

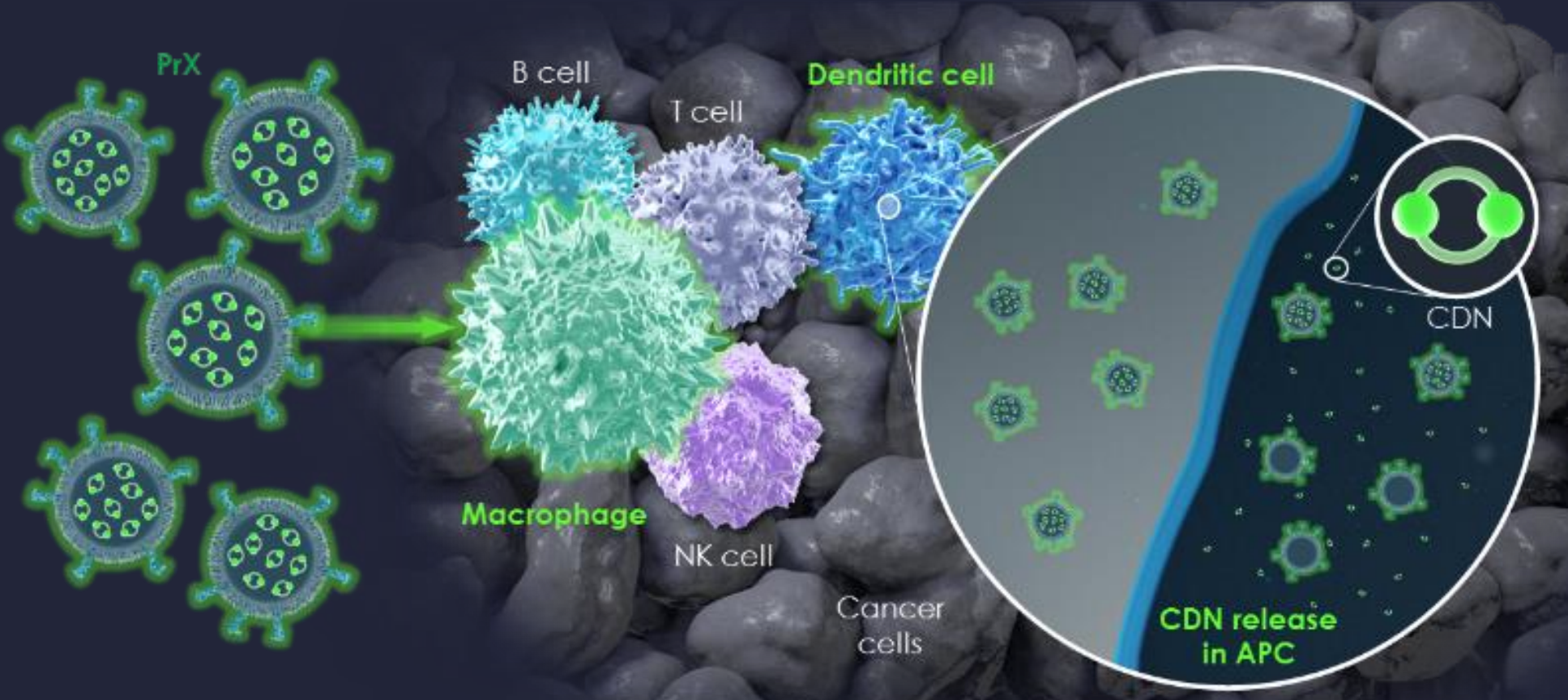
Selective Activation of Antigen Presenting Cells by exoSTING Enhances Tumor Antigen-Specific Immune Response

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Introduction

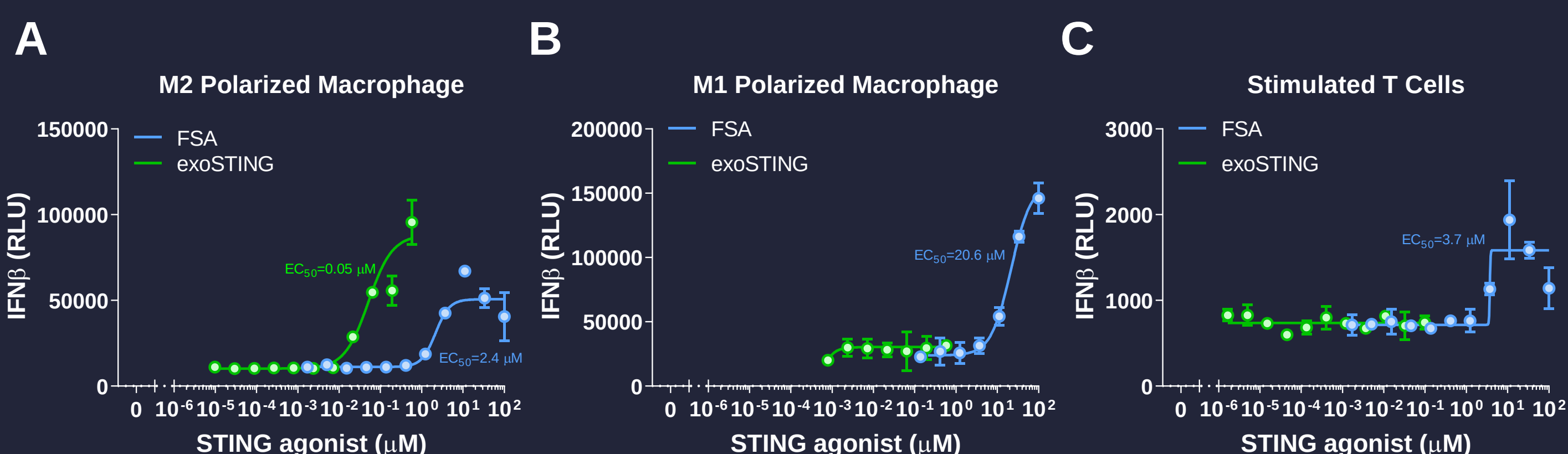


- Exosomes have shown potential to carry STING agonists to antigen presenting cells (APCs), resulting in an anti-tumor immune response.

- ExoSTING™ is composed of engineered exosomes overexpressing PTGFRN and loaded with a cyclic dinucleotide (CDN) small molecule STING agonist to leverage the inherent ability of exosomes to deliver STING agonists selectively to APCs.

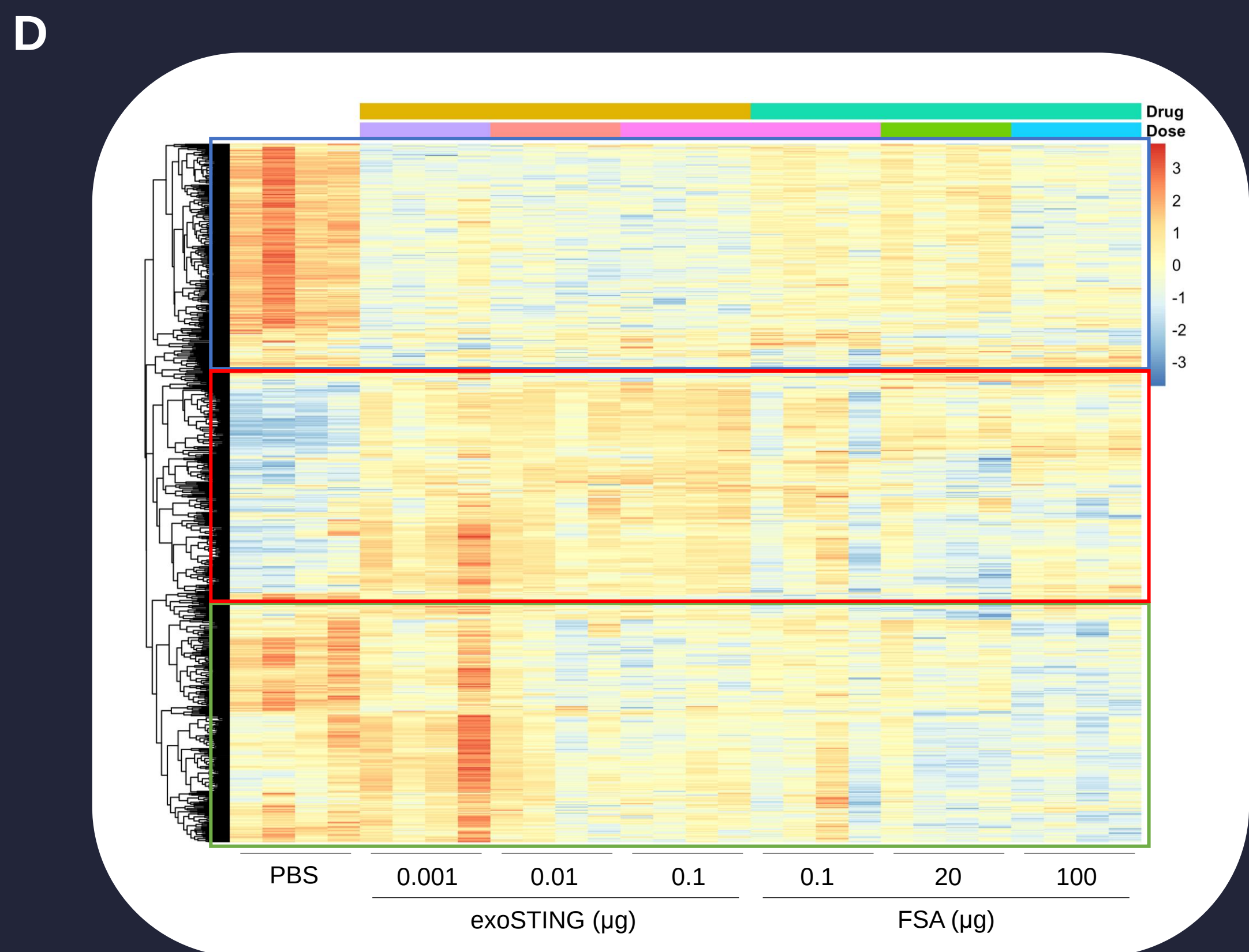
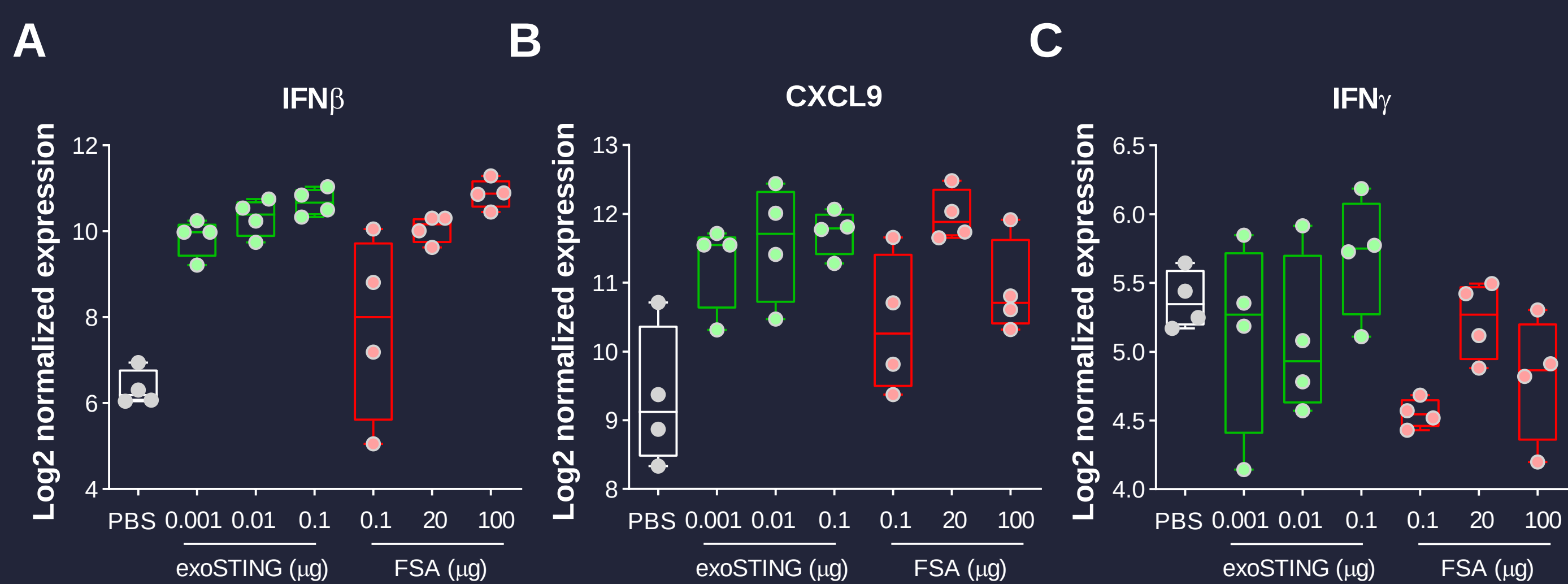
- ExoSTING selectively activates the STING pathway in tumor-resident APCs, demonstrating greater potency than free STING agonist (FSA) and without immune ablation.

exoSTING Selectively Activates M2 Immunosuppressive Macrophages



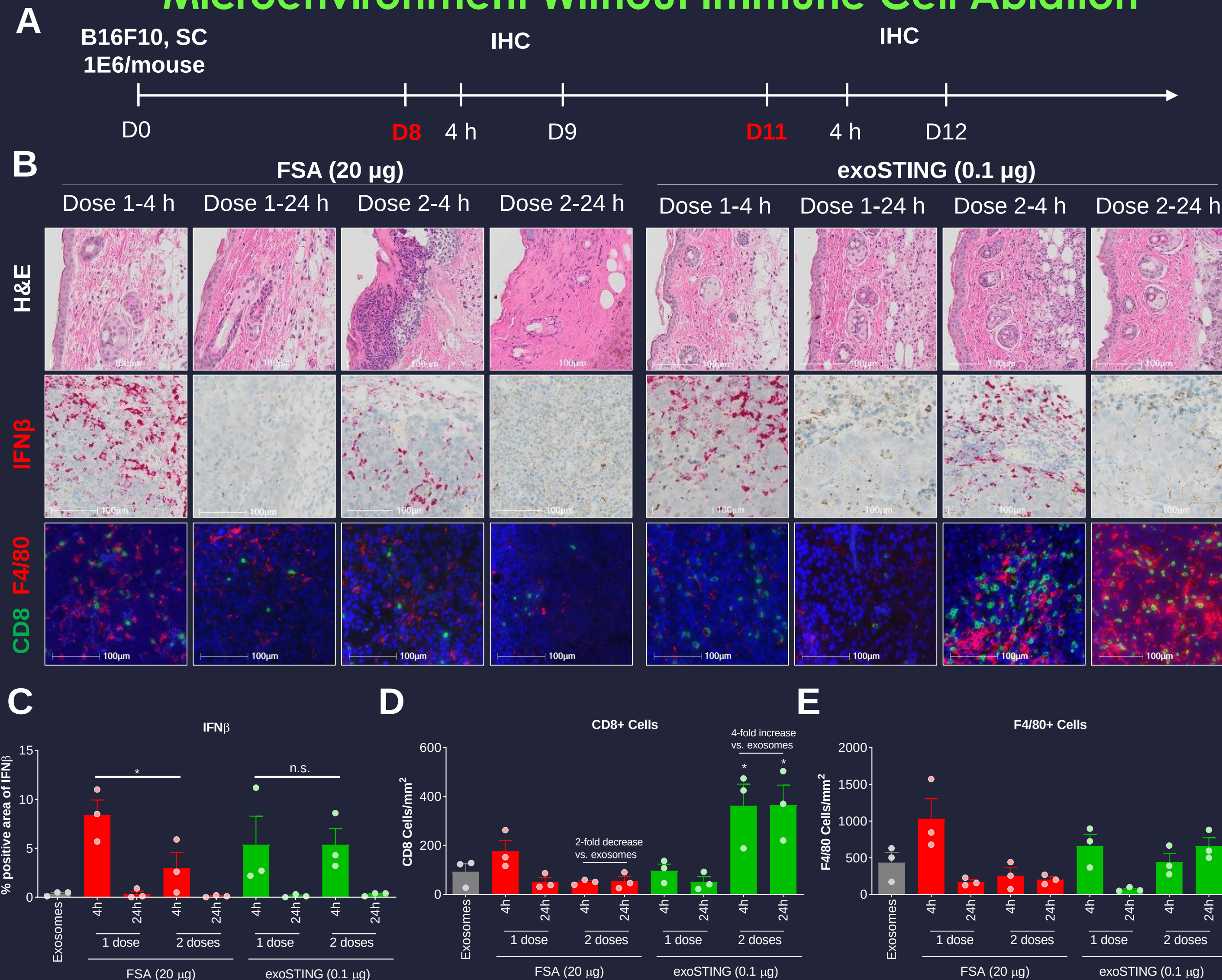
Various concentrations of FSA and exoSTING were incubated with M2 (A) or M1 (B) polarized human macrophages, or stimulated T cells (C), and production of IFN̢ in supernatant was measured. Monocytes were purified from human peripheral blood mononuclear cell (PBMCs) and differentiated into M1 or M2 macrophages by IFN̢ or combination of IL-4, TGF̢, and IL-10, respectively. T cells were purified from human PBMCs and stimulated with anti-CD3/anti-CD28.

exoSTING Induces Immune Activation Pathways in Tumor Without Bell-Shaped Dose Response



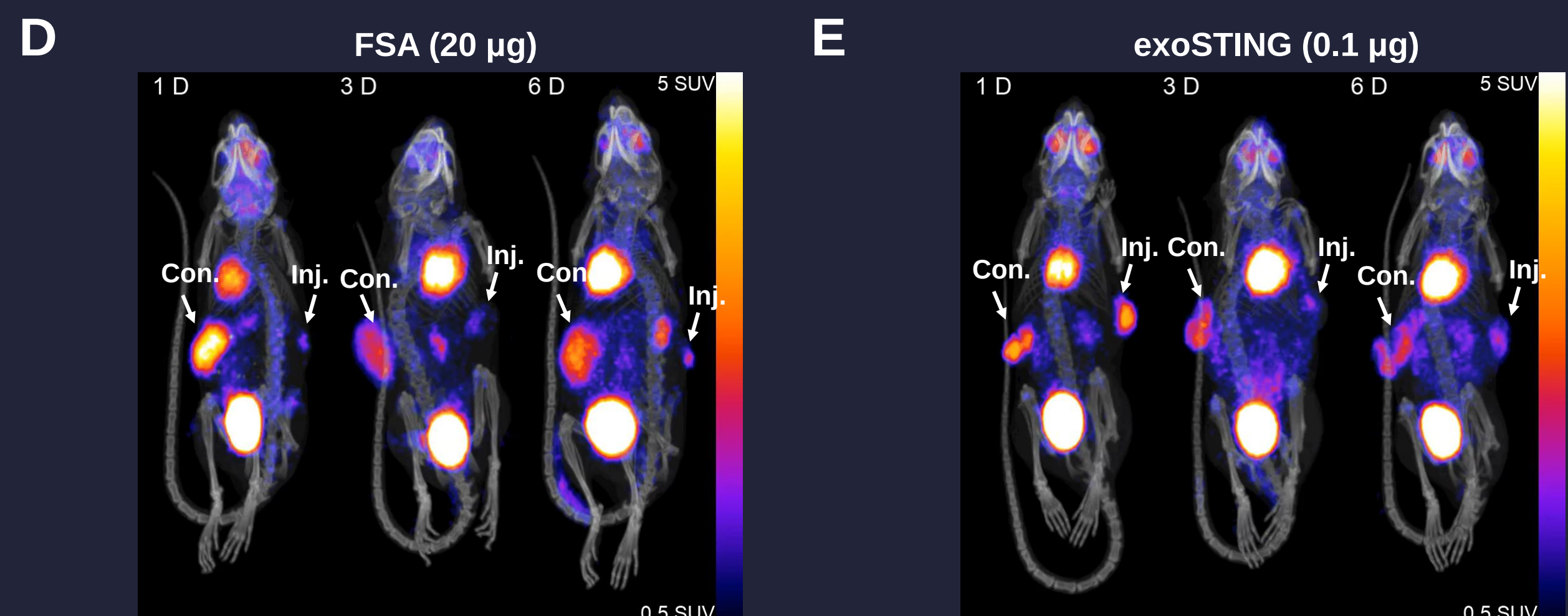
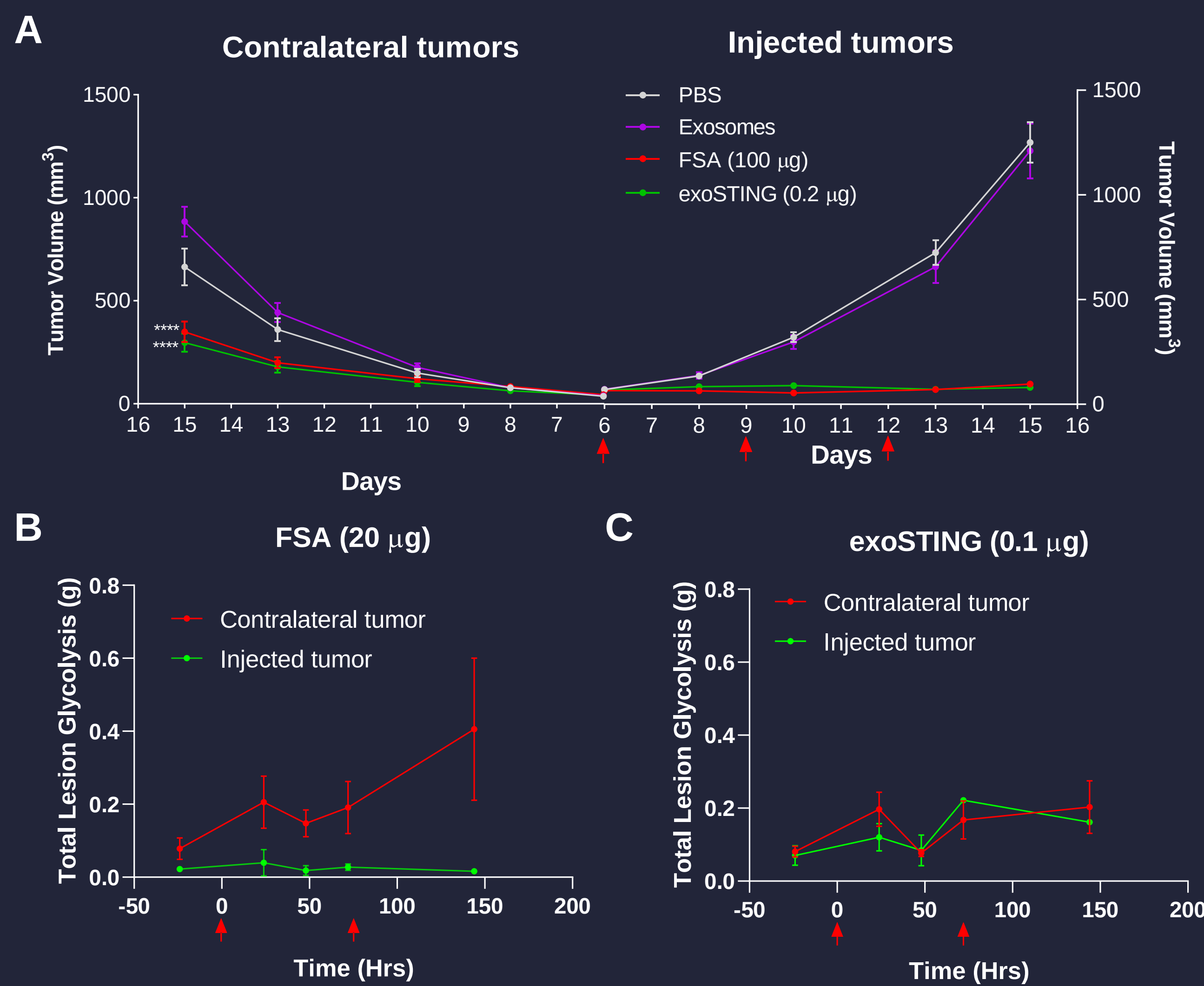
A-D. Four hours after intra-tumoral injection of PBS, FSA (0.1, 20, and 100 ̢g), and exoSTING (0.001, 0.01, and 0.1 ̢g) into B16F10 tumors ($n = 4$ per group), differentially expressed genes were analyzed using NanoString technology. Relative expression of IFN̢ (A), CXCL9 (B), and IFṆ (C). D. Heatmap of differentially expressed genes between FSA and exoSTING. Blue box; genes that are down-regulated by exoSTING more than FSA, Red box; genes that are up-regulated by exoSTING more than FSA, Green box; genes that are down-regulated by both exoSTING and FSA.

exoSTING Increases T Cell Infiltration in Tumor Microenvironment Without Immune Cell Ablation



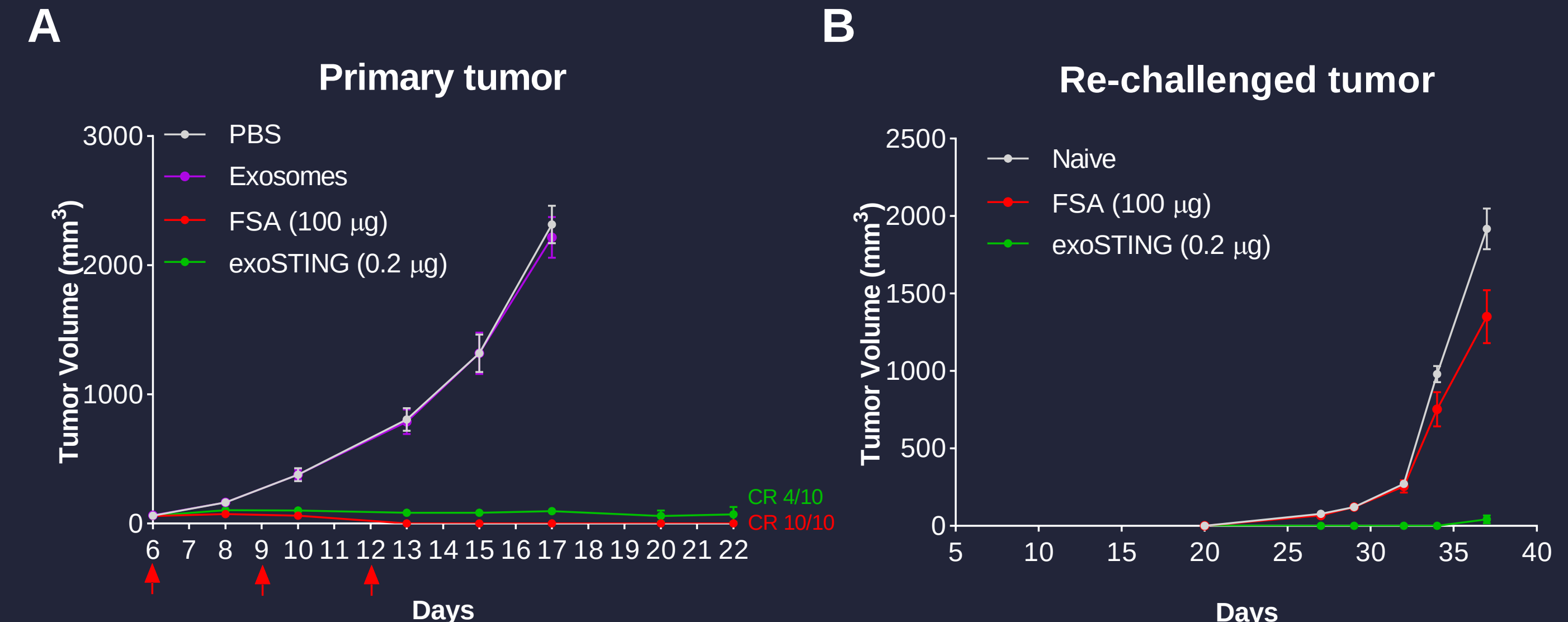
A-E. C57BL/6 mice were implanted subcutaneously with 1×10^6 B16F10 cell on the right flank of mice. FSA (20 ̢g) and exoSTING (0.1 ̢g) were injected intratumorally at Day 1 and Day 4 ($n = 5$ per group). Tumors were collected at 4 and 24 hours after the first dose and second doses. Tumor sections were stained with H&E, IFN̢ mRNA, CD8, and F4/80 (B). IFN̢ positive area (C), CD8 positive cells (D), and F4/80 positive cells (E) were measured. CD8 cells were 4-fold higher in exoSTING treated tumors than an exosome control. *, $P < 0.05$ by two-way ANOVA with Tukey's multiple comparison test, compared to exosome control. n.s.; non-significant

exoSTING Inhibits Tumor Growth in Both Injected and Non-Injected Contralateral Tumors



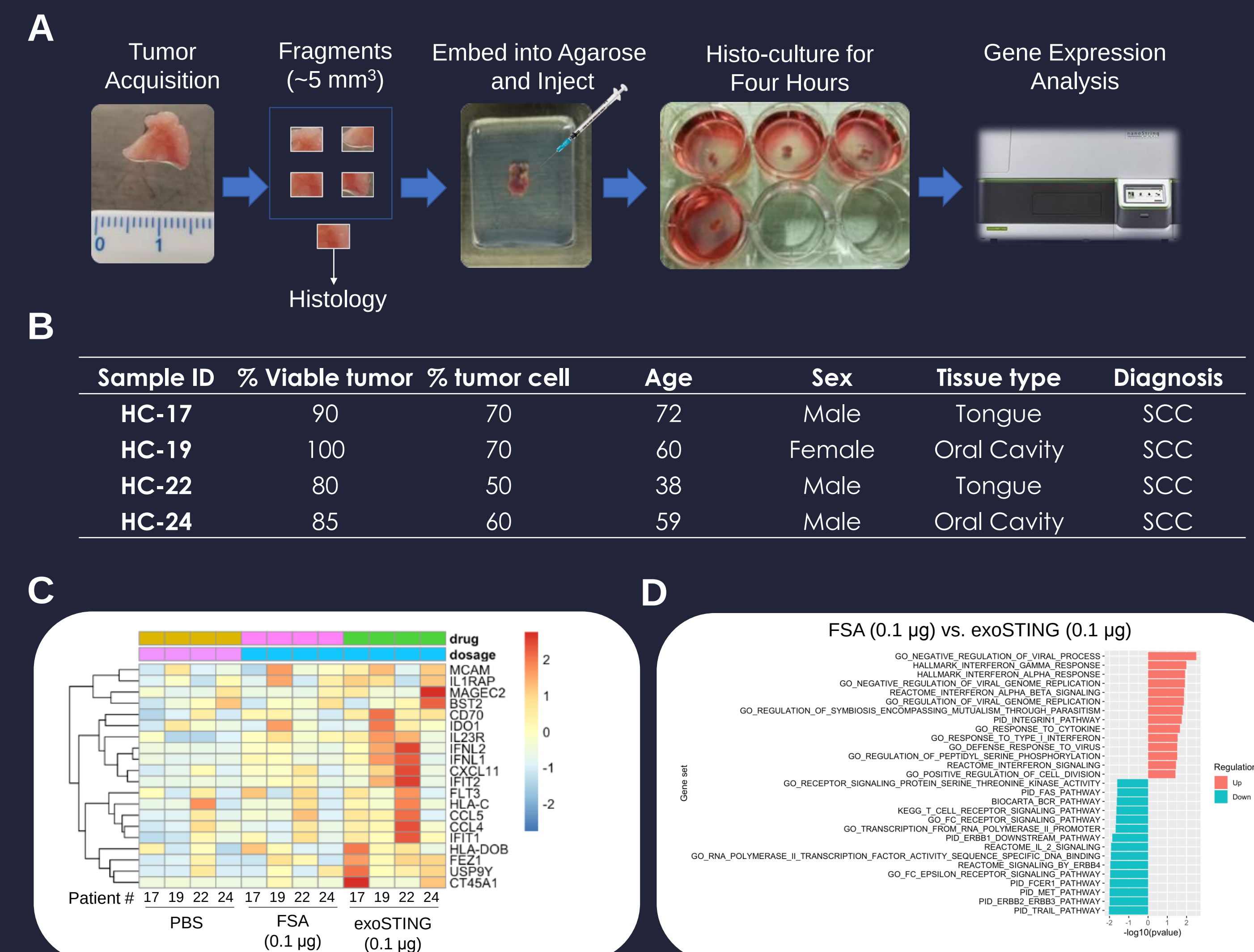
A, B16F10 tumor cells were implanted subcutaneously in the right (1×10^6 cells) and left (5×10^5 cells) flanks of mice ($n = 10$ per group). When tumor volumes reached 50 – 100 mm³, testing agents were injected intratumorally into right flank tumors 3 times at 3 days interval. Red arrows in the graph indicate intra-tumoral injection days. Both injected and non-injected contralateral tumor growth was measured. ****, $P < 0.0001$ by two-way ANOVA with Tukey's multiple comparison test, compared to both PBS and exosomes. B-E. When tumor volumes reached 50 – 100 mm³, testing agents were injected intratumorally into right flank tumors twice with 3 days interval. For PET-CT, ¹⁸F-FDG was injected intravenously at day -1, 1, 2, 3, and 6. Total lesion glycolysis was measured in both injected and non-injected contralateral tumors after FSA (20 ̢g) (B) and exoSTING (0.1 ̢g) (C) injection ($n = 3-8$ per group). Representative PET-CT images of FSA (20 ̢g) (D) and exoSTING (0.1 ̢g) (E) groups. Cont.; contralateral tumor, Inj.; injected tumor

exoSTING Preserves Memory Responses Against Tumors



B16F10 tumor cells were implanted subcutaneously in the right (1×10^6 cells) flank of mice ($n = 5-10$ per group). When tumor volumes reached 50 – 100 mm³, testing agents were injected intratumorally into right flank tumors. Red arrows in the graph indicate intra-tumoral injection days. B16F10 cells (1×10^6 cells) were implanted to mice that had CR and naive mice ($n = 5$) on day 20 to the left flank. Primary (A) and re-challenged (B) tumor growth were measured.

exoSTING Activates STING Pathway in ex vivo Human Tumor Tissues After Intra-Tumoral Administration



A, Fresh human tumor tissues were sliced into 5 x 5 mm size and injected with PBS, FSA (0.1 ̢g) or exoSTING (0.1 ̢g) intratumorally. After 4 hours treatment, RNA was isolated and analyzed by NanoString. B, Patient information and basic characterization of tumor tissue after H&E staining. C, The heatmap of top 20 up-regulated genes from NanoString analysis. D, Gene Set Enrichment Analysis of up- and down-regulated pathway in exoSTING (0.1 ̢g) treated tumors, compared to FSA (0.1 ̢g) treated tumors. E, Relative expression of IFN̢, CXCL9, CXCL10, IFṆ, CCL3, CCL4, and CCL3L1, measured by NanoString.

Summary

- ExoSTING was preferentially taken up by differentiated APCs. *In vitro* and *in vivo* studies showed more than a 100-fold increase in potency by exoSTING treatment compared to FSA.

- Intra-tumoral administration of exoSTING in B16F10 tumors preserves the viability of T cells and APCs, reduces collateral tissue damage, and eventually recruits more T cells into tumors, whereas FSA induces immune cell ablation.

- ExoSTING produced a systemic tumor-specific T cell response as demonstrated by growth inhibition of non-injected distal tumors and produced memory responses against tumors.

- ExoSTING activated interferon stimulated genes, including IFN̢, CXCL9, CXCL10, and IFṆ in ex vivo human tumor histo-culture, more than equivalent amounts of FSA.

- Clinical development for exoSTING is planned to begin in the first half of 2020.