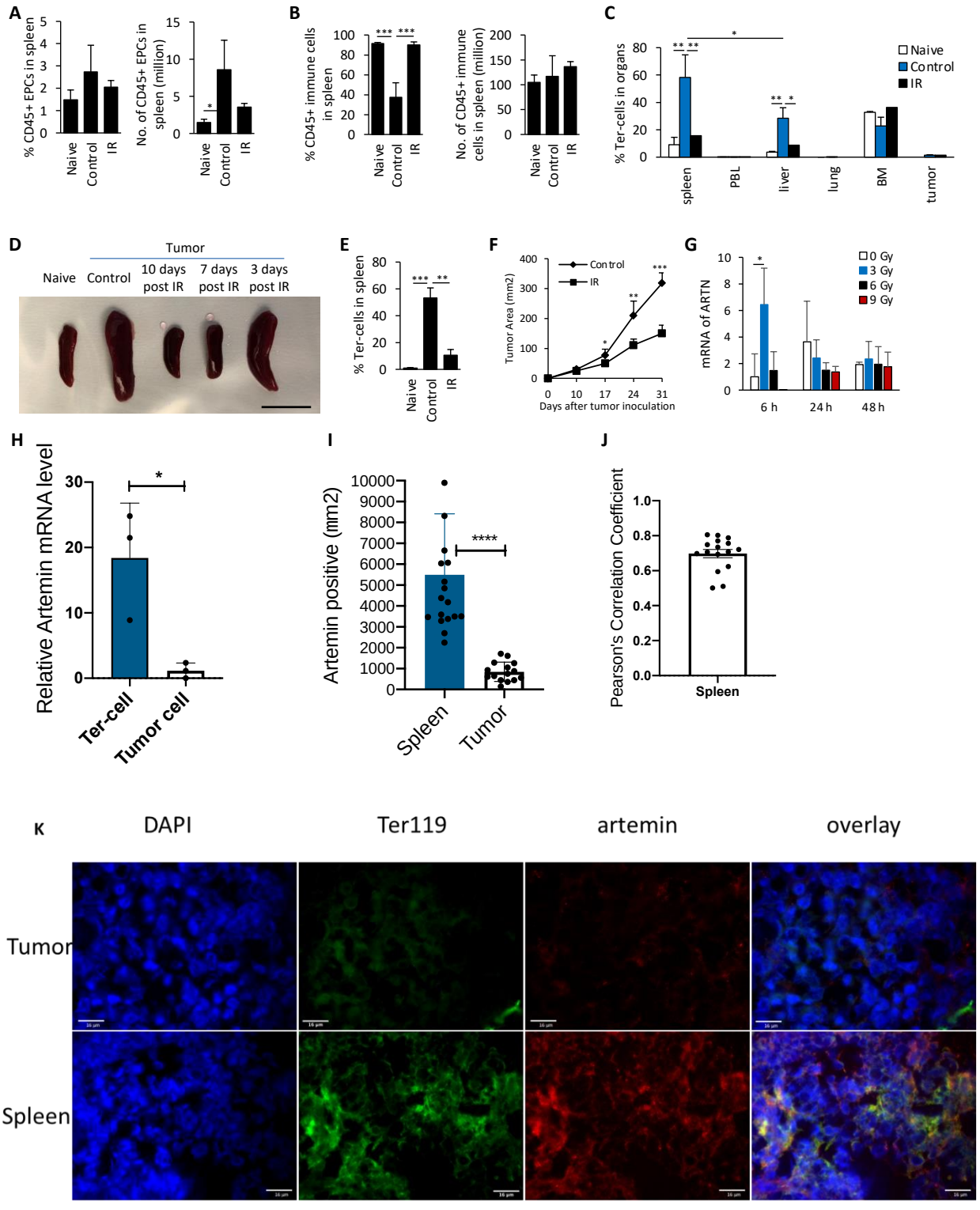
A
% CD45+ EPCs in spleen
No. of CD45+ EPCs in spleen (million)
Naive
Control
IR
B
% CD45+ immune cells in spleen
No. of CD45+ immune cells in spleen (million)
C
% Ter-cells in organs
Naive
Control
IR
spleen
PBL
liver
lung
BM
tumor
D
Tumor
Naive
Control
10 days post IR
7 days post IR
3 days post IR
E
% Ter-cells in spleen
F
Control
IR
Tumor Area (mm2)
Days after tumor inoculation
G
mRNA of ARTN
0 Gy
3 Gy
6 Gy
9 Gy
6 h
24 h
48 h
H
Relative Artemin mRNA level
Ter-cell
Tumor cell
I
Artemin positive (mm2)
Spleen
Tumor
J
Pearson's Correlation Coefficient
Spleen
K
DAPI
Ter119
artemin
overlay
Tumor
Spleen

Fig. S1. Local irradiation decreases tumor-induced Ter cell accumulation in the spleen. (A-F) C57BL/6 mice were inoculated subcutaneously with LLC cells on day 0. On day 10, tumors received one dose of 20 Gy ionizing radiation (IR). (A) The ratio and number of splenic $CD45^+$ erythroid progenitor cells (EPCs) on day 10 post IR. (B) The ratio and number of splenic $CD45^+$ immune cells on day 10 post IR. (C) The ratio of Ter-cells in spleen, peripheral blood (PBL), liver, lung, bone marrow (BM) and tumor tissue of tumor-bearing mice on day 10 post IR. (D) The size of spleen of tumor-bearing mice at indicated time post IR. Scale bar = 1cm. (E) The ratio of Ter-cells in spleen of mice on day 20 post IR. (F) The effect of IR on tumor growth. (G) Artemin mRNA expression in LLC tumor cells treated with irradiation. (H) Real-time PCR analysis of artemin expression in Ter-cells isolated from spleens of tumor-bearing mice and tumor cells isolated from tumors. (I) Quantification of areas stained positive for artemin in 100x field in spleen and tumor tissues. (J) Co-efficient of co-staining of artemin and Ter-cell in spleens of tumor-bearing animals, calculated by Image J. (K) Representative high-power magnification (100x) of tissue slides stained with DAPI(blue), Ter119(green), artemin (red) and 3-color overlay respectively. Scale bar=16μm. Representative data are shown from two or three experiments conducted with 3-5 mice or samples per group. Data are represented as mean ± SD. (A), (B), (C), (E), and (G) were calculated with one-way ANOVA with Bonferroni's multiple comparison tests. (F), (H), and (I) were analyzed with unpaired Student's t test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and ****$p<0.0001$.

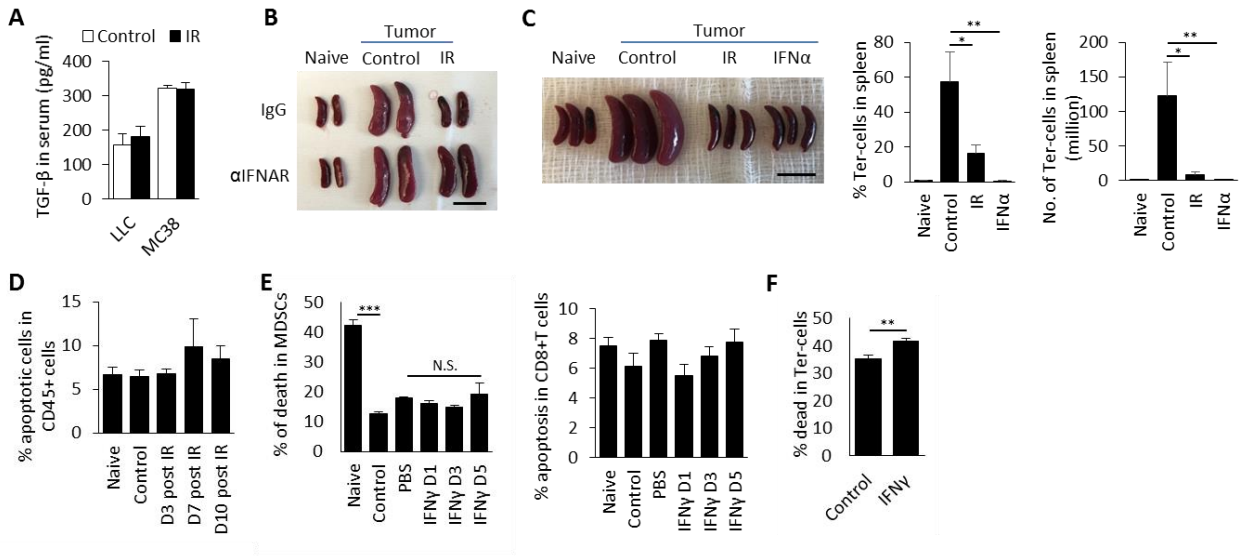

Fig. S2. Mechanism of IR-induced Ter cell reduction in the spleen. (A) Transforming growth factor-β1 (TGF-β1) in serum of Lewis Lung Carcinoma (LLC) or MC38 tumor-bearing mice on day 3 post IR. (B) LLC tumor-bearing mice were treated with IR, IFNAR blocking antibody, or a combination of both. The size of spleen was shown. (C) B16-SIY tumor-bearing mice were treated with IR, IFNα, or a combination of both. The ratio and number of Ter-cells in spleen were analyzed by flow cytometry. (D) Apoptosis of splenic $CD45^+$ immune cells were analyzed by flow cytometry at indicated time post IR. Annexin V and 7-AAD double positive cells were identified as apoptotic cells. (E) Apoptosis of splenic $CD45^+$ myeloid derived suppressor cells (MDSCs) and $CD8^+$ T cells were analyzed by flow cytometry at indicated time post intrasplenic injection of IFNγ. (F) Apoptosis of splenic Ter-cells were analyzed by flow cytometry at 48 hours post 100 ng/ml IFNγ treatment. Representative data are shown from two or three experiments conducted with 3-5 mice or samples per group. Data are represented as mean ± SD. (A) to (E) were calculated with one-way ANOVA with Bonferroni's multiple comparison tests. (F) was analyzed with unpaired Student's t test. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. N.S., not significant. Scale bar = 1cm.

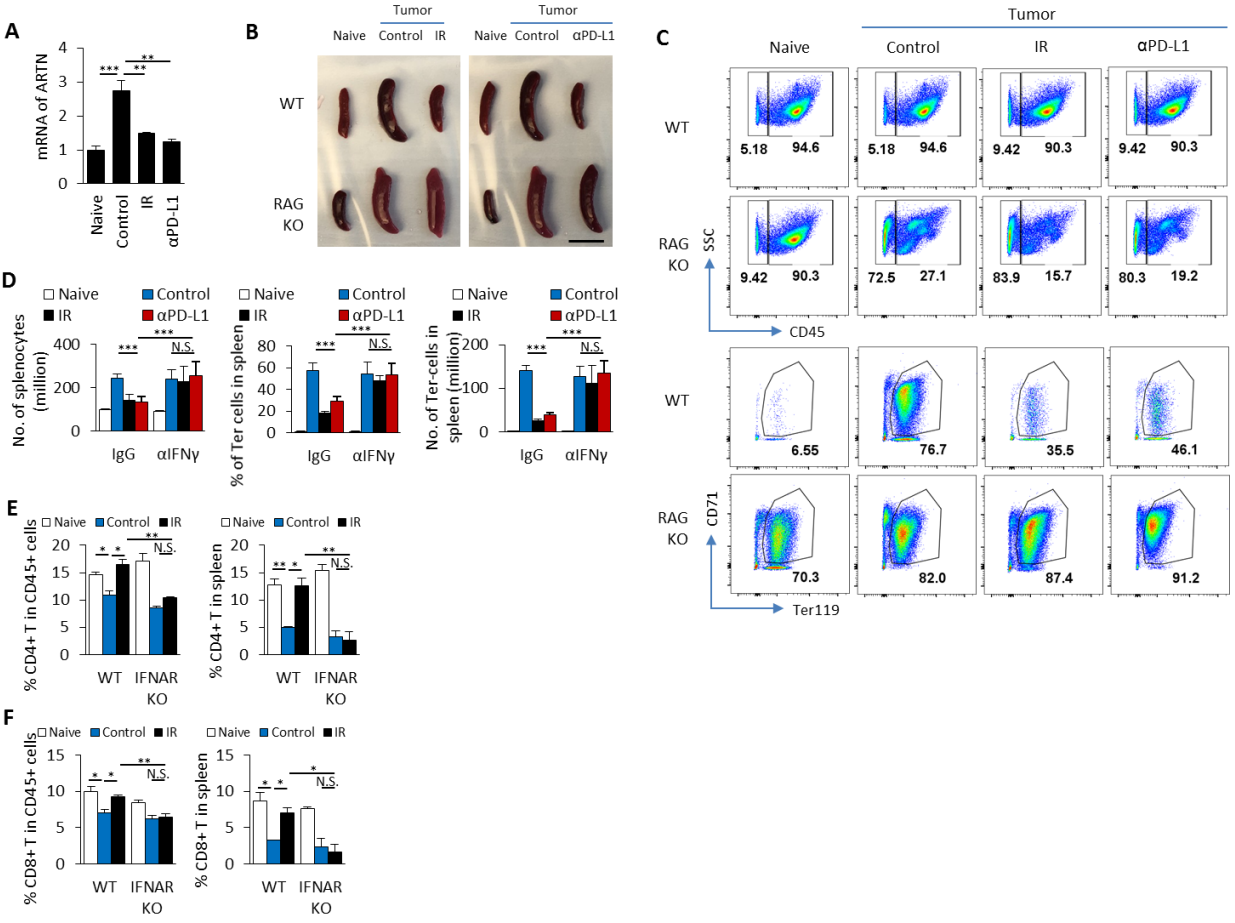

Fig. S3. PD-L1 blockade reduces tumor-induced Ter cell accumulation in the spleen. (A) Splenic artemin mRNA expression in LLC tumor-bearing mice was detected by qPCR on day 10 post treatment. (B) Spleen size of tumor-bearing wild-type (WT) and RAG knockout (KO) mice on day 10 post IR or PD-L1 blockade treatment, or IR plus PD-L1 blockade. (C) The ratio of Ter-cells in spleen of tumor-bearing WT and RAG KO mice was analyzed by flow cytometry. (D) The number and ratio of splenocytes and Ter-cells of tumor-bearing mice treated with IFNγ neutralizing antibody and either IR or PD-L1 blockade was analyzed by flow cytometry. (E) The ratio of splenic $CD4^+$ T cells in WT or IFNAR KO mice treated with IR was analyzed by flow cytometry. (F) The ratio of splenic $CD8^+$ T cells in WT or IFNAR KO mice treated with IR was analyzed by flow cytometry. Representative data are shown from two or three experiments conducted with 3-5 mice or samples per group. Data are represented as mean ± SD. All data in this figure were calculated with one-way ANOVA with Bonferroni's multiple comparison tests. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. N.S., not significant.

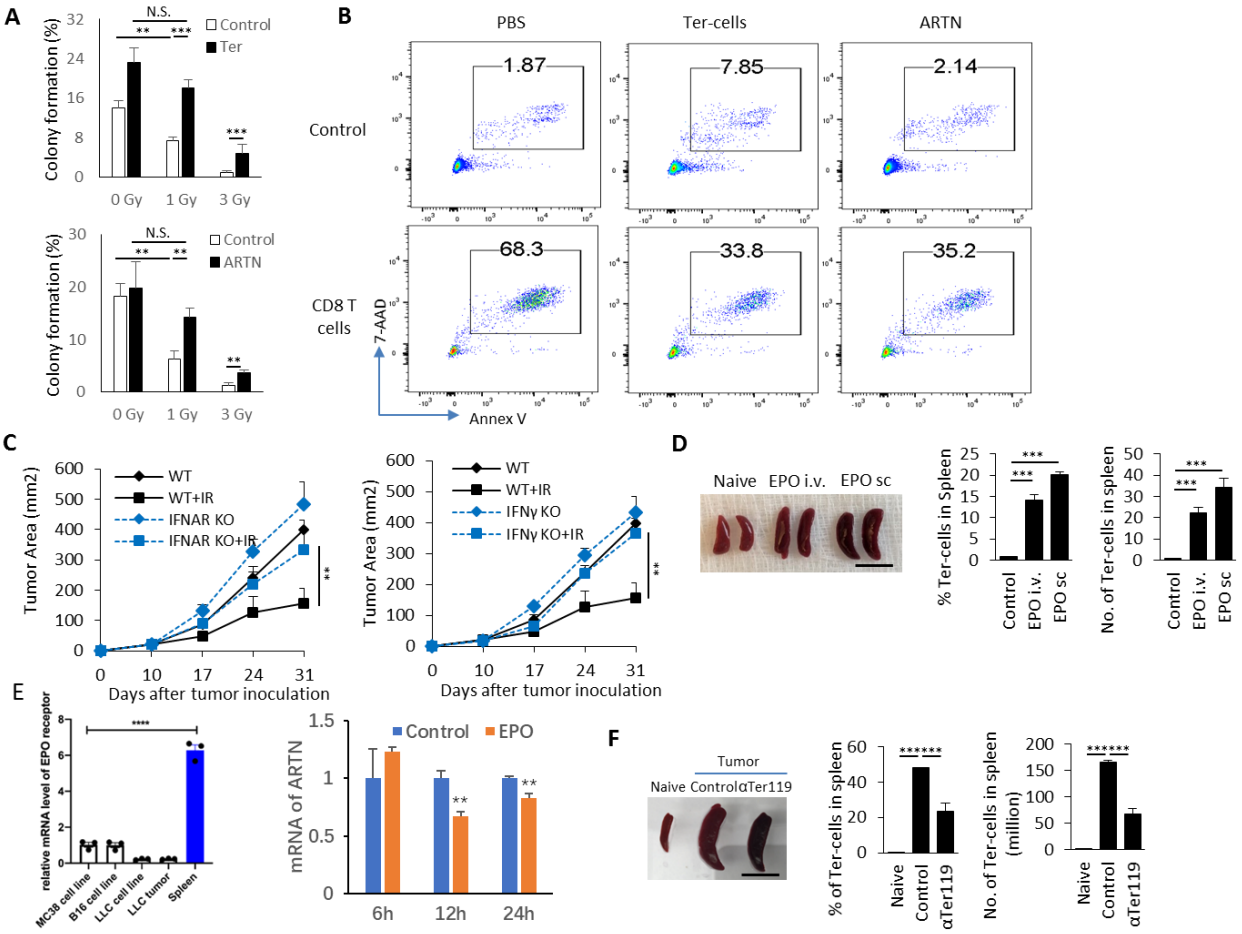

Fig. S4. Ter cells and artemin impair the efficacy of radiotheraphy and immunotherapy. (A) Colony formation assay of B16 cells treated 0, 1 or 3 Gy irradiation. B16 cells were either co-cultured with 2x10^6 Ter-cells sorted from spleens of tumor-bearing mice or treated with 100 ng/ml artemin. (B) 1x10^5 MC38-OTI-zsGreen cells were co-cultured with 2x10^5 CD8+ T cells purified from OTI mice in 96-well U bottom plate. Tumor cells were either co-cultured with 2x10^5 Ter-cells sorted from spleen of tumor-bearing mice or treated with 100 ng/ml artemin for 6 hours. Apoptosis of MC38 cells was analyzed by flow cytometry. (C) Tumor growth of LLC tumor-bearing WT, IFNAR KO and IFNγ KO mice treated with IR on day 10 post tumor inoculation. (D) C57BL/6 mice were treated intravenously or subcutaneously with 20 U/mouse erythropoietin (EPO) every other day for a total of 6 days. The ratio and number of Ter-cells in spleen were analyzed by flow cytometry. (E) Left, Expression of EPO receptor in cancer cell lines, LLC tumors and splenocytes. Right, expression of artemin in tumor cells in the presence of EPO (1U/ml) at indicated time point. (F) LLC tumor-bearing mice were treated via intraperitoneal injection with 20 μg/mouse anti-Ter119 every other day for a total of 6 days. The ratio and number of Ter-cells in spleen were analyzed by flow cytometry. Representative data are shown from two or three experiments conducted with 3-5 mice or samples per group. Data are

represented as mean ± SD. All data in this figure were calculated with one-way ANOVA with Bonferroni's multiple comparison tests. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. N.S., not significant. Scale bar = 1cm.

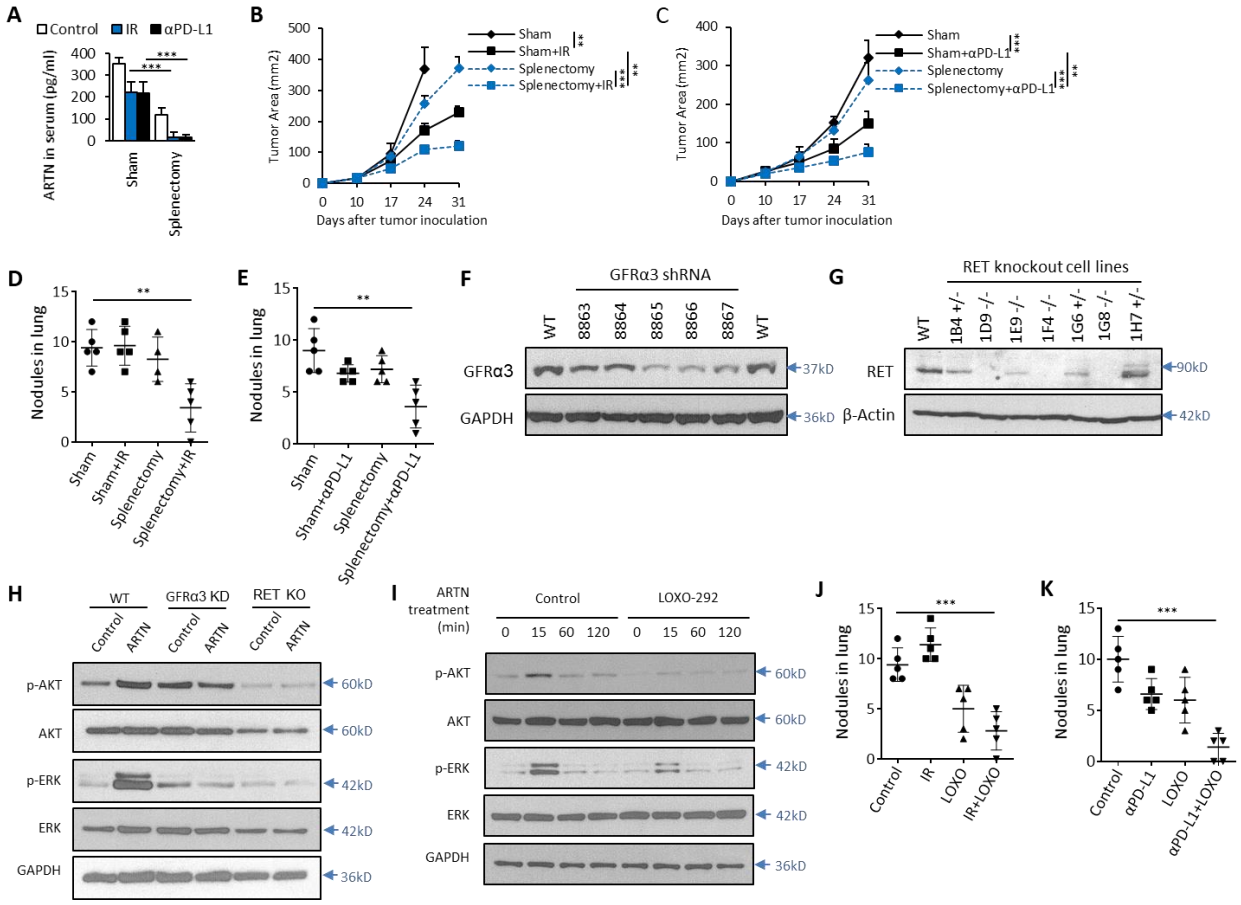

Fig. S5. Interfering with production of Ter cells or artemin promotes the efficacy of radiotherapy and immunotherapy in LLC tumor–bearing mice. (A-E) C57BL/6 mice were inoculated with LLC cells. On day 10 post-inoculation, tumor-bearing mice were treated with either IR or anti-PD-L1. Splenectomy was performed 1 day before treatments. (A) The artemin concentrations in serum were determined by ELISA on day 10 post-treatments. (B-C) Tumor growth of LLC tumor-bearing mice treated with splenectomy and either IR (B), or anti-PD-L1 (C). (D-E) The number of lung nodules were quantified in LLC tumor-bearing mice at the end of tumor growth measurement. (F) shRNA were used to generate GFRα3 Knockdown (KD) MC38 cell lines. The expression of GFRα3 in MC38 cells were analyzed with western blot. MC38 clone 8865 was used for tumor inoculation. (G) CRISPR/Cas9 were used to generate RET knockout (KO) MC38 cell lines. The expression of RET in MC38 cells was analyzed with western blot. MC38 clone 1F4 was used for tumor inoculations. (H) p-AKT and p-ERK in wild-type (WT), GFRα3 KD (8865), RET KO (1F4) tumor cells treated with 100ng/ml were detected by western blot. (I) p-AKT and p-ERK in LLC cells treated with 100ng/ml artemin and 1μg/ml LOXO-292 were detected by western blot. (J-K) C57BL/6 mice were inoculated with LLC cells. On day 10 post inoculation, tumor-bearing mice were treated with IR (J) or anti-PD-L1 (K). Tumor-bearing mice were treated with LOXO-292 from 1 day before IR or anti-PD-L1 treatments. The number of lung nodules were shown in LLC tumor-bearing mice at the end of tumor growth measurement. Representative data are shown from three experiments conducted with 3-5 mice or samples

per group. Data are represented as mean $\pm$ SD. All data in this figure were calculated with one-way ANOVA with Bonferroni's multiple comparison tests. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

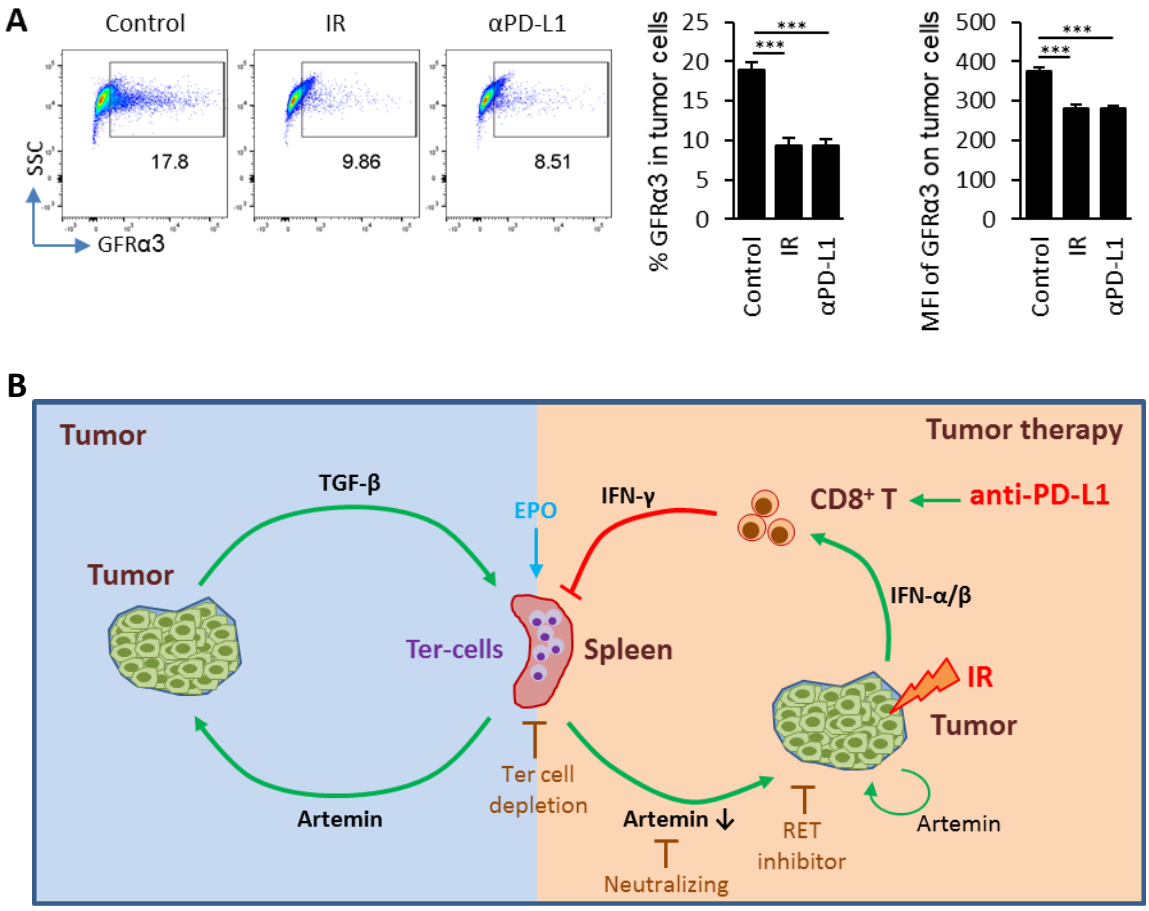

Fig. S6. Radiotheraphy and PD-L1 blockade reduce the expression of GRFα3 on tumor cells in vivo. (A) C57BL/6 mice were inoculated subcutaneously with LLC cells on day 0. On day 10, tumor-bearing mice were treated IR or PD-L1 blockade. Tumors were harvested and the expression of GFRα3 on $CD45^-$ tumor cells was detected by flow cytometry. Data are represented as mean ± SD. The data was calculated with one-way ANOVA with Bonferroni's multiple comparison tests. ***$p < 0.001$. (B) Schema of the interaction between IR and PD-L1 blockade and tumor-induced Ter-cells. Tumor induced Ter-cells accumulate in the spleen through TGF-β signaling and, in turn, Ter-cells promote tumor progression by secreting artemin. IR decreased Ter-cells in spleen through a Type I IFN – CD8 T cell – IFNγ axis. Similarly, PD-L1 blockade reduced the Ter-cells through CD8 T cells and IFNγ. In contrast, Ter-cells and artemin, which could be restored by erythropoietin (EPO) administration, impaired the efficacy of both IR and PD-L1 blockade. Furthermore, Ter-cell depletion, artemin neutralization, and artemin signaling partner inhibition facilitated the efficacy of IR and PD-L1 blockade.