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Supplemental Information

Modifying Antigen-Encapsulating Liposomes with KALA Facilitates MHC Class I Antigen Presentation and Enhances Anti-tumor Effects

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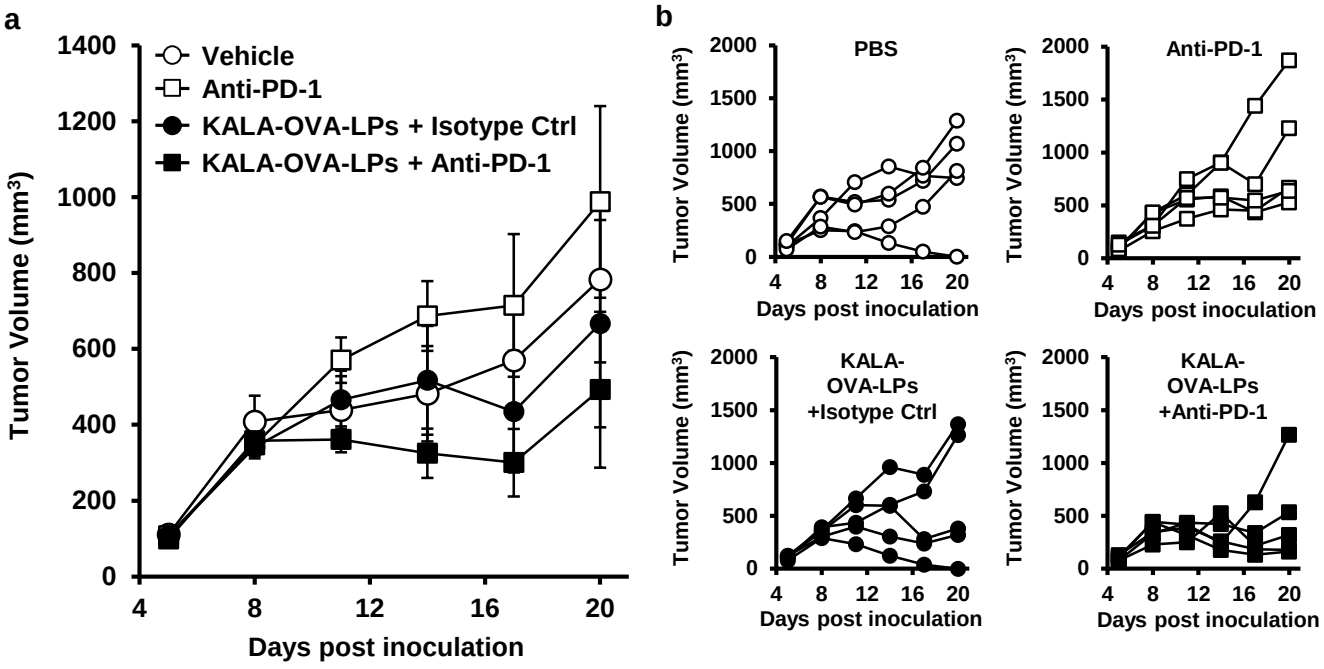
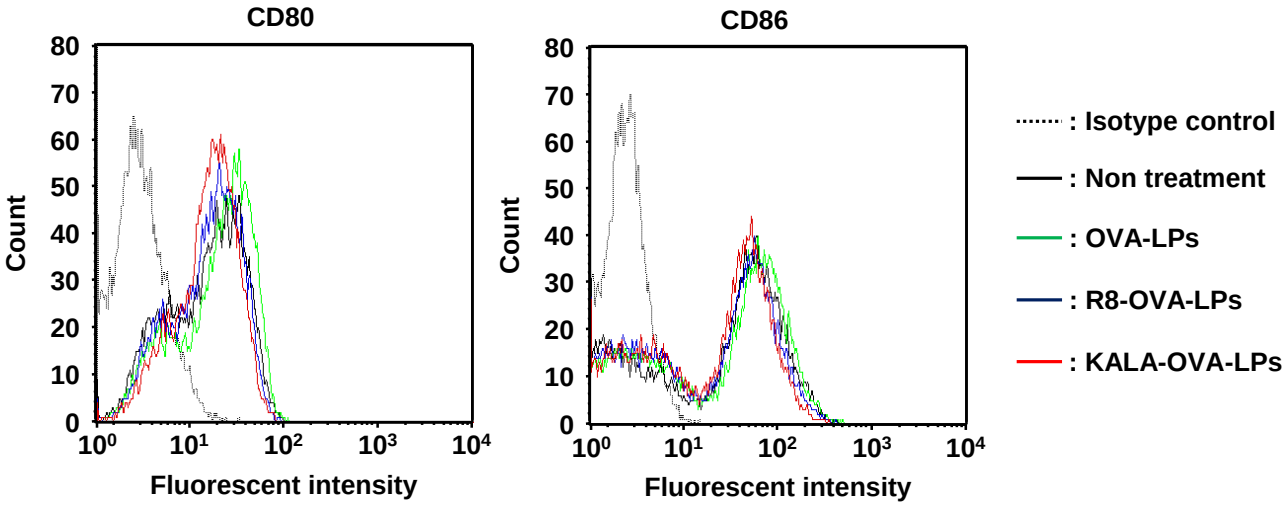
Supplementary Figure 1.



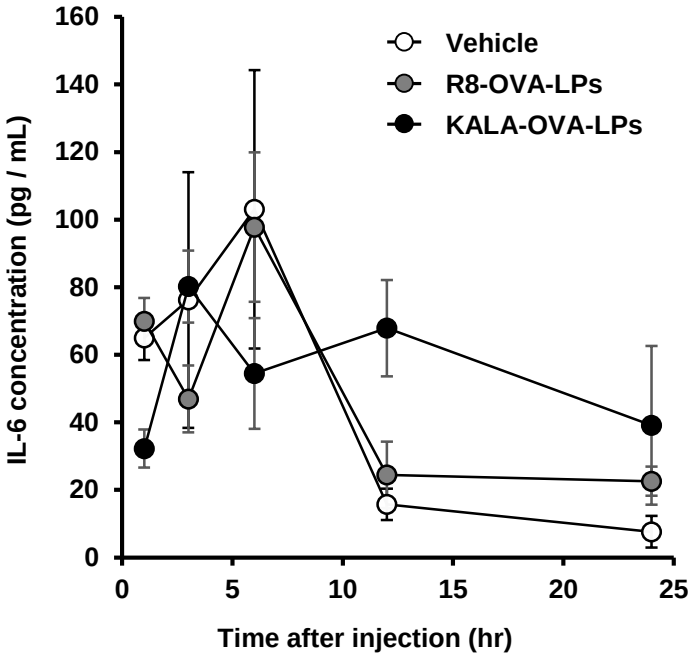
Supplementary Figure 1. Therapeutic anti-tumor effect combined with anti-PD-1 treatment
(a) C57BL/6 mice were inoculated with 8.0×10^5 cells of E.G7-OVA in the left flank. 5, 9, 13, 17 days after inoculation, mice were immunized subcutaneously with KALA-OVA-LPs at a dose of 25 μg OVA. Anti-PD-1 or Isotype Ctrl antibody was also administered intraperitoneally at a dose of 50 μg on every two days from first immunization. The tumor volume was measured up to 20 days after inoculation. The plots represent the mean ± SEM (n = 5). (b) Tumor volume of individual mouse of each group in the experiment (a).

Supplementary Figure 2.



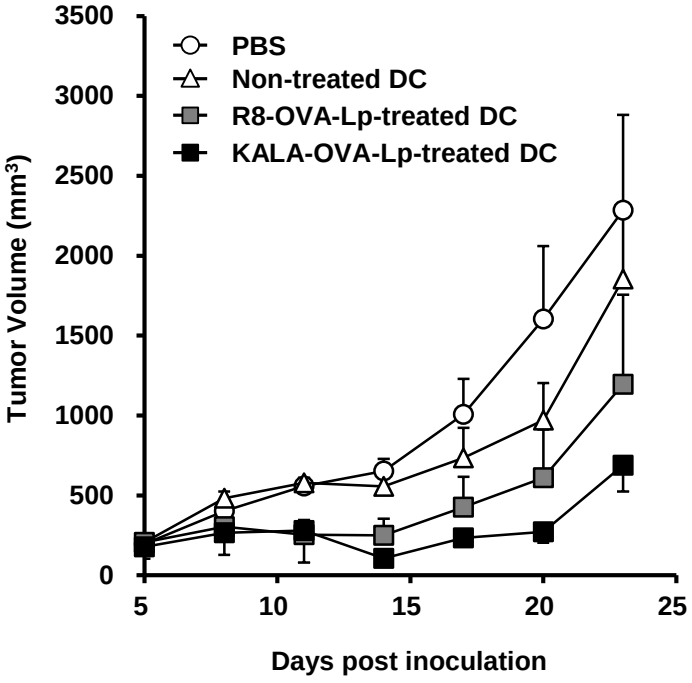
Supplementary Figure 2. CD80/86 expression in BMDCs that were treated with liposomes.
BMDCs (1.0×10^6 cells) were treated with the KALA-OVA-LPs, the R8-OVA-LPs or the non-modified OVA-LPs at a lipid dose of 32 μM. After 18 hours, the BMDCs were recovered and stained by PE-labeled anti-mouse CD80 and CD86 (Biolegend).

Supplementary Figure 3.



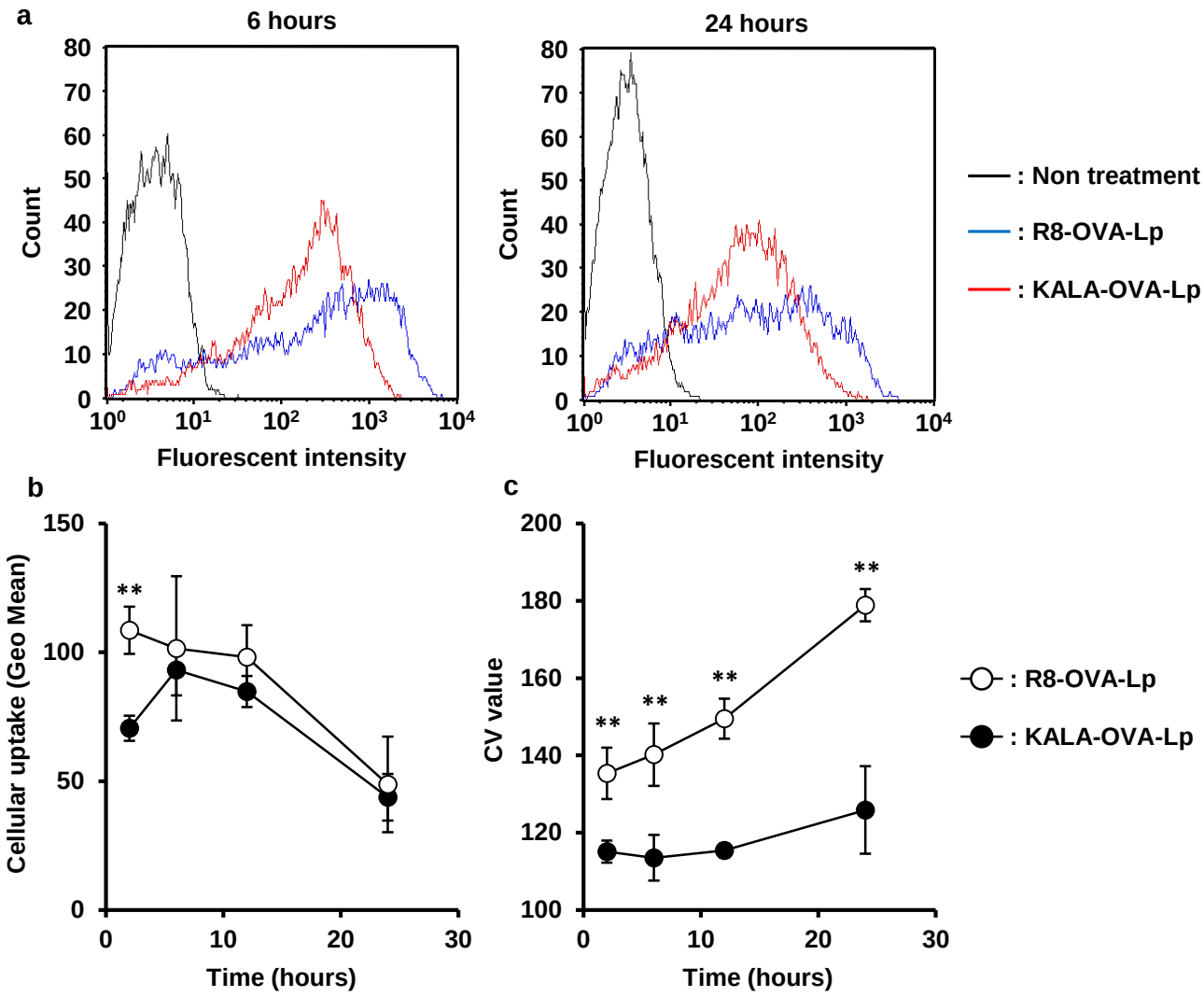
Supplementary Figure 3. IL-6 concentration in serum after liposome injection.
C57BL/6 mice were administered subcutaneously with KALA-OVA-LPs, R8-OVA-LPs at a dose of 25 μg OVA. Blood of these mice was collected at 1, 3, 6, 12 and 24 hrs after administration. Serum was separated from blood and the IL-6 concentration was measured by ELISA.

Supplementary Figure 4.



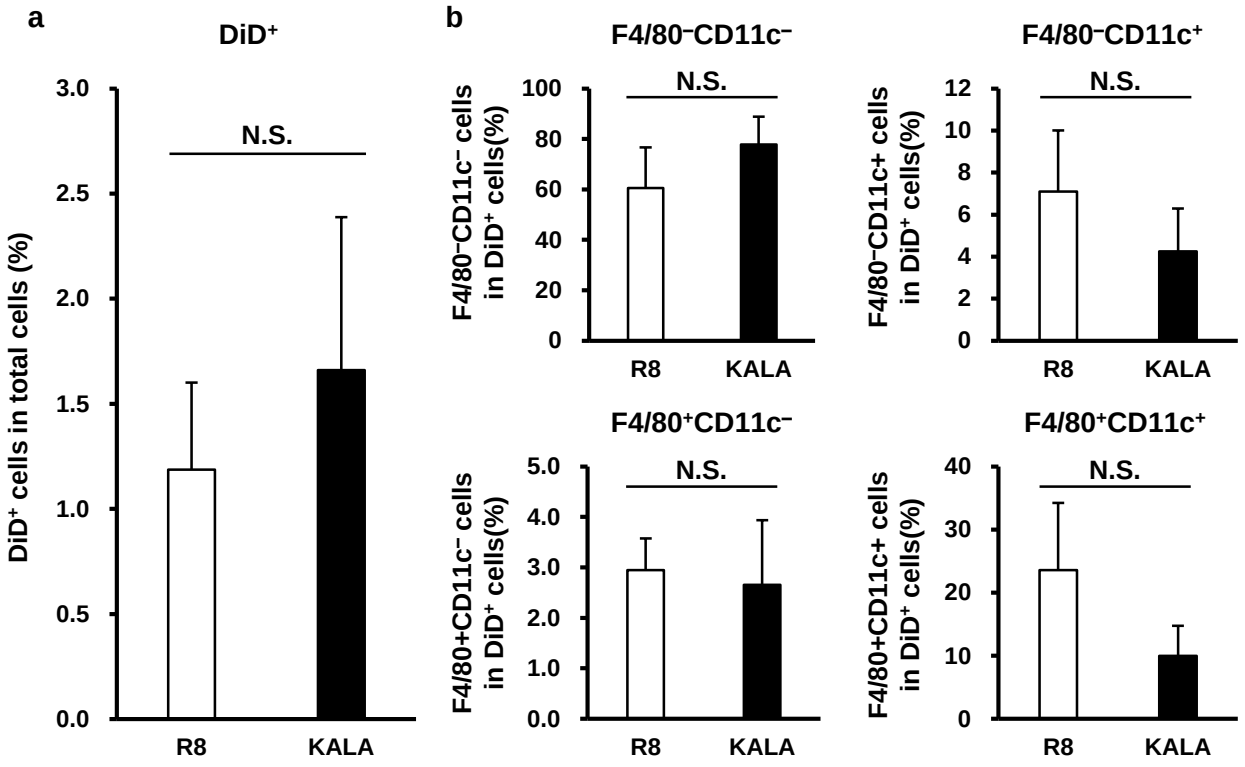
Supplementary Figure 4. *Ex vivo* anti-tumor experiment.
BMDCs (1.0×10^6 cells) were treated with the KALA-OVA-LPs or R8-OVA-LPs at a lipid dose of 32 μMd. After 6 hr incubation, the BMDCs were harvested. C57BL/6 mice were immunized with 5.0×10^5 cells of the harvested BMDCs treated with the KALA-OVA-LPs or the R8-OVA-LPs or non-treated BMDCs. At one week after immunization, mice were inoculated with 8.0×10^5 cells of E.G7-OVA in the left flank. Tumor volume was measured up to 23 days after inoculation. The plots represent the mean ± SEM (n = 4-5).

Supplementary figure 5.



Supplementary figure 5. Uptake of KALA-OVA-LPs and R8-OVA-LPs in BMDCs.
BMDCs were treated with KALA-OVA-LPs or R8-OVA-LPs encapsulating Alexa Flour 488-labeled OVA (25% of total OVA) at a lipid dose of 32 μM. After 2, 6, 12 or 24 hours, the BMDCs were recovered and measured the fluorescent intensity by flowcytometer. (a) Typical histogram of BMDCs treated with KALA-OVA-LPs or R8-OVA-LPs. (b) Average of fluorescence intensity (Geo mean, left) and coefficient variance (CV) value (right). Data are mean ± SD (n=3). Statistical analyses were performed by Student's t-test. **P < 0.01.

Supplementary figure 6.



Supplementary figure 6. Lymph node accumulation of KALA-OVA-LPs and R8-OBA-LPs
C57BL/6 mice were administered subcutaneously with KALA-OVA-LPs, R8-OVA-LP modified with 0.1 mol% DiD at a dose of 25 μg OVA in the both side flanks. After 24 hours, the draining lymph nodes (inguinal lymph nodes) were isolated and mashed. Nylon mesh-filtered cell suspension were analyzed by flow cytometry for uptake of the fluorescently-labeled liposomes and expression of F4/80 and CD11c. Data are mean + SEM (n=3). Statistical analyses were performed by Student's t-test. N.S.: Not significant.