Supplementary Information for

Anti-Cancer Effects of Glaucarubinone in the Hepatocellular Carcinoma Cell Line Huh7 Via Regulation of the Epithelial-to-Mesenchymal Transition-Associated Transcription Factor Twist1

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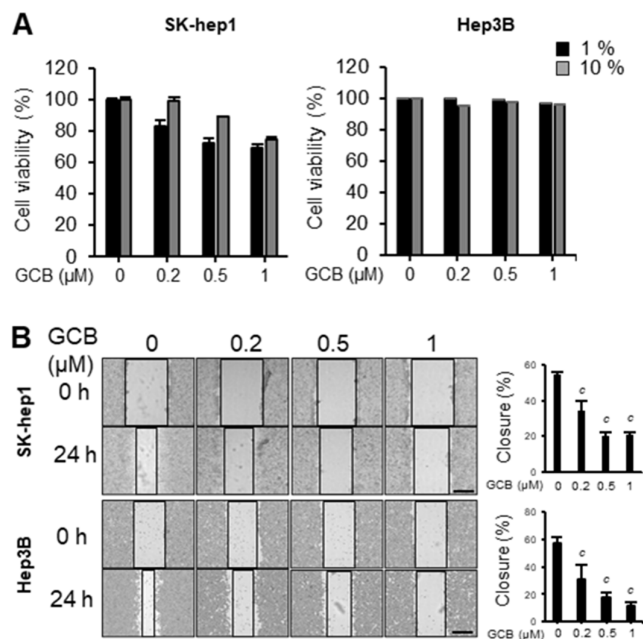
Supplementary Figure S1. Effects of GCB on other liver cancer cell lines. (A) SK-Hep1 and Hep3B cells were treated with GCB at the indicated concentration in cell culture medium containing 1% or 10% FBS for 24 h. The cell viability was measured using the EZ-CYTOX solution. The relative cell viability is shown using bar graphs in comparison with the untreated control (100%). Data are expressed as the mean ± SEM. (B) SK-Hep1 and Hep3B cells were incubated with GCB (0, 0.2, 0.5, and 1 μM). Microscopic images were taken at 24 h (scale bars, 0.5 mm). The wound closure values were quantified by measuring the percentage of wound closure in comparison with the 0 h point for each sample; the relative wound

closure is shown as a bar graph. Data were analyzed by one-way ANOVA; $^c p < 0.001$ relative to the untreated control.
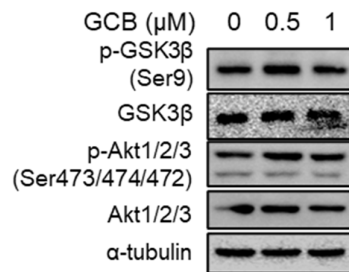
Supplementary Figure S2. Analysis of various intracellular signaling pathways. Huh7 cells treated with the indicated concentration of GCB were incubated for 24 h and then harvested. The protein samples were separated by SDS–PAGE, and the expression of other signaling pathways was evaluated using the indicated antibodies. This included p-Akt1/2/3 (Ser473/474/472), Akt1/2/3, p-GSK3β (Ser9), GSK3β, and α-tubulin. p-Akt1/2/3 is used for detection of Ser473 phosphorylated Akt1 and correspondingly Ser474 phosphorylated Akt2 and correspondingly Ser472 phosphorylated Akt3.

Supplementary Table S1. List of primers used for qRT-PCR.

Primer Name	5´ to 3´
CDH1 (F)	TACACTGCCCAGGAGCCAGA
CDH1 (R)	TGGCACCAGTGTCCGGATTA
TWIST1 (F)	GGCTCAGCTACGCCTTCTC
TWIST1 (R)	CTCCTTCTCTGGAAACAATGACAT
MMP2 (F)	AGCGAGTGGATGCCGCCTTTAA
MMP2 (R)	CATTCCAGGCATCTGCGATGAG
MMP9 (F)	GCCACTACTGTGCCTTTGAGTC
MMP9 (R)	CCCTCAGAGAATCGCCAGTACT
GAPDH (F)	GCTCTCTGCTCCTCCTGTTC
GAPDH (R)	ACGACCAAATCCGTTGACTC