Type I Interferons Induced by Radiation Therapy Mediate Recruitment and Effector Function of CD8$^+$ T Cells

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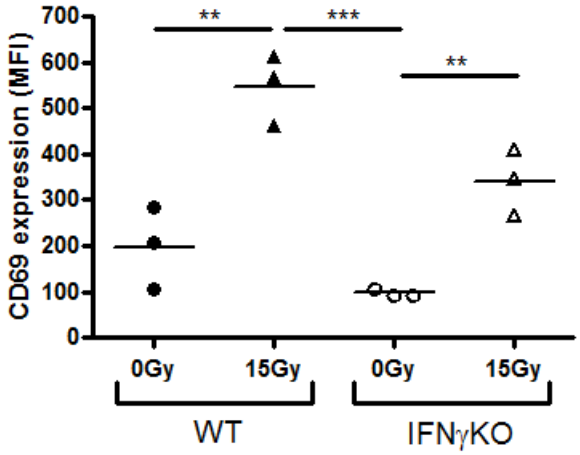
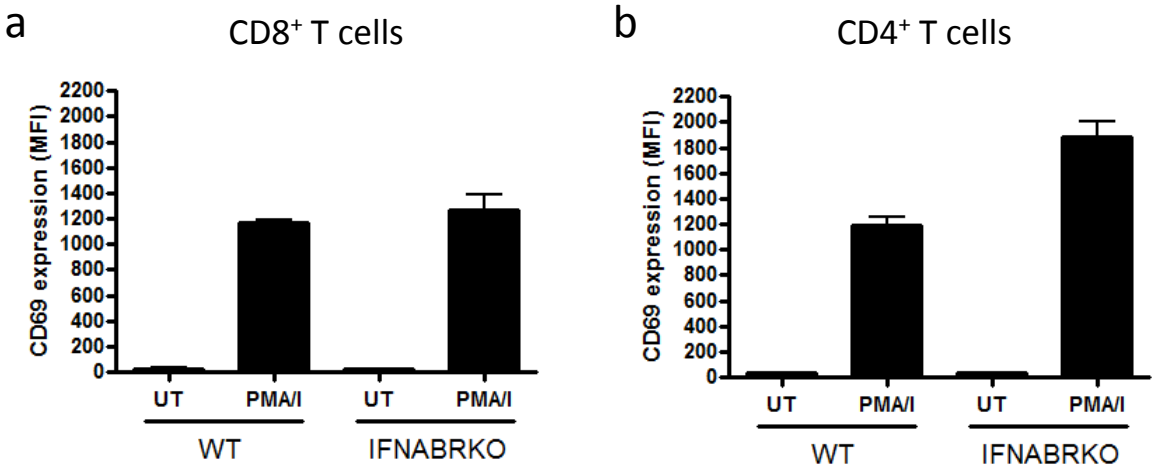
Online Resource 1



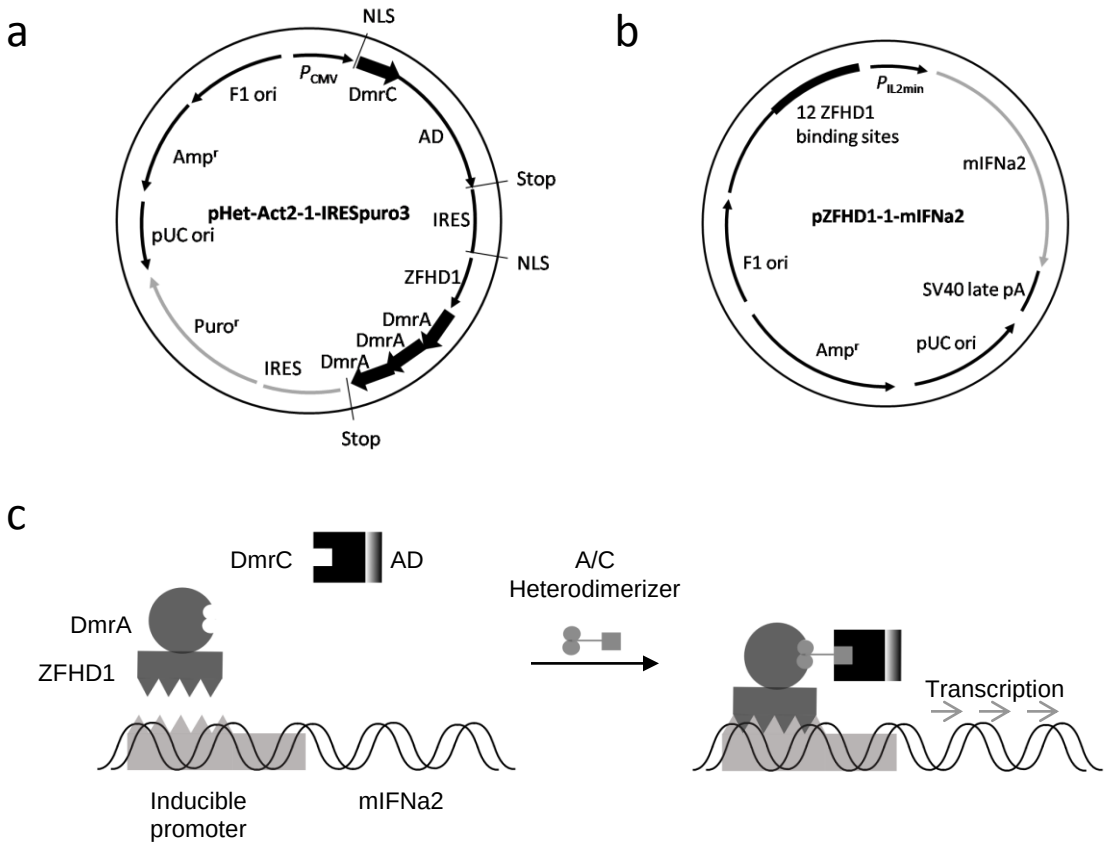
Online Resource 1. Tumor-infiltrating IFNγKO CD8$^+$ T cells upregulate cell surface expression of CD69 in response to radiation treatment. WT and IFNγKO C57BL/6 mice were injected intramuscularly with 1 x 10^5 B16 cells, treated as described before and CD69 expression on CD8$^+$ T cells was quantified using MFI values. Data are representative of two independent experiments.

Online Resource 2



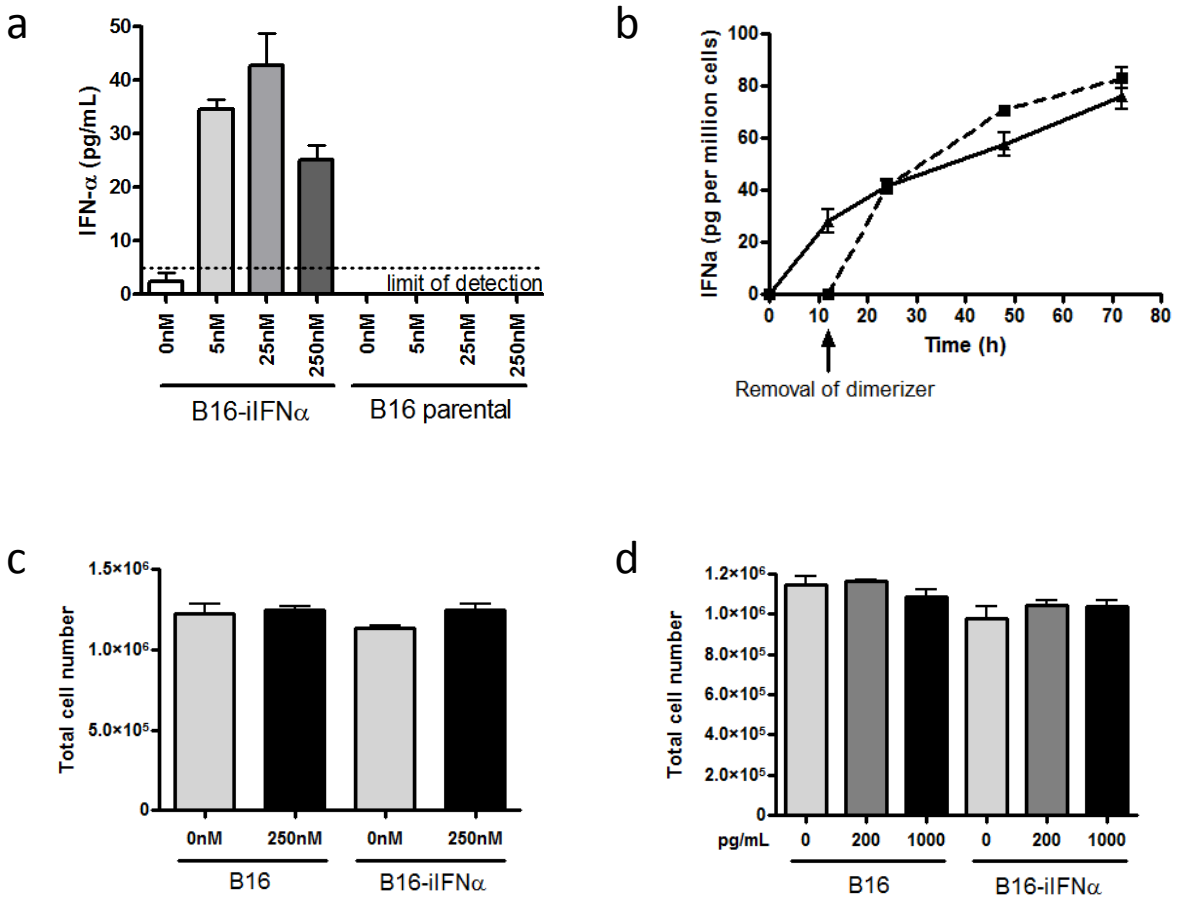
Online Resource 2. IFNABR-deficient T cells have normal ability to upregulate cell surface of expression CD69 upon activation. Splenocytes were harvested from B6-SJL (WT) mice and IFNABR-deficient (IFNABRKO) mice, and either left untreated or treated with 50ng/mL PMA and 500ng/mL Ionomycin for 6 hours. Cells were stained with an antibody cocktail containing fluorescence-labeled anti-CD45, CD3, CD4, CD8 and CD69 antibodies. CD69 expression on (a) $CD45^+$ $CD3^+$ $CD8^+$ and (b) $CD45^+$ $CD3^+$ $CD4^+$ cells were analyzed and expressed as mean fluorescence intensity values. (n=2)

Online Resource 3



Online Resource 3. Schematics for design of B16-iIFN-α clone and mechanism for induction of IFN-α transgene expression. (a) Transcription factor plasmid, pHet-Act2-1-IRESpuro3. It encodes two fusion proteins; one has a single dimerization domain (DmrC) fused to transcription activation domain (AD), the other has three dimerization domains (DmrA) fused to DNA-binding domain (ZFHD1). Under the control of a CMV promoter, these two fusion proteins are constituitively expressed by B16-iIFN-α cells, from a bicistronic transcript. (b) Target gene plasmid, pZFHD1-1-mIFNa2. It contains 12 ZFHD1-binding sites, the inducible promoter (P_{IL2min}) and murine IFN-α2 (mIFNa2) (c) Schematics of protein interactions before (left) and after (right) after treatment with A/C heterodimerizer (inducer) leading to transcription of target gene.

Online Resource 4



Online Resource 4. Characterization of B16-iIFN-α clone *in vitro*. (a) B16-iIFN-α and B16.F0 cells were seeded in 12-well plates at $2x10^5$ cells per well overnight and treated with indicated concentrations of the inducer. Supernatant was harvested 24 hours post-treatment and IFN-α concentration was assessed by ELISA. Data is combination of two experiments. (b) B16-iIFN-α cells were seeded in 12-well plates at $1x10^5$ cells per well and treated with 25nM of the inducer the next day. 12 hours later, inducer-containing media was either left in the wells (solid line) or removed and replaced with fresh media (dashed line). At various time points, supernatant was removed from individual wells and frozen down, until IFN-α quantification by ELISA. (c) B16-iIFN-α and B16.F0 cells were seeded as described in (a) and treated with 250nM of the inducer. Total number of cells per well was determined 48 hours after treatment. (d) B16-iIFN-α and B16.F0 cells were seeded as described in (a) and treated with 0, 200 or 1000pg/mL of recombinant IFN-α. Total number of cells per well was determined 48 hours after treatment. Error bars represent standard error of duplicate wells, and data are representative of two independent experiments.