Supporting Information for

Enhanced antitumor immunity by targeting dendritic cells with tumor cell lysate-loaded chitosan nanoparticles vaccine

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Experimental section

Preparation of FITC labeled tumor cell lysates (TCL-FITC)

Two mg of FITC in 1 mL of 20 mmol/L carbonate buffer (pH 9.5) added to a solution of TCL (1 mg/mL, 10 mL). The solution was incubated with continuous stirring at 4 ^{o}C for 18 h in the dark. The reaction mixture was dialyzed against distilled water (MWCO 1000) to obtain TCL-FITC.

Preparation and characterization of Man-CTS-TCL-FITC NPs

Three mL of TCL-FITC solution was added drop-by-drop into chitosan solution (1 mg/mL, 1% acetic acid) and mixed at 1:1 (w/w). The mixture was then agitated at 300 rpm for 30 min to obtain TCL-FITC loaded CTS nanoparticles (CTS-TCL-FITC NPs), which were collected by centrifugation and dissolved in PBS for experimental use. The Man-ALG solution (1 mg/mL) was added drop-by-drop into CTS-TCL-FITC NPs suspension to obtain mannose decorated CTS-TCL-FITC NPs (Man-CTS-TCL-FITC NPs) through electrostatic interaction. Man-CTS-TCL-FITC NPs were collected by centrifugation and suspended in PBS (pH 7.4) for further use.

Preparation of Cy7 labeled tumor cell lysates (TCL-Cy7)

Two mg of Cy7-NHS in 1 mL of 20 mmol/L carbonate buffer (pH 9.5) was added to a solution of TCL (1mg/mL, 10 mL). The solution was incubated with continuous stirring at 4 ^{o}C for 18 h in the dark. The reaction mixture was dialyzed against distilled water (MWCO 1000) to obtain TCL-Cy7.

Preparation and characterization of Man-CTS-TCL-Cy7 NPs

Three mL of TCL-Cy7 solution was added drop-by-drop into chitosan solution (1 mg/mL, 1% acetic acid) and mixed at 1:1 (w/w). The mixture was then agitated at 300 rpm for 30 min to obtain TCL-Cy7 loaded CTS nanoparticles (CTS-TCL-Cy7 NPs), which were collected by centrifugation and dissolved in PBS for experimental use. The Man-ALG solution (1 mg/mL) was added drop-by-drop into CTS-TCL-Cy7 NPs suspension to obtain mannose decorated CTS-TCL-Cy7 NPs (Man-CTS-TCL-Cy7 NPs) through electrostatic interaction. Man-CTS-TCL-Cy7 NPs were collected by centrifugation and suspended in PBS (pH 7.4) for further use.

Results section

Table S1. Characterization of nanoparticles (n=3).

Sample	Size (nm)	DPI	Zeta potentials (mV)
CTS-TCL NPs	127.46±6.73	0.114±0.031	-14.07±1.22
Man-CTS-TCL NPs	120.15±9.93	0.121±0.049	-12.07±1.36
CTS-TCL-FITC NPs	134.72±2.65	0.103±0.051	-15.41±1.25
Man-CTS-TCL-FITC NPs	136.18±7.63	0.131±0.047	-14.58±1.19
CTS-TCL-Cy7 NPs	139.16±10.03	0.119±0.039	-15.46±1.52
Man-CTS-TCL-Cy7 NPs	135.48±8.97	0.124±0.041	-14.92±1.43

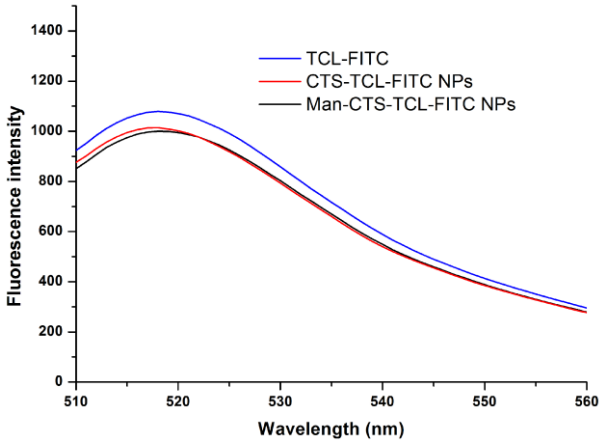

Figure S1The fluorescence spectra of TCL-FITC, CTS-TCL-FITC NPs and Man-CTS-TCL-FITC NPs in PBS, the excitation wavelength is 495 nm.

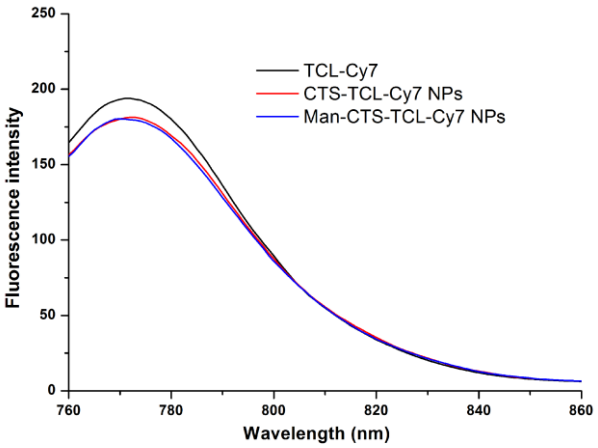

Figure S2 The fluorescence spectra of TCL-Cy7, CTS-TCL-Cy7 NPs and Man-CTS-TCL-Cy7 NPs in PBS, the excitation wavelength is 743 nm.

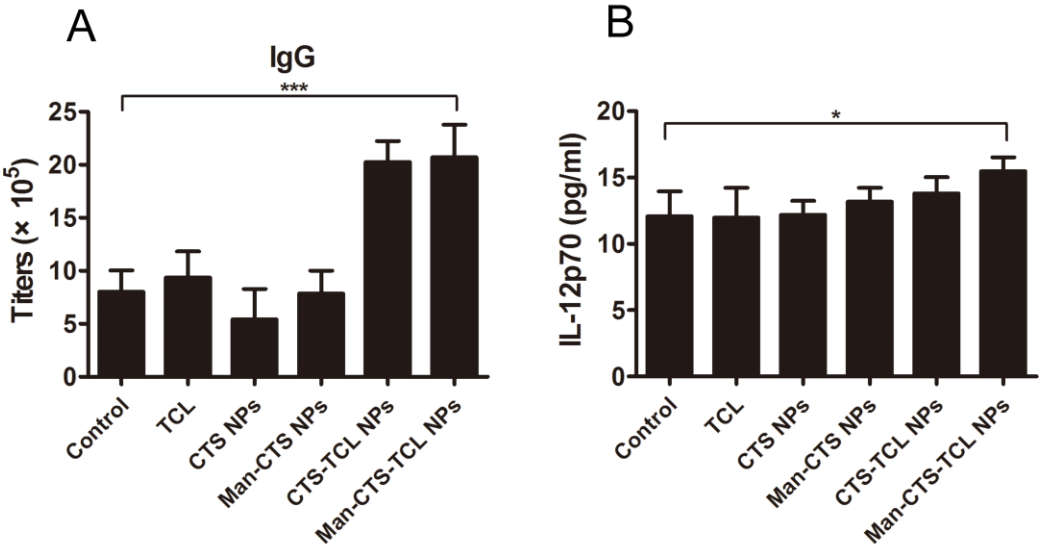

Figure S3 Immunization with Man-CTS-TCL NPs produces both cellular and humoral immune responses in mice. (A) Female C57BL/6 mice (n=6) 6-8 weeks old were subcutaneously immunized with PBS, TCL, CTS NPs, Man-CTS NPs, CTS-TCL NPs and Man-CTS-TCL NPs at days -14, -13, and -7. At day 0, serum of mice was collection and assayed for tumor specific IgG antibody (humoral immune response) by ELISA. (B) Serum of mice was used for measuring IL-12p70 (cellular immune response) by ELISA. Data are representative of three independent experiments. Bars shown are mean ± SD (n=6), and differences between PBS control

group and other groups are determined using one-way ANOVA analysis and Student's t test. Relative to control group: *P<0.05, **P<0.01 and ***P<0.001.

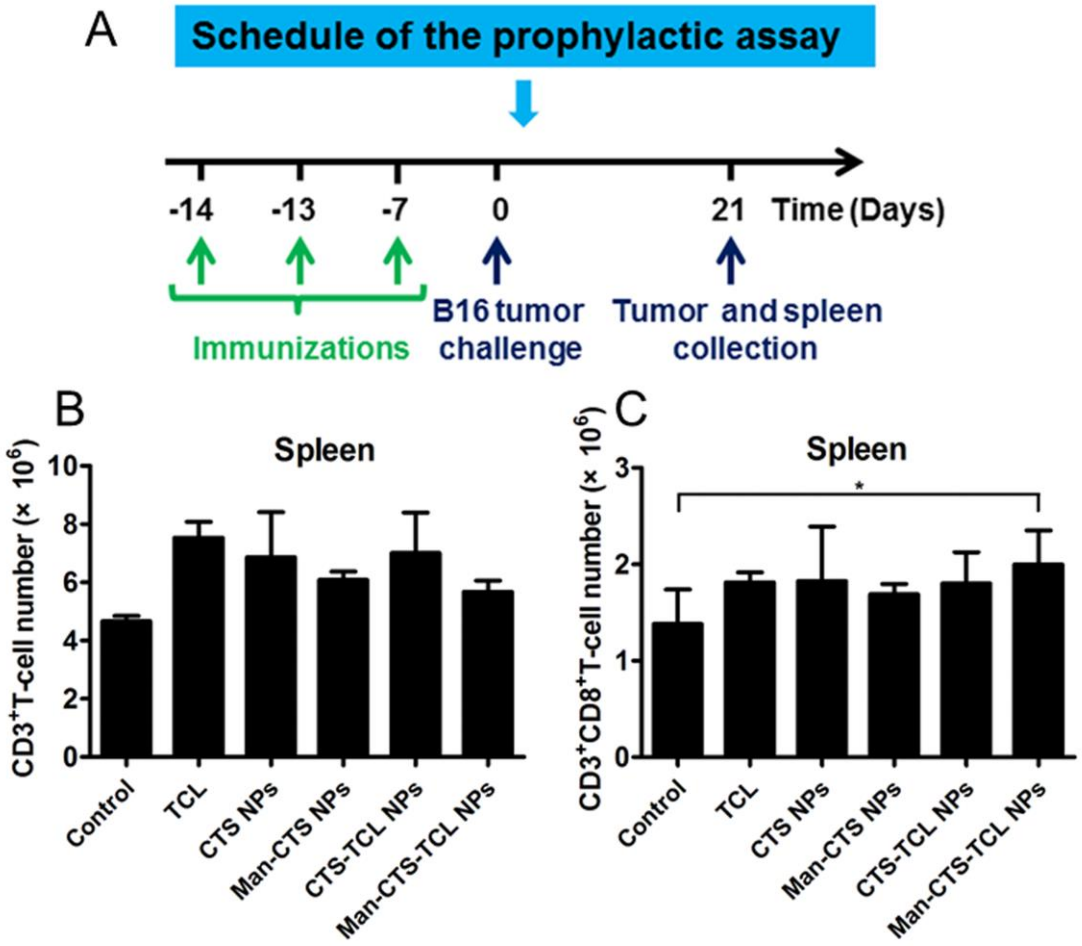

Figure S4 Immunization of mice with Man-CTS-TCL NPs enhances the absolute number of $CD3^+CD8^+$ T cells in mice spleen. (A) Schedule used for the prophylactic assay. (B) Number of $CD3^+$ T cells (C) and $CD3^+CD8^+$ T cells isolated from spleen 21 days after B16 tumor cells inoculation. Data are representative of three independent experiments. Bars shown are mean ± SD (n=6), and differences between PBS control group and other groups are determined using one-way ANOVA analysis and Student's t test. Relative to control group: *P<0.05, **P<0.01 and ***P<0.001.

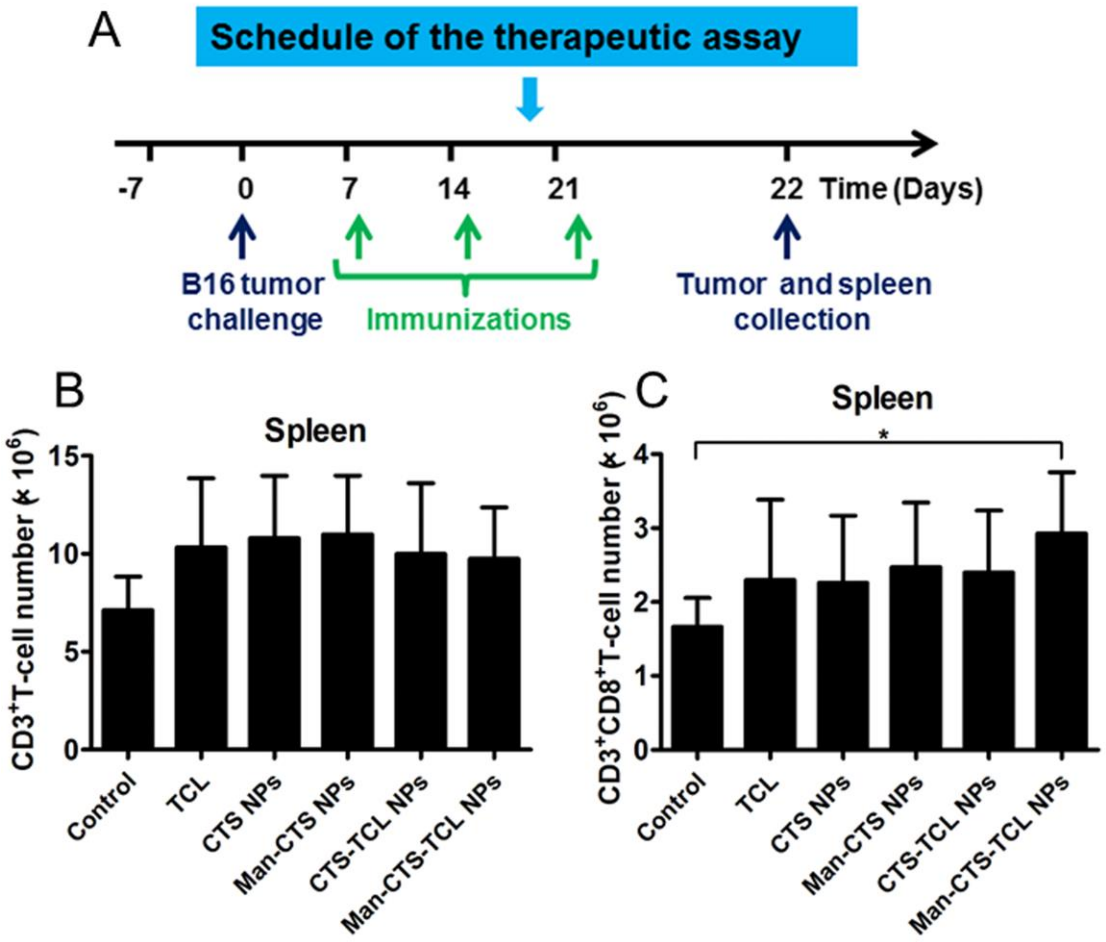

Figure S5 In the therapeutic tumor model, Man-CTS-TCL NPs treatment possesses higher level of the absolute number of $CD3^+CD8^+$ T cells in mice spleen. (A) Schedule used for the therapeutic assay. (B) Number of $CD3^+$ T cells (C) and $CD3^+CD8^+$ T cells isolated from spleen 22 days after B16 tumor cells inoculation. Data are representative of three independent experiments. Bars shown are mean ± SD (n=6), and differences between PBS control group and other groups are determined using one-way ANOVA analysis and Student's t test. Relative to control group: **P*<0.05, ***P*<0.01 and ****P*<0.001.