Supplementary Information

Preparation of glycopeptide-modified pH-sensitive liposomes for promoting antigen cross-presentation and induction of antigen-specific cellular immunity

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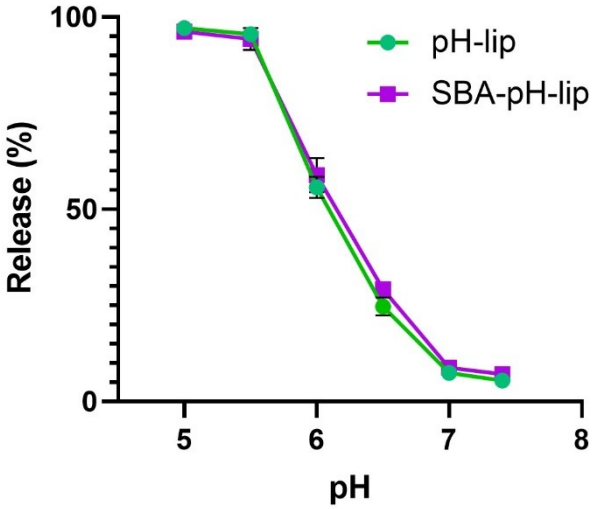

Figure S1. pH-Dependence of pyranine release after 30 min incubation at 37 °C from egg PC liposomes modified with or without SBA and/or pH-sensitive polysaccharide derivative (MGlu58-Dex-C10). Each point is the mean ± SEM (n = 3).

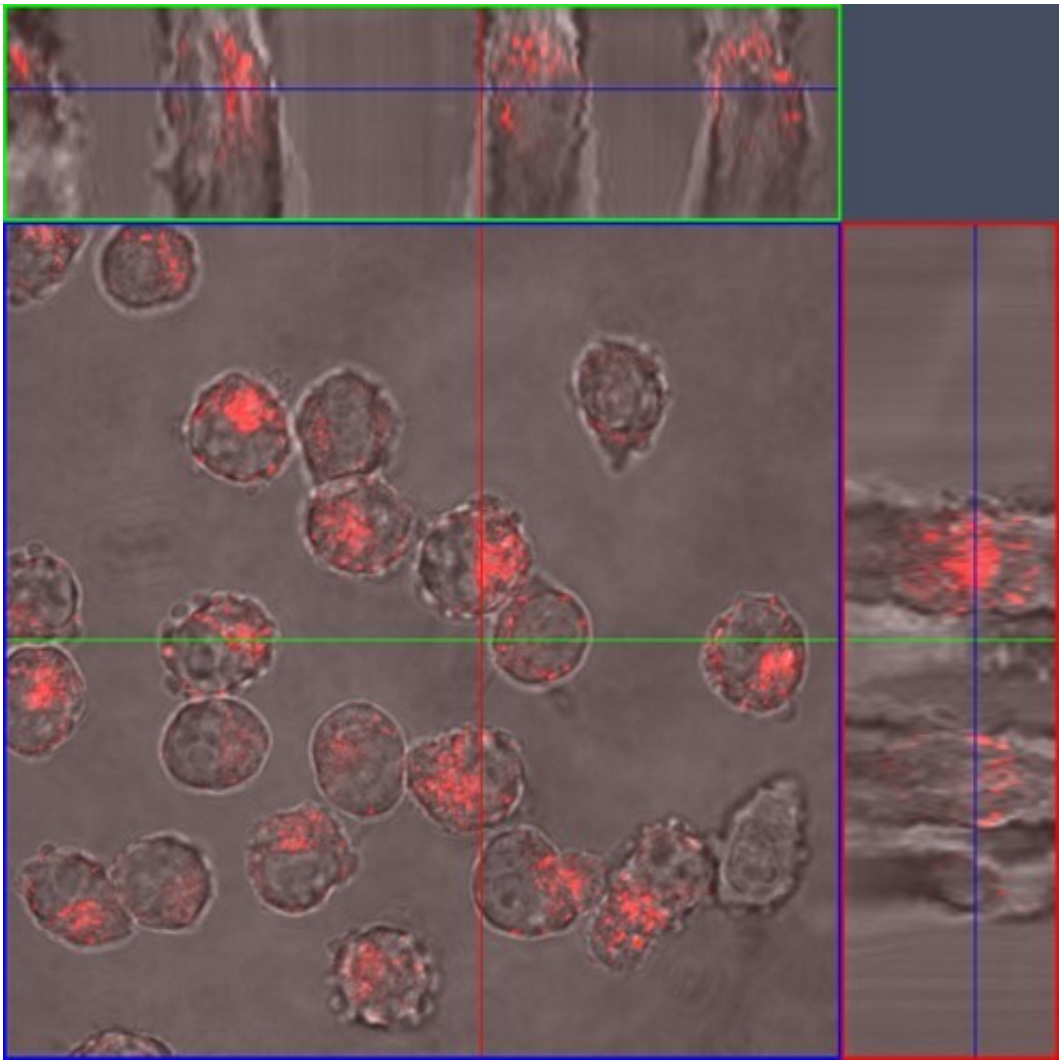

Figure S2. *Z*-stacked image of DC2.4 cells treated with SBA-pH-lip to confirm internalization of liposomes. DC2.4 cells were incubated in the presence of liposomes (0.3 mM lipids) for 5 h at 37 °C.

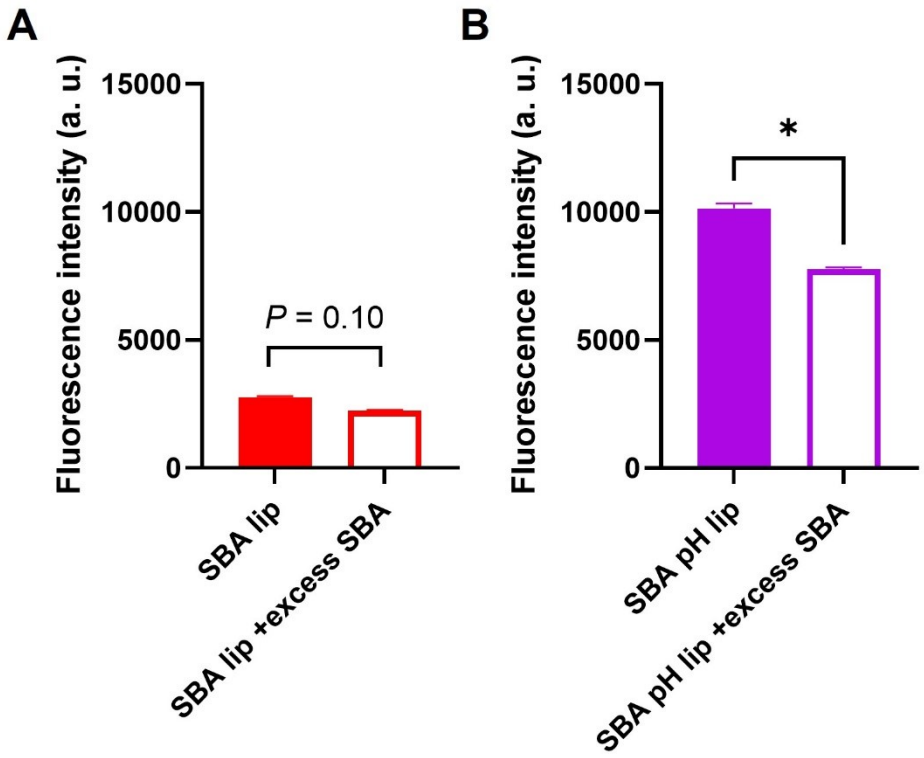

Figure S3. Effect of excess SBA for the cellular association of the liposomes. DiI-labeled SBA-lip (A) or SBA-pH-lip (B) were incubated with DC2.4 cells (lipid concentration: 0.3 mM) in the presence or absence of free SBA (1 mg/mL) for 4 h. Relative fluorescence intensity of DC2.4 cells were measured by a flow cytometry. Fluorescence intensity for untreated cells was subtracted. Statistical analyses were done using Student's *t*-test. **P* <0.05.

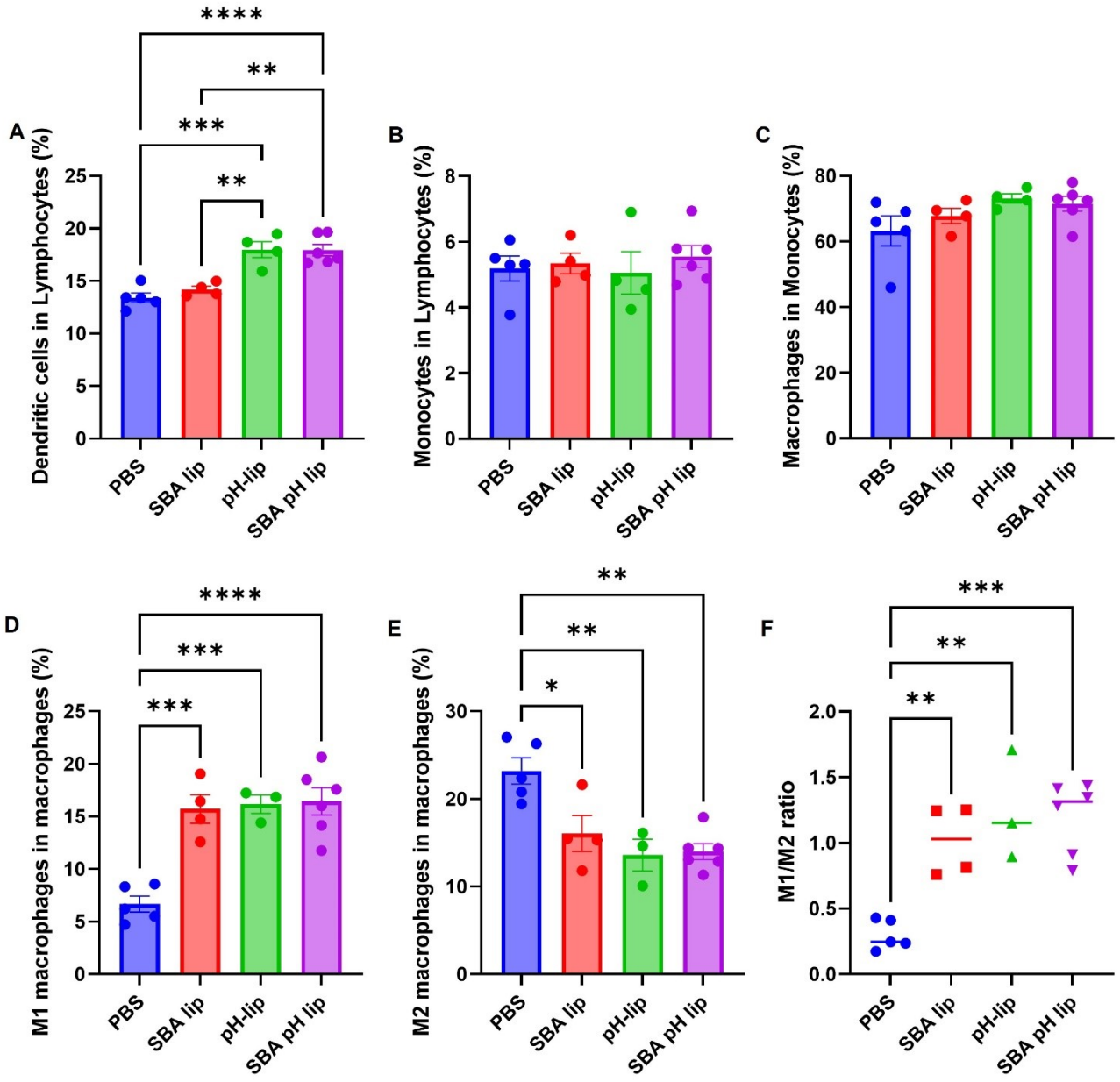

Figure S4. Effect of subcutaneous injection of liposomes on immune cell populations in the spleen. Mice were injected subcutaneously with PBS or liposomes on days 7 and 14. Single cells were extracted from the spleen on day 15, followed by flow cytometric analysis. Graphs depict the frequency of (A) CD11c+ dendritic cells within CD45+ lymphocytes, (B) CD11b+ monocytes within CD45+ lymphocytes, (C) F4/80+ macrophages within CD11b+ CD45+ monocytes. (D) MHC-II+ M1 macrophage within macrophages, (E) CD206+ M2 macrophage within macrophages and (F) M1/M2 ratio of macrophages. (mean ± SEM; n = 3-6) Statistical analyses were done using ANOVA with Tukey's test: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

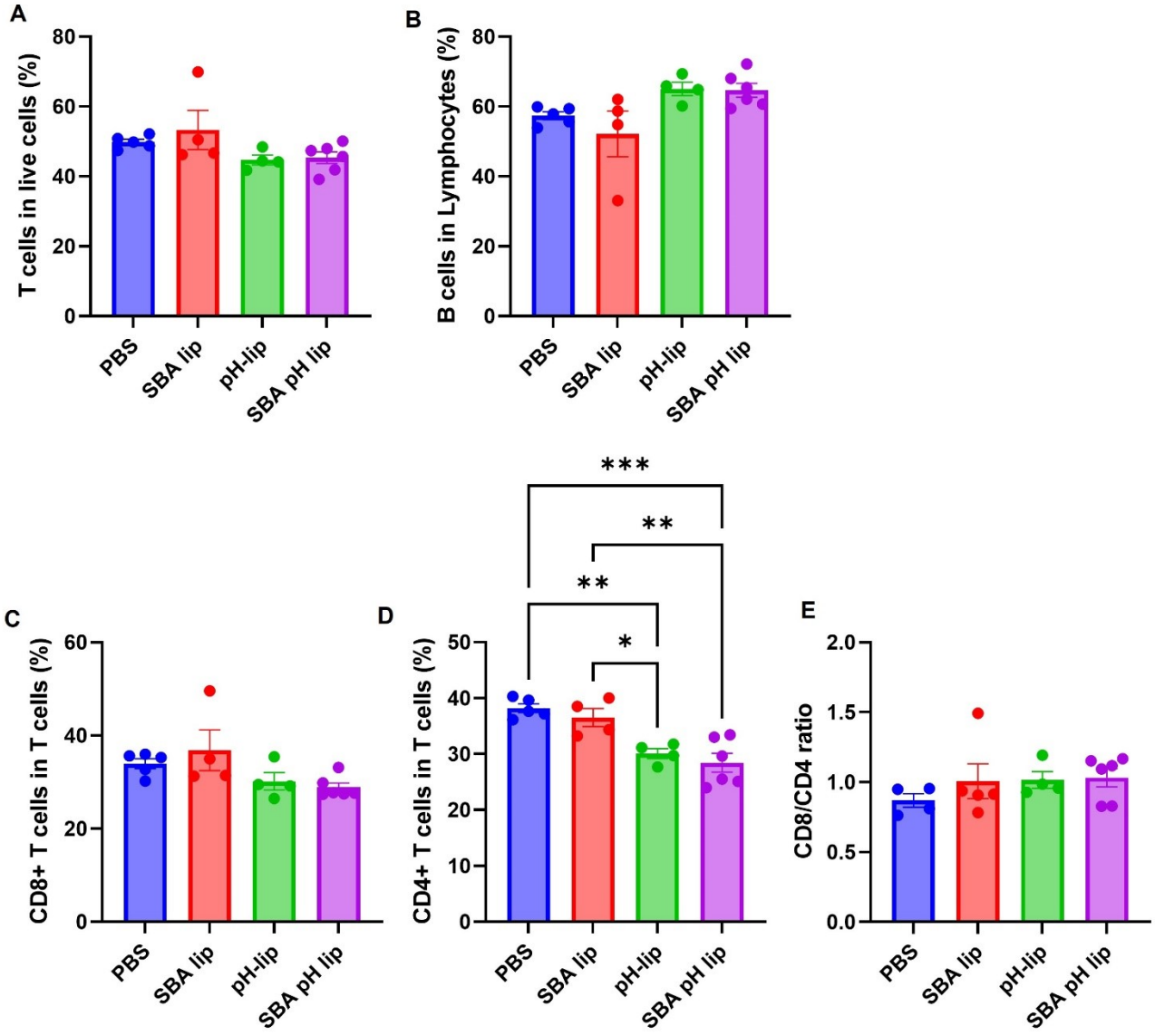

Figure S5. Effect of subcutaneous injection of liposomes on immune cell populations in the spleen. Mice were injected subcutaneously with PBS or liposomes on days 7 and 14. Single cells were extracted from the spleen on day 15, followed by flow cytometric analysis. Graphs depict the frequency of (A) CD3+ T cells within live cells, (B) B220+ B cells within CD45+ lymphocytes, (C) CD8+ T cells within CD3+ cells, (D) CD4+ T cells within CD3+ cells, and (E) CD8/CD4 ratios. (mean ± SEM; n = 4-6) Statistical analyses were done using ANOVA with Tukey's test: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

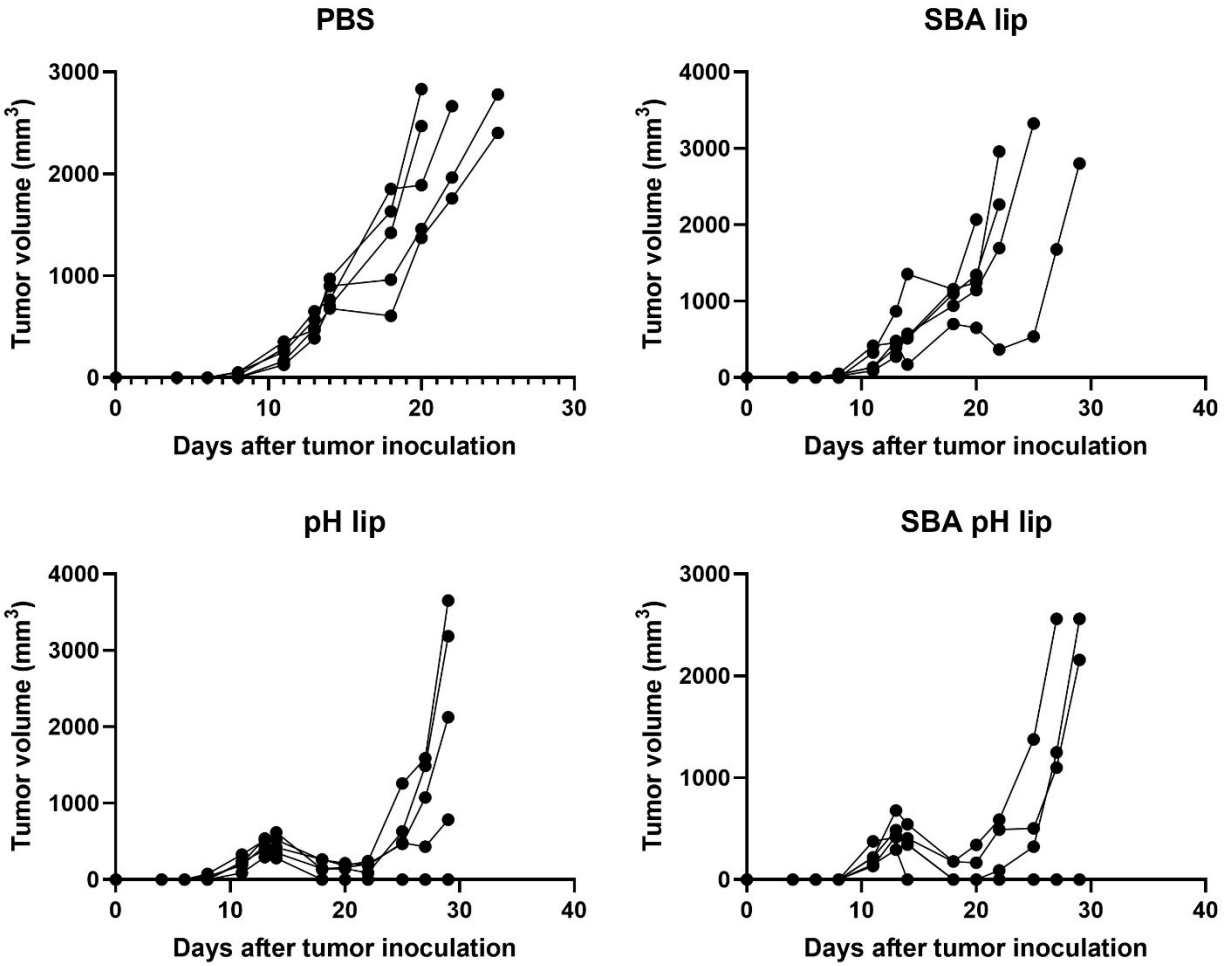

Figure S6. Individual tumor volume changes for Figure 8.

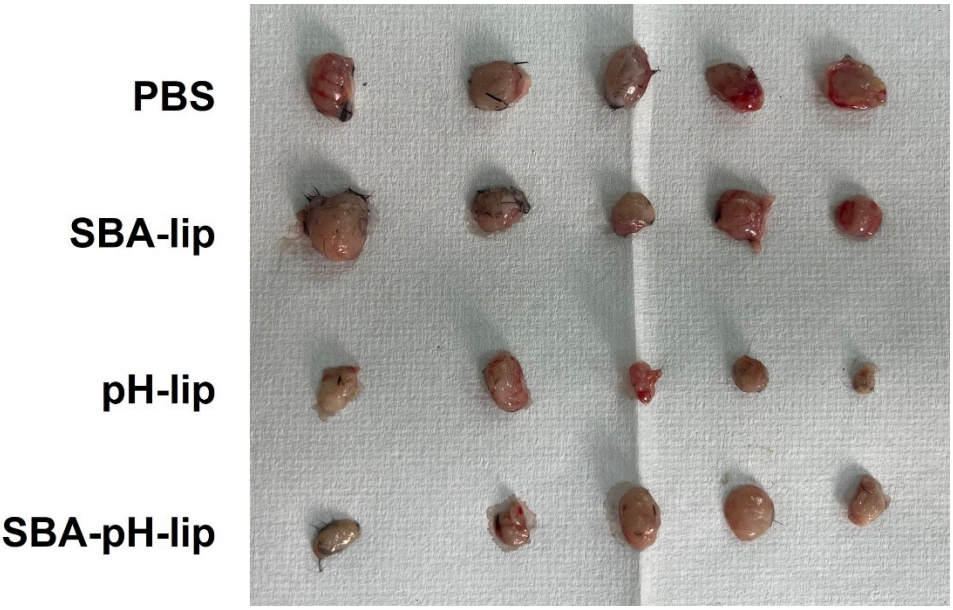

Figure S7. Images for excised tumor from tumor-bearing mice on Day 13. PBS or OVA-loaded liposomes were subcutaneously injected to the mice on Day 7.

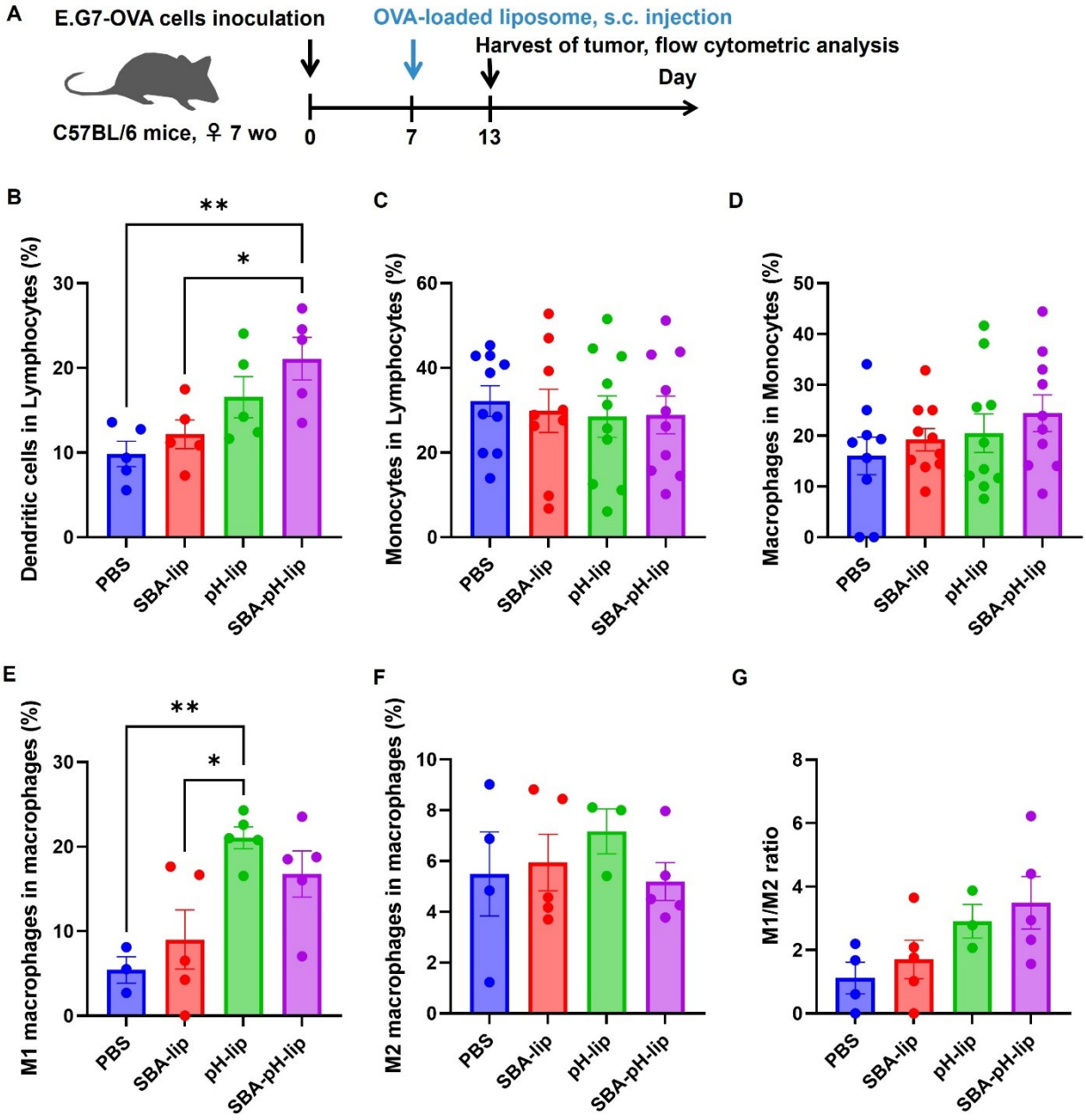

Figure S8. Effect of subcutaneous injection of liposomes on immune cell populations in the tumor. (A) Experimental schedule. E.G7-OVA (5×10^5/mouse) were subcutaneously inoculated into the left backs of C57BL/6 mice and tumor volume on day 0. Mice were subcutaneously injected 50 mg of OVA on day 7 with OVA-loaded liposomes. Single cells were extracted from the tumor on day 13, followed by flow cytometric analysis. Graphs depict the frequency of (B) CD11c+ dendritic cells within CD45+ lymphocytes, (C) CD11b+ monocytes within CD45+ lymphocytes, (D) F4/80+ macrophages within CD11b+ CD45+ monocytes. (E) MHC-II+ M1 macrophage within macrophages, (F) CD206+ M2 macrophage within macrophages and (G) M1/M2 ratio of macrophages. (mean ± SEM; n = 3-10) Statistical analyses were done using ANOVA with Tukey's test *P < 0.05 and **P<0.01.

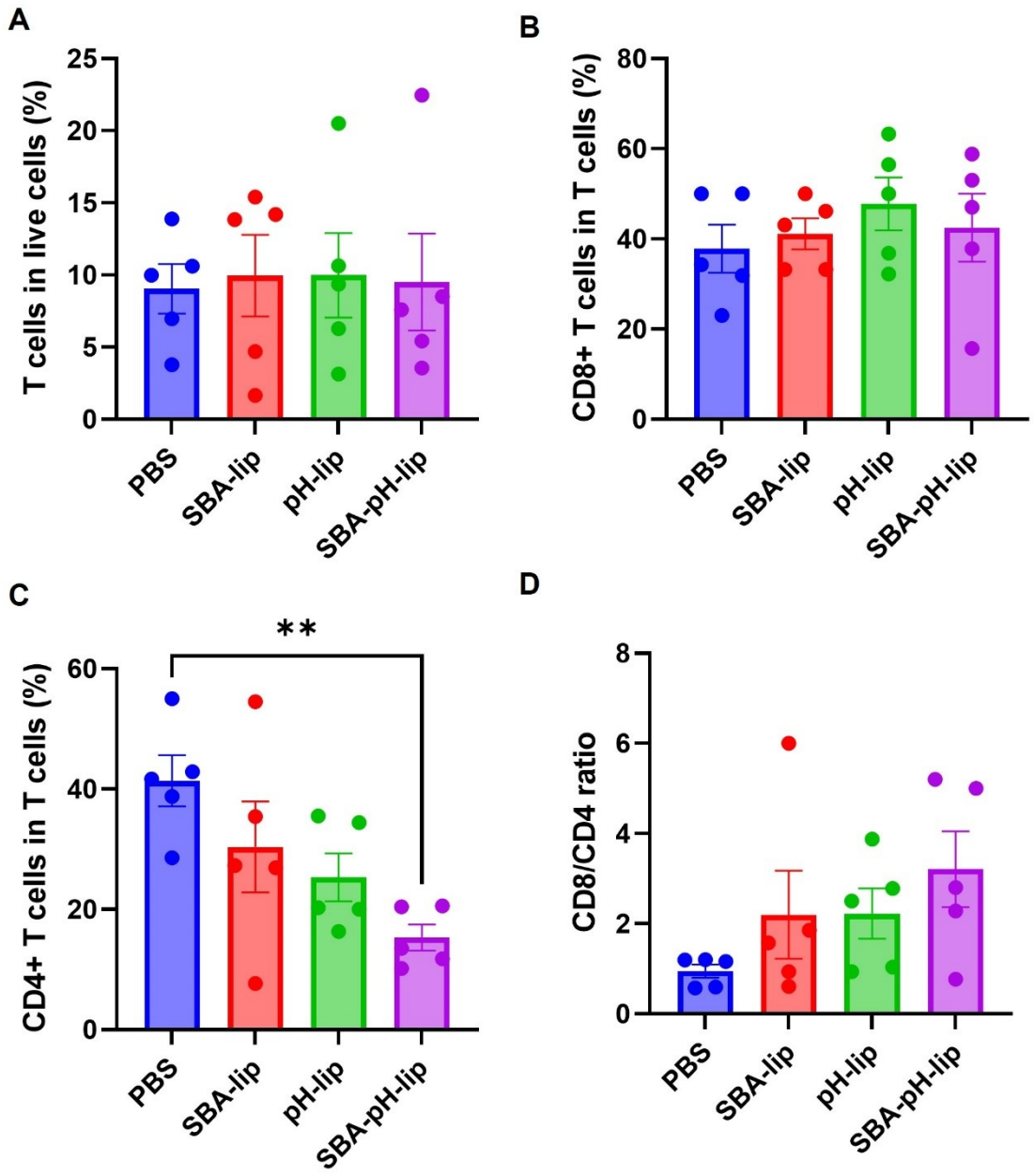

Figure S9. Effect of subcutaneous injection of liposomes on immune cell populations in the tumor. E.G7-OVA (5×10^5/mouse) were subcutaneously inoculated into the left backs of C57BL/6 mice and tumor volume on day 0. Mice were subcutaneously injected 50 mg of OVA on day 7 with OVA-loaded liposomes. Single cells were extracted from the tumor on day 13, followed by flow cytometric analysis. Graphs depict the frequency of (A) CD3+ T cells within live cells, (B) CD8+ T cells within CD3+ cells, (C) CD4+ T cells within CD3+ cells, and (D) CD8/CD4 ratios. (mean ± SEM; $n = 5$) Statistical analyses were done using ANOVA with Tukey's test. $^{**}P<0.01$.

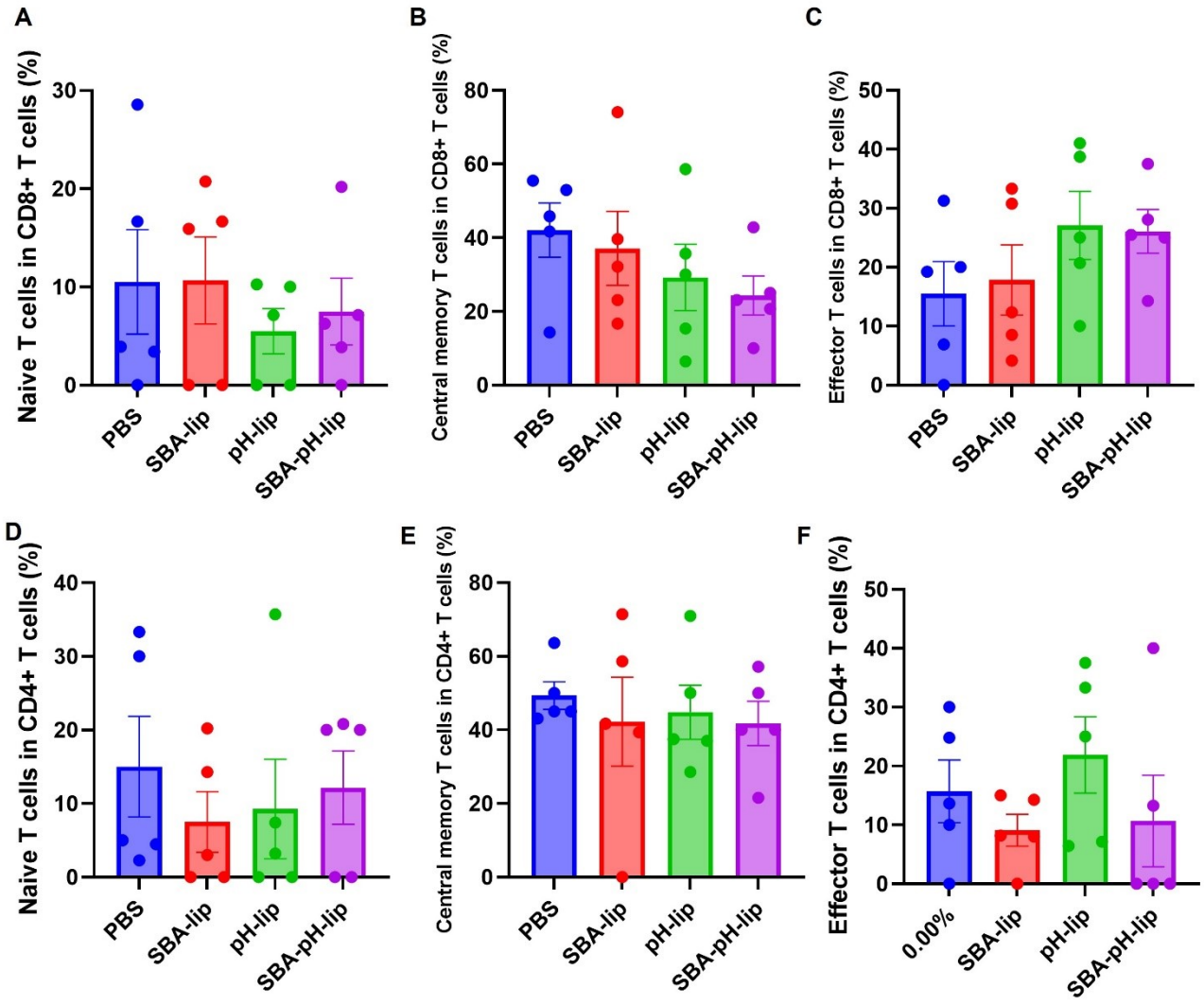

Figure S10. Effect of subcutaneous injection of liposomes on immune cell populations in the tumor. E.G7-OVA (5×10^5/mouse) were subcutaneously inoculated into the left backs of C57BL/6 mice and tumor volume on day 0. Mice were subcutaneously injected 50 mg of OVA on day 7 with OVA-loaded liposomes. Single cells were extracted from the tumor on day 13, followed by flow cytometric analysis. Graphs depict the frequency of (A) naïve CD8+ T cells (CD62L+ CD44-) of CD3+ cells, (B) central memory CD8+ T cells (CD62L+ CD44+) of CD3+ cells, (C) effector memory CD8+ T cells (CD62L- CD44+) of CD3+ cells, (D) naïve CD4+ T cells (CD62L+ CD44-) of CD3+ cells, (E) central memory CD4+ T cells (CD62L+ CD44+) of CD3+ cells, (F) effector memory CD4+ T cells (CD62L- CD44+) of CD3+ cells. (mean ± SEM; $n = 5$)