nature portfolio

https://doi.org/10.1038/s41596-022-00713-7

Supplementary information

Nanocarriers based on bacterial membrane materials for cancer vaccine delivery

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Nanocarriers based on bacterial membrane materials for cancer vaccine delivery

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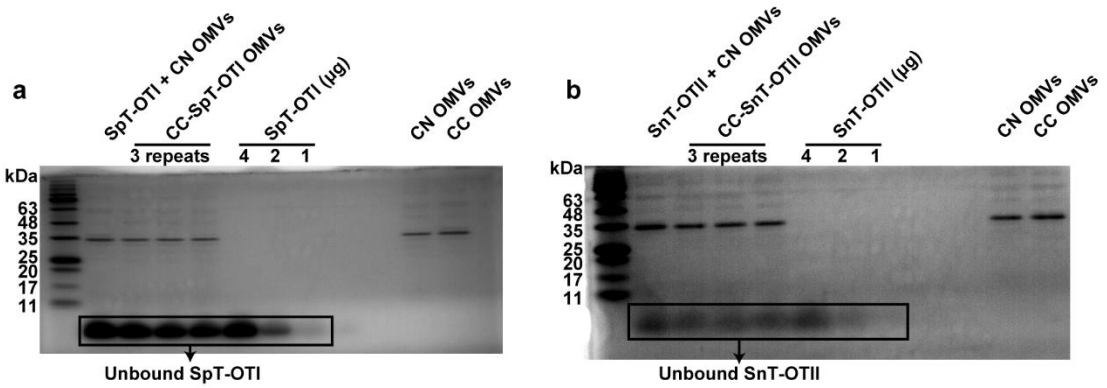
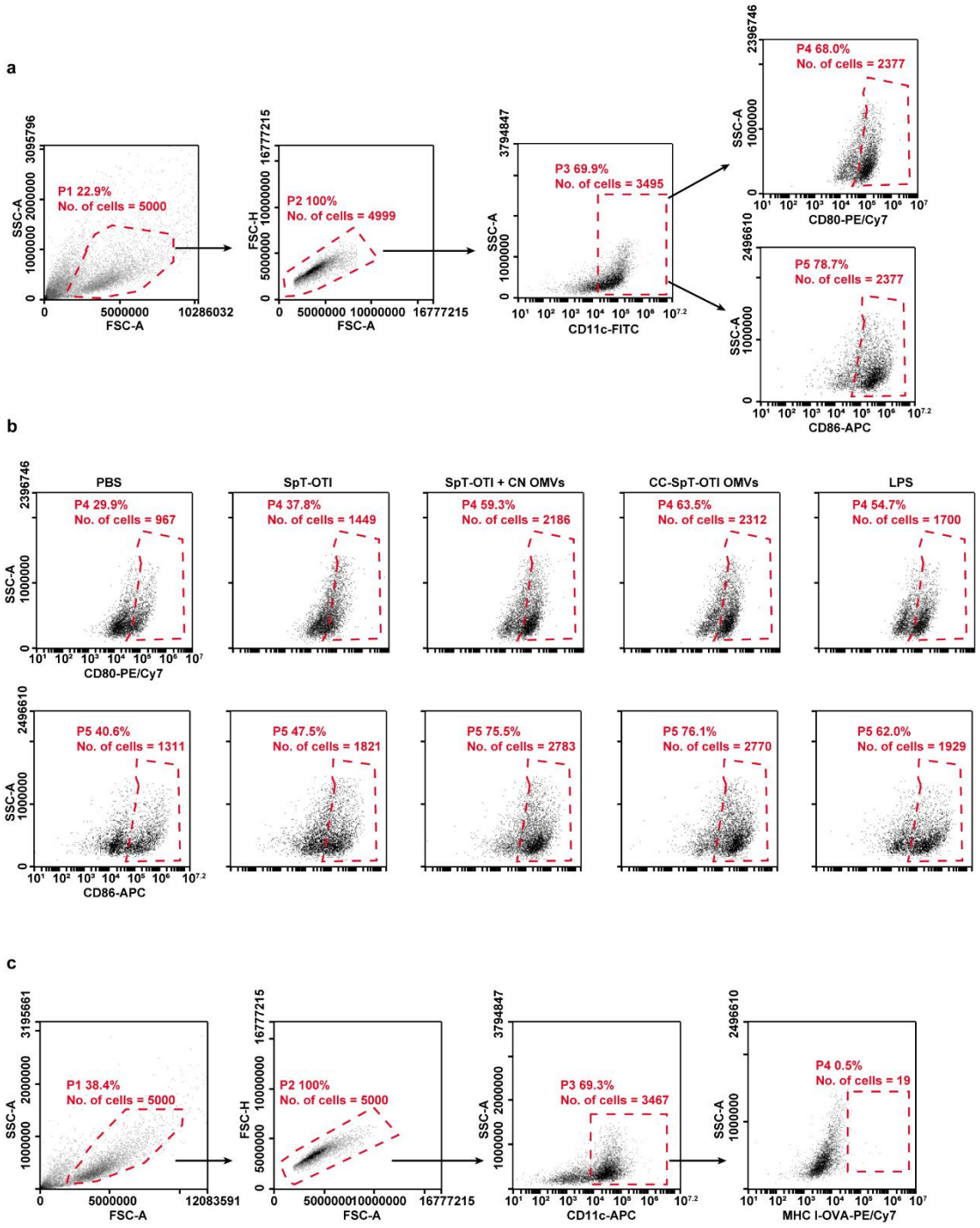
Associated publications

Cheng, K. et al. Bioengineered bacteria-derived outer membrane vesicles as a versatile antigen display platform for tumor vaccination via Plug-and-Display technology. Nat. Commun. 12, 2041 (2021). DOI: https://doi.org/10.1038/s41467-021-22308-8

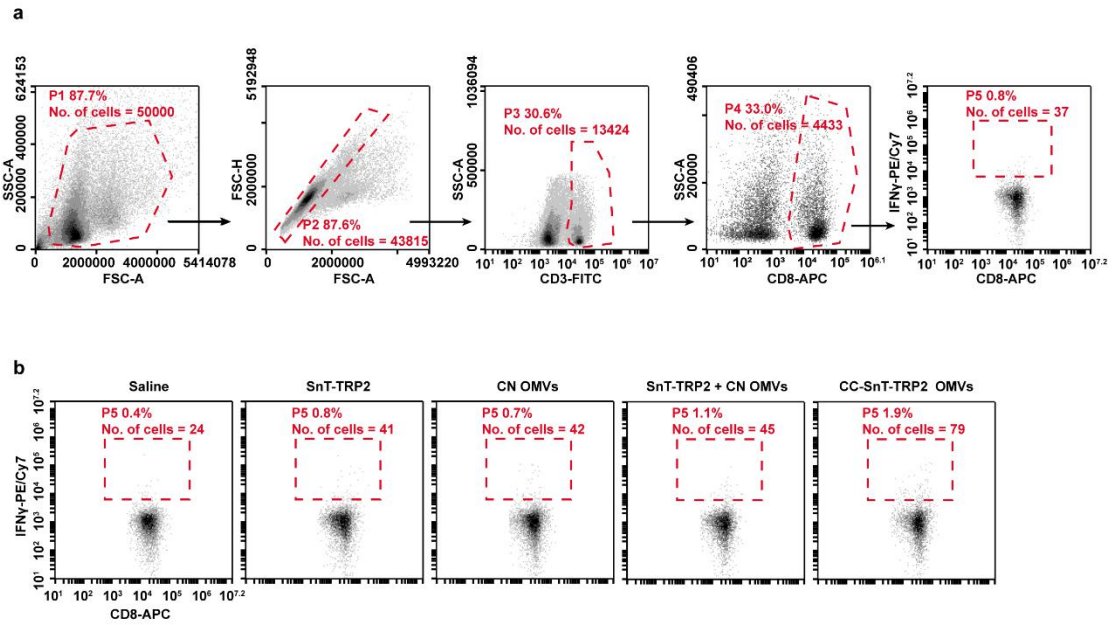
Chen, L. et al. Bacterial cytoplasmic membranes synergistically enhance the antitumor activity of autologous cancer vaccines. Sci. Transl. Med. 13, eabc2816 (2021). DOI: https://doi.org/ 10.1126/scitranslmed.abc2816



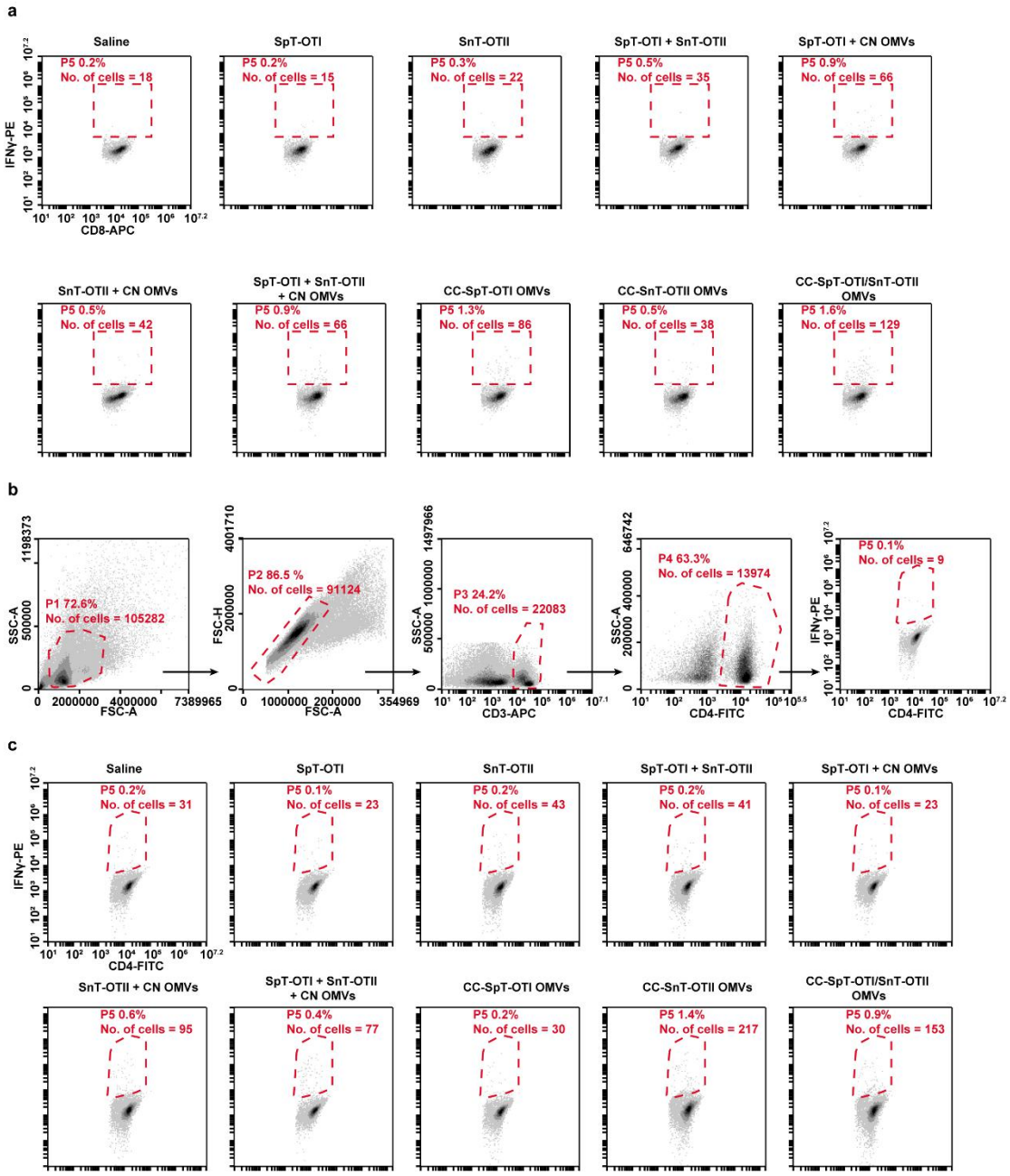
Supplementary Figure 1. **The evaluation of loading rates for the SpT or SnT-labelled antigens.** The SpT-OTI and SnT-OTII are displayed on the CC OMVs, respectively. CN OMVs are from the bacteria expressing ClyA without the fused Catchers (ClyA-none). The total proteins of the final OMV-based nanovaccines (containing 3 μg antigens and 3 μg CC OMVs) are extracted and analyzed by electrophoresis and Coomassie blue staining. According to the quantitative analysis of gray values of the bands in the gel using ImageJ software, a standard curve of gray values and antigen mass is established using the bands in the lane SpT-OTI (1, 2, 4 μg) and SnT-OTII (1, 2, 4 μg). Therefore, the mass of the unbound antigens in the CC-SpT-OTI OMVs and CC-SnT-OTII OMVs are calculated, ultimately evaluating the loading rates based on the total amount of antigen input (3 μg). **(a)** SpT-OTI. **(b)** SnT-OTII. Source data are provided as Supplementary information.



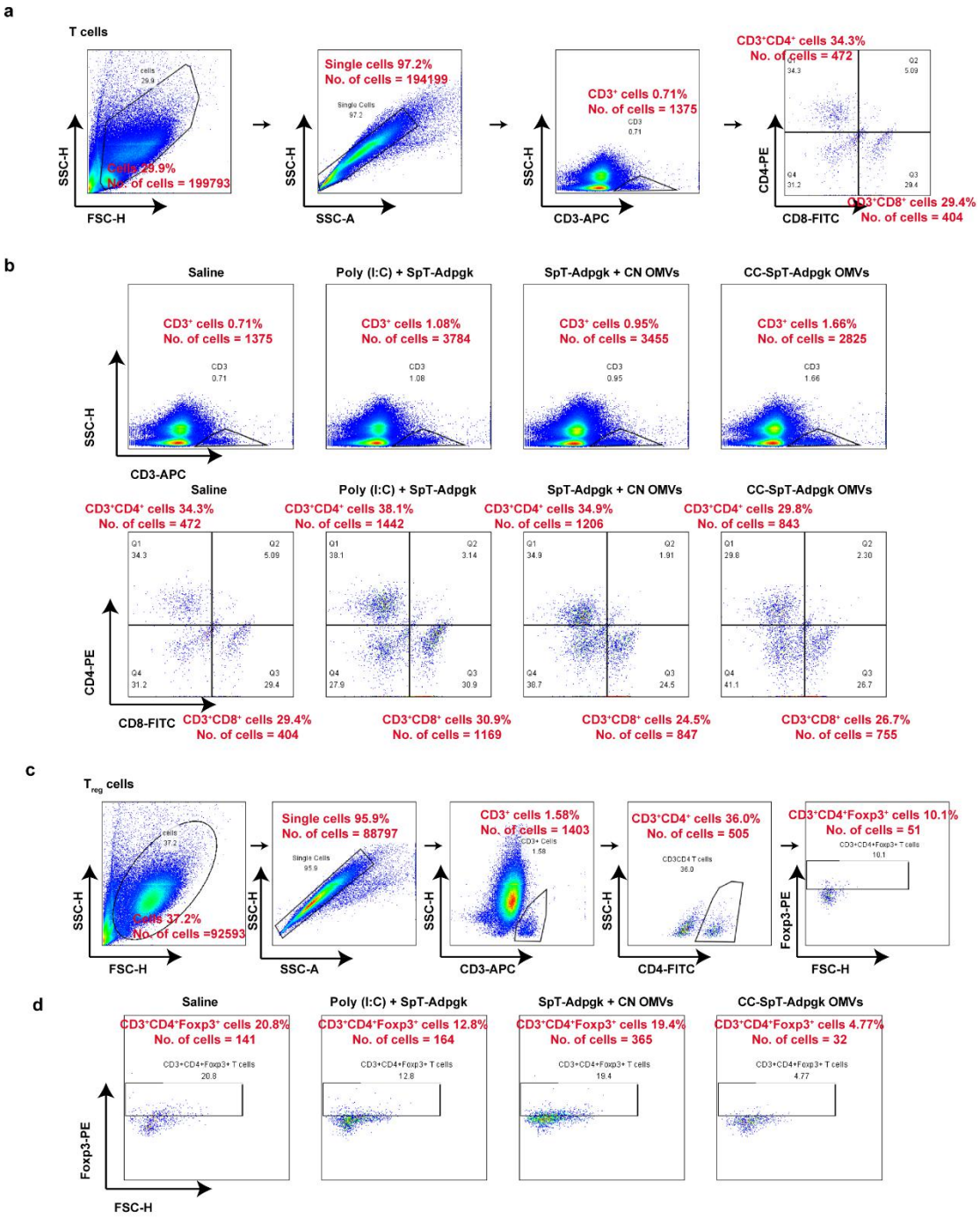
Supplementary Figure 2. **Gating strategy and representative scatter plots for analysis of maturation and antigen presentation in BMDCs (cf. Figure 3a-3c). (a)** Gating strategy for $CD80^+$ or $CD86^+$ BMDCs (cf. Figure 3a-3b). **(b)** Representative scatter plots for $CD80^+$ or $CD86^+$ BMDCs (cf. Figure3a-3b). **(c)** Gating strategy for MHCI-OVA^+ BMDCs (cf. Figure 3c). The data of flow cytometry in this protocol are collected using the BD Accuri C6 and analyzed using the BD Accuri C6 or FlowJo V10 software. Figure adapted with permission from Nat Commun 12, 2041 (2021).



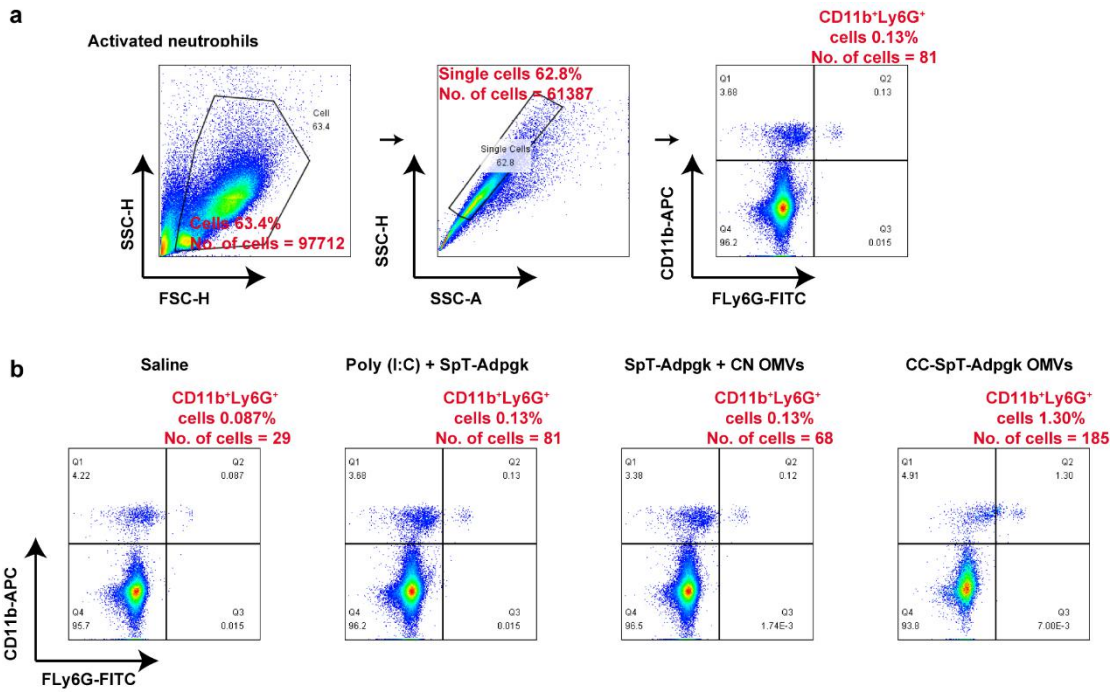
Supplementary Figure 3. **Gating strategy and representative scatter plots for antigen-specific CD8$^+$ T cells (cf. Figure 4c). (a)** Gating strategy for CD3$^+$CD8$^+$IFNγ$^+$ T cells (cf. Figure 4c). **(b)** Representative scatter plots for CD3$^+$CD8$^+$IFNγ$^+$ T cells (cf. Figure 4c). Figure adapted with permission from Nat Commun 12, 2041 (2021).



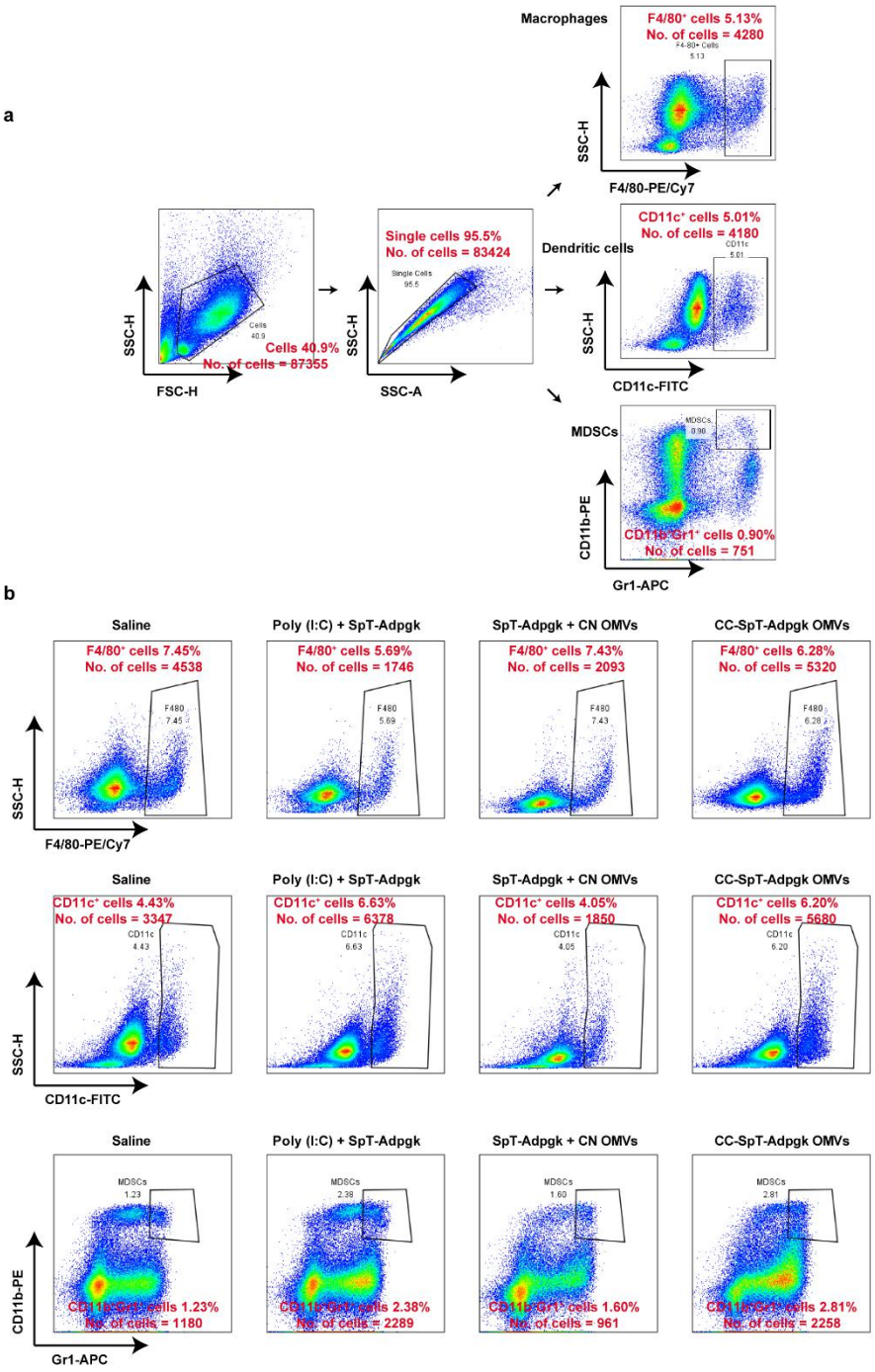
Supplementary Figure 4. **Gating strategy and representative scatter plots for antigen-specific CD8$^+$ and CD4$^+$ T cells (cf. Figure 5e-5f). (a)** Representative scatter plots for CD3$^+$CD8$^+$IFNγ$^+$ T cells. (cf. Figure 5e). **(b)** Gating strategy for CD3$^+$CD4$^+$IFNγ$^+$ T cells (cf. Figure 5f). **(c)** Representative scatter plots for CD3$^+$CD4$^+$IFNγ$^+$ T cells (cf. Figure 5f).



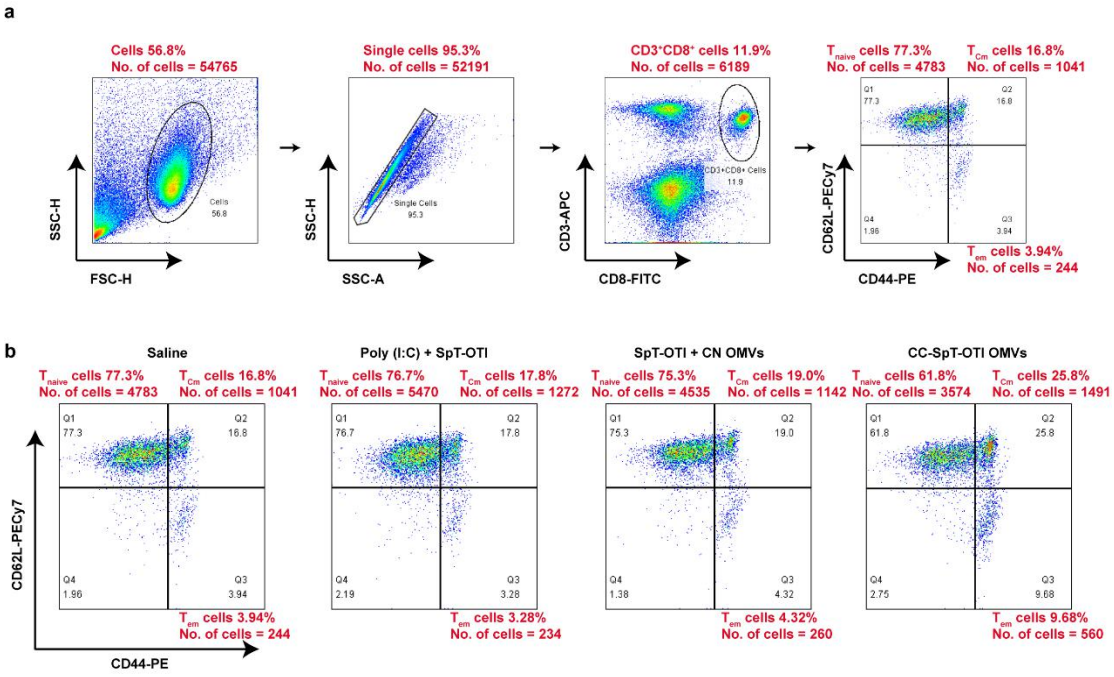
Supplementary Figure 5. **Gating strategy and representative scatter plots for T cells (cf. Figure 6c). (a)** Gating strategy for $CD3^+$, $CD3^+CD8^+$ and $CD3^+CD4^+$ T cells (cf. Figure 6c). **(b)** Representative scatter plots for $CD3^+$, $CD3^+CD8^+$ and $CD3^+CD4^+$ T cells (cf. Figure 6c). **(c)** Gating strategy for Treg cells ($CD3^+CD4^+Foxp3^+$ T cells) (cf. Figure 6c). **(d)** Representative scatter plots for Treg cells (cf. Figure 6c). The data in Figure 6c were the proportion of these cells in the "single cells" population. Figure adapted with permission from Nat Commun 12, 2041 (2021).



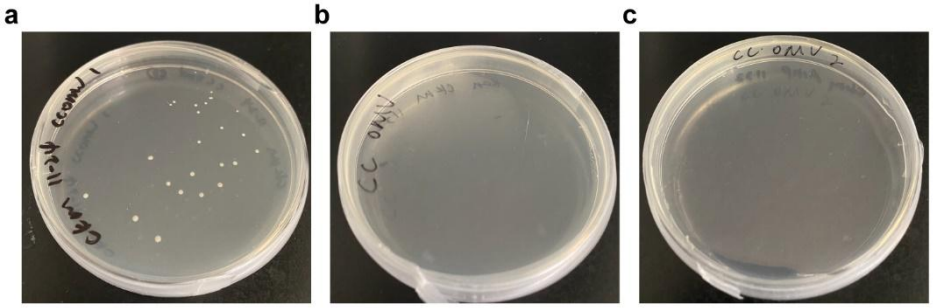
Supplementary Figure 6. Gating strategy and representative scatter plots for activated neutrophils (cf. Figure 6c). (a) Gating strategy for activated neutrophils ($CD11b^+Ly6G^+$ cells) (cf. Figure 6c). **(b)** Representative scatter plots (cf. Figure 6c). Figure adapted with permission from Nat Commun 12, 2041 (2021).



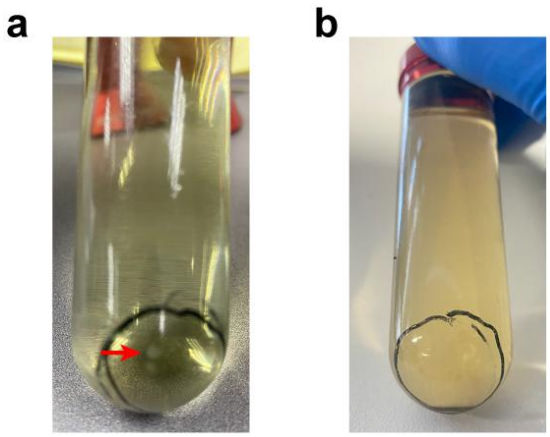
Supplementary Figure 7. **Gating strategy and representative scatter plots for macrophages, dendritic cells and MDSCs (cf. Figure 6c). (a)** Gating strategy for macrophages (F4/80$^+$ cells), dendritic cells (CD11c$^+$ cells), and MDSCs (CD11b$^+$Gr1$^+$ cells) (cf. Figure 6c). **(b)** Representative scatter plots (cf. Figure 6c). Figure adapted with permission from Nat Commun 12, 2041 (2021).



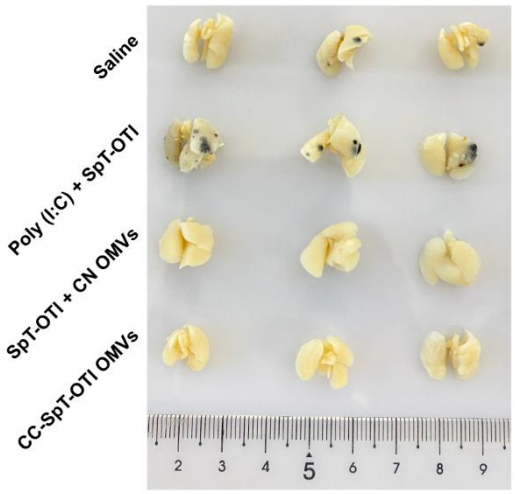
Supplementary Figure 8. **Gating strategy and representative scatter plots for immune memory cells (cf. Figure 7a). (a)** Gating strategy for naïve T cells ($T_{naïve}$, $CD3^+CD8^+CD44^-CD62L^+$), effector memory T cells (T_{em}, $CD3^+CD8^+CD44^+CD62L^-$) and central memory T cells (T_{cm}, $CD3^+CD8^+CD44^+CD62L^+$) (cf. Figure 7a). **(b)** Representative scatter plots (cf. Figure 7a). Figure adapted with permission from Nat Commun 12, 2041 (2021).



Supplementary Figure 9. **Representative images of problem 1 of Procedure 1 in the Troubleshooting. (a)** The correct bacteria plate. There are several dozens of colonies in a 10 cm plate. **(b) and (c)** There are very few bacteria colonies on the plate due to the using of wrong antibiotics (b) or the insufficient activity of competent cells (c).

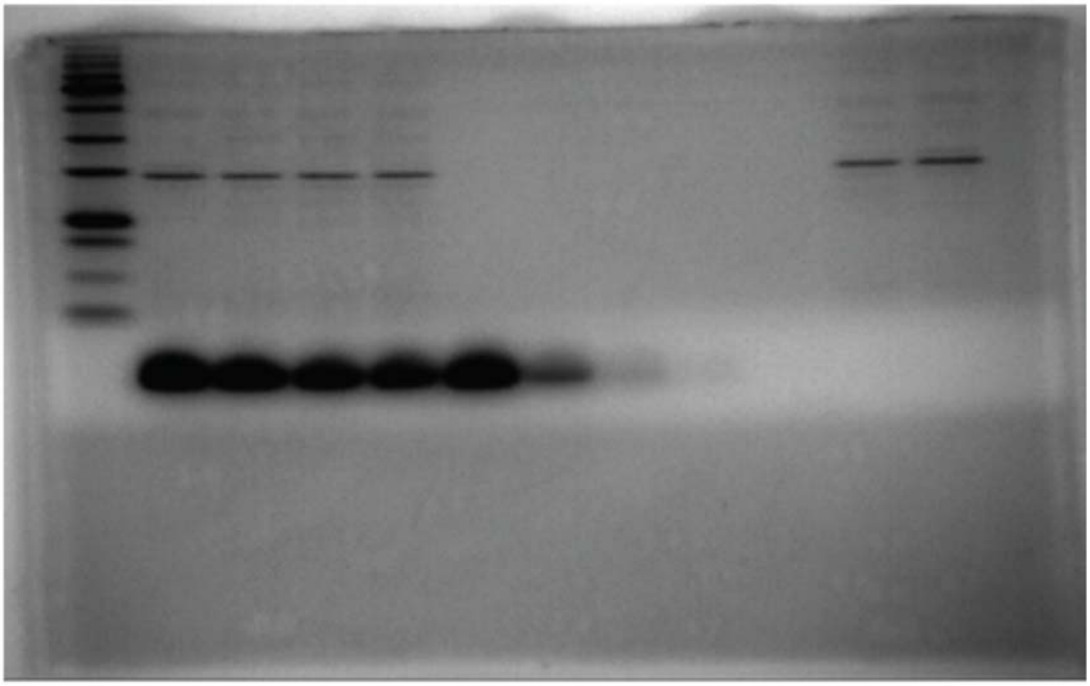


Supplementary Figure 10. **Representative images of problem 2 of Procedure 1 in the Troubleshooting. (a)** The correct extracted OMVs. After ultracentrifugation, there is an obvious white deposit at the bottom of the centrifuge tube as OMVs (red arrow). **(b)** The amount of extracted OMV is so low that no obvious white precipitate is visible.

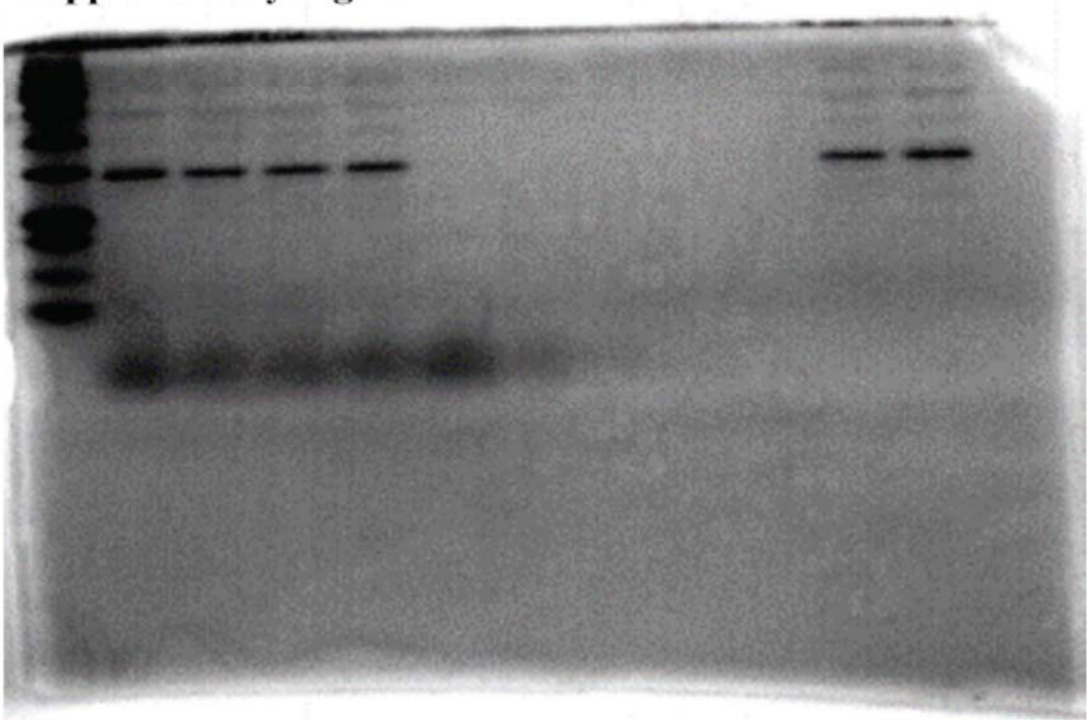


Supplementary Figure 11. **Representative images of problem 3 of Procedure 1 in the Troubleshooting.** Pulmonary metastases are so rare due to low viability of tumor cells.

Supplementary Fig. 1a



Supplementary Fig. 1b



Supplementary Data 1. Unprocessed blots for Supplementary Fig. 1a and 1b.