

1 **LncRNA FTX accelerates the progression of hepatocellular carcinoma by**  
2 **FTX/miR-374a-3p/HMGB1 pathway**

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4 Min Zhang, Songman Yu, Shang Gao, Haiyan Bu, Lihua Duan, Yan Huang

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## 14 **Supplementary Material and Methods Doc S1**

### 15 **RNA extraction and RT-PCR**

16 The total RNA Kit (Omega, USA) and the miRNeasy FFPE RNA kit (QIAGEN, German) were  
17 used for RNA extraction. The PrimeScript RT reagent kit (Takara, Japan) and TB Green  
18 Premix Ex Taq<sup>TM</sup> II (Takara, Japan) were used for reverse transcription and real-time PCR with  
19 Real-time fluorescence quantitative PCR instrument (BIO-RAD, USA). The transcriptional  
20 expression was determined using the value of  $2^{-\Delta\Delta CT}$ .

### 21 **Western blot**

22 RIPA Lysis Buffer (Beyotime, China) supplemented with a 100x Protease Inhibitor Cocktail  
23 (Bimake, USA) was used to obtain Cell lysates. Quantification of Protein Concentration Using  
24 a BCA Protein Assay Kit (Thermo, USA). Primary antibodies against HMGB1 (ab18256,  
25 Abcam, USA), RBMX (ab190352, Abcam, USA) and  $\beta$ -Tublin (A12289, ABclonal, China)  
26 were used. Following incubation with secondary antibodies (FSM0075, Fushen, China),  
27 membranes were developed with western ECL Substrate (1705062, BIO-RAD, USA) and the  
28 blots were detected with the enhanced chemiluminescence system (BIO-RAD, USA).

### 29 **Plasmids and transfection**

30 The expression vector pcDNA3.1 (+) containing full-length Lnc-FTX was purchased from  
31 Sangon Biotech (Shanghai, China) and transfected into MHCC-LM3 and Hep3B cells with  
32 Lipofectamine 2000 reagent (Invitrogen, USA) according to the manufacturer's instructions.  
33 We used 6.25 ug/ml and 100 ug/ml Geneticin<sup>TM</sup> (Thermo Fisher Scientific, USA) for a two-  
34 week selection of stable cell line respectively. We inserted NC shRNA or Lnc-FTX shRNA1-  
35 shRNA5 (Table S2) into the lentiviral vector PLV (Biosettia, USA). We transfected them using  
36 10 $\mu$ g/ml polybrene (Beyotime, Shanghai, China), and then selected stable cell lines with  
37 2.5 $\mu$ g/ml puromycin (Yeasen, Shanghai, China). Cells were transiently transfected with a

38 siRNA NC or siRNAs targeting Lnc-FTX by using Lipofectamine 8000 reagent (Beyotime,  
39 Shanghai, China). The efficiency of Lnc-FTX overexpression or knockdown was verified by  
40 qRT-PCR (Figure S1).

#### 41 **Cell viability and colony formation assays**

42 Cell proliferation was examined with the Cell Counting Kit 8 (CCK8) (Ecotop, China)  
43 according to the manufacturer's protocols. Cells were seeded in 96-well plates at a density of  
44  $5 \times 10^3$  cells/well and cultured for 4 days. CCK8 reagent was added to each well and incubated  
45 at 37°C for 2 h. The absorbance of each well was detected at 450 nm using a PerkinElmer  
46 spectrophotometer (Shanghai, China). For the colony formation assay, cells were plated in 6-  
47 well plates (1000cells/well) and cultured for 2 weeks. Colonies were fixed with 4%  
48 paraformaldehyde and stained with 0.1% crystal violet for 30 min.

#### 49 **Flow cytometry analysis of cell cycle and apoptosis**

50 Cell cycle assay was performed with the cell cycle staining kit (Multi sciences, China). Cells  
51 plated in 6-well plates were harvested after 24h starvation and adjusted to a concentration of  
52  $2 \times 10^5$ /ml. Cells were fixed overnight in 75% ethanol at 4 °C, then washed in PBS twice,  
53 incubated with the PI/RNase A (9:1) staining solution mixture for 30 minutes away from light.  
54 Flow cytometry was performed using a BD FACSCanto II Flow Cytometer (BD Biosciences,  
55 USA). For the apoptosis assay, cells were harvested and incubated with Annexin V FITC and  
56 PI according to the manufacturer's instructions (4A Biotech, China). The stained cells were  
57 detected by flow cytometry.

#### 58 **Wound-healing assay**

59 The gap between cells were created by a sterile pipette tip 200  $\mu$ l. Cells were cultured in serum-  
60 free medium, and images were captured at 0h, 24h and 48h by an inverted microscope

61 (CTR5000, LEICA, German). The migration rate was calculated by comparing the area at  
62 different time points to that at 0h.

### 63 **Transwell assay**

64 Transwell assay was conducted using the 8  $\mu$ m pore-size transwell plates (Corning, USA).  
65 Specifically, cells were seeded in upper chambers at a density of  $5 \times 10^4$  cells/well with FBS-  
66 free medium for migration, or 1:8 diluted Matrigel (Corning, USA) for invasion. The lower  
67 chambers were filled with medium containing 10% FBS. After 36 h, cells were fixed with 4%  
68 paraformaldehyde and stained with a 0.1% crystal violet solution (Beyotime, USA) for 30 min.

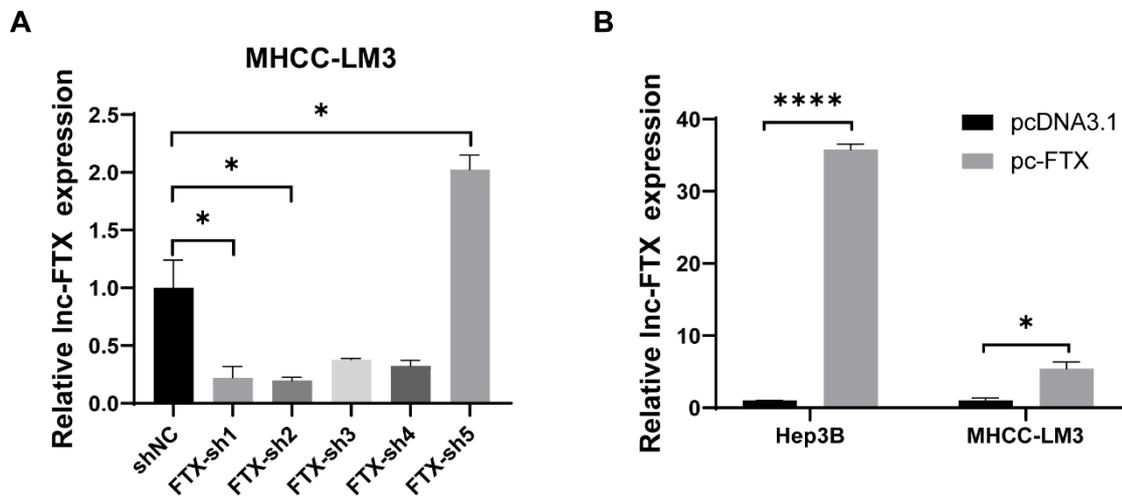
69

70 **Supplementary figure 1:**

71 **Validation of Knockdown and Overexpression Efficiency of Lnc-FTX**

72 (A) Detection of Lnc-FTX expression in MHCC-LM3 cell line 48 hours after transfection with  
73 shRNA (FTX-sh1~5) compared to ShNC-transfected cells by qRT-PCR. (B) Detection of Lnc-  
74 FTX expression 48 hours after transfection with pcDNA3.1 and pc-FTX plasmids in Hep3B  
75 and MHCC-LM3 cells by qRT-PCR.

**Figure S1**



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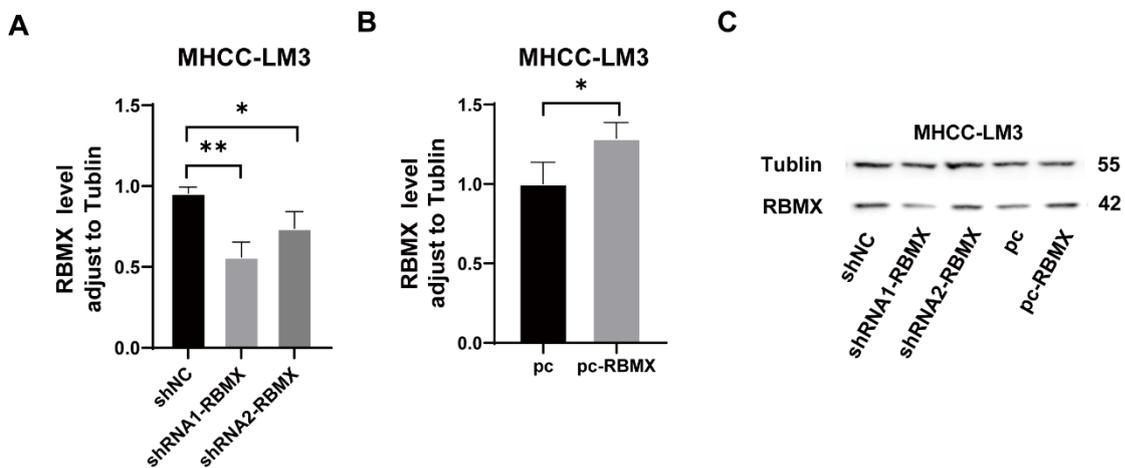
77

78 **Supplementary figure 2:**

79 **Validation of Knockdown and Overexpression Efficiency of RBMX**

80 (A) Detection of RBMX expression in MHCC-LM3 cell line 48 hours after transfection with  
81 shRNA (RBMX-sh1~2) compared to ShNC-transfected cells by Western Blot analysis. (B)  
82 Detection of RBMX expression 48 hours after transfection with pcDNA3.1 and pc-RBMX  
83 plasmids in MHCC-LM3 cells by Western Blot analysis. (C) Representative Western blot  
84 image showing Lnc-FTX knockdown or overexpression

**Figure S2**



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87 **Supplementary table 1:**

88 **Clinical and pathologic characteristics of patients with hepatocellular**

<b>characteristics</b>	<b>Value</b>
Age, mean (SD)	52.3 (12.8)
Sex(male/female)	24/3
Liver cirrhosis, no. (%)	16 (59.2)
Tumor size, mean (SD), mm	7.0 (5.6)
Tumor size $\geq$ 50 mm, no. (%)	13 (48.1)
Tumor number (solitary/multiple)	16/11
Edmondson-Steiner grade(II/II-III/III)	19/7/1
TNM stage(II/II-III/III)	13/4/7/3
BCLC stage, A/B/C	2/2/23
Capsular invasion, no. (%)	2 (7.4)
Vascular invasion, no. (%)	9 (33.3)

89 \*BCLC = Barcelona Clinic liver cancer; TNM = tumor node metastasis.

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91 **Supplementary table 2:**92 **Primers, shRNA and siRNA used in this study**

primer	Sequence (5' to 3')
GAPDH-homo (F)	AGCCACATCGCTCAGACAC
GAPDH-homo (R)	AATCCGTTGACTCCGACCT
Lnc-FTX-homo (F)	GGCGAGGATGAATTACGG
Lnc-FTX-homo (R)	CAAGGCTTCCCAACTGC
HMGB1-homo (F)	CCGTCTGTGCCTTCTCAT
HMGB1-homo (R)	CGTTCACGGTGGTCTCC
RBMX-homo (F)	GCCCAGCAGACGCTAAGGA
RBMX-homo (R)	TGGTTTGGTGGCTTGTTC
T7-Ftx(F)	TAATACGACTCACTATAGGGGGGAAGCACACTGGC
T7-Ftx (R)	GGGAAATAAGTTTATTAC
T7-AS-Ftx(F)	GGAAGCACACTGCG'
T7-AS-Ftx(R)	TAATACGACTCACTATAGGGGGGAAATAAGTTTATTA C
h-ShNC-(F)	GATCCGTTCTCCGAACGTGTCACGTTTTTCAAGAGAAA ACGTGACACGTTCGGAGAATTTTTTG
h-ShNC-(R)	AATTCAAAAAATTCTCCGAACGTGTCACGTTTTCTCTT GAAAAACGTGACACGTTCGGAGAACG
h-FTX (332)-sh1 (F)	GATCCGGCATCCTGCACCTAGTTATCATTCAAGAGATG ATAACTAGGTGCAGGATGCTTTTTTG
h-FTX (332)-sh1 (R)	AATTCAAAAAAGCATCCTGCACCTAGTTATCATCTCTT GAATGATAACTAGGTGCAGGATGCCG

h-FTX (432)-sh2 (F) GATCCGGCCTGTTACTCATACTGATGCTTCAAGAGAGC  
ATCAGTATGAGTAACAGGCTTTTTTG

h-FTX (432)-sh2 (R) AATTCAAAAAAGCCTGTTACTCATACTGATGCTCTCTT  
GAAGCATCAGTATGAGTAACAGGCCG

h-FTX (526)-sh3 (F) GATCCGGCTGGATTGTGAAGGATATTTTTCAAGAGAA  
AATATCCTTCACAATCCAGCTTTTTTG

h-FTX (526)-sh3 (R) AATTCAAAAAAGCTGGATTGTGAAGGATATTTTCTCTT  
GAAAATATCCTTCACAATCCAGCCG

h-FTX (1459)-sh4 (F) GATCCGGGGACAAATCCCATTGGAACATTCAAGAGAT  
GTTCAAATGGGATTTGTCCCTTTTTTG

h-FTX (1459)-sh4 (R) AATTCAAAAAAGGGACAAATCCCATTGGAACATCTCT  
TGAATGTTCAAATGGGATTTGTCCCG

h-FTX (2174)-sh5 (F) GATCCGGCTGAAACTTCAGTACTTAAGTTCAAGAGAC  
TTAAGTACTGAAGTTTCAGCTTTTTTG

h-FTX (2174)-sh5 (R) AATTCAAAAAAGCTGAAACTTCAGTACTTAAGTCTCTT  
GAACTTAAGTACTGAAGTTTCAGCCG

h-RBMX-shRNA (F) GATCCGGAGAAACGAATAAGTCAAGATTCAAGAGATC  
TTGACTTATTCGTTTCTC TTTTTT G

AATTCAAAAAA

h-RBMX-shRNA (R) GAGAAACGAATAAGTCAAGATCTCTTGAATCTTGACTT  
ATTCGTTTCTCCG

si-FTX-Homo-423 (F) GCCUGUACUCAUACUGAUGCTdT

si-FTX-Homo-423 (R) GCAUCAGUAUGAGUAAACAGGCTdT

Si-NC (F) Purchase from RiboBio (Sequence confidentiality)

Si-NC (R) Purchase from RiboBio (Sequence confidentiality)

HsnRNA U6 primer Purchase from FulenGen Cat.HmiRQP9001(Sequence  
confidentiality)

Has-miR-374a-3p primer Purchase from FulenGen Cat.HmiRQP0462(Sequence confidentiality)

Has-miR-545a-5p Primer Purchase from FulenGen Cat.HmiRQP0625(Sequence confidentiality)

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