Supplementary Figure 1. Combined RT+AZD0156 does not affect Treg infiltration in MOC2 tumor. (A) Gating strategy of Flow cytometry represented. (B) Flow cytometry was used to quantify the proportion of Treg (CD25+FOXP3+) among CD45+CD3+ tumor infiltrating CD4+ T cells. Significance was determined by one-way ANOVA with post hoc Tukey. Veh, vehicle. ns, not significant; *P ≤ 0.05.
Supplementary Figure 2. Combined RT+AZD0156 induces naïve CD8+ T cell priming. (A) MOC2 cells were radiated (0 or 8 Gy) 1 hour after DMSO vehicle or AZD0156 (50 nM) pre-treatment. After 24 hours of incubation, CD8+ T cells sorted from naïve mice spleen were co-cultured with pre-stimulated MOC2 cells. After 24 hours, the cells were collected and analyzed by flow cytometry to determine (B) live cells in CD8+ T cells. (C) The live+CD8+ T cells were further gated to check CD69- and IFNγ positive cells. Significance was determined by one-way ANOVA with post hoc Tukey. Veh, vehicle. ns, not significant; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.
Supplementary Figure 3.

Combined RT+AZD0156 stimulates STING-dependent type 1 interferon response in MOC2 and promotes CD8+ T cell migration. MOC2 cells were transduced to delete stimulator of interferon genes (STING) using electroporation of recombinant Cas9 and guide RNA. Then, the single sorted clone was expanded and sequenced. (A) STING genomic DNA sequence of WT and STING heterozygotic knockout (STING^{+/−}) alleles is shown. (B) MOC2 cells were radiated (0 or 8 Gy) 1 hour after DMSO vehicle or AZD0156 (50 nM) pre-treatment. After 0.5-hour of incubation, the cells were lysed, and western blot was performed to determine STING, γH2AX, and β-actin protein levels. C-F, MOC2-STING^{+/−} or MOC2-STING^{−/−} cells were treated with DMSO (Veh) or RT+AZD0156. On day 3, the cells or supernatants were collected and analyzed for (C) IFNβ production, (D) type I interferon-related mRNA expression (Ifnβ and Oas2), (E) MFI of PD-L1 and MHC I and (F) chemokine mRNA expression (CCL5, CXCL9, CXCL10, and CXCL12). (G) Tumor-secretome collected from RT+AZD0156-treated MOC2 cells or media (0.5% FBS in RPMI) was placed in the lower chamber (300 μl) of a 24-well transwell plate. Sorted CD8+ T cells prepared in 100 μl of the media were transferred into the upper chamber (5 μm-pore size). After 4 hours incubation, the number of cells that migrated to the lower chamber was determined. Significance was determined by one-way ANOVA with post hoc Tukey (C and D) and two-tailed student’s t-test (E, F, G). Veh, vehicle. ns, not significant; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.
Supplementary Figure 4.

A. MOC2 bilateral tumor

B. Distant tumor (LF) and Primary tumor (RF)

C. Tumor volume (mm$^3$)

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Supplementary Figure 4. Combined RT+AZD0156+αPD-L1 promotes antitumor response in non-radiated tumor. (A) C57BL/6 mice were engrafted subcutaneously with MOC2 in the right flank (RF) and the left flank (LF). When the bilateral tumor-bearing mice reached a mean tumor volume of 150-200 mm$^3$, the mice were randomized and treated on the RF-tumor with RT (0 or 8 Gy) 1 hour after control vehicle oil or AZD0156 (200 µg) administration. The mice were further treated with AZD0156, oil, intraperitoneal IgG or anti-PD-L1 (clone B7-H1, 200 µg) antibody as indicated. (A) Treatment regimen, (B) mean tumor volume, and (C) individual tumor volume curves are shown. ($n = 6$) Significance was determined by linear mixed-effects analysis with Tukey multiple comparisons. Veh, vehicle. ns, not significant; $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. 

Supplemental material

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