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**Supplemental Information**

**Combination PD-1 and PD-L1 Blockade Promotes  
Durable Neoantigen-Specific T Cell-Mediated  
Immunity in Pancreatic Ductal Adenocarcinoma**

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**Supplementary Table 1.** MHC class I binding scores and estimated affinity of candidate CB peptides, related to Figure 3.

Peptide	H-2	Sequence	BIMAS <sup>1</sup> Score (Rank)	SYFPEITHI <sup>2</sup> Score (Rank)	NETMHC 4.0 <sup>3</sup> Affinity <sup>4</sup> (Rank)	NETMHC 4.0 <sup>3</sup> Percent rank <sup>5</sup>
CB <sub>101-109</sub>	D <sup>b</sup>	VAPVNESYI	1020 (1)	32 (1)	3.6 (1)	0.01
CB <sub>173-181</sub>	D <sup>b</sup>	GNIANFKPL	720 (2)	23 (3)	663 (3)	0.30
CB <sub>325-333</sub>	D <sup>b</sup>	AKRLNLPGI	79 (3)	23 (4)	31177 (80)	15.0
CB <sub>280-289</sub>	D <sup>b</sup>	RSVINVPSV	39 (4)	20 (8)	105 (2)	0.07
CB <sub>396-405</sub>	D <sup>b</sup>	KGYYNNVEA	16 (7)	18 (13)	2216 (8)	0.70
Ova <sub>257-264</sub>	K <sup>b</sup>	SIINFEKL	17.4 (1)	25 (1)	19.4 (1)	0.06
GFP <sub>118-126</sub>	D <sup>b</sup>	DTLVNRIEL	720 (1)	26 (1)	1472 (1)	0.50

1 [https://www-bimas.cit.nih.gov/molbio/hla\\_bind/](https://www-bimas.cit.nih.gov/molbio/hla_bind/)

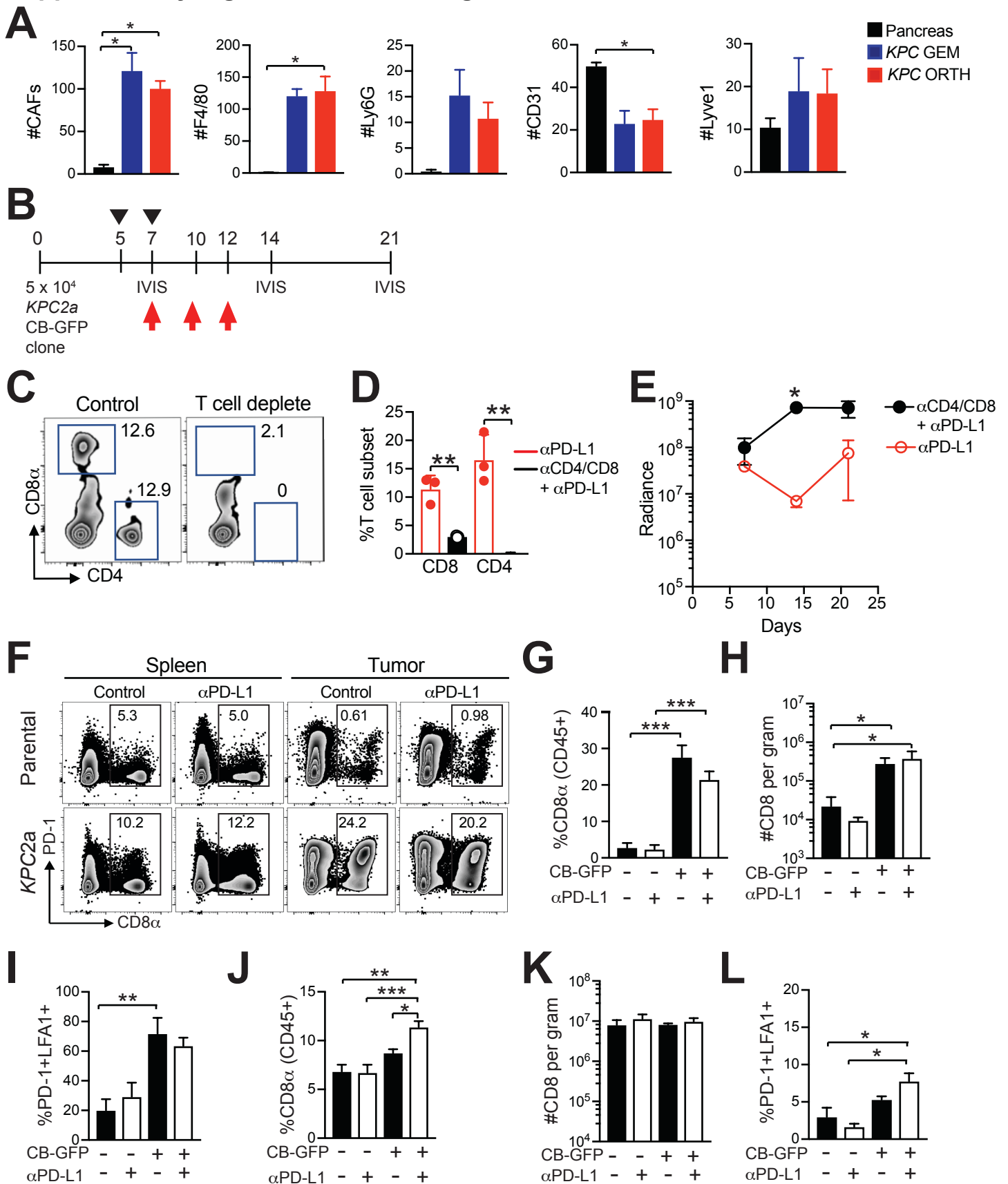
2 <http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm>

3 <http://www.cbs.dtu.dk/services/NetMHC/>

4 Predicted binding affinity in nanomolar (nM) units

5 Rank of predicted affinity compared a set of 400,000 random natural peptides. Stronger binders are defined as having a %rank of <0.5, and weak binders with %rank of <2.

## Supplementary Figure 1, related to Figure 1

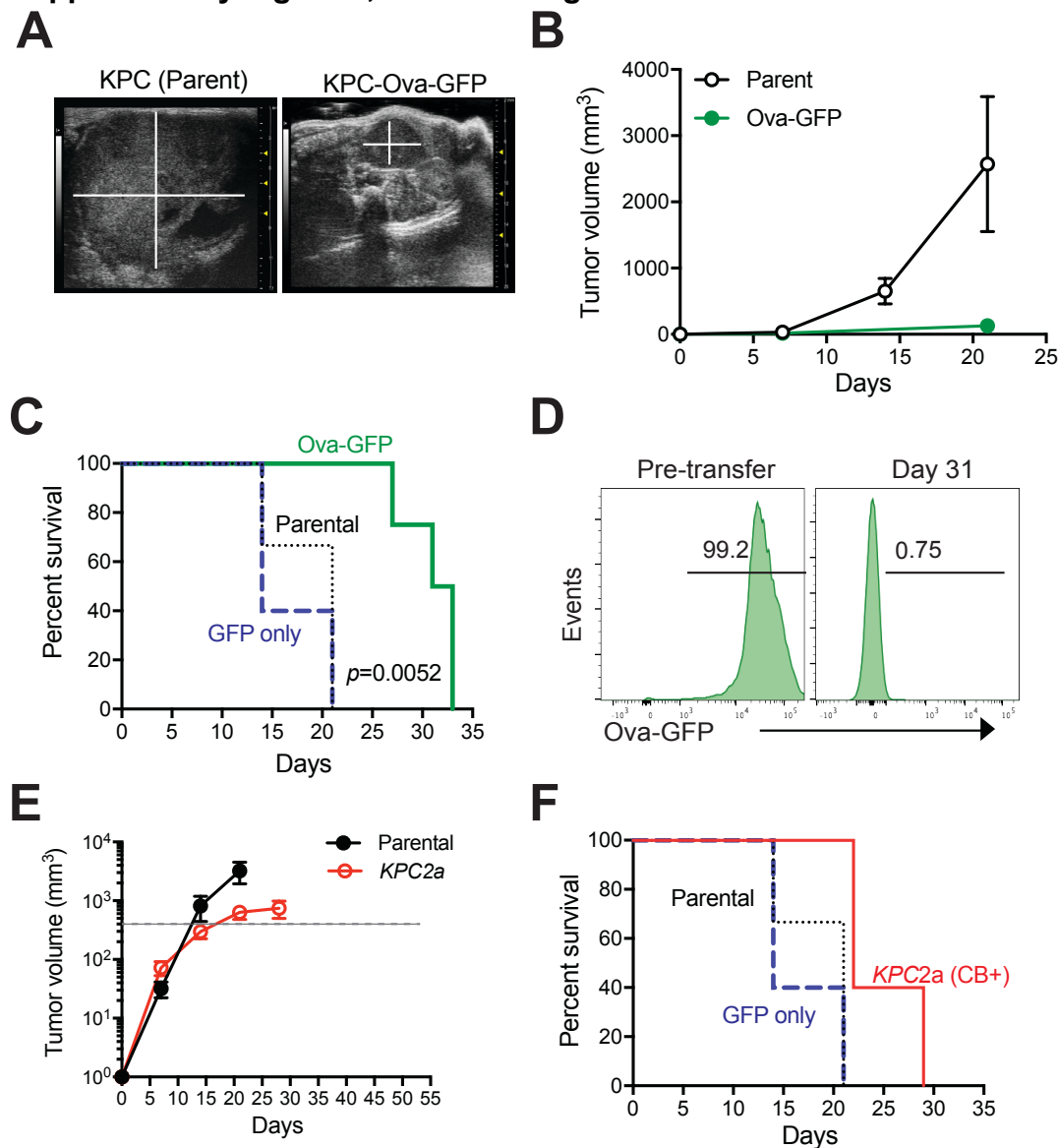


### Supplementary Figure 1, related to Figure 1. T cells are required for antitumor efficacy of PD-L1 blockade.

A) Number of immune cells per field of view in normal mouse pancreas, tumors that arise in the *KPC* genetically engineered mouse model (*KPC GEM*) and orthotopic *KPC* tumors (*KPC ORTH*). Data are mean  $\pm$  SEM. \*,  $p < 0.05$  (unpaired two-tailed unpaired students' T test).

- B)** Schematic of experimental set up. C57Bl/6 mice were surgically implanted with 20  $\mu$ l containing  $5 \times 10^4$  KPC2a cells in 60% matrigel into the pancreas. On days 5 and 7, mice received 200  $\mu$ g of anti-CD4 (GK1.5) and anti-CD8 (53-6.7) IP. Black arrowheads, T cell depleting antibodies. Red arrows, anti-PD-L1 (10F.9G2, 200  $\mu$ g IP).
- C)** Representative FACs of circulating CD4<sup>+</sup> and CD8<sup>+</sup> T cells following injection of T cell depletion antibodies on day 10.
- D)** Circulating T cell frequency in anti-CD4 + anti-CD8 treated mice on day 10 post tumor implantation. Data are mean  $\pm$  SEM and each dot is an independent mouse. \*\*,  $p < 0.005$  (unpaired two-tailed unpaired students' T test).
- E)** Radiance of orthotopic tumor growth. Data are mean  $\pm$  SEM. \*,  $p < 0.05$  (unpaired two-tailed unpaired students' T test).
- F)** Representative FACs of spleen and tumors at 3 weeks gated on live, CD45<sup>+</sup> cells.
- G)** Proportion of intratumoral CD8<sup>+</sup> T cells of total CD45<sup>+</sup> cells at 3 weeks. Data are mean  $\pm$  SEM. \*\*\*,  $p < 0.0005$  (unpaired two-tailed unpaired students' T test).
- H)** CD8<sup>+</sup> T cell number per gram tumor at 3 weeks. Data are mean  $\pm$  SEM. \*,  $p < 0.05$  (unpaired two-tailed unpaired students' T test).
- I)** Proportion of intratumoral CD8<sup>+</sup> T cells that co-express PD-1 and LFA-1 at 3 weeks. Data are mean  $\pm$  SEM. \*\*,  $p < 0.005$  (unpaired two-tailed unpaired students' T test).
- J)** Proportion of splenic CD8<sup>+</sup> T cells (of CD45<sup>+</sup> cells) at 3 weeks. Data are mean  $\pm$  SEM. \*,  $p < 0.05$ ; \*\*,  $p < 0.005$ ; \*\*\*,  $p < 0.0005$  (unpaired two-tailed unpaired students' T test).
- K)** CD8<sup>+</sup> T cell number per gram spleen at 3 weeks. Data are mean  $\pm$  SEM.
- L)** Proportion of splenic CD8<sup>+</sup> T cells that co-express PD-1 and LFA-1 at 3 weeks. Data are mean  $\pm$  SEM. \*,  $p < 0.05$  (unpaired two-tailed unpaired students' T test). All panels are from 3-5 mice per group.

Supplementary Figure 2, related to Figure 3



**Supplementary Figure 2, related to Figure 3. Ova expression in *KPC* cells drives immune editing and antigen loss variants independent of immunotherapy.**

**A)** Representative ultrasound of orthotopic tumor growth at day 19 (parent) and day 21 for *KPC2*-OVA-eGFP clone.

**B)** Tumor volume of parent (control *KPC2*) and *KPC2*-OVA-eGFP in normal B6 mice.

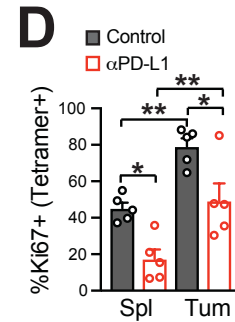
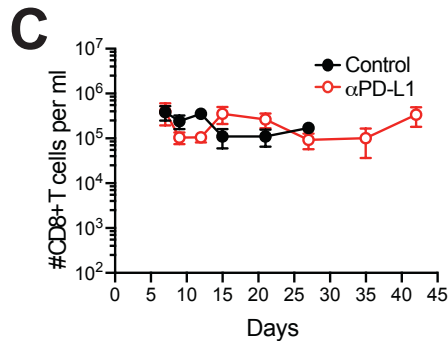
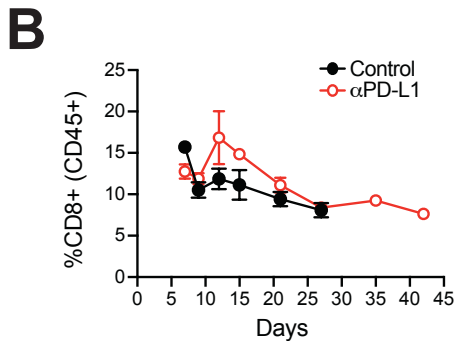
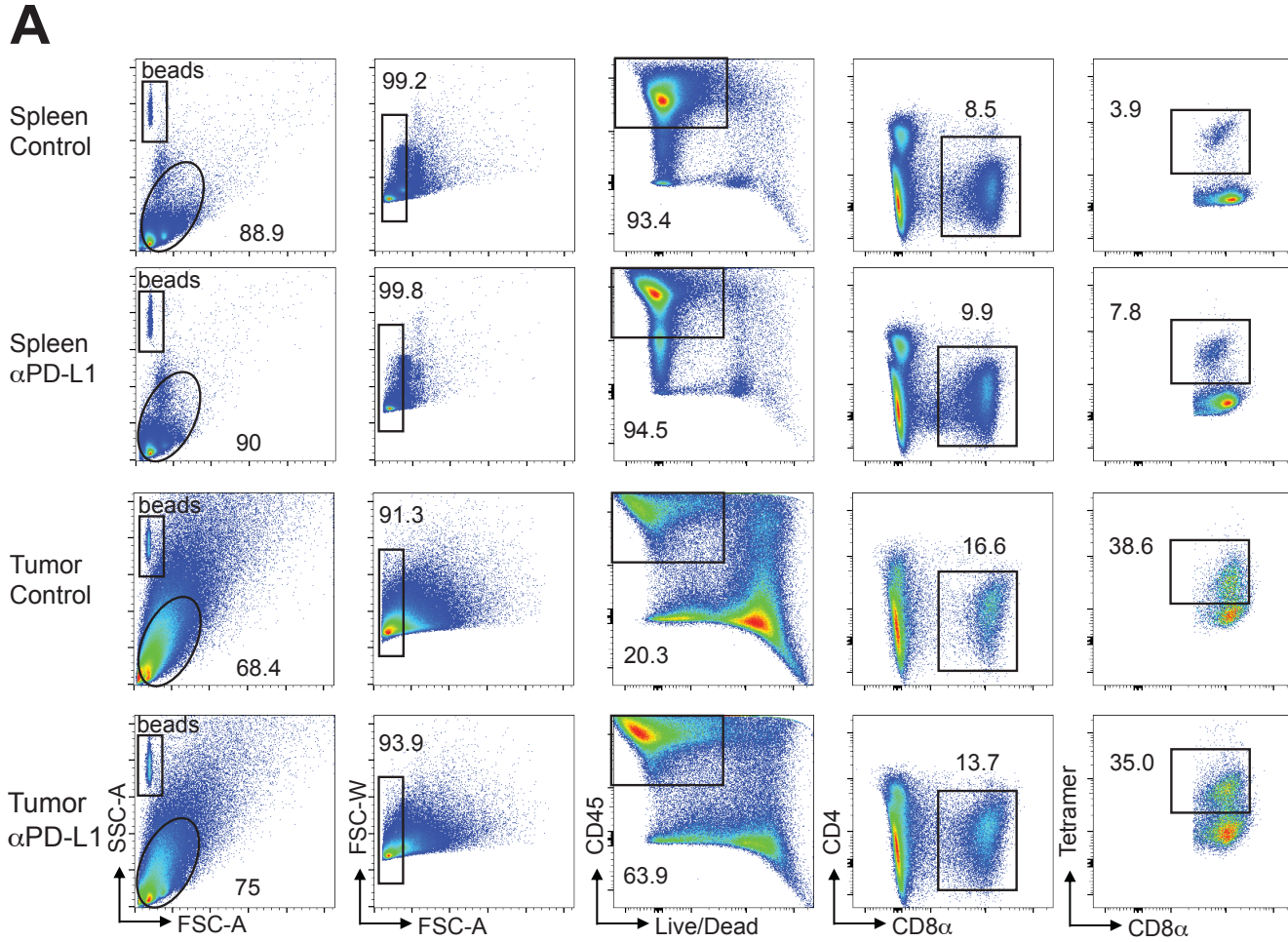
**C)** Kaplan-Meier survival curve of B6 mice orthotopically implanted with parental *KPC2*, *KPC2* expressing eGFP only, or the *KPC2*-OVA-eGFP clone. Significance was determined by a Mantel Cox Log-rank test ( $p=0.0052$ ) comparing Ova-eGFP+ cells to GFP+ cells.

**D)** Histograms of eGFP expression of *KPC*-Ova-GFP clones following single cell sort and prior to orthotopic injection (pre-transfer) and isolated following 31 days of orthotopic growth in syngeneic B6 mice.

**E)** Tumor volume in parental and *KPC2a* clone that expresses CB-GFP. Dashed bar represents required euthanasia criteria and data are mean  $\pm$  S.E.M.

**F)** Kaplan-Meier survival curve of B6 mice orthotopically injected with parental *KPC2*, *KPC2* expressing GFP only, or the *KPC2a* (CB-eGFP+) clone in untreated mice. All data are representative of  $n=3-5$  mice per group.

## Supplementary Figure 3, related to Figure 4



### Supplementary Figure 3, related to Figure 4. Validation of the CB<sub>101-109</sub>:H-2D<sup>b</sup> tetramer in PDA.

A) C57Bl/6J mice were surgically implanted into the pancreas with 20  $\mu$ l containing  $5 \times 10^4$  KPC2a cells in 60% matrigel. On days 7, 10 and 12, mice received 200  $\mu$ g of anti-PD-L1 (10F.9G2) IP. Flow cytometric analysis of tetramer+ T cells in spleen and tumors was determined at day 22 post tumor implantation. Lymphocytes are gated as follows: forward scatter (FSC) by side scatter (SSC), single cells, live CD45+ cells, CD8 $\alpha$ +CD4- cells. In some experiments, a dump gate (CD11b+, CD4+, CD19+ cells) was used instead of the CD4+ gate shown to remove non-CD8+ T cells. Cell counting beads (left plots) were added to quantify cell number per gram of tissue.

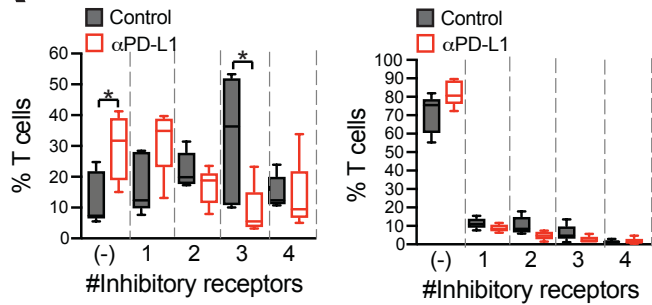
B) Proportion of CD8+ T cells (gated on live, CD45+ cells) in blood of B6 mice bearing CB+ KPC2a tumors  $\pm$  200  $\mu$ g of anti-PD-L1 (10F.9G2) IP on days 7, 10 and 12. Data are mean  $\pm$  SEM. n=3-6 mice per timepoint.

C) Circulating CD8+ T cell number from data Figure S3B. Data are mean  $\pm$  SEM. n=3-6 mice per timepoint.

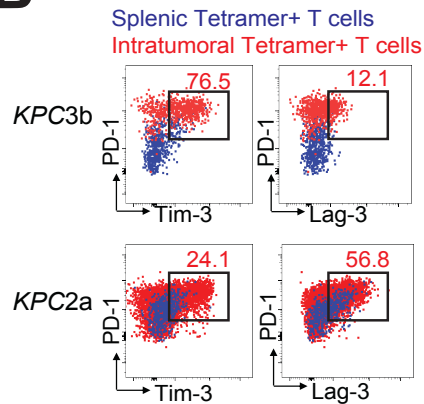
D) Ki67 expression by CB<sub>101-109</sub>:H-2D<sup>b</sup>-specific T cells in spleen (Spl) and tumors (Tum) from mice bearing CB+ KPC2a tumors  $\pm$  200  $\mu$ g of anti-PD-L1 (10F.9G2) IP on days 7, 10 and 12 and analyzed on day 22. Each dot represents an independent mouse. \*,  $p < 0.05$ ; \*\*,  $p < 0.005$ . Data are mean  $\pm$  SEM. Statistical significance was determined using a one-way Anova with a Tukey post-test to correct for multiple comparisons. All data are representative of n=3-6 mice per group.

Supplementary Figure 4, related to Figure 5

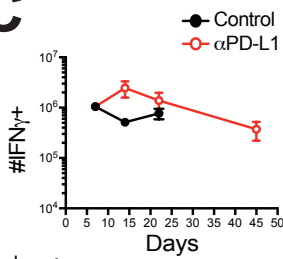
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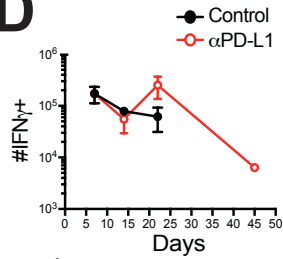
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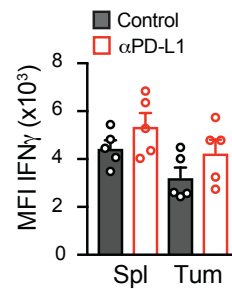
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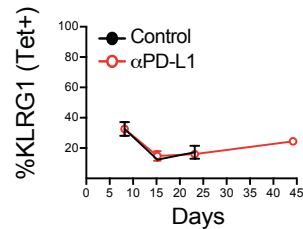
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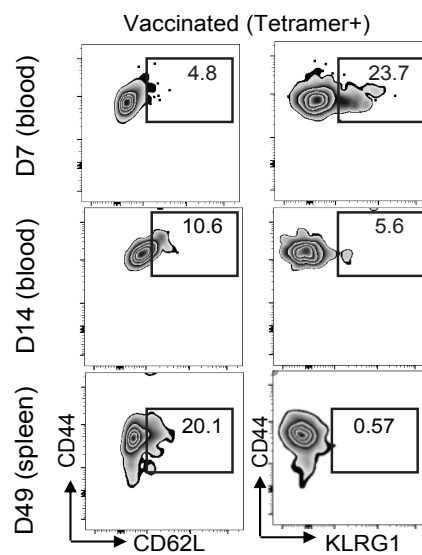
**E**



**F**



**G**

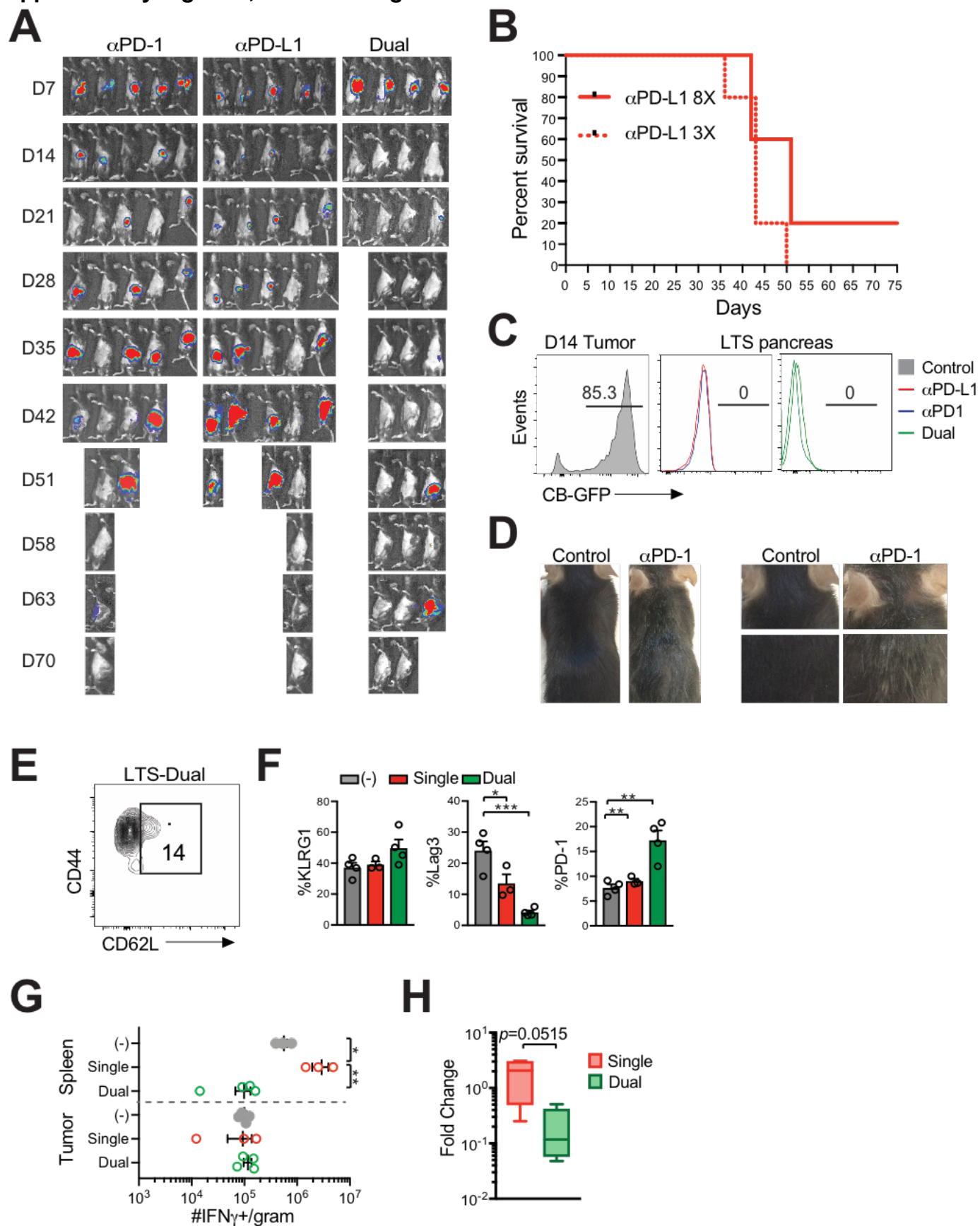


Supplementary Figure 4, related to Figure 5. Phenotype and function of PDA-specific T cells.

- A)** Proportion of CB<sub>101-109</sub>:H-2D<sup>b</sup>-specific T cells in PDA (left) and spleen (right) that expressed none (-), 1 or co-expressed multiple (2, 3, or 4) inhibitory receptors at day 22. Data are mean ± SEM. \*,  $p < 0.05$  (unpaired two-tailed students' T test). n=4-5 mice per group.
- B)** Representative plots of CB<sub>101-109</sub>:H-2D<sup>b</sup>-specific T cells in two distinct orthotopic tumors at day 22 post implantation. C57Bl/6 mice were surgically implanted into the pancreas with 20 μl containing  $5 \times 10^4$  *KPC3b* (top) or *KPC2a* (bottom) cells in 60% matrigel. FACs of tetramer+ T cells in spleen and PDA was determined at day 22 post tumor implantation. Lymphocytes are gated as follows: forward scatter (FSC) by side scatter (SSC), single cells, live CD45+ cells, CD8<sup>+</sup>CD4-Tetramer+ cells.
- C)** Number of marker+tetramer+ T cells normalized per gram spleen. Data are mean ± SEM.
- D)** Number of marker+tetramer+ T cells normalized per gram tumor. Data are mean ± SEM.
- E)** Mean fluorescence intensity (MFI) of IFN $\gamma$  expression by CB<sub>101-109</sub>:H-2D<sup>b</sup>-specific T cells at day 22 was determined by flow cytometry. Each dot is an independent mouse. Data are mean ± SEM.
- F)** Proportion of intratumoral tetramer+ T cells that express KLRG1. Data are mean ± SEM.
- G)** Representative FACs plots of tetramer+ T cells from vaccinated with a CB peptide + anti-CD40 + PolyIC as described in methods. Numbers in plots are frequency of CB<sub>101-109</sub>:H-2D<sup>b</sup>-specific T cells that co-express the indicated antigens. All data are representative of n=3-5 mice per group.



Supplementary Figure 5, related to Figure 6



Supplementary Figure 5, related to Figure 6. Impact of long-term blockade of the PD-1 and/or PD-L1 pathway in PDA.

A) Raw images of bioluminescent imaging of the treated cohorts at the indicated days (D) post-tumor implantation. Images are from 1 experiment. Experiment was performed 2 independent times.

- B)** Overall survival in mice with orthotopic *KPC2a* tumors treated with 3 doses (3X) or 8 doses (8X) of anti-PD-L1.
- C)** GFP expression gated on CD45<sup>-</sup> mononuclear cells isolated from orthotopic pancreatic tumors at day 14 (control) or pancreas from LTS mice (> day 70) treated with either dual or single PD-1/PD-L1 blockade.
- D)** Mild vitiligo in mice treated with 8X anti-PD-1 on day 45.
- E)** Phenotype of circulating tetramer<sup>+</sup> T cells from a LTS treated with dual PD-1+PD-L1 blockade. FACs plots are gated on live, CD45<sup>+</sup>CD8<sup>+</sup>Tetramer<sup>+</sup> T cells.
- F)** Proportion of splenic tetramer<sup>+</sup> T cells that express KLRG1 and/or Lag3 at day 14. Single, anti-PD-L1 only.
- G)** Number of CD8<sup>+</sup> T cells per gram that produce IFN $\gamma$  from mice treated with single or dual therapy at day 14. Each dot is an independent mouse. Data are mean  $\pm$  SEM. \*,  $p < 0.05$ , \*\* $p < 0.005$  (one-way Anova with a Tukey post-hoc test).
- H)** Fold change in pancreas tumor radiance from day 14 (prior to therapy) and day 21 (7 days post therapy) in mice treated with PD-L1 (single) or dual (PD-1+PD-L1) blockade. Data are mean  $\pm$  SEM. n=4 mice per group (unpaired two-tailed Student's t test). All data are representative of 3-8 mice per group.