Supplementary Materials

Bacterial membrane vesicles as effective immunotherapeutic agents in melanoma, colon, and breast cancer

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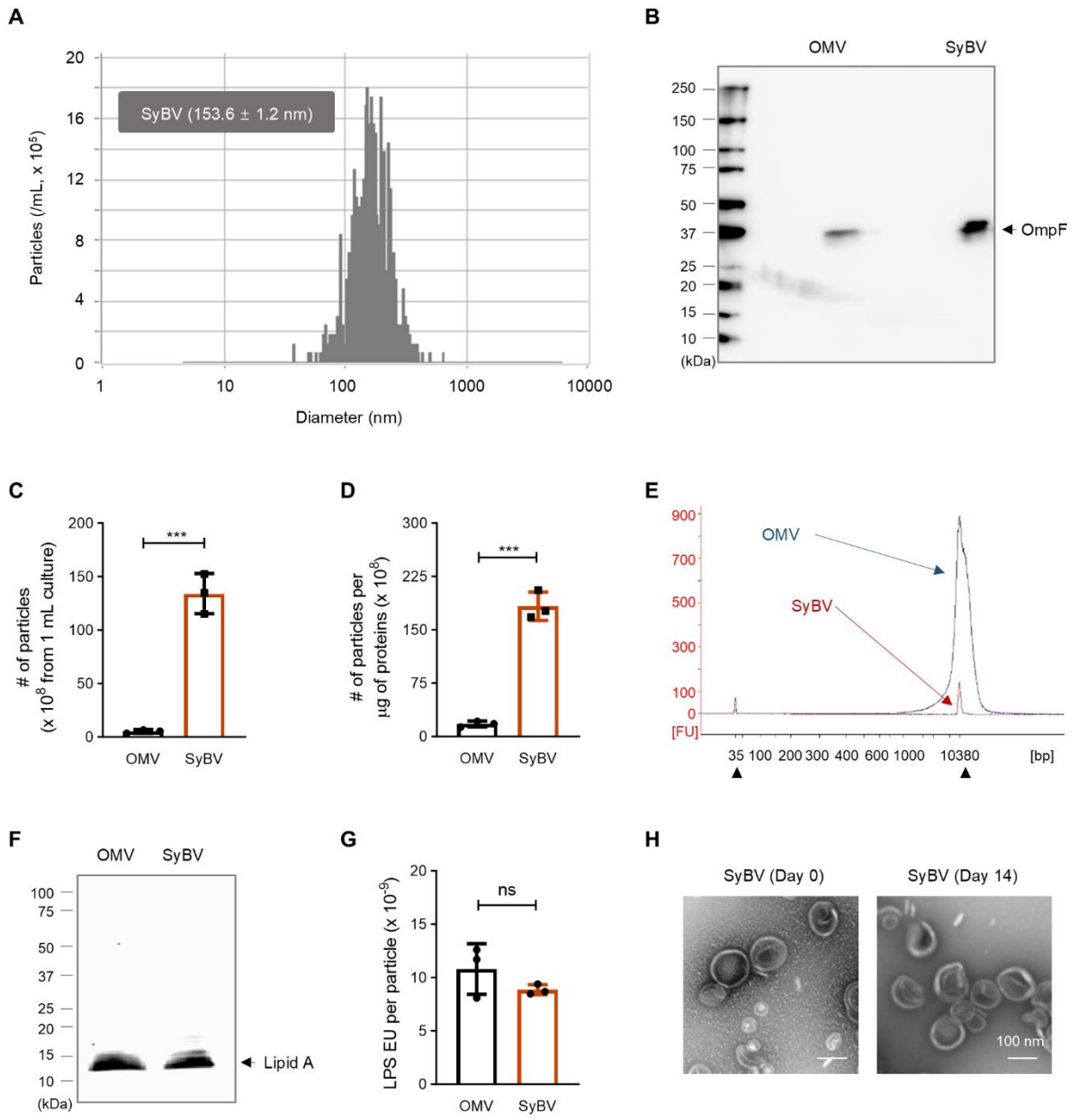
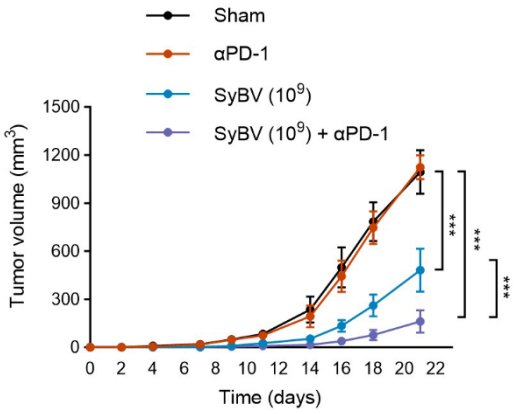
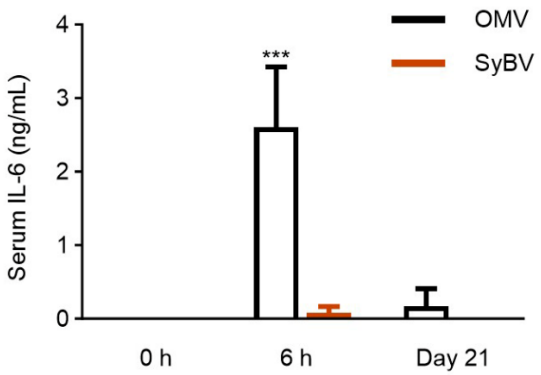


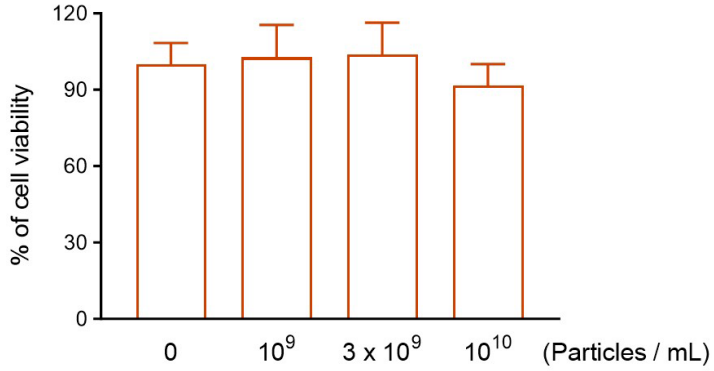
Supplementary Figure 1. Characterization of *E. coli*-derived SyBV. (A) Size distribution of SyBV measured by nanoparticle tracking analysisp; (B) Western blot analysis of OMV (10^9) and SyBV (10^9) with anti-OmpF antibody; (C and D) The number of particles derived from 1 mL bacterial culture (C) and the number of particles per one microgram of vesicular proteins (D) from OMV and SyBV. $n = 3$ / group. Data are presented as the mean ± SEM. $^{***}P < 0.001$ by unpaired two-tailed Student's *t*-test; (E) Representative electropherograms of DNA molecules isolated from SyBV in comparison to those from OMV. The filled triangles indicate internal markers; (F) Western blot analysis of OMV (10^9) and SyBV (10^9) with anti-lipid A antibody; (G) The LPS quantity as determined by the Limulus Amebocyte Lysate (LAL) assay. EU, enzyme unit. $n = 3$ / group. Data are presented as the mean ± SEM. ns, not significant by unpaired two-tailed Student's *t*-test; (H) TEM images comparing fresh SyBV (Day 0) with frozen SyBV after 14 days of storage. Scale bars, 100 nm.



Supplementary Figure 2. SyBV treatment synergizes with anti-PD-1 therapy to inhibit melanoma growth in mice. Mice were intratumorally immunized with SyBV (10^9) six times at 3-day intervals following B16F10 inoculation. Anti-PD-1 antibody (100 μg) was intraperitoneally given one day prior to immunization with SyBV. The tumor growth was monitored every 2 or 3 days. $n = 5$ / group. Data are presented as the mean ± SEM. $^{***}P < 0.001$ by two-way ANOVA with Tukey's post test.



Supplementary Figure 3. SyBV do not induce a systemic toxicity during immunization. Kinetics of serum interleukin (IL)-6 concentrations in mice administered with OMV (10^{10}) or SyBV (10^{10}). $n = 4$ / group. Data are presented as the mean ± SEM. $^{***}P < 0.001$ by two-way ANOVA with Tukey's post test versus 0 h group.



Supplementary Figure 4. SyBV do not directly affect the growth of tumor cells. The B16F10 cells were treated with various concentrations of SyBV for 24 h and cell viability was assessed by MTT assay. Results are expressed in percentage of control. n = 3 / group. Data are presented as the mean ± SEM.