

## Supplementary Figure 1

CD4 ${ }^{+}$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, $250 \mu \mathrm{~g}$ depletion antibody of CD4 ${ }^{+}$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.


## Supplementary Figure 2

MDSCs inhibit the proliferation of $\mathrm{CD8}^{+} \mathrm{T}$ cells. $\mathrm{Gr}^{+}$cells were purified from the spleen of three TUBO-bearing mice. Lymph nodes were obtained from naïve mice and then labeled with $5 \mu \mathrm{M}$ CFSE. Lymph node cells were stimulated with coated $10 \mu \mathrm{~g} / \mathrm{ml}$ anti-CD3 and $2 \mu \mathrm{~g} / \mathrm{ml}$ anti-CD28 in the presence of $\mathrm{Gr}^{+}$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 ( $\mathbf{A}$ ) and quantitative data ( $\mathbf{B}$ ) from two experiments conducted are shown.


## Supplementary Figure 3

The ratio of CD8 ${ }^{+}$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 ${ }^{+} \mathrm{Foxp}^{+}$) and $\mathrm{CD8}^{+}$T was gated on CD45 ${ }^{+}$cells. Representative data from two experiments conducted with 4-5 mice/group are shown.


## Supplementary Figure 4

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 ${ }^{+} \mathrm{Gr}^{+}$), macrophages (CD11b ${ }^{+}$F4/80 ${ }^{+}$), dendritic cells (CD11c ${ }^{+}$), B cells $\left(\mathrm{B}_{2} 2^{+}\right), \mathrm{CD} 8^{+} \mathrm{T}$ cells and $\mathrm{CD} 4^{+} \mathrm{T}$ cells. ${ }^{*} \mathrm{P}<0.05$. Representative data from two experiments conducted with 4 mice/group are shown.


## Supplementary Figure 5

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

