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Supplemental Information

siRNA Knockdown of RRM2 Effectively Suppressed Pancreatic Tumor Growth Alone or Synergistically with Doxorubicin

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RNase resistance assay

In order to evaluate the stability of unmodified and modified siRNA, an RNase resistance assay was performed. Unmodified siRNA-04 and modified siRNA-04M were incubated in 90% FBS (v/v, in 1×PBS) at 37°C. Samples were collected and immediately frozen at -20 °C at 0 h, 2 h, 4 h, 8 h, 24 h, 48 h and 72 h post incubation. Then all samples were diluted 2 times with DEPC water, and mixed with loading buffer, followed by separating in native 20% PAGE (polyacrylamide gel electrophoresis) for 120 min at constant voltage of 150 V. Finally, gels were stained with Sybr Gold for 20 min, exposed by Vtlber Lourmat imaging system (Frence).

***In vivo* toxicity evaluation**

Male CD-1 mice 6-8 weeks old, were used to evaluate the toxicity of siRNA in vivo. Animals were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Lipopolysaccharides (LPS) and siRNA was dosed at 5 mg/kg *via* intraperitoneal (i.p.) and intravenous (i.v.) injection, respectively. 1×PBS was also administered into the mice via i.v. injection. Ten animals were employed for each kind of treatment. Body weights of animals were recorded at 3 hours, 24 hours and 48 hours post injection, followed by collecting blood samples from the fundus venous plexus. Then animals were sacrificed *via* cervical dislocation, the main organs were isolated, and the weights of the liver and spleen were recorded. Serum specimens were prepared by centrifugation at 3000 rpm at room temperature, and sent to Beijing DIAN Clinical Laboratory Co. Ltd., Beijing, China (a subsidiary of Zhejiang DIAN Diagnostics Co., Ltd. China). Concentrations of AST (aspartate aminotransferase), ALT (alanine transaminase), CREA (Creatinine), UREA, TP (total protein), and TG (triglyceride) in serum were measured using biochemical analyzer. Meanwhile, the levels of several cytokines, including TNF-α (tumor necrosis factor alpha), IFN-γ (interferon gamma), IL-6 (interleukin 6), KC (keratinocyte-derived cytokine, or CXCL1, chemokine (C-X-C motif) ligand 1), MCP-1 (monocyte chemoattractant protein-1, or CCL2, chemokine (C-C motif) ligand 2), GM-CSF (granulocyte-macrophage colony-stimulating factor

(GM-CSF), or CSF2, colony stimulating factor 2), IL-12p70, IL-1β and IL-2 were measured using Luminex-detection technology (Beijing 4A Biotech Co., Ltd) according to the manufacturer's protocol. The organ coefficients of liver and spleen were also calculated by dividing the weight of liver or spleen to the weight of body.

In another assay, siRNA was dosed at 10 mg/kg. Serum samples were acquired at 24 hours post treatment. The concentrations of IL-6, TNF-α, ALT, AST, TP and LDH (lactate dehydrogenase) were determined with above-mentioned methods.

Figure S1. Stability of unmodified and modifies siRNA-04 in serum.

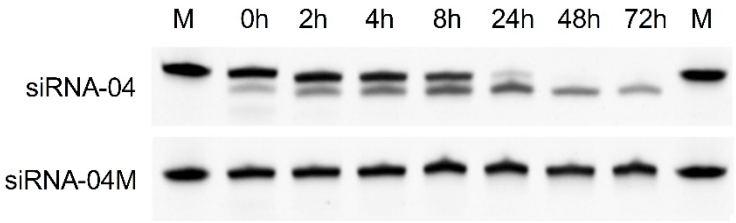

Figure S2. Cytotoxicity of siRNA-04M (siRRM2) in PNAC-1 cell. Data were shown as mean ± S.D.

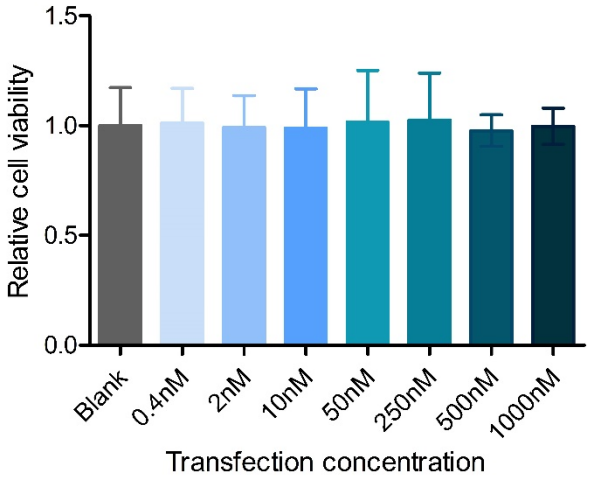

Figure S3. Cytokine inducement of siRNA *in vivo*. siRNA was dosed at 5 mg/kg *via* intravenous injection. Lipopolysaccharides was included as a positive control, which was also dosed at 5 mg/kg *via* intraperitoneal injection. Data were shown as mean ± S.D.

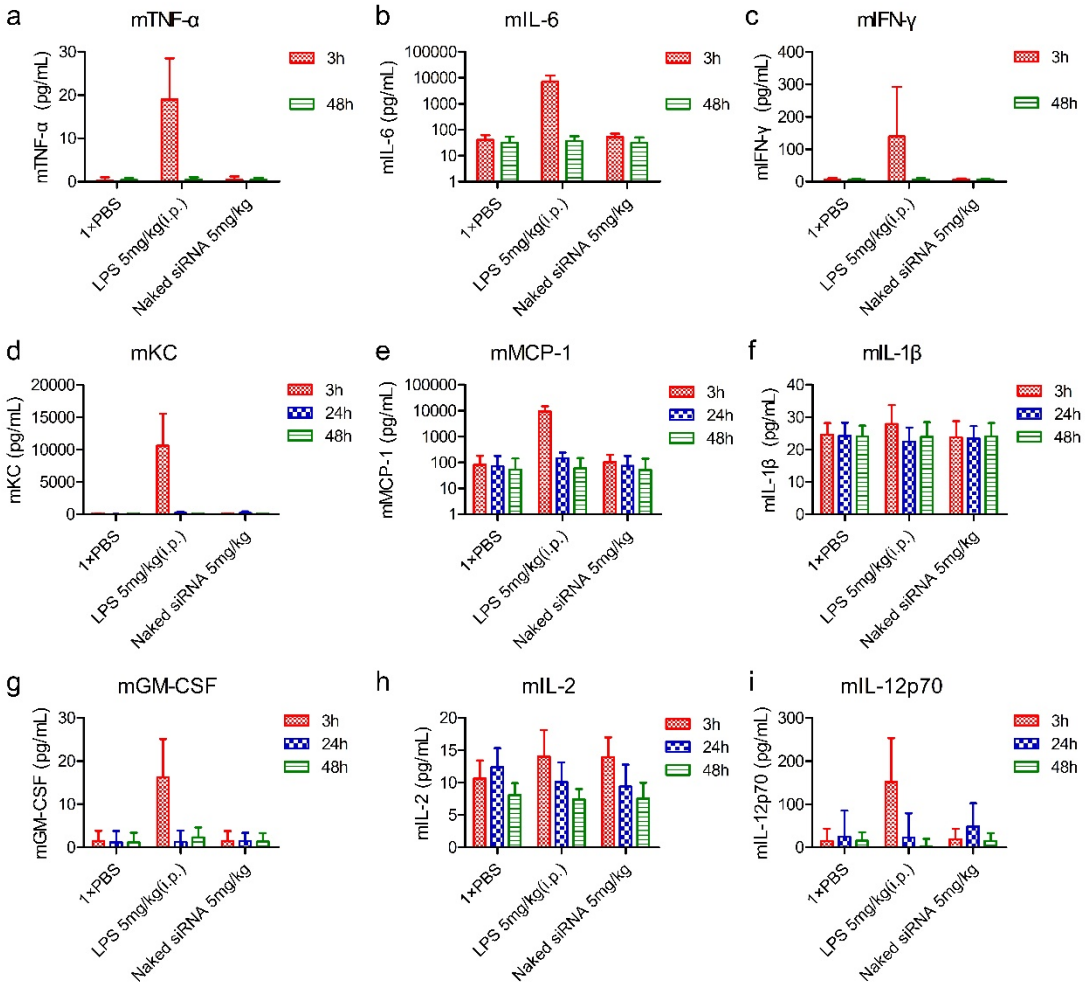

Figure S4. Serum biochemistry analysis of siRNA-treated mice *in vivo*. siRNA was dosed at 5 mg/kg *via* intravenous injection. Lipopolysaccharides was included as a positive control, which was also dosed at 5 mg/kg *via* intraperitoneal injection. Data were shown as mean ± S.D.

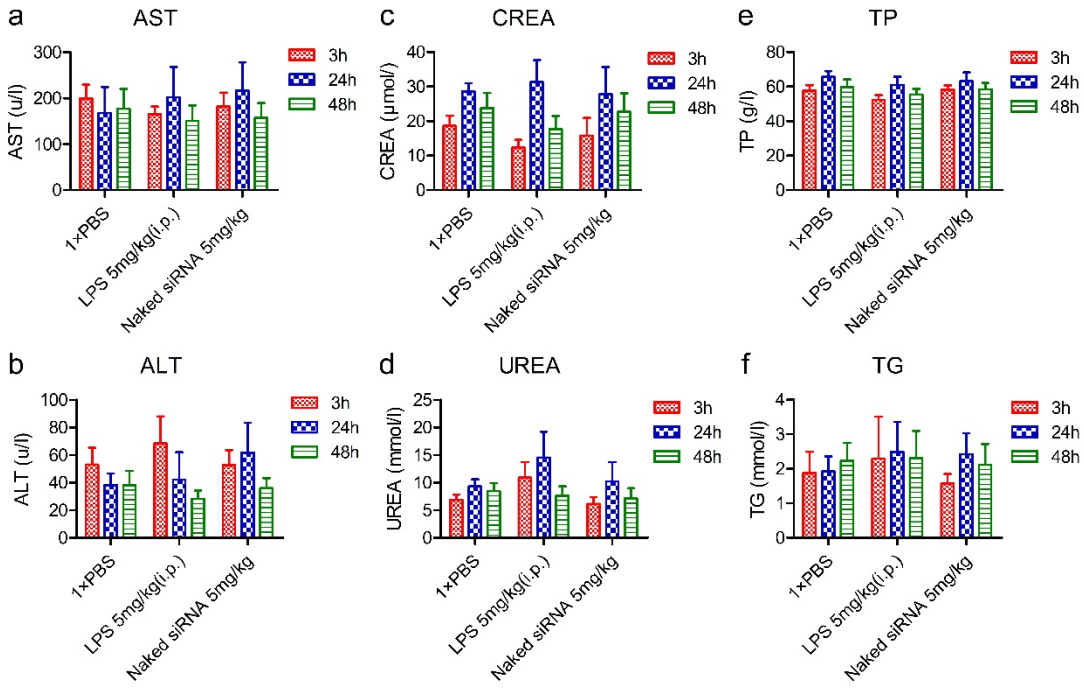

Figure S5. Organ coefficients of the liver and spleen of mice treated with 5 mg/kg of siRNA or LPS. Data were shown as mean ± S.D. ***, *p* value < 0.001.

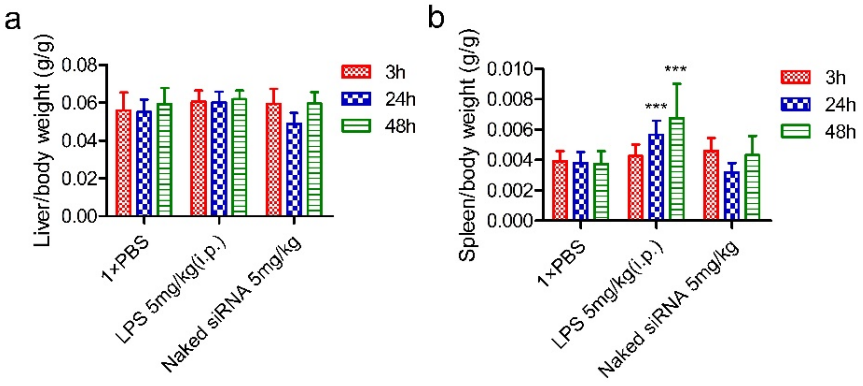

Figure S6. Cytokine inducement and serum biochemistry analysis of the mice treated with siRNA at a higher dose of 10 mg/kg. Data were shown as mean ± S.D.