Supporting Information for

ORIGINAL ARTICLE

An mRNA vaccine elicits STING-dependent antitumor immune responses

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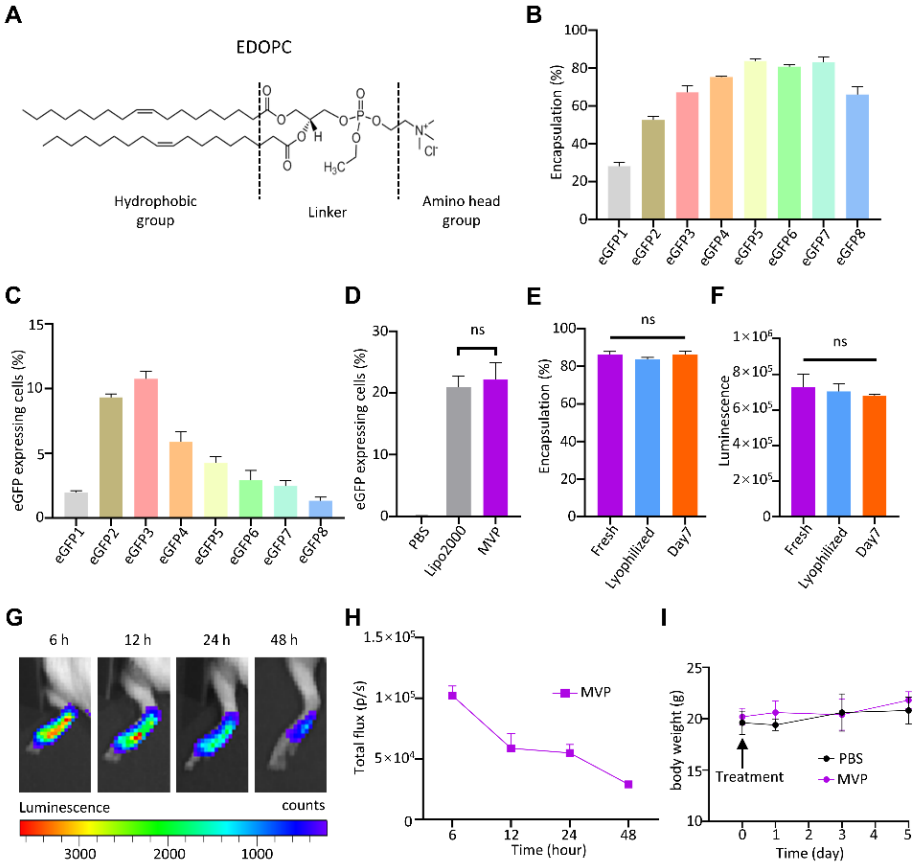

Figure S1 Characterization of mRNA-encapsulated nanoparticles. (A) Chemical structure of EDOPC. (B) Percentage of eGFP mRNA encapsulation by nanoparticles prepared with different lipid compositions (details in Table 1). (C) eGFP expression in DC2.4 cells treated with eGFP mRNA-encapsulated nanoparticles. (D) eGFP expression in DC2.4 cells transfected with eGFP mRNA using Lipofectamine 2000 (Lipo2000: mRNA 3:1) or treated with MVP-encapsulated eGFP mRNA. (E) Encapsulation efficiency of MVP-encapsulated Luc mRNA. (F) Samples were either freshly prepared (Fresh), lyophilized (Lyophilized), or frozen at −20 °C for 7 days (Day 7). (G and H) Time-dependent luciferase (Luc) expression in BALB/c mice treated with Luc mRNA-encapsulated MVP in the footpads. (I) Body weight change of BALB/c mice treated with PBS control or Luc mRNA-encapsulated MVP. Error bars: mean±SEM (*n*=3). ns, not significant.

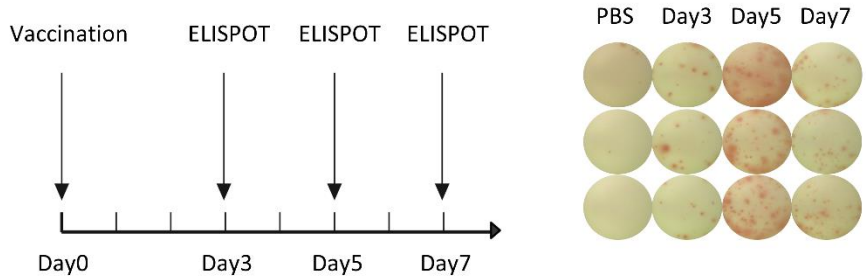

Figure S2 ELISpot assay. C57BL/6J mice were treated with OVA mRNA-encapsulated MVP, and lymph nodes were collected at the indicated time points. Cells were isolated from the tissues, and applied for ELISpot analysis to measure IFN-γ-expressing cells. Left panel: experimental design. Right panel: images of spots.

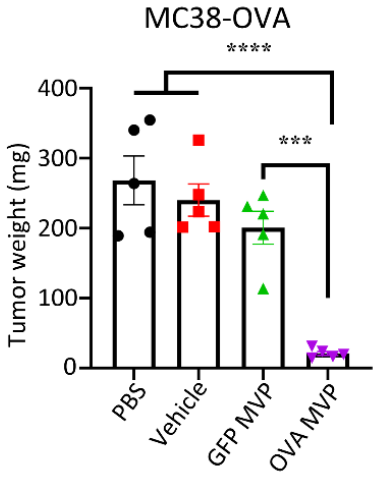

Figure S3 Inhibition of MC38-OVA tumor growth. Female C57BL/6J mice were inoculated subcutaneously with 5×10^5 MC38-OVA tumor cells/mouse on Day 0. They were treated with PBS control, mRNA-free vehicle control, GFP mRNA-encapsulated MVP, or OVA mRNA-encapsulated MVP on Days 3 and 10. Mice were euthanized on Day 13, and tumors were collected and weighed. Error bars: mean±SEM (n=5). $^{***}P<0.005$; $^{****}P<0.001$.

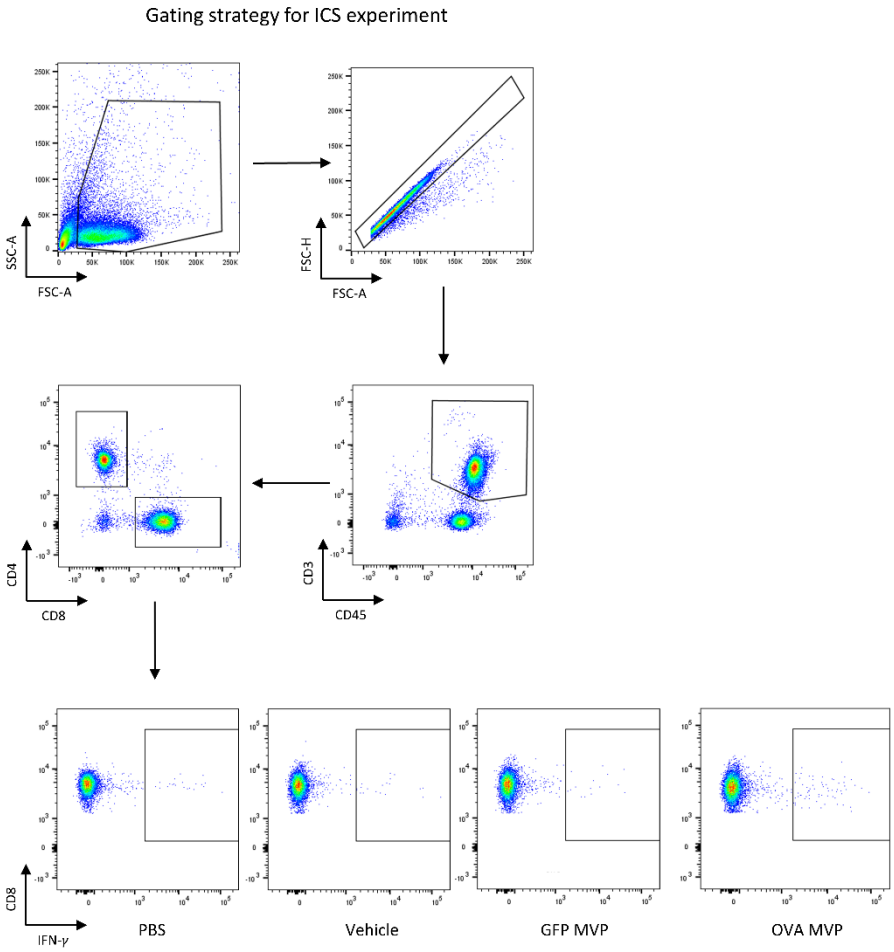

Figure S4 Gating strategy for flow cytometry analysis of IFN-γ-expressing T cells.

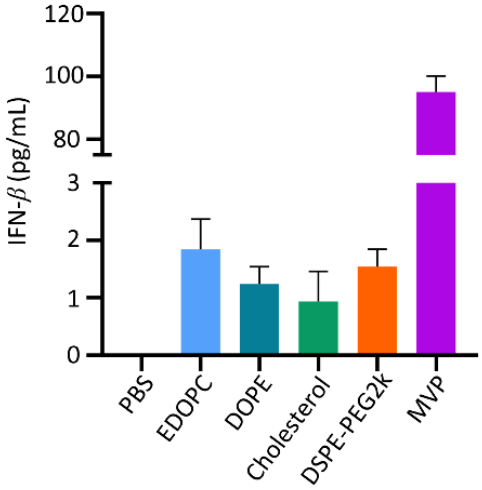

Figure S5 IFN-β expression in BMDCs treated with individual lipid components. BMDCs were treated either with 1 mg/mL mRNA-equivalent MVP (as positive control) or an equivalent dose of EDOPC, DOPE, cholesterol, or DSPE-PEG2k for 24 h. Cell growth media were collected to measure IFN-β expression with ELISA. Error bars: mean±SEM (n=3).

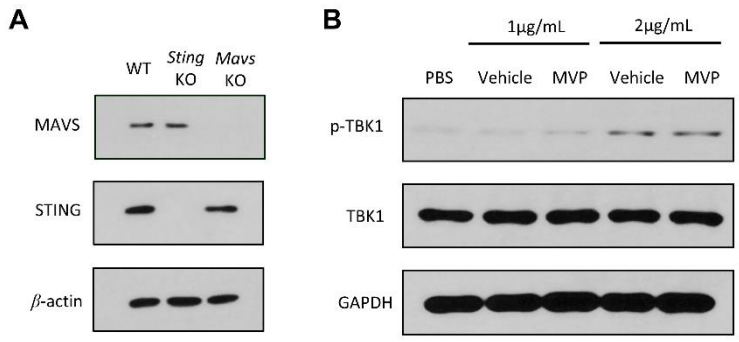

Figure S6 Western blot analysis. (A) Western blot analysis on MAVS and STING expression in BMDCs collected from WT, *Sting* KO and *Mavs* KO mice. (B) Western blot analysis on TBK1 phosphorylation after BMDCs from WT mice were treated with PBS control, vehicle, or OVA mRNA-encapsulated MVP for 2 h.