OMTO, Volume 25

Supplemental information

IFNAR blockade synergizes with oncolytic

VSV to prevent virus-mediated PD-L1

expression and promote antitumor T cell activity

Nader El-Sayes, Scott Walsh, Alyssa Vito, Amir Reihani, Kjetil Ask, Yonghong Wan, and Karen Mossman

Supplementary Figures



Figure S1. GFP and YFP expression following VSV Δ 51 and VacV infection. B16F10 cells were infected with VSV-GFP (VSV) or VacV-YFP (VacV) at MOI 1 for 24 hours then imaged using a fluorescence microscope.



Figure S2. VSV-induced PD-L1 upregulation in MC38 cells. MC38 cells were infected with VSV Δ 51 for 2, 4, 6 and 8 hours before RNA was harvested and used to assess PD-L1 mRNA expression via RT-PCR.



Figure S3. Type I IFN induces IFIT1 upregulation. (A) MC38 and (B) B16F10 parental and IFNAR knockout cells and were treated with 100 U/mL of IFN α or IFN β . RNA was harvested and used to assess mRNA expression of IFIT1 via RT-PCR.



Figure S4. Validation of VSV initiation of replication. (A) MC38 and B16F10 parental and IFNAR knockout cells were infected with VSV Δ 51-GFP at MOI 1 for 8, 16 and 24 hours then imaged using a fluorescence microscope. B16F10 parental and IFNAR knockout cells were infected with VSV Δ 51-GFP at MOI 1 for 24 hours then GFP fluorescence was measured by flow

cytometry. (**B**) Representative histogram and (**C**) MFIs of GFP expression in B16F10 parental and IFNAR ko cells 24 hours post infection. (**D**) MC38 parental/IFNAR ko cells were infected with VSV Δ 51-GFP at MOI 1 for 24 hours then viability was measured by flow cytometry.



Figure S5. Gating strategy used to characterize different leukocytes from PBMCs to assess PD-

L1 surface expression (corresponds to data from Figure 5).



Figure S6. Gating strategy used to characterize T cells from PBMCs (corresponds to data from Figure 6).