



Supplementary figure 3

BALB/c mice were injected with 1×10^5 4T1.2 cells into 4th mammary fat pad and palpable tumors were i.t. injected with vehicle or 3M-052 on day 7 post-inoculation. Mice were treated with four doses of isotype or anti-PD1 mAb before primary tumor resection on day 14 (shown in Figure 2a). Primary tumors were assessed for **(a)** proportion of tumor that is 4T1.2 cells (mCherry⁺); **(b)** proportion TCR β ⁺CD4⁺ that were CD25⁺FoxP3⁺ Tregs; **(c)** number of TCR β ⁺CD4⁺CD25⁺FoxP3⁺ Tregs; **(d)** Ly6G⁺CD11b⁺ cell number and **(e)** TNF α ⁺TCR β ⁺CD8⁺ T cell number by flow cytometry. **(f)** TCR β ⁺CD8⁺ cells from day 18 peripheral blood (PB) was assessed for IFN γ production with no stimulation, **(g)** representative flow cytometry plots shown from individual 3M-052 + anti-PD1 PB sample demonstrating 4T1.2-specificity. Experiments were independently repeated twice, and representative data are shown. $n = 8-9$ mice/group. Statistical analysis was performed by one-way ANOVA with post-hoc Tukey's multiple comparison test. Error bars are SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.