Supplementary Information

Surface engineering of oncolytic adenovirus for combinations of immune checkpoint blockade and virotherapy

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Experimental Section

Plasmid Construction. The PD-1 (mouse) gene with GFP gene sequence were subcloned to a PLV vector and expressed in L929 cell line, generating PD-1 gene stable expression cell line L929-PD1.

Localization of PD-1. L929-PD1 were seeded onto confocal glass-bottom dishes in DMEM medium supplemented with 10% FBS. After 24h, L929-PD1 cells were stained with DAPI for 15 min. Live cells were monitored with confocal laser scanning microscopes (Olympus). For the evaluation of PD-1 on the cell membrane surface, PD-1 antibodies were loaded on the surface of BeaverBeads™ Streptavidin

(BEAVER). PD1-BCMNs-GFP were mixed with BeaverBeads™ Streptavidin at room temperature for 30 min, balank EVs were used as control.

Real-time PCR analysis. After cells, tissues or plasma were collected, total RNA were isolated by Trizol (Sangon, SK1321). Reverse transcription was performed with RevertAid Premium Reverse Transcriptase kit (Thermo Scientific™ EP0733). Expression levels of PD-L1 mRNA was measured by SG Fast qPCR Master Mix (High Rox) (2X) (BBI) (ABI) (B639273). Primers for PD-L1 gene (5'ACTTGCTACGGGCGTTTACT3' and 5'ACTAACGCAAGCAGGTCCAG3') was used.

Results

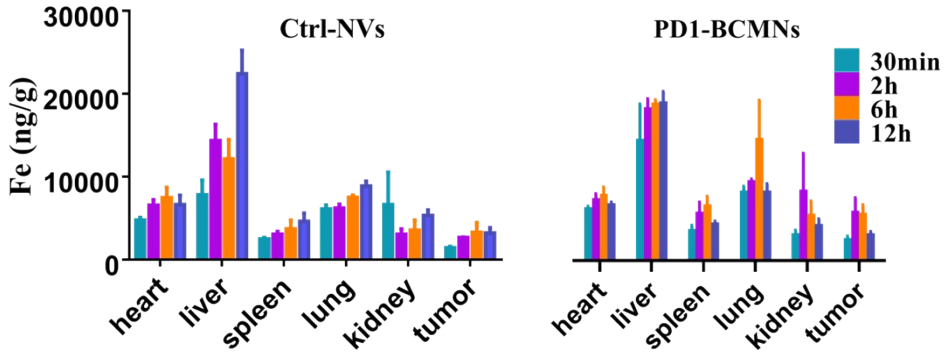

Figure S1. Distribution of Ctrl-NVs and PD1-BCMNs in tumor and major organs. Iron intensity per gram of tissue in tumor and major organs (n = 5). Error bar, mean ± s.d.

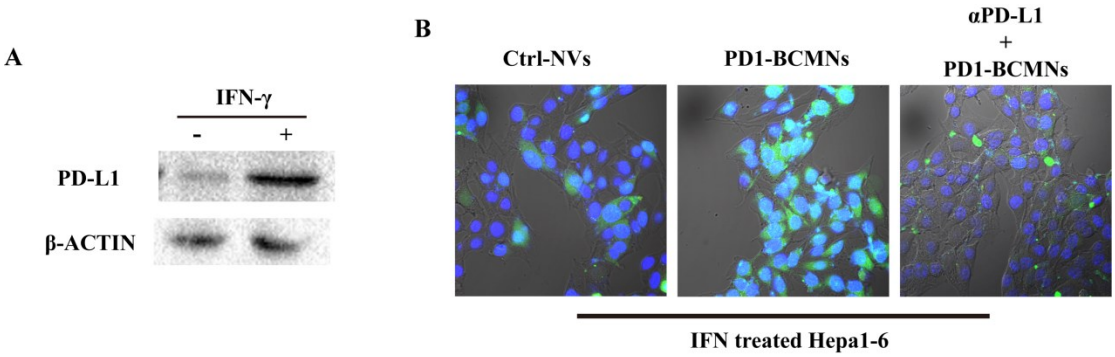

Figure S2. Characterization of PD1-BCMNs on Hepa1-6 cells with high levels of endogenous PD-L1. (A) Western blot analysis of PD-L1 expression of Hepa1-6 cells after treated with IFN-γ (200 U/mL). (B) (F) CLSM image for the binding capacity of PD1-BCMNs detection. PD1-BCMNs and Ctrl-NVs were stained with CFSE (green).

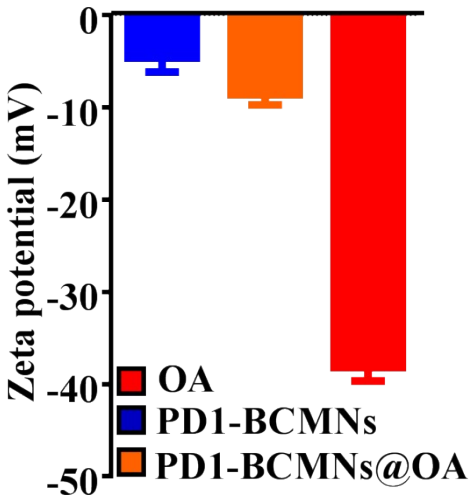

Figure S3. Zeta-potential of OA, PD1-BCMNs and PD1-BCMNs@OA measured by dynamic light scattering (DLS).

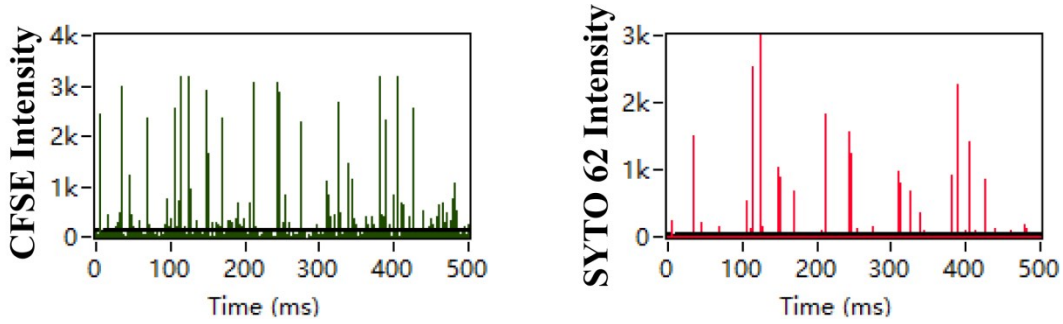

Figure S4. The encapsulation efficiency of PD1-BCMNs@OA was further evaluated by using a High Sensitivity Flow Cytometry (HSFCM). FITC-CFSE intensity, PC5.5-SYTO62 intensity represented PD1-BCMNs and OA, respectively.

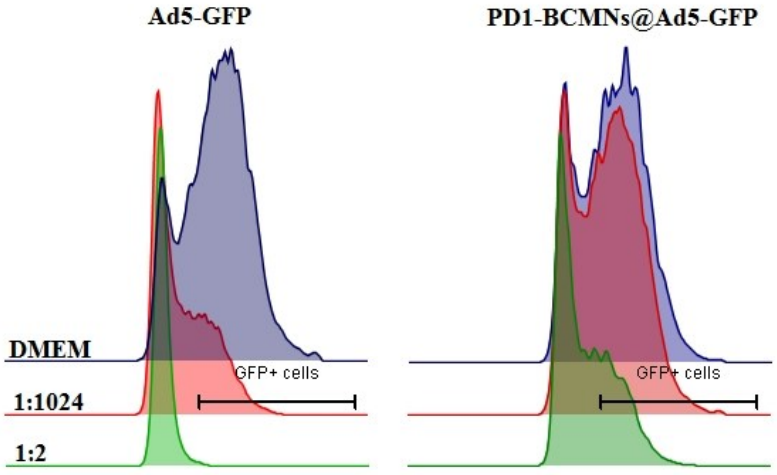

Figure S5. Flow cytometry assay of Ad5-GFP@BCMNs and Ad5-GFP in HEK 293 cells with anti-Ad5 serum at indicated dilutions.

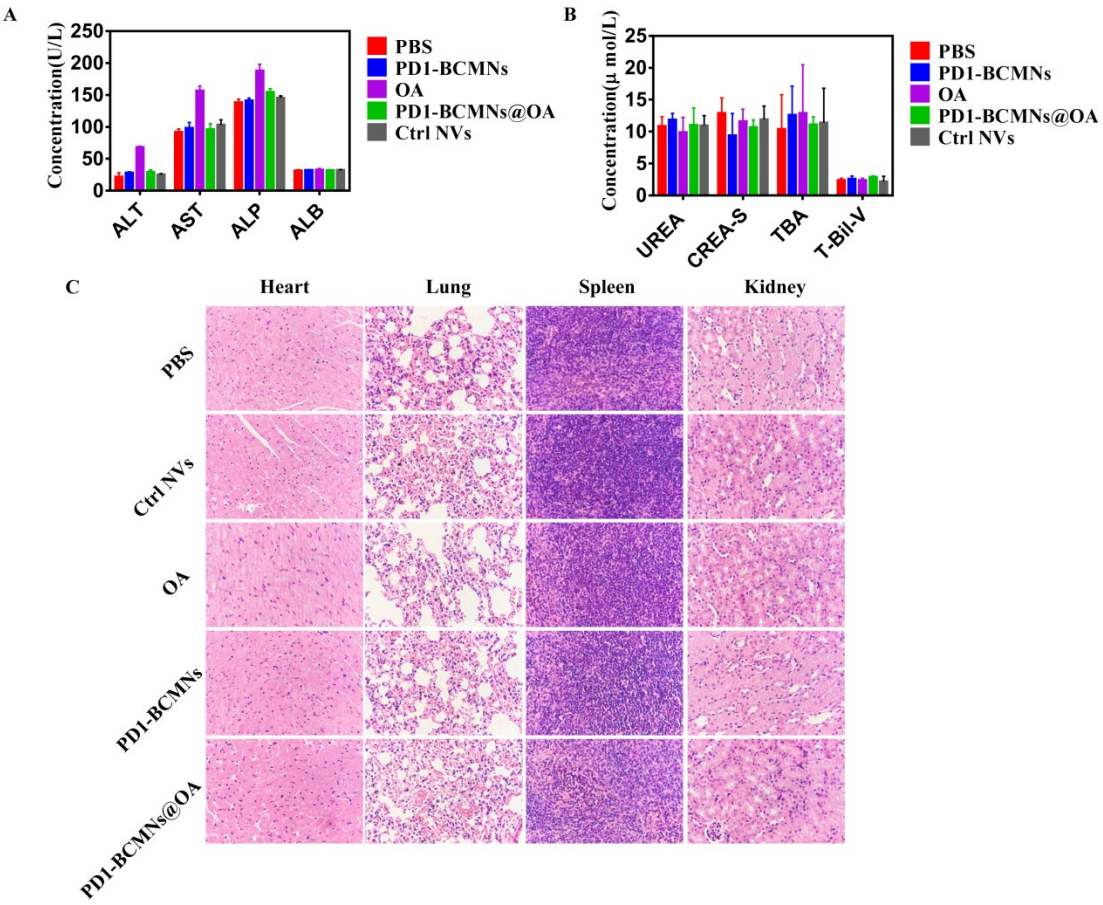

Figure S6. Biological safety evaluation of OA, PD1-BCMNs, Ctrl NVs, and PD1-BCMNs@OA after intravenous injection.

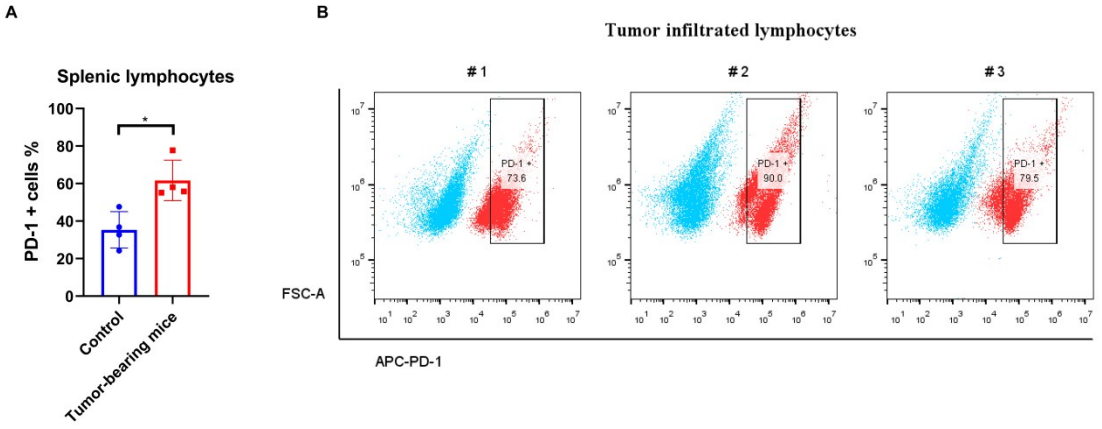

Figure S7. Quantitative analysis of PD1 expressing levels in splenic lymphocytes (A) and tumor infiltrated lymphocytes (B) by the flow cytometry. *p < 0.05, **p < 0.01, and ***p < 0.001.

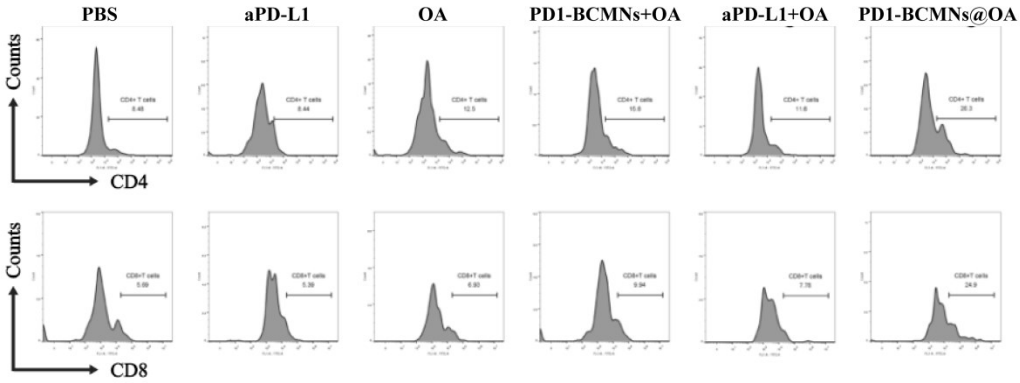

Figure S8. Representative plots of $CD4^+$ and $CD8^+$ T cells in different treatment groups analyzed by the flow cytometry.

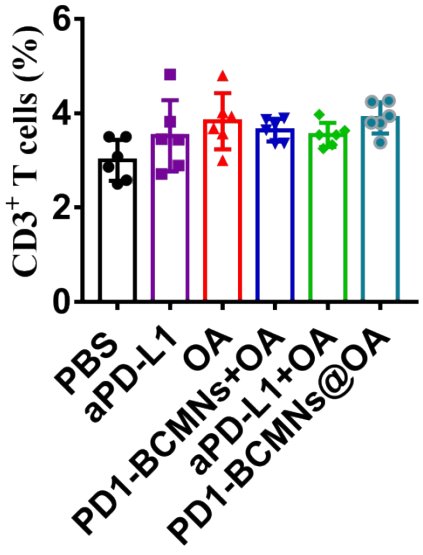

Figure S9. Quantitative analysis of infiltrating $CD3^+$ T cells in different treatment groups analyzed by the flow cytometry.

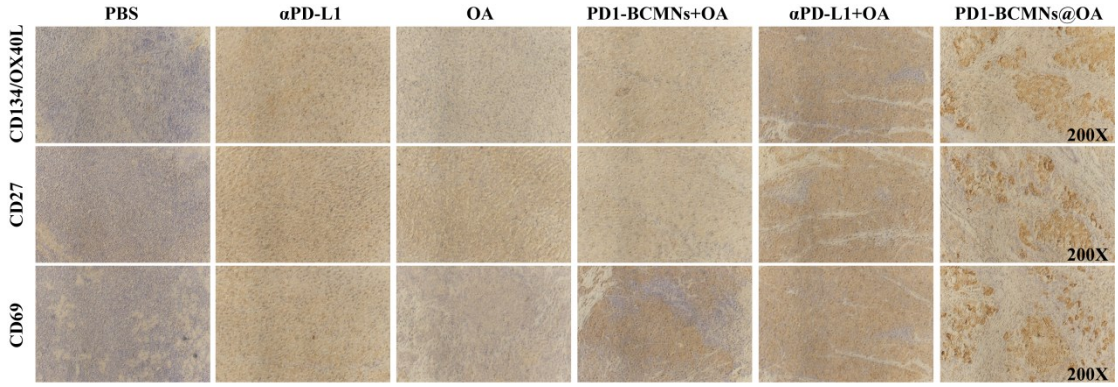

Figure S10. Representative images of T cell activation markers (CD134/OX40L, CD27, and CD69 proteins) immunohistochemistry staining for tumor from Hepa1-6 subcutaneous tumor models at day 23. Error bar, mean ± s.d.

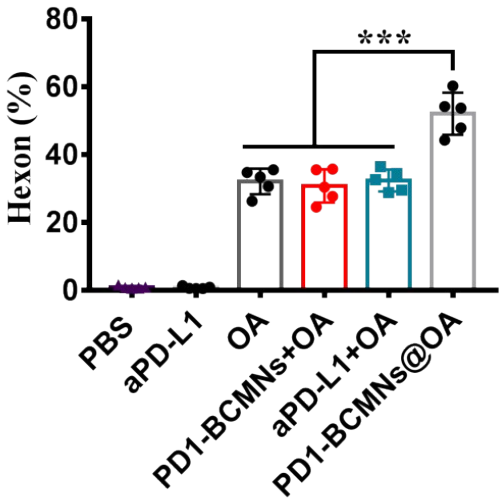

Figure S11. Quantitative analysis of Hexon rate in histology sections with anti-Hexon antibody staining, respectively. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.