

Supplementary Fig. S1

Supplementary Fig. S1: Low-dose irradiation induces tumor cell stress and elicits immune cell infiltration in ID8 ovarian tumors (A) In vitro immunogenic cell stress assay of ID8 cells and cellsurface expression of calreticulin (ecto-calreticulin) by flow cytometry (P < 0.0001 = ****) over time. **(B)** In vitro clonogenic ID8 cell survival assay following treatment by increasing doses of irradiation. (C) Quantification of γH2AX staining by immunofluorescence in LD-WART treated vs. control tumors at the indicated doses measured at 24hs. Five high-power fields (HPFs) were evaluated per slide. Right: representative tumoral yH2AX staining (pink). (D) Average body weight of tumor-bearing mice treated by escalating doses of LD-WART vs. untreated. (E) mRNA levels of Klrk1. (F) mRNA levels of rael(left) and protein expression by immunofluorescence staining (right) evaluated in five high-power fields (HPFs) per slide. Representative tumoral RAE1 staining (orange). (G-I) mRNA levels of H2Kb, Il7, Tgfb. (J) Representative immunohistochemical staining reveals CD8⁺, CD4⁺, and CD11b⁺ immune cells in tumor and stroma compartment 5 days after the administration of different doses of LD-WART. Five HPF were evaluated per slide for 5 mice per group. (K) Low dose irradiation weekly treatment schedule and evaluation of immune infiltration at cycle 1 days 2 (C1D2) and 7 (C1D7) as well as C2D2 and C3D2. Immune cell phenotypes were evaluated on single cell suspensions of ID8 tumors treated with control or 1 Gy (n=5-12 mice per group) with representative gating strategy. Anti-CD45 was used to define the lymphocyte population and anti-CD3 to define total T cells. T cells were then further subdivided by anti-CD4 and anti-CD8 antibody staining, and anti-CD161 was used to identify NK or NKT cells. Myeloid cells were identified with anti-CD11b, anti-CD11c, and anti-MHC class II antibodies. (L) Frequency of CD8⁺, CD4⁺, NK1.1⁺, CD11b⁺ cells in spleen and mesenteric lymph nodes measured by flow cytometry seven days after 1 Gy LD-WART. Data are representative of three independent experiments, each with n=5 mice per group. Statistical analysis was performed using Student's unpaired t-test, error bars represent mean \pm standard deviation. * $P \le 0.05$, **P < 0.01, ****P* < 0.001, *****P* < 0.0001.