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Supplemental Information

An Efficient Bivalent Cyclic RGD-PIK3CB siRNA

Conjugate for Specific Targeted Therapy

against Glioblastoma *In Vitro* and *In Vivo*

Bohong Cen, Yuanyi Wei, Wen Huang, Muzhou Teng, Shuai He, Jianlong Li, Wei Wang, Guolin He, Xin Bai, Xiaoxia Liu, Yawei Yuan, Xinghua Pan, and Aimin Ji

Supplemental figures and legends

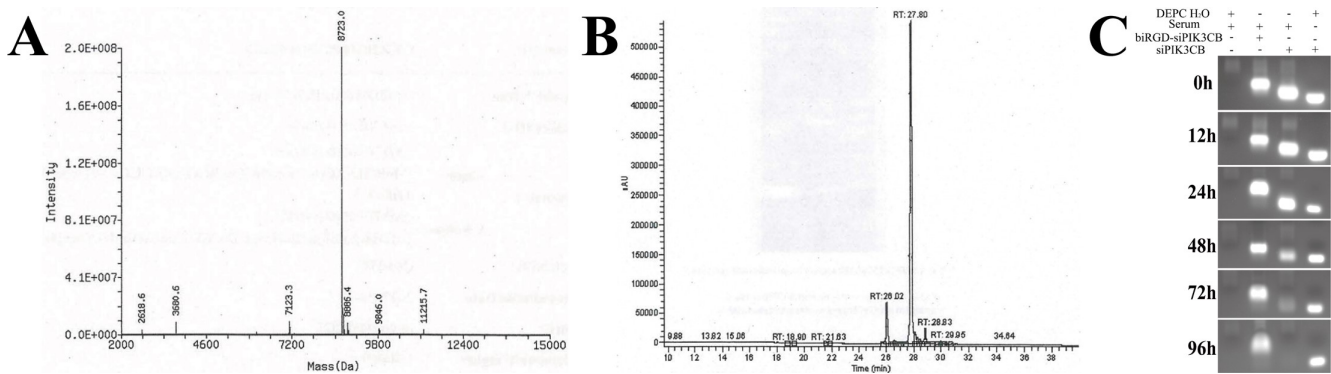


Figure S1. The characterization and nuclease resistance of biRGD-siPIK3CB conjugate. **(A)** The identification of biRGD-conjugated single-stranded PIK3CB RNA (biRGD-SS-PIK3CB-RNA) by HPLC-MS. The measured mass of sense strand conjugated with biRGD was 8723.0 Da (predicted mass was 8723.7 Da). **(B)** The analysis of purified biRGD-SS-PIK3CB-RNA by RP-HPLC. The purity of biRGD-SS-PIK3CB-RNA calculated by peak area normalization method was 90.59%. **(C)** The serum stability of biRGD-siPIK3CB. 20 μ M biRGD-siPIK3CB or siPIK3CB was mixed with 5 μ L of mouse serum and incubated at 37°C for 0, 12, 24, 48, 72 and 96 h, and then analyzed using agarose (1.2%) gel electrophoresis. siPIK3CB mixed with DEPC water was used as a marker.

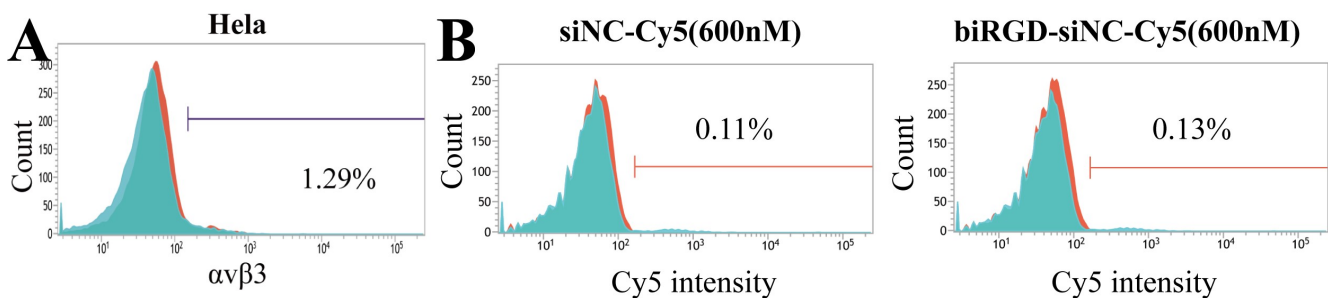


Figure S2. The expression of integrin α 5 β 3 receptors and the uptake of biRGD-siNC-Cy5 in HeLa cells. **(A)** Analysis of expression of α 5 β 3 receptors on HeLa cells by flow cytometry. **(B)** Cellular uptake levels of biRGD-siNC-Cy5 and siNC-Cy5 in HeLa cells incubated in Opti-MEM for 12 h at 37°C were measured by flow cytometry.

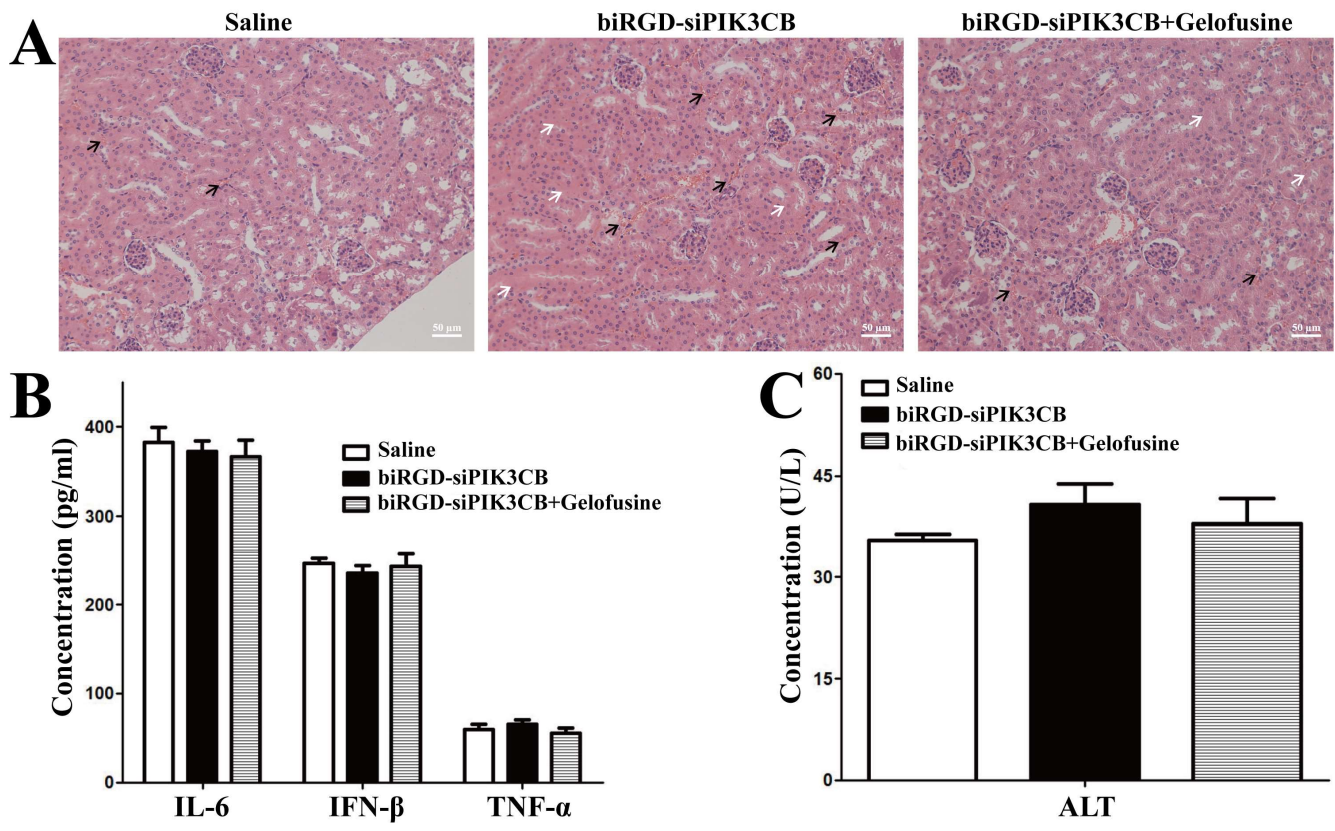


Figure S3. Immunogenicity and toxicity of biRGD-siPIK3CB. **(A)** Analysis of H&E stained sections of kidneys from in vivo anti-tumor assays. Black arrows indicate interstitial hyperemia and white arrows indicate renal edema. **(B)** Analysis of IL-6, IFN- β and TNF- α levels in the serum from Balb/c mice at 6 hours after the single injection of saline, biRGD-siPIK3CB (5 nmol/20g), or biRGD-siPIK3CB (5 nmol/20g) mixed with Gelofusine (4 mg/20 g) detected by ELISA kits. **(C)** Analysis of ALT (alanine aminotransferase) levels in the serum from in vivo anti-tumor assays. Bar=50 μ m.