Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Comparison of the uptake of untargeted and targeted immunostimulatory nanoparticles by immune cells in the microenvironment of metastatic breast cancer

Gil Covarrubias[1,2†], Taylor J. Moon[1†], Georgia Loutrianakis[1‡], Haley M. Sims[1‡], Mayura P. Umapathy[1], Morgan E. Lorkowski[1], Peter A. Bielecki[1,2], Michelle L. Wiese[1], Prabhani U. Atukorale[1,2§], Efstathios Karathanasis[1,2*]

[1] Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio 44106, USA

[2] Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio 44106, USA

[§] Current address: Department of Biomedical Engineering, University of Massachusetts, Amherst, MA 01003

[†] and [‡] Equal contribution

E-mail: stathis@case.edu

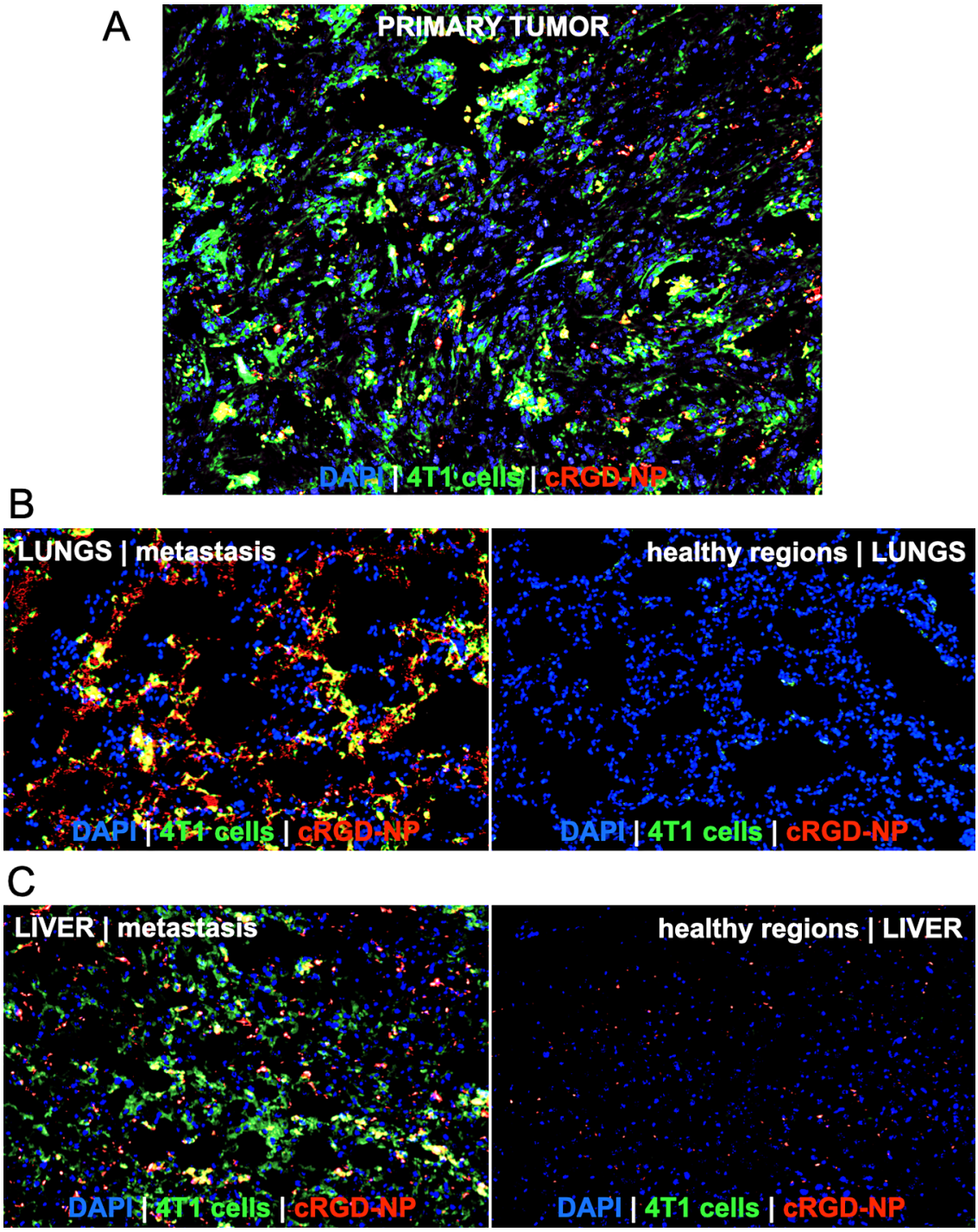

Fig. S1. Histological evaluation of the microdistribution of NPs in primary tumor and sites of metastasis and healthy tissue regions in liver and lungs. (A) Primary tumor, (B) lungs, and (C) liver. (20x magnification; scale bar = 100 μm; blue: nuclear stain, green: 4T1 cancer cells, red: cRGD-NP; metastatic tissue sections left and healthy tissue regions right).

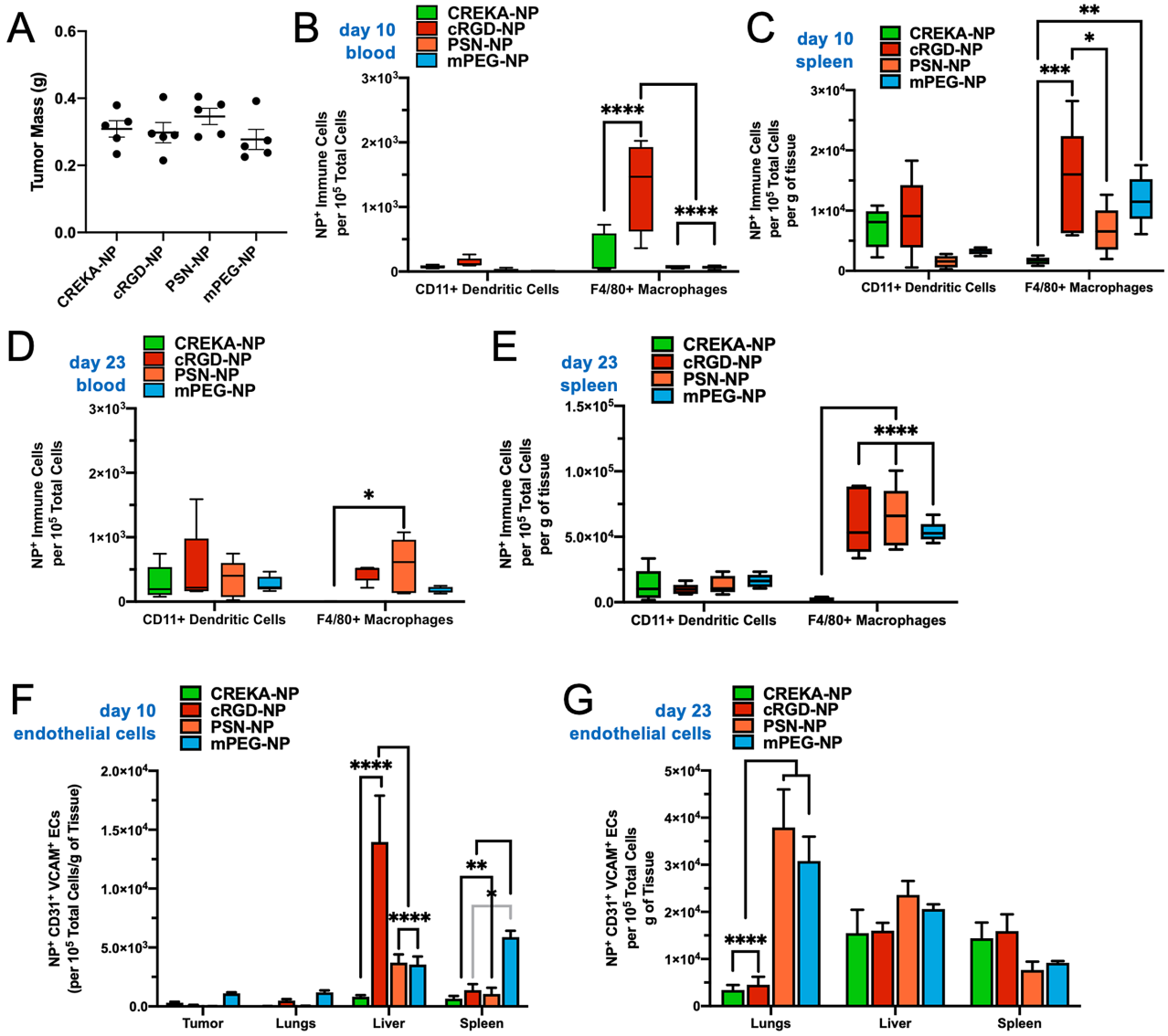

Fig. S2. Microdistribution and uptake of untargeted and targeted NPs by innate immune cells in the blood and the spleen and endothelial cells in tumor, lungs, liver and spleen. (A) Weight of primary tumors. (B–E) Flow cytometry analysis of nanoparticle uptake by APCs in the blood and the spleen on day 10 and day 23. Analysis of nanoparticle uptake by endothelial cells on (F) day 10 and (G) day 23. N=5 mice per condition. Box and whisker plots (5-95 percentile) with statistics by one-/two-way ANOVA with a post hoc Tukey or Sidak's test. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

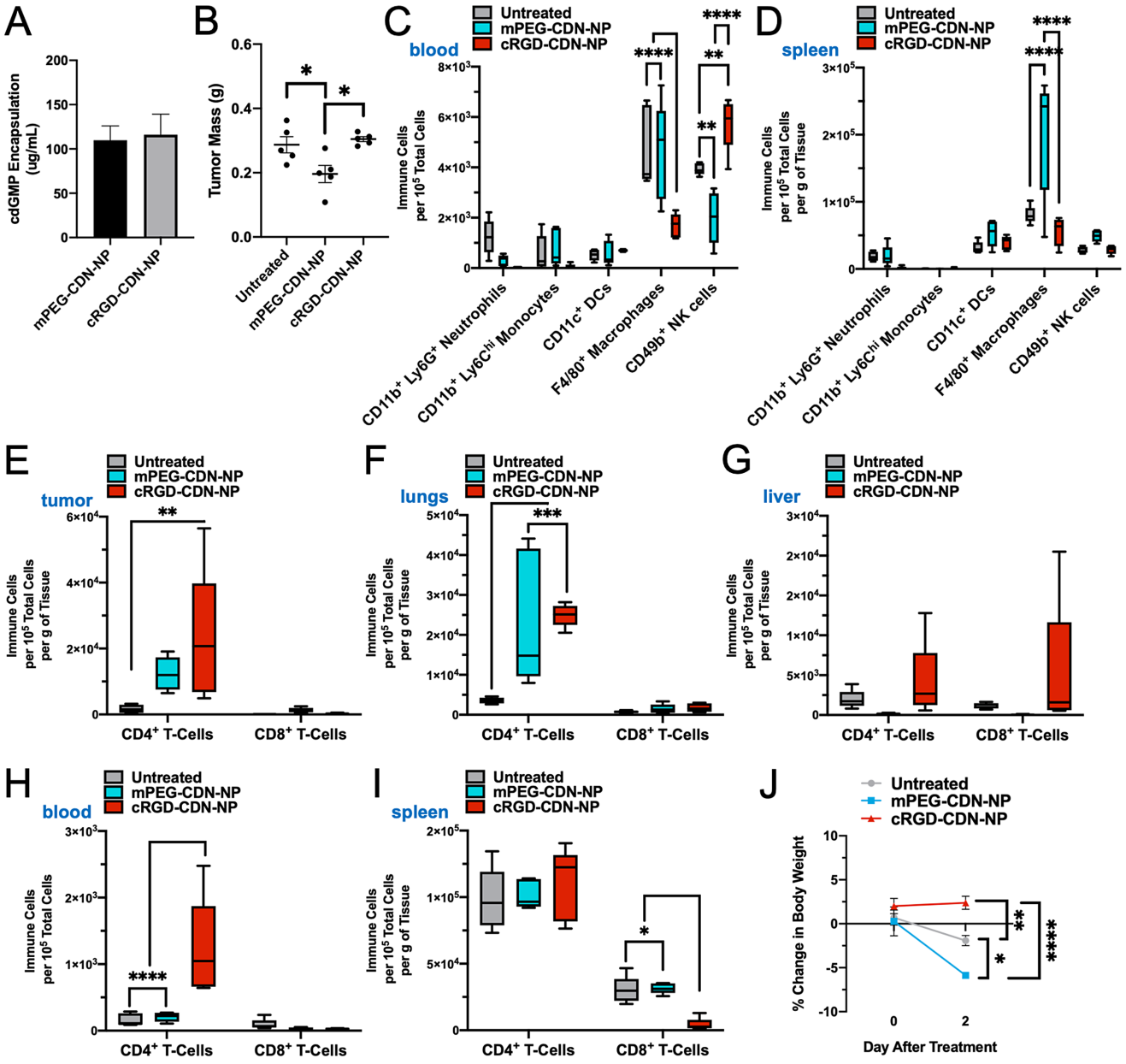

Fig. S3. Mechanistic study of the cellular immune response to immunostimulatory NPs 48 h after systemic delivery. (A) cdGMP encapsulation in untargeted and integrin-targeting NPs. (B) Weight of primary tumors was measured 2 days after NP treatment on day 10. Flow cytometry analysis of innate immune cells in (C) blood and (D) spleen, and $CD4^+$ and $CD8^+$ T cells in (E) Tumor, (F) lungs, (G) liver, (H) blood and (I) spleen. (J) Percent body weight change two days post-treatment. N=5 mice per condition. Box and whisker plots (5-95 percentile) both with statistics by one-/two-way ANOVA with a post hoc Tukey or Sidak's test. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

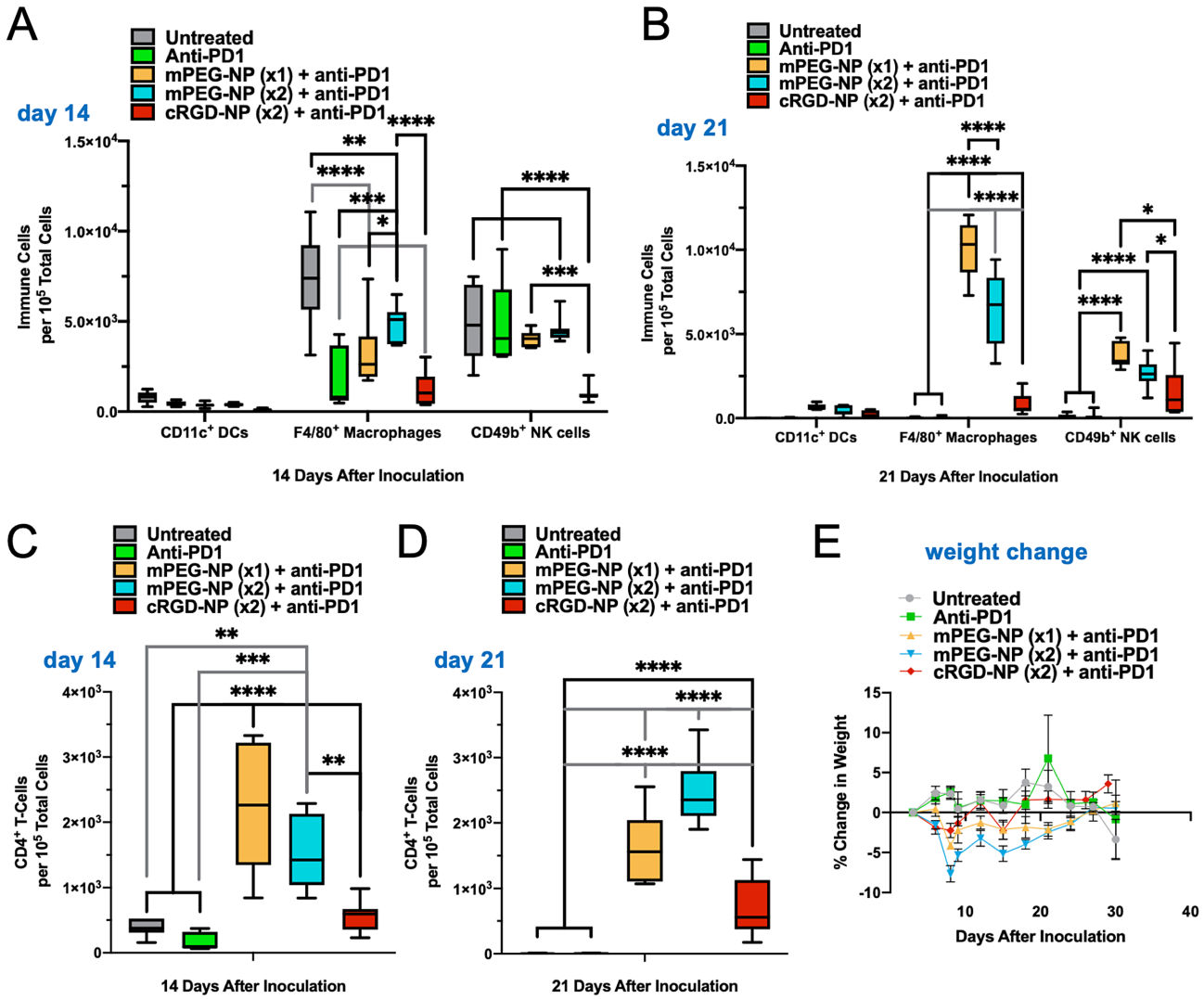

Fig. S4. Peripheral innate and adaptive immune cells in blood after treatment with immunostimulatory NPs in combination with surgery and anti-PD1. Flow cytometry analysis of circulating dendritic cells, macrophages and NK cells (A) 14 and (B) 21 days after tumor inoculation. Peripheral $CD4^+$ T cell counts (C) 14 and (D) 21 days after tumor inoculation. (E) Weight change of mice. N=7 mice per condition. Mean ± SEM are plotted with statistics by one-/two-way ANOVA with a post hoc Tukey or Sidak's test. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.