

Supplementary Information

In situ induction of multivalent PD-L1 antibody secretion to enhance cancer immunotherapy

Li-Li Chen,^{‡a} Shi-Kun Zhou,^{‡a} Shui-Qing Jiang,^{‡a} Yi-Qun Sun,^a Xin-Yu Tan,^a Yi-Fang Chen,^b Yang Liu,^{*a} Zi-Dong Lu,^{*c} and Cong-Fei Xu^{*a,d}

^a School of Biomedical Sciences and Engineering, South China University of Technology, Guangzhou International Campus, Guangzhou 511442, P. R. China

^b Department of Pharmacy and Pharmaceutic Sciences, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore

^c School of Medicine, South China University of Technology, Guangzhou 510006, P. R. China

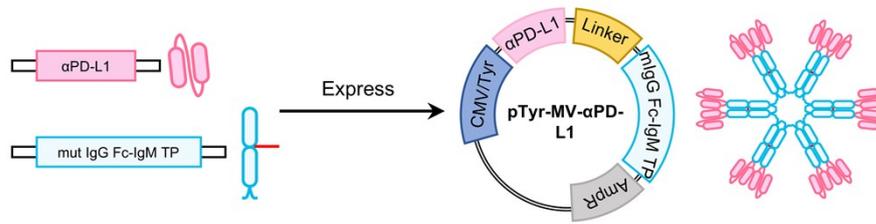
^d National Engineering Research Center for Tissue Restoration and Reconstruction, Guangdong Province Key Laboratory of Biomedical Engineering, South China University of Technology, Guangzhou 510006, P. R. China

[‡] These authors contributed equally.

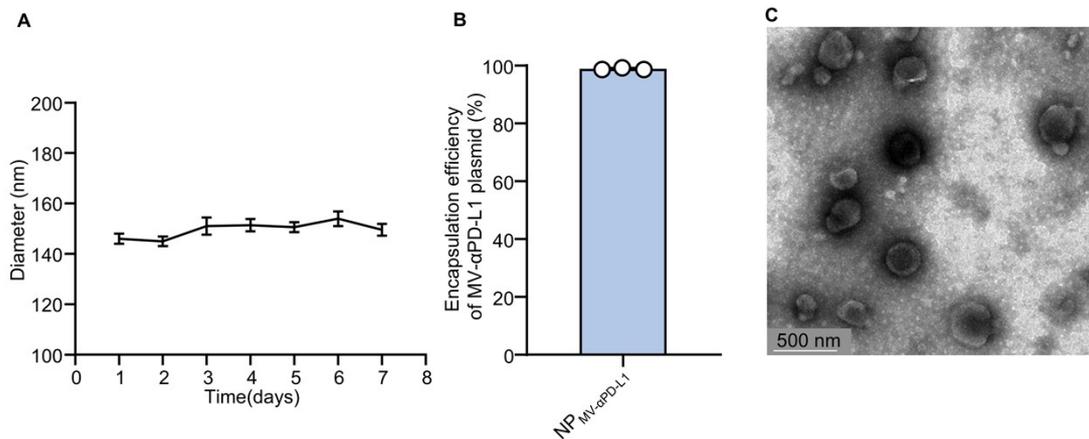
* Corresponding authors: Yang Liu, yaliu@scut.edu.cn; Zi-Dong Lu, luzd@scut.edu.cn;

Cong-Fei Xu, xucf@scut.edu.cn

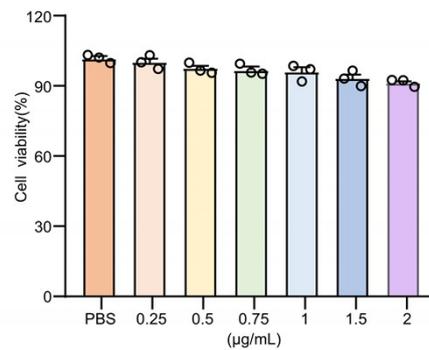
Supplementary Figures



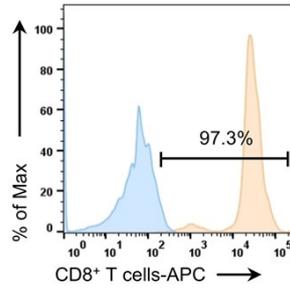
Supplementary Fig. 1. Construction of MV- α PD-L1 expression plasmids.



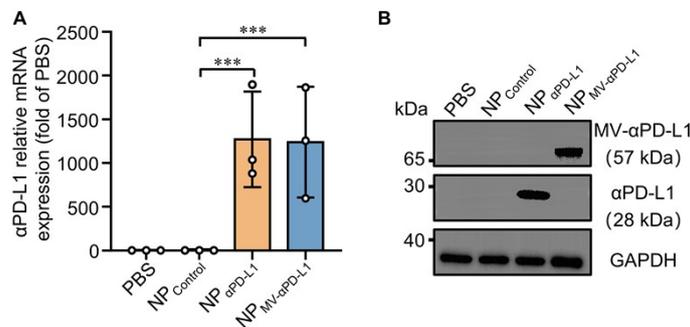
Supplementary Fig. 2. (A) The hydrodynamic diameter of the nanoparticles was measured by DLS continuously for a week. Data represent the mean \pm SD from three independent measurements. (B) The plasmid encapsulation efficiency of NP_{MV- α PD-L1}. (C) Representative TEM image of NP_{MV- α PD-L1} showing their overall uniformity and dispersity.



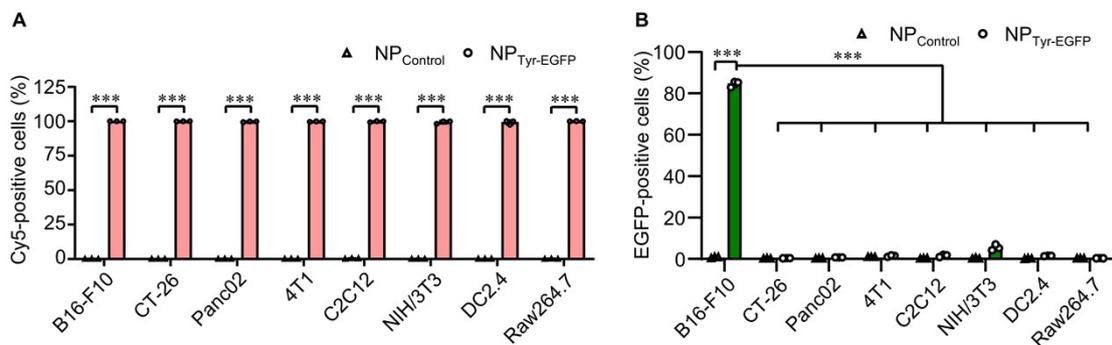
Supplementary Fig. 3. Cytotoxicity of NP_{MV- α PD-L1}. Cell viability of B16-F10 cells transfected with different concentrations of NP_{MV- α PD-L1}, as assessed by CCK-8 assay. Data are presented as mean \pm SD.



Supplementary Fig. 4. Flow cytometry analysis of the purity of isolated OVA-specific CD8⁺ T cells used in the CCK-8 cytotoxicity assay.



Supplementary Fig. 5. NP_{MV- α PD-L1} increases intratumoral α PD-L1 expression *in vivo*. (A) RT-qPCR analysis of α PD-L1 mRNA levels in the melanoma after different treatments. (B) Western blot analysis of the expression of α PD-L1 and MV- α PD-L1 in melanoma lysates. Data are presented as mean \pm SD (A) or as representative results (B). Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparisons test; *** $P < 0.001$.



Supplementary Fig. 6. Cellular uptake and expression specificity of NP_{Tyr-EGFP}. (A) Percentage of Cy5-positive cells and (B) EGFP-positive cells in B16-F10 cells and seven non-melanoma cell types following transfection with Cy5-labelled NP_{Tyr-EGFP} or NP_{Control}.

Data are presented as mean \pm SD. Statistical analyses were performed using a two-sided Student's *t*-test. ****P* < 0.001.

Supplementary Table 1. DNA sequences of Tyr promoter, α PD-L1, and MV- α PD-L1.

<p>Tyr promoter (546 bps)</p>	<p>CCTGCAGGTCATAGTTCCTGCCAGCTGACTTTGTCAAGA CAGTGATGTCTGTGTTCCAGCAGTTGTTCTGAGTATCCTT TTCATTATCCACTGTCCTTTCTTCTTAAATTCCACCCCA ACATTGTAAATAGCTTCTTTCTTAAACTCTGTTCAAAGAA CCAGCTTGAGTGTGTCAGCTGCTTCCTGCTGGGGTCTGG CAAACCACTAGTGACCTTTATTCATAAGAGATGATGTAT TCTTGATACTACTTCTCATTGCAAATTCCAATTATTATT AATTCATATCAATTAGAATAATATATCTTCCTTCAATTT AGTTACCTCACTATGGGCTATGTACAACTCCAAGAAA AGTTAGTCATGTGCTTTGCAGAAGATAAAAGCTTAGTGT AAAACAGGCTGAGAGTATTTGATGTAAGAAGGGGAGTG GTTATATAGGTCTTAGCCAAAACATGTGATAGTCACTCC AGGGGTTGCTGGAAAAGAAGTCTGTGACACTCATTAACC TATTGGTGCAGATTTTGTATGATCTAAAGGAGAC</p>
<p>αPD-L1 (795 bps)</p>	<p>ATGGAAACCGATAACCCTGCTGCTGTGGGTGCTGCTGCTG TGGGTGCCGGGCAGCACCGGGCGATGATATTCAGATGACC CAGAGCCCGAGCAGCCTGAGCGCGAGCGTGGGGCGATCG CGTGACCATTACCTGCCGCGGAGCCAGGATGTGAGCAC CGCGGTGGCGTGGTATCAGCAGAAACCGGGCAAAGCGC CGAAACTGCTGATTTATAGCGCGAGCTTTCTGTATAGCG GCGTGCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCG ATTTTACCCTGACCATTAGCAGCCTGCAGCCGGAAGATT TTGCGACCTATTATTGCCAGCAGTATCTGTATCATCCGGC GACCTTTGGCCAGGGCACCAAAGTGGAATTAACCGCG GAGGCGGTGGTTCCGGCGGAGGAGGCAGCGGTGGCGGA GGTTCCGAAGTGCAGCTGGTGGAAAGCGGCGGCGGCCT GGTGCAGCCGGGCGGCAGCCTGCGCCTGAGCTGCGCGGC GAGCGGCTTTACCTTTAGCGATAGCTGGATTCATTGGGT GCGCCAGGCGCCGGGCAAAGGCCTGGAATGGGTGGCGT GGATTAGCCCGTATGGCGGCAGCACCTATTATGCGGATA GCGTGAAAGGCCGCTTTACCATTAGCGCGGATAACCAGCA AAAACACCGCGTATCTGCAGATGAACAGCCTGCGCGCGG AAGATACCGCGGTGTATTATTGCGCGCGCCGCCATTGGC CGGGCGGCTTTGATTATTGGGGCCAGGGCACCCCTGGTGA CCGTGAGCGCGCTGAGCAGC</p>
<p>MV-αPD-L1</p>	<p>ATGGAAACCGATAACCCTGCTGCTGTGGGTGCTGCTGCTG</p>

(1458 bps)	TGGGTGCCGGGCAGCACCGGCGATGATATTCAGATGACC CAGAGCCCGAGCAGCCTGAGCGCGAGCGTGGGCGATCG CGTGACCATTACCTGCCGCGGAGCCAGGATGTGAGCAC CGCGGTGGCGTGGTATCAGCAGAAACCGGGCAAAGCGC CGAAACTGCTGATTTATAGCGCGAGCTTTCTGTATAGCG GCGTGCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCG ATTTTACCCTGACCATTAGCAGCCTGCAGCCGGAAGATT TTGCGACCTATTATTGCCAGCAGTATCTGTATCATCCGGC GACCTTTGGCCAGGGCACCAAAGTGGAATTAACCGCG GAGGCGGTGGTTCCGGCGGAGGAGGCAGCGGTGGCGGA GGTCCGAAGTGCAGCTGGTGGAAAGCGGCGGCGGCCT GGTGCAGCCGGGCGGCAGCCTGCGCCTGAGCTGCGCGGC GAGCGGCTTTACCTTTAGCGATAGCTGGATTCATTGGGT GCGCCAGGCGCCGGGCAAAGGCCTGGAATGGGTGGCGT GGATTAGCCCGTATGGCGGCAGCACCTATTATGCGGATA GCGTGAAAGGCCGCTTTACCATTAGCGCGGATAACCAGCA AAAACACCGCGTATCTGCAGATGAACAGCCTGCGCGCGG AAGATACCGCGGTGTATTATTGCGCGCGCCGCCATTGGC CGGGCGGCTTTGATTATTGGGGCCAGGGCACCCCTGGTGA CCGTGAGCGCGCTGAGCAGCGGTGGCGGCGGCTCTGGTG GCGGCGGCTCTGGTGGCGGCGGCTCTGAATTCATATCGG CCATGGTTAGATCTGACAAAACCTCACACATGCCACCGT GCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC TCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCC GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCC ACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCTGCCTCCAGGACTGGCTGAATGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATC GAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGA ACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGG CTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAA TGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGT GCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTA CACGCAGAAGAGCCTCTCCCTGTCTCCGGGTA
------------	--

Supplementary Table 2. RT-qPCR primer sequences for analyzing the expression of α PD-L1 and MV- α PD-L1.

Primer ID	Sequence
α PD-L1 forward primer	5'-GAATGGGTGGCGTGGATTAG-3'
α PD-L1 reverse primer	5'-GGCTGTTCATCTGCAGATACG-3'
MV- α PD-L1 forward primer	5'-TTCAACTGGTACGTGGACGG-3'
MV- α PD-L1 reverse primer	5'-GTACACCTGTGGTTCTCGGG-3'
GAPDH forward primer	5'-CATCACTGCCACCCAGAAGACTG-3'
GAPDH reverse primer	5'-ATGCCAGTGAGCTTCCCGTTCAG-3'