

## 1 **Supporting Figure Legend**

### 2 **Fig. S1**

3 H) RT-PCR validation results of the circRNAs in blank (left) and RNaseR treated  
4 (right) HCC-LM3 cells. B) Relative expression level of the 4 circRNAs in normal  
5 hepatocyte lines (THL-2) and HCC cell lines by real-time qRT-PCR analysis. C)  
6 RT-PCR and schematic illustration showed hsa\_circ\_0001727, hsa\_circ\_0006886 and  
7 hsa\_circ\_0071106 expression upon RNaseR treatment in HCC-LM3 cells, and  
8 sequencing showed hsa\_circ\_0001727, hsa\_circ\_0006886 and hsa\_circ\_0071106  
9 back-splice site. D) Relative Expression level of circZKSCAN1 in both HCC-LM3  
10 and Hep3B cells with RNaseR treatment. E) IC50 assay detected HCC-LM3 and  
11 Hep3B cells with or without circZKSaa transfection after different sorafenib treatment.  
12 F) Analysis of apoptosis in HCC-LM3 and Hep3B with or without knockdown  
13 circZKSCAN1 transfection by flow cytometry assays. Quantitative data from three  
14 independent experiments are presented as mean±SEM (error bars). T test was  
15 performed for significant analysis, significant differences are indicated with \* for  
16  $p<0.05$ , \*\* for  $p<0.01$  and \*\*\* for  $p<0.001$ .

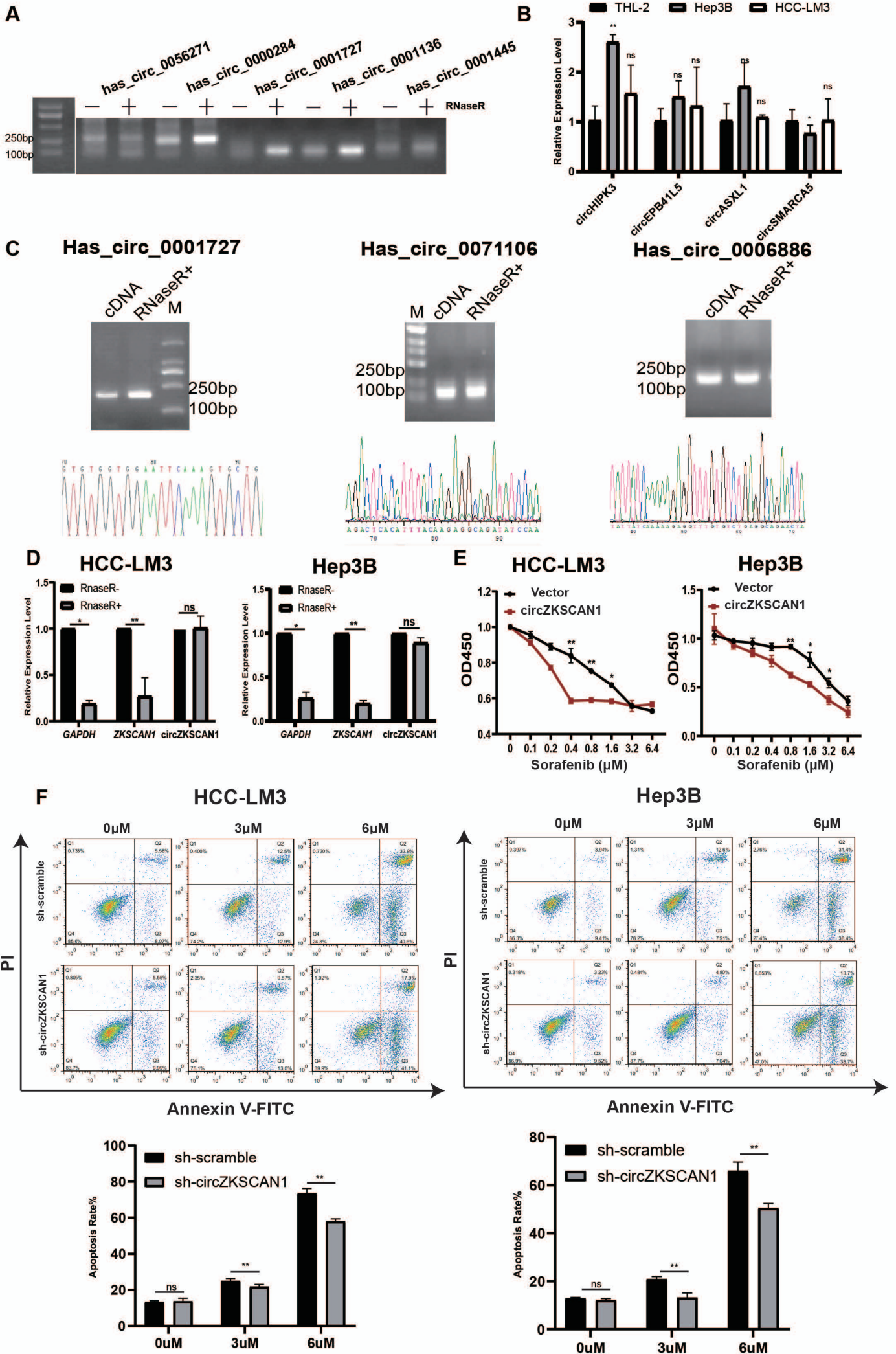
### 17 **Fig. S2**

18 A) CircInteractome website showed the potentially binding RBPs with  
19 circZKSCAN1. B) RIP analysis of circZKSCAN1, linZKSCAN1 using antibodies  
20 against AGO2. The RIP enrichment of the AGO2-associated circRNAs (as indicated)  
21 was measured by real time qRT-PCR, and each value was normalized to the level of

22 input RNA used in RIP analysis. linZKSCAN1 serve as the negative control and  
23 circHIPK3 as the positive control. C) circRNADb website datasets showed IRES  
24 regions and the potentially peptide translated by circZKSCAN1. D) Western blot  
25 showed expression of ZKSCAN1 in HCC-LM3 cells which transfected with  
26 circZKSCAN1-mut, circZKSCAN1, sh-scramble and sh-circZKSCAN1, Vector and  
27 circZKSaa. E) The circZKSaa content was tested via Western blot in 17 pairs of HCC  
28 and normal tissues.  $\beta$ -actin was used to indicate the amount of loading proteins.  
29 Quantitative data from three independent experiments are presented as mean $\pm$ SEM  
30 (error bars). T test was performed for significant analysis, significant differences are  
31 indicated with \* for  $p < 0.05$ , \*\* for  $p < 0.01$  and \*\*\* for  $p < 0.001$ .

32 **Fig. S3**

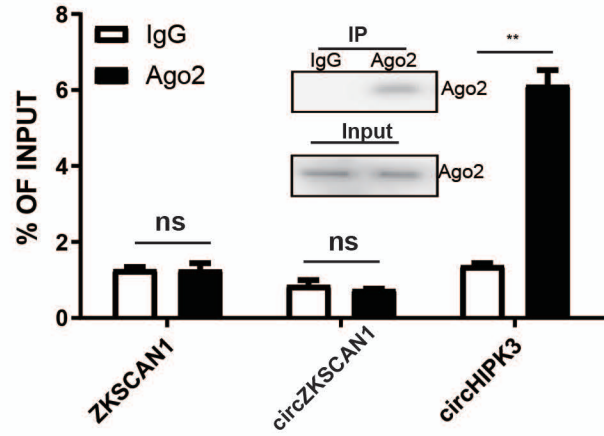
33 A) Analysis of apoptosis in HCC-LM3 and Hep3B with or without knockdown  
34 circZKSCAN1-mut transfection by flow cytometry assays. B) Cell cycle assays were  
35 used to detect cell cycle arrest levels of circZKSCAN1-mut, circZKSCAN1,  
36 sh-scramble, sh-circZKSCAN1, Vector and circZKSaa stable cell lines (HCC-LM3  
37 and Hep3B). C) Apoptosis flow cytometry analysis for circZKSCAN1-mut,  
38 circZKSCAN1, sh-scramble, sh-circZKSCAN1, Vector and circZKSaa stable cell  
39 lines (HCC-LM3 and Hep3B). D) The expression level of cyclin E were determined  
40 via Western blot, and TUBULIN expression level served to indicate the amount of  
41 loading proteins. Quantitative data from three independent experiments are presented  
42 as mean $\pm$ SEM (error bars). T test was performed for significant analysis, significant  
43 differences are indicated with \* for  $p < 0.05$ , \*\* for  $p < 0.01$  and \*\*\* for  $p < 0.001$ .



**A**

CircRNA ID	hsa_circ_0001727	Location	chr7:99621041-99621930
Genomic Length	889 bp	Spliced Seq Length	668 bp
Best Transcript	NM_003439 Primers	Gene Symbol	ZKSCAN1
Samples	HEK293, cd_19, cd_34, Hs68_RNase, Hs68_control, Nhek, K562, Huvec, Hsmm, Hepg2, Helas3, H1hesc, Gm12878, Bj, Ag04450, A549, Nhlf, Sknshra, Mcf7, SV5Y_exp2_D0, diencephalon, Sy5y_exp1_D4, cerebellum, Sy5y_exp1_D0, occipital lobe, frontal cortex, Sy5y_exp1_D2, parietal lobe, SV5Y_exp2_D8, temporal lobe, SV5Y_exp2_D4	Study	Jeck2013, Memczak2013, Rybak2015, Salzman2013
GenomicSeq	hsa_circ_0001727	Mature Seq	hsa_circ_0001727
RNA-binding protein sites matching to circRNAs			
RNA-binding protein sites matching flanking regions of circRNA			
RNA-binding Protein		# Tags	
EIF4A3		4	
FUS		1	
IDP43		1	

**B**



**C**

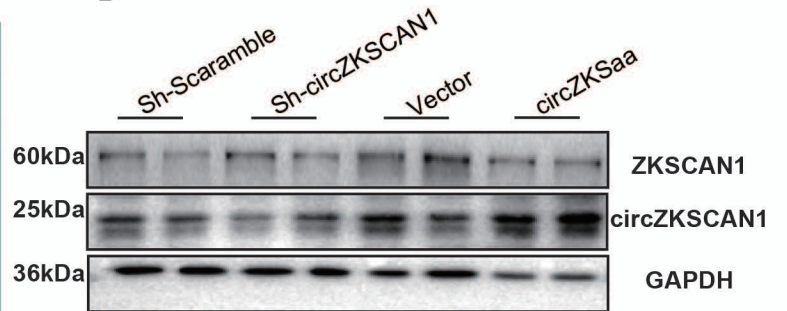
All Possible Transcripts

Transcript id	Exon information								
NM_003439	<table border="1"> <thead> <tr> <th>Spliced_jen</th> <th>Exon Number</th> <th>Exon Sizes</th> <th>Exon Offsets</th> </tr> </thead> <tbody> <tr> <td>666</td> <td>2</td> <td>514, 154</td> <td>0, 735</td> </tr> </tbody> </table>	Spliced_jen	Exon Number	Exon Sizes	Exon Offsets	666	2	514, 154	0, 735
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666	2	514, 154	0, 735						

Protein coding potential

IRES Elements	Parameter Index									
	<table border="1"> <thead> <tr> <th>Position (start-end)</th> <th>R Score</th> <th>With Pseudoknot (Y/N)</th> </tr> </thead> <tbody> <tr> <td>134-284</td> <td>1.627221</td> <td>Y</td> </tr> <tr> <td>143-293</td> <td>1.601725</td> <td>Y</td> </tr> </tbody> </table>	Position (start-end)	R Score	With Pseudoknot (Y/N)	134-284	1.627221	Y	143-293	1.601725	Y
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Start Position	End Position	Protein Length								
59	1141	206 aa								
	<p>Open Reading Frame (ORF)</p> <p>MTAASREAT GLSPQAQEK DGIVLVKVEE EDEEDHWGQ DSTLQTPFP DPEIFRQFR RFCYQTFGP REALSRLEKEL CHQHLRPEIN TKEQLLELV LEQFLSLPK ELQVWLQEVY PDSGEEAVTL LEDLELDLSG QQVPGQVHP EHLARQHVPL DPVQESSFFD LHHEATQSHF KHHSRKPALL QSRGIVKHI IKPFR*</p> <p>Note:            (*). nr represents n rounds(n&lt;3); (2). * represents a stop codon.</p>									

**D**



**E**

