

Supporting Information

**Recombinant cell-penetrating trichosanthin synergizes
anti-PD-1 therapy in colorectal tumor**

Table S1 The primers for RT-qPCR.

Target Gene	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
<i>Mus GAPDH</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Mus IL-1β</i>	CTTCAGGCAGGCAGTATCACTC	TGCAGTTGTCTAATGGGAACGT
<i>Mus IL-6</i>	GTCTGTAGCTCATTCTGCTCTG	GAAGGCAACTGGATGGAAGT
<i>Mus IL-10</i>	TTTCAAACAAAGGACCAG	GGATCATTTCGGATAAGG
<i>Mus IL-12</i>	AGACATCACACGGGACCAAAC	CCAGGCAACTCTCGTTCTTGT
<i>Mus IL-23</i>	TAATGCTATGGCTGTTGCCCT	TTCATATGTCCCGCTGGTGC
<i>Mus TBK1</i>	GGAGCCGTCCAATGCGTAT	GCCGTTCTCTCGGAGATGATTC
<i>Mus IRF3</i>	GAGAGCCGAACGAGGTTTCAG	CTTCCAGGTTGACACGTCCG
<i>Mus IP-10</i>	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
<i>Mus TNF-α</i>	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
<i>Mus iNOS</i>	CCCTTCAATGGTTGGTACATGG	ACATTGATCTCCGTGACAGCC

Figure S1 The in vitro biocompatibility of rTCS and rTCS-LMWP. The cell viability of BMDCs (A), M2-type BMDMs (B), bEnd3 (C), and HUVEC (D) after treatment with different concentrations of rTCS and rTCS-LMWP.

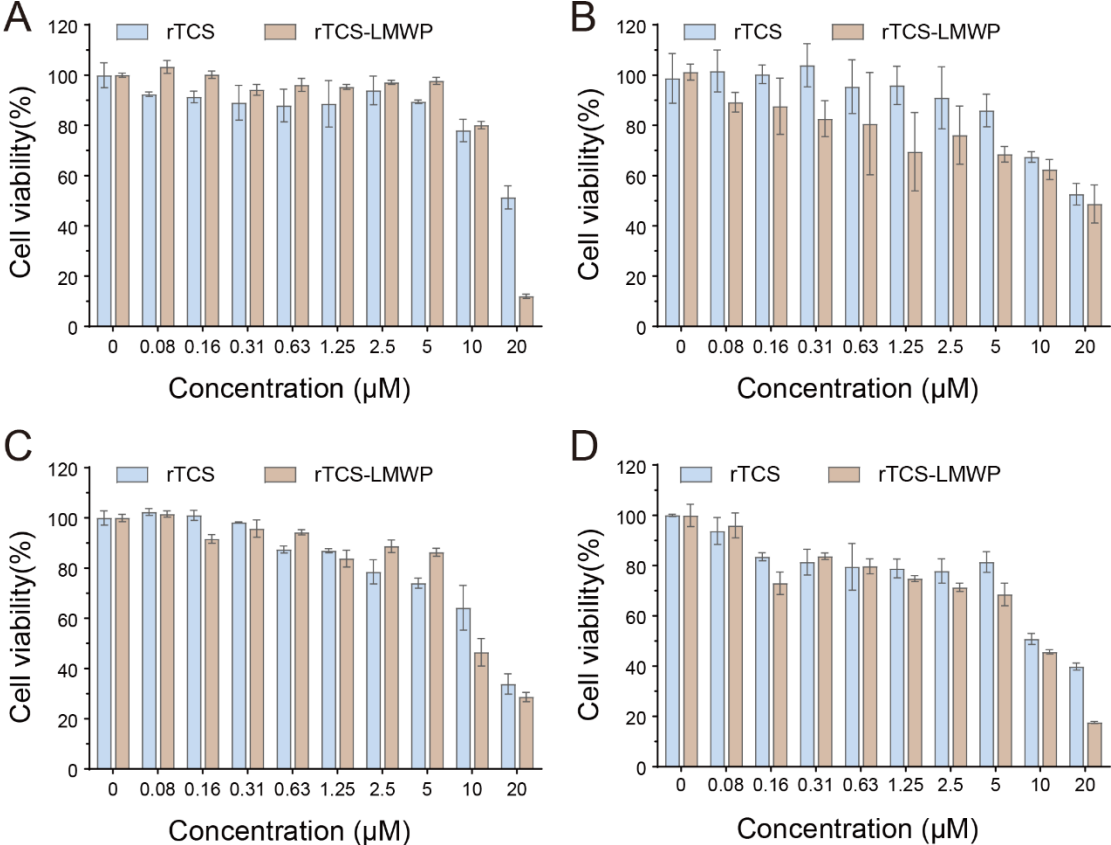


Figure S2 The stimulating effect of rTCS and rTCS-LMWP on BMDCs. **(A)** The mean fluorescence of MHC-II of BMDCs treated with rTCS and rTCS-LMWP by flow cytometry. The mRNA level of IL-1 β **(B)**, IL-6 **(C)**, and IL-10 **(D)** of BMDCs treated with rTCS and rTCS-LMWP by RT-qPCR. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

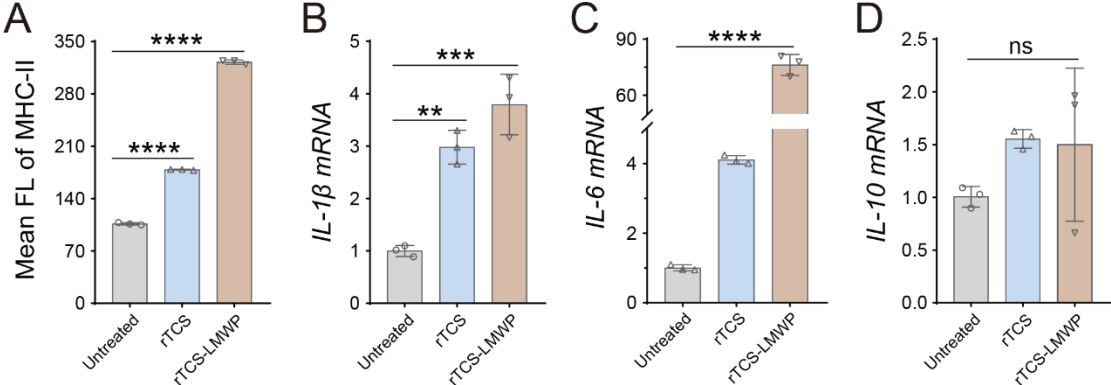


Figure S3 The in vivo remodeling of TIME on the 7th day after the last treatment. The intratumoral population of CD8⁺ IFN- γ ⁺ T cells (**A**), CD8⁺ GrzB⁺ T cells (**B**), F4/80⁺ CD86⁺ macrophages (**C**), and F4/80⁺ CD206⁺ macrophages (**D**).

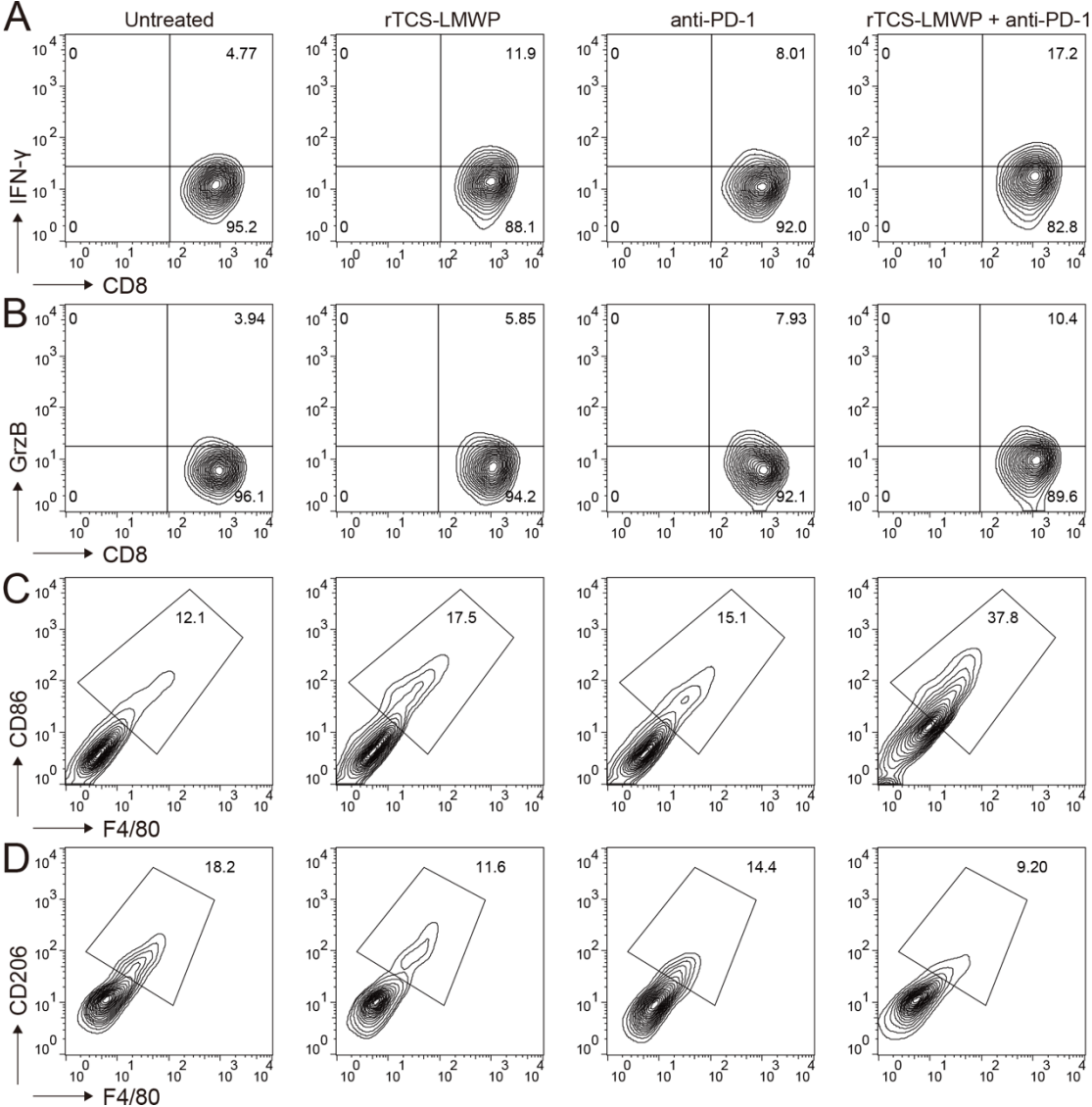


Figure S4 The in vivo immune regulation on the 7th day after the last treatment. **(A&B)** The population of CD8⁺ Ki67⁺ T cells in the tumor tissues. **(C&D)** The population of CD4⁺ T cells in the tumor tissues. **(E&F)** The population of CD4⁺ FoxP3⁺ T cells in the tumor tissues. **(G&H)** The population of F4/80⁺ CD169⁺ macrophages in the tumor tissues. *p < 0.05; **p < 0.01; ****p < 0.0001.

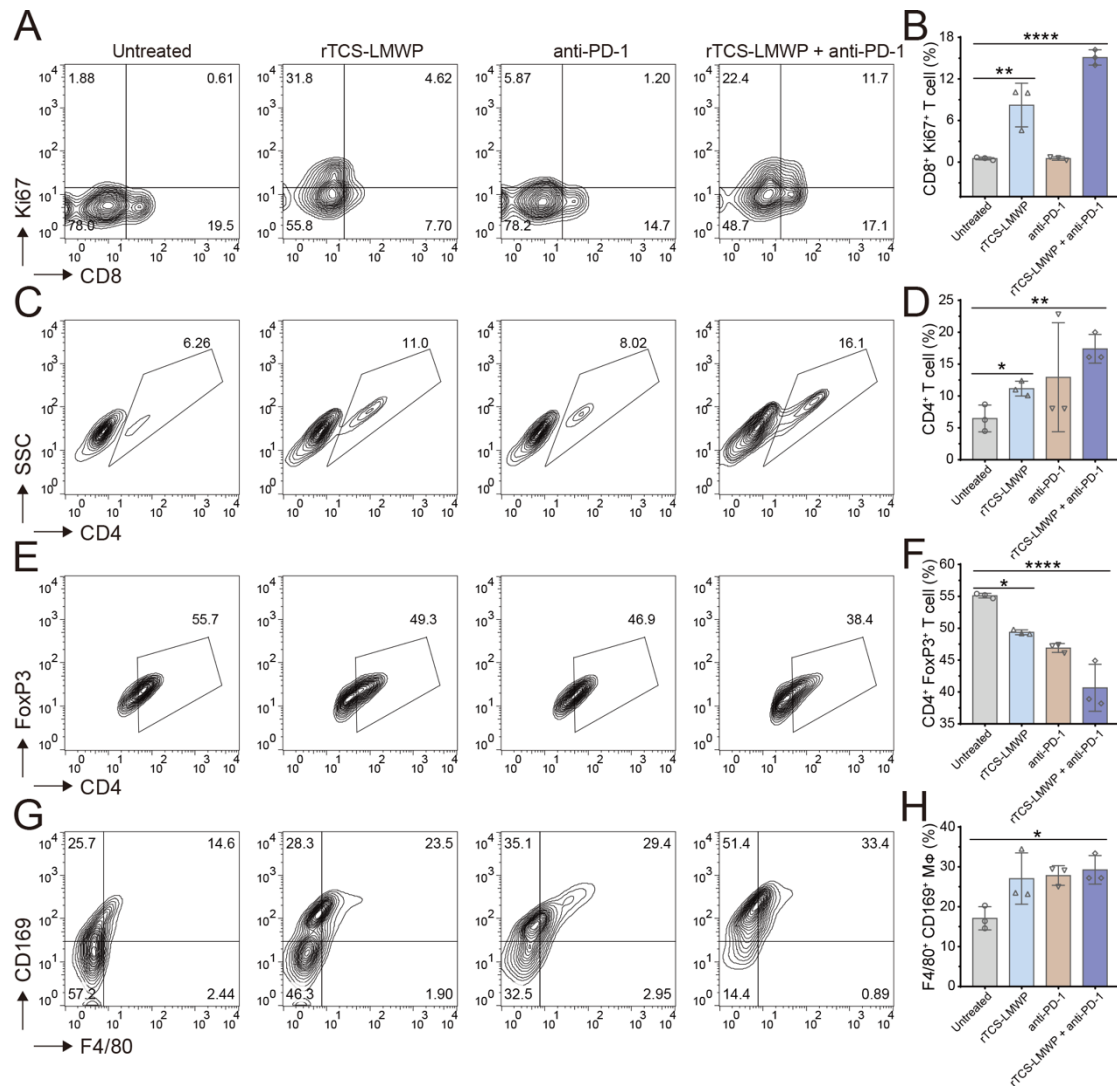


Figure S5 The in vivo immune regulation on the 7th day after the last treatment. **(A&C)** The population of CD8⁺ Ki67⁺ T cells in the tumor-draining lymph node. **(B&D)** The mean fluorescence intensity of MHC-II of CD11c⁺ cells in the tumor-draining lymph node. **p < 0.01; ****p < 0.0001.

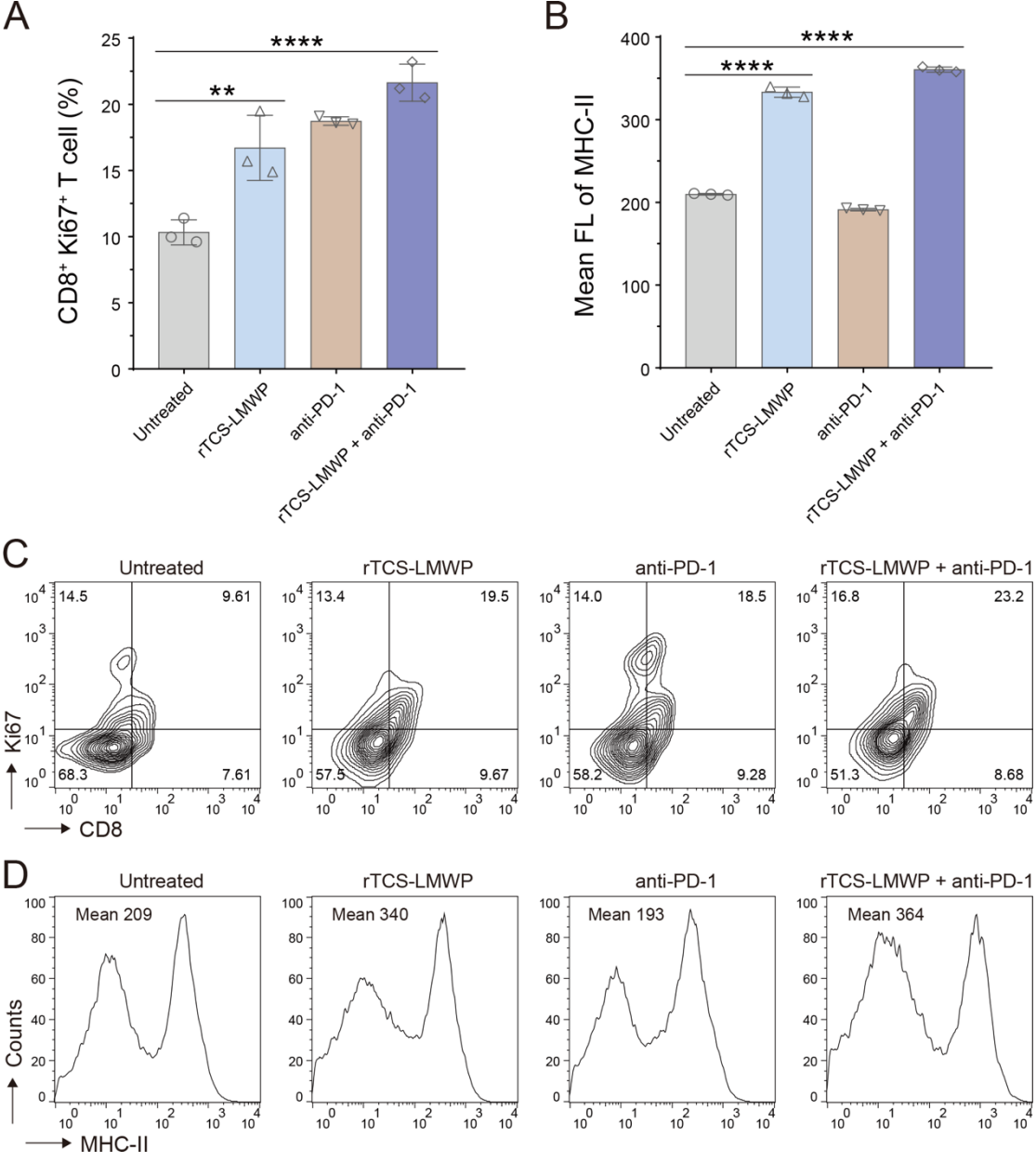


Figure S6 The preliminary in vivo biosafety of rTCS-LMWP in BALB/c mice. **(A)** The body weight. **(B)** The organ coefficient. **(C)** The levels of serum IL-6 and TNF- α . **(D)** The H&E staining of the skin tissue at the injected sites.

