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Supporting Information for

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

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2 **Experimental section**

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

4 Two mg of FITC in 1 mL of 20 mmol/L carbonate buffer (pH 9.5) added to a solution
5 of TCL (1 mg/mL, 10 mL). The solution was incubated with continuous stirring at 4
6 °C for 18 h in the dark. The reaction mixture was dialyzed against distilled water
7 (MWCO 1000) to obtain TCL-FITC.

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

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

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

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

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

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

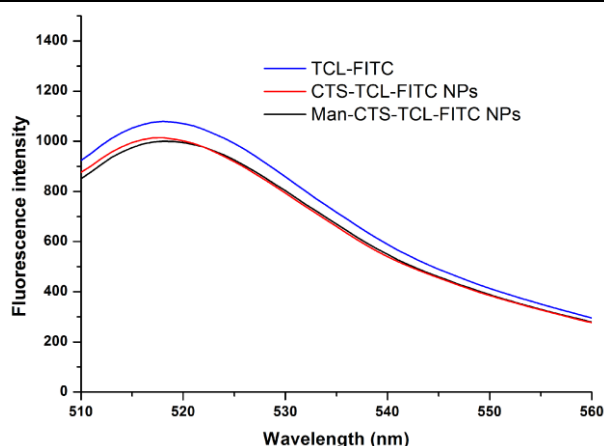
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10 Results section

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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



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13 **Figure S1**The fluorescence spectra of TCL-FITC, CTS-TCL-FITC NPs and
14 **Man-CTS-TCL-FITC NPs** in PBS, the excitation wavelength is 495 nm.

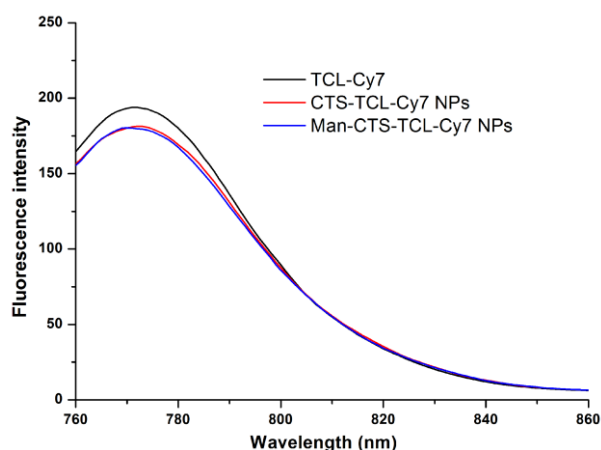


Figure S2 The fluorescence spectra of TCL-Cy7, CTS-TCL-Cy7 NPs and Man-CTS-TCL-Cy7 NPs in PBS, the excitation wavelength is 743 nm.

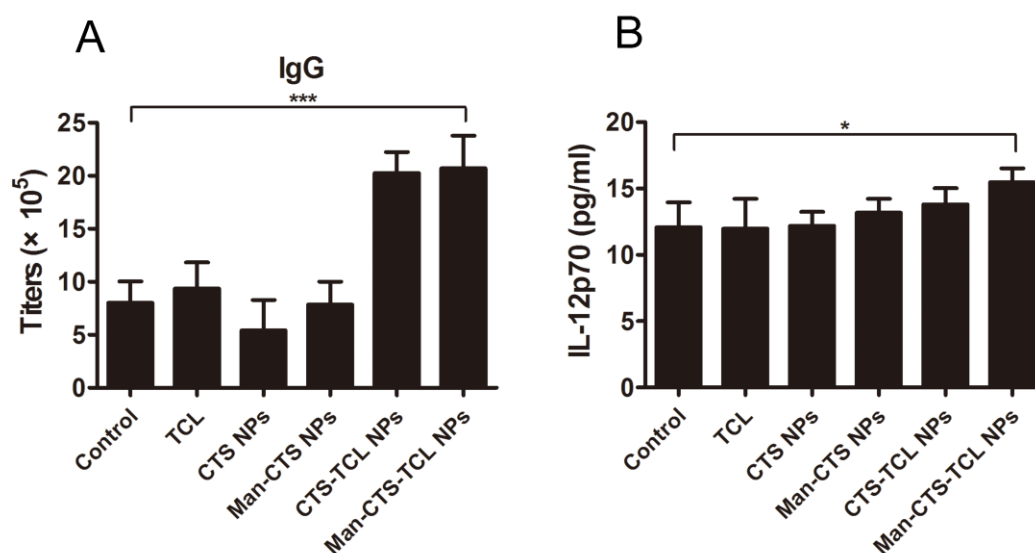


Figure S3 Immunization with Man-CTS-TCL NPs produces both cellular and humoral immune responses in mice. (A) Female C57BL/6 mice (n=6) 6-8 weeks old were subcutaneously immunized with PBS, TCL, CTS NPs, Man-CTS NPs, CTS-TCL NPs and Man-CTS-TCL NPs at days -14, -13, and -7. At day 0, serum of mice was 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 \pm 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 *** $P<0.001$.

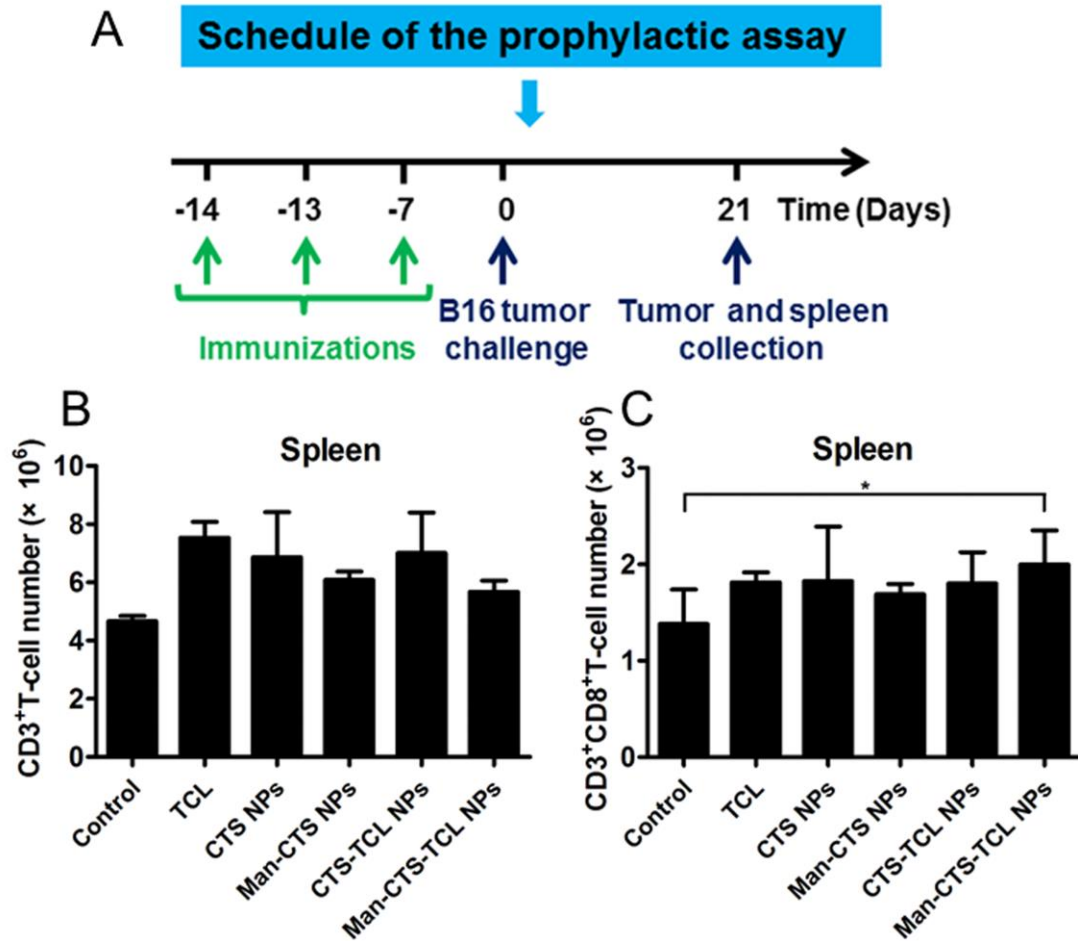


Figure S4 Immunization of mice with Man-CTS-TCL NPs enhances the absolute number of CD3⁺CD8⁺ T cells in mice spleen. (A) Schedule used for the prophylactic assay. (B) Number of CD3⁺ T cells (C) and CD3⁺CD8⁺ T cells isolated from spleen 21 days after B16 tumor cells inoculation. Data are representative of three independent experiments. Bars shown are mean \pm 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 *** $P<0.001$.

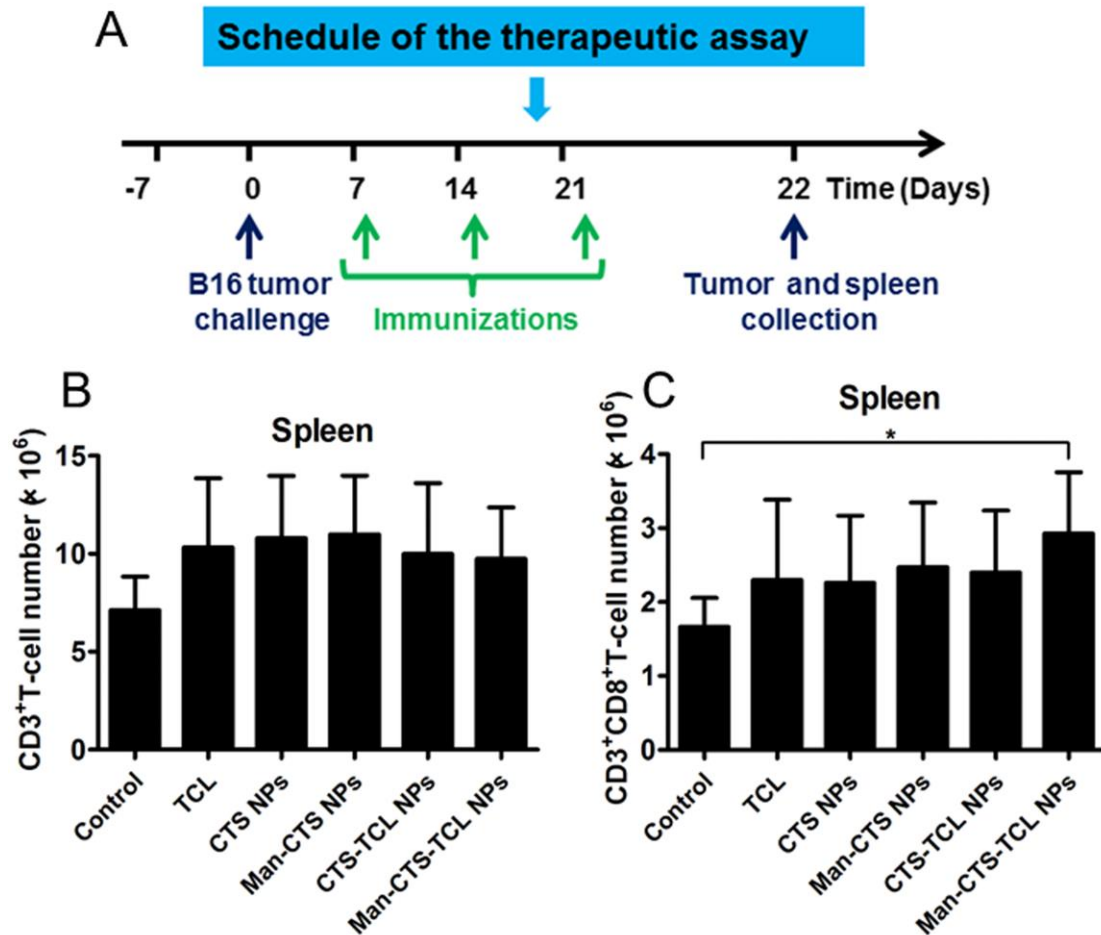


Figure S5 In the therapeutic tumor model, Man-CTS-TCL NPs treatment possesses higher level of the absolute number of CD3⁺CD8⁺ T cells in mice spleen.

(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 \pm 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 *** $P < 0.001$.