

Supporting Information

Sugar modified amphiphilic cationic nano-adjuvant ceased tumor immune suppression and rejuvenated peptide vaccine induced antitumor immunity in cervical cancer

Adityanarayan Mohapatra^{a‡}, Santhosh Kalash Rajendrakumar^{a‡}, Kondareddy Cherukula^a

Myong-Suk Park^b, Sathiyamoorthy Padmanaban^a, Arathy Vasukuty^a, Ayeskanta Mohanty^a,

Jae Young Lee^c, Woo Kyun Bae^b and In-kyu Park^{a}*

^aDepartment of Biomedical Science and BK21 PLUS Center for Creative Biomedical Scientists
at Chonnam National University, Chonnam National University Medical School,

Gwangju 61469, Republic of Korea.

^bDepartment of Hematology-Oncology, Chonnam National University Medical School,

Gwangju, 61469, South Korea.

^cSchool of Materials Science and Engineering, Gwangju Institute of Science and Engineering,

Gwangju 61005, Korea.

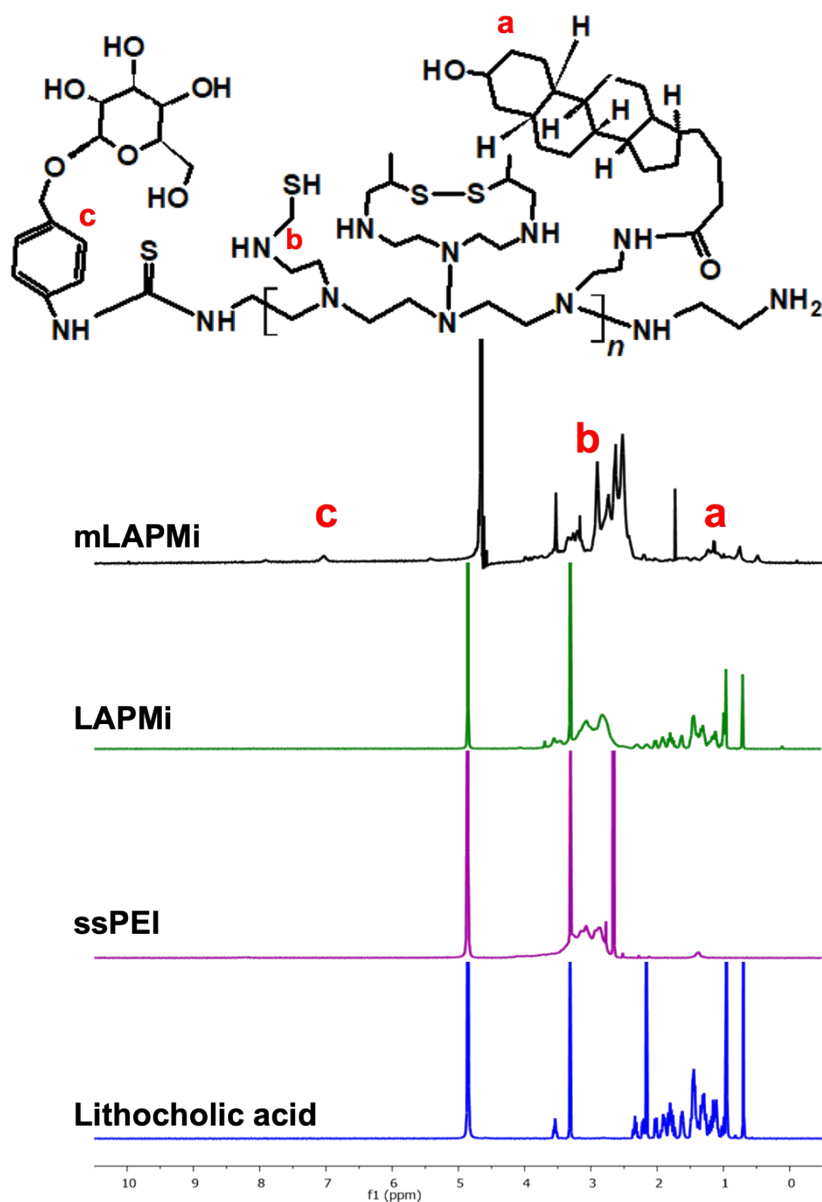
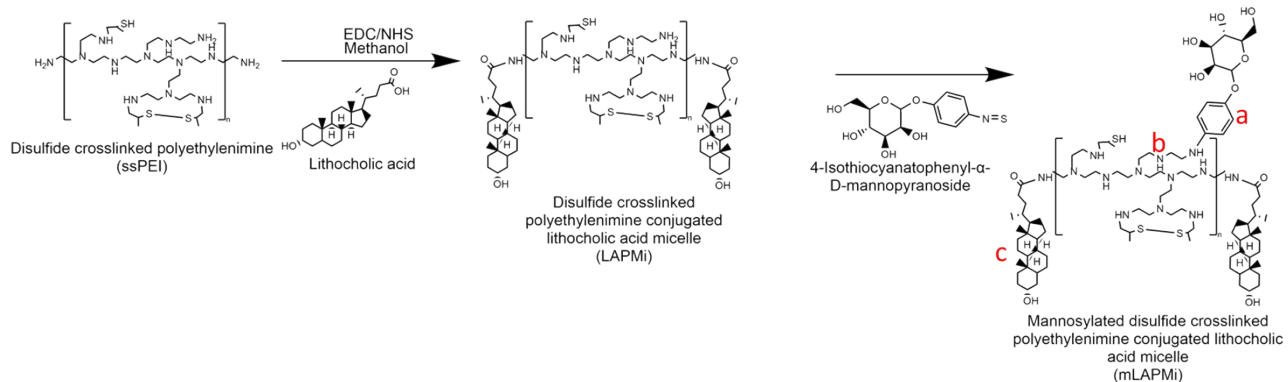


Figure S1: Schematic representation of mLAPMi synthesis and NMR analysis.

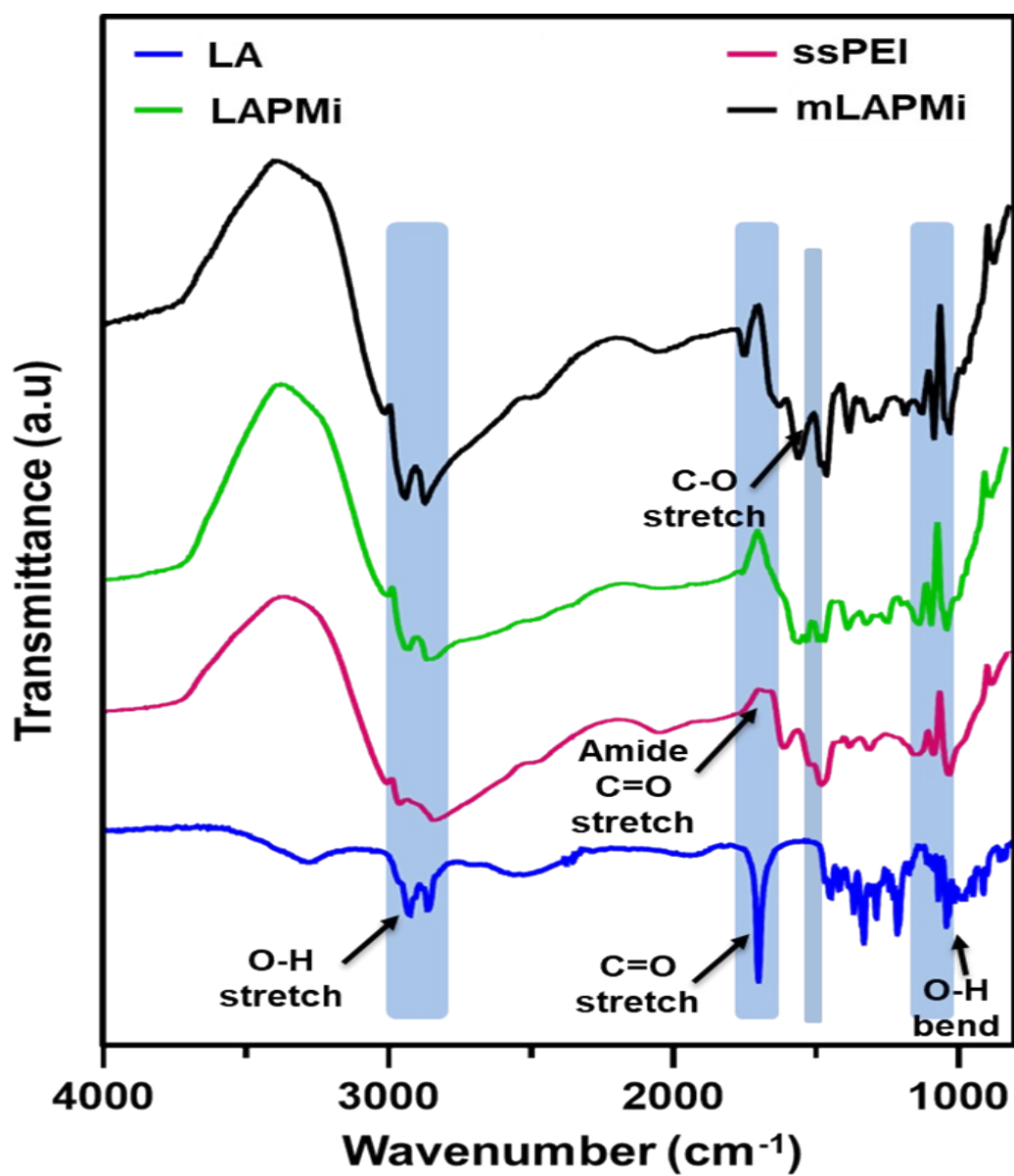


Figure S2: FTIR analysis of mLAPMi.

| Sample | [LAPMi]: [Mannose] (feed ratio) | Grafting ratio % (1HNMR) |
|--------|---------------------------------|--------------------------|
| mLAPMi | 1:0.1 | 39 |
| mLAPMi | 1:0.2 | 62 |
| mLAPMi | 1:0.25 | 65 |

Table S1: The chemical properties of mannose conjugated ssPEI-lithocholic acid micelle (mLAPMi).

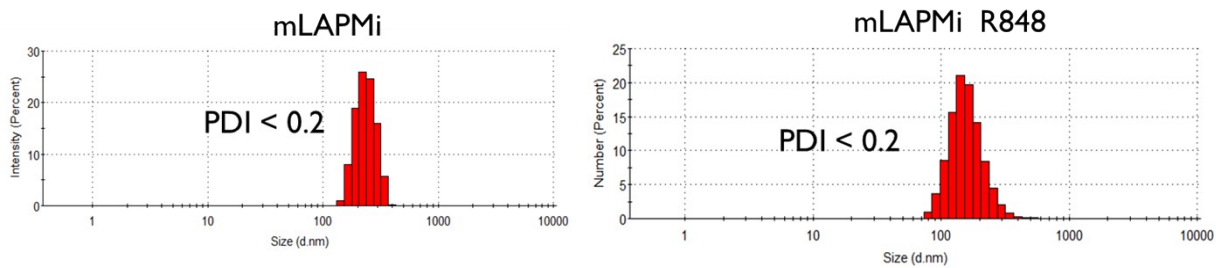


Figure S3: Dynamic light scattering analysis of mLAPMi and mLAPMi R848.

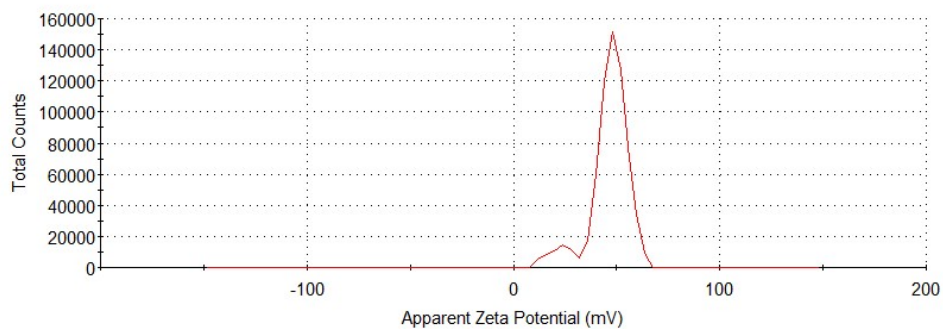


Figure S4: Zeta potential of mLAPMi-R848.

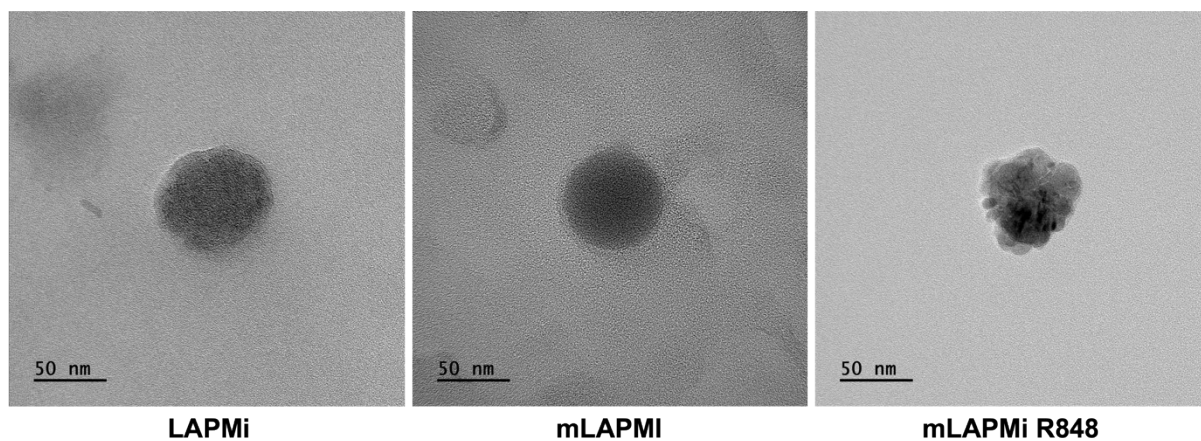


Figure S5- TEM images of mLAPMi and mLAPMi loaded with R848.

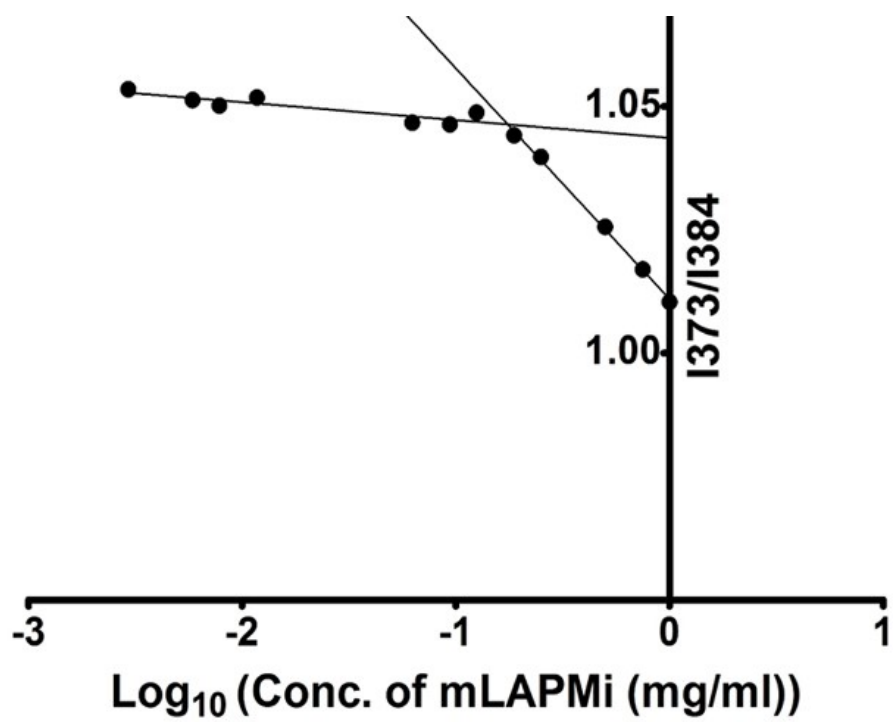


Figure S6- Critical micelle concentration of mLAPMi.

| Sample | Feed LAPMi (mg) | Feed R848 (mg) | Amount of R848 (μg)/ mg of micelle | Loading efficiency (%) | Loading content (%) |
|---------------|------------------------|-----------------------|---|-------------------------------|----------------------------|
| LAPMi | 10 | 2 | 46.4 | 22.6 | 4.64 |
| mLAPMi | 10 | 2 | 54.2 | 27.1 | 5.42 |

Table S2: Drug loading efficacy of mLAPMi

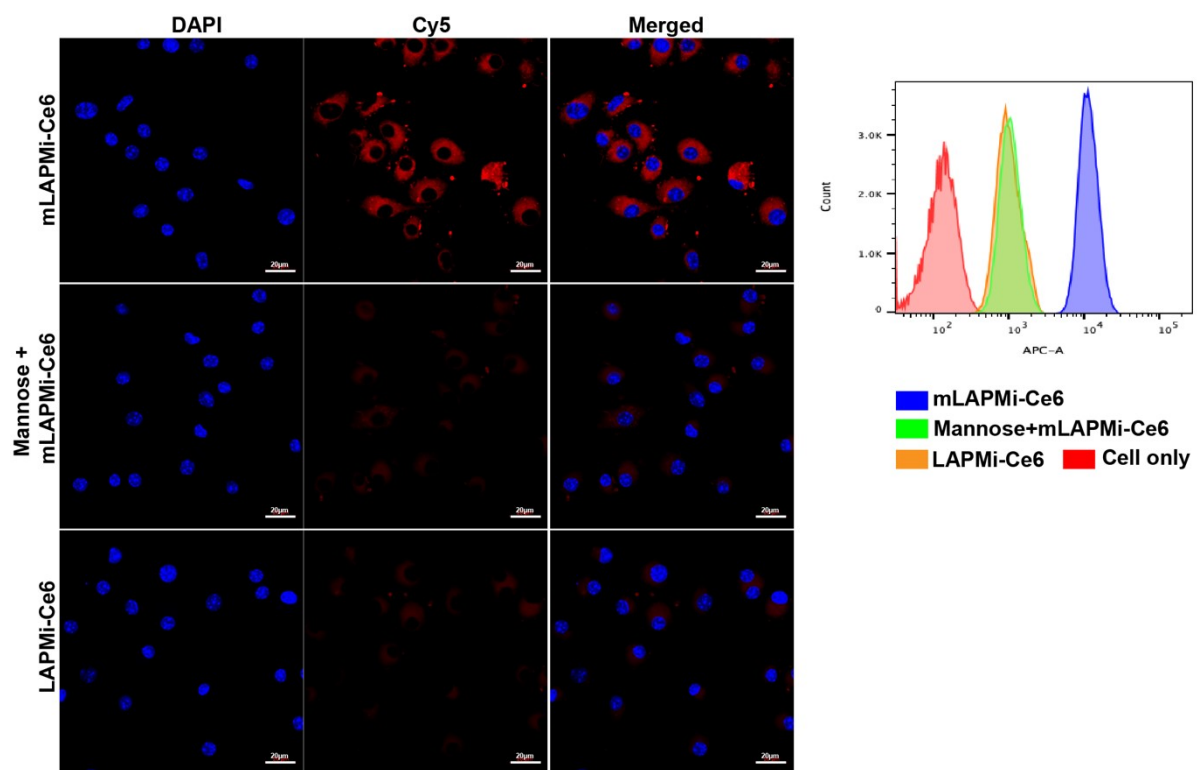


Figure S7: Intracellular uptake of mLAPMi-Ce6 in BMDCs. Scale bar is 20 μm.

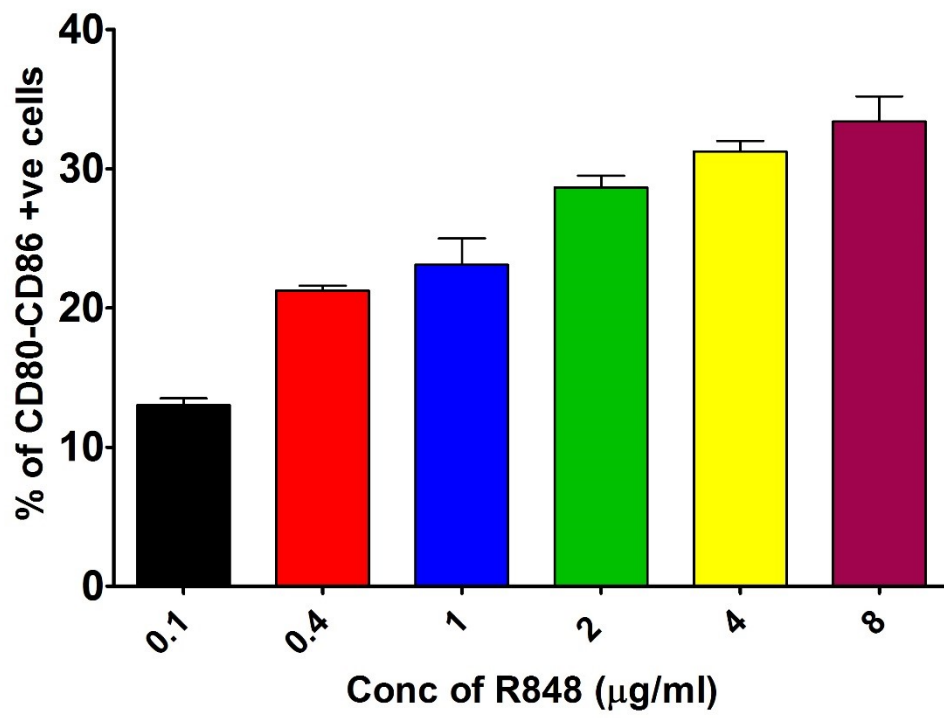


Figure S8: DC maturation in BMDCs treated with different concentration of R848.

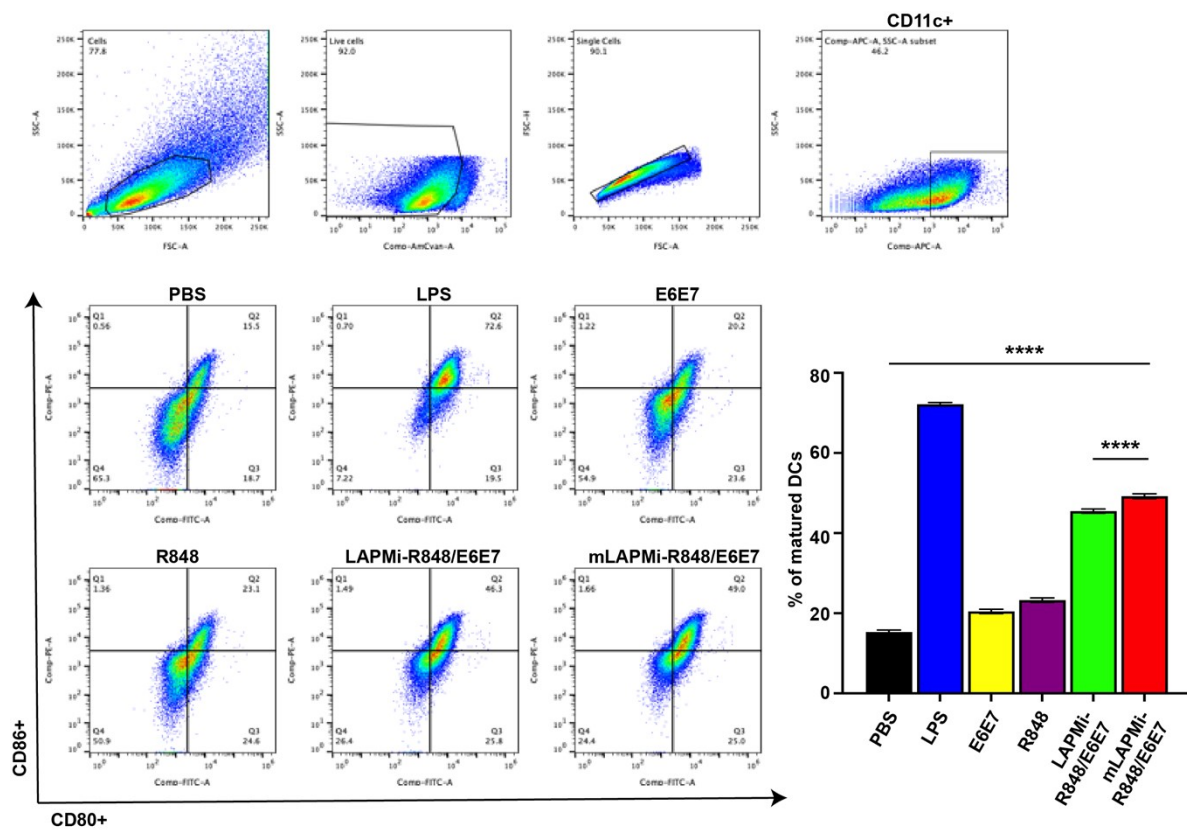


Figure S9: BMDCs maturation induced by mLAPMi-R848 nanoparticle and E6E7 peptide antigen in bone marrow derived dendritic cells, N = 4, SEM, ****, $p < 0.0001$

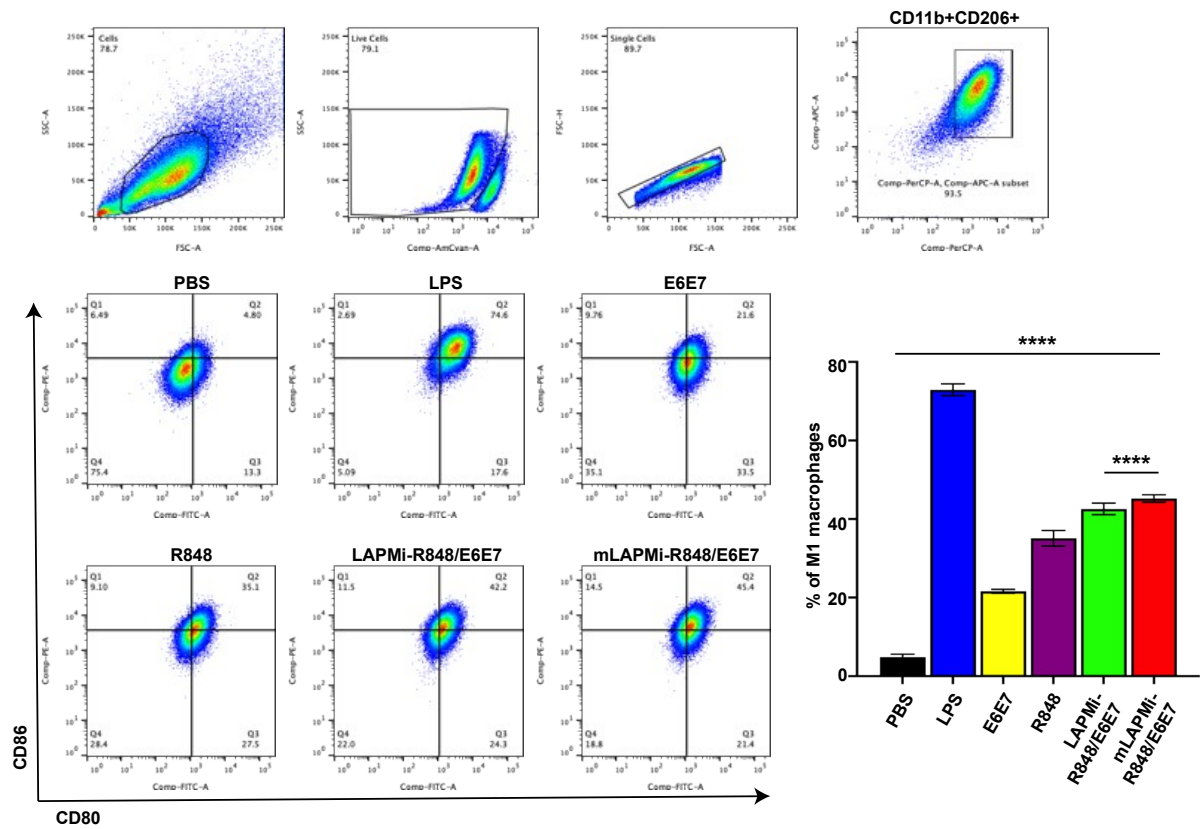


Figure S10- Macrophage polarization by mLAPMI-R848 nanoparticle and E6E7 peptide antigen in IL-4 induced M2 polarized J774A.1 macrophage cell lines. N = 4, SEM ****, $p < 0.0001$.

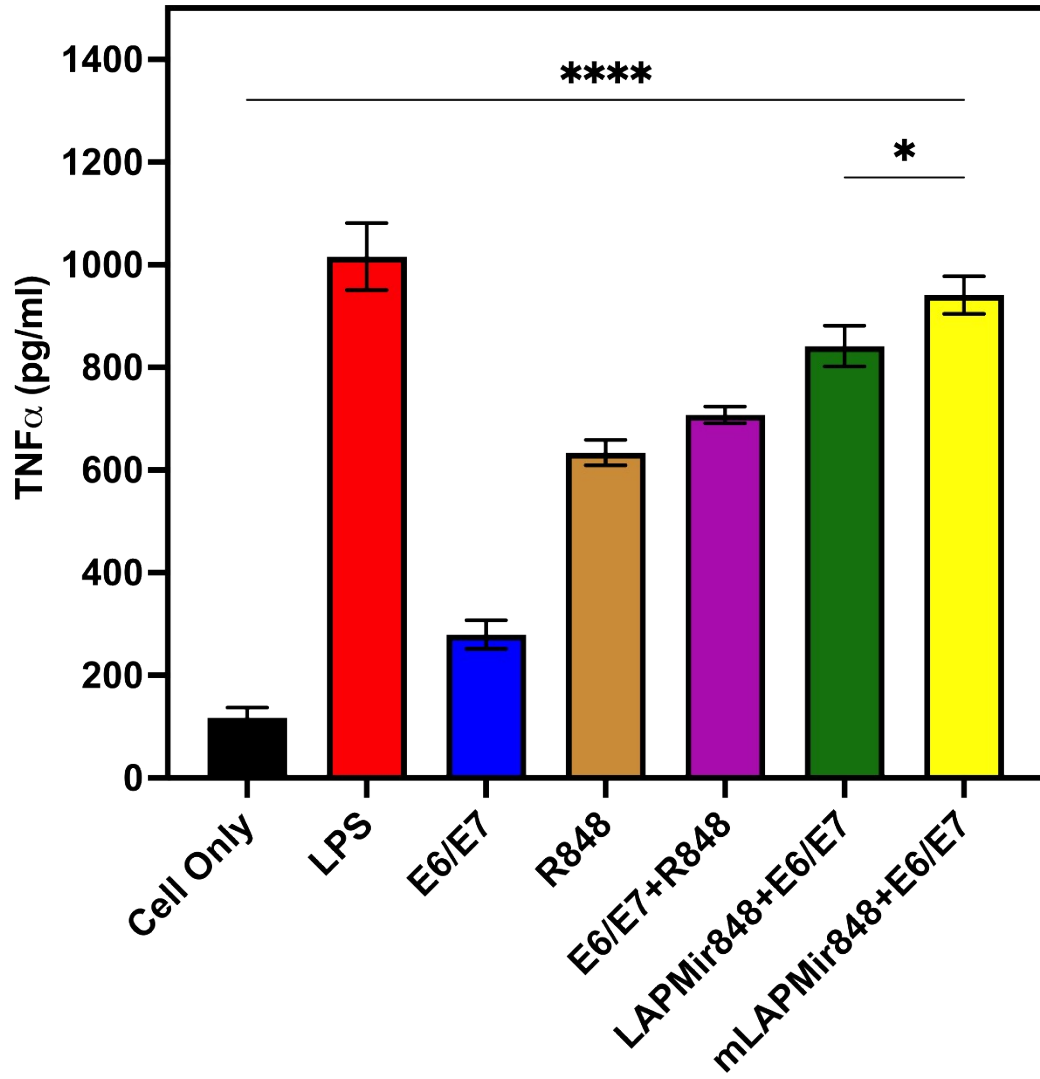


Figure S11: ELISA analysis of TNF α secreted from mLAPMi-R848 treated BMDCs. n=3, SEM, ****p<0.0001.

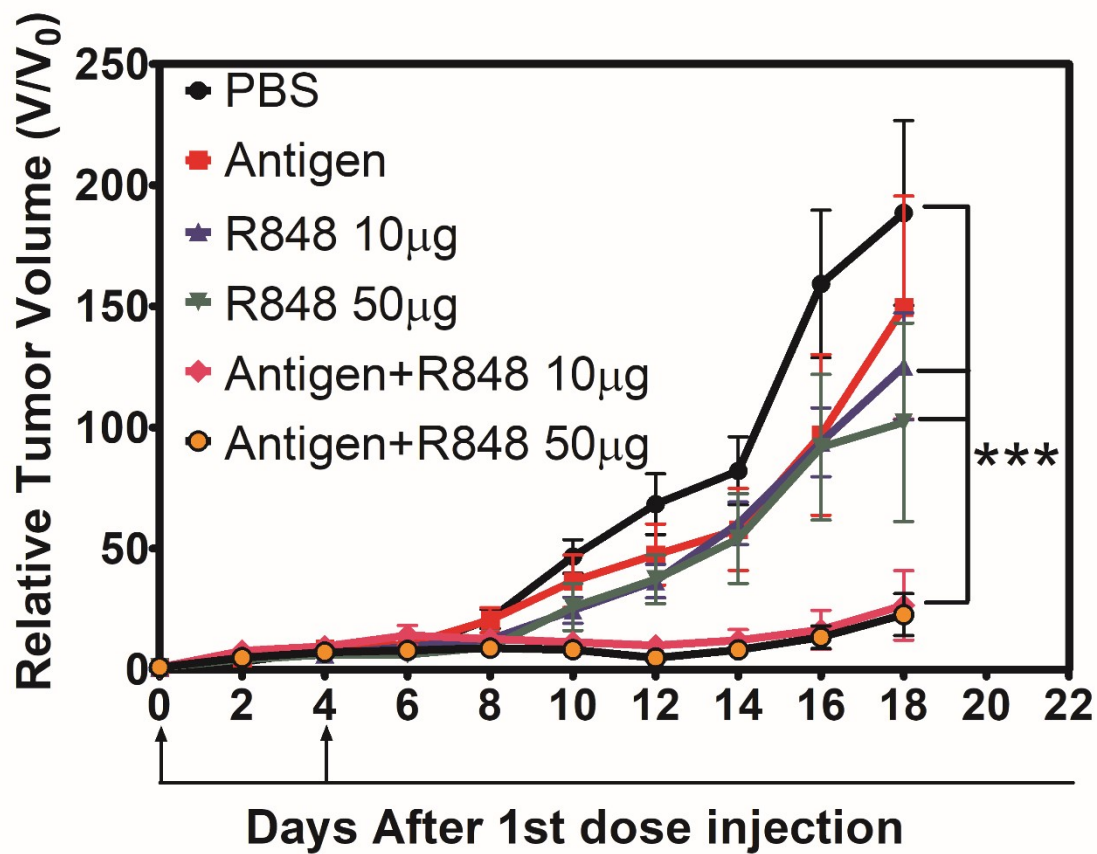


Figure S12: Antitumor effect of different dose of R848 in TC-1 tumor model. 10 μ g is equivalent to 0.5 mg/kg, 50 μ g is equivalent to 2.5 mg/kg. n=5, SEM, * p <0.05, *** p <0.001.

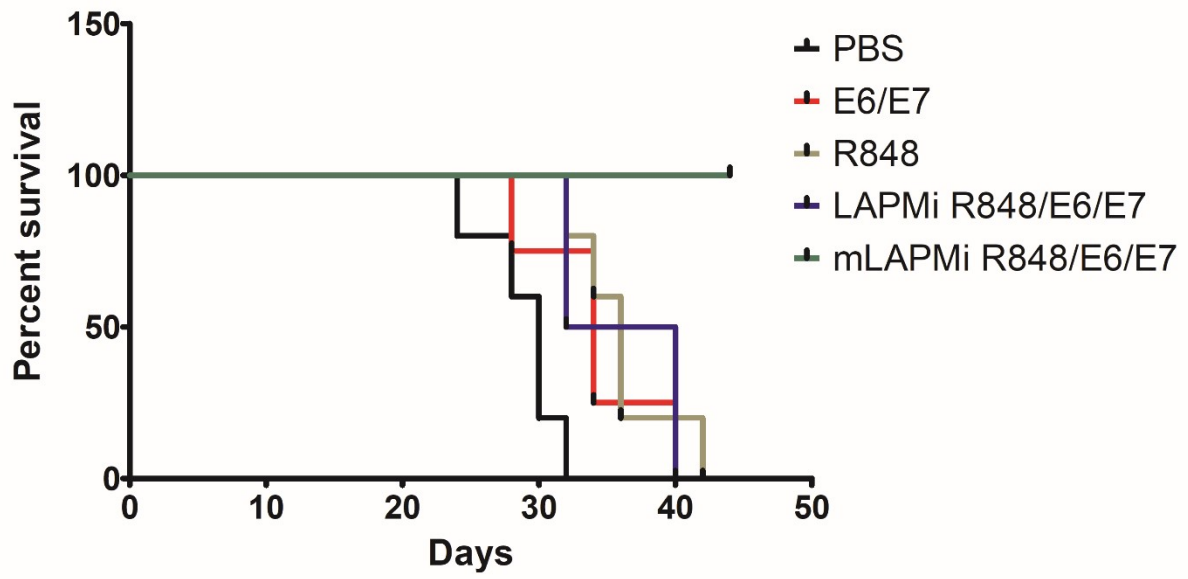


Figure S13: Survival rate of TC-1 tumor mice administered with mLAPMi-R848 and E6E7 peptide.

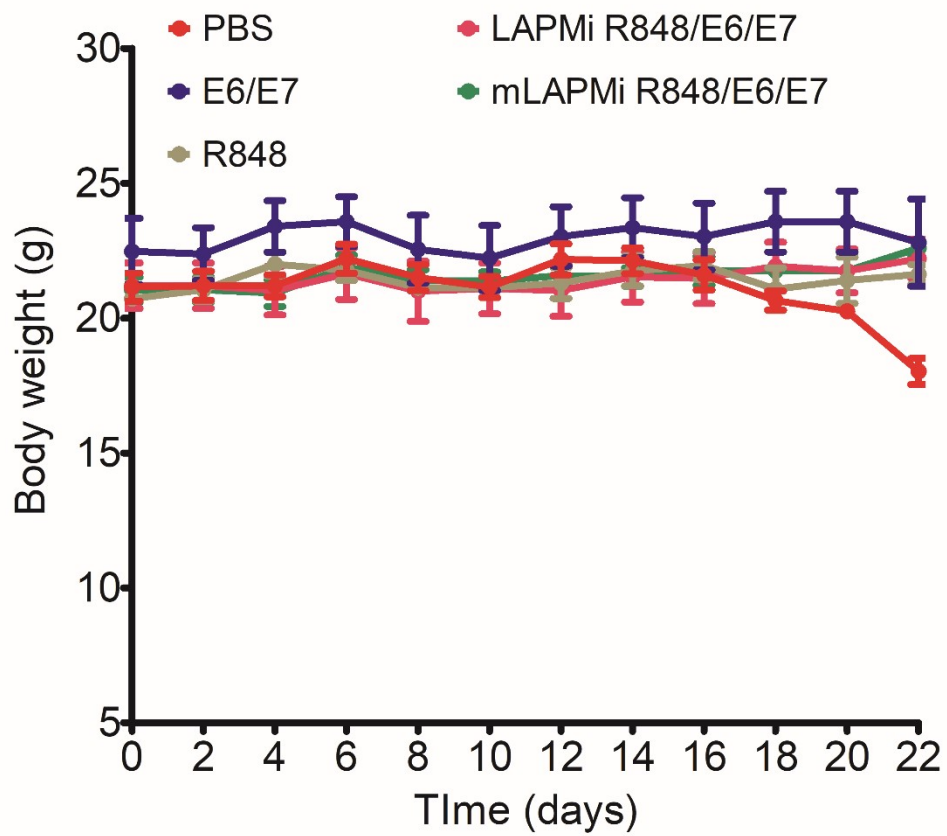


Figure S14: Body mass of TC-1 tumor mice administered with mLAPMi-R848 and E6E7 peptide.

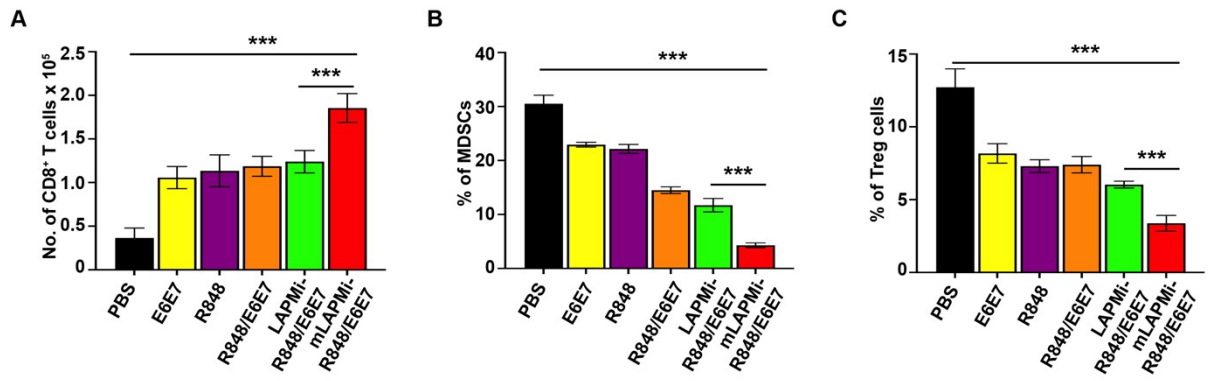


Figure S15: Immune phenotyping of cells isolated from primary tumor of mLAPMi-R848 and E6E7 peptide antigen vaccinated TC-1 tumor mice. A) Absolute numbers of CD8⁺ T cells (gated with CD3⁺CD8⁺), B) MDSCs (gated with CD11b⁺ Ly6g & Ly6cGR-1⁺) C) Tregs (gated with CD4⁺CD25⁺FoxP3⁺). N=4, ***p<0.001.

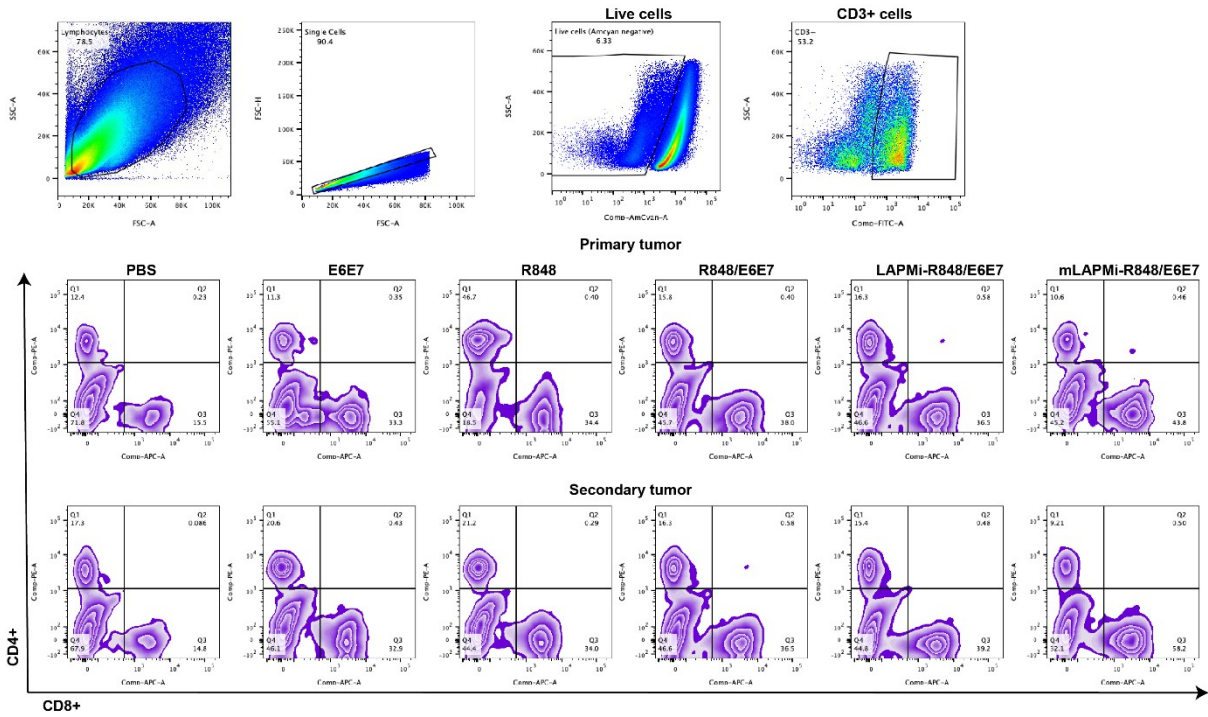


Figure S16: Gating strategy of CD8+ T cells isolated from the both primary and secondary tumors in mLAPMI-R848/E6E7 treated bilateral tumor model.

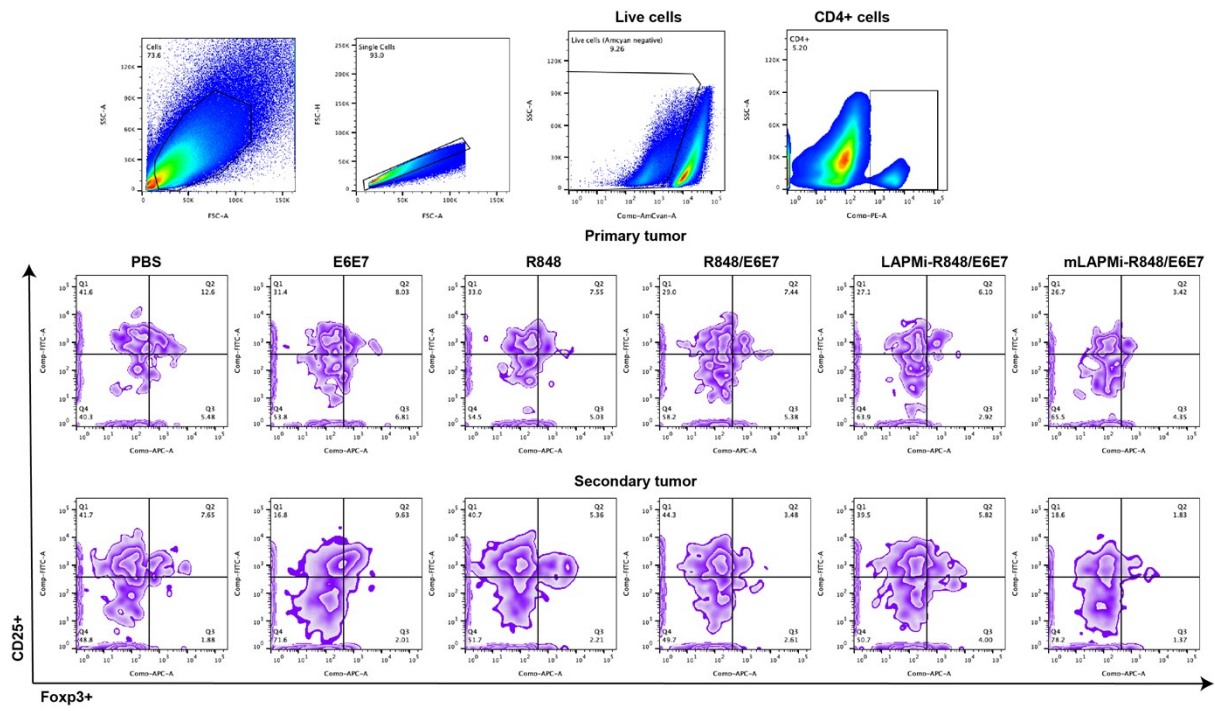


Figure S17: Gating strategy of Treg cells isolated from the both primary and secondary tumors in mLAPMI-R848/E6E7 treated bilateral tumor model.

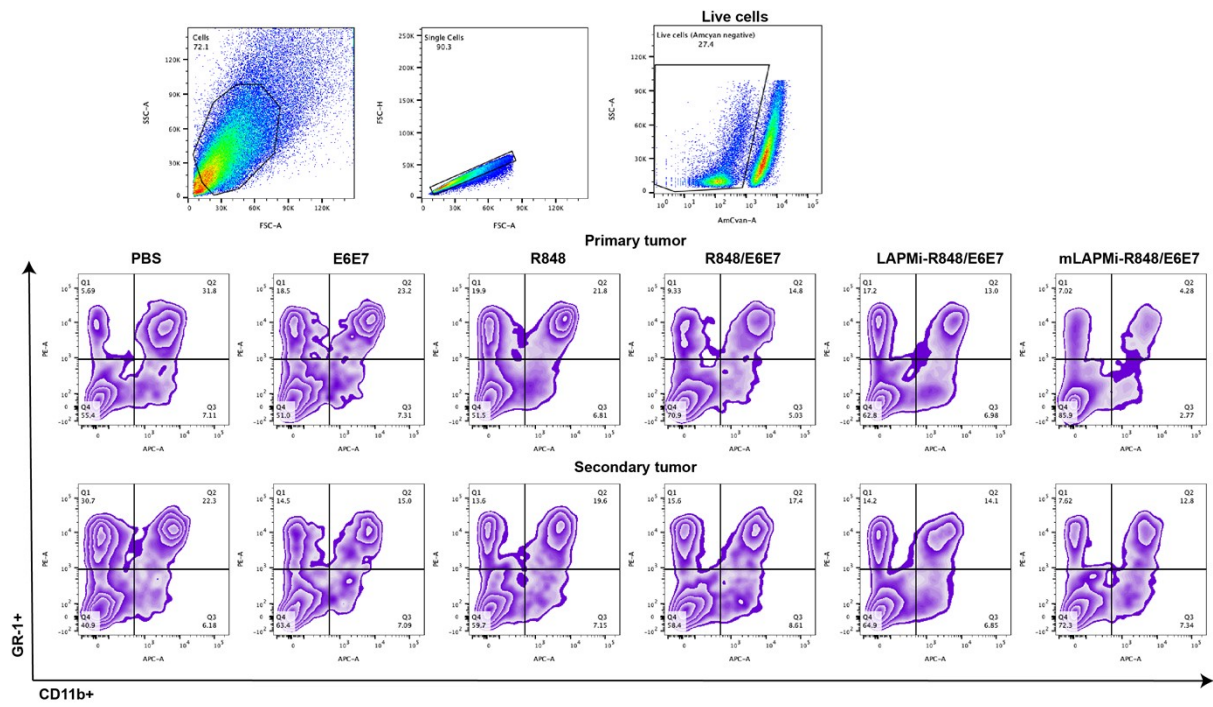


Figure S18: Gating strategy of MDSCs isolated from the both primary and secondary tumors in mLAPMI-R848/E6E7 treated bilateral tumor model.