

1 SUPPLEMENTARY MATERIAL

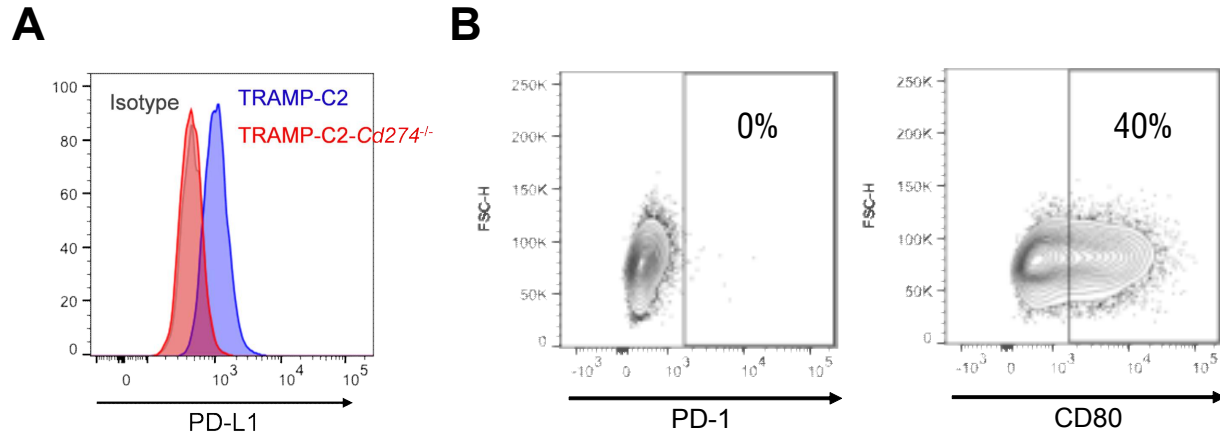


FIGURE S1

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3 **Figure S1: PD-L1 engagement promotes oncolytic virus infection.** (A) TRAMP-C2 cells (blue)
4 were transfected with Cas9 and gRNA targeting PD-L1 to generate TRAMP-C2-*Cd274*^{-/-} (red). A
5 representative plot depicting PD-L1 expression is shown. (B) Expression of PD-1 and CD80 in
6 TRAMP-C2 cells. Representative flow plots are shown.

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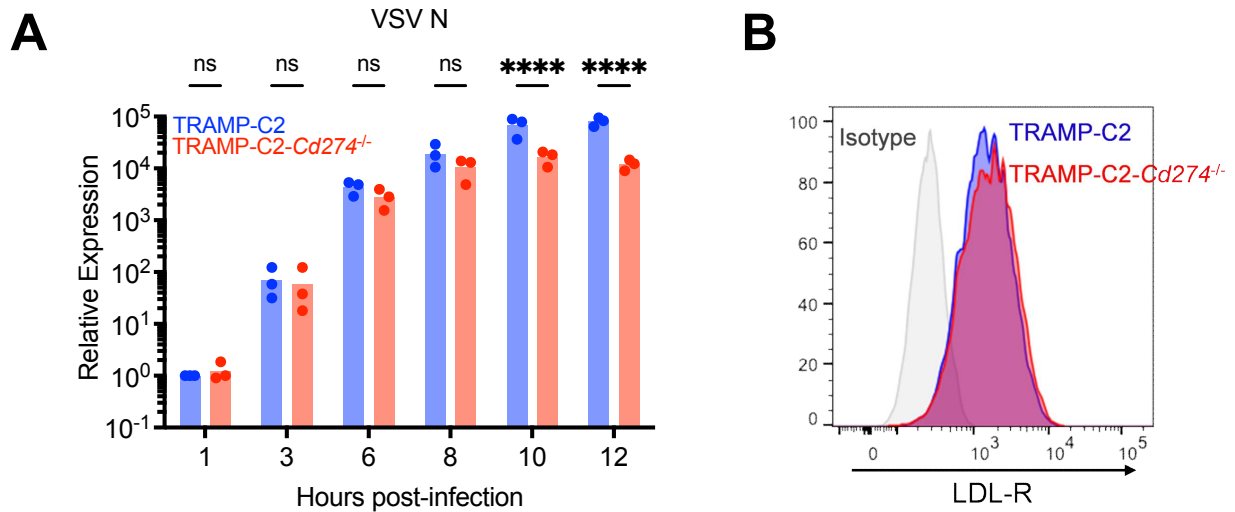


FIGURE S2

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10 **Figure S2: PD-L1 does not regulate viral entry.** (A) TRAMP-C2 or TRAMP-C2-*Cd274*^{-/-} were
 11 infected with VSV Δ 51-YFP at MOI 0.1, and RNA collected at indicated times post-infection for
 12 qPCR analysis of the viral nucleocapsid (N) transcript. n=3 biological replicates depicted.
 13 Statistical analysis by two-way ANOVA with Šídák's correction for multiple comparisons. (B)
 14 Expression of the VSV entry receptor LDL-R in TRAMP-C2 (blue) or TRAMP-C2-*Cd274*^{-/-} (red)
 15 cells. Representative histograms are shown.

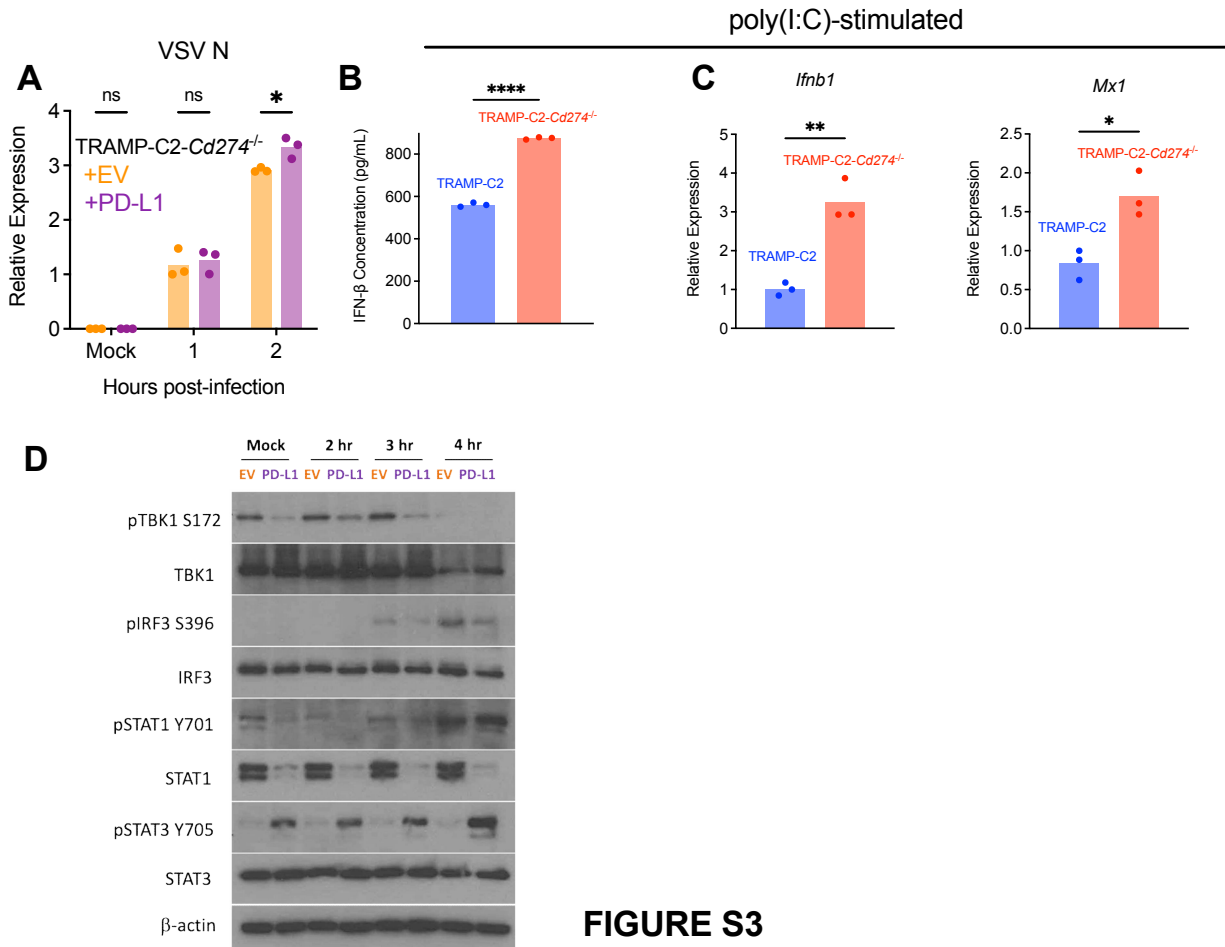


FIGURE S3

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17 **Figure S3: PD-L1 inhibits the type I interferon response to oncolytic viruses. (A)**

18 TRAMP-C2-*Cd274*^{-/-} cells transduced with PD-L1 or empty vector were infected with

19 VSVΔ51-YFP at MOI 0.1 and analyzed by qPCR at indicated times post-infection to quantify viral

20 N transcripts. The data depicted are representative of 3 performed with similar results. Statistical

21 analysis by two-way ANOVA with Šídák's correction for multiple comparisons. (**B** and **C**) Cells

22 were transfected with 1 ug of poly(I:C) for 8 hours prior to quantification of IFN-β (by ELISA, B)

23 and IFN-β and MX1 transcripts (by qPCR, C). The experiments depicted are representative of 3

24 performed with similar results. Statistical analysis by two-tailed unpaired Student's t-test. (**D**)

25 TRAMP-C2-*Cd274*^{-/-} cells transduced with PD-L1 or empty vector were infected with

26 VSV Δ 51-YFP at MOI 0.1 and analyzed by western blotting at indicated times post-infection. The
27 images depicted are representative of 3 performed with similar results.
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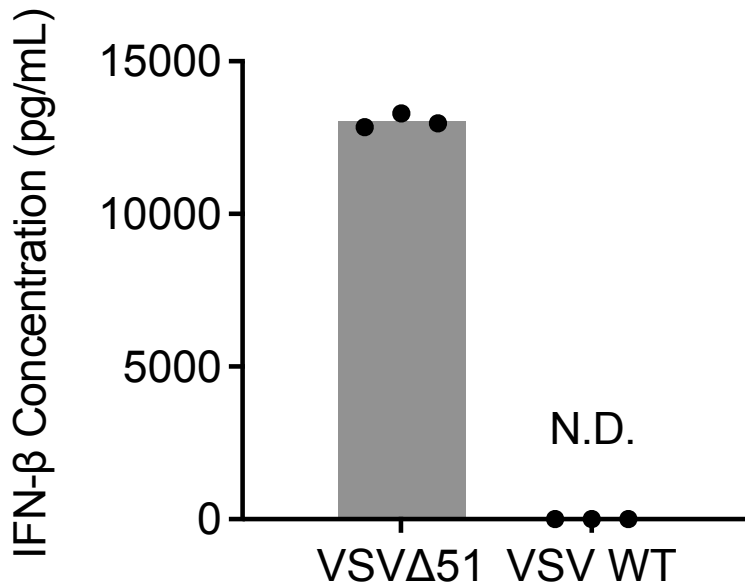


FIGURE S4

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31 **Figure S4: VSV WT efficiently blocks type I IFN responses.** TRAMP-C2 cells were infected
32 with VSVΔ51-YFP or VSV WT at MOI 0.1 for 12 hours prior to analysis by ELISA to quantify
33 IFN-β in supernatant. The experiment depicted is representative of 2 performed with similar
34 results. N.D. = not detected.

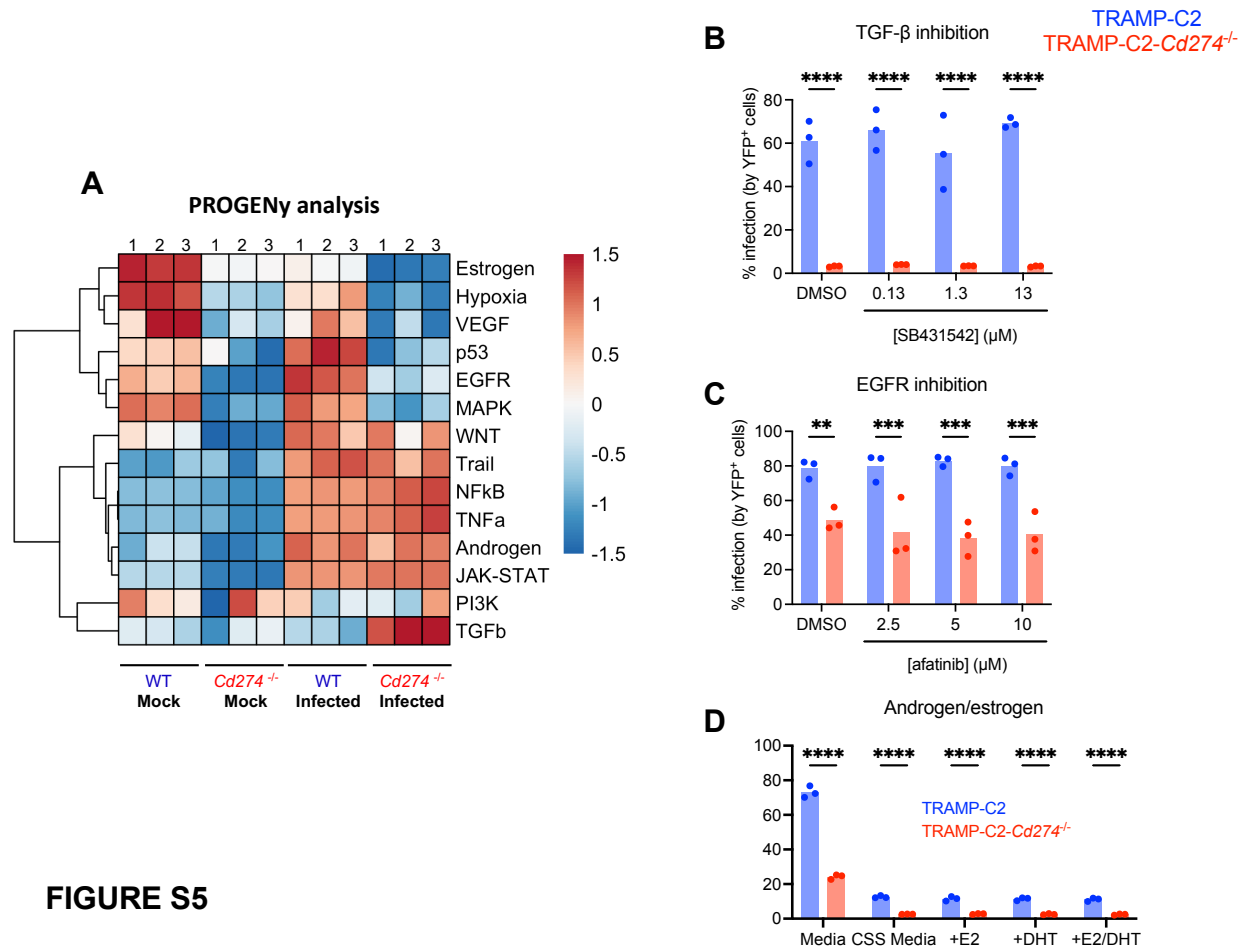


FIGURE S5

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36 **Figure S5: TGF-β, EGFR, and androgen/estrogen pathways are not involved in PD-L1's**

37 **promotion of viral infection. (A)** PROGENy pathway analysis of bulk RNA-seq performed on

38 TRAMP-C2 and TRAMP-C2-*Cd274*^{-/-} either mock-infected or infected with VSVΔ51-YFP at

39 MOI 0.1 for 8 hours (3 biological replicates per condition). **(B-C)** TRAMP-C2 and TRAMP-C2-

40 *Cd274*^{-/-} cells were pre-treated with the TGF-βRI inhibitor SB431452 for 24 hours **(B)** or the EGFR

41 inhibitor afatinib for 6 hours **(C)** at indicated concentrations (or DMSO as vehicle control),

42 followed by infection with VSVΔ51-YFP at MOI 0.1 for 24 hours prior to analysis by flow

43 cytometry for viral YFP reporter expression. Experiments depicted are representative of 2 with

44 similar results. Statistical analysis by two-way ANOVA with Šídák's correction for multiple

45 comparisons. **(D)** TRAMP-C2 and TRAMP-C2-*Cd274*^{-/-} cells were cultured in phenol red-free

46 DMEM supplemented with 10% charcoal-stripped serum (CSS) for 48 hours prior to treatment
47 with estradiol (E2) or dihydrotestosterone (DHT) for 24 hours. Following that, cells were infected
48 with VSV Δ 51-YFP at MOI 0.1 for 24 hours prior to analysis by flow cytometry for viral YFP
49 reporter expression. Experiments depicted are representative of 2 with similar results. Statistical
50 analysis by two-way ANOVA with Šídák's correction for multiple comparisons.

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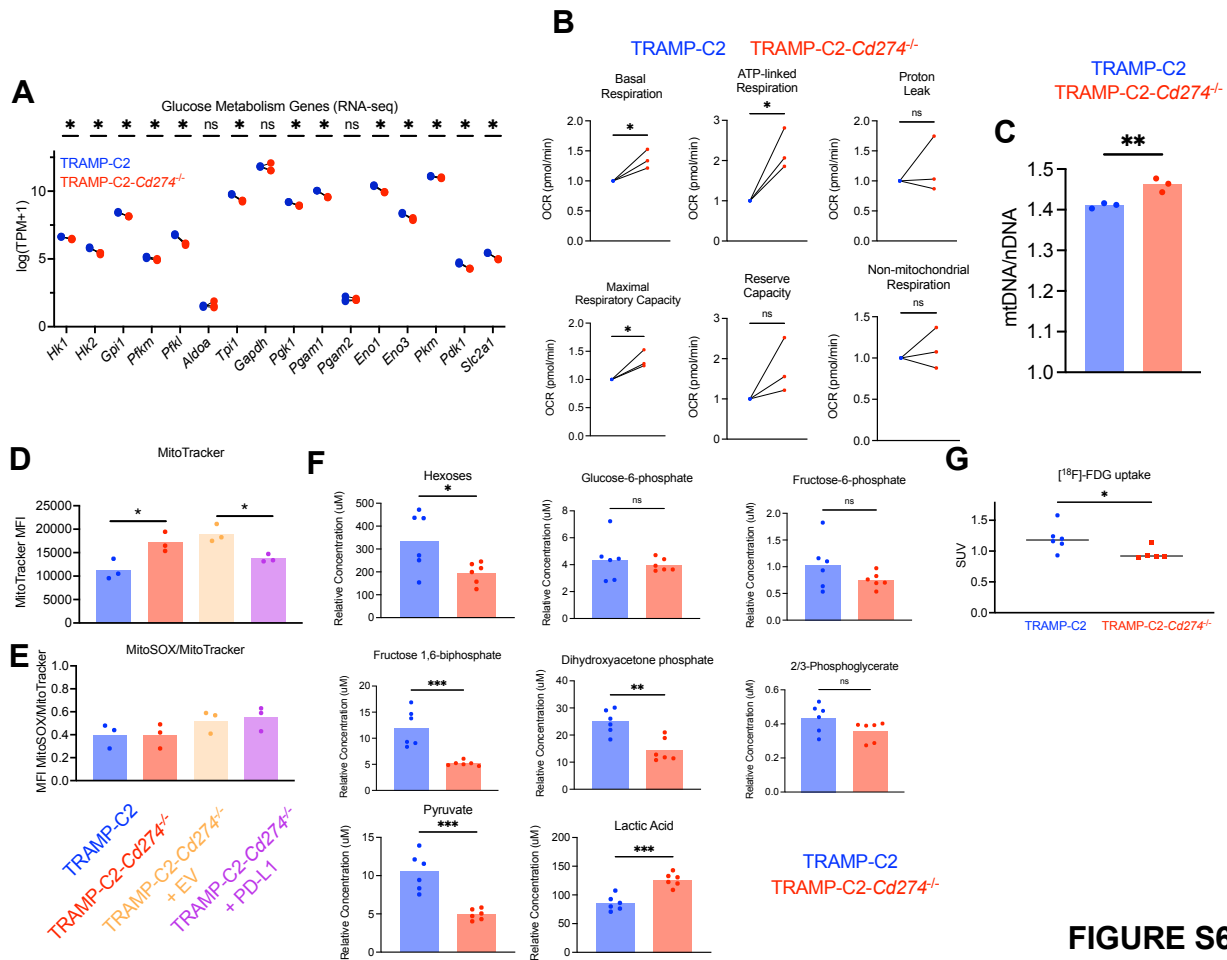


FIGURE S6

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54 **Figure S6: PD-L1 promotes Warburg metabolism in cancer cells.** (A) Transcripts per million

55 (TPM) for various glycolysis and glucose metabolism enzymes/transporters, from bulk RNA-seq

56 experiment. (B) Cellular respiration parameters calculated using Seahorse mitochondrial stress test

57 data. N=3 biological replicates are shown. For each replicate, data normalized to TRAMP-C2

58 values. Statistical analysis by two-tailed unpaired Student's t-test. (C) Relative quantification of

59 mitochondrial DNA/mtDNA by qPCR (normalized to nuclear DNA/nDNA in each sample), by

60 qPCR analysis. N=3 biological replicates. Statistical analysis by two-tailed unpaired Student's t-

61 test. (D) Mean fluorescence intensity (MFI) of MitoTracker and MitoSOX dyes by flow cytometry

62 for TRAMP-C2, TRAMP-C2-Cd274^{-/-}, TRAMP-C2-Cd274^{-/-} + EV, and TRAMP-C2-Cd274^{-/-} +

63 PD-L1 cells. MitoSOX MFI was corrected for total mitochondrial content by normalizing to
64 MitoTracker MFI. Statistical analysis by one-way ANOVA with Šídák's correction for multiple
65 comparisons. (E) Relative abundance of glycolysis metabolites in TRAMP-C2 and TRAMP-C2-
66 *Cd274^{-/-}* cells, measured in the untargeted metabolomics study. 6 biological replicates per cell line.
67 Statistical analysis by unpaired two-tailed Student's t-test. (F) Male NCG mice were implanted
68 with subcutaneous TRAMP-C2 or TRAMP-C2-*Cd274^{-/-}* tumors. Standardized uptake value (SUV)
69 of [¹⁸F]-fluorodeoxyglucose was assessed by PET imaging. Statistical analysis by two-tailed
70 unpaired Student's t-Test. *: p<0.05.

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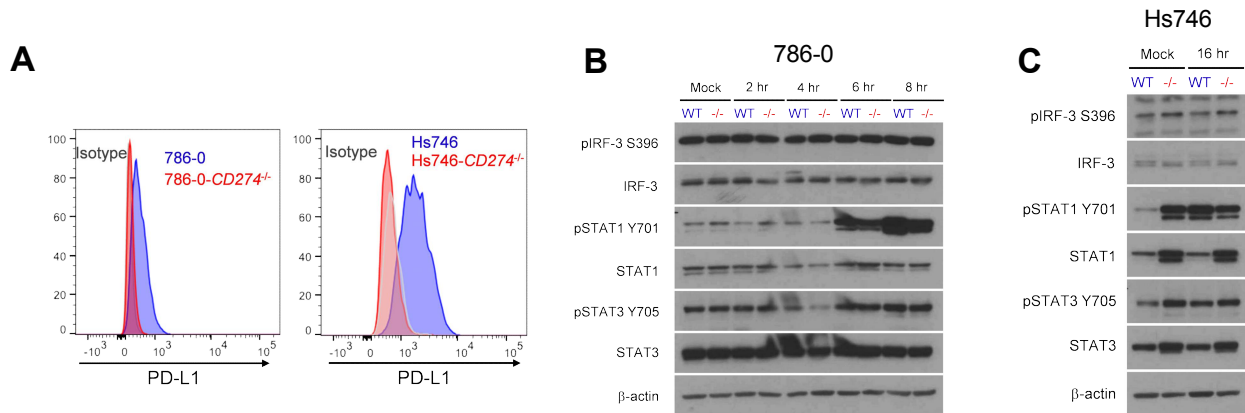


FIGURE S7

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74 **Figure S7: PD-L1 alters type I interferon responses in human cancer cells .** 786-0 (left) and
 75 Hs746 (right) cells were electroporated with Cas9 and gRNA targeting PD-L1 to generate *CD274*^{-/-}
 76 cells. Representative plots depicting PD-L1 expression are shown. **(B)** 786-0 and
 77 786-0-*CD274*^{-/-} cells infected with VSVΔ51-YFP at MOI 1 (or mock-infected) and analyzed by
 78 western blotting at indicated times post-infection. Images depicted are representative of 3 with
 79 similar results. **(C)** Hs746 and Hs746-*CD274*^{-/-} cells infected with VSVΔ51-YFP at MOI 0.1 (or
 80 mock-infected) for 16 hours prior to western blotting analysis. Images depicted are representative
 81 of 3 with similar results.

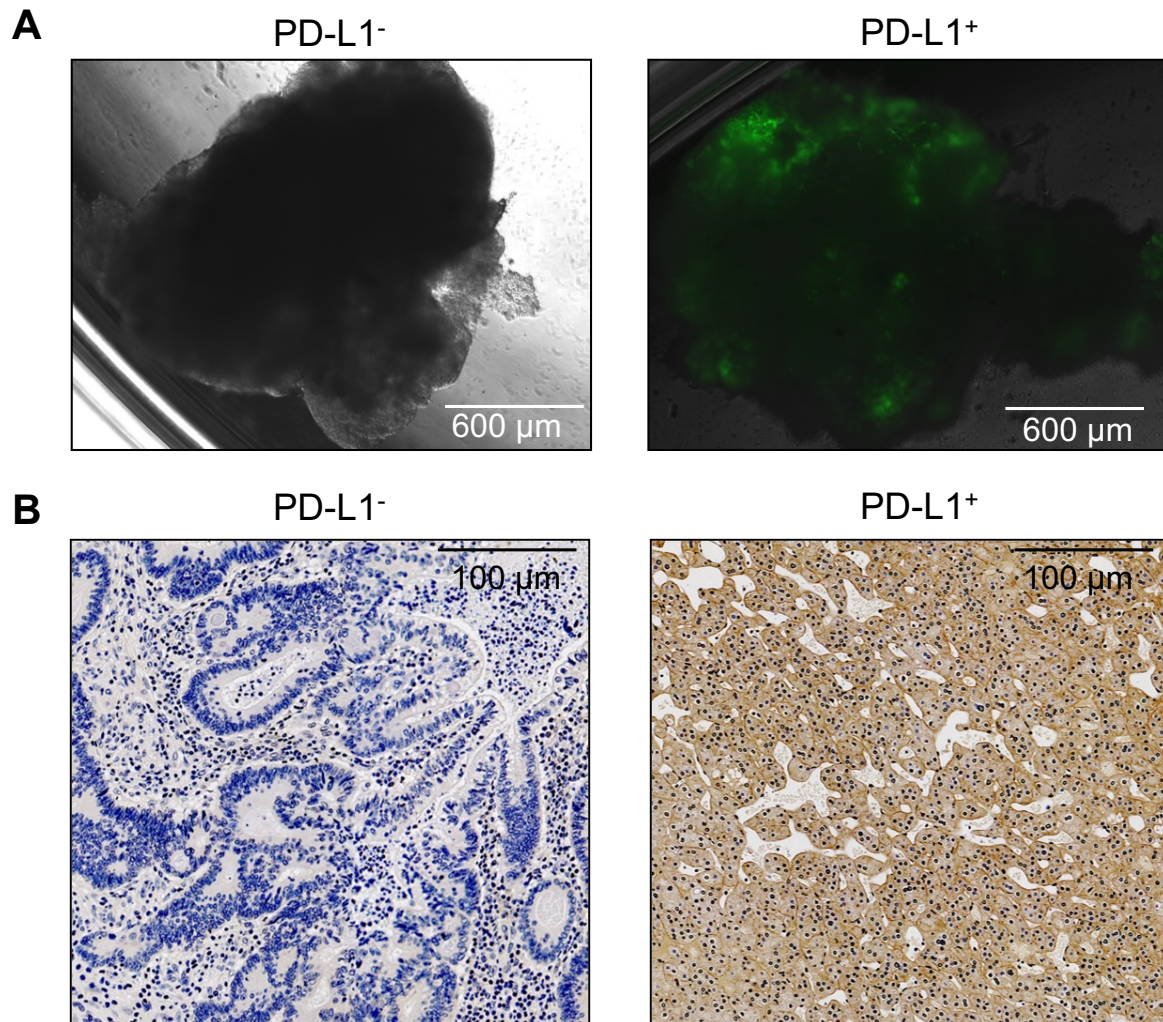


FIGURE S8

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83 **Figure S8: assessment of PD-L1 by immunohistochemistry and oncolytic virus infection by**

84 **fluorescent microscopy.** (A) Fluorescent imaging of tumor explant cores from patient biopsies

85 infected with VSV Δ 51-YFP for 48 hours prior to imaging for viral YFP reporter. Images of poorly

86 infected (left) and well infected (right) tumors are depicted. (B) Patient tumor biopsies subjected

87 to PD-L1 IHC to determine PD-L1 status of tumors. Images of PD-L1⁻ (left) and PD-L1⁺ (right)

88 tumors are depicted.

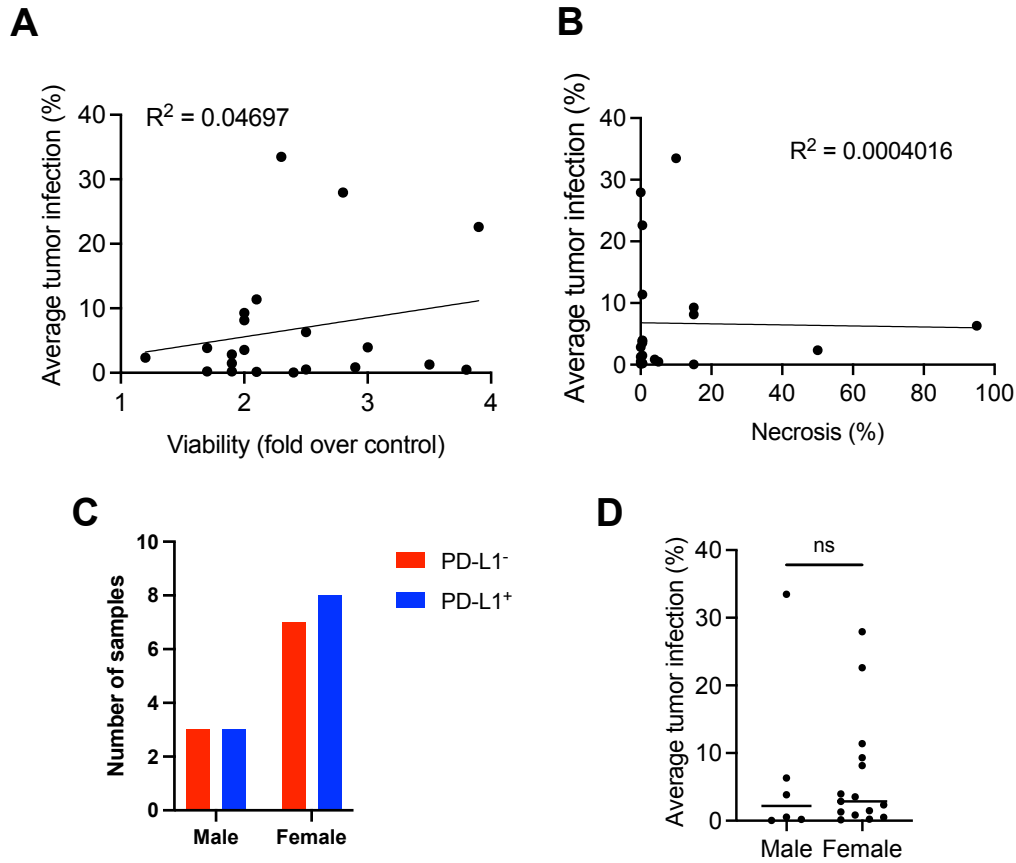


FIGURE S9

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90 **Figure S9: PD-L1 is a biomarker of patient tumors responsive to oncolytic virus. (A-B)**

91 Average tumor infection plotted against viability (assessed by alamarBlue) and necrosis (% of

92 tumor section necrotic, assessed by histology). (C-D) Distribution of PD-L1⁺ and PD-L1⁻ tumors,

93 and average tumor infection, between male and female donors. Statistical analysis by two-tailed

94 unpaired Student's t-test.

Table S1. Metadata for tumor scRNA-seq data analyzed for PD-L1 and glycolysis gene set score.

Cancer	Source	Accession	Platform	Sorted?	Patient # (>100 malignant cells)	Total cancer cell #	% mito threshold
Breast	Wu <i>et al.</i> (37)	ENA accession PRJEB35405	10x Genomics Chromium (3' v2)	NA	4	4452	20%
Breast	Qian <i>et al.</i> (28)	http://blueprint.lambrechtslab.org	10x Genomics Chromium (3')	NA	10	8766	20%
Breast	Bassez <i>et al.</i> (38)	http://biokey.lambrechtslab.org/	10x Genomics Chromium (5')	NA	62	38,765	15%
Colorectal	Lee <i>et al.</i> (26)	GEO Accession GSE144735 & GSE132465	10x Genomics Chromium (3' v2)	NA	25	18,058	20%
Colorectal	Uhlitz <i>et al.</i> (27)	Direct from authors	10x Genomics Chromium (3' v3)	NA	8	2659	20%
Colorectal	Qian <i>et al.</i> (28)	http://blueprint.lambrechtslab.org	10x Genomics Chromium (3')	NA	11	8766	25%
Gastric	Sathe <i>et al.</i> (29)	https://dna-discovery.stanford.edu	10x Genomics Chromium (3' v2)	NA	7	6909	20%
Lung	Kim <i>et al.</i> (32)	GEO Accession GSE131907	10x Genomics Chromium (3' v2)	NA	20	15,396	20%
Lung	Lambrechts <i>et al.</i> (31)	ArrayExpress Accessions E-MTAB-6149 & E-MTAB-6653	10x Genomics Chromium (3' v1/v2)	NA	9	8036	20%
Lung	Qian <i>et al.</i> (28)	http://blueprint.lambrechtslab.org	10x Genomics Chromium (3')	NA	24	6794	20%
Lung	Laughney <i>et al.</i> (24)	GEO Accession GSE123904	10x Genomics Chromium (3' v2)	Viability (scatter & DAPI)	8	3097	20%
Lung	Wu <i>et al.</i> (33)	GEO Accession GSE148071	GEXSCOPE (Singleron Biotechnologies)	NA	35	54,052	30%
Nasopharyngeal	Chen <i>et al.</i> (34)	GEO Accession GSE150430	10x Genomics Chromium (3' v2)	NA	9	7400	20%
Ovarian	Geistlinger <i>et al.</i> (33)	GEO Accession GSE154600	10x Genomics Chromium (3' v2)	NA	5	7479	20%
Ovarian	Qian <i>et al.</i> (28)	http://blueprint.lambrechtslab.org	10x Genomics Chromium (3')	NA	5	4967	20%
Pancreatic	Steele <i>et al.</i> (36)	GEO Accession GSE155698	10x Genomics Chromium (3')	NA	15	10,495	20%
Pancreatic	Peng <i>et al.</i> (54)	GSA: CRA001160	10x Genomics Chromium (3' v2)		24	41,986 (all cell types)	10%
Squamous cell carcinoma	Ji <i>et al.</i> (20)	GEO Accession GSE144236	10x Genomics Chromium (3' v2)	NA	9	12,154	10%

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96 **Table S1:** Metadata for tumour scRNA-seq data analyzed for PD-L1 expression and glycolysis

97 gene set score.

99 **Table S2: PD-L1 remodeling of the cancer cell metabolome.** Summary of unbiased
100 metabolomics study performed on cell lysate and culture supernatant from TRAMP-C2 and
101 TRAMP-C2-*Cd274*^{-/-} after 48 hours in culture. All data in units of relative concentration, measured
102 in μM . Culture supernatant data corrected using culture media without cells, to determine
103 production/depletion of media metabolites.
104

Table S3. Summary of patient cohort for PD-L1 biomarker study.

Sample ID	Sex	Age	Diagnosis	PD-L1 status	Average % infection
203	62	Female	lung squamous cell carcinoma	+	0.5
204	83	Female	endometrial cancer	-	4.0
205	63	Male	papillary renal cell carcinoma	+	33.5
215	57	Female	ovarian cancer	-	0.9
221	67	Female	endometrial cancer	+	22.6
223	68	Female	lung cancer	+	27.9
224	70	Female	hepatocellular carcinoma	-	1.3
225	73	Male	lung squamous cell carcinoma	+	6.3
226	48	Male	colon adenocarcinoma	-	0.5
227	74	Female	lung non-small cell carcinoma	-	2.3
241	57	Female	ovarian cancer	+	0.2
242	73	Female	chromophobe renal cell carcinoma	-	0.1
247	62	Female	colon adenocarcinoma	+	9.3
248	63	Female	ovarian cancer	-	2.9
249	56	Male	dermatofibrosarcoma protuberans	-	0.2
253	62	Male	neuroendocrine tumor	-	3.8
257	67	Female	uterine fibroid	+	3.5
260	61	Female	fibrous tumor of the abdomen	+	11.4
283	52	Female	breast carcinoma	+	1.5
289	79	Female	hepatic adenocarcinoma	-	8.2
291	59	Male	leiomyosarcoma	+	0.1

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106 **Table S3: Summary of patient cohort.**

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