Supplementary Figure S2



## Supplementary Fig. S2. Autophagy inhibition in cancer cells triggers T-cell-mediated immune response

(A–C) Western blotting was used to confirm knockdown efficiency of autophagy-related genes using shRNA transduction. Two types of shRNA targeting ATG7 (A), three types of shRNA targeting ATG5 (B), and three types of shRNA targeting ATG4B (C) were transduced into KPC1 and KPC2 cells.

(**D**) Results of a 24-hour proliferation assay, comparing the proliferation of KPC shNC cells with that of KPC shATG5 or shATG7 cells (KPC1, KPC2). All luminescence values at 24 hours were normalized to those at 0 hours. The shATG5 and shATG7 experiments were performed simultaneously and the same shNC values were used in the shNC/shATG5 and shNC/shATG7 comparisons.

(E–G) KPC1 shNC/shATG7 cells were subcutaneously implanted into BALB/c-nu mice (E) and C57BL/6 mice (F) to compare tumor growth. Tumor sections were then subjected to immunohistochemical (IHC) staining of CD8+ T cells (CD8a) and DCs (CD11c) (G). Representative IHC images are shown.

(H, I) Depletion of CD8<sup>+</sup> T cell was performed by anti-CD8a injection to assess the dependency of CD8<sup>+</sup> T cells in syngeneic subcutaneous PDAC tumors (KPC1 shNC and KPC1 shATG7). Flow cytometry of tumors and spleens in mice treated with anti-CD8a was performed to confirm the depletion of CD8<sup>+</sup> T cells (H). Tumor growth was compared among KPC1 shNC and KPC1 shATG7 treated with IgG/anti-CD8a (I).

Scale bars, 100  $\mu$ m (G). Bars, median; Error bars, mean  $\pm$  SD; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*p < 0.001; ns, not significant; analyzed using the one-way ANOVA (D, I) and Student's t-test (E–G).