## Supplementary Figures

**Fig. S1. Depletion confirmation.** Blood was collected at day 15 following treatment and cell populations were analyzed via flow cytometry.

**Fig. S2. Flow cytometry gating strategy**

**Fig. S3. MPL enhances efficacy of RT+C4 in B78 melanoma model.** Mice with a single B78 flank tumor were treated with PBS, External Beam Radiation (RT, 12 Gy), RT+anti-CTLA-4 (C4, 200 µg), RT+C4+MPL (MPL, 20 µg), RT+MPL, MPL+C4, C4 alone, or MPL alone. Tumor response, by group, and animal survival are shown for B78 (in **A**, and **B**). Mice with complete response to treatment with either RT+C4 or RT+C4+MPL (**C**) were rechallenged with the same tumor they initially rejected (**D**). N=10-16 mice per group. Significance determined by linear mixed effects regression analysis with Tukey multiple comparisons testing for tumor growth (significant differences, $p < 0.05$, demarcated by * with the color of the asterisk representing which group from which the sample is significantly different), Kaplan–Meier estimation with log-rank testing and Cox regression for survival analysis (significant differences, $p < 0.05$, demarcated by * with the color of the asterisk representing which group from which the sample is significantly different), and Chi-squared test for complete response rate.

**Fig. S4. MPL promotes Th1 antibody class switching.** To determine the presence of anti-tumor antibodies serum was isolated from mice bearing B78 tumors at day 15 following treatment initiation. Serum was incubated with B78 cells and antibody class was determined using secondary antibodies against IgG, IgG1, and IgG2c (**A**, **B**). Ratio of IgG2c:IgG1 at D15 was increased in RT+C4+MPL compared to other groups.

**Fig. S5. Radiation downregulates TLR4 expression on macrophages and MHC-II expression on B16 tumor cells and cytotoxic CD4 cells are not generated by combination therapy but B cells are modestly activated with radiation but not MPL or serum.**

Macrophages cultured in vitro were treated with PBS, anti-CTLA-4 (C4, 5 µg), radiation (RT, 12 Gy) or RT+C4 and expression of TLR4 was quantified using flow cytometry 24 hours following treatment (**A**). B16 melanoma cells cultured in vitro were treated with PBS or radiation (RT, 12 Gy) and expression of MHC-II was quantified using flow cytometry 3 days following treatment (**B**). B16 tumors were treated with PBS or RT+C4+MPL and expression of MHC-II was quantified using flow cytometry 15 days following treatment (**C**). CD4 cells were also harvested and co-cultured with B16 cells that had received either 0 Gy or 12 Gy of RT 7 days prior to co-culture and tumor cell killing was quantified using Annexin V staining (**D**). B cells cultured in vitro were treated with either PBS, MPL (100 µg), serum from disease free mice, or MPL+serum and activation markers were quantified using qPCR (**E**). B cells cultured in vitro were treated with either PBS, C4 (5 µg), RT (12 Gy), or RT+C4 and activation markers were quantified using qPCR (**F**).

**Table S1**. List of Taqman probes and primers utilized for quantitative RT-PCR experiments

**Table S2**. List of flow cytometry antibody targets, clones, and fluorophores