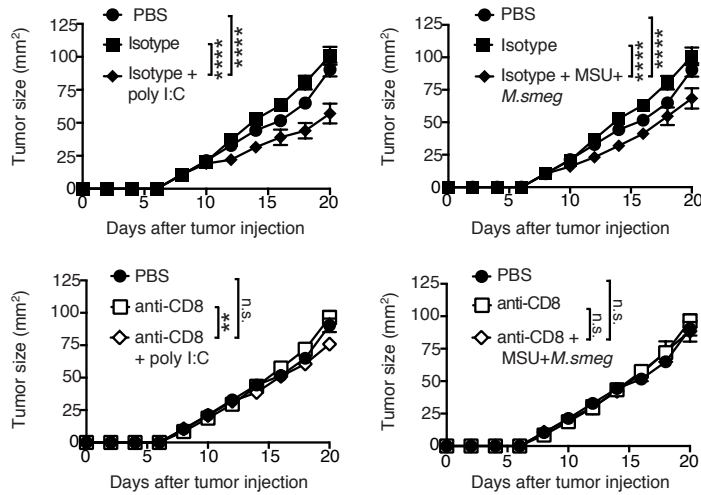


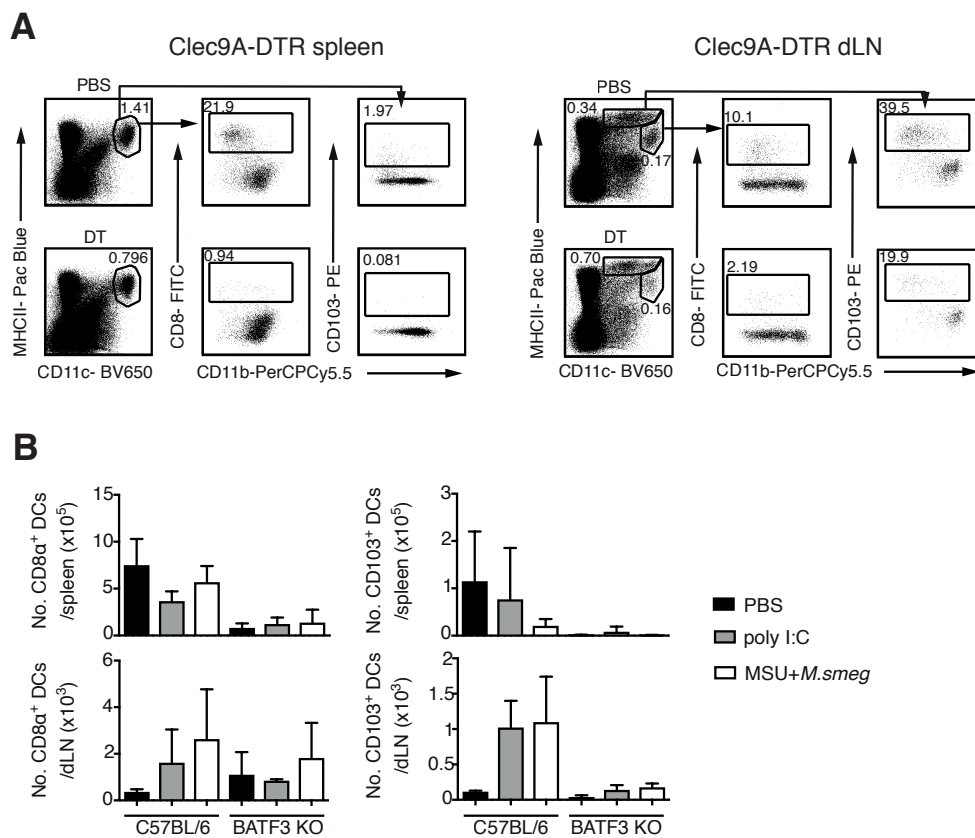
Supplemental Information

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Supplemental Figure 1: CD8⁺ T cells are required for the anti-tumor effect of poly I:C and MSU+*M.smeg*.

Tumor growth data for the experiments in Figure 1. Balb/cByJ mice bearing orthotopic 4T1 tumors were given peri-tumoral injections of the indicated treatments on days 8, 10, 12, and 14. CD8⁺ T cells were depleted by mAb treatment starting one day before tumor challenge. Tumor growth data are pooled from two independent experiments each with 4-5 mice/group, and refer to the same mice shown in Figure 1. Tumor growth curves were compared by two-way ANOVA with the Bonferroni correction. ** $p < 0.01$, *** $p < 0.001$. **** $p < 0.0001$.

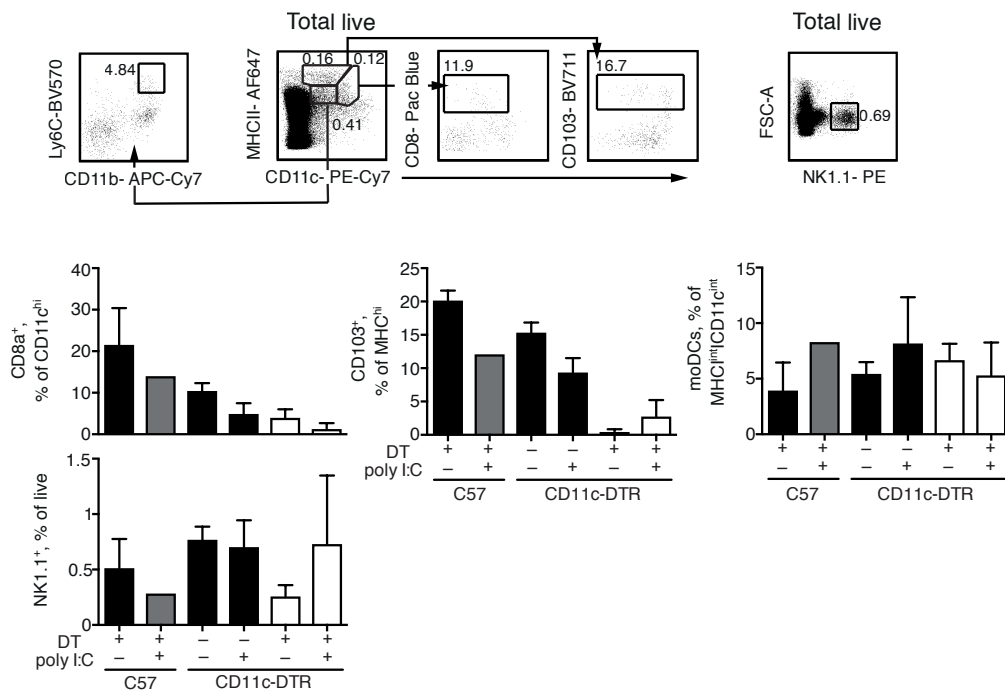


Supplemental Figure 2: Depletion of CD8 α ⁺ and CD103⁺ DC subsets in Clec9A-DTR and BATF3 KO mice.

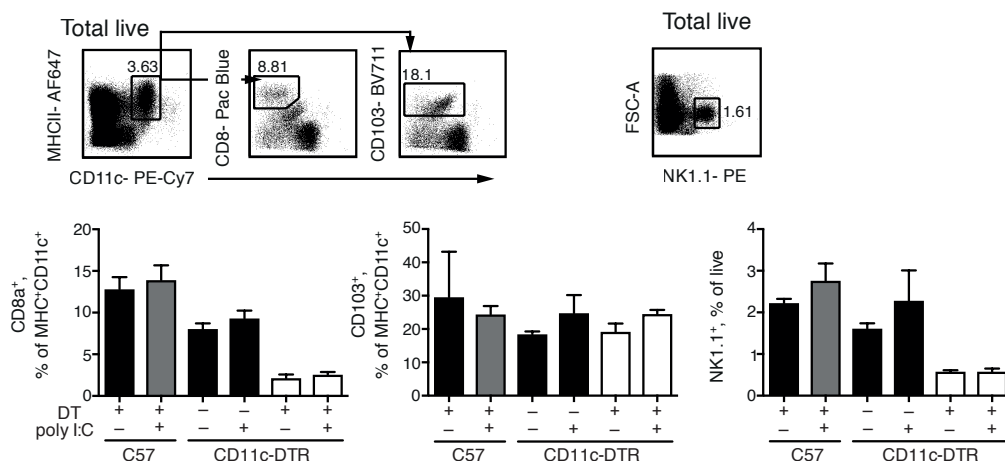
(A) Clec9A-DTR mice bearing orthotopic 4T1 tumors were treated with DT by i.p. injection on days 8, 10, 14, 18 and 20. Spleens and dLNs were harvested on day 21, and CD8 α ⁺ and CD103⁺ DCs were identified according to the gating strategy shown. Cells were previously gated for singlets, live, and CD45 expression.

(B) C57BL/6 and BATF3 KO mice bearing B16.F1 tumors were treated peri-tumorally as indicated on day 8, 10, 12, and 14. Spleens and dLNs were harvested on day 15 and DC subsets were identified by flow cytometry using the strategy shown in (A). Bar graphs show mean and SD for 2-4 mice/group.

dLN gating

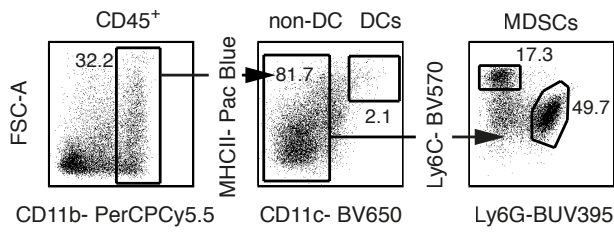


Spleen gating



Supplemental Figure 3: Analysis of cell populations in the dLN and spleen of CD11c-DTR chimeric mice

C57BL/6→C57BL/6 or CD11c-DTR→C57BL/6 BM chimeras were injected with B16.OVA tumors, treated poly I:C or PBS, and at the same time depleted of CD11c⁺ cells by DT injection 18 h before each treatment as described in Figure 2. Two days after the fourth treatment, 2-3 mice/group were sacrificed for flow cytometric analysis of cell populations in dLN and spleen. The remaining mice in each group were kept for assessment of tumor size and survival as shown in Figure 2D. Representative gating of dLN and spleen cell populations is shown on top, percentages of DC and NK cell populations in each tissue are shown below. Bar graphs show mean and SD for each group.



Supplemental Figure 4: Identification of MDSCs in 4T1 tumors

Tumors were harvested on day 21 from Clec9A-DTR mice bearing orthotopic 4T1 tumors. Ly6C⁺ and Ly6G⁺ MDSCs were identified by flow cytometry according to the gating strategy shown. CD45⁺ cells were previously gated for single cells and live (DAPI).