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Supplemental Material to:

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Dasatinib promotes the expansion of a therapeutically superior T-cell repertoire in response to dendritic cell vaccination against melanoma

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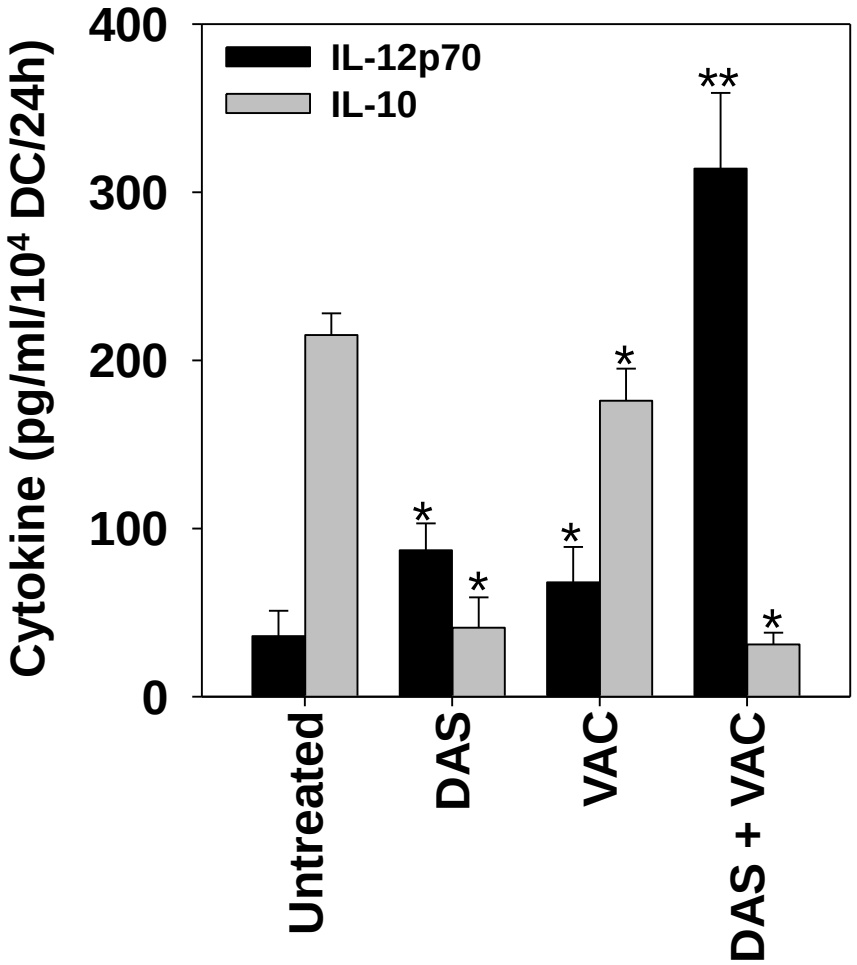
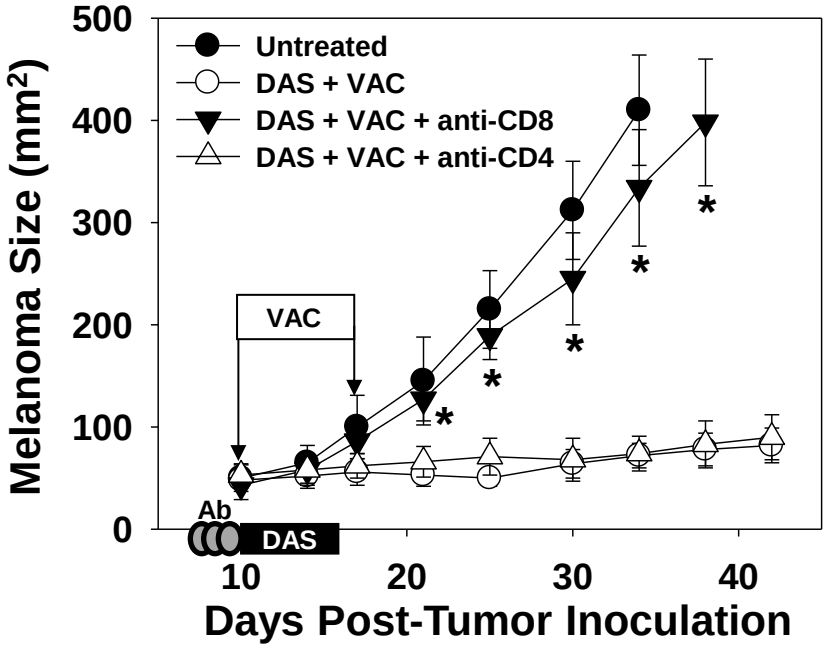
Fig. S1 Lowe et al.



DAS + VAC combined therapy results in improved DC1 function in the TME. Tumors were harvested on day 34 from M05 melanoma-bearing C57BL/6 mice treated as outlined in **Fig. 2A**, and enzymatically digested as described in Materials and Methods to yield a single-cell suspension. After CD11c-MACS isolation of DC from each treatment cohort, each DC population (10^4 DC/well) was stimulated for 24h with LPS (10 ng/ml; Sigma-Aldrich, St. Louis, MO). Cell-free supernatants were then recovered and analyzed by commercial ELISA (BD Biosciences, San Jose, CA) for levels of mIL-12p70 and mIL-10. Data are reported as mean +/- SD concentration of each cytokine based on triplicate determinations. Three independent experiments were performed, with each providing a comparable data set. *$p < 0.05$ versus untreated; **$p < 0.05$ versus all other groups (ANOVA).

Fig. S2 Lowe et al.



Superior anti-melanoma efficacy associated with combined DAS + VAC therapy is CD8⁺, but not CD4⁺ T cell-dependent. C57BL/6 mice bearing established day 8 s.c. M05 melanomas (5 mice/group) were left untreated, or they were treated with i.p. injection of 50 µg of monoclonal antibody (mAb) GK1.5 (ATCC) or 100 µg of mAb 53-6.7 (kindly provided by Dr. Zhaoyang Yu, University of Pittsburgh), respectively, in 100 µL PBS on days 8-10. Confirmation of specific T-cell depletion was performed by analyzing splenocytes from treated mice by flow cytometry using FITC-labeled anti-CD4 and anti-CD8 mAbs (both from BD Biosciences) that were not sterically blocked by the corresponding mAbs used for *in vivo* depletion. On day 10, 5 untreated mice and the mice depleted of T cell subsets were treated with combined DAS + VAC as described in **Fig. 2A**. Each cohort of animals was then monitored for mean +/- SD melanoma size through day 42 post-tumor inoculation. *$p < 0.05$ versus DAS + VAC control (no antibody; ANOVA).