## T<sub>H</sub>2-polarized CD4<sup>+</sup> T cells and macrophages limit efficacy of radiation therapy

## Supplemental Data

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## **Supplemental Figure Legends**

**Supplemental Figure 1: Radiation dose response and immune profile. A)** Orthotopic MMTV-PyMT-derived explant tumors were grown to a median diameter of 1.0 cm for study enrollment (day 0), and one day later received localized gamma irradiation (2 or 8 Gy). Total tumor burden/animal was then assessed every 3 days until endpoint. Treatment schematic is depicted at top and data are displayed as mean tumor burden  $\pm$  SEM (>5 mice/group). Significance was determined by two-way ANOVA. One of two experiments is shown. **B)** Number of CD3+CD4+, CD3+CD8+, and CD11b+F4/80+ cells within tumors 14 days following RT in groups that were untreated, treated with RT (2 Gy), RT (5 Gy) or RT (8 Gy). Data are depicted as mean number of cells  $\pm$  SEM as a % of CD45+ cells as analyzed by flow cytometry (>5 mice/group). Significance was determined by an unpaired t-test relative to untreated controls. For all panels, significance is shown as \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

Supplemental Figure 2: Immune profile with RT in combination with  $\alpha$ CSF-1 mAb or PLX3397. A) Number of CD3<sup>+</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD8<sup>+</sup>, and CD11b<sup>+</sup>F4/80<sup>+</sup> cells within tumors 4 days following RT (2 days after administration of  $\alpha$ CSF-1 mAb) in groups that were untreated, treated with  $\alpha$ CSF-1 mAb alone, RT (5 Gy) alone or treated with a combination of RT and  $\alpha$ CSF-1 mAb. B) Number of CD3<sup>+</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD8<sup>+</sup>, and CD11b<sup>+</sup>F4/80<sup>+</sup> cells within tumors 4 days following RT (2 days after administration of PLX3397) in groups that were untreated, treated with PLX3397 alone, RT (5 Gy) alone or treated with a combination of RT and PLX3397. Data are depicted as mean number of cells  $\pm$  SEM as a % of CD45<sup>+</sup> cells as analyzed by flow cytometry (>5 mice/group). For all figures significance is shown as \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



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