Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Layer-by-layer nanoparticle encapsulating all-trans retinoic acid and CpG as a mucosal adjuvant targeting colorectal cancer

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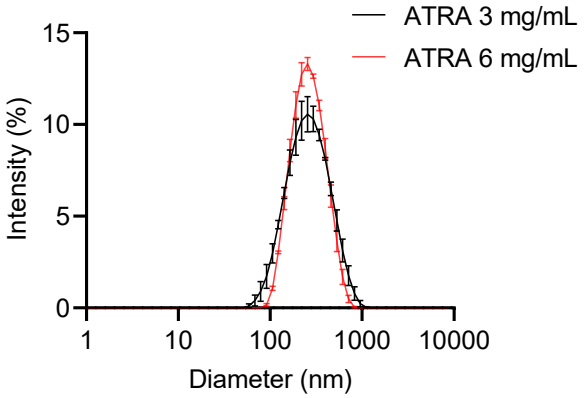

Figure S1. Representative size distribution of ATRA-NPs measured by DLS.

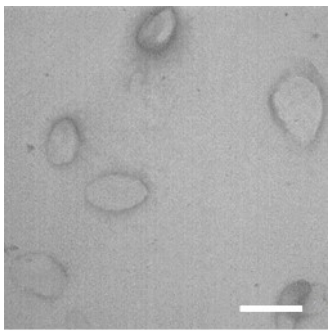

Figure S2. A representative TEM image of ATRA-NPs. The scale bar is 200 nm.

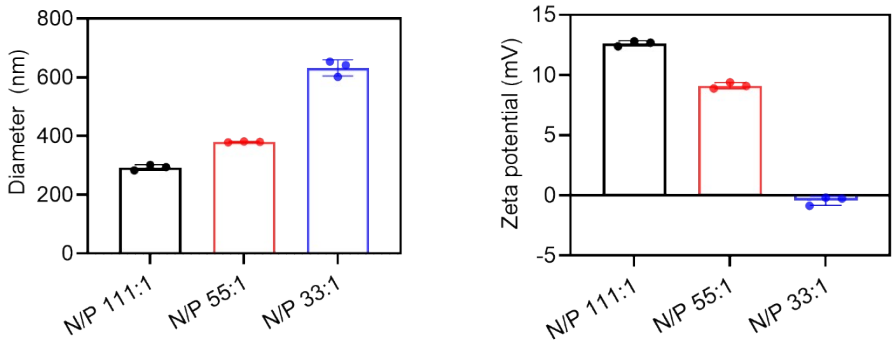

Figure S3. The hydrodynamic diameters and zeta potentials of ATRA@CpG-NPs with N/P ratios. n = 3 technical replicates. Data are shown as mean ± SD.

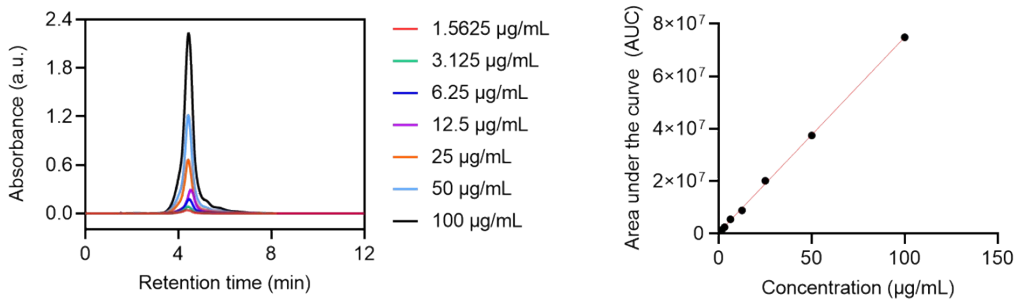

Figure S4. Reverse-phase HPLC chromatograms of the ATRA in acetonitrile (left). Standard curve of HPLC AUC over ATRA concentrations.

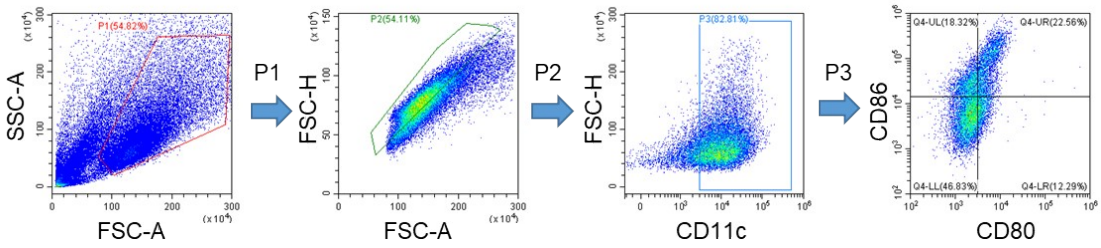

Figure S5. Gating strategy for flow cytometry analysis of $CD11c^{+}CD80^{+}CD86^{+}$ cells among BMDCs.

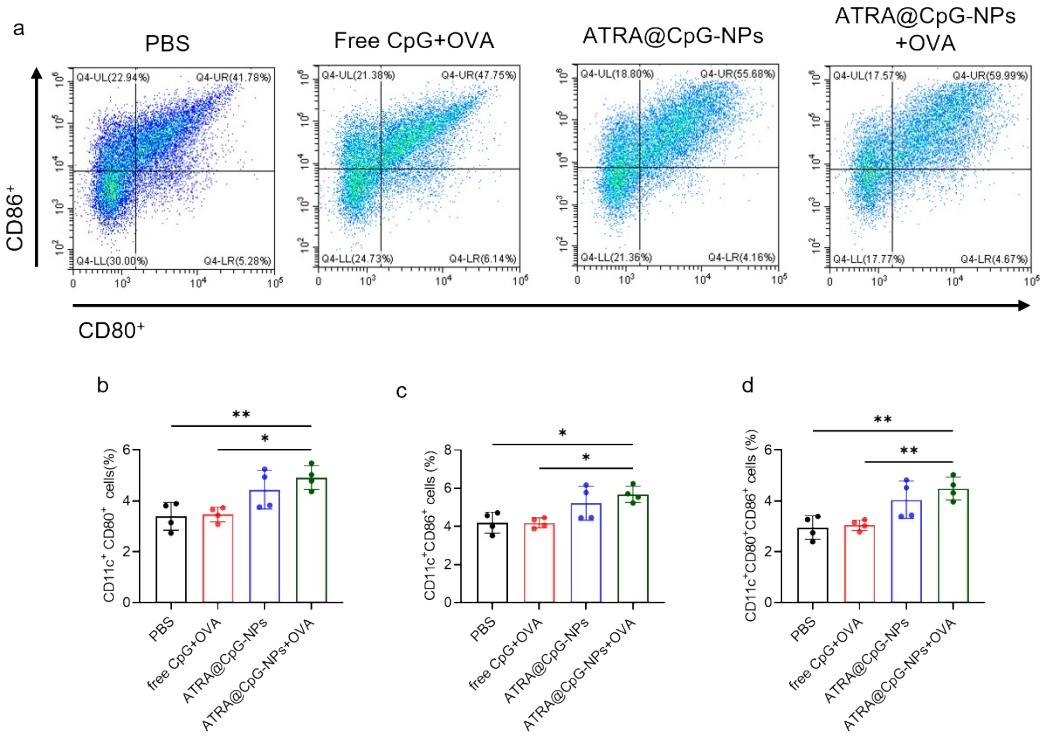

Figure S6. (a) Representative flow cytometry plots of $CD11c^{+}CD80^{+}CD86^{+}$ cells. Quantification analysis of $CD11c^{+}CD80^{+}$ cells (b), $CD11c^{+}CD86^{+}$ cells(c) and $CD11c^{+}CD80^{+}CD86^{+}$cells (d). n = 4 technical replicates. Data are shown as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons tests: *$p < 0.05$, **$p < 0.01$.

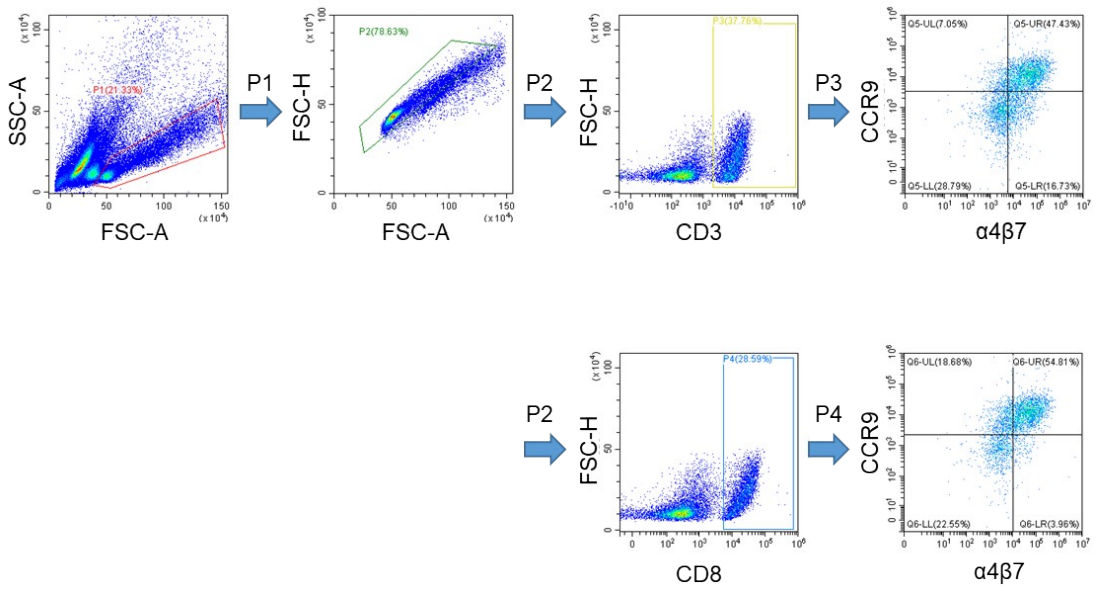

Figure S7. Gating strategy for flow cytometry analysis of α4β7⁺CCR9⁺CD3⁺ and α4β7⁺CCR9⁺CD8⁺ T cells among spleen cells.

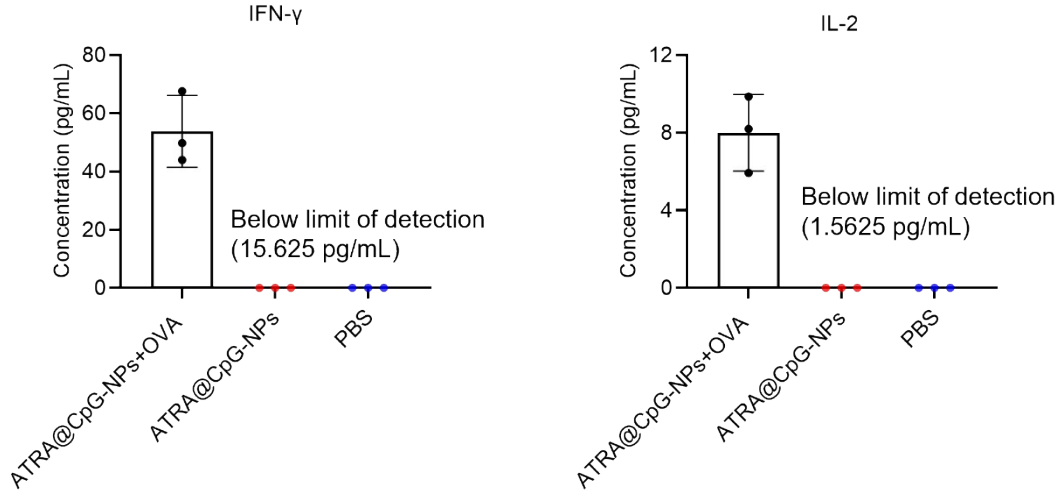

Figure S8. IFN-γ and IL-2 secretion by OT-1 CD8⁺ T cells after 48 h incubation with PBS, ATRA@CpG-NPs and ATRA@CpG-NPs. n = 3 technical replicates. Data are shown as mean ± SD

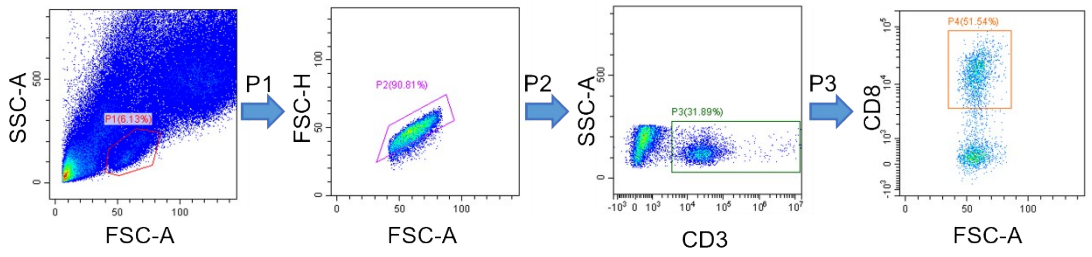

Figure S9. Gating strategy for flow cytometry analysis of CD3⁺CD8⁺ T cells among tumor cells.

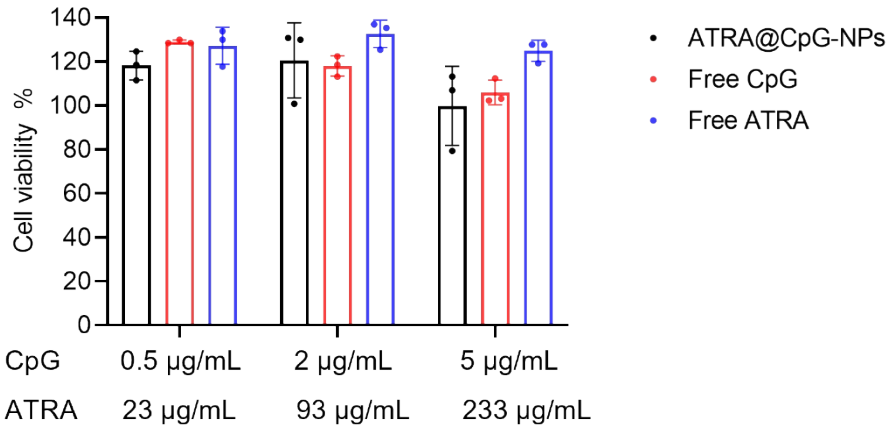

Figure S10. The viability of DC2.4 cells after incubation with different concentrations of free CpG, free ATRA and ATRA@CpG-NPs. Data are shown as mean ± SD.

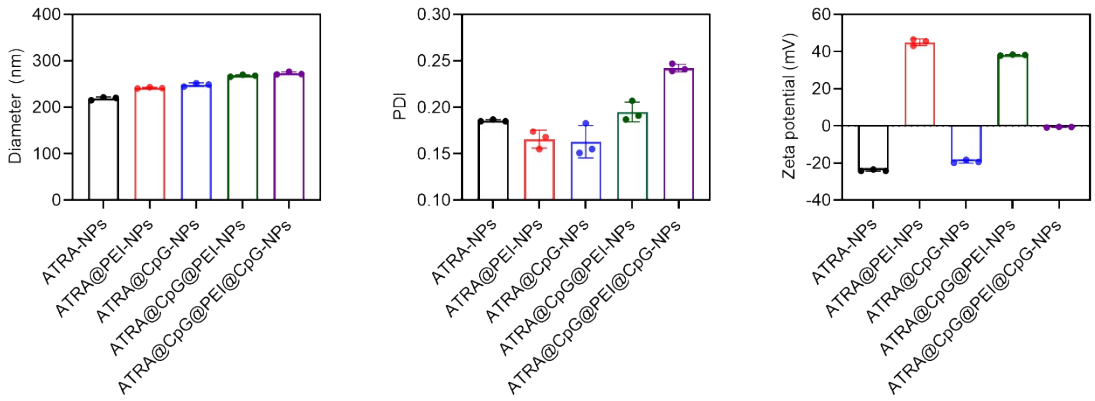

Figure S11. The hydrodynamic diameters, polydispersity index and zeta potentials of multiple layered nanoparticles. n = 3 technical replicates. Data are shown as mean ± SD.

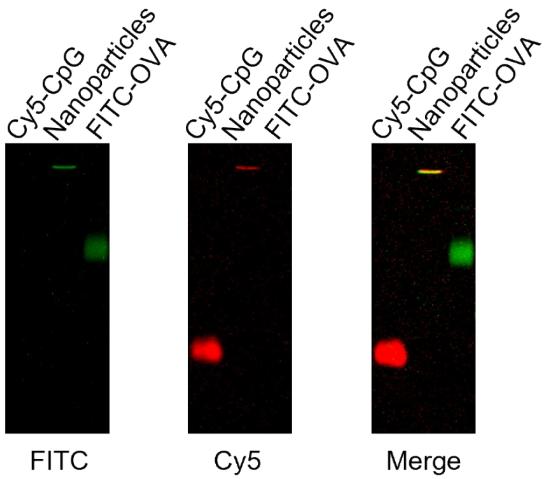

Figure S12. Agarose gel (1%) images of Cy5-CpG (red), FITC-OVA (green), and ATRA nanoparticles carrying both CpG and OVA.