shanghai GenePharma Co. Ltd., Shanghai, China; Table S3). On the day of transfection, the cells are plated in DMEM without FBS and transfected with mimics, inhibitors or si-RNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Quantitative Real-time PCR (qPCR)

QPCR is performed using a SYBR Green PCR Kit (Applied Biosystems, Foster City, CA) and ABI 7900HT Fast Real-Time PCR System (Applied Biosystems). The primers used in this research are listed (Table S4). All qPCR was repeated three times respectively.

Western blot

All the primary antibodies used in the study are against the following proteins listed in

Table S5. And the secondary antibody is HRP-IgG (1:10000; SantaCruz Biotechnology, SantaCruz, CA). Western blot was repeated twice respectively.

Immunofluorescence (IF) assay

IF staining of cell or liver slides is incubated with anti-LC3B, anti-COX4, anti-PINK1, anti-PCNA，anti-CD63 and anti-α-SMA primary antibodies (Table S4). DAPI is applied to show the nucleus. Representative images are captured via TCS SP8 CARS confocal fluorescent microscope (Leica Microsystems). Relative fluorescence values are measured via ImageJ 1.8.0 (Rawak Software Inc., Stuttgart, Germany). It was repeated three times.

EdU Staining

Cell proliferation is evaluated by the EdU assay Kit (RiboBio., Shanghai, China). First, cells with different treatments are incubated with EdU solution for approximately 2 hours. Then cells are fixed via 4% paraformaldehyde, followed by permeabilization with 0.5% Triton X-100 for approximately and 1%DAPI to distinguish the nuclei. Samples are viewed under a fluorescence microscope (Leica Microsystems). The number of EdU positive cells is measured via counting at least five random separate fields (including the 4 corner fields and the center filed). It was repeated three times.

Luciferase Assay

For the luciferase reporter assay, the psiCHECK-2 luciferase vector (Promega, Madison, WI) is used. The cells are co-transfected with PINK1 plasmids (wild type or mutant; GenePharma; primers for plasmids are listed in Table S3) and miR27a mimics/inhibitors (mi-NC, mi-miR, in-NC, in-MiR) for 24 hours. Luciferase activity is measured using the Promega Dual-Luciferase system, and the relative luciferase activity is calculated as Firefly to Renilla luciferase luminescence.

To further evaluate if miR27a could disturb the transcription stability of PINK1. Cells are transfected with 50nM mi-NC or mi-miR for 24 hours before addition of actinomycin D (5μg/mL, time 0, Sigma-Aldrich). Total cellular RNA is extracted at 0, 0.25, 0.5, 1, and 2 hours after treatment with actinomycin D. Relative mRNA levels of PINK1 at different time points are determined by qPCR and compared to time 0. The assay was repeated three times.

Reference

1. Liu T, Luo X, Li ZH, et al. Zinc-α2-glycoprotein 1 attenuates non-alcoholic fatty liver disease by negatively regulating tumor necrosis factor-α. World J Gastroenterol 2019; 25(36): 5451-5468.
2. Wu JC, Chen R, Luo X, et al. MicroRNA-194 inactivates hepatic stellate cells and alleviates liver fibrosis by inhibiting AKT2. World J Gastroenterol 2019; 25(31): 4468-4480.
3. Aparicio-Vergara M, Tencerova M, Morgantini C, et al. Isolation of Kupffer Cells and Hepatocytes from a Single Mouse Liver. Methods Mol Biol. 2017; 1639: 161-171.

Nonstandard Abbreviations:

4’6-Diamidino-2-phenylindole (DAPI)
advanced liver fibrosis (ALF)
alanine aminotransferase (ALT)
area under receiver operating characteristic (AUROC)
autophagy protein 7 (Atg7)
body mass index (BMI)
bovine serum albumin (BSA)
carbon tetrachloride (CCl4)
carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP)
chronic hepatitis B (CHB)
CollagenI(ColI)
differentiation 63 (CD63)
Ethynyl-20-Deoxyuridine (EdU)
exosomes (Exos)
exosomes derived from PA treated LO2 cells (Exo-PA)
hepatic stellate cells (HSCs)
hepatocytes (HCs)
high-fat diet (HFD)
immunofluorescence (IF)
liver/body weight (liver index)
low-fat diet (LFD)
lysosomal membrane protein (LAMP)
mammalian target of rapamycin (mTOR)
metabolic associated fatty liver disease (MAFLD)
methionine-choline deficient diet (MCD)
microRNAs (miRs)
microtubule-associated protein light chain 3B (LC3B)
miR negative control mimics (mi-NC)
miR negative inhibitors (in-NC)
miR27a inhibitors (in-miR/in-miR27a)
miR27a mimics (mi-miR/miR27a)
mitochondial transcription factor A (TFAM)
mitochondrial autophagy (mitophagy)
mitochondrial membrane potential (MMP)
mitoCMXRos (mtCMXRos)
mitoSOX (mtSOX)
moderate liver fibrosis (MLF)
nanoparticle tracking analysis (NTA)
nuclear respiratory factors (NRFs)
Oil Red O (ORO)
oxygen consumption rate (OCR)
palmitic acid (PA)

peroxisome proliferator-activated receptor (PPAR)
phosphatase and tensin homolog (PTEN)-induced putative protein kinase 1 (PINK1)
platelet-derived growth factor receptor (PDGFR)
primary HCs (PHCs)
primary HSCs (PHSCs)
primary Kupffer cells (PKCs)
proliferating cell nuclear antigen (PCNA)
quantitative real-time PCR (qPCR)
reactive oxygen species (ROS)
siRNA-PINK1 (siPINK1)
steatohepatitis (NASH)
transcription factor B1/2 mitochondrial (TFB1M/2M)
transmission electron microscopy (TEM)
wild type (WT)
α-smooth muscle actin (α-SMA)

Table S1：The clinic data of a cohort of 16 biopsy-proven MAFLD patients (n=16)

Parameter	MLF(n=8)	ALF(n=8)	P value
Gender			
Male	4	5	
Female	4	3	
Age (years)	48.38±5.13	51.25±7.65	P=0.260
ALT (U/L)	47.64±6.42	52.29±9.45	p=0.520
AST (U/L)	57.71±5.20	64.00±13.18	p=0.613
TBIL(umol/L)	28.05±9.26	35.73±11.87*	*p=0.016*
DBIL(umol/L)	12.62±4.84	19.00±6.19*	*p=0.045*
r-GT (U/L)	57.53±18.92	72.6±48.82*	*p=0.023*
ALB (g/L)	39.24±4.71	35.04±6.88	p=0.422
PLT (x109/L)	170.60±11.64	105.30±15.86*	*p=0.004*
BMI(kg/m2)	28.4±4.5	25.7±6.3*	*p<0.001*
LSM(kPa)	8.9±2.6	15.4±6.5*	*p<0.001*
CAP(dB/m)	285±39	273±23	p=0.722

NOTE:
MLF, mild liver fibrosis; ALF, sever liver fibrosis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direc bilirubin; r-GT, gamma glutamyl transpeptidase; ALB, albumin; PLT, platelet; LSM, liver stiffness measurement; CAP, controlled attenuation parameter; BMI, body mass index.
* means comparing with MLF, $p<0.05$.

Table S2：The clinic data of a cohort of 30 MAFLD patients by non-invasive diagnosis of FibroScan (n=30)

Parameter	Control(n=10)	MLF(n=10)	ALF(n=10)	P value
Gender				
Male	5	6	6	
Female	5	4	4	
Age (years)	45.10±6.54	36.40±4.86	50.20±3.92$^{\#}$	①VS②p=0.055 ②VS③p=0.019 ①VS③p=0.223
ALT (U/L)	29.00±5.35	79.45±13.12*	59.89±6.47*	①VS②p<0.001 ②VS③p=0.797 ①VS③p=0.025
AST (U/L)	30.51±6.79	47.26±5.60*	42.01±2.87	①VS②p=0.001 ②VS③p=0.813 ①VS③p=0.070
TBIL(umol/L)	19.50±4.85	26.69±8.45	28.66±6.91	①VS②p=0.013 ②VS③p=0.055 ①VS③p<0.001
DBIL(umol/L)	6.90±2.26	10.35±3.74	8.81±2.89	①VS②p=0.341 ②VS③p=0.261 ①VS③p=0.528
r-GT (U/L)	21.64±2.21	77.58±10.97*	55.06±10.18*	①VS②p<0.001 ②VS③p=0.150 ①VS③p=0.005
ALB (g/L)	41.07±4.14	49.83±1.82	38.11±4.16$^{\#}$	①VS②p=0.069 ②VS③p=0.019 ①VS③p=0.620
PLT (mmol/L)	217.20±32.83	218.80±26.41	102.40±15.37$^{*\#}$	①VS②p=0.912 ②VS③p<0.001 ①VS③p<0.001
BMI(kg/m^2)	21.3±5.7	29.3±5.1*	25.1±8.3$^{*\#}$	①VS②p<0.001 ②VS③p=0.016 ①VS③p=0.008
LSM(kPa)	4.8±2.4	10.3±4.3*	14.4±3.8$^{*\#}$	①VS②p=0.001 ②VS③p=0.017 ①VS③p<0.001
CAP(dB/m)	245±18	312±43*	284±38*	①VS②p<0.001 ②VS③p=0.093 ①VS③p=0.002

NOTE:
Control: ①, MLF: ②, ALF: ③
* means comparing with ①, p<0.05; # means comparing with ②, p<0.05

Table S3: Synthesized oligonucleotides in the transfection study

Gene		Primer Sequence
mi-miR		5'-UUCACAGUGGCUAAGUUCCGC-3'
mi-NC		5'-UUGUACUACACAAAAGUACUG-3'
in-miR		5'-AAGUGUCACCGAUUCAAGGCG-3'
in-NC		5'-CAGUACUUUUGUGUAGUACAA-3'
si-PINK1		F：5'-GCUAACCUGGAGUGUGAAATT-3'
		R：5'-UUUCACACUCCAGGUUAGCTT-3'
si-NC		F： 5'-UUCUCCGAACGUGUCACGUTT-3'
		R： 5'-ACGUGACACGUUCGGAGAATT-3'
PINK1 plasmid	Wild Type	F: 5'-CUGUGUCGUGAUGGUCUGUGAAU-3'
	Mutant	F: 5'-CUGUGUCGUGAUGGUGACACUAU-3'

Table S4: Primers used in qPCR

Gene	Primer Sequence	
	human	mouse
miR27a	F：5'-GCGCGTTCACAGTGGCTAAG -3'	F：5'-GCGCGTTCACAGTGGCTAAG -3'
	R：5'-AGTGCAGGGTCCGAGGTATT -3'	R：5'-AGTGCAGGGTCCGAGGTATT -3'
PINK1	F：5'-GCCTCATCGAGGAAAAACAGG-3'	F：5'-TTCTTCCGCCAGTCGGTAG-3'
	R：5'-GTCTCGTGTCCAACGGGTC-3'	R：5'-CTGCTTCTCCTCGATCAGCC-3'
PPAR-α	F：5'-ATGGTGGACACGGAAAGCC-3'	F：5'-AGAGCCCCATCTGTCCTCTC-3'
	R：5'-CGATGGATTGCGAAATCTCTTGG-3'	R：5'-ACTGGTAGTCTGCAAAACCAAA-3'
PPAR-δ	F：5'- GGGATCAGCTCCGTGGATCT-3'	F：5'- TCGCTGATGCACTGCCTATG-3'
	R：5'- TGCACTTTGGTACTCTTGAAGTT-3'	R：5'- GAGAGGTCCACAGAGCTGATT-3'
TFB1M	F：5'-GTTGCCCACGATTCGAGAAAT-3'	F：5'-CGGGAGATCATTAAGTTGTTCGG-3'
	R：5'-GCCCACTTCGTAAACATAAGCAT-3'	R：5'-GCCCAGGACCCACTTCATAAA-3'
TFB2M	F：5'-CCAAGGAAGGCGTCTAAGGC-3'	F：5'-GGCCCATCTTGCATTCTAGGG-3'
	R：5'-CTTTCGAGCGCAACCACTTTG-3'	R：5'-CAGGCAACGGCTCTATATTGAAG-3'
TFAM	F：5'-ATGGCGTTTCTCCGAAGCAT-3'	F：5'- ATTCCGAAGTGTTTTTCCAGCA-3'
	R：5'-TCCGCCCTATAAGCATCTTGA-3'	F：5'- TCTGAAAGTTTTGCATCTGGGT-3'
NRF-2A	F：5'-TTAAACCTGCGGACACTGTTG-3'	F：5'-GACAAACATTCAAGCCGATTAGAGG3'
	R：5'-GTATCCCAAGGCGTTCTTGTT-3'	R：5' -CACATTGGGATTCACGCATAGGA-3'
NRF-2B	F：5'-TCCACTTCATCTAGCAGCACA -3'	F：5' - GCTATGCAGAACCAAATCAACAC-3'
	R：5'-GTAATGGTGTTCGGTCCACTT -3'	R：5' - CCCCTCCAGGTCCAATGATAAA-3'
NRF-1	F：5'-AGGAACACGGAGTGACCCAA-3'	F：5'-AGCACGGAGTGACCCAAAC-3'
	R：5'-TATGCTCGGTGTAAGTAGCCA-3'	R：5'-TGTACGTGGCTACATGGACCT-3'
α-SMA	F：5'-TCATGGTCGGTATGGGTCAG-3′	F：5′ -GTCCCAGACATCAGGGAGTAA-3′
	R：5′ -CGTTGTAGAAGGTGTGGTGC-3′	R：5′ -TCGGATACTTCAGCGTCAGGA-3′
PDGFR-β	F：5'- TCTGGgACCAGCAGTCTTTC-3'	F：5'-TTCCAGGAGTGATACCAGCTT-3'
	R：5'- CCTCCAGGAAGTCCTCCTTAC-3'	R：5'-AGGGGGCGTGATGACTAGG-3'
Fibronectin	F：5'-GTGTGTTGGGAATGGTCGTG-3'	F：5'-GCCTCACCTGAGTGAAGATGG-3'
	R：5'-GACGCTTGTGGAATGTGTCG-3'	R：5'-CTGTGAGGCGTGGAATGTCTT-3'
Cyclin D1	F：5'- GCTGCGAAGTGGAAACCATC-3'	F：5'-GCGTACCCTGACACCAATCTC-3'
	R：5'- CCTCCTTCTGCACACATTTGAA-3'	R：5'-CTCCTCTTCGCACTTCTGCTC-3'
PCNA	F：5'-CCTGCTGGGATATTAGCTCCA-3'	F：5'-TTTGAGGCACGCCTGATCC-3'
	R：5'-CAGCGGTAGGTGTCGAAGC-3'	R：5'-GGAGACGTGAGACGAGTCCAT-3'
β-Actin	F：5'-CATGTACGTTGCTATCCAGGC-3'	F：5'-GGCTGTATTCCCCTCCATCG-3'
	R：5'-CTCCTTAATGTCACGCACGAT-3'	R：5'-CCAGTTGGTAACAATGCCATGT-3'
U6	F: 5'-GCTCGCTTCGGCAGCACATATAC-3'	F: 5'-GCTTCGGCAGCACATATACTAAAAT-3'
	R: 5'-AGTGCAGGGTCCGAGGTATT-3'	R: 5'-CGCTTCACGAATTTGCGTGTCAT-3'
U6-RT	F:5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCG CACTGGATACGACAAAATATGG-3'	F:5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCAC TGGATACGACAAAATATGG-3'
miR27a-RT	F:5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCG CACTGGATACGACGCGGAA-3'	F:5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCAC TGGATACGACGCGGAA-3'

Table S5: Antibodies used in western blot and IF

Antibody	Species	Manufacturer	Catalog #	Application
CD63	Rabbit	Abcam	ab134045	WB (1:1000)
TSG101	Rabbit	Abcam	ab125011	WB (1:1000)
PINK1	Rabbit	Abcam	ab23707	WB (1:1000)
Parkin	Rabbit	Cell Signaling Technology	CST#2132S	WB (1:1000)
LC3B	Rabbit	Abcam	ab192890	WB (1:1000)
p62	Rabbit	Abcam	ab109012	WB (1:1000)
α-SMA	Rabbit	Abcam	ab124964	WB(1:1000)
Col I	Rabbit	Cell Signaling Technology	CST#91144	WB(1:1000)
Cyclin D1	Rabbit	Abcam	ab16663	WB(1:1000)
PCNA	Rabbit	Abcam	ab92552	WB(1:1000)
LAMP1	Rabbit	Affinity	DF7033	WB(1:500)
LAMP2	Rabbit	Affinity	DF6719	WB(1:1000)
Beclin 1	Rabbit	Abcam	ab210498	WB (1:1000)
ATG7	Rabbit	Abcam	ab133528	WB(1:1000)
mTOR	Rabbit	Abcam	ab32028	WB(1:1000)
Rheb	Rabbit	Abcam	ab92313	WB(1:1000)
GAPDH	Rabbit	Abcam	ab9485	WB(1:2500)
LC3B	Rabbit	Abcam	ab192890	IF(1:100)
COX4	Mouse	Abcam	ab33985	IF(1:100)
PINK1	Mouse	Santa Cruz	sc-518052	IF(1:100)
CD63	Mouse	Abcam	ab1318	IF(1:100)
α-SMA	Rabbit	Abcam	ab124964	IF(1:100)
PCNA	Mouse	Abcam	ab265585	IF(1:100)

Table S6: The target genes of miR27a related to mitochondrial functions

Target gene	Representative transcript	Gene name	Representative miRNA	Cumulative weighted context++ score	Total context++ score	Aggregate PCT
SLC25A16	ENST00000609923.1	solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16	hsa-miR-27a-3p	-0.14	-0.41	0.74
ATP10B	ENST00000327245.5	ATPase, class V, type 10B	hsa-miR-27a-3p	-0.1	-0.1	0.58
FDX1	ENST00000260270.2	ferredoxin 1	hsa-miR-27a-3p	0	-0.46	0.45
PINK1	ENSG00000158828.8	PTEN induced kinase 1	hsa-miR-27a-3p	-0.05	-0.02	< 0.1

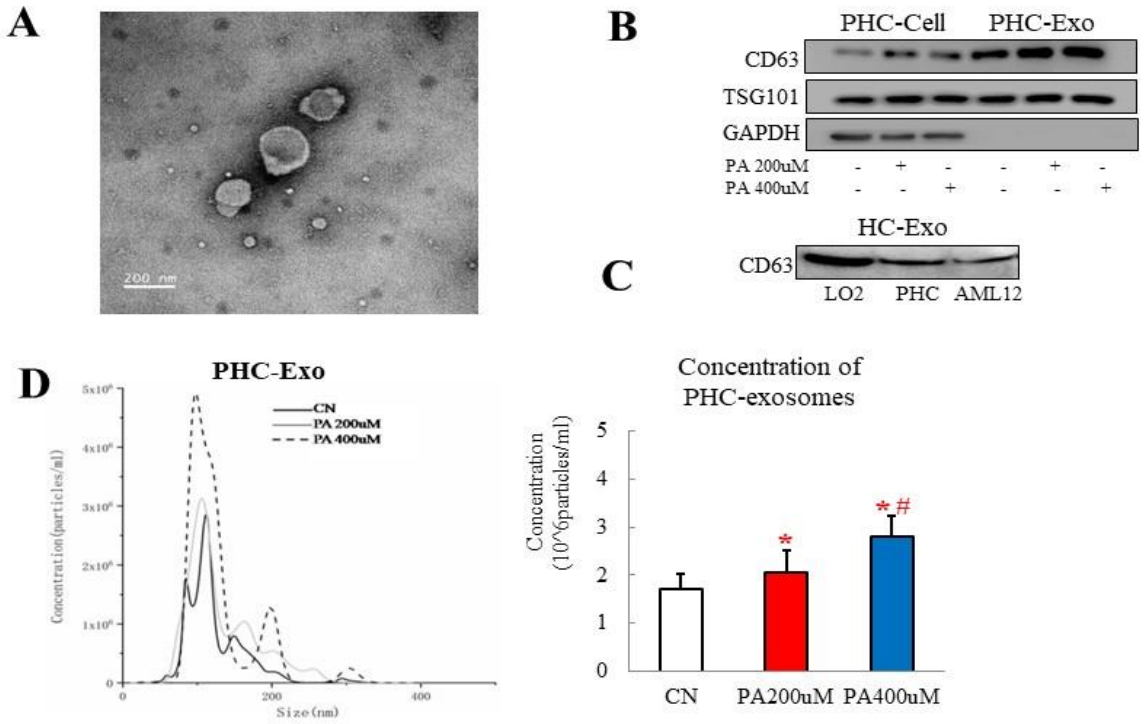

Figure S1 Identification of exosomes derived from HCs

(A) Representative morphology of PHC-exosomes treated with PA (200μM) under TEM (Scale bar: 2μm). (B) Protein levels of exosomal surface markers (CD63 and TSG101) from PHC-Exo and cells. (C) Three types of HC-Exo (incubated with PA 200μM) were measured by western blot. (D) Representative graph of size distribution and concentration of PHC-exosomes were examined by NTA analysis (CN, PA 200μM, PA 400μM groups).

Statistical significance: *p<0.05, compared to CN group; #p<0.05, compared to PA 200uM group.

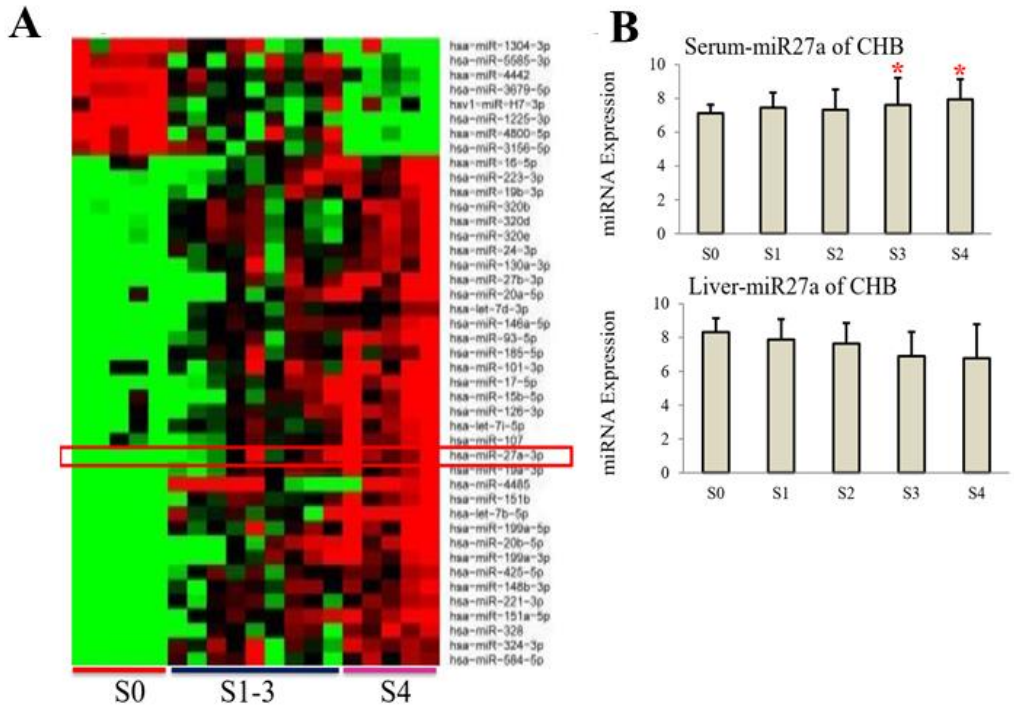

Figure S2 Serum miRNAs microarray in CHB patients

(A) Heatmap of serum miRNAs microarray.

(B) Trend analysis of serum miR27a: The expression trends of miR27a associated with the progression of hepatic fibrosis was shown in CHB patients.

Statistical significance: *p<0.05, compared to S0 group.

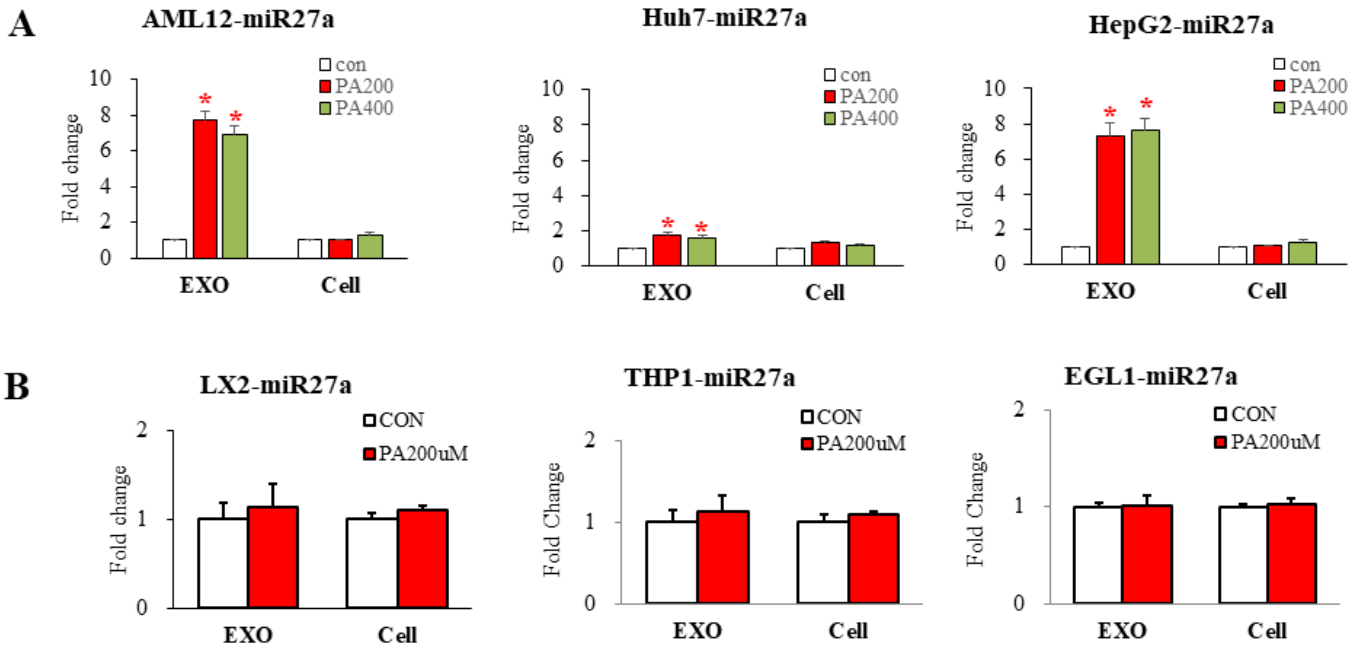

Figure S3 miR27a expression in hepatic exosomes and cells

(A) miR27a expressions in exosomes and cells of AML12, Huh7 and HepG2 were detected by PCR. (B) miR27a expressions in exosomes and cells of LX2, THP1 and EGL1 were detected by PCR.

Statistical significance: *p<0.05, compared to con group.

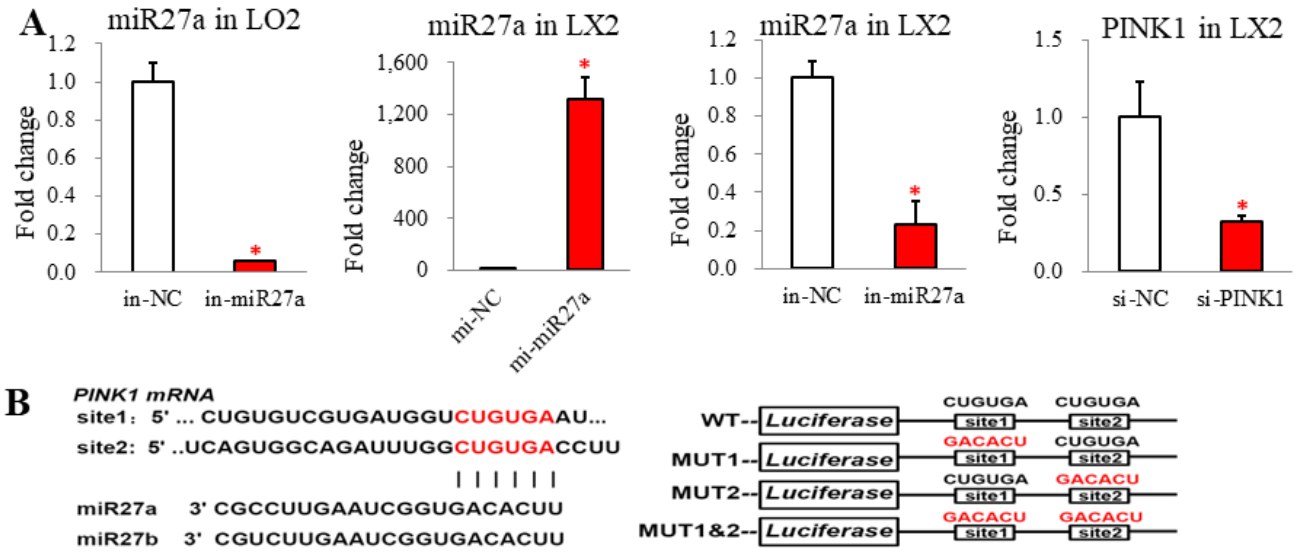

Figure S4 The transfective effects of cells and the binding site of miR27a-PINK1

(A) miR27a or PINK1 expressions in LO2 and LX2 cells were detected by PCR to confirm the transfective effect.

(B) The binding site of PINK1 mRNA with miR-27a and the mutant 3'-UTR of PINK1.

Statistical significance: *p<0.05, compared to in-NC/mi-NC/si-NC group.

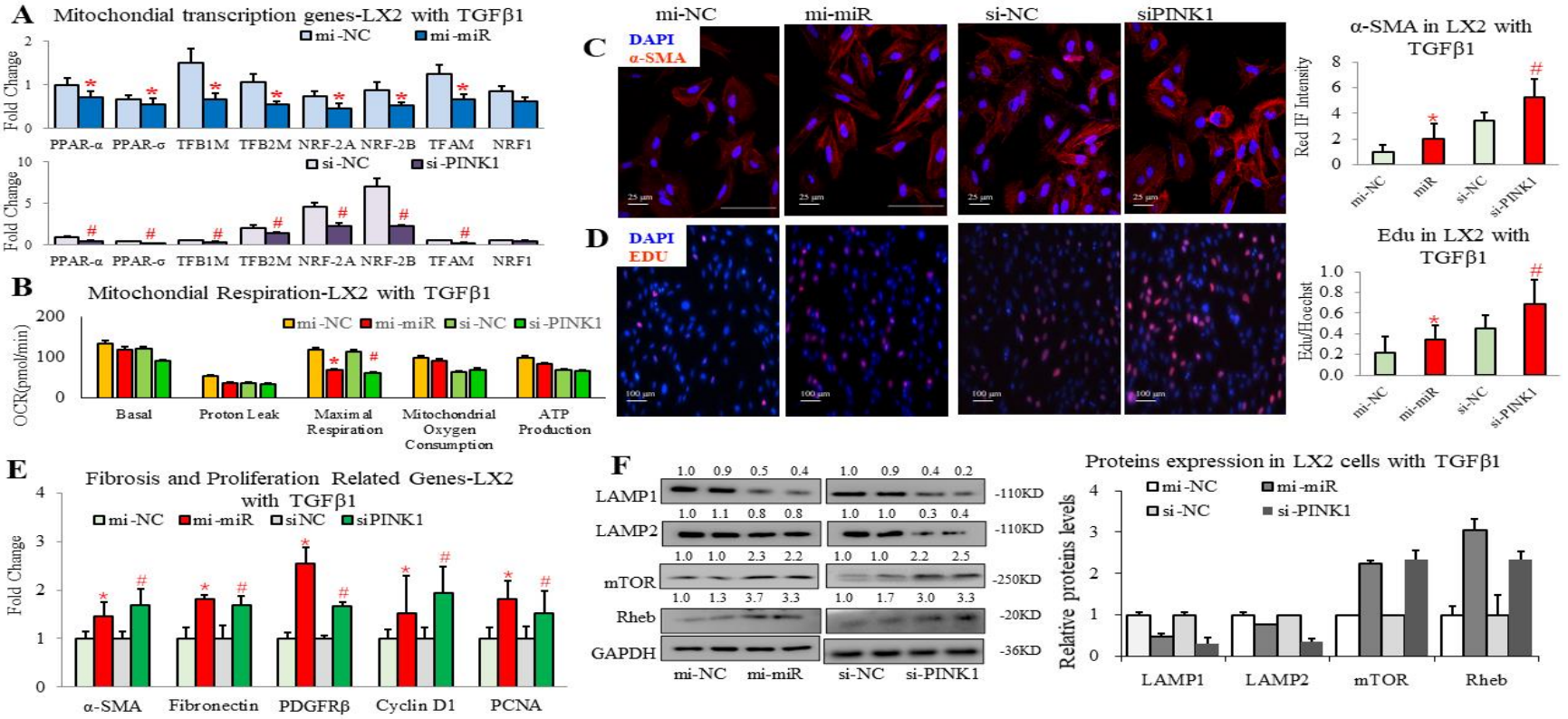

Figure S5 Mi-miR27a and si-PINK1 could significantly impair mitochondrial functions and promote activation of activated HSCs

Activated LX2 cells were transfected with mi-miR27a and si-PINK1. (A) mRNAs of mitochondrial transcription factors were detected by PCR. (B) Quantitative histogram of the mitochondrial respiration by Seahorse. (C) Proteins of α-SMA (red IF) and DAPI (blue IF) were imaged in activated LX2 cells by IF staining. Scale Bar=25 μm (D) Cell proliferation was monitored by EdU assay, and the representative images in activated LX2 cells were shown Scale Bar=100 μm. (E) Fibrosis genes (α-SMA, fibronectin and PDGFR-β) and proliferation genes (cyclin D1 and PCNA) in activated LX2 cells were detected via PCR. (F) Autophagy-related proteins (LAMP1/2, mTOR, and Rheb) were detected in activated LX2 cells via western blotting.

Statistical significance: *p<0.05, compared to mi-NC group; #p<0.05, compared to si-NC group.

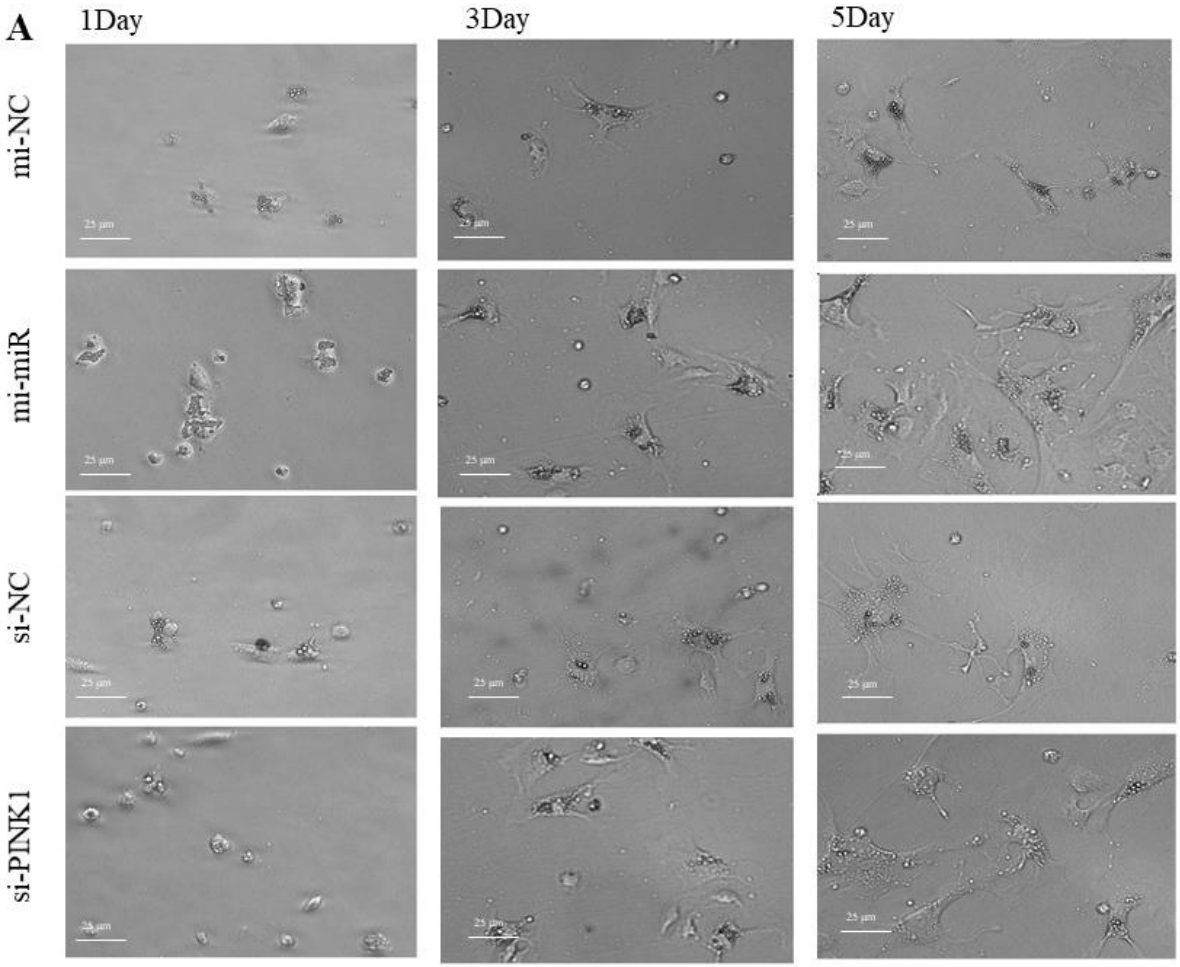

Figure S6 Mi-miR27a and si-PINK1 could significantly promote activation of activated HSCs

(A) Representative images of cell morphology changes in PHSCs at day 1, 3, 5 were shown. Scale Bar=25 μm

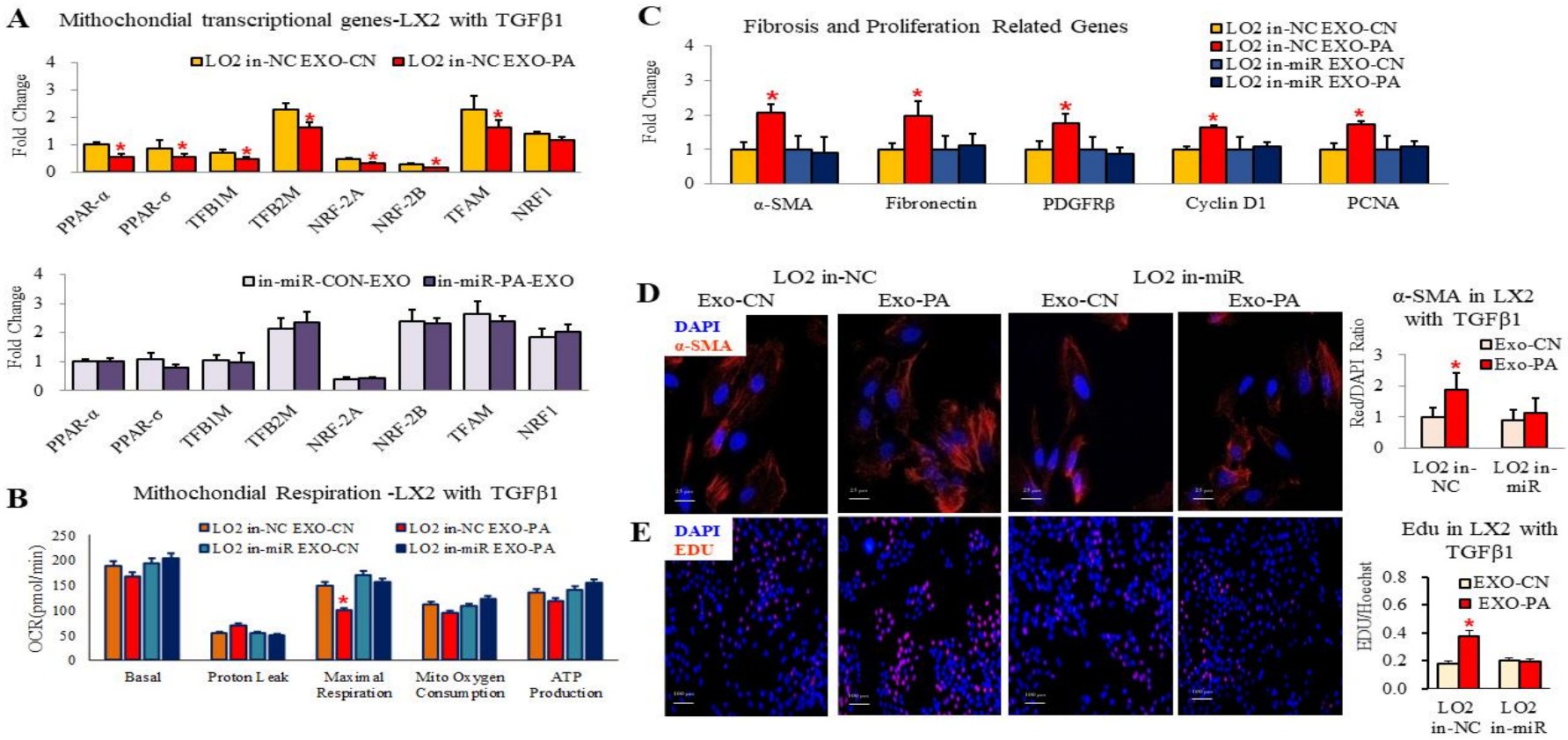

Figure S7 The functions of lipotoxic HC-exosomal miR27a on PINK1-related mitophagy and activation in HSCs

Activated LX2 cells or PHSCs were incubated with Exo-CN/Exo-PA derived from in-NC/in-miR27a LO2 cells were detected. (A) Mitochondrial transcription mRNAs of activated LX2 cells were detected by PCR. (B) Quantitative histogram of the mitochondial respiration by Seahorse. (C) Fibrosis genes (α-SMA, fibronectin, and PDGFR-β) and proliferation genes (cyclin D1 and PCNA) in activated LX2 cells were detected via PCR. (D) Proteins of α-SMA (red IF) and DAPI (blue IF) were imaged in activated LX2 cells by IF staining. Scale Bar=25 μm (E) Cell proliferation was monitored by EdU assay, and the representative images in activated LX2 cells were shown. Scale Bar=100 μm

Statistical significance: *p<0.05, compared to Exo-CN derived from in-NC LO2 cell group.

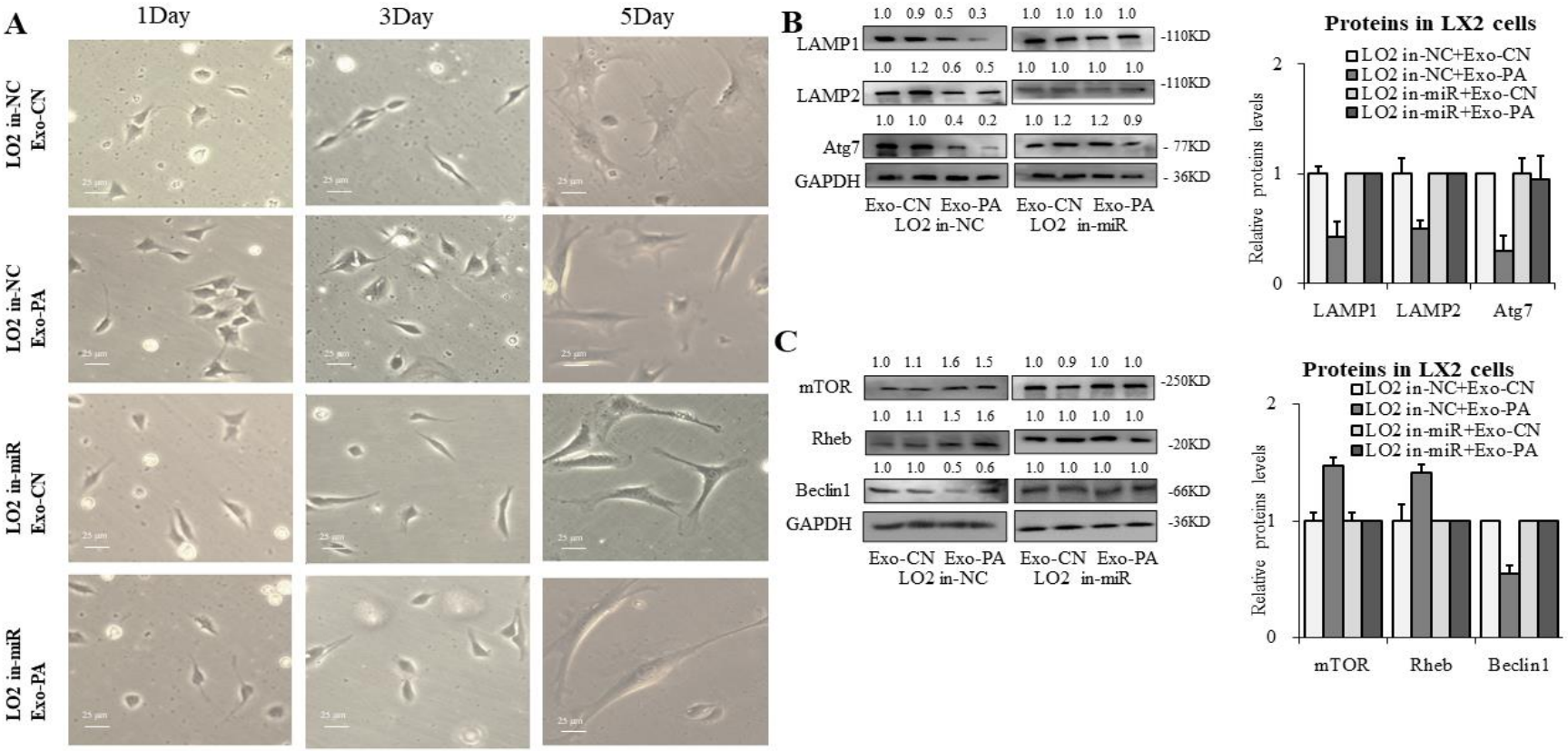

Figure S8 The functions of lipotoxic HC-exosomal miR27a on PINK1-related activation and proliferation in HSCs

(A) Representative images of cell morphology changes in PHSCs at day 1, 3, 5 were shown. Scale Bar=25 μm

(B-C) Autophagy-related (LAMP1/2, Atg7, mTOR, Rheb, and Beclin1) proteins were detected in activated LX2 cells via western blotting.

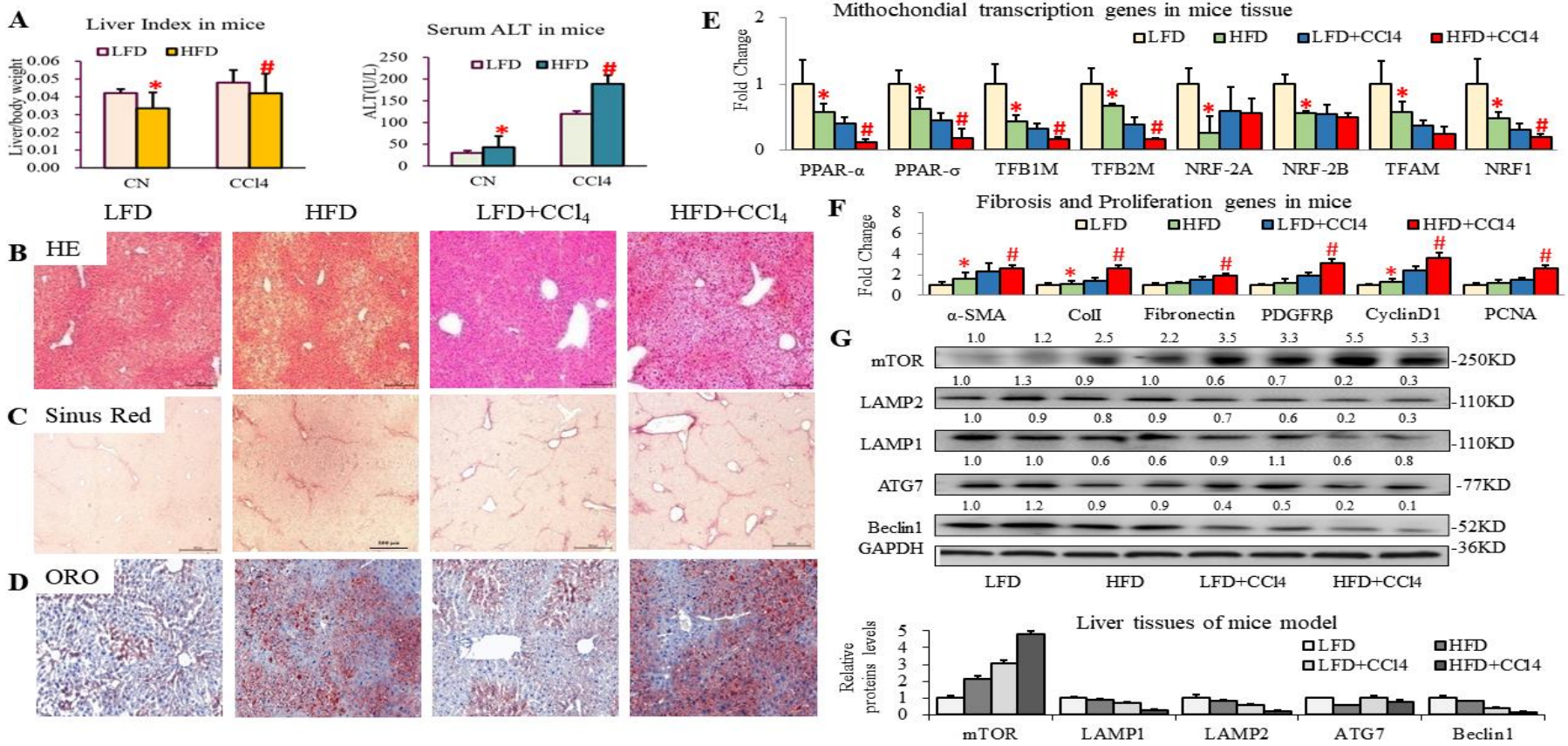

Figure S9 Up-regulating serum-miR27a could suppress hepatic PINK1-mitophagy and aggravate liver fibrosis in MAFLD mice

WT mice were divided to 4 groups: LFD, HFD, LFD+CCl_4, and HFD+CCl_4 (each group: n=10). (A)Liver index and serum ALT in mice was shown. (B-D) Representative images of H&E, Sinus Red, and ORO staining were shown. (E) Mitochondrial transcription mRNAs in mice livers were detected by PCR. (F) Fibrosis genes (α-SMA, ColI, fibronectin, and PDGFR-β) and proliferation genes (cyclin D1 and PCNA) in mouse livers were detected via PCR. (G) The protein levels of mTOR, LAMP1/2, Atg7 and Beclin1 were assessed via western blotting.

Statistical significance: *p<0.05, compared to mice of LFD group; #p<0.05, compared to mice of LFD+CCl_4 group.

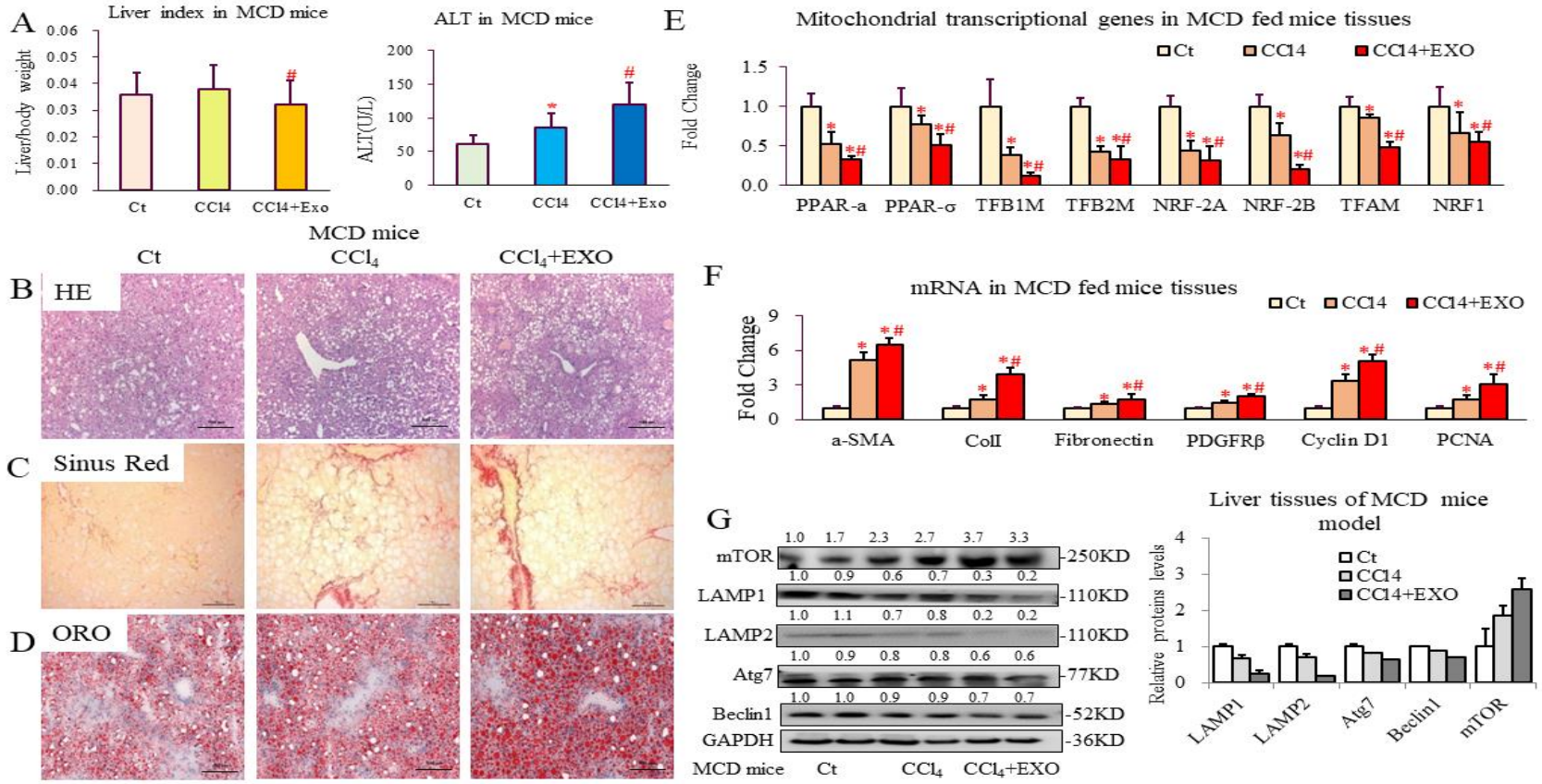

Figure S10 Transplantation of lipotoxic HC-exosomal miR27a exerted an aggravating effect on mitochondrial damage and fibrosis in MAFLD mice

MCD-fed WT mice were established and divided to 3 groups (Ct, CCl4 and CCl_4+Exo, each group: n=10). (A) Liver index and serum ALT in mice was shown. (B-D) Representative images of H&E, Sinus Red, and ORO staining were shown. (E) Mitochondrial transcription mRNAs in mice livers were detected by PCR. (F) Hepatic fibrosis- and proliferation-related mRNAs were detected via PCR. (G) The levels of proteins in the autophagy pathway (mTOR, LAMP1/2, Atg7, and Beclin 1) were assessed via western blotting.

Statistical significance: *p<0.05, compared to mice of Ct group; #p<0.05, compared to mice of CCl_4 group.

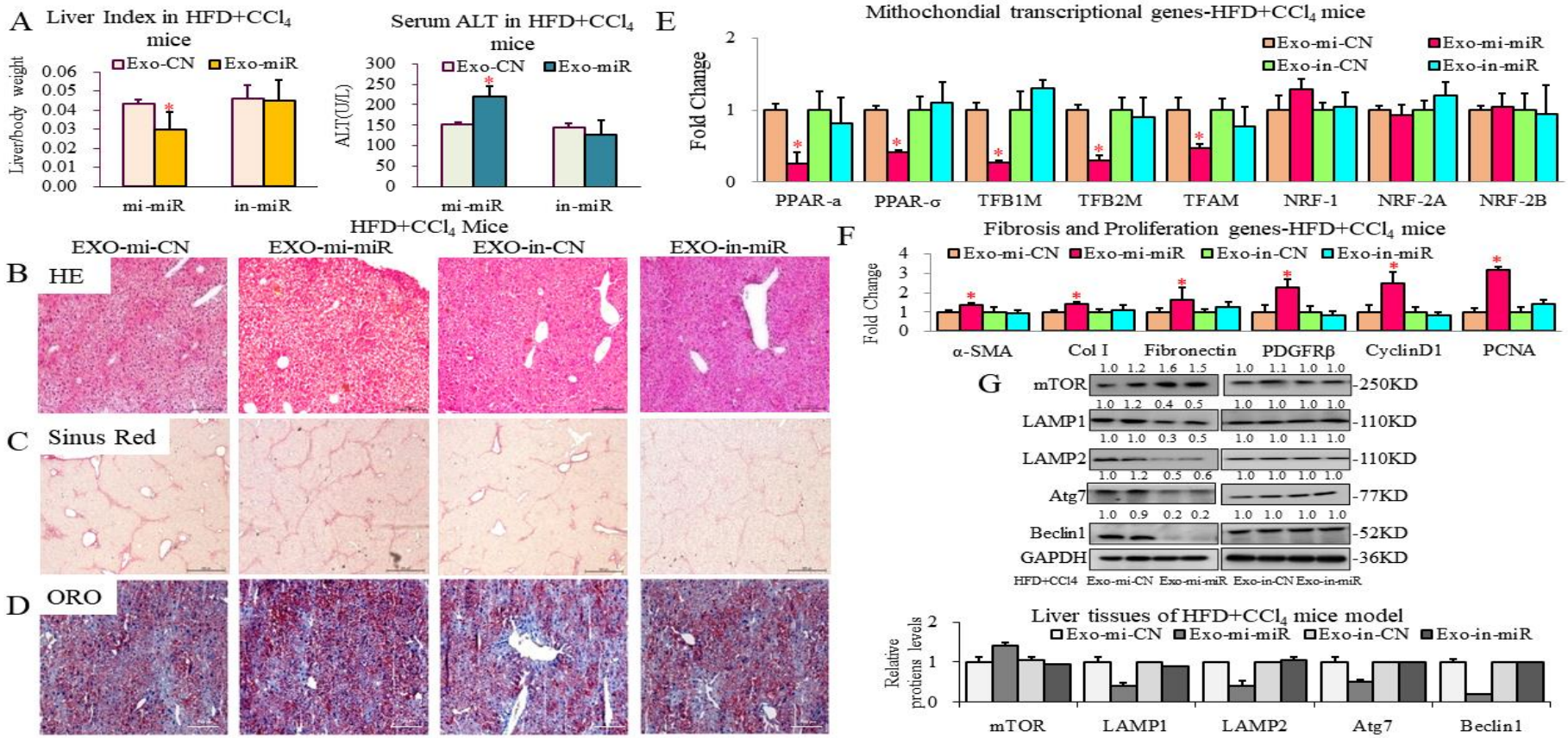

Figure S11 Lipotoxic HC-exosomal miR27a was the key player in mitochondrial and fibrotic liver injury in MAFLD mice

HFD+CCl_4 mice were transplanted with different purified exosomes and divided to 4 groups (Exo-mi-CN, Exo-mi-miR, Exo-in-CN, and Exo-in-miR, each group: n=10). (A) Liver index and serum ALT in mice was shown. (B-D) Representative images of H&E, Sinus Red, and ORO staining were shown. Scale Bar=500μm (E) Mitochondrial transcription mRNAs in mice livers were detected by PCR. (F) Fibrosis- and proliferation-related mRNAs were detected via PCR. (G) The levels of proteins in the autophagy pathway (mTOR, LAMP1/2, Atg7, Beclin1) in HFD+CCl_4 mouse livers.

Statistical significance: *p<0.05, compared to HFD+CCl_4 mice of the Exo-mi-CN group.