Supplemental Figures

Targeting Antigen Presenting Cells by Anti PD-1 Nanoparticles Augments Antitumor Immunity

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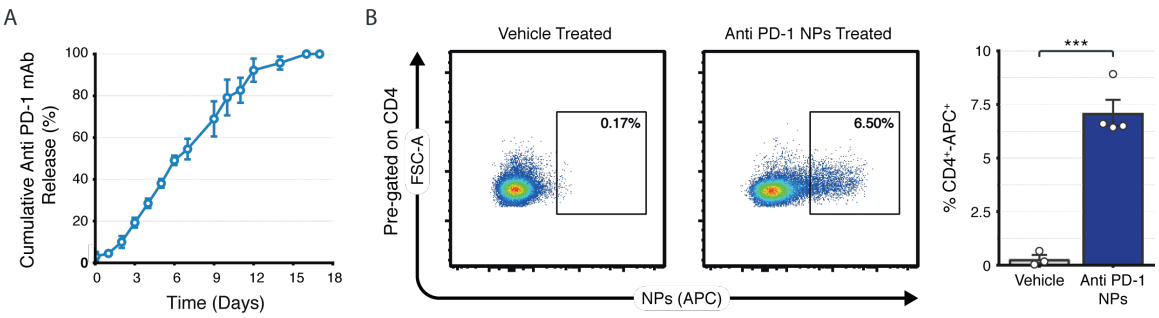
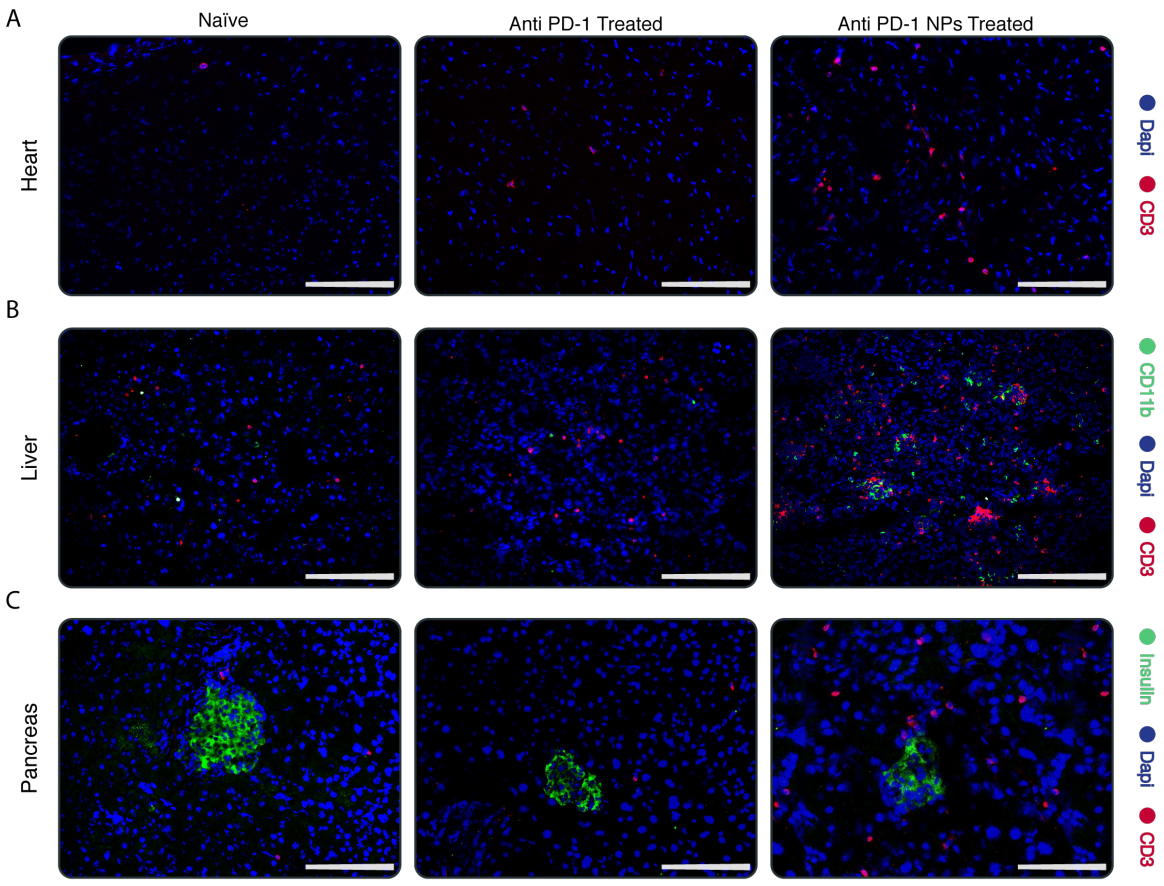
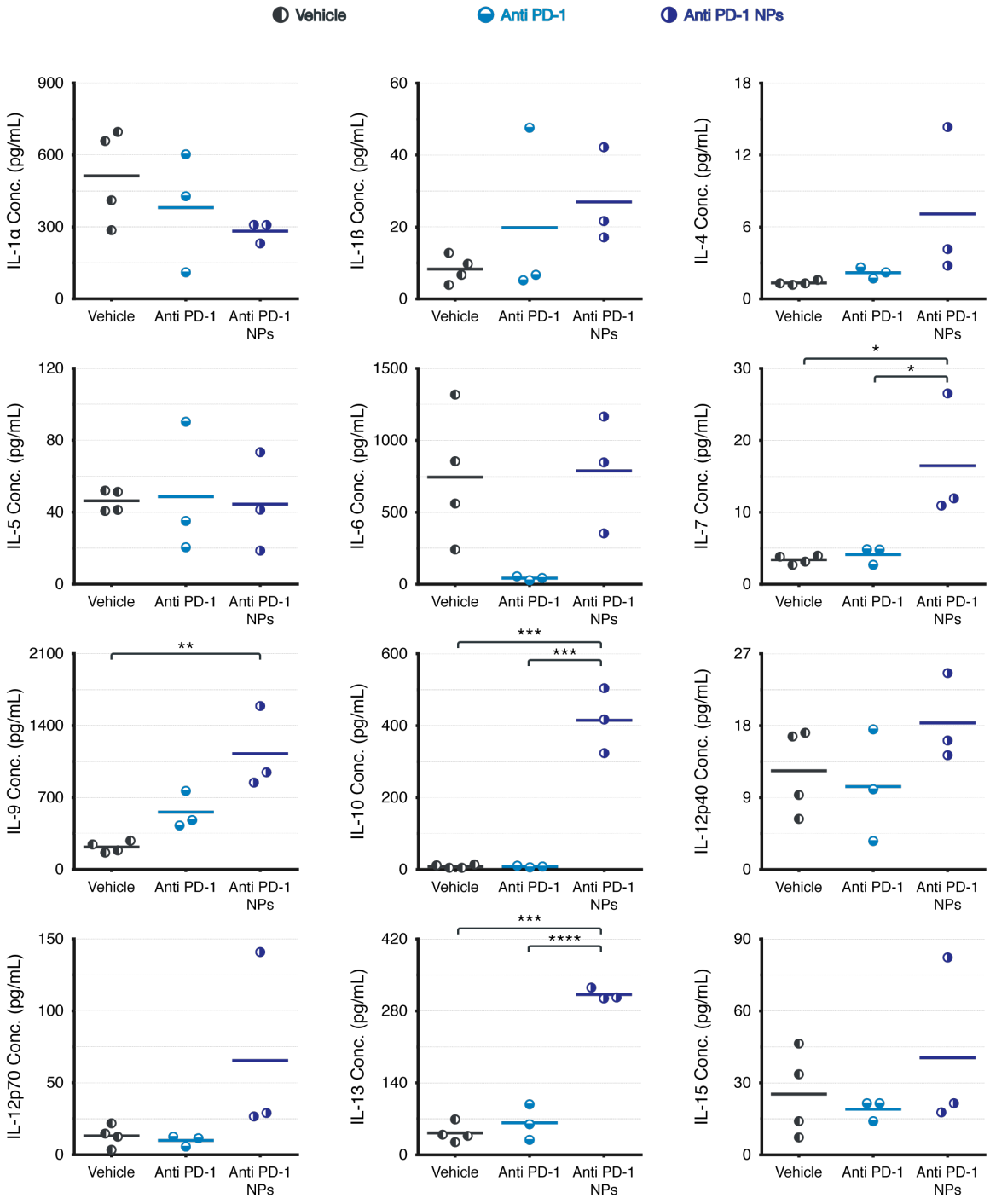


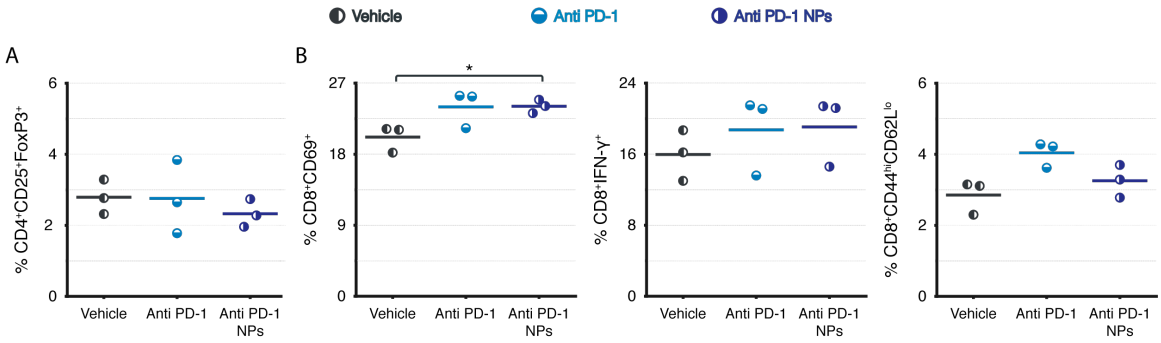
Supplemental Figure 1: (a) Release kinetics of Anti PD-1 mAb from PLGA NPs revealing sustained release of Anti PD-1 mAb over time with activity maintained for two weeks. Data are mean ± SEM of three preparations. (b) A high percentage of expression in $CD4^+$ T cells, labeled with the fluorochrome APC, was observed in the group that was co-incubated with DCs that were precultured with APC-labeled Anti PD-1-NPs (***P<0.005, n=4/group, calculated by two-tailed Student's t-test).



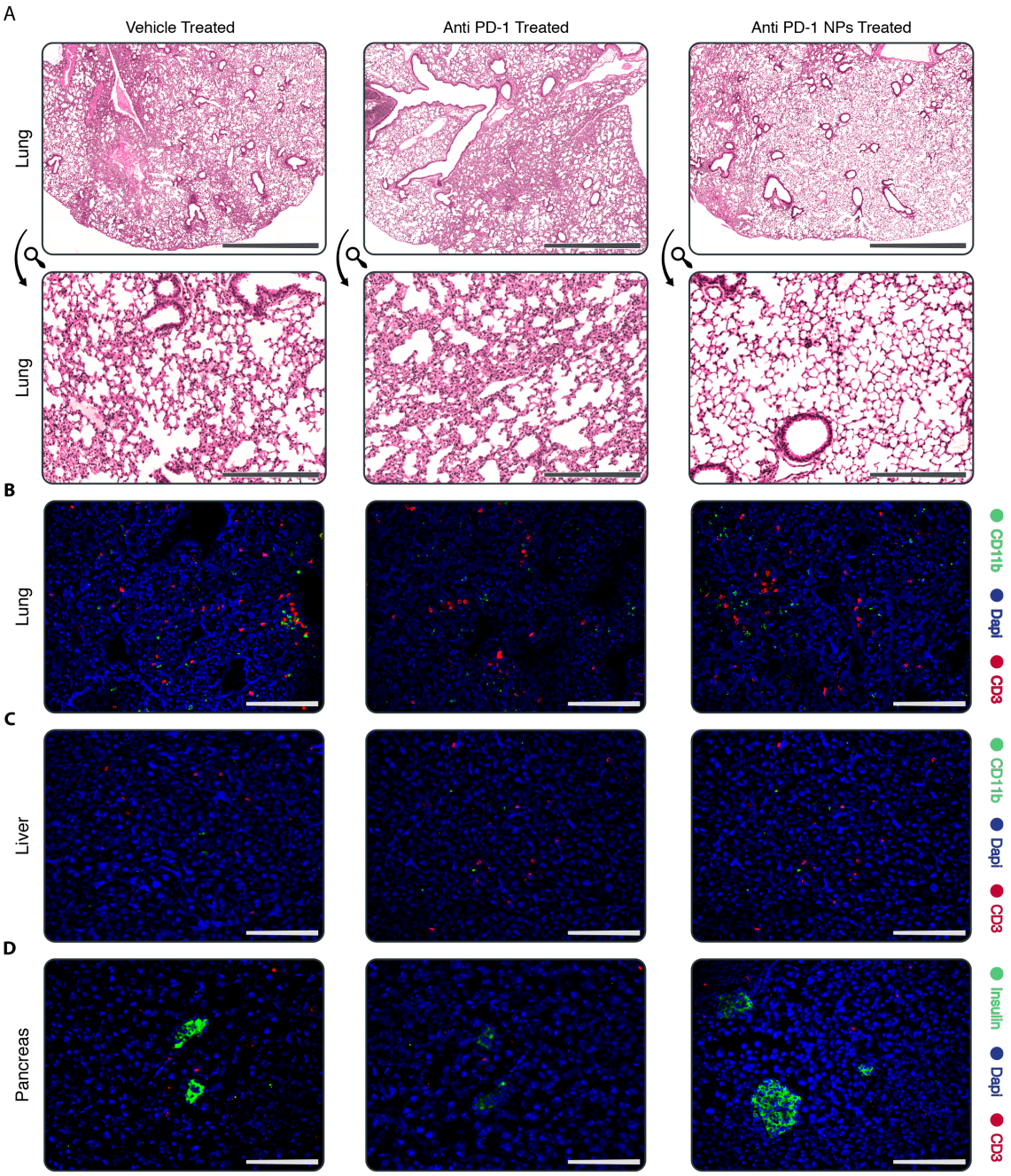
Supplemental Figure 2. Infiltration of lymphocytes in non-lymphoid tissues following high-dose Anti-PD-1-NPs treatment. Immunofluorescence staining of (A) heart, (B) liver, and (C) pancreas tissues show extreme infiltration of lymphocytes in non-lymphoid tissues of mice receiving high-dose Anti PD-1-NPs. These images are representative of 3 sections of 3-4 different mice in each group. (Scale bar represents 100 μm.)



Supplemental Figure 3. Cytokine profiles of mice following treatment with high-dose Anti PD-1-NPs. Mice received Anti PD-1-NPs every other day for 10 days. Data represent mean ± SEM (n = 3-4 mice/group). *P values: *P<0.05; **P<0.01; ***P<0.005;* calculated by one-way ANOVA.



Supplemental Figure 4: T cell immune activation status of mice following treatment with high-dose Anti PD-1-NPs. Mice received Anti PD-1-NPs every other day for 10 days. (A) No difference in the frequency of T_{Regs} was observed between the three groups. (B) The splenocytes from Anti PD-1-NPs-treated mice had a significantly higher proportion of $CD8^+CD69^+$ compared to vehicle treated mice, while no difference was observed in $CD8^+$ IFN-γ^+ and $CD8^+CD44^{hi}$ $CD62L^{lo}$ effector memory T cells compared to free Anti PD-1 (Anti PD-1) and vehicle-treated mice**.** The data are representative of two independent experiments. Data represent mean ± SEM (n = 3-4 mice/group). *P* values: **P*<0.05; ***P*<0.01; ****P*<0.005; calculated by one-way ANOVA.



Supplemental Figure 5. Infiltration of lymphocytes in non-lymphoid tissues following low-dose Anti-PD-1-NPs treatment. (A) Light micrographs of H&E-stained lung tissue from low-dose Anti PD-1-NPs-, Anti PD-1-, and vehicle-treated B16-F10 melanoma tumor-bearing mice demonstrate no difference in cellular infiltrates. From top to bottom, scale bar represents 1500 μm and 375 μm

respectively. Immunofluorescence micrograph of (B) lung, (C) liver, and (D) pancreas tissues show no differences in lymphocytic infiltration. The images are representative images from 2-3 sections of 3-4 mice in each group. (Scale bar represents 100 μm.)