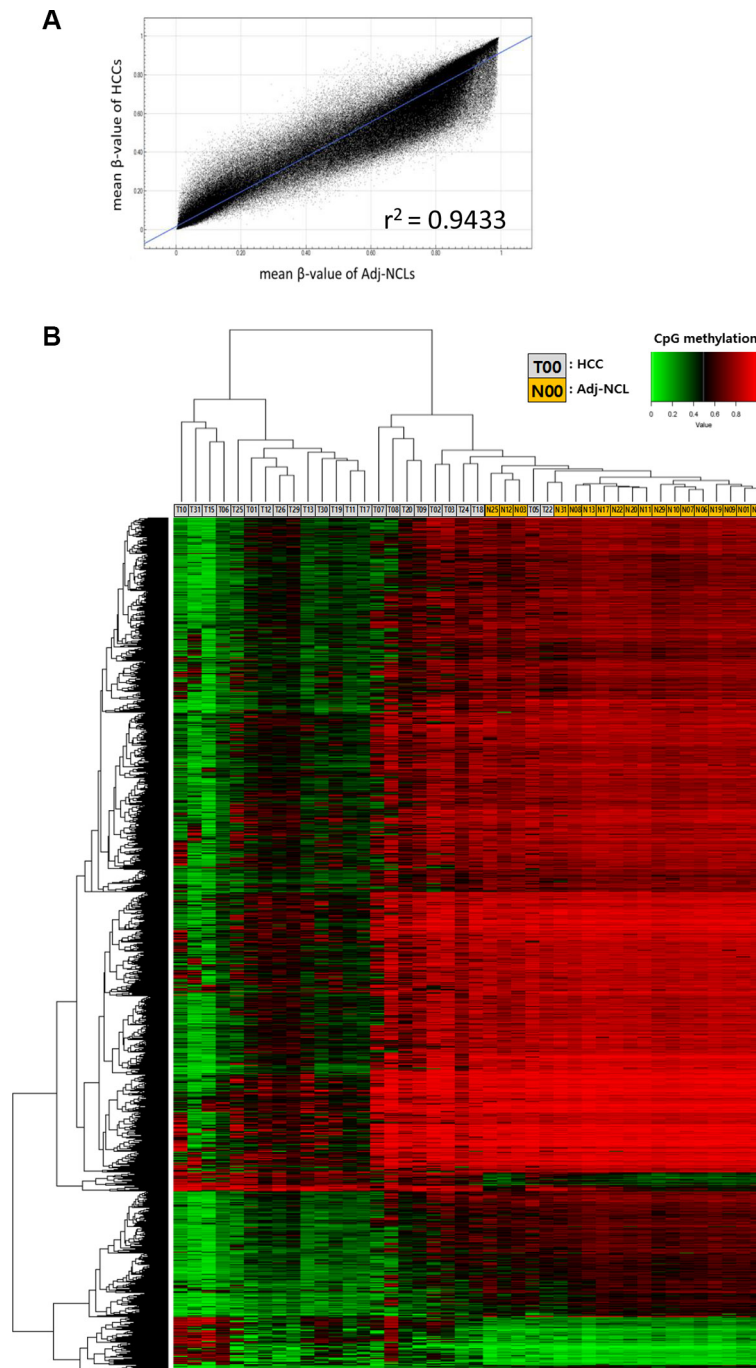
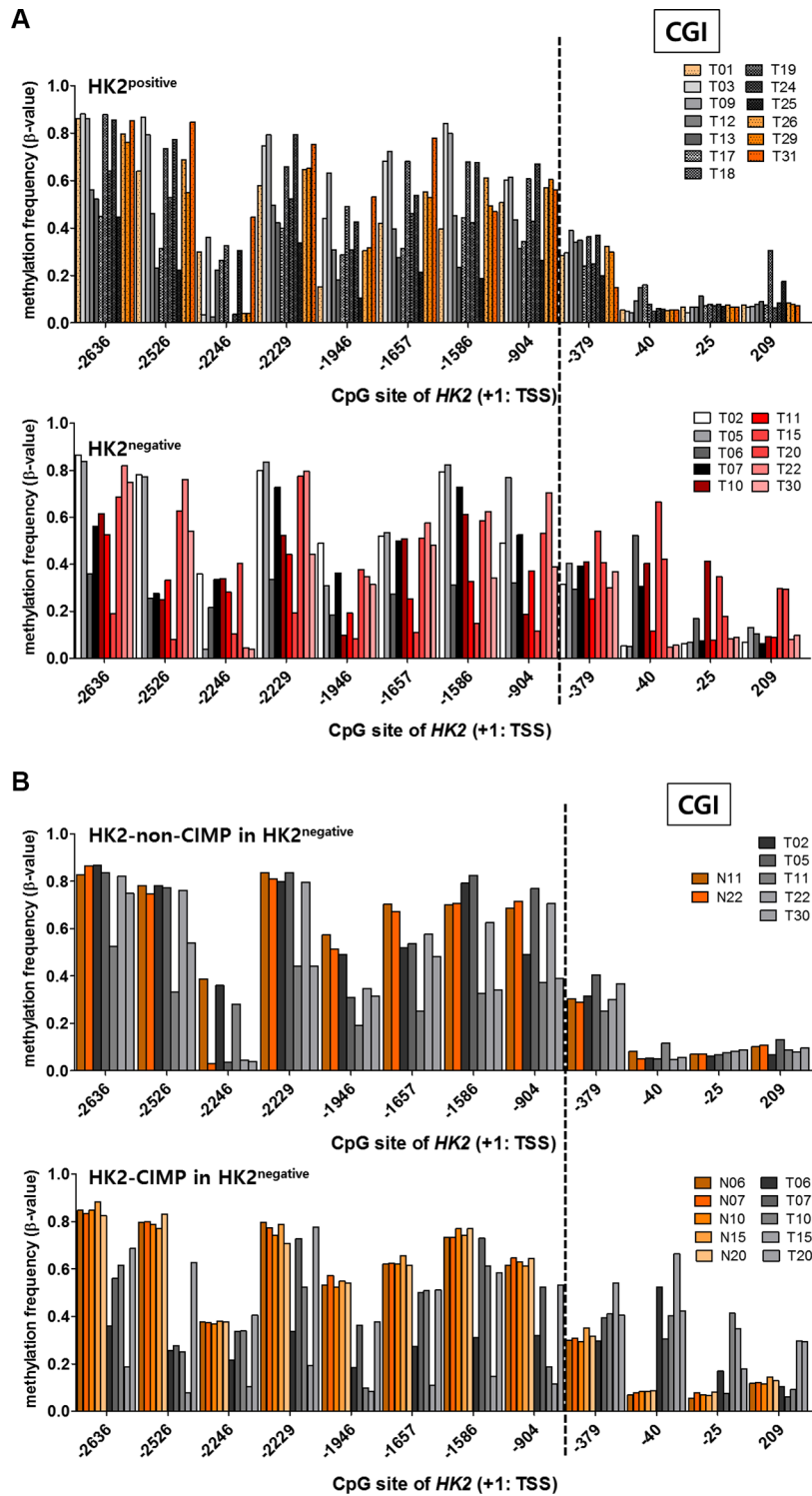


Regulation of *HK2* expression through alterations in CpG methylation of the *HK2* promoter during progression of hepatocellular carcinoma

Supplementary Materials



Supplementary Figure S1: Global hypomethylation of CpG DNAs in the HCCs. (A) The mean β -values of HCCs and Adj-NCLs were plotted. (B) Unsupervised hierarchical clustering of 24 HCCs and 18 Adj-NCLs, by samples and by CpGs showing delta mean β -values between HCC and its Adj-NCLs $> \pm 0.2$ (total 25,330 CpG sites).



Supplementary Figure S2: The methylation status of the *HK2* promoter according to *HK2* expression in HCCs. The methylation status of the *HK2* promoter CpGs was plotted according to *HK2* expression: (A) *HK2*^{positive} and *HK2*^{negative}, (B) *HK2*-CIMP HCCs (T06-T20) and *HK2*-non-CIMP HCCs (T02-T30). Their Adj-NCL tissues were co-plotted, respectively.

Supplementary Table S1: Primer sequences

	Name of Primer		sequences
ChIP	-1135F (CGI-shore)	F	5'-AAGCCTCCTCACCCACCCCA-3'
	-963R (CGI-shore)	R	5'-GGCCCTATTCGCTTGCCCA-3'
	-302F (CGI)	F	5'-ACCCATAGCCGAGCCTGAC-3'
	-114R (CGI)	R	5'-GCAGCCAATCATCGCTCAC-3'
	+480F (control)	F	5'-TCACGGAGCTCAACCATGAC-3'
	+792R (control)	R	5'-GGAAAAGGGACTGGGGGAG-3'
Bisulfite Sequencing¹	HK2-BS-F	F	5'-TTATAATAGGTAAGAATTAGGATGTTTTAG-3'
	HK2-BS-1st R	R	5'-TCATACAACAATATAACTTATTAATAAAACT-3'
	HF2-BS-2nd R	R	5'-CCTAAAATAAAAACGTATAATCCGTA-3'

¹Nested PCR was performed due to the low efficiency of PCR

Supplementary Table S2: Primer sequences used to construct luciferase reporter vectors

Name of Primer		sequences
-965F	F	5'-GCCTTAAAGTAGAGGGACAAGGG-3'
-407F	F	5'-GATCCCGAGATGCCAGACAG-3'
-337F	F	5'-GCCCCACACC CCGGGTCC-3'
-305F	F	5'-CCCACCCATAGCCGAGCCTGAC-3'
-268F	F	5'-TG CGCCTTCCGTCCAGCC-3'
-255F	F	5'-CCAGCCTT TAGCCACGGACCACA-3'
-175F	F	5'-AGGCTGCC GGCTCCGGTGTCTG-3'
+106R	R	5'-CTCTCCGATTCACAGGCGCGTGG-3'

Supplementary Table S3: siRNA sequences

Target	No. of siRNA	sequences
<i>HIF1A</i>	1	5'-GGAAGAACUAUGAACAUAAAAGUCTG-3'
	2	5'-ACAAUACCCUAUGUAGUUGUGGAAG-3'
	3	5'-CCAGCAGACUCAAUACAAGAACCT-3'