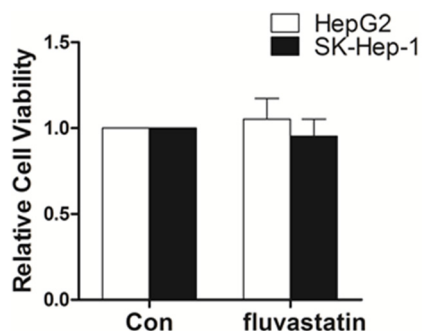
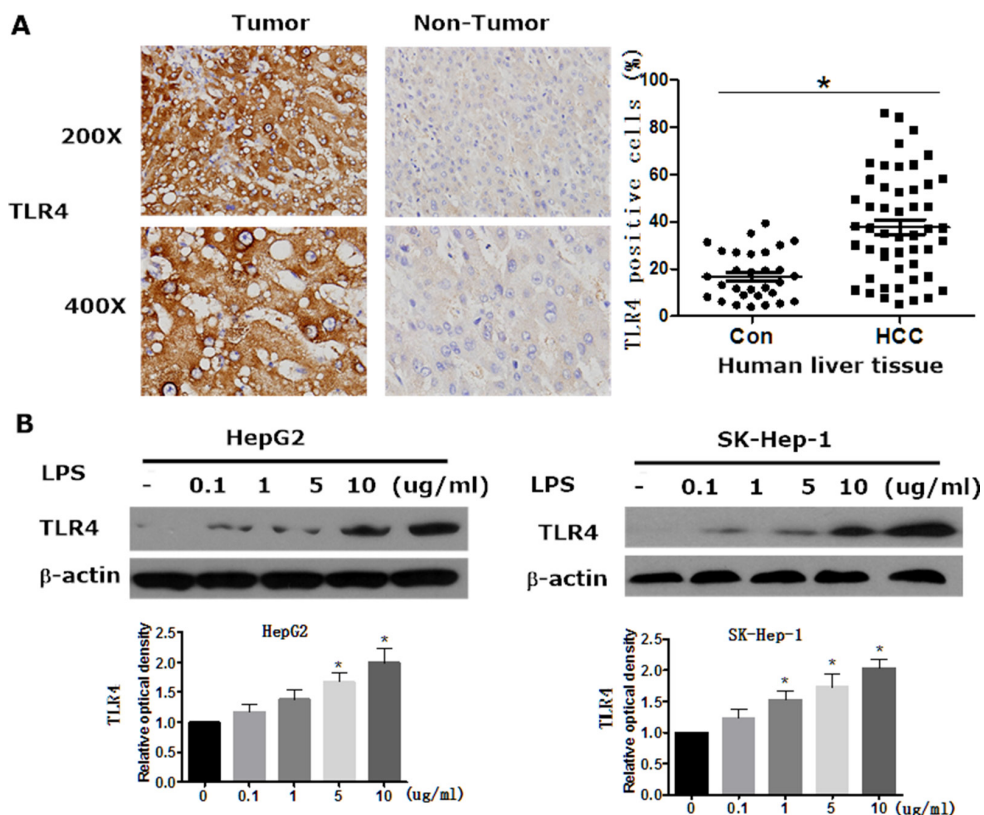


Synergistic anti-tumor efficacy of sorafenib and fluvastatin in hepatocellular carcinoma

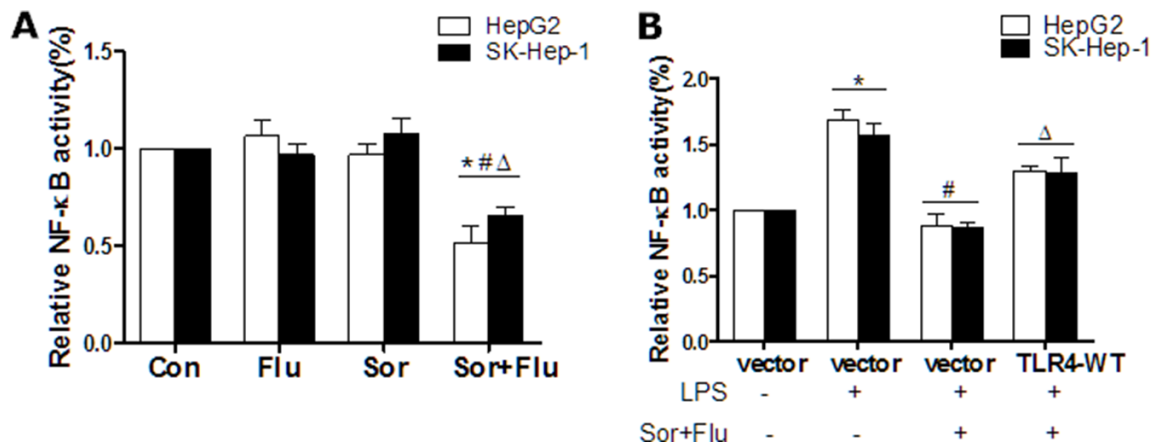
Supplementary Materials



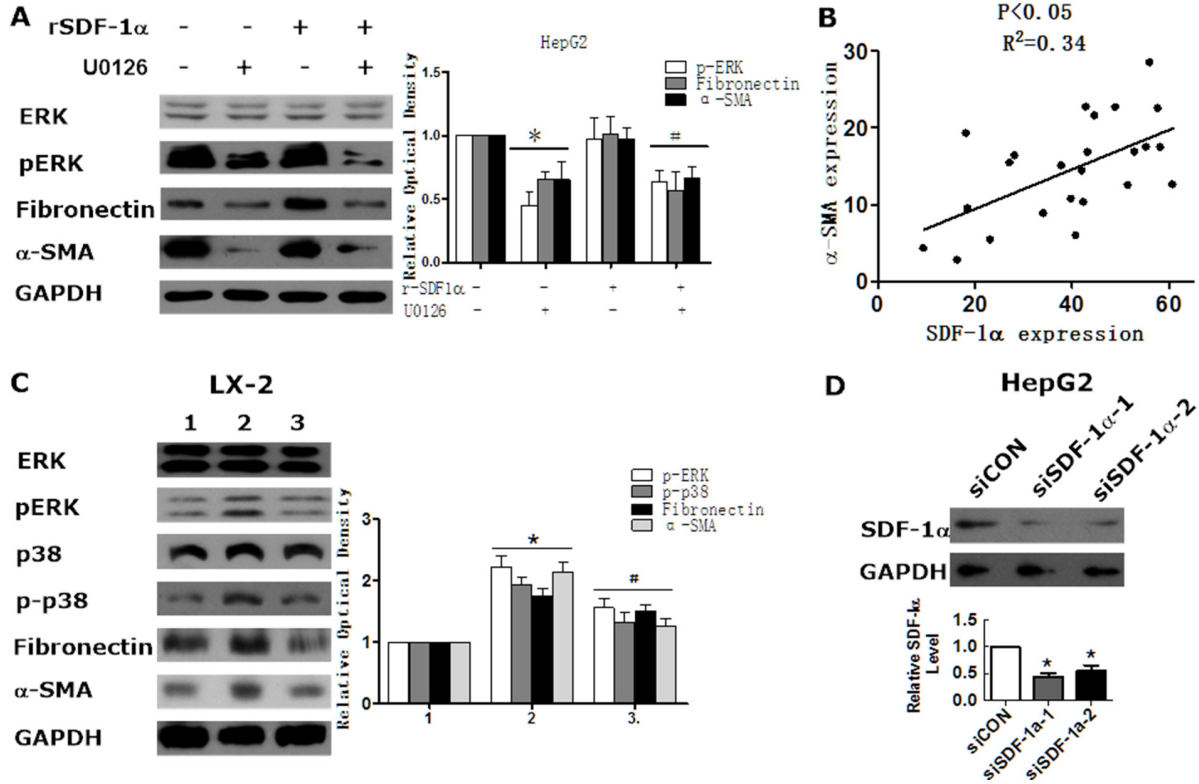
Supplementary Figure 1: HepG2 and SK-Hep-1 cells were incubated with 1 μ M fluvastatin or vehicle for 72 h. Cell viability was evaluated by CCK8 assays. * $P < 0.05$ vs. control group. Data are expressed as the mean \pm SEM of three independent experiments.



Supplementary Figure 2: (A) IHC staining of TLR4 in human HCC tissues and normal liver tissues. * $P < 0.05$ vs. non-tumor samples. (B) The serum-starved HepG2 and SK-Hep-1 cell lines were treated with LPS (0, 0.1, 1, or 10 μ g/ml) for 24 h. The protein level of TLR4 was determined by western blot analysis. Data are expressed as the mean \pm SEM of three independent experiments. * $P < 0.05$ vs control.



Supplementary Figure 3: (A) HepG2 and SK-Hep-1 cells were pretreated with sorafenib (1 μ M), fluvastatin (1 μ M) or both drugs for 24 h. The protein level of p65 in the nucleus was determined by ELISA. * P < 0.05 vs. Con, # P < 0.05 vs. Flu and ΔP < 0.05 vs. Sor. (B) HepG2 and SK-Hep-1 cells were transfected with control vector or TLR4-WT. Twenty-four hours after transfection, HCC cells were pre-treated with DMSO or sorafenib (1 μ M) plus fluvastatin (1 μ M) for 30 min and then induced with 10 μ g/mL LPS for 24 h. Changes in p65 activity were determined by ELISA. Data are expressed as the mean \pm SEM of three independent experiments. * P < 0.05 vs. control, # P < 0.05 vs. LPS alone and ΔP < 0.05 vs. LPS+Sor+Flu.



Supplementary Figure 4: (A) LX-2 cells were cultured with U0126 (2 μ M) or rSDF1- α (40 ng/ml) as indicated for 30 min and 24 h. The expressions of p-ERK, p-p38, fibronectin and α -SMA were measured by western blotting. * P < 0.05 vs. control, # P < 0.05 vs. rSDF1- α treated group. (B) The correlation between SDF-1 α and PCNA expression in liver tumor tissues of HCC rats was determined by Pearson's χ^2 test. (C) LX-2 cells were treated with control medium (lane 1), culture supernatant of HepG2 cells (lane 2), or supernatant of HepG2 cells plus neutralizing antibody of SDF-1 α (lane 3) for 30 min or 24 h. Western blot analysis was performed to detect the expression of p-ERK, p-p38 (30 min), ERK, p38, fibronectin and α -SMA (24 h). * P < 0.05 compared to lane 1, # P < 0.05 vs. lane 2. (D) HepG2 cells were transfected with control siRNA, SDF-1 α siRNA-1 and SDF-1 α siRNA-2. The protein level of SDF-1 α was detected by Western blotting. * P < 0.05 vs. siCON. Data are expressed as the mean \pm SEM of three independent experiments.

Supplementary Table 1: Sequence of primers for qRT-PCR

Gene	Forward primer	Reverse primer
<i>Tlr4</i>	5'- TGTATCGGTGGTCAGTGTGC -3'	5'-CAGCTCGTTTCTCACCCAGT -3'
<i>Gapdh</i>	5'-TGTTTCGTCATGGGTGTGAAC-3'	5'-ATGGCATGGACTGTGGTCAT-3'