

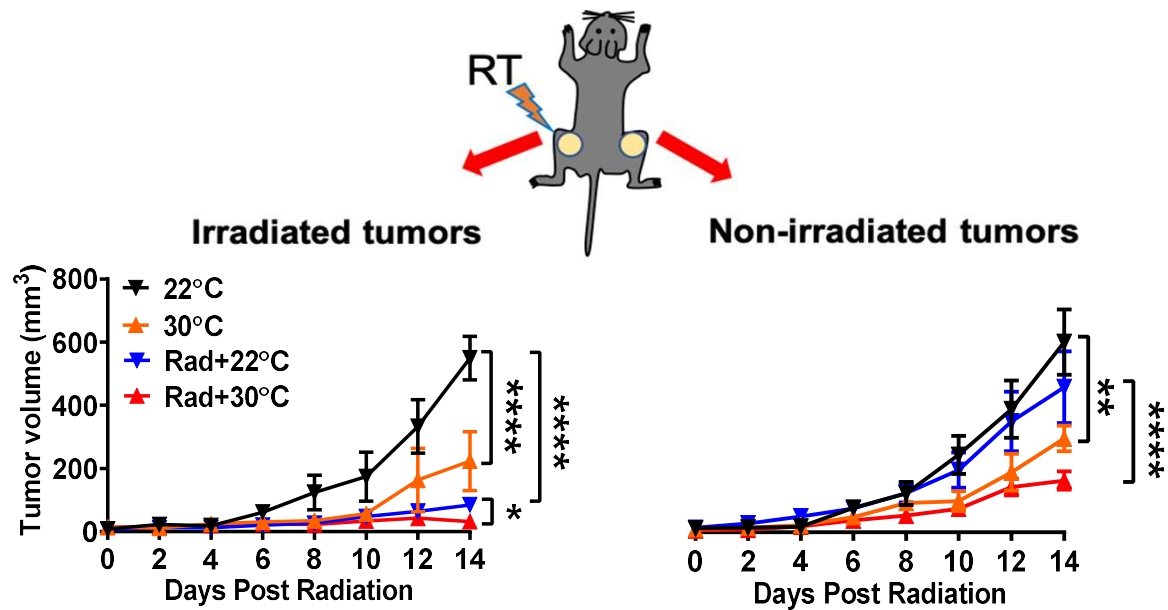
Supplementary Information

Adrenergic stress constrains the development of anti-tumor immunity and abscopal responses following local radiation

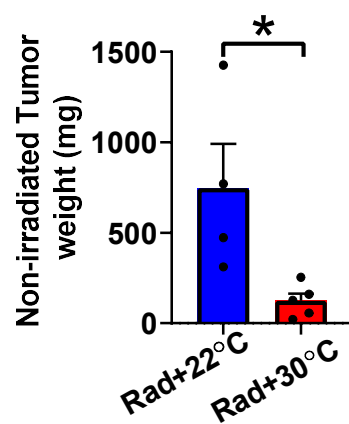
Chen et al.

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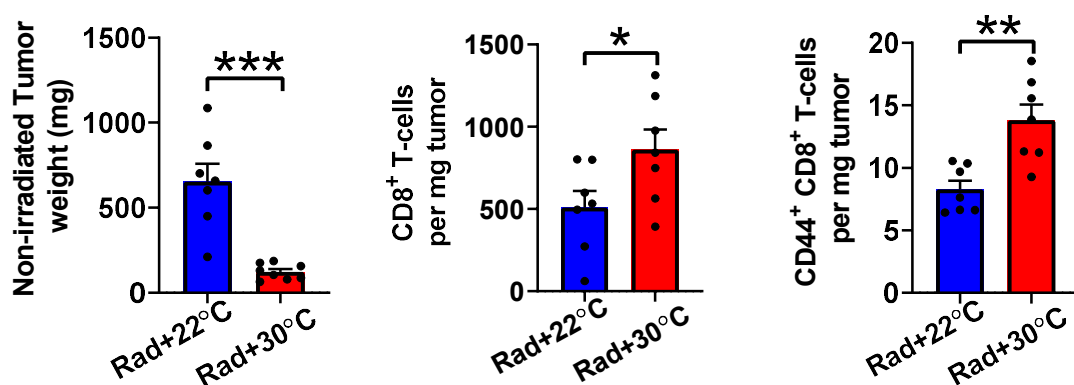
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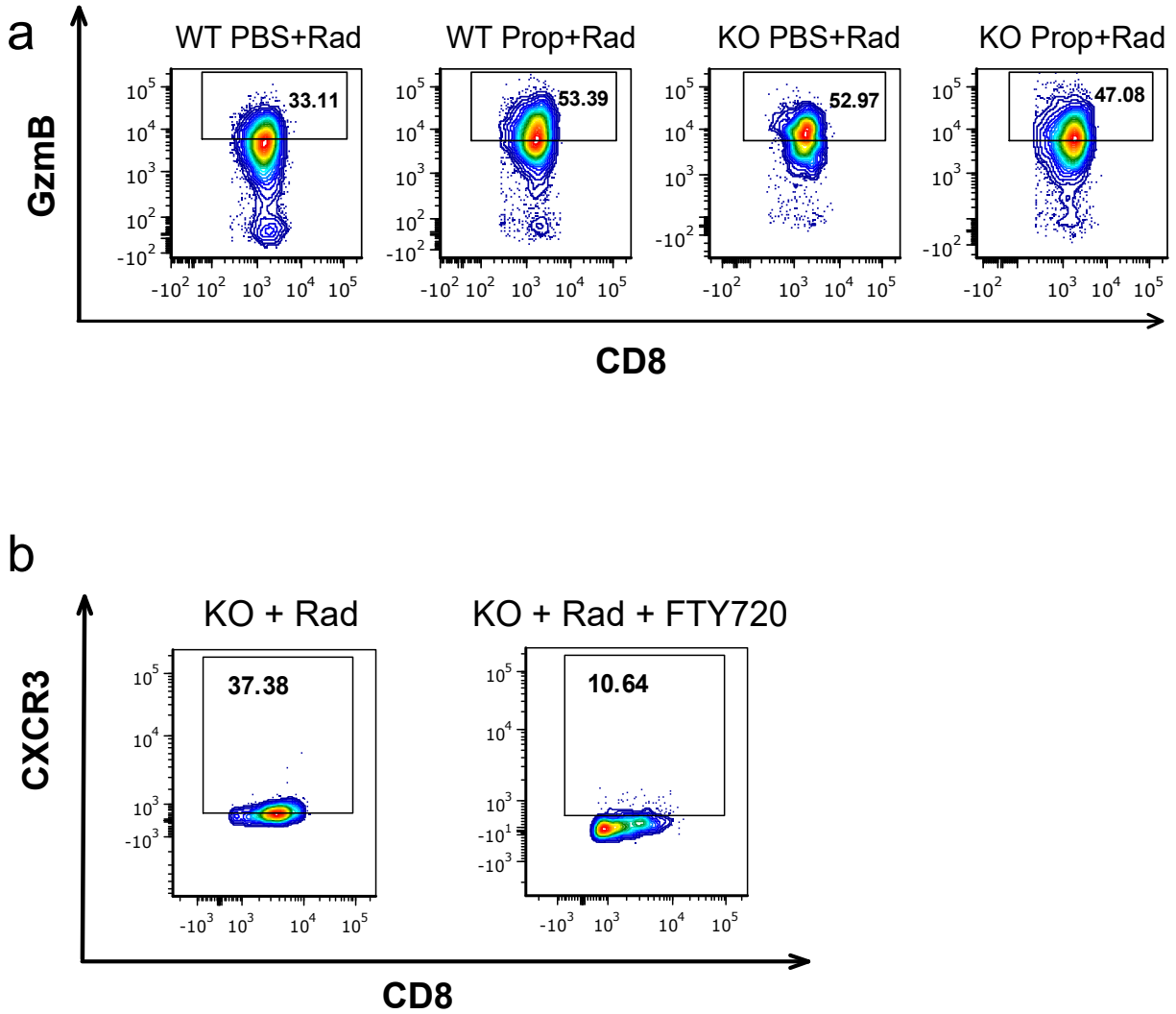
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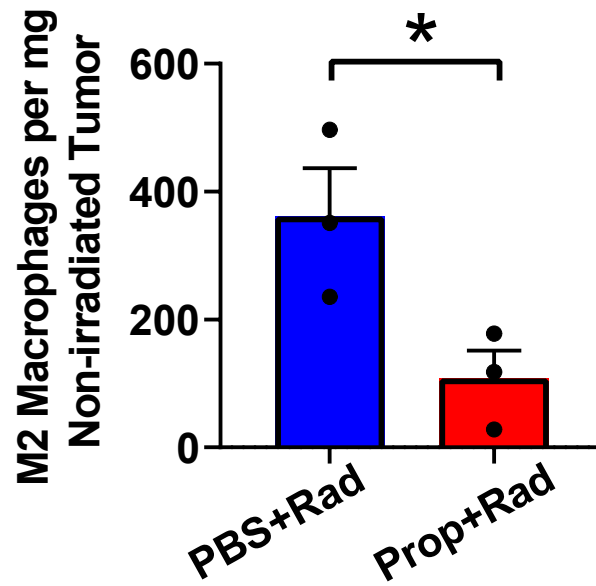
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Supplementary Figure 1. Housing mice at a thermoneutral temperature results in an abscopal effect against a tumor contralateral to an irradiated tumor. **a-b** The B16 tumor model. Growth of irradiated tumors (left) and non-irradiated tumors (right) (**a**) and the weight of non-irradiated tumors (**b**) were measured in C57BL/6 mice housed at 22°C or 30°C treated with or without radiation. Data are presented as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ (two-way ANOVA analysis for **a**; Student's *t* test analysis for **b**). $n = 4-7$ biologically independent mice. (**c**) BALB/c mice were implanted bilaterally with CT26 tumors and housed at 22°C or 30°C. One tumor was irradiated and the non-irradiated tumor was assessed for weight (left), numbers of intratumoral CD8⁺ T cells (middle), and number of CD44⁺ CD8⁺ T cells (right). Data are presented as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (Student's *t* test analysis). For **a**, $n = 5$ biologically independent mice in 22°C group, $n = 4$ biologically independent mice in 30°C group, $n = 7$ biologically independent mice in Rad+22°C and Rad+30°C groups; for **b**, $n = 4$ biologically independent mice in Rad+22°C group, $n = 5$ biologically independent mice in Rad+30°C group; for **c**, $n = 7$ biologically independent mice in Rad+22°C and Rad+30°C groups except for $n = 8$ biologically independent mice in Rad+30°C group in left panel.

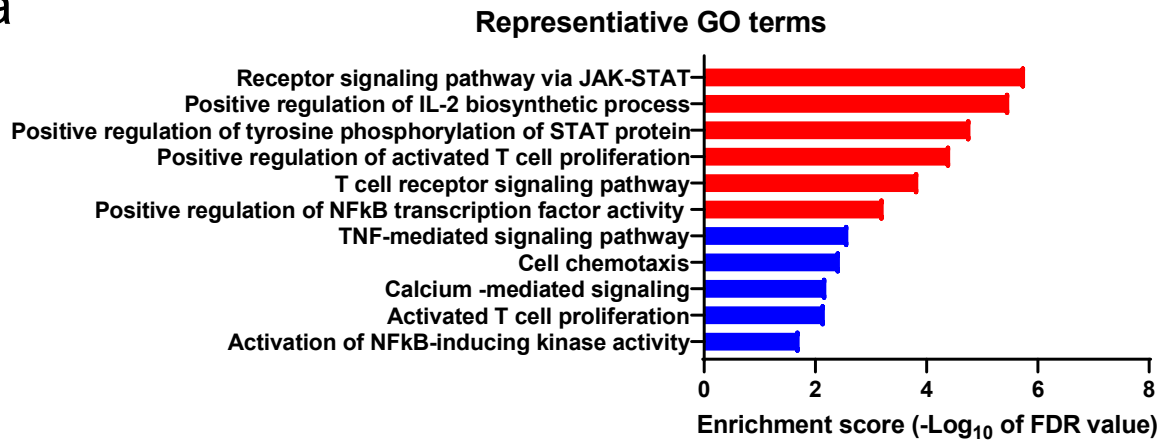


Supplementary Figure 2. Representative flow plots for the data from the CT26 model. Tumors were implanted bilaterally and one tumor was irradiated. **(a)** Representative flow plots of GzmB expression in CD8⁺ T cells in non-irradiated tumors from WT or β 2-AR KO mice which were or were not treated with propranolol. **(b)** Representative flow plots of CXCR3 expression in CD8⁺ T cells in non-irradiated tumors of β 2-AR KO treated with or without FTY720.

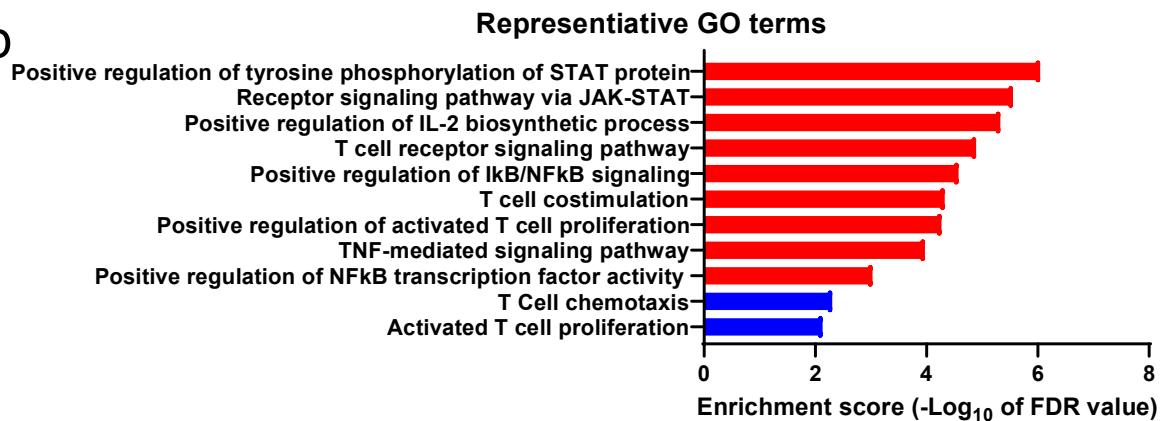


Supplementary Figure 3. β -blocker decreases M2 macrophages ($CD45^+CD11b^+F4/80^{hi}Ly6G^-$) in non-irradiated CT26.CL25 tumors of mice treated with propranolol and radiation compared to the control group. Data are presented as mean $\pm$ SEM. *, $P < 0.05$ (Student's t test analysis). $n = 3$ biologically independent mice in two groups.

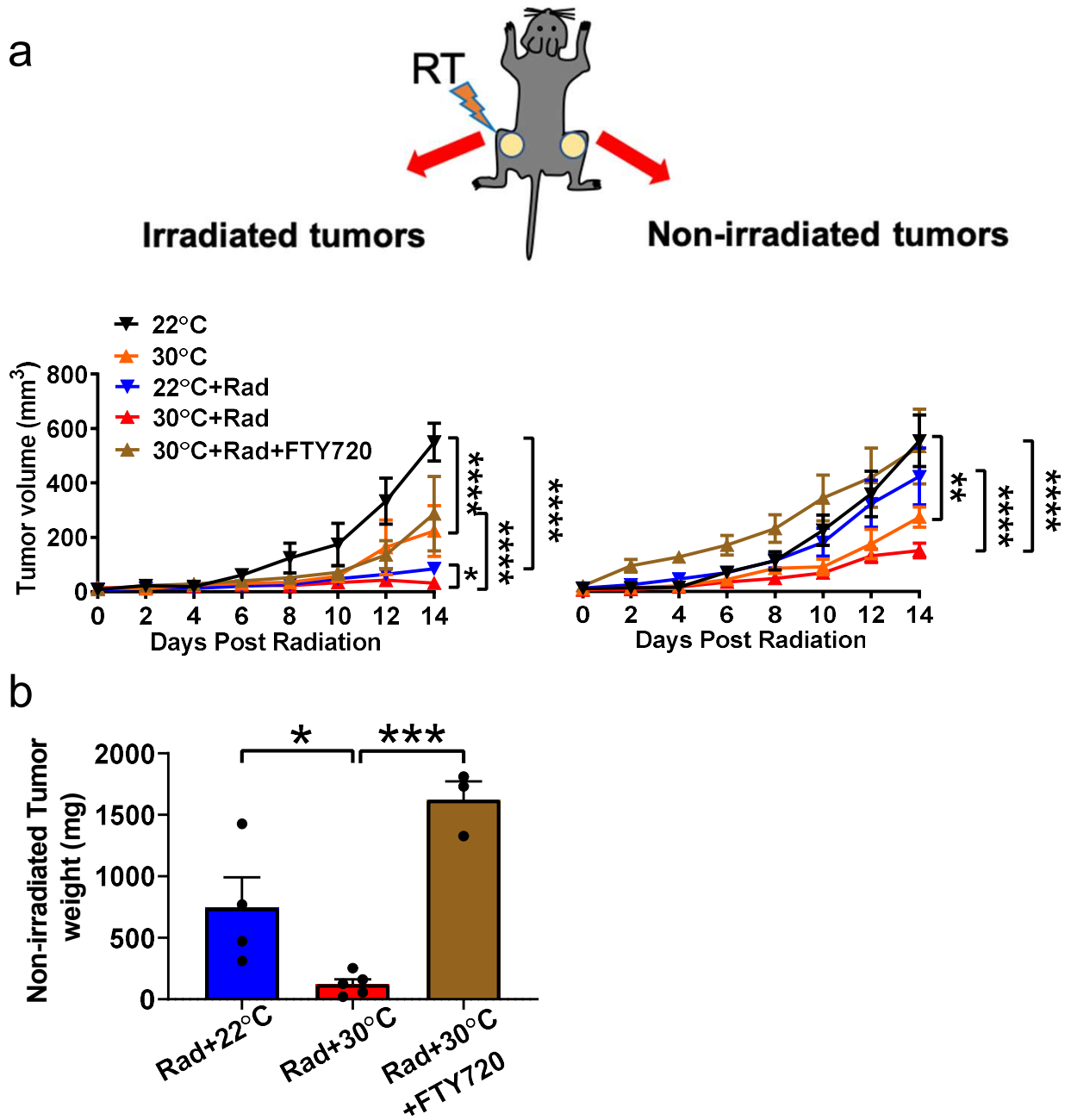
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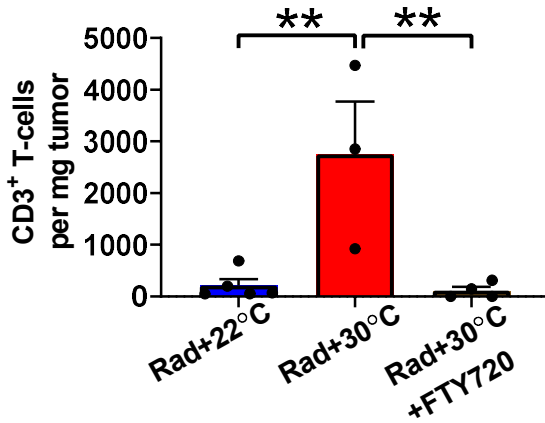


Supplementary Figure 4. Functional annotation of immune-related genes in irradiated β2-AR KO mice. The GO terms (FDR<0.05) of upregulated genes of non-irradiated tumors (**a**) and irradiated tumors (**b**) from irradiated β2-AR KO mice represented as horizontal bars are listed on the y axis and values of -Log₁₀(FDR) are shown on the x axis. The red bars represent FDR<10⁻⁴. The blue bars represent FDR<0.05.

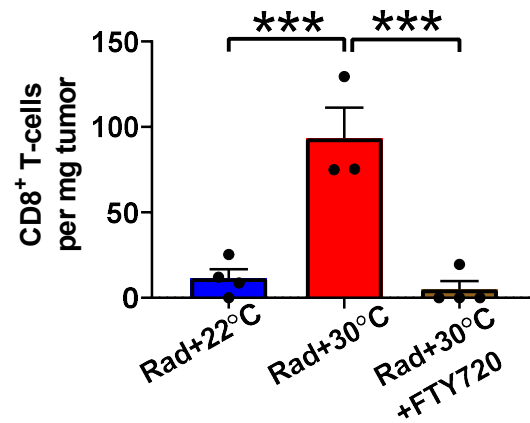


Supplementary Figure 5. Prevention of T-cell migration negates thermoneutral temperature improved abscopal effect of local radiotherapy in B16 melanoma model. The B16 tumor growth of irradiated tumors (left) and non-irradiated tumors (right) (**a**) and the weight of non-irradiated tumors (**b**) were measured in irradiated C57BL/6 mice housed at 22°C or 30°C treated with or without FTY720. Data are presented as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ (two-way ANOVA analysis for **a**; one-way ANOVA analysis for **b**). For **a**, $n = 5$ biologically independent mice in 22°C group, $n = 4$ biologically independent mice in 30°C group, $n = 7$ biologically independent mice in Rad+22°C and Rad+30°C groups; for **b**, $n = 4$ biologically independent mice in Rad+22°C group, $n = 5$ biologically independent mice in Rad+30°C group, $n = 3$ biologically independent mice in Rad+30°C+FTY720 group.

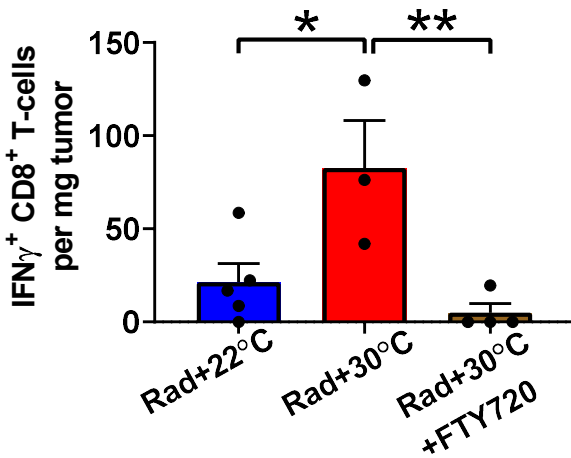
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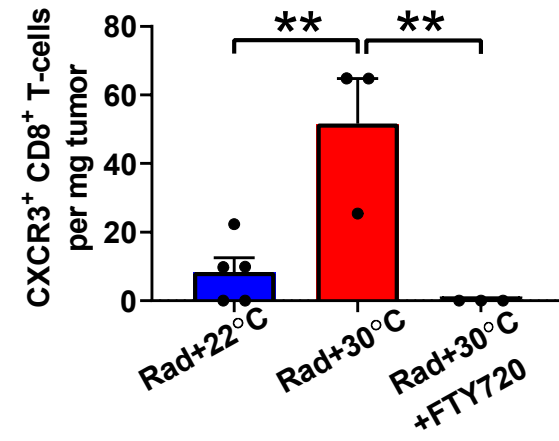
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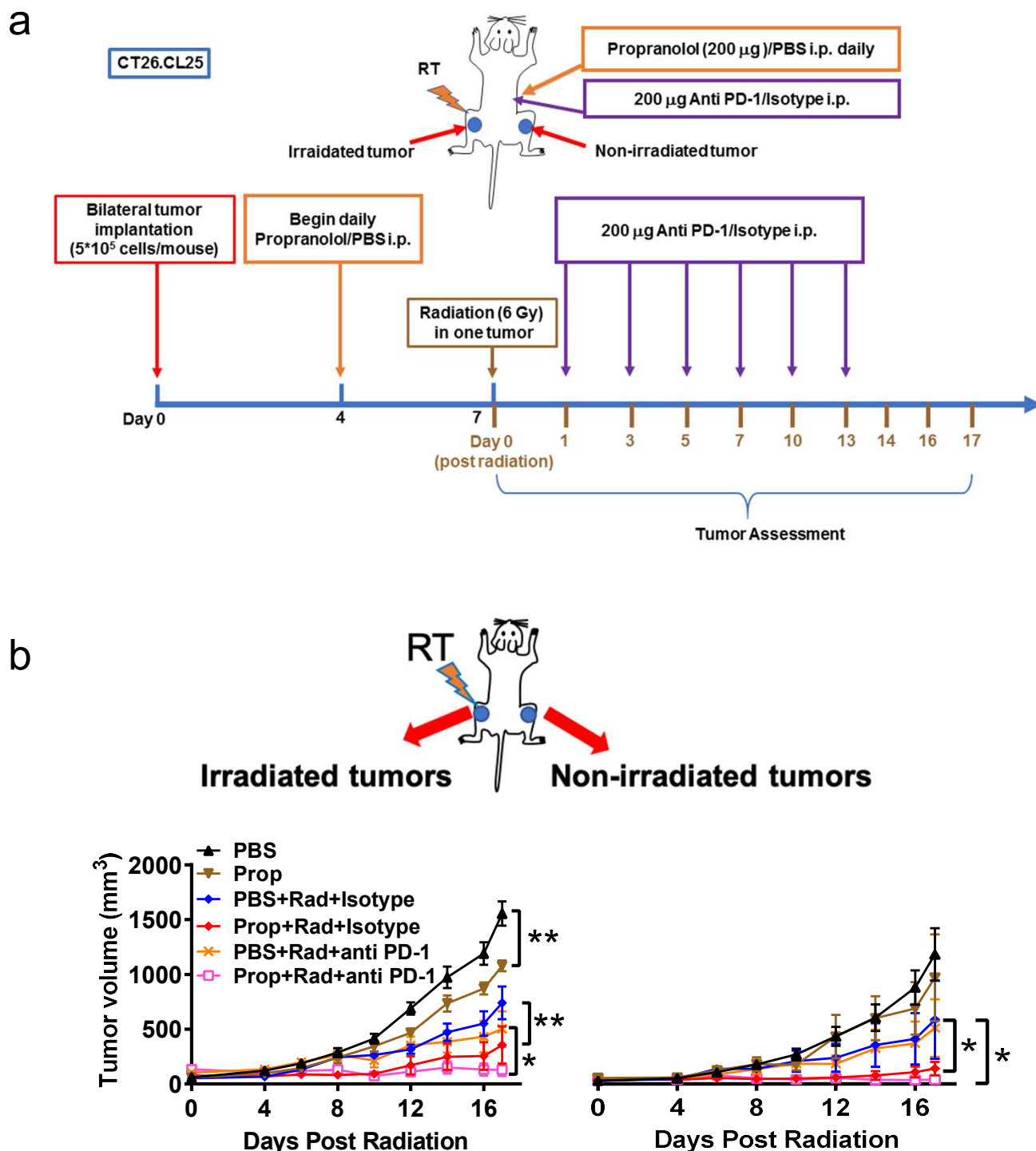
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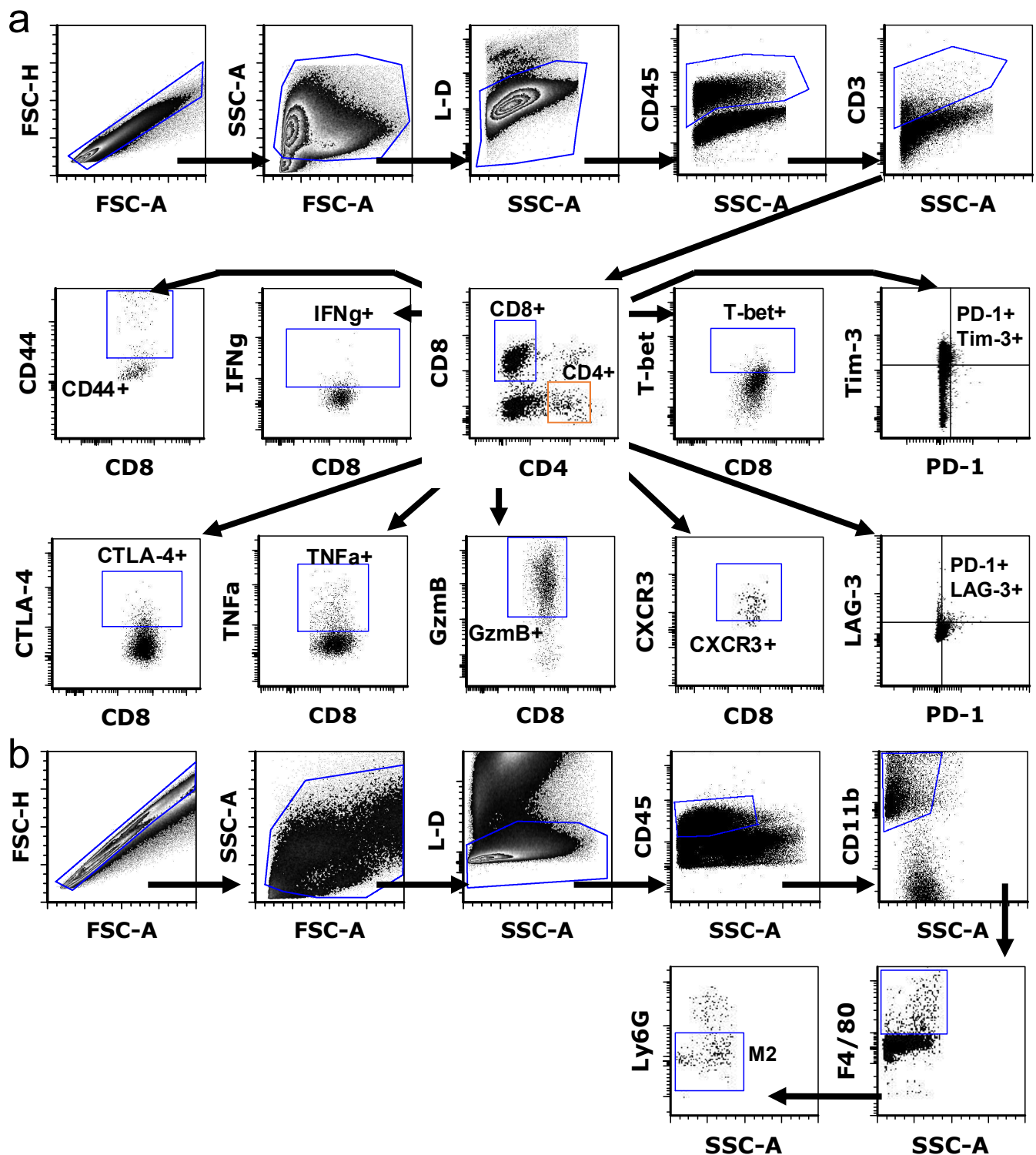
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Supplementary Figure 6. Thermoneutral temperature improved abscopal effect of local radiotherapy via enhancing anti-tumor immune response and T-cell egress in B16 melanoma model. CD3⁺ T cells (a), CD8⁺ T cells (b), IFN γ ⁺ CD8⁺ T cells (c) and CXCR3⁺ CD8⁺ T cells (d) were detected in non-irradiated tumors (B16) from irradiated mice housed at 22°C or 30°C treated with or without FTY720. Data are presented as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (two-way ANOVA analysis). For **a**, $n=5$ biologically independent mice in Rad+22°C group, $n=3$ biologically independent mice in Rad+30°C group, $n=4$ biologically independent mice in Rad+30°C+FTY720 group; for **b**, $n=4$ biologically independent mice in Rad+22°C group, $n=3$ biologically independent mice in Rad+30°C group, $n=4$ biologically independent mice in Rad+30°C+FTY720 group; for **c**, $n=5$ biologically independent mice in Rad+22°C group, $n=3$ biologically independent mice in Rad+30°C group, $n=4$ biologically independent mice in Rad+30°C+FTY720 group; for **d**, $n=5$ biologically independent mice in Rad+22°C group, $n=3$ biologically independent mice in Rad+30°C group, $n=3$ biologically independent mice in Rad+30°C+FTY720 group.



Supplementary Figure 7. β -blocker increases the efficacy of radiation combined with anti PD-1 in CT26 model. **(a)** Experimental design for combination of radiation, propranolol and anti PD-1. Mice housed at 22°C were implanted with CT26.CL25 tumor cells and then randomly received 6 doses of 200 μ g anti PD-1 or isotype antibody (Day 1, 3, 5, 7, 10, 13) with or without daily 200 μ g propranolol/PBS treatment. **(b)** Tumor growth is shown for irradiated tumors (left) and non-irradiated tumors (right). Data are presented as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$ (two-way ANOVA analysis). $n = 7$ biologically independent mice in PBS group; $n = 4$ biologically independent mice in Prop group; $n = 6$ biologically independent mice in PBS+Rad+isotype group; $n = 9$ biologically independent mice in Prop+Rad+isotype group; $n = 5$ biologically independent mice in PBS+Rad+anti PD-1 group; $n = 6$ biologically independent mice in Prop+Rad+anti PD-1 group.



Supplementary Figure 8. Immune profiling of T lymphocytes and M2 macrophages using multiparametric flow cytometry. (a) Bivariate plots representing sequential gating approach used for a functional characterization of CD8⁺ and CD4⁺ T lymphocytes in non-irradiated tumors and lymph nodes from the mice with specific treatment mentioned in the Methods. Leukocytes that were singlets, debris-free, viable, and CD45⁺ were identified. Within the leukocyte population, cytotoxic T cells were defined as CD3⁺CD8⁺/CD4⁻ (Fig. 2a, b, and d; Fig. 3i and j; Fig. 4c; Supplementary Figure 1 and 6), whereas helper T cells were defined as CD3⁺CD4⁺/CD8⁻ (Fig. 2d). The expression profiles of IFNγ⁺, T-bet⁺, GzmB⁺, TNFα⁺, CXCR3⁺ (Fig. 3 a-d, g; Fig. 4 d-f, j; Supplementary Figure 2, 6), CTLA-4⁺, PD-1⁺Tim-3⁺, PD-1⁺LAG-3⁺ (Fig. 4 g-i) and CD44⁺ (Supplementary Figure 1c) were evaluated in CD8⁺ T cells. (b) Identification of M2 macrophages. Myeloid cells that were singlets, debris-free, viable, CD45⁺, and CD11b⁺ were identified. Within myeloid cells, M2 macrophages were defined as F4/80⁺, Ly6G⁻ (Supplementary Figure 3).

Supplementary Table 1 Detailed information on commercial antibodies

Number	Name	Company	Cat.No.	Dilution
1	anti-CD8 α BUV395	BD	563786	1:160
2	anti-CD4 BV786	BD	563331	1:100
3	anti-CD3 BV786	BD	564010	1:160
4	anti-CD3 BV605	BD	564009	1:160
5	anti-CD45 FITC	BD	553080	1:100
6	anti-CD45 BV605	BD	563053	1:100
7	anti-CD11b BUV737	BD	564443	1:100
8	anti-F4/80 Ax647	BD	565853	1:100
9	anti- Ly6G BV711	BD	563979	1:80
10	anti-PD-1 BV605	BD	563059	1:50
11	anti-CTLA-4 PerCP/Cy5.5	Biolegend	106316	1:80
12	anti-Tim3 PE	Biolegend	134004	1:80
13	anti-LAG3 APC	BD	562346	1:50
14	anti-CXCR3 BV650	Biolegend	126531	1:50
15	anti-FoxP3 PE	BD	560408	1:80
16	anti-T-bet Ax647	BD	561264	1:20
17	anti-IFN- γ APC	eBiosciences	17-7311-82	1:160
18	anti-TNF α PerCP/Cy5.5	Biolegend	506322	1:80
19	anti-granzyme B Ax647	Biolegend	515406	1:20
20	BV605 Hamster IgG2, k Isotype Control	BD	563012	1:50
21	PerCP/Cy5.5 Armenian Hamster IgG Isotype Ctrl Antibody	Biolegend	400931	1:80
22	PE Rat IgG1, k Isotype Ctrl Antibody	Biolegend	400407	1:80
23	BV650 Armenian Hamster IgG Isotype Ctrl Antibody	Biolegend	400945	1:50
24	PE Rat IgG1 k Isotype Control	BD	551979	1:80
25	Ax647 mouse IgG1 k Isotype Control	BD	557783	1:20
26	APC Rat IgG1 k Isotype Control	eBioscience	17-4301-81	1:160
27	PerCP/Cy5.5 Rat IgG1 k Isotype Ctrl antibody	Biolegend	400425	1:80
28	Ax647 Mouse IgG1 k Isotype Ctrl antibody	Biolegend	400135	1:20