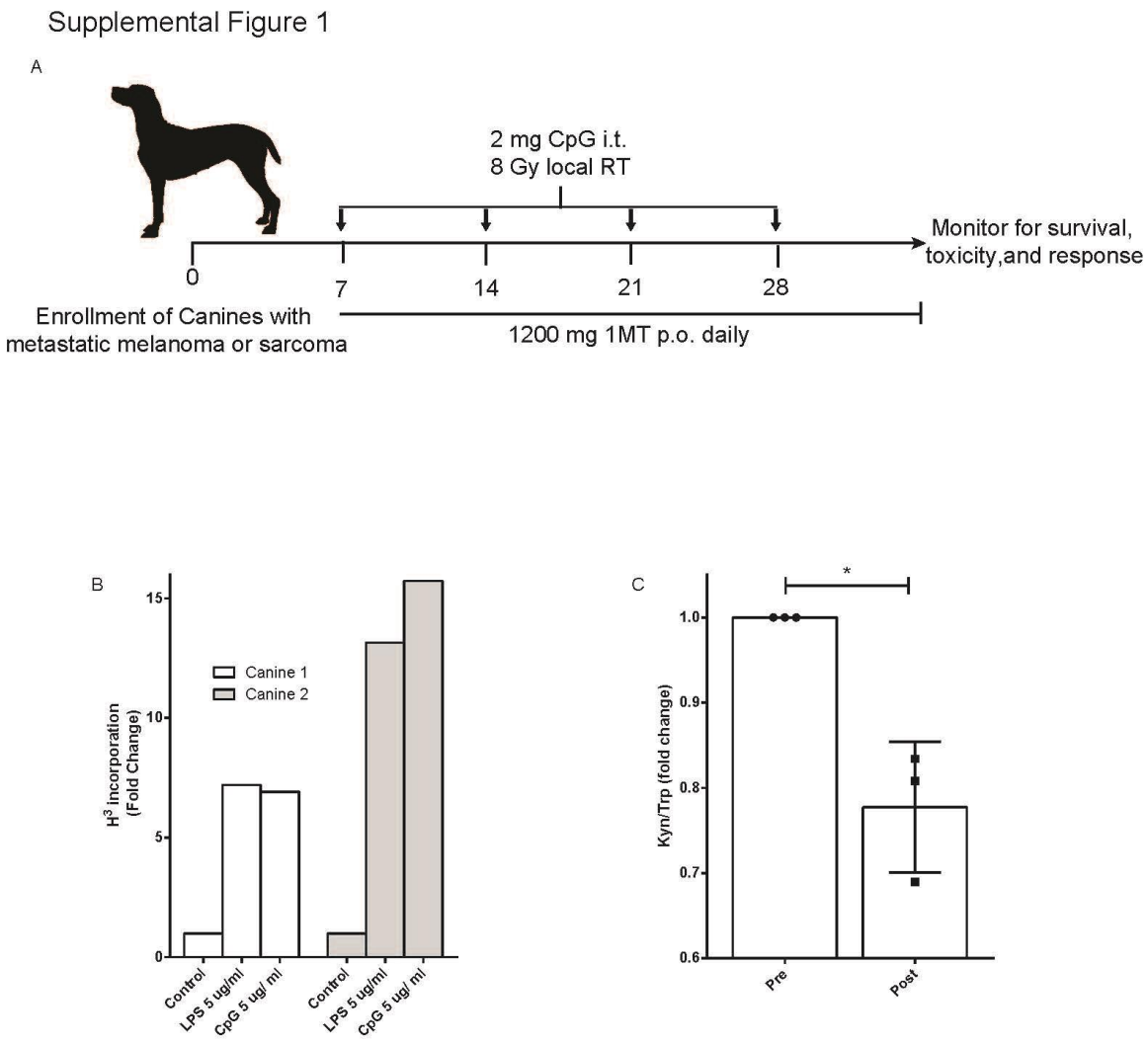
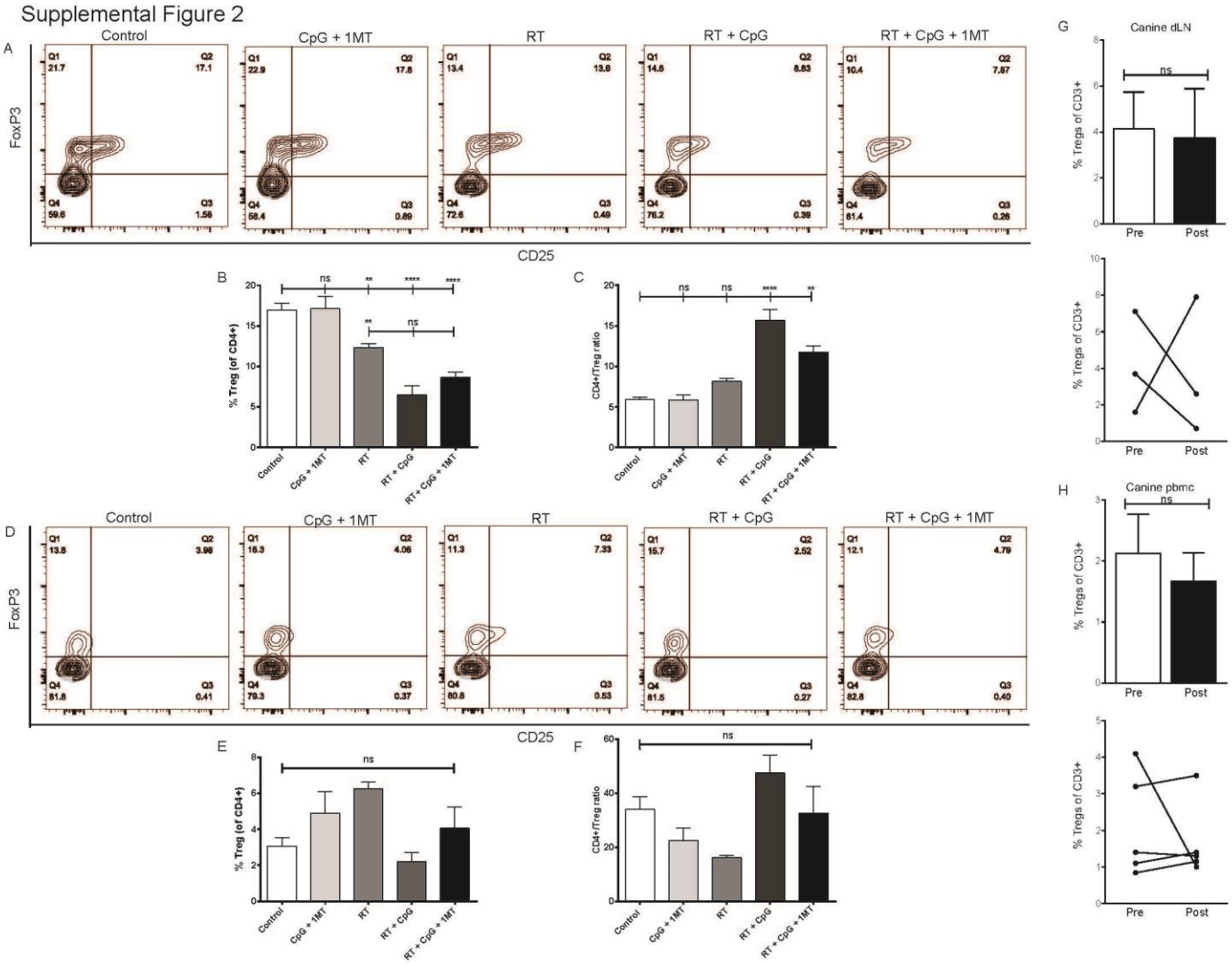
Supplemental Figures:

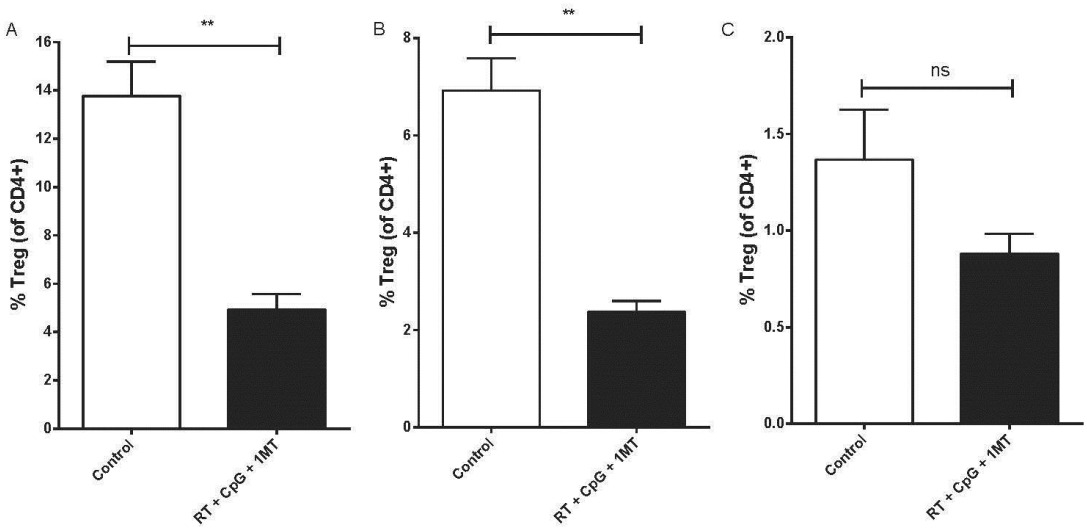


Supplemental Figure 1. Canine clinical trial. Schema of canine clinical trial (**A**). Proliferation of control ODN treated, LPS treated (positive control) or CpG ODN treated canine peripheral blood mononuclear cells as measured by tritium incorporation expressed as fold change over control (**B**). IDO enzymatic activity as measured by serum kynurenine to tryptophan ratio in the serum of three canines pre- and post- therapy (**C**). Results analyzed by student's t-test (* $p < 0.05$).



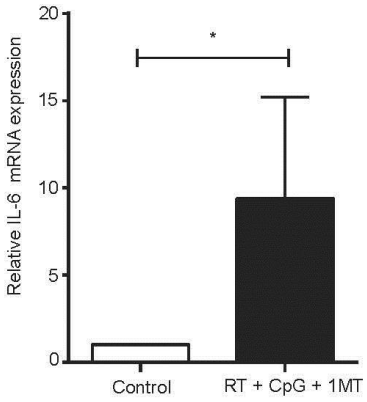
Supplemental Figure 2. Radiation + CpG + 1MT effects on peripheral and draining lymph node regulatory CD4+ T-cells in mice and canines. Levels of regulatory CD4+ T-cells as assessed by flow cytometry in 4T1 bearing mice (**A-F**) or canine patients (**G-H**) treated with RT + CpG + 1MT. Representative flow cytometry contour plots demonstrating staining of draining lymph node CD4+ cells for FoxP3 and CD25 (**A**). Flow cytometry data represented as a bar graph expressed as %Treg (CD4+CD25+FoxP3+) of CD4+ cells (**B**). Bar graph representation of CD4+ to Treg ratio in the draining lymph node as measured by flow cytometry (**C**). Representative flow cytometry contour plots demonstrating staining of splenic CD4+ cells for FoxP3 and CD25 (**D**). Flow cytometry data represented as a bar graph expressed as %Treg (CD4+,CD25+,FoxP3+) of all CD4+ cells (**E**). Bar graph representation of CD4+ to Treg ratio in the spleen as measured by flow cytometry (**F**). Bar graph representation of flow cytometric analysis of Tregs, expressed as a percentage of CD3+ cells, in canine draining lymph nodes (**G**) and peripheral blood (**H**) pre- and post- RT + CpG + 1MT therapy. Line graphs demonstrates changes in Treg levels in individual patients as assessed by flow cytometry (**G,H**). n=3-4 mice per group and 3-5 canines patients. Bar graphs represent mean +/- standard error of mean. Results analyzed by one-way ANOVA or student's t-test between the indicated groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Supplemental Figure 3



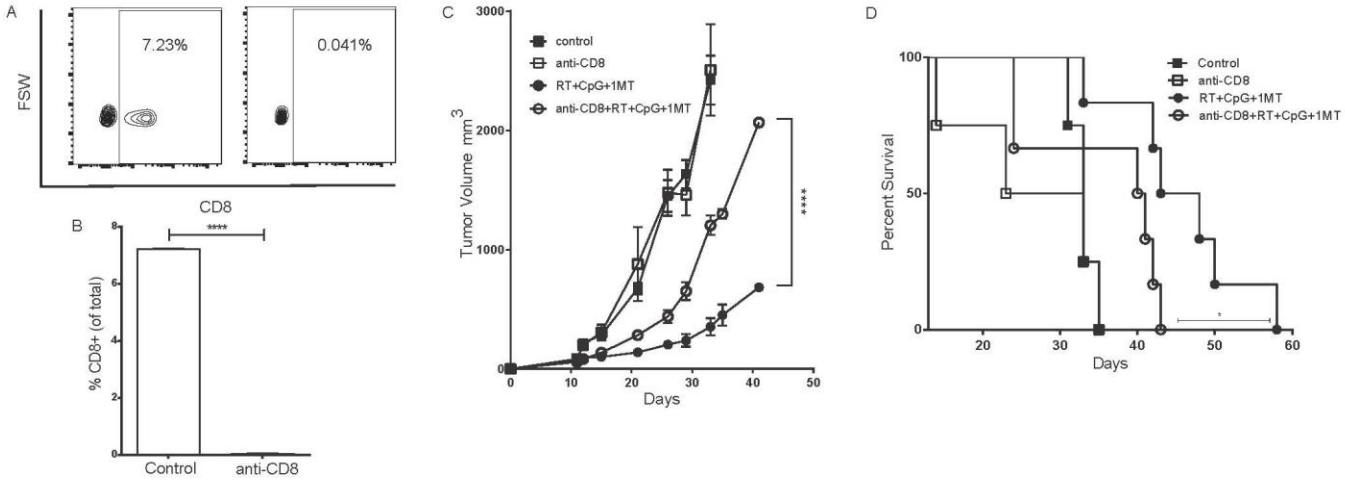
Supplemental Figure 3. Radiation + CpG + 1MT effects on tumor infiltrating, draining lymph node, and splenic regulatory CD4+ T-cells in mice at Day 21. Levels of regulatory CD4+ T-cells at Day 21 (7 days post therapy) in 4T1 bearing mice treated with RT + CpG + 1MT as assessed by flow cytometry (**A-C**). Data represented as bar graphs expressed as %Treg (CD4+,CD25+,FoxP3+) of CD4+ cells in the tumor (**A**), draining lymph node (**B**), and spleen (**C**). n=3-4 mice per group. Bar graphs represent mean +/- standard error of mean. Results analyzed by student's t-test (** $p < 0.01$).

Supplemental Figure 4

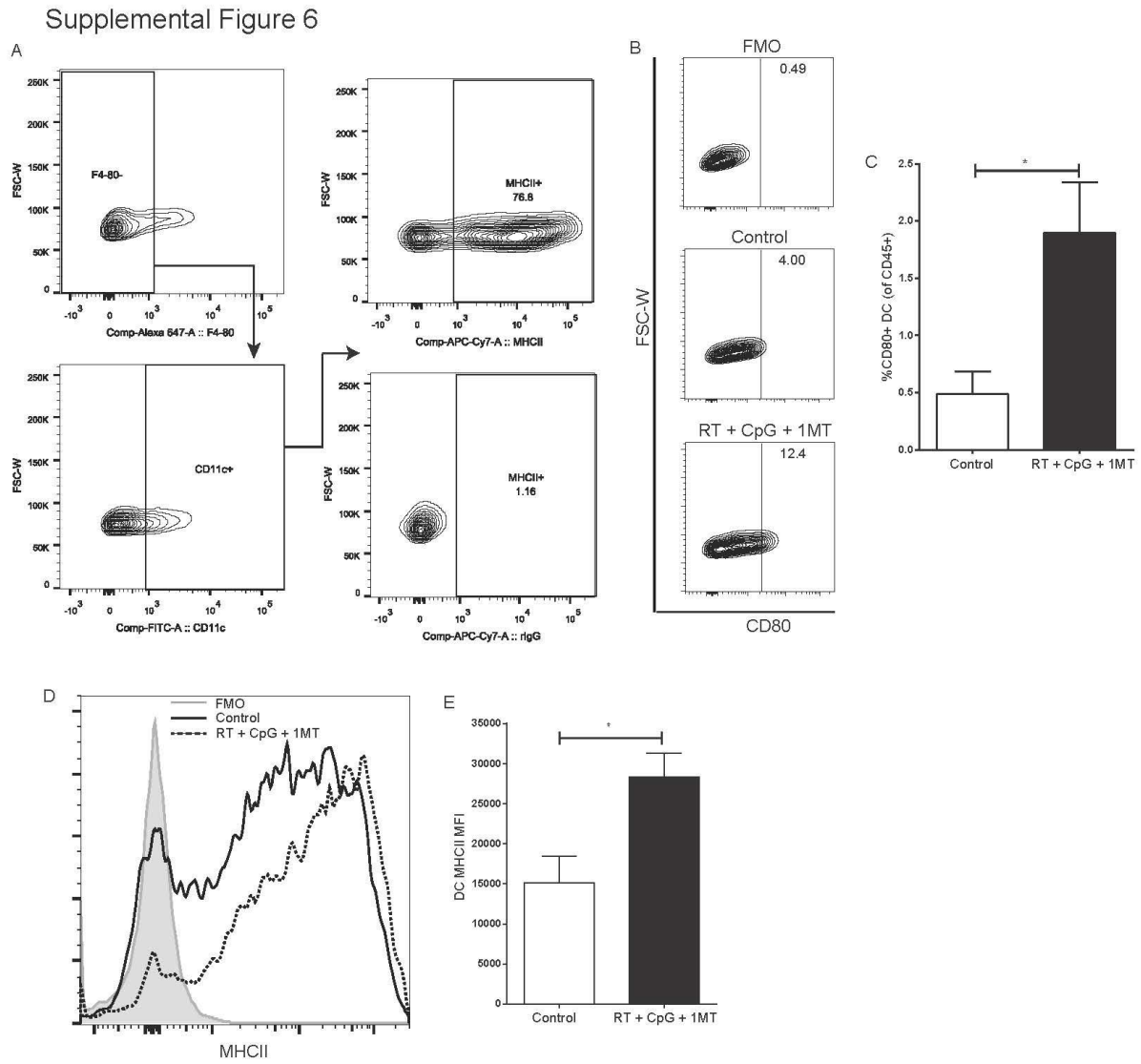


Supplemental Figure 4. Radiation + CpG + 1MT increases intratumoral IL-6 mRNA expression in 4T1 bearing mice. Relative intratumoral IL-6 mRNA expression in control or triple therapy treated mice. n=3-4 mice per group. Bar graphs represent mean +/- standard error of mean. Results analyzed by student's t-test (* $p < 0.05$).

Supplemental Figure 5



Supplemental Figure 5. Anti-tumor effects of radiation + CpG + 1MT are CD8+ T cell dependent. Balb/c mice bearing orthotopic 4T1 breast tumors were depleted of CD8+ T cells with 500ug i.p. injections of anti-CD8 anti-body administered once weekly (**A-D**). Levels of circulating CD8+ T cells as assessed by flow cytometry in 4T1 bearing mice (**A-B**). Representative flow cytometry contour plots demonstrating staining of CD45+CD3+ cells for CD8 (**A**). Flow cytometry data represented as a bar graph expressed as % CD8+ cells of all peripheral blood mononuclear cells (**B**). Tumor growth (**C**) and survival (**D**) of 4T1 bearing mice treated with triple therapy and / or CD8 depletion. n=6-10 mice per group. Bar graphs represent mean +/- standard error of mean. Results analyzed by ANOVA, student's t-test, or kaplan-meier analysis between the indicated groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).



Supplemental Figure 6. Radiation + CpG + 1MT increases intratumoral dendritic cell activation in 4T1 bearing mice. Flow cytometry gating strategy for dendritic cells defined as CD45+F4/80-CD11c+MHCII+ with MHCII fluorescence minus one staining control depicted in the bottom right panel (**A**). Representative flow cytometry contour plots demonstrating staining for CD80 expression on intratumoral dendritic cells (**B**). Bar graph representation of intratumoral CD80+ dendritic cells expressed as a percentage of CD45+ cells (**C**). Representative flow cytometry histogram plot demonstrating mean fluorescence intensity of MHC II staining on intratumoral dendritic cells (**D**). Bar graph representation of MHC II mean fluorescence intensity on intratumoral dendritic cells (**E**). n=3-4 mice per group. Bar graphs represent mean +/- standard error of mean. Results analyzed by student's t-test (* $p < 0.05$).