THE LONG NON-CODING RNA *H19* SUPPRESSES CARCINOGENESIS AND CHEMORESISTANCE IN HEPATOCELLULAR CARCINOMA

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Supplementary information

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SUPPLEMENT

Supplemental Table S1: Target gene-specific primer information

mRNA	forward primer sequence 5' \rightarrow 3'	reverse primer sequence 5' \rightarrow 3'	gene bank accession no.	AT [°C]	product size [bp]	primer concen- tration [µM]
hu ABCB1/ hu MDR1	GCTATAATGCGA CAGGAGATAGGCT	CATTCCAATTTTGT CACCAATAACTT	NM_001348946.1;NM_0013 48945.1;NM_001348944.1	56	116	0.2
hu ACTB	TGCGTGACATTAA GGAGAAG	GTCAGGCAGCTC GTAGCTCT	NM_001101.3	60	107	0.2
hu ELAVL1	GGTGACATCGGG AGAACGAA	CCAAGCTGTGTCC TGCTACT	NM_001419.2	60	142	0.2
hu H19	TTCAAAGCCTCCA CGACTCT	CTGAGACTCAAGG CCGTCTC	NR_131224.1;NR_131223.1 ;NR_002196.2;NM_0012931 71.2	60	100	0.2
hu H19 IP	GCTCCCAGAACCC ACAACAT	CCTTCCAGAGCCG ATTCCTG	NR_131224.1;NR_131223.1 ;NR_002196.2;NM_0012931 71.2	61	149	0.2
hu IGF2	GGACTTGAGTCCC TGAACCA	TGAAAATTCCCGT GAGAAGG	NM_000612.5;NM_0010071 39.5;NM_001127598.2;NM_ 001291861.2;NM_00129186 2.2	56	100	0.25
mu H19	CAGAGGTGGATGT GCCTGCC	CGGACCATGTCAT GTCTTTCTGTC	NR_001592.1	60	80	0.25
mu lgf2	GGAAGTCGATGTT GGTGCTTCTC	CGAACAGACAAAC TGAAGCGTGT	NM_010514.3	60	121	0.2
mu Csnk2a2	GTAAAGGACCCTG TGTCAAAGA	GTCAGGATCTGGT AGAGTTGCT	NM_009974.3	60	85	0.4
miRNA	forward primer sequence 5' \rightarrow 3'	reverse primer sequence 5' \rightarrow 3'	accession no.	AT [°C]		primer concen- tration [µM]
hsa-miR- 675	TGGTGCGGAAAG GGCCCACAGT	GAATCGAGCACCA GTTACGCAT	MIMAT0004284	64		0.2
RNU6B	ACGCAAATTCGTG AAGCGTT	GAATCGAGCACCA GTTACG	e.g. NR_125730.1	55		0.5



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AAGCTTGGGAGGGGGGGGGGGGGGGGGGGGGGGGGGGAAACTGGGGGAAGTGGGGAACCGAGGGGCAACCAGG GGAAGATGGGGTGCTGGAGGAGAGCTTGTGGGAGCCCAAGGAGCACCTTGGACATCTGGAGTCTGGCAGGAGTGATGA CGGGTGGAGGGGCTAGCTCGAGGCAGGGCTGGTGGGGCCTGAGGCCAGTGAGGAGTGTGGAGTAGGCGCCCAGGCA TCGTGCAGACAGGGCGACATCAGCTGGGGACGATGGGCCTGAGCTAGGGCTGGAAAGAAGGGGGGAGCCAGGCATTCA CTGGGCAACGGAGGTGTAGCTGGCAGCAGCGGGCAGGTGAGGACCCCATCTGCCGGGCAGGTGAGTCCCTTCCCTCC CCAGGCCTCGCTTCCCCAGCCTTCTGAAAGAAGGAGGTTTAGGGGATCGAGGGCTGGCGGGGGAGAAGCAGACACCCT CCCAGCAGAGGGGCAGGATGGGGGCAGGAGAGTTAGCAAAGGTGACATCTTCTCGGGGGGGAGCCGAGACTGCGCAA GGTCCAGCACGTGGGGTGGTACCCCAGGCCTGGGTCAGACAGGGACATGGCAGGGGACACAGGACAGAGGGGTCCC CAGCTGCCACCTCACCCACCGCAATTCATTTAGTAGCAGGCACAGGGGCAGCTCCGGCACGGCTTTCTCAGGCCTATG CCGGAGCCTCGAGGGCTGGAGAGCGGGAAGACAGGCAGTGCTCGGGGGAGTTGCAGCAGGACGTCACCAGGAGGGCG AAGCGGCCACGGGAGGGGGGCCCCCGGGACATTGCGCAGCAAGGAGGCTGCAGGGGCTCGGCCTGCGGGCGCCGGT CCCACGAGGCACTGCGGCCCAGGGTCTGGTGCGGAGAGGGCCCACAGTGGACTTGGTGACGCTGTATGCCCTCACCG CTCAGCCCCTGGGGCTGGCTTGGCAGACAGTACAGCATCCAGGGGAGTCAAGGGCATGGGGCGAGACCAGACTAGGC GCCTCAGCGTTCGGGCTGGAGACGAGGCCAGGTCTCCAGCTGGGGTGGACGTGCCCACCAGCTGCCGAAGGCCAAG GGCCTTCCTGAACACCTTAGGCTGGTGGGGCTGCGGCAAGAAGCGGGTCTGTTTCTTTACTTCCTCCACGGAGTCGGC AATCCGGACACAAAACCCTCTAGCTTGGAAATGAATATGCTGCACTTTACAACCACTGCACTACCTGACTCAGGAATCGG CTCTGGAAGGTGAAGCTAGAGGAACCAGACCTCATCAGCCCCAACATCAAAGACACCATCGGAACAGCAGCGCCCGCAG CACCCACCCGCACCGGCGACTCCATCTTCATGGCCACCCCCTGCGGCGGACGGTTGACCACCAGCCACCACATCATC CCAGAGCTGAGCTCCTCCAGCGGGATGACGCCGTCCCCACCACCTCCTTCTTCTTTTTCATCCTTCTTGTCTCTTTGT TTCTGAGCTTTCCTGTCTTTCCTGTTTTCTGAGAGATTCAAAGCCTCCACGACTCTGTTTCCCCCCGTCCCTTCTGAATTTA

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Supplemental Figure S1: (A) Plasmid map (pcDNa3.1(+)_A009) and (B) *H19*-sequence (2334 bp).



Supplemental Figure S2: H19 expression in HCC tissues from Saarland University Medical Center determined by qPCR (each, n=32, Mann-Whitney U test; see Figure 1D). Highlighted are the two highest expressing HCC tissues and corresponding normal tissues, from which hepatocytes were microdissected and compared with hepatocytes from three low expressing HCC tissues.



Supplemental Figure S3: Effect of H19 overexpression on colony formation ability in stably H19 overexpressing (H19) and vector control (control, co) HepG2 (left panels), Plc/Prf/5 (middle panels), and Huh7 (right panels) cells. (A, B) Colony formation ability of control or H19 cells normalized to their respective untreated controls after (A) doxorubicin (n≥3, duplicates) or (B) sorafenib (n=3, triplicates) treatment. The p values were calculated by two-sample t-test or Mann-Whitney *U* test depending on the data distribution. * p < 0.05, ** p < 0.01, *** p < 0.001.