**A Virus-Infected, Reprogrammed Somatic cell-derived Tumor cell (VIReST) regime can prevent initiation and progression of pancreatic cancer.**

**Running Title: Stem-cell-based prophylactic vaccination for pancreatic cancer**

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**Supplementary Figure Legends**

**Supplementary Figure 1. Tail-tip fibroblasts from WT or KPC mice can be converted to pluripotency and homologous recombination can effectively introduce KRasG12D and P53R172H mutations into iPSCs.** LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1-Cre (KPC) or wild-type (WT) littermates (129J/Bl6) tail tip fibroblasts were reprogrammed using retroviral transduction of Oct4, Sox2 and Klf4 on day 0. (**A**) KRas was modified to KrasG12D/+ in WT iPSCs using the homologous recombination targeting vector shown (arrows indicate genotyping primer location; \* indicates the mutation site in the construct). PCR primers were used to specifically detect mutated KRas. \*WT-K indicates the selected clone heterozygous for mutated KRas; + indicates the KrasG12D positive control from KPC fibroblasts. (**B**) P53 was modified to p53R172H/+ in WT-K cells through TALEN-mediated homologous recombination with the vector shown (arrows indicate genotyping primer location; \* indicates the mutation site in the construct). PCR primers were used to specifically detect mutated p53. \*WT-KP indicates the selected clone heterozygous for mutated KRas and p53; + indicates the p53R172H/+ positive control from KPC fibroblasts. (**C**) Presence of heterozygous mutant KRas (GGT-GAT) and p53 (CGC-CAC) in the selected WT-KP clone was confirmed using sequencing. The arrows indicate the heterozygous mutant sites.(**D**) Pseudopregnant ICR mice were used to generate chimeric mice using WT, WT-KP or KPC iPSCs. A representative example of chimeras generated is shown.

**Supplementary Figure 2. iPSCs derived from KPC or WT mice can be effectively differentiated and transformed into tumor cells.**(**A**) iPS cells were differentiated into definitive endoderm (DE) and then pancreatic progenitor like cells (PPLCs) following the protocol shown and detailed in the Methods. Briefly, Activin (Act) and Chir99021 (Chir) were used in medium 1 (Med1) to induce DE formation. Incubation in Medium 2 (Med2) containing Retinoic acid (RA), Cyclopamin (cyclo), Noggin (Nog), A83-01 (A83) and Vitamin C (VC) induced PPLCs. Finally, PPLCs derived from WT-KP iPSCs were infected with non-replicating AdV-Cre to remove the LSL cassette flanking the KrasG12D and p53R172H modified genes to create KP-AC tumor cells. (**B**) iPSC markers (Oct4 and Nanog), DE markers (Foxa2 and Sox17) and PPLC markers (Sox9 and Pdx1) were analysed in WT, KPC and WT-KP iPSCs undergoing pancreatic specification using qPCR. Samples were analysed at days 0 (iPSC), 5 (DE) and 9 (PPLC) during transformation from iPSCs into PPLCs. Results were normalised using Actin. (**C**) The indicated cell lines were passaged to observe proliferation or senescence *in vitro* and light microscope images depicted. (**F**) Differentiation of LSL-KrasG12D/+; LSL-Trp53R172H/+ iPSCs to lung cancer cells. The protocol is listed in the Methods. iPSCs were differentiated to embryoid bodies (1 day), endoderm (3 days), anterior foregut endoderm (2 days) and lung progenitors (9-10 days). After this stage, cells were infected with Ad5-Cre to remove the LSL cassette flanking the KrasG12D and p53R172H modified genes to create KP-LC tumor cells.

**Supplementary Figure 3. AdV and VV can infect and replicate in transformed iPSCs and mitomycin-C treatment inhibits ongoing replication and tumor cell proliferation.**(**A and B**) Cytotoxicity of Ad5 (**A**) or VVL15-RFP (**B**) on PPLCs derived from KPC or KP-AC iPSCs and in TB11381 pancreatic tumor cells. Cell death was determined by MTS assay 144 hours post-infection. Mean EC50 values ± SEM are shown. (**C-F**) Production of infectious AdV (**C-D**) or VV (**E-F**) virions in PPLCs derived from KPC or KP-AC iPSCs. Cells were infected with virus and were untreated or treated with mitomycin C. Mean viral replication ± SEM was determined at 24 hour intervals for 96 hours by TCID50 assay on CV1 cells for VV or JH293 cells for Ad5. (**G-H**) Cell proliferation of KPC (**G**) or KP-AC (**H**) after infection and mitomycin-C treatment was determined using MTS assay at 24 and 72 hours post-mitomycin C treatment. Mean OD490nm values ± SEM are shown. (**I**) Viral protein expression was determined in KPC or KP-AC PPLCs at 24 and 72 hours post-infection +/- mitomycin C treatment of cells. Anti-E1A was used to confirm AdV protein expression and anti-VV coat proteins used to confirm VV protein expression. Actin was used as a loading control in each case.

**Supplementary Figure 4. Immunization with virus-infected non-transformed iPSCs does not induce tumor-specific immunity against pancreatic cancer.** (**A**)Four weeks-old transgenic mice were immunized with AdV-infected, mitomycin C -treated KPC or KP-AC iPSC-derived PPLCs, followed by a booster 4 weeks later using VV-infected cell lines. CD8+ cells and CD4+ T cell infiltrate in the pancreas was assessed using IHC at two weeks following the prime vaccination. Original objective magnification is 10x. CD8+ cells from IHC sections were counted and the average number per high power field (HPF) ± SEM plotted. Three to four mice per time-point per group were analyzed and the whole section analyzed. CD4+ T cell presence in the whole tissue slice was graded and is shown graphically. Grade 1: 0-25 positive cells in the 10x field; grade 2: 25-50 positive cells; Grade 3: 50-75 positive cells; Grade 4: More than 75 positive cells. Significance was analyzed using one-way ANOVA with Tukey post-test \*\* p<0.01, \*\*\* p<0.001. (**B**)T cell subsets in spleens (Sp) and draining lymph nodes (LN) from KPC mice 1 week after VIReST. Naïve T cell (CD44lo;CD62Lhi), TCM: Central memory T cell (CD44hi;CD62Lhi) and TEM: Effector memory T cell (CD44hi;CD62Llo) were assessed. Significance was analysed using one-way ANOVA with Tukey post-test. (n=3-4/group)(**C**) Representative IHC staining and quantification of FoxP3 cells in the pancreas of VIReST treated animals at 2 weeks post-prime and 2 weeks post prime-boost VIReST treatment. (**D**)Representative IHC staining of CD8+ T cells in pancreas tissue of KPC mice 2 and 3 months after VIReST treatment. (**E**) Representative IHC staining of CD8+ T cells in pancreas tissue of KPC mice one week or two weeks after immunization with virus-infected iPSCs using a VICCV regime in which non-transformed iPSCs were infected with AdV or VV and used in a prime-boost vaccination regime. Scale bar 100µm. CD8+ T cells infiltrate into the pancreas was quantified and analyzed using one-way ANOVA with Tukey post-test \* p<0.05. (**F**) IFN-γ production of splenic cells of KPC mice 1 week after VICCV non-transformed iPSC immunization. The splenocytes from the immunized KPC mice or PBS treated mice were stimulated with mitomycin-C treated tumor cells derived from transgenic KC or KPC pancreatic cancer model. The IFN-γ production was detected by ELISA and analyzed using one-way ANOVA with Tukey post-test \* p<0.05.

**Supplementary Figure 5. VIReST delays the progression of PanINs.** Tumors were excised from animals 1 week post-prime and 1 week post-boost injections using KPC or KP-AC VIReST. Tumors were stained using H&E and the presence of PanINs and invasive disease scored. N=3/group. (**A**) Representative images of tumor tissue one week post-boost. (**B** and **C**) Scoring of positive PanINs 1 week post-prime (**B**) and 1 week post-boost (**C**). Data was analysed using a One-way ANOVA with Bonferoni post-hoc test. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

**Supplementary Figure 6. iPSC-derived tumor cells are as effective as syngeneic tumor cells and virus infection is required for treatment efficacy.** (**A**) Four-week-old KPC transgenic mice were immunized using a VIResT regime using KPC VIReST or syngeneic TB11381 cells. Kaplan-Meier survival analysis followed by Log rank (Mantel-Cox) tests was used to determine significance of long-term survival. (**B**) Four-week-old KPC transgenic mice were immunized using a VIResT regime using KPC VIReST or KPC cells treated with MMC only and not virus-infected. Kaplan-Meier survival analysis followed by Log rank (Mantel-Cox) tests was used to determine significance of long-term survival. (**C**) Four-week-old male C57/BL6 mice were immunized using a KPC VIReST regime or KPC cells treated with MMC only and not virus-infected. One week later, splenocytes were re-stimulated *ex-vivo* with growth-arrested PDAC cells, or KRas or mesothelin (Meso) peptides and IFNγ production measured using ELISA. Mean IFNγ production **±** SEM is shown (n=3/group). Significance was analyzed using one-way ANOVA with Tukey post-test.