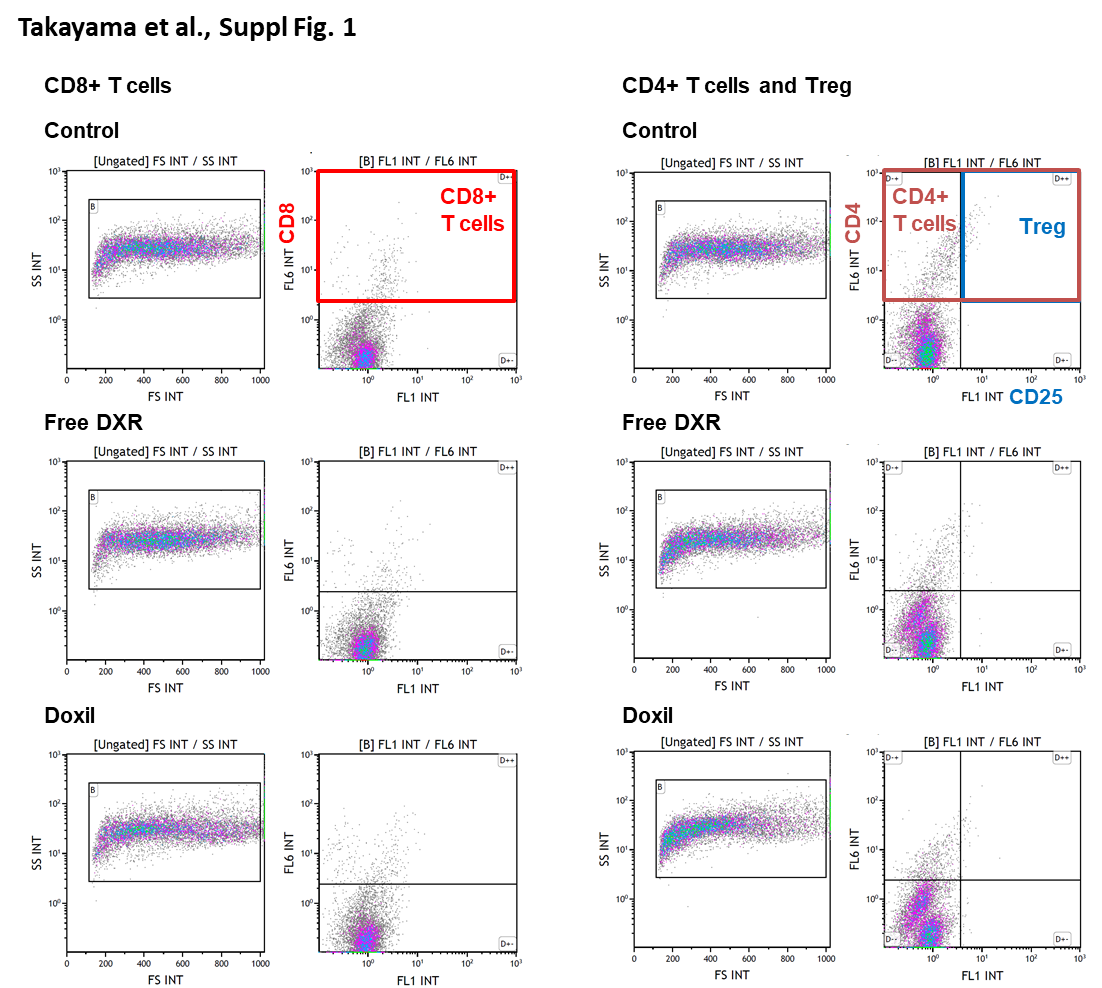
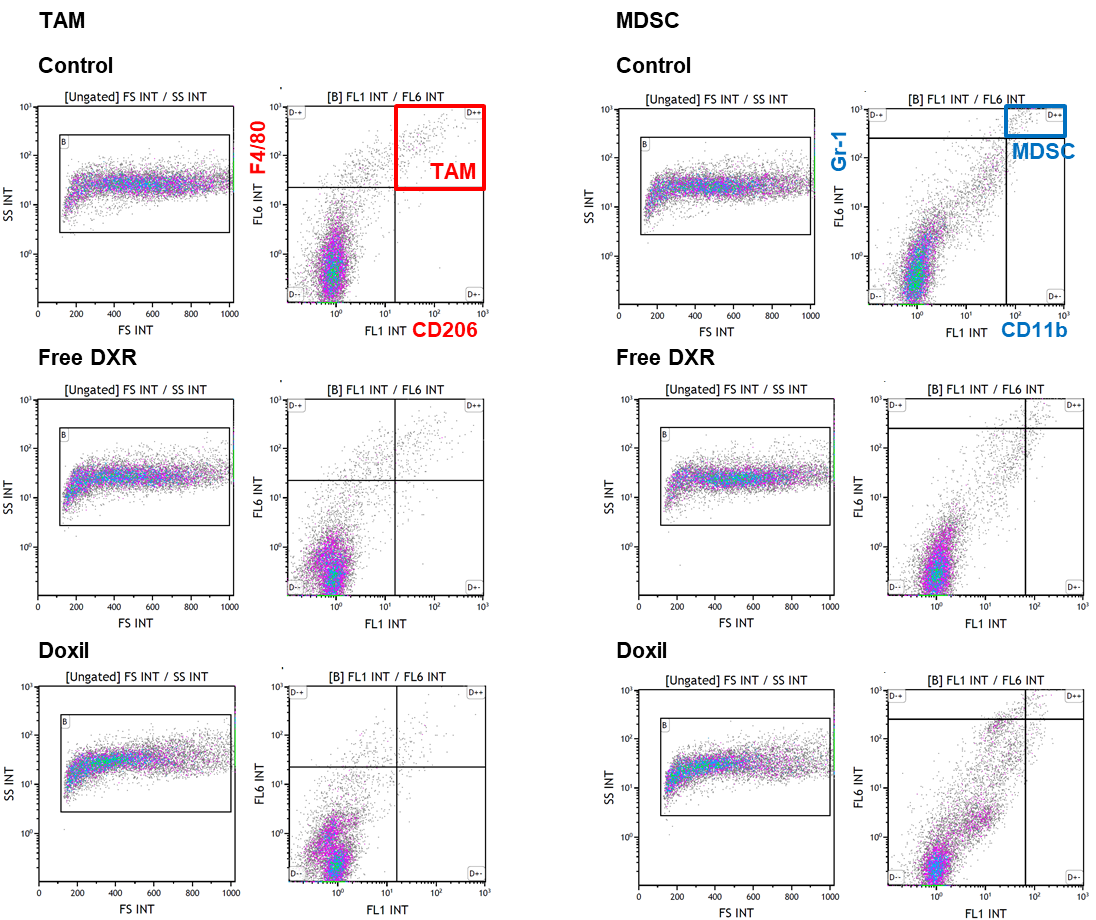
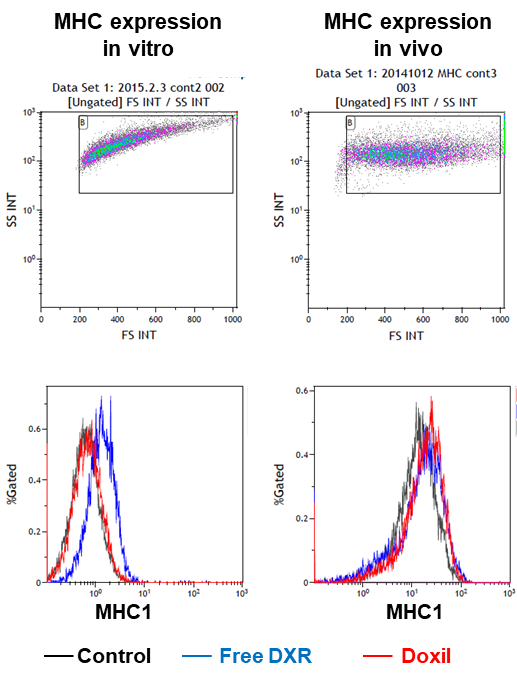
Supplementary Materials: Adjuvant Antitumor Immunity Contributes to the Overall Antitumor Effect of Pegylated Liposomal Doxorubicin (Doxil®) In C26 Tumor-Bearing Immunocompetent Mice

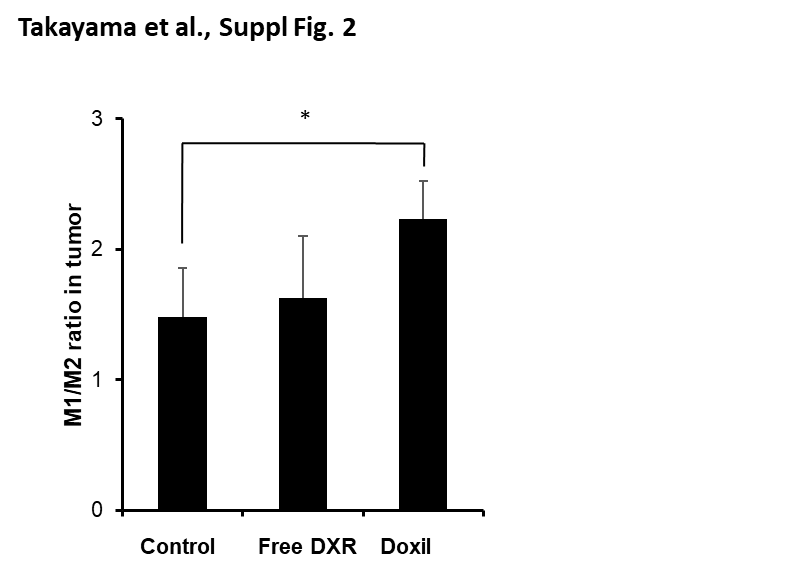
Takuma Takayama 1, Taro Shimizu 1, Amr S. Abu Lila 1,2,3, Yuki Kanazawa 1, Hidenori Ando 1,   
Yu Ishima 1 and Tatsuhiro Ishida 1,\*



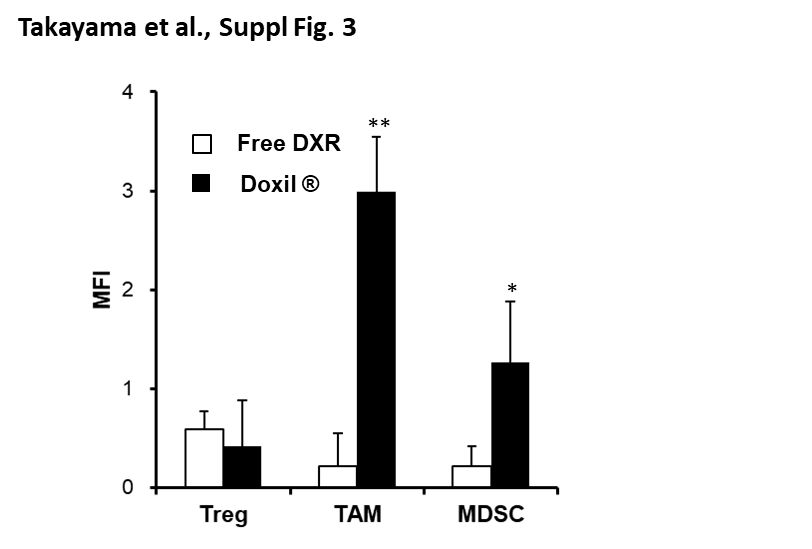




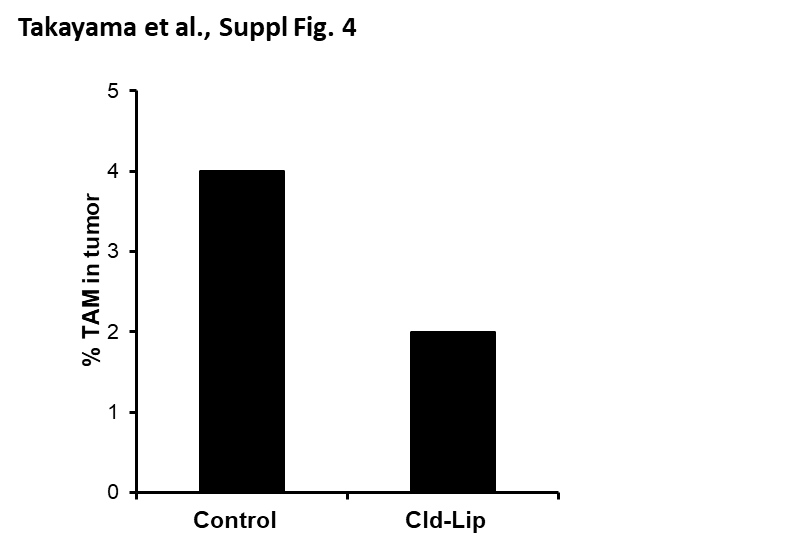
**Figure S1.** Gating strategies and representative plot of flow cytometry analysis.



**Figure S2.** Polarization of Tam in tumor tissues after treatment with Doxil. BALB/c mice were inoculated s.c. with C26 cells (2 × 106 cells). The day when tumor volumes reached 100–150 mm3 was set at Day 0. Mice were injected i.v. with PBS, free DXR (5 mg/kg) or Doxil® (5 mg DXR/kg) on Day 0 and Day 5. Tumor was harvested on Day 11. The ratio of M1 macrophages (CD206-, F4/80+ cells) to M2 macrophages (CD206+, F4/80+ cells) were analyzed by flow cytometry. Each value represents the mean ± SD. \* *p* < 0.05.



**Figure S3.** Cellular uptake of Doxil® by immune cells in tumor tissue. BALB/c mice were inoculated s.c. with C26 cells (2 × 106 cells). The day when tumor volume reached 100–150 mm3 was set at Day 0. Mice were injected i.v. with either free DXR or Doxil® (10 mg DXR/kg) on Day 0. Tumor was harvested on Day 2. Fluorescence derived from DXR in Treg, TAM or MDSC was detected by flow cytometry. Each value represents the mean ± SD. \* *p* < 0.05, \*\* *p* < 0.01 vs. DXR.



**Figure S4.** Effect of treatment with clodronate liposomes on TAMs in tumor tissues. C26 tumor-bearing immunocompetent mice were injected i.v. with Cld-Lip (6.6 µmol/mouse). Tumors were excised on Day 4. The proportion of TAMs in total tumor cells of tumor was analyzed by flow cytometry. Each value represents the mean ± SD.