

Supplementary Figure S1

Folic acid facilitated the transfection of mIP-10 plasmid

Hepa1-6 cells were transfected with FA-chitosan/mIP-10. The culture medium was changed 8 h after transfection and 48 h later EGFP was observed under a fluorescence microscope. EGFP expression of FA-chitosan/mIP-10 group was significantly higher than that of chitosan/mIP-10 group and naked plasmid mIP-10 group. Green represented EGFP-positive cells. (A) The naked plasmid mIP-10; (B) chitosan/mIP-10; (C) FA-chitosan/mIP-10; (D) After blocking with folic acid, EGFP expression in FA-chitosan/mIP-10 group decreased. Original magnification, ×400.



Supplementary Figure S2

Combination of FA-chitosan/mIP-10 and FC vaccine increases IP-10 expression in the tumor tissues: (A) PBS; (B) FC; (C) FA-chitosan/CP; (D) FA-chitosan/mIP-10; (E) FA-chitosan/mIP-10 + FC; (F) Quantitative analysis. IP-10 protein was expressed in the tumor site (brown). Five sections of tumors were taken from each group and calculated as mean optical density. The experiment was repeated three times. * P < 0.05; *** P < 0.001.



Supplementary Figure S3

Treatment with FA-chitosan/mIP-10 and FC vaccine decreases the frequency of CD11b⁺LY6G⁺ granulocytic cells in splenocytes, bone marrow cells from different groups of tumor-bearing mice. The frequency of CD11b⁺LY6G⁺ granulocytic cells was determined by flow cytometry. Data are presented as representative images or expressed as mean \pm SD of each group (n = 5) of cells from three separate experiments. (A-B) The frequency of CD11b⁺LY6G⁺ granulocytes in splenocytes were determined by flow cytometry. (C-D) The frequency of CD11b⁺LY6G⁺ granulocytes in bone marrow cells were determined by flow cytometry. * P < 0.05; ** P < 0.01; *** P < 0.001.