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Supplemental Information

Irradiation Enhances Abscopal Anti-tumor Effects of Antigen-Specific Immunotherapy through Regulating Tumor Microenvironment

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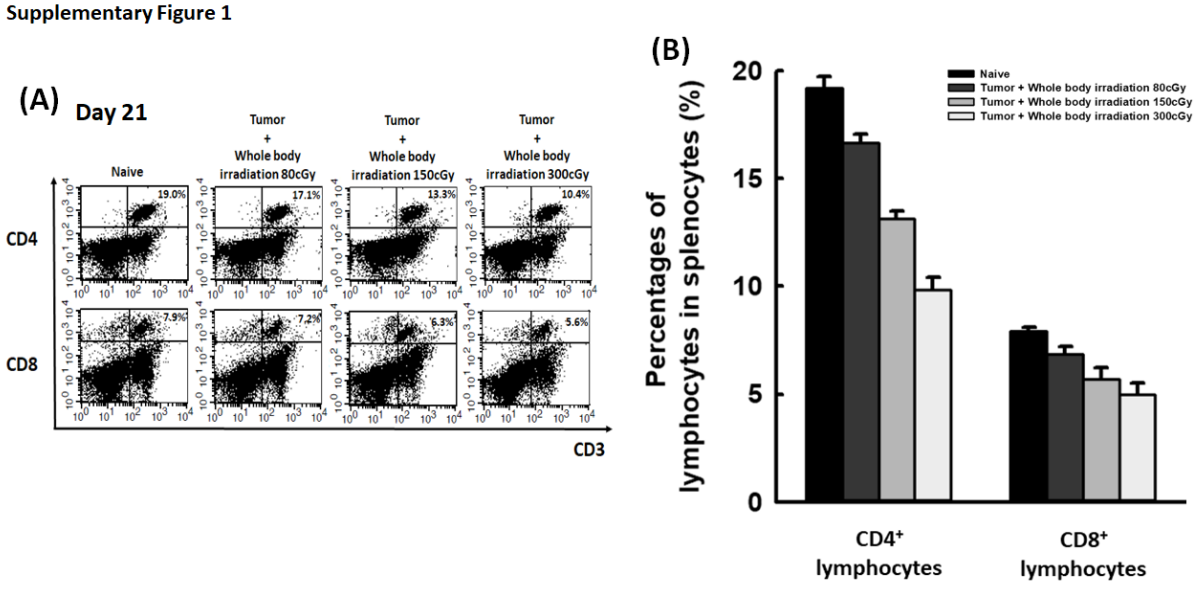
Supplementary Figures and Legends

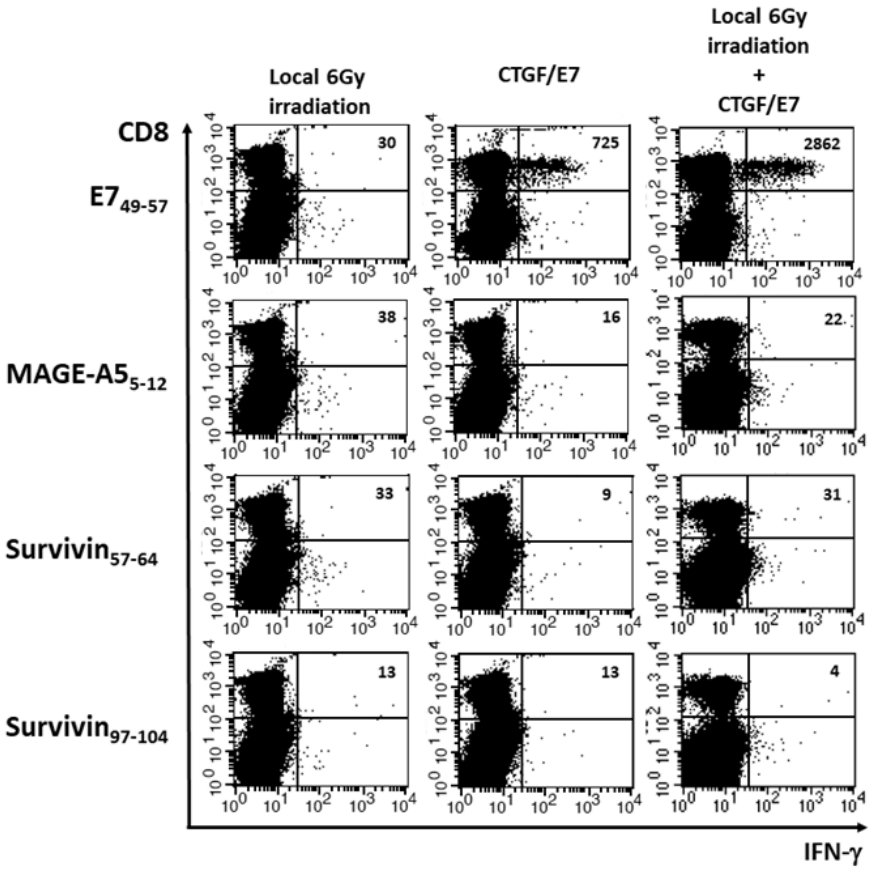
Supplementary Figure 1. Alterations of $CD4^+$ and $CD8^+$ lymphocytes in splenocytes of mice with tumors treated using various doses of whole-body irradiation. Representative images **(A)** and bar figures **(B)** showing flow cytometry analysis of $CD4^+$ and $CD8^+$ lymphocytes at day 21 in various experimental groups: naïve (no tumor and no irradiation), tumor + whole-body irradiation 80 cGy, tumor + whole-body irradiation 150 cGy, tumor + whole-body irradiation 300 cGy (n = 5 per group). Bar figure data are shown as mean ± SD.

Note: The percentages of $CD4^+$ and $CD8^+$ lymphocytes, respectively, in splenocytes at day 35 were as follows: naïve, 19.2 ± 0.5%, 7.9 ± 0.1%; tumor + whole-body irradiation 80 cGy, 16.7 ± 0.4%, 6.7 ± 0.3%; tumor + whole-body irradiation 150 cGy, 13.1 ± 0.4%, 5.7 ± 0.5%; and tumor + whole-body irradiation 300 cGy, 9.8 ± 0.6%, 5.0 ± 0.6% ($P < 0.001$, $P = 0.002$, one-way ANOVA).

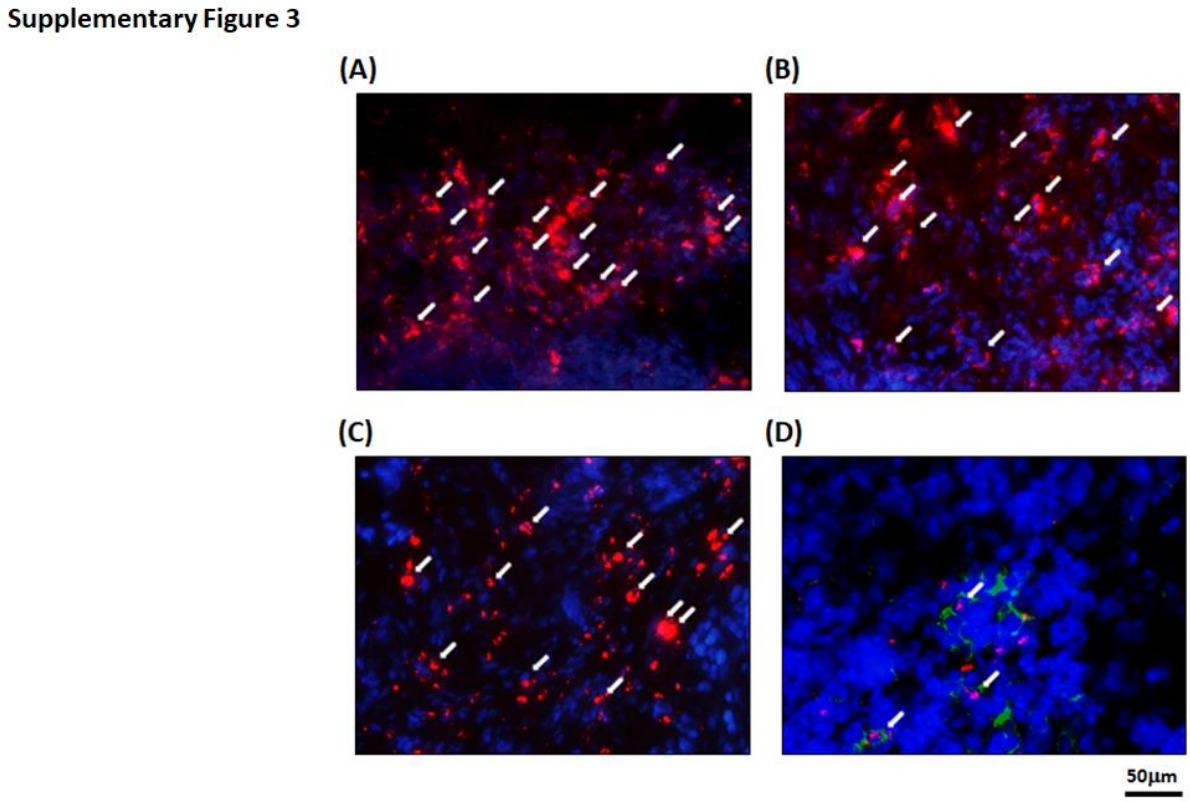


Supplementary Figure 2. Antigen/epitope-specific IFN-γ-secreting CD8$^+$ cytotoxic T cells in various treatment groups. Representative figures of the flow cytometry analysis of different antigen/epitope-specific IFN-γ-secreting CD8$^+$ cytotoxic T cells in various treatment groups (n = 5 per group). *Note:* Only E7-specific IFN-γ-secreting CD8$^+$ T cells were induced in the mice treated with the CTGF/E7 DNA vaccine alone or with the CTGF/E7 DNA vaccine plus 6-Gy local irradiation.

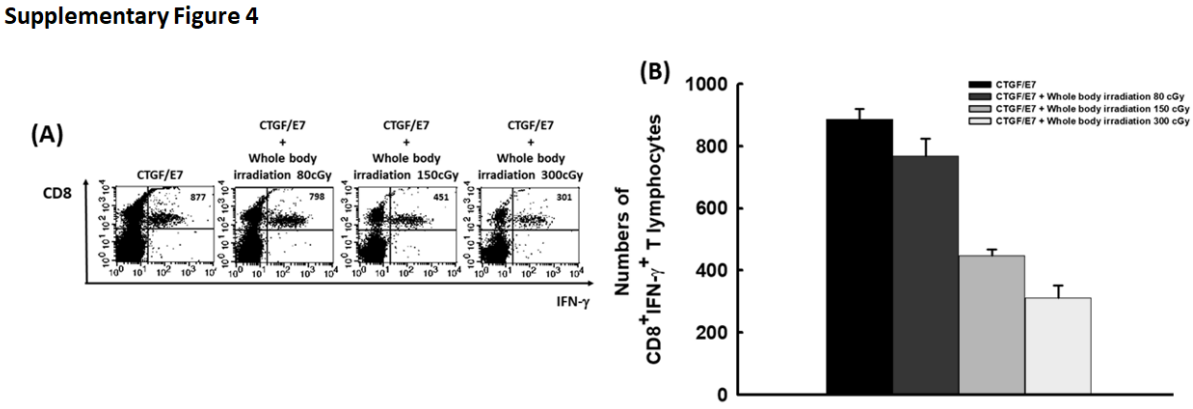
Supplementary Figure 2



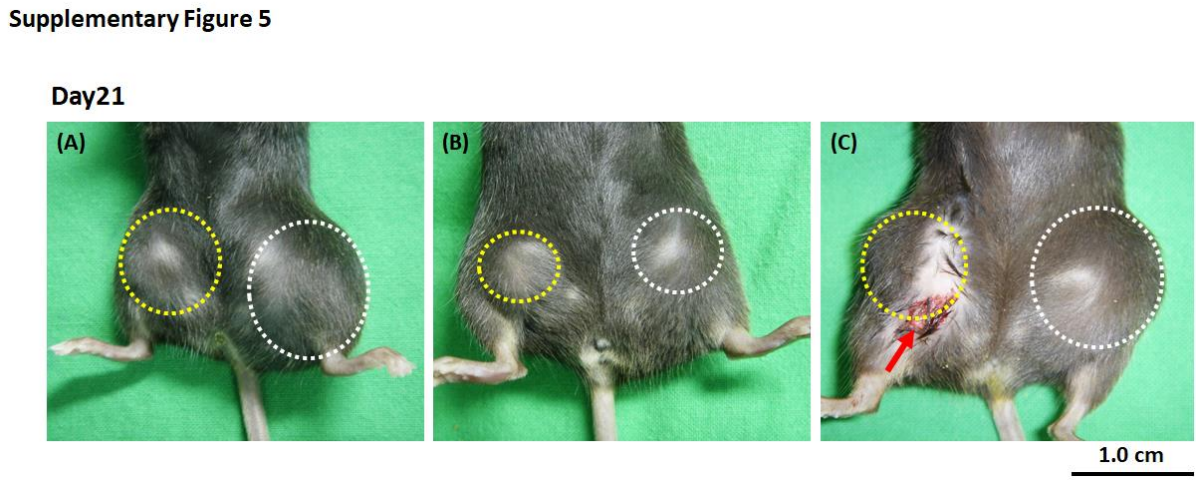
Supplementary Figure 3. Other infiltrating immunocytes following treatment within index tumors. Representative images showing immunofluorescence staining of other infiltrating immunocytes in the index tumor treated with 6-Gy local irradiation plus the CTGF/E7 DNA vaccine. A, macrophage (blue, DAPI; red, F4/80); B, neutrophil (blue, DAPI; red, $Ly6g^{+}$); C, B lymphocytes (blue, DAPI; red, CD19); D, Tregs (blue, DAPI; red, FoxP3; green, CD4) (*n* = 5 per group).



Supplementary Figure 4. The numbers of E7-specific IFN-γ-secreting CD8$^+$ T cells in tumor-bearing mice treated with whole-body irradiation (overall treatment period = 2 weeks) and/or CTGF/E7 DNA vaccine. (A) Representative figures of flow cytometry analysis of the numbers of E7-specific IFN-γ-secreting CD8$^+$ T cells in mice treated with whole-body irradiation and/or CTGF/E7 DNA vaccine (n = 5 per group). **(B)** Bar figures indicating the numbers of E7-specific IFN-γ-secreting CD8$^+$ T cells in mice treated with whole-body irradiation and/or CTGF/E7 DNA vaccine (n = 5 per group). Data are shown as mean ± SD. *Note:* The numbers of E7-specific IFN-γ-secreting CD8$^+$ T cells in splenocytes were as follows: CTGF/E7, 885.1 ± 35.1%; CTGF/E7 + whole-body irradiation 80 cGY, 767.7 ± 55.9%; CTGF/E7 + whole-body irradiation 150 cGY, 446.3 ± 21.0%; and CTGF/E7 + whole-body irradiation 300 cGY, 310.7 ± 39.6% (P < 0.001, one-way ANOVA).



Supplementary Figure 5. Mouse index and distant tumors. Representative images showing mouse index tumors (yellow circle, treated with DNA vaccine plus local irradiation) and distant tumors (white circle, untreated) on day 21. A, CTGF/E7 + 3-Gy local RT; B, CTGF/E7 + 6-Gy local RT; C, CTGF/E7 + 12-Gy local RT. (*n* = 5 per group). *Note:* The skin wound (red arrow) with poor healing and progression was observed starting on day 16.



Supplementary Figure 6. MHC class I-expressing TC-1 tumor cells in different treatment groups. Bar figures indicating the percentage of MHC class I-expressing TC-1 tumor cells in groups treated without or with irradiation (3, 6, or 12 Gy) at the indicated intervals (0 and 24 h) (n = 3 per group). Data are shown as mean ± SD. *Note:* The percentages of MHC class I-expressing cells were as follows: 0-h group: 0-Gy irradiation, 82.1 ± 1.8%; 3-Gy irradiation, 81.1 ± 1.2%; 6-Gy irradiation, 83.3 ± 1.5%; 12-Gy irradiation, 83.8 ± 1.2% (P = 0.09, one-way ANOVA); 24-h group: 0-Gy irradiation, 81.4 ± 1.5%; 3-Gy irradiation, 94.1 ± 1.7%; 6-Gy irradiation, 94.6 ± 1.6%; 12-Gy irradiation, 92.6 ± 0.9% (P < 0.001, one-way ANOVA).

Supplementary Figure 6