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Supplemental information

IFN-I-tolerant oncolytic Semliki Forest virus in combination with anti-PD1 enhances T cell response against mouse glioma

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Supplemental methods

Mouse CT-2A and NXS2 cell lines

CT-2A-Fluc cells (provided by Jan Brun, Children's Hospital of Eastern Ontario) were cultured in Gibco RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 10 U/ml Penicillin-Streptomycin, and 1mM sodium pyruvate (Thermo Fischer Scientific). NXS2 cells were cultured in Gibco DMEM Glutamax (Thermo Fischer Scientific) supplemented with 10% FBS, PEST, and sodium pyruvate. For cell viability assay cells were seeded on 96-well plate (20,000 cells/well) and infected on the next day with virus (diluted in complete cell culture medium) at different MOIs. Cell viability was measured using CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI, US) according to manufacturer's instructions 72h after infection.

SFV production and titration

BHK-21 cells were seeded in a 6-well plate and transfected with virus cDNA plasmid using Lipofectamine 3000 reagent (Thermo Fischer Scientific). 48 hours after transfection, p0 virus stock was harvested. 200µl p0 stock was added to a confluent T175 flask of BHK-21 cells to produce the p1 stock, which was harvested 24h later and concentrated by ultracentrifugation on a 20% sucrose cushion (2h, 140,000 x g, 4°C).

Virus was titrated by infecting BHK-21 cells seeded on 6 well-plate (500,000 cells/well) were with 200µl of virus diluted in culture medium. 30mins later the cells were covered with 0.6% CMC-containing medium. The cell layer was stained two days later with crystal violet to visualize the plaques. Titer was counted as plaque forming units (PFU)/ml.

xCELLigence assay

50µl culture medium was added into each well and incubated at room temperature for 30 min for the impedance baseline measurement. 10,000 GL261 cells were added into each well in 100µl volume and incubated at room temperature for 30min. Virus (MOI = 0.01) was added at

indicated timepoint in 50µl volume. The plates were monitored in the system at 37°C under 5% CO_2 at 15-min time intervals.

Staining of slice cultures

Blocking overnight (2% BSA, 0.25% Triton X-100 in PBS), primary antibody staining overnight at +4°C with rabbit anti-SFV (kind gift from Ari Hinkkanen, University of Eastern Finland, Finland) and hamster anti-CD31 (Thermo Fisher Scientific, 2H8) diluted in blocking buffer, washing 5x10 min at room temperature with 0.25% Triton X-100, Secondary antibody staining overnight at +4°C with Life Technologies Alexa Fluor 488-conjugated donkey anti-rabbit and Alexa Fluor 647-conjugated goat Anti-hamster (Thermo Fischer Scientific), washing as before, staining with Invitrogen Hoechst 33342 (Thermo Fischer Scientific), washing as before, mounting on microscope cover slide (SuperFrost) using Fluoromount-G (Thermo Fischer Scientific). Imaging of the slides was done with confocal microscope (Leica, Germany).

Table S1. Primers used in RT-qPCR analysis.

mIFNb-forw	AAGAGTTACACTGCCTTTGCCATC
mIFNb-rev	CACTGTCTGCTGGTGGAGTTCATC
mPD-L1-forw	TGCTGCATAATCAGCTACGG
mPD-L1-rev	GCTGGTCACATTGAGAAGCA
mTNFa-forw	TGGCCCAGACCCTCACACTCAG
mTNFa-rev	ACCCATCGGCTGGCACCACT
mIFIT1-forw	CCATAGCGGAGGTGAATATC
mIFIT1-rev	GGCAGGACAATGTGCAAGAA
mISG15-forw	GGTGTCCGTGACTAACTCCAT
mISG15-rev	CTGTACCACTAGCATCACTGTG
mCXCL10-forw	GGATGGCTGTCCTAGCTCTG
mCXCL10-rev	TGAGCTAGGGAGGACAAGGA
mGAPDH-frow	CAAGGAGTAAGAAACCCTGGACC
mGAPDH-rev	CGAGTTGGATAGGGCCTCT
mISG20-forw	CAATGCCCTGAAGGAGGATA
mISG20-rev	TGTAGCAGGCGCTTACACAG

Table S2. Antibodies used in FACS analysis

Marker	Flurocrome	Clone	Company
CD45	BV510	30-F11	BD
CD3e	BV421	145-2C11	BD
CD8	APC-Cy7	53-6.7	BD
CD4	BUV496	GK1.5	BD
CD44	FITC	IM7	Biolegend
CD62L	PE-Cy7	MEL-14	BD
CD69	BB700	H1.2F3	BD
CD127	PE-Cy5	A7R34	Biolegend
PD1	BV785	29F.1A12	Biolegend
TIM-3	BV605	RMT3-23	Biolegend
LAG-3	BV711	C9B7W	BD
Ki67	PE dazzle594	16A8	Biolegend
CX3CR1	BV650	SA011F11	Biolegend
KLRG1	BUV737	2F1	BD
Foxp3	A647	150D	Biolegend
CD25	PE	3C7	Biolegend
Livedead	Fixable dye 700		BD
CD45	BV510	17A2	BD
B220	APC-Cy7	RA3-6B2	Biolegend
IA/IE	BB700	M5/114.15.2	BD
CD11b	BUV395	M1/70	BD
Ly6G	BV421	1A8	BD
CD11c	PE-Cy5	N418	Biolegend
Ly-6C	BV785	HK1.4	Biolegend
NK1.1	BUV737	PK136	BD
Livedead	Fixable dye 700		BD
H-2Kb MuLV p15E Tetramer-KSPWFTTL	APC		MBL International Corporation

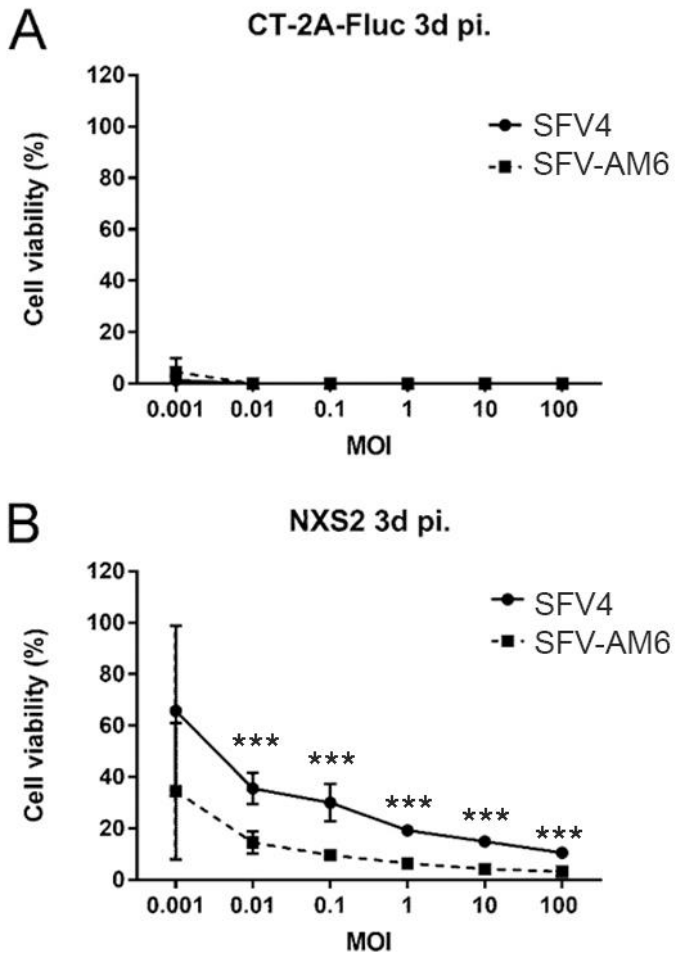

Figure S1. Cell viability of infected glioma CT-2A-Fluc **(A)** and neuroblastoma NXS2 **(B)** cells measured with MTS assay (mean ± SD) 72 hours after infection using different MOIs. Statistical analysis was performed by two-tailed, unpaired t-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

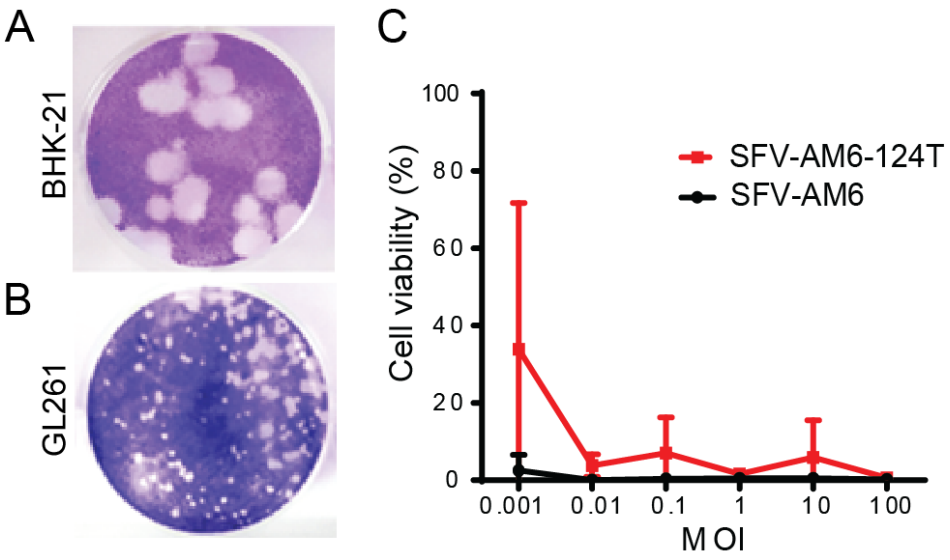

Figure S2. miRNA-124 de-targeted SFV-AM6-124T retains oncolytic potency in GL261 cells. A: Plaque phenotype of SFV-AM6-124T in BHK-21 cells. **B:** Plaque phenotype of SFV-AM6-124T in GL261 cells. **C:** Viability of GL261 cells measured with MTS assay 72h after infection (mean ± SD).

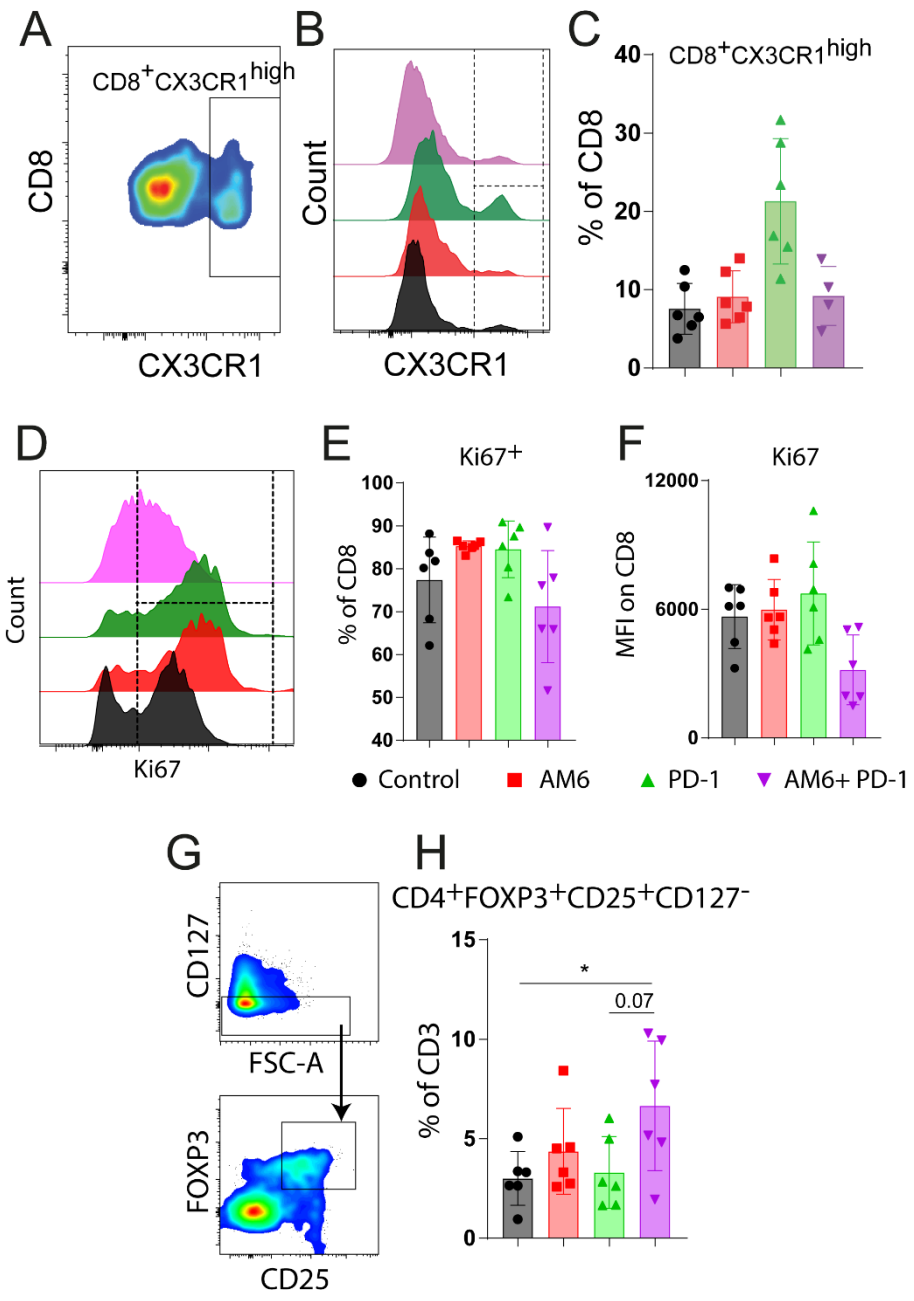

Figure S3. T-cell phenotype after SFV-AM6-124T and anti-PD1 treatments: A: Flow cytometry-gating strategy for quantification of CX3CR1 expressing $CD8^+$ T cells. **B:** Histograms showing CX3CR1 on $CD8^+$ T cells in the different treatment groups, and **C:** quantification of CX3CR1 in $CD8^+$ T cells. **D:** Histograms showing Ki67 expression on $CD8^+$ T cells in the different treatment groups. **E:** quantification of $Ki67^+$ $CD8^+$ T cells and **F:** MFI of Ki67 on $CD8^+$ T cells. **G:** Flow cytometry-gating strategy for quantification of Tregs and **H:** quantification of $CD4^+$ $CD25^+FOXP3^+CD127^-$ Tregs. Data plotted as mean ± SD.