

**OMTO, Volume 18**

**Supplemental Information**

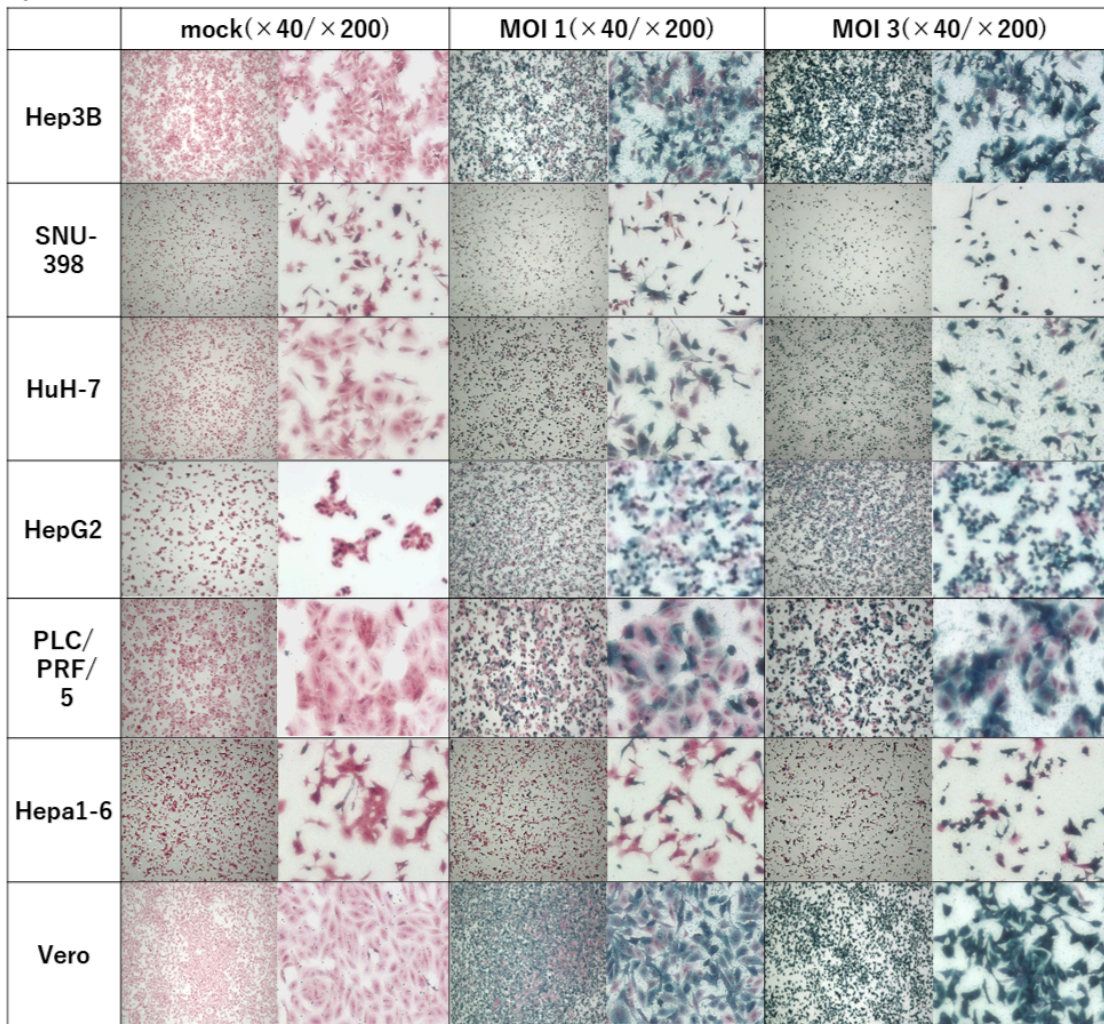
**Neoadjuvant Use of Oncolytic Herpes**

**Virus G47 $\Delta$  Enhances the Antitumor**

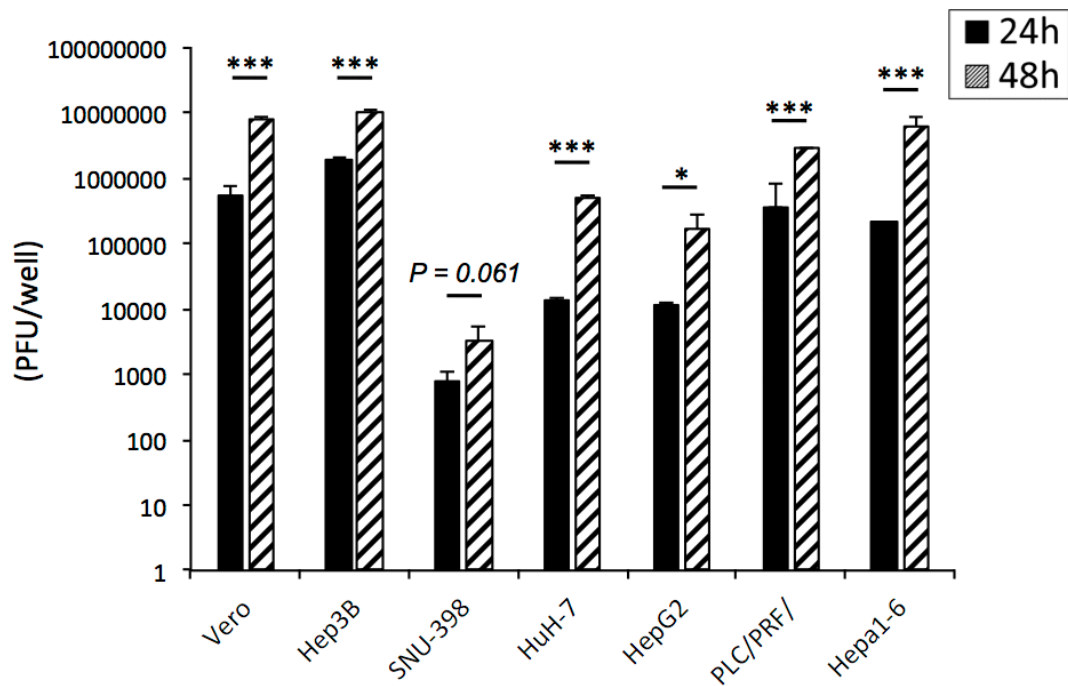
**Efficacy of Radiofrequency Ablation**

**Tomoharu Yamada, Ryosuke Tateishi, Miwako Iwai, Kazuhiko Koike, and Tomoki Todo**

(A)



(B)



## Supplementary Fig. S1

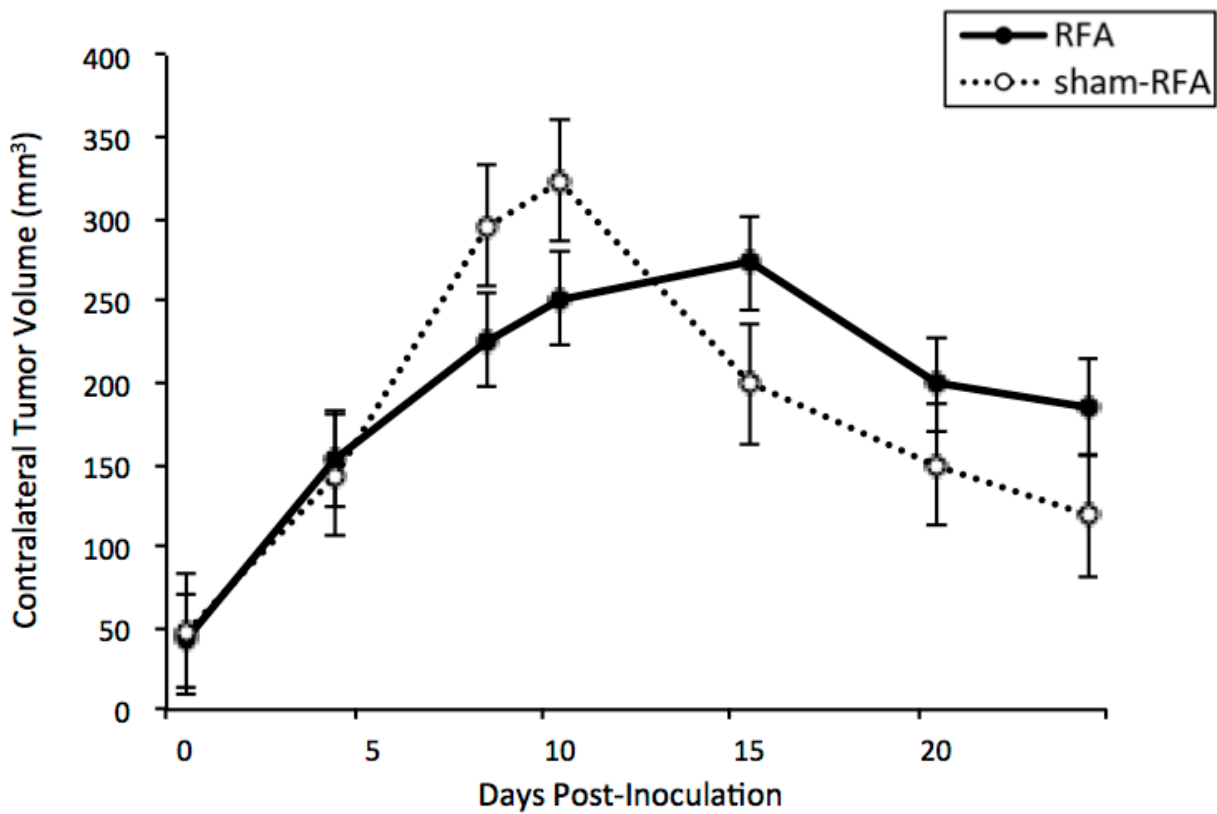
### *In vitro X-gal staining and virus replication assay*

(A) Hep3B, SNU-398, HuH-7, HepG2, PLC/PRF/5, Hepa1-6 and Vero cell monolayers were infected with G47 $\Delta$  at a MOI of 1 or 3, incubated for 6 hours, fixed, and stained with X-gal. (B)

The cell monolayers were infected with G47 $\Delta$  ( $3 \times 10^3$  pfu) and incubated at 37°C for 24 or 48 hours.

Virus yields were evaluated by plaque assay on Vero cells. The data are means (n = 3); bars

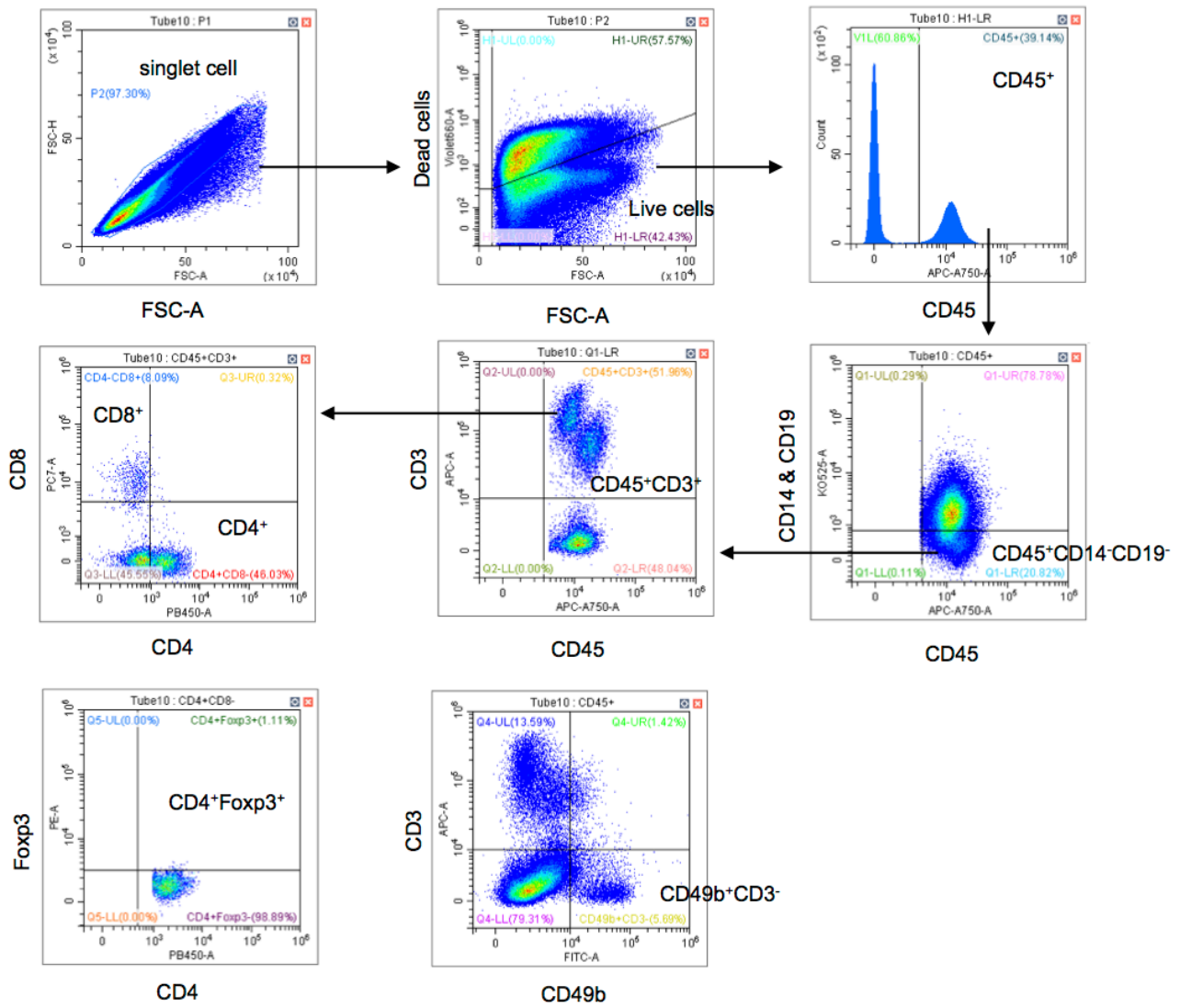
represent SD. \*,  $P < 0.05$ . \*\*\*,  $P < 0.001$ .



**Supplementary Fig. S2**

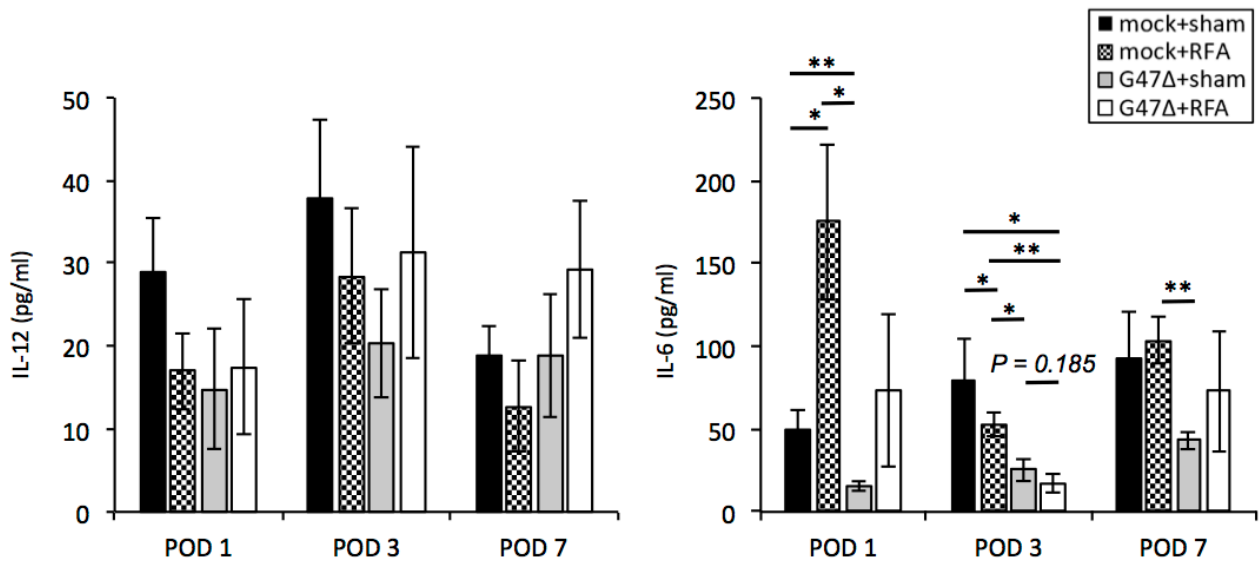
*MH134-TC is too immunogenic to evaluate the antitumor efficacy of G47Δ in combination with RFA*

MH134-TC was inoculated subcutaneously on bilateral flanks, and when tumors reached 6–8mm in diameter, the left tumors were treated with RFA or sham-RFA on day 4. Contralateral tumor volume was evaluated. Regardless of whether RFA was performed, all contralateral tumors eventually shrank. The data are means (n = 7); bars represent SEM.



**Supplementary Fig. S3**

*Determination of tumor-infiltrating lymphocytes (TILs) subsets by a flow cytometry gating strategy*



### Supplementary Fig. S4

#### *Serum cytokine analyses of the G47Δ and RFA combination therapy*

The left subcutaneous Neuro2a tumors (average 78 mm<sup>3</sup>) were treated as described in Fig. 2A. The serum was collected on days 13 (POD 1), 15 (POD 3) and 19 (POD 7). Levels of interleukin (IL)-12 and IL-6 were measured using the BioPlex system. For IL-12, the combination therapy did not cause any significant difference between other therapies. The combination therapy significantly decreased the IL-6 level compared with RFA monotherapy on POD 3. The data are means (n = 5); bars represent SEM. \*,  $P < 0.05$ . \*\*,  $P < 0.01$ .

Description	Fluorochromes	company
anti-mouse CD49b	FITC	BioLegend
anti-mouse Foxp3	PE	eBioscience
anti-mouse CD8a	PE/cy7	BioLegend
anti-mouse CD3	APC	BioLegend
anti-mouse CD45	APC/cy7	BioLegend
anti-mouse CD4	BV421	BioLegend
anti-mouse CD14	BV510	BioLegend
anti-mouse CD19	BV510	BioLegend
Fixable Viability Dye	BV570	BioLegend
Purified Anti-mouse CD16/32		BioLegend

### **Supplementary Table S1**

*The antibodies used in the flow cytometric analysis*