Supplementary materials and methods

Cell culture and determination of the minimal effective concentration of VPA

Human hepatic stellate cell line LX2 was maintained in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, USA) with 10% fetal bovine serum (Invitrogen Co, USA) in an atmosphere of 5% CO_2 at 37°C. Cells were serially passaged at a dilution of 1:3 when the cell confluence reached 70%. In order to determine the minimal effective concentration of VPA used in the present experiments, the cytotoxicity of VPA was determined by Cell Counting Kit-8 (CCK8, Beyotime, Nantong, China). Stock solution of 200 mM VPA (Sigma, St. Louis, MO) were prepared by dissolving 33.22 mg VPA in 1 ml PBS. LX2 cells were seeded at 5000 cells per well in 96-well plate for 24 hr, and then treated with VPA at the concentrations of 0, 0.5, 1.0, 2.5, 5.0, 10, or 20 mM for 0 hr, 24 hr, 36 hr 48 hr or 72 hr; 10 μl CCK8 solution was added into each well and then incubated in 37°C for 2 hr. The optical density readings at 450 nm were determined by a microplate reader (Bio-Rad, Tokyo, Japan). The inhibition rate was calculated as (1 - Absorption of experimental group / Absorption of control group) × 100%.

Cell proliferation and migration assay

Cell proliferation was measured by 5-ethynyl-2'-deoxyuridine (EdU) incorporation assay by EdU assay kit (RiboBio, Guangzhou, China) according to the manufacturers' instructions. Forty-six hours after 2.5mM VPA treatment, LX2 cells were further incubated with 10 μM EdU for 2 hours. Hoechst 33258 (Beyotime) was used for counter-staining of the nuclei, the cells were visualized under a fluorescent microscope (Olympus, Tokyo, Japan), EdU positive cells (red) were counted manually. The results were expressed as labeling index according to the following formula: number of EdU-positive nuclei × 100 / number of total nuclei.

For migration assay, LX2 cells were treated with 2.5mM VPA for 24 hr, 20,000 VPA treated or untreated LX2 cells resuspended in 200 μl serum-free DMEM were plated in the upper chambers (Millicell, 0.8 μm; Millipore, Bedford, MA), DMEM medium containing 2.5% FBS was added as a chemoattractant in the lower chambers. After 16 hr, nonmigrating cells were removed from the upper surface softly by a cotton swab. The cells that migrated through the membrane to the lower surface were fixed with 2% paraformaldehyde and stained with 0.5% crystal violet, and then counted under a

microscope (Olympus) at 200-fold magnification. At least 5 random fields were examined for each analysis.

Western blotting

For western blot analysis, LX2 cells were incubated with complete DMEM with or without 2.5 mM VPA respectively for 48 hr, whole cell proteins were extracted by RIPA lysis buffer (Beyotime) according to the manufacturer's instructions. Equal amounts of protein (30 μg) were separated on 10% SDS PAGE gel and transferred onto polyvinyldifluoride (PVDF) membranes (Millipore). PVDF membranes were blocked with 5% non-fat milk for 1 hr, then incubated with specific primary antibodies for α-smooth muscle actin(α-SMA, Abcam, Cambridge, UK), Collagen I (R&D Systems, Minneapolis, MN), pan acetyl-lysine (PTM BioLabs, Hangzhou, China) or acetyl-histone H3 (Lys27) (Cell Signaling Technology, Danver, MA) and GAPDH (Beyotime) at 4°C overnight, and then incubated with horseradish peroxidase-conjugated secondary antibody for an additional 1 hr at room temperature. The protein expression was visualized with the ECL chemiluminescence detection system (Pierce Chemical Co., Rockford, IL).

Plasmid construction and Luciferase assay

The putative miR-103a-3p anchor element from 1168 to 1194 of HMGA1 mRNA 3'UTR (5'-GCCCCTAGGATGCTGCAGCAGAGTGA-3') was termed as miR-103a recognition element (MRE103a) , and the putative miR-195-5p anchor element from 1007 to 1034 of HMGA1 mRNA 3'UTR (5'-GACATCCGTCATTGCTGCTGCTACCAGC-3') was termed as miR-195 recognition element (MRE195). As previously reported, the synthesized HMGA1 3' UTR fragment containing three copies of MRE103a or MRE195 with flanking Xho1 and Not1 restriction enzyme cohesive end were annealed and ligated into the psiCHECK-2 Vector (Promega, Madison, WI, USA) between the XhoI and NotI sites located 3' to the renilla luciferase translational stop codon, resulting in single insertion as psiCHECK-2/HMGA1MRE103a×3 or psiCHECK-2/HMGA1MRE195×3. In these vectors, the post-transcriptional regulation of renilla luciferase was potentially regulated by HMGA1MRE103a or HMGA1MRE195 fragment respectively. The activity of renilla luciferase was normalized by the internal firefly luciferase activity. The nucleotide sequences of constructed plasmids were confirmed by DNA

sequencing (Invitrogen).

The 293T cells were seeded into 24-well plates 24 hr before transfection and were transiently cotransfected with psiCHECK-2/HMGA1MRE103a×3 reporter plasmid and miR-103a mimic (RiboBio) by Lipofectamine 2000 (Invitrogen), and mir-neg provided by RioBio served as control. Luciferase assays were performed 42 hr later using the Dual-Luciferase reporter system (Promega). Renilla and firefly luciferase signals were detected using BioTek Microplate Reader (BioTek, Winooski, VT). The luciferase assays for psiCHECK-2/HMGA1MRE195×3 reporter plasmid and miR-195 mimic were carried out in the same way.

Supplementary Figures

Figure S1

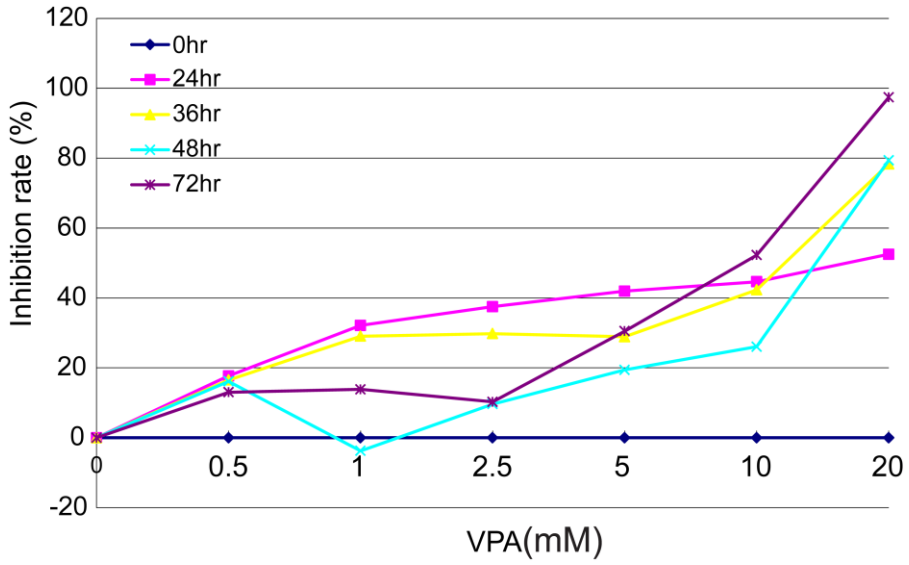

Figure S1 The minimal effective concentration of VPA for LX2 cells. LX2 cells were exposed to different concentrations of VPA ranging from 0 to 20mM for 0hr, 24hr, 36hr 48hr or 72hr. Cell proliferation was determined by Cell Counting Kit-8. The optical density readings at 450 nm were determined by a microplate reader. The inhibition rate was calculated as (1 - absorption of experimental group / absorption of control group) × 100%.

Figure S2

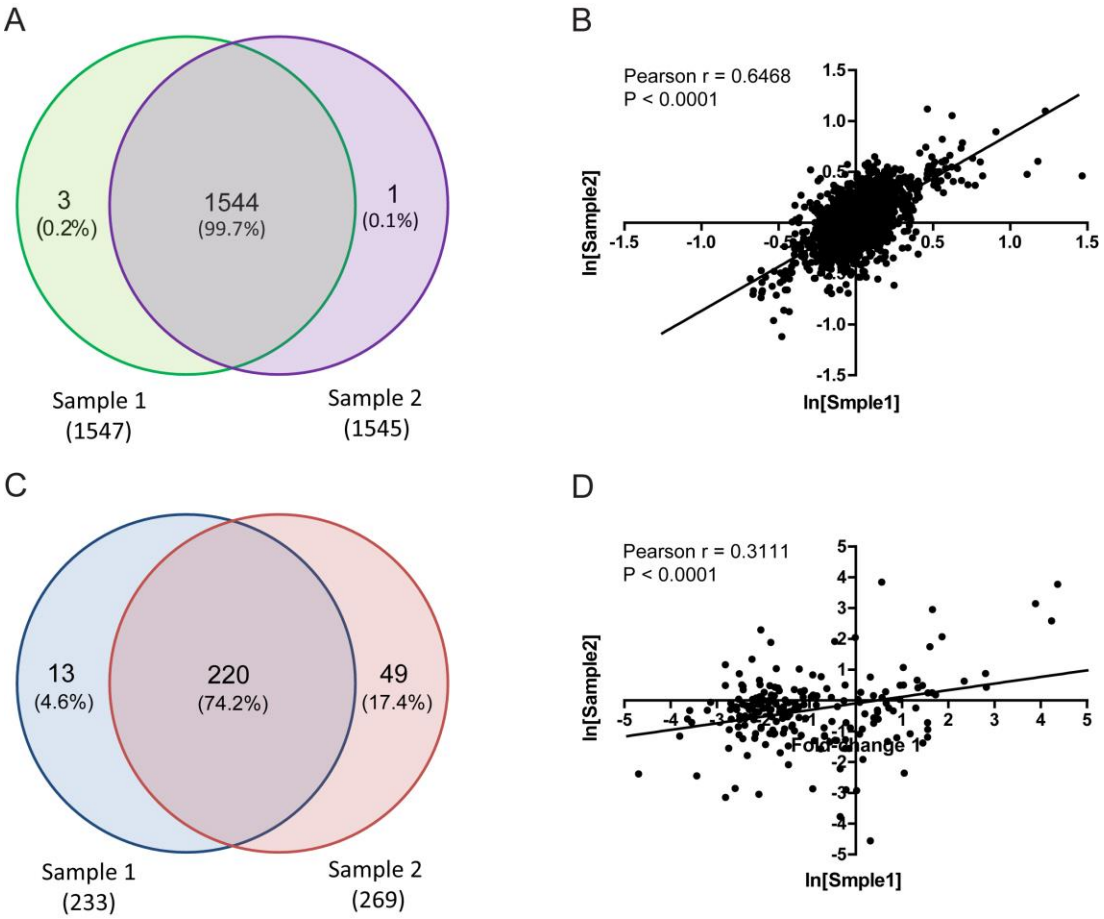

Figure S2 Biological reproducibility. (A) Venn diagram depicting the overlap of proteins identified in two independent experiments. Numbers in parentheses indicate the number of proteins passed filter for each sample. (B) To examine the biological reproducibility, linear regression analyses were performed on ln-transformed 115/114 (Sample1) and 117/116 (Sample2). Pearson correlation coefficients between samples 1 and 2 are 0.6468, P (two-tailed) < 0.0001. (C) Venn diagram depicting the overlap of miRNAs identified in two independent miRNA array experiments. Numbers in parentheses indicate the number of miRNAs passed filter for each sample. (D) To examine the biological reproducibility, linear regression analyses were performed on ln-transformed relative expression of two independent analyses. Pearson correlation coefficients between samples 1 and 2 are 0.3111, P (two-tailed) < 0.0001.

Supplementary Tables

Table S1 Primers for qRT-PCR

Table S1 Sequence of primers used for PCR analyses

ID		Primer Sequence（5'→3'）
α-SMA	Sense	CTTGGACTCTGTGCCCTCAATC
	Antisense	TGGCTCTTTCATCGGGTTTAG
ACAD9	Sense	CTGGAACTGGCGGGTATTC
	Antisense	TGGTTGAGCAGAAATGGCTA
ACTN1	Sense	CTGGGCATCCACAATGAGGT
	Antisense	AAACTGCTTGCGTAGCCTCT
BAG3	Sense	CCCTTCTGCTGTCCCCTCTT
	Antisense	TGGCTTCCACTTTCAGCACTC
Collagen Ⅰ	Sense	CTGCGATGACGTGATCTGTGA
	Antisense	TTGGTCGGTGGGTGACTCTG
CPSF6	Sense	TCTGGATCAAGACGTGAACGAT
	Antisense	ACGATGACGATATTCGCGCT
HMGA1	Sense	TGCGCTCCTCTAATTGGGAC
	Antisense	GCTGGTAGCAAATGCGGATG
HDAC2	Sense	ACCTGGTGTCCAGATGCAAG
	Antisense	ATCCGCTTGTCTGATGCTCG
HDAC3	Sense	GAGTGGCCGCTACTACTGTC
	Antisense	ATTCAACGCATTCCCCATGC
HNRNPA1	Sense	AGCCCTGTCAAAGCAAGAGA
	Antisense	GTTTCCTCCACGACCGAAGT
HNRNPA2B1	Sense	ATTCTGCTGCCACAAAGACTG
	Antisense	CCAACACCACTGAAGGAACC
18S	Sense	GGCCCTGTAATTGGAATGAGTC
	Antisense	CCAAGATCCAACTACGAGCTT
U6snRNA	Sense	CTCGCTTCGGCAGCACA
	Antisense	AACGCTTCACGAATTTGCGT
miR-103a-3p	Sense*	AGCAGCAUUGUACAGGGCUAUGA
miR-155-5p	Sense*	CGCTTAATGCTAATCGTGATAGG
miR-195-5p	Sense*	UAGCAGCACAGAAAUAUUGGC

miR-26a-5p	Sense*	UUCAAGUAAUCCAGGAUAGGCU
miR-27a-3p	Sense*	UUCACAGUGGCUAAGUUCCGC
miR-30b-5p	Sense*	GCGCTGTAAACATCCTACACTC

Sense primers for mature miRNAs were provided here, antisense primer was provided by Invitrogen as Universal q-PCR Primer.

Table S2 Differentially expressed proteins in VPA treated LX2 cells

Submitted in a separate excel file (28642q_suppl2.xls).

Table S3 Biofunctions of differentially expressed proteins in VPA treated LX2 cells

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Category	p-value	Molecules
Cell Death and Survival	5.01E-07-5.53E-04	S100A11,FYN,CUL1,HLA-B, BAG3,SFPQ,CBX5,CD47,CYFIP2,ANXA11,FASN,NEK6, CAV1,BID,ILKAP,NUP205,MYBBP1A,KRT10,PDXK,TYMS,TUBB3,MIF,THY1,ANXA2,HMGA1,F3,TLDC1,ALB,PRDX3,TUBA1A,RRAS2,ANXA6,CTNNBL1,HNRNPA1,ALCAM,ATP2B4
Cellular Movement	1.32E-06-5.37E-04	FYN,S100A11,CUL1,HNRNPA2B1,BAG3,CD47,FASN,CAV1,NRDC,BID,ACTN1,KRT10,MYL12A,MIF,NUP85,AHSG,THY1,ANXA2,HMGA1,F3,ALB,TUBA1A,RRAS2,CORO1B,CNN3,ANXA3,HECTD1,ALCAM,ATP2B4,MYO1E
RNA Post-Transcriptional Modification	1.8E-06-1.63E-04	CPSF6,ALB,NONO,HNRNPA1,RBMX,HNRNPA2B1,SFPQ,HNRNPM
Cellular Development	4.86E-06-8.08E-04	FYN,S100A11,CUL1,HNRNPA2B1,SLC7A1,SFPQ,ADSL, CD47,FASN,NRDC,CAV1,BID,ILKAP,MYBBP1A,KRT10, ACTN1,TYMS,OGFR,TUBB3,MIF,AHSG,THY1,ANXA2,HMGA1,F3,ALB,ANXA6,HNRNPA1,HECTD1,VMP1,ALCAM
Cellular Growth and Proliferation	4.86E-06-8.08E-04	FYN,S100A11,CUL1,HNRNPA2B1,SLC7A1,SFPQ,ADSL, CD47,FASN,NRDC,CAV1,BID,ILKAP,MYBBP1A,KRT10, ACTN1,TYMS,OGFR,TUBB3,MIF,AHSG,THY1,ANXA2,HMGA1,F3,ALB,ANXA6,HNRNPA1,HECTD1,VMP1,ALCAM
Cell-To-Cell Signaling and Interaction	4.79E-05-4.79E-04	FYN,ALB,CD47,MIF,RRAS2,ANXA11,ANXA3,AHSG,CAV1,THY1,F3,MYL12B,MYL12A
Cellular Compromise	4.79E-05-4.79E-04	CD47,AHSG,THY1,F3
Molecular Transport	7.2E-05-3.96E-04	FYN,S100A11,ALB,ANXA6,HNRNPA1,NUP133,HNRNPA2B1,CAV1,BID,ATP2B4,XPO6
Cellular Function and Maintenance	7.61E-05-5.97E-04	FYN,CD47,MIF,ANXA6,RRAS2,ANXA11,ANXA3,AHSG,CAV1,SFPQ
Cell Morphology	8.08E-05-8.08E-05	FYN,FASN

DNA Replication, Recombination, and Repair	1.23E-04-1.23E-04	FYN,S100A11,CD47,HNRNPA1,ANXA3,CUL1,CAV1,VMP1,HMGA1
Cell Signaling	2.01E-04-3.35E-04	S100A11,CD47,ANXA6,CAV1,BID,F3
Lipid Metabolism	2.01E-04-2.01E-04	CAV1,BID
Small Molecule Biochemistry	2.01E-04-4.79E-04	TYMS,CD47,CAV1,BID,F3,NUDT15
Protein Trafficking	3.13E-04-3.13E-04	ADSL,CPSF6,ALB,BAG3,CAV1,ANXA2,HMGA1
Vitamin and Mineral Metabolism	3.35E-04-3.35E-04	S100A11,ANXA6,CAV1,BID
Nucleic Acid Metabolism	4.79E-04-4.79E-04	TYMS,NUDT15
Cell Cycle	5E-04-5.02E-04	TYMS,FYN,MIF,TUBB3,CUL1,AHSG,SFPQ,ANXA2,NUDT15,PPP2CB,TUBA1A,RRAS2,FASN,NEK6,CAV1,BID,MYBBP1A,KRT10
Cellular Assembly and Organization	5.97E-04-5.97E-04	ANXA6,CAV1

Table S4 Differentially expressed miRNAs in VPA treated LX2 cells

Submitted in a separate excel file (28642q_suppl4.xls).

Table S5 Biofunctions of paired miRNA targets in differentially expressed proteins in VPA treated LX2 cells

Table S5 Biofunctions of paired miRNA targets in differentially expressed proteins in VPA treated LX2 cells

Category	p-value	Molecules
Cellular Movement	1.29E-05-1.53E-04	S100A11,NUP85,AHSG,CUL1,BAG3,THY1,ANXA2,HMGA1,ALB,CD47,RRAS2,TUBA1A,HECTD1,ALCAM,BID,ATP2B4,MYO1E,KRT10,MYL12A
Cell Death and Survival	3.44E-05-2.21E-04	TYMS,S100A11,TUBB3,HLA-B,CUL1,BAG3,THY1,ANXA2,HMGA1,TLDC1,CD47,PRDX3,ALB,TUBA1A,RRAS2,ANXA6,ANXA11,HNRNPA1,ALCAM,BID,ATP2B4,KRT10
Cell-To-Cell Signaling and Interaction	1.48E-04-1.87E-04	CD47,AHSG,THY1
Cellular Compromise	1.48E-04-1.87E-04	CD47,AHSG,THY1
Nucleic Acid Metabolism	1.48E-04-1.48E-04	TYMS,NUDT15
Small Molecule Biochemistry	1.48E-04-1.48E-04	TYMS,NUDT15
Cell Cycle	2.56E-04-4.27E-04	PPP2CB,BID,ANXA2
Cell Morphology	3.02E-04-3.7E-04	PPP2CB,CD47,PRDX3,AHSG,BID,HMGA1
Cellular Assembly and Organization	3.02E-04-3.02E-04	PPP2CB,PRDX3,BID,HMGA1