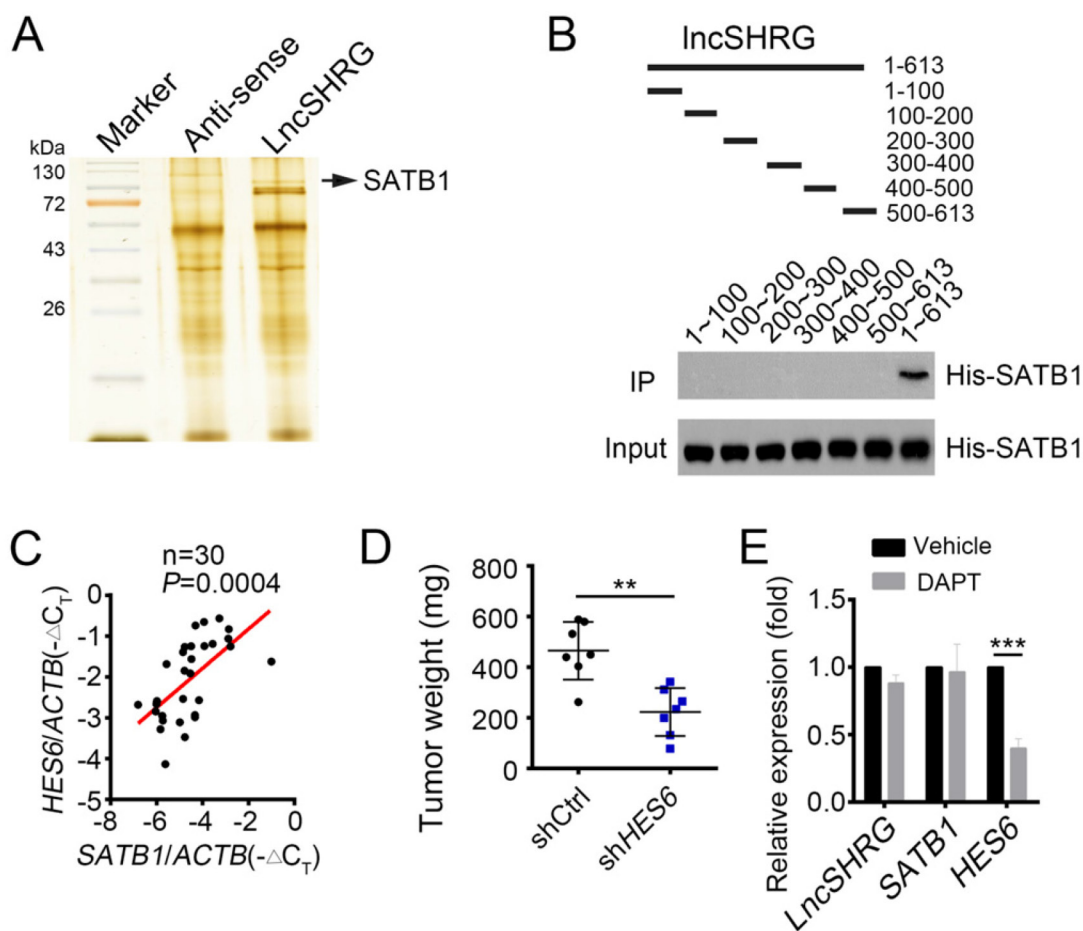


LncSHRG promotes hepatocellular carcinoma progression by activating *HES6*

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: LncSHRG interacts with SATB1. (A) Biotin-RNA pull-downs were performed using biotin-labeled LncSHRG or anti-sense control. Eluted fractions were resolved by SDS-PAGE, followed by silver staining and mass spectrometry. SATB1 was identified as a potential interactive protein of LncSHRG. (B) Analysis of the interactive region in LncSHRG with SATB1 by domain mapping and RNA pull-down assays. LncSHRG truncates were obtained by T7 transcription *in vitro* and biotin labeled. (C) *SATB1* expression was positively correlated with that of *HES6* in HCC samples. (D) Analysis of mRNA levels of LncSHRG, SATB1 and *HES6*. HCC sample cells were cultured with in the presence of N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) (1 μ M) or not. DAPT was an inhibitor of Notch signaling. (E) 2×10^6 *HES6*-depleted or control Hep3B cells were injected into nude mice. The weights of tumors were measured on week 5 after injection. ** $p < 0.01$ by two-tailed Student's *t* test. All data presented are shown as means \pm SD collected from three independent experiments.

Supplementary Table 1: Information of clinical patients

Sample no.	Age	Gender	Diagnosis	Tumor size(cm)	Stage
1#	40	Male	HCC	20*12.5*8.5	advanced
2#	60	Female	HCC	2.5*2.5*1.5	middle-ad
3#	54	Male	HCC	8.4*8.2*7.6	middle-ad
4#	62	Male	HCC	2.6*2.3*1.8	early-mid
5#	37	Male	HCC	10*8*8	early-mid
6#	56	Male	HCC	5*4.5*4	advanced
7#	53	Male	HCC	9*9*6.5	middle-ad
8#	54	Male	HCC	6*5.8*5.5	middle-ad
9#	31	Female	HCC	9.5*8.5*5.8	middle-ad
10#	41	Male	HCC	2.5*1.5*2	early
11#	36	Female	HCC	2.2*1.2*1	early
12#	54	Male	HCC	13*11*9	middle-ad
13#	68	Female	HCC	2.5*2.4*1.8	early
14#	64	Female	HCC	1.9*1.5*1	early
15#	51	Male	HCC	2.2*1.7*1.6	early-mid
16#	50	Male	HCC	6.5*5.5*4	early-mid
17#	43	Male	HCC	3*2.5*2	early-mid
18#	53	Male	HCC	3*3*2	early-mid
19#	49	Male	HCC	8.5*5.5*4.1	middle-ad
20#	71	Male	HCC	4*3*3	early-mid
21#	76	Male	HCC	11*8*7.5	middle-ad
22#	60	Male	HCC	3*3*2.5	early-mid
23#	60	Male	HCC	1.2*1*0.9	early
24#	61	Male	HCC	7*7*5.5	middle-ad
25#	42	Male	HCC	4.3*4.5*4	early-mid
26#	49	Male	HCC	4*2.5*3	early
27#	61	Male	HCC	10*7*6	middle-ad
28#	57	Male	HCC	4*3*3	advanced
29#	61	Female	HCC	3*2.5*2	advanced
30#	49	Male	HCC	2.5*2.2*2.1	early

Supplementary Table 2: Real time PCR primers used in this study

Genes	Forward	Reverse
<i>lncSHRG</i>	5'-TCAACAATTAAGACTCTTTGGCAGT-3'	5'-CTGCCATCTTGACACGAGGT-3'
<i>Actb</i>	5'-TCCATCATGAAGTGTGACGT-3'	5'-GAGCAATGATCTTGATCTTCAT-3'
<i>HIF1a</i>	5'-GAACGTCGAAAAGAAAAGTCTCG-3'	5'-CCTTATCAAGATGCGAACTCACA-3'
<i>VEGF</i>	5'-ATCACGAAGTGGTGAAGTTC-3'	5'-TGCTGTAGGAAGTCTCATCTC-3'
<i>TWIST1</i>	5'-GTCCGCAGTCTTACGAGGAG-3'	5'-GCTTGAGGGTCTGAATCTTGCT-3'
<i>MYC</i>	5'-GGCTCCTGGCAAAGGTCA-3'	5'-CTGCGTAGTTGTGCTGATGT-3'
<i>CCND2</i>	5'-TTTGCCATGTACCCACCGTC-3'	5'-AGGGCATCACAAGTGAGCG-3'
<i>TCF1</i>	5'-CTGGCTTCTACTCCCTGACCT-3'	5'-ACCAGAACCTAGCATCAAGGA-3'
<i>HES6</i>	5'-AGCAGGAGCCTGACTCAGTT-3'	5'-AGCTCCTGAACCATCTGCTC-3'
<i>HEY1</i>	5'-GTTCGGCTCTAGGTTCCATGT-3'	5'-CGTCGGCGCTTCTCAATTATTC-3'
<i>NRARP</i>	5'-TCAACGTGAACTCGTTTCGGG-3'	5'-ACTTCGCCTTGGTGTGATGAGAT-3'
<i>GLI1</i>	5'-TGGATATGATGGTTGGCAAGTG-3'	5'-ACAGACTCAGGCTCAGGCTTCT-3'
<i>PTCH1</i>	5'-CCACAGAAGCGCTCCTACA-3'	5'-CTGTAATTCGCCCCTTCC-3'
<i>GLI3</i>	5'-GAAGTGCTCCACTCGAACAGA-3'	5'-GTGGCTGCATAGTGATTGCG-3'
<i>Satb1</i>	5'-TGATTTGTGACGCACCCTCA-3'	5'-AGCAAGGCAGAAACATCCCC-3'

Supplementary Table 3: Primers used for *HES6* promoter analysis in this study

Region	Forward	Reverse
-2000~-1800	5'-TTAATGTACACAAATCAGTAGC-3'	5'-CATCGTAGAGGTCTTTTGATTCC-3'
-1700~-1500	5'-ACAAGAGCGAAACTCCCTCTG-3'	5'-GGGATTGCATTGAATTTGTAGATT3'
-1400~-1200	5'-AAGGCCATAGTTACCAAAACAG-3'	5'-ATGTGTTGAAAAGGGTGTCCCTT-3'
-1100~-900	5'-CTAGAAGATAAACTGGATAA-3'	5'-TTACTCTACTGACTGTTCCCT-3'
-800~-600	5'-TAATCCCTAACCCCAACCT-3'	5'-AAGGTTGGTGTTAGGGGTAA-3'
-500~-300	5'-AATGCTGCATTTGTCTTCCGAGGC-3'	5'-AGTGTGGAAAACAGGAAACCA-3'
-200~0	5'-AGGAGAACGCTGCTCTGCCTTAC-3	5'-CGAGGGCGGAGCAGTGTCT-3'