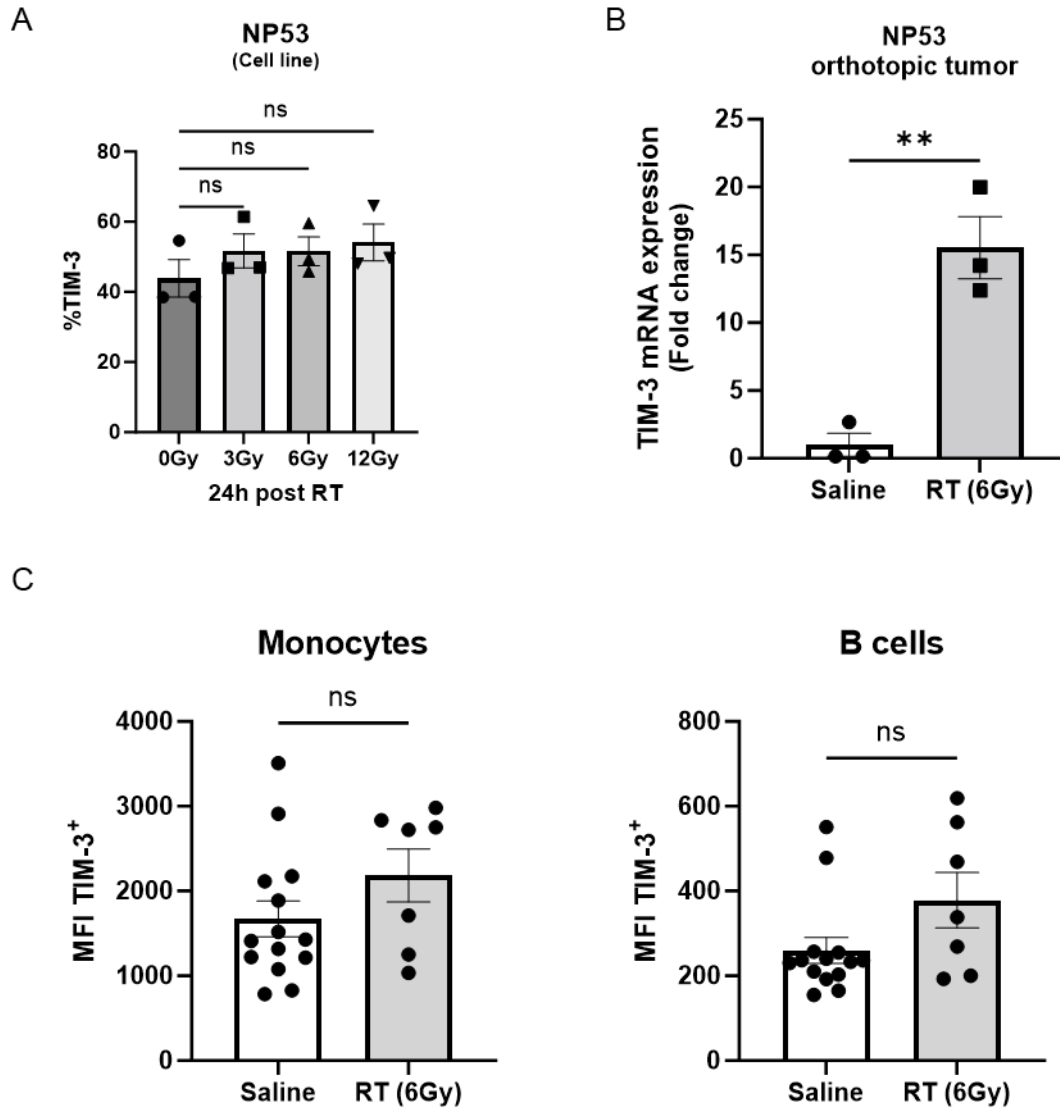
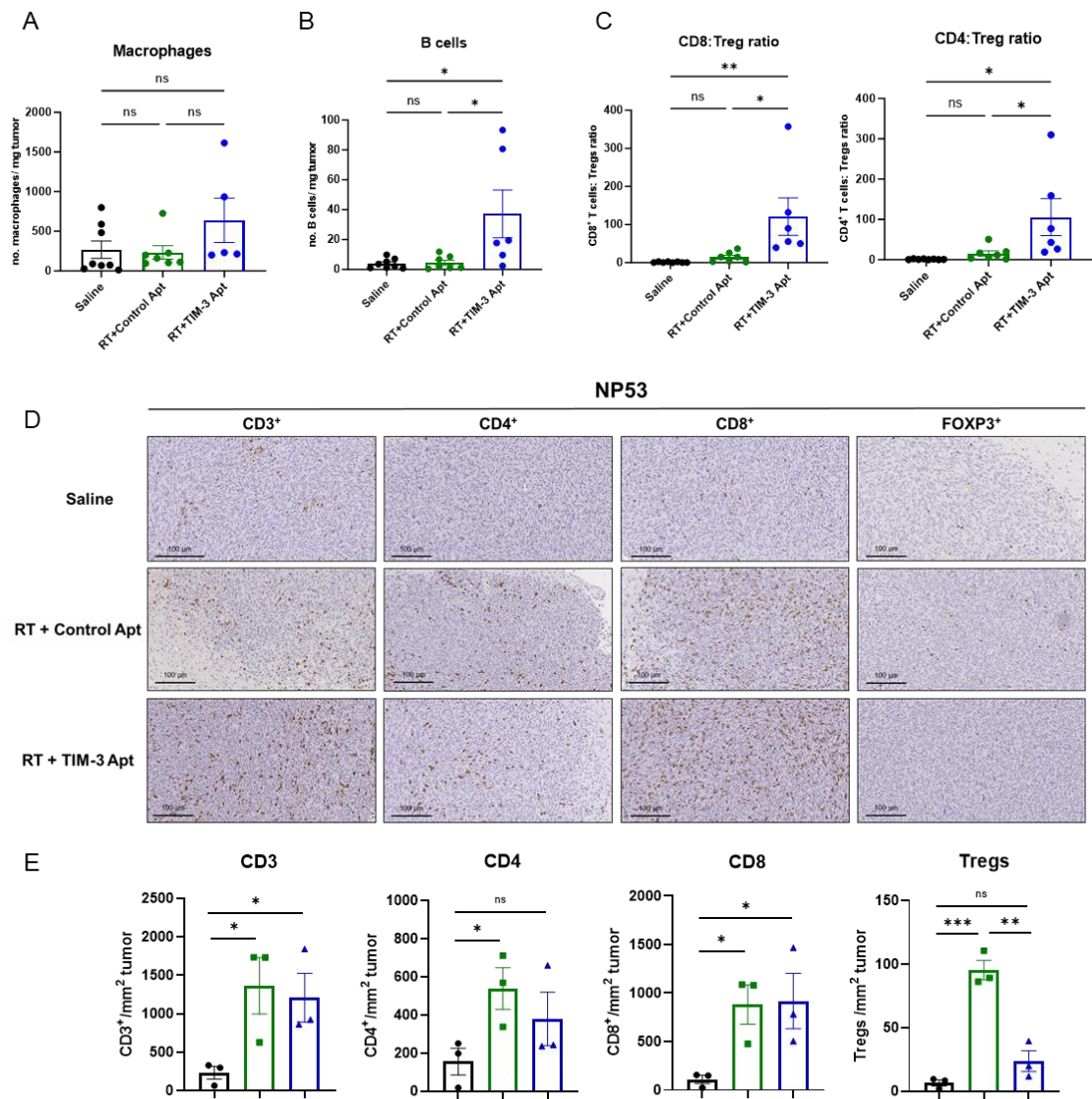


## Supplemental data

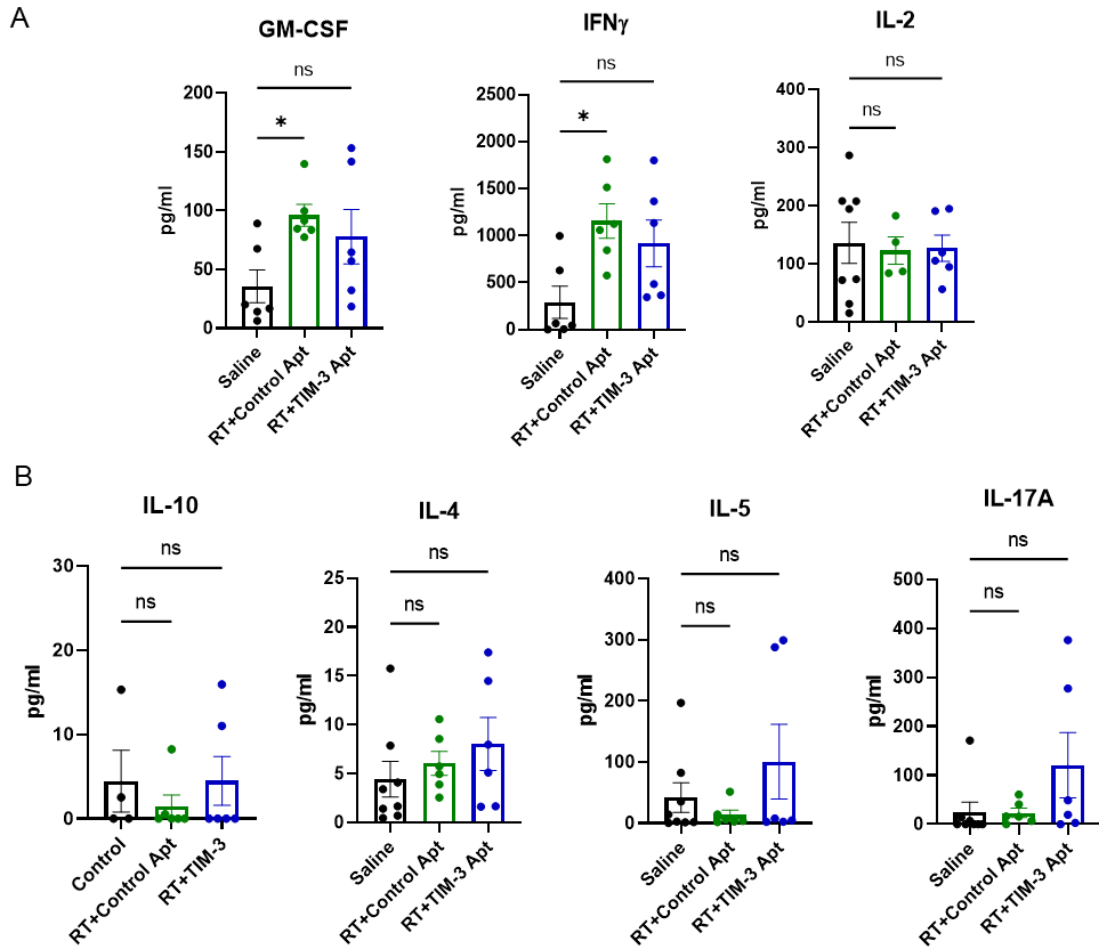


**Figure S1: Evaluation of TIM-3 expression after radiotherapy in DMG models. (A)** Flow cytometry analysis of TIM-3 expression in NP53 tumor cells 24h post 3, 6, and 12Gy of radiotherapy. **(B)** Real-time-PCR analysis of TIM-3 expression in mice bearing NP53 tumor cells comparing saline and radiotherapy groups. **(C)** Flow cytometry analysis of TIM-3 expression MFI on monocytes and B cells after radiotherapy (6Gy) in a DMG orthotopic model. Data were analyzed with One-way ANOVA (A) and student t-test (B-C) Bar graphs indicate the mean  $\pm$  SEM (ns,  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ ).



**Figure S2: Characterization of inflammatory response produced by radiotherapy and TIM-3 blockade.** NP53 cells were engrafted and animals were treated with saline (n=8), radiotherapy (6Gy) control Apt (n=7), or radiotherapy and TIM-3 (n=6). Animals were sacrificed 15 days after the tumor implantation and 8 days after radiotherapy. **(A-B)** Flow cytometry analyses of **(A)** macrophages and **(B)** B cells per mg of tumor on the indicated groups. **(C)** Analyses of the CD8: Treg and CD4: Treg pro-inflammatory ratios in the tumor microenvironment of NP53 mice treated with radiotherapy and TIM-3 Apt. **(D)** CD3, CD4, and CD8 immune-staining representative images from NP53 tumors 15

days after the tumor implantation on indicated treatments. Scale bars: 100 $\mu$ m. **(E)** CD3<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>+</sup> and Tregs (FOXP3<sup>+</sup>) cell infiltration per mm<sup>2</sup> of NP53 tumors. Graph showing the quantification of positive cells infiltrating the brain after the indicated treatments per mm<sup>2</sup> (Saline, RT+Control apt, and RT+TIM-3 Apt; n=3-4). Data were analyzed with One-way ANOVA. Bar graphs indicate the mean  $\pm$  SEM (ns, P>0.05; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; \*\*\*\*P<0.0001).



**Figure S3: Characterization of inflammatory chemokines and cytokines produced by radiotherapy and TIM-3 blockade.** (A) MACSplex analysis in serum of immune innate and Th1 cytokines 15 days after tumor implantation between saline, RT+Control apt, and RT+TIM-3 Apt groups. (B) MACSplex analysis in serum of Th2 and Th17 cytokines 15 days after tumor implantation between saline, RT+Control apt, and RT+TIM-3 Apt groups. Data were analyzed with One-way ANOVA. Bar graphs indicate the mean  $\pm$  SEM (ns,  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ ).