**Figure S1. Radiation plan dosimetry and dose-volume histograms. (A)** Representative images in coronal, axial and sagittal orientation of tumor-bearing mouse with radiation target volumes (DLN, green; tumor, red; tumor isocenter, cyan) contoured on cone-beam CT images imported into Pinnacle3 planning software (Philips, Fitchburg, WI). **(B-C)** Representative dose-volume histogram (DVH) and corresponding dosimetry to tumor and/or DLN (mean, minimum and maximum radiation dose [cGy]) for Tumor RT (left panel) and T+LN RT (right panel) treatment plans. **(D)** Representative radiation dose distribution for Tumor RT (upper panel) and T+LN RT (lower panel) with colorimetric isodose lines as % of prescription dose (12 Gy x 1).

**Figure S2. Multi-parametric flow cytometry gating schemas. (A)** Immunophenotyping panel and flow gates used for characterization and downstream analysis of CD11b+ Gr-1 (myeloid-derived suppressor cells, Fig 2c), CD4+ FoxP3+ (Tregs, Fig 2B, 2D, 6B) and CD8+ CD44+ (CD8 effectors, Fig 2A, 2C, 4C, 6A, 7A) immune cells. **(B-C)** Tetramer H-2Kb OVA (SIINFEKL) and OT-1 AT flow gates used for characterization and downstream analysis of OVA-specific CD8+ T-cells (Fig 4C,7A) and intracellular cytokine staining (Fig 4B, 4C).

**Figure S3. Quantitative analysis of radiation-induced intratumoral chemokine and cytokine expression over time. (A)** Absolute number of live CD45+ immune cells and **(B)** Tumor lysates analyzed at timepoints described in Fig. 3A, 3B were analyzed by Luminex® multiplex immunoassay. Quantitative bar graphs represent concentration [pg/mL] of chemokine/cytokine target of interest at distinct timepoints by treatment group. Data supplement colorimetric heat map data in Fig 3B. Error bars represent SEM. **(C)** Histogram of CFSE dilution in AT CD45.2+ CD8+ T-cells in the DLN, corresponding to data in Figure 4A-C.

**Figure S4. Quantitative analysis of radiation-induced changes in peripheral blood subsets over time. (A)** Peripheral blood collected and analyzed at serial timepoints (baseline, 48 hrs and 240 hrs after treatment) were analyzed by automated GenesisTM veterinary hematology platform. Quantitative scatter plots and grouped linear plots demonstrating change over time in absolute [109/L] number of cells (leukocytes, lymphocytes, neutrophils, platelets), % change in cell subsets (lymphocytes, neutrophils) and ratio of cell subsets (platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio) by treatment group. **(B)** Measurement of IL-7 (pg/mL) in serum and DLN lysate at pre- and post-RT timepoints shown as quantitative scatter plots stratified by treatment group. Error bars represent SEM, \*\*\*: p < 0.001, \*\*: p < 0.01, \*: p < 0.05, peripheral blood subsets and IL-7 time course experiments analyzed by two-way ANOVA and post-hoc Tukey’s multiple comparison test.

**Figure S5. Comparison of intratumoral Treg depletion by anti-CTLA4 Ig2Ga and IgG2b isotypes. (A-B)** Images of C56BL/6J mice treated with αCTLA4 IgG2a alone or in combination with Tumor RT; depicting presence or absence of vitiligo at site of initial tumor implant. **(C-D)** Tumor-naïve and tumor-bearing mice injection s.c. with 2.0x106 MC38 cells were treated with no antibody (negative control), isotype and anti-CTLA4 IgG2a or IgG2b (i.p., 200μg) on days 7,10,13. Positive control for Treg depletion are FoxP3DTR (DTR) mice treated every other day x 2 with diphtheria toxin. Tumors, spleen, tumor-draining DLN (TDLN) and non-TDLN were harvested on day 14. Representative flow plots and quantitative bar graphs of percentage FoxP3+ Tregs in lymphoid tissues and tumors.