Supporting Information for

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

Gao-Na Shi\textsuperscript{a,1}, Chuang-Nian Zhang\textsuperscript{a,1}, Rong Xu\textsuperscript{b}, Jin-Feng Niu\textsuperscript{c}, Hui-Juan Song\textsuperscript{a}, Xiu-Yuan Zhang\textsuperscript{a}, Wei-Wei Wang\textsuperscript{a}, Yan-Ming Wang\textsuperscript{c}, Chen Li\textsuperscript{a,**}, Xiao-Qing Wei\textsuperscript{d,**}

*, De-Ling Kong\textsuperscript{a,***}

\textsuperscript{a} Tianjin Key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Science & Peking Union Medical College, Tianjin, 300192, China.

\textsuperscript{b} Department of Clinical Infection Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, L69 7BE, UK.

\textsuperscript{c} State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, 300353, China.

\textsuperscript{d} Cardiff Institute of Tissue Engineering & Repair, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, UK.

\textsuperscript{e} Key Laboratory of Bioactive Materials, Ministry of Education, Nankai University, Tianjin, 300071, China.

\textsuperscript{1} Gaona Shi and Chuangnian Zhang contributed equally to this work.

\* Corresponding authors

\** Corresponding authors

\*** Corresponding authors
Email address: kongdeling@nankai.edu.cn (D.Kong); cli0616826@126.com (C.Li); weiX1@cardiff.ac.uk (X.Wei).
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 °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 °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>
<thead>
<tr>
<th>Sample</th>
<th>Size (nm)</th>
<th>DPI</th>
<th>Zeta potentials (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTS-TCL NPs</td>
<td>127.46±6.73</td>
<td>0.114±0.031</td>
<td>-14.07±1.22</td>
</tr>
<tr>
<td>Man-CTS-TCL NPs</td>
<td>120.15±9.93</td>
<td>0.121±0.049</td>
<td>-12.07±1.36</td>
</tr>
<tr>
<td>CTS-TCL-FITC NPs</td>
<td>134.72±2.65</td>
<td>0.103±0.051</td>
<td>-15.41±1.25</td>
</tr>
<tr>
<td>Man-CTS-TCL-FITC NPs</td>
<td>136.18±7.63</td>
<td>0.131±0.047</td>
<td>-14.58±1.19</td>
</tr>
<tr>
<td>CTS-TCL-Cy7 NPs</td>
<td>139.16±10.03</td>
<td>0.119±0.039</td>
<td>-15.46±1.52</td>
</tr>
<tr>
<td>Man-CTS-TCL-Cy7 NPs</td>
<td>135.48±8.97</td>
<td>0.124±0.041</td>
<td>-14.92±1.43</td>
</tr>
</tbody>
</table>

**Figure S1** The 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
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.