# Supplemental Materials and Data

## Supplemental Methods:

### Cell culture

Panc02, pancreatic adenocarcinoma cells (RRID:CVCLD627) were acquired from Dr. John Bell (University of Ottawa). Panc-1 (RRID:CVCL_0480), AsPC1 (RRID:CVCL_0152), and Capan-2 (RRID:CVCL_0026) cells were acquired from Dr. Jeanette Boudreau (Dalhousie University). Vero (RRID:CVCL_0059) and B16-F10 (RRID:CVCL_0159) cells were originally purchased from ATCC. Cell lines were cultured at 37°C, 5% CO2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL of penicillin (1%), and 100µg/mL of streptomycin (1%) (Hyclone).

### Virus Purification

Oncolytic VSV (VSVΔM51) was previously modified to express green fluorescent protein (VSV-GFP)[19] or IL-15 (VSV-IL-15).[27] The VSV-IL-15 construct encodes human IL-15 with an Igκ leader sequence to increase secretion, and is biologically active in mice.[27] To propagate viruses, Vero cells at ~95% confluency were infected with VSV at a MOI of ~0.1 in serum free DMEM for 48 hours. Supernatants were collected and filtered through a 45 µm filter before layering on 1.1 mL of 20% sucrose in PBS. Tubes were centrifuged at 36,000 rpms for 90 minutes at 4°C. Virus was resuspended in 15% glucose in PBS and stored at -80°C. Virus titers were determined by counting plaques 48 hrs after plating dilutions on confluent Vero cells.

### Flow cytometry & immune phenotyping

For both the subcutaneous and orthotopic pancreatic tumor models, mice were sacrificed 7 days after DC injection (Day 38 and 26 respectively). Additional mice with subcutaneous tumors were harvested 21 days after anti-PD-1 treatment (Day 55). Spleens and tumors were isolated and dispersed into single cell

suspensions via mechanical dispersion through 70µm stainless steel mesh. Tumor infiltrating lymphocytes were enriched using a 30% Percoll gradient (GE Healthcare). Red blood cells were lysed using ammonium chloride buffer and cells were washed in PBS containing 2% FBS. Lymphoid and myeloid populations were examined by flow cytometry.

The following antibodies were obtained from eBioscience or BioLegend: fluorescein isothiocyanate-labeled NK1.1 (clone PK136) and Ly6C (clone HK1.4); phycoerythrin-labeled TCRβ (clone H57-597), Ly6G (clone 1A8), PD-L1 (10F.9G2), and PD-1 (29F.1A12); Peridinin-chlorophyll-protein complex-cyanine5.5-labeled CD11c (clone N418) and CD8α (clone 53-6.7); Phycoerythrin-cyanine-7-labeled CD4 (clone RM4-5) and CD11b (clone M1/70); Allophycocyanin-labeled F4/80 (clone BM8) and TCRβ (clone H57-597); and Allophycocyanin-eFlour 780-labelled MHC II (clone M5/114.15.2). Allophycocyanin- and phycoerythrin-labeled CD1d tetramers loaded with the synthetic glycolipid PBS57 were obtained from the NIH Tetramer Core Facility (Emory University). Cells were incubated for 20 mins at room temperature with eflour450 fixable viability dye (Thermofisher). Cells were incubated 30 min at 4°C with antibody panels to stain cell subsets, washed, and fixed in 2% paraformaldehyde (Fisher Scientific). For FoxP3 intracellular staining, cells were fixed with 4% paraformaldehyde after staining cell-surface antigens. Cells were resuspended in permeabilization buffer (BD Biosciences) and incubated for 20mins at 4°C with phycoerythrin-labeled FoxP3 antibody (clone FJK-16s). Samples were acquired using a three-laser FACSCanto cytometer and analyzed using Flowjo software (version 10.5; BD Biosciences). A two-laser FACSAria III cell sorter with FACSDiva software (BD Biosciences) was used to sort cell populations (>90% purity) for *ex vivo* cytotoxicity and cytokine assays.

**VSV-IL-15 infection and cytokine production *in vivo***

Subcutaneous Panc02 tumors were treated with VSV-GFP or VSV-IL-15 (it. 5 x $10^8$ PFU) on Days 26,28, and 30. Twenty-four hours after the final virus infection, tumors were harvested and homogenized. Viral titers

were determined by plaque assay as described above. Human IL-15 production was determined in tumor homogenates and serum by ELISA (Invitrogen).

**VSV-IL-15 infection and cytokine production *in vitro***

Panc02, Panc-1, ASPC1, and Capan-2 cells were infected at an MOI of 10, 1, or 0.1 with VSV-GFP, VSV-IL-15, or UV inactivated VSV (UV-VSV) for 24, 48, or 72 hours. To assess viability, 0.5mg/ml MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma) diluted in PBS was added to each well. After 2 hours at 37°C, MTT reagent was removed and 100ul of DMSO was added. Formazan production was measured using an Epoch plate reader ( Bioteck), subtracting the 690 nm value from the 540 nm value to determine metabolic activity.

Panc02, Panc-1, AsPC1, and Capan-2 cells were infected at an MOI of 1 for 24 hours. Culture supernatants were collected, and human IL-15 production was determined by ELISA (Invitrogen).

## Supplemental Figures:

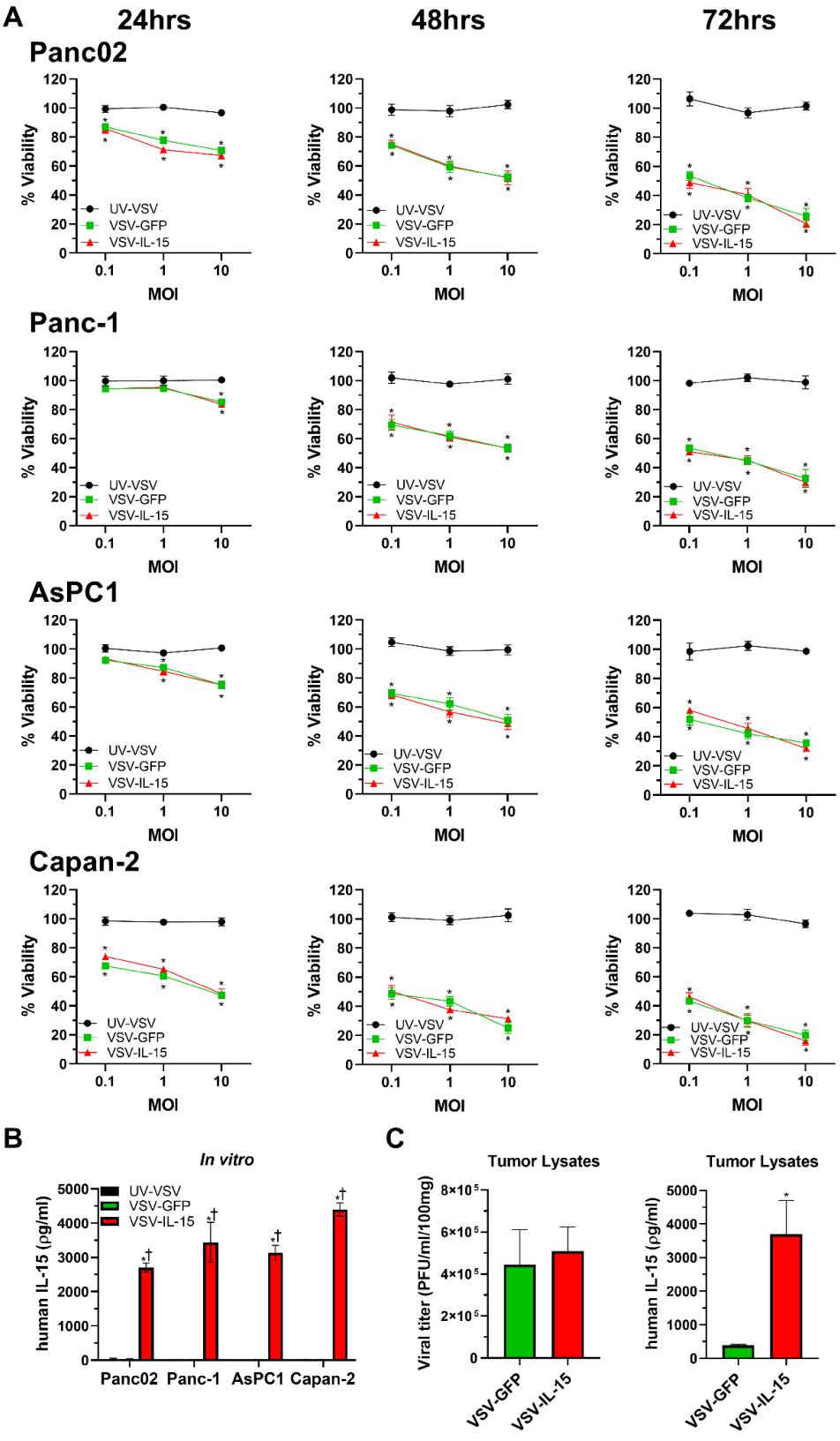
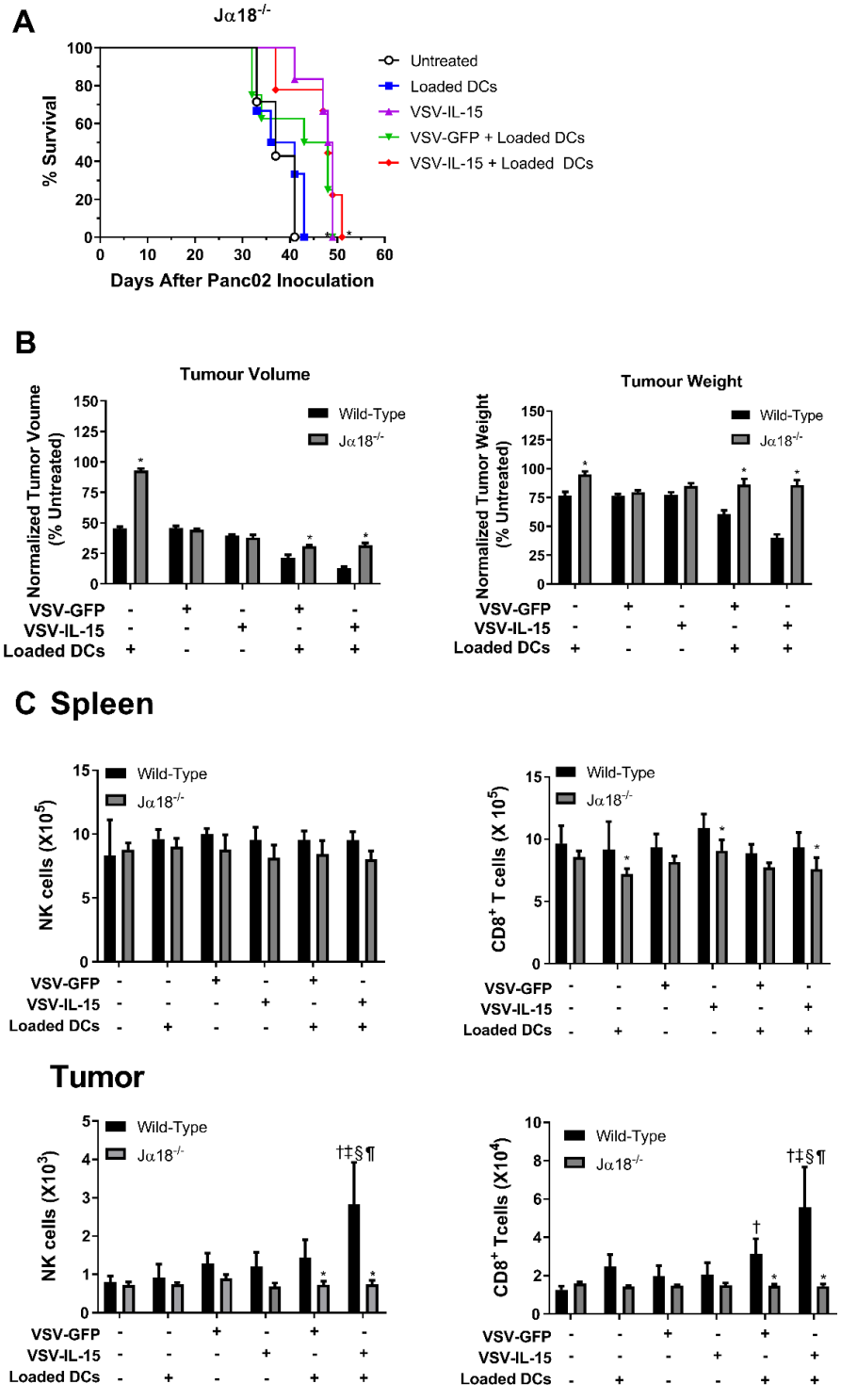

A
24hrs
48hrs
72hrs
Panc02
Panc-1
AsPC1
Capan-2
% Viability
MOI
UV-VSV
VSV-GFP
VSV-IL-15
B
In vitro
human IL-15 (pg/ml)
Panc02
Panc-1
AsPC1
Capan-2
C
Tumor Lysates
Viral titer (PFU/ml/100mg)
Tumor Lysates
human IL-15 (pg/ml)
VSV-GFP
VSV-IL-15

**Figure S1: VSV-IL-15 infection and cytokine production by pancreatic cancer cells *in vitro* and in *vivo*.** A) Murine Panc02 and human Panc-1, AsPC1, and Capan-2 pancreatic adenocarcinoma lines were treated with VSV-IL-15, VSV-GFP, or UV-inactivated VSV. Cell viability was analyzed at different multiplicities of infection (MOI) using an MTT cell viability assay at 24, 48- and 72-hours following infection. Cell viability is expressed relative to untreated media conditions for each time point (n=3 per group). *$p < 0.05$ compared to UV-inactivated VSV. B) Murine Panc02 and human Panc-1, AsPC1, and Capan-2 pancreatic adenocarcinoma lines were treated with VSV-IL-15, VSV-GFP, or UV-inactivated VSV at an MOI of 1 for 24 hours. Culture supernatants were collected for measurement of IL-15 by ELISA (n=3 per group). *$p < 0.05$ compared to UV-inactivated VSV. $^{\dagger}p<0.05$ compared to VSV-GFP. C) Mice with subcutaneous Panc02 tumors were treated with VSV-GFP or VSV-IL-15 as in Figure 1A. Tumors were isolated 24 hours after final virus injection. Viral titers in tumor homogenates were measured by plaque assay on Vero cells. IL-15 production was measured by ELISA (n=4-5). Viral titers and IL-15 concentration in tumors were normalized per 100mg of tumor weight. $^{*}p<0.05$ compared to VSV-GFP.


A
Jα18-/-
% Survival
Days After Panc02 Inoculation
Untreated
Loaded DCs
VSV-IL-15
VSV-GFP + Loaded DCs
VSV-IL-15 + Loaded DCs
B
Tumour Volume
Normalized Tumor Voume (% Untreated)
Wild-Type
Jα18-/-
VSV-GFP
VSV-IL-15
Loaded DCs
Tumour Weight
Normalized Tumor Weight (% Untreated)
C Spleen
NK cells (X10^5)
CD8+ T cells (X 10^5)
Tumor
NK cells (X10^3)
CD8+ Tcells (X10^4)

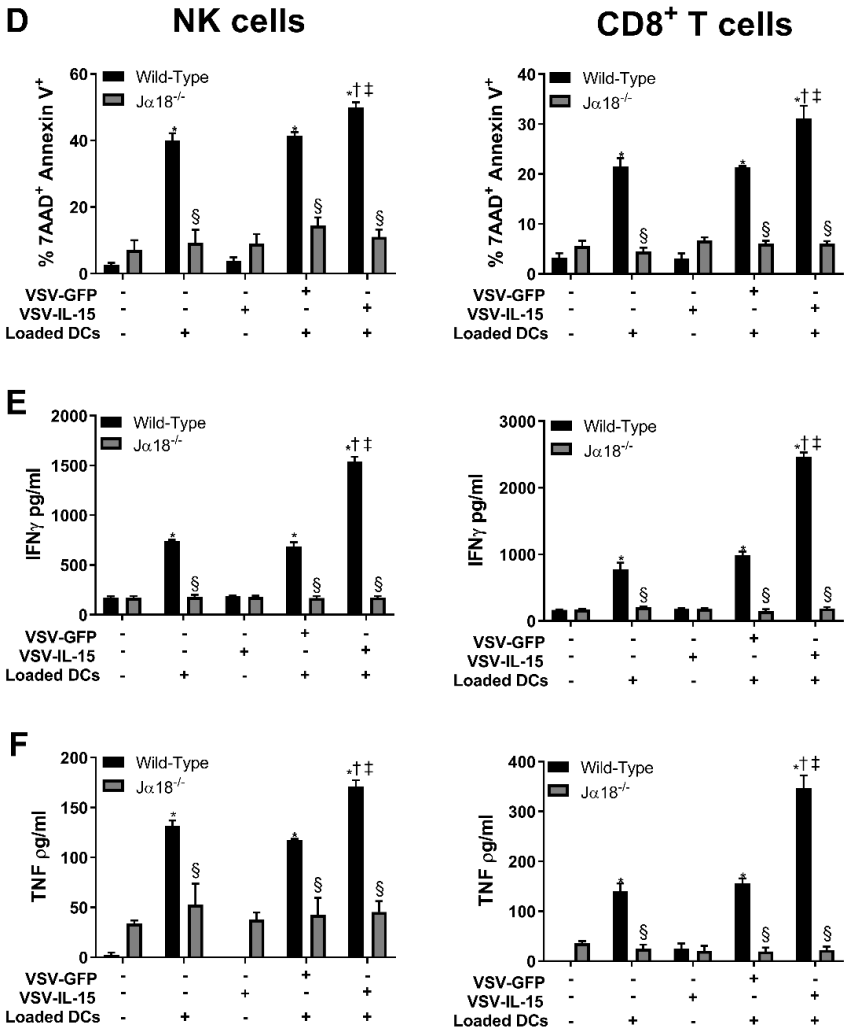

D
NK cells
CD8+ T cells
% 7AAD+ Annexin V+
Wild-Type
Jα18-/-
VSV-GFP
VSV-IL-15
Loaded DCs
E
IFNγ pg/ml
F
TNF ρg/ml

**Figure S2: Therapy-induced survival, tumor regression, and immune cell recall responses are impaired in NKT deficient (Jα18$^{-/-}$) mice.** Wild-type and NKT cell-deficient Jα18$^{-/-}$ mice were inoculated subcutaneously with Panc02 tumor cells and treated as in Fig. 1A. A) Survival was assessed in Jα18$^{-/-}$ mice inoculated with Panc02 cells and treated with NKT cell activation, VSV-IL-15, combined therapy with VSV-GFP and NKT cell activation, or combined therapy with VSV-IL-15 and NKT cell activation. (n=6-9 per group). *p<0.05 compared to untreated. B) Tumor volumes and weights were measured at day 38. Measurements were normalized to the untreated group of each mouse strain (n=4-11 per group). *p<0.05 compared to wild-type. C) Spleens and tumors of mice were isolated and dispersed into single cell suspensions. Flow cytometry was used to assess NK (NK1.1$^{+}$ TCRβ$^{-}$) and CD8$^{+}$ T cells (TCRβ$^{+}$ CD8α$^{+}$). Immune cell populations in tumors were normalized per100mg of tumor weight. *p<0.05 compared to wild-type. †p<0.05 compared to untreated. ‡P<0.05 compared to glycolipid-loaded DCs. §p<0.05 compared to VSV-IL-15. ¶P<0.05 compared to combined treatment with VSV-GFP plus glycolipid-loaded DCs. E-F) Panc02 cells were incubated for 18 hours at a 1:1 ratio with NK cells, CD8$^{+}$ T cells isolated from the spleens of wild-type and Jα18$^{-/-}$ mice receiving different therapies. D) Cytotoxicity was measured by staining Panc02 cells with Annexin V and 7AAD. E) IFNγ and F) TNF were determined in culture supernatants by ELISA (n=3 per group). *p<0.05 compared to untreated. †P<0.05 compared to glycolipid-loaded DCs. ‡P<0.05 compared to combined treatment with VSV-GFP plus glycolipid-loaded DCs. §p<0.05 compared to wild-type.

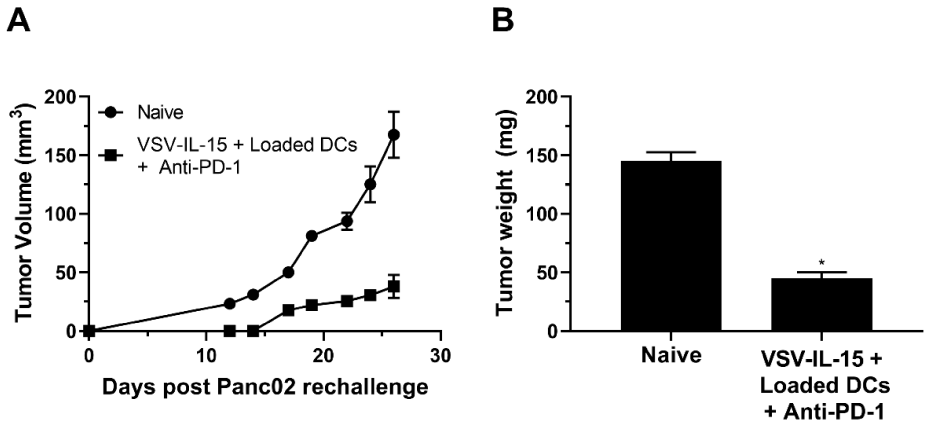



**Figure S3: Rechallenged mice exhibited reduced tumor growth compared to naïve mice.** A) Panc02 cells (2X $10^6$) were inoculated subcutaneously into naïve mice and mice who survived original tumor challenge following treatment with VSV-IL-15, glycolipid-loaded DCs and PD-1 blockade as in Fig 4E. A) Tumor volume was assessed over time and B) tumor weight was measured 26 days after Panc02 cell injection (n=2-6 per group). *p<0.05 compared to naive.

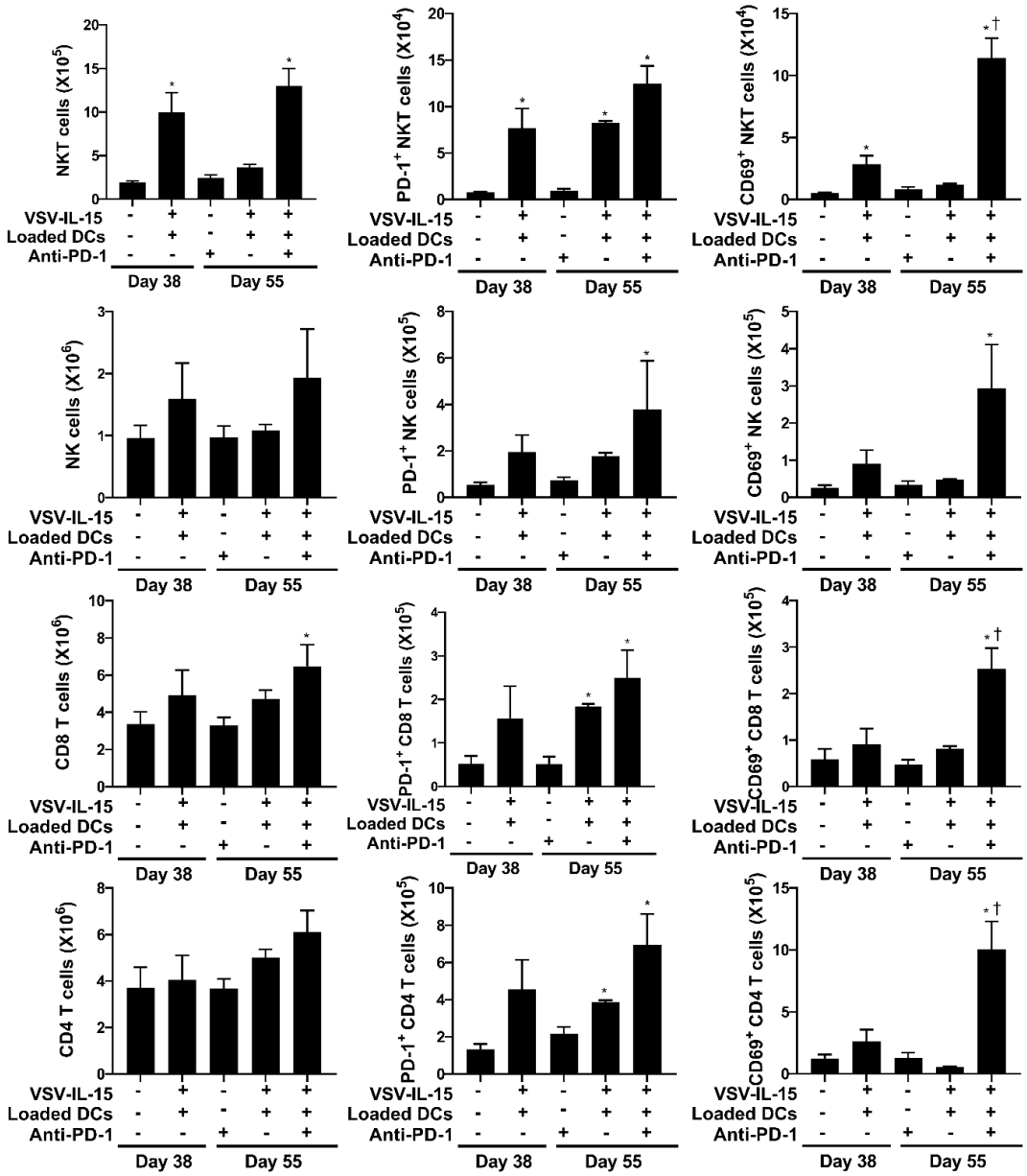

NKT cells (X10^5)
PD-1+ NKT cells (X10^4)
CD69+ NKT cells (X10^4)
NK cells (X10^6)
PD-1+ NK cells (X10^5)
CD69+ NK cells (X10^5)
CD8 T cells (X10^6)
PD-1+ CD8 T cells (X10^5)
CD69+ CD8 T cells (X10^5)
CD4 T cells (X10^6)
PD-1+ CD4 T cells (X10^5)
CD69+ CD4 T cells (X10^5)
VSV-IL-15
Loaded DCs
Anti-PD-1
Day 38
Day 55

**Figure S4: PD-1 blockade increased activation of splenic lymphocytes following combined VSV-IL-15 and NKT cell activation therapies.** Mice were inoculated subcutaneously with Panc02 cells ($2X10^6$). Mice were treated with VSV-GFP or VSV-IL-15 (it. $5X10^8$ PFU) on days 26, 28, and 30 and/or $\alpha$-GalCer-loaded DCs (iv. $6X10^5$) on day 31. Mice were given anti-PD-1 (ip.300μg) weekly for three weeks starting on day 34. Spleens of untreated and treated mice were harvested and dispersed into single cell suspensions on day 38 or 55. Flow cytometry was used to assess infiltration of NKT cells (CD1d tetramer$^+$ TCRβ$^+$), NK cells (NK1.1$^+$ TCRβ$^-$), CD8$^+$ T cells (TCRβ$^+$ CD8α$^+$), and CD4$^+$ T cells (TCRβ$^+$ CD4$^+$) (n=3-6 per group). *p<0.05 compared to untreated. †p<0.05 compared to VSV-IL-15 plus glycolipid-loaded DCs (Day 38).

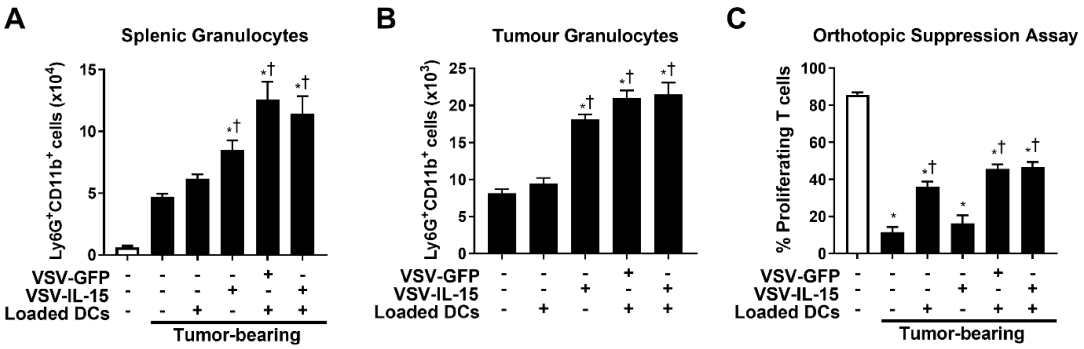



**Figure S5: NKT cell activation decreased MDSC immunosuppression in an orthotopic model of pancreatic cancer.** Mice were inoculated orthotopically with Panc02 cells ($5X10^5$). Mice were treated with VSV-GFP or VSV-IL-15 (it. $5X10^8$ PFU) on days 14, 16, and 18 and/or $\alpha$-GalCer-loaded DCs (iv. $6X10^5$) on day 19. A) Spleens and (B) tumors of orthotopic Panc02 mice were harvested and dispersed into single cell suspensions on day 26. Flow cytometry was used to assess accumulation of granulocytic ($Ly6G^+$ $CD11b^+$) cells (n=6 per group). Immune cell populations in tumors were normalized per 100mg of tumor weight. *p<0.05 compared to untreated. †p<0.05 compared to glycolipid-loaded DCs. C) Naïve splenocytes from WT mice were stained with Oregon green and cultured with CD3/CD28 beads. Blood granulocytes from untreated and treated mice were added to the coculture for 72 hours. T cells ($TCR\beta^+$) were stained and examined for proliferation (n=3-4 per group). $^*p<0.05$ compared to naïve. $^\dagger p<0.05$ compared to untreated.