

## Figure S1: Radiation therapy is insufficient to optimally prime robust T cell responses in PDAC

(A) IHC analysis of CK19<sup>+</sup> tumor cell number in surgical resection samples from patients who received neoadjuvant Chemo/SBRT, Chemo/RT, or no neoadjuvant therapy. n = 8-32 patients/group. (B) Schematic of SARRP machine with CT-guided multi-beam radiation planning utilized for precise RT targeting. (C) Representative US images from KPC vehicle or RT-treated mice at days 0, 7, and 14 for tumor growth kinetics in Fig. 1D. (D) Representative gross tissue images and day 7 pancreas weight from KPC vehicle or RT-treated mice from Fig. 1C. n = at least 7 mice/group. (E) Representative gross tissue and hematoxylin and eosin (H&E) images from KPC vehicle or RT-treated mice at day 14. (F) Representative Ki67 and CC3 IHC images from Fig. **1H**. **(G)** Flow cytometry quantification of total CD4<sup>+</sup> T effector and CD4<sup>+</sup> Foxp3<sup>+</sup> T regulatory cells in KPC mice at days 7 and 14. Flow cytometry quantification of total CD8<sup>+</sup> T cells and percentage of Ki67<sup>+</sup> or CD44<sup>+</sup> CD8<sup>+</sup> T cells out of total CD8<sup>+</sup> T cells in KPC mice at day 7. n = at least 7 mice/group. (H) Flow cytometry quantification of various myeloid infiltrates: (i) monocytes (CD45<sup>+</sup> CD3<sup>-</sup> CD19<sup>-</sup> Ly6C<sup>+</sup>), (ii) TAMs (CD45<sup>+</sup> CD3<sup>-</sup> CD19<sup>-</sup> Ly6C<sup>-</sup> Ly6G<sup>-</sup> CD11b<sup>+</sup> F4/80<sup>+</sup> MHC-II<sup>+</sup>), (iii) granulocytes (CD45<sup>+</sup> CD3<sup>-</sup> CD19<sup>-</sup> Ly6C<sup>-</sup> Ly6G<sup>+</sup>), and (iv) eosinophils (CD45<sup>+</sup> CD3<sup>-</sup> CD19<sup>-</sup> Ly6C<sup>-</sup> Ly6G<sup>-</sup> CD11b<sup>+</sup> F4/80<sup>-</sup> MHC-II<sup>-</sup>) in KPC vehicle or RT-treated mice at days 7 and 14. n = at least 7 mice/group. (I) Frequency distribution of CD8<sup>+</sup> T cell proximity to nearest CK19<sup>+</sup> tumor cell in KPC-OG mice treated as indicated. n = at least 7 mice/group. (J) IHC analysis of Ki67 on tissues from KPS-OG mice at day 14 post-RT. n = at least 7 mice/group. (K) Representative CD8 $\alpha$ /CK19 IHC in KPC-OG mice and CD8 $\alpha$  IHC in KPS-OG mice from Fig. 1J-K. (L) Analysis of collagen density in KPC-OG and KPS-OG vehicle mice in Fig. **1J-K**. n = at least 7 mice/group.

All graphs depict mean +/- SEM. For comparisons between two groups, "\*" denotes p < 0.05 by two-tailed t-test. "ns" denotes not significant.