

Supplementary Data

**Dual-Armed Oncolytic Myxoma Virus Encoding IFN- γ
and CD47 Promotes Lymphocyte Infiltration and
Tumor Suppression of Syngeneic Murine Melanoma**

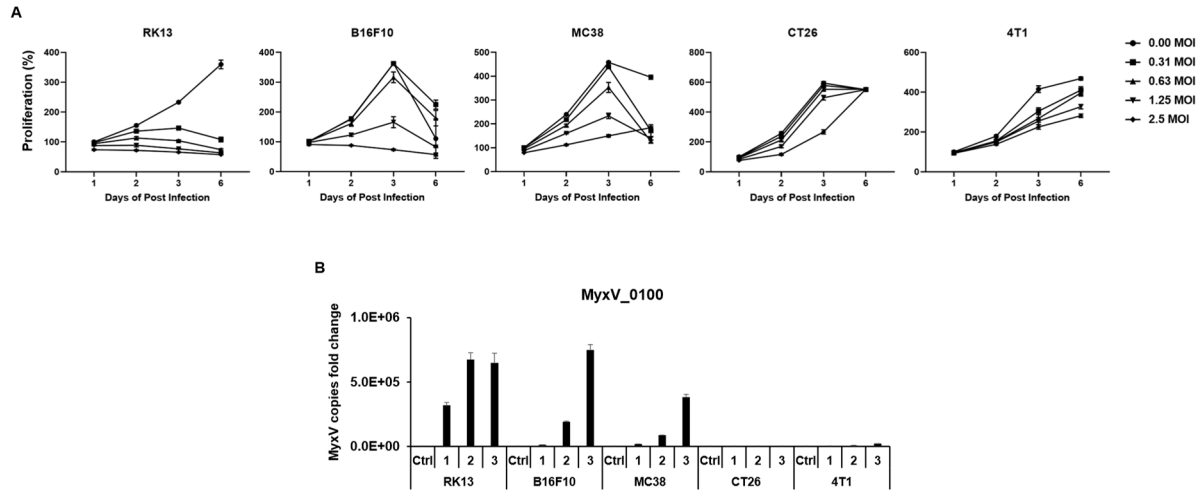


Figure S1. Oncolytic and replication activity of MyxV_0100 in murine cancer cell lines

A. Graphs show the relative proliferation rates of four different murine cancer cell lines and a rabbit-derived cell line RK13 when infected with different MOI of MyxV_0100 for 1-, 2-, 3-, and 6- days post-infection, respectively. **B.** RK13 and murine cancer cell lines B16F10, MC38, CT26, and 4T1 were used for virus amplification. The cells were infected with 0.2MOI of MyxV_0100, and PCR analysis was conducted at 1, 2, and 3 days of post-infection. Virus amplification was compared with the uninfected control. β -actin was used as an internal control.

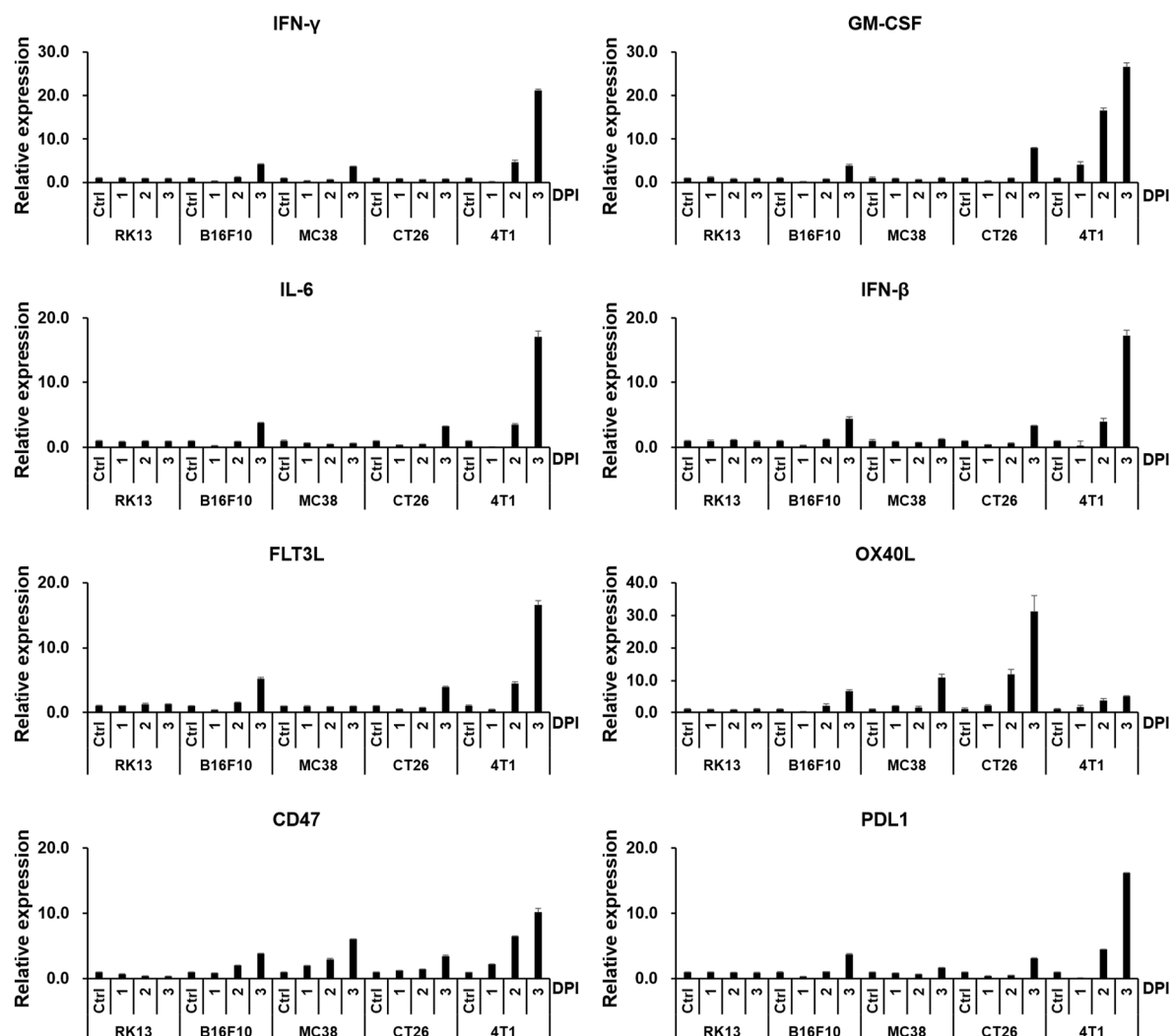


Figure S2. Expression of cytokines and the immune checkpoint-related genes in MyxV_0100 infected murine cancer cell lines

RK13 and murine cancer cell lines B16F10, MC38, CT26, and 4T1 were used for qPCR analysis. The cells were infected with 0.2MOI of MyxV_0100, and qPCR analysis was conducted at 1-, 2-, and 3-days post-infection. The expression of cytokines or cell receptors was compared with the uninfected control. β -actin was used as an internal control.

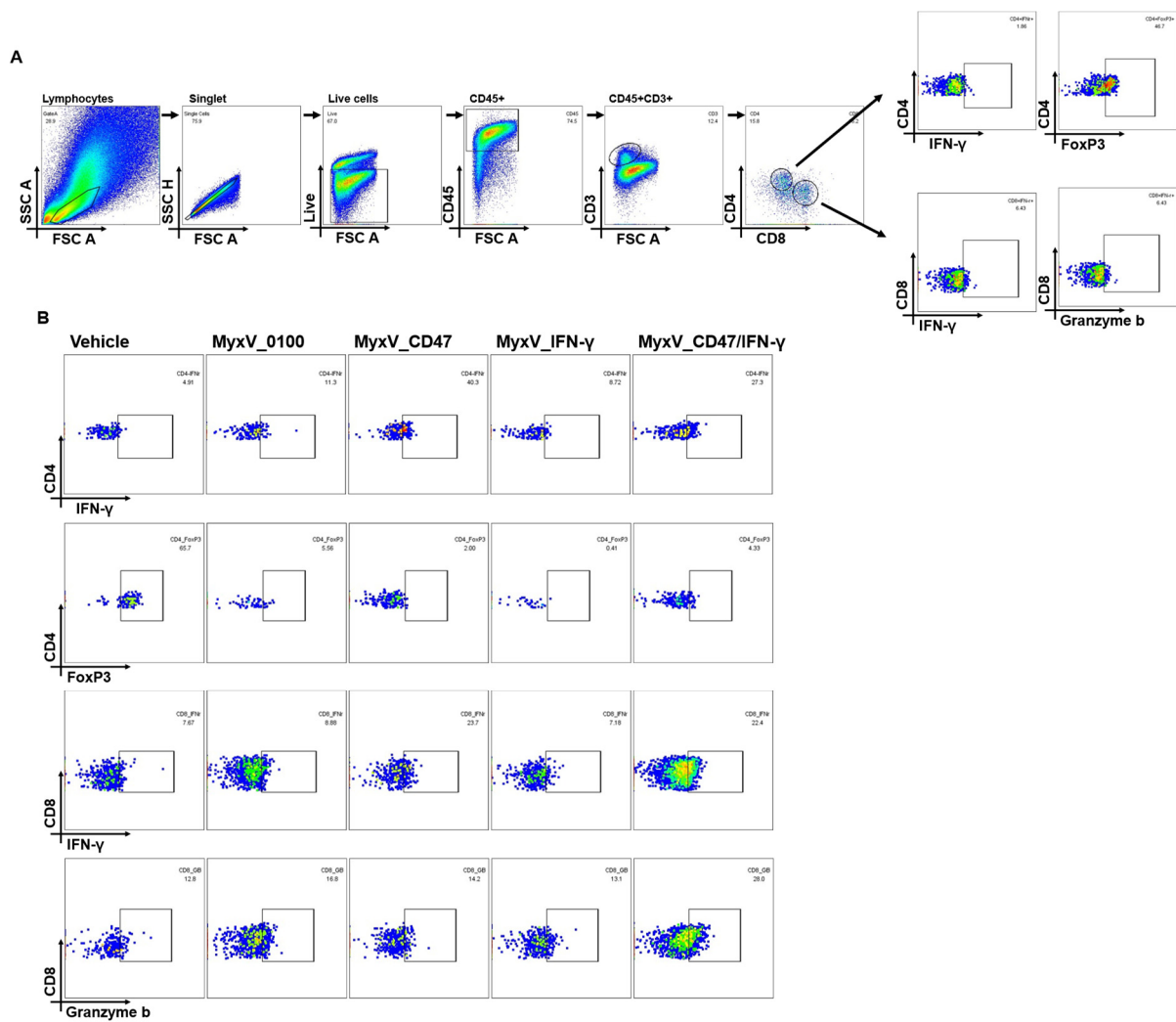


Figure S3. FACS analyses of tumor-infiltrated T-cell subsets in tumor.

A. Gating strategy for phenotypic analysis of T-cell subsets in the tumor. B. Representative FACS plot of T-cell subsets (CD4⁺ IFN-γ⁺, CD4⁺ Foxp3⁺, CD8⁺ IFN-γ⁺, and CD8⁺ Granzyme b⁺).

Table S1. Primer List

Gene	5' → 3'	Sequence	Size (bp)	Gene ID
Myxoma virus	Forward	GTTGGTAATTTCAATTCGTACCA	119	NC_001132.2
	Reverse	GGTAAACAATTAATCCGTTGTAA		
IFN- γ	Forward	AGCCTCAGGAAGCGGAAAAG	354	NM_008337.4
	Reverse	TGAGTTCAGTCAGCCGCTTG		
GM-CSF	Forward	GGCTGCAGAATTTACTTTTCCTGGG	366	NM_009969.4
	Reverse	GGCTGTCTATGAAATCCGCATAGG		
IL-6	Forward	GAGGATACTCACTCCCAACAGACC	141	NM_031168.2
	Reverse	AAGTGCATCATCGTTGTTCATACA		
IFN- β	Forward	CAACAGGTGGATCCTCCACG	446	NM_010510.2
	Reverse	GTTTAAGGTACCTTTGCACCCTCC		
FLT3L	Forward	CAGCCTGGAGCCCAAATTCC	599	NM_001402831.1
	Reverse	CAGCAGCACCAGTGTGAGAGG		
OX40L	Forward	GGAAGGGGTTCAACCCCTGG	543	U12763.1
	Reverse	GCACAGTATCCAGGCGTTAGC		
CD47	Forward	CCATATTGATCACTCAAGTGC	129	AB012693.1
	Reverse	GTTCTGCTAGAGCTATGATC		
PD-L1	Forward	GCTGGCATTATATTCACAGC	378	GQ904196.1
	Reverse	GACTTTCAGCGTGATTCTG		
β -Actin	Forward	TGGAATCCTGTGGCATCCATGAAAC	349	NM_007393.5
	Reverse	TAAAACGCAGCTCAGTAACAGTCCG		

Table S2. Antibody List

Antibody Name	Clone	Company	Cat No.	Usage
Anti-CD47 APC	miap301	eBioscience	17-0471-82	IF
Anti-CD47 FITC	miap301	eBioscience	11-0471-82	FACS
Anti-IFN- γ PE	XMG1.2	eBioscience	12-7311-82	IF
Anti-IFN- γ APC	XMG1.2	eBioscience	17-7311-82	FACS
Anti-CD45 Super Bright™ 780	30-F11	eBioscience	78-0451-82	FACS
Anti-CD3e APC-eFluor™ 780	145-2C11	eBioscience	47-0031-82	FACS
Anti-CD4 FITC	GK1.5	eBioscience	11-0041-82	FACS, IF
Anti-CD8a Super Bright™ 645	53-6.7	eBioscience	64-0081-82	FACS, IF
Anti-FoXP3 eFluor™ 450	FJK-16s	eBioscience	48-5773-82	FACS
Anti-Granzyme B PerCP-eFluor™ 710	NGZB	eBioscience	46-8898-82	FACS
Anti-CD8 alpha antibody	EPR21769	abcam	ab217344	IHC
Anti-CD4 antibody	EPR19514	abcam	ab183685	IHC
Anti-CD11c antibody Alexa Fluor® 594	N418	Biolegend	117346	IF