

Supplementary information

Hypofractionated radiotherapy combined with lenalidomide improves systemic antitumor activity in mouse solid tumor models

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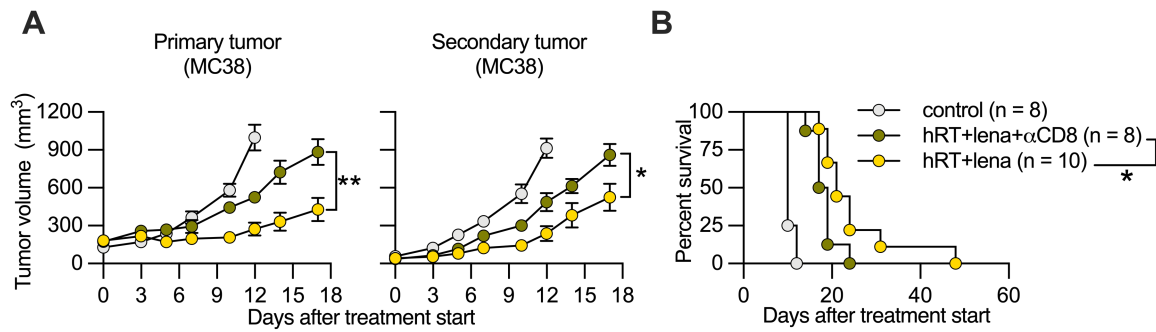
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Running title: Lenalidomide enhances the RT-induced abscopal effect

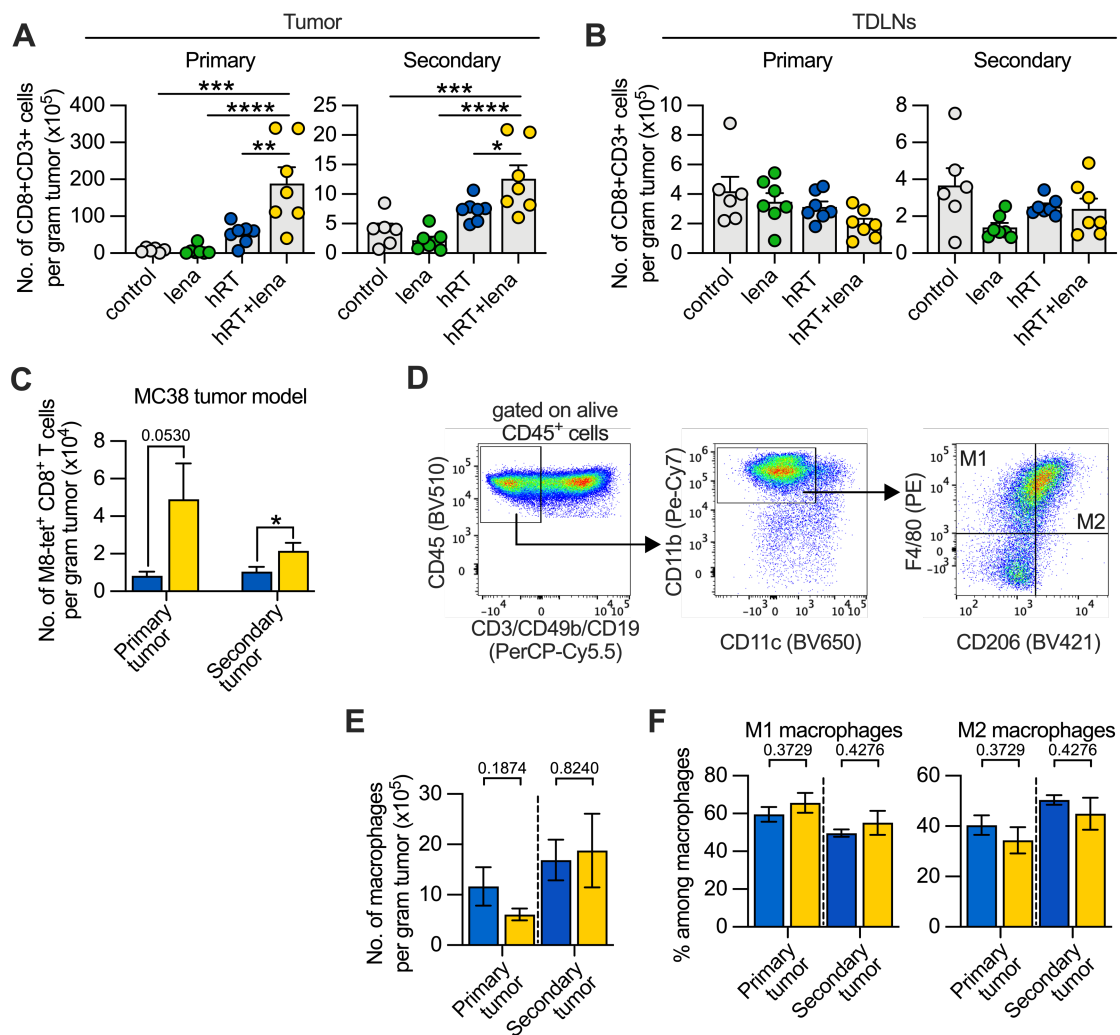
Keywords: radiotherapy, abscopal effect, lenalidomide, dendritic cell cross-presentation

Supplementary figure 1



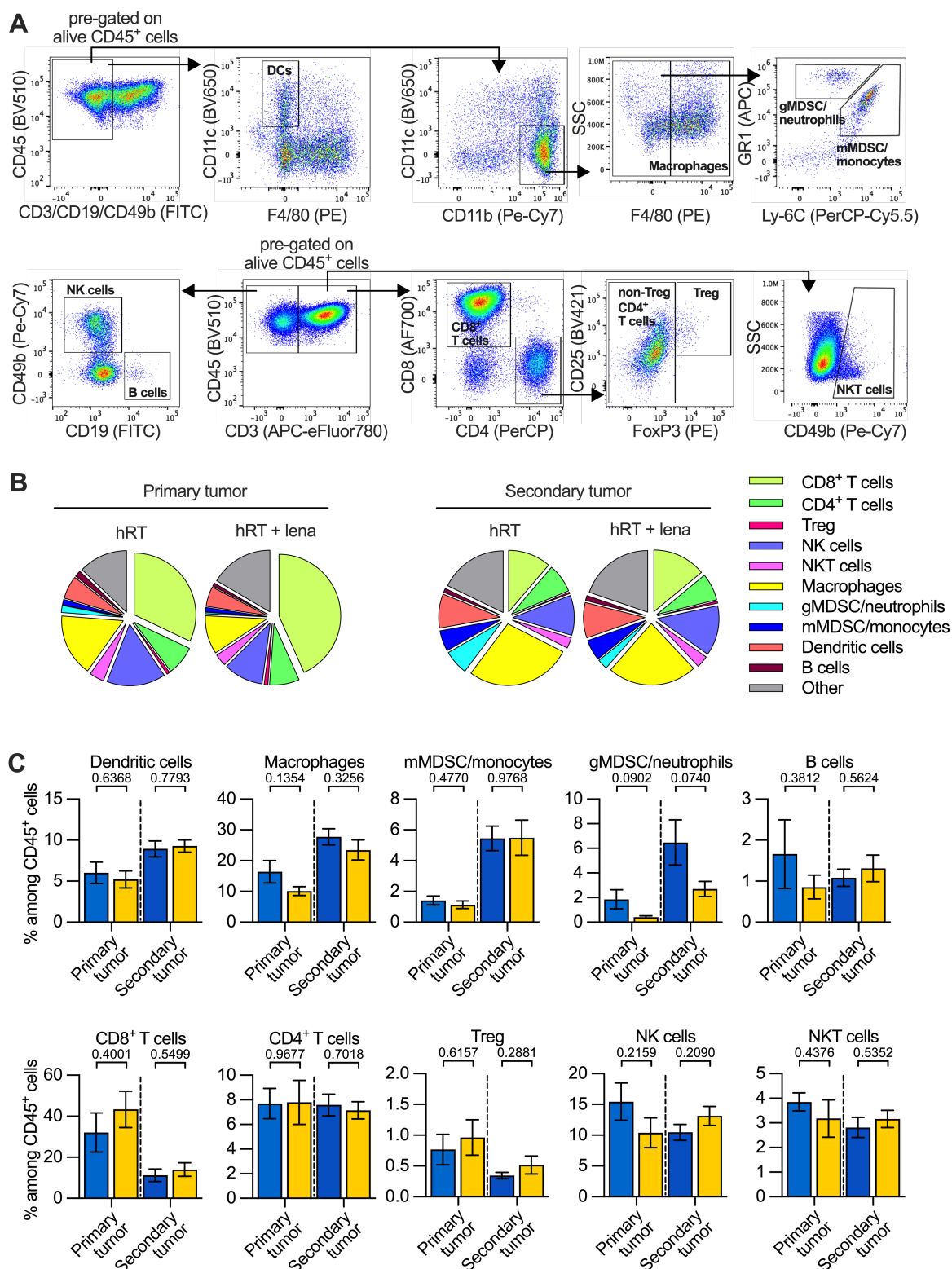
Supplementary figure 1. hRT/lena-mediated local and abscopal tumor control in MC38 colon cancer model depends on CD8⁺ cells. **A.** Growth of irradiated primary (left) and nonirradiated secondary (right) MC38 tumors. **B.** Survival of mice. Data are presented as mean with SEM and were collected from 2 independent experiments. *P* values (ns, not significant; * *P* < 0.05; ** *P* < 0.01) were determined by unpaired two-tailed Student's *t*-test (A) or Kaplan–Meier analysis (B).

Supplementary figure 2



Supplementary figure 2. hRT/lena treatment increases the number of CD8⁺ T cells in tumors and TDLNs but does not affect macrophages. Numbers of bulk CD8⁺ T cells in primary and secondary tumor (**A**) and TDLNs (**B**) at day 8 after treatment start; untreated control (n=6, grey), lena (n=7, green), hRT (n=7, blue) or hRT/lena (n=7, yellow). **D**, Gating strategy. **E**, Numbers of macrophages (F4/80⁺ CD11b⁺) per gram tumor in the B16-CD133 model at day 8 after treatment start. **F**, Percentage of M1 (CD206⁻) and M2 (CD206⁺) macrophages in primary and secondary tumors of hRT- (n=7) and hRT+lena (n=7)- treated mice. Data are presented as mean with SEM and were collected from 3 independent experiments. *P* values (ns, not significant; * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001) were determined one-way ANOVA with Tukey's multiple comparisons test (A, B) or unpaired two-tailed Student's t-test (C, E, F).

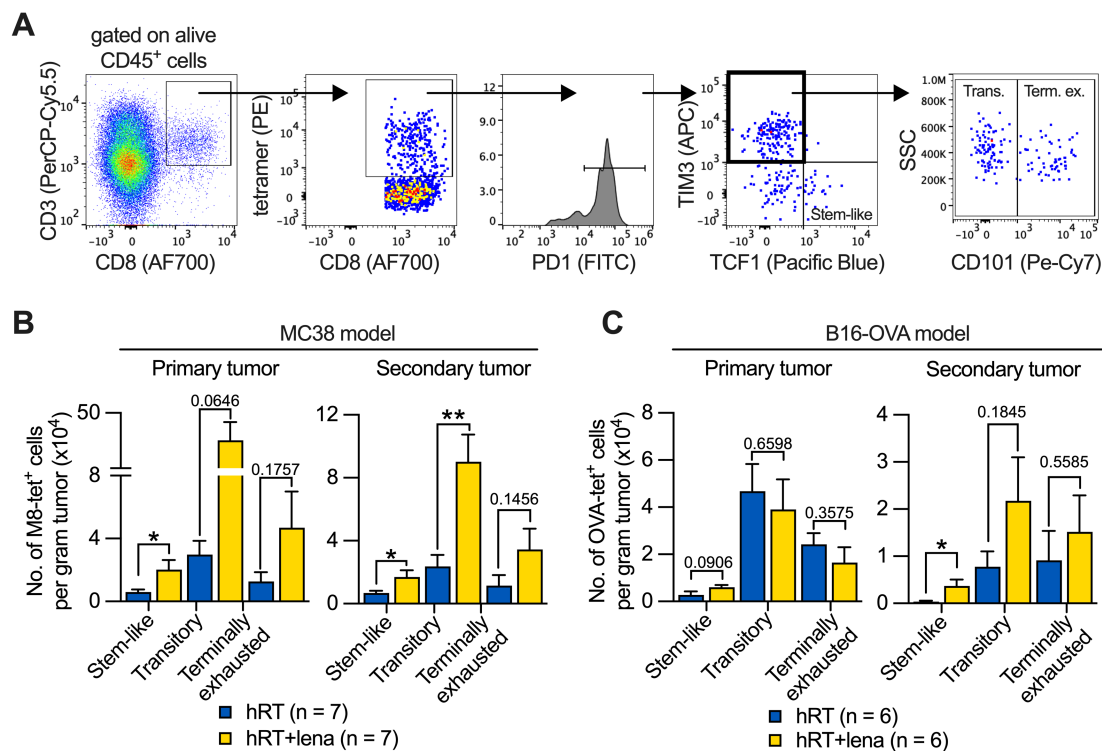
Supplementary figure 3



Supplementary figure 3. Lymphoid and myeloid cell composition in primary and secondary tumors of hRT- and hRT+lena-treated B16-CD133 tumor-bearing mice. A, Gating strategy. B,

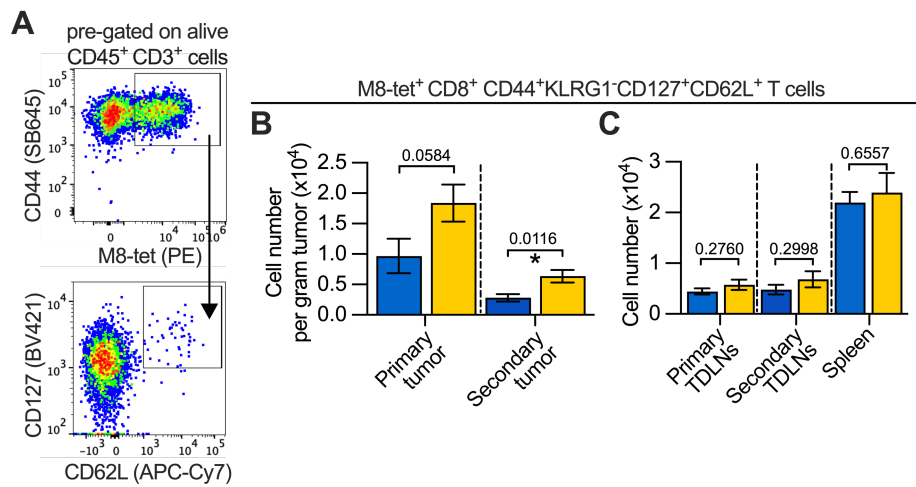
Pie chart summarizing the cell composition within the tumors of hRT- (n=7) and hRT+lana (n=7) -treated mice at day 8 after treatment start. C, Percentage of different cell types among CD45⁺ cells in primary and secondary tumors. Data are presented as mean with SEM and were collected from 3 independent experiments. P values are indicated in the figure and were determined by unpaired two-tailed Student's t-test.

Supplementary figure 4



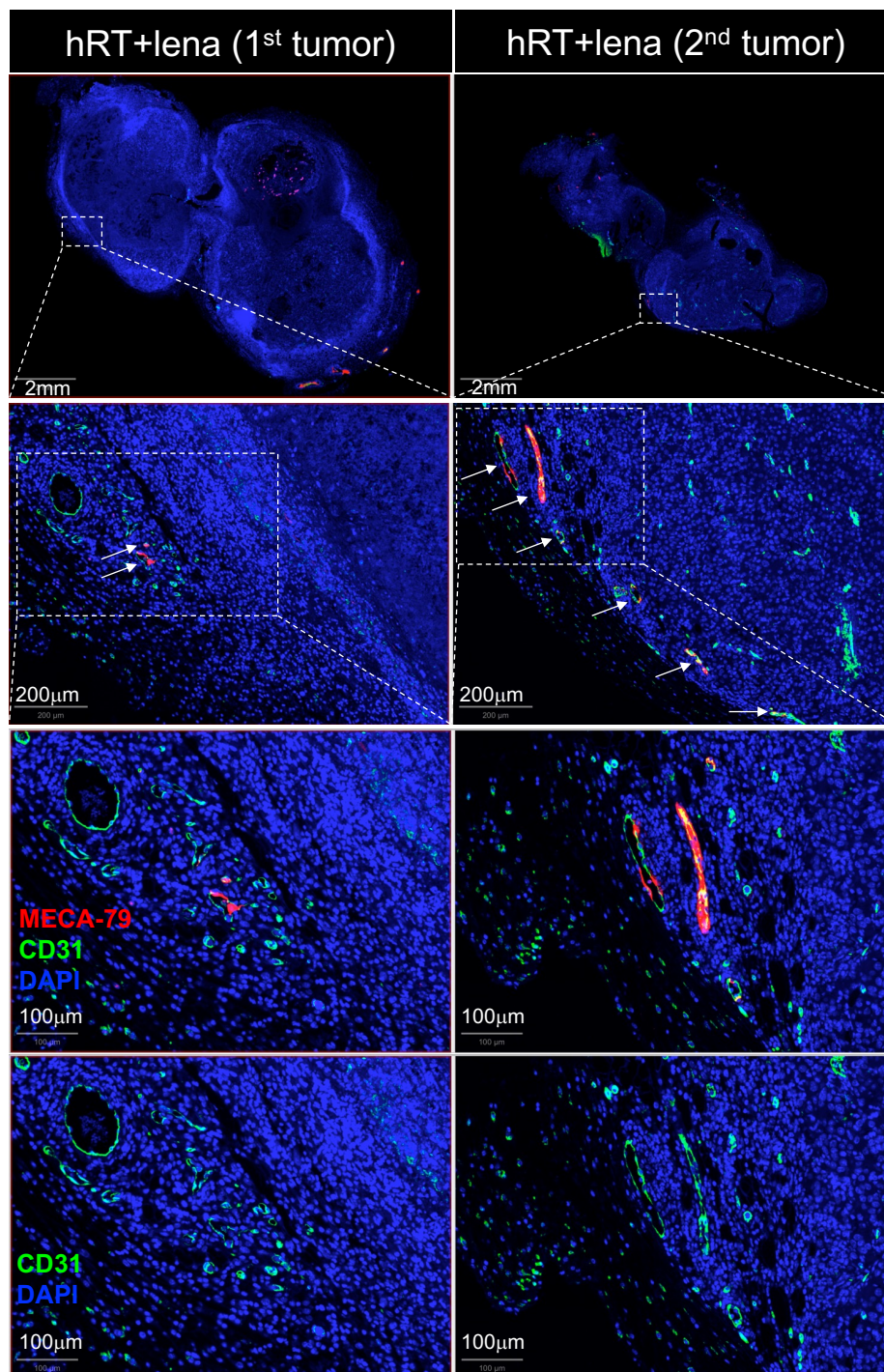
Supplementary figure 4. Adding lena to hRT increases the number of stem- and effector-like exhausted TILs. A, Gating strategy to characterize exhausted subsets of tumor-specific T cells. **B-C,** Cell number of stem-like (TCF1⁺TIM3⁻PD1⁺), transitory (CD101⁻TCF1⁻TIM3⁺PD1⁺), and terminally exhausted (CD101⁺TCF1⁻TIM3⁺PD1⁺) tetramer⁺ CD8⁺ T cells in primary and secondary tumors of MC38 (**B**) and B16-OVA (**C**) tumor-bearing mice. Data are presented as mean with SEM and were collected from 2 independent experiments. P values (ns, not significant; * P < 0.05; ** P < 0.01) were determined by unpaired two-tailed Student's t-test.

Supplementary figure 5



Supplementary figure 5. Adding lena to hRT increases the numbers of CD8⁺ cells with memory phenotype in secondary tumor. A, Gating strategy. B-C, Numbers of M8-tet⁺ cells with memory phenotype (CD44⁺KLRG1⁻CD127⁺CD62L⁺) in primary and secondary tumors (B), TDLNs, and spleen (C) at day 8 after treatment start (n=7 mice per group). Data are presented as mean with SEM and were collected from 3 independent experiments. P values are indicated in the figure and were determined by unpaired two-tailed Student's t-test.

Supplementary figure 6



Supplementary figure 6. HEVs in tumors of hRT/lana-treated mice. Representative multiplex immunohistochemistry images showing CD31 (green), MECA-79 (red), DAPI (blue) in primary (left) and secondary (right) tumor of hRT/lana-treated mice at day 8 after treatment start. Arrows indicate MECA-79⁺ vessels (TA-HEVs).