**Site-dependent immune escape due to impaired dendritic cell cross-priming**

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**Supplementary Figure S1.** **Characterization of Ova-expressing PDAC.** (**A**) 4662 cells, clones derived from 4662 (C9, C10), or clones expressing full-length Ova fused to Td-tomato (V6.Ova, G10.Ova) were analyzed by flow cytometry for expression of Td-tomato, SIINFEKL bound to H-2Kb (H-2Kb-SIIN), and MHC class I molecules (H-2Kb, H-2Db) at baseline or following IFN treatment for 48 hrs. (**B**) Growth kinetics *in vitro* of 4662 and V6.Ova cells. Plots represent mean cell number (± S.D.) for triplicate samples. (**C**) *In vivo* growth of 4662 and V6.Ova cells transplanted s.c. at 1.25x105 cells/mouse into *Rag2-/-* hosts. Mean tumor diameters (± S.E.M.) for n=4 mice/group are shown. (**D**) Expression of Td-tomato and H-2Kb-SIIN in untreated or IFN-treated 4662, V6.Ova, or long-term (>2 months) *in vitro* passaged V6.Ova. (**E**) Representative H&E-stained sections from 4662 and V6.Ova tumors grown s.c. in *Rag2-/-* mice. (**F**) *In vivo* growth of C9, C10, and G10.Ova tumor cells in WT mice following s.c., orth., or i.p. injection at 1.25x105 cells/mouse (C9, C10) or 1x106 cells/mouse (G10.Ova). Survival curves include n=5-13 mice/group.

**Supplementary Figure S2.** **Ova tolerance abrogates rejection of orth. PDAC.Ova tumors and the development of lung metastases after i.v. challenge.** (**A**) Schema for induction of Ova tolerance by oral gavage with Ova or PBS weekly for 3 doses followed one week later by PDAC.Ova injection. (**B**-**C**) Mice treated as in A were challenged with 1.25x105 PDAC.Ova tumor cells by orthotopic injection and tumor growth followed by ultrasound. Tumor volumes in individual mice for n=6-7 mice/group are shown in B, and the corresponding survival curves in C. \*\*, p<0.01 by log-rank test. (**D**) Representative H&E stained lung tissue from control mice, WT mice injected i.v. with PDAC (day 40) or PDAC.Ova (day 90), and Act-mOVA mice injected i.v. with PDAC.Ova (day 25), shown at 4X magnification. (**E**) Survival curves following PDAC.Ova i.v. injection into WT mice (n=2, 5x105 cells/mouse) or Act-mOVA mice (n=4, 1.25x105 cells/mouse).

**Supplementary Figure S3. TIL analysis of s.c. and i.p. PDAC and PDAC.Ova tumors.** (**A**-**D**) Cohorts of WT, Act-mOVA, *Batf3-/-*, *CD40-/-*, *CXCR3-/-*, WT mice treated with serial OVA oral gavage, or WT mice treated with depleting anti-NK1.1 (n=4-11 mice/group) were injected s.c. with 1x106 PDAC.Ova or PDAC cells and tumors were harvested at day 9 for flow cytometric analysis. Tumor weights are shown in A, and quantitation of the specified immune populations in B-D. (**E**-**G**) WT mice (n=5-8 mice/group) were injected i.p. with 1x106 PDAC or PDAC.Ova cells and late tumors were harvested for analysis. Tumor weights are shown in E, cell density of the indicated immune populations in F, and quantitation of additional immune subsets in G. Group means are indicated, and statistical significance compared to PDAC tumors is designated by asterisks where appropriate. \*\*\*\*, p<0.0001; \*\*\*, p<0.001; \*\*, p<0.01; \*, p<0.05, by one-way ANOVA or unpaired t test; N.T., not tested. Data reflect at least 2 independent experiments.

**Supplementary Figure S4. TIL analysis of late orth. PDAC and PDAC.Ova tumors.** (**A**-**C**)Cohorts of WT, Act-mOVA, *Batf3-/-*, *CD40-/-*, and WT mice pretreated with serial OVA oral gavage (n=2-7 mice/group) were injected orthotopically with PDAC.Ova or PDAC cells and late tumors were harvested for flow cytometric analysis. Cell density is shown in A, Ova tet+ T cells in the spleen or tumor in B, and quantitation of the indicated immune populations in C. (**D**-**F**) Immunophenotyping of T cells isolated from s.c. (D) or orth. (E-F) tumors for the indicated markers. Group means are denoted, error bars in F reflect S.E.M, and statistical significance compared to PDAC tumors in WT mice is designated by asterisks where appropriate. \*\*\*\*, p<0.0001; \*\*, p<0.01; \*, p<0.05, by one-way ANOVA. Data include at least 2 independent experiments.

**Supplementary Figure S5.** **Expression of maturation markers in tumor-associated cDC1s.** (**A**-**B**) Tumors and tumor-draining lymph nodes (LNs) were harvested from WT mice following implantation with PDAC or PDAC.Ova tumor cells via the specified route. Expression of the indicated immune markers in cDC1s are shown for n= 2-4 mice/group. Plots represent median MFI of staining minus isotype control, with gating strategy for cDC1s as described in Methods. Panels A and B represent independent experiments and MFIs are not directly comparable. Naïve LN in B included mLN and ppLN. Spl, spleen; iLN, inguinal LN, mLN, mesenteric LN, ppLN, peripancreatic LN.

**Supplementary Figure S6. Differential requirement for CD4+ cells in the rejection of s.c. vs. orthotopic PDAC.Ova tumors.** WT mice were injected s.c. (**A**-**B**) or orthotopically (**C**-**D**) with 1.25x105 PDAC.Ova tumor cells and treated with ctrl Ig, anti-CD4, or anti-CD8 (n=8-10 mice/group). Tumor growth and survival were monitored. Mean tumor diameters or tumor volumes from individual mice by ultrasound are shown in A and C, with the corresponding survival curves in B and D. One non-tumor bearing mouse in the s.c. anti-CD4 group was censored at day 56 due to dermatitis requiring euthanasia. \*\*\*, p<0.001; n.s., not significant, by log-rank test. Data reflect 2 independent experiments with group sizes as indicated.

**Supplementary Figure S7.** **Efficacy of CD40 agonist monotherapy is dependent on tumor antigenicity.** (**A**-**B**) WT mice were injected orthotopically with 1x106 PDAC cells and treated on day 7 with either ctrl Ig or agonistic anti-CD40. Tumor growth kinetics (A) or survival (B) are shown for n=4-5 mice/group. (**C**) WT mice were injected i.p. with 1x106 PDAC cells and treated on day 7 with either ctrl Ig or CD40 agonist and survival was monitored for n=5 mice/group. \*\*, p<0.01; n.s., not significant, by log-rank test.

**Supplementary Figure S8.** **Lack of neoantigen or MHC class I loss in PDAC.Ova escape tumors from anti-PD-1 treated mice.** PDAC, PDAC.Ova, and cell lines generated from orth. escape tumors in anti-PD-1 treated WT mice (n=2, PD-1 orth. ‘escape’) were analyzed by flow cytometry for expression of Td-tomato (**A**), H-2Kb-SIIN (**B**), MHC class I molecules (H-2Kb, H-2Db) (**C**), and PD-L1 (**D**) in untreated cells (black lines) or following IFN treatment for 48 hours (blue lines). Isotype control staining is shown in shaded gray lines. (**E**) Comparison of MHC class I, MHC class II, and PD-L1 levels by flow cytometry on *ex vivo* s.c. PDAC and PDAC.Ova tumors at day 9 (n=5 mice each) with untreated and IFN-treated *in vitro* tumor cells. Plots reflect geometric mean fluorescence intensity (gMFI) of staining minus isotype control staining.