Supporting information

Lipid Platform Increases the Activity of STING Agonist to Synergize Checkpoint Blockade Therapy Against Melanoma

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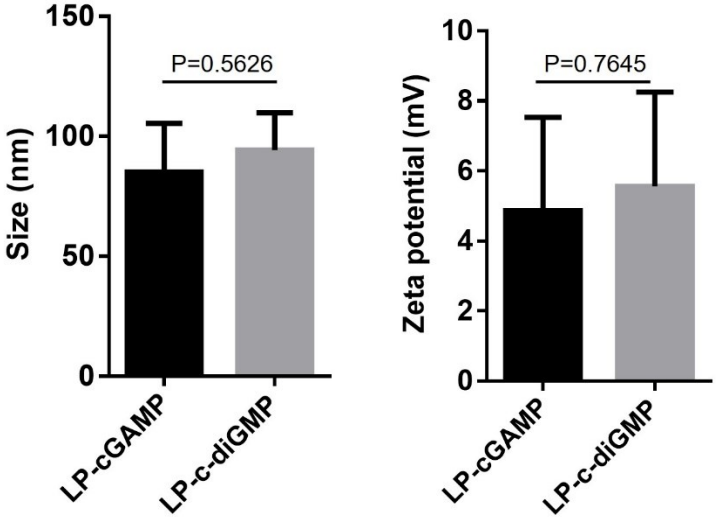
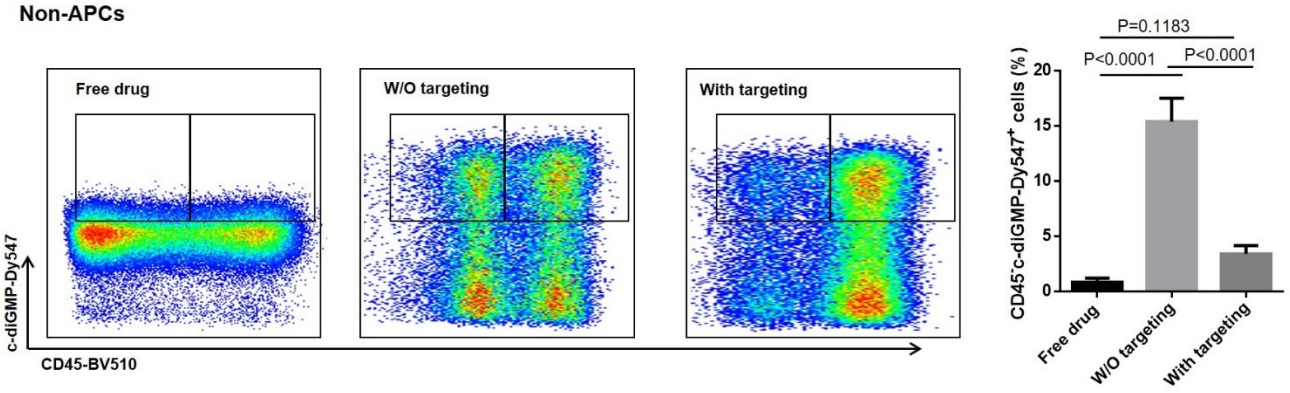
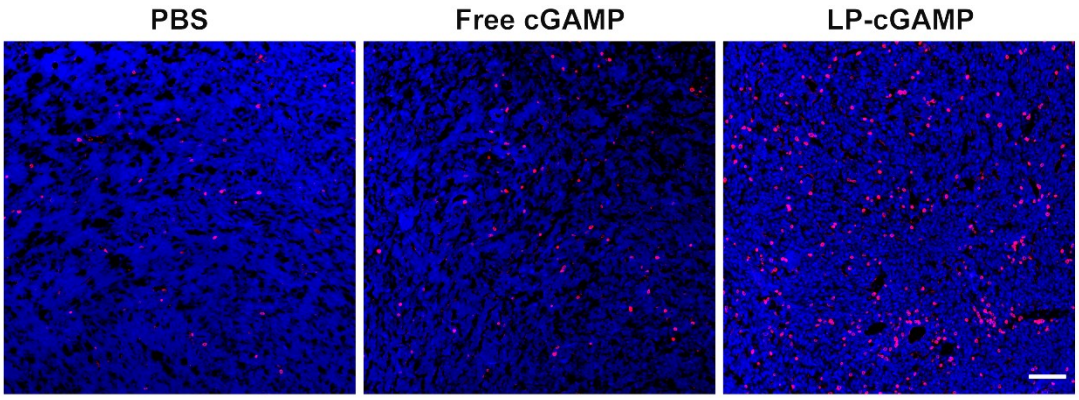


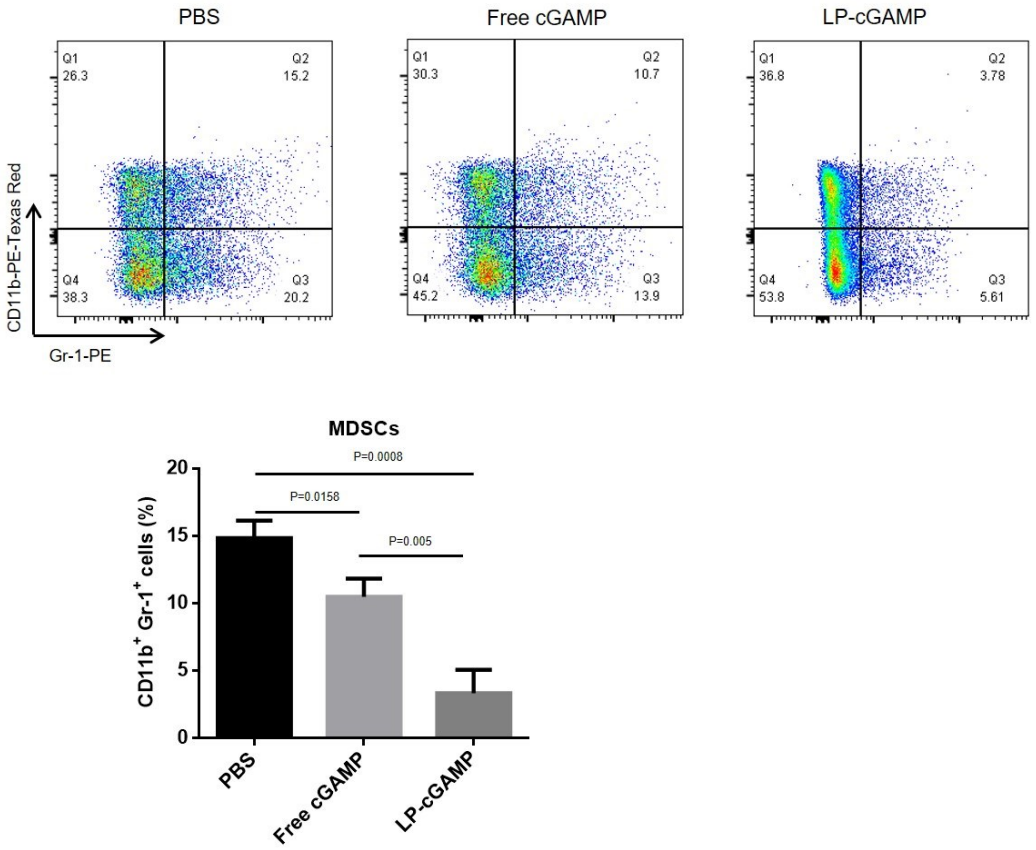
Supplementary Figure 1. Particle size and zeta potential characterization of LP-cGAMP and LP-c-diGMP by DLS (n=3). Results are presented as mean (SD).



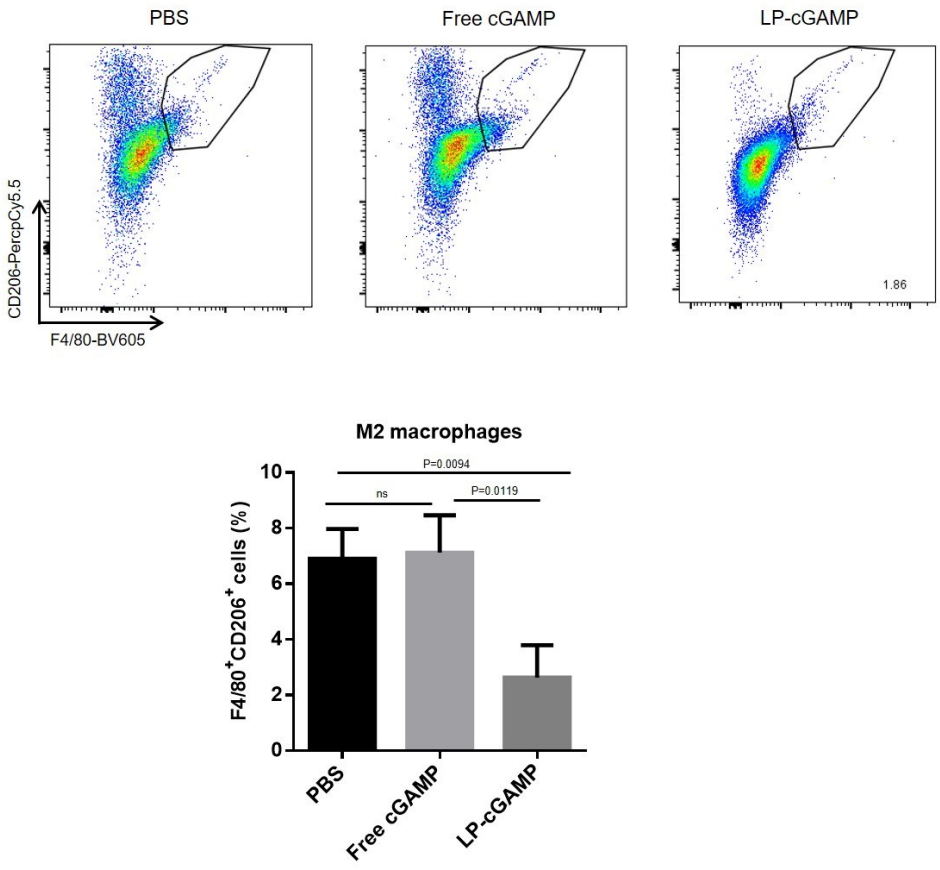
Supplementary Figure 2. Cellular uptake of c-diGMP in non-APC ($CD45^-$) cells inside the tumor tissue analyzed by flow cytometry after administration with targeting or non-tarteing LP nanoparticles. The tumor tissues were collected after single injection of indicated formulations 4 hours, as a dose corresponding to 5 μg/mL of c-diGMP (i.t.). BV510 anti-CD45 was used to label lymphocytes. Representative flow cytometry dot was shown in Left and the quantification was shown in Right for each treated group.



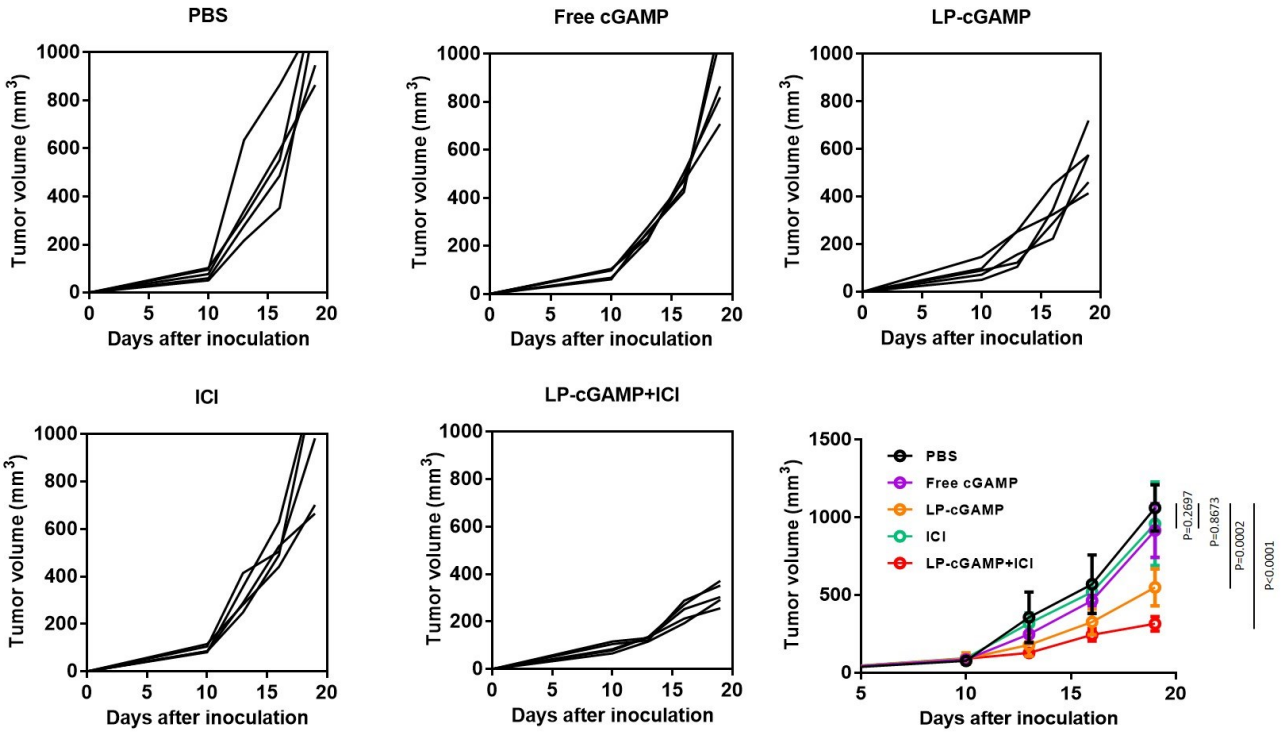
Supplementary Figure 3. Immunofluorescence staining of V600E BRAF melanoma on day 7 after PBS, free cGAMP, or LP-cGAMP treatments using anti-CD3 (red) and DAPI (blue). Bar represents 100 μm.



Supplementary Figure 4. Representative flow cytometry dots and quantification of the percentage of MDSCs ($CD11b^+Gr1^+$) after the indicated administration (n=3 biologically independent samples, respectively; Significant differences were assessed using *t* test).



Supplementary Figure 5. Representative flow cytometry dots and quantification of the percentage of M2 macrophages ($F4/80^+CD206^+$) after the indicated administration (n=3 biologically independent samples, respectively; Significant differences were assessed using *t* test).



Supplementary Figure 6. Combination of LP-cGAMP and ICI therapy synergistically suppresses the progression of B16F10 melanoma. Spider plots of individual tumor growth curves in each treatment group as well as average tumor volumes in different treatment groups. Mice with about 80 mm^3 subcutaneous tumors were administered with PBS, free cGAMP, LP-cGAMP, ICI, and LP-cGAMP plus ICI therapy every 3 days, in total 3 doses. n=5 biologically independent samples; two-way ANOVA with multiple comparisons.

Table S1. Antibodies used in this Study

Antibodies	Company	Catalog #	Application
PE anti-Gr-1	Biolegend	108409	Flow cyt
PE-Texas Red anti-CD11b	BD	562317	Flow cyt
PercpCy5.5 anti-CD206	Biolegend	141715	Flow cyt
BV605 anti-F4/80	Biolegend	123133	Flow cyt
PE anti-CD11c	Biolegend	117307	Flow cyt
PE-Cy7 anti-CD11c	Biolegend	117317	Flow cyt
PercpCy5.5 anti-MHCII	Biolegend	116415	Flow cyt
PercpCy5.5 anti-CD45	Biolegend	103131	Flow cyt
APC-Cy7 anti-CD3	Biolegend	100221	Flow cyt
FITC anti-CD4	Biolegend	100405	Flow cyt
Pacific Blue anti-CD8	Biolegend	100728	Flow cyt
Alexa 488 anti-CD274	Invitrogen	53-5982-82	Flow cyt

Table S2. Primers used in the study

Primer	Applied Biosystems
Mouse IFN-β	Mm00439552_s1
Mouse IL12	Mm00434169_m1
Mouse CD86	Mm00444540_m1
Mouse GAPDH	Mm99999915_g1