# Long non-coding RNA HULC promotes tumor angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1)

**Supplementary Material**

**Supporting materials and methods**

**Plasmid construction**

The 5' flanking region (nucleotides -1531 to +202) of SPHK1 was amplified by PCR from the genomic DNA of HepG2 using specific primers and cloned into the upstream of the pGL3-Basic vector (Promega) via KpnI and HindIII sites. The resulting plasmid was sequenced and named pGL3-1733. To construct various lengths luciferase reporter plasmids of SPHK1 promoter, the regions (-1531/-951, -970/+202, -500/+202, -300/+202, -300/+20, +1/+202) of SPHK1 were amplified from pGL3-1733, then they were inserted into the pGL3-Basic vector to generate pGL3-1172, pGL3-581, pGL3-702, pGL3-502, pGL3-320, pGL3-202, respectively. Mutant construct of pGL3-320, named as pGL3-320-mut, which had a deletion of eight nucleotides (-147 to -140) within the binding sites of E2F1, was carried out using overlap extension PCR. 3′UTR of E2F1 mRNA containing miR-107 target was cloned into pGL3-control, termed pGL3-E2F1. HULC with a deletion of miR-107 binding site was constructed to pcDNA3.1 (termed HULC-107-mut). All primers are listed in Supporting Table 3.

**Cell transfection**

The cells were cultured in a 6-well or 24-well plate for 24 h and then transfected with plasmids, miRNA or siRNAs, respectively. All transfections were performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. siRNA oligonucleotides, including targeting HULC (or SPHK1 and E2F1) and a non-specific scrambled control, miR-107 (or anti-miR-107), and a miRNA control were synthesized by RiboBio (Guangzhou, China). The siRNA duplexes sequences are all listed in Supporting Table 4.

**Tumor xenograft in mice and hemoglobin content analysis**

Four-week-old male BALB/c athymic nude mice (Experiment Animal Center of Peking, China) (each group, n=6) were subcutaneously injected with $4 \times 10^5$ HepG2 (or Huh7) cells transfected with pcDNA3.1 and si-control (named NC), or pcDNA3.1-HULC and si-control, or pcDNA3.1-HULC and si-SPHK1-1, respectively. The growth of solid tumors after 5 days from the injected cells was monitored every five days for up to 30 days. The animals were sacrificed to remove tumors for analysis. The tumors were measured and calculated with the formula $(L \times W^2) \times 0.5$. Hemoglobin concentration was determined using the method described previously [1]. The use of mice was approved by the Animal Care and Use Committee of Nankai University.

**Immunohistochemistry**

Immunohistochemical staining of samples were performed as previously reported [2]. The HCC tissue and normal liver tissue microarrays (NO. 03C03) were obtained from the Xi'an Aomei Biotechnology Co., Ltd. (Xi'an, China). These microarrays were composed of 143 HCC tissues, 5 paratumor liver tissues (supporting Table 2). The primary antibody of rabbit anti-SPHK1 (Proteintech Group, USA) or anti-E2F1 (Proteintech Group, USA) was used. The extents of cytosolic and nuclear staining were considered in the scoring. Categorization of immunostaining intensity was performed by three independent observers.

**References**

1. Liu F, You X, Wang Y, Liu Q, Liu Y, Zhang S, Chen L, Zhang X and Ye L. The oncoprotein HBXIP enhances angiogenesis and growth of breast cancer through modulating FGF8 and VEGF. Carcinogenesis. 2014; 35(5):1144-1153.
2. Zhang X, Dong N, Yin L, Cai N, Ma H, You J, Zhang H, Wang H, He R and Ye L. Hepatitis B virus X protein upregulates survivin expression in hepatoma tissues. J Med Virol. 2005; 77(3):374-381.

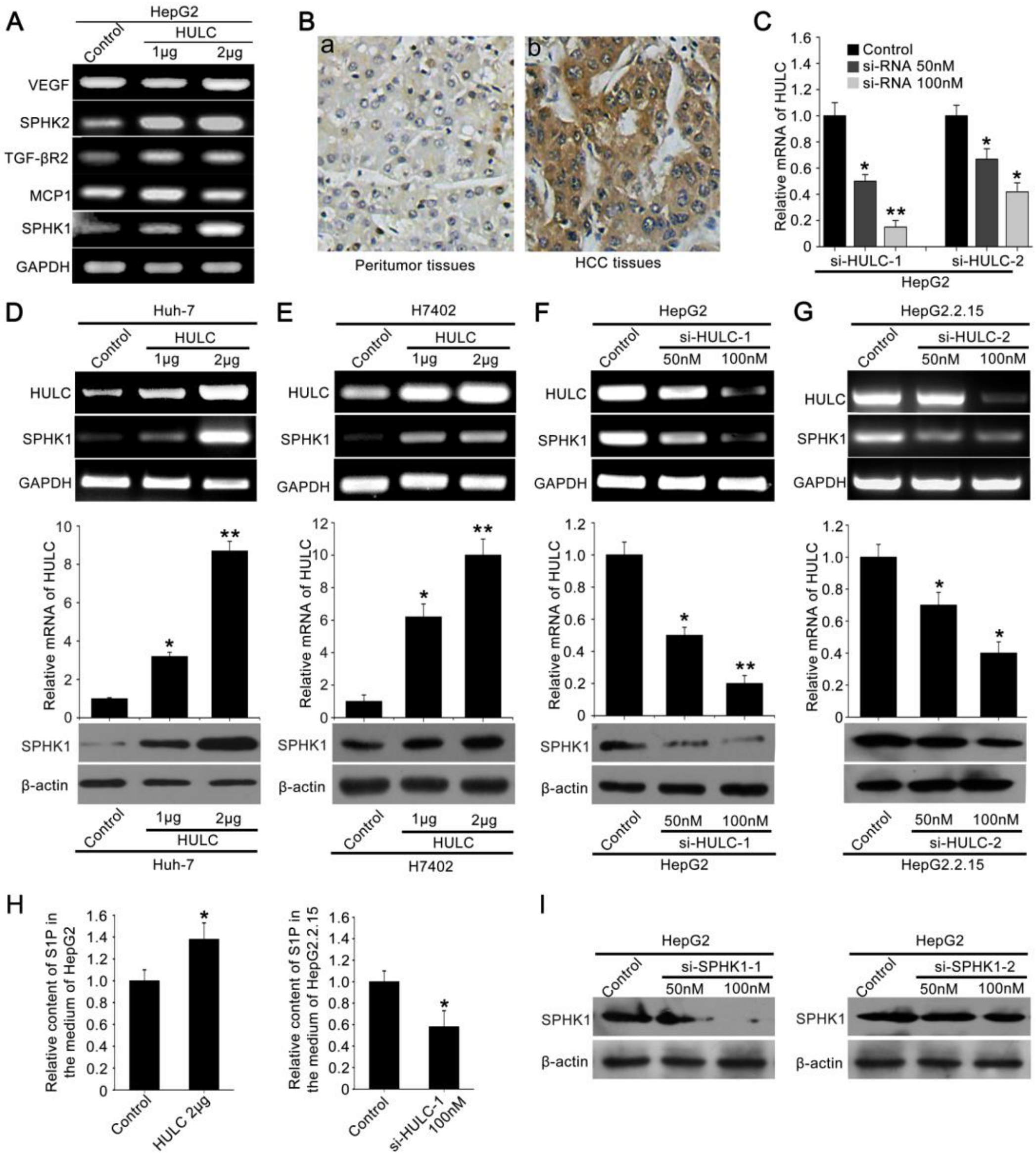


**Figure S1: HULC is able to up-regulate SPHK1.** (**A**) The effect of HULC on the expression of candidate angiogenic genes was examined by RT-PCR in HepG2 cells. (**B**) The expression of SPHK1 was examined by IHC staining in clinical tissues. A representative example of IHC observed in peritumoral tissues (a) and HCC tissues (b) using rabbit anti-SPHK1 Ab is presented. (**C**) The interference efficiency of si-HULC-1 and si-HULC-2 was detected by qRT-PCR. (**D, E**) The expression levels of SPHK1 were detected by RT-PCR and Western blot analysis in Huh7 (or H7402) cells transfected with pcDNA3.1-HULC. (**F, G**) The expression levels of SPHK1 were detected by RT-PCR and

Western blot analysis in HepG2 (or HepG2.2.15) cells transfected with HULC siRNA. (**H**) The relative level of S1P in the medium of HepG2 (HepG2.2.15) cells was measured by ELISA. (**I**) The interfering efficiency of si-SPHK1-1 and si-SPHK1-2 was detected by Western blot analysis in HepG2 cells. (*$P < 0.05$, **$P < 0.01$; Student's *t* test)

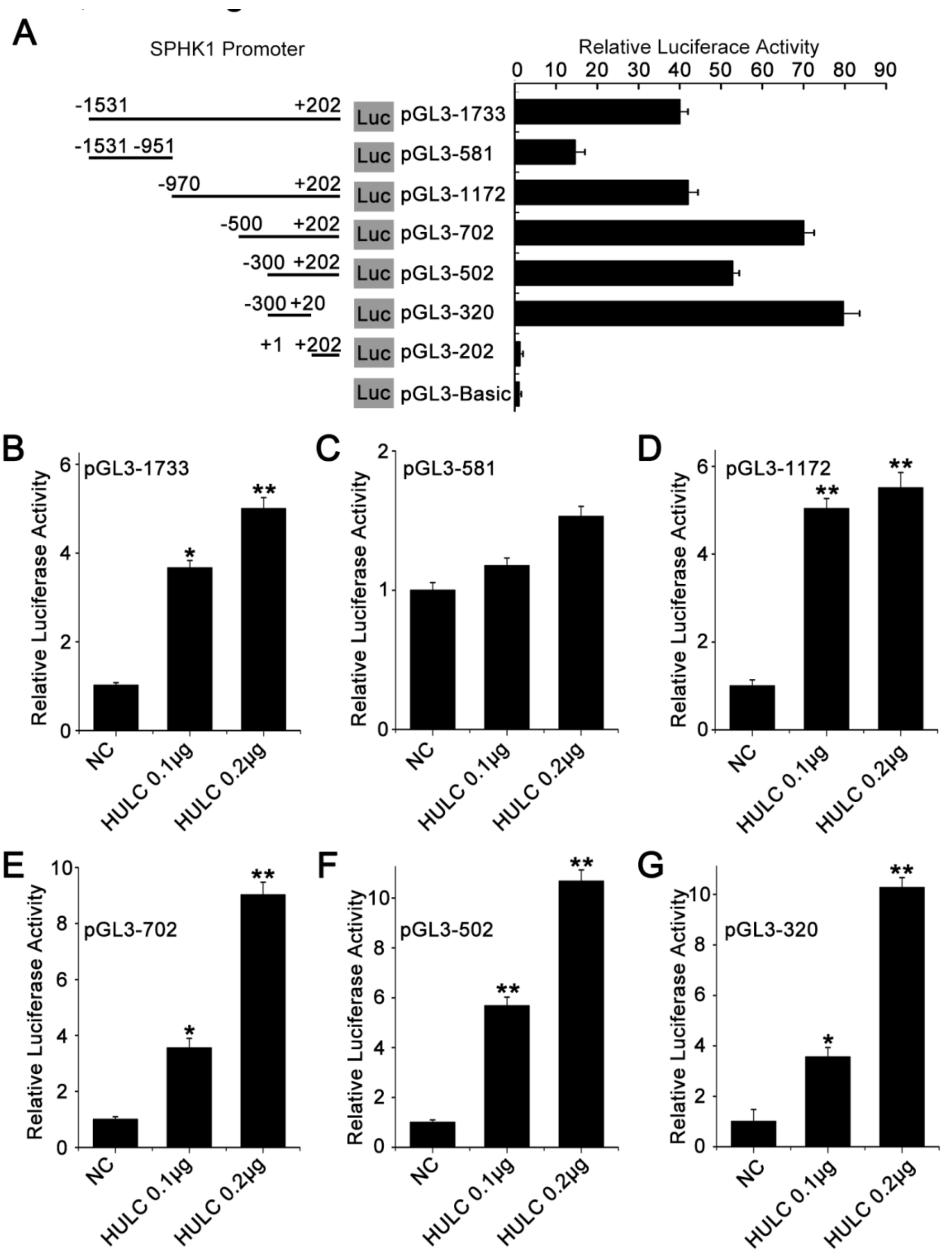


**Figure S2: HULC is able to activate the promoter of SPHK1.** (**A**) 293T cells were transiently transfected with pGL3-Basic (0.2 μg/well) or reporter constructs containing various lengths of the 5' -flanking region of the SPHK1 gene, as indicated (pGL3-1733, pGL3-581, pGL3-1172, pGL3-702, pGL3-502 and pGL3-320 0.2 μg/well, respectively). Results were obtained as relative luciferase activity against the activity of pGL3-Basic. (**B-G**) 293T cell lines were co-transfected with reporter constructs (0.2 μg/well) and HULC expression plasmid (pcDNA3.1-HULC), respectively. Promoter activities of SPHK1 were measured by luciferase reporter gene assays. Data are shown as mean ± SD of three independent experiments. (*$P < 0.05$; **$P < 0.01$; Student's *t* test)

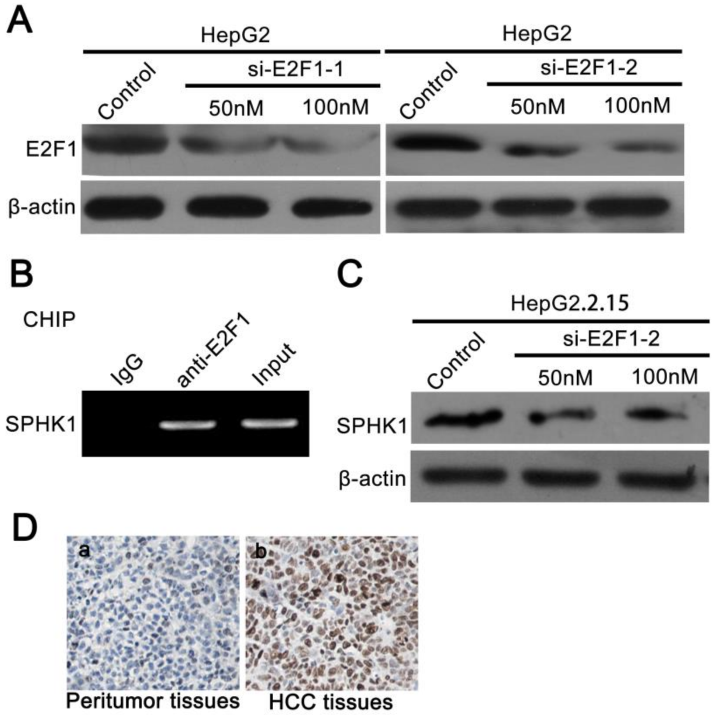


**Figure S3: HULC activates SPHK1 promoter through transcription factor E2F1.** (**A**) The interfering efficiency of si-E2F1-1 and si-E2F1-2 was detected by Western blot in HepG2 cells. (**B**) The interaction of E2F1 and SPHK1 promoter region was validated by ChIP assays using clinical HCC tissues. (**C**) The expression levels of SPHK1 were detected by Western blot in HepG2.2.15 cells transfected with si-E2F1-2. (**D**) The expression of E2F1 was examined by IHC staining in clinical tissues using tissue microarray. A representative example of IHC observed in peritumoral tissues (a) and HCC tissues (b) using rabbit anti-E2F1 Ab is presented.

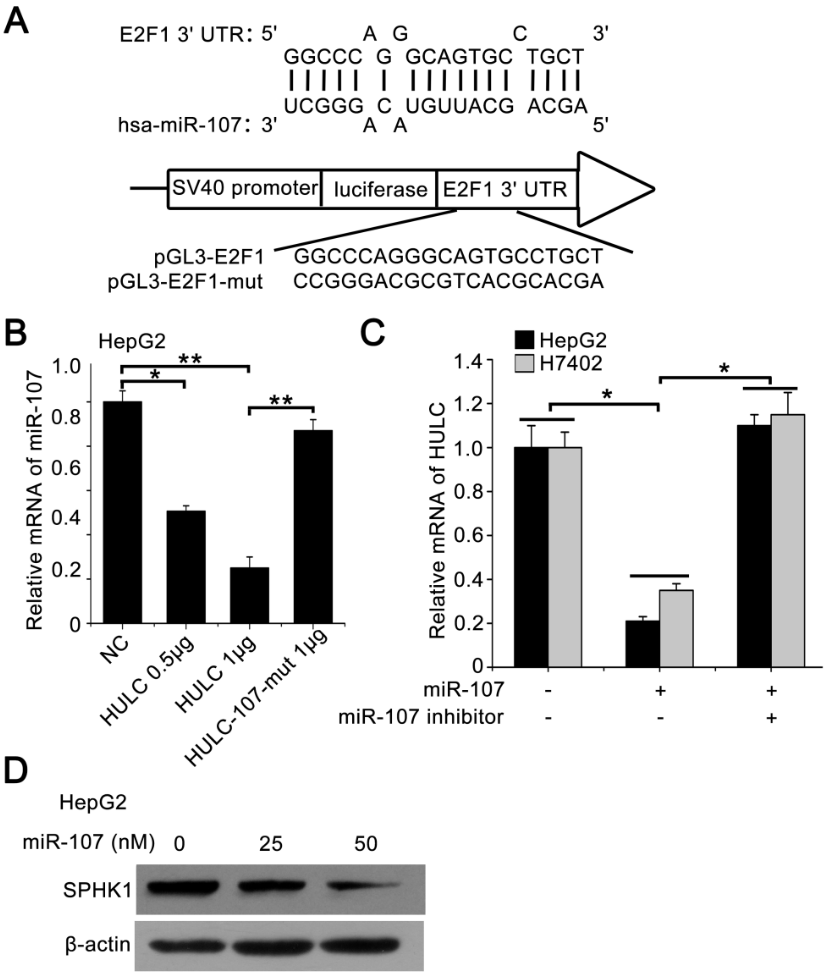


**Figure S4: HULC increases E2F1 by sequestering miR-107. (A)** The model demonstrated the predicted conserved miR-107 binding site at nucleotides 1872-1895 of the E2F1 3′UTR. The generated mutant site at the E2F1 3′UTR is indicated. The wild type E2F1 3′UTR (or mutant) was inserted into the downstream of luciferase reporter gene in the pGL3-Control vector. (**B**) The expression levels of SPHK1 were examined by Western blot analysis in HepG2 cells transfected with miR-107. (**C**) MiR-107 was examined by qRT-PCR in HepG2 cells transfected with pcDNA3.1, pcDNA3.1-HULC or pcDNA3.1-HULC-107-mut. (**D**) The expression levels of HULC were tested by qRT-PCR in HepG2 (or H7402) cells treated with miR-107 or miR-107 and miR-107 inhibitor. Data are shown as mean ± SD of three independent experiments. (**P* < 0.05; ***P* < 0.01; Student's *t* test)

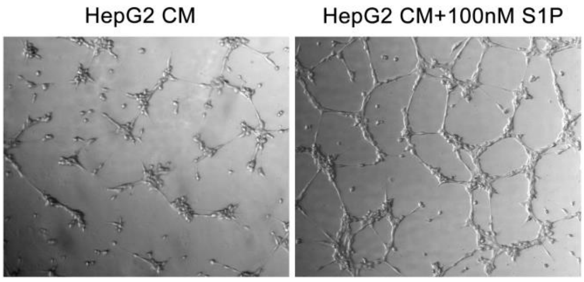


**Figure S5: HULC accelerates tumor angiogenesis *in vitro*.** Representative examples of tube formation were shown as a low-power image (magnification, ×10) when HUVECs were cultured with conditioned medium of HepG2 cells or conditioned medium of HepG2 cells with 100 nM S1P.

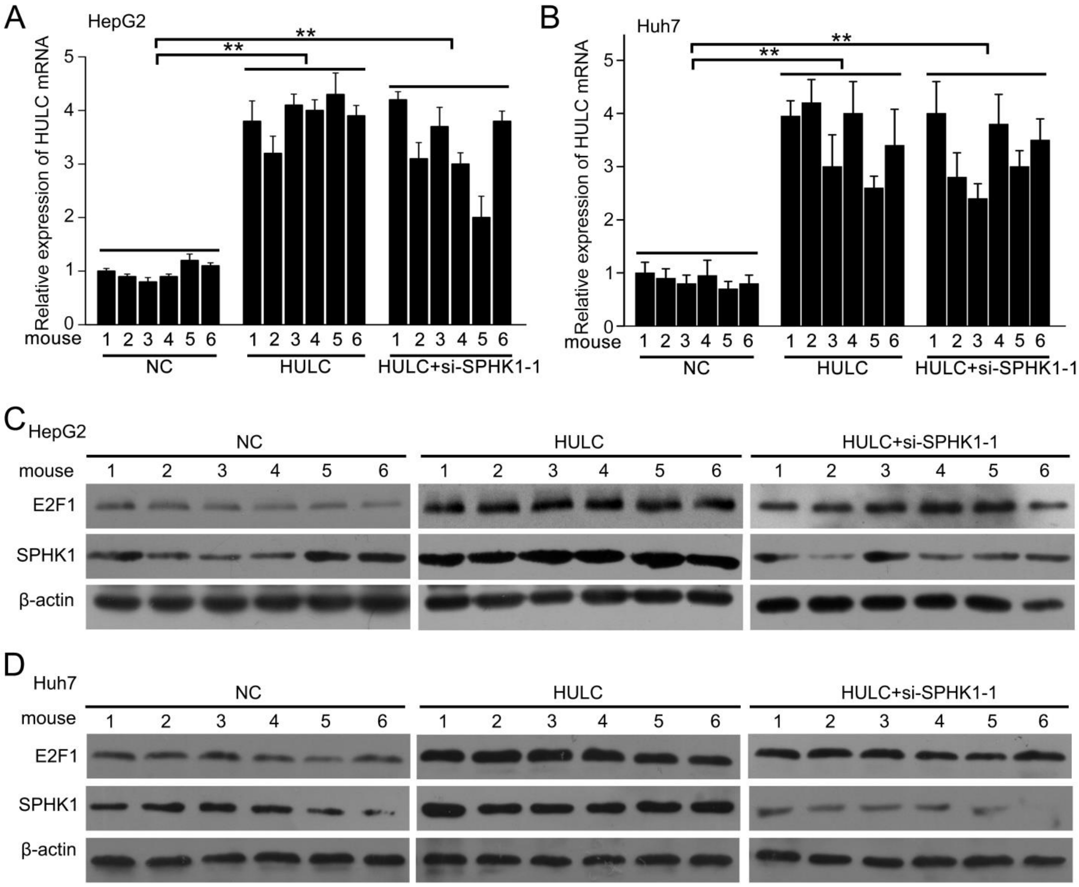


**Figure S6: HULC promotes tumor angiogenesis *in vivo.*** (**A, B**) The expression levels of HULC were detected by qRT-PCR in the tumor tissues transplanted with HepG2 (or Huh7) cells pretreated with pcDNA3.1 and si-control, pcDNA3.1-HULC and si-control, or pcDNA3.1-HULC and si-SPHK1. (**C, D**) The protein levels of E2F1 and SPHK1 in the tumors were detected by Western blot analysis, respectively. (**P* < 0.05; ***P* < 0.01; Student's *t* test)

**Supporting Table 1: characteristics of HCC patients**

| Case No. | Age (yr) | Gender | Diagnosis | HBV Infection | Survival(m) |
|---|---|---|---|---|---|
| 1 | 55 | M | HCC | + | 3 |
| 2 | 45 | M | HCC | + | 3 |
| 3 | 40 | M | HCC | + | 8 |
| 4 | 60 | F | HCC | + | 9 |
| 5 | 73 | M | HCC | + | 11 |
| 6 | 46 | M | HCC | + | N/A |
| 7 | 60 | M | HCC | + | N/A |
| 8 | 59 | M | HCC | + | N/A |
| 9 | 57 | F | HCC | + | N/A |
| 10 | 51 | M | HCC | + | N/A |
| 11 | 38 | M | HCC | + | N/A |
| 12 | 56 | M | HCC | + | N/A |
| 13 | 49 | M | HCC | + | N/A |
| 14 | 58 | F | HCC | + | N/A |
| 15 | 46 | M | HCC | + | N/A |
| 16 | 56 | M | HCC | + | N/A |
| 17 | 54 | M | HCC | + | N/A |
| 18 | 39 | M | HCC | + | N/A |
| 19 | 64 | M | HCC | + | N/A |
| 20 | 61 | M | HCC | + | N/A |
| 21 | 59 | F | HCC | + | N/A |
| 22 | 60 | M | HCC | + | N/A |
| 23 | 65 | M | HCC | + | N/A |
| 24 | 43 | M | HCC | + | N/A |
| 25 | 60 | F | HCC | + | N/A |
| 26 | 41 | M | HCC | + | N/A |
| 27 | 45 | M | HCC | + | N/A |
| 28 | 56 | M | HCC | + | N/A |
| 29 | 70 | M | HCC | + | N/A |
| 30 | 67 | M | HCC | + | N/A |
| 31 | 59 | M | HCC | + | N/A |
| 32 | 57 | M | HCC | + | N/A |
| 33 | 61 | F | HCC | + | N/A |
| 34 | 51 | F | HCC | + | N/A |
| 35 | 56 | M | HCC | + | N/A |
| 36 | 60 | M | HCC | + | N/A |
| 37 | 54 | M | HCC | + | N/A |
| 38 | 36 | M | HCC | + | N/A |

| | | | | | |
|---|---|---|---|---|---|
| 39 | 62 | F | HCC | - | N/A |
| 40 | 57 | M | HCC | + | N/A |
| 41 | 45 | M | HCC | + | N/A |
| 42 | 54 | M | HCC | + | N/A |
| 43 | 68 | M | HCC | + | N/A |
| 44 | 52 | M | HCC | + | N/A |
| 45 | 68 | F | HCC | + | N/A |
| 46 | 59 | M | HCC | + | N/A |
| 47 | 63 | M | HCC | + | N/A |
| 48 | 61 | M | HCC | + | N/A |
| 49 | 56 | F | HCC | - | N/A |
| 50 | 35 | F | HCC | + | N/A |
| 51 | 40 | M | HCC | - | N/A |
| 52 | 75 | M | HCC | - | N/A |
| 53 | 53 | M | HCC | + | N/A |
| 54 | 60 | F | HCC | + | N/A |
| 55 | 26 | M | HCC | + | N/A |
| 56 | 51 | M | HCC | + | N/A |
| 57 | 72 | F | HCC | - | N/A |
| 58 | 43 | M | HCC | + | N/A |
| 59 | 26 | M | HCC | + | N/A |
| 60 | 56 | M | HCC | + | N/A |

Abbreviations: “yr” refers to year. “m” refers to month. “N/A” refers not available

**Supporting Table 2: clinical characteristics of liver cancer and normal tissue**

| No | Age | Sex | Organ | Pathology diagnosis | Grade | Grade of SPHK1 | Grade of E2F1 |
|---|---|---|---|---|---|---|---|
| **1** | 55 | F | Liver | Hepatocellular carcinoma | II | + | ++ |
| **2** | 32 | F | Liver | Hepatocellular carcinoma | I | ++ | ++ |
| **3** | 38 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **4** | 63 | F | Liver | Hepatocellular carcinoma | I | ++ | + |
| **5** | 35 | M | Liver | Hepatocellular carcinoma | I | – | + |
| **6** | 42 | F | Liver | Hepatocellular carcinoma | I | + | ++ |
| **7** | 46 | M | Liver | Hepatocellular carcinoma | I | + | + |
| **8** | 40 | M | Liver | Hepatocellular carcinoma | I | + | + |
| **9** | 48 | F | Liver | Hepatocellular carcinoma | I | – | + |
| **10** | 40 | M | Liver | Hepatocellular carcinoma | I | – | – |
| **11** | 41 | F | Liver | Hepatocellular carcinoma | I | + | + |
| **12** | 49 | M | Liver | Hepatocellular carcinoma | I | – | + |
| **13** | 42 | M | Liver | Hepatocellular carcinoma | I | – | + |
| **14** | 18 | M | Liver | Hepatocellular carcinoma | I | + | – |
| **15** | 55 | M | Liver | Hepatocellular carcinoma | I | ++ | + |
| **16** | 71 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **17** | 43 | F | Liver | Hepatocellular carcinoma | II | – | + |
| **18** | 50 | M | Liver | Hepatocellular carcinoma | I | + | + |
| **19** | 98 | F | Liver | Hepatocellular carcinoma | II | + | ++ |
| **20** | 48 | F | Liver | Hepatocellular carcinoma | III | + | ++ |
| **21** | 58 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **22** | 70 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **23** | 49 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **24** | 52 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **25** | 56 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **26** | 58 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **27** | 61 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **28** | 50 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **29** | 39 | M | Liver | Hepatocellular carcinoma | II | – | – |
| **30** | 37 | F | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **31** | 45 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **32** | 56 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **33** | 64 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **34** | 69 | F | Liver | Hepatocellular carcinoma | II | + | ++ |
| **35** | 58 | M | Liver | Hepatocellular carcinoma | II | ++ | + |
| **36** | 47 | M | Liver | Hepatocellular carcinoma | II | – | + |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **37** | 49 | F | Liver | Hepatocellular carcinoma | II | – | + |
| **38** | 48 | M | Liver | Hepatocellular carcinoma | II | + | – |
| **39** | 49 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **40** | 43 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **41** | 42 | M | Liver | Hepatocellular carcinoma | II | ++ | + |
| **42** | 35 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **43** | 50 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **44** | 40 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **45** | 33 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **46** | 57 | M | Liver | Hepatocellular carcinoma | III | ++ | + |
| **47** | 55 | M | Liver | Hepatocellular carcinoma | II | – | ++ |
| **48** | 36 | F | Liver | Hepatocellular carcinoma | II | – | + |
| **49** | 63 | M | Liver | Hepatocellular carcinoma | II | ++ | + |
| **50** | 19 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **51** | 68 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **52** | 40 | M | Liver | Hepatocellular carcinoma | II | – | – |
| **53** | 52 | F | Liver | Hepatocellular carcinoma | II | – | – |
| **54** | 38 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **55** | 48 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **56** | 53 | M | Liver | Hepatocellular carcinoma | II | + | – |
| **57** | 41 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **58** | 35 | F | Liver | Hepatocellular carcinoma | II | – | – |
| **59** | 27 | M | Liver | Hepatocellular carcinoma | III | – | ++ |
| **60** | 65 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **61** | 39 | F | Liver | Hepatocellular carcinoma | II | + | + |
| **62** | 41 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **63** | 46 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **64** | 60 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **65** | 41 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **66** | 45 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **67** | 48 | F | Liver | Hepatocellular carcinoma | II | – | + |
| **68** | 47 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **69** | 47 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **70** | 75 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **71** | 25 | M | Liver | Hepatocellular carcinoma | II | – | – |
| **72** | 51 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **73** | 55 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **74** | 65 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **75** | 45 | M | Liver | Hepatocellular carcinoma | II | – | – |
| **76** | 46 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **77** | 46 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **78** | 43 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **79** | 62 | F | Liver | Hepatocellular carcinoma | III | – | ++ |
| **80** | 35 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **81** | 47 | F | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **82** | 47 | M | Liver | Hepatocellular carcinoma | II | – | ++ |
| **83** | 67 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **84** | 63 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **85** | 45 | M | Liver | Hepatocellular carcinoma | II | ++ | + |
| **86** | 52 | F | Liver | Hepatocellular carcinoma | II | – | ++ |
| **87** | 48 | F | Liver | Hepatocellular carcinoma | II | ++ | – |
| **88** | 37 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **89** | 69 | F | Liver | Hepatocellular carcinoma | I | – | + |
| **90** | 60 | M | Liver | Hepatocellular carcinoma | II | – | ++ |
| **91** | 62 | F | Liver | Hepatocellular carcinoma | II | + | – |
| **92** | 70 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **93** | 40 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **94** | 45 | M | Liver | Hepatocellular carcinoma | II | ++ | ++ |
| **95** | 40 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **96** | 74 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **97** | 45 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **98** | 48 | F | Liver | Hepatocellular carcinoma | II | – | – |
| **99** | 40 | M | Liver | Hepatocellular carcinoma | II | ++ | + |
| **100** | 47 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **101** | 32 | M | Liver | Hepatocellular carcinoma | II | + | ++ |
| **102** | 58 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **103** | 47 | M | Liver | Hepatocellular carcinoma | II | – | – |
| **104** | 26 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **105** | 65 | F | Liver | Hepatocellular carcinoma | II | + | + |
| **106** | 52 | M | Liver | Hepatocellular carcinoma | III | + | ++ |
| **107** | 54 | M | Liver | Hepatocellular carcinoma | II | – | + |
| **108** | 48 | M | Liver | Hepatocellular carcinoma | II | + | + |
| **109** | 63 | M | Liver | Hepatocellular carcinoma | II | ++ | – |
| **110** | 63 | M | Liver | Hepatocellular carcinoma | III | – | + |
| **111** | 67 | M | Liver | Hepatocellular carcinoma | III | + | – |
| **112** | 43 | M | Liver | Hepatocellular carcinoma | III | + | ++ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **113** | 46 | M | Liver | Hepatocellular carcinoma | III | _ | + |
| **114** | 35 | M | Liver | Hepatocellular carcinoma | III | ++ | + |
| **115** | 38 | M | Liver | Hepatocellular carcinoma | III | + | ++ |
| **116** | 58 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **117** | 41 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **118** | 56 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **119** | 72 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **120** | 65 | M | Liver | Hepatocellular carcinoma | III | + | ++ |
| **121** | 56 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **122** | 38 | M | Liver | Hepatocellular carcinoma | III | ++ | ++ |
| **123** | 43 | F | Liver | Hepatocellular carcinoma | III | _ | ++ |
| **124** | 51 | M | Liver | Hepatocellular carcinoma | I | _ | _ |
| **125** | 51 | M | Liver | Hepatocellular carcinoma | III | ++ | + |
| **126** | 52 | M | Liver | Hepatocellular carcinoma | III | _ | + |
| **127** | 68 | M | Liver | Hepatocellular carcinoma | III | ++ | _ |
| **128** | 52 | M | Liver | Hepatocellular carcinoma | III | + | ++ |
| **129** | 50 | M | Liver | Hepatocellular carcinoma | III | + | _ |
| **130** | 49 | M | Liver | Hepatocellular carcinoma | III | _ | _ |
| **131** | 56 | F | Liver | Hepatocellular carcinoma | III | _ | + |
| **132** | 68 | M | Liver | Hepatocellular carcinoma | III | _ | + |
| **133** | 55 | M | Liver | Hepatocellular carcinoma | III | + | + |
| **134** | 54 | M | Liver | Hepatocellular carcinoma | III | _ | _ |
| **135** | 62 | M | Liver | Hepatocellular carcinoma | III | + | + |
| **136** | 53 | M | Liver | Hepatocellular carcinoma | III | _ | _ |
| **137** | 66 | M | Liver | Hepatocellular carcinoma | III | ++ | + |
| **138** | 56 | M | Liver | Hepatocellular carcinoma | III | ++ | + |
| **139** | 32 | F | Liver | Hepatocellular carcinoma | III | + | ++ |
| **140** | 47 | M | Liver | Hepatocellular carcinoma | III | + | ++ |
| **141** | 39 | M | Liver | Hepatocellular carcinoma | III | _ | ++ |
| **142** | 56 | F | Liver | Hepatocellular carcinoma | III | ++ | + |
| **143** | 68 | M | Liver | Hepatocellular carcinoma | III | _ | + |
| **144** | 38 | F | Liver | Normal | — | + | + |
| **145** | 60 | M | Liver | Normal | — | _ | _ |
| **146** | 46 | M | Liver | Normal | — | _ | _ |
| **147** | 63 | M | Liver | Normal | — | _ | + |
| **148** | 63 | M | Liver | Normal | — | _ | _ |

**Supporting Table 3: primers used in the paper**

| Gene | Primer | Sequence (5′-3′) |
|---|---|---|
| Primers for SPHK1 promoter | | |
| pGL3-1733 | forward | CGGGGTACCCTCGGAGGTGCAGGACCCAT |
| pGL3-1172 | forward | CGGGGTACCTGGCAACTTCTTCCTCCGTC |
| pGL3-702 | forward | CGGGGTACCCTCGAATTTCGGGTGGGCTA |
| pGL3-502 | forward | CGGGGTACCCGCGCGCCCCAGACACTGCG |
| pGL3-202 | forward | CGGGGTACCAGTGCCCTCCCCGCTCCGCG |
| | reverse | CCCAAGCTTCTACCCAGTCGGTCCGGTTT |
| pGL3-581 | forward | CGGGGTACCCTCGGAGGTGCAGGACCCAT |
| | reverse | CCCAAGCTTGACGGAGGAAGAAGTTGCCA |
| pGL3-320 | forward | CGGGGTACCCGCGCGCCCCAGACACTGCG |
| | reverse | CCCAAGCTTCGCGGAGCGGGGAGGGCACT |
| Primers for qRT-PCR | | |
| HULC | forward | ATGGGGGTGGAACTCATGATGG |
| | reverse | AAGAATGGACATCATTTTATTTCA |
| SPHK1 | forward | CTGTCACCCATGAACCTGCT |
| | reverse | TACAGGGAGGTAGGCCAGTC |
| E2F1 | forward | AAACAAGGCCCGATCGATGT |
| | reverse | GGTGGGGAAAGGCTGATGAA |
| GAPDH | forward | GGGAGCCAAAAGGGTCATCA |
| | reverse | TGATGGCATGGACTGTGGTC |
| miRNA-107 | forward | AGCAGCATTGTACAGGGCTATCA |
| | reverse | GCGAGCACAGAATTAATACGAC |
| U6 | forward | AGAGCCTGTGGTGTCCG |
| | reverse | CATCTTCAAAGCACTTCCCT |
| VEGF | forward | ACTGCCATCCAATCGAGACC |
| | reverse | CAGGGCATTAGACAGCAGCG |
| SPHK2 | forward | TCGTTCTGTGTCTGACCTGC |
| | reverse | CATGAGCACAAAGTCCCCCT |
| TGF-βR2 | forward | TAGGACTGCCCATCCACTGA |
| | reverse | GAGGCTGATGCCTGTCACTT |
| MCP1 | forward | CTCGCTCAGCCAGATGCAAT |
| | reverse | TTCTTTGGGACACTTGCTGC |
| Primers for CHIP | | |
| | forward | CAGACGCCTAGGACGAGC |
| | reverse | CCGGGGGTGGAACCTGA |
| Nucleotide sequence for EMSA (-152/-135) | | |
| | forward | CCCGGGCGGGAACCAGCT |
| | reverse | AGCTGGTTCCCGCCCGGG |
| E2F1 3'UTR | forward | GCTCTAGACGGGGAATGAAGGTGAACATAC |
| | reverse | GGGGGCCGGCCTATGGGGCAGAAGAACAGCTCA |
| E2F1 3'UTR-mut | forward | CTGGGGGTCCCACAACCGGGTCCCGTCAC GGACGTCCCAGAATCTGGTG |

| | | |
|---|---|---|
| | reverse | CACCAGATTCTGGGACGTCCGTGACGGGA CCCGGTTGTGGGACCCCCAG |
| HULC-107-mut | forward | AAATTTGTACTTTCTGGGACTTAATACAAC AAATCAAAGAAAAAAAT |
| | reverse | ATTTTTTTCTTTGATTTGTTGTATTAAGTCC CAGAAAGTACAAATTT |

**Supporting Table 4: list of siRNA and miRNA mimics**

| Gene | | Sequence (5′-3′) |
|---|---|---|
| **siRNA Duplexes** | | |
| si-HULC-1 | sense | CCUCCAGAACUGUGAUCCAdTdT |
| si-HULC-2 | sense | AGCUCUUGUCUCUUCUUCCCdTdT |
| si-E2F1-1 | sense | UGGACCACCUGAUGAAUAUdTdT |
| si-E2F1-2 | sense | UUUGUUCUCCGAAGAGUCCACdTdT |
| si-SPHK1-1 | sense | GGGCAAGGCCUUGCAGCUCUdTdT |
| si-SPHK1-2 | sense | AUACUUCUCACUCUCUAGGUCdTdT |
| Negative control | sense | UUCUUCGAAGGUGUGACGUdTdT |
| has-miR-107 | sense | AGCAGCAUUGUACAGGGCUAUCA |
| miR-107 inhibitor | sense | UGAUAGCCCUGUACAAUGCUGCU |