IRF1 inhibits antitumor immunity through the upregulation of PD-L1 in the tumor cell.

Lulu Shao[1,3], Weizhou Hou[1], Nicole E. Scharping[2,4], Frank P. Vendetti[5], Rashmi Srivastava[1,3], Chandra N. Roy[1,3] Ashley V. Menk[2,4], Yiyang Wang[2,4], Joe-Marc Chauvin[4], Pooja Karukonda[5], Stephen H. Thorne[1], Veit Hornung[6], Hassane M. Zarour[4], Christopher J. Bakkenist[5], Greg M. Delgoffe[2,4] and Saumendra N. Sarkar*,[1,3,4]

SUPPLEMENTATRY FIGURE LEGENDS:

Fig. S1: Loss of IRF1 in tumor cells caused slower tumor growth rate in mice.

(A), (B) and (C) Validation of IRF1 deficiency in IRF1-KO MC38, B16-F10 and CT26 cells. Cell lysates from mouse IFNγ treated WT and IRF1-KO cells were analyzed for IRF1 expression by immunoblotting.

(D), (E) and (F) Tumor growth rate of MC38, B16-F10 and CT26 WT and IRF1-KO cells in each mouse. Mice were subcutaneously or intradermally injected with 10^6 or 5×10^5 cells, followed by tumor growth measurements.

Fig. S2: Depletion of $CD8^+$ T cells enables B16-F10 IRF1-KO cells to establish tumor.

(A) Percentage of $CD8^+$ T cell of total $CD3^+$ splenic cells. MC38 IRF1-KO inoculated mice were injected with anti-CD8 Ab or rat IgG2b isotype control. After finishing tumor growth experiment, mice were sacrifice. Spleens were collected and splenic cells suspension was prepared for $CD8^+$ T

cell staining. For each column mean and SEM were plotted, statistical significance calculated by unpaired student t test, and represented as **** $P < 0.0001$.

(B) Individual tumor growth in each mouse re-challenged with WT MC38 cells (fig. 2B). Following complete regression of MC38 IRF-1 KO tumors (n=8), mice were re-challenged with MC38 WT tumor cells on day 47 of post-injection. 7 out of 8 mice showed complete tumor regression.

(C) Tumor growth of B16-F10 IRF1-KO cells in $CD8^+$ T cell depletion C57BL/6 mice. 5×10^5 of B16-F10 WT or IRF1-KO cells were intradermally injected into C57BL/6 mice (n=5). IRF1-KO cell injection groups of mice were intraperitoneally injected with 250 µg of anti-CD8 antibody or rat IgG2b isotype control 2-day before tumor cell injection, then followed by antibody or isotype control injections twice a week.

(D) Percentage of $CD8^+$ T cell of total $CD3^+$ splenic cells in mice. Mice were sacrificed at the end of the experiment. spleens were collected and splenic cells suspension was prepared for $CD8^+$ T cell staining. For each column mean and SEM were plotted, statistical significance calculated by unpaired student t test, and represented as **** $P < 0.0001$.

(E) 10^6 of CT26 IRF1-KO cells were subcutaneously injected into BALB/c mice (n=5). 4 out of 5 mice had complete tumor regression by day 15 of post-injection and were sacrificed at 30 days after complete tumor regression. 1 out of 5 mouse which had tumor was sacrificed as a positive control. Naïve mice were used as negative control. Representative flow cytogram (left) and percentage of $CD8^+$ $tetramer^+$ cells in T_{EM} (right) in the spleen and dLNs. For each column mean and SEM were plotted, statistical significance calculated by unpaired student t test. * $P < 0.05$.

Fig. S3: IRF1 deficiency in tumor cells have no significant impact on and the frequencies of MDSCs and NK cells.

5×10^5 of B16F10 WT or IRF1-KO cells were intradermally injected into C57BL/6 mice (n=5). Tumors were collected around size $100mm^3$. Flow cytometry analysis of infiltrated granulocytic MDSC (A), monocytic MDSC (B), NK cells (C) has been conducted. For each column mean and SEM were plotted.

Fig. S4: Mouse IFNγ-induced mRNA expression of STAT1 and GBP2 in B16-F10 WT and IRF1-KO cells.

(A-B) The mRNA expression levels of STAT1 and GBP2 in B16-F10 WT and IRF1-KO cells were detected using TaqMan real-time PCR after 0-8 hrs mouse IFNγ induction. For each time point, mean and SEM were plotted, statistical significance calculated by two-way ANOVA with Sidak's multiple comparison test, and presented as *** $P < 0.001$, **** $P < 0.0001$. (C) The geometric mean (MFI) of PD-L1 in B16-F10 WT and IRF1-KO cells after 6 hrs treatment of mouse IFNα, IFNγ, TGFβ1 and TNFα.