

Supplementary Information for:

Nanoparticulate STING agonists are potent lymph node-targeted vaccine adjuvants

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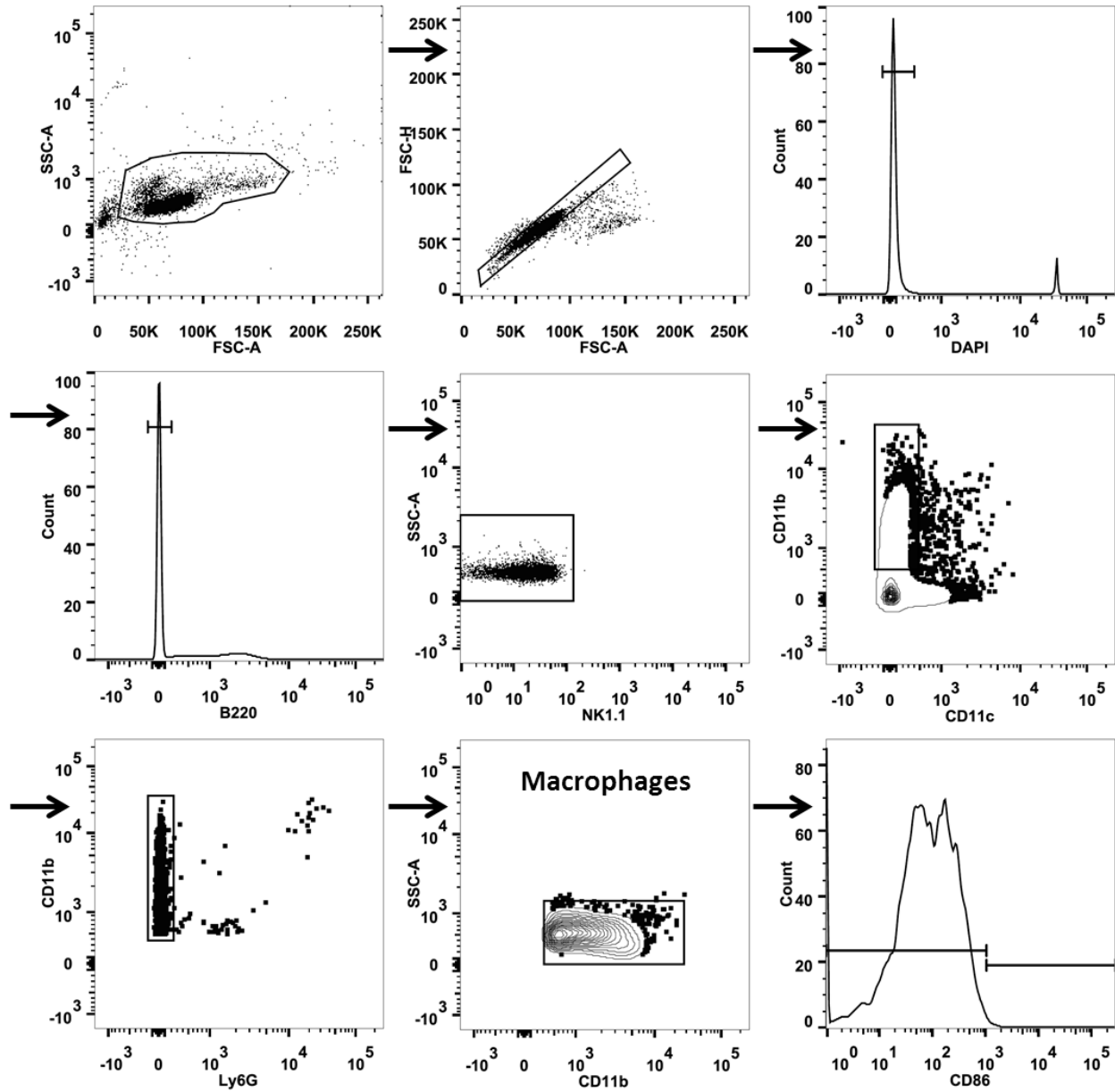
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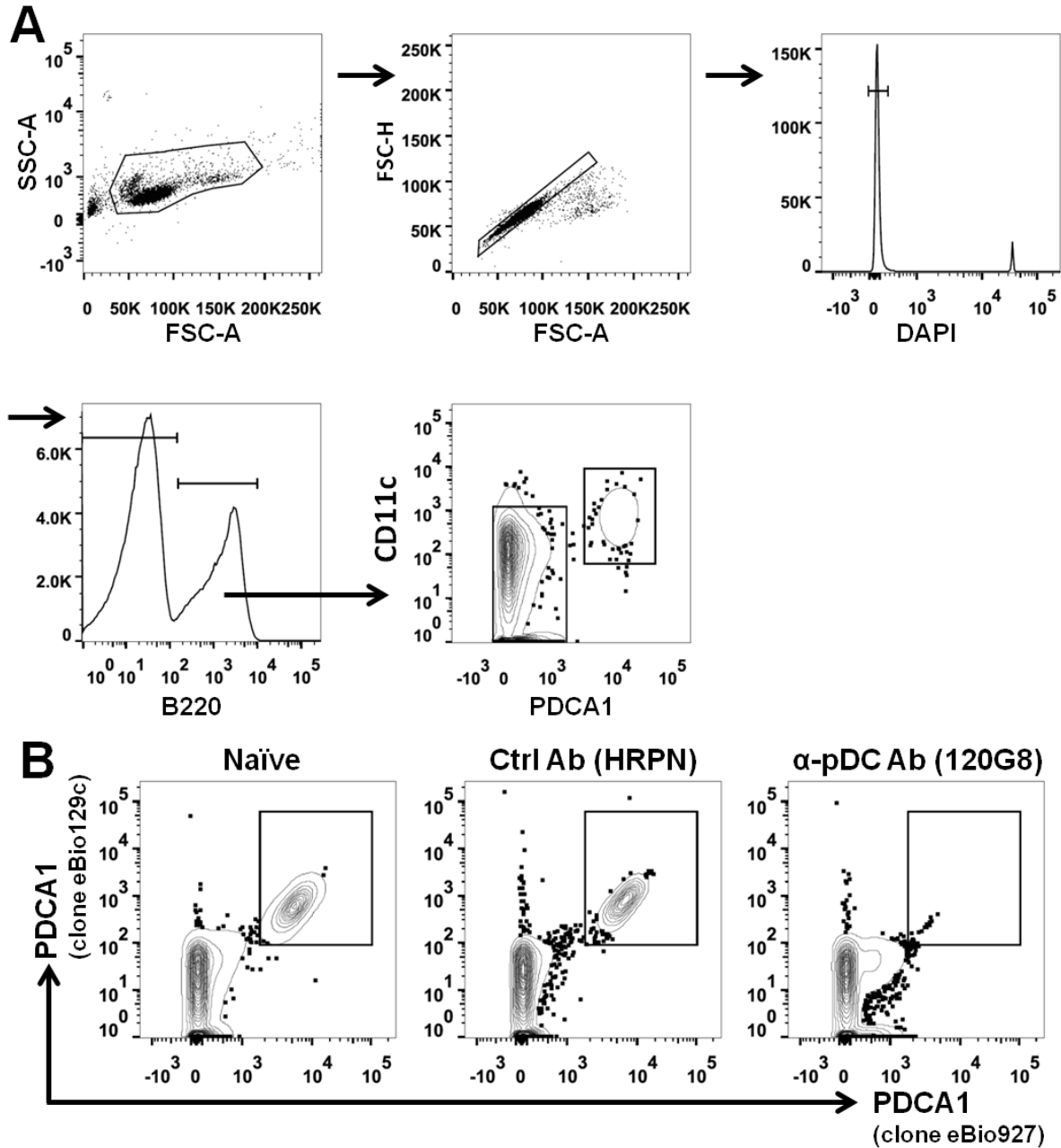
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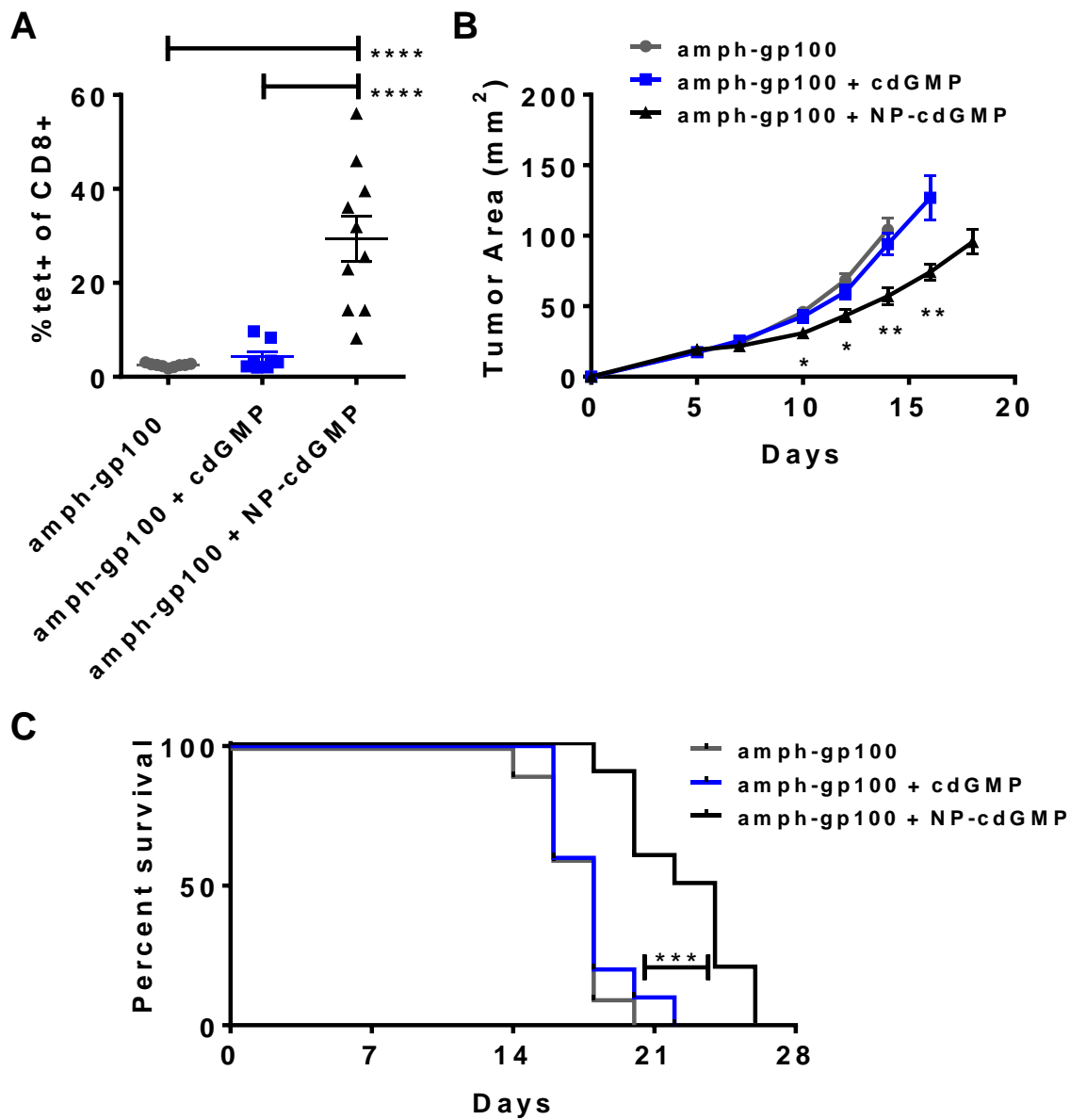
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Supplemental Figure 1: Gating to identify lymph node macrophages. Representative staining of naïve balb/c mice for lymph node macrophages, which were defined as B220⁻NK1.1⁻CD11c⁻CD11b⁺Ly6G⁻SSC^{lo} of single live cells. For activation studies, CD86⁺ macrophages were defined by histogram gating.



Supplemental Figure 2: Flow cytometry gating and depletion of plasmacytoid dendritic cells. (A) Representative gating of naïve balb/c mice for identification of plasmacytoid dendritic cells, which were defined as PDCA1⁺ CD11c⁺ of B220⁺ of live cells of single cells of total cells. (B) Representative dual PDCA1 staining of pDCs to confirm depletion of pDCs in mice treated with 500 μg 120G8 i.p. at 24 hours prior to necropsy and staining. pDCs were defined as PDCA1(clone eBio927)⁺ PDCA1(clone eBio129c)⁺ of CD11c⁺ B220⁺ live cells.



Supplemental Figure 3: NP-cdGMP delays B16.F10 tumor growth. C57BL/6 mice ($n=10/\text{group}$) were inoculated with 2.5×10^5 B16.F10 cells s.c. on day 0 and vaccinated on days 5, 12, and 19 with $10 \mu\text{g}$ amph-gp100 alone or adjuvanted with $5 \mu\text{g}$ cdGMP or $5 \mu\text{g}$ NP-cdGMP. **(A)** Percent SIINFEKL-tetramer positive CD8^+ T-cells among PBMCs on day 18. **(B)** Mean tumor size over time (compared to OVA alone). **(C)** Survival curves over time. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, ****, $p < 0.0001$.