Supporting Information

A Combined Antitumor Strategy Mediated by a New Targeted Nanosystem to Hepatocellular Carcinoma

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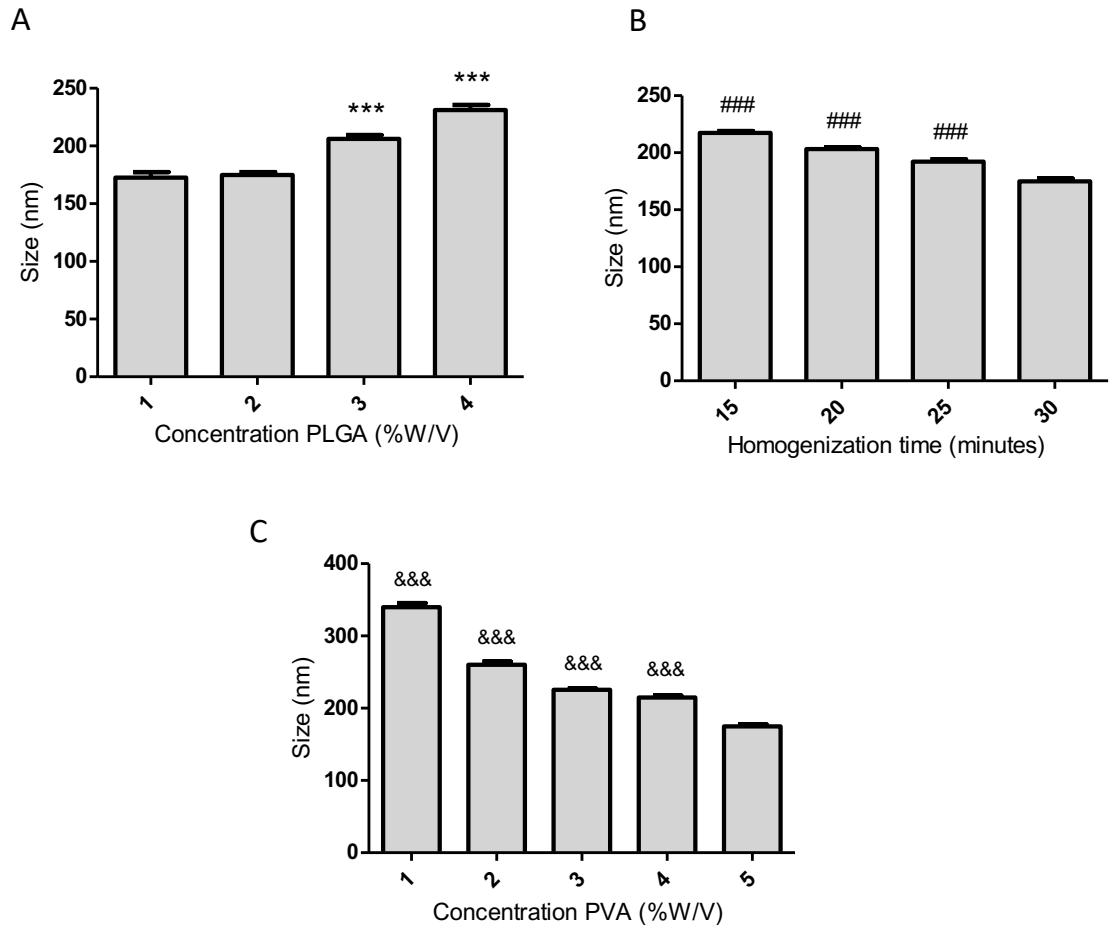

Figure S1 Size optimization of PLGA nanoparticles. (A) Effect of PLGA polymer concentration on the size of nanoparticles, maintaining the PVA concentration at 5 % and homogenization time of 30 minutes. (B) Effect of homogenization time on the size of nanoparticles, maintaining the concentration of PLGA at 2 % and PVA at 5 %. (C) Effect of surfactant concentration on the size of nanoparticles, maintaining the PLGA concentration at 2 % and the homogenization time of 30 minutes. Data are expressed as mean ± SD obtained from four independent samples (n= 4). (***) (P< 0.001) Denotes statistically significant difference when compared to PLGA nanoparticles produced with 2 % of PLGA. (###) (P< 0.001) Denotes statistically significant difference when compared to PLGA nanoparticles produced after 30 minutes of homogenization. (&&&) (P< 0.001) Denotes statistically significant difference when compared to PLGA nanoparticles produced with 5 % of PVA.

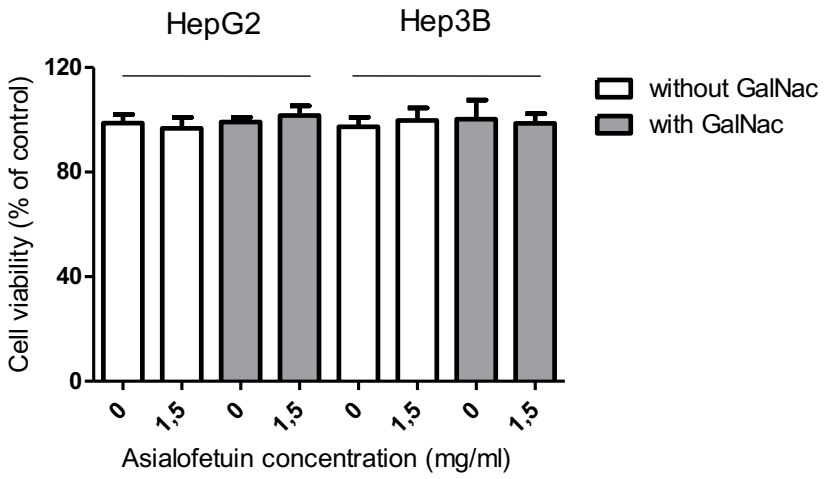

Figure S2 Evaluation of cytotoxicity of hybrid nanosystems in HepG2 and Hep3B cells in the presence or absence of asialofetuin. Cells were treated with 0.5 µg/ml of HNP, loaded with coumarin-6 and nile red and prepared with or without the GalNAc cluster, in the presence or absence of 1.5 mg/ml asialofetuin for 4 hours. Cell viability is expressed as a percentage of untreated control cells and is the mean ± SD obtained from triplicates of three independent experiments (n=3)

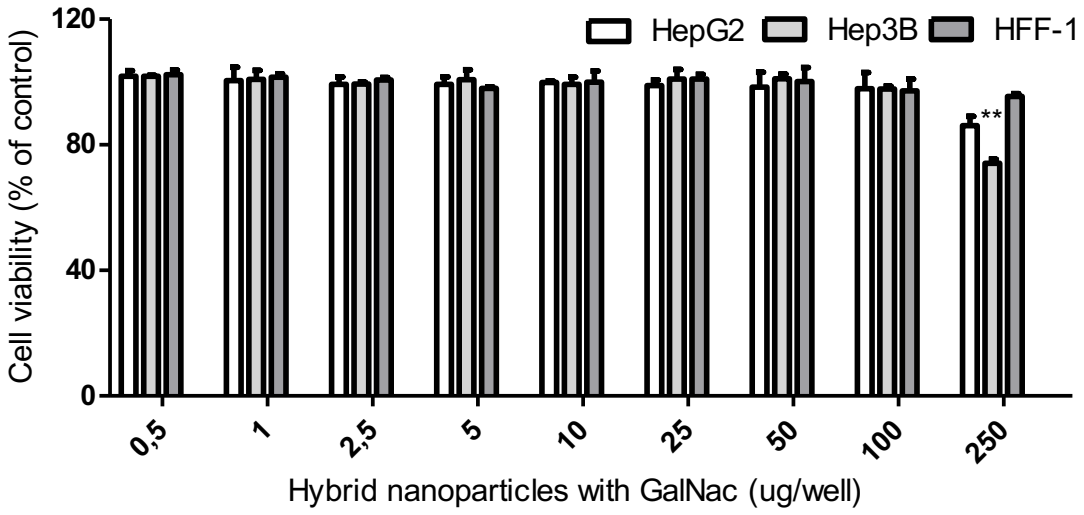

Figure S3 Cytotoxicity assessment of empty hybrid nanosystems in different cell lines. HepG2, Hep3B and HFF-1 cells were treated with different amounts of HNP (0–250 µg/well) for 72 hours. Data are expressed as mean ± SD obtained from triplicates of three independent

experiments (n=3). (**) (P< 0.01) Denotes statistically significant difference in the cell viability when compared with HFF-1 cell line treated with the same amount (µg/well) of HNP.

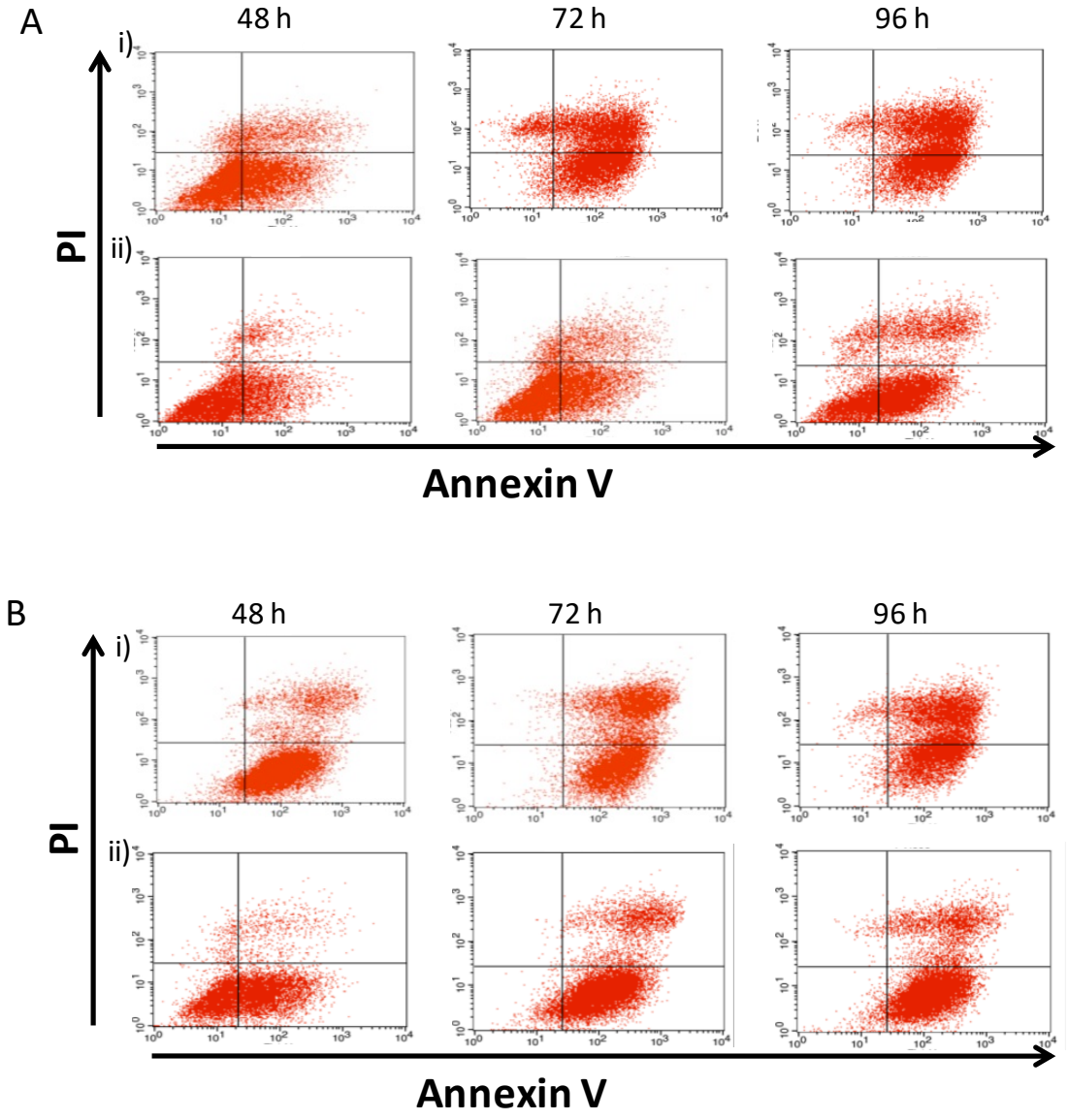

Figure S4 Apoptosis levels promoted by the therapeutic strategies in HCC cells. HepG2 (A) and Hep3B (B) cells were stained with Annexin V and PI double-staining for 15 min after being treated, for 48, 72 and 96 hours, with: (i) HNP containing sorafenib and selumetinib; or (ii) the combination of both free drugs.

Table S1 - Optimization of conjugation conditions of GalNAc cluster to hybrid nanosystems.

Optimization of Conjugation Conditions			
DSPE-PEG Concentration	**% acetone**	**Temperature (°C)**	**Conversion (%)**
1.1mM	50%	25	---
1.1mM	30%	25	93%
1.1mM	30%	35	90%
1.1mM	20%	25	30%

DSPE-PEG(2000)-amine dissolved in 0.1 M sodium tetraborate, pH 8.5, and 3 equiv of GalNAc PFP ester dissolved in acetone were mixed for overnight.