



Supplementary Fig. S2

**Supplementary Fig. S2: Low-dose radiation and combinatorial immunotherapy (RACIM) is required for tumor control.** (A) NanoString analysis of LD-WART treated *vs.* control tumors. Costimulatory molecule and immune checkpoint expression in tumors 5 days post-LDRT is displayed as heatmaps; n=5 tumors treated by 1Gy LDRT, and n=4 control tumors. (B) Frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> Treg cells assessed by flow cytometry analysis of dissociated ID8 tumors, spleens and tumor draining lymph nodes following one dose of low-dose cyclophosphamide (CP), or in control untreated mice (n=5 mice per group). (C) Tumor growth curves evaluated by bioluminescence; pie charts depict percent of mice with complete tumor response. (D) Mouse weight measurements over the time course of treatments. (E) Cytokine/chemokine bead array performed in the serum of ID8 tumor bearing mice treated or not with RACIM at cycle 2 day 5. (F) Kaplan-Meier analysis of overall survival of mice treated with RACIM in which LDRT is delivered only at cycle 1, only at cycles 1 and 2, or at all 3 cycles. (G) Evaluation of immune infiltration in the subcutaneous (s.c) Lewis Lung Carcinoma (LLC) model when tumor volume reached 100, 200 or 400 mm<sup>3</sup>. (H) RACIM treatment schema in LLC tumors implanted s.c. Tumor burden measured by caliper and Kaplan-Meier analysis of overall survival. (I) mRNA levels of *Nos2* in sorted CD11b<sup>+</sup> cells. Data are representative of 2 to 3 independent experiments with n=5 to 10 mice per group. *P* values for overall survival were determined by a one-sided log-rank Mantel–Cox test and the remaining statistical analyses were performed using Student’s unpaired *t*-test, error bars represent mean ± SEM. \**P* ≤ 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001.