

Supplementary Figures

Figure S1:

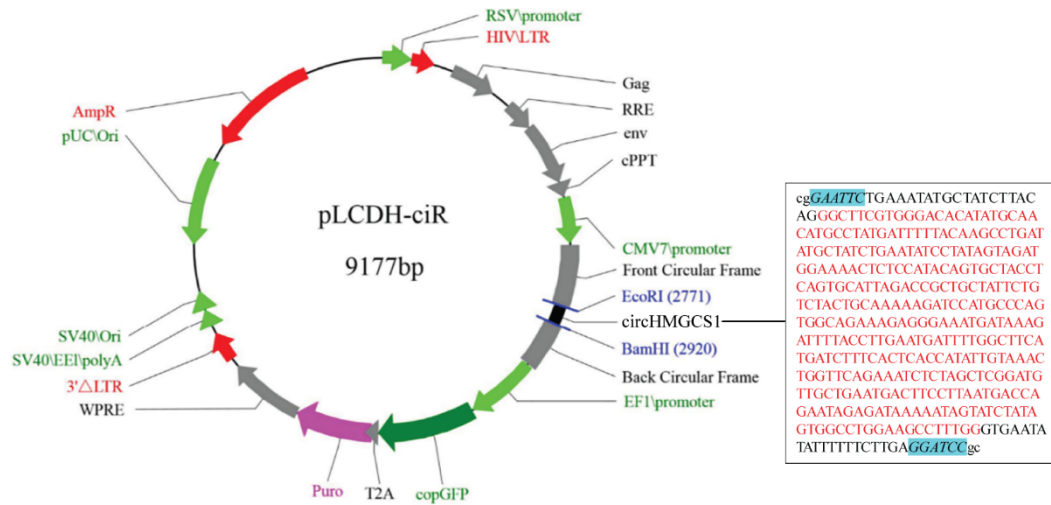


Figure S1: The profile of circHMGCS1 overexpression vector. CircHMGCS1 sequence was constructed into pLCDH-ciR vector.

Figure S2:

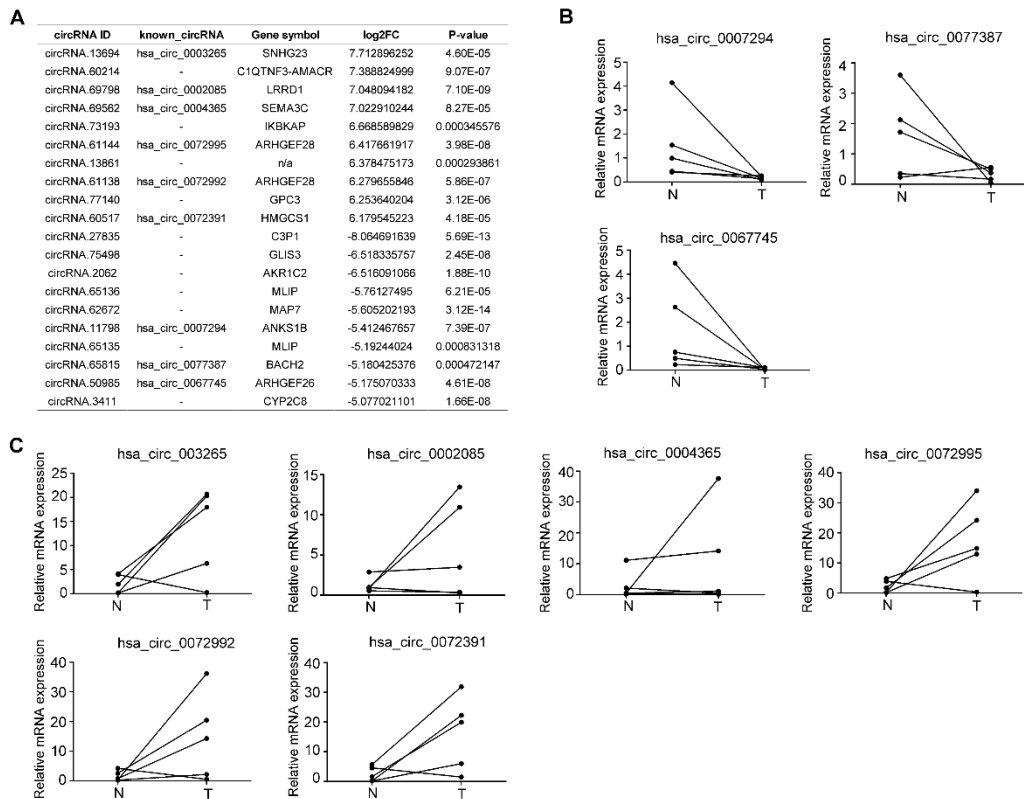


Figure S2. Validation of circRNA sequencing results by qRT-PCR of three downregulated known_circRNA and six upregulated known_circRNA in five paired HB samples. (A) The

top 10 upregulated circRNAs and the top 10 downregulated circRNAs. The log₂FC of has_circ_0072391 is 6.179545223. (B) Expression of three downregulated known_circRNA detected by qRT-PCR in five paired samples. (C) Expression of six upregulated known_circRNA detected by qRT-PCR in five paired samples. Data from qRT-PCR are acquired from three independent experiments.

Figure S3:

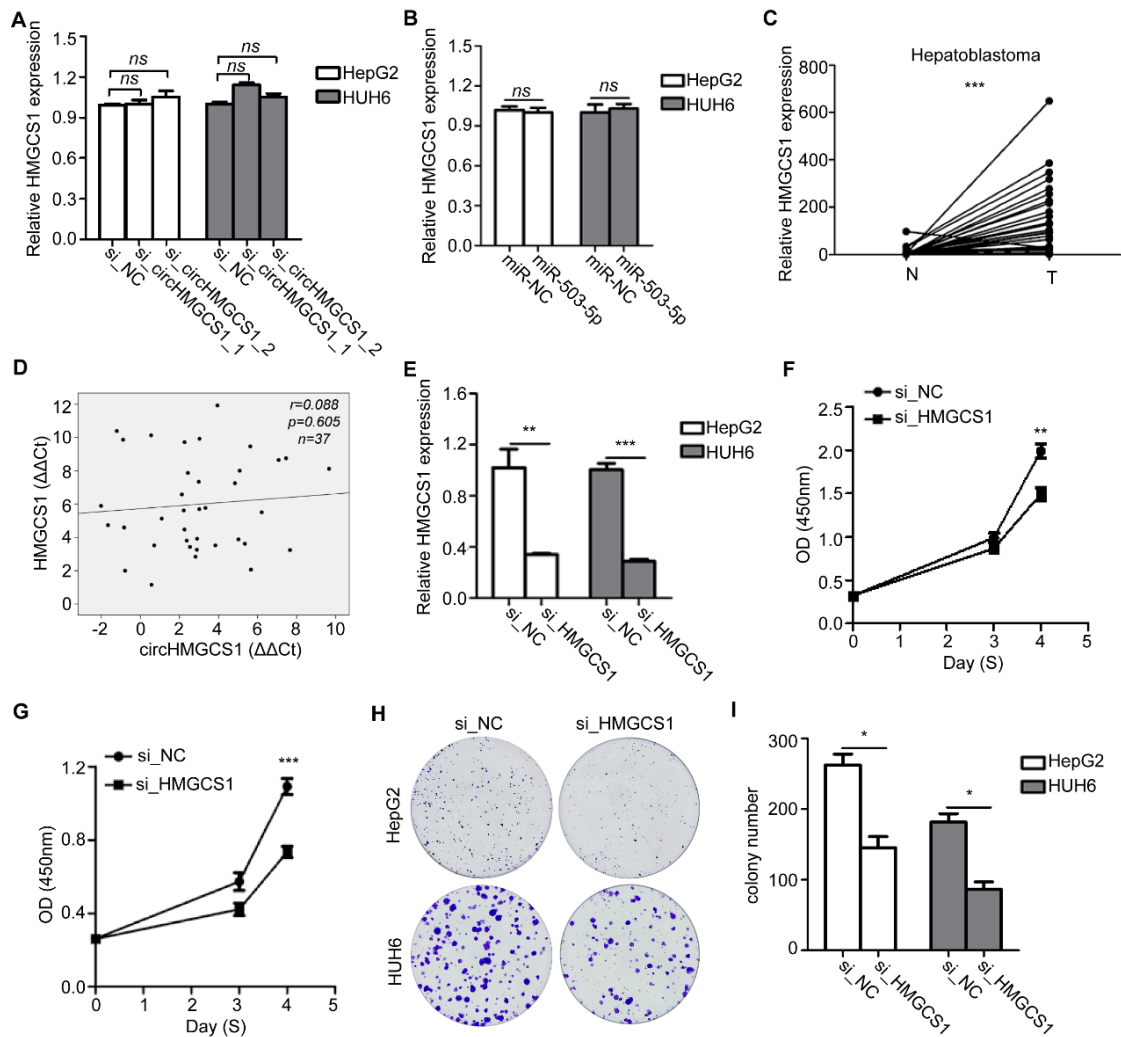


Figure S3. The relationship between circHMGCS1 and HMGCS1. (A) qRT-PCR assays were performed to detect the expression level of HMGCS1 in siRNA transfected HepG2 and HUH6 cells (one-way analysis of variance, Dunnett's test). (B) qRT-PCR assays were performed to detect the expression level of HMGCS1 in miR-503-5p transfected HepG2 cells and HUH6 cells (independent-samples t test). (C) The mRNA level of HMGCS1 in HB tissues was detected by qRT-PCR (paired samples t-test). (D) The correlation between circHMGCS1 and HMGCS1 was

analysed by Pearson correlation confidences. (E) The interference efficiency of siHMGCS1 were detected by qRT-PCR(independent-samples t-test). (F-I) The effect of HMGCS1 knockdown on cell proliferation was detected by CCK8 assays (F and G) and colony formation assay (H-I) (independent-samples t-test). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S4:

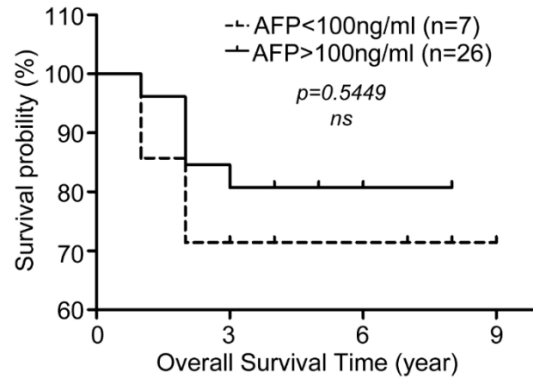


Figure S4 Kaplan-Meier’s analyses of correlations between AFP level at diagnosis and OS of 33 HB patients (log-rank test).

Figure S5:

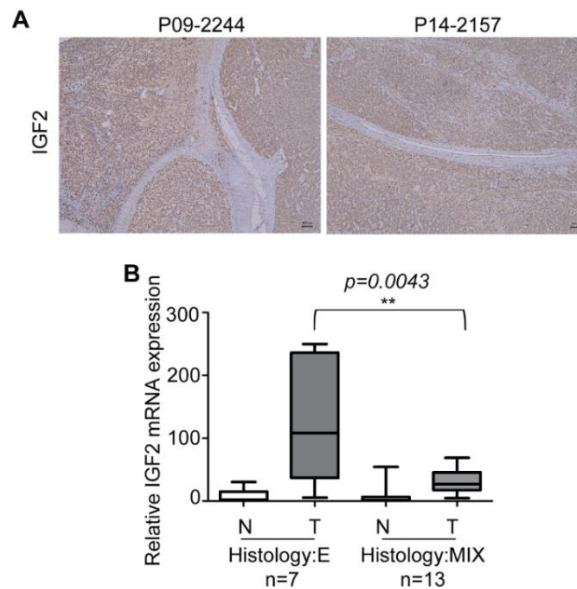


Figure S5 The expression pattern of IGF2 in HB tissues. (A) IHC staining of IGF2 in HB tissue sections. (B) IGF2 expression was detected by qRT-PCR, and IGF2 expression was higher in Hepatoblastoma (epithelial) than that in Hepatoblastoma mixed epithelial and mesenchymal (independent-samples t test).

Supplementary Tables

Supplementary Table 1 clinical patients information.

Sample	Patient Number	Admission time	Sex	Age at diagnosis (month)	AFP at diagnosis (ng/ml)	Histology	PRETEXT	Tumor Size (cm ³)	Metastasis	AFP at Final test	Event	Detection Method	Pathological section number
S1	291465	20170408	M	20	1094.3	MIX	I	1080	NO	NA	NA	qPCR	
S2	293467	20170428	F	20	26243.2	MIX	III	1080	NO	5.92 (201780418)	NA	qPCR	
S3	298082	20170614	F	18	3330.1	NA	III	556.79925	NO	9.06 (20180312)	NA	qPCR	
S4	318998	20180104	M	77	>120000	E	III	480	NO	133.05 (20180531)	NA	qPCR	
S5	325997	20180317	M	19	88178.5	MIX	I	1950	NO	9.78 (20180606)	NA	qPCR	
S6	299192	20170524	M	27	>120000	NA	III	906.40431	YES	1.35(20180607)	NA	qPCR	
S7	300376	20170708	M	30	1394.16	E	II	720	NO	2.14 (20180423)	NA	qPCR	
S8	299060	20170623	F	41	>120000	MIX	II	960	YES	40.65 (20180604)	NA	qPCR	
S9	305761	20170819	M	29	165.1	MIX	II	60	YES	1.11 (20180425)	NA	qPCR	
S10	311532	20171018	F	17	12639.6	MIX	II	532.44	NO	7.32 (20180326)	NA	qPCR	
S11	236250	20150629	M	88	>3000	NA	III-IV	299.88	NO	NA	Death	qPCR	
S12	245611	20151023	F	94	11044	MIX	III-IV	720.00	NO	1.25 (20180605)	Alive	qPCR	
S13	241094	20150822	M	88	2882.96	NA	III-IV	299.88	NO	349.32 (20160615)	Alive	qPCR	
S14	242769	20150914	M	37	23098.5	MIX	I	1080	NO	2.19 (20180319)	NA	qPCR	
S15	243878	20150928	M	37	126000	NA	III	445.718	NO	1.18 (20180416)	Alive	qPCR	
S16	271526	20160822	F	39	1691.93	NA	III	1042.81814	NO	NA	NA	qPCR	
S17	247365	20151114	M	42	2753.13	NA	III-IV	240	NO	4.19 (20180531)	Alive	qPCR	
S18	290459	20151224	M	30	35986	E	I-II	45.325	NO	5.24 (20181205)	NA	qPCR	
S19	290610	20151226	M	34	52.98	E	NA	675.50616	NO	NA	NA	qPCR	
S20	262581	20160121	M	86	120000	NA	III-IV	725.3316	YES	1.24(20160429)	NA	qPCR	
S21	253463	20160202	M	66	3064.24	E	NA	480	NO	348.63 (20160216)	NA	qPCR	
S22	253871	20160211	F	50	9.8	E	III	1371.04	NO	1.90 (20180315)	NA	qPCR	
S23	253883	20160211	F	32	1036.51	NA	IV	1442.65023	NO	4.81 (20180314)	NA	qPCR	
S24	259035	20160414	F	48	3.71	MIX	NA	1512	NO	4.38 (20180316)	NA	qPCR	
S25	261638	20160514	M	32	8.38	NA	III	1128.8816	YES	2.13 (20180118)	NA	qPCR	
S26	264135	20160610	M	28	44.7	NA	NA	1016.64158	NO	NA	Death	qPCR	
S27	267627	20160716	M	34	2076.16	E	NA	576.238551	NO	NA	NA	qPCR	
S28	268381	20160723	M	38	83080.4	NA	III-IV	1016.64158	NO	3.17 (20180409)	NA	qPCR	
S29	271332	20160820	F	27	1364.47	MIX	NA	727.25255	NO	5.99 (20180409)	NA	qPCR	
S30	272355	20160906	M	25	1293.17	NA	NA	114.9812	NO	1.92 (20170710)	NA	qPCR	
S31	276132	20161015	M	29	1088.14	MIX	NA	92.736	NO	1.47 (20180103)	NA	qPCR	
S32	277441	20161029	M	54	>120000	MIX	I	1302.32448	NO	2.43 (20180205)	NA	qPCR	
S33	279778	20161109	M	23	1464.89	NA	NA	2115.34158	NO	NA	NA	qPCR	
S34	290534	20161203	M	121	3332.98	NA	NA	664.44	NO	0.67 (20180418)	NA	qPCR	
S35	290824	20170401	F	15	83927	NA	NA	139.99314	NO	432.52 (20180621)	NA	qPCR	
S36	291465	20170408	M	20	1742	MIX	I	821.24394	YES	6.28 (20180403)	NA	qPCR	
S37	291967	20170410	F	41	25944.3	NA	IV	1793.464	NO	4.00 (20180226)	NA	qPCR	
S38	229082	20150328	M	20	45.25	MIX	II-III	964.8608	NO	2.51 (20180317)	Alive	ISH and IHC	P15-966A
S39	163497	20130924	M	48	>3000	E	III-IV	2288.286	NO	894.61	Alive	ISH and IHC	P13-255B
S40	242768	20150914	M	4.5	23908.5	MIX	I	708.084	NO	2.00 (20180319)	Alive	ISH and IHC	P15-2989A
S41	226740	20150225	M	30	>3000	MIX	I	435.1994	NO	1.01 (20151116)	Alive	ISH and IHC	P15-587A
S42	234511	20150606	M	27	>3000	MIX	I-II	200	NO	1.10 (20170711)	Alive	ISH and IHC	P15-1760A
S43	231145	20150425	F	14	2996.21	NA	III	1429.25598	NO	1.95 (20171225)	Alive	ISH and IHC	P15-1274
S44	097948	20090818	F	11	3.74	MIX	NA	985.536	NO	1.16 (20100920)	Alive	ISH and IHC	P09-1761
S45	151638	20120628	F	9	>3000	E	III	597.702	NO	>3000 (20120711)	Death	ISH and IHC	P12-1406
S46	149863	20120429	M	6	>3000	MIX	III-IV	640.32	NO	2.03 (20130925)	Alive	ISH and IHC	P12-201A
S47	117479	20101009	F	48	1210	E	NA	118.8	NO	0.71 (20160506)	Alive	ISH and IHC	P10-2165
S48	109786	20100514	M	15	1210000ng	NA	IV	NA	NA	5.01 (20110224)	Alive	ISH and IHC	P10-985
S49	133632	20110719	F	48	2.38	E	II	185.724	NO	1.03 (20120521)	Alive	ISH and IHC	P11-1967B
S50	238248	20150721	F	21	10931.7	MIX	III	24.19	NO	3.75 (20180321)	Alive	ISH and IHC	P15-2330A
S51	226975	20150228	M	19	>3000	MIX	III-IV	309.514	NO	3.84 (20150804)	Alive	ISH and IHC	P15-626A
S52	229142	20150329	F	20	>3000	MIX	III	650.87	NO	2.14 (20180407)	Alive	ISH and IHC	P15-1020
S53	236250	20150629	M	48	>3000	NA	III-IV	291	YES	349.32	Death	ISH and IHC	P15-1978
S54	165739	20121227	M	24	>3000	E	III	722.304	NO	0.95 (20170717)	Alive	ISH and IHC	P12-3391
S55	176888	20130624	M	16	>3000	E	NA	NA	NO	>3000(20130621)	Alive	ISH and IHC	P13-1588
S56	209061	20140705	M	8	918.74	E	II	277.4772	NO	1.24 (20181026)	Alive	ISH and IHC	P14-2157
S57	210766	20140725	F	18	>3000	MIX	I-II	105.722412	NO	1.99 (20141229)	Alive	ISH and IHC	P14-2366
S58	156095	20120727	M	18	33.85	E	IV	853.86	NO	10.88 (20130506)	Alive	ISH and IHC	P12-2009
S59	153654	20120625	M	9	6.49	E	IV	252.992	NO	6.49 (20121031)	Death	ISH and IHC	P12-1602
S60	100472	20090524	F	23	1865.49	MIX	NA	NA	NA	1.11 (20111115)	Alive	ISH and IHC	P09-2244
S61	194371	20140303	F	4.5	>3000	MIX	IV	755.969456	NO	2.95 (20170515)	Alive	ISH and IHC	P14-567
S62	124565	20110225	F	72	>1210.00	NA	IV	93.95	NO	>1210(20121123)	Death	ISH and IHC	P11-439
S63	124142	20100520	F	20	9.42	NA	II	688.7	NO	11.71 (20111120)	Alive	ISH and IHC	P11-1307
S64	200832	20140321	M	15	>3000	MIX	III-IV	1095.12	NO	>3000(20150915)	Death	ISH and IHC	P14-793

NA means no data; M means male; F means female; MIX means mixed epithelial and mesenchymal; E means epithelial.

Supplementary Table 2 Primers and RNA sequences used in this study

List of oligonucleotide sequences

5'→ 3'

qRT-PCR

circHMGCs1-F-1 TCTAGCTCGGATGTTGCTGA
 circHMGCs1-R-1 TCAGGCTTGAAAAATCATAGGC
 has_circ_0003265-F-1 TGTGCAATGACCGTGAACCTG
 has_circ_0003265-R-1 CAGTCTTTCTGCTCCTTAGTCCA
 has_circ_0002085-F-1 ACAGAAAAACAGTCTGTGGAGAG
 has_circ_0002085-R-1 TCAATTGGACTTTTCCCAAATGCT
 has_circ_0004365-F-1 TTCTCGGAACAGGACCAAGT
 has_circ_0004365-R-1 ACGTTGGGGTTGAAAGAGCA
 has_circ_0072995-F-1 TCCAGCGGTGCAGAAACTG
 has_circ_0072995-R-1 CCCGGGAGACACAAGAAGAAC
 has_circ_0072992-F-1 TGCTCTAGAGTGGACTGTGT
 has_circ_0072992-R-1 ATGTGCTGATGAGATCCGT
 has_circ_0007294-F-1 GCCAGAGTGTAAACAGAAAAGGG
 has_circ_0007294-R-1 TCGTACAACATCCACCTTTCCA
 has_circ_0077387-F-1 TCCTAAGACTCGAAGGCAGC
 has_circ_0077387-R-1 CTGTGCTTCAAGGGCTCATC
 has_circ_0067745-F-1 GAGCTGAGACGCAATGGTTC
 has_circ_0067745-R-1 TCCAGCTGGGAGTTAATTGTGT
 IGF2-F CTTGGACTTTGAGTCAAATTGG
 IGF2-R GGTCTGCCAATTACATTTCA
 miR-503-5p-RT GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATACGACCTGCAG
 miR-503-5p-F CGTAGCAGCGGGGAACAGTT
 miR-503-5p-R AGTGCAGGGTCCGAGGTATT
 miR-490-5p-RT GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATACGACACCCAC
 miR-490-5p-F CGCGCCATGGATCTCCAG
 miR-490-5p-R AGTGCAGGGTCCGAGGTATT
 miR-615-3p-RT GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATACGACAAAGAGG
 miR-615-3p-F CGTCCGAGCCTGGGTCTC
 miR-615-3p-R AGTGCAGGGTCCGAGGTATT
 U6-RT AAAAAATATGGAACGCTCACGAATTTG
 U6-F GTGCTCGCTTTCGGCAGCACATATAC
 U6-R AAAAAATATGGAACGCTCACGAATTTG
 GLS-F CTGGAGGAAGGTTGCAGATTA
 GLS-R GAATGCCTCTGTCCATCTACTG
 PLAG1-F ATCACCTCCATACACACGACC
 PLAG1-R AGCTTGGTATTGTAGTTCTTGGC
 BCL2-F TTGCCAGCCGGAACCTATG
 BCL2-R GAAAGCGACCCAGCAATGATA
 E2F3-F AGAAAGCGGTCTCAGTACCT
 E2F3-R TGGACTTCGTAGTGCAGCTCT
 IGF1R-F TCGACATCCGCAACGACTATC
 IGF1R-R CCAGGGCGTAGTTGTAGAAGAG
 VEGFA-F AGGGCAGAATCATCACGAAGT
 VEGFA-R GGGTCTCGATTGGATGGCA
 HMGCs1-F GATGTGGGAATTGTTGCCCTT
 HMGCs1-R ATGTCTCTGTTCCAACTTCCAG
 IGF1-F GCTCTTCAAGTTCGTGTGTGGA
 IGF1-R GCCTCCTTAGATCACAGCTCC
 18S-F cagccaccgagattgagca
 18S-R tagtagcgaaggcggtgtg
 GAPDH-F TGTGGCCATCAATGACCCCTT
 GAPDH-R CTCCACGACGTAAGTACAGCG
 U1-F GGCTTGTGAACTTG
 U1-R CGCTACCATAAATAATACGC

luciferase PCR

luciferase-circHMGCs1-F CCGCTCGAGGGCTTCGTGGGACACATATGCAA
 luciferase-circHMGCs1-R ATAAAGAAATGCGGCCGCCAAAGGCTTCCAGGCCACTA
 luciferase-IGF2-3'UTR(500-849)-F TGAAGAACGAGCAGTAATTTCTAGGCGATCGCTCGAGACCCCCCTCTTTCTCTTCCCTT
 luciferase-IGF2-3'UTR(500-849)-R AATGAAAAATAAGATATTTTATTGCGGCCAGCGGCCGCTTAGGATGGGAATTGAGATGT
 luciferase-IGF2-3'UTR(2617-2916)-F TGAAGAACGAGCAGTAATTTCTAGGCGATCGCTCGAGGCCCACTGAGCCCCCTTGGAGAAC
 luciferase-IGF2-3'UTR(2617-2916)-R AATGAAAAATAAGATATTTTATTGCGGCCAGCGGCCCAAGATGTACCCGAGGGAGAGG

siRNA

si_NC-S sense (5'-3')
 si_NC-AS UUCUCCGAACGUGUCACGUTT
 si_circHMGCs1-1-S ACGUGACACGUUCGGAGAATT
 si_circHMGCs1-1-AS UGGAAGCCUUUGGGGCUUCGUdTdT
 si_circHMGCs1-2-S ACGAAGCCCAAAGGCUUCCAdTdT
 si_circHMGCs1-2-AS GCCUGGAAGCCUUUGGGGCUUdTdT
 siHMGCs1-S AAGCCCAAAGGCUUCCAGGCdTdT
 siHMGCs1-AS GCACAGCUGCUGUCUUAATT
 UUGAAGACAGCAGCUGUGCTT

shRNA

sh_NC plvx-shRNA1
 sh_circHMGCs1 GGATCC TGAAGCCTTTGGGGCTTCGT CTCGAG ACGAAGCCCAAAGGCTTCCA TTTTTGAATTC

Primers for overexpression plasmid

circHMGCs1-oe-F cgGAATTCGAAATATGCTATCTTACAGGGCTTCGTGGGACACATATGCA
 circHMGCs1-oe-R cgGGATCCTCAAGAAAAAATATTTCCAAAGGCTTCCAGGCCACTAT

circHMGCs1 ISH probe

5'-DIG-ATGTGTCCCACGAAAGCCCAAAGGCTTCCAGGCCA-3'

List of Antibodies

anti-IGF2 abcam, ab9574
 anti-IGF1R abcam, ab182408
 anti-Akt cell signaling technology, 9272
 anti-p-Akt(S473) cell signaling technology, 4060
 anti-p-Akt(T308) cell signaling technology, 2965
 anti-BCL2 cell signaling technology, 2872
 anti-VEGFA abcam, ab1316
 anti-GLS abcam, ab156876

Supplementary Table 3 experimentally validated target gene of miR-503-5p extracted from the literature.

Name	Reference(Pubmed ID)
LARP1	29969631
IGF1R	29917206/ 24636986 /24378652 /28810619 /27366090 /18986336
HNRNPA1	29872500
VEGFA	27619772 /26268439 /23352645 /23352645
Apelin	29800588
cyclin D1	29571017/ 28602785/23731275 /19538740 /26882816
PRKACA	29568867
D52 like 2	29375699
PI3K p85	29327155/28900284 /24550137
WEE1	29019284
E2F3	23967867/26722476 / 29169421
BCL2	24636986/23355742/28343379/23856992 /23355742
CDKN2A	28942143
ALK	28915608
PDCD4	28912531
eIF4G	28599480
CD40	28566713
PUMA	28423513
Smurf1	28341855
EIF4E	27840964
insulin receptor (INSR)	27829550
ZNF217	27539783/27267060
c-myb	26768615
Apelin-13.	26756969
FGF2	26461038/23352645
RNF31	26231797
PRMT1	26163260
CCND1	26047605
DDHD2	25630602
L1CAM	25536034
FBXW7	25269767
Smurf2	24554769
IKK- β	24550137
FANCA	24486548
RANK	23821519
CD40	22429276
CUGBP1	22072795
Cdc25A	20462953
Cyclin E	26882816/ 22814423