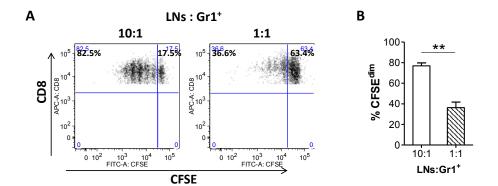
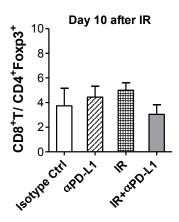


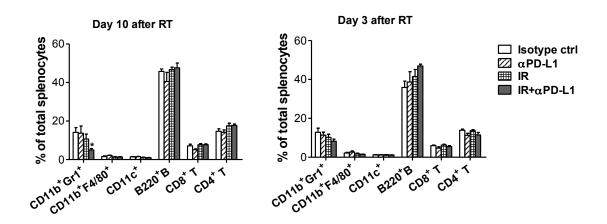
CD4<sup>+</sup> T cells are dispensable for the efficacy of combination of IR and anti-PD-L1. Tumors received 12 Gy and mice were treated with anti-PD-L1 as described in Figure 2(**A**). Starting from one day prior to IR, 250µg depletion antibody of CD4<sup>+</sup> T cells (clone GK1.5) was injected intraperitoneally every three days for a total four times. Representative data from two experiments conducted with 6-8 mice/group are shown.



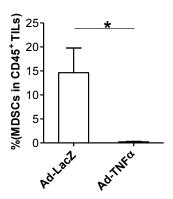
MDSCs inhibit the proliferation of CD8 $^{+}$  T cells. Gr1 $^{+}$  cells were purified from the spleen of three TUBO-bearing mice. Lymph nodes were obtained from naïve mice and then labeled with 5µM CFSE. Lymph node cells were stimulated with coated 10µg/ml anti-CD3 and 2µg/ml anti-CD28 in the presence of Gr1 $^{+}$  cells at a ratio of 10:1 or 1:1 in triplicate. Three days later, the cells were harvest and subjected to staining. \*\*P<0.01. Representative data (**A**) and quantitative data (**B**) from two experiments conducted are shown.



The ratio of CD8<sup>+</sup>T/Treg is unaffected by IR and PD-L1 blockade. Tumors received 12 Gy and mice were treated with anti-PD-L1 as described in Figure 2(**A**). Ten days later after IR, tumors were removed to obtain cell suspensions for surface and intracellular staining. Flow cytometry analysis of Treg (CD4<sup>+</sup>Foxp3<sup>+</sup>) and CD8<sup>+</sup> T was gated on CD45<sup>+</sup> cells. Representative data from two experiments conducted with 4-5 mice/group are shown.



Quantitative data of the percentage of immune cell populations relative to total splenocytes on day 10 (*left*) and on day 3 (*right*) after IR. Mice were treated as described in Figure 2(**A**). Three days or ten days after IR, spleens were removed to obtain cell suspensions for surface staining of MDSCs (CD11b<sup>+</sup>Gr1<sup>+</sup>), macrophages (CD11b<sup>+</sup>F4/80<sup>+</sup>), dendritic cells (CD11c<sup>+</sup>), B cells(B220<sup>+</sup>), CD8<sup>+</sup>T cells and CD4<sup>+</sup>T cells. \*P<0.05. Representative data from two experiments conducted with 4 mice/group are shown.



TNF- $\alpha$  promotes the reduction of MDSCs in vivo. Tumors were allowed to grow for 14 days. The recombinant Ad-TNF- $\alpha$  (1.5x10<sup>10</sup> VP) was infected intratumorally. 1.5x10<sup>10</sup> VP Ad-LacZ was used as negative control. Three days after adenovirus treatment, the tumors were harvested and processed for flow cytometry assay. \*P<0.05. Representative data from two experiments conducted with 4 mice/group are shown.